SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL ACTIVITY STUDIES OF NITROGEN AND SULPHUR HETEROCYCLIC COMPOUNDS

THESIS
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BY

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CHAPTER-I

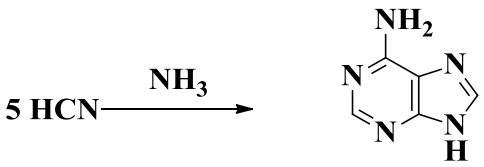
INTRODUCTION

1. Multicomponent Reactions:

Multicomponent reactions (MCRs)[1-3] are powerful synthetic organic reactions in which three or more different functionalized organic molecules are transformed to give the target product in one pot reaction. Multicomponent reactions generate the products with high chemical and structural diversity. The MCRs possess ideal synthetic properties such as high atom economy incorporating the essential functional groups of the starting reactants into sophisticated target product, mild reaction conditions, energy efficient, eco friendly, time efficient, cost effective and operation simplicity. Recently MCRs are widely used in the field of organic synthesis[4], agro chemistry[5-7], combinatorial chemistry[8-12], polymer chemistry[13-17], medicinal chemistry[18-21] and natural product[22,23] synthesis.

1.1 History of Multicomponent Reactions:

Multicomponent reaction itself exist in nature in the evolution process[24]. DNA and RNA purine base (adenine) is formed by the multicomponent reaction method through ammonia catalysed condensation reaction of hydrogen cyanide (Scheme 1).

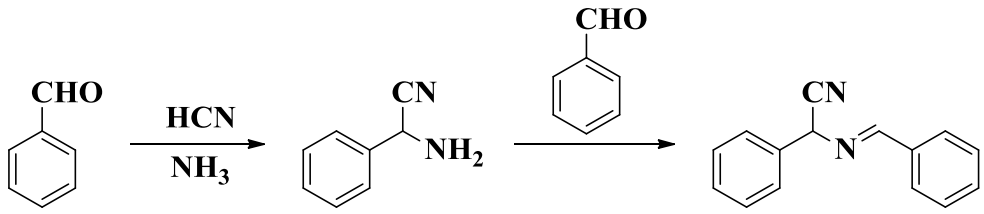


Scheme.1

The following are some important multicomponent reactions based on named organic reactions.

Laurent and Gerhardt multicomponent reaction:

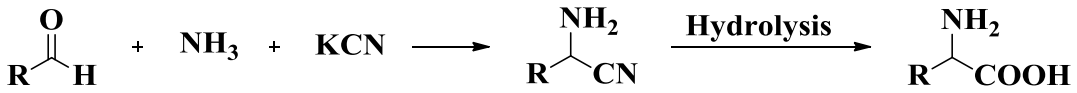
Laurent and Gerhardt[25] reported the multicomponent synthesis of "benzoyl azotid". The reaction of bitter almond oil in presence of ammonia and hydrogen cyanide generates the amino benzyl cyanide followed by reaction of with another mole of bitter almond oil to give the Schiff's base of benzyl cyanide i.e. "benzoyl azotid" (**Scheme 2**).



Scheme.2

Strecker's three component amino acid synthesis (S-3CR):

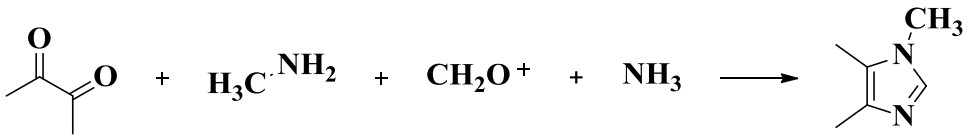
The first official report on multicomponent reactions was reported by Strecker[26] in 1850. Strecker developed a one pot three component reaction for α – amino nitriles by the reaction of aldehyde, hydrogen cyanide and ammonia and subsequent hydrolysis of α - amino nitriles.



Scheme.3

Radziszewski imidazole synthesis:

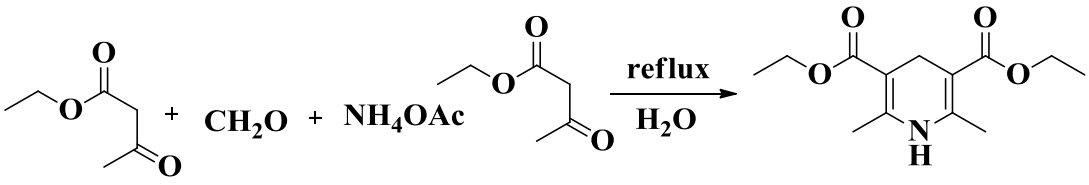
One pot four component reaction was developed by Radziszewski for the synthesis of substituted imidazoles[27]. The reaction of 1,2-dicrbonyl compounds with formaldehyde, primary amine and ammonia gave the substituted imidazoles as shown below.



Scheme.4

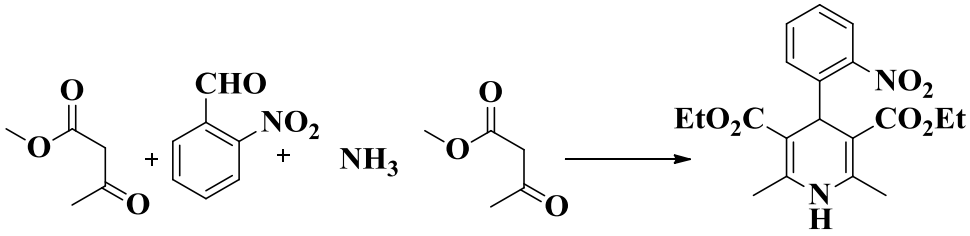
Hantzsch dihydropyridine synthesis:

Hantzsch[28] developed a four component reaction for the synthesis of substituted 1,4-dihydropyridines. Cyclocondensation reaction between two equivalent ethylacetoacetate, aldehyde and ammonium acetate generate the dihydropyridines.



Scheme.5

Hantzsch dihydropyridine methodology was successfully applied in the medicinal chemistry for synthesize the cardiovascular drug "Nefedipin".

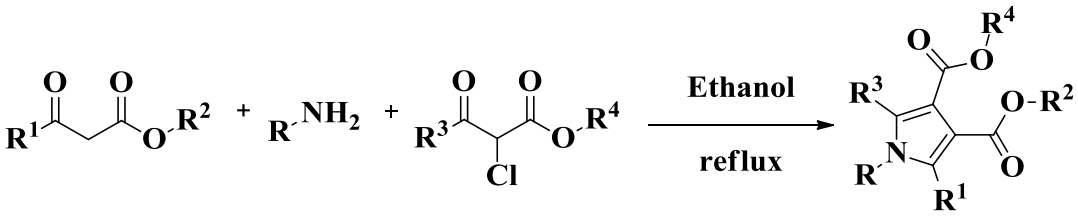


Nefedipin

Scheme.6

Hantzsch pyrrole synthesis:

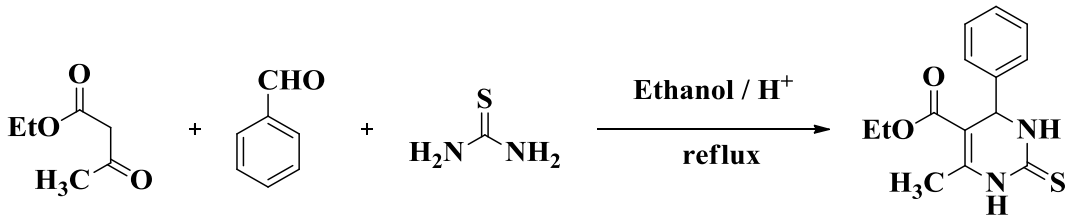
Another multicomponent reaction was developed by Hantzsch for the synthesis of pyrroles. Pyrroles[29] are synthesized by the reaction of β–keto esters with primary amines and α-halo β – keto esters.



Scheme.7

Biginelli Reaction:

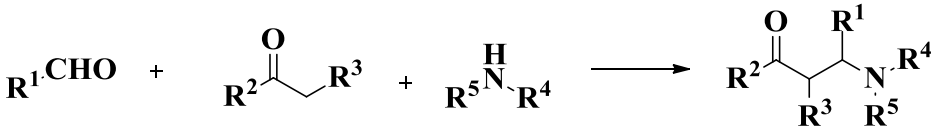
An Italian chemist, Pietro Biginelli[30] developed a multicomponent reaction for the synthesis of dihydropyrimidines. Acid catalysed three component reaction of β–keto esters with aldehydes and urea in presence of ethanol under reflux condition gave the dihydropyrimidine derivatives.



Scheme.8

Mannich reaction:

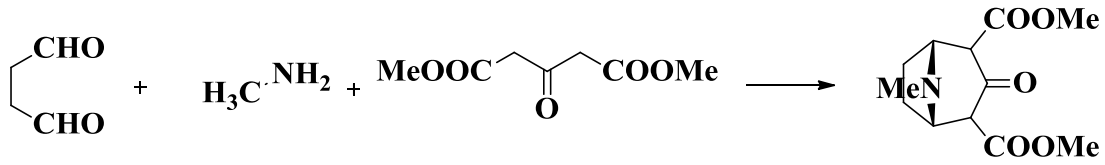
Carl Mannich[31] introduced a one pot multicomponent C-C and C-N bond formation reaction**.** Condensation of aldehyde, amine with active methylenic carbonyl compounds generate the corresponding derivatives.



Scheme.9

Robinson's synthesis of tropinone:

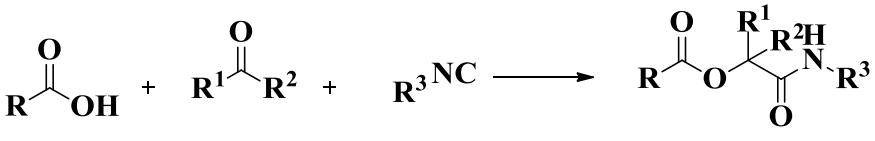
Mannich reactions are extensively utilized in organic synthesis particularly in natural product synthesis. Robinson[32] synthesized tropinone alkaloid by using double Mannich reaction of succinaldehyde, methylamine and dimethyl 3-oxopentanedioate.



Scheme.10

Passerini reaction:

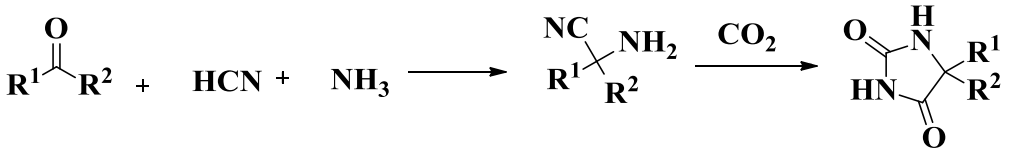
Passerini[33] developed first isocyanides based multicomponent reaction. α- Acyloxy carbamides were synthesized by the reaction of carboxylic acid, carbonyl compound and an isocyanide.



Scheme.11

Bucherer Bergs Hydantoin synthesis:

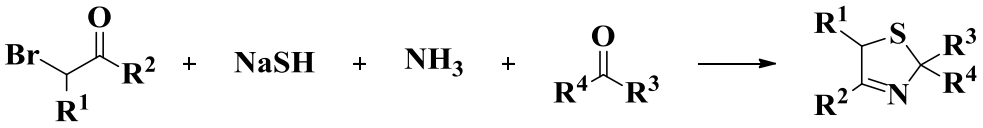
Bucherer and Bergs[34] described the four component reaction for hydantoin synthesis. The reaction involves the cyclocondensation of hydrogen cyanide, carbonyl compounds, ammonia and carbon dioxide to generate the hydantoin.



Scheme.12

Asinger reaction:

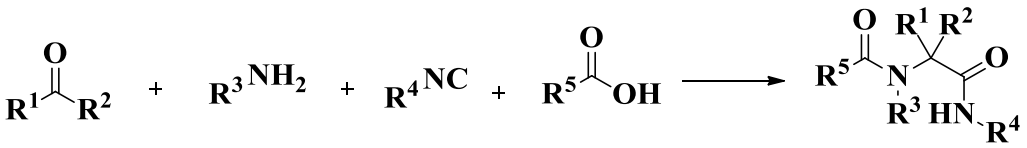
Asinger[35] developed a multi component reaction for the synthesis of thiazoline. The synthesis involves the reaction of alpha halogenated carbonyl compounds with sodium hydrosulphide and ammonia to form thiazoline as shown in **Scheme 13**.



Scheme.13

Ugi reaction:

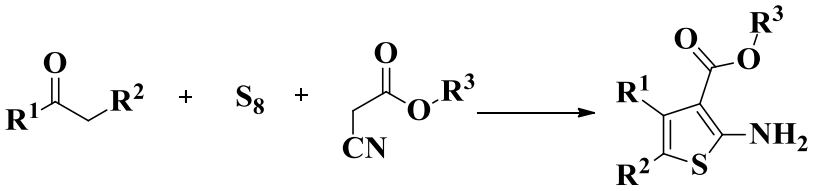
Ivar Karl Ugi[36] developed one of the most important four component reaction. Ugi synthesized α-acylamino amides by the condensation reaction of aldehydes, amines, carboxylic acids and isonitriles (**Scheme-14**). The ugi reaction is the most extensively studied and applied in organic synthesis.



Scheme.14

Gewald Reaction:

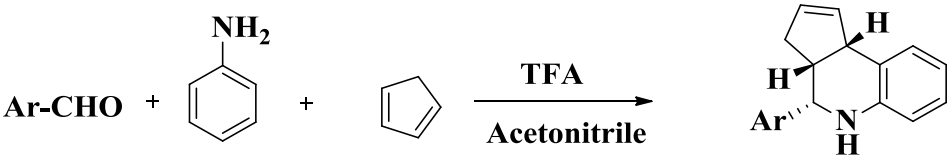
Karl Gewald[37] developed an efficient method for the synthesis of poly substituted 2-amino thiophenes via a three component reaction. The target compound was obtained by the reaction of α-methylene carbonyl compounds, α-cyanoesters and elemental sulphur in presence of base (**Scheme 15**).



Scheme.15

Grieco three component synthesis of tetrahydroquinolines:

Grieco[38] reported the three component reaction for the synthesis of tetrahydroquinolines. The target compounds were obtained by the cycloaddition reaction of aldehydes, amines and cyclopentadiene in presence of trifluroacetic acid.



Scheme.16

1.2 Classifications of Multicomponent reactions

Multicomponent reactions can be classified based on the reactants involved in the synthetic route as three component reactions, four component reactions, five component reactions etc.

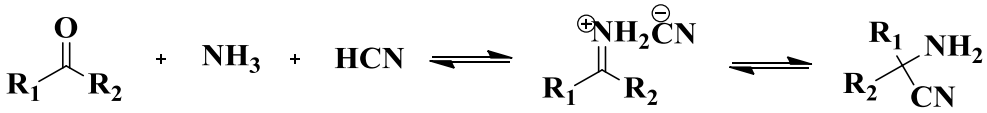
Multicomponent reactions can be classified based on the functional groups available on the reactants as following.

1) Imine based multicomponent reactions
2) Isocyanide based multicomponent reactions

Many known multicomponent reactions belong to the first type of classification multicomponent reactions. In this type of reactions condensation of carbonyl functional group containing compounds with amines lead to the formation of imine functional group in the products. Where as in isocyanide based multicomponent reactions isocyanide is the starting material for the reaction.

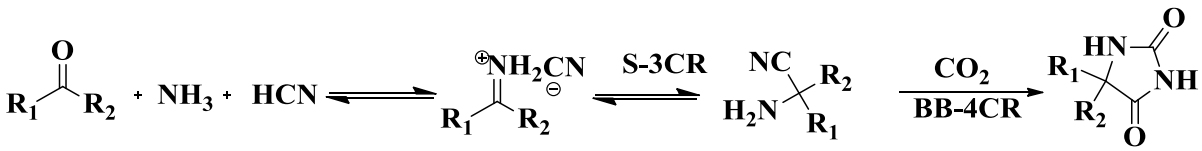
Multicomponent reactions may be classified into three types based on the reaction kinetic paths i.e. reactivity and isolability of the products (reversible or irreversible).

Type-I: In this type of multicomponent reactions starting materials, intermediates and products are equilibrium to each other, the isolability and yield of the reaction depends on the thermodynamics of the reaction.



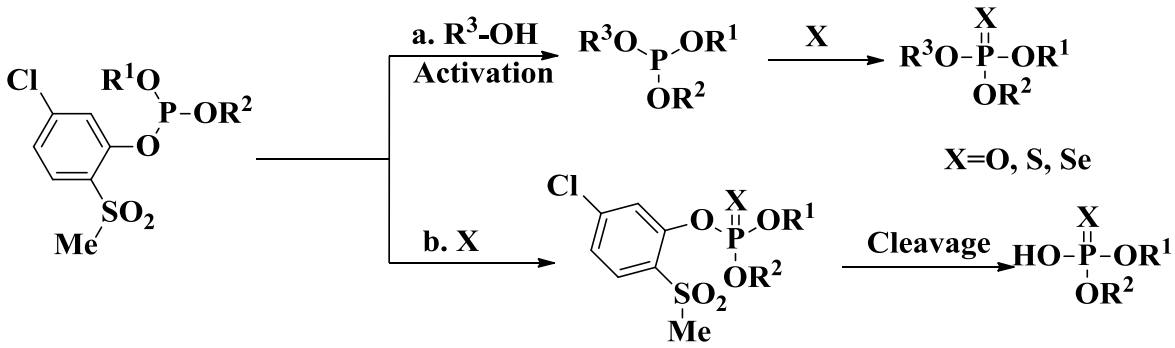
Scheme.17

Type-II: In this kind of reactions equilibrium exist between reactants and intermediates and the reaction is terminated by irreversible step between intermediate and product.



Scheme.18

Type-III: In this type of reactions one product is formed by all sub reactions involving irreversible steps.



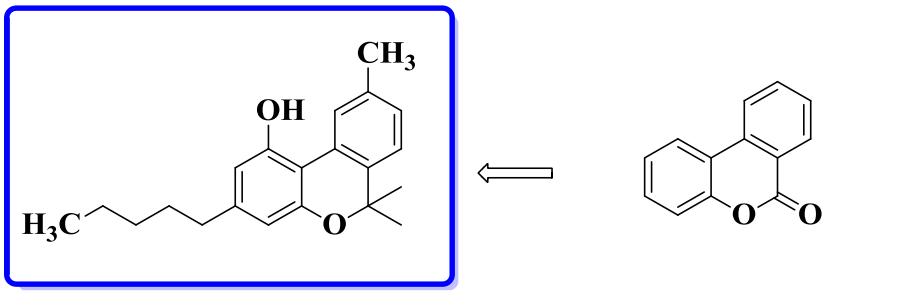
Scheme.19

1.3 Applications of Multicomponent reactions

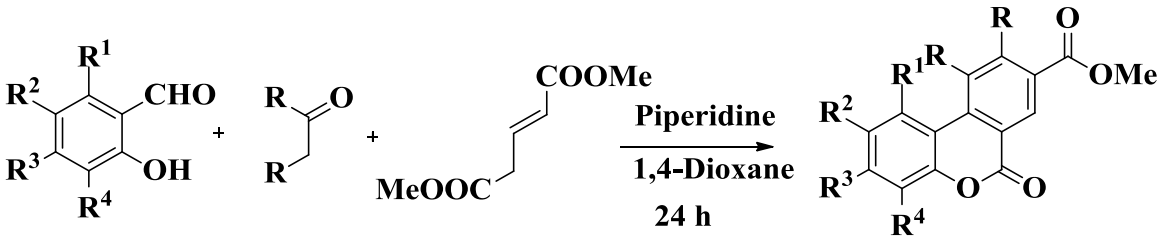
Applications in natural product synthesis:

A variety of natural products were extensively used in therapeutics. Due to their limited availability, their synthesis with quantitative yields is so important that they can be utilized for various purposes. In this direction, multi-component reactions are essential in the synthesis of natural products.

The cannabinols are important natural products which are used as central nervous system G-protein agonists. Cannabinol synthesis involves 6*H*-dibenzo[*b*,*d*]pyranone as one of the intermediate. Penchal Reddy[39] *et al.* developed an efficient multicomponent reaction method for the synthesis of 6*H*-dibenzo[*b*,*d*]pyranone (**Scheme - 20**).

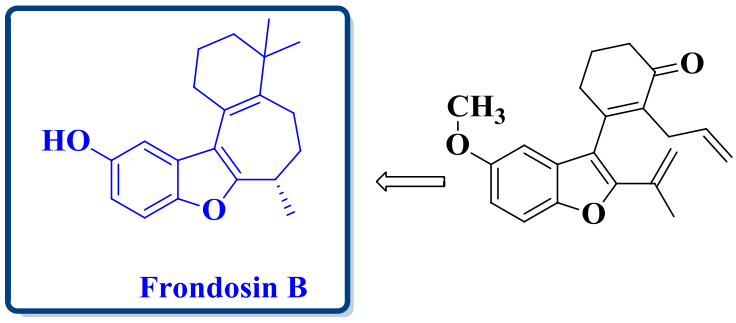


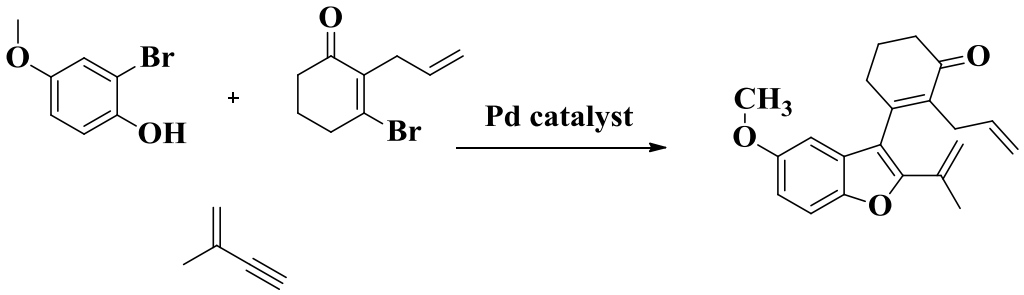
Cannabinol **6*H*-dibenzo[*b*,*d*]pyranone**



Scheme.20

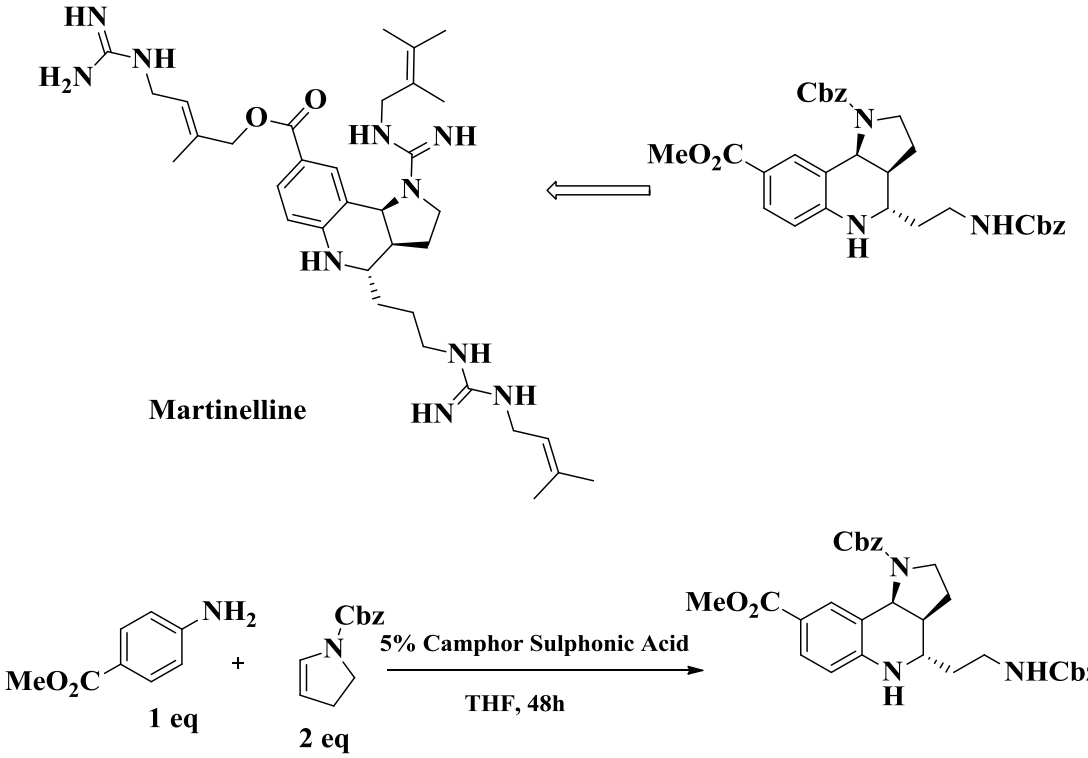
Frondosin B is a marine sesquiterpenoid isolated from marine sponge *Dysidea frondosa.* It acts as a interleukin-8 receptor antagonist. Due to its therapeutic significance, many natural product chemists inspired to synthesize the Frondosin B. Flynn *et al.*[40] developed a multicomponent reaction strategy starting from substituted 2-bromophenol, alkyne and bromoenone by palladium catalysis to provide the benzofuran derivative.





Scheme.21

Martinelline is the naturally occurring nonpeptidic bradykinin B2 receptor antagonist, isolated from the *Martinella iquitosensis* vine roots. The alkaloid contains hexahydropyrrolo[3,2-*c*]quinoline core structure with three pendant isoprenyl-derived guanidine moieties. David A. Powell and Robert A. Batey[41] developed multicomponent Povarov strategy to synthesized Martinelline precursor.

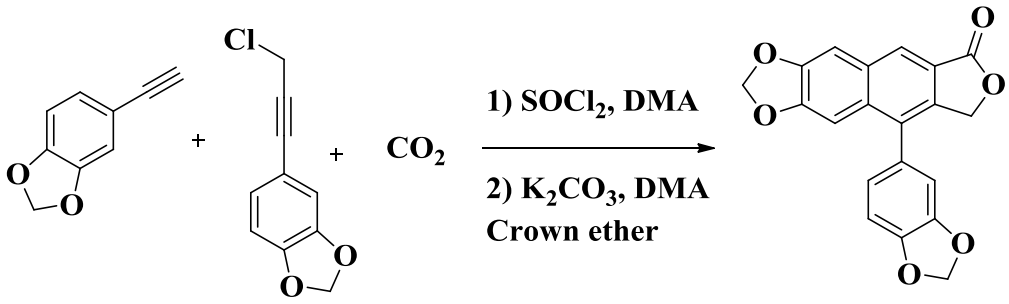


Scheme.22

Justicidine E is a naturally occurring Lignan class of arylnaphthalene lactone ring compounds. Foley *et al.*[42] developed a one pot multicomponent reaction for Justicidine. Justicidine E was synthesized from 5-ethynylbenzo[*d*][1,3]dioxole, 5-(3-chloroprop-1-yn-1-yl)benzo[*d*][1,3]dioxole and carbon dioxide.



Justicidin E



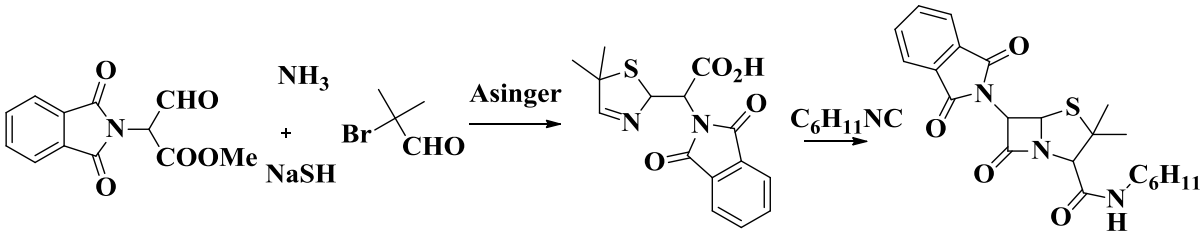
Scheme.23

Multicomponent reaction applications in synthesis of drugs:

Multi-component reactions are powerful organic transformation reactions for the synthesis of library of molecules and they are also used for the synthesis of drug like molecules. They are potent enough to synthesize the well known drugs like pencillin derivative, Crixivan, (S)-clopidogrel and bicalutamide.

Synthesis of Pencillin derivatives via Asinger and Ugi multicomponent reaction

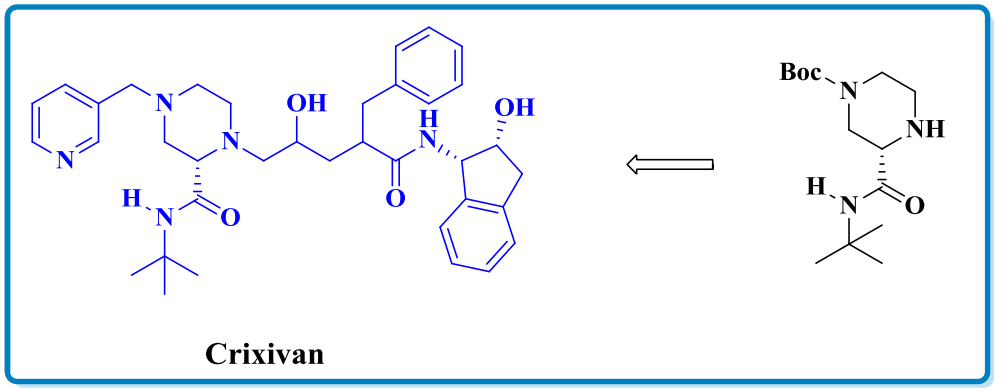
Ugi developed a multicomponent reaction for the synthesis of pencillin derivatives[43] by coupling Asinger and ugi reaction as shown in **Scheme 24**.

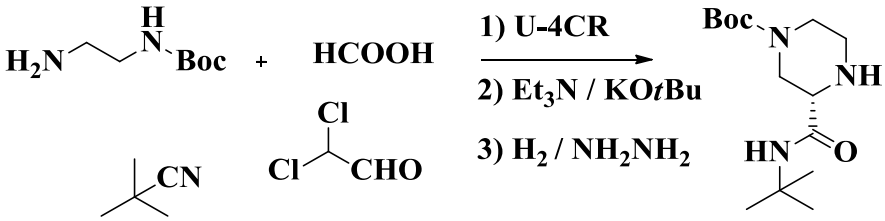


Scheme.24

Synthesis of HIV-Protease inhibitot Crixivan intermediate piperazine intermediate:

Rossen *et al*.[44] developed an efficient synthetic method for the synthesis Crixivan intermediate. The piperizine intermediate was synthesized using *N*-Boc-ethylenediamine, dichloroacetaldehyde, *tert*-butylisocyanide and formicacid using Ugi multicomponent reaction.

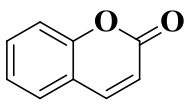




Scheme.25

2. Coumarins:

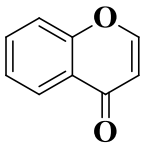
Coumarin is an important natural as well as synthetic heterocyclic compound which comes under oxygen heterocyclic system of benzopyran-2-one. A large variety of coumarins were ubiquitous in nature and isolated from many plants (Umbelliferae, Orchidaceae, Luguminoceae, Rutaceae etc.) and microorganisms by different extraction methods with tedious job. Coumarin was first isolated by Vogel[55] from Tonka beans. Coumarin was first synthesized by Henry perkin in 1868. Chemically coumarins can be synthesized by Pechmann[56], Knoevenagel[57,58], Perkin[59], Reformatsky[60,61], Claisen[62,63], Hoesch[64] and Wittig[65] cyclization reaction methods.



1

Benzo α-pyrone

(Coumarin)



2

Benzo-γ-pyrone

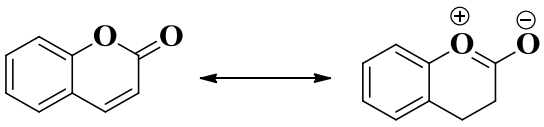
(Chromone)

Coumarins are classified into five groups

i. **Non-condensed coumarins**:
   Eg: Hydroxy coumarins, alkyl /aryl coumarins, alkoxy coumarins
ii. **C-prenylated coumarins**:
   Eg: Rutamarin, Umbelliprenin and Ostruthin
iii. Eg: Psoralen, Angelicin, Xanthotoxin and Spondin
iv. **Pyrano coumarins**
   Eg: Samidin, Visnadin and Xanthyetin
v. **Dimeric coumarins**
   Eg: 11-Methylgarberinol and Dapnoretin

2.1 General characteristics of coumarins:

The aromatic nature of heterocyclic ring of coumarin is under dispute due to its dual chemical reaction nature i.e. it exhibits both aliphatic and aromatic compounds characteristic properties. The lactone carbonyl (-O-C=O) functional group donates two lone pair of electrons to the ring system to exhibit 10 π-electron aromatic system.



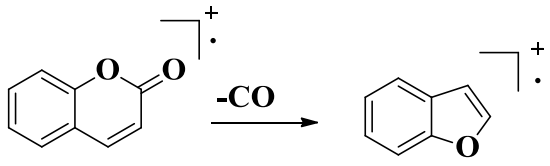
3

The UV spectrum[66,67] of coumarin and substituted coumarins exhibit characteristic absorption values. Introduction of substituents at different positions does not change the nature of UV spectrum. The λ_{max} and ε values of coumarin are 273 nm (40,368) and 309 nm (37,449).

The IR spectrum[68] of coumarin exhibits characteristic stretching frequency for lactone carbonyl at 1705, C=C stretching frequency at 1608 and 1450 cm^{-1}.

Dharmatti[69] *et al.* reported ^{1}H NMR spectrum of coumarin. The proton NMR shows characteristic resonance peaks for C3-H and C4-H at 6.45 and 7.80 ppm respectively.

Barnes and Occolowitz[70] studied the electron impact ionization mass spectra of coumarin. The molecular ion peak and fragmentation peaks shows the transformation of coumarin to benzofuran.



m/z 146 **m/z 118**

4

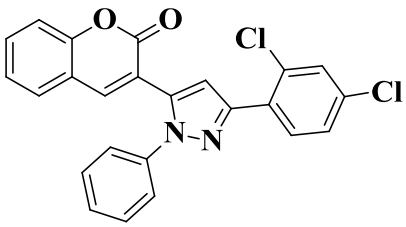
2.2 Applications of 3-heteryl coumarins:

Recently natural as well as synthetic 3-substituted coumarin derivatives have drawn remarkable attention of scientific community due to their wide applications in the field of therapeutics, optoelectronics, dyes, agrochemicals and material science. Moreover a variety of 3-substituted coumarin derivatives as a privileged heterocyclic scaffolds with diverse bioactivities such as antitumor, antimicrobial, carbonic anhydrase inhibitors, antioxidant, anti-coagulant, antitubercular and MAO-B inhibitor activities. So development of synthetic and semi synthetic coumarin heterocyclic scaffolds with multifunctional applications is essential. The concept of molecular hybridization is a useful method to develop the heterocyclic compounds and their derivatives with potential therapeutic values.

Antibacterial activity of 3-substituted coumarins:

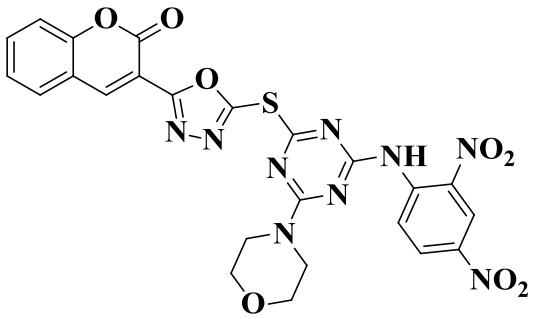
A series of 3-[3-(substituted phenyl)-1-phenyl-1*H*-pyrazol-5-yl]-2*H*chromen-2-one derivatives (**5**) were synthesized by Argade[71] *et al.* and studied their antibacterial activity against gram positive bacteria *Staphylococcus aureus*, *Bacillus subtilis* and gram

negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa*. Compound **5** exhibited excellent potency against all the tested bacteria.



5

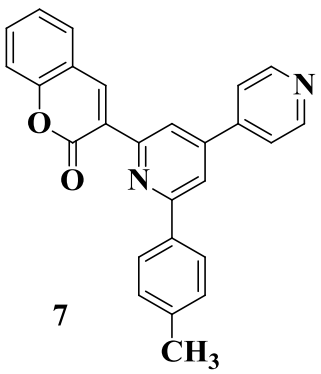
Modh[72] *et al*. synthesized a novel series of 3-substituted coumarinyl triazine (**6**) derivatives starting from coumarin-3-carboxylicacid and studied their antibacterial activity. The compounds exhibited promising antibacterial activity against gram positive (*S. aureus* and *Bacillus cereus*) and gram negative bacteria (*E.coli* and *S. typhi*).



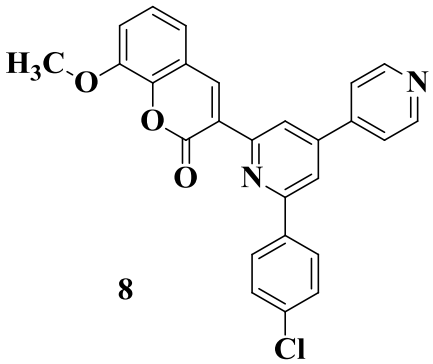
6

Antifungal activity of 3-substituted coumarins:

Lad[73] *et al*. studied antifungal activity of 3-bipyridyl substituted coumarins against *Candida albicans* and *Aspergillus niger* using Greseofulvin as antifungal standard drug. The compound 7 and 8 exhibited equipotent antifungal activity as compared to Greseofulvin standard drug.

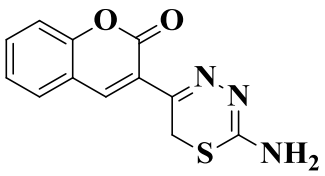


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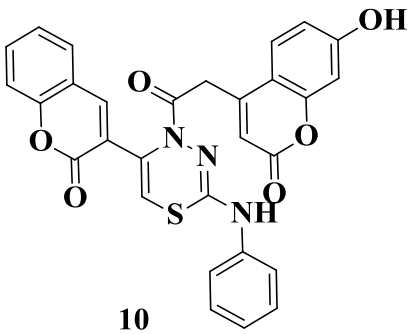


8

A series of coumarinyl 1,3,4-thiadiazines were synthesized by Cacic[74] *et al*. utilizing 3-(2-bromoacetyl)coumarins and thiosemicarbazide. The newly prepared compounds were evaluated for antifungal property against *A. ochraceus, A. flavus, F.verticillioides and F.graminearum.* The compounds 9 and 10 were exhibited potential antifungal activity.



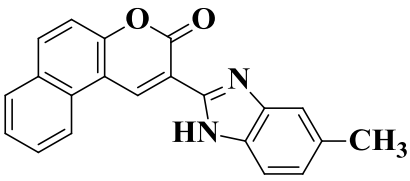
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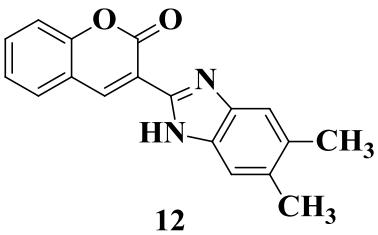
10

Antiviral activity of 3-substituted coumarins

A new series of benzimidazolyl – coumarins were synthesized by Tsay[75] *et al*. by using substituted phenylenediamine and coumarin-3-carboxylate in presence of *ortho*-phosphoric acid. The title compounds were screened for anti hepatitis-C- virus activity (HCV) and the compounds 11 and 12 exhibited excellent activity with high selectivity index value.

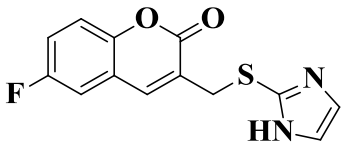


11



12

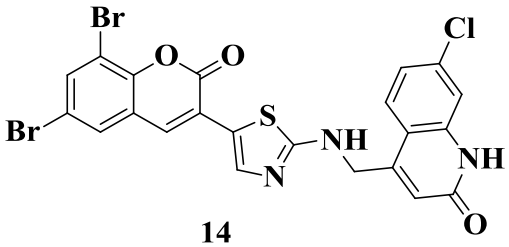
3-Imidazolylthio methyl-coumarin compounds were synthesized and studied their anti hepatitis C virus activity by Tsay[76] *et al*. The compound **13** has shown excellent antiviral activity with high significant index values.



13

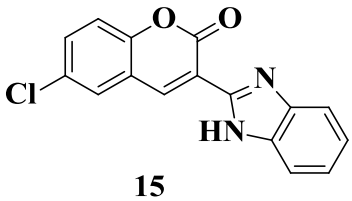
Anti-inflammatory activity of 3-substituted coumarins

Kalkhambkar[77] *et al*. synthesized thiazolyl coumarin heterocyclic compounds starting from the reaction of 3-(2-bromoacetyl)coumarins and studied their anti-inflammatory properties. The compound 14 has shown excellent activity as comparable with the standard drug.



14

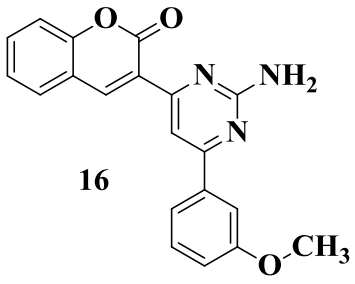
Novel coumarinyl benzimidazole derivatives were synthesized by Radha Krishna[78] *et al*. starting from the reaction of coumarin -3-carboxylicacid with *o*-phenylenediamine and 2-amino benzimidazole. The newly synthesized compounds were evaluated for anti-inflammatory activity using indomethacin as a standard drug and the compound 15 shown good anti-inflammatory activity.



15

Analgesic activity of 3-heteryl coumarins:

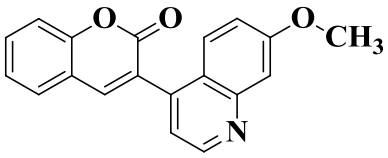
2-Amino-4-(coumarin-3-yl)-6-aryl pyrimidine derivatives were synthesized by Gupta[79] *et al*. starting from 3-acetyl coumarin. The newly synthesized compounds were evaluated for *in vivo* analgesic property using diclophenac sodium as reference drug. The compound 16 exhibited potential activity as compare to standard drug.



16

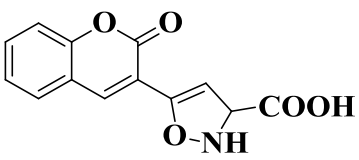
Antioxidant activity of 3-substituted coumarins

3-(7-Methoxyquinolin-4-yl)-2*H*-chromen-2-one was synthesized by Hamama[80] *et al.* starting from the reaction of 3-acetylcoumarin, dimethylformamide-dimethylacetal in presence of dioxane followed by the reaction of 3-methoxy aniline and phosphorous pentachloride. The newly synthesized compound was evaluated for antioxidant activity using ascorbic acid as reference drug. The compound **17** exhibited moderate antioxidant property.

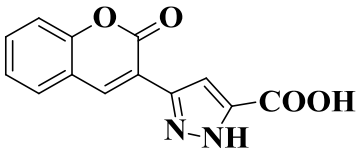


17

5-(2-Oxo-2*H*-chromen-3-yl)-2,3-dihydroisoxazole-3-carboxylic acid (**18**) / 3-(2-oxo-2*H*-chromen-3-yl)-1*H*-pyrazole-5-carboxylic acid (**19**) were synthesized by Salem[81] *et al.* using 4-oxo-4-(2-oxo-2*H*-chromen-3-yl)but-2-enoic acid and hydroxylamine hydrochloride or hydrazine hydrate. The target compounds were evaluated for their antioxidant activity and the compound **19** exhibited excellent activity.



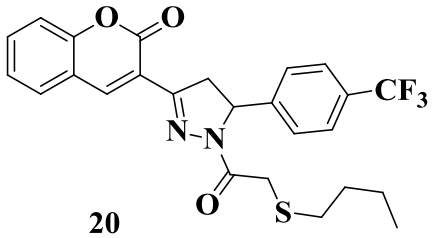
18



19

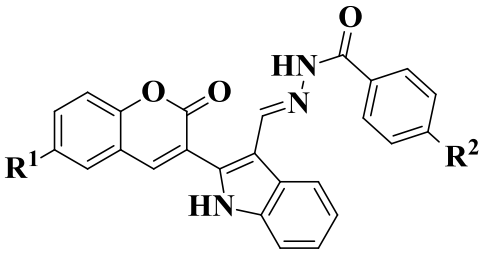
Anticancer activity of 3-substituted coumarins

Wu[82] *et al*. synthesized a series of 1-(3-substituted-5-phenyl-4,5-dihydropyrazol-1-yl)-2-thio-ethanone derivatives starting from substituted coumarin chalcones, hydrazine hydrate, bromoacetyl bromide and substituted thiols. The title compounds were evaluated for antiproliferative activity against SGC-7901, MGC-803, Bcap-37 and HEPG-2 cell lines. The compound **20** exhibited excellent inhibition efficiency against the tested cancer cell line.



20

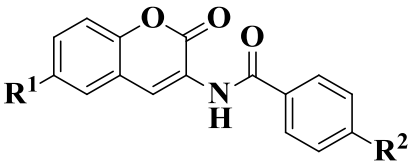
Kamath[83] *et al*. studied the anticancer property of *N*'-((2-(2-oxo-2*H*-chromen-3-yl)-1*H*-indol-3-yl)methylene)benzohydrazides **21** against human breast adenocarcinoma cells (MCF-7). The compounds were found to be potential for breast cancer chemotherapy with apoptosis properties.



21

Acetylcolinesterase inhibitor activity of 3-substituted coumarins

A series of *N*-(2-oxo-2*H*-chromen-3-yl)benzamide **22** derivatives have been synthesized by Vina[84] *et al*. from the reaction of 3-aminocoumarin and substituted acid chlorides in presence of dichloromethane and pyridine as base. These compounds were evaluated for acetylcholine esterase inhibition activity and the compounds were exhibited moderate AChE activity.



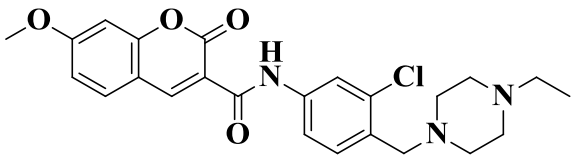
22

R^1 = H, CH_3

R_2 = H, CH_3, -OCH_3, Cl, NO_2

Yao[85] *et al.* synthesized 2-oxo-*N*-(4-(2-(piperazin-1-yl)ethyl)phenyl)-2*H*-chromene-3-carboxamide derivatives by the reaction of 2-oxo-2*H*-chromene-3-carbonyl chloride with 3-chloro-4-((4-ethylpiperazin-1-yl)methyl)aniline and studied acetylcholine esterase

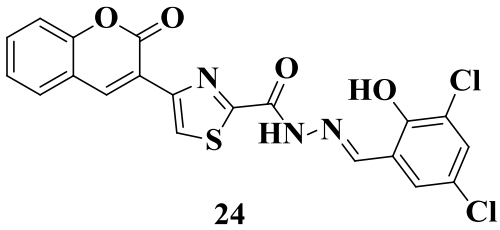
inhibition. The compound **23** exhibited excellent AChE inhibition activity when compare to the standard drug Huperzine A.



23

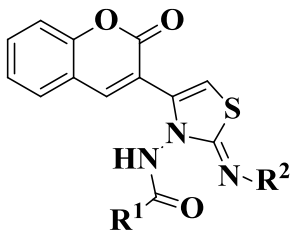
Glucosidase inhibitor activity of 3-acetyl coumarin

Wang[86] et al synthesized a series of thiazolyl coumarins derivatives by the reaction of ethyl 4-(2-oxo-2*H*-chromen-3-yl)thiazole-2-carboxylate, hydrazine hydrate and substituted aldehydes. The newly synthesized compounds were evaluated for their α-glucosidase inhibitory activity and most of the compounds exhibited potential inhibition activity and the compound **24** shown excellent inhibitory activity among the synthesized molecules.



24

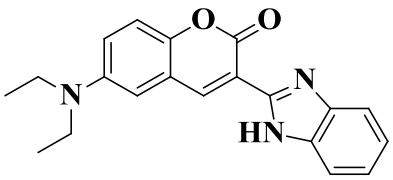
Salar[87] *et al*. reported the synthesis of 3-thiazolylcoumarins **25** through one pot reaction method by the reaction of 2-benzoyl-*N*-arylhydrazinecarbothioamide and 3-(2-bromoacetyl)coumarins in presence of ethanol. The target compounds were screened for *in vitro* α-glucosidase inhibitory activity and the compounds were exhibited good inhibitory activity.



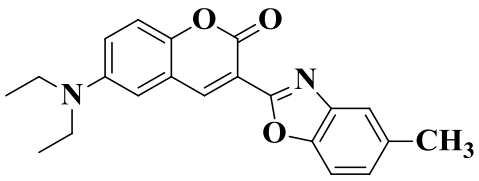
25

Ataeefard, and Nourmohammadian[88] developed coumarin dyes as fluorescent ink for digital printing. Benzimidazolyl / benzoxazoly coumarin derivatives (**26** and **27**) were

incorporated into polystyrene-acrylic acid and studied the emission and reflectance properties of fluorescent laser printing ink.

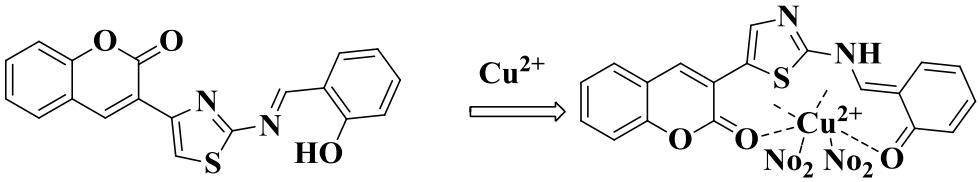


26



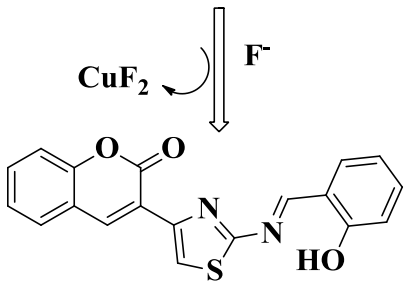
27

Razi[89] *et al*. developed an efficient coumarin-thiazole molecular probe **28** to detect the metal ions and anions through fluorescent spectroscopy. Naked eye On-Off-On strong fluorescence was observed with Cu^{2+} ions and only F^- ions displace the Cu^{2+} ions leading to the fluorescence enhancement.



Fluroscence **Fluroscence OFF**

28



Fluroscence ON

2.3 Preparation of coumarins

In the literature several methods are available to synthesize the coumarins. Coumarins can be synthesized Pechmann condensation, Claisen rearrangement, Perkin reaction, as well as the Knoevenagel condensation.

Preparation of various substituted 3-(2-bromoacetyl)coumarins:

In the present study 3-acetylcoumarins and 3-(2-bromoacetyl) coumarins were used as starting materials for the synthesis of heterocyclic compounds. 3-(2-Bromoacetyl) coumarins were prepared from salicylaldehydes in three steps.

(i) Preparation of substituted salicylaldehydes

(ii) Preparation of substituted 3-acetylcoumarins

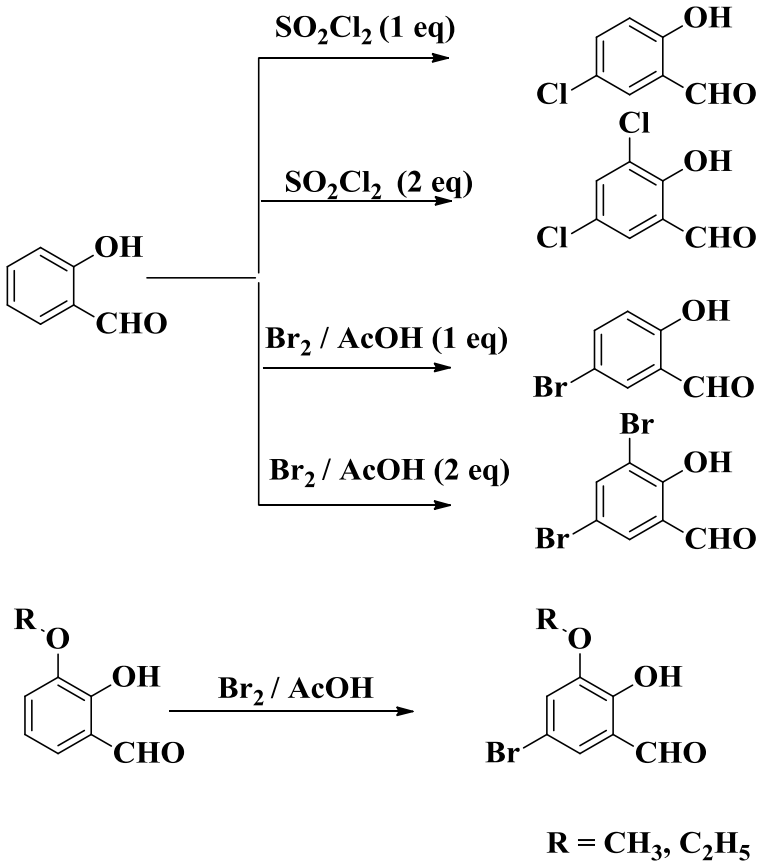
(iii) Preparation of substituted 3-(2-bromoacetyl)coumarins

Step 1: Preparation of substituted salicylaldehydes:

Substituted salicylaldehydes required for the synthesis of 3-acetyl coumarin were prepared according to the literature procedures. Resorcinol or 2-methyl resorcinol was formylated to get the 2,4-dihydroxy benzaldehydes[90] or 2,4-dihydroxy-3-methylbenzaldehyde. The mono halo salicylaldehydes and di halo salicylaldehydes have been prepared by halogenation. 5-chlorosalicylaldehyde[91] was prepared by reaction of one equivalent $SOCl_2$ with salicylaldehydes. 3,5-dichlorosalicylaldehyde was prepared by the reaction of salycylaldehyde with 2 equivalent of $SOCl_2$. An equivalent amount of bromine and salicylaldehyde in presence of acetic acid medium gave exclusively 5-bromo salicylaldehyde[92], 3, 5-dibromo salicylaldehyde[93] is prepared by bromination of salicylaldehyde with two equivalent of bromine. Similarly bromination of o-vanillin and 3-ethoxy salicylaldehyde gave 5-bromo *o*-vanillin and 5-bromo-3-ethoxy salicylaldehyde. Nitration of salicylaldehyde[94] with two equivalent of nitration mixture gave 3,5-dinitro salicylaldehyde. Salicylaldehyde, 2-hydroxy-1-naphthaldehyde, *o*-vanillin and 3-ethoxy salicylaldehydes were procured from commercial sources and further used without any purification (**Scheme-33**).



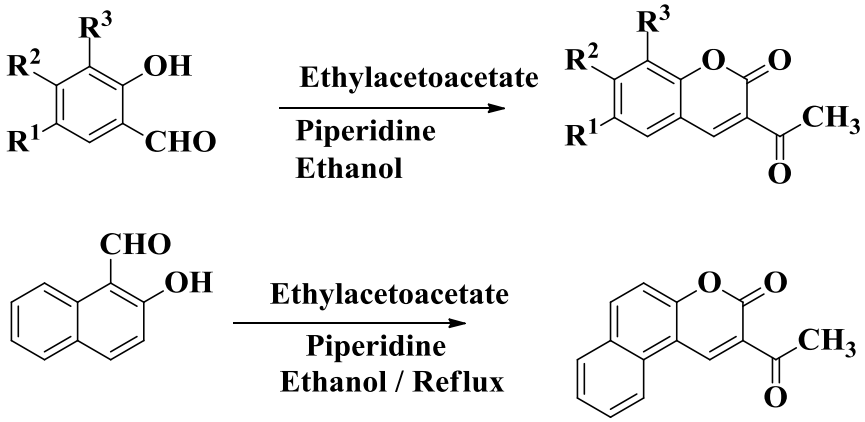
R = H, CH_3



Scheme - 33

Step 2: Preparation of substituted 3-acetyl coumarins:

3-Acetyl coumarin[95, 96] and substituted 3-acetyl coumarins were prepared by the condensation of substituted salicylaldehydes with ethylacetoacetate in ethanol in presence of piperidine. 3-Acetyl coumarin was prepared in cold condition (0 - 10°C) and substituted 3-acetyl coumarins were prepared at reflux temperature of ethanol (**Scheme-34**).



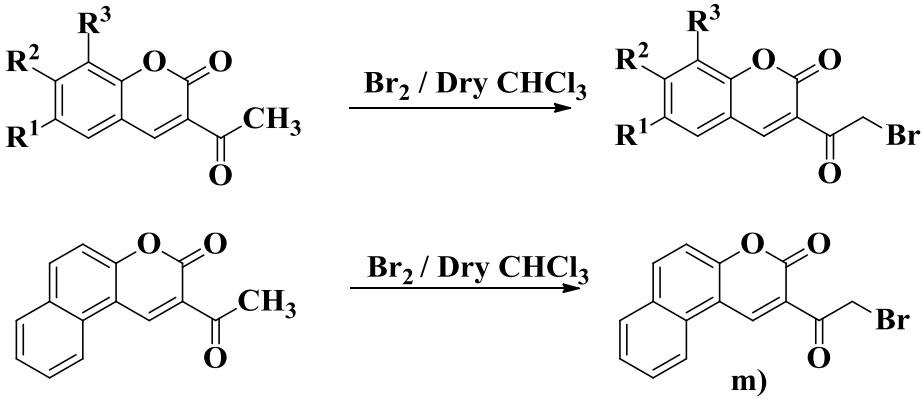
R^1	R^2	R^3

H	H	H
Cl	H	H
Cl	H	Cl
Br	H	H
Br	H	Br
H	H	OCH_3
Br	H	OCH_3
H	H	OC_2H_5
Br	H	OC_2H_5
H	OH	H
H	OH	CH_3
$N(C_2H_5)$	H	H
NO_2	H	NO_2
$C(CH_3)_3$	H	$C(CH_3)_3$

Scheme-34

Step 3: **Preparation of substituted 3-(2-bromoacetyl)-2*H*-chromen-2-ones:**

3-(2-Bromoacetyl)-2*H*-chromen-2-ones[97-99] were prepared by taking equivalent amounts 3-acetyl coumarins and bromine in presence of dry chloroform under heating condition (**Scheme-35**).



R^1	R^2	R^3
H	H	H
Cl	H	H
Cl	H	Cl
Br	H	H
Br	H	Br
H	H	OCH_3
Br	H	OCH_3
H	H	OC_2H_5
Br	H	OC_2H_5

H	OH	H
H	OH	CH_3
$N(C_2H_5)$	H	H
NO_2	H	NO_2
$C(CH_3)_3$	H	$C(CH_3)_3$

Scheme-35

In the present work, the various 3-(2-bromoacetyl)coumarins were prepared by modified literature procedure.

PRESENT WORK:

From the preceding review, Multicomponent reactions (MCRs) are the important for the synthesis of new heterocyclic compounds. MCRs provide rapid access to high-quality compound libraries. Multi-component reactions ideally suited the new demand, and this, in turn, fueled more interest in the earlier developed reactions and in the invention of similar or even fundamentally new ones.

This research work is aimed at developing efficient synthetic methods to synthesize the new heterocyclic systems by the use of multi-component reaction (MCR) approach and studies on biological activity applications.

OBJECTIVES OF THE RESEARCH:

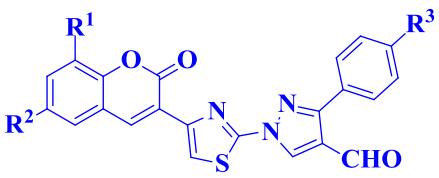
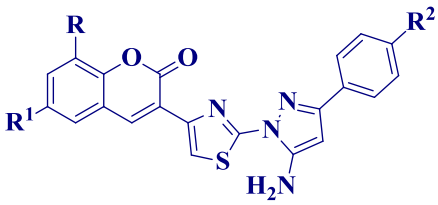
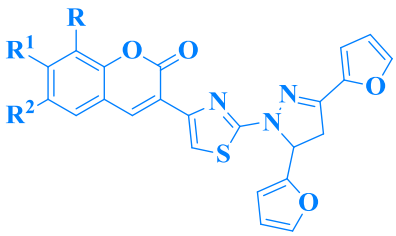
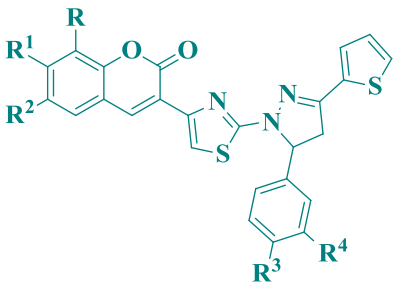
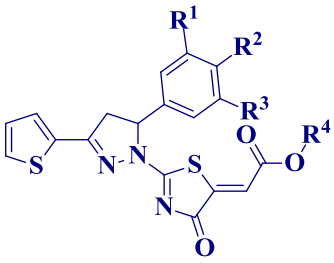
1. To develop an efficient, environmentally benign, facile methods for the synthesis of biologically potent molecules.
2. Evaluation of pharmacological activities of the newly synthesized heterocyclic compounds.

The present research work briefly covers the synthesis of nitrogen and sulphur heterocyclic compounds and their biological activity studies. The target heterocyclic compounds were synthesized by taking readily available starting materials such as substituted 3-acetyl coumarins, 3-(2-bromoacetyl)coumarins, thiosemicarbazide, thiocarobohydrazide, 4-amino-4*H*-1,2,4-triazole-3,5-dithiol and morpholine-4-carbothiohydrazide.

Chapter-I deals with introduction to multicomponent reactions and synthesis and biological activity applications of 3-substituted coumarins.

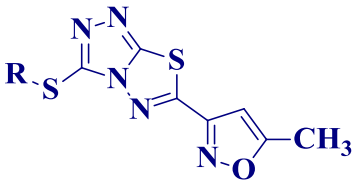
Chapter-II describes an efficient one pot multi component synthesis of substituted thiazolyl-pyrazole 4-carbaldehydes, substituted 5-amino pyrazoly-thiazoles, substituted

dihydro pyrazolyl-thiazoles and substituted dihydro pyrazolyl-thiazolidine acetate derivatives and their biological activity studies.

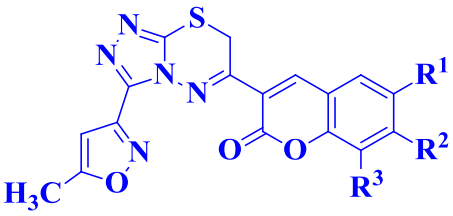
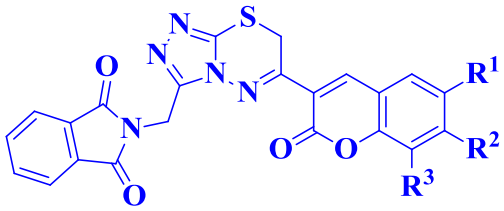
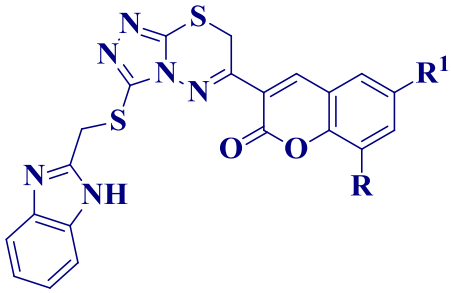






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Chapter-III deals with the synthesis of new 6-(5-methyl-isoxazol -3yl)-3-alkyl sulfanyl-[1,2,4]triazolo-[3,4-*b*][1,3,4]thiadiazoles and their antibacterial activity

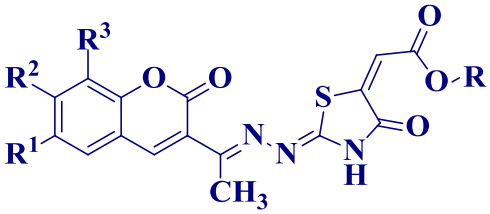


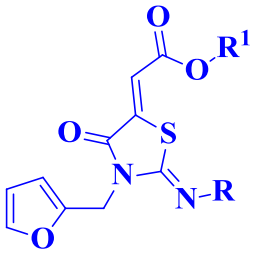
Chapter-IV reports the synthesis and biological applications of 3,6-di substituted 7*H*-[1,2,4]-triazolo [3,4-*b*][1,3,4] thiadiazine derivatives.

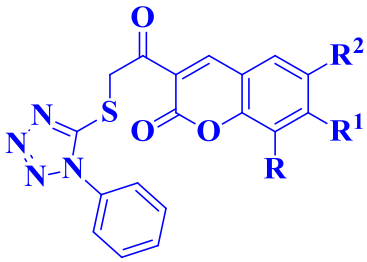
Chapter-V comprises of a facile one pot multi component synthesis of alkyl 4-oxo-coumarinyl ethylidene hydra zono-thiazolidin-5-ylidene acetates and their biological

activity studies and facile one pot multicomponent synthesis of alkyl 2-(2-((aryl) imino)-3-(furan-2-ylmethyl)-4-oxothiazolidin-5-ylidene) acetate derivatives and their anticancer activity.

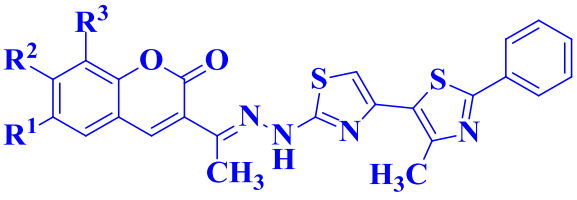


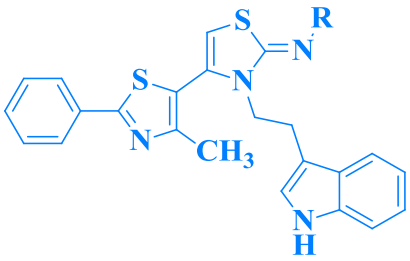


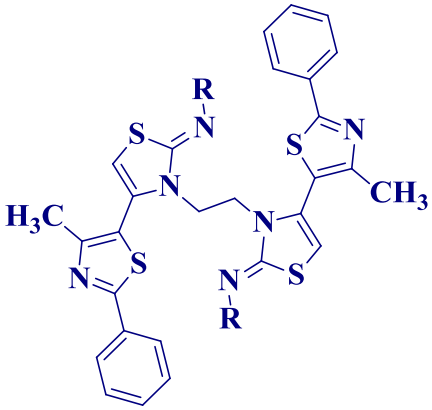
Chapter-VI describes the multicomponent synthesis of 3-(2-((1-phenyl-1*H*-tetrazol-5-yl) thio) acetyl)-2*H*-coumarin derivatives and their studies of anticancer property.



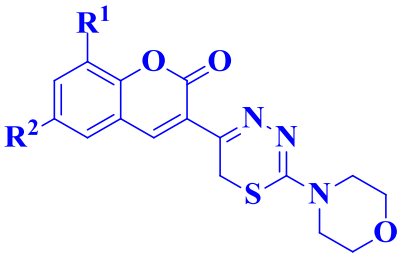
Chapter-VII consists of a facile one pot multicomponent synthesis of 3-(1-(2-(4'-methyl-2'-phenyl-[4,5'-bithiazol]-2-yl) hydrazono)ethyl)-2*H*-chromen-2-one derivatives, a facile one pot multicomponent Synthesis of *N*-(3-(2-(1*H*-indol-3-yl)ethyl)-4'-methyl-2'-phenyl-[4,5'-bithiazol]-2(3*H*)-ylidene) aniline derivatives and a facile one pot pseudo five component reaction of 3,3''-(ethane-1,2-diyl)bis(4'-methyl-2'-phenyl-[4,5'-bithiazole]-3(3*H*)-yl-2(3*H*)-ylidene)) dianiline derivatives and their antiviral applications.

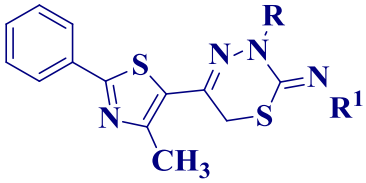






Chapter-VIII describes the synthesis of 3-(2-morpholino-6*H*-1,3,4-thiadiazin-5-yl)-2*H*-chromen-2-one derivatives, an efficient one pot multicomponent synthesis of 2, 5 substituted 3,6 dihydro 1,3,4-thiadiazine derivatives and their antiviral activity.





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CHAPTER-II

AN EFFICIENT ONE POT MULTICOMPONENT SYNTHESIS AND BIOLOGICAL ACTIVITY EVALUATION OF

- SUBSTITUTED THIAZOLYL –PYRAZOLE 4-CARBALDEHYDES,
- SUBSTITUTED 5-AMINO PYRAZOLY-THIAZOLES,
- SUBSTITUTED DIHYDRO PYRAZOLYL-THIAZOLES
- SUBSTITUTED DIHYDRO PYRAZOLYL-THIAZOLIDINE ACETATE DERIVATIVES

INTRODUCTION:

In recent years medicinal chemists focused to develop efficient therapeutic agents mainly heterocyclic compounds with varied biological applications. Heterocyclic compounds containing more than one hetero atom with diversified functional units are consider greater utility in medicinal chemistry. Among the heterocyclic compounds, pyrazoles are considered as effective therapeutic agents with diversified medicinal, material, agrochemical applications. Pyrazoles are an important aromatic five membered heterocyclic compound having two nitrogen atoms at adjacent position in a cyclic ring. Consistent interest has been taken by the medicinal chemists to synthesize the pyrazoles or *N*- substituted pyrazoles. Numerous pyrazoles and substituted pyrazoles exhibit antimicrobial[1-4], antiviral[5-7], antifungal[8,9], antipyretic[10,11], hypnotic[12], anti-inflammatory[13-15] and herbicidal[16-19]properties. Pyrazole nucleus was found to be the core pharmacophore unit available in numerous biologically active compounds (**Fig.1)**.

N- Substituted pyrazoles with aryl or heteryl functional groups were found to be the most effective biological properties. Among the heteryl compounds, thiazoles are the most promising compounds having diversified applications[20-29]. The molecular hybridization of thiazoles and pyrazoles would expect to exhibit enhanced therapeutic applications. Synthesis of substituted thiazolyl pyrazoles or pyrazolyl thiazoles attracted much attention of scientific community because of their combined pharmacological applications. Thiazolyl pyrazoles or pyrazolyl thiazoles exhibit diversified therapeutic[30-34] and agrochemical applications. Hence the development of novel, environmentally benign, efficient synthetic protocols for thiazolylpyrazoles is the main goal for the organic chemist.

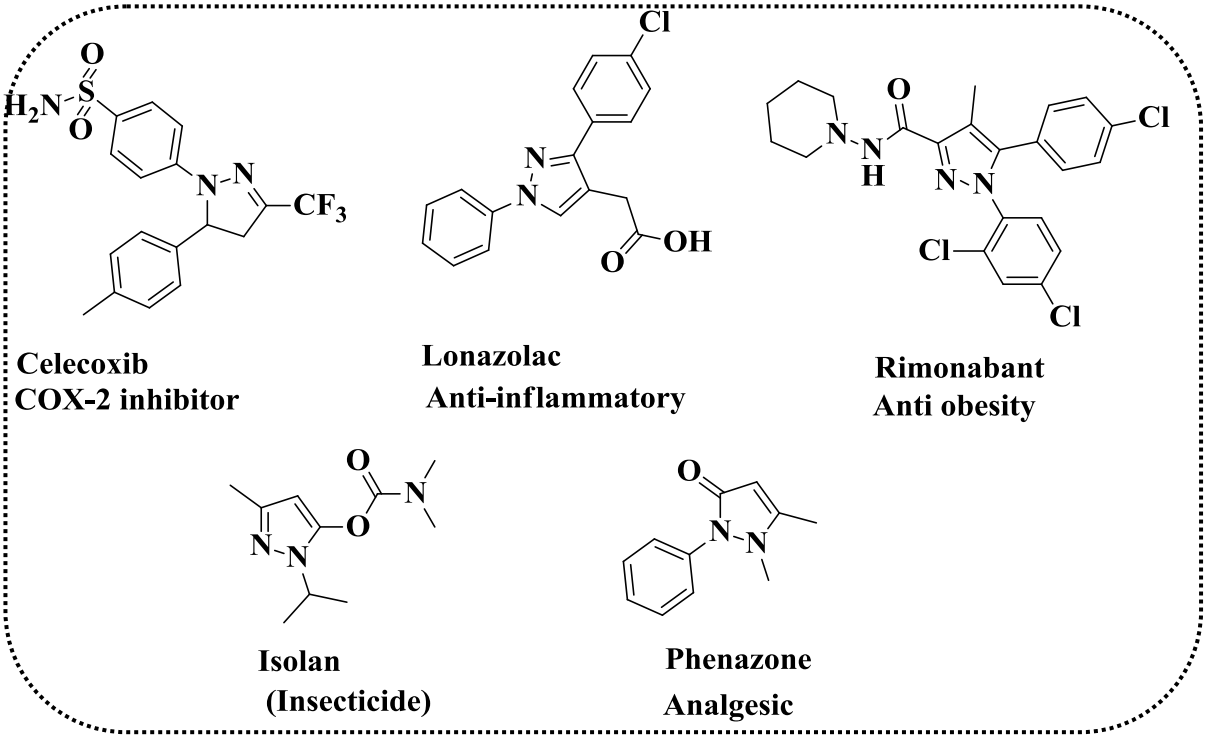
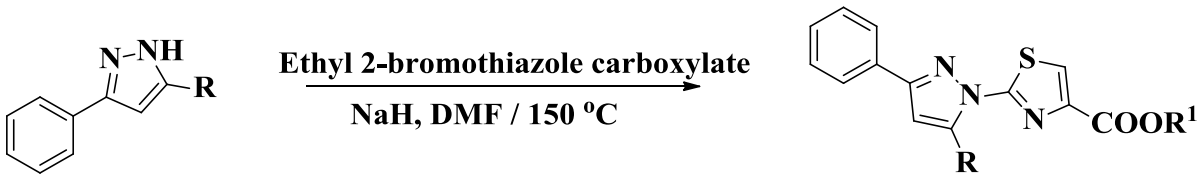


Fig. 1 Some of the therapeutic drugs containing pyrazoles unit

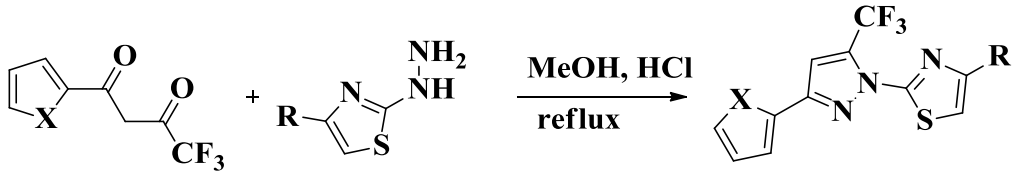
The following are brief literature review on synthesis of thiazolylpyrazoles and pyrazolylthiazoles.

Atobe[35] *et al.* described the synthesis of pyrazol-1-yl-thiazole derivatives (**2**) via aromatic nucleophilic substitution reaction of 3,5-disubstituted pyrazoles (**1)** with ethyl-2-bromo thiazolecarboxylate in presence of sodium hydride and dimethyl formamide solvent under reflux conditions. The products were isolated with moderate yields.



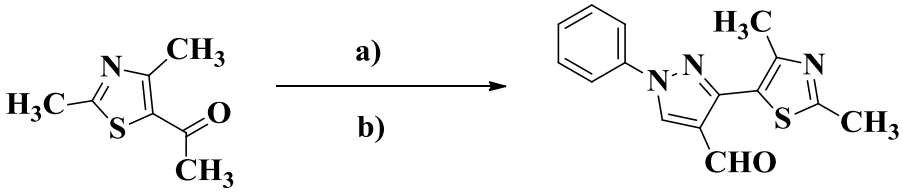
1 **2**

Denisova[36] *et al.* reported the regioselctive synthesis of 2-pyrazolyl thiazoles (**5**) starting from 2-hydrazinothiazoles (**4**) and 1-thienyl and 1-furyl 1,3-butanedione (**3**) through hydrochloric acid catalyst in presence of methanol. The target compounds were obtained with good yields.



3 **4** **5**

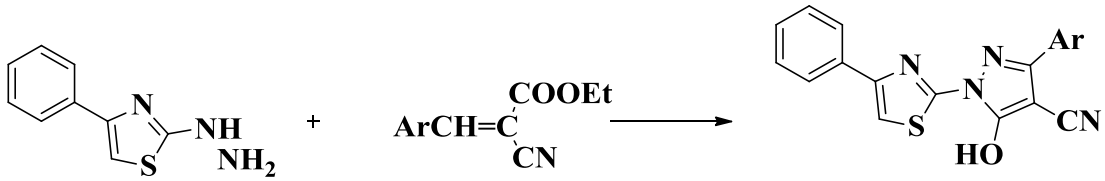
Khillare[37] *et al.* synthesized thiazolyl pyrazole-4-carbaldehydes (**7**) in a step wise manner by using 1-(2,4-dimethylthiazol-5-yl)ethanone (**6**) as a starting reactant. The compound 6 was treated with phenyl hydrazine to give the intermediate 2,4-dimethyl-5-(1-(2-phenylhydrazono)ethyl)thiazole, which was further converted into the target compound via Vilsmeier-Haack reaction condition with good yields.



6 **7**

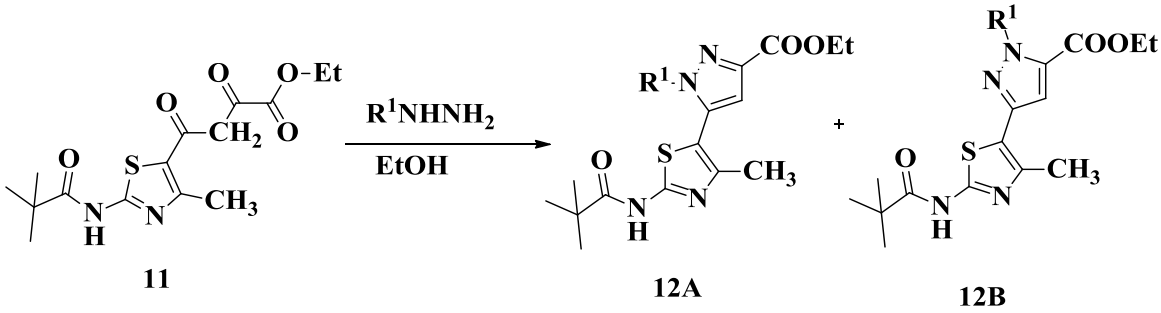
a) = $PhNHNH_2$ / Ethanol, b) $DMF\text{-}POCl_3$ / 80 ^oC, 10 h

Amer[38] reported the synthesis of thiazolylpyrazole (**10**) derivatives by the reaction of 2-hydrazino-4-phenylthiazole (**8**) and ethyl 2-cyano-3-arylacrylate (**9**) in presence of triethylamine catalyst by using ethanol solvent under reflux condition. The final compounds were obtained in moderate to good yields.

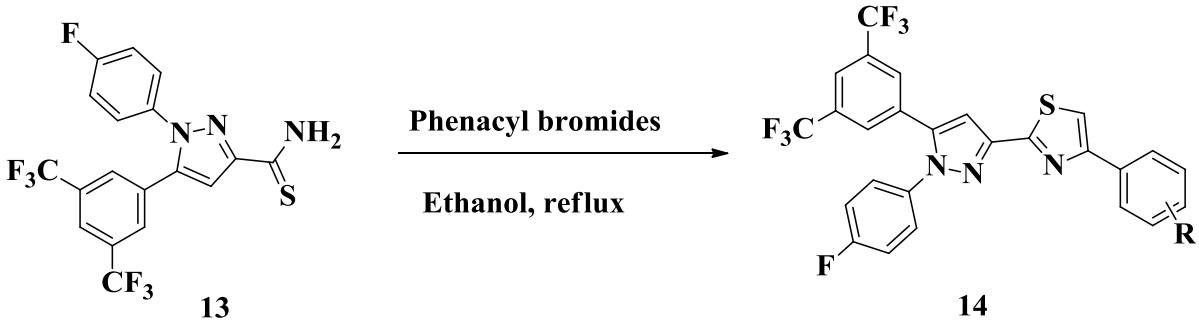


8 **9** **10**

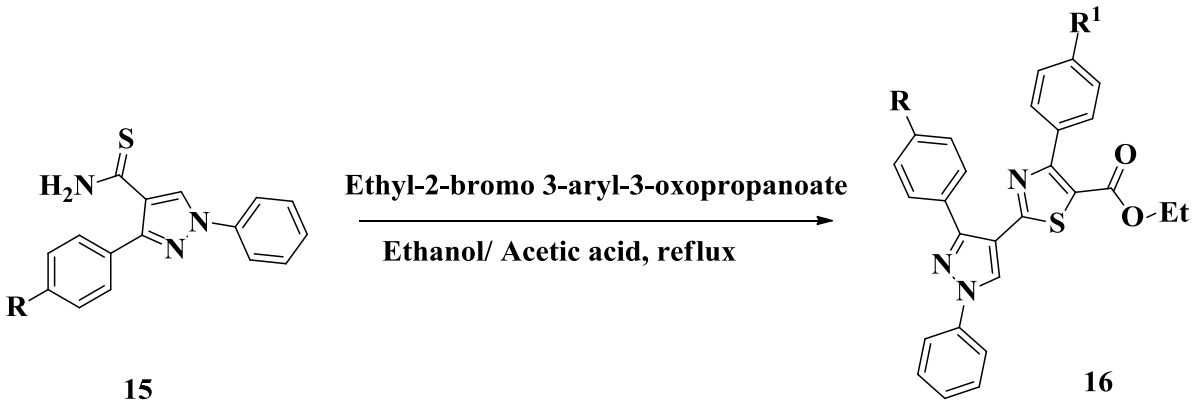
Ye[39] *et al.* described the synthesis of ethyl 5-(4-methyl-2-pivalamidothiazol-5-yl)-1-phenyl-1*H*-pyrazole-3-carboxylate (**12A**) and ethyl 3-(4-methyl-2-pivalamidothiazol-5-yl)-1-phenyl-1*H*-pyrazole-5-carboxylate (**12B**) by the reaction of ethyl 4-(4-methyl-2-pivalamidothiazol-5-yl)-2,4-dioxobutanoate (**11**) and substituted hydrazine in presence of ethanol at ambient temperature.



Patil[40] *et al.* synthesized 5-(3,5-bis(trifluoromethyl)phenyl)-1-(4-fluorophenyl)-3-(5-arylthiazol-2-yl)-1*H*-pyrazole (**14**) derivatives starting from 5-(3,5-bis (trifluoromethyl) phenyl)-1-(4-fluorophenyl)-1*H*-pyrazole-3-carbothioamide (**13**) and substituted phenacyl bromides in presence of ethanol under reflux condition. The target molecules were obtained with good yields through shorter reaction time and easy reaction workup.

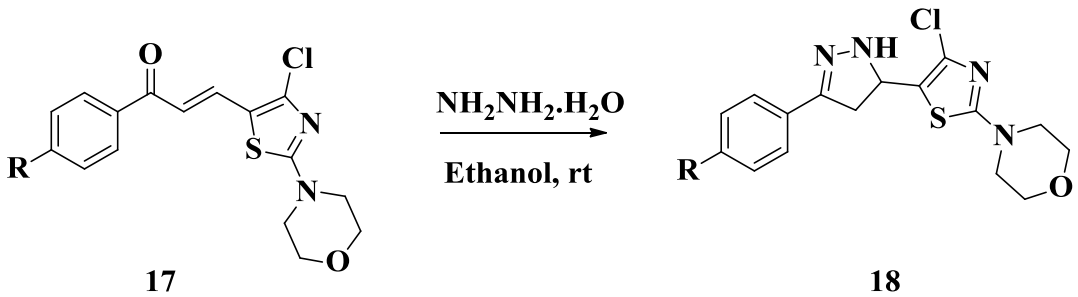


Pyrazolylthiazole carboxylates (**16**) were synthesized by Khloya[41] *et al.* through condensation reaction of 1,3-diaryl-1*H*-pyrazole-4-carbothioamide (**15**) with ethyl-2-bromo-3-aryl-3-oxo-propanoate in presence of ethanol and catalytic amount of acetic acid. The target compounds were obtained in moderate to good yields with simple reaction workup.



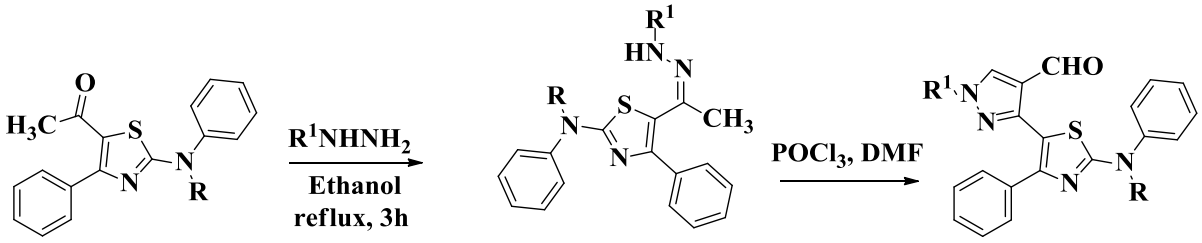
15 16

4-5, Dihydropyrazoles (**18**) were synthesized by J.Ramirez[42] *et al.* by the reaction of hydrazine hydrate with α, β – unsaturated carbonyl compounds (**17**) under mild reaction condition at ambient temperature. The target compounds were obtained with moderate yields.



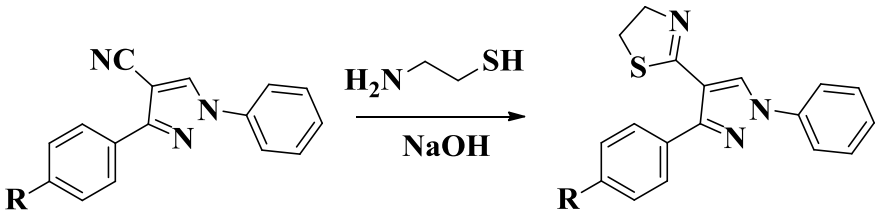
17 18

Yuvaraj and Manju[43] reported the synthesis of 3-(substituted 1,3-thiazol-5-yl)-1-substituted 1*H*-pyrazole-4-carbaldehydes (**21**) starting from *N*-substituted-1-[4-phenyl-2-(phenylamino)-1,3-thiazol-5-yl]ethanone (**19**). The compound 19 was converted into the intermediate (**20**) by the reaction of arylhydrazine, followed by Vielsmeir-Haack formylation into the final product in two steps with good yields.



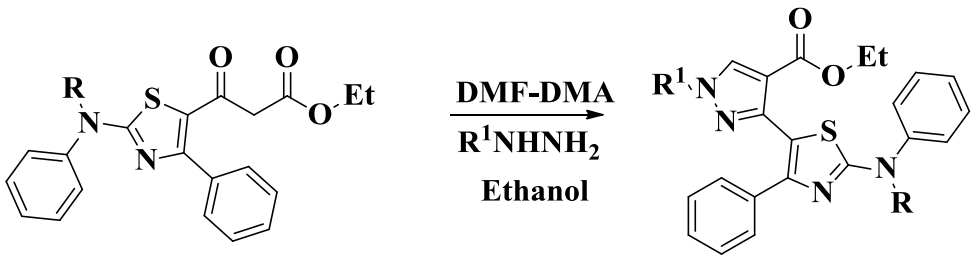
19 20 21

2-(3-Aryl-1-phenyl-1H-pyrazole-4-yl)-4,5-dihydrothiazoles (**23**) were prepared by Bekhit[44] *et al*. by the reaction of 3-aryl-1-phenyl-1*H*-pyrazole-4-carbonitrile (**22**) with cysteamine hydrochloride in presence of ethanolic sodium hydroxide solution under reflux condition. The target compounds were obtained with good yields through simple reaction work up.



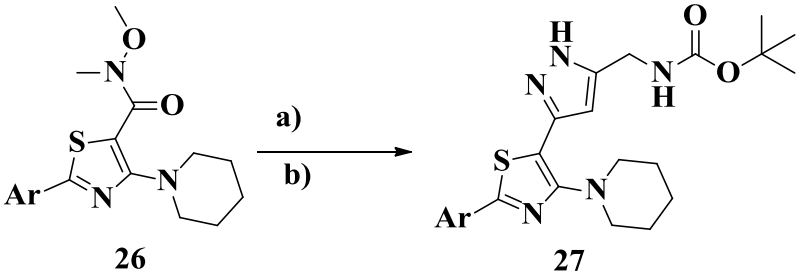
22 **23**

Yuvaraj[45] *et al.* described the synthesis of novel 3-(substituted 1,3-thiazol-5-yl)-1-substituted 1*H*-pyrazole-4-carboxylates (**25**) starting from the reaction of *N*-substituted-1-[4-phenyl-2-(phenylamino)-1,3-thiazol-5-yl] 3-oxo-propionic acid ethyl ester (**24**) with N,N-dimethylformamide dimethylacetal (DMF-DMA) followed by substituted hydrazine derivatives in presence of ethanol under reflux condition with moderate to good yields.



24 **25**

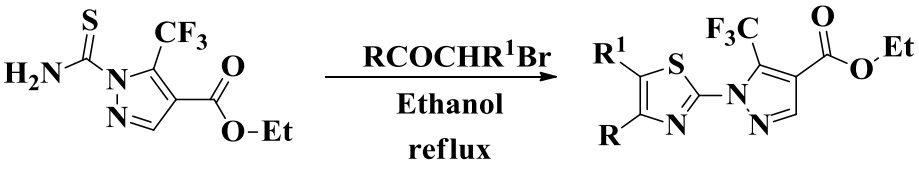
Ronkin[46] *et al.* described the synthesis of pyrazolthiazoles (**27**) through efficient synthetic method. The target compound was obtained by the reaction of *N*-methoxy-*N*-methyl-2-phenyl-4-(piperidin-1-yl)thiazole-5-carboxamide (**26**) with *tert*-butyl prop-2-ynylcarbamate followed by hydrazine hydrate in two steps.



26 **27**

a) *tert*-butyl prop-2-ynylcarbamate, *n*-butyllithium, THF, -15 ^{o}C to -10 ^{o}C;
b) Hydrazine hydrate, ethanol

Donohue[47] *et al.* reported the synthesis of ethyl 1-(4-Phenyl-2-thiazolyl)-5-(trifluoromethyl)pyrazole-4-carboxylate (**29**) by the reaction of ethyl 1-carbamothioyl-5-(trifluoromethyl)-1H-pyrazole-4-carboxylate (**28**) with α-bromoketones in presence of ethanol. The target compounds were obtained with good yields by simple reaction workup.



28 **29**

Bondock[48] *et al.* synthesized a novel ethyl 5-formyl-3-methyl-1-(4-phenylthiazol-2-yl)-1*H*-pyrazole-4-carboxylates (**31**) by the reaction of ethyl 3-(2-(4-phenylthiazol-2-yl)hydrazono)butanoate (**30**) through Vilsmeier-Haack reaction condition. The formylation occur selectively on pyrazole-C_5 carbon in good yields.



30 **31**

The present chapter has been divided into part A to part-D. Part A describes the synthesis, biological activity evaluation and molecular docking studies of novel Coumarin substituted thiazolyl-3-aryl-pyrazole-4-carbaldehydes, Part-B describes 3-(2-(5-amino-3-aryl-1*H*-pyrazol-1-yl) thiazol-4-yl)-2*H*-chromen-2-ones as potential

anticancer agents: Synthesis, anticancer activity evaluation and molecular docking studies, Part-C describes facile one pot multicomponent synthesis of 3-(2-(3,5-di(furan-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)thiazol-4-yl)-2*H*-chromen-2-ones and their biological activity and part-D describes a facile one pot multicomponent synthesis of alkyl 2-(4-oxo-2-(5-aryl-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)thiazol-5(4*H*)-ylidene)acetate and their biological applications.

CHAPTER-II
PART-A

SYNTHESIS, BIOLOGICAL ACTIVITY EVALUATION AND MOLECULAR DOCKING STUDIES OF NOVEL COUMARIN SUBSTITUTED THIAZOLYL-3-ARYL-PYRAZOLE-4-CARBALDEHYDES

PART-A

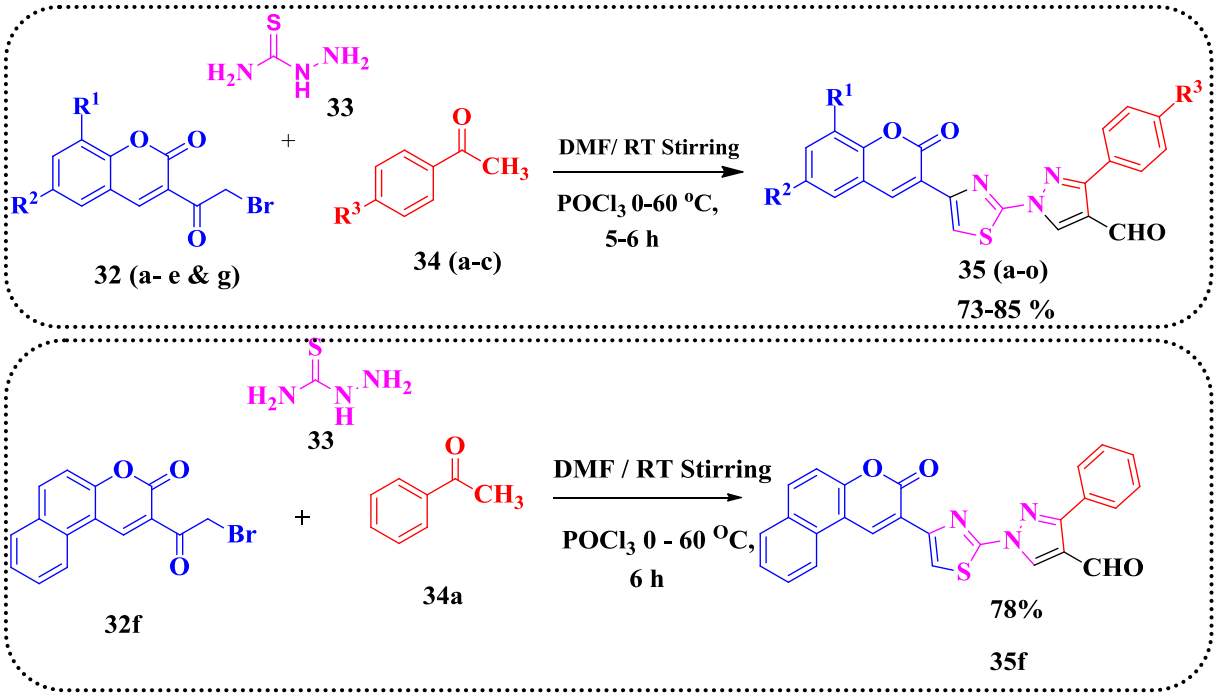
PRESENT WORK:

Various substituted 3-(2-bromoacetyl)coumarins were prepared by bromination of 3-acetyl coumarins in presence of dry chloroform according to the literature procedure as described in the chapter-I (Reference 97-99). Thiosemicarbazide, different substituted acetophenones, dimethylformamide and phosphorous oxychloride were procured from commercial source.

In view of the importance of coumarins, thiazoles and pyrazoles in the field of medicinal chemistry and also to explore the scope of these motifs, we have focused on the design of a novel structural entity that comprises these three structural moieties along with a potential functional group into a single molecular framework like aldehyde.

An efficient one pot multicomponent synthesis of coumarin substituted thiazolyl-3-aryl-pyrazol-4-carbaldehydes and their biological applications described in the present chapter.

The title compounds (**35a-o**) were obtained by the reaction of various 3-(2-bromo acetyl) coumarins (**32a-g**) with thiosemicarbazide (**33**) and acetophenones (**34a-c**) under Hanztsch thiazole synthesis followed by Vilsmeier-Haack reaction conditions with good yields. The general schematic representation is outlined in **Scheme 1**.



Scheme1. Synthesis of coumarin substituted thiazolyl-3-aryl-pyrazole-4-carbaldehydes **35 (a-o)**.

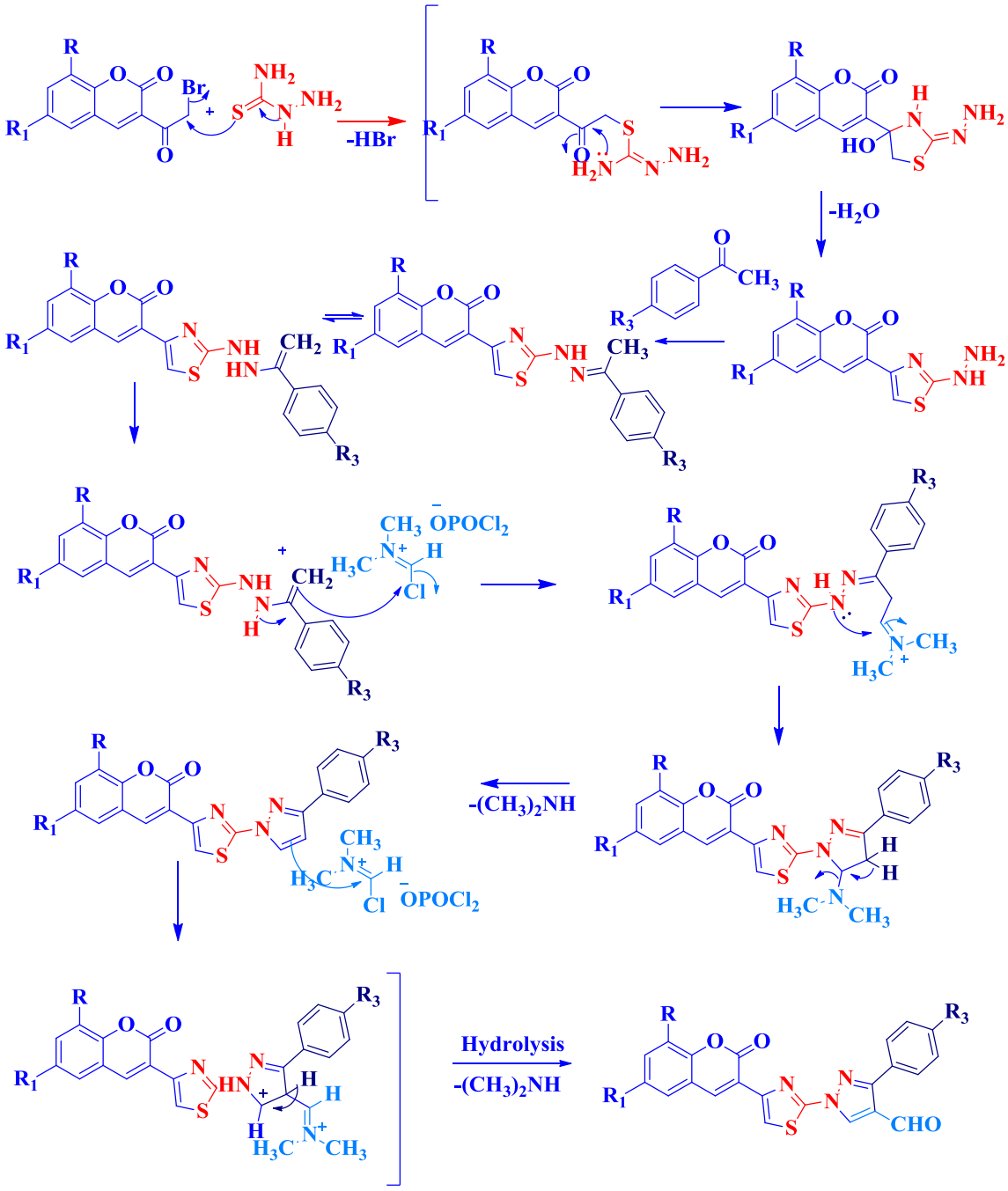
To know the feasibility of the reaction, was carried out the reaction in one –pot multicomponent approach of 3-(2-bromoacetyl)-2*H*-chromen-2-one (**32a**), thiosemicarbazide (**33**) and acetophenone (**34a**) in presence of dimethylformamide. In this step substituted 3-(2-bromoacetyl)coumarins react with thiosemiarbazide and form the Hanztsch thiazole product and on further reaction with substituted acetophenones lead to the formation of an intermediate 3-(2-hydrazino-4-thiazolyl)coumarino)phenyl methyl methane. Without isolation of this intermediate, the compound is subjected to Vilsmeier-Haack formylation reaction condition to generate the target product. This method is simple and found to be easier to synthesize the thiazolyl pyrazole carbaldehyde derivatives in shorter reaction time with good yields. **Table 1**

During these reactions in one step both the thiazole and pyrazole rings are formed at a time along with a potential functional group (-CHO) on pyrazole ring (**Scheme 2**). The formylation is highly regioselective, it took place only on pyrazole ring and not on thiazole ring. It is a novel observation, and as far as our knowledge is concerned, no such report is so far available in one-pot multicomponent synthesis of coumarinyl thioazolo-pyrazole-4-carbaldehydes in the literature. This may trigger a new and interesting area of research in chemistry with reference to Vilsmeier-Haack formylation reaction.

Table 1. A facile one-pot synthesis of coumarin substituted thiazolyl-3-aryl-pyrazole-4-carbaldehydes **(35a-o).**

Product	R^1	R^2	R^3	Time	Yield (%)[a]
35a	H	H	H	5.0	84
35b	H	Cl	H	5.5	80
35c	Cl	Cl	H	6.0	75
35d	H	Br	H	5.5	80
35e	Br	Br	H	6.0	85
35g	H	Cl	Cl	6.0	77
35h	Cl	Cl	Cl	5.5	73
35i	H	Br	Cl	5.0	83
35j	OCH_3	H	Cl	6.0	82
35k	H	H	CH_3	5.0	77
35l	H	Br	CH_3	6.0	82
35m	Cl	Cl	CH_3	5.5	81
35n	Br	Br	CH_3	5.0	85
35o	OCH_3	H	CH_3	6.0	82

Reaction condition:- 3-(2-Bromoacetyl)coumarin (**32a-g**, 1 mmol), thiosemicarbazide (**33**, 1 mmol), acetophenone (**34a-c**, 1 mmol), DMF (5 mL) and $POCl_3$ (5 mmol), 0 - 60^{o}C, 5-6 h; [a,]Isolated yields



Scheme.2: Plausible reaction mechanism for the formation of target molecule.

The newly synthesized coumarin substituted thiazolyl pyrazole- carbaldehyde compounds having different substituents on coumarin as well as on phenyl ring, were evaluated for their cytotoxic activity against three human cancer cell lines (DU-145, MCF-7 and HeLa,) by adopting the MTT assay method[49]. Doxorubicin was used as a standard reference drug. Initially all the fifteen coumarin derivatives were taken to evaluate the single dose (10 μM) screening. From this screening some of the compounds like 35g, 35i, 35m and 35n showed good inhibitory effect against HeLa cell line.

Subsequently all the synthesized compounds were screened at three different concentrations (2.5, 5, 100 µM) to evaluate their cytotoxicity. All the synthesized compounds showed moderate to good anticancer activity with IC_{50} values ranging from 5.75 µM to 100 µM. The compound 35m showed excellent cytotoxic activity against HeLa cell line, having IC_{50} value 5.75 µM. Similarly compounds 35g, 35h, 35i and 35n also exhibited good cytotoxic activity against HeLa cell line. Additionally the results obtained from MTT assay against DU-145 cell line, compound 35f and 35n exhibited moderate cytotoxic activity. On the other hand, compounds 35 (a-o) exhibited poor activities against MCF-7 cell lines. From these anticancer biological studies, we were able to identify potent molecules which exhibited good anticancer activity in human cancer cell lines (**Table 2)**.

Table 2. IC_{50} values for compounds **35** (a- o) against human cancer cell lines:

Compound	IC_{50} values (µM)		
	DU-145	MCF-7	HeLa
35a	NA	NA	NA
35b	NA	NA	**12.82**
35c	41.05	76.33	**13.75**
35d	35.01	18.16	**11.02**
35e	27.97	22.23	**13.69**
35f	11.91	39.46	**13.11**
35g	**14.86**	18.67	**9.51**
35h	30.90	21.74	**10.29**
35i	22.32	85.03	**9.46**
35j	38.18	71.68	41.89
35k	50.23	69.45	37.36
35l	20.86	35.17	14.13
35m	**14.71**	**14.56**	**5.75**
35n	**10.81**	**24.52**	**6.25**
35o	31.42	42.57	28.19
Doxorubicin	2.49	3.18	3.92

NA = Not Active (IC_{50} Values > 100 µM)

Apoptosis[50] is an important process of cell death of undesirable cells during development or homeostasis in multicellular organisms and during apoptosis, chromatin condensation takes place. To see whether coumarin derivatives (35m and 35n) induced cytotoxicity occurs through apoptosis, HeLa cervical cancer cells were treated with 10 µM concentration of these compounds for a period of 24 h. Hoechst 33258 staining was used to visualize nuclear condensation. It was found that both these compounds caused a significant nuclear condensation as shown in **Figure 1**.

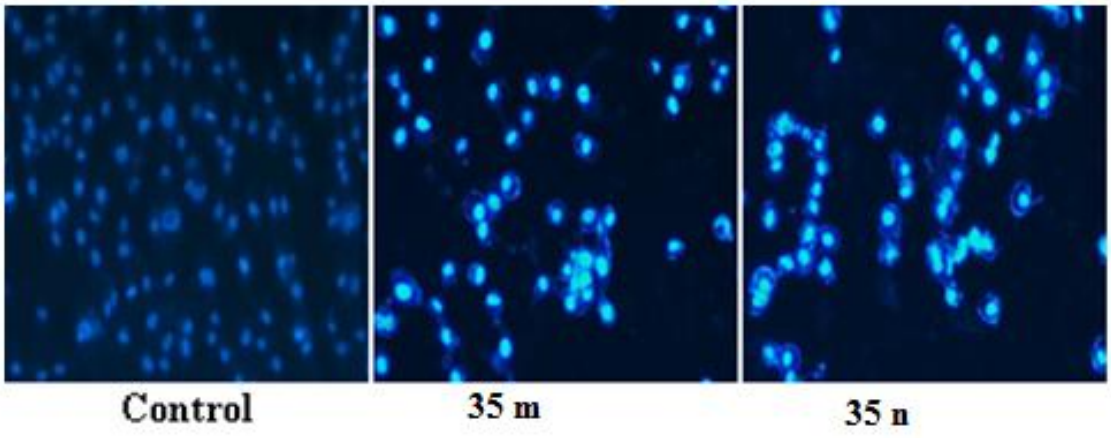


Figure.1 Coumarin derivatives cause apoptosis in HeLa cells. Cells were treated with **35m** and **35n** at 10µM concentration for 24 h and washed with PBS, incubated with Hoechst-33258 stain (4 mg/mL) for 20 min to measure chromatin condensation. Images were taken using fluorescenece microscopy equipped with DAPI filter.

To evaluate the biological activities further, the title compounds were screened against various gram positive (*B. Subtilis [ATCC 6633]*, *B. cereus [ATCC 14579]* and Methcillin- resistant *Staphylococcus aureus [*NCTC 13616]) and gram negative bacterial (*E. Coli[ATCC 8739]*, *K. Pneumoniae[ATCC 43816]* and *P. Vulgaris [ATCC 13315]*) strains by using agar well diffusion method[51]. Gentamycin Sulfate was used as standard drug. The anti bacterial activity of the synthesized compounds exhibited significant zones of inhibition against tested gram positive and gram negative strains at 10, 20, 100, 200 and 300 µg/ml concentrations. The zone of inhibition (mm) of synthesized molecules at 100 µg/ml concentration was shown in **Figure 2.**

However, some of the strains exhibited resistance to tested compounds**.** Among, the tested compounds, compound 35n exhibited highest activity against gram negative *E. coli, K. penumoniae* and *P. Vulgaris* 22, 24 and 20 mm zone of inhibition at100 µg/ml respectively. On the other hand, compound 4m also exhibited significant antibacterial activity with zone of inhibition 18, 20 and 18 mm against *E. coli, K. penumoniae* and *P. Vulgaris* at 100 µg/ml concentration. From the above results it is clear that the compounds 4n, 4m and 4h are found to exhibit greater inhibition efficiency against various strains. Apart from these, compounds 35c, 35d, 35e, 35g, 35i, 35j and 35l also showed significant to moderate antibacterial activitiy. On the other hand compounds 35a, 35b, 35k and 35o failed to exhibit activity against Methcillin- resistant *Staphylococcus aureus* [MRSA].

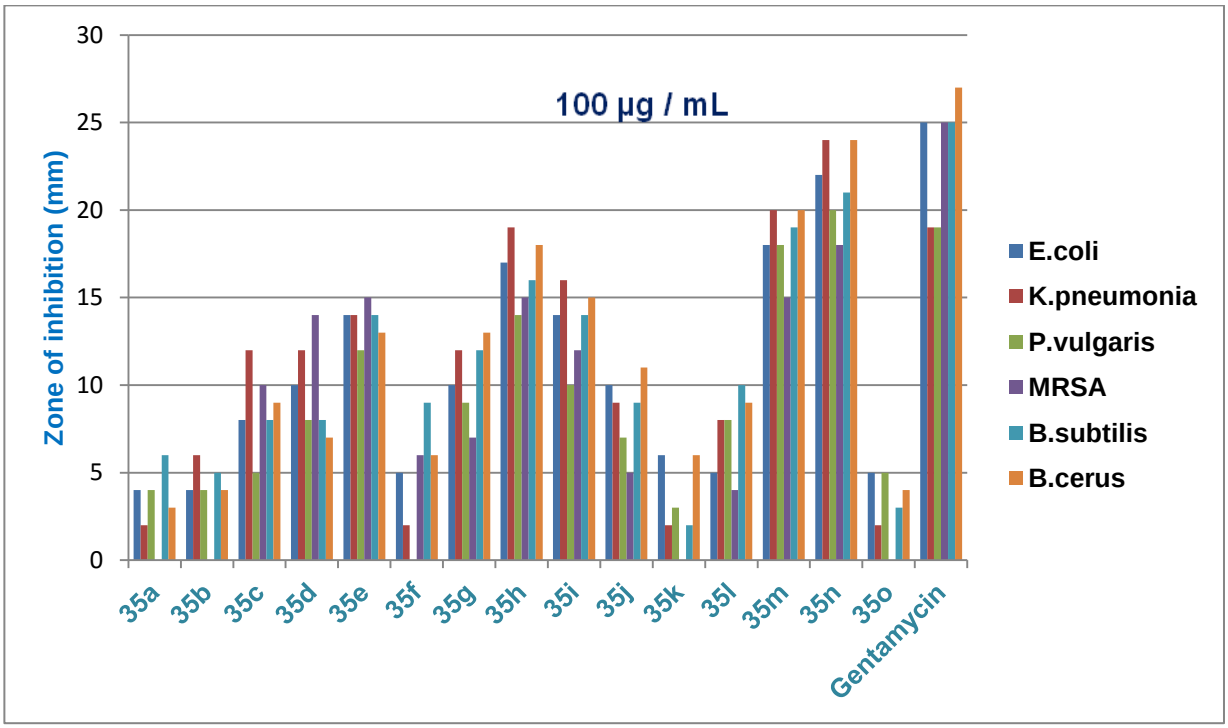


Figure.2a Antibacterial activity (Zone of inhibition in mm) of compounds **35**a-o

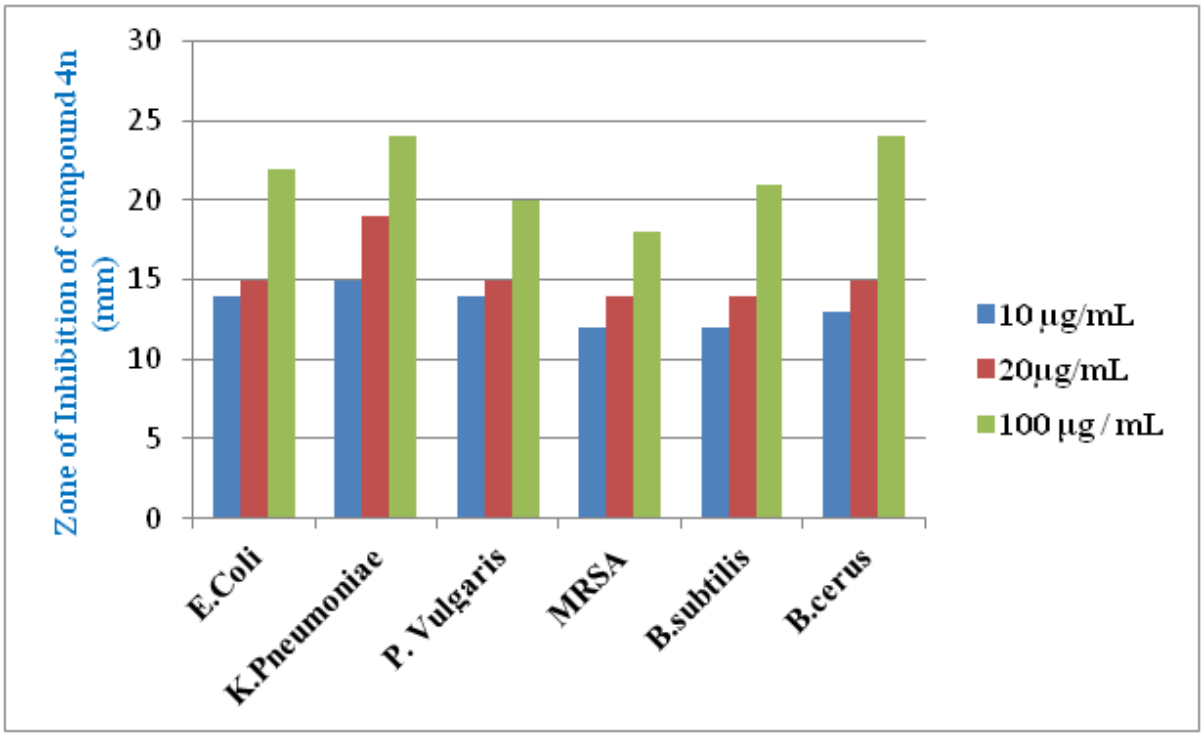


Figure.2b Antibacterial activity (Zone of inhibition in mm) of compounds **35m**

In order to evaluate the anti bacterial properties, apart from the zone of inhibition method we have calculated the minimum inhibitory concentration [MIC] and minimum bactericidal concentration [MBC] values for the fifteen synthesized molecules against various Gram- positive and Gram-negative bacterial strains by using standard MIC and MBC protocol[52]. Gentamycin sulphate and ampicillin were used as standard reference drugs. The MIC values were calculated after 24 h of incubation. Compound **35n** produced significant MIC values of 86.5, 79.1 and 72.8 µg/mL concentrations against *E.coli*, *K.pnuemonia* and *B. cerus* respectively. The corresponding MBC values of 130, 115 and 110 µg/mL against *E.coli*, *K.pnuemonia* and *B.Cerus.* Similarly the compound **35m** also exhibited moderate MIC value of 98.2 µg/mL concentration against *B.subtilis.* Whereas the remaining compounds exhibited moderate to poor antimicrobial properties in the tested concentration range. The measured MIC and MBC values are shown in **Table 3.**

Table.3 Minimum inhibitory concentration (µg/mL) and minimum bactericidal concentration (µg/mL) values of novel Coumarinyl thiazolyl pyrazole carbaldehyde derivaties (**35** a- o)

	Gram Negative						Gram Positive					
	E.Coli		*K.pnuemoniae*		*p.vulgaris*		*MRSA*		*B.subtilis*		*B.Cerus*	
Compound	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
35a	135	270	150	225	133	200	150	300	132	260	145	290
35b	134.6	265	129	190	130	260	150	300	129.5	190	138.6	275
35c	119.6	240	118	175	131.5	260	115	175	123	250	117	175
35d	118.1	240	116.8	175	124	250	116	175	125	250	129	260
35e	115.6	170	119	240	120	240	116.8	175	118	240	119	240
35f	132.8	260	150	225	150	300	135	270	122.8	240	138.9	275
35g	126.3	250	117.4	240	130.7	260	134	265	112.2	165	110.8	165
35h	110.9	220	105	155	113	225	112.5	225	110.7	165	112	220
35i	117.5	230	115	172	127.5	250	122.9	185	119.7	175	115	170
35j	125.3	185	129	260	132.3	260	139	275	132.6	260	126	250
35k	132.5	260	150	300	150	300	150	300	150	300	135	270
35l	134.6	270	124	250	121.8	240	138.1	275	125	250	126	250
35m	101.3	150	100.9	150	105	210	115.6	230	98.2	145	100.1	150
35n	86.5	130	79.1	115	100.7	150	105.9	175	92.4	150	72.8	110
35o	132.7	260	150	300	142	285	150	300	150	300	136	275
Gentamycin	2	8	4	16	4	8	22	50	45	80	10	22
Ampicillin	10	18	NT	NT	NT	NT	4	6	5	10	4	8

NT = Not Tested

According to the data obtained in the present study, the compounds exhibited antibacterial activity in a concentration dependent manner. It has been clear that, the

compounds are showing good activity against gram negative species compared with that from gram positive species. There are several reasons that can be speculated for immense activity of the compounds against gram negative strains.

In order to explain the structure activity relationship of antimicrobial and anti cancerous properties of the newly synthesized coumarin substituted thiazolyl pyrazole-carbaldehydes, these compounds were examined by changing the functional groups such as Cl, Br, CH_3, -Ph and $–OCH_3$ at R^1 R^2 and R^3 positions and these were divided into two groups: group one was un-substituted coumarins and un-substituted phenyl ring on pyrazole nucleus and group two was substituted coumarins and substituted phenyl ring on pyrazole nucleus. The SAR studies clearly indicated the presence of substituents on both coumarin as well as on the benzene ring which are crucial for anticancer and antibacterial properties of the tested compounds. From the MIC values Br substitution at R^1, R^2 and $–CH_3$ substitution at R^3 position exhibit good antibacterial property. Similarly the presence of chlorine substituent at R^1, R^2 and $–CH_3$ substituent at R^3 position also showed good cytotoxic activity and antibacterial activity. On the other hand unsubstituted coumarin ring and unsubstituted phenyl ring on thiazolyl pyrazole carbaldehydes did not show any biological activities. From the above results, the presence of two halogen atoms (Br or Cl) on coumarin nucleus and methyl substituent on phenyl ring may be said to drastically enhance the properties.

For understanding the biological properties of the synthesized molecules, the lipophilicity[53] values were determined through experimental procedure. Any molecule which exhibits pharmacological properties should have affinity for fat and high lipid solubility. Lipophilicity is a physicochemical property which can explain the drug like nature of the molecule and describe the partition equilibrium of solute molecule between aqueous medium and immiscible organic solvent. Experimentally, lipophilicity of the biologically active compounds were measured by shake – flask method using n- Octanol – Phosphate buffer (50 mM, P^H 7.4) biphasic system. Partition coefficient (*P*) values were calculated for all the active molecules and log *P* values(which is considered as lipophilicity) were measured. All the active molecules were found to show good lipophilicity values in the range of 3.0 to 3.6 **Table 4**.

Table 4. Lipophilicity values of novel coumarinyl thiazolyl pyrazole carbaldehyde derivaties by shake flask method

Compound	Wave length (nm)	Conc. of stock added (mM)	Conc. obtained (mM)	Absorbance	*P*	Log *p*
35d	325	0.2857	0.01025	0.1251	2015.48	3.304
35e	330	0.2857	0.01563	0.1949	1295.92	3.112
35f	393	0.2857	0.00608	0.0913	3449.25	**3.537**
35g	335	0.2857	0.01754	0.2171	1146.63	3.059
35h	340	0.2857	0.01981	0.2483	1006.65	3.002
35i	329	0.2857	0.01723	0.2144	1168.61	3.067
35m	340	0.2857	0.00525	0.0773	4000.21	**3.602**
35n	335	0.2857	0.00955	0.1183	2168.71	**3.336**

In the current scenario the use of computational approach is very important in the field of computer aided drug designing to elucidate the molecular level interaction and active site residues property in relation to activity. Therefore, we elicit synthesis, molecular docking and biological evaluation of coumarin substituted thiazolyl-3-aryl-pyrazole-4-carbaldehydes.

The docking simulation study was carried out using AutoDock[54] to validate the in vitro result and elucidate the importance of different types of interactions to inhibit the function of probable target human microsomal cytochrome P450 2A6 (1z11.pdb) enzyme[55,56]. The docking simulation results shows that the binding affinity of all the novel fifteen coumarin derivatives have stronger binding energies than that of the Methoxsalen (co-crystallized ligand of PDB molecule) and coumarin itself. The order of binding affinity of docked coumarin derivatives against the receptor is 35f> 35l>35n> 35o>35m>35g> 35h> 35j> 35e> 35d> 35k> 35a> 35b> and 35c with the range of binding energy being-10 to -12.61 k cal/mol **Table 5.**

The *in silico* study results show that the catalytic unit of Microsomal cytocrome P450 2A6 protein (1z11.pdb) active site residues Thr 305, Gln 360, Val 365, Pro 431, Phe 432, Cys439 and Phe 440 are key interacting amino acids and common among 15, 15, 14, 15, 15, 16 and 14 ligand molecules. The number of hydrogen bonds vary from zero to three with the receptor molecule that show other interactions like electrophilic and hydrophobic which are responsible for forming strong bonds between ligands and receptor (1z11.pdb) to inhibit the function of enzyme and are responsible for the cytotoxic effect.

Table 5: Docking results of novel coumarin derivatives with the Human Microsomal cytochrome P450 CYP2A6 [1z11.pdb] protein

Ligand name	Binding energy (kcal/ mol)	Inhibition Constant (nM)	No. of H bonds
Coumarin	-6.37	----	--
35a	-10.76	12.96	2
35b	-10.75	2.58	3
35c	-10.61	3.07	1
35d	-11.22	6.02	0
35e	-11.55	3.41	1
35f	-12.61	$5.70X\ 10^{-2}$	1
35g	-11.61	16.62	1
35h	-11.6	2.7	2
35i	-11.72	13.2	1
35j	-11.58	3.27	2
35k	-10.86	10.97	1
35l	-12.02	1.54	1
35m	-11.72	2.57	1
35n	-11.95	1.73	1
35o	-11.89	1.93	2

The obtained binding energies for the coumarin derivatives show similarity with the cytotoxic activity of among all the tested derivatives except **35**f , the good inhibitory potential as seen for the 35g, 35i, 35m and 35n demonstrated lowest binding energies (-11.61, -11.72, -11.72 and -11.95 kcal/mol) than the coumarin and methoxsalane (-6.37 and -7.77 kcal/mol). Particularly, the lowest cytotoxic activity of compound **35**n showed the lowest binding energy of -11.95 kcal/mol and other active compounds interestingly have nearly equal binding energies. The docking poses of these compounds revealed that they could also occupy the same active site as shown in **Figure 3** and able to form strong hydrophobic and electrostatic interaction with the active site residues other than hydrogen bonding (**Figure 4**). Among the most key interacting residues, Ile366 and Cys 439 actively participate in the formation of hydrogen bonding with the compounds 35g, 35m and 35n.

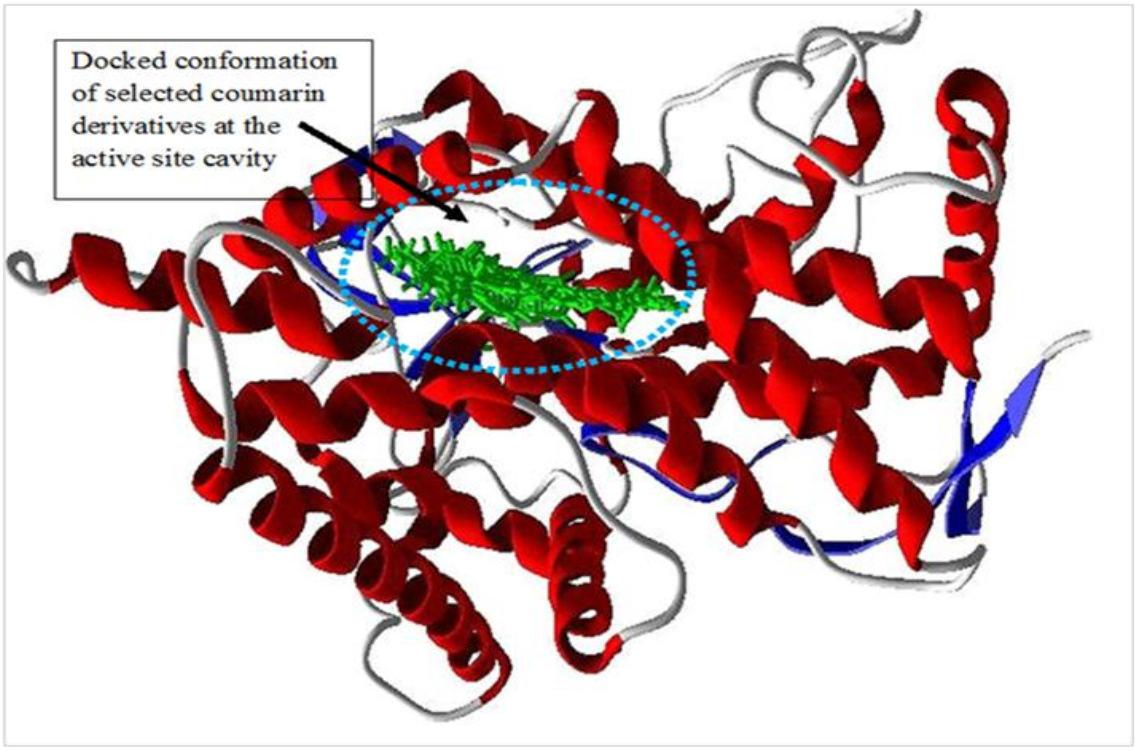


Figure.3 Secondary structure (cartoon) representation of target protein Human Microsomal cytochrome P450 2A6 together with the docked conformation of novel coumarin derivatives at the common active site.

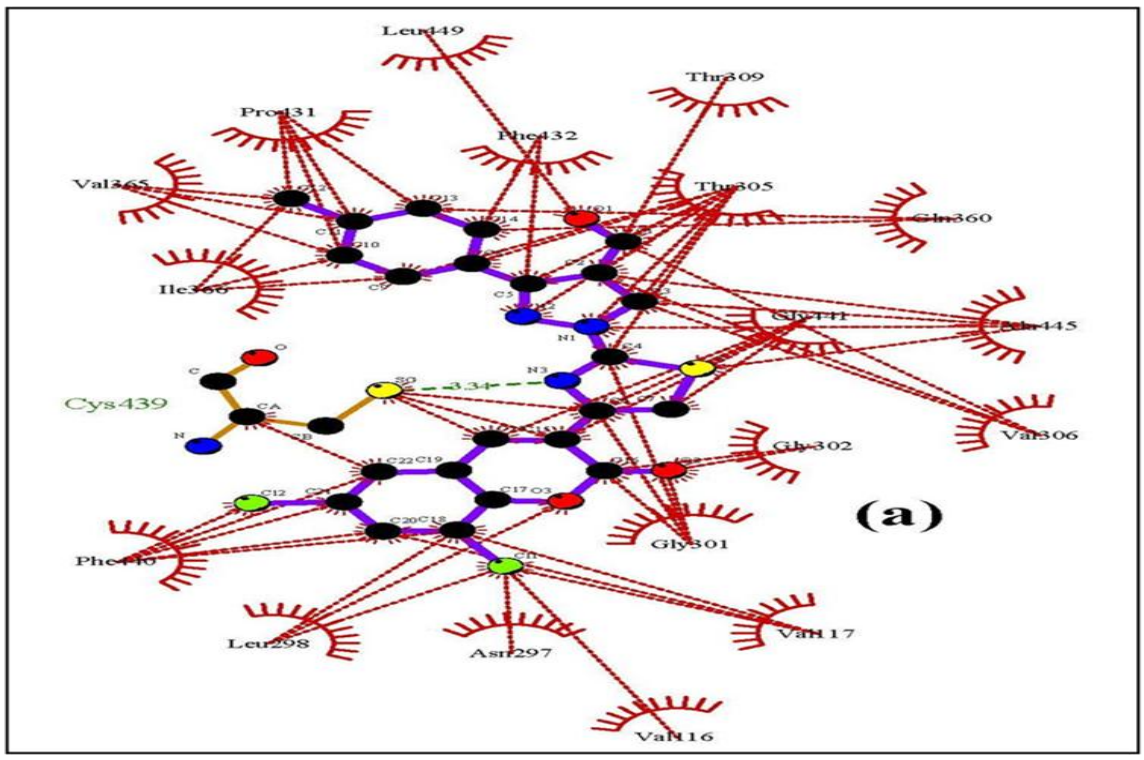


Figure.4 (a) Docked conformation of compound **35**m with hydrogen bonding view in form of 2-D using LigPlus[57].

In summary, we have developed a facile method for the synthesis of substituted thiazolyl pyrazole carbaldehyde derivatives under Vilsmeier-Haack reaction conditions *via* a multicomponent approach and evaluated molecular interactions as well as cytotoxic activities against human cancer cell lines and antibacterial activity against gram positive and gram negative bacteriae. Among the tested compounds, compound 4m, 4n exhibited significant cytotoxic activity against HeLa cell line. This is due to the presence of pharmacologically active rings like coumarin, thiazole and pyrazole along with a potential functional group like aldehyde in their structures. They seem to be suitable candidates for further structural modification and may be biologically active and useful as ligands in coordination chemistry.

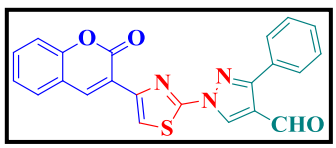
EXPERIMENTAL:

General procedure for the synthesis of coumarin substituted thiazolyl-3-aryl-pyrazole-4-carbaldehydes (35a-o):

A reaction mixture of substituted 3-(2-bromoacetyl)-2*H*-chromen-2-ones (1 mmol), thiosemicarbazide (0.0911 g, 1 mmol) and substituted acetophenones (1.1 mmol) was stirred for 15 minutes at room temperature. To this reaction mixture dimethyl formamide (5 mL) was added and stirred for about 30 minutes. The reaction mixture was cooled 0 °C and phosphorous oxychloride (0.7675 g, 5 mmol) was added drop wise by maintaining the temperature around 0 – 5 °C. After complete addition of $POCl_3$ the reaction mixture was stirred at room temperature for about 1 hour and further continued stirring at 60 °C for 4-6 h. After completion of the reaction (moniterde by TLC), the contents were poured into crushed ice and neutralized with aqueous sodium bicarbonate. The solid obtained was filtered, washed several times with water, dried and re crystallized from DMF solvent.

SPECTRAL DATA:

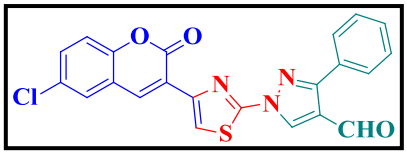
1-(4-(2-Oxo-2*H*-chromen-3-yl)thiazol-2-yl)-3-phenyl-1*H*-pyrazole-4-carbaldehyde (35a):



White solid; m.p.: 254-256 ^{o}C; **FT-IR (KBr, υ_{max}, cm^{-1}):** 1548 (C=N), 1638 (CHO), 1713 (C=O); **^{1}H NMR (400 MHz, DMSO-d_6, ppm):** δ 7.44-7.52 (m, 5H, Ar-H), 7.68 (d, 1H, *J* = 7.6 Hz, Ar-H), 7.89-7.94 (m, 3H, Ar-H), 8.37 (s, 1H, C_4-H of coumarin), 8.96 (s,

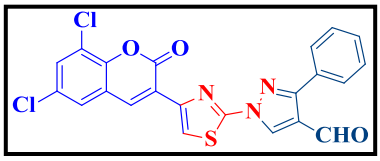
1H, C_5-H of pyrazole), 9.48 (s, 1H, C_5-H of thiazole), 10.07 (s, 1H, -CHO); **MS (LC-MS ESI) *m/z*:** 400 $[M+H]^+$; Anal. calcd. for $C_{22}H_{13}N_3O_3S$: C, 66.15; H, 3.28; N, 10.52; S, 8.03. Found: C, 66.19; H, 3.34; N, 10.43; S, 8.14%.

1-(4-(6-Chloro-2-oxo-2*H*-chromen-3-yl)thiazol-2-yl)-3-phenyl-1*H*-pyrazole-4-carbaldehyde (35b):



White solid; m.p.: 262-264 °C; **FT-IR (KBr, υ_{max}, cm^{-1}):** 1636 (-C=N), 1683 (-CHO), 1726 (-C=O); **^{1}H NMR (400 MHz, DMSO-d_6, ppm):** δ 7.54 (t, 3H, *J* = 6.0 Hz, Ar-H), 7.70 (d, 1H, *J* = 2.0 Hz, Ar-H), 7.93 (t, 1H, *J* = 3.6 Hz, Ar-H), 8.01 (d, 1H, *J* = 2.4 Hz, Ar-H), 8.22 (s, 2H, Ar-H), 8.39 (s, 1H, C_4-H of coumarin), 8.92 (s, 1H, C_5-H of pyrazole), 9.38 (s, 1H, C_5-H of thiazole), 10.07 (s, 1H, -CHO); **^{13}C NMR (100 MHz, DMSO-d_6, ppm):** δ 117.95, 118.96, 120.35, 121.32, 123.13, 127.64, 128.91, 129.00, 129.95, 130.17, 130.29, 131.55, 131.90, 138.47, 145.30, 151.47, 158.94, 163.21, 184.78; **HRMS (ESI) *m/z*** calculated for $C_{22}H_{12}ClN_3O_3S$ $[M+Na]^+$: 456.0186, found 456.0199. Anal. calcd. for $C_{22}H_{12}ClN_3O_3S$: C, 60.90; H, 2.79; N, 9.69; S, 7.39. Found: C, 60.84; H, 2.84; N, 9.63; S, 7.42 %.

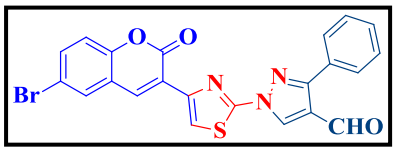
1-(4-(6,8-Dichloro-2-oxo-2*H*-chromen-3-yl)thiazol-2-yl)-3-phenyl-1*H*-pyrazole-4-carbaldehyde (35c):



White solid; m.p.: 270-272 °C; **FT-IR (KBr, υ_{max}, cm^{-1}):** 1637 (-C=N), 1685 (-CHO), 1727 (-C=O); **^{1}H NMR (400 MHz, DMSO-d_6, ppm):** δ 7.55 (d, 3H, *J* = 3.2 Hz, Ar-H), 7.71 (d, 1H, *J* = 8.4 Hz, Ar-H), 7.93 (d, 1H, *J* = 3.2 Hz, Ar-H), 8.00 (s, 1H, Ar-H), 8.18 (s, 1H, Ar-H), 8.39 (s, 1H, C_4-H of coumarin), 8.92 (s, 1H, C_5-H of pyrazole), 9.38 (s, 1H, C_5-H of thiazole), 10.07 (s, 1H, -CHO); **^{13}C NMR (100 MHz, DMSO-d_6, ppm):** 121.97, 123.02, 126.37, 128.54, 128.94, 129.02, 129.93, 130.33, 130.44, 131.37, 133.49, 135.60, 146.08, 152.92, 155.74, 159.18, 159.58, 185.09; **MS (ESI) *m/z*:** 468 $[M+2]^+$; Anal. calcd. for $C_{22}H_{11}Cl_2N_3O_3S$: C, 56.42; H, 2.37; N, 8.97; S, 6.85. Found: C, 56.46; H, 2.34; N, 8.99; S, 6.81 %.

1-(4-(6-Bromo-2-oxo-2*H*-chromen-3-yl)thiazol-2-yl)-3-phenyl-1*H*-pyrazole-4-carbaldehyde (35d):

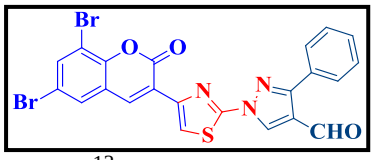
Yellow solid; m.p.: 285-287 °C; **FT-IR (KBr, υ_{max}, cm^{-1}):** 1636 (-C=N), 1682 (-CHO), 1728 (-C=O); **^{1}H NMR (400 MHz, DMSO-d_6, ppm):** δ 7.46 (d, 1H, *J* = 8.8 Hz, Ar-H),



7.54 (t, 3H, *J* = 3.6 Hz, Ar-H), 7.79-7.82 (m, 1H, Ar-H), 7.92(t, 2H, *J* = 4.0 Hz, Ar-H), 8.11 (d, 1H, *J* = 2.0 Hz, Ar-H), 8.37 (s, 1H, C_4-H of coumarin), 8.90 (s, 1H, C_5-H of pyrazole), 9.33 (s, 1H, C_5-H of thiazole), 10.06 (s, 1H, -CHO); **^{13}C NMR (100 MHz, DMSO-*d*$_6$, ppm):** 117.47, 118.24, 118.97, 123.14, 128.91, 129.01, 130.71, 131.55, 134.70, 138.36, 145.92, 152.86, 158.48, 163.52, 184.76; **HRMS (ESI)** ***m/z*** calculated for $C_{22}H_{12}BrN_3O_3S$ $(M + Na)^+$ 499.9680, found. 499.9567; Anal. calcd. for $C_{22}H_{12}BrN_3O_3S$: C, 55.24; H, 2.53; N, 8.78; S, 6.70. Found: C, 55.29; H, 2.59; N, 8.84; S, 6.77 %.

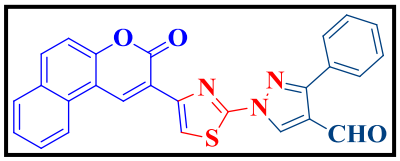
1-(4-(6,8-Dibromo-2-oxo-2*H*-chromen-3-yl)thiazol-2-yl)-3-phenyl-1*H*-pyrazole-4-carbaldehyde (35e):

Yellow solid; m.p.: 268-270 °C; **FT-IR (KBr, υ_{max}, cm^{-1}):** 1637(-C=N), 1681 (-CHO), 1737 (-C=O); ^{1}H NMR (400 MHz, DMSO-*d*$_6$, ppm): δ 7.54 (t, 2H, *J* = 3.6 Hz, Ar-H),



7.92 (t, 1H, *J* = 4.0 Hz, Ar-H), 8.14-8.18 (m, 4H, Ar-H), 8.38 (s, 1H, C_4-H of coumarin), 8.87 (s, 1H, C_5-H of pyrazole), 9.33 (s, 1H, C_5-H of thiazole), 10.06 (s, 1H, -CHO); ^{13}C NMR (100 MHz, DMSO-*d*$_6$, ppm): δ 111.01, 117.37, 119.64, 121.63, 123.19, 128.92, 128.99, 129.92, 129.98, 131.51, 137.28, 137.87, 155.74, 157.94, 163.22, 184.76; **MS (ESI) *m/z*:** 559 $[M+2]^+$; Anal. calcd. for $C_{22}H_{11}Br_2N_3O_3S$: C, 47.42; H, 1.99; N, 7.54; S, 5.75. Found: C, 47.37; H, 1.91; N, 7.50; S, 5.71 %.

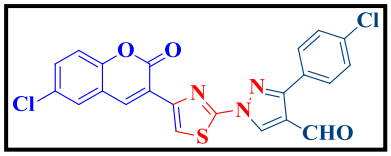
1-(4-(3-Oxo-3*H*-benzo[*f*]chromen-2-yl)thiazol-2-yl)-3-phenyl-1*H*-pyrazole-4-carbaldehyde (35f):



Yellow solid; m.p.: 257-259 °C; **FT- IR (KBr, υ_{max}, cm^{-1}):** 1635 (-C=N), 1683 (-CHO), 1725 (-C=O); **^{1}H NMR (400 MHz, DMSO-*d*$_6$, ppm):** δ 7.55 (t, 2H, *J* = 3.6 Hz, Ar-H), 7.65-7.70 (m, 2H, Ar-H), 7.85 (t, 1H, *J* = 7.6 Hz, Ar-H), 7.94 (t, 2H, *J* = 3.6 Hz, Ar-H), 8.10 (d, 1H, *J* = 8.4 Hz, Ar-H), 8.25 (d, 1H, *J* = 9.2 Hz, Ar-H), 8.40 (s, 2H, Ar-H), 8.80 (s, 1H, C_4-H of coumarin), 9.64 (s, 1H, C_5-H of pyrazole), 9.78 (s, 1H, C_5-H of thiazole), 10.09 (s, 1H, -CHO); **^{13}C NMR (100 MHz, DMSO-*d*$_6$, ppm):** δ 113.20, 116.45, 118.47, 118.88, 122.41, 122.82, 126.31, 128.50, 128.63, 128.75, 128.97, 129.72, 130.05, 130.21, 133.65, 135.04, 135.71, 145.22, 152.45, 153.96, 158.63, 158.87, 184.87; **HRMS (ESI)** ***m/z*** calculated for $C_{26}H_{15}N_3O_3S$ $(M + Na)^+$ 472.0732,

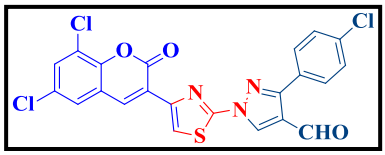
found.472.0784; Anal. calcd. for $C_{26}H_{15}N_3O_3S$: C, 69.48; H, 3.36; N, 9.35; S, 7.13, Found: C, 69.51;H, 3.32; N, 9.31; S, 7.17 % .

1-(4-(6-Chloro-2-oxo-2*H*-chromen-3-yl)thiazol-2-yl)-3-(4-chlorophenyl)-1*H*-pyrazole-4-carbaldehyde (35g):



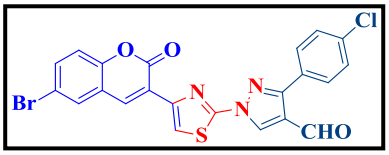
White solid; m.p.: 293-295 °C; **FT-IR (KBr, υ_{max}, cm^{-1}):** 1634 (-C=N), 1682 (-CHO), 1734 (-C=O); **^{1}H NMR (400 MHz, DMSO-d_6, ppm):** δ 7.50-7.70 (m, 4H, Ar-H), 7.97 (d, 3H, *J* = 7.6 Hz, Ar-H), 8.37 (s, 1H, C_4-H of coumarin), 8.85 (s, 1H, C_5-H of pyrazole), 9.38 (s, 1H, C_5-H of thiazole), 10.05 (s, 1H, -CHO); **HRMS (ESI)** *m/z* calculated for $C_{22}H_{11}Cl_2N_3O_3S$ $(M + Na)^+$ 489.9796, found.489.9712; Anal. calcd. for $C_{22}H_{11}Cl_2N_3O_3S$: C, 56.42; H, 2.37; N, 8.97; S, 6.85. Found: C, 56.40; H, 2.39; N, 8.97; S, 6.83 %.

1-(4-(6,8-Dichloro-2-oxo-2*H*-chromen-3-yl)thiazol-2-yl)-3-(4-chlorophenyl)-1*H*-pyrazole -4-carbaldehyde (35h):



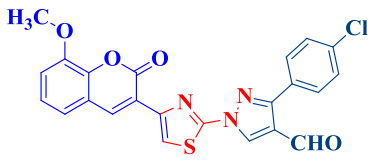
White solid; m.p.: 272-274 °C; **FT-IR (KBr, υ_{max}, cm^{-1}):** 1635 (-C=N), 1683 (-CHO), 1735 (-C=O); **^{1}H NMR (400 MHz, DMSO-d_6, ppm):** δ 7.61 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.70 (d, 1H, *J* = 8.8 Hz, Ar-H), 7.99 (d, 3H, *J* = 8.0 Hz, Ar-H), 8.39 (s, 1H, C_4-H of coumarin), 8.89 (s, 1H, C_5-H of pyrazole), 9.41 (s, 1H, C_5-H of thiazole), 10.06 (s, 1H, -CHO); **MS (ESI) *m/z*:** 501 $[M+H]^+$; Anal. calcd. for $C_{22}H_{10}Cl_3N_3O_3S$: C, 52.56; H, 2.00; N, 8.36; S, 6.38. Found: C, 52.52; H, 1.96; N, 8.40; S, 6.45 %.

1-(4-(6-Bromo-2-oxo-2*H*-chromen-3-yl)thiazol-2-yl)-3-(4-chlorophenyl)-1*H*-pyrazole-4-carbaldehyde (35i):



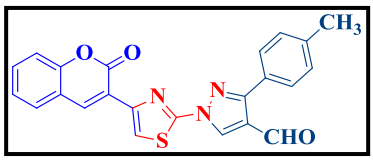
Yellow solid; m.p.: 297-299 °C; **FT-IR (KBr, υ_{max}, cm^{-1}):** 1553 (-C=N), 1678 (-CHO), 1735(-C=O); **^{1}H NMR (400 MHz, DMSO-d_6, ppm):** δ 7.47 (d, 1H, *J* = 8.8 Hz, Ar-H), 7.61 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.81 (d, 1H, *J* = 6.8 Hz, Ar-H), 7.99 (d, 2H, *J* = 8.4 Hz, Ar-H), 8.12 (s, 1H, Ar-H), 8.38 (s, 1H, C_4-H of coumarin), 8.88 (s, 1H, C_5-H of pyrazole), 9.39 (s, 1H, C_5-H of thiazole), 10.06 (s, 1H, -CHO); **HRMS (ESI)** *m/z* calculated for $C_{22}H_{11}BrClN_3O_3S$ $(M + Na)^+$ 533.9291, found.533.9246; Anal. calcd. for $C_{22}H_{11}BrClN_3O_3S$: C, 51.53; H, 2.16; N, 8.19; S, 6.25. Found: C, 51.59; H, 2.00; N, 8.24; S, 6.19 %.

3-(4-Chlorophenyl)-1-(4-(8-methoxy-2-oxo-2*H*-chromen-3-yl)thiazol-2-yl)-1*H*-pyrazole-4-carbaldehyde (35j):



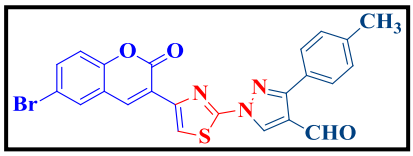
White solid; m.p.: 260-262 °C; **FT-IR (KBr, υ_{max}, cm^{-1}):** 1272 (C-O-C), 1570 (-C=N), 1617 (-CHO), 1722 (-C=O); **^{1}H NMR (400 MHz, DMSO-d_6, ppm):** δ 3.93 (s, 3H, -OCH_3), 7.30-7.34 (m, 4H, Ar-H), 7.48 (d, 1H, *J* = 8.0 Hz, Ar-H), 7.58-7.61 (m, 1H, Ar-H), 7.79 (t, 1H, *J* = 2.4 Hz, Ar-H), 8.53 (s, 1H, C_4-H of coumarin), 8.85 (s, 1H, C_5-H of pyrazole), 9.47 (s, 1H, C_5-H of thiazole), 10.04 (s, 1H, -CHO); ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ 56.02, 119.64, 119.80, 119.95, 120.66, 124.58, 127.27, 128.34, 128.51, 128.78, 129.23, 130.02, 130.29, 133.35, 136.54, 138.08, 141.51, 143.90, 145.46, 146.22, 158.36, 169.05, 185.13; MS (ESI) *m/z*: 465 $[M+2H]^+$; Anal. calcd. for $C_{23}H_{14}ClN_3O_4S$: C, 59.55; H, 3.04; N, 9.06; S, 6.91. Found: C, 59.51; H, 3.05; N, 9.15; S, 6.86 %.

1-(4-(2-Oxo-2*H*-chromen-3-yl)thiazol-2-yl)-3-*p*-tolyl-1*H*-pyrazole-4-carbaldehyde (35k):



Yellow solid; m.p.: 275-277 °C; **FT-IR (KBr, υ_{max}, cm^{-1}):** 1552 (-C=N), 1636 (-CHO), 1714 (-C=O); **^{1}H NMR (400 MHz, DMSO-d_6, ppm):** δ 2.40 (s, 3H, -CH_3), 7.35(d, 2H, *J* = 8.0 Hz, Ar-H), 7.45 (t, 2H, *J* = 7.6 Hz, Ar-H), 7.51 (d, 1H, *J* = 8.8 Hz, Ar-H), 7.68 (d, 2H, *J* = 7.2 Hz, Ar-H), 7.83-7.91 (m, 1H, Ar-H), 8.36 (s, 1H, C_4-H of coumarin), 8.96 (s, 1H, C_5-H of pyrazole), 9.45 (s, 1H, C_5-H of thiazole), 10.06 (s, 1H, -CHO); **MS (ESI) *m/z*:** 414 $[M+H]^+$; Anal. calcd. for $C_{23}H_{15}N_3O_3S$: C, 66.82; H, 3.66; N, 10.16; S, 7.76. Found: C, 66.87; H, 3.82; N, 10.22; S, 7.80 %.

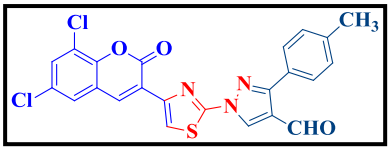
1-(4-(6-Bromo-2-oxo-2*H*-chromen-3-yl)thiazol-2-yl)-3-*p*-tolyl-1*H*-pyrazole-4-carbaldehyde (35l):



Yellow solid; m.p.: 263-265 °C; **FT-IR(KBr, υ_{max}, cm^{-1}):** 1547 (-C=N), 1636 (-CHO), 1729 (-C=O); **^{1}H NMR (400 MHz, DMSO-d_6, ppm):** δ 2.39 (s, 3H, -CH_3), 7.35 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.47 (d, 1H, *J* = 8.8 Hz, Ar-H), 7.81-7.84 (m, 2H, Ar-H), 8.14 (d, 1H, *J* = 2.4 Hz, Ar-H), 8.19 (s, 1H, Ar-H), 8.38 (s, 1H, C_4-H of coumarin), 8.92 (s, 1H, C_5-H of pyrazole), 9.34 (s, 1H, C_5-H of thiazole), 10.05 (s, 1H, -CHO); **MS (ESI)**

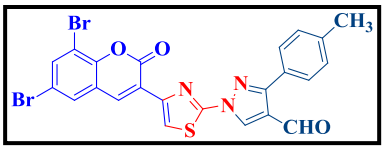
***m/z*:** 493 $[M+H]^+$; Anal. calcd. for $C_{23}H_{14}BrN_3O_3S$: C, 56.11; H, 2.87; N, 8.53; S, 6.51. Found: C, 56.18; H, 2.94; N, 8.58; S, 6.00 %.

1-(4-(6,8-Dichloro-2-oxo-2*H*-chromen-3-yl)thiazol-2-yl)-3-*p*-tolyl-1*H*-pyrazole-4-carbaldehyde (35m):



Yellow solid; m.p.: 172-174 °C; **FT-IR (KBr, υ_{max}, cm^{-1}):** 1556 (-C=N), 1631 (-CHO), 1728 (-C=O); **^{1}H NMR (400 MHz, DMSO-d_6, ppm):** 2.31 (s, 3H, -CH_3), 7.23 (d, 1H, *J* = 8.0 Hz, Ar-H), 7.34 (d, 1H, *J* = 6.4 Hz, Ar-H), 7.65-7.69 (m, 1H, Ar-H), 7.81 (d, 1H, *J* = 1.2 Hz, Ar-H), 7.96 (s, 1H, Ar-H), 8.37 (s, 1H, Ar-H), 8.49 (s, 1H, C4-H of coumarin), 8.88 (s, 1H, C4-H of pyrazole), 9.32 (s, 1H, C5-H of thiazole), 10.05 (s, 1H, -CHO); **^{13}C NMR (100 MHz, DMSO-d_6, ppm):** δ 20.75, 111.84, 117.80, 120.59, 121.62, 125.58, 127.52, 127.80, 128.56, 128.94, 129.17, 131.04, 134.98, 136.49, 138.30, 143.73, 147.09, 150.81, 158.27, 169.42, 184.87; **HRMS (ESI) *m/z*** calculated for $C_{23}H_{14}Cl_2N_3O_3S$ $(M + H)^+$ 482.0133, found.482.1837; Anal. calcd. for $C_{23}H_{13}Cl_2N_3O_3S$: C, 57.27; H, 2.72; N, 8.71; S, 6.65. Found: C, 57.32; H, 2.69; N, 8.76; S, 6.63 %.

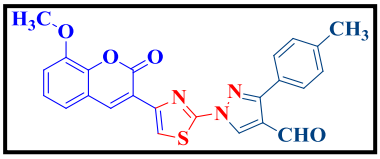
1-(4-(6,8-Dibromo-2-oxo-2*H*-chromen-3-yl)thiazol-2-yl)-3-*p*-tolyl-1*H*-pyrazole-4-carbaldehyde (35n):



Yellow solid; m.p.: 170-172 °C; **FTIR (KBr, υ_{max}, cm^{-1}):** 1611 (-C=N), 1676 (-CHO), 1737(-C=O); **^{1}H NMR (400 MHz, DMSO-d_6, ppm):** δ 2.31 (s, 3H, -CH_3), 7.22 (d, 1H, *J* = 8.0 Hz, Ar-H), 7.43 (d, 1H, *J* = 8.0 Hz, Ar-H), 7.67 (d, 1H, *J* = 8.4 Hz, Ar-H), 7.82 (s, 1H, Ar-H), 8.10-8.14 (m, 2H, Ar-H), 8.43 (s, 1H, C_4-H of coumarin), 8.85 (s, 1H, C_5-H of pyrazole), 9.30 (s, 1H, C_5-H of thiazole), 10.04 (s, 1H, -CHO); **^{13}C NMR (DMSO- d_6, ppm):** δ 20.76; 112.51; 116.38; 122.12; 125.58; 128.21; 128.55; 128.94; 129.17; 130.13; 130.47; 134.94; 135.72; 136.02; 138.33; 143.42; 147.18; 148.05; 157.60; 162.25; 169.52; 184.47; **MS (ESI) *m/z*:** 591 $[M+Na]^+$; Anal. calcd. for $C_{23}H_{13}Br_2N_3O_3S$: C, 48.36; H, 2.29; N, 7.36; S, 5.61. Found: C, 48.30; H, 2.23; N, 7.31; S, 5.54 %.

1-(4-(8-Methoxy-2-oxo-2*H*-chromen-3-yl)thiazol-2-yl)-3-*p*-tolyl-1*H*-pyrazole-4-carbaldehyde (35o):

Yellow solid; m.p.: 276-278 °C; **FTIR (KBr, υ_{max}, cm^{-1}):** 1274 (C-O-C), 1637 (-C=N), 1673 (-CH0), 1720 (C=O); **^{1}H NMR (400 MHz, DMSO-d_6, ppm):** δ 2.39 (s, 3H, -CH_3),



3.94 (s, 3H, -OCH_3), 7.33-7.40 (m, 6H, Ar-H), 7.83(d, 1H, *J* = 7.6 Hz, Ar-H), 8.34 (s, 1H, C_4-H of coumarin), 8.89 (s, 1H, C_5-H of pyrazole), 9.42 (s, 1H, C_5-H of thiazole), 10.04 (s, 1H, -CHO); **MS (ESI) *m/z*:** 444 $[M+H]^+$; Anal. calcd. for $C_{24}H_{17}N_3O_4S$: C, 65.00; H, 3.86; N, 9.48; S, 7.23. Found: C, 65.12; H, 3.81; N, 9.57; S, 7.29 %.

ANTICANCER ACTIVITY EVALUATION COUMARINYL THIAZOLYL PYRAZOLE CARBALDEHYDE DERIVATIES USING MTT ASSAY

The different human cancer cell lines viz., breast cancer (MCF-7), prostate cancer (DU-145) and human epithelial cervical cancer (HeLa) cells were procured from American Type Culture Collection (ATCC). These cells were grown in Dulbecco's modified Eagle's medium (DMEM) with 10 % fetal bovine serum (FBS) supplemented with antibiotics and treated with test compounds at 2.5, 5, 10 and 100 µM concentrations for a period of 48 h and cytotoxicity was measured by standard MTT assay[49].

At the end of the treatment, the medium was removed and the cells were washed with Dulbecco's phosphate buffered saline (DPBS) and 10 µL of 5 mg/ml MTT solution in 200 µL of culture medium was added and incubated for 1 h at 37 ^{0}C. Cells were solubilized with 200 µL of DMSO and absorbance was measured at 562 nm in a spectrophotometer. The absorbance of the wells containing treated cells was compared with that of the wells in which the drug is omitted (control). Assays were performed in triplicate and the results were showed as the percent reduction in cell viability compared to untreated control cultures.

HOECHST-33258 STAINING

Hela cells were incubated for a period of 24 h in the presence or absence of test compounds **35m** and **35n** (10 µM). At the end of treatment, the medium was aspirated, cells were washed with medium without FBS, and Hoechst-33258 stain (Invitrogen cat. no. H3570) was added to the cells for 20 min at 37 $^\circ$C under humidified atmosphere. Cells were washed again with the medium. The cells were covered with medium and observed under a fluorescence microscope equipped with DAPI filter.

Anti Bacterial Activity evaluation of Coumarinyl thiazolyl pyrazole carbaldehyde derivaties

Preparation of standard bacterial suspensions:

The bacterial strains were inoculated into sterilized nutritive broth and incubated at 35 ± 2°C for 24 h. The turbidity of the resulting suspensions are diluted with same nutritive broth to obtain a transmittance of 25% at 580 nm, this percentage was calculated spectrophotometrically using Bausch & Lomb spectrophotometer comparable to McFarland turbidity standard. This level of turbidity is equivalent to approximately 3.0 × 108 CFU/ml (a stock standard from which a working standard was drawn with concentration of 1 × 108 CFU/ml).

The antibacterial activity of these synthesized compounds was carried out according to the literature method. Each selective medium was inoculated with the test organism suspended in nutritive broth. Once the agar was solidified, it was punched with a six millimeter diameter wells and filled with various concentrations and corresponding wells with positive and negative control. The concentration of the synthesized compounds 10, 20 ,100, 200 and 300 µg/ml simultaneously, gentamycin sulfate (10 µg/ml) is used as positive control. The test was carried out in triplicate. The plates were incubated at 35 ± 2°C for 24 h. The inhibition zone diameter was measured in mm.

Determination of Minimum Inhibitory Concentration and Minimum Bactericidal Concentrations

The assay was conducted to assess the minimum inhibitory concentration of synthesized compounds through microtiter plate method. The assay reaction mixture consisted of PBS (50mM sodium phosphate, 150 mM NaCl [pH 7.0]), the test compounds at various concentrations and the bacterial strains, were prepared in sterile 96-well microtiter plates (Nunc, Inc). The wells are filled with 100µl diluted test compounds in PBS and 50 µl of the diluted bacterial strains. The wells were incubated with gentle shaking (140 rpm) at 37 °C for 24 h. For positive and negative controls, a separate microtiter plate was prepared and screened 24 h. Following incubation, a 20-µl aliquot from each well was spotted at the top of a square plate containing Nutrient agar medium. The plate was labelled and tapped gently to facilitate the movement of the liquid. There were approximately 200 cells in the spotted (20-µl) sample. Plates were placed uncovered in bio hood until the sample liquid dried (ca. 10 min) and incubated overnight at 37^{o}C. The experiments were performed in duplicate, and CFU for each streak was enumerated after 24 h using a colony counter.

The number of CFU at each dilution of test compounds was compared with the average of positive control value to determine the percentage of bacteria killed per well. The percentage of the bacteria killed was plotted graphically, and the percentage of the test compound resulting in maximum decrease in the number of CFU (MIC) was determined.

Minimum bactericidal concentration [MBC] is defined as the lowest concentration of a test sample required to kill the 99.9% of bacteria after a define time of incubation. To determine the MBC values of the fifteen synthesized compounds, the nutrient agar was spread on to petri dish in the initial step. Finally the bacterial strains along with various concentrations of samples were spread over the plates. The coated petri plates with bacterial strains were incubated at 37 ^{o}C for about 24 h. The number of surviving colonies was counted to determine the minimum bactericidal concentration values for the synthesized molecules.

Lipophilicity

Shake flask method:

The lipophilicity values of all the tested compounds were determined experimentally by shake flask method using n-Octanol – Phosphate buffer (0.15M, P^{H} = 7.4) solution. Initially 1mM concentration of all the synthesized compounds stock solution was prepared in dimethylsulphoxide solution. The experiment was carried out using n-octanol : phosphate buffer in 1: 75 volume ration. The stock solution was added to saturated octanol- phosphate buffer system and the phase was shaken on a mechanical stirrer (labtech) for about 30 minutes. The phase was centrifuged at 9000 rpm 15 minutes to afford complete separation of n-octanol – phosphate buffer system. The upper layer of n-octanol was removed and the absorbance of buffer was measured by using Perkin elmer UV-spectrophotometer. Partition coefficient values were calculated using the following formula.

P = (b-a/a) X (V_{buffer}/ $V_{n\text{-}octanol}$)

b = Conc. Of stock solution added

a = Conc. Of solution found

Computation

Protein and ligands structure selection and preparation

Three-dimensional X-ray crystal structure of Human Microsomal cytochrome P450 2A6 (PDB ID: 1Z11, resolution=2.05Å, Chains: A, B, C and D) was downloaded from the Protein Data Bank. The downloaded protein complexed with 4 Methoxsalen as a ligand, 4 Hem (Protoporphyrin IX Containing Fe) as a co-factor and 504 water molecules for crystallization ChemSketch was used to draw the 2-D structure of ligands and converted into energy minimized 3-D structure using chimera software.

The MGLTool 1.5.6 program was used for preparation of protein structure and the ligands were selected as an input file for grid generation and docking. The co-crystallized heteroatoms (cofactors, ligands and water molecules) were removed. The missing hydrogen atom and charge were added to the receptor protein. The final input file generated in the form of .pdbqt format was used for docking simulation. The active site was selected on the basis of already bound ligand (Methoxsalen binding site) to the receptor. Fifteen novel chemically synthesized coumarin derivatives have been selected for anti cancerous activity using *in silico* approach to validate the in vitro results and elucidate the important SAR properties.

Grid generation and docking simulation

For the grid generation, AutoGrid 4 was used by selecting the active site region of interest (grid box of limit 100 × 100 × 100 points, grid centre X=57.947; Y=76.735; Z=61.289 and default (0.375 Å grid spacing) in the macromolecule. For the docking simulation AutoDcok 4 was used with all the rotatable bonds of ligands which were allowed to rotate freely and Lamarckian genetic algorithm (LGA) was selected to search the energy landscape. The docked conformation of selected ligands having good binding affinity with receptor has been demonstrated in two and three dimensional form using LigPlus and Molegro Molecular Viewer[58].

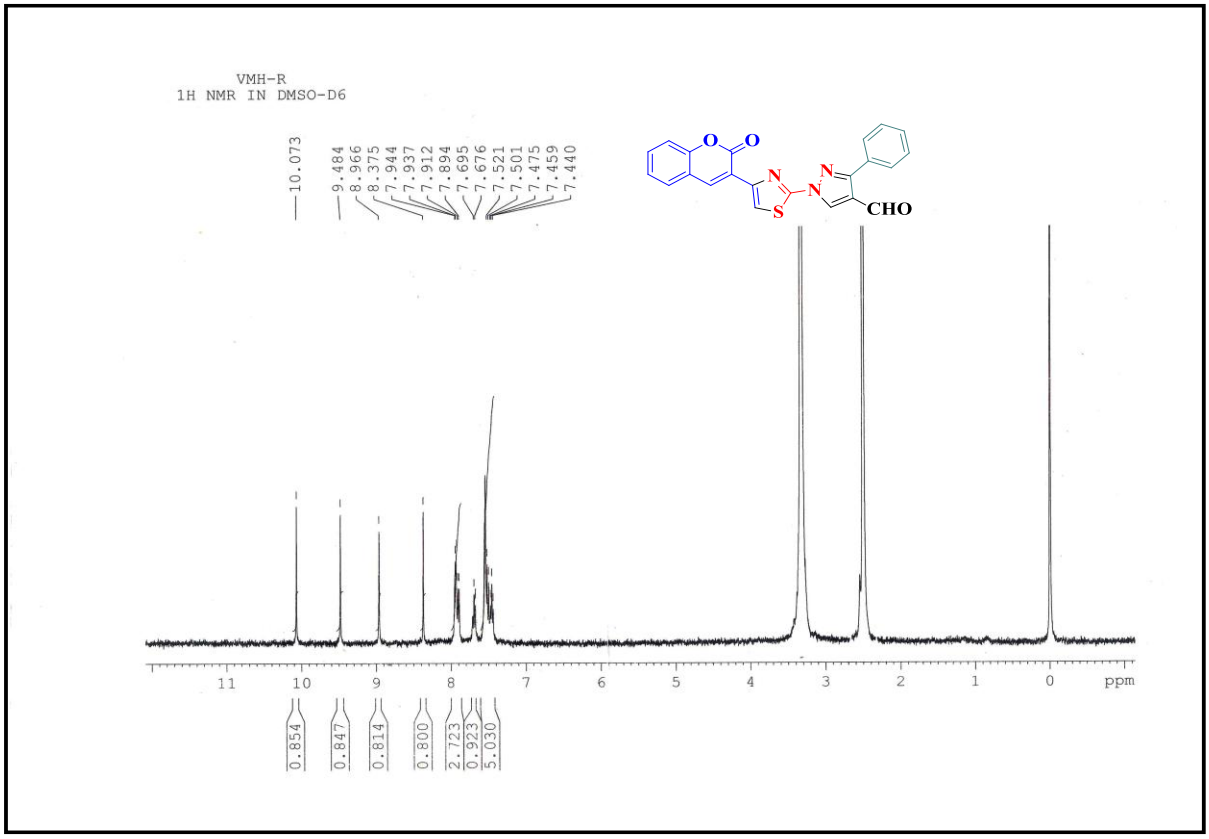


Fig: ^{1}H NMR spectrum of compound **35a**

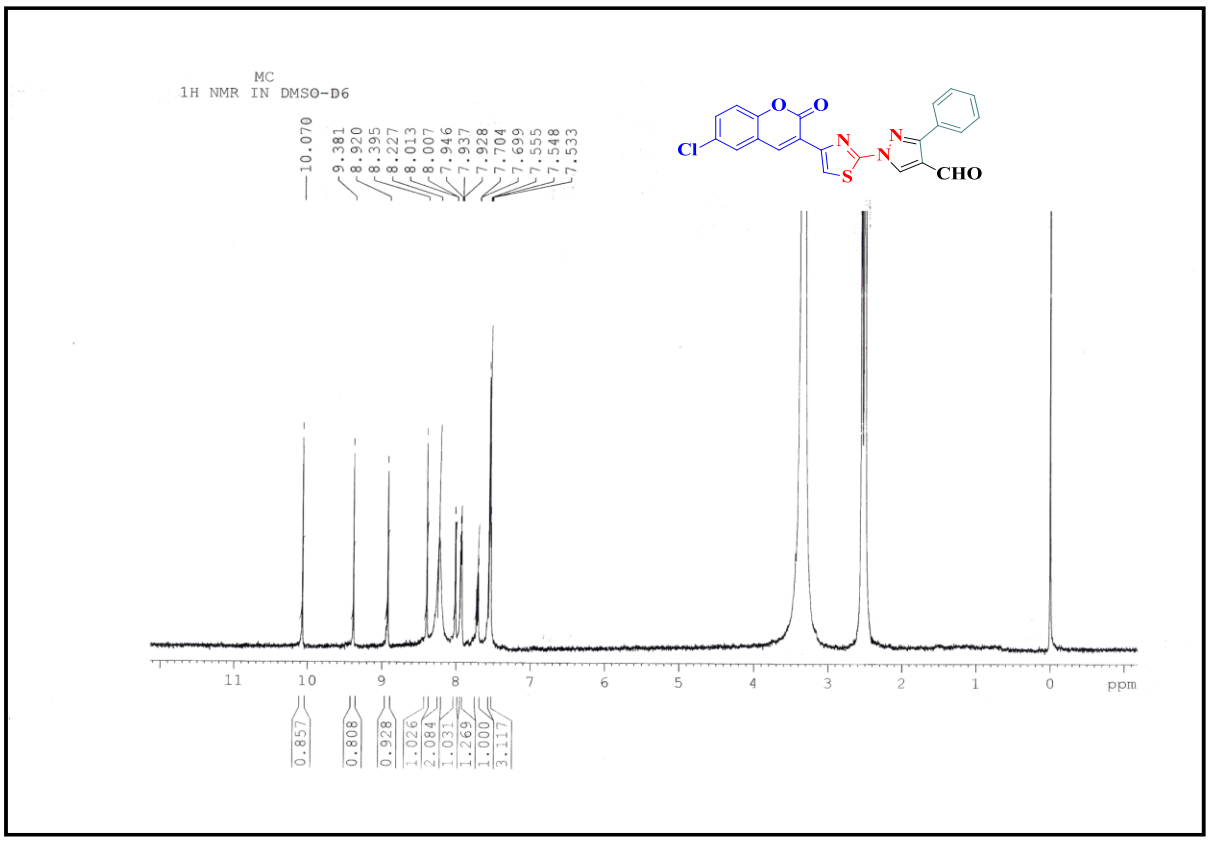


Fig: ^{1}H NMR spectrum of compound **35b**

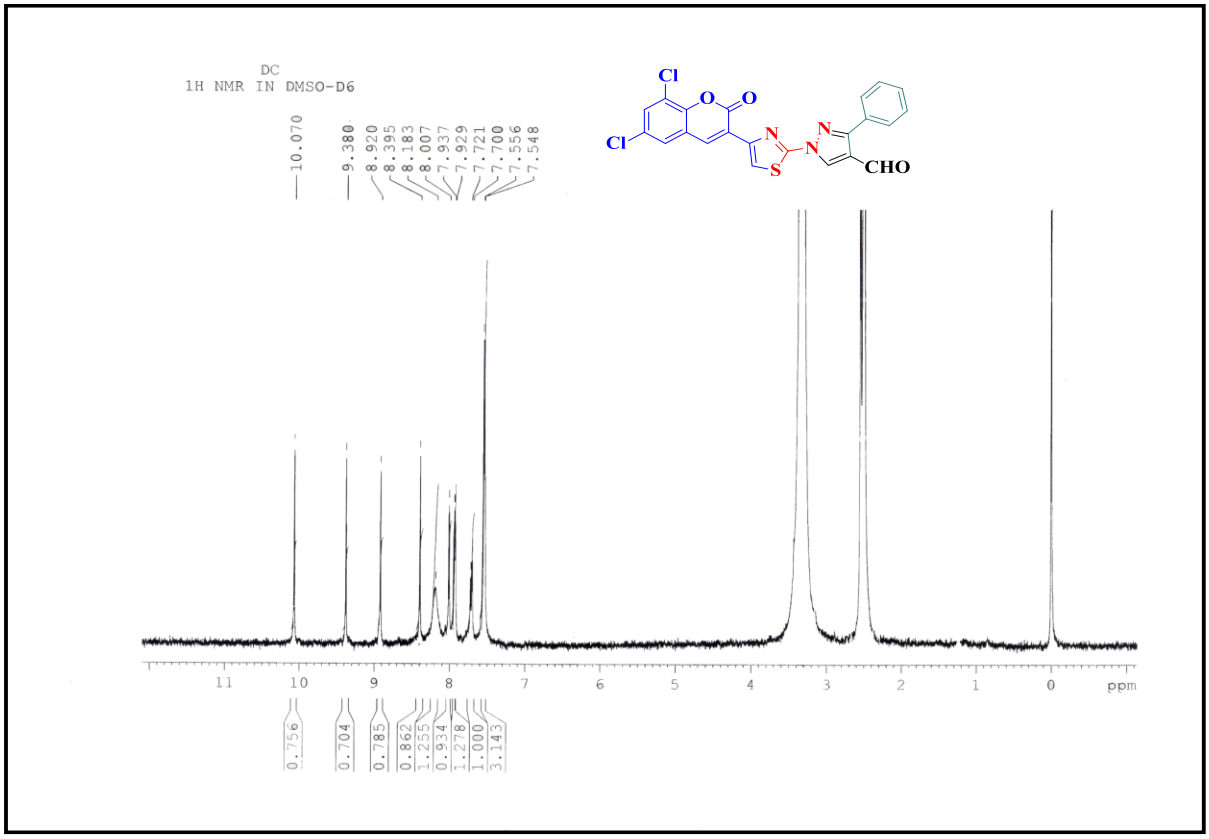


Fig: ^{1}H NMR spectrum of compound **35c**

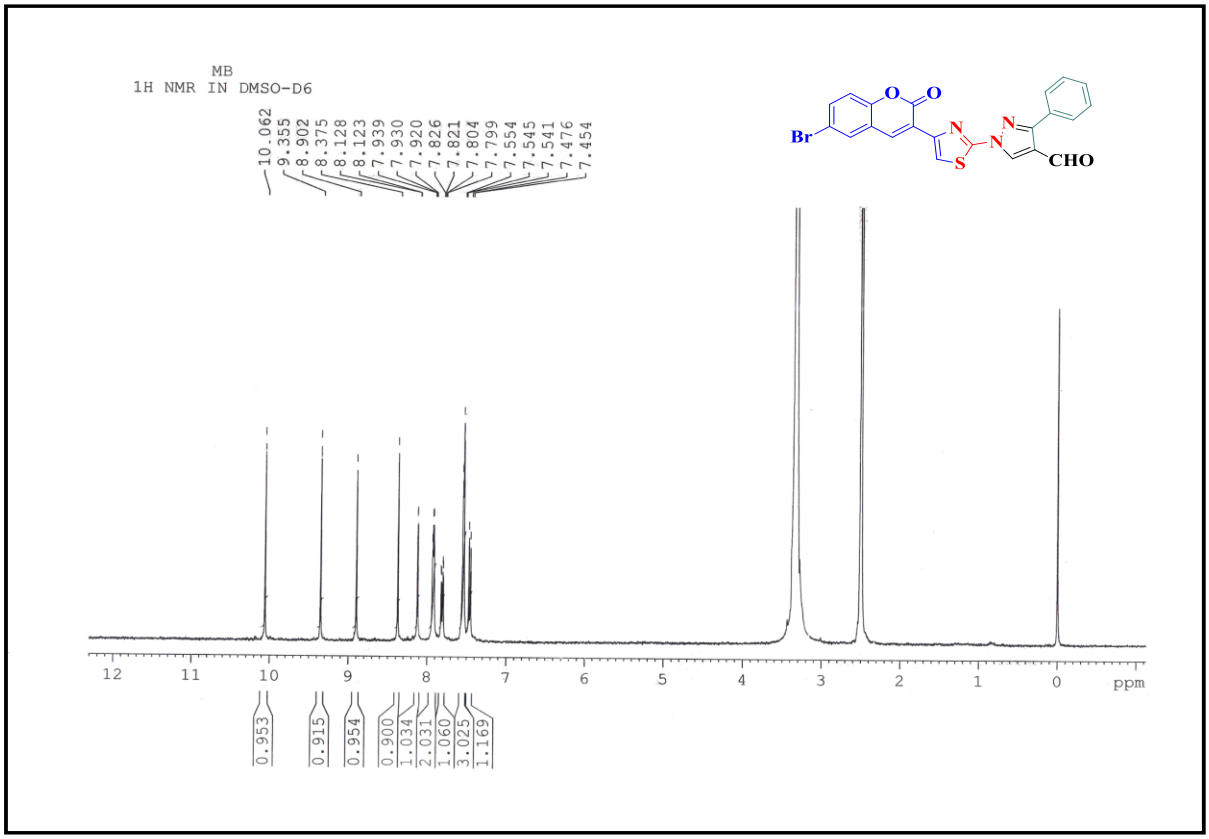


Fig: ^{1}H NMR spectrum of compound **35d**

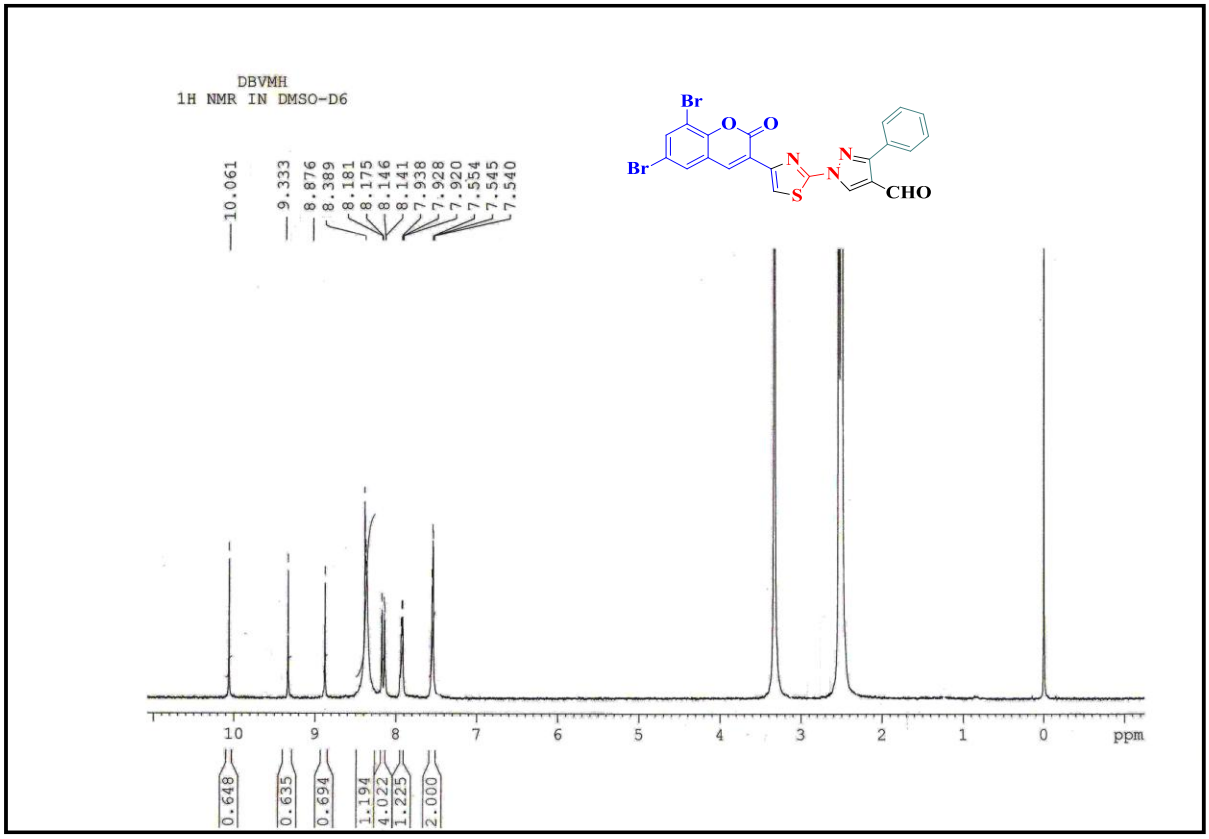


Fig: ^{1}H NMR spectrum of compound **35e**

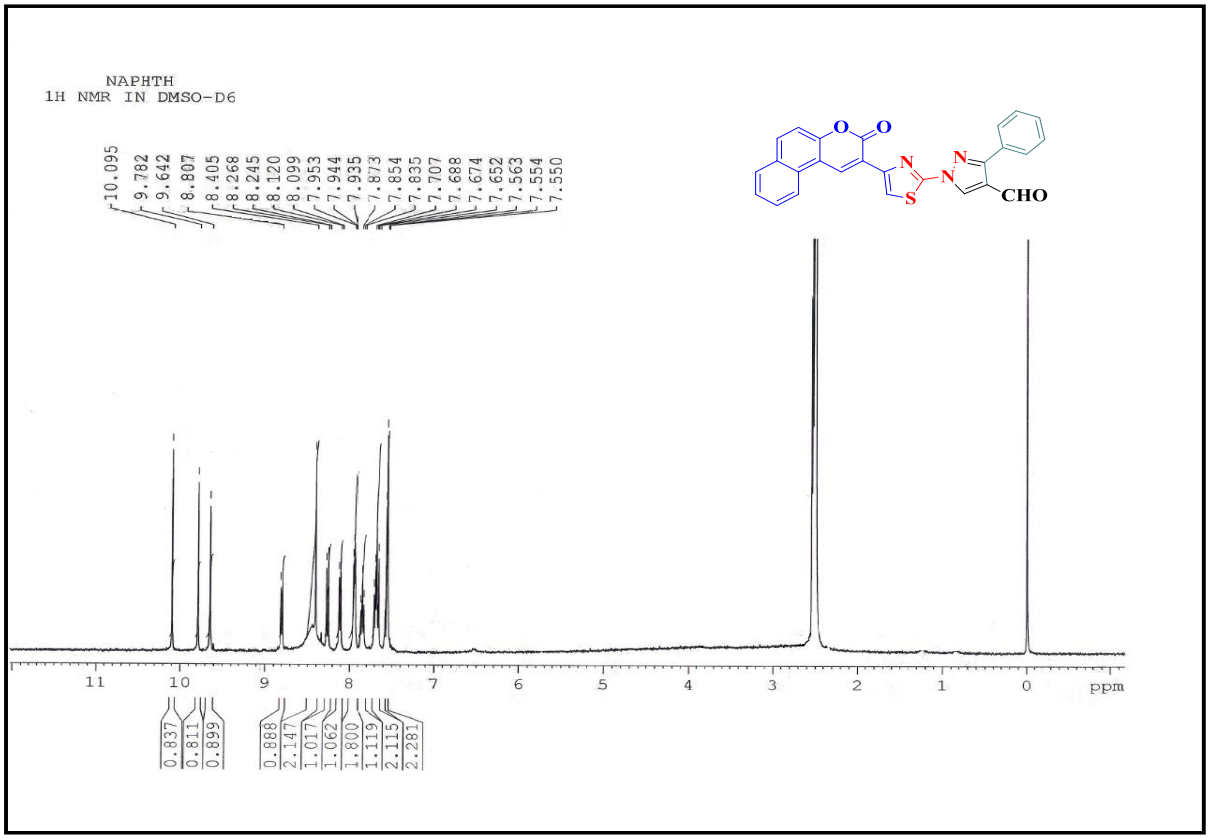


Fig: ^{1}H NMR spectrum of compound **35f**

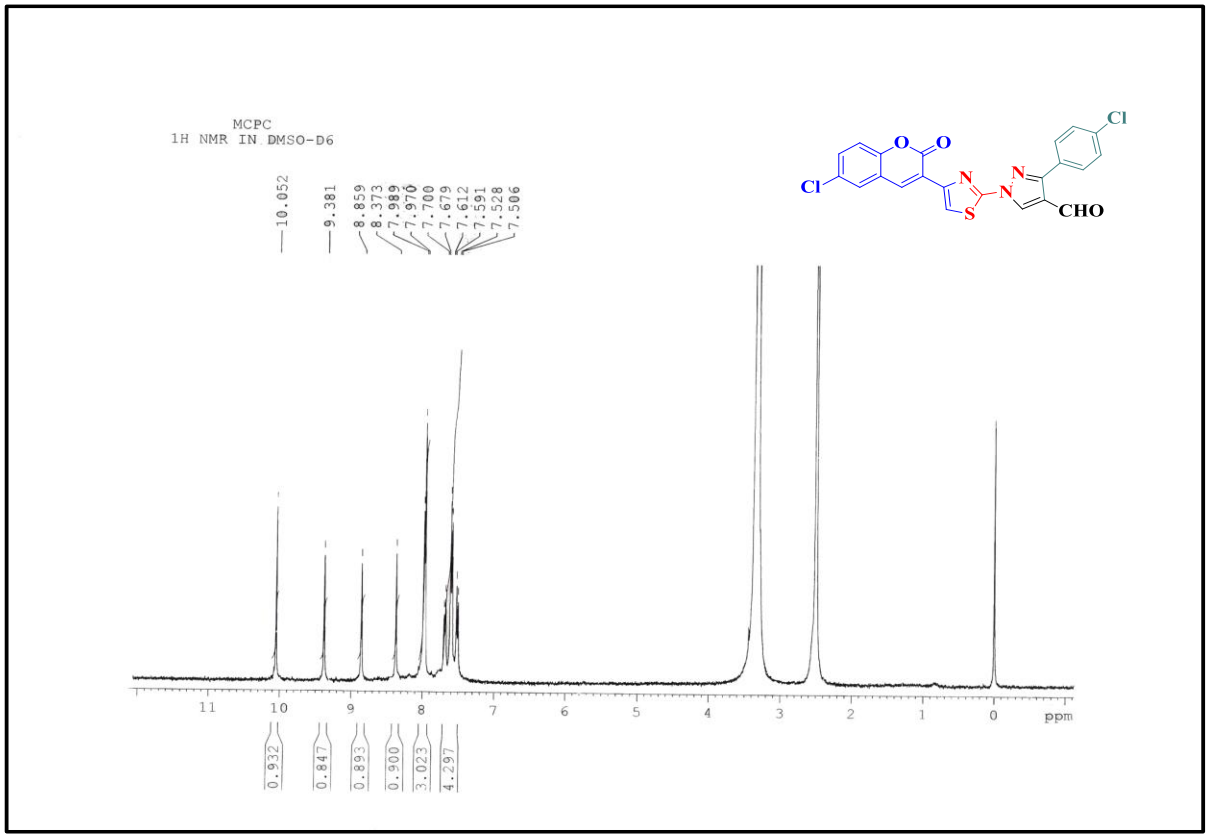


Fig: ^{1}H NMR spectrum of compound **35g**

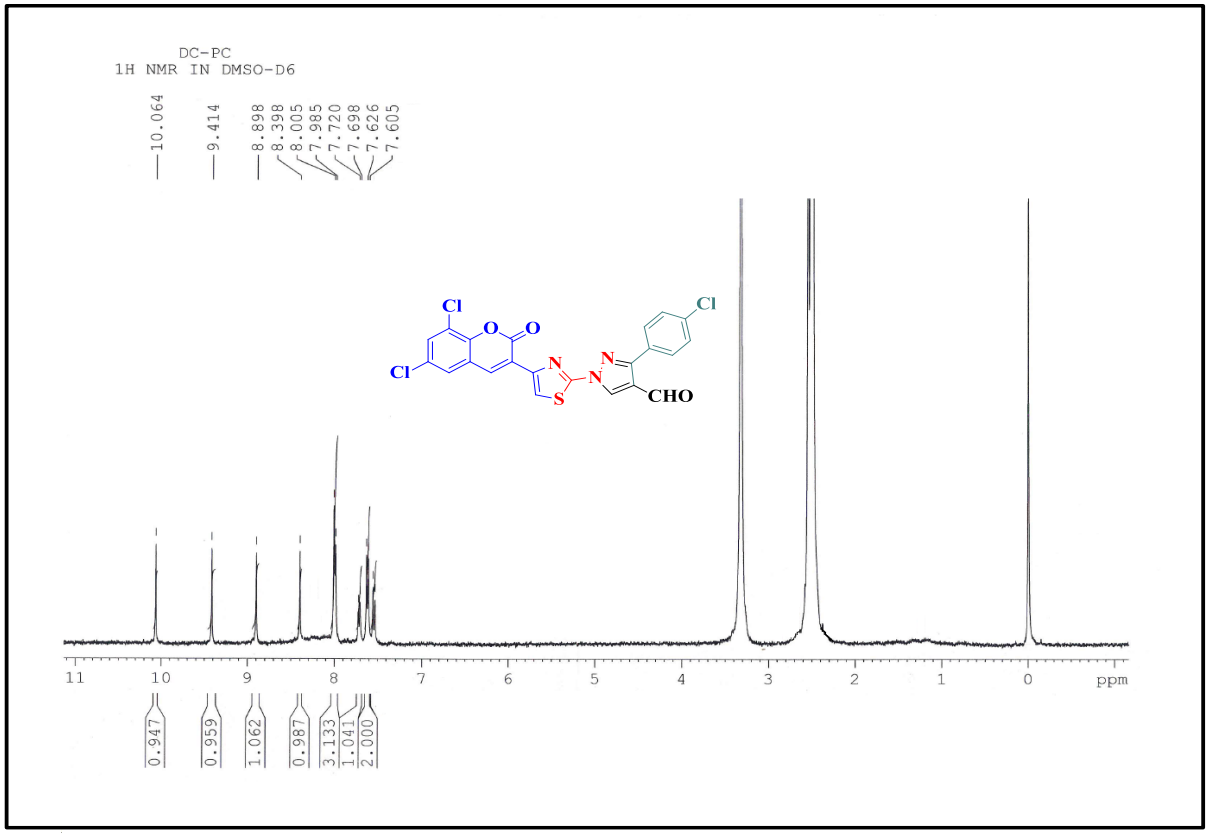


Fig: ^{1}H NMR spectrum of compound **35h**

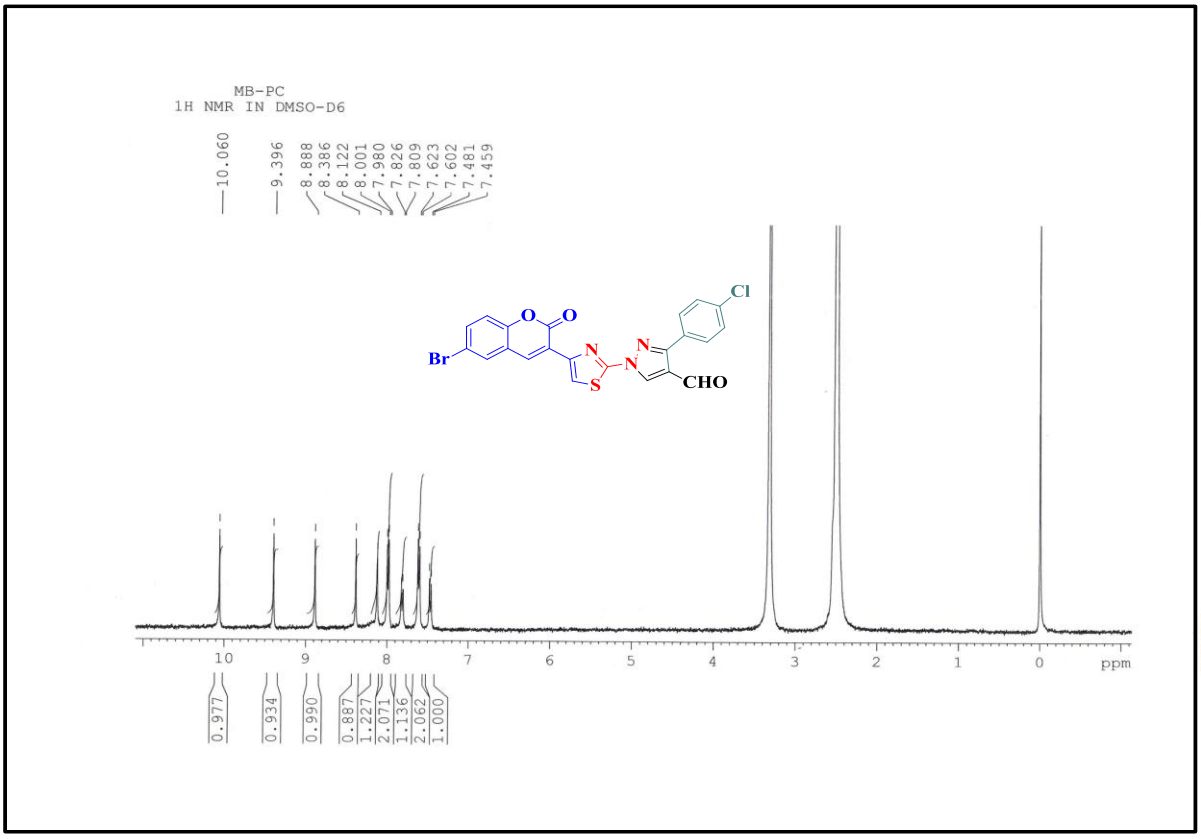


Fig: ^{1}H NMR spectrum of compound **35i**

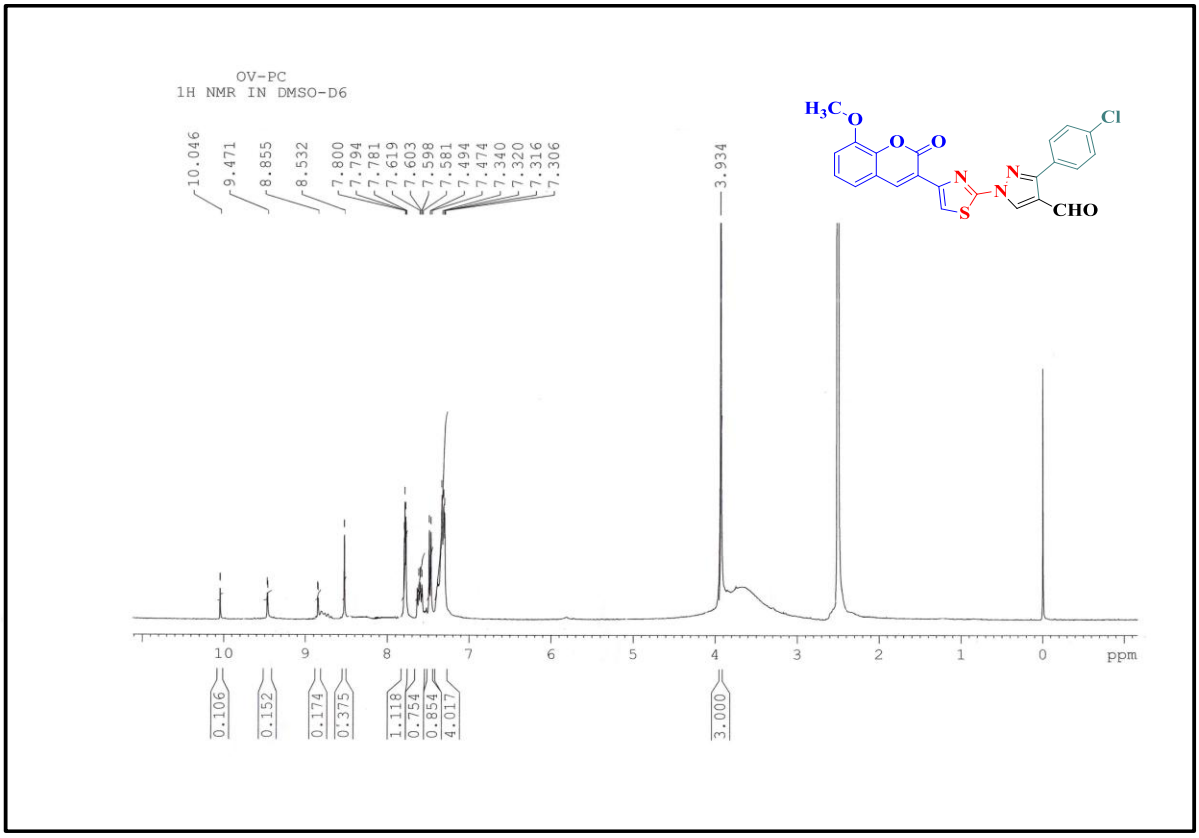


Fig: ^{1}H NMR spectrum of compound **35j**

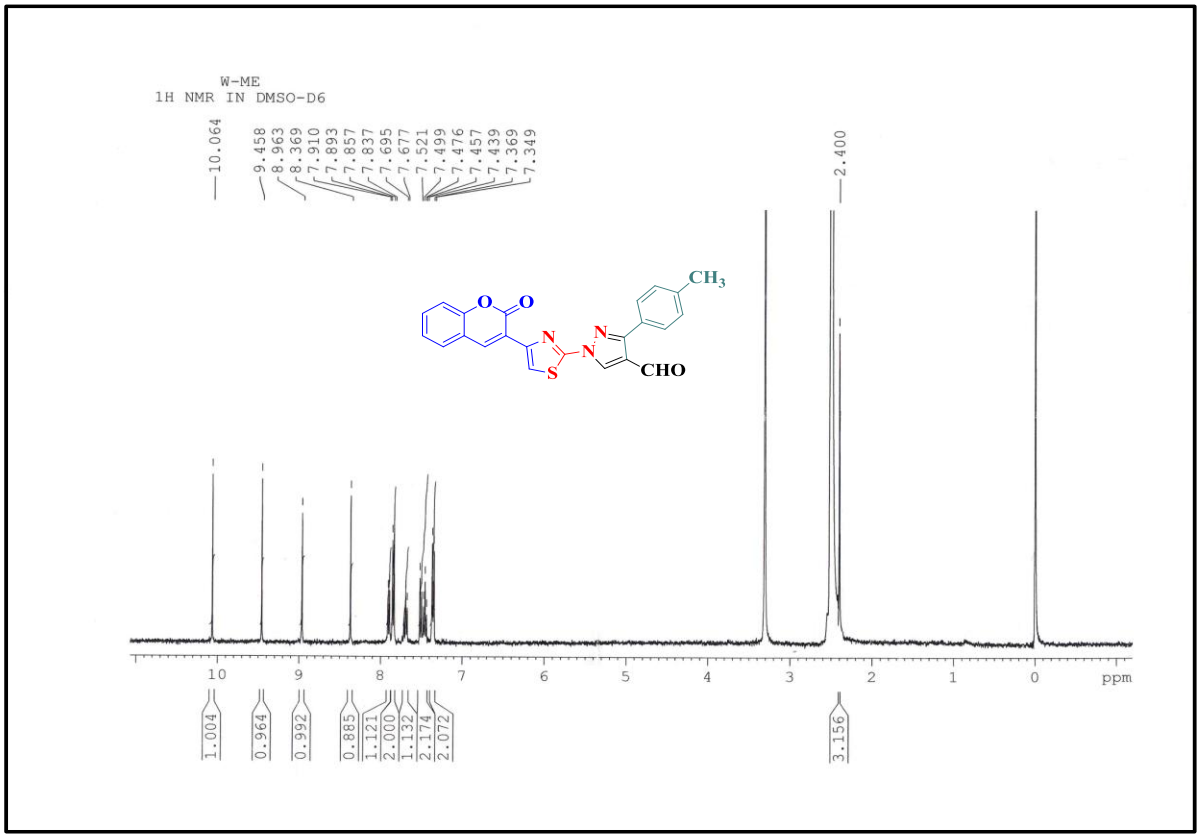


Fig: ^{1}H NMR spectrum of compound **35k**

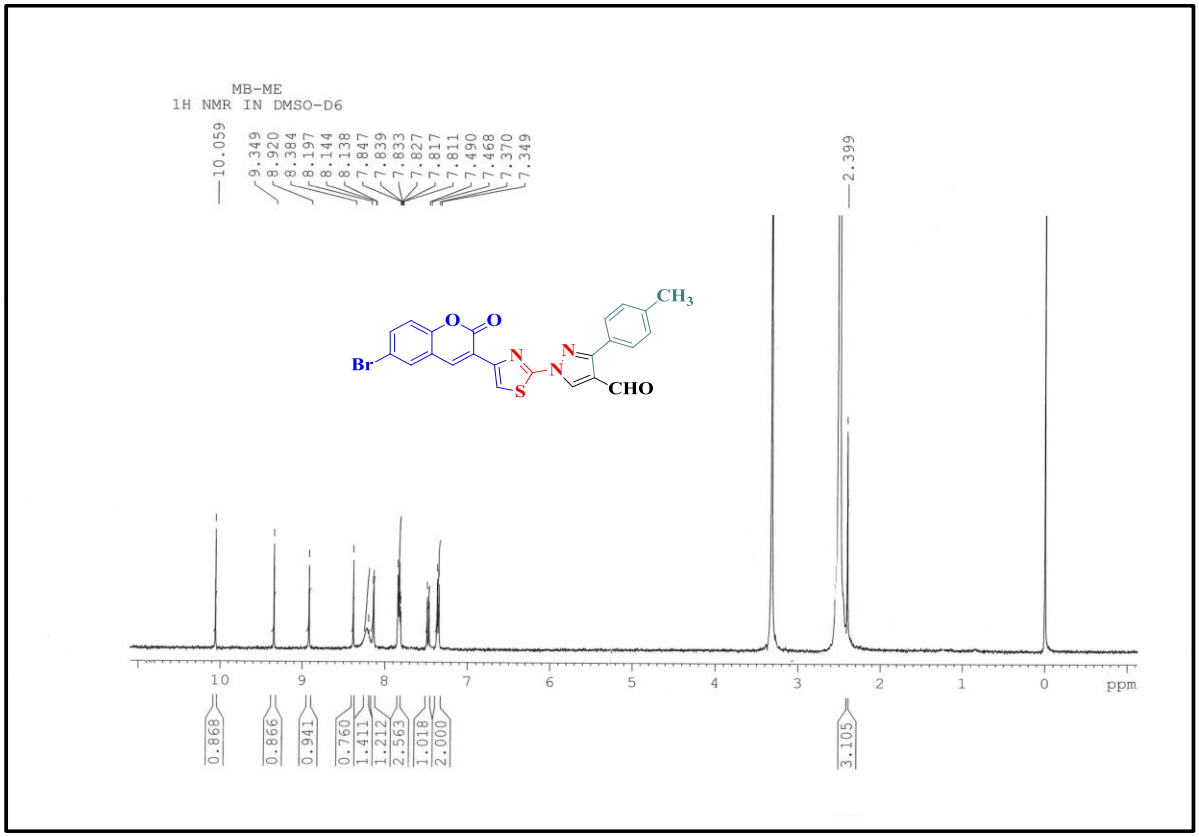


Fig: ^{1}H NMR spectrum of compound **35l**

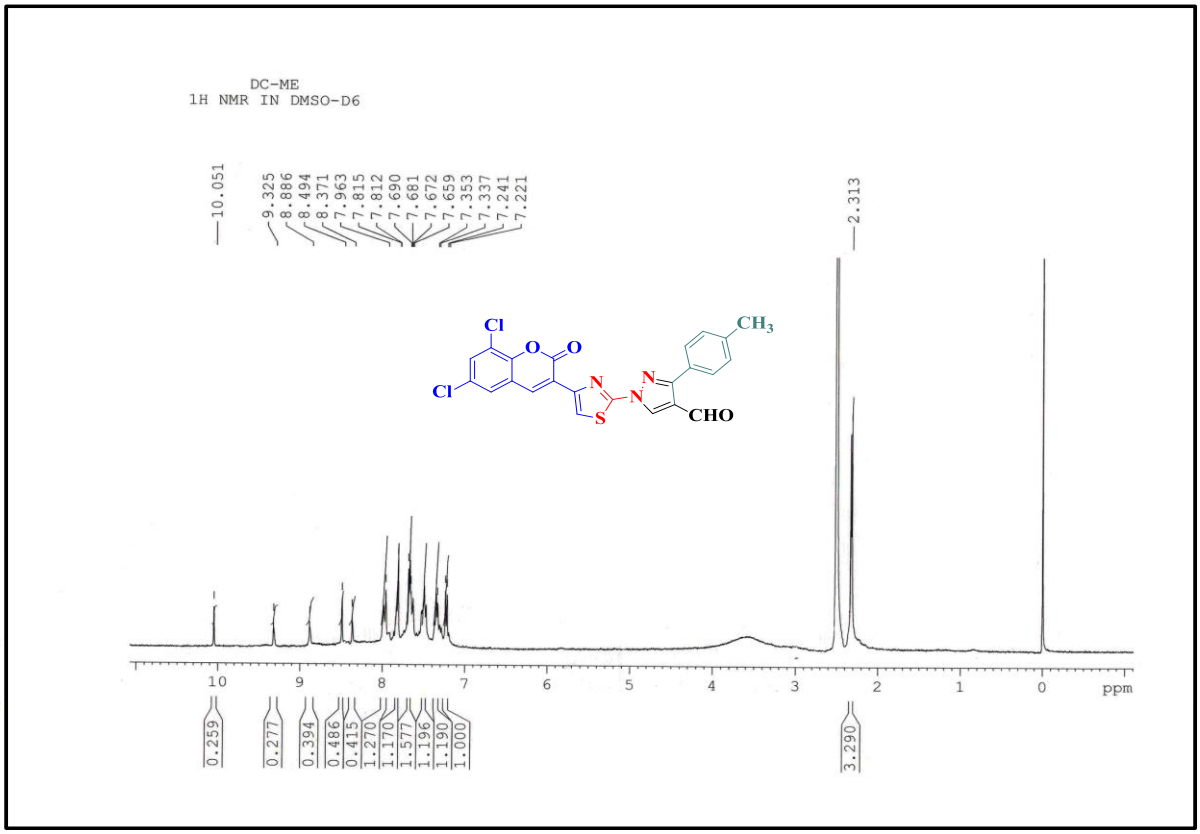


Fig: ^{1}H NMR spectrum of compound **35m**

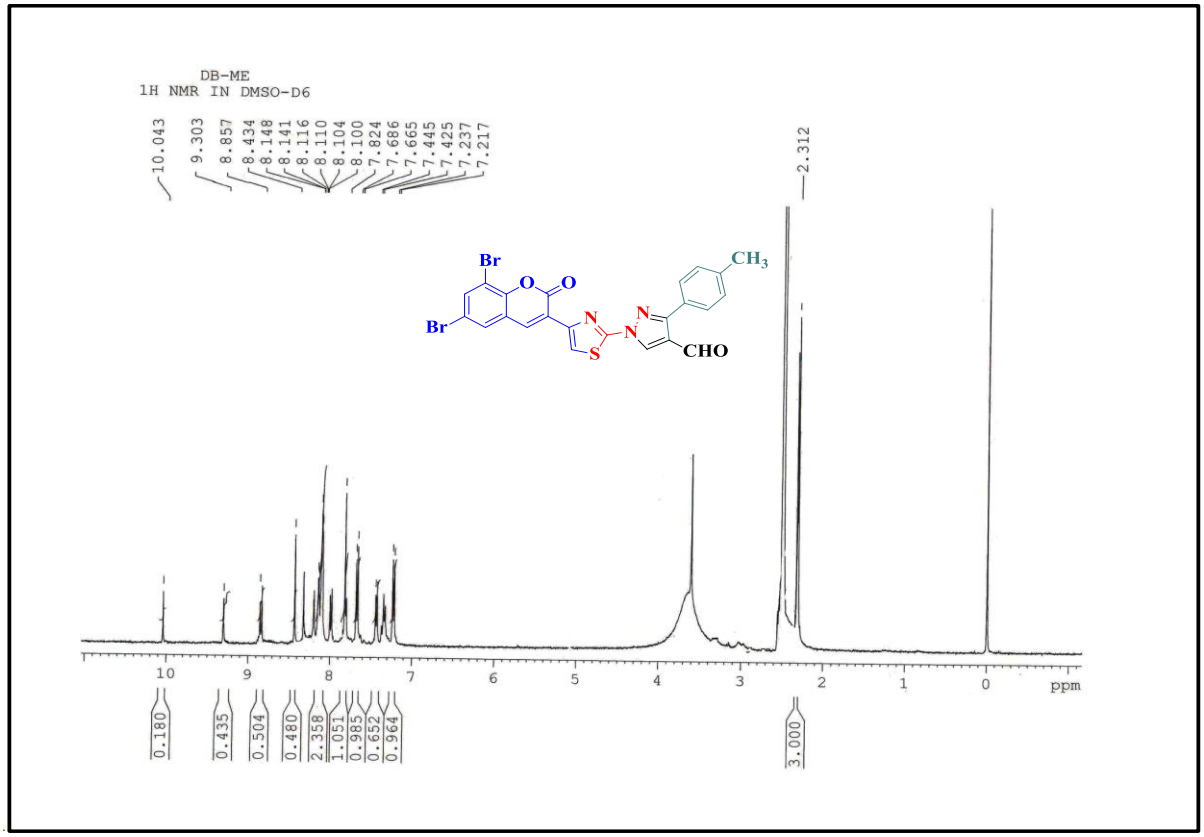


Fig: ^{1}H NMR spectrum of compound **35n**

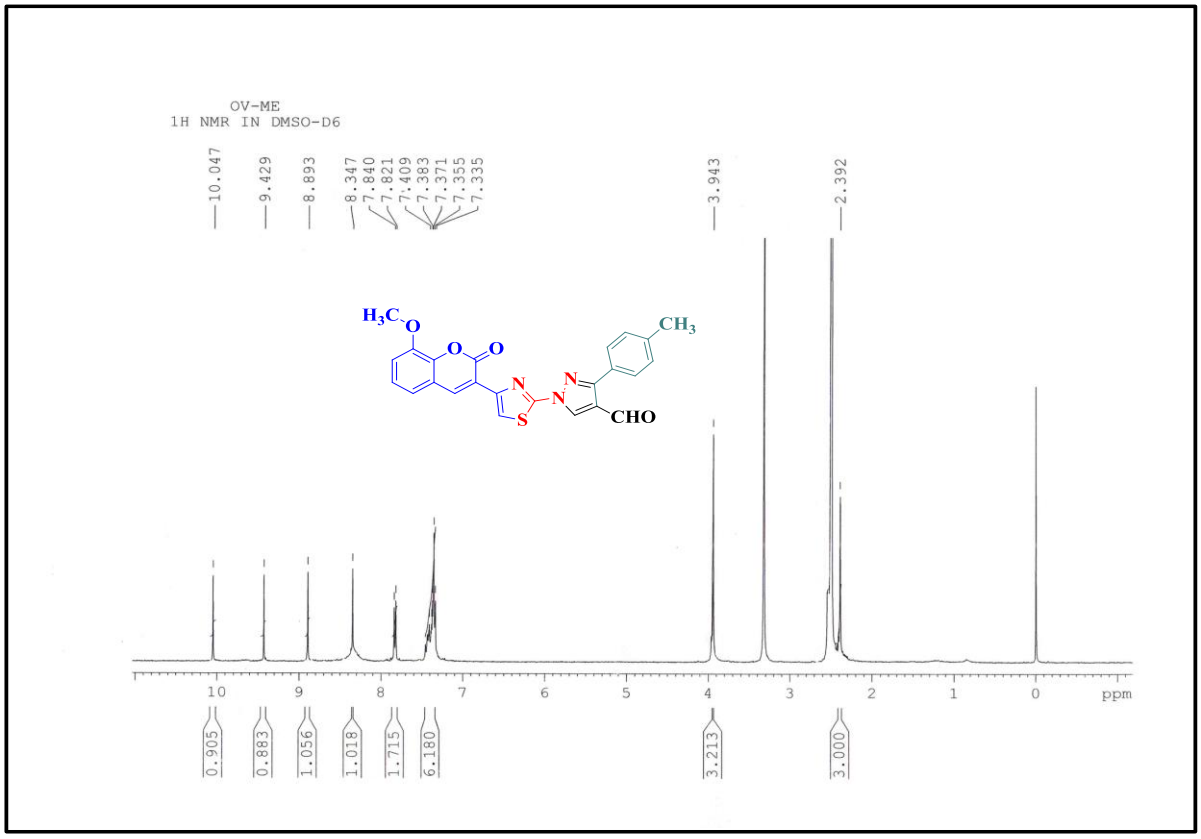


Fig: ^{1}H NMR spectrum of compound **35o**

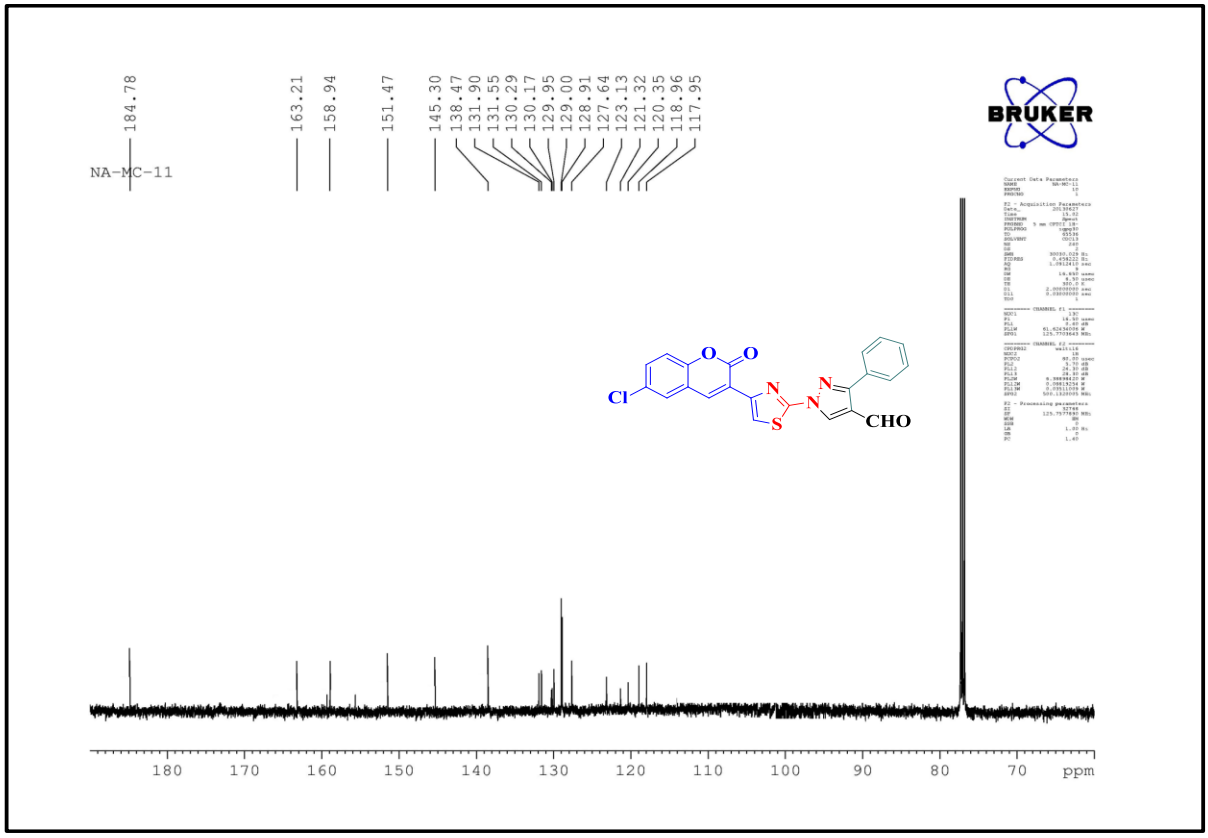


Fig: ^{13}C NMR spectrum of compound **35b**

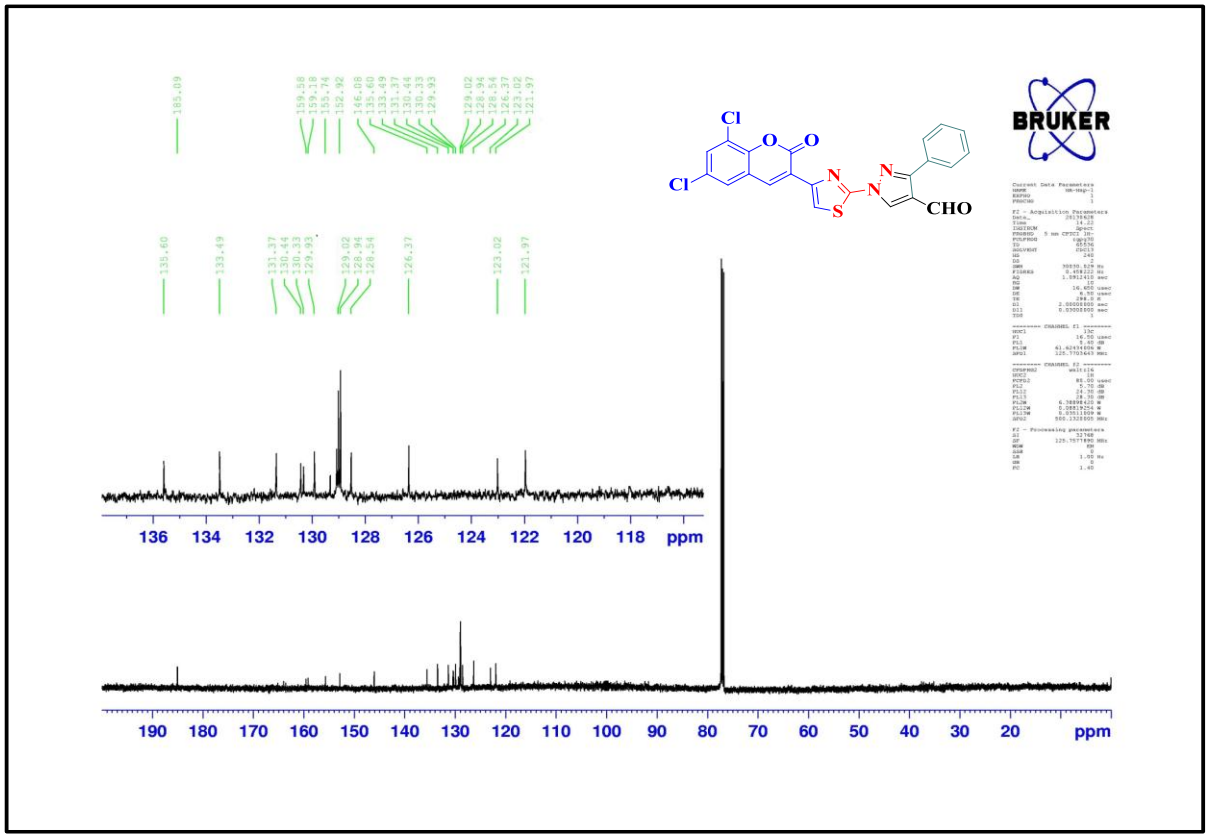


Fig: ^{13}C NMR spectrum of compound **35c**

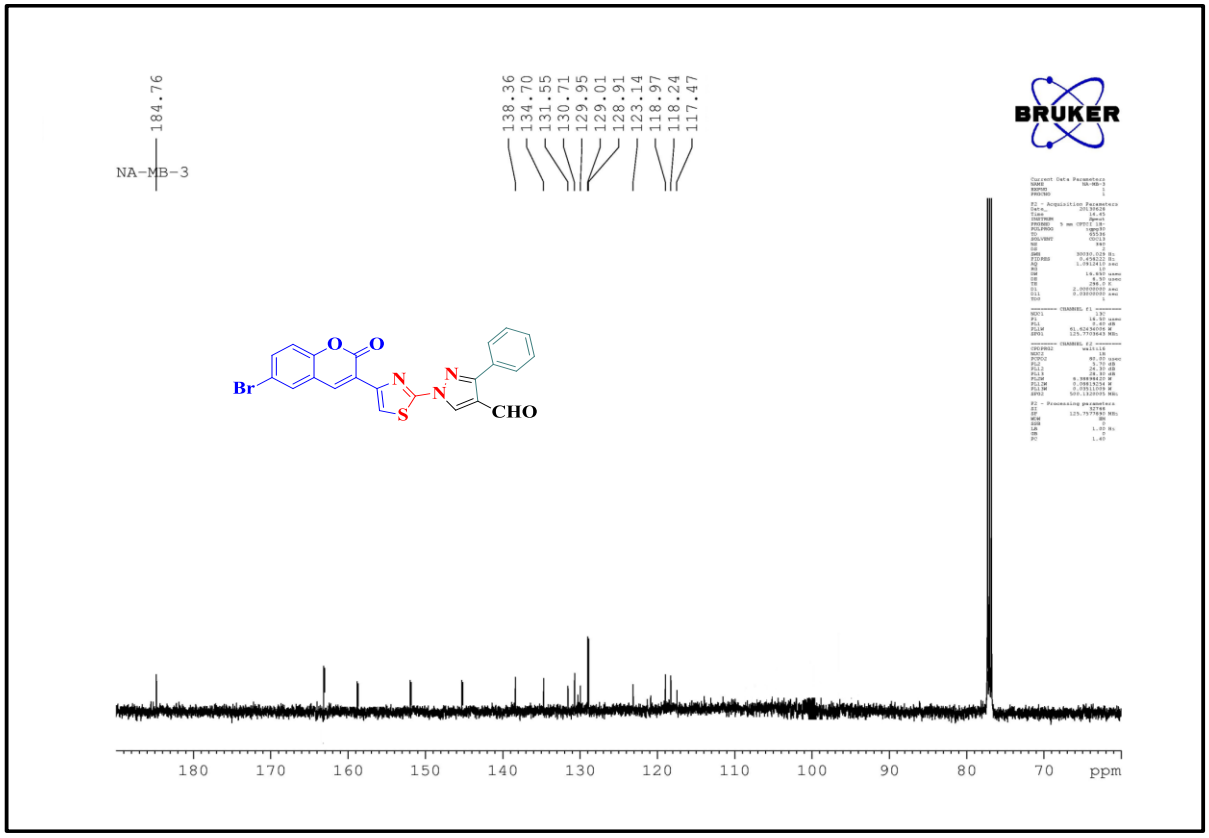


Fig: ^{13}C NMR spectrum of compound **35d**

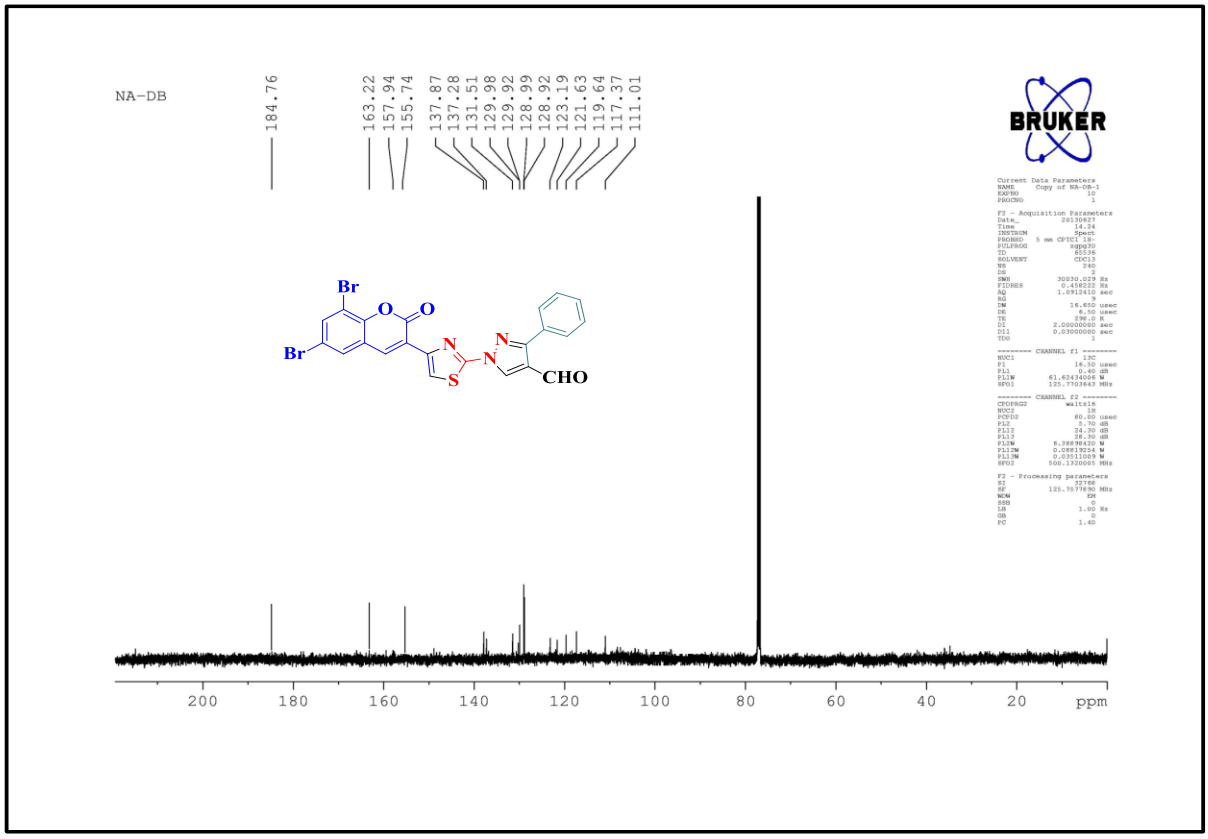


Fig: ^{13}C NMR spectrum of compound **35e**

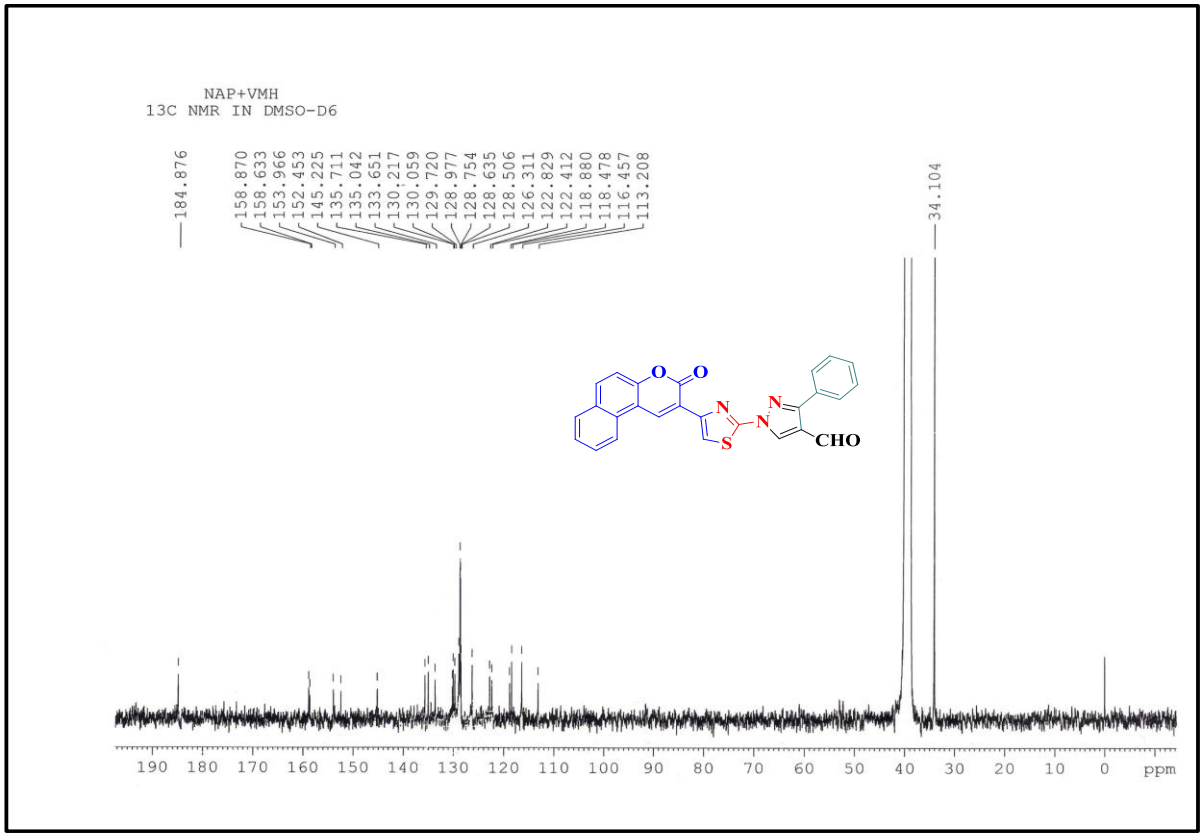


Fig: ^{13}C NMR spectrum of compound **35f**

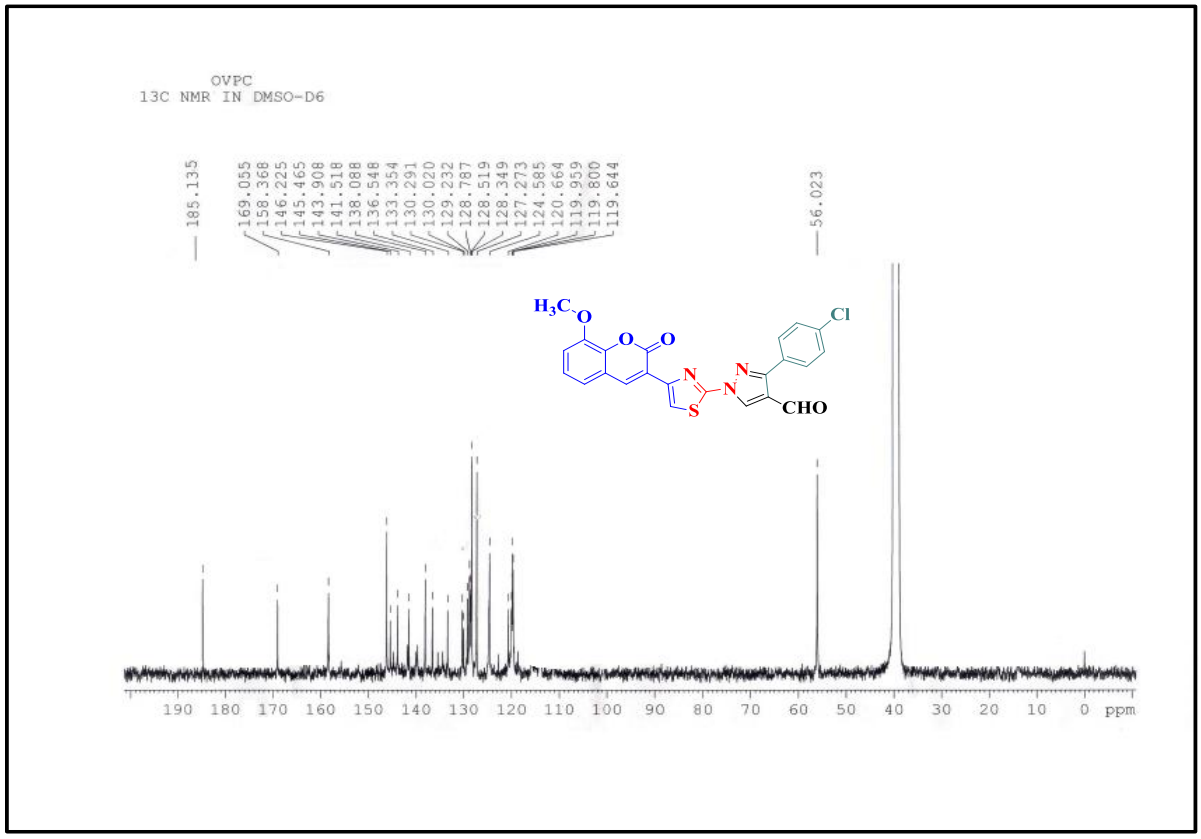


Fig: ^{13}C NMR spectrum of compound **35j**

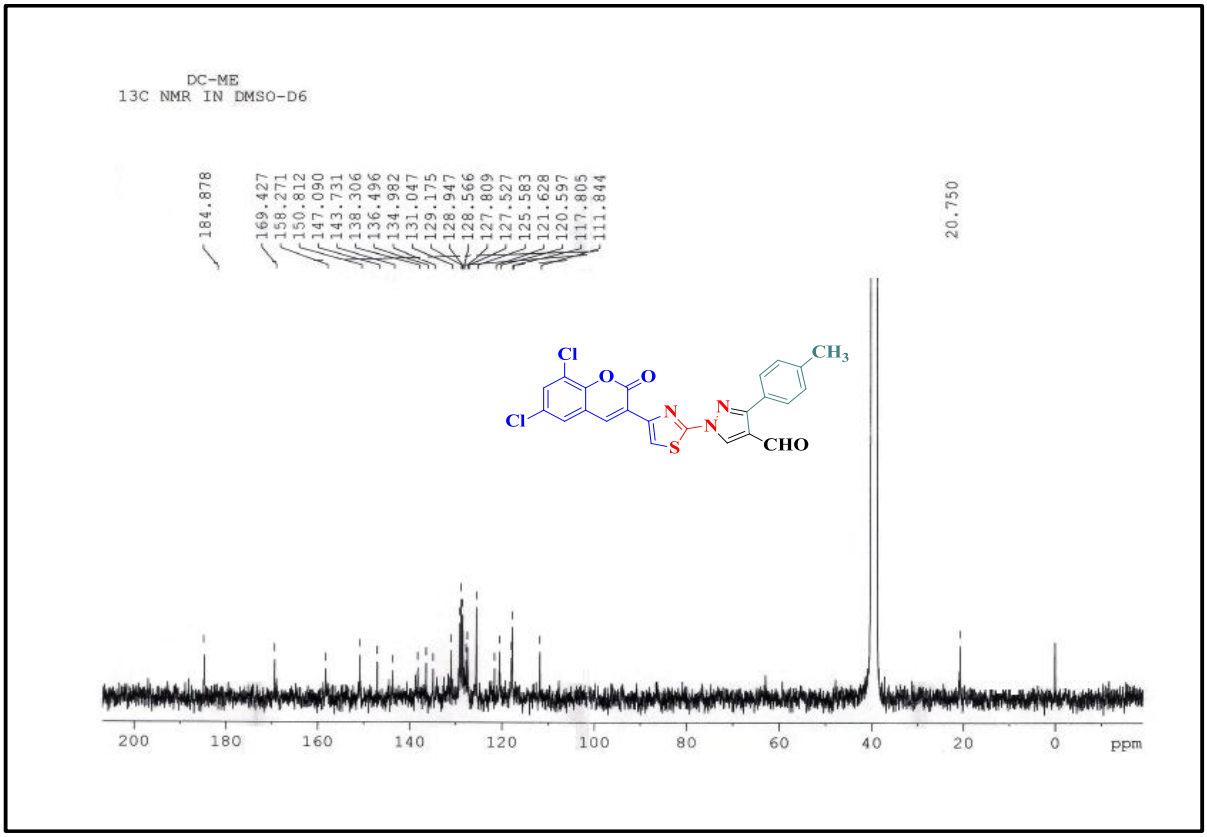


Fig: ^{13}C NMR spectrum of compound **35m**

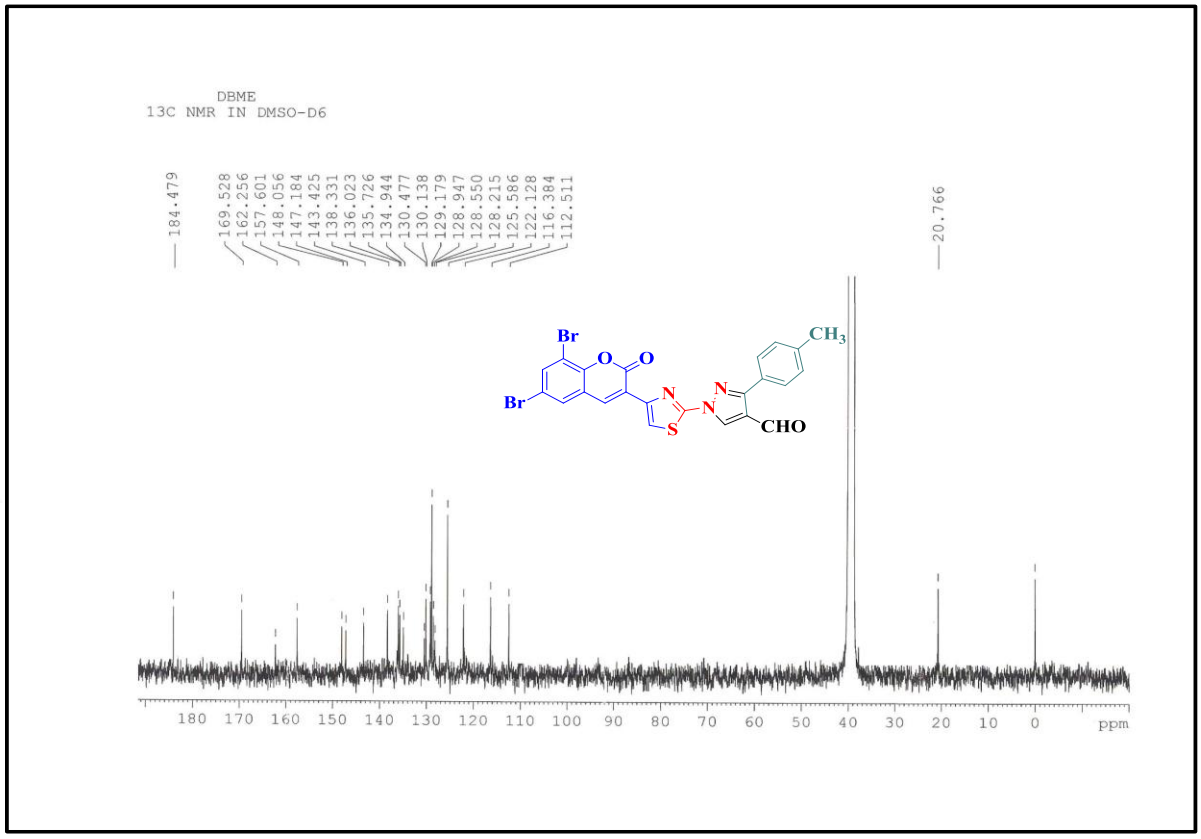


Fig: ^{13}C NMR spectrum of compound **35n**

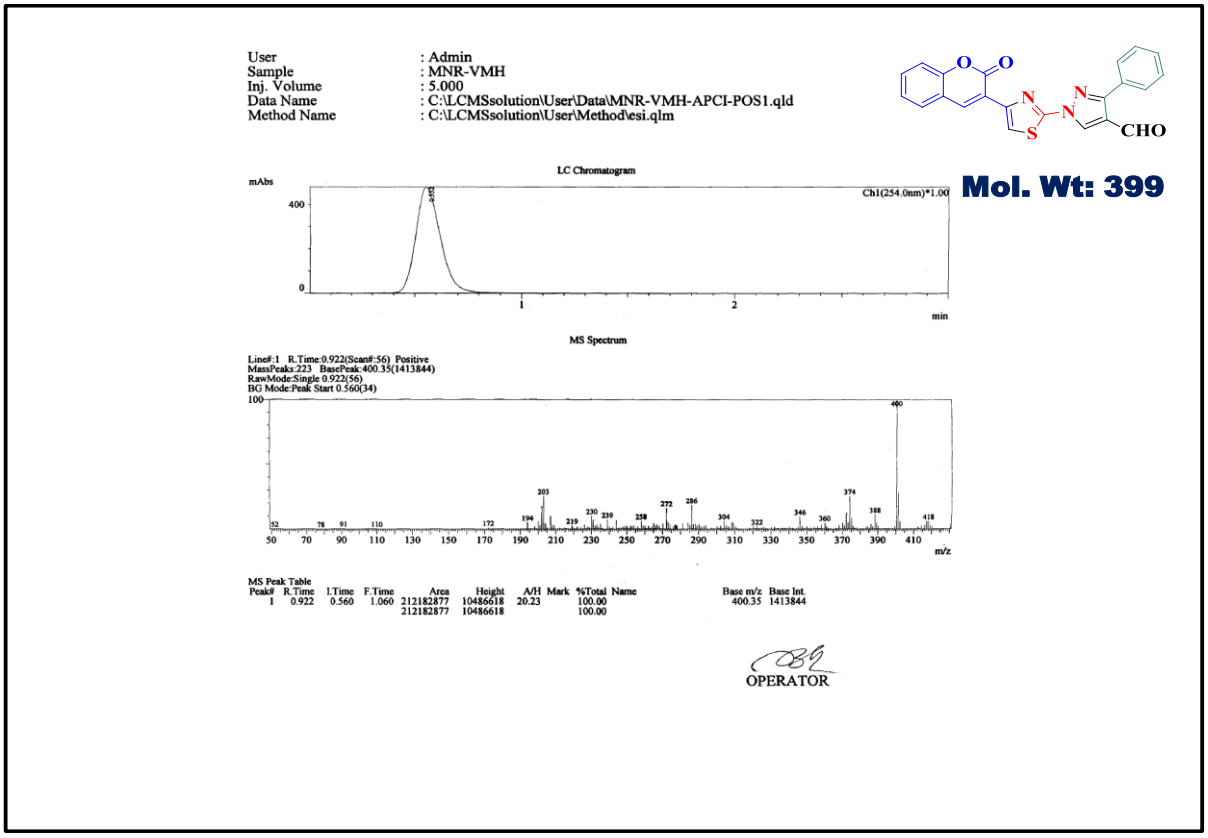


Fig: Mass spectrum of compound **35a**

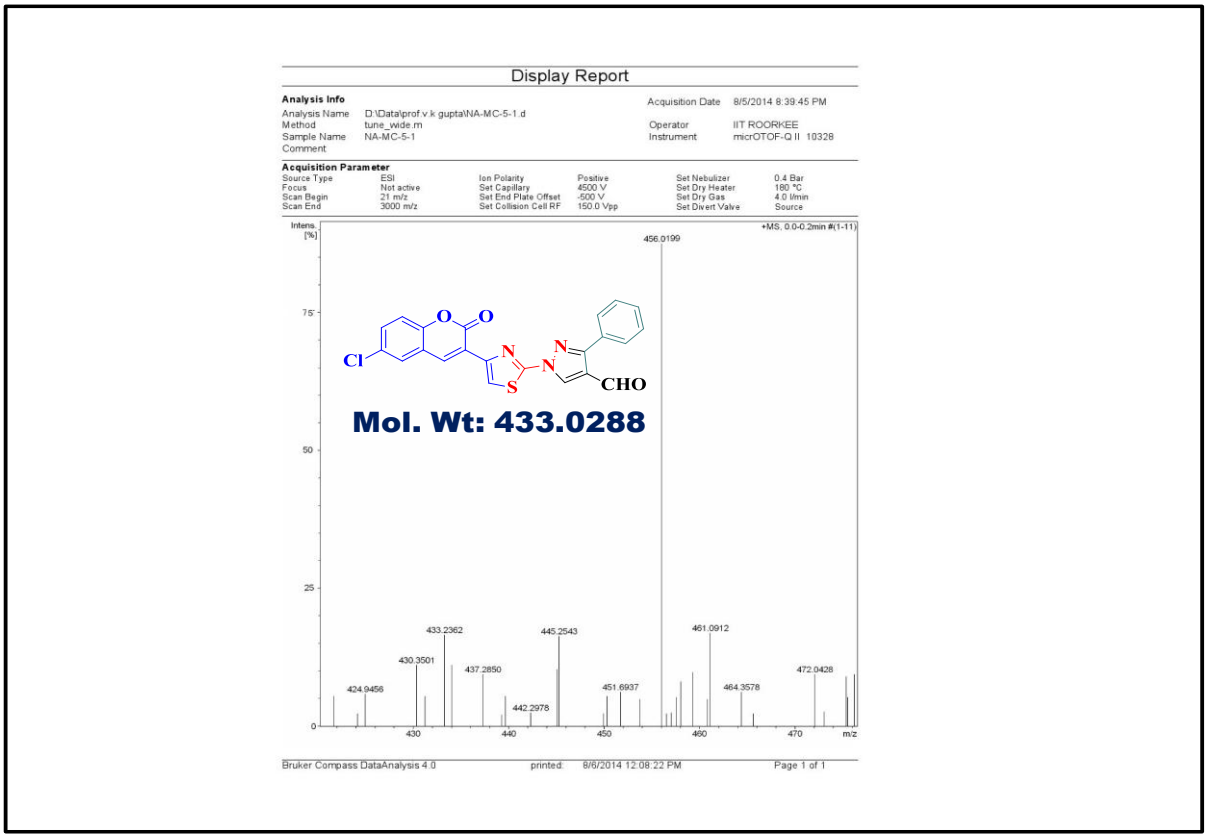


Fig: Mass spectrum of compound **35b**

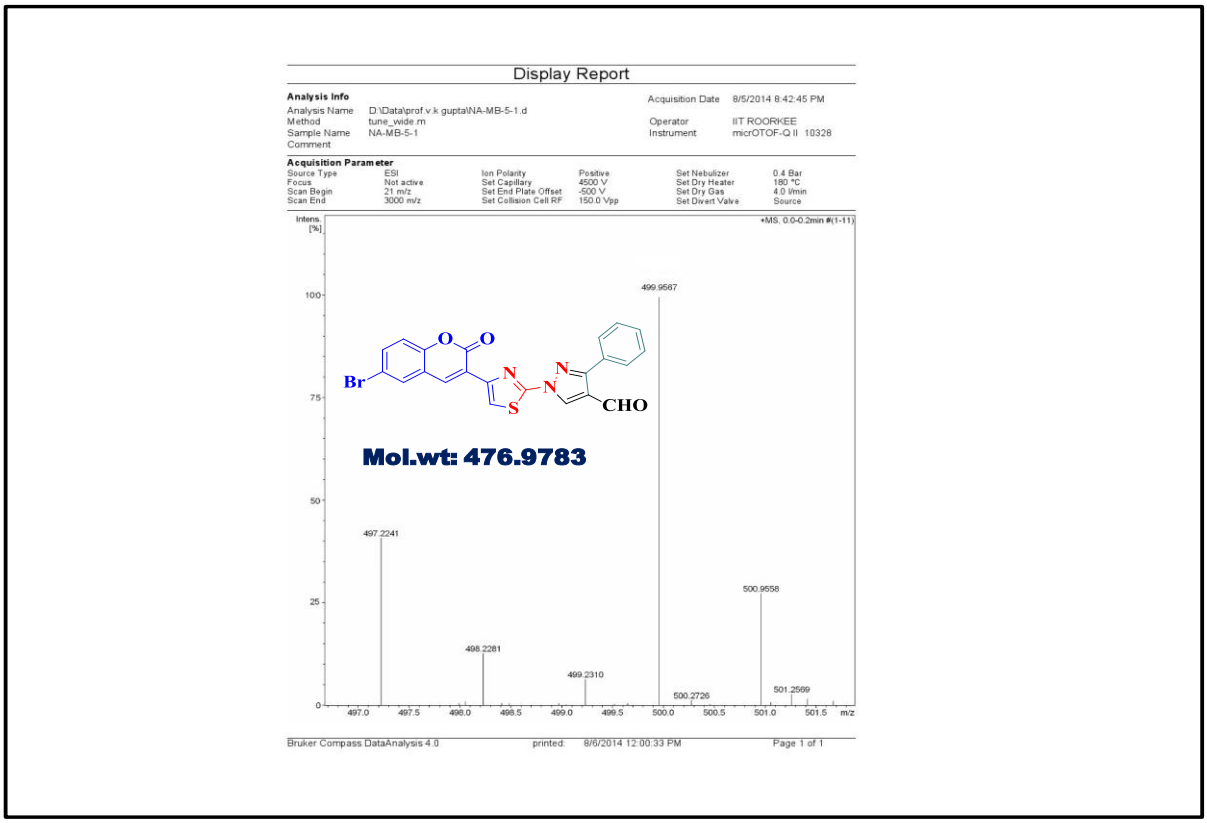


Fig: Mass spectrum of compound **35d**

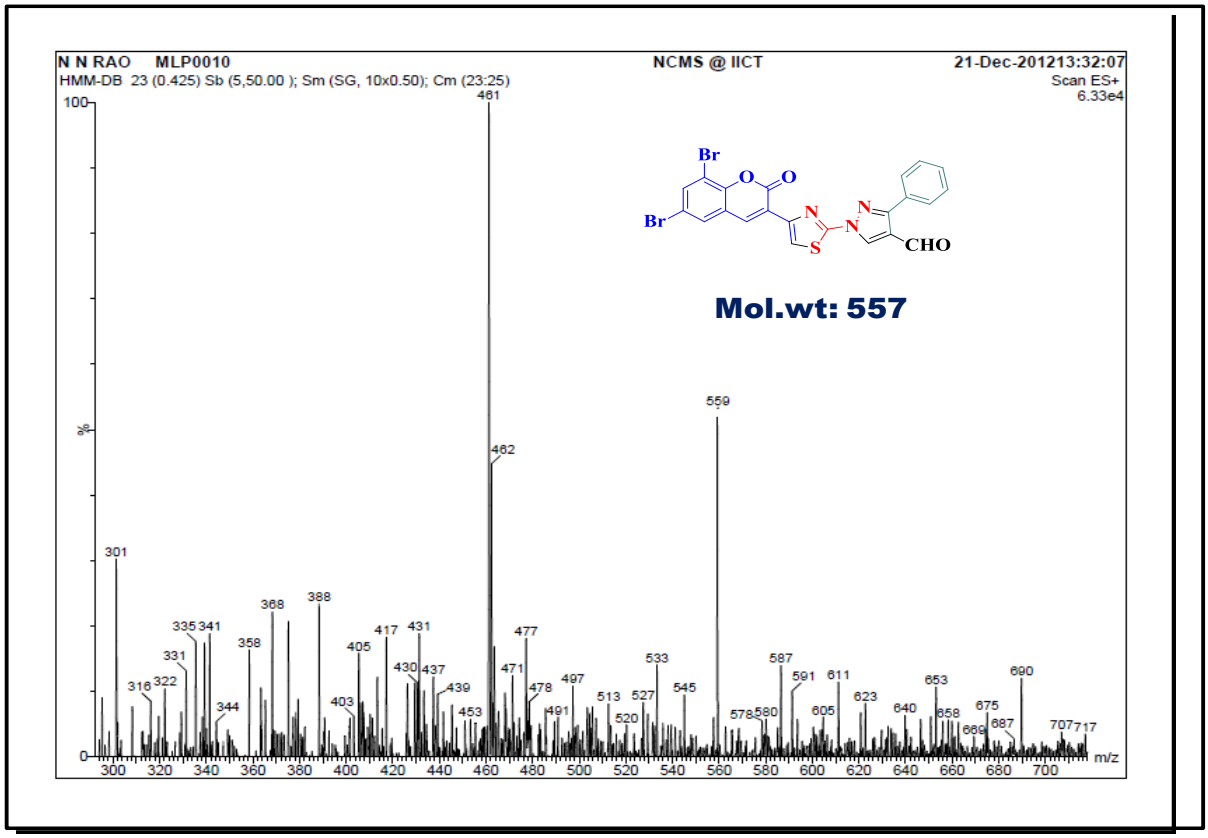


Fig: Mass spectrum of compound **35e**

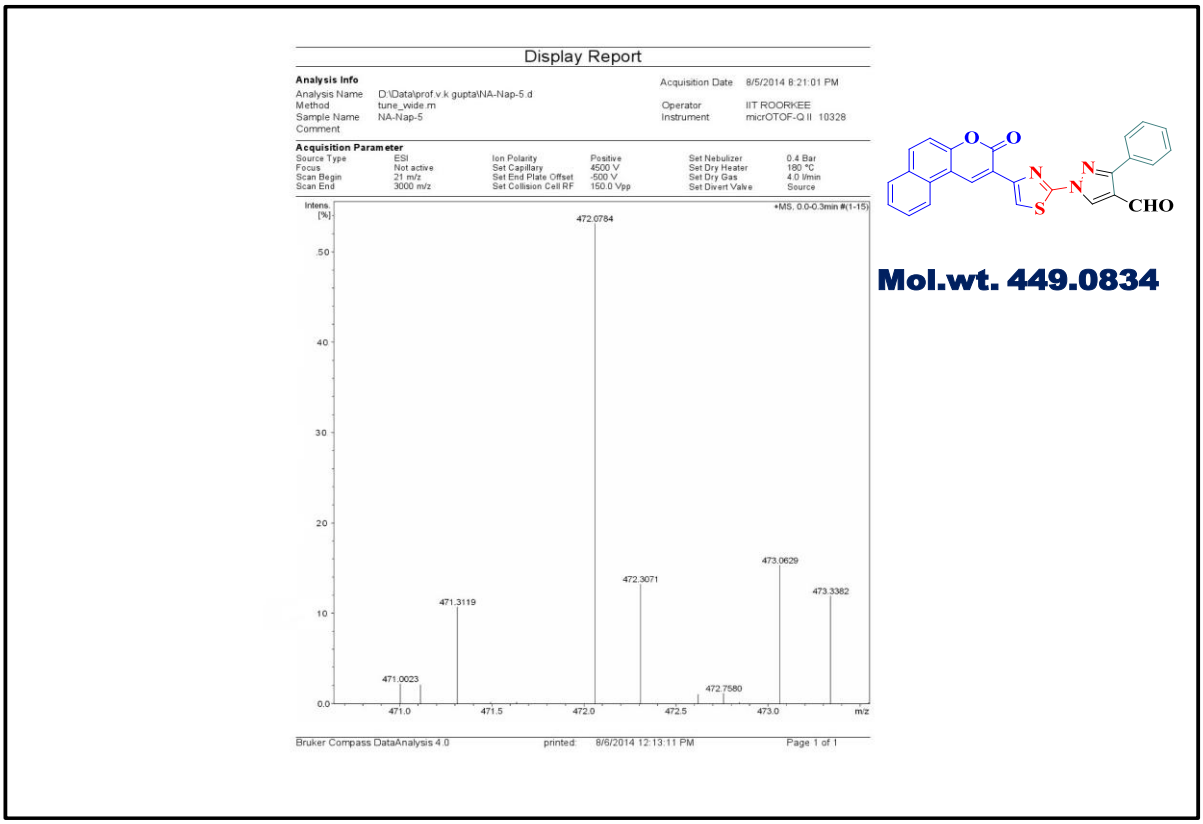


Fig: Mass spectrum of compound **35f**

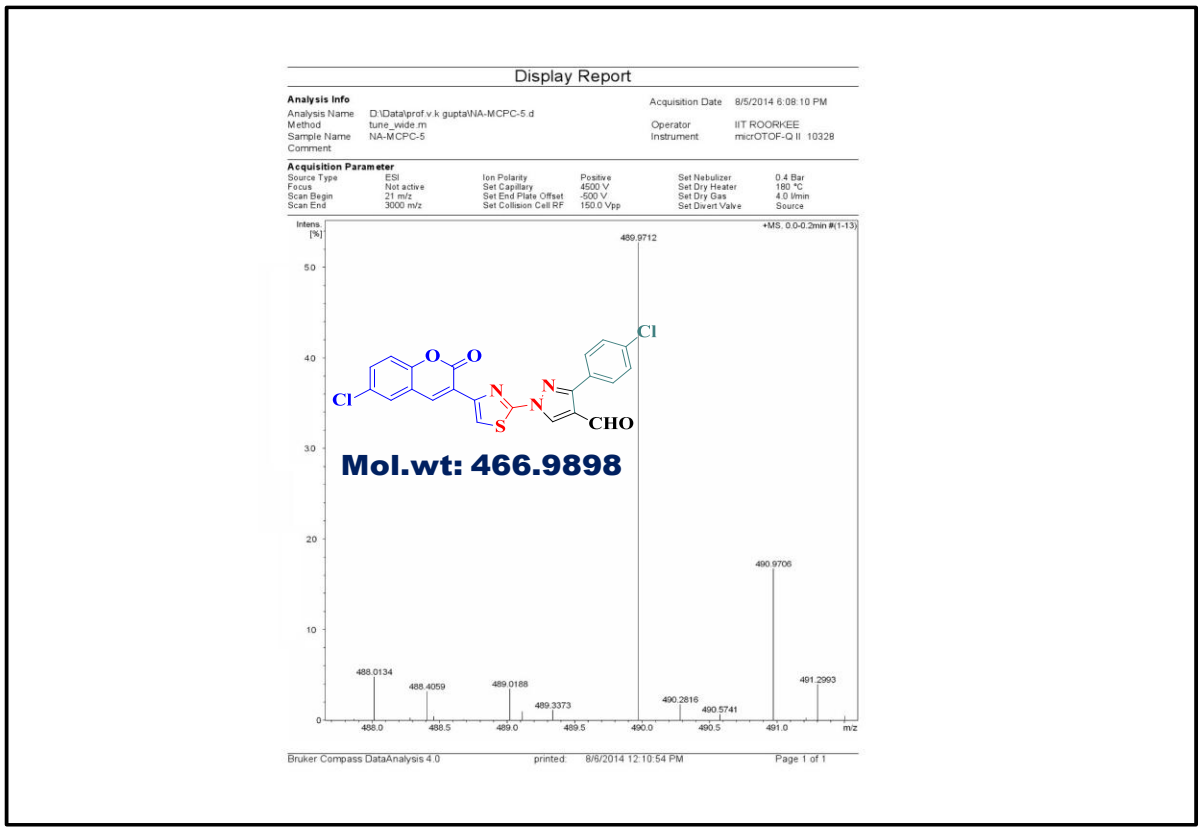


Fig: Mass spectrum of compound **35g**

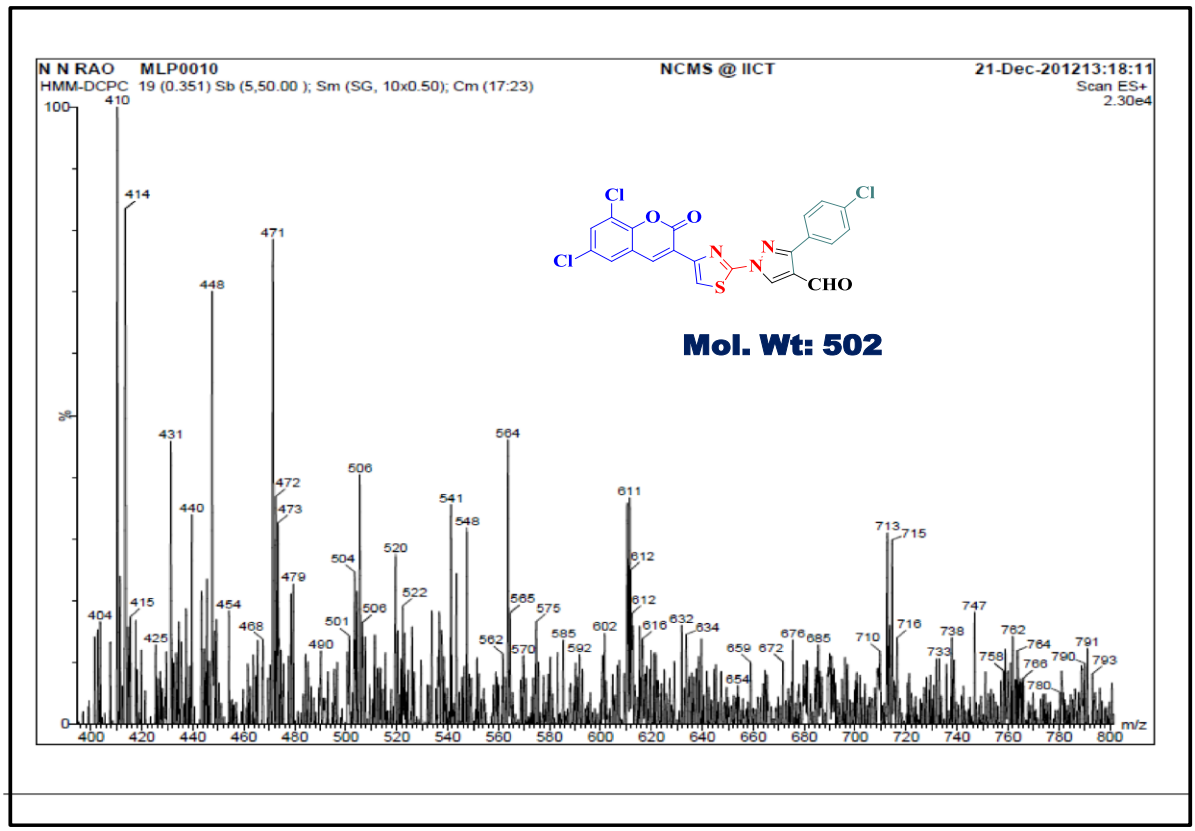


Fig: Mass spectrum of compound **35h**

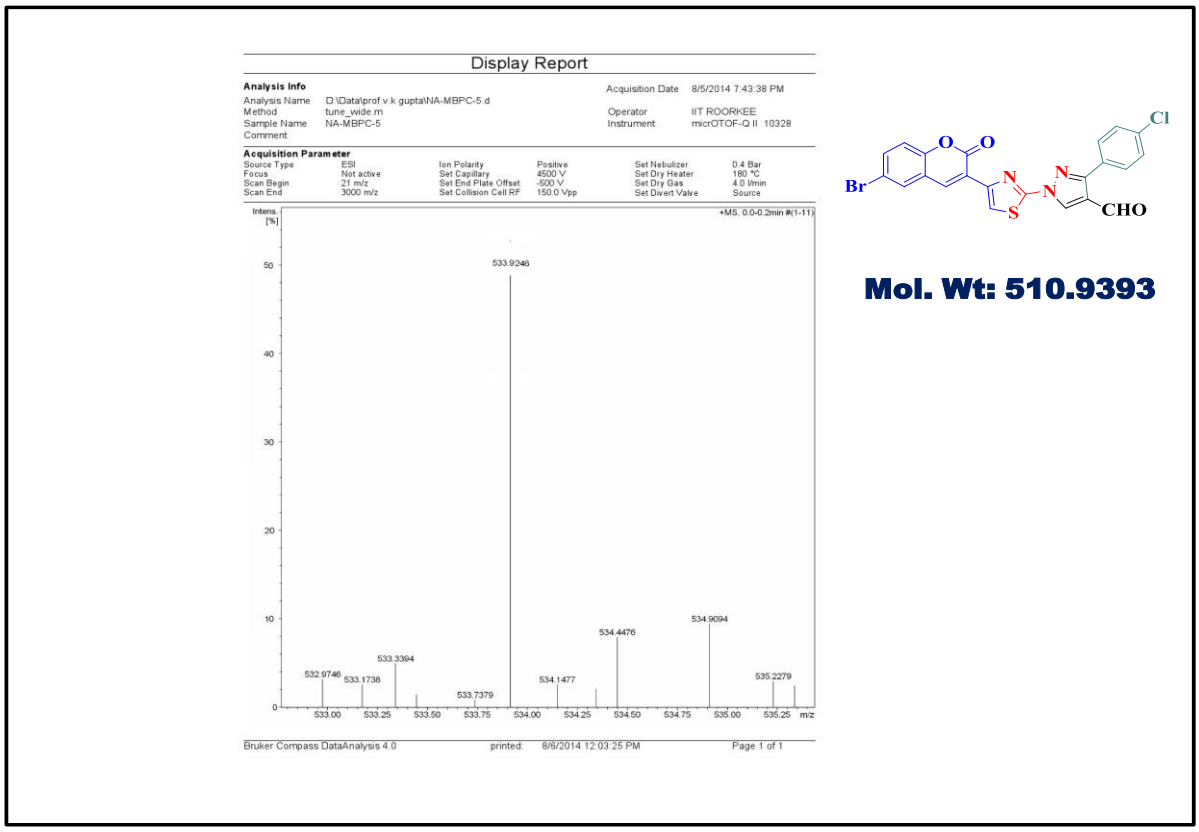


Fig: Mass spectrum of compound **35i**

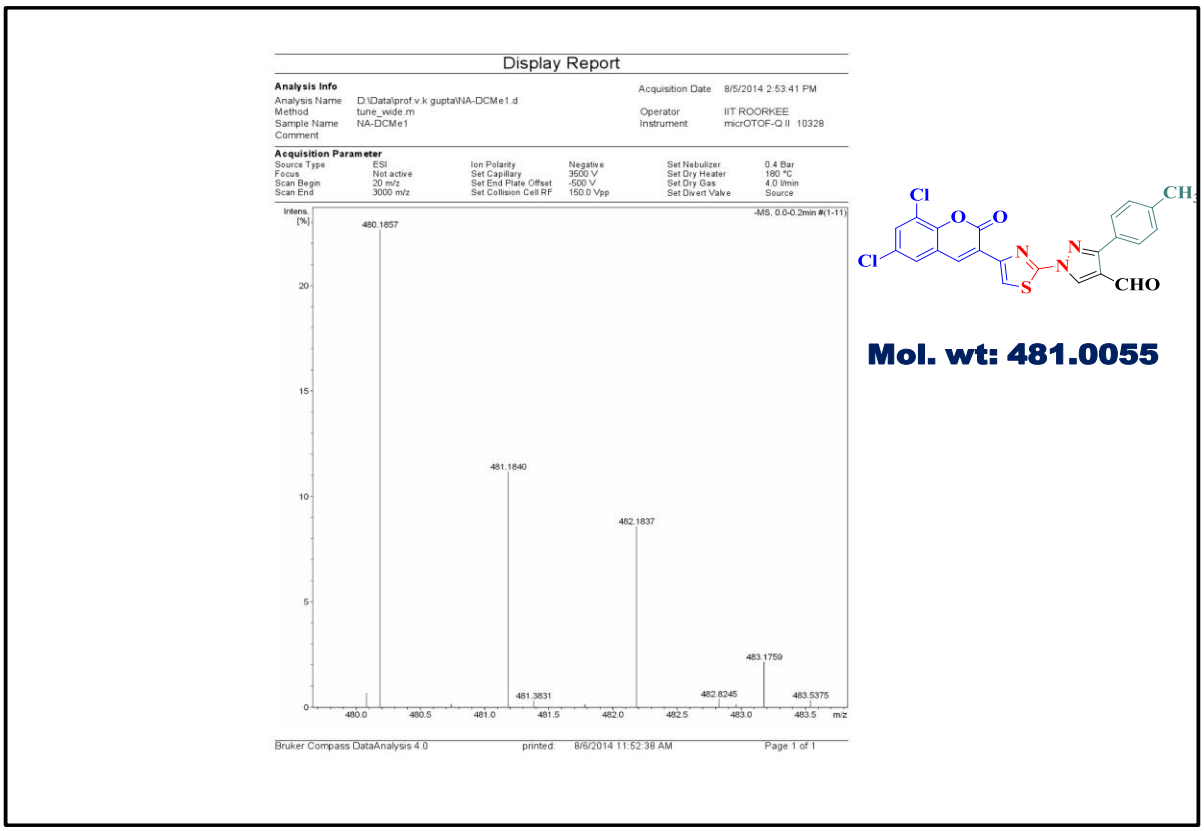


Fig: Mass spectrum of compound **35m**

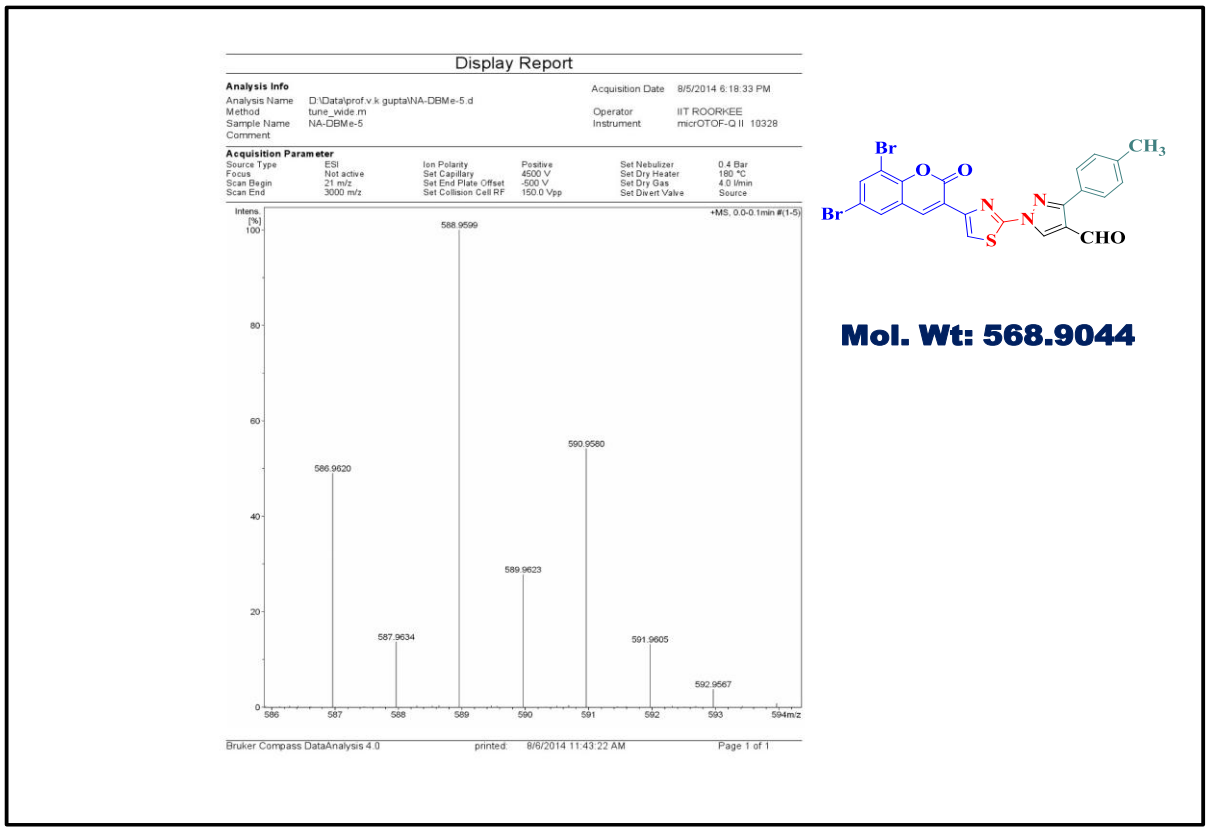


Fig: Mass spectrum of compound **35n**

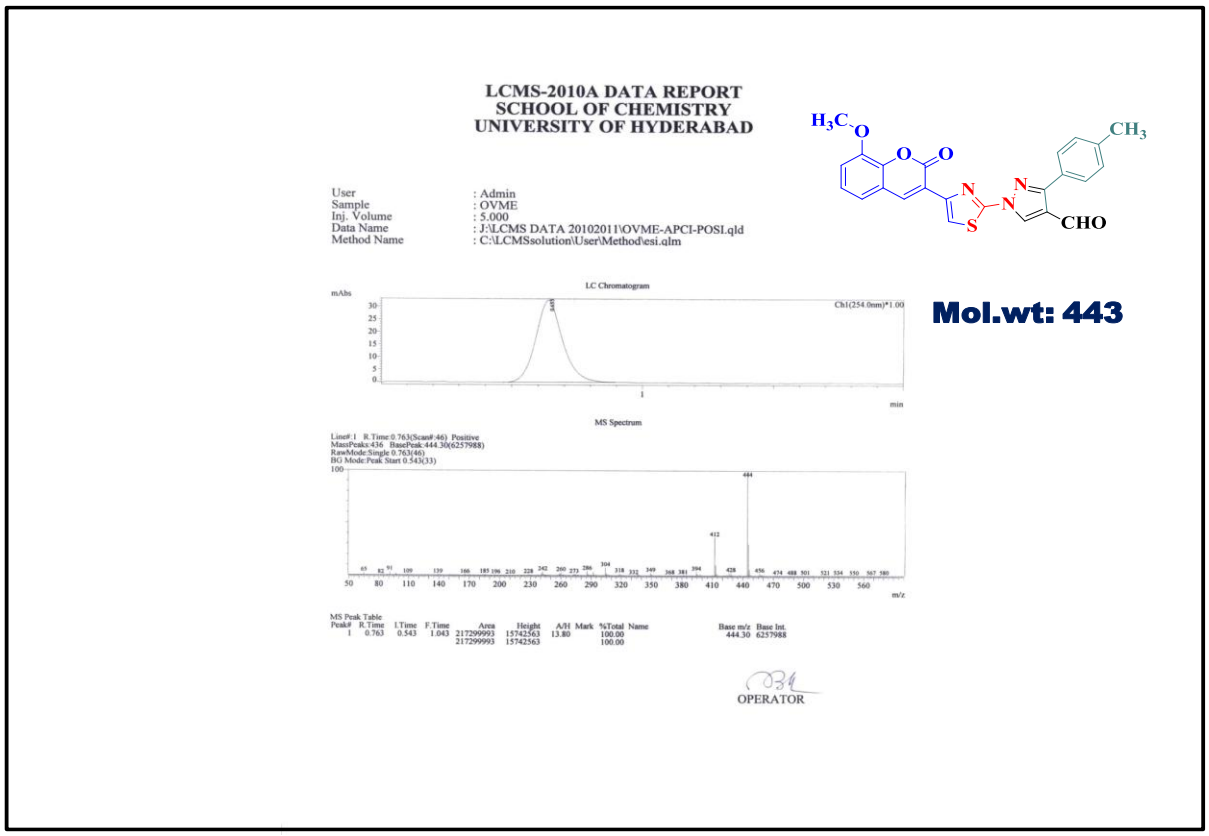


Fig: Mass spectrum of compound **35o**

CHAPTER-II
PART-B

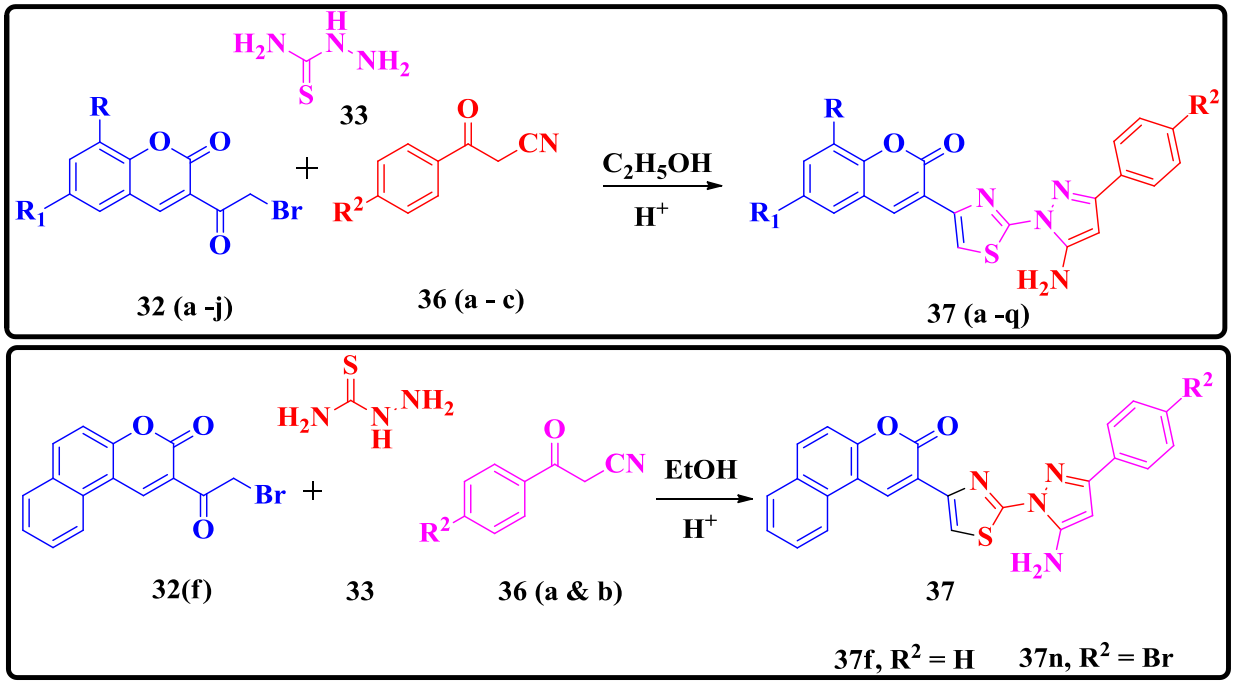
3-(2-(5-AMINO-3-ARYL-1H-PYRAZOL-1-YL) THIAZOL-4-YL)-2*H*-CHROMEN-2-ONES AS POTENTIAL ANTICANCER AGENTS: SYNTHESIS, ANTICANCER ACTIVITY EVALUATION AND MOLECULAR DOCKING STUDIES

PART-B

PRESENT WORK:

The present chapter describes the synthesis of 3-(2-(5-amino-3-aryl-1*H*-pyrazol-1-yl)thiazol-4-yl)-2*H*-chromene-2-ones via an efficient one pot multicomponent reaction approach, their anticancer activity and molecular docking studies.

The target compounds were synthesized by the reaction of substituted 3-(2-bromoacetyl) coumarins (**32a-j**), thiosemicarbazide (**33**) and benzoylactonitrile[59] (**36a-c**) in presence of ethanol with excellent yields. The schematic representation of the reaction is outlined in the **Scheme 2**. The importance of this method is that two heterocyclic systems like thiazole and pyrazoles were formed at a time with a potential amino functionality. Thus this method involves in the first step classical Hantzsch thiazole synthesis followed by pyrazole formation. Initially we have carried out the reaction in presence of absolute ethanol solvent at 60 °C for 10 – 12 h. The yield of the reaction was low and the time taken for converting the starting reactants in to final product was very long. So we have tried with ethanol solvent with catalytic amount of acetic acid at 60 °C The yield of the reaction was increased and the reaction time also decreased to 4 – 6 hrs. By using ethanol / catalytic amount of acetic acid solvent medium we have synthesized various 3-(2-(5-amino-3-aryl-1*H*-pyrazol-1-yl)thiazol-4-yl)-2*H*-chromene-2-ones with excellent yields (**Table.6**).



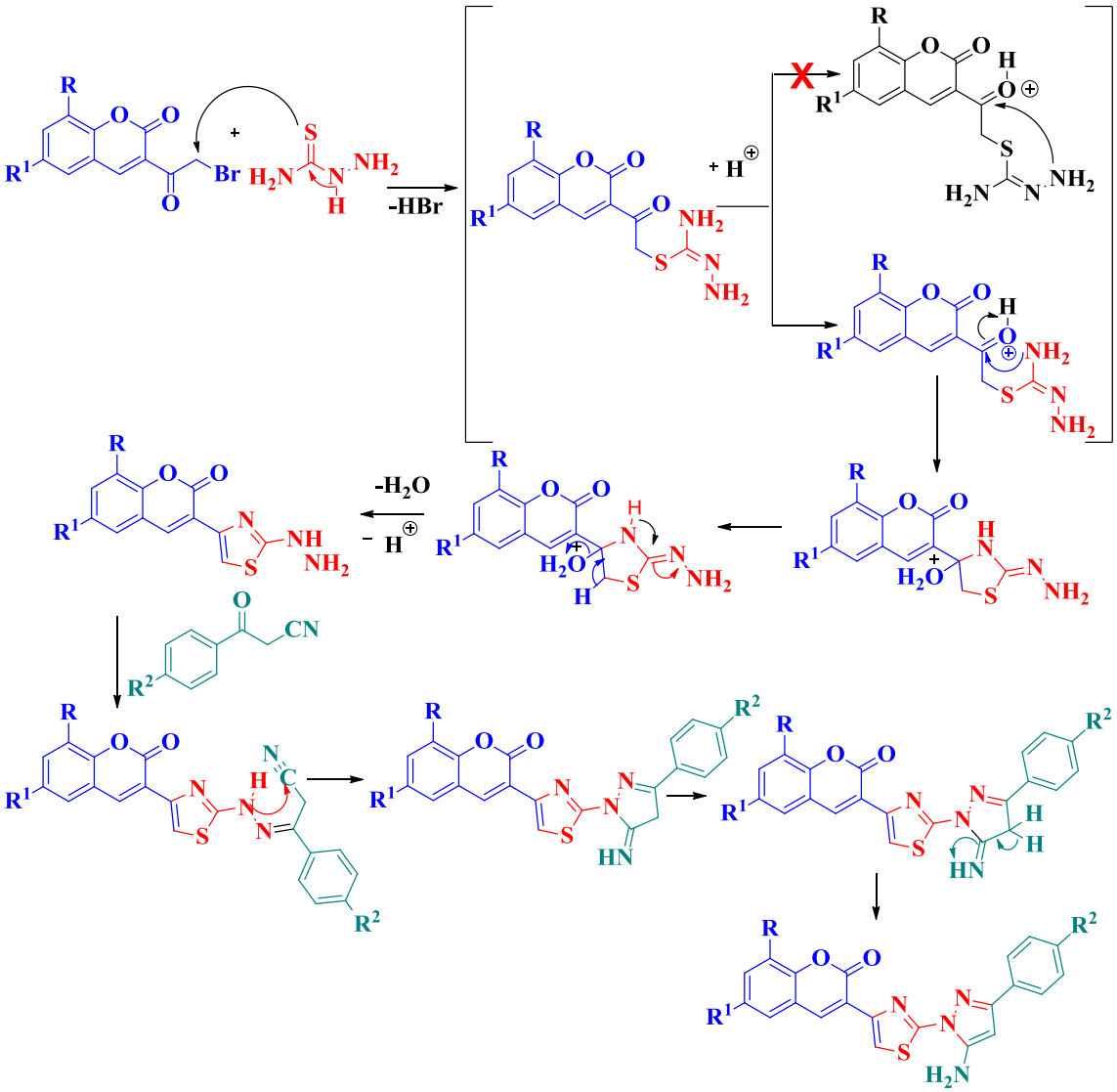
Scheme-2: Synthesis of 3-(2-(5-amino-3-phenyl-1*H*-pyrazol-1-yl)thiazol-4-yl)-2*H*-chromene -2-ones 37 (a-q)

Table 6: A facile one pot multicomponent synthesis of 3-(2-(5-amino-3-phenyl-1*H*-pyrazol-1-yl)thiazol-4-yl)-2*H*-chromene-2-ones.

S. No.	Product	R	R^1	R^2	Time (h)	Yield (%) [a]
1	37a	H	H	H	4.5	83
2	37b	H	Cl	H	5	86
3	37c	Cl	Cl	H	5	84
4	37d	H	Br	H	5.5	88
5	37e	Br	Br	H	6	92
6	37g	$-OCH_3$	H	H	5	82
7	37h	$-OCH_3$	Br	H	5.5	88
8	37i	$-OCH_2CH_3$	H	H	5.5	85
9	37j	$-C(CH_3)_3$	$-C(CH_3)_3$	H	4.5	79
10	37k	H	$-N(CH_2CH_3)_2$	H	5	81
11	37l	H	H	Br	6	84
12	37m	Br	Br	Br	6	91
13	37o	$-OCH_3$	H	Br	6	90
14	37p	$-OCH_3$	Br	Br	6	93
15	37q	H	H	$-C_6H_5$	5.5	81

[a] Isolated yields

Structural assignment of newly synthesized molecules (37a-q) was established on the basis of physical and analytical spectral data (IR, NMR and Mass Spectra).



Anticancer activity of novel coumarinyl thiazolyl pyrazole amines by MTT Assay[49]:

In order to determine the anticancer potency, the synthesized 3-(2-(5-amino-3-phenyl-1*H*-pyrazol-1-yl)thiazol-4-yl)-2*H*-chromene-2-ones (**37 a - q**), were evaluated *in vitro* against a panel of five different human cancer cell lines, Murine leukemia (L1210), human T-lymphocyte cells (CEM), prostate cancer (DU-145), human cervix carcinoma cells (HeLa), and breast cancer (MCF-7) cells by using standard MTT anticancer screening protocol. The preliminary anticancer activity for all the synthesized compounds was done at 100 µM concentration and cell inhibition was determined. Majority of the analogues were exhibited profound growth inhibition of more than 50 percent at the preliminary anticancer evaluation. From the preliminary anticancer activity results, all the compounds were further assayed at various concentrations (5 -

150 µM) in order to generate the IC_{50} values and the IC_{50} values were shown in **Table 7**. The experiment was repeated thrice and the average values were taken.

Table.7: In vitro anticancer activity IC_{50} (µM) values of novel 3-(2-(5-amino-3-phenyl-1*H*-pyrazol-1-yl)thiazol-4-yl)-2*H*-chromene-2-ones (**37 a - q**).

Compound	IC_{50}^{*} (µM)				
	L1210	**CEM**	**Du 145**	**HeLa**	**MCF-7**
37a	46 ± 7	48 ± 9	29 ± 10	35 ± 4	**14 ± 7**
37b	35 ± 1	40 ± 2	35 ± 1	73 ± 2	**13 ± 4**
37c	45 ± 3	59 ± 3	**10± 3**	53 ± 2	**9 ± 5**
37d	62 ± 8	70 ± 5	32 ± 1	75 ± 4	55 ± 3
37e	35 ± 3	48 ± 8	30 ± 3	55 ± 2	**20 ± 8**
37f	123±1	92±5	59±1	115±8	70 ±6
37g	102 ± 5	167 ± 18	**18 ± 1**	74 ± 2	**10 ± 6**
37h	108 ± 1	139 ± 5	**18 ± 4**	126 ± 1	**16 ± 1**
37i	130 ± 1	133 ± 1	**14 ± 5**	116 ± 1	75 ± 8
37j	47 ± 2	41 ± 2	**7 ± 1**	102 ± 1	**9 ± 6**
37**k**	**24 ± 1**	**21 ± 4**	**8 ± 1**	81 ± 2	**8 ± 2**
37l	79 ± 4	65 ± 2	**11 ± 2**	55 ± 4	**10 ± 4**
37m	54 ± 4	82 ± 3	23 ± 9	87 ± 3	45 ± 6
37n	NT	NT	NT	NT	NT
37o	110 ± 5	148 ± 6	75 ± 7	136 ± 8	89 ± 4
37p	51 ± 2	112 ± 6	68 ± 5	87 ± 4	25 ± 10
37q	NT	NT	NT	NT	NT
Doxorubicin	**6**	**8**	**4.2**	**2.5**	**3.0**

From the anticancer activity results, all the synthesized molecules exhibited broad spectrum of activity against five human cancer cell lines in a dose dependent manner. Among the seventeen derivatives, compound **37k** exhibited impressive activity against all the tested cell lines of L1210, CEM, DU-145 and MCF-7 with the IC_{50} values of 24±1, 21±4, 8±2 and 8±1 µM concentration. Similarly compounds 37a, 37b, 37c, 37e, 37g, 37h, 37j, 37k, 37l and 37p were found to show excellent activity against the MCF-7 cancer cell line with IC_{50} values of 14±7, 13±4, 9±5, 20±8, 10±6, 16±1, 9±6, 8±2, 10±4, 25±10 µM concentration and compounds 37c, 37g, 37h, 37i, 37j, 37k, 37l and 37m exhibited good anticancer property against DU-145 cell line with IC_{50} values of 10±3, 18±1, 18±4, 14±5, 7±1, 8±1, 11±2 and 23±9 µM concentration. Whereas remaining compounds exhibited appreciable anticancer activity against tested cancer cell lines.

Cell cyle arrest of 3-(2-(5-amino-3-phenyl-1*H*-pyrazol-1-yl)thiazol-4-yl)-2*H*-chromene -2-ones in DU-145 Cell line:

To understand the anticancer activity mechanism of the synthesized 3-(2-(5-amino-3-phenyl-1*H*-pyrazol-1-yl)thiazol-4-yl)-2*H*-chromene -2-ones derivatives, we analysed the cell cycle alteration phase distribution of the cells by treating DU-145 cells with 10 µM concentration of the compounds 37b, 37c, 37j, 37k, and 37l for 24h were harvested and analyzed by flow cytometry. Treatment of cells with compounds 37b, 37c, 37j, 37k, and 37l caused an accumulation of 87.44%, 88.75%, 75.32%, 86.18% and 76.43% cells in the G0/G1 phase respectively as compared to 68.34% in untreated cells All the tested compounds exhibited promising cell cycle arrest at G0/G1 phase.(Fig. 5 and Table 8).

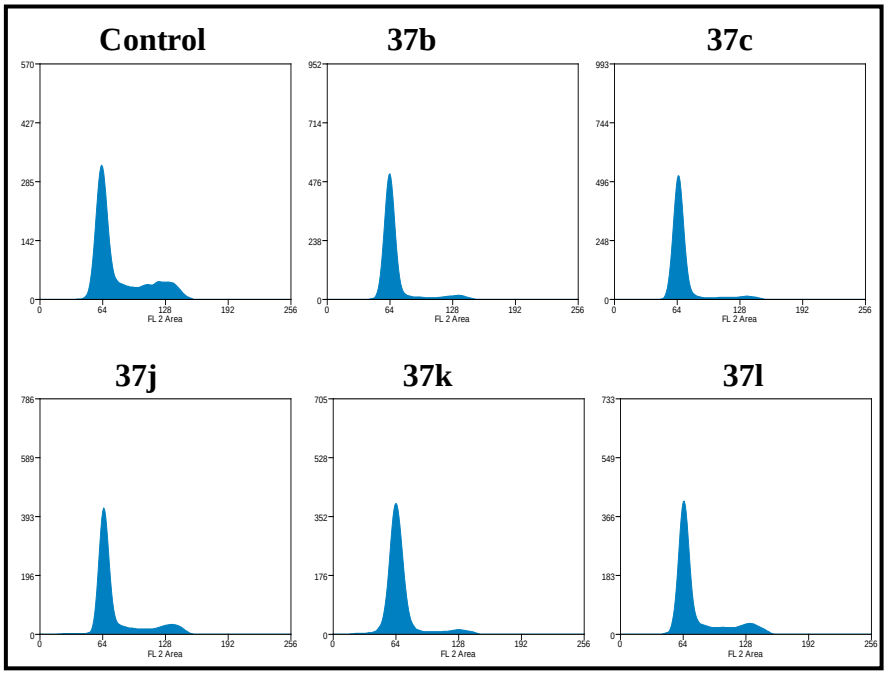


Fig. 5 The cell cycle phase distribution analysis by FACS

Table 8: Cell cycle analysis of DU-145 cell line with 3-(2-(5-amino-3-phenyl-1*H*-pyrazol-1-yl)thiazol-4-yl)-2*H*-chromene -2-ones derivatives.

Compound	Phase		
	G0/G1	S	G2/M
Control	68.34	8.33	23.42
37b	87.44	2.73	9.08
37c	88.75	2.84	8.10
37j	75.32	5.77	17.42
37k	86.18	2.77	8.45

37l	76.43	6.24	17.50

Effect on chromatin condensation:

Apoptosis[50] is a crucial and continuous destruction process of undesirable cells during development or homeostasis in multicellular organisms. During apoptosis, chromatin condensation takes place. In order to examine the synthesized compounds induces cytotoxicity through apoptosis, DU-145 cells were treated with compounds 37b, 37c, 37j, 37k, and 37l of 10 µM concentration and harvested for a period of 24 h. To visualize the nuclear condensation Hoechst 33258 stain was used. The compounds 37b, 37c, 37j, 37k, and 37l caused significant chromatin condensation in DU-145 cells (**Fig. 6**).

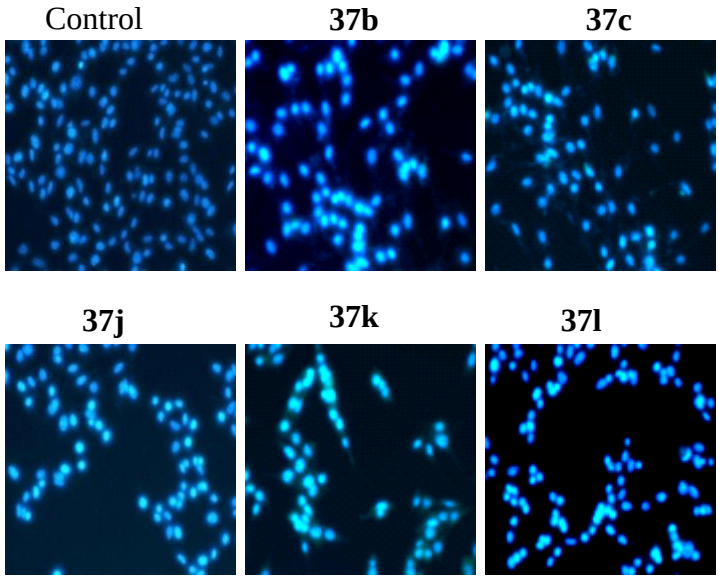


Fig.6. 3-(2-(5-amino-3-phenyl-1*H*-pyrazol-1-yl)thiazol-4-yl)-2*H*-chromene-2-one derivatives cause apoptosis in DU- 145 cells. Cells were treated with 37**b,** 37**c,** 37**j,** 37**k** and 37**l** at 10µM concentration for 24 h. Images were taken by using DAPI filter.

Molecular docking studies:

In order to validate With a view to determine the possible binding modes and corresponding interaction energies between 3-(2-(5-amino-3-phenyl-1*H*-pyrazol-1-yl)thiazol-4-yl)-2*H*-chromene-2-ones and their target Epidermal growth factor receptor (EGFR), molecular docking studies were carried out. Overlay of dock poses for all the compounds is represented in **Fig. 7**. EGFR was chosen as target for the following three

reasons[60]. 1) It is an attractive anticancer drug target since long time. 2) Previous studies have shown that there exists a moderate correlation between the anti-proliferative activity against HeLa and the EGFR inhibitory activity. 3) The similarity between co-crystallized ligand[61] (5EM8.pdb) and the best active compound **37k** was found to be 54.4%.

The docking results revealed that the amine group attached to pyrazole ring and carbonyl group of chromene ring play a vital role in inhibiting the activity of EGFR. The *in silico* studies also disclosed that residues Lys 745, Gln 791 and Met 793 surrounding the active site are the key amino acids and play a crucial role in binding of coumarin derivatives to the target EGFR. From in vitro studies it is clear that compound 37k is the best active compound (**Table 9**). Compound 37k, which is having good inhibitory potential against all the cancer cell lines showed back bone hydrogen bonding interaction with Met 793, with a distance of 1.77 A^{o} (**Fig. 8 and 9**). The hydrogen bond in compound 37k was formed by the carbonyl group of chromene with amine group of Met 793. Compounds 37f and 37n exhibited similar hydrogen bonding interaction as that of compound 37k, whereas in other compounds the amine group attached to pyrazole ring showed hydrogen bonding interaction with carboxylic acid of Gln 791. In compounds 37i and 37p the hydrogen bond was formed by sulphur of thiazole ring with that of Met 793. Further compound 37g was the only compound in which hydrogen bonding interactions were completely absent. These compounds therefore showed hydrogen bonding interactions with same amino acid residues as that of co-crystallized ligand. Another noteworthy thing is that in most of the compounds another hydrogen bonding interaction was observed with Lys 745 apart from Gln 791 and Met 793. This hydrogen bonding interaction is seen between carbonyl group of chromene and amine group of Lys 745. Furthermore compound 37i was the only compound which showed π-cationic interactions with Lys 716.

The binding affinity results showed that some of the newly synthesized coumarin derivatives had comparable binding energies to that of the standard doxorubicin. The binding energy values varied from -72.733 to -84.377 Kcal/mol. From binding energy values (Table 9) it is clear that the compound 37k which is having good inhibitory potential against all cancer cell lines has a binding energy value of -84.377 Kcal/mol. Further compound **37j** which exhibited good inhibitory activity against DU-145 and

MCF-7 cancer cell lines also showed binding energy value of -82.408 K.cal/mol. Therefore both compounds 37k and 37j can serve as most promising inhibitors against EGFR and can be used for further lead optimization for design and synthesis of novel molecules as anticancer agents. On the whole from the docking studies we can say that the new series of coumarin derivatives were stabilized with target EGFR which is evident from receptor-ligand interactions and their binding energy values.

Table.9 The binding energy values of new 3-(2-(5-amino-3-phenyl-1*H*-pyrazol-1-yl)thiazol-4-yl)-2*H*-chromene -2-one derivatives with their target EGFR.

Compound	**Binding energies (Kcal/mol)**
37a	-72.874
37b	-78.977
37c	-81.226
37d	-78.874
37e	-73.866
37f	-80.363
37g	-72.733
37h	-76.993
37i	-81.221
37j	-82.408
37k	**-84.377**
37l	-75.493
37m	-82.364
37n	-81.539
37o	-75.424
37p	-78.519
37q	-79.811
Doxorubicin	-79.464

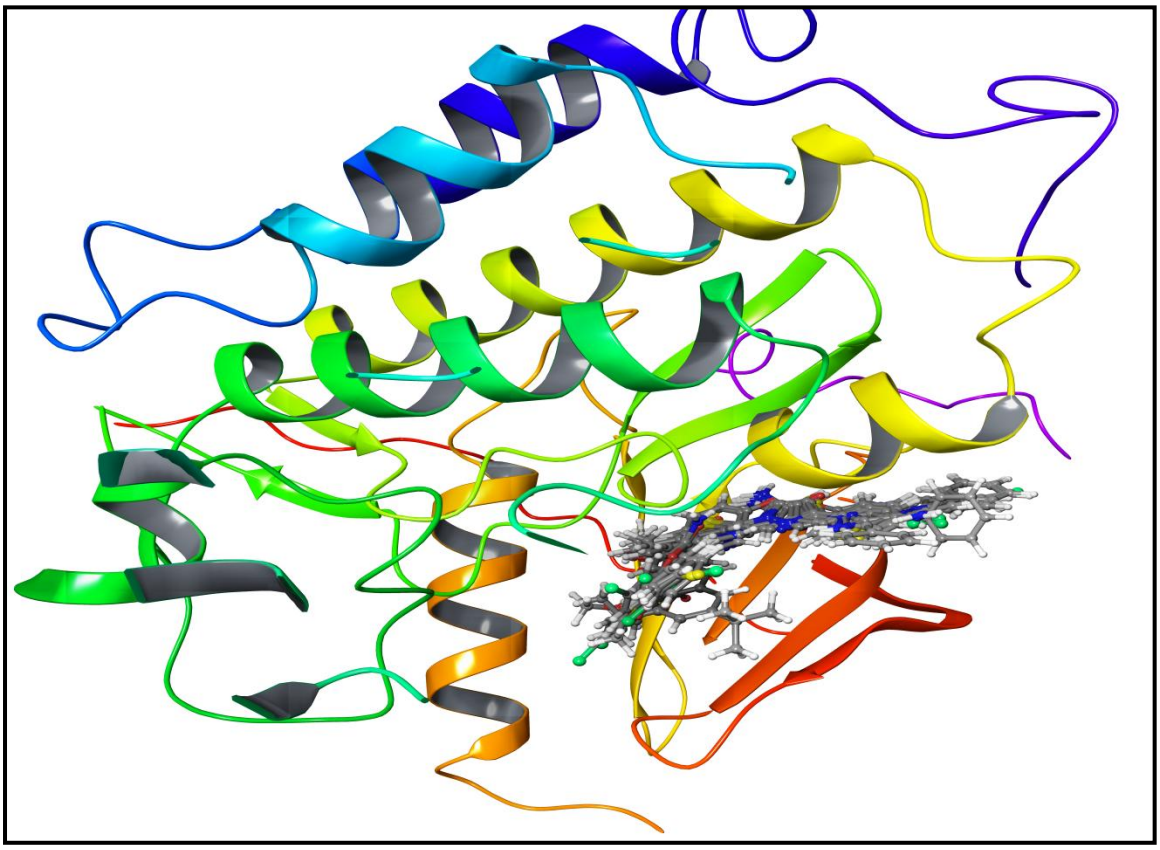


Fig: 7. Overlay of dock poses for 3-(2-(5-amino-3-phenyl-1*H*-pyrazol-1-yl)thiazol-4-yl)-2*H*-chromene-2-ones

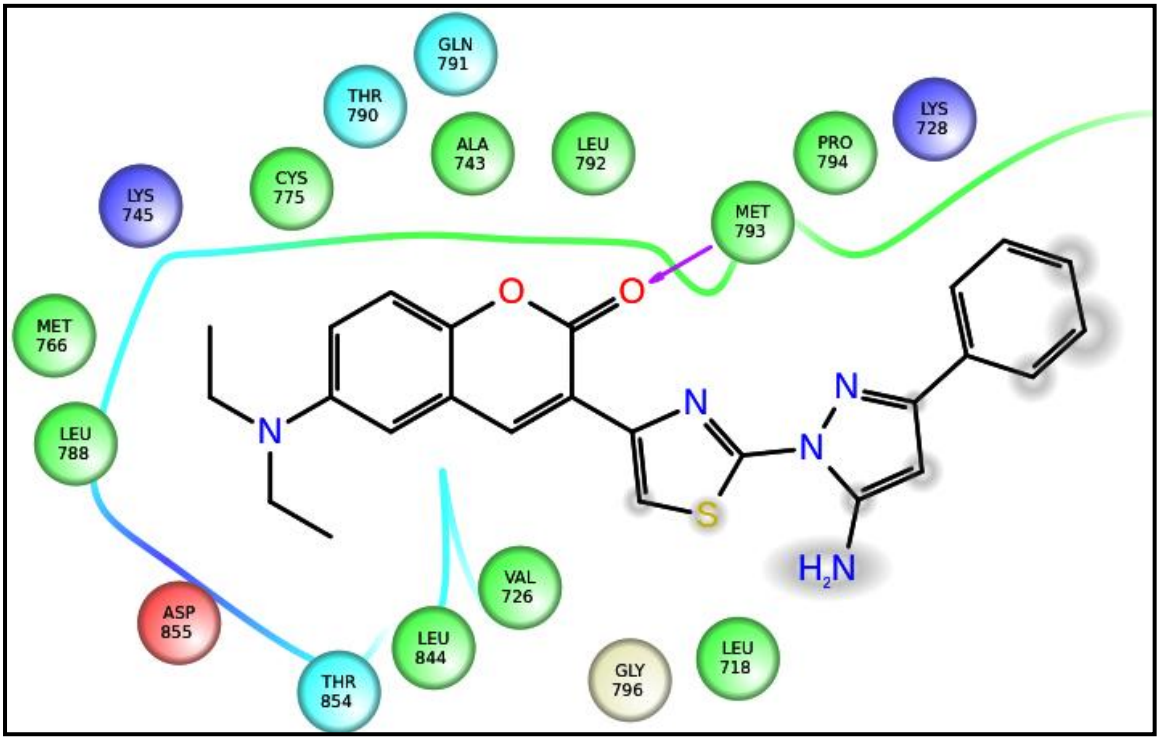


Fig.8

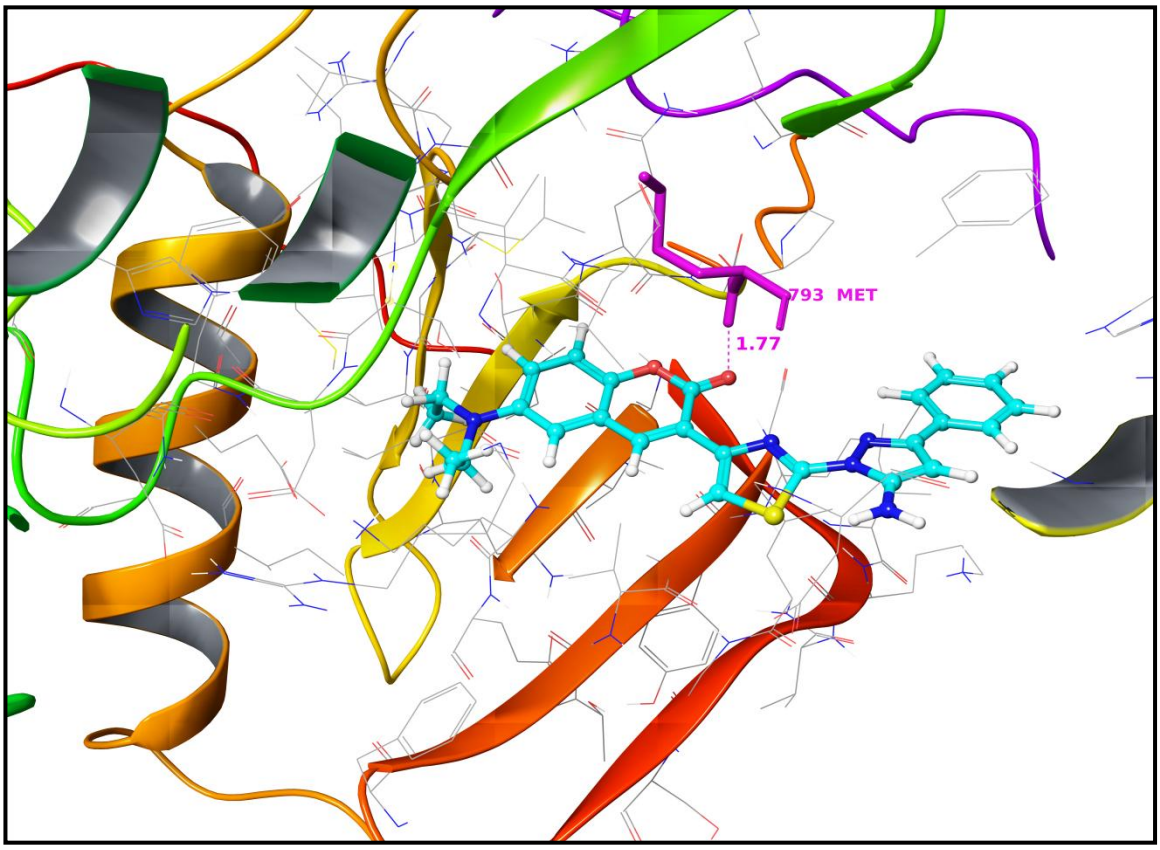


Fig.9

In conclusion, we have developed an efficient method of multicomponent protocol for the synthesis of 3-(2-(5 amino-3-phenyl -1*H*-pyrazol-1yl)thiazol-4-yl)-2*H*-chromene-2-ones and screened for their anticancer activity against five representative human cancer cell lines. All the synthesized compounds exhibited moderated to appreciable activity against the tested cancer cell lines. Among the tested compounds, compound **37k** exhibited a broad spectrum of anti cancer activity where as compound **37j** shown excellent activity against DU-145 and MCF-7 cancer cell lines with IC_{50} values of 7±1and 9±6 µM concentrations. The molecular docking studies with target Epidermal growth factor receptor (EGFR) further supports the anticancer activity.

EXPERIMENTAL:

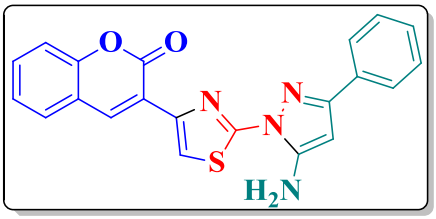
Typical procedure of one-pot three component reaction for the synthesis of 3-(2-(5-amino-3-aryl-1H-pyrazol-1-yl)thiazol-4-yl)-2H-chromene-2-ones:

An equivalent reaction mixture of substituted 3-(2-bromoacetyl)-2*H*-chromen-2-ones **32a-j** (1 mmol), thiosemicarbazide **33** (0.091 g, 1mmol) and substituted benzoylacetonitrile **36 a-c** (1 mmol) were taken in a round bottom flask containing

ethanol solvent (5 mL). To this reaction mixture catalytic amount of acetic acid was added. The temperature of the reaction mixture was slowly raised to 60 - 65 °C and maintained for about 4 - 6 h. After completion of the reaction (as detected in TLC), the reaction mixture was cooled to room temperature. The isolated product was filtered, washed with ethanol, dried and recrystallized from methanol solvent.

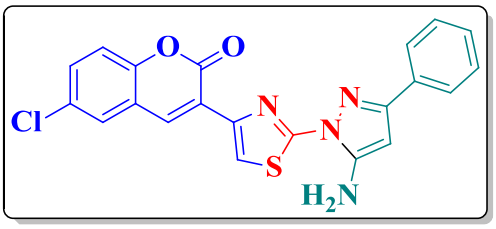
SPECTRAL DATA

3-(2-(5-Amino-3-phenyl-1*H*-pyrazol-1-yl)thiazol-4-yl)-2*H*-chromen-2-one (37a):



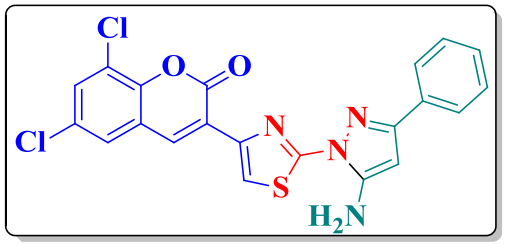
IR (KBr υ_{max}, cm^{-1}): 3329 (NH_2); 1708 (C=O); 1607 (C=N); **^{1}H NMR(400 MHz, DMSO-d_6, δ ppm):** 5.92 (s, 1H, C_4-H of Pyrazole); 6.94 (bs, 2H, -NH_2); 7.37-7.49 (m, 5H, Ar-H); 7.64-7.68 (m, 1H, Ar-H); 7.82 (d, *J* = 7.2Hz, 2H, Ar-H); 7.96 (d, *J* = 8 Hz, 1H, Ar-H); 8.20 (s, 1H, C_4-H of coumarin); 9.02 (s, 1H, thiazole); **^{13}C NMR (100 MHz, DMSO-d_6, ppm):** δ 85.13; 115.38; 115.84; 119.19; 119.55; 124.65; 125.59; 128.60; 129.02; 131.92; 132.08; 139.68; 144.51; 149.39; 152.46; 153.68; 161.64. **MS (ES m/z):** 387 $[M+H]^+$; *Anal.* calcd for $C_{21}H_{14}N_4O_2S$: C, 65.27; H, 3.65; N, 14.50; S, 8.30%. Found: C, 65.19; H, 3.71; N, 14.58; S, 8.22%.

3-(2-(5-Amino-3-phenyl-1*H*-pyrazol-1-yl)thiazol-4-yl)-6-chloro-2*H*-chromen-2-one (**37b**):



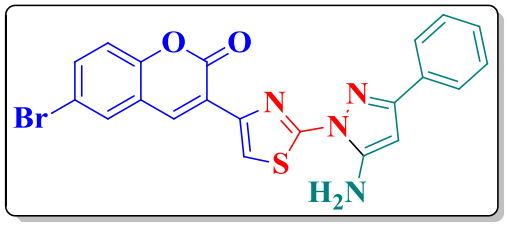
IR (KBr υ_{max}, cm^{-1}): 3320 (NH_2); 1717 (C=O); 1607 (C=N); **^{1}H NMR(400 MHz, DMSO-d_6, δ ppm):** 5.13 (bs, 2H, -NH_2); 7.39-7.47 (m, 1H, Ar-H); 7.53 (t, *J* = 6 Hz, 2H, Ar-H); 7.71-7.74 (m, 2H, Ar-H); 8.09 (d, *J* = 2.4 Hz, 1H, Ar-H); 8.13 (s, 1H, Ar-H); 8.38 (s, 1H, Ar-H); 8.97(S, 1H, C_4-H of coumarin); 9.16 (s, 1H, C_4-H of pyrazole); 9.73 (s, 1H, thiazole); **^{13}C NMR (100 MHz, DMSO-d_6, ppm):** δ 85.14; 117.90; 120.59; 120.71; 125.61; 127.56; 127.90; 128.37; 128.63; 130.76; 131.36; 136.32; 138.36; 143.85; 144.20; 149.42; 150.71; 151.10; 153.42; 158.29; 161.76; **MS (HRMS m/z):** 443.0402 $[M+Na]^+$; *Anal.* calcd for $C_{21}H_{13}ClN_4O_2S$: C, 59.93; H, 3.11; N, 13.31; S, 7.62%; Found: C, 59.88; H,3.17; N,13.38; S, 7.69%;

3-(2-(5-Amino-3-phenyl-1*H*-pyrazol-1-yl)thiazol-4-yl)-6,8-dichloro-2*H*-chromen-2-one (37c):



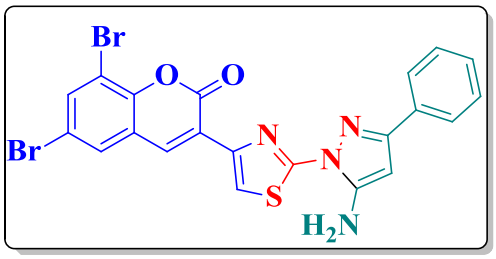
IR (KBr υ_{max}, cm^{-1}): 3324 (NH_2); 1717 (C=O); 1607 (C=N); **^{1}H NMR(400 MHz, DMSO-d_6, δ ppm):** 5.13 (bs, 2H, -NH_2); 7.45-7.54 (m, 3H, Ar-H); 7.66-7.80 (m, 2H, Ar-H); 8.09 (d, *J* = 2Hz, 1H, Ar-H); 8.20 (d, *J* = 7.2 Hz, 1H, Ar-H); 8.38 (s, 1H, C_4-H of coumarin); 9.17 (s, 1H, C_4-H of pyrazole); 9.74 (s, 1H, thiazole); **^{13}C NMR (100 MHz, DMSO-d_6, ppm):** δ 92.83; 117.93; 120.04; 125.58; 126.14; 128.29; 128.59; 132.21; 142.25; 152.30; 158.10; 169.48; *Anal.* calcd for $C_{21}H_{12}Cl_2N_4O_2S$: C, 55.40; H, 2.66; N, 12.31; S, 7.04%. Found: C, 55.34; H, 2.62; N, 12.38; S, 7.12%.

3-(2-(5-Amino-3-phenyl-1*H*-pyrazol-1-yl)thiazol-4-yl)-6-bromo-2*H*-chromen-2-one (37d):



IR (KBr, υ_{max}, cm^{-1}): 3324 (NH_2); 1731 (C=O); 1602 (C=N); **^{1}H NMR(400 MHz, DMSO-d_6, δ ppm):** 5.13 (bs, 2H, -NH_2); 7.47 (d, *J* = 8.8 Hz, 5H, Ar-H); 7.84 (d, *J* = 8.8 Hz, 1H, Ar-H); 8.14 (d, *J* = 8Hz, 1H, Ar-H); 8.22 (s, 1H, Ar-H); 8.37 (s, 1H, C_4-H of coumarin); 9.16(s, 1H, C_4-H of pyrazole); 9.73 (s, 1H, thiazole); **MS (ES m/z):** 466 $[M+H]^+$, 467$[M+2]^+$; *Anal.* calcd for $C_{21}H_{13}BrN_4O_2S$: C, 54.20; H, 2.82; N, 12.04; S, 6.89%. Found: C, 54.24; H, 2.88; N, 12.12; S, 6.93%;

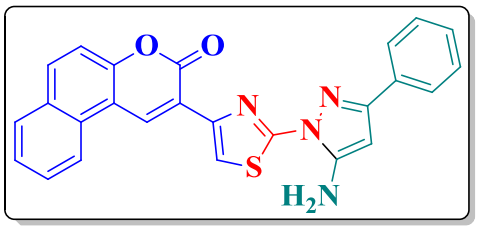
3-(2-(5-Amino-3-phenyl-1*H*-pyrazol-1-yl)thiazol-4-yl)-6,8-dibromo-2*H*-chromen-2-one (37e):



IR (KBr, υ_{max}, cm^{-1}): 3343 (NH_2); 1735 (C=O); 1607 (C=N); **^{1}H NMR(400 MHz, DMSO-d_6, δ ppm):** 5.91 (s, 1H, C_4-H of pyrazole); 6.92 (bs, 2H, -NH_2); 7.37-7.46 (m, 3H, Ar-H); 7.81 (d, *J* = 7.2 Hz, 2H, Ar-H); 8.14 (d, *J* = 2Hz, 1H, Ar-H); 8.21 (t, *J* = 5.2 Hz, 2H, Ar-H); 8.92 (s, 1H, C_4-H of coumarin); **^{13}C NMR (100 MHz, DMSO-d_6, ppm):** δ 85.13; 109.75; 116.17;

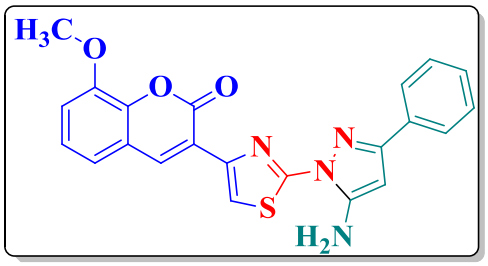
116.61; 121.00; 122.13; 125.54; 128.53; 130.45; 131.98; 135.88; 137.76; 143.78; 148.27; 149.33; 153.33; 157.54; 161.74; *Anal.* calcd for $C_{21}H_{12}Br_2N_4O_2S$: C, 46.35; H, 2.22; N, 10.29; S, 5.89%. Found: C, 46.39; H, 2.28; N, 10.34; S, 5.93%; **MS (ES m/z):** 544.5 $[M]^+$.

2-(2-(5-Amino-3-phenyl-1*H*-pyrazol-1-yl)thiazol-4-yl)-3*H*-benzo[*f*]chromen-3-one (37f):



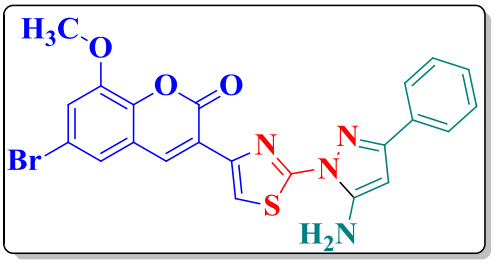
IR (KBr, υ_{max}, cm^{-1}): 3421(NH_2); 1716(C=O); 1607(C=N); **^{1}H NMR(400 MHz, DMSO-d_6, δ ppm):** 4.26 (bs, 2H, -NH_2); 7.39-7.47 (m, 1H, Ar-H); 7.67 (d, *J* = 8.8 Hz, 4H, Ar-H); 7.80 (t, *J* = 7.2Hz, 3H, Ar-H); 8.11 (d, *J* = 8Hz, 2H, Ar-H); 8.25(t, *J* = 8.8 Hz, 1H, Ar-H); 8.34(s, 1H, C_4-H of coumarin); 8.66 (s, 1H, C_4-H of pyrazole); 9.23 (s, 1H, thiazole); **^{13}C NMR (100 MHz, DMSO-d_6, ppm):** δ 112.71; 116.42; 120.88; 122.34; 125.56; 126.46; 128.59; 128.82; 128.96; 129.95; 135.16; 139.91; 154.18; 158.57; 164.84; **MS (HRMS m/z):** 459.0894 $[M+Na]^+$; *Anal.* calcd for $C_{25}H_{16}N_4O_2S$: C, 68.79; H, 3.69; N, 12.84; S, 7.35%. Found: C, 68.84; H, 3.72; N, 12.87; S, 7.39%.

3-(2-(5-Amino-3-phenyl-1*H*-pyrazol-1-yl)thiazol-4-yl)-8-methoxy-2*H*-chromen-2-one (37g):



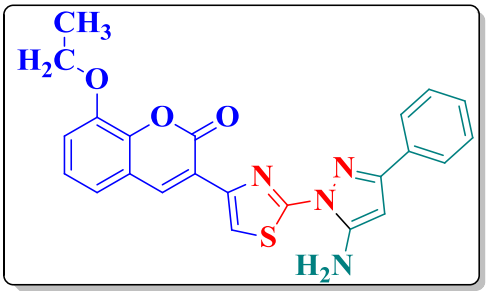
IR (KBr, υ_{max}, cm^{-1}): 3358 (NH_2); 1710(C=O); 1604(C=N); 1276 (-C-O-C). **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 3.94 (s, 3H,-OCH_3); 5.92 (s, 1H, C_4-H of pyrazole); 6.96 (bs, 2H, -NH_2); 7.34 (d, *J* = 6.8 Hz, 3H, Ar-H); 7.39-7.49 (m, 3H, Ar-H); 7.82 (d, *J* = 7.2Hz, 2H, Ar-H); 8.20 (s, 1H, C_4-H of coumarin); 8.97(s, 1H, thiazole); **^{13}C NMR (100 MHz, DMSO-d_6, ppm):** δ 55.92; 85.89; 115.74; 118.97; 119.76; 120.21; 120.74; 125.60; 128.62; 132.08; 139.88; 141.76; 143.12; 144.14; 144.47; 146.34; 149.40; 153.32; 158.42; 161.65; **MS (HR-MS m/z):** 417.1040 $[M+H]^+$; *Anal.* calcd for $C_{22}H_{16}N_4O_3S$: C, 63.45; H, 3.87; N, 13.45; S, 7.70%. Found: C, 63.49; H, 3.81; N, 13.49; S, 7.63%.

3-(2-(5-Amino-3-phenyl-1*H*-pyrazol-1-yl)thiazol-4-yl)-6-bromo-8-methoxy-2*H*-chromen-2-one (37h) :



IR (KBr, υ_{max}, cm^{-1}): 3364(-NH_2); 1738 (C=O); 1610 (C=N); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 3.95(s, 3H, -OCH_3); 5.92 (s, 1H, C_4-H of pyrazole); 6.92 (bs, 2H, -NH_2); 7.41 (t, *J* = 7.2 Hz, 1H, Ar-H); 7.48-7.58 (m, 1H, Ar-H); 7.62 (d, *J* = 2Hz, 1H, Ar-H); 7.69 (t, *J* = 5.6Hz, 1H, Ar-H), 7.76 (d, *J* = 2Hz, 1H, Ar-H); 7.82 (t, *J* = 7.2 Hz, 2H, Ar-H); 8.39 (s, 1H, -C_4-H of coumarin); 8.93 (s, 1H, thiazol); **^{13}C NMR (100 MHz, DMSO-d_6, ppm):** δ 56.61; 85.12; 116.04; 116.15; 116.66; 120.68; 121.11; 121.66; 122.05; 125.60; 128.62; 132.04; 138.51; 141.08; 144.16; 147.13; 149.40; 153.38; 157.93; 161.74; **MS (HR-MS m/z):** 516.9944 $[M+Na]^+$; *Anal.* calcd for $C_{22}H_{15}BrN_4O_3S$: C, 53.34; H, 3.05; N, 11.31; S, 6.47%. Found: C, 53.39; H, 3.14; N, 11.35; S, 6.52%.

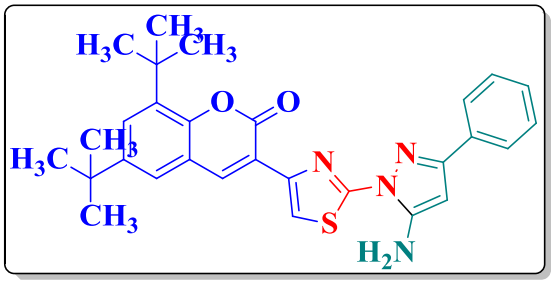
3-(2-(5-Amino-3-phenyl-1*H*-pyrazol-1-yl)thiazol-4-yl)-8-ethoxy-2*H*-chromen-2-one(37i):



IR (KBr, υ_{max}, cm^{-1}): 3344(NH_2); 1710(C=O); 1607(C=N); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 1.43 (t, *J* = 2Hz, 3H, -CH_3); 4.20 - 4.22 (m, 2H, -O-CH_2-); 5.92 (s, 1H, C_4-H of pyrazole); 6.93 (bs, 2H, -NH_2); 7.33-7.48 (m, 5H, Ar-H); 7.82 (t, *J* = 6.8Hz, 2H, Ar-H); 8.20 (s, 1H, Ar-H); 8.46 (s, 1H, C_4-H of coumarin); 8.99 (s, 1H, thiazole); **^{13}C NMR (100 MHz, DMSO-d_6, ppm):** δ 14.50; 64.35; 85.12; 120.16; 120.69; 122.03; 124.55; 125.03; 125.58; 128.59; 132.07; 139.91; 141.86; 143.21; 144.16; 144.46; 145.43; 145.56; 148.90; 149.38; 153.20; 158.47; 161.64; **MS (HR-MS m/z):** 431.1163 $[M+Na]^+$; *Anal.* calcd for $C_{23}H_{18}N_4O_3S$: C, 64.17; H, 4.21; N, 13.01; S, 7.45%. Found: C, 64.21; H, 4.26; N, 13.14; S, 7.49%.

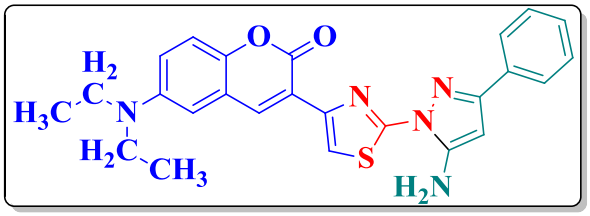
6,8-Di-*tert*-butyl-3-(2-(5-amino-3-phenyl-1*H*-pyrazol-1-yl)thiazol-4-yl)-2*H*-chromen-2-one (37j):

IR (KBr, υ_{max}, cm^{-1}): 3331(NH_2); 1727(C=O); 1610(C=N); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 1.39 (s, 9H, *t*-Bu); 1.55 (s, 9H, *t*-Bu); 5.79 (bs, 2H, -NH_2); 5.90 (s, 1H, C_4-H of pyrazole); 7.34-7.38 (m, 1H, Ar-H); 7.40-7.44 (m, 3H, Ar-H); 7.59 (d, *J* = 1.6Hz, 1H, Ar-H); 7.83-7.85 (m, 2H, Ar-H); 8.22 (S, 1H, C_4-H of coumarin); 8.44 (s,



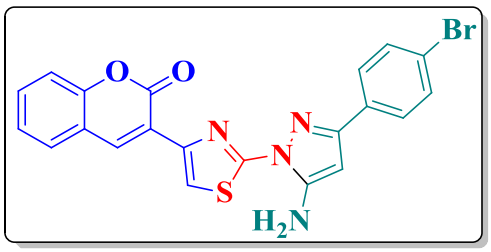
1H, thiazole); **^{13}C NMR (100 MHz, DMSO-d_6, ppm):** δ 31.08; 34.45; 85.13; 115.06; 118.53; 119.18; 123.85; 124.57; 125.60; 126.54; 128.10; 128.34; 128.62; 128.83; 132.09; 134.18; 135.69; 136.06; 140.98; 144.64; 145.10; 146.32; 146.81; 149.16; 149.37; 150.51; 153.33; 158.49; 161.79; **MS (ES m/z):** 499 $[M+H]^+$; *Anal.* calcd for $C_{29}H_{30}N_4O_2S$: C, 69.85; H, 6.06; N, 11.24; S, 6.43%. Found: C, 69.89; H, 6.13; N, 11.29; S, 6.48%.

***3-(2-(5-amino-3-phenyl-1H-pyrazol-1-yl)thiazol-4-yl)-6-(diethylamino)-2H-chromen-2-one* (37k):**



IR (KBr, υ_{max}, cm^{-1}): 3338 (NH_2); 1721(C=O); 1605 (C=N); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 1.15 (t, *J* = 3.6 Hz, 6H, -N-CH_2-CH_3); 3.45 – 3.49 (m, 4H, -N-CH_2-); 4.17 (s, 2H, -NH_2); 5.91 (s, 1H, C_4-H Pyrazole); 6.61 (t, *J* = 5.6 Hz, 2H, -Ar-H); 6.80 (t, *J* = 8.8 Hz, 2H, -Ar-H); 7.44 (d , *J* = 7.6 Hz, 1H, -Ar-H); 7.60 – 7.68 (m, 1H, -Ar-H); 7.81 (d, *J* = 6.8Hz, 1H, -Ar-H); 7.96 (s, 1H, -Ar-H); 8.30 (s, 1H, C_4-H of coumarin); 8.79 (s, 1H, -Thiazole-H); **^{13}C NMR (100 MHz, DMSO-d_6, ppm):** δ 12.29; 44.11; 96.09; 107.87; 109.47; 115.88; 130.37; 142.12; 144.91; 149.48; 151.05; 156.53; 160.24. *Anal*. Calcd for $C_{25}H_{23}N_5O_2S$: C, 65.63; H, 5.07; N, 15.31; S, 7.01%; Found. C, 65.66; H, 5.10; N, 15.25; S, 7.04%.

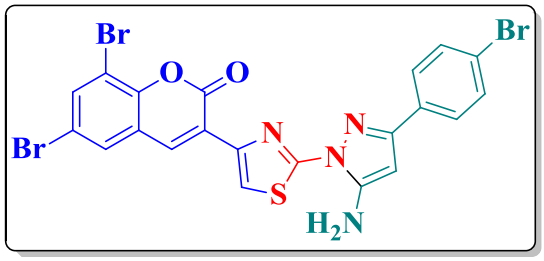
3-(2-(5-Amino-3-(4-bromophenyl)-1*H*-pyrazol-1-yl)thiazol-4-yl)-2*H*-chromen-2-one (37l):



IR (KBr, υ_{max}, cm^{-1}): 3329 (NH_2); 1712 (C=O); 1608 (C=N); **^{1}HNMR (400 MHz, DMSO-d_6, δ ppm):** 5.94 (s, 1H, C_4-H Pyrazole); 6.98 (bs, 2H, -NH_2); 7.41- 7.49 (m, 2H, Ar-H); 7.63 – 7.66 (m, 3H, Ar-H); 7.78 (d, *J* = 8.4Hz, 2H, Ar-H); 7.94 – 7.97 (m, 1H, -Ar-H); 8.21 (s, 1H, C_4-H of coumarin); 9.02 (s, 1H, Thiazole-H); **^{13}C NMR (100 MHz, DMSO-d_6, ppm):** δ 85.12; 115.54; 115.88;

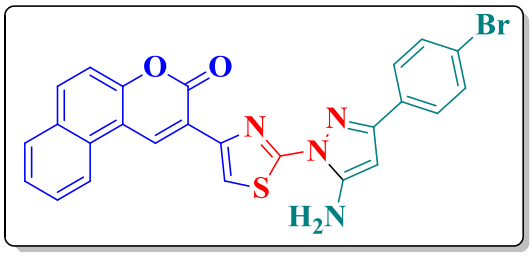
119.20; 119.53; 121.78; 124.69; 127.60; 129.05; 131.30; 131.58; 131.97; 139.76; 144.55; 149.53; 152.21; 152.49; 158.69; 161.56; **MS (ES m/z):** 467 $[M+2]^+$; 488 $[M+Na]^+$; *Anal*. Calcd. For $C_{21}H_{13}BrN_4O_2S$: C, 54.20; H, 2.82; N, 12.04; S, 6.89%. Found: C, 54.25; H, 2.78; N, 12.08; S, 6.86%.

3-(2-(5-Amino-3-(4-bromophenyl)-1*H*-pyrazol-1-yl)thiazol-4-yl)-6,8-dibromo-2*H*-chromen-2-one (37m):



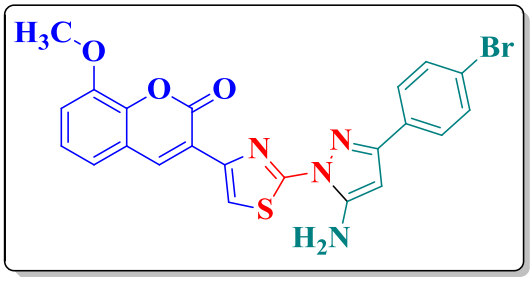
IR (KBr, υ_{max}, cm^{-1}): 3307 (NH_2); 1729 (C=O); 1606 (C=N); **^{1}H NMR (DMSO-d_6, δ ppm):** 5.14 (bs, 2H, -NH_2); 7.64 (d, *J* = 8.4Hz, 1H, Ar-H); 7.77 (d, *J* = 8.4Hz, 1H, Ar-H); 7.84 (t, *J* = 10.8Hz, 1H, Ar-H); 8.13 – 8.19 (m, 1H, Ar-H); 8.23 – 8.26 (m, 2H, Ar-H); 8.36 (s, 1H, C_4-H of coumarin); 9.20 (s, 1H, C_4-H Pyrazole); 9.76 (s, 1H, thiazole-H); **^{13}C NMR (100 MHz, DMSO-d_6, ppm):** δ 92.69; 109.86; 116.20; 121.49; 126.99; 130.31; 131.00; 131.93; 136.96; 141.90; 149.54; 157.47; 169.54; *Anal*. Calcd. For $C_{21}H_{11}Br_3N_4O_2S$: C, 40.48; H, 1.78; N, 8.99; S, 5.15%; Found: C, 40.45; H, 1.83; N, 8.95; S, 5.19%.

2-(2-(5-Amino-3-(4-bromophenyl)-1*H*-pyrazol-1-yl)thiazol-4-yl)-3*H*-benzo[*f*] chromen-3-one (37n):



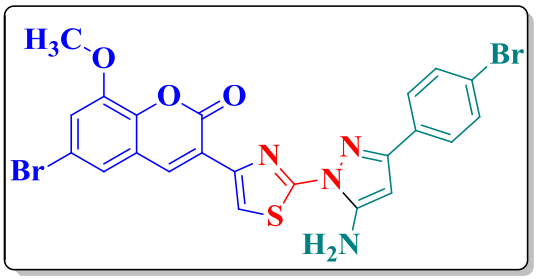
IR (KBr, υ_{max}, cm^{-1}): 3250 (NH_2); 1713 (C=O); 1645 (C=N); **^{1}HNMR (400 MHz, DMSO-d_6, δ ppm):** 5.17 (bs, 2H, -NH_2); 7.67 (t, *J* = 8.8 Hz, 3Hz, Ar-H); 7.80 (d, *J* = 8 Hz, 2H, Ar-H); 8.12 (d, *J* = 7.6 Hz, 2H, Ar-H); 8.23 (s, 1H, Ar-H); 8.30 (d, *J* = 9.2 Hz, 1H, Ar-H); 8.50 (d, *J* = 8.8 Hz, 1H, Ar-H); 9.08 (s, 1H, C_4-H of coumarin); 9.19 (s, 1H, C_4-H pyrazole); 9.75 (s, 1H, thiazole-H); **^{13}C NMR (100 MHz, DMSO-d_6, ppm):** δ 93.22; 116.45; 121.79; 123.79; 126.24; 126.52; 127.61; 128.77; 128.86; 129.05; 129.83; 129.99; 130.32; 131.60; 131.94; 134.17; 135.24; 138.88; 139.98; 153.69; 154.23; 158.51; 158.64; 164.89; *Anal*. Calcd. For $C_{25}H_{15}BrN_4O_2S$: C, 58.26; H, 2.93; N, 10.87; S, 6.22%; Found: C, 58.30; H, 2.96; N, 10.82; S, 6.18%.

3-(2-(5-Amino-3-(4-bromophenyl)-1*H*-pyrazol-1-yl)thiazol-4-yl)-8-methoxy-2*H*-chromen-2-one (37o):



IR (KBr, υ_{max}, cm^{-1}): 3436 (NH_2); 1712 (C=O); 1608 (C=N); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 3.94 (s, 3H, -OCH_3); 4.18 (bs, 2H, -NH_2); 5.93 (s, 1H, C_4-H of Pyrazole); 7.33 – 7.39 (m, 3H, Ar-H); 7.42 – 7.45 (m, 2H, Ar-H); 7.64 (d, *J* = 8.8 Hz, 1H, Ar-H); 7.78 (t, *J* = 7.2 Hz, 1H, Ar-H); 8.47 (s, 1H, C_4-H of coumarin); 8.98 (s, 1H, thiazole-H); **^{13}C NMR (100 MHz, DMSO-d_6, ppm):** δ 56.74; 92.81; 115.94; 117.46; 120.43; 122.34; 127.60; 128.38; 130.31; 131.58; 131.93; 133.64; 147.16; 157.76; 169.50; **MS (ES m/z):** 495 $[M]^+$; *Anal*. Cald. For $C_{22}H_{15}BrN_4O_3S$: C, 53.34; H, 3.05; N, 11.31; S, 6.47%. Found: C, 53.29; H, 3.10; N, 11.34; S, 6.43%.

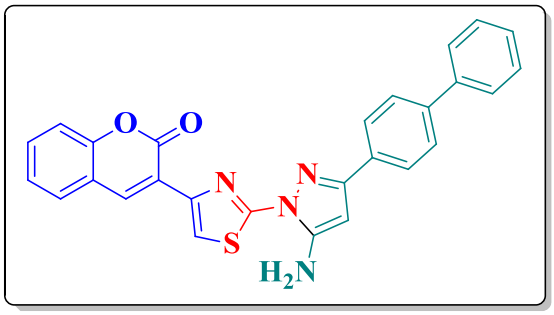
3-(2-(5-Amino-3-(4-bromophenyl)-1*H*-pyrazol-1-yl)thiazol-4-yl)-6-bromo-8-methoxy-2*H*-chromen-2-one (37p):



IR (KBr, υ_{max}, cm^{-1}): 3365 (NH_2); 1721 (C=O); 1605 (C=N); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 3.96 (s, 3H, -OCH_3); 5.12 (bs, 2H, -NH_2); 7.46 – 7.49 (m, 1H, Ar-H); 7.54 (d, *J* = 2 Hz, 1H, Ar-H); 7.64 (d, *J* = 8.8 Hz, 1H, Ar-H); 7.76 (t, *J* = 9.6 Hz, 1H, Ar-H); 7.84 (t, *J* = 8.4Hz, 1H, Ar-H); 8.12 (s, 1H, Ar-H); 8.34 (s, 1H, C_4-H of coumarin); 9.17 (s, 1H, C_4-H Pyrazole); 9.74 (s, 1H, Thiazole-H); **^{13}C NMR (100 MHz, DMSO-d_6, ppm):** δ 56.73; 92.80; 115.92; 117.46; 120.40; 122.33; 126.31; 127.57; 130.28; 131.55; 131.90; 142.38; 147.14; 157.73; 169.49; *Anal*. Calcd for $C_{22}H_{14}Br_2N_4O_3S$: C, 46.01; H, 2.46, N, 9.76; S, 5.58%. Found: C, 46.05; H, 2.40; N, 9.80; S, 5.60%.

3-(2-(3-([1,1'-Biphenyl]-4-yl)-5-amino-1*H*-pyrazol-1-yl)thiazol-4-yl)-2*H*-chromen-2-one (37q):

IR (KBr, υ_{max} , cm^{-1}): 3332 (-NH_2); 1715 (C=O); 1605 (C=N); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 5.97 (s, 1H, C_4-H of Pyrazole), 6.96 (bs, 2H, -NH2); 7.39 – 7.49 (m,



5H, Ar-H); 7.67 (t, *J* = 8.4 Hz, 1H, Ar-H); 7.72 – 7.77 (m, 4H, Ar-H); 7.91 – 7.97 (m, 3H, Ar-H); 8.21 (s, 1H, C_4-H of coumarin); 9.03 (s, 1H, thiazole-H); **^{13}C NMR (100 MHz, DMSO-d_6, ppm):** δ 85.20; 115.44; 115.87; 119.22; 119.56; 124.68; 126.18; 126.53; 126.85; 127.58; 128.94; 129.05; 131.16; 131.95; 139.55; 139.71; 140.21; 144.55; 149.44; 152.48; 152.94; 158.70; 161.64; **MS (ES m/z):** 463 [M +H]$^+$; *Anal*. Calcd for $C_{27}H_{18}N_4O_2S$: C, 70.11; H, 3.92; N, 12.11; S, 6.93%; Found: C, 70.08; H, 3.96; N, 12.15; S, 6.89%.

Anticancer activity evaluation of 3-(2-(5 amino-3-phenyl-1*H*-pyrazol-1-yl)thiazol-4-yl)-2*H*-chromen-2-ones by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide [MTT] assay:

The novel 3-(2-(5 amino-3-phenyl-1*H*-pyrazol-1-yl)thiazol-4-yl)-2*H*-chromen-2-ones were screened by using standard MTT protocol in a 96 well plate for their anticancer activity against a panel of five human cancer cell lines viz. Murine leukemia (L1210), human T-lymphocyte cells (CEM), human cervix carcinoma cells (HeLa), prostate cancer (DU-145) and breast cancer cancer (MCF-7) cell lines. All the five cancer cell lines were procured from American Type Culture Collection (ATCC) and cells were grown in Dulbecco's modified Eagle medium (DMEM) with 10% fatal bovine serum (FBS) supplemented with 1% antibiotics and incubated at 37 °C in a humidified incubator with 5% CO_2. All the synthesized compounds were dissolved in cell culture grade dimethyl sulphoxide solution. The five cancerous cell lines were taken in a 96 well tissue culture plate (10^4 cells/ each well) and the cells were treated with different concentrations of synthesized compounds and incubated for 48 h. Finally the medium was removed, washed with Dulbecco's phosphate buffer saline (DPBS) and treated with 10 µL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) solution in 200 µL of culture medium. The MTT solution treated cells were incubated for 1 h and absorbance was measured at 562 nm using spectrophotometer. The absorbance of the treated cells was compared with control. The MTT assay was performed in triplicate and the results were shown as percentage reduction in cell viability.

Cell cycle analysis:

The (DU-145) was treated with the active compounds of 4b, 4c, 4j, 4k, and 4l at 10 µM concentration and incubated for about 24 h. The DU-145 cancer cells were harvested with trypsin EDTA and fixed in ice cold ethanol solvent at 4 °C for about 30 minutes. The ethanol was removed and cells were treated with 1 mL of DNA staining solution (2 mg of RNase and 0.2 mg of propidium iodide (PI)) for 30 minutes. The DNA content was measured by flow cytometry (BD FACS C anto II). Histograms were plotted and the results were analyzed using FCS express 4 plus.

Hoechst-33258 staining:

The DU-145 cancer cell line was treated with the potent molecules of 4b, 4c, 4j, 4k, and 4l at 10 µM concentration and incubated for 24 h. After 24 h incubation the medium was aspirated and washed without FBS. Finally the DU-145 cells were stained with Hoechst-33258 for 20 min at 37 °C under humidified atmosphere. Cells were washed again with medium. DU-145 cells were covered with medium and observed under a fluorescence microscope equipped with DAPI filter.

Docking study:

The docking studies were carried out using Schrodinger suite 2010 and binding energy values were calculated using Prime MM/GBSA module which is available in the same suite in order to understand possible mode of interactions and find a correlation between anticancer activity of 3-(2-(5-amino-3-phenyl-1*H*-pyrazol-1-yl)thiazol-4-yl)-2*H*-chromen-2-ones and their binding with target Epidermal growth factor receptor (5EM8.pdb). The importance of receptor ligand interactions in inhibiting the function of EGFR and binding affinities of newly synthesized coumarin derivatives were identified after docking studies.

We have taken the crystal structure of EGFR with PDB ID: 5EM8 from the protein data bank[61]. Initially the protein obtained from protein data bank was prepared, refined and minimized using OPLS-AA force field. Later receptor grid was generated using GLIDE 5.6 and all the low energy conformers of prepared ligands were docked into the active site using extra precision docking mode as recommended by Schrodinger. During grid generation the receptor van der Waals[62] scaling for the non-polar atoms was set to 0.9 with a partial charge cut-off of 0.25. The pose viewer file that was obtained after docking was taken as such to calculate the binding affinity values. The similarity

between co-crystallized ligand and the best active compound was established by phase shape program available in the Schrodinger suite where similarity is computed based on overlapping hard sphere volumes. Default parameters were applied in shape screening panel while running phase shape program.

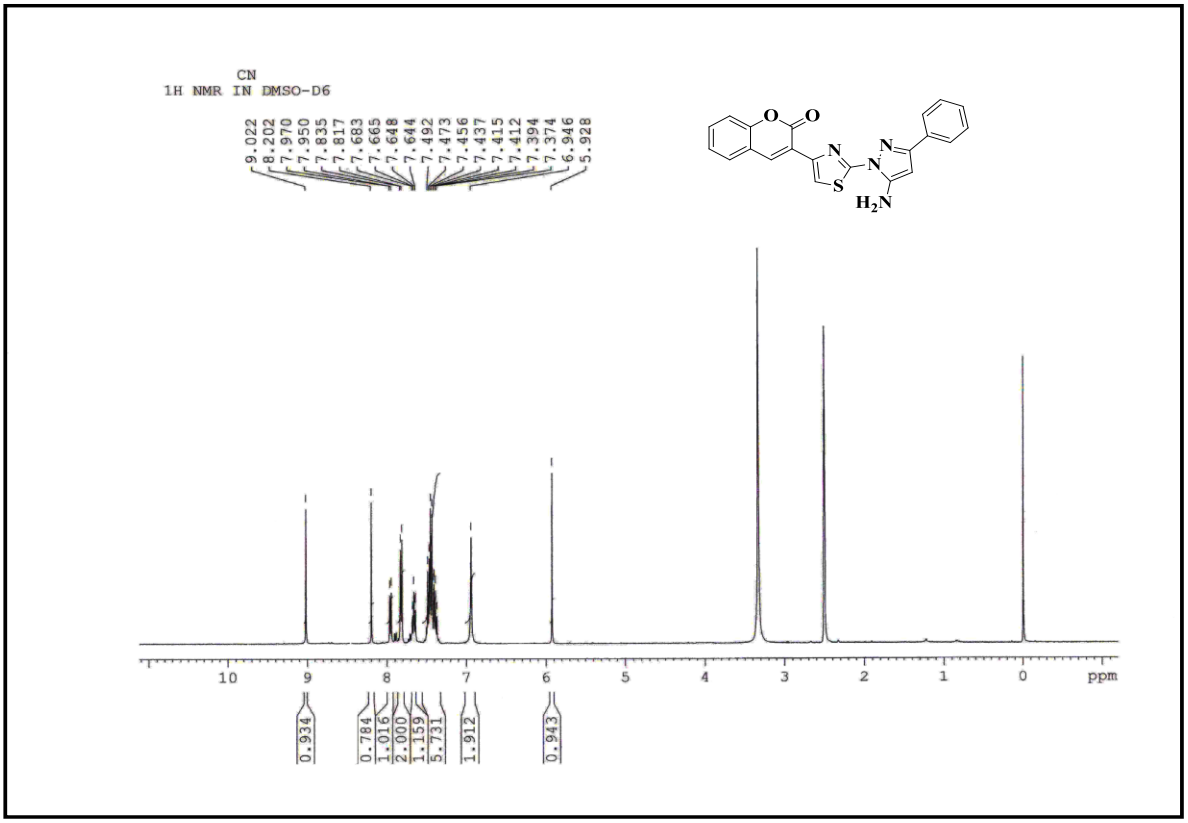


Fig: [1]H NMR spectrum of compound **37a**

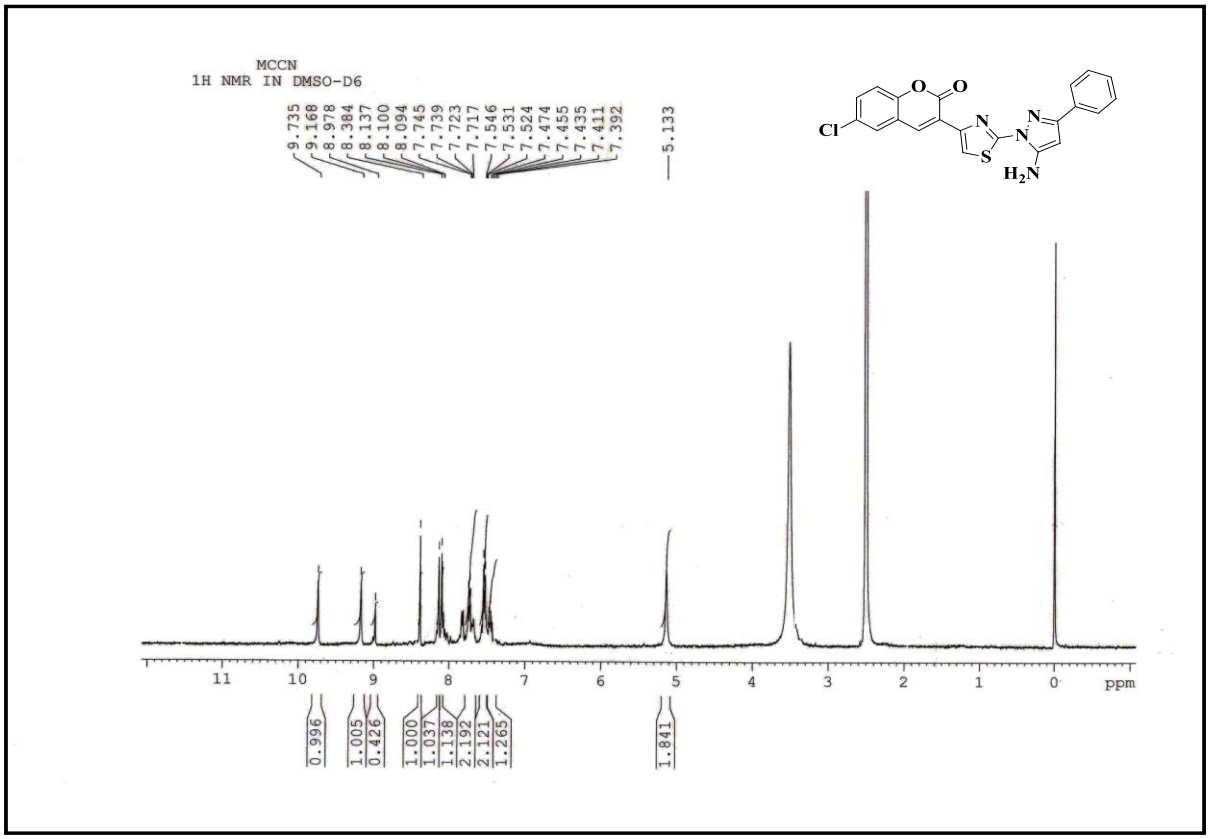


Fig: [1]H NMR spectrum of compound **37b**

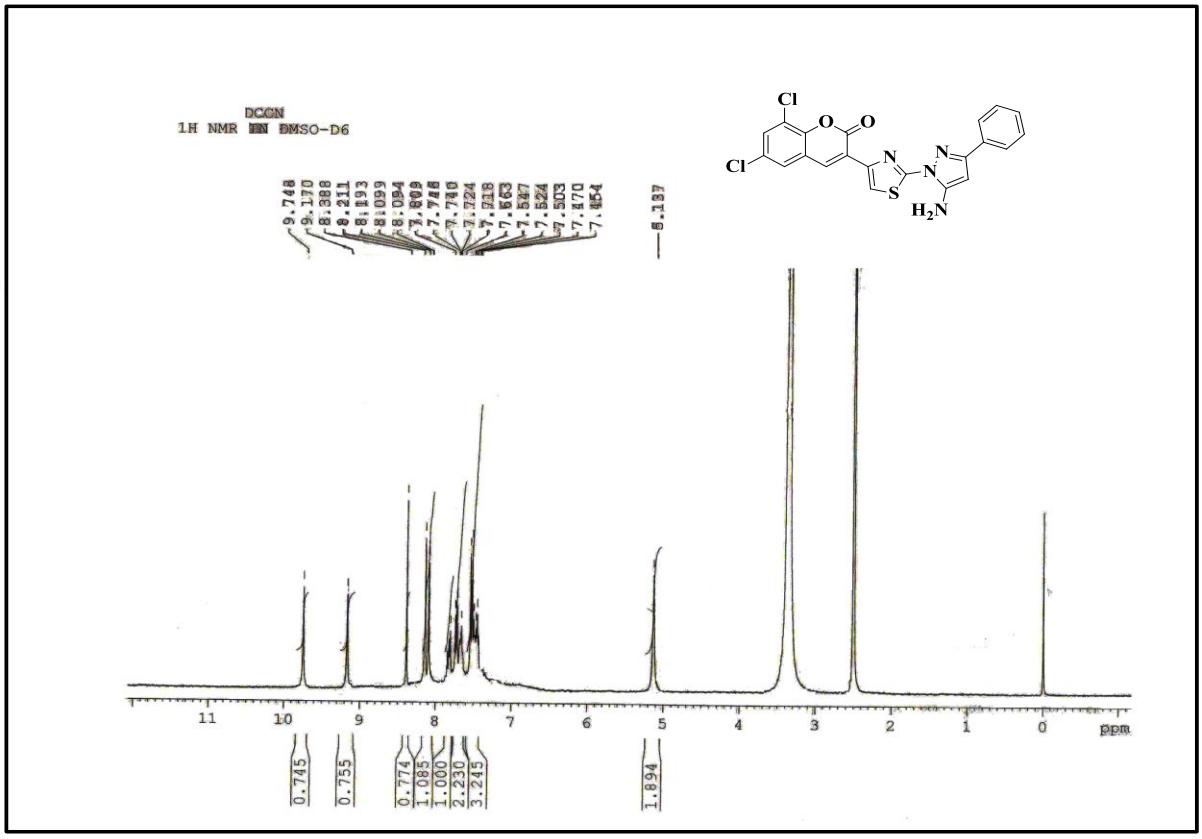


Fig: ^{1}H NMR spectrum of compound **37c**

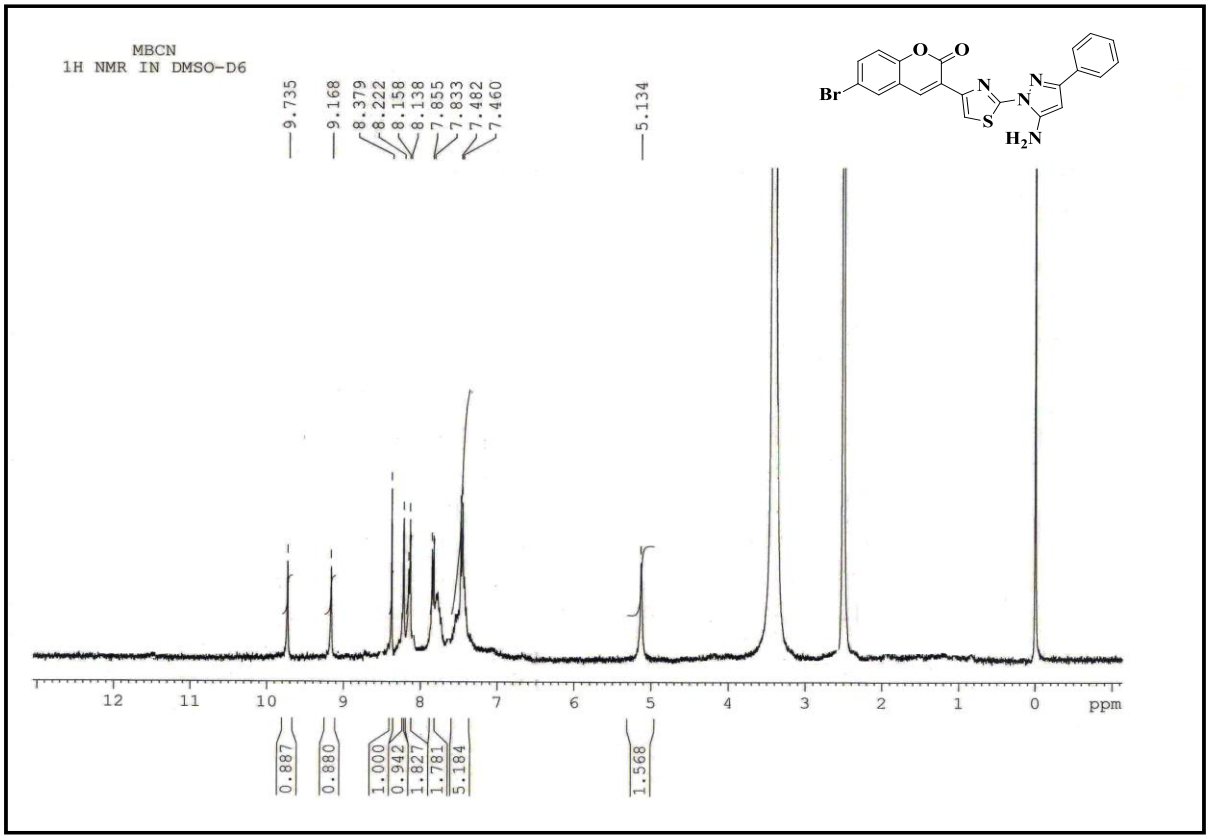


Fig: ^{1}H NMR spectrum of compound **37d**

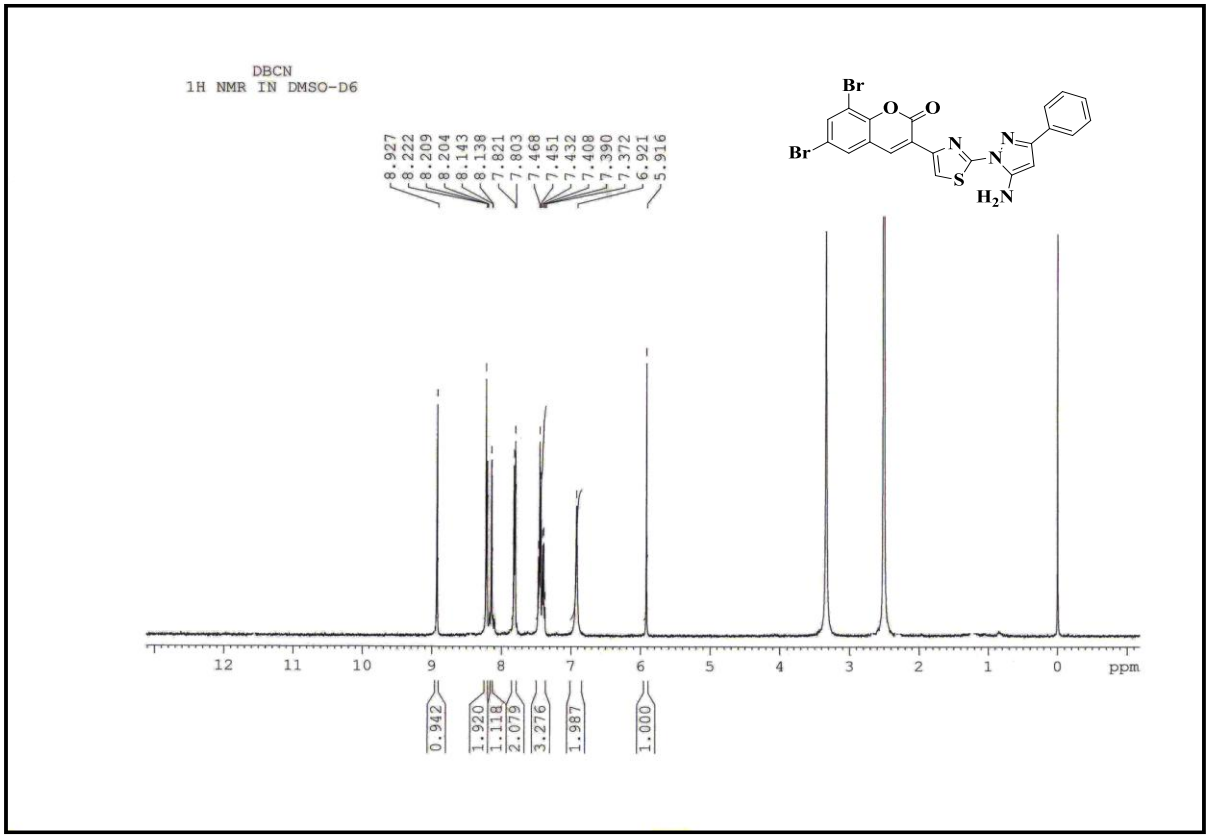


Fig: ^{1}H NMR spectrum of compound **37e**

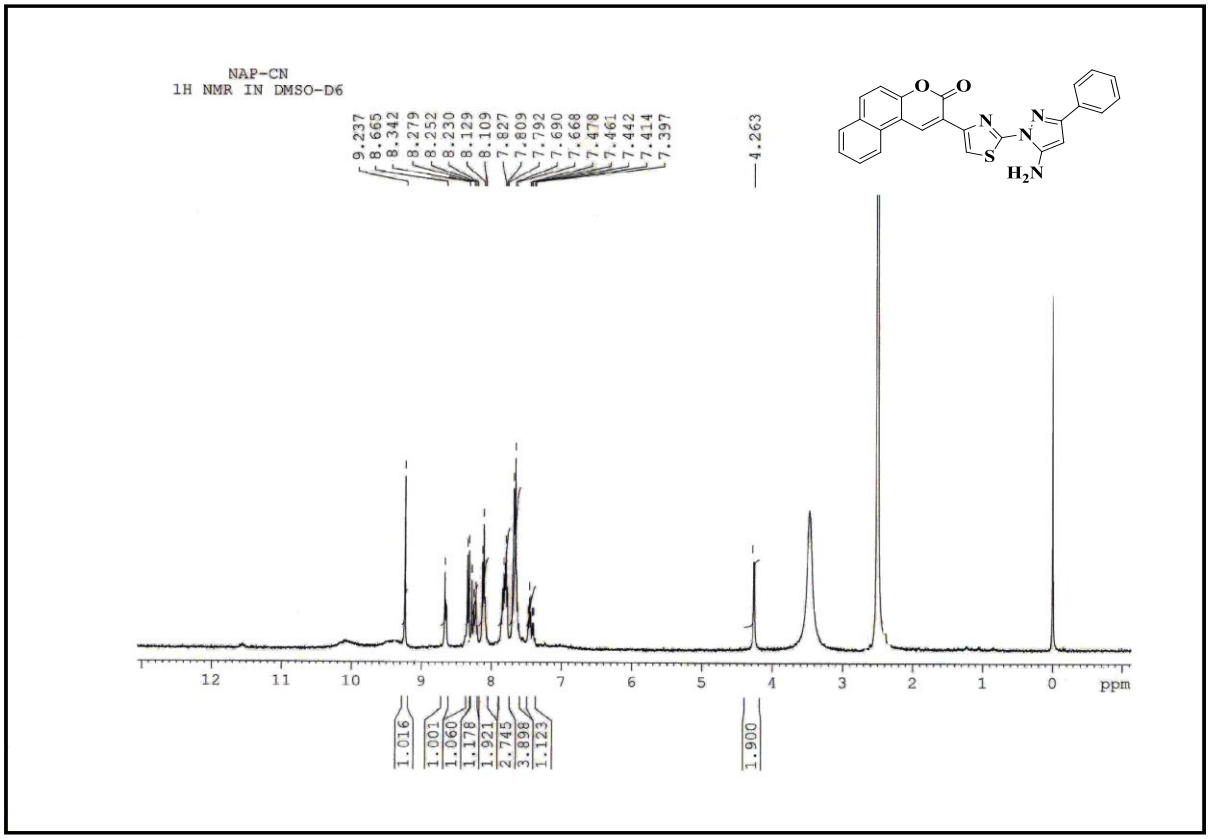


Fig: ^{1}H NMR spectrum of compound **37f**

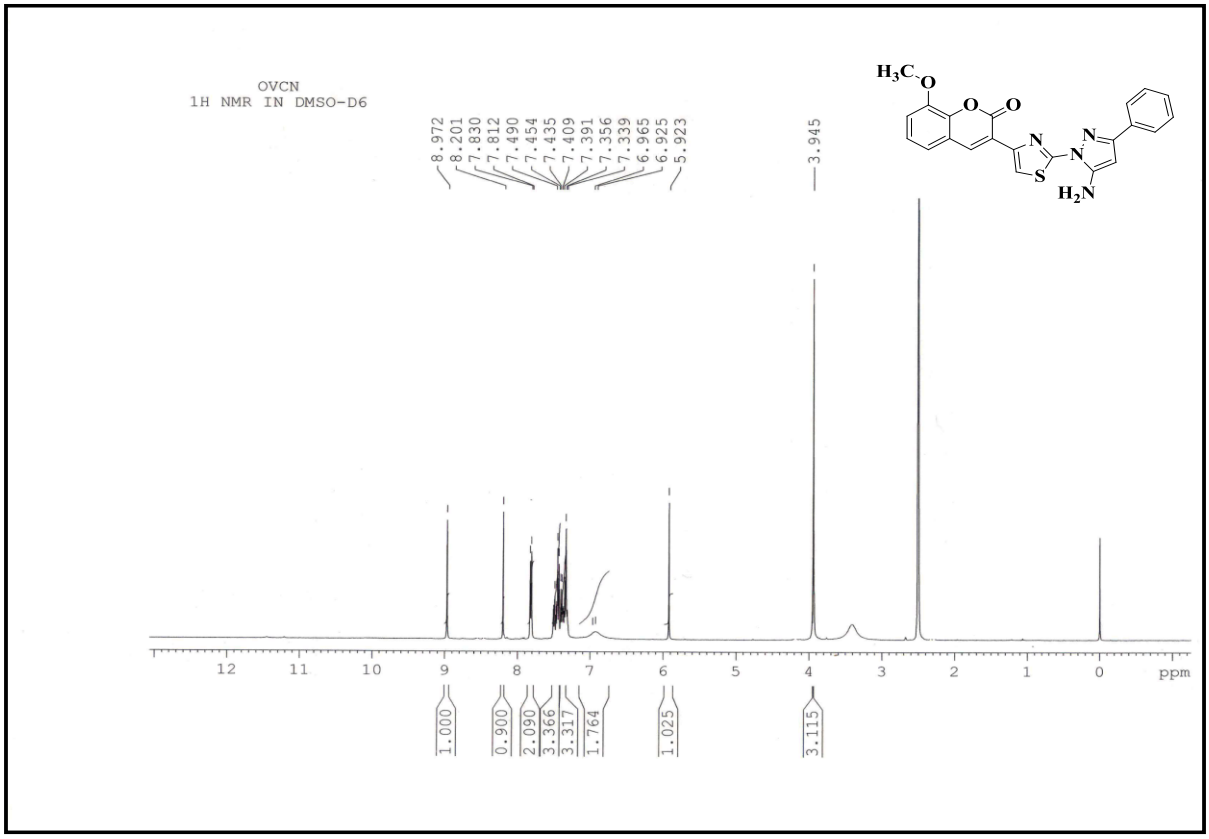


Fig: ^{1}H NMR spectrum of compound **37g**

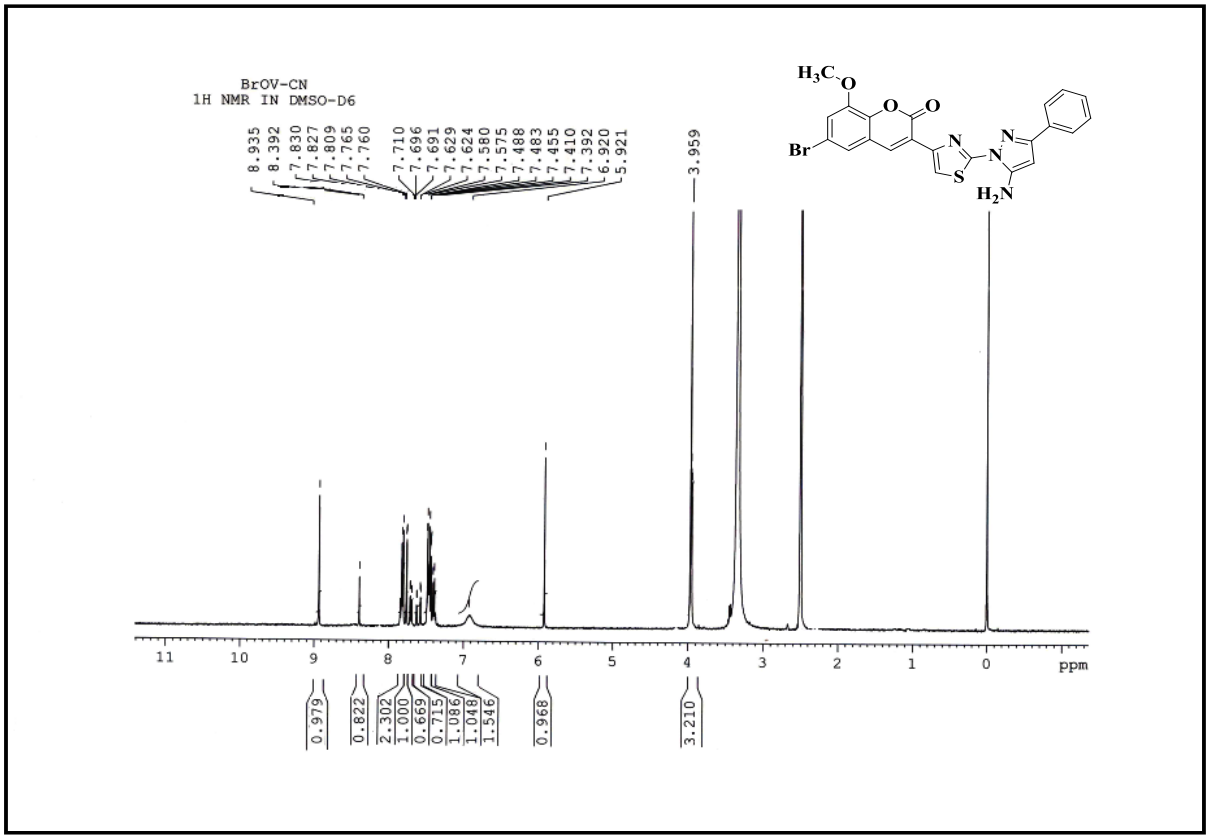


Fig: ^{1}H NMR spectrum of compound **37h**

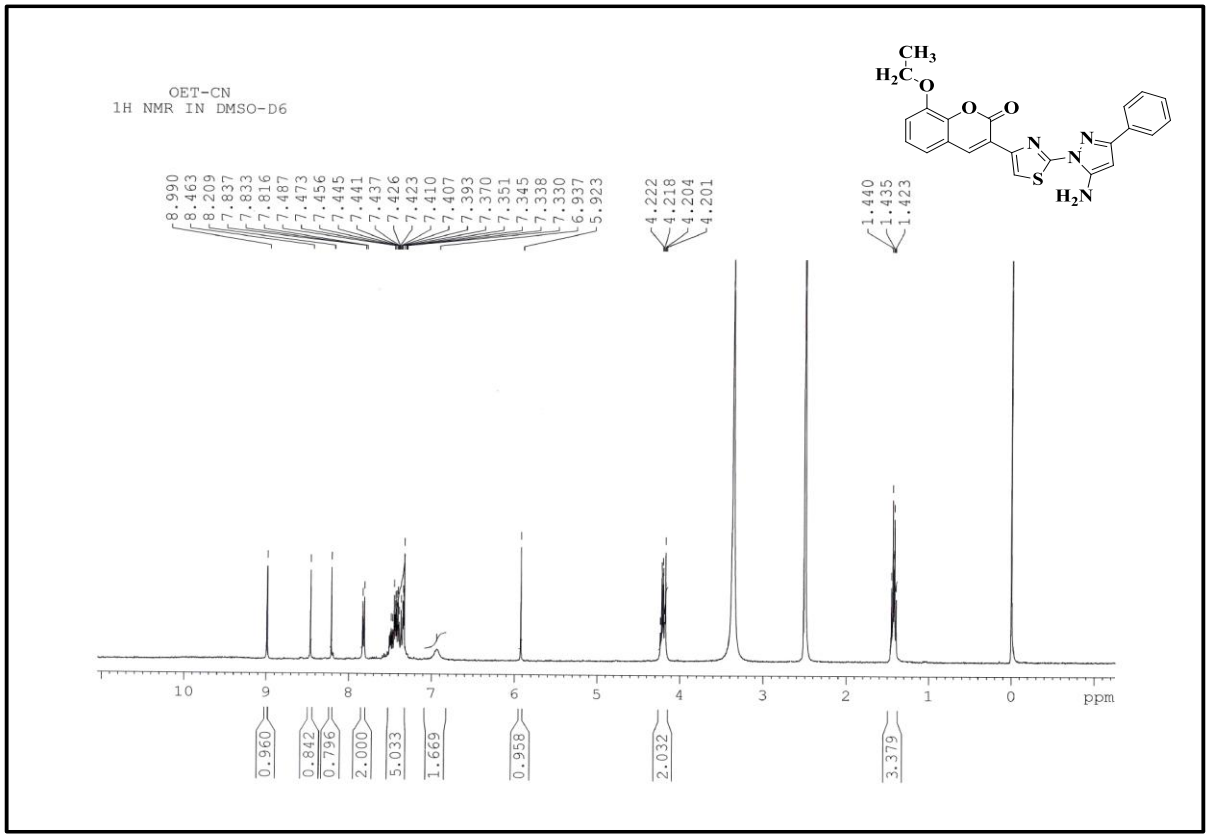


Fig: ^{1}H NMR spectrum of compound **37i**

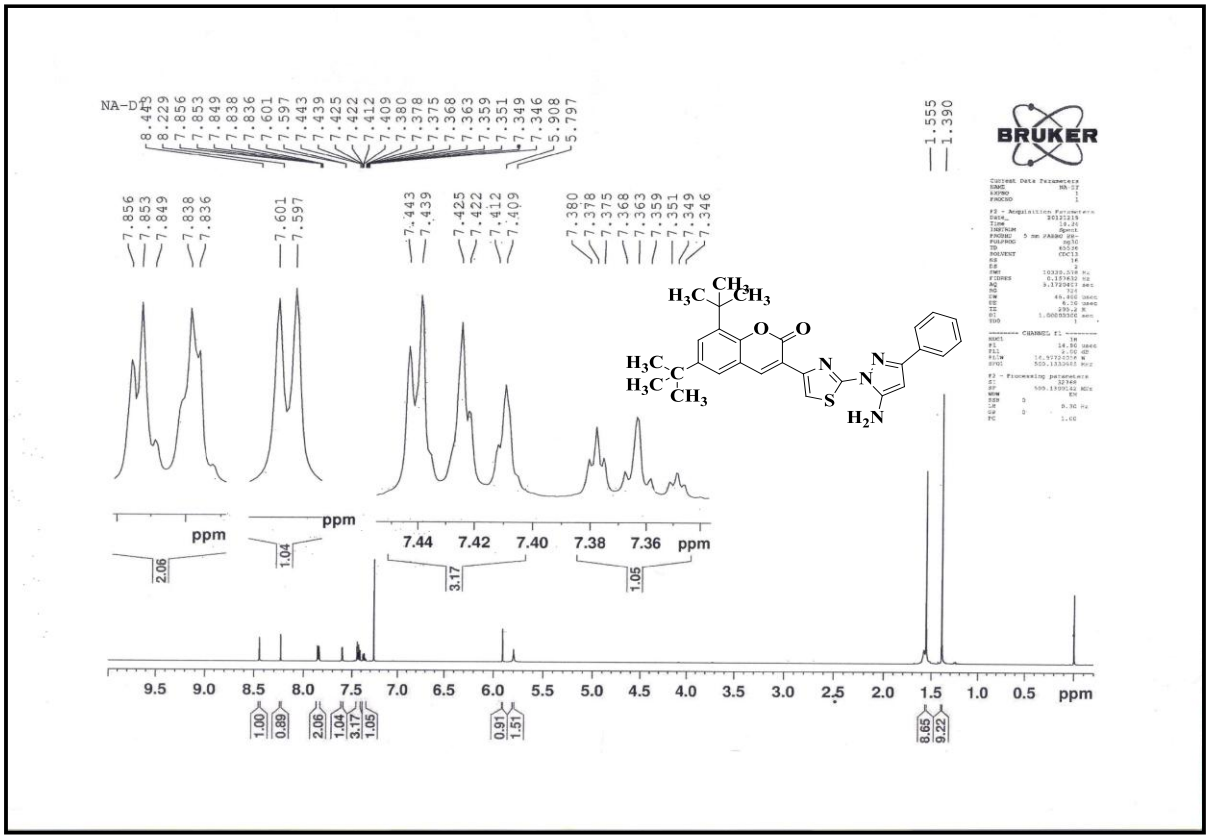


Fig: ^{1}H NMR spectrum of compound **37j**

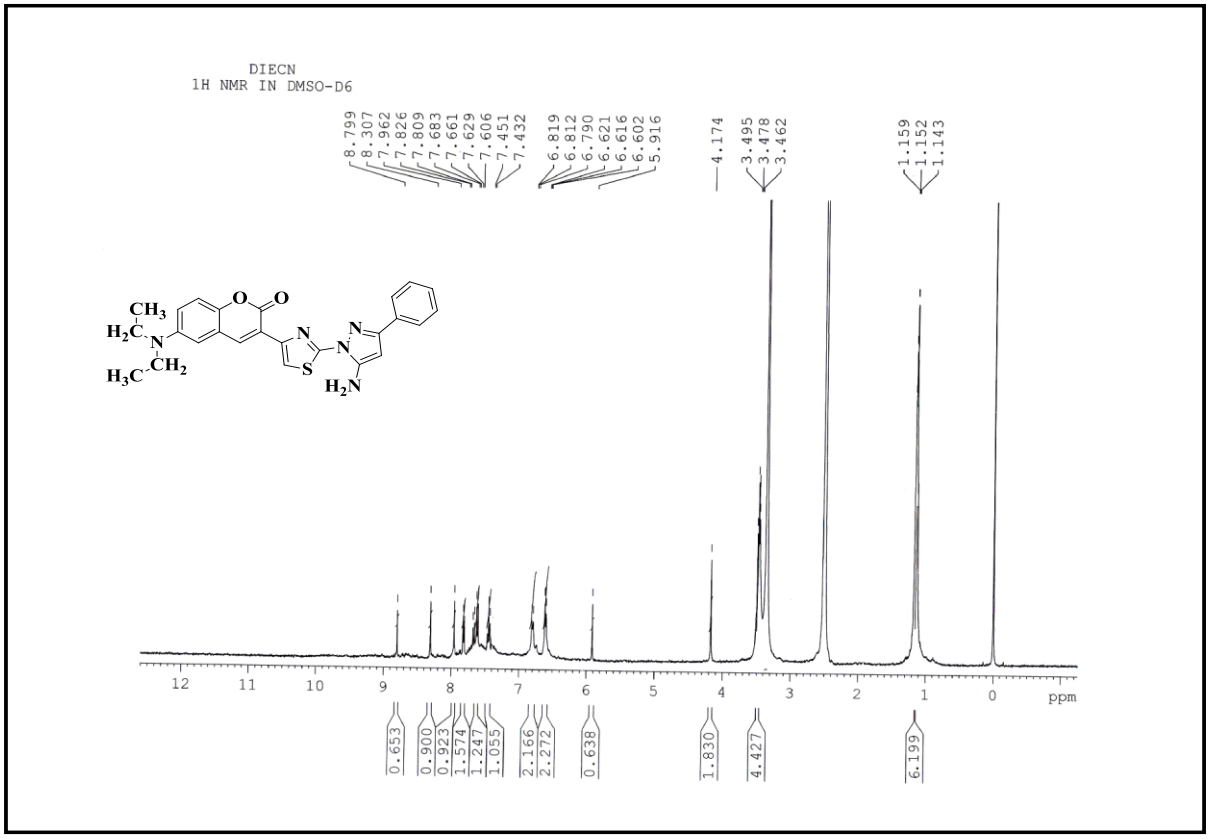


Fig: ^{1}H NMR spectrum of compound **37k**

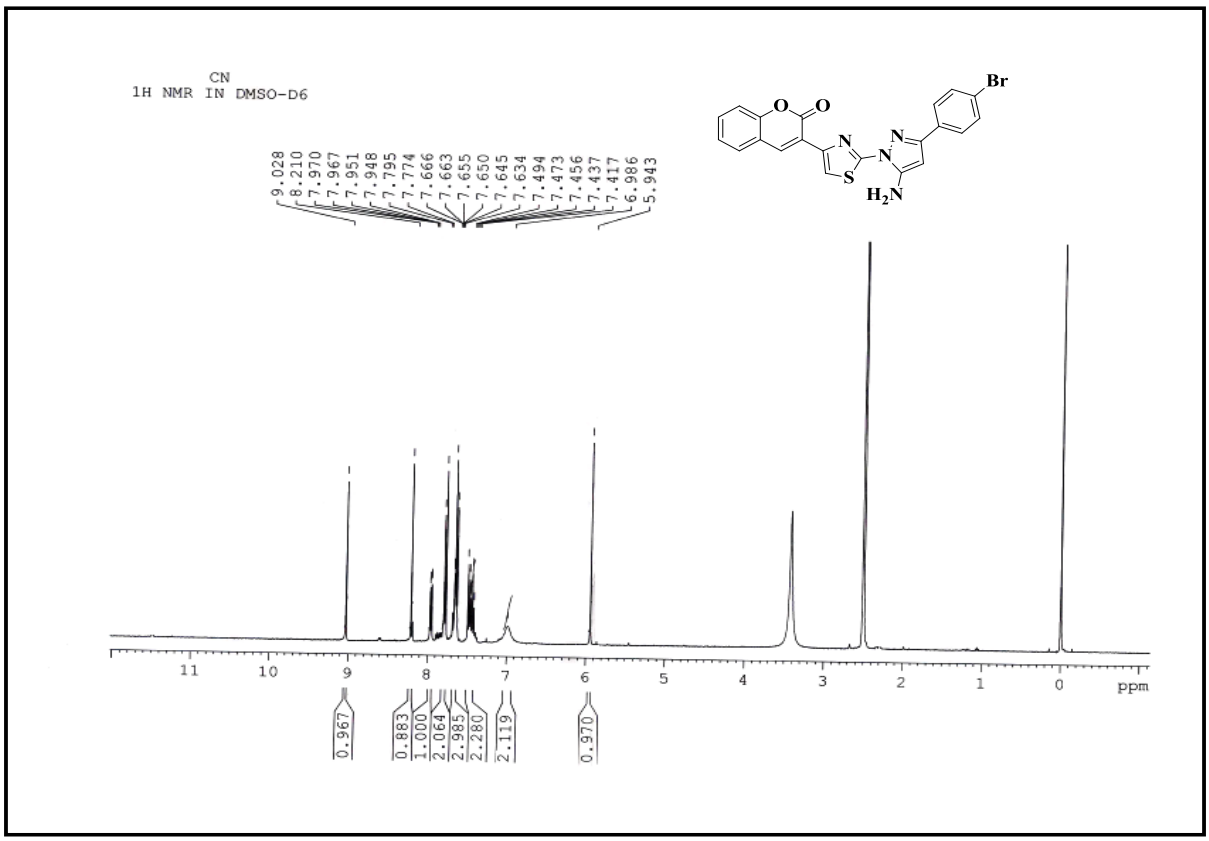


Fig: ^{1}H NMR spectrum of compound **37l**

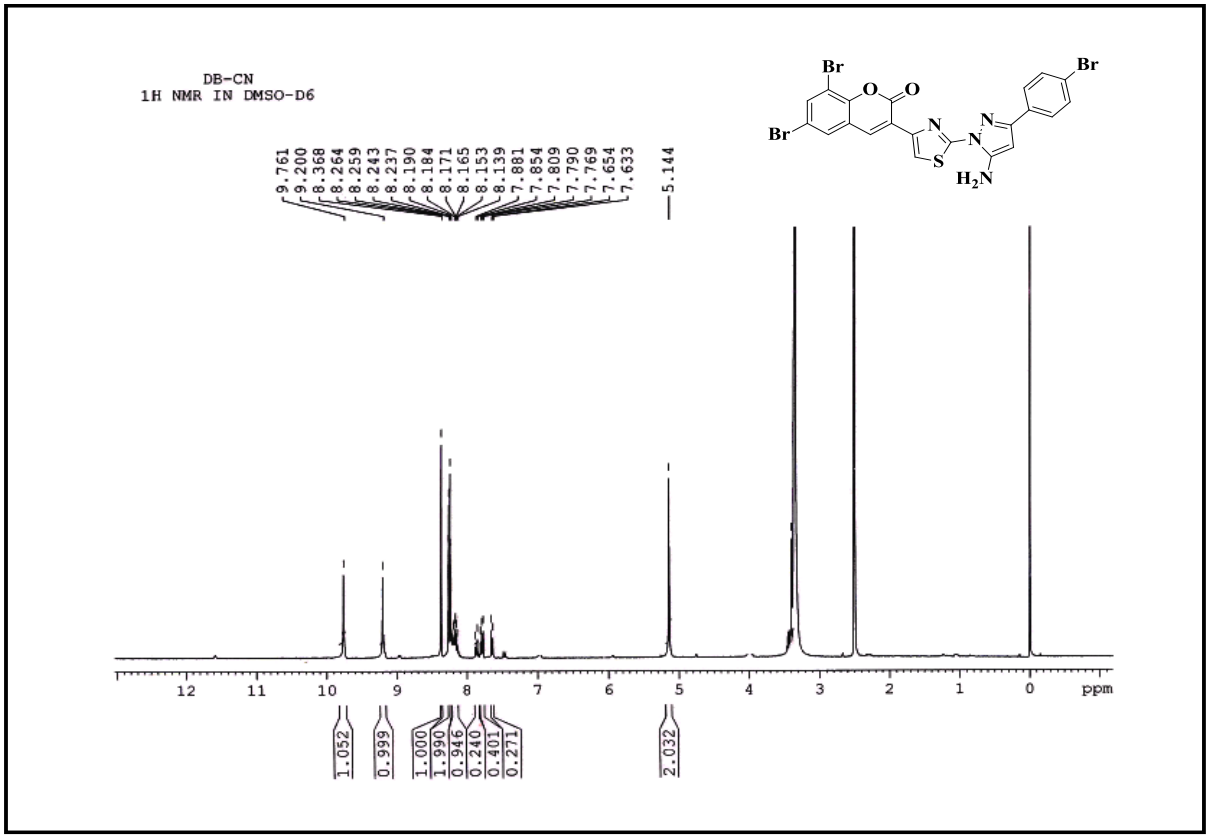


Fig: ^{1}H NMR spectrum of compound **37m**

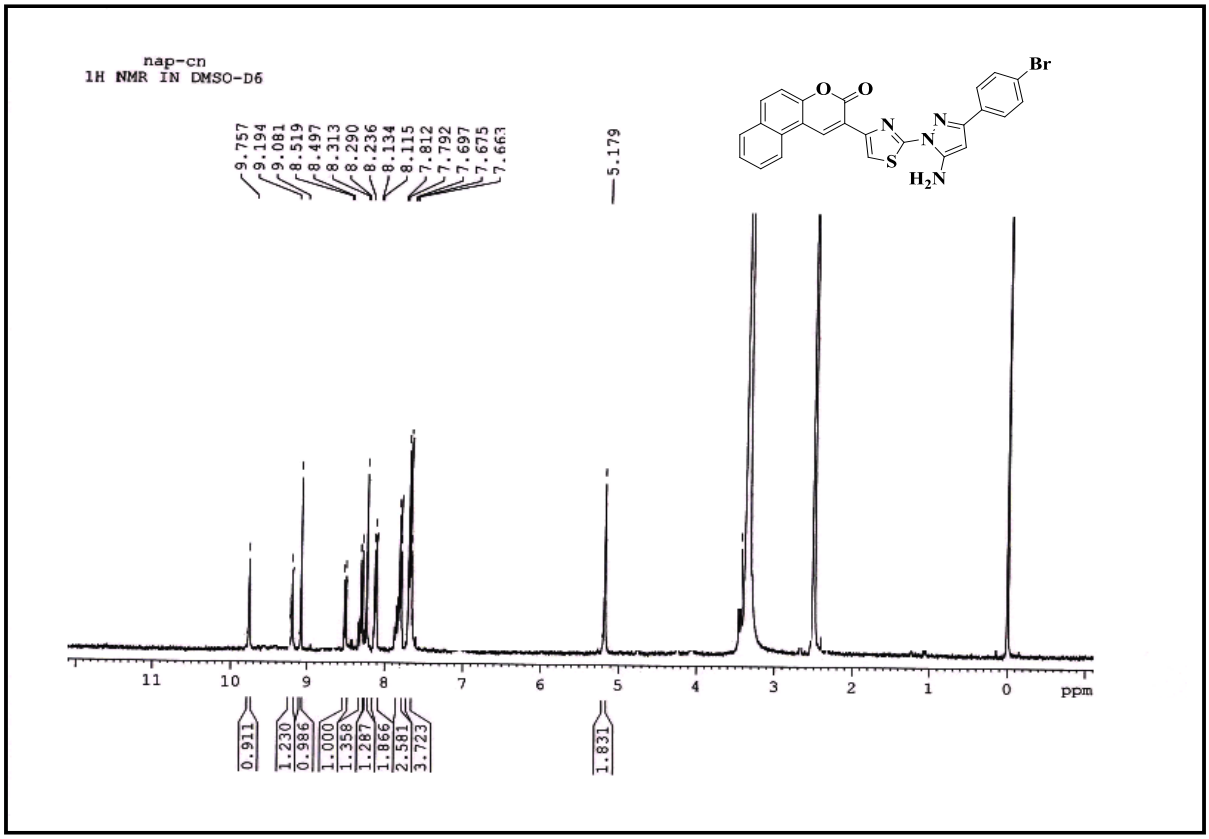


Fig: ^{1}H NMR spectrum of compound **37n**

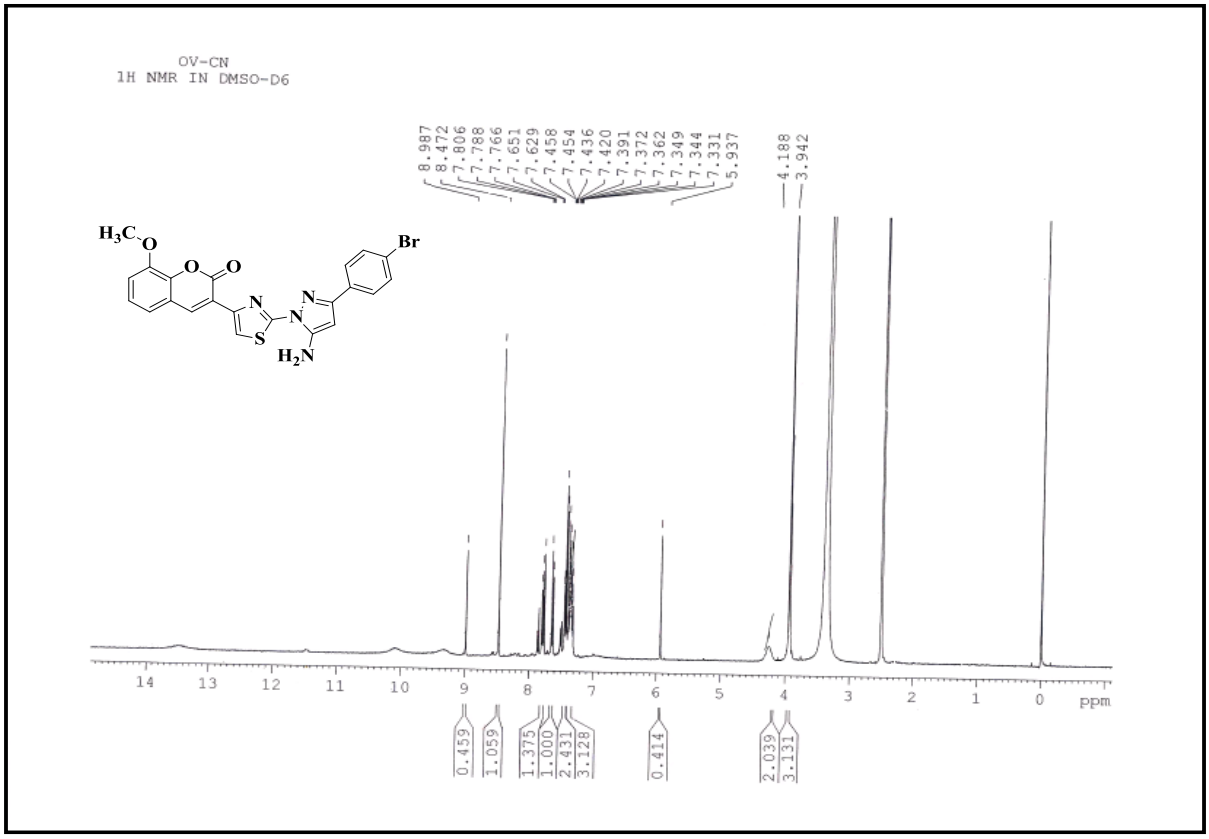


Fig: ^{1}H NMR spectrum of compound **37o**

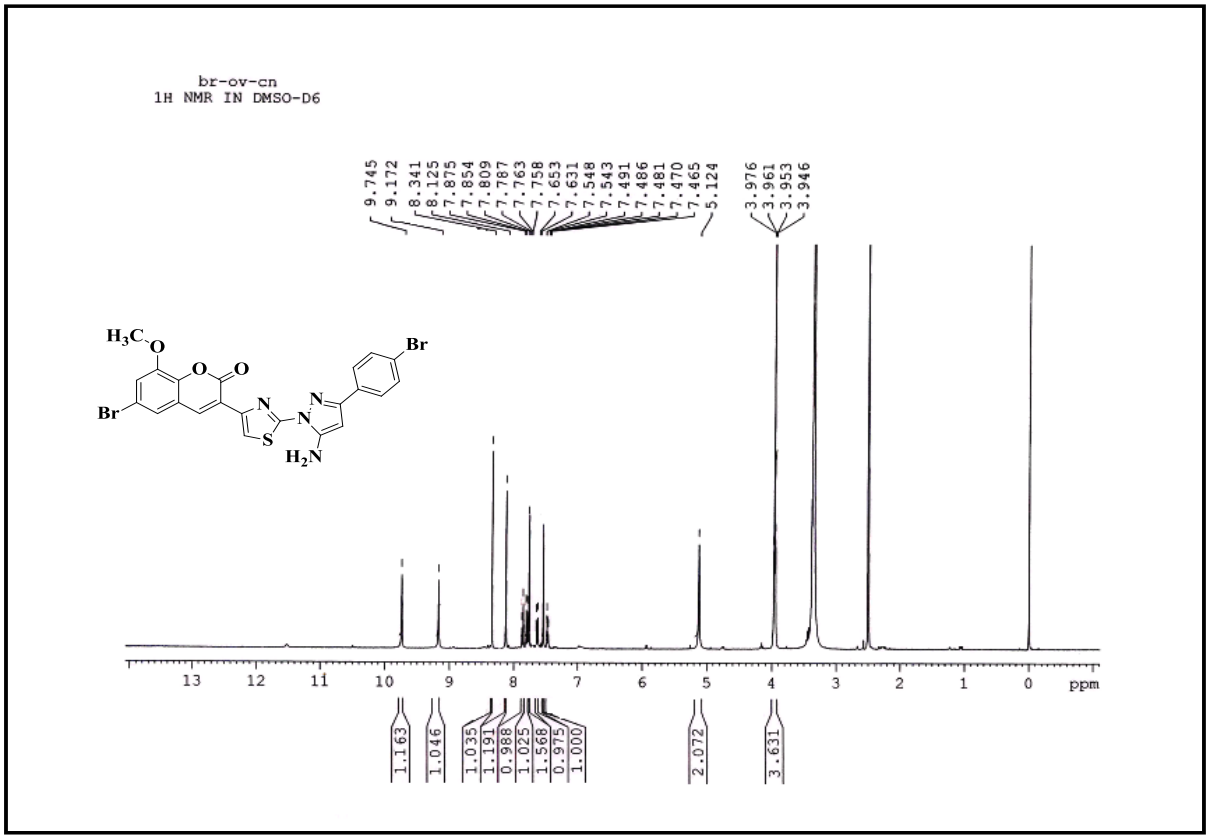


Fig: ^{1}H NMR spectrum of compound **37p**

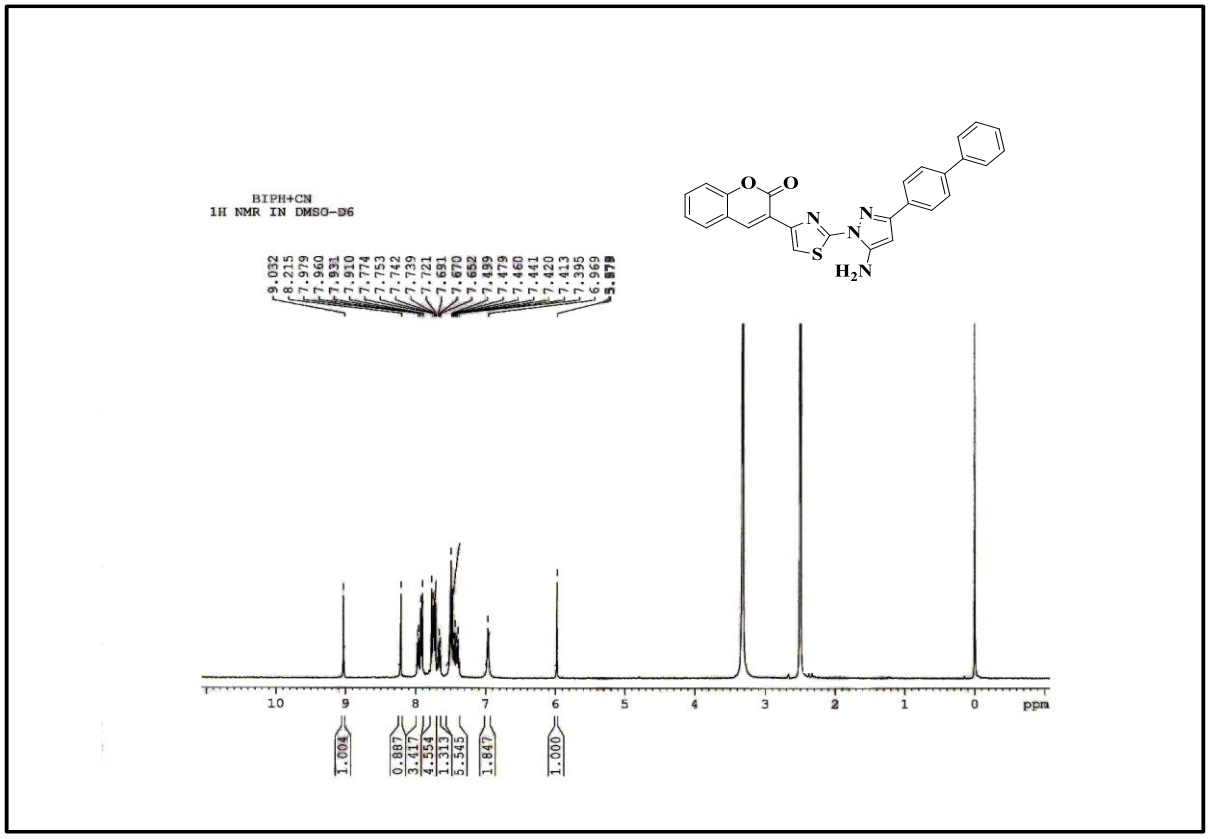


Fig: ^{1}H NMR spectrum of compound **37q**

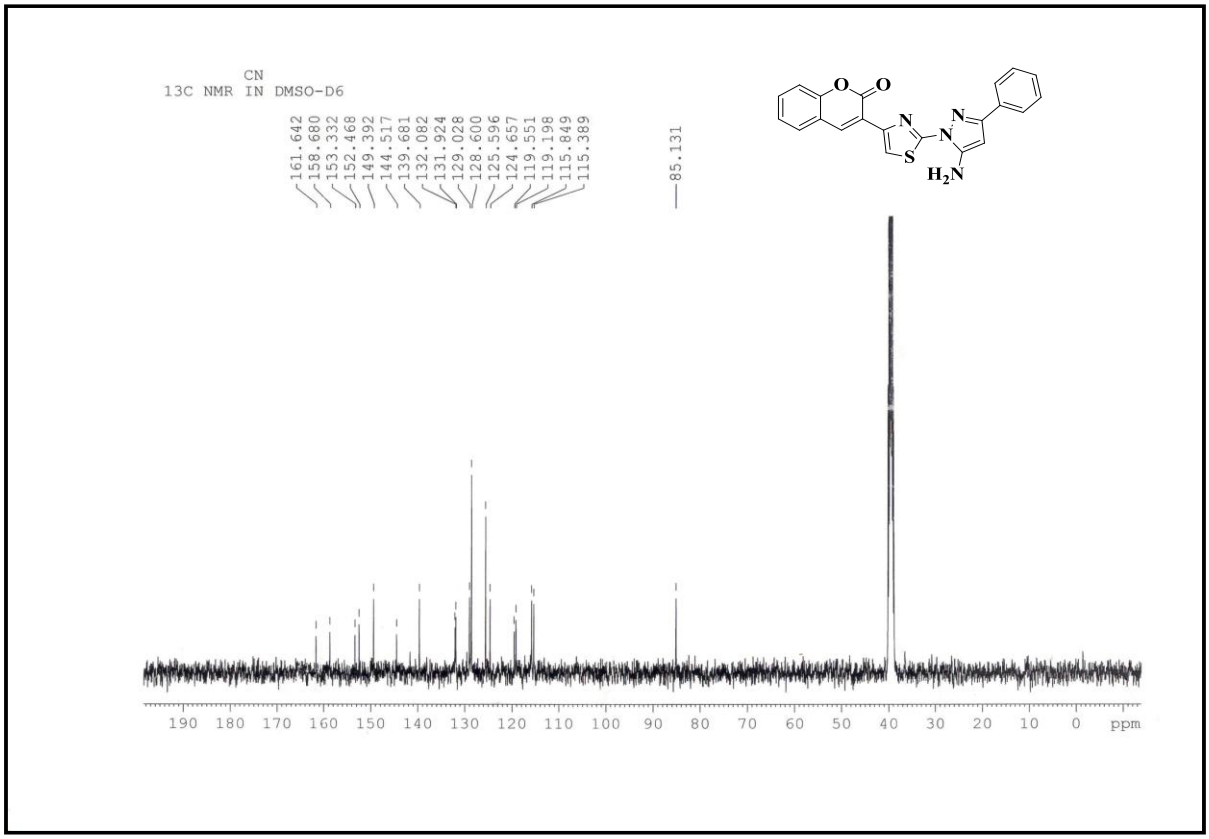


Fig: ^{13}C NMR spectrum of compound **37a**

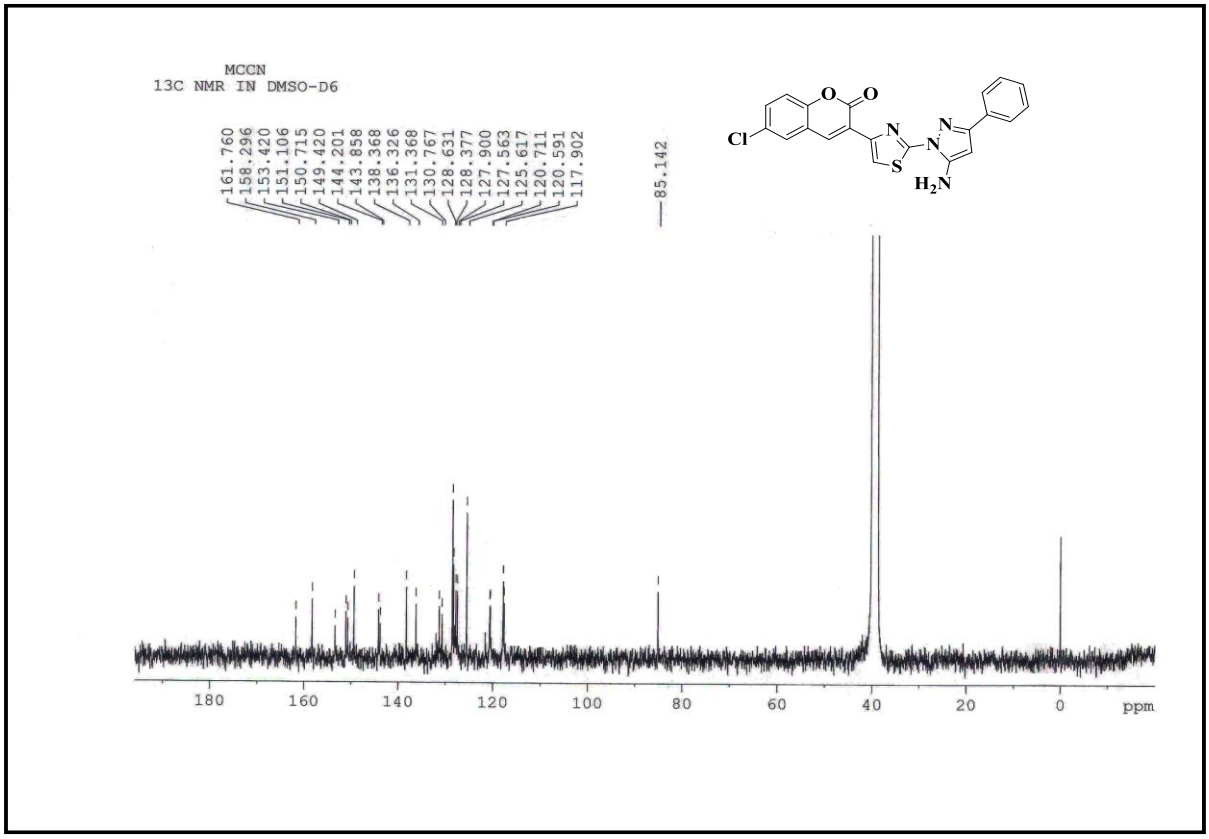


Fig: ^{13}C NMR spectrum of compound **37b**

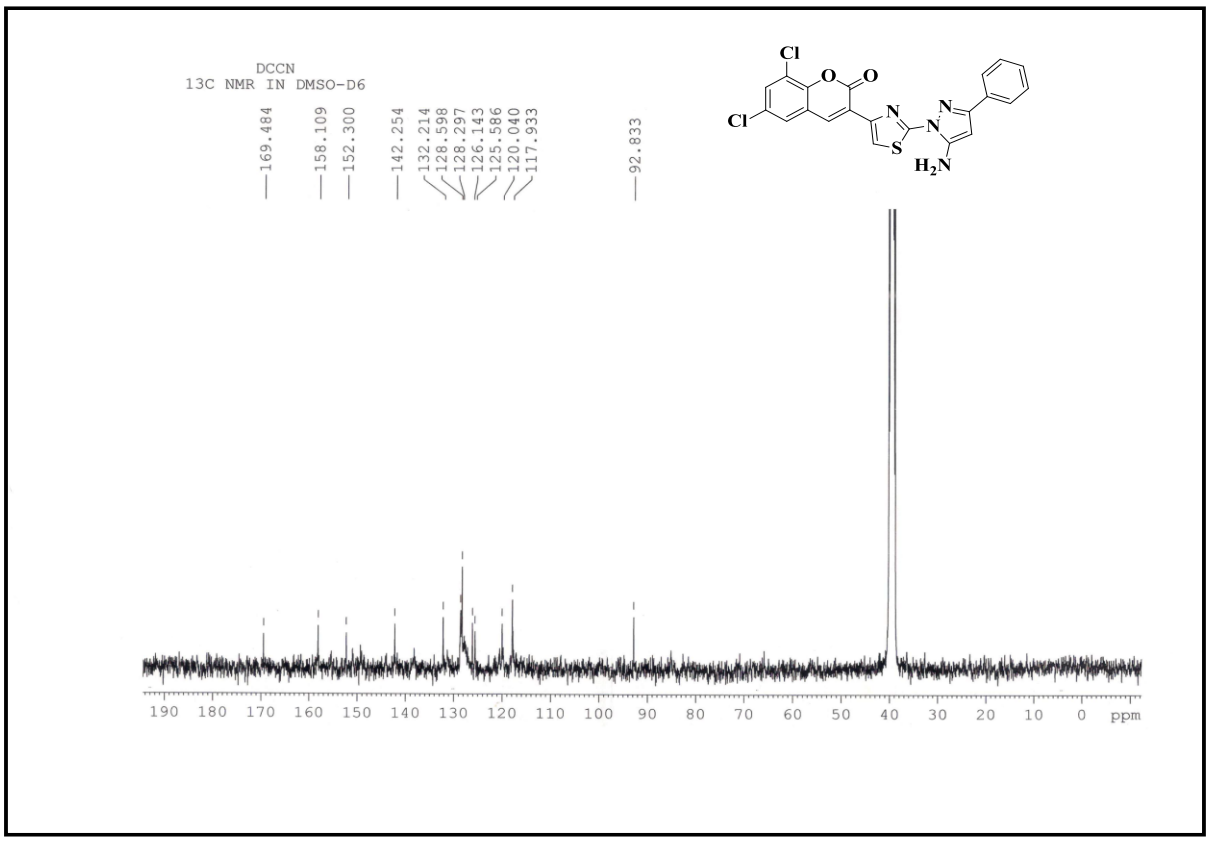


Fig: ^{13}C NMR spectrum of compound **37c**

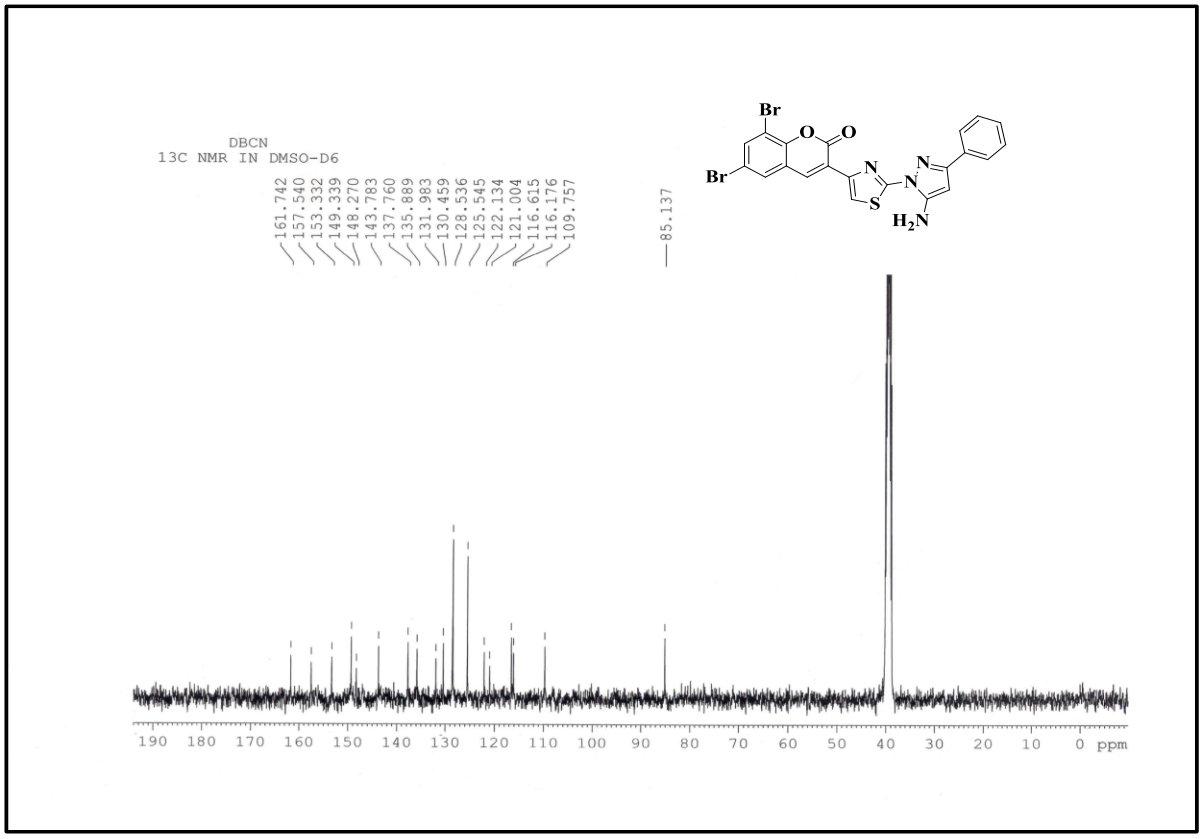


Fig: ^{13}C NMR spectrum of compound **37e**

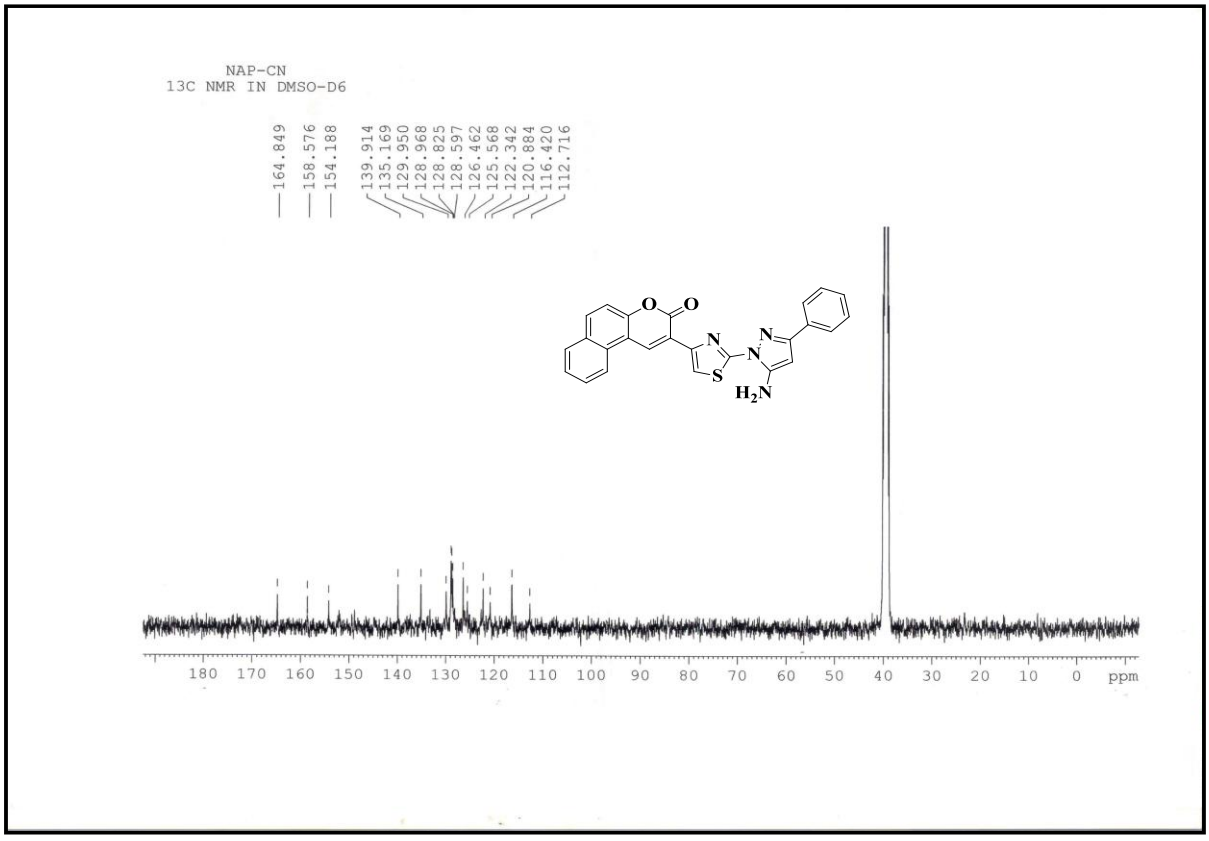


Fig: ^{13}C NMR spectrum of compound **37f**

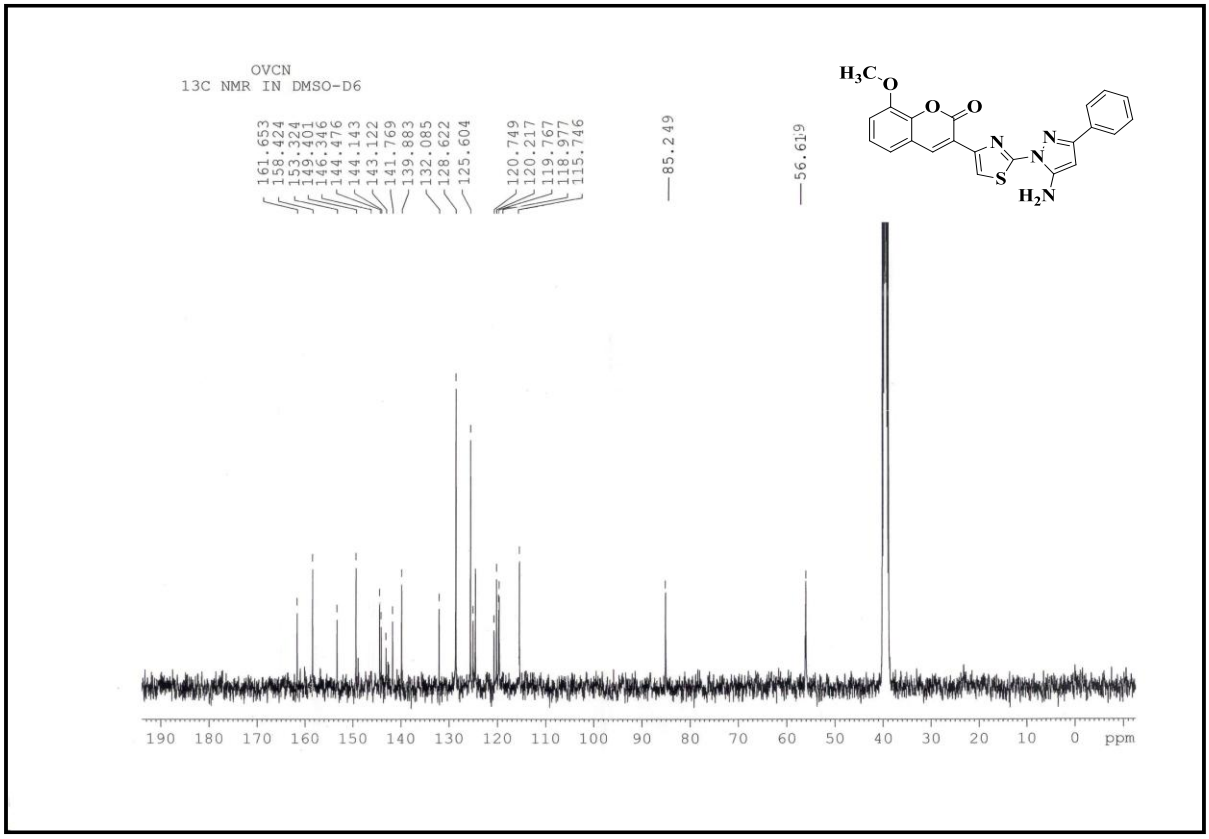


Fig: ^{13}C NMR spectrum of compound **37g**

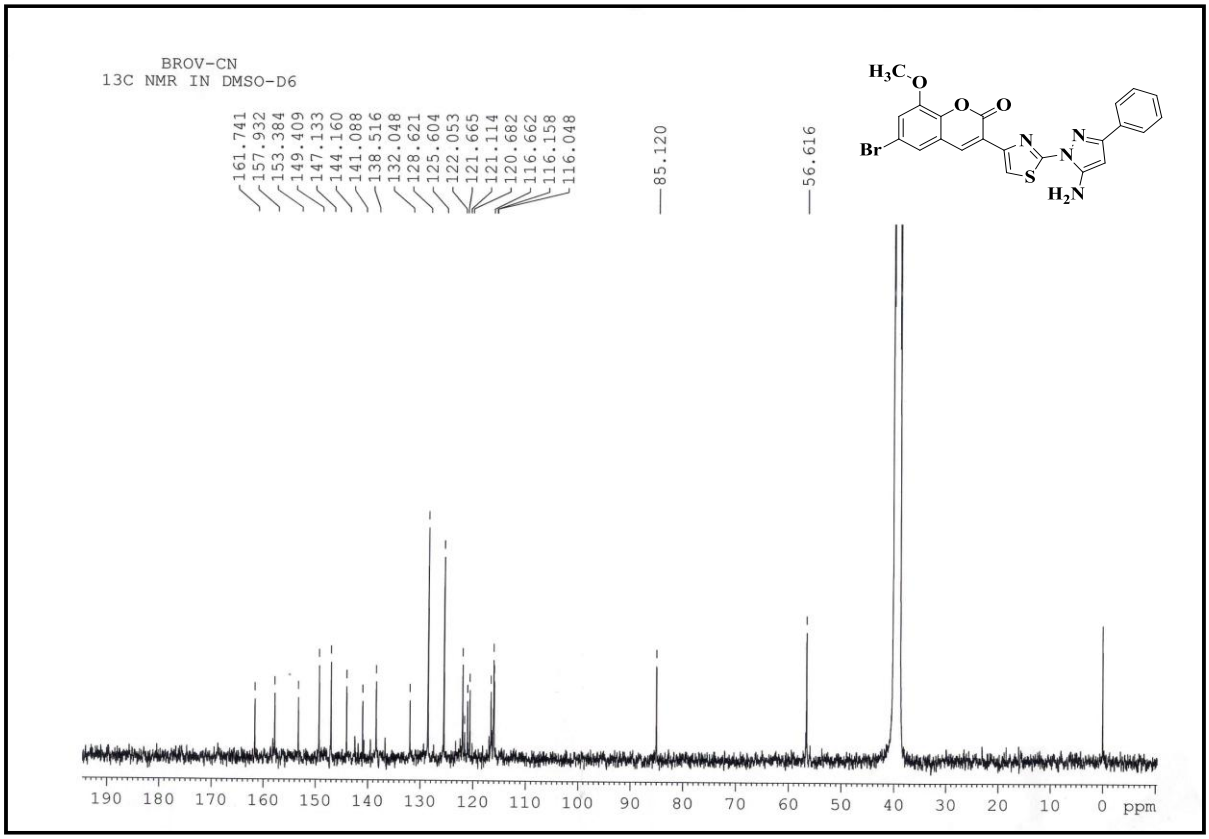


Fig: ^{13}C NMR spectrum of compound **37h**

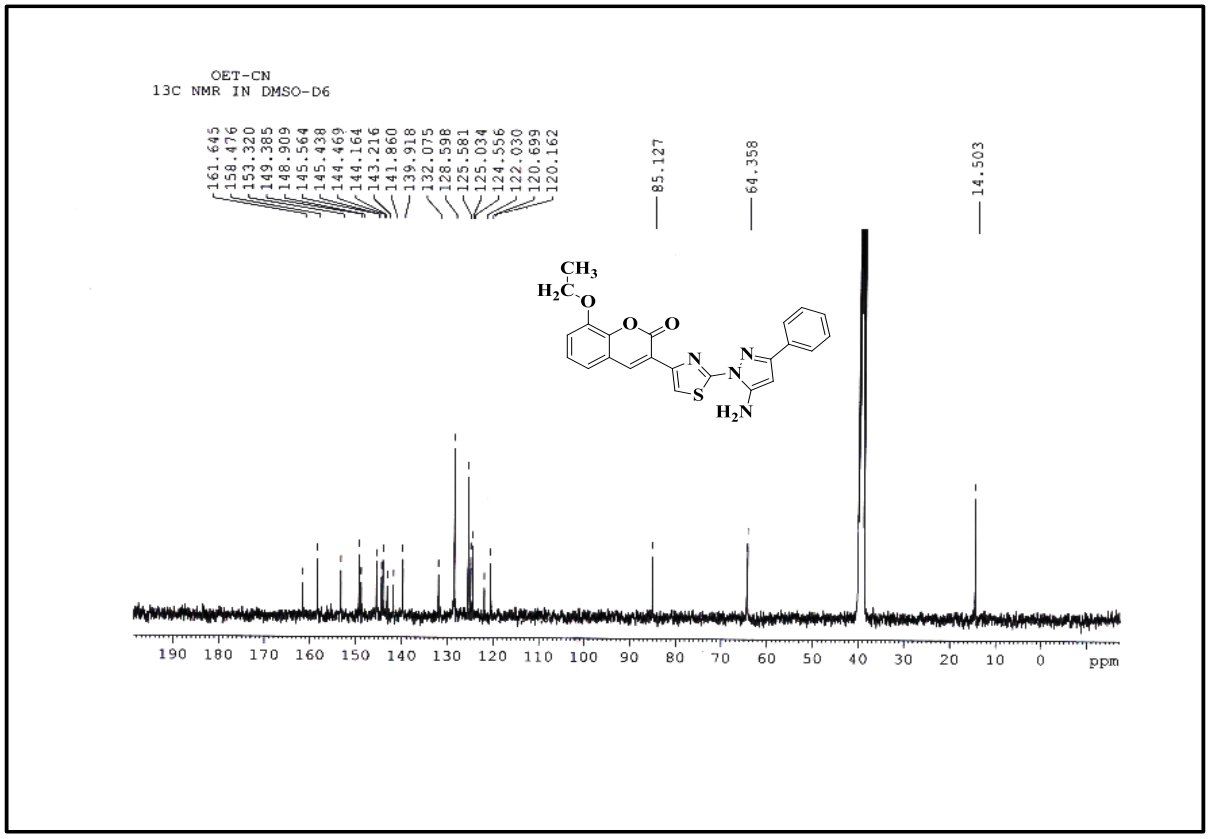


Fig: ^{13}C NMR spectrum of compound **37i**

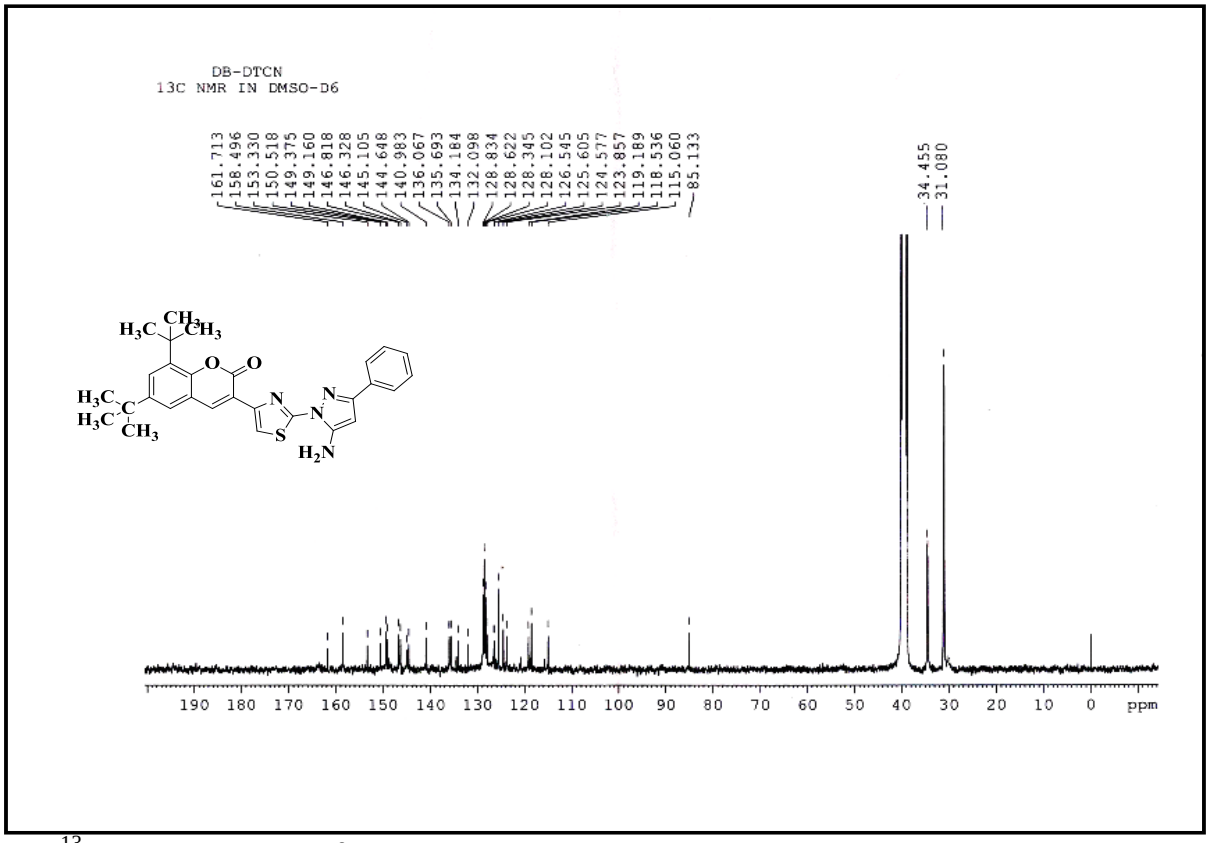


Fig: ^{13}C NMR spectrum of compound **37j**

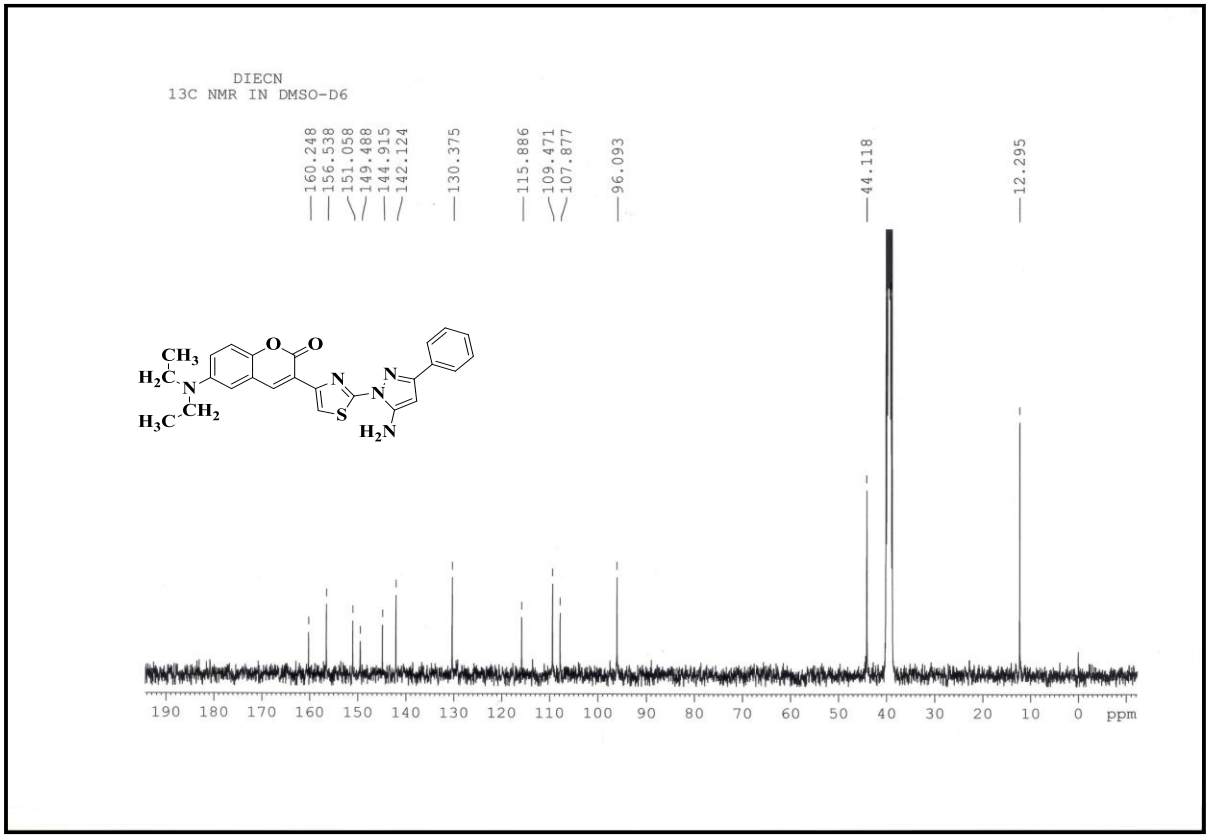


Fig: ^{13}C NMR spectrum of compound **37k**

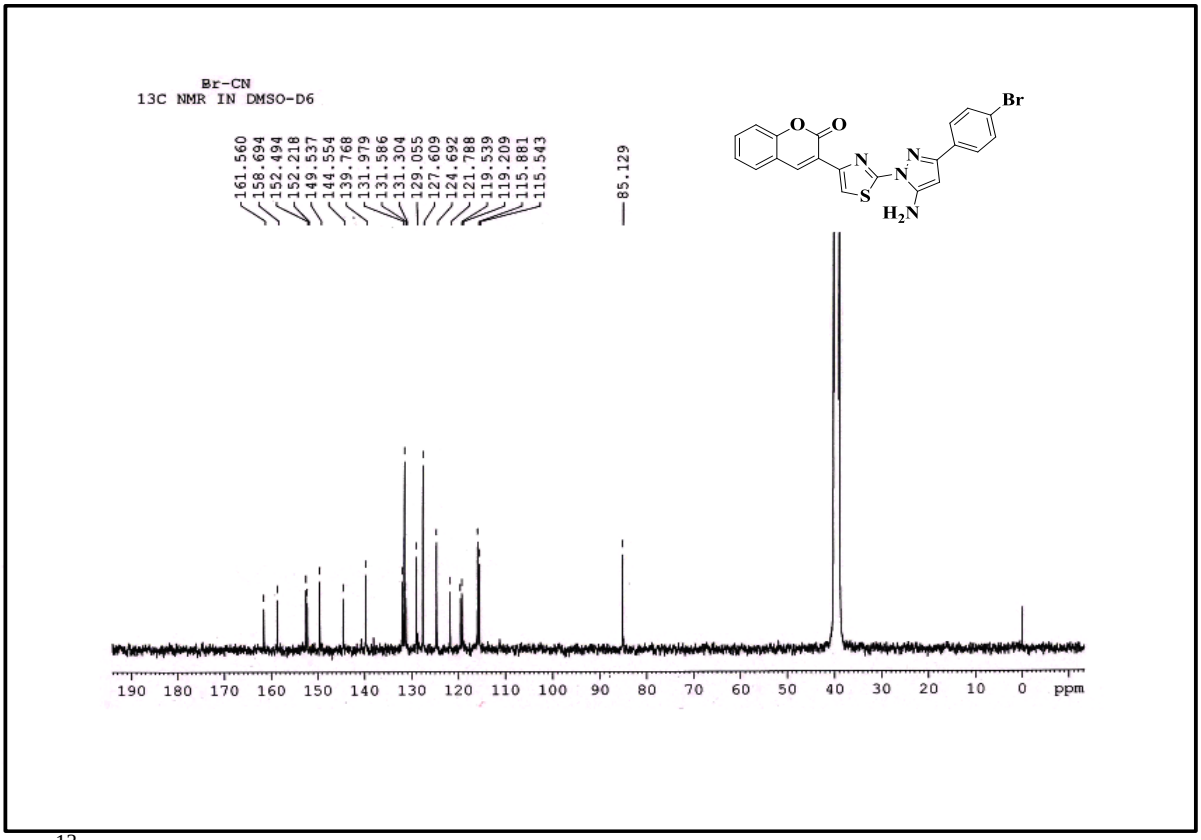


Fig: ^{13}C NMR spectrum of compound **37l**

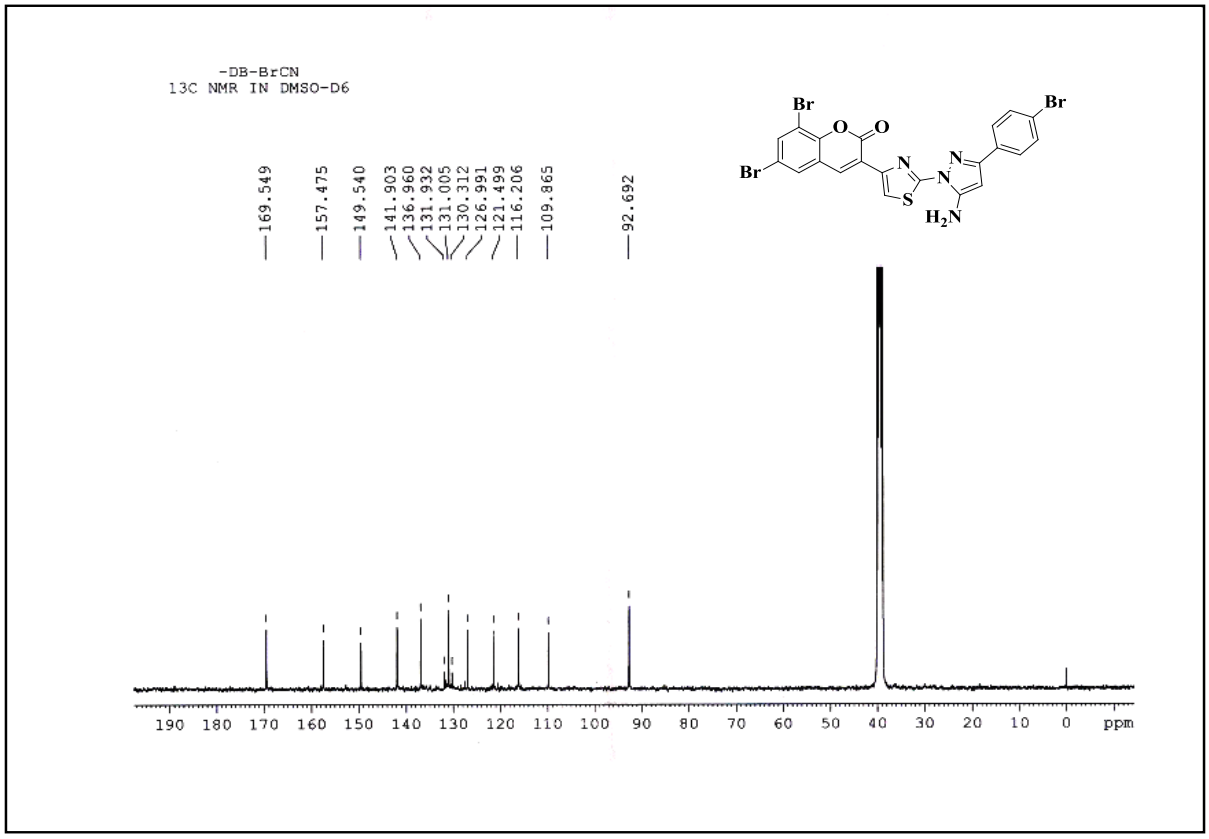


Fig: ^{13}C NMR spectrum of compound **37m**

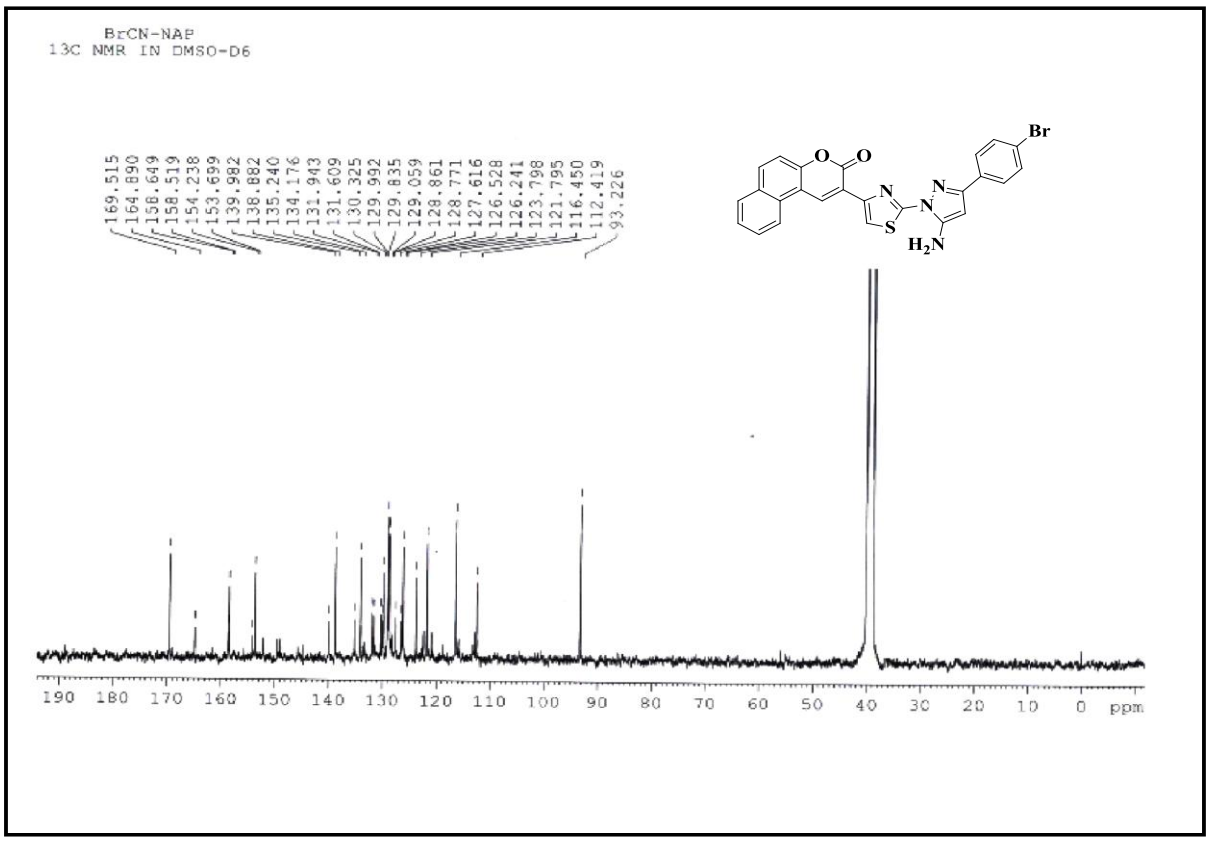


Fig: ^{13}C NMR spectrum of compound **37n**

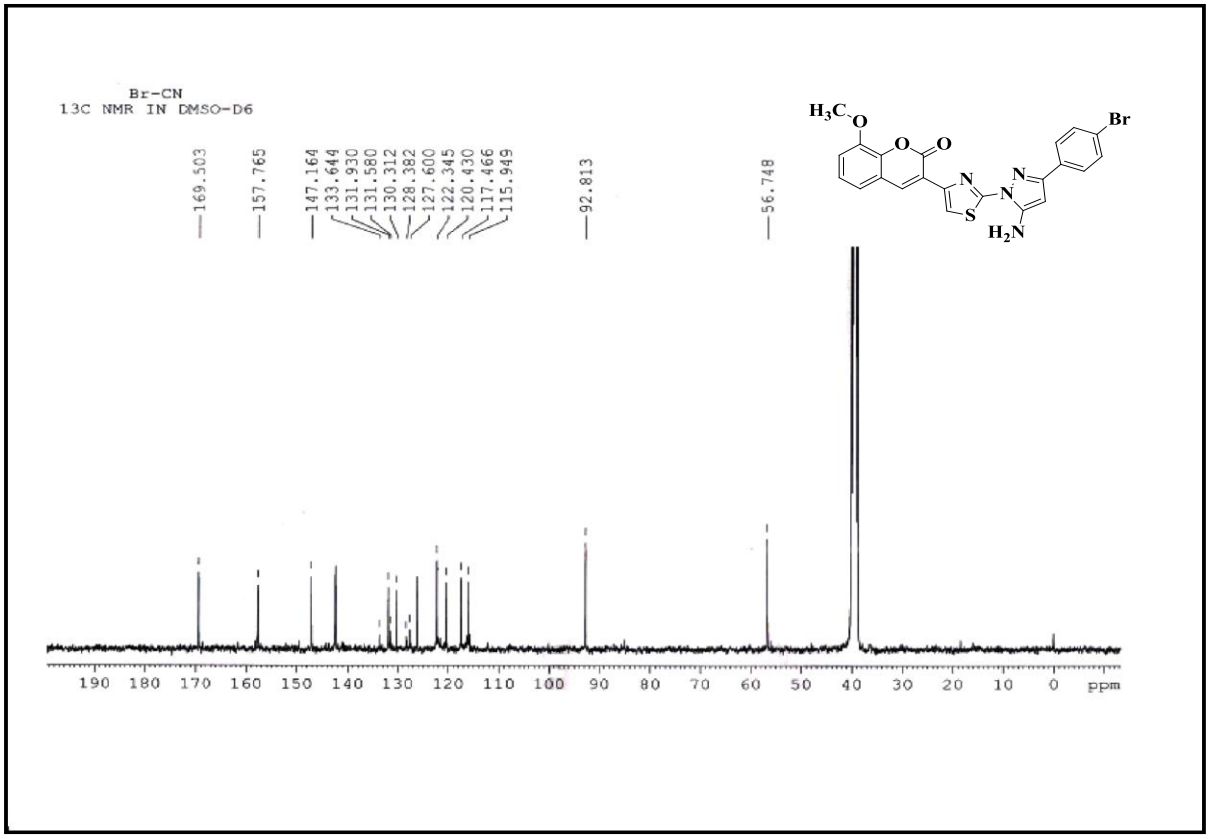


Fig: ^{13}C NMR spectrum of compound **37o**

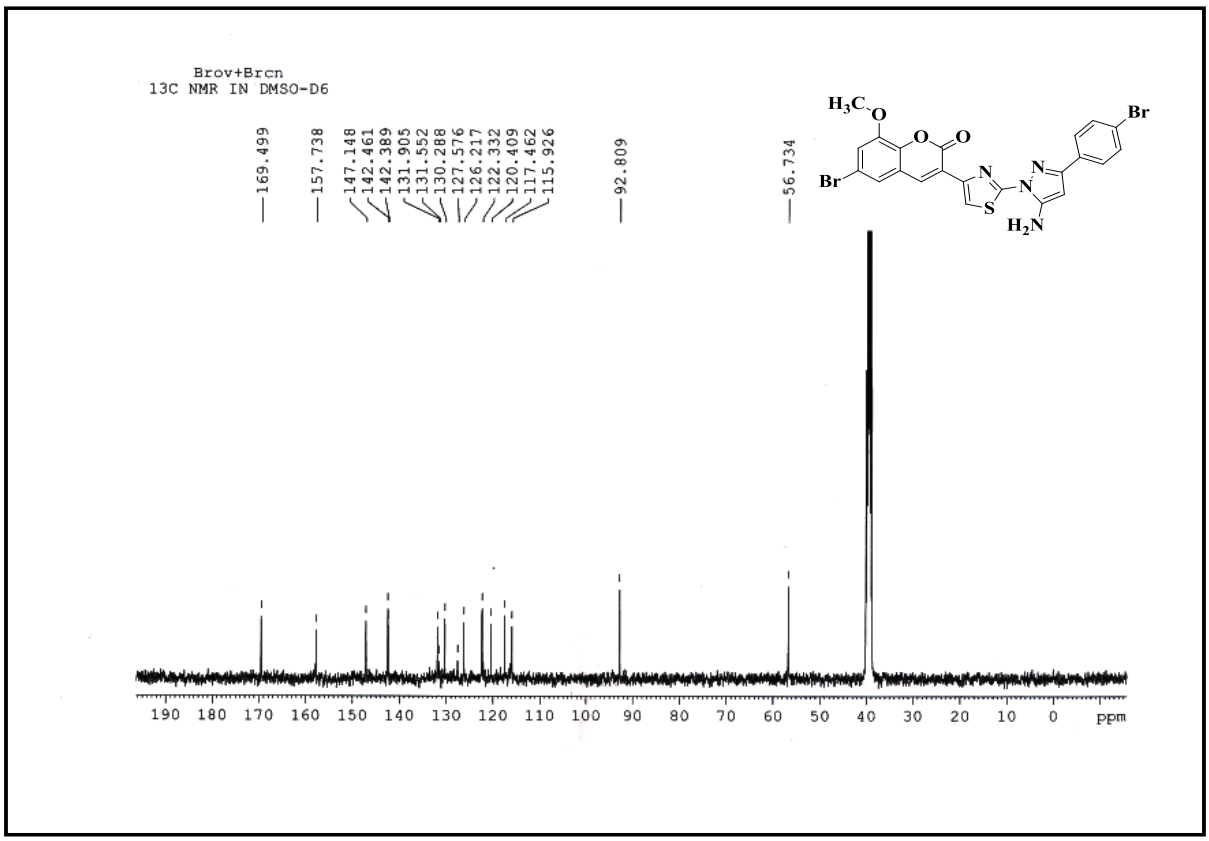


Fig: ^{13}C NMR spectrum of compound **37p**

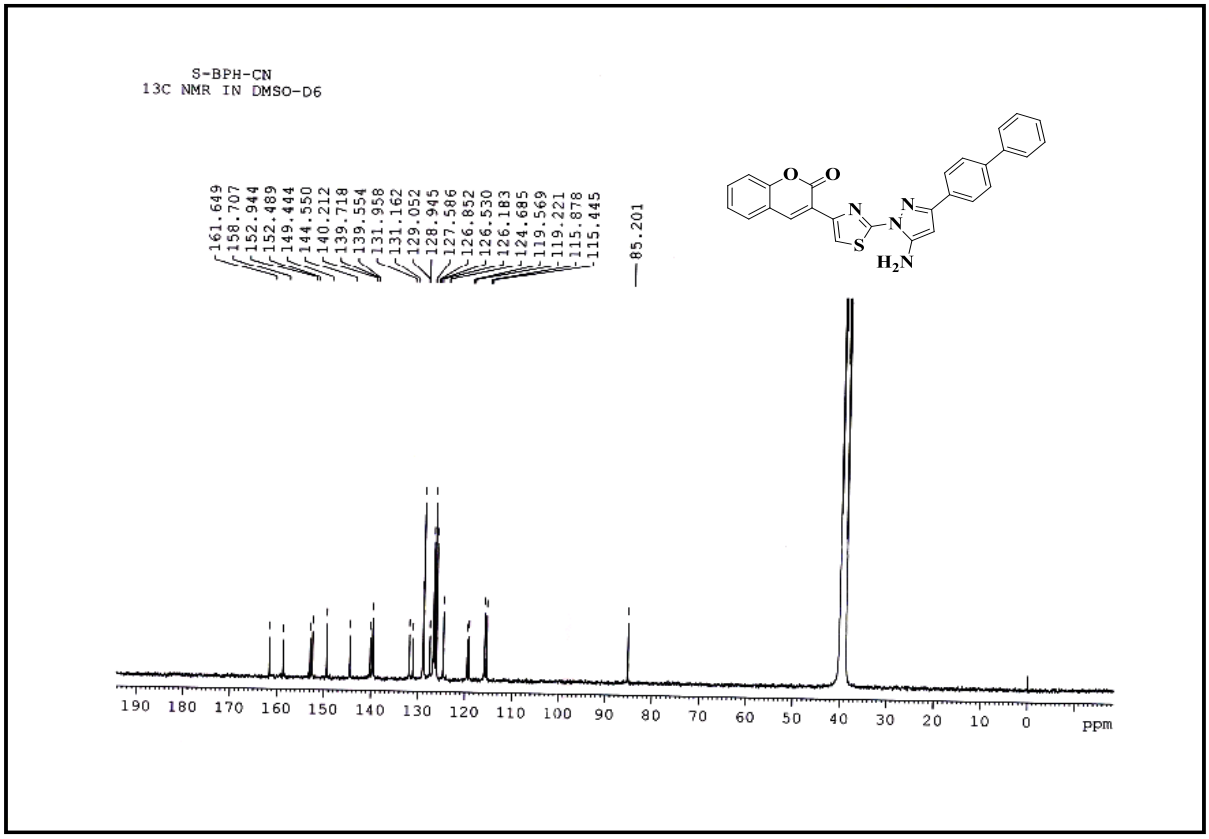


Fig: ^{13}C NMR spectrum of compound **37q**

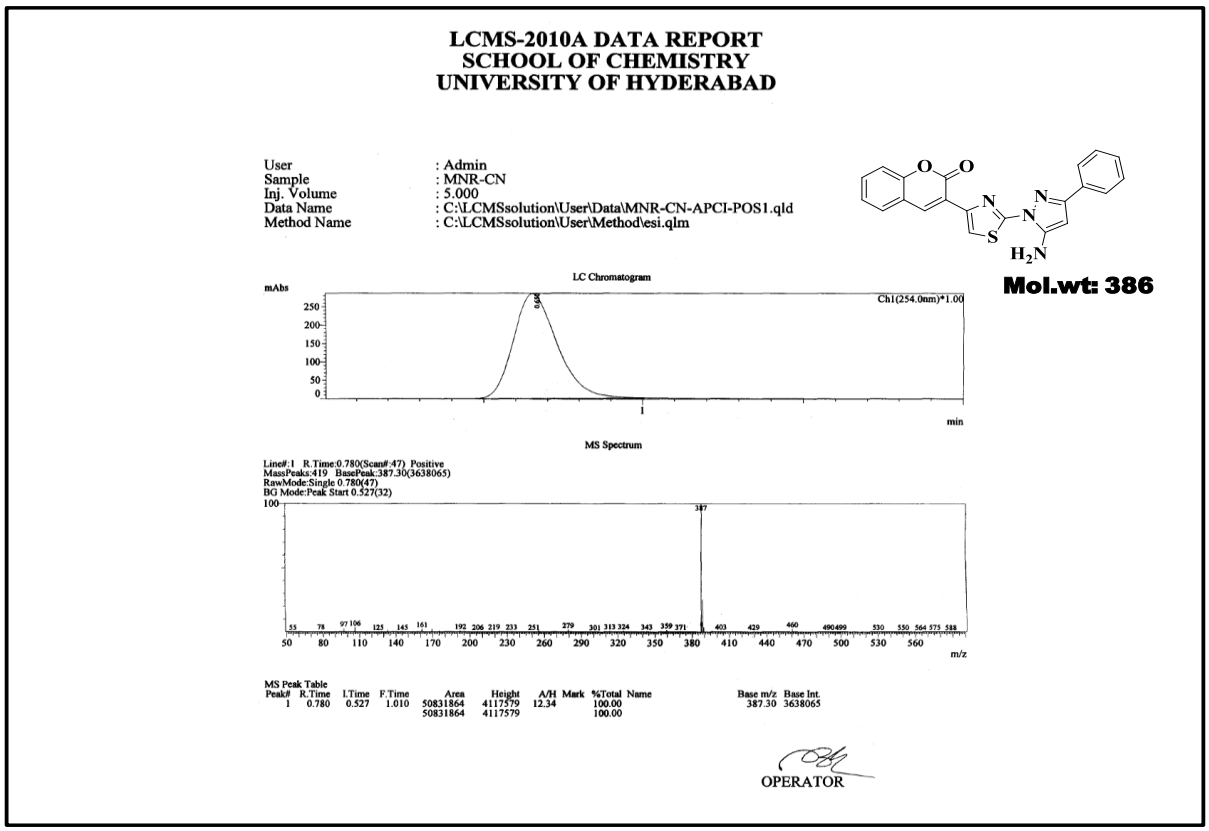


Fig: Mass Spectrum of compound **37a**

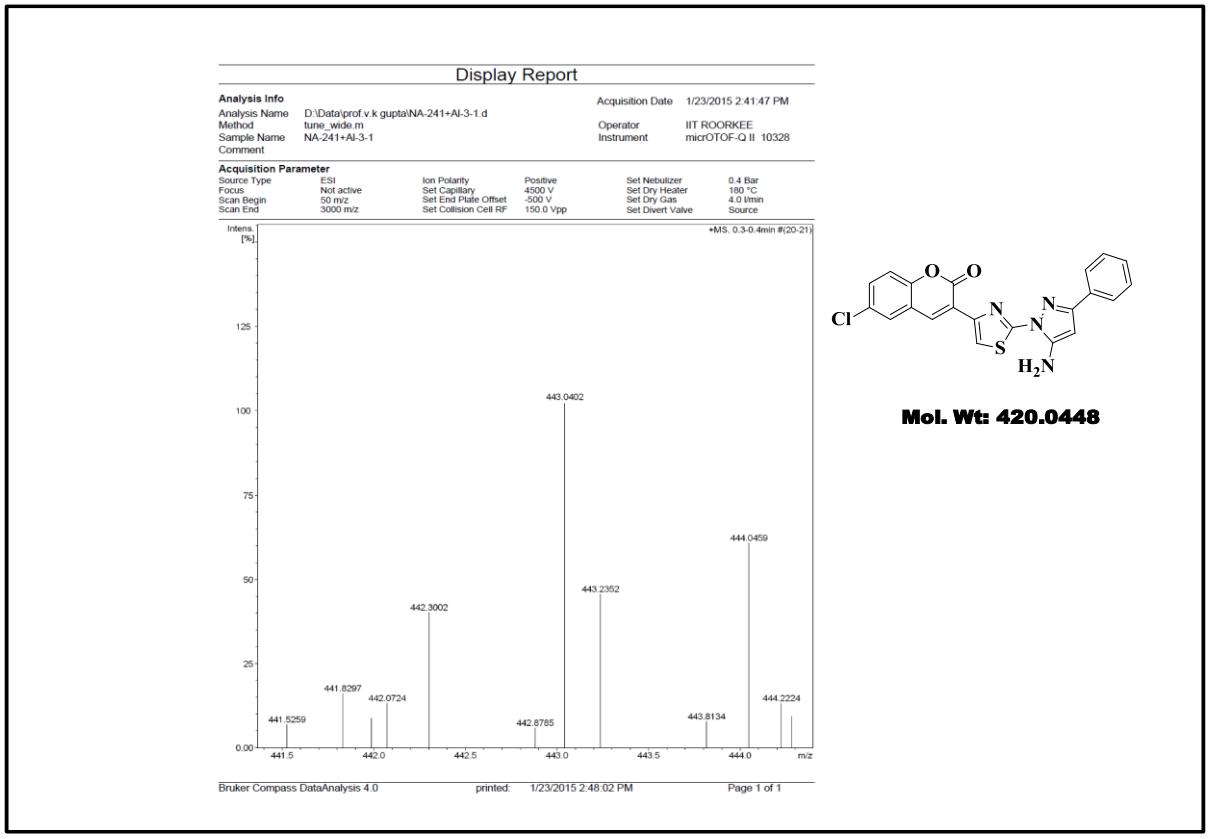


Fig: Mass Spectrum of compound **37b**

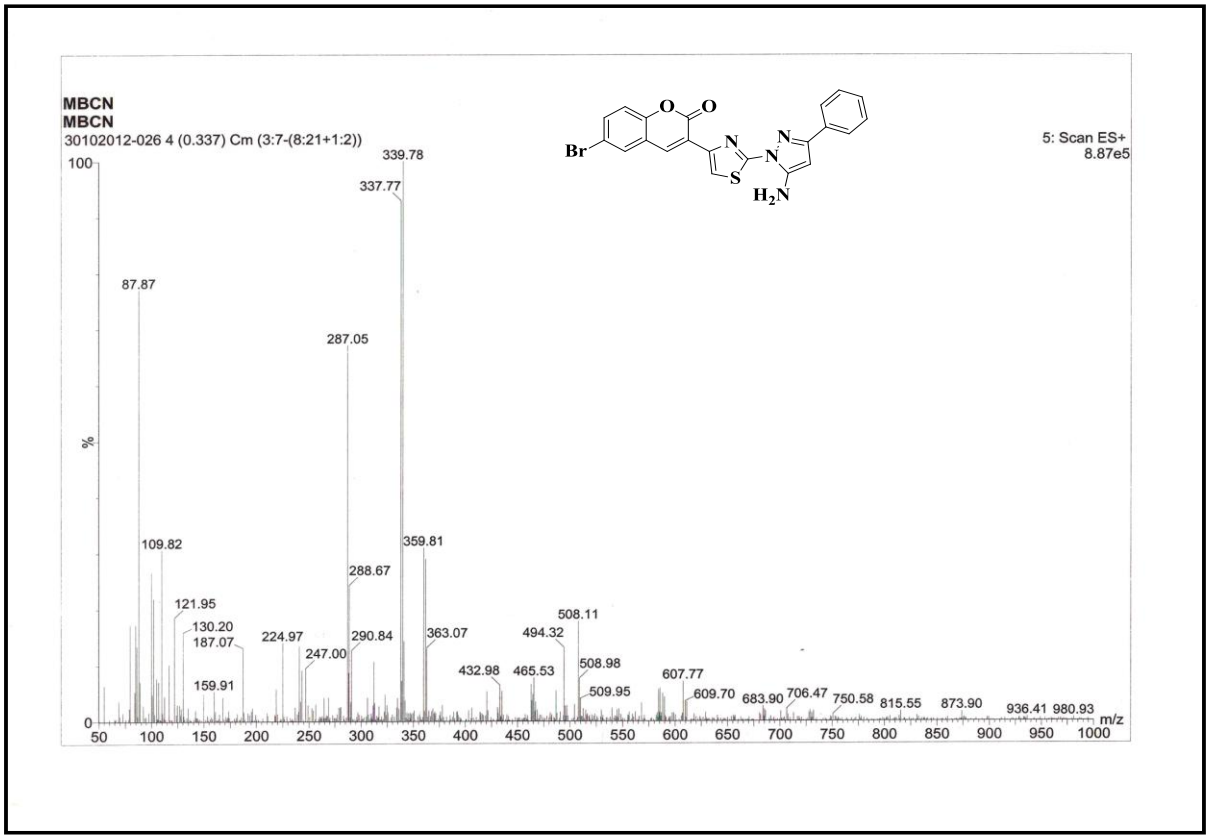


Fig: Mass Spectrum of compound **37d**

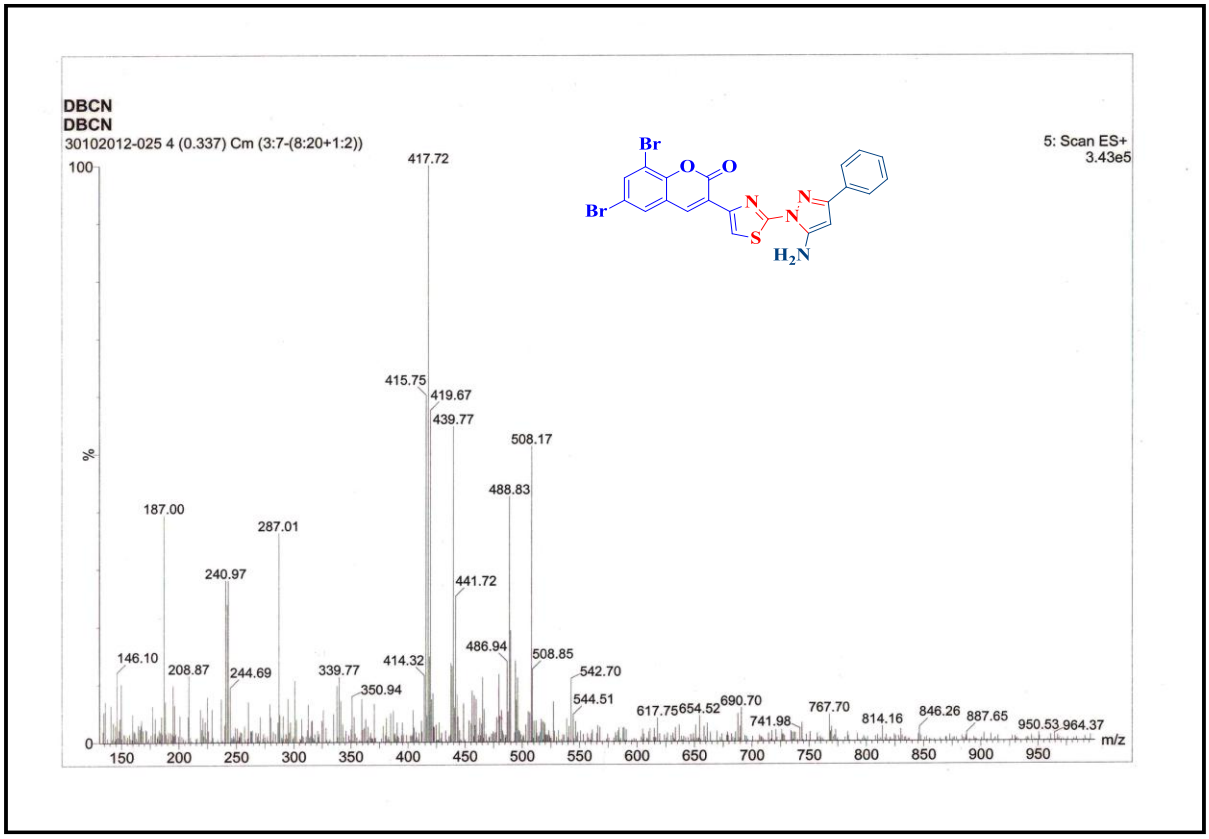


Fig: Mass Spectrum of compound **37e**

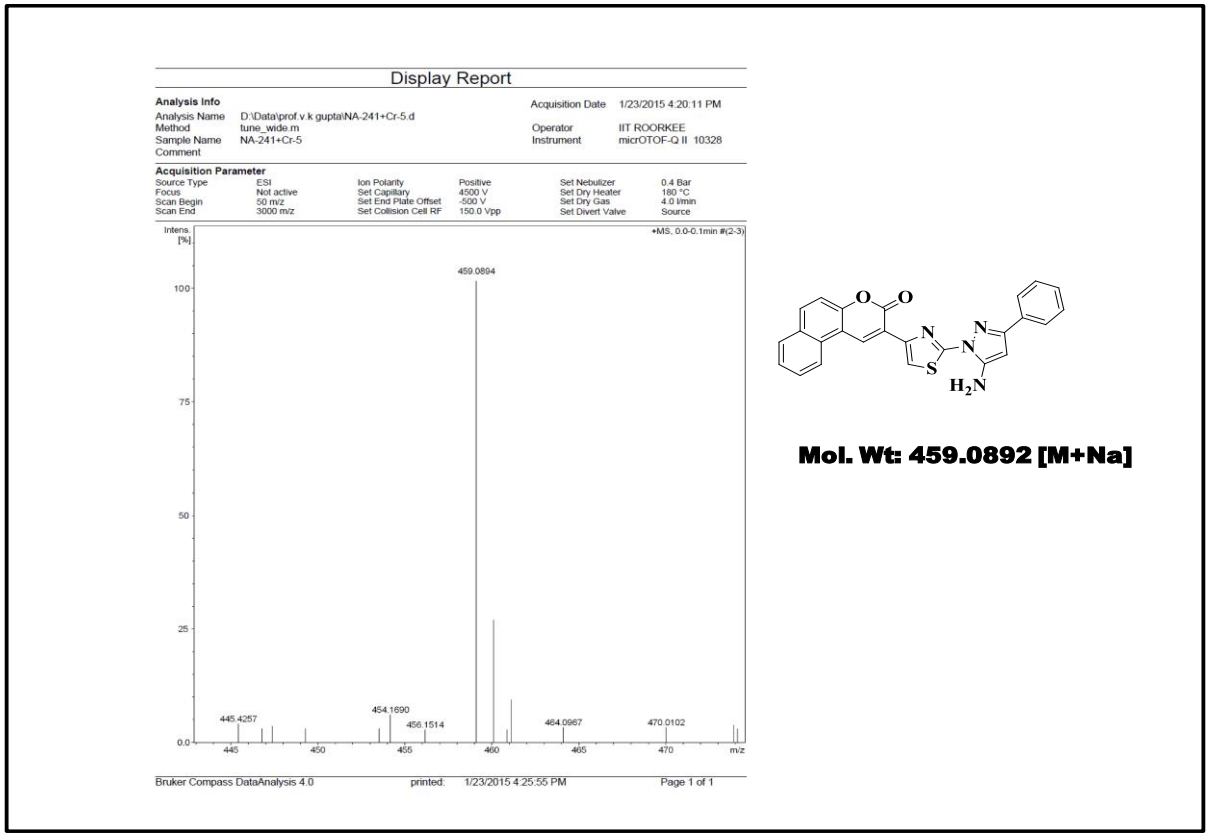


Fig: Mass Spectrum of compound **37f**

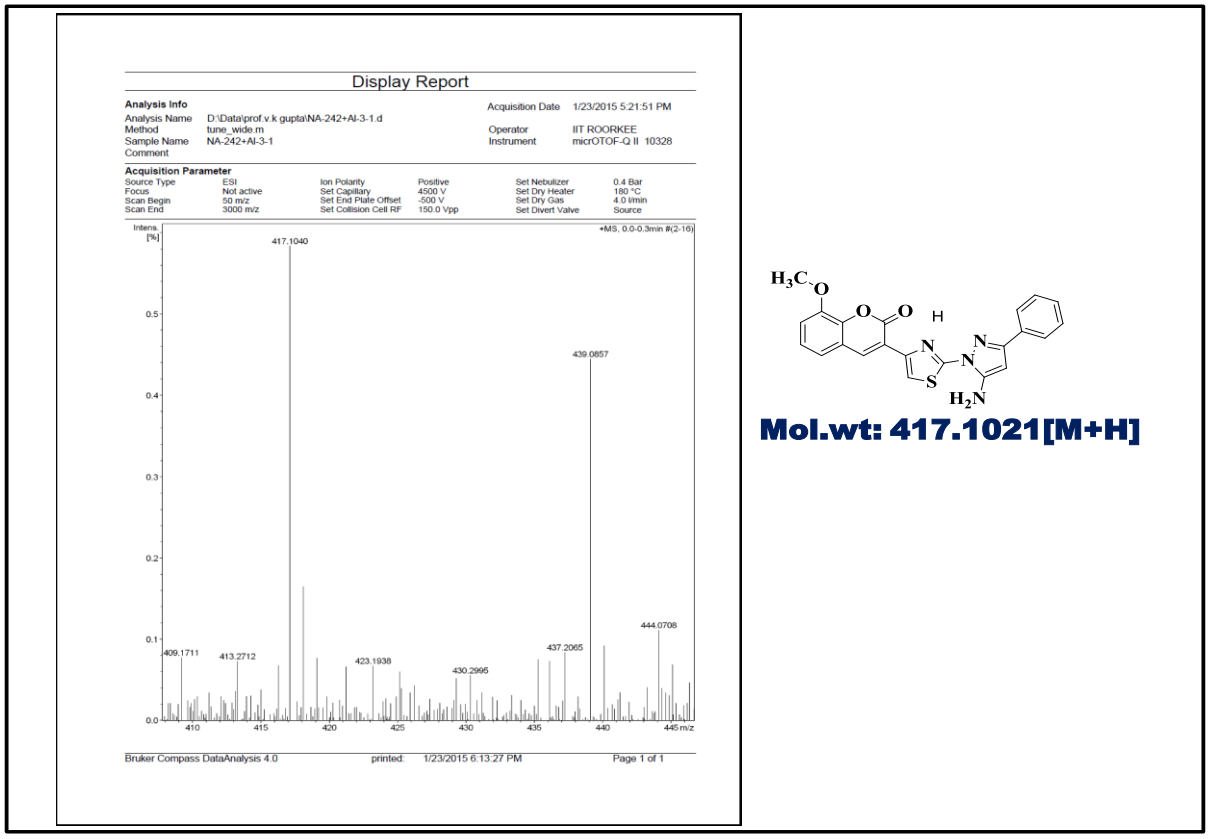


Fig: Mass Spectrum of compound **37g**

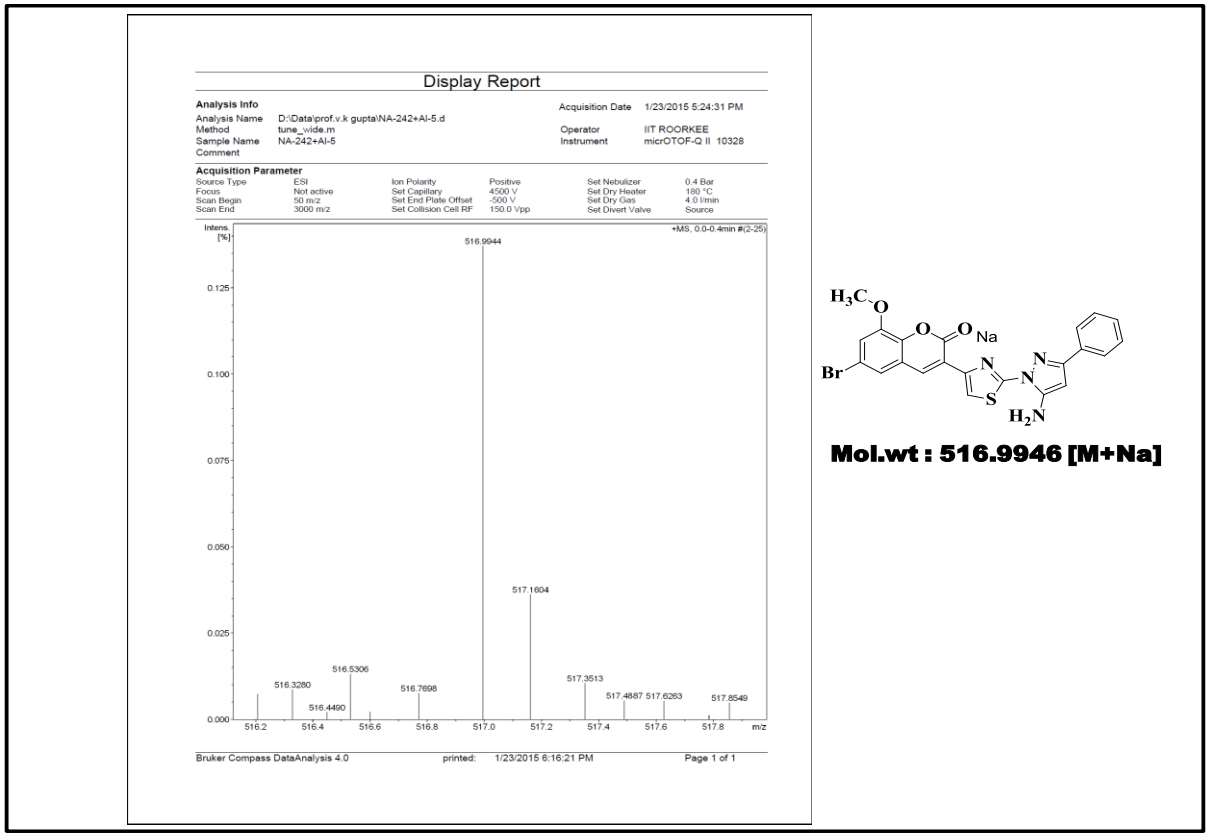


Fig: Mass Spectrum of compound **37h**

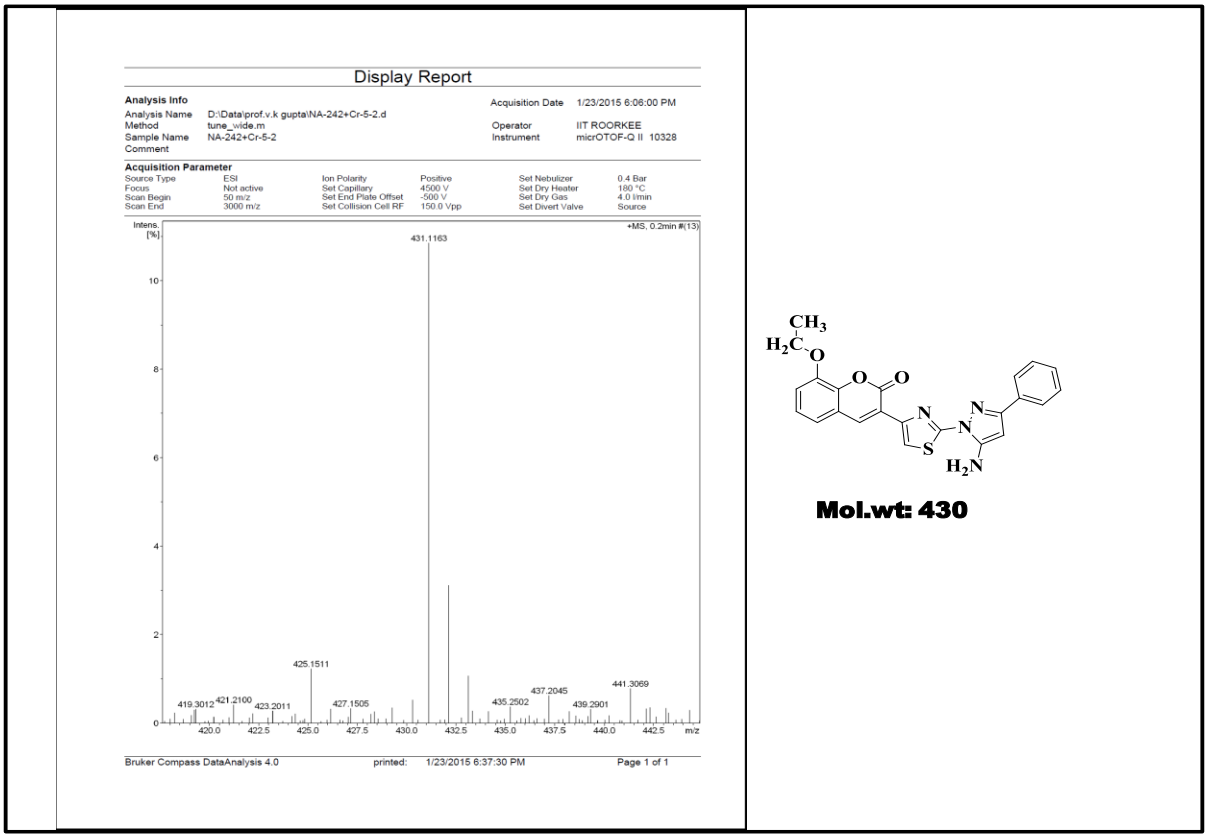


Fig: Mass Spectrum of compound **37i**

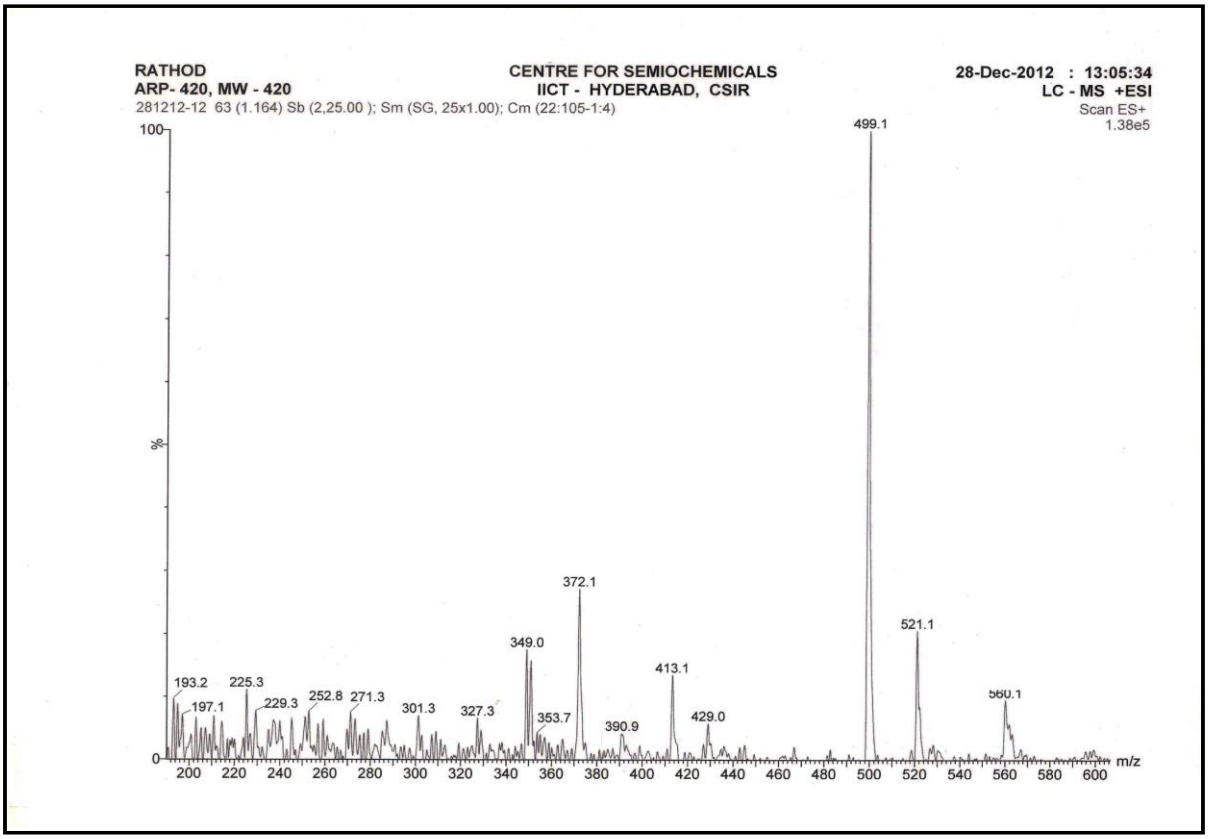


Fig: Mass Spectrum of compound **37j**

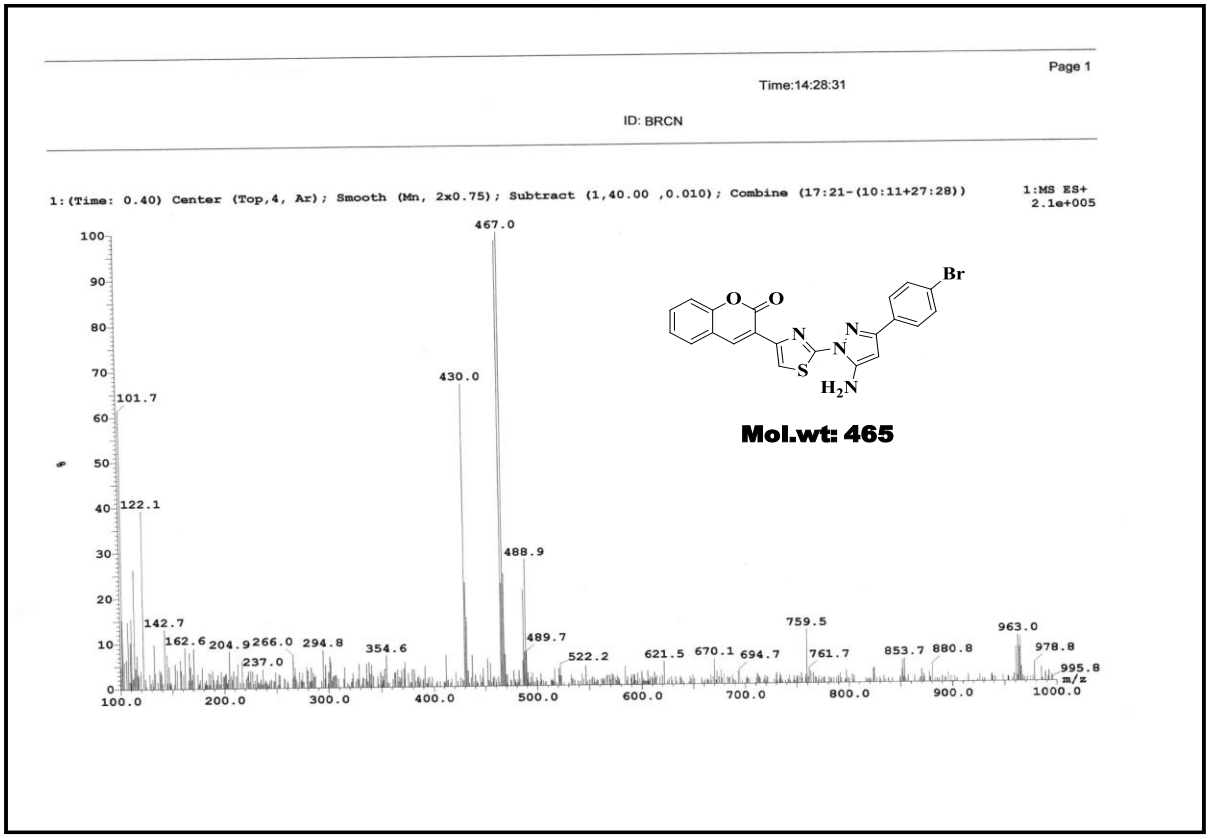


Fig: Mass Spectrum of compound **37l**

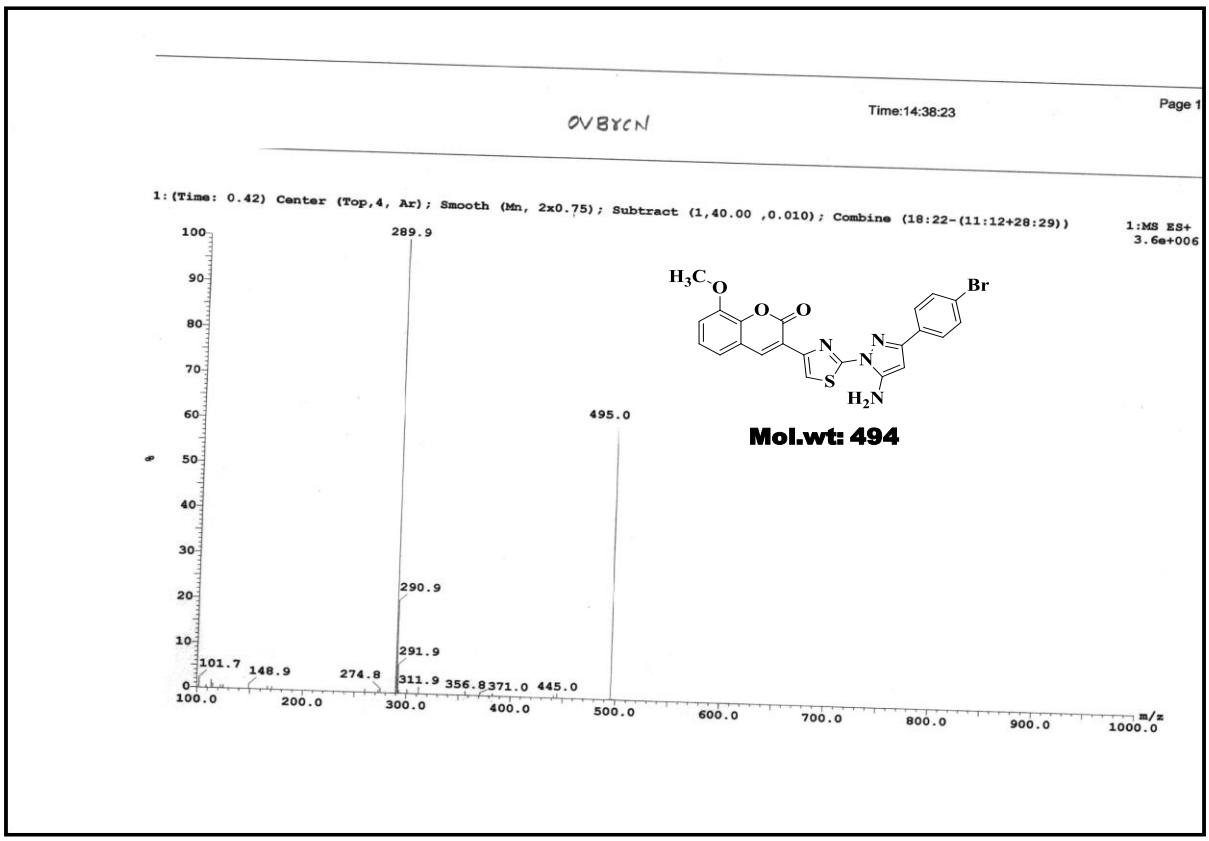


Fig: Mass Spectrum of compound **37o**

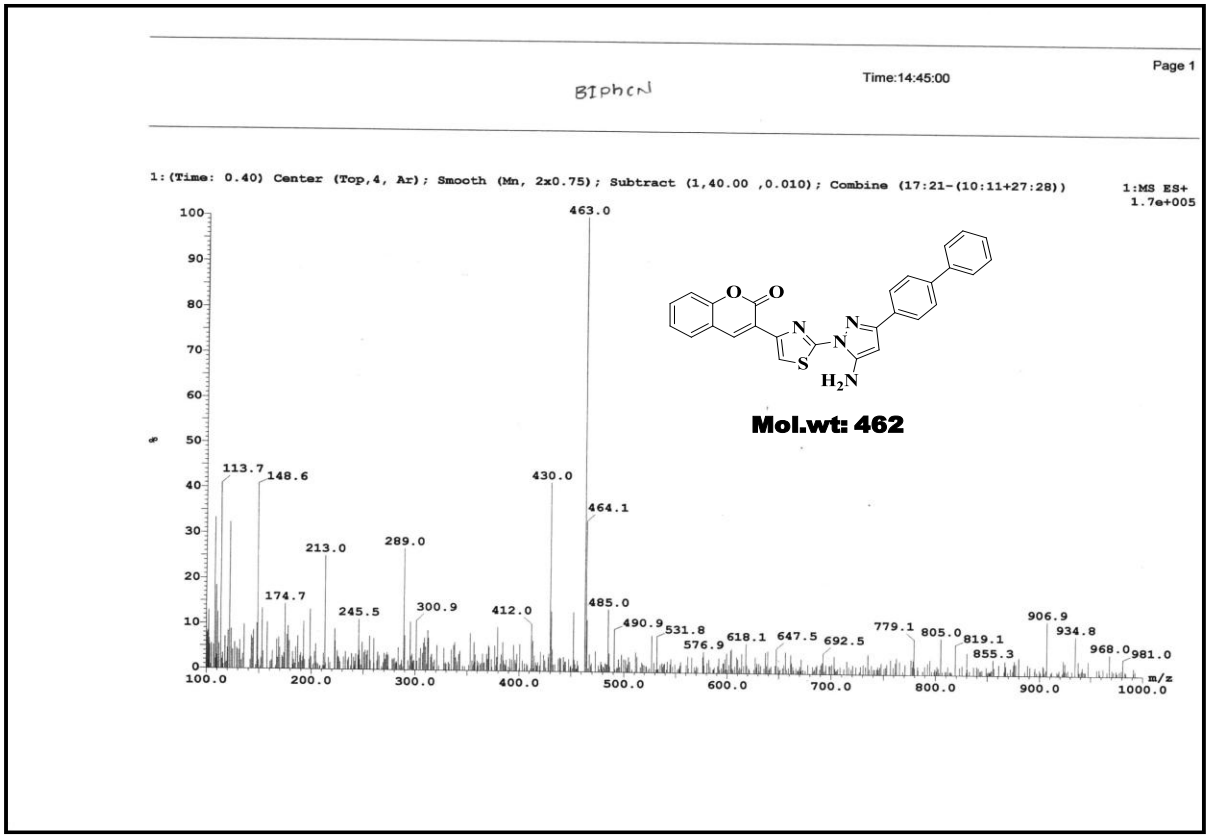


Fig: Mass Spectrum of compound **37q**

CHAPTER-II
PART-C

FACILE ONE POT MULTICOMPONENT SYNTHESIS 3, 5-DI SUBSTITUTED-4,5-DIHYDRO-1*H*-PYRAZOL-1-YL)THIAZ OL-4-YL)-2*H*-CHROMEN-2-ONES AND THEIR BIOLOGICAL ACTIVITY

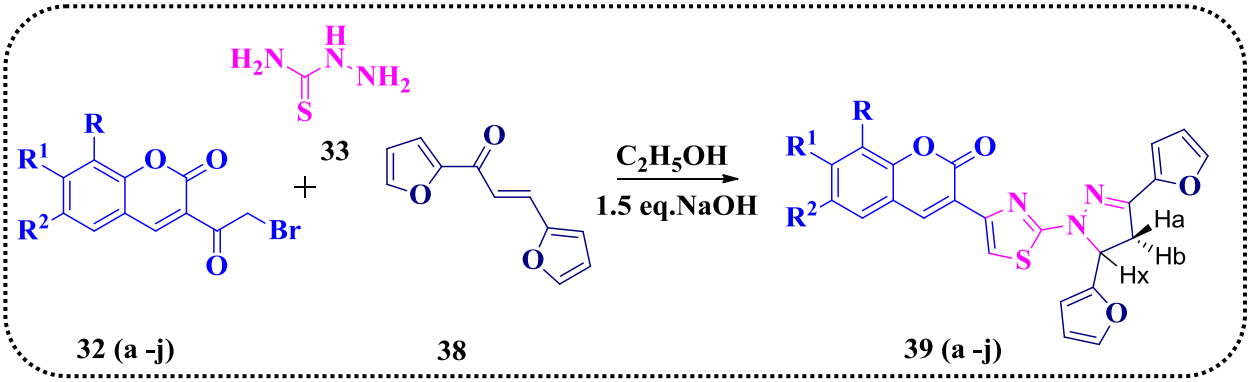
PART-C

PRESENT WORK:

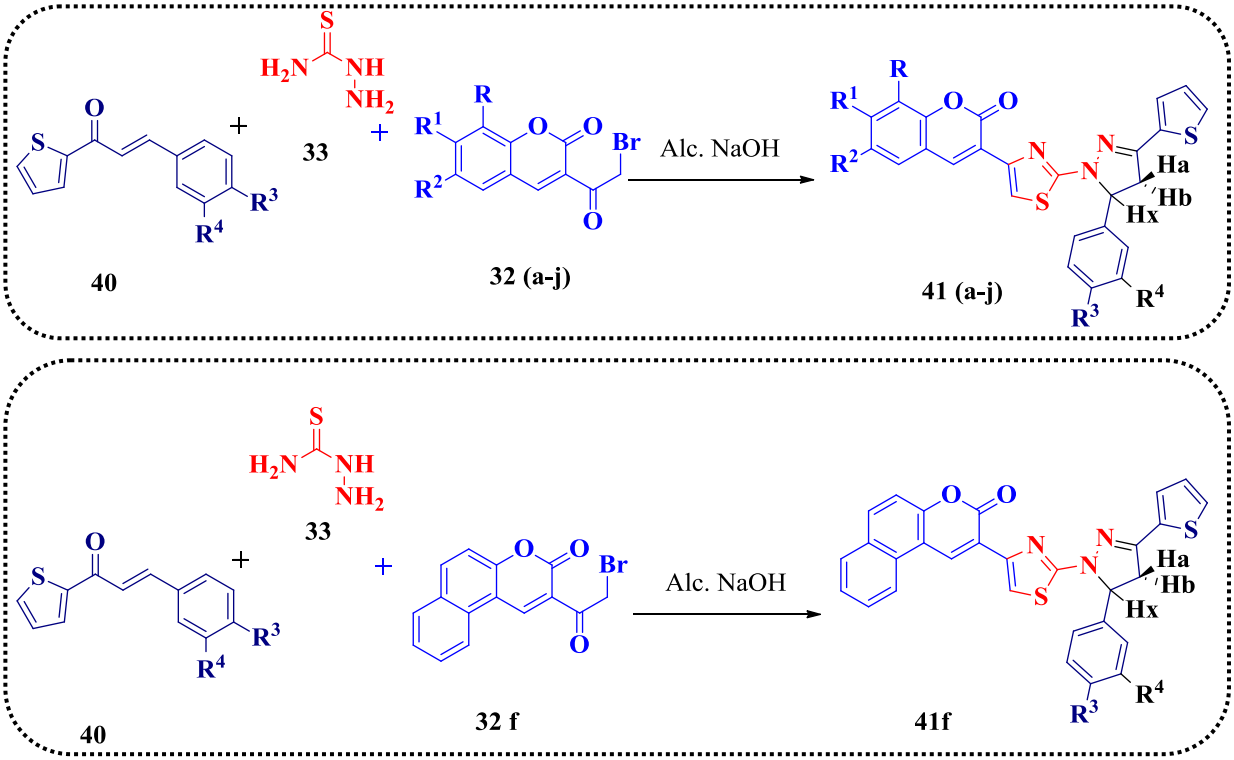
STARTING MATERIALS:

The present chapter describes the synthesis and biological activity evaluation of dihydropyrazolyl thiazolyl coumarins. The starting materials required for the synthesis of the title compounds are substituted 3-(2-bromoacetyl)coumarins, thiosemicarbazide, 1,3-di(furan-2-yl)prop-2-en-1-one and 3-aryl-1-(thiophen-2-yl)prop-2-en-1-ones. Substituted 3-(2-bromoacetyl)coumarins were prepared from substituted salicylaldehydes as described in the preceding chapter. 1,3-di(furan-2-yl)prop-2-en-1-one[63] and 3-aryl-1-(thiophen-2-yl)prop-2-en-1-one[64] derivatives were prepared according to the literature procedure. Thiosemicarbazide was procured from commercial source.

Dihydropyrazoles are considered as important pharmacophores in many biological applications. In the present chapter 3-(2-(3,5-di(furan-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)thiazol-4-yl)-2*H*-chromen-2-ones **39(a-j)** and 3-(2-(5-phenyl-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)thiazol-4-yl)-2*H*-chromen-2-one derivatives **41(a-q)** were synthesized in presence of alcoholic NaOH solution starting from the reaction of 1,3-di(furan-2-yl)prop-2-en-1-one **38** or 3-aryl-1-(thiophen-2-yl)prop-2-en-1-one **40 (a-c)** with thiosemicarbazide **33** and substituted 3-(2-bromoacetyl)coumarins **32 (a-j)** under reflux condition (**Scheme 3 & 4**) with satisfactory yields (**Table.10**).



Scheme.3 A facile one pot multicomponent synthesis of 3-(2-(3,5-di(furan-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)thiazol-4-yl)-2*H*-chromen-2-ones.



Scheme.4 An efficient synthesis of 3-(2-(5-phenyl-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)thiazol-4-yl)-2*H*-chromen-2-one derivatives.

Table.10: Facile one pot synthesis of dihydropyrazolylthiazoly coumarins

Compound	R	R^1	R^2	R^3	R^4	Reaction Time (h)	Yield (%)
39a	H	H	H	-	-	4.0	78
39b	H	H	Cl	-	-	5.0	81
39c	H	H	Br	-	-	4.5	85
39d	Br	H	Br	-	-	6.0	89
39f	OCH_3	H	H	-	-	4.0	80
39g	OCH_3	H	Br	-	-	5.5	83
39h	OC_2H_5	H	H	-	-	4.0	81
39i	OC_2H_5	H	Br	-	-	5.5	86
39j	H	H	$N(C_2H_5)_2$	-	-	4.0	77
39k	$C(CH_3)_3$	H	$C(CH_3)_3$	-	-	4.5	79
41a	H	H	H	H	H	5.0	79
41b	H	H	Cl	H	H	6.0	83
41c	H	H	Br	F	H	4.5	85
41d	H	H	H	Cl	H	5.0	80
41e	H	H	Cl	OCH_3	H	4.5	81
41f	H	H	Br	OCH_3	H	6.0	82
41h	OCH_3	H	H	OCH_3	H	3.0	84
41i	OC_2H_5	H	H	OCH_3	H	4.0	82
41j	CH_3	OH	H	OCH_3	H	5.0	85
41k	H	H	$N(C_2H_5)_2$	OCH_3	H	5.0	77
41l	H	H	Cl	OCH_3	OCH_3	6.0	88
41m	H	H	Br	OCH_3	OCH_3	5.0	89

41o	OCH_3	H	H	OCH_3	OCH_3	4.0	84
41p	OC_2H_5	H	H	OCH_3	OCH_3	4.5	88
41q	CH_3	OH	H	OCH_3	OCH_3	5.0	83

All the newly synthesized compounds **39a-k** and **41a-q** have been characterized by using analytical techniques like IR, ^{1}H NMR, ^{13}C NMR, ESI-Mass and Single crystal X-ray analysis. The proton NMR spectrum of the title compounds exhibited characteristic ABX splitting pattern for pyrazoline three protons present at fourth and fifth positions. The three protons appeared as three double doublets (dd). The compound **41b** was further confirmed by single crystal X-ray analysis.

In the title compound (**Fig.10**), the 2*H*-chromene ring system which is planar with a r.m.s. deviation of 0.003 Å, is nearly co-planar with the 1,3-thiazole ring, being a dihedral angle of 4.08(11) °. The central 4,5-dihydro-1*H*-pyrazole ring makes dihedral angles of 5.57 (15), 10.06 (14) and 68.78 (14) °, with the thiophene ring, the 1,3-thiazole ring and the chloro-phenyl ring, respectively. Selected geometric parameters are given in **Table 11**.

Molecular conformation of the title compound is stabilized by an intra-molecular C---H…O hydrogen bond, forming a S(6) motif. In the crystal, one inter-molecular C---H…O contact links the molecules, into layers along the c-axis (**Table 12** & **Fig. 11**). The other inter-molecular C---H…O contact connects the mentioned layers to each other, forming a 3D network. Furthermore, one C---H…π interaction and two π-π interactions [centroid-to-centroid distances = 3.7883(17) Å between the thiophen rings; centroid-to-centroid distances = 3.5258 (16) Å between the 2*H*-pyran and benzene rings of the 2*H*-chromene ring system] contribute to the stabilization of molecular packing.

Table. 11. Crystal data and structure refinement for the compound **41b**

Identification code	shelxl
Chemical formula	$C_{25}H_{16}ClN_3O_2S_2$
Formula weight M_r	489.98
Temperature	296(2) K
Wavelength	0.71073 A
Crystal system, space group	**Triclinic, $P\bar{1}$**
Temperature (K)	296
Unit cell dimensions	a= 9.1476 (6) (Å), α= 61.620 (2) (°), b =11.6523 (6) (Å), β = 84.686 (3) (°), c =12.1075 (6) (Å). ɣ = 81.914 (3) (°).
Volume V (Å^3)	1123.66 (11)
Z, Calculated density	2, 1.448 Mg/m^3
Absorption coefficient	0.385 mm^-1
F(000)	504
Crystal size	0.30 x 0.25 x 0.20 mm
Theta range for data collection	2.25 to 28.41 deg.
Limiting indices	-12<=h<=11, -15<=k<=11, -16<=l<=15
Reflections collected / unique	9030 / 5396 [R(int) = 0.0189]
Completeness to theta	= 28.41, 95.3 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.9270 and 0.8932
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	5396 / 0 / 298
Goodness-of-fit on F^2	1.022
Final R indices [I>2sigma(I)]	R1 = 0.0530, wR2 = 0.1468
R indices (all data)	R1 = 0.0901, wR2 = 0.1759
Largest diff. peak and hole	0.433 and -0.455 e.A^-3

Table 12. Hydrogen Bonds (Å, °)

D—H···A	D—H	H···A	D···A	D—H···A
C11--- H12. ..O2	0.93	2.30	2.858(4)	118
C20---H20A...O2i	0.97	2.58	3.514(3)	163
C20---H20B...O2^{ii}	0.97	2.55	3.456(3)	155
C25---H25...Cg6^{iii}	0.93	2.92	3.768(4)	153

Cg6 is a centroid of the C14—C19 cholorobenzene ring.
Symmetry codes: (i) 1-x, -y, 1-z; (ii) x, -1+y, z; (iii) 2-x, -1-y, 1-z.

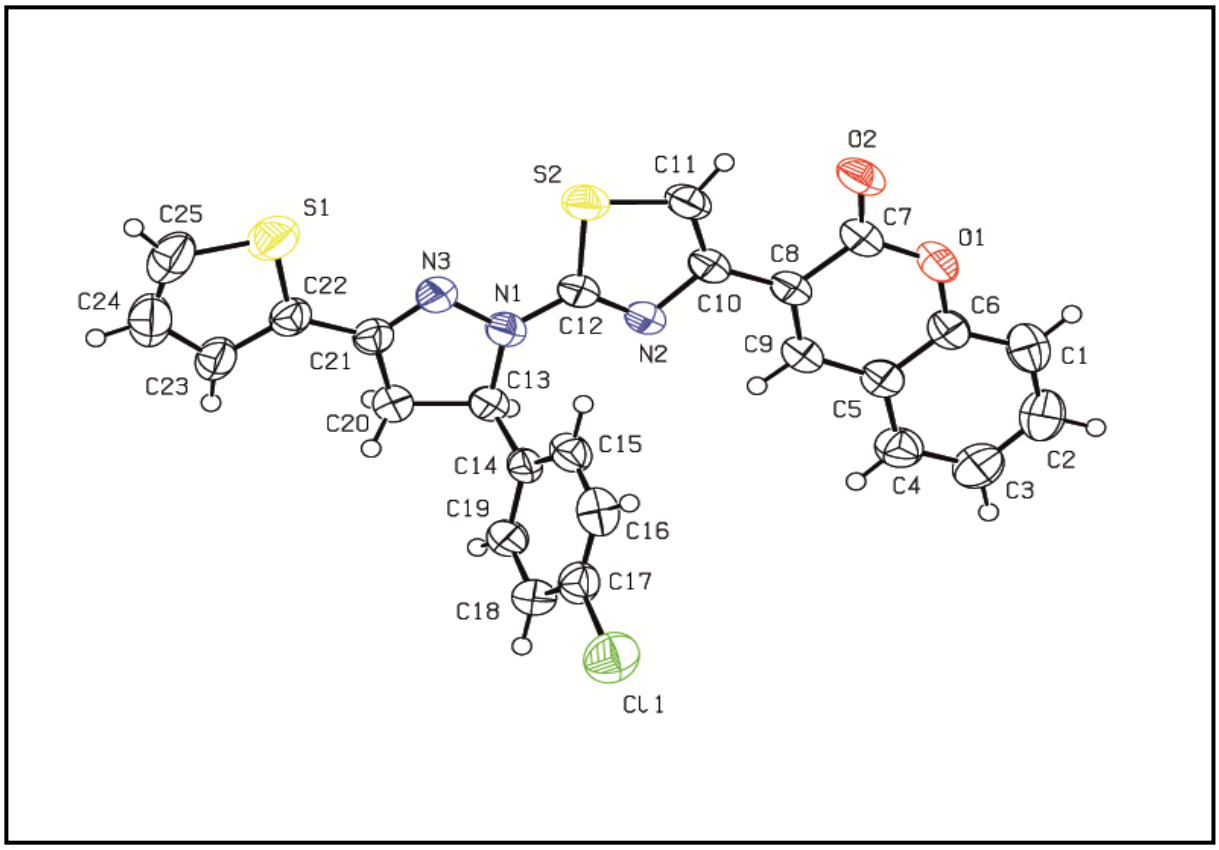


Figure.10. The title molecule with labelling scheme and 50% probability ellipsoids.

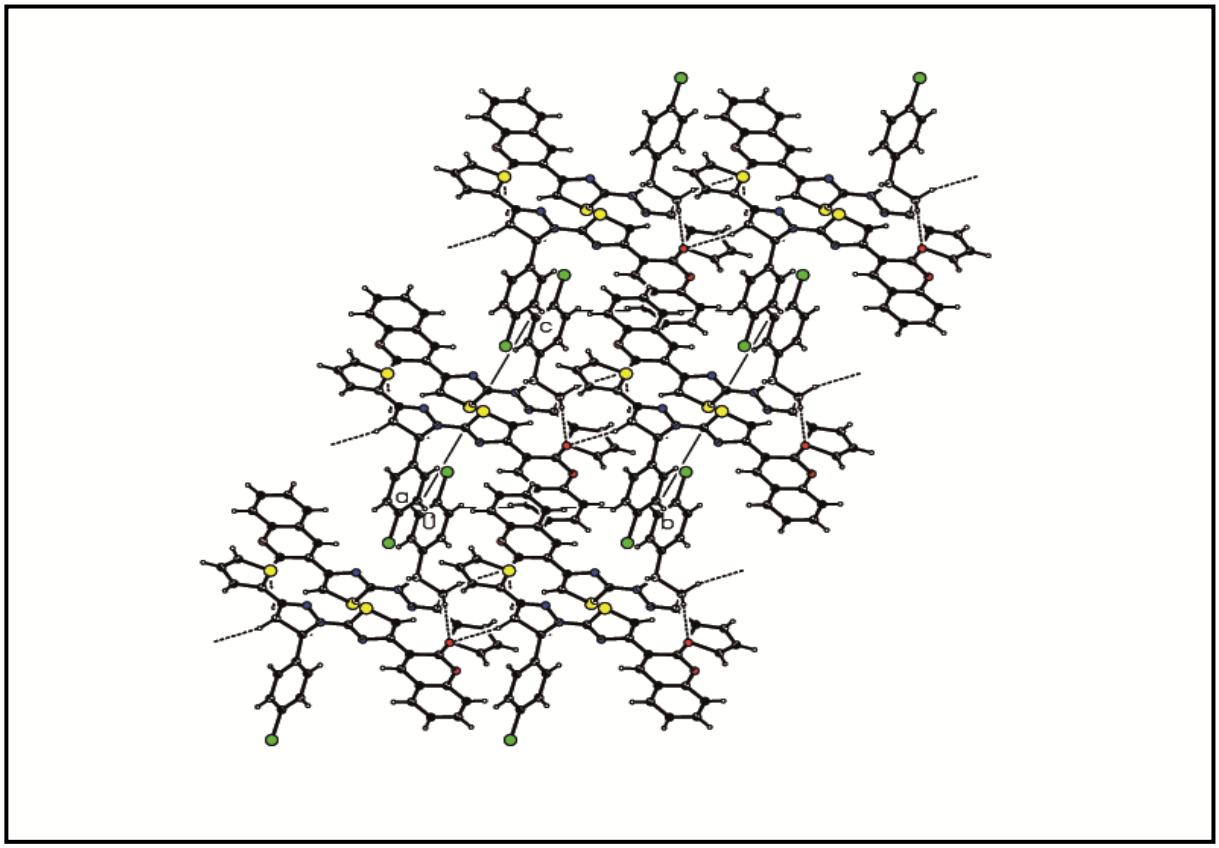


Figure 11. A view of the molecular packing of the title compound down the a-axis.

in vitro antiviral activity of dihydro-1*H*-pyrazol-1-yl)thiazol-4-yl)-2*H*-chromen-2-one derivatives

The newly synthesized compounds were evaluated for their antiviral activity against various human viruses. The HEL cell culture was used to evaluate the compounds against *Herpes simplex* virus type I (KOS) [HSV-1 KOS], *Herpes simplex* virus type 2 (G) [HSV-2 G], *Vaccinia* virus [VV], *Vesicular stomatitis* virus (VSV), thymidine kinase-deficient *Herpes simplex* virus type 1 (HSV-1 TK^- KOS ACV^r) and *Adeno* virus type 2 (AV-2), the HeLa cell culture was used for *Vesicular stomatitis*, *Coxsackie* virus B4, *Respiratory syncytial* virus, Vero cells were utilized to evaluate the compounds against *Parainfluenza* type 3 virus (PI-3V), *Reo* virus type 1 (RV-1), *Sindbis* virus (SV), *Coxsackie* virus B4 (CV B4) and *Punto toro* virus (PTV), The MDCK cell line was used to infect with *Influenza* A virus type H1N1 (strain A/Ned/378/05) and type H3N2 (strain A/HK/7/87), and *Influenza* B virus (strain B/Ned/537/05), the CRFK cell culture medium was used for *Feline Corona* Virus (FIPV) and *Feline Herpes* Virus. Based on the microscopical analysis of the virus-induced cytopathicity (CPE) the antiviral potency of the compounds was determined and compared to different reference antiviral drugs (brivudin, cidofovir, acyclovir, ganciclovir, zalcitabine and alovudine). The antiviral activity was shown in **Table 13-17**.

The antiviral activity data of the tested compounds clearly shown that the compounds are active against influenza viruses. The compounds 39b, 39e, 39g, 39h, 39i, 39k, 41c, 41e and 41l are potent antiviral compounds. The remaining compounds did not exhibit any potency against the tested viruses.

Table 13. Cytotoxicity and antiviral activity in: HEL cell cultures

Compound	Minimum cytotoxic concentration[a] (μM)	EC_{50}[b] (μM)					
		Herpes simplex virus-1 (KOS)	*Herpes simplex* virus-2 (G)	*Herpes simplex* virus-1 TK-KOS ACVr	*Vaccinia* virus	*Adeno* virus-2	*Human Corona* virus (229E)
39a	≥20	>100	>100	>100	>100	>100	>100
39b	≥100	>100	>100	>100	>100	>100	>100
39c	≥100	>100	>100	>100	>100	>100	>100
39d	≥100	>100	>100	>100	>100	>100	>100
39e	≥100	>100	>100	>100	>100	>100	>100
39f	>100	>100	>100	>100	>100	>100	>100
39g	≥20	>100	>100	>100	>100	>100	>100
39h	≥4	>100	>100	>100	>100	>100	>100
39i	100	>100	>100	>100	>100	>100	>100
39j	100	>100	>100	>100	>100	>100	>100
39k	≥20	>100	>100	>100	>100	>100	>100
41a	≥100	>100	>100	>100	>100	>100	>100
41b	≥100	>100	>100	>100	>100	>100	>100
41c	≥100	>100	>100	>100	>100	>100	>100
41d	≥100	>100	>100	>100	>100	>100	>100
41e	>100	>100	>100	>100	>100	>100	>100
41f	≥20	>100	>100	>100	>100	>100	>100
41g	≥4	>100	>100	>100	>100	>100	>100
41h	100	>100	>100	>100	>100	>100	>100
41i	20	>100	>100	>100	>100	>100	>100
41j	100	>100	>100	>100	>100	>100	>100
41k	≥4	>100	>100	>100	>100	>100	>100
41l	100	>100	>100	>100	>100	>100	>100
41m	20	>100	>100	>100	>100	>100	>100
41n	100	>100	>100	>100	>100	>100	>100
41o	20	>100	>100	>100	>100	>100	>100
41p	100	>100	>100	>100	>100	>100	>100
41q	100	>100	>100	>100	>100	>100	>100
Brivudin	>250	0.008	112	10	10	-	-
Cidofovir	>250	4.5	2	3.4	22	29	-
Acyclovir	>250	0.7	0.2	85	>250	-	-
Ganciclovir	>250	0.16	0.06	4	>100	-	-
Zalcitabine	>250	-	-	-	-	19	-
Alovudine	>250	-	-	-	-	50	-
UDA	>100	-	-	-	-	-	1.8
Ribavirin	>250	-	-	-	-	-	112

[a] Required to cause a microscopically detectable alteration of normal cell morphology
[b] Required to reduce virus-induced cytopathogenicity by 50 %.

Table.14 Cytotoxicity and antiviral activity in: **HeLa cell cultures**

Compound	Minimum cytotoxic concentration[a]	EC50[b] *Vesicular stomatitis* virus	EC50[b] *Coxsackie* virus B4	EC50[b] *Respiratory syncytial* virus
39a	≥100	>100	>100	>100
39b	>100	>100	>100	>100
39c	>100	>100	>100	>100
39d	>100	>100	>100	>100
39e	>100	>100	>100	>100
39f	>100	>100	>100	>100
39g	>100	>100	>100	>100
39h	≥100	>100	>100	>100
39i	>100	>100	>100	>100
39j	>100	>100	>100	>100
39k	>100	>100	>100	>100
41a	>100	>100	>100	>100
41b	>100	>100	>100	>100
41c	>100	>100	>100	>100
41d	>100	>100	>100	>100
41e	≥100	>100	>100	>100
41f	>100	>100	>100	>100
41g	≥20	>100	>100	>100
41h	>100	>100	>100	>100
41i	>100	>100	>100	>100
41j	>100	>100	>100	>100
41k	>100	>100	>100	>100
41l	≥100	>100	>100	>100
41m	>100	>100	>100	>100
41n	≥20	>100	>100	>100
41o	>100	>100	>100	>100
41p	≥20	>100	>100	>100
41q	>100	>100	>100	>100
DS-10.000	>100	0.4	6.8	0.4
Ribavirin	>250	25	50	10

[a]Required to cause a microscopically detectable alteration of normal cell morphology.
[b]Required to reduce virus-induced cytopathogenicity by 50 %.

Table.15 Cytotoxicity and antiviral activity in: Vero cell cultures

Compound	Minimum cytotoxic conc.[a] (µM)	EC_{50}[b] (µM)					
		Para influenza-3 virus	*Reo* virus-1	*Sindbis* virus	*Coxsackie* virus B4	*Punta Toro* virus	*Yellow Fever* virus
39a	>100	>100	>100	>100	>100	>100	>100
39b	4	>100	>100	>100	>100	>100	>100
39c	20	>100	>100	>100	>100	>100	>100
39d	4	>100	>100	>100	>100	>100	>100
39e	100	>100	>100	>100	>100	>100	>100
39f	>100	>100	>100	>100	>100	>100	>100
39g	100	>100	>100	>100	>100	>100	>100
39h	≥20	>100	>100	>100	>100	>100	>100
39i	100	>100	>100	>100	>100	>100	>100
39j	100	>100	>100	>100	>100	>100	>100
39k	100	>100	>100	>100	>100	>100	>100
41a	>100	>100	>100	>100	>100	>100	>100
41b	100	>100	>100	>100	>100	>100	>100
41c	100	>100	>100	>100	>100	>100	>100
41d	100	>100	>100	>100	>100	>100	>100
41e	>100	>100	>100	>100	>100	>100	>100
41f	100	>100	>100	>100	>100	>100	>100
41g	100	>100	>100	>100	>100	>100	>100
41h	100	>100	>100	>100	>100	>100	>100
41i	>100	>100	>100	>100	>100	>100	>100
41j	100	>100	>100	>100	>100	>100	>100
41k	100	>100	>100	>100	>100	>100	>100
41l	100	>100	>100	>100	>100	>100	>100
41m	>100	>100	>100	>100	>100	>100	>100
41n	100	>100	>100	>100	>100	>100	>100
41o	≥20	>100	>100	>100	>100	>100	>100
41p	100	>100	>100	>100	>100	>100	>100
41q	≥4	>100	>100	>100	>100	>100	>100
DS.10.000	>100	>100	>100	100	45	2.3	0.8
Ribavirin	>250	146	>250	>250	>250	125	>250
Mycophenolic acid	≥100	0.4	0.5	20	45	4.0	0.8

[a]Required to cause a microscopically detectable alteration of normal cell morphology.
[b]Required to reduce virus-induced cytopathogenicity by 50 %.

Table.16 Cytotoxicity and antiviral activity in: **MDCK cell cultures**

Compound	Cytotoxicity		Antiviral EC50[c]					
	CC_{50}[a]	Minimum cytotoxic concentration[b]	*Influenza* A/H1N1 A/Ned/378/05		*Influenza* A/H3N2 A/HK/7/87		*Influenza* B B/Ned/537/05	
			visual CPE score	MTS	visual CPE score	MTS	visual CPE score	MTS
39a	>100	>100	>100	>100	>100	>100	>100	>100
39b	>100	>100	>100	>100	**19.8**	**11.1**	>100	>100
39c	>100	100	>100	>100	>100	>100	>100	>100
39d	>100	>100	>100	>100	>100	>100	>100	>100
39e	>100	>100	**20**	**4.2**	**12**	**5.8**	>100	>100
39f	>100	4	>100	>100	>100	>100	>100	>100
39g	>100	>100	>100	>100	**75**	**19.65**	>100	>100
39h	>100	20	>100	>100	**22**	**3.35**	>100	>100
39i	>100	>100	**54.45**	**10.7**	**15.85**	**7.35**	>100	>100
39j	>100	≥100	>100	>100	>100	>100	>100	>100
39k	>100	20	**50**	**12.7**	**32.5**	**14.95**	**50**	**73**
41a	>100	>100	>100	>100	>100	>100	>100	>100
41b	>100	>100	>100	>100	>100	>100	>100	>100
41c	>100	>100	**15**	**3.8**	**25**	**4.56**	**22**	**5.65**
41d	>100	>100	>100	>100	>100	>100	>100	>100
41e	>100	>100	**55.26**	**11.85**	**32.8**	**12.61**	>100	>100
41f	>100	>100	>100	>100	>100	>100	>100	>100
41g	>100	>100	>100	>100	>100	>100	>100	>100
41h	>100	>100	>100	>100	>100	>100	>100	>100
41i	>100	>100	>100	>100	>100	>100	>100	>100
41j	>100	>100	>100	>100	>100	>100	>100	>100
41k	>100	>100	>100	>100	>100	>100	>100	>100
41l	>100	>100	**25.25**	**8.54**	**32.10**	**11.25**	>100	>100
41m	>100	>100	>100	>100	>100	>100	>100	>100
41n	>100	>100	>100	>100	>100	>100	>100	>100
41o	>100	>100	>100	>100	>100	>100	>100	>100
41p	>100	>100	>100	>100	>100	>100	>100	>100
41q	>100	>100	>100	>100	>100	>100	>100	>100
Zanamivir	>100	>100	0.4	0.1	0.8	0.1	0.5	0.4
Ribavirin	>100	100	8.9	4.4	4.0	1.0	8.9	8.4
Amantadine	>100	>100	100	>100	0.2	0.1	>100	>100
Rimantadine	>200	>200	>200	>200	0.02	0.01	>200	>200

[a]50% Cytotoxic concentration, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay.
[b]Minimum compound concentration that causes a microscopically detectable alteration of normal cell morphology.
[c]50% Effective concentration, or concentration producing 50% inhibition of virus-induced cytopathic effect, as determined by visual scoring of the CPE, or by measuring the cell viability with the colorimetric formazan-based MTS assay.
MDCK cells: Madin Darby canine kidney cells

Table.17 Cytotoxicity and antiviral activity in: CRFK cell cultures

Compound	CC_{50} (μM) [a]	EC_{50} (μM)[b]	
		Feline Corona Virus (FIPV)	*Feline Herpes* Virus
39a	>100	>100	>100
39b	>100	>100	>100
39c	>100	>100	>100
39d	>100	>100	>100
39e	>100	>100	>100
39f	>100	>100	>100
39g	67.0	>100	>100
39h	17.4	>100	>100
39i	>100	>100	>100
39j	>100	>100	>100
39k	>100	>100	>100
41a	>100	>100	>100
41b	>100	>100	>100
41c	>100	>100	>100
41d	>100	>100	>100
41e	>100	>100	>100
41f	>100	>100	>100
41g	>100	>100	>100
41h	>100	>100	>100
41i	>100	>100	>100
41j	>100	>100	>100
41k	>100	>100	>100
41l	>100	>100	>100
41m	>100	>100	>100
41n	>100	>100	>100
41o	>100	>100	>100
41p	>100	>100	>100
41q	>100	>100	>100
HHA	>100	>100	>100
UDA	>100	5.7	2.0
Ganciclovir	>100	>100	3.4

[a]50% Cytotoxic concentration, as determined by measuring the cell viability with the colorimetric formazanb50%
[b]Effective concentration, or concentration producing 50% inhibition of virus-induced cytopathic effect, as
CRFK cells: Crandell-Rees Feline Kidney cells.

In conclusion we have synthesized dihydropyrazoly thiazoly coumarins via a facile one pot multicomponent reaction method and the compounds were evaluated against different human viruses. The compounds 39b, 39e, 39g, 39h, 39i, 39k, 41c, 41e and 41l were potent against influenza viruses.

EXPERIMENTAL:

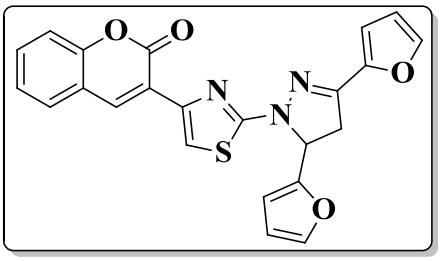
General procedure for the synthesis of 4,5-dihydro-1*H*-pyrazol-1-yl)thiazol-4-yl)-2*H*-chromen-2-one (39a-k and 41 a-q):

An ethanolic solution of 1,3-di(furan-2-yl)prop-2-en-1-one **38** (0.188 g, 1.0 mmol) or 3-aryl-1-(thiophen-2-yl)prop-2-en-1-one **40 a-e** (1.0 mmol), thiosemicarbazide **33** (0.091g, 1.0 mmol) and substituted 3-(2-bromoacetyl)-2*H*-chromen-2-ones **32 a-j** (1.0 mmol) was stirred at room temperature for about 10 min. To this reaction mixture sodium hydroxide (0.060 g, 1.5 mmol) pellet was added. The temperature of the reaction was slowly raised to 60 – 65 °C and maintained for about 4 h. The progress of the reaction was monitored through thin layer chromatography. After completion of the reaction, the temperature of the reaction mixture was cooled to room temperature. The isolated product was filtered, washed with ethanol, dried and recrystallized from ethanol solvent.

PHYSICAL AND SPECTRAL DATA

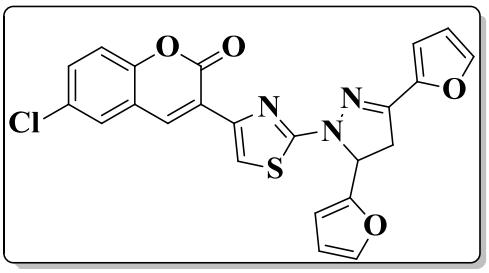
3-(2-(3,5-Di(furan-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)thiazol-4-yl)-2*H*-chromen-2-one (39a):

Yellow solid; m.p.: 188 – 190 °C; **FTIR (KBr, υ_{max}, cm^{-1})**: 1721 (-C=O), 1602 (-C=N); **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm):** 3.54 – 3.59(dd, *J* = 5.6 Hz, 1H, Pyrazole-Ha), 3.69 – 3.75 (dd, *J* = 9.6Hz, 1H, pyrazole-Hb), 5.74 – 5.77 (dd, *J* = 5.6Hz, 1H, pyrazole-Hx), 6.36 (s, 1H, Ar-H); 6.50 – 6.53 (dd, *J* = 2Hz, 2H, Furan-H), 6.79 (d, *J* = 2.8Hz, 1H, Ar-H), 7.29 (t, *J* = 6 Hz, 1H,-Ar-H), 7.33 (d, *J* = 6.8 Hz, 1H, -Ar-H), 7.38 (s, 1H, -Ar-H), 7.49 (t, *J* = 6.4 Hz, 1H, -Ar-H), 7.57 (d, *J* = 6.8 Hz, 2H, -Ar-H), 7.86 (s, 1H, -Ar-H), 8.44 (s, 1H, -C_4-H of coumarin); **^{13}C NMR (100 MHz, DMSO-d_6, ppm):** δ 31.74, 57.04, 109.15, 110.65, 111.24, 112.24, 113.62, 115.88, 119.13, 120.43, 124.72, 128.82, 131.71, 138.46, 142.85, 143.83, 144.68, 145.55, 145.91, 151.45, 152.30, 158.64, 163.42; **MS: (ESI – m/z):** 430.08 $[M+H]^+$; *Anal*.Calcd. for $C_{23}H_{15}N_3O_4S$: C, 64.33, H, 3.52, N, 9.78, S, 7.47%; Found: C, 64.39, H, 3.55, N, 9.72; S, 7.45%.



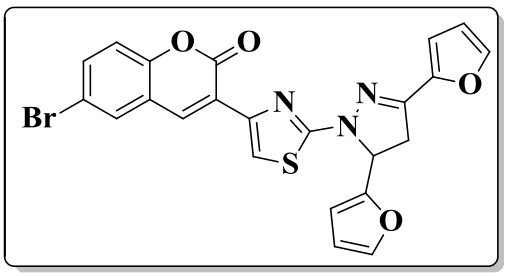
6-Chloro-3-(2-(3,5-di(furan-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)thiazol-4-yl)-2*H*-chromen-2-one (39b):

Yellow solid; m.p: 175 -177 °C; **FTIR (KBr, υ_{max}, cm^{-1})**: 1725 (-C=O), 1606 (-C=N); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 3.47 – 3.53 (dd, *J* = Hz, 1H, -CH_2), 3.84 – 3.91 (dd, *J* = Hz, 1H, -CH_2), 5.76 – 5.80 (dd, *J* =Hz, 1H, CH), 6.45 – 6.46 (dd, *J* = Hz, 1H, Ar-H), 6.69 – 6.70 (dd, *J* = Hz, 1H, Ar-H), 6.81 (d, *J* = 2.8 Hz, 1H, Ar-H), 7.03 (d, *J* = 3.2 Hz, 1H, Ar-H), 7.47 (d, *J* = 8.8 Hz, 1H, Ar-H), 7.63 – 7.65 (dd, *J* = Hz, 1H, Ar-H), 7.78 (s, 1H, Ar-H), 7.84 (s, 1H, Ar-H), 7.91 (d, *J* = 1.6 Hz, 1H, Ar-H), 8.08 (d, *J* = 2.4Hz, 1H, Ar-H), 8.48 (s, 1H, C_4-H of coumarin); **^{13}C NMR (100 MHz, DMSO-d_6, ppm):** δ 31.12, 56.88, 112.25, 113.64, 117.82, 118.12, 120.65, 121.40, 127.77, 128.50, 129.47, 131.12, 133.79, 137.09, 142.89, 143.60, 144.78, 145.57, 145.89, 150.89, 151.16, 158.25, 163.41; *Anal.*Calcd. for $C_{23}H_{14}ClN_3O_4S$: C, 59.55, H, 3.04, N, 9.06, S, 6.91%; Found: C, 59.60, H, 2.97, N, 9.12, S, 6.87%.



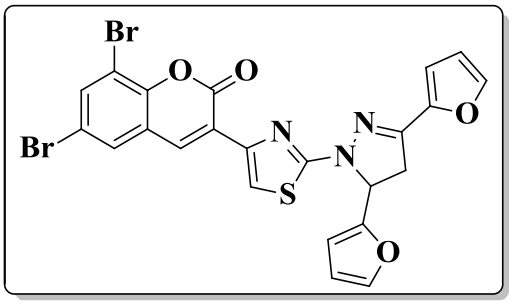
6-Bromo-3-(2-(3,5-di(furan-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)thiazol-4-yl)-2*H*-chromen-2-one (39c):

Red solid; m.p.: 211 – 213 °C; **FTIR (KBr, υ_{max}, cm^{-1})**: 1723 (-C=O), 1606 (-C=N); **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm):** 3.54 – 3.59 (dd, *J* = Hz, 1H,-CH_2), 3.70 – 3.76 (dd, *J* = Hz, 1H,-CH_2), 5.70 – 5.73 (dd, *J* = Hz, 1H, -CH), 6.37 (d, *J* = 1.6 Hz, 1H, Ar-H), 6.48 (d, *J* = 2.4 Hz, 1H, Furan-H), 6.53 (t, *J* = 1.6 Hz, 1H, Ar-H), 6.79 (d, *J* = 2.8 Hz, 1H, Ar-H), 7.21 (d, *J* = 7.2 Hz, 1H, Ar-H), 7.38 (s, 1H, Ar-H), 7.57 (t, *J* = 4.4 Hz, 2H, Ar-H), 7.69 (d, *J* = 1.6 Hz, 1H, Ar-H), 7.87 (s, 1H, Ar-H), 8.33 (s, 1H, C_4-H of coumarin); **^{13}C NMR (100 MHz, DMSO-d_6, ppm):** δ 31.09, 57.20, 110.05, 111.12, 112.49, 112.70, 114.17, 116.78, 118.53, 118.80, 121.58, 131.17, 132.91, 134.32, 136.98, 137.43, 143.35, 144.00, 145.20,146.03, 151.71, 158.65, 163.76; **MS(ESI - m/z):** 509 $[M+H]^+$; *Anal*. Calcd. for $C_{23}H_{14}BrN_3O_4S$: C, 54.34, H, 2.78, N, 8.27, S, 6.31%; Found: C, 54.29, H, 2.74, N, 8.32, S, 6.35%.



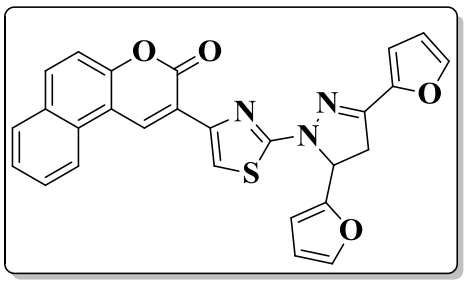
6,8-Dibromo-3-(2-(3,5-di(furan-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)thiazol-4-yl)-2*H*-chromen-2-one (39d):

Red solid; m.p.: 191 - 193 °C; **FTIR (KBr, υ_{max}, cm^{-1})**: 1724 (-C=O), 1602 (-C=N); **^{1}H NMR (400 MHz, $CDCl_3$-*d_6*, δ ppm):** 3.54 – 3.59 (dd, *J* = Hz, 1H, -CH_2), 3.70 – 3.76 (dd, *J* = Hz, 1H, -CH_2), 5.68 – 5.72 (dd, *J* = Hz, 1H, -CH), 6.38 (s, 1H, -Ar-H), 6.46 – 6.48 (dd, *J* = Hz, 1H, Furan-H), 6.53 (t, *J* = 1.6 Hz, 1H, Ar-H), 6.79 (d, *J* = 2.8 Hz, 1H, Ar-H), 7.22 (d, *J* = 6.8 Hz, 1H, Ar-H), 7.39 (s, 1H, Ar-H), 7.56 – 7.58 (dd, *J* = Hz, 1H, Furan-H), 7.62 – 7.69 (dd, *J* = Hz, 1H, Furan-H), 7.89 (d, *J* = 9.6 Hz, 1H, Ar-H), 8.32 (s, 1H, C_4-H of coumarin); **^{13}C NMR (100 MHz, DMSO-*d_6*, ppm):** δ 31.12, 57.32, 113.70, 116.39, 118.14, 121.19, 130.76, 132.16, 133.92, 137.04, 138.37, 142.93, 143.63, 144.81, 145.61, 151.15, 151.33, 158.24, 163.41; *Anal*.Calcd. for $C_{23}H_{13}Br_2N_3O_4S$: C, 47.04, H, 2.23, N, 7.16, S, 5.46%; Found: C, 46.99, H, 2.20, N, 7.22, S, 5.49%.



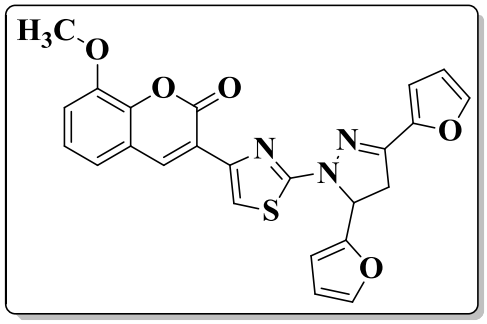
2-(2-(3,5-Di(furan-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)thiazol-4-yl)-3*H*-benzo[*f*] chromen-3-one (39e):

Yellow solid; m.p.: 279 – 281 °C; **FTIR (KBr, υ_{max}, cm^{-1})**: 1704 (-C=O), 1604 (-C=N); **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm):** 3.56 – 3.62 (dd, *J* = 8 Hz, 1H, Pyrazole-H_a), 3.72 – 3.79 (dd, *J* = 11.6 Hz, 1H, Pyrazole-H_b), 5.77 – 5.82 (dd, *J* = 8.8 Hz, 1H, Pyrazole-H_x), 6.43 – 6.44 (dd, *J* = 2 Hz, 1H, Ar-H), 6.53 – 6.54 (dd, *J* = 2Hz, 1H, Ar-H), 6.64 (d, *J* = 3.2 Hz, 1H, Ar-H), 6.80 (d, *J* = 3.2 Hz, 1H, Ar-H), 7.43 – 7.47 (m, 2H, Ar-H), 7.57 – 7.60 (m, 2H, Ar-H), 7.74 (t, *J* = 8.4 Hz, 1H, Ar-H), 7.89 – 7.95 (m, 3H, Ar-H), 8.37 (d, *J* = 8.4 Hz, 1H, Ar-H), 9.24 (s, 1H, C_4-H of coumarin); *Anal*.Calcd. for $C_{27}H_{17}N_3O_4S$: C, 67.63, H, 3.57, N, 8.76, S, 6.69%; Found: C, 67.66, H, 3.59, N, 8.70, S, 6.68%.



3-(2-(3,5-Di(furan-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)thiazol-4-yl)-8-methoxy-2*H*-chromen-2-one (39f):

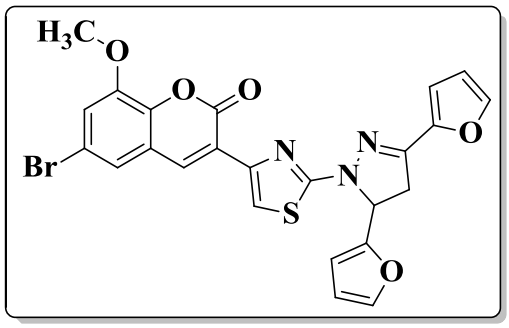
Yellow soild; m.p.: 167 – 169 °C; **FTIR (KBr, υ_{max}, cm^{-1})**: 1721 (-C=O), 1576 (-C=N), 1271 (-C-O-C); ^{1}H NMR (400 MHz, $CDCl_3$-*d_6*, δ ppm): 3.53 – 3.58 (dd, *J* = Hz, 1H, -



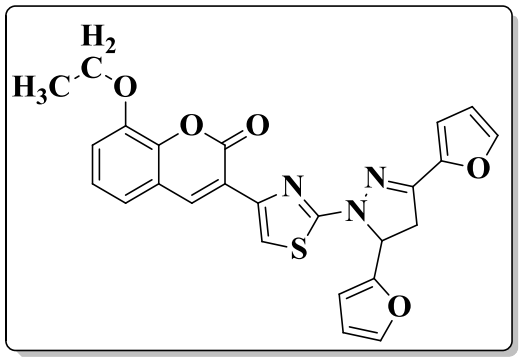
CH_2), 3.69 – 3.74 (dd, *J* = Hz, 1H, -CH_2), 3.96 (s, 3H, -OCH_3), 5.70 – 5.74 (dd, *J* = Hz, 1H, -CH), 6.36 (s, 1H, -Ar-H), 6.48 (d, *J* = 2.4 Hz, 1H, -Ar-H), 6.52 (d, *J* = 1.6 Hz, 1H, -Ar-H), 6.78 (d, *J* = 2.8 Hz, 1H, -Ar-H), 7.04 (d, *J* = 6.4 Hz, 1H, -Ar-H), 7.14 (d, *J* = 6.4 Hz, 1H, -Ar-H), 7.21 (t, *J* = 6.4 Hz, 1H, -Ar-H), 7.37 (s, 1H, -Ar-H), 7.55 (s, 1H, -Ar-H), 7.87 (s, 1H, -Ar-H), 8.39 (s, 1H, C_4-H of coumarin); **^{13}C NMR (100 MHz, DMSO-*d*$_6$, ppm):** δ 31.06, 56.06, 57.06, 109.12, 110.65, 111.33, 112.25, 113.63, 113.94, 119.70, 119.99, 120.56, 124.66, 138.66, 141.59, 142.85, 143.79, 144.69, 145.56, 145.92, 146.25, 151.49, 158.37, 163.46; **MS (ESI – m/z):** 460.05 $[M+H]^+$; *Anal*.Calcd. for $C_{24}H_{17}N_3O_5S$: C, 62.74, H, 3.73, N, 9.15, S, 6.98%; Found: C, 62.77, H, 3.69, N, 9.10, S, 7.04%.

6-Bromo-3-(2-(3,5-di(furan-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)thiazol-4-yl)-8-methoxy-2*H*-chromen-2-one (39g):

Red solid; m.p.: 193 – 195 °C; **FTIR (KBr, υ_{max}, cm^{-1}):** 1717 (-C=O), 1611 (-C=N), 1266 (-C-O-C); **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm):** 3.53 – 3.59 (dd, *J* = 6.8 Hz, 1H, Pyrazole-H_a), 3.69 – 3.77 (dd, *J* = 12 Hz, 1H, Pyrazole-H_b), 3.96 (s, 3H, -OCH_3), 5.71 – 5.75 (dd, *J* = 6.8 Hz, 1H, Pyrazole-H_x), 6.37 – 6.38 (dd, *J* = 2 Hz, 1H, Ar-H), 6.48 (d, *J* = 3.2 Hz, 1H, Ar-H), 6.52 – 6.53 (dd, *J* = 2 Hz, 1H, Ar-H), 6.79 (d, *J* = 3.6 Hz, 1H, Ar-H), 7.12 (d, *J* = 2 Hz, 1H, Ar-H), 7.28 (d, *J* = 2 Hz, 1H, Ar-H), 7.38 – 7.39 (dd, *J* = 0.4 Hz, 1H, Ar-H), 7.56 (d, *J* = 1.6 Hz, 1H, Ar-H), 7.89 (s, 1H, Ar-H), 8.30 (s, 1H, C_4-H of coumarin); **MS (ESI m/z):** 539.9 $[M+H]^+$; *Anal*.Calcd. for $C_{24}H_{16}BrN_3O_5S$: C, 53.54, H, 3.00, N, 7.81, S, 5.96%; Found: C, 53.56, H, 3.05, N, 7.76, S, 5.93%.

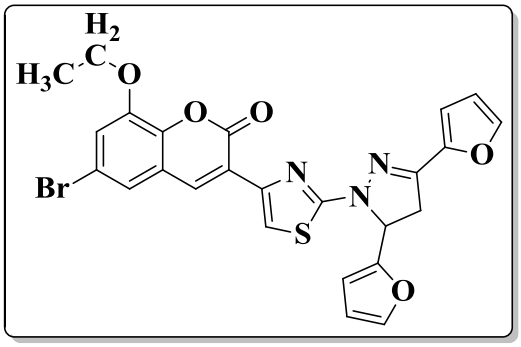


3-(2-(3,5-Di(furan-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)thiazol-4-yl)-8-ethoxy-2*H*-chromen-2-one (39h):



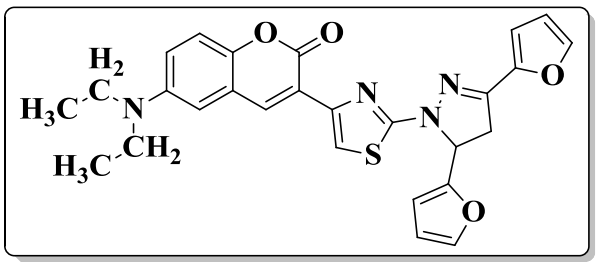
Yellow solid; m.p.: 174 – 176 °C; **FTIR (KBr, υ_{max}, cm^{-1})**: 1714 (-C=O), 1602 (-C=N), 1272 (-C-O-C); **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm):** 1.51 (t, *J* = 6.8 Hz, 3H, -CH_3), 3.53 – 3.59 (dd, *J* =6.8 Hz, 1H, Pyrazole-H_a), 3.68 – 3.75 (dd, *J* = 11.6 Hz, 1H, Pyrazole-H_b), 4.17 – 4.22 (m, 2H, -OCH_2), 5.71 – 5.76 (dd, *J* = 6.8 Hz, 1H, Pyrazole-H_x), 6.35 – 6.37 (dd, *J* = 2 Hz, 1H, Ar-H), 6.49 (d, *J* = 3.2 Hz, 1H,Ar-H), 6.52 – 6.53 (dd, *J* = 2 Hz, 1H, Ar-H), 6.78 (d, *J* = 3.6 Hz, 1H, Ar-H), 7.02 – 7.05 (dd, *J* = 1.6 Hz, 1H, Ar-H), 7.12 – 7.19 (dd, *J* = 6.4 Hz, 2H,Ar-H), 7.37 – 7.38 (dd, *J* = 0.8 Hz, 1H, Ar-H), 7.56 (d, *J* = 2 Hz, 1H, Ar-H), 7.88 (s, 1H, Ar-H), 8.40 (s, 1H, C_4-H of coumarin); **MS (ESI m/z):** 474.06 $[M+H]^+$; *Anal*.Calcd. for $C_{25}H_{19}N_3O_5S$: C, 63.41, H, 4.04, N, 8.87, S, 6.77%; Found: C, 63.46, H, 3.99, N, 8.84, S, 6.82%.

6-Bromo-3-(2-(3,5-di(furan-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)thiazol-4-yl)-8-ethoxy-2*H*-chromen-2-one (39i):



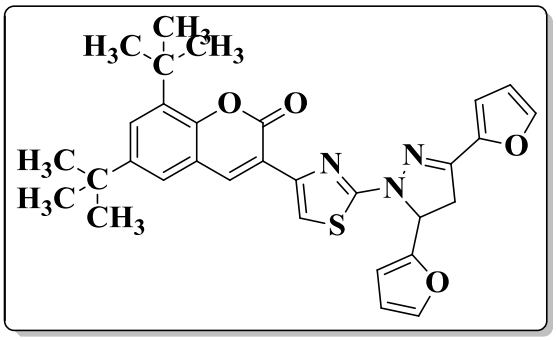
Red solid; m.p.: 166 – 168 °C; **FTIR (KBr, υ_{max}, cm^{-1})**: 1718 (-C=O), 1604 (-C=N), 1265 (-C-O-C); **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm):** 1.51(t, *J* = 5.2 Hz, 3H, -CH_3), 3.53 – 3.59 (dd, *J* = 6.8 Hz, 1H, Pyrazole-H_a), 3.69 – 3.76 (dd, *J* = 11.6 Hz, 1H, Pyrazole-H_b), 4.14 – 4.20 (m, 2H, -OCH_2), 5.68 – 5.73 (dd, *J* = 6.8 Hz, 1H, Pyrazole-H_x), 6.37 – 6.38 (dd, *J* = 2 Hz, 1H, Ar-H), 6.47 (d, *J* = 3.2 Hz, 1H, Ar-H), 6.52 – 6.53 (dd, *J* = 2 Hz, 1H, Ar-H), 6.79 (d, *J* = 3.6 Hz, 1H, Ar-H), 6.93 – 6.95 (dd, *J* = 2 Hz, 1H, Ar-H), 7.09 – 7.12 (m, 1H, Ar-H), 7.38 – 7.39 (dd, *J* = 0.8 Hz, 1H, Ar-H), 7.56 (d, *J* = 1.6 Hz, 1H, Ar-H), 7.89 (s, 1H, Ar-H), 8.28 (s, 1H, C_4-H of coumarin); **MS (ESI – m/z):** 553.9 $[M+H]^+$; *Anal*.Calcd. for $C_{25}H_{18}BrN_3O_5S$: C, 54.36, H, 3.28, N, 7.61, S, 5.80%; Found: C, 54.30, H, 3.36, N, 7.64, S, 5.76%.

3-(2-(3,5-Di(furan-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)thiazol-4-yl)-6-(diethylamino)-2*H*-chromen-2-one (39j):



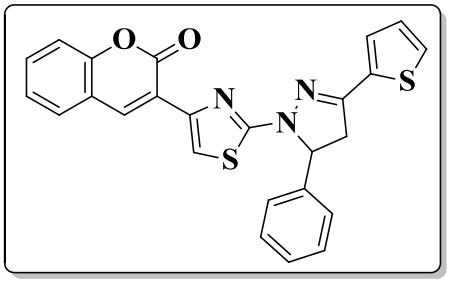
Green solid; m.p.: 201 -203 °C; **FTIR (KBr, υ_{max}, cm^{-1}):** 1708 (-C=O), 1617 (-C=N); **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm):** 1.22 (t, *J* = 5.6 Hz, 6H, $-CH_3$), 3.39 – 3.48 (m, 4H, $-CH_2$), 3.52 – 3.57 (dd, *J* = Hz, 1H, $-CH_2$), 3.66 – 3.72 (dd, *J* = Hz, 1H, $-CH_2$), 5.69 – 5.73 (dd, *J* = Hz, 1H, -CH), 6.35 (s, 1H, Ar-H), 6.45 – 6.50 (m, 3H, Ar-H), 6.60 (t, *J* = 6.8 Hz, 1H, Ar-H), 6.76 (d, *J* = 2.4 Hz, 1H, -Ar-H), 7.32 – 7.41 (m, 2H, Ar-H), 7.54 (s, 1H, Ar-H), 7.66 (s, 1H, Ar-H), 8.29 (s, 1H, C_4-H of coumarin); *Anal*. Calcd. for $C_{27}H_{24}N_4O_4S$: C, 64.78, H, 4.83, N, 11.19, S, 6.41%; Found: C, 64.81, H, 4.87, N, 11.15, S, 6.36%.

6,8-Di-*tert*-butyl-3-(2-(3,5-di(furan-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)thiazol-4-yl)-2*H*-chromen-2-one (39k):



Yellow solid; m.p.: 147 – 149 °C; **FTIR (KBr, υ_{max}, cm^{-1})**: 1722 (-C=O), 1551 (-C=N); **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm):** 1.38 (s, 9H, *t*-butyl) 1.53 (s, 9H, *t*-butyl), 3.55 – 3.60 (dd, *J* = Hz, 1H, $-CH_2$), 3.69 – 3.74 (dd, *J* = Hz, 1H, $-CH_2$), 5.74 – 5.78 (dd, *J* = Hz, 1H, -CH), 6.35 (s, 1H, Ar-H), 6.48 (d, *J* = 2.4 Hz, 1H, Ar-H), 6.52 (d, *J* = 1.2 Hz, 1H, Ar-H), 6.78 (d, *J* = 2.8 Hz, 1H, Ar-H), 7.37 (d, *J* = 2.8 Hz, 2H, Ar-H), 7.54 (d, *J* = 1.6 Hz, 1H, Ar-H), 7.56 (s, 1H, Ar-H), 7.85 (s, 1H, Ar-H), 8.42 (s, 1H, C_4-H of coumarin); **MS (ESI – m/z):** 542.20 $[M+H]^+$; *Anal*. Calcd. for $C_{31}H_{31}N_3O_4S$: C, 68.74, H, 5.77, N, 7.76, S, 5.92%; Found: C, 68.76, H, 5.82, N, 7.70, S, 5.94%.

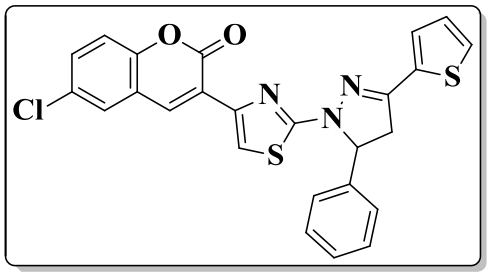
3-(2-(5-Phenyl-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)thiazol-4-yl)-2*H*-chromen-2-one (41a):



Yellow solid; m.p.: 189 – 191 °C; **IR (KBr, υ_{max}, cm^{-1}):** 1716 (-C=O), 1601 (-C=N), **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm):** 3.27 – 3.33 (dd, *J* = 7.2 Hz, 1H, $-CH_2$), 3.90 - 3.97 (dd, *J* = 11.6 Hz, 1H, $-CH_2$), 5.57 – 5.61 (dd, *J* = 7.2 Hz, 1H, -CH), 7.06

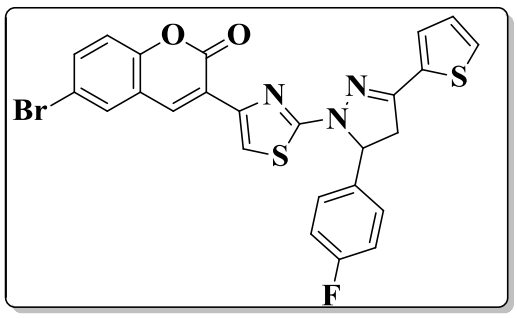
– 7.08 (m, 1H, Ar-H), 7.21 (d, *J* = 4 Hz, 1H, Ar-H), 7.37 (s, 4H, Ar-H), 7.40 (d, *J* = 2.4 Hz, 2H, Ar-H), 7.43 (d, *J* = 5.2Hz, 3H, Ar-H), 7.84 (s, 1H, Ar-H), 8.05 (s, 1H, Ar-H), 8.41 (s,1H, C_4-H of coumarin), Anal. Calcd for $C_{25}H_{17}N_3O_2S_2$ (455.55): C, 65.91; H, 3.76; N, 9.22, S, 14.08%, Found: C, 65.87; H, 3.73; N, 9.26, S, 14.12%.

6-Chloro-3-(2-(5-phenyl-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)thiazol-4-yl)-2*H*-chromen-2-one (41b)



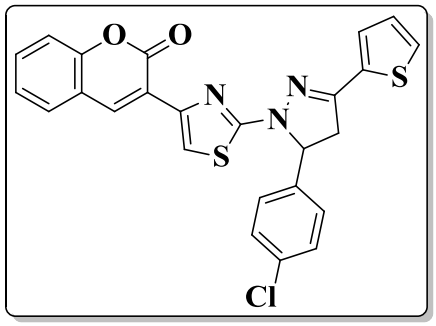
Yellow solid; m.p.: 157 – 159 °C; **IR (KBr, υ_{max}, cm^{-1}):** 1722 (-C=O), 1612 (-C=N), **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm):** 3.43 – 3.49 (dd, *J* = 6.4Hz, 1H, CH_2), 4.07 – 4.14 (dd, *J* = 11.6 Hz, 1H, CH_2), 5.66 – 5.71 (dd, *J* = 6.8 Hz, 1H, Ar-H), 7.17 – 7.20 (m, 1H, Ar-H), 7.45 – 7.49 (m, 5H, Ar-H), 7.53 (d, *J* = 8.8 Hz, 1H, Ar-H), 7.63 – 7.77 (m, 3H, Ar-H), 7.98 (d, *J* = 2.4Hz, 2H, Ar-H), 8.23 (s, 1H, C_4-H of coumarin); Anal. Calcd for $C_{25}H_{16}ClN_3O_2S_2$ (489.99): C, 61.28; H, 3.29; N, 8.58; S, 13.09%, Found: C, 61.33; H, 3.26; N, 8.55, S, 13.11%.

6-Bromo-3-(2-(5-(4-fluorophenyl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl) thiazol-4-yl)-2*H*-chromen-2-one (41c):



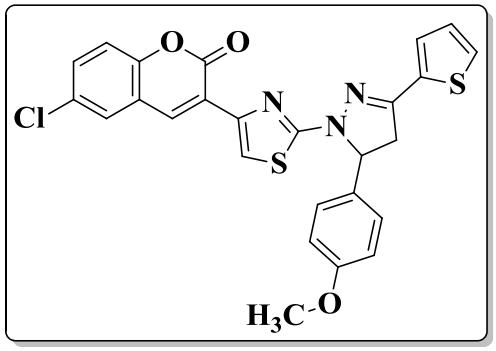
Red solid; m.p.: 225 - 227 °C; **IR (KBr, υ_{max}, cm^{-1}):** 1712 (-C=O), 1604 (-C=N); **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm):** 3.89 – 3.96 (dd, *J* = 11.2 Hz, 1H, -CH_2), 4.07 – 4.14 (dd, *J* = 11.6 Hz, 1H, CH_2), 5.67 – 5.71 (dd, *J* = 6.8 Hz, 1H, CH), 7.14 – 7.19 (m, 3H, Ar-H), 7.25 (t, *J* = 8.8 Hz, 3H, Ar-H), 7.49 (t, *J* = 3.2 Hz, 2H, Ar-H), 7.75 – 7.79 (m, 2H, Ar-H), 8.11 (s, 1H, Ar-H), 8.26 (s, 1H, C4-H of coumarin); **MS (ESI m/z):** 552 $[M+H]^+$; Anal. Calcd for $C_{25}H_{15}BrFN_3O_2S_2$ (552.43): C, 54.35; H, 2.74; N, 7.61, S, 11.61%, Found: C, 54.32; H, 2.79; N, 7.64, S, 11.58%.

3-(2-(5-(4-Chlorophenyl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)thiazol-4-yl)-2*H*-chromen-2-one (41d):



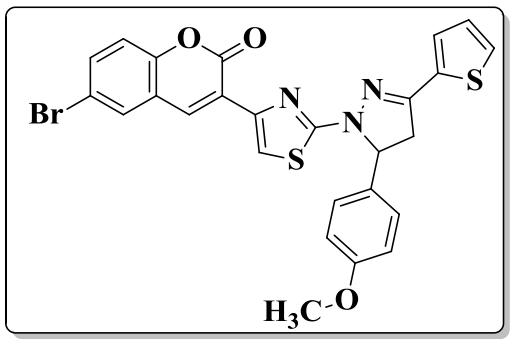
Yellow solid; m.p.: 162- 164 °C; **IR (KBr, υ_{max}, cm^{-1}):** 1716 (-C=O), 1614 (-C=N); **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm):** 3.41 – 3.47 (dd, *J* = 7.2 Hz, 1H, CH_2), 4.05 – 4.13 (dd, *J* = 12 Hz, 1H, CH_2), 5.66 – 5.71 (dd, *J* = 6.8 Hz, 1H, CH), 7.18 (t, *J* = 4 Hz, 1H, Ar-H), 7.38 – 7.43 (m, 2H, Ar-H), 7.48 (t, *J* = 6.8 Hz, 3H, Ar-H), 7.54 (d, *J* =8.4 Hz, 2H, Ar-H), 7.62 (t, *J* = 8 Hz, 1H, Ar-H), 7.72 (s, 1H, Ar-H), 7.77 (t, *J* = 6 Hz, 2H, Ar-H), 8.23 (s, 1H, C_4-H of coumarin); Anal. Calcd for $C_{25}H_{16}ClN_3O_2S_2$ (489.99): C, 61.28; H, 3.29; N, 8.58, S, 13.09%, Found: C, 61.24; H, 3.31; N, 8.62, S, 13.06%.

6-Chloro-3-(2-(5-(4-methoxyphenyl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)thiazol-4-yl)-2*H*-chromen-2-one (41e):



Yellow solid; m.p.: 178 – 180 °C; **IR (KBr, υ_{max}, cm^{-1}):** 1724 (-C=O), 1606 (-C=N), 1248 (-C-O-C); **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): HR-MS (ESI m/z):** Exact Mass calculated for $C_{26}H_{19}ClN_3O_3S_2$ $[M+H]^+$: 520.0556, Found: 520.0553; Anal. Calcd for $C_{26}H_{18}ClN_3O_3S_2$ (520.02): C, 60.05; H, 3.49; N, 8.08, S, 12.33%, Found: C, 60.07; H, 3.52; N, 8.05, S, 12.30%.

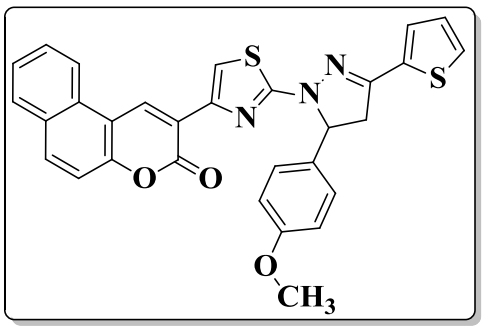
6-Bromo-3-(2-(5-(4-methoxyphenyl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl) thiazol-4-yl)-2*H*-chromen-2-one (41f):



Red solid; m.p.: 216 - 218°C; **IR (KBr, υ_{max}, cm^{-1}):** 1722 (-C=O), 1610 (-C=N), 1245 (-C-O-C); **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm):** 3.41 – 3.47 (dd, *J* = 6.8 Hz, 1H, $-CH_2$), 3.73 (s, 3H, $-OCH_3$), 4.04 – 4.11 (dd, *J* = 11.6 Hz, 1H, CH_2), 5.62 – 5.67 (dd, *J* = 6.8 Hz, 1H, -CH), 6.97 (d, *J* = 8.8 Hz, 1H, Ar-H), 7.40 – 7.47 (m, 5H, Ar-H), 7.75 – 7.78 (m, 3H, Ar-H), 7.88 – 7.91 (dd, *J* = 2.4 Hz, 1H, Ar-H), 8.22 (s, 1H, Ar-H), 8.61 (s, 1H, C_4-H of coumarin); **HR-MS (ESI m/z):** Exact Mass calculated for $C_{26}H_{19}BrN_3O_3S_2$ $[M+H]^+$: 564.0051, Found: 566.0024; Anal.Calcd for

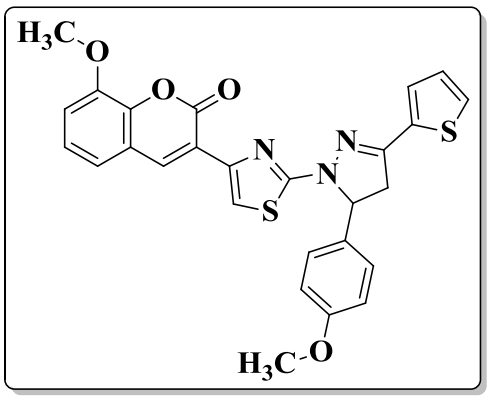
$C_{26}H_{18}BrN_3O_3S_2$ (564.47): C, 55.32; H, 3.21; N, 7.44, S, 11.36%, Found: C, 55.35; H, 3.18; N, 7.48, S, 11.33%.

2-(2-(5-(4-Methoxyphenyl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)thiazol-4-yl)-3*H*-benzo[*f*]chromen-3-one (41g):



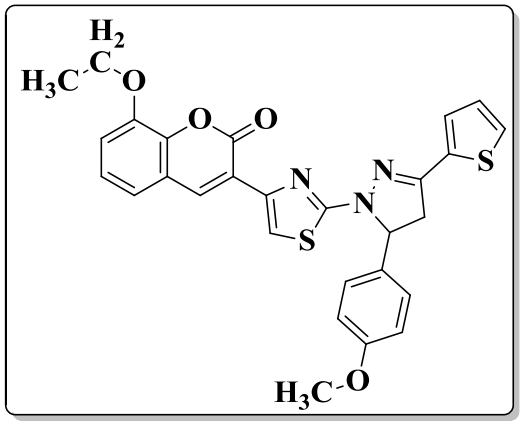
Yellow solid; m.p.: >300 °C; **IR (KBr, υ_{max}, cm^{-1}):** 1711 (-C=O), 1607 (-C=N), 1245 (-C-O-C); **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm):** 3.48 – 3.54 (dd, *J* = 7.2 Hz, 1H, CH_2), 3.85 (s, 3H, -OCH_3), 4.13 – 4.18 (dd, *J* = 7.2 Hz, 1H, CH2), 5.17 – 5.31 (dd, *J* = 14.8 Hz, 1H, -CH), 6.72 (d, *J* = 8.8Hz, 1H, Ar-H), 6.80 (d, *J* = 8.8Hz, 1H, Ar-H), 6.90 (d, *J* = 8.8Hz, 1H, Ar-H), 7.12 (d, *J* = 8.8 Hz, 1H, Ar-H), 7.19 (d, *J* = 8.8Hz, 1H, Ar-H), 7.53 (t, *J* = 6 Hz, 3H, Ar-H), 7.72 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.85 (d, *J* = 6 Hz, 3H, Ar-H), 8.05 (s, 1H, Ar-H), 8.64 (s, 1H, C_4-H of coumarin); **HR-MS (ESI m/z):** Exact Mass calculated for $C_{30}H_{22}N_3O_3S_2$ $[M+H]^+$: 536.1103, Found: 536.1093; Anal. Calcd for $C_{30}H_{21}N_3O_3S_2$ (535.63): C, 67.27; H, 3.95; N, 7.84, S, 11.97%, Found: C, 67.25; H, 3.99; N, 7.80, S, 11.95 %.

8-Methoxy-3-(2-(5-(4-methoxyphenyl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)thiazol-4-yl)-2*H*-chromen-2-one (41h):



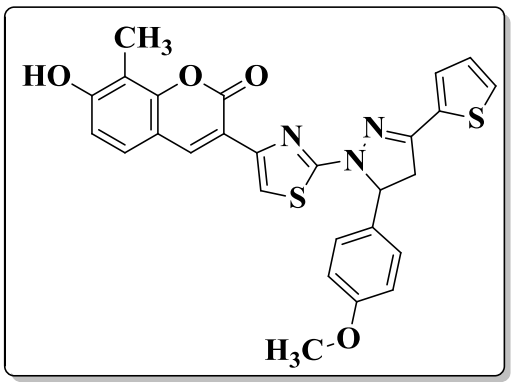
Yellow solid; m.p.: 197 - 199 °C; **IR (KBr, υ_{max}, cm^{-1}):** 1723 (-C=O), 1608 (-C=N), 1273 (-C-O-C); **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm):** 3.72 (s, 3H, -OCH_3), 3.79 – 3.83 (dd, *J* = 9.2 Hz, 1H, CH2), 3.92 (s, 3H, -OCH_3), 4.02 – 4.09 (dd, *J* = 10.4 Hz, 1H, CH2), 5.61 – 5.66 (dd, *J* = 6 Hz, 1H, -CH), 6.98 (s, 2H, Ar-H), 7.20 (s, 1H, Ar-H), 7.33 (s, 3H, Ar-H), 7.45 (t, *J* = 7.2 Hz, 3H, Ar-H), 7.71 (s, 1H, Ar-H), 7.76 (s, 1H, Ar-H), 8.27 (s, 1H, C_4-H of coumarin); **HR-MS (ESI m/z):** Exact Mass calculated for $C_{27}H_{22}N_3O_4S_2$ $[M+H]^+$: 516.1052, Found: 516.1057; Anal.Calcd for $C_{27}H_{21}N_3O_4S_2$ (515.60): C, 62.90; H, 4.11; N, 8.15, S, 12.44%; Found: C, 62.94; H, 4.08; N, 8.18, S, 12.41%.

8-Ethoxy-3-(2-(5-(4-methoxyphenyl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)thiazol-4-yl)-2*H*-chromen-2-one (41i):



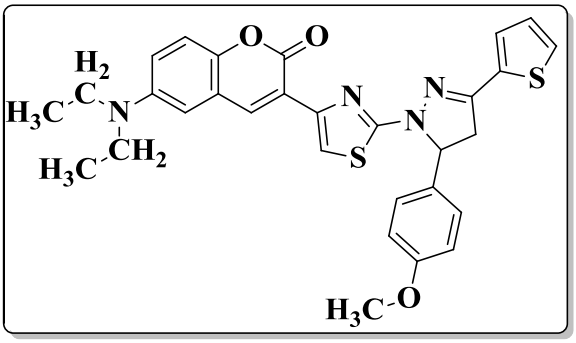
Yellow solid; m.p.: 218 – 220 °C; **IR (KBr, υ_{max}, cm^{-1}):** 1726 (-C=O), 1612 (-C=N), 1275 (-C-O-C); **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm):** 1.40 (t, *J* = 6.8 Hz, 3H, -CH3), 3.37 – 3.44 (dd, *J* = 8.4 Hz, 1H, CHa), 3.71 (s, 3H, -OCH_3), 4.00 – 4.07 (dd, *J* = 12 Hz, 1H, CHb), 4.13 – 4.15 (m, 2H, -OCH_2), 5.59 – 5.63 (dd, *J* = 6.8 Hz, 1H, -CHx), 6.96 (d, *J* = 8.4 Hz, 2H, Ar-H), 7.17 (t, *J* = 4 Hz, 1H, Ar-H), 7.24 – 7.30 (m, 3H, Ar-H), 7.41 (d, *J* = 8.4 Hz, 2H, Ar-H), 7.46 (d, *J* = 3.6 Hz, 1H, Ar-H), 7.69 (s, 1H, Ar-H), 7.74 (d, *J* = 5.2 Hz, 1H, Ar-H), 8.23 (s, 1H, C_4-H of coumarin); **^{13}C NMR (100 MHz, DMSO-d6, ppm):** δ 15.06, 44.12, 55.54, 64.47, 64.80, 111.56, 114.35, 115.14, 120.19, 120.96, 125.17, 128.54, 128.89, 129.76, 130.25, 133.74,134.47, 139.04, 142.08, 144.23, 145.97, 149.71, 158.88, 159.24, 163.79; **HR-MS (ESI m/z):** Exact Mass calculated for $C_{28}H_{24}N_3O_4S_2$ $[M+H]^+$: 530.1208, Found: 530.1196; Anal. Calcd for $C_{28}H_{23}N_3O_4S_2$ (529.62): C, 63.50; H, 4.38; N, 7.93, S, 12.11%, Found: C, 63.45; H, 4.40; N, 7.90, S, 12.13%.

7-Hydroxy-3-(2-(5-(4-methoxyphenyl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)thiazol-4-yl)-8-methyl-2*H*-chromen-2-one (41j)



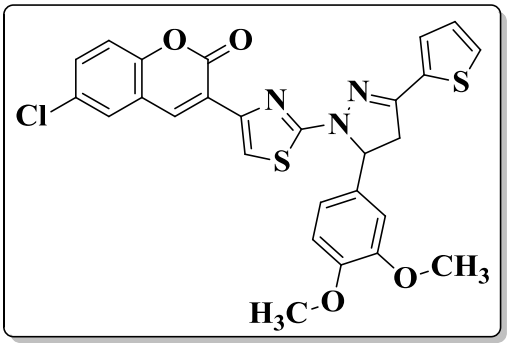
Yellow solid; m.p.: 257 – 259 °C; **IR (KBr, υ_{max}, cm^{-1}):** 3269 (-OH), 1722 (-C=O), 1607 (-C=N), 1252 (-C-O-C); **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm):** ; **^{13}C NMR (100 MHz, DMSO-d6, ppm):** δ 7.87, 43.62, 55.07, 63.99, 108.85, 110.39, 111.48, 112.62, 113.87, 115.75, 126.75, 128.06, 128.39, 129.21, 129.70, 133.36, 134.05, 139.59, 144.27, 149.06, 152.29, 158.74, 159.21, 163.27; **HR-MS (ESI m/z):** Exact Mass calculated for $C_{27}H_{22}N_3O_4S_2$ $[M+H]^+$: 516.1052, Found: 516.1036; Anal. Calcd for $C_{27}H_{21}N_3O_4S_2$ (515.60): C, 62.90; H, 4.11; N, 8.15, S, 12.44%, Found: C, 62.93; H, 4.15; N, 8.12, S, 12.40%.

6-(Diethylamino)-3-(2-(5-(4-methoxyphenyl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)thiazol-4-yl)-2*H*-chromen-2-one (41k):



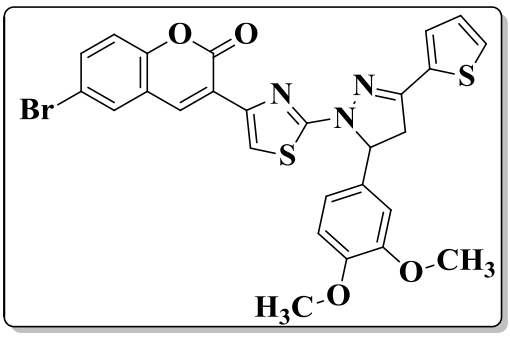
Green solid; m.p.: 236 - 238 °C; **IR (KBr, υ_{max}, cm^{-1}):** 1722 (-C=O), 1609 (-C=N), 1256 (C-O-C); **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm):** 1.21 (t, *J* = 5.6 Hz, 6H, -CH_3), 3.29 – 3.34 (dd, *J* = 6 Hz, 1H, CH2), 3.40 – 3.41 (m, 4H, -NCH_2), 3.78 (s, 3H, -OCH_3), 3.85 – 3.92 (dd, *J* = 11.6 Hz, 1H, CH2), 5.64 – 5.60 (dd, *J* = 7.6 Hz, 1H, CH), 6.48 (s, 1H, Ar-H), 6.59 (d, *J* = 7.6 Hz, 1H, Ar-H), 6.90 (d, *J* = 7.2 Hz, 2H, Ar-H), 7.06 (s, 1H, Ar-H), 7.20 (s, 1H, Ar-H), 7.38 (d, *J* = 8 Hz, 4H, Ar-H), 7.61 (s, 1H, Ar-H), 8.13 (s, 1H, C_4-H of coumarin); **MS (ESI m/z):** 557.16 $[M+H]^+$; Anal. Calcd for $C_{30}H_{28}N_4O_3S_2$ (556.69): C, 64.72; H, 5.07; N, 10.06, S, 11.62%, Found: C, 64.68; H, 5.05; N, 10.10, S, 11.55%.

6-Chloro-3-(2-(5-(3,4-dimethoxyphenyl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)thiazol-4-yl)-2*H*-chromen-2-one (41l):



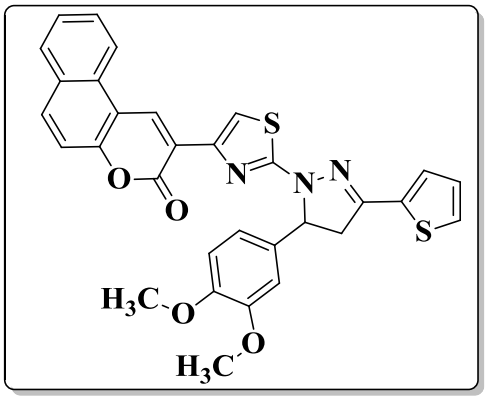
Yellow solid; m.p.: 278 - 280 °C; **IR (KBr, υ_{max}, cm^{-1}):** 1723 (-C=O), 1605 (-C=N), 1259 (C-O-C); **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm):** 3.45 – 3.51 (dd, *J* = 6.4 Hz, 1H, -CHa), 3.71 (s, 3H, -OCH_3), 3.77 (s, 3H, -OCH_3), 4.02 – 4.09 (dd, *J* = 11.6 Hz, 1H), 5.59 – 5.64 (dd, *J* = 6.4 Hz, 1H, Ar-H), 6.97 (s, 2H, Ar-H), 7.14 (s, 1H, Ar-H), 7.18 (t, *J* =3.6 Hz, 1H, Ar-H), 7.47 (t, *J* = 8.8 Hz, 2H, Ar-H), 7.62 – 7.65 (dd, *J* = 2.4 Hz, 1H, Ar-H), 7.75 (d, *J* = 4 Hz, 2H, Ar-H), 7.86 (d, *J* = 2.8 Hz, 1H, Ar-H), 8.34 (s, 1H, C_4-H of coumarin); **^{13}C NMR (100 MHz, DMSO-d6, ppm):** δ 43.99, 55.95, 56.12, 64.71, 111.93, 112.39, 118.41, 119.61, 121.09, 121.93, 127.80, 128.56, 128.97, 129.80, 130.32, 131.57, 134.00, 134.46, 137.45, 144.04, 148.87, 149.09, 149.97, 151.38, 158.69, 163.95; **MS (ESI m/z):** 550.04 $[M+H]^+$; Anal. Calcd for $C_{27}H_{20}ClN_3O_4S_2$ (549.05): C, 58.96; H, 3.66; N, 7.64, S, 11.66%, Found: C, 59.00; H, 3.60; N, 7.66, S, 11.68%.

6-Bromo-3-(2-(5-(3,4-dimethoxyphenyl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl) thiazol-4-yl)-2*H*-chromen-2-one (41m):



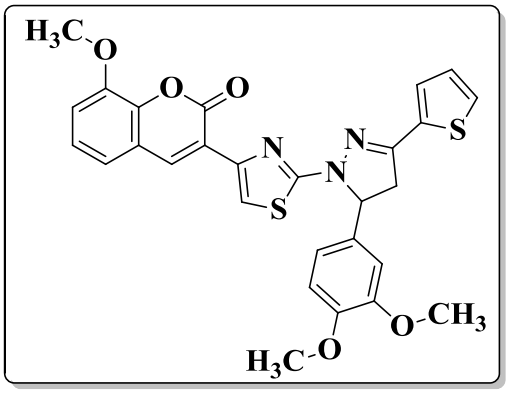
Red solid; m.p.: 246 - 248 °C; **IR (KBr, υ_{max}, cm^{-1}):** 1720 (-C=O), 1603 (-C=N), 1259 (C-O-C); **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm):** 3.45 – 3.51 (dd, *J* = 6.8 Hz, 1H, -CHa), 3.71 (s, 3H, $-OCH_3$), 3.77 (s, 3H, $-OCH_3$), 4.02 – 4.09 (dd, *J* = 11.6 Hz, 1H, -CHb), 5.59 – 5.63 (dd, *J* = 6.8 Hz, 1H, CHx), 6.97 (s, 2H, Ar-H), 7.14 (s, 1H, Ar-H), 7.18 (t, *J* = 4 Hz, 1H,Ar-H), 7.40 (d, *J* = 8.8 Hz, 1H, Ar-H), 7.48 (d, *J* = 3.2 Hz, 1H, Ar-H), 7.74 (t, *J* = 3.2 Hz, 3H, Ar-H), 7.99 (d, *J* = 2.4 Hz, 1H, Ar-H), 8.34 (s, 1H, C4-H of coumarin); **^{13}C NMR (100 MHz, DMSO-d6, ppm):** δ 43.98, 55.95, 56.11, 64.71, 111.97, 112.42, 116.81, 118.69, 119.62, 120.54, 121.60, 121.88, 128.57, 129.81, 130.80, 134.00, 134.46, 137.40, 144.04, 148.87, 149.97, 151.79, 158.65, 163.93; **MS (ESI m/z):** 596.02 $[M+2H]^+$; Anal. Calcd for $C_{27}H_{20}BrN_3O_4S_2$ (594.49): C, 54.55; H, 3.39; N, 7.07, S, 10.79%, Found: C, 54.59; H, 3.36; N, 7.10, S, 10.75%.

2-(2-(5-(3,4-Dimethoxyphenyl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl) thiazol-4-yl)-3*H*-benzo[*f*]chromen-3-one (41n)



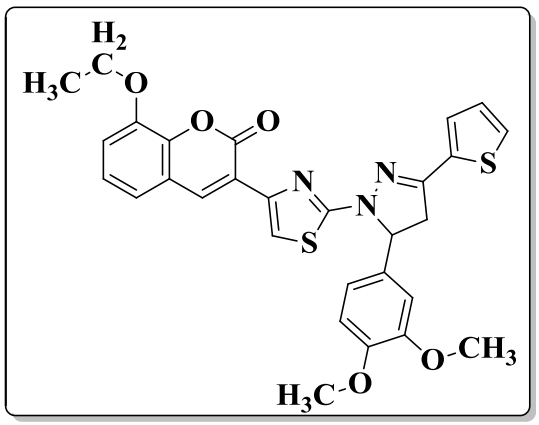
Yellow solid; m.p.: 291 – 293 °C; **IR (KBr, υ_{max}, cm^{-1}):** 1707 (-C=O), 1596 (-C=N), 1258 (C-O-C); **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm):** 3.39 – 3.46 (dd, *J* = 8.4Hz, 1H, CHa), 3.71 (s, 3H, $-OCH_3$), 3.79 (s, 3H, $-OCH_3$), 4.01 – 4.08 (dd, *J* = 11.6 Hz, 1H, -CHb), 5.59 – 5.64 (dd, *J* = 8.8 Hz, 1H, -CHx), 7.05 – 7.10 (m, 2H, Ar-H), 7.19 (t, *J* = 4.8 Hz, 1H, Ar-H), 7.23 (s, 1H, Ar-H), 7.48 (d, *J* = 2.8 Hz, 1H, Ar-H), 7.55 (d, *J* = 8.8 Hz, 1H, Ar-H), 7.67 (t, *J* = 7.2 Hz, 1H, Ar-H), 7.75 (t, *J* = 5.6 Hz, 2H, Ar-H), 7.88 (t, *J* = 7.6 Hz, 1H, Ar-H), 8.06 (d, *J* = 8.4 Hz, 1H, Ar-H), 8.13 – 8.18 (dd, *J* = 9.2 Hz, 2H, Ar-H), 8.91 (s, 1H, C_4-H of coumarin); **MS (ESI m/z):** 566.10 $[M+H]^+$; Anal. Calcd for $C_{31}H_{23}N_3O_4S_2$ (565.66): C, 65.82; H, 4.10; N, 7.43, S, 11.34%, Found: C, 65.86; H, 4.05; N, 7.46, S, 11.30%.

3-(2-(5-(3,4-Dimethoxyphenyl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl) thiazol-4-yl)-8-methoxy-2*H*-chromen-2-one (41o)



Yellow solid; m.p.: 202 - 204 °C; **IR (KBr, υ_{max}, cm^{-1}):** 1724 (-C=O), 1574 (-C=N), 1277 (C-O-C); **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm):** 3.45 – 3.50 (dd, *J* = 5.6 Hz, 1H, CH2), 3.71 (s, 3H, -OCH_3), 3.79 (s, 3H, -OCH_3), 3.92 (s, 3H, -OCH_3), 4.02 – 4.07 (dd, *J* = 9.2 Hz, 1H, CH2), 5.59 – 5.63 (dd, *J* = 5.6 Hz, 1H, -CH), 6.97 (s, 2H, Ar-H), 7.18 (s, 2H, Ar-H), 7.25 (d, *J* = 6 Hz, 1H, Ar-H), 7.29 – 7.34 (m, 2H, Ar-H), 7.48 (d, J = 3.6 Hz, 1H, Ar-H), 7.72 (s, 1H, Ar-H), 7.75 (d, *J* = 4.8 Hz, 1H, Ar-H), 8.32 (s, 1H, C_4-H of coumarin); **MS (ESI m/z):** 546.10 $[M+H]^+$; Anal.Calcd for $C_{28}H_{23}N_3O_5S_2$ (545.62): C, 61.64; H, 4.25; N, 7.70, S, 11.75 %, Found: C, 61.61; H, 4.23; N, 7.74, S, 11.77%.

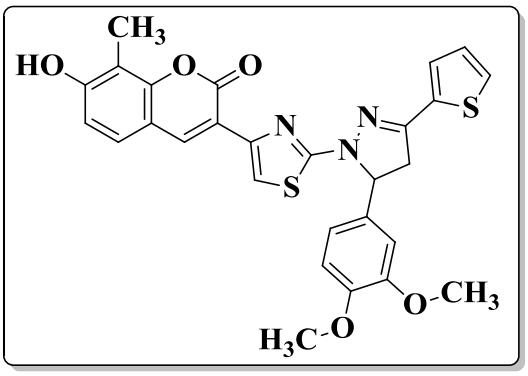
3-(2-(5-(3,4-Dimethoxyphenyl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)thiazol-4-yl)-8-ethoxy-2*H*-chromen-2-one (41p)



Yellow solid; m.p.: 227 – 229 °C; **IR (KBr, υ_{max}, cm^{-1}):** 1729 (-C=O), 1586 (-C=N), 1272 (C-O-C); **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): , ^{13}C NMR (100 MHz, DMSO-d6, ppm):** δ 14.59, 43.55, 55.50, 55.63, 64.38, 111.21, 111.59, 111.90, 114.71, 118.97, 119.43, 119.73, 124.79, 128.08, 129.30, 129.81, 133.69, 134.01, 138.55, 141.64, 143.74, 145.55, 148.31, 148.53, 149.38, 158.52, 163.50; **MS (ESI m/z):** 560.13 $[M+H]^+$; Anal. Calcd for $C_{29}H_{25}N_3O_5S_2$ (559.65): C, 62.24; H, 4.50; N, 7.51, S, 11.46 %, Found: C, 62.27; H, 4.46; N, 7.54, S, 11.43%.

3-(2-(5-(3,4-Dimethoxyphenyl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)thiazol-4-yl)-7-hydroxy-8-methyl-2*H*-chromen-2-one (41q)

Yellow solid; m.p.: 278 – 280 °C; **IR (KBr, υ_{max}, cm^{-1}):** 3426 (-OH), 1716 (-C=O), 1608 (-C=N), 1258 (C-O-C); **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): ; HR-MS (ESI m/z):**



Exact Mass calculated for $C_{28}H_{23}N_3O_5S_2$ $[M+H]^+$: 546.1157, Found: 546.1145; Anal. Calcd for $C_{28}H_{23}N_3O_5S_2$ (545.62): C, 61.64; H, 4.25; N, 7.70, S, 11.75%, Found: C, 61.69; H, 4.20; N, 7.73, S, 11.71%.

Antiviral activity evaluation

The antiviral activity of the new compounds was determined in different cell types. (i) HEL cells were seeded in 96-well plates and incubated for 6 days at 37°C until confluency was reached. Medium was aspirated and replaced by serial dilutions of the test compounds (100 µl per well). One hundred microliters of the virus (*Herpes simplex virus type 1, Herpes simplex virus type 2, Herpes simplex virus type 1 TK- ACV^r, Vaccinia virus, Adeno virus-2* or *Vesicular stomatitis virus*), diluted in medium to obtain a virus input of 100 $CCID_{50}$ (1 $CCID_{50}$ being the virus dose that is able to infect 50% of the cell cultures), was added to each well. Mock-treated cell cultures receiving solely the test compounds were included, to determine the cytotoxicity. After 4 days of incubation at 37°C, microscopical analysis was performed to score the virus-induced cytopathicity. (ii) Vero cells were seeded in 96-well plates at 30,000 cells per well and incubated for 1 day at 37°C until confluency was reached. Medium was aspirated and replaced by serial dilutions of the test compounds (100 µl per well). One hundred microliters of the virus (*Coxsackie virus B4, Sindbis virus, Parainfluenza virus 3, Punta Toro virus* or *Reovirus*) diluted in medium to obtain a virus input of 100 $CCID_{50}$ was added to each well. Mock-treated cell cultures receiving solely the test compounds were included, to determine the cytotoxicity. After 3 days (for Coxsackie virus B4 and Sindbis virus) or 6 days (for Parainfluenza virus 3, Punta Toro virus and Reovirus) of incubation at 37°C microscopical analysis was performed to score the virus-induced cytopathicity. (iii) HeLa cells were seeded in 96-well plates at 15,000 cells per well and incubated for 1 day at 37°C until confluency was reached. Medium was aspirated and replaced by serial dilutions of the test compounds (100 µl per well). One hundred microliters of the virus (*Coxsackie virus B4, Vesicular stomatitis virus* or *Respiratory syncytial virus*) diluted in medium to obtain a virus input of 100 $CCID_{50}$ was added to each well. Mock-treated cell

cultures receiving solely the test compounds were included, to determine the cytotoxicity. After 3 days (for Coxsackie virus B4 and Vesicular stomatitis virus) or 6 days (for Respiratory syncytial virus) of incubation at 37°C microscopical analysis was performed to score the virus-induced cytopathicity. (iv) MDCK cells were seeded in 96-well plates at 7,500 cells per well and incubated for 1 day at 35°C. Medium was aspirated and replaced by serial dilutions of the test compounds (100 µl per well). One hundred microliters of the virus (*Influenza A/H1N1 A/Ned/378/05, Influenza A/H3N2 A/HK/7/87* or *Influenza B B/Ned/537/05*) diluted in medium to obtain a virus input of 100 $CCID_{50}$ was added to each well. Mock-treated cell cultures receiving solely the test compounds were included, to determine the cytotoxicity. After 4 days of incubation at 35°C the virus-induced cytopathicity was determined by visual scoring of the CPE, as well as by measuring the cell viability with the colorimetric formazan-based MTS assay. (v) CRFK cells were seeded in 96-well plates at 30,000 cells per well and incubated for 1 day at 37°C until confluency was reached. Medium was aspirated and replaced by serial dilutions of the test compounds (100 µl per well). One hundred microliters of the virus (*Feline herpes virus* or *Feline corona virus*) diluted in medium to obtain a virus input of 100 $CCID_{50}$ was added to each well. Mock-treated cell cultures receiving solely the test compounds were included, to determine the cytotoxicity. After 4 days of incubation at 37°C the virus-induced cytopathicity was determined by measuring the cell viability with the colorimetric formazan-based MTS assay[65-67].

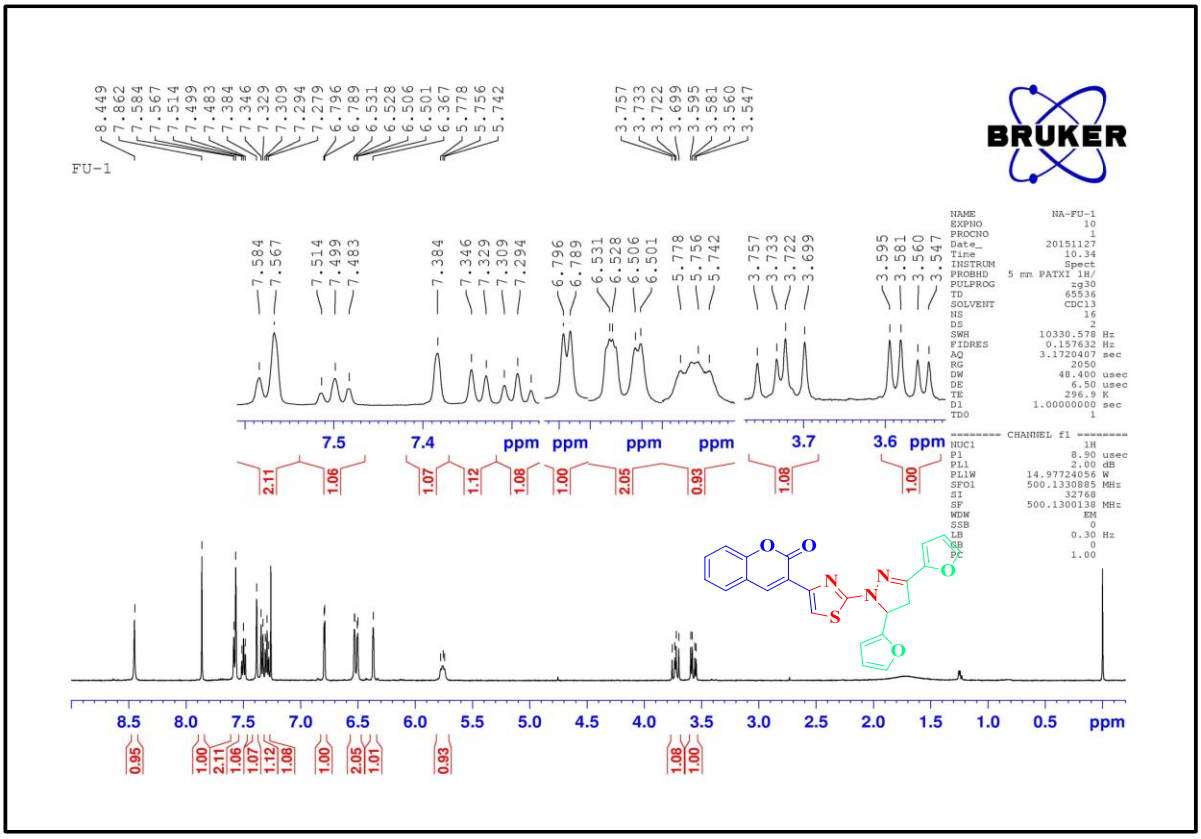


Fig: ^{1}H NMR spectrum of compound **39a**

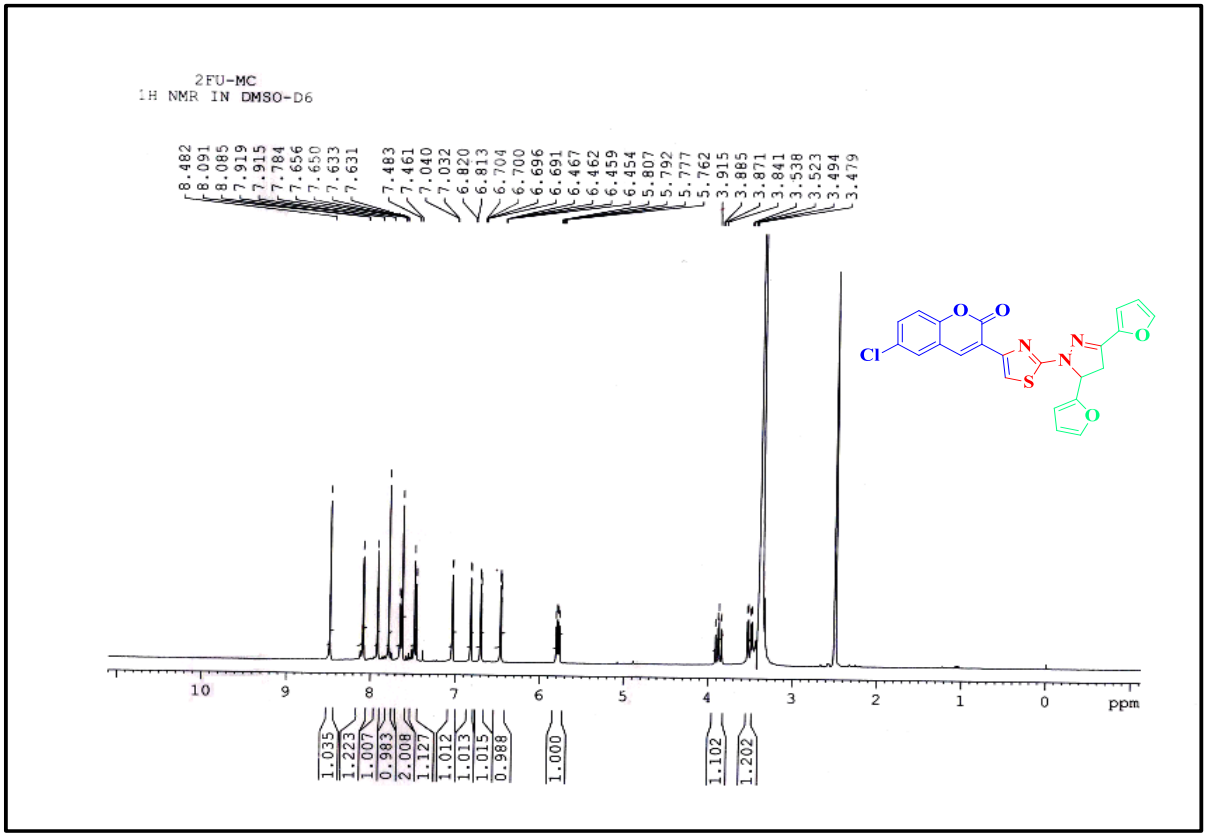


Fig: ^{1}H NMR spectrum of compound **39b**

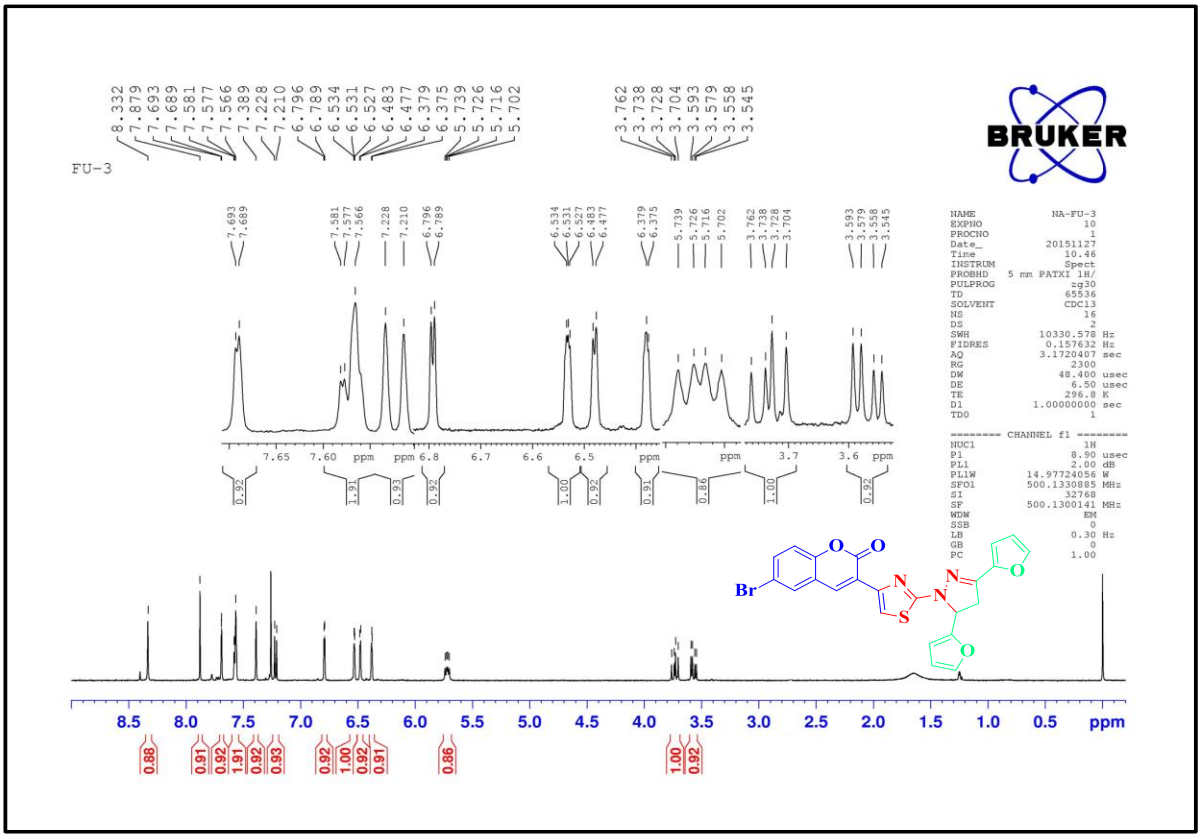


Fig: ^{1}H NMR spectrum of compound **39c**

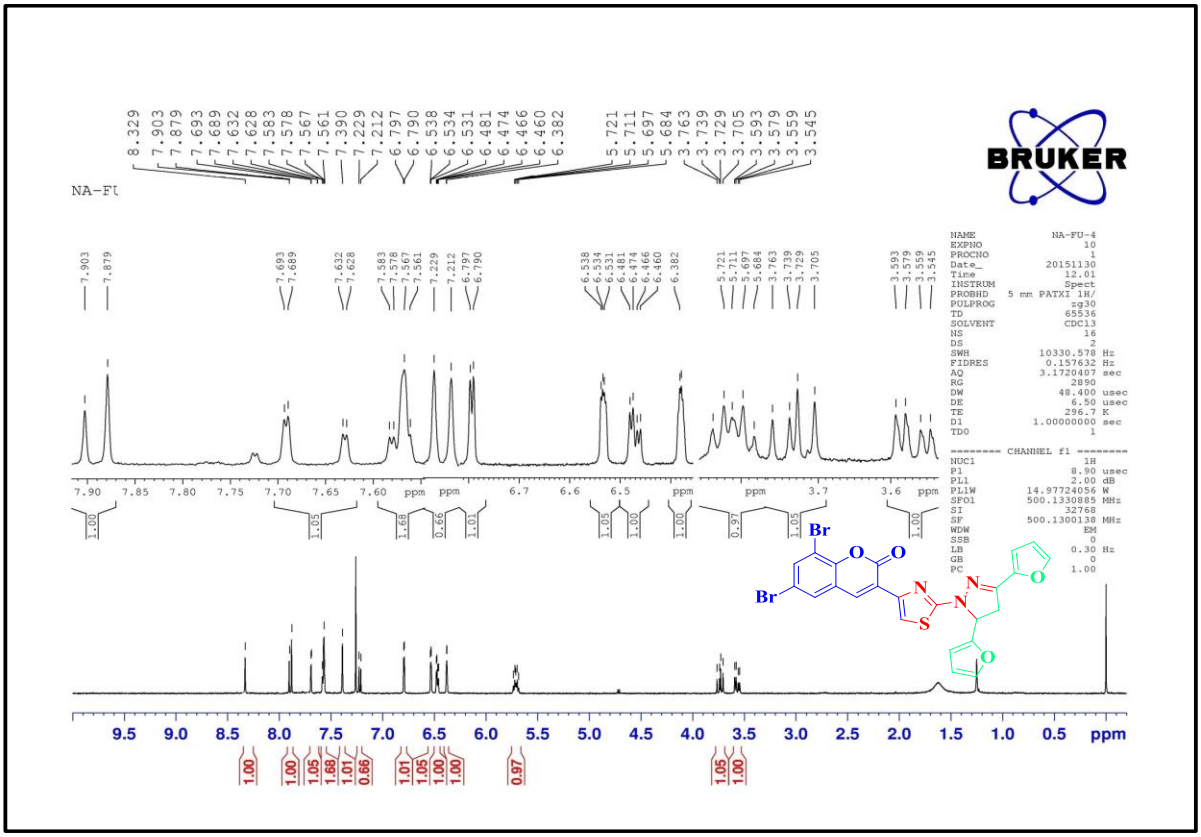


Fig: ^{1}H NMR spectrum of compound **39d**

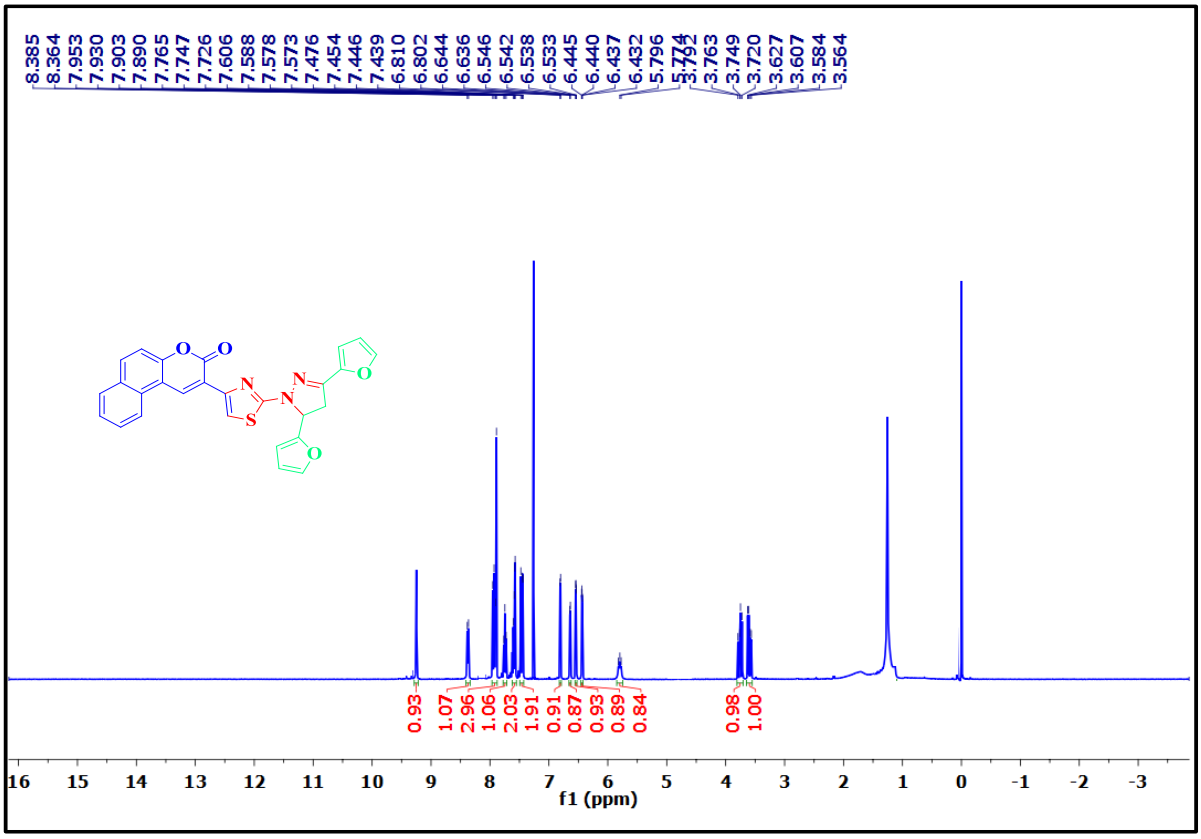


Fig: ^{1}H NMR spectrum of compound **39e**

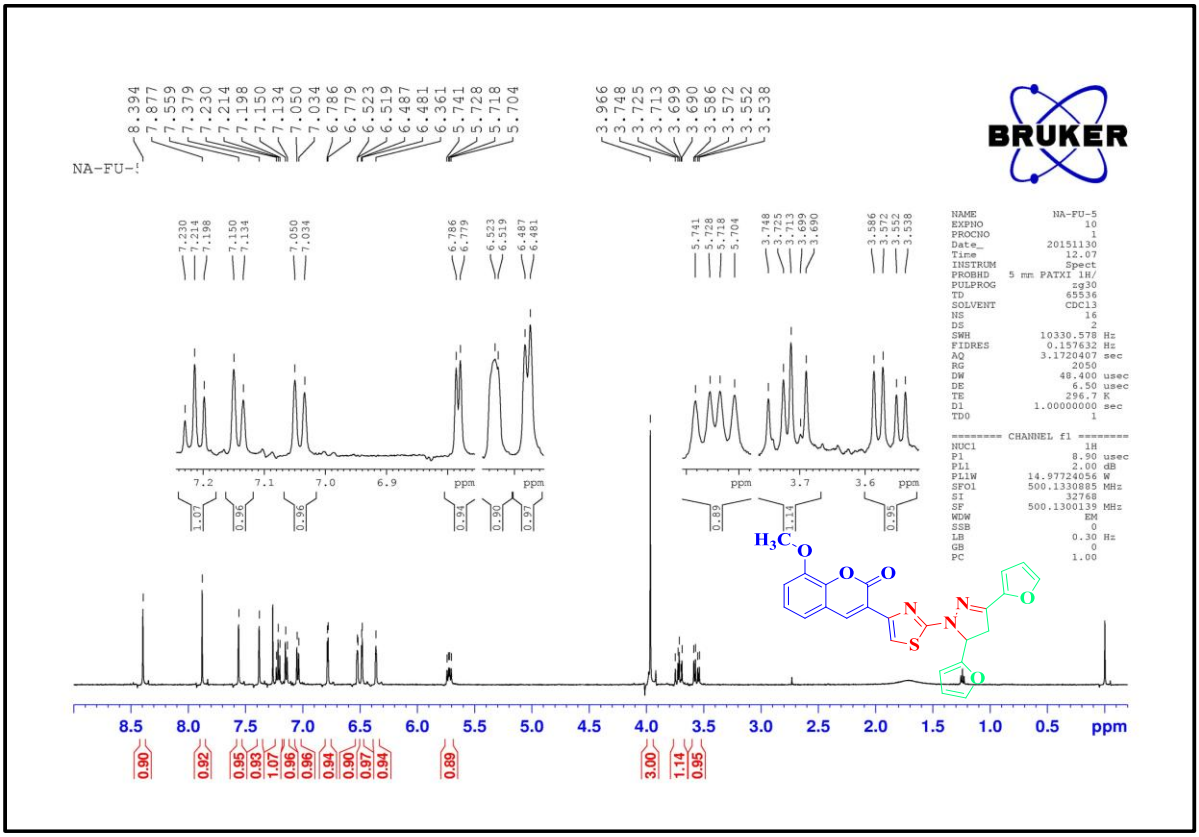


Fig: ^{1}H NMR spectrum of compound **39f**

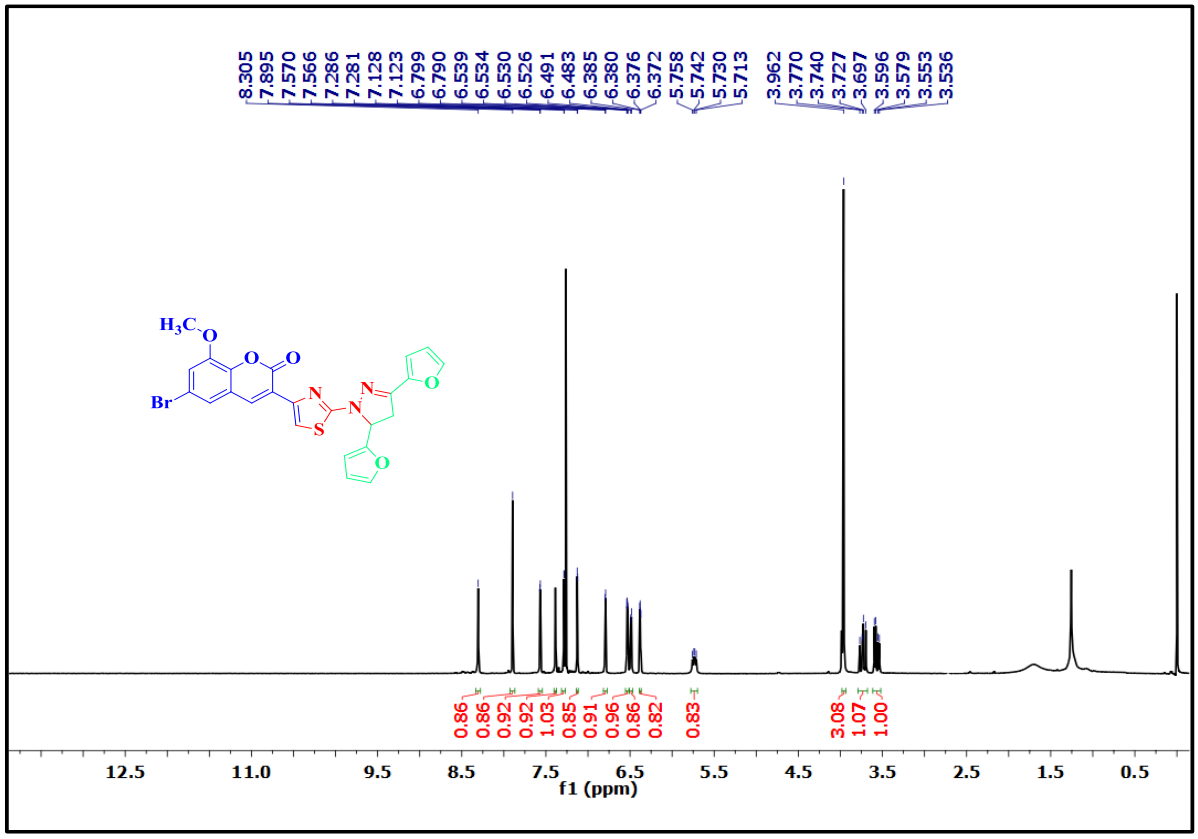


Fig: ^{1}H NMR spectrum of compound **39g**

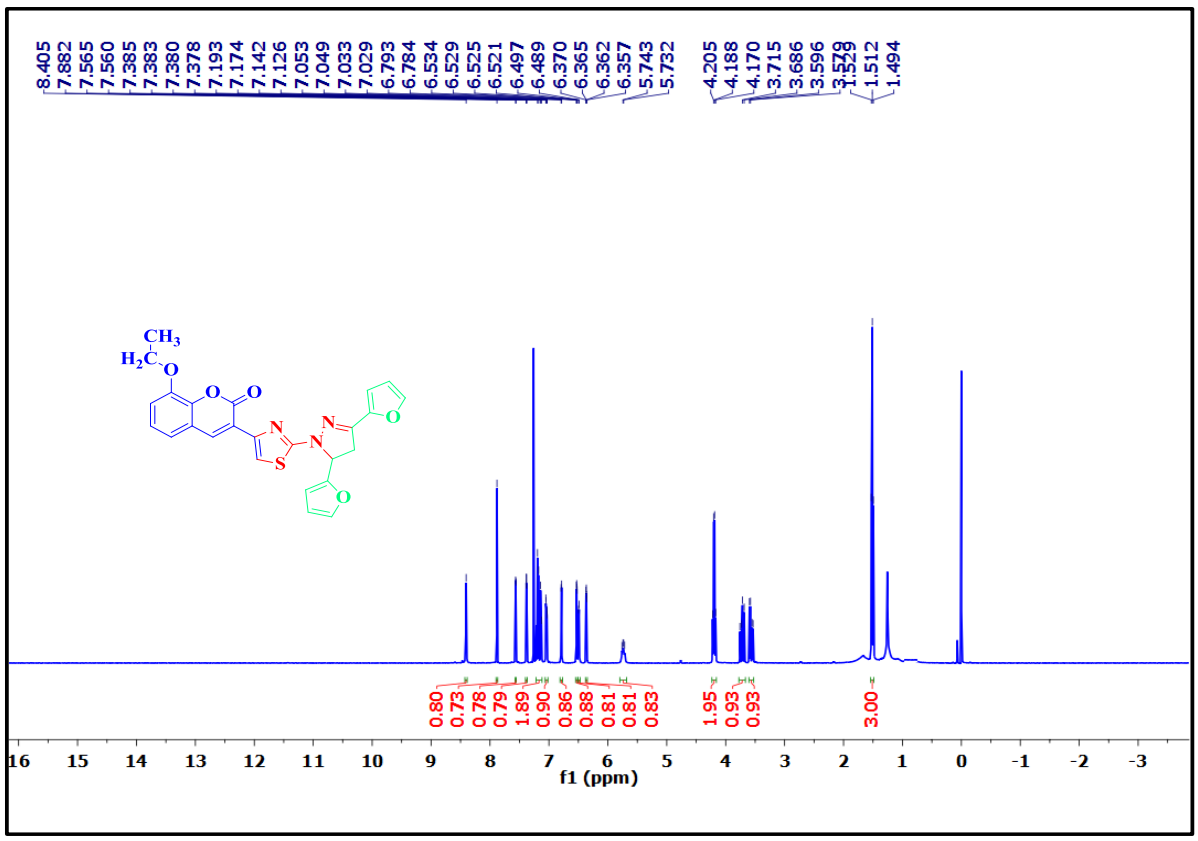


Fig: ^{1}H NMR spectrum of compound **39h**

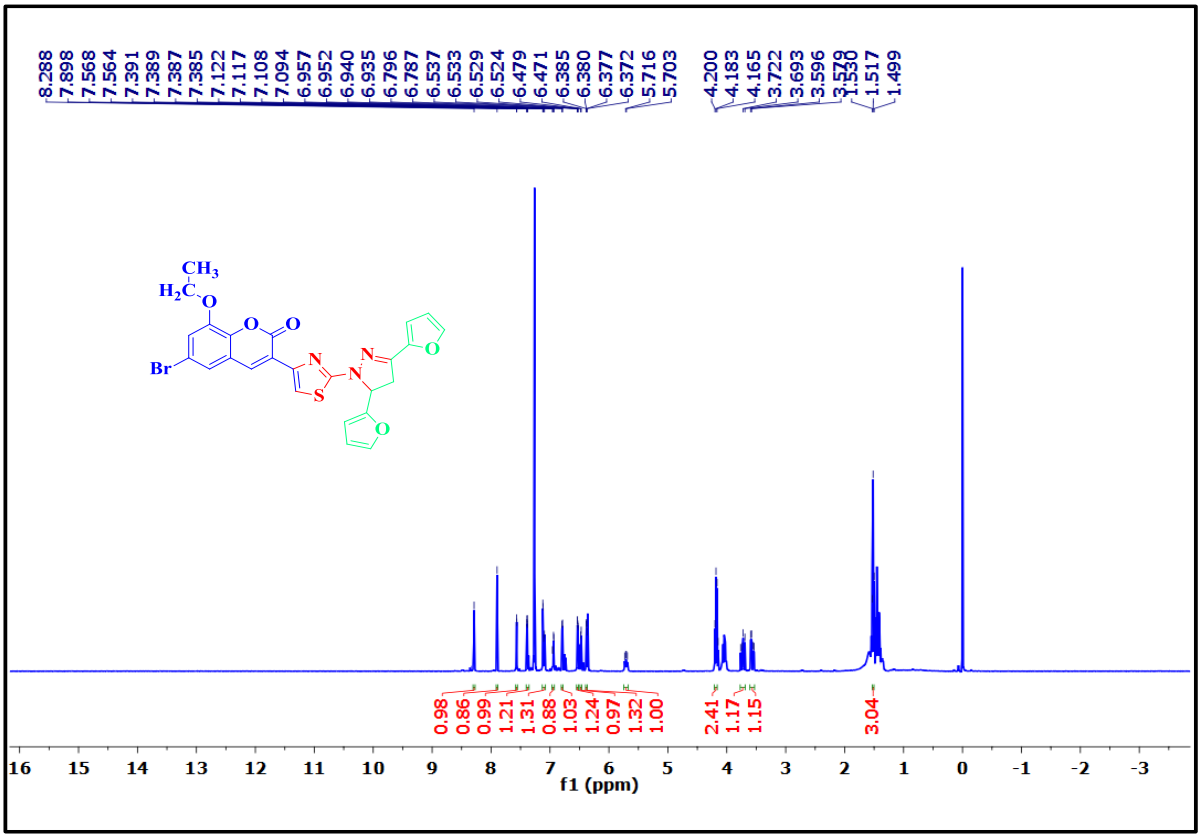


Fig: ^{1}H NMR spectrum of compound **39i**

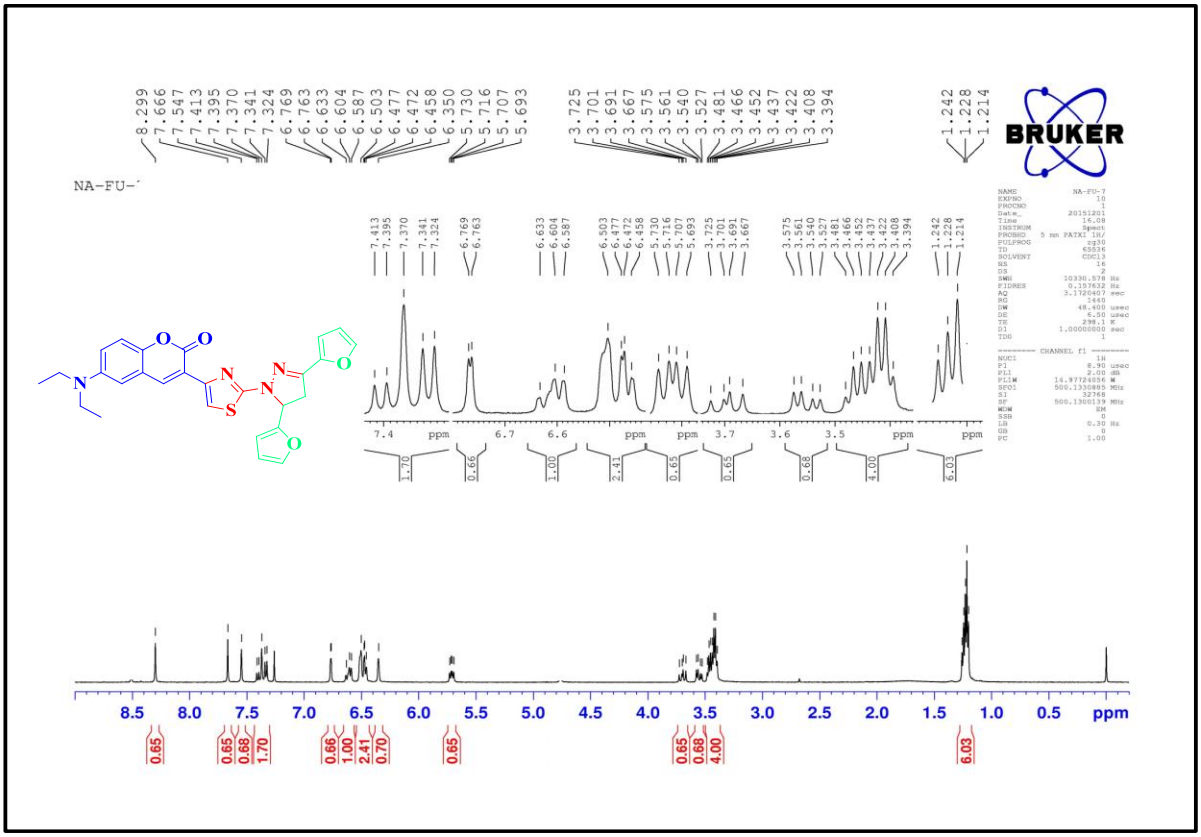


Fig: ^{1}H NMR spectrum of compound **39j**

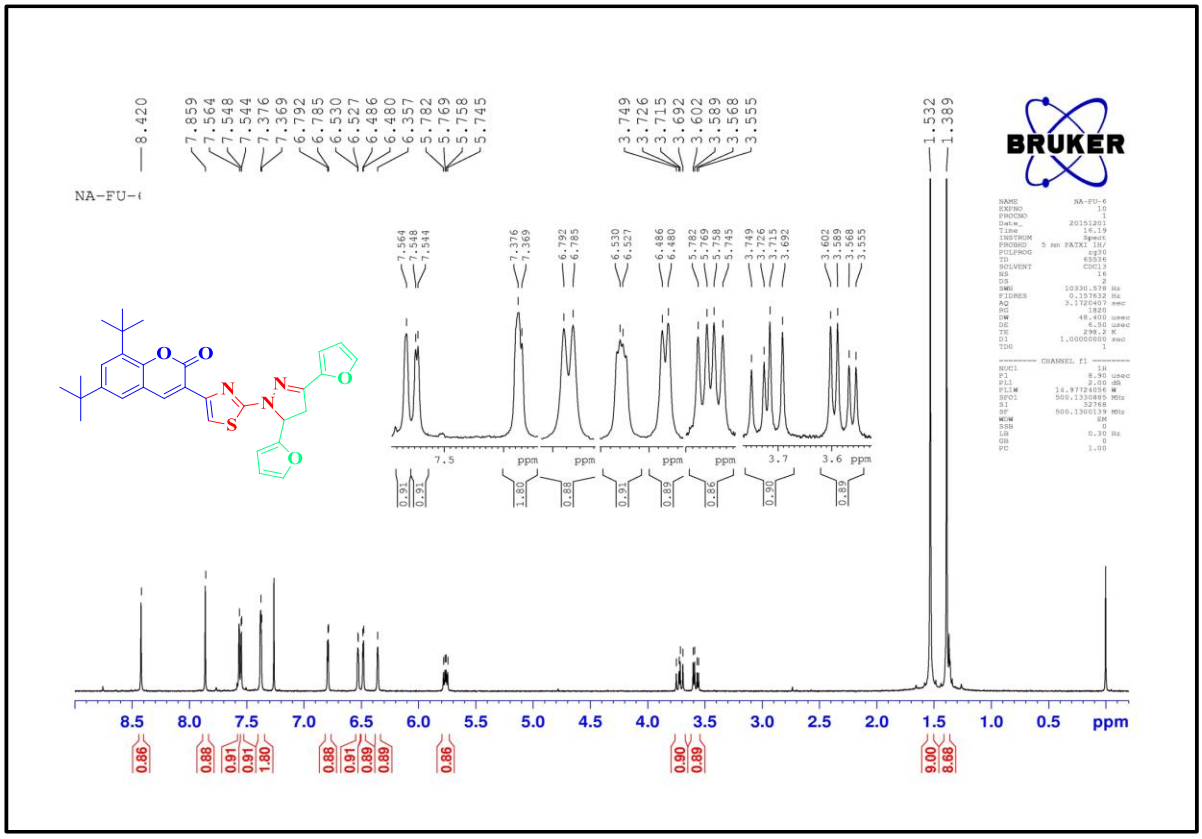


Fig: ^{1}H NMR spectrum of compound **39k**

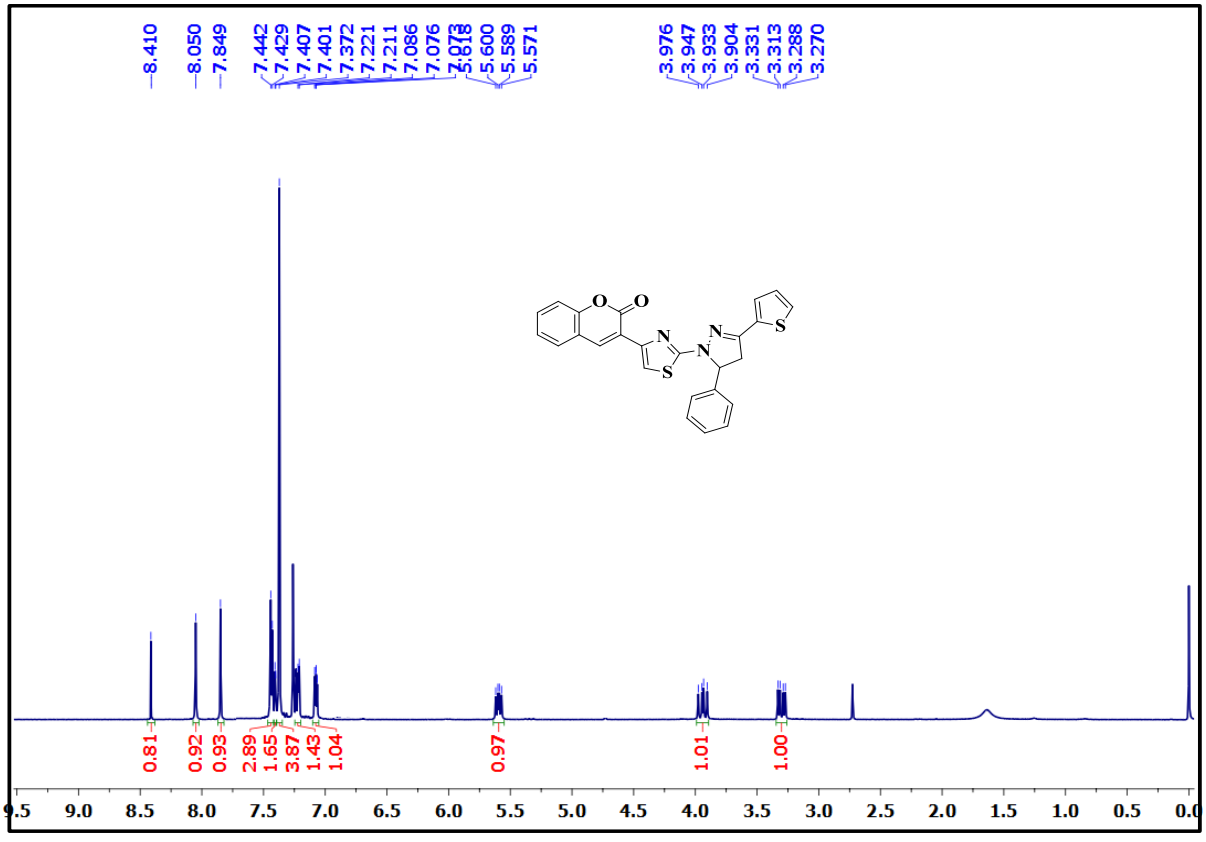


Fig: ^{1}H NMR spectrum of compound **41a**

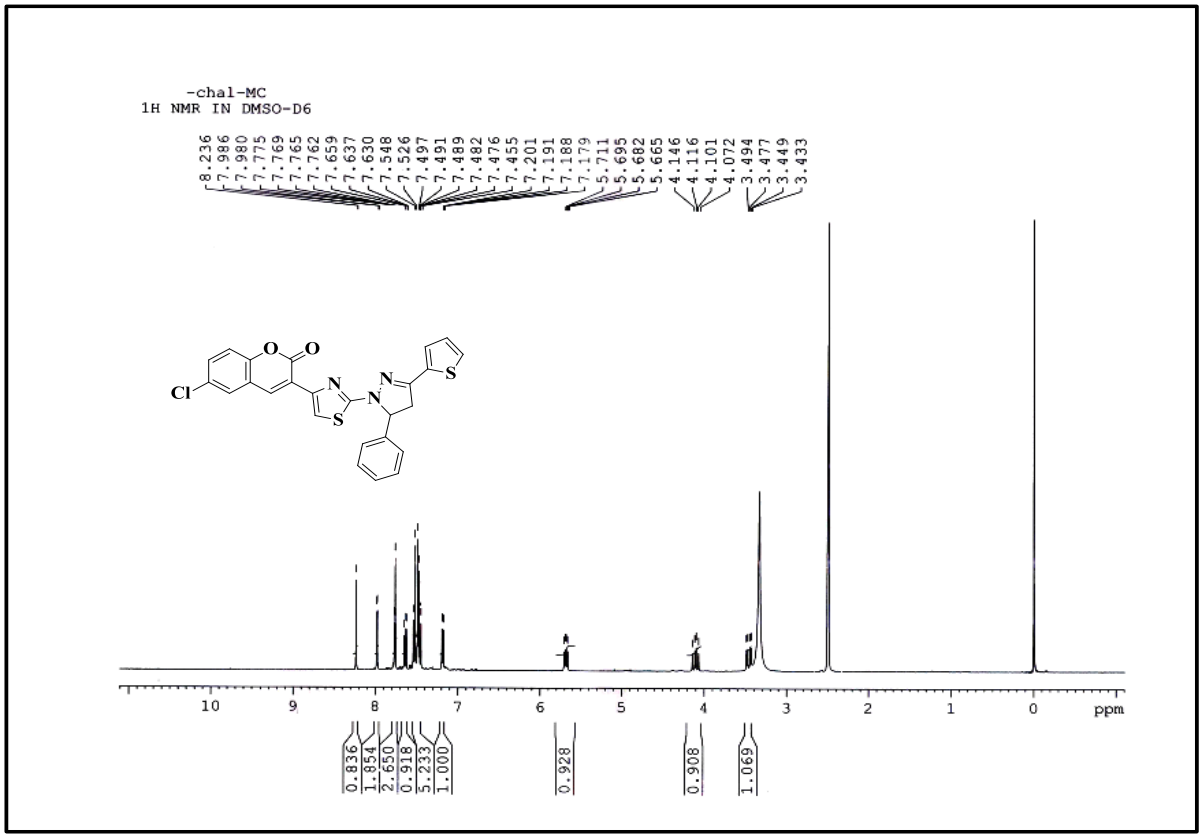


Fig: ^{1}H NMR spectrum of compound **41b**

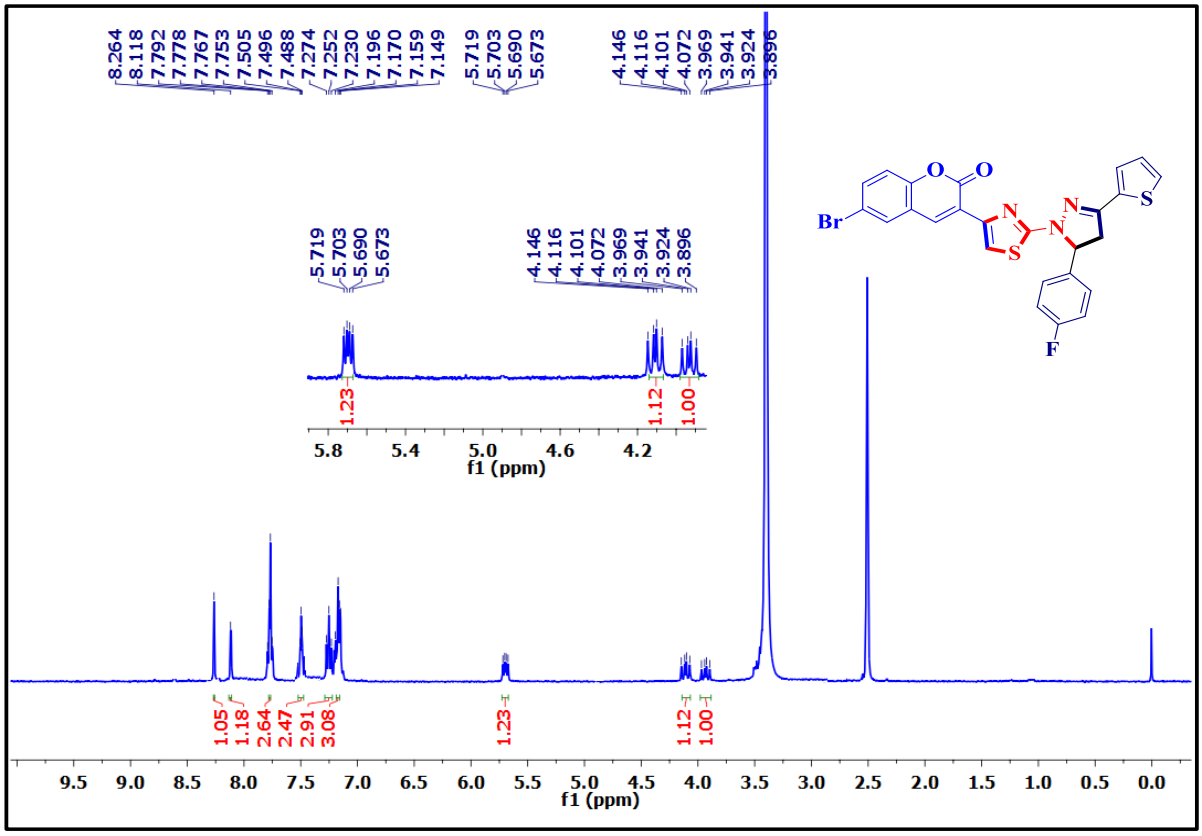


Fig: ^{1}H NMR spectrum of compound **41c**

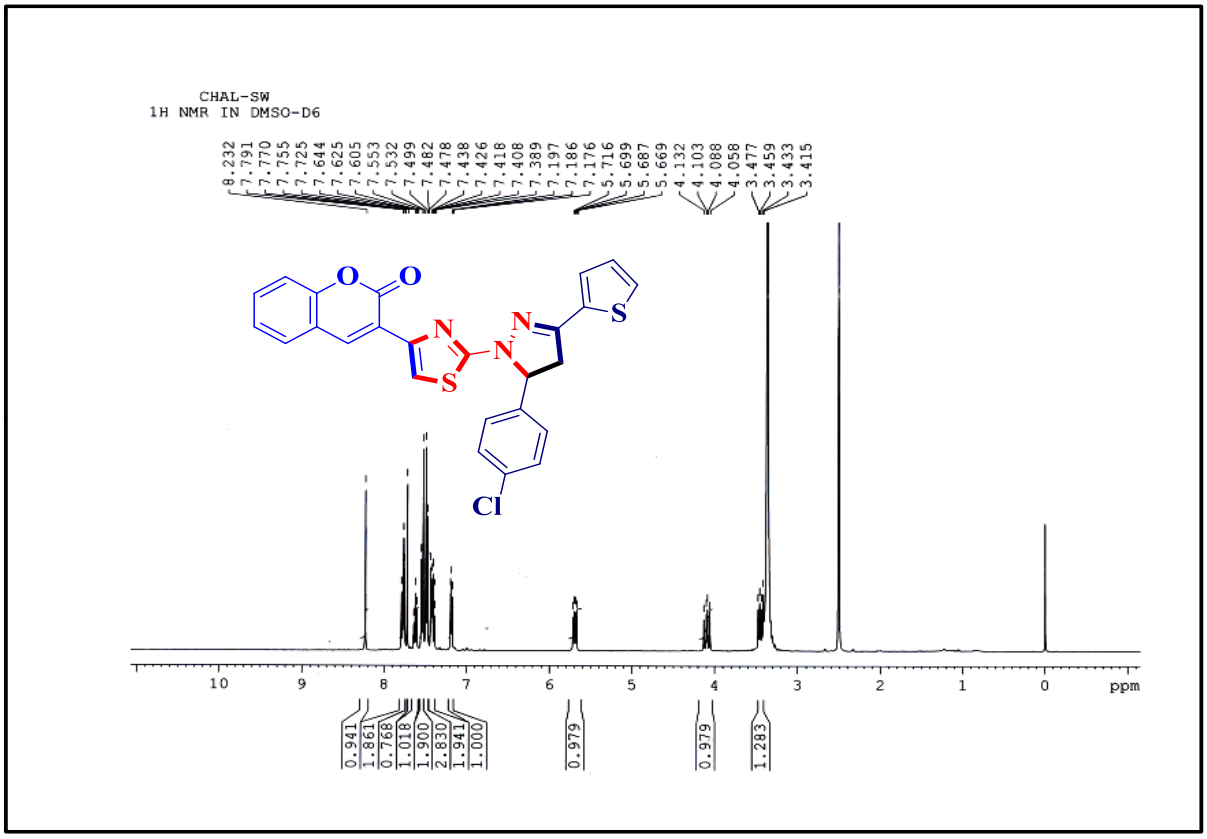


Fig: ^{1}H NMR spectrum of compound **41d**

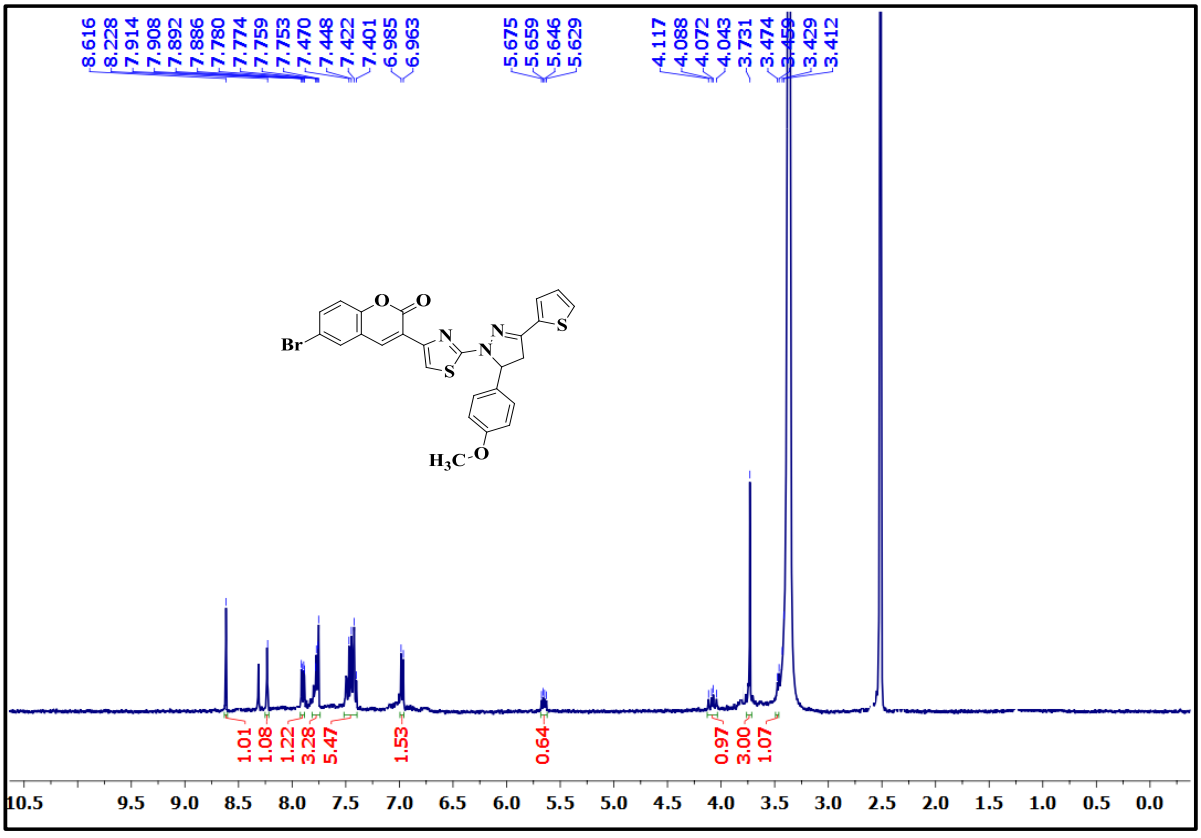


Fig: ^{1}H NMR spectrum of compound **41f**

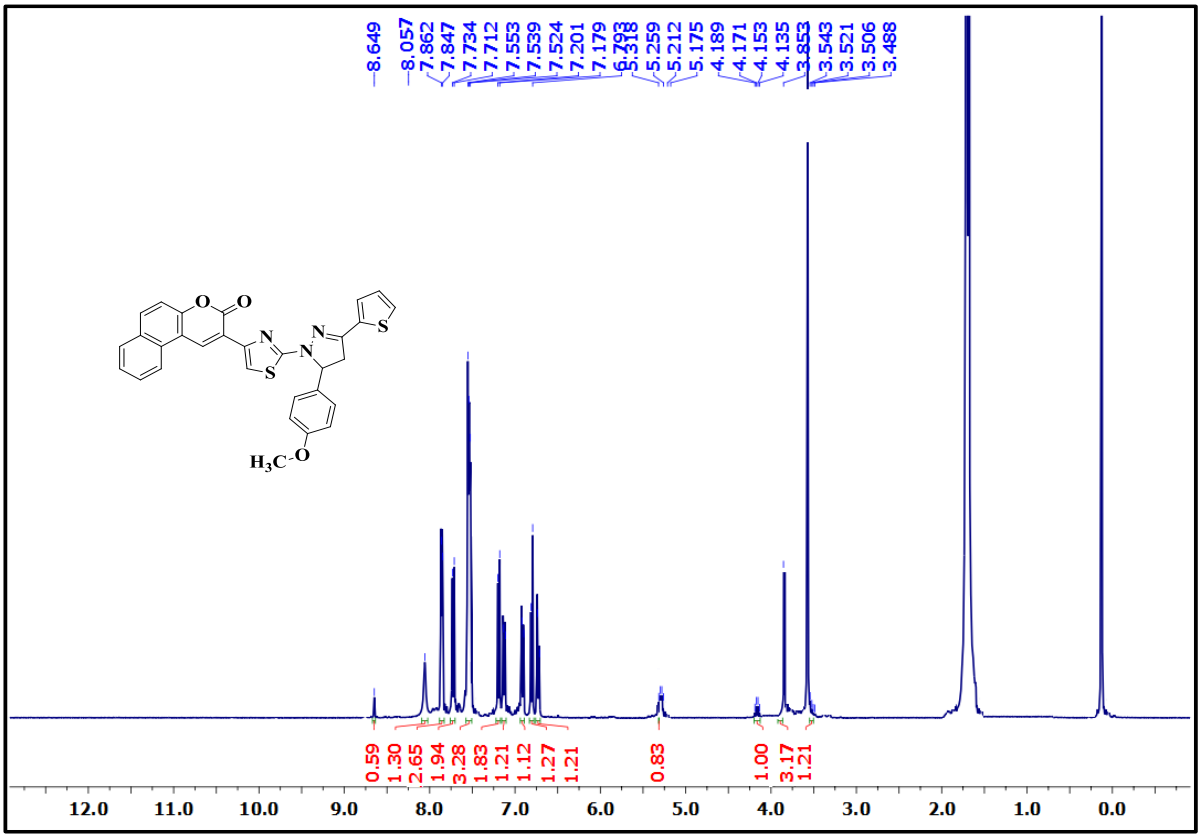


Fig: ^{1}H NMR spectrum of compound **41g**

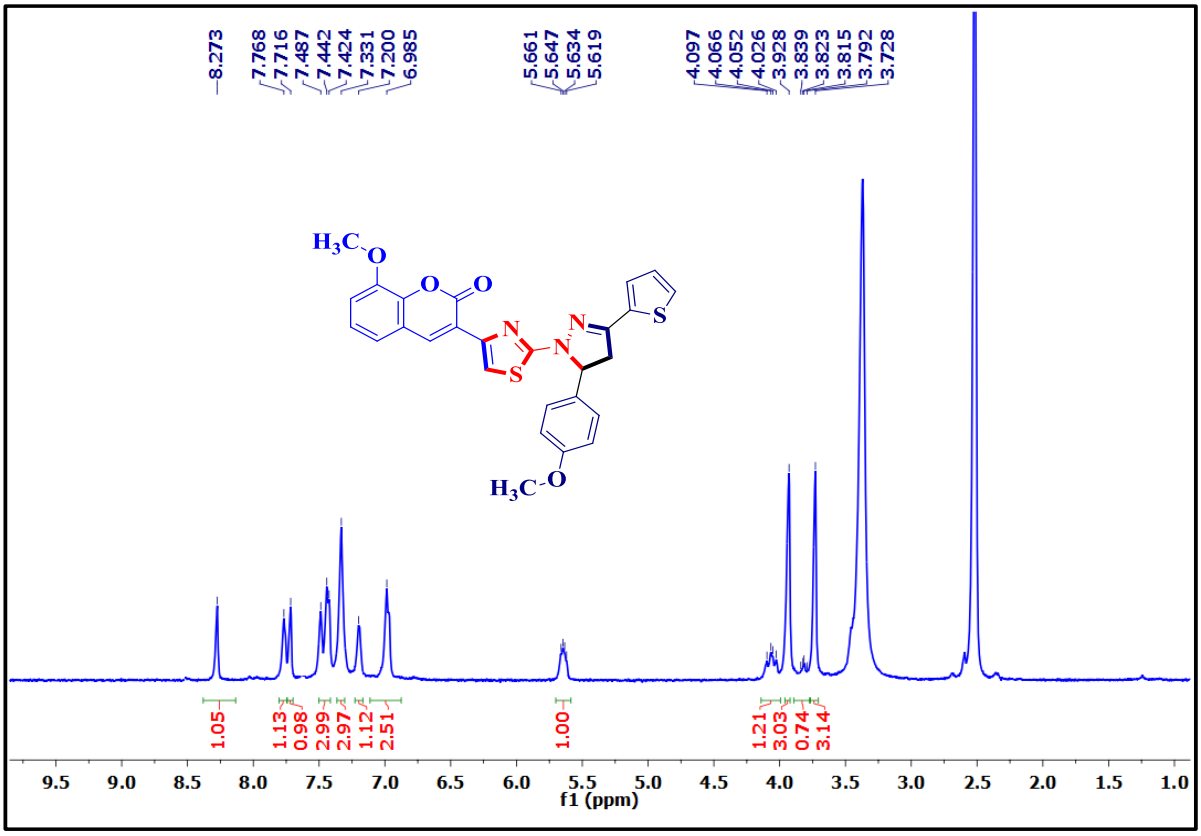


Fig: ^{1}H NMR spectrum of compound **41h**

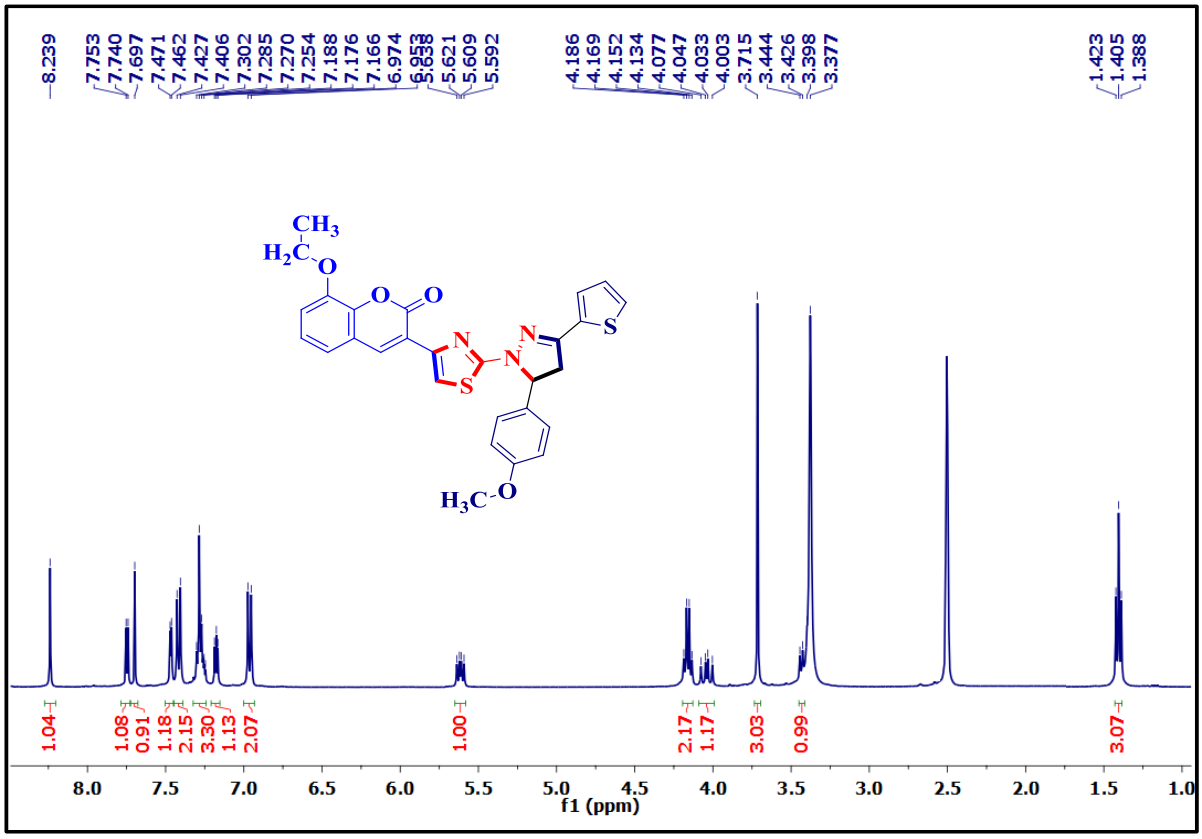


Fig: [1]H NMR spectrum of compound **41i**

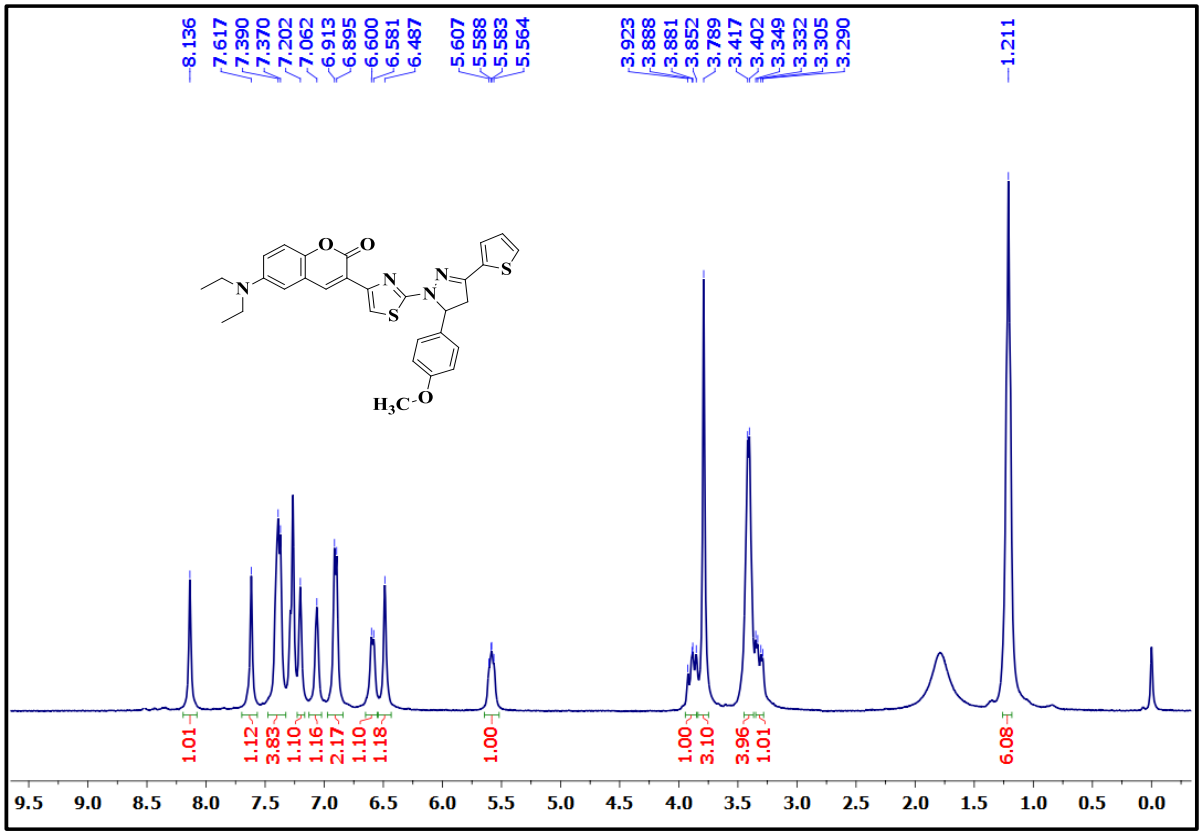


Fig: [1]H NMR spectrum of compound **41k**

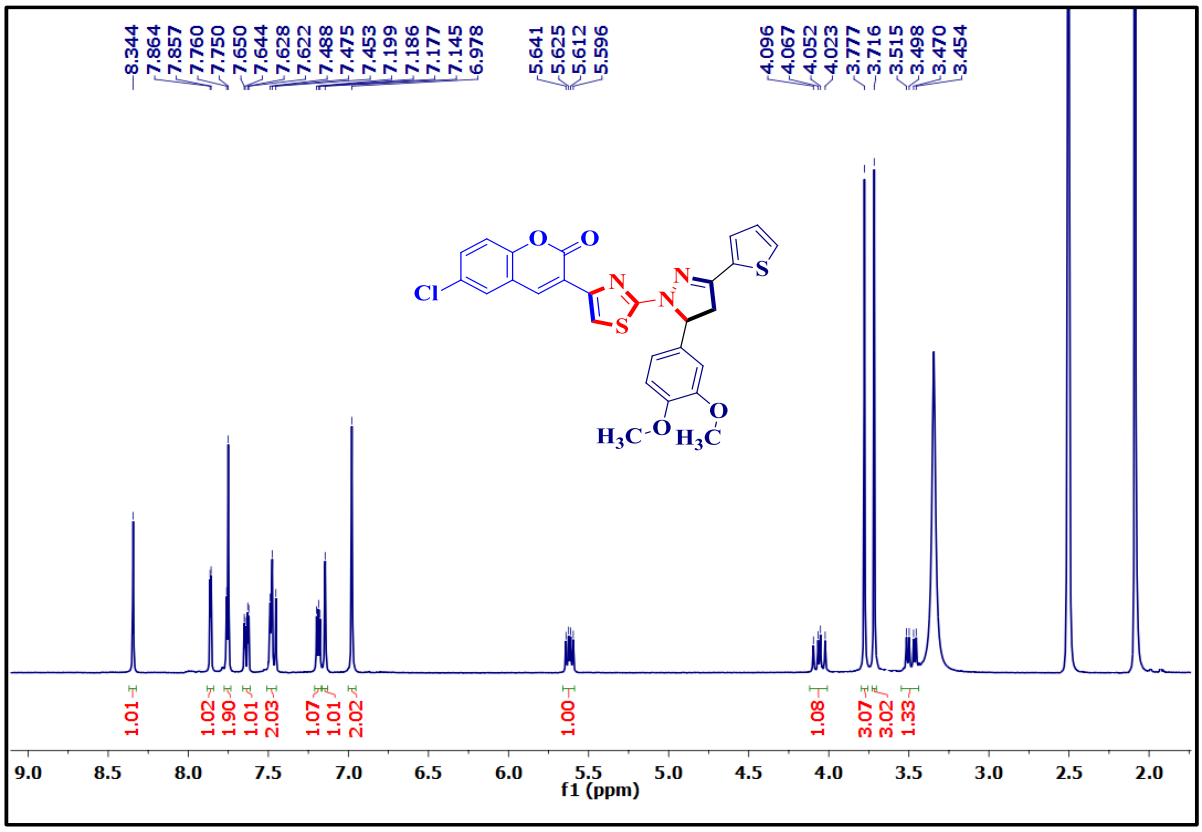


Fig: ^{1}H NMR spectrum of compound **41l**

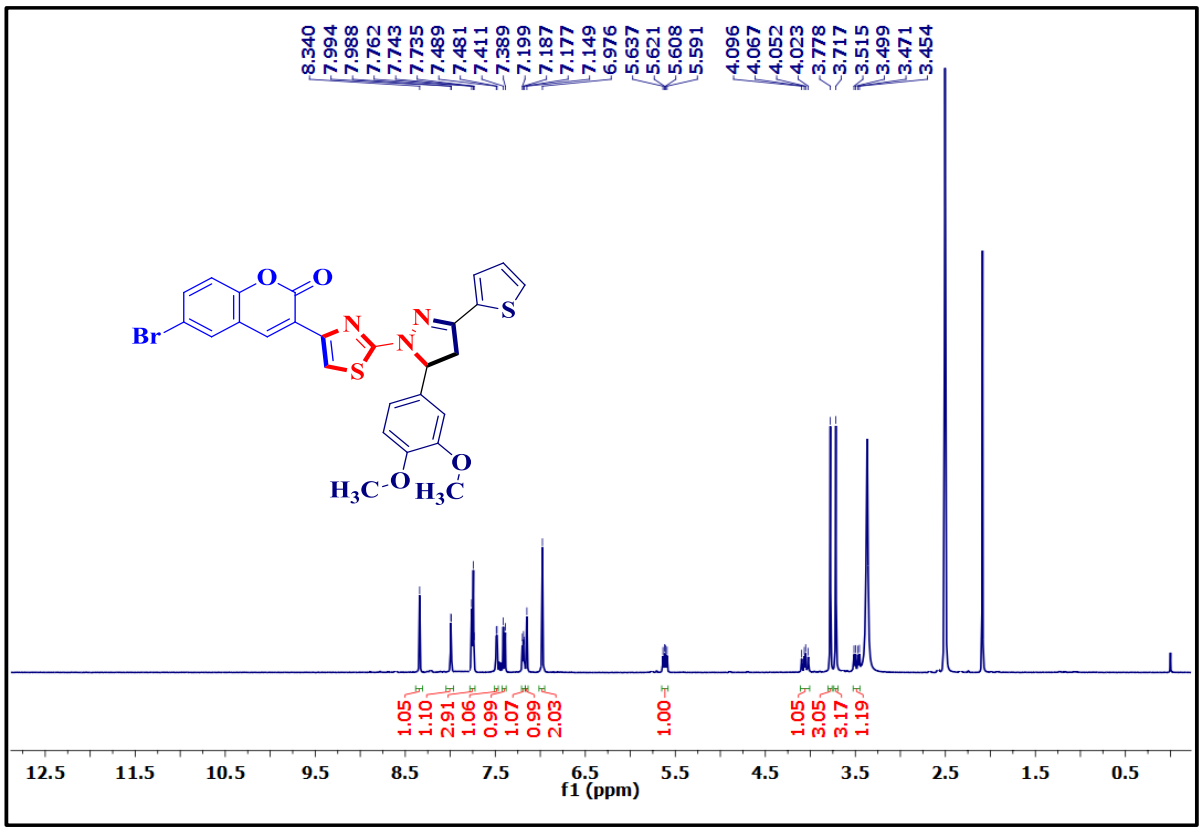


Fig: ^{1}H NMR spectrum of compound **41m**

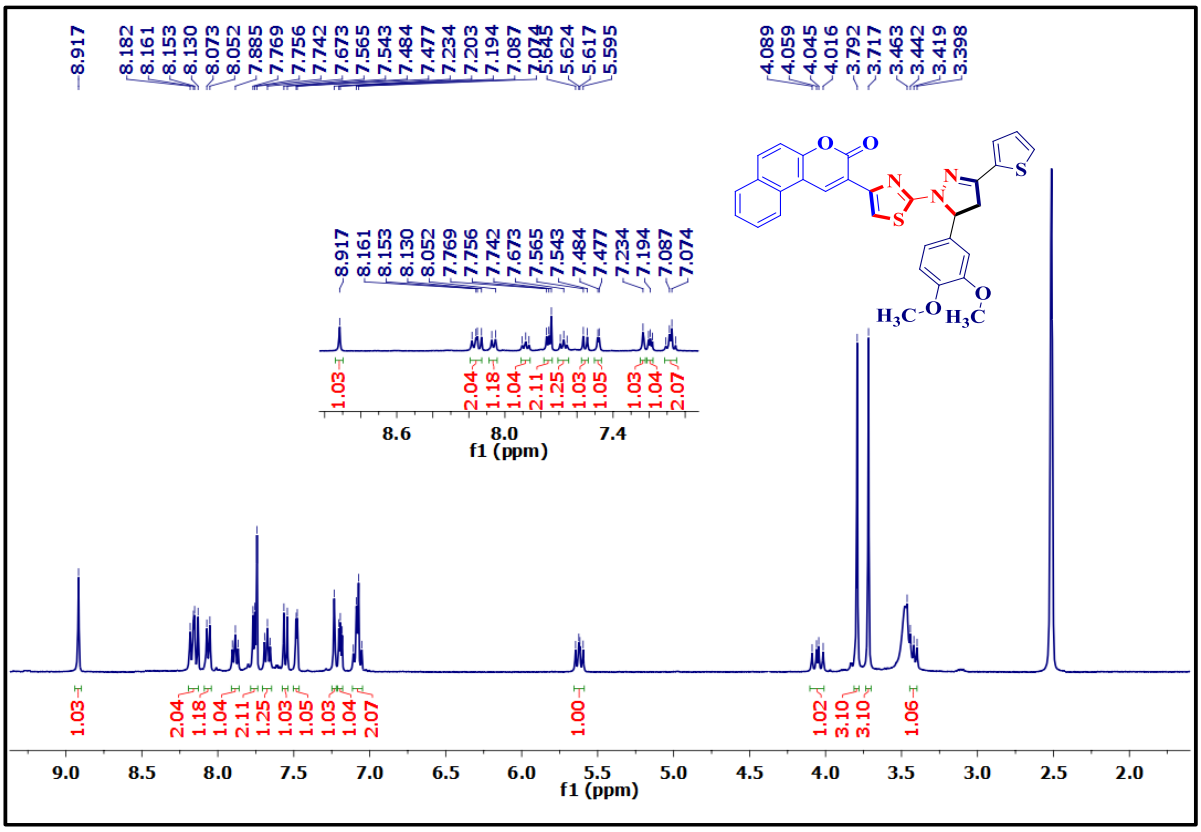


Fig: ^{1}H NMR spectrum of compound **41n**

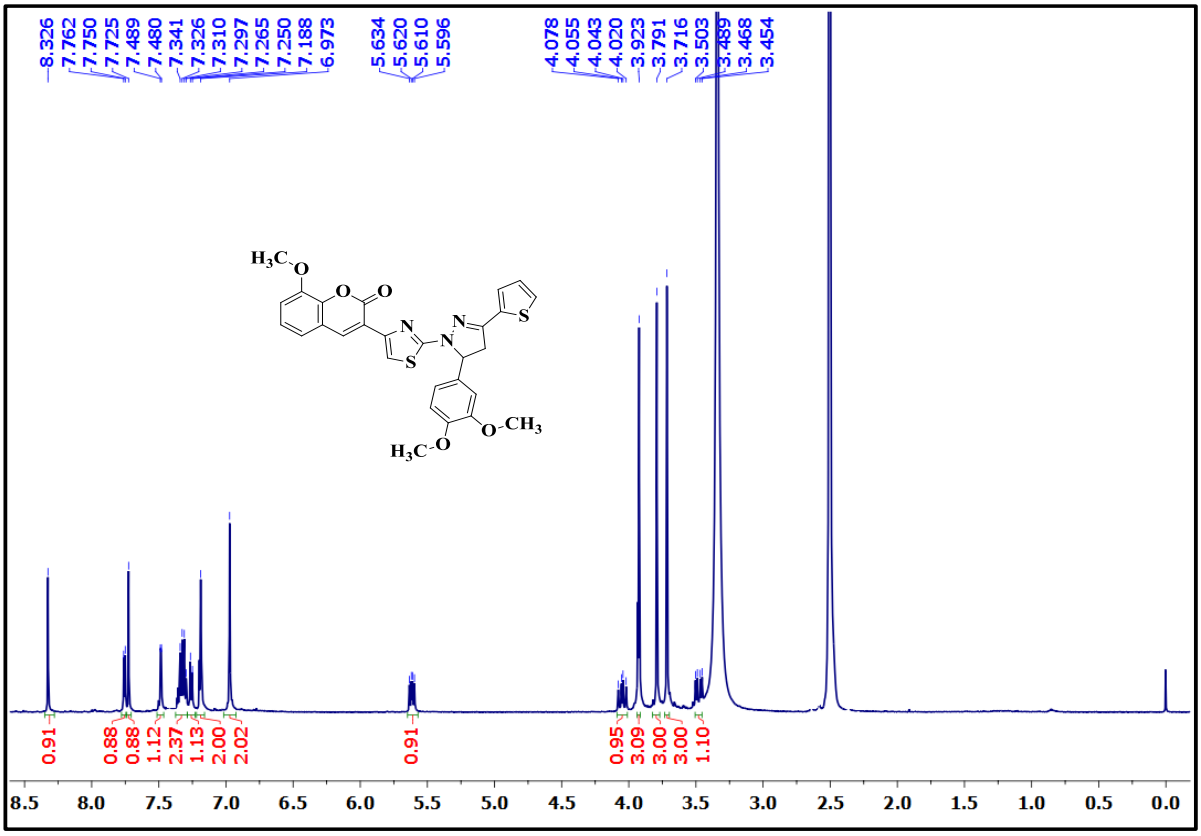


Fig: ^{1}H NMR spectrum of compound **41o**

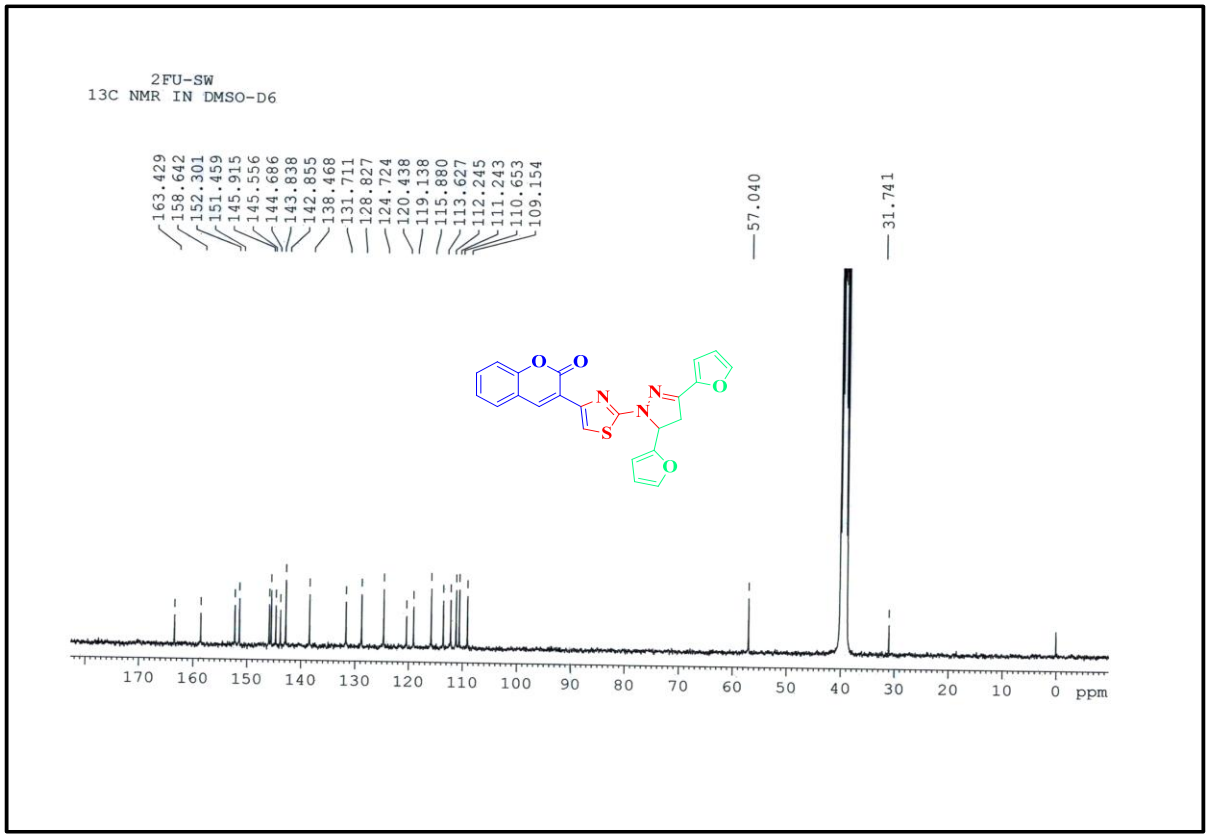


Fig: ^{13}C NMR spectrum of compound **39a**

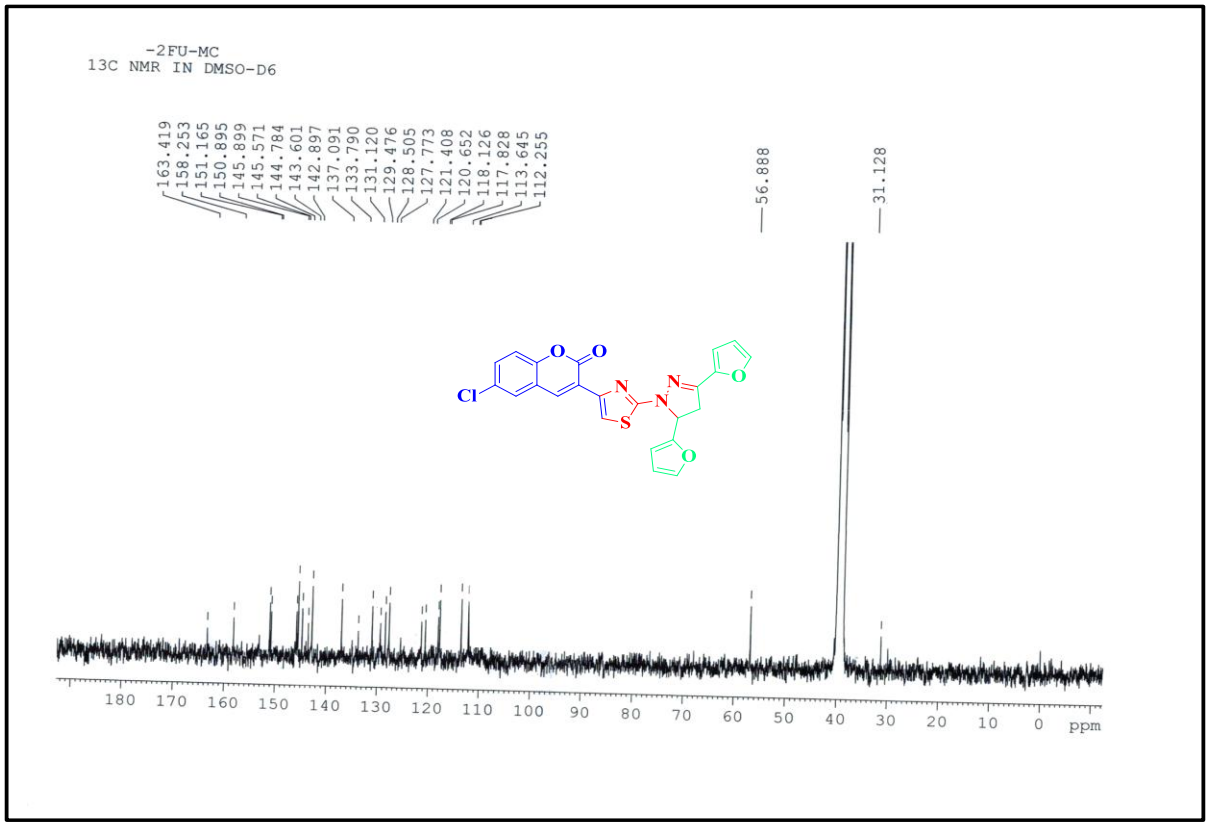


Fig: ^{13}C NMR spectrum of compound **39b**

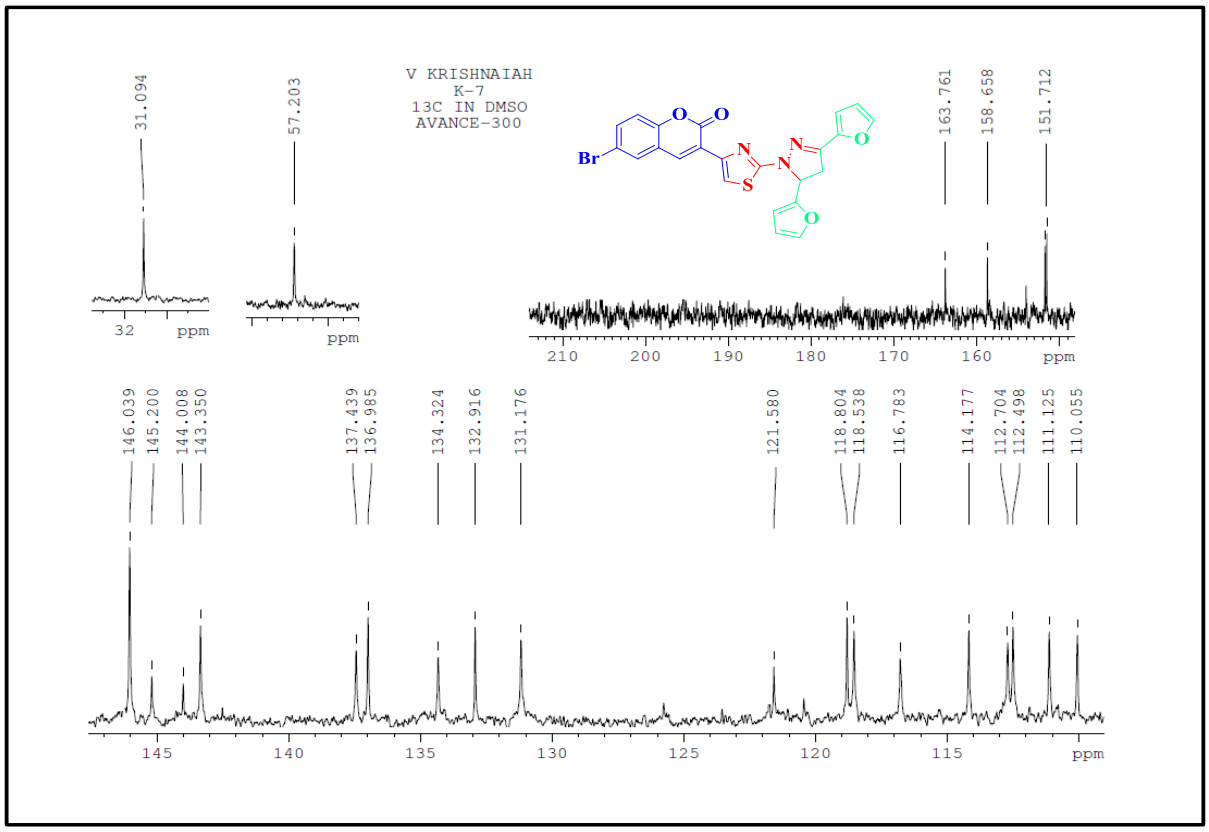


Fig: ^{13}C NMR spectrum of compound **39c**

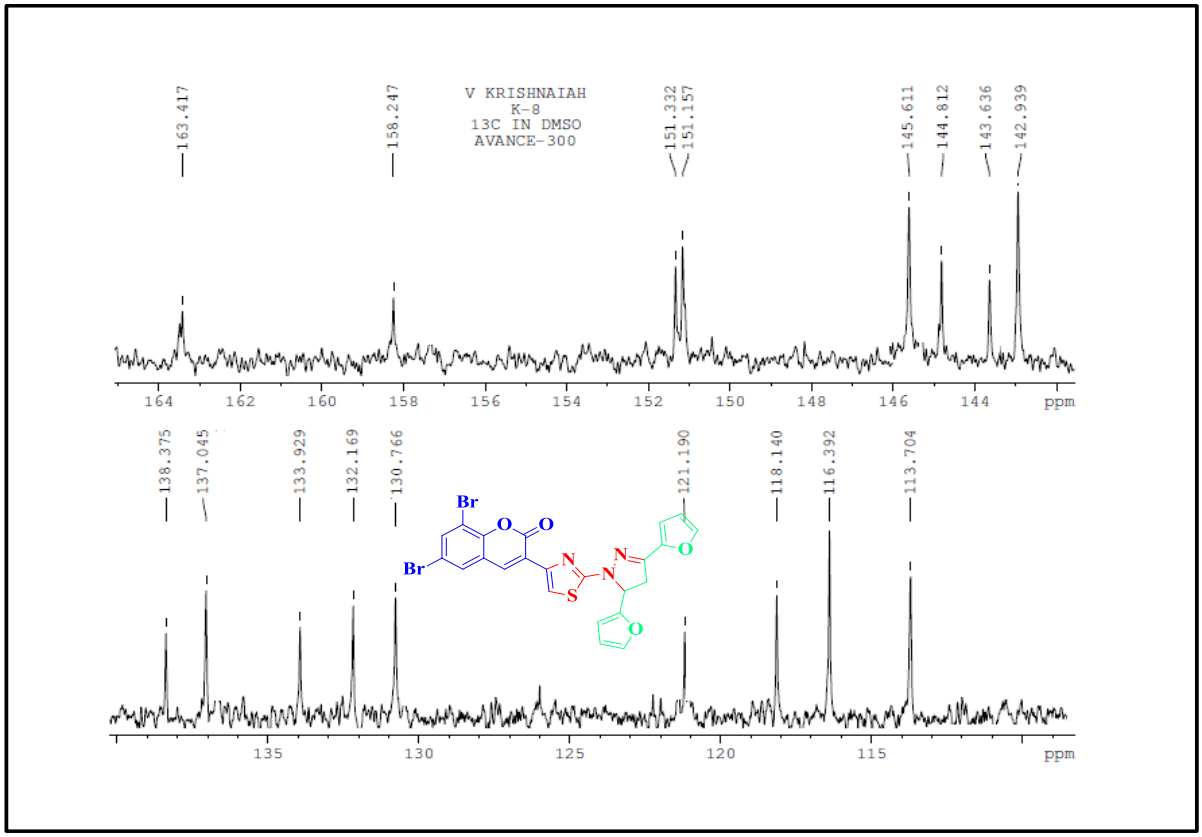


Fig: ^{13}C NMR spectrum of compound **39d**

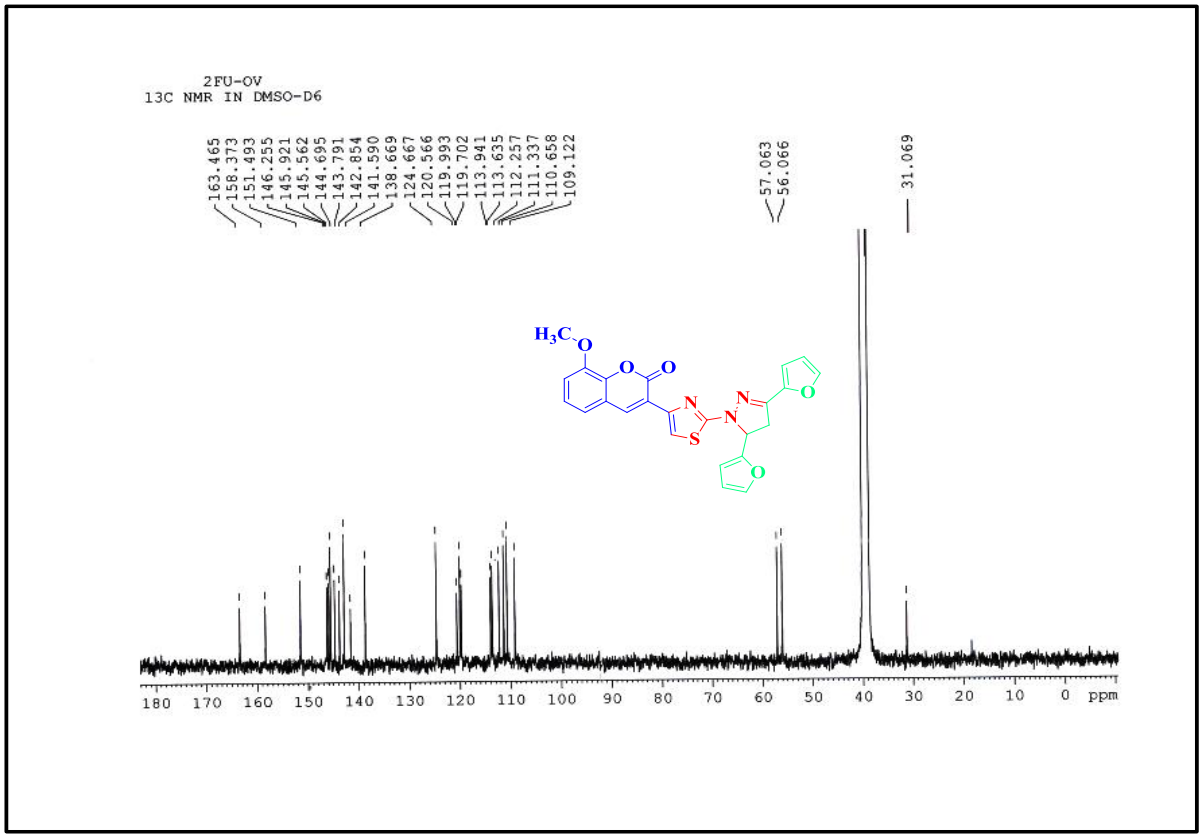


Fig: ^{13}C NMR spectrum of compound **39f**

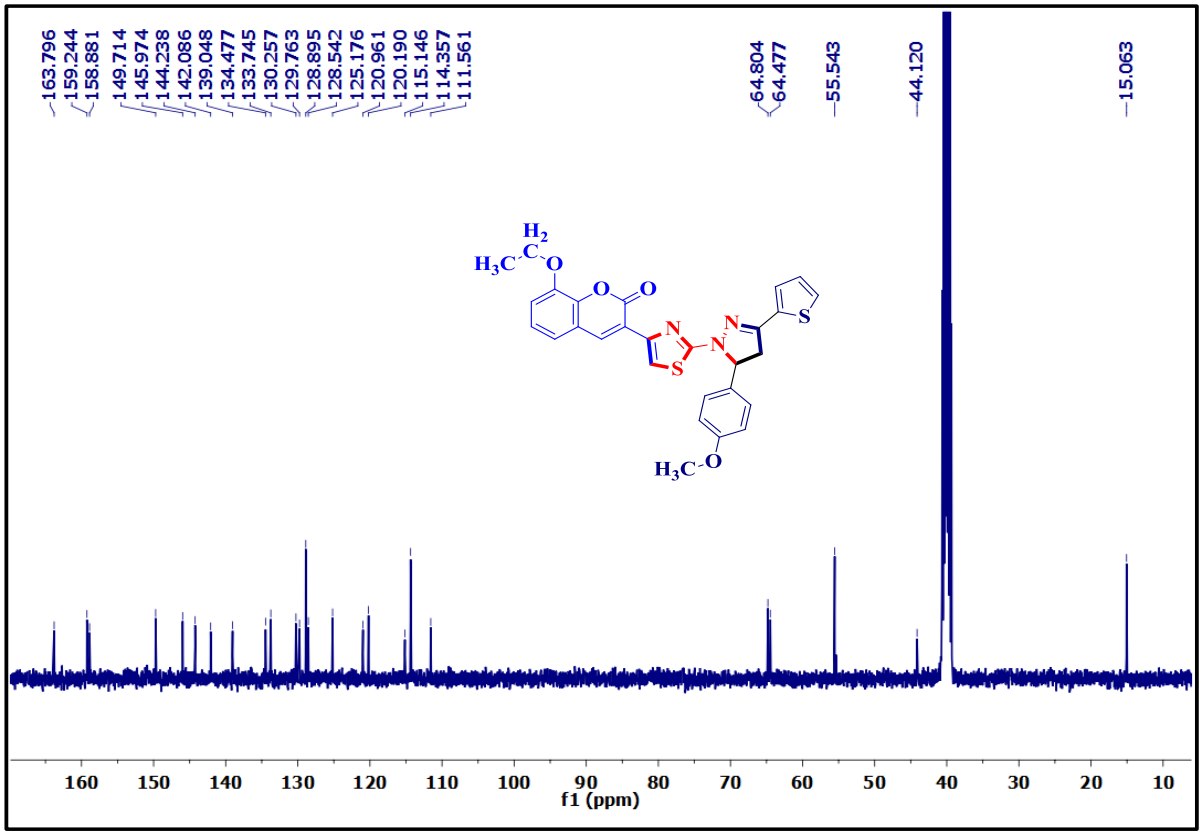


Fig: ^{13}C NMR spectrum of compound **41i**

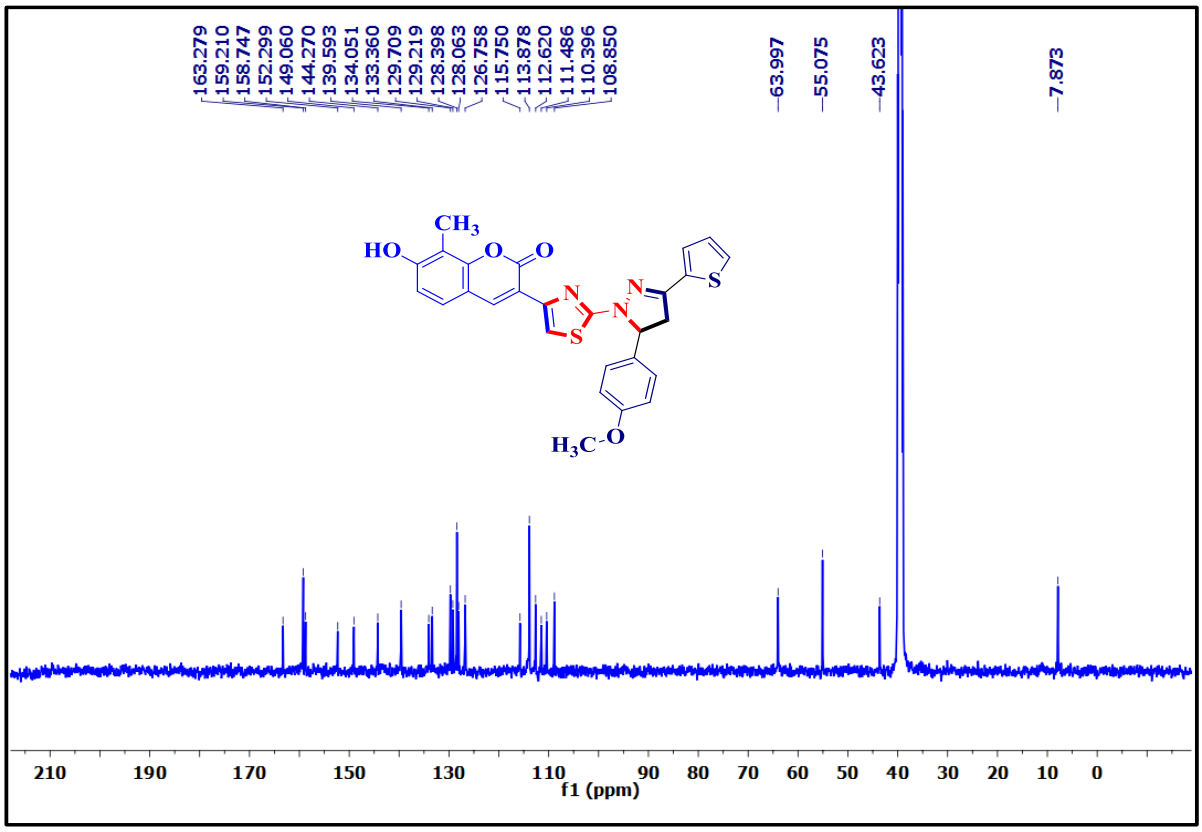


Fig: ^{13}C NMR spectrum of compound **41j**

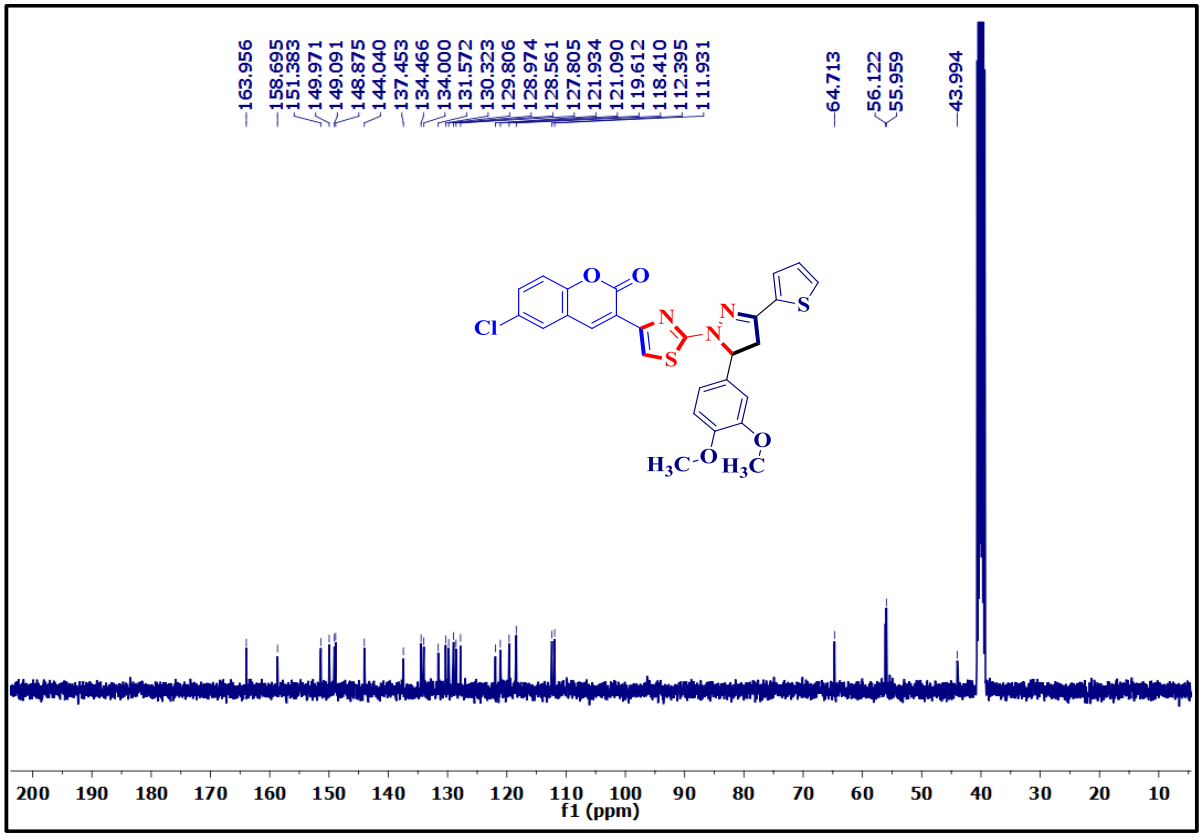


Fig: ^{13}C NMR spectrum of compound **41l**

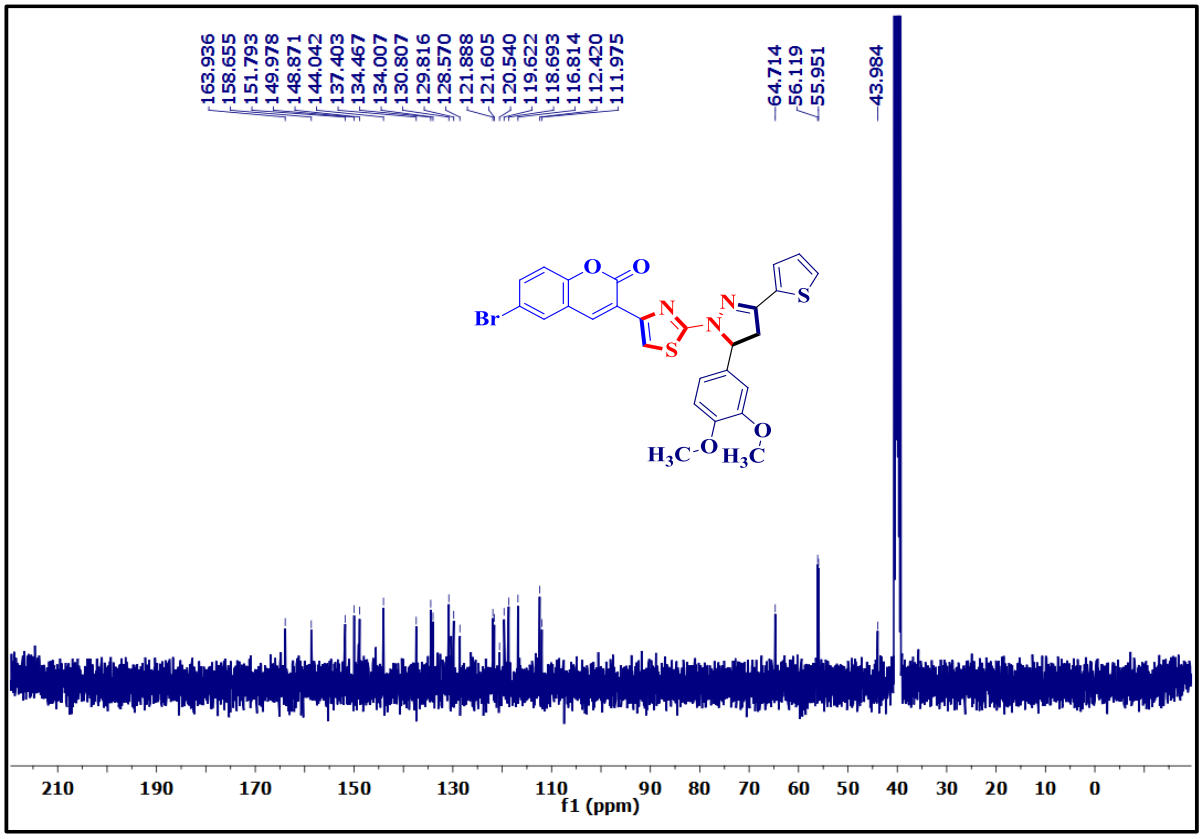


Fig: ^{13}C NMR spectrum of compound **41m**

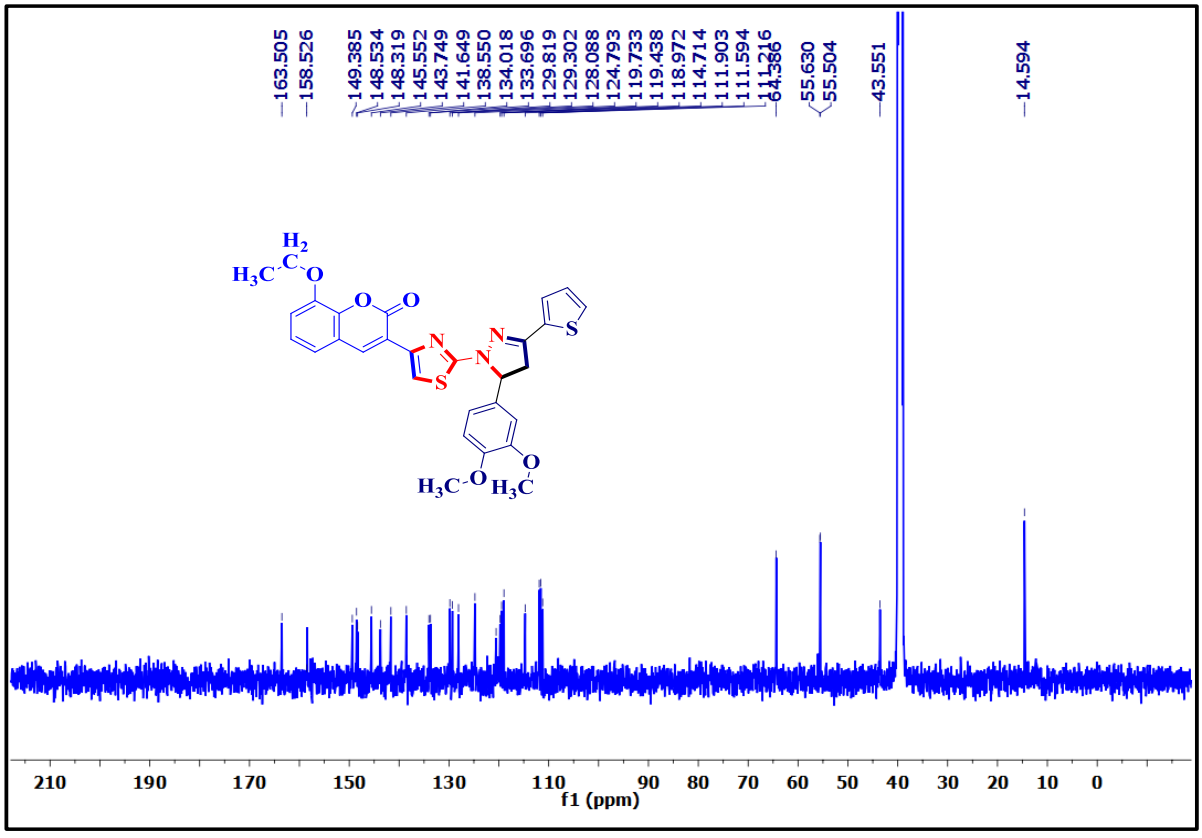


Fig: ^{13}C NMR spectrum of compound **41p**

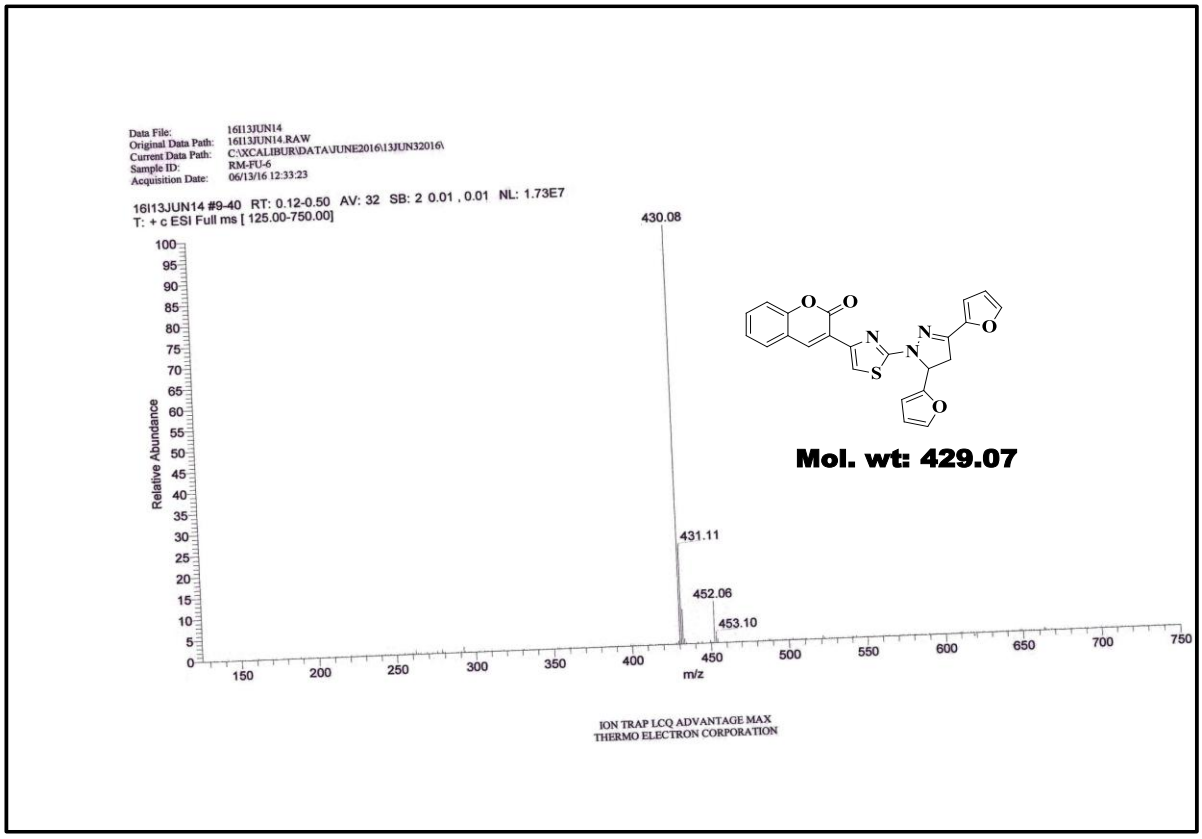


Fig: Mass spectrum of compound **39a**

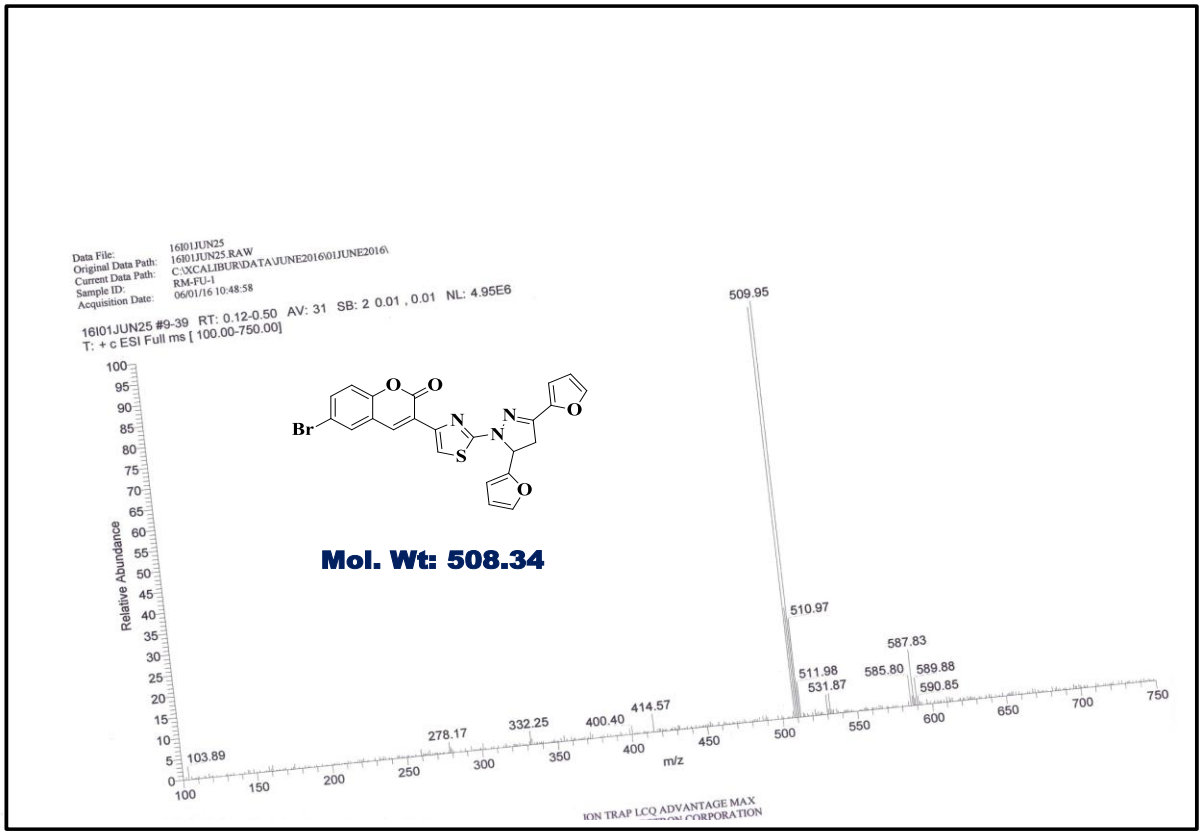


Fig: Mass spectrum of compound **39c**

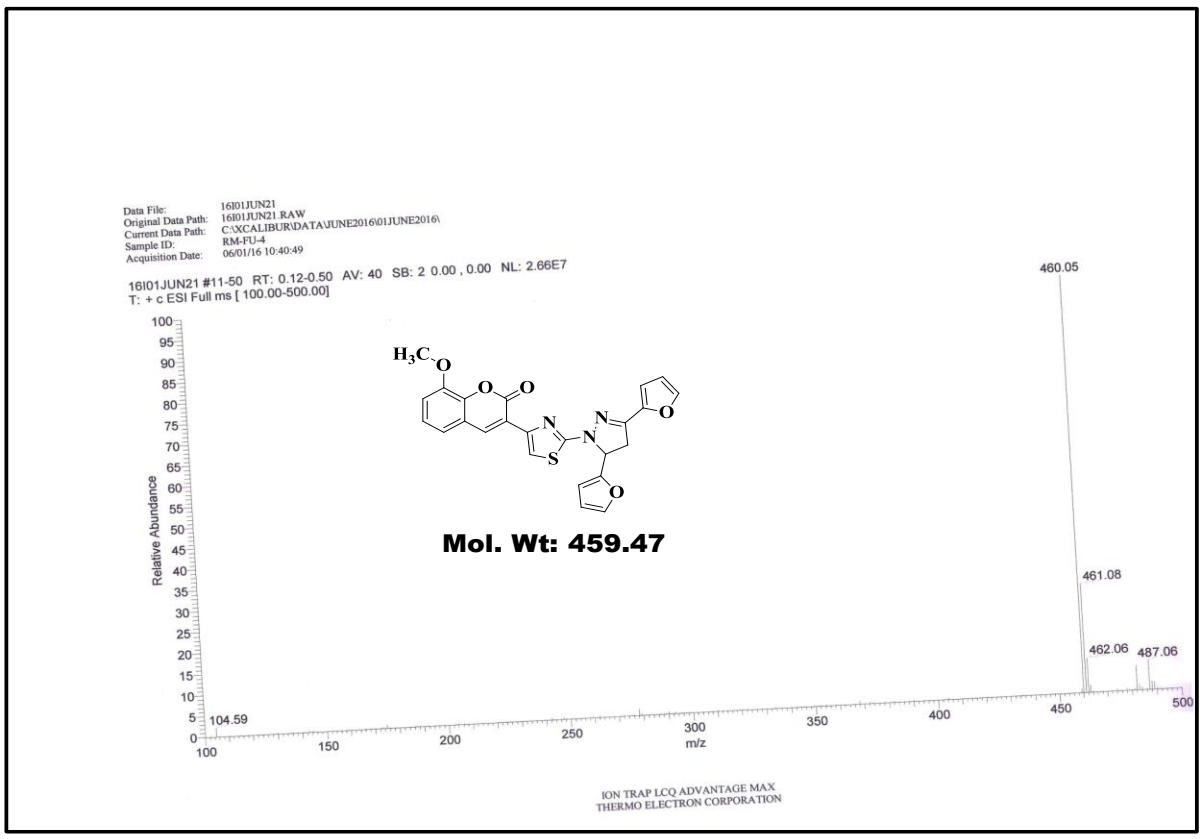


Fig: Mass spectrum of compound **39f**

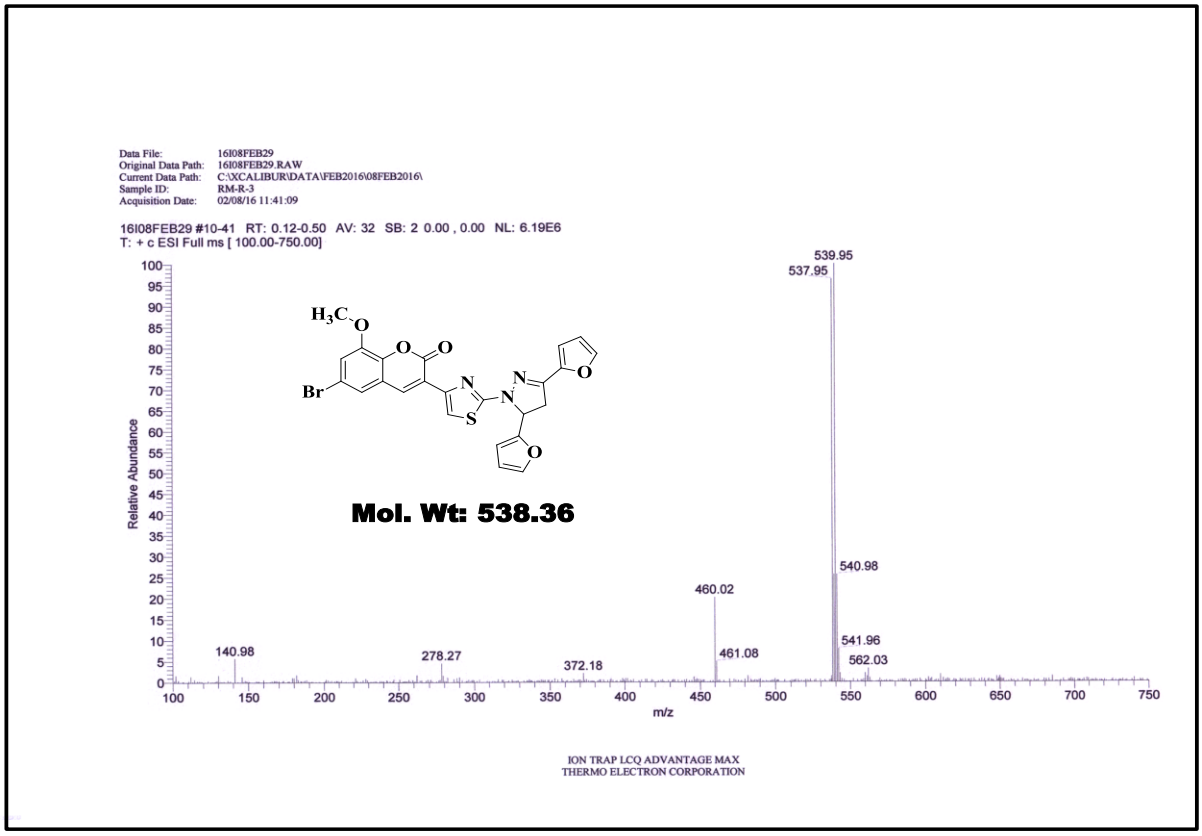


Fig: Mass spectrum of compound **39g**

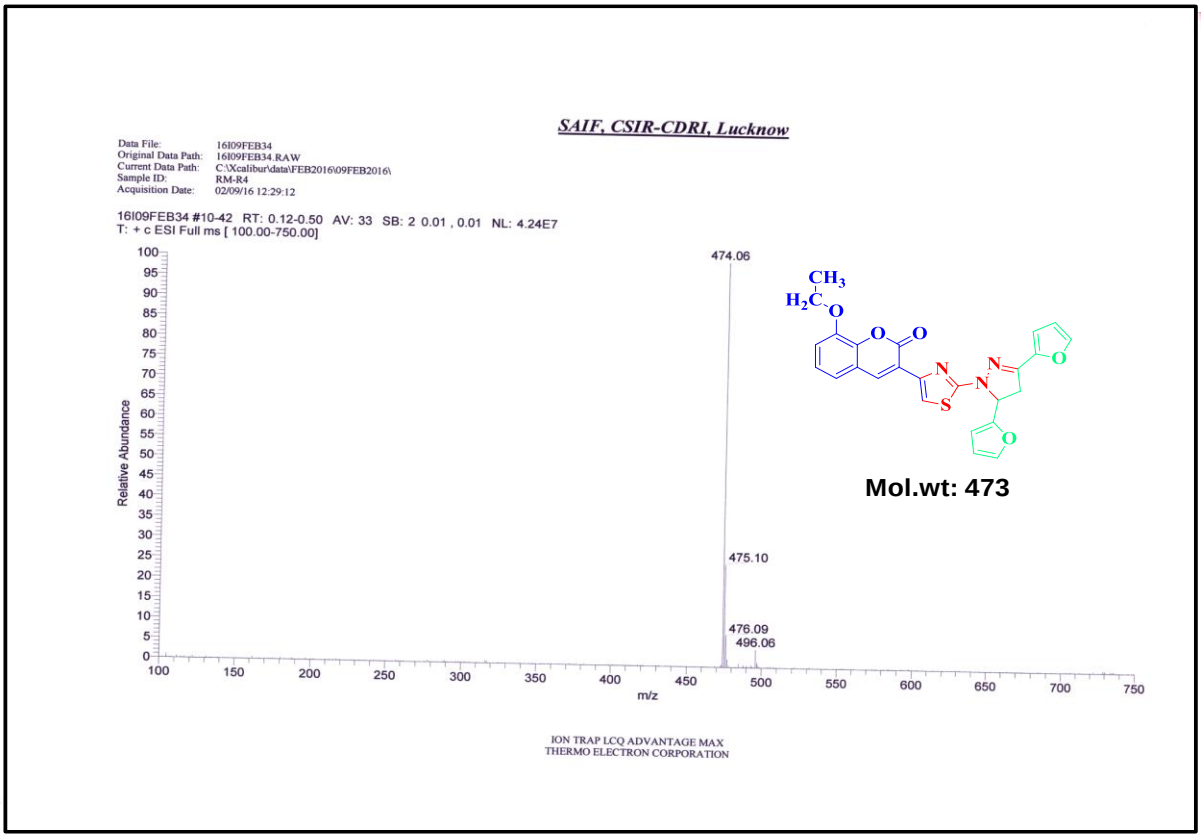


Fig: Mass spectrum of compound **39h**

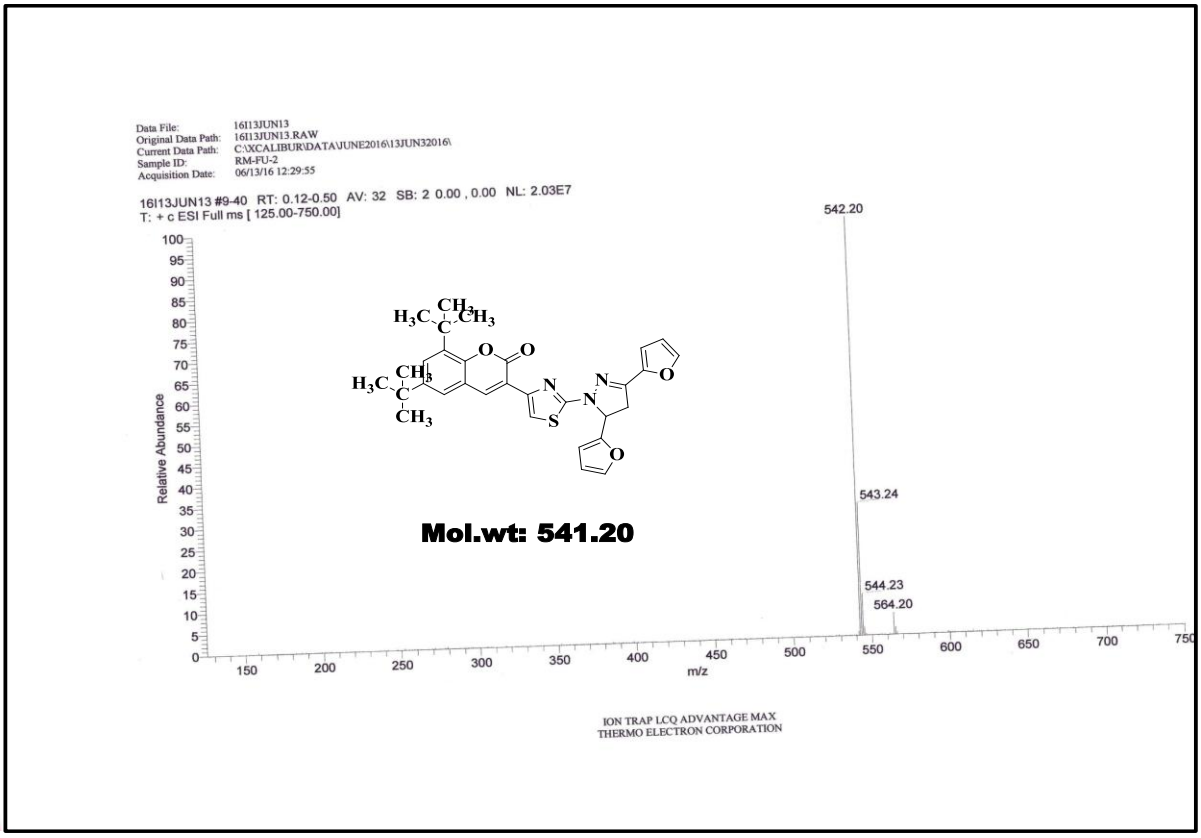


Fig: Mass spectrum of compound **39k**

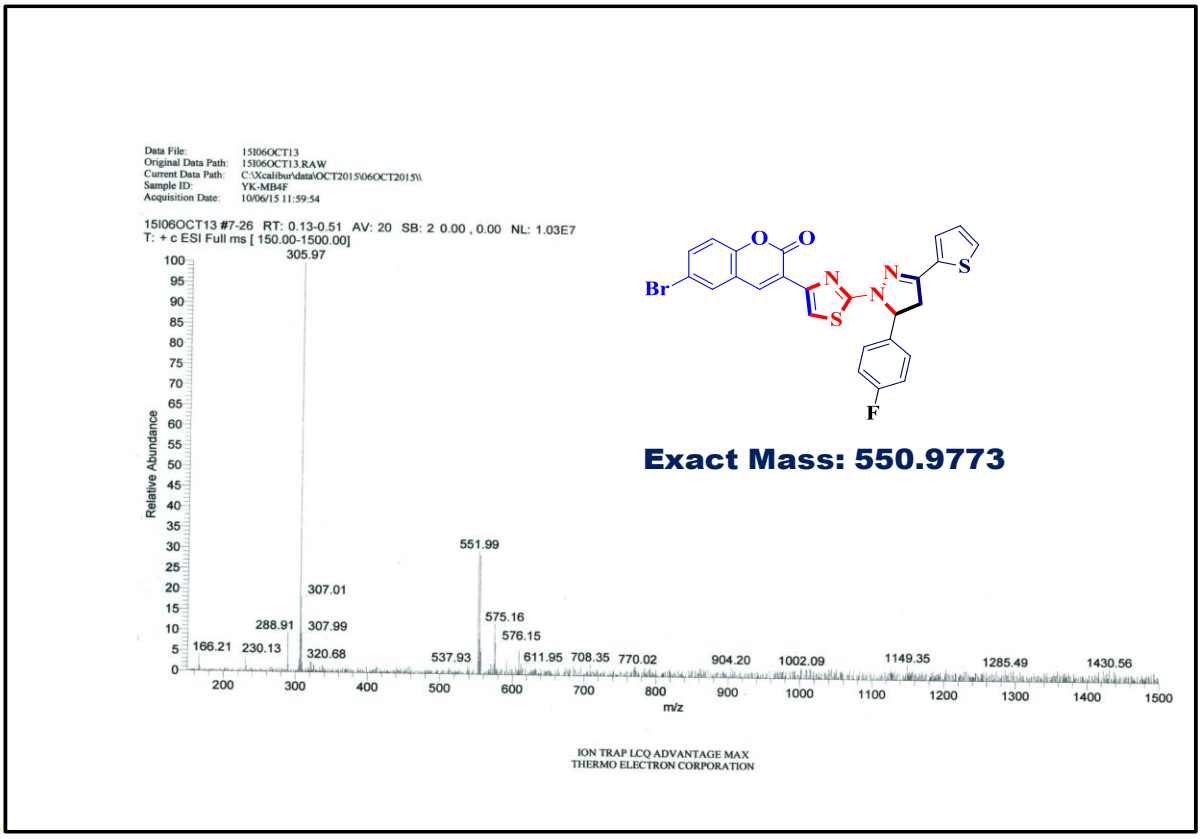


Fig: Mass spectrum of compound **41c**

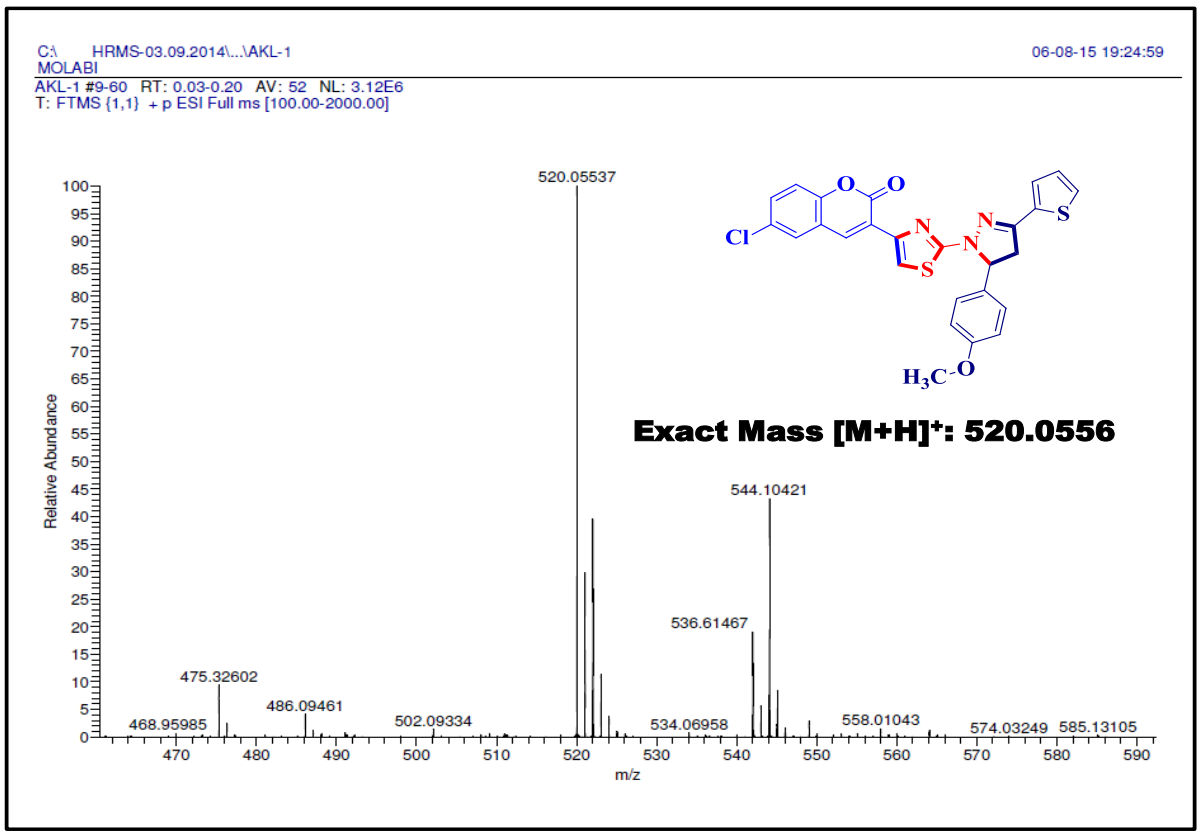


Fig: Mass spectrum of compound **41e**

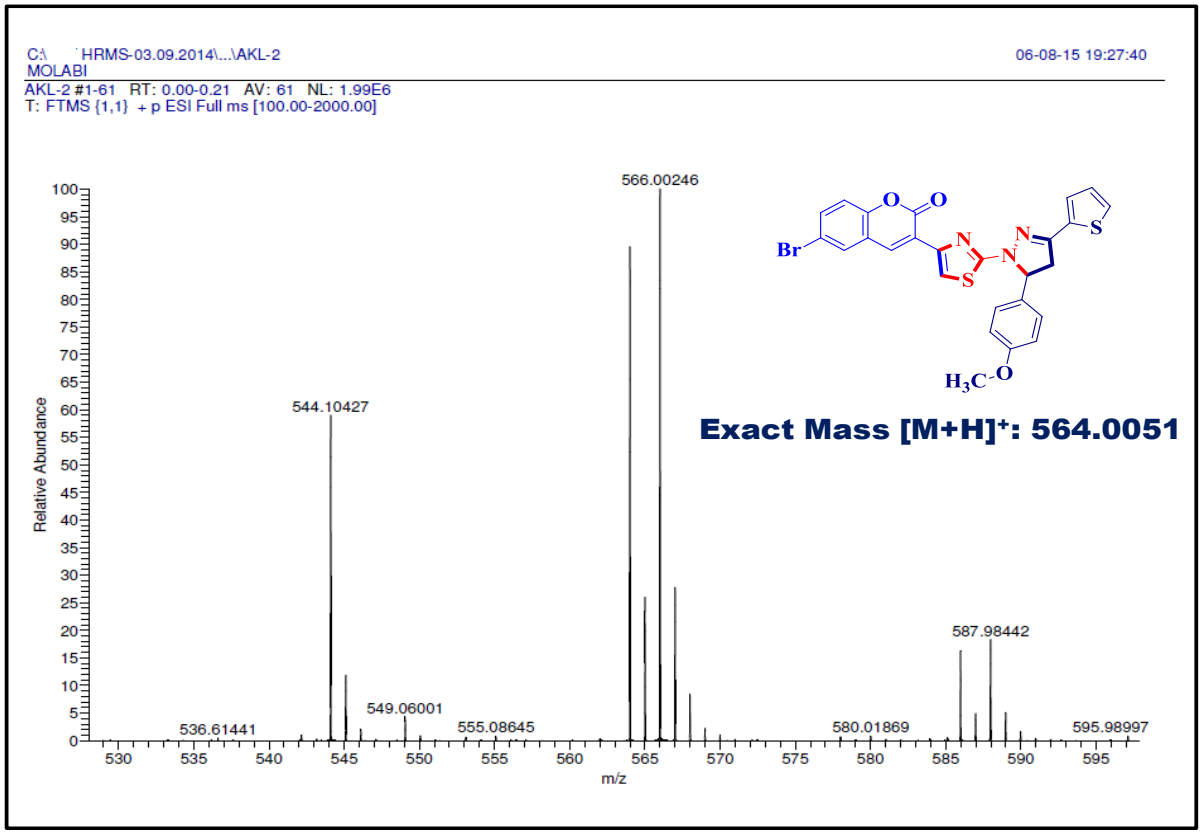


Fig: Mass spectrum of compound **41f**

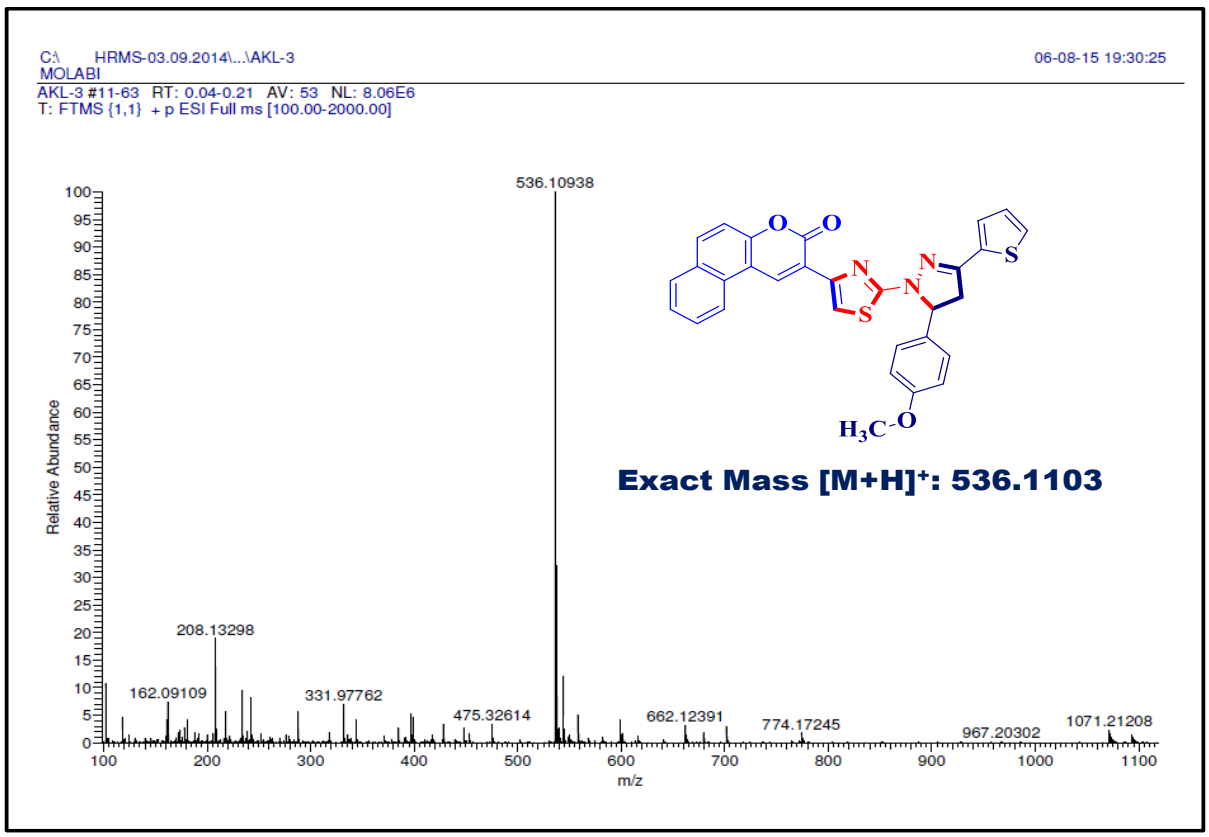


Fig: Mass spectrum of compound **41g**

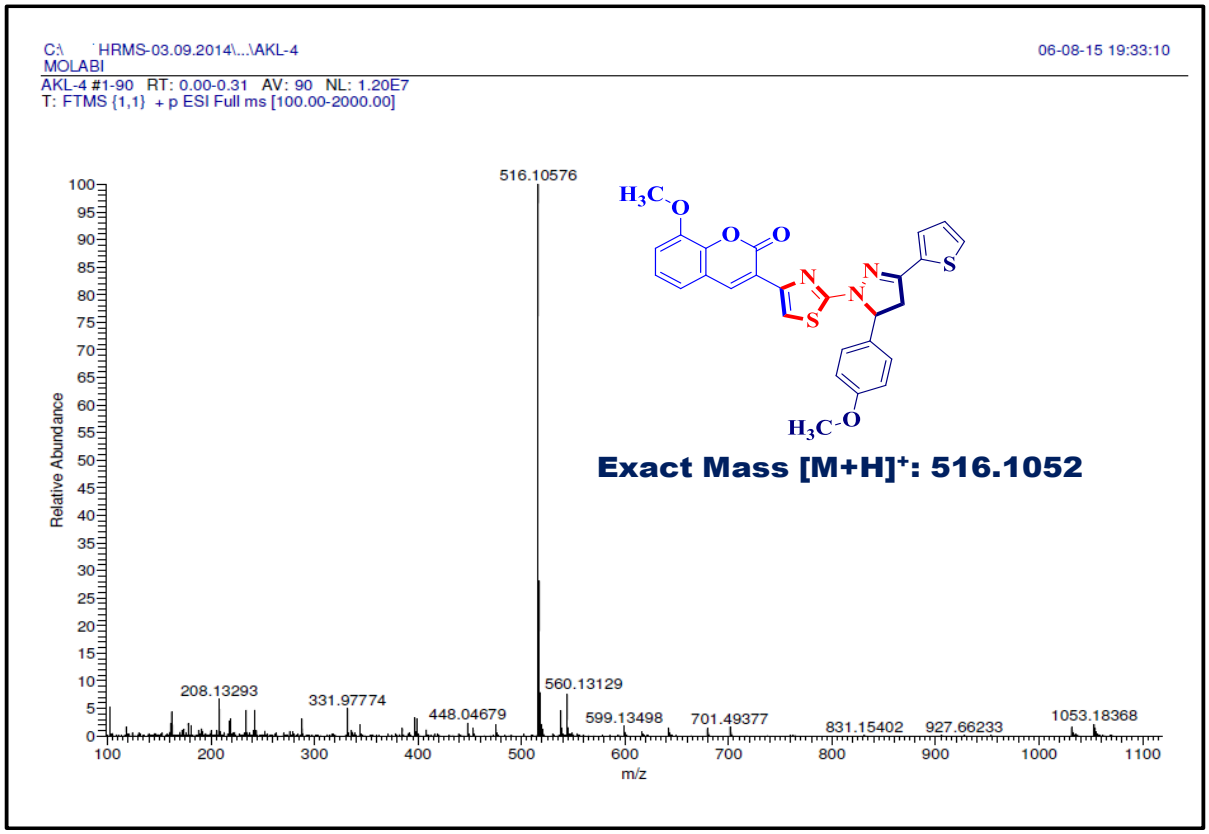


Fig: Mass spectrum of compound **41h**

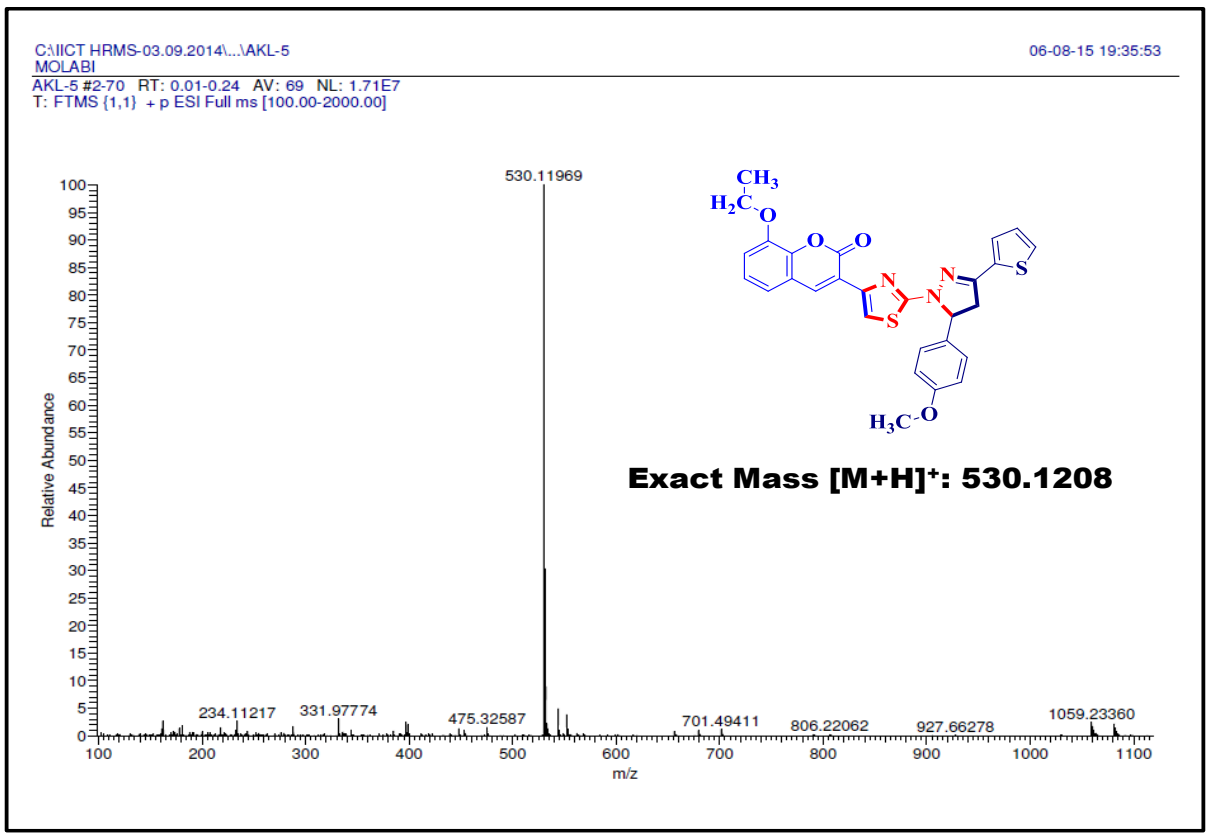


Fig: Mass spectrum of compound **41i**

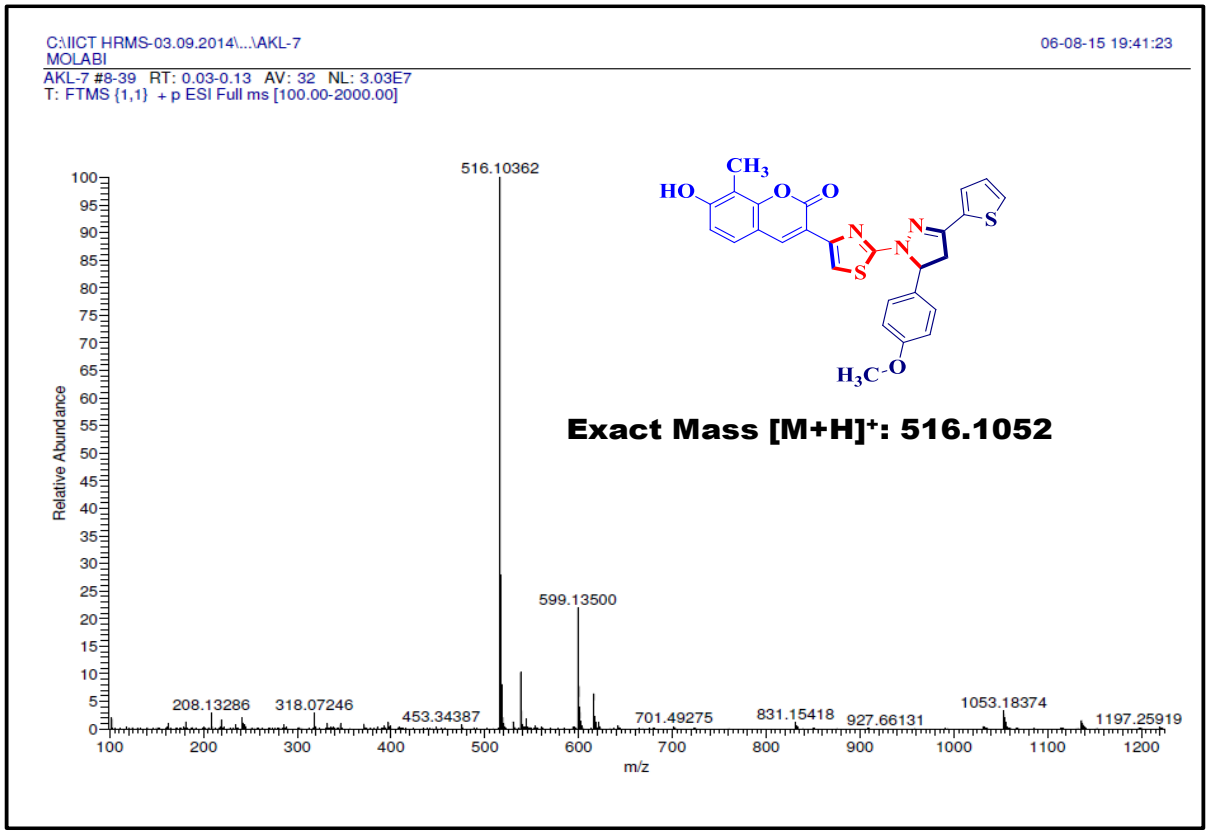


Fig: Mass spectrum of compound **41j**

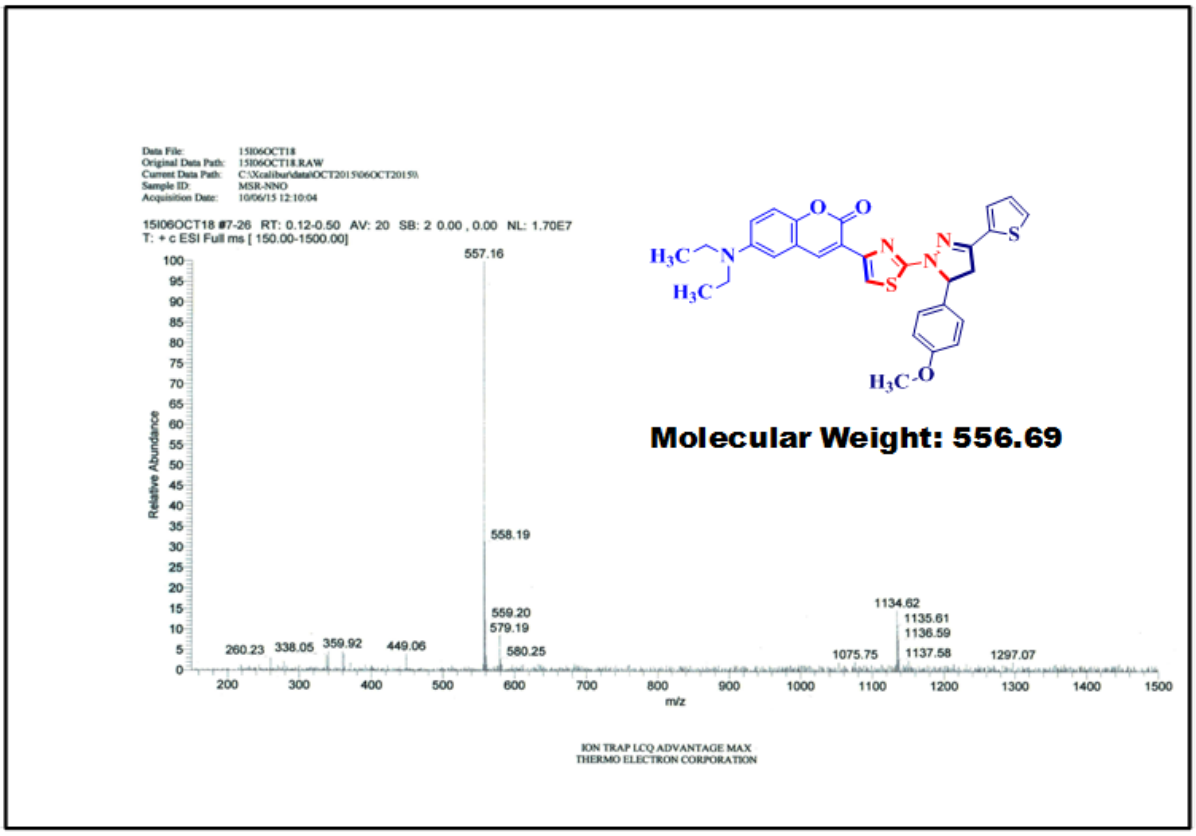


Fig: Mass spectrum of compound **41k**

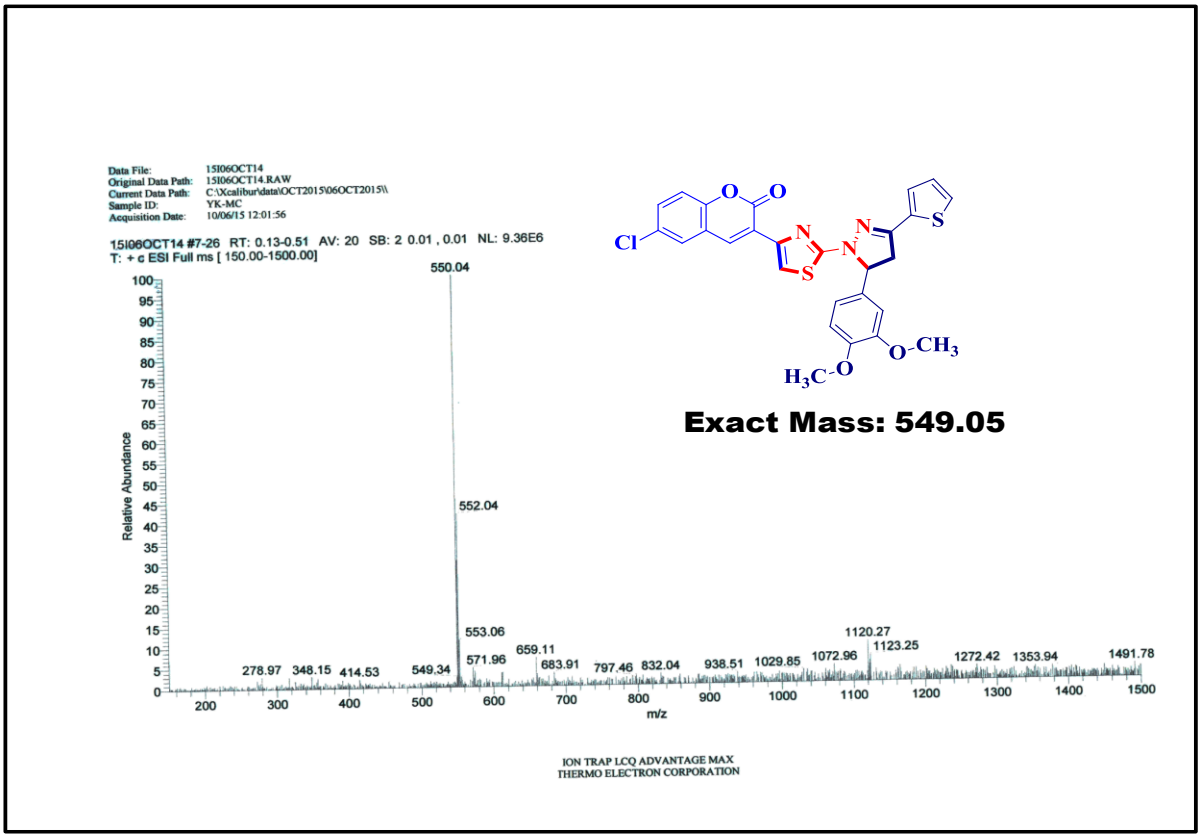


Fig: Mass spectrum of compound **41l**

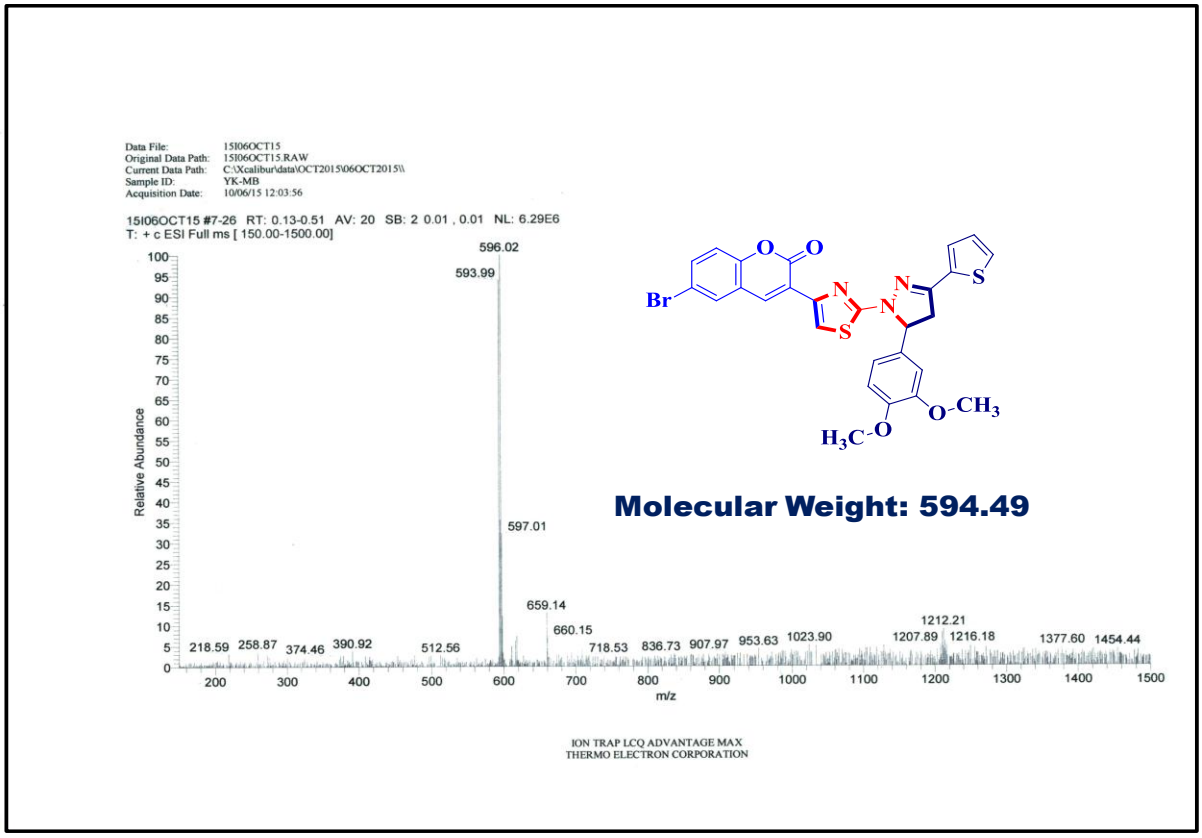


Fig: Mass spectrum of compound **41m**

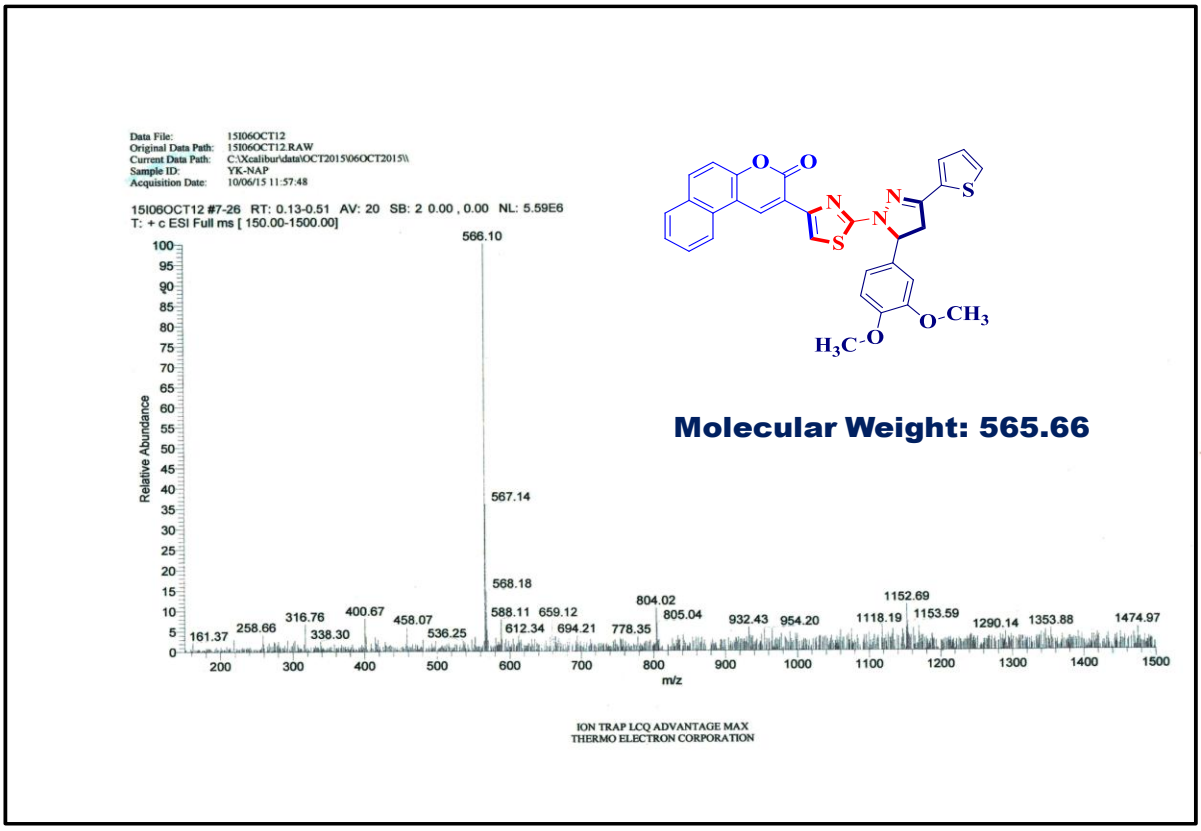


Fig: Mass spectrum of compound **41n**

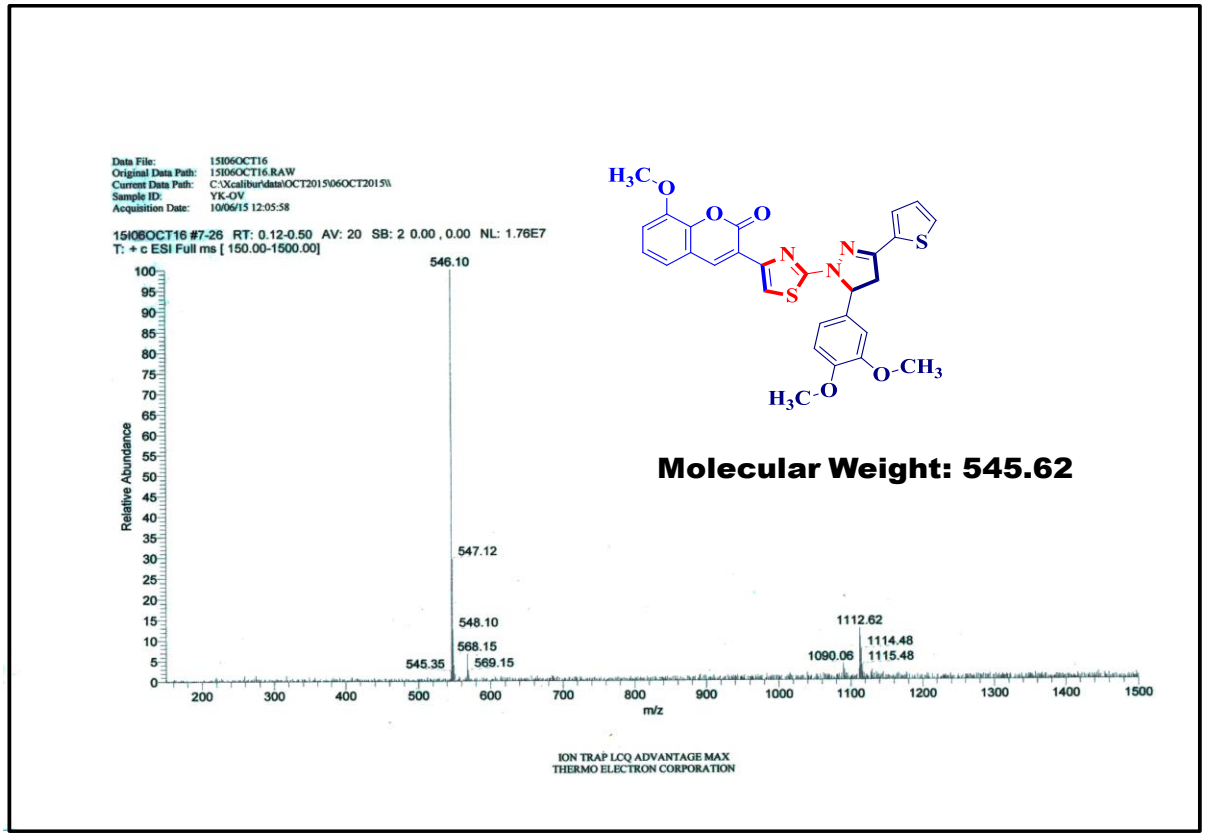


Fig: Mass spectrum of compound **41o**

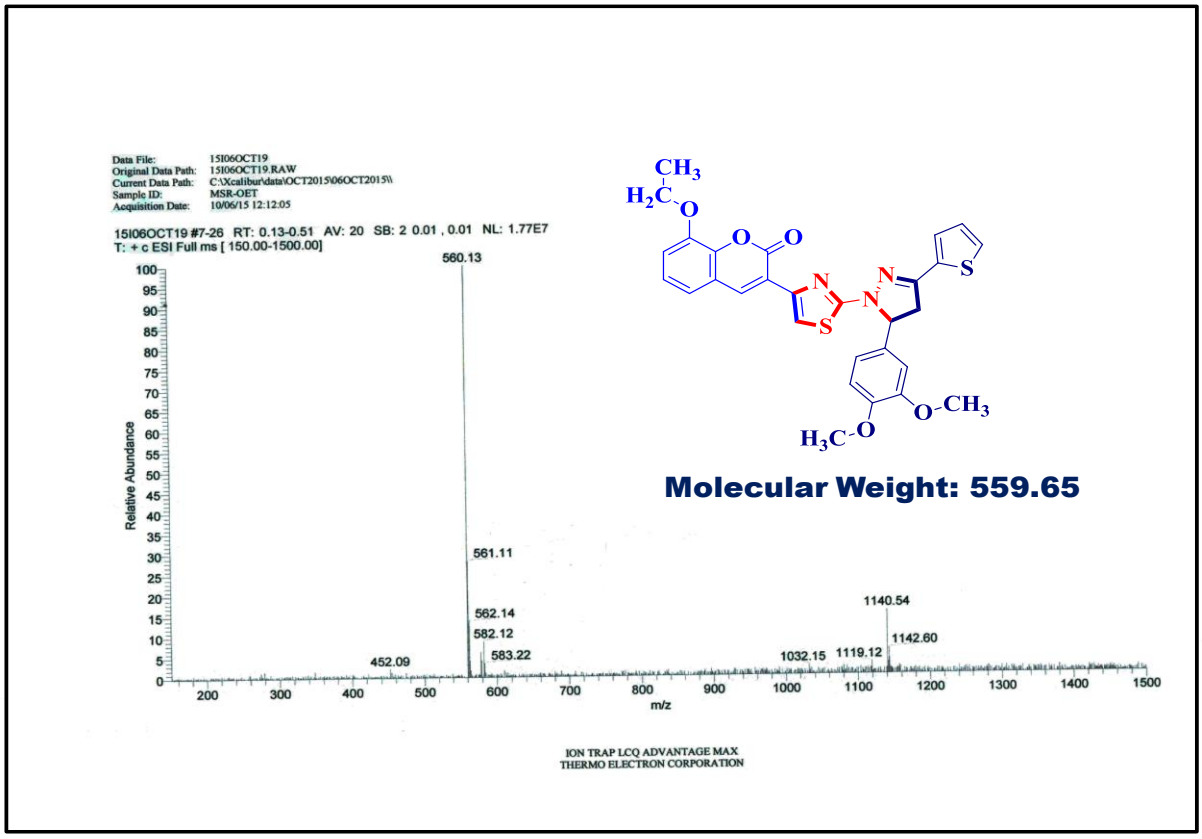


Fig: Mass spectrum of compound **41p**

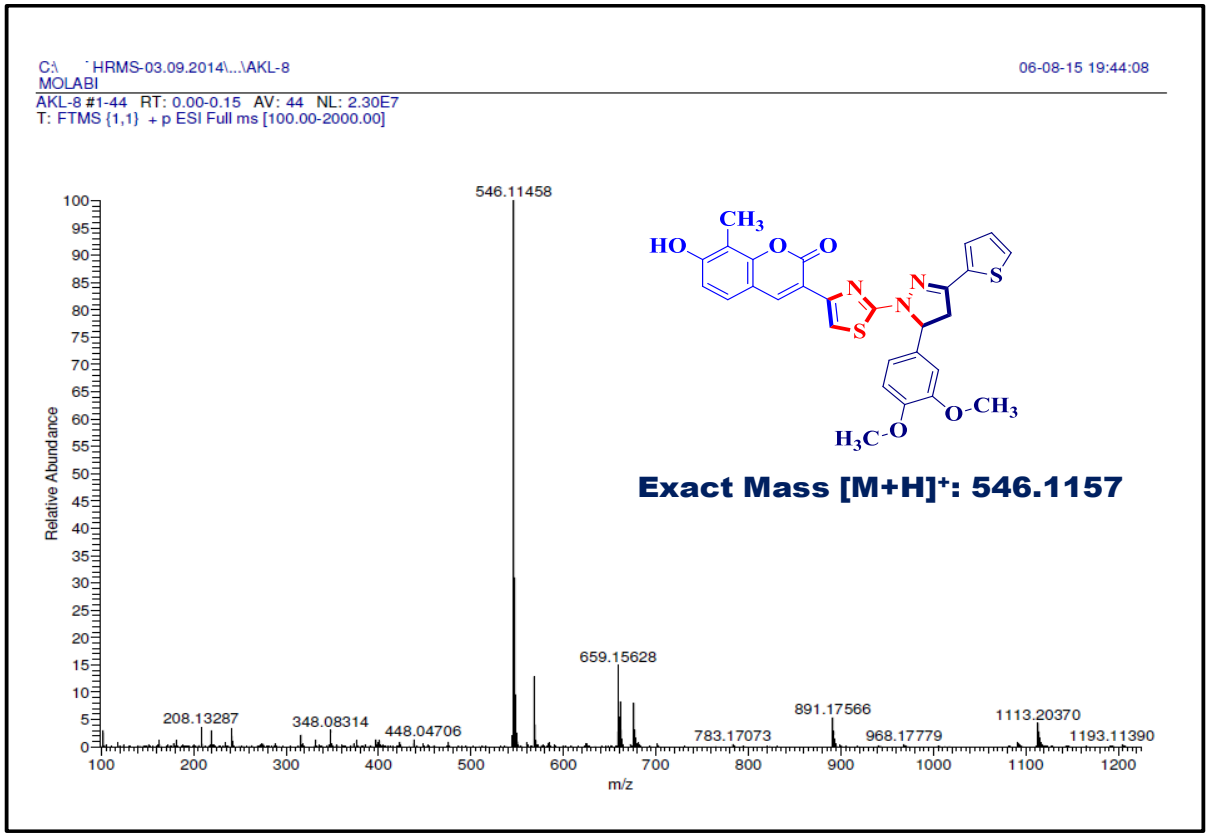


Fig: Mass spectrum of compound **41q**

CHAPTER-II
PART-D

A FACILE ONE POT MULTICOMPONENT SYNTHESIS OF ALKYL 2-(4-OXO-2-(5-ARYL-3-(THIOPHEN-2-YL)-4,5-DIHYDRO-1*H*-PYRAZOL-1-YL)THIAZOL-5(4*H*)-YLIDENE)ACETATE DERIVATIVES AND THEIR BIOLOGICAL ACTIVITY EVALUATION

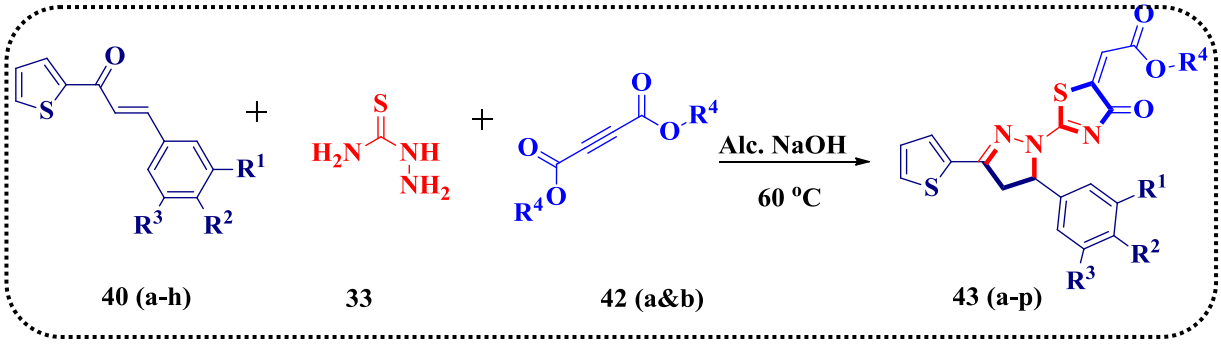
PART-D

PRESSENT WORK:

STARTING MATERIALS:

To synthesize the alkyl 2-(4-oxo-2-(5-aryl-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)thiazol-5(4*H*)-ylidene)acetate derivatives **43(a-p)**, 3-aryl-1-(thiophen-2-yl)prop-2-en-1-ones, thiosemicarbazide and dialkylacetylene dicarboxylates were used as starting materials. Various substituted 3-aryl-1-(thiophen-2-yl)prop-2-en-1-ones were prepared by using 2-acetyl thiophene and substituted benzaldehydes according to the literature procedure[64]. Thiosemicarbazide and dialkylacetylene dicarboxylates were procured from commercial source.

In view of the biological applications of dihydropyrazoles and 4-thiazolidinones, we have planned to synthesize the heterocyclic compounds having dihydropyrazoles and thiazolidinones through a multicomponent reaction method. The present chapter describes the synthesis and anticancer activity of 2-(4-oxo-2-(5-aryl-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)thiazol-5(4*H*)-ylidene)acetate derivatives. The title compounds were synthesized by the reaction of an equimolar amount of substituted 3-aryl-1-(thiophen-2-yl)prop-2-en-1-ones, thiosemicarbazide and dialkylacetylene dicarboxylates in presence of alcoholic NaOH solution at 60 °C (**Scheme-5**). The target molecules were obtained through simple reaction workup in a shorter reaction time with good yields (**Table 18**).



Scheme: 5 One pot synthesis of alkyl 2-(4-oxo-2-(5-aryl-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)thiazol-5(4*H*)-ylidene)acetate derivatives.

Table 18: Synthesis of alkyl 2-(4-oxo-2-(5-aryl-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)thiazol-5(4*H*)-ylidene)acetate derivatives (43 a-p).

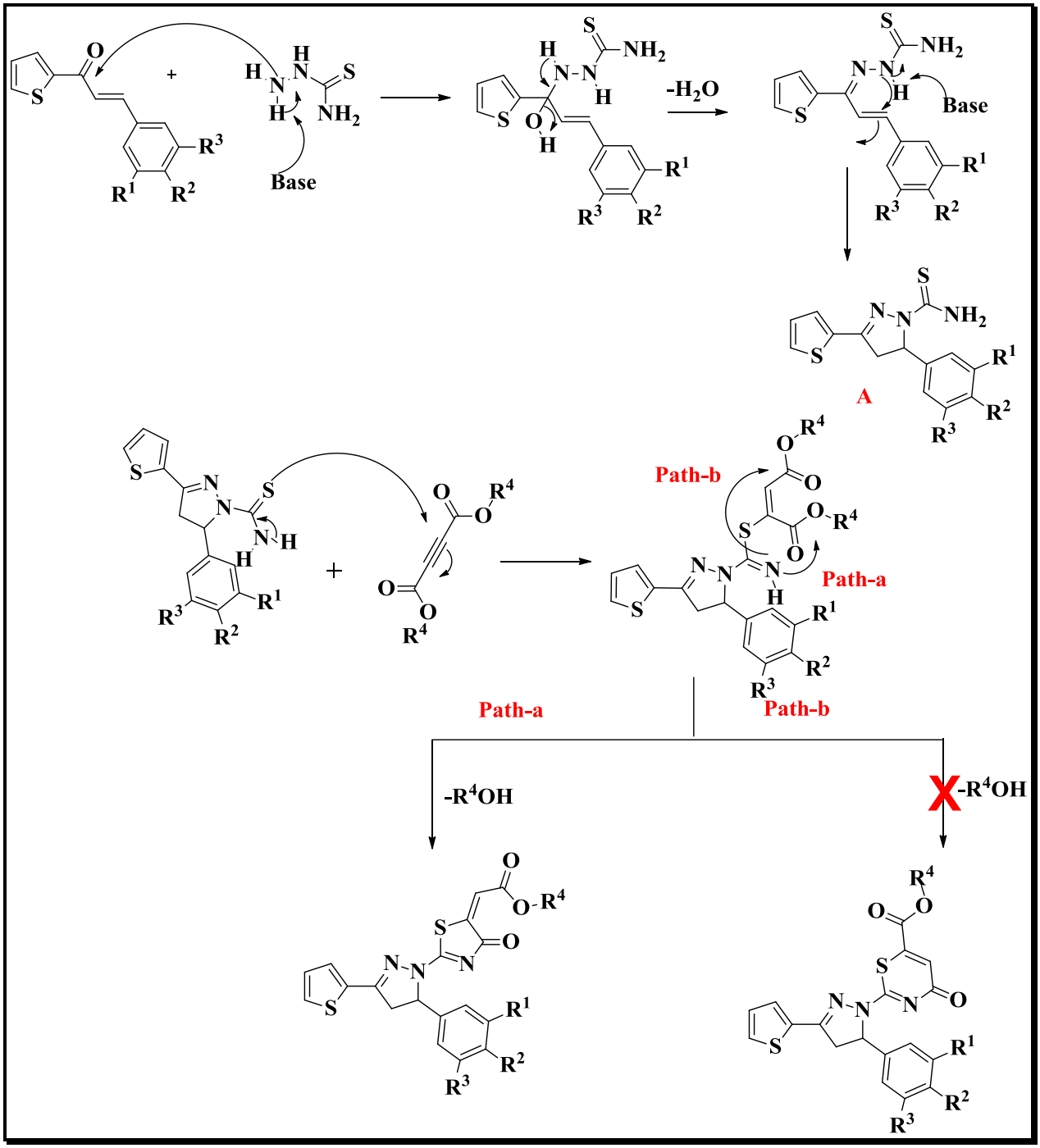
S. No.	Compound [a]	R^1	R^2	R^3	R^4	Reaction Time (h)	Yield (%) [b]
1	**43a**	H	H	H	$-CH_3$	4	78
2	**43b**	H	F	H	$-CH_3$	5.5	75
3	**43c**	H	Cl	H	$-CH_3$	4.5	84
4	**43d**	H	Br	H	$-CH_3$	5.0	89
5	**43e**	H	$N(CH_3)_2$	H	$-CH_3$	5.5	87
6	**43f**	H	$-OCH_3$	H	$-CH_3$	4.5	79
7	**43g**	$-OCH_3$	$-OCH_3$	H	$-CH_3$	5.0	81
8	**43h**	$-OCH_3$	$-OCH_3$	$-OCH_3$	$-CH_3$	5.0	84
9	**43i**	H	H	H	$-C_2H_5$	4.5	81
10	**43j**	H	F	H	$-C_2H_5$	5.5	90
11	**43k**	H	Cl	H	$-C_2H_5$	5.5	91
12	**43l**	H	Br	H	$-C_2H_5$	4.5	93
13	**43m**	H	$N(CH_3)_2$	H	$-C_2H_5$	5.0	88
14	**43n**	H	$-OCH_3$	H	$-C_2H_5$	4.5	82
15	**43o**	$-OCH_3$	$-OCH_3$	H	$-C_2H_5$	5.5	86
16	**43p**	$-OCH_3$	$-OCH_3$	$-OCH_3$	$-C_2H_5$	5.0	81

[a] **Reaction condition:** 3-aryl-1-(thiophen-2-yl) prop-2-en-1-one **40(a-h)** (1.0 mmol), Thiosemicarbazide **33** (1.0 mmol) and dialkylacetylene dicarboxylate **42(a&b)** (1.0 mmol) and Sodium hydroxide (1.5 mmol) in ethanol at 60 °C. [b] Isolated yields

Mechanism:

A plausible reaction mechanism for the synthesis of target compounds **43(a-p)** was proposed as shown in **Scheme-6**. The base catalysed cyclocondensation reaction of substituted 3-aryl-1-(thiophen-2-yl)prop-2-en-1-ones with thiosemicarbazide generates the intermediate 5-aryl-3-(thiophen-2-yl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (**A**). In the next step more nucleophilic sulphur atom of intermediate further reacts with dialkylacetylene dicarboxylates and forms dialkyl 2-((imino(5-phenyl-3-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl) methyl) thio)maleate (**B**). The intermediate undergoes cyclization through **Path a** leading to the formation of target compound.

The newly synthesized compounds were well characterized by IR, ^{1}H NMR, ^{13}C NMR, Mass and single crystal X-ray data. All the analytical spectral data was consistent with the assigned structures of the title compounds.



Scheme: 6 Plausible reaction mechanism for the formation of target compounds

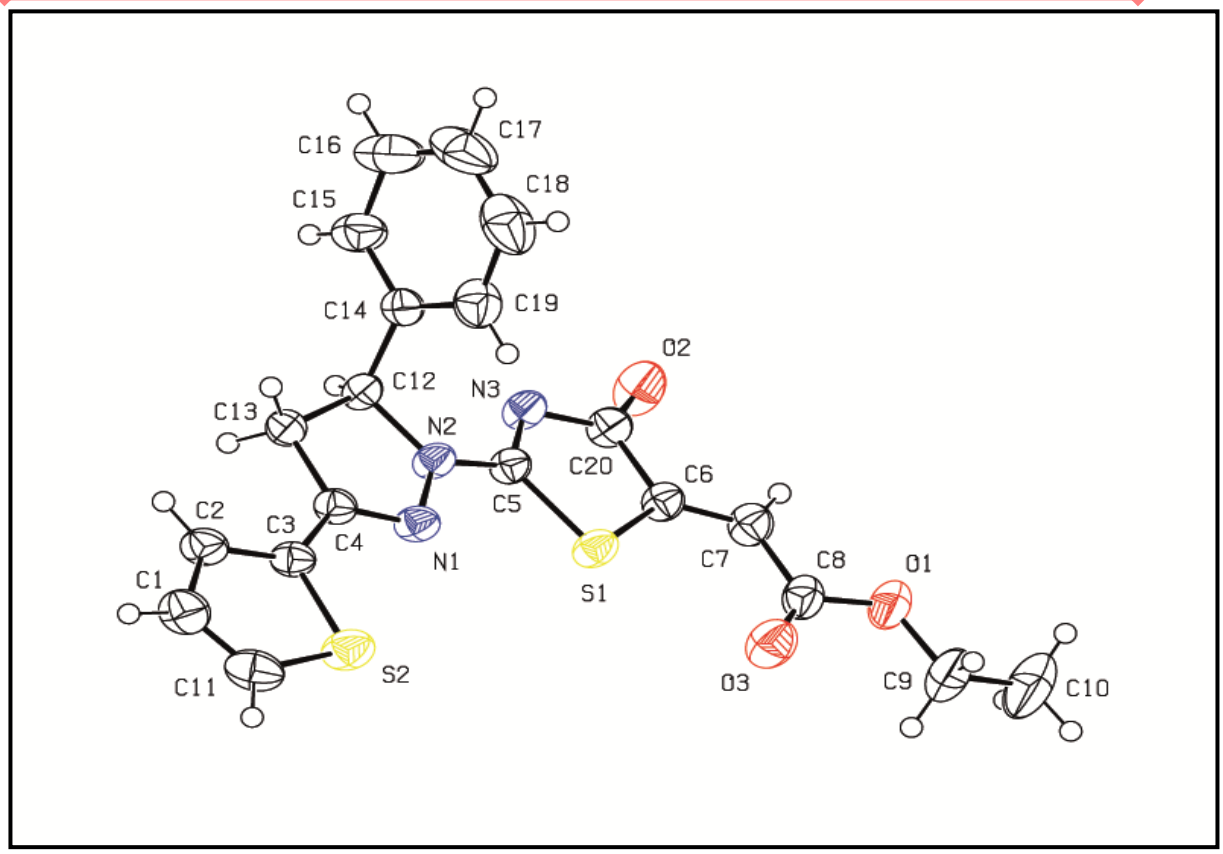


Fig.12: The title molecule **43i** with labelling scheme and 50% probability ellipsoids

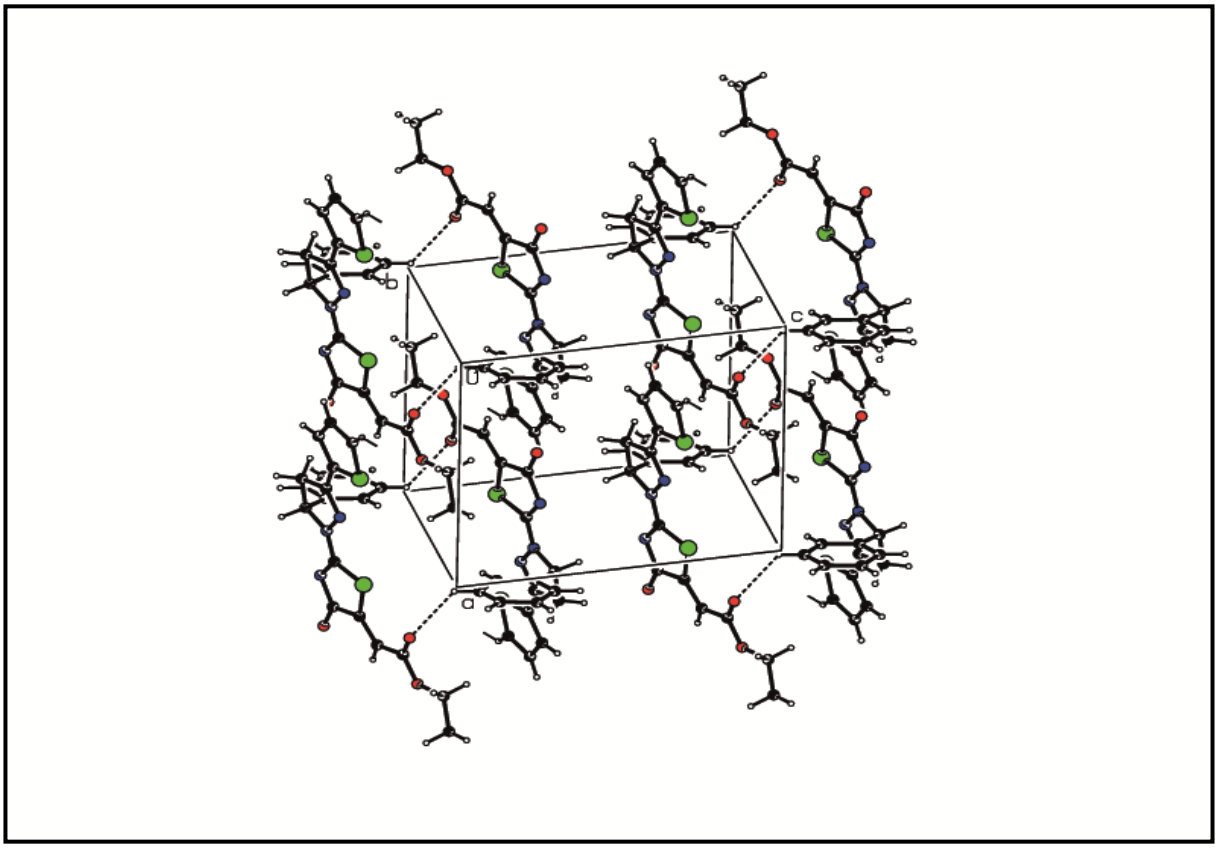


Fig.13: View of the packing and hydrogen bonding down the b axis.

As sown **Fig.12**, the central 4,5-dihydro-1*H*-pyrazole ring of the title compound makes dihedral angles of 5.48 (11), 1.35 (11) and 78.27 (14) °, with the 4,5-dihydro-1,3-thiazole

ring, the thiophene ring and the phenyl ring, respectively. The S1---C6---C7---C8, C6---C7—C8—O1, C7---C8---O1---C9 and C8---O1---C9---C10 torsion angles are 1.6(3), -178.2(2), -179.9(2) and -166.2(2)°, respectively. Therefore, the atoms of the ethyl acetate group is co-planar with. the 4,5-dihydro-1,3-thiazole ring attached to it. Crystal data, data collection and structure refinement details are summarized in **Table 19** and Selected geometric parameters are given in **Table 20**.

In the crystal, C---H…O hydrogen bonds connect to the adjacent molecules, into layers parallel to (0 0 1) (**Table 21** and **Fig.13**). In addition, π-π interactions [centroid-to-centroid distances = 3.6770 (13) Å between the thiophene ring and the 4,5-dihydro-1,3-thiazole ring] further stabilize the molecular packing of the title compound.

Table 19. Single crystal X-ray diffraction experimental details

Chemical formula	$C_{20}H_{17}N_3O_3S_2$
M_r	411.49
Crystal system, space group	Triclinic, $P\bar{1}$
Temperature (K)	296
a, *b*, *c* (Å)	9.1574 (6), 10.0040 (5), 11.9699 (7)
α, β, γ (°)	101.213 (2), 98.293 (3), 110.921 (2)
V (Å^3)	977.02 (10)
Z	2
F(000)	428
D_x (Mg m^{-3})	1.399
Radiation type	Mo *K*α
No. of reflections for cell measurement	2792
θ range (°) for cell measurement	4.5–54.3
μ (mm^{-1})	0.30
Crystal shape	Block
Colour	Yellow
Crystal size (mm)	0.35 × 0.30 × 0.20
Diffractometer	Bruker axs kappa apex2 CCD Diffractometer
Radiation source	fine-focus sealed tube
Monochromator	Graphite

Scan method	ω and ϕ scan
Absorption correction	Multi-scan *SADABS* (Bruker, 1999)
T_{min}, T_{max}	0.903, 0.943
No. of measured, independent and observed [$I > 2\sigma(I)$] reflections	8223, 4742, 3475
R_{int}	0.021
θ values (°)	θ_{max} = 28.3, θ_{min} = 1.8
$(\sin\theta/\lambda)_{max}$ (Å^{-1})	0.668
Range of *h*, *k*, *l*	*h* = -12→11, *k* = -13→11, *l* = -15→15
Refinement on	F^2
$R[F^2 > 2\sigma(F^2)]$, $wR(F^2)$, *S*	0.046, 0.157, 1.11
No. of reflections	4742
No. of parameters	254
No. of restraints	0
H-atom treatment	H atoms treated by a mixture of independent and constrained refinement
Weighting scheme	$w = 1/[\sigma^2(F_o^2) + (0.0836P)^2 + 0.0571P]$ where $P = (F_o^2 + 2F_c^2)/3$
$(\Delta/\sigma)_{max}$	0.001
$\Delta\rangle_{max}$, $\Delta\rangle_{min}$ (e Å^{-3})	0.36, -0.47

Table 20. Selected geometric parameters (Å, °)

C3—S2 1.719(2) C4—N1 1.285(3) C4—C13 1.513(3) C5—N3 1.320(2) C5—N2 1.324(3) C5—S1 1.772(2) C6—S1 1.744(2) C7—C8 1.470(3)	C8—O3 1.202(3) C8—O1 1.339(3) C9—O1 1.456(3) C11—S2 1.702(3) C12—N2 1.476(3) C20—O2 1.222(2) C20—N3 1.374(3) N1—N2 1.399(2)
N1—C4—C3 122.43(19) N1—C4—C13 115.11(17) N3—C5—N2 121.14(19) N3—C5—S1 119.47(16) N2—C5—S1 119.39(14) C7—C6—S1 127.33(17) C20—C6—S1 110.27(15) O3—C8—O1 125.2(2) O3—C8—C7 124.2(2) O2—C20—N3 124.8(2) O2—C20—C6 122.2(2) N3—C20—C6 113.02(17)	C5—N2—N1 120.35(17) C5—N2—C12 125.61(16) N1—N2—C12 114.03(16) C5—N3—C20 110.02(17) O1—C8—C7 110.7(2) O1—C9—C10 107.2(3) C1—C11—S2 113.05(17) N2—C12—C14 112.33(16) N2—C12—C13 101.18(14) C8—O1—C9 115.8(2) C6—S1—C5 87.02(9) C11—S2—C3 91.32(12)

C4—N1—N2 106.66(17)	
C2—C3—C4—N1 -178.0(2) S2—C3—C4—C13 178.40(15) C20—C6—C7—C8 -179.55(19) S1—C6—C7—C8 1.6(3) C6—C7—C8—O3 2.2(4)	C6—C7—C8—O1 -178.2(2) N2—C12—C14—C15 156.13(19) O3—C8—O1—C9 -0.3(4) C7—C8—O1—C9 -179.9(2) C10—C9—O1—C8 166.2(2)

Table 21. Hydrogen Bonds (Å, °)

D—H···A	D—H	H···A	D···A	D—H···A
C18---H18…O3^{i}	0.93	2.58	3.260(4)	130

Symmetry codes: (i) 2-x, 1-y, 2-z.

***In vitro* antiviral activity of alkyl 2-(4-oxo-2-(5-aryl-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)thiazol-5(4*H*)-ylidene)acetate derivatives (43 a-p).**

The title compounds were evaluated for antiviral activity against various human viruses. The HEL cell culture was used to evaluate the compounds against *Herpes simplex* virus type I (KOS) [HSV-1 KOS], *Herpes simplex* virus type 2 (G) [HSV-2 G], *Vaccinia* virus [VV], *Vesicular stomatitis* virus (VSV), thymidine kinase-deficient *Herpes simplex* virus type 1 (HSV-1 TK^{-} KOS ACV^{r}) and *Adeno* virus type 2 (AV-2), Vero cells were utilized to evaluate the compounds against *Parainfluenza* type 3 virus (PI-3V), *Reo* virus type 1 (RV-1), *Sindbis* virus (SV), *Coxsackie* virus B4 (CV B4) and *Punto toro* virus (PTV), the CRFK cell culture medium was used for *Feline Corona* Virus (FIPV) and *Feline Herpes* Virus. The MDCK cell line was used to infect with *Influenza* A virus type H1N1 (strain A/Ned/378/05) and type H3N2 (strain A/HK/7/87), and *Influenza* B virus (strain B/Ned/537/05). Based on the microscopical analysis of the virus-induced cytopathicity (CPE) the antiviral potency of the compounds was determined and compared to different reference antiviral drugs (brivudin, cidofovir, acyclovir, ganciclovir, zalcitabine and alovudine).

From the antiviral activity experimental results, the compounds **43b** exhibited excellent activity against *Feline Corona* Virus at EC_{50} of **2.6 µM** and compound **43l** exhibited excellent antiviral activity against *Feline Herpes* Virus at EC_{50} of **1.45 µM** concentration. The compounds **43b** and **43l** are more potent when compared to

Ganciclovir, HHA and UDA antiviral standard drugs (**Table.22**). The remaining compounds did not exhibit any antiviral activity against the tested viruses.

Table.22: Cytotoxicity and antiviral activity in: CRFK cell cultures

Compound	CC_{50} (μM) [a]	EC_{50} (μM)[b]	
		Feline Corona Virus (FIPV)	*Feline Herpes* Virus
43a	>100	>100	>100
43b	29.0	**2.6**	>100
43c	20.5	>100	>100
43d	>100	>100	>100
43e	9.1	>100	>100
43f	9.9	>100	>100
43g	>100	>100	>100
43h	37.1	>100	>100
43i	8.5	>100	>100
43j	8.05	>100	>100
43k	8.4	>100	>100
43l	9.0	>100	**1.45**
43m	1.8	>100	>100
43n	>100	>100	>100
43o	43.7	>100	>100
43p	5.0	>100	>100
HHA	>100	9.4	2.3
UDA	>100	5.7	2.0
Ganciclovir	>100	>100	3.4

[a]50% Cytotoxic concentration, as determined by measuring the cell viability with the colorimetric formazanb50%
[b]Effective concentration, or concentration producing 50% inhibition of virus-induced cytopathic effect, as
CRFK cells: Crandell-Rees Feline Kidney cells.

***In vitro* anticancer activity alkyl 2-(4-oxo-2-(5-aryl-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)thiazol-5(4*H*)-ylidene)acetate derivatives (43 a-p).**

The newly synthesized alkyl 2-(4-oxo-2-(5-aryl-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)thiazol-5(4*H*)-ylidene)acetate derivatives were submitted to National Cancer Institute –USA (NCI) for anticancer activity screening against a panel of nine different cancer types of sixty cell lines at one dose concentration (10 μM). The nine types of cancer cells include Leukemia, Non-Small Cell Lung, Colon, CNS, Melanoma, Ovarian, Renal, Prostate and Breast cancer cells. The compounds **43b** and **43f** were selected by NCI for anticancer screening. The preliminary anticancer activity was done

according to NCI protocol[68-70]**.** The cells were treated with the tested compounds at single dose concentration (10 μM) and incubated for 48 h. The percentage cell growth was calculated using protein binding dye, sulforhodamine B (SRB) under UV-Visible spectrophotometer. The compound **43b** exhibited good growth inhibition against the tested cancer cell lines.

The compound **43b** was selected for further screening at five dose concentration levels (100, 10, 1, 0.1 and 0.01 μM). From the cytotoxicity experiment three anticancer activity dose response parameters were measured against each cancer cell line. GI_{50} is the concentration of the test compound required to inhibit 50% net cell growth. TGI is the concentration of test compound requires inhibiting the total cells. LC_{50} is the concentration of compound required to 50% cell death.

The NCI anticancer activity screening data reveals that the compound **43b** was found to be the potent anticancer agent *in vitro*. The compound **43b** was found to be a highly active growth inhibitor for **renal cancer cell** UO-31 (GI_{50} 1.49 μM), **melanoma** UACC-62 cell (GI_{50} 1.57 μM), **CNS** SNB-75 cell (GI_{50} 1.64 μM), **colon cancer** KM12 cell (GI_{50} 1.92 μM) and **Non-small cell lung cancer** HOP -62 cell (GI_{50} 1.87 μM). The mean *p*GI_{50}, *p*TGI and *p*LC_{50} of the compound **43b** was shown in **Fig.14**

In conclusion, we have synthesized alkyl 2-(4-oxo-2-(5-aryl-3-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl)thiazol-5(4H)-ylidene)acetate derivatives via a multicomponent reaction approach with good yields. The newly synthesized compounds were evaluated for their antiviral and anticancer activity and the compound **43b** exhibited potential antiviral activity against Feline Herpes virus (EC_{50} 2.6 μM) and compound **43l** against Feline corona virus (EC_{50} 1.45 μM). The compound **43b** exhibited excellent anticancer against NCI-60 cancer cell lines.

EXPERIMENTAL:

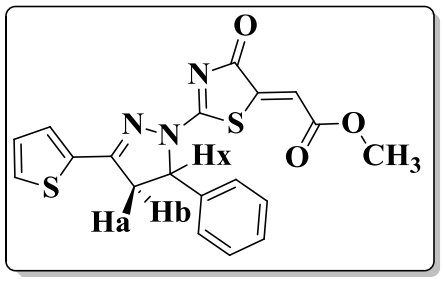
General procedure for the synthesis of alkyl 2-(4-oxo-2-(5-aryl-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)thiazol-5 (4*H*)-ylidene)acetate 43a-p:

Substituted 3-aryl-1-(thiophen-2-yl) prop-2-en-1-ones **40a-h** (1.0 mmol); thiosemicarbazide **33** (0.091 g, 1.0 mmol) and sodium hydroxide (0.060 g, 1.5 mmol) in ethanol (5 mL) solvent were charged in a round bottom flask equipped with condenser

and mechanical stirrer. The temperature of reaction mixture was slowly raised to 60 – 65 °C and maintained the temperature for about 2.5 h. To this hot reaction mixture dialkyl acetylenedicarboxylate **42 a & b** (1.0 mmol) was added and refluxed for about 1.5 h. After completion of the reaction (as detected by TLC) the reaction mixture was cooled to room temperature and the isolated solid product was filtered, washed with ethanol, dried and recrystallized from ethanol.

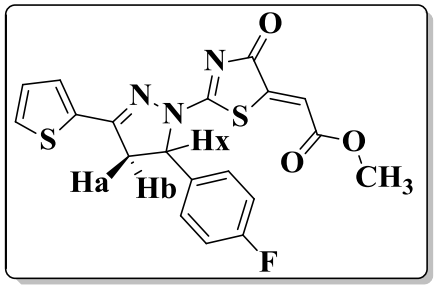
SPECTRAL DATA

Methyl 2-(4-oxo-2-(5-phenyl-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)thiazol-5(4*H*)-ylidene)acetate (43a):



Yellow solid; m.p.: 115 – 117 °C; **FTIR (KBr, υ_{max}, cm^{-1}):** 1707 (Ester –C=O); 1667 (amide-C=O), 1612 (C=N), 1252 (-C-O-C); **^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm):** 3.39 - 3.44 (dd, *J* =3.42 Hz, 1H, Pyrazole-Ha); 3.86 (s, 3H, -OCH_3); 3.97 – 4.02 (dd, *J* = 4.01Hz, 1H, Pyrazole-H_b); 5.85 – 5.89 (dd, *J* = 5.87, 1H, Pyrazole –Hx); 6.91 (s, 1H, =CH); 7.12 – 7.14 (dd, *J* = 7.14 Hz, 1H, Thiophene-H); 7.24 -7.27 (m, 1H, Ar-H); 7.30 – 7.37 (m, 4H, Ar-H); 7.39 - 7.40 (dd, *J* = 7.40 Hz, 1H, Thiophene-H); 7.59 - 7.60 (dd, *J* = 7.60 Hz, 1H, Thiophene-H); **^{13}C NMR (100 MHz, DMSO-d_6, ppm):** δ 44.67, 53.13, 64.58, 116.75, 126.23, 128.63, 129.14, 129.54, 132.51, 133.08, 134.05, 140.05, 147.03, 158.84, 166.77, 171.86, 177.68; Anal.Calcd for $C_{19}H_{15}N_3O_3S_2$ (397.47): C, 57.41; H, 3.80; N, 10.57%, Found: C, 57.46; H, 3.76; N, 10.59 %.

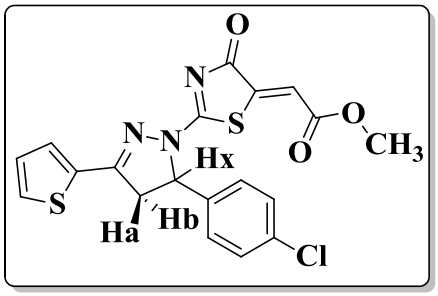
Methyl 2-(2-(5-(4-fluorophenyl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)-4-oxo thiazol-5(4*H*)-ylidene)acetate (43b):



Yellow solid; m.p.: 110 – 112 °C; **FTIR (KBr, υ_{max}, cm^{-1}):** 1686 (Ester –C=O); 1658 (amide –C=O), 1588 (-C=N); **^{1}H NMR (400 MHz, $CDCl_3$, ppm):** 3.37 – 3.43 (dd, *J* = 3.40 Hz, 1H, pyrazole-Ha); 3.86 (s, 3H, -OCH_3); 3.97- 4.04 (dd, *J* = 4.01 Hz, 1H, pyrazole-H_b); 5.83 – 5.86 (dd, *J* = 5.85 Hz,

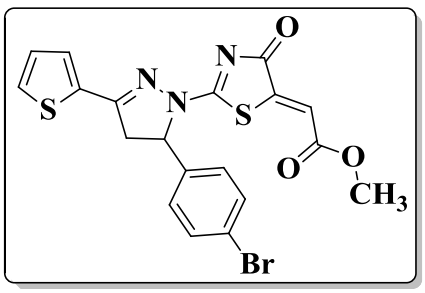
1H, pyrazole-H_x); 6.92 (s, 1H, =CH,); 7.03 (t, *J* = 7.03 Hz, 2H, Ar-H); 7.13 – 7.15 (dd, *J* = 7.15 Hz, 1H, Thiophene-H); 7.24 – 7.27 (m, 2H, Ar-H); 7.40 – 7.41 (dd, *J* = 7.41 Hz, 1H, Thiophene-H); 7.60 – 7.62 (dd, *J* = 7.61 Hz, 1H, Thiophene-H); **^{13}C NMR (100 MHz, DMSO-d_6, ppm):** 44.50, 53.08, 63.90, 116.17, 116.39, 116.76, 128.63, 128.71, 129.09, 132.49, 133.03, 133.97, 146.96, 158.73, 161.05, 166.78, 171.92, 177.62; **MS (ESI m/z):** 416 $[M+H]^+$; Anal. Calcd for $C_{19}H_{14}FN_3O_3S_2$ (415.46): C, 54.93; H, 3.40; N, 10.11 %, Found: C, 54.96; H, 3.35; N, 10.09%.

Methyl 2-(2-(5-(4-chlorophenyl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)-4-oxo thiazol-5(4*H*)-ylidene)acetate (43c):



Yellow solid; m.p.: 145 – 147 °C; **FTIR (KBr, υ_{max}, cm^{-1}):** 1692 (Ester –C=O), 1674 (amide –C=O), 1610 (-C=N); **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm):** 3.36 – 3.42 (dd, *J* = 4 Hz, 1H, -CH_2), 3.86 (s, 3H, -OCH_3), 3.98 – 4.06 (dd, *J* = 12 Hz, 1H, -CH_2), 5.82 – 5.86 (dd, *J* = 4 Hz, 1H, -CH), 6.92 (s, 1H, =CH), 7.14 (t, *J* = 8 Hz, 1H, Ar-H), 7.21 (d, *J* = 8 Hz, 2H, Ar-H), 7.32 (d, *J* = 8 Hz, 2H, Ar-H), 7.40 (s, 1H, Ar-H), 7.61 (d, *J* = 4 Hz, 1H, Ar-H); **^{13}C NMR (100 MHz, $CDCl_3$, ppm):** δ 43.94, 52.62, 63.40, 117.60, 127.40, 128.14, 129.46, 131.22, 131.75, 132.77, 134.62, 137.38, 146.92, 155.97, 167.07, 173.31, 178.57; Anal. Calcd for $C_{19}H_{14}ClN_3O_3S_2$ (431.91): C, 52.84; H, 3.27; N, 9.73%, Found: C, 52.79; H, 3.30; N, 9.77%.

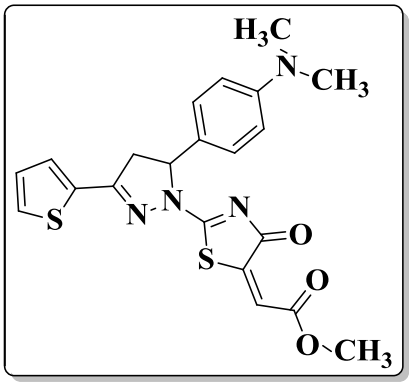
Methyl 2-(2-(5-(4-bromophenyl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)-4-oxo thiazol-5(4*H*)-ylidene)acetate (43d):



Brown solid; m.p.: 178 – 180 °C; **FTIR (KBr, υ_{max}, cm^{-1}):** 1689 (Ester-C=O), 1646 (amide-C=O), 1602 (-C=N); **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm):** 3.35 – 3.41 (dd, *J* = 3.6 Hz, 1H, Pyrazole-H); 3.86 (s, 3H, -OCH_3); 3.97 – 4.04 (dd, *J* = 11.2 Hz, 1H, Pyrazole-H); 5.80 – 5.84 (dd, *J* = 4 Hz, 1H, Pyrazole-H); 6.93 (s, 1H, =CH); 7.13 – 7.15 (dd, *J* = 3.6 Hz, 1H, Thiophene-H); 7.20 (t, *J* = 1.6Hz, 1H, Ar-H), 7.22 (s, 1H, Ar-H), 7.37 (t, *J* = 1.6Hz, 1H, Ar-H); 7.39 – 7.40 (dd, *J* = 1.2 Hz, 1H, Thiophene-H); 7.43 – 7.45 (m, 1H, Ar-H); 7.60 – 7.62 (dd, 1H, *J* = 1.2

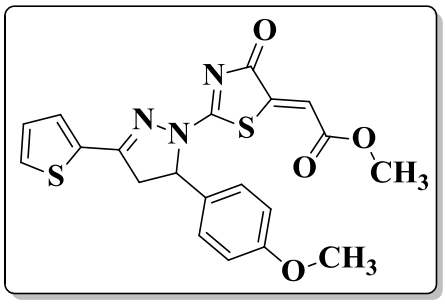
Hz, Thiophene-H). Anal Calcd for $C_{19}H_{14}BrN_3O_3S_2$: C, 47.90; H, 2.96; N, 8.82%; Found: C, 47.95; H, 2.92; N, 8.85 %.

Methyl 2-(2-(5-(4-(dimethylamino)phenyl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)-4-oxothiazol-5(4*H*)-ylidene)acetate (43e):



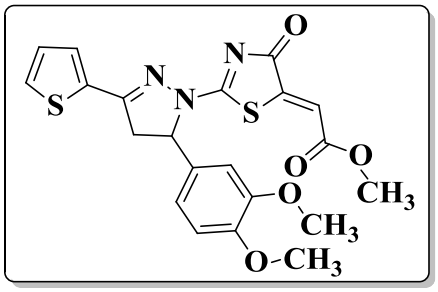
Red solid; m.p.: 248 – 250 °C; **FTIR (KBr, υ_{max}, cm^{-1}):** 1709 (Ester –C=O), 1688 (amide-C=O), 1612 (-C=N); **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm):** 2.92 (s, 6H, -N-CH_3), 3.41 – 3.46 (dd, *J* = 3.6 Hz, 1H, -CH_2), 3.85 (s, 3H, -OCH_3), 3.90 – 3.97 (dd, *J* = 10.8 Hz, 1H, -CH_2), 5.76 – 5.80 (dd, *J* = 3.6H, 1H, -CH), 6.64 (d, *J* = 9.2 Hz, 2H, Ar-H), 6.90 (s, 1H, =CH), 7.14 – 7.17 (m, 3H, Ar-H), 7.40 – 7.41 (dd, *J* = 1.2 Hz, 1H, Ar-H), 7.58 – 7.60 (dd, *J* = 1.2 Hz, 1H, Ar-H); Anal. Calcd for $C_{21}H_{20}N_4O_3S_2$ (440.53): C, 57.25; H, 4.58; N, 12.72%; Found: C, 57.29; H, 4.60; N, 12.68 %.

Methyl 2-(2-(5-(4-methoxyphenyl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)-4-oxothiazol-5(4*H*)-ylidene)acetate (43f):



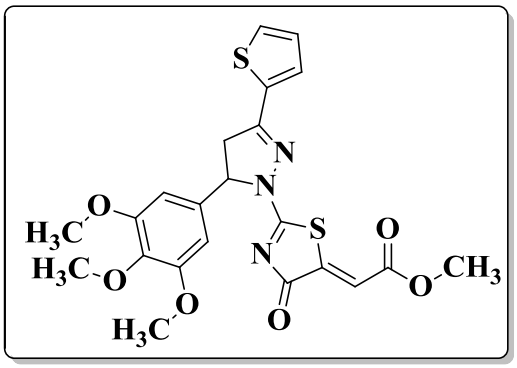
Yellow solid; m.p.: 122 – 124 °C; **FTIR (KBr, υ_{max}, cm^{-1}):** 1686 (Ester-C=O); 1667 (amide-C=O), 1590 (-C=N); 1248 (-C-O-C); **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm):** 3.39 – 3.44 (dd, *J* = 4Hz, 1H, Pyrazole-Ha); 3.77 (s, 3H, -$COOCH_3$); 3.85 (s, 3H, -OCH_3); 3.93 – 4.00 (dd, *J* = 10.8 Hz, 1H, Pyrazole-Hb); 5.79 – 5.83 (dd, *J* = 3.6 Hz, 1H, Pyrazole-Hx); 6.85 (d, *J* = 8.8 Hz, 2H, Ar-H); 6.90 (s, 1H, =CH); 7.13 -7.15 (dd, *J* = 3.6 Hz, 1H, Thiophene-H); 7.21 (d, *J* = 8.8 Hz, 2H, Ar-H); 7.40 – 7.41 (dd, *J* = 1.2Hz, 1H, Thiophene-H); 7.59 – 7.60 (dd, *J* = 1.2 Hz, 1H, Thiophene-H); Anal. Calcd for $C_{20}H_{17}N_3O_4S_2$ (427.49): C, 56.19; H, 4.01; N, 9.83%; Found: C, 56.23; H, 3.98; N, 9.80 %.

Methyl 2-(2-(5-(3,4-dimethoxyphenyl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)-4-oxothiazol-5(4*H*)-ylidene)acetate (43g)



Yellow solid; m.p.: 146 – 148 °C; **FTIR (KBr, υ_{max}, cm^{-1}):** 1696 (Ester-C=O); 1677 (amide –C=O), 1612 (-C=N); 1246 (-C-O-C); **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm):** 3.39 – 3.45 (dd, *J* = 3.6 Hz, 1H, Pyrazole-Ha); 3.84 (s, 6H, $-OCH_3$); 3.86 (s, 3H, $-OCH_3$); 3.94 – 4.01 (dd, *J* = 10.8 Hz, 1H, Pyrazole-Hb); 5.80 – 5.83 (dd, *J* = 3.6 Hz, 1H, Pyrazole-H); 6.79 (d, *J* = 4 Hz, 3H, Ar-H); 6.91 (s, 1H, =CH); 7.13 – 7.15 (dd, *J* = 3.6 Hz, 1H, Thiophene-H); 7.40 – 7.41 (dd, *J* = 1.2 Hz, 1H, Thiophene-H); 7.59 – 7.60 (dd, *J* = 1.2 Hz, 1H, Thiophene-H); **^{13}C NMR (100 MHz, DMSO-d_6, ppm):** δ 44.56, 53.06, 55.99, 64.48, 110.49, 112.52, 116.61, 118.05, 129.08, 132.27, 132.63, 132.93, 133.85, 147.08, 149.10, 149.43, 158.78, 166.75, 171.82, 177.68; **MS (ESI m/z):** 458 $[M+H]^+$; Anal. Calcd for $C_{21}H_{19}N_3O_5S_2$ (457.52): C, 55.13; H, 4.19; N, 9.18%; Found: C, 55.09; H, 4.25; N, 9.15%.

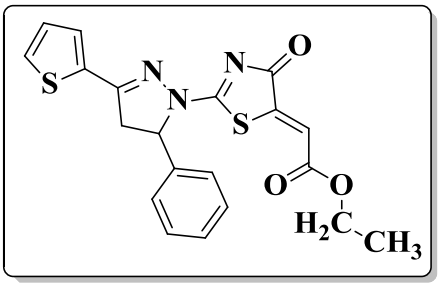
Methyl 2-(4-oxo-2-(3-(thiophen-2-yl)-5-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)thiazol-5(4*H*)-ylidene)acetate (43h):



Yellow solid; m.p.: 166 – 168 °C; **FTIR (KBr, υ_{max}, cm^{-1}):** 1692 (Ester-C=O); 1654 (amide –C=O); 1590 (-C=N); 1234 (-C-O-C); **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm):** 3.37 – 3.42 (dd, *J* = 3.6 Hz, 1H, CH_a), 3.81 (s, 9H, $-OCH_3$), 3.87 (s, 3H, $-COOCH_3$), 3.94 – 3.40 (dd, *J* = 11.2 Hz, 1H, CH_b), 5.78 – 5.82 (dd, *J* = 3.6 Hz, 1H, CH_x), 6.41 (s, 1H, Ar-H), 6.94 (s, 1H, =CH), 7.13 – 7.15 (dd, *J* = 4 Hz, 1H, Thiophene-H), 7.39 – 7.41 (dd, *J* = 1.2 Hz, 1H, Thiophene-H), 7.60 (d, *J* = 5.2 Hz, 1H, Ar-H), 7.67 – 7.78 (dd, *J* = 1.2 Hz, 1H, Thiophene-H), Anal. Calcd for $C_{22}H_{21}N_3O_6S_2$ (487.54): C, 54.20; H, 4.34; N, 8.62 %; Found: C, 54.25; H, 4.30; N, 8.60 %.

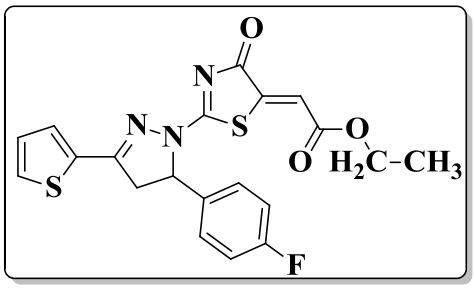
Ethyl 2-(4-oxo-2-(5-phenyl-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)thiazol-5(4*H*)-ylidene)acetate (43i):

Yellow solid; m.p.: 194 – 196 °C; **FTIR (KBr, υ_{max}, cm^{-1}):** 1704 (Ester –C=O); 1689 (amide-C=O); 1557 (-C=N); **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm):** 1.34 (t, *J* =1.35 Hz, 3H, $-CH_3$); 3.39 – 3.44 (dd, *J* = 3.42 Hz, 1H, Pyrazole-Ha); 3.96 – 4.03 (dd, *J* = 4.00 Hz,



1H, Pyrazole Hb); 4.28 – 4.34 (m, 2H, -CH_2); 5.85 – 5.89 (dd, *J* = 5.87 Hz, 1H, Pyrazole- Hx); 6.90 (s, 1H, =CH); 7.12 – 7.14 (dd, *J* = 7.13 Hz, 1H, Thiophene-H); 7.30 -7.34 (m, 5H, Ar-H); 7.38 – 7.39 (dd, *J* = 7.39Hz, 1H, Thiophene-H); 7.58 – 7.60 (dd, *J* = 7.60 Hz, 1H, Thiophene-H); **^{13}C NMR (100 MHz, $CDCl_3$):** δ 14.22; 44.10; 61.70; 64.05; 117.87; 125.81; 128.09; 128.64; 129.24; 131.09; 131.53; 132.99; 138.95; 146.80; 156.08; 166.66, 173.31, 178.71; **HR-Mass (ESI m/z):** Calculated for $C_{20}H_{18}N_3O_3S_2$ $[M+H]^+$: 412.0790, Found: 412.0775; Anal. Calcd for $C_{20}H_{17}N_3O_3S_2$ (411.49): C, 58.38; H, 4.16; N, 10.21%; Found: C, 58.35; H, 4.12; N, 10.26%.

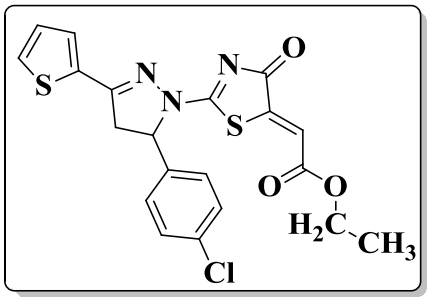
Ethyl 2-(2-(5-(4-fluorophenyl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)-4-oxo thiazol-5(4*H*)-ylidene)acetate (43j):



Yellow solid; m.p.: 210 – 212 °C; **FTIR (KBr, υ_{max}, cm^{-1}):** 1687 (Ester –C=O); 1654 (amide –C=O), 1587 (-C=N); **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm):** 1.34 (t, *J* = 7.2 Hz, 3H, -CH_3); 3.37 – 3.42 (dd, *J* = 3.6 Hz, 1H, pyrazole-H); 3.96 – 4.04 (dd, *J* = 11.2 Hz, 1H, Pyrazole-H); 4.28 – 4.34 (m, 2H, -CH_2); 5.82 – 5.86 (dd, *J* = 4 Hz, 1H, Pyrazole-H); 6.91 (s, 1H, =CH); 7.03 (t, *J* = 8.4 Hz, 2H, Ar-H); 7.13 – 7.15 (dd, *J* = 3.6 Hz, 1H, thiophene-H), 7.24 – 7.28 (m, 2H, Ar-H); 7.39 – 7.41 (dd, *J* = 1.2 Hz, 1H, Thiophene-H); 7.60 – 7.61 (dd, *J* = 1.2 Hz, 1H, Thiophene-H); **^{13}C NMR (100 MHz, DMSO-d_6, ppm):** δ 14.50, 44.53, 62.03, 63.90, 116.21, 116.42, 117.06, 128.72, 129.13, 132.51, 133.07, 134.03, 136.23, 146.88, 158.75, 161.02, 163.45, 166.27, 171.98, 177.66; **MS (ESI m/z):** 430 $[M+H]^+$; Anal. Calcd for $C_{20}H_{16}FN_3O_3S_2$ (429.48): C, 55.93; H, 3.75; N, 9.78%; Found: C, 55.98; H, 3.72; N, 9.80 %.

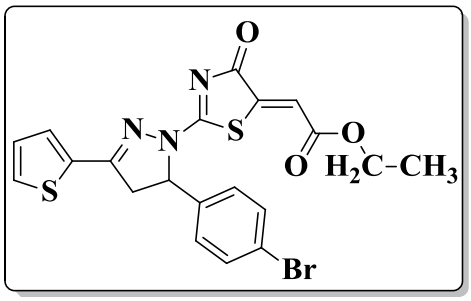
Ethyl 2-(2-(5-(4-chlorophenyl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)-4-oxo thiazol-5(4*H*)-ylidene)acetate (43k):

Yellow solid; m.p.: 200 – 202 °C; **FTIR (KBr, υ_{max}, cm^{-1}):** 1697 (Ester –C=O), 1647 (amide –C=O), 1603 (-C=N); **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm):** 1.35 (t, *J* = 7.2 Hz, 3H, -CH_3), 3.35 – 3.41 (dd, *J* = 3.6 Hz, 1H, -CH_2), 3.97 – 4.04 (dd, *J* = 10.8 Hz, 1H, -



CH_2), 4.29 – 4.34 (m, 2H, -OCH_2), 5.81 – 5.85 (dd, *J* = 3.6 Hz, 1H, -CH), 6.92 (s, 1H, =CH), 7.13 – 7.15 (dd, *J* = 3.6 Hz, 1H, Ar-H), 7.21 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.32 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.39 – 7.40 (dd, *J* = 0.8 Hz, 1H, Ar-H), 7.60 – 7.62 (dd, *J* = 1.2 Hz, 1H, Ar-H); **^{13}C NMR (100 MHz, DMSO-*d*$_6$, ppm):** δ 13.99, 43.93, 61.52, 63.40, 116.60, 127.90, 128.61, 128.97, 131.96, 132.57, 132.71, 133.49, 138.44, 146.34, 158.17, 165.75, 171.52, 177.11; **HR-MS (ESI m/z):** Calculated for $C_{20}H_{17}ClN_3O_3S_2$ $[M+H]^+$: 446.0400; Found: 446.0380; Anal. Calcd for $C_{20}H_{16}ClN_3O_3S_2$ (445.94): C, 53.87; H, 3.62; N, 9.42 %; Found: C, 53.91; H, 3.59; N, 9.44%.

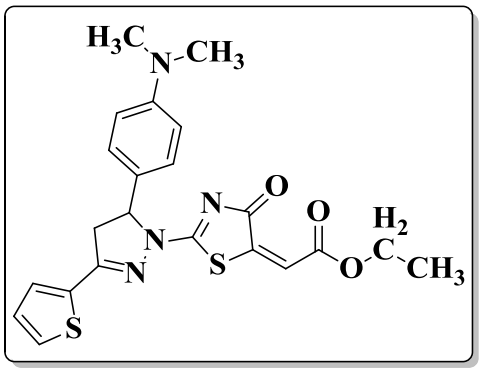
Ethyl 2-(2-(5-(4-bromophenyl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)-4-oxo thiazol-5(4*H*)-ylidene)acetate (43l):



Brown solid; m.p.: 237 – 239 °C; **FTIR (KBr, υ_{max}, cm^{-1}):** 1712 **(**Ester –C=O**),** 1687 (amide-C=O); 1621 (-C=N); **^{1}H NMR (400 MHz, $CDCl_3$,δ ppm):** 1.35 (t, *J* = 7.2 Hz, 3H,-CH_3); 3.35 – 3.40 (dd, *J* = 4 Hz, 1H,Pyrazole-H); 3.97 -4.04 (dd, *J* = 10.8 Hz, 1H, Pyrazole-H); 4.29 – 4.34 (m, 2H, -CH_2); 5.80 – 5.84 (dd, *J* = 4 Hz, 1H, Pyrazole-H); 6.92 (s, 1H, =CH); 7.13 -7.15 (dd, *J* = 4 Hz, 1H, Thiazole-H); 7.17 – 7.22 (m, 2H, Ar-H); 7.36 (t, *J* = 2 Hz, 1H, Ar-H); 7.38 – 7.39 (dd, *J* = 1.2 Hz, 1H, Thiophene-H); 7.42 – 7.45 (m, 1H, Ar-H); 7.60 – 7.61 (dd, *J* = 1.2 Hz, 1H, Thiophene-H); **^{13}C NMR (100 MHz, DMSO-*d*$_6$, ppm):** δ 14.47, 44.48, 62.01, 63.82, 117.14, 122.52, 125.18, 129.09, 129.37, 131.54, 131.78, 132.43, 133.06, 134.03, 142.56, 146.81, 158.65, 166.24, 172.14, 177.62; Anal. Calcd for $C_{20}H_{16}BrN_3O_3S_2$ (490.39): C, 48.98; H, 3.29; N, 8.57 %; Found: C, 48.92; H, 3.33; N, 8.62 %.

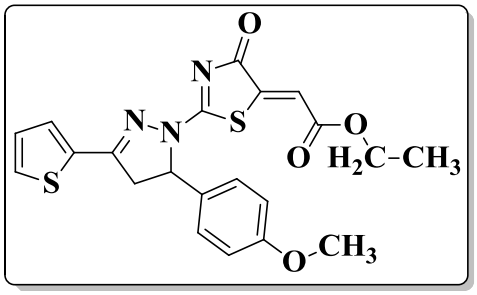
Ethyl 2-(2-(5-(4-(dimethylamino)phenyl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)-4-oxothiazol-5(4*H*)-ylidene)acetate (43m)

Red solid; m.p.: 249 – 251 °C; **FTIR (KBr, υ_{max}, cm^{-1}):** 1705 (Ester –C=O), 1688 (amide –C=O), 1611 (-C=N); **^{1}H NMR (400 MHz, $CDCl_3$,δ ppm):** 1.33 (t, *J* = 6.8 Hz, 3H, -CH_3) 2.92 (s, 6H, -NCH_3), 3.40 – 3.46 (dd, *J* = 3.6 Hz, 1H, -CH_2), 3.89 – 3.97 (dd,



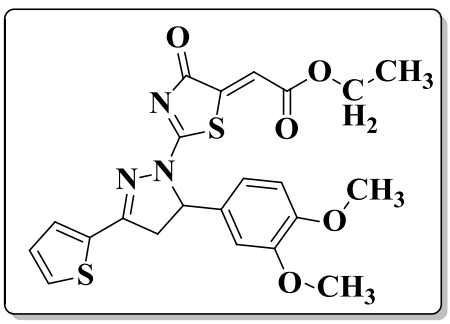
J = 11.2 Hz, 1H, -CH_2), 4.27 – 4.33 (m, 2H, -OCH_2), 5.76 – 5.80 (dd, *J* = 3.6 Hz, 1H, -CH), 6.63 (d, *J* = 8.8 Hz, 2H, Ar-H), 6.88 (s, 1H, =CH), 7.12 – 7.17 (m, 3H, Ar-H), 7.40 – 7.41 (dd, *J* = 1.2 Hz, 1H, Ar-H), 7.57 – 7.59 (dd, *J* = 1.2 Hz, 1H, Ar-H); Anal. Calcd for $C_{22}H_{22}N_4O_3S_2$ (454.56): C, 58.13; H, 4.88; N, 12.33 %; Found: C, 58.10; H, 4.93; N, 12.30 %.

Ethyl 2-(2-(5-(4-methoxyphenyl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)-4-oxo thiazol-5(4*H*)-ylidene)acetate (43n):



Yellow solid; m.p.: 179 – 181 °C; **FTIR (KBr, υ_{max}, cm^{-1}):** 1704 (Ester-C=O), 1693 (amide-C=O), ; 1244 (-C-O-C); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 1.26 (t, *J* = 7.2 Hz, 3H, -CH_3), 3.49 – 3.55 (dd, *J* = 4 Hz, 1H, -CH_2), 3.76 (s, 3H, -OCH_3), 4.12 – 4.19 (dd, *J* = 11.2 Hz, 1H, -CH_2), 4.22 – 4.28 (m, 2H, -CH_2), 5.85 – 5.88 (dd, *J* = 4 Hz, 1H, -CH), 6.66 (s, 1H, =CH), 6.92 – 6.94 (m, 1H, Ar-H), 7.19 – 7.21 (m, 2H, Ar-H), 7.24 – 7.27 (m, 2H, Ar-H), 7.71 – 7.72 (m, 1H, Ar-H), 7.95 – 7.97 (m, 1H, Ar-H); **MS (ESI m/z):** 442 $[M+H]^+$; Anal. Calcd for $C_{21}H_{19}N_3O_4S_2$ (441.52): C, 57.13; H, 4.34; N, 9.52%; Found: C, 57.18; H, 4.30; N, 9.55%.

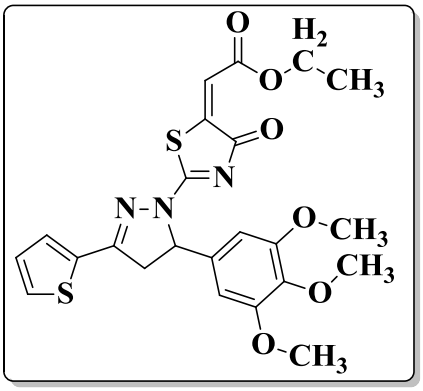
Ethyl 2-(2-(5-(3,4-dimethoxyphenyl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)-4-oxothiazol-5(4*H*)-ylidene)acetate (43o):



Yellow solid; m.p.: 188 – 190 °C; **FTIR (KBr, υ_{max}, cm^{-1}):** 1742 (Ester –C=O); 1719 (amide –C=O); 1257 (-C-O-C); **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm):** 1.34 (t, *J* = 7.2 Hz, 3H, -CH_3); 3.39 – 3.44 (dd, *J* = 3.6 Hz, 1H, Pyrazole-Ha); 3.84 (s, 6H, -OCH_3); 3.94 -4.01 (dd, *J* = 11.2 Hz, 1H, Pyrazole-Hb); 4.28 - 4.34 (m, 2H, -OCH_2); 5.80 – 5.84 (dd, *J* = 3.6 Hz, 1H, Pyrazole-Hx); 6.79 (d, *J* = 2.8 Hz, 3H, Ar-H); 6.90 (s,

1H, =CH); 7.13 – 7.15 (dd, *J* = 4 Hz, 1H, Thiophene-H); 7.40 – 7.41 (dd, *J* = 1.2 Hz, 1H, Thiophene-H); 7.59 – 7.60 (dd, *J* = 1.2 Hz, 1H, Thiophene-H); **^{13}C NMR (100 MHz, DMSO-*d*$_6$):** δ 13.99, 44.09, 55.55, 61.49, 63.97, 109.98, 112.02, 116.40, 117.53, 128.60, 131.79, 132.14, 132.44, 133.36, 146.48, 148.93, 158.27, 165.78, 171.39, 172.08, 177.20; **MS (ESI m/z):** 472 $[M+H]^+$; Anal. Calcd for $C_{22}H_{21}N_3O_5S_2$ (471.54): C, 56.04; H, 4.49; N, 8.91%; Found: C, 56.08; H, 4.43 N, 8.95%.

Ethyl 2-(4-oxo-2-(3-(thiophen-2-yl)-5-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)thiazol-5(4*H*)-ylidene)acetate (43p):



Yellow solid; m.p.: 204 – 206 °C; **FTIR (KBr, υ_{max}, cm^{-1}):** 1689 (Ester -C=O); 1592 (-C=N); 1234 (-C-O-C); **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm):** 1.35 (t, *J* = 7.2 Hz, 3H, -CH_3); 3.36 – 3.41 (dd, *J* = 3.6 Hz, 1H, Pyrazole-Ha); 3.81 (s, 9H, -OCH_3); 3.93 – 4.01 (dd, *J* = 10.8 Hz, 1H, Pyrazole-Hb); 4.29 - 4.35 (m, 2H, -CH_2); 5.78 – 5.82 (dd, *J* = 4 Hz, 1H, Pyrazole-Hx); 6.93 (s, 1H, =CH); 7.10 – 7.15 (m, 1H, Ar-H); 7.39 – 7.40 (dd, *J* = 1.2 Hz, 1H, Thiophene-H); 7.59 – 7.60 (dd, *J* = 1.2 Hz, 1H, Thiophene-H); 7.74 – 7.78 (m, 1H, Ar-H); 7.95 – 8.01 (m, 1H, Ar-H); **MS (ESI m/z):** 502 $[M+H]^+$; Anal. Calcd for $C_{23}H_{23}N_3O_6S_2$ (501.57): C, 55.08; H, 4.62; N, 8.38%; Found: C, 55.05; H, 4.64; N, 8.42%.

Antiviral activity experiment for the alkyl 2-(4-oxo-2-(5-aryl-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)thiazol-5(4*H*)-ylidene)acetate derivatives (43 a-p).

All the synthesized compounds were screened for their antiviral activity and the experimental procedure was used as described in the preceding chapter. After incubation at 37°C the virus-induced cytopathicity was determined by measuring the cell viability with the colorimetric formazan-based MTS assay[65-67].

Anticancer activity experiment for the alkyl 2-(4-oxo-2-(5-aryl-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)thiazol-5(4*H*)-ylidene)acetate derivatives (43b and 43f).

All the synthesized compounds were submitted for primary anticancer activity evaluation. The selected compounds were screened for primary anticancer activity at 10

µM concentration in accordance to the National Cancer Institute (NCI), Bethesda, USA of Drug Evaluation Branch[68-70]. The compounds were added to the cell culture at single dose concentration (10 µM) and the cultures were incubated for 48 h. After incubation sulforhodamine B (SRB) protein binding dye was added and absorbance values were measured. The cell percentage growth was calculated for the test compounds with respected to the untreated control cells.

The compounds which are exhibiting the excellent cell percentage growth inhibition were tested for five dose concentrations (10^{-4} to 10^{-8} µM) and measured the cytotoxicity and growth inhibitory effects in vitro against 60 human cancer cell lines after 48 h incubation and measured the absorbance values using sulforhodamine B dye.

Using the seven absorbance values (time zero (Tz), the percentage growth was calculated using the test growth in presence of the compound (T*i*) and control growth in absence of the compound (C).

$[(Ti - Tz) / (C - Tz)] \times 100$ for concentrations for which $Ti \geq Tz$,

$[(Ti - Tz) / Tz] \times 100$ for concentrations for which $Ti < Tz$.

From the five dose concentration experiment dose response parameters GI_{50}, TGI, LC_{50} were calaculated. Percentage growth inhibition (GI_{50}) of compound was calculated using $[(Ti - Tz)/(C - Tz)] \times 100 = 50$. The test compound concentration required for total growth inhibition (TGI) was calculated from T*i* = Tz. The LC_{50} (net loss of cells by treatment of the test compound) was calculated from $[(Ti - Tz)/ Tz] \times 100 = -50$. The compounds which are having GI_{50} <100 µM were considered as anticancer active compounds.

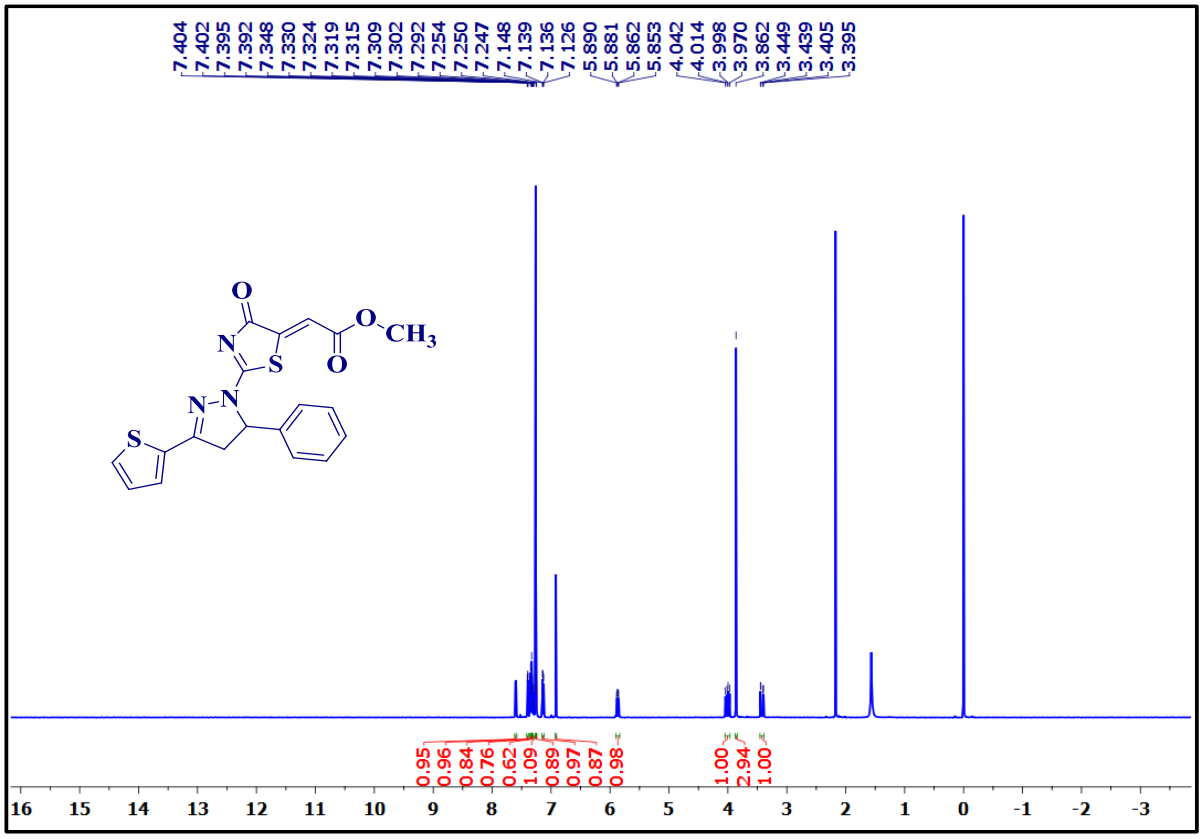


Fig: ^{1}H NMR Spectrum of compound **43a**

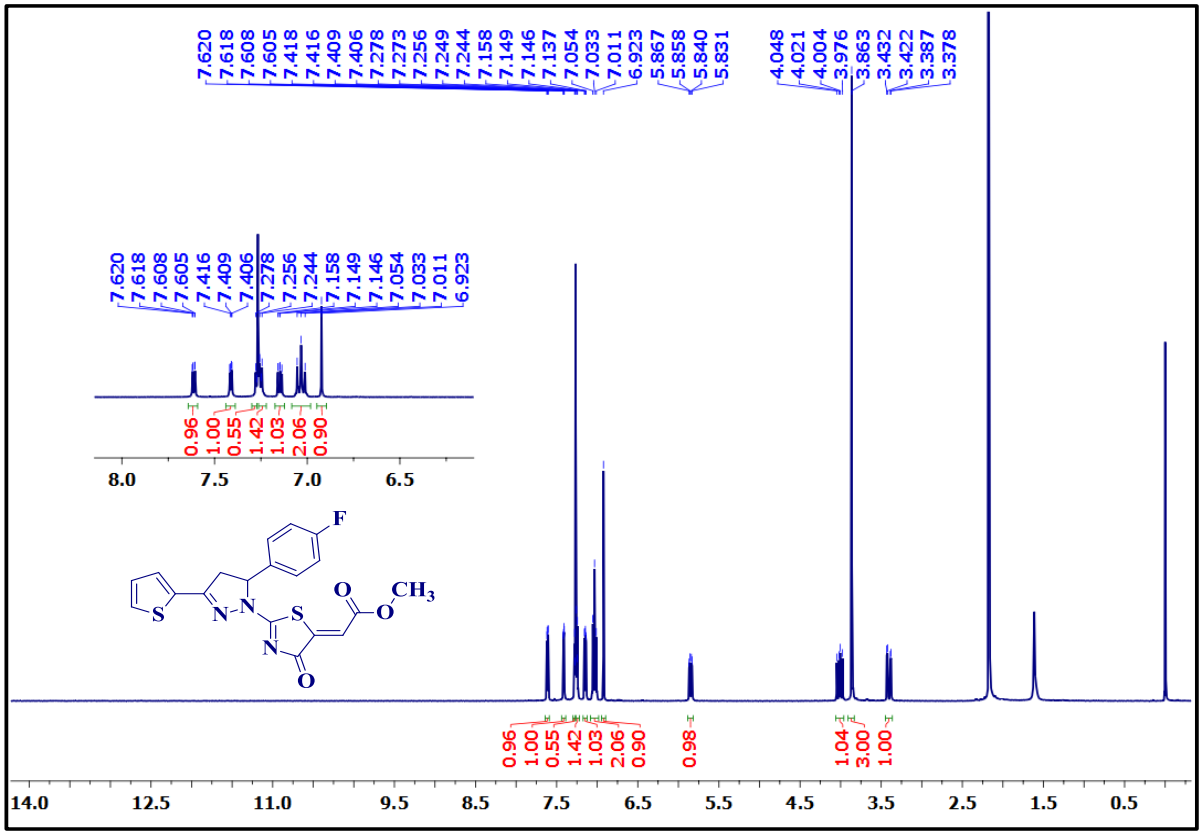


Fig: ^{1}H NMR Spectrum of compound **43b**

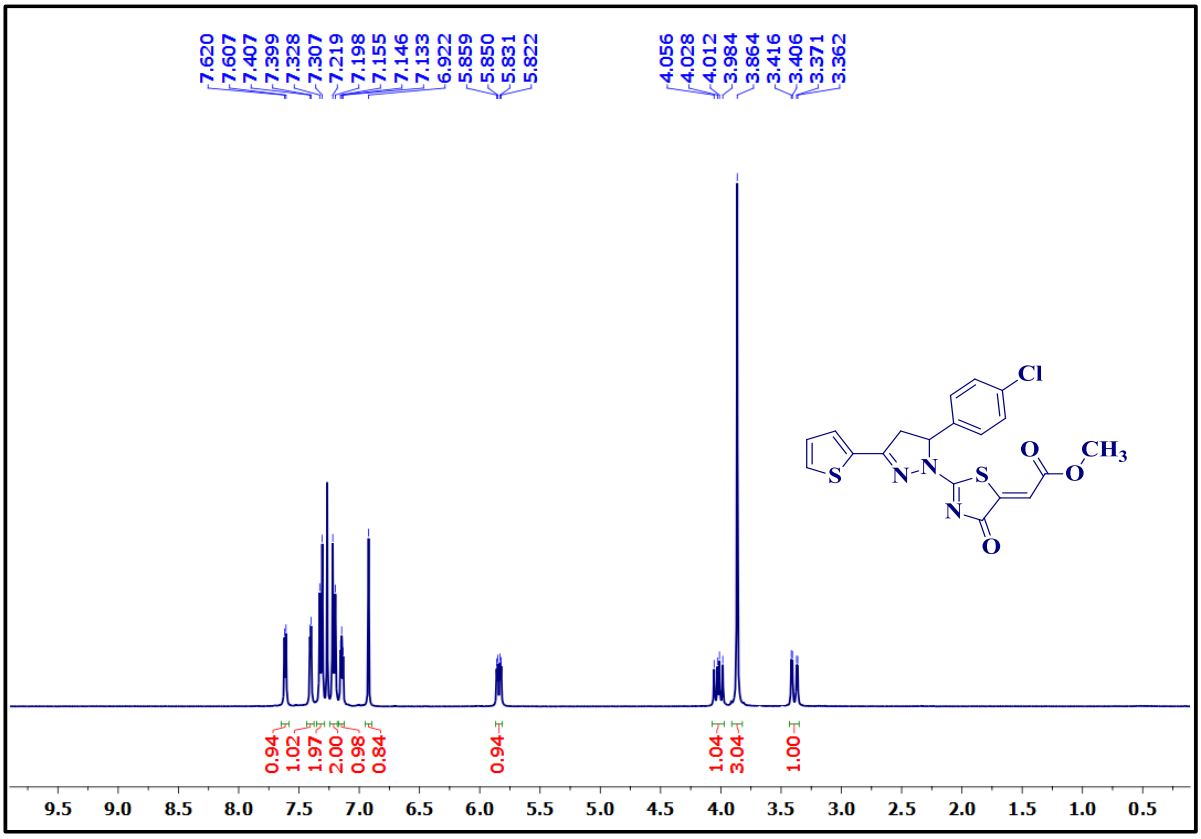


Fig: ^{1}H NMR Spectrum of compound **43c**

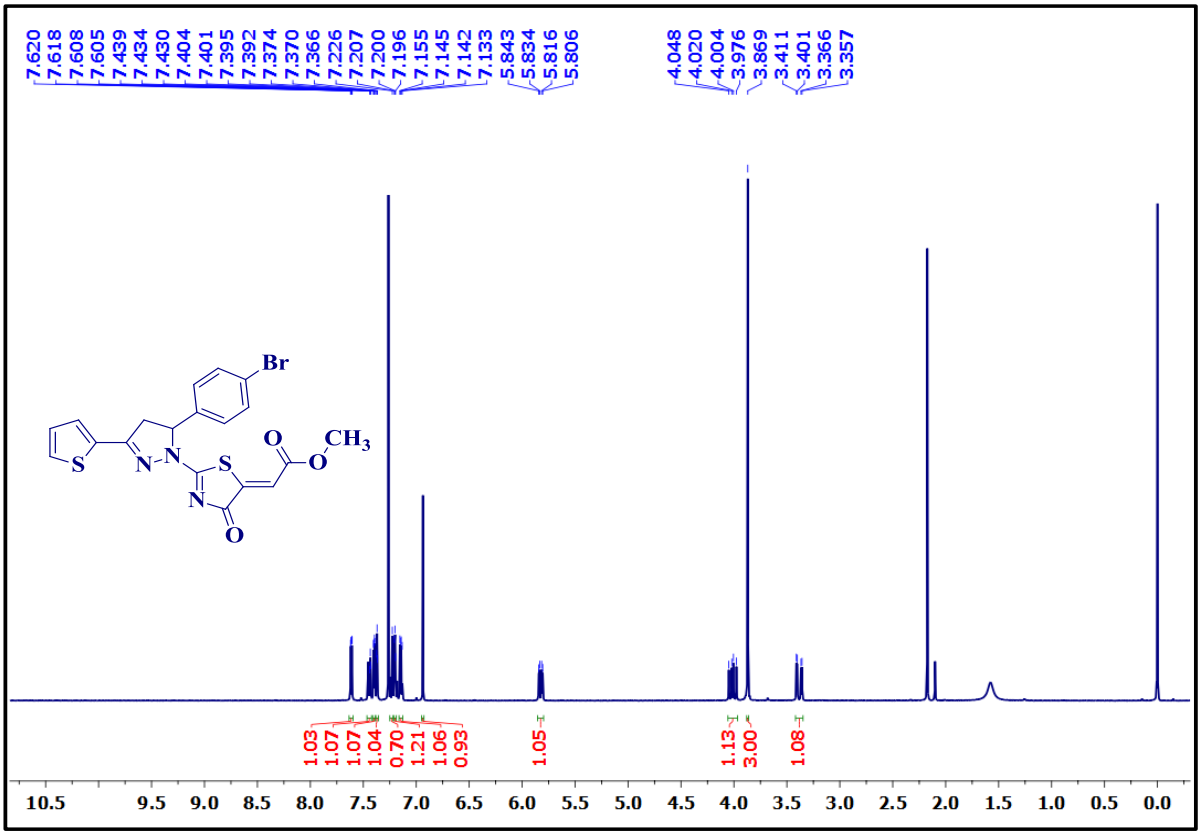


Fig: ^{1}H NMR Spectrum of compound **43d**

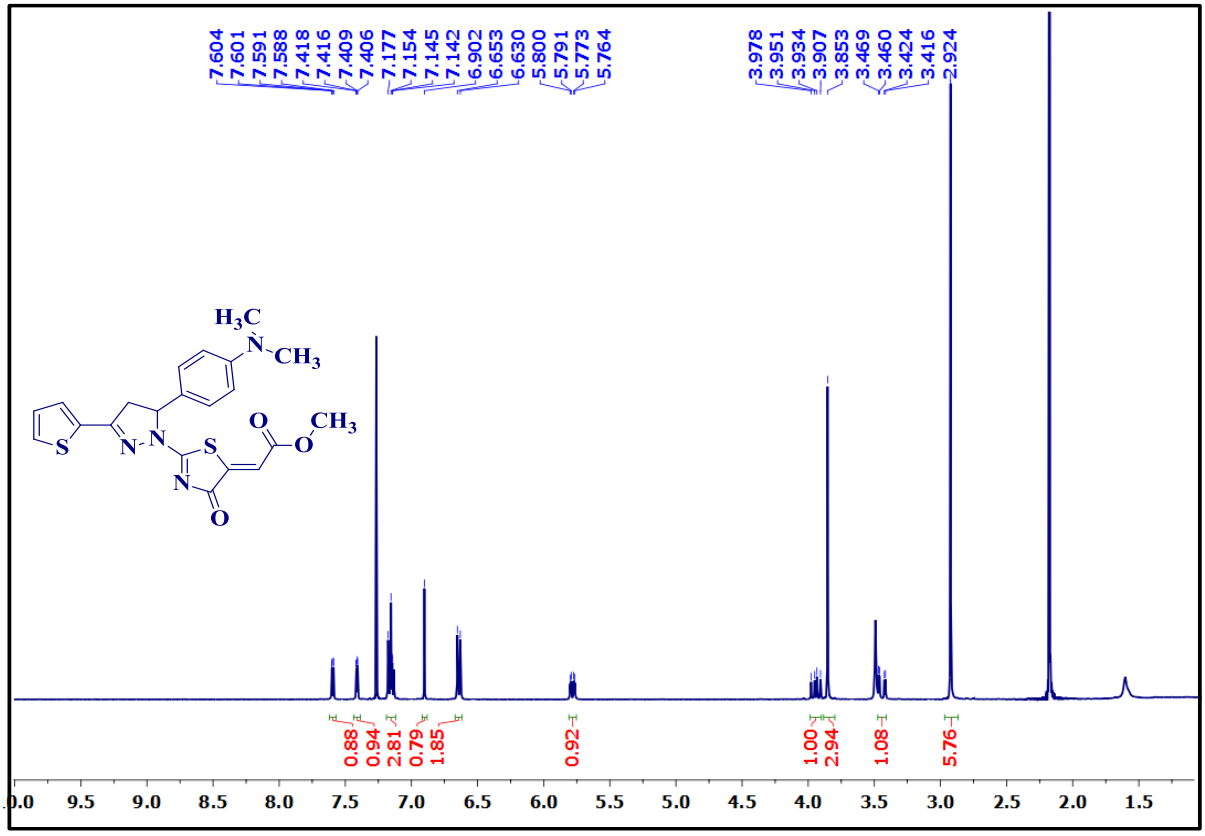


Fig: ^{1}H NMR Spectrum of compound **43e**

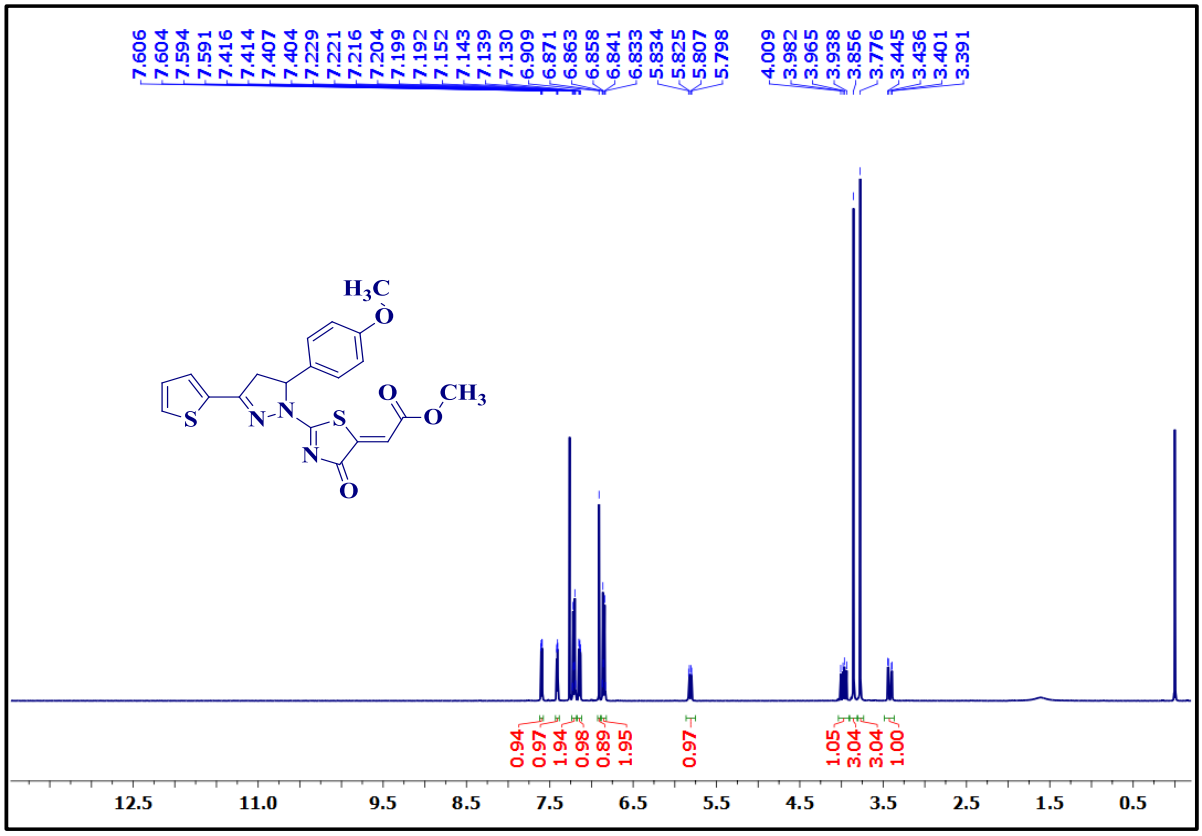


Fig: ^{1}H NMR Spectrum of compound **43f**

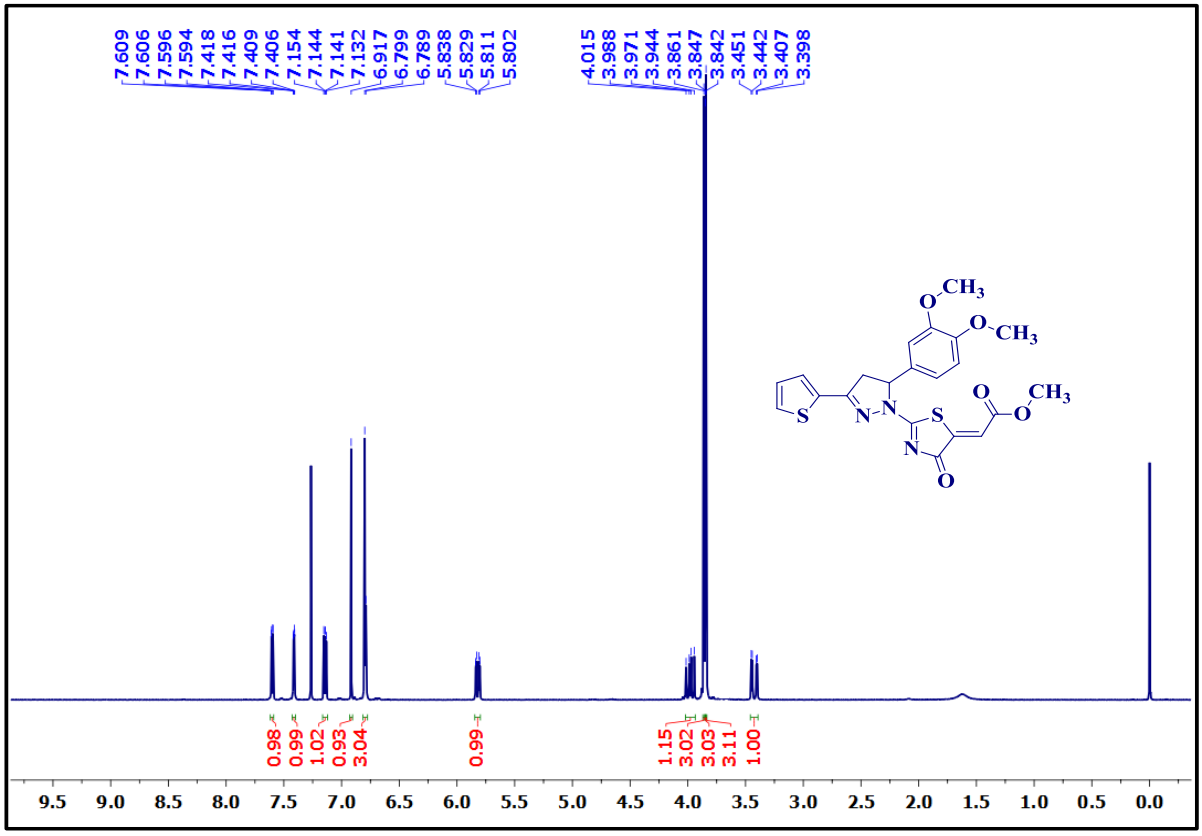


Fig: ^{1}H NMR Spectrum of compound **43g**

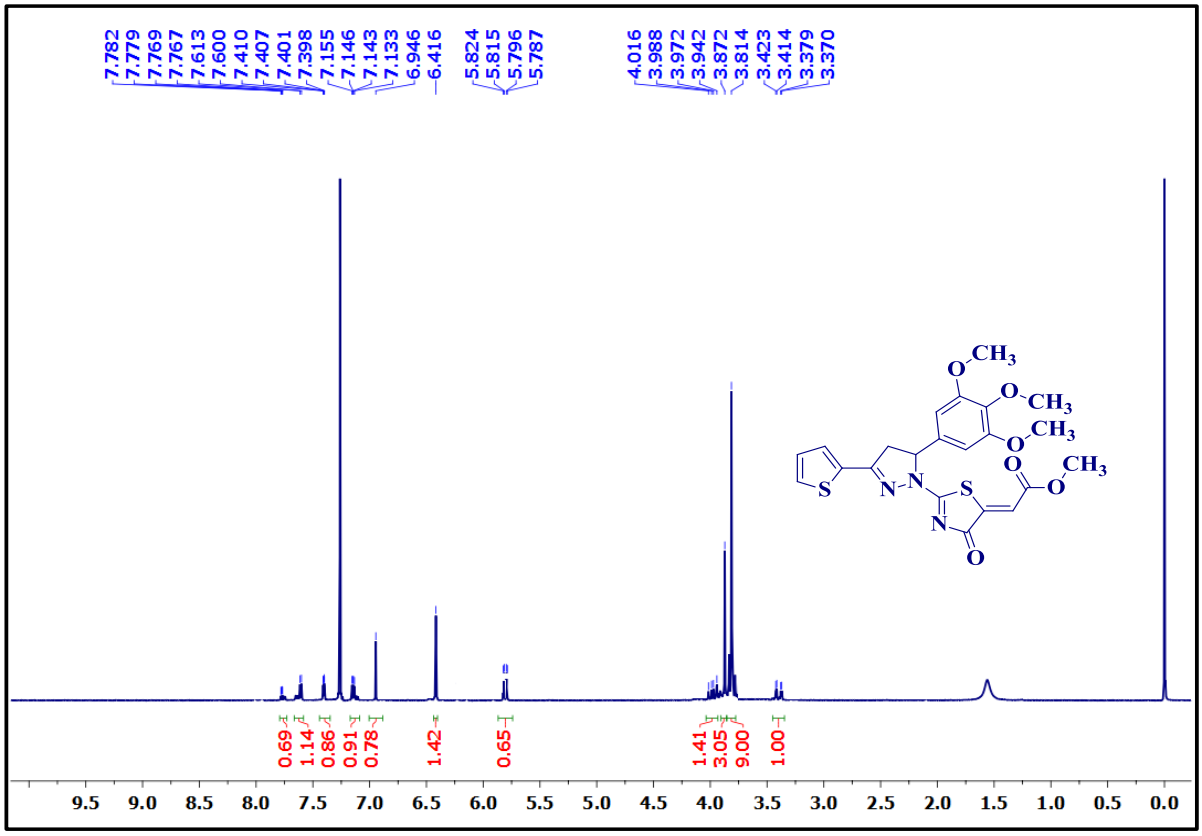


Fig: ^{1}H NMR Spectrum of compound **43h**

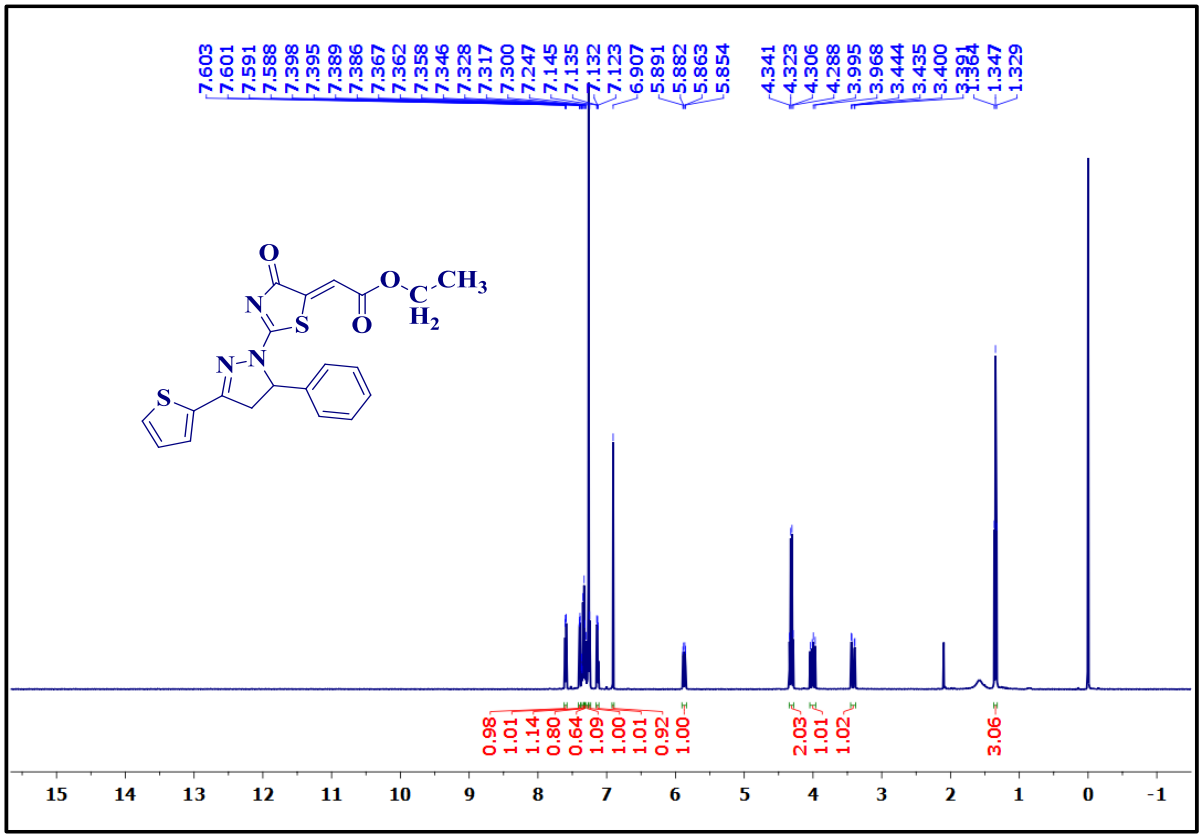


Fig: ^{1}H NMR Spectrum of compound **43i**

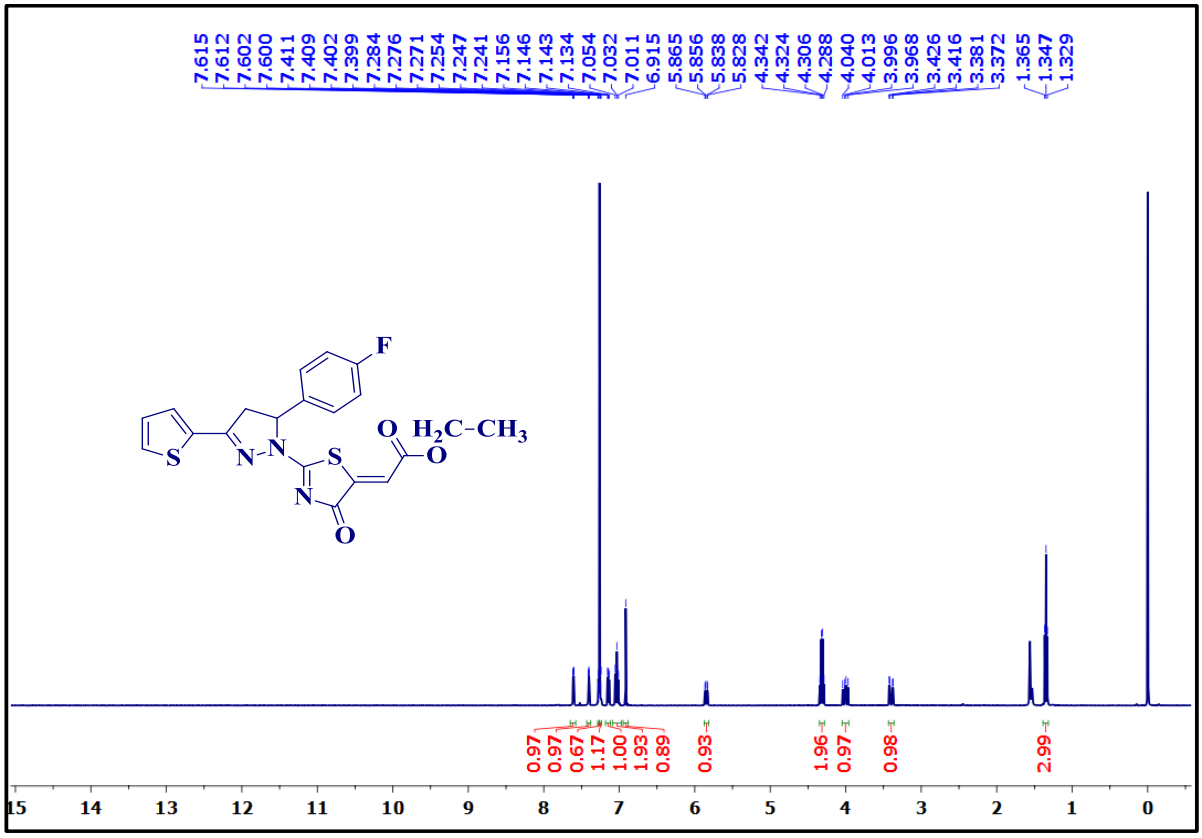


Fig: ^{1}H NMR Spectrum of compound **43j**

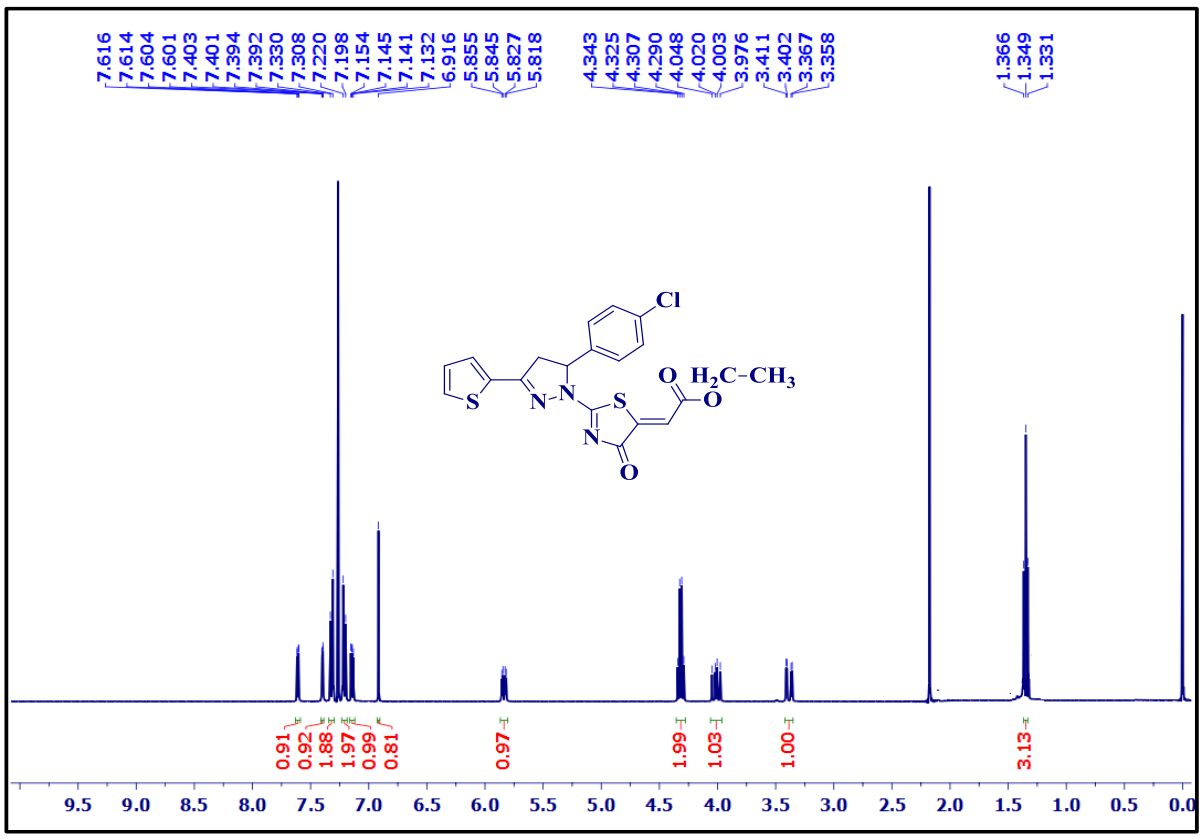


Fig: ^{1}H NMR Spectrum of compound **43k**

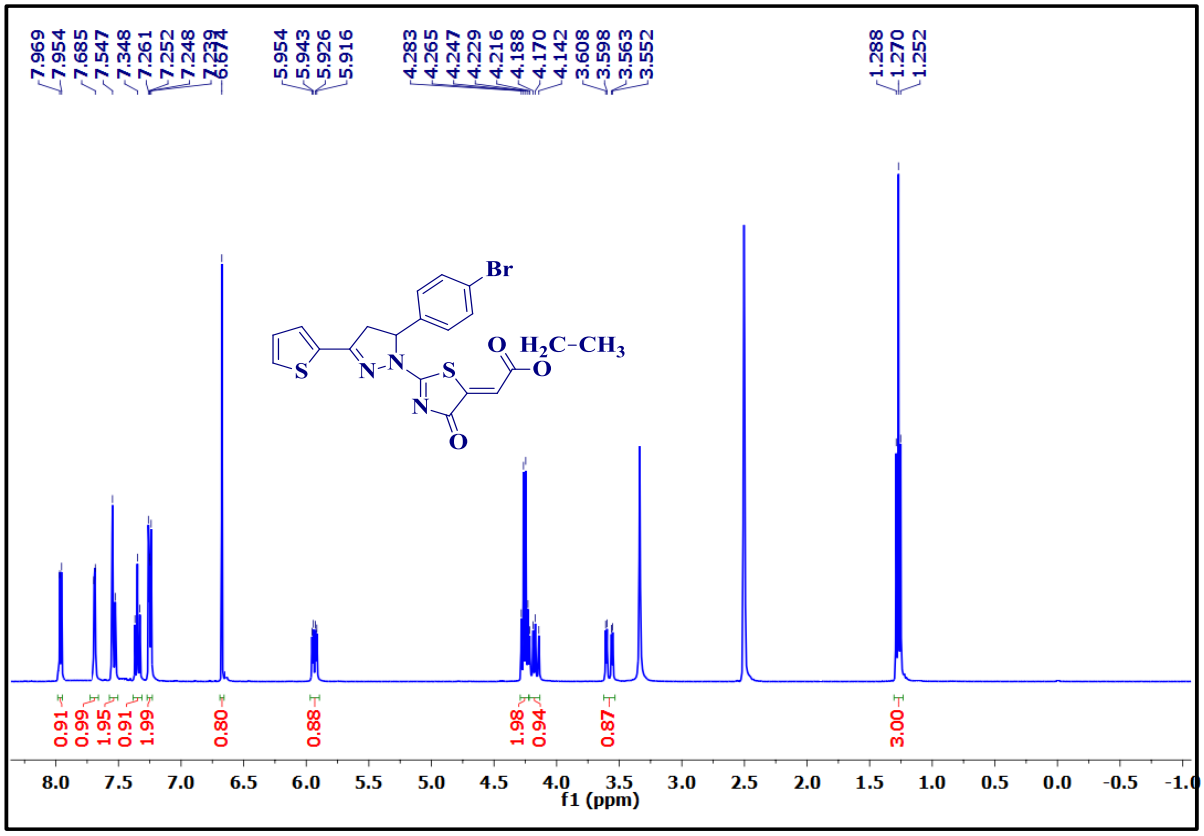


Fig: ^{1}H NMR Spectrum of compound **43l**

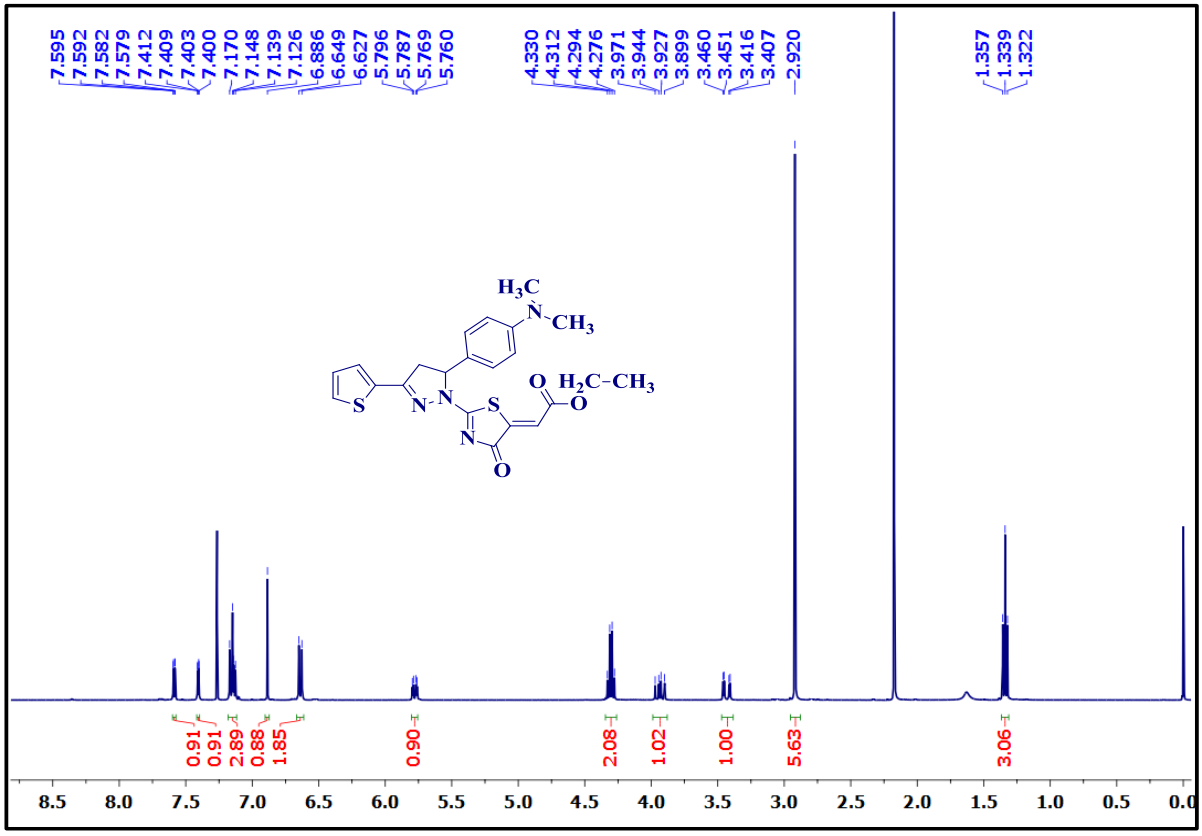


Fig: ^{1}H NMR Spectrum of compound **43m**

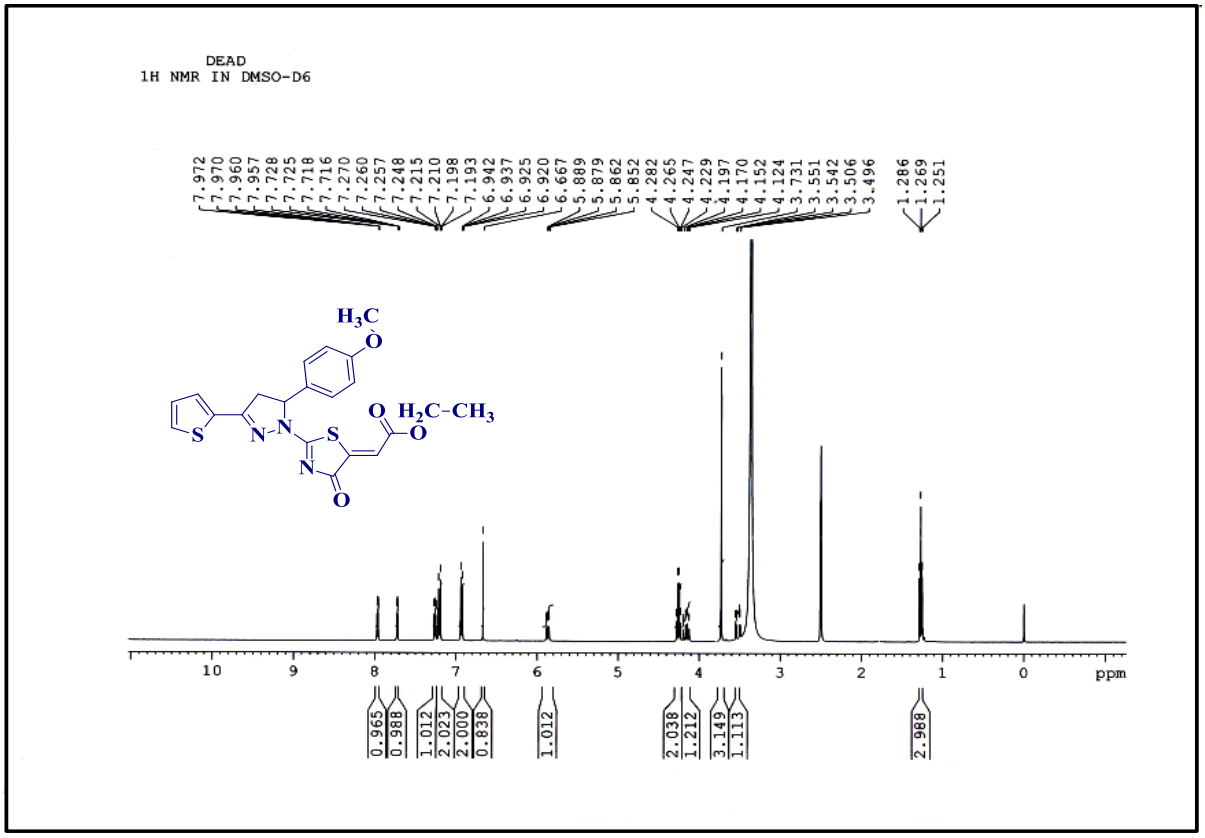


Fig: ^{1}H NMR Spectrum of compound **43n**

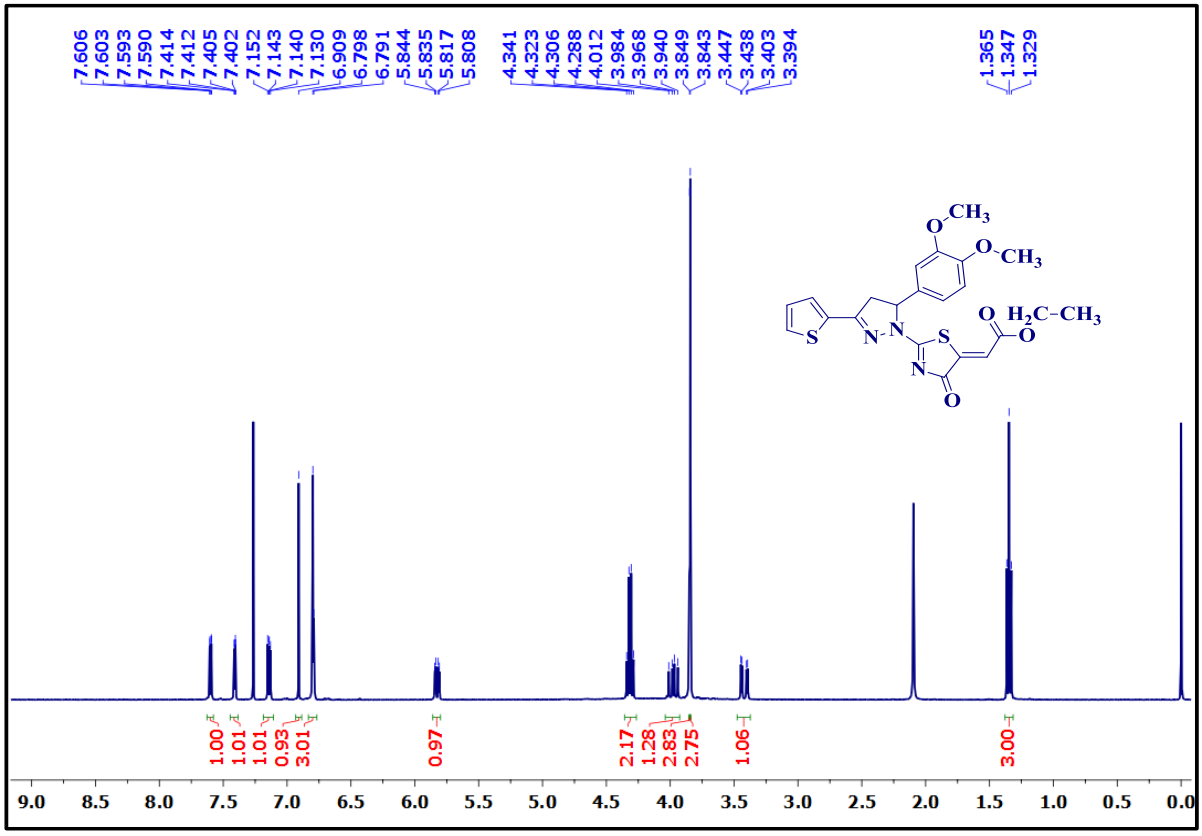


Fig: ^{1}H NMR Spectrum of compound **43o**

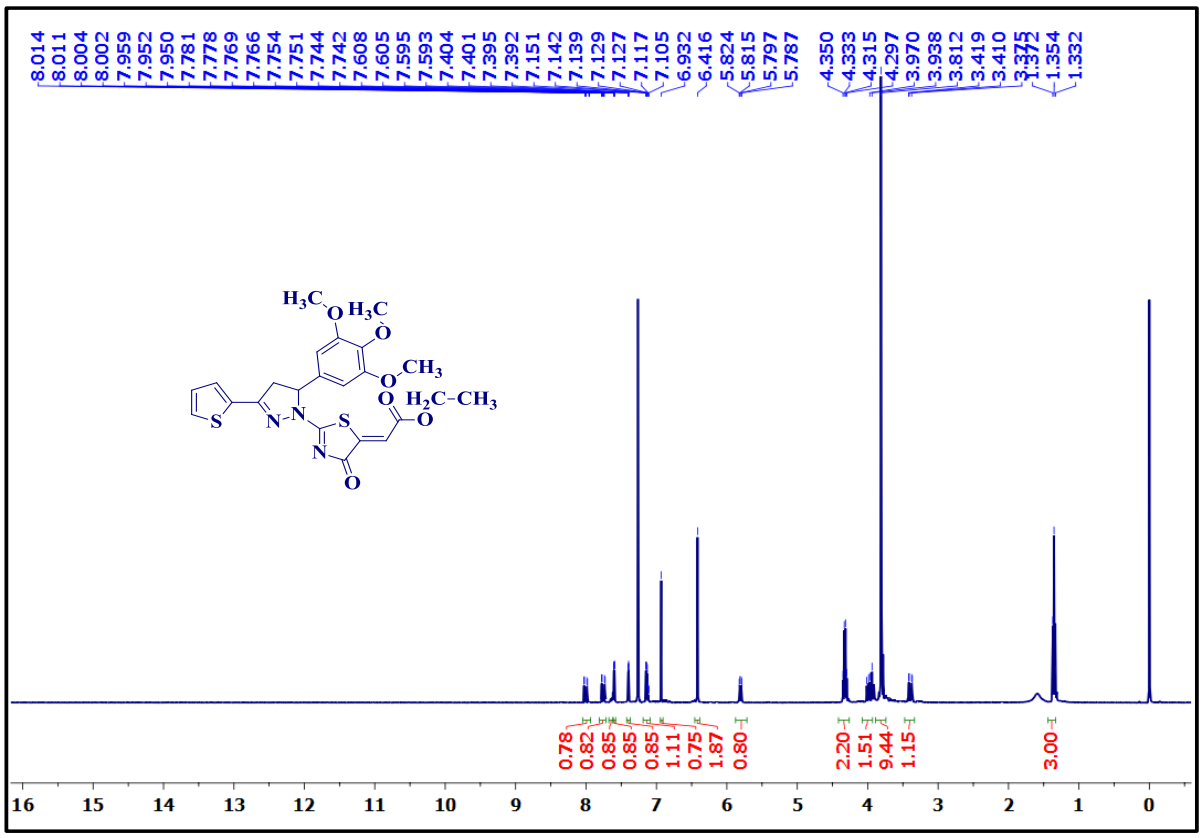


Fig: ^{1}H NMR Spectrum of compound **43p**

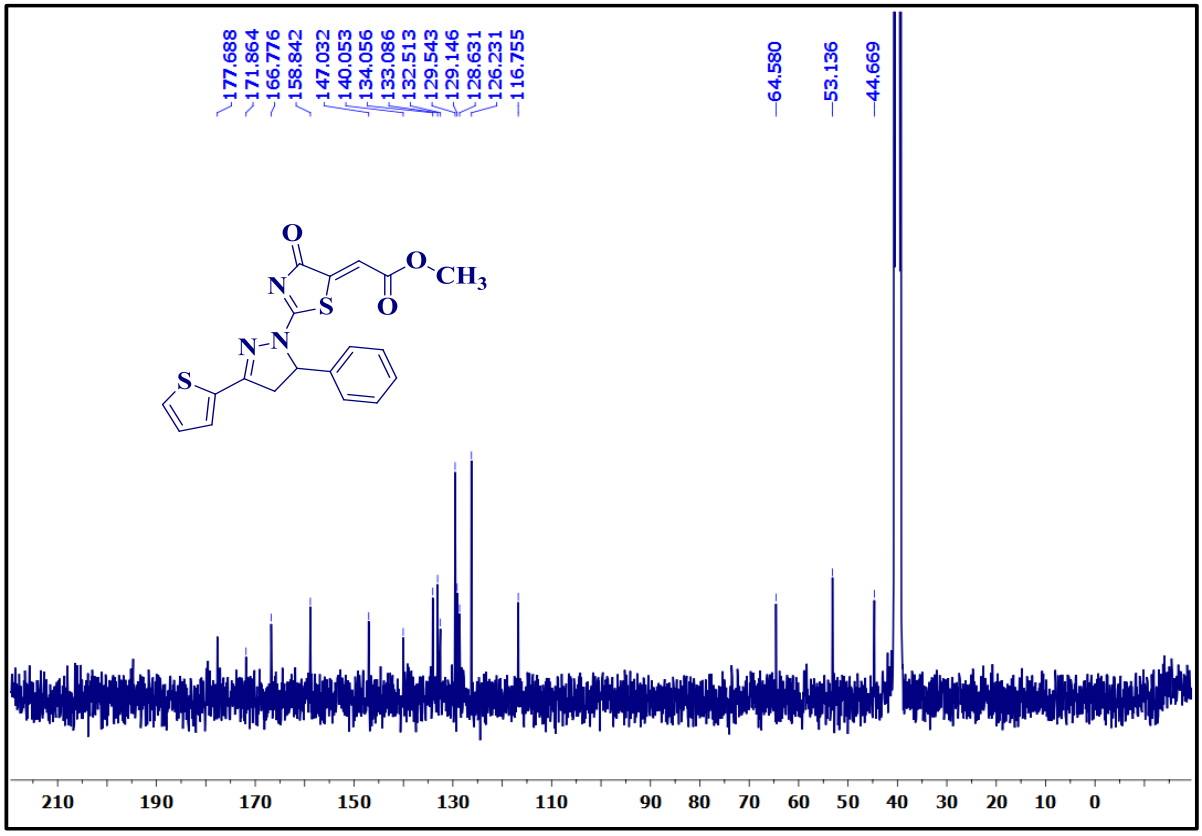


Fig: ^{13}C NMR Spectrum of compound **43a**

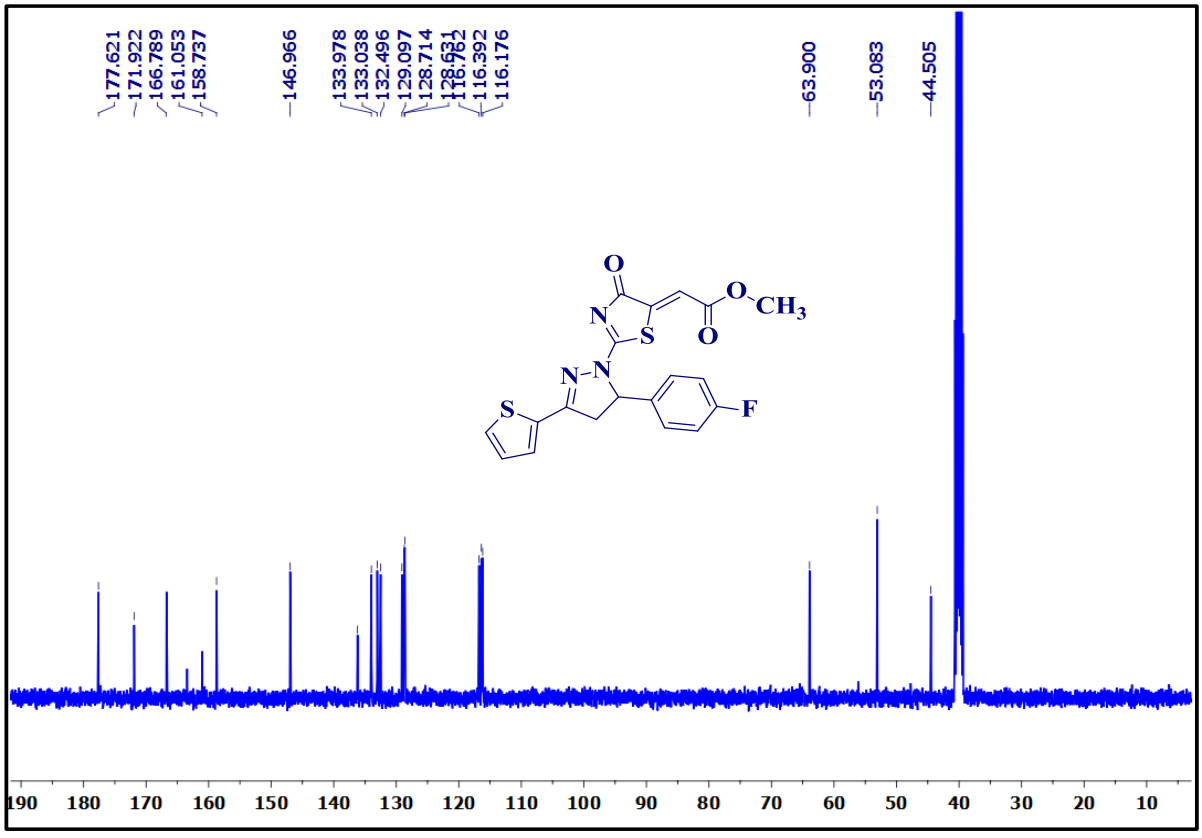


Fig: ^{13}C NMR Spectrum of compound **43b**

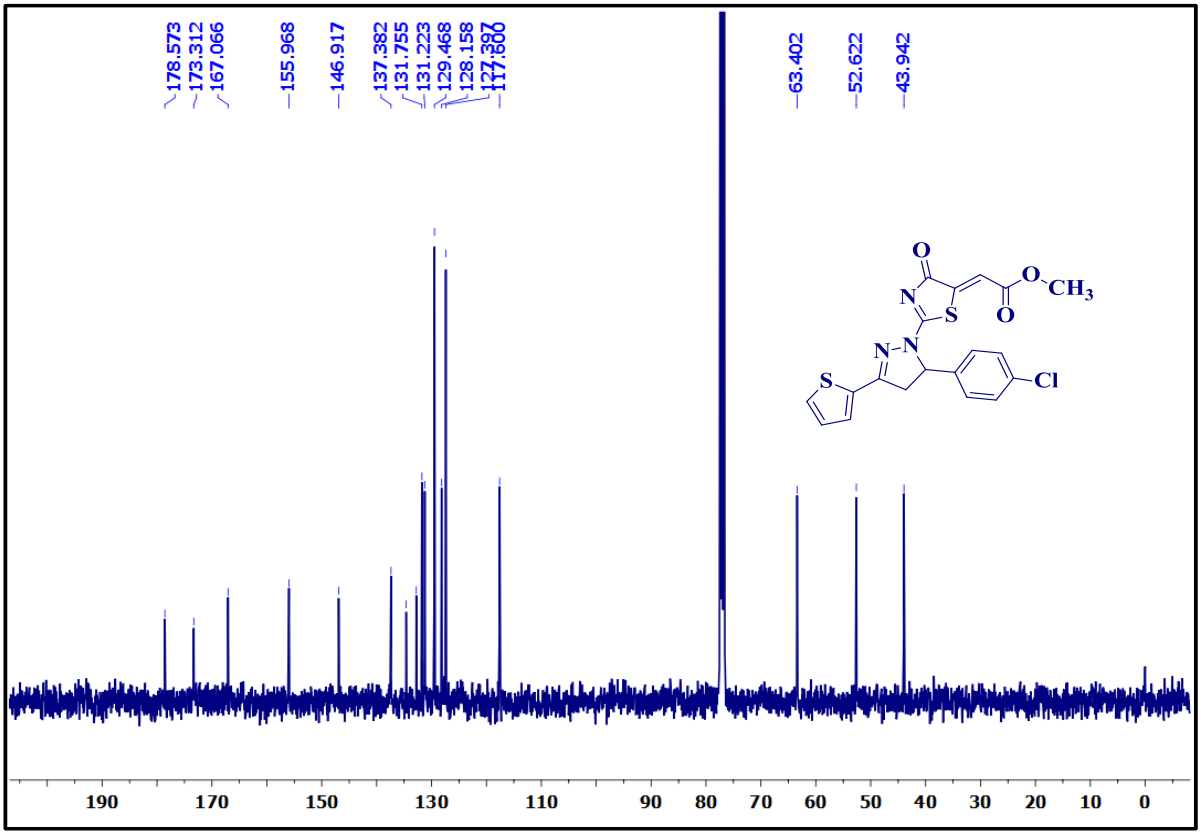


Fig: ^{13}C NMR Spectrum of compound **43c**

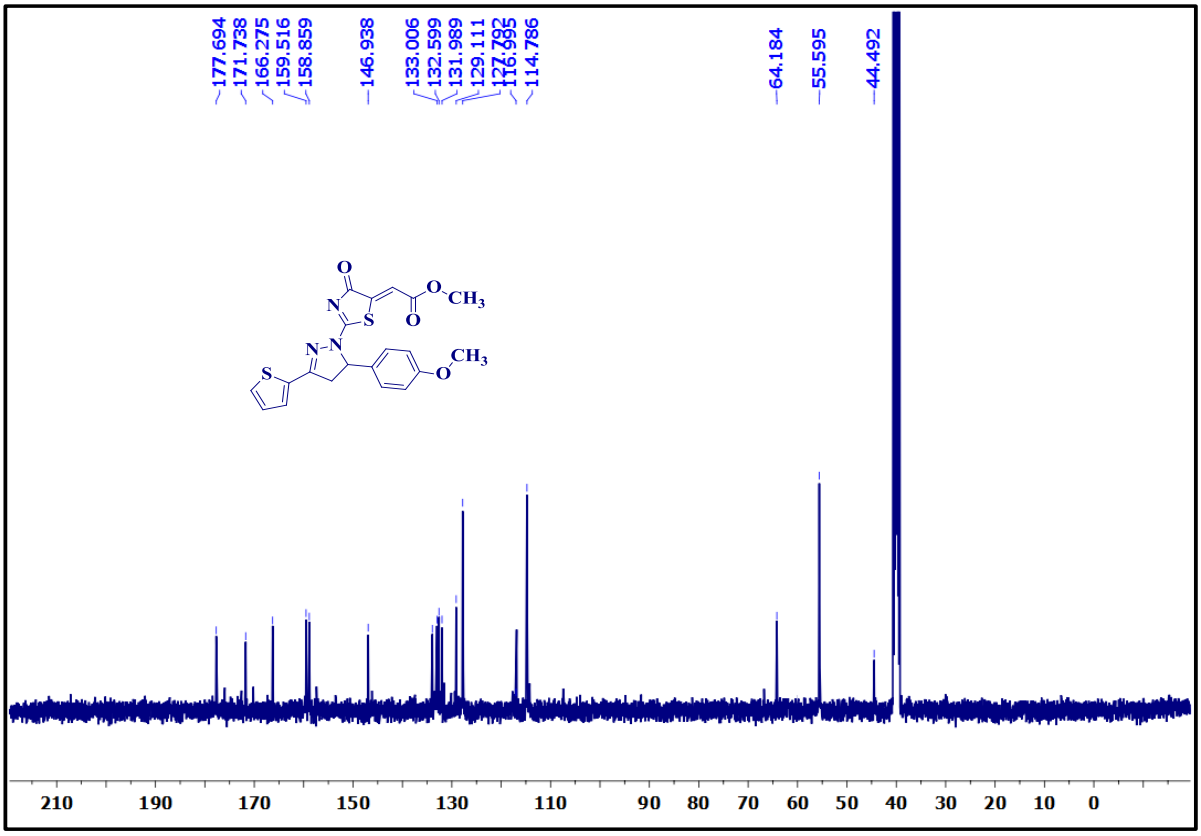


Fig: ^{13}C NMR Spectrum of compound **43f**

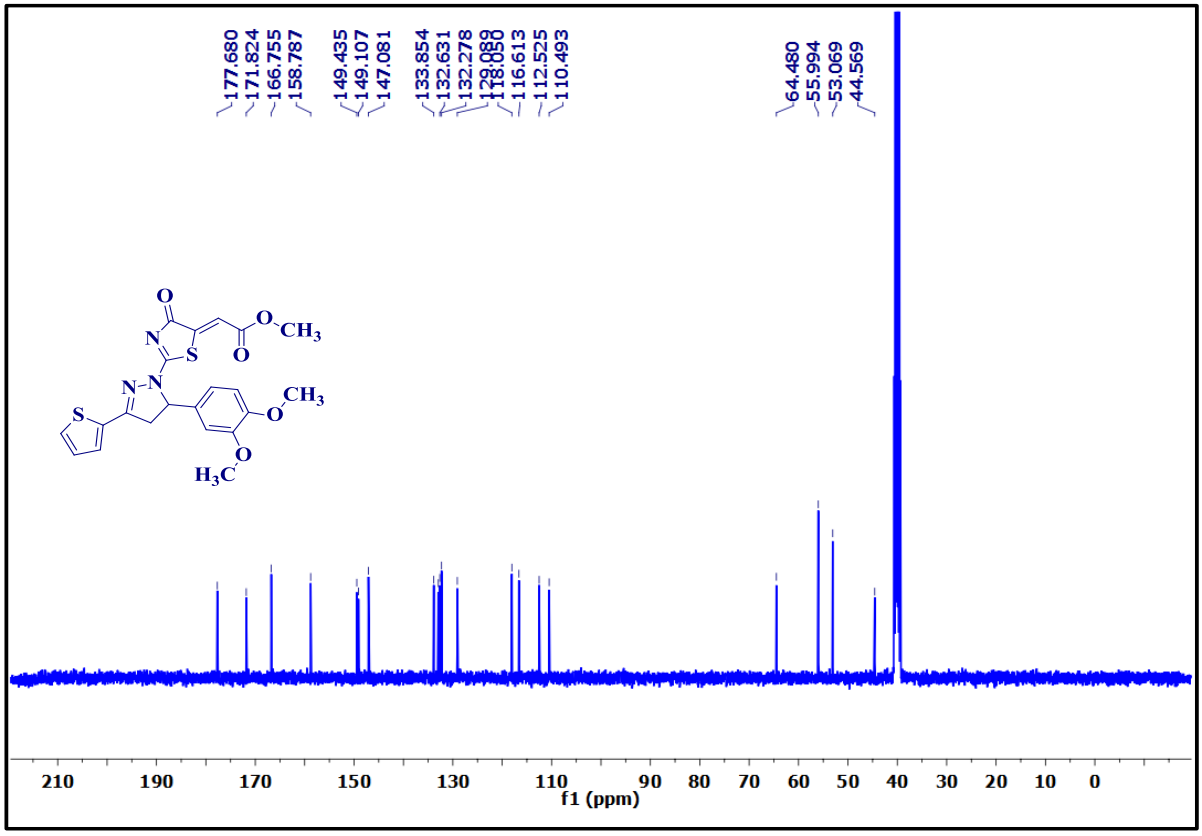


Fig: ^{13}C NMR Spectrum of compound **43g**

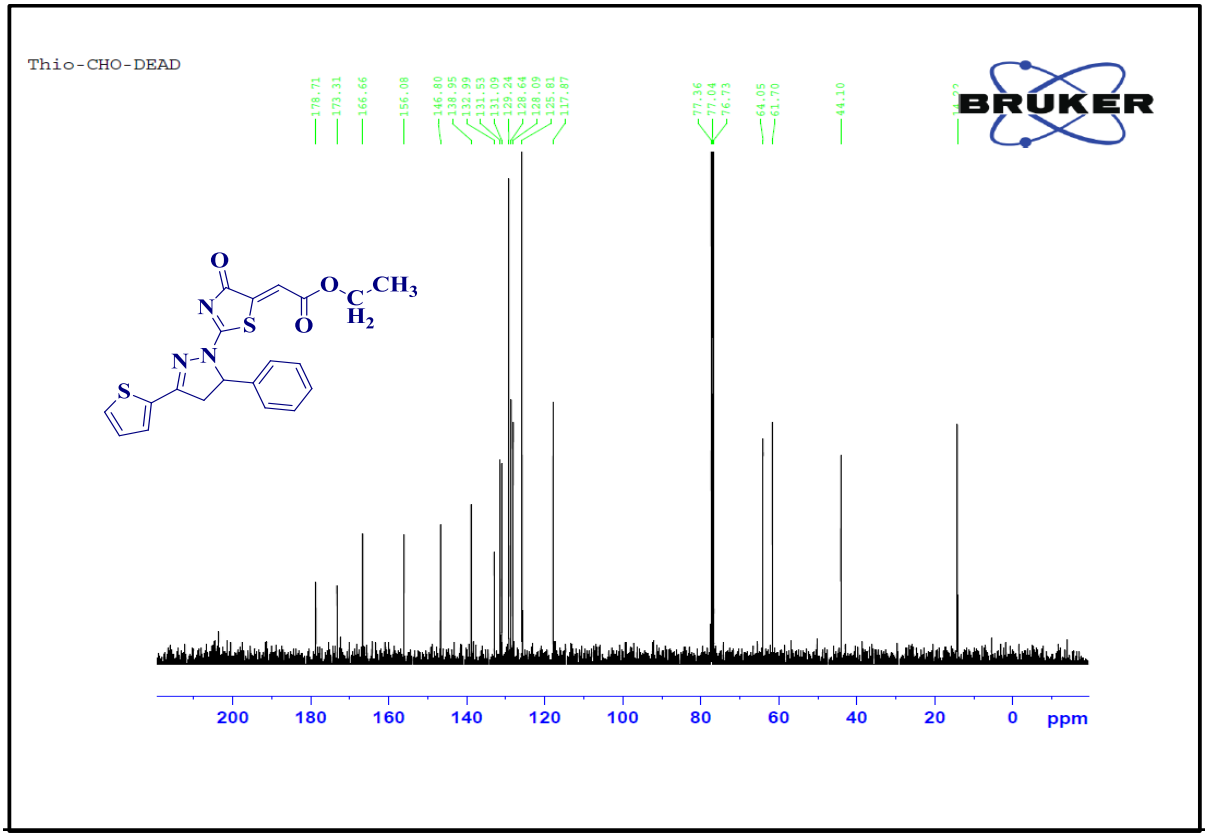


Fig: ^{13}C NMR Spectrum of compound **43i**

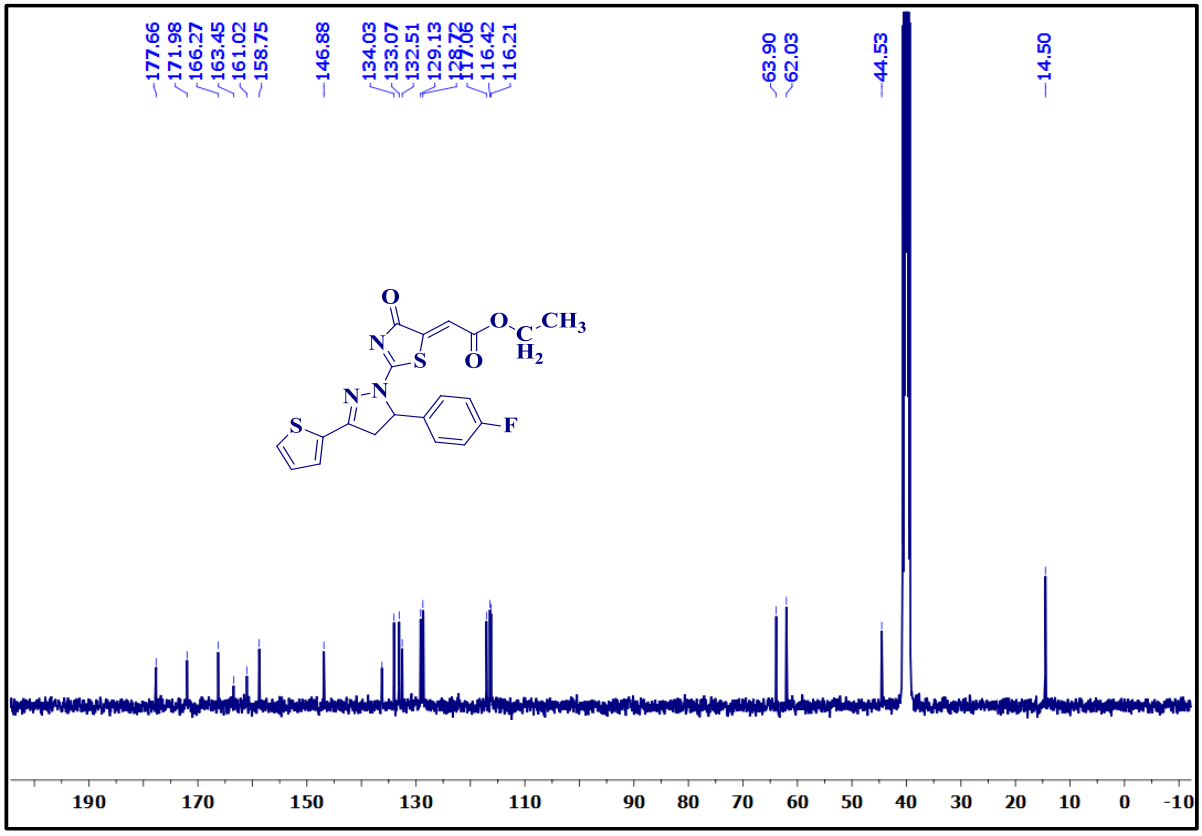


Fig: ^{13}C NMR Spectrum of compound **43j**

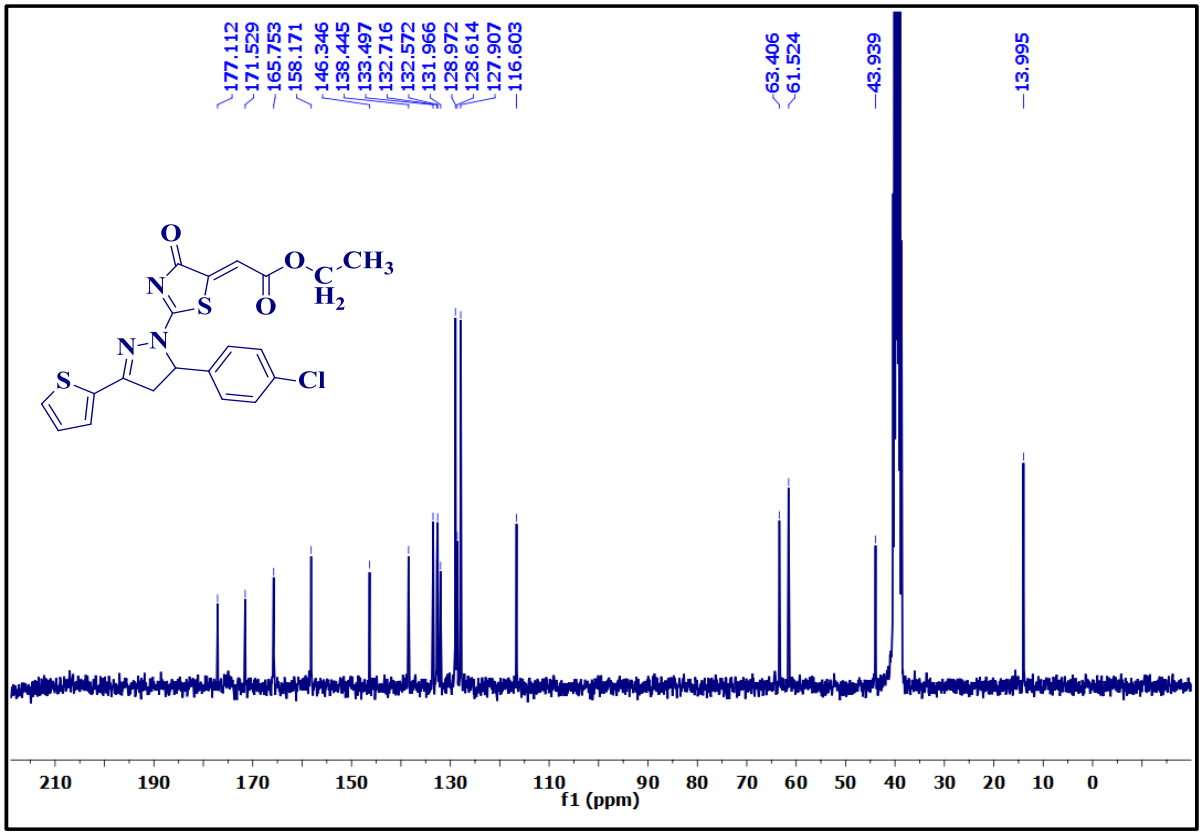


Fig: ^{13}C NMR Spectrum of compound **43k**

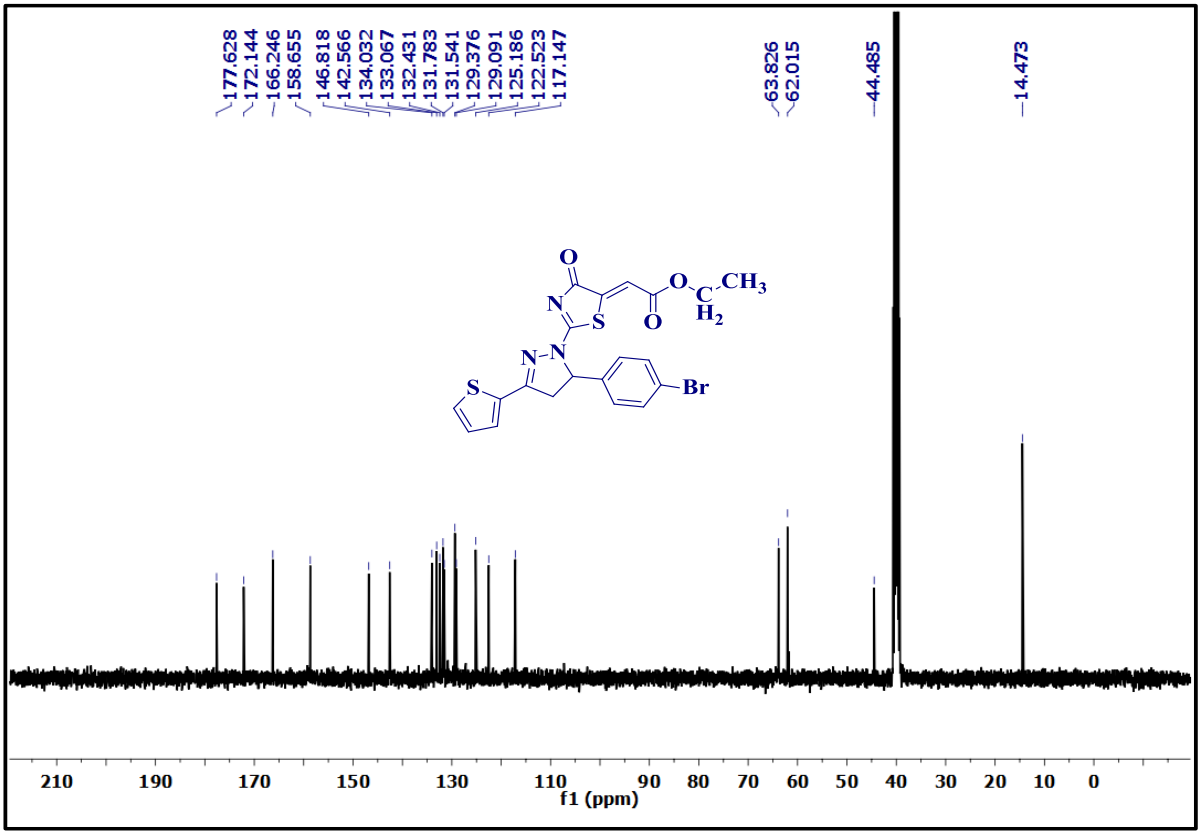


Fig: ^{13}C NMR Spectrum of compound **43l**

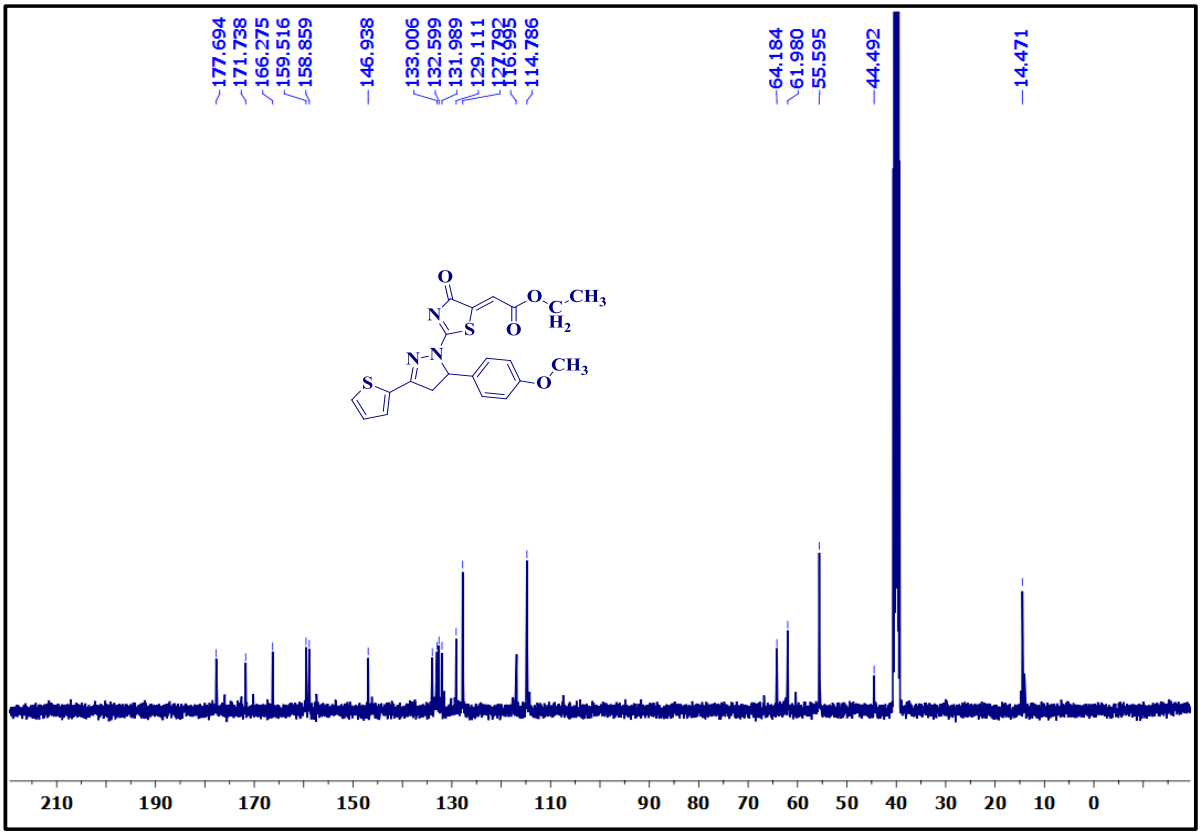


Fig: ^{13}C NMR Spectrum of compound **43n**

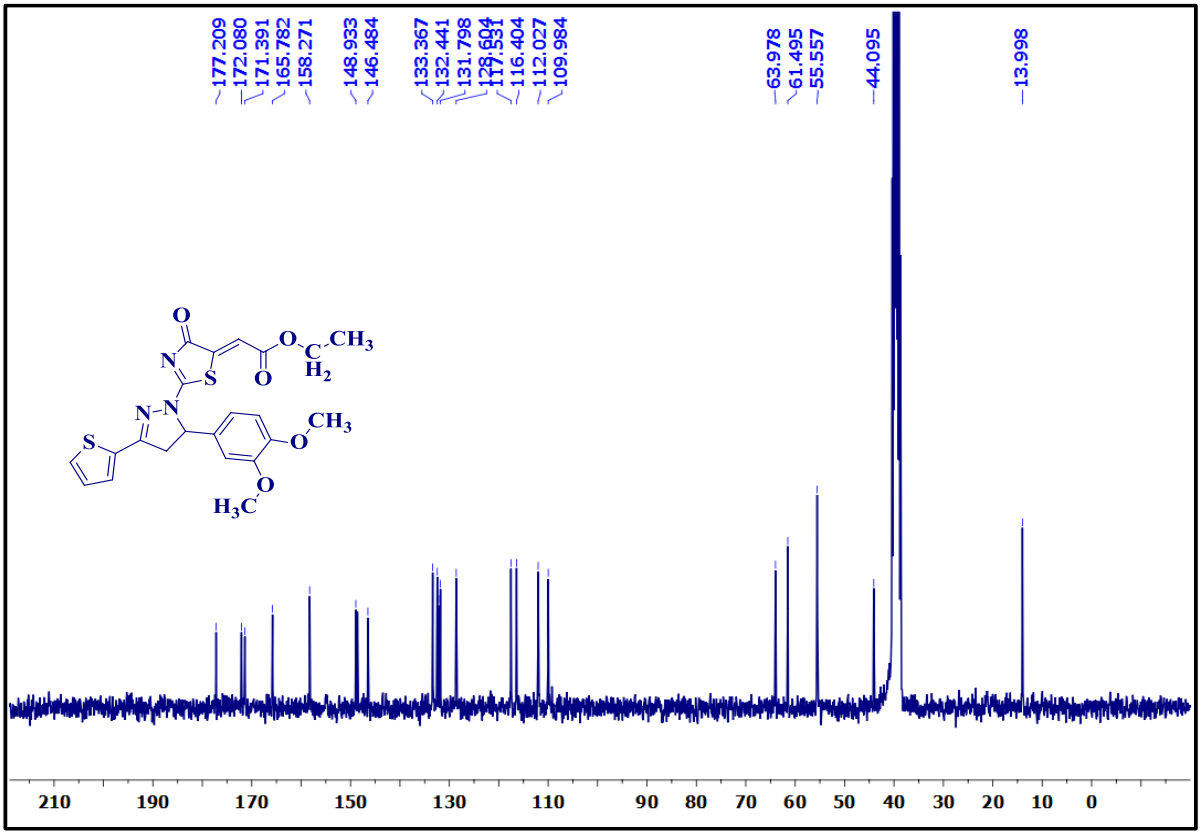


Fig: ^{13}C NMR Spectrum of compound **43o**

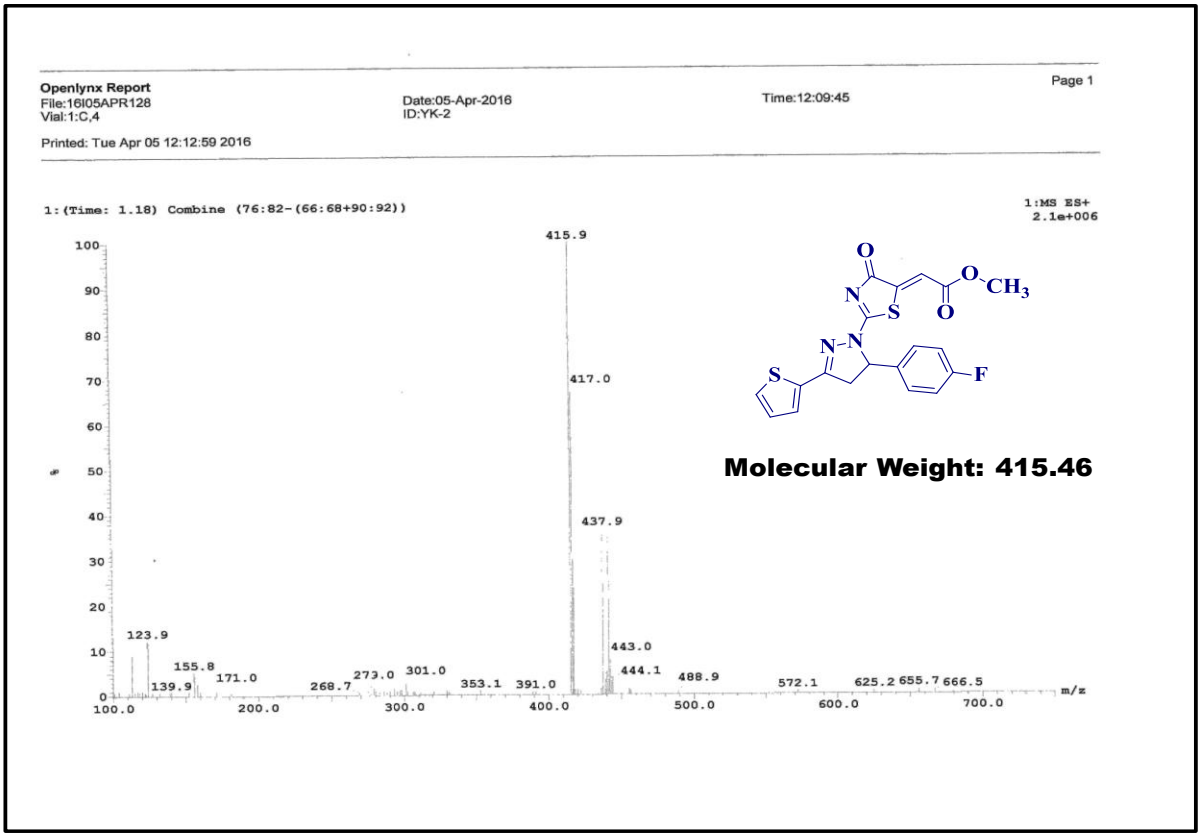


Fig: Mass spectrum of compound **43b**

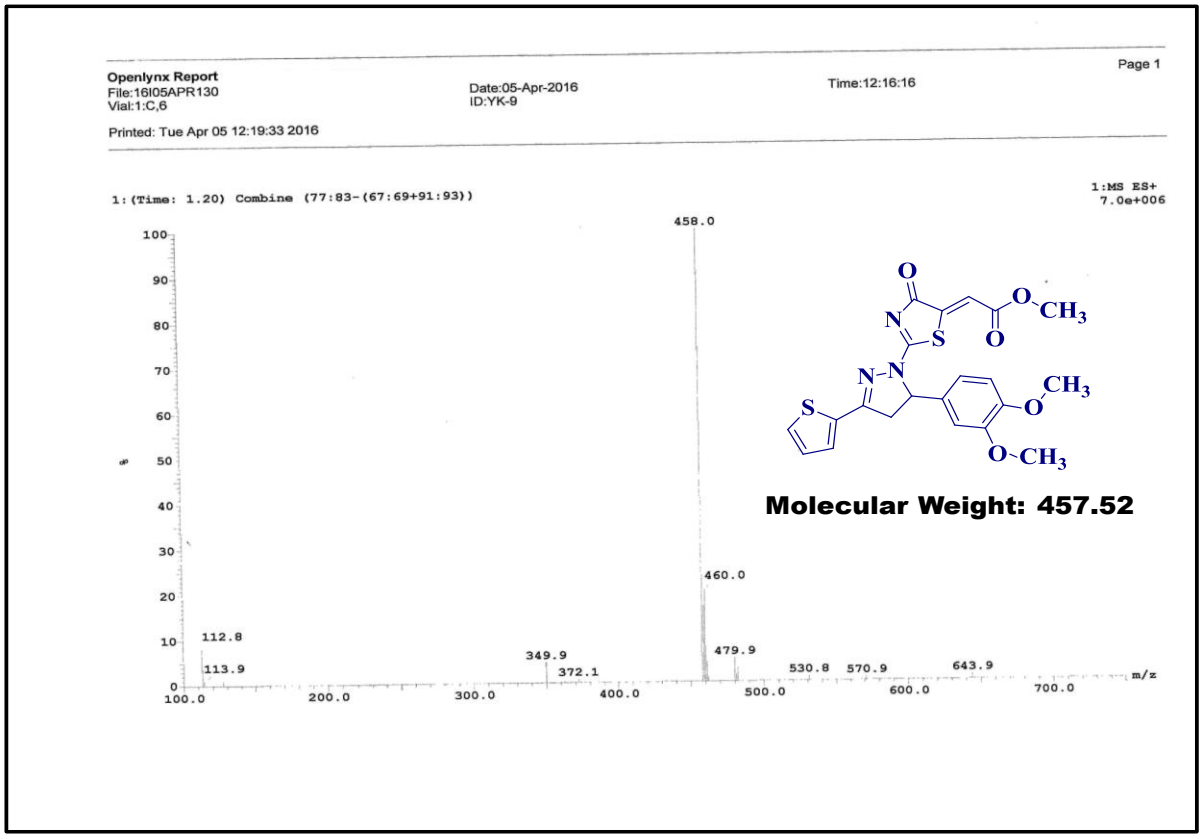


Fig: Mass spectrum of compound **43g**

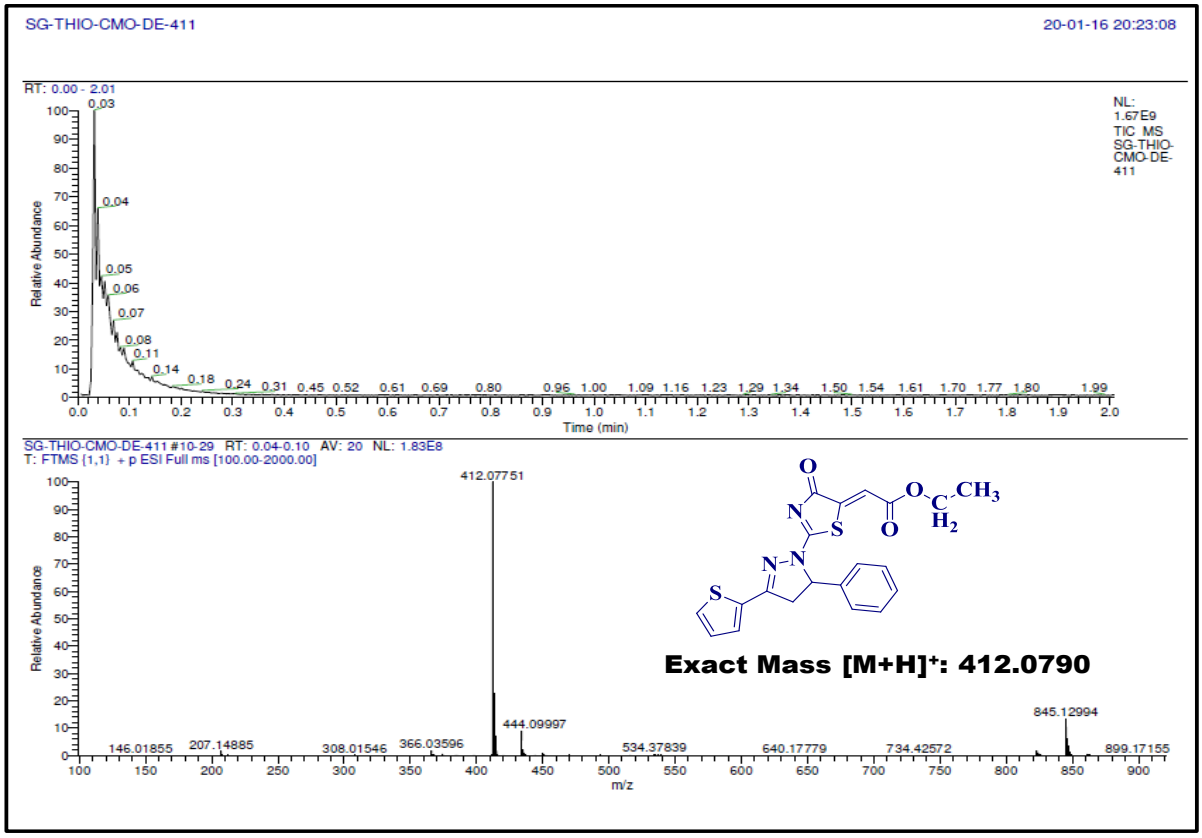


Fig: Mass spectrum of compound **43i**

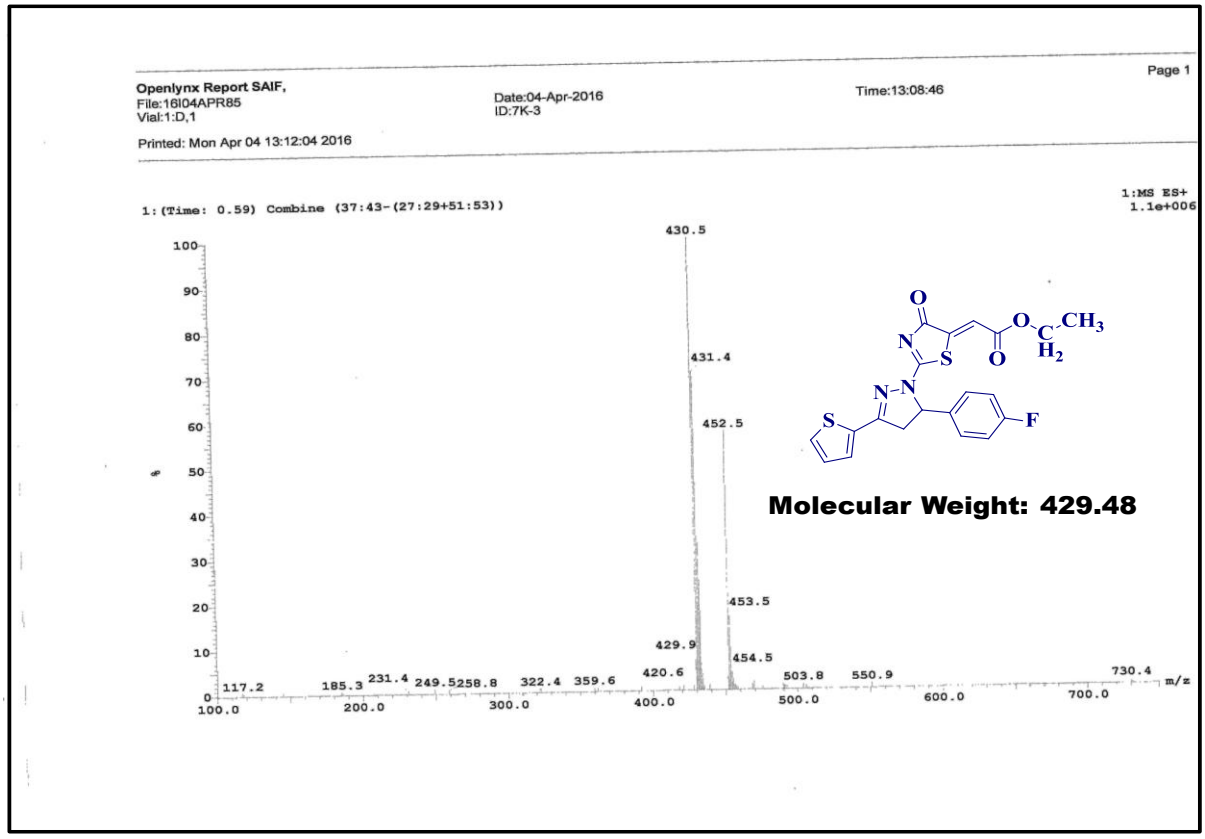


Fig: Mass spectrum of compound **43j**

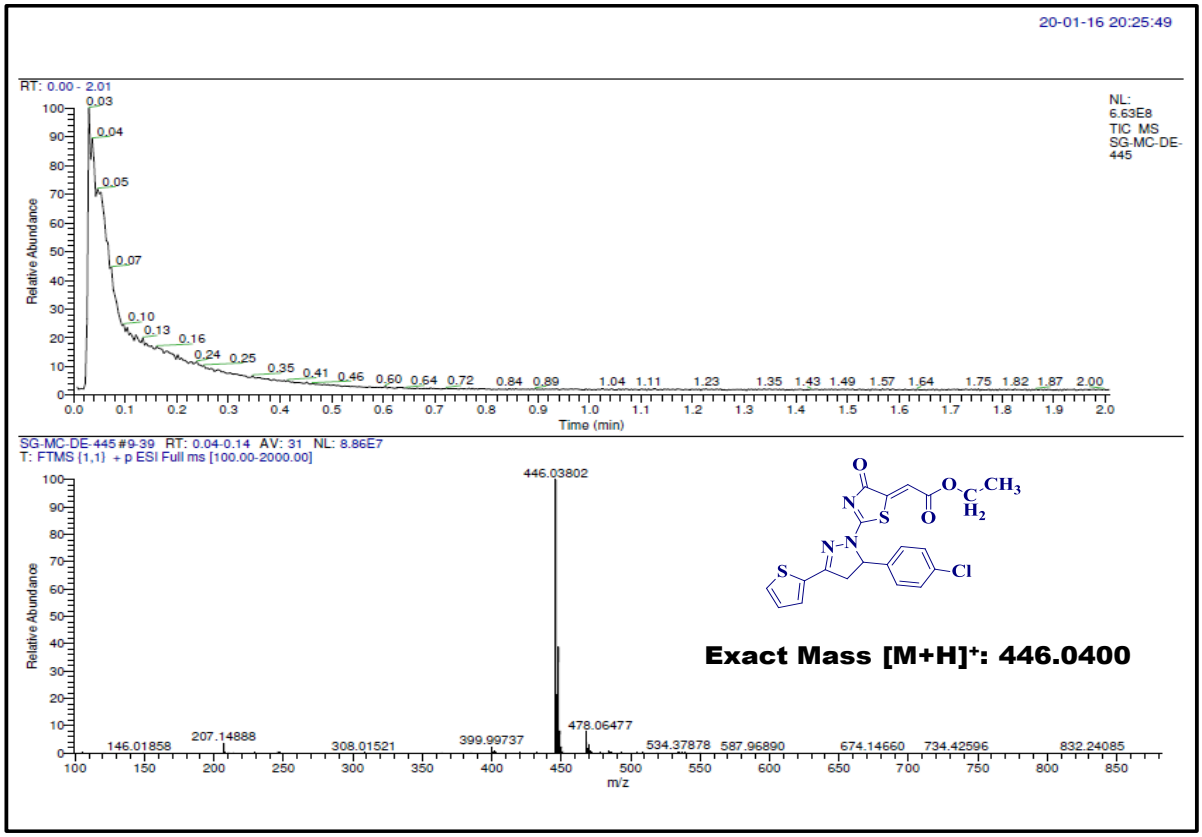


Fig: Mass spectrum of compound **43k**

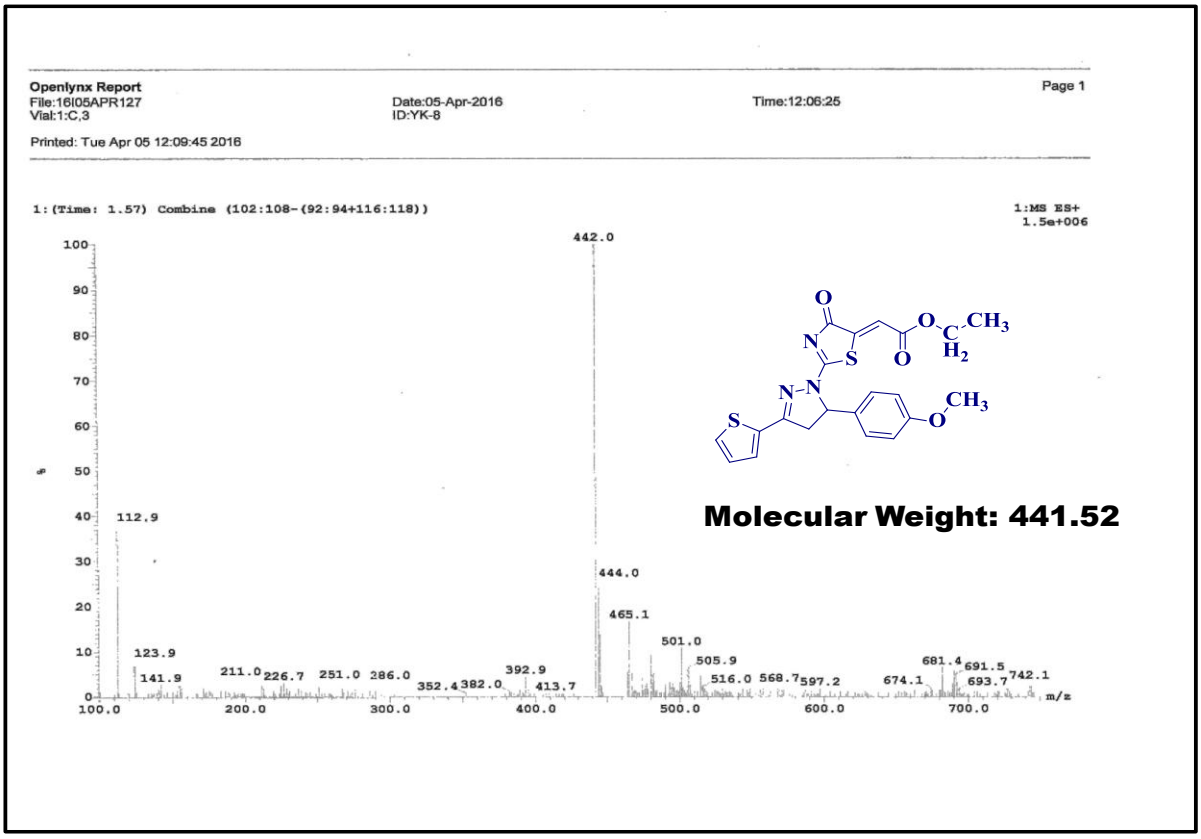


Fig: Mass spectrum of compound **43n**

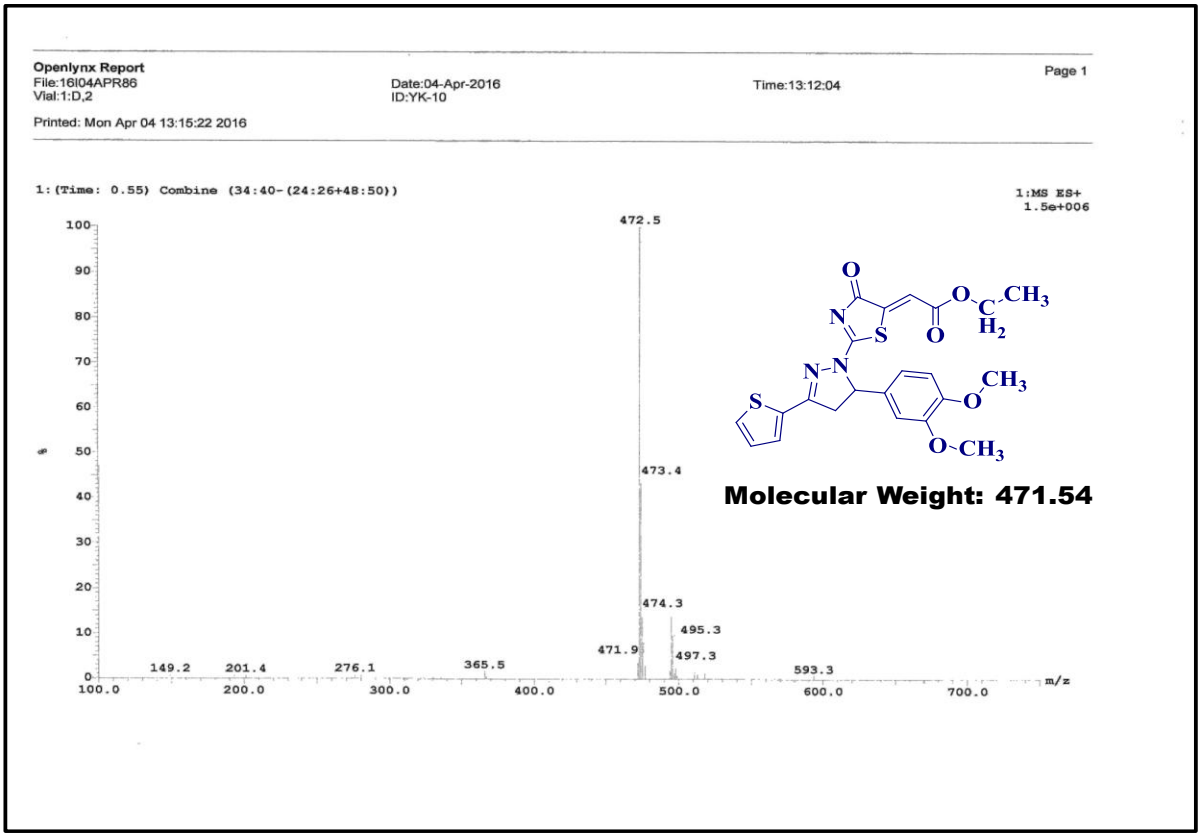


Fig: Mass spectrum of compound **43o**

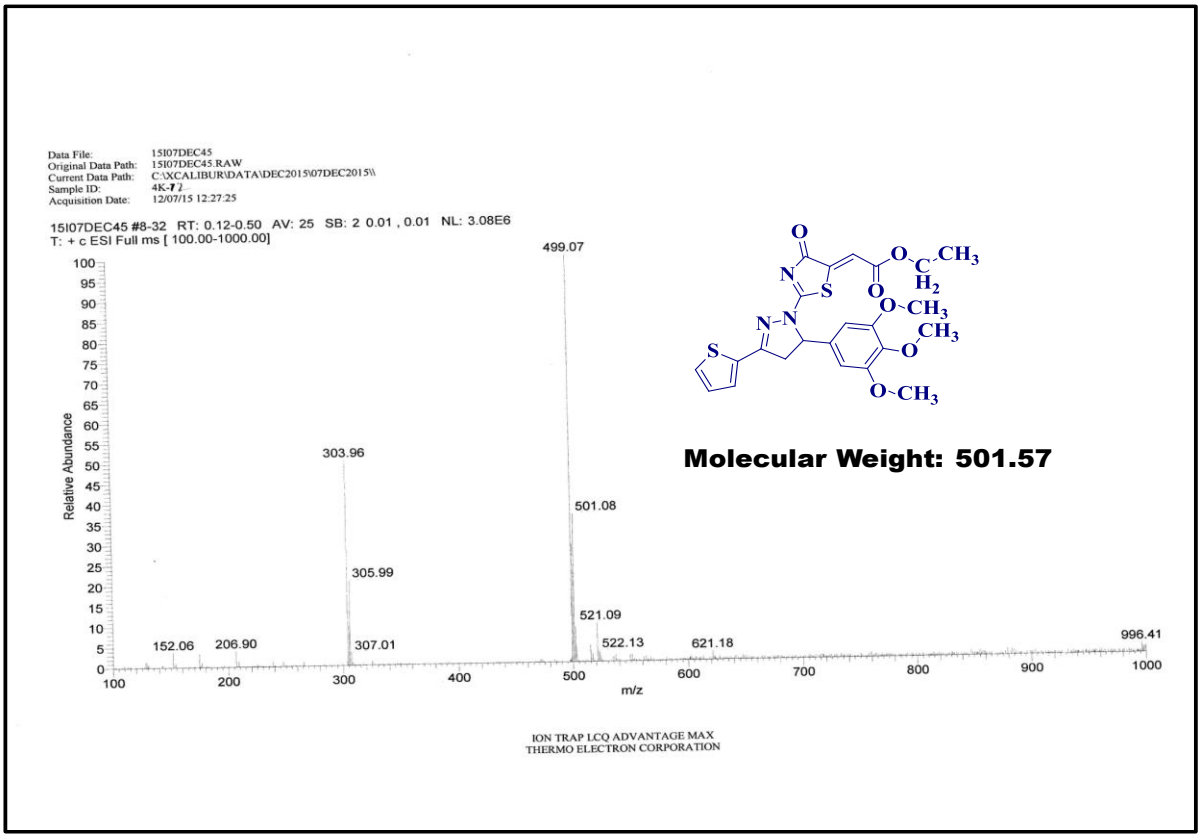


Fig: Mass spectrum of compound **43p**

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CHAPTER-III

SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF 6-(5-METHYL-ISOXAZOL-3YL)-3-ALKYL SULFANYL-[1,2,4]TRIAZOLO-[3,4-*b*][1,3,4]THIADIAZOLES

Introduction:

The heterocyclic compounds belong to the largest and most varied family of organic molecules. Heterocyclic compounds are a rich source of various chemical, biological and physical properties. Most of the drug molecules contain heterocyles as core unit. The design of new pharmacologically active molecules has always driven the attention of synthetic and medicinal chemists throughout the world. The compounds having nitrogen and sulphur as hetero atoms have significant pharmacological properties. Among these nitrogen and sulphur possessing five membered heterocycles display exceptional chemical properties and diverse pharmacological applications.

Thiadiazole is a prevalent and most vital of the five-membered heterocyclic compound possessing one sulphur and two nitrogen hetero atoms in the ring structure. Thiadiazole exhibits various isomeric structures such as 1,2,3-thiadiazole, 1,2,4-thiadiazole, 1,2,5-thiadiazole and 1,3,4-thiadiazole (**Fig.1**). Among these isomers of thiadiazole, 1,3,4-thiadiazole is a well studied polar symmetric molecule with pseudoaromatic property and has varied applications in the field of medicine, agriculture, optics, electrochemistry and material science. In particular, the 1,3,4-thiadiazoles exhibits varied biological activities including antibacterial[1,2], anticancer[3,4], antifungal[5-7], diuretic[8] and antitubercular[9-11]. Several thiadiazole heterocyclic compounds are available as drugs in the market, such as Acetazolamide as Diuretic, Megazole as antiparasitic, Sulphamethazole as antibacterial and Cefazoline as antibiotic (**Fig 2**).

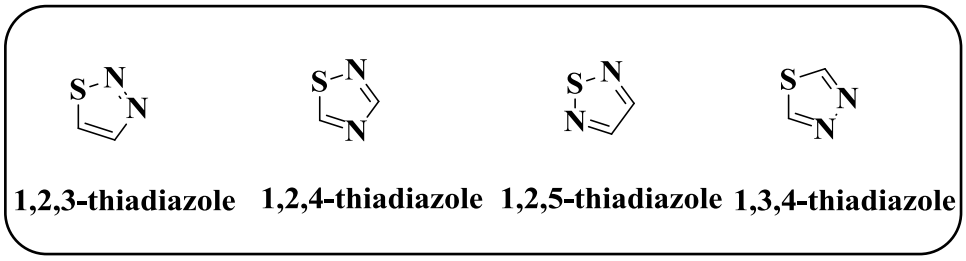


Fig.1: Isomeric structures of thiadiazole

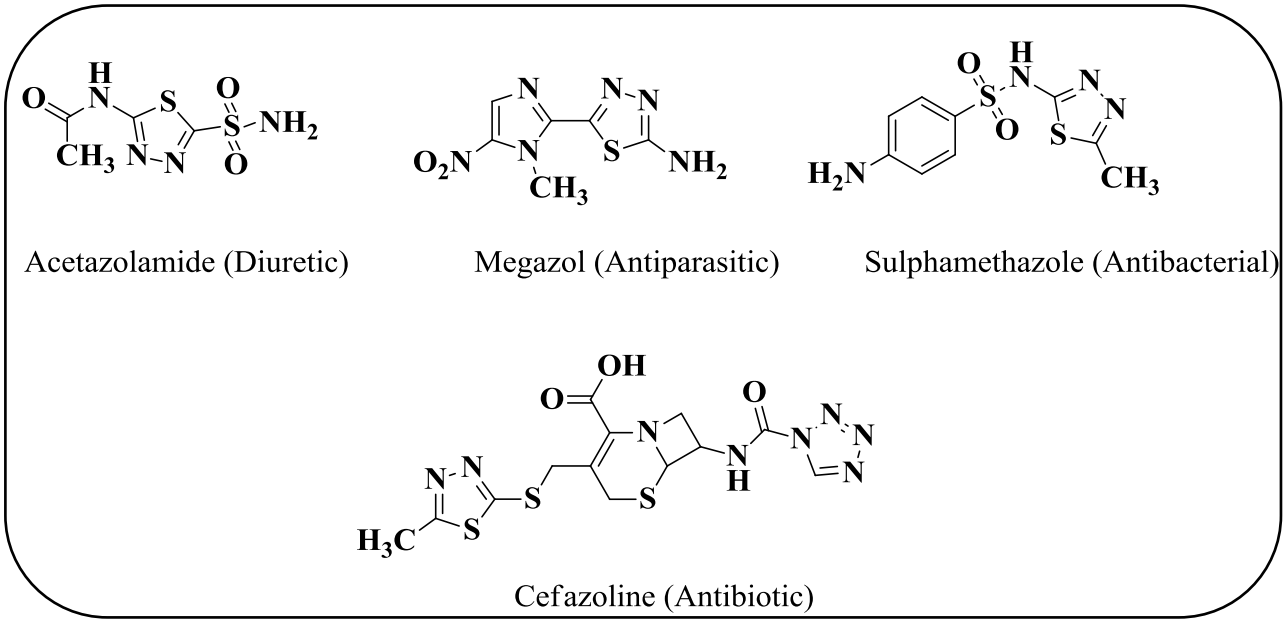


Fig. 2: Some of thiadiazole heterocycic ring containing drug molecules

Triazole is an important heterocyclic compound with three nitrogen atoms in a five membered ring system having two isomeric forms i.e.1,2,3-triazole and 1,2,4-triazole (**Fig.3**). A large number of 1,2,4-triazoles and their fused heterocyclic compounds have drawn considerable attention of the scientific community due to their synthetic and versatile pharmacological applications. Various 1,2,4-triazole heterocyclic compounds exhibit vast therapeutic properties such as antibacterial[12-14], antiviral[15-17], antifungal[18,19], anti-tubercular[20], anticancer[21,22], anti-inflammatory[23-25]. Furthermore, a large number of therapeutic drugs such as Etizolam (Anticonvulsant), Flucanazole (Antifungal), Ribavarin (Antiviral), Letrazole (Aromatase inhibitor), Rizatriptan (Antimigrane), Paclobutrazole (Plant growth regulator), Diniconazole (Plant fungicide) contain 1,2,4-triazole nucleus as heterocyclic core unit (**Fig.4**).

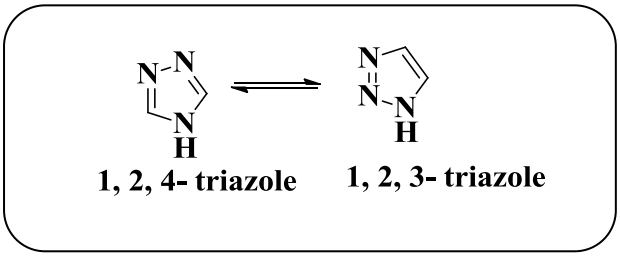


Fig.3: Two isomeric structures of triazole

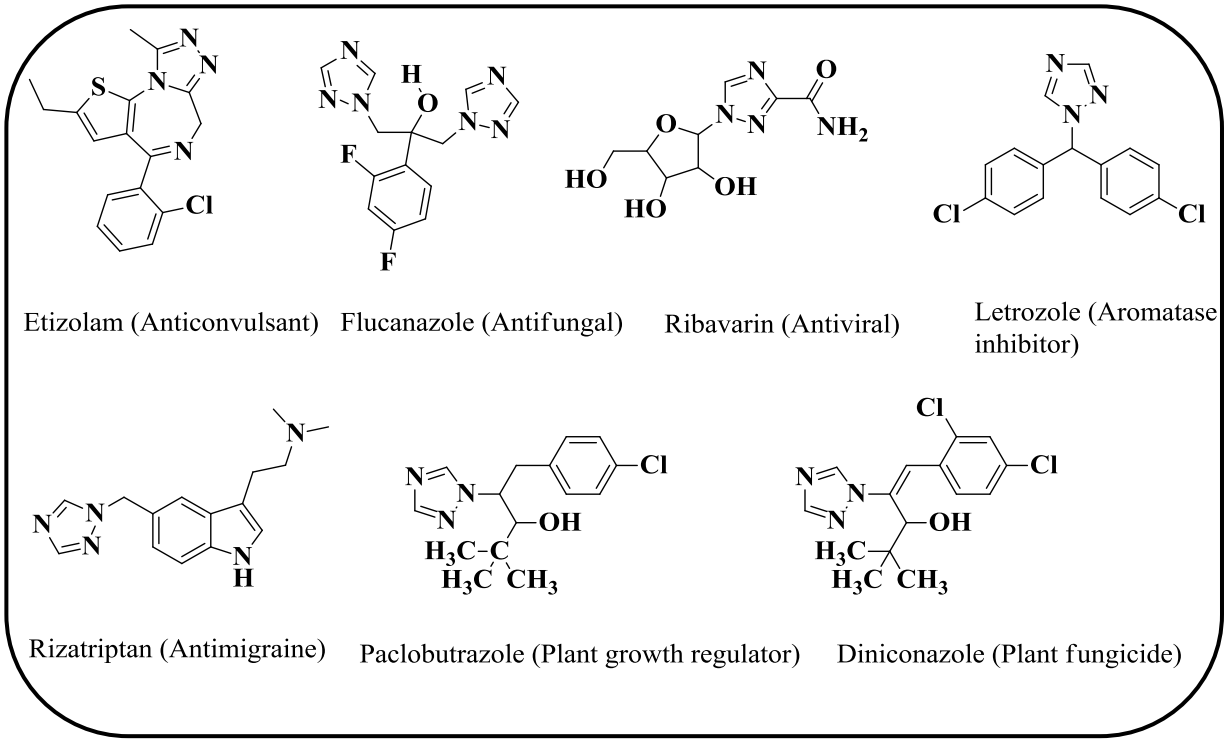


Fig.4: Some of the therapeutic drugs with 1,2,4-triazole nucleus.

Development of new therapeutic molecules through the combination of hybrid pharmacophores may lead to increased biological properties. The current literature is replete with N-bridged heterocyclic systems and their synthesis and biological properties. These N-bridged heterocyclics often a variety of applications in the field of medicine, agriculture and industry.

Much attention has been paid to symmetrical triazoles fused with 1,3,4 thiadiazoles due to their diverse pharmacological applications. The heterocyclic compound triazolothiadiazole is a fused motif possessing the 1, 2, 4-triazole and 1,3,4-thiadiazole nucleus (Fig.5). Triazolo-thiadiazole was first synthesized by kanaoka[26] by using 4-amino-5-mercapto-3-phenyl-1,2,4-triazole and various carboxylic acids in presence of phosphorous oxychloride. Triazolo-thiadiazole heterocyclic ring system is viewed as cyclic analogue of thiosemicarbazide and biguanide[27]. These heterocyclic compounds exhibit diverse pharmacological activities, possibly due to the presence of an important functional toxophore -N-C=S[28,29]. These triazolo-thiadiazoles exhibit a variety of pharmaceutical applications[30-35].

There are three major synthetic routes for the effective preparation of *s*-triazolo [3,4-*b*] [1,3,4]-thidiazole heterocyclic ring system. The first route involves the cyclocondensation reaction of 4-amino-5-alkyl/aryl -1,2,4-triazolo-3-thiones with various aromatic carboxylic acid in presence of phosphorous oxychloride[36,37]. The second method consists of ring formation reaction of 2-hydrazino 1,3,4-thiadiazoles in presence of toluene or xylene with carboxylic acids and phosphorous oxychloride[38-40]. The third method is a one – step cyclization of carboxylic acids with thiocarbohydrazide by silica supported dichlorophosphate as cyclodehydrant under microwave reaction condition[41].

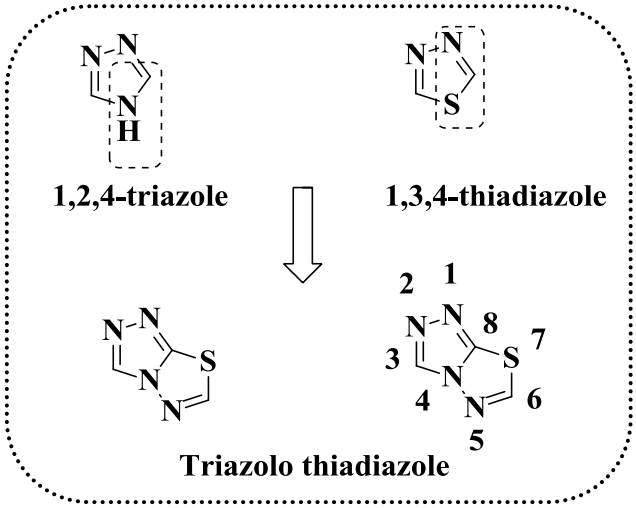
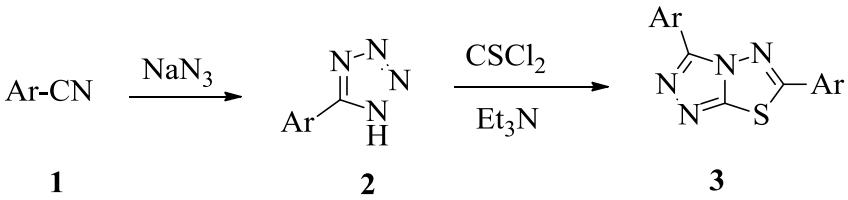


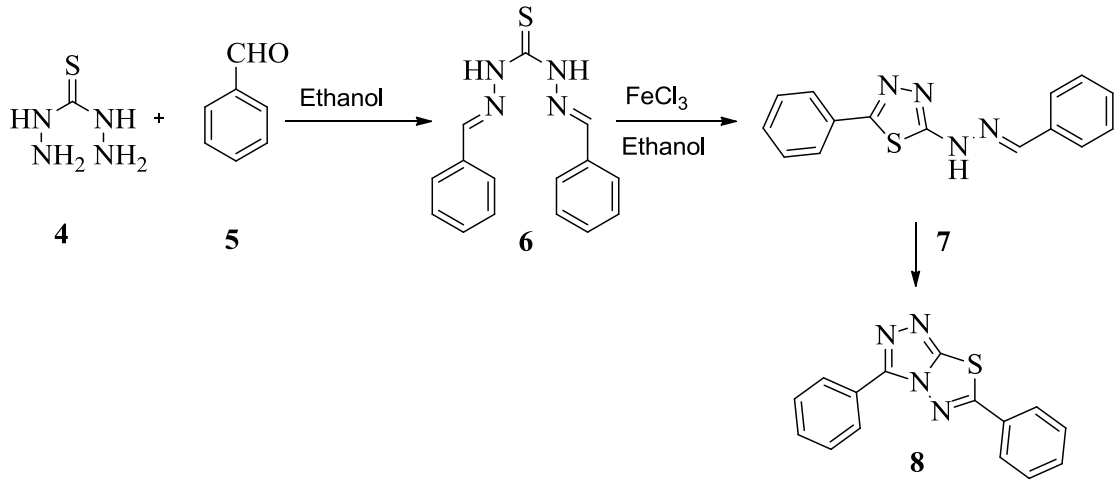
Fig.5: Numbering of [1, 2, 4] triazolo [3, 4-*b*][1, 3,4]thiadiazole ring system

A brief literature review on the synthesis of condensed N – bridged 3, 6-substituted triazolo thiadiazole is presented below.

Uher and Berkes[42] described a new simple one-pot method for the synthesis of 3,6-disubstituted 1,2,4-traizolo[3,4-b][1,3,4]thiadiazoles (**3**) by reacting 5-aryl tetrazole (**2**) in presence of thiophosgene and triethylamine in anhydrous dioxane solution. Compound 2 was obtained by the reaction of aryl cyanide (**1**) with sodium azide.

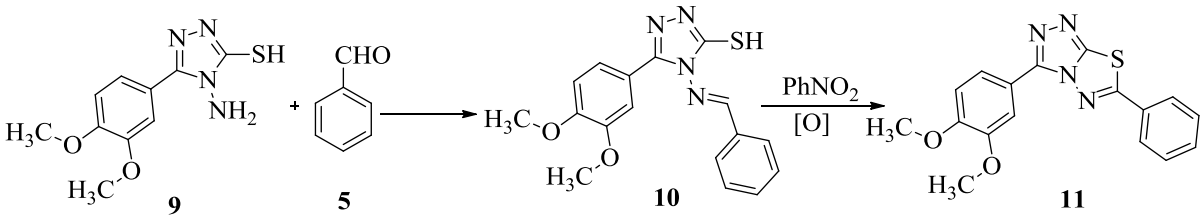


Shawali and co-workers[43] reported a convenient method for the synthesis of [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles (**8**) by oxidative electro cyclization of 2,2-bis (arylmethylene) carbonothioic dihydarzides (**6**) with easily available starting materials, shorter reaction time and higher overall yields. Compound 6 was prepared by the reaction of thiocarbohydrazide (**4**) with benzaldehyde (**5**) in presence of ethanol solvent.



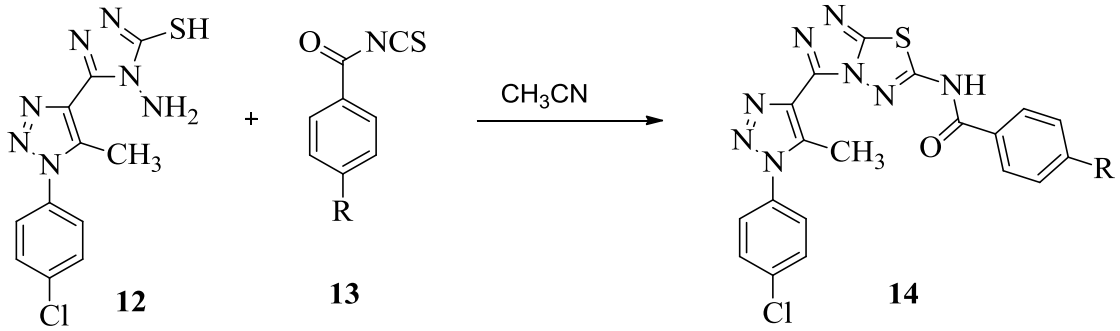
4 **5** **6** **7** **8**

El-Emam *et al*[44] described a facile and efficient method for the synthesis of 2-aryl -5-(3,4-dimethoxyphenyl)triazolo thiadiazoles (**11**) by oxidative cyclization of 4-(arylideneamino)-5-(3,4-dimethoxyphenyl)-4H-1,2,4-triazole-3-thiol (**10**) in presence of nitrobenzene with shorter reaction time and high overall yield. Compound 10 was synthesized by the reaction of 4-amino-5-(3,4-dimethoxyphenyl)-4H-1,2,4-triazole-3-thiol (**9**) and benzaldehyde (**5**).

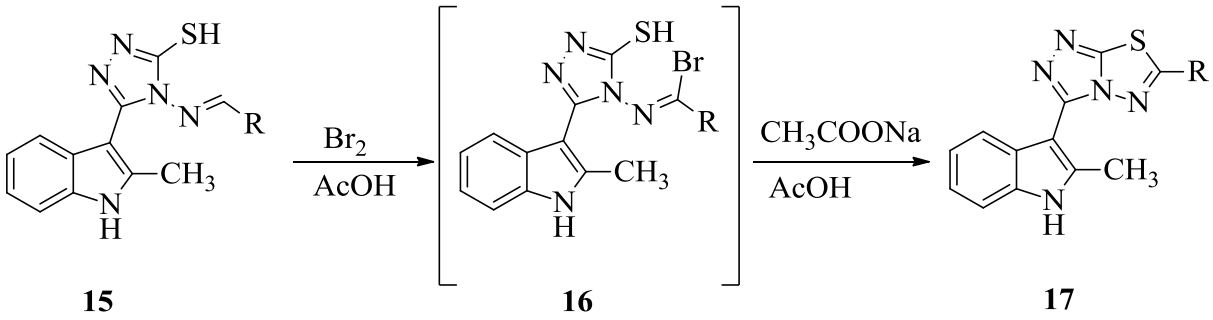


9 **5** **10** **11**

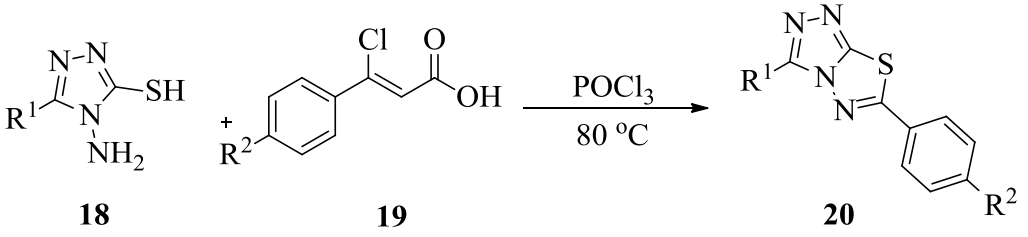
Sun *et al*[45] synthesized *N*-(3-(1-(4-chlorophenyl)-5-methyl-1H-1,2,3-triazol-4-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-yl)benzamides (**14**) by the reaction of various aroyl isothiocyanates (**13**) with 4-amino-5-(1-(4-chlorophenyl)-5-methyl-1H-1,2,3-triazol-4-yl)-4H-1,2,4-triazole-3-thiol (**12**) in presence of anhydrous acetonitrile under reflux condition with good yields.



Gomha and Riyadh[46] synthesized [1,2,4-triazolo][3,4-b][1,3,4]thiadiazoles (**17**) by bromination of 4-(arylideneamino)-5-mercapto-4H-[1,2,4] triazoles (**15**) followed by the elimination of hydrogen bromide (without isolation of the intermediate **16**) with simple and convenient route.

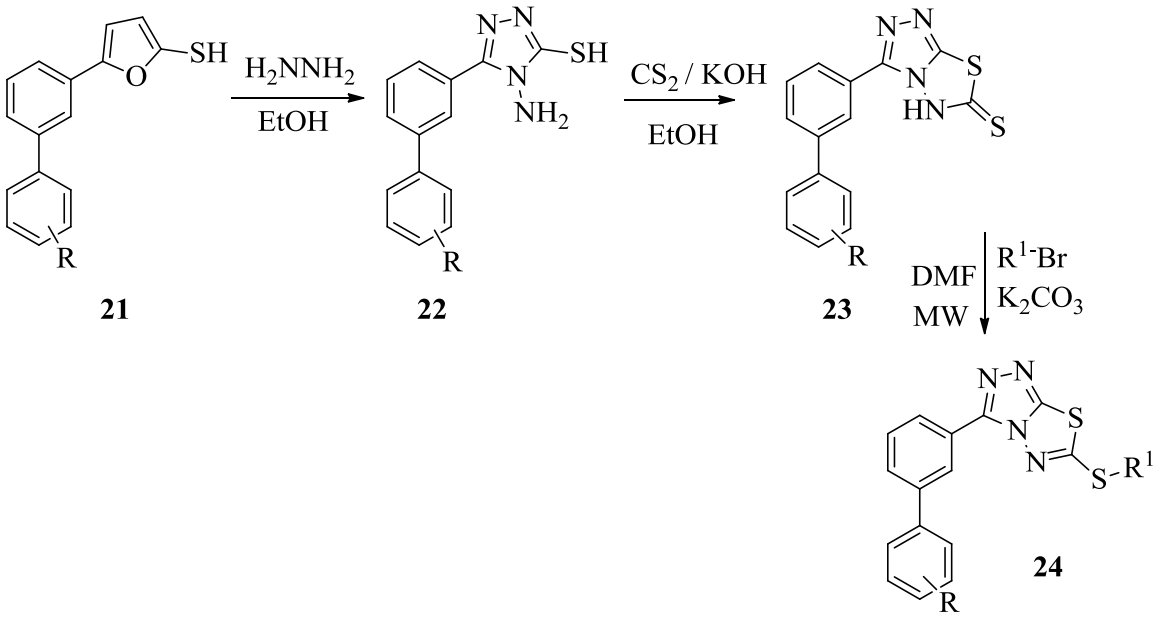


Sahi *et al*[47] developed unprecedent method for the synthesis of s- triazolo-thiadiazoles (**20**) by the reaction of 4- amino – 3- mercapto -5-alkyl/ aryl -1,2,4-triazole (**18**) with (*Z*)-3-chloro-(3-aryl) acrylic acid (**19**) in presence of phosphorous oxychloride at 80 °C. During this reaction the product isolated was 6-aryl-3-alkyl/aryl-s-triazolo-[3,4-*b*] [1,3,4]thiadiazole instead of (*Z*)-6-(2-chloro-2-phenylvinyl)-3-alkyl/aryl-[1,2,4] triazolo [3,4-*b*][1,3,4]thiadiazole.

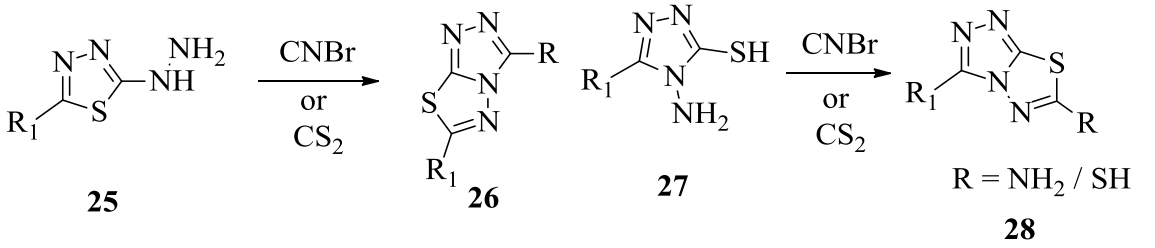


Ram prasad *et al*[48] reported regioselective synthesis of 3-([1,1'-biphenyl]-3-yl)-6-(alkylthio)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazoles (**24**) with high product yield and

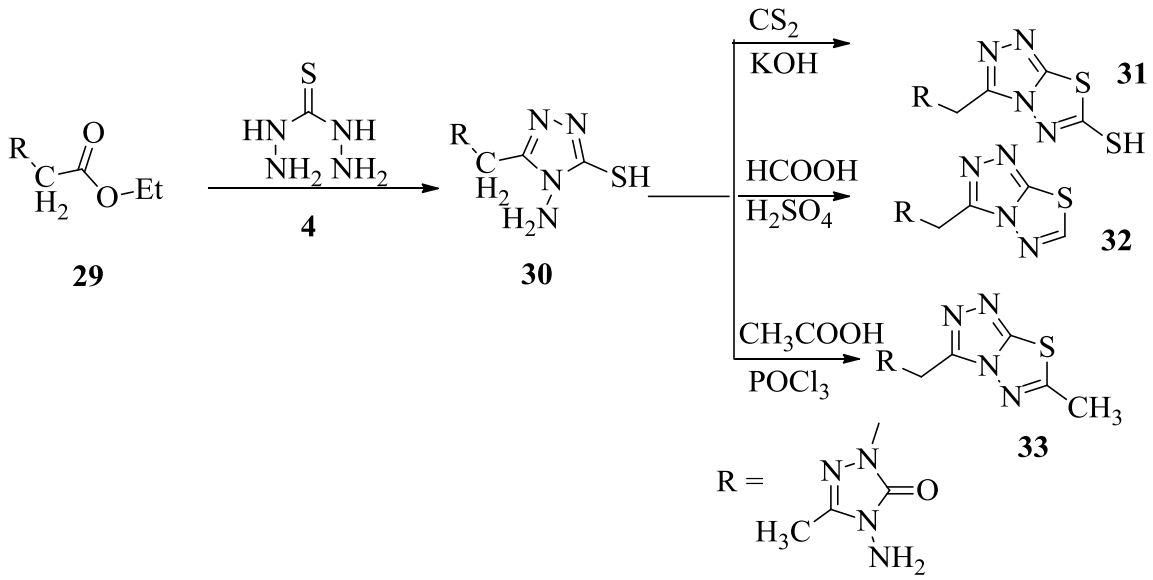
shorter reaction time by the reaction of 3-([1,1'-biphenyl]-3-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole-6-thiol (**23**) with various alkyl halides by using potassium carbonate in presence of dimethyl formamide solvent under microwave reaction condition. Compound 23 was obtained in two steps via ring transformation of compound **21** followed by ring formation of compound **22.**



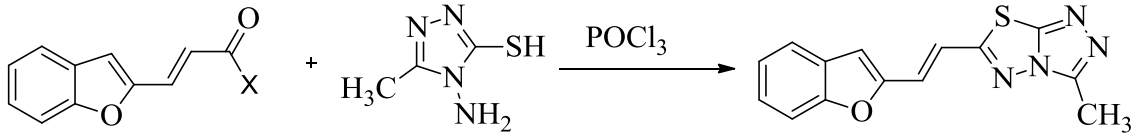
Potts and Huseby[49] prepared [1,2,4]triazolo[3,4-b] [1,3,4] thiadiazole heterocyclic ring systems (**26** & **28**) with good yields by the reaction of cyanogens bromide / carbon disulphide with 2-hydrazinyl-5-phenyl-1,3,4-thiadiazole (**25**) / 4-amino-3-alkyl-s-triazole-5-thiols (**27**) under alkaline reaction condition.



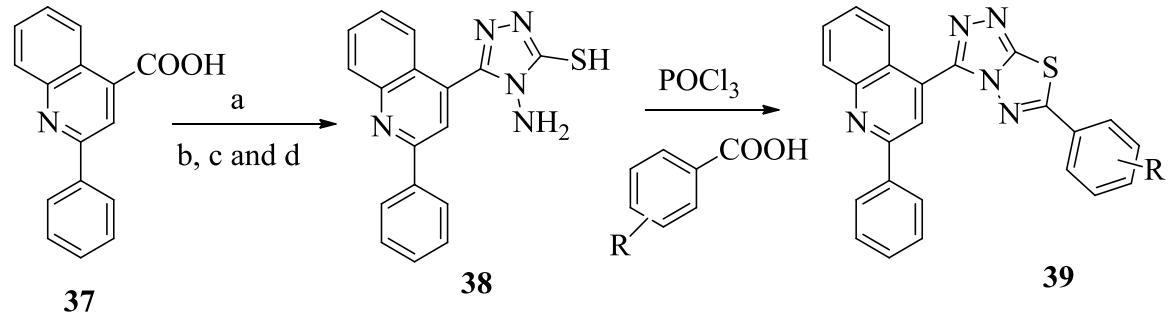
Demirbas *et al*[50] synthesized traizolothiadiazoles (**31 - 33**) from the reaction of ethyl 2-(4-amino-3-alkyl/aryl-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)acetates (**29**) with thiocarbohydrazide (**4**) followed by condensation of 4-amino-1-((4-amino-5-mercapto-4H-1,2,4-triazol-3-yl)methyl)-3-alkyl/aryl-1H-1,2,4-triazol-5(4H)-one (**30**) with acetic acid/ Formic acid or carbon disulphide. These novel triazolothiadiazoles were evaluated for antimicrobial activity.



Obushak *et al*[51] reported synthesis of (E)-6-(2-(benzofuran-2-yl)vinyl)-3-alkyl/aryl-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles (**36**) by the reaction of 4-amino-5-methyl-4H-1,2,4-triazole-3-thiol (**35**) with (E)-3-(benzofuran-2-yl)acrylic acid or (E)-3-(benzofuran-2-yl)acryloyl chloride (**34**) in presence of phosphorous oxychloride under reflux condition for about 1 h with good yields.

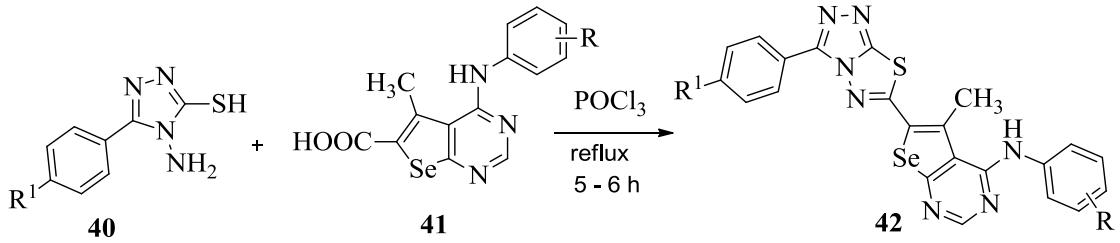


Xu *et al*[52] described synthesis of 6-aryl-3-(2-phenylquinolin-4-yl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles (**39**) and studied their antimicrobial activity. The target product was synthesized using 2-phenylquinoline-4-carboxylic acid (**37**) as starting material. 2-phenylquinoline-4-carboxylic acid was converted into 4-amino-5-(2-phenylquinolin-4-yl)-4*H*-1,2,4-triazole-3-thiol (**38**) in four steps. Compound **38** was treated with various substituted aromatic carboxylic acids in presence of phosphorous oxychloride to yield the target compound **39**.

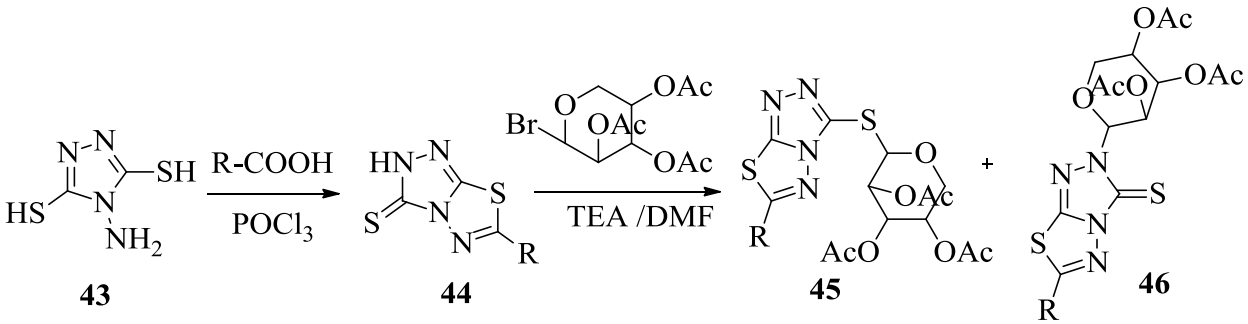


a = EtOH / H_2SO_4 b = $H_2NNH_2.H_2O$ c = CS_2 / KOH/ EtOH d = $H_2NNH_2.H_2O$/EtOH

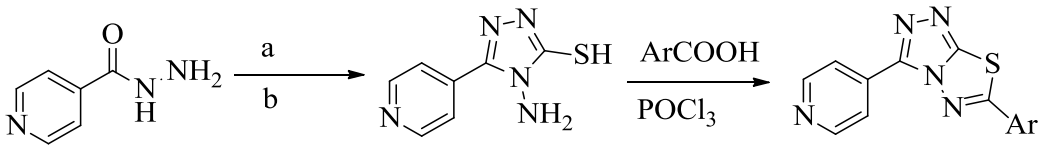
Kotaiah *et al*[53] reported the synthesis and antimicrobial activity of 1,2,4-(triazolo[3,4-*b*][1,3,4]thiadiazol-6-yl)selenopheno[2,3-*d*]pyrimidines (**42**). The title compounds were synthesized by the reaction of various 4-amino-5-aryl-4*H*-1,2,4-triazole-3-thiol (**40**) with 5-methyl-4-(phenylamino)selenopheno[2,3-*d*]pyrimidine-6-carboxylic acid (**41**) in presence of phosphorous oxychloride with good yields.



Khalil[54] reported synthesis of novel regioisomeric *N*- and *S*-α-L-arabinopyranosyl [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles (**45 & 46**). The target compound was synthesized in two steps via cyclization of 4-amino-4*H*-1,2,4-triazole-3,5-dithiol (**43**) with aromatic carboxylic acids in presence of phosphorous oxychloride followed by arabinosidation of 6-aryl-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole-3-thiol (**44**) with 2,3,5-tri-*O*-acetyl-*β*-L-arabinopyranosyl bromide which yielded two regioisomeric products.



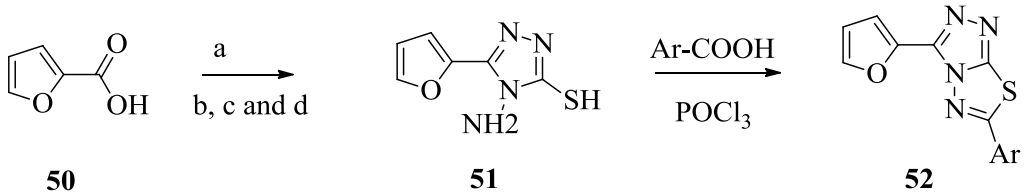
Khan *et al*[55] synthesized 3,6-disubstituted 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazoles (**49**) by using isonicotinic acid hydrazide (**47**) as starting material. Cyclocondensation of 4-amino-5-(pyridin-4-yl)-4*H*-1,2,4-triazole-3-thiol (**48**) with various substituted aromatic carboxylic acids in presence of phosphorous oxychloride under refluxed condition gave the target compounds with good yields.



47 **48** **49**

a = CS_2 / KOH / Methanol b = $H_2NNH_2.H_2O$ / Ethanol

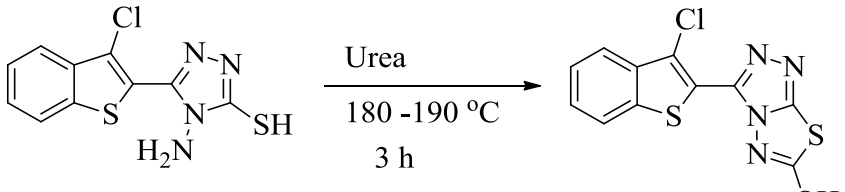
Zhang *et al*[56] reported the synthesis of 3-(furan-2-yl)-6-aryl-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles (**52**) from 4-amino-5-(furan-2-yl)-4*H*-1,2,4-triazole-3-thiol (**51**) and different aromatic carboxylic acids in phosphorous oxychloride. Compound **51** was obtained via a multi step reaction condition using furoic acid (**50**) as starting material.



50 **51** **52**

a) EtOH/ H_2SO_4 b) $H_2NNH_2.H_2O$/ EtOH c) CS_2/KOH/ EtOH d) $H_2NNH_2.H_2O$/ EtOH

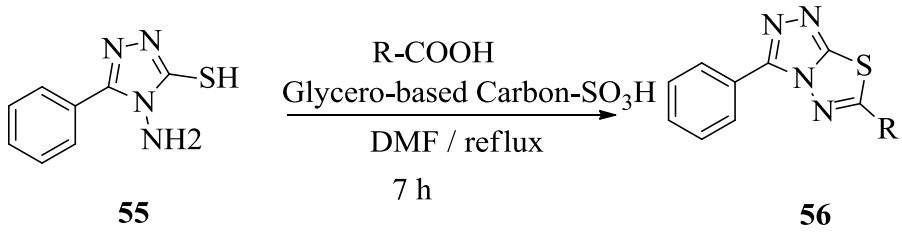
Ashry *et al*[57] described the synthesis of 3-(3-chlorobenzo[*b*]thiophen-2-yl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-6-ol (**54**) by the reaction of 4-amino-5-(3-chlorobenzo[*b*]thiophen-2-yl)-4*H*-1,2,4-triazole-3-thiol (**53**) with urea. The reaction mixture was fused at 180 -190 °C for about 3 h. The isolated product was obtained sufficiently high quantity.



53 **54**

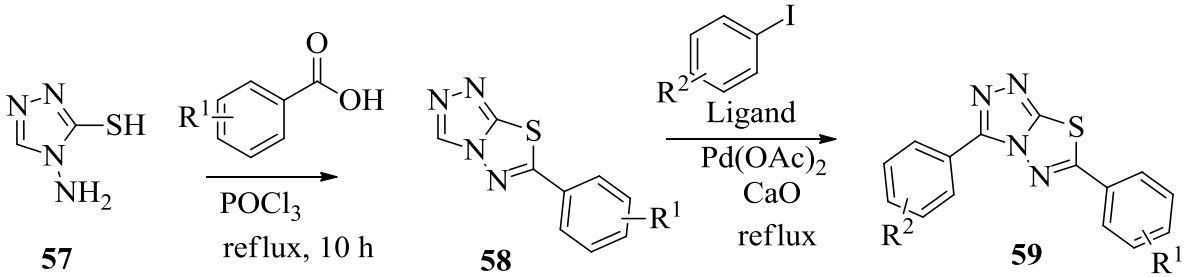
Baburajeev *et al*[58] synthesized novel condensed bicylic 3,6-disubstituted traizolothiadiazoles (**56**) by an efficient green heterogeneous solid catalytic method by

using 4-amino-5-phenyl-4*H*-1,2,4-triazole-3-thiol (**55**) and various carboxylic acids, and studied the effective inhibition activity against Protein tyrosine phosphatase (PTP_1B) *in vitro*.

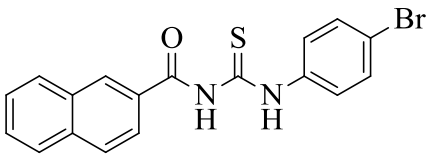


55 **56**

Saeed *et al*[59] described application of acyl thiourea to the synthesis of 3, 6- di subtituted triazolothiadiazole (**59**) by C-H arylation method. A series of traizolo-thiadiazoles were synthesized by the condensation 4-amino 1,2,4-triazole-3-thiol (**57**) with aromatic acids followed by arylation of 6-aryl-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (**58**) with substituted aryliodides by using 1-(2-naphthoyl)-3-(4-bromophenyl)thiourea (**60**) as a ligand with good yield.

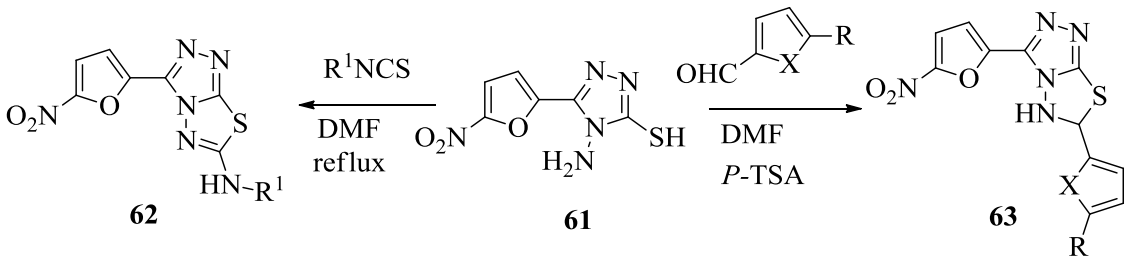


57 **58** **59**

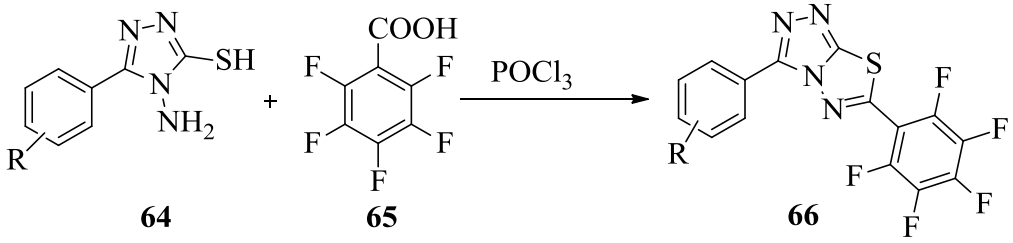
Ligand = 

60

Badr and Barwa[60] reported the synthesis of triazolo thiadiazoles (**62 & 63**) by the reaction of 4-amino-5-(5-nitrofuran-2-yl)-4*H*-1,2,4-triazole-3-thiol (**61**) with various heterocyclic carbaldehydes and alkyl / aryl isothiocyanates in presence of dimethyl formamide solvent. The final products were obtained in good yields and were recrystallized from ethanol/ dioxane or ethanol/ petroleum ether solvent medium.



Chowrasia *et al*[61] synthesized fluorinated 3,6-diaryl-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles (**66**) with good yields by condensation of 4-amino-5-phenyl-4*H*-1,2,4-triazole-3-thiol (**64**) with 2,3,4,5,6 penta fluorobenzoic acid (**65**) in presence of phosphorous oxychloride solution. The target compounds were evaluated for anti cancer activity using MCF-7(human breast cancer cell line), K562 (human myeloid leukemia cell line) and SaOS-2 (human osteosarcoma cell line). The screened compounds have shown moderate to good anticancer potency against tested cancer cell lines.

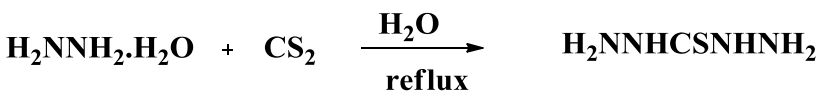


STARTING MATERIALS

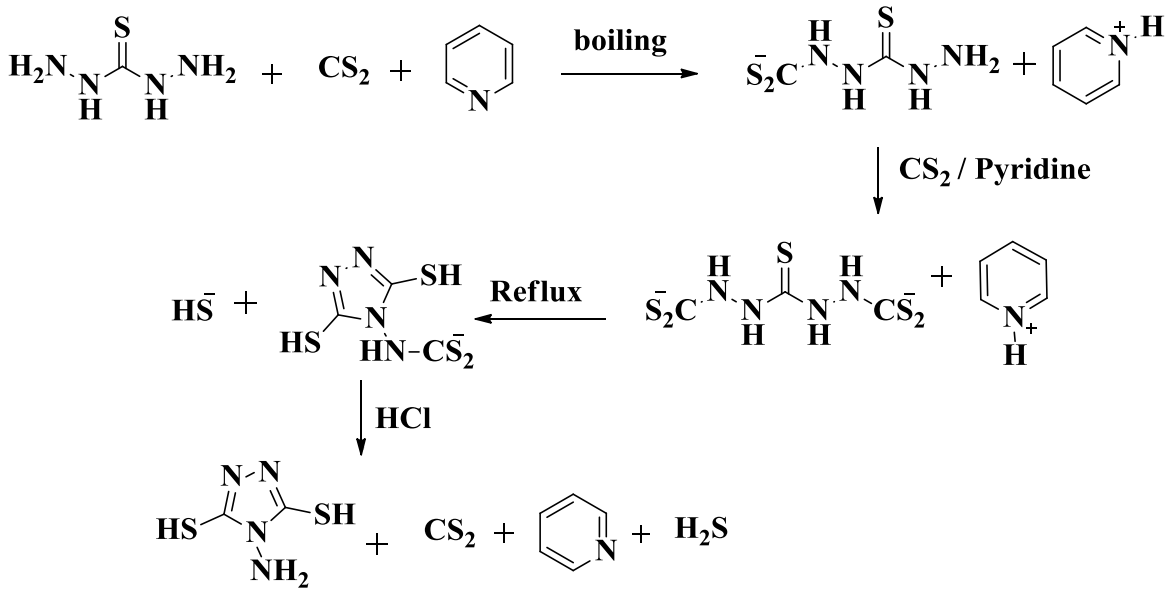
The starting material 4-Amino-4*H*-1,2,4-triazole 3,5-dithiol was prepared according to the procedure detailed in the literature[62]. 5-Methylisoxazole-3-carboxylic acid, substituted phenacyl bromides and different alkyl halides were procured from commercial sources.

4-Amino-4*H*-[1, 2, 4] triazole-3, 5-dithiol:

4-Amino-4*H*-[1,2,4]triazole-3,5-dithiol was synthesized in two steps by using carbon disulphide and hydrazine hydrate. In the first step, thiocarbohydrazide was prepared according to the procedure recommended in the literature[63] by the reaction of hydrazine hydrate with carbon disulphide in presence of water solvent medium.



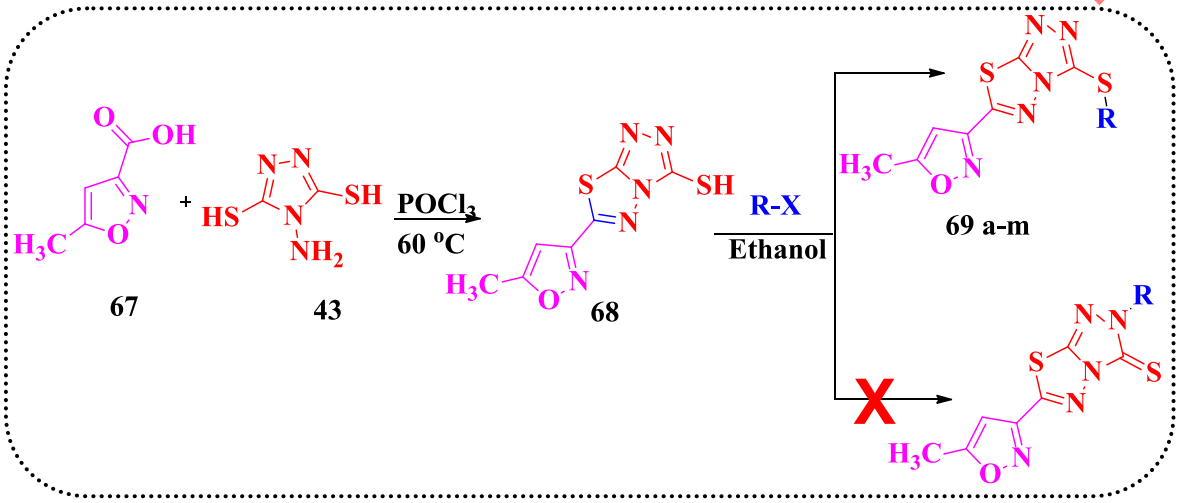
In the second step thiocarbohydrazide was converted in to 4-amino-4*H*-[1,2,4]triazole-3,5-dithiol by using carbon disulphide in presence of pyridine solvent. Reaction of thiocarbohydrazide with carbon disulphide yielded yellow pyridinium salt of amino-4*H*-[1,2,4]triazole-3,5-dithiol. This pyridinium salt was dissolved in hot water and treated with Conc. HCl to yield the 4-amino-4*H*-[1,2,4]triazole-3,5-dithiol (**Scheme.1**).



Scheme1: Preparation of 4-Amino-4*H*-[1, 2, 4] triazole-3, 5-dithiol

PRESENT WORK:

In view of the synthesis and biological importance of the triazolo-thiadiazoles, it was decided to synthesize *s*-triazolo-thiadiazole core unit having isoxazole and thioalkyl substituents. In this chapter a series of 5-methyl-3-(3-(alkyl/phenacylthio)-[1,2,4] triazolo [3,4-b][1,3,4]thiadiazol-6-yl)isoxazoles were designed and synthesized in two steps by the combination of bioactive substructures and screened for their in vitro anti bacterial activity. The first step is cyclo condensation of 5-methyl isoxazole-3-carboxylic acid with 4-amino-4H-1,2,4-triazole-3,5-dithiol in presence of phosphorous oxychloride and the second step is a regioselective alkylation of 6-(5-methylisoxazol-3-yl)-[1,2,4]triazolo[3,4-*b*][1,3,4] thiadiazole-3-thiol with different alkyl / phenacyl bromides in presence of ethanol solvent to report good yield (**Scheme-2**).



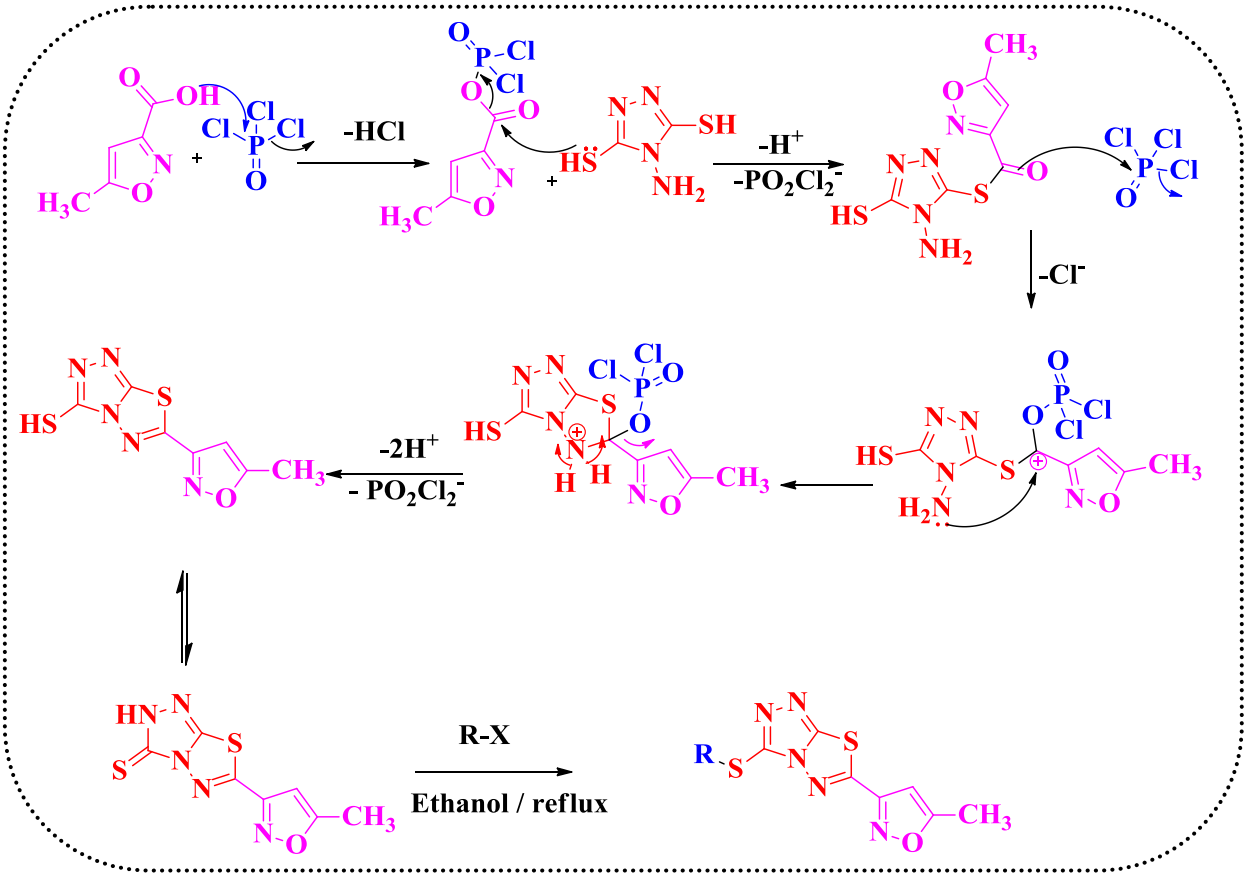
Scheme.2: Synthesis of 5-methyl-3-(3-(alkyl/phenacylthio)-[1,2,4] triazolo [3,4-*b*] [1,3,4] thiadiazol-6-yl)isoxazoles (69 a -m)

Mechanism:

The plausible mechanism for the target compound 5-methyl-3-(3-(alkyl/phenacylthio)-[1,2,4] triazolo [3,4-*b*][1,3,4]thiadiazol-6-yl)isoxazole was proposed as shown in **Scheme-3**. The two nucleophilc amine and thiol functional groups of 4-amino 1,2,4-traizole 3,5-dithiol reacts with 5-methyl isoxazole-3-carboxylic acid in presence of phosphorous oxychloride to yield the intermediate 6-(5-methylisoxazol-3-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole-3-thiol (**68**). The key step for condensation is the formation of phosphate ester followed by the removal of $OPOCl_2$ as anion. The nucleophilic thiol functional group of the intermediate reacts with different alkyl/ phenacyl bromides regioselectively to generate the title product by elimination of hydrogen bromide.

The structures of newly synthesized compounds were confirmed by their analytical and spectral data (IR, ^{1}H NMR, ^{13}C NMR, Mass and Single crystal X-ray). The intermediate 6-(5-methylisoxazol-3-yl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole-3-thiol (**68**) in ^{1}H NMR spectrum showed a peak at 14.37 δ ppm which corresponds to the thiol functional group, which disappeared in the next step confirming the s-alkylation. The alkylation of **68** with different alkyl halides may result in different types of products, such as N-alkylated, S-alkylated or a mixture of both. In the present investigation a mixture of products are not formed (as evidenced by TLC). The formation of S- alkylated products in preference to N- alkylated products can be explained as being due to high nucleophilicity of thiol group. The formation of S-alkylated product was confirmed by

spectral data. All the product yield along with respective melting points are shown in **Table 1.**



Scheme: 3 Plausible mechanism for the synthesis of 6-(5-methylisoxazol-3-yl)-[1,2,4] triazolo[3,4-*b*][1,3,4]thiadiazole-3-thiol

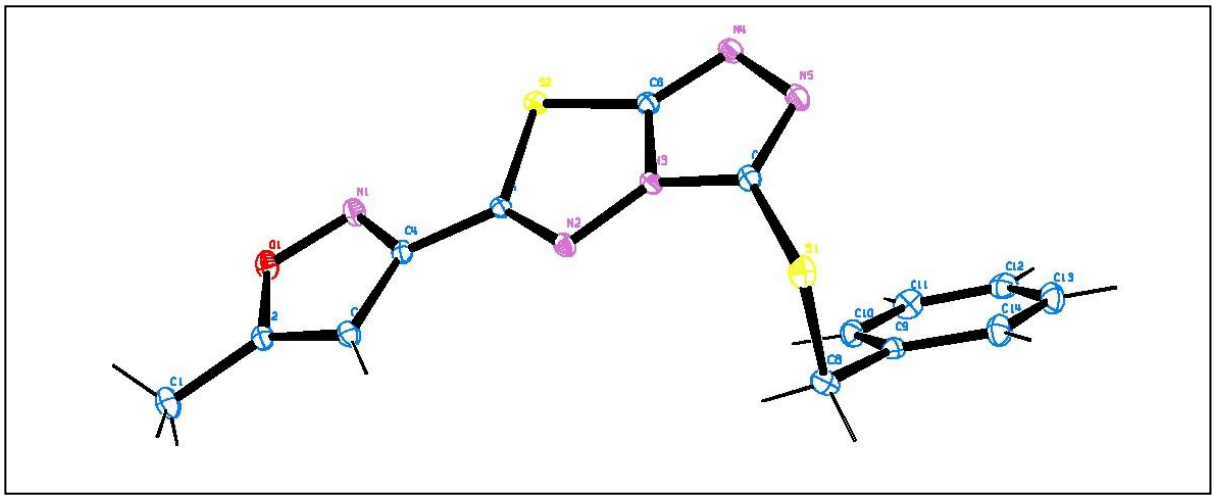
Table 1: Synthesis of 5-methyl-3-(3-(alkyl/phenacylthio)-[1,2,4] triazolo [3,4-*b*] [1,3,4] thiadiazol-6-yl)isoxazoles (69 a -m)

S. No.	Product	R	Yield (%)	M.P. (°C)
1	**69a**	CH_3	79	220-222
2	**69b**	C_2H_5	82	174-176
3	**69c**	CH_2-CH=CH_2	78	131- 133
4	**69d**	*n*-C_4H_9	77	109-111
5	**69e**	-CH_2COOH	75	249-251
6	**69f**	Benzyl	85	222-224
7	**69g**	Ph-CO-CH_2	88	172 -174
8	**69h**	4-Cl-Ph-CO-CH_2	85	184 -186
9	**69i**	4-Br-Ph-CO-CH_2	90	198 - 200
10	**69j**	4-CH_3- Ph-CO-CH_2	80	183 -185
11	**69k**	4-OCH_3- Ph-CO-CH_2	93	180 -182
12	**69l**	4-Ph-Ph-CO-CH_2-	87	197 - 199
13	**69m**	4-NO_2-Ph-CO-CH_2	86	201 – 203

Single Crystal X-ray crystallographic data of 3-(benzylthio)-6-(5-methylisoxazol-3-yl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (69f):

The single crystal X-ray data of the molecule **69f** is shown in **Fig. 6**. The compound has orthorhombic $Pca2_1$ crystal system. The plane of the triazolo-thiadiazole system [r.m.s. deviation = 0.002 Å] forms dihedral angles of 6.33(12) and 42.95(14) ° with the isoxazole (O1/N1/C2–C4) and phenyl (C9–C14) rings, respectively. All the bond lengths and bond angles in the compound are within normal ranges.

The crystal structure is stabilized by face-to-face π-π interactions [Cg1...Cg2(x, -1+y, z) = 3.4707(18) Å and Cg2...Cg1(x, 1+y, z) = 3.4707(18) Å] between the centers Cg1 and Cg2 of the thiadiazole and isoxazole rings, respectively. There are also intermolecular non-bonded O...H contacts of 2.86 Å, which may cause steric hindrance. **Fig. 7** show the molecular packing of the title compound down a- and b-axes, respectively.



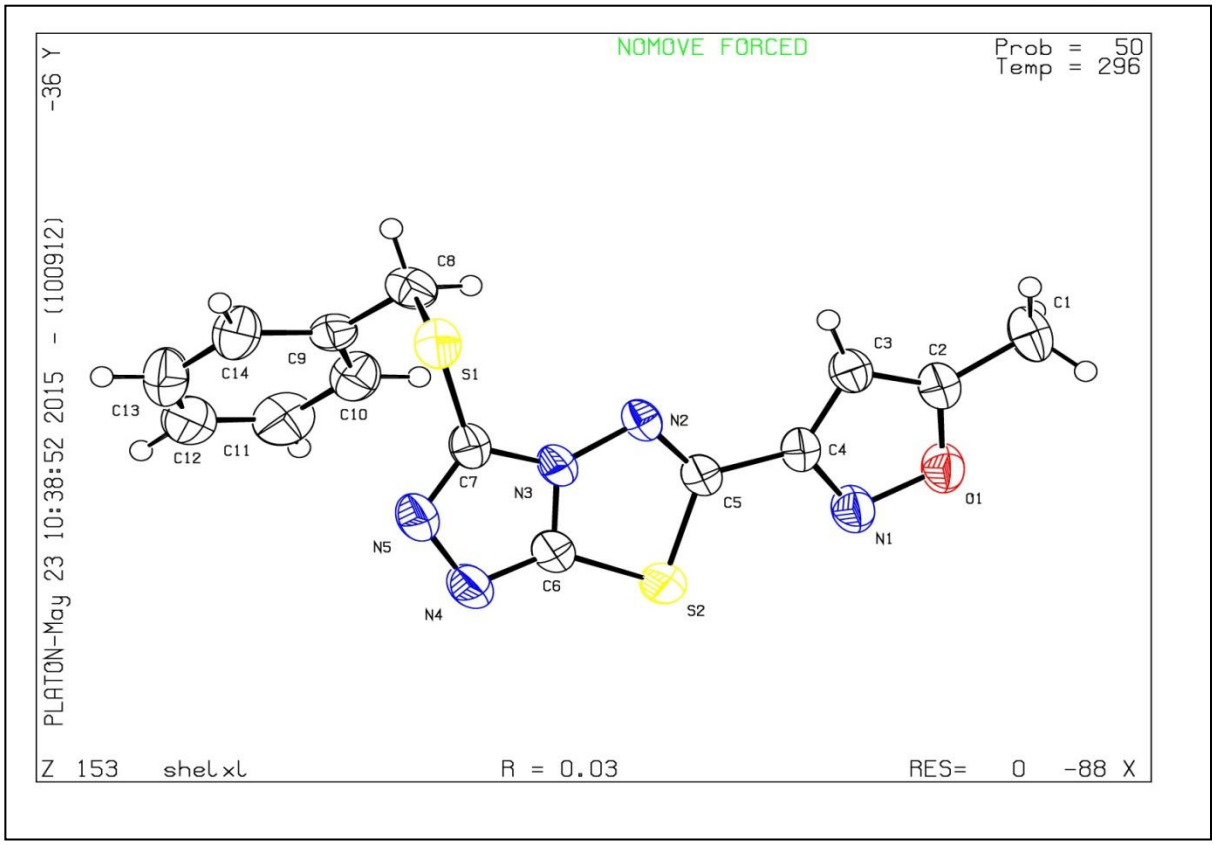


Fig. 6. The title molecule with the atom numbering scheme. Displacement ellipsoids for non-H atoms are drawn at the 30% probability level.

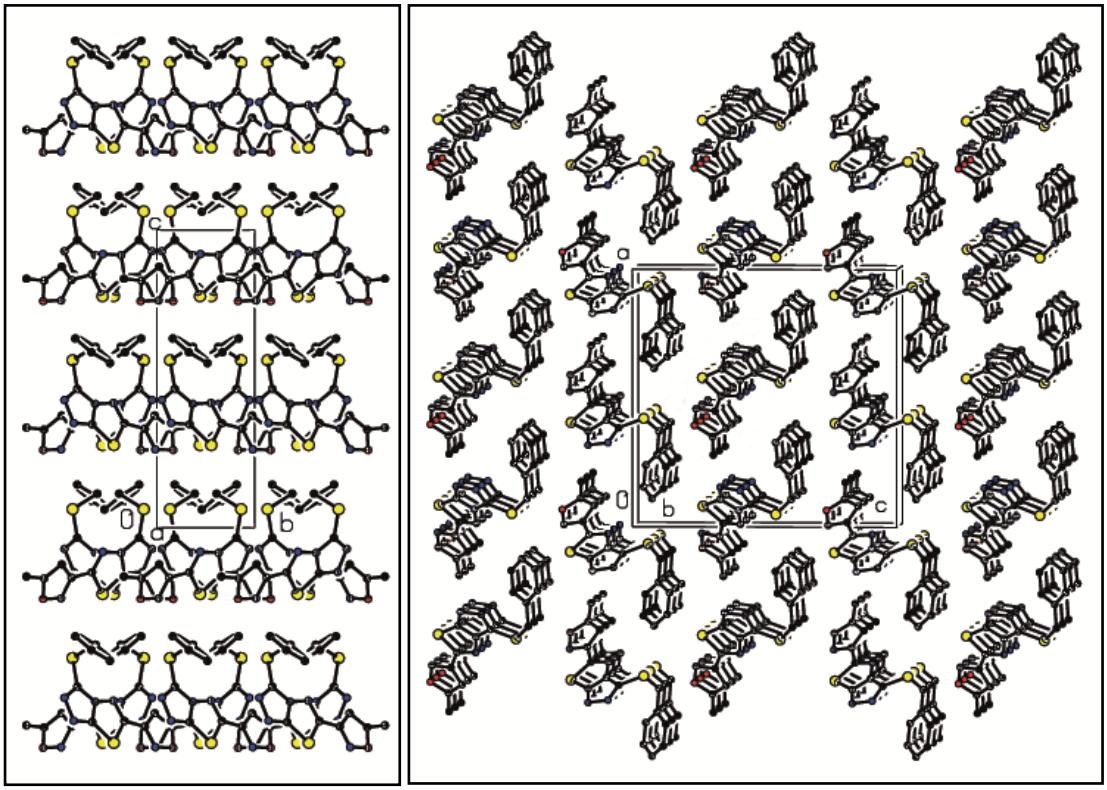


Fig. 7. (A) View of the molecular packing of the title compound down a-axis.
(B) View of the molecular packing of the title compound down b-axis.
All H atoms have been omitted for clarity.

Crystal data

$C_{14}H_{11}N_5OS_2$	D_x = 1.497 Mg m^{-3}
M_r = 329.40	Mo $K\alpha$ radiation
Orthorhombic, $Pca2_1$	Cell parameters from 5610 reflections
a = 16.271 (5) Å	θ = 5.0–55.6°
b = 5.3804 (13) Å	μ = 0.37 mm^{-1}
c = 16.700 (4) Å	T = 296 (2) K
V = 1462.0 (7) Å^3	Block, colorless
Z = 4	0.50 × 0.45 × 0.30 mm

Data collection

Bruker axs kappa apex2 CCD Diffractometer	2905 reflections with $I > 2\sigma(I)$
ω and ϕ scan	R_{int} = 0.027
Absorption correction: multi-scan (based on symmetry-related measurements)	θ_{max} = 28.4°
T_{min} = 0.836, T_{max} = 0.896	h = -20 → 21
10433 measured reflections	k = -6 → 7
3117 independent reflections	l = -16 → 21

Refinement

Refinement on F^2	Calculated weights $w = 1/[\sigma^2(F_o^2) + (0.0411P)^2 + 0.1925P]$ where $P = (F_o^2 + 2F_c^2)/3$
$R[F^2 > 2\sigma(F^2)]$ = 0.031	$(\Delta/\sigma)_{max}$ = 0.001
$wR(F^2)$ = 0.077	$\Delta\rho_{max}$ = 0.18 e Å^{-1}
S = 1.08	$\Delta\rho_{min}$ = -0.20 e Å^{-1}
3117 reflections	Extinction correction: none
200 parameters	Absolute structure: Flack (1983)
H atoms constrained to parent site	Flack parameter: 0.02 (2)

Table 2 Fractional atomic coordinates and isotropic or equivalent isotropic displacement parameters (Å^2)

	x	y	z	Uiso*/Ueq
S1	0.58876 (4)	0.55792 (12)	0.27749 (5)	0.0430 (2)
S2	0.56435 (4)	0.13539 (13)	0.56223 (5)	0.0480 (2)
O1	0.41434 (13)	1.1668 (4)	0.26095 (13)	0.0513 (7)
N1	0.47539 (15)	0.9833 (5)	0.26294 (16)	0.0500 (9)
N2	0.51832 (12)	0.5539 (4)	0.41898 (14)	0.0359 (6)
N3	0.57592 (12)	0.3670 (4)	0.41555 (14)	0.0348 (6)
N4	0.66919 (15)	0.1462 (4)	0.35159 (18)	0.0478 (8)
N5	0.65754 (15)	0.0517 (4)	0.42870 (17)	0.0464 (8)
C1	0.30250 (19)	1.3389 (5)	0.3361 (2)	0.0539 (10)
C2	0.37083 (16)	1.1579 (4)	0.32957 (17)	0.0396 (8)
C3	0.40056 (18)	0.9771 (5)	0.37679 (18)	0.0415 (8)
C4	0.46538 (15)	0.8755 (4)	0.33223 (17)	0.0350 (7)
C5	0.52042 (14)	0.6671 (4)	0.35064 (17)	0.0342 (7)
C6	0.61891 (16)	0.3354 (4)	0.34707 (18)	0.0381 (7)
C7	0.60185 (15)	0.1833 (5)	0.46600 (18)	0.0388 (8)
C8	0.61793 (19)	0.3882 (5)	0.6143 (2)	0.0488 (9)
C9	0.70934 (17)	0.3711 (4)	0.60716 (16)	0.0391 (8)
C10	0.7528 (2)	0.5388 (5)	0.5610 (2)	0.0523 (9)
C11	0.8368 (2)	0.5174 (7)	0.5528 (3)	0.0639 (11)
C12	0.8789 (2)	0.3276 (7)	0.5891 (2)	0.0603 (11)
C13	0.8372 (2)	0.1623 (6)	0.6353 (3)	0.0628 (11)
C14	0.7532 (2)	0.1828 (6)	0.6448 (2)	0.0556 (11)
H1A	0.324	1.4987	0.3507	0.0810*
H1B	0.2644	1.284	0.3763	0.0810*
H1C	0.2748	1.3511	0.2855	0.0810*
H3	0.3824	0.9302	0.4274	0.0500*
H8A	0.5998	0.5459	0.5924	0.0590*
H8B	0.603	0.3847	0.6705	0.0590*
H10	0.7251	0.6672	0.5353	0.0630*
H11	0.8654	0.6333	0.5222	0.0770*
H12	0.9353	0.3121	0.5822	0.0730*
H13	0.8654	0.0344	0.6607	0.0750*
H14	0.7256	0.06900	0.67690	0.0670*

Table 3: Atomic displacement parameters (Å^2)

	*U*11	*U*22	*U*33	*U*12	*U*13	*U*23
S1	0.0443 (3)	0.0412 (3)	0.0434 (4)	0.0021 (3)	0.0010 (3)	-0.0021 (3)
S2	0.0399 (3)	0.0460 (3)	0.0582 (4)	-0.0038 (3)	0.0005 (4)	0.0125 (3)
O1	0.0461 (11)	0.0513 (11)	0.0566 (15)	0.0077 (8)	-0.0012 (9)	0.0149 (10)
N1	0.0457 (13)	0.0496 (13)	0.0546 (18)	0.0096 (10)	0.0017 (11)	0.0107 (12)
N2	0.0286 (10)	0.0314 (10)	0.0476 (13)	0.0009 (7)	-0.0017 (9)	-0.0029 (9)
N3	0.0276 (9)	0.0299 (10)	0.0470 (13)	0.0001 (7)	-0.0024 (9)	-0.0055 (9)
N4	0.0436 (13)	0.0434 (12)	0.0564 (15)	0.0092 (10)	-0.0002 (12)	-0.0080 (11)
N5	0.0415 (12)	0.0367 (12)	0.0611 (16)	0.0062 (9)	-0.0050 (12)	-0.0029 (11)
C1	0.0434 (15)	0.0392 (14)	0.079 (2)	0.0064 (11)	-0.0022 (16)	0.0071 (15)
C2	0.0334 (12)	0.0312 (12)	0.0543 (17)	-0.0041 (9)	-0.0055 (11)	-0.0018 (12)
C3	0.0426 (14)	0.0345 (12)	0.0474 (17)	0.0023 (10)	-0.0002 (12)	0.0018 (12)
C4	0.0322 (11)	0.0280 (10)	0.0449 (15)	-0.0040 (8)	-0.0077 (10)	-0.0004 (10)
C5	0.0303 (11)	0.0272 (11)	0.0450 (15)	-0.0033 (8)	-0.0048 (11)	-0.0058 (10)
C6	0.0332 (12)	0.0334 (11)	0.0478 (15)	-0.0024 (9)	-0.0036 (12)	-0.0068 (11)
C7	0.0312 (12)	0.0342 (12)	0.0509 (16)	-0.0024 (9)	-0.0054 (11)	-0.0011 (11)
C8	0.0520 (17)	0.0423 (14)	0.0522 (18)	0.0040 (12)	0.0088 (14)	-0.0035 (13)
C9	0.0451 (14)	0.0368 (12)	0.0354 (14)	-0.0027 (10)	0.0008 (11)	-0.0051 (11)
C10	0.0605 (17)	0.0420 (14)	0.0544 (17)	-0.0026 (12)	-0.0032 (17)	0.0069 (14)
C11	0.059 (2)	0.0688 (19)	0.064 (2)	-0.0199 (16)	0.0027 (18)	0.0050 (18)
C12	0.0470 (17)	0.072 (2)	0.062 (2)	-0.0033 (15)	-0.0081 (15)	-0.0165 (18)
C13	0.057 (2)	0.0575 (19)	0.074 (2)	0.0046 (15)	-0.0232 (18)	0.0005 (17)
C14	0.0602 (19)	0.0467 (16)	0.060 (2)	-0.0040 (13)	-0.0082 (15)	0.0126 (15)

Table 4: Geometric parameters (Å, º)

S1—C5	1.753 (3)	C8—C9	1.495 (4)
S1—C6	1.739 (3)	C9—C10	1.382 (4)
S2—C7	1.738 (3)	C9—C14	1.390 (4)
S2—C8	1.835 (3)	C10—C11	1.378 (5)
O1—N1	1.401 (3)	C11—C12	1.371 (5)
O1—C2	1.348 (4)	C12—C13	1.359 (5)
N1—C4	1.305 (4)	C13—C14	1.380 (5)
N2—N3	1.376 (3)	C1—H1A	0.96
N2—C5	1.294 (4)	C1—H1B	0.96
N3—C6	1.351 (4)	C1—H1C	0.96
N3—C7	1.366 (4)	C3—H3	0.93
N4—N5	1.397 (4)	C8—H8A	0.97
N4—C6	1.308 (3)	C8—H8B	0.97
N5—C7	1.308 (4)	C10—H10	0.93
C1—C2	1.482 (4)	C11—H11	0.93
C2—C3	1.342 (4)	C12—H12	0.93

C3—C4	1.402 (4)	C13—H13	0.93
C4—C5	1.468 (3)	C14—H14	0.93
C5—S1—C6	86.79 (13)	C10—C9—C14	117.8 (3)
C7—S2—C8	99.29 (14)	C9—C10—C11	120.6 (3)
N1—O1—C2	109.1 (2)	C10—C11—C12	120.9 (3)
O1—N1—C4	104.2 (2)	C11—C12—C13	119.3 (3)
N3—N2—C5	106.8 (2)	C12—C13—C14	120.5 (3)
N2—N3—C6	118.7 (2)	C9—C14—C13	121.0 (3)
N2—N3—C7	135.6 (2)	C2—C1—H1A	110.00
C6—N3—C7	105.7 (2)	C2—C1—H1B	109
N5—N4—C6	104.6 (2)	C2—C1—H1C	109
N4—N5—C7	109.6 (2)	H1A—C1—H1B	109
O1—C2—C1	115.7 (2)	H1A—C1—H1C	109
O1—C2—C3	109.6 (2)	H1B—C1—H1C	109
C1—C2—C3	134.7 (3)	C2—C3—H3	128
C2—C3—C4	104.0 (3)	C4—C3—H3	128
N1—C4—C3	113.0 (2)	S2—C8—H8A	109
N1—C4—C5	116.7 (2) S	2—C8—H8B	109
C3—C4—C5	130.2 (3)	C9—C8—H8A	109
S1—C5—N2	118.27 (17)	C9—C8—H8B	109
S1—C5—C4	119.8 (2)	H8A—C8—H8B	108
N2—C5—C4	121.9 (2)	C9—C10—H10	120
S1—C6—N3	109.44 (17)	C11—C10—H10	120
S1—C6—N4	138.7 (2)	C10—C11—H11	120
N3—C6—N4	111.9 (3)	C12—C11—H11	119
S2—C7—N3	124.7 (2)	C11—C12—H12	120
S2—C7—N5	127.1 (2)	C13—C12—H12	120
N3—C7—N5	108.2 (3)	C12—C13—H13	120
S2—C8—C9	112.91 (19)	C14—C13—H13	120
C8—C9—C10	120.9 (2)	C9—C14—H14	120
C8—C9—C14	121.3 (2)	C13—C14—H14	119
C5—S1—C6—N4	178.0 (3)	N5—N4—C6—N3	−0.3 (3)
C6—S1—C5—N2	−0.6 (2)	N5—N4—C6—S1	−178.5 (2)
C6—S1—C5—C4	−178.2 (2)	C6—N4—N5—C7	0.5 (3)
C5—S1—C6—N3	−0.21 (18)	N4—N5—C7—N3	−0.4 (3)
C8—S2—C7—N5	104.8 (3)	N4—N5—C7—S2	178.5 (2)
C8—S2—C7—N3	−76.5 (2)	C1—C2—C3—C4	178.8 (3)
C7—S2—C8—C9	−58.4 (2)	O1—C2—C3—C4	−0.1 (3)
N1—O1—C2—C1	−179.0 (2)	C2—C3—C4—C5	−178.1 (3)
C2—O1—N1—C4	−0.2 (3)	C2—C3—C4—N1	−0.1 (3)
N1—O1—C2—C3	0.1 (3)	C3—C4—C5—N2	−5.1 (4)

O1—N1—C4—C3	0.1 (3)	N1—C4—C5—S1	−5.5 (3)
O1—N1—C4—C5	178.5 (2)	N1—C4—C5—N2	176.9 (2)
C5—N2—N3—C6	−1.3 (3)	C3—C4—C5—S1	172.5 (2)
N3—N2—C5—C4	178.7 (2)	S2—C8—C9—C10	109.4 (3)
N3—N2—C5—S1	1.1 (3)	S2—C8—C9—C14	−68.8 (3)
C5—N2—N3—C7	−178.4 (3)	C8—C9—C10—C11	−178.1 (3)
N2—N3—C7—S2	−1.4 (4)	C14—C9—C10—C11	0.2 (5)
N2—N3—C6—N4	−177.8 (2)	C8—C9—C14—C13	177.4 (3)
C6—N3—C7—N5	0.2 (3)	C10—C9—C14—C13	−1.0 (5)
C7—N3—C6—N4	0.1 (3)	C9—C10—C11—C12	1.0 (6)
C7—N3—C6—S1	178.79 (17)	C10—C11—C12—C13	−1.6 (6)
N2—N3—C6—S1	0.9 (3)	C11—C12—C13—C14	0.9 (6)
N2—N3—C7—N5	177.6 (3)	C12—C13—C14—C9	0.4 (6)
C6—N3—C7—S2	−178.7 (2)		

Table 5: Atomic coordinates ($x10^4$) and equivalent isotropic displacement parameters (A^2 x 10^3) for isox U(eq) are defined as one third of the trace of the orthogonalized Uij tensor.

	x	y	z	U(eq)
C(1)	-1975(2)	-3392(4)	5390(2)	53(1)
C(2)	-1292(1)	-1580(3)	5326(1)	39(1)
C(3)	-995(1)	232(4)	5799(2)	41(1)
C(4)	-347(1)	1244(3)	5352(1)	35(1)
C(5)	204(1)	3329(3)	5536(1)	34(1)
C(6)	1189(1)	6645(3)	5500(1)	38(1)
C(7)	1019(1)	8165(4)	6690(1)	38(1)
C(8)	1180(2)	6120(4)	8174(2)	48(1)
C(9)	2093(1)	6286(3)	8101(1)	39(1)
C(10)	2529(2)	4611(4)	7640(2)	52(1)
C(11)	3367(2)	4824(6)	7558(2)	64(1)
C(12)	3789(2)	6726(5)	7921(2)	60(1)
C(13)	3371(2)	8377(5)	8382(2)	63(1)
C(14)	2533(2)	8173(4)	8478(2)	55(1)
N(1)	-246(1)	166(4)	4659(1)	50(1)
N(2)	183(1)	4461(3)	6220(1)	35(1)
N(3)	759(1)	6331(3)	6186(1)	35(1)
N(4)	1692(1)	8539(3)	5545(2)	47(1)
N(5)	1576(1)	9482(3)	6317(1)	46(1)
O(1)	-857(1)	-1669(3)	4639(1)	51(1)
S(1)	644(1)	8646(1)	7652(1)	47(1)
S(2)	888(1)	4421(1)	4805(1)	42(1)

Table 6: Bond lengths [A] and angles [deg] for isox.

Bond	Bond length (A°)	Bond	Bond Angle (°)
C(1)-C(2)	1.482(3)	C(2)-C(1)-H(1A)	109.5
C(1)-H(1A)	0.9600	C(2)-C(1)-H(1B)	109.5
C(1)-H(1B)	0.9600	H(1A)-C(1)-H(1B)	109.5
C(1)-H(1C)	0.9600	C(2)-C(1)-H(1C)	109.5
C(2)-C(3)	1.345(3)	H(1A)-C(1)-H(1C)	109.5
C(2)-O(1)	1.349(3)	H(1B)-C(1)-H(1C)	109.5
C(3)-C(4)	1.402(3)	C(3)-C(2)-O(1)	109.64(19)
C(3)-H(3)	0.9300	C(3)-C(2)-C(1)	134.8(2)
C(4)-N(1)	1.304(3)	O(1)-C(2)-C(1)	115.60(19)
C(4)-C(5)	1.469(3)	C(2)-C(3)-C(4)	103.9(2)
C(5)-N(2)	1.296(3)	C(2)-C(3)-H(3)	128.1
C(5)-S(2)	1.752(2)	C(4)-C(3)-H(3)	128.1
C(6)-N(4)	1.310(3)	N(1)-C(4)-C(3)	113.20(19)
C(6)-N(3)	1.352(3)	N(1)-C(4)-C(5)	116.6(2)
C(6)-S(2)	1.738(2)	C(3)-C(4)-C(5)	130.2(2)
C(7)-N(5)	1.308(3)	N(2)-C(5)-C(4)	121.80(18)
C(7)-N(3)	1.364(3)	N(2)-C(5)-S(2)	118.29(14)
C(7)-S(1)	1.738(2)	C(4)-C(5)-S(2)	119.87(16)
C(8)-C(9)	1.494(3)	N(4)-C(6)-N(3)	111.9(2)
C(8)-S(1)	1.835(2)	N(4)-C(6)-S(2)	138.6(2)
C(8)-H(8A)	0.9700	N(3)-C(6)-S(2)	109.50(14)
C(8)-H(8B)	0.9700	N(5)-C(7)-N(3)	108.2(2)
C(9)-C(10)	1.381(3)	N(5)-C(7)-S(1)	127.05(17)
C(9)-C(14)	1.392(3)	N(3)-C(7)-S(1)	124.73(16)
C(10)-C(11)	1.376(4)	C(9)-C(8)-S(1)	112.97(15)
C(10)-H(10)	0.9300	C(9)-C(8)-H(8A)	109
C(11)-C(12)	1.373(4)	S(1)-C(8)-H(8A)	109
C(11)-H(11)	0.9300	C(9)-C(8)-H(8B)	109
C(12)-C(13)	1.358(4)	S(1)-C(8)-H(8B)	109
C(12)-H(12)	0.9300	H(8A)-C(8)-H(8B)	107.8
C(13)-C(14)	1.378(4)	C(10)-C(9)-C(14)	117.6(2)
C(13)-H(13)	0.9300	C(10)-C(9)-C(8)	121.1(2)
C(14)-H(14)	0.9300	C(14)-C(9)-C(8)	121.2(2)
N(1)-O(1)	1.401(2)	C(11)-C(10)-C(9)	120.7(2)
N(2)-N(3)	1.376(2)	C(11)-C(10)-H(10)	119.7
N(4)-N(5)	1.398(3)	C(9)-C(10)-H(10)	119.7
		C(12)-C(11)-C(10)	120.9(3)
		C(12)-C(11)-H(11)	119.6
		C(10)-C(11)-H(11)	119.6
		C(13)-C(12)-C(11)	119.3(3)

C(13)-C(12)-H(12)	120.4
C(11)-C(12)-H(12)	120.4
C(12)-C(13)-C(14)	120.6(2)
C(12)-C(13)-H(13)	119.7
C(14)-C(13)-H(13)	119.7
C(13)-C(14)-C(9)	121.0(2)
C(13)-C(14)-H(14)	119.5
C(9)-C(14)-H(14)	119.5
C(4)-N(1)-O(1)	104.22(18)
C(5)-N(2)-N(3)	106.77(17)
C(6)-N(3)-C(7)	105.80(17)
C(6)-N(3)-N(2)	118.62(18)
C(7)-N(3)-N(2)	135.54(19)
C(6)-N(4)-N(5)	104.48(19)
C(7)-N(5)-N(4)	109.67(18)
C(2)-O(1)-N(1)	109.07(16)
C(7)-S(1)-C(8)	99.29(11)
C(6)-S(2)-C(5)	86.80(10)

Antibacterial activity:

The preliminary antimicrobial activity of the synthesized compounds against various bacterial strains, including *E.coli* TG1, *S. aureus* ATCC 6538P, *B.subtilis* ATCC 6633 and *P. Aeruginosa* PAO1, was tested using the broth micro dilution method[64,65] and the percentage of OD600 of bacterial strains are shown in **Fig.8**.

The optical density data (OD600) of anti bacterial activity reveals that the tested compounds (**69 a-m**) exhibited moderate inhibition effect against *S. aureus* ATCC 6538P, *B. subtilis* ATCC 6633 and *P. aeruginosa* PAO1 bacterial strains and good inhibition effect against *E. coli* TG1 at 100 µM concentration. Among the screened compounds, compounds having hydrophobic alkyl chains like methyl, ethyl, allyl, *n*-butyl and acetic acid are showing inhibition effect against *S. aureus* ATCC 6538P, *B. subtilis* ATCC 6633 and *P. aeruginosa* PAO1 and the compounds having substituted phenacyl groups were not exhibiting any bacterial activity.

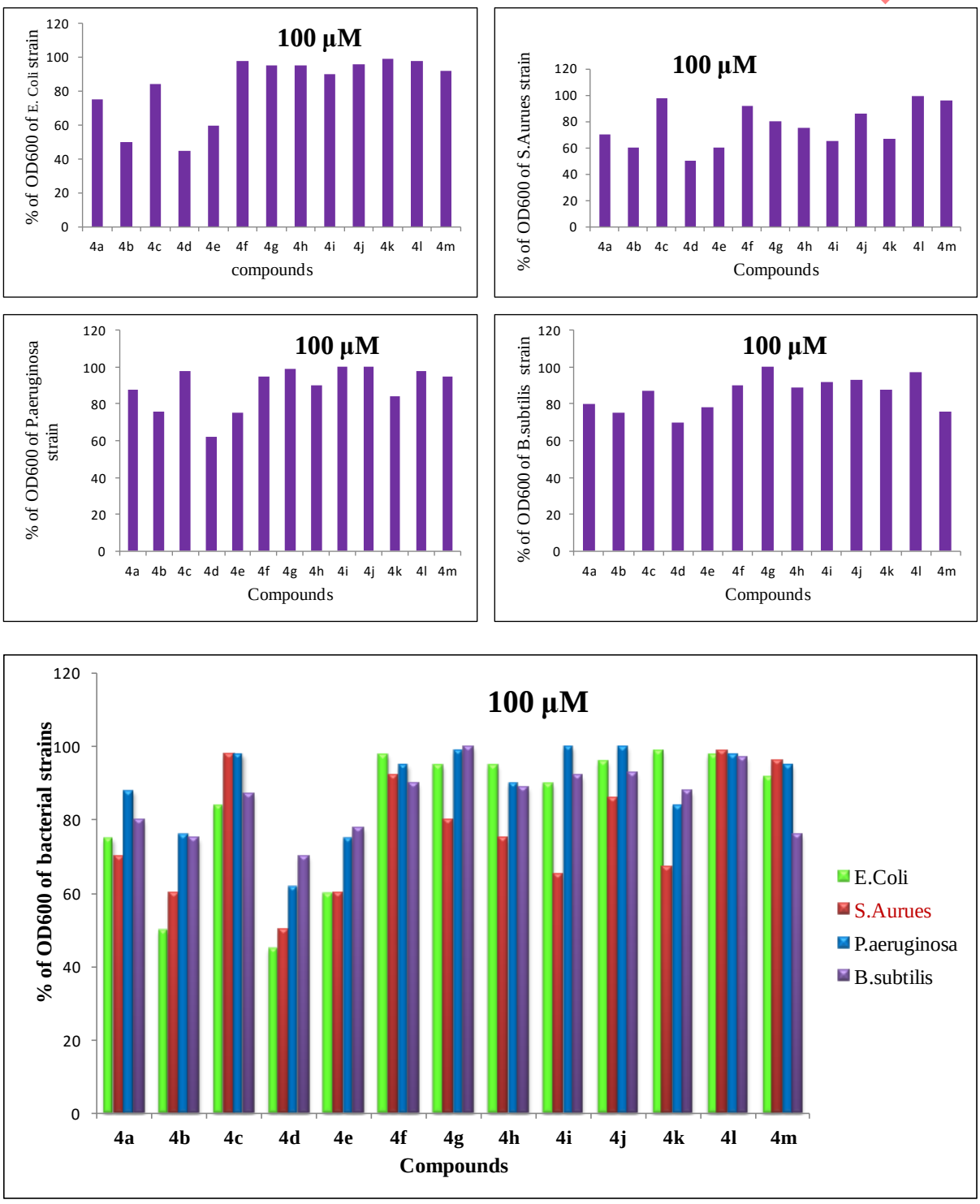


Fig. 8 The percentage of OD_{600} of bacterial strains.

In summary we have synthesized a series of 5-methyl-3-(3-(alkyl/phenacylthio)-[1,2,4] triazolo [3,4-*b*][1,3,4]thiadiazol-6-yl)isoxazoles and studied their antibacterial activity. All the synthesized compounds structures were confirmed by analytical and

spectral data. From the antibacterial activity results, it was clear that the compounds exhibited moderate activity against *S.aurues* at 100 µg/mL concentrations and weak activity against *E.coli*, *P.aeruginosa*, *B.subtilis* bacterial strains.

EXPERIMENTAL:

Synthesis of 6-(5-methylisoxazol-3-yl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole-3-thiol (68):

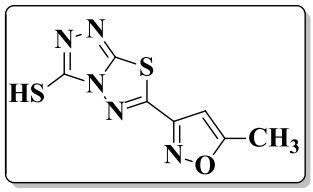
A mixture of 5-methylisoxazole-3-carboxylic acid (1.27 g, 10 mmol) and 4-amino-4*H*-[1,2,4]triazole-3,5-dithiol (1.48 g, 10 mmol) was taken in a round bottom flask containing phosphorous oxy chloride (15 mL). The mixture was stirred at room temperature for 30 min and then heated at 60 °C for about five hours. After completion of the reaction (monitored with TLC), the reaction mixture was cooled to room temperature and gradually poured into crushed ice with stirring. Then it was neutralized by using 5% sodium bicarbonate solution. The white solid which separated was then filtered, washed thoroughly with cold water and re crystallized from methanol. Yield: (1.864 g, 78%), mp: 292 – 294 ^{o}C.

Synthesis 6-(5-Methyl-Isoxzol-3yl)-3-alkyl/ phenacyl sulfanyl-[1,2,4]triazolo-[3,4 *b*][1,3,4] thiadiazole: (69 a-m):

6-(5-Methylisoxazol-3-yl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole-3-thiol **68** (0.239 g, 1 mmol) was dissolved in ethanol (5 mL) then alkyl halide (1.1 mmol) was added and refluxed for 6 h. After completion of the reaction (monitored with TLC) the reaction mixture was allowed to cool to room temperature. The solid which separated was then filtered. The compound so obtained was re crystallized from methanol.

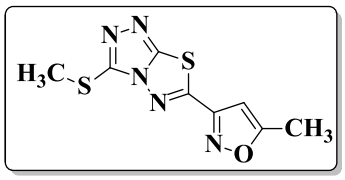
PHYSICAL AND SPECTRAL DATA:

6-(5-Methylisoxazol-3-yl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole-3-thiol (68):



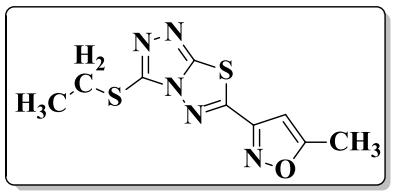
Colorless solid; **FTIR (KBr, ν_{max}, cm^{-1})**: 1593 (C=N); 1263 (C-O-N); 2880 (S-C); ^{1}H **NMR (DMSO-d_6, δ ppm):** 2.56 (s, 3H, CH_3), 7.03 (s, 1H, Ar-H), 14.37 (s, 1H, S-H).

6-(5-Methylisoxazol-3-yl)-3-(methylthio)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (69a):



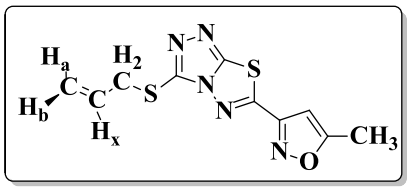
White solid; **FTIR (KBr, ν_{max}, cm^{-1})**: 1599 (C=N); 1255 (C-O-N); 1191 (S-C); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 2.57 (s, 3H, CH_3), 2.81 (s, 3H, -S-CH_3), 6.64 (s, 1H, Ar-H); **^{13}C NMR (100 MHz, DMSO-d_6, δ ppm):** 11.87, 14.76, 100.05, 143.31, 153.71, 155.27, 156.71, 173.05, **MS (ESI m/z):** 275.95 $[M+Na]^+$; 253.95 $[M+H]^+$; *Anal.* calcd for $C_8H_7N_5OS_2$: C, 37.93, H, 2.79, N, 27.65, S, 25.32. Found: C, 37.87, H, 2.83, N, 27.70, S, 25.27.

3-(Ethylthio)-6-(5-methylisoxazol-3-yl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (69b):



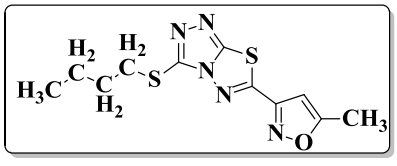
Yellow solid; **FTIR (KBr, ν_{max}, cm^{-1})**: 1598 (C=N); 1262 (C-O-N); 1153 (S-C); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 1.34 (t, *J* = 7.6 Hz, 3H, CH_3); 2.55 (s, 3H, CH_3); 3.24 (q, *J*= 7.2 Hz, 2H, CH_2); 7.02 (s, 1H, Ar-H); **^{13}C NMR (100 MHz, DMSO-d_6, δ ppm):** 11.88; 15.07; 27.12; 100.05; 142.18; 153.82; 155.28; 156.79; 173.05; **MS (ESI m/z):** 289.95 $[M+ Na]^+$; 267.93 $[M+H]^+$; *Anal*. Calcd for $C_9H_9N_5OS_2$: C, 40.44; H, 3.39; N, 26.20; S, 23.99. Found: C, 40.49; H, 3.34; N, 26.26; S, 23.93.

3-(Allylthio)-6-(5-methylisoxazol-3-yl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (69c):



White solid; **FTIR (KBr, ν_{max}, cm^{-1})**: 1591(C=N); 1254 (C-O-N); 1151 (S-C); **^{1}H NMR (400 MHz, DMSO-d_6; δ ppm)**: 2.57 (s, 3H, CH_3); 3.92 (d, *J*= 7.2 Hz, 2H, S-CH_2); 5.13 (d, *J* H_x,H_A = 10 Hz, 1H, H_A); 5.25 (d, *J* H_x,H_B = 17.2 Hz, 1H, H_B); 5.94-6.03 (m, 1H, H_X); 6.66 (s, 1H, Ar-H); **MS (ESI m/z):** 280 $[M+H]^+$; *Anal*. Calcd for $C_{10}H_9N_5OS_2$: C, 43.00; H, 3.25; N, 25.07; S, 22.96. Found: C, 42.89; H, 3.30; N, 25.15; S, 22.89.

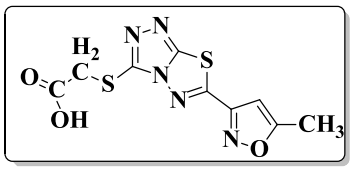
3-(Butylthio)-6-(5-methylisoxazol-3-yl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (69d):



White solid; **FTIR (KBr, ν_{max}, cm^{-1})**: 1592 (C=N); 1259 (C-O-N); 1187 (S-C); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm)**: 0.88 (t, *J* = 7.4 Hz, 3H, -CH_3);

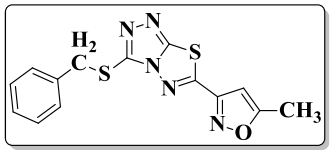
1.37-1.46 (m, 2H, -CH_2); 1.63-1.70 (m, 2H, -CH_2); 2.55 (s, 3H, Ar-CH_3); 3.23 (t, *J* = 7.2 Hz, 2H, S-CH_2); 7.01 (s, 1H, Ar-H); **^{13}C NMR (100 MHz, DMSO-*d*$_6$, δ ppm):** 11.86; 13.27; 20.88; 31.20; 32.22; 99.99; 142.34; 153.74; 155.26; 156.75; 173.05; **MS (ESI m/z):** 318.02 $[M+Na]^+$; 296.01 $[M+H]^+$; *Anal*. Calcd for $C_{11}H_{13}N_5OS_2$: C, 44.73; H, 4.44; N, 23.71; S, 21.71. Found: C, 44.67; H, 4.38; N, 23.78; S, 21.66.

2-(6-(5-Methylisoxazol-3-yl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-ylthio)acetic acid (69e):



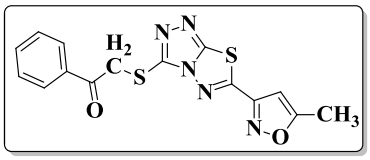
White solid; **FTIR (KBr, ν_{max}, cm^{-1})**: 3406 (-OH); 1697 (-C=O); 1606 (C=N); 1245 (C-O-N); 1196 (S-C); **^{1}H NMR (400 MHz, DMSO-*d*$_6$, δ ppm):** 2.55 (s, 3H,-CH_3); 3.85 (s, 2H, -S-CH_2); 7.02 (s, 1H, Ar-H); 12.46 (s, 1H, -OH); **MS (ESI m/z):** 319.88 $[M + Na]^+$; 297.89 $[M+H]^+$; .*Anal*. Calcd for $C_9H_7N_5O_3S_2$: C, 36.36; H, 2.37; N, 23.56; S, 21.57. Found: C, 36.33; H, 2.31; N, 23.52; S, 21.63.

3-(Benzylthio)-6-(5-methylisoxazol-3-yl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (69f):



Yellow solid; **FTIR (KBr, ν_{max}, cm^{-1})**: 1684 (-C=O); 1609 (C=N); 1235 (C-O-N) ; 1186 (S-C); **^{1}H NMR (400 MHz, DMSO-*d*$_6$, δ ppm):** 2.56 (s, 3H,-CH_3); 4.47 (s, 2H, -S-CH_2); 6.99 (s, 1H, Ar-H); 7.22-7.33 (m, 5H, Ar-H); **^{13}C NMR (100 MHz, DMSO-*d*$_6$, δ ppm):** 12.38; 37.75; 99.73; 127.89; 128.64; 129.08; 135.97; 143.17; 154.14; 155.35; 156.80; 172.23; **LC-MS (ESI m/z):** 330 $[M+H]^+$; *Anal*. Calcd for $C_{14}H_{11}N_5OS_2$: C, 51.05; H, 3.37; N, 21.26; S, 19.47. Found: C, 51.16; H, 3.29; N, 21.31; S, 19.41.

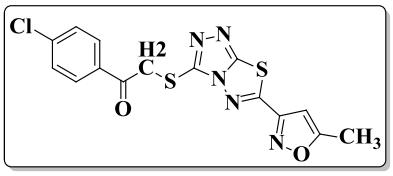
2-(6-(5-Methylisoxazol-3-yl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-ylthio)-1-phenylethanone (69g)



Yellow solid; **FTIR (KBr, ν_{max}, cm^{-1})**: 1680 (C=O); 1602 (C=N); 1257 (C-O-N); 1194 (S-C); **^{1}H NMR (400 MHz, DMSO-*d*$_6$, δ ppm):** 2.57 (s, 3H, -CH_3); 4.97 (s, 2H, -S-CH_2); 6.62 (s, 1H, Ar-H); 7.48-7.52 (m, 2H, Ar-H); 7.60-7.65 (m, 1H, Ar-H); 8.02 (t, *J* = 7.2 Hz, 2H, Ar-H); **^{13}C NMR (100 MHz, DMSO-*d*$_6$, δ ppm):** 11.84; 40.17; 99.90; 128.33; 128.65; 133.65; 134.92; 141.67;

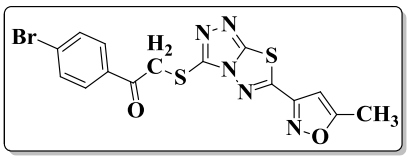
153.72; 155.11; 156.75; 172.82; 192.90; **MS (ESI m/z):** 379.93 $[M+ Na]^+$; 357.94 $[M+H]^+$; *Anal*. Calcd for $C_{15}H_{11}N_5O_2S_2$: C, 50.41; H, 3.10; N, 19.59; S, 17.94. Found: C, 50.52; H, 3.05; N, 19.52; S, 17.99.

2-(6-(5-Methylisoxazol-3-yl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-ylthio)-1-(4-chlorophenyl)ethanone (69h):



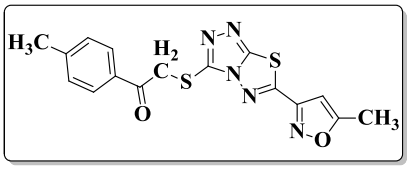
Yellow solid; **FTIR (KBr, ν_{max}, cm^{-1})**: 1694 (-C=O); 1592 (C=N); 1261 (C-O-N); 1200 (S-C); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 2.55 (s, 3H,-CH_3); 4.99 (s, 2H,S-CH_2); 6.90 (s, 1H, Ar-H); 7.63 (d, *J* = 8.4Hz, 2H, Ar-H,); 8.02 (d, *J* = 8.4Hz, 2H, Ar-H); **MS (ESI m/z):** 413.90 $[M+Na]^+$; 391.93 $[M+H]^+$; *Anal*. Calcd for $C_{15}H_{10}ClN_5O_2S_2$: C, 45.98; H, 2.57; N, 17.87; S, 16.37. Found: C, 45.90; H, 2.64; N, 17.82; S, 16.31.

2-(6-(5-Methylisoxazol-3-yl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-ylthio)-1-(4-bromophenyl)ethanone (69i):



Yellow solid; **FTIR (KBr, ν_{max}, cm^{-1})**: 1679 (-C=O); 1586 (C=N); 1257 (C-O-N); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 2.55 (s, 3H, -CH_3); 4.98 (s, 2H, S-CH_2); 6.89 (s, 1H, Ar-H); 7.77 (d, *J* = 8.4Hz, 2H, Ar-H); 7.94 (d, *J* = 8.4Hz, 2H, Ar-H); **^{13}C NMR (100 MHz, DMSO-d_6, δ ppm):** 11.92; 40.10; 100.01; 127.99; 130.45; 131.88; 134.03; 141.53; 153.92; 155.23; 156.91; 173.08; 192.52; **MS (ESI m/z):** 459.86 $[M+Na]^+$; 435.86 $[M]^+$; *Anal*. Calcd for $C_{15}H_{10}BrN_5O_2S_2$: C, 41.29; H, 2.31; N, 16.05; S, 14.70. Found: C, 41.35; H, 2.26; N, 15.97; S, 14.76.

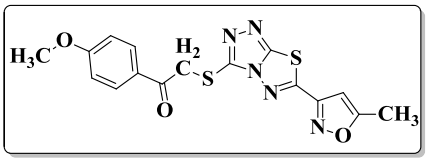
2-(6-(5-Methylisoxazol-3-yl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-ylthio)-1-*p*-tolyl ethanone (69j):



Yellow solid; **FTIR (KBr, ν_{max}, cm^{-1})**: 1675 (-C=O); 1601 (C=N); 1255 (C-O-N); 1190 (S-C); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 2.42 (s, 3H, -CH_3); 2.57 (s, 3H,-CH_3); 4.94 (s, 2H, S-CH_2); 6.62 (s, 1H, Ar-H); 7.28 (d, *J* = 8Hz, 2H, Ar-H,); 7.91 (d, *J* = 8Hz, 2H, Ar-H); **^{13}C NMR (100MHz,**

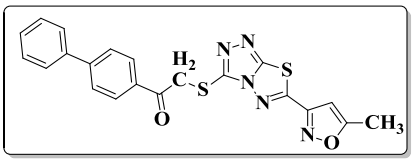
DMSO-d_6, δ ppm): 11.86; 21.12; 40.20; 99.98; 128.52; 129.29; 132.48; 141.70; 144.34; 153.81; 155.21; 156.80; 173.02; 192.63; **MS (ESI m/z):** 394.00 $[M+ Na]^+$; 371.99 $[M+H]^+$; *Anal*. Calcd for $C_{16}H_{13}N_5O_2S_2$: C, 51.74; H, 3.53; N, 18.85; S, 17.27. Found: C, 51.66; H, 3.47; N, 18.91; S, 17.34.

2-(6-(5-Methylisoxazol-3-yl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-ylthio)-1-(4-methoxyphenyl)ethanone (69k):



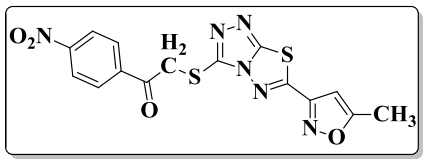
Yellow solid; **FTIR (KBr, ν_{max}, cm^{-1})**: 1673 (-C=O); 1599 (C=N); 1258 (C-O-N); 1180 (S-C); **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm):** 2.56 (s, 3H, $-CH_3$); 3.88 (s, 3H, $-OCH_3$); 4.93 (s, 2H, $S-CH_2$); 6.62 (s, 1H, Ar-H); 6.95 (d, *J* = 7.2Hz, 2H, Ar-H); 7.20 (d, *J* = 7.2 Hz, 2H, Ar-H); **MS (ESI m/z):** 388 $[M+H]^+$; *Anal*. Calcd for $C_{16}H_{13}N_5O_3S_2$: C, 49.60; H, 3.38; N, 18.08; S, 16.55. Found: C, 49.72; H, 3.31; N, 18.12; S, 16.60.

1-Biphenyl-4-yl-2-[6-(5-methyl-isoxazol-3-yl)[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-ylsulfanyl]-ethanone (69l):



White solid; **FTIR (KBr, ν_{max}, cm^{-1})**: 1672 (-C=O); 1601 (C=N); 1260 (C-O-N); 1194 (S-C); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 2.51 (s, 3H, $-CH_3$); 5.03 (s, 2H, $-CH_2$); 6.88 (s, 1H, Ar-H); 7.44 (t, *J* = 7.4Hz, 1H, Ar-H,); 7.52 (t, *J* = 7.6Hz, 2H, Ar-H); 7.76 (d, *J* = 8Hz, 2H, Ar-H); 7.85 (d, *J* = 7.6Hz, 2H, Ar-H); 8.09 (d, *J* = 8 Hz, 2H, Ar-H) **^{13}C NMR (100 MHz, DMSO-d_6, δ ppm):** 11.87; 40.15; 100.01; 126.92; 126.97; 128.50; 129.07; 129.20; 133.78; 138.64; 141.65; 145.10; 153.89; 155.23; 156.86; 173.04; 192.75; **MS (ESI m/z):** 433.98 $[M+H]^+$; *Anal*. Calcd for $C_{21}H_{15}N_5O_2S_2$: C, 58.18; H, 3.49; N, 16.16; S, 14.79. Found: C, 58.24; H, 3.54; N, 16.02; S, 14.84.

2-(6-(5-Methylisoxazol-3-yl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-ylthio)-1-(4-nitrophenyl) ethanone (69m):



Red solid; **FTIR (KBr, ν_{max}, cm^{-1})**: 1699 (-C=O); 1601 (C=N); 1257 (C-O-N); 1198 (S-C); **^{1}H NMR (400MHz, DMSO-d_6, δ ppm):** 2.55 (s, 3H, $-CH_3$);

5.06 (s, 2H, -CH_2); 6.93 (s, 1H, Ar-H); 8.24 (d, *J* = 8Hz, 2H, Ar-H); 8.36 (d, *J* = 7.6Hz, 2H, Ar-H); **MS (ESI m/z):** 424.94 $[M+ Na]^+$; 402.95 $[M+H]^+$; *Anal*. Calcd for $C_{15}H_{10}N_6O_4S_2$: C, 44.77; H, 2.50; N, 20.88; S, 15.94. Found: C, 44.68; H, 2.56; N, 20.92; S, 15.90.

Antibacterial activity experiment:

The antibacterial activity of synthesized molecules against various gram positive and gram negative bacterial strains were tested using broth microdilution method. An overnight culture of bacterial species was 200 times diluted in LB medium and incubated at 37 °C to get the $OD_{600\ nm}$ to 0.3. Again the cultures were diluted to an $OD_{600\ nm}$ of 0.01. Each well of a 96-well microtiter plate was filled with 15 µL culture and a compound concentration of 100 µM (final DMSO concentration of 2.5%), corresponding to approximately 1 X 10^4 cells/well in a final volume of 150 µL. The microtiter plates were incubated at 37 °C for 20 h. Finally the $OD_{600\ nm}$ of the cultures was measured and the data was normalized against the control (0 µM compound, 2.5% DMSO).

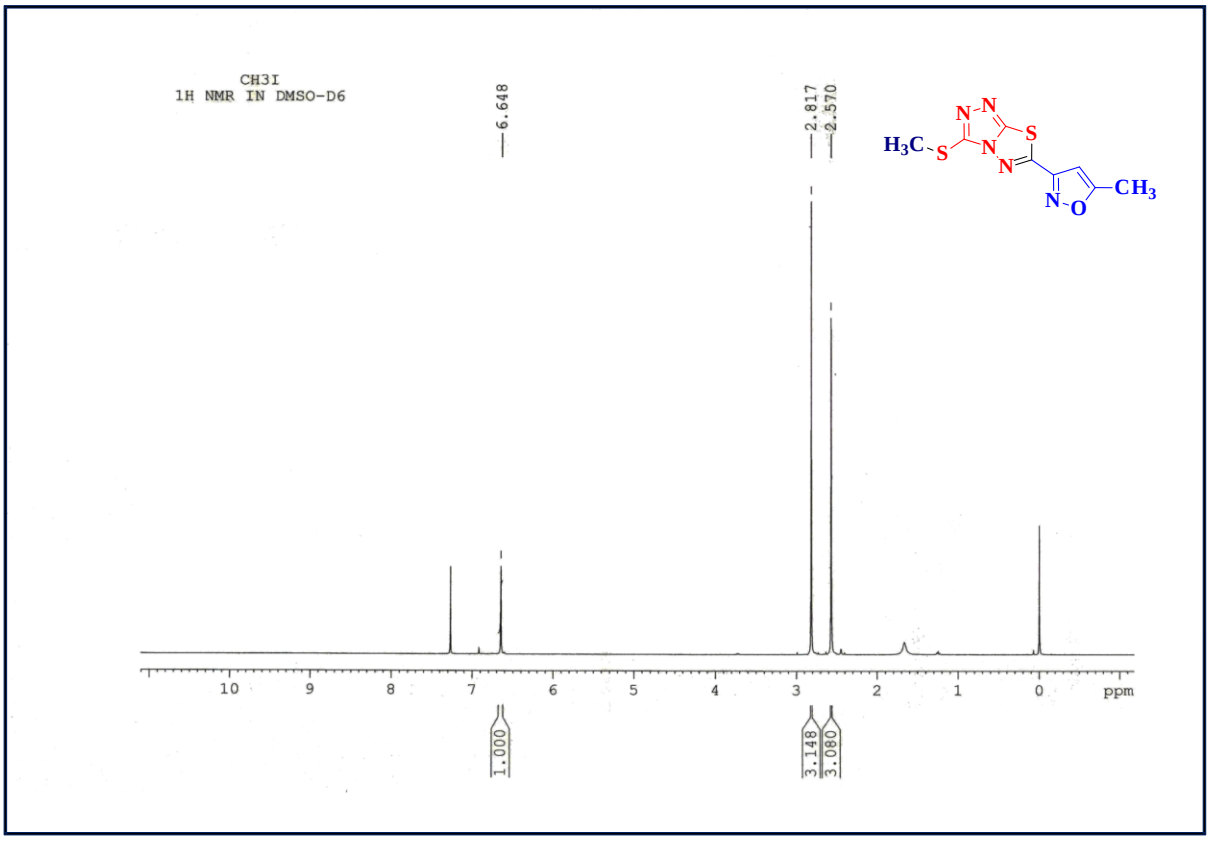


Fig: ^{1}H NMR Spectrum of compound 69a

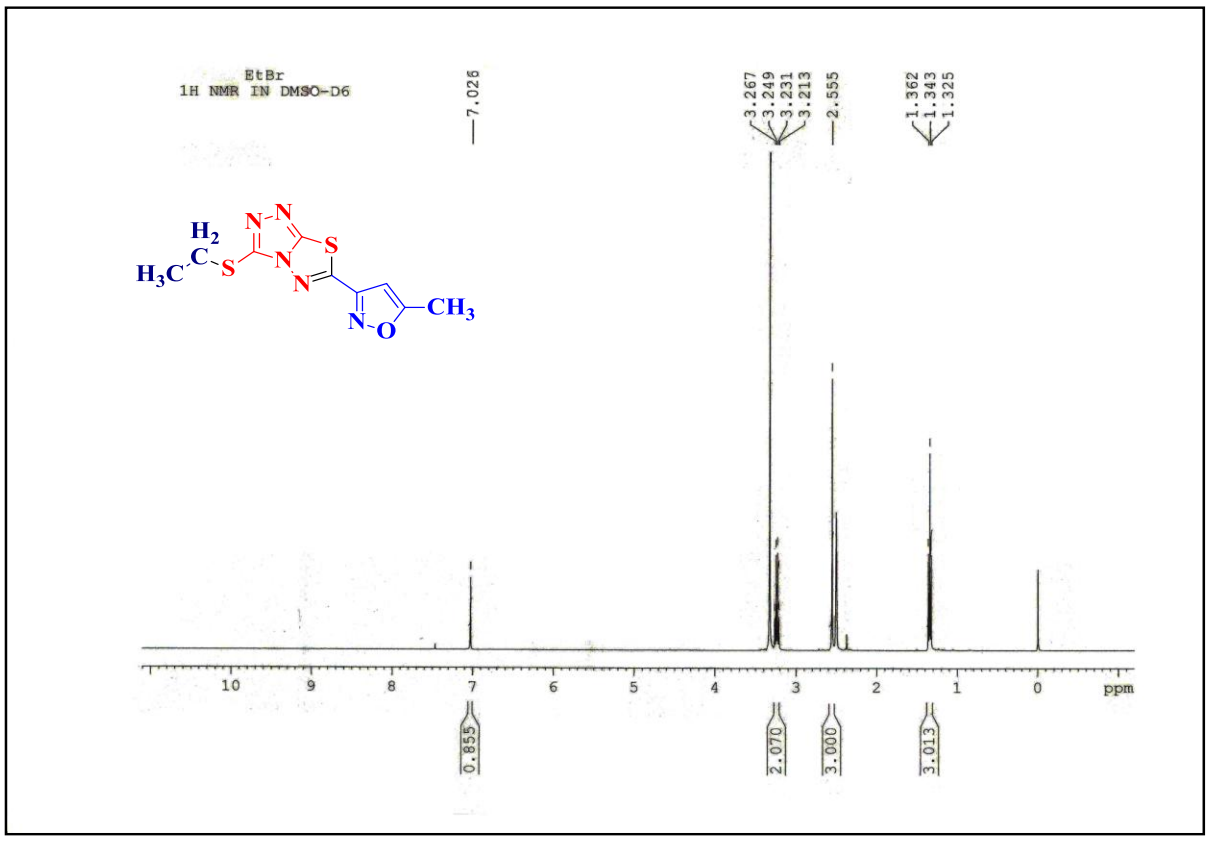


Fig: ^{1}H NMR Spectrum of compound 69b

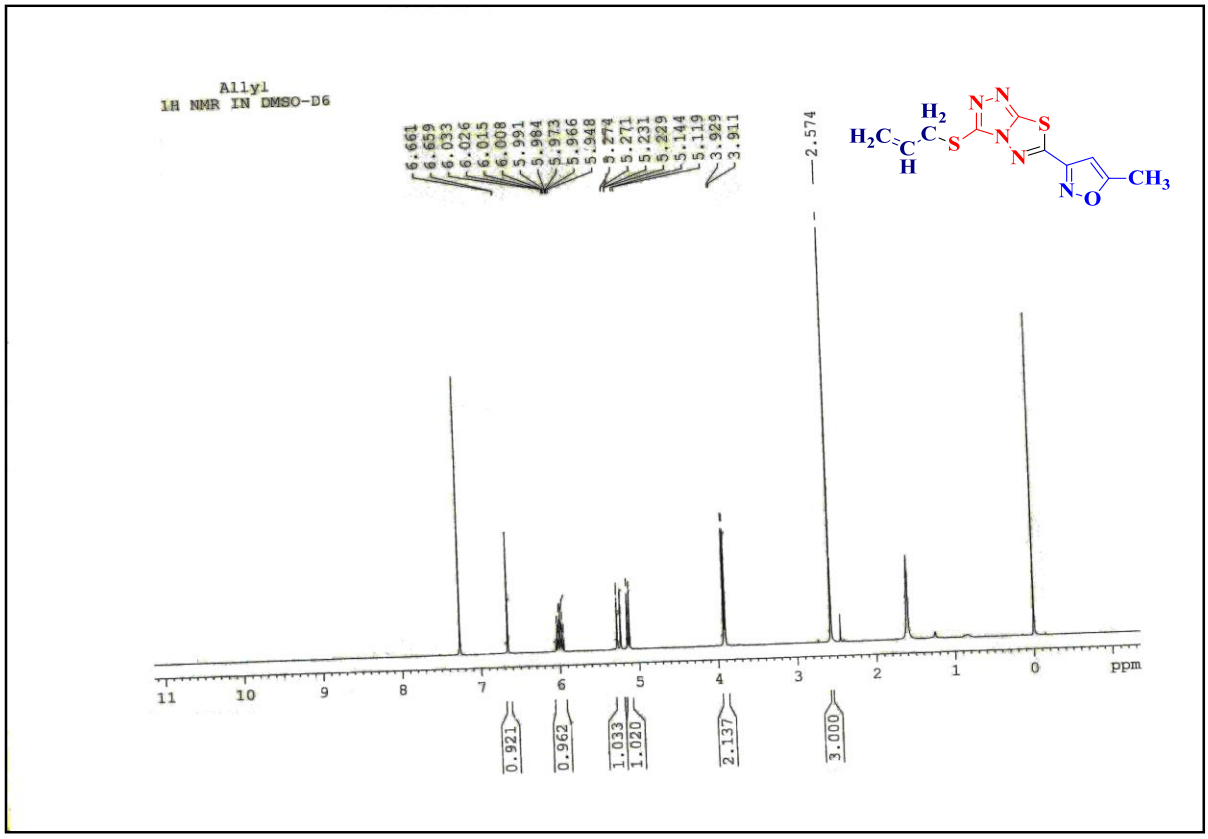


Fig: ^{1}H NMR Spectrum of compound 69c

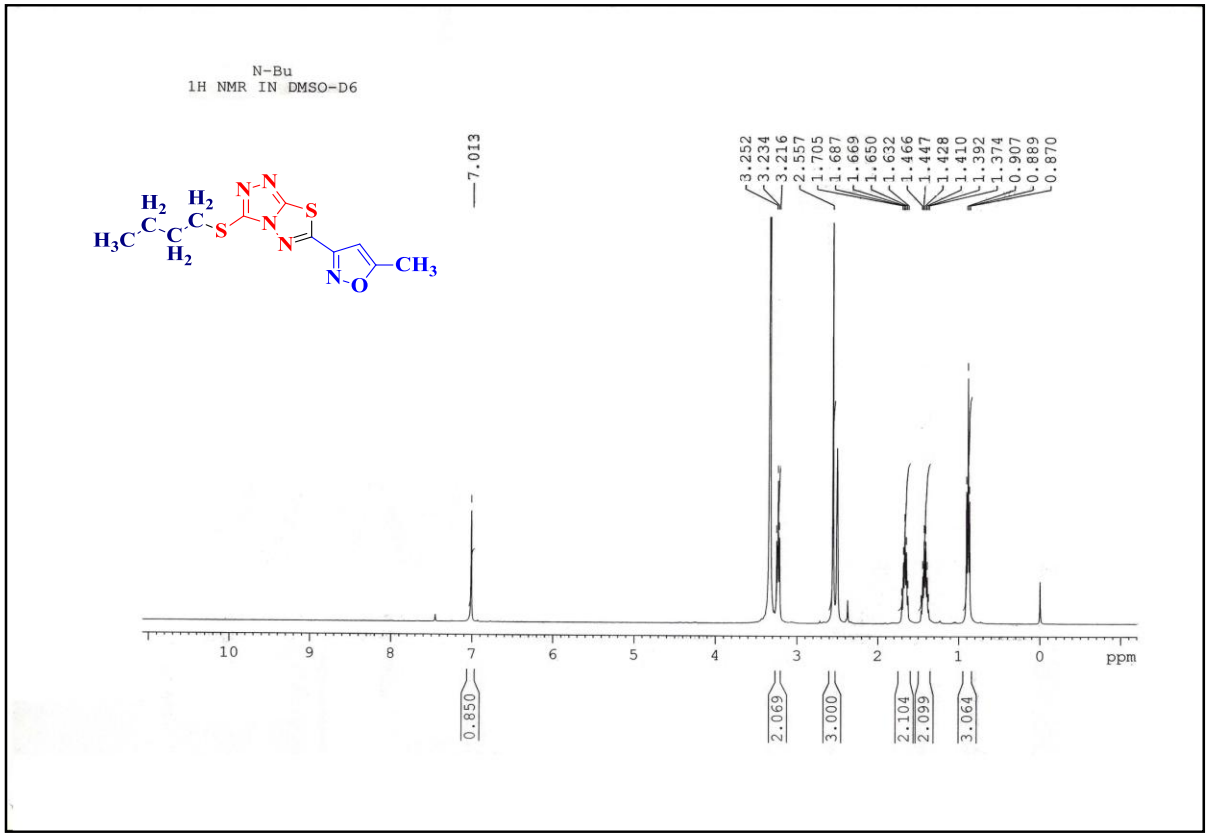


Fig: ^{1}H NMR Spectrum of compound 69d

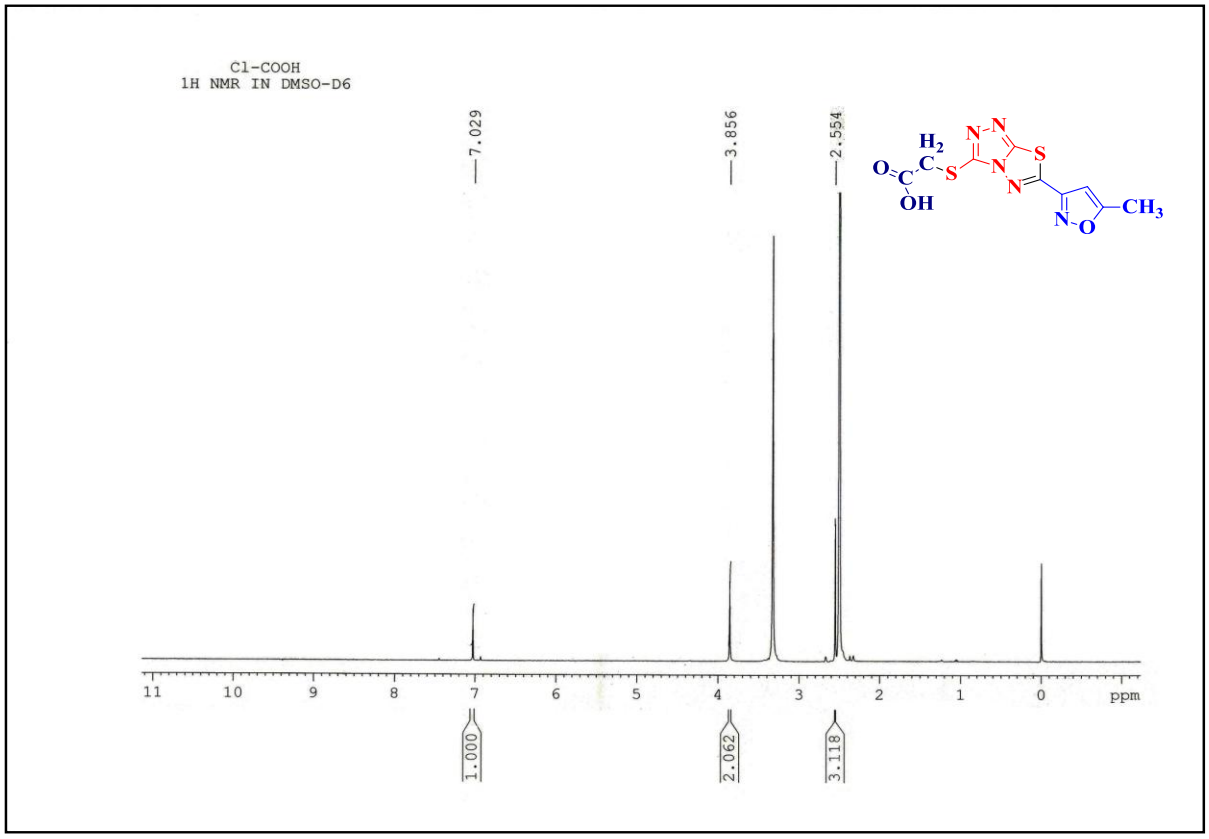


Fig: ^{1}H NMR Spectrum of compound 69e

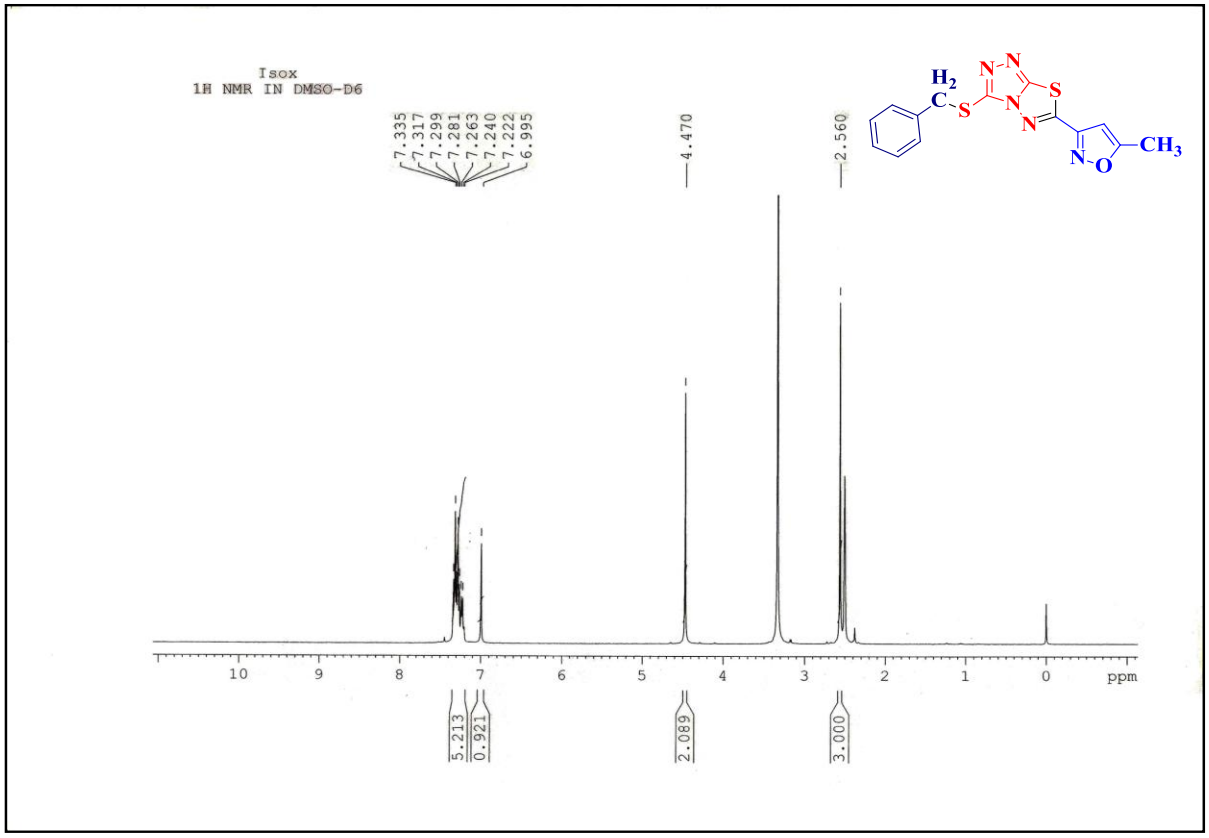


Fig: ^{1}H NMR Spectrum of compound 69f

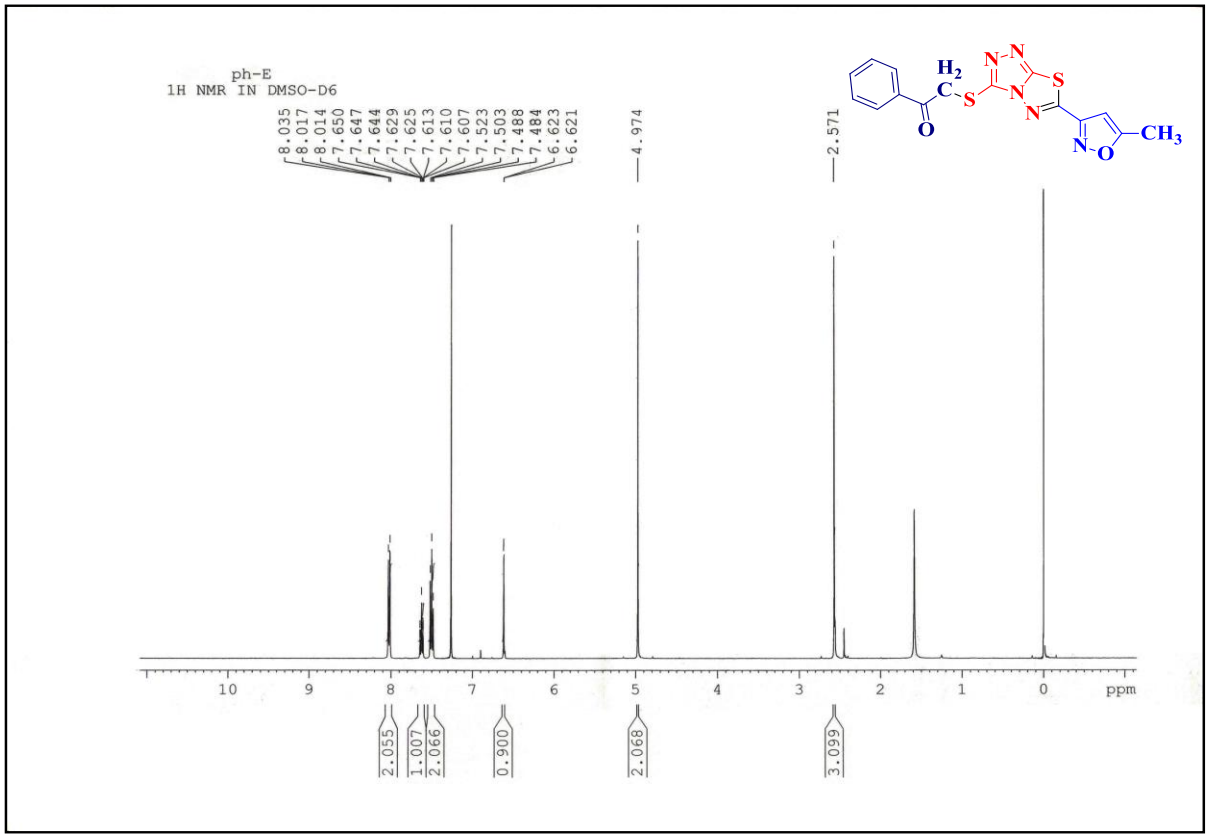


Fig: ^{1}H NMR Spectrum of compound 69g

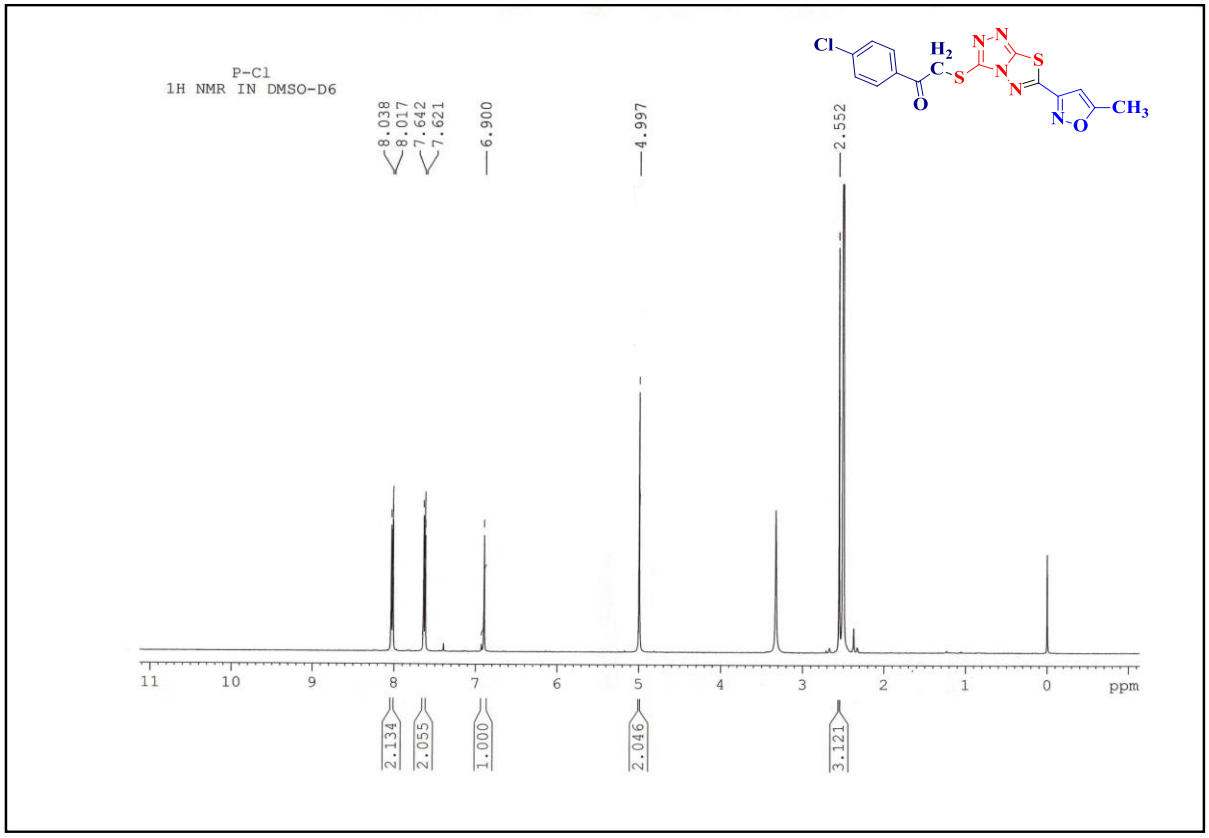


Fig: ^{1}H NMR Spectrum of compound 69h

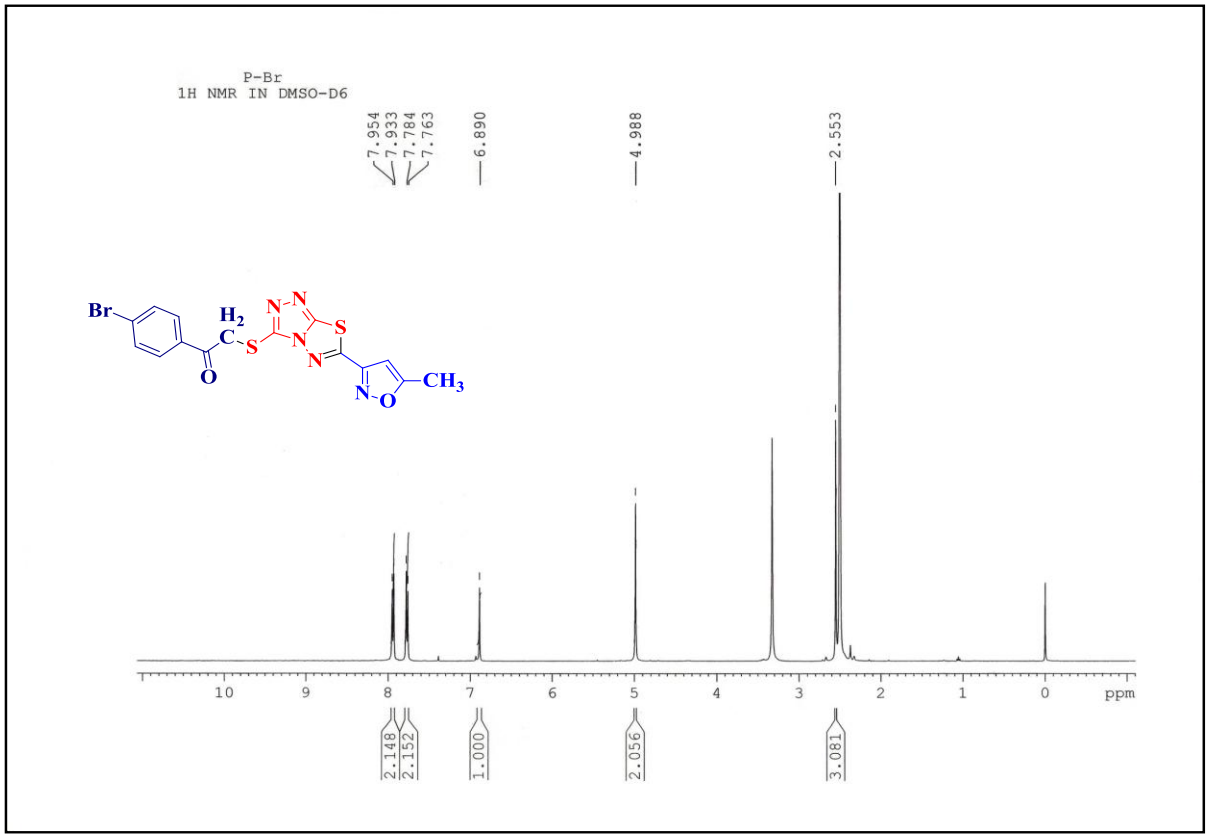


Fig: ^{1}H NMR Spectrum of compound 69i

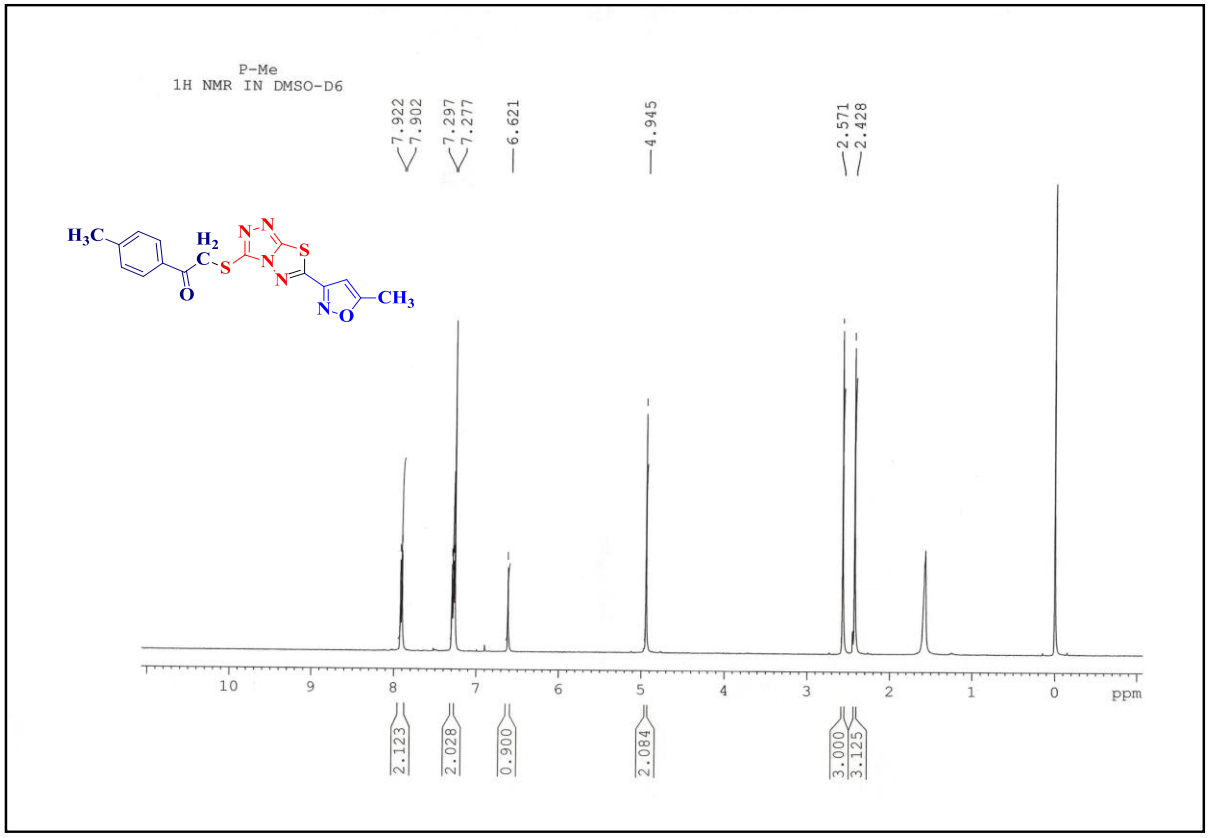


Fig: ^{1}H NMR Spectrum of compound 69j

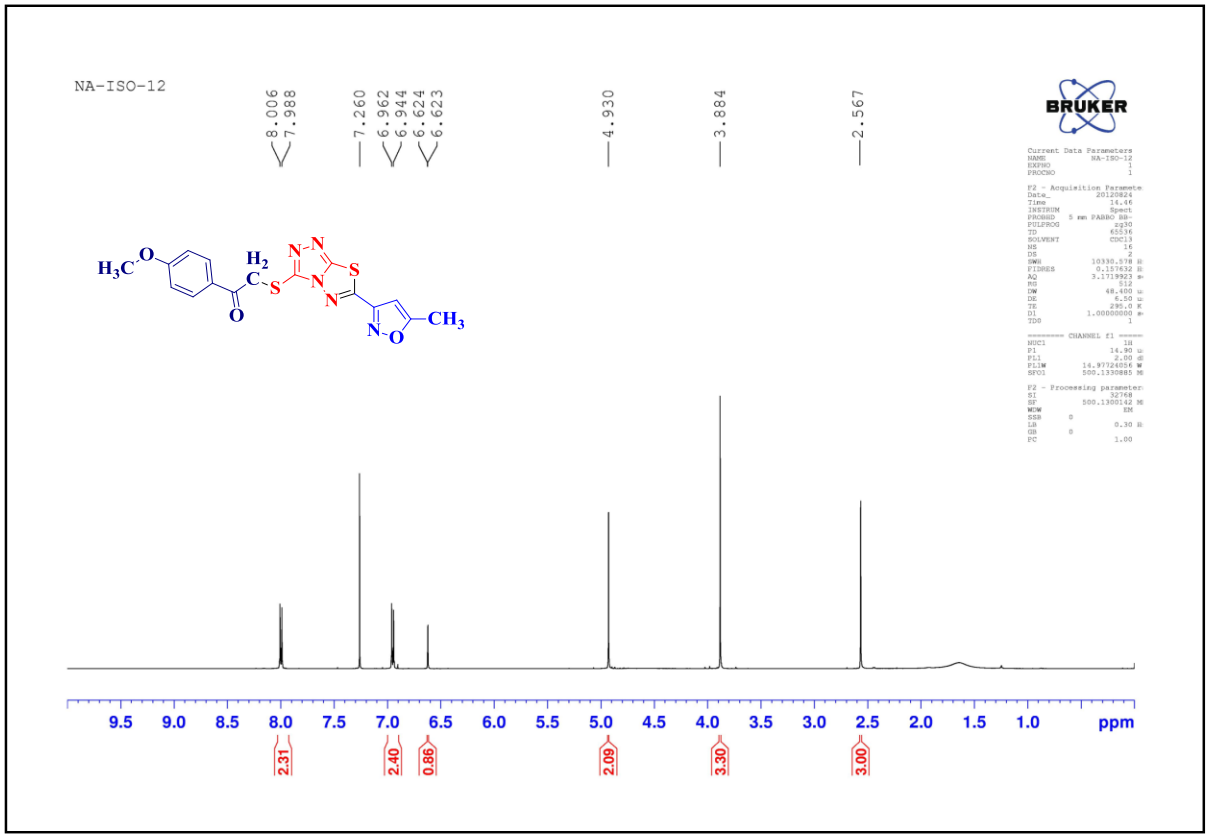


Fig: ^{1}H NMR Spectrum of compound 69k

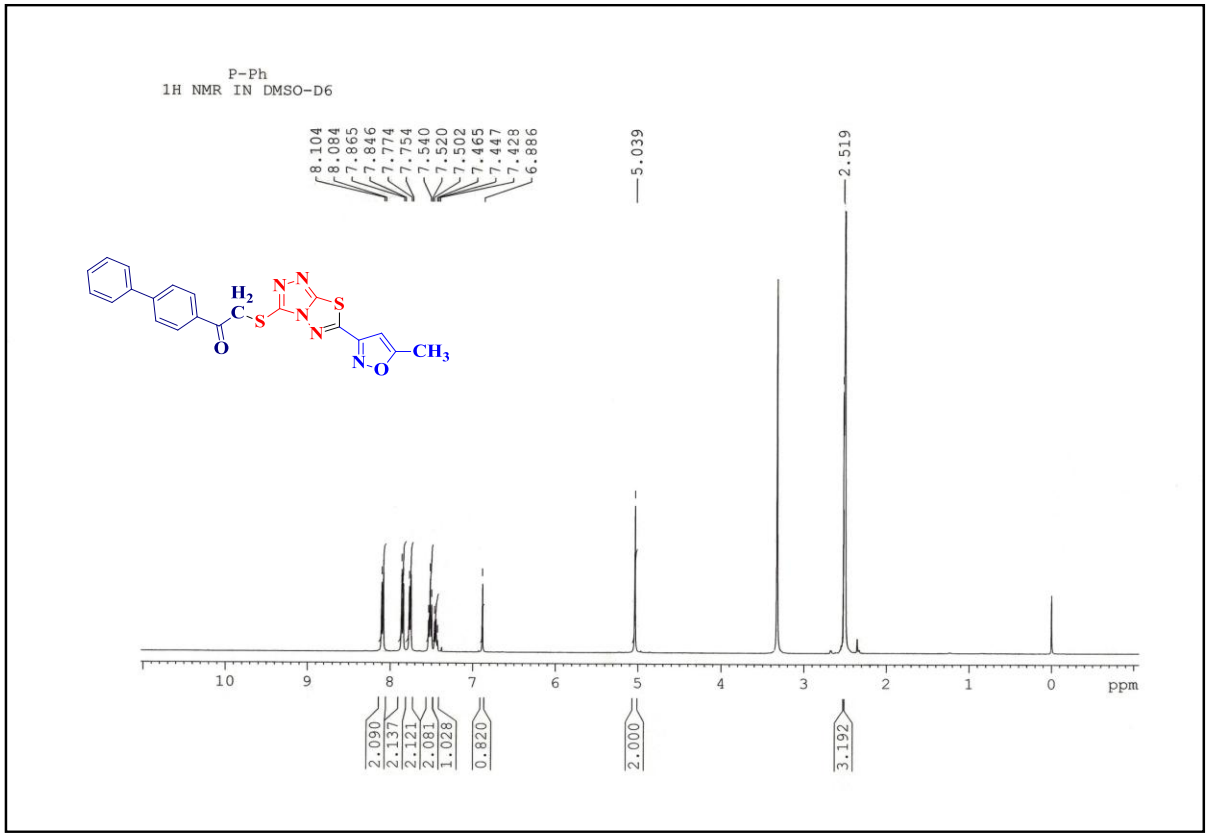


Fig: ^{1}H NMR Spectrum of compound 69l

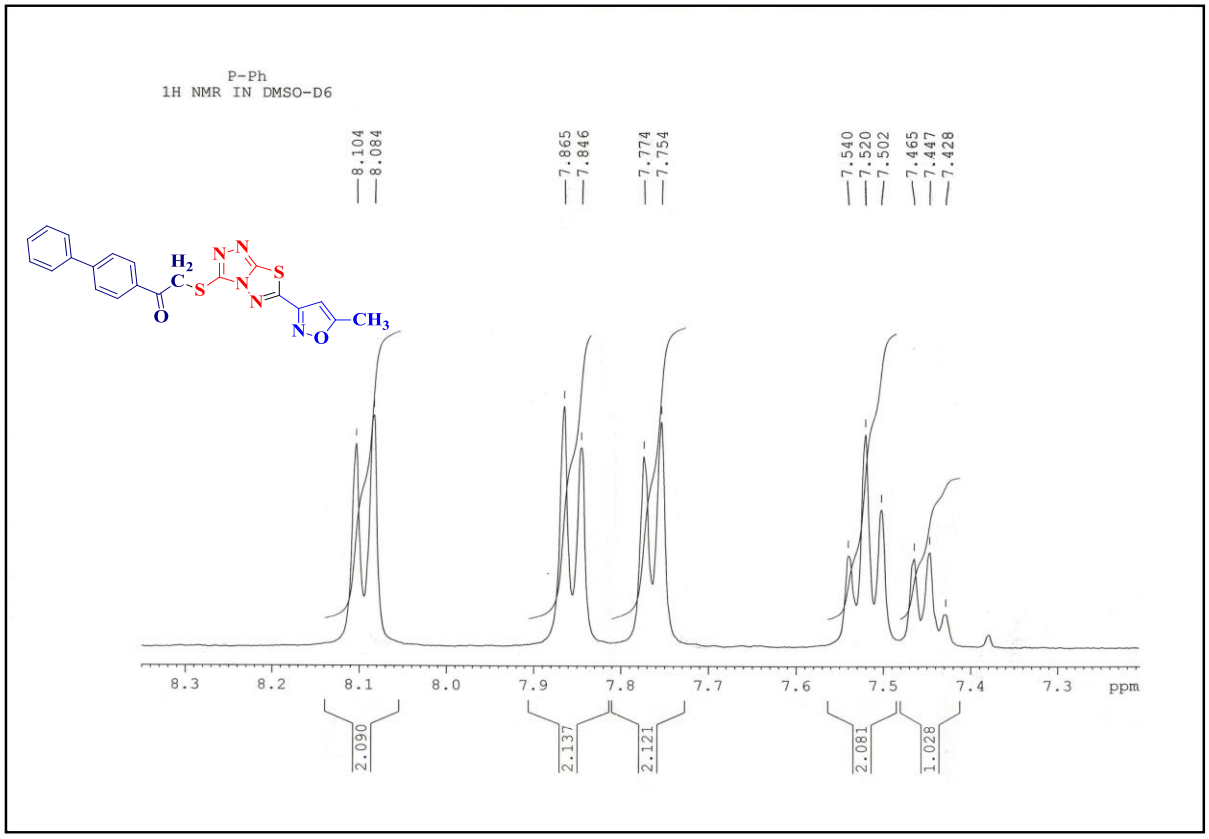


Fig: ^{1}H NMR Spectrum of compound 69l

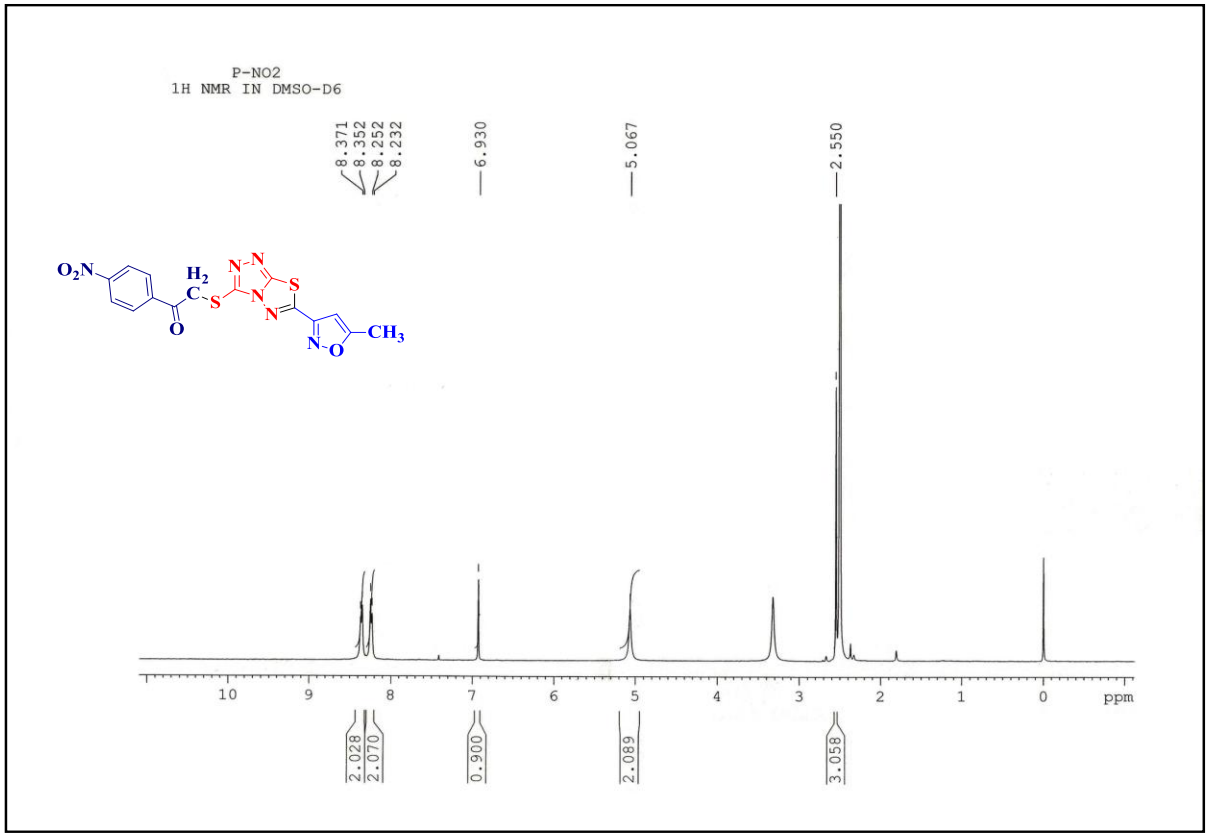


Fig: ^{1}H NMR Spectrum of compound 69m

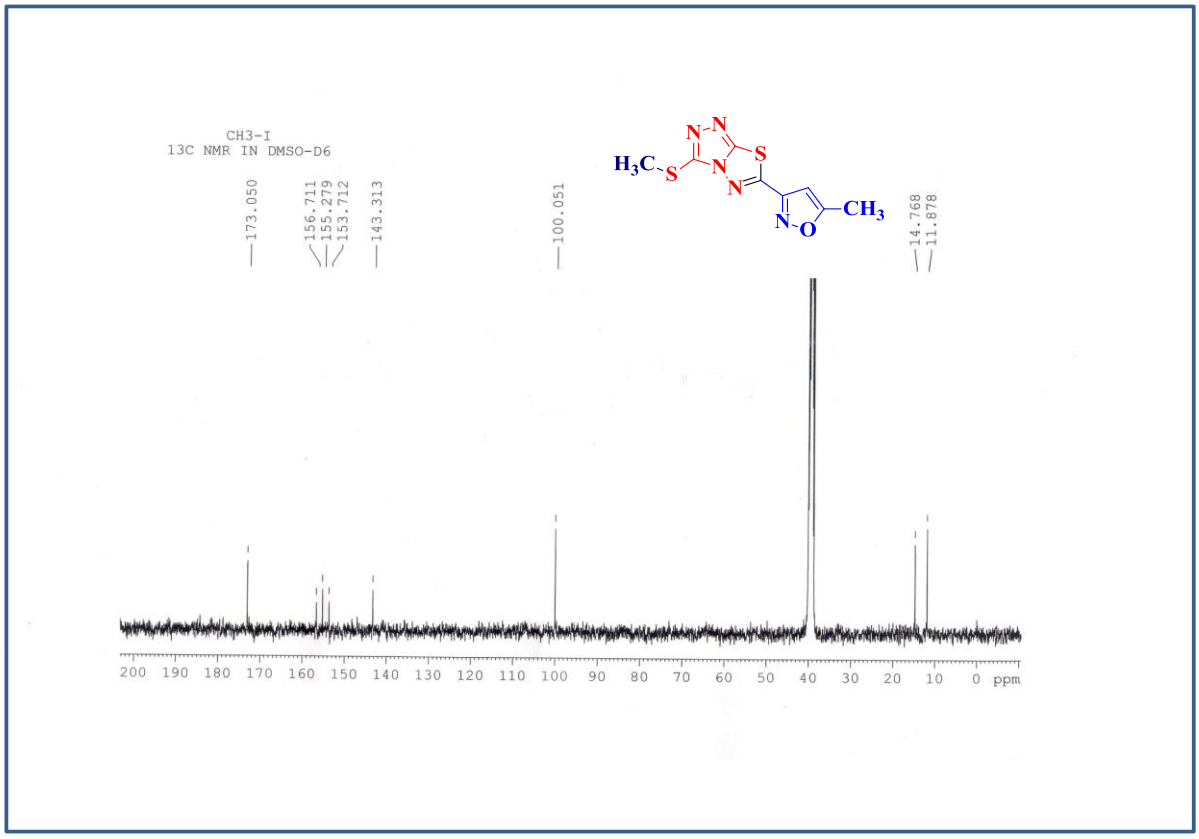


Fig: ^{13}C NMR Spectrum of compound 69a

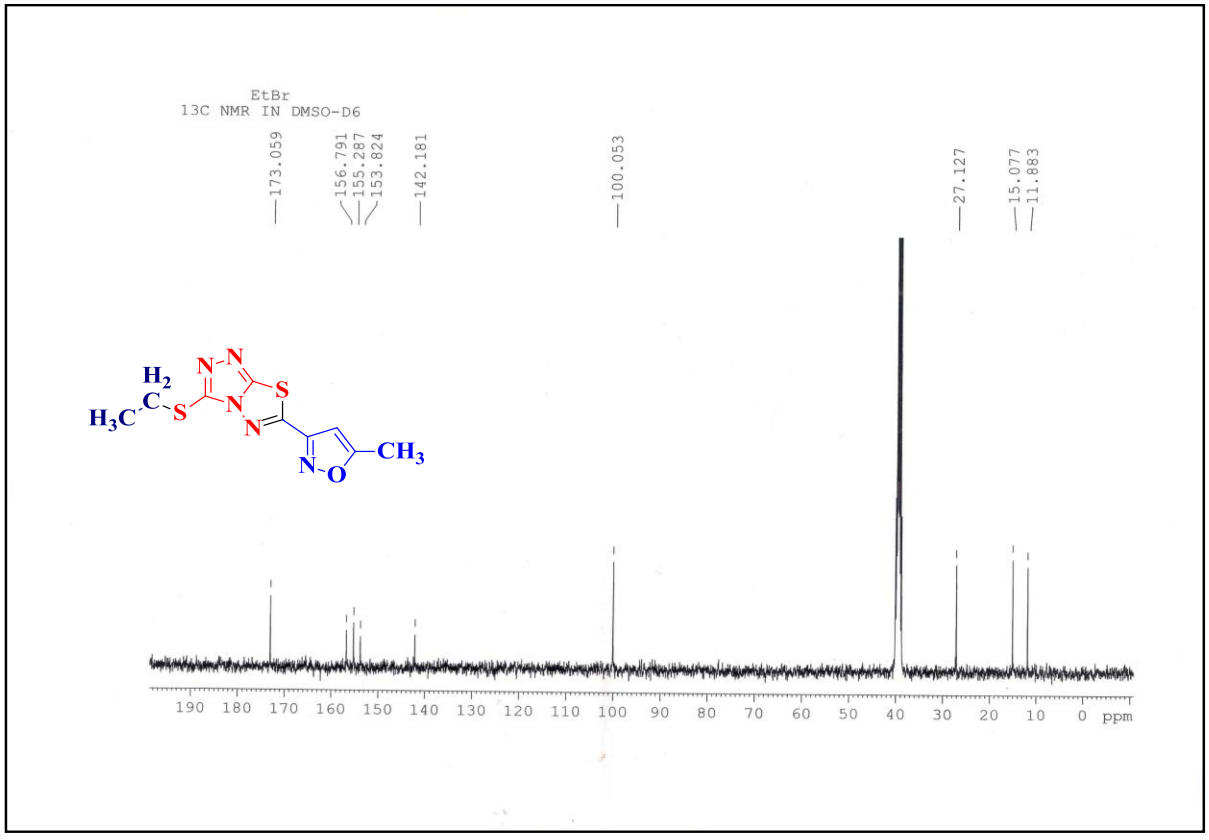


Fig: ^{13}C NMR Spectrum of compound 69b

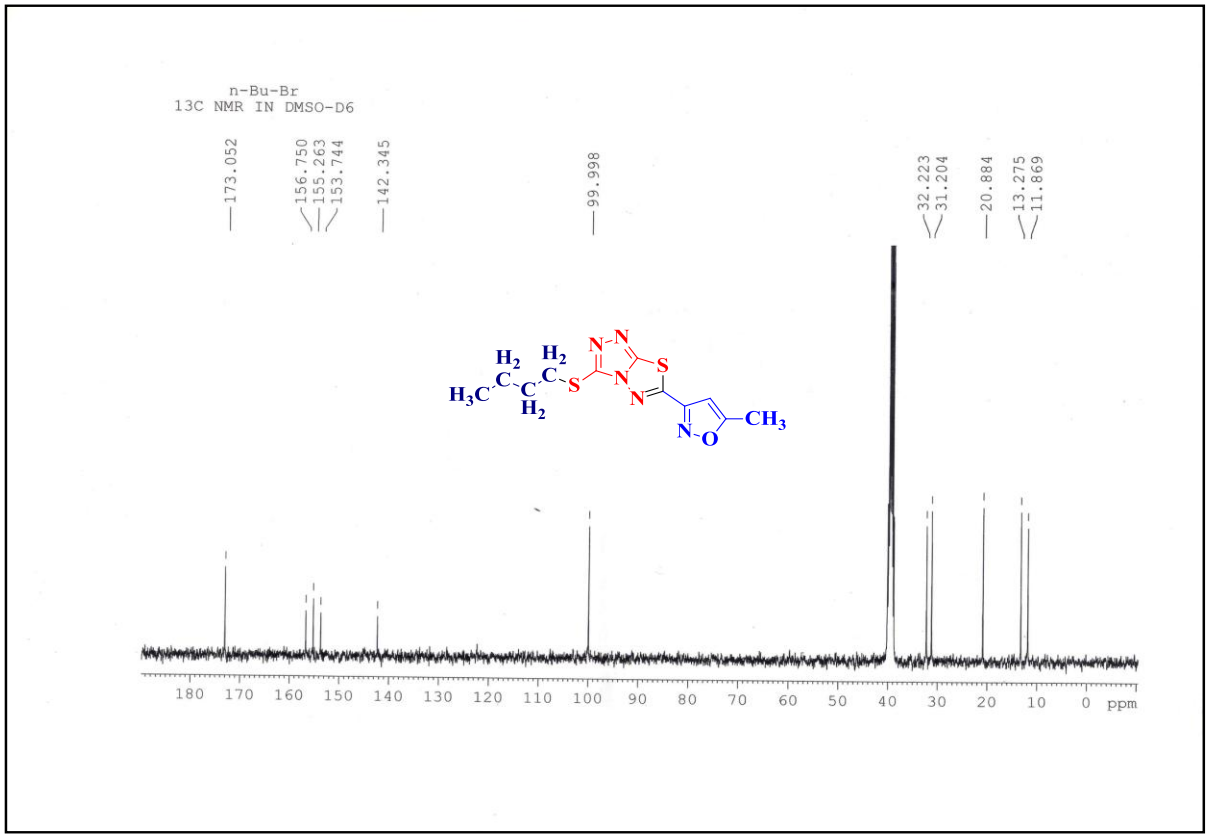


Fig: ^{13}C NMR Spectrum of compound 69d

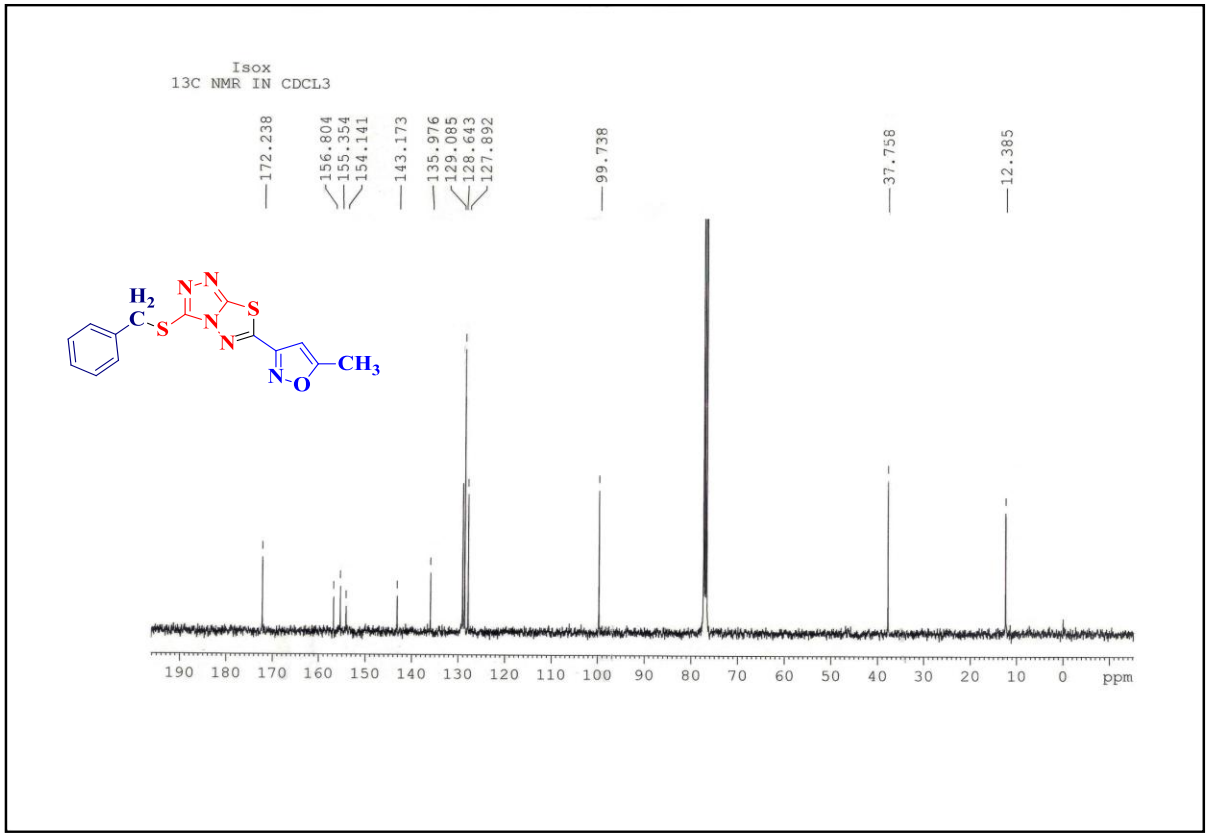


Fig: ^{13}C NMR Spectrum of compound 69f

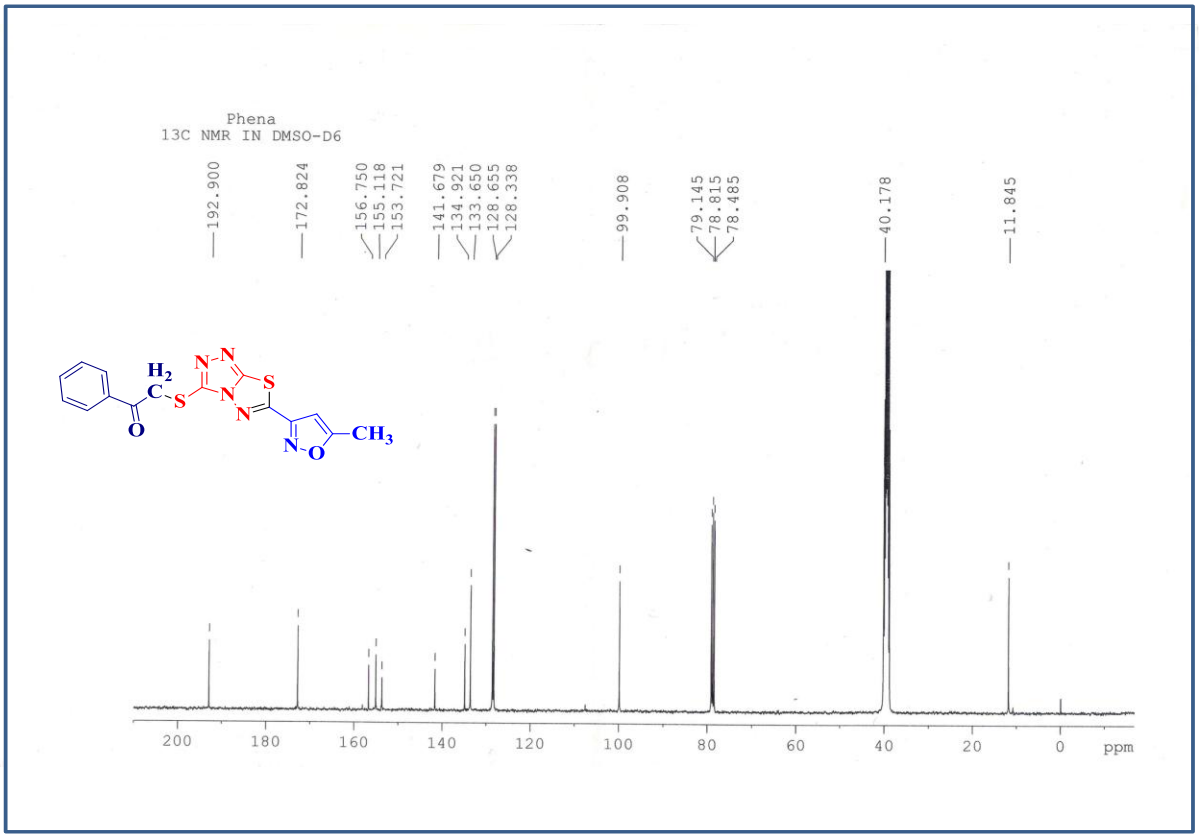


Fig: ^{13}C NMR Spectrum of compound 69g

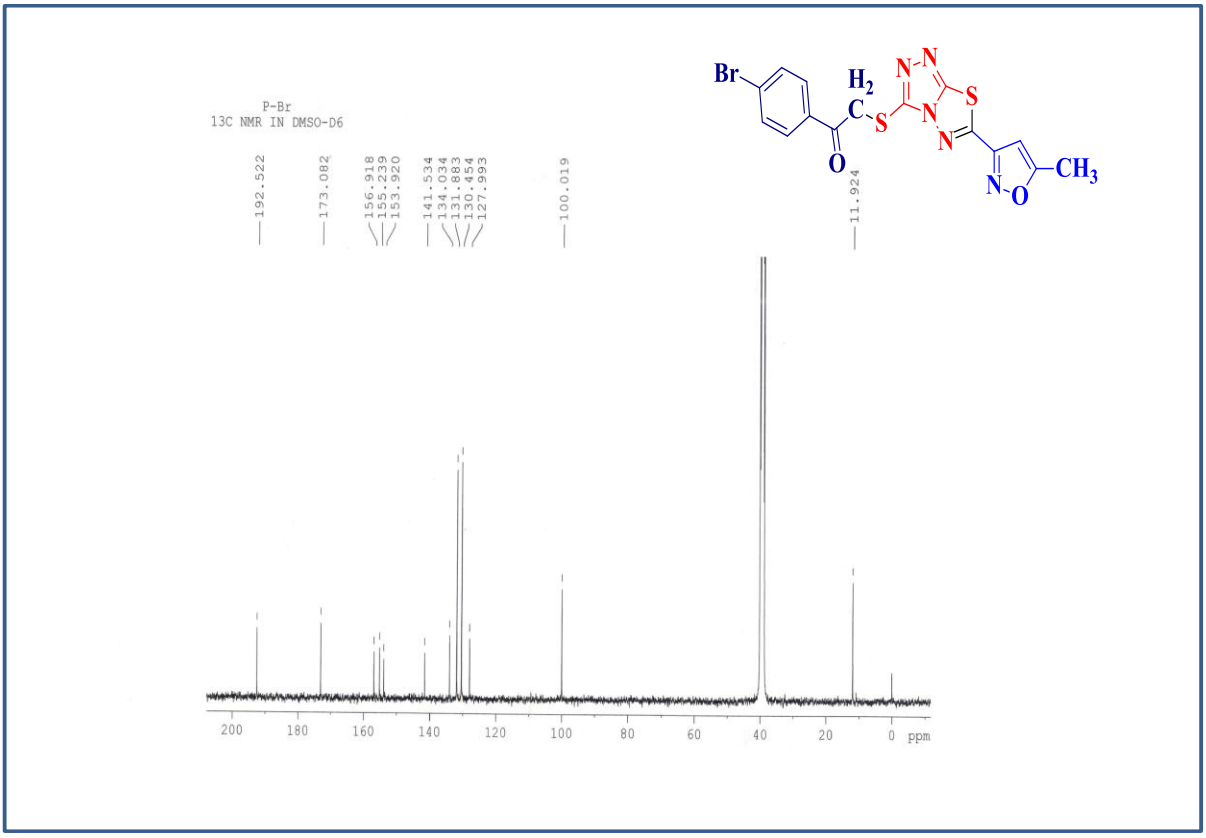


Fig: ^{13}C NMR Spectrum of compound 69i

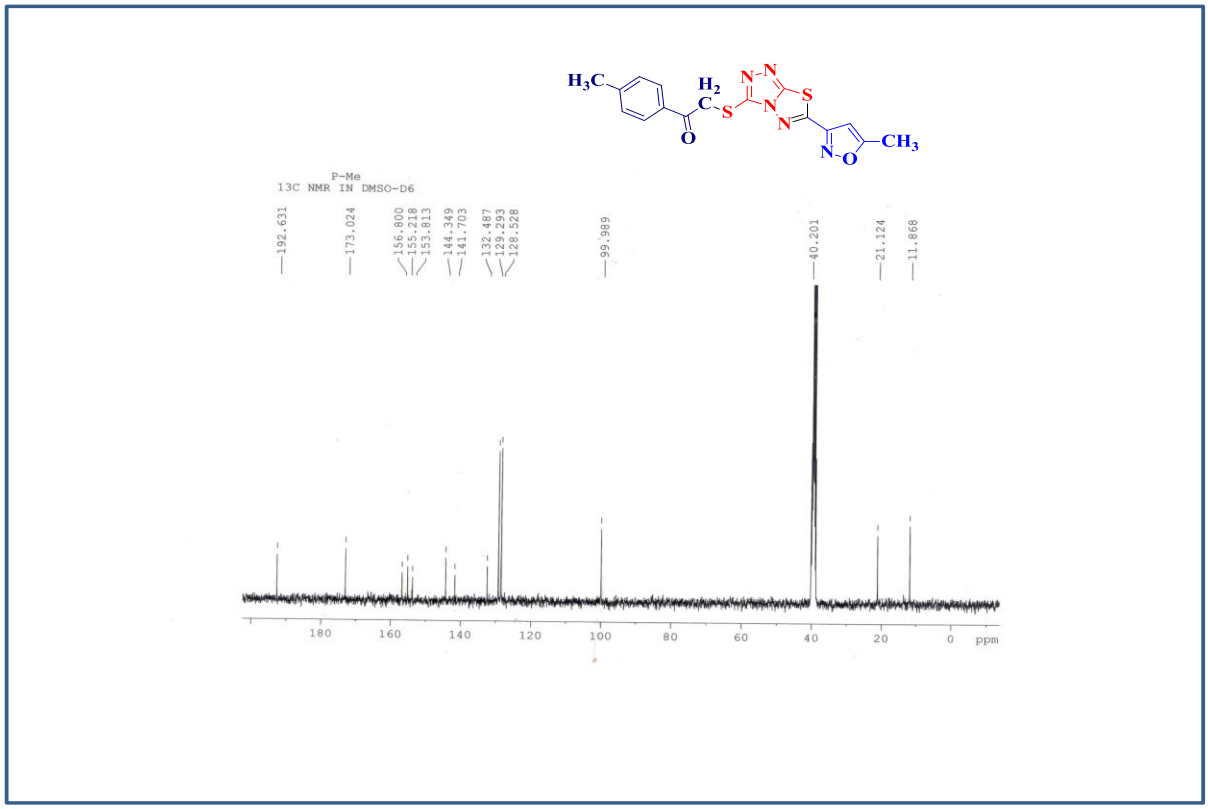


Fig: ^{13}C NMR Spectrum of compound 69j

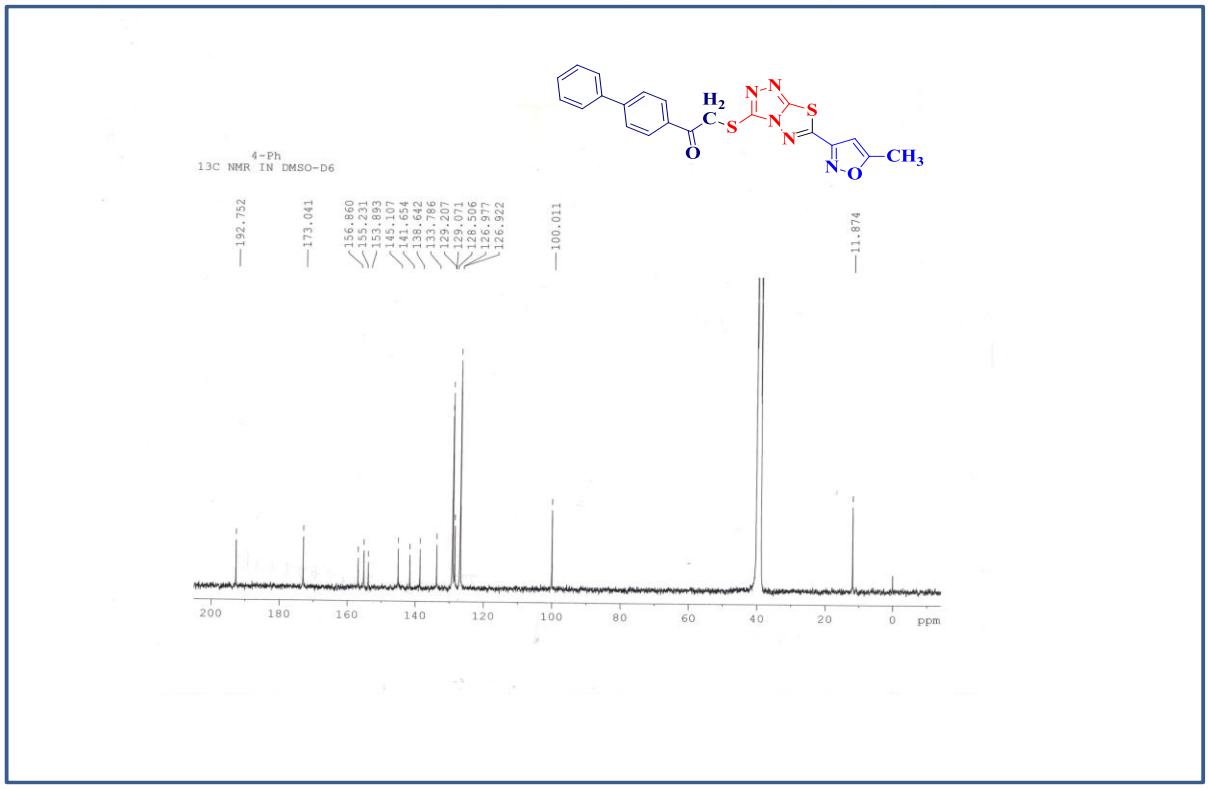


Fig: ^{13}C NMR Spectrum of compound 69l

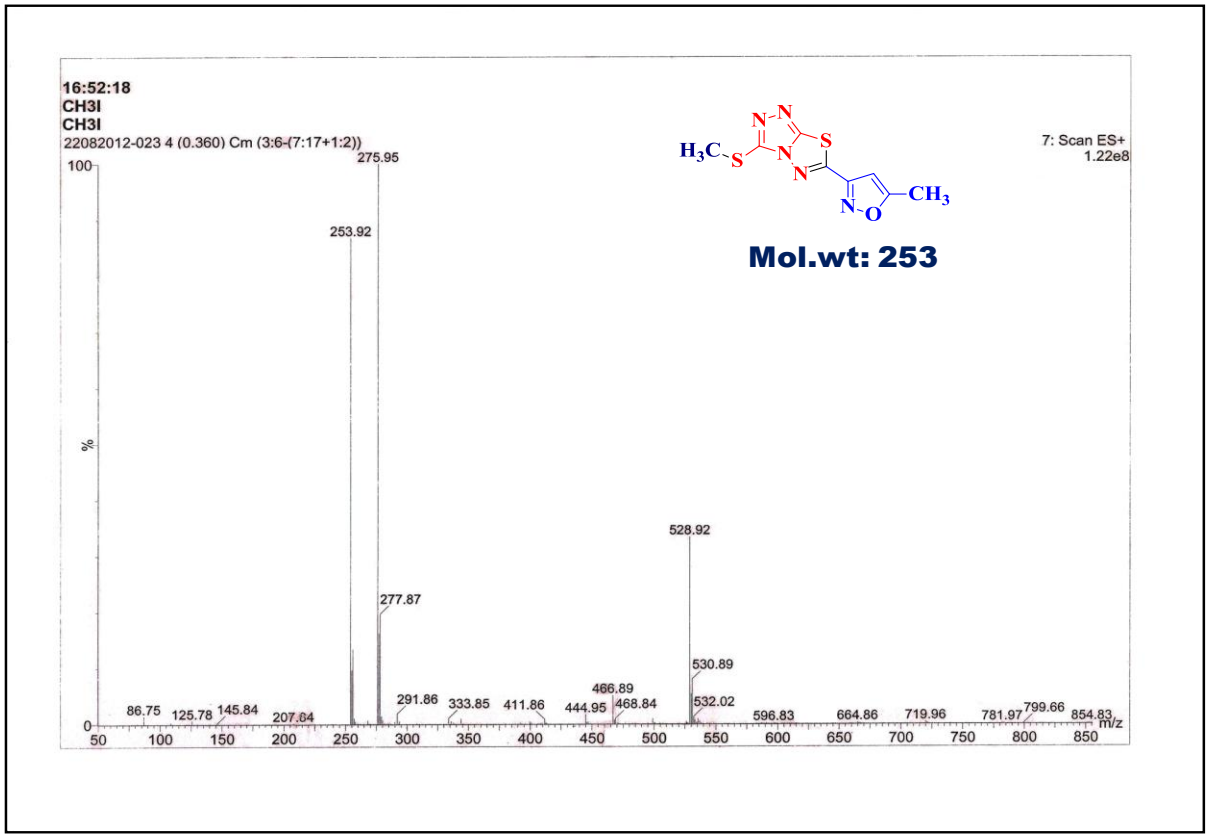


Fig: Mass spectrum of compound 69a

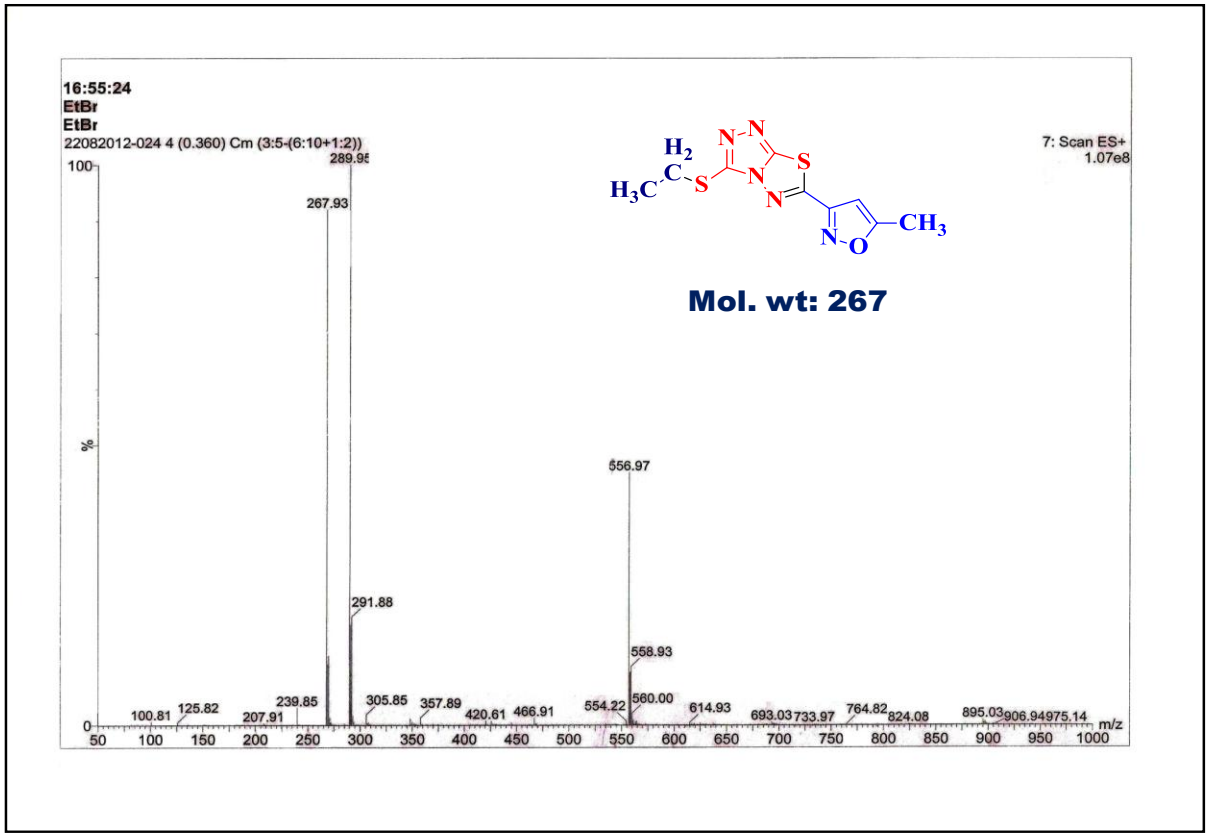


Fig: Mass spectrum of compound 69b

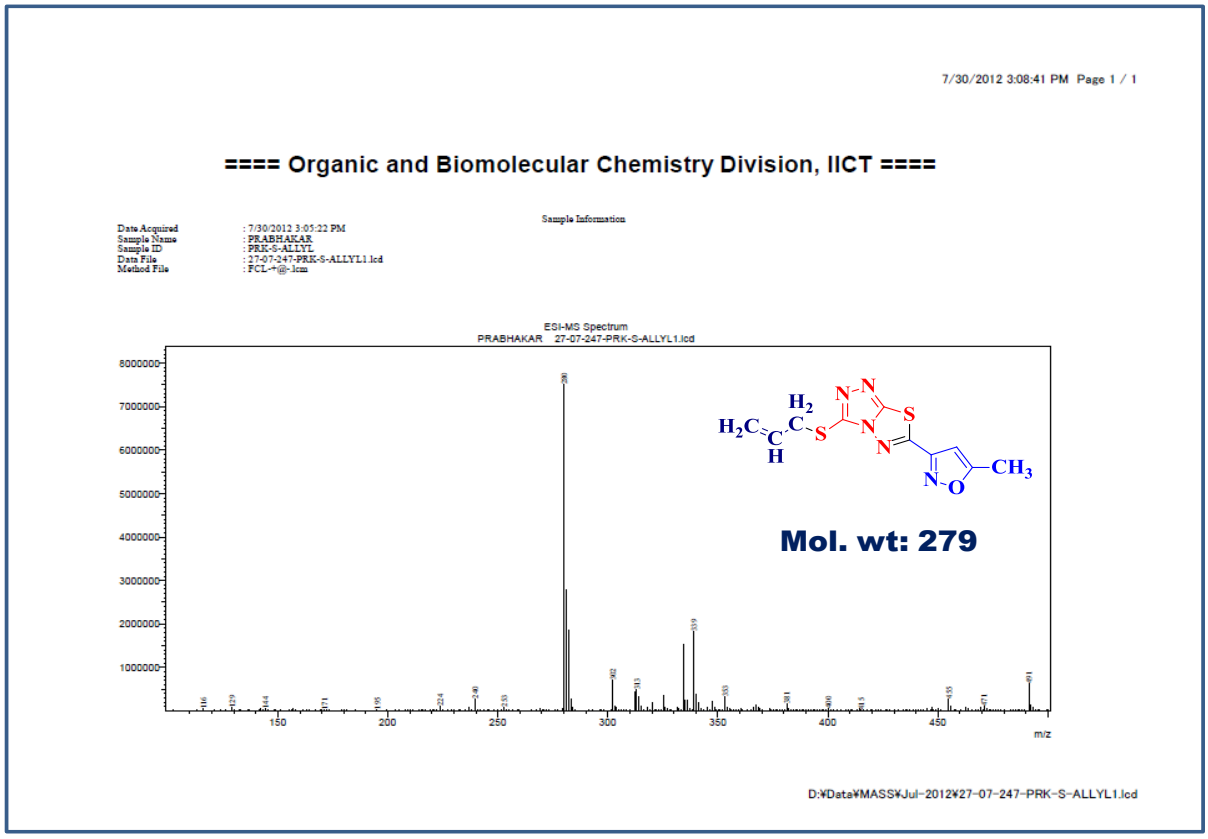


Fig: Mass spectrum of compound 69c

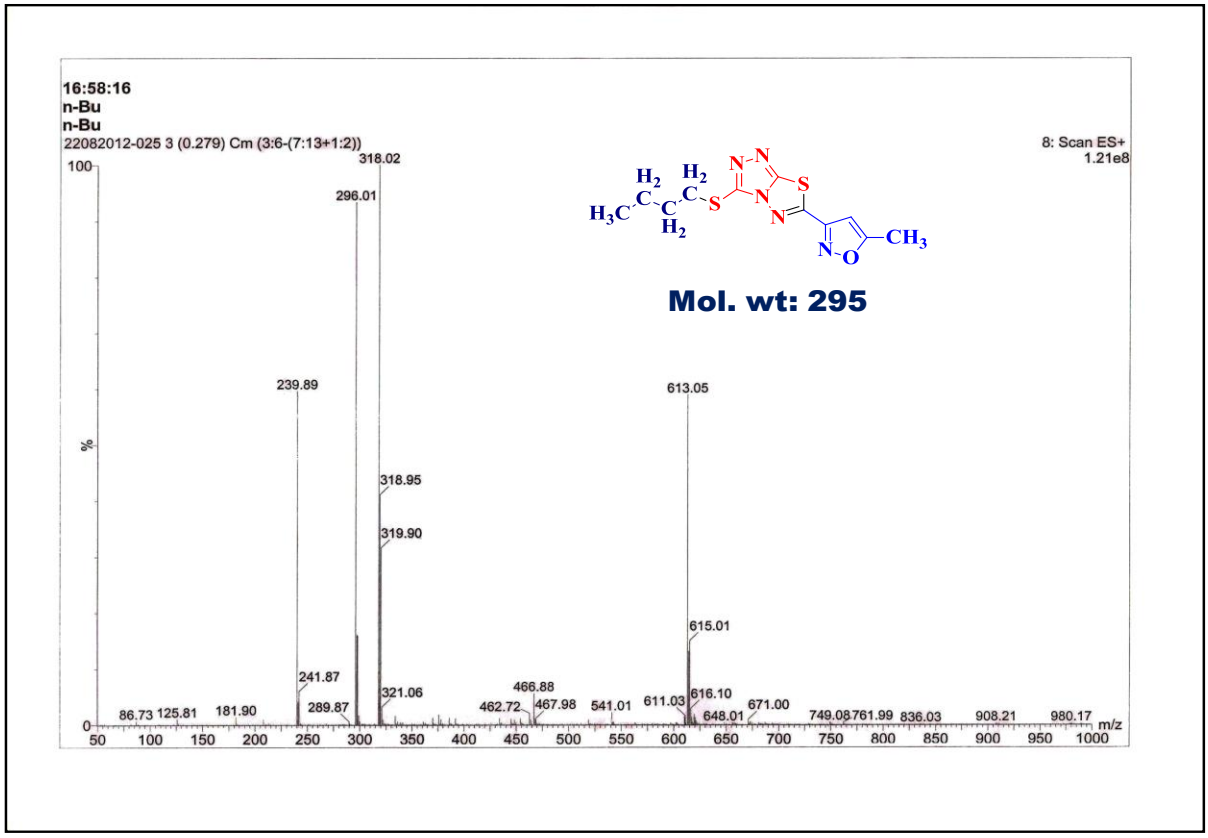


Fig: Mass spectrum of compound 69d

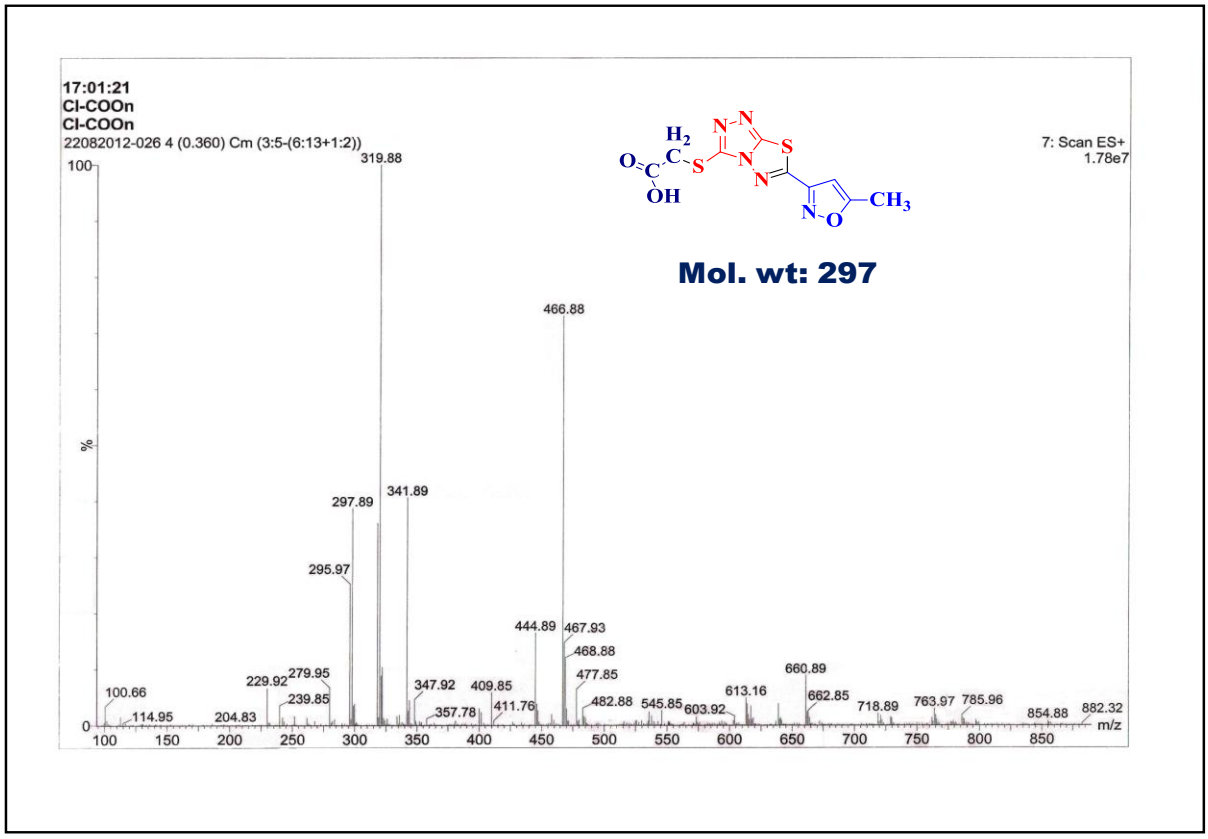


Fig: Mass spectrum of compound 69e

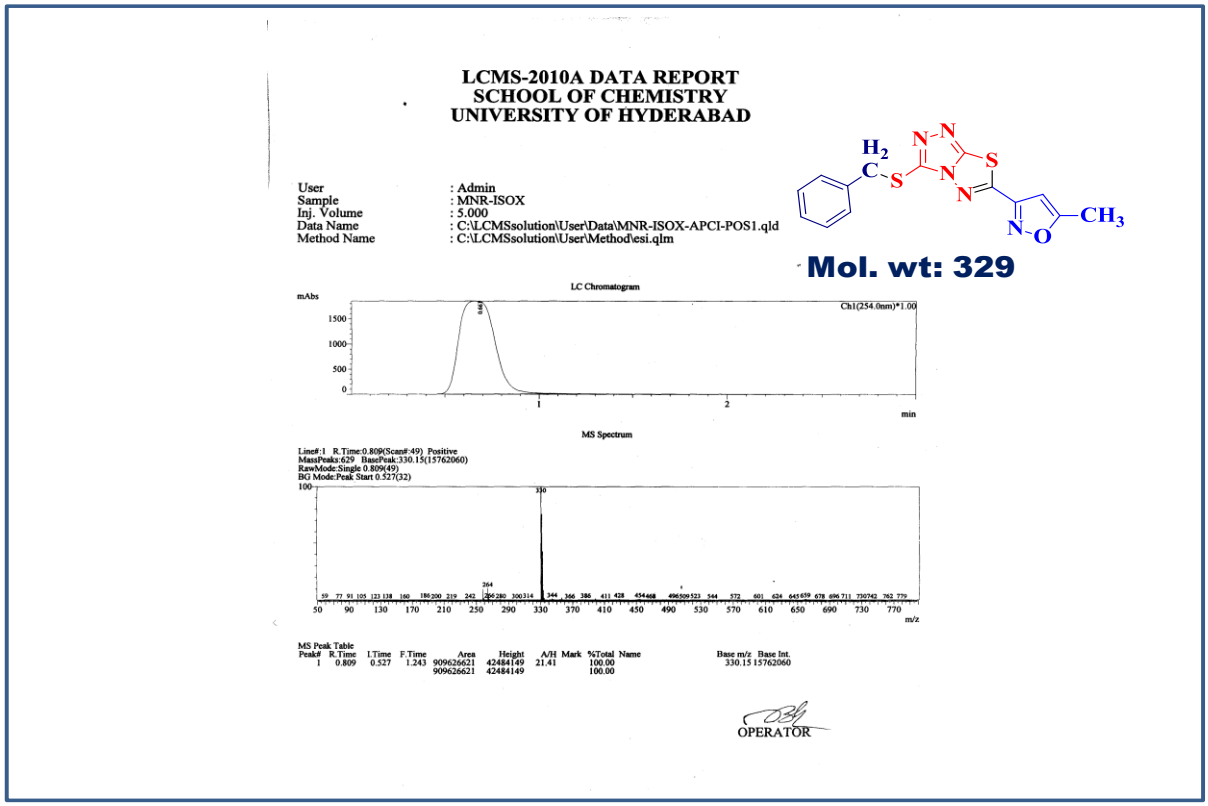


Fig: Mass spectrum of compound 69f

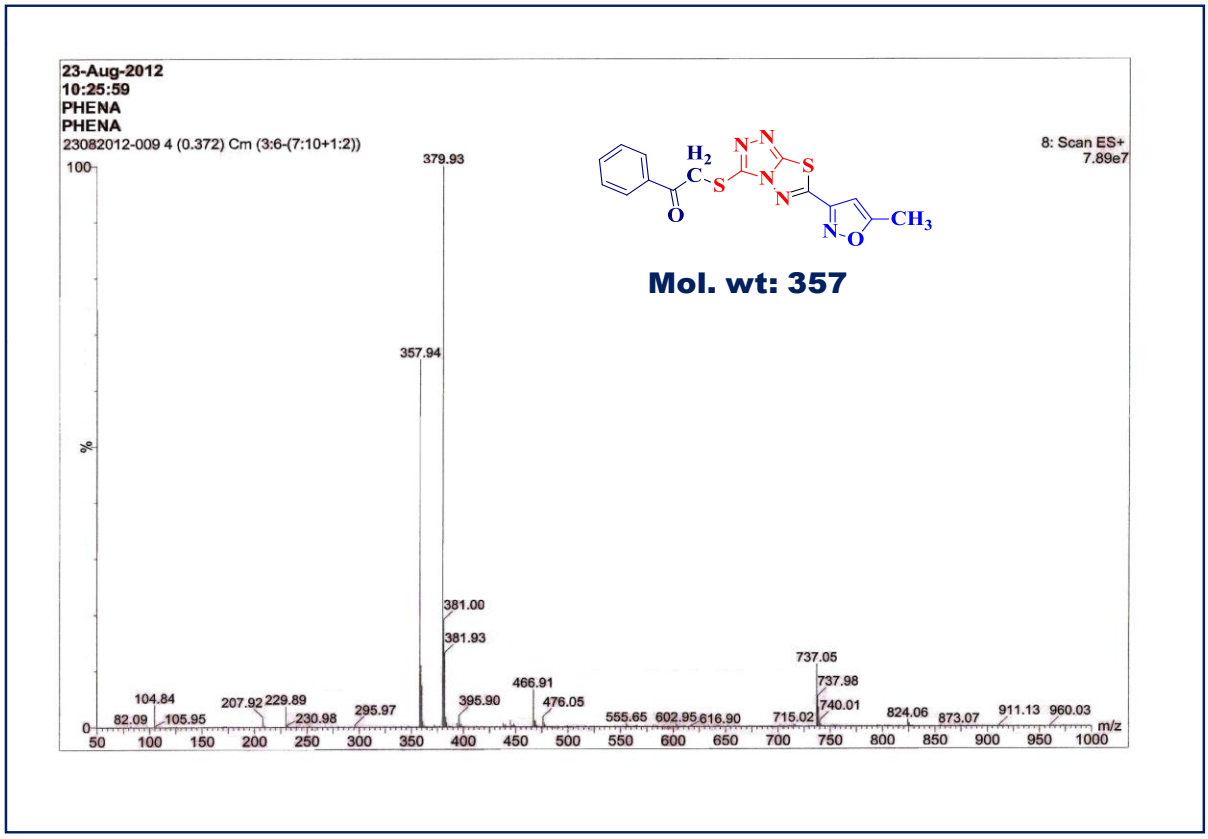


Fig: Mass spectrum of compound 69g

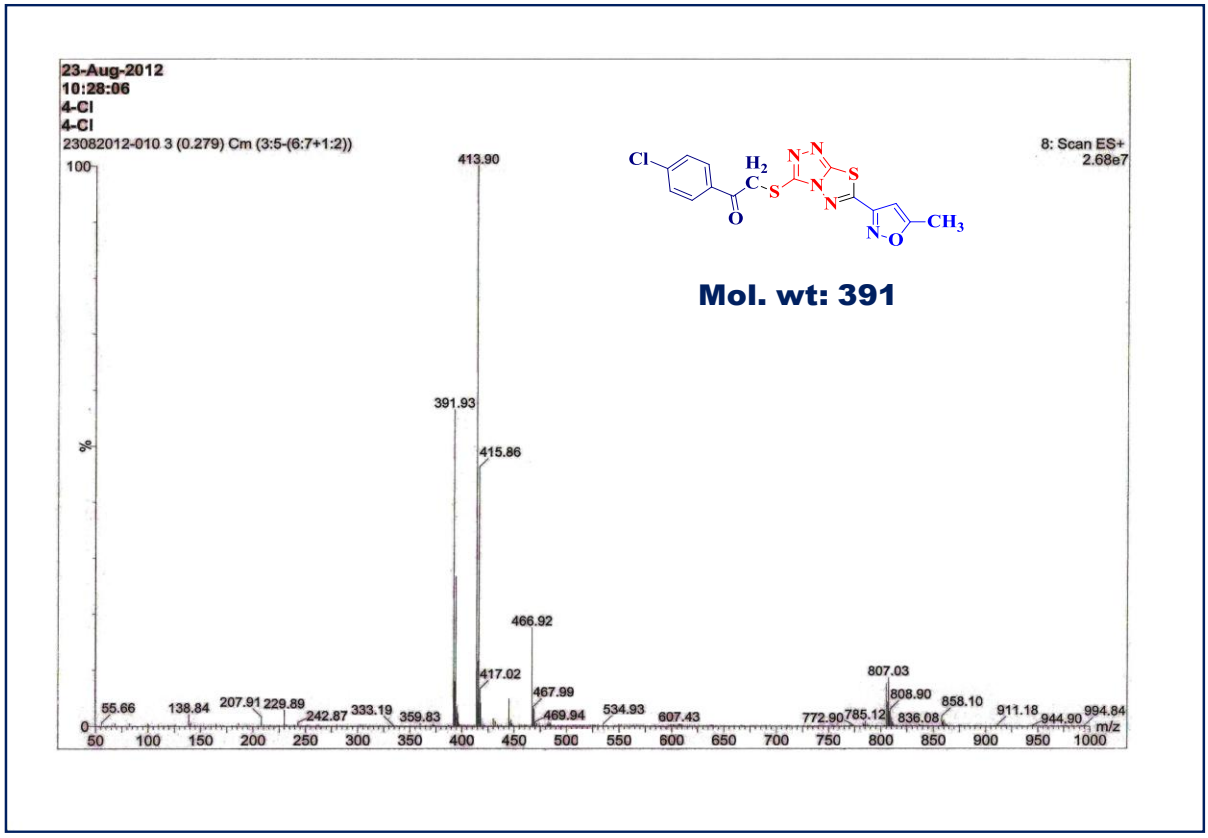


Fig: Mass spectrum of compound 69h

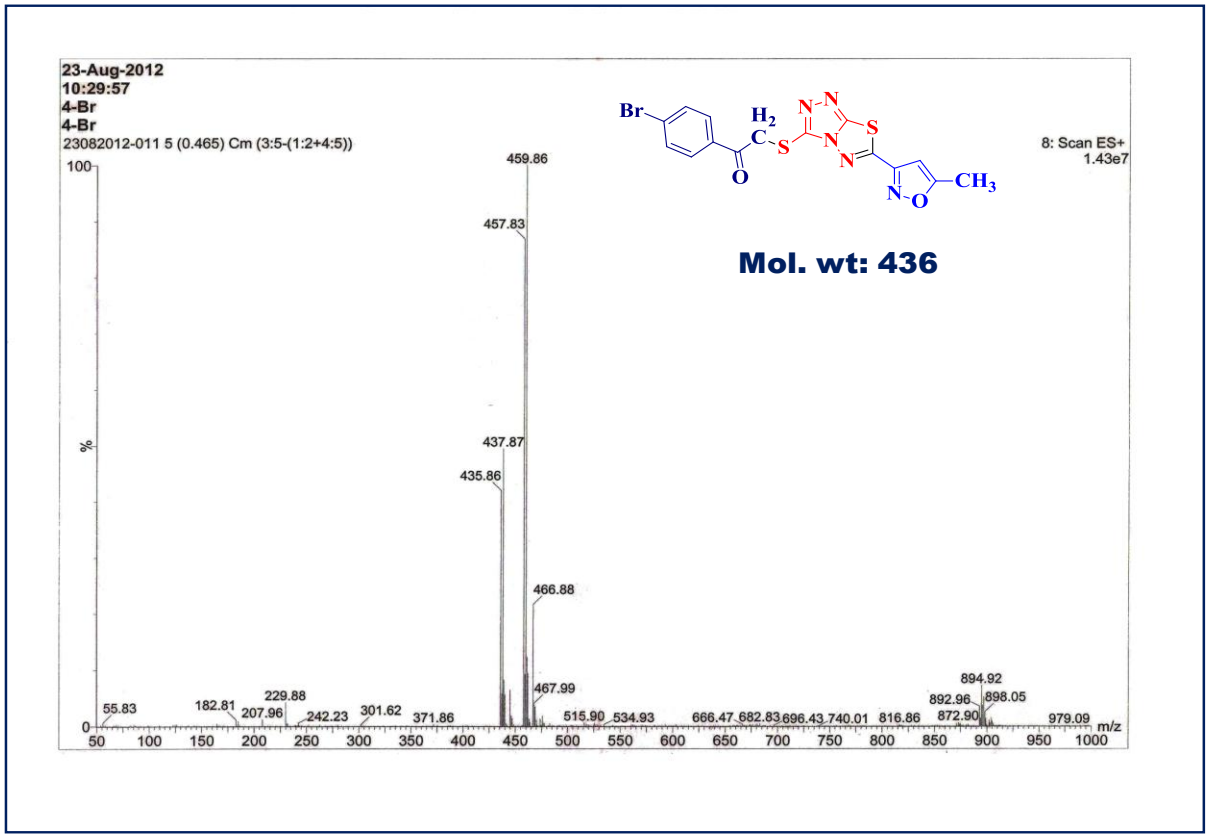


Fig: Mass spectrum of compound 69i

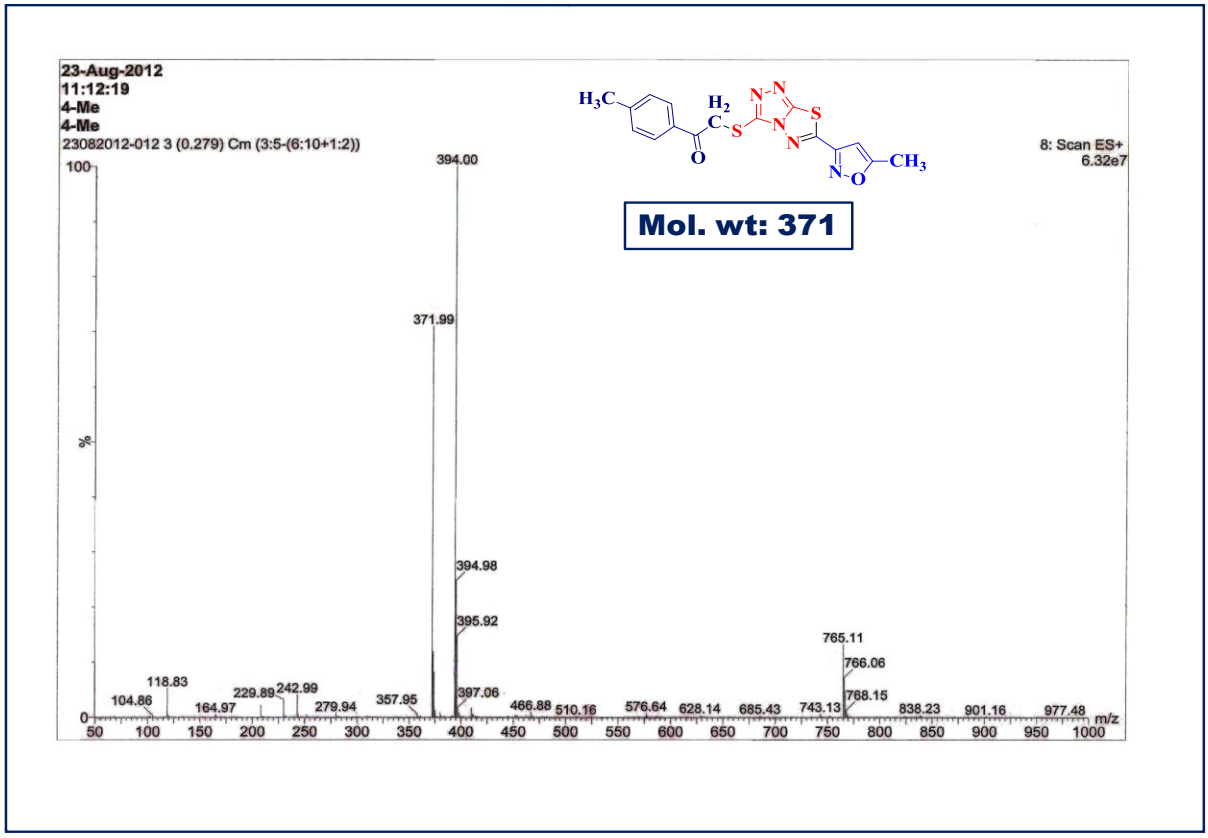


Fig: Mass spectrum of compound 69j

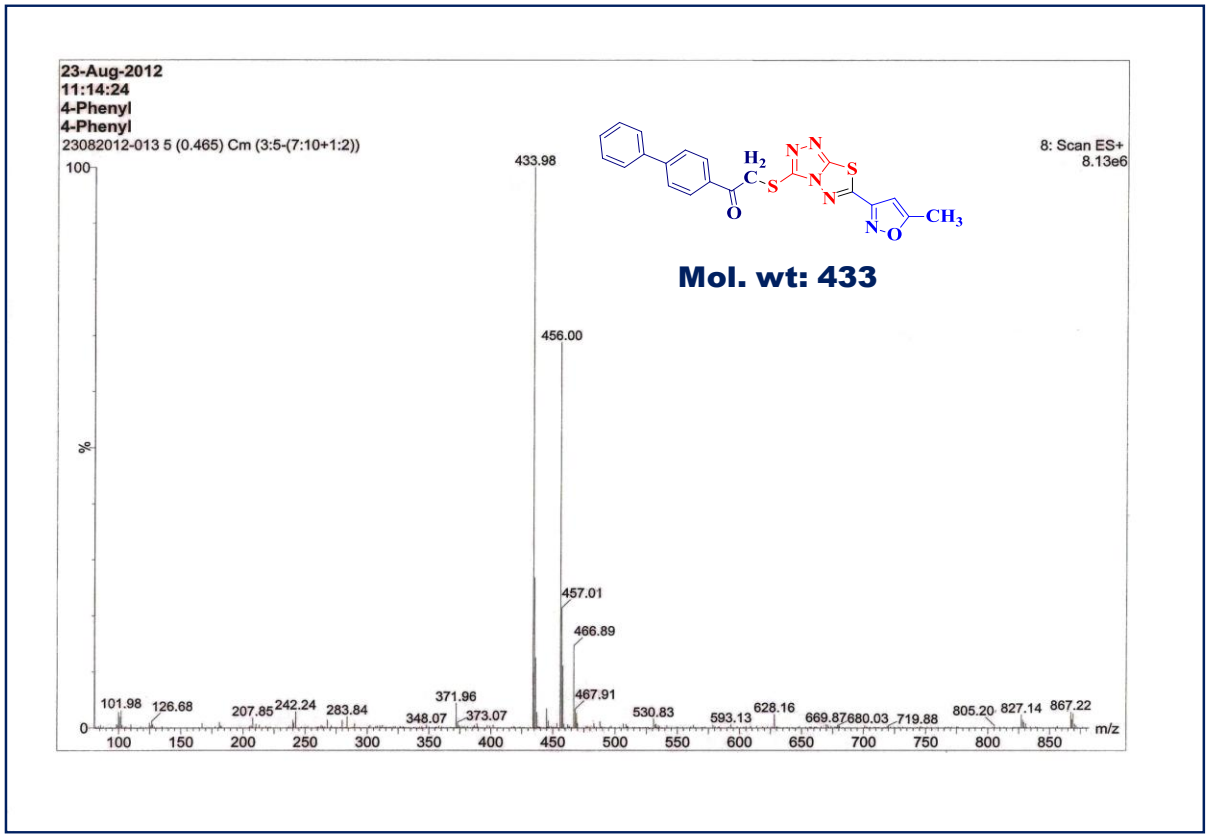


Fig: Mass spectrum of compound 69l

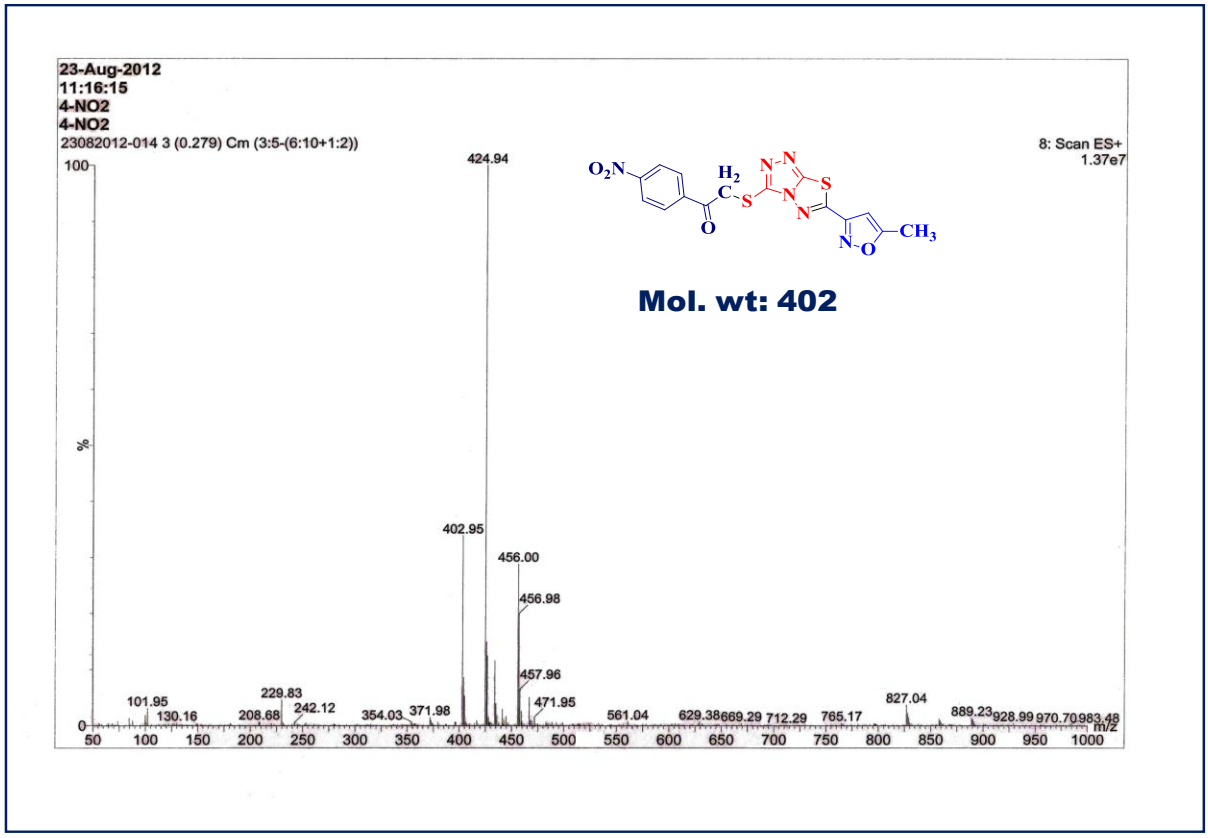


Fig: Mass spectrum of compound 69m

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CHAPTER-IV

SYNTHESIS AND BIOLOGICAL ACTIVITY EVALUATION OF 3, 6-DISUBSTITUTED 7*H*-[1,2,4]-TRIAZOLO [3,4-*b*][1,3,4]THIADIAZINES

INTRODUCTION:

Design, synthesis and screening for biological activity application of compounds having therapeutic importance is the major challenging objective for the researchers across the globe. During the past years majority of the compounds having nitrogen and sulphur as hetero atoms with therapeutic values have received considerable attention. Nitrogen containing heterocyclic system is the most privileged and most abundant integral scaffolds that occur ubiquitously in nature in the form of bioactive natural products and these are also available as large number of synthetic drugs, pharmaceuticals and agro chemicals.

The 1, 2, 4-triazole heterocyclic compound is one of the well studied and privileged compound with a large variety of therapeutic applications. Moreover, 1,2,4-triazoles and their fused heterocyclic compounds have received remarkable attention due their synthetic and effective therapeutic values. The heterocyclic compounds with 1, 2, 4-triazole core unit will exhibit broad spectrum of antibacterial[1,2], antiviral[3-5], antifungal[6,7], antidepressant[8], antitumor[9-11], anti-inflammatory[12], anticonvulsant[13-15], insecticidal[16], herbicidal[17,18] and pharmacological properties.

4-Amino-4*H*-1,2,4-triazole-3-thiol is a versatile heterocyclic system having two ambient nucleophilic centres. It is a good starting material for the synthesis of a large variety of N and S containing condensed heterocyclic compounds such as triazolothiadiazoles, triazolothiadiazine and triazolothiazipines. The most common route for the synthesis of 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine was the reaction of 4-amino 1,2,4-triazole-3-thiol with α-halo carbonyl compounds, haloacetonitriles, haloacetic acid, benzil and benzoin.

Triazolothiadiazine heterocyclic system is a fused motif of triazole and thiadiadizine core unit. These triazolothiadiazines will exist in various structural isomeric forms such as [1,2,3]triazolo[5,1-*b*][1,3,4]thiadiazine, [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine, [1,2,4] triazolo[5,1-*b*][1,3,5]thiadiazine and [1,2,4]triazolo[1,5-*c*][1,3,5]thiadiazine (**Fig. 1**). Among, these structural isomers, 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine is a privileged scaffold owing to its great importance in the field of medicinal chemistry. The triazolothiadiazine heterocyclic system having substituents at 3rd and 6th position exhibit broad spectrum of therapeutic applications such as antibacterial[19,20], antifungal[21,22], antiviral[23], antitubercular[24], anti-inflammatory[25], antitumor[26], PDE4 inhibitors[27,28] and plant growth regulators[29].

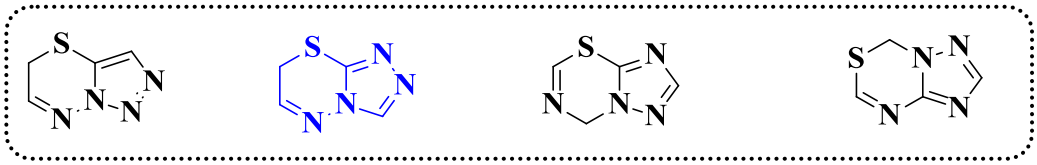
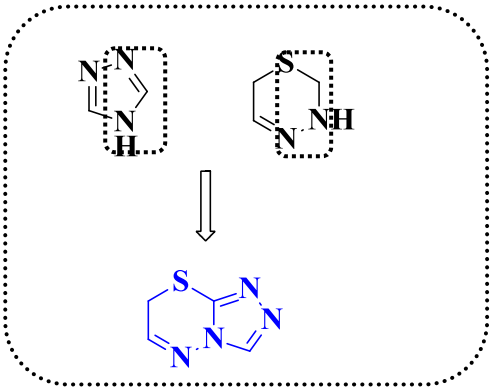
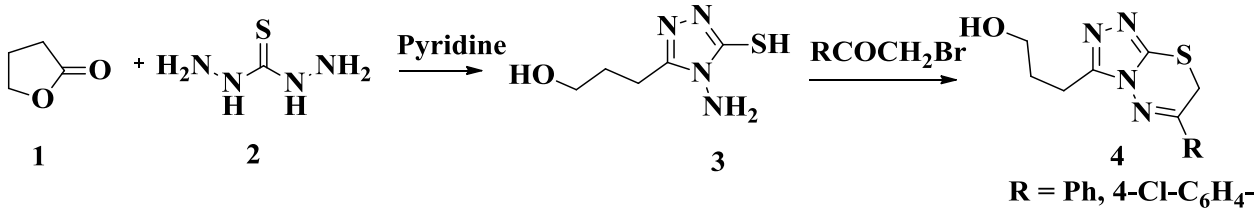


Fig. 1 Isomeric Forms of various triazolothiadiazines

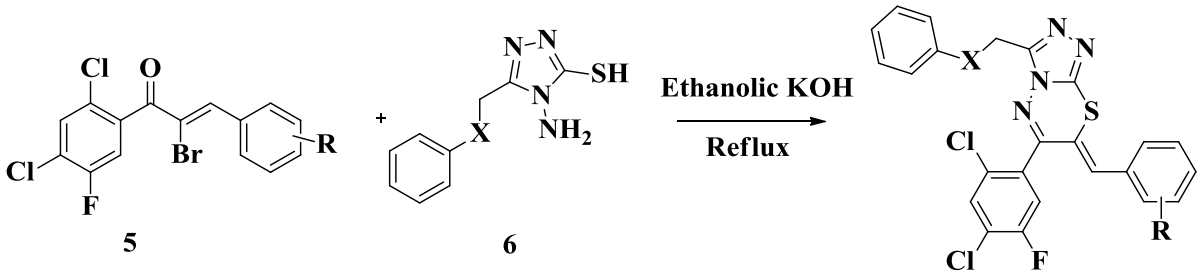


Here in, a brief review on synthesis and biological applications of 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines is presented.

Jin *et al*[30] reported the synthesis of 3-(6-aryl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)propan-1-ols by the reaction of 3-(4-amino-5-mercapto-4*H*-1,2,4-triazol-3-yl)propan-1-ol (3) with various substituted phenacyl bromides in presence of ethanol solvent with good yields. 3-(4-Amino-5-mercapto-4*H*-1,2,4-triazol-3-yl)propan-1-ol was obtained by the reaction of thiocarohydrazide with ɣ-butyrolactone in presence of pyridine solvent.

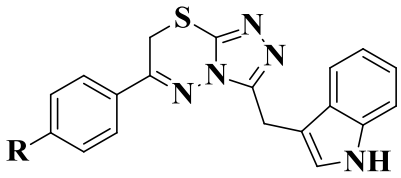


Holla *et al*[31] described the synthesis of 7-benzylidene-6-(2,4-dichloro-5-fluorophenyl)-3 (phenoxymethyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine or 7-benzylidene-6-(2,4-dichloro-5-fluorophenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)methyl)aniline by condensation reaction of 2-bromo-1-(2,4-dichloro-5-fluorophenyl)-3-phenylprop-2-en-1-one (5) with 4-amino-5-(aryloxy/phenylamino methyl)-4*H*-1,2,4-triazole-3-thiol under alkaline reaction condition with good yields.



X = O , NH

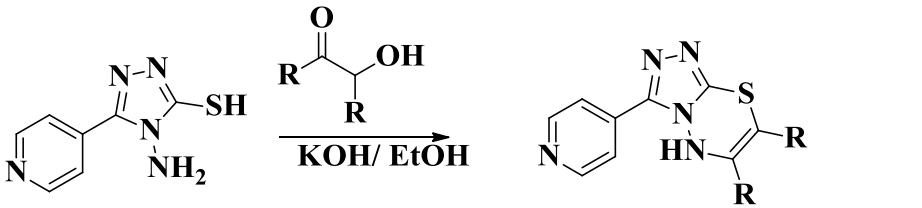
Kaplancikli *et al*[32] synthesized 3-((1*H*-indol-3-yl)methyl)-6-phenyl-7*H*-[1,2,4] triazolo[3,4-*b*][1,3,4]thiadiazine derivatives via condensation of 2-(1*H*-indol-3-yl)acetic acid with thiocarobohydrazide followed by reaction with substituted phenacyl halides in presence of ethanol solvent.



8

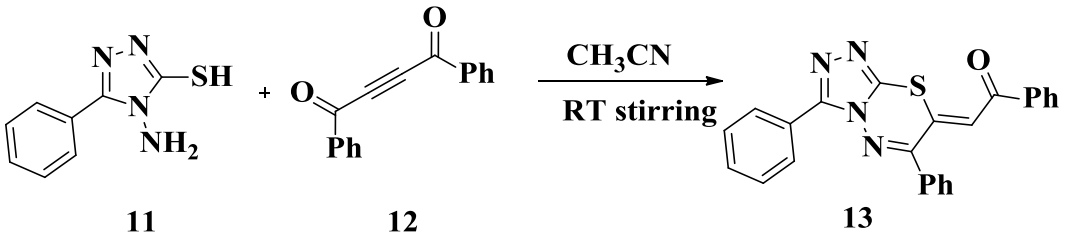
R = H, Cl, CH_3, NO_2 and $N(CH_3)_2$

Deohate[33] developed an efficient method for the synthesis of 6,7-di-substitutedphenyl-3-(pyridin-4-yl)-5*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine derivatives by the reaction of 4-amino-5-(pyridin-4-yl)-4*H*-1,2,4-triazole-3-thiol with various substituted benzoin derivatives in presence of ethanolic potassium hydroxide solution with shorter reaction time. 4-Amino-5-(pyridin-4-yl)-4*H*-1,2,4-triazole-3-thiol was synthesized by the interaction of isoniazide with carbon disulphide and hydrazine hydrate in two steps.



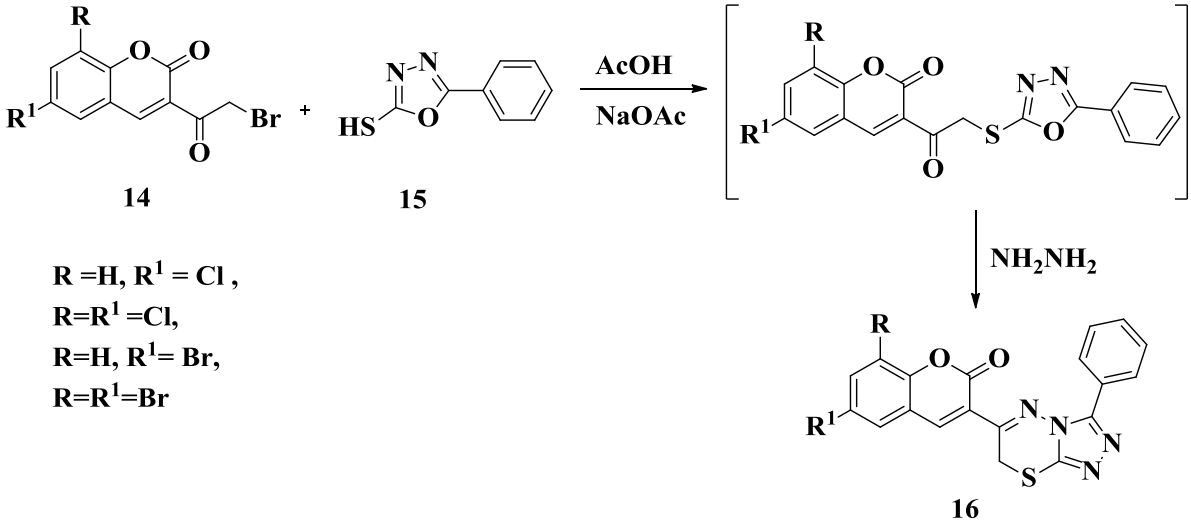
9 **10** **R = Ph**

Shah *et al*[34] synthesized 2-(3,6-diphenyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-7-ylidene)-1- by using 4-amino-5-phenyl-4*H*-1,2,4-triazole-3-thiol and 1,4-diphenylbut-2-yne-1,4-dione in a shorter reaction time in presence of acetonitrile solvent at room temperature with excellent yield.



11 **12** **13**

Chunduru and Rao[35] synthesized 3-(3-Phenyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-6-yl)-2*H*-chromen-2-ones via a facile one pot multicomponent synthetic approach. The target compound was synthesized by condensation of substituted 3-(2-bromoacetyl) coumarins with 5-phenyl-1,3,4-oxadiazole-2-thiol in acetic acid to give corresponding un isolable 3-(2-(5-phenyl-1,3,4-oxadiazol-2-ylthio)-2*H*-chromen-2-ones. These on reaction with hydrazine hydrate gave the final products with good yields.

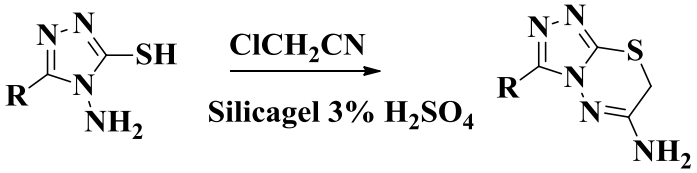


14 **15**

R =H, R^1 = Cl ,
R=R^1 =Cl,
R=H, R^1= Br,
R=R^1=Br

16

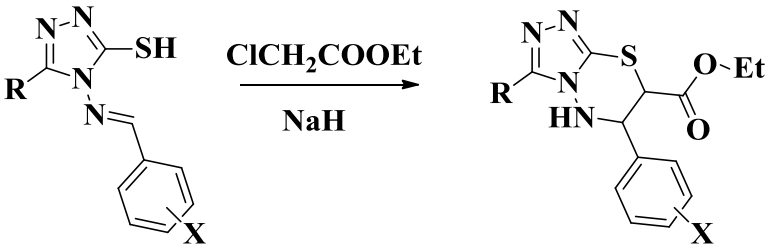
Heravi *et al*[36] developed an efficient silica gel – sulphuric acid catalyzed one – pot cyclocondensation method for the synthesis of 3-alkyl / aryl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-6-amines. The target compound was synthesized by the reaction of 4-amino-5-alkyl / aryl -4*H*-1,2,4-triazole-3-thiol with chloroacetonitrile in presence of ethanol solvent with satisfactory yield.



17 **18**

R = H, CH_3, C_6H_4-CH_3

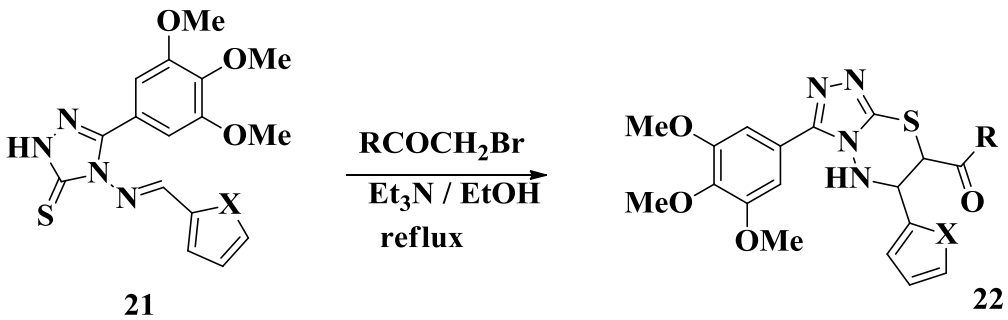
Foroughifar *et al*[37] synthesized ethyl 6-phenyl-3-(pyridin-3-yl / pyridin-4-yl)-6,7-dihydro-5*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine-7-carboxylates via cyclization of 4-(benzylideneamino)-5-(pyridin-3-yl / pyridin-4-yl)-4*H*-1,2,4-triazole-3-thiol with ethyl chloro acetate in presence of sodium hydride at room temperature with good yields.



19 **20**

R = 3-pyridyl / 4- pyridyl

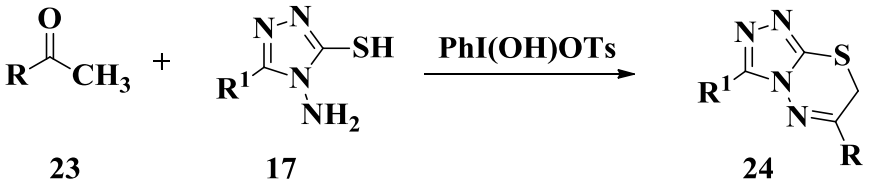
Zhang *et al*[38] reported the synthesis and anti proliferative activity of 1,2,4-triazolo [3,4-*b*] [1,3,4] thiadiazines. The target compounds (6-(furan -2-yl)-3-(3,4,5-trimethoxy phenyl)-6,7-dihydro-5*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-7-yl)(phenyl)methanones were synthesized by utilizing 4-((furan / thiophene-2-ylmethylene)amino)-3-(3,4,5-trimethoxy phenyl)-1*H*-1,2,4-triazole-5(4*H*)-thione and various α-bromoketones in presence of absolute ethanol using triethylamine as base with good yields. All the synthesized molecules were evaluated against human cancer cell lines (PC-3, HepG2, A549 and MCF-7). The tested compounds exhibited moderate to good antiproliferative activity.



21 **22**

X = O, S & R = Aryl / Heteryl

Singh *et al*[39] developed a method of hydroxy(tosyloxy)iodo]benzene mediated synthesis of triazolothiadiazines. In this method various acetophenones were treated with 4-amino-5-aryl/alkyl-4*H*-1,2,4-triazole-3-thiols in the presence of hydroxyl (tosyloxy) iodo]benzene to yield the target compounds with excellent yields.

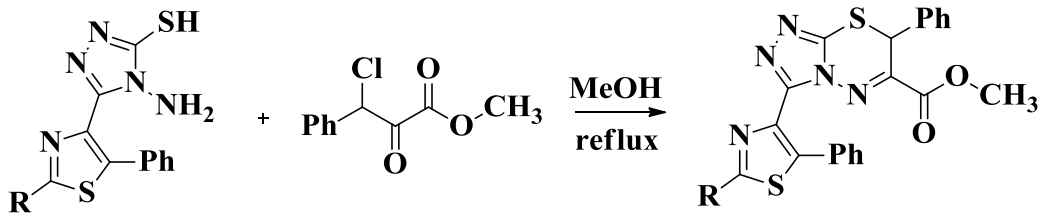


23 **17** **24**

R = 2-Thiophene/ 2-Furan

R^1 = H / CH_3

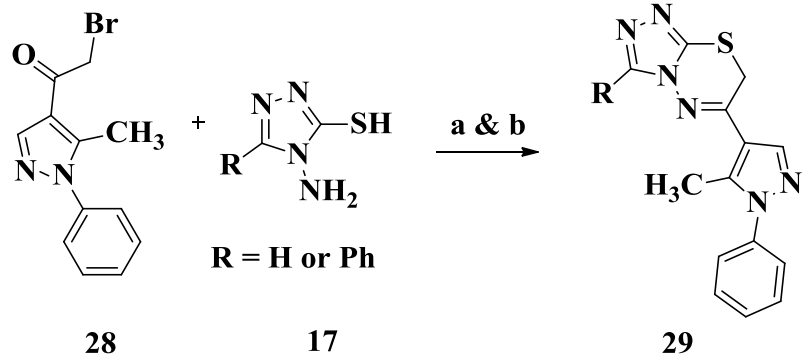
Mamedov *et al*[40] synthesized methyl 3-(2-methyl/phenyl-5-phenylthiazol-4-yl)-7-phenyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine-6-carboxylates by the reaction of 4-amino-5-(2-methyl/phenyl-5-phenylthiazol-4-yl)-4*H*-1,2,4-triazole-3-thiol with methyl 3-chloro-2-oxo-3-phenylpropanoate in presence of methanol solvent under reflux condition. The final product structure was confirmed by single crystal X-ray crystallography data.



25 **26** **27**

R = CH_3 or Ph

Salem *et al*[41] described the synthesis of novel pyrazolyl (5,6-dihydro -s-triazolo[3,4-*b*] thiadiazines by the reaction of 2-bromo-1-(5-methyl-1-phenyl-1*H*-pyrazol-4-yl)ethanone and 4-amino-3-mercapto 1,2,4-triazole in presence of ethanol, triethyl amine followed by condensation in acetic acid with good yields.

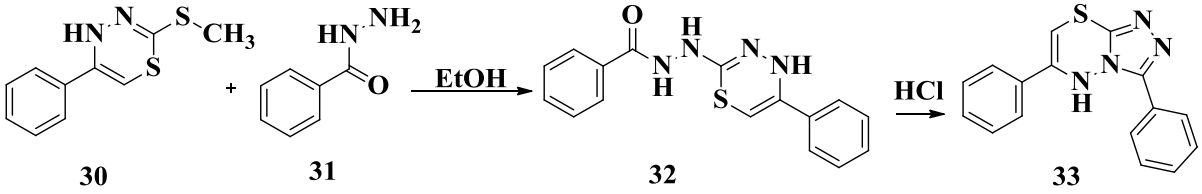


28 **17** **29**

R = H or Ph

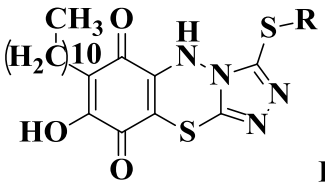
a = Et_3N / Ethanol / reflux **b = Acetic acid / reflux**

Usui[42] developed an efficient method for the synthesis of 3,6-diphenyl-5*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines. The target compound was synthesized by the reaction of 2-(methylthio)-5-phenyl-4*H*-1,3,4-thiadiazine with benzohydrazide in presence of ethanol followed by treatment with hydrochloric acid with good yields.



30 31 32 33

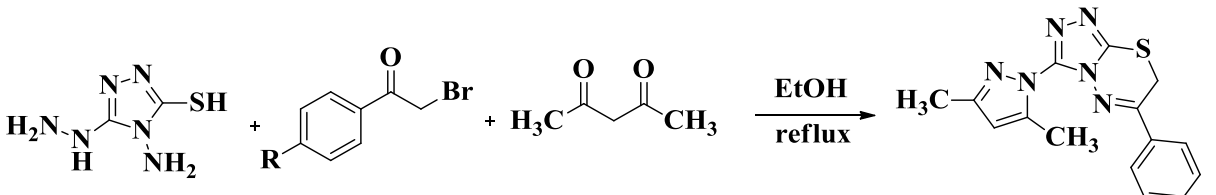
Srinivas and Rao[43] described a highly regioselective synthesis of 8-hydroxy-3-sulfanyl-7-undecyl-5*H*-benzo[*e*][1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine-6,9-diones by the condensation reaction of 2-bromo-3,6-dihydroxy-5-undecylcyclohexa-2,5-diene-1,4-dione, 4-amino-4*H*-1,2,4-triazole-3,5-dithiol in presence of ethanol followed by alkylation with different alkyl, aralkyl and phenacyl halides with satisfactory yields.



R = alkyl / Phenacyl

34

Tewodros and Rao[44] reported the synthesis of triazolothiadiazinyl pyrazolones via an efficient one pot multicomponent reaction method. The target compounds were synthesized with excellent yields by an equivalent reaction mixtures of 4-amino-5-hydrazinyl-4*H*-1,2,4-triazole-3-thiol, various substituted phenacylbromides and acetylacetone in presence of absolute ethanol under reflux condition



35 36 37 38

R = H , Cl, Br, -OCH$_3$, Ph

CHAPTER-IV
PART-A

SYNTHESIS AND ANTIVIRAL ACTIVITY OF 3-HETERYL / ARYL/ ALKYL-7*H*-[1,2,4]TRIAZOLO[3,4-*b*][1,3,4]THIADIAZIN-6-YL)-2*H*-CHROMEN-2-ONES

Present Work

Starting materials:

Thiocarbohydrazide was prepared according to the literature procedure by using hydrazine hydrate and carbon disulphide as described in the preceding chapter. Various substituted 3-(2-bromoacetyl)coumarins were prepared as per the literature procedure by bromination of substituted 3-acetylcoumarins in presence of chloroform. 2-(1,3-Dioxoisoindolin-2-yl)acetic acid[45] was prepared by using literature procedure and 5-methyl isoxazole-3-carboxylic acid was procured from commercial source.

Thiocarbohydrazide(TCH):

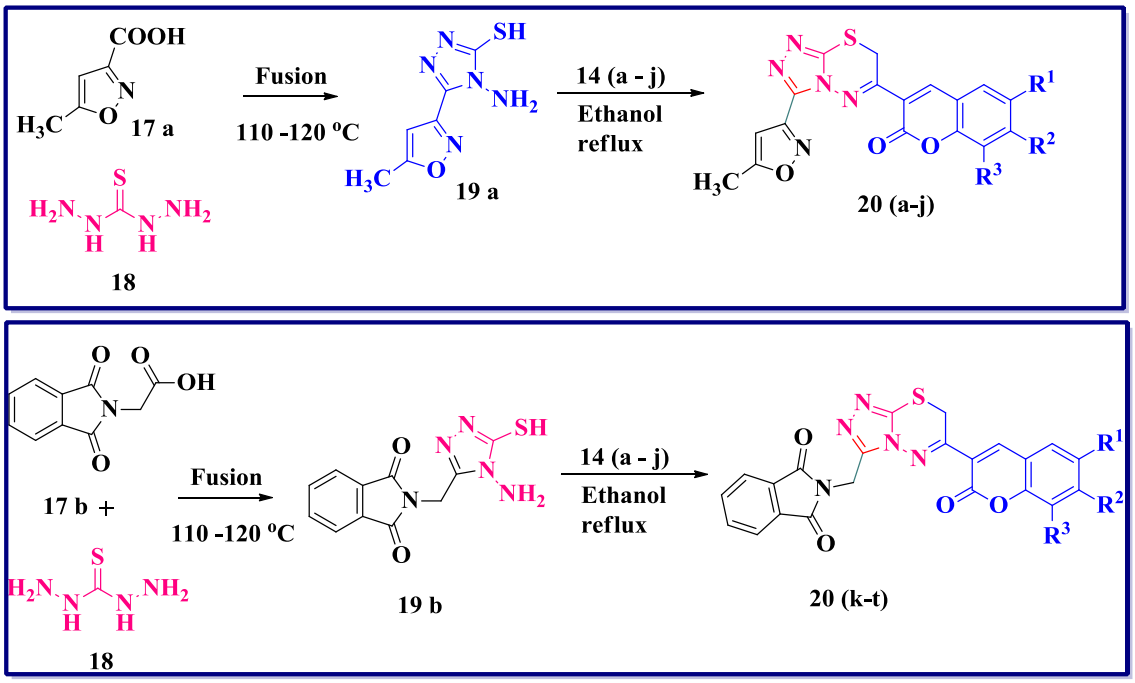


In view of the importance of triazolothiadiazines and various substituted coumarins as human therapeutic applications, we planned to synthesize these compounds for their biological activity.

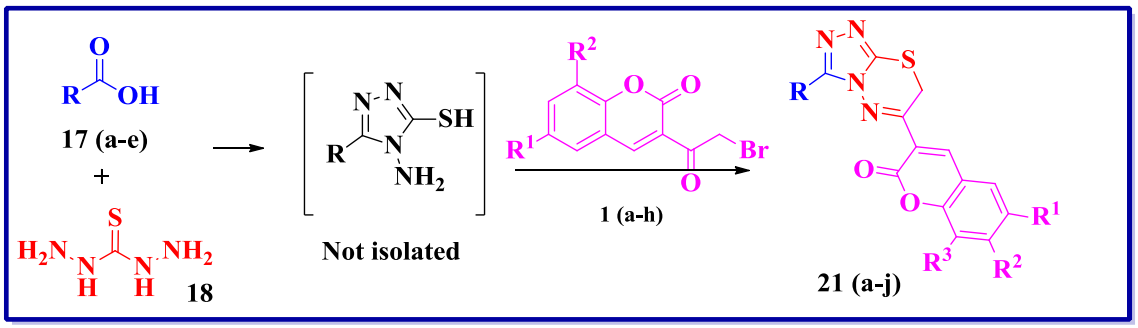
The present chapter describes the synthesis and antiviral activity of 3- heteryl / aryl/ alkyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-6-yl)-2*H*-chromen-2-ones (Part-A) and synthesis and biological activity evaluation of 3-(3-(((1*H*-benzo[*d*]imidazol-2-yl)methyl)thio)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-6-yl)-6,8-disubstituted-2*H*-chromen-2-ones as possible therapeutic agents (Part-B).

3-aryl/ hetery-triazolothiadiazinyl coumarin derivatives were synthesized in an efficient method by taking aromatic / heterocyclic carboxylic acids, thiocarbohydrazide and various substituted 3-(2-bromo acetyl) coumarins in two steps with good yields as shown in **scheme-1**. In the first step the reaction of carboxylic acids (**3 a-c)**, (5-methyl isoxazole-3-carboxylic, 2-(1,3-dioxoisoindolin-2-yl)acetic acid and 2-(4-methoxyphenyl)acetic acid) were fused with thiocarbohydrazide at 110 – 120 °C for about 15 minutes to get the intermediate (**40 a-c**). In the second step the isolated intermediate was treated with various substituted 3-(2-bromo acetyl) coumarins in presence of absolute ethanol at 60 °C for about 3 – 5 h to get the title products (**41a-t**). Similarly 3-(3-alkyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-6-yl)-2*H*-chromen-2-one were synthesized via a facile one pot tandem multicomponent approach with excellent

yields (**Scheme-2**). The expected target coumarinyl triazolo-thiadiazines (**43 a-j**) were synthesized by the condensation of aliphatic carboxylic acids (**42 a-c**) with thiocarbohydrazide (which generates the 5-alkyl substituted 4-amino 1,2,4-triazolo-3-thione) followed by further addition of substituted 3-(2-bromoacetyl) coumarins (**14a-j**) in a single step with good yields (**Table 1**). All the synthesized compounds were well characterized by physical and analytical spectral data. The compound **43d** was further characterized by single crystal X-Ray crystallography. The thermo ellipsoidal plot for the compound was shown in **Fig.2.** The single crystal data was shown in **Table 2-6**.



Scheme 1: Synthesis of 3- heteryl / aryl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-6-yl) -2*H*-chromen-2-ones



Scheme-2: A facile one pot synthesis of 3-(3-alkyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-6-yl)-2*H*-chromen-2-one.

Table.1 Synthesis of 3- heteryl / aryl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-6-yl)-2*H*-chromen-2-ones

S.No	Product	R	R^1	R^2	R^3	Yield (%)	M.P. (°C)
1	**41 a**	5-methylisoxazole	H	H	H	85	232-234
2	**41 b**	5-methylisoxazole	Cl	H	H	88	258-260
3	**41 c**	5-methylisoxazole	Cl	H	Cl	90	262-264
4	**41 d**	5-methylisoxazole	Br	H	H	87	235-237
5	**41 e**	5-methylisoxazole	Br	H	Br	92	274-276
6	**41 f**	5-methylisoxazole	H	H	OCH_3	80	211-213
7	**41 g**	5-methylisoxazole	Br	H	OCH_3	89	242-244
8	**41 h**	5-methylisoxazole	*t*-Bu	H	*t*-Bu	80	239-241
9	**41 i**	5-methylisoxazole	H	OH	H	82	294-296
10	**41 j**	5-methylisoxazole	NO_2	H	NO_2	86	260-262
11	**41 k**	2-methyl isoindoline-1,3 -dione	H	H	H	83	175-177
12	**41 l**	2-methyl isoindoline-1,3 -dione	Cl	H	H	91	245-247
13	**41m**	2-methyl isoindoline-1,3 -dione	Cl	H	Cl	92	260-262
14	**41 n**	2-methyl isoindoline-1,3 -dione	Br	H	H	80	230-232
15	**41 o**	2-methyl isoindoline-1,3 -dione	Br	H	Br	78	212-214
16	**41 q**	2-methyl isoindoline-1,3 -dione	H	H	OCH_3	80	236-238
17	**41 r**	2-methyl isoindoline-1,3 -dione	H	H	O-Et	89	252-254
18	**41 s**	4-methoxybenzyl	H	H	H	82	212-214
19	**41 t**	4-methoxybenzyl	Cl	H	H	88	189-191
20	**43 a**	methyl	H	H	H	85	266-268
21	**43 b**	methyl	Br	H	Br	90	259-261
22	**43 d**	ethyl	H	H	H	89	205-207
23	**43 e**	ethyl	Cl	H	H	86	262-264
24	**43 f**	ethyl	Br	H	H	91	257-259
25	**43 g**	ethyl	Br	H	Br	90	263-265
26	**43 i**	ethyl	Br	H	OCH_3	87	213-215
27	**43 j**	trifluoromethyl	H	H	OCH_3	85	198-200
28	**41 p**	2-methyl isoindoline-1,3 -dione	benzo[*f*]chromen			84	222-224
29	**43 c**	methyl	benzo[*f*]chromen			87	270-272
30	**43 h**	ethyl	benzo[*f*]chromen			82	251-253

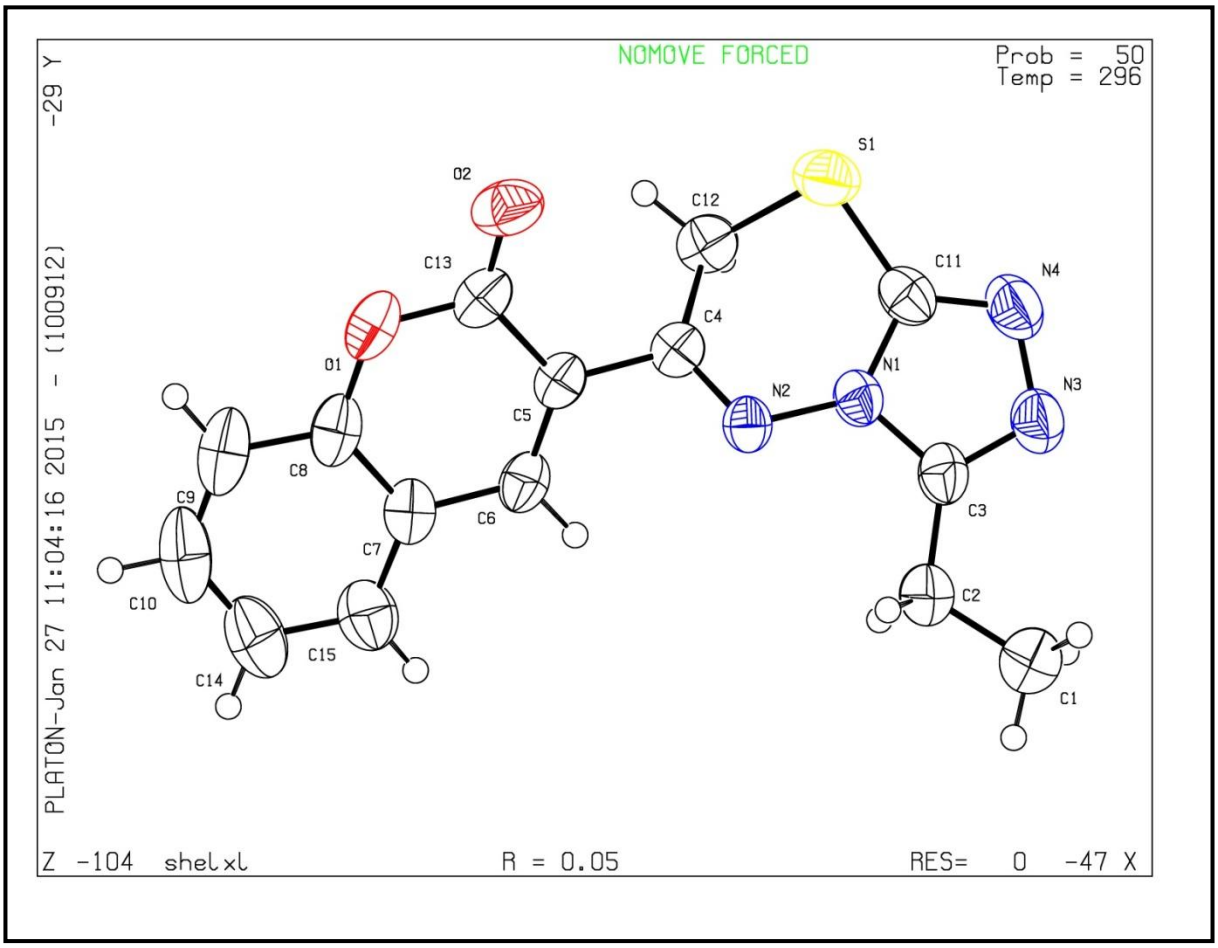


Fig: 2a. Platon representation of compound 43d.

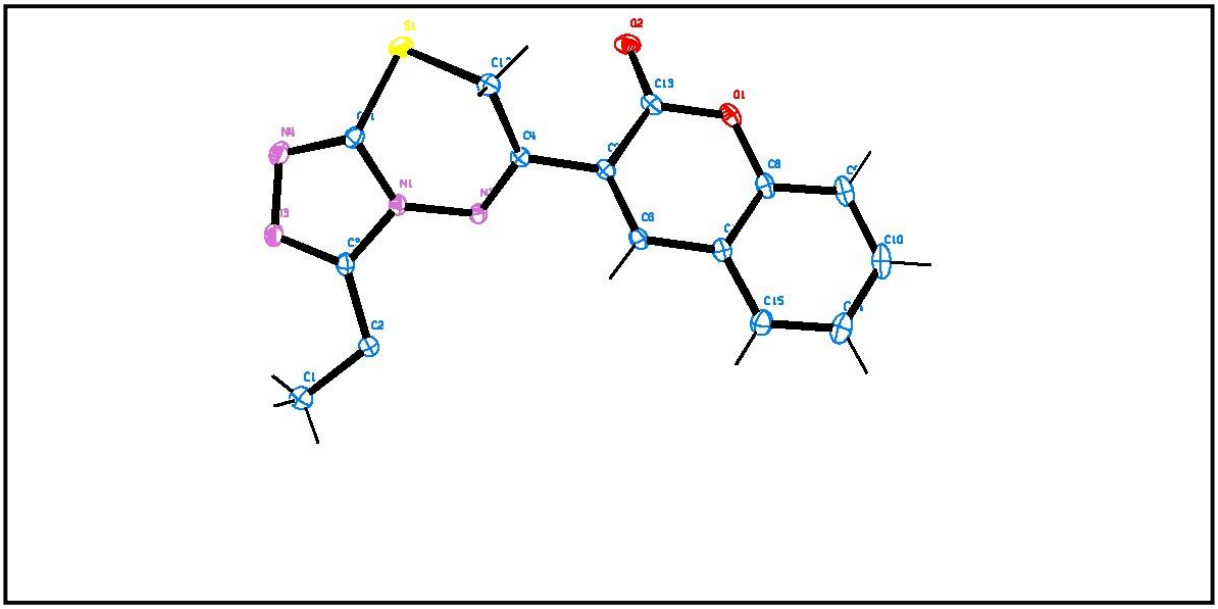


Fig. 2b Thermo ellipsoidal representation of compound **43d**

Table 2. Crystal data and structure refinement for compound **43d**.

Identification code	shelxl
Empirical formula	C_{15} H_{12} N_4 O_2 S
Formula weight	312.35
Temperature	296(2) K
Wavelength	0.71073 A
Crystal system, space group	**Monoclinic, P21/c**
Unit cell dimensions	a = 8.1256(4) A alpha = 90 deg. b = 19.8813(11) A beta = 112.939(2) deg. c = 9.4961(5) A gamma = 90 deg.
Volume	1412.76(13) A^3
Z, Calculated density	4, 1.469 Mg/m^3
Absorption coefficient	0.242 mm^-1
F(000)	648
Crystal size	0.35 x 0.30 x 0.20 mm
Theta range for data collection	2.05 to 28.24 deg.
Limiting indices	-10<=h<=10, -26<=k<=26, -12<=l<=10
Reflections collected / unique	12375 / 3462 [R(int) = 0.0284]
Completeness to theta	= 28.24 99.0 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.9532 and 0.9200
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	3462 / 0 / 200
Goodness-of-fit on F^2	0.931
Final R indices [I>2sigma(I)]	R1 = 0.0455, wR2 = 0.1325
R indices (all data)	R1 = 0.0793, wR2 = 0.1692
Largest diff. peak and hole	0.309 and -0.292 e.A^-3

Table 3. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters (A^2 x 10^3) for **43d**. U(eq) is defined as one third of the trace of the orthogonalized Uij tensor.

	x	y	z	U(eq)
S(1)	9364(1)	8579(1)	12333(1)	63(1)
O(1)	6438(2)	10675(1)	7805(2)	55(1)
O(2)	8299(2)	10332(1)	10068(2)	65(1)
N(1)	5911(2)	8214(1)	11165(2)	43(1)
N(2)	5330(2)	8764(1)	10186(2)	41(1)
N(3)	5731(3)	7367(1)	12550(2)	58(1)
N(4)	7528(3)	7565(1)	13050(3)	63(1)
C(1)	1906(4)	7195(2)	11007(4)	79(1)
C(2)	2835(3)	7744(1)	10556(3)	61(1)
C(3)	4789(3)	7762(1)	11430(3)	49(1)
C(4)	6534(3)	9049(1)	9839(2)	41(1)
C(5)	5891(3)	9601(1)	8720(2)	41(1)
C(6)	4350(3)	9548(1)	7502(2)	47(1)
C(7)	3757(3)	10062(1)	6364(2)	48(1)
C(8)	4851(3)	10620(1)	6556(3)	51(1)
C(9)	4381(5)	11138(1)	5501(3)	71(1)
C(10)	2808(5)	11095(2)	4250(4)	82(1)
C(11)	7592(3)	8073(1)	12219(3)	50(1)
C(12)	8424(3)	8805(1)	10332(3)	51(1)
C(13)	6980(3)	10207(1)	8955(3)	48(1)
C(14)	1709(5)	10540(2)	4005(3)	76(1)
C(15)	2183(4)	10027(1)	5061(3)	63(1)

Table 4. Bond lengths [A] and angles [deg] for compound **43d**.

Bond	Bond length (A)	Bond	Bond Angle (degrees)
S(1)-C(11)	1.725(2)	C(11)-S(1)-C(12)	94.50(11)
S(1)-C(12)	1.807(2)	C(8)-O(1)-C(13)	122.40(17)
O(1)-C(8)	1.374(3)	C(11)-N(1)-C(3)	106.06(17)
O(1)-C(13)	1.370(3)	C(11)-N(1)-N(2)	129.00(17)
O(2)-C(13)	1.202(3)	C(3)-N(1)-N(2)	124.08(17)
N(1)-C(11)	1.371(3)	C(4)-N(2)-N(1)	115.18(16)
N(1)-C(3)	1.372(3)	C(3)-N(3)-N(4)	108.0(2)
N(1)-N(2)	1.393(2)	C(11)-N(4)-N(3)	107.23(18)
N(2)-C(4)	1.280(3)	C(2)-C(1)-H(1A)	109.5
N(3)-C(3)	1.304(3)	C(2)-C(1)-H(1B)	109.5
N(3)-N(4)	1.404(3)	H(1A)-C(1)-H(1B)	109.5
N(4)-C(11)	1.296(3)	C(2)-C(1)-H(1C)	109.5
C(1)-C(2)	1.482(4)	H(1A)-C(1)-H(1C)	109.5
C(1)-H(1A)	0.9600	H(1B)-C(1)-H(1C)	109.5
C(1)-H(1B)	0.9600	C(1)-C(2)-C(3)	114.3(2)

C(1)-H(1C)	0.9600	C(1)-C(2)-H(2A)	108.7
C(2)-C(3)	1.478(3)	C(3)-C(2)-H(2A)	108.7
C(2)-H(2A)	0.9700	C(1)-C(2)-H(2B)	108.7
C(2)-H(2B)	0.9700	C(3)-C(2)-H(2B)	108.7
C(4)-C(5)	1.474(3)	H(2A)-C(2)-H(2B)	107.6
C(4)-C(12)	1.500(3)	N(3)-C(3)-N(1)	108.9(2)
C(5)-C(6)	1.338(3)	N(3)-C(3)-C(2)	127.2(2)
C(5)-C(13)	1.461(3)	N(1)-C(3)-C(2)	123.89(18)
C(6)-C(7)	1.428(3)	N(2)-C(4)-C(5)	115.04(18)
C(6)-H(6)	0.9300	N(2)-C(4)-C(12)	124.65(18)
C(7)-C(8)	1.388(3)	C(5)-C(4)-C(12)	119.84(18)
C(7)-C(15)	1.392(3)	C(6)-C(5)-C(13)	120.25(19)
C(8)-C(9)	1.383(3)	C(6)-C(5)-C(4)	120.97(18)
C(9)-C(10)	1.367(5)	C(13)-C(5)-C(4)	118.78(19)
C(9)-H(9)	0.9300	C(5)-C(6)-C(7)	121.65(19)
C(10)-C(14)	1.381(5)	C(5)-C(6)-H(6)	119.2
C(10)-H(10)	0.9300	C(7)-C(6)-H(6)	119.2
C(12)-H(12A)	0.9700	C(8)-C(7)-C(15)	118.5(2)
C(12)-H(12B)	0.9700	C(8)-C(7)-C(6)	117.5(2)
C(14)-C(15)	1.376(4)	C(15)-C(7)-C(6)	124.0(2)
C(14)-H(14)	0.9300	O(1)-C(8)-C(9)	117.8(2)
C(15)-H(15)	0.9300	O(1)-C(8)-C(7)	120.98(19)
		C(9)-C(8)-C(7)	121.2(3)
		C(10)-C(9)-C(8)	118.9(3)
		C(10)-C(9)-H(9)	120.5
		C(8)-C(9)-H(9)	120.5
		C(9)-C(10)-C(14)	121.4(3)
		C(9)-C(10)-H(10)	119.3
		C(14)-C(10)-H(10)	119.3
		N(4)-C(11)-N(1)	109.8(2)
		N(4)-C(11)-S(1)	130.35(18)
		N(1)-C(11)-S(1)	119.78(16)
		C(4)-C(12)-S(1)	111.76(16)
		C(4)-C(12)-H(12A)	109.3
		S(1)-C(12)-H(12A)	109.3
		C(4)-C(12)-H(12B)	109.3
		S(1)-C(12)-H(12B)	109.3
		H(12A)-C(12)-H(12B)	107.9
		O(2)-C(13)-O(1)	117.42(19)
		O(2)-C(13)-C(5)	125.7(2)
		O(1)-C(13)-C(5)	116.8(2)
		C(15)-C(14)-C(10)	119.4(3)
		C(15)-C(14)-H(14)	120.3
		C(10)-C(14)-H(14)	120.3
		C(14)-C(15)-C(7)	120.7(3)
		C(14)-C(15)-H(15)	119.7
		C(7)-C(15)-H(15)	119.7

Table 5. Anisotropic displacement parameters (A^2 x 10^3) for compound **43d**. The anisotropic displacement factor exponent takes the form: -2 pi^2 [h^2 a^{*2} U11 + ... + 2 h k a* b* U12]

	U11	U22	U33	U23	U13	U12
S(1)	49(1)	63(1)	63(1)	-3(1)	9(1)	-2(1)
O(1)	75(1)	39(1)	66(1)	-4(1)	43(1)	-11(1)
O(2)	51(1)	52(1)	87(1)	-13(1)	22(1)	-8(1)
N(1)	48(1)	36(1)	44(1)	4(1)	16(1)	5(1)
N(2)	47(1)	35(1)	43(1)	2(1)	20(1)	4(1)
N(3)	65(1)	45(1)	58(1)	10(1)	17(1)	2(1)
N(4)	65(1)	51(1)	59(1)	11(1)	11(1)	8(1)
C(1)	72(2)	79(2)	77(2)	16(2)	19(2)	-13(1)
C(2)	60(2)	53(1)	65(2)	13(1)	17(1)	-2(1)
C(3)	59(1)	37(1)	49(1)	5(1)	20(1)	2(1)
C(4)	46(1)	36(1)	44(1)	-6(1)	21(1)	0(1)
C(5)	48(1)	34(1)	48(1)	-4(1)	26(1)	-2(1)
C(6)	59(1)	35(1)	50(1)	-2(1)	26(1)	-7(1)
C(7)	64(1)	40(1)	47(1)	1(1)	30(1)	4(1)
C(8)	77(2)	39(1)	55(1)	0(1)	45(1)	4(1)
C(9)	116(2)	48(1)	73(2)	9(1)	63(2)	2(1)
C(10)	132(3)	66(2)	69(2)	25(2)	62(2)	29(2)
C(11)	52(1)	46(1)	46(1)	0(1)	14(1)	9(1)
C(12)	49(1)	46(1)	61(1)	-5(1)	25(1)	3(1)
C(13)	53(1)	37(1)	64(1)	-8(1)	35(1)	-4(1)
C(14)	96(2)	83(2)	52(2)	14(1)	31(2)	25(2)
C(15)	76(2)	61(2)	53(1)	2(1)	24(1)	5(1)

Table 6. Hydrogen coordinates (x 10^4) and isotropic displacement parameters (A^2 x 10^3) for compound 43d.

	X	Y	Z	U(eq)
H(1A)	2313	6769	10794	119
H(1B)	640	7234	10438	119
H(1C)	2162	7228	12081	119
H(2A)	2333	8171	10686	74
H(2B)	2602	7696	9478	74
H(6)	3647	9167	7393	56
H(9)	5122	11510	5641	85
H(10)	2471	11446	3549	98
H(12A)	9147	9156	10149	61
H(12B)	8452	8417	9724	61
H(14)	658	10513	3135	92
H(15)	1446	9653	4902	76

The compounds 3- heteryl / aryl/ alkyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-6-yl)-2*H*-chromen-2-ones (**41 a-t** and **43 a-j**) were evaluated against a wide range of human RNA and DNA viruses in different types of cell cultures. The HEL cell culture was used to evaluate the compounds against *herpes simplex virus type I* (KOS) [HSV-1 KOS], *herpes simplex virus type 2* (G) [HSV-2 G], *vaccinia virus* [VV], *vesicular stomatitis virus* (VSV), *thymidine kinase-deficient herpes simplex virus type 1* (HSV-1 TK^- KOS ACV^r) and *adeno virus type 2* (AV-2). Based on the microscopical analysis of the virus-induced cytopathicity (CPE) the antiviral potency of the compounds was determined and compared to different reference antiviral drugs (brivudin, cidofovir, acyclovir, ganciclovir, zalcitabine and alovudine). As summarized in **Table 7**, none of the compound exhibited any antiviral activity against tested viral strains.

Vesicular stomatitis virus (VSV) was also tested in the HeLa cell culture, together with the RNA viruses *coxsackie virus B4* (CV B4) and respiratory *syncytial virus* (RSV). Here, dextran sulphate (DS-10000) and ribavirin were included as reference compounds (**Table 8**).

Next, Vero cells were utilized to evaluate the compounds against *parainfluenza* type 3 virus (PI-3V), *reovirus* type 1 (RV-1), *sindbis virus* (SV), *coxsackie virus B4* (CV B4) and *punto toro virus* (PTV), together the reference compounds DS-10000 and ribavirin. As depicted in **Table 9**

Finally, the same compounds were tested for their potency against *influenza* virus (IV). The MDCK cell line was used to infect with *influenza A* virus type H1N1 (strain A/Ned/378/05) and type H3N2 (strain A/HK/7/87), and *influenza B* virus (strain B/Ned/537/05). As reference compounds, zanamivir, ribavirin, amantadine and rimantadine were selected. As summarized in **Table 10.**

Evaluation of these 3- heteryl / aryl/ alkyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-6-yl)-2*H*-chromen-2-ones (**41 a-t** and **43 a-j**) against *feline corona virus* and feline herpes virus was less successful as none of the compounds showed any detectable activity against these feline viruses (**Table 11**).

Table.7:
Cytotoxicity and antiviral activity of compounds in HEL cell cultures.

Compound	Minimum Cytotoxic Concentration (μM)[a]	EC_{50}[b] (μM)					
		Herpes simplex virus-1 (KOS)	*Herpes simplex virus*-2 (G)	*Vaccinia virus*	*Vesicular stomatitis virus*	*Herpes simplex virus*-1 TK^- KOS ACV^r	*Adeno virus*-2
41a	>100	>100	>100	>100	>100	>100	>100
41b	>100	>100	>100	>100	>100	>100	>100
41c	100	>20	>20	>20	>20	>20	>20
41d	100	>20	>20	>20	>20	>20	>20
41e	100	>20	>20	>20	>20	>20	>20
41f	100	>20	>20	>20	>20	>20	>20
41g	>100	>100	>100	>100	>100	>100	>100
41h	>100	>100	>100	>100	>100	>100	>100
41i	>100	>100	>100	>100	>100	>100	>100
41j	100	>20	>20	>20	>20	>20	>20
41k	>100	>100	>100	>100	>100	>100	>100
41l	>100	>100	>100	>100	>100	>100	>100
41m	>100	>100	>100	>100	>100	>100	>100
41n	>100	>100	>100	>100	>100	>100	>100
41o	>100	>100	>100	>100	>100	>100	>100
41p	>100	>100	>100	>100	>100	>100	>100
41q	100	>100	>100	>100	>100	>100	>100
41r	>100	>100	>100	>100	>100	>100	>100
41s	100	>100	>100	>100	>100	>100	>100
41t	>100	>100	>100	>100	>100	>100	>100
43a	>100	>100	>100	>100	>100	>100	>100
43b	>100	>100	>100	>100	>100	>100	>100
43c	100	>20	>20	>20	>20	>20	>20
43d	>100	>100	>100	>100	>100	>100	>100
43e	100	>20	>20	>20	>20	>20	>20
43f	>100	>100	>100	>100	>100	>100	>100
43g	100	>20	>20	>20	>20	>20	>20
43h	≥20	>20	>20	>20	>20	>20	>20
43i	100	>20	>20	>20	>20	>20	>20
43j	>100	>100	>100	>100	>100	>100	>100
Brivudin	>250	0.05	181	20	>250	150	-
Cidofovir	>250	1.6	1.0	22	>250	1.6	7.9
Acyclovir	>250	0.4	0.2	>250	>250	98	-
Ganciclovir	>100	0.06	0.06	>100	>100	2.9	-
Zalcitabine	>250	-	-	-	-	-	7.5
Alovudine	>250	-	-	-	-	-	16

[a] Required to cause a microscopically detectable alteration of normal cell morphology.
[b] Required to reduce virus-induced cytopathogenicity by 50 %.

Table 8:
Cytotoxicity and antiviral activity of compounds in HeLa cell cultures.

Compound	Minimum Cytotoxic Concentration (μM)[a]	EC 50[b] (μM)		
		Vesicular stomatitis virus	*Coxsackie virus* B4	*Respiratory syncytial virus*
41a	>100	>100	>100	>100
41b	>100	>100	>100	>100
41c	100	>20	>20	>20
41d	>100	>100	>100	>100
41e	>100	>100	>100	>100
41f	>100	>100	>100	>100
41g	≥100	>100	>100	>100
41h	100	>20	>20	>20
41i	>100	>100	>100	>100
41j	>100	>100	>100	>100
41k	>100	>100	>100	>100
41l	>100	>100	>100	>100
41m	>100	>100	>100	>100
41n	>100	>100	>100	>100
41o	>100	>100	>100	>100
41p	>100	>100	>100	>100
41q	>100	>100	>100	>100
41r	>100	>100	>100	>100
41s	>100	>100	>100	>100
41t	>100	>100	>100	>100
43a	>100	>100	>100	>100
43b	>100	>100	>100	>100
43c	>100	>100	>100	>100
43d	>100	>100	>100	>100
43e	100	>20	>20	>20
43f	>100	>100	>100	>100
43g	>100	>100	>100	>100
43h	>100	>100	>100	>100
43i	100	>20	>20	>20
43j	>100	>100	>100	>100
DS-10000 (μg/ml)	>100	1.8	27	3.0
Ribavirin	>250	22	81	7.5

[a] Required to cause a microscopically detectable alteration of normal cell morphology.
[b] Required to reduce virus-induced cytopathogenicity by 50 %.

Table 9
Cytotoxicity and antiviral activity of compounds in Vero cell cultures.

Compound	Minimum Cytotoxic Concentration[a] (μM)	EC50[b](μM)				
		*Para-influenza-*3 virus	*Reovirus-*1	*Sindbis virus*	*Coxsackie virus* B4	*Punta Toro virus*
41a	>100	>100	>100	>100	>100	>100
41b	100	>20	>20	>20	>20	>20
41c	≥20	>20	>20	>20	>20	>20
41d	>100	>100	>100	>100	>100	>100
41e	100	>20	>20	>20	>20	>20
41f	100	>20	>20	>20	>20	>20
41g	100	>20	>20	>20	>20	>20
41h	100	>20	>20	>20	>20	>20
41i	≥100	>100	>100	>100	>100	>100
41j	>100	>100	>100	>100	>100	>100
41k	>100	>100	>100	>100	>100	>100
41l	>100	>100	>100	>100	>100	>100
41m	>100	>100	>100	>100	>100	>100
41n	>100	>100	>100	>100	>100	>100
41o	>100	>100	>100	>100	>100	>100
41p	>100	>100	>100	>100	>100	>100
41q	>100	>100	>100	>100	>100	>100
41r	>100	>100	>100	>100	>100	>100
41s	>100	>100	>100	>100	>100	>100
41t	>100	>100	>100	>100	>100	>100
43a	>100	>100	>100	>100	>100	>100
43b	>100	>100	>100	>100	>100	>100
43c	>100	>100	>100	>100	>100	>100
43d	>100	>100	>100	>100	>100	>100
43e	100	>20	>20	>20	>20	>20
43f	>100	>100	>100	>100	>100	>100
43g	100	>20	>20	>20	>20	>20
43h	100	>20	>20	>20	>20	>20
43i	>100	>100	>100	>100	>100	>100
43j	>100	>100	>100	>100	>100	>100
DS-10.000 (μg/ml)	>100	>100	>100	50	8.9	20
Ribavarin	>250	126	>250	250	>250	112

[a] Required to cause a microscopically detectable alteration of normal cell morphology.
[b] Required to reduce virus-induced cytopathogenicity by 50 %.

Table 10

Cytotoxicity and antiviral activity of compounds in MDCK cell cultures.

Compound	Cytotoxicity (μM)		Antiviral EC_{50}[c] (μM)					
	Minimum Cytotoxic Conc.[a]	CC_{50}[b]	*Influenza* A/H1N1 A/Ned/378/05		*Influenza*A/H3 N2 A/HK/7/87		*Influenza* B B/Ned/537/05	
			CPE	MTS	CPE	MTS	CPE	MTS
41a	>100	>100	>100	>100	>100	>100	>100	>100
41b	100	>100	>20	>20	>20	>20	>20	>20
41c	20	9.8	>4	>4	>4	>4	>4	>4
41d	100	44.7	>20	>20	>20	>20	>20	>20
41e	100	>100	>20	>20	>20	>20	>20	>20
41f	100	>100	>20	>20	>20	>20	>20	>20
41g	100	>100	>20	>20	>20	>20	>20	>20
41h	20	>100	>4	>4	>4	>4	>4	>4
41i	100	50.6	>20	>20	>20	>20	>20	>20
41j	>100	>100	>100	>100	>100	>100	>100	>100
41k	>100	>100	>100	>100	>100	>100	>100	>100
41l	>100	>100	>100	>100	>100	>100	>100	>100
41m	>100	≥100	>100	>100	>100	>100	>100	>100
41n	>100	100	>100	>100	>100	>100	>100	>100
41o	>100	100	>100	>100	>100	>100	>100	>100
41p	>100	100	>100	>100	>100	>100	>100	>100
41q	59.8	≥20	>100	>100	>100	>100	>100	>100
41r	>100	≥100	>100	>100	>100	>100	>100	>100
41s	56.6	100	>100	>100	>100	>100	>100	>100
41t	>100	>100	>100	>100	>100	>100	>100	>100
43a	>100	>100	>100	>100	>100	>100	>100	>100
43b	>100	>100	>100	>100	>100	>100	>100	>100
43c	>100	>100	>100	>100	>100	>100	>100	>100
43d	>100	>100	>100	>100	>100	>100	>100	>100
43e	48.8	100	>20	>20	>20	>20	>20	>20
43f	51.0	100	>20	>20	>20	>20	>20	>20
43g	>100	100	>20	>20	>20	>20	>20	>20
43h	0.3	0.8	>0.16	>0.16	>0.16	>0.16	>0.16	>0.16
43i	29.1	20	>4	>4	>4	>4	>4	>4
43j	>100	100	>20	>20	>20	>20	>20	>20
Zanamivir	>100	>100	0.2	0.3	0.5	0.6	0.4	0.4
Ribavirin	≥100	>100	8.9	8.2	8.9	5.3	6.8	8.4
Amantadie	>200	>200	8.0	19.1	0.8	1.6	>200	>200
Rimantadie	>200	>200	200	>200	0.3	0.1	>200	>200

[a] Minimum compound concentration that causes a microscopically detectable alteration of normal cell morphology.

[b] 50% Cytotoxic concentration, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay.

[c] 50% Effective concentration, or concentration producing 50% inhibition of virus-induced cytopathic effect, as determined by visual scoring of the CPE, or by measuring the cell viability with the colorimetric formazan-based MTS assay.

Table 11.
Anti-Feline Corona Virus (FIPV) and anti-Feline Herpes Virus activity and cytotoxicity in CRFK cell cultures.

Compound	CC_{50}[a] (µM)	EC_{50}[b] (µM)	
		Feline CoronaVirus (FIPV)	*Feline Herpes Virus*
41a	>100	>100	>100
41b	>100	>100	>100
41c	55.1	>20	>20
41d	61.7	>20	>20
41e	>100	>100	>100
41f	>100	>100	>100
41g	>100	>100	>100
41h	>100	>100	>100
41i	>100	>100	>100
41j	>100	>100	>100
41k	>100	>100	>100
41l	>100	>100	>100
41m	>100	>100	>100
41n	>100	>100	>100
41o	>100	>100	>100
41p	>100	>100	>100
41q	>100	>100	>100
41r	>100	>100	>100
41s	>100	>100	>100
41t	>100	>100	>100
43a	>100	>100	>100
43b	>100	>100	>100
43c	>100	>100	>100
43d	>100	>100	>100
43e	>100	>100	>100
43f	>100	>100	>100
43g	>100	>100	>100
43h	>100	>100	>100
43i	53.9	>20	>20
43j	>100	>100	>100
HHA (µg/ ml)	>100	24.3	15.1
UDA (µg/ ml)	>100	7.3	4.3
Ganciclovir	>100	>100	8.6

[a] 50% Cytotoxic concentration, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay.
[b] 50% Effective concentration, or concentration producing 50% inhibition of virus-induced cytopathic effect, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay.

CRFK cells: Crandell-Rees Feline Kidney cells.

In conclusion we have synthesized 3- heteryl / aryl/ alkyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-6-yl)-2*H*-chromen-2-ones (**41 a-t** and **43 a-j**) via an efficient method. The main advantages of the method are mild reaction conditions, short reaction time and easy work up with high yields. All the synthesized compounds were screened against broad panel of human viral strains in different cell cultures. But none of the compounds exhibited potency against the tested viral strains.

EXPERIMENTAL:

General procedure for the synthesis of 4-amino-5-(heteryl / aryl)-4*H*-1,2,4-triazole-3-thiol (40 a-c):

A mixture of 5-methylisoxazole-3-carboxylicacid **39a** (0.635g, 5 mmol) or 2-(1,3-dioxoisoindolin-2-yl)acetic acid **39b** (1.025g, 5 mmol) or 2-(4-methoxyphenyl)acetic acid **39c** (0.830g, 5 mmol) and thiocarbohydrazide **2** (0.530g, 5 mmol) were taken in a round bottom flask and the contents were mixed thoroughly. The reaction contents were slowly fused by raising the temperature up to 110 ° - 120 °C. The molten reaction mass was kept at the same temperature for about 10 – 15 minutes by thorough mixing. Finally the temperature of the reaction mass slowly decreased to room temperature and stirred with distilled water for few minutes. The separated solid intermediate was filtered and dried. Colour: Pale yellow solid;

Yield: **40a** (0.552 g, 56%); m.p. 185 – 187 ^{o}C.

40b (0.990 g, 72%); m.p.: 192 – 194 ^{o}C. [Literature 189 -192 ^{o}C] [46]

40c (0.755 g, 64%); m.p. 176 – 178 ^{o}C.

Typical synthetic ptocedure for the synthesis of 3-aryl/ heteroaryl -7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-6-yl)-2*H*-chromen-2-ones (41a-t):

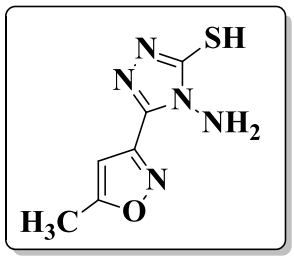
A mixture of 4-amino-5-(5-methylisoxazol-3-yl)-4H-1,2,4-triazole-3-thiol **40a** (0.197g, 1 mmol) or 2-((4-amino-5-mercapto-4H-1,2,4-triazol-3-yl)methyl)isoindoline-1,3-dione (0.275 1 mmol) or 4-amino-5-(4-methoxybenzyl)-4H-1,2,4-triazole-3-thiol (0.236g, 1 mmol) and substituted 3-(2-bromoacetyl)-2*H*-chromen-2-one 1**4a-j** (1 mmol) in ethanol (5 ml) were taken in a round bottomed flask, stirred for 10 minutes at room temperature and finally the reaction mixture was refluxed for about 3 h. After completion of the reaction (monitored by TLC), the reaction mixture was cooled to room temperature. The solid product obtained was filtered, dried and purified by recrystallization from ethanol.

A facile one pot multicomponent synthesis of 3-alkyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-6-yl)-2*H*-chromen-2-one derivatives (43a-j):

A mixture of thiocarbohydrazide **2** (0.106 g, 1 mmol) and acetic acid/ propionic acid/ trifluoroacetic acid (2 mL) was taken in a round bottom flask equipped with condenser and mechanical stirrer and the contents were heated at 75 – 80 ^{o} C for about one hour. To this hot reaction mixture substituted 3-(2-bromoacetyl)-2*H*-chromen-2-ones **43a-j** (1 mmol) were added. The reaction mixture was further heated for about 2 h. The progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was cooled to room temperature and poured into water. The solid separated was filtered washed thoroughly with water, dried and recrystallized from ethanol solvent.

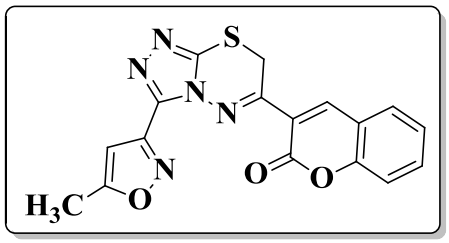
SPECTRAL DATA:

4-Amino-(5-methylisoxazol-3-yl)-4-triazoleo3-thiol (40a):



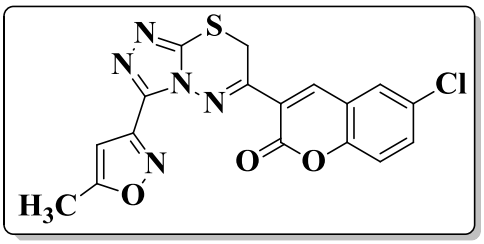
White solid; **FTIR (KBr, ν_{max}, cm^{-1}):** 3273 (-NH2); 1695 (C=N); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 2.51 (s, 3H, -CH_3), 5.85 (s, 2H, -NH_2), 6.84 (s, 1H, C_4-H of isoxazole), 14.19 (s, 1H, -SH).

3-(3-(5-Methylisoxazol-3-yl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-6-yl)-2*H*-chromen-2-one (41a):



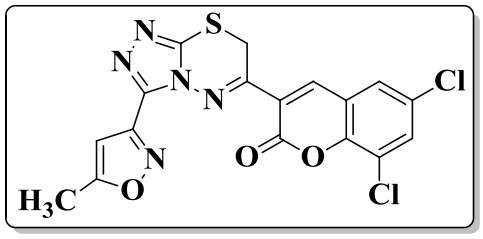
Red solid; **FTIR (KBr, ν_{max}, cm^{-1}):** 1707 (lactone C=O); 1608 (C=N); **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm)**: 2.54 (s, 3H,-CH_3), 4.16 (s, 2H, -S-CH_2), 6.70 (s, 1H, C_4-H of isoxazole), 7.41 (t, *J* = 8.8 Hz, 2H, Ar-H), 7.66- 7.70 (m, 2H, Ar-H), 8.50 (s, 1H, C_4-H of coumarin); **^{13}C NMR (100 MHz, DMSO-d_6, δ ppm)**: 11.80, 24.28, 101.95, 116.35, 118.36, 122.53, 125.21, 130.07, 134.03, 144.20, 144.24, 144.98, 151.14, 154.03, 154.55, 158.57, 170.97; *Anal.* calcd for $C_{17}H_{11}N_5O_3S$: C, 55.88; H, 3.03; N, 19.17; S, 8.78%. Found: C, 55.82; H, 3.14; N, 19.24; S, 8.71%.

6-Chloro-3-(3-(5-methylisoxazol-3-yl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-6-yl)-2*H*-chromen-2-one (41b):



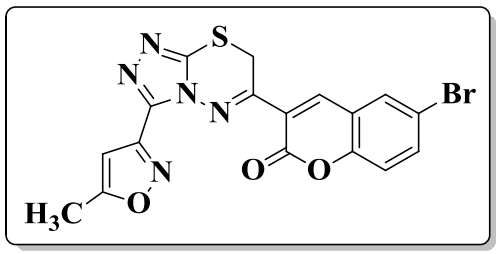
White solid; **FTIR (KBr, υ_{max} cm^{-1}):** 1716 (lactone, -C=O), 1602 (-C=N), 1241 (-C-O-N), **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm):** 2.55 (s, 3H- CH_3), 4.16 (s, 2H, -S-CH_2), 6.71 (s, 1H, C_4-H of isoxazole), 7.38 (d, *J* = 9.2 Hz, 1H, Ar-H), 7.62 (t, *J* = 9.2 Hz, 1H, Ar-H), 7.67 (d, *J* = 2Hz, 1H, Ar-H), 8.42 (s, 1H, C_4-H of coumarin). **MS (ESI m/z):** 399.95 $[M]^+$, 401.75 $[M+2H]^+$; *Anal.* calcd for: $C_{17}H_{10}ClN_5O_3S$: C, 51.07; H, 2.52; N, 17.52; S, 8.02%. Found: C, 51.14; H, 2.56; N, 17.56; S, 8.14%.

6,8-Dichloro-3-(3-(5-methylisoxazol-3-yl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-6-yl)-2*H*-chromen-2-one (41c):



Yellow solid; **FTIR (KBr, υ_{max} cm^{-1}):** 1723 (lactone -C=O), 1601 (-C=N), 1241 (-C-O-N), **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 2.51 (s, 3H, -CH_3), 4.35 (s, 2H, -S-CH_2), 6.86 (s, 1H, C_4-H of isoxazole), 7.78-7.80 (m, 1H, Ar-H), 8.06 (d, *J* = 2Hz, 1H, Ar-H), 8.55 (s, 1H, C_4-H of coumarin). *Anal.* calcd for $C_{17}H_9Cl_2N_5O_3S$: C, 47.02; H, 2.09; N, 16.13; S, 7.38%. Found: C, 47.14; H, 2.14; N, 16.18; S, 7.29%.

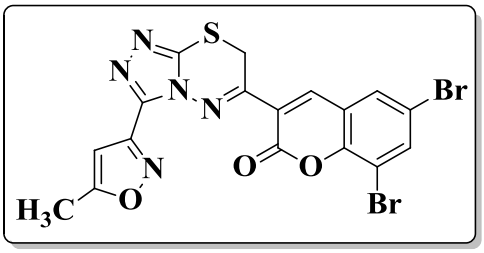
6-Bromo-3-(3-(5-methylisoxazol-3-yl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-6-yl)-2*H*-chromen-2-one (41d):



Red solid; **FTIR (KBr, ν_{max} cm^{-1}):** 1717 (lactone C=O); 1599 (C=N); 1241 (-C-O-N); **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm):** 2.55 (s, 3H, -CH_3), 4.16 (s,2H,-S-CH_2), 6.71 (s, 1H, C_4-H of isoxazole), 7.30 (t, *J* = 8.8Hz, 1H, Ar-H), 7.48-7.77 (m, 1H, Ar-H), 7.82 (d, *J* = 2.4Hz, 1H, Ar-H), 8.42 (s, 1H, C_4- H of coumarin). **MS (ESI m/z):** 443.90 $[M]^+$, 445.88 $[M+2H]^+$; *Anal.* calcd for

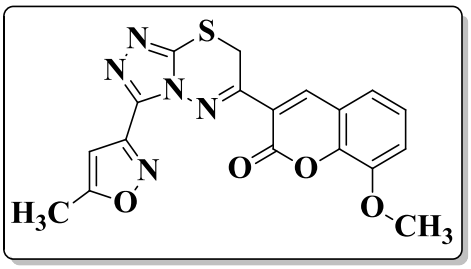
$C_{17}H_{10}BrN_5O_3S$: C, 45.96; H, 2.27; N, 15.76; S, 7.22%. Found: C, 45.91; H, 2.32; N, 15.70; S, 7.27%.

6,8-Dibromo-3-(3-(5-methylisoxazol-3-yl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-6-yl)-2*H*-chromen-2-one (41e):



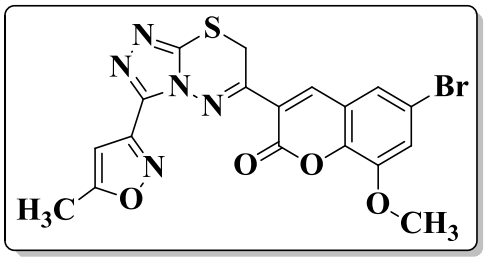
Red solid; **FTIR (KBr, ν_{max} cm^{-1}):** 1748 (lactone C=O); 1605 (-C=N); 1255 (-C-O-N); **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm):** 2.55 (s, 3H, -CH_3), 4.16 (s, 2H,-S-CH_2), 6.71 (s, 1H, C_4-H of isoxazole), 7.52 (s, 1H, Ar-H), 7.78 (d *J* = 2.4Hz, 1H, Ar-H), 8.39 (s, 1H, C_4-H of coumarin); **^{13}C NMR (100 MHz, DMSO-d_6, δ ppm):** 12.19, 24.55, 102.32, 116.41, 118.20, 121.83, 125.62, 131.91, 138.40, 143.48, 149.15; 151.22, 154.28, 158.42, 171.34; **MS (ESI m/z):** 523.75 $[M]^+$, 525.62 $[M+ 2H]^+$; *Anal.* calcd for $C_{17}H_9Br_2N_5O_3S$: C, 39.03; H, 1.73; N, 13.39; S, 6.13%. Found: C, 39.14; H, 1.77; N, 13.31; S, 6.18%.

8-Methoxy-3-(3-(5-methylisoxazol-3-yl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-6-yl)-2*H*-chromen-2-one (41f):



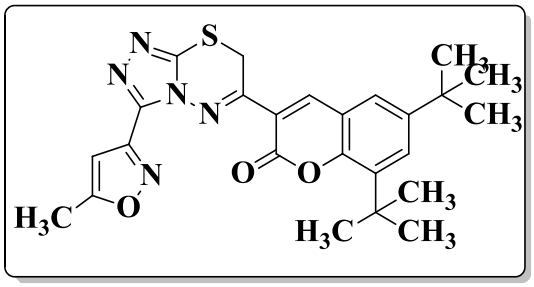
Yellow solid; FTIR (KBr, ν_{max} cm^{-1}): 1738 (lactone C=O); 1609 (-C=N); 1275 (C-O-C); 1260 (-C-O-N); **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm):** 2.53 (s, 3H, -CH_3), 4.00 (s, 3H, -OCH_3), 4.16 (s, 2H, -S-CH_2), 6.70 (s, 1H, C_4-H of isoxazole), 7.19- 7.25 (m ,2H, Ar-H), 7.30 (t, *J* = 6.4 Hz, 1H, Ar-H), 8.47 (s, 1H, C_4-H of coumarin). **^{13}C NMR (100 MHz, DMSO-d_6, δ ppm):** 11.75, 24.22, 56.23, 101.90, 116.06, 118.85, 120.95, 122.55, 125.14, 143.30, 144.20, 145.22, 146.37, 151.10, 154.40, 158.24, 170.93. **MS (ESI m/z):** 395.97$[M+H]^+$; *Anal.* calcd for $C_{18}H_{13}N_5O_4S$: C, 54.68; H, 3.31; N, 17.71; S, 8.11%. Found: C, 54.61; H, 3.35; N, 17.82; S, 8.17%.

6-Bromo-8-methoxy-3-(3-(5-methylisoxazol-3-yl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4] thiadiazin-6-yl)-2*H*-chromen-2-one (41g):



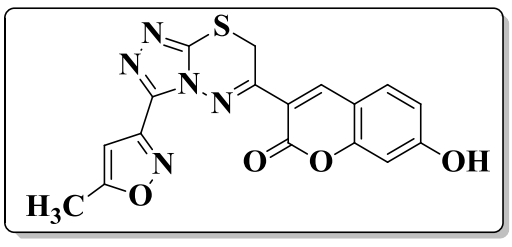
Red solid; FTIR (KBr, ν_{max} cm^{-1}): 1720 (lactone-C=O); 1604 (-C=N); 1273 (C-O-C); 1241 (-C-O-N); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 2.51 (s, 3H, $-CH_3$), 3.97 (s, 3H, $-OCH_3$), 4.34 (s, 2H, $-S-CH_2$), 6.86 (s, 1H, C_4-H of isoxazole), 7.60 (d, *J* = 2Hz, 1H, Ar-H), 7.72 (d, *J* = 1.6 Hz, 1H, Ar-H), 8.52 (s, 1H, C_4-H of coumarin). **^{13}C NMR (100 MHz, DMSO-d_6, δ ppm):** 16.61, 24.28, 56.78, 117.27, 118.67, 120.55, 122.86, 123.39, 126.72, 140.60, 142.05, 144.04, 145.99, 147.13, 155.60, 157.70, 158.04. **MS (ESI m/z):** 473.90 $[M]^+$, 475.86 $[M+2H]^+$; *Anal.* calcd for $C_{18}H_{12}BrN_5O_4S$: C, 45.58; H, 2.55; N, 14.77; S, 6.76%. Found: C, 45.51; H, 2.58; N, 14.71; S, 6.79%.

6,8-Di-*tert*-butyl-3-(3-(5-methylisoxazol-3-yl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-6-yl)-2*H*-chromen-2-one (41h):



White solid; **FTIR (KBr, ν_{max} cm^{-1}):** 1730 (lacone -C=O); 1610 (-C=N); 1242 (-C-O-N); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 1.33 (s, 9H, *t*-Bu), 1.49 (s, 9H, *t*-Bu), 2.51 (s, 3H, $-CH_3$), 4.38 (s, 2H, $-S-CH_2$), 6.88 (s, 1H, C_4-H of isoxazole), 7.67 (d, *J* = 2Hz, 1H, Ar-H), 7.81 (d, *J* = 2Hz, 1H, Ar-H), 8.60 (s, 1H, C_4-H of coumarin). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 11.77, 24.23, 29.52, 30.99, 34.49, 34.67, 101.93, 118.37, 121.17, 124.83, 128.55, 136.21, 144.19, 144.25, 146.32, 146.95, 150.74, 151.11, 154.47, 158.26, 170.92. *Anal.* calcd for $C_{25}H_{27}N_5O_3S$: C, 62.87; H, 5.70; N, 14.66; S, 6.71%. Found: C, 62.81; H, 5.76; N, 14.61; S, 6.78%.

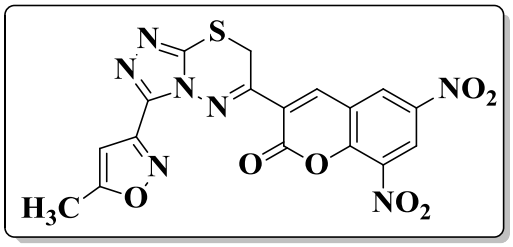
7-Hydroxy-3-(3-(5-methylisoxazol-3-yl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-6-yl)-2*H*-chromen-2-one (41i) :



White solid; **FTIR (KBr, ν_{max} cm^{-1}):** 3415 (-OH); 1717 (lactone –C=O); 1608 (-C=N); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 2.51(s, 3H, $-CH_3$), 4.33 (s, 2H, $-S-CH_2$), 6.82 (d, *J* = 2Hz, 1H, C_4-H of isoxazole), 6.86 - 6.90 (m,

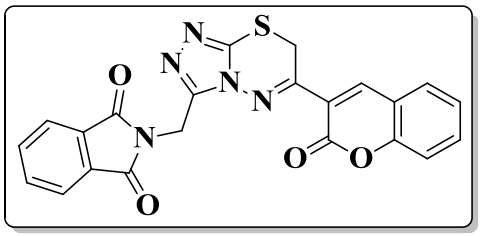
2H, Ar-H), 7.76 (d, *J* = 8.8 Hz, 1H, Ar-H), 8.52 (s, 1H, C_4-H of coumarin), 11.10 (s, 1H, -OH); **^{13}C NMR (100 MHz, DMSO-d_6, δ ppm):** 11.77, 24.18, 101.90, 102.02, 110.93, 114.27, 117.13, 131.81, 144.07, 144.27, 145.59, 151.15, 154.82, 156.42, 158.97, 163.50, 170.92; *Anal.* calcd for $C_{17}H_{11}N_5O_4S$: C, 53.26; H, 3.42; N, 18.27; S, 8.36%. Found: C, 53.21; H, 3.48; N, 18.24; S, 8.42%.

3-(3-(5-Methylisoxazol-3-yl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-6-yl)-6,8-dinitro-2*H*-chromen-2-one (41j) :



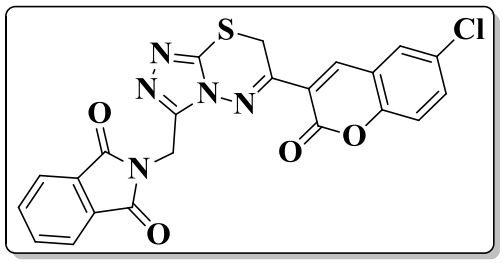
Red solid; FTIR (KBr, ν_{max} cm^{-1}): 1731 (lactone C=O); 1615 (-C=N); 1243 (-C-O-N); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 2.52 (s, 3H, $-CH_3$), 4.37 (s, 2H, -S-CH_2), 6.88 (s, 1H, C_4-H of isoxazole), 7.75 (d, *J* = 7.2 Hz, 1H, Ar-H), 8.51-8.54 (m, 1H, Ar-H), 8.76 (s, 1H, C_4-H of coumarin). **^{13}C NMR (100 MHz, DMSO-d_6, δ ppm):** 11.80, 24.15, 101.94, 117.90, 118.74, 124.44, 125.74, 128.10, 143.59, 143.88, 144.11, 144.20, 151.02, 153.99, 157.45, 157.74, 171.02; *Anal.* calcd for $C_{17}H_9N_7O_7S$: C, 44.84; H, 1.99; N, 21.53; S, 7.04%. Found: C, 44.88; H, 2.04; N, 21.50; S, 6.99%.

2-((6-(2-Oxo-2*H*-chromen-3-yl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)methyl)isoindoline-1,3-dione (41k):



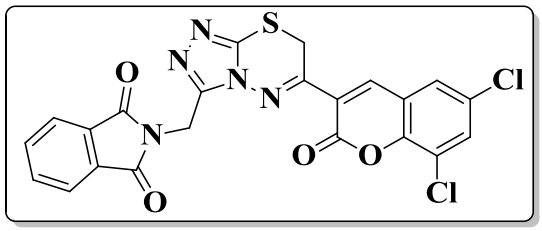
White solid; IR (KBr, ν_{max}, cm^{-1}): 1713 (lactone -C=O); 1663 (amide-C=O); 1606 (-C=N); **^{1}H NMR (400MHz, DMSO-d_6, δ ppm):** 4.28 (s, 2H, -S-CH_2); 5.10 (s, 2H, -N-CH_2); 7.46 (t, *J* = 7.2 Hz, 1H, Ar-H); 7.52 (d, *J* = 8.4 Hz, 1H, -Ar-H); 7.74 – 7.80 (m, 2H, -Ar-H); 7.86 – 7.89 (m, 3H, -Ar-H); 7.93 – 7.95 (m, 1H, -Ar-H); 8.48 (s, 1H, -C_4-H of coumarin); **^{13}C NMR (100 MHz, DMSO-d_6, ppm):** δ 24.23, 32.42, 116.34, 118.24, 122.44, 123.41, 125.21, 129.87, 131.46, 132.55, 133.97, 134.80, 141.98, 144.70, 148.32, 153.49, 153.94, 158.54, 167.11; *Anal.*Calcd. for $C_{22}H_{13}N_5O_4S$: C, 59.59; H, 2.95; N, 15.79; S, 7.23%; Found: C, 59.65; H, 2.99; N, 15.70; S, 7.20%.

2-((6-(6-Chloro-2-oxo-2*H*-chromen-3-yl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)methyl)isoindoline-1,3-dione (41l):



Yellow solid; **FTIR (KBr, ν_{max}, cm^{-1}):** 1726 (lactone-C=O); 1709(amide-C=O); 1603 (-C=N); **^{1}H NMR (400MHz, DMSO-d_6, δ ppm):** 4.28 (s, 2H, -S-CH_2); 5.08 (s, 2H, -N-CH_2); 7.57 (d, *J* = 8.8 Hz, 1H, Ar-H); 7.78 – 7.81 (m, 1H, Ar-H); 7.87 – 7.89 (m, 3H, Ar-H); 7.93 – 7.95 (m, 2H, Ar-H); 8.45 (s, 1H, C_4-H of coumarin); **^{13}C NMR (100 MHz, DMSO-d_6, ppm):** δ 24.68, 32.85, 118.88, 120.07, 123.94, 124.12, 129.14, 129.32, 131.92, 133.87, 135.29, 143.84, 148.88, 153.72, 158.66, 167.63; **HRMS (ESI m/z)** calculated for $C_{22}H_{13}ClN_5O_4S$ $[M+H]^+$ 478.0377, found: 478.0391; *Anal*. Calcd. for $C_{22}H_{12}ClN_5O_4S$: C, 55.29; H, 2.53; N, 14.66; S, 6.71%; Found: C, 55.26; H, 2.59; N, 14.70; S, 6.65%.

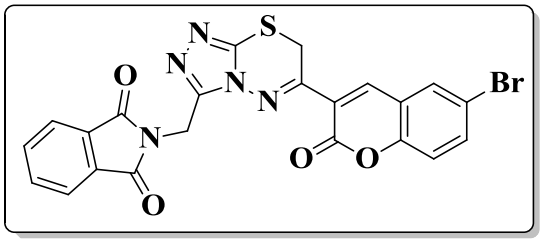
2-((6-(6,8-Dichloro-2-oxo-2*H*-chromen-3-yl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4] thiadiazin-3-yl)methyl)isoindoline-1,3-dione (41m):



Yellow solid; **FTIR (KBr, ν_{max}, cm^{-1}):** 1726 (lactone-C=O), 1710 (amide –C=O), 1605 (-C=N); **^{1}H NMR (400MHz, DMSO-d_6, δ ppm):** 4.28 (s, 2H, -CH_2); 5.08 (s, 2H, -N-CH_2); 7.57 (d, *J* = 8.8 Hz, 1H, Ar-H); 7.76 – 7.81 (m, 1H, Ar-H); 7.87 – 7.89 (m, 2H, Ar-H); 7.93 – 7.95 (m, 1H, Ar-H); 8.08 – 8.13 (m, 1H, Ar-H); 8.45 (s, 1H, C_4-H of coumarin); *Anal*. Calcd. for $C_{22}H_{11}Cl_2N_5O_4S$: C, 51.58, H, 2.16; N, 13.67; S, 6.26%; Found: C, 51.52; H, 2.14; N, 13.71; S, 6.29%.

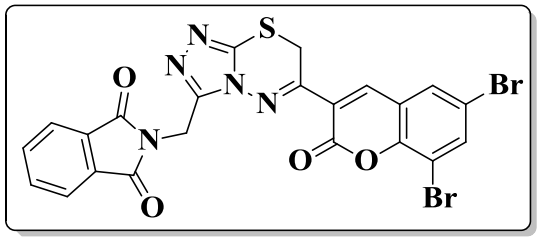
2-((6-(6-Bromo-2-oxo-2*H*-chromen-3-yl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)methyl)isoindoline-1,3-dione (41n):

Red solid; **FTIR (KBr, ν_{max}, cm^{-1}):** 1715 (lactone-C=O); 1601 (-C=N). **^{1}H NMR (400MHz, DMSO-d_6, δ ppm):** 4.28 (s, 2H, -S-CH_2); 5.08 (s, 2H, -N-CH_2); 7.45 (d, *J* = 8.8 Hz, 1H, Ar-H); 7.50 (d, *J* = 8.8 Hz, 1H, Ar-H); 7.87 – 7.89 (m, 3H, Ar-H); 7.91 – 8.01 (m, 1H, Ar-H); 8.22 (d, *J* = 2.4 Hz, 1H, Ar-H); 8.44 (s, 1H, C_4-H of coumarin); **^{13}C**



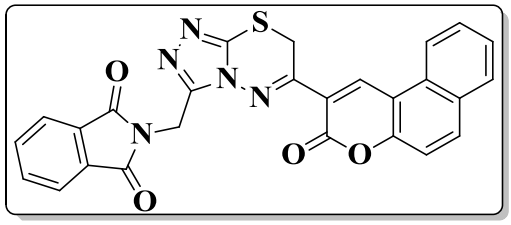
NMR (100 MHz, DMSO-d_6, ppm): δ 24.73, 32.90, 116.83, 118.73, 122.93, 123.89, 125.70, 130.35, 131.95, 133.04, 134.45, 135.28, 142.44, 145.18, 148.81, 153.96, 154.43, 159.02, 167.59; **HRMS (ESI m/z)** calculated for $C_{22}H_{13}BrN_5O_4S$ $[M + H]^+$ 521.9872, found: 521.9829; *Anal.* Calcd for $C_{22}H_{12}BrN_5O_4S$: C, 50.59; H, 2.32; N, 13.41; S, 6.14%; Found: C, 50.63; H, 2.26; N, 13.45; S, 6.18%.

2-((6-(6,8-Dibromo-2-oxo-2*H*-chromen-3-yl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4] thiadiazin-3-yl)methyl)isoindoline-1,3-dione (41o):



Red solid; **FTIR (KBr, ν_{max}, cm^{-1}):** 1715 (lactone-C=O); 1612 (amide –C=O); 1600 (-C=N); **^{1}H NMR (400MHz, DMSO-d_6, δ ppm):** 4.28 (s, 2H, -S-CH_2); 5.08 (s, 2H, -N-CH_2); 7.50 (d, *J* = 8.8Hz, 1H, Ar-H); 7.87 – 7.89 (m, 1H, Ar-H); 7.93 (t, *J* = 6 Hz, 1H, Ar-H); 8.00 (d, *J* = 2.4 Hz, 1H, Ar-H); 8.23 (t, *J* = 2 Hz, 1H, Ar-H); 8.30 (t, *J* = 7.6 Hz, 1H, Ar-H); 8.44 (s, 1H, C_4-H of coumarin); MS (ESI m/z): 602 $[M+H]^+$; *Anal.* Calcd. for $C_{22}H_{11}Br_2N_5O_4S$: C, 43.95; H, 1.84; N, 11.65; S, 5.33%; Found: C, 43.99; H, 1.79; N, 11.62; S, 5.39%.

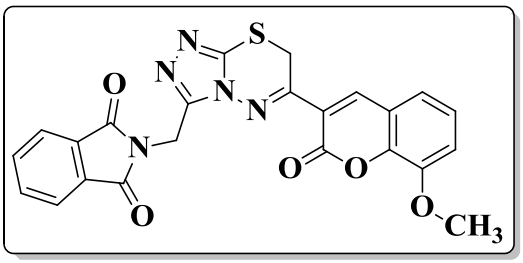
2-((6-(3-Oxo-3*H*-benzo[*f*]chromen-2-yl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)methyl)isoindoline-1,3-dione (41p):



Red solid; IR (KBr, ν_{max}, cm^{-1}): 1710 (lactone-C=O); 1625 (amide –C=O); 1603 (-C=N); **^{1}H NMR (300MHz, DMSO-d_6, δ ppm):** 4.14 (s, 2H, -S-CH_2); 5.22 (s, 2H, -N-CH_2); 7.47 (d, *J* = 14.88, 1H, Ar-H); 7.58 (s, 3H, Ar-H); 7.73 (s, 3H, Ar-H); 7.90 (s, 1H, Ar-H), 8.08 (s, 1H, Ar-H), 8.55 (s, 1H, Ar-H), 9.27 (s, 1H, C_4-H of coumarin); **^{13}C NMR (100 MHz, DMSO-d_6, ppm):** δ 24.48; 32.45; 113.29; 116.53; 120.09; 122.18; 123.65; 126.95; 129.32; 129.39; 129.58; 130.47; 131.97; 134.31; 136.13; 141.59; 152.56; 155.38; 167.42; **MS (ESI m/z):**

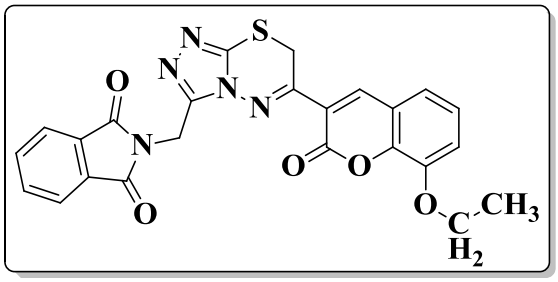
494.0921[M+H]$^+$; *Anal*.Calcd. for $C_{26}H_{15}N_5O_4S$: C, 63.28; H, 3.06; N, 14.19; S, 6.50%; Found: C, 63.25; H, 3.02; N, 14.24; S, 6.54%.

2-((6-(8-Methoxy-2-oxo-2*H*-chromen-3-yl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4] thiadiazin-3-yl)methyl)isoindoline-1,3-dione (41q):



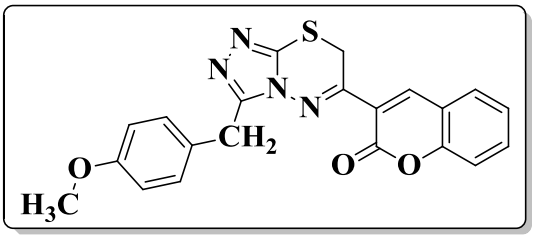
Yellow solid; FTIR (KBr, ν_{max}, cm^{-1}): 1715 (lactone-C=O); 1701 (amide –C=O); 1609 (-C=N); 1281 (-C-O-C); **^{1}H NMR (400MHz, DMSO-*d*$_6$, δ ppm):** 3.95 (s, 3H, -OCH_3); 4.28 (s, 2H,-S-CH_2); 5.09 (s, 2H, -N-CH_2); 7.31 – 7.34 (m, 1H, Ar-H), 7.38 (d, *J* = 8 Hz, 1H, Ar-H), 7.41 – 7.46 (m, 1H, Ar-H), 7.86 – 7.89 (m, 2H, Ar-H), 7.92 -7.95 (m, 2H, Ar-H), 8.46 (s, 1H, C_4-H of coumarin); **^{13}C NMR (100 MHz, DMSO-*d*$_6$, ppm):** δ 24.70, 32.90, 56.74, 116.56, 119.31, 121.32, 123.08, 123.89, 125.67, 131.96, 135.29, 142.44, 143.76, 145.44, 146.90, 148.82, 153.93, 158.76, 167.60; **MS (ESI m/z):** 474.0923 [M+H]$^+$; *Anal*. Calcd. for $C_{23}H_{15}N_5O_5S$: C, 58.35; H, 3.19; N, 14.79; S, 6.77%; Found: C, 58.41; H, 3.23; N, 14.70; S, 6.74%.

2-((6-(8-Ethoxy-2-oxo-2*H*-chromen-3-yl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)methyl)isoindoline-1,3-dione (41r):



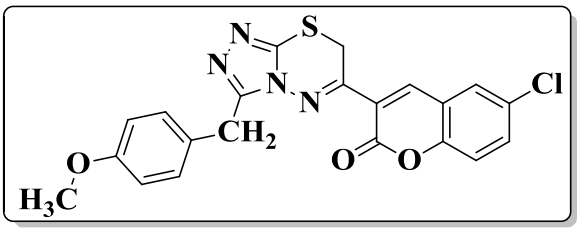
Yellow solid; **FTIR (KBr, ν_{max}, cm^{-1}):** 1724 (lactone –C=O); 1664 (amide –C=O); 1604 (-C=N); 1279 (-C-O-C); **^{1}H NMR (400MHz, DMSO-*d*$_6$, δ ppm):** 1.42 (t, *J* = 6.8 Hz, 3H, -CH_3); 4.19 – 4.24 (m, 2H, -OCH_2); 4.27 (s, 2H, -S-CH_2); 5.09 (s, 2H, -N-CH_2); 7.31 (d, *J* = 7.6Hz, 1H, Ar-H); 7.37 (t, *J* = 7.6 Hz, 1H, Ar-H); 7.43 (d, *J* = 8 Hz, 1H, Ar-H); 7.86 – 7.88 (m, 2H, Ar-H); 7.93 – 7.95 (m, 2H, Ar-H); 8.45 (s, 1H, C_4-H of coumarin); *Anal*. Calcd. for $C_{24}H_{17}N_5O_5S$: C, 59.13; H, 3.51; N, 14.37; S, 6.58%; Found: C, 59.05; H, 3.60; N, 14.41; S, 6.60%.

3-(3-(4-Methoxybenzyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-6-yl)-2*H*-chromen-2-one (41s):



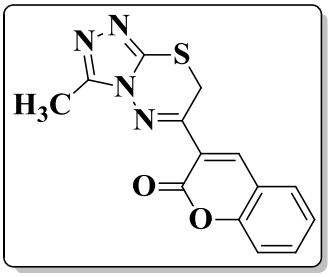
Brown solid; **FTIR (KBr, ν_{max}, cm^{-1}):** 1716 (-C=O); 1606 (-C=N); 1237 (-C-O-C); **^{1}H NMR (400MHz, DMSO-d_6, δ ppm):** 3.72 (s, 3H, -OCH_3); 4.20 (s, 2H, Ph-CH_2); 4.27 (s, 2H, -S-CH_2); 6.88 (d, *J* = 8.8 Hz, 2H, Ar-H); 7.24 (d, *J* = 8.4 Hz, 2H, Ar-H); 7.45 – 7.49 (m, 1H, Ar-H); 7.53 (d, *J* = 8 Hz, 1H, Ar-H); 7.74 – 7.78 (m, 1H, Ar-H); 7.90 – 7.93 (m, 1H, Ar-H); 8.54 (s, 1H, C_4-H of Coumarin); **^{13}C NMR (100 MHz, DMSO-d_6, ppm):** δ 24.14, 28.98, 55.05, 113.60, 113.96, 116.31; 118.34, 122.52, 125.19, 127.27, 129.96, 130.01, 133.96, 141.25, 144.73, 152.83, 153.43, 153.96, 158.17, 158.53; **MS (ESI m/z):** 405.4 $[M+H]^+$; *Anal*. Calcd. for $C_{21}H_{16}N_4O_3S$: C, 62.36; H, 3.99; N, 13.85; S, 7.93%; Found: C, 62.29; H, 4.05; N, 13.88; S, 7.96%.

6-Chloro-3-(3-(4-methoxybenzyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-6-yl)-2*H*-chromen-2-one (41t):



Brown solid; **FTIR (KBr, ν_{max}, cm^{-1}):** 1729 (-C=O); 1588 (-C=N); 1245 (-C-O-C); **^{1}H NMR (400MHz, DMSO-d_6, δ ppm):** 3.72 (s, 3H, -OCH_3); 4.19 (s, 2H, Ph-CH_2); 4.26 (s, 2H, -S-CH_2); 6.88 (d, *J* = 8.8 Hz, 2H, Ar-H); 7.24 (d, *J* = 8.4 Hz, 2H, Ar-H); 7.57 (d, *J* = 8.8 Hz, 1H, Ar-H); 7.77 – 7.80 (m, 1H, Ar-H); 8.05 (d, *J* = 2.4 Hz, 1H, Ar-H); 8.50 (s, 1H, C_4-H of coumarin); **^{13}C NMR (100 MHz, DMSO-d_6, ppm**: δ **):** 24.11, 28.96, 55.02, 113.59, 113.95, 118.33, 119.73, 123.77, 127.38, 128.74, 129.95, 133.28, 141.03, 143.22, 152.58, 152.81, 153.00, 158.14; *Anal*.Calcd. for $C_{21}H_{15}ClN_4O_3S$: C, 57.47, H, 3.44, N, 12.77, S, 7.31%; Found: C, 57.41, H, 3.48, N, 12.74; S, 7.36%.

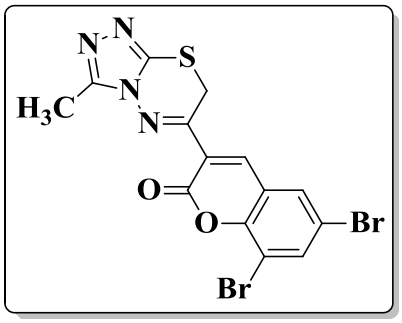
3-(3-Methyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-6-yl)-2*H*-chromen-2-one(43a):



Yellow solid; **FTIR (KBr, ν_{max}, cm^{-1}):** 1724 (-C=O), 1606 (-C=N); **^{1}H NMR (400MHz, DMSO-d_6, δ ppm):** 2.51(s, 3H, -CH_3); 4.26 (s, 2H, -CH_2); 7.44-7.54 (m, 2H, Ar-H); 7.73-7.78 (m, 1H, Ar-H); 7.92-7.94 (m, 1H, Ar-H); 8.63 (s,

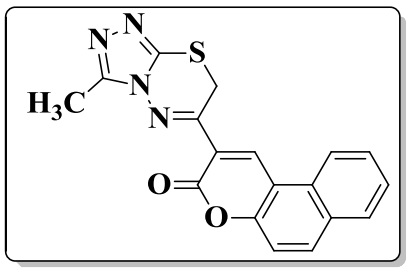
1H, C_4-H of Coumarin); **^{13}C NMR (100 MHz, DMSO-d_6; δ ppm):** 9.82, 24.24, 116.32, 118.33, 122.49, 125.19, 129.99, 133.98, 141.36, 144.80; 150.66, 153.99, 158.54; **MS (ESI m/z):** 299.6 $[M+H]^+$; *Anal*. Calcd for $C_{14}H_{10}N_4O_2S$: C, 56.37; H, 3.38; N, 18.78; S, 10.75%; Found: C, 56.45; H, 3.41; N, 10.64; S, 10.78%.

6,8-Dibromo-3-(3-methyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-6-yl)-2H-chromen-2-one (43b):



Red solid; **FTIR (KBr, ν_{max}, cm^{-1}):** 1715 (-C=O), 1599 (-C=N); **^{1}H NMR (400MHz, DMSO-d_6, δ ppm):** 2.48 (s, 3H, -CH_3); 4.25 (s, 2H, -CH_2); 8.19 (d, *J* = 2.4 Hz, 1H, Ar-H); 8.29 (d, *J* = 2.4 Hz, 1H, Ar-H); 8.54 (s, 1H, C_4-H of coumarin); **^{13}C NMR (DMSO-d_6; δ ppm):** 9.85, 24.11, 116.68, 121.23, 124.53, 132.38, 137.22, 137.80, 140.50, 142.67, 149.83, 150.42, 152.31, 157.54; **MS (ESI m/z):** 457 $[M+H]^+$; *Anal.* Calcd for $C_{14}H_8Br_2N_4O_2S$: C, 36.87; H, 1.77; N, 12.28; S, 7.03%. Found: C, 36.94; H, 1.81; N, 12.22; S, 6.97%.

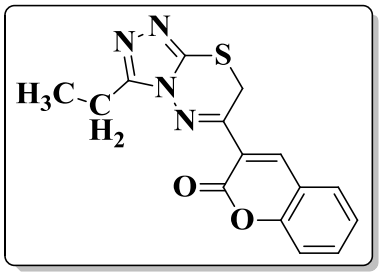
2-(3-Methyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-6-yl)-3*H*-benzo[*f*]chromen-3-one (43c):



Yellow solid; FTIR (KBr, ν_{max}, cm^{-1}): 1716 (-C=O), 1625 (-C=N); **^{1}H NMR (400MHz, DMSO-d_6, δ ppm):** 2.55 (s, 3H, -CH_3); 4.33 (s, 2H, -CH_2); 7.69 (t, *J* = 8.8 Hz, 2H, Ar-H); 7.80 (t, *J* = 8 Hz, 1H, Ar-H); 8.13 (d, *J* = 8 Hz, 1H, Ar-H); 8.35 (d, *J* = 8.8 Hz, 1H, Ar-H); 8.72 (d, *J* = 8.4 Hz, 1H, Ar-H); 9.38 (s, 1H, C_4 H of coumarin); **^{13}C NMR (DMSO-d_6, δ ppm):** 9.92, 24.19, 112.75, 116.54, 122.54, 126.56, 128.92, 129.07, 130.00, 135.50, 140.76, 158.51; **MS (ESI m/z)**: 371.0558 $[M+Na]^+$; *Anal*. alcd for $C_{18}H_{12}N_4O_2S$: C, 62.06; H, 3.47; N, 16.08; S, 9.20%. Found: C, 61.99; H, 3.52; N, 16.05; S, 9.26%.

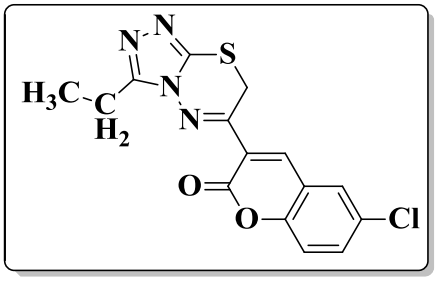
3-(3-Ethyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-6-yl)-2*H*-chromen-2-one (43d):

Yellow solid; **FTIR (KBr, ν_{max}, cm^{-1}):** 1722 (-C=O), 1604 (-C=N); **^{1}H NMR (400MHz, DMSO-d_6, δ ppm):** 1.29 (t, *J* = 7.6Hz, 3H, -CH_3); 2.85 – 2.90 (m, 2H, -CH_2); 4.25 (s, 2H, -S-CH_2); 7.45 (t, *J* = 7.6Hz, 1H, Ar-H); 7.52 (d, *J* = 8.4Hz, 1H, Ar-H); 7.73 – 7.77



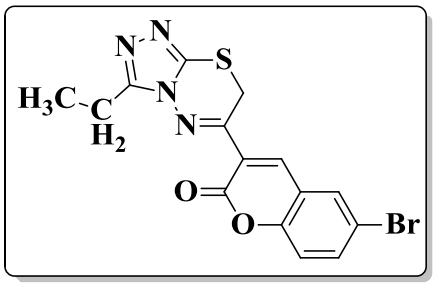
(m, 1H, Ar-H); 7.93 (d, *J* = 7.6Hz, 1H, Ar-H); 8.61 (s, 1H, C_4- H of coumarin); **^{13}C NMR (DMSO-d_6, δ ppm):** 11.05, 17.67, 24.22, 116.27, 118.38, 122.83, 125.11, 129.88, 133.76, 140.62, 144.43, 152.76, 153.92, 154.49, 158.59; **HR-MS (ESI m/z)** calculated for $C_{15}H_{12}N_4NaO_2S$ $[M+Na]^+$: 335.0579 ; Found: 335.0560 $[M + Na]^+$; *Anal*. Calcd. for $C_{15}H_{12}N_4O_2S$: C, 57.68; H, 3.87; N; 17.94; S, 10.27%. Found: C, 57.73; H, 3.81; N, 17.98; S, 10.29%.

6-Chloro-3-(3-ethyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-6-yl)-2*H*-chromen-2-one(43e):



Yellow solid; **FTIR (KBr, ν_{max}, cm^{-1}):** 1726 (-C=O), 1601 (-C=N); **^{1}H NMR (400MHz, DMSO-d_6, δ ppm):** 1.29 (t, *J* = 3.2Hz, 3H, $-CH_3$); 2.86 – 2.89 (m, 2H, $-CH_2$); 4.26 (s, 2H, -S-CH_2); 7.57 (d, *J* = 8.8Hz, 1H, Ar-H); 7.78 (t, *J* = 2.8 Hz, 1H, Ar-H); 8.06 (d, *J* = 2.8Hz, 1H, Ar-H); 8.56 (s, 1H, C_4-H of coumarin); **^{13}C NMR (100 MHz, DMSO-d_6, δ ppm):** 11.00, 17.65, 24.17, 118.28, 119.76, 120.25, 128.72, 133.17, 140.69, 143.07, 144.02, 152.67, 154.51, 158.71; **MS (ESI m/z):** 347.6 $[M+H]^+$; *Anal*. Calcd. for $C_{15}H_{11}ClN_4O_2S$: C, 51.95; H, 3.20; N, 16.16; S, 9.25%; Found: C,51.90; H, 3.22; N, 16.12; S, 9.34%.

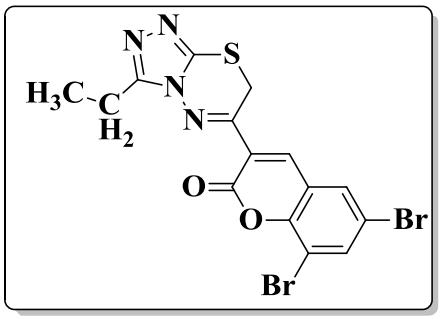
6-Bromo-3-(3-ethyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-6-yl)-2*H*-chromen-2-one (43f):



Brown solid; **IR (KBr, ν_{max}, cm^{-1}):** 1726 (-C=O), 1595 (-C=N); **^{1}H NMR (400MHz, DMSO-d_6, δ ppm):** 1.30 (t, *J* = 7.2Hz, 3H, $-CH_3$); 2.85 – 2.91 (m, 2H, $-CH_2$); 4.26 (s, 2H, -S-CH_2); 7.50 (d, *J* = 8.8Hz, 1H, Ar-H); 7.88- 7.91 (m, 1H, Ar-H); 8.20 (d, *J* = 2Hz, 1H, Ar-H); 8.56 (s, 1H, C_4-H of coumarin); **^{13}C NMR (100 MHz, DMSO-d_6, δ ppm):** 11.04, 17.65, 24.17, 116.53, 118.31, 118.53, 120.73, 123.89, 131.71, 135.91, 140.63, 142.98, 152.96, 154.50, 158.14; **HR-MS (ESI m/z)** calculated for $C_{15}H_{11}BrN_4NaO_2S$ $[M+Na]^+$: 412.9684; Found:

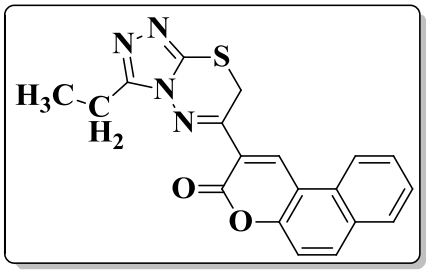
412.9683; *Anal*. Calcd. for $C_{15}H_{11}BrN_4O_2S$: C, 46.05; H, 2.83; N, 14.32; S, 8.20%; Found: C, 46.10; H, 2.85; N, 14.28; S, 8.22%.

6,8-Dibromo-3-(3-ethyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-6-yl)-2*H*-chromen-2-one(43g):



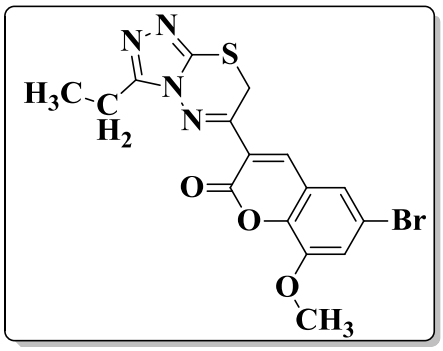
Red solid; **FTIR (KBr, ν_{max}, cm^{-1}):** 1715 (-C=O), 1602 (-C=N); **^{1}H NMR (400MHz, DMSO-d_6, δ ppm):** 1.30 (t, *J* = 7.6 Hz, 3H, -CH_3); 2.85-2.90 (m, 2H, -CH_2); 4.26 (s, 2H, -S-CH_2); 8.21 (d, *J* = 2.4Hz, 1H, Ar-H); 8.28 (d, *J* = 2.4Hz, 1H, Ar-H); 8.54 (s, 1H, C_4 - H of coumarin); **^{13}C NMR (100 MHz, DMSO-d_6, δ ppm):** 10.94, 17.66, 24.11, 110.23, 116.63, 121.27, 124.51, 131.43, 137.88, 140.98, 142.80, 149.89, 152.69, 154.58, 157.56; **MS (ESI m/z):** 471 $[M+H]^+$; *Anal*.Calcd. for $C_{15}H_{10}Br_2N_4O_2S$: C, 38.32; H, 2.14; N, 11.92; S, 6.82%; Found: C, 38.36; H, 2.12; N, 11.96; S, 6.78%.

2-(3-Ethyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-6-yl)-3*H*-benzo[*f*]chromen-3-one(43h):



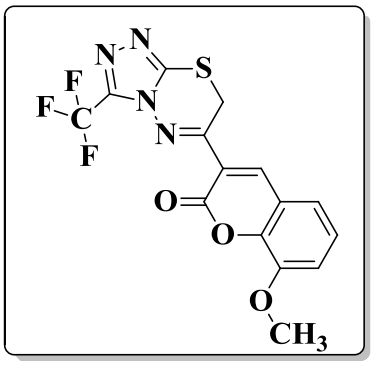
Brown solid; **FTIR (KBr, ν_{max}, cm^{-1}):** 1714 (-C=O), 1625 (-C=N); **^{1}H NMR (400MHz, DMSO-d_6, δ ppm):** 1.33 (t, *J* = 7.6Hz, 3H, -CH_3); 2.92-2.97 (m, 2H, -CH_2); 4.33 (s, 2H, -S-CH_2); 7.66 -7.70 (m, 2H, Ar-H); 7.78 -7.82 (m, 1H, Ar-H); 8.12 (d, *J* = 8Hz, 1H, Ar-H); 8.34 (d, *J* = 8.8Hz, 1H, Ar-H); 8.71 (d, *J* = 8.4Hz, 1H, Ar-H); 9.36 (s, 1H, C_4- H of coumarin); **^{13}C NMR (100 MHz, DMSO-d_6, δ ppm):** 10.83, 17.67, 24.18, 112.73, 116.52, 121.61, 122.50,126.54, 128.91, 129.05, 129.98, 135.46, 140.70, 141.17, 153.00, 154.44, 154.74, 158.50; **MS (ESI m/z):** 363.0884 $[M+H]^+$; *Anal*.Calcd. for $C_{19}H_{14}N_4O_2S$: C, 62.97; H, 3.89; N, 15.46; S, 8.85%; Found: C, 62.99; H, 3.94; N, 15.42; S, 8.82%.

6-Bromo-3-(3-ethyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-6-yl)-8-methoxy-2*H*-chromen-2-one (43i):



Red solid; **FTIR (KBr, ν_{max}, cm^{-1}):** 1733 (-C=O), 1568 (-C=N), 1273 (-C-O-C); **^{1}H NMR (400MHz, DMSO-*d*$_6$, δ ppm):** 1.29 (t, *J* = 7.6Hz, 3H, -CH_3); 2.84 - 2.90 (m, 2H, -CH_2); 3.97 (s, 3H, -OCH_3); 4.25 (s, 2H, -S-CH_2); 7.59(d, *J* = 2.4Hz, 1H, Ar-H); 7.73 (d, *J* = 2Hz, 1H, Ar-H); 8.53 (s, 1H, C_4- H of coumarin); **^{13}C NMR (100 MHz, DMSO-*d*$_6$, δ ppm):** 11.06, 17.67, 24.15, 56.76, 116.44, 118.34, 120.18, 122.61, 124.06, 140.58, 142.64, 143.27, 147.26, 152.45, 154.51, 157.86; **MS (ESI m/z):** 420.99 $[M + H]^+$; *Anal*.Calcd. for $C_{16}H_{13}BrN_4O_3S$: C, 45.62; H, 3.11; N, 13.30; S, 7.61%; Found: C, 45.68; H, 3.08; N, 13.34; S, 7.58%.

8-Methoxy-3-(3-(trifluoromethyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-6-yl)-2*H*-chromen-2-one (43j):



Yellow solid; **FTIR (KBr, ν_{max}, cm^{-1}):** 1715 (-C=O), 1608 (-C=N); **^{1}H NMR (400MHz, DMSO-*d*$_6$, δ ppm):** 3.93 (s, 3H, -OCH_3); 4.41 (s, 2H, -S-CH_2); 7.36 – 7.51 (m, 3H, Ar-H); 8.55 (s, 1H, C_4- H of coumarin); **^{13}C NMR (100 MHz, DMSO-*d*$_6$, δ ppm):** 24.31, 56.23, 116.28, 118.76, 121.10, 122.27, 125.13, 143.42, 145.37, 146.00, 146.37, 155.85, 158.11; **MS (ESI m/z):** 353 $[M+ H]^+$; *Anal*.Calcd. for $C_{15}H_9F_3N_4O_3S$: C, 47.12; H, 2.37; N, 14.65; S, 8.39; Found: C, 47.16; H, 2.41; N, 14.58; S, 8.36%.

Antiviral activity of 3- heteryl / aryl / alkyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4] thiadiazin-6-yl)-2*H*-chromen-2-ones (41a-t and 43a-j)

The antiviral activity of the new compounds was determined by measuring the cell viability with the colorimetric formazan-based MTS assay (Chapter-II reference 65-67) in different cell types. One hundred microliters of the virus diluted in medium to obtain a virus input of 100 $CCID_{50}$ was added to each well. Mock-treated cell cultures receiving solely the test compounds were included, to determine the cytotoxicity. After 4 days of incubation at 37°C the virus-induced cytopathicity was determined by measuring the cell viability with the colorimetric assay.

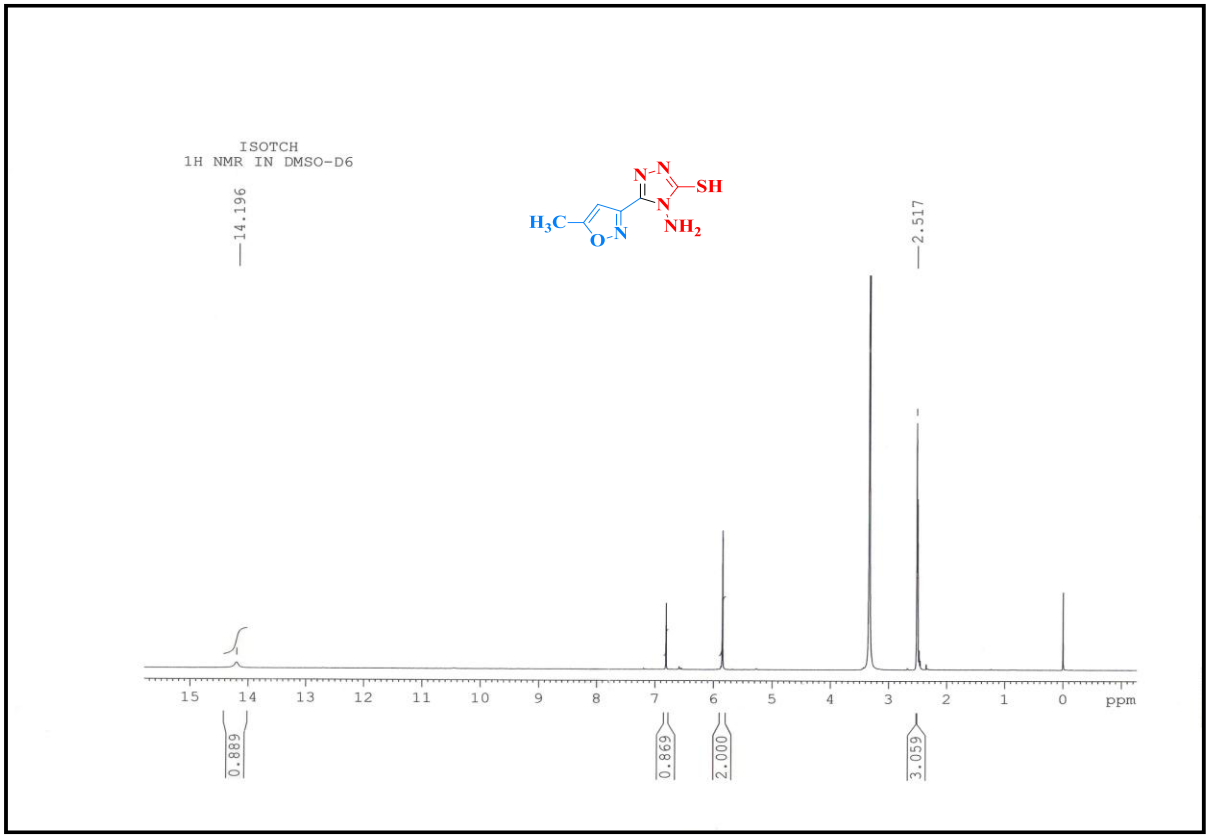


Fig: ^{1}H NMR spectrum of compound **40a**

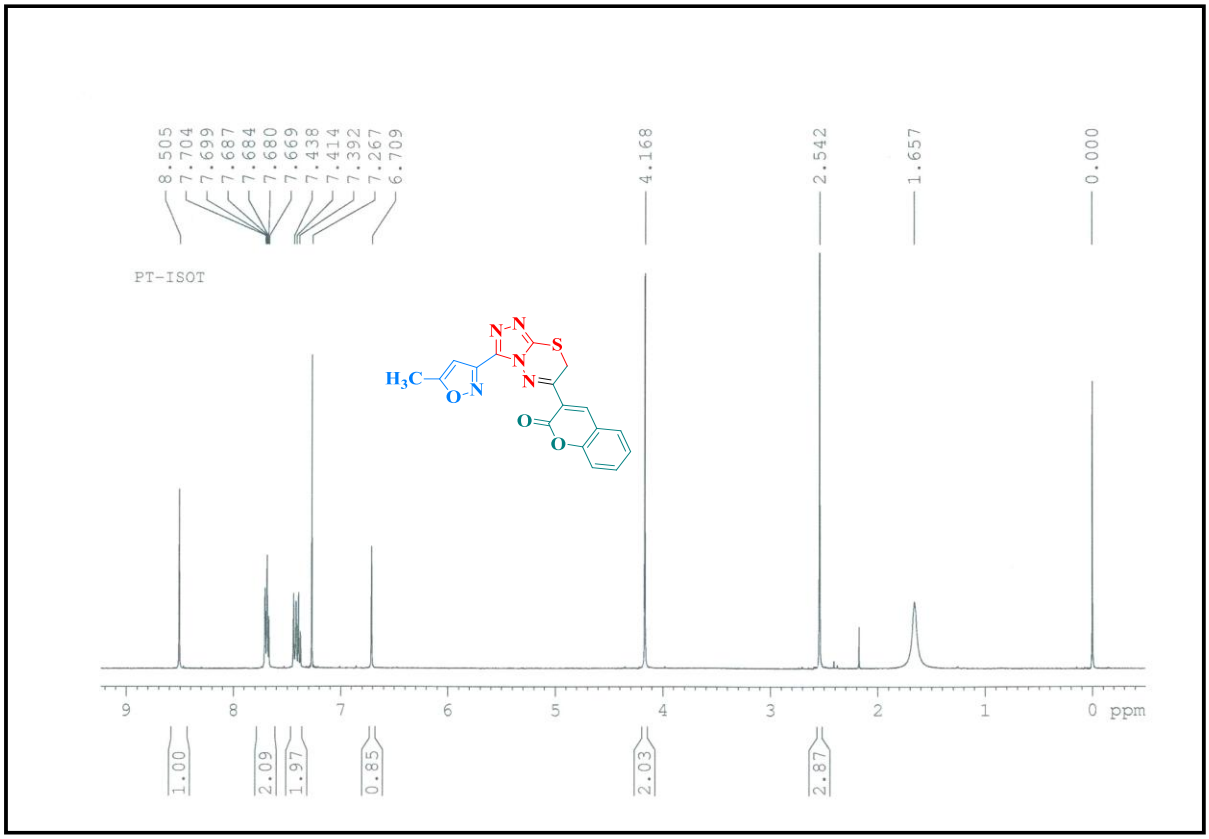


Fig: ^{1}H NMR spectrum of compound **41a**

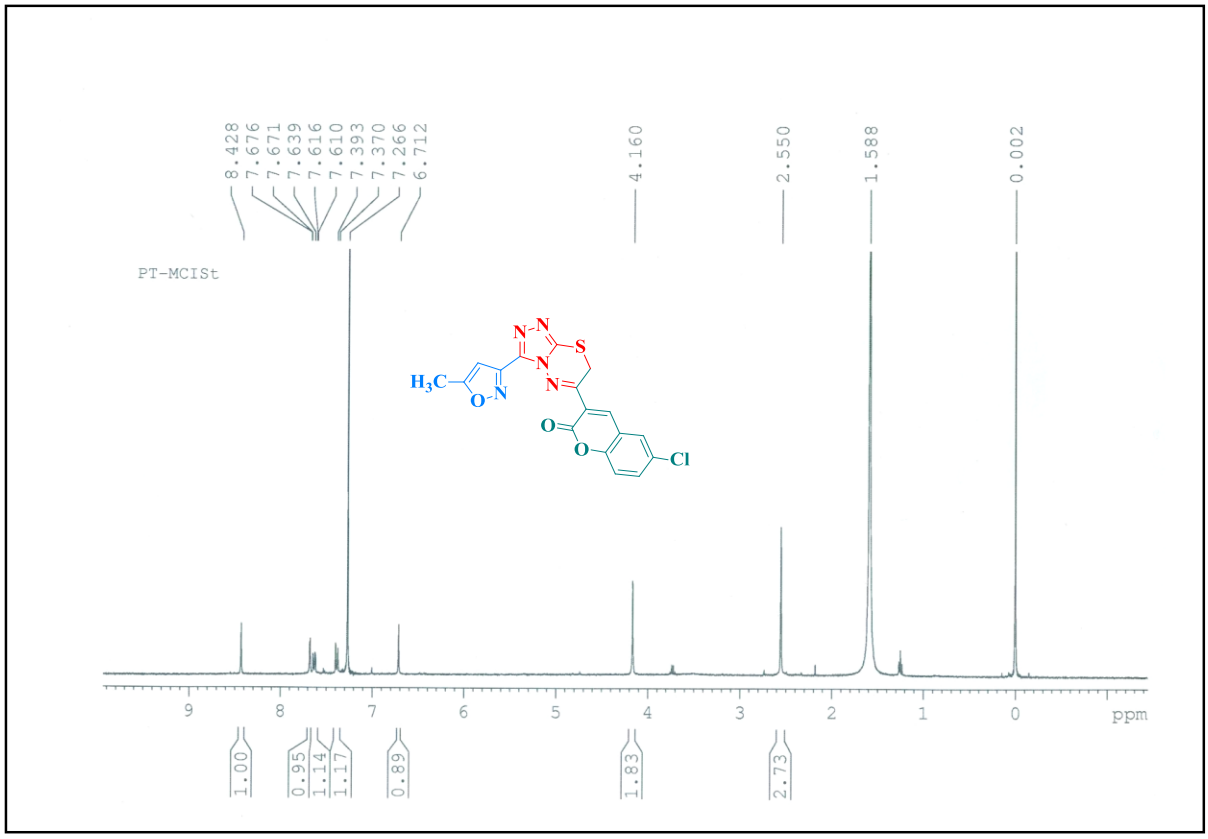


Fig: ^{1}H NMR spectrum of compound **41b**

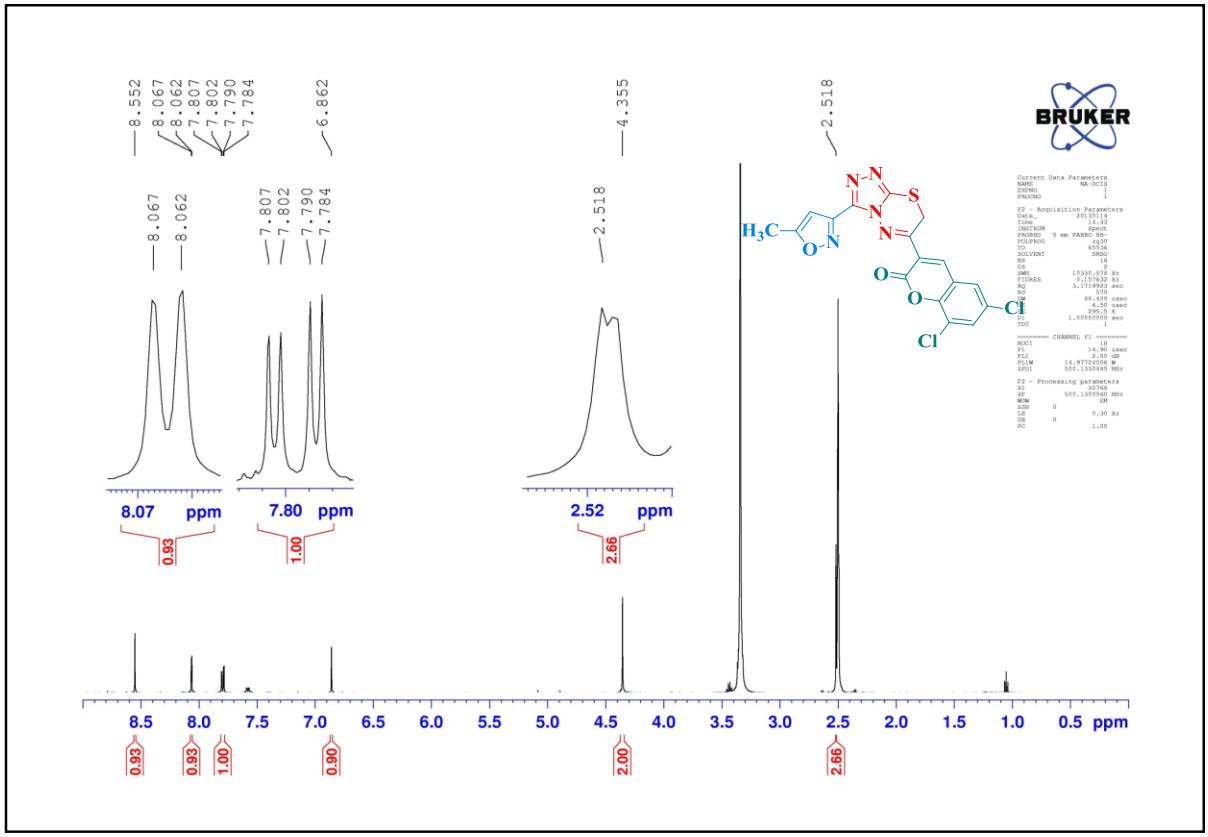


Fig: ^{1}H NMR spectrum of compound **41c**

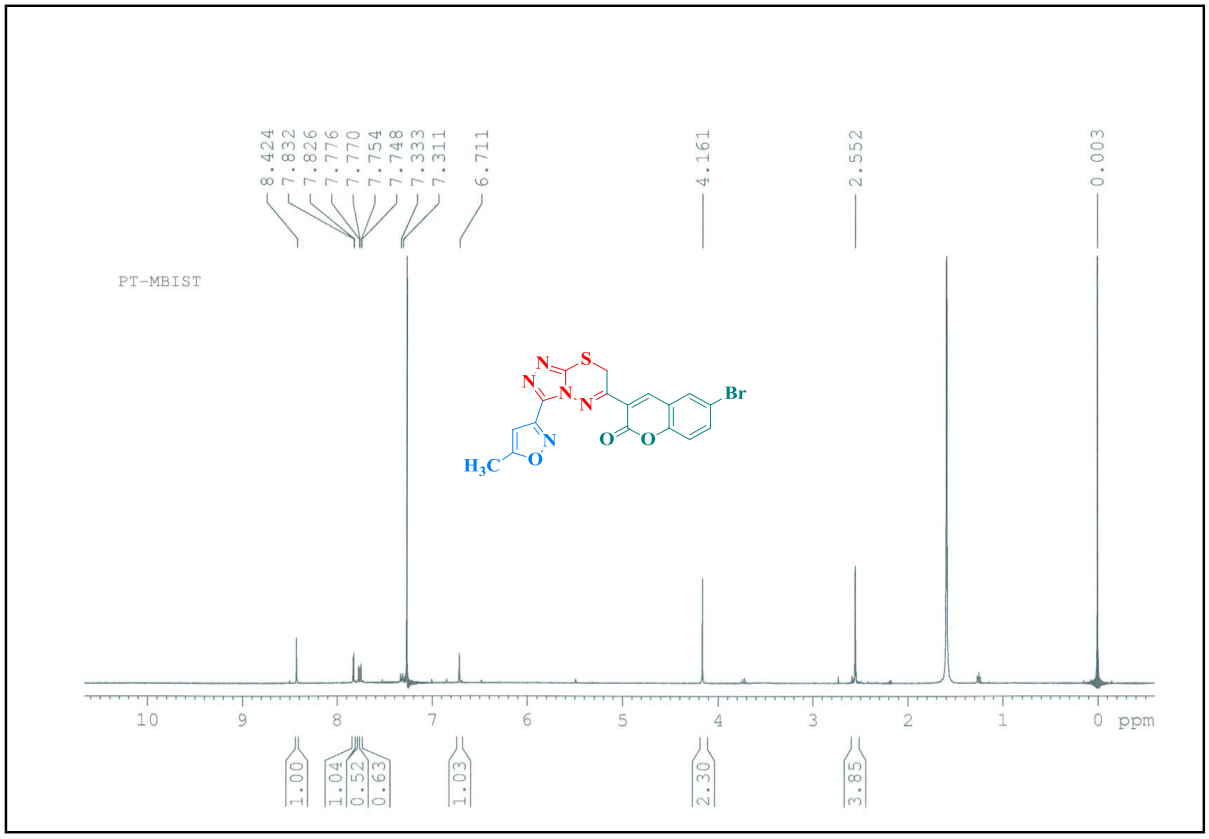


Fig: ^{1}H NMR spectrum of compound **41d**

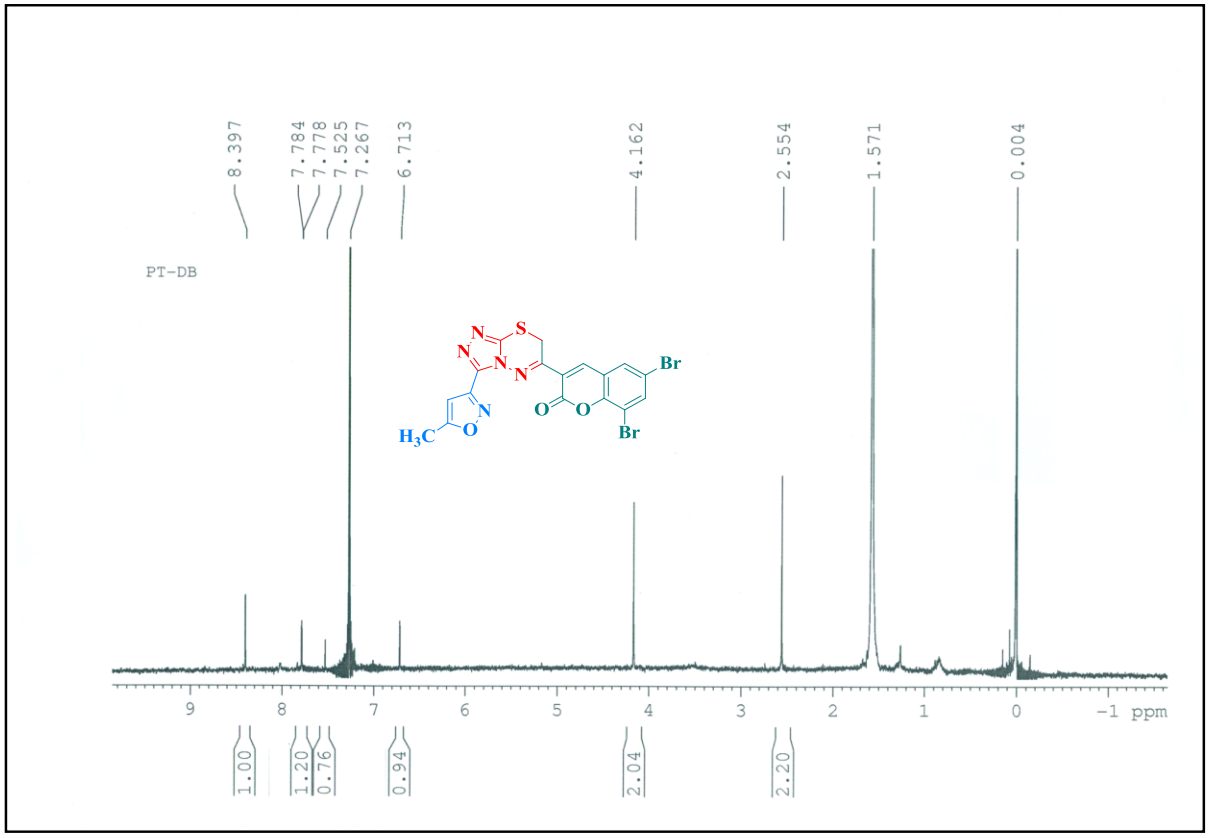


Fig: ^{1}H NMR spectrum of compound **41e**

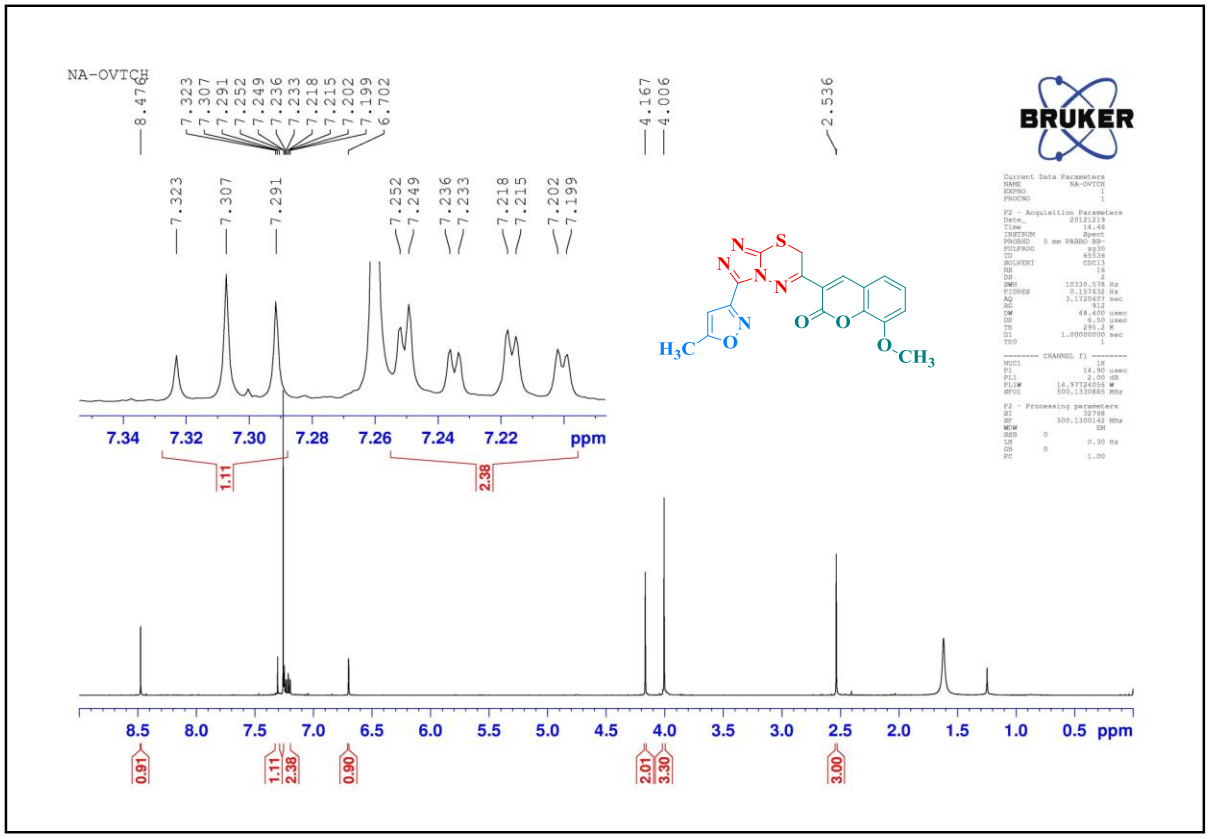


Fig: ^{1}H NMR spectrum of compound **41f**

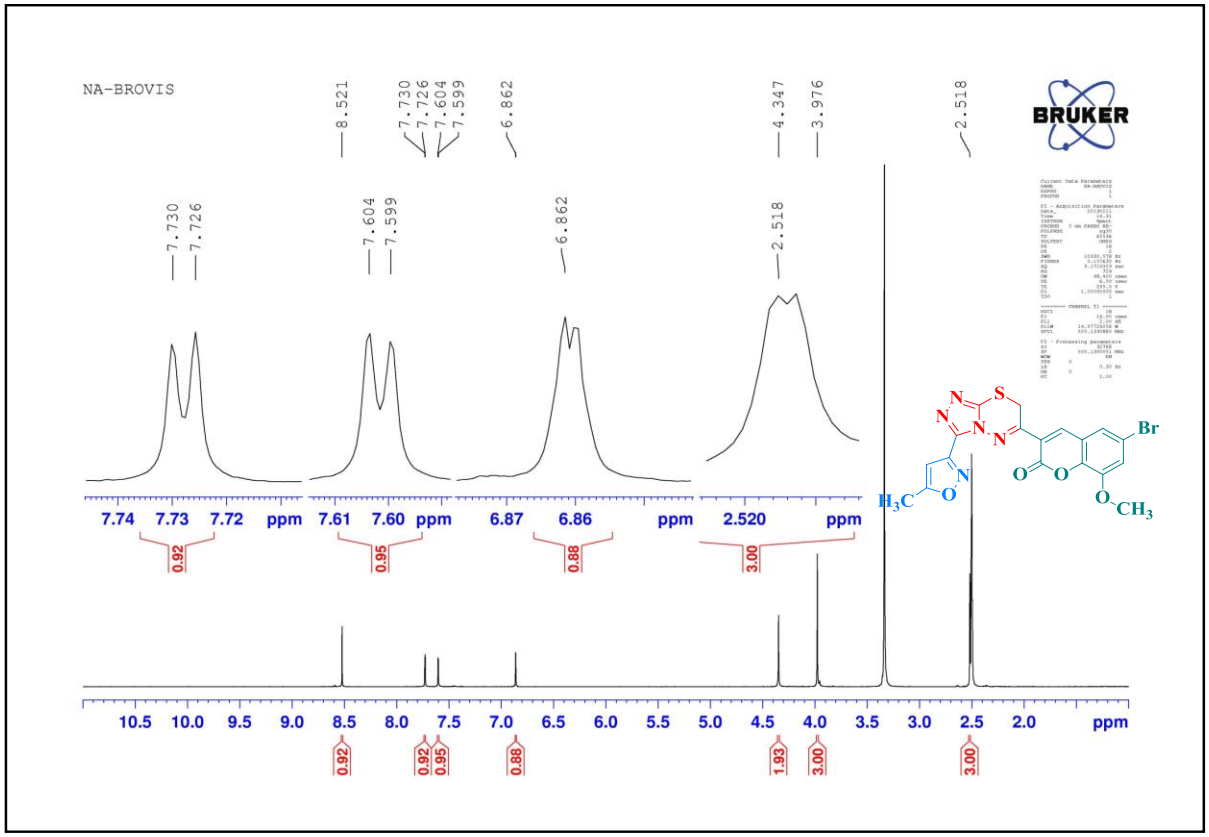


Fig: ^{1}H NMR spectrum of compound **41g**

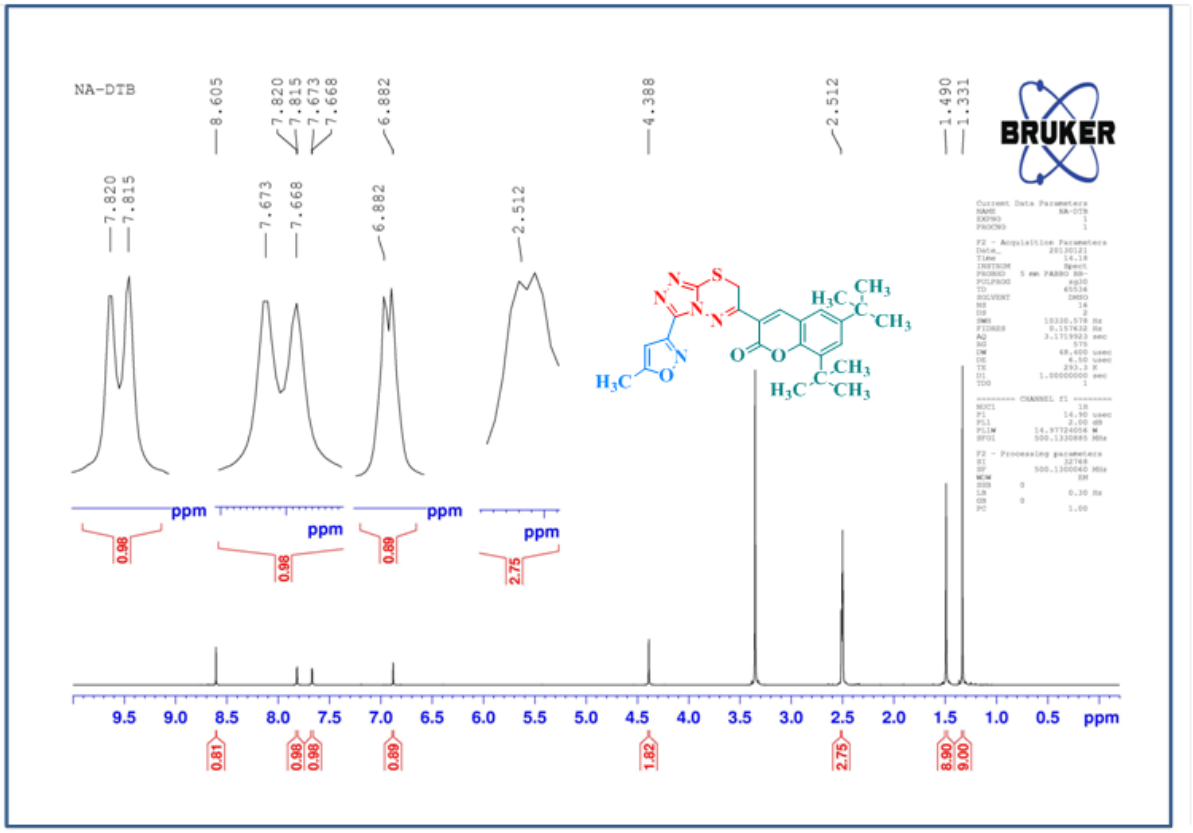


Fig: ^{1}H NMR spectrum of compound **41h**

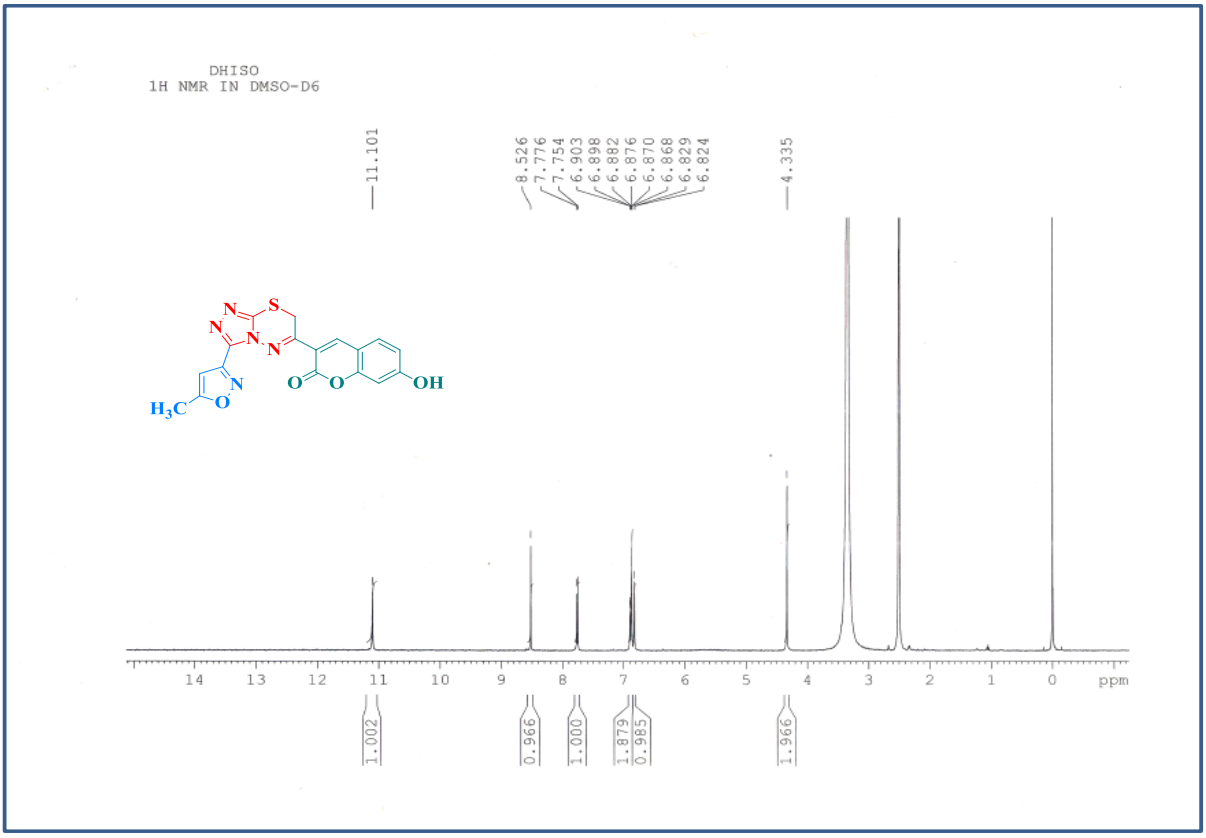


Fig: ^{1}H NMR spectrum of compound **41i**

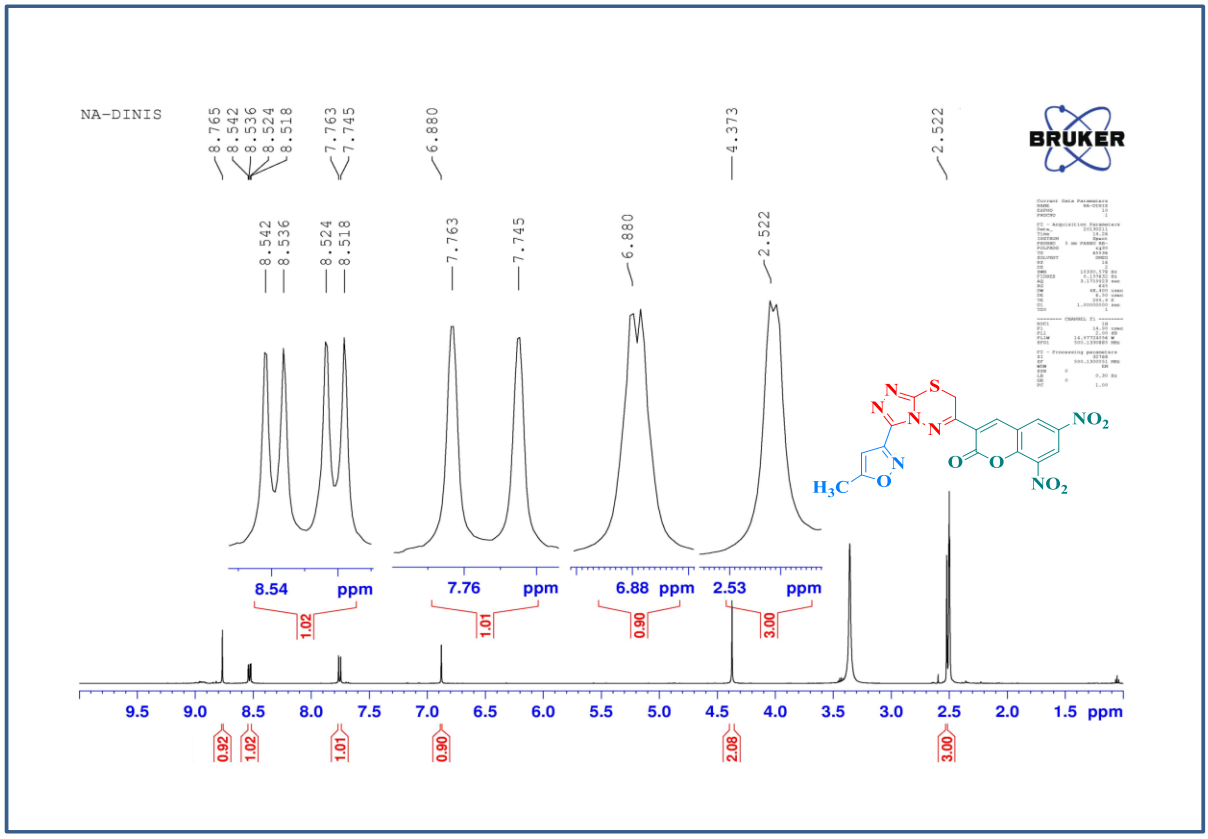


Fig: ^{1}H NMR spectrum of compound **41j**

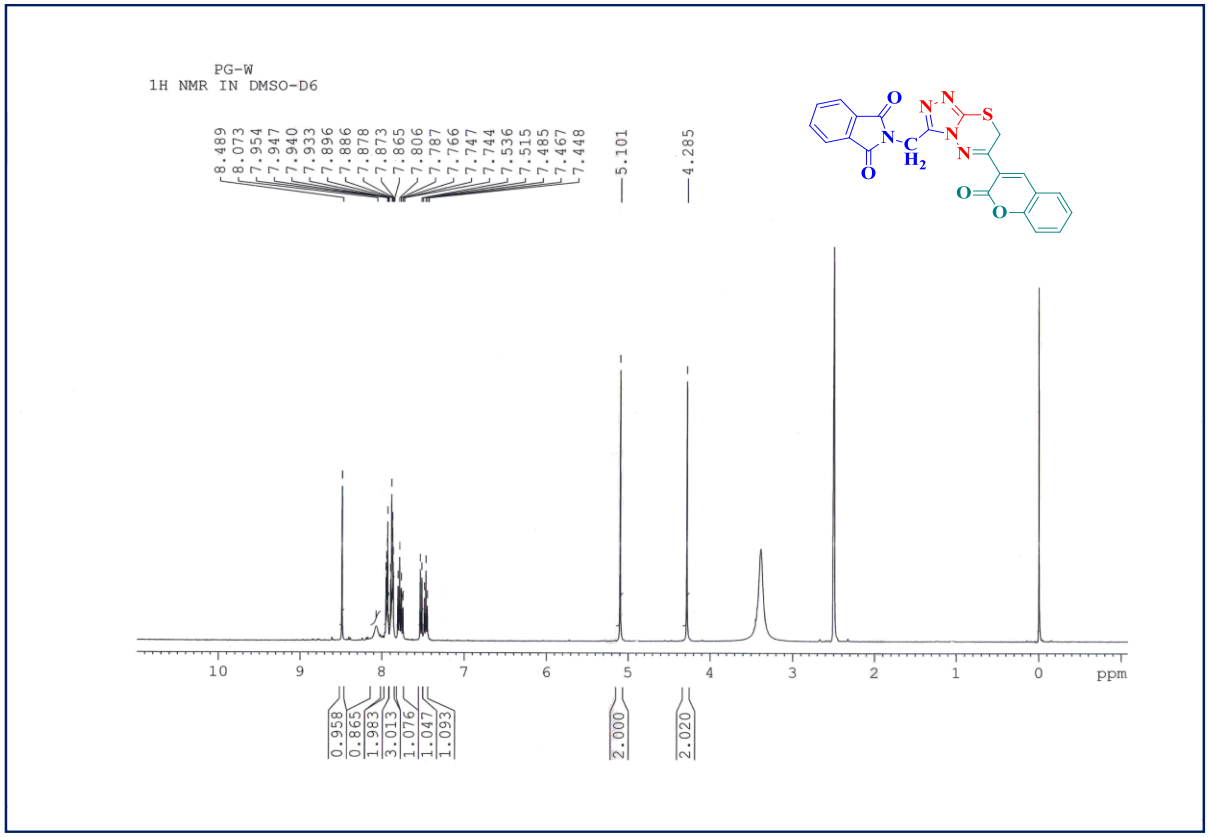


Fig: ^{1}H NMR spectrum of compound **41k**

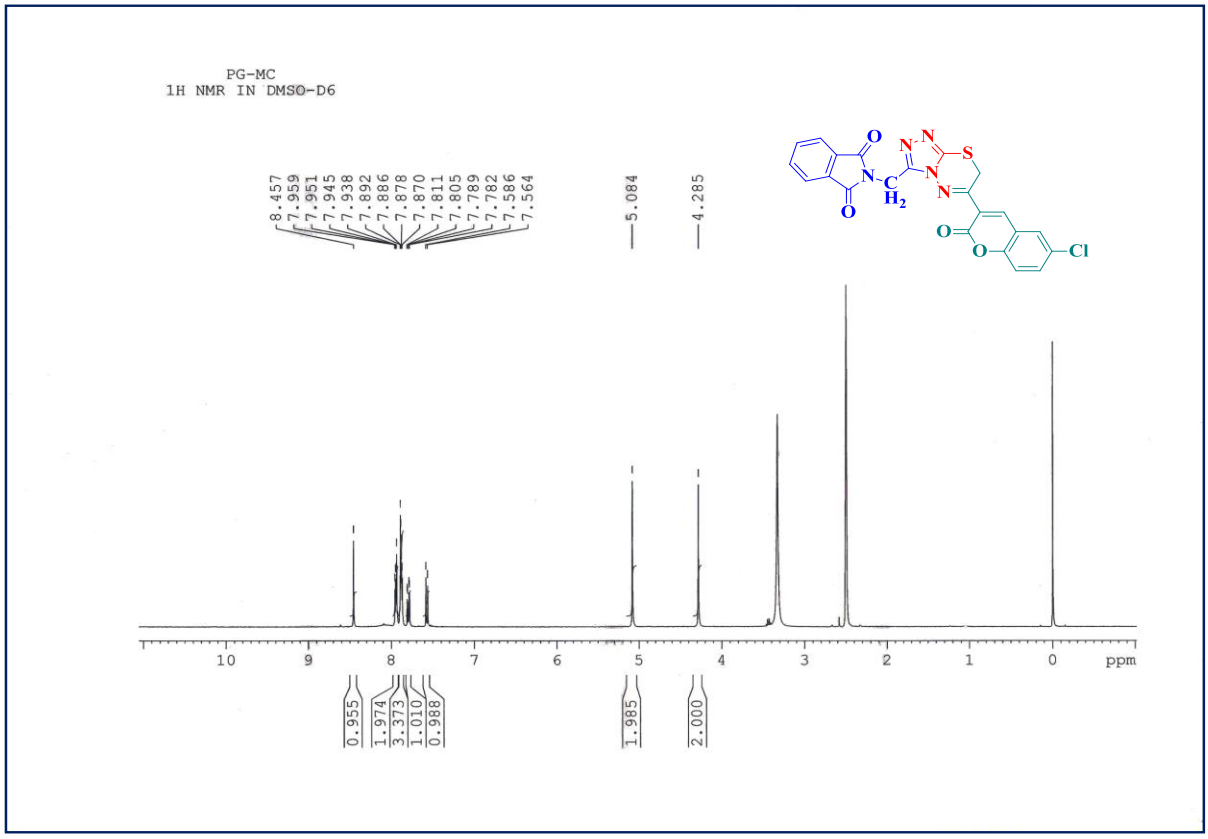


Fig: ^{1}H NMR spectrum of compound **41l**

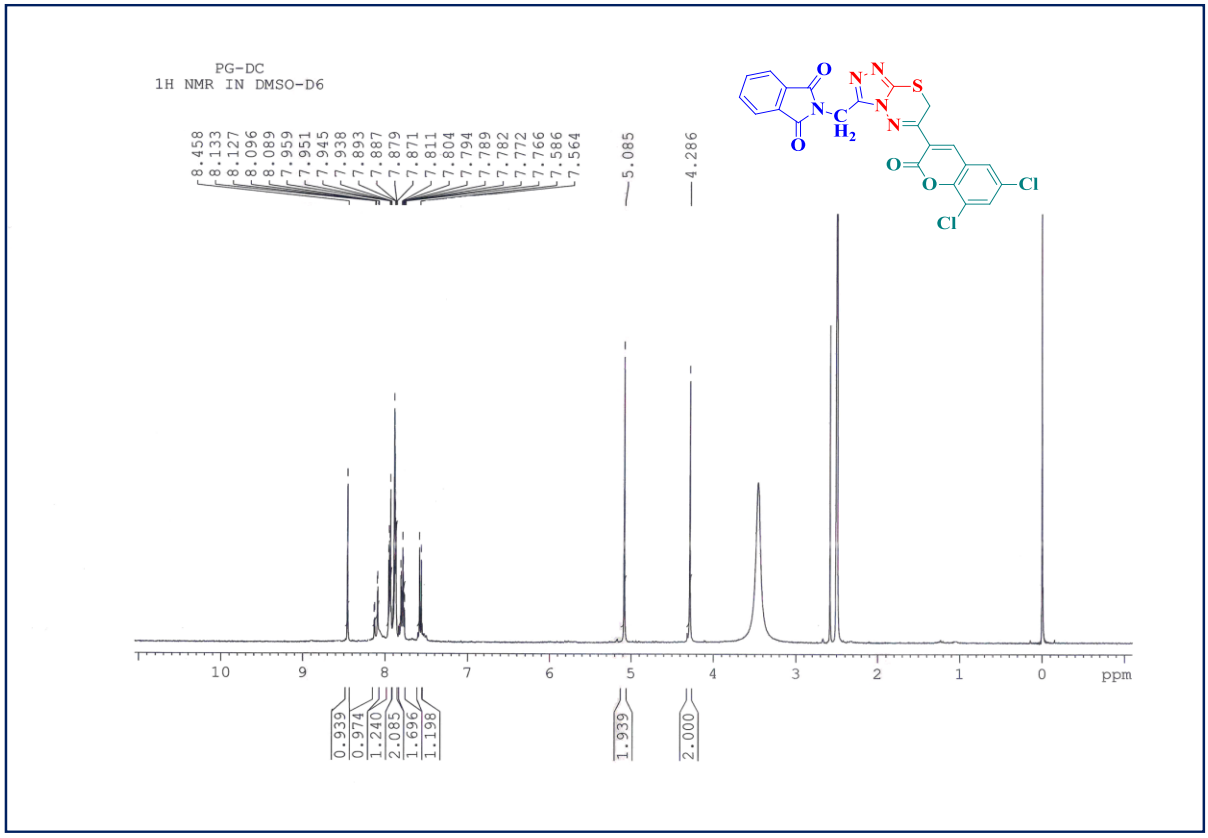


Fig: ^{1}H NMR spectrum of compound **41m**

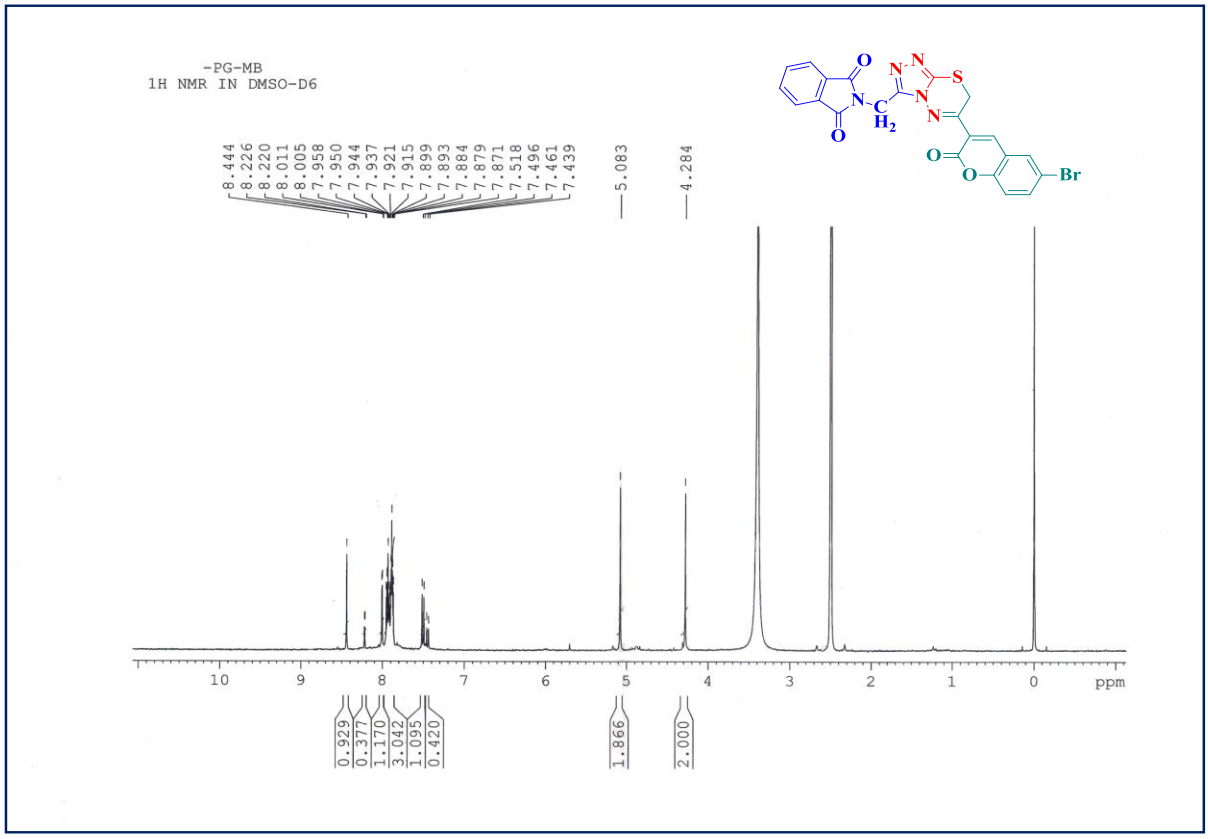


Fig: ^{1}H NMR spectrum of compound **41n**

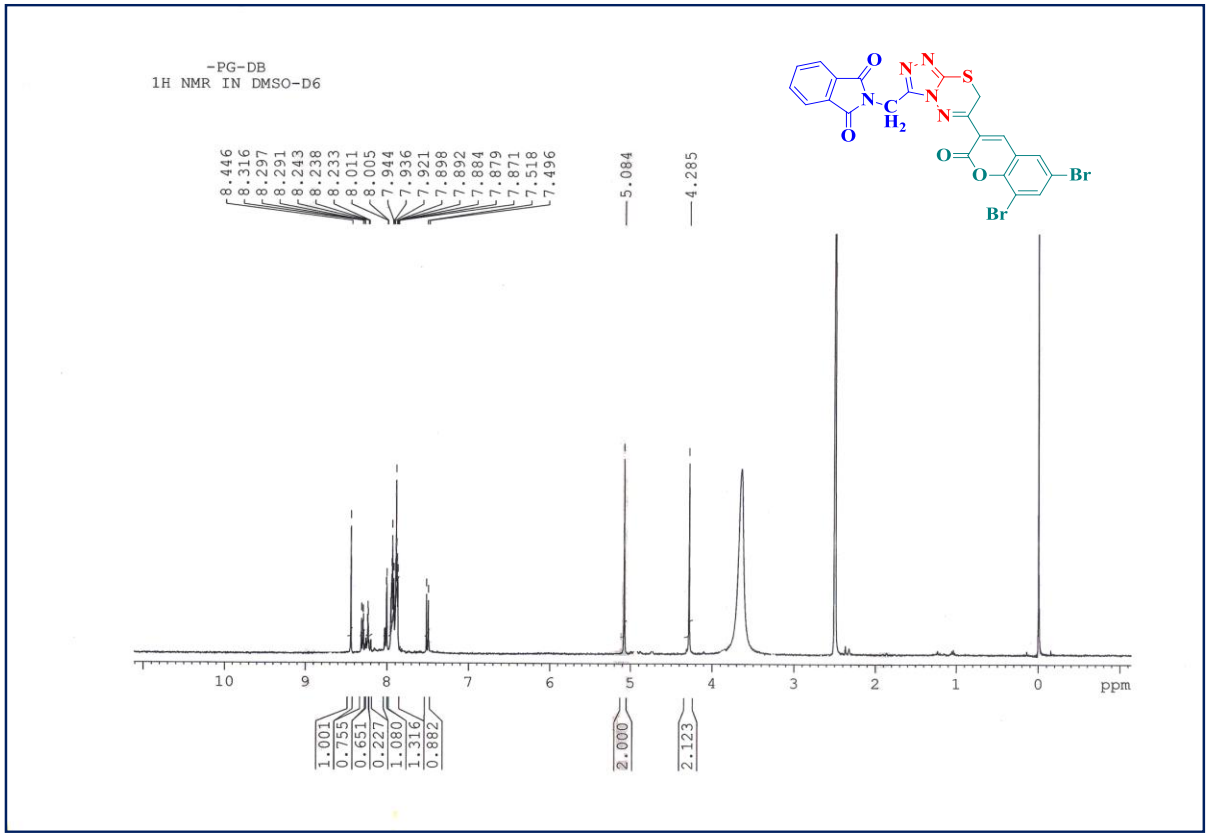


Fig: ^{1}H NMR spectrum of compound **41o**

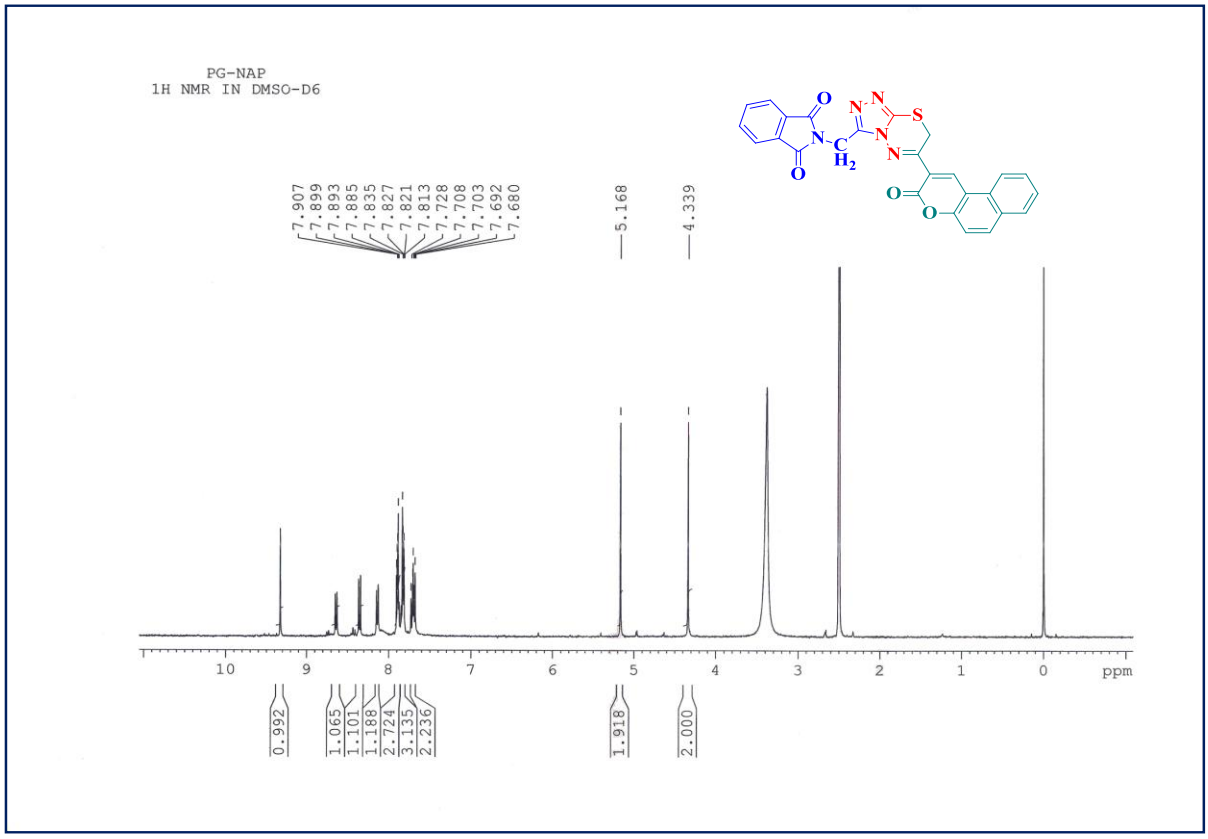


Fig: ^{1}H NMR spectrum of compound **41p**

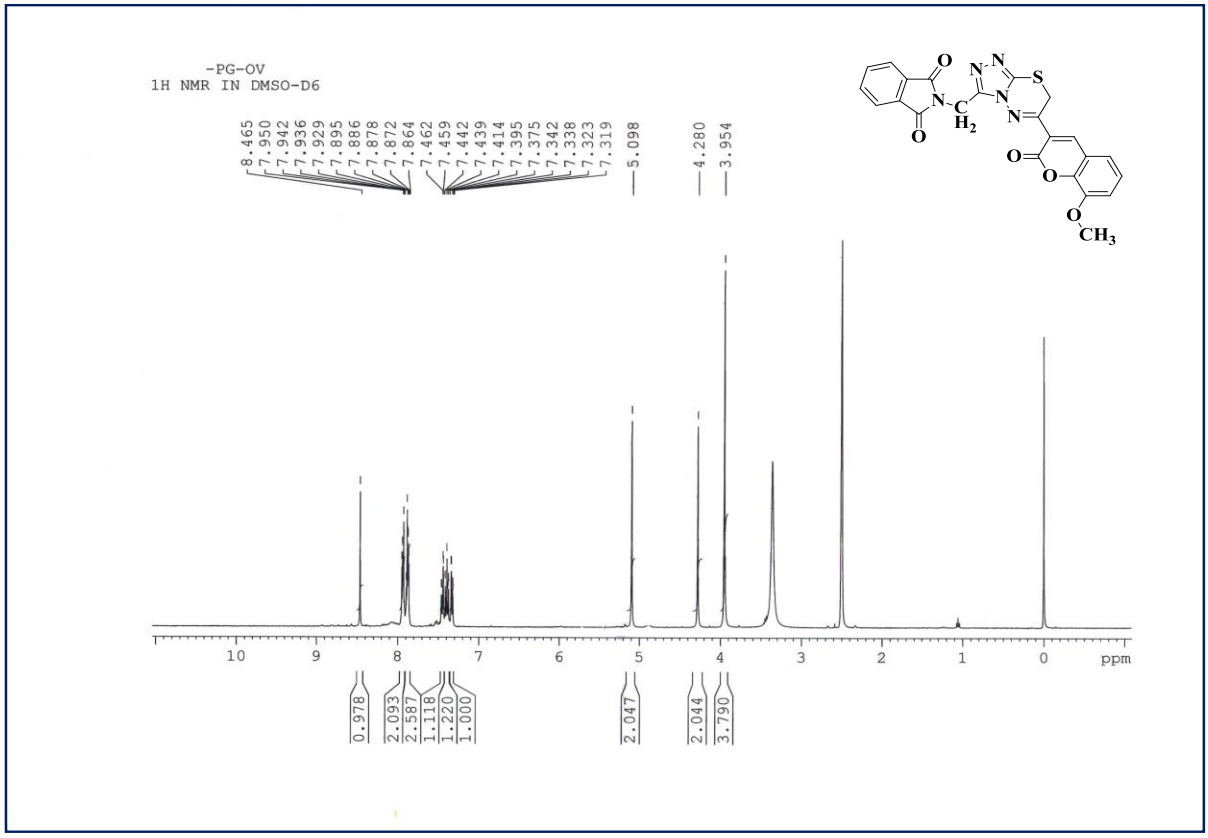


Fig: ^{1}H NMR spectrum of compound **41q**

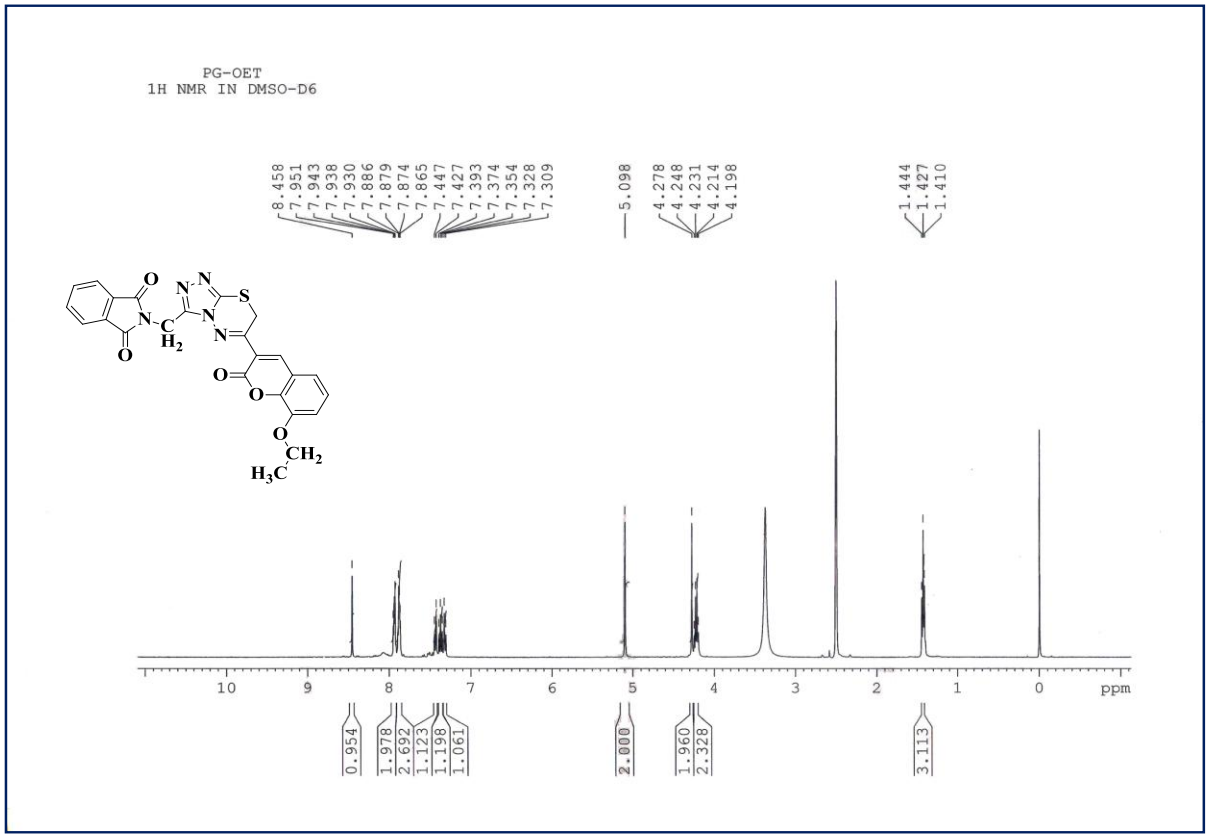


Fig: ^{1}H NMR spectrum of compound **41r**

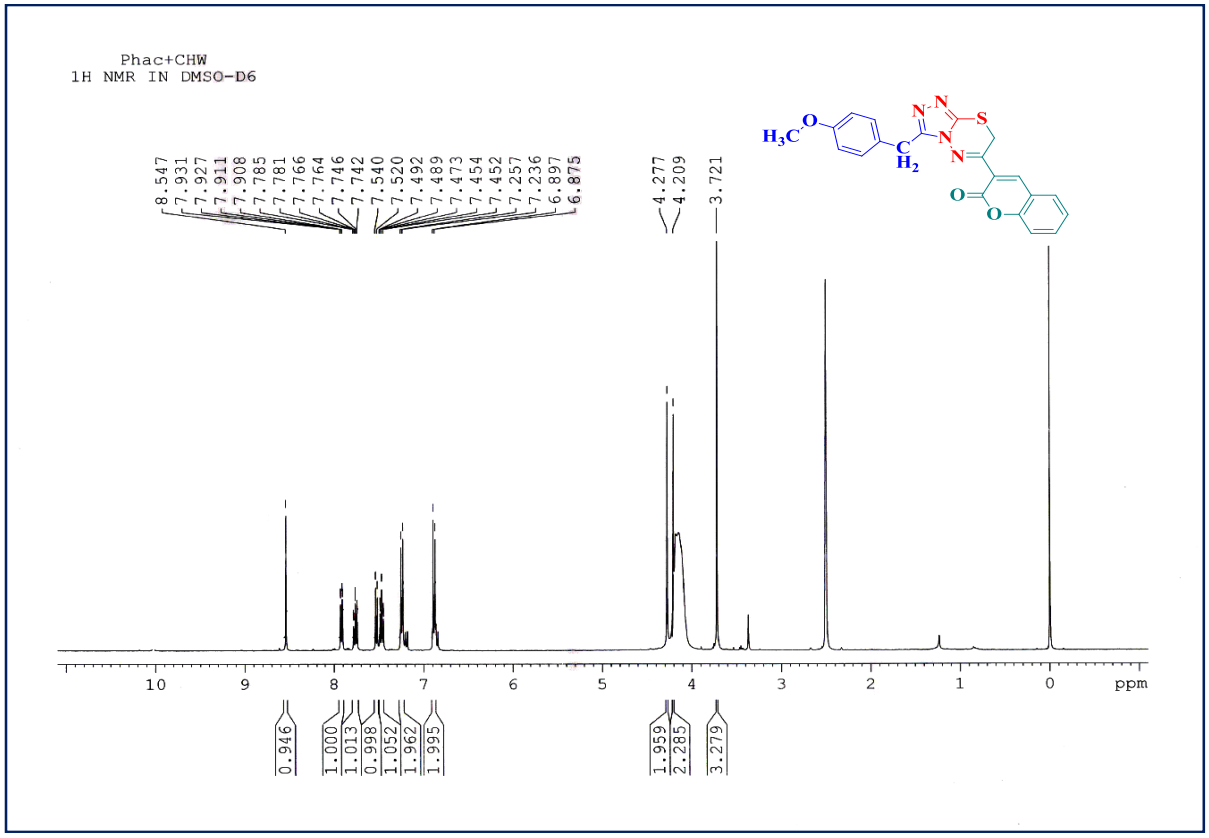


Fig: ^{1}H NMR spectrum of compound **41s**

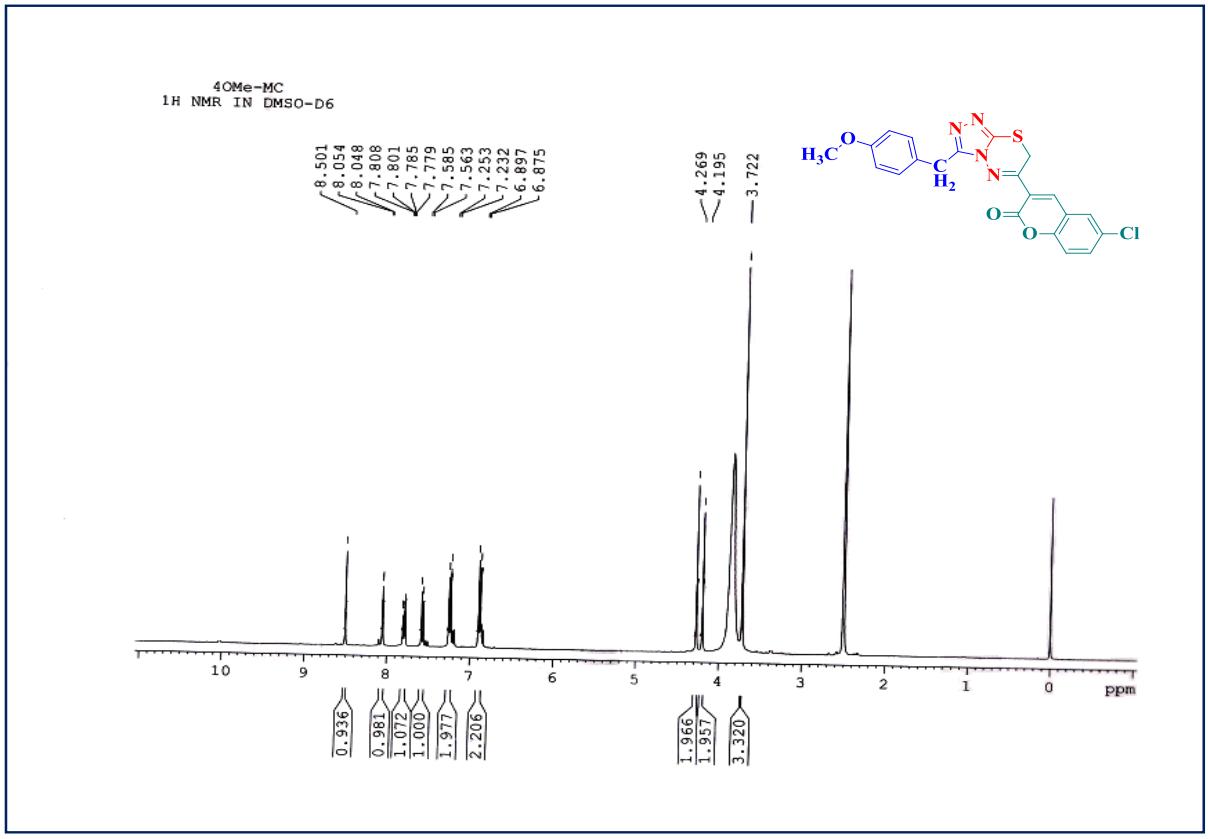


Fig: ^{1}H NMR spectrum of compound **41t**

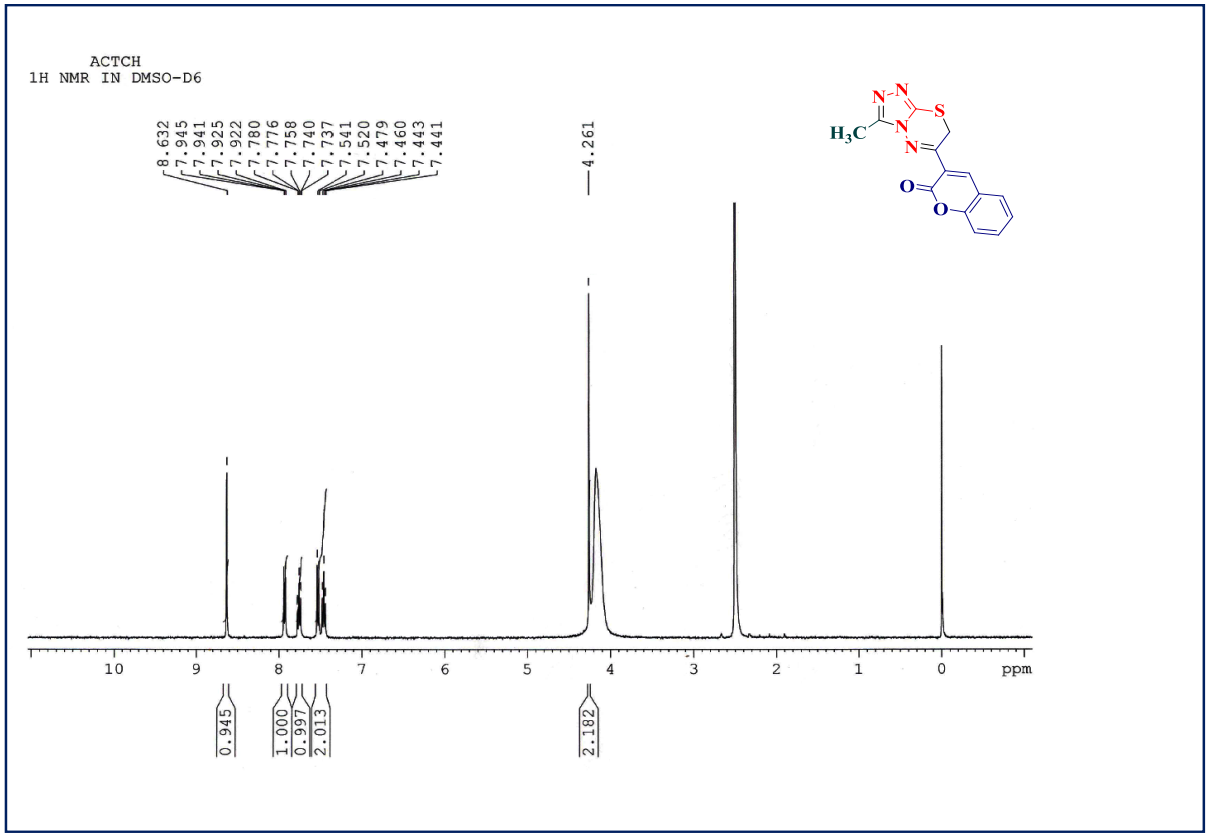


Fig: ^{1}H NMR spectrum of compound **43a**

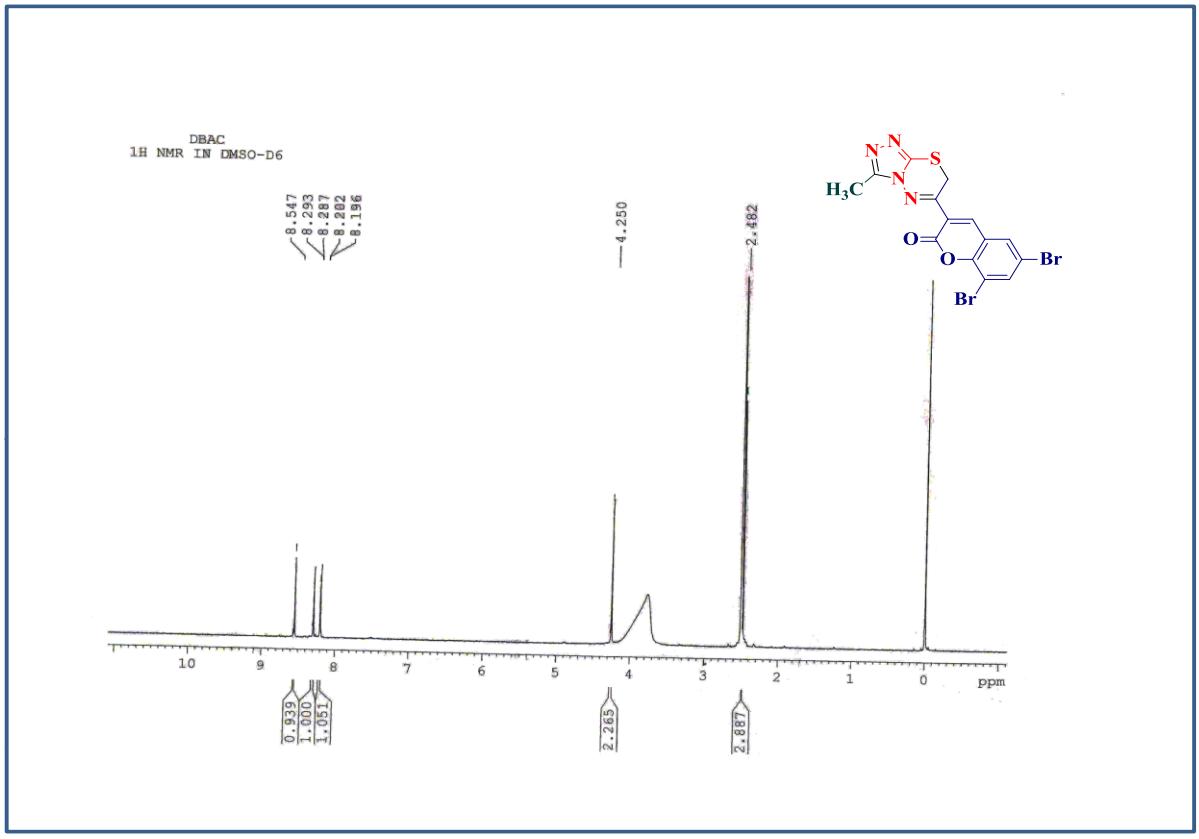


Fig: ^{1}H NMR spectrum of compound **43b**

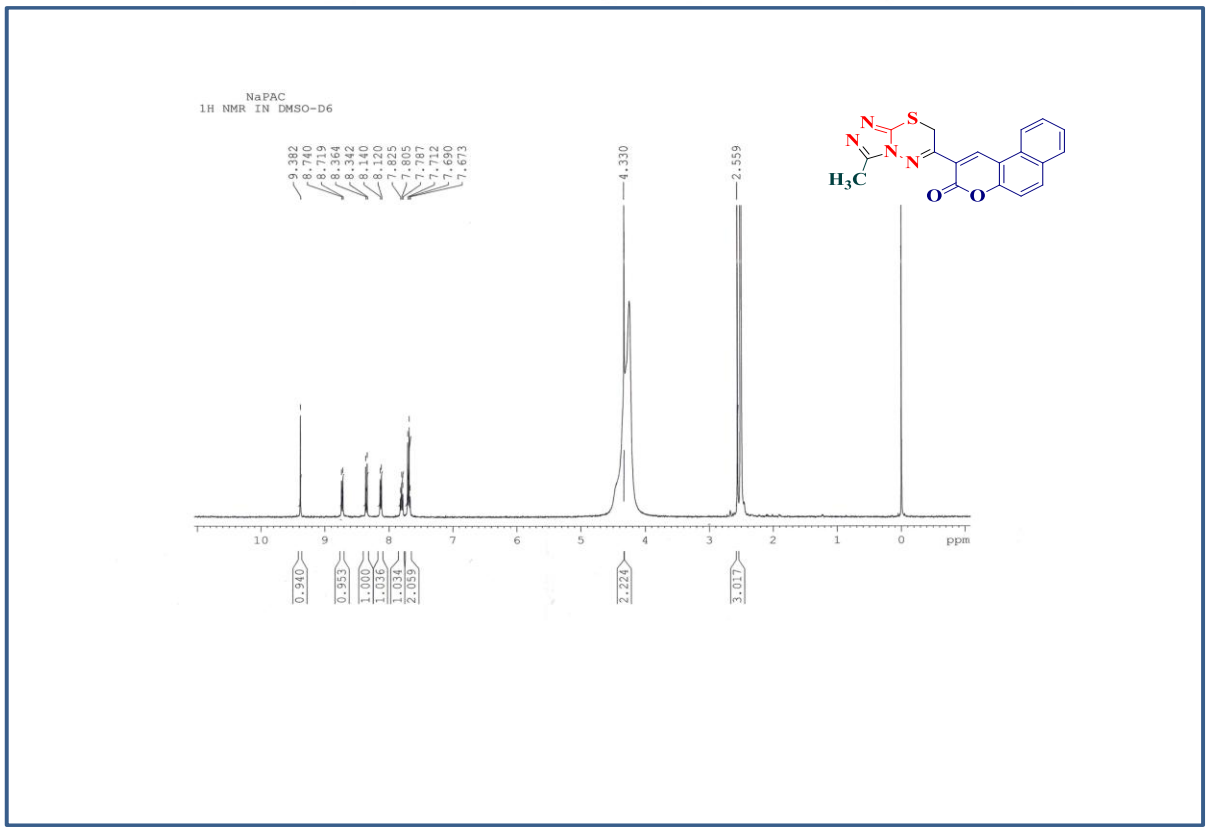


Fig: ^{1}H NMR spectrum of compound **43c**

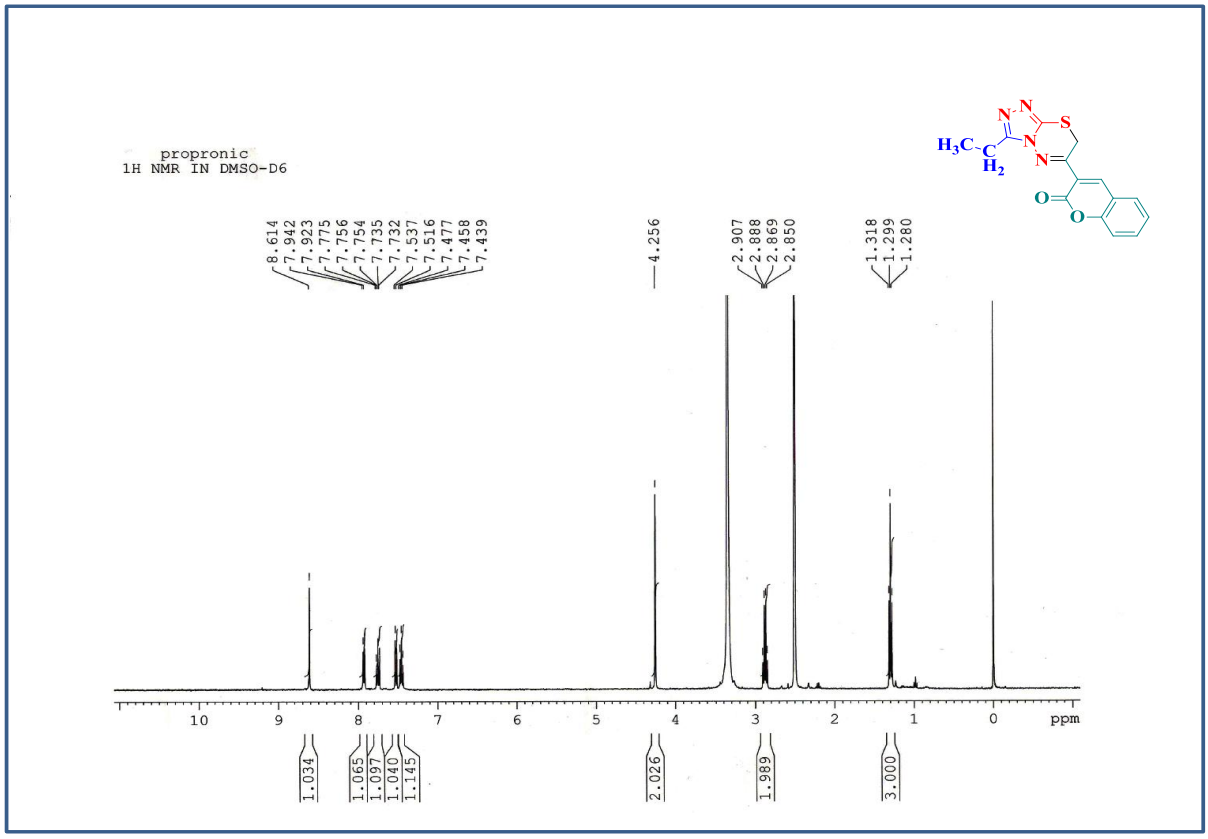


Fig: ^{1}H NMR spectrum of compound **43d**

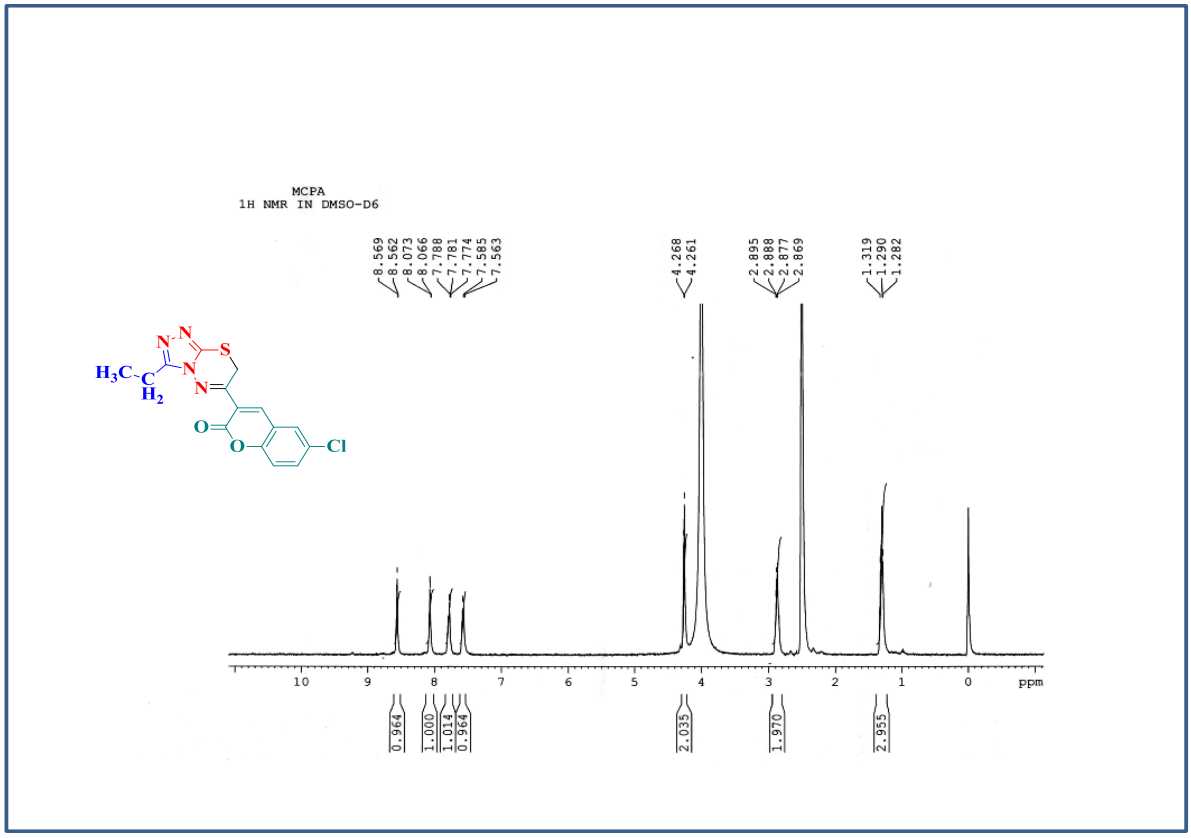


Fig: ^{1}H NMR spectrum of compound **43e**

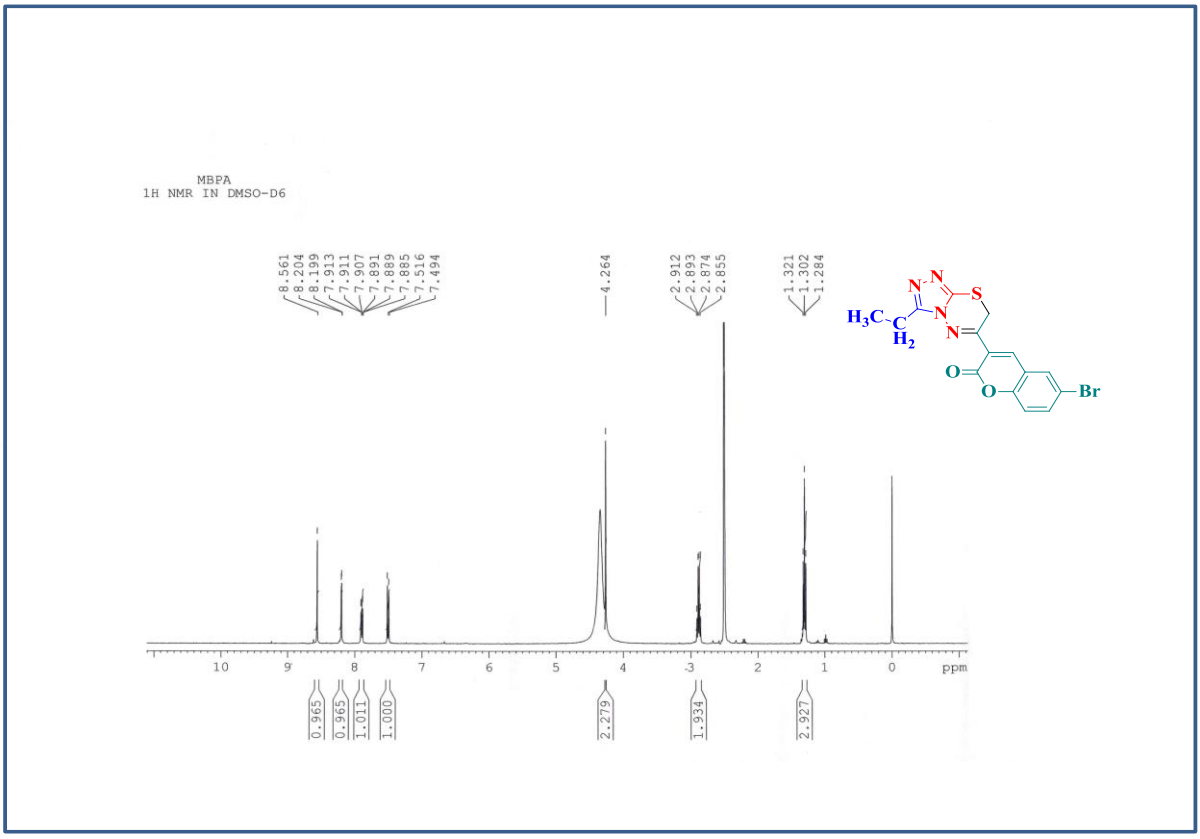


Fig: ^{1}H NMR spectrum of compound **43f**

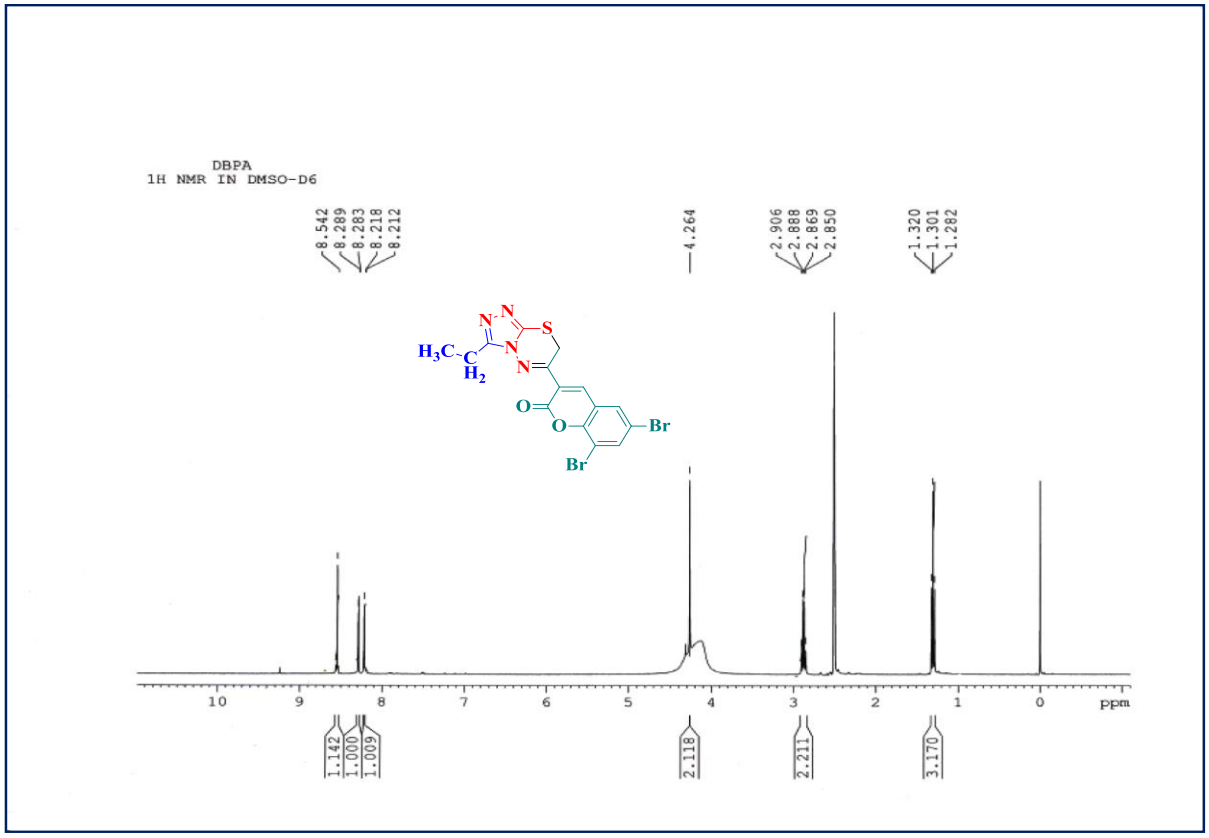


Fig: ^{1}H NMR spectrum of compound **43g**

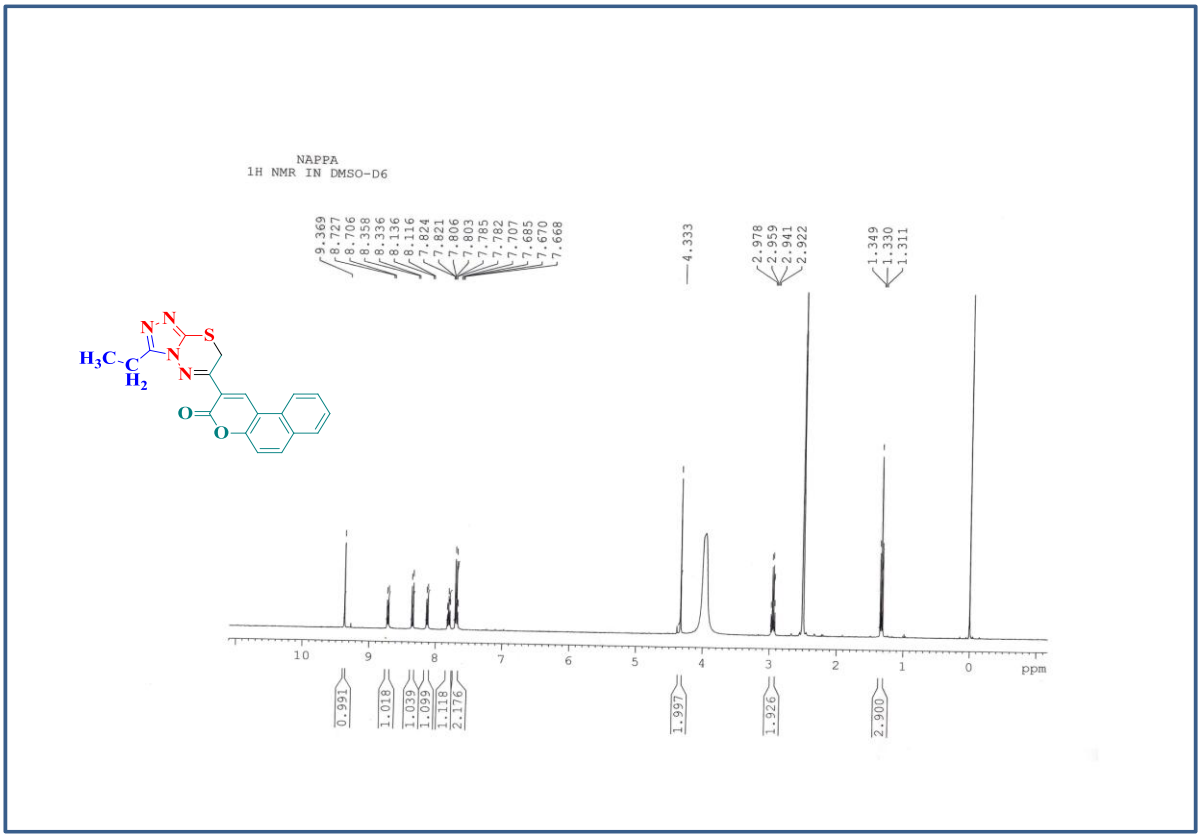


Fig: ^{1}H NMR spectrum of compound **43h**

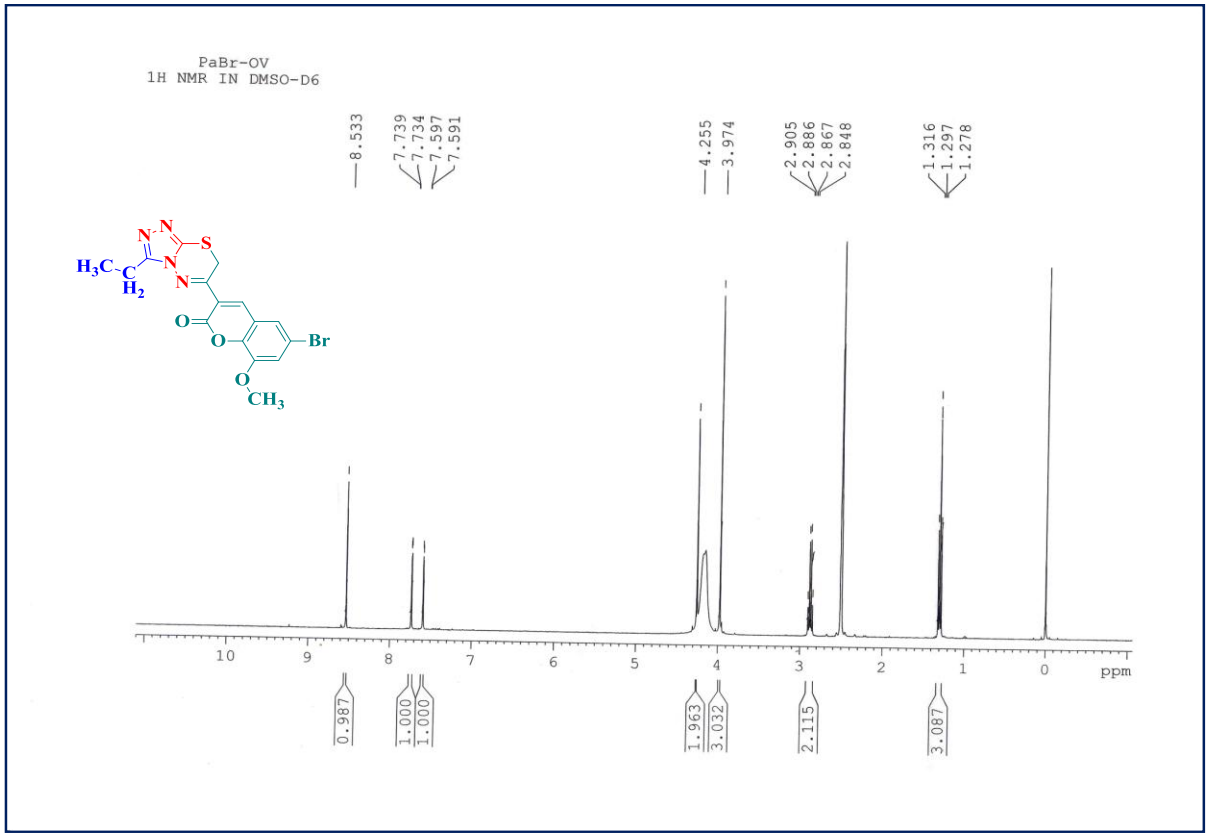


Fig: ^{1}H NMR spectrum of compound **43i**

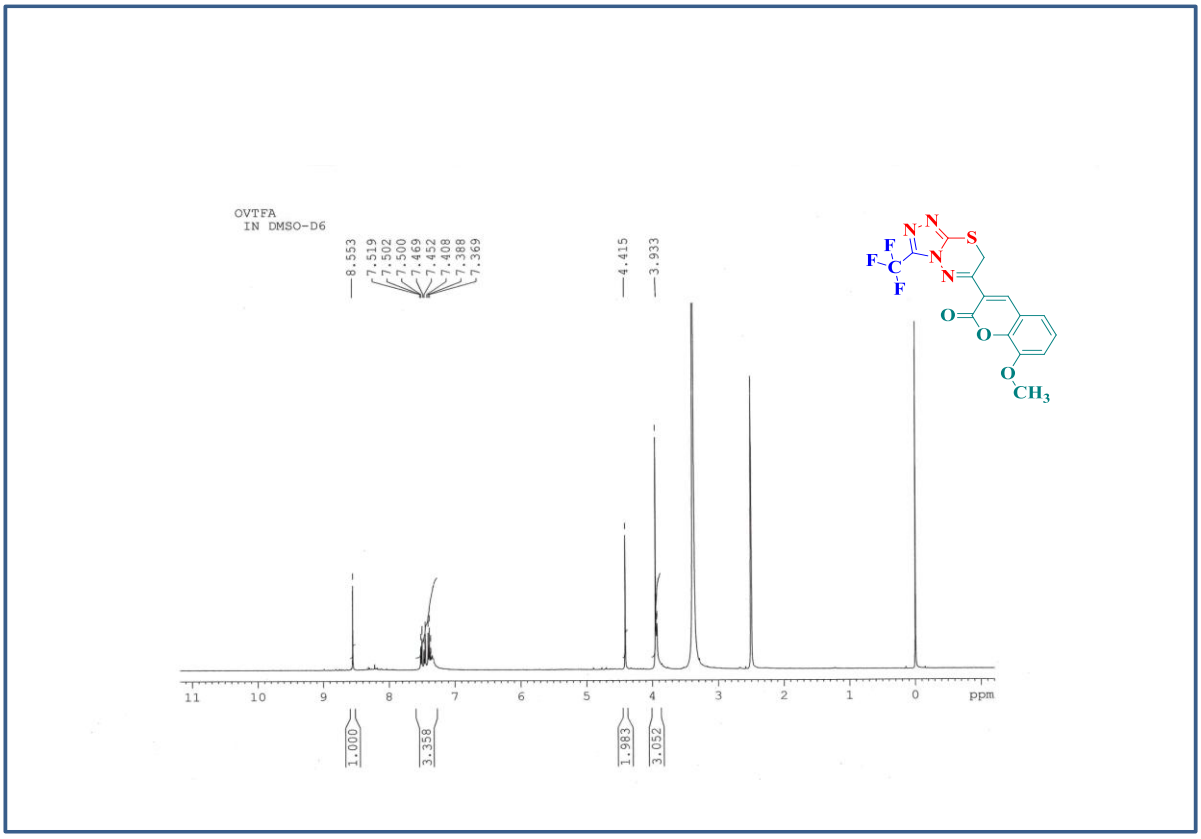


Fig: ^{1}H NMR spectrum of compound **43j**

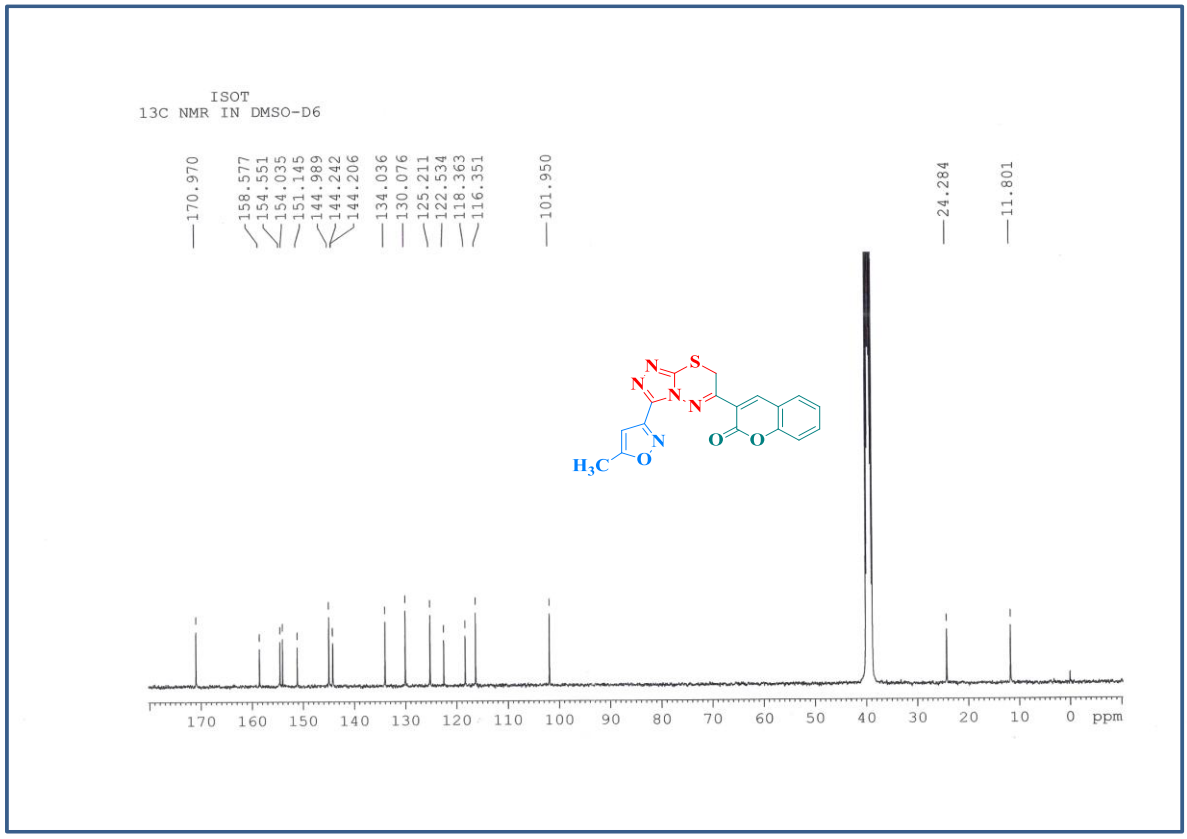


Fig: ^{13}C NMR spectrum of compound **41a**

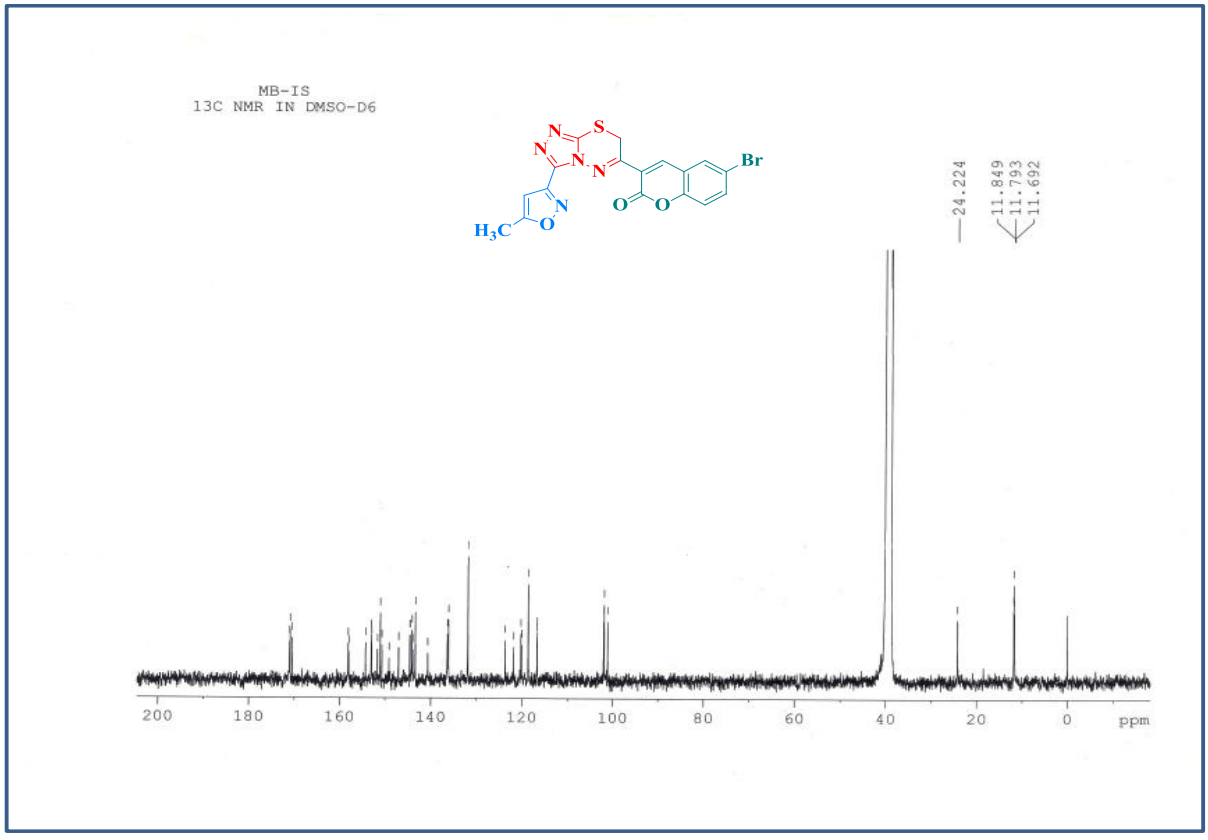


Fig: ^{13}C NMR spectrum of compound **41d**

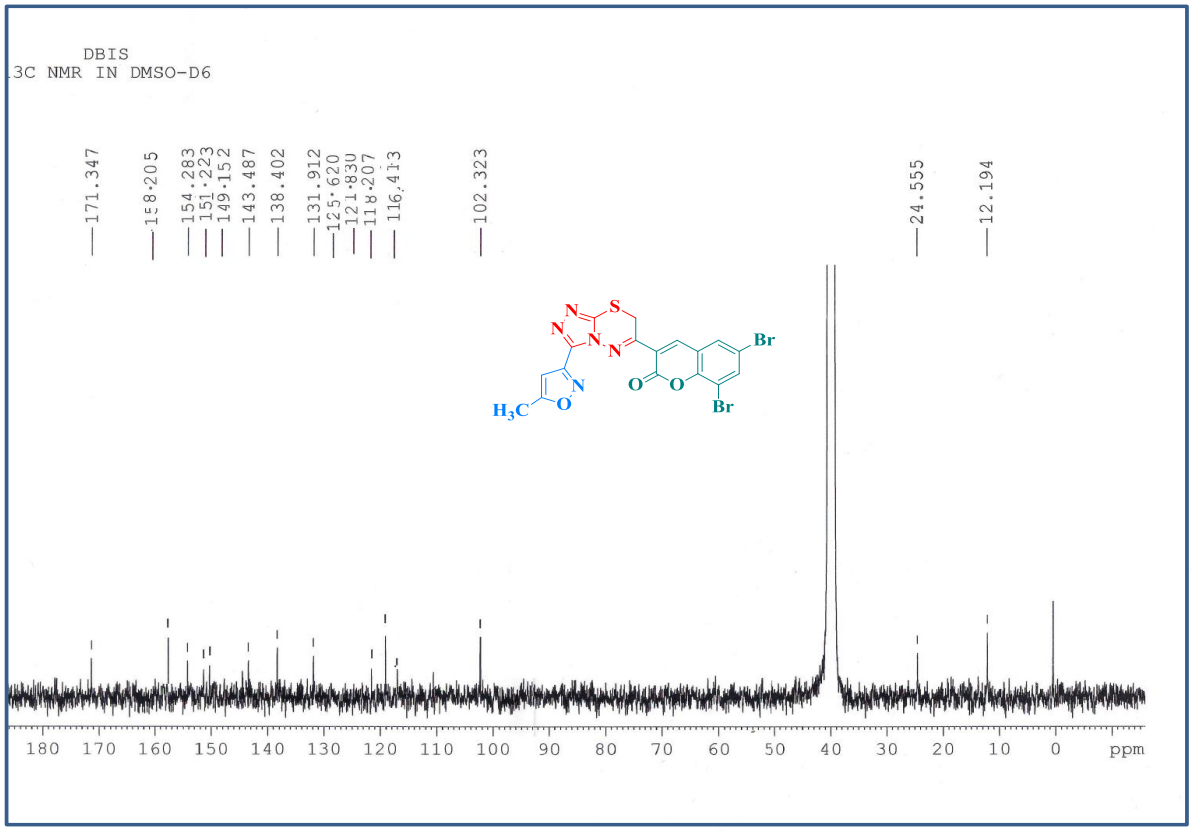


Fig: ^{13}C NMR spectrum of compound **41e**

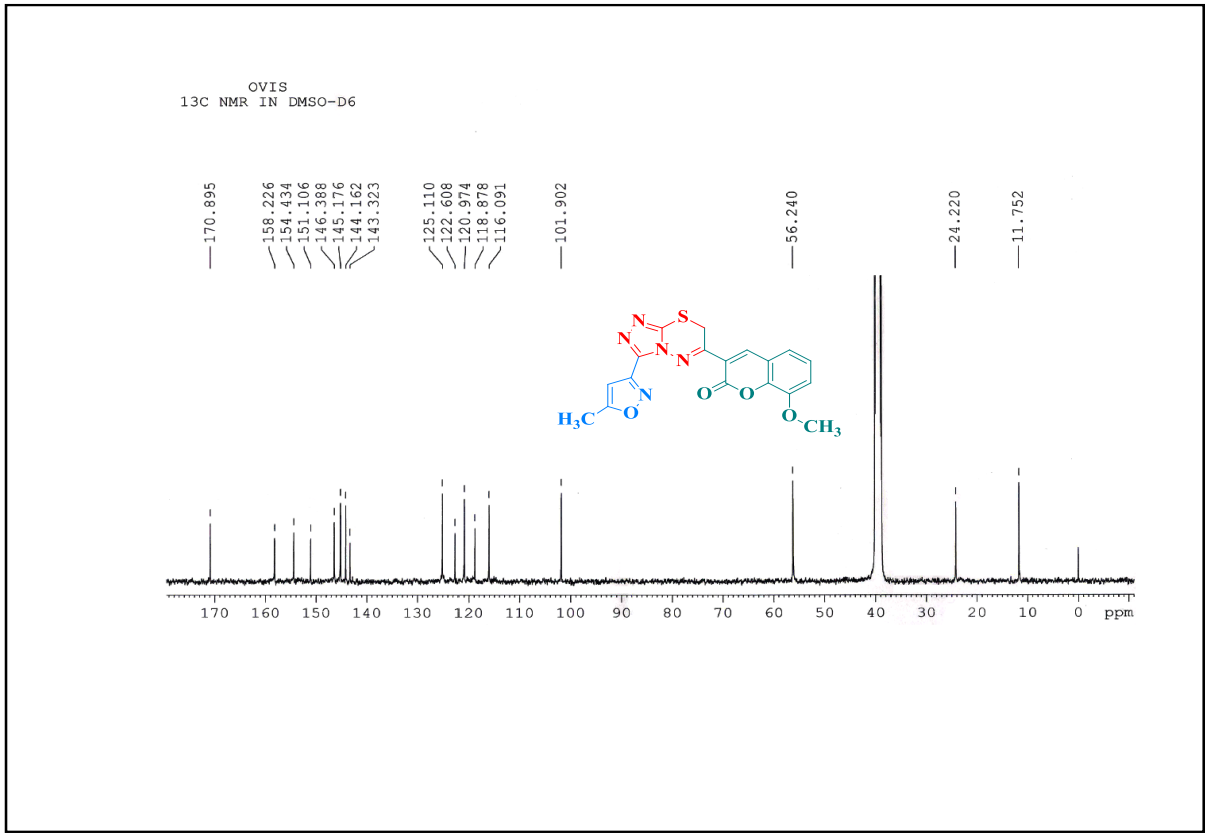


Fig: ^{13}C NMR spectrum of compound **41f**

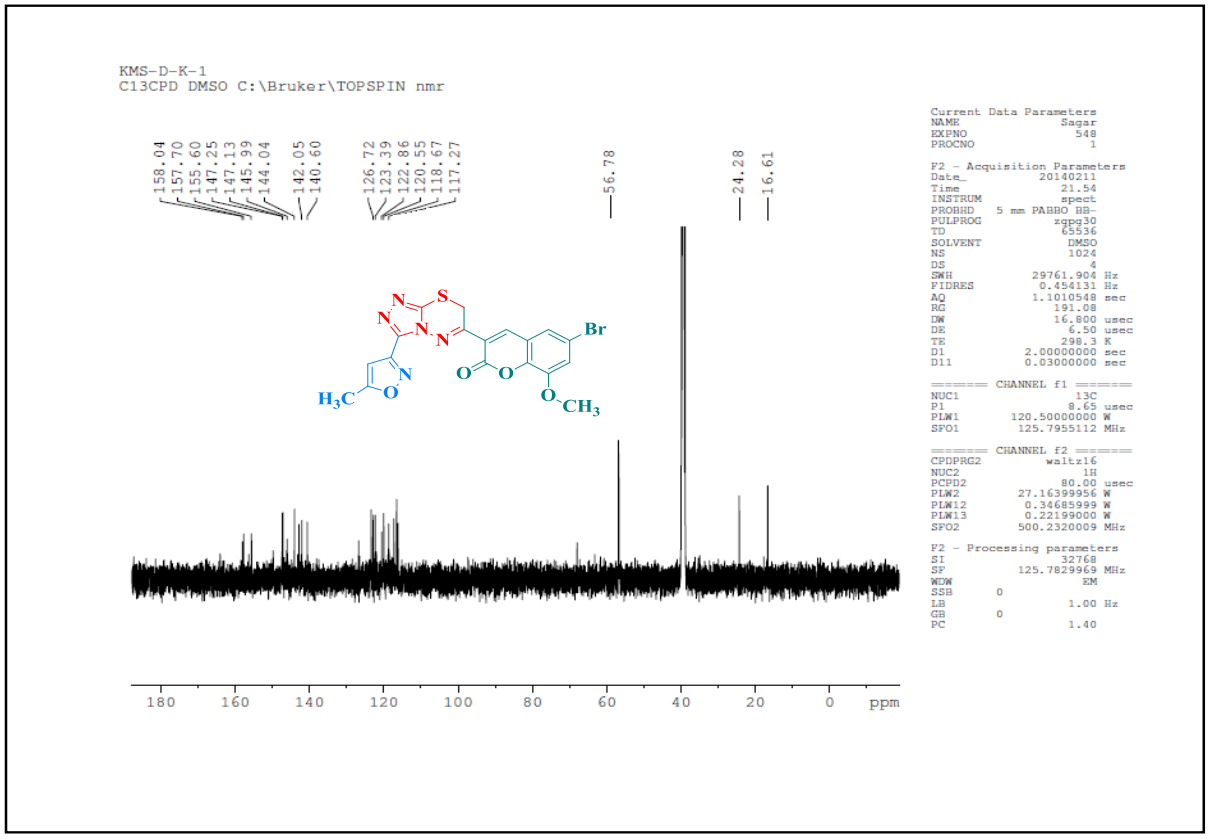


Fig: ^{13}C NMR spectrum of compound **41g**

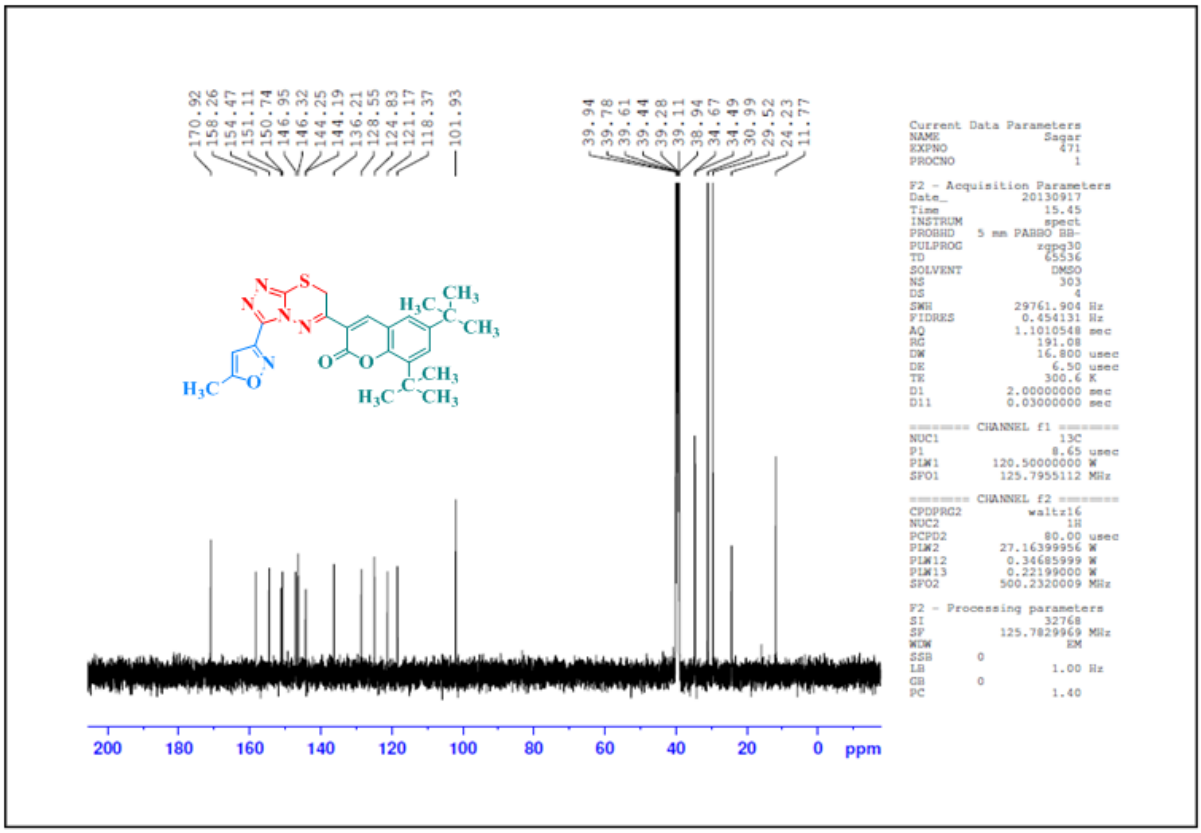


Fig: ^{13}C NMR spectrum of compound **41h**

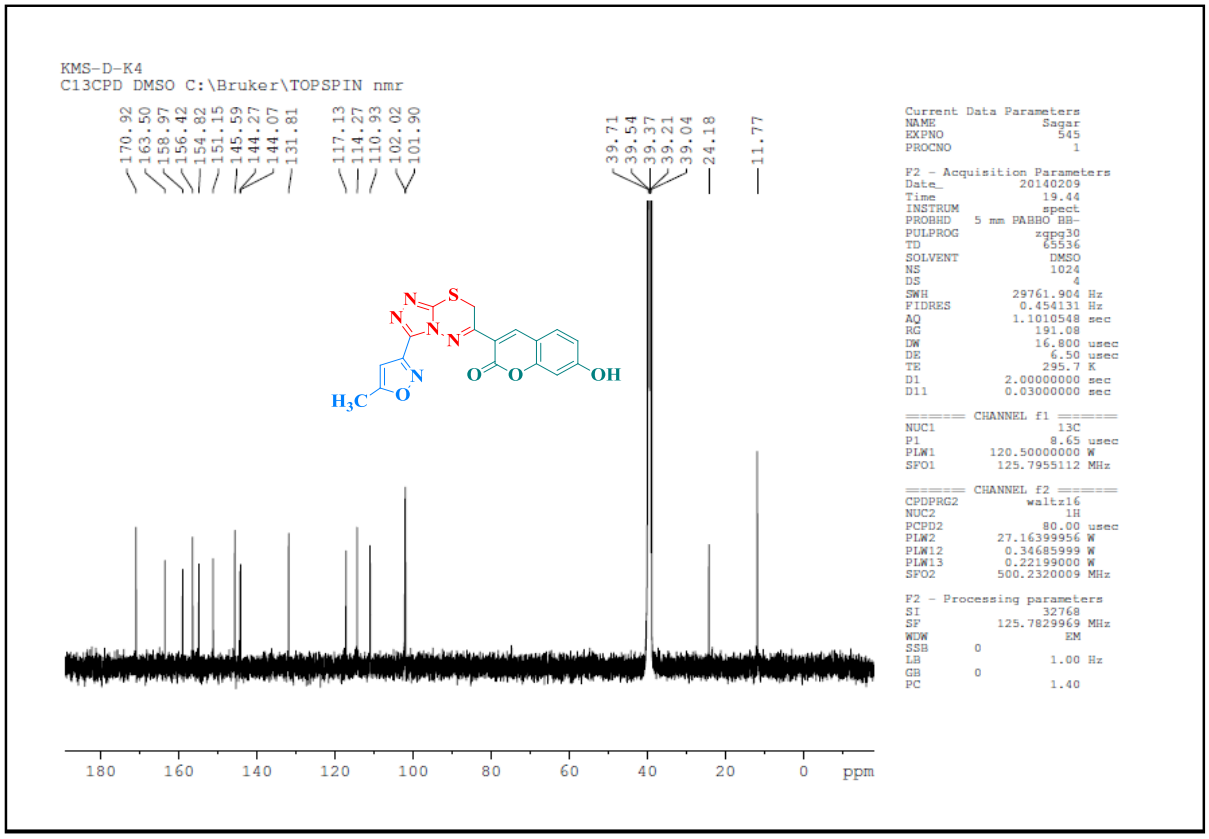


Fig: ^{13}C NMR spectrum of compound **41i**

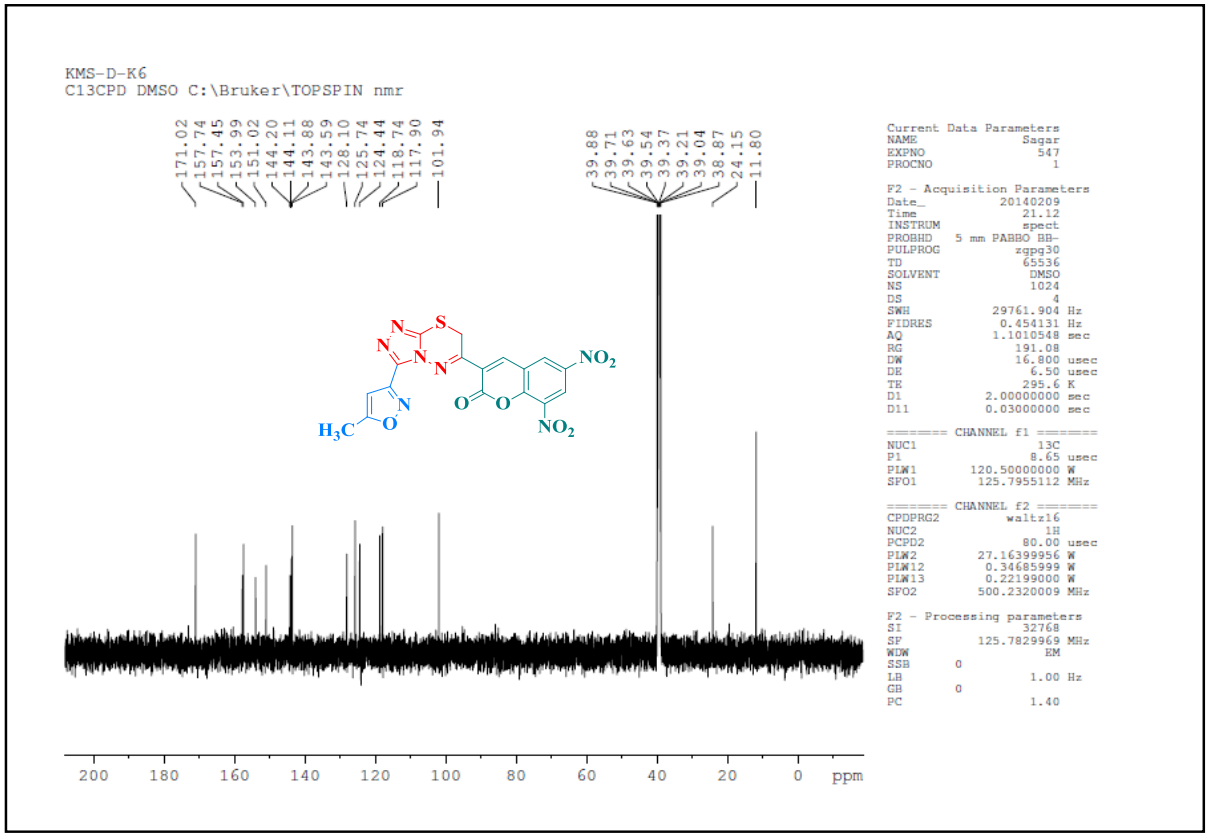


Fig: ^{13}C NMR spectrum of compound **41j**

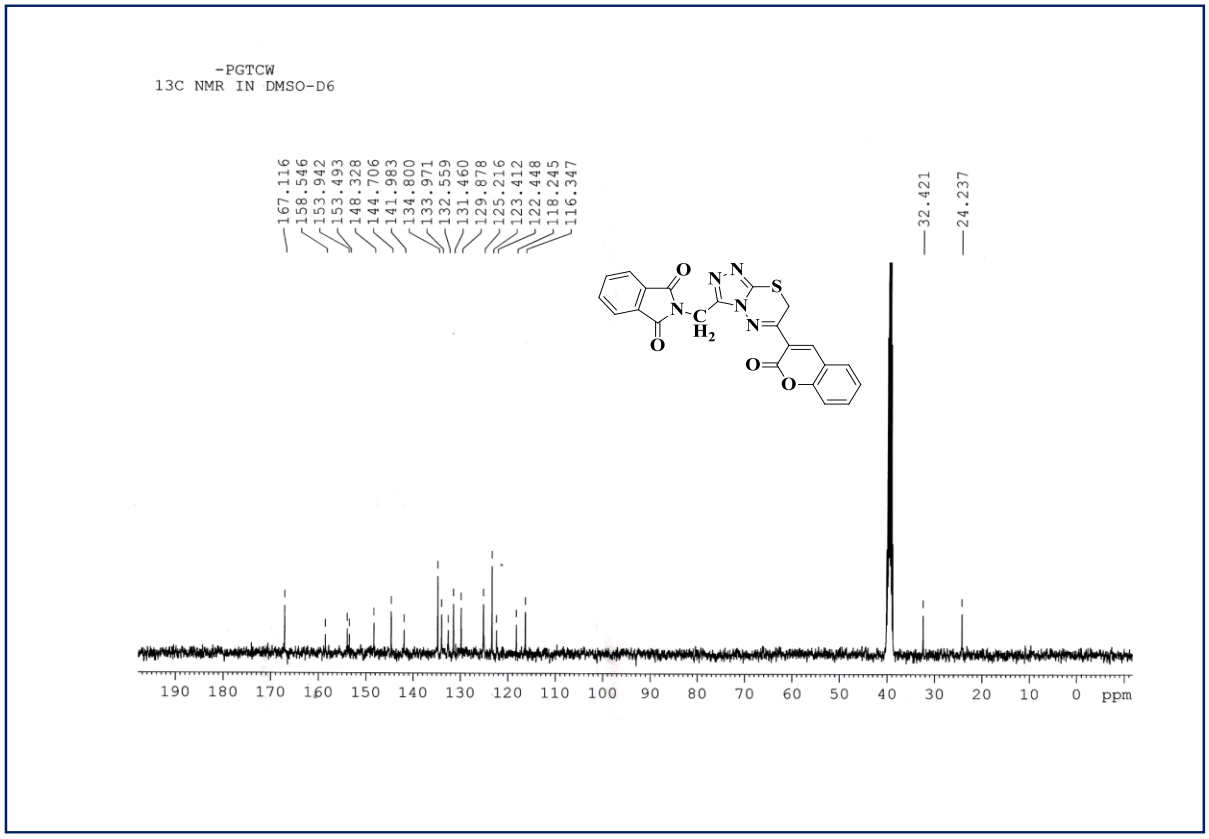


Fig: ^{13}C NMR spectrum of compound **41k**

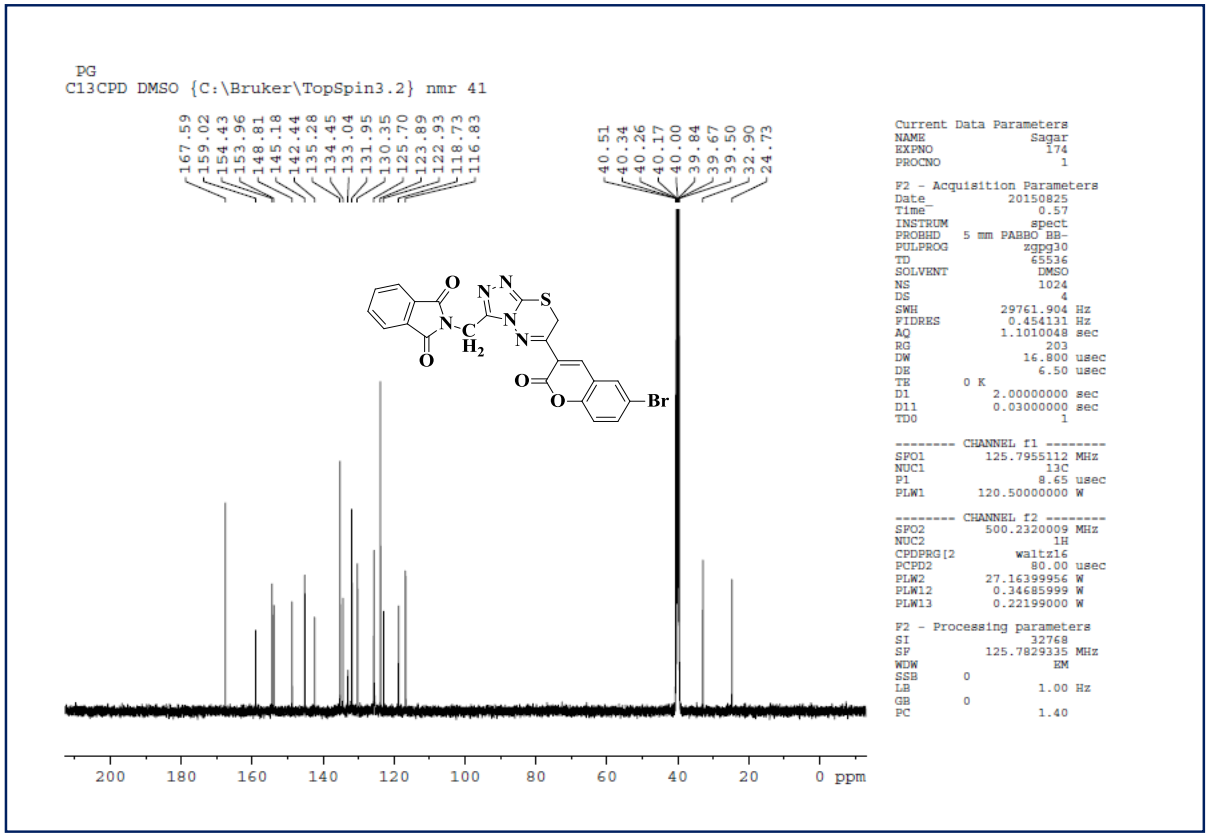


Fig: ^{13}C NMR spectrum of compound **41n**

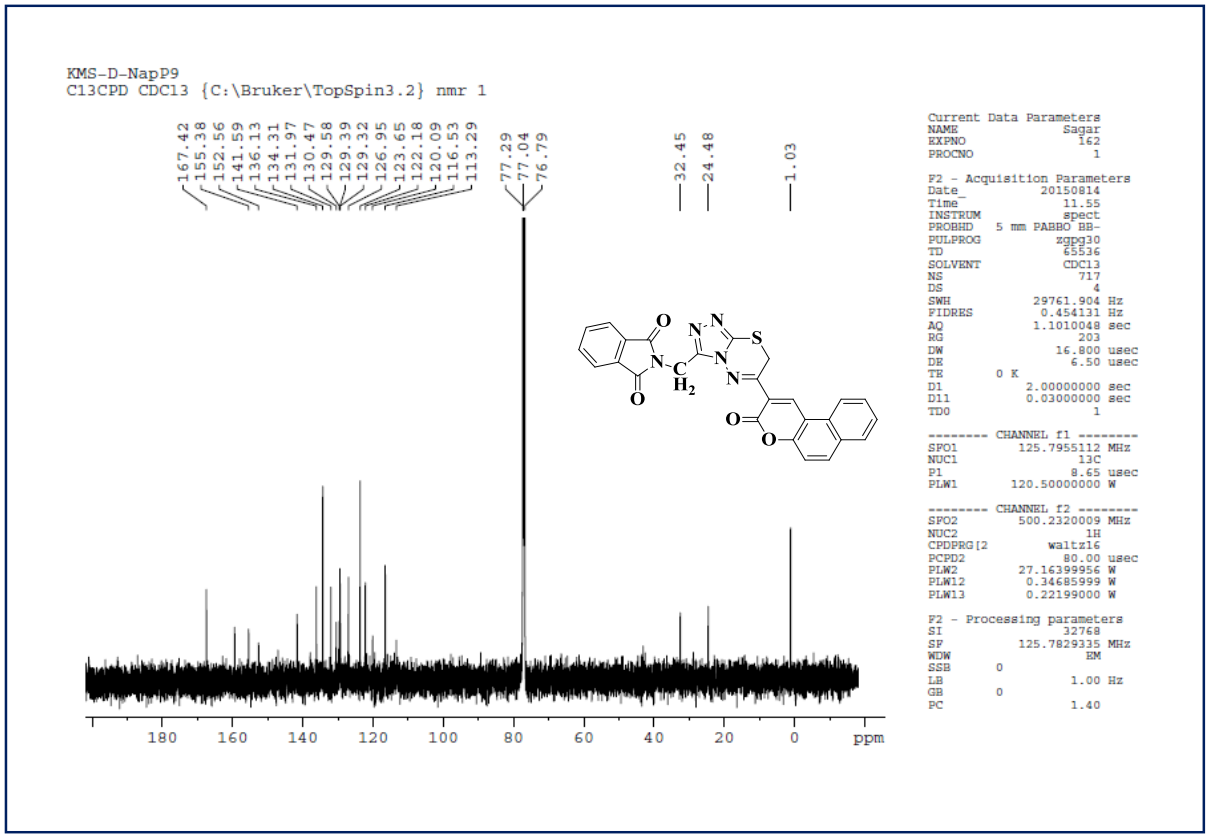


Fig: ^{13}C NMR spectrum of compound **41p**

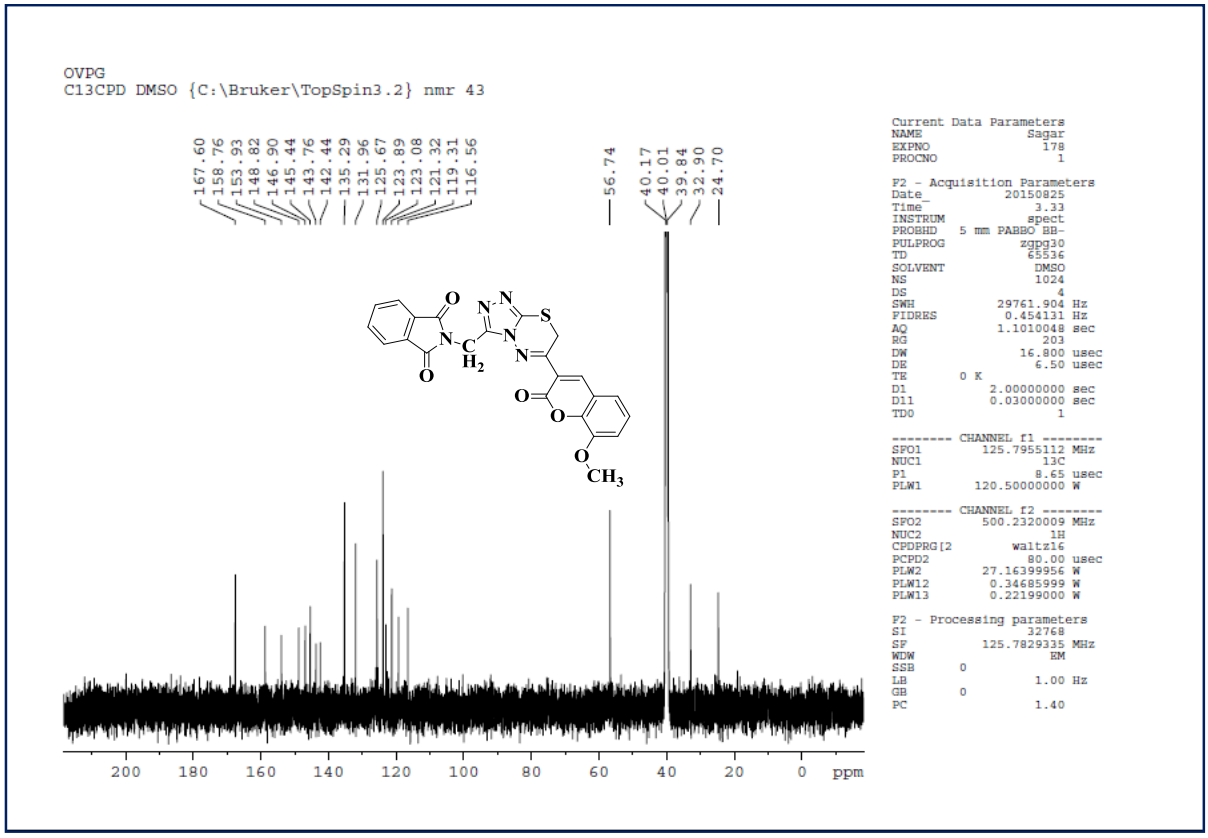


Fig: ^{13}C NMR spectrum of compound **41q**

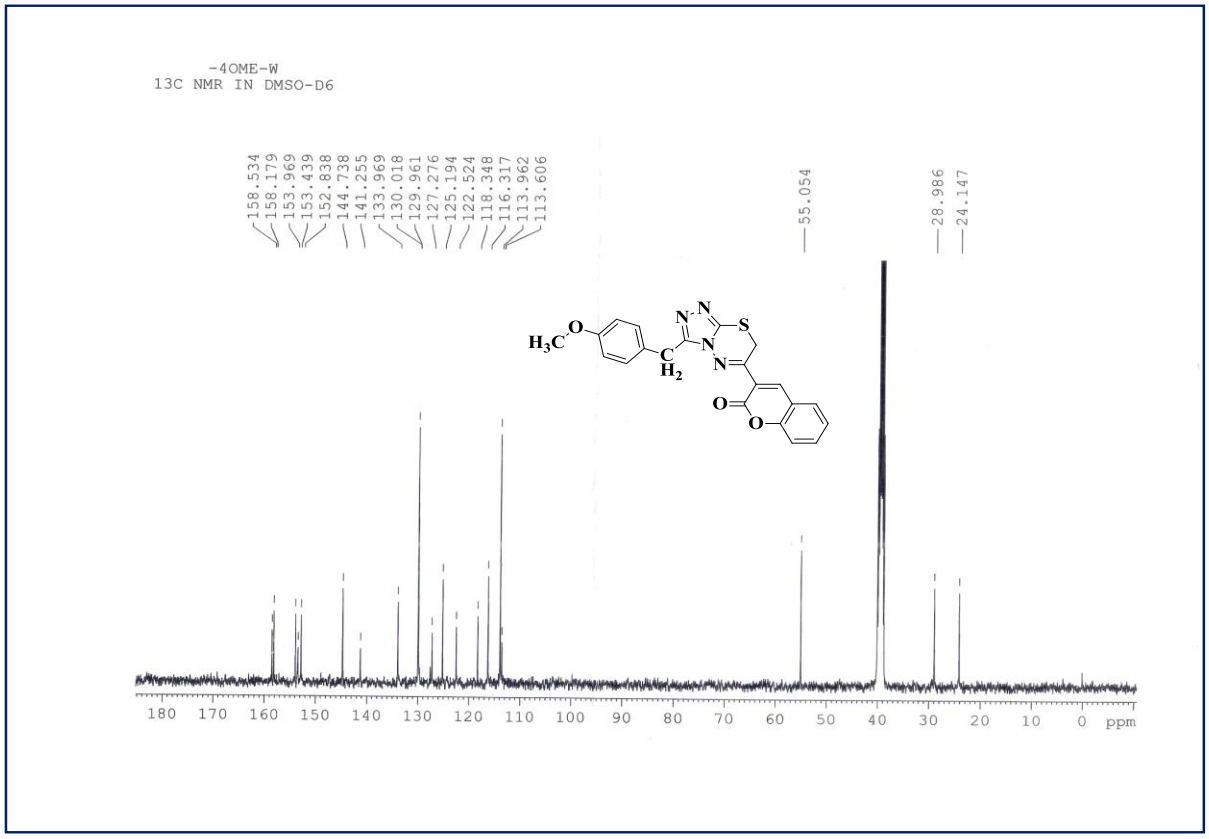


Fig: ^{13}C NMR spectrum of compound **41s**

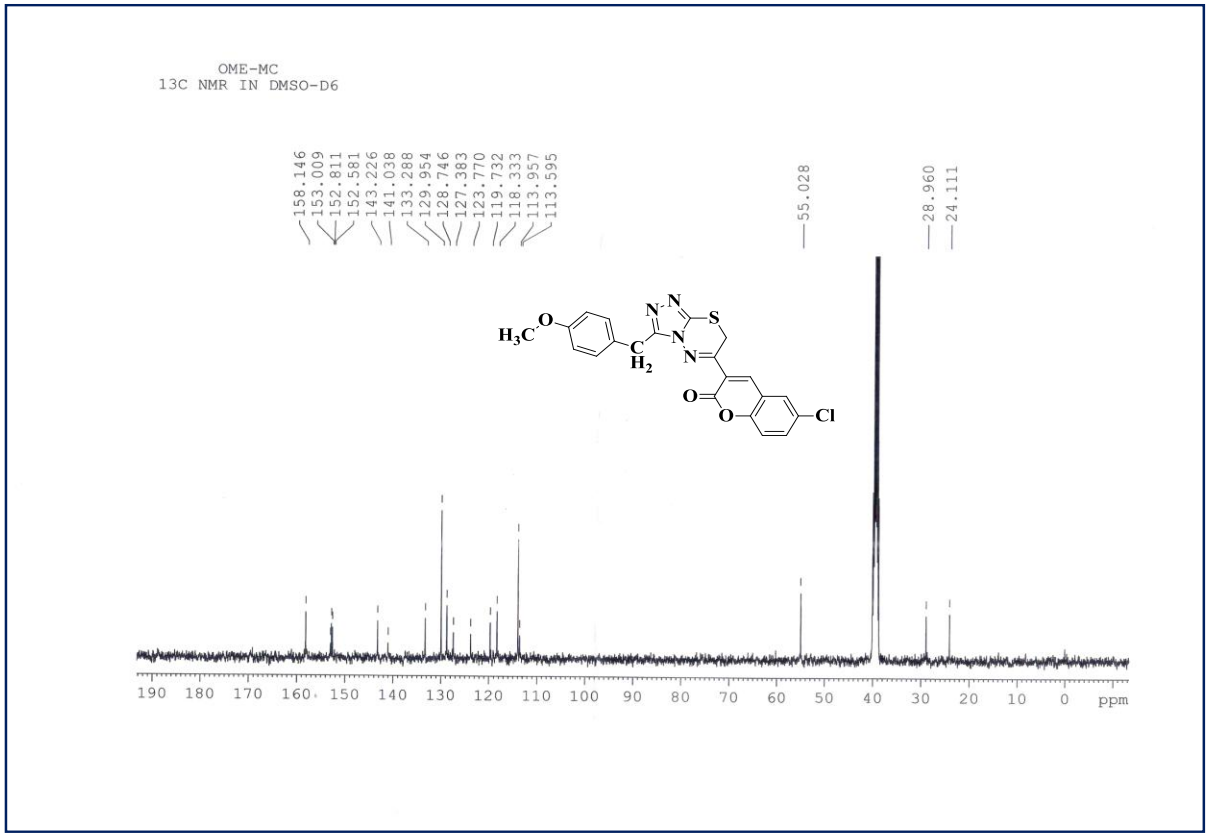


Fig: ^{13}C NMR spectrum of compound **41t**

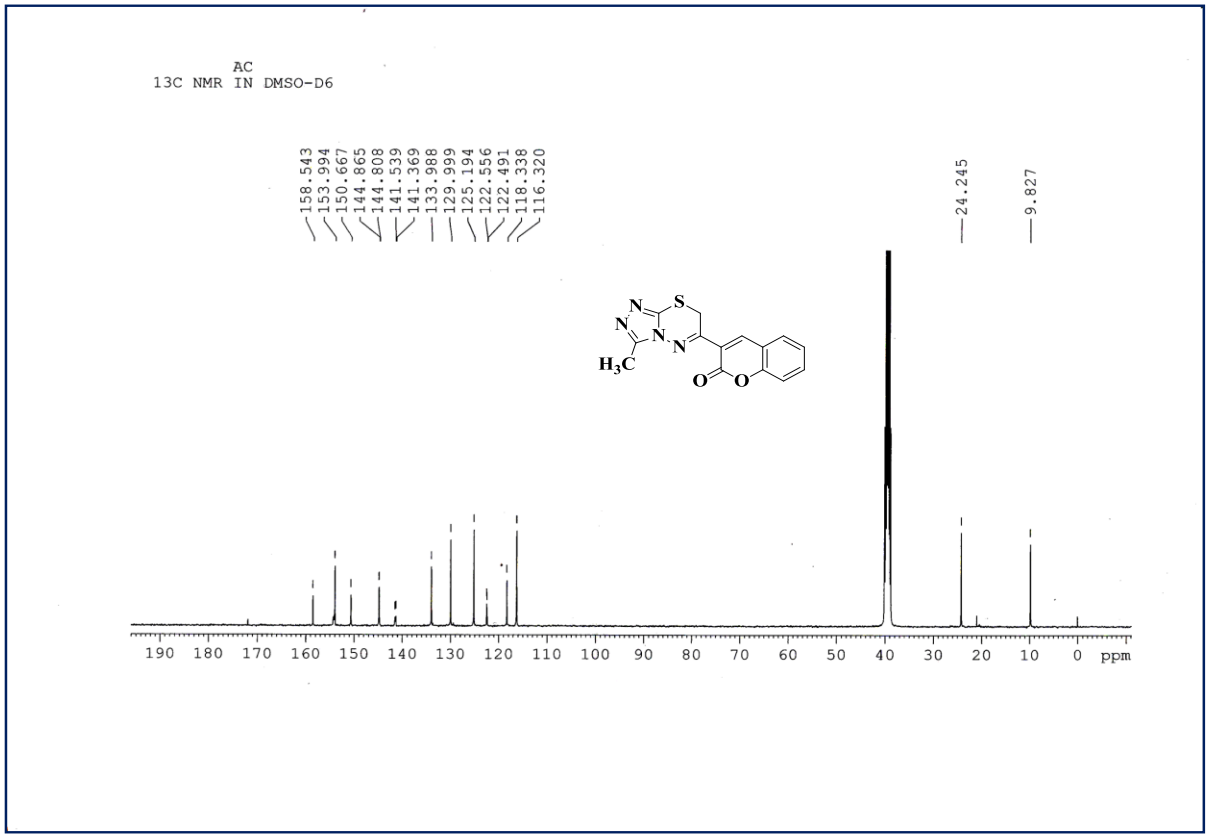


Fig: ^{13}C NMR spectrum of compound **43a**

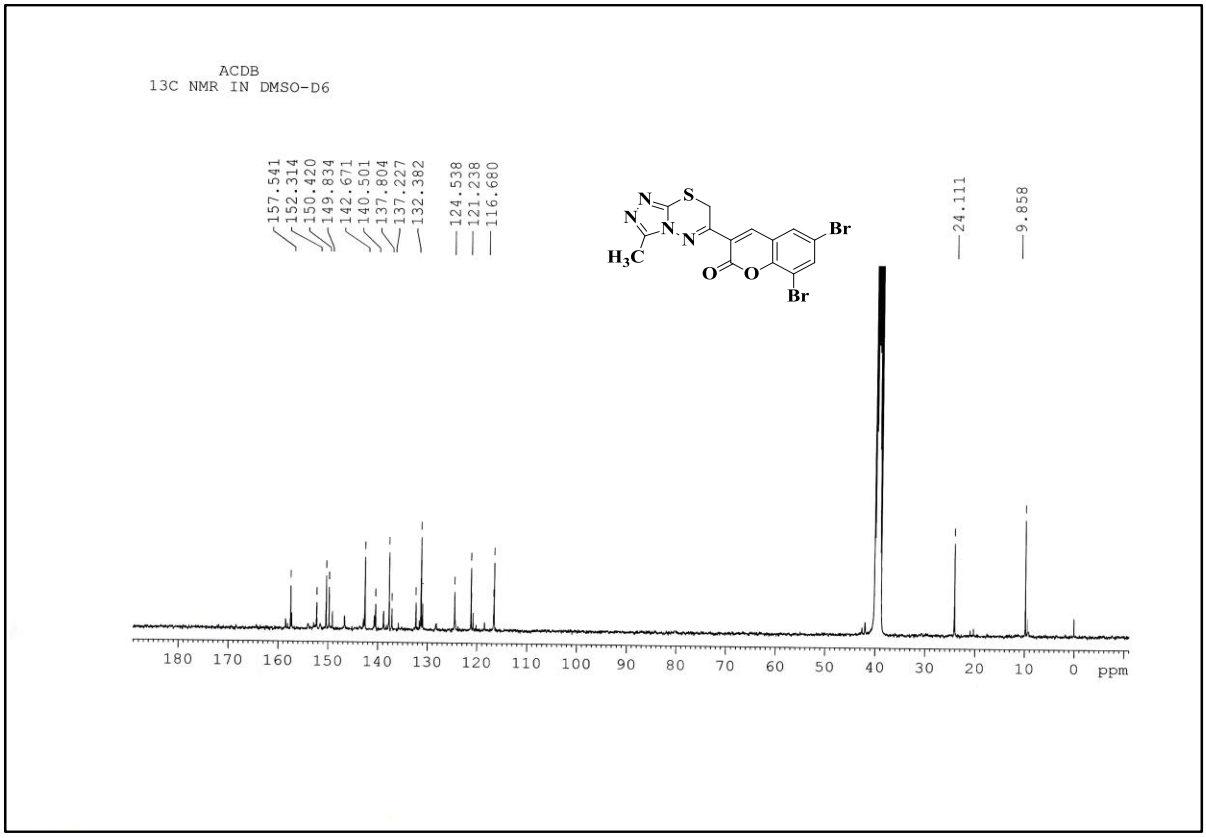


Fig: ^{13}C NMR spectrum of compound **43b**

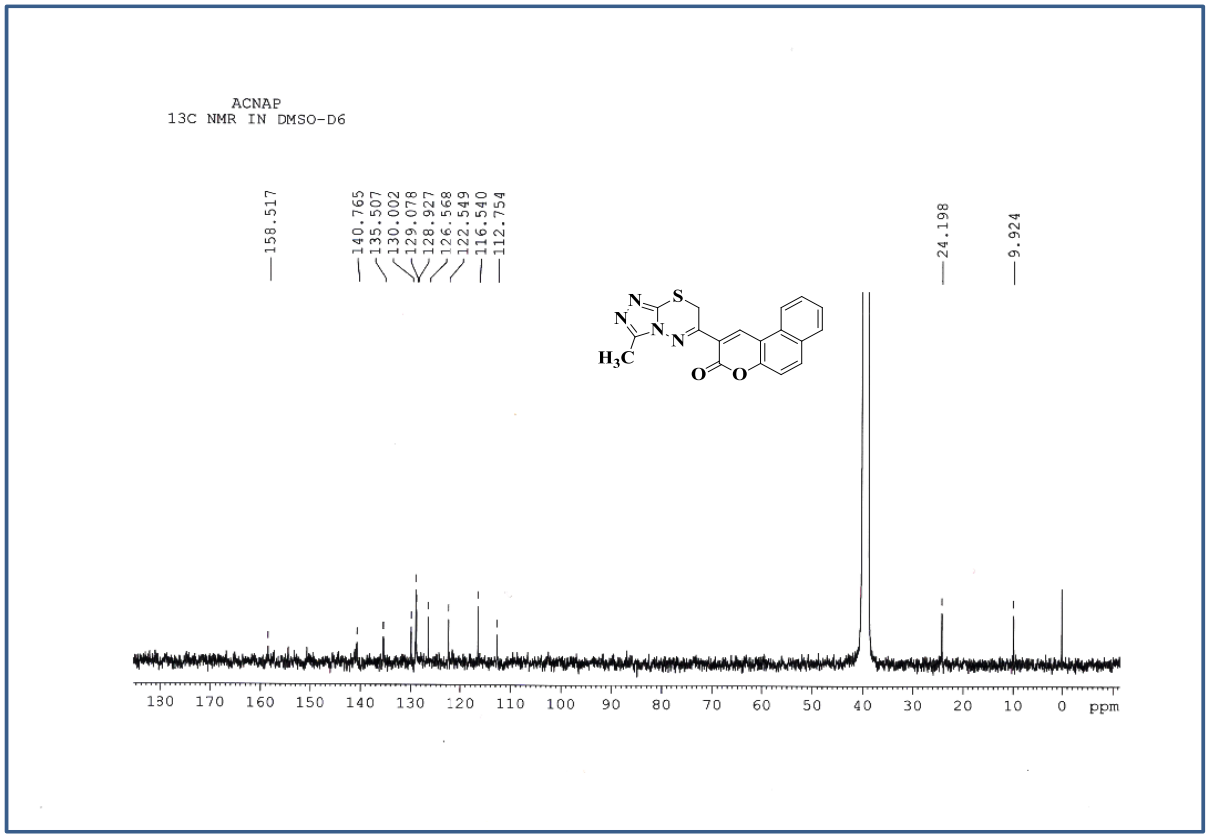


Fig: ^{13}C NMR spectrum of compound **43c**

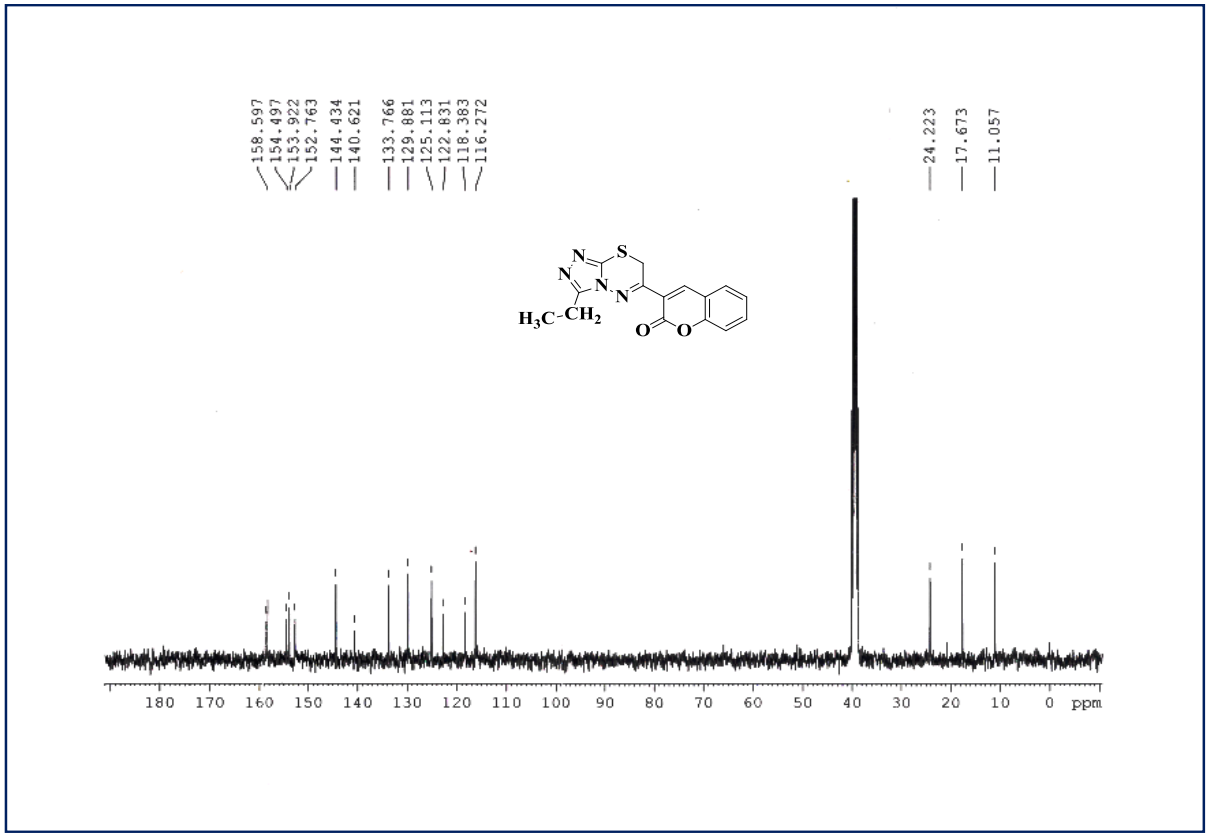


Fig: ^{13}C NMR spectrum of compound **43d**

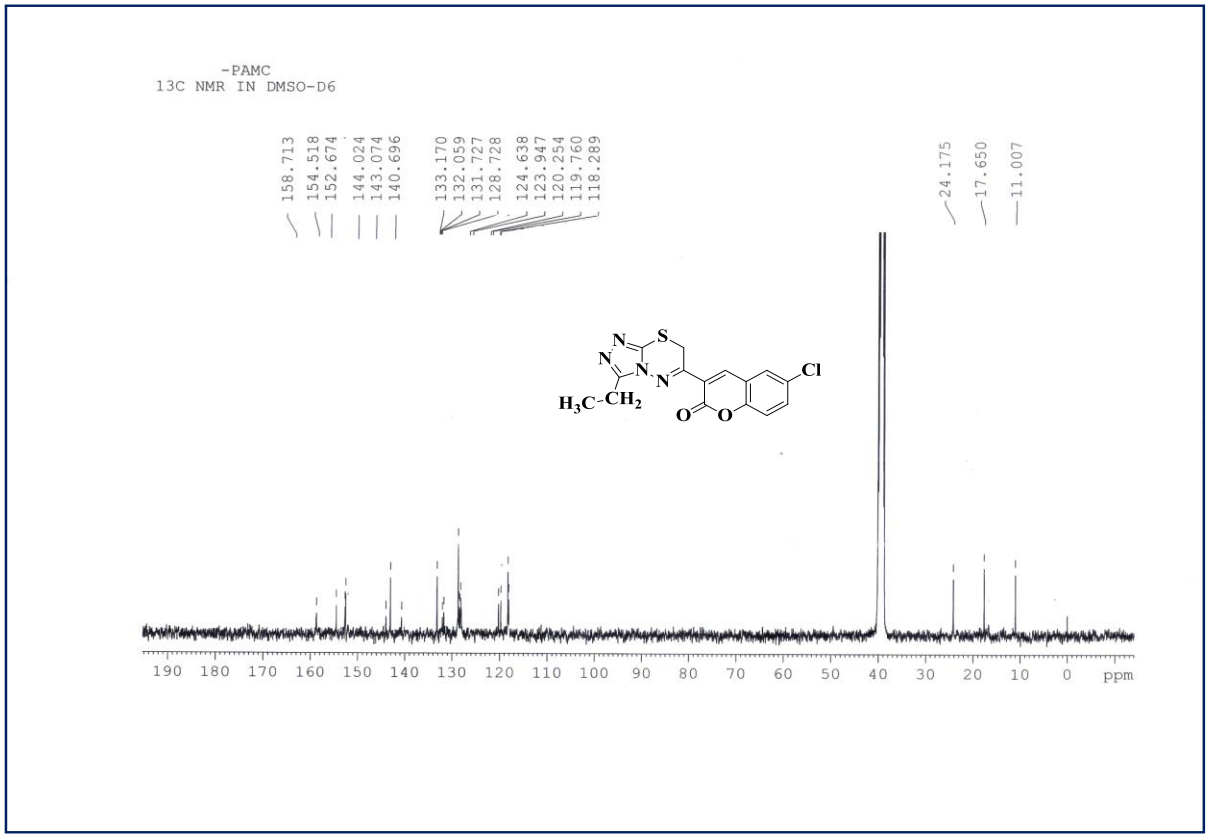


Fig: ^{13}C NMR spectrum of compound **43e**

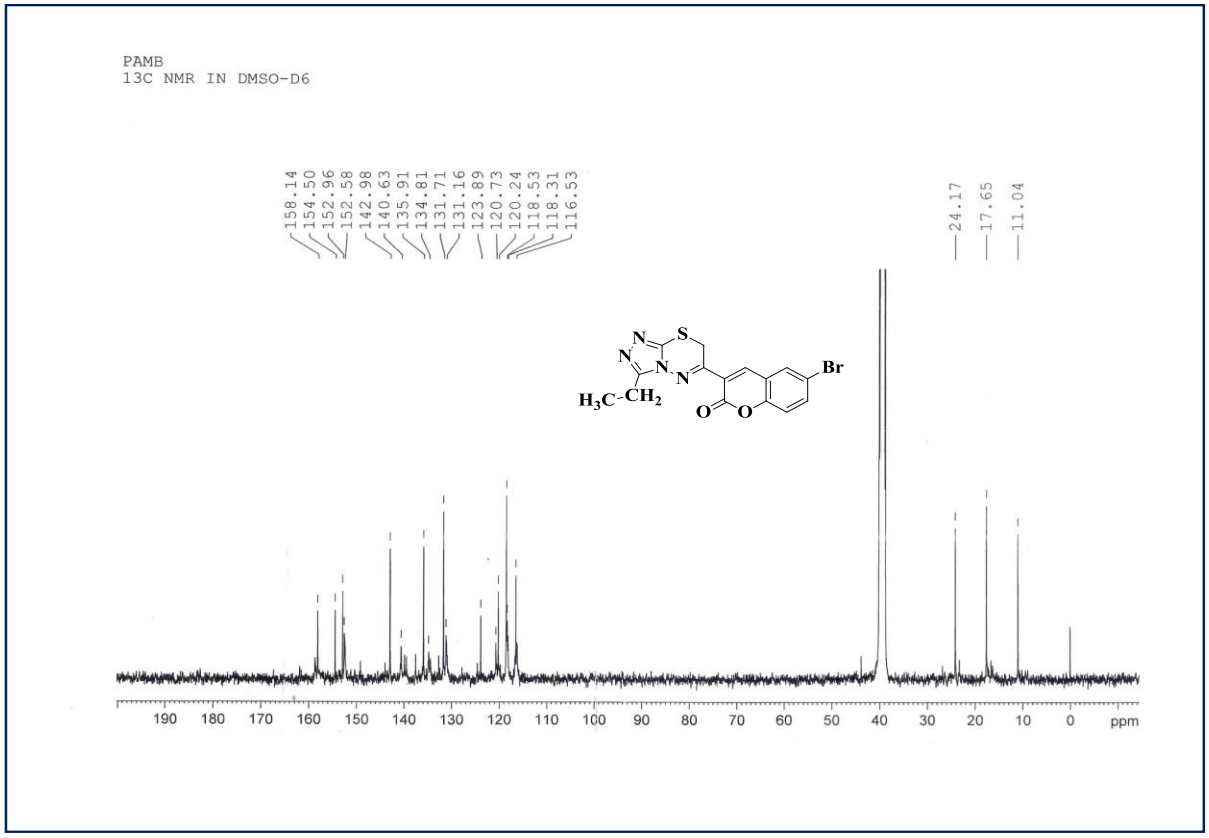


Fig: ^{13}C NMR spectrum of compound **43f**

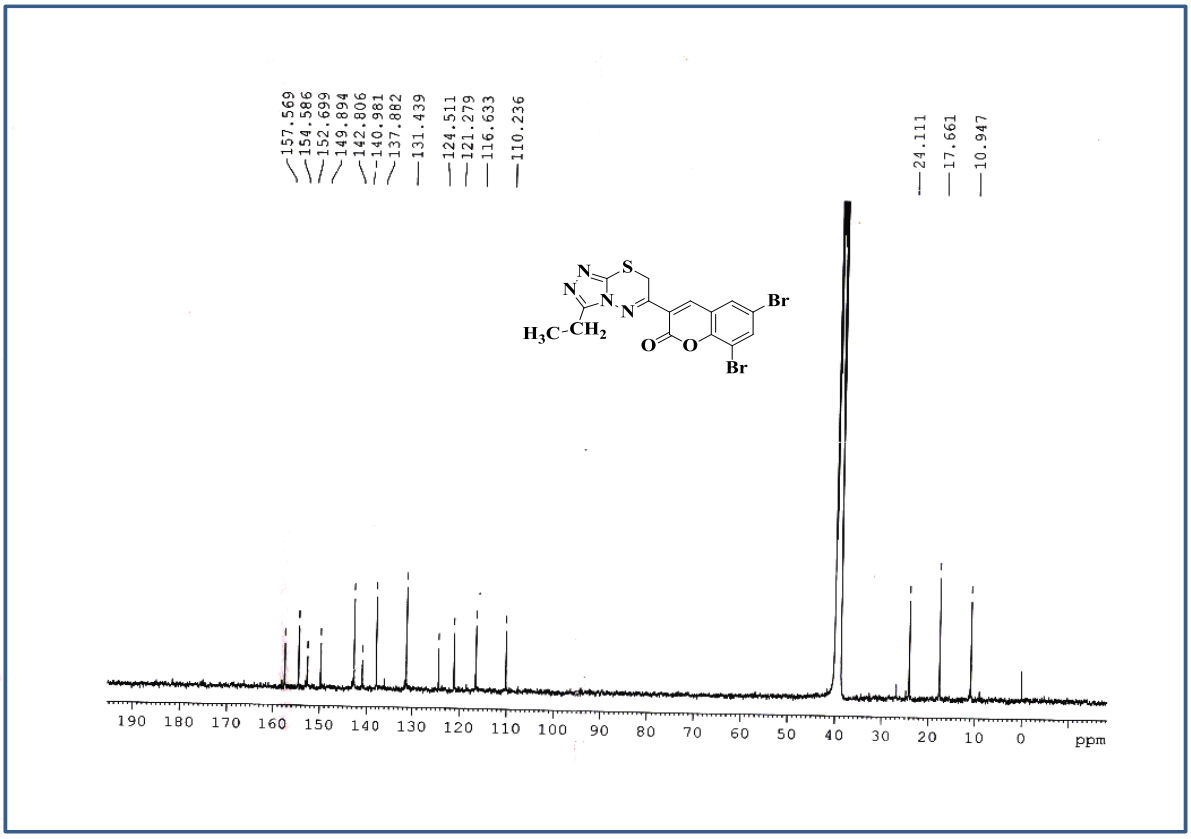


Fig: ^{13}C NMR spectrum of compound **43g**

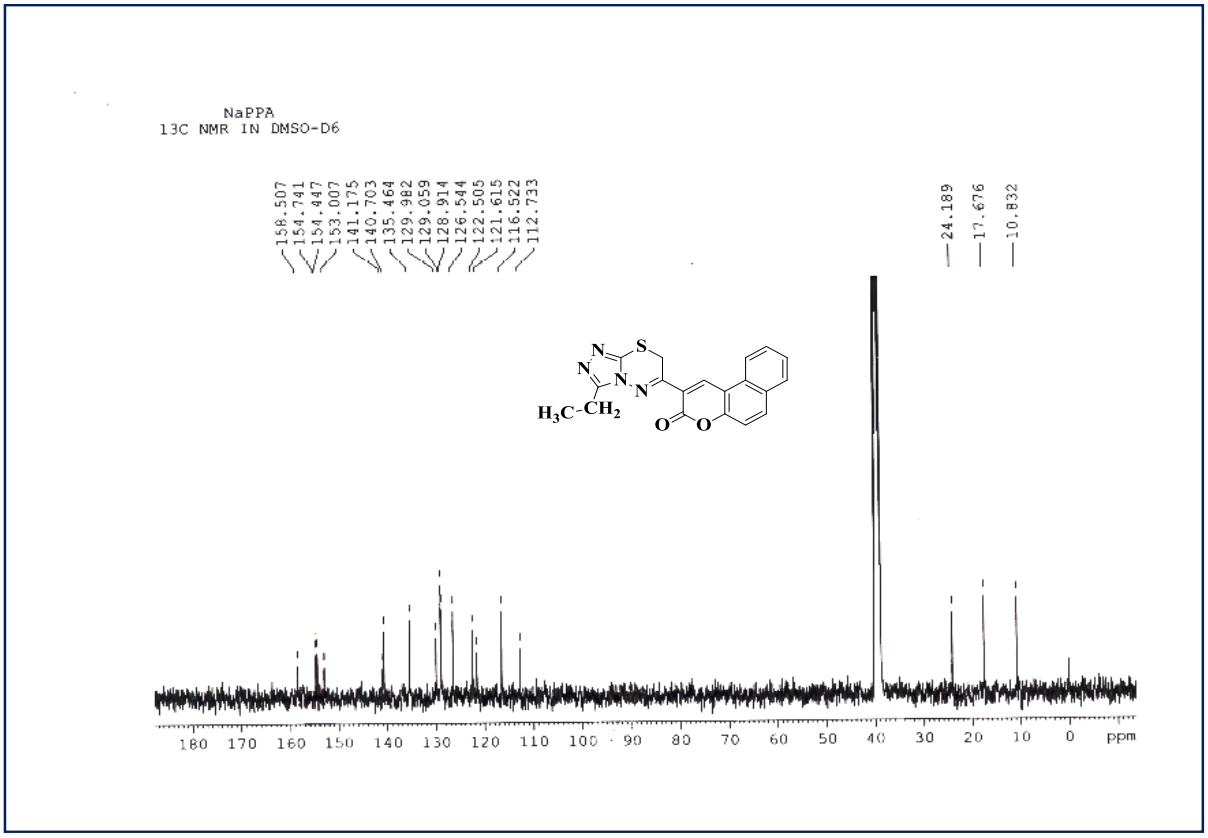


Fig: ^{13}C NMR spectrum of compound **43h**

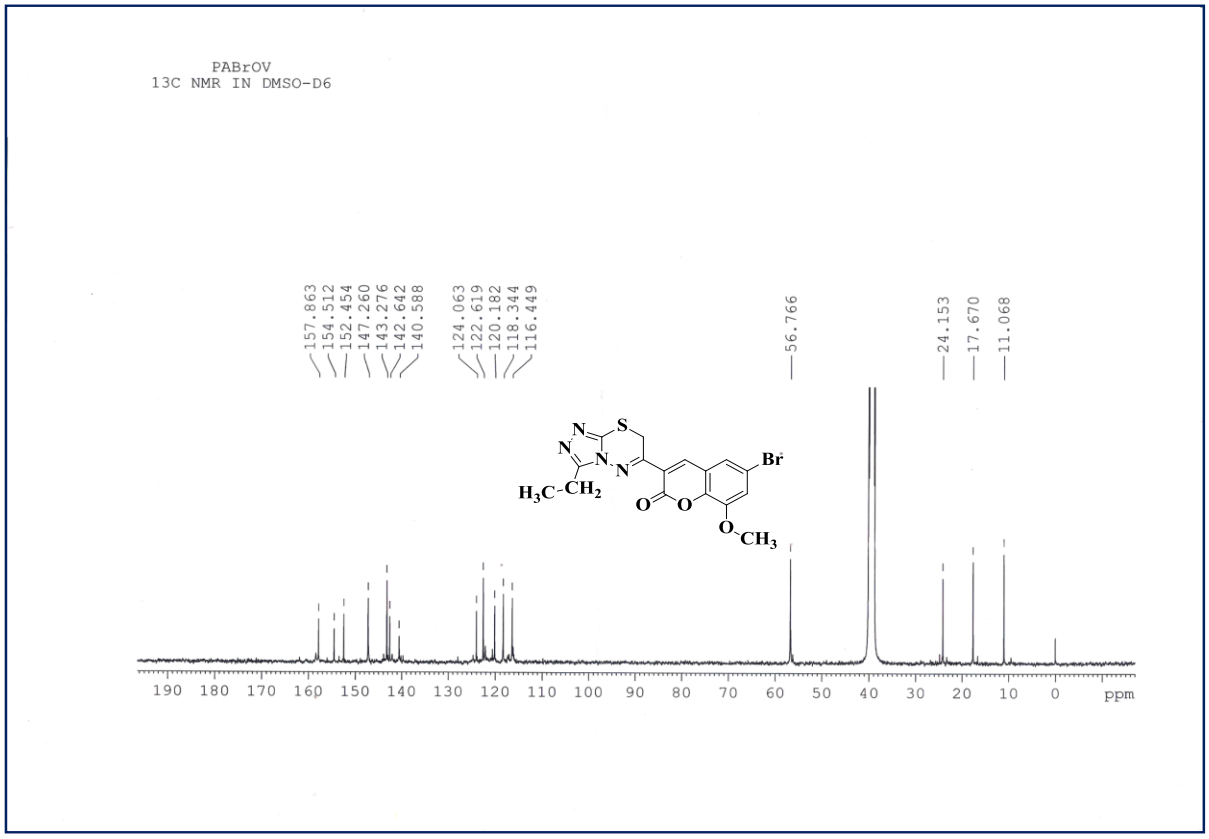


Fig: ^{13}C NMR spectrum of compound **43i**

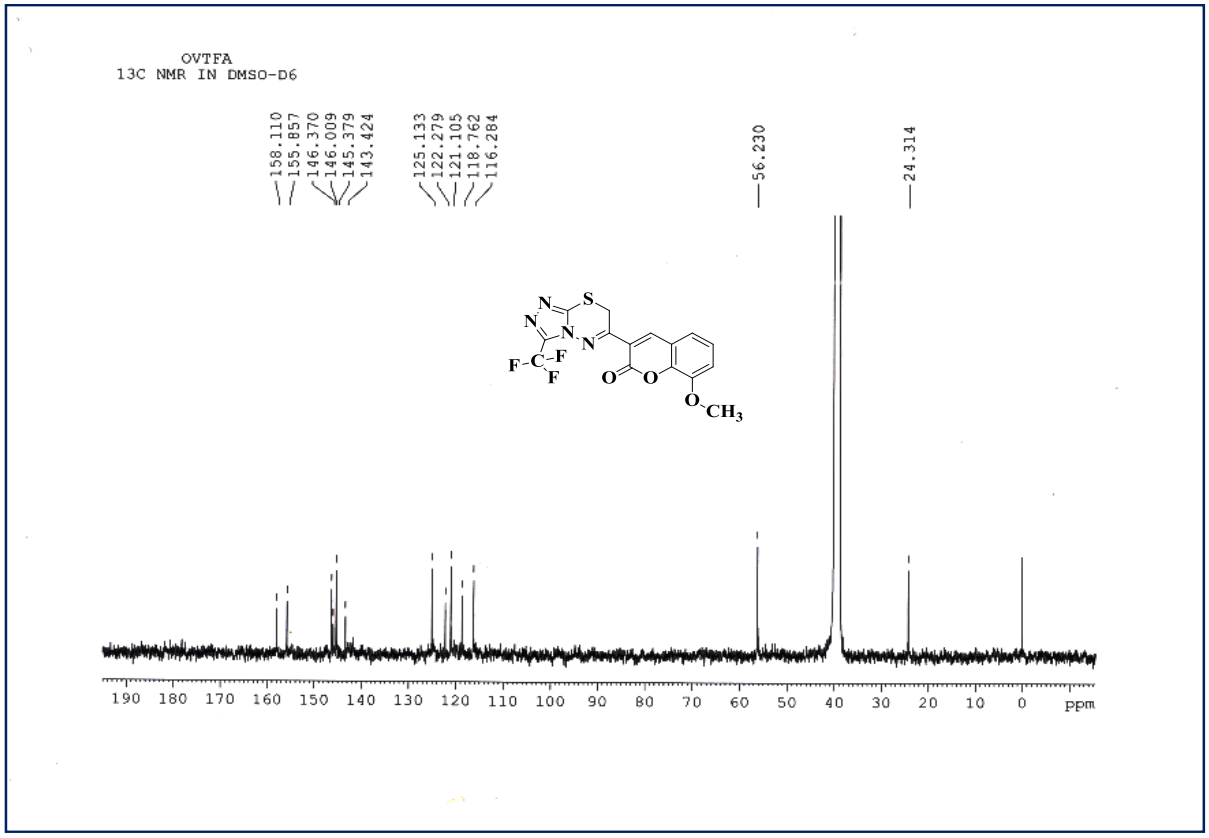


Fig: ^{13}C NMR spectrum of compound **43j**

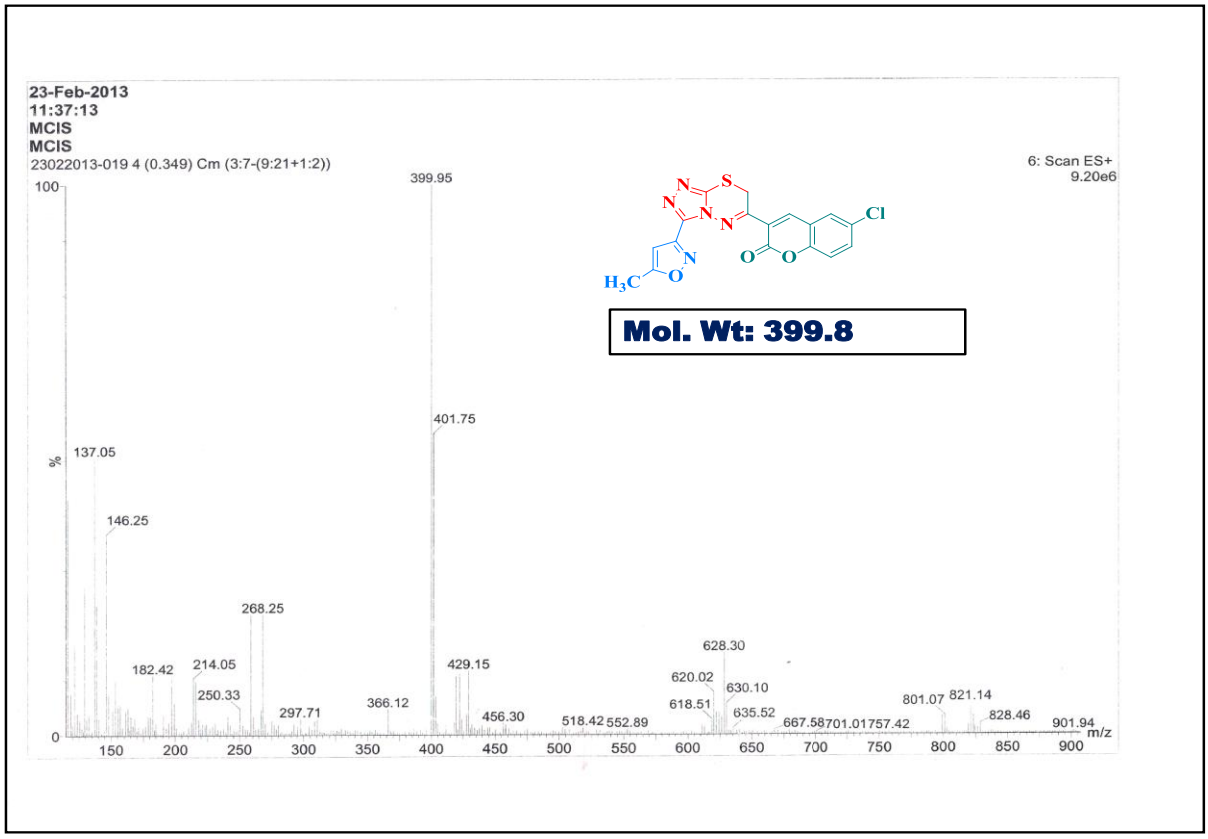


Fig: Mass spectrum of compound **41b**

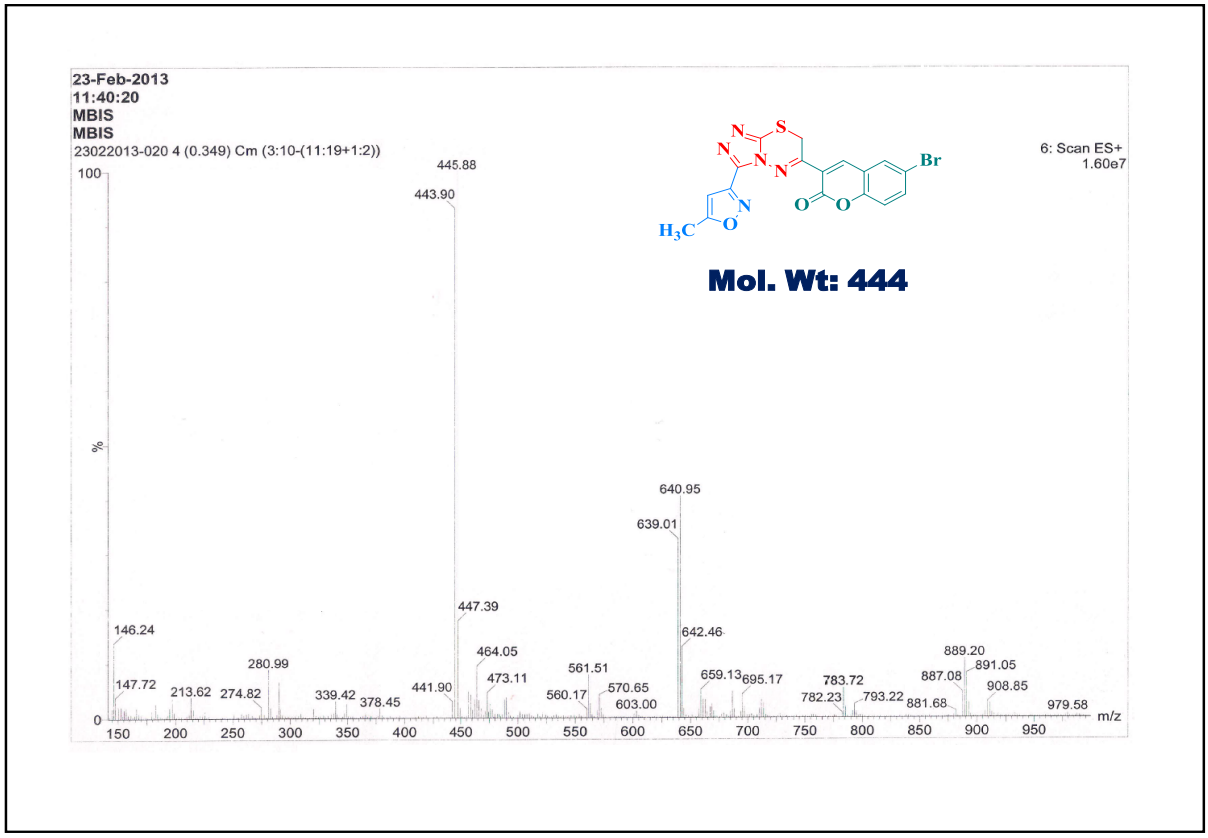


Fig: Mass spectrum of compound **41d**

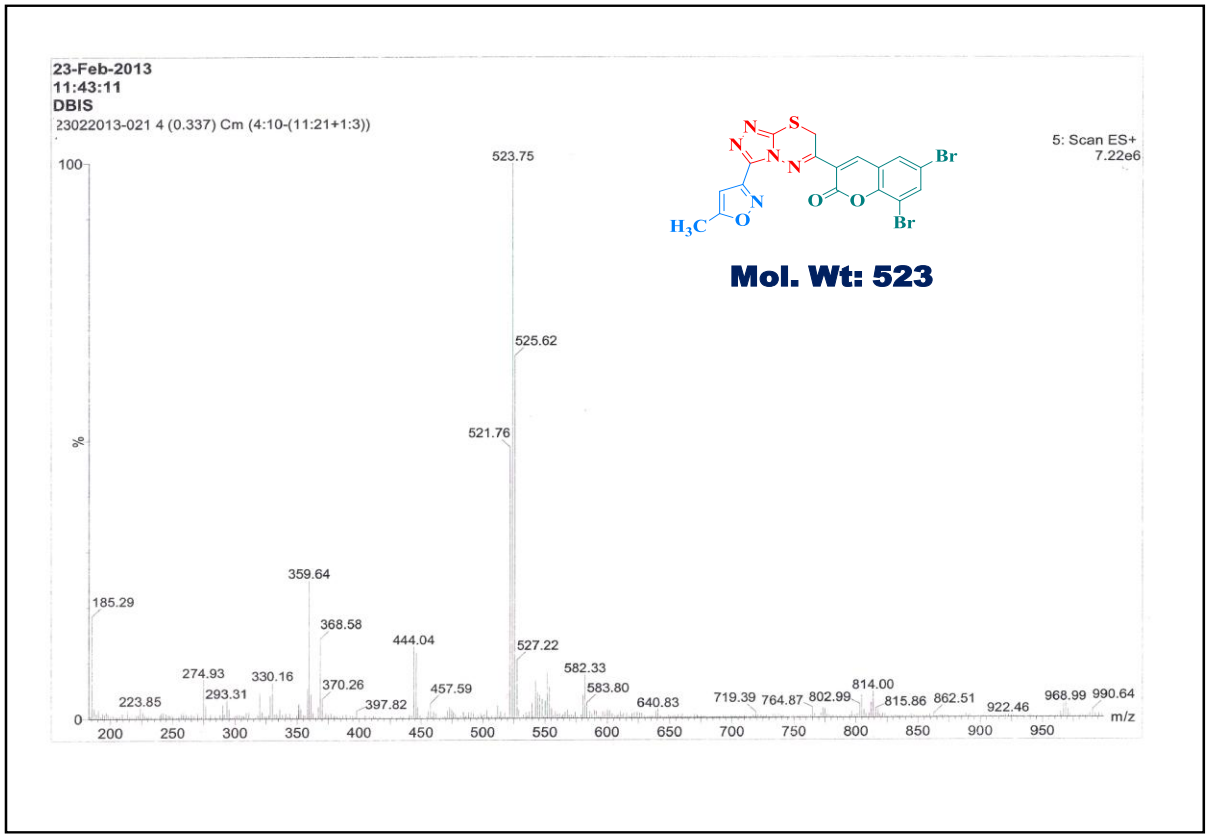


Fig: Mass spectrum of compound **41e**

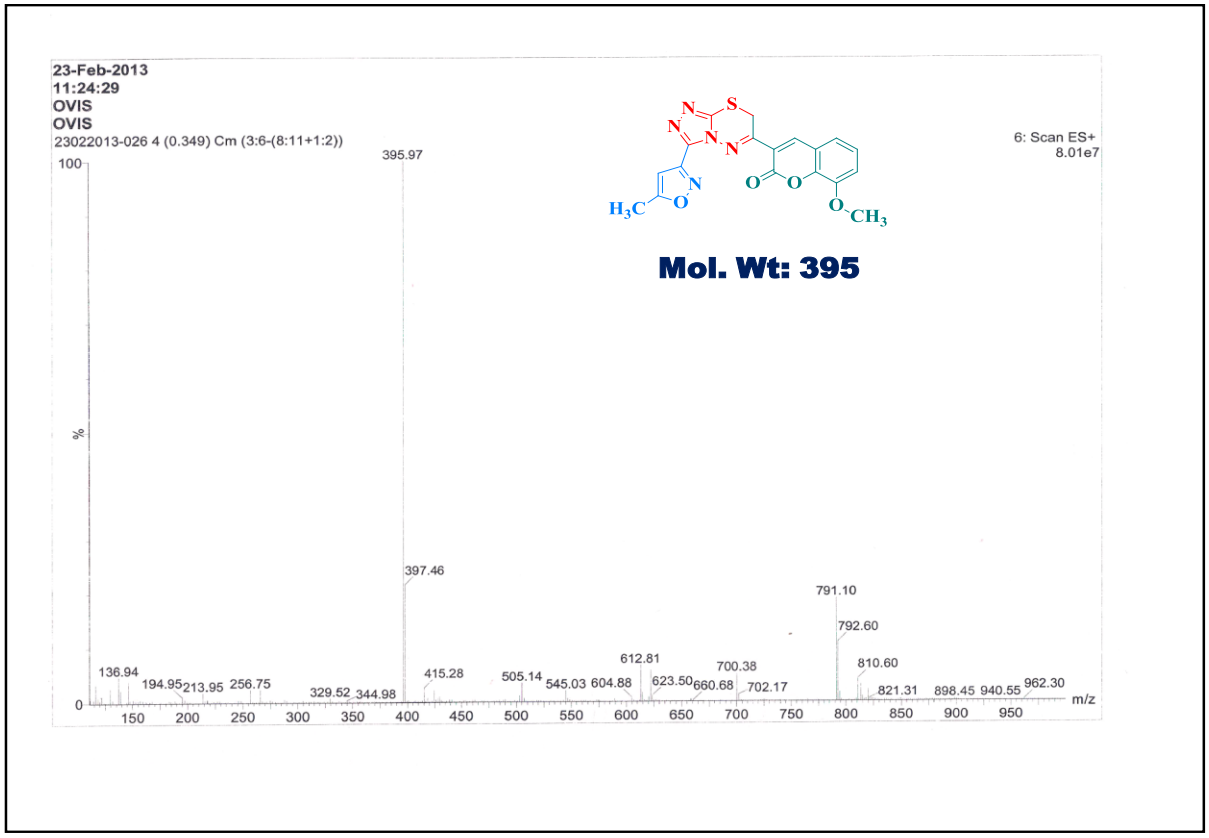


Fig: Mass spectrum of compound **41f**

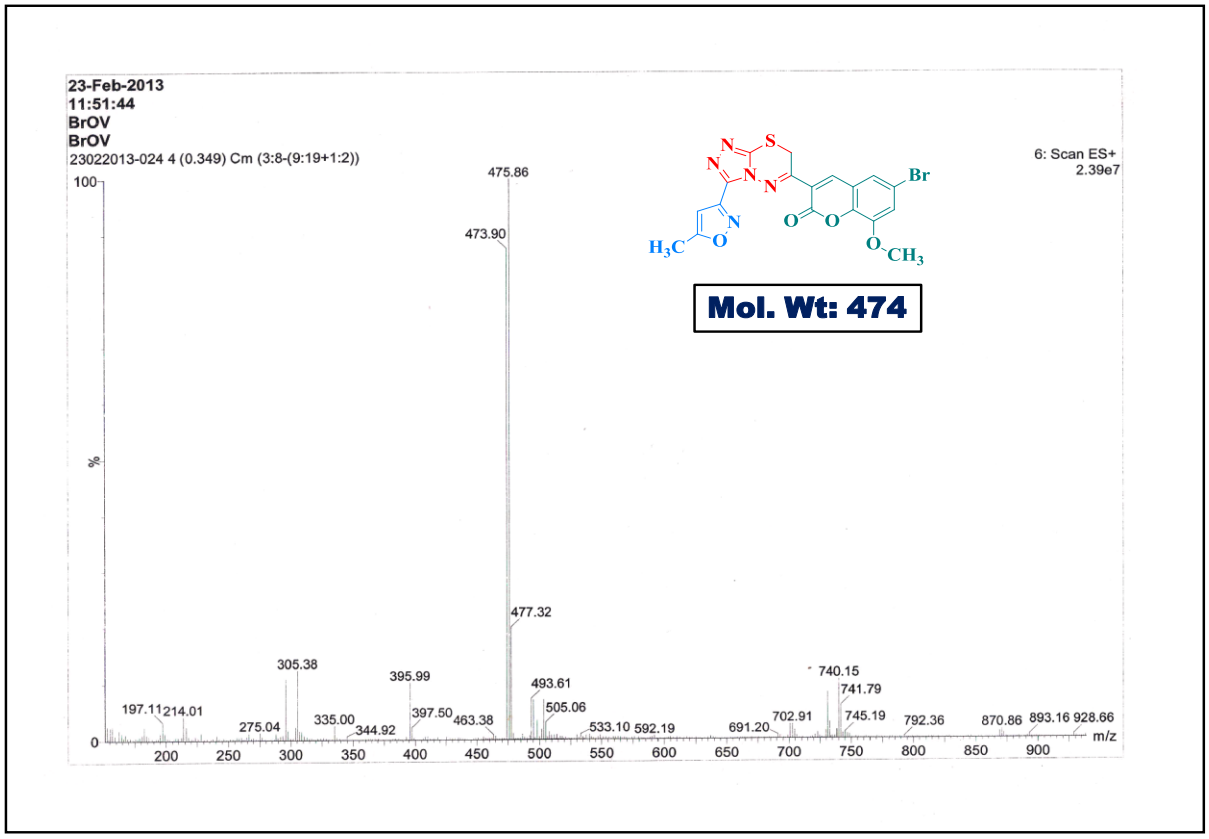


Fig: Mass spectrum of compound **41g**

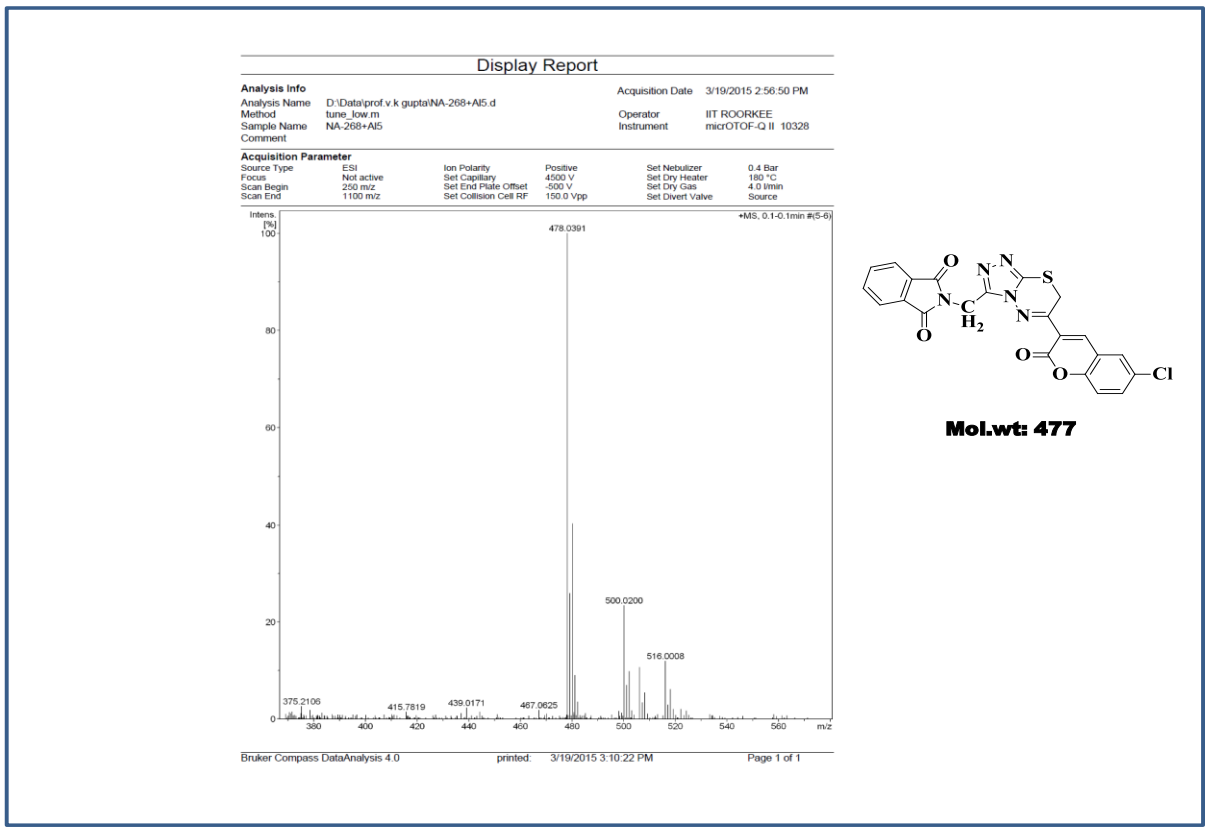


Fig: Mass spectrum of compound **41l**

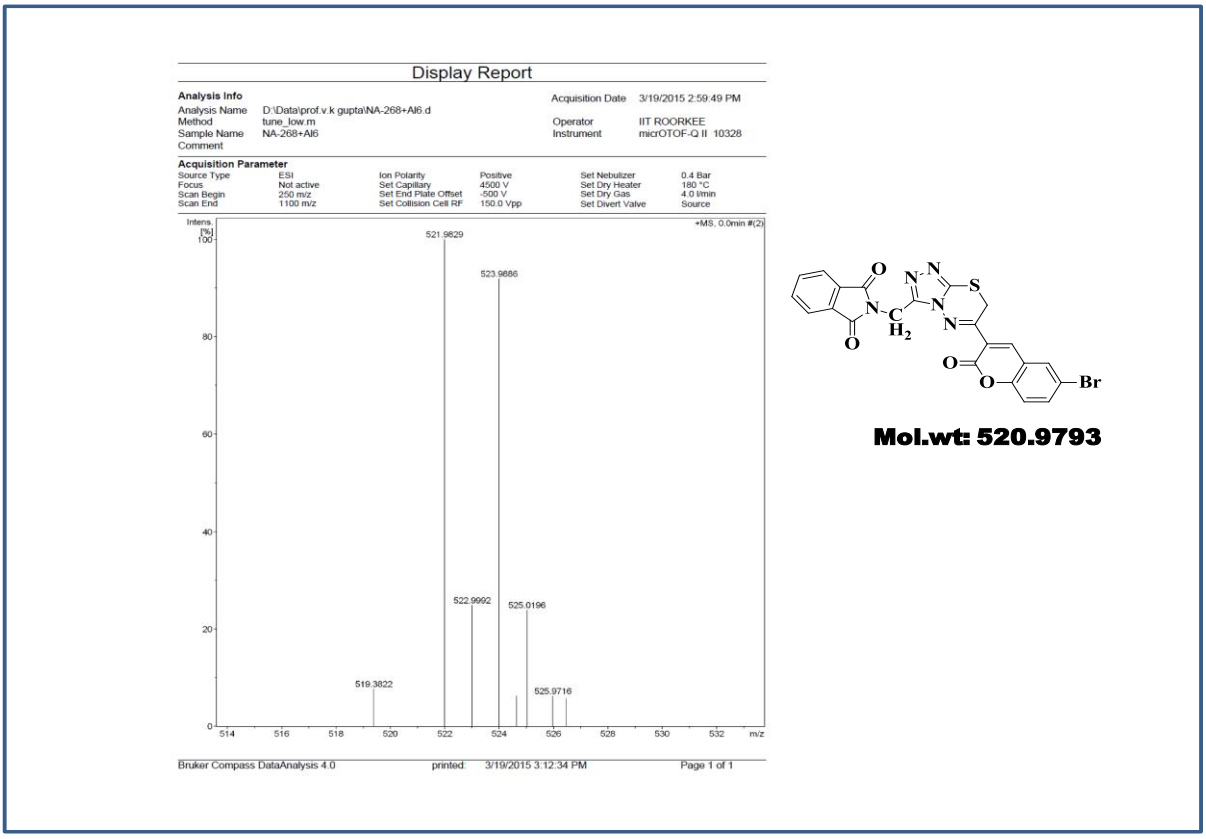


Fig: Mass spectrum of compound **41n**

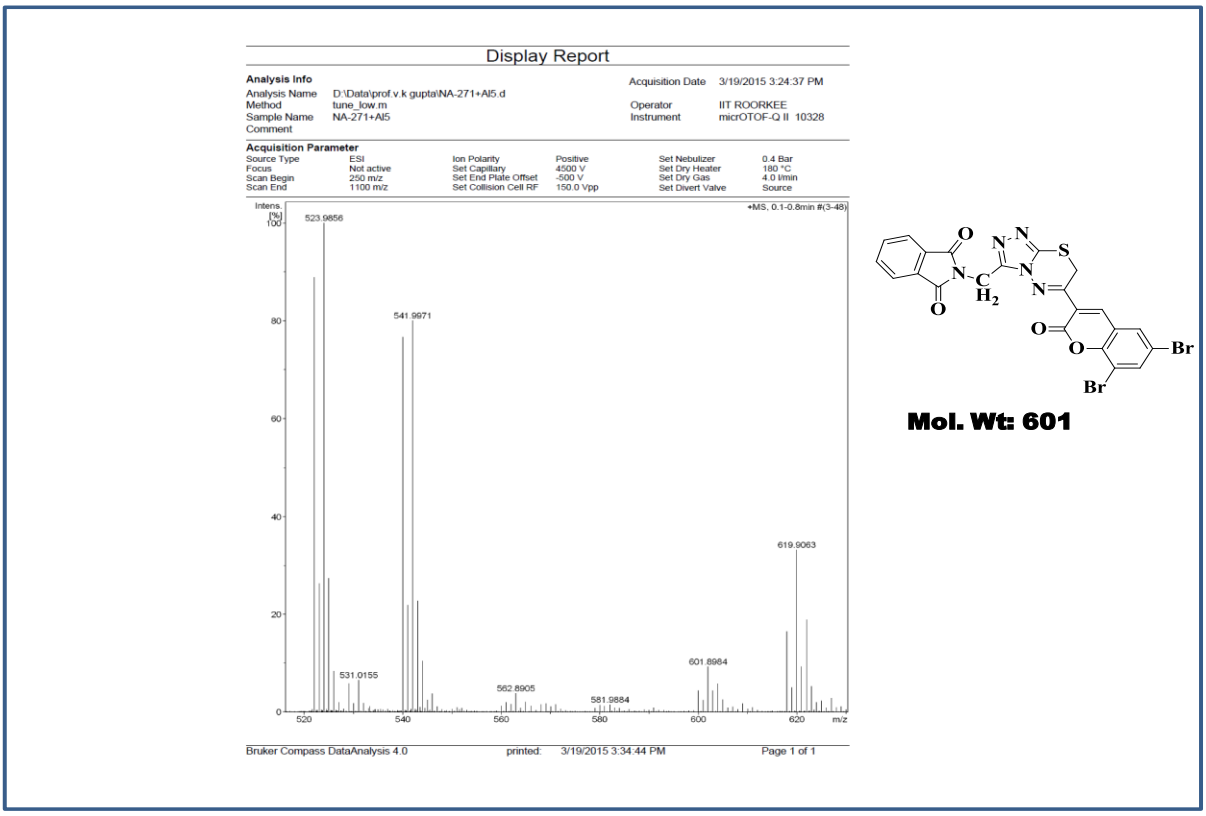


Fig: Mass spectrum of compound **41o**

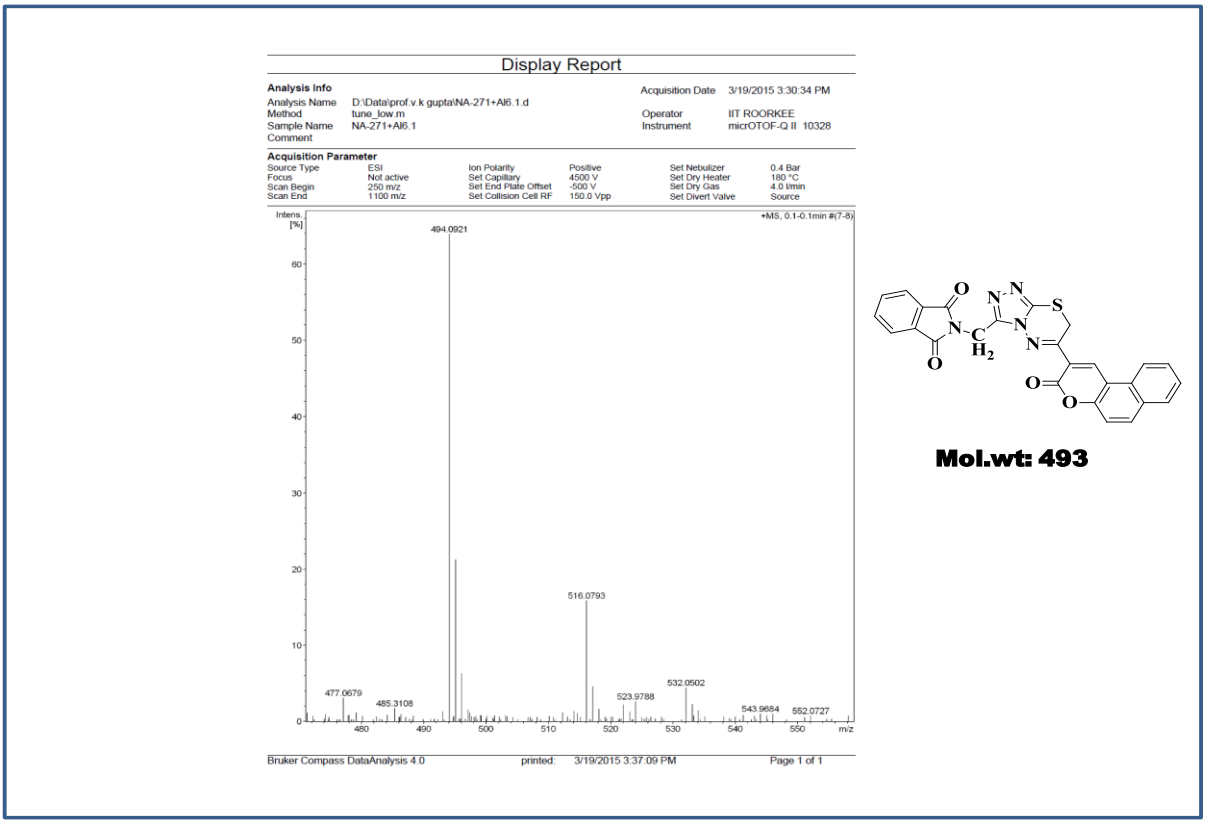


Fig: Mass spectrum of compound **41p**

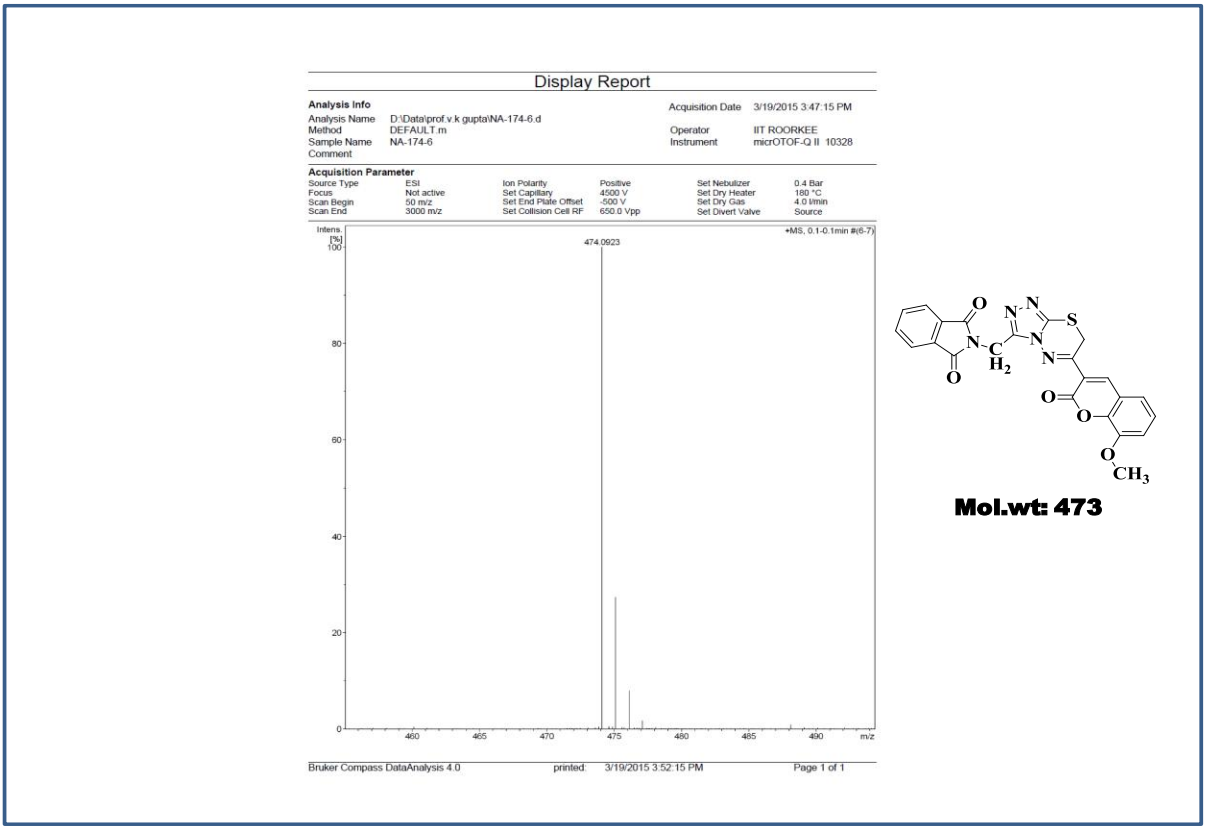


Fig: Mass spectrum of compound **41q**

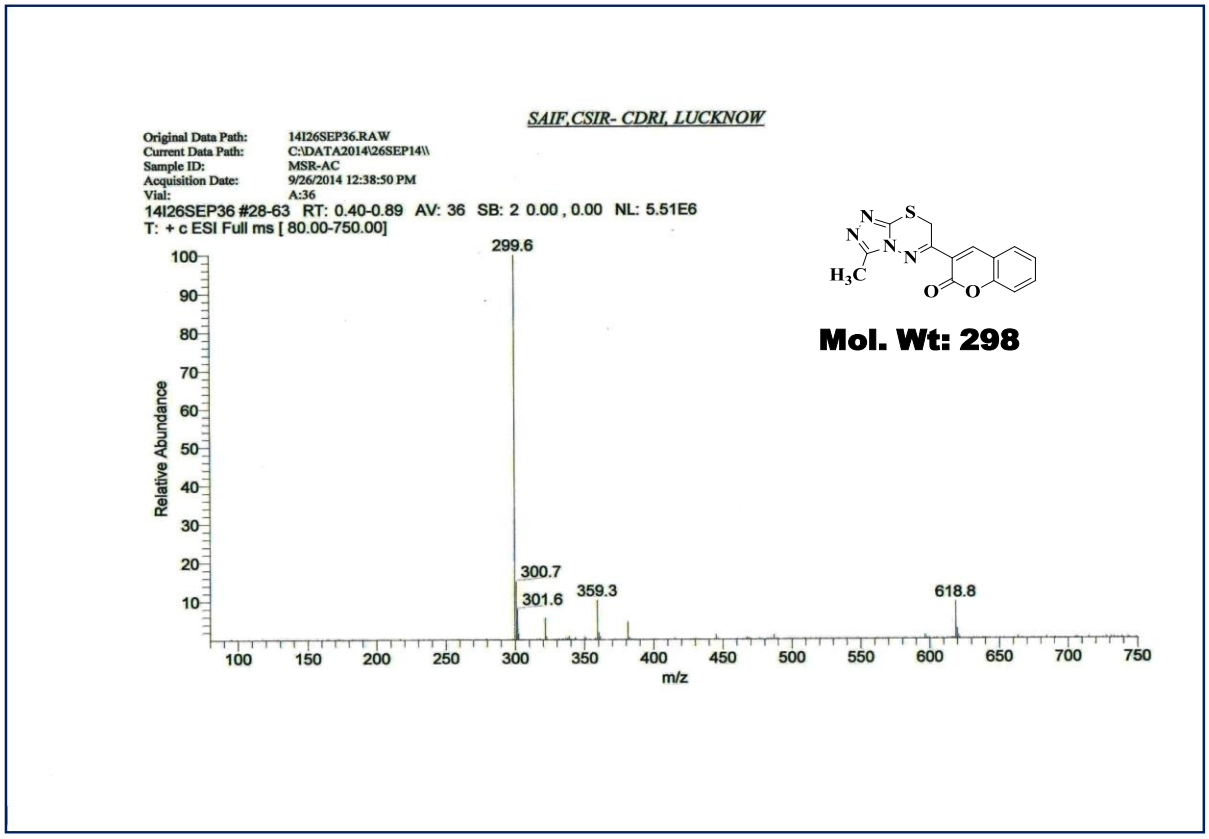


Fig: Mass spectrum of compound **43a**

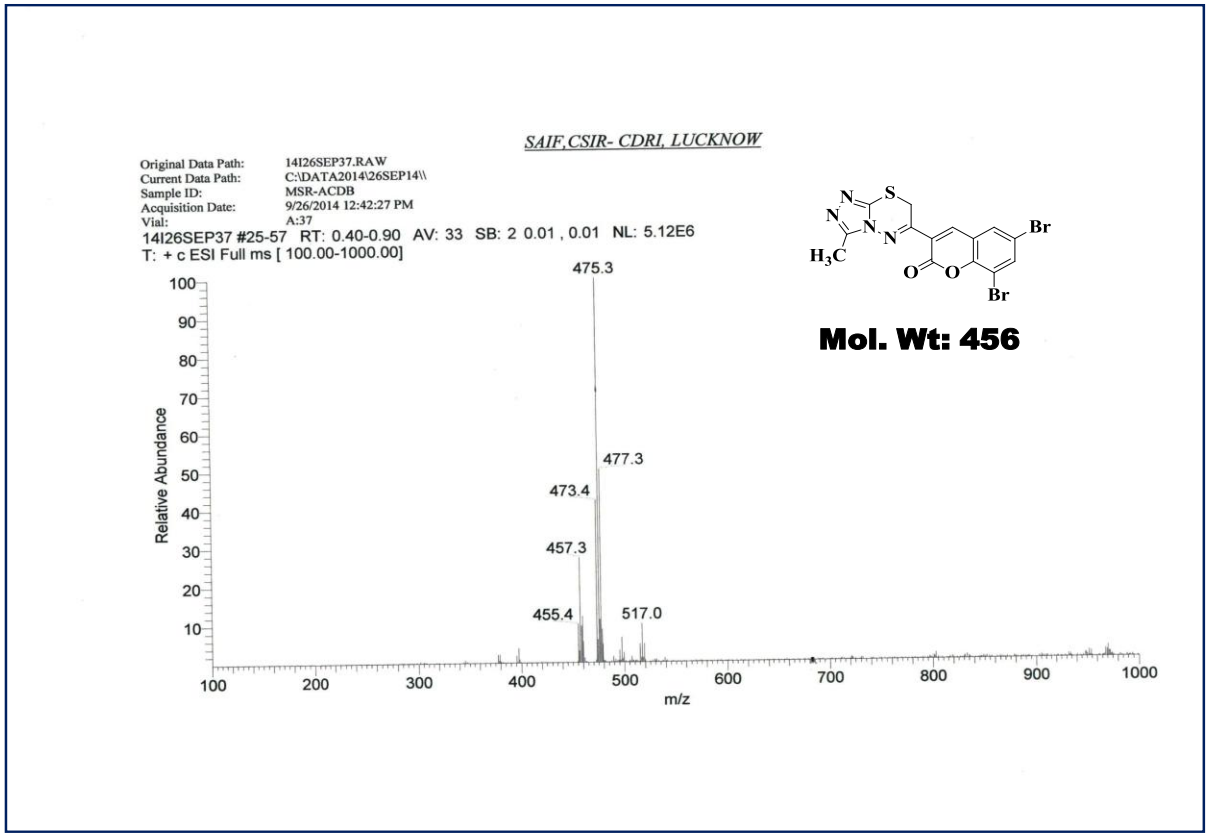


Fig: Mass spectrum of compound **43b**

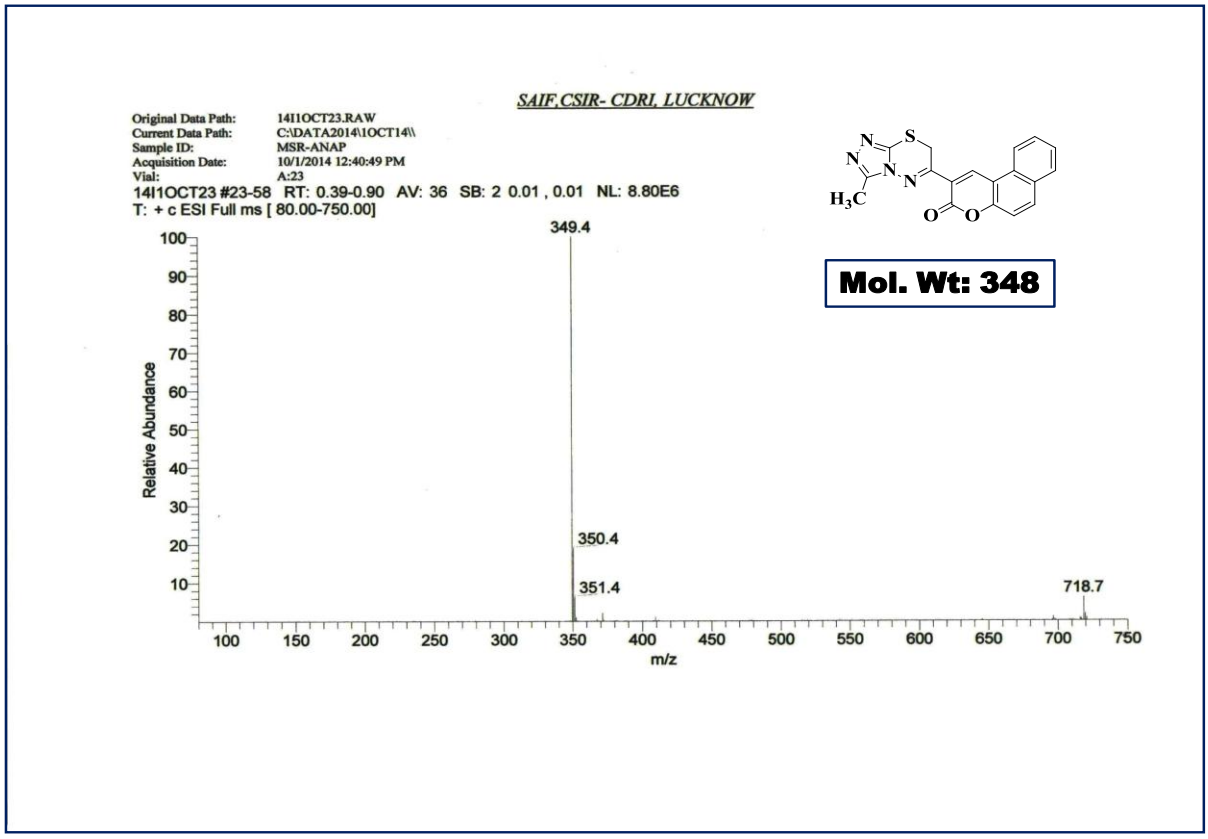


Fig: Mass spectrum of compound **43c**

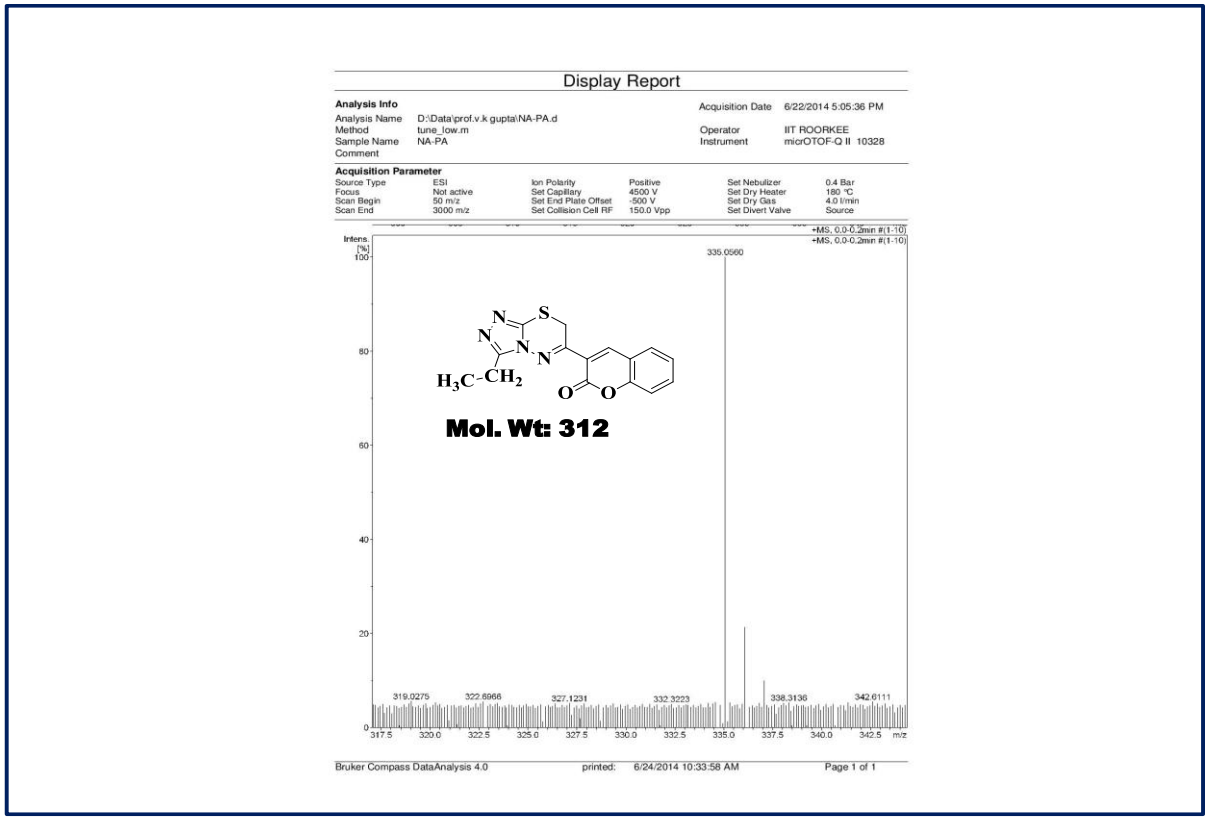


Fig: Mass spectrum of compound **43d**

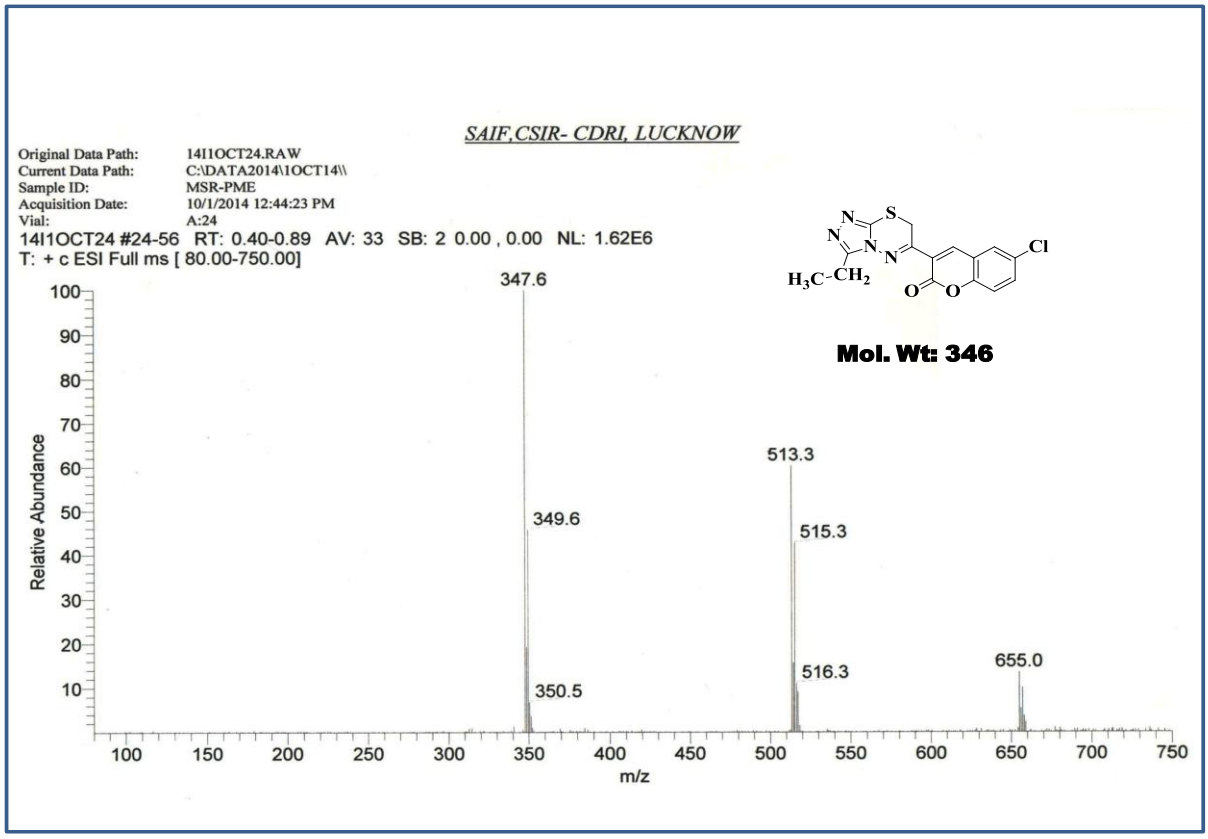


Fig: Mass spectrum of compound **43e**

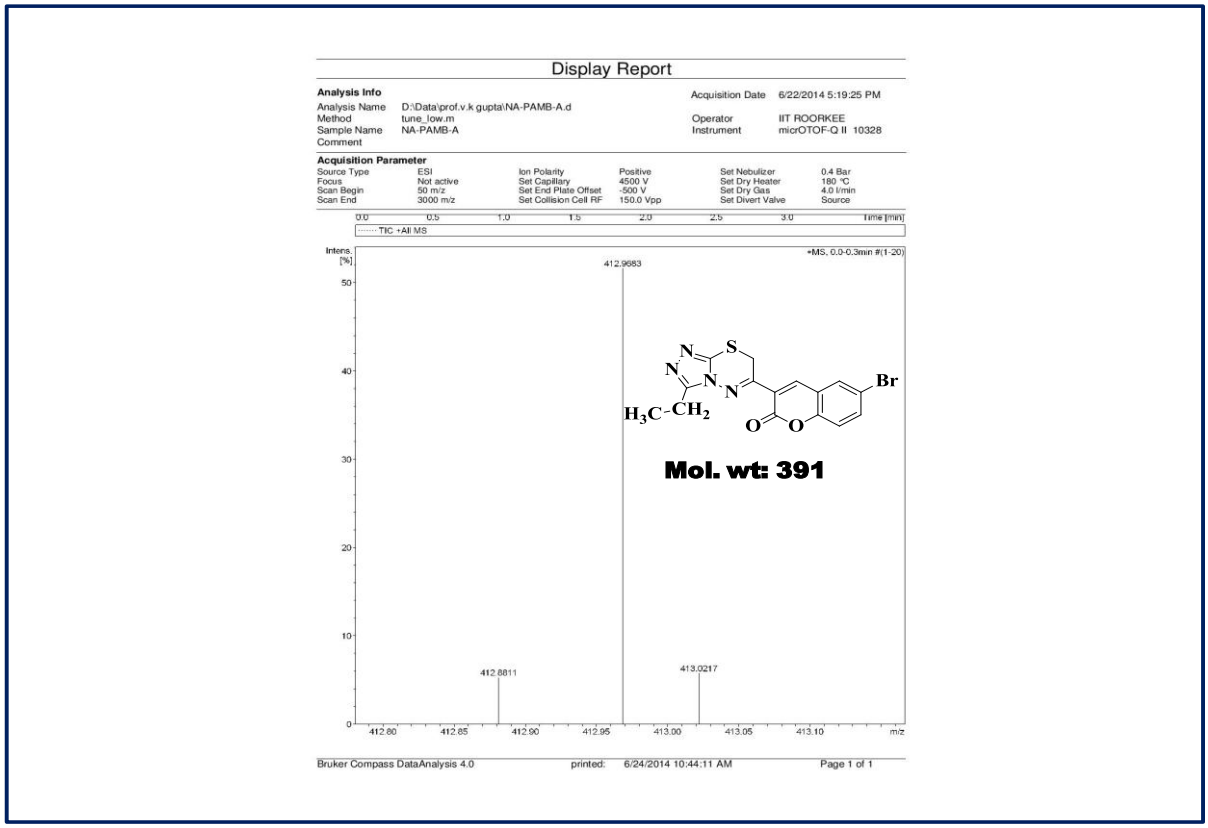


Fig: Mass spectrum of compound **43f**

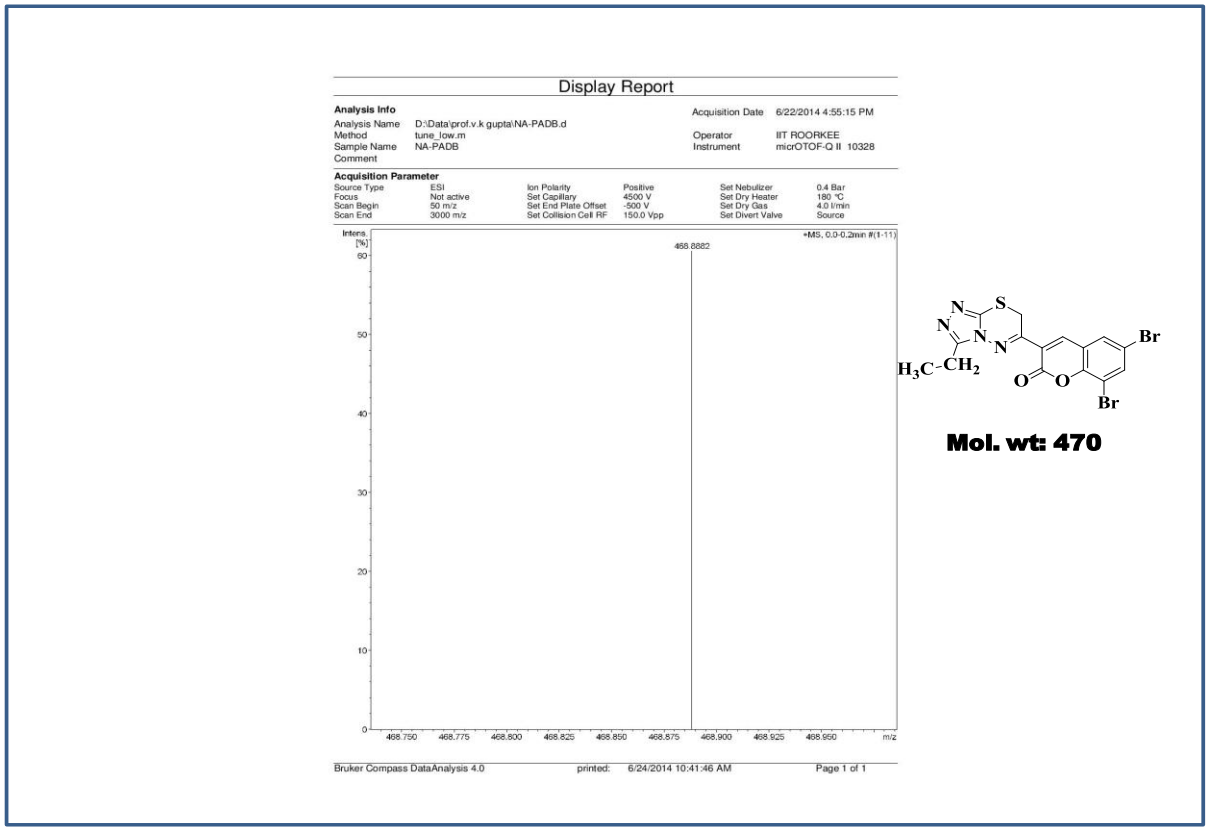


Fig: Mass spectrum of compound **43g**

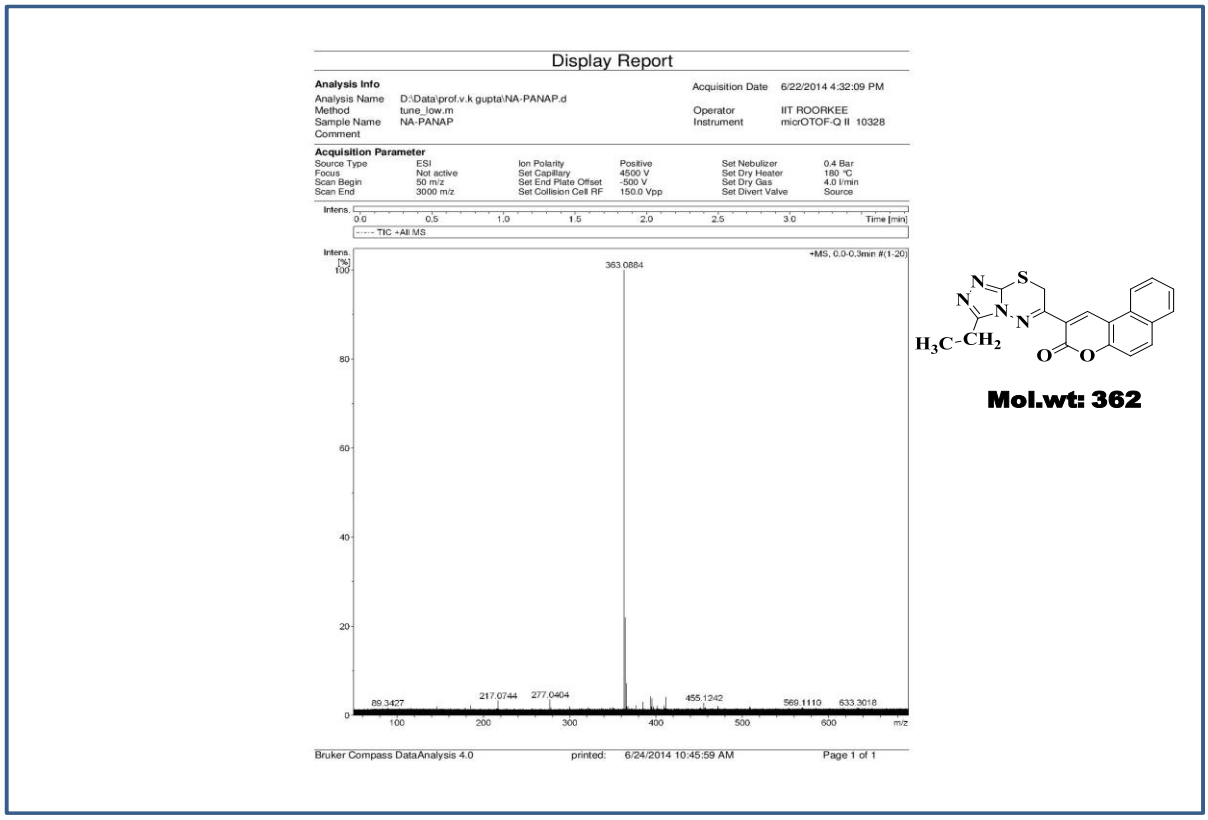


Fig: Mass spectrum of compound **43h**

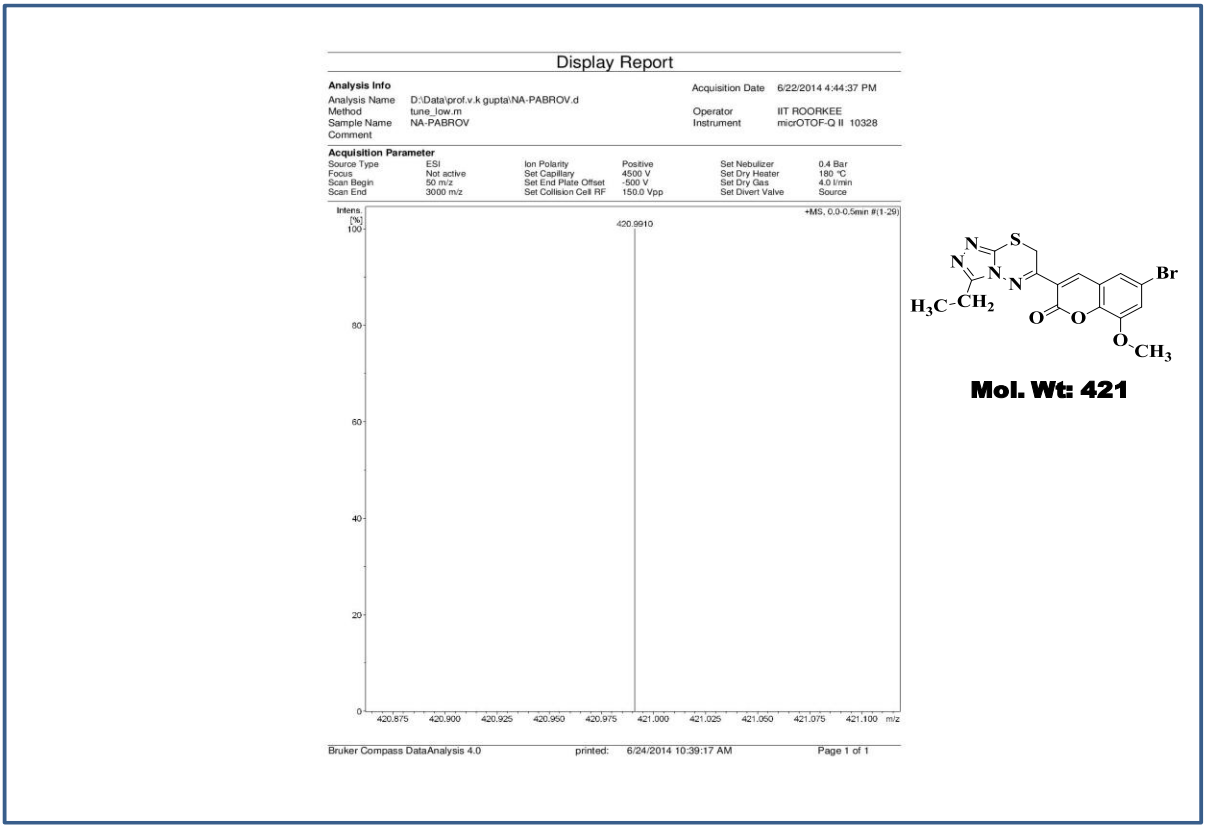


Fig: Mass spectrum of compound **43i**

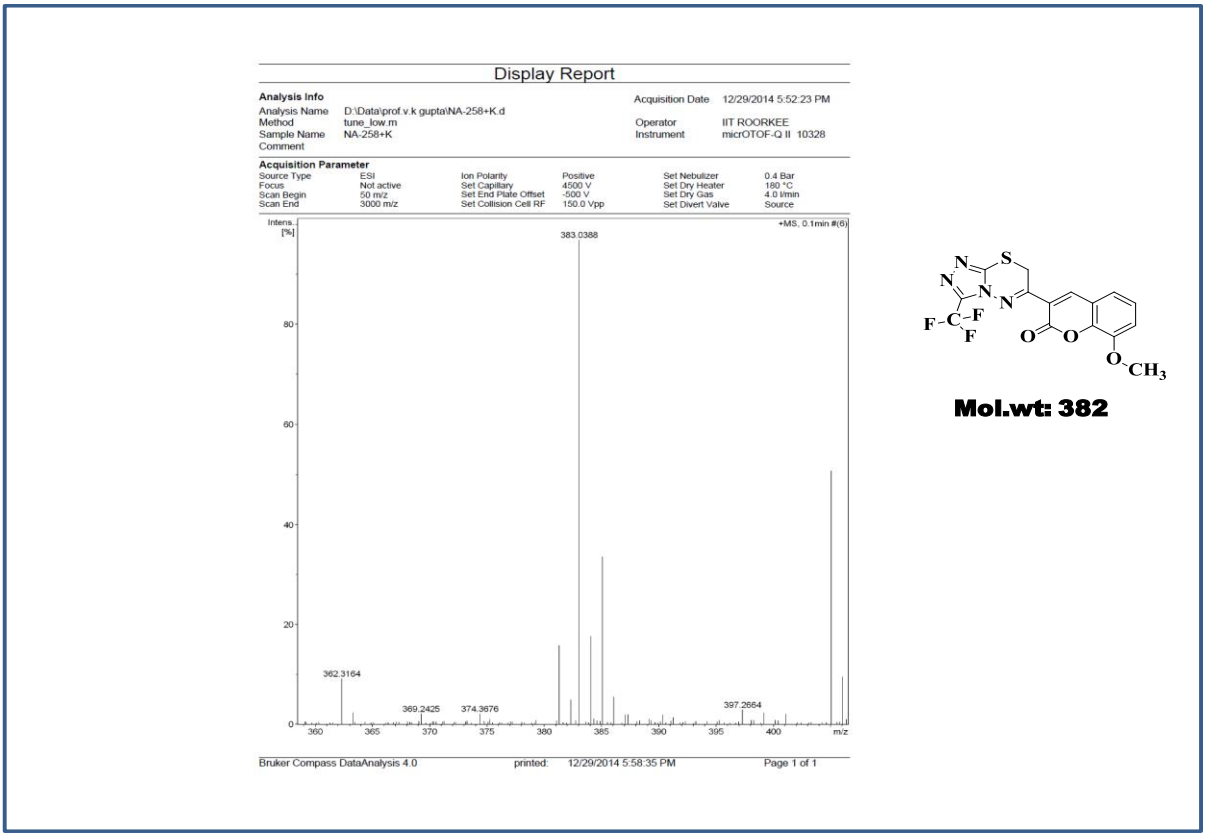


Fig: Mass spectrum of compound **43j**

CHAPTER-IV
PART-B

SYNTHESIS AND BIOLOGICAL ACTIVITY EVALUATION OF 3-(3-(((1*H*-BENZO[*D*]IMIDAZOL-2-YL)METHYL)THIO)-7*H*-[1,2,4]TRIAZOLO[3,4-*b*][1,3,4]THIADIAZIN-6-YL)-6,8-DISUBSTITUTED-2*H*-CHROMEN-2-ONES AS POSSIBLE THERAPEUTIC AGENTS

PART-B

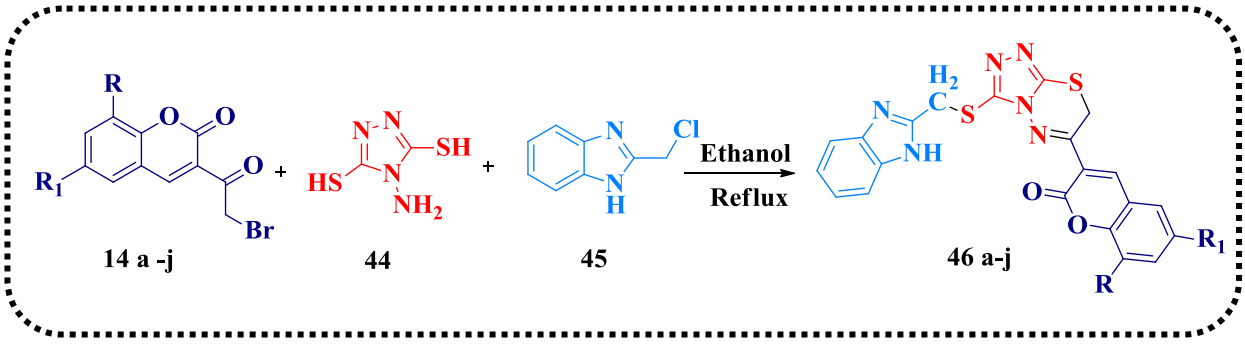
Starting materials:

4-Amino-4*H*-1,2,4-triazole-3,5-dithiol, various substituted 3-(2-bromoacetyl) coumarins, 2-chloro methyl benzimidazoles were used as starting materials for the synthesis of 3-(3-(((1*H*-benzo[*d*] imidazol-2-yl)methyl)thio)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-6-yl)-6,8-disubstituted-2*H*-chromen-2-ones. 4-Amino-4*H*-1,2,4-triazole-3,5-dithiol was prepared according to literature procedure as described in the preceding chapter. Substituted 3-(2-bromoacetyl)coumarins were prepared in two steps by using substituted salicylaldehydes as starting material as described in the preceding chapter. 2-Chloromethyl benzimidazole[47-49] was prepared by using *o*-Phenelene diamine and chloroacetic acid in presence of 4N hydrochloric acid solution by Phillip's condensation.

In view of the therapeutic applications of coumarins, triazolothiadiazines and benzimidazoles, an efficient one pot multicomponent reaction was developed to synthesize the 3, 6 substituted triazolothiadiazines.

PRESENT WORK

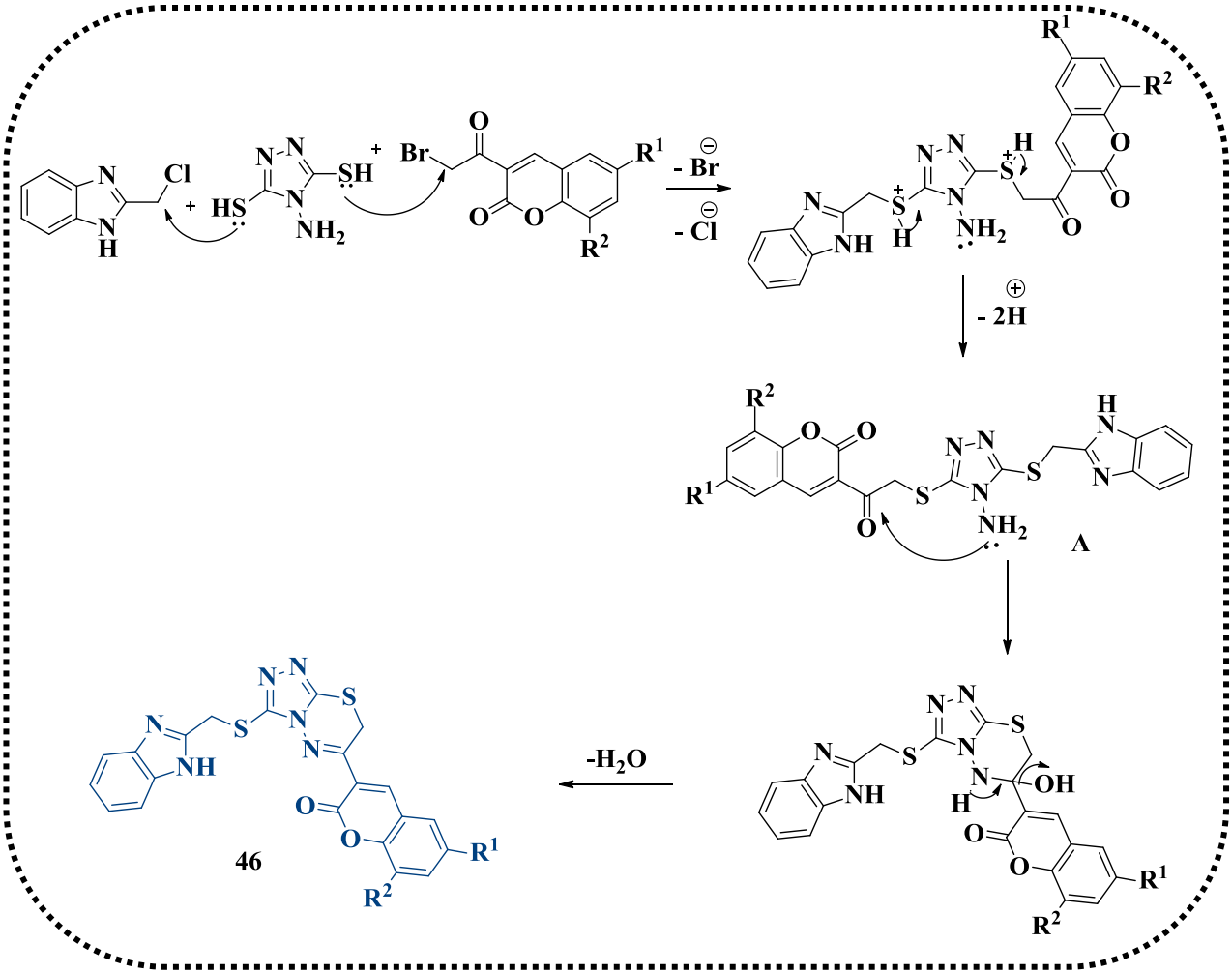
The present chapter describes an efficient one pot multicomponent synthesis of 3-(3-(((1*H*-benzo[*d*]imidazol-2-yl)methyl)thio)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-6-yl)-6,8-di substituted-2*H*-chromen-2-ones and their biological activity applications. The target product was synthesized by the reaction of 4-amino-4*H*-1,2,4-triazole-3,5-dithiol, substituted 3-(2-bromoacetyl)coumarins and 2-(chloromethyl)-1*H*-benzo[*d*]imidazole in presence of absolute ethanol under reflux condition with excellent yields (**Scheme-3**). The plausible mechanism for the synthesis of target compounds was shown in **Scheme-4**.



Scheme-3: A facile one pot multicomponent synthesis of 3-(3-(((1*H*-benzo[*d*]imidazol-2-yl)methyl)thio)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-6-yl)-2*H*-chromen-2-ones

Mechanism:

The formation of the designed molecule can be explained by the following mechanism (**Scheme-4**).The starting material 4-Amino-4*H*-1,2,4-triazole-3,5-dithiol is having three nucleophilic groups (1-NH_2 and 2-SH). The highly nucleophilic thiol (-SH) functional group readily displaces the bromine of 3-(2-bromoacetyl)coumarin as well as chlorine atom of 2-chloro methyl benzimidazole to give the intermediate A. This intermediate undergoes cyclocondensation to give the final product 46.



Scheme-4: Plausible mechanism for the synthesis of compounds **46 (a-l)**.

The above reaction is highly regioselective and only S-alkylated products are formed. No N-alkylated products are detected.

The structures of the prepared compounds were confirmed by analytical and spectral data (IR, ^{1}H NMR, ^{13}C NMR and Mass spectra).

All the synthesized compounds were evaluated for their antiviral and anticancer activities. The target compounds were screened against a broad panel of human RNA and DNA viruses in different cell cultures as described in chapter-II (reference 65-67). From the antiviral activity results, the compounds exhibited potent antiviral activity against *Feline Corona Virus* and *Feline Herpes virus* in CRFK cell culture (**Table.12**). The compound **46h** is more potent against *Feline Corona Virus* with EC_{50} of 4.1µM concentration and *Feline herpes virus* with EC_{50} of 8.6 µM concentration when compared to the standard antiviral drugs HHA, UDA and Ganciclovir. Similarly the compounds 46e and 46j are also exhibited excellent activity against *Feline corona virus* with EC_{50} of 30.1 and 15.95 µM concentrations and *Feline herpes virus* with EC_{50} of 50.45 and 10.95 µM concentrations.

Table 12: Cytotoxicity and antiviral activity in CRFK Cell culture.

Compound	Cytotoxic Conc (CC_{50}) µM	EC_{50} µM	
		Feline Corona Virus	*Feline Herpes Virus*
46a	31.0	>100	>100
46b	NT	NT	NT
46c	57.1	>100	>100
46d	>100	21.45	>100
46e	>100	**30.1**	**50.45**
46f	>100	13.55	>100
46g	44.1	>100	>100
46h	>100	**4.1**	**8.6**
46i	>100	>100	>100
46j	>100	**15.95**	**10.9**
46k	>100	>100	>100
46l	>100	>100	>100
HHA	>100	39.3	9.7
UDA	>100	6.9	8.1
Ganciclovir	>100	>100	0.8

The target compounds 3-(3-(((1*H*-benzo[d]imidazol-2-yl)methyl)thio)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-6-yl)-2*H*-chromen-2-ones (**46 a-l**) were evaluated for anticancer activity against murine leukemia cells (L1210), human T –Lymphocytes cells (CEM) and human cervix carcinoma cells (HeLa). From the anticancer activity results (**Table 13**), the compound **46g** is exhibiting promising activity against the tested cancer cell lines with IC_{50} values 22±0, 19±2 and 29±10 µM concentrations. The

compound **46j** is exhibiting moderate activity against the tested cell lines and the remaining compounds are shown poor activity.

Table 13. Inhibitory effect values (IC_{50}) of compounds against cancer cell lines

Compound	IC_{50} (μM)		
	L1210	CEM	HeLa
46a	141±1	106±3	96±30
46b	NT	NT	NT
46c	115±17	112±28	103±28
46d	102±12	100±0	95±19
46e	106±2	128±36	210±42
46f	103±6	199±63	206±23
46g	**22±0**	**19±2**	**29±10**
46h	199±72	≥250	121±11
46i	145±9	>250	>250
46j	84±25	84±5	67±14
46k	178±101	>250	134±39
46l	108±6	117±31	172±87

In conclusion we have synthesized 3-(3-(((1*H*-benzo[*d*]imidazol-2-yl)methyl)thio)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-6-yl)-2*H*-chromen-2-ones via an efficient one pot multicomponent reaction approach with simple reaction conditions, shorter reaction time, and excellent yields. All the compounds synthesized were evaluated for their antiviral and anticancer activity applications. The compound **46h** exhibited excellent antiviral property and compound **46g** is potent anticancer agent.

EXPERIMENTAL:

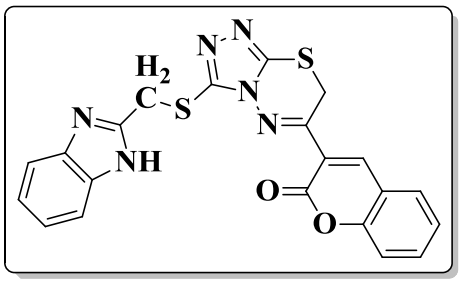
Typical procedure for the synthesis of 3-(3-(((1*H*-Benzo[*d*]imidazol-2-yl)methyl)thio)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4] thiadiazin-6-yl)-2*H*-chromen-2-one derivatives (46a-l):

A mixture of substituted 3-(2-bromoacetyl)-2*H*-chromen-2-ones (1 mmol), 4-amino-4*H*-1,2,4-triazole-3,5-dithiol (0.148 g, 1 mmol) and 2-(chloromethyl)-1*H*-benzo[*d*]imidazole (0.166 g, 1 mmol) was charged in a round bottom flask along with ethanol (5 mL). The reaction mixture was stirred at room temperature for about 10 min, heated to 60 – 65 °C and maintained the temperature for about 4-6 h. After completion of the reaction (as detected by TLC), the reaction mixture was cooled to room

temperature and the solid obtained was filtered washed with ethanol and dried. The product was recrystallized from ethanol.

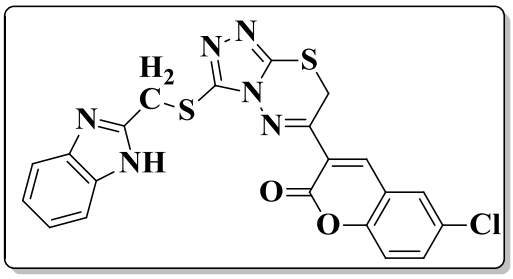
SPECTRAL DATA

3-(3-(((1*H*-Benzo[*d*]imidazol-2-yl)methyl)thio)-7*H*-[1,2,4]triazolo[3,4-*b*] [1,3,4] thiadiazin-6-yl)-2*H*-chromen-2-one (46a):



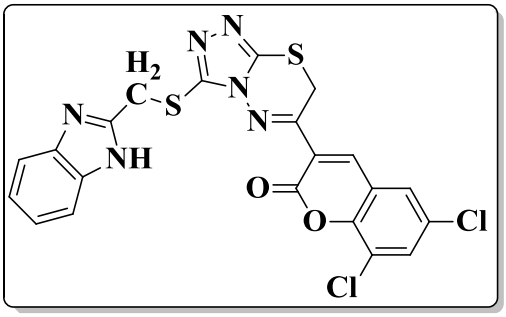
Yellow solid; yield: 77%; m.p.: 254 - 256 °C; **FTIR (KBr, ν_{max} cm^{-1}):** 3038 (-NH), 1727 (lactone –C=O), 1605 (-C=N); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 4.31 (s, 2H, -SCH_2), 4.93 (s, 2H, -NCH_2), 7.43 – 7.52 (m, 4H, -Ar-H), 7.73 – 7.80 (m, 2H, -Ar-H), 7.92 – 7.99 (m, 2H, Ar-H), 8.58 (s, 1H, C_4-H of coumarin), 8.81 (bs, 1H, -NH); **^{13}C NMR (100 MHz, DMSO-d_6, ppm):** δ 24.53, 43.99, 114.01, 116.29, 118.19, 118.38, 122.19, 122.95, 125.16, 125.64, 129.99, 133.78, 134.04, 140.48, 144.35, 144.55, 150.36, 153.87, 153.94, 158.72, 165.30. **MS** (ESI *m/z*): 447 $[M+H]^+$; Anal. Calcd. for $C_{21}H_{14}N_6O_2S_2$ (446.50): C, 56.49; H, 3.16; N, 18.82%; Found: C, 56.53; H, 3.13; N, 18.84 %.

3-(3-(((1*H*-Benzo[*d*]imidazol-2-yl)methyl)thio)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4] thiadiazin-6-yl)-6-chloro-2*H*-chromen-2-one (46b):



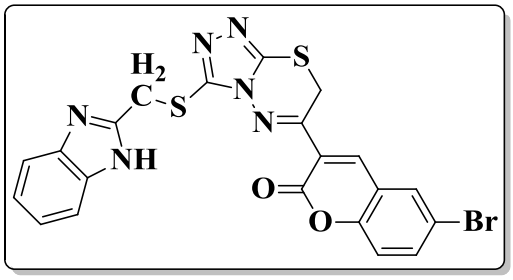
Yellow solid; yield: 80%; m.p.: 230 - 232 °C; **FTIR (KBr, ν_{max} cm^{-1}):** 3064 (-NH); 1727 (lactone –C=O); 1608 (-C=N); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 4.27 (s, 2H, -SCH_2), 4.90 (s, 2H, -NCH_2), 7.39 (s, 1H, Ar-H); 7.52 – 7.57 (m, 2H, Ar-H), 7.68 (s, 1H, Ar-H), 7.78 (t, *J* = 8.4Hz, 1H, Ar-H), 8.02 (d, *J* = 2Hz, 1H, Ar-H), 8.07 (d, *J* = 2.4Hz, 1H, Ar-H), 8.50 (s, 1H C_4-H of coumarin), 8.75 (s, 1H, -NH); **MS** (ESI *m/z*): 481 $[M+H]^+$; Anal. Calcd. for $C_{21}H_{13}ClN_6O_2S_2$ (480.94): C, 52.44; H, 2.72; N, 17.47%; Found: C, 52.39; H, 2.75; N, 17.50%.

3-(3-(((1*H*-Benzo[*d*]imidazol-2-yl)methyl)thio)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4] thiadiazin-6-yl)-6,8-dichloro-2*H*-chromen-2-one (46c):



Yellow solid; yield: 82%; m.p.: 226 - 228 °C; **IR (KBr, ν_{max} cm^{-1}):** 3062 (-NH); 1729 (lactone –C=O); 1607 (-C=N); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 4.30 (s, 2H, -SCH_2), 4.90 (s, 2H, -NCH_2), 7.52 – 7.55 (m, 2H, Ar-H); 7.75 – 7.80 (m, 2H, Ar-H), 8.02 (d, *J* = 2.8 Hz, 1H, Ar-H), 8.07 (d, *J* = 2.4 Hz, 1H, Ar-H), 8.50 (s, 1H, C_4-H of coumarin), 8.75 (s, 1H, -NH); **^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ** 24.53, 43.78, 119.68, 123.66, 123.77, 123.88, 128.78, 129.66, 129.78, 133.29, 134.37, 134.46, 142.66, 143.23, 147.17, 147.27, 149.28, 152.56, 153.25, 156.99, 158.09; **MS** (ESI *m/z*): 516 $[M+H]^+$; Anal. Calcd. for $C_{21}H_{12}Cl_2N_6O_2S_2$ (515.39): C, 48.94; H, 2.35; N, 16.31%; Found: C, 48.90; H, 2.33; N, 16.36%.

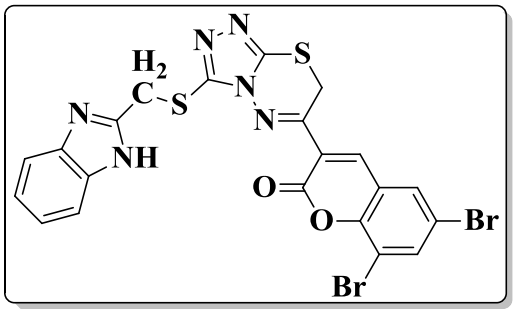
3-(3-(((1*H*-Benzo[*d*]imidazol-2-yl)methyl)thio)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4] thiadiazin-6-yl)-6-bromo-2*H*-chromen-2-one (46d):



Red solid; yield: 82%; m.p.: 248 - 250 °C; **FTIR (KBr, ν_{max} cm^{-1}):** 3042 (-NH); 1703 (lactone –C=O); 1601 (-C=N); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 4.75 (s, 2H, -SCH_2), 4.85 (s, 2H, -NCH_2), 7.46 (t, *J* = 8.8 Hz, 2H, Ar-H), 7.61 (s, 1H, Ar-H), 7.90 – 7.95 (m, 2H, Ar-H), 8.23 (t, *J* = 2Hz, 2H, Ar-H), 8.60 (s, 1H, C_4-H of coumarin), 8.75 (s, 1H, Ar-H); **^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ** 30.00, 43.79, 116.60, 118.37, 118.53, 119.89, 123.71, 132.50, 132.80, 136.55, 137.21, 145.60, 147.21, 153.59, 153.70, 156.99, 157.99; **MS** (ESI *m/z*): 525 $[M+H]^+$, 527 $[M+2H]^+$; Anal. Calcd. for $C_{21}H_{13}BrN_6O_2S_2$ (525.40): C, 48.01; H, 2.49; N, 16.00%; Found: C, 48.05; H, 2.44; N, 15.98 %.

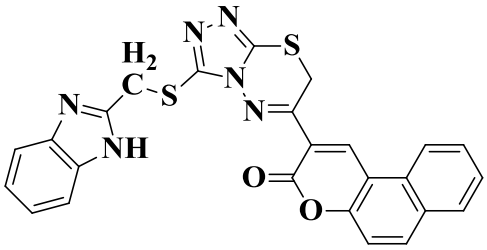
3-(3-(((1*H*-Benzo[*d*]imidazol-2-yl)methyl)thio)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4] thiadiazin-6-yl)-6,8-dibromo-2*H*-chromen-2-one (46e):

Brown solid; yield: 85%; m.p.: 260 - 262 °C; **FTIR (KBr, ν_{max} cm^{-1}):** 3262 (-NH); 1731 (lactone –C=O); 1595 (-C=N); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 4.30 (s, 2H, -SCH_2), 4.85 (s, 2H,-NCH_2), 7.50 (bs, 1H, -NH), 8.16 – 8.33 (m, 5H, Ar-H), 8.46 (s, 1H, Ar-H), 8.72 (s, 1H, C_4-H of coumarin); **MS** (ESI *m/z*): 605 $[M+H]^+$; Anal. Calcd. for



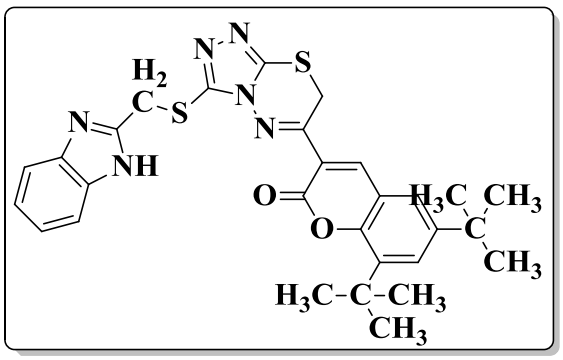
$C_{21}H_{12}Br_2N_6O_2S_2$ (604.29): C, 41.74; H, 2.00; N, 13.91%; Found: C, 41.79; H, 1.97; N, 13.88%.

2-(3-(((1*H*-Benzo[*d*]imidazol-2-yl)methyl)thio)-7*H*-[1,2,4]triazolo[3,4-*b*] [1,3,4] thiadiazin-6-yl)-3*H*-benzo[*f*]chromen-3-one (46f):



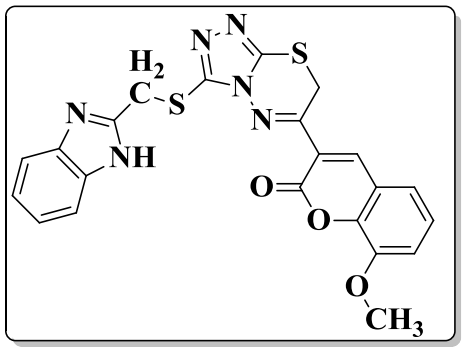
Red solid; yield: 77%; m.p.: 310 - 312 °C. **FTIR (KBr, ν_{max} cm^{-1}):** 3059 (-NH); 1719 (lactone-C=O); 1596 (-C=N); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 4.36 (s, 2H, -CH_2), 4.94 (s, 2H, -NCH_2), 7.53 (t, *J* = 8.8 Hz, 1H, Ar-H), 7.62 – 7.83 (m, 5H, Ar-H), 8.03 – 8.06 (m, 2H, Ar-H), 8.12 (d, *J* = 8 Hz, 1H, Ar-H), 8.23 – 8.41 (m, 1H, Ar-H), 8.52 (d, *J* = 8.4 Hz, 1H, Ar-H), 8.59 (s,1H, C_4-H of coumarin); **MS** (ESI*m/z*): 497 $[M+H]^+$; Anal. Calcd. for $C_{25}H_{16}N_6O_2S_2$ (496.56): C, 60.47; H, 3.25; N, 16.92%; Found: C, 60.44; H, 3.28; N, 16.89%.

3-(3-(((1*H*-Benzo[*d*]imidazol-2-yl)methyl)thio)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4] thiadiazin-6-yl)-6,8-di-*tert*-butyl-2*H*-chromen-2-one (46g):



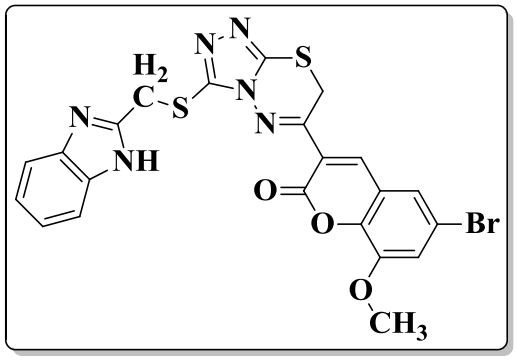
White solid; yield: 78%; m.p.: 186 - 188 °C. **IR (KBr, ν_{max} cm^{-1}):** 3054 (-NH); 1726 (lactone –C=O); 1573 (-C=N); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 1.34 (s, 9H, -*t*-butyl-H), 1.47 (s, 9H, -*t*-butyl-H), 4.29 (s, 2H, -SCH_2), 4.68 (s, 2H, -NCH_2), 7.10 – 7.12 (m, 2H, Ar-H), 7.46 – 7.48 (m, 2H, Ar-H), 7.66 (d, *J* = 2Hz, 1H, Ar-H), 7.79 (d, *J* = 2.4 Hz, 1H, Ar-H), 8.44 (s, 1H, C_4-H of coumarin), 8.84 (s, 1H, -NH); **^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ** 28.95, 29.51, 31.02, 34.49, 114.84, 118.17, 118.35, 120.88, 121.98, 124.87, 125.86, 128.43, 136.04, 143.06, 145.83, 146.83, 149.96, 150.68, 153.32, 158.13; **MS (ESI *m/z*):** 559 $[M+H]^+$. Anal. Calcd. for $C_{29}H_{30}N_6O_2S_2$ (558.71): C, 62.34; H, 5.41; N, 15.04%; Found: C, 62.40; H, 5. 38; N, 15.02%.

3-(3-(((1*H*-Benzo[*d*]imidazol-2-yl)methyl)thio)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4] thiadiazin-6-yl)-8-methoxy-2*H*-chromen-2-one (46h):



Yellow solid; yield: 81%; m.p.: 226 - 228 °C. **FTIR (KBr, ν_{max} cm^{-1}):** 3075 (-NH); 1717 (lactone –C=O); 1606 (-C=N); 1276 (-C-O-C); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 3.94 (s, 3H, -OCH_3); 4.23 (s, 2H, -SCH_2), 4.29 (s, 2H, -NCH_2), 7.34 – 7.40 (m, 2H, Ar-H), 7.45 – 7.48 (m, 1H, Ar-H), 7.50 – 7.53 (m, 2H, Ar-H), 7.70 – 7.73 (m, 2H, Ar-H), 8.46 (s, 1H, C_4-H of coumarin), 8.87 (bs, 1H, -NH); **MS** (ESI *m/z*): 477 $[M+H]^+$; Anal. Calcd. for $C_{22}H_{16}N_6O_3S_2$ (476.53): C, 55.45; H, 3.38; N, 17.64%, Found: C, 55.48; H, 3.33; N, 17.68%.

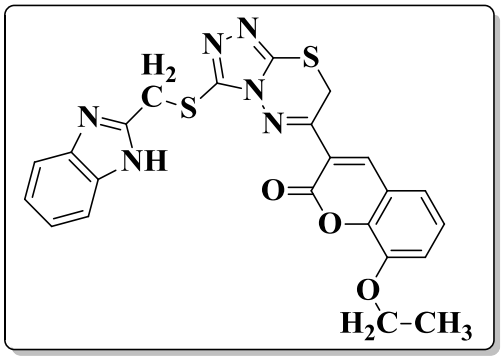
3-(3-(((1*H*-Benzo[*d*]imidazol-2-yl)methyl)thio)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4] thiadiazin-6-yl)-6-bromo-8-methoxy-2*H*-chromen-2-one (46i):



Red solid; yield: 86%; m.p.: 215 - 217 °C. **FTIR (KBr, ν_{max} cm^{-1}):** 3076 (-NH); 1735 (lactone –C=O); 1601 (-C=N); 1273 (-C-O-C); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 3.97 (s, 3H, -OCH_3), 4.26 (s, 2H, -SCH_2), 4.89 (s, 2H, -NCH_2), 7.39 (bs, 1H, -NH), 7.60 (t, *J* = 2.4 Hz, 3H, Ar-H), 7.67 – 7.79 (m, 1H, Ar-H), 8.35 (s, 1H, Ar-H), 8.44 (s, 1H, Ar-H), 8.70 (s, 1H, C_4-H of coumarin); **^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ** 24.50, 26.75, 56.85, 114.15, 116.53, 118.60, 122.67, 123.55, 125.50, 131.65, 136.69, 143.39, 147.29, 153.55, 157.73, 159.70, 164.13; **MS** (ESI *m/z*): 555$[M+H]^+$, 556 $[M+2H]^+$; Anal. Calcd. for $C_{22}H_{15}BrN_6O_3S_2$ (555.42): C, 47.57; H, 2.72; N, 15.13%; Found: C, 47.53; H, 2.74; N, 15.16 %.

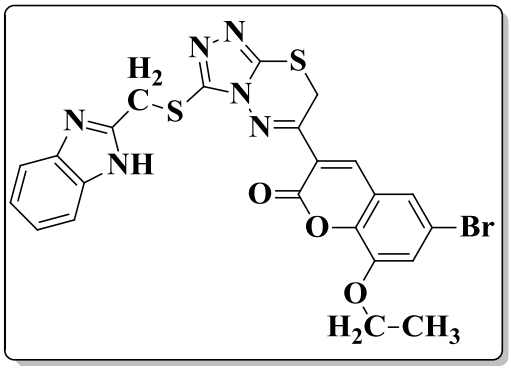
3-(3-(((1*H*-Benzo[*d*]imidazol-2-yl)methyl)thio)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4] thiadiazin-6-yl)-8-ethoxy-2*H*-chromen-2-one (46j):

Yellow solid; yield: 80%; m.p.: 250 - 252 °C; **FTIR (KBr, ν_{max} cm^{-1}):** 3085 (-NH); 1728 (lactone –C=O); 1601 (-C=N); 1280 (-C-O-C); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 1.30 (t, *J* = 5.2 Hz, 3H, -CH_3), 4.17 – 4.22 (m, 2H, -OCH_2); 4.30 (s, 2H, -SCH_2),



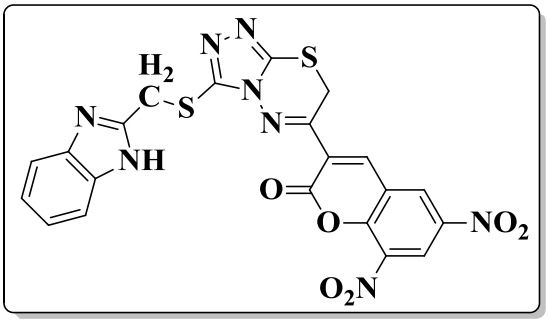
4.91 (s, 2H, -NCH_2), 7.32 – 7.47 (m, 6H, Ar-H), 7.69 – 7.70 (m, 1H, Ar-H), 8.52 (s, 1H, C_4-H of coumarin), 8.75 (s, 1H, -NH); **MS (ESI *m/z*):** 491 $[M+H]^+$; Anal. Calcd. for $C_{23}H_{18}N_6O_3S_2$ (490.55): C, 56.31; H, 3.70; N, 17.13%; Found: C, 56.35; H, 3.68; N, 17.09%.

3-(3-(((1*H*-Benzo[*d*]imidazol-2-yl)methyl)thio)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4] thiadiazin-6-yl)-6-bromo-8-ethoxy-2*H*-chromen-2-one (46k):



Red solid; yield: 84%; m.p.: 238 - 240 °C. **FTIR (KBr, ν_{max} cm^{-1}):** 3297 (-NH); 1737 (lactone –C=O); 1604 (-C=N); 1238 (-C-O-C); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 1.29 (t, *J* = 4.8 Hz, 3H, -CH_3), 3.99 – 4.04 (m, 2H, -OCH_2), 4.34 (s, 2H, -SCH_2), 4.84 (s, 2H, -NCH_2), 6.61 – 6.73 (m, 1H, Ar-H), 6.97 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.15 – 7.20 (m, 2H, Ar-H), 7.49 (t, *J* = 3.2 Hz, 1H, Ar-H), 7.73 (t, *J* = 3.2 Hz, 1H, Ar-H), 8.52 (s, 1H, C_4-H of coumarin), 13.77 (bs, 1H, -NH); **^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ** 14.50, 25.43, 43.38, 64.66, 112.70, 114.09, 115.59, 116.57, 117.83, 121.56, 125.01, 125.63, 131.48, 141.76, 146.44, 147.95, 148.42, 150.24, 167.97; **MS** (ESI *m/z*): 570 $[M+H]^+$; Anal. Calcd. for $C_{23}H_{17}BrN_6O_3S_2$ (569.45): C, 48.51; H, 3.01; N, 14.76%, Found: C, 48.56; H, 2.99; N, 14.75%.

3-(3-(((1*H*-Benzo[*d*]imidazol-2-yl)methyl)thio)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4] thiadiazin-6-yl)-6,8-dinitro-2*H*-chromen-2-one (46l):



Yellow solid; yield: 76%; m.p.: 266 - 268 °C; **FTIR (KBr, ν_{max} cm^{-1}):** 3227 (-NH); 1748 (lactone –C=O); 1617 (-C=N); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 4.28 (s, 2H, -SCH_2), 4.86 (s, 2H, -NCH_2), 7.69 (t, *J* = 8.8 Hz, 3H, Ar-H), 8.50 – 8.56 (m,

2H, Ar-H), 8.81 (s, 1H, C_4-H of coumarin), 8.95 - 8.99 (m, 2H, Ar-H); **^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ** 29.90, 43.67, 114.01, 117.67, 117.84, 118.37, 118.51, 118.74, 124.51, 125.68, 126.08, 126.42, 126.73, 128.46, 129.02, 143.71, 143.88, 145.78, 147.36, 157.44, 158.01; **MS (ESI *m/z*):** 537 $[M+H]^+$; Anal. Calcd. for $C_{21}H_{12}N_8O_6S_2$ (536.50): C, 47.01; H, 2.25; N, 20.89%, Found: C, 46.97; H, 2.23; N, 20.94%.

Antiviral activity of 3-(3-(((1*H*-Benzo[*d*] imidazol-2-yl)methyl)thio)-7*H*-[1,2,4] triazolo[3,4-*b*][1,3,4]thiadiazin-6-yl)-6,8-disubstituted-2*H*-chromen-2-ones.

The newly synthesized compounds were screened against human viruses and anti viral activity were determined by measuring the cell viability with the colorimetric formazan-based MTS assay as described in the chapter-II (reference 65-67).

Anticancer activity of 3-(3-(((1*H*-Benzo[*d*] imidazol-2-yl)methyl)thio)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-6-yl)-6,8-disubstituted-2*H*-chromen-2-ones.

The compounds 3-(3-(((1*H*-benzo[*d*] imidazol-2-yl)methyl)thio)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-6-yl)-6,8-disubstituted-2*H*-chromen-2-ones were evaluated for their anticancer properties against three human cancer cell lines, murine leukemia cells (L1210), human T –Lymphocytes cells (CEM) and human cervix carcinoma cells (HeLa) by using MTT protocol as described in the chapter-II (reference 49) and the IC_{50} values in micromolar concentration were calculated.

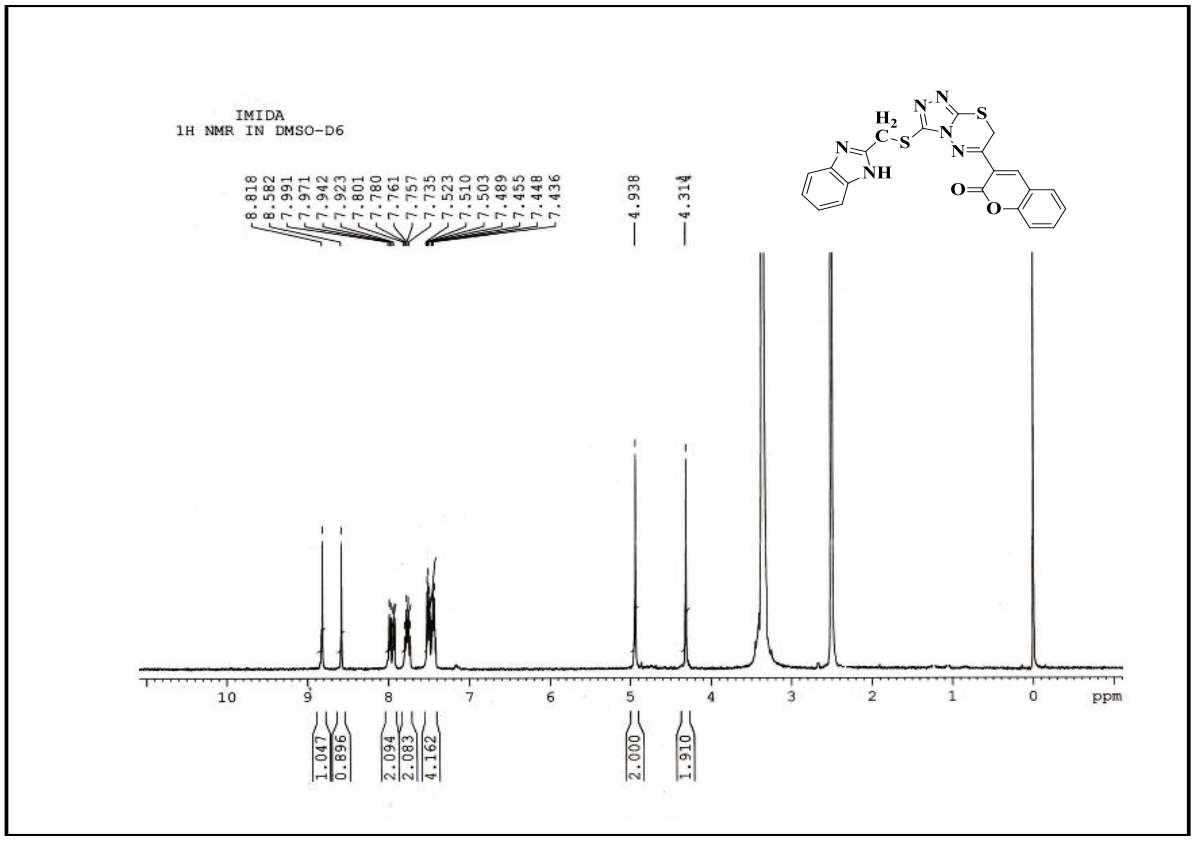


Fig: ^{1}H NMR spectrum of compound **46a**

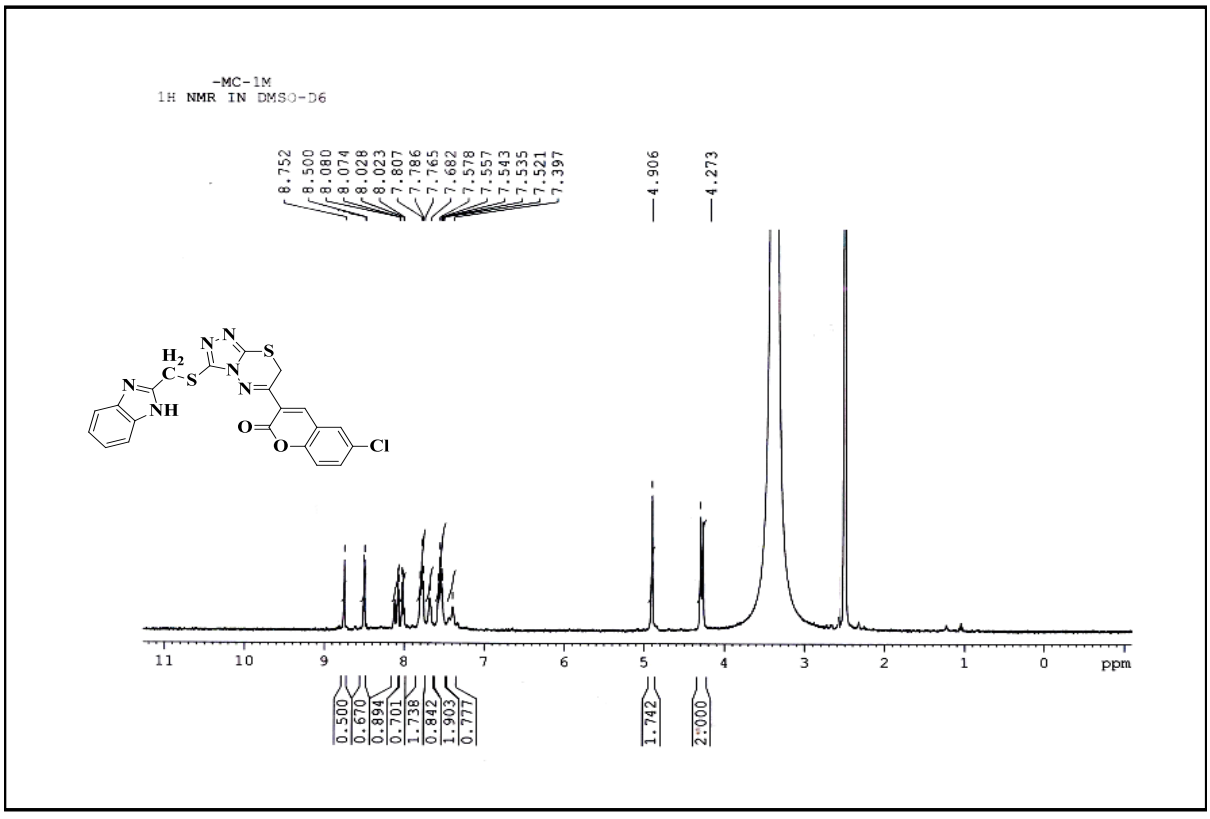


Fig: ^{1}H NMR spectrum of compound **46b**

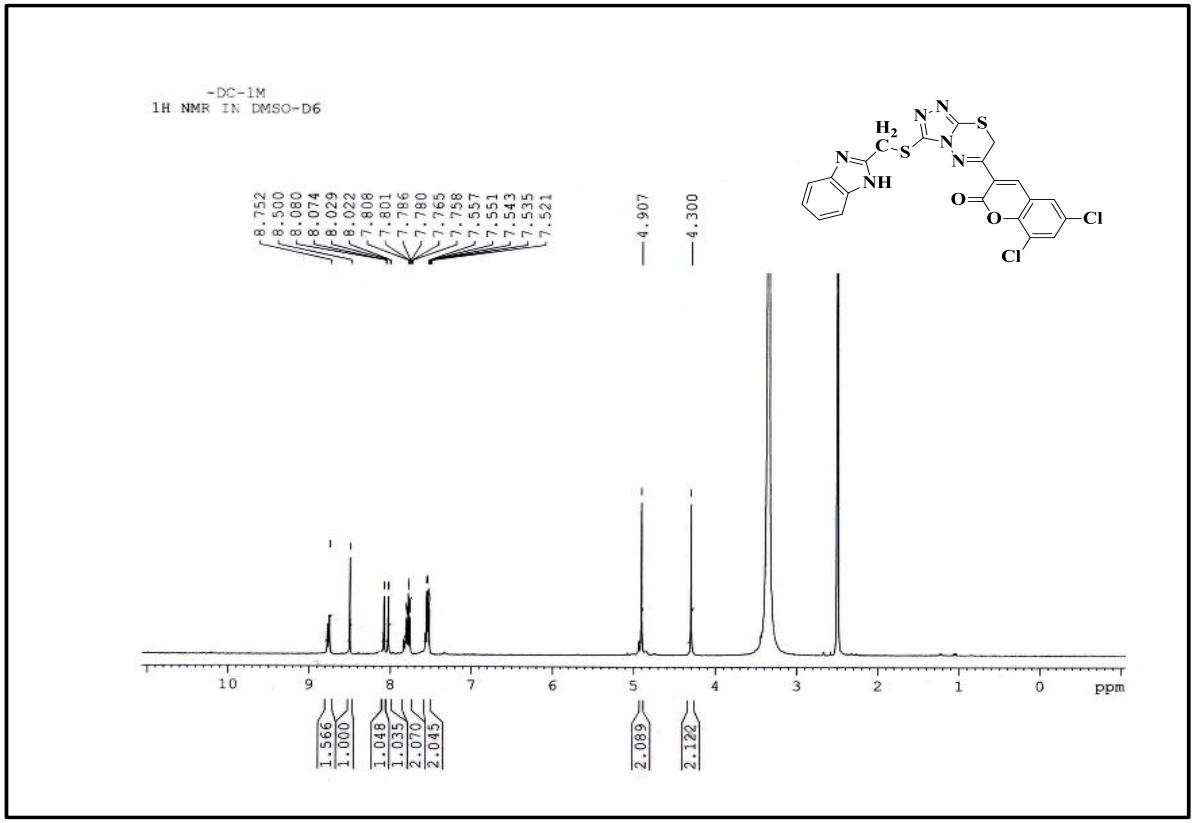


Fig: ^{1}H NMR spectrum of compound **46c**

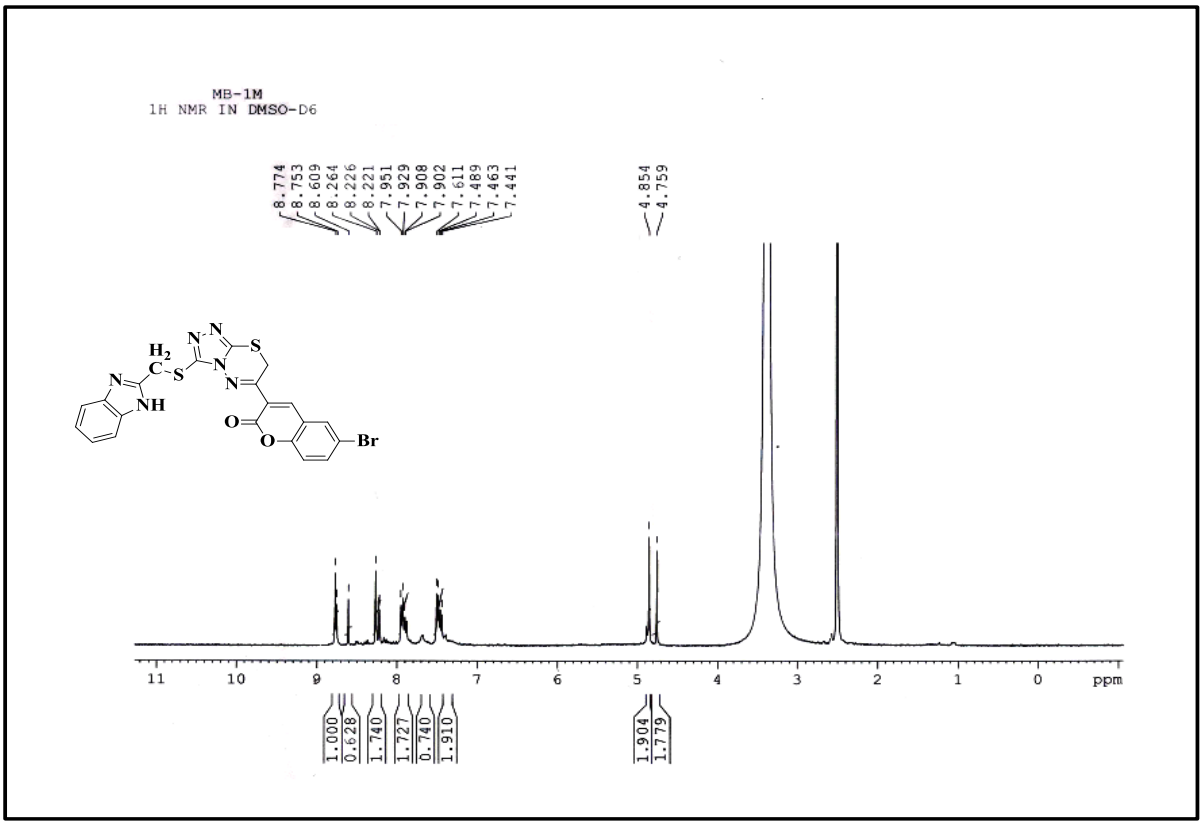


Fig: ^{1}H NMR spectrum of compound **46d**

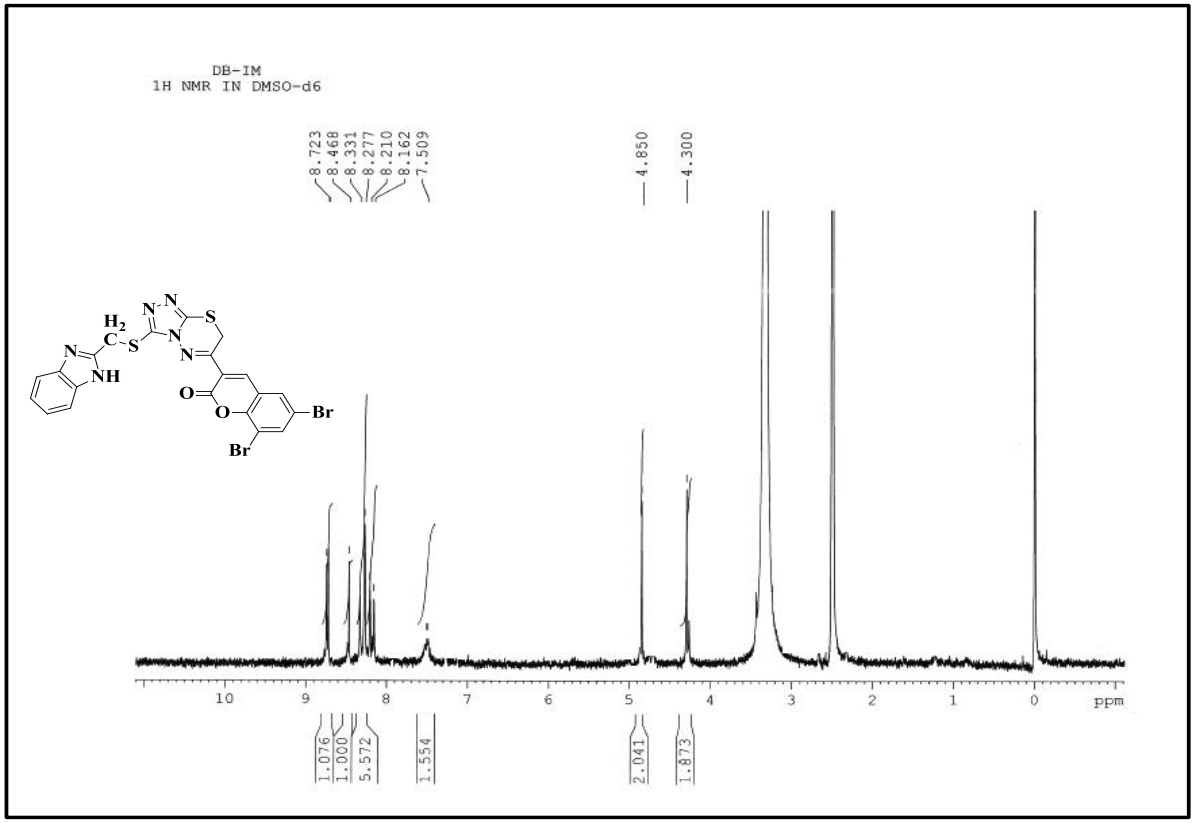


Fig: ^{1}H NMR spectrum of compound **46e**

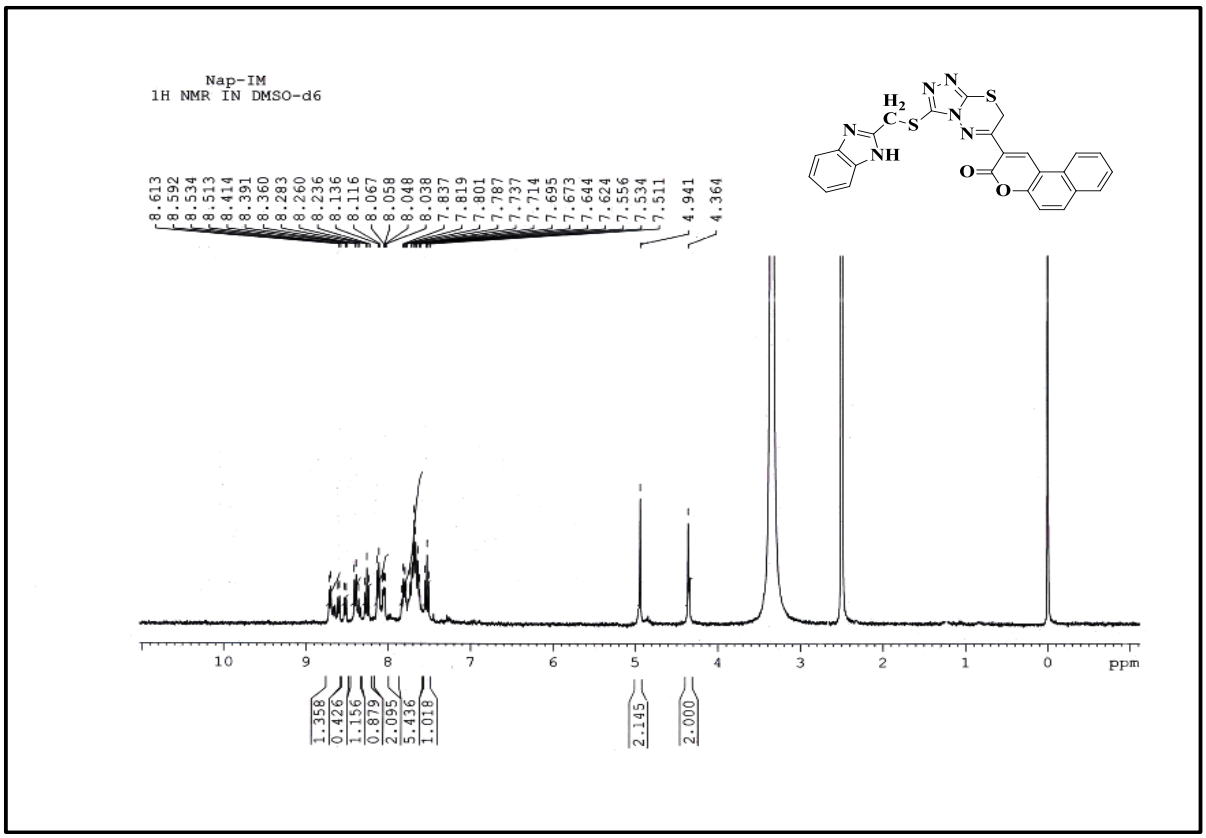


Fig: ^{1}H NMR spectrum of compound **46f**

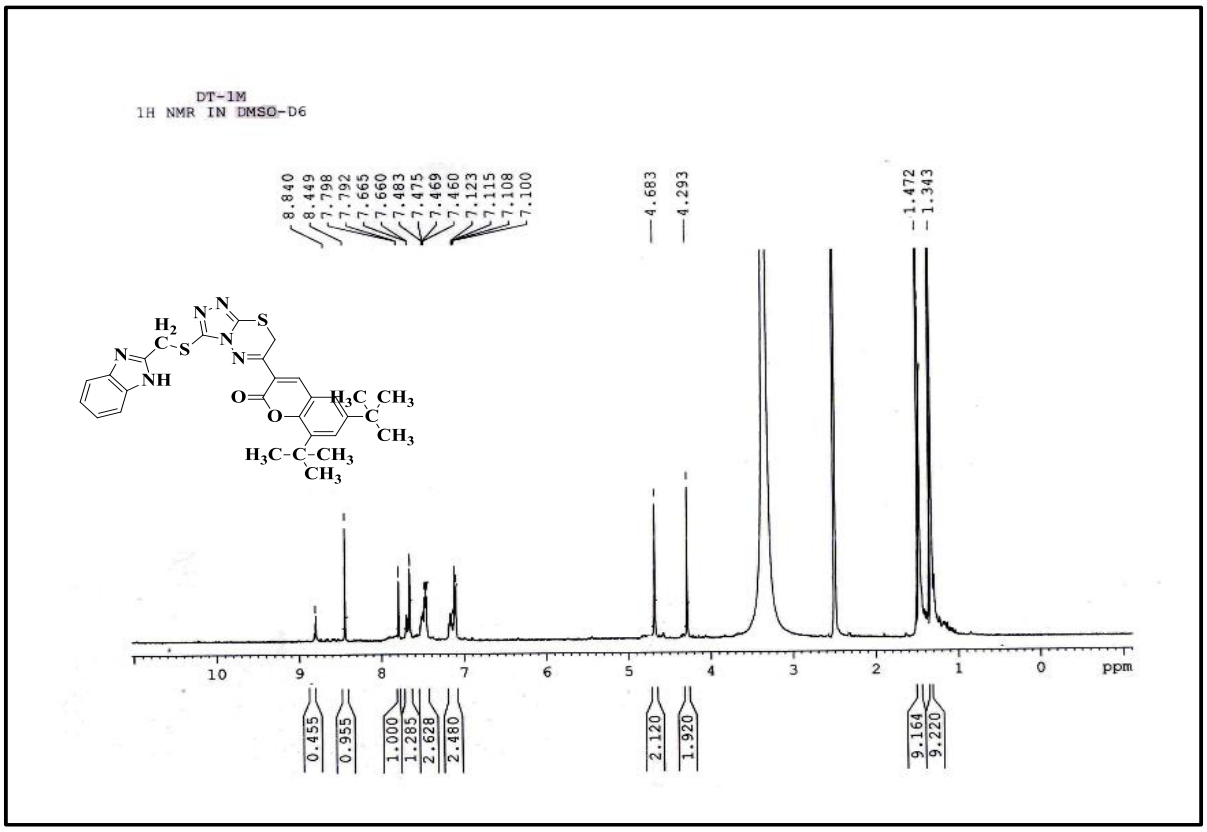


Fig: ^{1}H NMR spectrum of compound **46g**

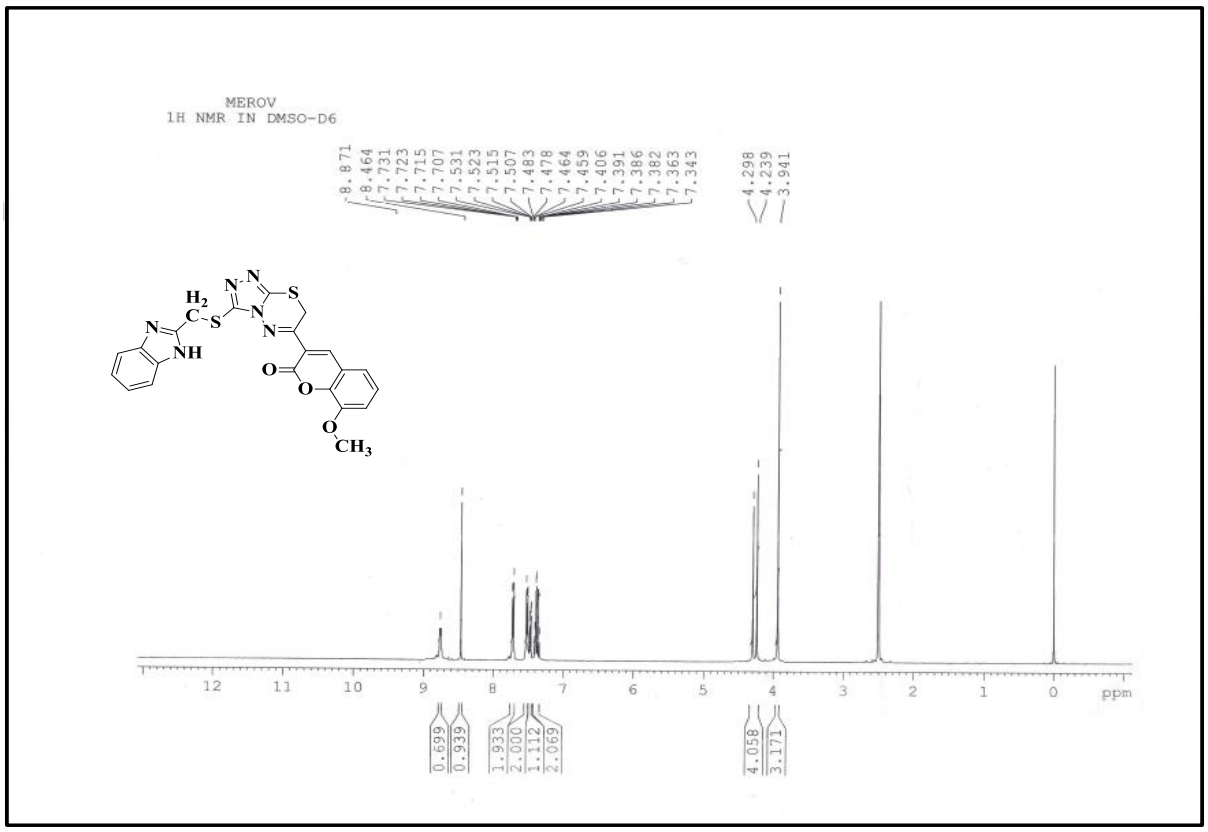


Fig: ^{1}H NMR spectrum of compound **46h**

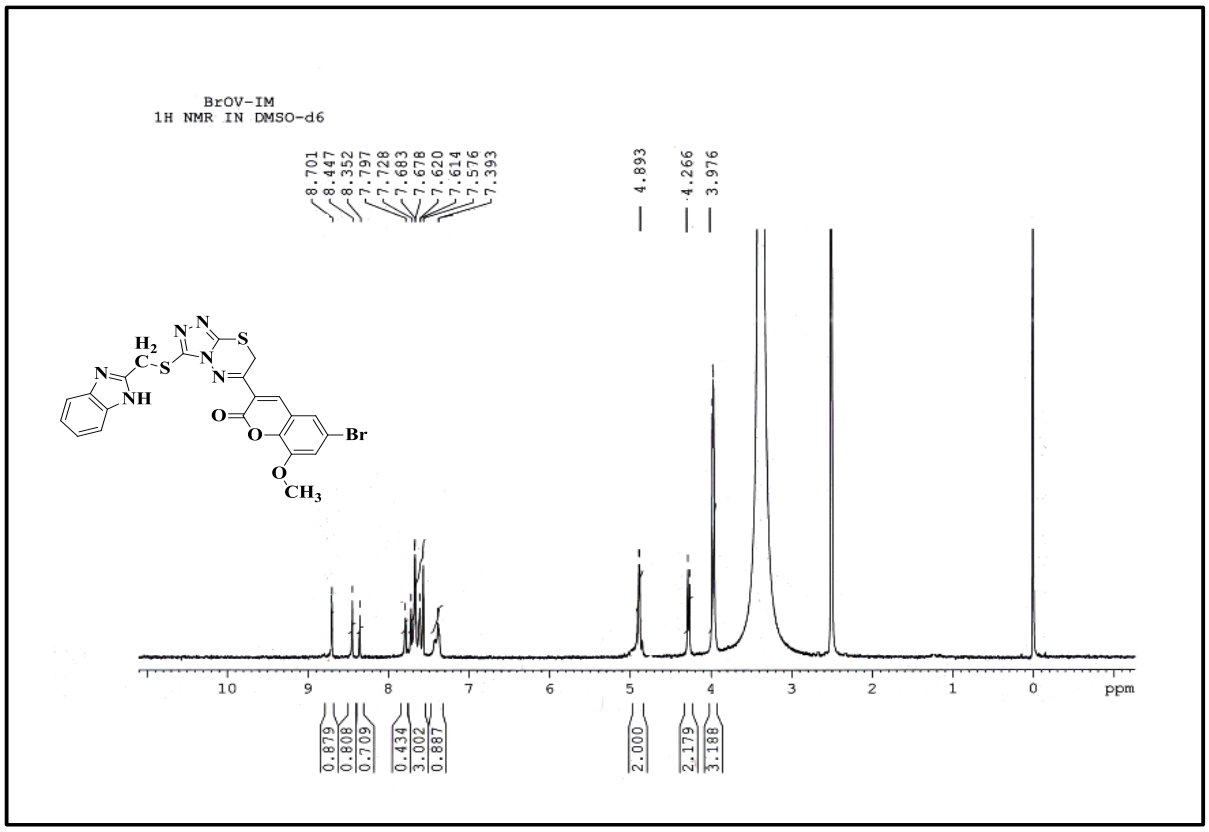


Fig: ^{1}H NMR spectrum of compound **46i**

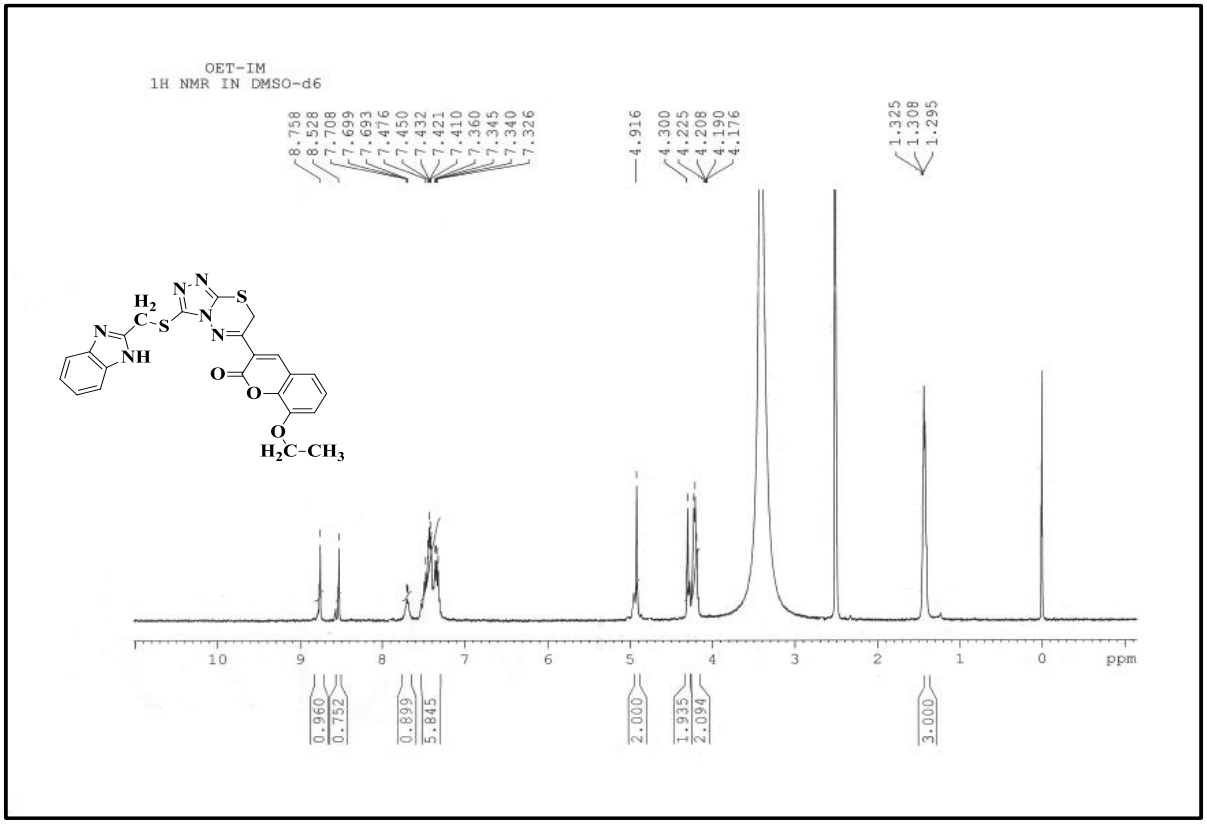


Fig: ^{1}H NMR spectrum of compound **46j**

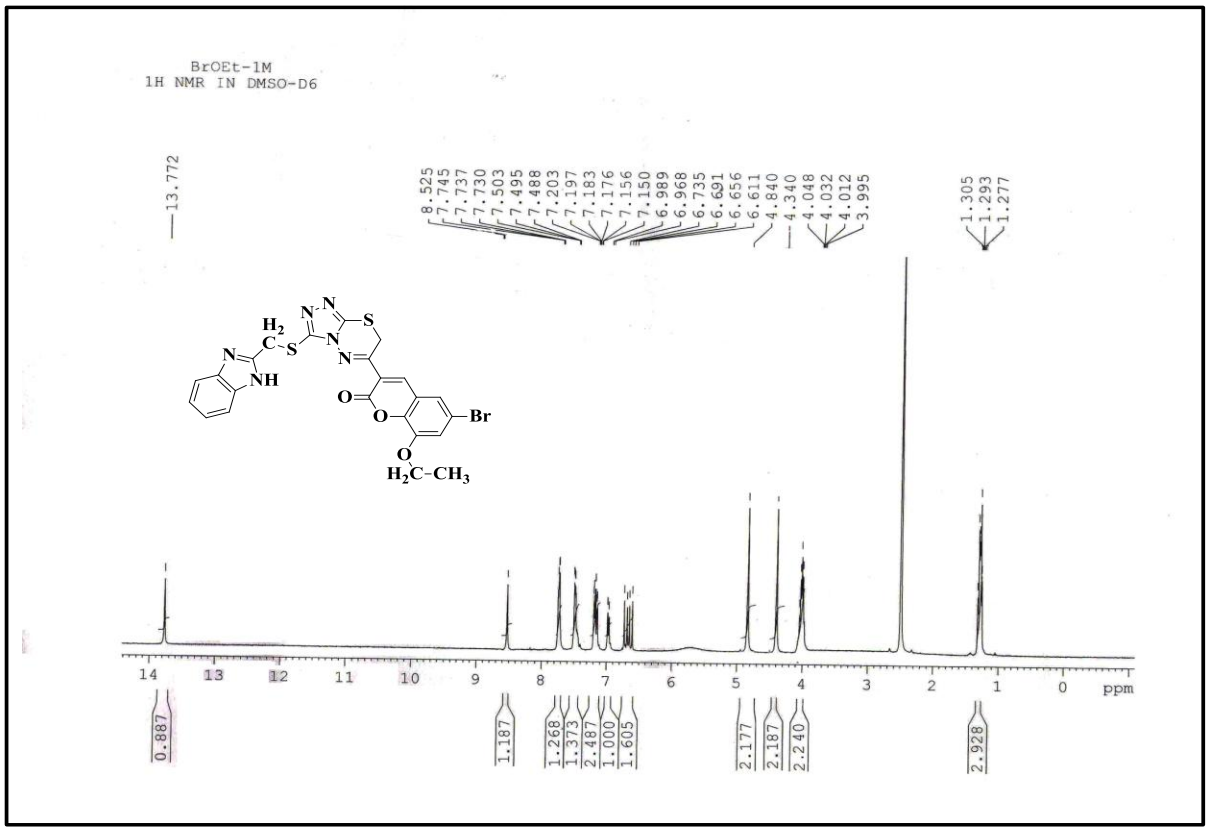


Fig: ^{1}H NMR spectrum of compound **46k**

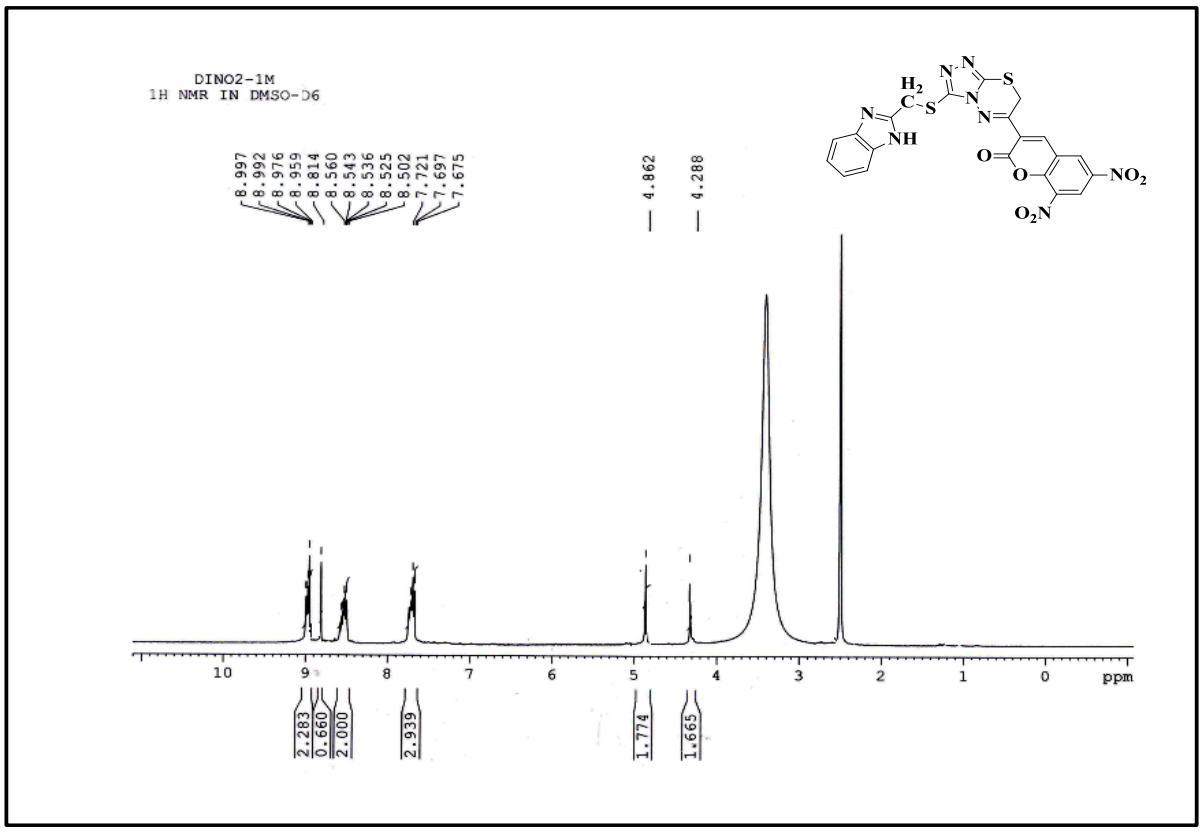


Fig: ^{1}H NMR spectrum of compound **46l**

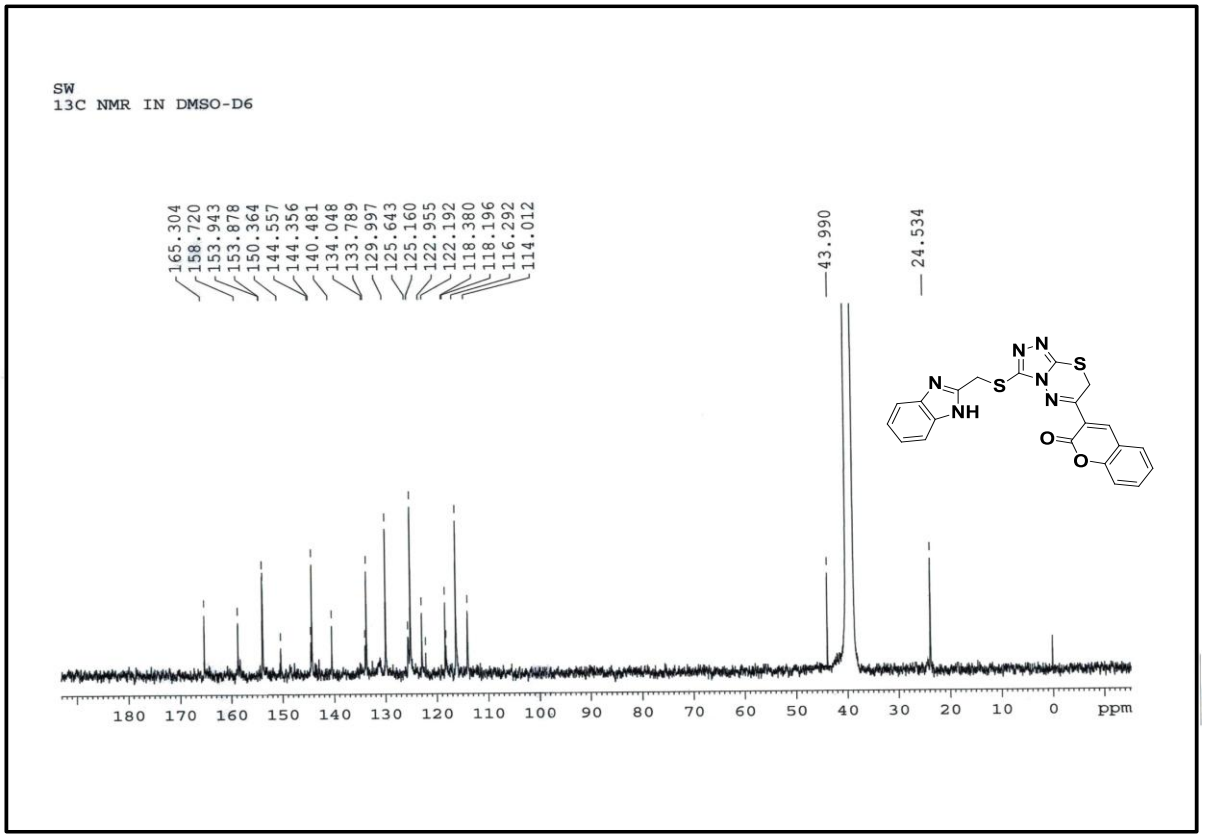


Fig: ^{13}C NMR spectrum of compound **46a**

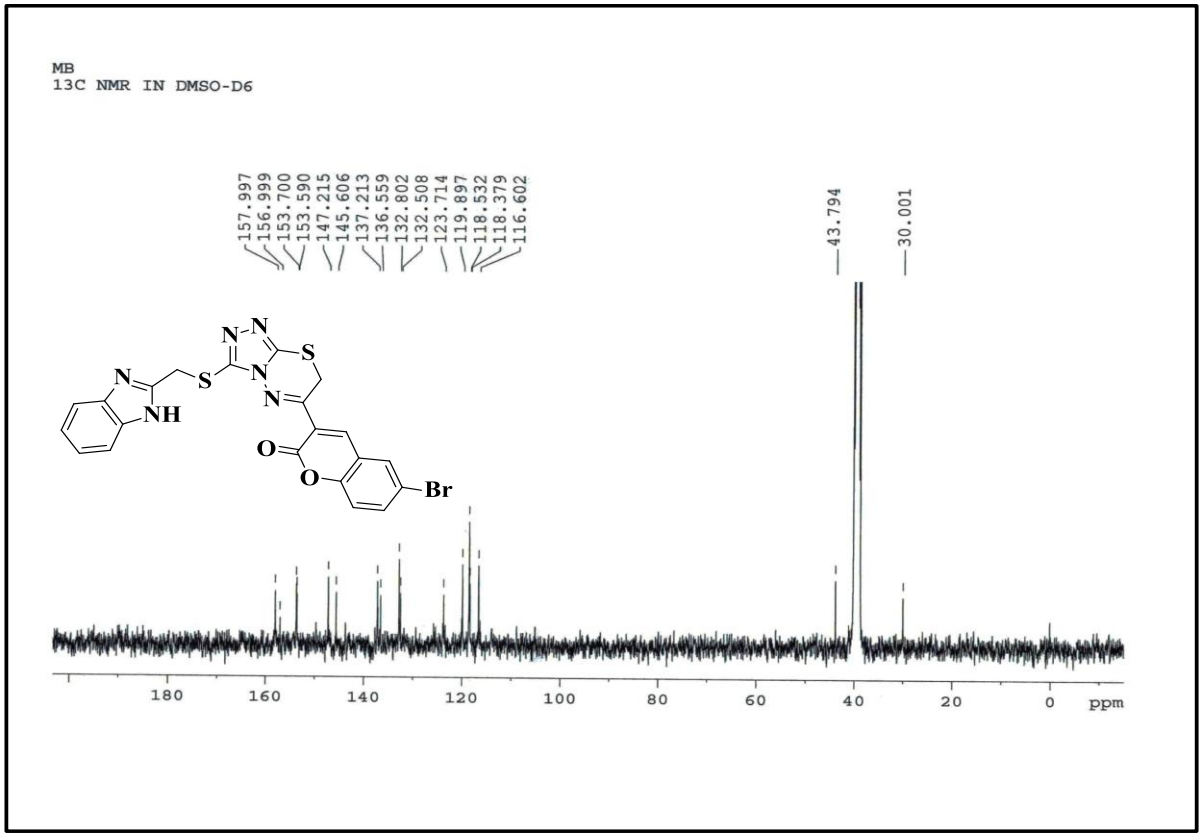


Fig: ^{13}C NMR spectrum of compound **46**

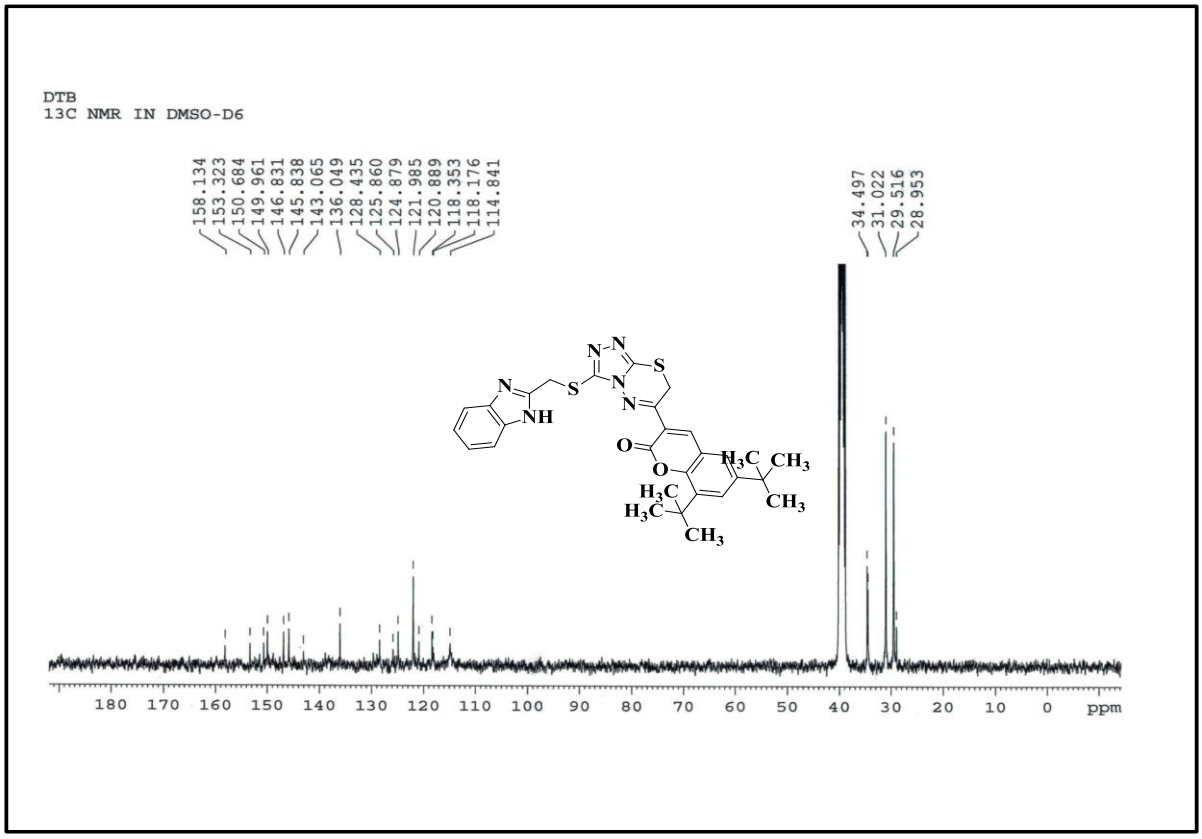


Fig: ^{13}C NMR spectrum of compound **46**

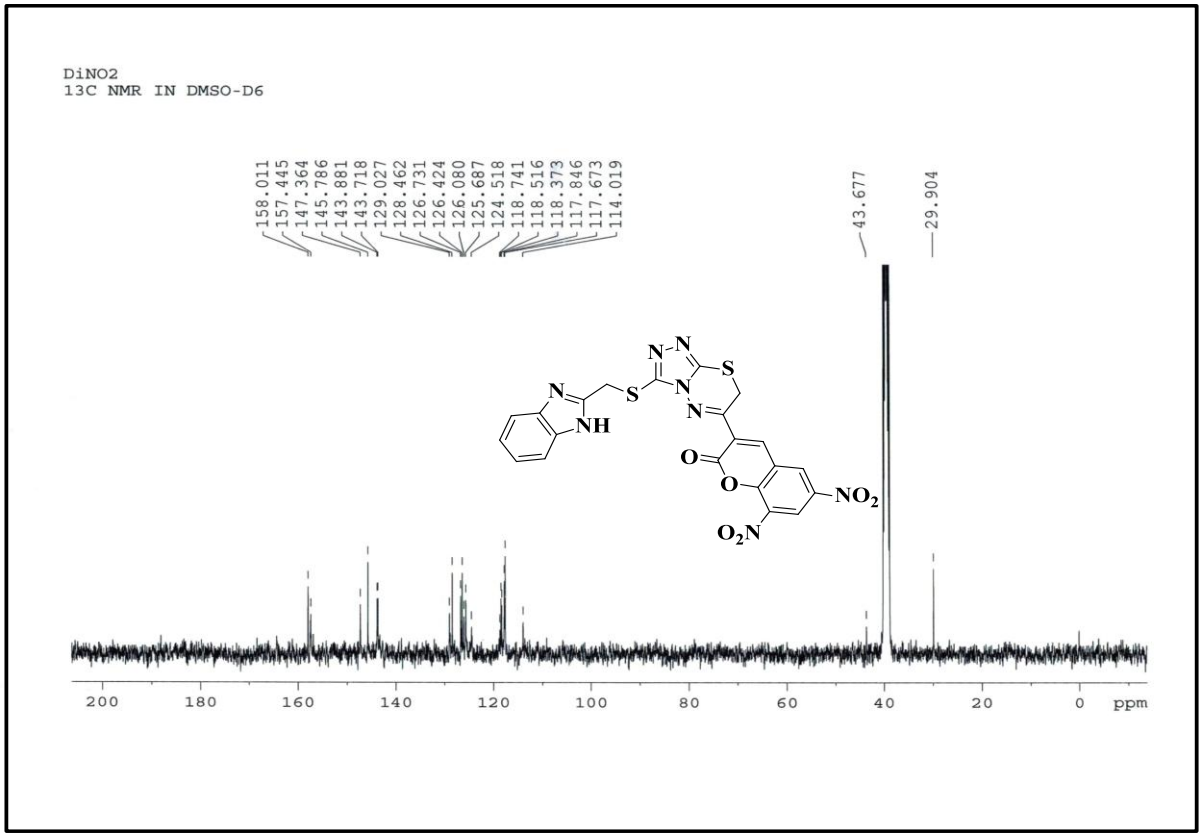


Fig: ^{13}C NMR spectrum of compound **46**

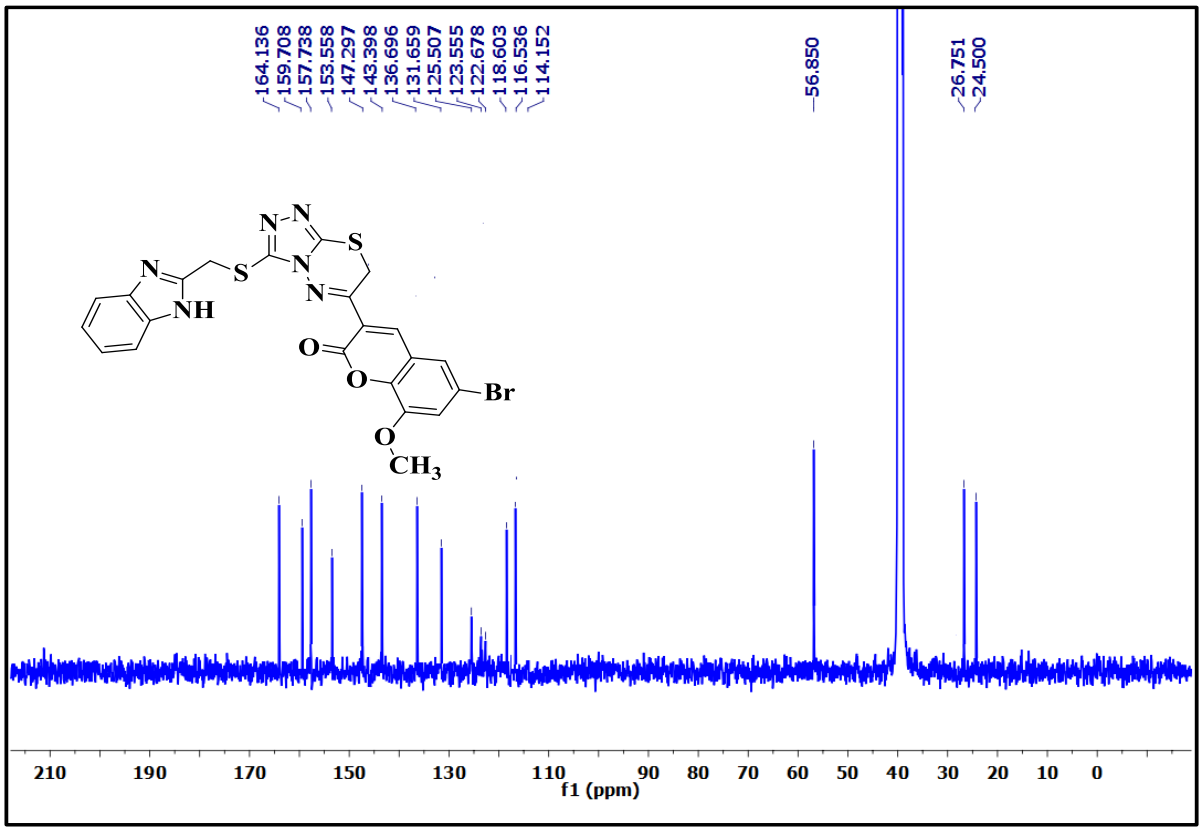


Fig: ^{13}C NMR spectrum of compound **46**

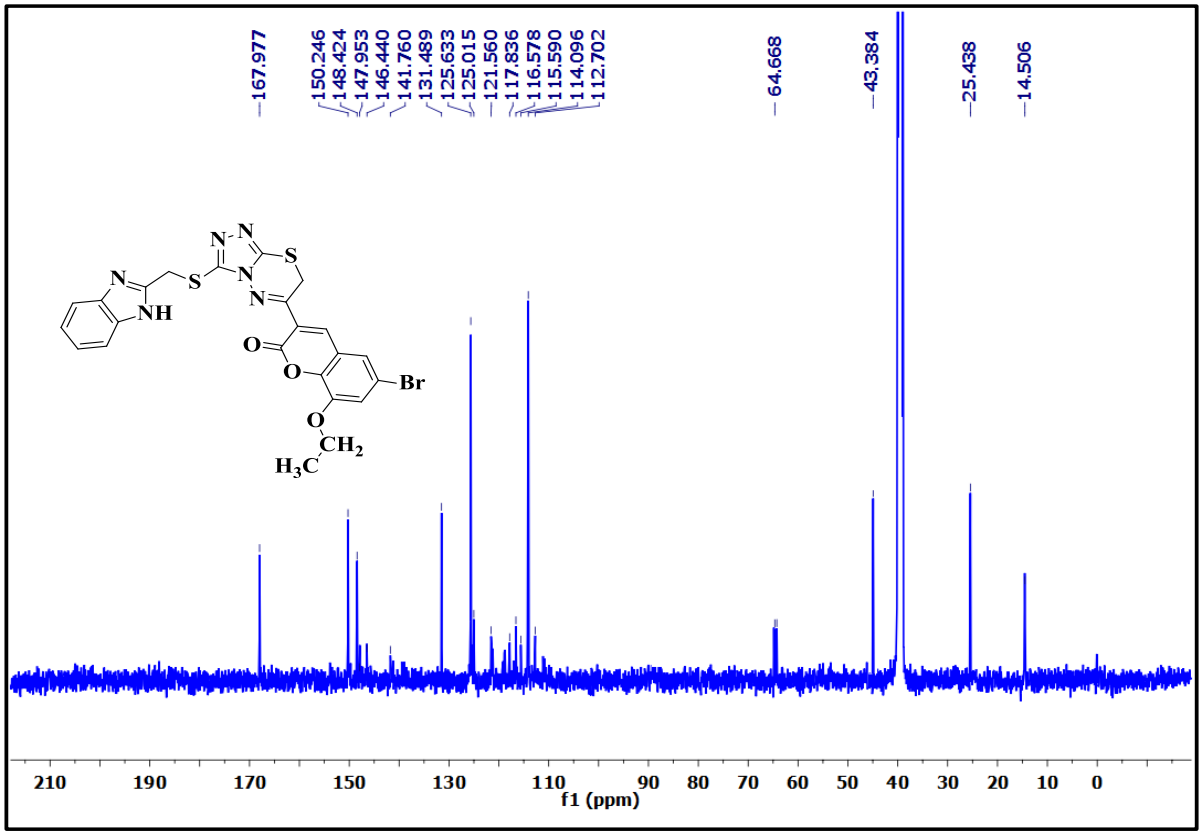


Fig: ^{13}C NMR spectrum of compound **46**

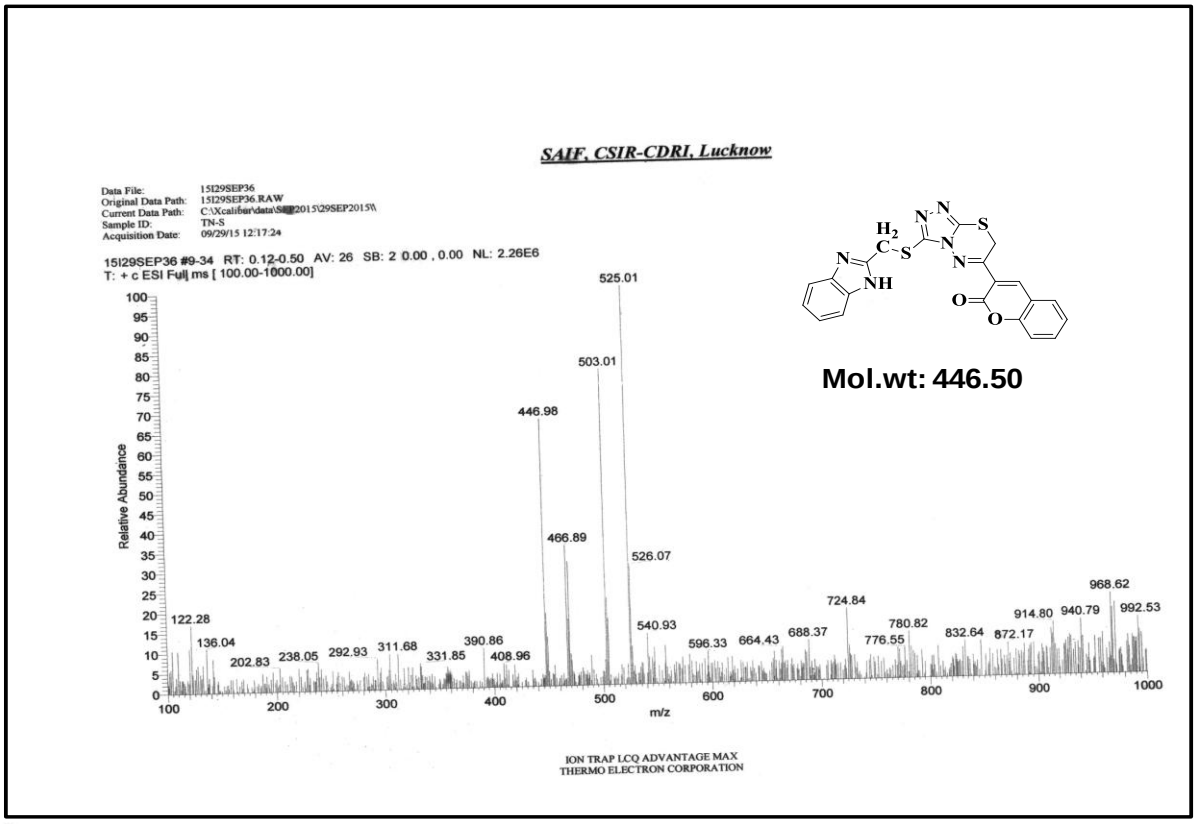


Fig: Mass spectrum of compound **46a**

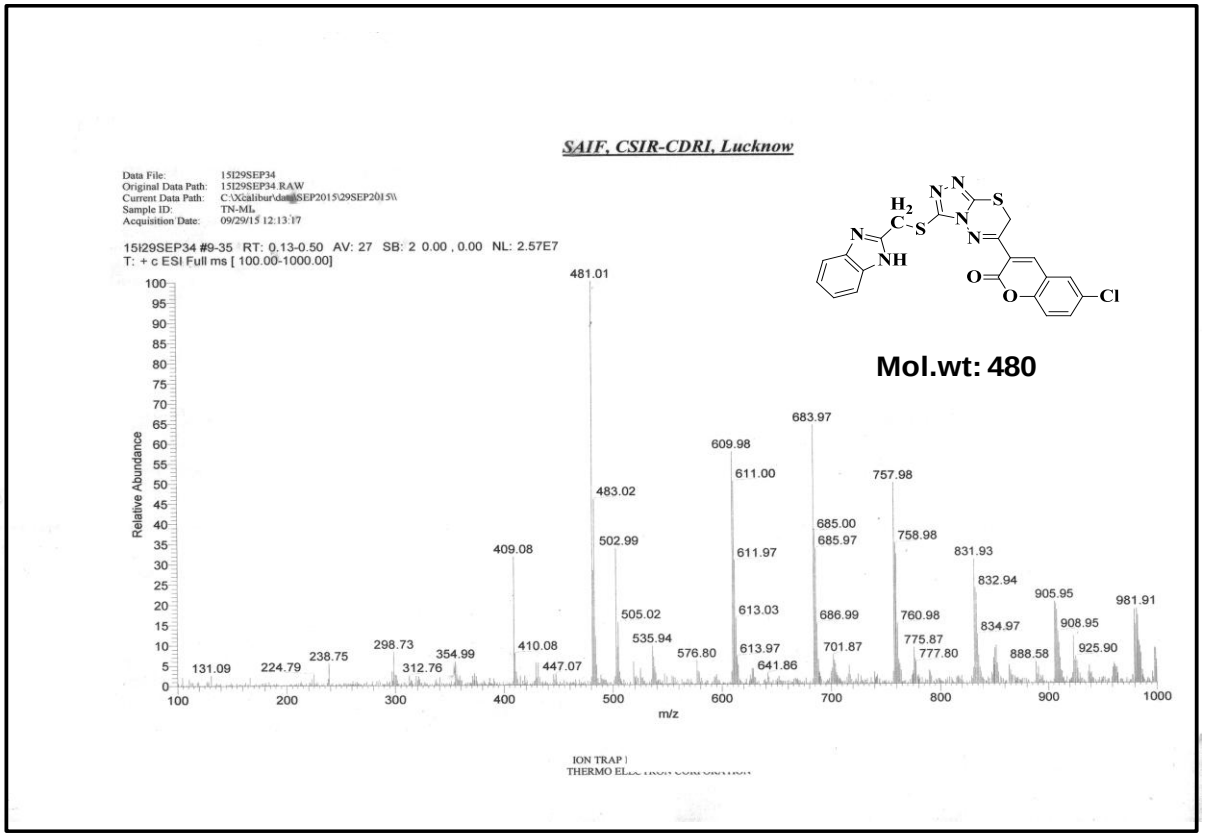


Fig: Mass spectrum of compound **46b**

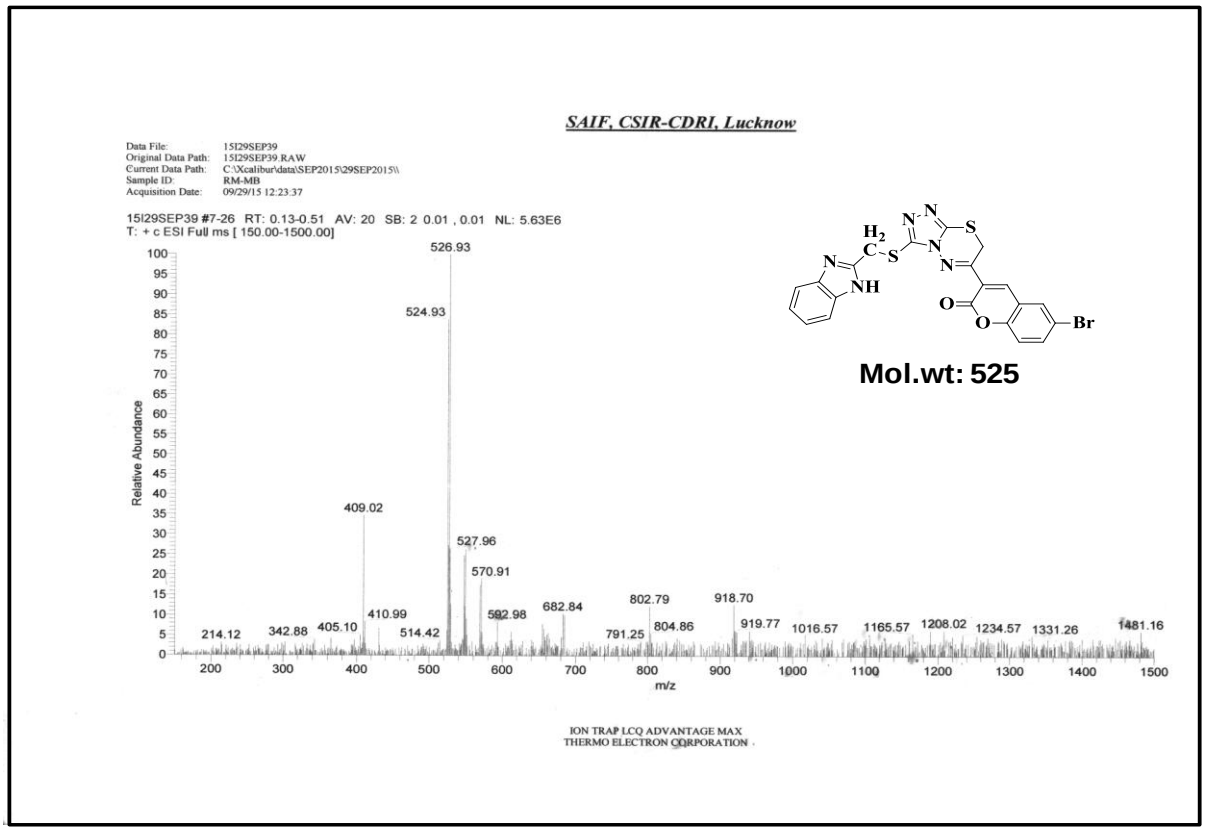


Fig: Mass spectrum of compound **46d**

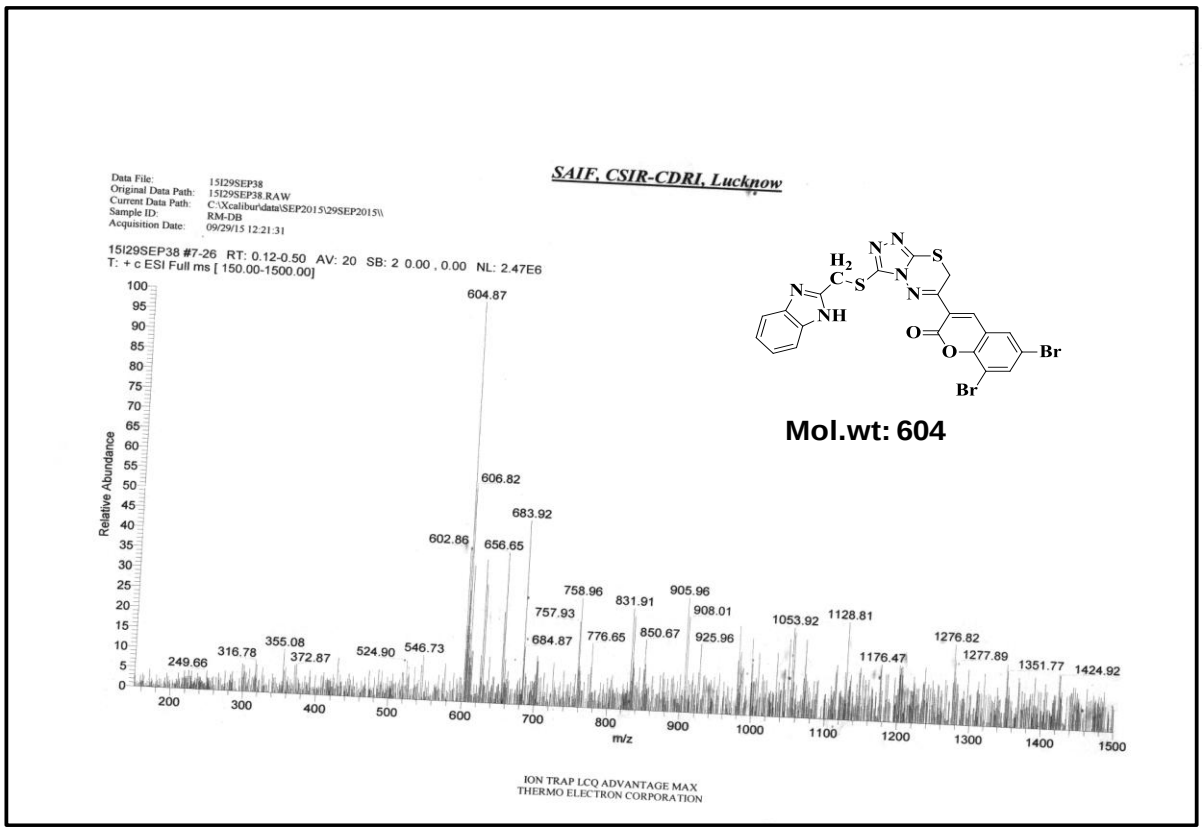


Fig: Mass spectrum of compound **46e**

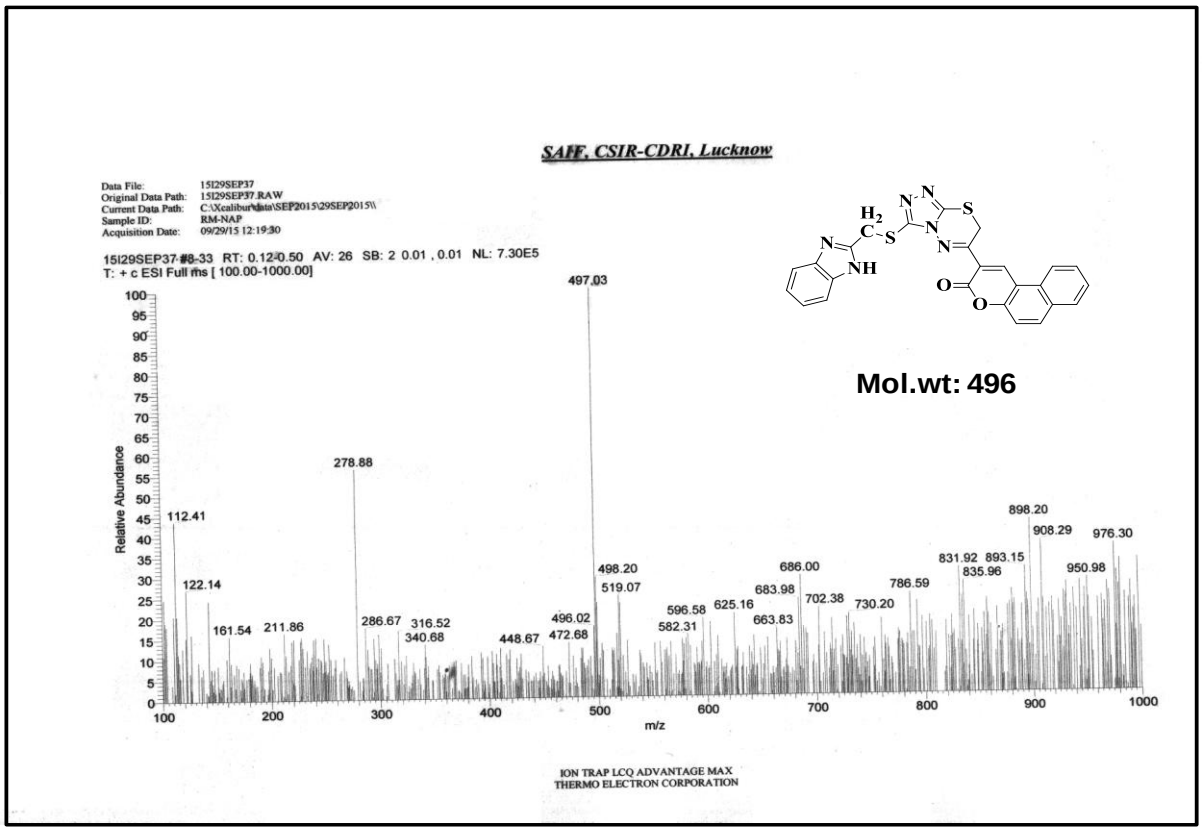


Fig: Mass spectrum of compound **46f**

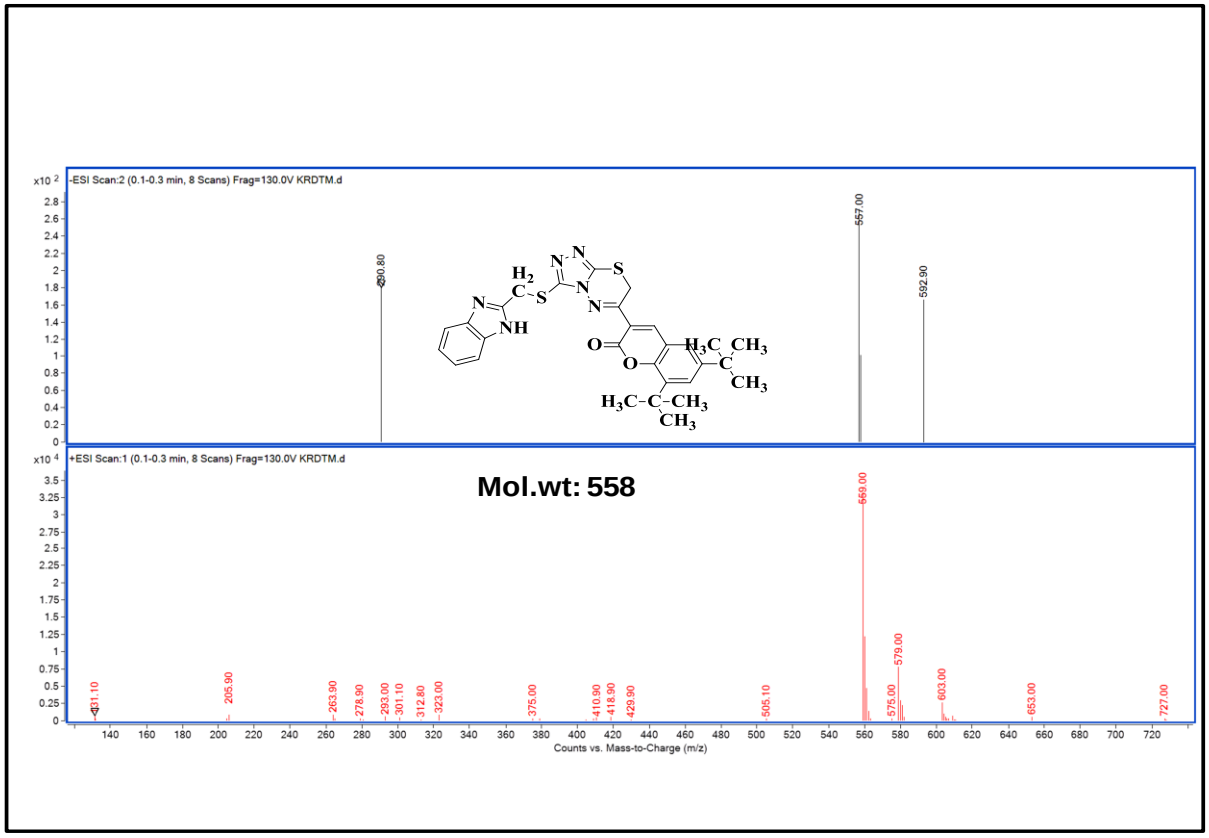


Fig: Mass spectrum of compound **46g**

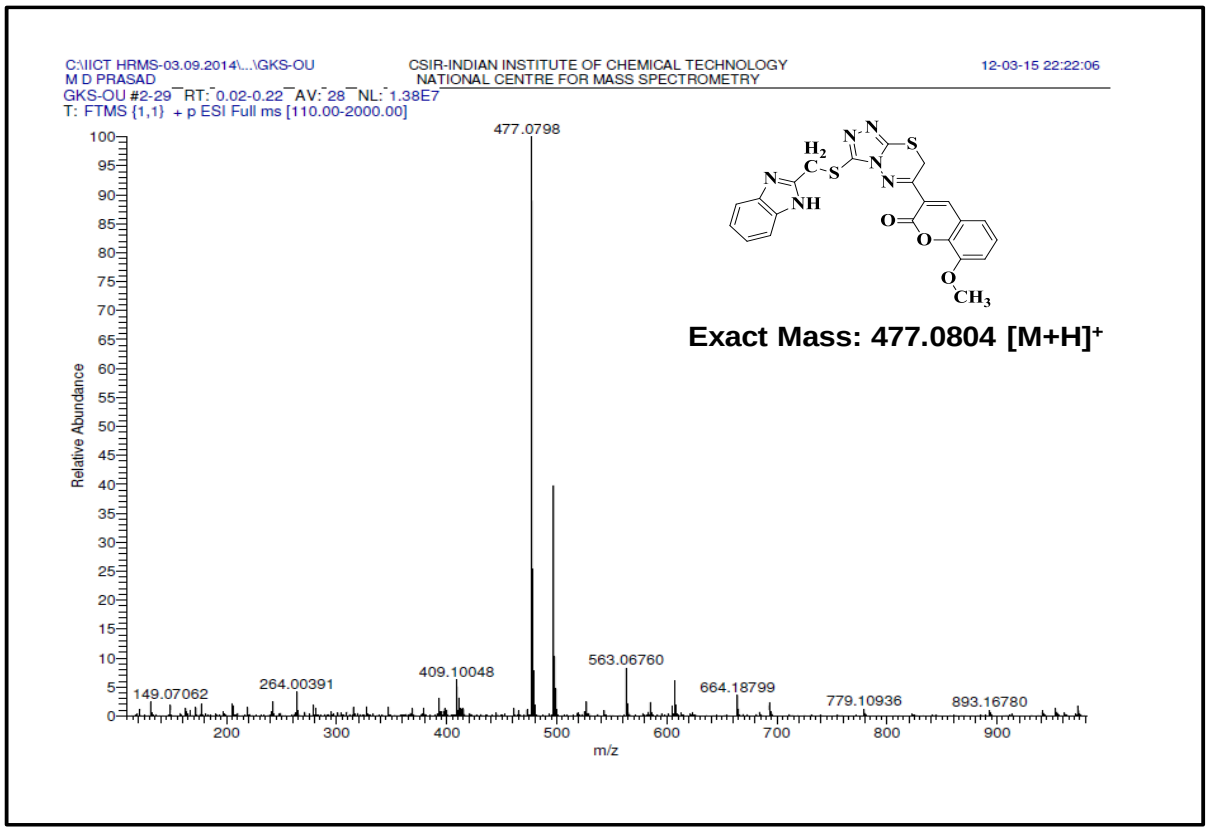


Fig: Mass spectrum of compound **46h**

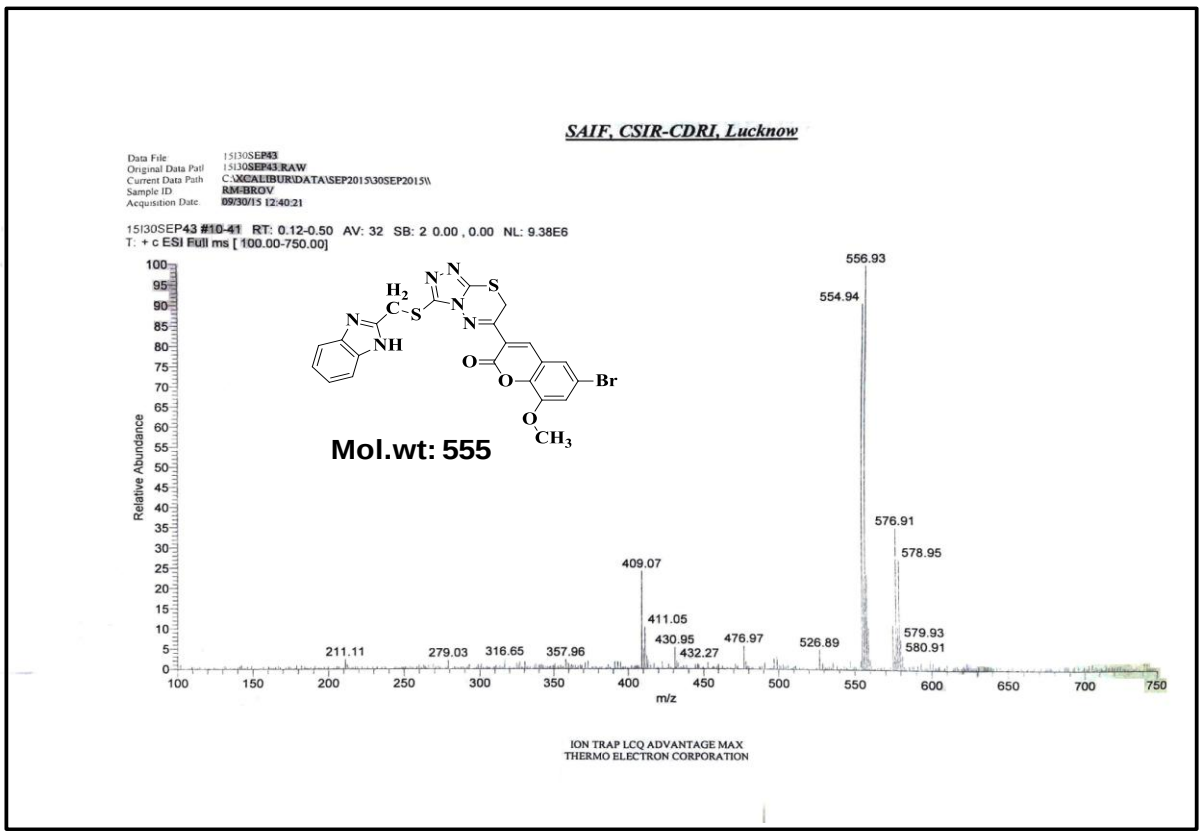


Fig: Mass spectrum of compound **46i**

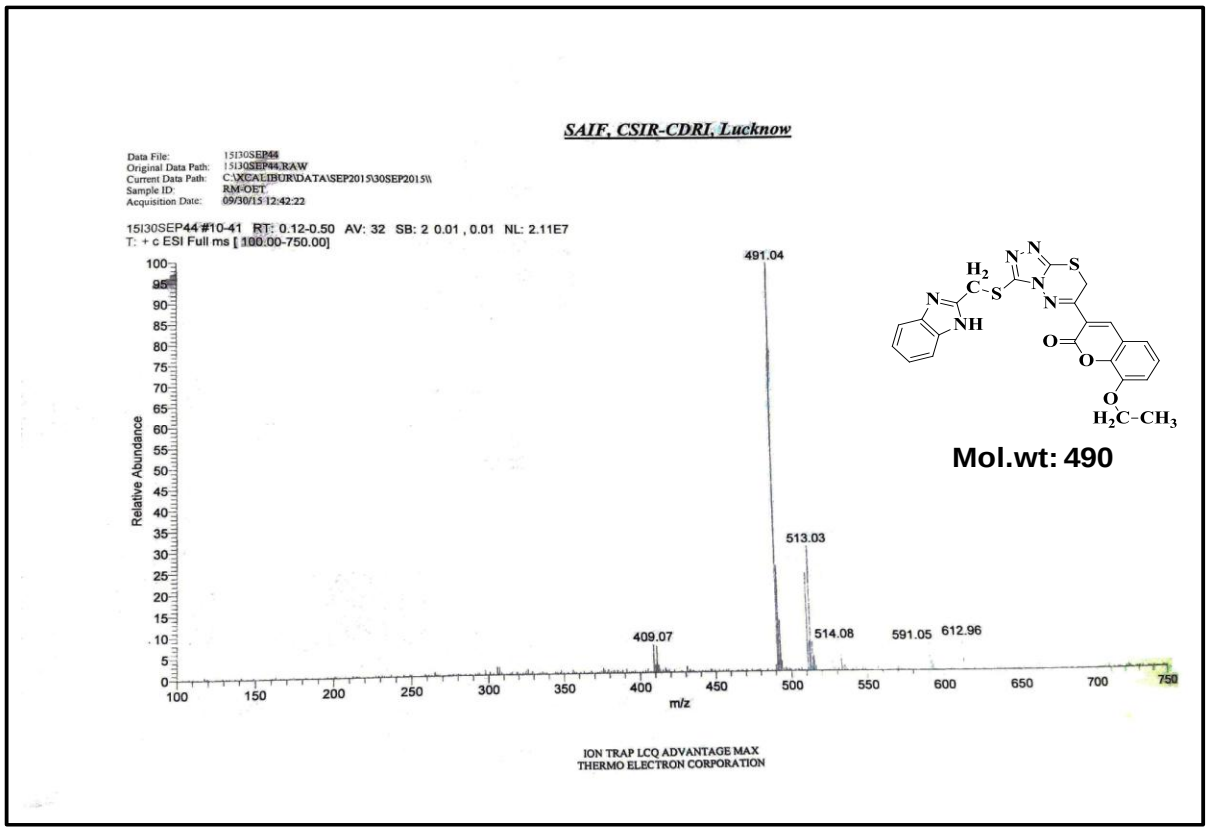


Fig: Mass spectrum of compound **46j**

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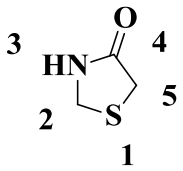
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CHAPTER-V

A facile one pot synthesis of 2, 5 and 2, 3 ,5 substituted 4-thiazolidinones and their biological applications

INTRODUCTION:

The main aim of the organic and medicinal chemist is the target molecule design, synthesis and evaluation of biological properties of the target molecule. In medicinal chemistry the utility of heterocyclic compounds have been proven with enormous therapeutic values. A large number of heterocyclic compounds with two hetero atoms in a five member ring are remarkable pharmacological applications. 4-thiazolidinone is a five membered heterocyclic system having one sulphur and one nitrogen atom at 1st and 3rd position and carbonyl functional group at 4th position. 4-thiazolidinone is a prevalent scaffold associated with a variety of medicinal applications.



4-Thiazolidinone

4-thiazolidinones have emerged as an important heterocyclic compounds with diversified biological applications such as antimicrobial[1,2], antifungal[3-5], antiviral[6-8], anti tubercular[9,10], anti protozoan[11-13], anti inflammatory[14-16], anti convulsant[17,18], antioxidant[19,20], anticancer[21-24], cardiovascular[25], anti diabetic[26-28], antihistaminic[29], osteoarthritis[30], calcium ion channel blockers[31,32], hypnotic[33,34] and follicle stimulating hormone receptors[35]. Some of the biologically active 4-thiazolidinones are shown in **Fig.1.** The synthesis and biological applications of thiazolidinones are well investigated and still research is going on to develop the efficient synthetic methods and explore biological properties.

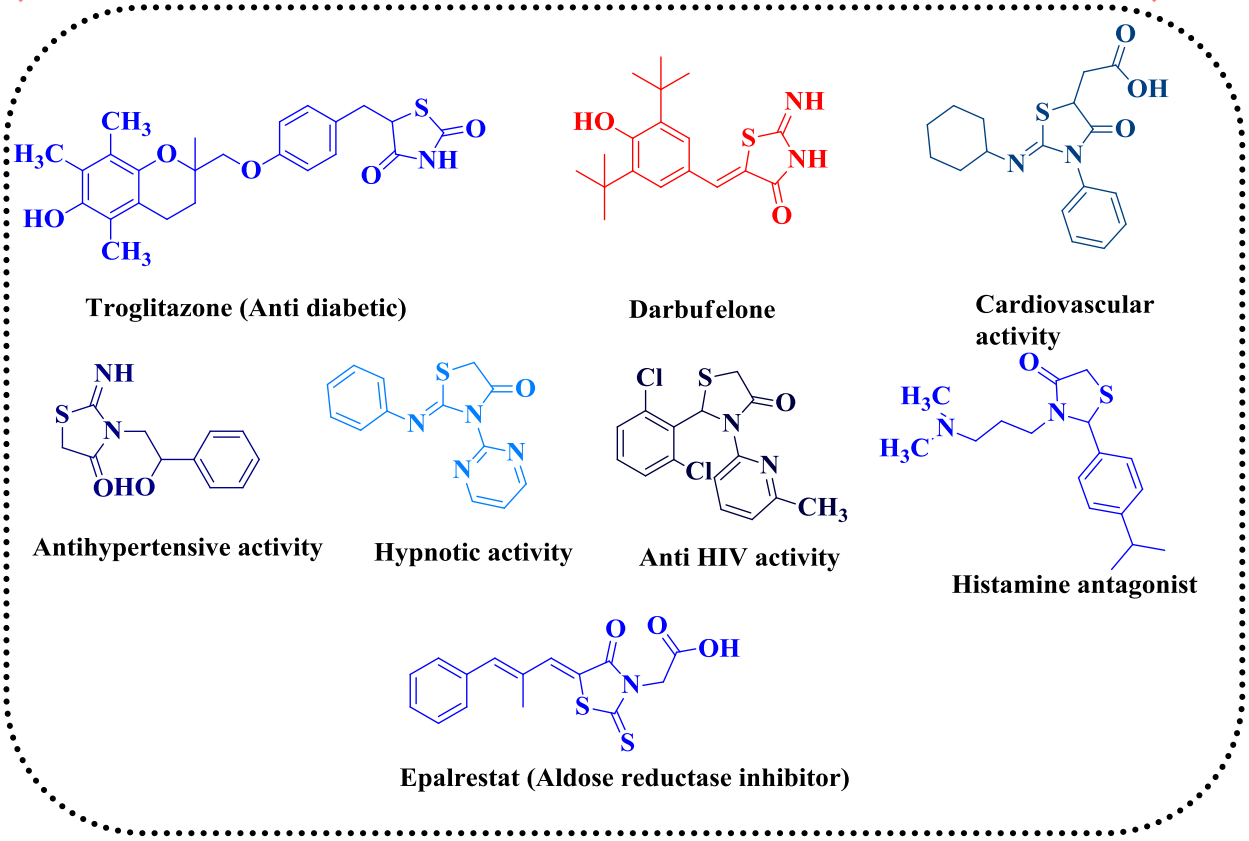
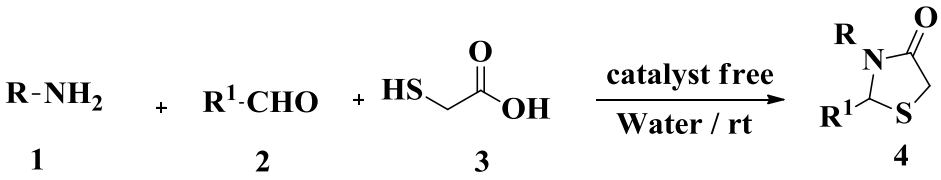


Fig. 1 Biologically potent 4-thiazolidinones

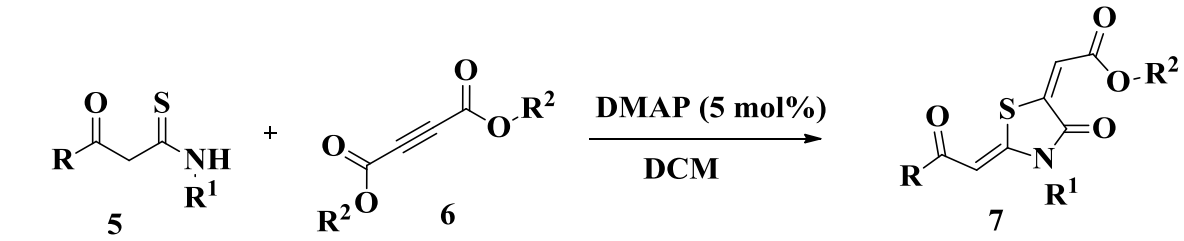
The following is a brief review of literature on synthetic methods on 4-thiazolidinones.

Manoj P Thakare[36] *et al.* reported the synthesis of 4-thiazolidinones (4) under catalytic free reaction conditions using aliphatic/ aromatic amines (1), aromatic aldehydes (2) and thioglycolic acid (3) in aqueous medium at room temperature with moderate to good yields.

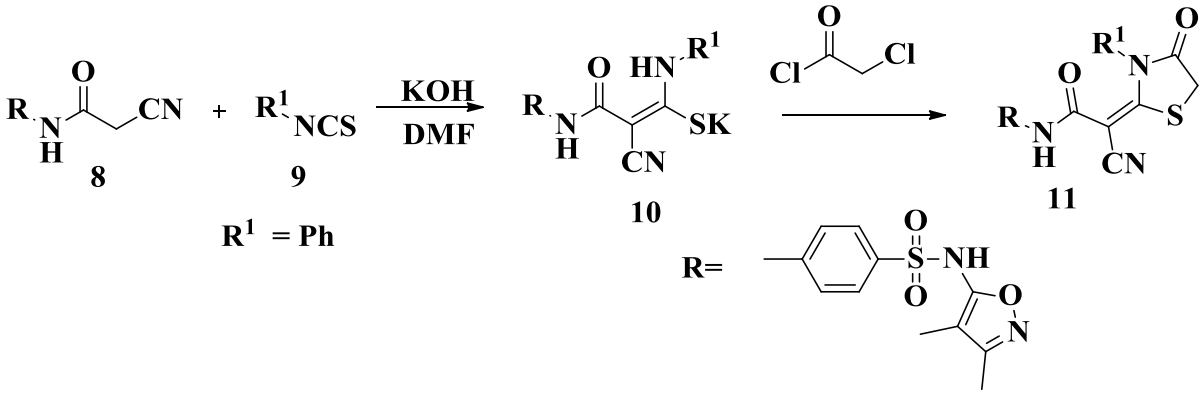


R = Aliphatic / aromatic /heterocyclic R^1 = Aromatic

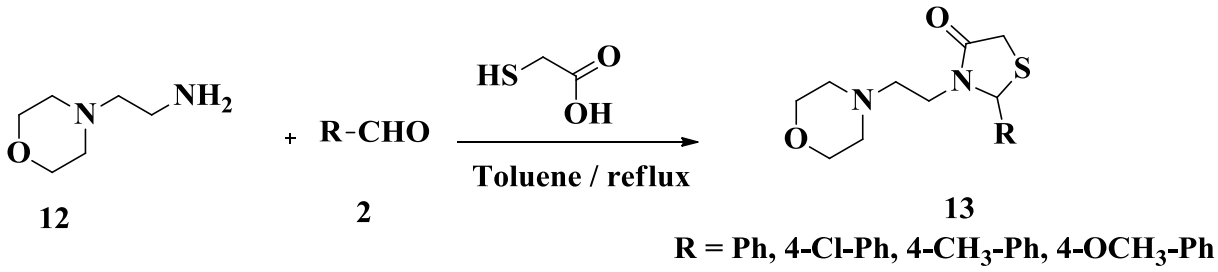
G. K. Verma[37] *et al.* described a regioselective synthesis of functionalized thiazolidinones (7) through catalytic reaction conditions. The target compounds were synthesized by the reaction of β-ketothiomides (5) with dialkyacetylene dicarboxylates (6) through DMAP catalyzed reaction conditions.



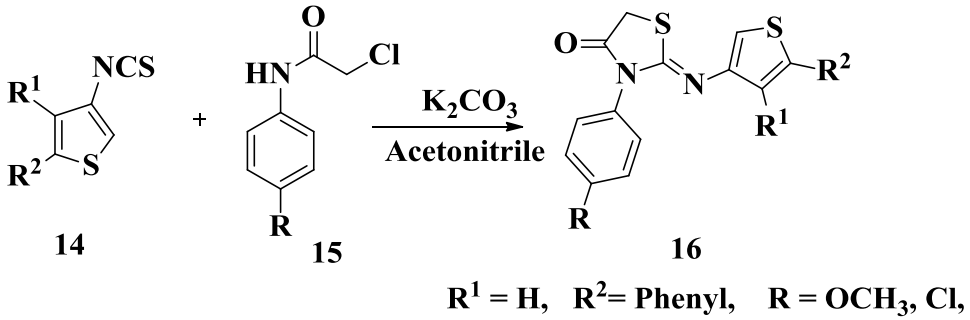
2-cyano-*N*-(4-(*N*-(3,4-dimethylisoxazol-5-yl)sulfamoyl)phenyl)-2-(4-oxo-3-aryl thiazoli din-2-ylidene)acetamide derivatives (11) were prepared in a stepwise reaction method by T. Nasr[38] *et al.* starting from the reaction of 2-cyano-*N*-(4-(*N*-(3,4-dimethylisoxazol-5-yl) sulfamoyl) phenyl)acetamide (8) with arylisothiocyanates (9) in presence of KOH. This reaction forms an intermediate of potassium salt of 2-cyano-3-((4-(*N*-(3,4-dimethylisoxazol-5-yl)sulfamoyl) phenyl)amino)-3-oxo-1-(phenylamino)prop-1-ene-1-thiolate (10). This intermediate was further treated with chloroacetylchloride and stirred at room temperature to generate the target compound 2-cyano-*N*-(4-{[(3,4-dimethylisoxazol-5-yl)amino]sulfonyl}phenyl)-2-(3-aryl-4-oxothiazolidin-2-ylidene)-acetamide.



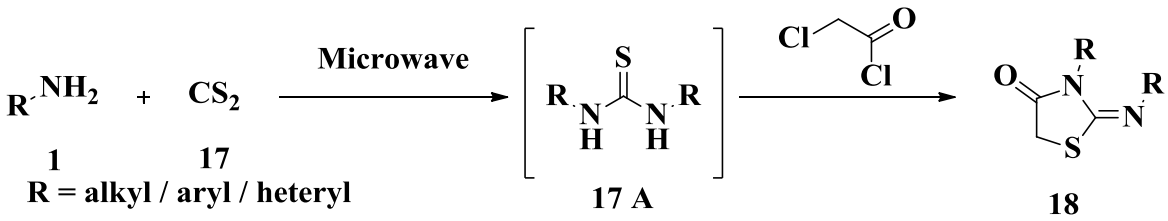
D. P. Gouvea[39] *et al.* described the synthesis of 2-aryl-3-(2-morpholinoethyl)thiazolidin-4-ones (13) through one pot reaction condition of 4-(2-aminoethyl)morpholine (12) with aryl aldehydes (2) and thioglycolic acid in presence of toluene under reflux condition. The target compounds were obtained with moderate to good yields.



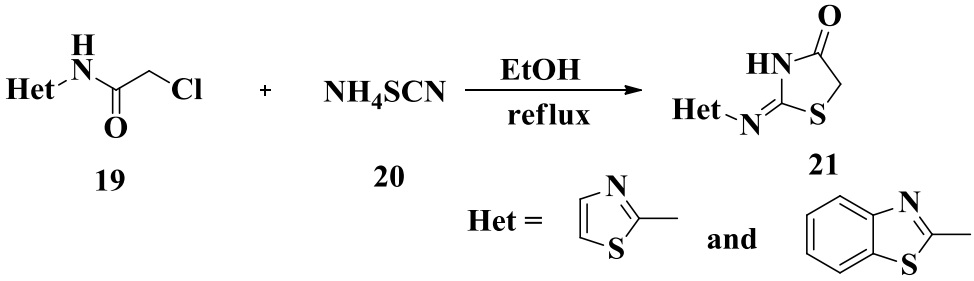
G. Revelant[40] *et al.* synthesized 2-heteroarylimino-4-thiazolidinones by the reaction of 2-chloro-*N*-arylacetamide with 3-isothiocyanatothiophene in presence of potassium carbonate using acetonitrile as a solvent with good yields.



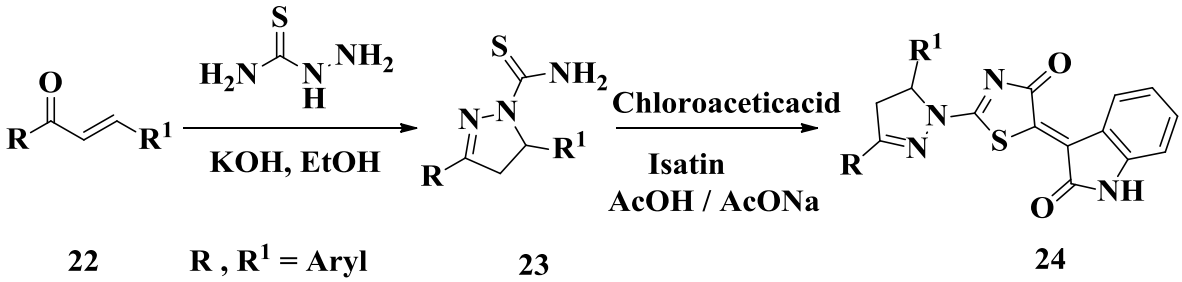
C. M. Chau[41] *et al.* reported an efficient synthetic method for the synthesis of 2-imino-4-thiazolidinones (18) through microwave reaction conditions. The target molecules were obtained from simple starting reactants like aliphatic / aromatic amines (1), carbon disulphide (17) and chloroacetylchloride in a sequential addition and the product was obtained in a satisfactory yields.



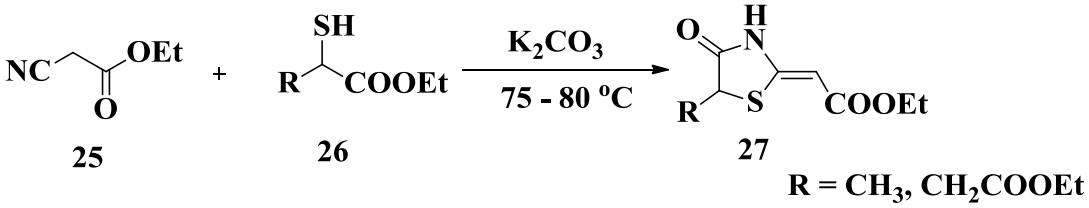
Geronikaki[42] *et al.* described the synthesis of 2-heteroarylimino-4-thiazolidinones (21) by the reaction of 2-chloro-N-(thiazole/benzo[d]thiazole)acetamides (19) with ammonium thiocyanate (20) in presence of ethanol under reflux conditions.



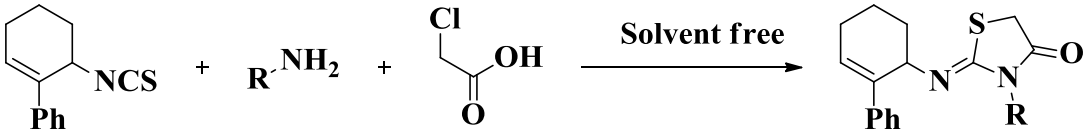
D. Havrylyuk[43] et al synthesized 3-[2-(3,5-diaryl-4,5-dihydropyrazol-1-yl)-4-oxo-4,5-dihydro-1,3-thiazol-5-ylidene]-2,3-dihydro-1*H*-indol-2-ones (24) starting from the reaction of chalcones (22) with thiosemicarbazide in alcoholic KOH to generate the intermediate 3,5-diphenyl-4,5-dihydro-1H-pyrazole-1-carbothioamide (23) which further undergoes cyclocondensation with chloroaceticacid followed by the reaction of isatin in presence of sodium acetate and acetic acid.



M. Stojanovic[44] *et al.* described the solvent free synthesis of 4-oxo-thiazolidine (27) by the reaction of ethyl cyanoacetate (25) and α-mercaptoesters (26) in presence of potassium carbonate at 75 – 80 °C with short reaction time. The products were obtained with satisfactory yields.



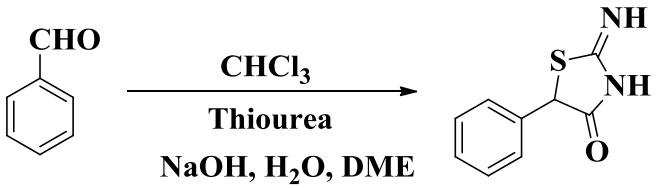
An efficient, rapid one pot solvent free synthesis was developed by M. Sathishkumar[45] *et al.* to synthesize the 2-iminothiazolidin-4-ones (30). The title compounds were obtained by the reaction of 2-isothiocyanato-2,3,4,5-tetrahydro-1,1'-biphenyl (28) with aliphatic/aromatic amines (1) and chloroaceticacid (29) with good yields.



28 1 29 30

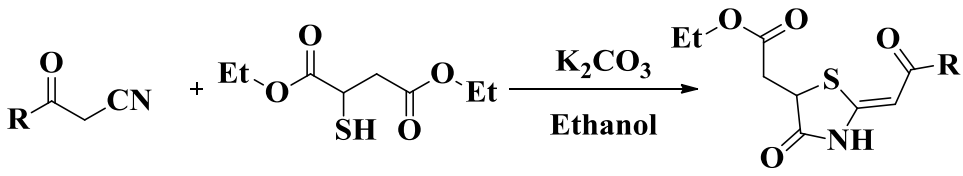
R = Ph, 4-CH_3-Ph, 4-F-Ph, 4-Cl-Ph

J. Blanchet and J.Zhu[46] developed a three component reaction for the synthesis of 2-imino-4-thiazolidinones (32) from readily available aliphatic /aromatic aldehydes (31), chloroform and thiourea. The base catalysed reaction generated the title products with reasonable yields.



31 32

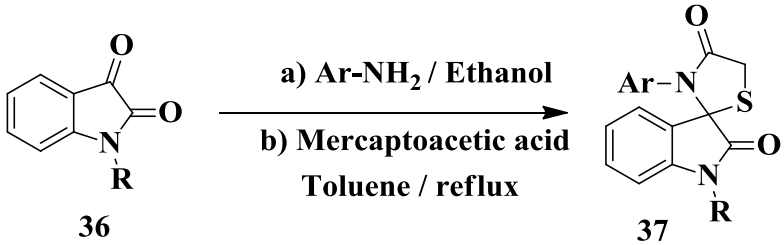
R. Markovic[47] et al. reported a regioselective hetero cyclization reaction method to give the 5-substituted-4-oxothiazolidine derivatives (35). The base catalysed reaction of β-oxo-nitriles (33) with diethyl mercaptosuccinate (34) in presence of ethanol under reflux condition afforded the target molecules with satisfactory yields.



33 34 35

R = Ph, NHPh, NHC_2H_4Ph

V. V. Vintonyak[48] described the synthesis of indolin-2-on-3-spirothiazolidinones (37) in step wise reaction method. Condensation reaction of 1-benzylindoline-2,3-dione (36) with various substituted anilines in presence of ethanol resulted in the formation of an intermediate isatin-3-imines. The intermediate underwent cyclization with mercaptoacetic acid under reflux condition to generate the target compounds with good yields.



36

R = H, Ph-CH_2-

37

CHAPTER-V

PART-A

A FACILE ONE POT MULTI COMPONENT SYNTHESIS OF ALKYL 4-OXO-COUMARINYL ETHYLIDENE HYDRAZONO-THIAZOLIDIN-5-YLIDENE CARBOXYLATES AND THEIR ANTIVIRAL AND CYTOTOXIC BIOLOGICAL ACTIVITIES

PART-A

PRESENT WORK:

STARTING MATERIALS:

To synthesize alkyl 4-oxo-coumarinyl ethylidene hydrazono-thiazolidin-5-ylidene acetate derivatives, various substituted 3-acetylcoumarins, thiosemicarbazide and dialkylacetylene dicarboxylates were used as starting materials. Substituted 3-acetylcoumarins were prepared as described in the preceding chapter, starting from substituted salicylaldehydes with ethylacetoacetate in presence of ethanol using piperidine as a base (Chapter-I, Ref.95 & 96). Thiosemicarbazide and dialkylacetylene dicarboxylates were procured from commercial sources and used further without any purification.

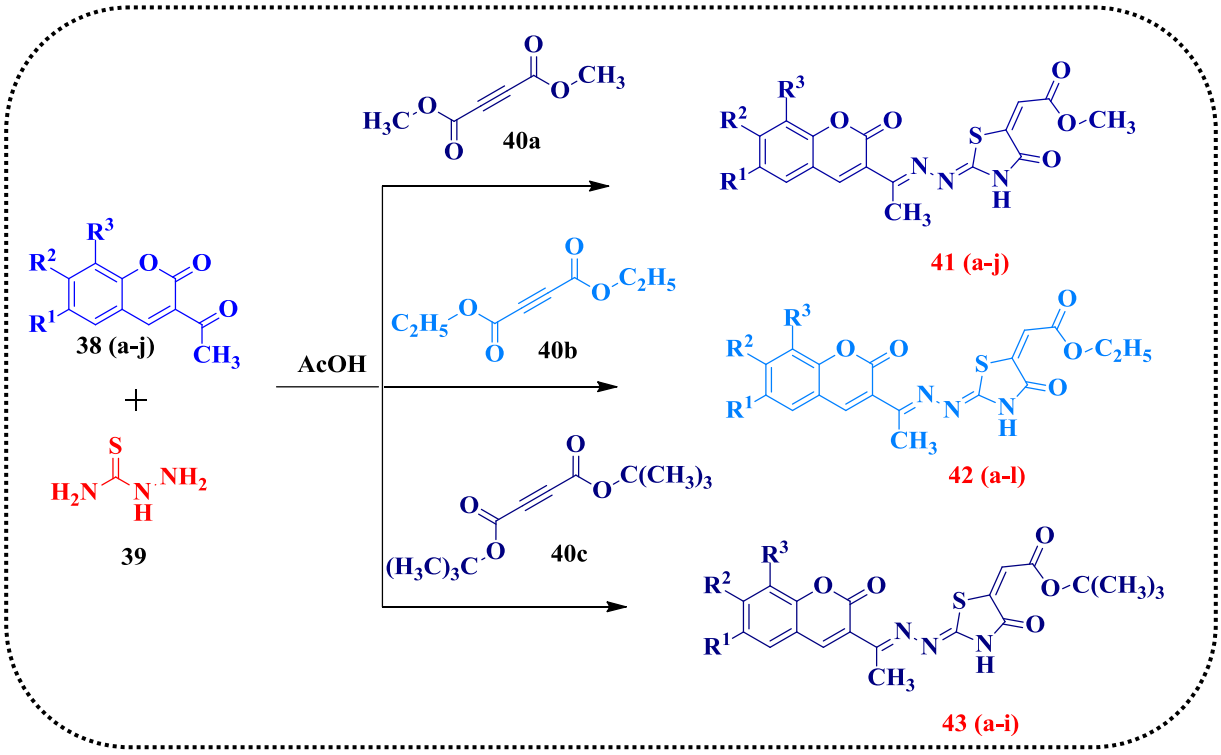
In view of the biological significance of the coumarins, 4-thiazolidinones, there is a huge demand to explore the synthesis and therapeutic applications of these heterocyclic compounds. Multicomponent reactions are useful in the synthetic chemistry to develop the efficient methods. So development of facile methods to synthesis of 4-thiazolidinones is crucial for the exploration of biological applications. Hence we have undertaken the present work to develop an efficient one pot multicomponent synthesis of alkyl 4-oxo-coumarinyl ethylidene hydrazono-thiazolidin-5-ylidene acetate derivatives and their antiviral activity evaluation.

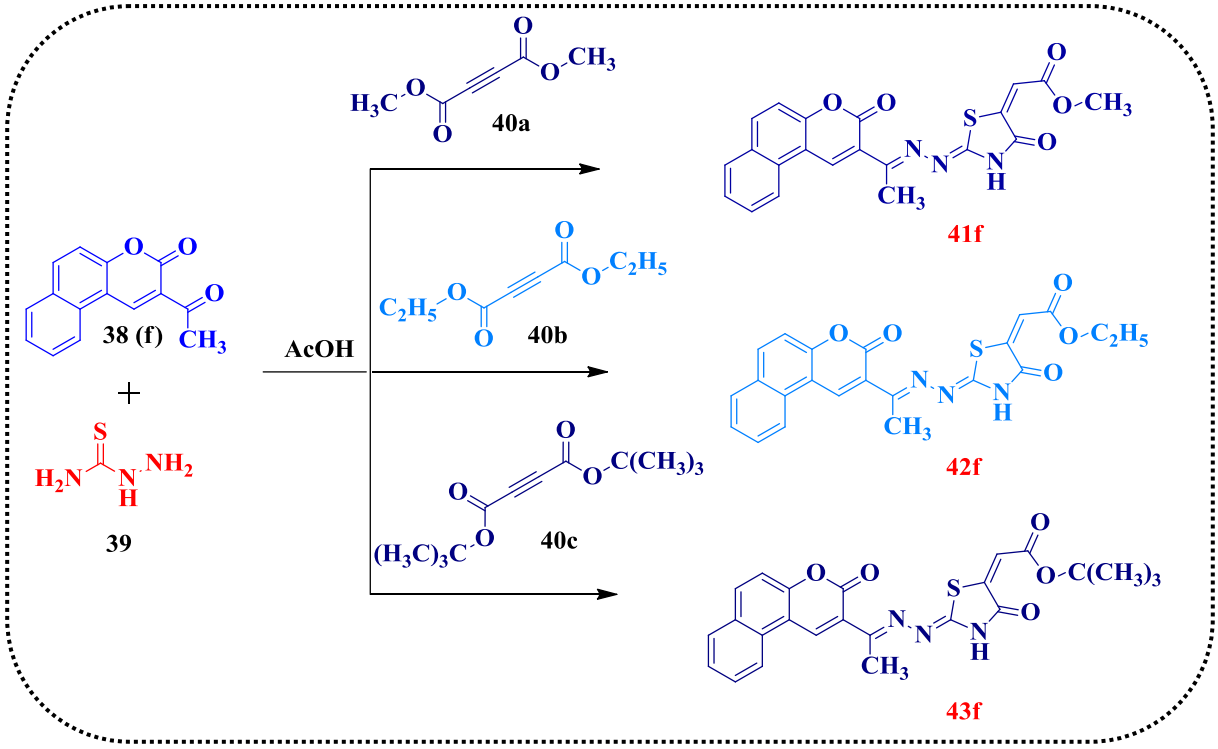
The title compounds were synthesized by the reaction of substituted 3-acetyl coumarins with thiosemicarbazide and dialkylacetylene dicarboxylates in presence of acetic acid through simple, efficient one pot multicomponent reaction approach with good yields (**Scheme-1**). The present scheme describes the formation of 4-thiazolidinone ring in a single reaction step.

Mechanism:

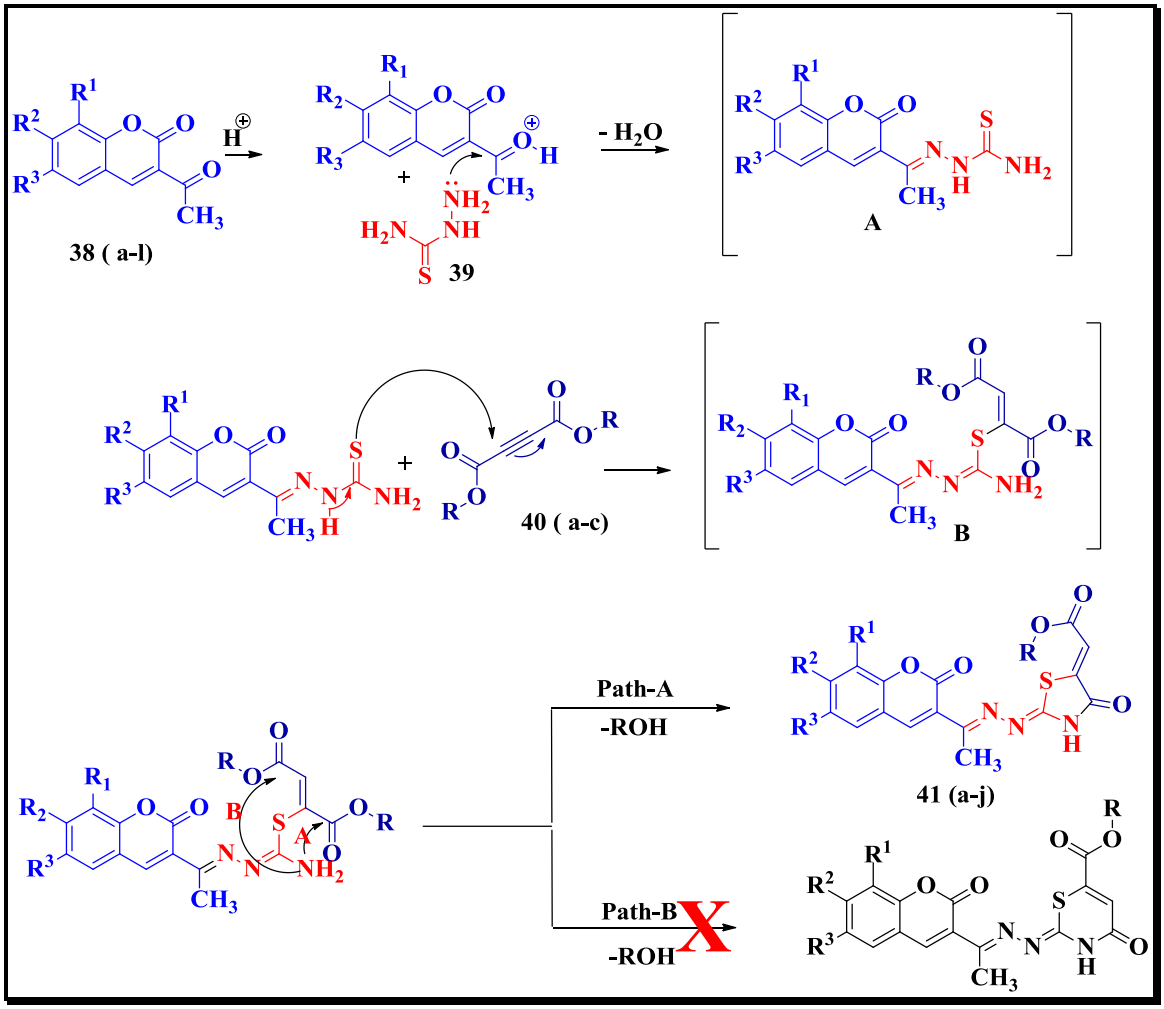
The mechanism for the formation of alkyl 4-oxo-coumarinyl ethylidene hydrazono-thiazolidin-5-ylidene acetate derivatives was proposed as shown in **Scheme-2**. In this mechanism the first step involves the nucleophilic attack of thiosemicarbazide with 3-acetyl coumarin to form the 2-(1-(2-oxo-2H-chromen-3-yl) ethylidene) hydrazinecarbothioamide intermediate. In the second step the intermediate undergoes

cyclization with dialkyacetylene dicarboxylate and forms the five membered 4-thiazolidinone heterocyclic ring.





Scheme: 1 An efficient one pot multicomponent synthesis of alkyl 4-oxo-coumarinyl ethylidene hydrazono-thiazolidin-5-ylidene acetate derivatives



Scheme-2: Plausible reaction mechanism for the formation of 4-thiazolidinone

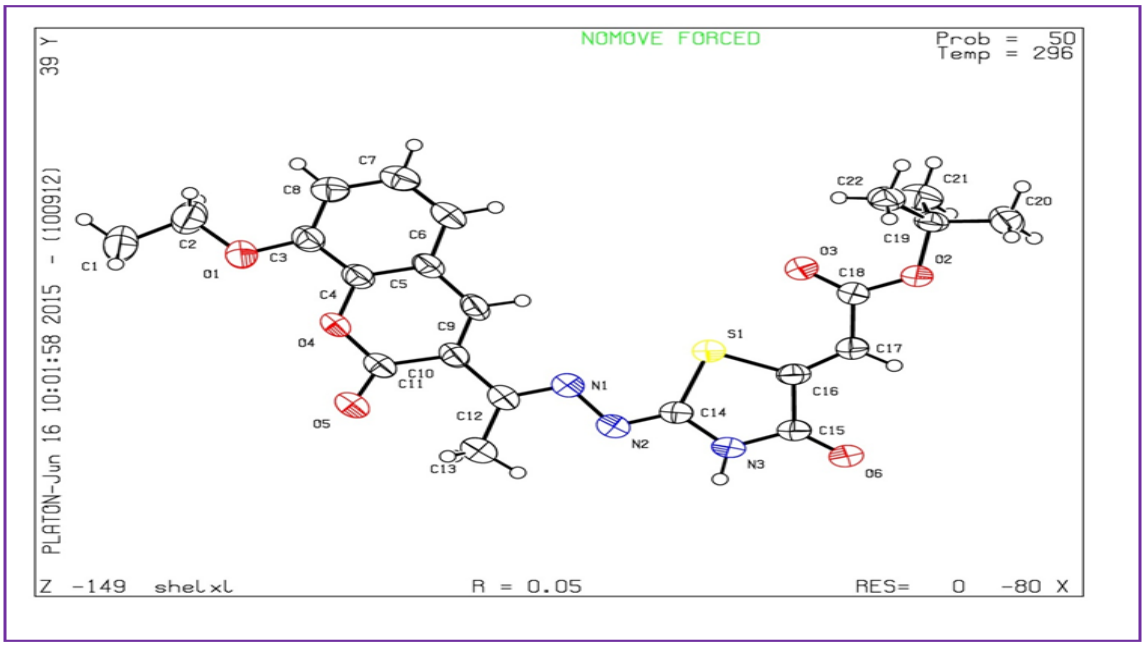


Fig.1: ORTEP diagram of compound **43i**

Table: 1 One pot multicomponent synthesis of 4-thiazolidinone derivatives

S.No.	Product	R	R^1	R^2	R^3	Time (h)	Yield (%) [b]
1	**41a**	$-CH_3$	H	H	H	1.5	85
2	**41b**	$-CH_3$	Cl	H	H	2.5	88
3	**41c**	$-CH_3$	Cl	H	Cl	2.5	87
4	**41d**	$-CH_3$	Br	H	H	3	92
5	**41e**	$-CH_3$	Br	H	Br	3	95
6	**41g**	$-CH_3$	H	H	$-OCH_3$	2.5	82
7	**41h**	$-CH_3$	Br	H	$-OCH_3$	2.5	90
8	**41i**	$-CH_3$	H	H	$-OCH_2CH_3$	2	83
9	**41j**	$-CH_3$	Br	H	$-OCH_2CH_3$	2.5	90
10	**42a**	$-C_2H_5$	H	H	H	2	82
11	**42b**	$-C_2H_5$	Cl	H	H	2.5	86
12	**42c**	$-C_2H_5$	Cl	H	Cl	2.5	89
13	**42d**	$-C_2H_5$	Br	H	H	2	88
14	**42e**	$-C_2H_5$	Br	H	Br	2.5	91
15	**42g**	$-C_2H_5$	H	H	$-OCH_3$	2	80
16	**42h**	$-C_2H_5$	Br	H	$-OCH_3$	2.5	85
17	**42i**	$-C_2H_5$	H	H	$-OCH_2CH_3$	2.5	82
18	**42j**	$-C_2H_5$	Br	H	$-OCH_2CH_3$	2.5	88
19	**42k**	$-C_2H_5$	H	-OH	H	1.5	82
20	**42l**	$-C_2H_5$	H	-OH	$-CH_3$	2	86
21	**43a**	$-C(CH_3)_3$	H	H	H	1.5	80
22	**43b**	$-C(CH_3)_3$	Cl	H	H	2	85
23	**43c**	$-C(CH_3)_3$	Cl	H	Cl	2	85
24	**43d**	$-C(CH_3)_3$	Br	H	H	2.5	89
25	**43e**	$-C(CH_3)_3$	Br	H	Br	2.5	92
26	**43g**	$-C(CH_3)_3$	H	H	$-OCH_3$	2	84
27	**43h**	$-C(CH_3)_3$	Br	H	$-OCH_3$	2	88
28	**43i**	$-C(CH_3)_3$	H	H	$-OCH_2CH_3$	2	81

[b] Isolated yields

All the synthesized compounds were characterized by physical and analytical spectral data. Compound **43i** was further confirmed by single crystal X-ray diffraction data. In the title compound (Fig. 1), the 2*H*-chromene ring system is essentially planar [r.m.s deviation = 0.003 Å] and are twisted with a little angle of 2.70(10)° to the 1,3-thiazolidine ring. The C8---C3---O1---C2, C3---O1---C2---C1, C9---C10---C12---N1, C10---C12---N1---N2, S1—C14---N2---N1, C14---N2---N1---C12, S1---C16---C17---C18 and C16---C17---C18---O2 torsion angles are 8.5(4), - 174.6(3), 5.1(4), -179.9(2), -0.4(4), -172.8(2), 0.9(4) and 166.7(2) ° respectively. Selected geometric parameters are given in **Table 2**.

In the crystal, there exist two intramolecular C—H···O hydrogen bonds. N—H···O hydrogen bonds link the molecules to each other, forming centrosymmetric $R^2_2(8)$ dimers (**Table 3, Fig. 2**). Furthermore, C---H…π and π-π interactions are not observed.

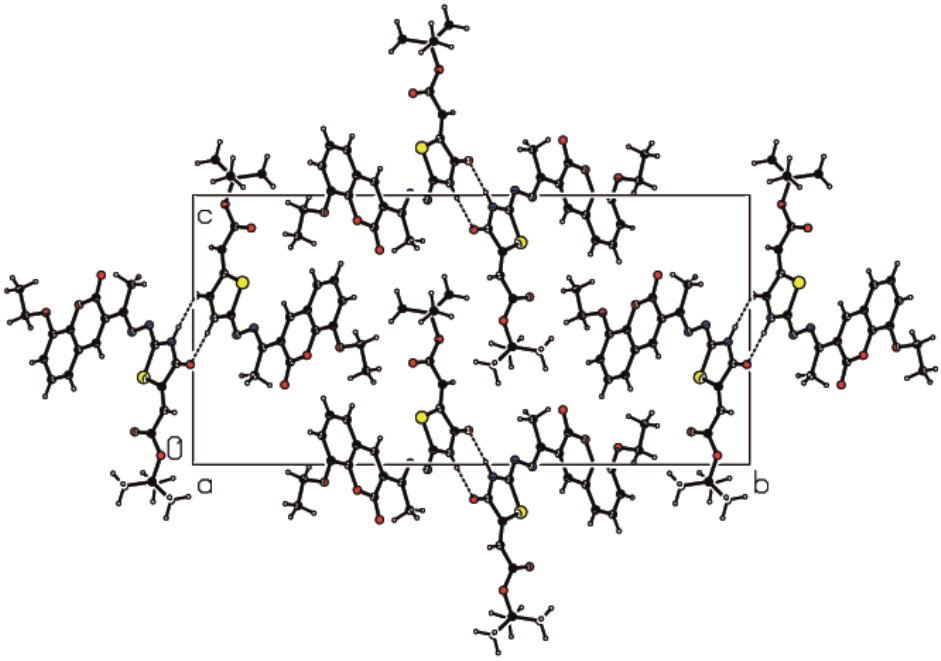


Fig.2 View of the dimers formed by the pairs of N-H---O hydrogen bonds with $R^2_2(8)$ motifs, down the a axis.

Table 2: Single crystal X-ray diffraction data of compound 43

Chemical formula	$C_{22}H_{23}N_3O_6S$
M_r	457.49
Crystal system, space group	Monoclinic, $P2_1/c$
Temperature (K)	296
a, *b*, *c* (Å)	5.6858 (3), 28.274 (2), 13.7996 (10)
β (°)	97.095 (3)
V (Å^3)	2201.5 (3)
Z	4
F(000)	960
D_x (Mg m^{-3})	1.380
Radiation type	Mo *Kα*
No. of reflections for cell measurement	3354
θ range (°) for cell measurement	5.2–45.5
μ (mm^{-1})	0.19

Crystal shape	Block
Colour	Yellow
Crystal size (mm)	0.60 × 0.30 × 0.20
Diffractometer	Bruker axs kappa apex2 CCD Diffractometer
Radiation source	fine-focus sealed tube
Monochromator	Graphite
Scan method	ω and ϕ scan
Absorption correction	Multi-scan *SADABS* (Bruker, 1999)
T_{min}, T_{max}	0.894, 0.963
No. of measured, independent and observed [$I > 2\sigma(I)$] reflections	17534, 5392, 3321
R_{int}	0.039
θ values (°)	θ_{max} = 28.4, θ_{min} = 1.4
$(\sin\theta/\lambda)_{max}$ (Å^{-1})	0.670
Range of *h*, *k*, *l*	*h* = -7→7, *k* = -35→37, *l* = -18→18
Refinement on	F^2
$R[F^2 > 2\sigma(F^2)]$, $wR(F^2)$, *S*	0.054, 0.177, 1.07
No. of reflections	5392
No. of parameters	294
No. of restraints	0
H-atom treatment	H-atom parameters constrained
Weighting scheme	$w = 1/[\sigma^2(F_o^2) + (0.091P)^2 + 0.0395P]$ where $P = (F_o^2 + 2F_c^2)/3$
$(\Delta/\sigma)_{max}$	< 0.001
$\Delta\rangle_{max}$, $\Delta\rangle_{min}$ (e Å^{-3})	0.40, -0.34

Table 3. Hydrogen Bonds (Å, °)

D—H···A	D—H	H···A	D···A	D—H···A
N3---H3N…O6	0.86	1.98	2.828(3)	170
C21-H21C…O3	0.96	2.55	3.104 (3)	117
C22---H22B...O3	0.96	2.36	2.908 (3)	115

The newly synthesized compounds were evaluated for antiviral activity against *Herpes simplex* virus-1 (KOS), *Herpes simplex* virus-2 (G), *Vaccinia* virus, *Vesicular stomatitis* virus, *Herpes simplex* virus-1 TK$^-$ KOS ACV^r and *Adeno* virus-2 in HEL cell culture medium using Brivudin, Cidofovir, Acyclovir, Ganciclovir, Zalcitabine and Alovudine as reference drugs. Compound **42i** exhibited good antiviral activity against *Herpes simplex* virus-1 (KOS), *Herpes simplex* virus-2 (G) and *Herpes simplex* virus-1 TK$^-$

KOS ACV^r at **11**, **10** and **10** µM concentrations (**Table 4**). The remaining compounds did not exhibit any activity against the tested viruses in HEL medium.

Similarly the compounds were evaluated for antiviral activity against *Vesicular stomatitis* virus, *Coxsackie* virus B4 and *Respiratory syncytial* virus in HeLa cell culture medium using Dextron sulphate (DS-10.000) and Ribavirin as standard drugs. Compound **43c**, compound **43b** and compound **42i** exhibited excellent activity against *Vesicular stomatitis* virus at EC_{50} of **9.5**, **20** and **9.5** µM. The compounds are more potent than the reference drug Ribavirin. Similarly compounds **43b** and **43c** also exhibited good activity against *Respiratory syncytial* virus at **11** and **10** µM concentrations (**Table 5**).

The compounds were screened against *Para-influenza*-3 virus, *Reo* virus-1, *Sindbis* virus, *Coxsackie* virus B4 and *Punta Toro* virus in Vero cell culture medium using Dextron sulphate (DS-10.000) and Ribavirin as reference drugs. Majority of the compounds exhibited excellent activity against *Punta Toro* virus when compared to reference drugs (**Table 6**).

The compounds were evaluated for their antiviral activity against *Influenza* A/H1N1 A/Ned/378/05, *Influenza* A/H3N2 A/HK/7/87 and *Influenza* B B/Ned/537/05 in MDCK cell culture medium by using Zanamivir, Ribavirin, Amantidine and Rimantadine as standard drugs. Compounds **41g**, **41h** and **42k** expressed good antiviral activity and the remaining compounds did not exhibit any antiviral activity (**Table 7**).

Newly synthesized compounds were tested for antiviral activity against *Feline Corona* Virus and *Feline Herpes* Virus in CRFK cell culture medium. The activity was compared with respect to HHA, UDA and Ganciclovir standard drugs. The tested compounds did not exhibited any activity (**Table 8**).

4-Thiazolidinone derivatives were further evaluated for their anti cancer activity against murine Leukemia cells (**L1210**), human-T-Lymphocyte cells (**CEM**) and human cervix carcinoma cell (**HeLa**). Compounds **43h** and **42l** shown excellent activity against L1210 cancer cell lines and the compounds **43e**, **43f**, **43g**, **43i**, **43b**, **42k, 42l** and **41i** exhibited good anticancer activity against the tested cancer cells (**Table 9**).

Table 4.Cytotoxicity and antiviral activity of compounds in HEL cell cultures.

Compound	Minimum cytotoxic concentration[a] (µM)	EC_{50}[b] (µM)					
		Herpes simplex virus-1 (KOS)	*Herpes simplex* virus-2 (G)	*Vaccinia* virus	*Vesicular stomatitis* virus	*Herpes simplex* virus-1 TK^- KOS ACV^r	*Adeno* virus-2
41a	100	>20	>20	>20	>20	>20	>20
41b	≥100	>100	>100	>100	>100	>100	>100
41c	100	>20	>20	>20	>20	>20	>20
41d	100	>20	>20	>20	>20	>20	>20
41e	100	>20	>20	>20	>20	>20	>20
41f	100	>20	>20	>20	>20	>20	>20
41g	100	>20	>20	>20	>20	>20	>20
41h	100	>20	>20	>20	>20	>20	>20
41i	≥20	>20	>20	>20	>20	>20	>20
41j	20	>4	>4	>4	>4	>4	>4
42a	100	>20	>20	>20	>20	>20	>20
42b	100	>20	>20	>20	>20	>20	>20
42c	100	>20	>20	>20	>20	>20	>20
42d	100	>20	>20	>20	>20	>20	>20
42e	20	>4	>4	>4	>4	>4	>4
42f	100	>20	>20	>20	>20	>20	>20
42g	100	>20	>20	>20	>20	>20	>20
42h	100	>20	>20	>20	>20	>20	>20
42i	100	**11**	**10**	>20	>20	**10**	>20
42j	100	>20	>20	>20	>20	>20	>20
42k	100	>20	>20	>20	>20	>20	>20
42l	≥20	>20	>20	>20	>20	>20	>20
43a	>100	>100	>100	>100	>100	>100	>100
43b	20	>4	>4	>4	>4	>4	>4
43c	20	>4	>4	>4	>4	>4	>4
43d	>100	>100	>100	>100	>100	>100	>100
43e	20	>4	>4	>4	>4	>4	>4
43f	≥4	>4	>4	>4	>4	>4	>4
43g	20	>4	>4	>4	>4	>4	>4
43h	≥4	>4	>4	>4	>4	>4	>4
43i	20	>4	>4	>4	>4	>4	>4
Brivudin	>250	0.05	181	20	>250	150	-
Cidofovir	>250	1.6	1.0	22	>250	1.6	7.9
Acyclovir	>250	0.4	0.2	>250	>250	98	-
Ganciclovir	>100	0.06	0.06	>100	>100	2.9	-
Zalcitabine	>250	-	-	-	-	-	7.5
Alovudine	>250	-	-	-	-	-	16

[a] Required to cause a microscopically detectable alteration of normal cell morphology.
[b] Required to reduce virus-induced cytopathogenicity by 50 %.

Table 5. Cytotoxicity and antiviral activity of compounds in HeLa cell cultures.

Compound	Minimum cytotoxic concentration[a] (µM)	EC_{50}[b] (µM)		
		Vesicular stomatitis virus	*Coxsackie* virus B4	*Respiratory syncytial* virus
41a	100	>20	>20	>20
41b	100	>20	>20	>20
41c	100	>20	>20	>20
41d	100	>20	>20	>20
41e	100	>20	>20	>20
41f	100	>20	>20	>20
41g	100	>20	>20	>20
41h	100	>20	>20	>20
41i	100	>20	>20	>20
41j	100	>20	>20	>20
42a	100	>20	>20	>20
42b	>100	>100	>100	>100
42c	100	>20	>20	>20
42d	≥100	>100	>100	>100
42e	>100	>100	>100	>100
42f	≥100	>100	>100	>100
42g	100	>20	>20	>20
42h	100	>20	>20	>20
42i	100	**9.5**	>20	>20
42j	100	>20	>20	>20
42k	>100	>100	>100	>100
42l	>100	>100	>100	>100
43a	100	>20	>20	>20
43b	100	**20**	>20	**11**
43c	100	**9.5**	>20	**10**
43d	>100	>100	>100	>100
43e	20	>4	>4	>4
43f	100	>20	>20	>20
43g	20	>4	>4	>4
43h	20	>4	>4	>4
43i	20	>4	>4	>4
DS-10000 (µg/ml)	>100	1.8	27	3.0
Ribavirin	>250	22	81	7.5

[a] Required to cause a microscopically detectable alteration of normal cell morphology.
[b] Required to reduce virus-induced cytopathogenicity by 50 %.

Table: 6 Cytotoxicity and antiviral activity of compounds in Vero cell cultures.

Compound	Minimum cytotoxic concentration[a] (µM)	EC_{50}[b] (µM)				
		*Para-influenza-*3 virus	*Reo* virus-1	*Sindbis* virus	*Coxsackie* virus B4	*Punta Toro virus*
41a	100	>20	>20	>20	>20	>20
41b	≥100	**33**	>100	**39**	**39**	**9.5**
41c	≥100	**45**	>100	**46**	>100	**8.9**
41d	100	>20	>20	>20	>20	**9.5**
41e	100	**10**	>20	>20	>20	>20
41f	100	>20	>20	>20	>20	>20
41g	≥100	>100	>100	>100	>100	>100
41h	100	>20	>20	>20	>20	**3.2**
41i	≥20	>20	>20	>20	>20	>20
41j	100	>20	>20	>20	>20	**3.0**
42a	100	>20	>20	>20	>20	>20
42b	100	>20	>20	>20	>20	**8.9**
42c	100	>20	>20	>20	>20	**8.4**
42d	100	>20	>20	>20	>20	**7.0**
42e	100	>20	>20	>20	>20	>20
42f	100	>20	>20	>20	>20	>20
42g	100	>20	>20	>20	**7.0**	**1.9**
42h	≥20	>20	>20	>20	>20	**1.9**
42i	100	**10**	>20	>20	**7.0**	**8.3**
42j	≥20	>20	>20	>20	>20	**6.2**
42k	≥20	>20	>20	>20	>20	>20
42l	≥20	>20	>20	>20	>20	>20
43a	≥20	>20	>20	>20	**8.9**	>20
43b	≥20	>20	>20	>20	>20	>20
43c	≥20	>20	>20	>20	>20	>20
43d	>100	>100	>100	>100	>100	>100
43e	20	>4	>4	>4	>4	>4
43f	≥20	>20	>20	>20	>20	>20
43g	≥20	>20	>20	>20	>20	**8.9**
43h	20	>4	>4	>4	>4	>4
43i	≥20	>20	**9.5**	>20	>20	>20
DS-10000 (µg/ml)	>100	>100	>100	50	8.9	20
Ribavirin	>250	126	>250	250	>250	112

[a] Required to cause a microscopically detectable alteration of normal cell morphology.
[b] Required to reduce virus-induced cytopathogenicity by 50 %.

Table: 7 Cytotoxicity and antiviral activity of compounds in MDCK cell cultures.

Compound	Cytotoxicity (µM)		Antiviral EC_{50}[c] (µM)					
	Minimum cytotoxic concentration[a]	CC_{50}[b]	*Influenza* A/H1N1 A/Ned/378/05		*Influenza* A/H3N2 A/HK/7/87		*Influenza* B B/Ned/537/05	
			CPE	MTS	CPE	MTS	CPE	MTS
41a	100	>100	>20	>20	>20	>20	>20	>20
41b	20	8.6	>4	>4	>4	>4	>4	>4
41c	20	9.5	>4	>4	>4	>4	>4	>4
41d	≥100	>100	>100	>100	>100	>100	>100	>100
41e	20	37.7	>4	>4	>4	>4	>4	>4
41f	100	>100	>20	>20	>20	>20	>20	>20
41g	100	>100	**11.0**	**7.4**	**6.5**	**4.1**	**27.0**	**13.8**
41h	20	72.6	>4	>4	>4	>4	>4	>4
41i	≥20	39.0	**10.5**	**6.3**	**7.9**	**4.2**	>20	>20
41j	≥20	38.0	>20	>20	>20	>20	>20	>20
42a	≥20	40.8	>20	>20	>20	>20	>20	>20
42b	100	>100	>20	>20	>20	>20	>20	>20
42c	≥20	>100	>20	>20	>20	>20	>20	>20
42d	100	57.2	>20	>20	>20	>20	>20	>20
42e	20	12.4	>4	>4	>4	>4	>4	>4
42f	20	38.8	>4	>4	>4	>4	>4	>4
42g	20	43.7	>4	>4	>4	>4	>4	>4
42h	20	48.4	>4	>4	>4	>4	>4	>4
42i	20	10.1	>4	>4	>4	>4	>4	>4
42j	≥4	27.2	>4	>4	>4	>4	>4	>4
42k	≥100	>100	**20.9**	**9.1**	**34**	**8.7**	**29.5**	**24.9**
42l	100	>100	>20	>20	>20	>20	>20	>20
43a	≥4	44.7	>4	>4	>4	>4	>4	>4
43b	4	2.1	>0.8	>0.8	>0.8	>0.8	>0.8	>0.8
43c	4	8.5	>0.8	>0.8	>0.8	>0.8	>0.8	>0.8
43d	>100	>100	>100	>100	>100	>100	>100	>100
43e	0.8	0.6	>0.16	>0.16	>0.16	>0.16	>0.16	>0.16
43f	0.8	1.8	>0.16	>0.16	>0.16	>0.16	>0.16	>0.16
43g	≥4	9.0	>4	>4	>4	>4	>4	>4
43h	0.8	1.9	>0.16	>0.16	>0.16	>0.16	>0.16	>0.16
43i	20	9.4	>4	>4	>4	>4	>4	>4
Zanamivir	>100	>100	0.2	0.3	0.5	0.6	0.4	0.4
Ribavirin	≥100	>100	8.9	8.2	8.9	5.3	6.8	8.4
Amantadine	>200	>200	8.0	19.1	0.8	1.6	>200	>200
Rimantadine	>200	>200	200	>200	0.3	0.1	>200	>200

[a] Minimum compound concentration that causes a microscopically detectable alteration of normal cell morphology.
[b] 50% Cytotoxic concentration, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay.

[c] 50% Effective concentration, or concentration producing 50% inhibition of virus-induced cytopathic effect, as determined by visual scoring of the CPE, or by measuring the cell viability with the colorimetric formazan-based MTS assay.

Table 8 Anti-Feline Corona Virus (FIPV) and anti-Feline Herpes Virus activity and cytotoxicity in CRFK cell cultures**:**

Compound	CC_{50}[a] (µM)	EC_{50}[b] (µM)	
		Feline Corona Virus (FIPV)	Feline Herpes Virus
41a	62.5	>20	>20
41b	>100	>100	>100
41c	>100	>100	>100
41d	47.0	>20	>20
41e	73.4	>20	>20
41f	>100	>100	>100
41g	>100	>100	>100
41h	55.4	>20	>20
41i	>100	>100	>100
41j	46.7	>20	>20
42a	50.0	>20	>20
42b	>100	>100	>100
42c	>100	>100	>100
42d	>100	>100	>100
42e	>100	>100	>100
42f	>100	>100	>100
42g	85.0	>20	>20
42h	>100	>100	>100
42i	52.2	>20	>20
42j	48.8	>20	>20
42k	>100	>100	>100
42l	>100	>100	>100
43a	50.0	>20	>20
43b	20.3	>20	>20
43c	37.6	>20	>20
43d	>100	>100	>100
43e	9.5	>4	>4
43f	23.1	>20	>20
43g	13.7	>4	>4
43h	9.7	>4	>4
43i	39.1	>20	>20
HHA (µg/ml)	>100	24.3	15.1
UDA (µg/ml)	>100	7.3	4.3
Ganciclovir	>100	>100	8.6

[a] 50% Cytotoxic concentration, as determined by measuring the cell viability with the colorimetric formazan- based MTS assay.
[b] 50% Effective concentration, or concentration producing 50% inhibition of virus-induced cytopathic effect, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay
CRFK cells: Crandell-Rees Feline Kidney cells.

Table 9: Anticancer activity of 4-thiazolidinone derivatives

Compound	IC_{50} (µM)		
	L1210	**CEM**	**HeLa**
41a	54 ± 45	83 ± 9	94 ± 9
41b	28 ± 6	112 ± 2	116 ± 1
41c	66 ± 51	98 ± 3	90 ± 26
41d	100 ± 7	105 ± 4	97 ± 38
41e	94 ± 26	95 ± 4	33 ± 16
41f	111 ± 1	122 ± 9	60 ± 18
41g	30 ± 1	136 ± 18	86 ± 2
41h	85 ± 10	109 ± 15	77 ± 1
41i	**20 ± 0**	38 ± 10	79 ± 10
41j	57 ± 30	88 ± 22	88 ± 10
42a	71 ± 0	67 ± 7	78 ± 15
42b	118 ± 9	128 ± 4	100 ± 25
42c	97 ± 3	99 ± 7	72 ± 32
42d	104 ± 9	111 ± 6	83 ± 20
42e	94 ± 11	107 ± 6	92 ± 50
42f	122 ± 1	123 ± 3	112 ± 37
42g	21 ± 4	55 ± 5	68 ± 0
42h	81 ± 27	108 ± 2	89 ± 15
42i	28 ± 4	72 ± 22	62 ± 20
42j	43 ± 0	75 ± 5	76 ± 20
42k	**19 ± 1**	≥ 250	136 ± 47
42l	**9.9 ± 0.1**	**16 ± 8**	46 ± 29
43a	94 ± 7	78 ± 14	118 ± 50
43b	**23 ± 1**	**21 ± 2**	58 ± 8
43c	31 ± 3	36 ± 7	55 ± 10
43d	NT	NT	NT
43e	**16 ± 1**	**19 ± 2**	26 ± 11
43f	**18 ± 5**	**23 ± 1**	**20 ± 5**
43g	**22 ± 4**	31 ± 20	**23 ± 6**
43h	**5.6 ± 0.6**	**15 ± 2**	**20 ± 3**
43i	**17 ± 2**	**17 ± 5**	22 ± 3

In summary we have developed an efficient method for the synthesis of alkyl 4-oxo-coumarinyl ethylidene hydrazono-thiazolidin-5-ylidene acetate derivatives via a one pot multicomponent reaction approach with reasonable good yields. All the synthesized compounds were evaluated for antiviral and anticancer activity. The compounds were found to be excellent antiviral compounds.

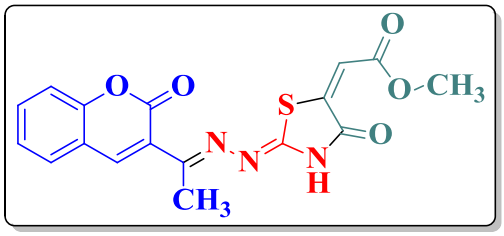
EXPERIMENTAL:

An efficient one pot multicomponent synthesis of alkyl 4-oxo-coumarinyl ethylidene hydrazono-thiazolidin-5-ylidene acetate derivatives:

A mixture of substituted 3-acetyl-2*H*-chromen-2-ones **38a-j** (1.0 mmol) and thiosemicarbazide **39** (0.091 g, 1.0 mmol) was taken in a round bottom flask having acetic acid (5 mL). The reaction mixture was stirred at room temperature for about ten minutes. To this reaction mixture dialkylacetylene dicarboxylate **40a-c** (1.0 mmol) were added slowly. The temperature of the reaction was raised to 60 -65 °C and maintained for about 2-3 h. The progress of the reaction was monitored through TLC using hexane and ethyl acetate in 2:1 volume ratio. After completion of the reaction, the temperature of the reaction was cooled to room temperature. The solid separated was filtered, washed with cold methanol and dried. The product was recrystallized from methanol.

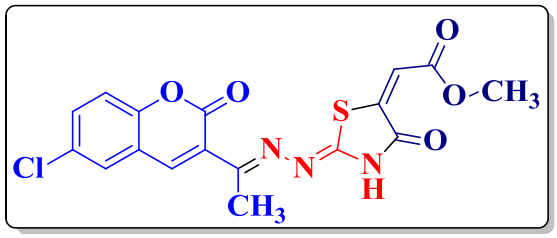
SPECTRAL DATA

Methyl 2-(-4-oxo-2-(-(1-(2-oxo-2*H*-chromen-3-yl) ethylidene) hydrazono) thiazolidin-5-ylidene)acetate (41a):



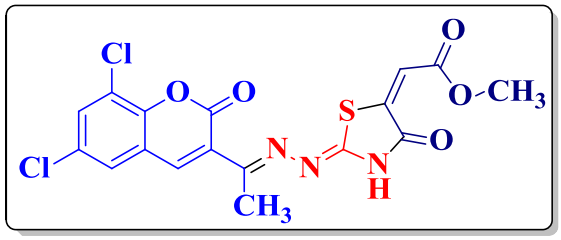
Yellow solid; m.p.: 247 – 249 ^{o}C; **FTIR (KBr, υ_{max} cm^{-1}):** 3431(-NH), 1723(lactone -C=O), 1643 (ester –C=O);1517 (-C=N); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 2.38 (s, 3H, -CH_3); 3.76 (s, 3H, -OCH_3); 6.68 (s, 1H, -C=H); 7.40 (t, *J* = 7.6 Hz, 1H, Ar-H); 7.43 – 7.47 (m, 1H, Ar-H); 7.66 – 7.71 (m, 1H, Ar-H); 7.91 – 7.93 (m, 1H, Ar-H); 8.27(s, 1H, C_4-H of coumarin); 12.98 (s, 1H, -NH); **^{13}C NMR (100 MHz, DMSO-d_6, ppm):** δ 22.49; 57.67; 119.75; 121.24; 123.86; 130.02; 131.35; 134.75; 138.05; 147.23; 147.35; 148.01; 158.85; 164.06; 165.68; 167.41; 170.93; **HRMS (ESI) m/z** calculated for $C_{17}H_{13}N_3NaO_5S$ $[M + Na]^+$: 394.0474, found. 394.0472; *Anal*. Calcd for $C_{17}H_{13}N_3O_5S$: C, 54.98; H, 3.53; N, 11.31; S, 8.63%; Found: C, 54.92; H, 3.58; N, 11.26; S, 8.56%.

Methyl 2-(-2-(-(1-(6-chloro-2-oxo-2*H*-chromen-3-yl)ethylidene) hydrazono)-4-oxo thiazolidin-5-ylidene)acetate (41b):



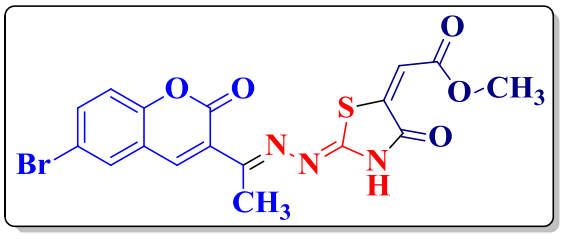
Yellow solid; m.p.: 267 – 269 °C; **FTIR (KBr, υ_{max} cm^{-1}):** 3152 (-NH), 1722 (lactone – C=O), 1623 (ester – C=O), 1505 (-C=N); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 2.36 (s, 1H, -CH_3); 3.79 (s, 1H, -OCH_3); 6.67 (s, 1H, -C=H); 7.50 (d, *J* = 8.8 Hz, 1H, Ar-H) 7.70 – 7.72 (m, 1H, Ar-H); 8.08 (d, *J* = 2.4 Hz, 1H, Ar-H); 8.23 (s, 1H, C_4-H of coumarin); 12.98 (s, 1H, -NH); **HRMS (ESI) m/z** calculated for $C_{17}H_{12}ClN_3NaO_5S$ $[M + Na]^+$: 428.0084, found: 428.0083; *Anal*. Calcd for $C_{17}H_{12}ClN_3O_5S$: C, 50.31; H, 2.98; N, 10.35; S, 7.90%; Found: C, 5037; H, 2.93; N, 10.31; S, 7.84%.

Methyl 2-(-2-(-(1-(6,8-dichloro-2-oxo-2*H*-chromen-3-yl)ethylidene) hydrazono)-4-oxothiazolidin-5-ylidene)acetate (41c):



Yellow solid; m.p.: 265 – 267 °C; 3152 (-NH), **FTIR (KBr, ν_{max}, cm^{-1}):** 1725 (lactone –C=O), 1614 (ester –C=O); **^{1}H NMR(400MHz, DMSO-d_6, δ ppm):** 2.36 (s, 3H, -CH_3); 3.76 (s, 3H, -OCH_3), 6.66 (s, 1H, -C=H); 7.70 (t, *J* = 6.4 Hz, 1H, Ar-H); 8.08 (s, 1H, Ar-H); 8.23 (s, 1H, C_4-H of coumarin); 12.99 (s, 1H, -NH); MS (ESI m/z): 441 $[M+ H]^+$; *Anal.* Calcd for $C_{17}H_{11}Cl_2N_3O_5S$: C, 46.38; H, 2.52; N, 9.54; S, 7.28%; Found: C, 46.32; H, 2.58; N, 9.59; S, 7.22%.

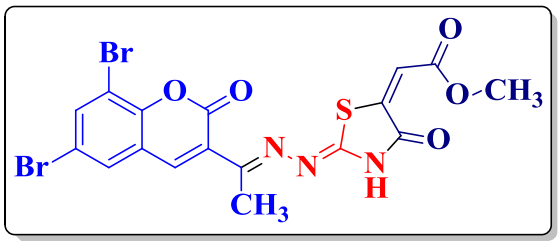
Methyl 2-(-2-(-(1-(6-bromo-2-oxo-2*H*-chromen-3-yl)ethylidene) hydrazono)-4-oxo thiazo lidin-5-ylidene)acetate (41d):



Yellow solid; m.p.: 258 - 260 °C; **FTIR (KBr, ν_{max}, cm^{-1}):** 3132 (-NH), 1708 (lactone-C=O),1708 (ester –C=O), 1602 (-C=N); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 2.35 (s, 3H, -CH_3); 3.77 (s, 3H, -OCH_3); 6.93 (s, 1H, -C=H); 7.42 – 7.45 (m, 1H, Ar-H); 7.81 – 7.84 (m, 1H, Ar-H); 8.16 (d, *J* = 2.4Hz, 1H, Ar-H); 8.23 (s, 1H, C_4-H of coumarin); 12.97 (s, 1H, -NH); **^{13}C NMR (100 MHz, DMSO-d_6, ppm):** δ 17.11; 52.43; 116.30; 118.31; 120.58; 122.51; 131.32; 135.00; 140.68; 152.63; 171.97; **HRMS (ESI) m/z** calculated for $C_{17}H_{12}BrN_3NaO_5S$

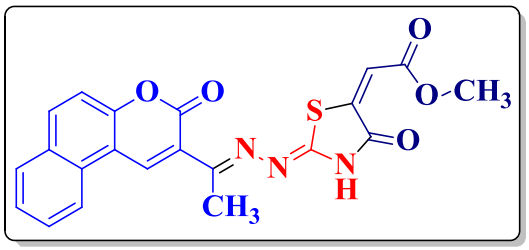
$[M + Na]^+$: 471.9579, found: 471.9575; *Anal*. Calcd for $C_{17}H_{12}BrN_3O_5S$: C, 45.35; H, 2.69; N, 9.33; S, 7.12%; Found:, C, 45.39; H, 2.74; N, 9.38; S, 7.16%.

Methyl 2-(-2-(-(1-(6,8-dibromo-2-oxo-2*H*-chromen-3-yl) ethylidene) hydrazono)-4-oxo thiazolidin-5-ylidene)acetate (41e):



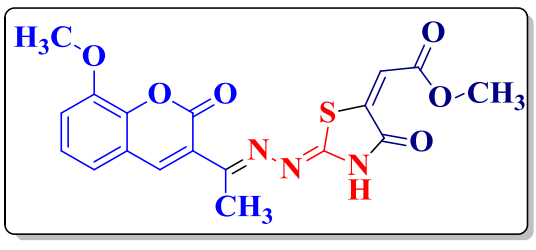
Yellow solid; m.p.: 246 - 248 °C; **FTIR (KBr, ν_{max}, cm^{-1}):** 3150 (-NH), 1728 (lactone -C=O), 1614 (-C=N); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 2.36 (s, 3H, $-CH_3$); 3.77 (s, 3H, $-OCH_3$); 6.68 (s, 1H, -C=H); 7.45 (s, 1H, Ar-H); 7.83 (s, 1H, Ar-H); 8.23 (s, 1H, C_4-H of coumarin); 12.99 (s, 1H, -NH); **MS (ESI m/z):** 530 $[M+H]^+$; *Anal*. Calcd for $C_{17}H_{11}Br_2N_3O_5S$: C, 38.59; H, 2.10; N, 7.94; S, 6.06%; Found: C, 38.63; H, 2.15; N, 7.90; S, 6.01%.

Methyl 2-(-4-oxo-2-(-(1-(3-oxo-3*H*-benzo[*f*]chromen-2-yl) ethylidene) hydrazono) thiazo lidin-5-ylidene)acetate (41f):



Yellow solid; m.p.: 312 - 314 °C; **FTIR (KBr, ν_{max}, cm^{-1}):** 3138 (-NH), 1717 (lactone –C=O), 1617 (amide –C=O), 1570 (-C=N); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 2.31(s, 3H,$-CH_3$); 3.71 (s, 3H, $-OCH_3$); 7.65 – 7.67 (m, 2H, Ar-H); 7.77 (t, *J* = 7.6 Hz, 2H, Ar-H); 8.08 (d, *J* = 8.8 Hz, 1H, Ar-H); 8.23 (d, *J* = 9.2 Hz, 1H, Ar-H); 8.43 (s, 1H, Ar-H); 9.11 (s, 1H, C_4-H of coumarin); 10.43 (s, 1H, -NH); **MS (ESI m/z):** 422 $[M+H]^+$; *Anal*. Calcd. for $C_{21}H_{15}N_3O_5S$: C, 59.85; H, 3.59; N, 9.97; S, 7.61%; Found: C, 59.80; H, 3.64; N, 9.91; S, 7.65%.

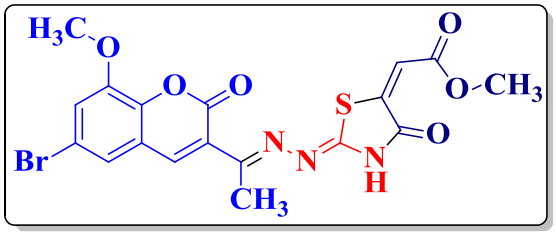
Methyl 2-(-2-(-(1-(8-methoxy-2-oxo-2*H*-chromen-3-yl)ethylidene) hydrazono)-4-oxo thiazolidin-5-ylidene)acetate (41g):



Yellow solid; m.p.: 260 – 262 °C; **FTIR (KBr, ν_{max}, cm^{-1}):** 3155 (-NH), 1705 (lactone –C=O), 1611 (amide –C=O), 1523 (-C=N); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 2.37 (s, 3H, $-CH_3$); 3.76 (s, 3H, $-COOCH_3$); 3.93 (s, 3H, Ar-OCH_3); 6.68 (s, 1H,

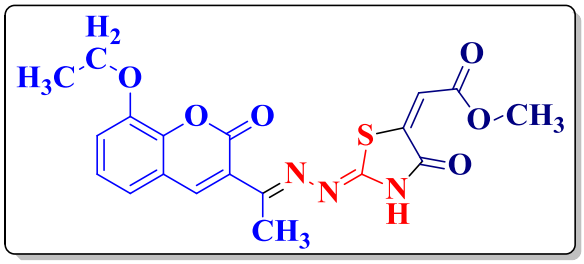
=CH); 7.31 – 7.38 (m, 2H, Ar-H); 7.44 – 7.46 (m, 1H, Ar-H); 8.24 (s, 1H,C_4-H of coumarin); 12.97 (s, 1H, -NH); **^{13}C NMR (100 MHz, DMSO-d_6, ppm):** δ 17.13; 52.41; 56.14; 114.50; 114.98; 119.17; 120.43; 122.43; 124.75; 126.11; 142.18; 142.89; 146.28; 158.48; 165.79; **HRMS (ESI) m/z** calculated for $C_{18}H_{15}N_3O_6S$ $[M + H]^+$: 402.0760, found: 402.0763; *Anal*. Calcd for $C_{18}H_{15}N_3O_6S$: C, 53.86; H, 3.77; N, 10.47; S, 7.99%; Found: C, 53.81; H, 3.72; N, 10.52; S, 7.93%.

Methyl 2-(-2-(-(1-(6-bromo-8-methoxy-2-oxo-2*H*-chromen-3-yl)ethylidene) hydrazono)-4-oxothiazolidin-5-ylidene)acetate (41h):



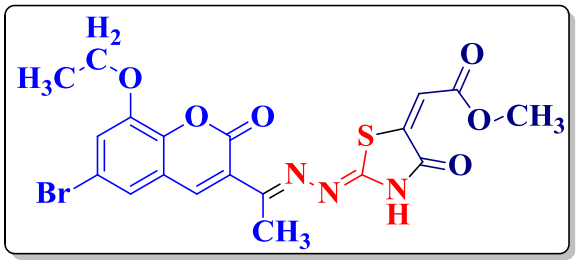
Yellow solid; m.p.: 267 -269 °C; **FTIR (KBr, ν_{max}, cm^{-1}):** 3420 (-NH), 1717 (lactone –C=O), 1634 (ester –C=O), 1587 (-C=N); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 2.36 (s, 3H, -CH_3); 3.77 (s, 3H, - $COOCH_3$); 3.96 (s, 3H, Ar-OCH_3); 6.68 (s, 1H, -C=H); 7.52 (d, *J* = 2Hz, 1H, Ar-H); 7.74 (d, *J* = 2 Hz, 1H, Ar-H); 8.20 (s, 1H, C_4-H of coumarin); 12.98 (s, 1H, -NH); HRMS (ESI) m/z calculated for $C_{18}H_{15}BrN_3O_6S$ $[M+H]^+$: 479.9865, found: 479.9870; *Anal*. Calcd for $C_{18}H_{14}BrN_3O_6S$: C, 45.01; H, 2.94; N, 8.75; S, 6.68%; Found: C, 45.05; H, 2.89; N, 8.79; S, 6.63%.

Methyl 2-(-2-(-(1-(8-ethoxy-2-oxo-2*H*-chromen-3-yl)ethylidene) hydrazono)-4-oxo thiazolidin-5-ylidene)acetate (41i):



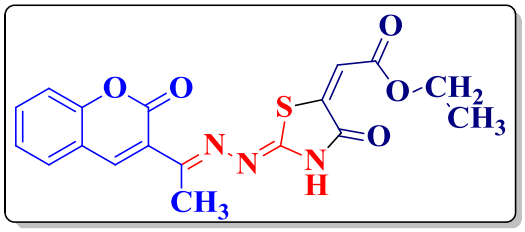
Yellow solid; m.p.: 255 – 257 °C; **FTIR (KBr, ν_{max}, cm^{-1}):** 3150 (-NH), 1711 (lactone -C=O), 1605 (amide –C=O); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 1.42 (t, *J* = 6.8 Hz, 3H, -CH_2CH_3); 2.37 (s, 3H, CH_3); 3.76 (s, 3H, -OCH_3); 4.17 – 4.23 (m, 2H, CH_2); 6.68 (s, 1H, =CH); 7.29 – 7.37 (m, 2H, Ar-H); 7.43 – 7.45 (m, 1H, Ar-H); 8.23 (s, 1H, C_4-H of coumarin); 12.97 (s, 1H, -NH); **MS (ESI m/z):** 416 $[M+H]^+$; *Anal*. Calcd for $C_{19}H_{17}N_3O_6S$: C, 54.93; H, 4.12; N, 10.12; S, 7.72%; Found: C, 54.98; H, 4.08; N, 10.09; S, 7.75%.

Methyl 2-(-2-(-(1-(6-bromo-8-ethoxy-2-oxo-2*H*-chromen-3-yl)ethylidene) hydrazono)-4-oxothiazolidin-5-ylidene)acetate (41j):



Yellow solid; m.p.: 257 – 259 °C; **FTIR (KBr, ν_{max}, cm^{-1}):** 3141 (-NH), 1740 (lactone -C=O), 1702 (ester -C=O), 1605 (-C=N); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 1.40 (t, *J* = 6.8 Hz, 3H, -CH_2CH_3); 2.36 (s, 3H, -CH_3); 3.77 (s, 3H, -OCH_3); 4.20 – 4.25 (m, 2H, -OCH_2); 6.68 (s, 1H, =CH); 7.50 (d, *J* = 2 Hz, 1H, Ar-H); 7.73 (d, *J* = 2 Hz, 1H, Ar-H); 8.19 (s, 1H, C_4-H of coumarin); 12.98 (s, 1H, -NH); **^{13}C NMR (100 MHz, DMSO-d_6, ppm):** δ 14.41; 17.17; 52.40; 65.07; 114.56; 116.16; 118.25; 120.53; 122.37; 127.25; 140.97; 142.37; 142.65; 146.42; 161.89; 165.76; **MS (ESI m/z):** 496 $[M+2H]^+$; Anal.Calcd for $C_{19}H_{16}BrN_3O_6S$: C, 46.17; H, 3.26; N, 8.50; S, 6.49%; Found: C, 46.11; H, 3.30; N, 8.48; S, 6.54%.

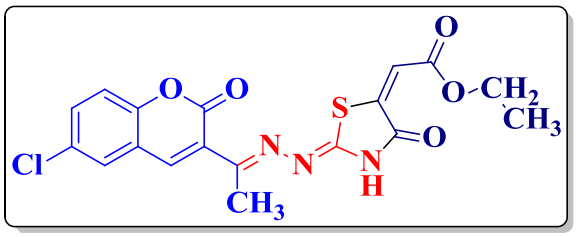
Ethyl 2-(-4-oxo-2-(-(1-(2-oxo-2*H*-chromen-3-yl) ethylidene) hydrazono) thiazolidin-5-ylidene)acetate (42a):



Yellow solid; m.p.: 236 - 238 °C; **FTIR (KBr, ν_{max}, cm^{-1}):** 3247 (-NH); 1738 (lactone –C=O); 1713 (ester –C=O); 1688 (amide –C=O); 1614 (-C=N); 1233 (C-O-C); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 1.25 (t, *J* = 6.8 Hz, 3H, -CH_2-CH_3); 2.38(s, 3H, -CH_3); 4.20 – 4.25 (m, 2H, - CH_2); 6.65 (s, 1H, =CH); 7.39 – 7.48 (m, 2H, Ar-H); 7.69 (t, *J* = 7.2 Hz, 1H, Ar-H)7.92 (d, *J* = 6.8 Hz, 1H, Ar-H); 8.27 (s, 1H, C_4-H of coumarin); 12.96 (s, 1H, NH); **^{13}C NMR (100 MHz, DMSO-d_6, ppm):** δ 13.96, 17.19, 61.26, 114.64, 115.81, 116.01, 118.51, 123.97, 124.79, 126.10, 129.44, 131.72, 132.79, 141.10, 141.99, 153.58, 158.82, 171.92; **HRMS (ESI) m/z** calculated for $C_{18}H_{15}N_3NaO_5S$ $[M + Na]^+$: 408.0630, found: 408.0637; *Anal.* Calcd for $C_{18}H_{15}N_3O_5S$: C, 56.10; H, 3.92; N, 10.90; S, 8.32%; Found: C, 56.15; H, 3.94; N, 10.85; S, 8.29%.

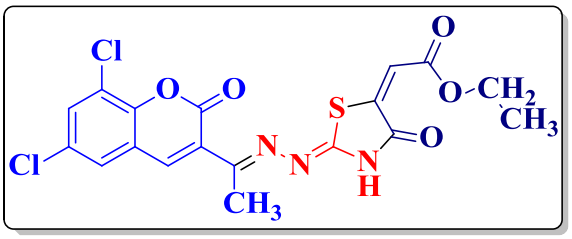
Ethyl 2-(-2-(-(1-(6-chloro-2-oxo-2*H*-chromen-3-yl)ethylidene)hydrazono)-4-oxo thiazo lidin-5-ylidene)acetate (42b):

Yellow solid; m.p.: 256 - 258 °C; **FTIR (KBr, ν_{max}, cm^{-1}):** 3245 (-NH); 1715 (lactone-C=O); 1630 (ester –C=O); 1603 (amide –C=O); 1572 (-C=N); 1286 (-C-O-C); **^{1}H NMR (400 MHz, DMSO-d_6,δ ppm):** 1.26 (t, *J* = 6.8 Hz, 3H, -CH_2-CH_3); 2.33 (s, 3H, -CH_3);



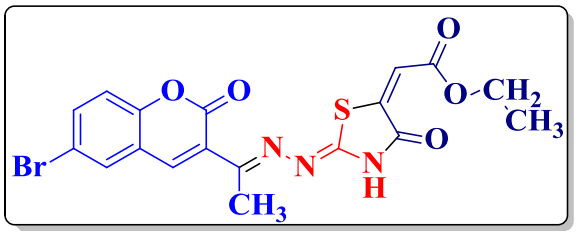
4.22 – 4.27 (m, 2H, -CH_2); 6.62 (s, 1H, =CH); 7.51 (d, *J*= 8.8 Hz, 1H, Ar-H); 7.70 – 7.73 (m, 1H, Ar-H); 7.86 (d, *J* = 2.8 Hz, 1H, Ar-H); 8.24 (s, 1H, C_4-H of coumarin); 12.64 (s, 1H, -NH); **^{13}C NMR (100 MHz, DMSO-*d*$_6$, ppm):** δ 14.01; 23.04; 61.33; 114.73;118.26; 119.62; 127.01; 127.77; 128.60; 132.08; 140.36; 142.49; 151.72; 156.74; 159.91; 165.37; **MS (ESI m/z):** 420 $[M]^+$; *Anal.* Calcd. for $C_{18}H_{14}ClN_3O_5S$: C, 51.49; H, 3.36; N, 10.01; S, 7.64%; Found: C, 51.54; H, 3.33; N, 9.96; S, 7.69%.

Ethyl 2-(-2-(-(1-(6,8-dichloro-2-oxo-2*H*-chromen-3-yl)ethylidene) hydrazono)-4-oxo thiazolidin-5-ylidene)acetate (42c):



Yellow solid; m.p.: 254 - 256°C; **FTIR (KBr, ν_{max}, cm^{-1}):** 3251 (-NH); 1741 (lactone -C=O); 1697 (ester –C=O); 1607 (amide -C=O); 1567 (-C=N); 1240 (-C-O-C); **^{1}H NMR (400 MHz, DMSO-*d*$_6$,δ ppm):** 1.25 (t, *J* = 6.4 Hz, 3H, -CH_2CH_3); 2.36 (s, 3H, -CH_3); 4.20 - 4.24 (m, 2H, -CH_2); 6.65 (s, 1H, =CH); 7.86 (d, *J* = 2.4Hz, 1H, Ar-H); 8.06 (t, *J* = 2.8 Hz, 1H, Ar-H); 8.23 (s, 1H, -C_4-H of coumarin); 12.69 (s, 1H, NH); **^{13}C NMR (100 MHz, DMSO-*d*$_6$, ppm):** δ 13.98; 23.01; 61.28; 114.67; 118.23; 119.59; 126.97; 127.74; 128.30; 128.57; 132.04; 140.33; 142.47; 151.69; 156.71; 165.33; MS (ESI m/z): 454 $[M]^+$; 456$[M+2H]^+$; *Anal.* Calcd for $C_{18}H_{13}Cl_2N_3O_5S$: C, 47.59; H, 2.88; N, 9.25; S, 7.06%; Found: C, 47.56; H, 2.92; N, 9.22; S, 7.10%.

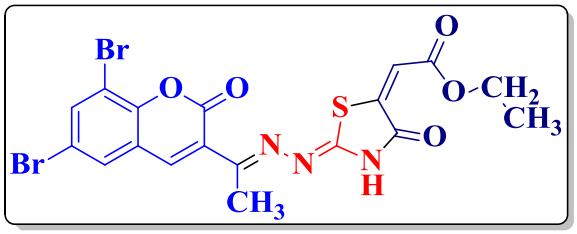
Ethyl 2-(-2-(-(1-(6-bromo-2-oxo-2*H*-chromen-3-yl)ethylidene)hydrazono)-4-oxo thiazol idin-5-ylidene)acetate (42d):



Yellow solid; m.p.: 258 - 260 °C; **FTIR (KBr, ν_{max}, cm^{-1}):** 3165 (-NH); 1728 (lactone-C=O); 1697 (ester –C=O); 1635 (amide –C=O); 1614 (-C=N); 1246 (C-O-C); **^{1}H NMR (400 MHz, DMSO-*d*$_6$,δ ppm):** 1.26 (t, *J* = 6.8 Hz, 3H, -CH_2CH_3); 2.36 (s, 3H, CH_3); 4.18 – 4.27 (m, 2H,- CH_2); 6.63 (s, 1H, =CH); 7.44 (d, *J*= 8.8 Hz, 1H, Ar-H); 7.82 (d, *J* = 9.2 Hz, 1H, Ar-H); 8.00

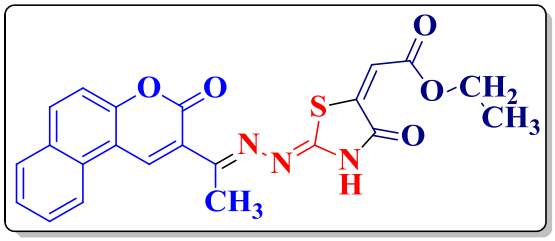
(t, *J* = 2 Hz, 1H, Ar-H); 8.21 (s, 1H, C_4-H of coumarin); 12.96 (s, 1H,-NH); **MS (ESI m/z):** 464 $[M+H]^+$; *Anal.* Calcd for $C_{18}H_{14}BrN_3O_5S$: C, 46.56; H, 3.04; N, 9.05; S, 6.91%; Found: C, 46.50; H, 3.10; N, 9.12; S, 6.85%.

Ethyl 2-(-2-(-(1-(6,8-dibromo-2-oxo-2*H*-chromen-3-yl)ethylidene) hydrazono)-4-oxo thiazolidin-5-ylidene)acetate (42e):



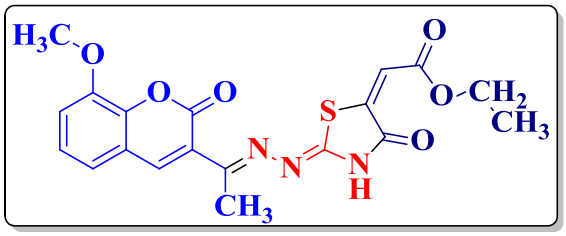
Yellow solid; m.p.: 246 - 248 °C; **FTIR (KBr, ν_{max}, cm^{-1}):** 3249 (-NH); 1741 (lactone-C=O); 1697 (ester –C=O); 1634 (amide –C=O); 1614 (-C=N); 1241 (-C-O-C); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 1.24 (t, *J* = 4.4 Hz, 3H, -CH_3); 2.36 (s, 3H, -CH_3); 4.18 – 4.23 (m, 2H, -CH_2); 6.92 (s, 1H,=CH); 7.44 (d, *J* = 8.8 Hz, 1H, Ar-H); 7.81- 7.84 (m, 1H, Ar-H); 8.58 (s, 1H, C_4-H of coumarin); 12.99 (s, 1H, -NH); MS (ESI m/z): 544 $[M + H]^+$; *Anal.* Calcd for $C_{18}H_{13}Br_2N_3O_5S$: C, 39.80; H, 2.41; N, 7.74; S, 5.90%; Found: C, 39.74; H, 2.46; N, 7.70; S, 5.95%.

Ethyl 2-(-4-oxo-2-(-(1-(3-oxo-3*H*-benzo[*f*]chromen-2-yl)ethylidene) hydrazono) thiazo lidin-5-ylidene)acetate (42f):



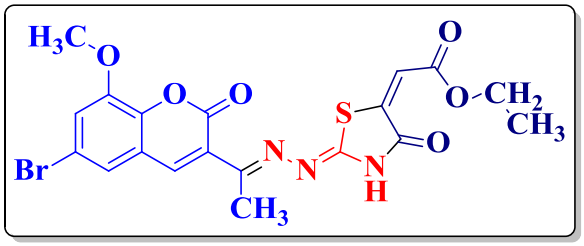
Yellow solid; m.p.: 278 - 280 °C; **FTIR (KBr, ν_{max}, cm^{-1}):** 3154 (-NH); 1711 (lactone-C=O); 1632 (ester –C=O); 1607 (-amide –C=O); 1565 (-C=N); 1238 (-C-O-C); **^{1}H NMR (400 MHz, DMSO-d_6,δ ppm):** 1.24 (t, *J* = 6.8 Hz, 3H, -CH_2CH_3); 2.45 (s, 3H, -CH_3); 4.18 – 4.23 (m, 2H, -CH_2); 6.61(s, 1H, =CH); 7.63 – 7.68 (m, 2H, Ar-H); 7.79 (t, *J* = 8 Hz, 1H, Ar-H); 8.10 (d, *J* = 8 Hz, 1H, Ar-H); 8.27 (d, *J* = 8.8 Hz, 1H, Ar-H); 8.55 (d, *J* = 8 Hz, 1H, Ar-H); 9.00 (s, 1H, C_4-H of coumarin); 12.97 (s, 1H, -NH); **MS (ESI m/z):** 436 $[M+H]^+$; *Anal.* Calcd for $C_{22}H_{17}N_3O_5S$: C, 60.68; H, 3.93; N, 9.65; S, 7.36%; Found: C, 60.62; H, 3.97; N, 9.61; S, 7.40%.

Ethyl 2-(-2-(-(1-(8-methoxy-2-oxo-2*H*-chromen-3-yl)ethylidene) hydrazono)-4-oxo thiazolidin-5-ylidene)acetate (42g):



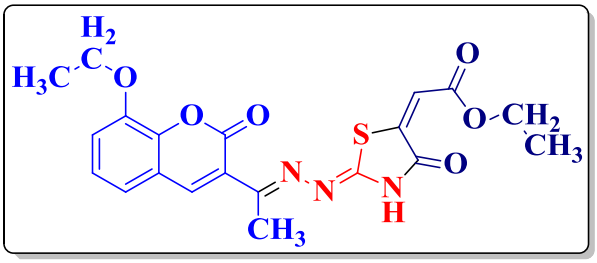
Yellow solid; m.p.: 252 -254 °C; **FTIR (KBr, ν_{max}, cm^{-1}):** 3436 (-NH); 1708 (lactone-C=O); 1643 (ester –C=O); 1614 (amide –C=O); 1578 (-C=N); 1235 (-C-O-C); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 1.24 (t, *J* = 6.8 Hz, 3H, -CH_2CH_3); 2.37(s, 3H, -CH_3); 3.93 (s, 3H, -OCH_3); 4.20 – 4.26 (m, 2H, -CH_2); 6.65 (s, 1H, =CH); 7.26 – 7.28 (m, 1H, Ar-H); 7.33 – 7.36 (m, 1H, Ar-H); 7.44 – 7.46 (m, 1H, Ar-H); 8.24 (s, 1H, -C_4-H of coumarin); 12.95 (s, 1H, -NH); **^{13}C NMR (100 MHz, DMSO-d_6, δ ppm):** 13.98; 17.19; 56.16; 61.30; 114.68; 115.03; 118.82; 119.18; 119.87; 120.54; 124.76; 124.85; 126.02; 126.21; 142.23; 142.36; 146.29; 158.52; 165.35; **MS (ESI m/z):** 416 $[M + H]^+$; *Anal.* Calcd for $C_{19}H_{17}N_3O_6S$: C, 54.93; H, 4.12; N, 10.12; S, 7.72%; Found: C, 54.91; H, 4.16; N, 10.08; S, 7.75%.

Ethyl 2-(-2-(-(1-(6-bromo-8-methoxy-2-oxo-2*H*-chromen-3-yl)ethylidene) hydrazono)-4-oxothiazolidin-5-ylidene)acetate (42h):



Yellow solid; m.p.: 264 - 266 °C; **FTIR (KBr, ν_{max}, cm^{-1}):** 3081 (-NH); 1724 (lactone-C=O); 1632 (ester –C=O); 1618 (amide –C=O); 1600 (-C=N); 1235 (-C-O-C); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 1.25 (t, *J* = 6.8 Hz, 3H, -CH_3); 2.36 (s, 3H, -CH_3); 3.96 (s, 3H, -OCH_3); 4.20 – 4.25 (m, 2H, -CH_2); 6.65 (s, 1H, =CH); 7.52 (d, *J* = 2.4 Hz, 1H, Ar-H); 7.74 (d, *J* =2 Hz, 1H, Ar-H); 8.20 (s, 1H, C_4-H of coumarin); 12.97 (s, 1H, -NH); **MS (ESI m/z):** 496 $[M+2H]^+$; *Anal.* Calcd for $C_{19}H_{16}BrN_3O_6S$: C, 46.17; H, 3.26; N, 8.50; S, 6.49%; Found: C, 46.13; H, 3.23; N, 8.55, S, 6.51%.

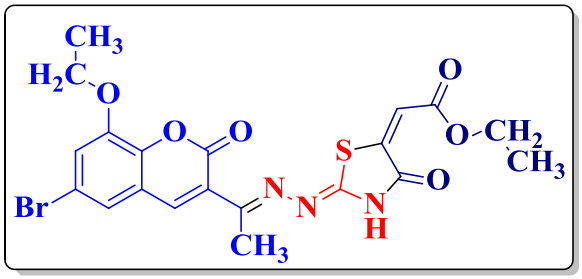
Ethyl 2-(-2-(-(1-(8-ethoxy-2-oxo-2*H*-chromen-3-yl)ethylidene)hydrazono)-4-oxo thiazo lidin-5-ylidene)acetate (42i):



Yellow solid; m.p.: 244 - 246 °C; **FTIR (KBr, ν_{max}, cm^{-1}):** 3426 (-NH); 1735 (lactone-C=O); 1708 (ester –C=O); 1696 (amide –C=O); 1607 (-C=N); 1240 (-C-O-C); **^{1}H NMR (400**

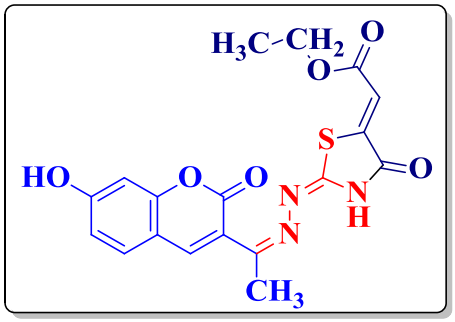
MHz, DMSO-d_6, δ ppm): 1.25 (t, *J* = 7.2 Hz, 3H, -CH_2-CH_3); 1.42 (t, *J* = 6.8 Hz, 3H, -CH_2CH_3); 2.37 (s, 3H, -CH_3); 4.17 – 4.25 (m, 4H, -CH_2), 6.65 (s, 1H, =CH); 7.29 – 7.35 (m, 2H, Ar-H); 7.37 – 7.45 (m, 1H, Ar-H); 8.23 (s, 1H, C4-H of coumarin); 12.96 (s, 1H, -NH); **^{13}C NMR (100 MHz, DMSO-d_6, δ ppm):** 13.98; 14.57; 17.20; 61.30; 64.48; 114.73; 115.93; 119.28; 120.51; 124.77; 126.17; 142.25; 142.72; 143.05; 145.52; 158.61; 165.36; **MS (ESI m/z):** 452 $[M + Na]^+$; *Anal.* Calcd for $C_{20}H_{19}N_3O_6S$: C, 55.94; H, 4.46; N, 9.78; S, 7.47%; Found: C, 55.90; H, 4.49; N, 9.75; S, 7.51%.

Ethyl 2-(-2-(-(1-(6-bromo-8-ethoxy-2-oxo-2*H*-chromen-3-yl)ethylidene) hydrazono)-4-oxothiazolidin-5-ylidene)acetate (42j):



Yellow solid; m.p.: 236 - 238°C; **FTIR (KBr, ν_{max}, cm^{-1}):** 3251 (-NH); 1726 (lactone –C=O); 1709 (ester-C=O); 1613 (amide –C=O); 1567 (-C=N); 1237 (-C-O-C); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 1.25 (t, *J* = 7.2 Hz, 3H, -CH_2-CH_3); 1.40 (t, *J* = 6.8 Hz, 3H, -CH_2-CH_3); 2.36 (s, 3H, -CH_3); 4.00 – 4.03 (m, 2H, -O-CH_2); 4.21 – 4.25 (m, 2H, -OCH_2); 6.69 (s, 1H, -C=H); 7.50 (d, *J* = 2Hz, 1H, Ar-H); 7.73 (d, *J* = 2 Hz, 1H, Ar-H); 8.19 (s, 1H, C_4-H of coumarin); 12.97 (s, 1H, -NH); **MS (ESI m/z):** 510 $[M+2H]^+$; *Anal.* Calcd for $C_{20}H_{18}BrN_3O_6S$: C, 47.25; H, 3.57; N, 8.27; S, 6.31%; Found: C, 47.21; H, 3.62; N, 8.31; S, 6.28%.

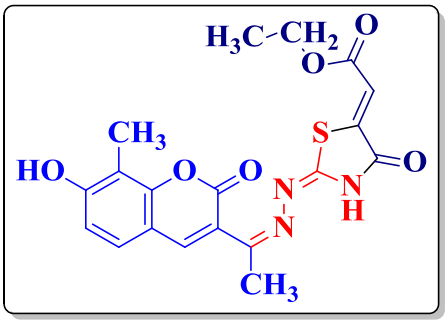
Ethyl 2-(-2-(-(1-(7-hydroxy-2-oxo-2*H*-chromen-3-yl)ethylidene) hydrazono)-4-oxo thiazolidin-5-ylidene)acetate (42k):



Yellow solid; m.p.: 260 – 262 °C; **FTIR (KBr, ν_{max}, cm^{-1}):** 3212 (-OH); 3162 (-NH); 1728 (lactone-C=O); 1693 (ester -C=O); 1609 (amide –C=O); 1216 (C-O-C); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 1.25 (t, *J* = 7.2 Hz, 3H, -CH_2CH_3); 2.36 (s, 3H, -CH_3); 4.20 – 4.25 (m, 2H, -CH_2); 6.64 (s, 1H, =CH); 6.76 (d, *J* = 2.4 Hz, 1H, -Ar-H); 6.82 – 6.85 (m, 1H, Ar-H); 7.73 (d, *J* = 8.8Hz, 1H, Ar-H); 8.18 (s, 1H, C_4-H of coumarin); 10.83 (s, 1H, -OH); 12.91 (s, 1H, -NH); **^{13}C NMR (100 MHz, DMSO-d_6,**

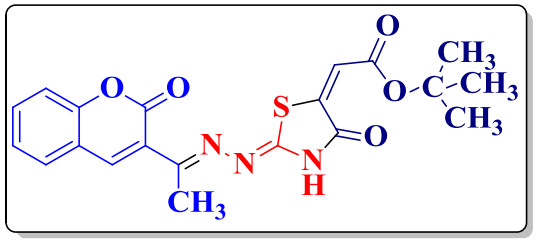
ppm): 14.44; 17.68; 61.79; 110.99; 111.57; 113.13; 115.08; 120.96; 128.25; 143.19; 143.47; 154.18; 159.87; 160.18; 160.68; 162.83; 165.84; 166.15; **MS (ESI m/z):** 402 $[M+H]^+$; *Anal*. Calcd for $C_{18}H_{15}N_3O_6S$: C, 53.86; H, 3.77; N, 10.47; S, 7.99%; Found: C, 53.83; H, 3.81; N, 10.50; S, 7.93%.

Ethyl 2-(-2-(-(1-(7-hydroxy-8-methyl-2-oxo-2*H*-chromen-3-yl) ethylidene) hydrazono)-4-oxothiazolidin-5-ylidene)acetate (42l):



Yellow solid; m.p.: 273 – 275°C; **FTIR (KBr, ν_{max}, cm^{-1}):** 3315 (-OH); 3175 (-NH); 1730 (lactone –C=O); 1678 (ester –C=O); 1633 (amide – C=O)1605 (-C=N); 1230 (-C-O-C); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 1.25 (t, *J* = 6.8 Hz, 3H, -CH_2-CH_3); 2.18 (s, 3H, Ar-CH_3); 2.37 (s, 3H, -CH_3); 4.20 – 4.25 (m, 2H, -CH_2); 6.64 (s, 1H, =CH); 6.89 (d, *J* = 8.4 Hz, 1H, Ar-H); 7.57 (d, *J* = 8.4Hz, 1H, Ar-H); 8.16 (s, 1H, C_4-H of coumarin); 10.75 (s, 1H, -OH); 12.92 (s, 1H, -NH); **^{13}C NMR (100 MHz, DMSO-d_6, ppm):** δ 7.80; 13.97; 17.22; 61.26; 110.46; 111.09; 112.63; 114.51; 120.52; 127.78; 142.80; 142.96; 153.72; 159.35; 159.76; 160.19; 162.32; 165.35; 165.66; MS (ESI m/z): 416 $[M+ H]^+$; *Anal*. Calcd for $C_{19}H_{17}N_3O_6S$: C, 54.93; H, 4.12; N, 10.12; S, 7.72%; Found: C, 54.96; H, 4.16; N, 10.08; S, 7.71%.

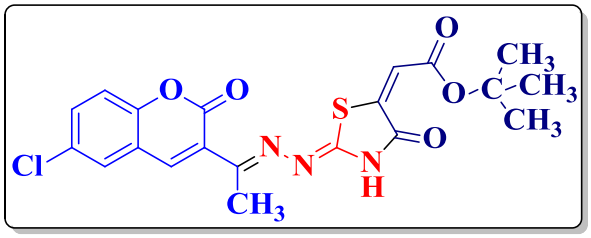
***tert*-Butyl 2-(-4-oxo-2-(-(1-(2-oxo-2*H*-chromen-3-yl) ethylidene) hydrazono) thiazolidin-5-ylidene)acetate (43a):**



Yellow solid; m.p.: 240 - 242 °C; **FTIR (KBr, ν_{max}, cm^{-1}):** 3122 (-NH); 1721 (lactone –C=O); 1685 (ester –C=O); 1637 (amide –C=O); 1602 (-C=N); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 1.47 (s, 9H, *tert* butyl); 2.38 (s, 3H, -CH_3); 6.54 (s, 1H, =CH); 7.39 – 7.43 (m, 1H, Ar-H); 7.47 (d, *J* = 8.4 Hz, 1H, Ar-H); 7.66 – 7.71 (m, 1H, Ar-H); 7.90 – 7.93 (m, 1H, Ar-H); 8.28 (s, 1H, C_4-H of coumarin); 12.91 (s, 1H, -NH); **^{13}C NMR (100 MHz, DMSO-d_6, δ ppm):** 17.13; 27.59; 82.12; 116.01; 116.49; 118.61; 124.79; 126.05; 129.46; 132.80; 141.66; 142.00; 153.58; 158.78; 160.72; 161.86; 164.56; 165.70; **HRMS (ESI) m/z**

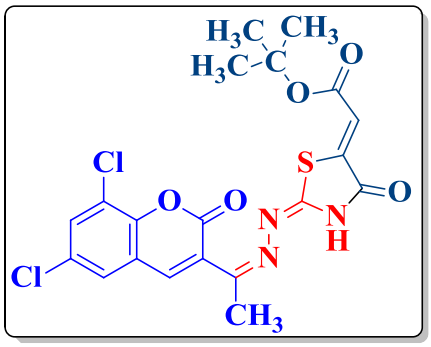
calculated for $C_{20}H_{19}N_3NaO_5S[M + Na]^+$: 436.0943, found: 436.0942; *Anal*. Calcd for $C_{20}H_{19}N_3O_5S$: C, 58.10; H, 4.63; N, 10.16; S, 7.76%; Found: C, 58.16; H, 4.60; N, 10.13; S, 7.78%.

tert-Butyl 2-(-2-(-(1-(6-chloro-2-oxo-2*H*-chromen-3-yl)ethylidene) hydrazono)-4-oxo thiazolidin-5-ylidene)acetate (43b):



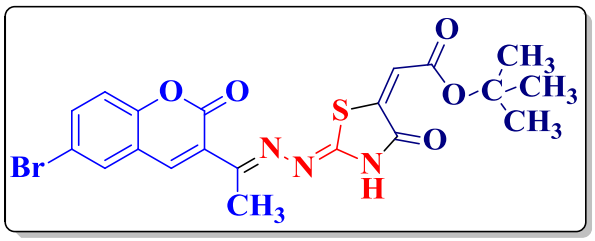
Yellow solid; m.p.: 272 - 274 °C; **FTIR (KBr, ν_{max}, cm^{-1}):** 3232 (-NH), 1728 (lactone -C=O), 1605 (-amide –C=O); **^{1}H NMR (400 MHz, DMSO-d_6, ppm):** 1.47 (s, 9H, *tert* butyl); 2.36 (s, 3H, -CH_3); 6.55 (s, 1H, =CH); 7.50 (d, *J* = 8.8 Hz, 1H, Ar- H); 7.70 – 7.72 (m, 1H, Ar-H); 8.07 (d, *J* = 2.4 Hz, 1H, Ar-H); 8.24 (s, 1H, C_4-H of coumarin); 12.92 (s, 1H, -NH); **^{13}C NMR (100 MHz, DMSO-d_6, ppm):** δ 17.09; 27.59; 82.10; 116.45; 118.01; 120.07; 127.20; 128.41; 128.47; 132.21; 140.72; 152.21; 158.41; 161.58; 164.61; **MS (ESI m/z):** 470 $[M+Na]^+$; *Anal*. Calcd for $C_{20}H_{18}ClN_3O_5S$: C, 53.63; H, 4.05; N, 9.38; S, 7.16%; Found: C, 53.67; H, 4.02; N, 9.35; S, 7.20%.

tert-Butyl2-(-2-(-(1-(6,8-dichloro-2-oxo-2*H*-chromen-3-yl)ethylidene) hydrazono)-4-oxo thiazolidin-5-ylidene)acetate (43c):



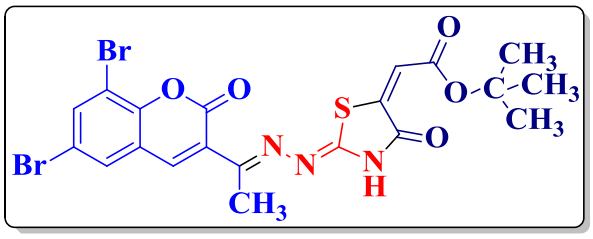
Yellow solid; m.p.: 269 - 270 °C; **FTIR (KBr, ν_{max}, cm^{-1}):** 3141 (-NH); 1732 (lactone –C=O); 1675 (ester –C=O); 1621 (amide –C=O); 1601 (-C=N); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 1.47 (s, 9H, *tert* butyl); 2.36 (s, 3H, -CH_3); 6.55 (s, 1H, =CH); 7.50 (d, *J* = 8.8 Hz, 1H, Ar-H); 7.70 – 7.73 (m, 1H, Ar-H); 8.29 (s, 1H, C_4-H of coumarin); 12.92 (s, 1H, -NH). **MS (ES m/z):** 483 $[M+H]^+$; *Anal*. Calcd for $C_{20}H_{17}Cl_2N_3O_5S$: C, 49.80; H, 3.55; N, 8.71; S, 6.65%; Found: C, 49.84; H, 3.50; N, 8.72; S, 6.67%.

tert-Butyl 2-(-2-(-(1-(6-bromo-2-oxo-2*H*-chromen-3-yl)ethylidene) hydrazono)-4-oxo thiazolidin-5-ylidene)acetate (43d):



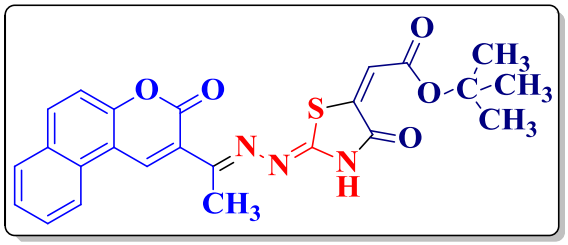
Yellow solid; m.p.: 274 – 276 °C; **FTIR (KBr, ν_{max}, cm^{-1}):** 3202 (-NH); 1722 (lactone –C=O); 1605 (-C=N); 1241 (-C-O-C); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 1.47 (s, 9H, *tert*. butyl); 2.36 (s, 3H, -CH_3); 6.55 (s, 1H, =CH); 7.44 (d, *J* = 8.8 Hz, 1H, -Ar-H); 7.81 – 7.84 (m, 1H, -Ar-H); 8.20 (d, *J* = 2.4 Hz, 1H); 8.24 (s, 1H, C_4-H of coumarin); 12.92 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ 17.10; 27.58; 82.07; 115.96; 116.43; 124.73; 125.98; 129.40; 132.74; 141.59; 141.95; 153.50; 158.72; 160.68; 161.82; 164.50; 165.61; MS (ESI m/z): 494 $[M+2H]^+$; *Anal*. Calcd for $C_{20}H_{18}BrN_3O_5S$: C, 48.79; H, 3.69; N, 8.53; S, 6.51%; Found: C, 48.82; H, 3.65; N, 8.55; S, 6.53%.

tert-Butyl 2-(-2-(-(1-(6,8-dibromo-2-oxo-2*H*-chromen-3-yl)ethylidene) hydrazono)-4-oxo thiazolidin-5-ylidene)acetate (43e):



Yellow solid; m.p.: 242 - 244 °C; **FTIR (KBr, ν_{max}, cm^{-1}):** 3064 (-NH); 1725 (lactone –C=O); 1617 (amide –C=O); 1235 (-C-O-C); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 1.47 (s, 9H, *tert* butyl); 2.36 (s, 3H, -CH_3); 6.54 (s, 1H, =CH); 7.44 (d, *J* = 8.8 Hz, 1H, Ar-H); 7.81 – 7.83 (m, 1H, Ar-H); 8.20 (s, 1H, C_4-H of coumarin); 12.92 (s, 1H, -NH); **MS (ESI m/z):** 572 $[M+H]^+$; *Anal*. Calcd for $C_{20}H_{17}Br_2N_3O_5S$: C, 42.05; H, 3.00; N, 7.36; S, 5.61%; Found: C, 42.10; H, 2.96; N, 7.32; S, 5.66%.

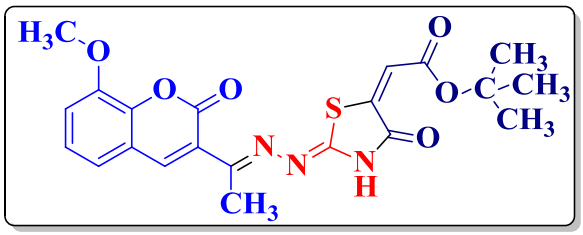
tert-Butyl 2-(-4-oxo-2-(-(1-(3-oxo-3*H*-benzo[*f*]chromen-2-yl)ethylidene) hydrazono) thiazolidin-5-ylidene)acetate (43f):



Yellow solid; m.p.: 318 – 320 °C; **FTIR (KBr, ν_{max}, cm^{-1}):** 3085 (-NH); 1720 (lactone -C=O); 1626 (ester –C=O); 1612 (amide –C=O); 1238 (-C-O-C); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 1.46 (s, 9H, *tert* butyl- H); 2.45 (s, 3H, -CH_3); 6.54 (s, 1H, =CH); 7.64 – 7.69 (m, 2H, Ar-H); 7.78 (t, *J* = 8 Hz, 1H, Ar-H); 8.11 (d, *J* = 8.4 Hz, 1H, Ar-H); 8.28 (d, *J* = 8.8 Hz, 1H, Ar-

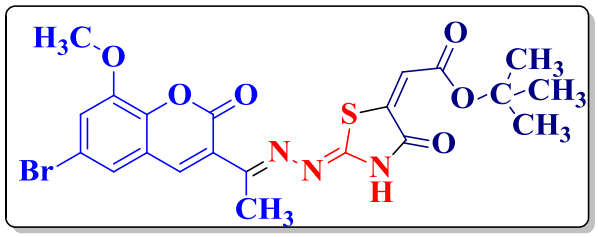
H); 8.56 (d, *J* = 8.4 Hz, 1H, Ar-H); 9.01 (s, 1H, C_4-H of coumarin); 12.95 (s, 1H, -NH); **MS (ESI m/z):** 464 $[M+H]^+$; *Anal*. Calcd for $C_{24}H_{21}N_3O_5S$: C, 62.19; H, 4.57; N, 9.07; S, 6.92%; Found: C, 62.14; H, 4.60; N, 9.05; S, 6.96%.

***tert*-Butyl 2-(-2-(-(1-(8-methoxy-2-oxo-2*H*-chromen-3-yl)ethylidene) hydrazono)-4-oxo thiazolidin-5-ylidene)acetate (43g):**



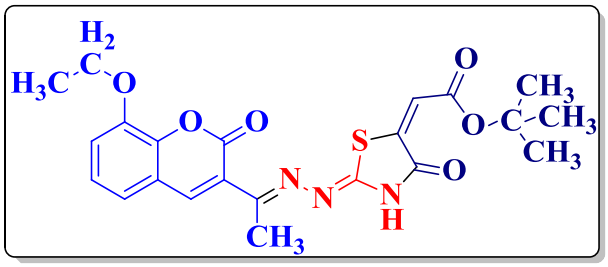
Yellow solid; m.p.: 138 - 140 °C; **FTIR (KBr, ν_{max}, cm^{-1}):** 3215(-NH); 1722 (-lactone – C=O); 1614 (amide – C=O); 1247 (-C-O-C); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 1.47 (s, 9H, *tert* butyl - H); 2.37 (s, 3H, $-CH_3$); 3.93 (s, 3H, $-OCH_3$); 6.54 (s, 1H, =CH); 7.31 – 7.38 (m, 2H, Ar-H); 7.43 – 7.45 (m, 1H, Ar-H); 8.29 (s, 1H, C_4-H of coumarin); 12.91 (s, 1H, -NH); **^{13}C NMR (100 MHz, DMSO-d_6, ppm):** δ 17.68; 28.15; 56.69; 82.68; 115.53; 117.03; 119.72; 121.07; 125.29; 126.72; 142.21; 142.28; 143.47; 146.83; 159.07; 162.30; 165.12; 166.26; **MS (ESI m/z):** 444 $[M+H]^+$; *Anal*. Calcd for $C_{21}H_{21}N_3O_6S$: C, 56.87; H, 4.77; N, 9.48, S, 7.23%; Found: C, 56.84; H, 4.79; N, 9.45; S, 7.26%.

***tert*-Butyl 2-(-2-(-(1-(6-bromo-8-methoxy-2-oxo-2*H*-chromen-3-yl) ethylidene) hydrazono)-4-oxothiazolidin-5-ylidene)acetate (43h):**



Yellow solid; m.p.: 249 – 250 °C; **FTIR (KBr, ν_{max}, cm^{-1}):** 3196 (-NH); 1734 (lactone-C=O); 1685 (ester – C=O); 1627 (amide –C=O); 1603 (-C=N); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 1.47 (s, 9H, *tert* butyl-H), 2.35 (s, 3H, $-CH_3$); 3.95 (s, 3H, $-OCH_3$); 6.54 (s, 1H, =CH); 7.52 (d, *J* = 2 Hz, 1H, Ar-H); 7.74 (d, *J* = 2 Hz, 1H, Ar-H); 8.21 (s, 1H, C_4-H of coumarin); 12.91 (s, 1H, -NH); **^{13}C NMR (100 MHz, DMSO-d_6, ppm):** δ 17.06; 27.59; 56.67; 82.10; 116.15; 116.47; 117.51; 120.45; 122.39; 127.25; 140.91; 141.67; 142.27; 147.15; 158.02; 161.05; 161.54; 164.56; 165.71; **MS (ESI m/z):** 523 $[M+H]^+$; *Anal*. Calcd for $C_{21}H_{20}BrN_3O_6S$: C, 48.28; H, 3.86; N, 8.04; S, 6.14%; Found: C, 48.32; H, 3.81; N, 8.10; S, 6.10%.

***tert*-Butyl 2-(-2-(-(1-(8-ethoxy-2-oxo-2*H*-chromen-3-yl)ethylidene) hydrazono)-4-oxo thiazolidin-5-ylidene)acetate (43i):**



Yellow solid; m.p.: 225 - 227 °C; **FTIR (KBr, ν_{max}, cm^{-1}):** 3147 (-NH); 1717 (lactone –C=O); 1608 (-C=N); 1250 (-C-O-C); **^{1}H NMR (400 MHz, DMSO- d_6, δ ppm):** 1.42 (t, *J* = 6.8 Hz, 3H, $-CH_2CH_3$); 1.47 (s, 9H, *tert* butyl-H); 2.37 (s, 3H, $-CH_3$); 4.17 – 4.22 (m, 2H, $-OCH_2$); 6.54 (s, 1H, =CH); 7.29 – 7.37 (m, 2H, Ar-H); 7.43 (d, *J* = 7.2Hz, 1H, Ar-H); 8.24 (s, 1H, C_4-H of coumarin); 12.91 (s, 1H, -NH); **^{13}C NMR (100 MHz, DMSO-d_6, ppm):** δ 14.58; 17.17; 27.61; 64.47; 82.16; 115.89; 116.51; 119.28; 120.49; 124.77; 126.14; 141.70; 142.26; 143.03; 145.53; 158.62; 160.81; 161.86; 164.59; 165.76; **HRMS ESI m/z** calculated for $C_{22}H_{23}N_3NaO_6S$ $[M + Na]^+$: 480.1205, found: 480.1204; *Anal*. Calcd for $C_{22}H_{23}N_3O_6S$: C, 57.76; H, 5.07; N, 9.18; S, 7.01%; Found: C, 57.79; H, 5.02; N, 9.24; S, 6.97%.

Antiviral activity evaluation of alkyl 4-oxo-coumarinyl ethylidene hydrazono-thiazo lidin-5-ylidene acetate derivatives:

All the synthesized compounds were screened for their antiviral activity as described in chapter-II (reference 65-67). After incubation at 37°C the virus-induced cytopathicity was determined by measuring the cell viability with the colorimetric formazan-based MTS assay.

Anticancer activity experiment for alkyl 4-oxo-coumarinyl ethylidene hydrazono-thiazo lidin-5-ylidene acetate derivatives:

Alkyl 4-oxo-coumarinyl ethylidene hydrazono-thiazo lidin-5-ylidene acetate derivatives were tested for anticancer activity against murine Leukemia cells (L1210), human-T-Lymphocyte cells (CEM) and human cervix carcinoma cell (HeLa) using standard MTT protocol as described in the chapter-II (reference-49).

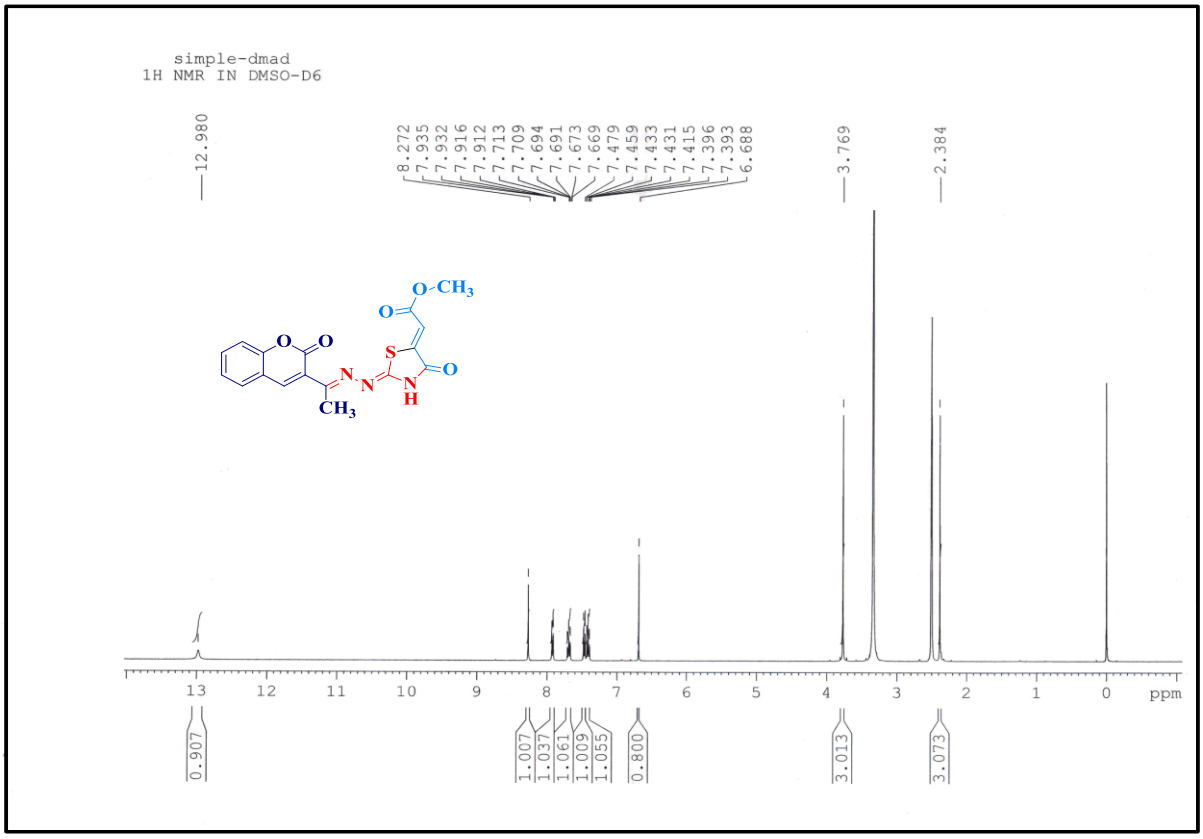


Fig. [1]H NMR spectrum of compound **41a**

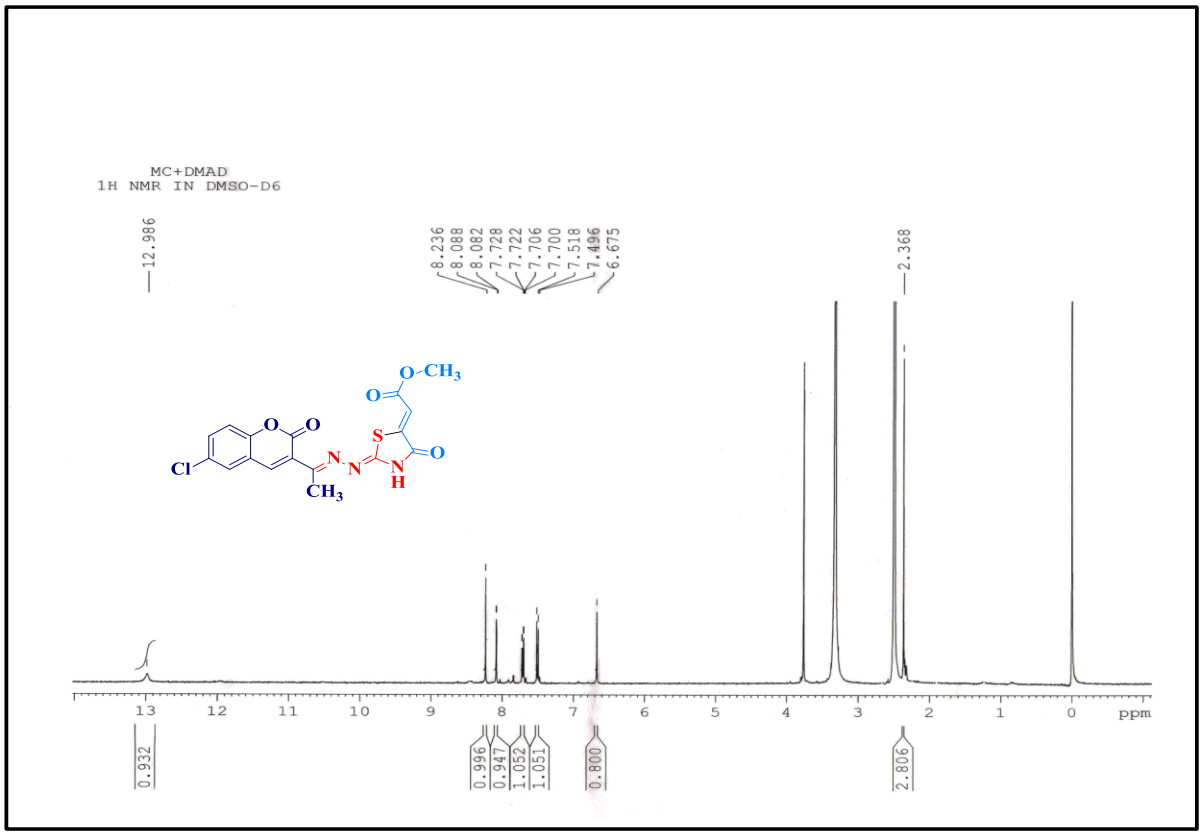


Fig. [1]H NMR spectrum of compound **41b**

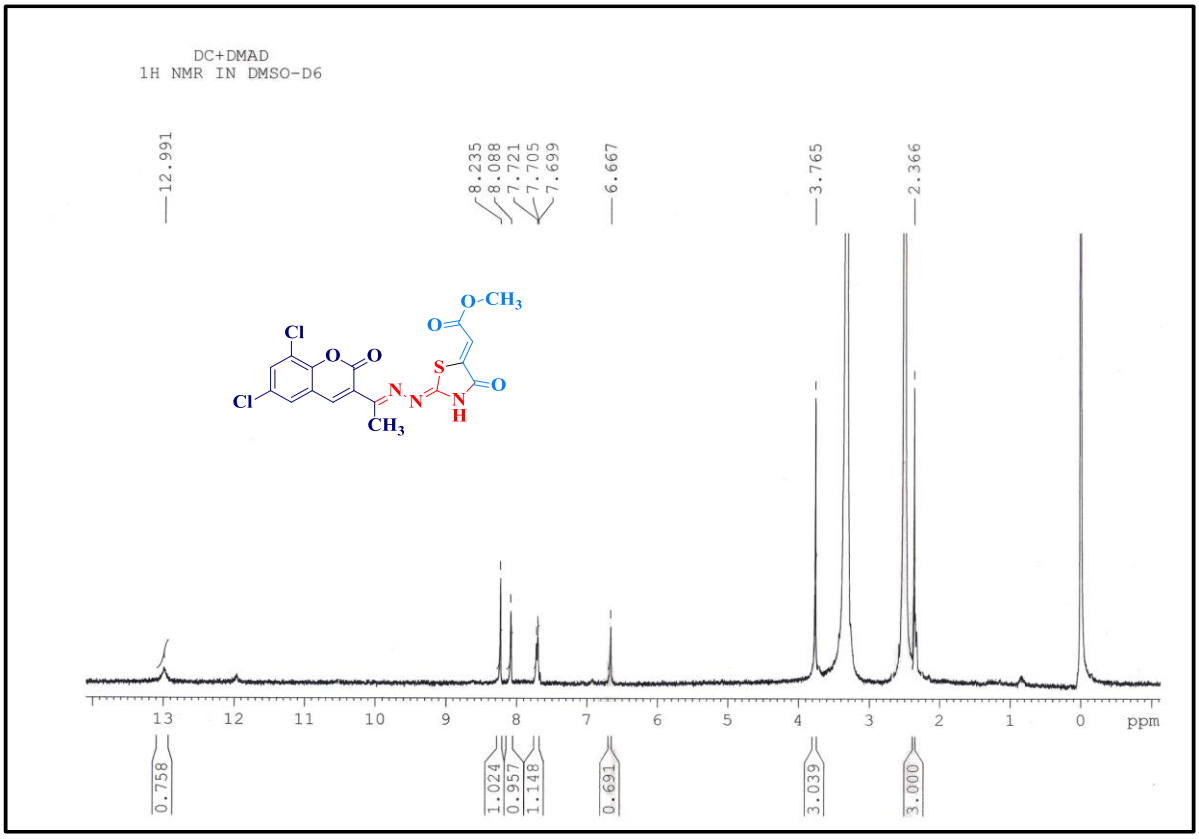


Fig. [1]H NMR spectrum of compound **41c**

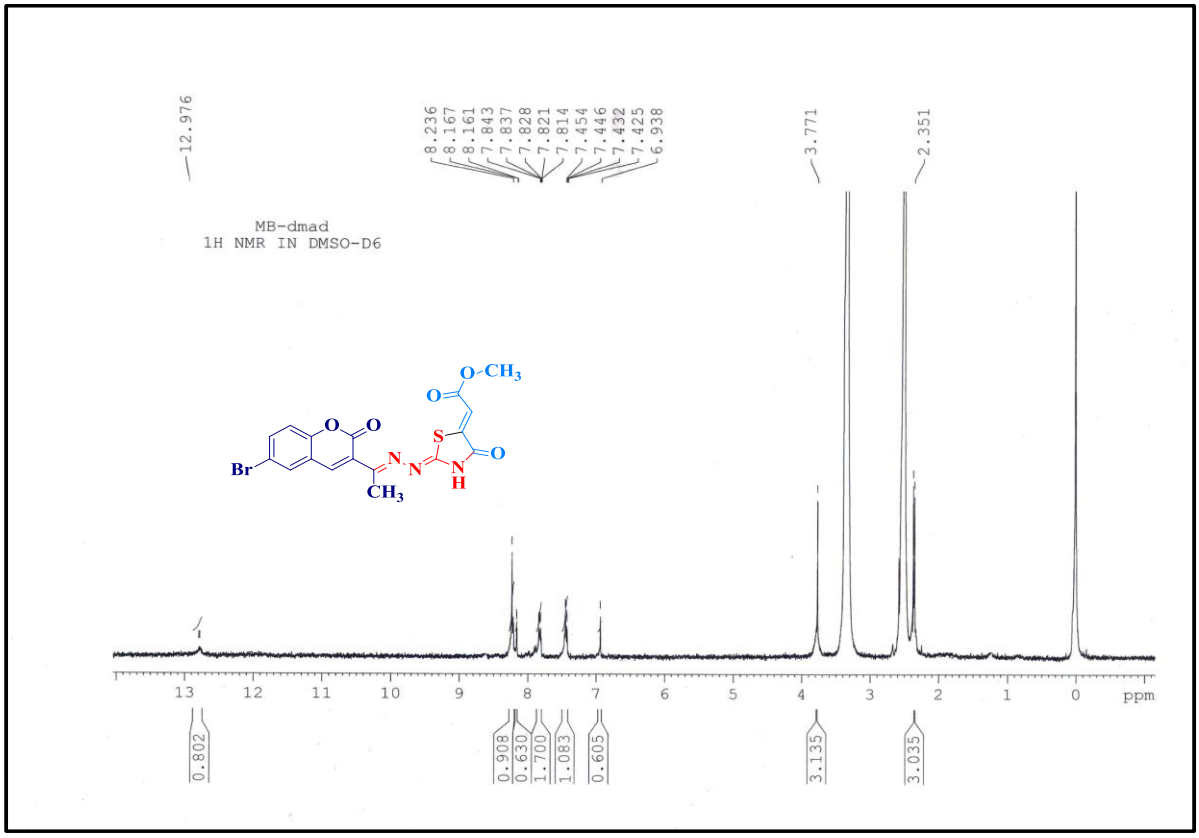


Fig. [1]H NMR spectrum of compound **41d**

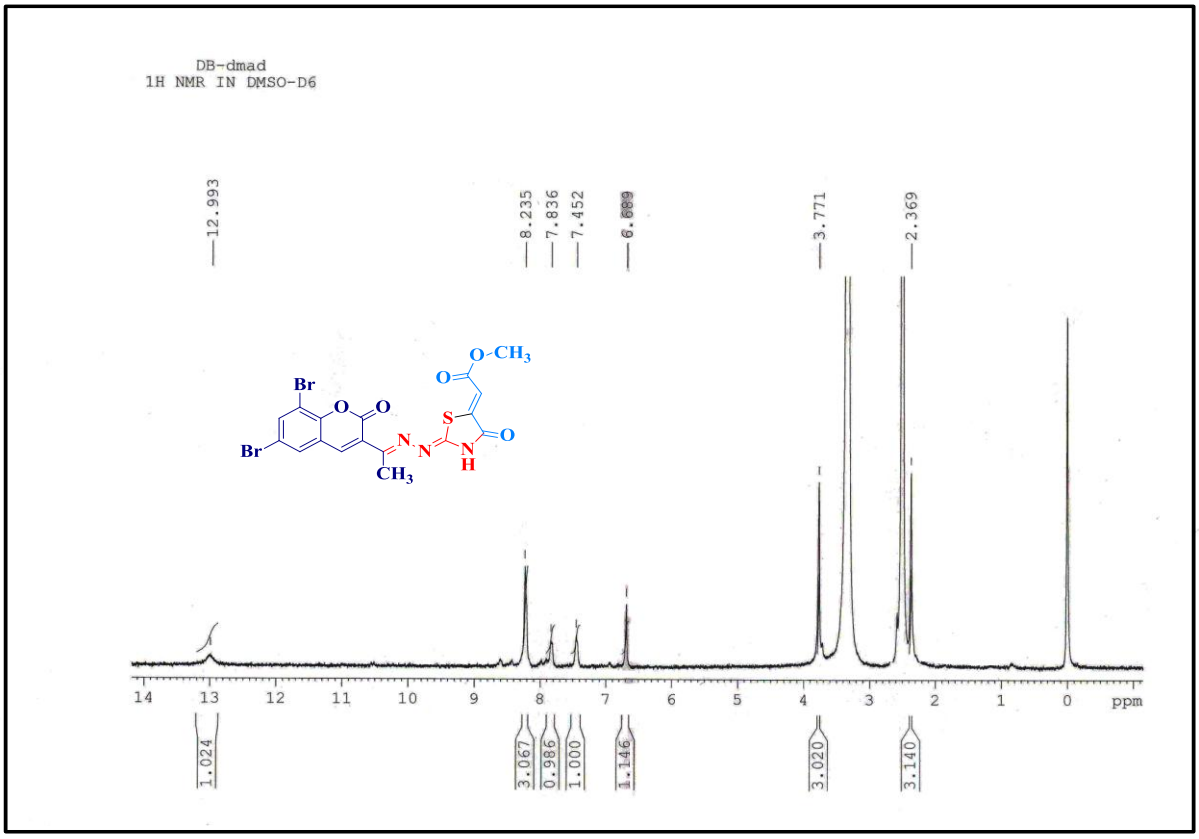


Fig. [1]H NMR spectrum of compound **41e**

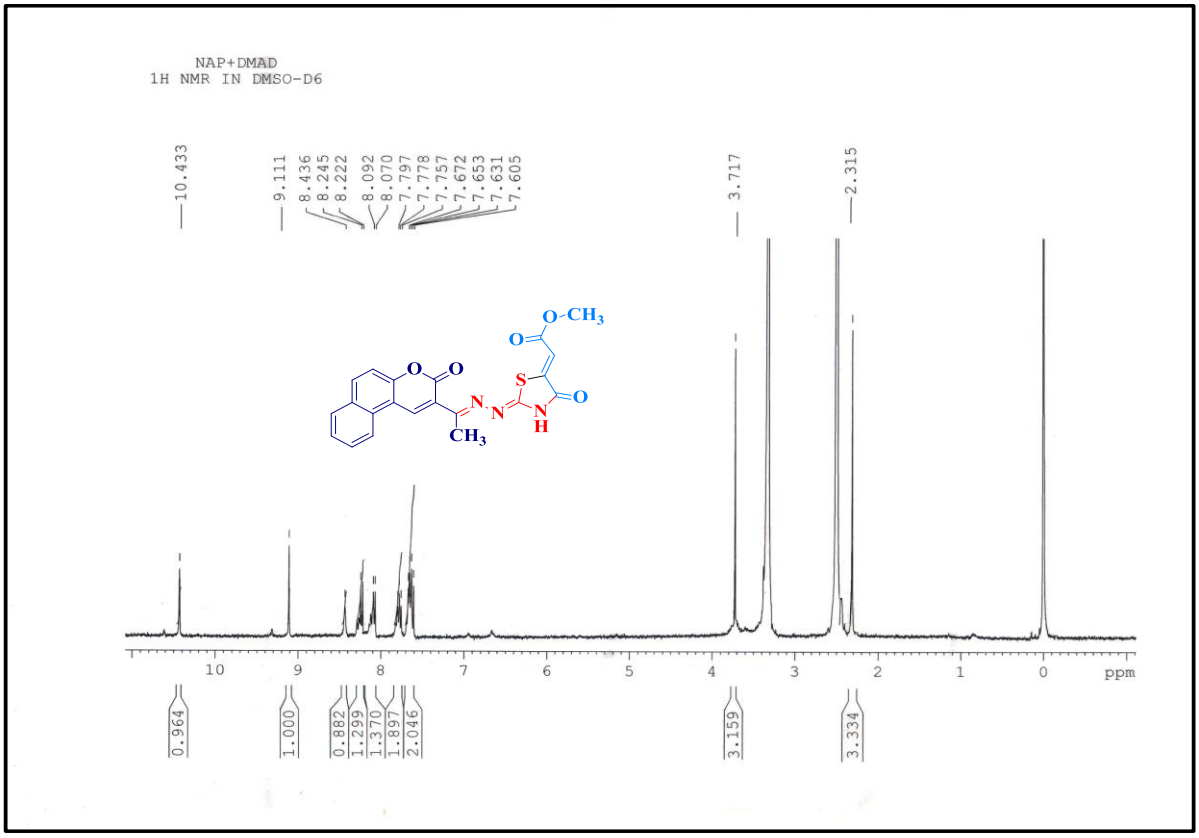


Fig. [1]H NMR spectrum of compound **41f**

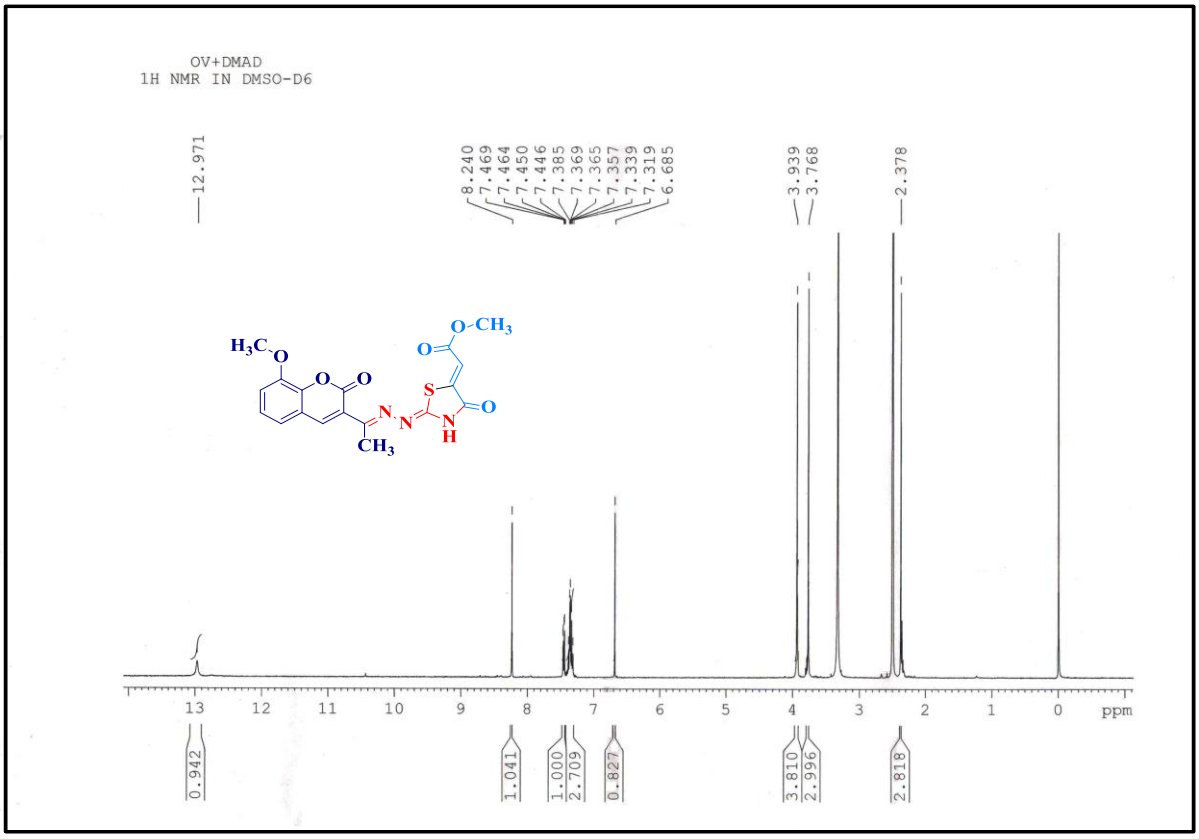


Fig. [1]H NMR spectrum of compound **41g**

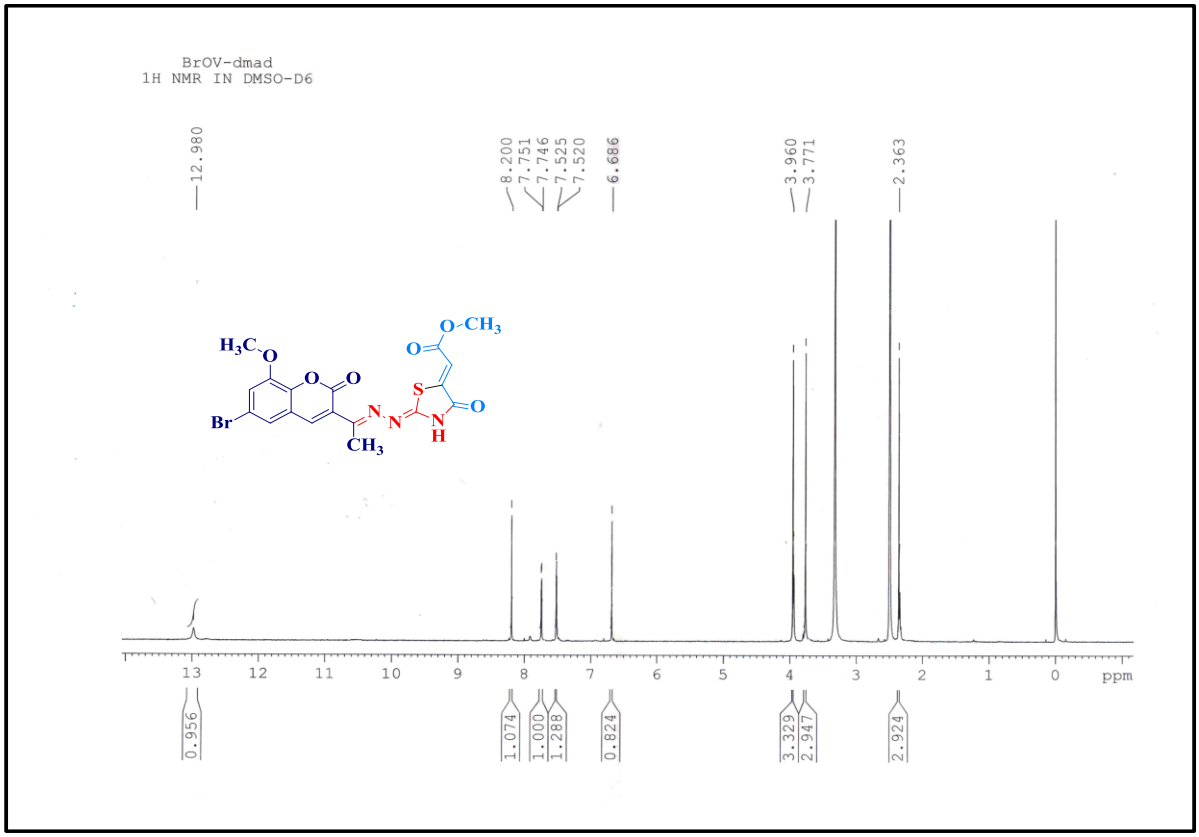


Fig. [1]H NMR spectrum of compound **41h**

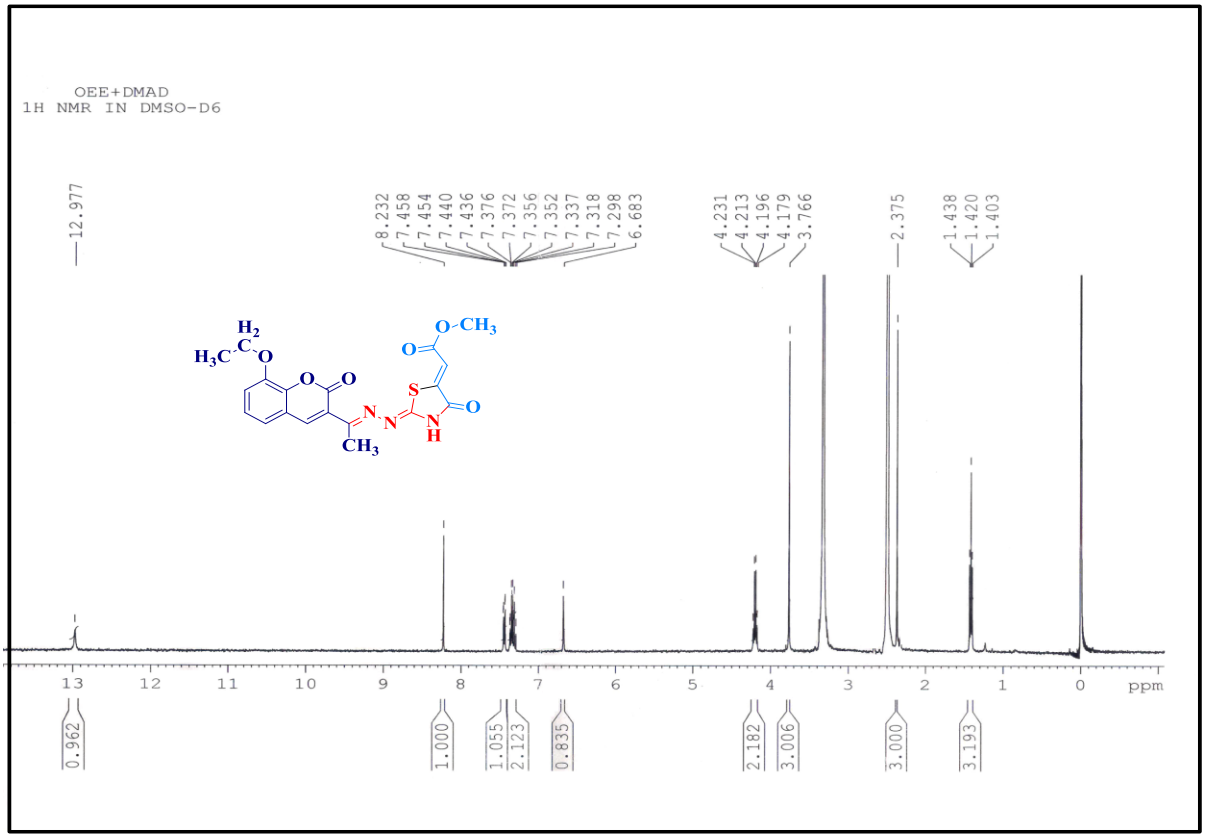


Fig. ^{1}H NMR spectrum of compound **41i**

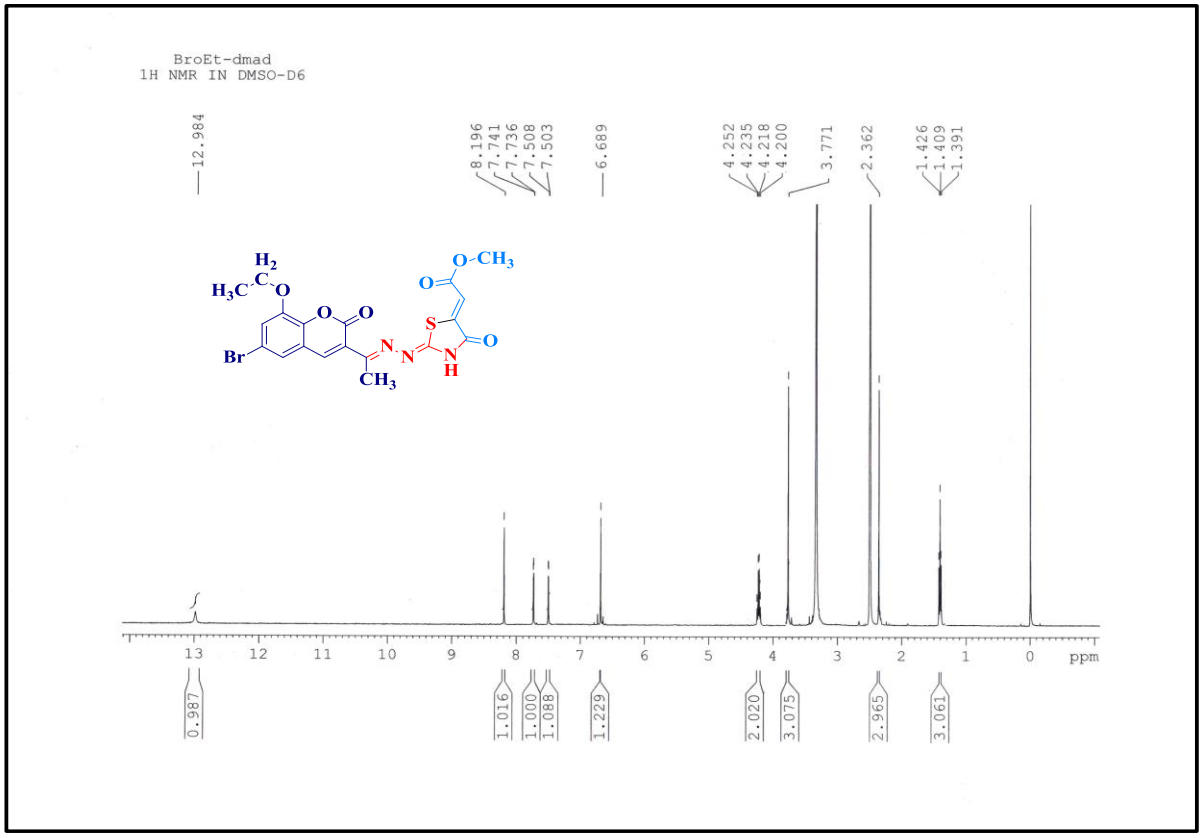


Fig. ^{1}H NMR spectrum of compound **41j**

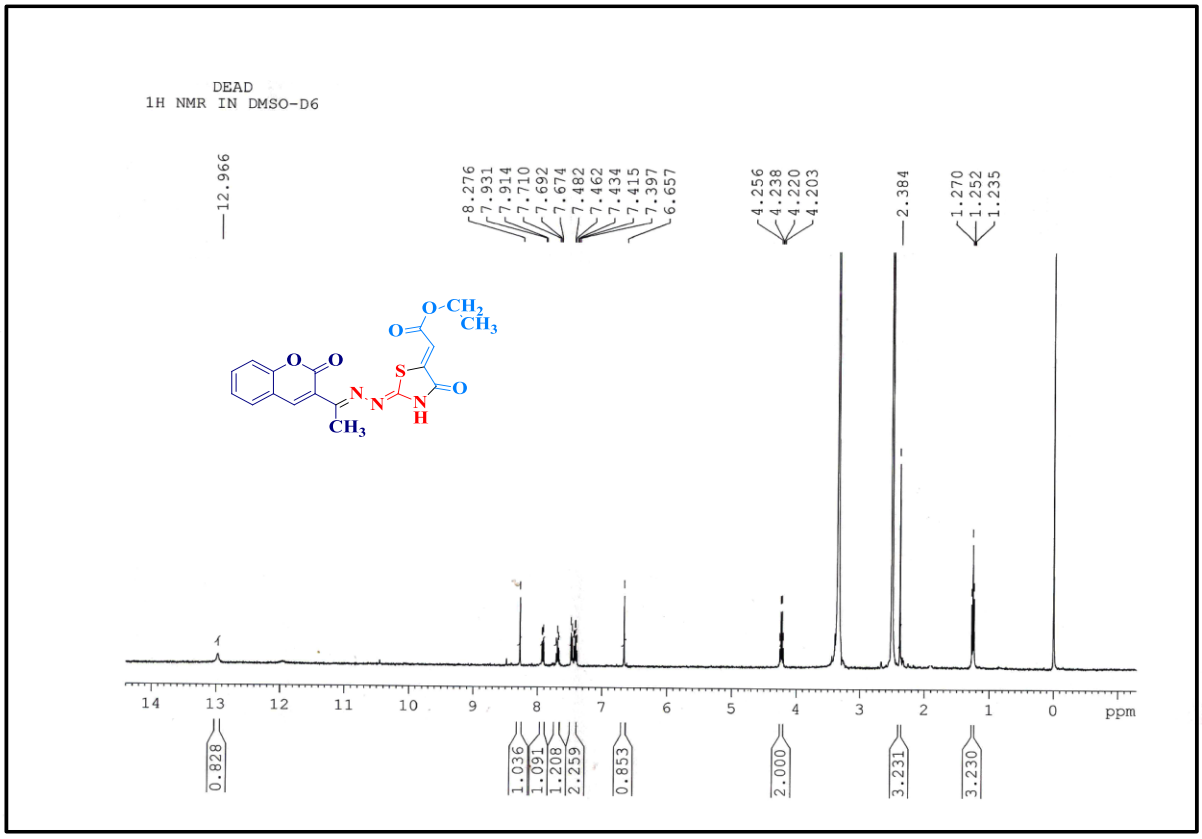


Fig. [1]H NMR spectrum of compound **42a**

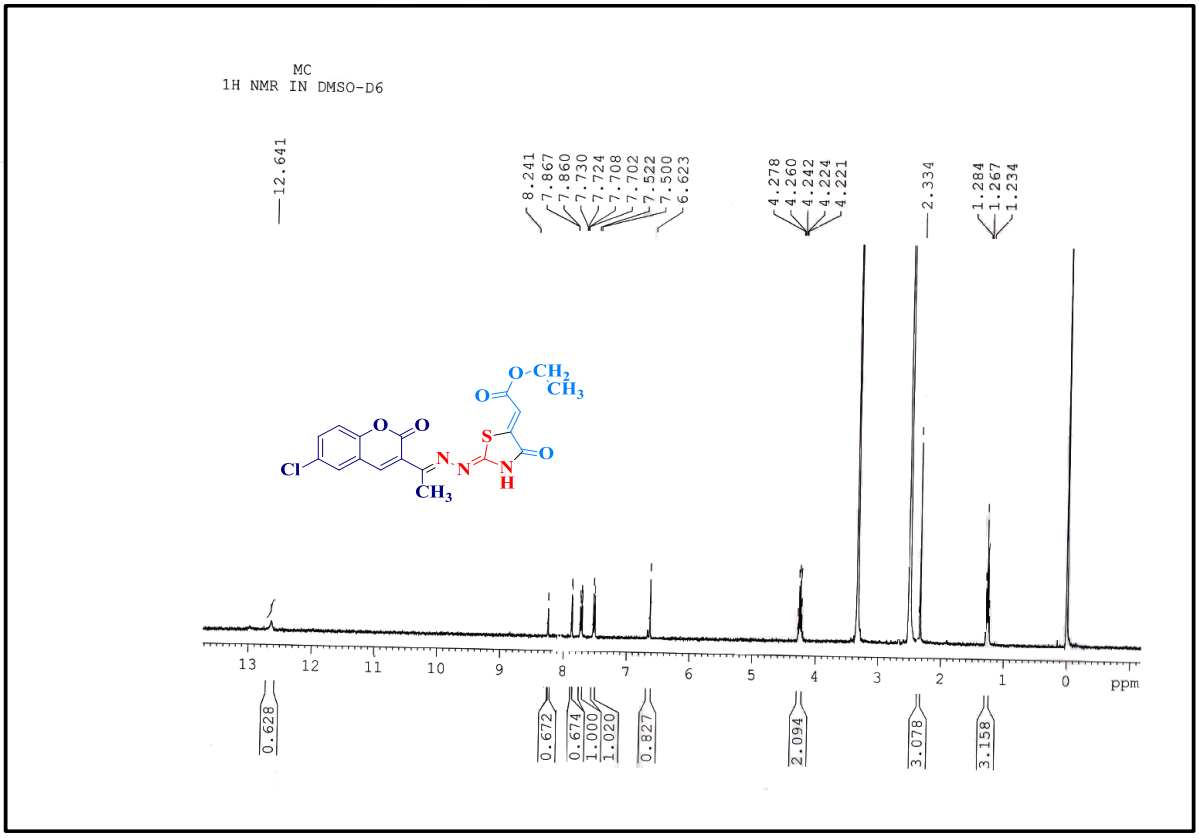


Fig. [1]H NMR spectrum of compound **42b**

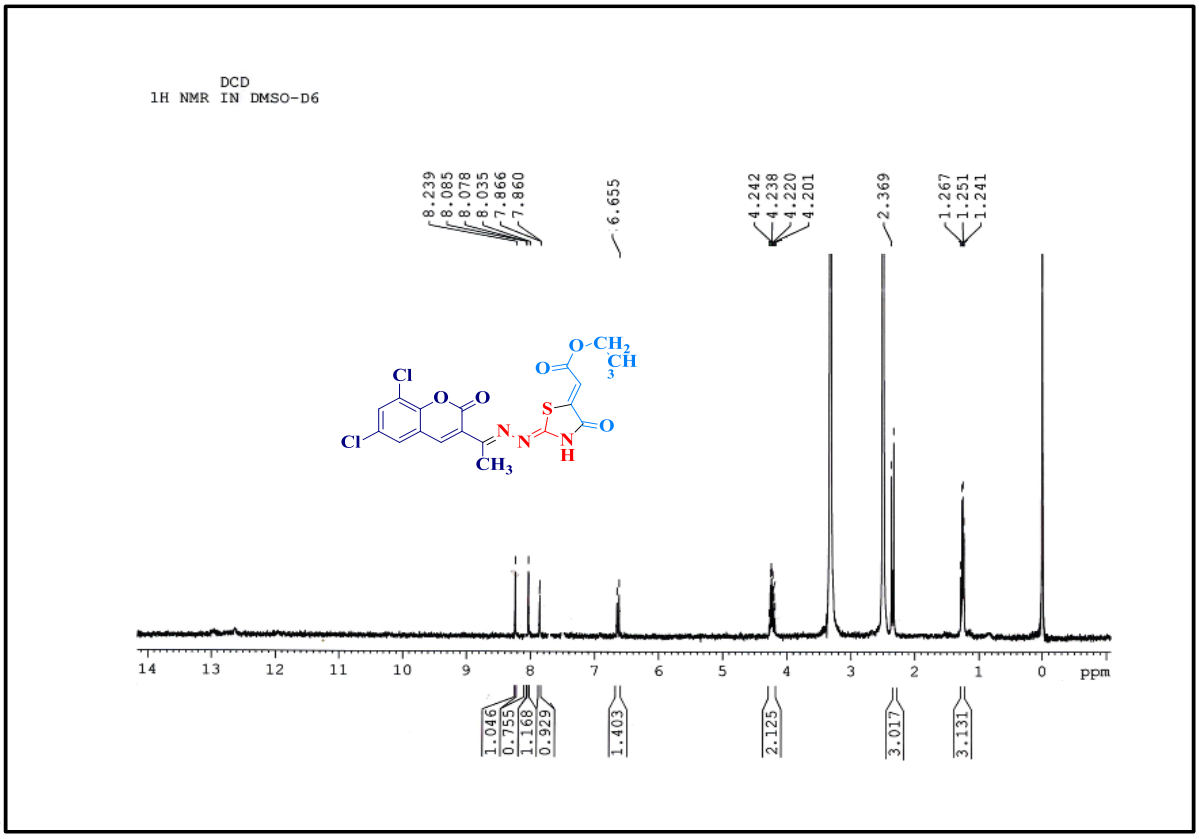


Fig. [1]H NMR spectrum of compound **42c**

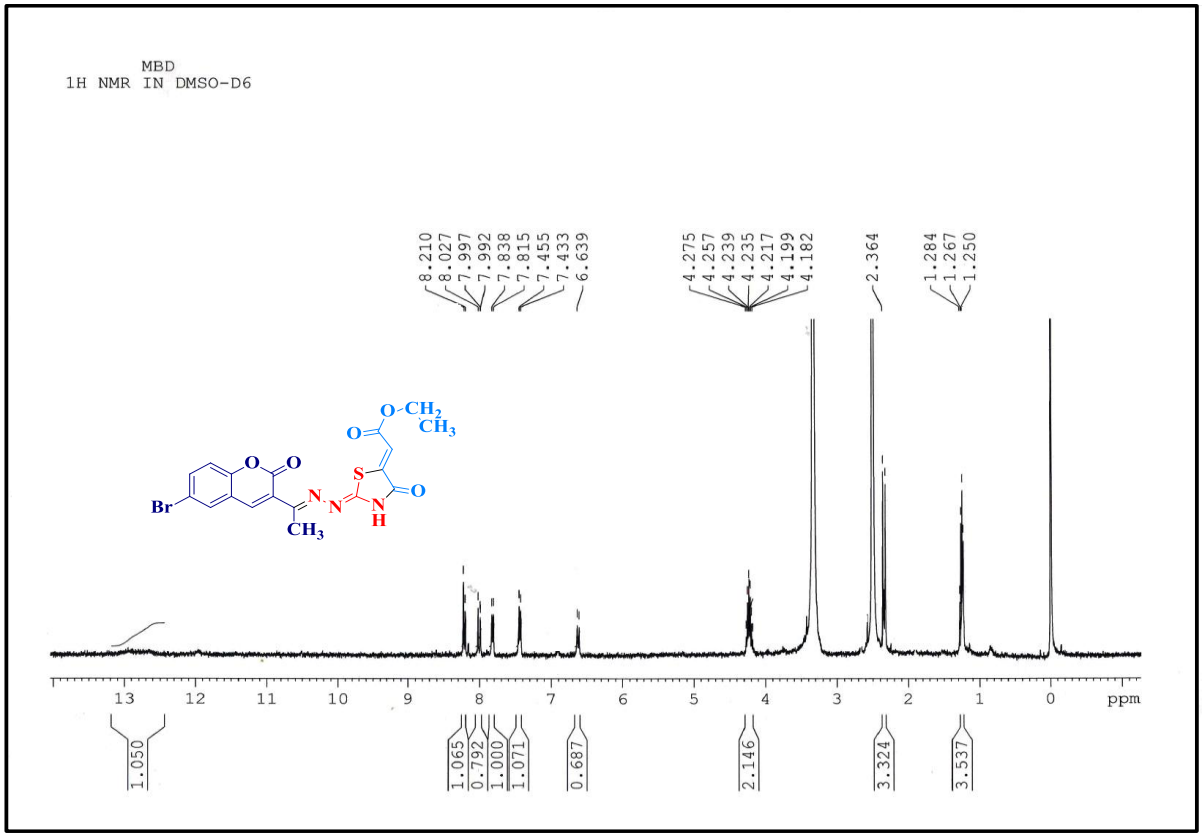


Fig. [1]H NMR spectrum of compound **42d**

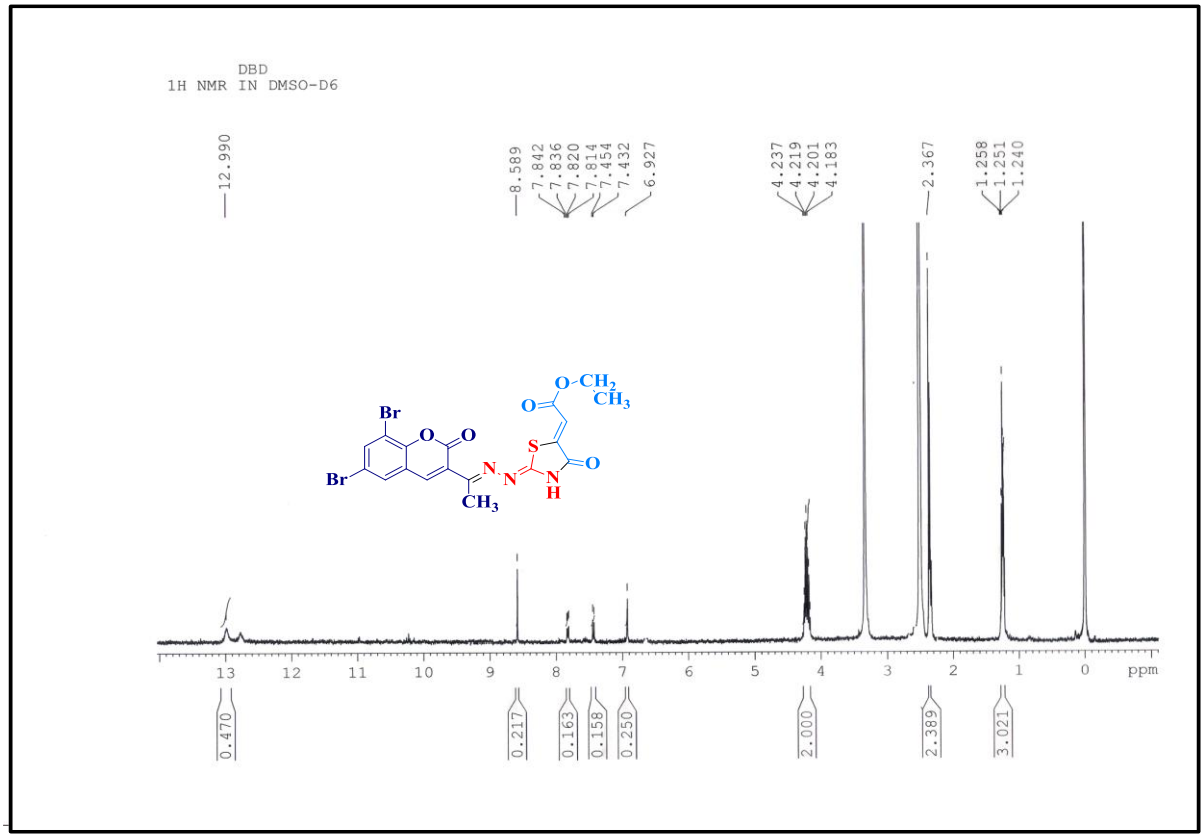


Fig. [1]H NMR spectrum of compound **42e**

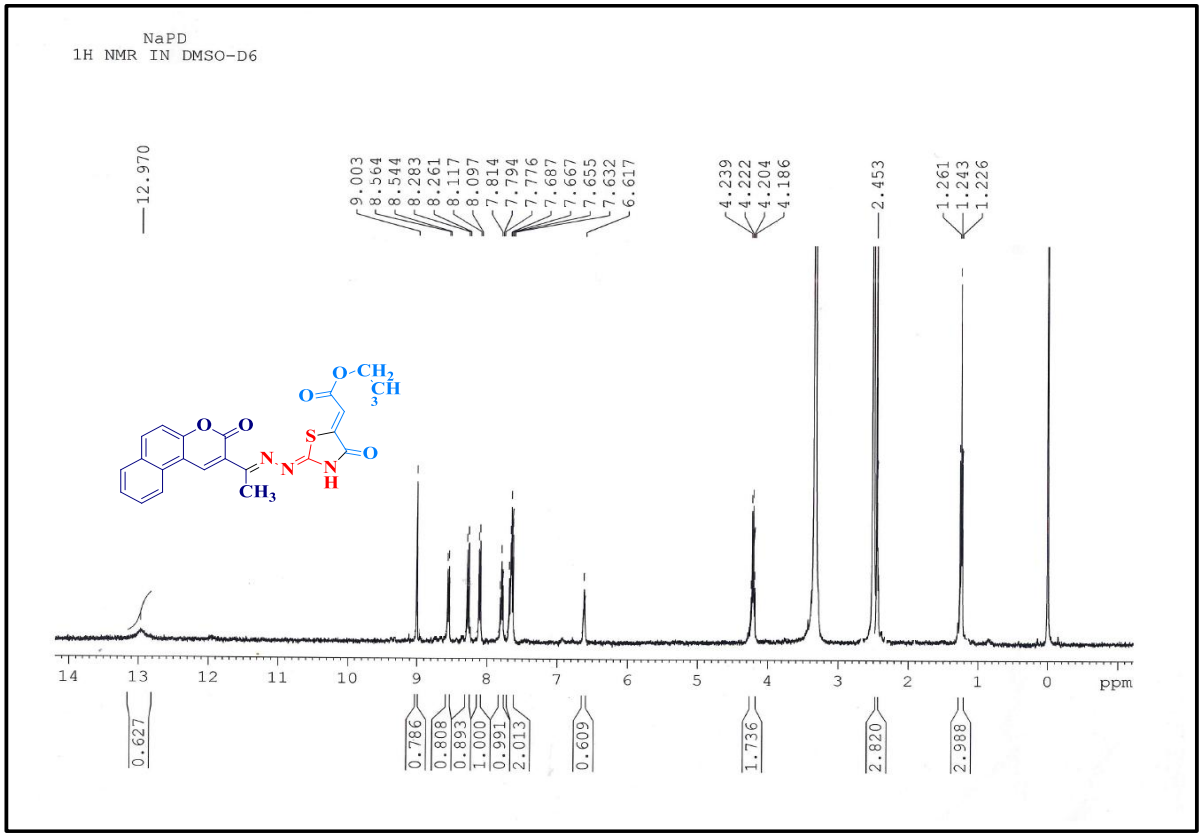


Fig. [1]H NMR spectrum of compound **42f**

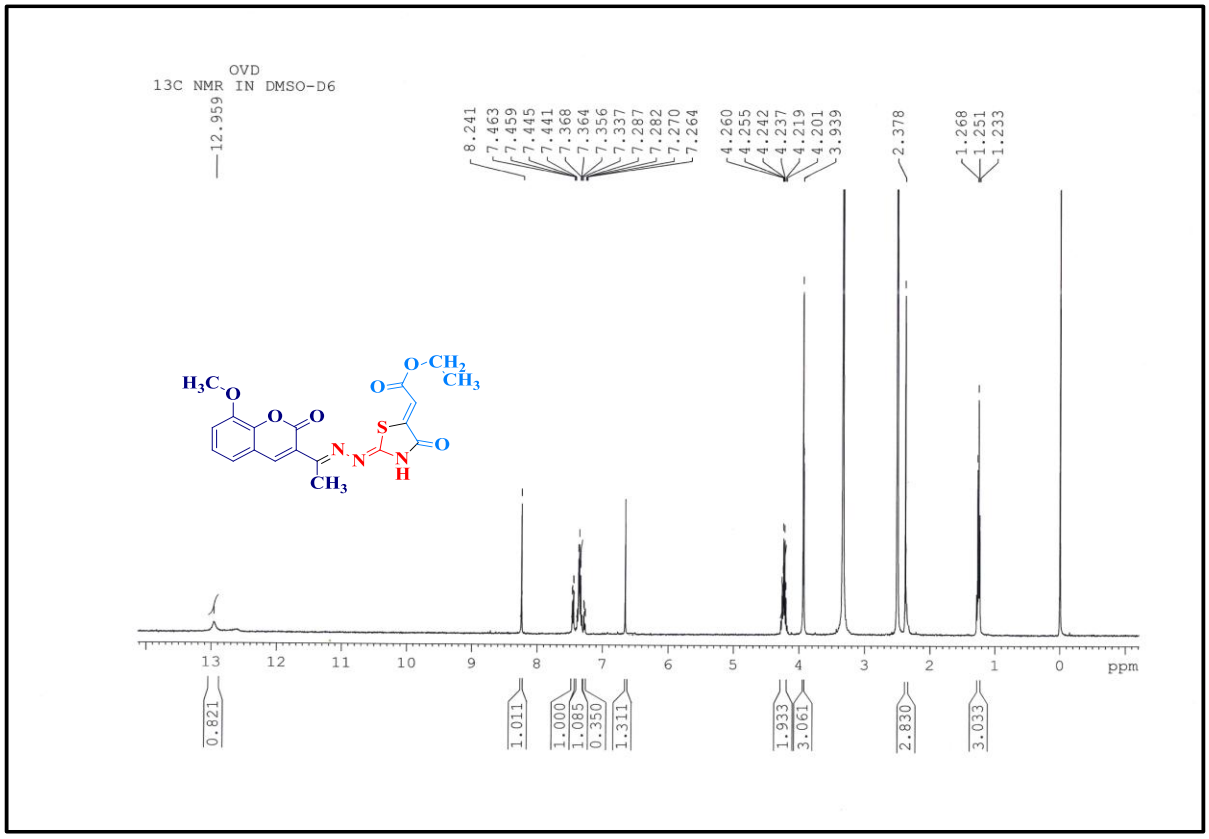


Fig. [1]H NMR spectrum of compound **42g**

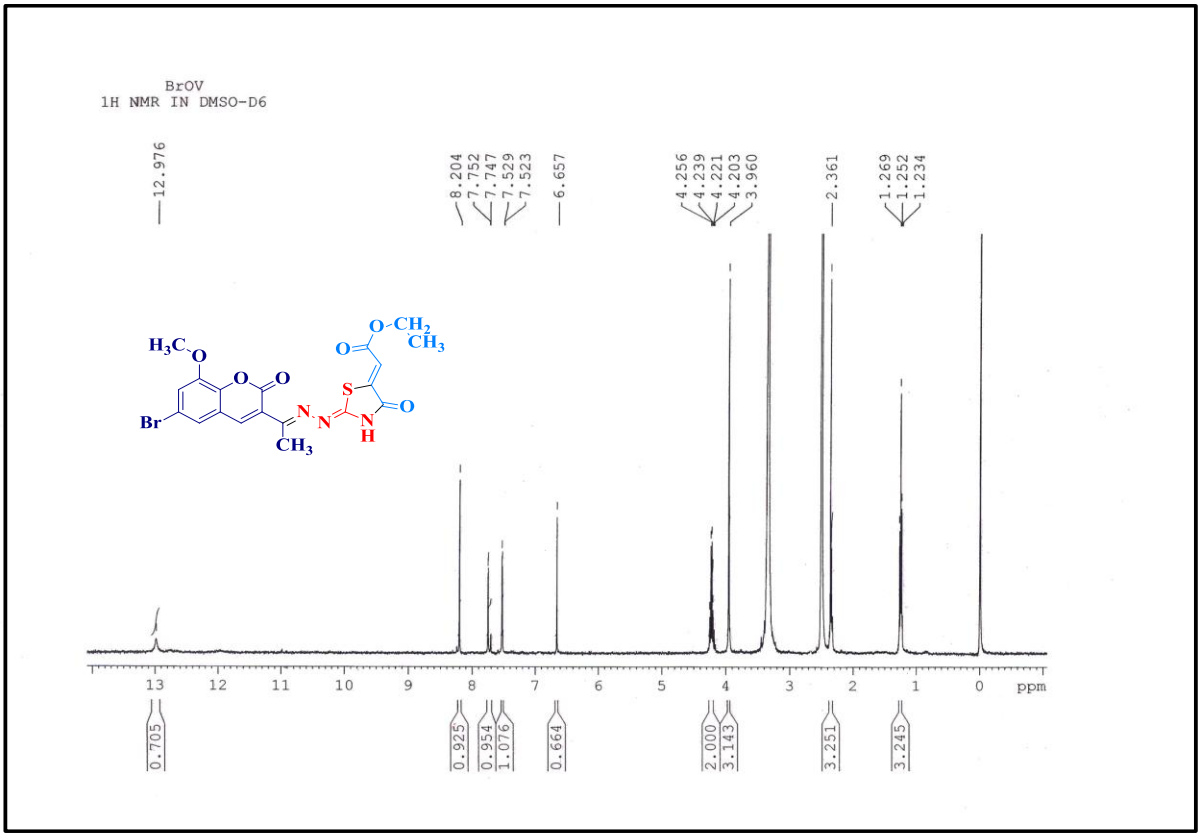


Fig. [1]H NMR spectrum of compound **42h**

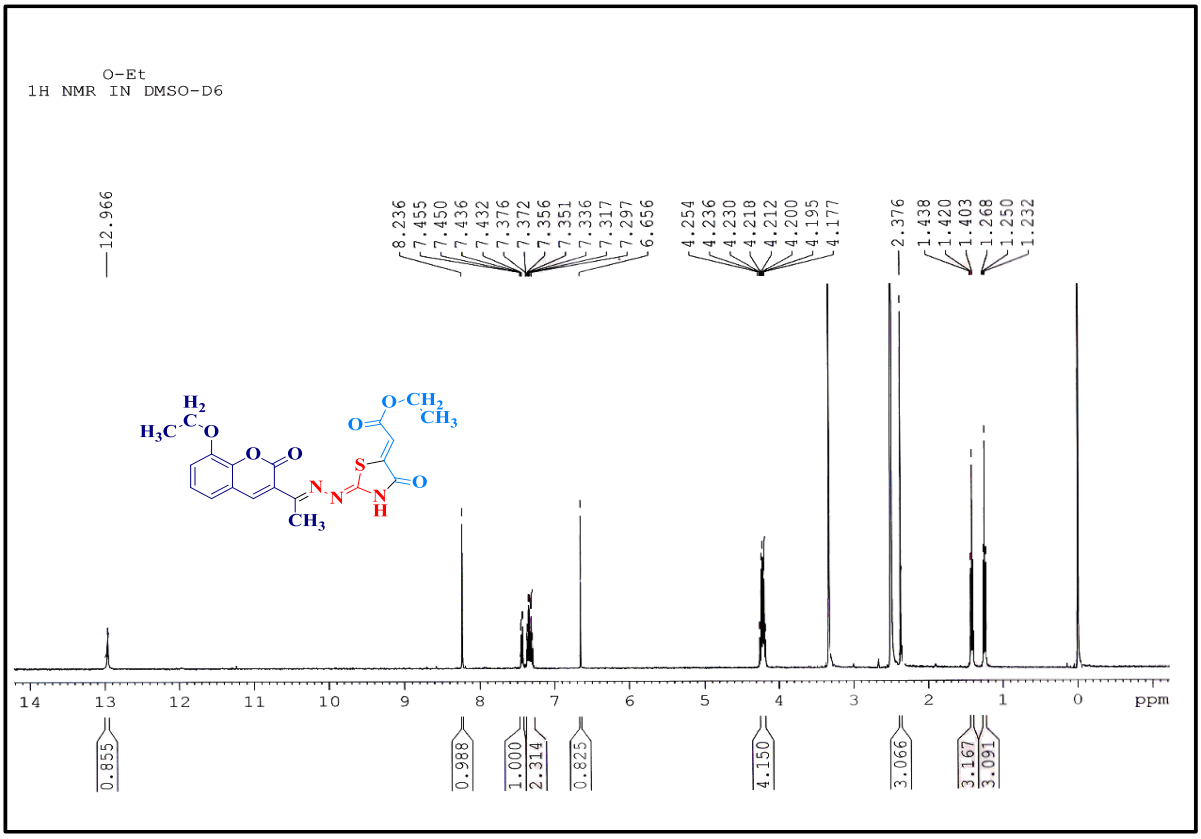


Fig. [1]H NMR spectrum of compound **42i**

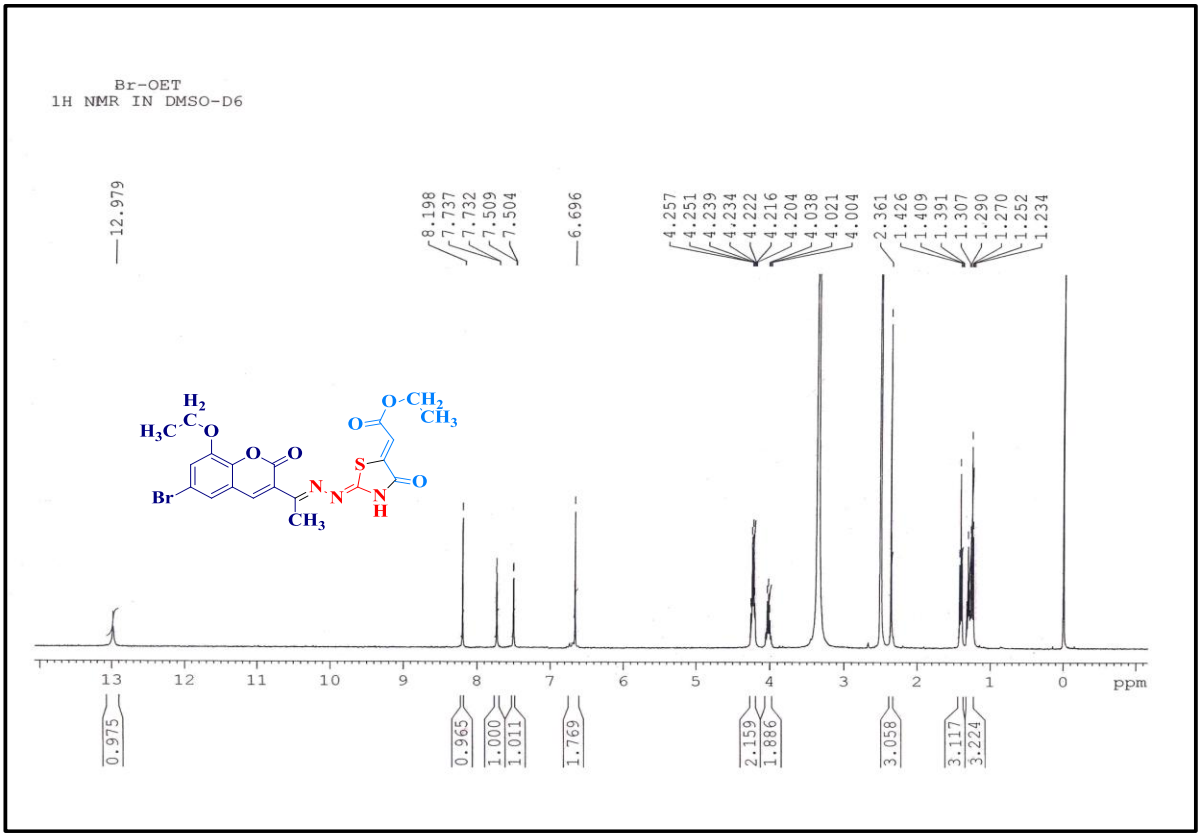


Fig. [1]H NMR spectrum of compound **42j**

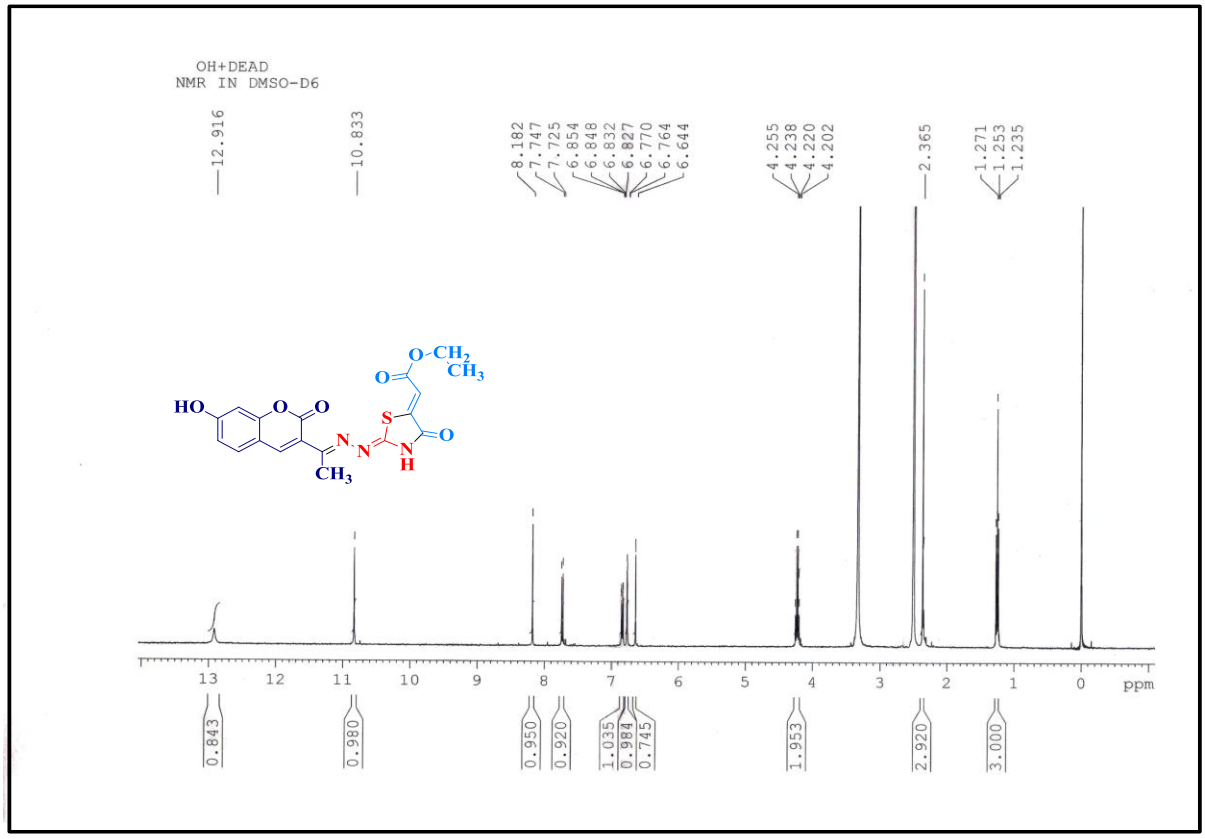


Fig. [1]H NMR spectrum of compound **42k**

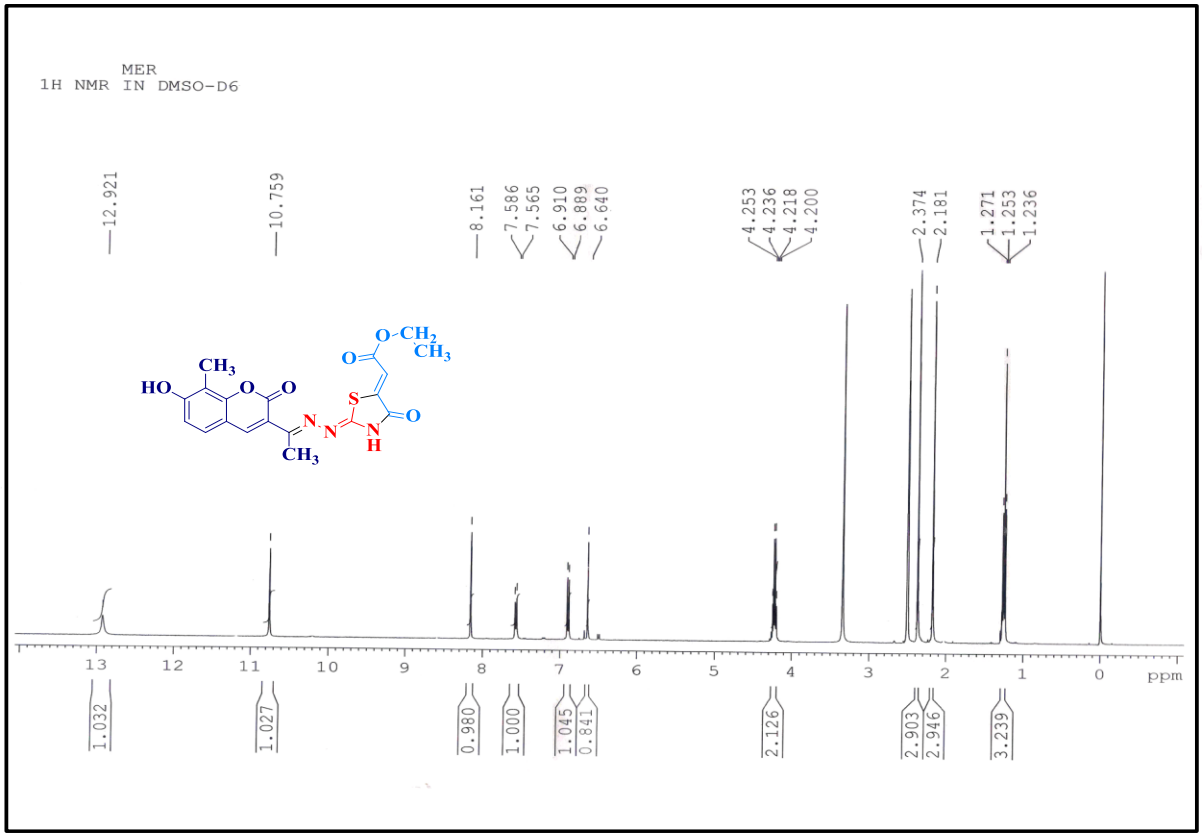


Fig. [1]H NMR spectrum of compound **42l**

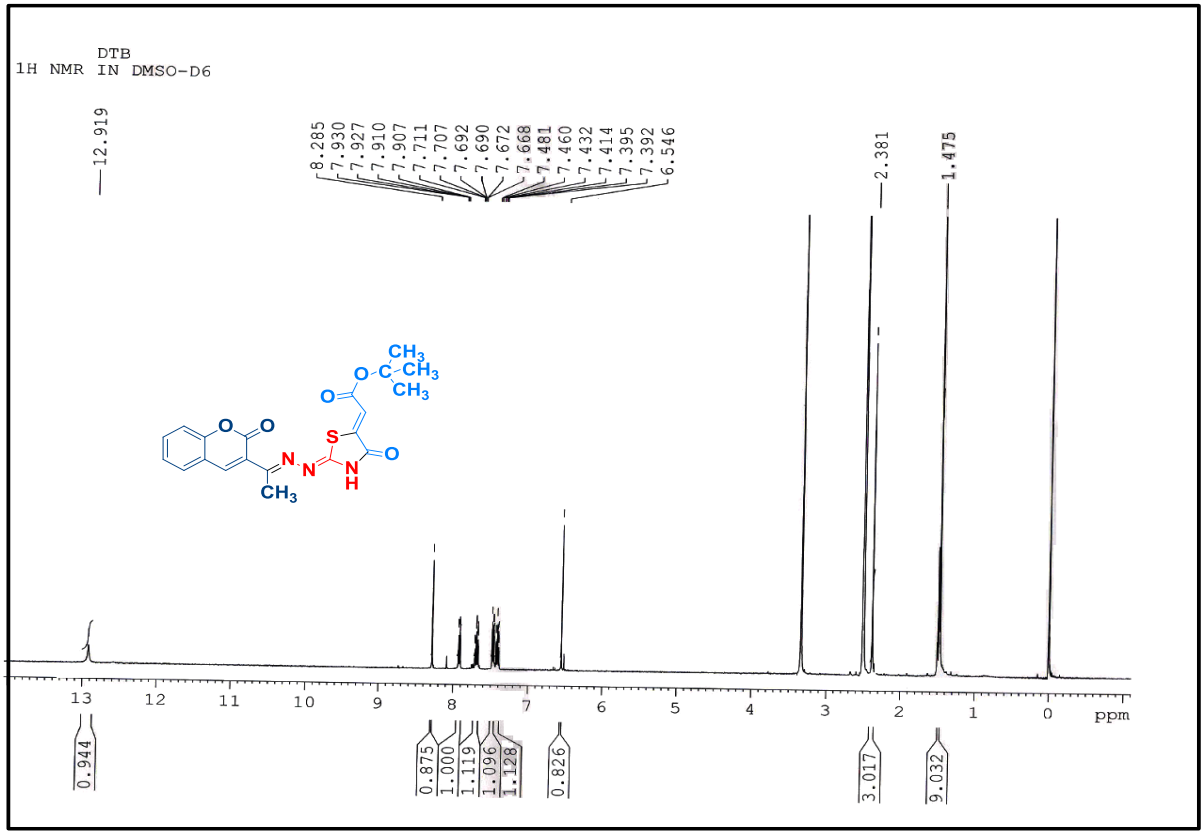


Fig. [1]H NMR spectrum of compound **43a**

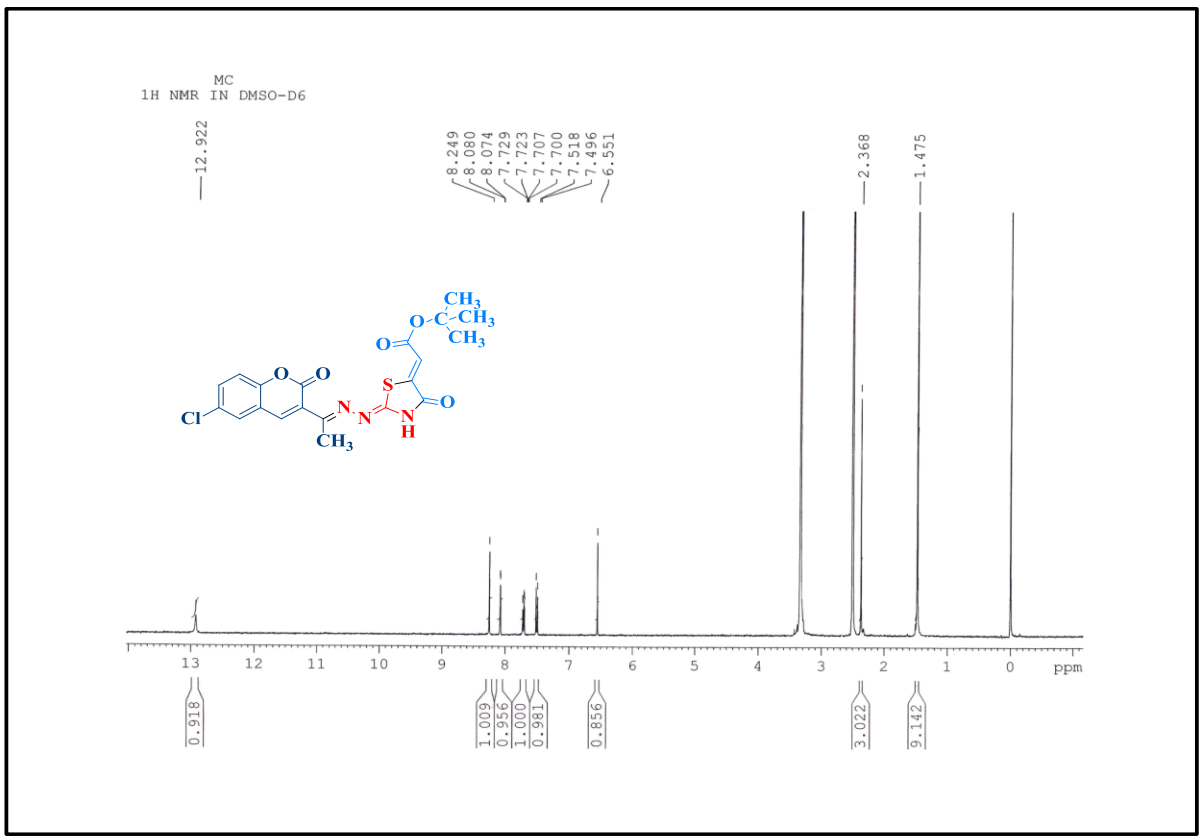


Fig. [1]H NMR spectrum of compound **43b**

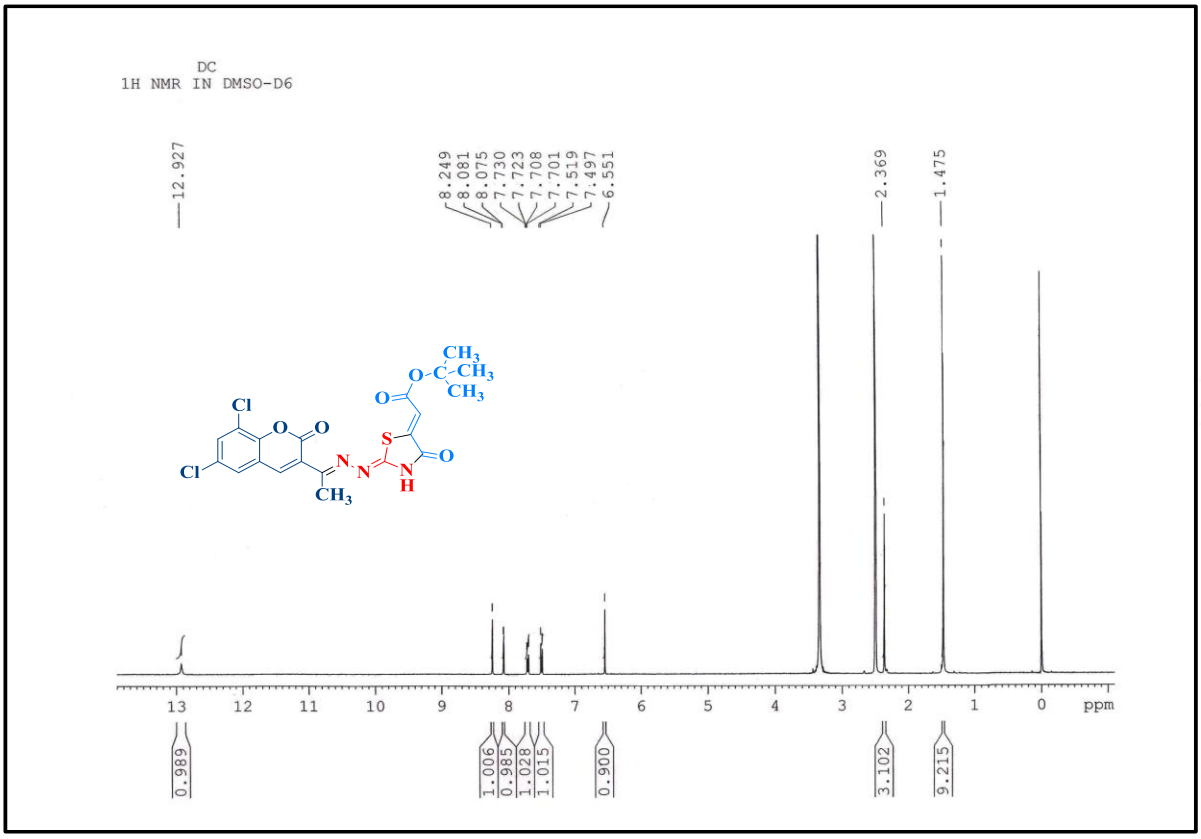


Fig. [1]H NMR spectrum of compound **43c**

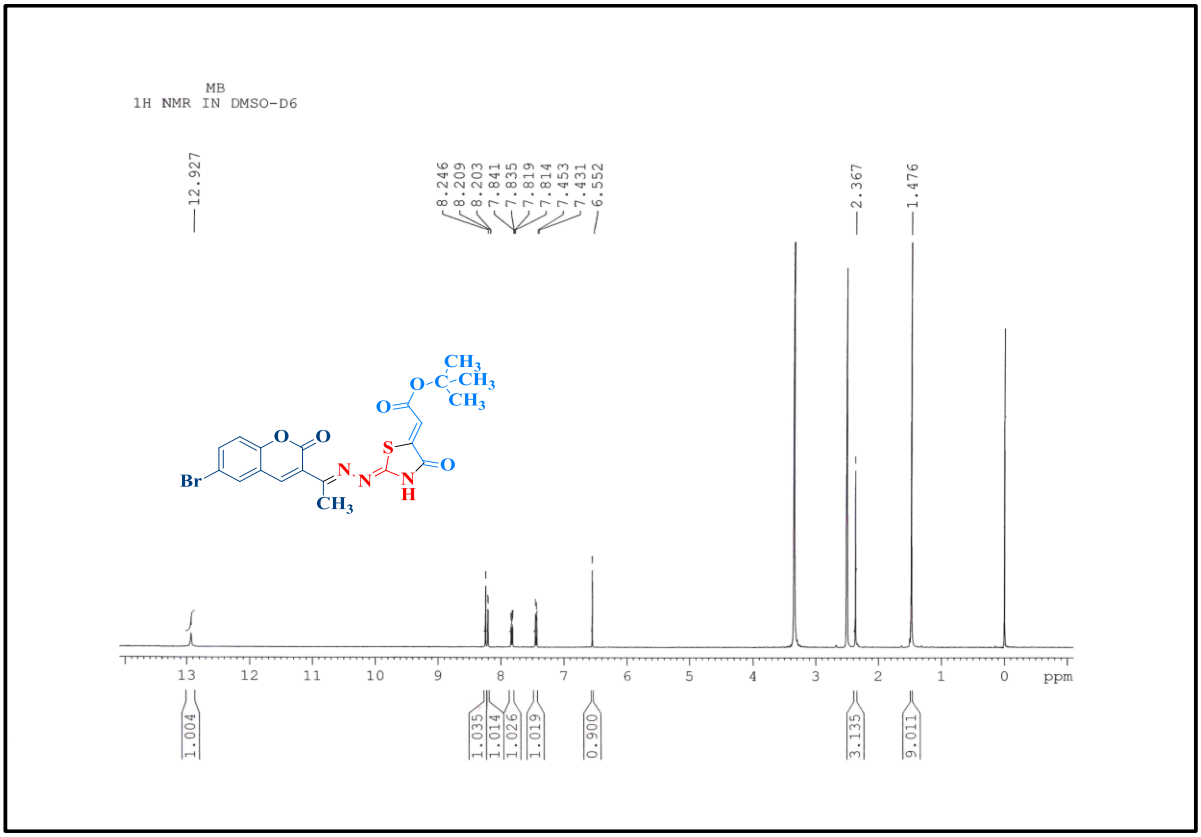


Fig. [1]H NMR spectrum of compound **43d**

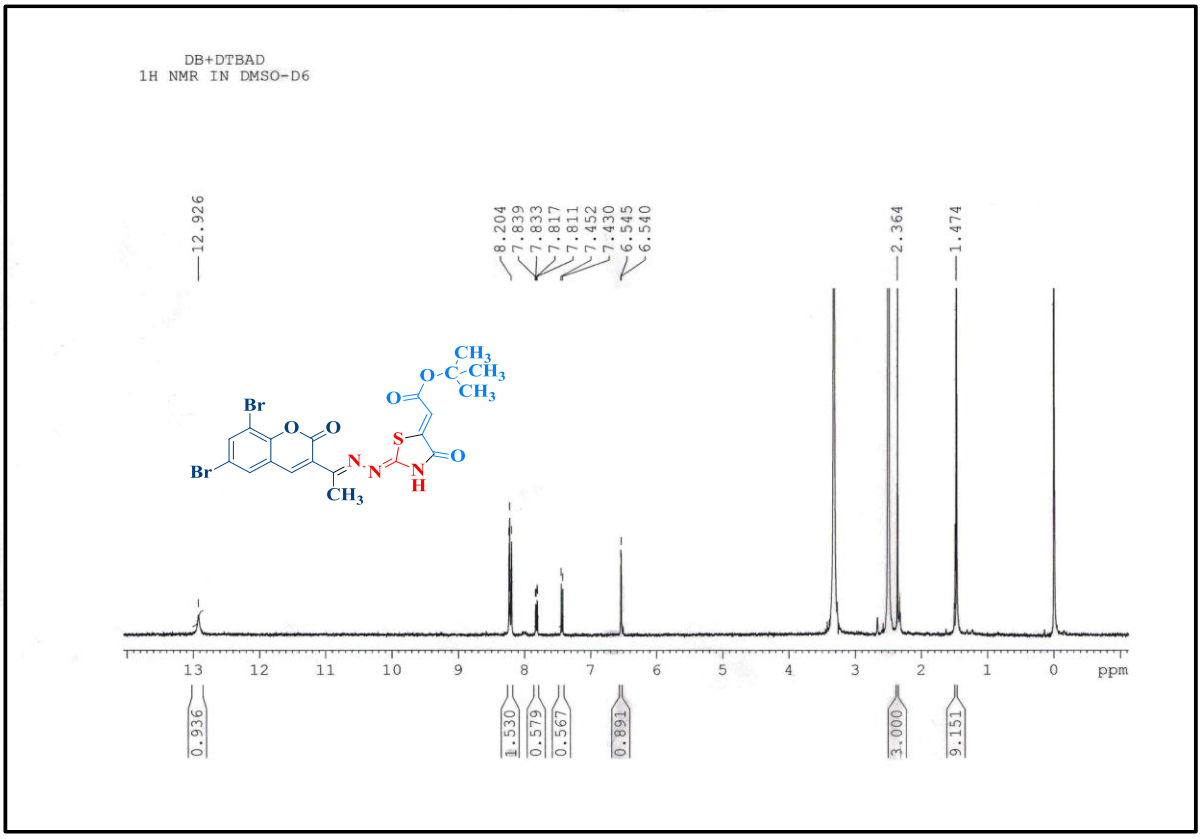


Fig. ^{1}H NMR spectrum of compound **43e**

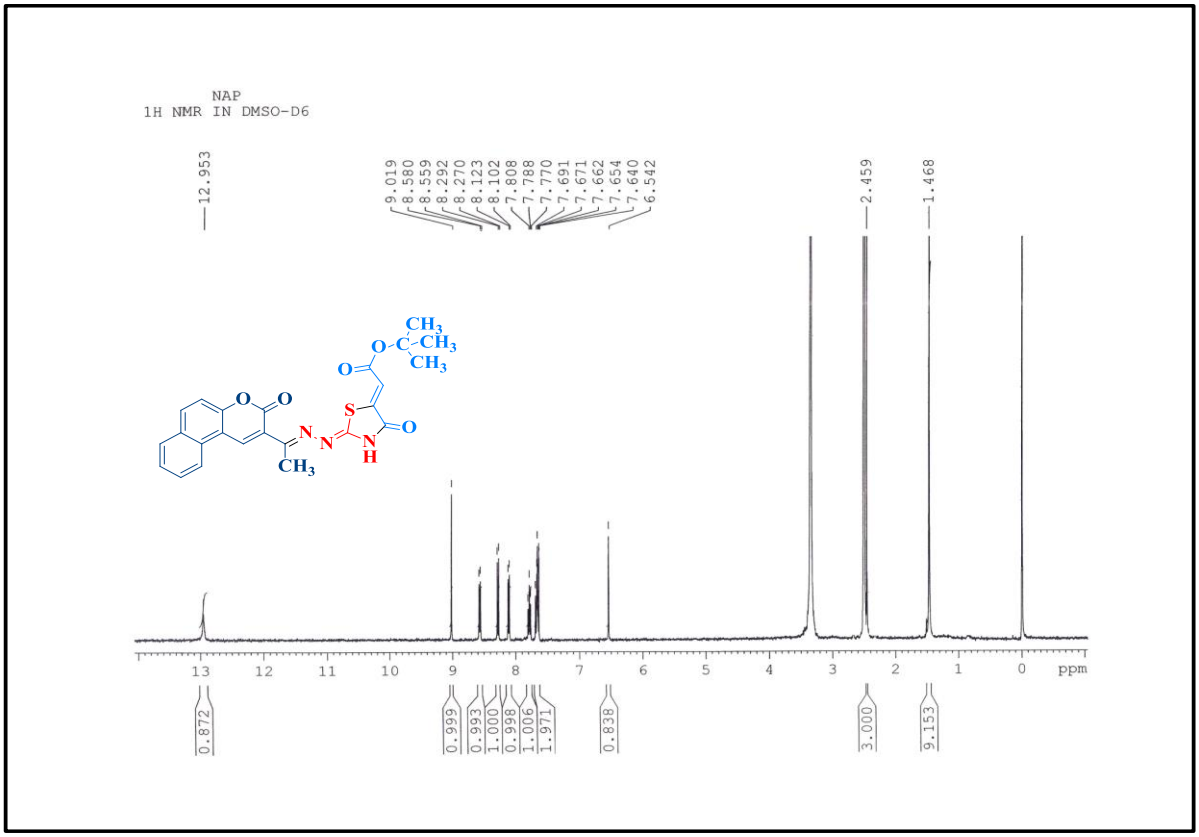


Fig. ^{1}H NMR spectrum of compound **43f**

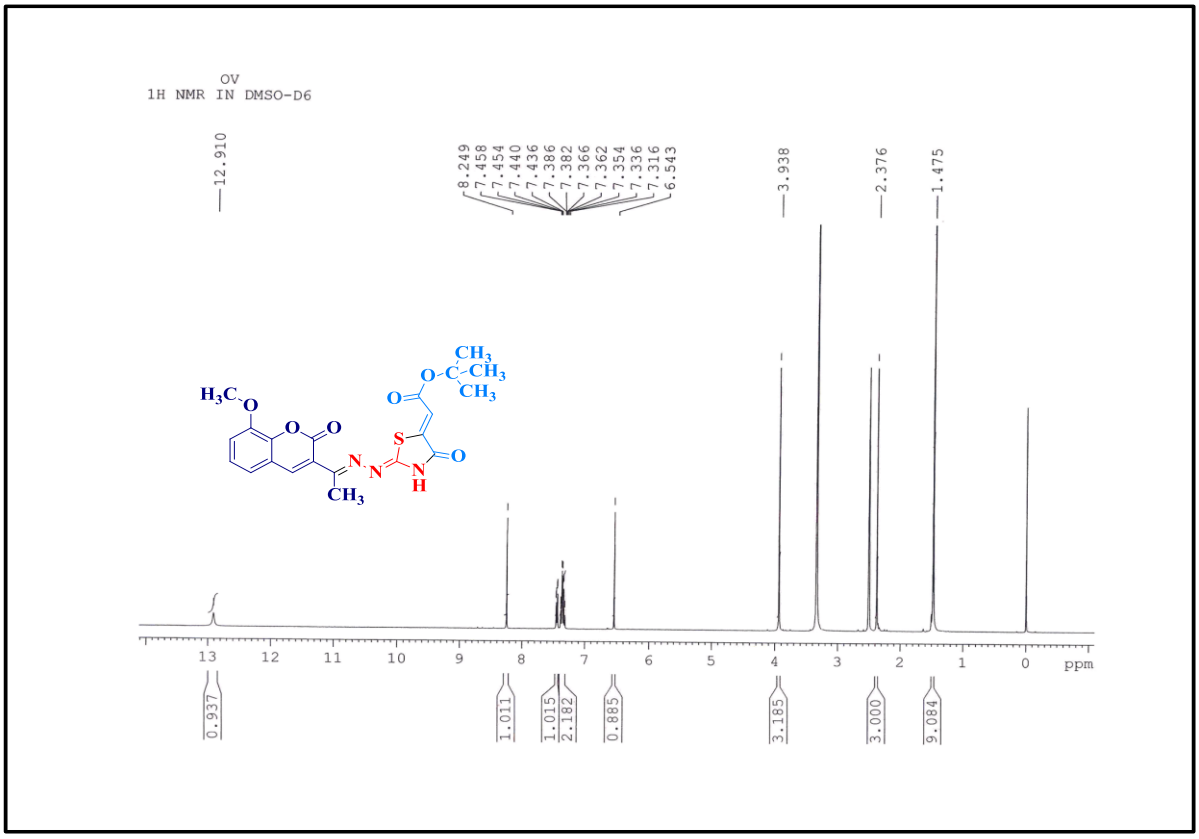


Fig. [1]H NMR spectrum of compound **43g**

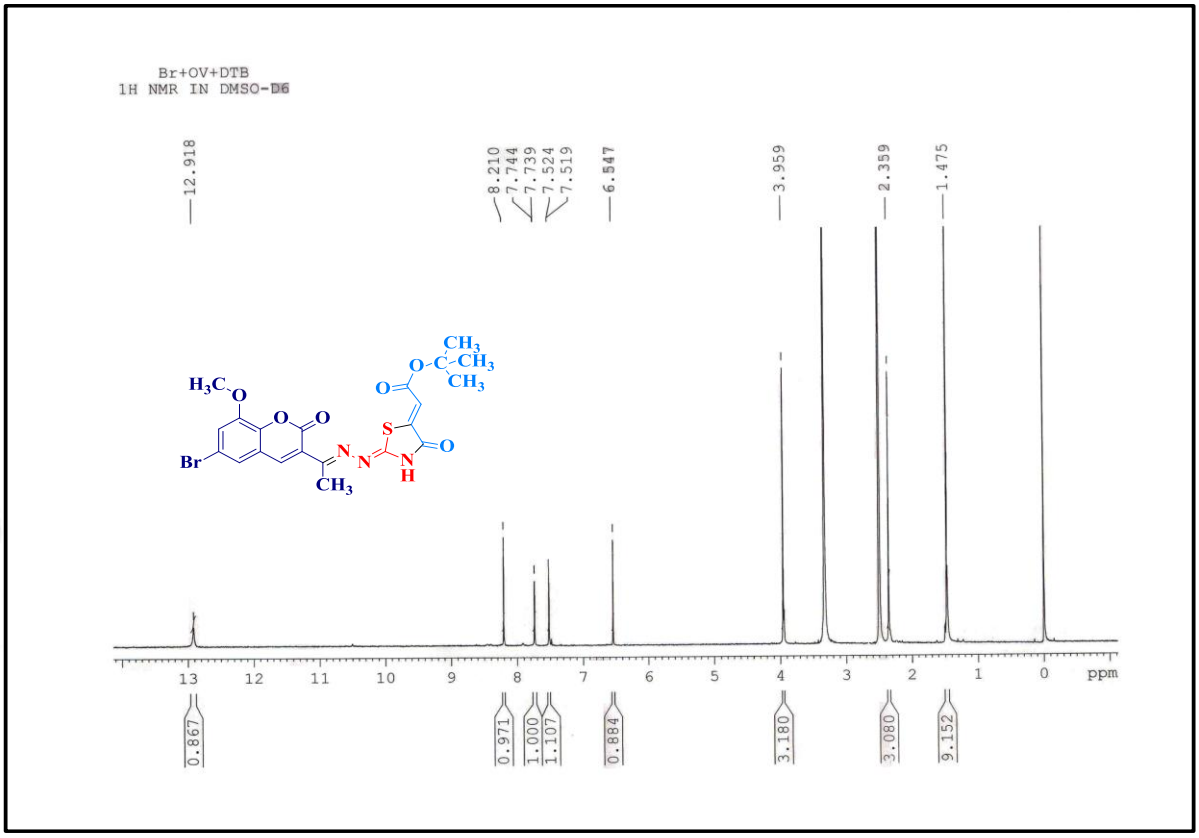


Fig. [1]H NMR spectrum of compound **43h**

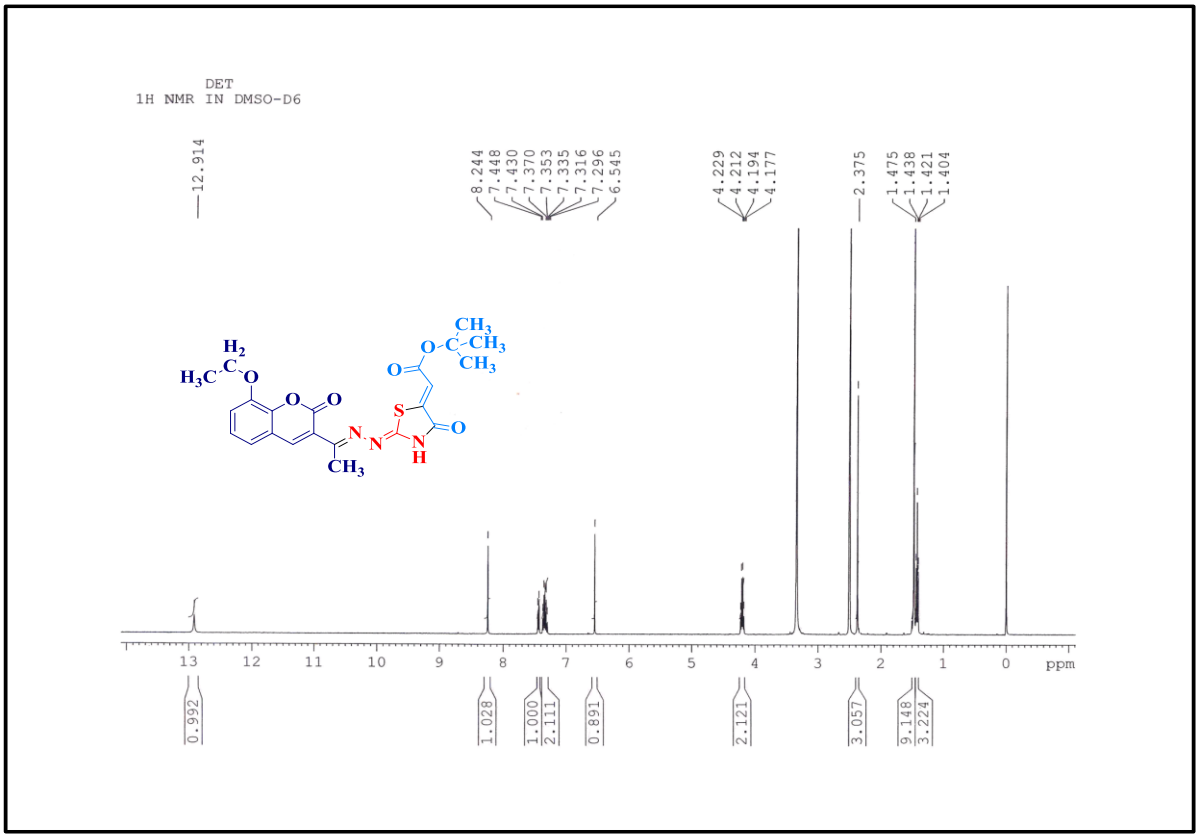


Fig. [1]H NMR spectrum of compound **43i**

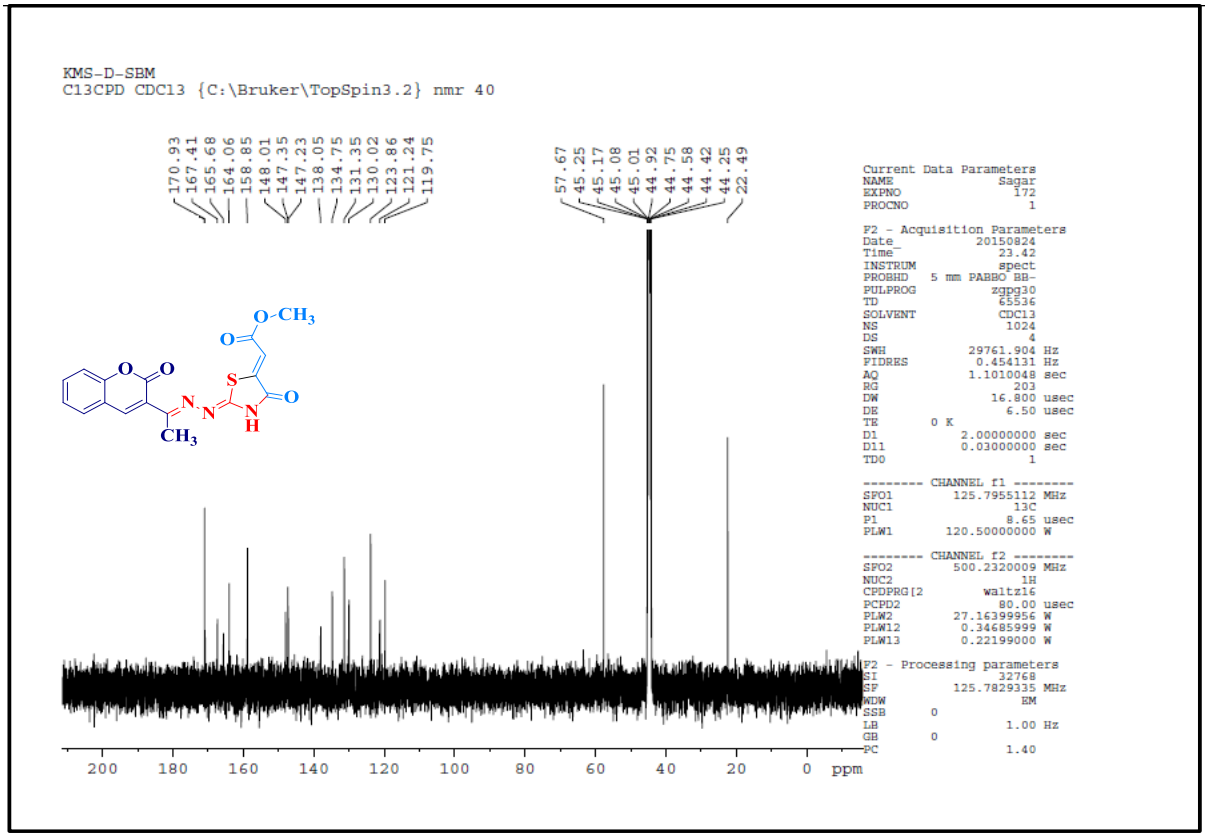


Fig. [13]C NMR spectrum of compound **41a**

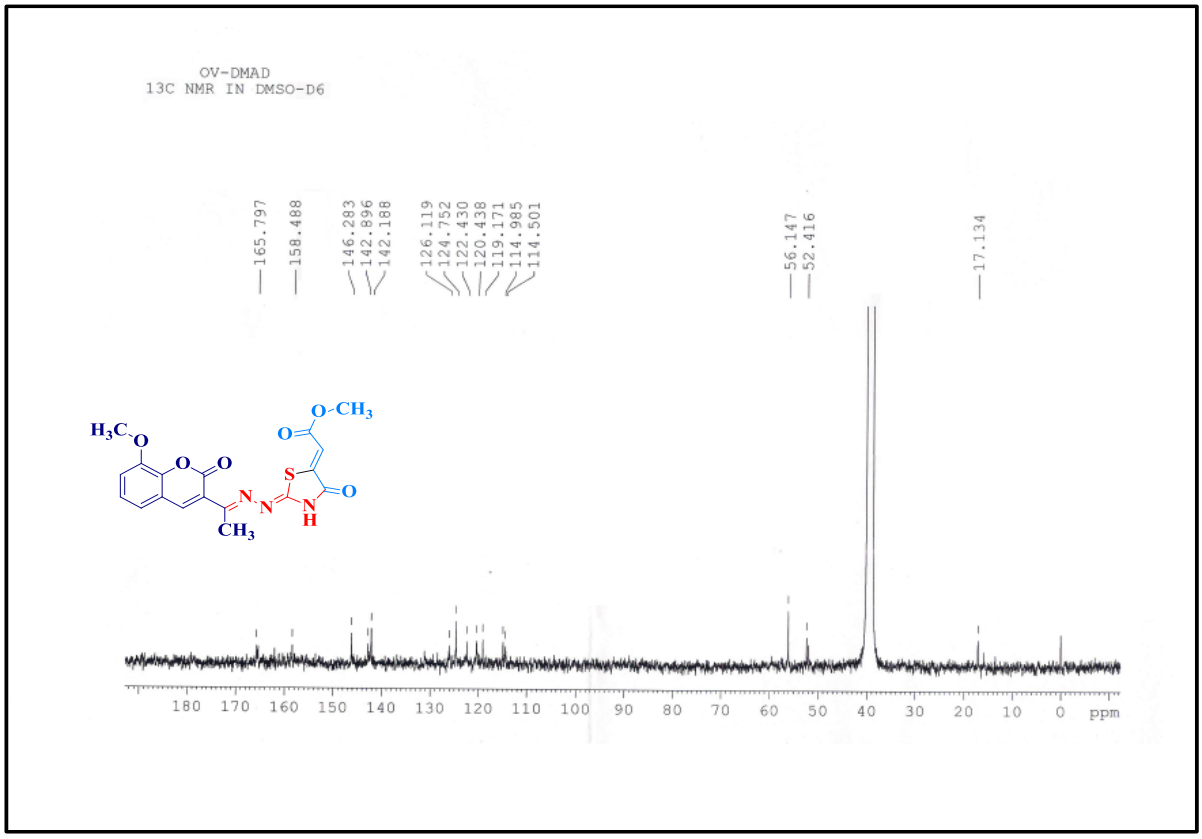


Fig. ^{13}C NMR spectrum of compound **41g**

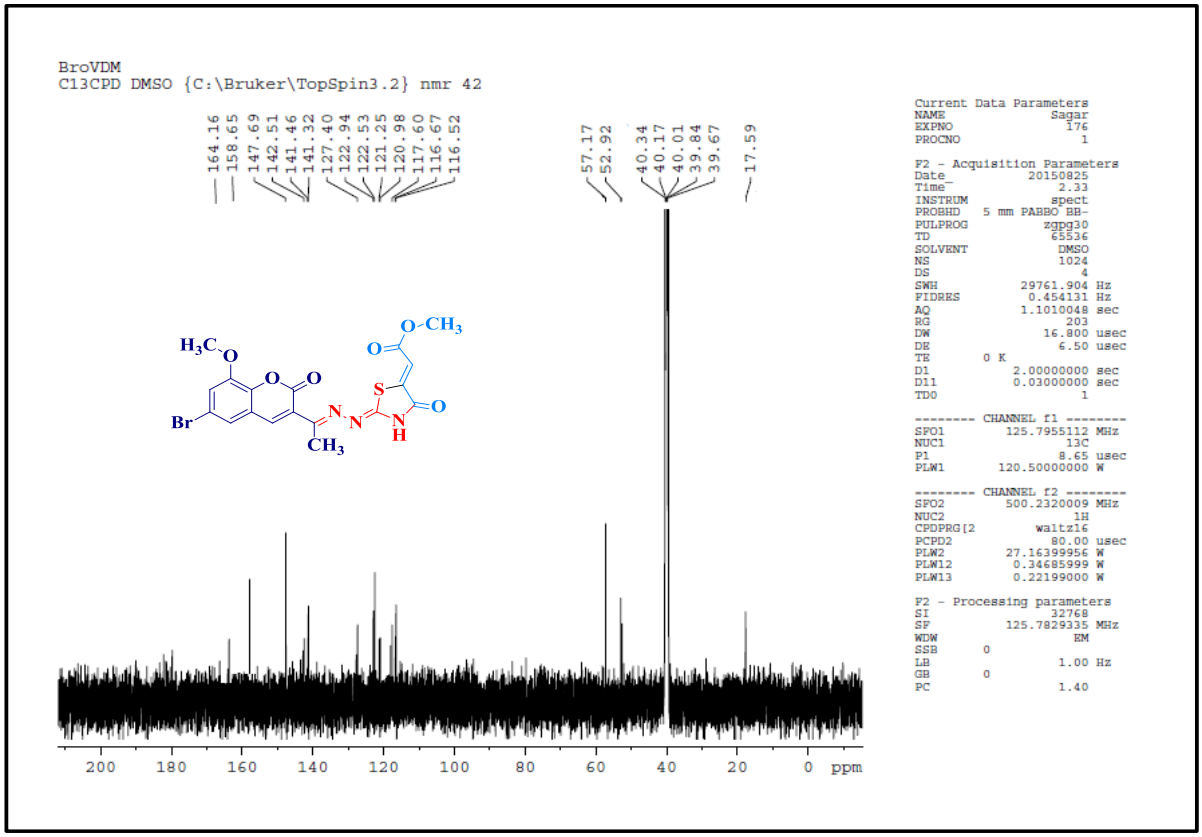


Fig. ^{13}C NMR spectrum of compound **41h**

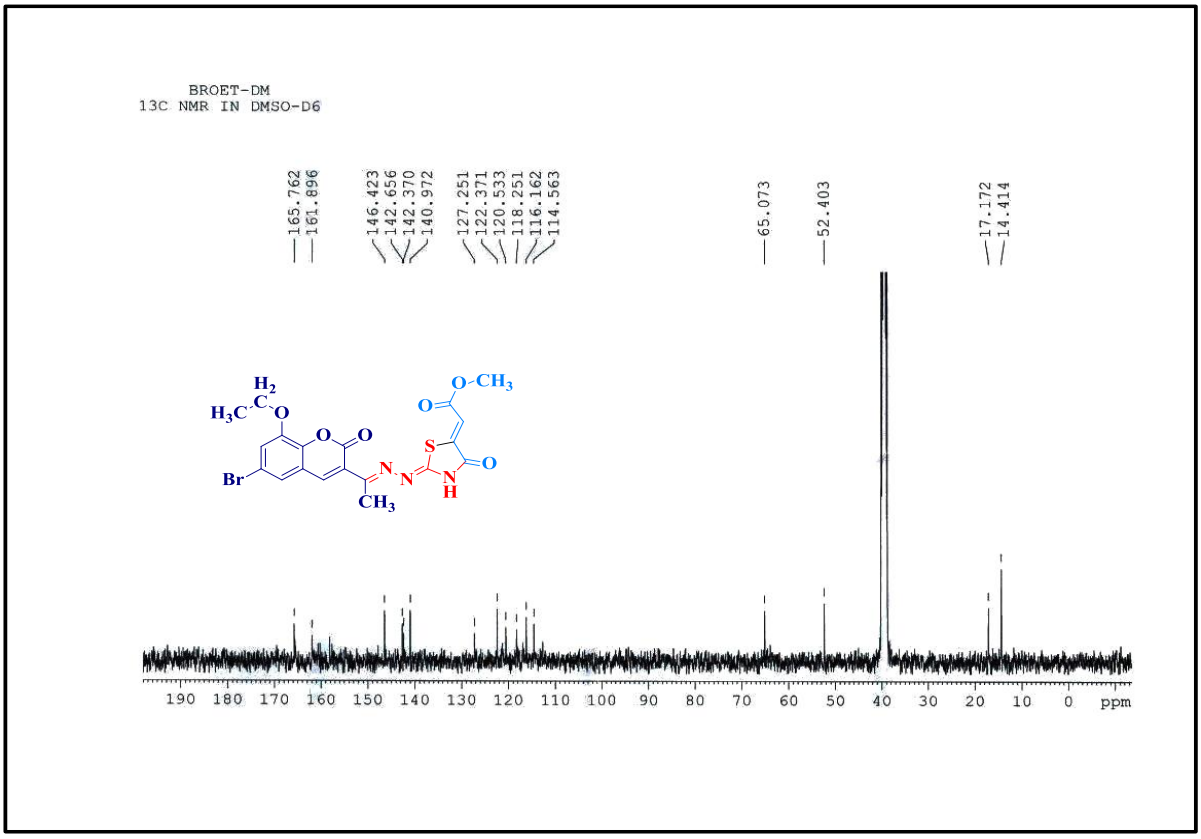


Fig. ^{13}C NMR spectrum of compound **41j**

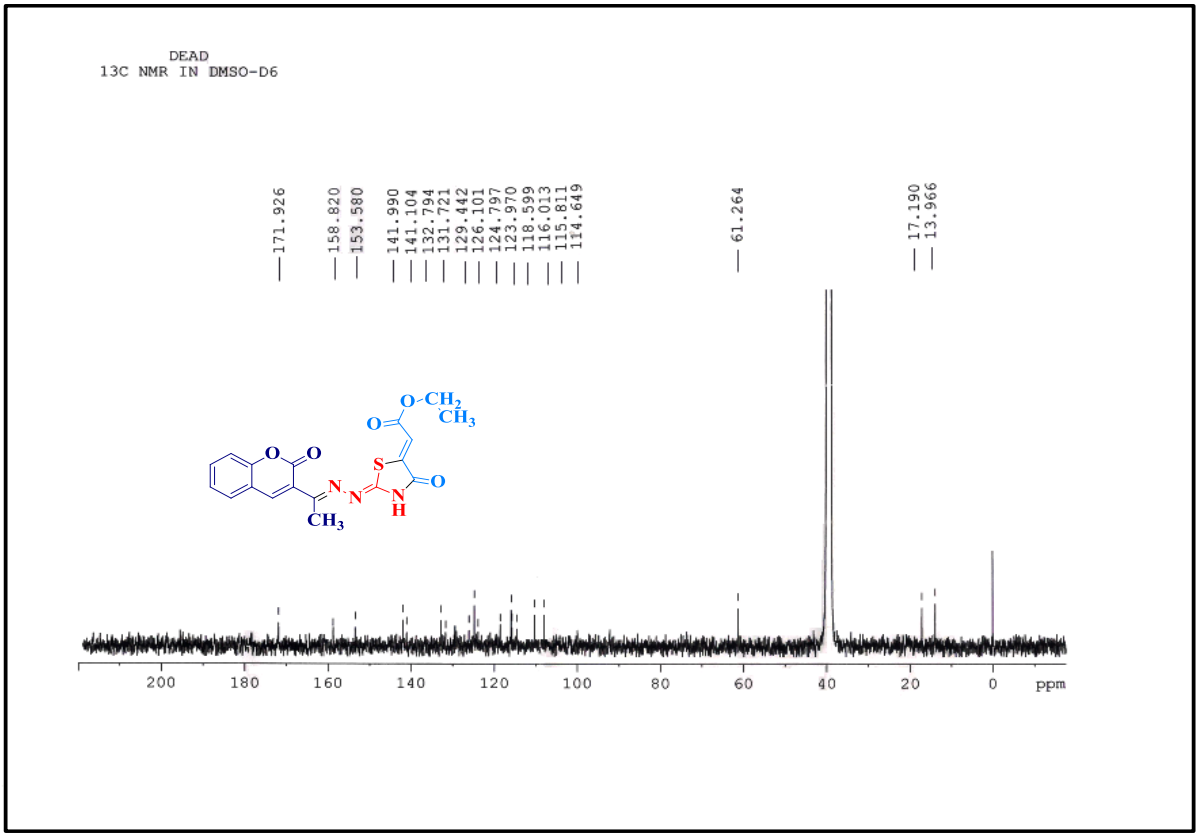


Fig. ^{13}C NMR spectrum of compound **42a**

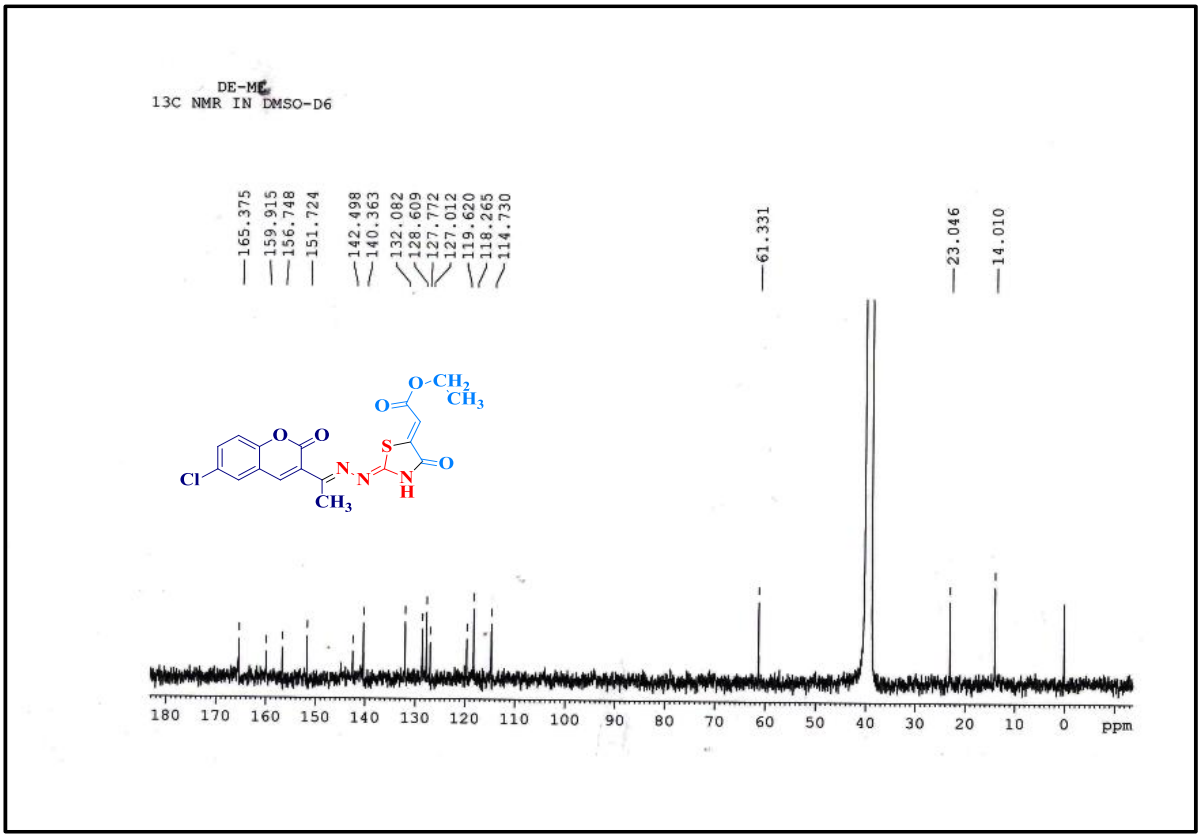


Fig. ^{13}C NMR spectrum of compound **42b**

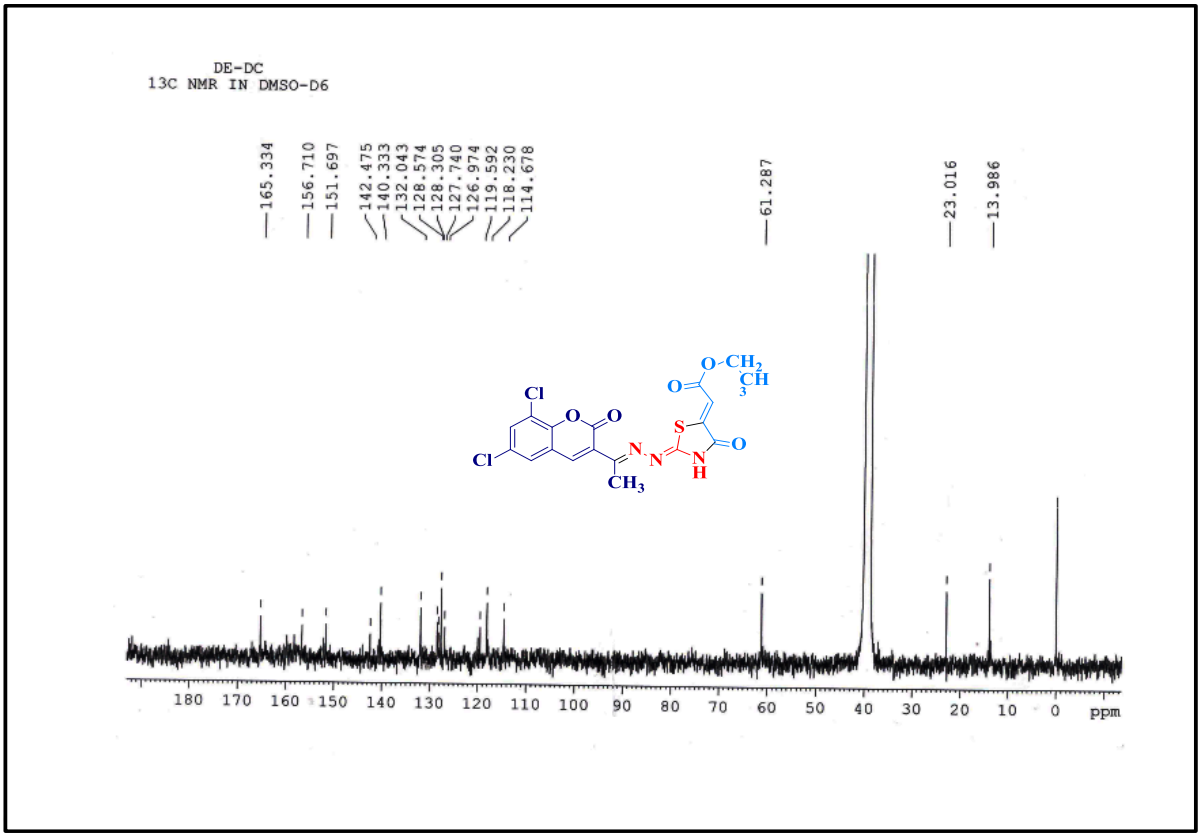


Fig. ^{13}C NMR spectrum of compound **42c**

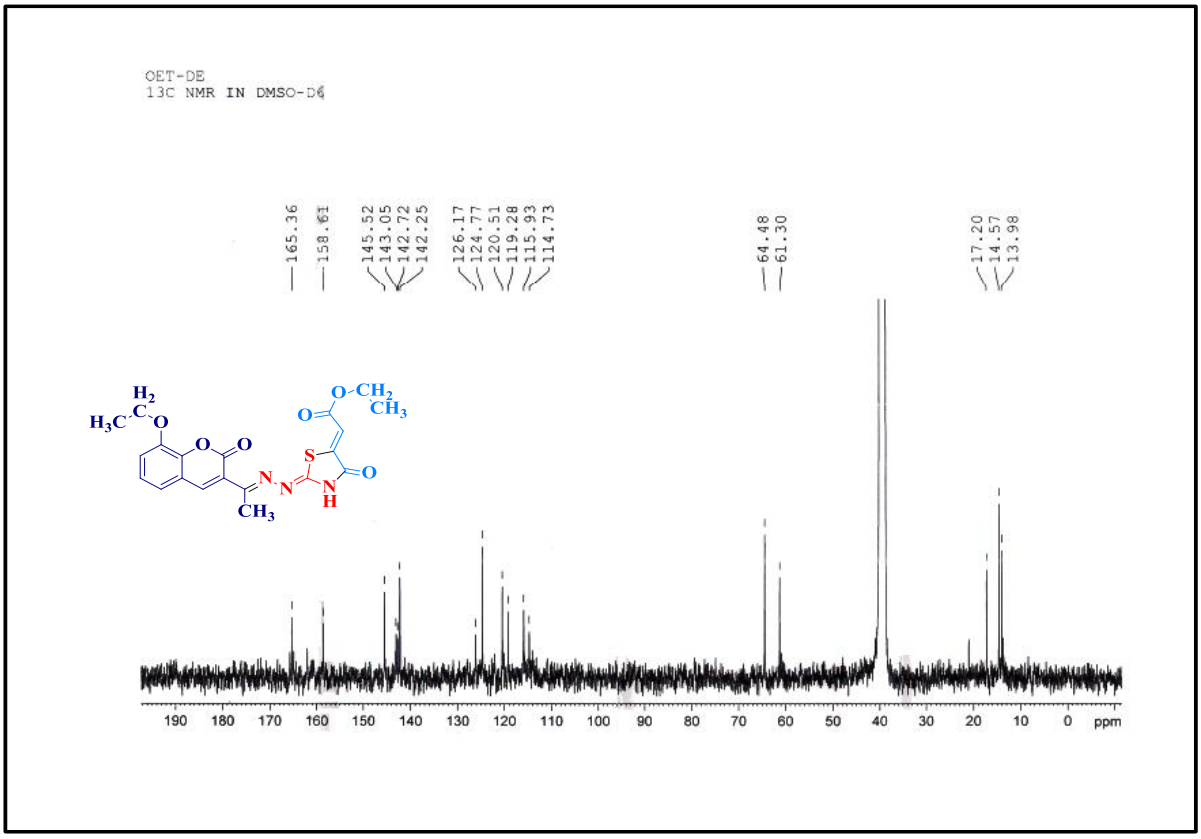


Fig. ^{13}C NMR spectrum of compound **42i**

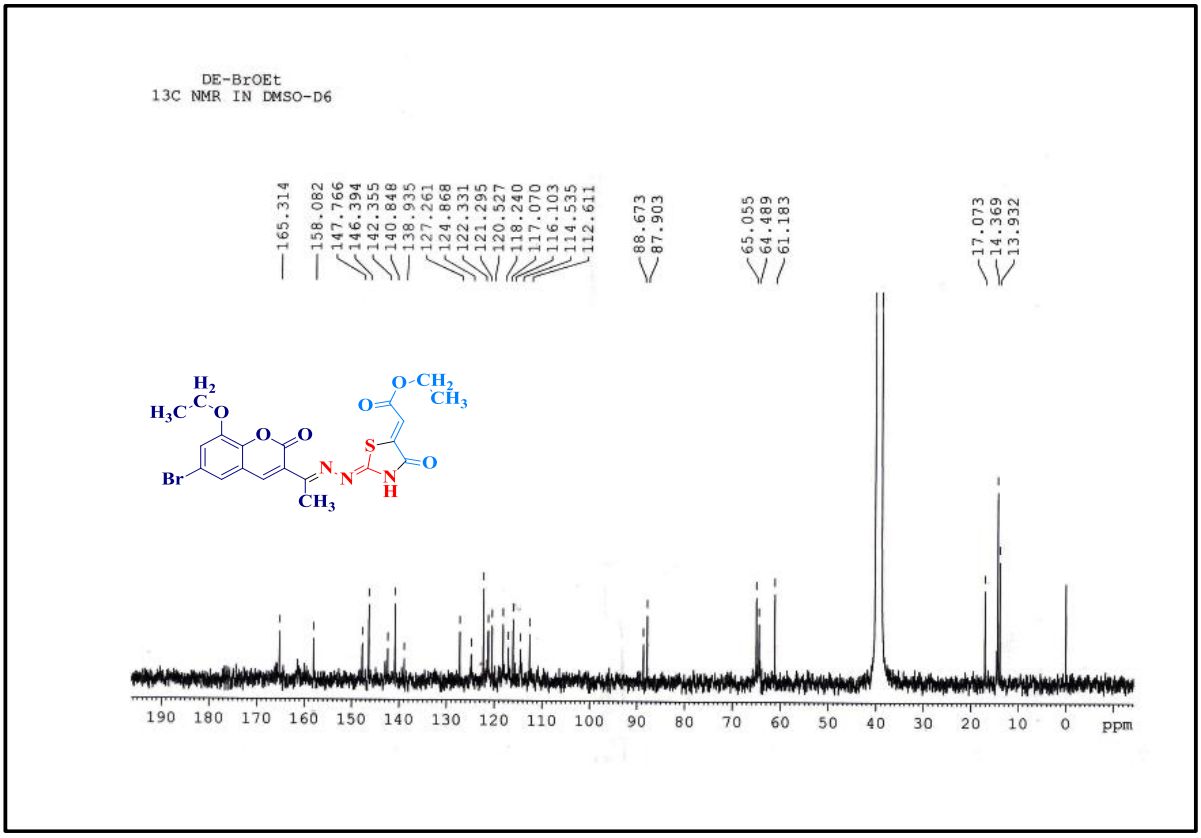


Fig. ^{13}C NMR spectrum of compound **42j**

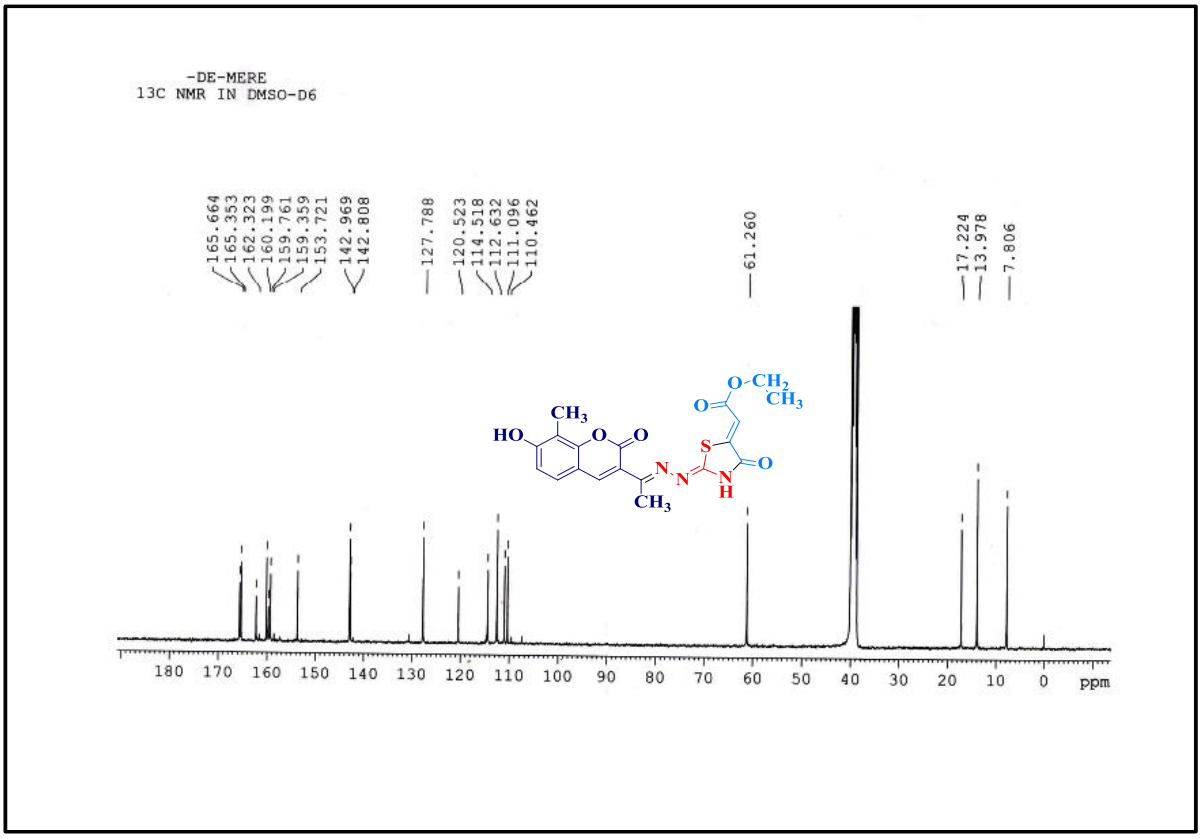


Fig. ^{13}C NMR spectrum of compound **42l**

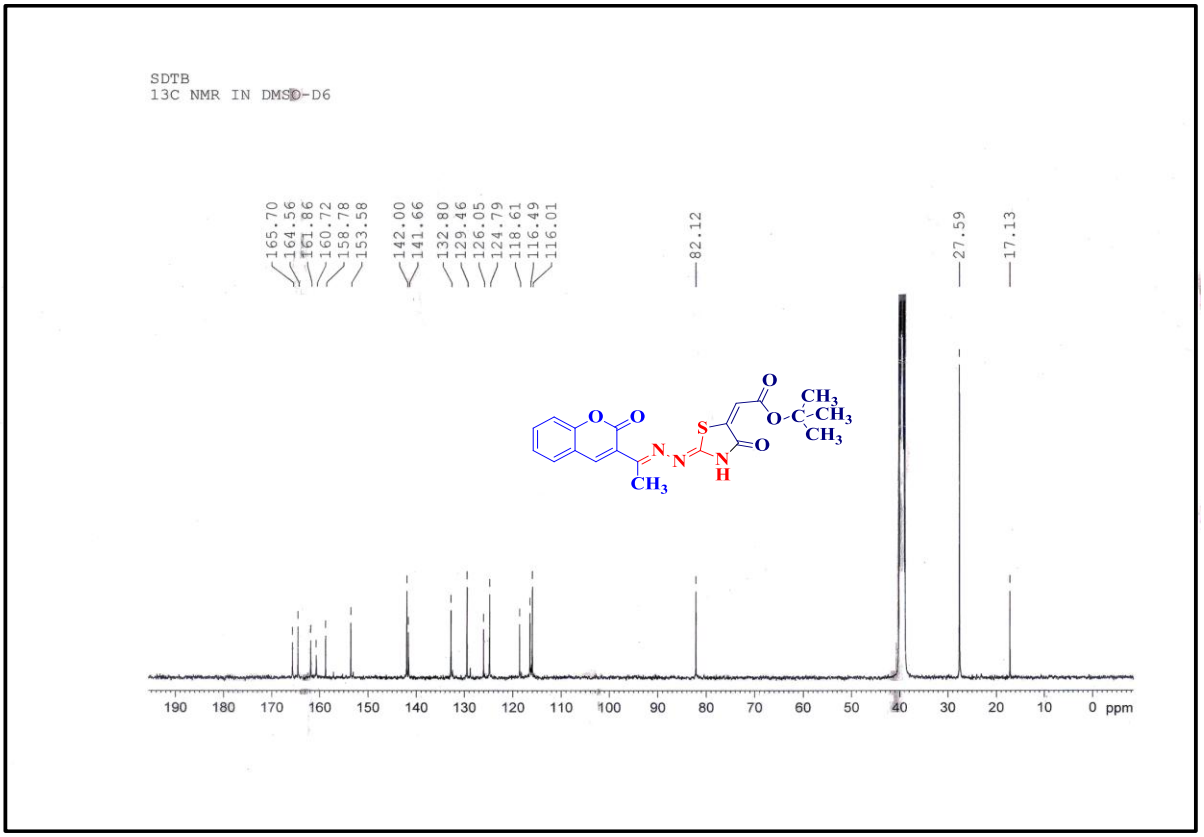


Fig. ^{13}C NMR spectrum of compound **43a**

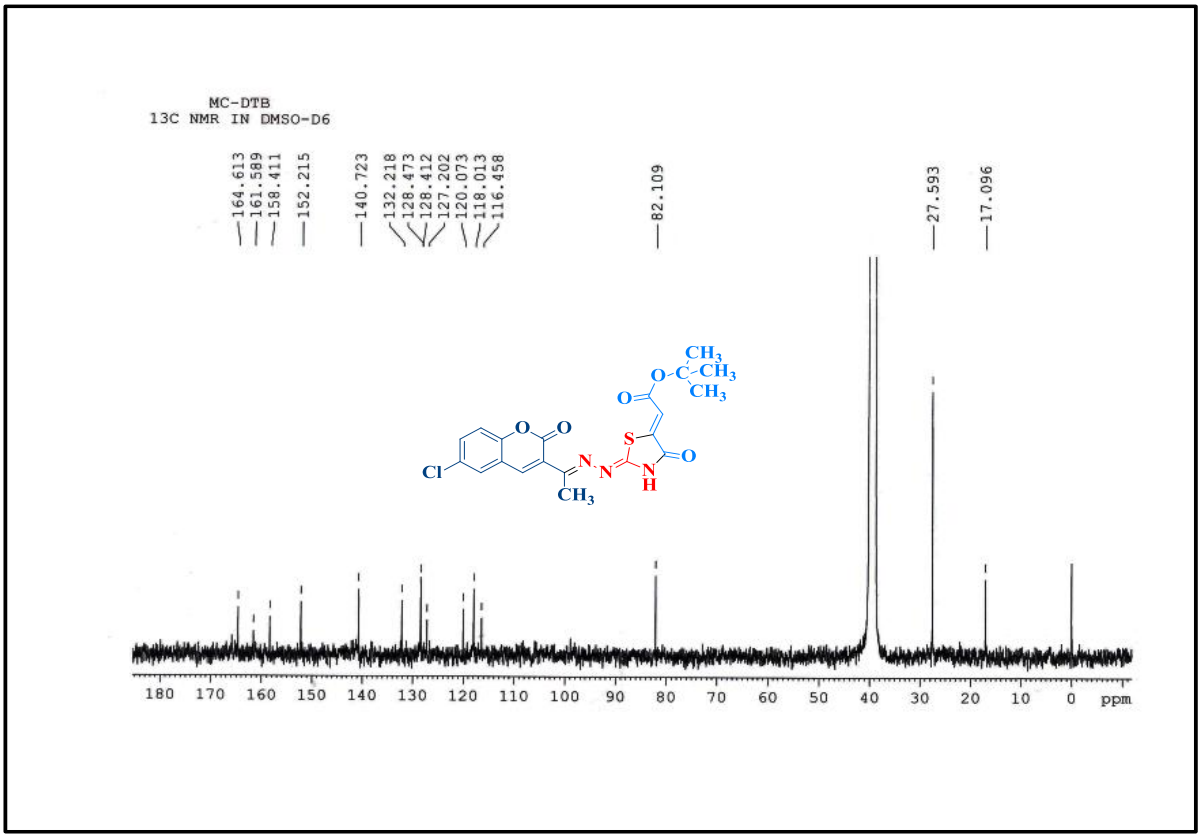


Fig. ^{13}C NMR spectrum of compound **43b**

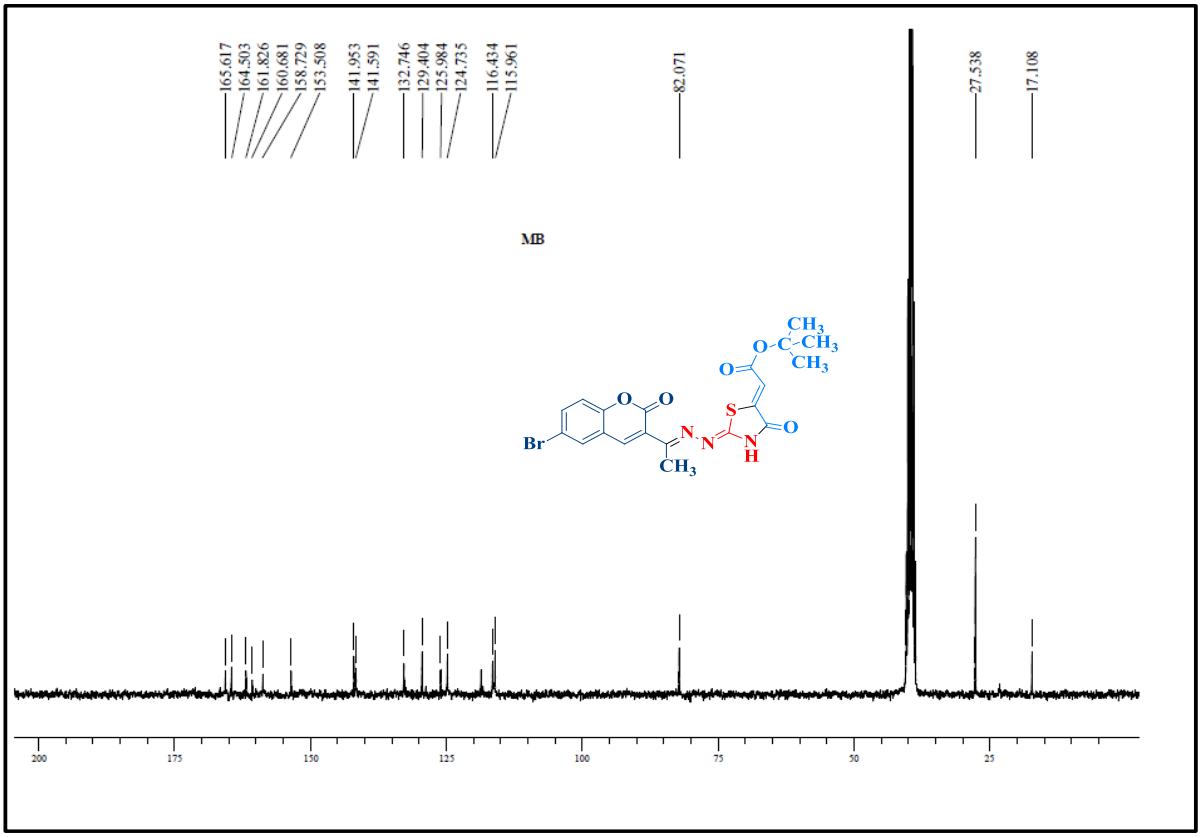


Fig. ^{13}C NMR spectrum of compound **43d**

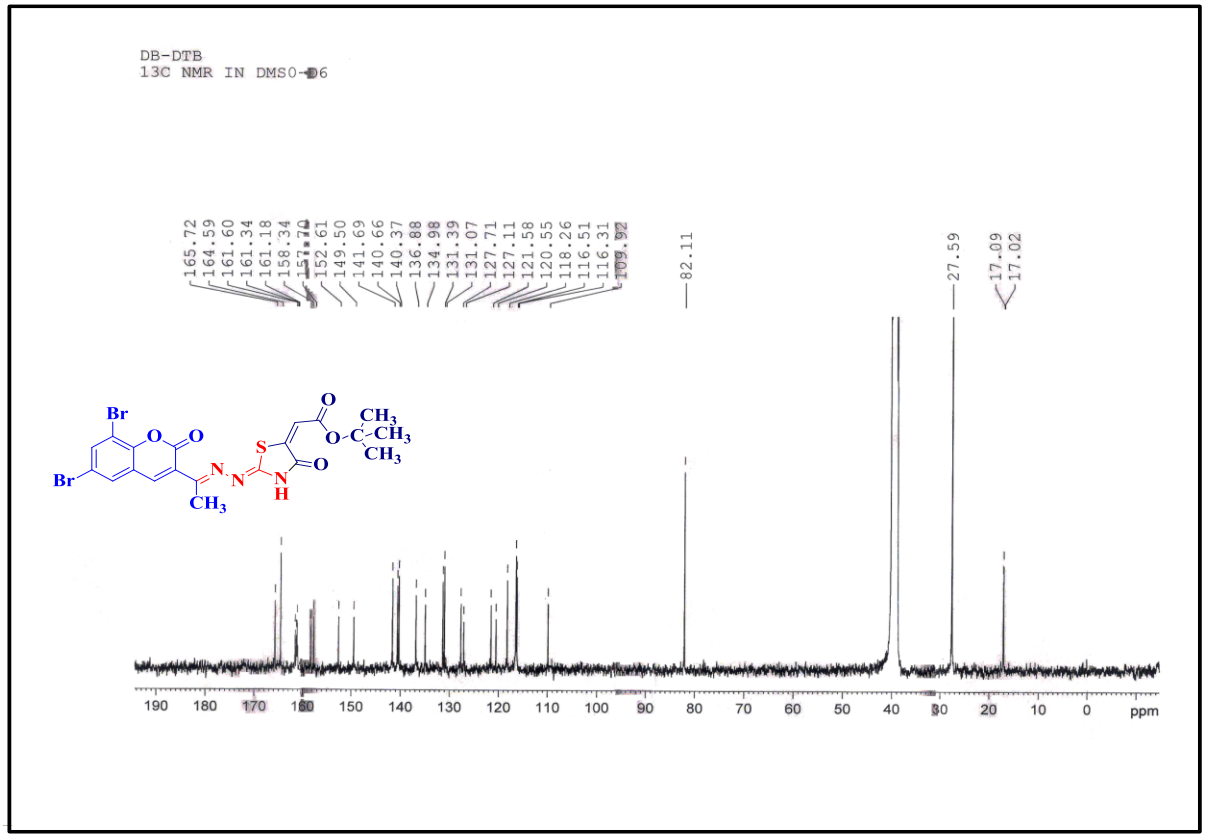


Fig. ^{13}C NMR spectrum of compound **43e**

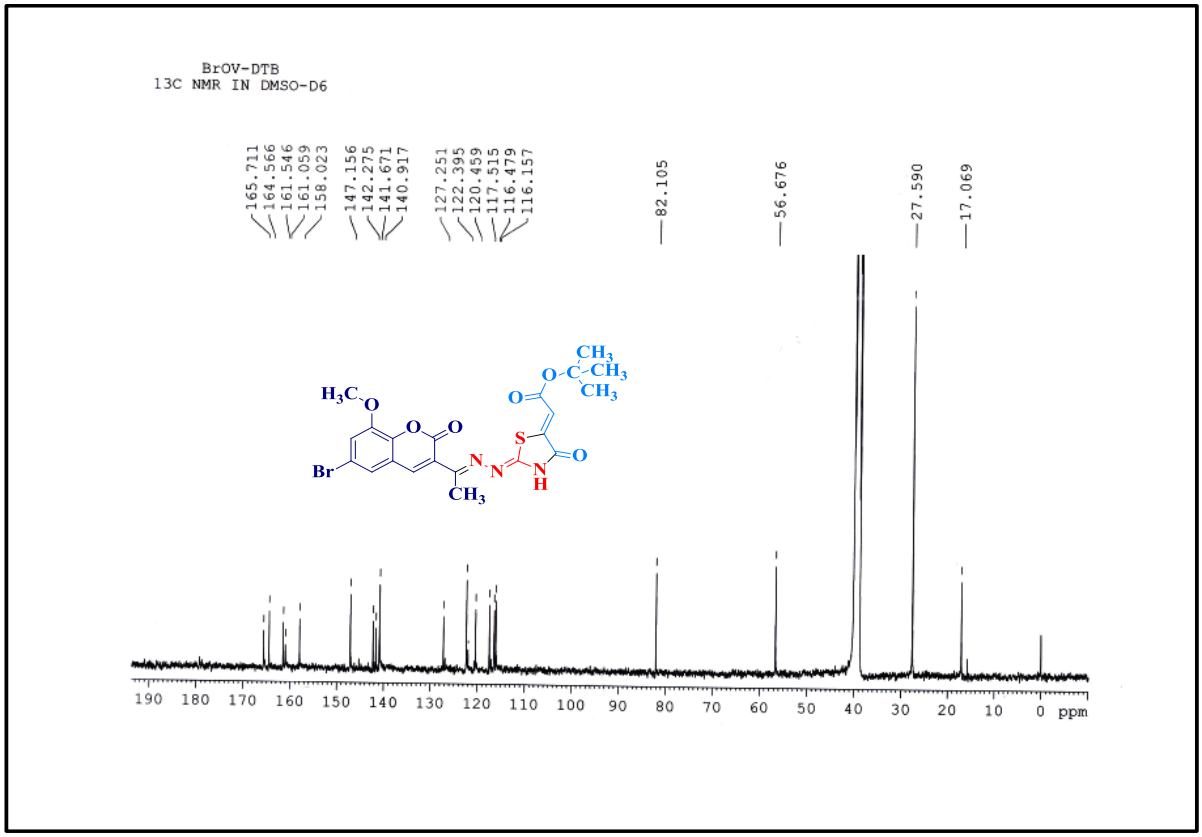


Fig. ^{13}C NMR spectrum of compound **43h**

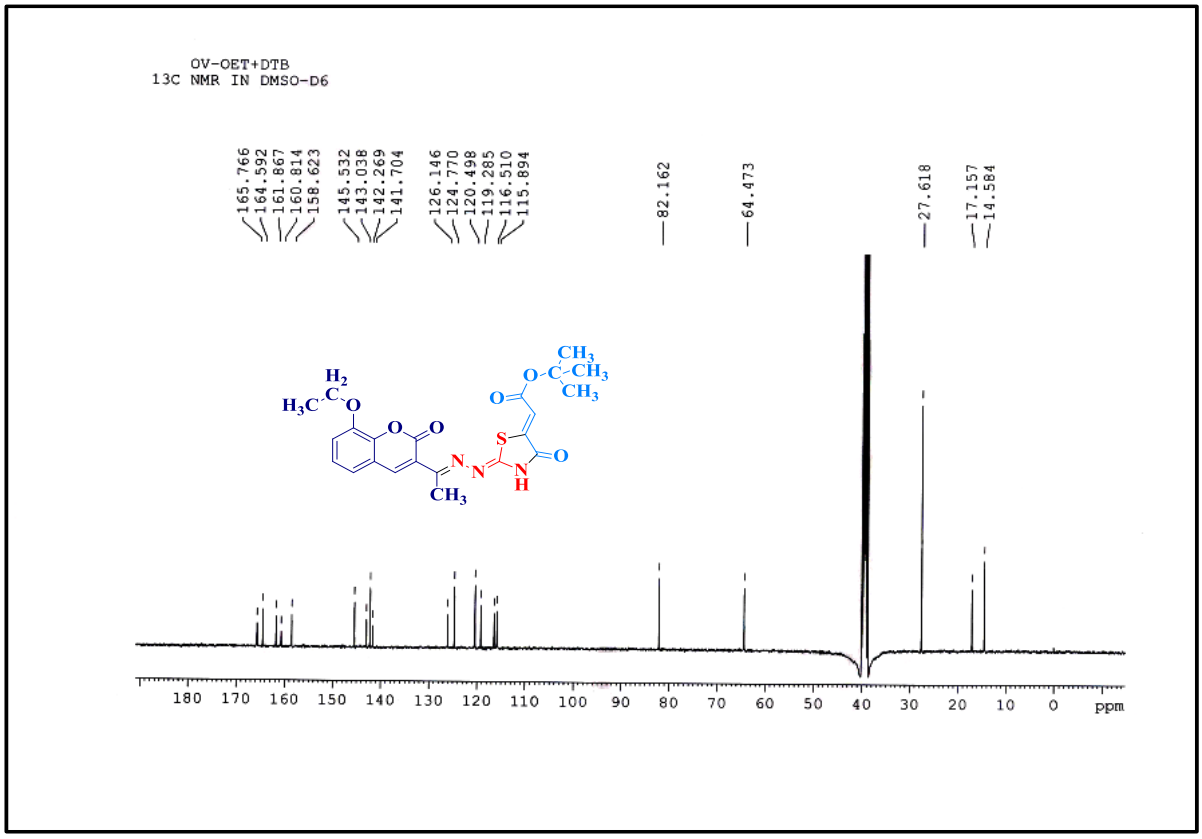


Fig. ^{13}C NMR spectrum of compound **43i**

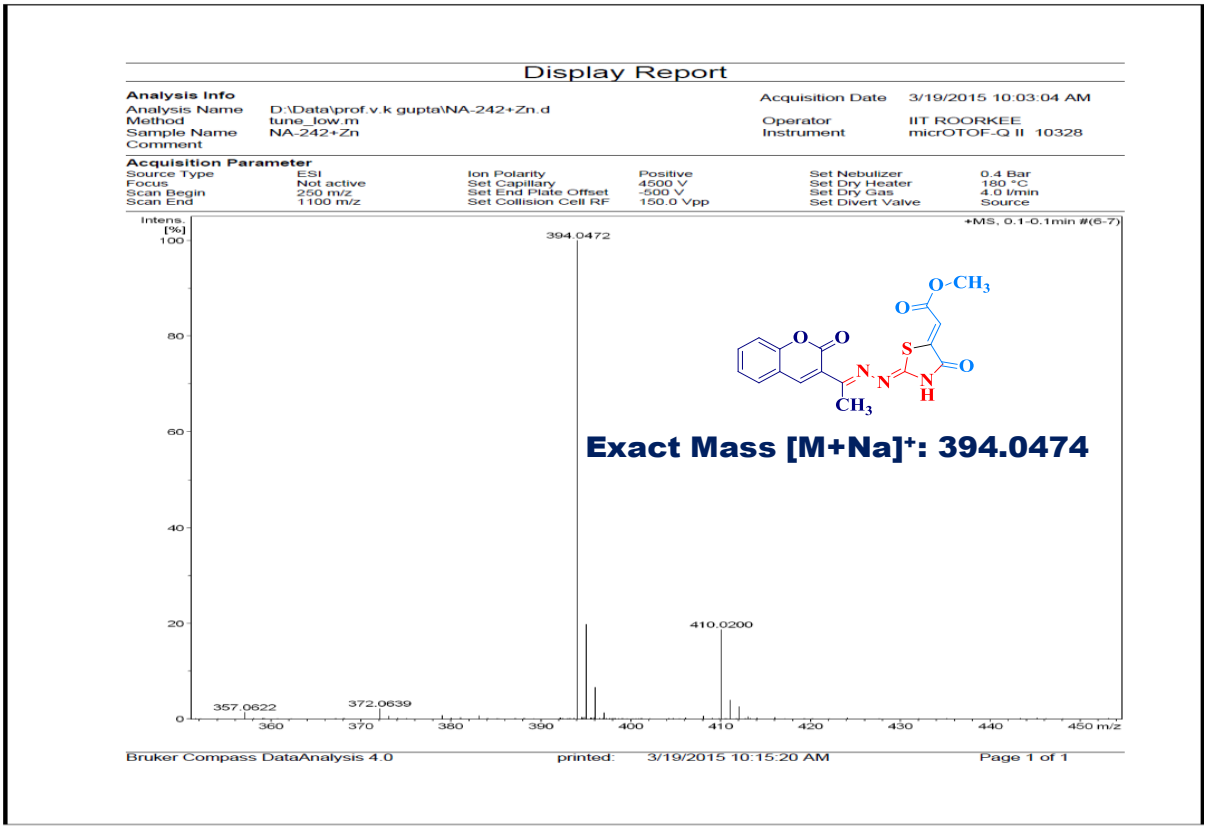


Fig. Mass spectrum of compound **41a**

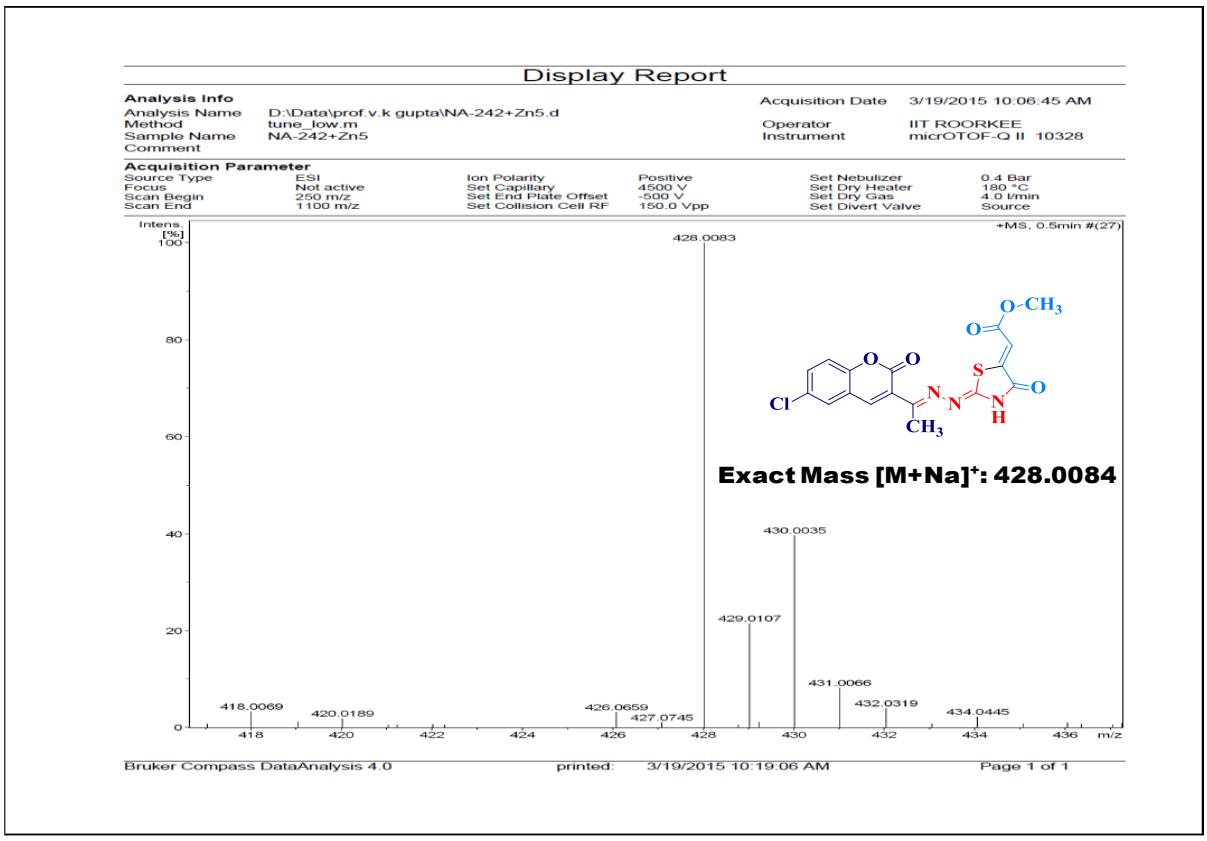


Fig. Mass spectrum of compound **41b**

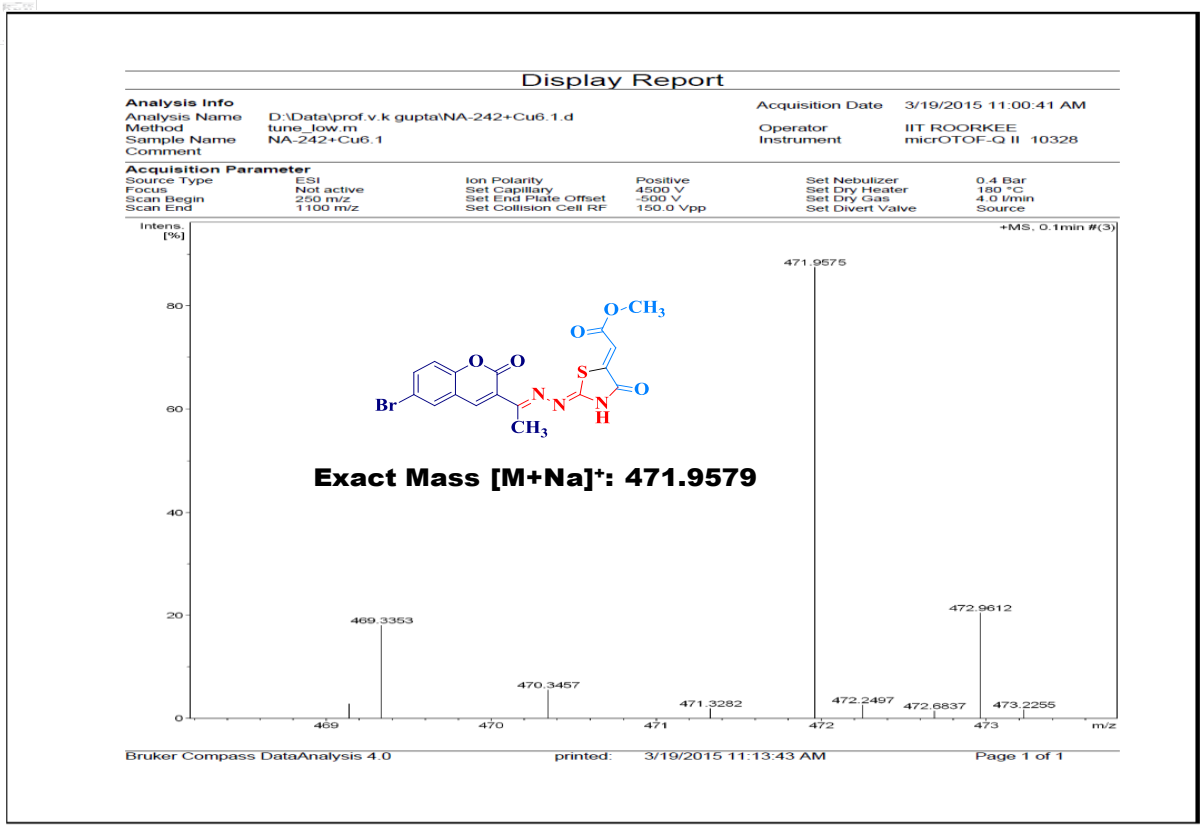


Fig. Mass spectrum of compound **41d**

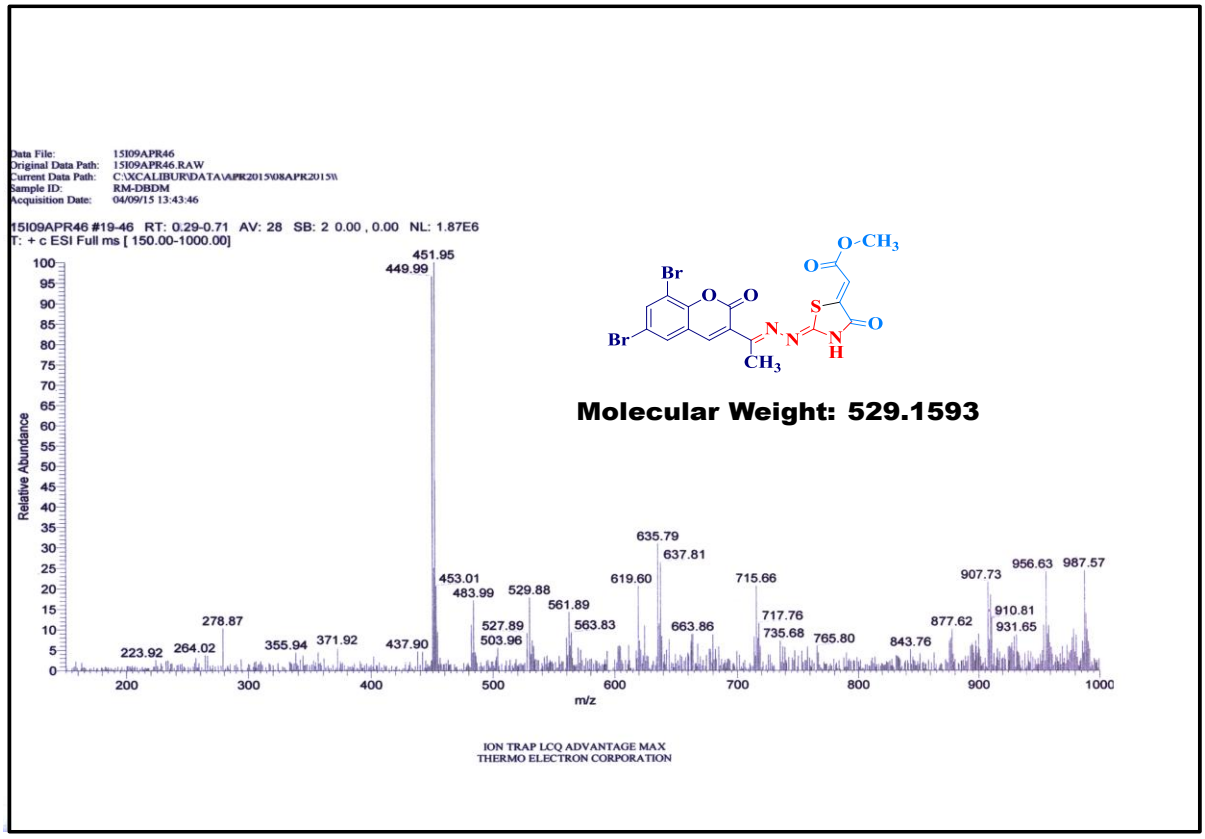


Fig. Mass spectrum of compound **41e**

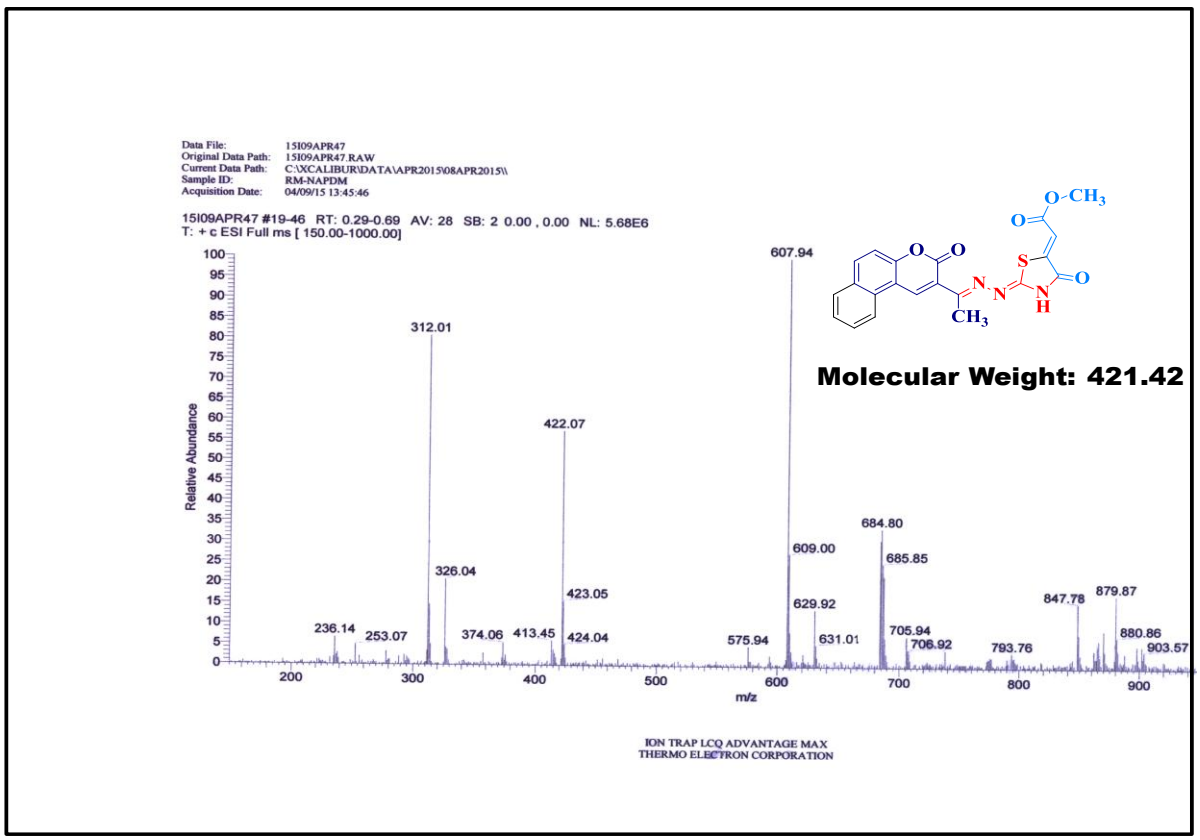


Fig. Mass spectrum of compound **41f**

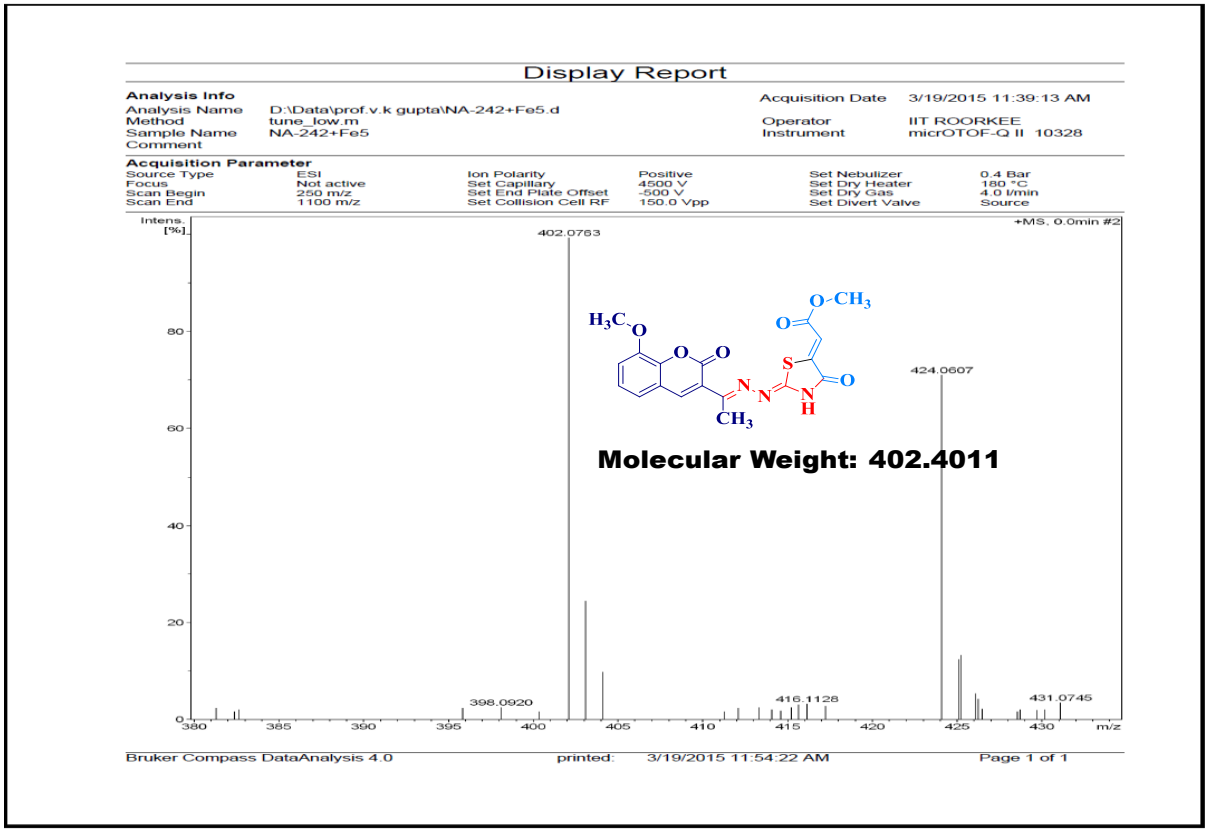


Fig. Mass spectrum of compound **41g**

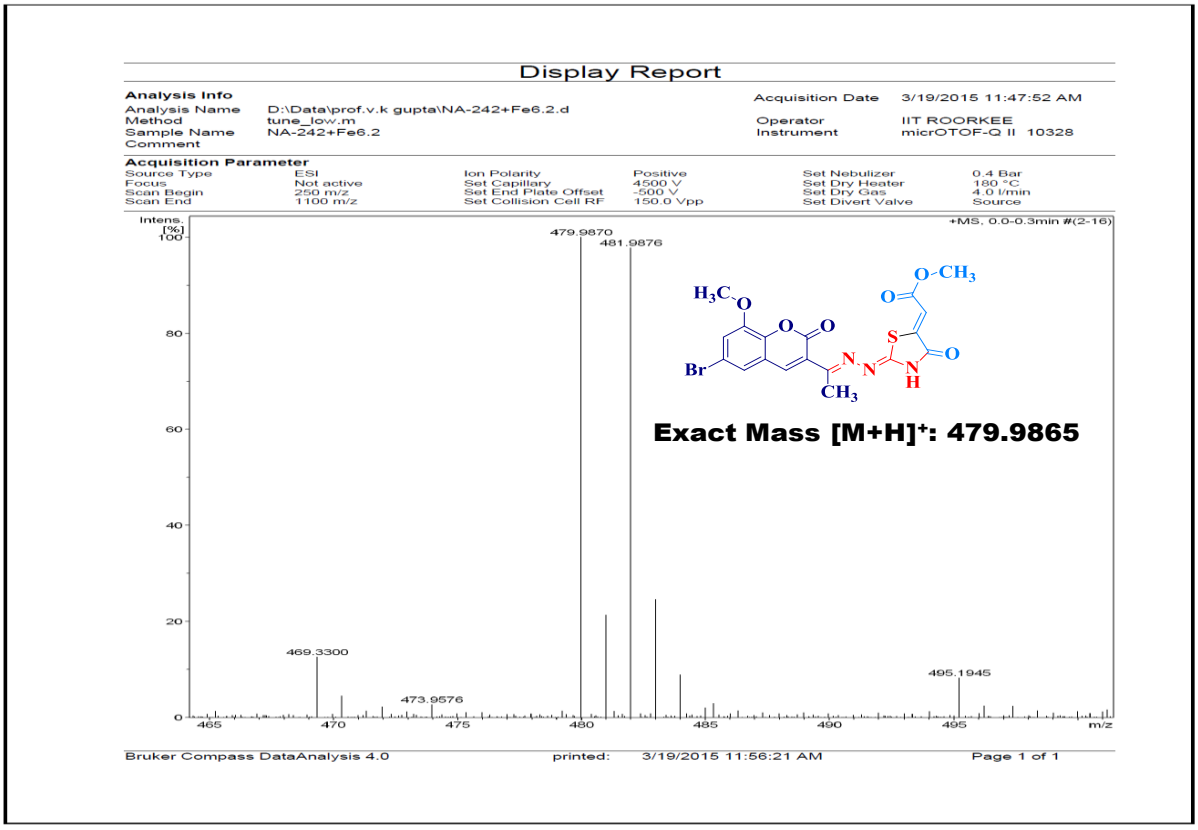


Fig. Mass spectrum of compound **41h**

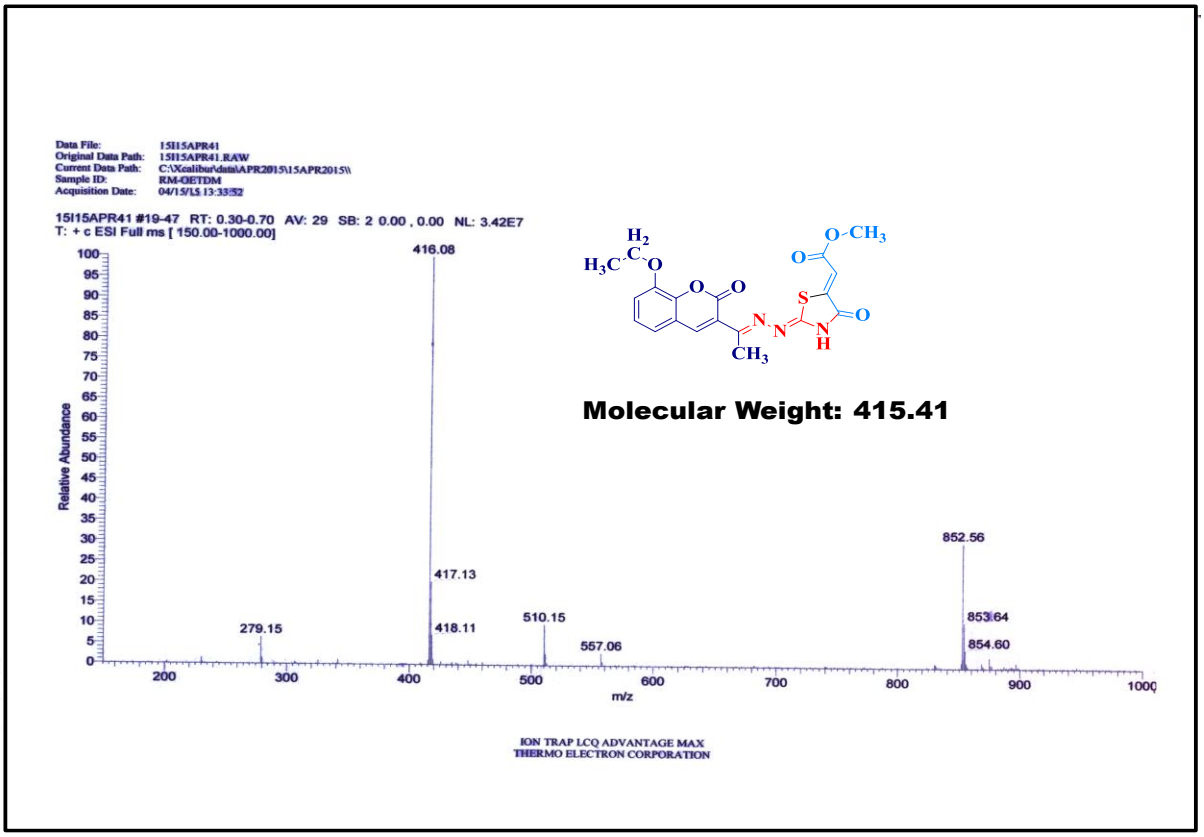


Fig. Mass spectrum of compound **41i**

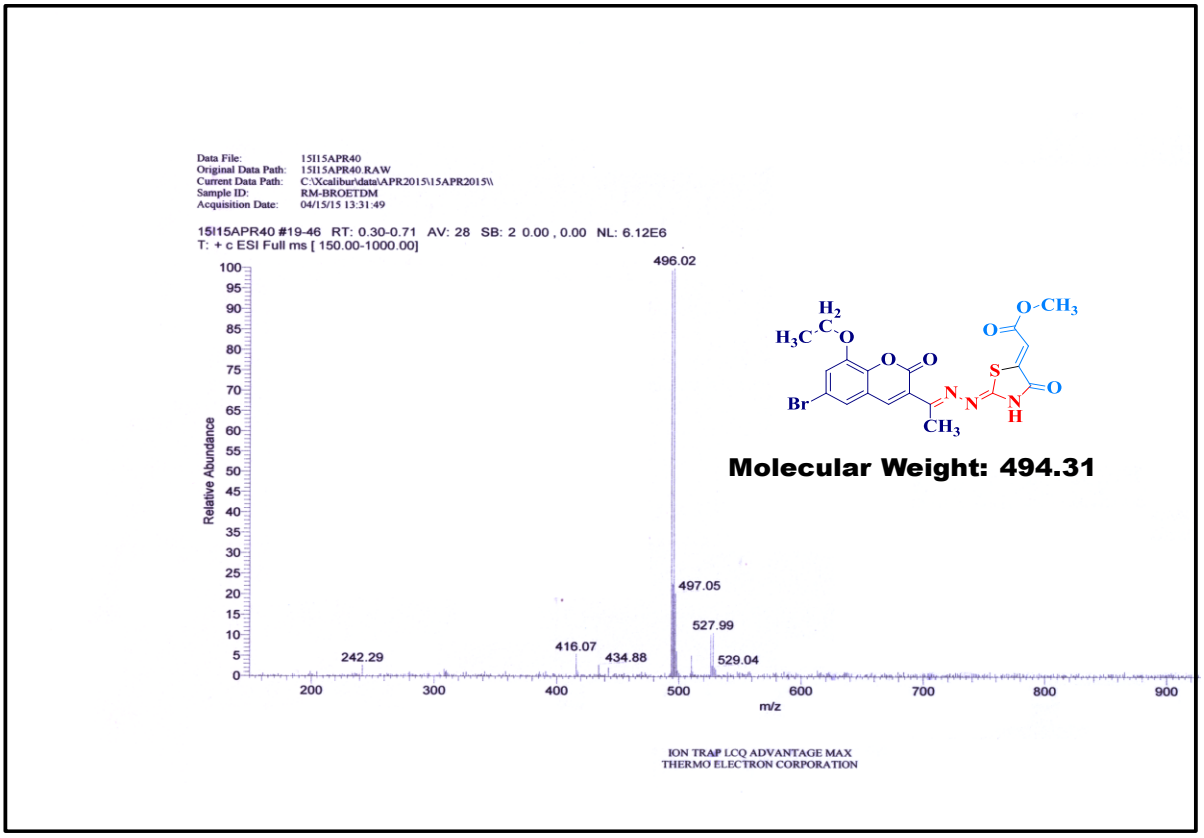


Fig. Mass spectrum of compound **41j**

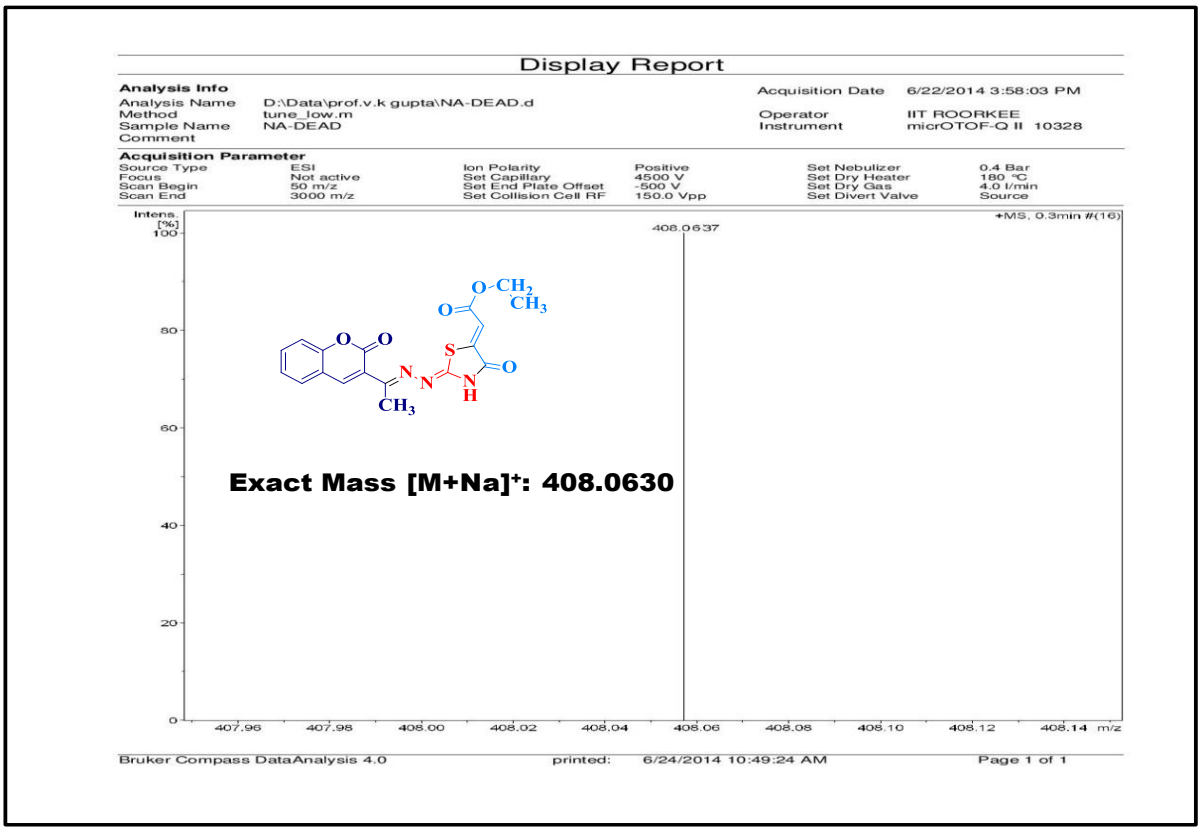


Fig. Mass spectrum of compound **42a**

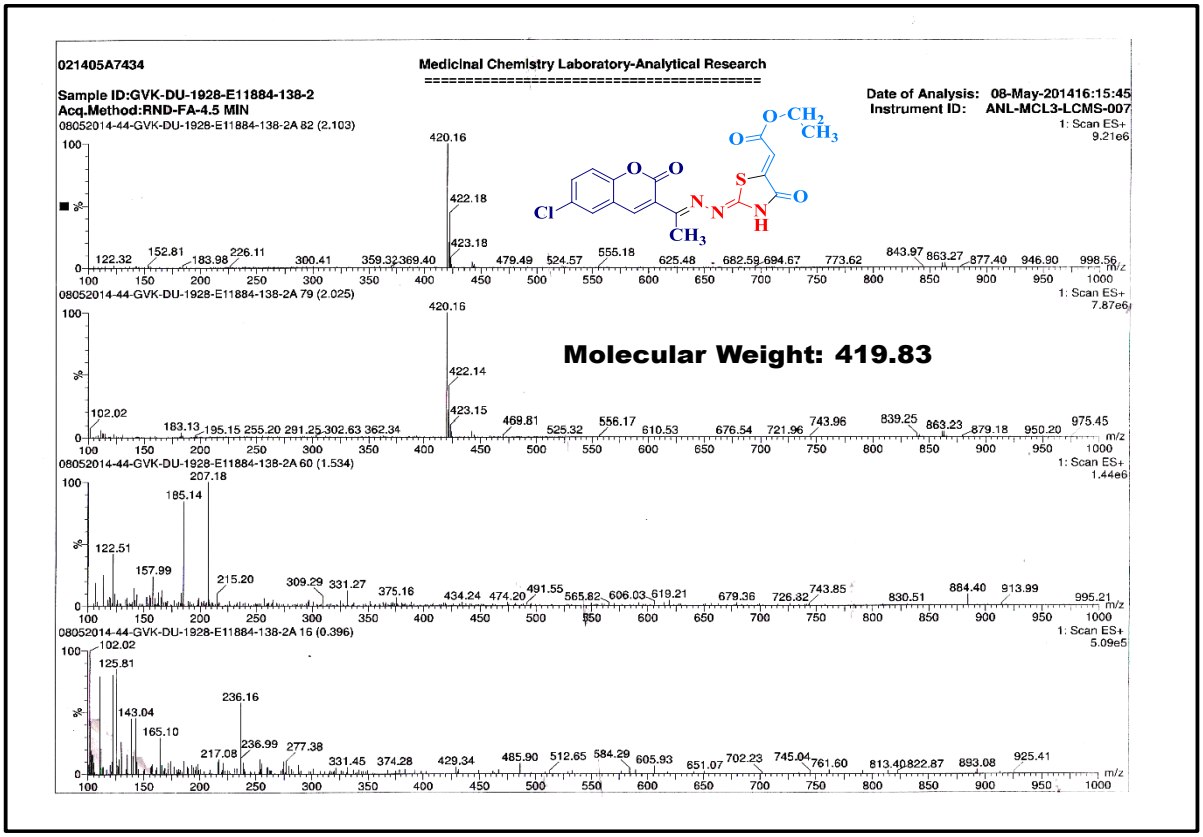


Fig. Mass spectrum of compound **42b**

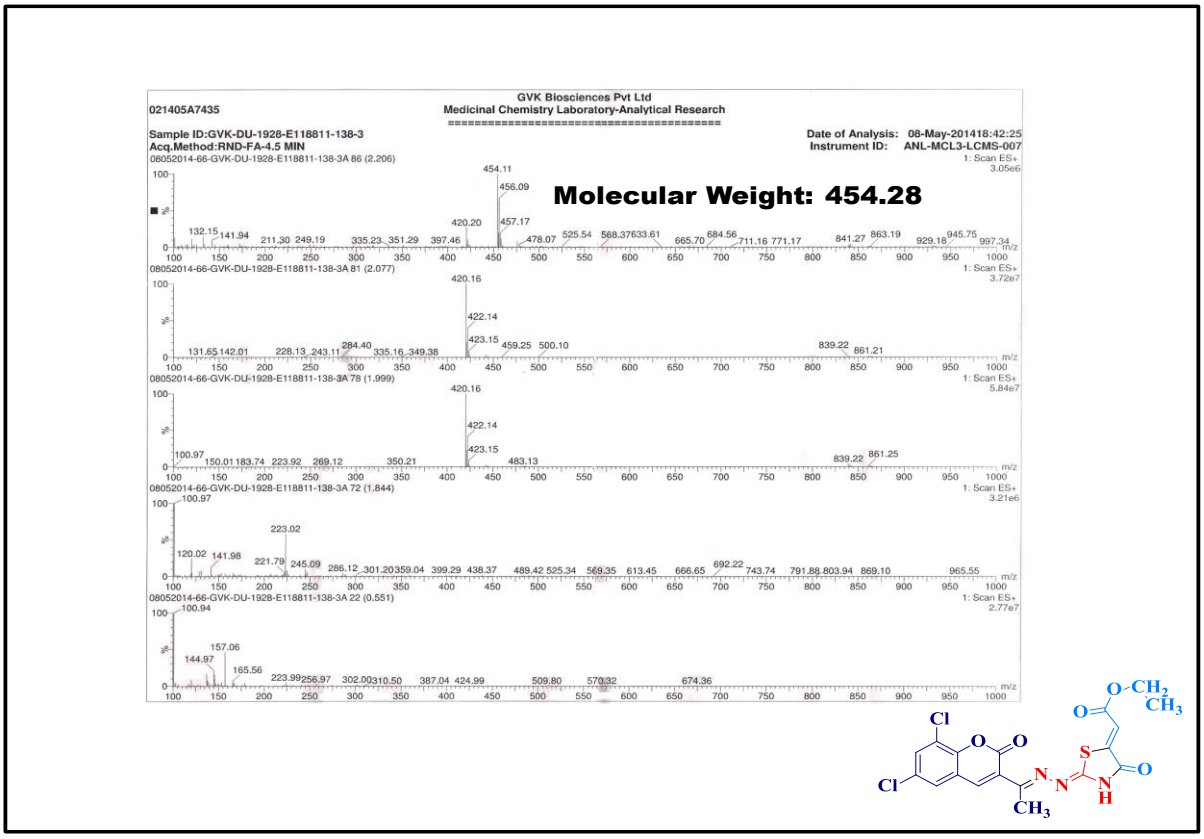


Fig. Mass spectrum of compound **42c**

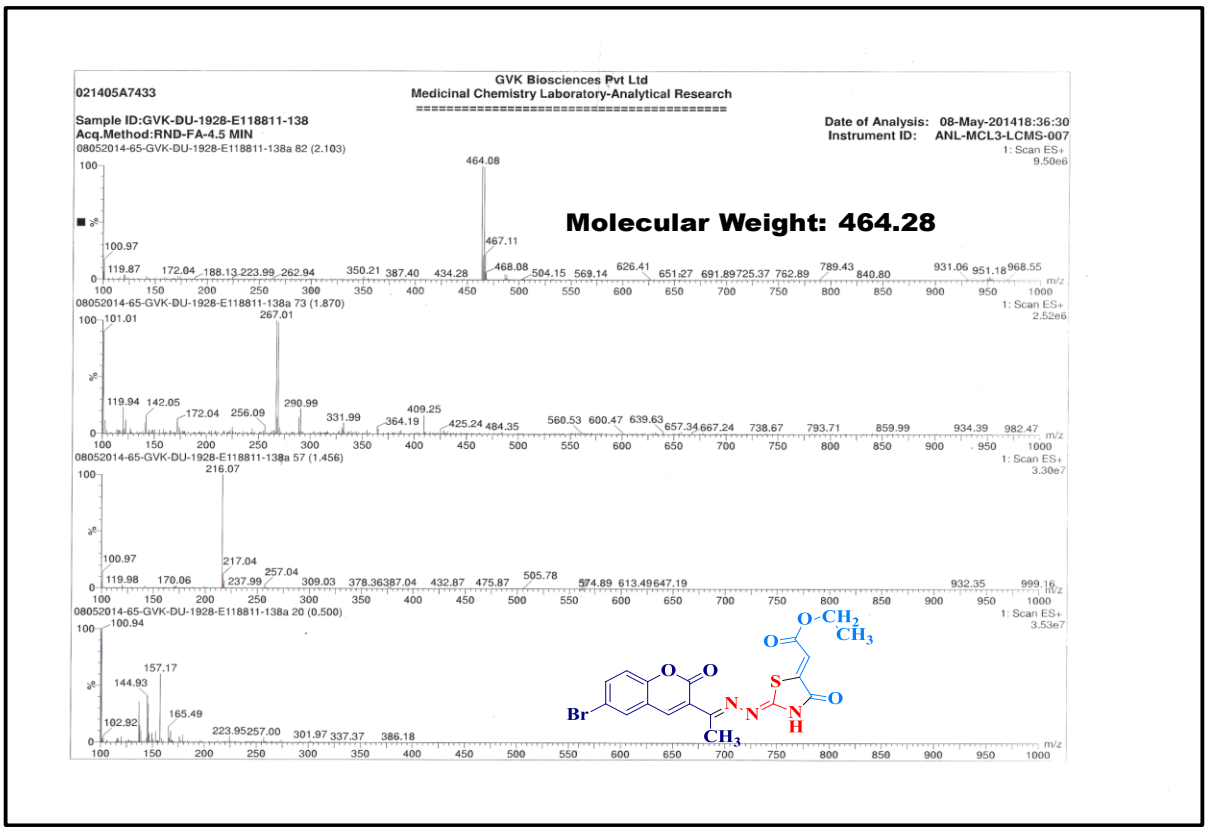


Fig. Mass spectrum of compound **42d**

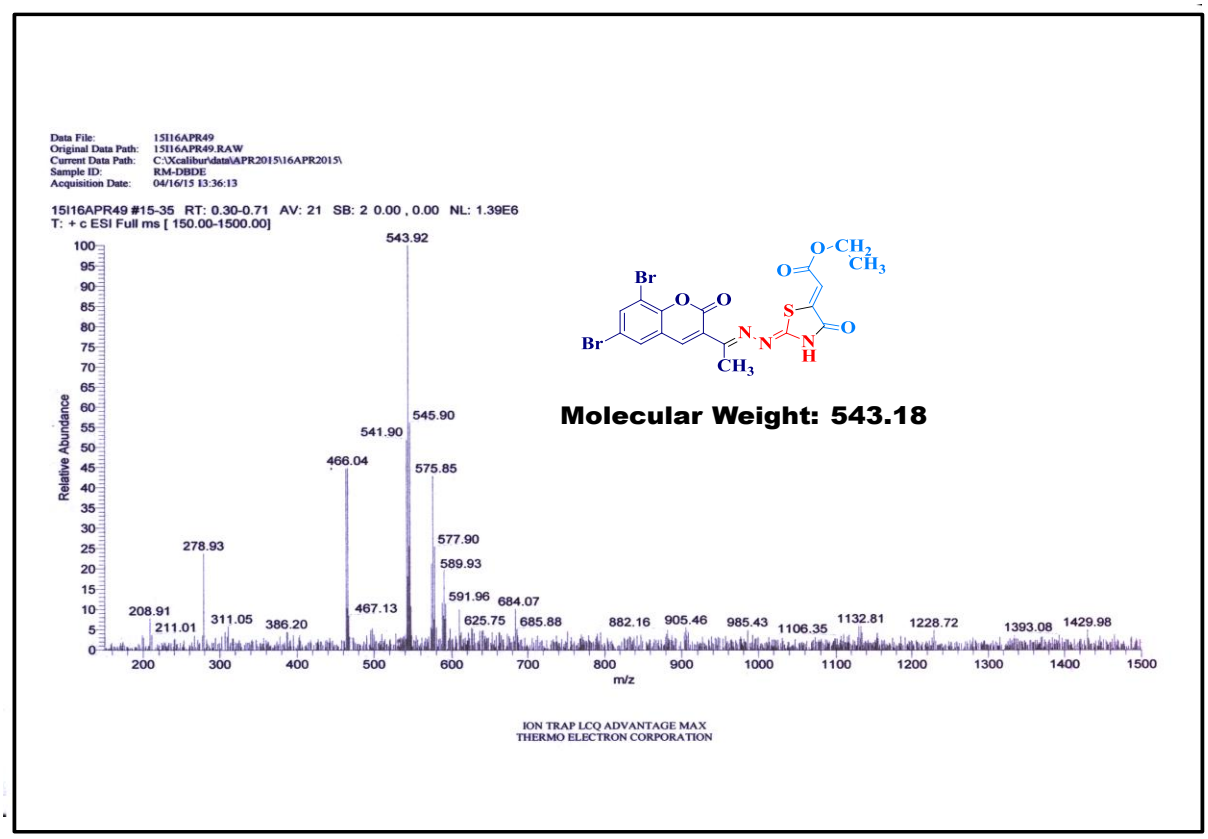


Fig. Mass spectrum of compound **42e**

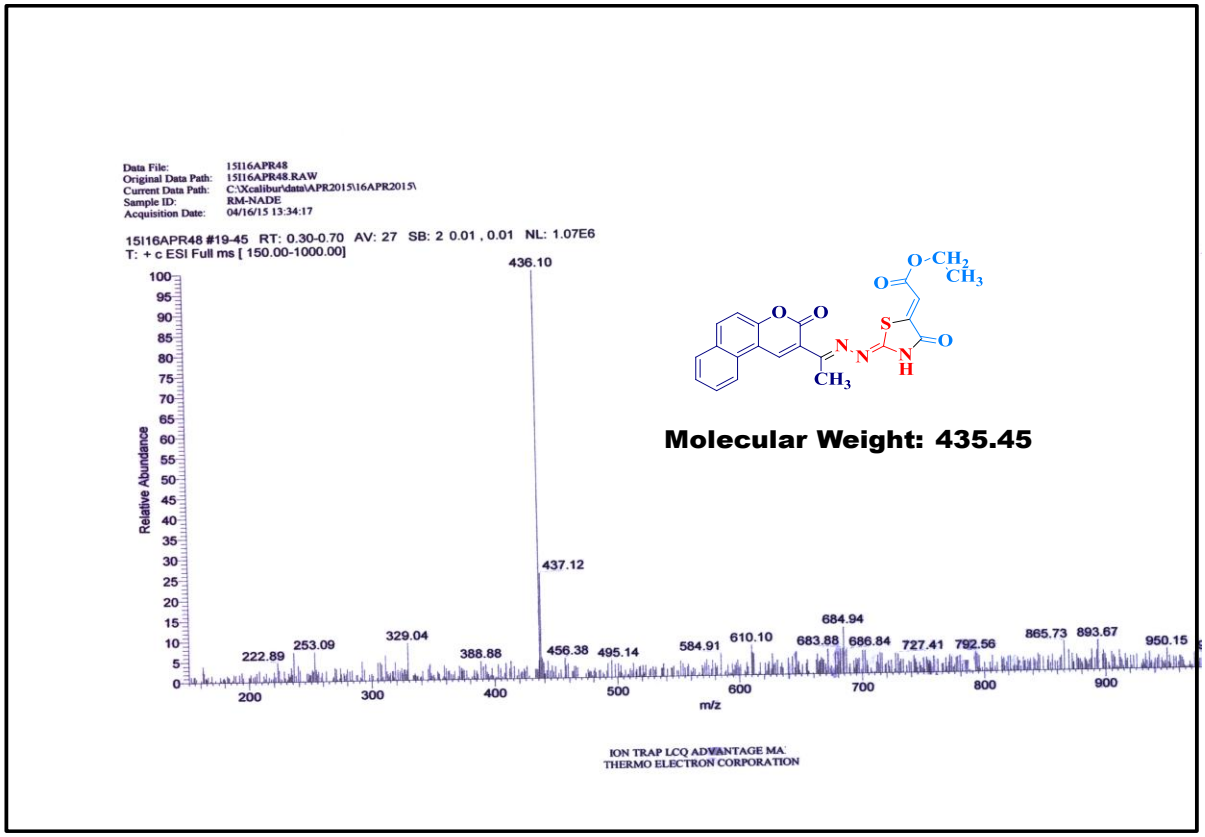


Fig. Mass spectrum of compound **42f**

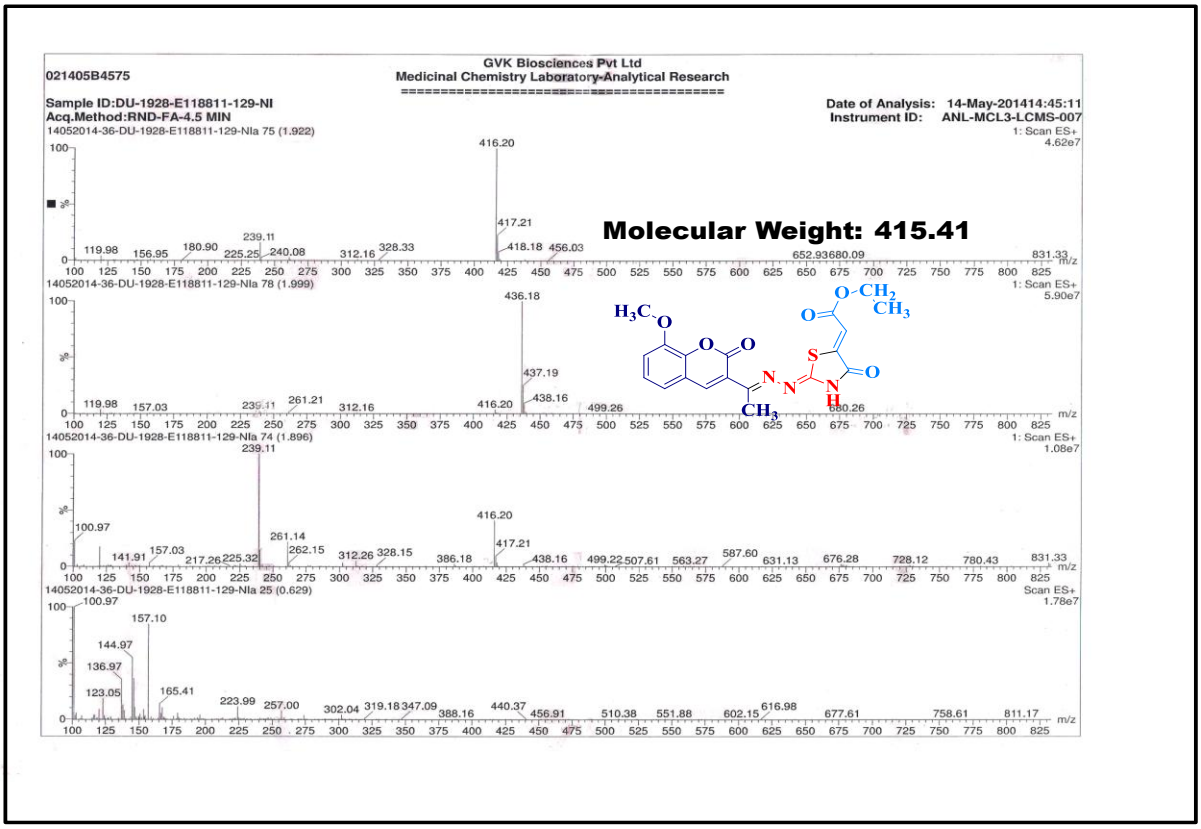


Fig. Mass spectrum of compound **42g**

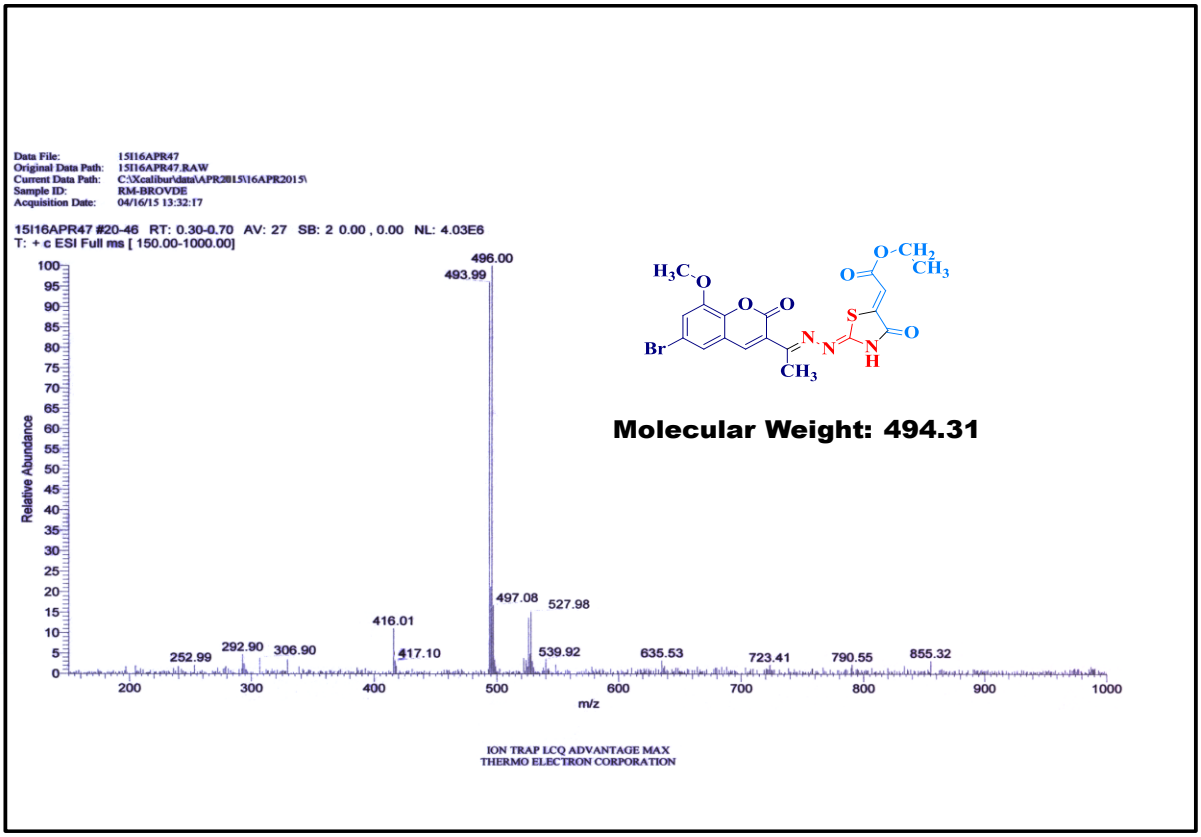


Fig. Mass spectrum of compound **42h**

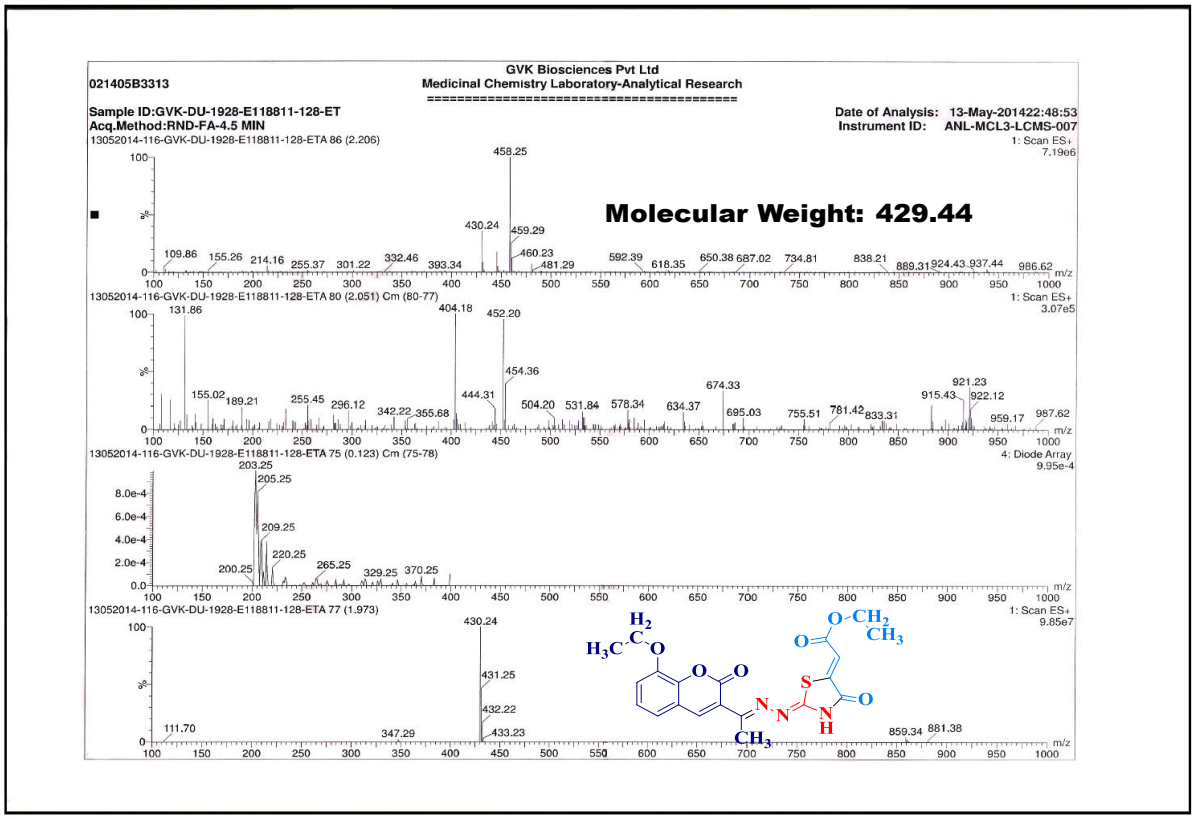


Fig. Mass spectrum of compound **42i**

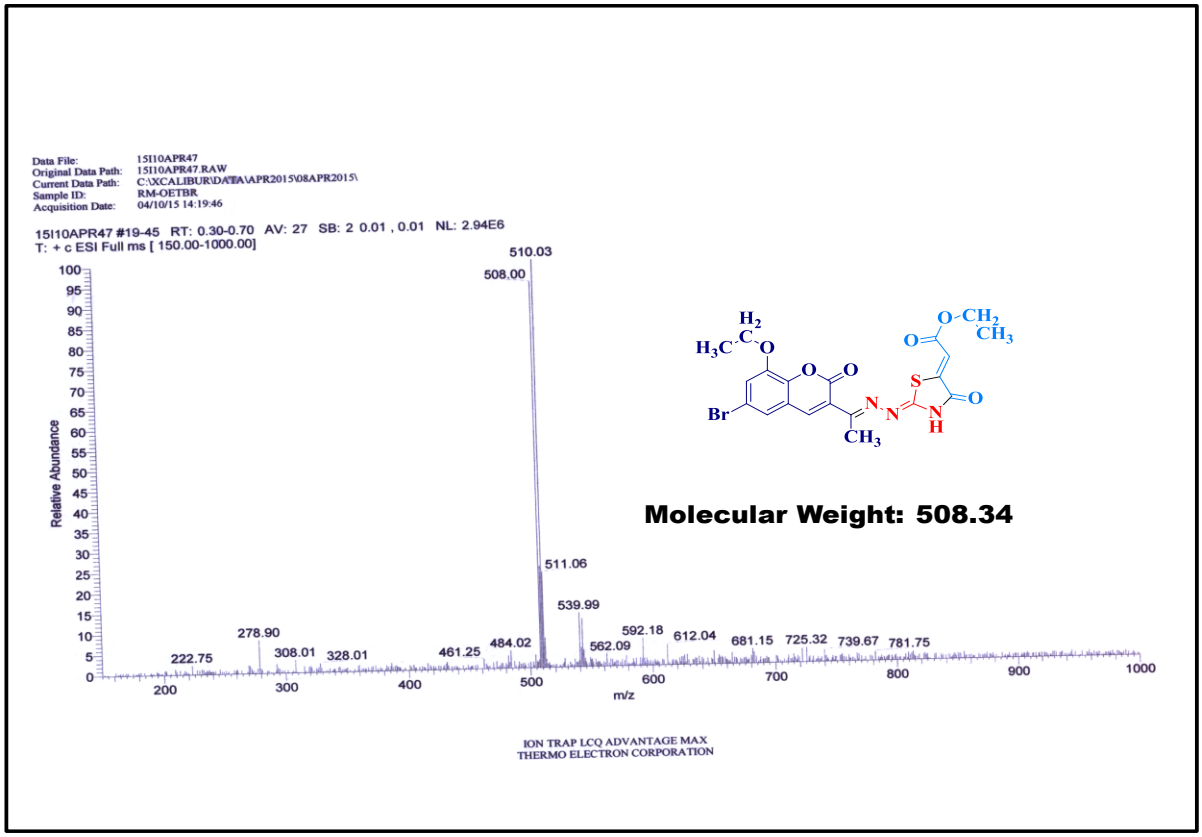


Fig. Mass spectrum of compound **42j**

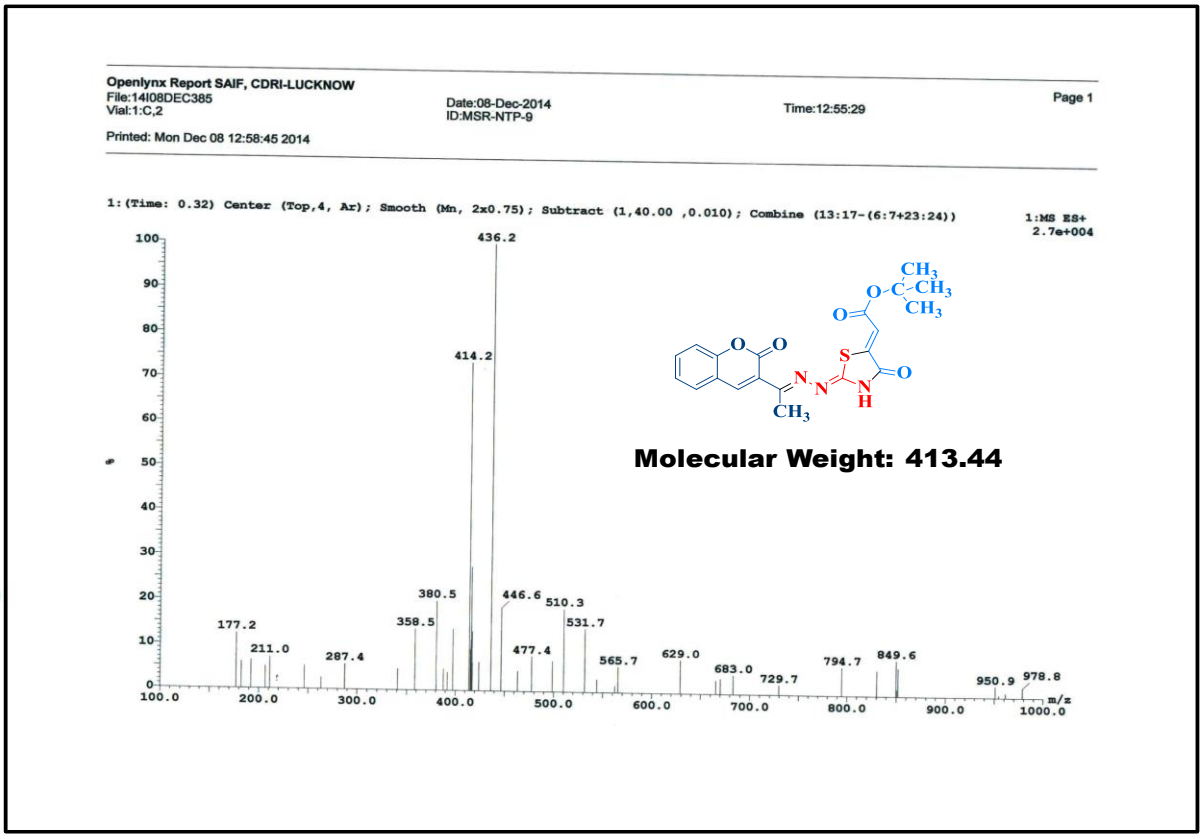


Fig. Mass spectrum of compound **43a**

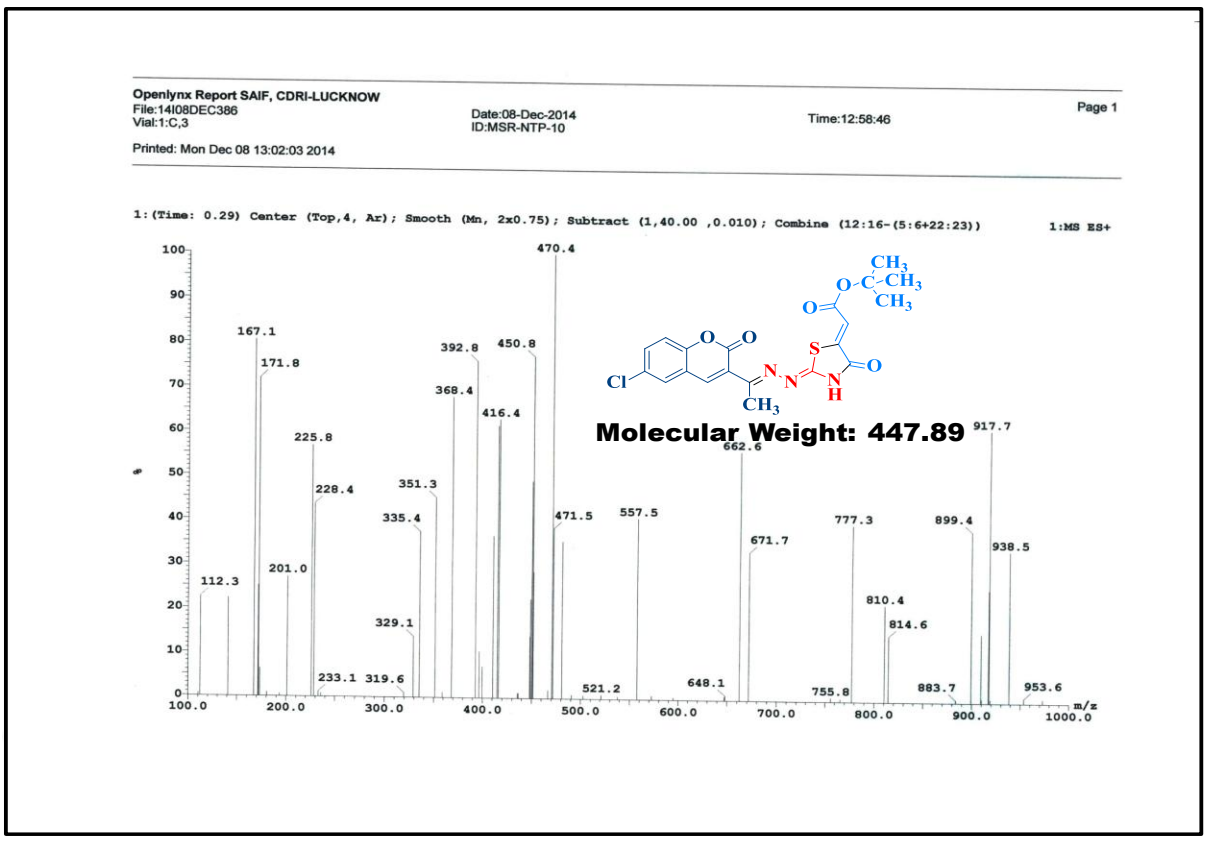


Fig. Mass spectrum of compound **43b**

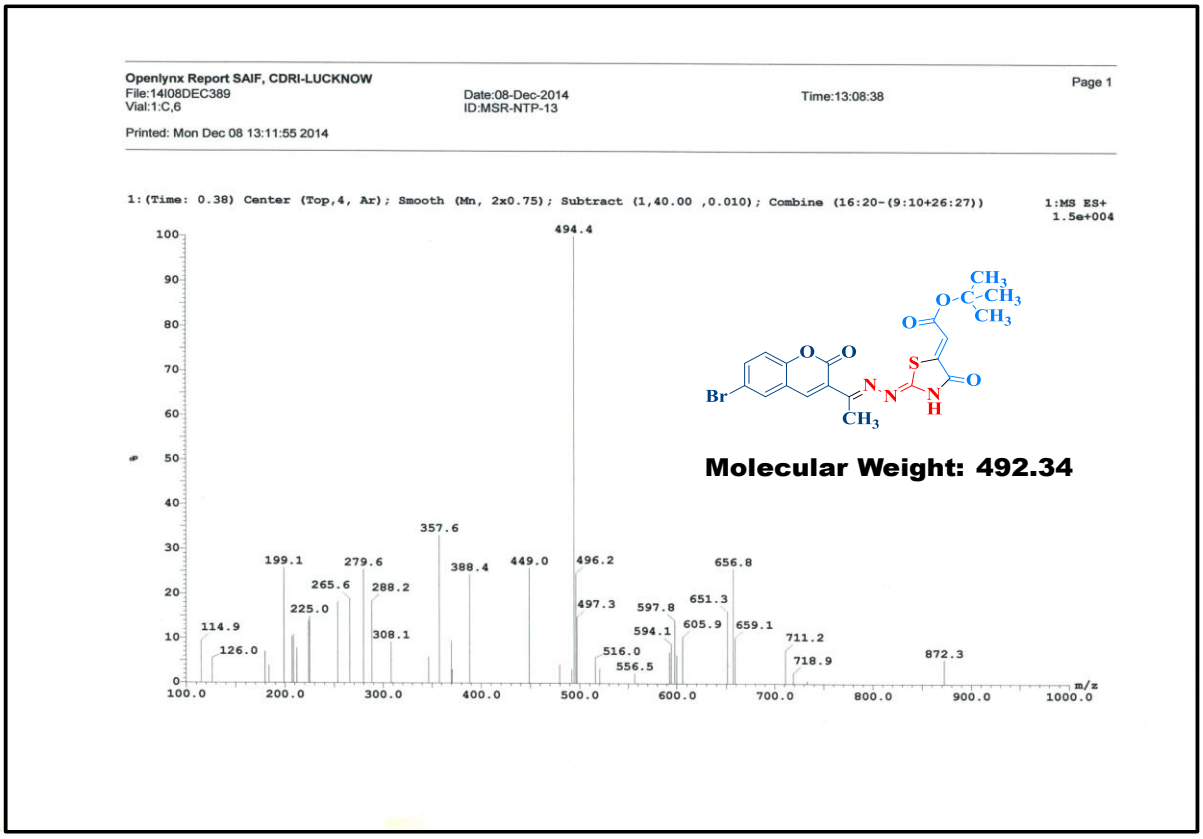


Fig. Mass spectrum of compound **43d**

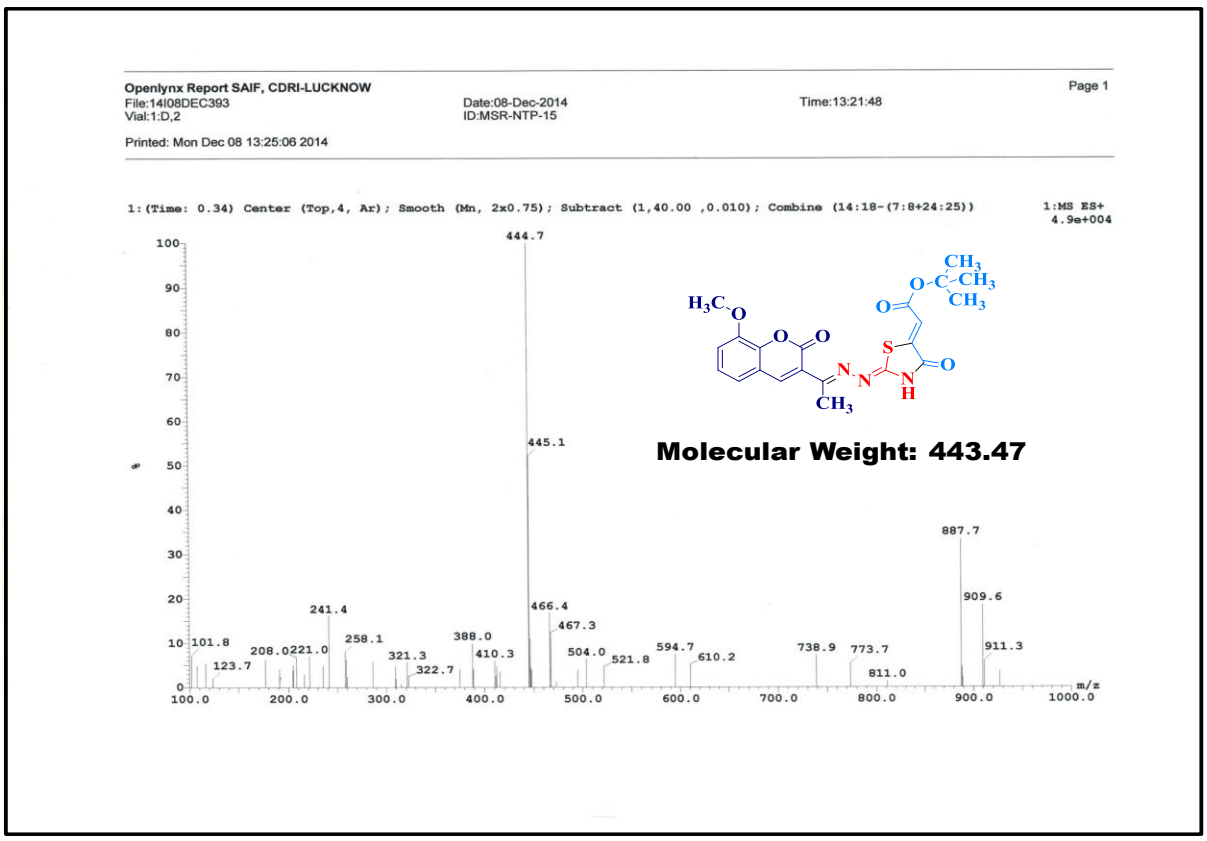


Fig. Mass spectrum of compound **43g**

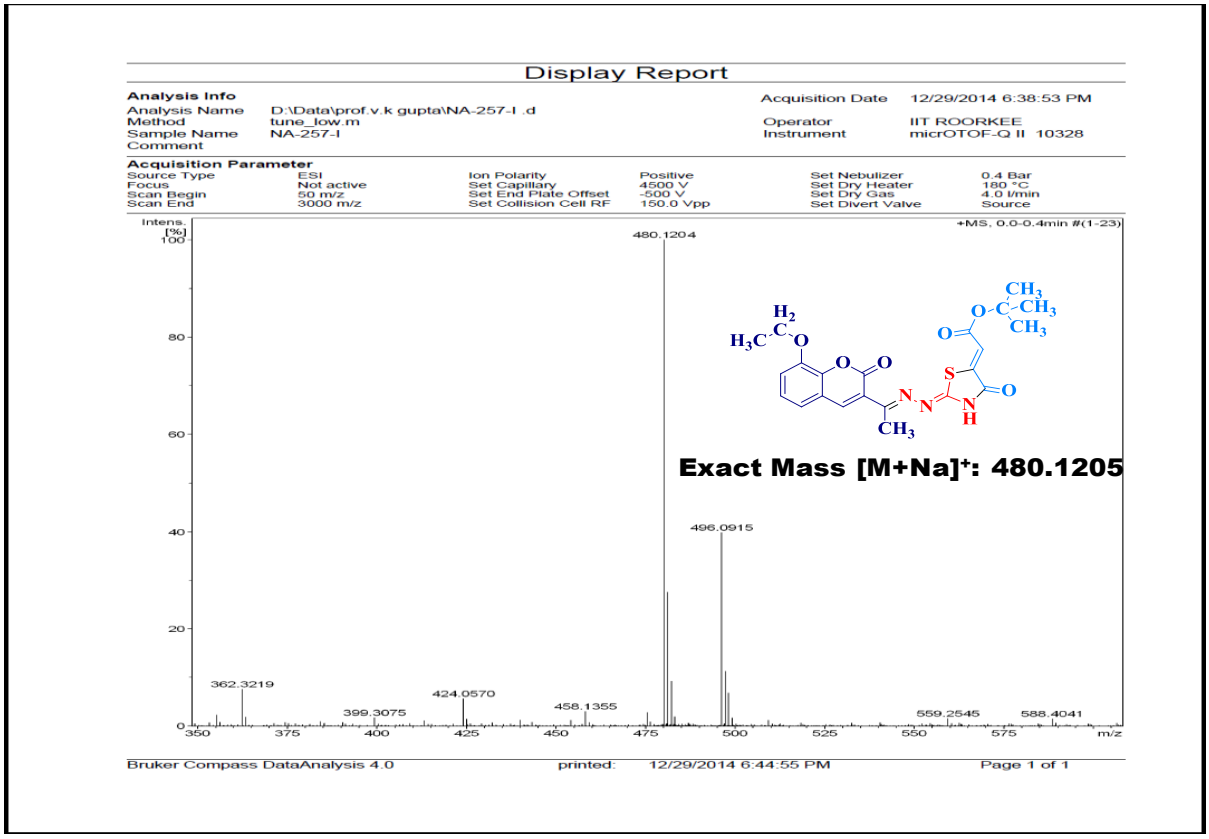


Fig. Mass spectrum of compound **43i**

CHAPTER-IV
PART-B

FACILE ONE POT MULTICOMPONENT SYNTHESIS OF (Z)-ALKYL 2-((Z)-2-((ARYL)IMINO)-3-(FURAN-2-YLMETHYL)-4-OXOTHIAZOLIDIN-5-YLIDENE)ACETATE

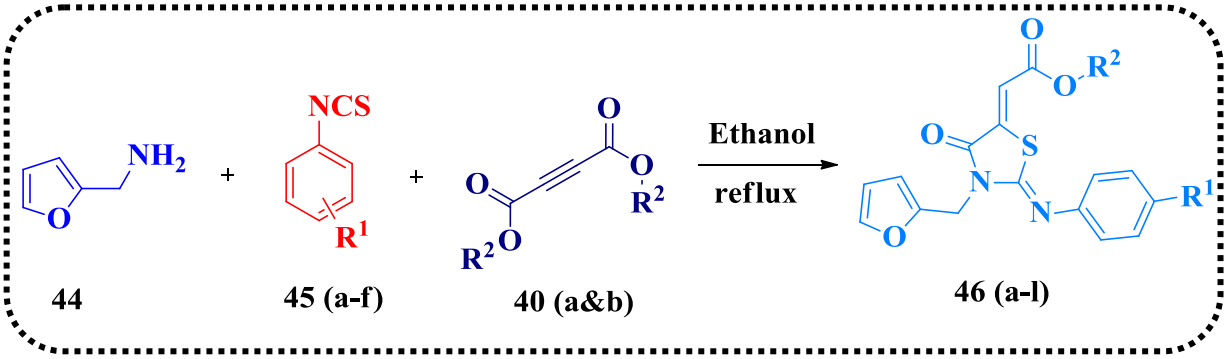
PART-B

PRESENT WORK:

Alkyl 2-(-2-((aryl)imino)-3-(furan-2-ylmethyl)-4-oxothiazolidin-5-ylidene)acetate derivatives were synthesized starting from the reaction of furfurylamine with aryl isothiocyanates and dialkylacetylene dicarboxylates. The reactants used for the synthesis of these heterocyclic compounds were procured from commercial sources and used further without any purification.

In the literature enormous importance was given to 4-thiazolidinones due to their synthesis and wide range of biological applications. In recent years, much attention was given to explore the synthesis and biological applications of 4-thiazolidinones by the medicinal chemists. In view of the pharmacological applications of furan and thiazolidinones, an efficient multicomponent reaction method was developed for the synthesis of substituted thiazolidinone having furan heterocylic moiety. Synthesis of substituted thiazolidinone ring with furan moiety would result in enhanced therapeutic activity that might be provide the lead molecules for the development of potent therapeutic molecules.

In this present chapter, an efficient one pot multicomponent reaction of alkyl 2-(-2-((aryl)imino)-3-(furan-2-ylmethyl)-4-oxothiazolidin-5-ylidene)acetate derivatives and their anticancer activity was described. The target molecules were obtained via a multicomponent reaction approach by the reaction of furfurylamine with substituted aryl isothiocyantes followed by the reaction of dialkylacetylene dicarboxylates in presence of ethanol, through simple reaction workup with shorter reaction time. When this method was used for the synthesis of title compounds they gave good yields (**Scheme 3**).



Scheme 3: A facile one pot multicomponent synthesis of alkyl 2-(-2-((aryl)imino)-3-(furan-2-ylmethyl)-4-oxothiazolidin-5-ylidene)acetate derivatives.

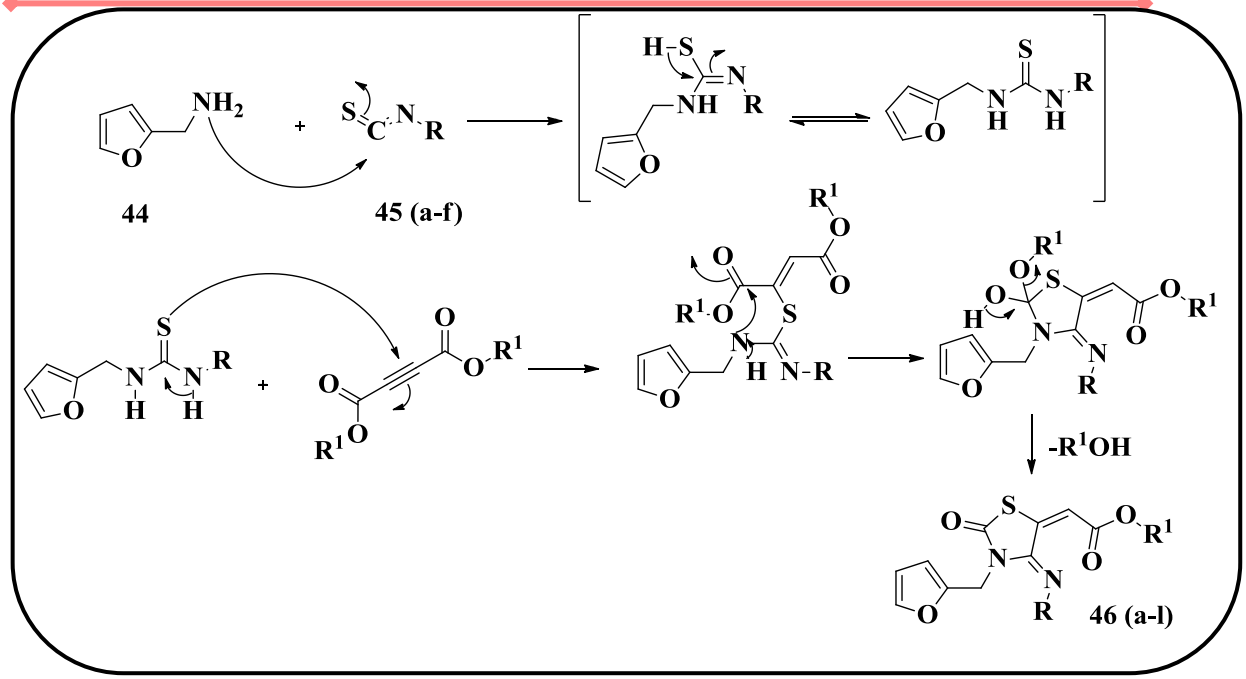
Table.10: A facile one pot multicomponent synthesis of alkyl 2-(-2-((aryl)imino)-3-(furan-2-ylmethyl)-4-oxothiazolidin-5-ylidene)acetate derivatives.

S. No.	Compound	R^1	R^2	Reaction Time (h)	Yield (%)
1	**46a**	Phenyl	$-CH_3$	1	81
2	**46b**	Benzyl	$-CH_3$	1	85
3	**46c**	*p*-Tolyl	$-CH_3$	1.5	84
4	**46d**	4-Cyanophenyl	$-CH_3$	2.0	88
5	**46e**	4-Fluorophenyl	$-CH_3$	2.5	89
6	**46f**	3-Chlorophenyl	$-CH_3$	2.5	88
7	**48g**	Phenyl	$-C_2H_5$	1.5	86
8	**46h**	Benzyl	$-C_2H_5$	2.0	89
9	**46i**	*p*-Tolyl	$-C_2H_5$	2.0	87
10	**46j**	4-Cyanophenyl	$-C_2H_5$	3.0	88
11	**46k**	4-Fluorophenyl	$-C_2H_5$	3.0	90
12	**46l**	3-Chlorophenyl	$-C_2H_5$	3.5	92

Mechanism:

The plausible reaction mechanism for the synthesis of alkyl 2-(-2-((aryl)imino)-3-(furan-2-ylmethyl)-4-oxothiazolidin-5-ylidene)acetate derivatives **46 (a-l)** can be explained by the following mechanism (Scheme-6). The nucleophilic functional group of furfurylamine **44** readily reacts with electrophilic centre of aryl isothiocyante **45 (a-f)** and forms an unsymmetrical thiourea intermediate i.e. 1-(furan-2-ylmethyl)-3-arylthiourea. The more nucleophilic centre (S) of resulting intermediate reacts with dialkylacetylene dicaroxylate and forms dialkyl 2-((-*N*-(furan-2-ylmethyl)-*N'*-phenylcarbamimidoyl)thio)maleate which further undergoes cyclization to generate the title compound.

All the newly synthesized compounds were well characterized by their analytical (IR, ^{1}H NMR, ^{13}C NMR and Mass) and spectral data. All the analytical spectral data consists with assigned structure of the title compounds.



Scheme 4: Plausible synthetic mechanism for the formation of 2-(-2-((aryl)imino)-3-(furan-2-ylmethyl)-4-oxothiazolidin-5-ylidene)acetate derivatives.

The selected compounds by NCI were initially evaluated at single dose concentration (10 µM) against 60 human cancer cell lines. Only the compounds which exhibited significant predetermined threshold growth inhibition were selected for five dose screening (10 nM to 100 µM).

The primary screening data of the selected compounds **46h, 46i and 46j** are shown in **Table 11**. Compound **46j** exhibited remarkable antitumor activity against all the tested cancer cell lines. Based on the primary screening data, compound 46h was selected for five dose screening and the five dose report of GI_{50}, TGI and LC_{50} values were shown in **Table 12**. The GI_{50} values is the concentration of the compound inhibiting the 50% growth in net cell growth, the TGI value is the concentration of the compound resulting in net total cell growth inhibition and the LC_{50} value is the concentration of compound causing net 50% cell loss of initial cells at 48 h incubation period.

Compound **46h** exhibited remarkable antitumor activity (GI50 and TGI <100 µM) against the most of the tested cancer cell lines. Compound **46j** exhibited a broad spectrum of anticancer activity noticeably: Leukemia SR cell line (**GI_{50} 2.71 µM),** Non-Small Cell Lung Cancer NCI-H522 (**GI_{50} 1.83 µM**), Colon Cancer HCT-15 (**GI_{50} 2.02 µM**), CNS Cancer U251 (**GI_{50} 2.07 µM**), Melanoma LOX IMVI (**GI_{50} 1.72 µM**), Ovarian Cancer OVCAR-3 (**GI_{50} 1.82 µM**), Renal Cancer 786-0 (**GI_{50} 1.93 µM**),

ACHN (**GI_{50} 1.78 µM**), RXF 393 (**GI_{50} 1.48 µM**), UO-31 (**GI_{50} 1.45 µM**), Prostate Cancer PC-3 (**GI_{50} 3.11 µM**), Breast Cancer T-47D (**GI_{50} 2.57 µM**).

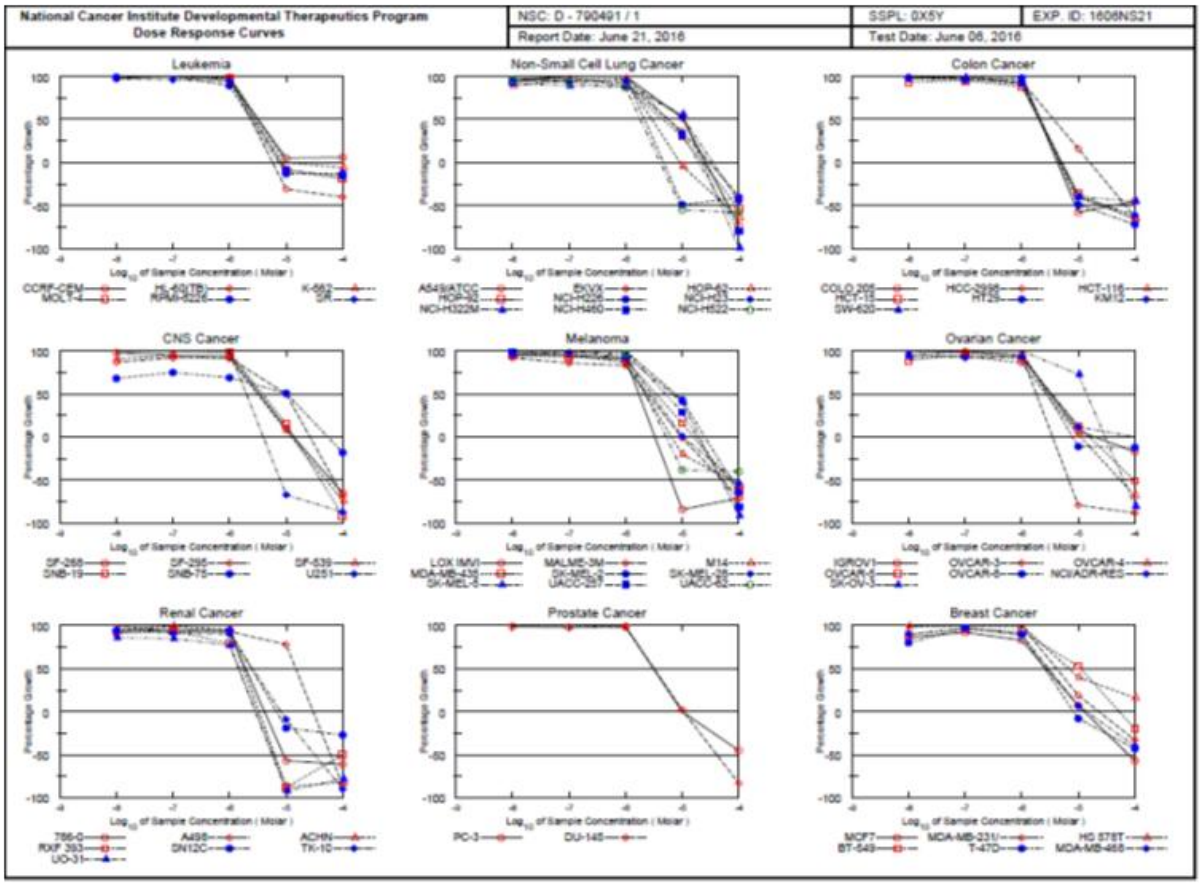


Fig3. Dose response graph of compound **46j**

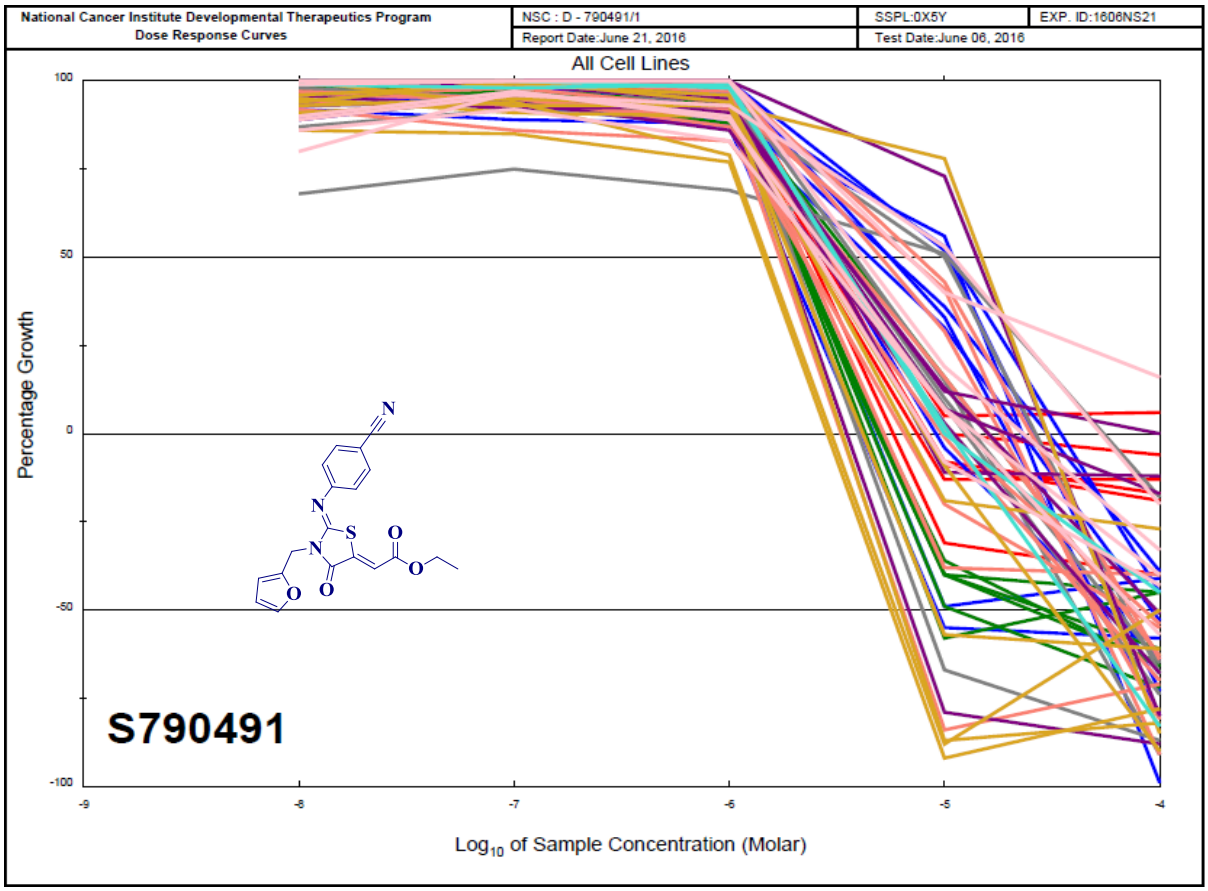


Fig4. Dose response curve of all tested cell lines of compound **46j**

Table 11. One dose mean growth percentage of selected compounds**.**

Panel/Cell Line	46h	46i	46j
	Growth Percentage at 10 µM		
Leukemia			
CCRF-CEM	89.72	**24.76**	**0.58**
HL-60(TB)	97.96	85.51	**-26.63**
K-562	92.34	52.02	8.76
MOLT-4	94.08	29.73	**-6.94**
RPMI-8226	92.55	36.33	**-33.75**
SR	91.76	32.16	0.97
Non-Small Cell Lung Cancer			
A549/ATCC	75.13	68.76	67.19
EKVX	107.13	87.97	73.98
HOP-62	104.62	71.46	60.33
HOP-92	47.30	56.71	37.23
NCI-H226	102.02	83.02	70.27
NCI-H23	100.54	87.93	70.57
NCI-H322M	93.80	84.82	95.49
NCI-H460	113.31	75.24	38.92
Colon Cancer			
COLO 205	99.21	85.13	50.20

HCC-2998	108.51	93.13	64.73
HCT-116	87.19	34.00	**-61.63**
HCT-15	102.44	45.40	**-4.10**
HT29	110.05	62.02	35.02
KM12	102.78	74.15	60.86
SW-620	107.67	70.13	**12.02**
CNS Cancer			
SF-268	107.67	82.63	73.21
SF-295	109.86	88.83	88.83
SF-539	106.03	95.44	38.06
SNB-19	104.36	86.97	67.12
SNB-75	82.00	70.85	83.28
U251	100.41	70.92	59.83
Melanoma			
LOX IMVI	102.26	59.54	**-73.17**
MALME-3M	100.58	81.46	47.64
M14	95.22	70.45	**25.75**
MDA-MB-435	95.84	74.57	50.52
SK-MEL-2	95.07	73.47	78.55
SK-MEL-28	112.10	85.63	38.49
SK-MEL-5	104.32	73.62	62.47
UACC-257	80.69	52.14	54.88
UACC-62	90.84	59.25	35.50
Ovarian Cancer			
IGROV1	111.35	79.12	**26.26**
OVCAR-3	108.04	59.58	**17.92**
OVCAR-4	100.75	74.45	51.16
OVCAR-5	111.98	107.10	37.41
OVCAR-8	94.07	70.47	54.22
NCI/ADR-RES	109.92	77.23	40.93
SK-OV-3	99.58	95.45	91.66
Renal Cancer			
786-0	99.06	79.60	34.58
A498	110.44	90.21	87.04
ACHN	111.36	87.66	**-78.64**
RXF 393	110.91	74.19	**-23.86**
SN12C	105.18	80.26	52.49
UO-31	83.18	65.35	31.55
Prostate Cancer			
PC-3	65.10	56.53	**20.18**
DU-145	109.06	69.94	39.19
Breast Cancer			
MCF-7	90.33	**26.15**	**12.55**
MDA-MB-231/ATCC	107.83	88.45	57.00
HS578T	104.18	85.41	65.38
BT-549	86.72	56.13	44.77
T-47D	97.96	45.48	**12.08**
MDA-MB-468	101.87	62.68	27.46

Table 12. Growth inhibition, total growth inhibition, loss of cells concentration values of compound **46j**

Panel/Cell Line	GI_{50} (µM)	TGI (µM)	LC_{50} (µM)
Leukemia			
CCRF-CEM	3.17	>100	>100
HL-60(TB)	2.38	5.76	>100
K-562	3.16	11.4	>100
MOLT-4	2.81	8.31	>100
RPMI-8226	2.40	7.46	>100
SR	2.71	8.41	>100
Non-Small Cell Lung Cancer			
A549/ATCC	10.4	26.1	65.3
EKVX	5.66	30.2	>100
HOP-62	2.94	9.21	58.0
HOP-92	4.51	22.9	91.4
NCI-H226	2.22	4.76	>100
NCI-H23	10.6	34.5	>100
NCI-H322M	10.9	23.1	48.6
NCI-H460	5.66	19.7	54.7
NCI-H522	1.83	4.12	9.27
Colon Cancer			
COLO 205	2.09	4.30	
HCC-2998	3.67	15.8	67.9
HCT-116	2.06	4.97	25.2
HCT-15	2.02	5.13	29.4
HT29	2.11	4.61	10.5
KM12	2.13	5.01	29.4
SW-620	2.06	4.97	>100
CNS Cancer			
SF-268	3.38	13.7	63.1
SF-295	9.99	26.6	70.8
SF-539	3.09	12.4	51.0
SNB-19	3.83	13.8	40.8
SNB-75	10.3	55.4	>100
U251	2.07	4.06	7.98
Melanoma			
LOX IMVI	1.72	3.32	6.40
MALME-3M	2.45	9.65	50.8
M14	2.22	6.55	70.9
MDA-MB-435	3.44	15.8	68.9
SK-MEL-2	7.65	25.3	74.0
SK-MEL-28	2.92	10.5	84.3
SK-MEL-5	6.71	20.4	48.9
UACC-257	5.34	18.4	52.1
UACC-62	2.23	5.25	>100
Ovarian Cancer			
IGROV1	3.22	20.2	>100
OVCAR-3	1.82	3.52	6.83

OVCAR-4	2.74	11.2	56.6
OVCAR-5	3.36	16.1	95.6
OVCAR-8	2.83	7.94	>100
NCI/ADR-RES	3.42	90.8	>100
SK-OV-3	14.1	29.9	63.6
Renal Cancer			
786-0	1.93	4.17	9.00
A498	14.8	30.0	60.9
ACHN	1.78	3.33	6.25
RXF 393	1.48	2.96	100
SN12C	2.45	6.76	>100
TK-10	2.54	8.19	32.4
UO-31	1.45	2.86	5.64
Prostate Cancer			
PC-3	3.11	9.96	>100
DU-145	3.17	10.5	40.6
Breast Cancer			
MCF-7	2.70	12.7	78.1
MDA-MB-231/ATCC	4.29	23.5	>100
HS578T	6.96	>100	>100
BT-549	10.8	52.6	>100
T-47D	2.57	8.24	>100
MDA-MB-468	2.97	14.2	>100

Panel/Cell Line	$Log_{10}GI_{50}$	$Log_{10}TGI$	$Log_{10}LC_{50}$
Leukemia			
CCRF-CEM	-5.50	-4.00	> -4.00
HL-60(TB)	-5.62	-5.24	> -4.00
K-562	-5.50	-4.94	> -4.00
MOLT-4	-5.55	-5.08	> -4.00
RPMI-8226	-5.62	-5.13	> -4.00
SR	-5.57	-5.08	> -4.00
Non-Small Cell Lung Cancer			
A549/ATCC	-4.98	-4.58	-4.19
EKVX	-5.25	-4.52	> -4.00
HOP-62	-5.53	-5.04	-4.24
HOP-92	-5.35	-4.64	-4.04
NCI-H226	-5.65	-5.32	> -4.00
NCI-H23	-4.97	-4.46	> -4.00
NCI-H322M	-4.96	-4.64	-4.31
NCI-H460	-5.25	-4.71	-4.26
NCI-H522	-5.74	-5.39	-5.03
Colon Cancer			
COLO 205	-5.68	-5.37	
HCC-2998	-5.44	-4.80	-4.17
HCT-116	-5.69	-5.30	-4.60
HCT-15	-5.69	-5.29	-4.53
HT29	-5.68	-5.34	-4.98
KM12	-5.67	-5.30	-4.53

SW-620	-5.69	-5.30	-4.00
CNS Cancer			
SF-268	-5.47	-4.86	-4.20
SF-295	-5.00	-4.58	-4.15
SF-539	-5.51	-4.91	-4.29
SNB-19	-5.42	-4.86	-4.39
SNB-75	-4.99	-4.26	-4.00
U251	-5.68	-5.39	-5.10
Melanoma			
LOX IMVI	-5.76	-5.48	-5.19
MALME-3M	-5.61	-5.02	-4.29
M14	-5.65	-5.18	-4.15
MDA-MB-435	-5.46	-4.80	-4.16
SK-MEL-2	-5.12	-4.60	-4.13
SK-MEL-28	-5.53	-4.98	-4.07
SK-MEL-5	-5.17	-4.69	-4.31
UACC-257	-5.27	-4.74	-4.28
UACC-62	-5.65	-5.28	> -4.00
Ovarian Cancer			
IGROV1	-5.49	-4.70	>-4.00
OVCAR-3	-5.74	-5.45	-5.17
OVCAR-4	-5.56	-4.95	-4.25
OVCAR-5	-5.47	-4.79	-4.02
OVCAR-8	-5.55	-5.100	>-4.00
NCI/ADR-RES	-5.47	-4.04	>-4.00
SK-OV-3	-4.85	-4.52	-4.20
Renal Cancer			
786-0	-5.71	-5.38	-5.05
A498	-4.83	-4.52	-4.22
ACHN	-5.75	-5.48	-5.20
RXF 393	-5.83	-5.53	-4.00
SN12C	-5.61	-5.17	> -4.00
TK-10	-5.59	-5.09	-4.49
UO-31	-5.84	-5.54	-5.25
Prostate Cancer			
PC-3	-5.51	-5.00	-4.00
DU-145	-5.50	-4.98	-4.39
Breast Cancer			
MCF-7	-5.57	-4.90	-4.11
MDA-MB-231/ATCC	-5.37	-4.63	> -4.00
HS578T	-5.16	>-4.00	>-4.00
BT-549	-4.96	-4.28	>-4.00
T-47D	-5.59	-5.08	>-4.00
MDA-MB-468	-5.53	-4.85	>-4.00

In conclusion, we have developed an efficient one pot multicomponent synthesis for 2-(-2-((aryl)imino)-3-(furan-2-ylmethyl)-4-oxothiazolidin-5-ylidene)acetate derivatives **46 (a-l)**. The method applied for the synthesis of the title was simple, with simple reaction work up, shorter reaction time and high yields. The newly synthesized compounds were screened against a broad panel of 60 human cancer cell lines. Compound 46h exhibited excellent potency against all the tested cancer cell lines.

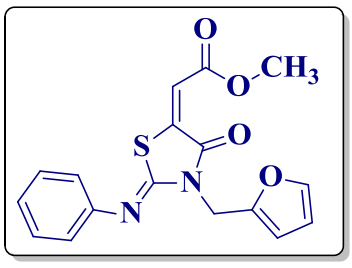
EXPERIMENTAL:

General procedure for the synthesis of alkyl 2-(-2-((aryl)imino)-3-(furan-2-ylmethyl)-4-oxothiazolidin-5-ylidene)acetate derivatives 48a-l:

A mixture of furfural amine **44** (0.097 g, 1.0 mmol) and aryl isothiocyantes **45 a-f** (1.0 mmol) was taken in a round bottom flask having absolute ethanol (5 mL). The reaction mixture was stirred at room temperature for about ten minutes. To this reaction mixture, dialkylacetylene dicarboxylate **40 a&b** (1.0 mmol) was added slowly. The temperature of the reaction was raised to 60 - 65 °C and maintained for about 1.0 – 3.5 h. The progress of the reaction was monitored through TLC using hexane and ethyl acetate in 2:1 volume ratio. After completion of the reaction, the temperature of the reaction was brought to room temperature. The solid separated was filtered, washed with cold ethanol and dried. The product was recrystallized from methanol.

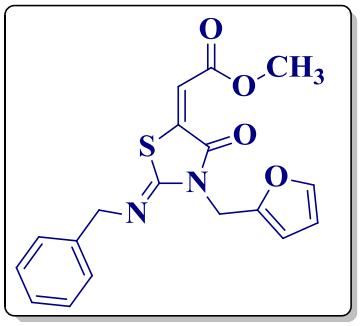
SPECTRAL DATA:

Methyl 2-(-3-(furan-2-ylmethyl)-4-oxo-2-(phenylimino)thiazolidin-5-ylidene) acetate (46a):



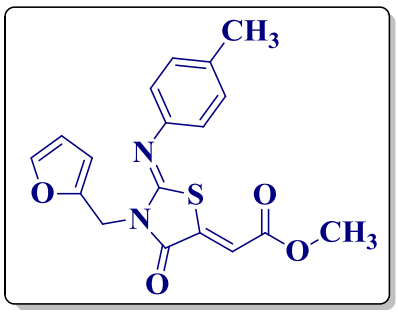
Yellow solid; m.p.: 136 – 138 °C; **FTIR (KBr, ν_{max}, cm^{-1}):** 1712 (Ester –C=O), 1634 (amide-C=O), 1613 (-C=N); **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm):** 3.79 (s, 3H, -$COOCH_3$), 5.15 (s, 2H, -CH_2), 6.33 – 6.34 (dd, *J* = 1.6Hz, 1H, Ar-H), 6.43 – 6.44 (dd, *J* = 3.2 Hz, 1H, Ar-H), 6.93 (s, 1H, =CH), 6.96 – 6.98 (dd, *J* = 1.2 Hz, 2H, Ar-H), 7.18 (t, *J* = 7.6 Hz, 1H, Ar-H), 7.36 (t, *J* = 7.6 Hz, 3H, Ar-H); **^{13}C NMR (100 MHz, $CDCl_3$, ppm):** δ 38.95, 52.55, 109.82, 110.53, 116.31, 121.06, 125.31, 127.84, 129.39, 141.52, 142.59, 147.11, 148.58, 150.10, 164.51, 166.31; Anal. Calcd for $C_{17}H_{14}N_2O_4S$: C, 59.64, H, 4.12, N, 8.18%; Found: C, 59.61; H, 4.14; N, 18.20%.

Methyl 2-(-2-(benzylimino)-3-(furan-2-ylmethyl)-4-oxothiazolidin-5-ylidene)acetate (46b):



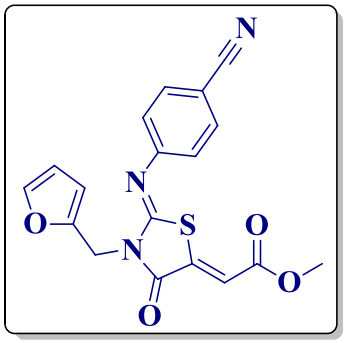
White solid; m.p.: 148 – 150 °C; **FTIR (KBr, ν_{max}, cm^{-1}):** 1718 (Ester-C=O), 1650 (Amide-C=O), 1612 (-C=N); **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm):** 3.85 (s, 3H, -$COOCH_3$), 4.69 (s, 2H, -CH_2), 5.08 (s, 2H, -CH_2), 6.30 – 6.36 (dd, *J* = 4 Hz, 2H, Ar-H), 6.94 (s, 1H, =CH), 7.28 (t, *J* = 4Hz, 1H, Ar-H), 7.34 (t, *J* = 4 Hz, 4H, Ar-H), 7.41 – 7.44 (dd, *J* = 4 Hz, 1H, Ar-H); **^{13}C NMR (100 MHz, $CDCl_3$, ppm):** δ 38.82, 52.56, 55.89, 109.56, 110.44, 115.85, 127.43, 128.49, 135.65, 138.57, 141.03, 142.43, 148.82, 149.04, 150.82, 151.71, 164.41, 166.39; Anal. Calcd for $C_{18}H_{16}N_2O_4S$: C, 60.66, H, 4.53, N, 7.86%; Found: C, 60.62, H, 4.55, N, 7.89%.

Methyl 2-(-3-(furan-2-ylmethyl)-4-oxo-2-(*p*-tolylimino)thiazolidin-5-ylidene)acetate (46c):



Yellow solid; m.p.: 165 -167 °C; **FTIR (KBr, ν_{max}, cm^{-1}):** 1721 (Ester-C=O), 1689 (Amide-C=O), 1602 (-C=N); **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm):** 2.35 (s, 3H, -CH_3), 3.80 (s, 3H, -$COOCH_3$), 5.15 (s, 2H, -CH_2), 6.33 – 6.34 (m, 1H, Ar-H), 6.43 (d, *J* = 4Hz, 1H, Ar-H), 6.88 (d, *J* = 8 Hz, 2H, Ar-H), 6.92 (s, 1H, =CH), 7.16 (d, *J* = 8Hz. 2H, Ar-H), 7.37 – 7.38 (m, 1H, Ar-H); **^{13}C NMR (100 MHz, $CDCl_3$, ppm):** δ 20.96, 38.94, 52.51, 109.78, 110.51, 116.12, 120.93, 129.95, 134.97, 141.65, 142.55, 144.56, 148.64, 149.79, 164.54, 166.32; Anal. Calcd for $C_{18}H_{16}N_2O_4S$: C, 60.66, H, 4.53, N, 7.86%; Found: C, 60.71, H, 4.50, N, 7.83%.

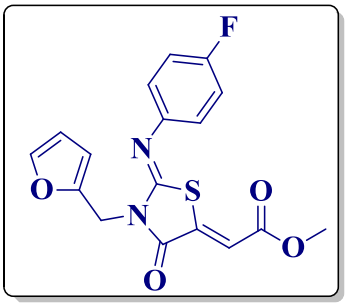
Methyl 2-(-2-((4-cyanophenyl)imino)-3-(furan-2-ylmethyl)-4-oxothiazolidin-5-ylidene) acetate (46d):



Yellow solid; m.p.: 226 – 228 °C; **IR (KBr, ν_{max}, cm^{-1}):** 2224 (-CN), 1733 (Ester –C=O), 1650 (-Amide C=O), 1598 (-C=N); **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm):** 3.83 (s, 3H, -$COOCH_3$), 5.15 (s, 2H, -CH_2), 6.36 – 6.37 (dd, *J* =

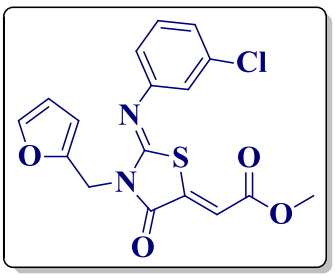
2 Hz, 1H, Ar-H), 6.44 (d, *J* = 2.8 Hz, 1H, Ar-H), 6.99 (s, 1H, Ar-H), 7.07 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.40 (s, 1H, Ar-H), 7.68 (d, *J* = 8.4 Hz, 2H, Ar-H); **MS (ESI m/z):** 367.80 $[M+H]^+$; Anal. Calcd for $C_{18}H_{13}N_3O_4S$: C, 58.85, H, 3.57, N, 11.44%; Found: C, 58.81, H, 3.54, N, 11.49%.

Methyl 2-(-2-((4-fluorophenyl)imino)-3-(furan-2-ylmethyl)-4-oxothiazolidin-5-ylidene) acetate (46e):



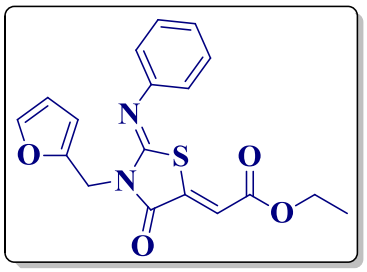
Yellow solid; m.p.: 184 – 188 °C; **FTIR (KBr, ν_{max}, cm^{-1}):** 1714 (Ester-C=O), 1635 (Amide-C=O), 1612 (-C=N), 1011 (C-F); **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm):** 3.81 (s, 3H, $-COOCH_3$), 5.14 (s, 2H, $-CH_2$), 6.33 – 6.35 (dd, *J* = 4Hz, 1H, Ar-H), 6.43 – 6.44 (dd, *J* = 4 Hz, 1H, Ar-H), 6.93 – 6.96 (m, 3H, Ar-H), 7.06 (t, *J* = 8 Hz, 2H, Ar-H), 7.37 – 7.38 (dd, *J* = 4Hz, 1H, Ar-H); **^{13}C NMR (100 MHz, $CDCl_3$, ppm):** δ 38.96, 52.60, 109.83, 110.54, 116.05, 116.55, 122.51, 122.59, 141.18, 142.62, 143.18, 148.49, 150.71, 159.26, 161.64, 164.44, 166.28; Anal. Calcd for $C_{17}H_{13}FN_2O_4S$: C, 56.66, H, 3.64, N, 7.77%; Found: C, 56.70, H, 3.59, N, 7.80%**.**

Methyl 2-(-2-((3-chlorophenyl)imino)-3-(furan-2-ylmethyl)-4-oxothiazolidin-5-ylidene) acetate (46f):



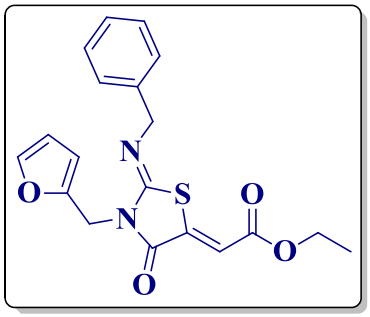
Yellow solid; m.p.: 191 – 192 °C; **FTIR (KBr, ν_{max}, cm^{-1}):** 1712 (Ester-C=O), 1693 (Amide –C=O), 1611 (-C=N), 768 (C-Cl); **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm):** 3.81 (s, 3H, $-COOCH_3$), 5.13 (s, 2H, $-CH_2$), 6.34 – 6.35 (dd, *J* = 4 Hz, 1H, Ar-H), 6.43 (d, *J* = 4Hz, 1H, Ar-H), 6.85 – 6.87 (dd *J* = 0.8 Hz, 1H, Ar-H), 6.95 (s, 1H,=CH), 6.97 (t, *J* = 4 Hz, 1H, Ar-H), 7.15 – 7.18 (dd, *J* = 4 Hz, 1H, Ar-H), 7.29 (t, *J* = 8Hz, 1H, Ar-H) 7.37 – 7.38 (m, 1H, Ar-H); **^{13}C NMR (100 MHz, $CDCl_3$, ppm):** δ 38.94, 52.67, 109.94, 110.57, 116.82, 119.20, 121.47, 125.35, 130.44, 134.94, 141.01, 142.69, 148.35, 151.26, 164.40, 166.26; Anal. Calcd for $C_{17}H_{13}ClN_2O_4S$: C, 54.19, H, 3.48, N, 7.43%; Found: C, 54.25, H, 3.45, N, 7.39%.

Ethyl 2-(-3-(furan-2-ylmethyl)-4-oxo-2-(phenylimino)thiazolidin-5-ylidene)acetate (46g):



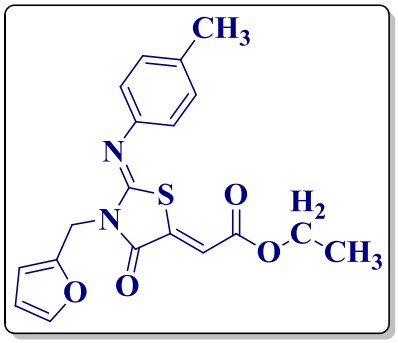
Yellow solid; m.p.: 178 – 180 °C; **FTIR (KBr, ν_{max}, cm^{-1}):** 1726 (Ester -C=O), 1688 (Amide –C=O), 1609 (-C=N); **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm):** 1.29 (t, *J* = 4 Hz, 3H, $-CH_3$), 4.21 – 4.27 (m, 2H, $-OCH_2$), 5.15 (s, 2H, $-CH_2$), 6.33 – 6.34 (dd, *J* = 1.6 Hz, 1H, Ar-H), 6.43 (d, *J* = 4 Hz, 1H, Ar-H), 6.92 (s, 1H, =CH), 6.97 (d, *J* = 7.2 Hz, 2H, Ar-H), 7.17 (t, *J* = 7.6 Hz, 1H, Ar-H), 7.33 (s, 1H, Ar-H), 7.35 (s, 1H, Ar-H), 7.37 (s, 1H, Ar-H); **^{13}C NMR (100 MHz, $CDCl_3$, ppm):** δ 14.14, 38.91, 61.73, 109.79, 110.52, 116.78, 121.06, 125.27, 129.37, 141.24, 142.57, 147.14, 148.62, 150.27, 164.56, 165.92; **MS (ESI m/z):** 356.91 $[M+H]^+$; Anal. Calcd for $C_{18}H_{16}N_2O_4S$: C, 60.66, H, 4.53, N, 7.86%; Found: C, 60.62, H, 4.58, N, 7. 83%.

Ethyl 2-(-2-(benzylimino)-3-(furan-2-ylmethyl)-4-oxothiazolidin-5-ylidene)acetate (46h):



White solid; yield: (0.329 g, 89%); m.p.: 204 -206 °C; **FTIR (KBr, ν_{max}, cm^{-1}):** 1715 (Ester C=O), 1693 (Amide –C=O); **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm):** 1.33 (t, *J* = 7.2 Hz, 3H, $-CH_3$), 4.27 – 4.32 (m, 2H, $-OCH_2$), 4.68 (s, 2H, Ph-CH_2), 5.07 (s, 2H, Furan-CH_2), 6.29 – 6.30 (m, 1H, Ar-H), 6.32 – 6.35 (m, 1H, Ar-H), 6.92 (s, 1H, =CH), 7.27 – 7.28 (m, 1H, Ar-H), 7.33 (t, *J* = 3.2 Hz, 3H, Ar-H), 7.37 – 7.38 (m, 1H, Ar-H), 7.41 – 7.43 (m, 1H, Ar-H); Anal. Calcd for $C_{19}H_{18}N_2O_4S$ (370.42): C, 61.61, H, 4.90, N, 7.56%; Found: C, 61.64, H, 4.86, N, 7.59%.

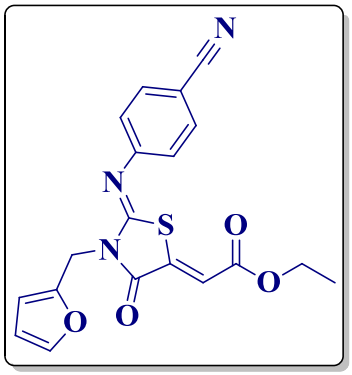
Ethyl 2-(-3-(furan-2-ylmethyl)-4-oxo-2-(*p*-tolylimino)thiazolidin-5-ylidene)acetate (46i):



Yellow solid; m.p.: 215- 217 °C; **FTIR (KBr, ν_{max}, cm^{-1}):** 1726 (Ester –C=O), 1689 (Amide –C=O), 1605 (-C=N); **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm):** 1.29 (t, *J* = 7.2 Hz, 3H, -CH3), 2.34 (s, 3H, Ar-CH_3), 4.21 – 4.26 (m, 2H, $-OCH_2$), 5.14 (s, 2H, $-CH_2$), 6.32 – 6.33 (dd, *J* = 1.6 Hz, 1H, Ar-H), 6.43 (d, *J* = 3.6 Hz, 1H, Ar-H), 6.87 (d, *J* = 8 Hz, 2H, Ar-H), 6.91 (s, 1H,

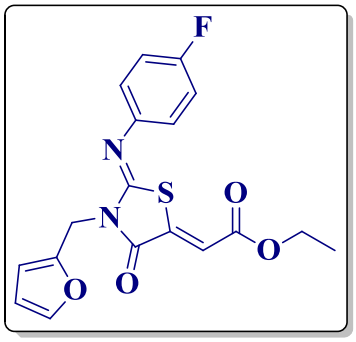
=CH), 7.15 (d, *J* = 8Hz, 2H,Ar-H), 7.36 – 7.37 (dd, *J* = 0.8 Hz, 1H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 14.15, 20.96, 38.90, 61.69, 109.74, 110.51, 116.59, 120.94, 129.93, 134.93, 141.38, 142.53, 144.60, 148.68, 149.97, 164.59, 165.93; **MS (ESI m/z):** 370.93 $[M+H]^+$; Anal. Calcd for $C_{19}H_{18}N_2O_4S$: C, 61.61, H, 4.90, N, 7.56%; Found: C, 61.64, H, 4.86, N, 7.59%.

Ethyl 2-(-2-((4-cyanophenyl)imino)-3-(furan-2-ylmethyl)-4-oxothiazolidin-5-ylidene) acetate (46j):



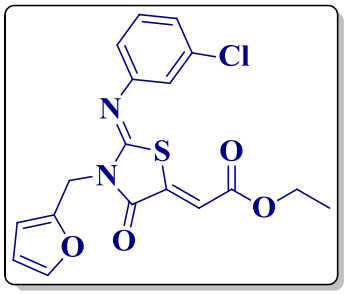
Yellow solid; m.p.: 195 – 197 °C; **FTIR (KBr, ν_{max}, cm^{-1}):** 2227 (-CN), 1729 (Ester –C=O), 1687 (Amide –C=O), 1596 (-C=N); **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm):** 1.30 (t, *J* = 7.2 Hz, 3H, $-CH_3$), 4.23 – 4.28 (m, 2H,$-OCH_2$), 5.13 (s, 2H, $-CH_2$), 6.34 – 6.35 (dd, *J* = 1.6 Hz, 1H, Ar-H), 6.43 (d, *J* = 4 Hz, 1H, Ar-H), 6.96 (s, 1H =CH), 7.06 (d, *J* = 8.8 Hz, 2H,Ar-H), 7.37 – 7.38 (dd, *J* = 0.8 Hz, 1H, Ar-H), 7.66 (d, *J* = 8.8 Hz, 2H, Ar-H); **^{13}C NMR (100 MHz, $CDCl_3$, ppm):** δ 14.12, 38.96, 62.01, 108.68, 109.99, 110.59, 117.90, 118.79, 122.04, 133.55, 140.09, 142.75, 148.18, 151.17, 151.99, 164.23, 165.75; **MS (ESI m/z):** 381.80 $[M+H]^+$; Anal. Calcd for $C_{19}H_{15}N_3O_4S$: C, 59.83, H, 3.96, N, 11.02%; Found: C, 59.85; H, 3.92; N, 11.05%.

Ethyl 2-(-2-((4-fluorophenyl)imino)-3-(furan-2-ylmethyl)-4-oxothiazolidin-5-ylidene) acetate (46k):



Yellow solid; m.p.: 236 – 238 °C; **FIR (KBr, ν_{max}, cm^{-1}):** 1727 (Ester –C=O), 1689 (Amide –C=O), 1612 (-C=N), 1019 (C-F); **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm):** 1.30 (t, *J* = 7.2 Hz, 3H, $-CH_3$), 4.22 – 4.28 (m, 2H, $-OCH_2$), 5.13 (s, 2H, $-CH_2$), 6.33 – 6.34 (dd, *J* = 2 Hz, 1H, Ar-H), 6.42 (d, *J* = 4 Hz, 1H, Ar-H), 6.93 (s, 1H, =CH), 6.95 (d, *J* = 4.8 Hz, 2H, Ar-H), 7.05 (t, *J* = 8.4 Hz, 2H, Ar-H), 7.37 (s, 1H, Ar-H); **MS (ESI m/z):** 374.88 $[M+H]^+$; Anal. Calcd for $C_{18}H_{15}FN_2O_4S$ (374.38): C, 57.75; H, 4.04; N, 7.48%; Found: C, 57.73; H, 4.08; N, 7.45%.

Ethyl 2-(-2-((3-chlorophenyl)imino)-3-(furan-2-ylmethyl)-4-oxothiazolidin-5-ylidene) acetate (46l):



Yellow solid; m.p.: 222 – 224 °C; **FTIR (KBr, ν_{max}, cm^{-1}):** 1726 (Ester –C=O), 1687 (Amide –C=O), 1606 (-C=N), 860 (C-Cl); **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm):** 1.30 (t, *J* = 7.2 Hz, 3H, -CH_3), 4.23 – 4.28 (m, 2H, -OCH_2), 5.12 (s, 2H, Furan-CH_2), 6.33 – 6.35 (m, 1H, Ar-H), 6.42 (d, *J* = 4 Hz, 1H, Ar-H), 6.84 – 6.86 (dd, *J* = 2 Hz, 1H, Ar-H), 6.94 (s, 1H, =CH), 6.97 (t, *J* = 2 Hz, 1H, Ar-H), 7.14 – 7.16 (dd, *J* = 2 Hz, 1H, Ar-H), 7.29 (d, *J* = 8 Hz, 1H, Ar-H), 7.37 – 7.38 (dd, *J* = 0.8 Hz, 1H, Ar-H); Anal. Calcd for $C_{18}H_{15}ClN_2O_4S$ (390.84): C, 55.31; H, 3.87; N, 7.17%; Found: C, 55.36; H, 3.84; N, 7.14%.

Anticancer activity evaluation of 2-(-2-((aryl)imino)-3-(furan-2-ylmethyl)-4-oxo thiazolidin-5-ylidene)acetate derivatives:

All the synthesized compounds were submitted for primary anticancer activity evaluation. The selected compounds were screened for primary anticancer activity at 10 µM concentration in accordance with the National Cancer Institute (NCI), Bethesda, USA of Drug Evaluation Branch (Chapter-II Reference 68 - 70).

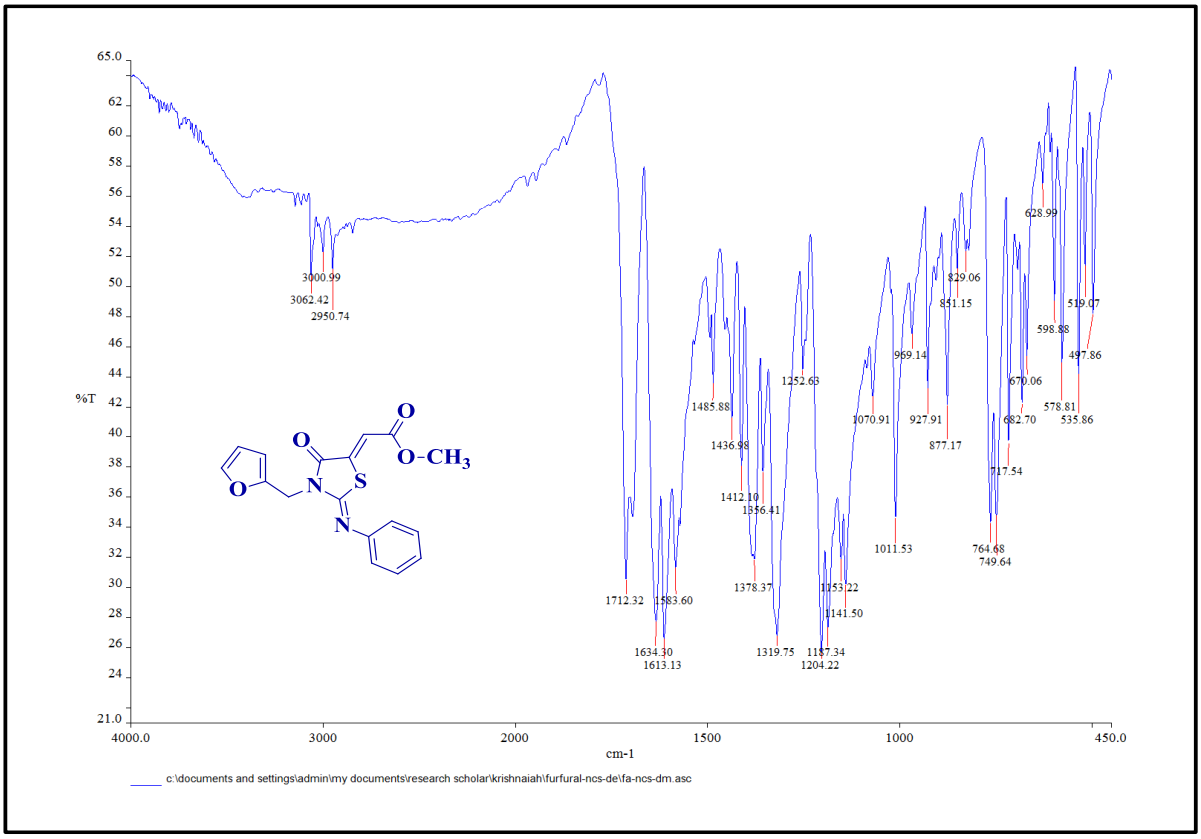


Fig. IR Spectrum of compound **48a**

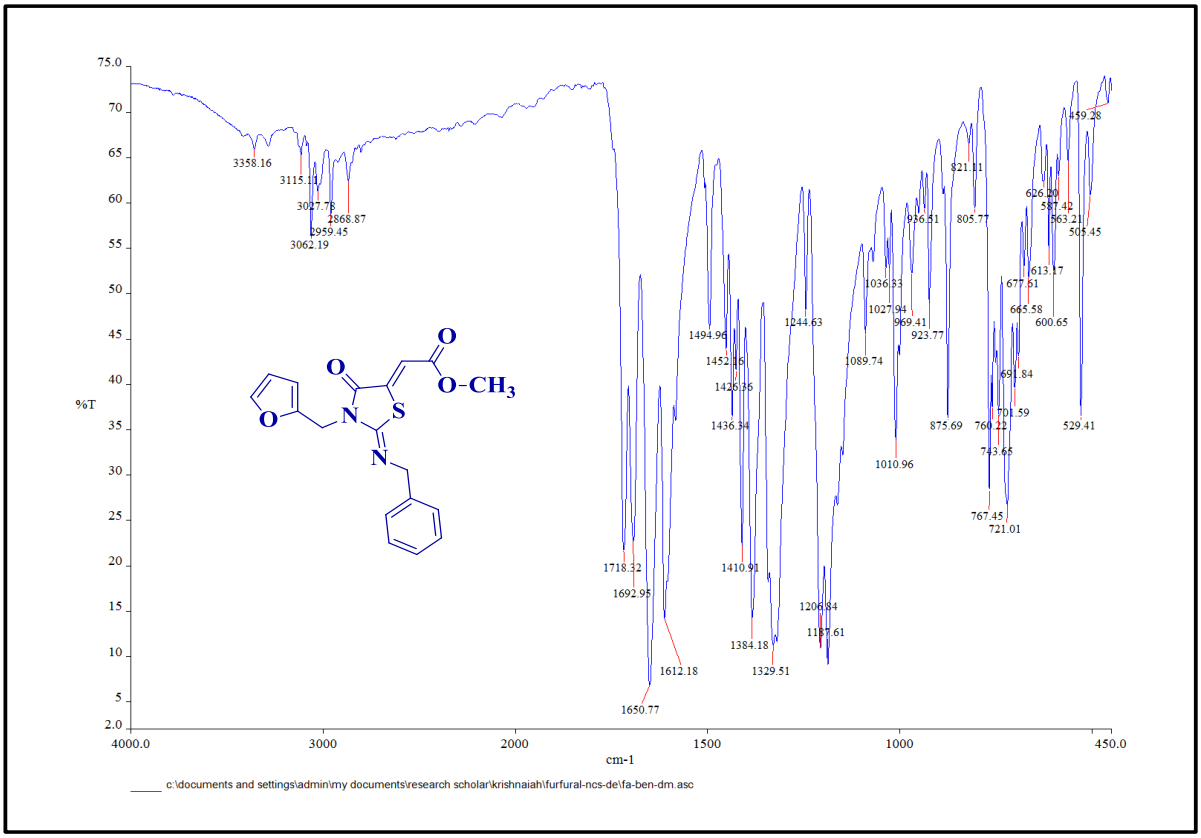


Fig. IR Spectrum of compound **48b**

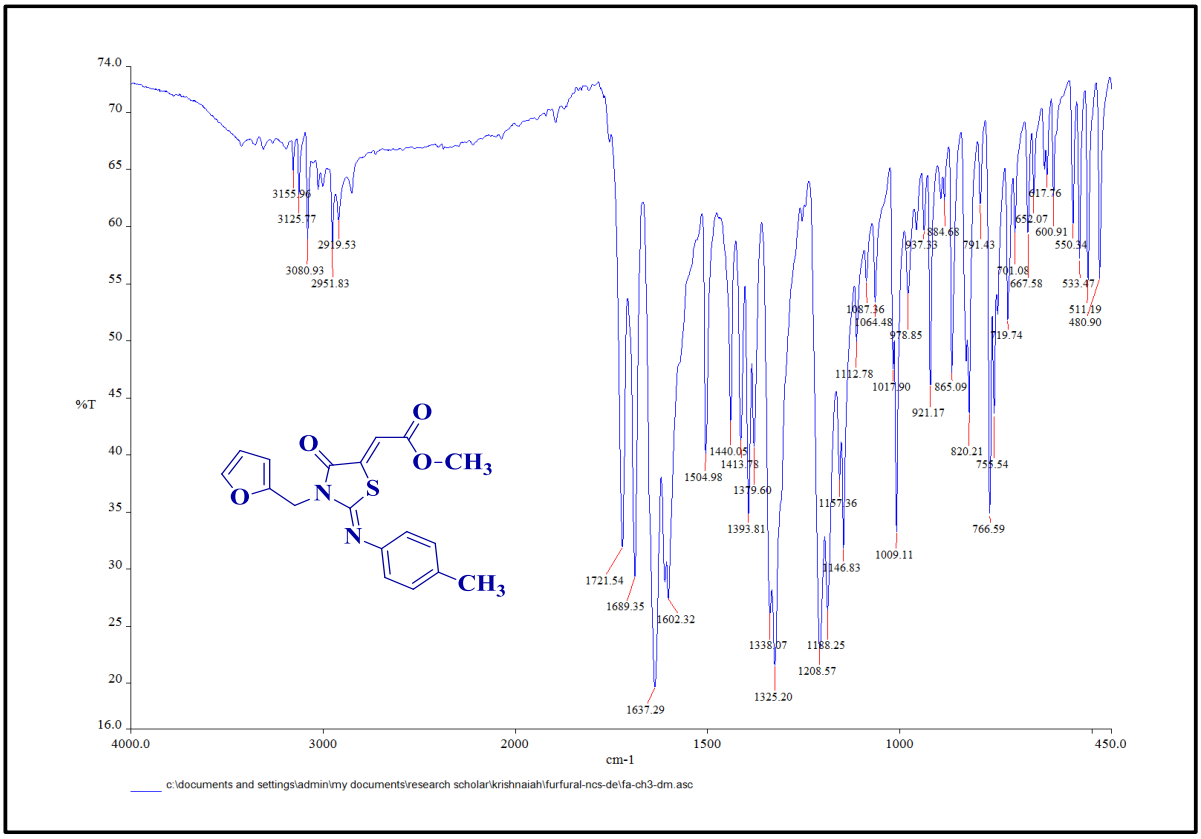


Fig. IR Spectrum of compound **48c**

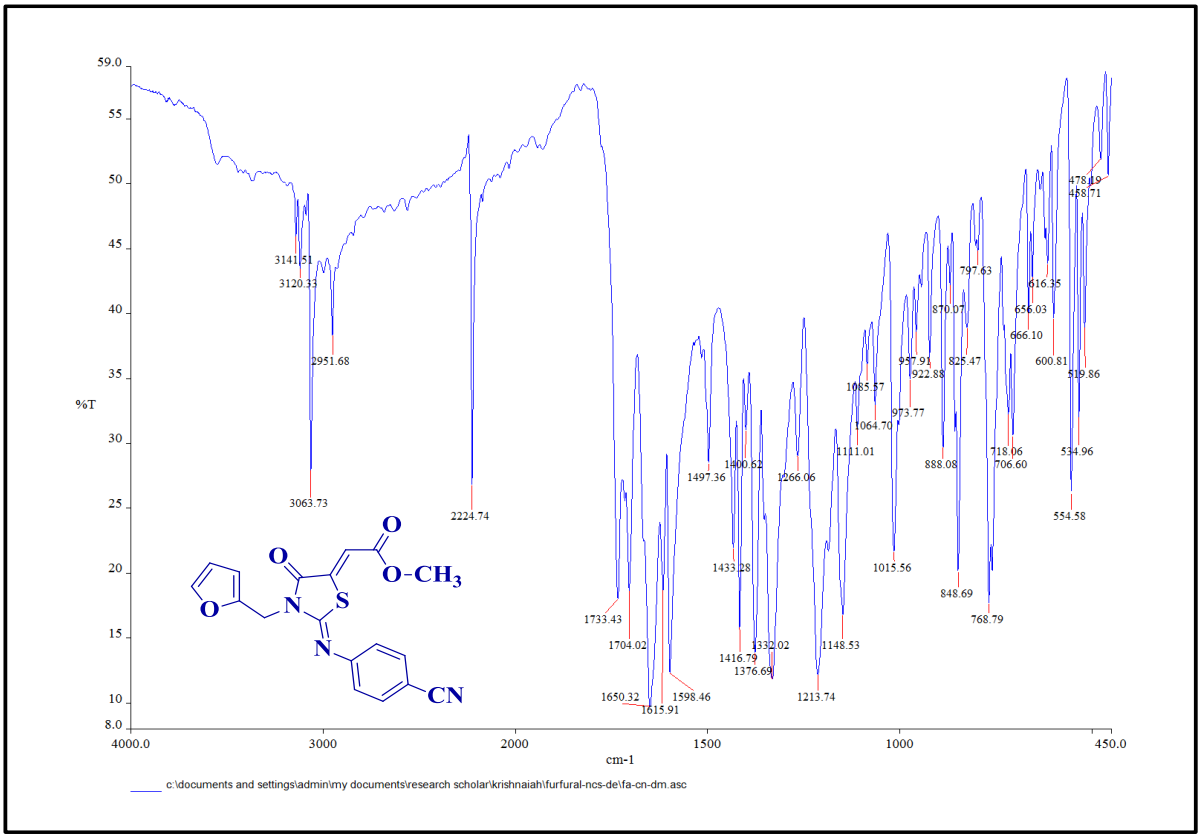


Fig. IR Spectrum of compound **48d**

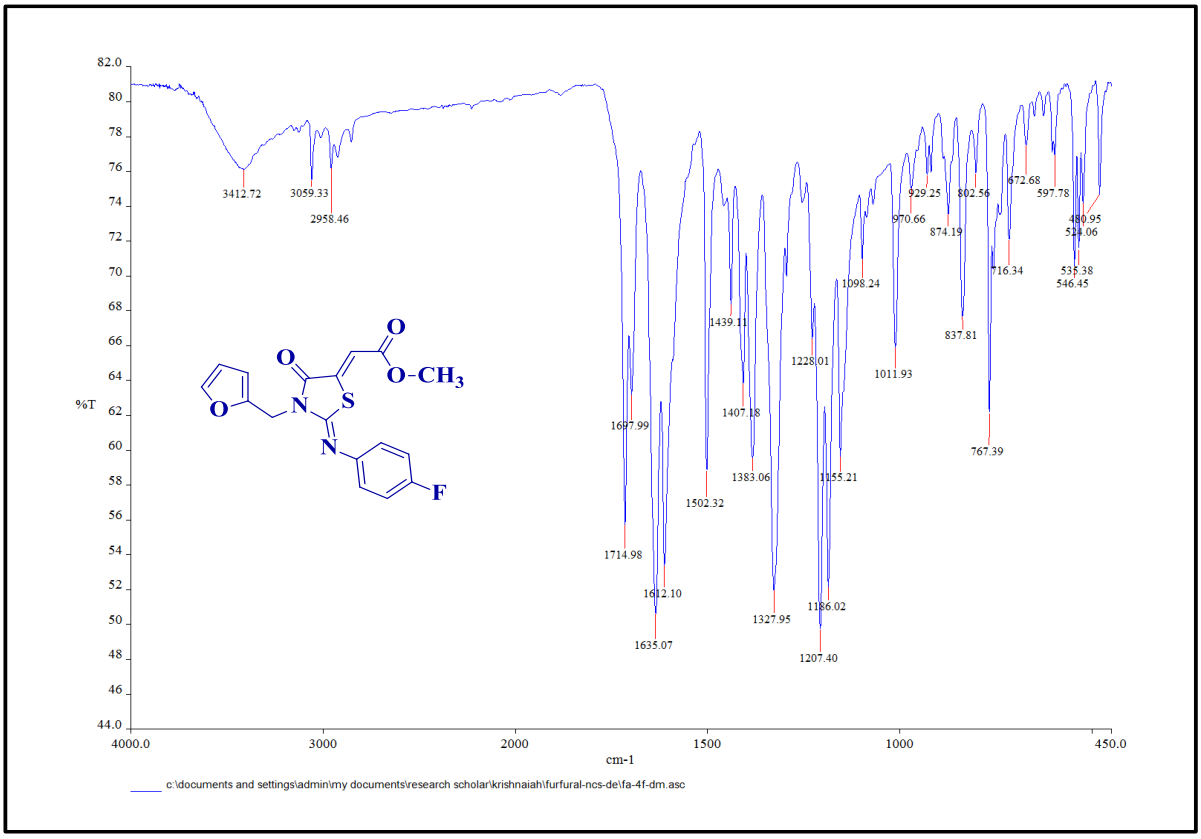


Fig. IR Spectrum of compound **48e**

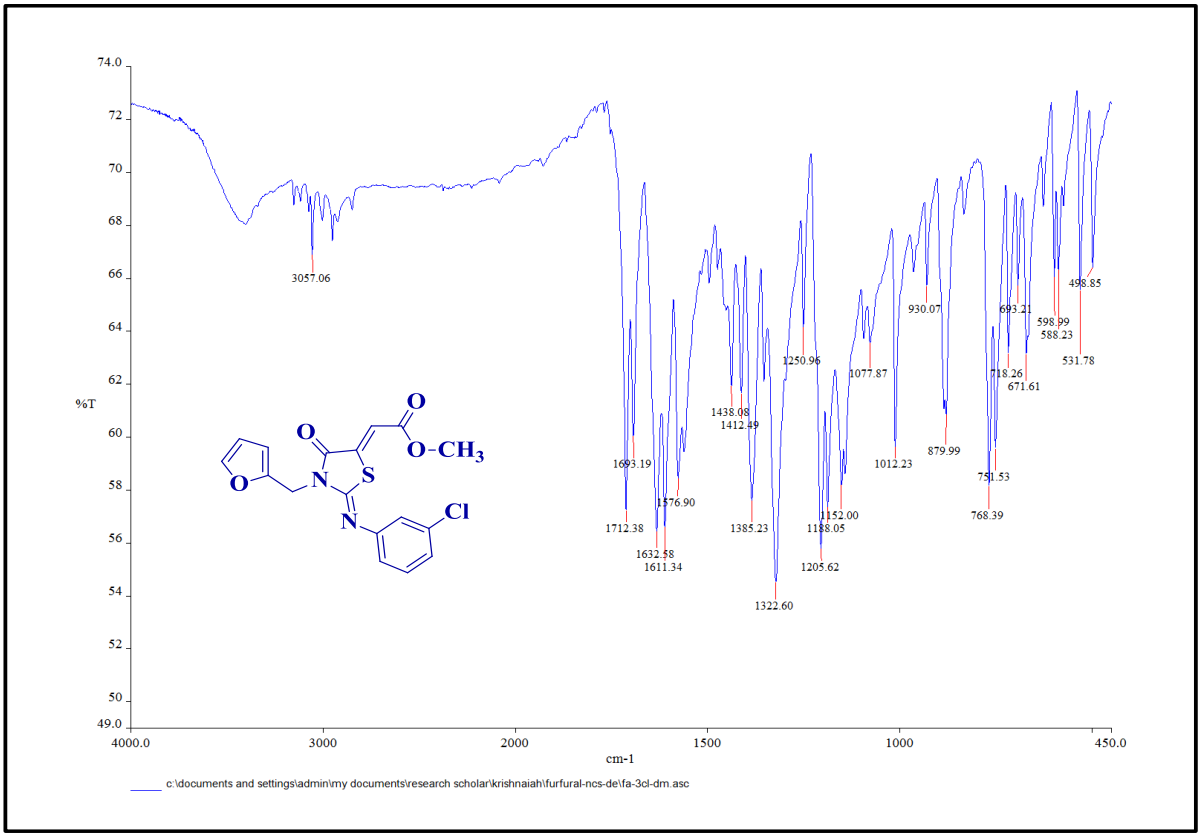


Fig. IR Spectrum of compound **48f**

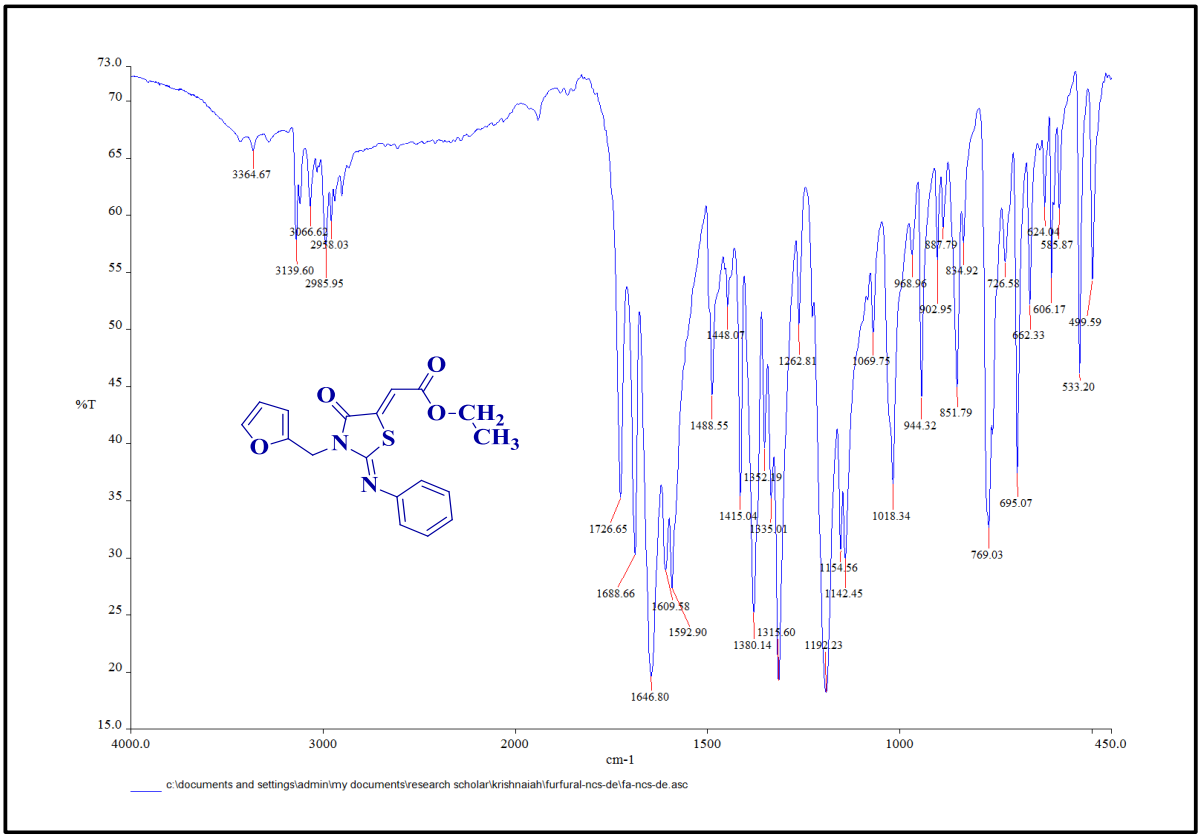


Fig. IR Spectrum of compound **48g**

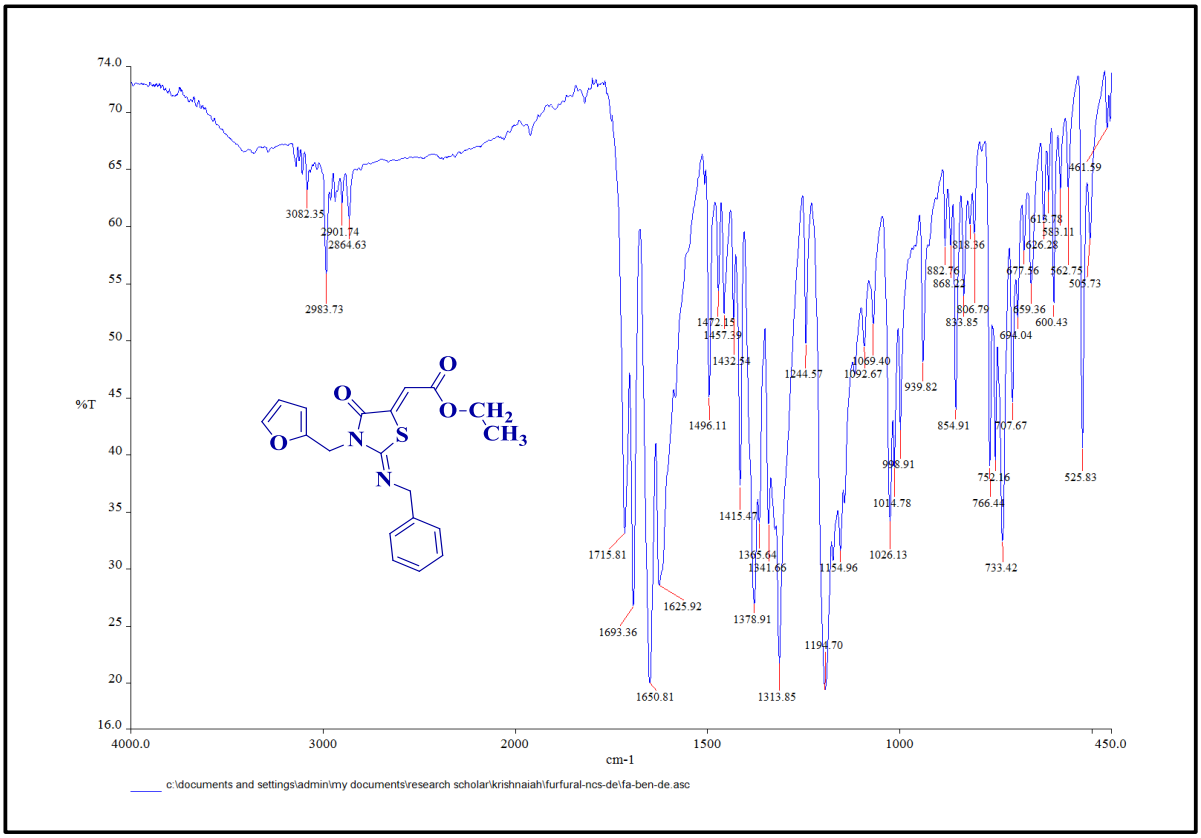


Fig. IR Spectrum of compound **48h**

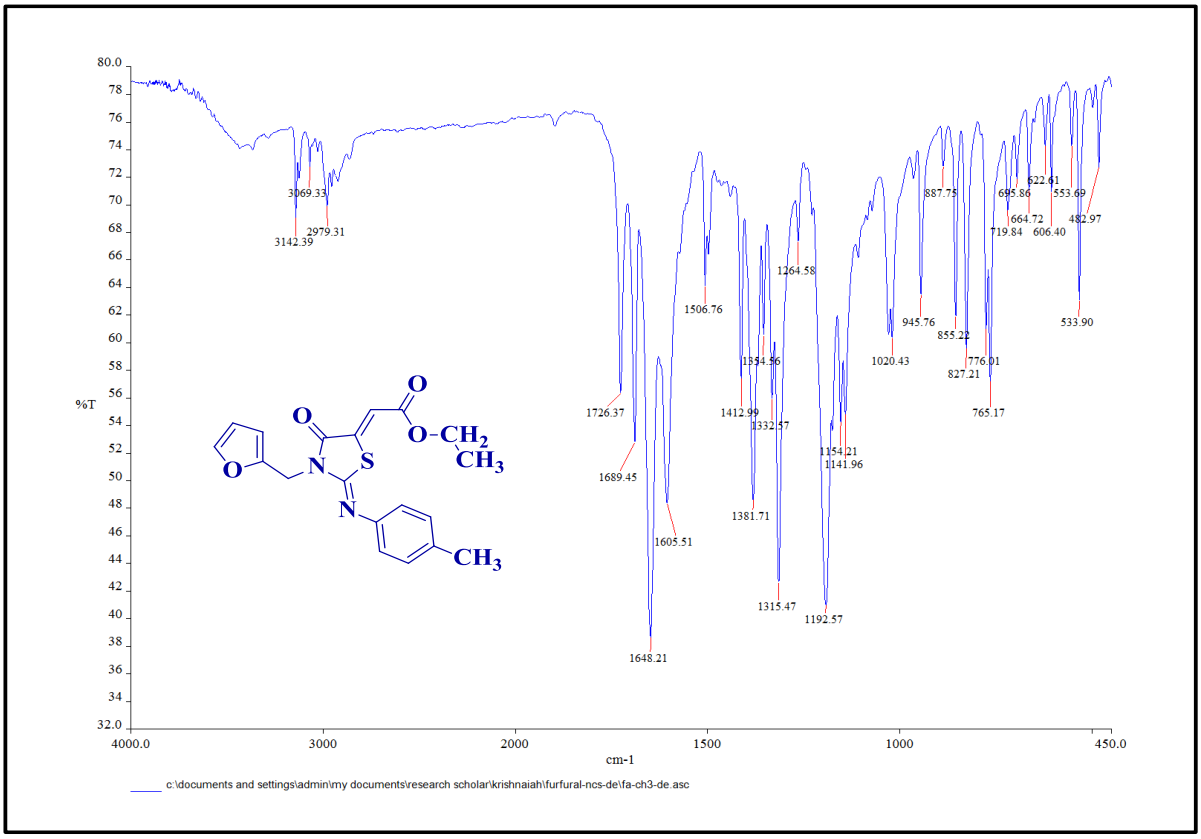


Fig. IR Spectrum of compound **48i**

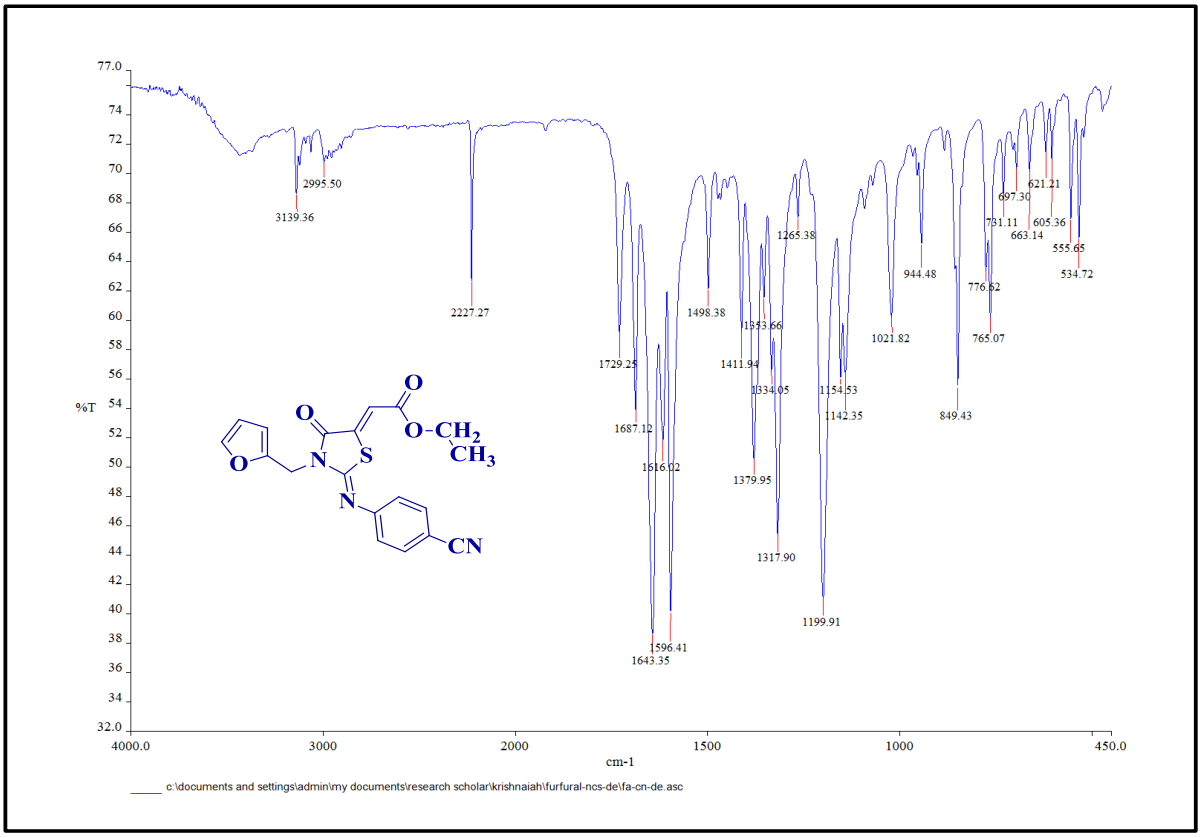


Fig. IR Spectrum of compound **48j**

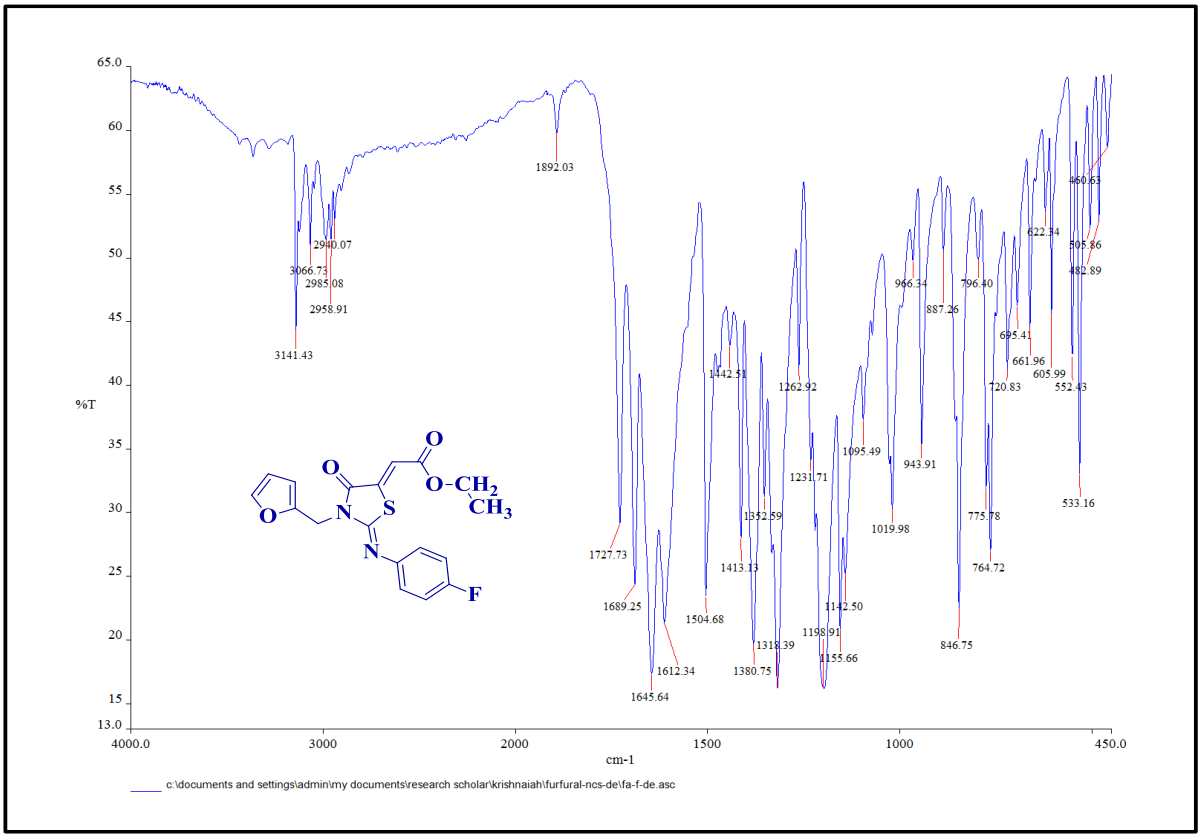


Fig. IR Spectrum of compound **48k**

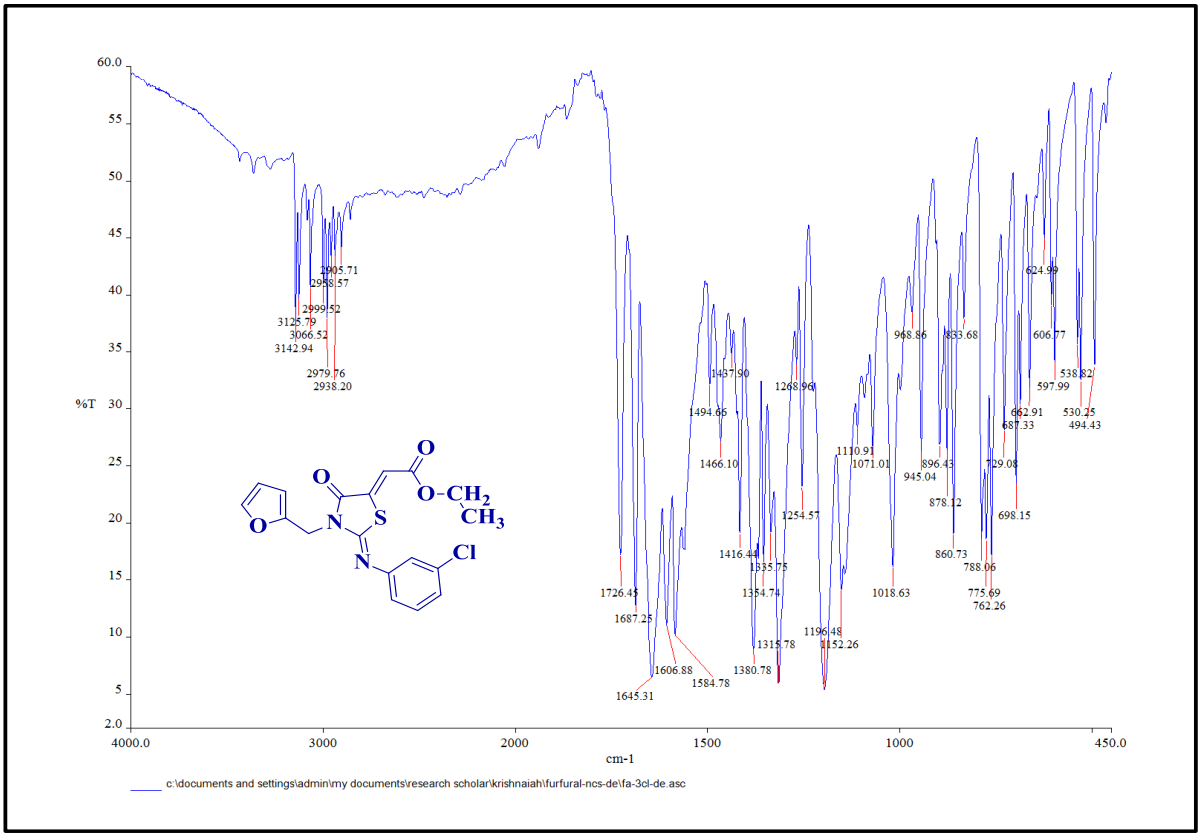


Fig. IR Spectrum of compound **48l**

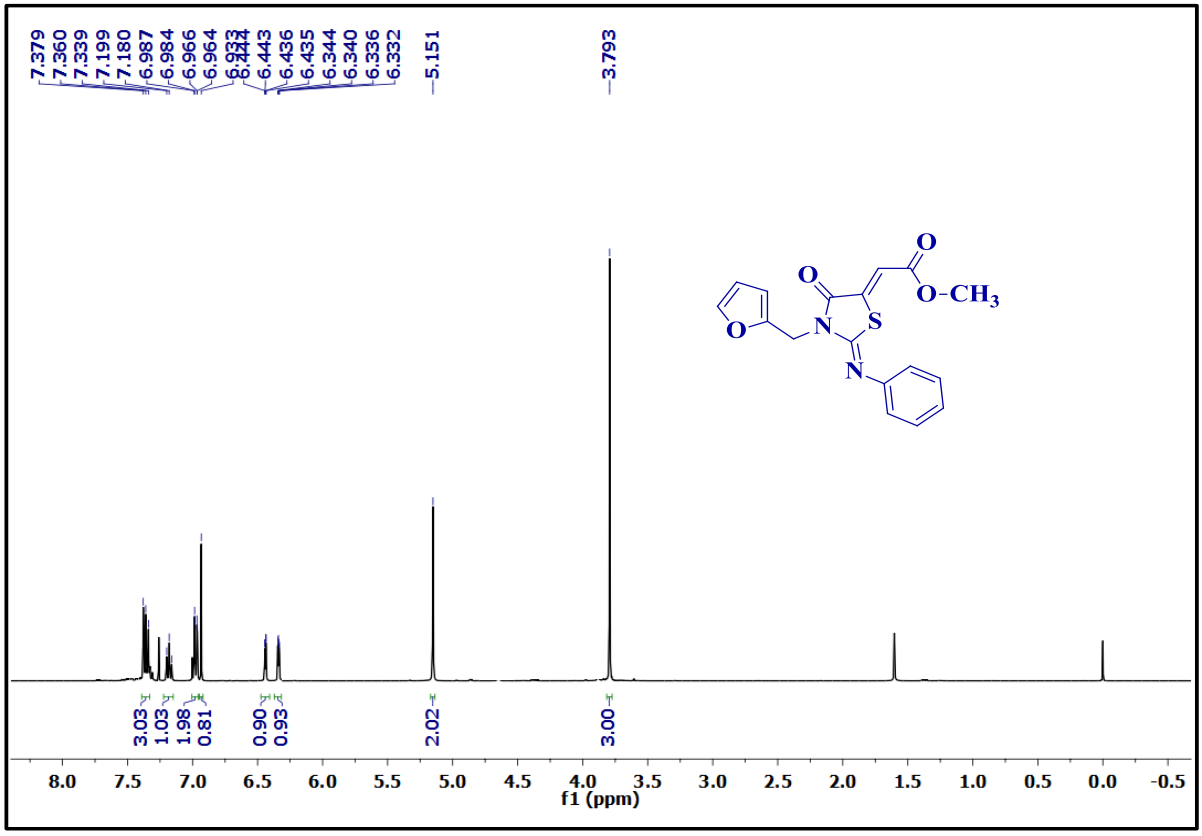


Fig. [1]H NMR Spectrum of compound **48a**

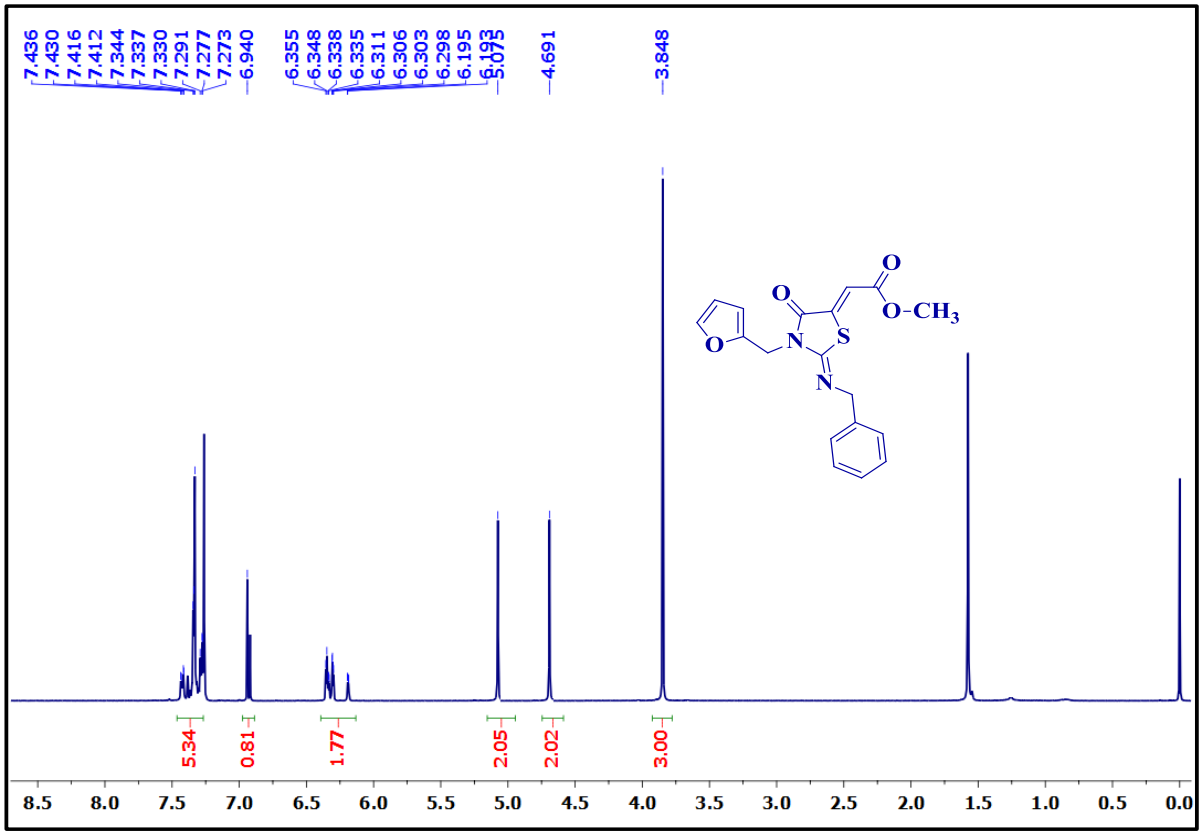


Fig. [1]H NMR Spectrum of compound **48b**

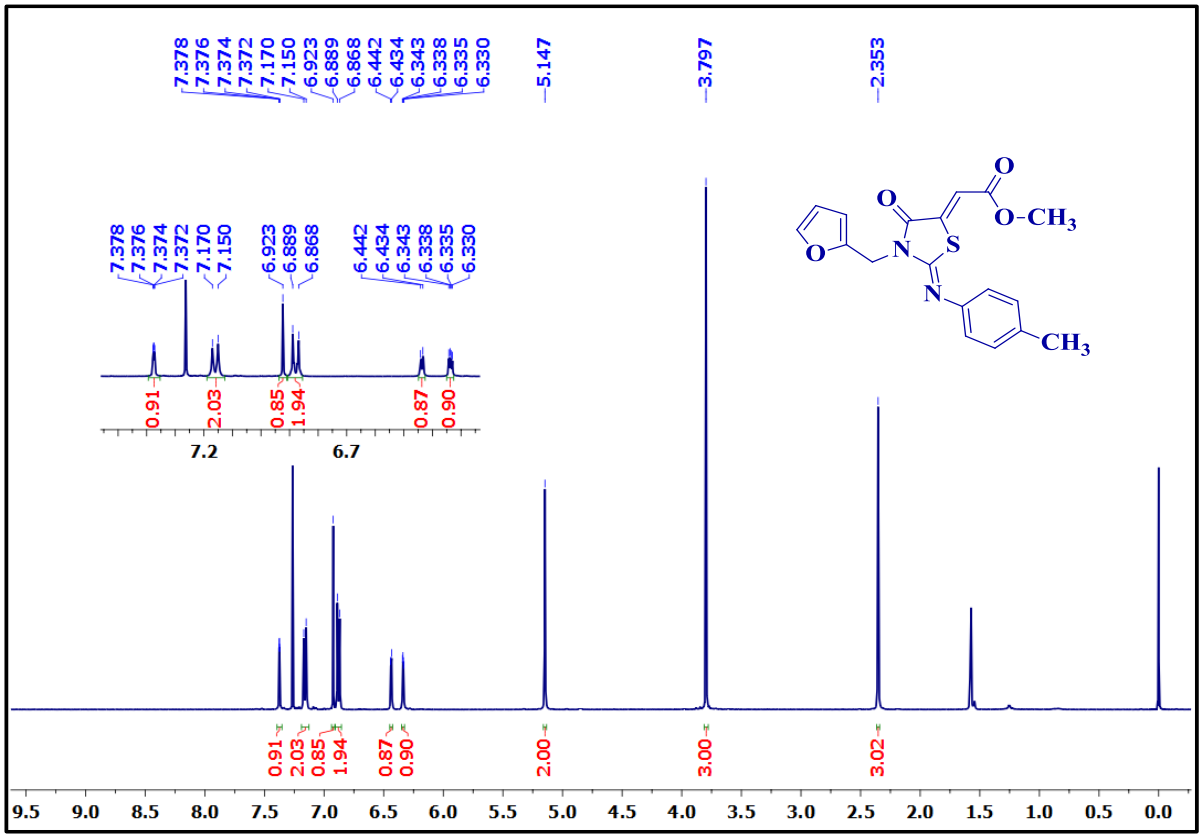


Fig. [1]H NMR Spectrum of compound **48c**

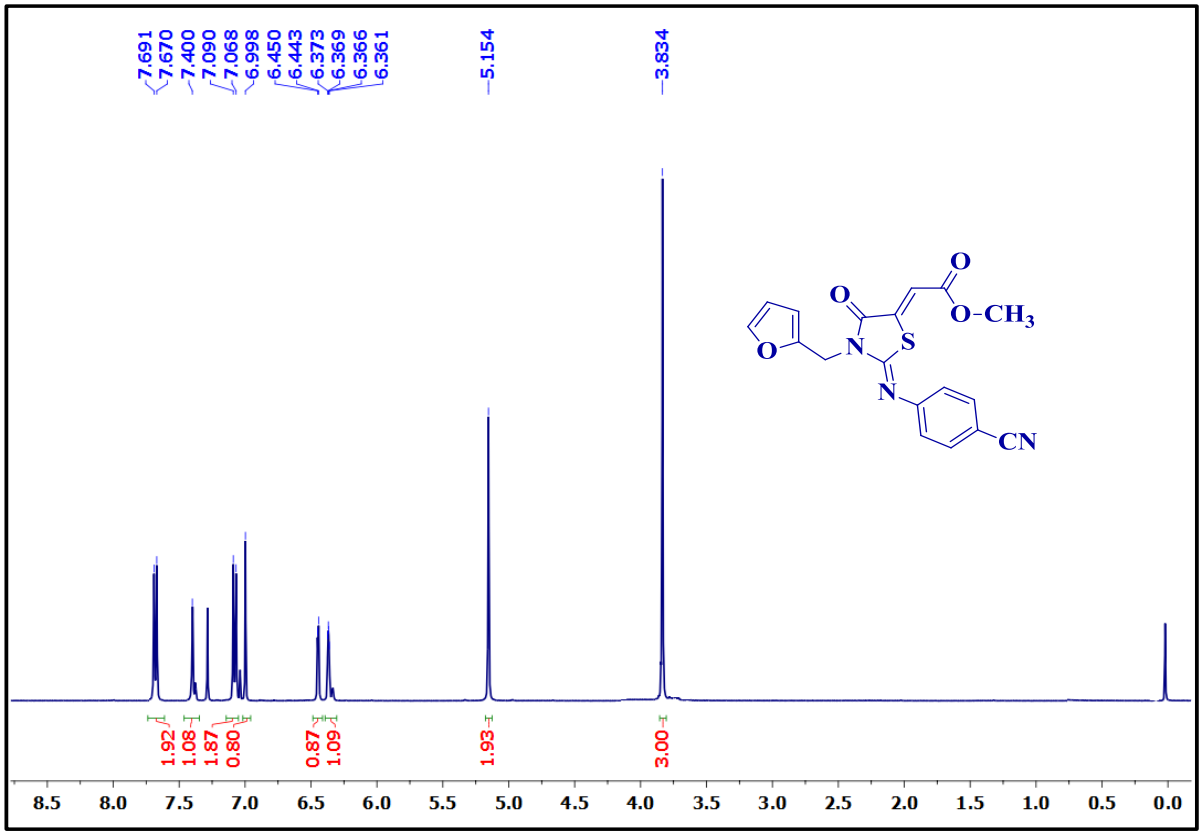


Fig. [1]H NMR Spectrum of compound **48d**

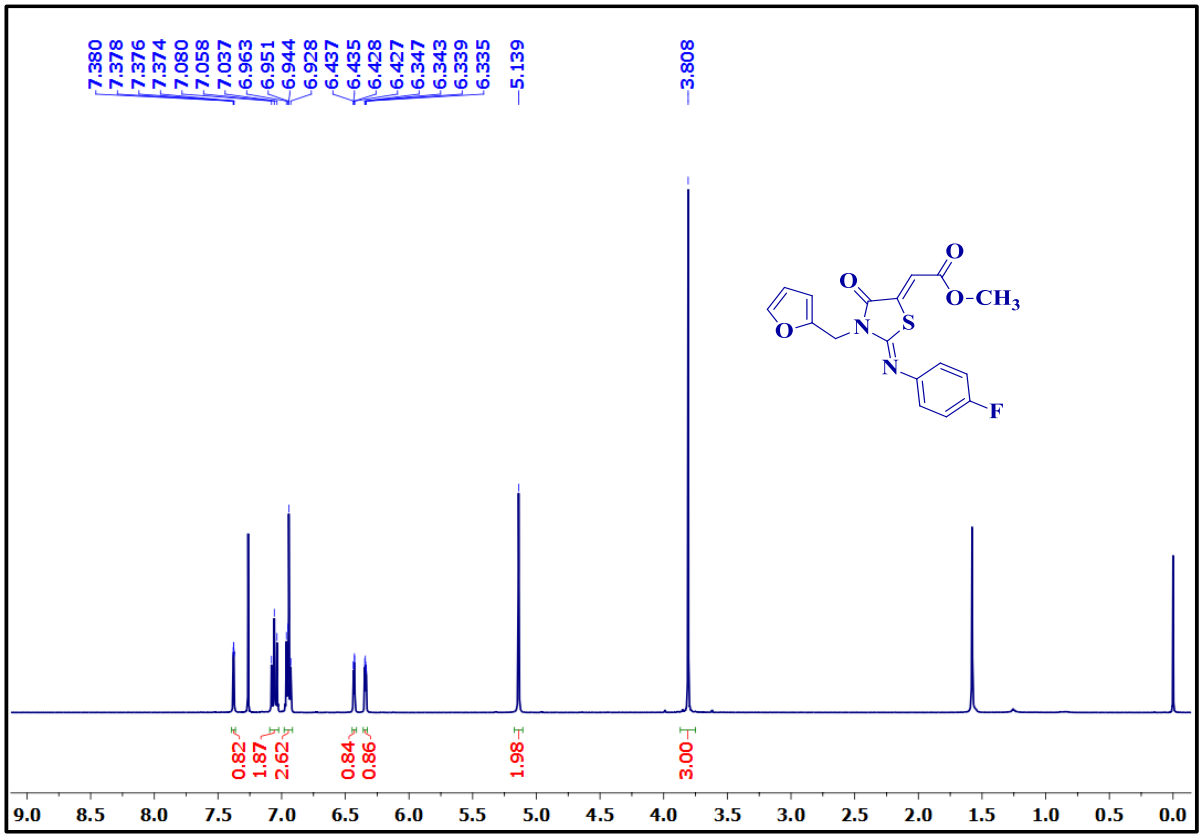


Fig. [1]H NMR Spectrum of compound **48e**

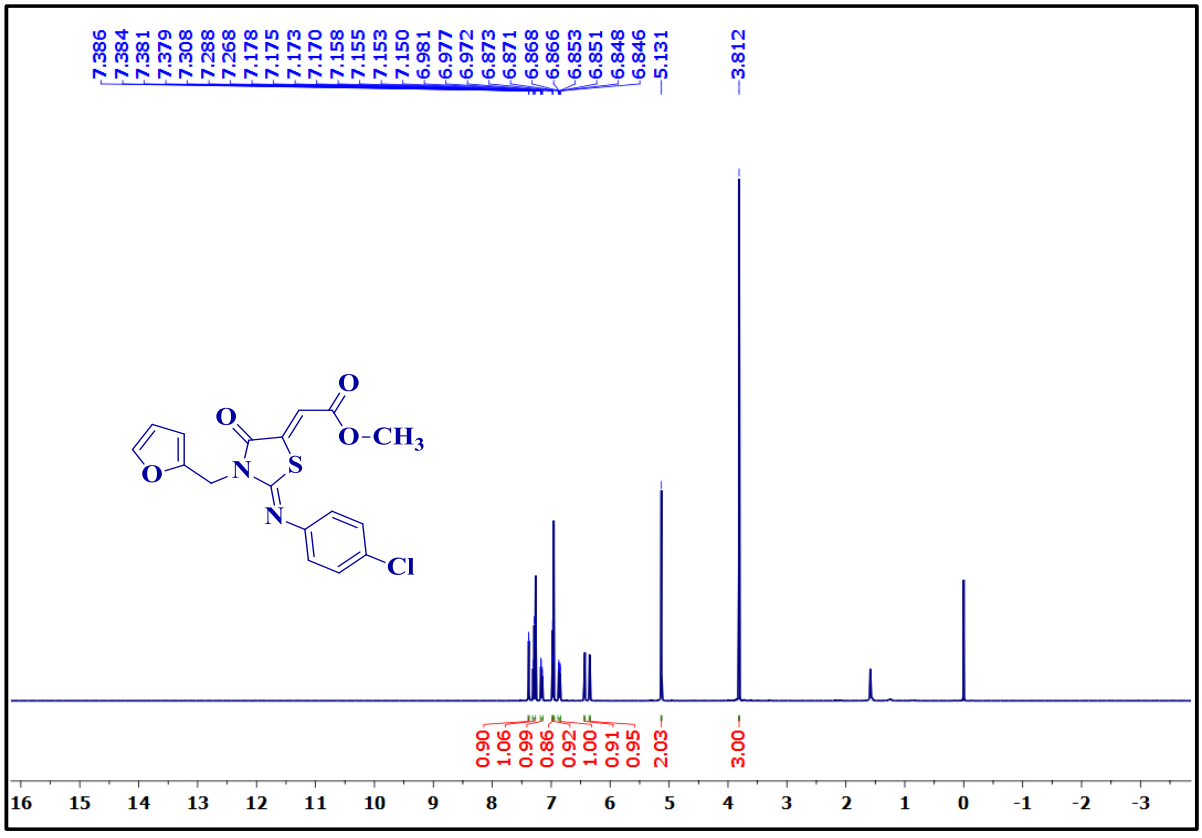


Fig. [1]H NMR Spectrum of compound **48f**

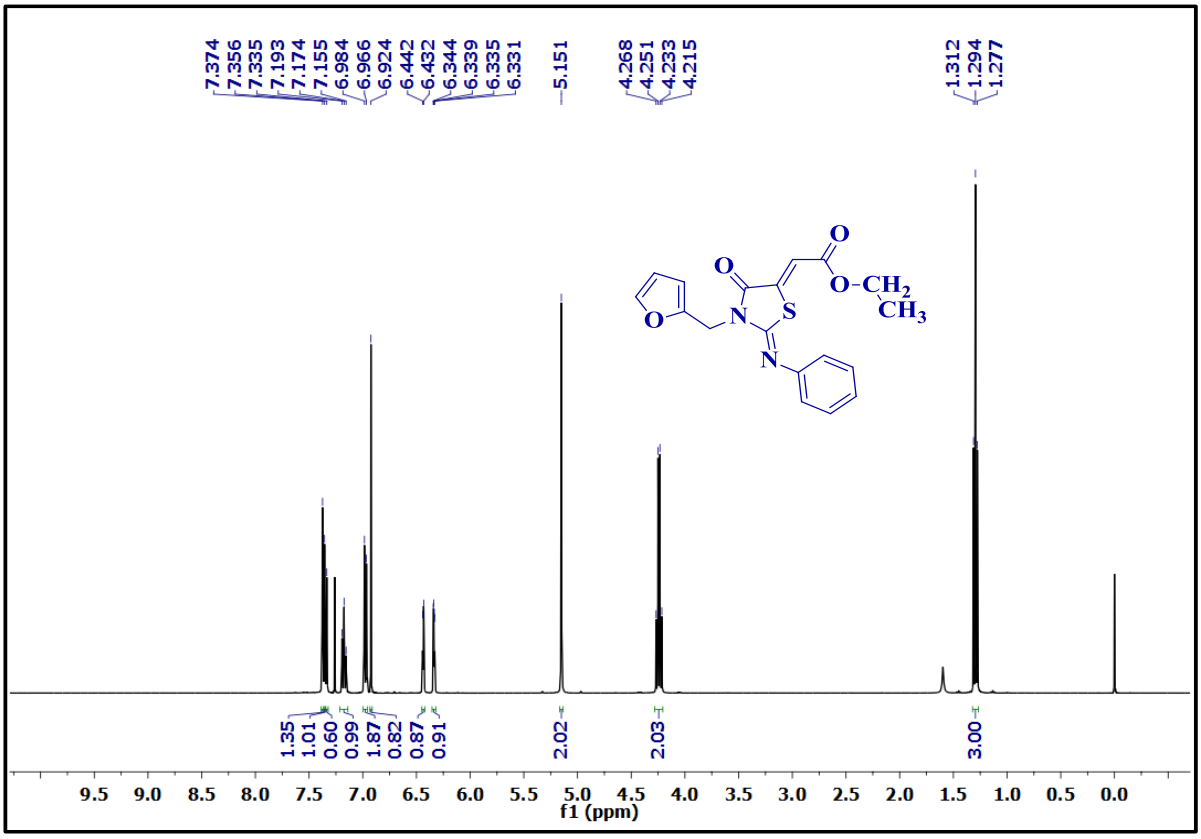


Fig. [1]H NMR Spectrum of compound **48g**

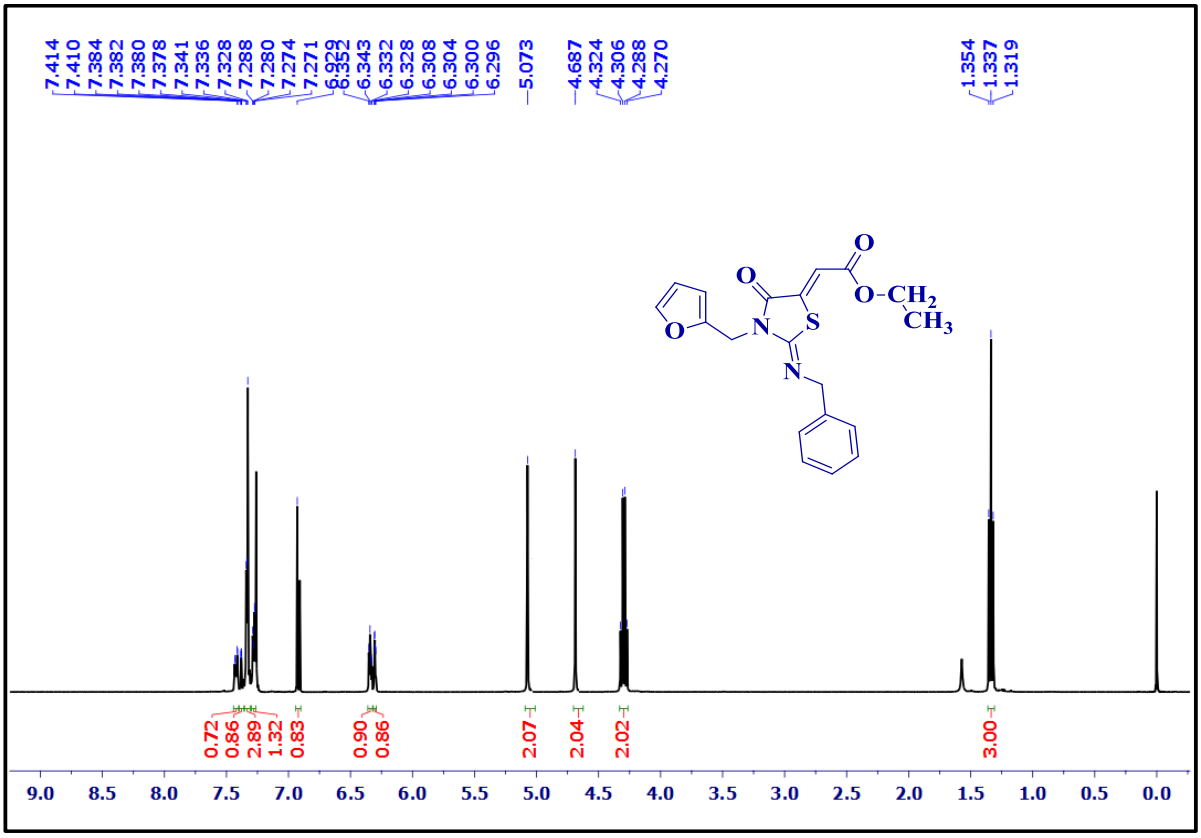


Fig. [1]H NMR Spectrum of compound **48h**

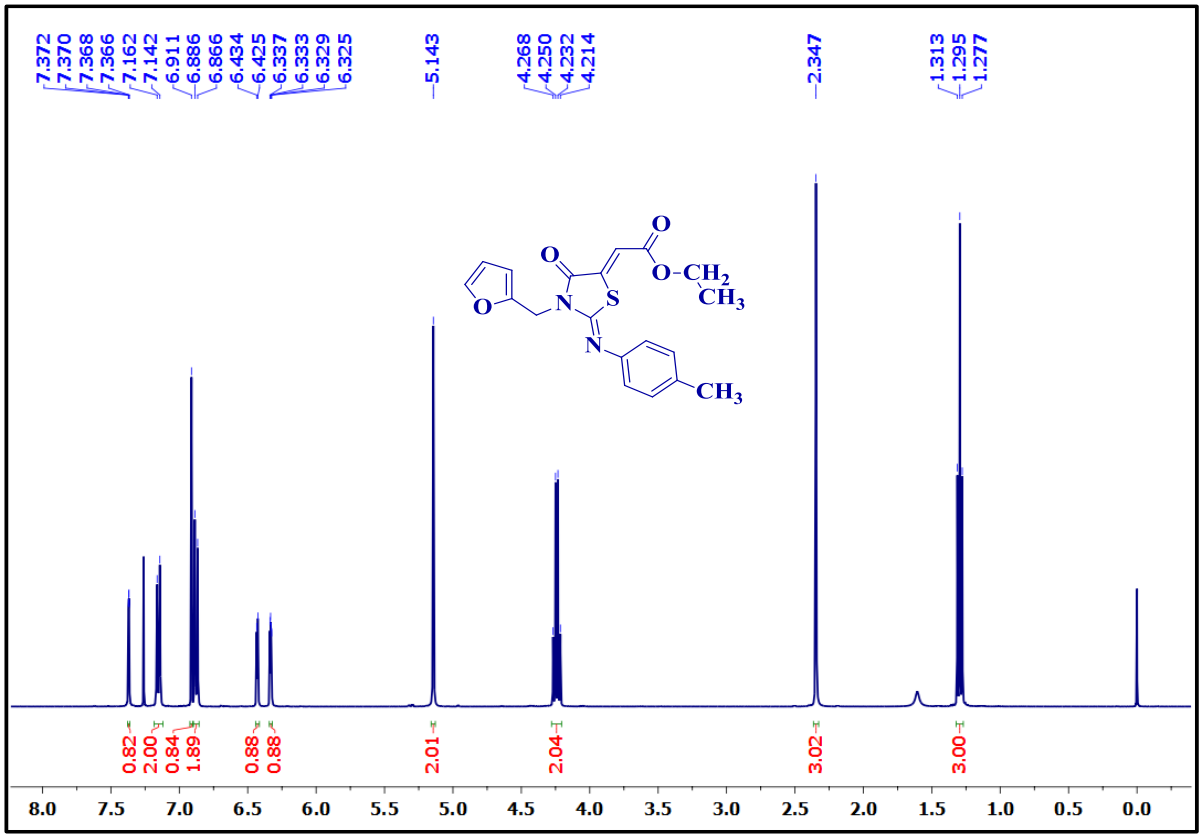


Fig. [1]H NMR Spectrum of compound **48i**

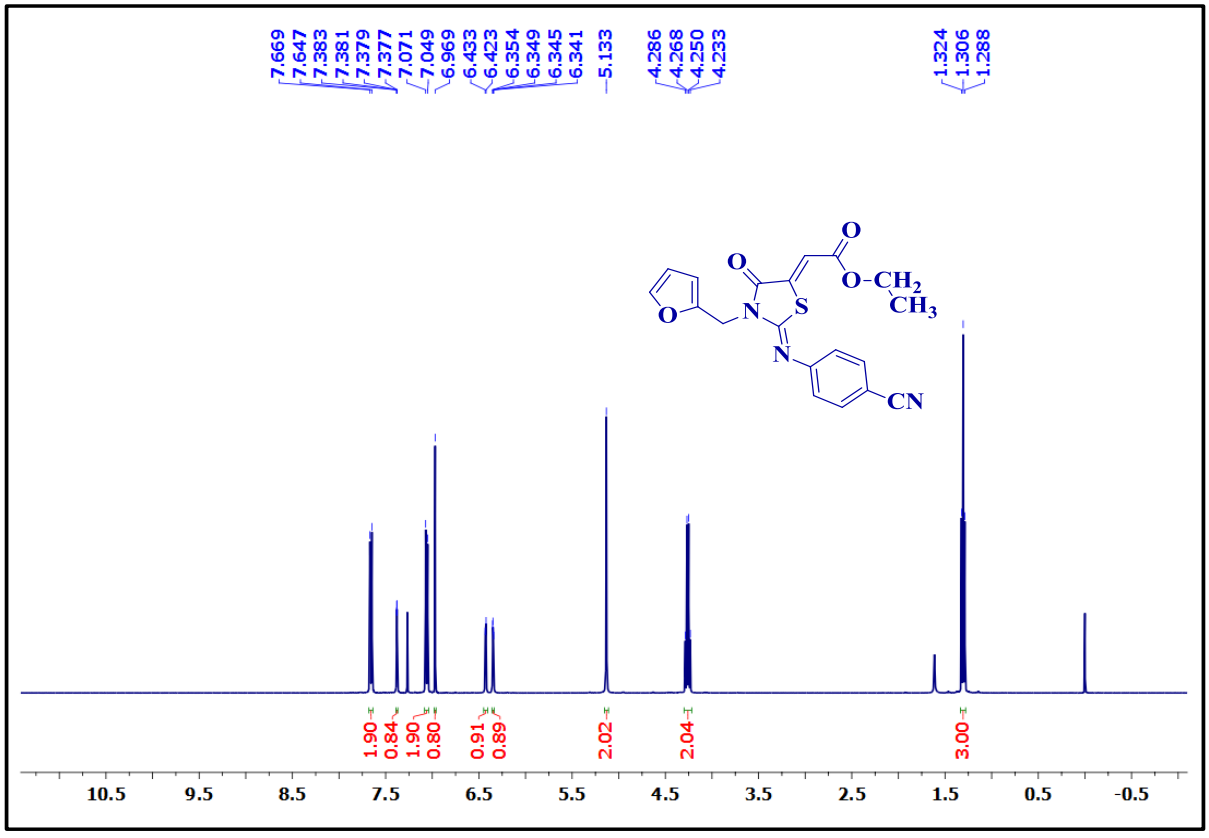


Fig. [1]H NMR Spectrum of compound **48j**

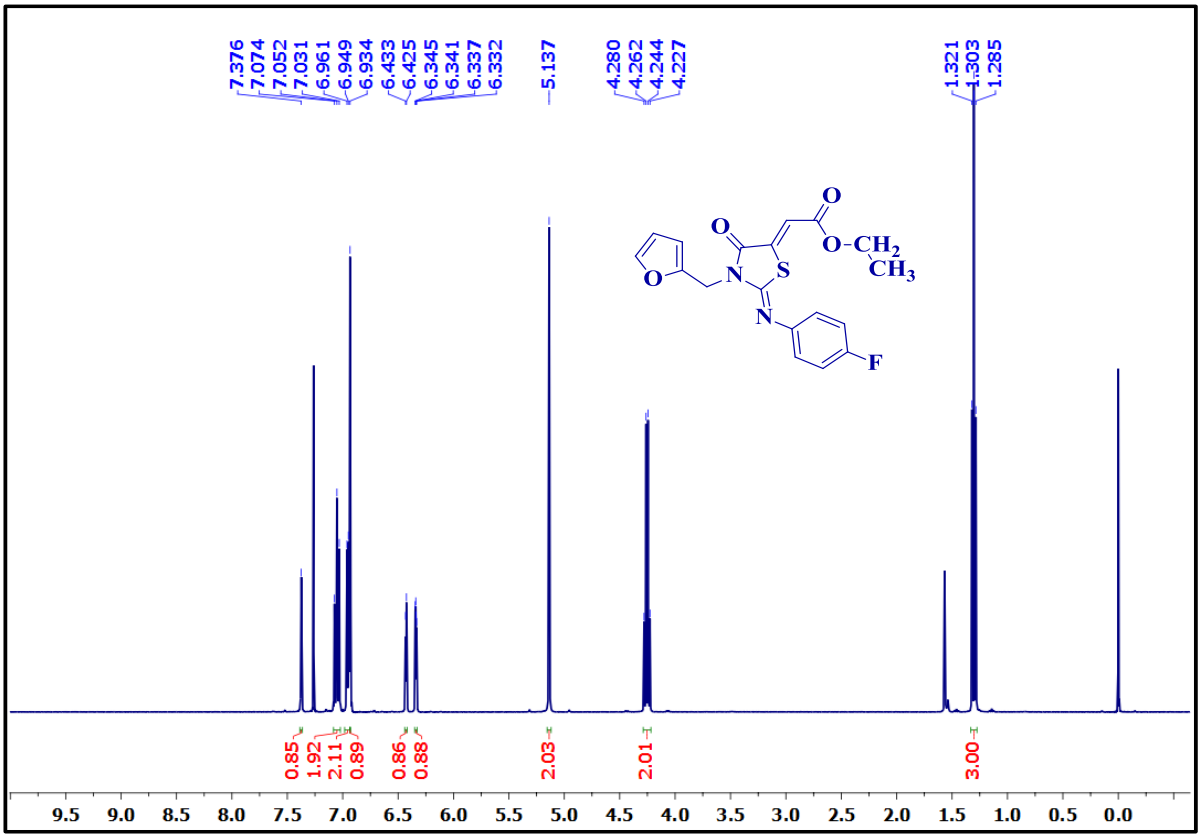


Fig. [1]H NMR Spectrum of compound **48k**

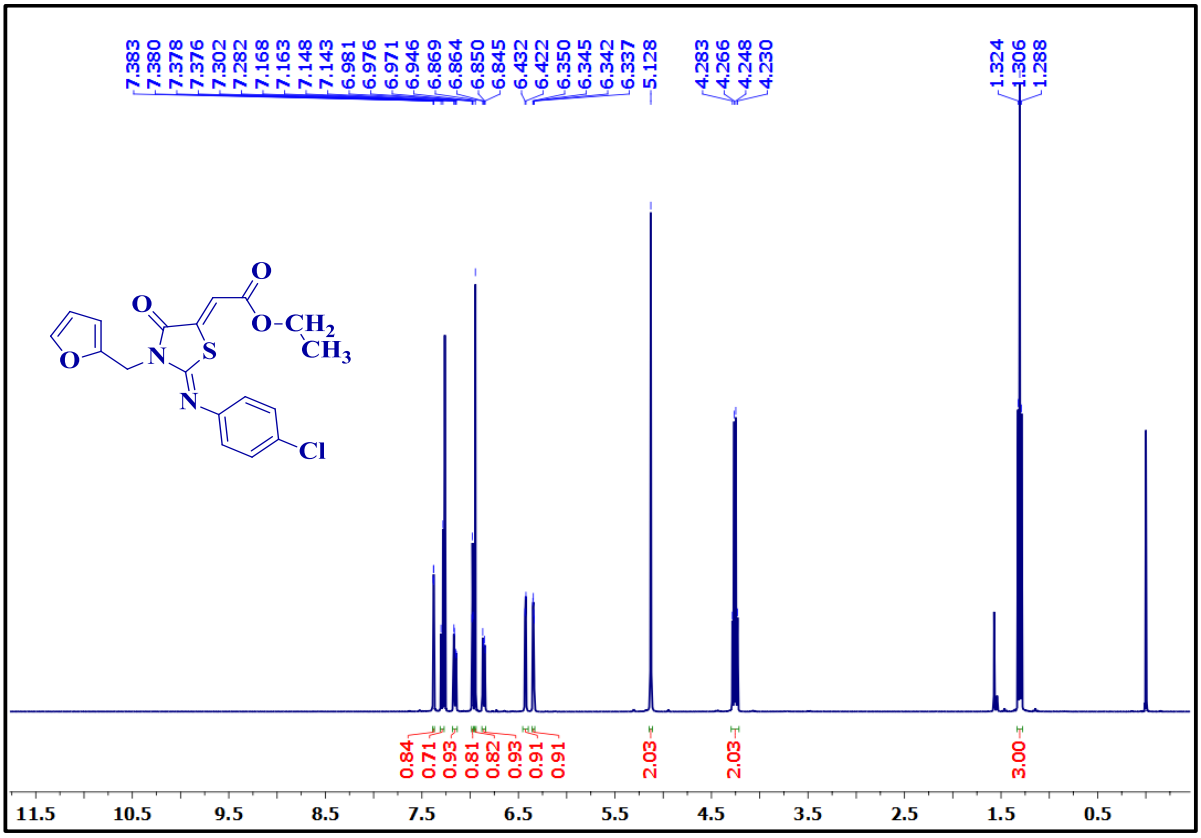


Fig. [1]H NMR Spectrum of compound **48l**

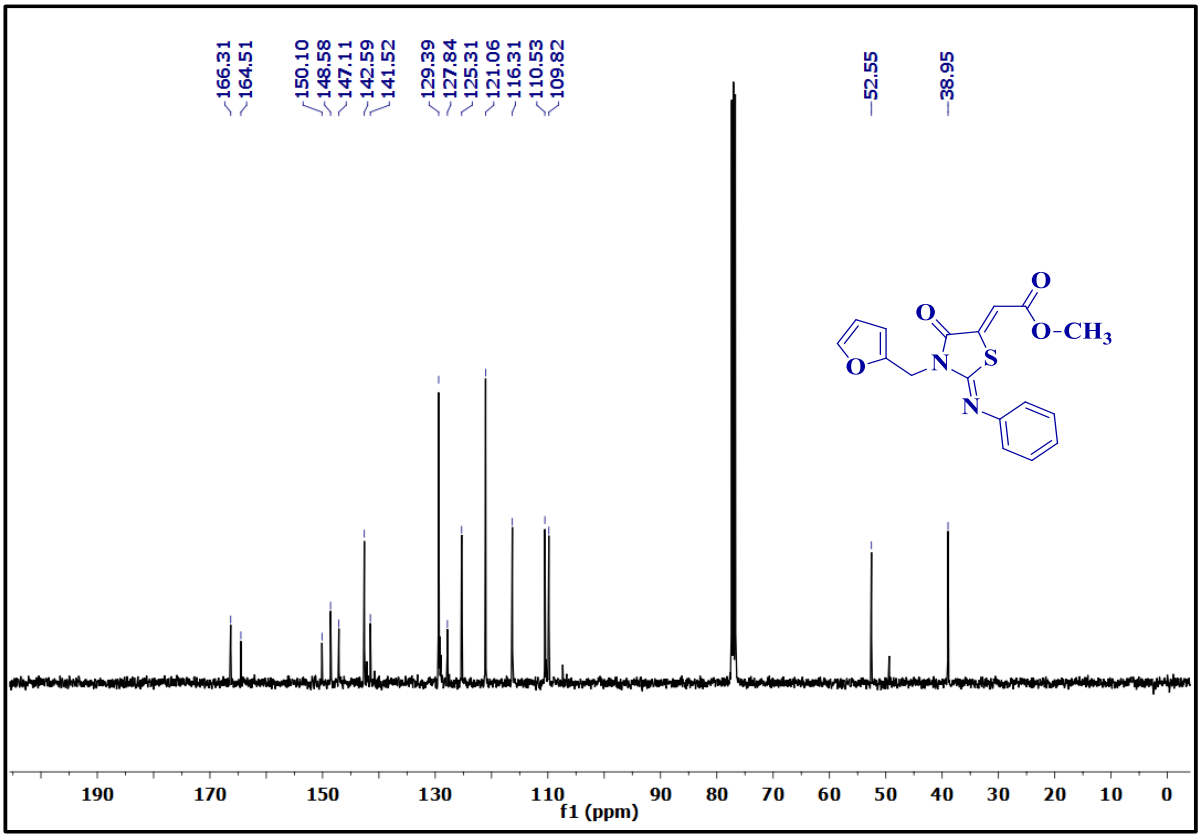


Fig. ^{13}C NMR Spectrum of compound **48a**

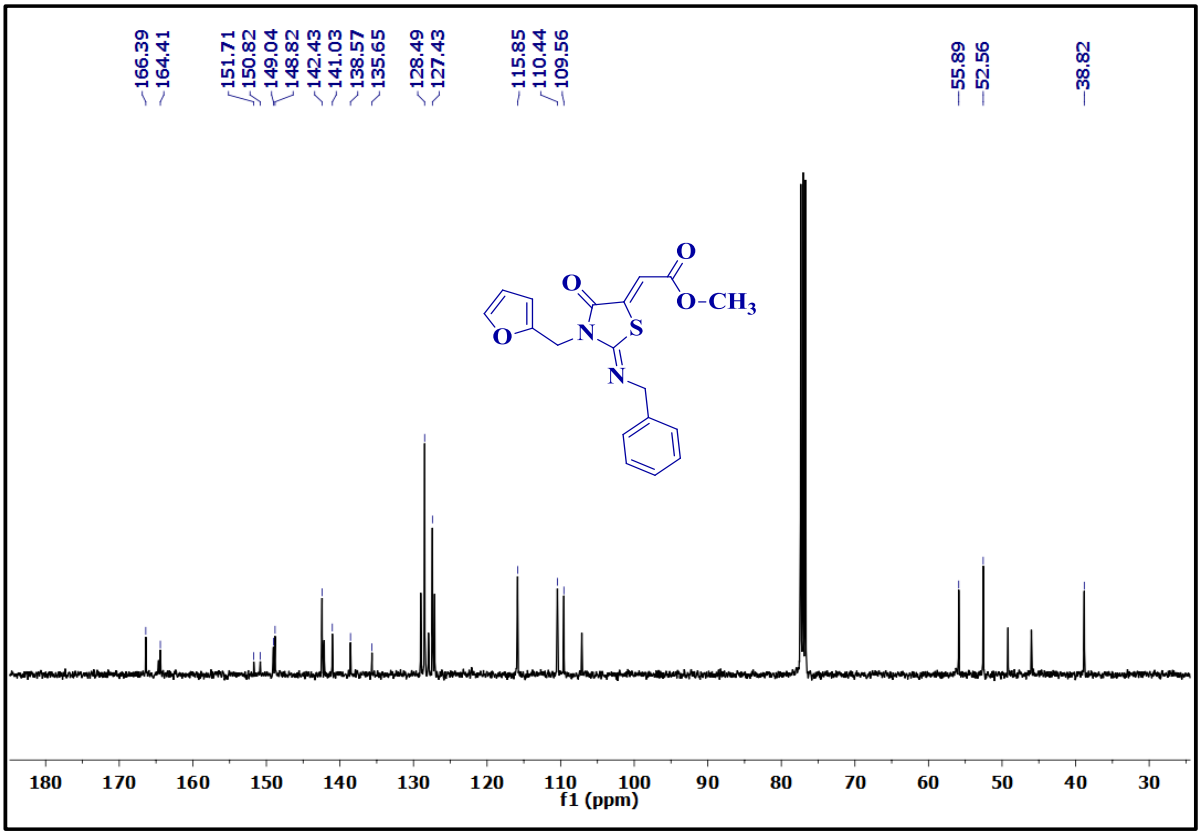


Fig. ^{13}C NMR Spectrum of compound **48b**

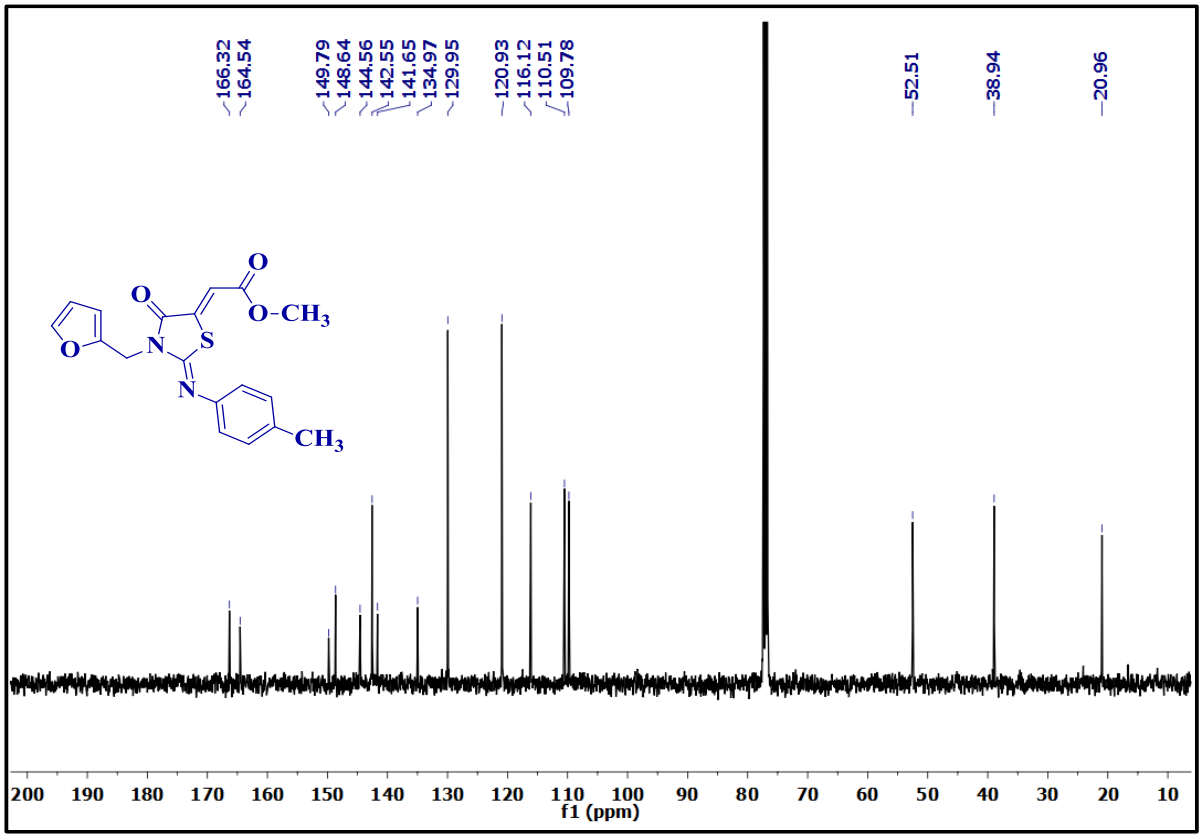


Fig. ^{13}C NMR Spectrum of compound **48c**

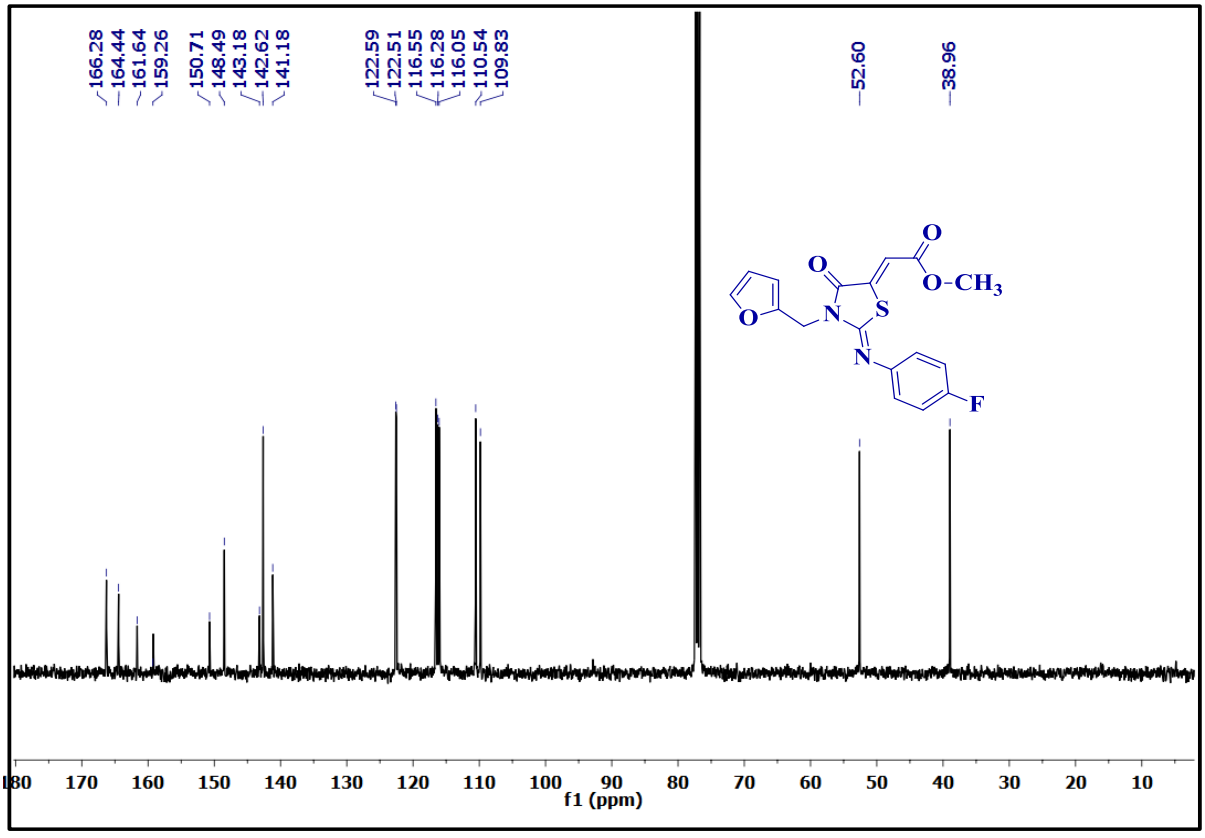


Fig. ^{13}C NMR Spectrum of compound **48e**

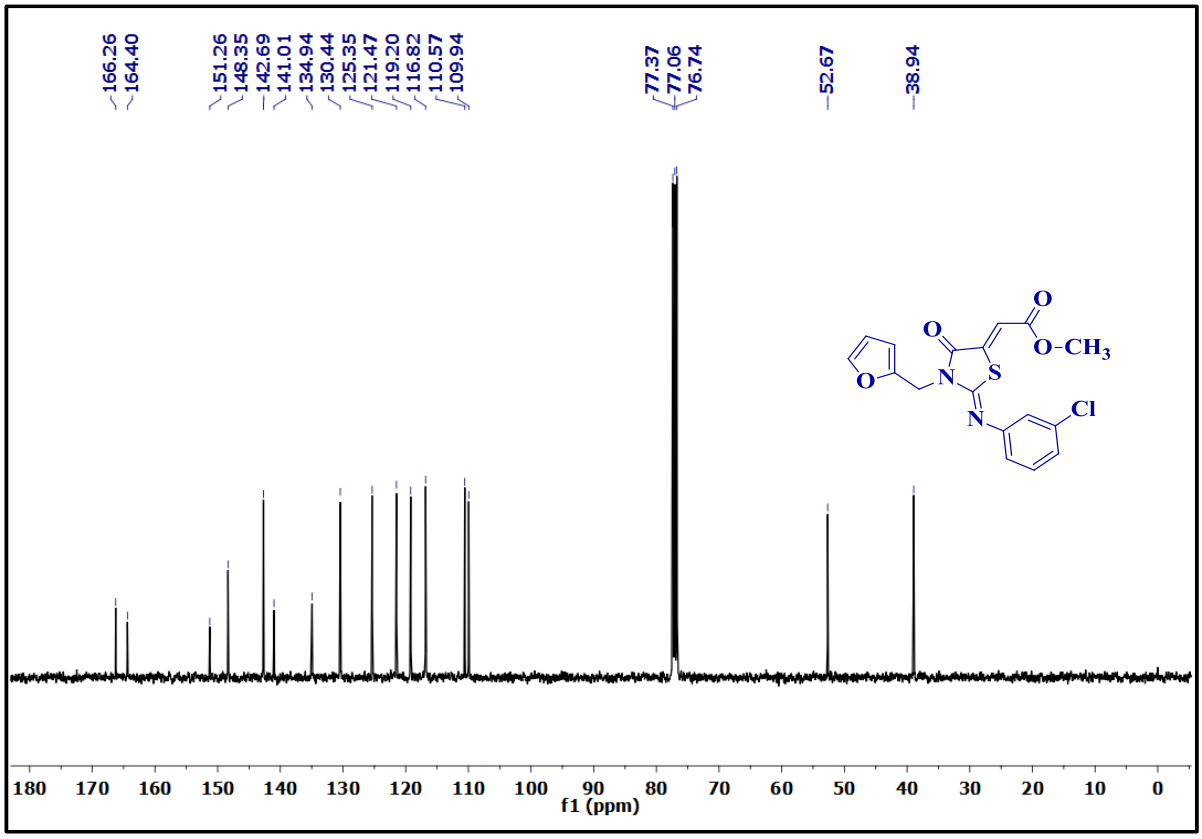


Fig. ^{13}C NMR Spectrum of compound **48f**

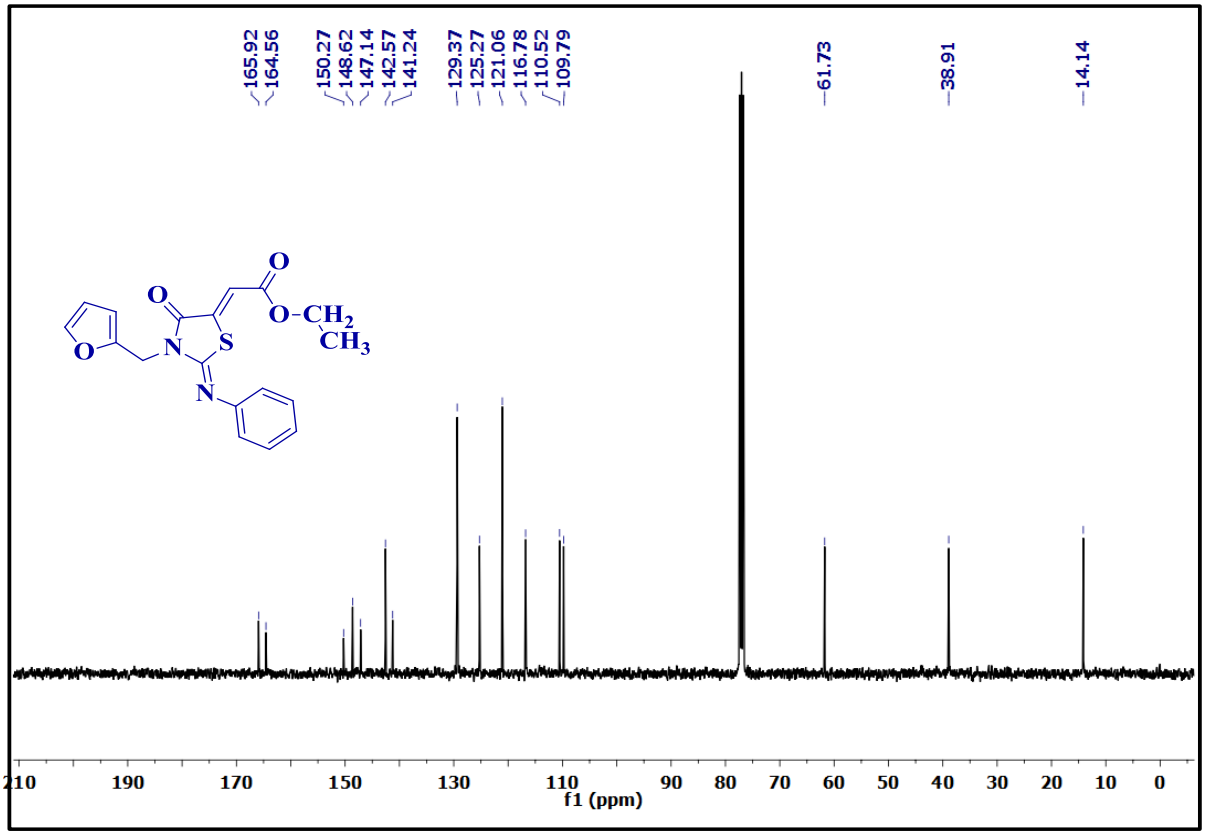


Fig. ^{13}C NMR Spectrum of compound **48g**

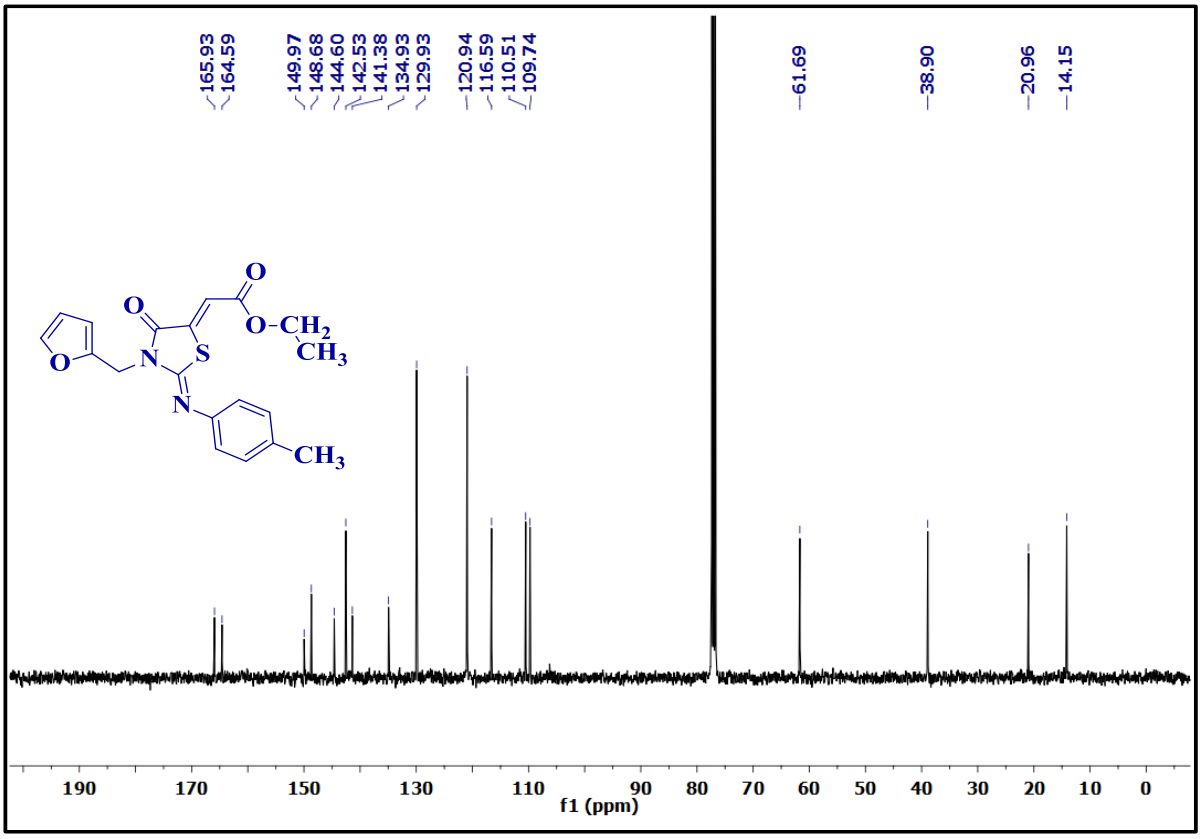


Fig. ^{13}C NMR Spectrum of compound **48i**

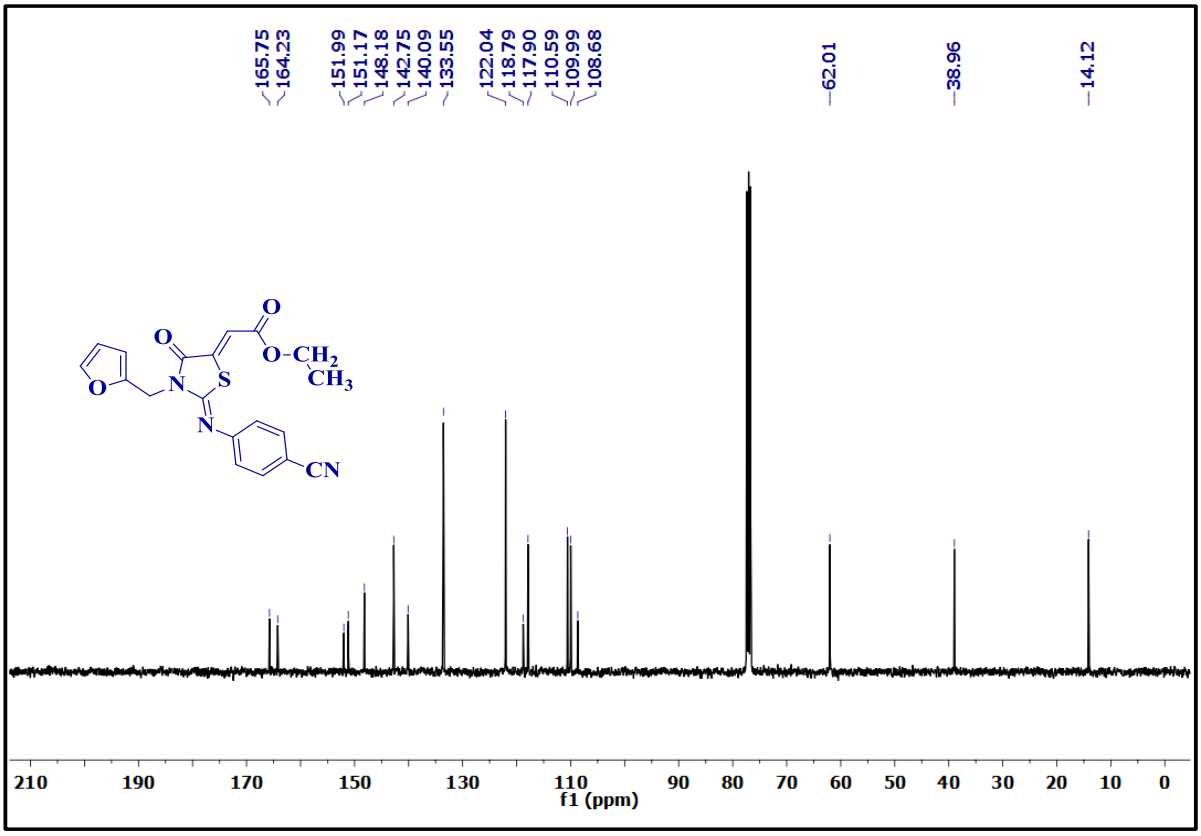


Fig. ^{13}C NMR Spectrum of compound **48j**

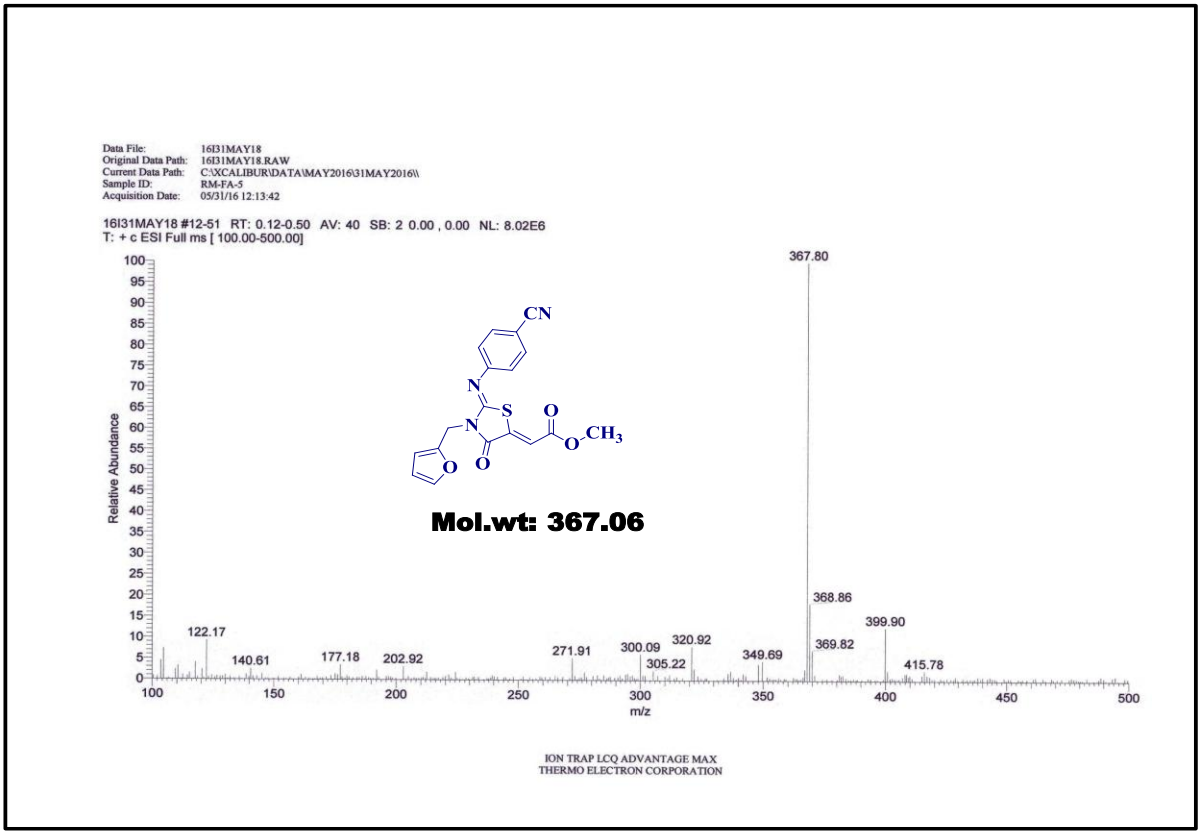


Fig. Mass Spectrum of compound **48d**

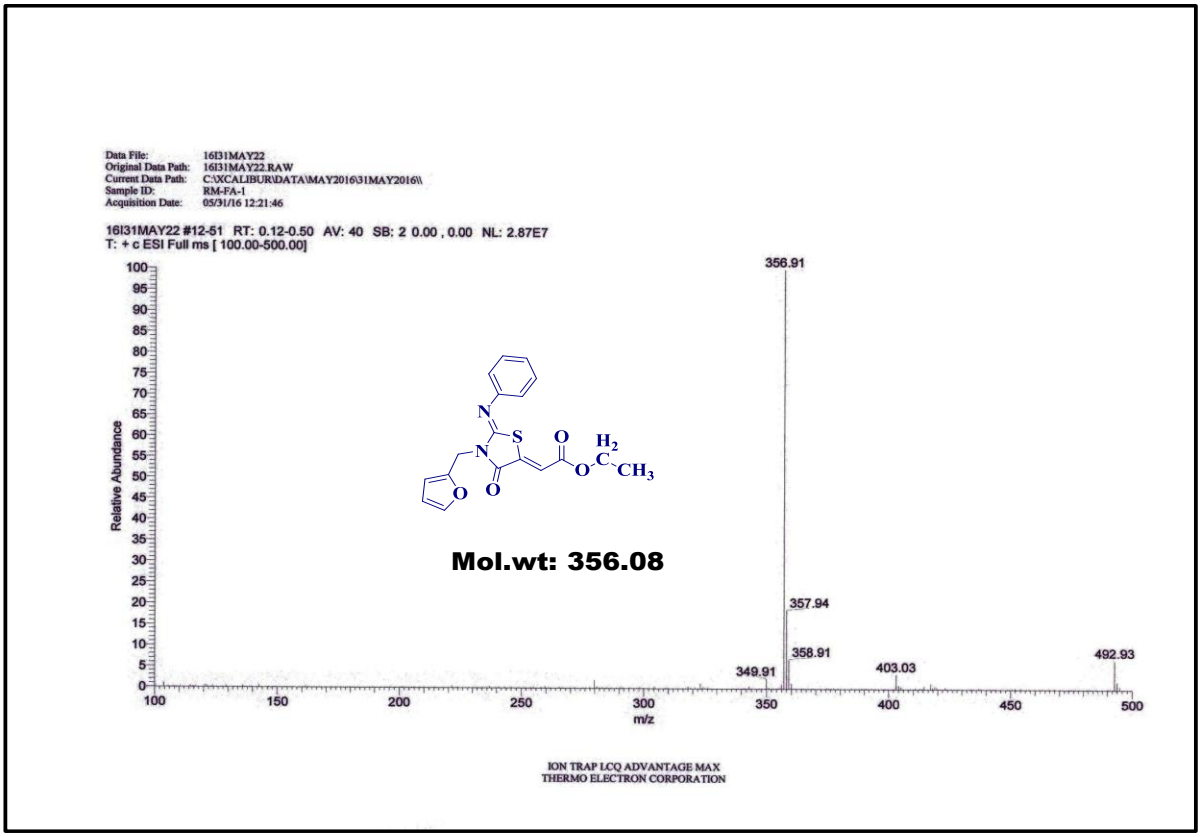


Fig. Mass Spectrum of compound **48g**

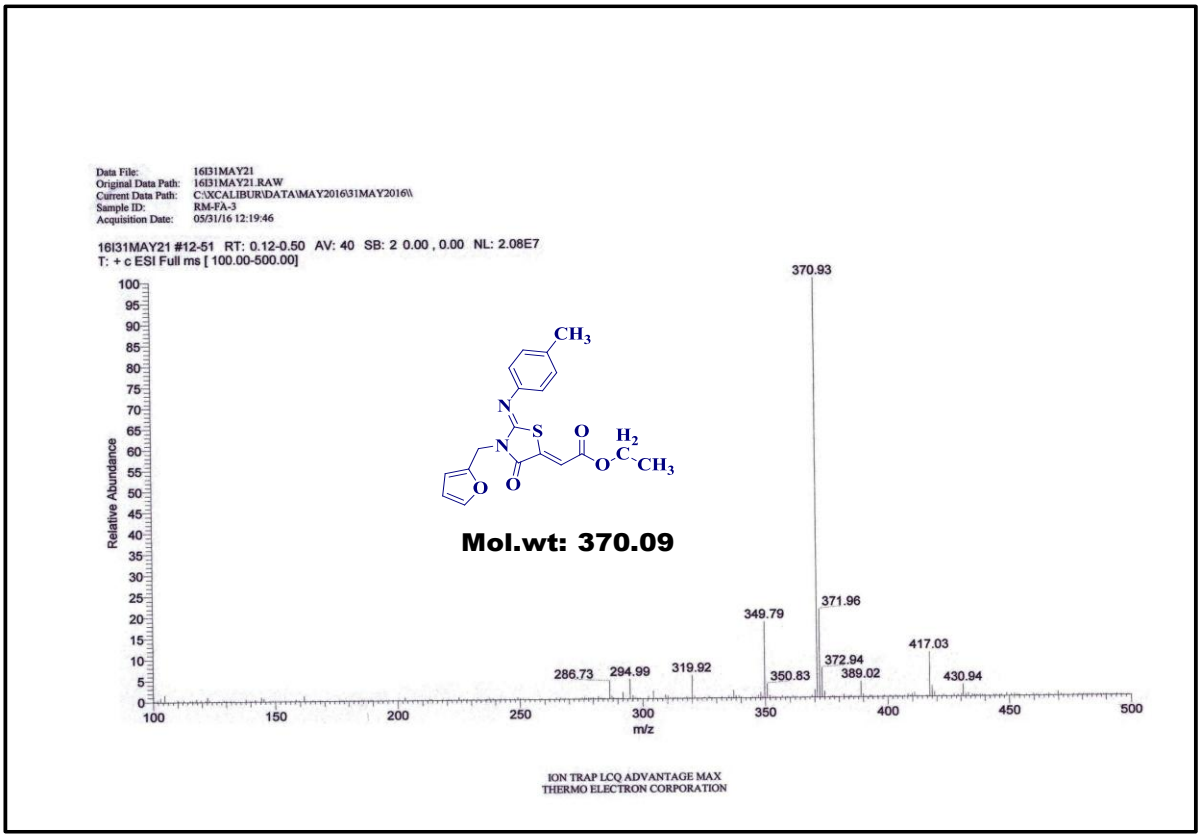


Fig. Mass Spectrum of compound **48i**

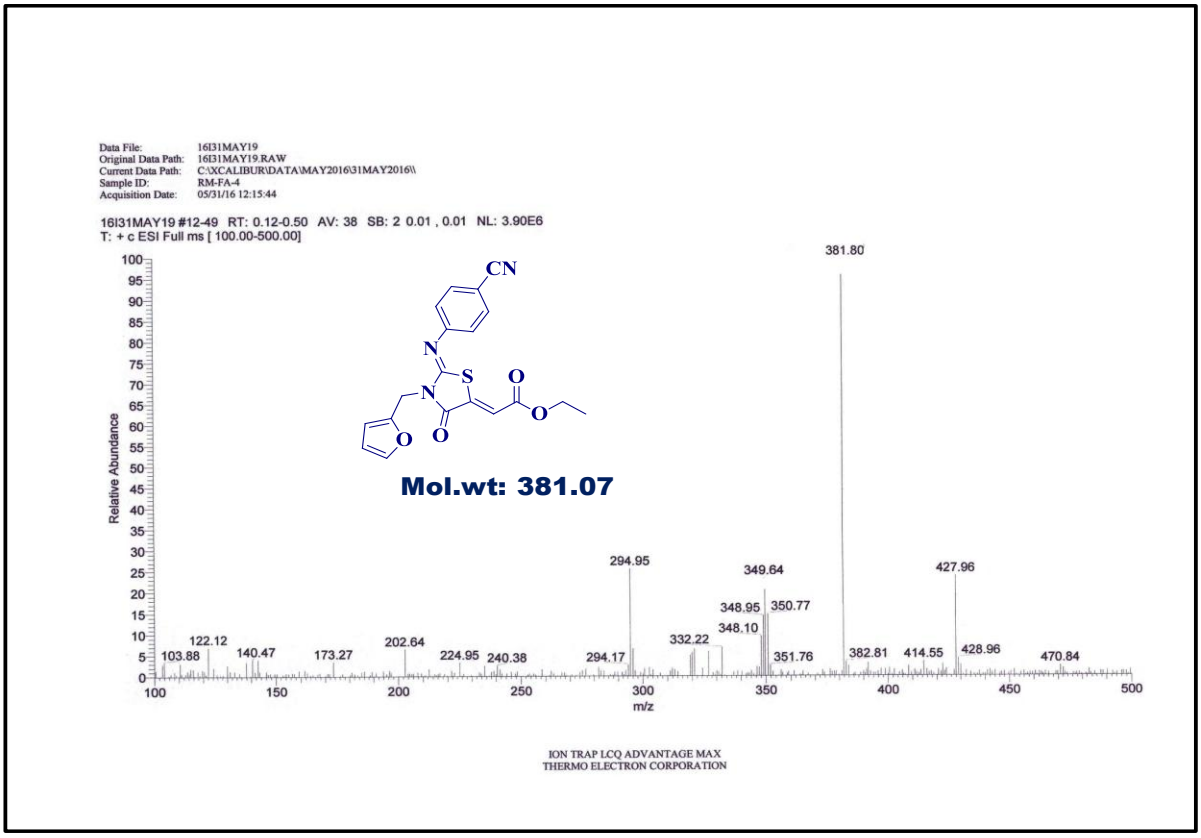


Fig. Mass Spectrum of compound **48j**

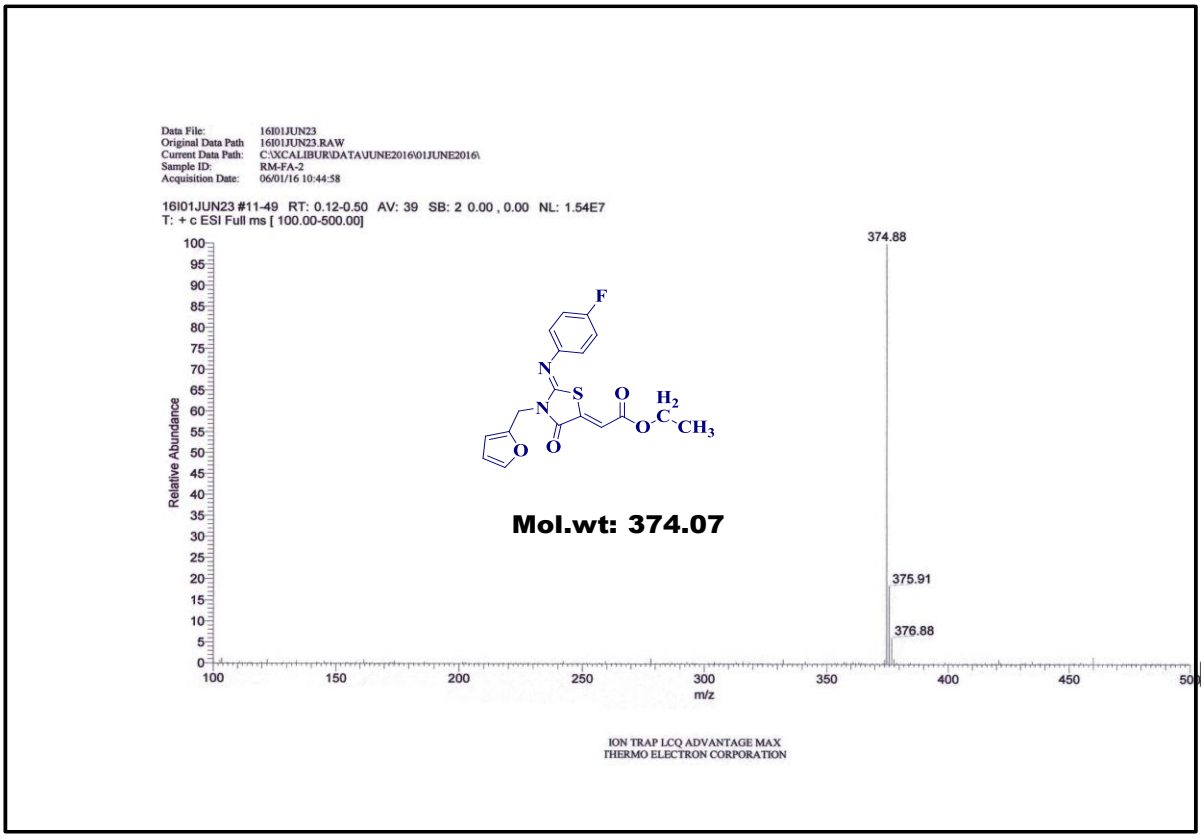


Fig. Mass Spectrum of compound **48k**

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CHAPTER-VI

SYNTHESIS AND ANTICANCER ACTIVITY OF 3-(2-((1-PHENYL-1*H*-TETRAZOL-5-YL) THIO) ACETYL)-2*H*-COUMARIN DERIVATIVES

INTRODUCTION:

Synthesis of heterocyclic compounds having nitrogen as hetero atom, received greater importance in the field of organic and bio molecular chemistry. Majority of the therapeutic molecules contain nitrogen heterocyclic rings as core pharmacophores.

Azoles are nitrogen atom containing heterocyclic compounds which are extensively available in various natural products and medicinal compounds. These azole heterocyclic compounds have various applications in the field of medicine, agriculture and material science. Among the various azole heterocyclic compounds reported, tetrazoles and their derivatives have received remarkable attention of scientific community across the globe.

Tetrazoles contain one carbon atom and four nitrogen atoms in a five membered aromatic 6π electronic ring system. Tetrazoles obey Huckel's aromaticity rule by donating the lone pair of nitrogen electrons to the ring system and remaining four π electrons from other four atoms of the tetrazole ring. These tetrazoles exist in three regioisomeric forms i.e. 1*H*-tetrazole, 2*H*-Tetrazole and 5*H*-tetrazole (**Fig.1**).

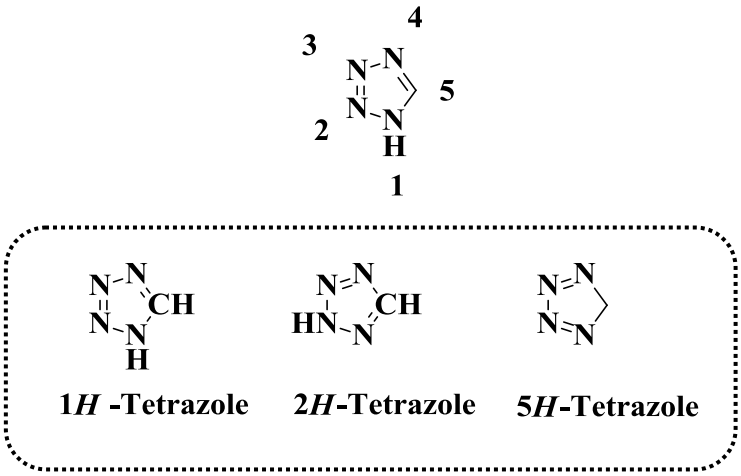


Fig. 1 Regioisomeric structures of tetrazole nucleus

The chemistry of azoles especially tetrazole heterocyclic compounds acquired an immense importance in the field of medicinal chemistry. The first tetrazole was synthesized by J. A. Bladin[1] by the reaction of dicyanophenylhydrazine with nitrous acid. Tetrazoles are considered as non classical bioisosteric form of carboxylic acids[2,3] in terms of acidic character and these are metabolically more stable in biological transformations and chemical oxidants. The replacement of carboxylic acid functional group with tetrazoles enhance the therapeutic activity and bio availability. An efficient

drug should have sufficient lipophilic in nature in order to reach the target effectively through cell membrane. The anionic tetrazoles are more lipophilic in nature than the carboxylates. The close similarity of acidic character with carboxylic acids, metabolic stability, enhanced biological property, bioavailability and high lipophilic nature inspired the medicinal chemists to develop the novel tetrazole derivatives as potential therapeutic agents.

Among the tetrazoles, 1, 5-substituted tetrazoles and their derivatives represent a useful *cis*-amide isoster of peptide bond[4,5] (**Fig.2**). They exhibit significant stability towards chemical, biological degradation and environmental redox conditions. Due to this 1,5 substituted tetrazoles received significant importance in the field of biochemistry, pharmaceutical chemistry, material science[6-11], agriculture[12,13] and explosives[14]. A large number of tetrazoles are available in the market as potential drugs including Valsartan, Pemirolast, Cilostazole, Losartan, candesartan and cefoperazone and also several tetrazole based heterocyclic compounds have reported as anti-inflammatory[15], anti microbial[16-18], anti viral[19-21], anti-hypertensive[22], CNS stimulants[23], analgesic[24], antiulcer[25], anticancer[26-30], antitubercular[31], COX-2 inhibitor properties[32,33]. Tetrazoles with therapeutic activities are shown in **Fig. 3**.



1,5-Substituted Tetrazole ***cis*- amide bond of peptide**

Fig. 2 Bioisosteres of 1, 5-substituted tetrazoles with *cis* –amide bond of peptide.

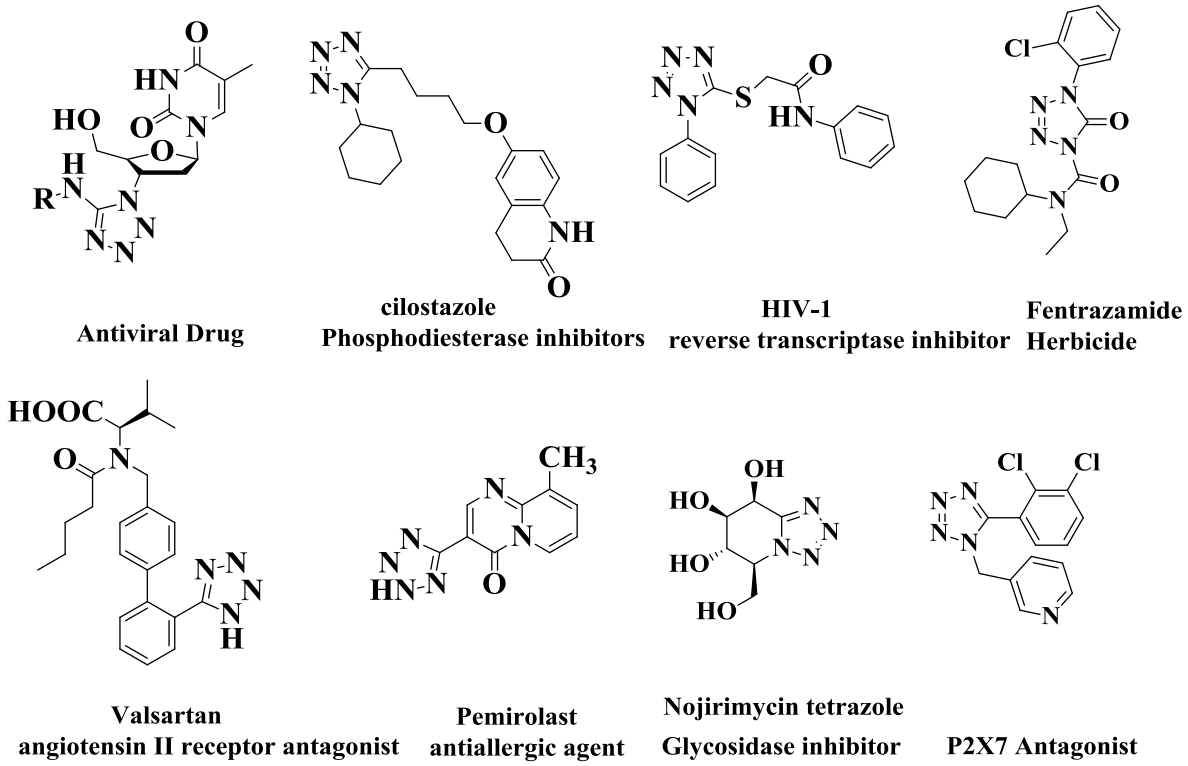
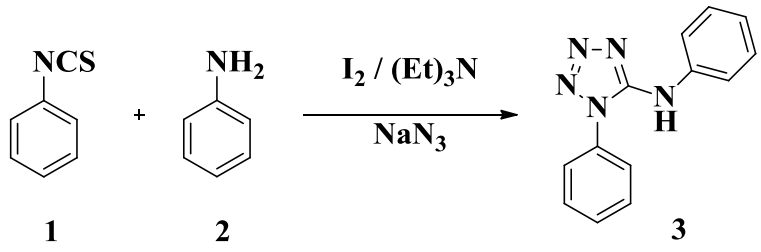


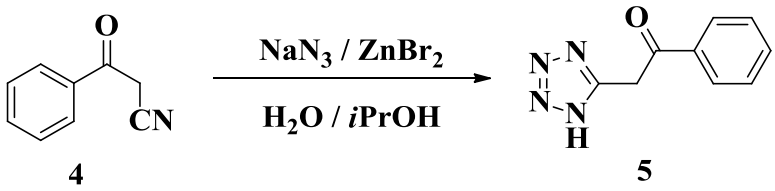
Fig. 3 Pharmaceutically active tetrazole heterocyclic compounds.

The following is a brief review of literature on synthetic methods on tetrazoles.

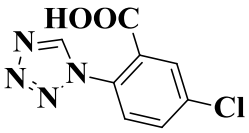
Ramesh *et al* [34] developed an efficient one pot multicomponent reaction for the synthesis of amino tetrazoles (3) by utilizing various substituted aryl isothiocyanates (1) and aryl amines (2) in presence of molecular Iodine followed by treatment with sodium azide at room temperature in presence of DMF with good yields.



A. L. Cardoso *et al* [35] synthesized 1-phenyl-2-(1*H*-tetrazol-5-yl)ethanone (5) with excellent yields in a sealed tube with high temperatures by using 3-oxo-3-phenylpropanenitrile (4) in presence of sodium azide, zinc bromide and water / iso propanol medium.

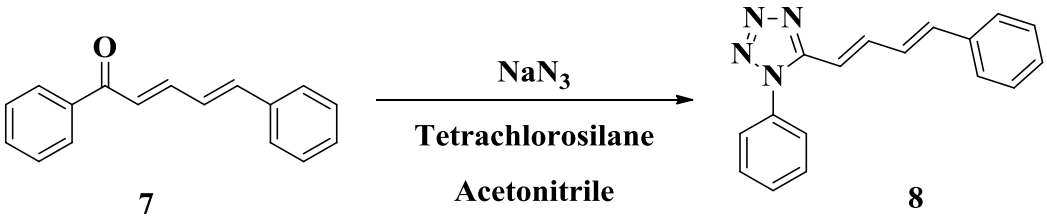


5-Chloro-2-(1*H*-tetrazol-1-yl)benzoic acid (6) was prepared by D. Varadaraji *et al* [36] by taking 2-amino-5-chlorobenzoic acid, sodium azide and trimethyl orthoformate at room temperature with good yield.



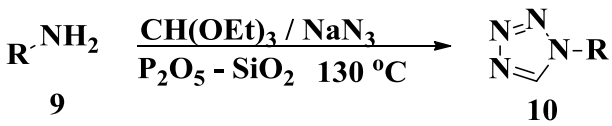
6

T. A. Salama *et al* [37] reported regiospecific synthesis of 1-phenyl-5-((1*E*,3*E*)-4-phenylbuta-1,3-dien-1-yl)-1*H*-tetrazole (8) from the reaction of 1,5-diphenylpenta-2,4-dien-1-one (7) with tetrachlorosilane – sodium azide reagent in presence of acetonitrile at room temperature with quantitative yield.



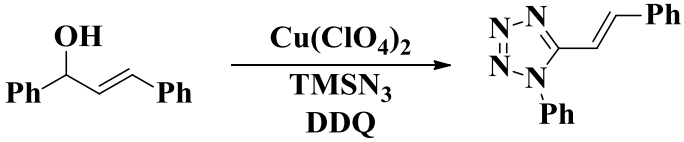
7 **8**

D. Habibi *et al* [38] synthesized 1-substituted tetrazoles (10) via an efficient solvent free P_2O_5-SiO_2 heterogeneous catalysed ultrasound irradiation condition. The target compounds were obtained in a shorter reaction time under conventional and as well as ultra sound irradiation method by the reaction of primary amines (9), sodium azide and triethy orthoformate with high yields.



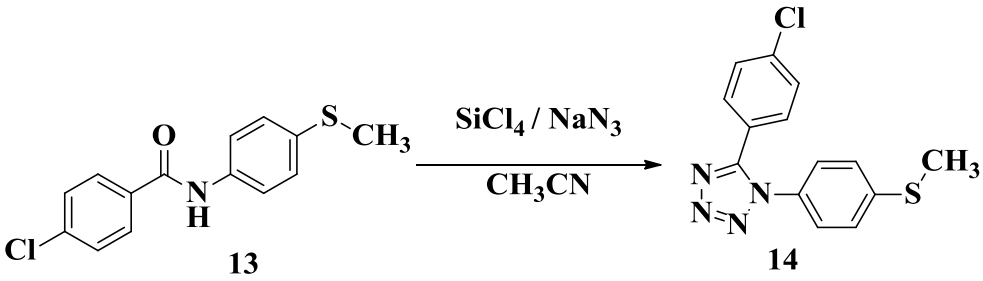
9 **10**

1, 5 Di substituted tetrazoles (12) were synthesized by B. V. Rokade *et al* [39] with excellent yields at ambient temperature by oxidative transformation of secondary alcohols (11) in presence of trimethylsilylazide, copper perchlorate hexahydrate and 2,3-dichloro-5,6-dicyano-*para*-benzoquinone in a shorter reaction time.

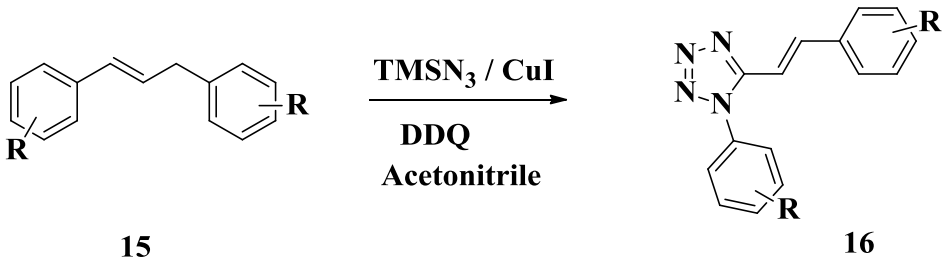


11 **12**

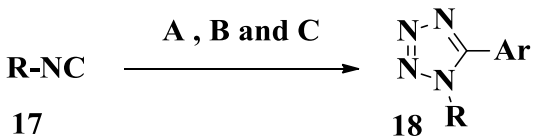
B. Jawabrah Al-Hourani *et al* [40] described the synthesis of 5-(4-chlorophenyl)-1-(4-(methylthio) phenyl)-1*H*-tetrazole (14) by the reaction of 4-chloro-N-(4-(methylthio) phenyl) benzamide (13) with tetrachlorosilane – sodium azide in presence of acetonitrile at higher temperature with good yield.



Y. Liang *et al* [41] developed copper iodide catalysed novel and efficient method to synthesize the 1-aryl-5-styryl-1*H*-tetrazoles (16) by the reaction of various substituted 1,3-diaryl prop-1-enes (15) with trimethylsilylazide in presence of DDQ and acetonitrile solvent medium at high temperature in good yields.

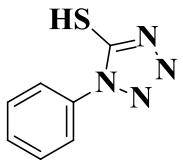


L. El Kaim *et al* [42] developed one pot three step synthesis of 1,5-substituted tetrazoles (18) by utilizing isocyanides (17) as a reactant. Initially isocyanides were converted into isocyanide dibromides by the reaction of bromine in acetonitrile at room temperature. The isocyanide dibromide was transformed into tetrazoles by using sodium azide in acetonitrile. In the final step the tetrazoles were treated with arylboronic acid in presence of potassium carbonate by using tetrakis(triphenylphosphine)palladium as a catalyst to get the substituted tetrazoles with good yields.



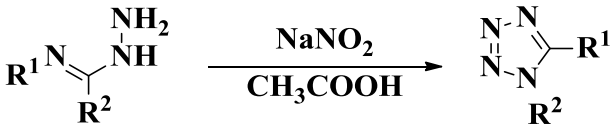
A = Br_2 / CH_3CN B = NaN_3 / CH_3CN
C = $ArB(OH)_2$ / K_2CO_3 / $Pd(PPh_3)_4$ / toluene

I. C. Richards and P. S. Thomas [43] synthesized a series of 1-aryl tetrazole-5-thiones (**19**) in two steps. In the first step aniline (2) was converted into aryl isothiocyante by the reaction of thiophosgene. In the second step aryl isothiocyante was converted in to 1-aryl tetrazole-5-thione by [3+2] dipolar cycloaddition of sodium azide.



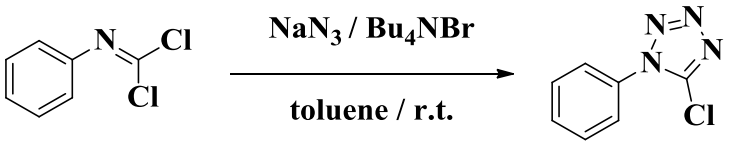
19

C. Wei *et al* [44] reported the synthesis of 1,5-substituted tetrazoles (21) by the reaction of compound 20 with sodium nitrite in presence of acetic acid under cold condition .



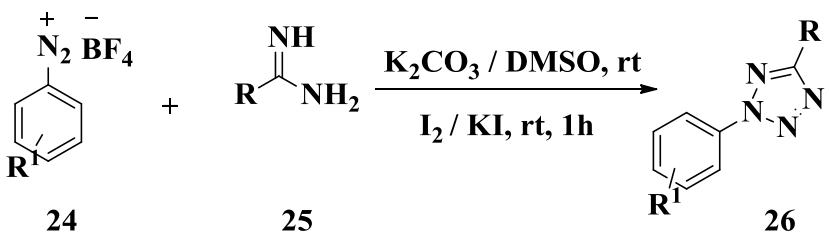
20 **21**

Alves and Johnstone [45] reported the improved synthesis of 5-chloro-1-phenyl-1*H*-tetrazoles by the reaction of phenylcarbonimidic dichloride (22) with sodium azide in presence of tetrabutyl ammonium bromide and toluene at room temperature with excellent yields.



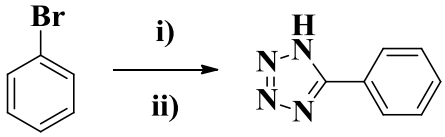
22 **23**

M. Ramanathan *et al* [46] developed an efficient method to synthesize the 2,5-substituted tetrazoles (26). The target compounds were synthesized with good yields at room temperature by one pot reaction of aryldiazonium salts (24) with amidines (25) followed by oxidative transformation with iodine/ potassium iodide in presence of DMSO solvent.



24 **25** **26**

Alterman and Hallberg [47] prepared aryl tetrazoles (28) in pot reaction condition with shorter reaction time starting from bromo benzene (27). The bromo benzene was converted into aryl nitrile under microwave reaction condition by using zinc cyanide and tetrakis (triphenylphosphine) palladium in DMF. In the second step aryl nitrile was converted into 5-phenyl tetrazole by cycloaddition reaction of sodium azide in presence of ammonium chloride and DMF with excellent yields.



27 **28**

i) = $Zn(CN)_2$ / $Pd(PPh_3)_4$ DMF / MW 60 W
ii) = NaN3 / NH4Cl / DMF / MW

Present work

Starting materials

Different substituted 3-(2-bromoacetyl) coumarins were prepared according to the literature procedure as described in the chapter-I. Phenyl isothiocyanate and sodium azide were procured from commercial source and used directly.

In general tetrazoles were prepared by the reaction of hydazoic acid with alkyl / aryl nitriles in presence of inert organic solvent at high temperatures. When tin azides or silicon azides were used for the preparation of tetrazoles, strong Lewis acids with acidic environment is required. The methods applied for the synthesis of tetrazoles always require harsh reaction condition such as high temperature, longer reaction time and organic solvents in particular dimethylformamide as a solvent. So these methods always have their own limitations such as efficiency and environmental issues. In view of the synthesis, various biological and material applications of tetrazole derivatives, significant efforts are required to develop the efficient synthetic methods.

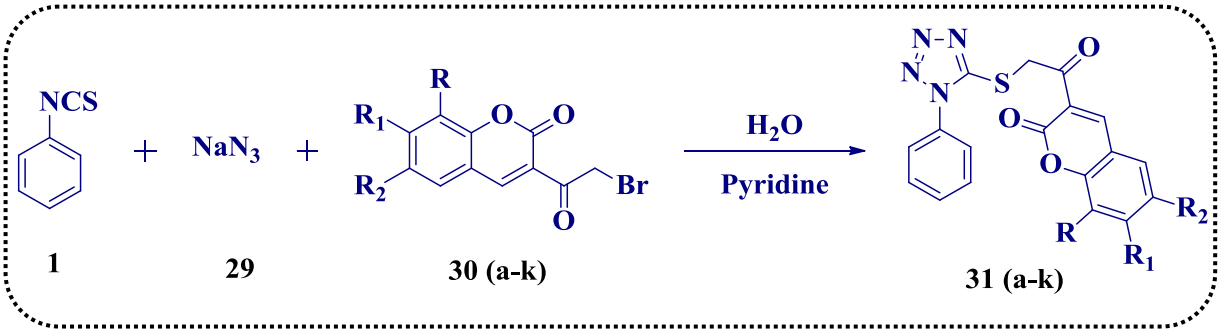
By considering the importance of both synthesis and biological applications of tetrazoles and coumarins, we developed an efficient one pot multicomponent reaction for the synthesis of 1, 5 substituted tetrazoles. The combination of two biologically significant

pharmacophores like tetrazoles and coumarins in a single molecular frame work would expected to exhibit good biological activity.

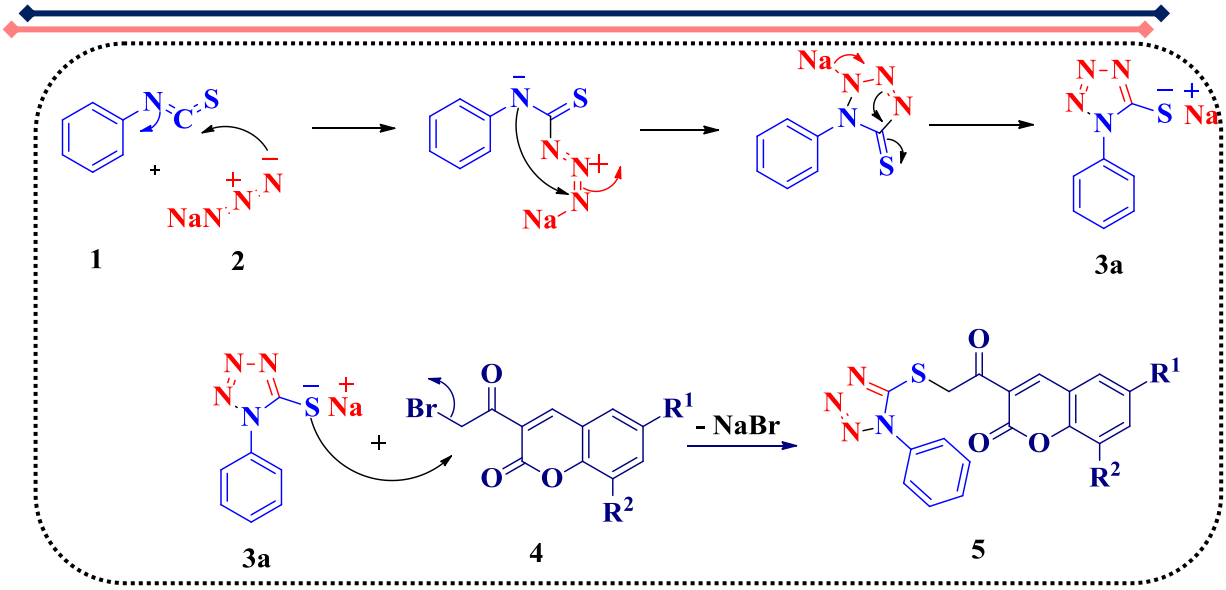
The present work describes the synthesis of 3-(2-((1-phenyl-1*H*-tetrazol-5-yl) thio) acetyl)-2*H*-coumarin derivatives via a facile one pot multicomponent approach and their anticancer activity. The title compounds were obtained by the reaction of phenyl isothiocyante, sodium azide and substituted 3-(2-bromoacetyl) coumarins in presence of water at room temperature in a shorter reaction time with excellent yields (**Scheme-1**). The reaction proceeds through the formation of tetrazole heterocyclic ring followed by thiol alkylation in presence of pyridine as a base in a single operation.

Mechanism:

The formation of tetrazole ring followed by alkylation of thiol was explained through the following plausible mechanism (**Scheme-2**). In the first step the nucleophilic nitrogen of the sodium azide (**2**) reacts with the electrophilic thio-carbonyl carbon of phenyl isothiocyanate (**1**) to form an intermediate sodium (azidocarbonothioyl)(phenyl)amide. The nucleophilic nitrogen of sodium (azidocarbonothioyl)(phenyl)amide reacts with electrophilic nitrogen intra molecularly to form an intermediate sodium 1-phenyl-1*H*-tetrazole-5-thiolate (**3a**). The nucleophilic sulphur of intermediate sodium 1-phenyl-1*H*-tetrazole-5-thiolate displaces the bromine atom of 3-(2-bromo acetyl) coumarin to give the title compounds. During the reaction three different types of bonds (one C-N, one N-N and one C-S bond) are formed in one pot.



Scheme-1: A facile one pot multicomponent synthesis of 3-(2-((1-phenyl-1*H*-tetrazol-5-yl) thio) acetyl)-2*H*-coumarin derivatives.



Scheme-2: The plausible reaction mechanism for the synthesis of compound

The structures of the newly synthesized 3-(2-((1-phenyl-1*H*-tetrazol-5-yl) thio) acetyl)-2*H*-coumarin derivatives were confirmed by their physical, analytical and spectral data (IR, ^{1}H NMR, ^{13}C NMR and Mass spectra). All the spectral data was consistent with the assigned structures. The compound **4i** structure was further confirmed by single crystal X-ray data (Table 1 - 6). The compound is showing monoclinic crystal system. The platoon and ORTEP diagram of the compound 4i is shown in **Fig 4 and 5**.

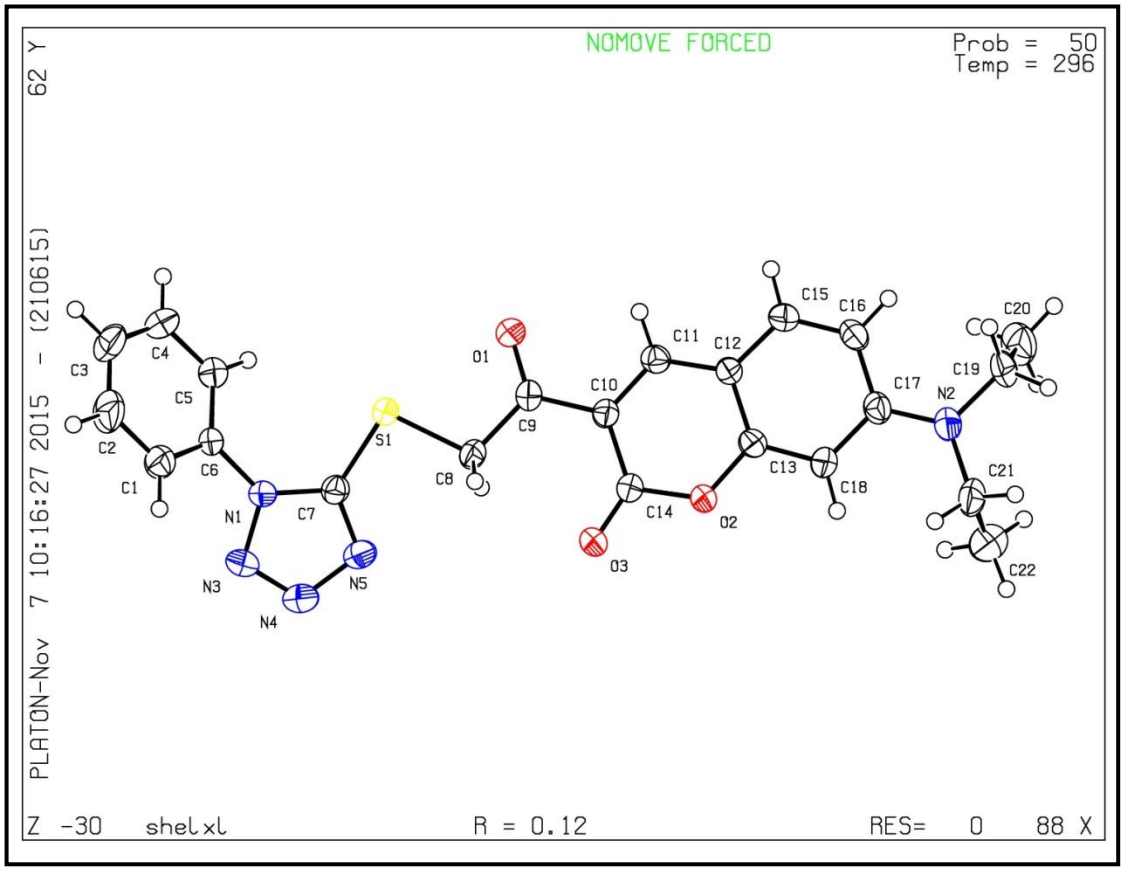


Fig. 4 The platoon diagram of compound **31i**

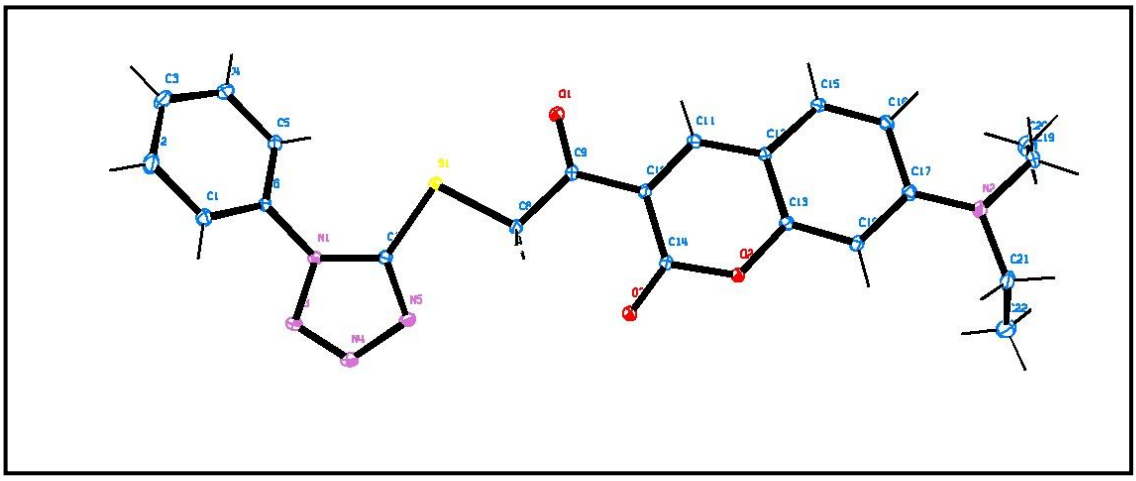


Fig. 5 The ORTEP diagram of compound **31i**

Table 1. Crystal data and structure refinement for compound **31i**

Identification code	shelxl
Empirical formula	$C_{22}H_{21}N_5O_3S$
Formula weight	435.50
Temperature	296(2) K
Wavelength	0.71073 A
Crystal system, space group	**Monoclinic, P21/c**
Unit cell dimensions	a = 8.2432(8) A alpha = 90 deg. b = 12.0982(15) A beta = 93.738(6) deg. c = 20.811(2) A gamma = 90 deg.
Volume	2071.0(4) A^3
Z, Calculated density	4, 1.397 Mg/m^3
Absorption coefficient	0.192 mm^-1
F(000)	912
Crystal size	0.80 x 0.20 x 0.20 mm
Theta range for data collection	1.95 to 29.96 deg.
Limiting indices	-11<=h<=10, -16<=k<=16, -19<=l<=29
Reflections collected / unique	17555 / 5920 [R(int) = 0.0515]
Completeness to theta	= 29.96 98.4 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.9626 and 0.8616
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	5920 / 0 / 282
Goodness-of-fit on F^2	1.142
Final R indices [I>2sigma(I)]	R1 = 0.1219, wR2 = 0.3784
R indices (all data)	R1 = 0.1602, wR2 = 0.3993
Largest diff. peak and hole	1.204 and -0.514 e.A^-3

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters (A^2 x 10^3) for nnd. U(eq) is defined as one third of the trace of the orthogonalized Uij tensor.

	X	Y	Z	U (eq)
C(1)	-604(10)	-1005(6)	11524(4)	43(2)
C(2)	-272(12)	-1380(7)	12150(4)	57(2)
C(3)	-367(11)	-676(8)	12661(4)	52(2)
C(4)	-786(11)	407(7)	12557(3)	48(2)
C(5)	-1139(9)	799(6)	11939(3)	38(2)
C(6)	-1030(7)	79(5)	11432(3)	29(1)
C(7)	-902(7)	1364(5)	10480(3)	29(1)
C(8)	852(8)	3058(5)	10107(3)	31(1)
C(9)	1687(8)	4132(5)	10290(3)	34(1)
C(10)	2409(8)	4810(5)	9795(3)	29(1)
C(11)	3200(7)	5766(5)	9972(3)	29(1)
C(12)	3901(7)	6464(5)	9540(3)	28(1)
C(13)	3778(7)	6159(5)	8882(3)	29(1)
C(14)	2270(8)	4500(5)	9117(3)	34(1)
C(15)	4744(8)	7461(5)	9689(3)	34(1)
C(16)	5363(8)	8086(5)	9227(3)	35(1)
C(17)	5253(8)	7765(5)	8567(3)	33(1)
C(18)	4421(9)	6776(5)	8403(3)	36(1)
C(19)	6658(9)	9465(6)	8271(4)	41(2)
C(20)	5418(11)	10353(7)	8377(5)	56(2)
C(21)	6022(11)	8007(7)	7448(4)	50(2)
C(22)	4567(13)	8325(8)	7017(4)	63(2)
N(1)	-1397(6)	454(4)	10791(2)	29(1)
N(2)	5937(8)	8386(5)	8114(3)	40(1)
N(3)	-2264(7)	-189(5)	10359(3)	40(1)
N(4)	-2288(8)	323(6)	9816(3)	45(2)
N(5)	-1471(7)	1305(5)	9871(3)	38(1)
O(1)	1788(8)	4429(5)	10850(2)	56(2)
O(2)	2994(6)	5202(4)	8693(2)	37(1)
O(3)	1638(8)	3685(4)	8879(2)	53(2)
S(1)	220(2)	2434(1)	10838(1)	33(1)

Table 3. Bond lengths [A] and angles [deg] for **31i**.

Bond	Bond lengths [A]	Bond	Bond angles [deg]
C(1)-C(6)	1.367(9)	C(6)-C(1)-C(2)	118.5(7)
C(1)-C(2)	1.390(11)	C(6)-C(1)-H(1)	120.8
C(1)-H(1)	0.9300	C(2)-C(1)-H(1)	120.8
C(2)-C(3)	1.369(12)	C(3)-C(2)-C(1)	120.5(8)
C(2)-H(2)	0.9300	C(3)-C(2)-H(2)	119.7
C(3)-C(4)	1.369(12)	C(1)-C(2)-H(2)	119.7
C(3)-H(3)	0.9300	C(4)-C(3)-C(2)	119.9(7)
C(4)-C(5)	1.383(10)	C(4)-C(3)-H(3)	120.1
C(4)-H(4)	0.9300	C(2)-C(3)-H(3)	120.1
C(5)-C(6)	1.376(9)	C(3)-C(4)-C(5)	120.8(7)
C(5)-H(5)	0.9300	C(3)-C(4)-H(4)	119.6
C(6)-N(1)	1.423(8)	C(5)-C(4)-H(4)	119.6
C(7)-N(5)	1.324(8)	C(6)-C(5)-C(4)	118.4(7)
C(7)-N(1)	1.354(8)	C(6)-C(5)-H(5)	120.8
C(7)-S(1)	1.730(6)	C(4)-C(5)-H(5)	120.8
C(8)-C(9)	1.508(9)	C(1)-C(6)-C(5)	122.0(6)
C(8)-S(1)	1.805(6)	C(1)-C(6)-N(1)	118.2(6)
C(8)-H(8A)	0.9700	C(5)-C(6)-N(1)	119.8(6)
C(8)-H(8B)	0.9700	N(5)-C(7)-N(1)	108.3(5)
C(9)-O(1)	1.216(8)	N(5)-C(7)-S(1)	126.7(5)
C(9)-C(10)	1.474(8)	N(1)-C(7)-S(1)	124.9(5)
C(10)-C(11)	1.367(8)	C(9)-C(8)-S(1)	107.4(4)
C(10)-C(14)	1.457(9)	C(9)-C(8)-H(8A)	110.2
C(11)-C(12)	1.387(8)	S(1)-C(8)-H(8A)	110.2
C(11)-H(13)	0.9300	C(9)-C(8)-H(8B)	110.2
C(12)-C(13)	1.415(8)	S(1)-C(8)-H(8B)	110.2
C(12)-C(15)	1.416(8)	H(8A)-C(8)-H(8B)	108.5
C(13)-O(2)	1.371(7)	O(1)-C(9)-C(10)	120.0(6)
C(13)-C(18)	1.379(9)	O(1)-C(9)-C(8)	119.9(6)
C(14)-O(3)	1.206(8)	C(10)-C(9)-C(8)	120.1(6)
C(14)-O(2)	1.387(7)	C(11)-C(10)-C(14)	119.1(6)
C(15)-C(16)	1.348(9)	C(11)-C(10)-C(9)	119.3(6)
C(15)-H(15)	0.9300	C(14)-C(10)-C(9)	121.6(6)
C(16)-C(17)	1.425(9)	C(10)-C(11)-C(12)	123.4(6)
C(16)-H(16)	0.9300	C(10)-C(11)-H(13)	118.3
C(17)-N(2)	1.356(8)	C(12)-C(11)-H(13)	118.3
C(17)-C(18)	1.410(9)	C(11)-C(12)-C(13)	117.6(5)
C(18)-H(18)	0.9300	C(11)-C(12)-C(15)	126.5(6)
C(19)-N(2)	1.462(9)	C(13)-C(12)-C(15)	115.9(5)
C(19)-C(20)	1.509(11)	O(2)-C(13)-C(18)	116.5(6)
C(19)-H(19A)	0.9700	O(2)-C(13)-C(12)	120.0(5)
C(19)-H(19B)	0.9700	C(18)-C(13)-C(12)	123.5(6)
C(20)-H(20A)	0.9600	O(3)-C(14)-O(2)	115.6(6)
C(20)-H(20B)	0.9600	O(3)-C(14)-C(10)	127.8(6)

C(20)-H(20C)	0.9600	O(2)-C(14)-C(10)	116.6(5)
C(21)-N(2)	1.467(10)	C(16)-C(15)-C(12)	121.7(6)
C(21)-C(22)	1.499(13)	C(16)-C(15)-H(15)	119.2
C(21)-H(21A)	0.9700	C(12)-C(15)-H(15)	119.2
C(21)-H(21B)	0.9700	C(15)-C(16)-C(17)	122.0(6)
C(22)-H(22A)	0.9600	C(15)-C(16)-H(16)	119.0
C(22)-H(22B)	0.9600	C(17)-C(16)-H(16)	119.0
C(22)-H(22C)	0.9600	N(2)-C(17)-C(18)	121.3(6)
N(1)-N(3)	1.356(7)	N(2)-C(17)-C(16)	121.0(6)
N(3)-N(4)	1.288(8)	C(18)-C(17)-C(16)	117.7(6)
N(4)-N(5)	1.367(9)	C(13)-C(18)-C(17)	119.2(6)
		C(13)-C(18)-H(18)	120.4
		C(17)-C(18)-H(18)	120.4
		N(2)-C(19)-C(20)	113.5(6)
		N(2)-C(19)-H(19A)	108.9
		C(20)-C(19)-H(19A)	108.9
		N(2)-C(19)-H(19B)	108.9
		C(20)-C(19)-H(19B)	108.9
		H(19A)-C(19)-H(19B)	107.7
		C(19)-C(20)-H(20A)	109.5
		C(19)-C(20)-H(20B)	109.5
		H(20A)-C(20)-H(20B)	109.5
		C(19)-C(20)-H(20C)	109.5
		H(20A)-C(20)-H(20C)	109.5
		H(20B)-C(20)-H(20C)	109.5
		N(2)-C(21)-C(22)	113.5(7)
		N(2)-C(21)-H(21A)	108.9
		C(22)-C(21)-H(21A)	108.9
		N(2)-C(21)-H(21B)	108.9
		C(22)-C(21)-H(21B)	108.9
		H(21A)-C(21)-H(21B)	107.7
		C(21)-C(22)-H(22A)	109.5
		C(21)-C(22)-H(22B)	109.5
		H(22A)-C(22)-H(22B)	109.5
		C(21)-C(22)-H(22C)	109.5
		H(22A)-C(22)-H(22C)	109.5
		H(22B)-C(22)-H(22C)	109.5
		C(7)-N(1)-N(3)	108.2(5)
		C(7)-N(1)-C(6)	130.9(5)
		N(3)-N(1)-C(6)	120.6(5)
		C(17)-N(2)-C(19)	121.4(6)
		C(17)-N(2)-C(21)	122.1(6)
		C(19)-N(2)-C(21)	116.5(6)
		N(4)-N(3)-N(1)	106.4(5)
		N(3)-N(4)-N(5)	111.4(6)
		C(7)-N(5)-N(4)	105.6(6)
		C(13)-O(2)-C(14)	123.3(5)
		C(7)-S(1)-C(8)	97.2(3)

Table 4. Anisotropic displacement parameters (A^2 x 10^3) for nnd. The anisotropic displacement factor exponent takes the form: -2 pi^2 [h^2 a*^2 U11 + ... + 2 h k a* b* U12]

	U11	U22	U33	U23	U13	U12
C(1)	62(5)	31(3)	37(4)	0(3)	9(3)	-1(3)
C(2)	83(6)	36(4)	53(5)	17(4)	7(4)	4(4)
C(3)	64(5)	60(5)	32(4)	15(4)	1(3)	-5(4)
C(4)	66(5)	53(5)	27(3)	-3(3)	6(3)	-10(4)
C(5)	49(4)	33(3)	33(3)	0(3)	5(3)	-6(3)
C(6)	33(3)	28(3)	26(3)	1(2)	4(2)	-8(2)
C(7)	28(3)	32(3)	28(3)	0(2)	5(2)	-1(2)
C(8)	36(3)	31(3)	26(3)	2(2)	8(2)	-2(2)
C(9)	38(3)	32(3)	31(3)	4(2)	4(2)	-7(3)
C(10)	35(3)	26(3)	27(3)	2(2)	4(2)	-1(2)
C(11)	31(3)	27(3)	30(3)	-1(2)	0(2)	2(2)
C(12)	29(3)	26(3)	27(3)	1(2)	0(2)	-3(2)
C(13)	33(3)	25(3)	31(3)	-1(2)	2(2)	0(2)
C(14)	45(4)	30(3)	27(3)	1(2)	5(3)	-7(3)
C(15)	37(3)	34(3)	30(3)	-1(3)	-3(2)	-5(3)
C(16)	37(3)	27(3)	40(4)	-2(3)	2(3)	-7(2)
C(17)	36(3)	26(3)	37(3)	5(2)	0(3)	-1(2)
C(18)	49(4)	30(3)	29(3)	5(2)	2(3)	-5(3)
C(19)	40(4)	36(4)	49(4)	5(3)	7(3)	-14(3)
C(20)	66(5)	35(4)	65(6)	9(4)	2(4)	4(4)
C(21)	68(5)	42(4)	42(4)	5(3)	22(4)	-13(4)
C(22)	87(7)	57(5)	45(5)	1(4)	4(5)	-6(5)
N(1)	33(3)	30(2)	25(2)	-2(2)	2(2)	-5(2)
N(2)	50(3)	33(3)	38(3)	2(2)	10(3)	-10(2)
N(3)	47(3)	38(3)	34(3)	-6(2)	-4(2)	-11(3)
N(4)	53(4)	49(4)	32(3)	-7(3)	0(3)	-8(3)
N(5)	43(3)	45(3)	27(3)	-3(2)	2(2)	-5(3)
O(1)	90(4)	52(3)	28(2)	-6(2)	13(3)	-35(3)
O(2)	58(3)	27(2)	28(2)	-2(2)	7(2)	-12(2)
O(3)	92(4)	37(3)	32(3)	-5(2)	10(3)	-30(3)
S(1)	41(1)	33(1)	25(1)	1(1)	3(1)	-10(1)

Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters (A^2 x 10^3) for **31i**.

	X	Y	Z	U(eq)
H(1)	-538	-1480	11175	52
H(2)	17	-2115	12222	69
H(3)	-147	-933	13079	63
H(4)	-833	885	12905	58
H(5)	-1443	1532	11868	46
H(8A)	1593	2571	9899	37
H(8B)	-84	3194	9811	37
H(13)	3273	5959	10406	35
H(15)	4873	7691	10115	41
H(16)	5877	8747	9343	42
H(18)	4308	6543	7976	43
H(19A)	7313	9691	7923	50
H(19B)	7376	9395	8657	50
H(20A)	4740	10126	8711	84
H(20B)	4759	10471	7985	84
H(20C)	5966	11028	8501	84
H(21A)	6129	7209	7446	60
H(21B)	6988	8315	7274	60
H(22A)	3616	7974	7165	94
H(22B)	4724	8091	6585	94
H(22C)	4431	9112	7026	94

Table 6. Torsion angles[deg] for **31i**.

C(6)-C(1)-C(2)-C(3)	-0.2(13)
C(1)-C(2)-C(3)-C(4)	-0.2(14)
C(2)-C(3)-C(4)-C(5)	0.9(14)
C(3)-C(4)-C(5)-C(6)	-1.2(12)
C(2)-C(1)-C(6)-C(5)	-0.1(11)
C(2)-C(1)-C(6)-N(1)	-178.9(7)
C(4)-C(5)-C(6)-C(1)	0.8(11)
C(4)-C(5)-C(6)-N(1)	179.6(6)
S(1)-C(8)-C(9)-O(1)	-1.6(9)
S(1)-C(8)-C(9)-C(10)	177.1(5)
O(1)-C(9)-C(10)-C(11)	0.5(10)
C(8)-C(9)-C(10)-C(11)	-178.2(6)
O(1)-C(9)-C(10)-C(14)	-178.1(7)
C(8)-C(9)-C(10)-C(14)	3.2(10)
C(14)-C(10)-C(11)-C(12)	-0.6(10)

C(9)-C(10)-C(11)-C(12)	-179.2(6)
C(10)-C(11)-C(12)-C(13)	0.1(9)
C(10)-C(11)-C(12)-C(15)	-179.7(6)
C(11)-C(12)-C(13)-O(2)	-0.2(9)
C(15)-C(12)-C(13)-O(2)	179.5(6)
C(11)-C(12)-C(13)-C(18)	-179.9(6)
C(15)-C(12)-C(13)-C(18)	-0.1(9)
C(11)-C(10)-C(14)-O(3)	178.9(7)
C(9)-C(10)-C(14)-O(3)	-2.5(12)
C(11)-C(10)-C(14)-O(2)	1.2(9)
C(9)-C(10)-C(14)-O(2)	179.8(6)
C(11)-C(12)-C(15)-C(16)	-179.2(6)
C(13)-C(12)-C(15)-C(16)	1.1(9)
C(12)-C(15)-C(16)-C(17)	-2.1(11)
C(15)-C(16)-C(17)-N(2)	-177.5(7)
C(15)-C(16)-C(17)-C(18)	2.0(10)
O(2)-C(13)-C(18)-C(17)	-179.5(6)
C(12)-C(13)-C(18)-C(17)	0.2(10)
N(2)-C(17)-C(18)-C(13)	178.5(6)
C(16)-C(17)-C(18)-C(13)	-1.0(10)
N(5)-C(7)-N(1)-N(3)	1.2(7)
S(1)-C(7)-N(1)-N(3)	179.1(5)
N(5)-C(7)-N(1)-C(6)	175.1(6)
S(1)-C(7)-N(1)-C(6)	-7.1(10)
C(1)-C(6)-N(1)-C(7)	-133.0(7)
C(5)-C(6)-N(1)-C(7)	48.2(9)
C(1)-C(6)-N(1)-N(3)	40.2(9)
C(5)-C(6)-N(1)-N(3)	-138.6(6)
C(18)-C(17)-N(2)-C(19)	173.8(6)
C(16)-C(17)-N(2)-C(19)	-6.7(10)
C(18)-C(17)-N(2)-C(21)	-7.4(11)
C(16)-C(17)-N(2)-C(21)	172.1(7)
C(20)-C(19)-N(2)-C(17)	-72.8(9)
C(20)-C(19)-N(2)-C(21)	108.3(8)
C(22)-C(21)-N(2)-C(17)	89.4(9)
C(22)-C(21)-N(2)-C(19)	-91.7(8)
C(7)-N(1)-N(3)-N(4)	-0.4(7)
C(6)-N(1)-N(3)-N(4)	-175.0(6)
N(1)-N(3)-N(4)-N(5)	-0.6(8)
N(1)-C(7)-N(5)-N(4)	-1.5(7)
S(1)-C(7)-N(5)-N(4)	-179.3(5)
N(3)-N(4)-N(5)-C(7)	1.3(8)
C(18)-C(13)-O(2)-C(14)	-179.3(6)

C(12)-C(13)-O(2)-C(14)	1.0(9)
O(3)-C(14)-O(2)-C(13)	-179.4(6)
C(10)-C(14)-O(2)-C(13)	-1.5(9)
N(5)-C(7)-S(1)-C(8)	-18.2(6)
N(1)-C(7)-S(1)-C(8)	164.4(5)
C(9)-C(8)-S(1)-C(7)	172.3(5)

Anticancer activity of 3-(2-((1-phenyl-1*H*-tetrazol-5-yl) thio) acetyl)-2*H*-coumarin derivatives (31 a-k).

The newly synthesized compounds were screened in vitro against five different human cancer cell lines Murine leukemia (L1210), human T-lymphocyte cells (CEM), prostate cancer (DU-145), human cervix carcinoma cells (HeLa), and breast cancer (MCF-7) cells by using standard MTT anticancer screening protocol. The preliminary anticancer activity of the newly synthesized compounds was done at 100 µM concentration to determine the cancer cell growth inhibition. From the preliminary anticancer activity data, it was confirmed that the majority of the compounds were exhibiting good cancer cell growth inhibition. All the compounds were assayed at various concentrations (5 – 150 µM) in order to determine the 50% growth inhibition (IC_{50}) values. The IC_{50} values of all the newly synthesized compounds were shown in **Table 7**.

The anticancer activity results have shown that the compounds were exhibited broad spectrum of anticancer activity in a dose dependent manner. Majority of the compounds were exhibited excellent activity against MCF-7, DU-145 and HeLa cancer cell lines. The compound 31j exhibited excellent activity against all the tested cancer cell lines in the range of 10 – 18 µM concentration and the compounds 31i and 31d exhibited good anticancer activity against HeLa, DU-145 and MCF-7 cancer cell lines at low concentration values (6 – 8 µM). The remaining compounds exhibited good to moderate anticancer activity.

Table. 7 *In vitro* anticancer activity IC_{50} (µM) values of 3-(2-((1-phenyl-1*H*-tetrazol-5-yl) thio) acetyl)-2*H*-coumarin derivatives (**31 a-k**).

Compound	**$IC_{50\%}$ (µM)**				
	L1210	**CEM**	**HeLa**	**DU-145**	**MCF-7**
31a	75±6	53±2	50 ±7	76.56±5	19.89±4
31b	29±4	25±8	22±2	**13.3±2**	**13.52±2**

31c	28±7	30±6	23±1	15.09±4	**9.48±3**
31d	24±5	22±3	**6.11**±3	**8.17**±3	**7.48**±2
31e	26±3	26±4	21±9	14.51±6	39.06±5
31f	75±5	65±6	30±8	**9**±3	**11.26**±3
31g	33±5	47±5	40±5	65.35±8	35.26±6
31h	80±9	43±1	65±6	24.22±5	15.35±3
31i	65±4	75±3	**7.1**±2	**6.89**±3	**8.79**±2
31j	**15±7**	**18±5**	**10.16**±3	**12.94±4**	**12.08±4**
31k	75±3	65±7	65±4	27.3±6	**7.98±3**
31l	95±12	99±13	86±2	NT	NT

Cell cycle arrest of 3-(2-((1-phenyl-1*H*-tetrazol-5-yl) thio) acetyl)-2*H*-coumarin derivatives in DU-145 Cell line

To understand the anticancer activity mechanism of 3-(2-((1-phenyl-1*H*-tetrazol-5-yl) thio) acetyl)-2*H*-coumarin derivatives, we analysed the cell cycle alteration phase distribution of the cells by treating the DU-145 cancer cells with 10 µM concentration of the compounds 31b, 31d and 31i for 24h were harvested and analyzed by flow cytometry. Treatment of cells with compounds 31b, 31d, and 31i caused an accumulation of 74.91%, 79.55%, and 78.84%, cells in the G0/G1 phase respectively as compared to 68.10% in untreated cells. All the tested compounds exhibited promising cell cycle arrest at G0/G1 phase (**Fig. 6 and Table 8**).

Table. 8 Cell cycle alteration phase distribution analysis

Compound	Phase		
	G0/G1	S	G2/M
Control	68.10	8.37	23.53
31b	74.91	6.89	18.40
31d	79.55	5.94	14.45
31i	78.84	5.38	15.56

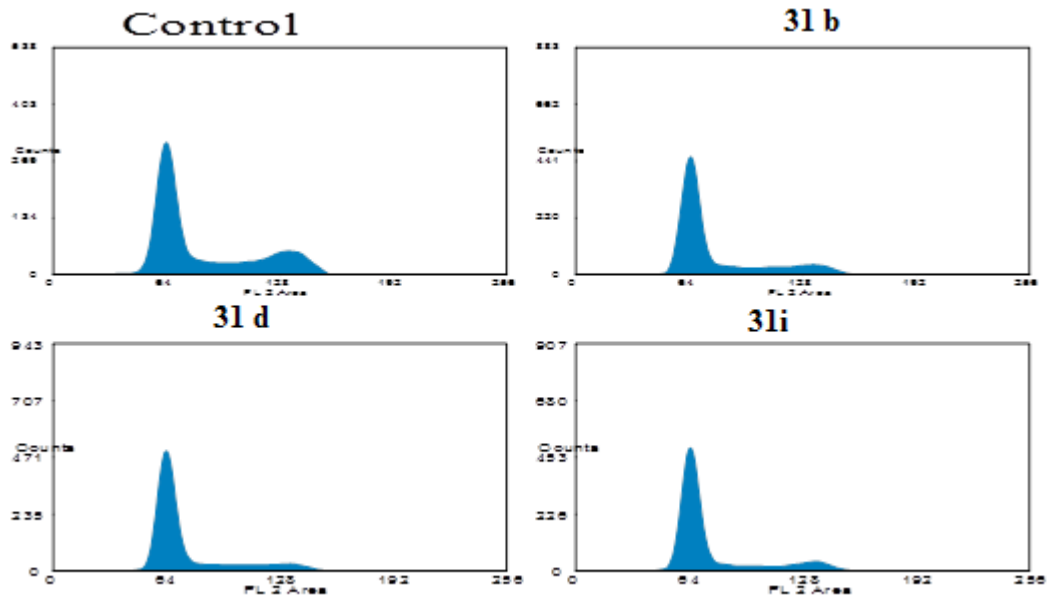


Fig. 6 The cell cycle phase distribution analysis by FACS

Effect on chromatin condensation:

Apoptosis is a crucial and continuous destruction process of undesirable cells during development or homeostasis in multicellular organisms. During apoptosis, chromatin condensation takes place. In order to examine the synthesized compounds induces cytotoxicity through apoptosis, DU-145 cells were treated with compounds 31b, 31d, and 31i of 10 µM concentration and harvested for a period of 24 h. To visualize the nuclear condensation Hoechst 33258 stain was used. The compounds 31b, 31d, and 31i caused significant chromatin condensation in DU-145 cells (**Fig. 7**).

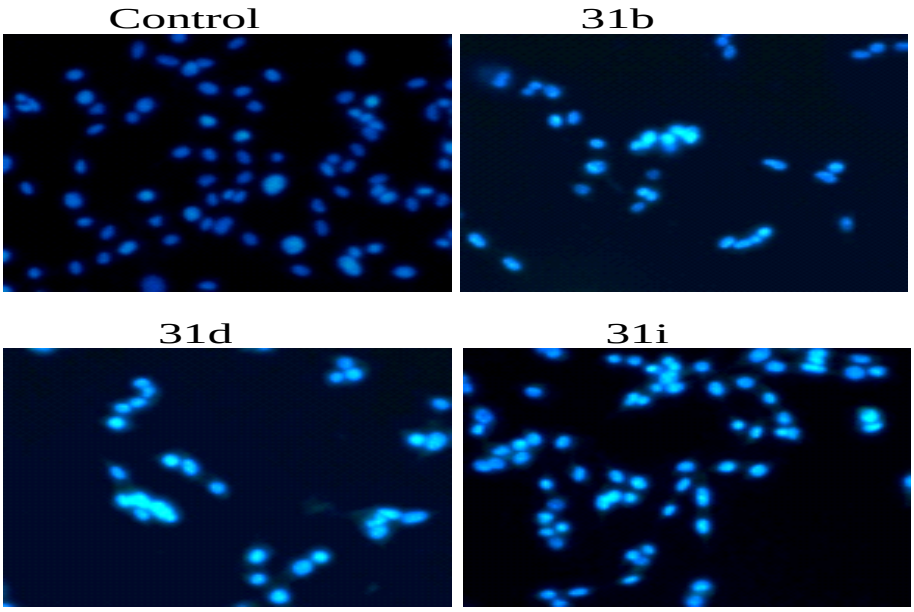


Fig 7. 3-(2-((1-phenyl-1*H*-tetrazol-5-yl) thio) acetyl)-2*H*-coumarin derivatives cause apoptosis in DU- 145 cells. Cells were treated with **31b, 31d,** and **31i** at 10µM concentration for 24 h. Images were taken by using DAPI filter.

In conclusion, we have developed an efficient method for the synthesis of 3-(2-((1-phenyl-1*H*-tetrazol-5-yl) thio) acetyl)-2*H*-coumarin derivatives (31 a-l) via a facile one pot multicomponent approach with excellent yields at ambient temperature. The main advantages of the method are shorter reaction time, mild reaction conditions and easy workup. All the synthesized compounds were screened against five different human cancer cell lines, the compound **31J** was found to be potent anticancer activity against the tested cell lines and majority of the compounds were exhibited excellent anticancer property against MCF-7 cancer cells and DU-145 cells.

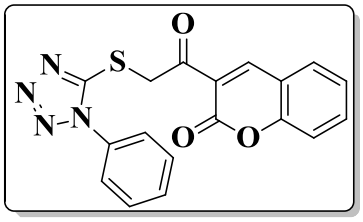
EXPERIMENTAL:

Facile one pot multicomponent synthesis of 3-(2-((1-phenyl-1*H*-tetrazol-5-yl) thio) acetyl)-2*H*-coumarin derivatives (31a-l):

A mixture of phenyl isothiocyante (0.135 g, 1 mmol), sodium azide (0.071 g, 1.5 mmol) and pyridine (0.079 g, 1 mmol) was taken in a round bottom flask containing water. The reaction mixture was stirred at room temperature for about 1 h. To this reaction mixture substituted 3-(2-bromoacetyl)-2*H*-chromen-2-one derivatives (1 mmol) were added and stirring was continued for 4-5 h. After completion of the reaction (monitored by TLC), the reaction mixture was neutralized with acetic acid and separated product was filtered, washed with water, dried and recrystallized from ethanol.

SPECTRAL DATA

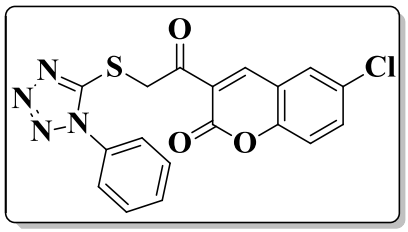
3-(2-((1-Phenyl-1*H*-tetrazol-5-yl)thio)acetyl)-2*H*-chromen-2-one (31a):



White solid; yield: 89%, m.p.: 201-203 °C; **FTIR (KBr, ν_{max} cm^{-1}):** 1721 (lactone-C=O); 1698 (-C=O); 1610 (-C=N); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):**): 5.09 (s, 2H, -S-CH_2), 7.45 (t, *J* = 7.6 Hz, 1H, Ar-H), 7.52 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.65 – 7.70 (m, 5H, Ar-H), 7.80 (t, *J* = 8.4 Hz, 1H, Ar-H), 8.01 (d, *J* = 8Hz, 1H, Ar-H), 8.83 (s, 1H, C_4-H of coumarin); **^{13}C NMR (100 MHz, DMSO-d_6, δ ppm):** 43.56, 116.86, 118.14, 123.02, 123.78, 125.30,

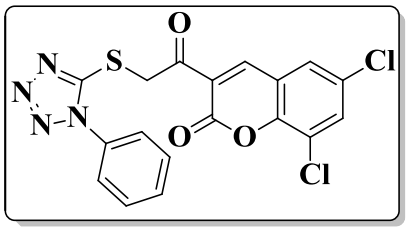
129.85, 130.21, 130.52, 133.56, 135.10, 149.32, 153.29, 155.38, 159.26, 189.53; **MS (ESI m/z):** 364.9 $[M+H]^+$; *Anal*. Calcd for $C_{18}H_{12}N_4O_3S$: C, 59.33; H, 3.32; N, 15.38% Found: C, 59.37; H, 3.26; N, 15.42%.

6-Chloro-3-(2-((1-phenyl-1*H*-tetrazol-5-yl)thio)acetyl)-2*H*-chromen-2-one (31b):



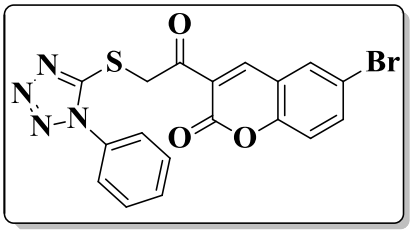
Yellow solid; yield: 91%; m.p.: 198 – 200 °C; **FTIR (KBr, ν_{max} cm^{-1}):** 1741 (lactone-C=O); 1681 (-C=O); 1607 (-C=N); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 5.08 (s, 2H, -S-CH2), 7.57 (d, *J* = 8.8 Hz, 1H, Ar-H), 7.67 – 7.71 (m, 5H, Ar-H), 7.82 – 7.85 (m, 1H, Ar-H), 8.15 (d, *J* = 2.8 Hz, 1H, Ar-H), 8.77 (s, 1H, C_4-H of Coumarin); **^{13}C NMR (100 MHz, DMSO-d_6, δ ppm)**: 44.29, 118.30, 119.41, 123.75, 124.50, 128.78, 129.80, 130.05, 130.67, 133.02, 134.47, 147.26, 153.33, 153.70, 158.09, 189.72; **MS (ESI m/z):** 398.9 $[M+H]^+$; *Anal*. Calcd for $C_{18}H_{11}ClN_4O_3S$: C, 54.21; H, 2.78; N, 14.05%; Found: C, 54.26; H, 2.72; N, 14.08%.

6,8-Dichloro-3-(2-((1-phenyl-1*H*-tetrazol-5-yl)thio)acetyl)-2*H*-chromen-2-one (31c):



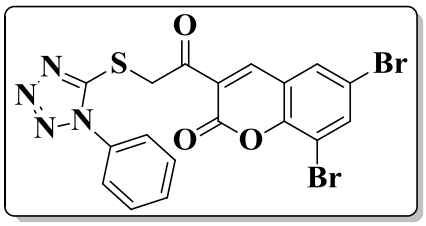
Yellow solid; yield: 92%; m.p.: 181 – 183 °C; **FTIR (KBr, ν_{max} cm^{-1}):** 1744 (lactone-C=O); 1681 (-C=O); 1610 (-C=N); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 5.08 (s, 2H, -S-CH2), 7.57 (d, *J* = 8.8 Hz, 1H, Ar-H), 7.69 (s, 3H, Ar-H), 7.76 – 7.82 (m, 1H, Ar-H), 7.84 (d, *J* = 2.4Hz, 1H), 8.12 (s, 1H, Ar-H), 8.77 (s, 1H, C_4-H of Coumarin); Anal. Calcd for $C_{18}H_{10}Cl_2N_4O_3S$: C, 49.90; H, 2.33; N, 12.93%; Found: C, 49.86; H, 2.36; N, 12.95%.

6-Bromo-3-(2-((1-phenyl-1*H*-tetrazol-5-yl)thio)acetyl)-2*H*-chromen-2-one (31d):



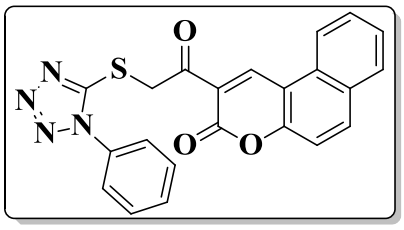
Red solid; yield: 95%; m.p.: 171 - 173 ^oC; **IR (KBr, ν_{max} cm^{-1}):** 1730 (lactone-C=O); 1673 (-C=O); 1599 (-C=N); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 5.08 (s, 2H, -S-CH_2), 7.67 – 7.70 (m, 5H, Ar-H), 7.88 (d, *J* = 2.4 Hz, 1H, Ar-H), 7.90- 7.98 (m, 2H, Ar-H), 8.22 (s, 1H, C_4- H of coumarin); **MS (ESI m/z):** 444.9 $[M+H]^+$; Anal. Calcd for $C_{18}H_{11}BrN_4O_3S$: C, 48.77; H, 2.50; N, 12.64%; Found: C, 48.82; H, 2.46; N, 12.62%.

6,8-Dibromo-3-(2-((1-phenyl-1*H*-tetrazol-5-yl)thio)acetyl)-2*H*-chromen-2-one (31e):



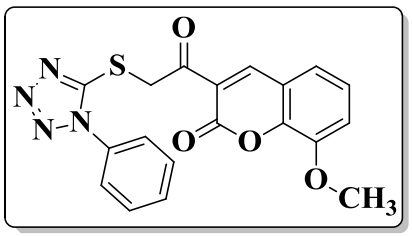
Brown solid; yield: 95%; m.p.: 193 - 195 °C; **FTIR (KBr, υ_{max} cm^{-1}):** 1735 (lactone-C=O); 1676 (-C=O); 1595 (-C=N); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 5.08 (s, 2H, -S-CH_2), 7.67 – 7.71 (m, 5H, Ar-H), 8.29 (d, *J* = 2 Hz, 1H, Ar-H), 8.33 (d, *J* = 2 Hz, 1H, Ar-H), 8.74 (s, 1H, C_4-H of coumarin); **MS (ESI m/z):** 522.8 $[M+H]^+$; 524.8 $[M+2H]^+$; Anal. Calcd for $C_{18}H_{10}Br_2N_4O_3S$: C, 41.40; H, 1.93; N, 10.73%; Found: C, 41.44; H, 1.95; N, 10.69%.

2-(2-((1-Phenyl-1*H*-tetrazol-5-yl)thio)acetyl)-3*H*-benzo[*f*]chromen-3-one (31f):



Yellow solid; yield: 90%; mp: 184 - 186 °C; **FTIR (KBr, υ_{max} cm^{-1}):** 1715 (lactone-C=O); 1676 (-C=O); 1555 (-C=N); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 5.16 (s, 2H, -S-CH_2), 7.67 – 7.71 (m, 7H, Ar-H), 7.80 (d, *J* = 7.6 Hz, 1H, Ar-H), 8.13 (d, *J* = 7.6 Hz, 1H, Ar-H), 8.41 (d, *J* = 9.2 Hz, 1H, Ar-H), 8.70 (d, *J* = 8.4 Hz, 1H, Ar-H), 9.45 (s, 1H, C_4-H of coumarin) **^{13}C NMR (100 MHz, DMSO-d_6, δ ppm):** 44.29, 112.42, 116.45, 121.08, 122.46, 124.51, 124.75, 126.68, 129.30, 129.55, 129.89, 130.41, 130.63, 133.02, 137.07, 137.49, 144.04, 145.72, 153.75, 155.74, 158.43, 189.63; **MS (ESI m/z):** 415.0 $[M+H]^+$; *Anal*. Calcd for $C_{22}H_{14}N_4O_3S$: C, 63.76; H, 3.40; N, 13.52; Found: C, 63.72; H, 3.46; N, 13.54%.

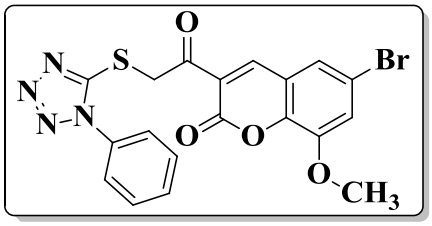
8-Methoxy-3-(2-((1-phenyl-1*H*-tetrazol-5-yl)thio)acetyl)-2*H*-chromen-2-one (31g):



Yellow solid; yield: 89%; m.p.: 197 - 199 °C; **FTIR (KBr, υ_{max} cm^{-1}):** (lactone-C=O); 1678 (-C=O); 1605 (-C=N); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 3.94 (s, 3H, -OCH_3), 5.08 (s, 2H, -S-CH_2), 7.36 – 7.49 (m, 1H, Ar-H), 7.53 – 7.55 (m, 1H, Ar-H), 7.67 – 7.69 (m, 1H, Ar-H), 7.70 (d, *J* = 1.6Hz, 5H, Ar-H), 8.79 (s, 1H, C_4-H of coumarin); **^{13}C NMR (100 MHz, DMSO-d_6, δ ppm):** 44.37, 56.27, 117.12, 118.60, 121.96, 122.76, 124.67, 125.12, 130.07, 130.68, 133.03, 144.06, 146.30, 148.90, 153.76,

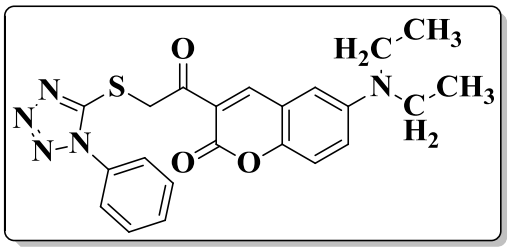
158.22, 189.84; **MS (ESI m/z):** 394.9 $[M+H]^+$; Anal. Calcd for $C_{19}H_{14}N_4O_4S$: C, 57.86; H, 3.58; N, 14.21; S, 8.13; Found: C, 57.89; H, 3.52; N, 14.25%.

6-Bromo-8-methoxy-3-(2-((1-phenyl-1*H*-tetrazol-5-yl)thio)acetyl)-2*H*-chromen-2-one (31h):



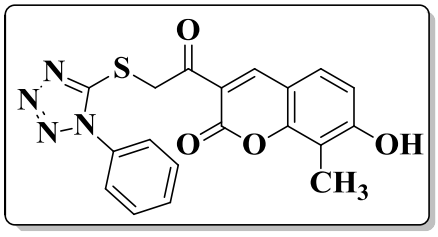
Red solid; yield: 94%; m.p.: 202 - 204 °C; **FTIR (KBr, ν_{max} cm^{-1}):** 1731 (lactone C=O); 1678 (-C=O); 1602 (-C=N); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 3.97 (s, 3H, $-OCH_3$), 5.07 (s, 2H, S-CH_2), 7.63 (d, *J* = 2 Hz, 1H, Ar-H), 7.67 – 7.70 (m, 5H, Ar-H), 7.81 (d, *J* = 2 Hz, 1H, Ar-H), 8.74 (s, 1H, C4-H of coumarin); **MS (ESI m/z):** 474.9 $[M+H]^+$; Anal. Calcd for $C_{19}H_{13}BrN_4O_4S$: C, 48.22; H, 2.77; N, 11.84; Found: C, 48.19; 2.83; H, N, 11.80%.

6-(Diethylamino)-3-(2-((1-phenyl-1*H*-tetrazol-5-yl)thio)acetyl)-2*H*-chromen-2-one (31i):



Green solid; yield: 87%; m.p.: 181 - 183 °C; **FTIR (KBr, ν_{max} cm^{-1}):** 1715 (lactone-C=O); 1614 (-C=O); 1566 (-C=N); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 1.15 (t, *J* = 6.8 Hz, 6H, $-CH_3$), 3.49 – 3.54 (m, 4H, -CH_2), 5.03 (s, 2H, S-CH_2), 6.63 (d, *J* = 2 Hz, 1H, Ar-H), 6.82 – 6.85 (m, 1H, Ar-H), 7.67 – 7.72 (m, 6H, Ar-H), 8.59 (s,1H, C_4-H of coumarin); **MS (ESI m/z):** 436.0 $[M+H]^+$; Anal. Calcd for $C_{22}H_{21}N_5O_3S$: C, 60.67; H, 4.86; N, 16.08; Found: C, 60.71; H, 4.80; N, 16.12%.

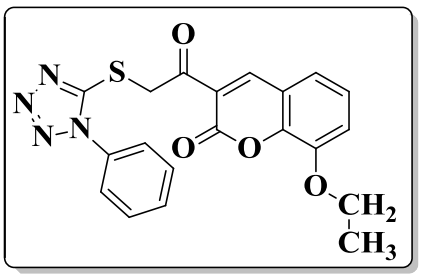
7-Hydroxy-8-methyl-3-(2-((1-phenyl-1*H*-tetrazol-5-yl)thio)acetyl)-2*H*-chromen-2-one (31j):



Yellow solid; yield: 89%; m.p.: 223 - 225 °C; **FTIR (KBr, ν_{max} cm^{-1}):** 3365 (-OH), 1722 (lactone –C=O), 1624 (-C=O), 1583 (-C=N); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 2.18 (s, 3H, $-CH_3$), 5.05 (s, 2H, -S-CH_2), 6.95 (d, *J* = 8.4

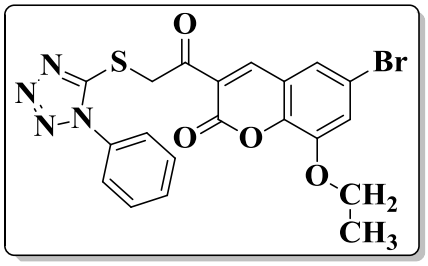
Hz, 1H, Ar-H), 7.76 – 7.72 (m, 6H, Ar-H), 8.72 (s, 1H, C_4-H of coumarin), 11.26 (s, 1H, -OH); **MS (ESI m/z):** 395.0 $[M+H]^+$; Anal. Calcd for $C_{19}H_{14}N_4O_4S$: C, 57.86; H, 3.58; N, 14.21; Found: C, 57.82; H, 3.64; N, 14.23%.

8-Ethoxy-3-(2-((1-phenyl-1*H*-tetrazol-5-yl)thio)acetyl)-2*H*-chromen-2-one (31k):



Yellow solid; yield: 88%; mp: 207 - 209 °C; **FTIR (KBr, ν_{max} cm^{-1}):** 1708 (lactone-C=O), 1628 (-C=O), 1596 (-C=N); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 1.42 (t, *J* = 6.8 Hz, 3H, $-CH_3$), 4.19 – 4.24 (m, 2H, $-O-CH_2$), 5.08 (s, 2H, $-S-CH_2$), 7.36 (t, *J* = 8 Hz, 1H, -Ar-H), 7.47 (d, *J* = 8 Hz, 1H, Ar-H), 7.54 (d, *J* = 7.6 Hz, 1H, Ar-H), 7.67 – 7.69 (m, 5H, Ar-H), 8.79 (s, 1H, C_4-H of coumarin); **^{13}C NMR (100 MHz, DMSO-d_6, δ ppm):** 14.52, 44.35, 64.59, 117.97, 118.70, 121.92, 122.72, 124.51, 125.10, 130.04, 130.66, 133.01, 144.14, 145.51, 148.92, 153.75, 158.27, 189.87; **MS (ES m/z):** 409.0 $[M+H]^+$; Anal. Calcd for $C_{20}H_{16}N_4O_4S$: C, 58.81; H, 3.95; N, 13.72; Found: C, 58.85; H, 3.92; N, 13.74%.

6-Bromo-8-ethoxy-3-(2-((1-phenyl-1*H*-tetrazol-5-yl)thio)acetyl)-2*H*-chromen-2-one (31l):



Brown solid; yield: 91%; m.p.: 185 - 187 °C; **FTIR (KBr, ν_{max} cm^{-1}):** 1734 (lactone-C=O); 1696 (-C=O); 1602 (-C=N); 1273 ($-OCH_2$); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 1.29 (t, *J* = 4Hz, 3H, $-CH_3$), 4.22 – 4.25 (m, 2H, $-OCH_2$), 4.89 (s, 2H, $-S-CH_2$), 6.99 (d, *J* = 8.8 Hz, 1H, Ar-H), 7.15 – 7.21 (m, 1H, Ar-H), 7.60 (d, *J* = 6.4 Hz, 1H, Ar-H), 7.68 (t, *J* = 6.4 Hz, 3H, Ar-H), 7.79 (t, *J* = 4.4 Hz, 1H, Ar-H), 8.72 (s, 1H, C_4-H of coumarin); **MS (ESI m/z):** 486.9 $[M+H]^+$; Anal. Calcd for $C_{20}H_{15}BrN_4O_4S$: C, 49.29; H, 3.10; N, 11.50; Found: C, 49.34; H, 3.06; N, 11.52%.

Anticancer activity of 3-(2-((1-phenyl-1*H*-tetrazol-5-yl) thio) acetyl)-2*H*-coumarin derivatives (31 a-l).

The newly synthesized 3-(2-((1-phenyl-1*H*-tetrazol-5-yl) thio) acetyl)-2*H*-coumarin derivatives were screened against five different human cancer cell lines Murine leukemia

(L1210), human T-lymphocyte cells (CEM), prostate cancer (DU-145), human cervix carcinoma cells (HeLa), and breast cancer (MCF-7) cells by using standard MTT anticancer screening protocol as described in chapter-II (Reference 49).

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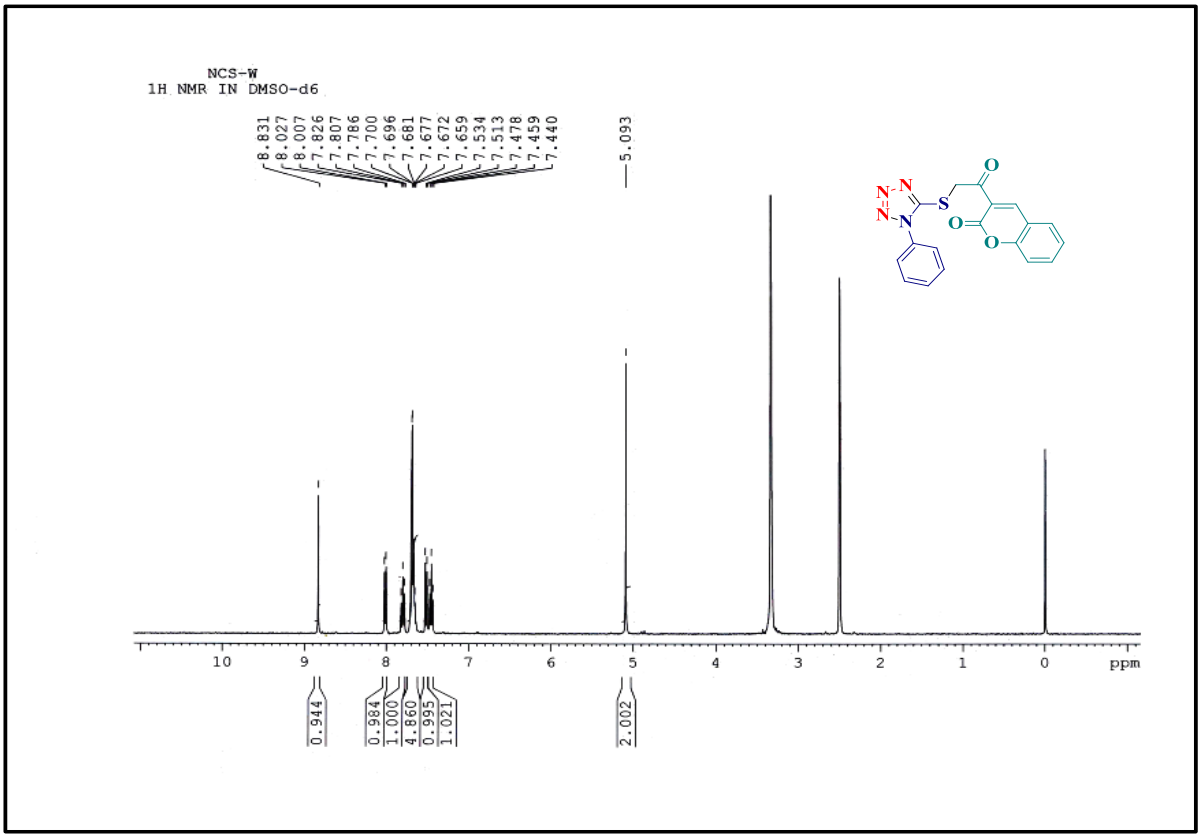


Fig. ^{1}H NMR of compound 31a

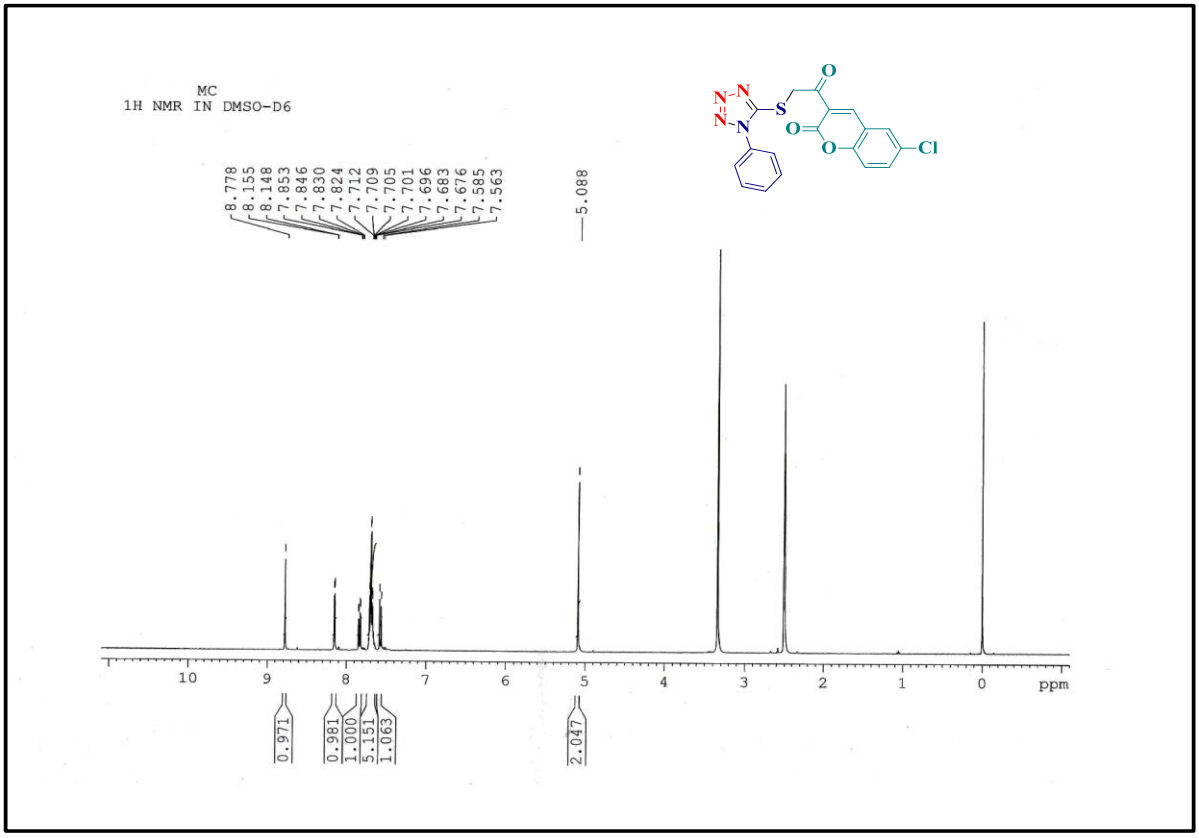


Fig. ^{1}H NMR of compound 31b

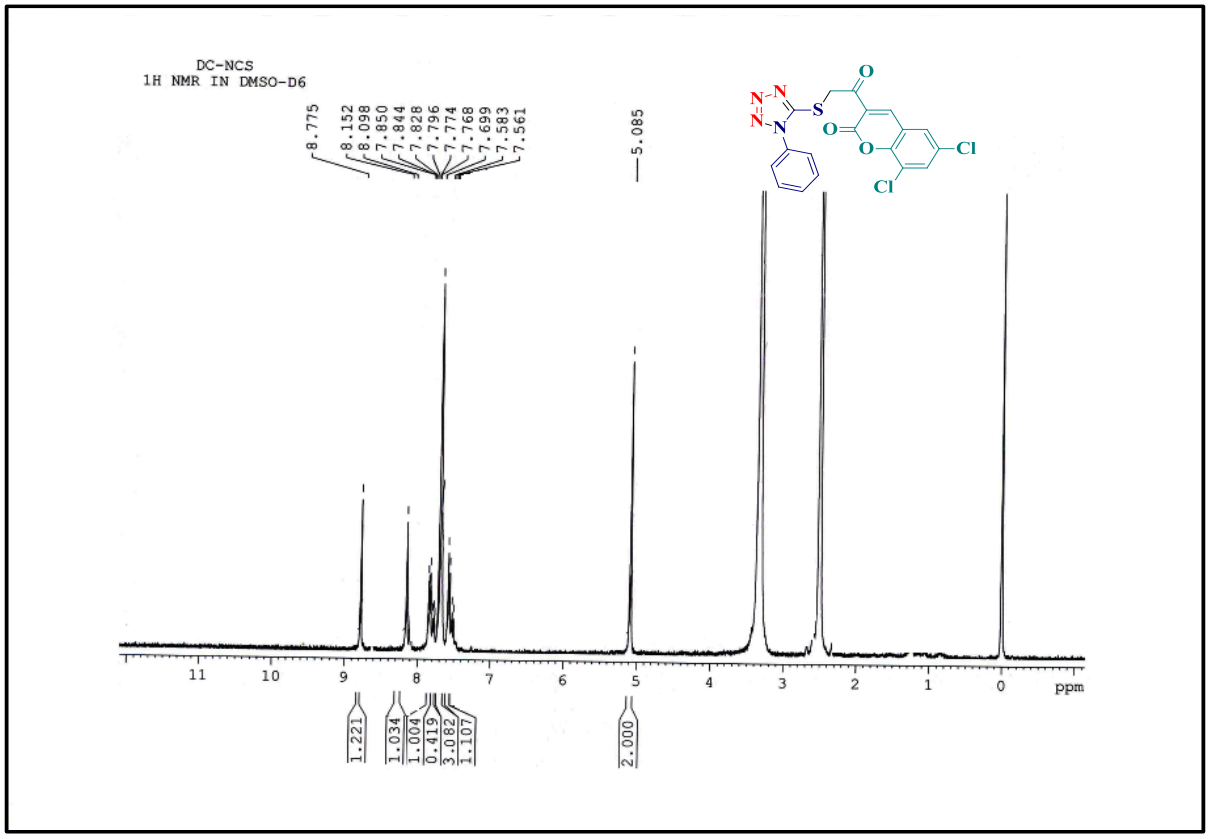


Fig. ^{1}H NMR of compound 31c

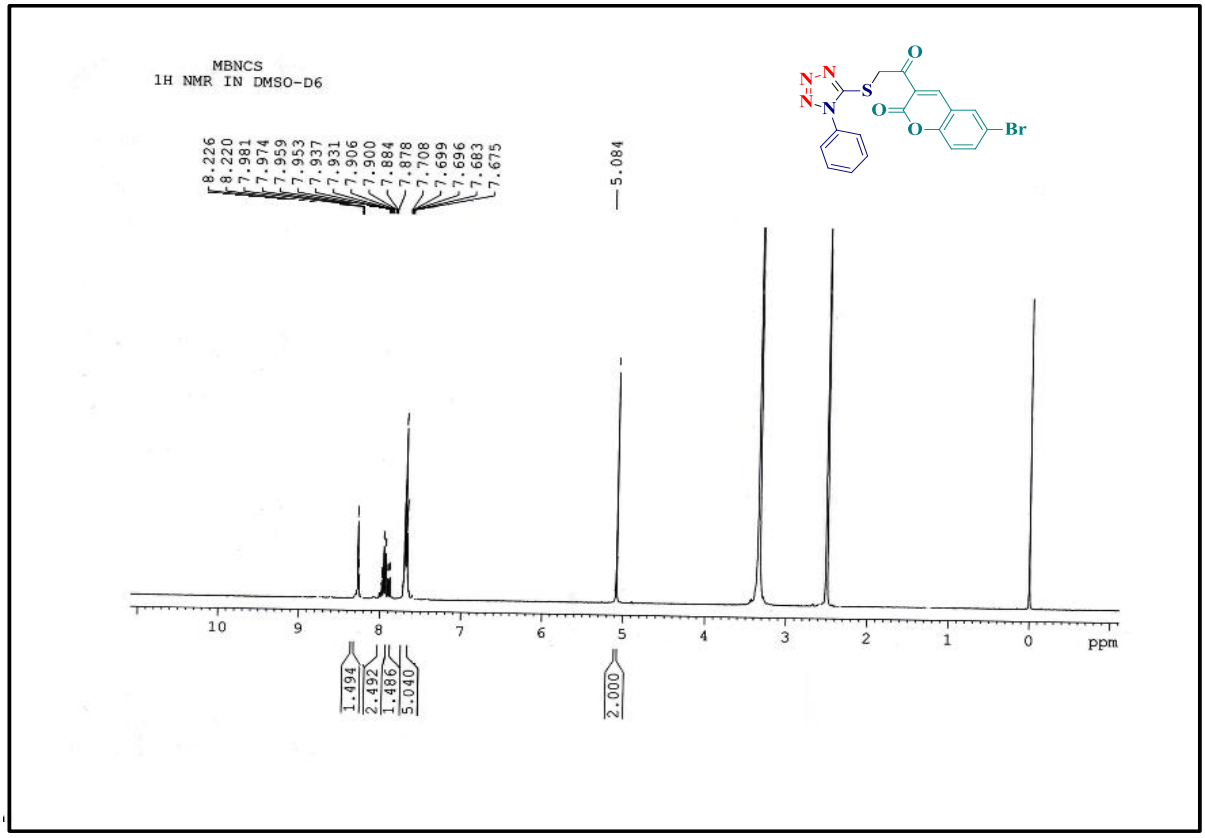


Fig. ^{1}H NMR of compound 31d

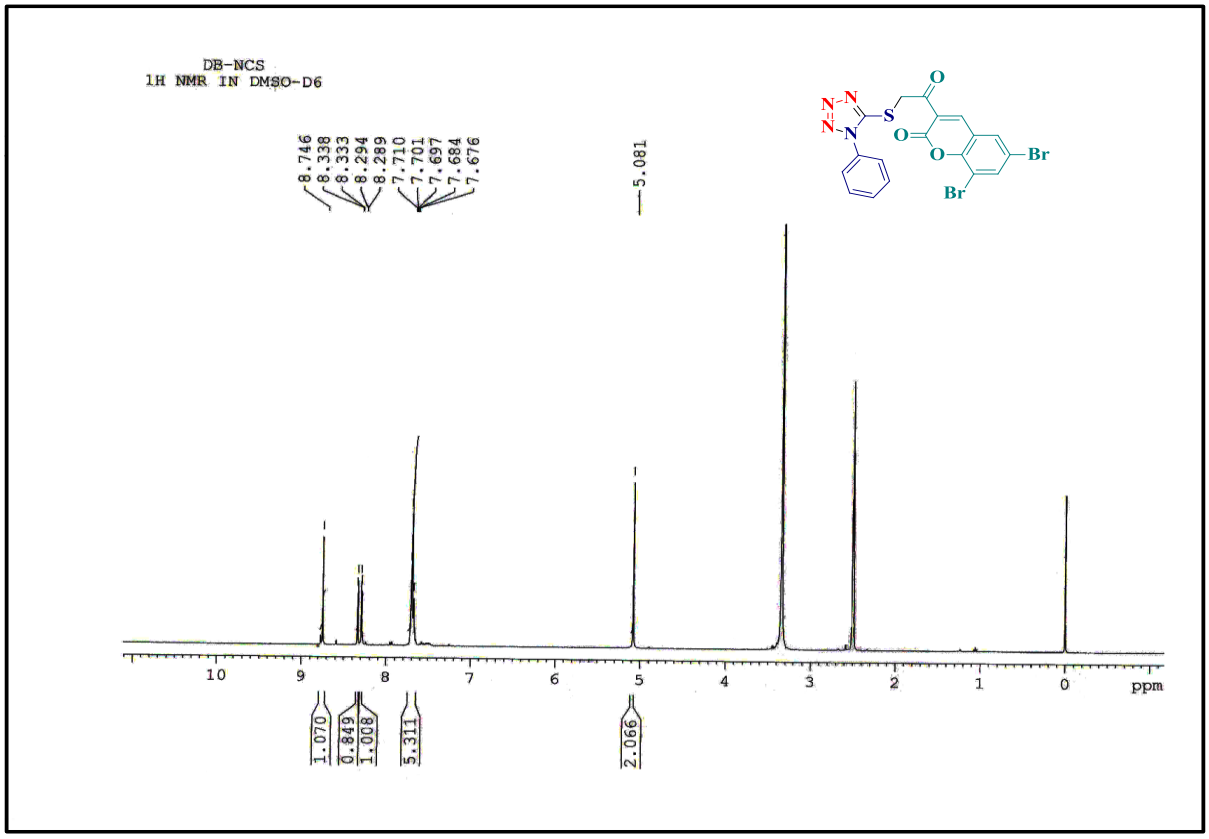


Fig. ^{1}H NMR of compound 31e

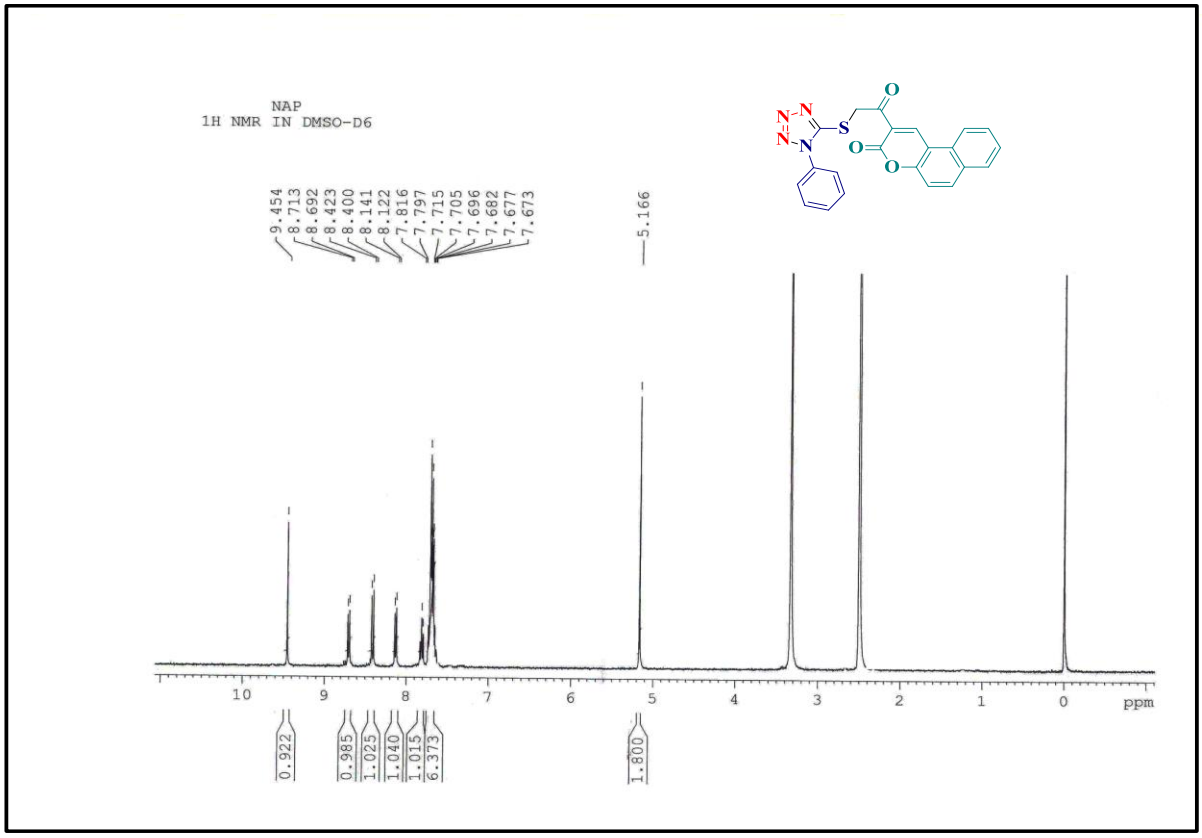


Fig. ^{1}H NMR of compound 31f

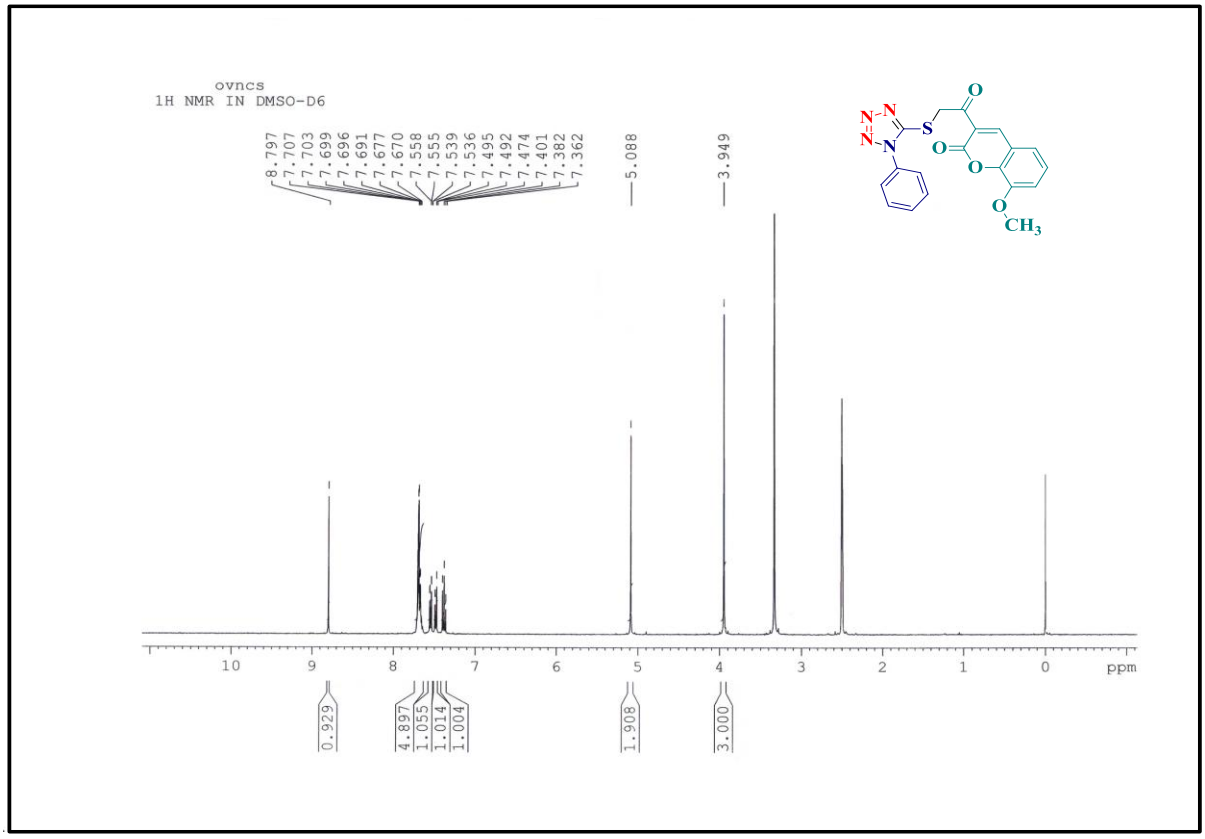


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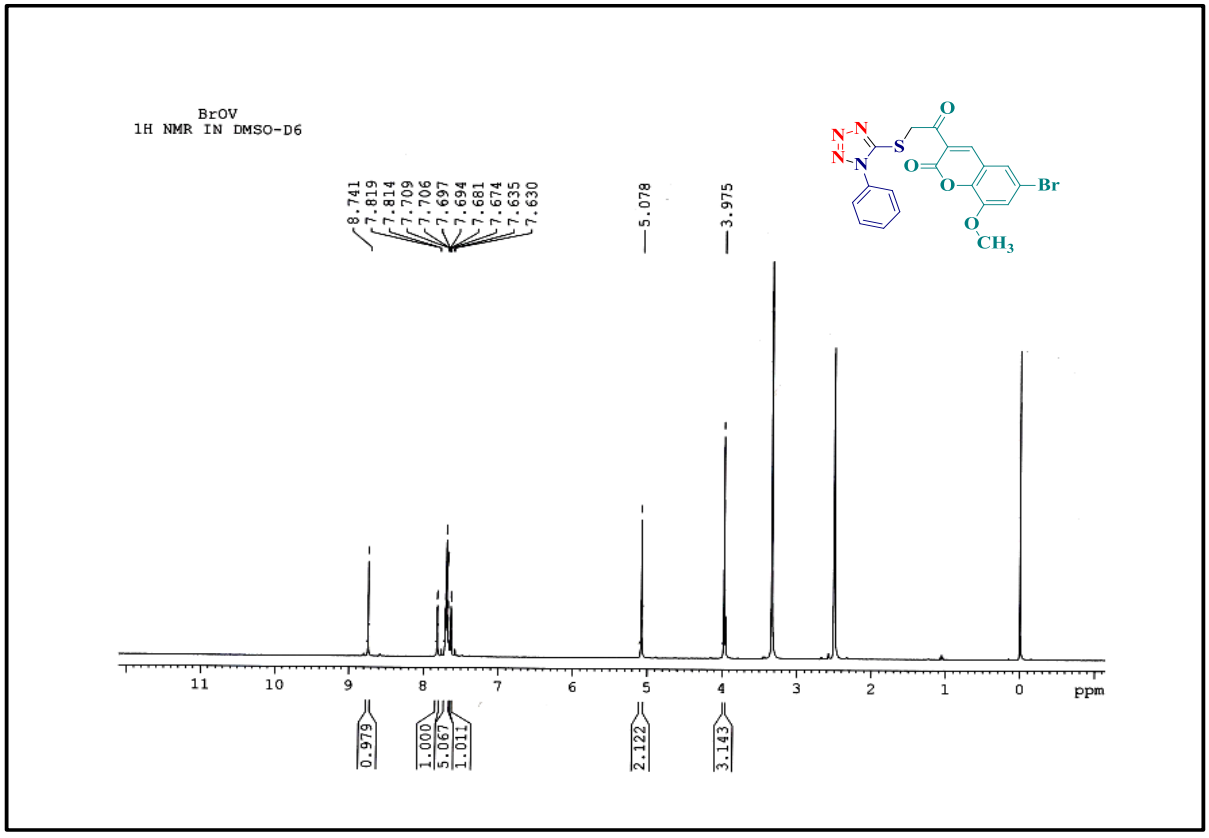


Fig. ^{1}H NMR of compound 31h

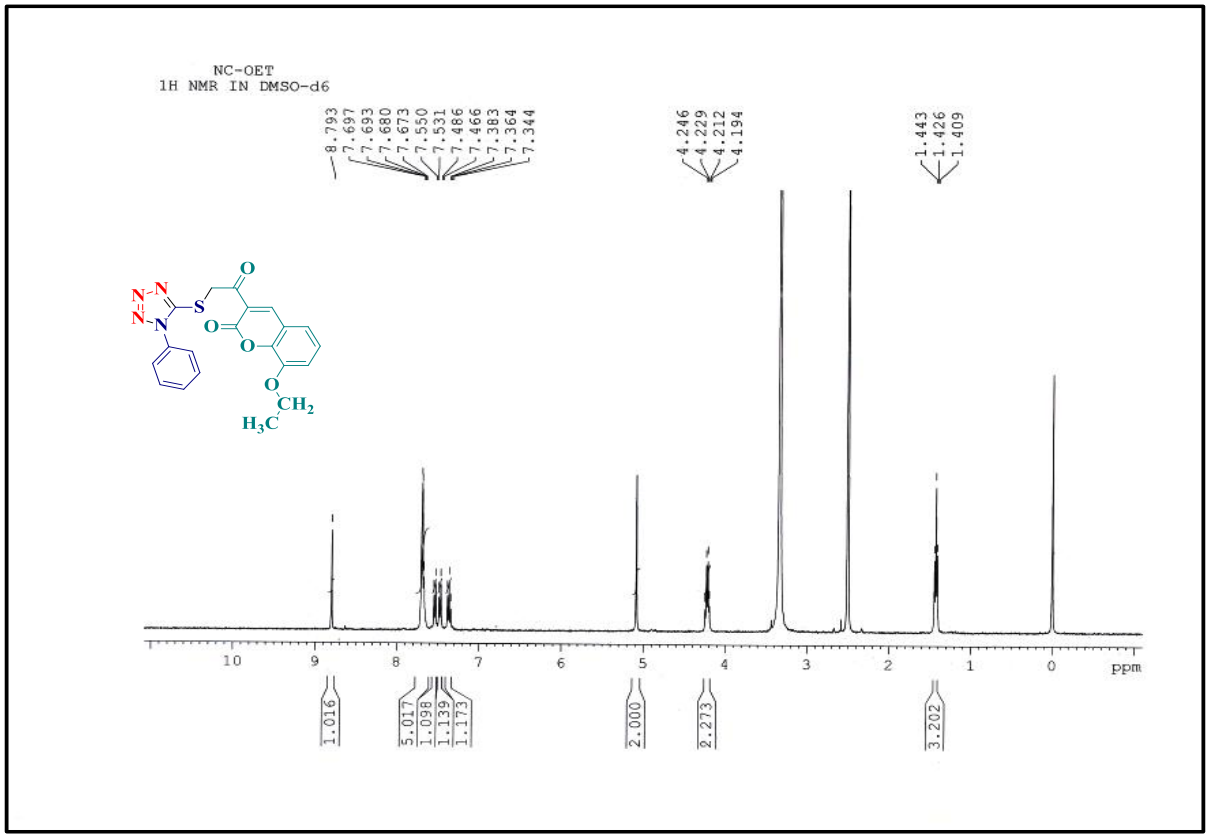


Fig. ^{1}H NMR of compound 31k

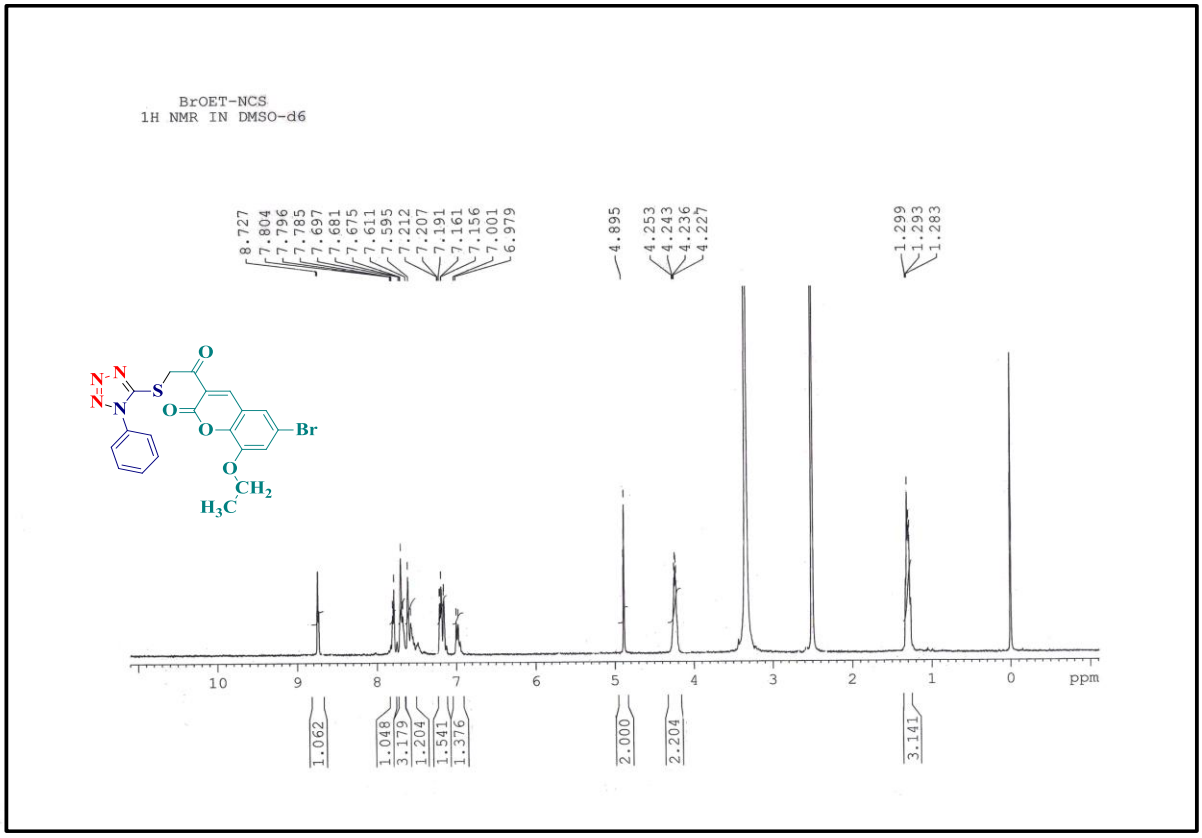


Fig. ^{1}H NMR of compound 31l

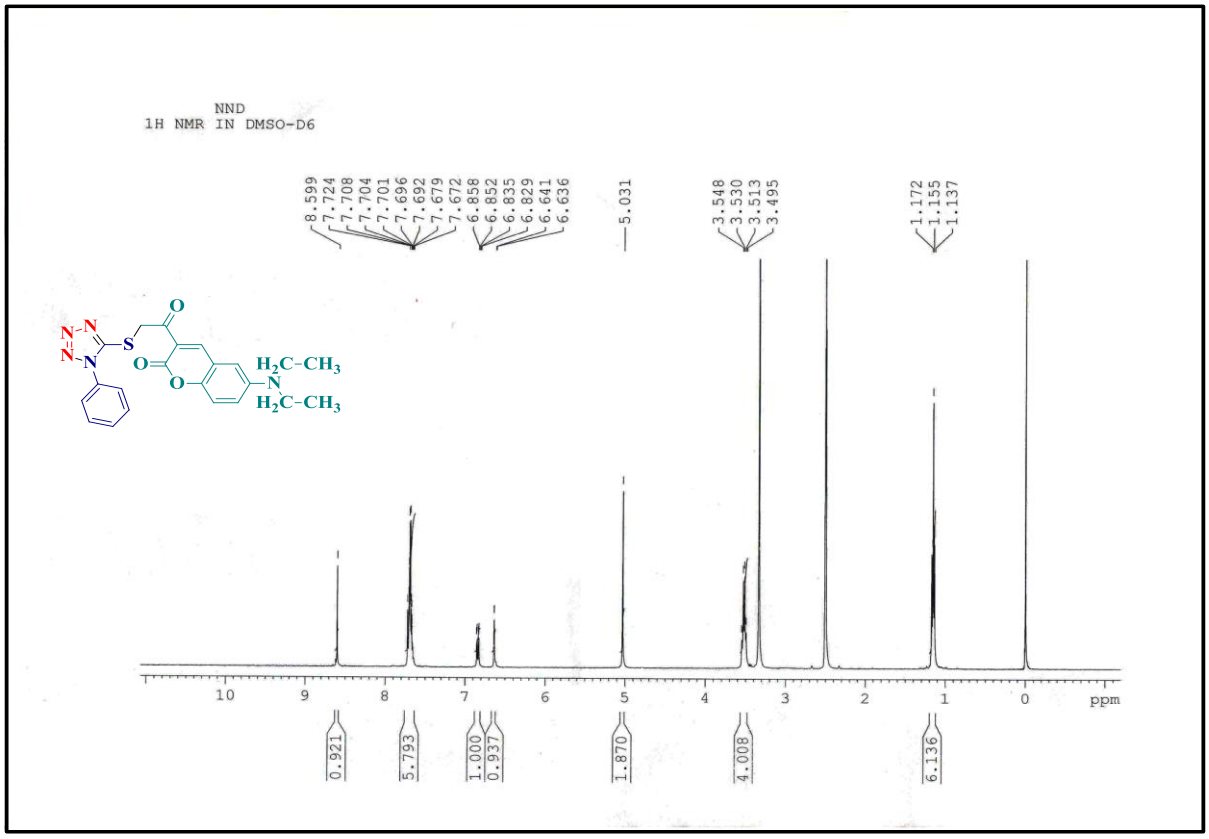


Fig. ^{1}H NMR of compound 31i

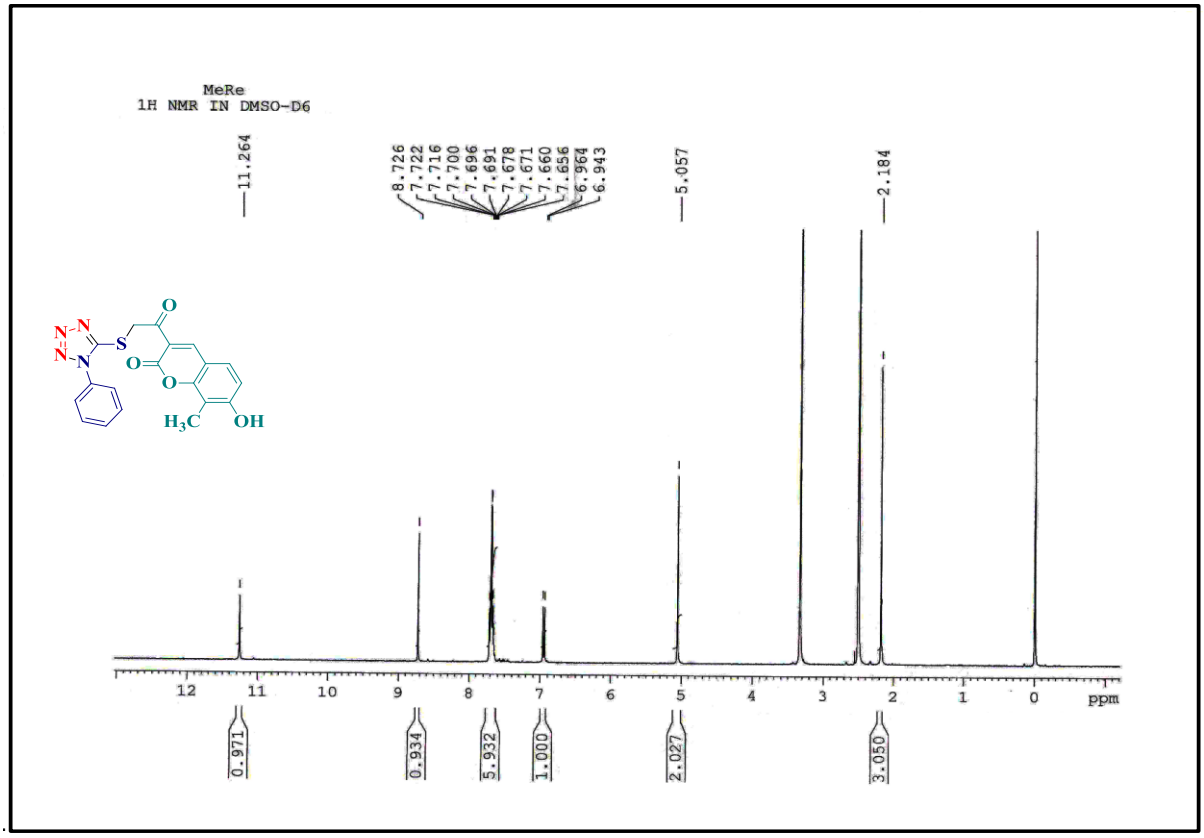


Fig. ^{1}H NMR of compound 31j

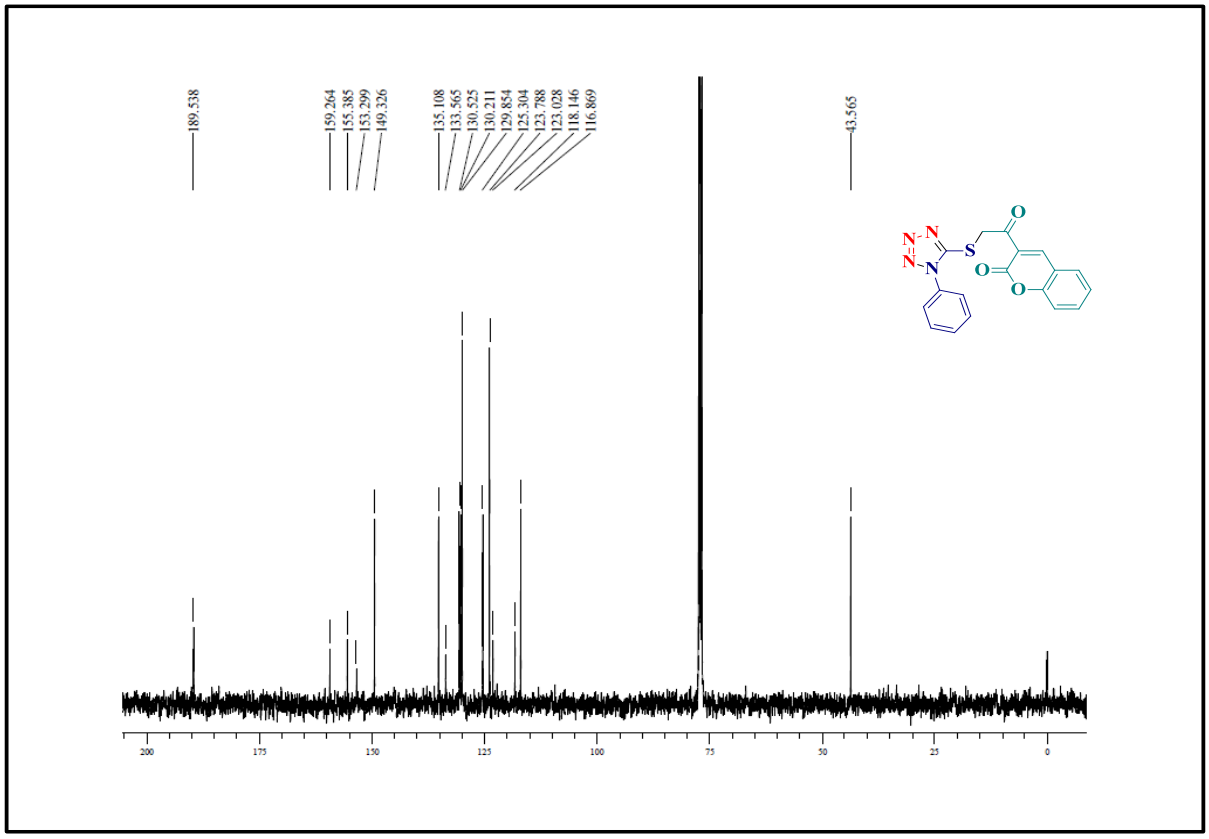


Fig. ^{13}C NMR of compound 31a

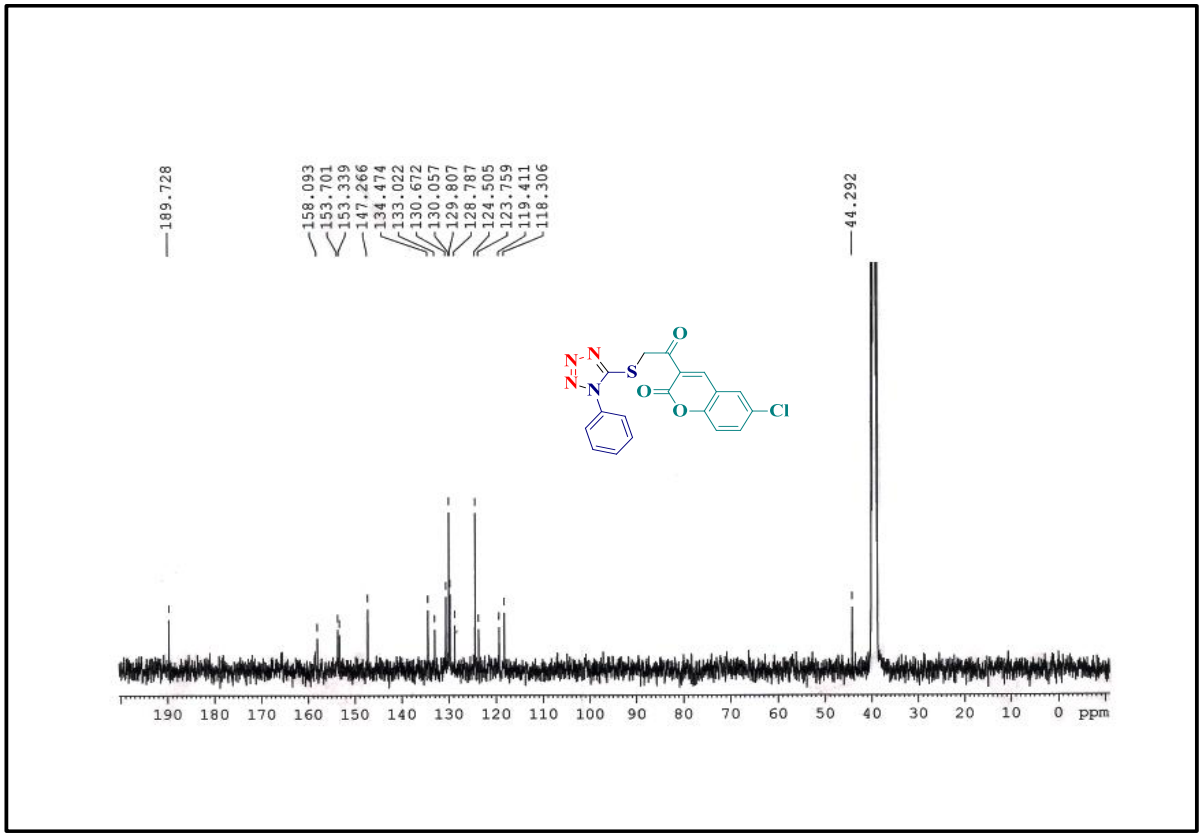


Fig. ^{13}C NMR of compound 31b

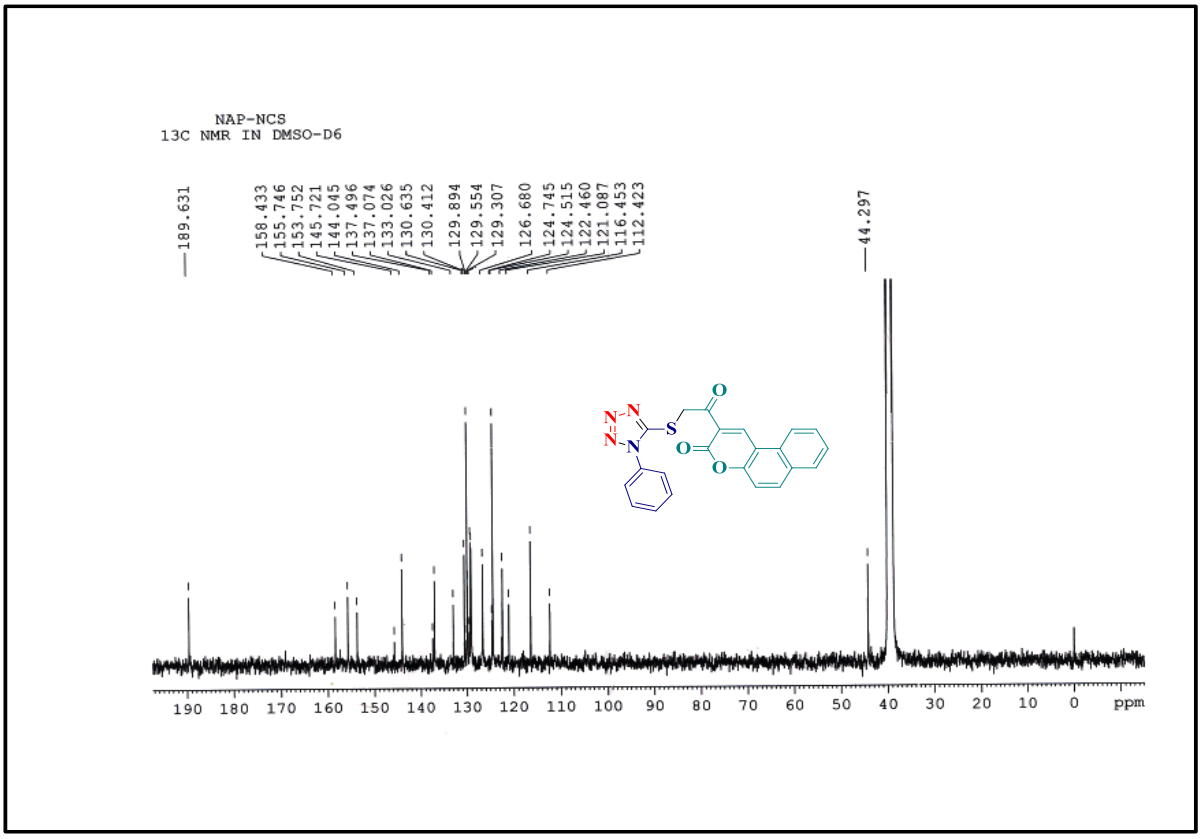


Fig. ^{13}C NMR of compound 31f

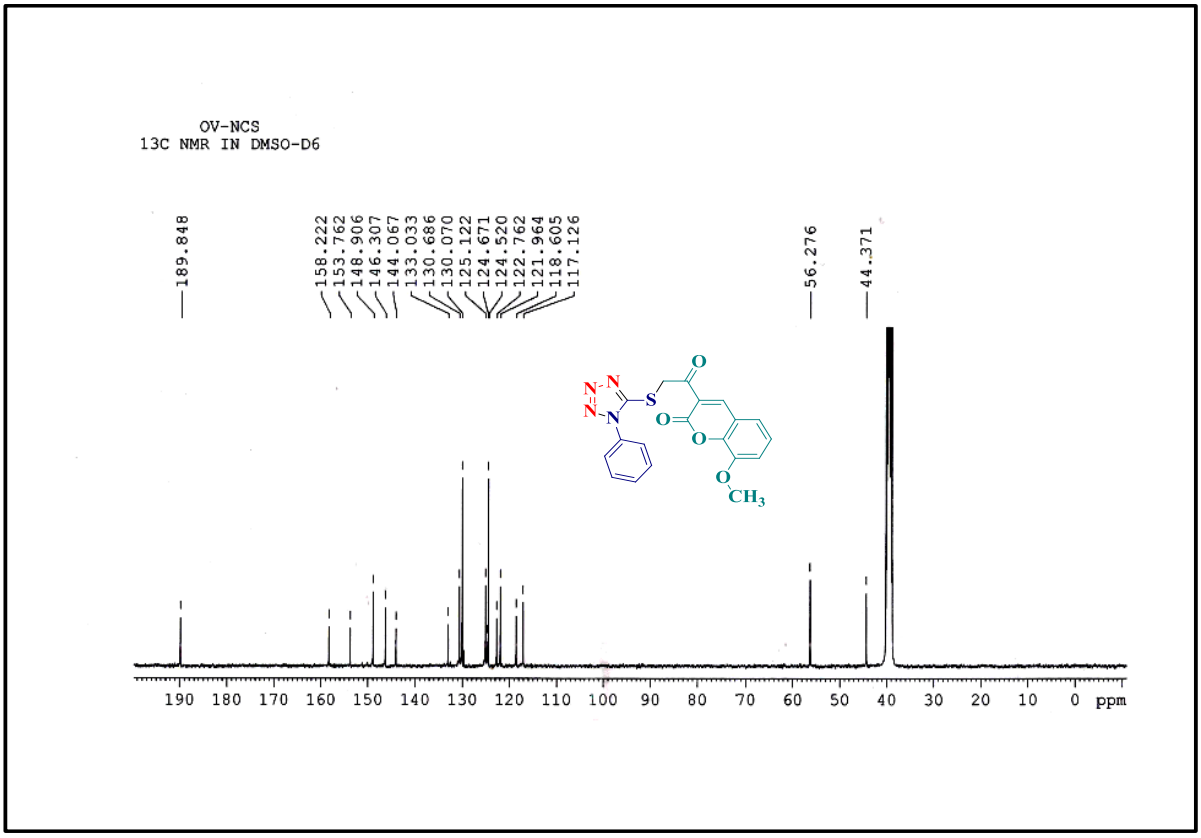


Fig. ^{13}C NMR of compound 31g

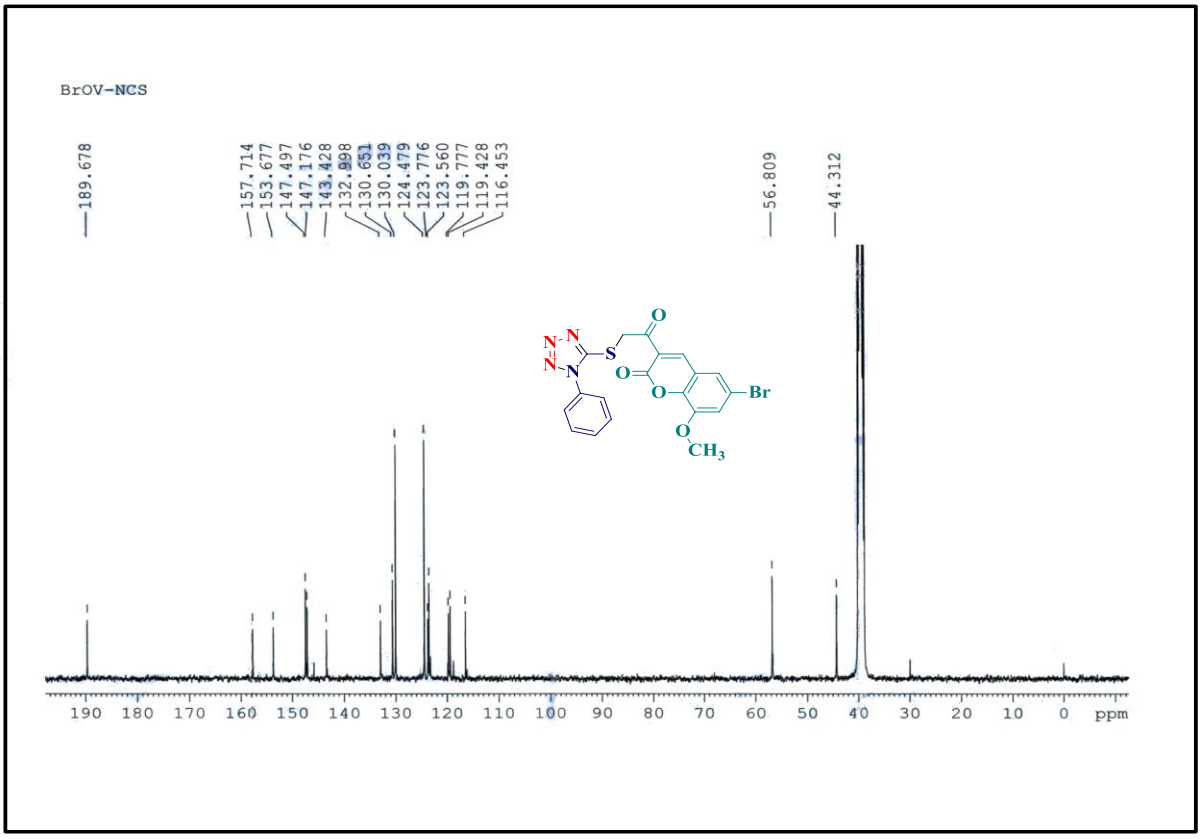


Fig. ^{13}C NMR of compound 31h

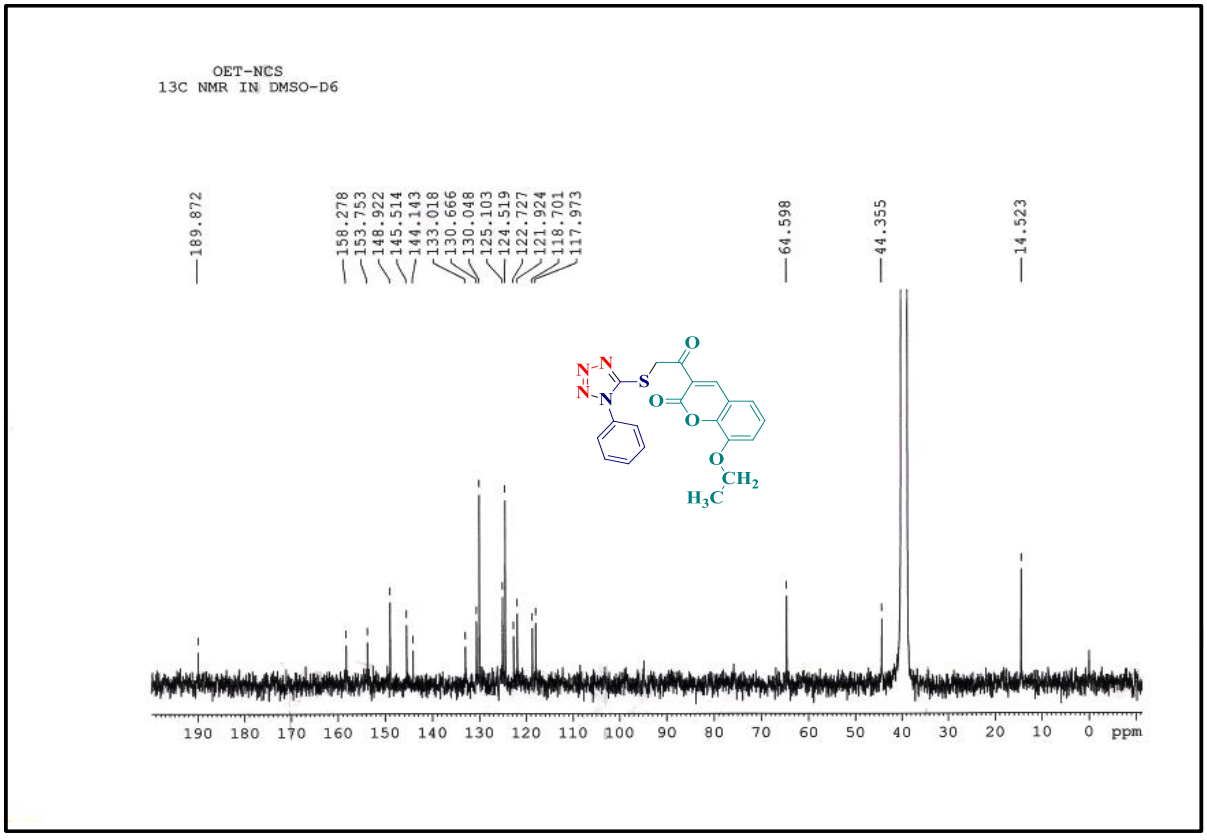


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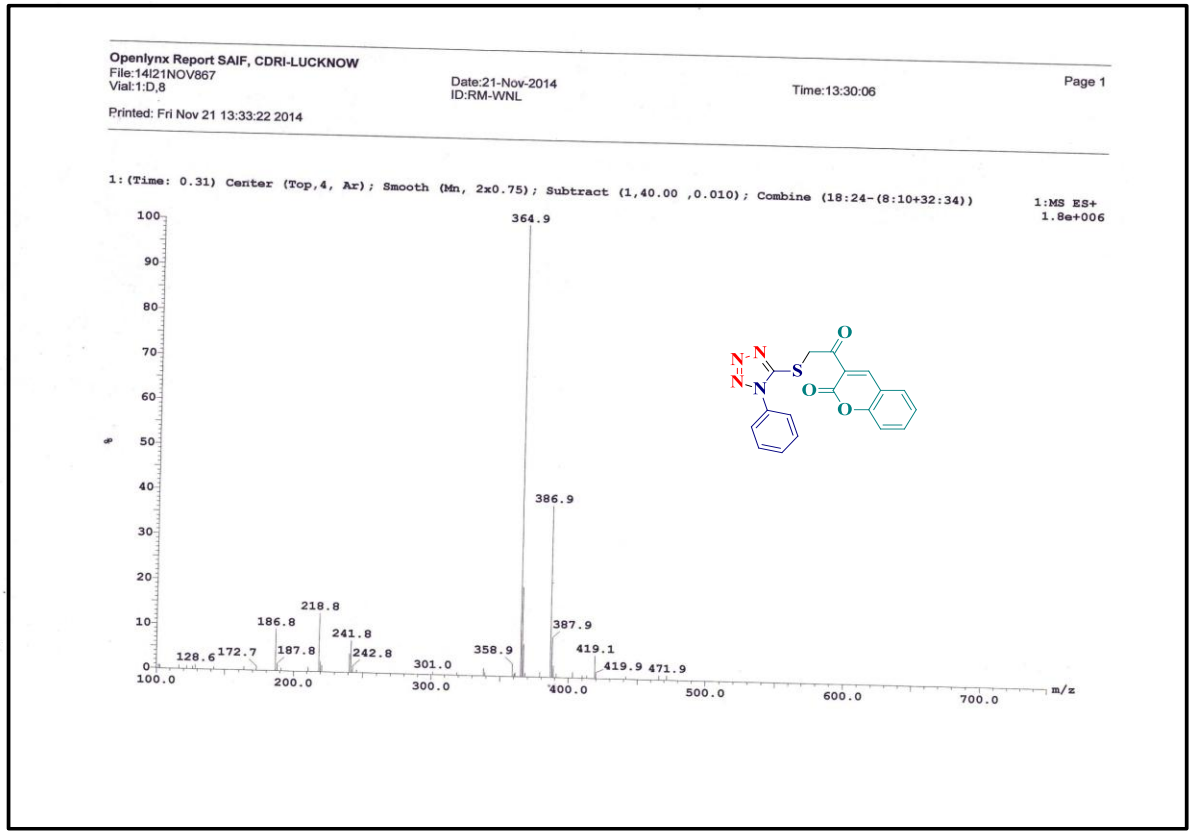


Fig. Mass spectrum of compound 31a

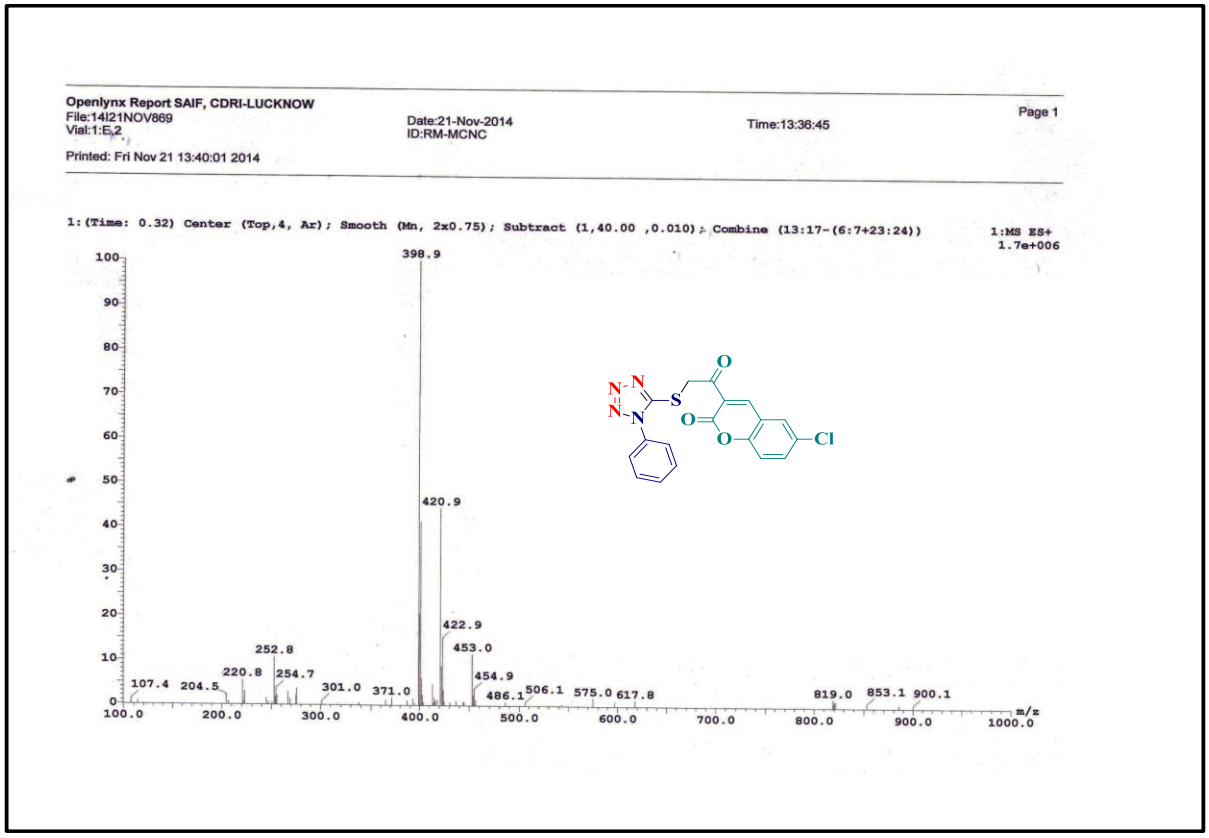


Fig. Mass spectrum of compound 31b

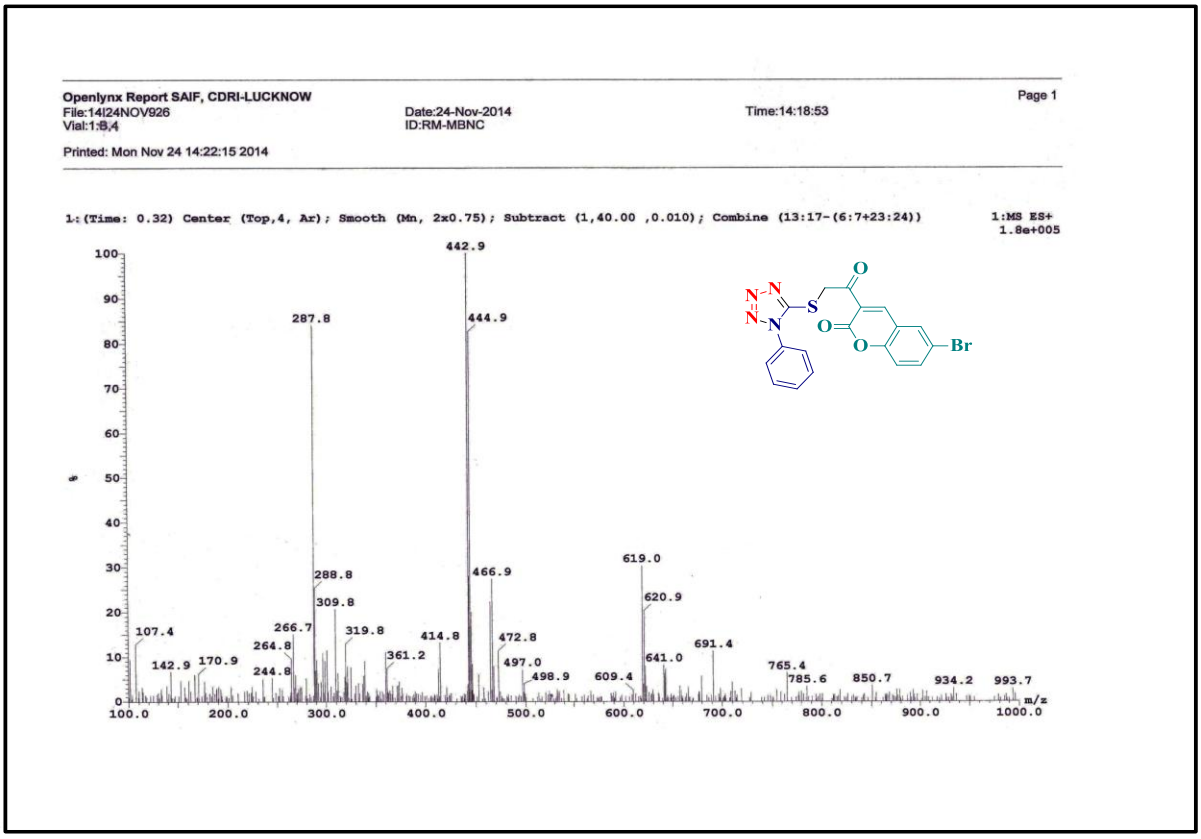


Fig. Mass spectrum of compound 31d

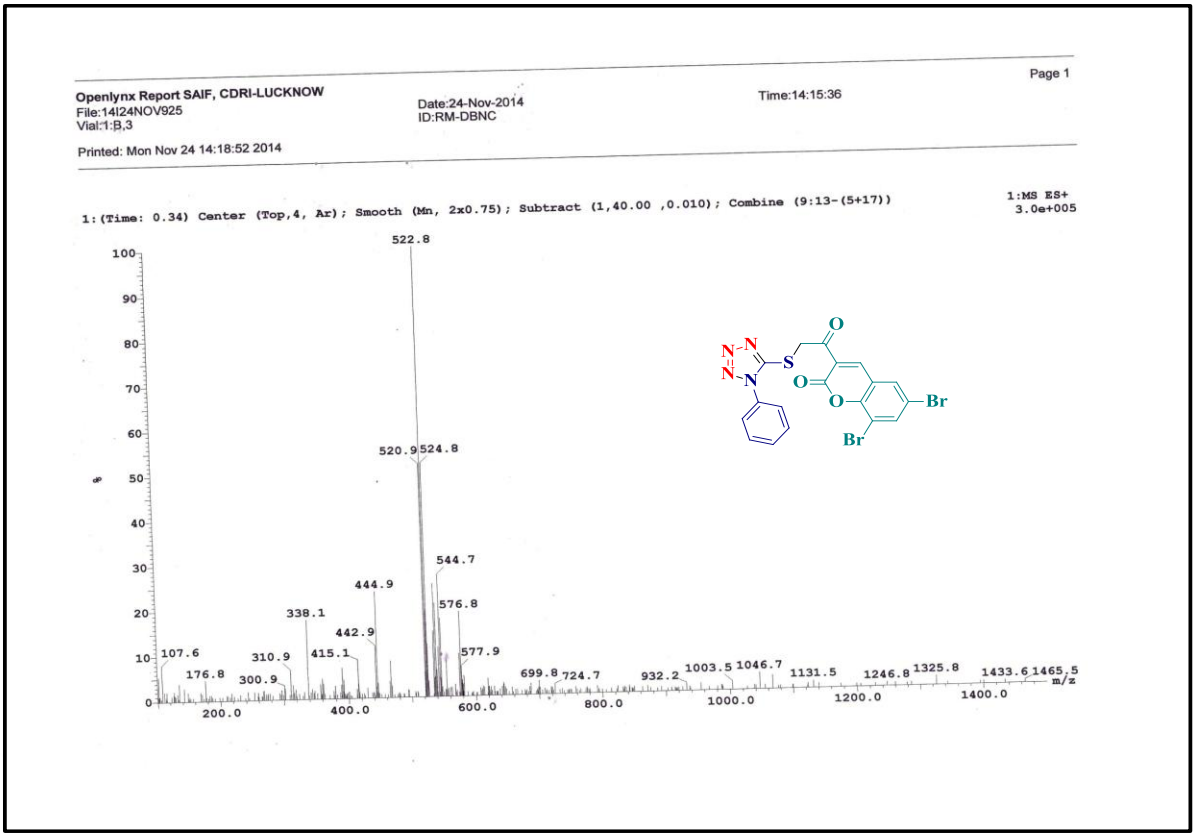


Fig. Mass spectrum of compound 31e

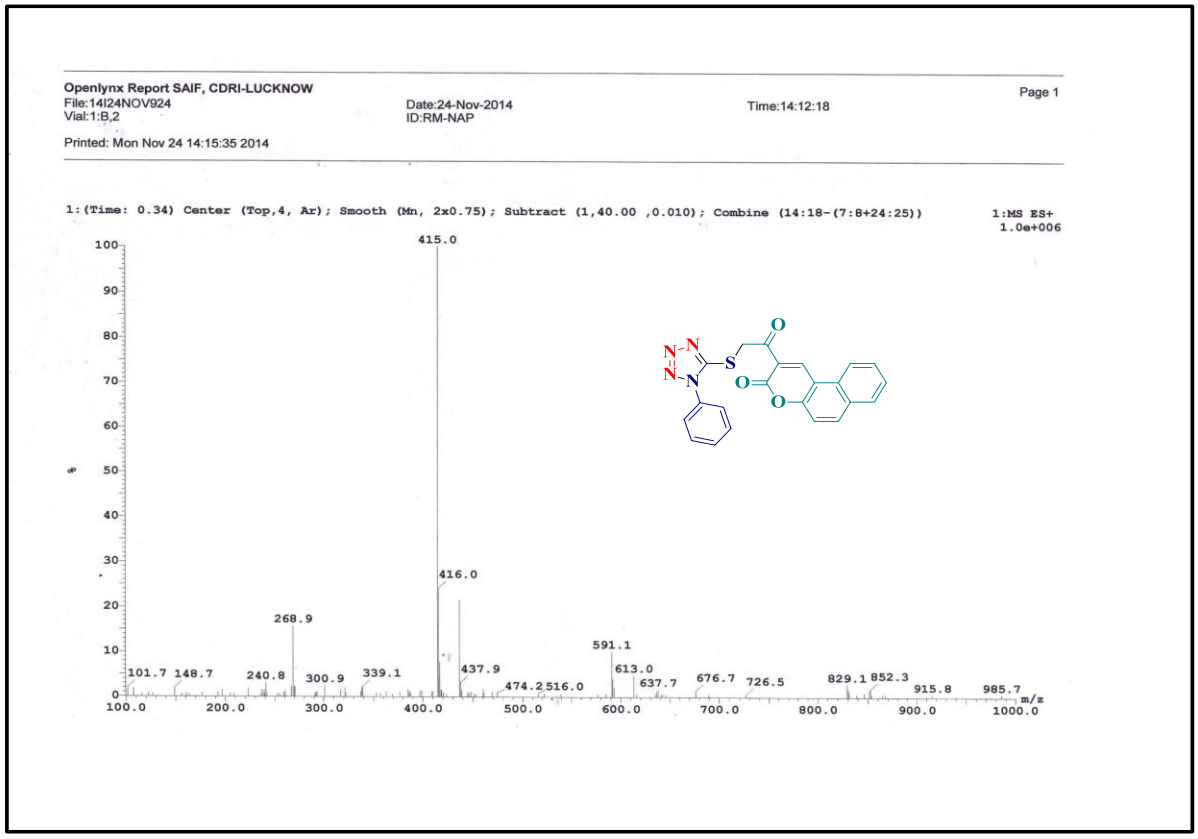


Fig. Mass spectrum of compound 31f

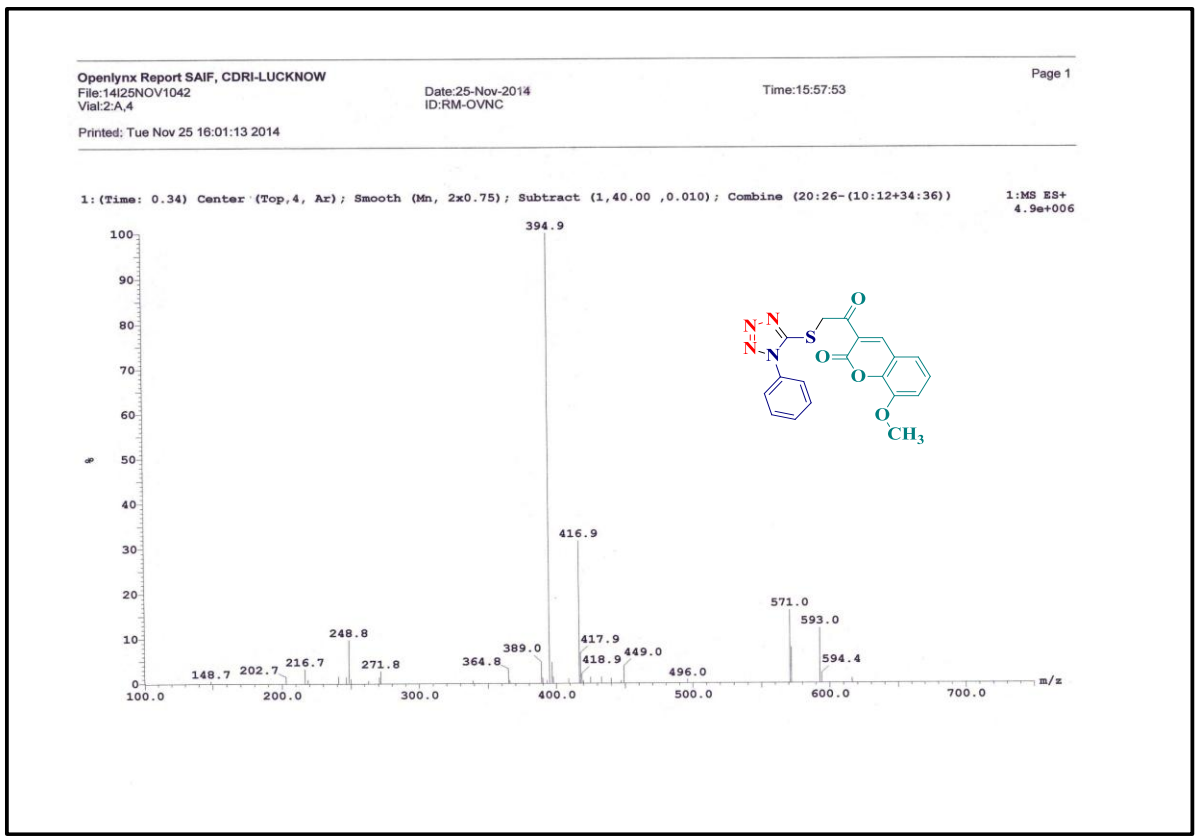


Fig. Mass spectrum of compound 31g

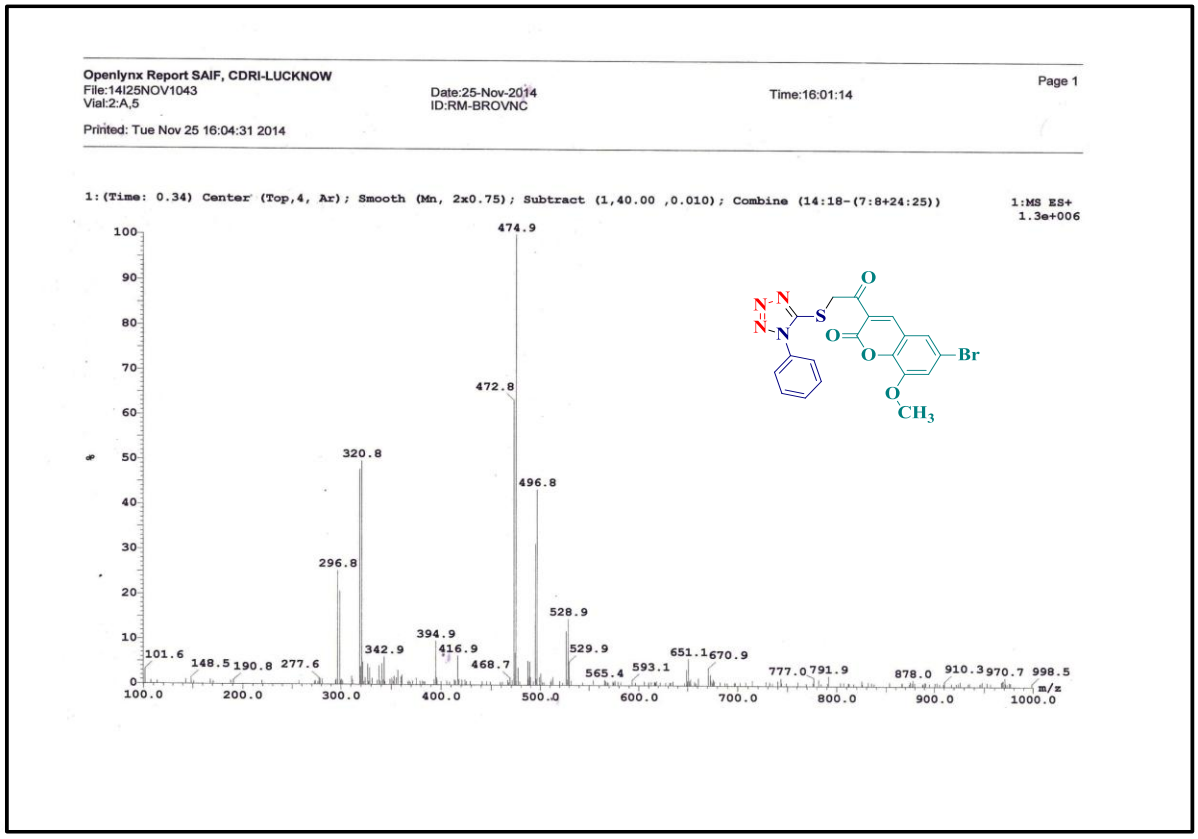


Fig. Mass spectrum of compound 31h

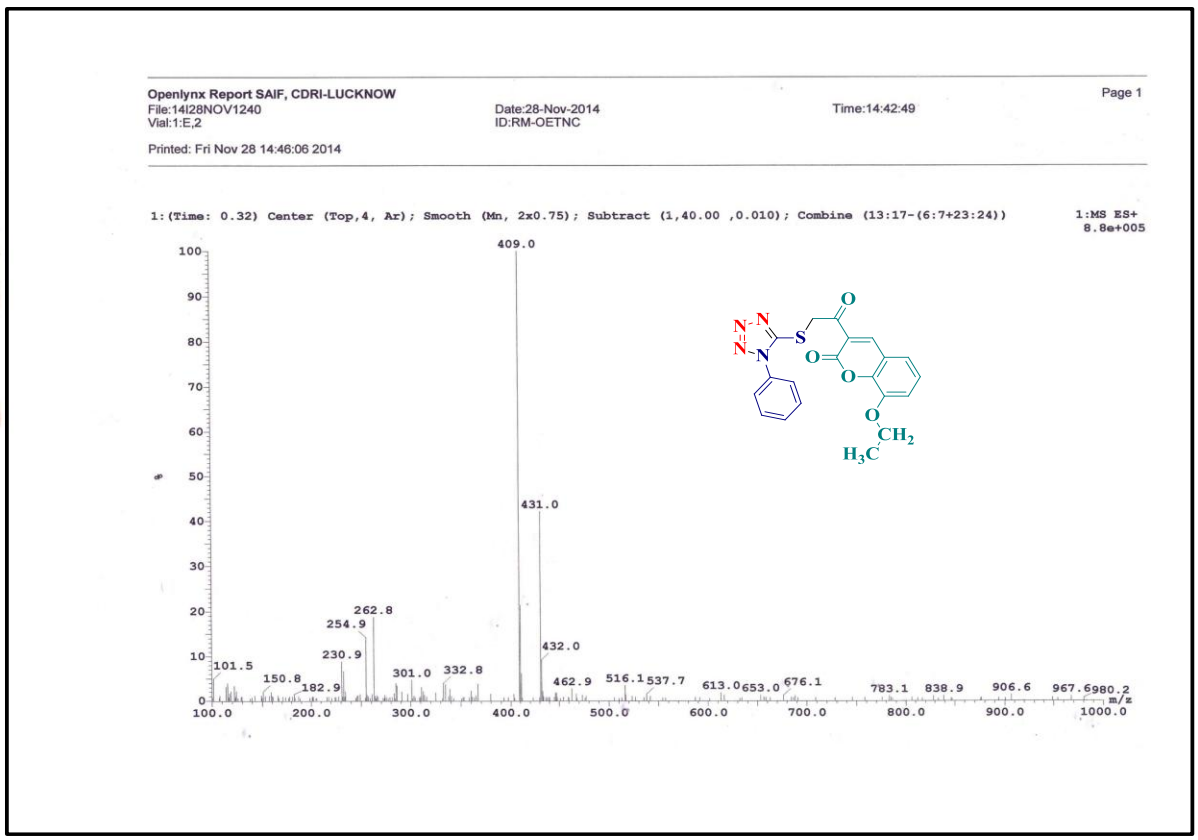


Fig. Mass spectrum of compound 31k

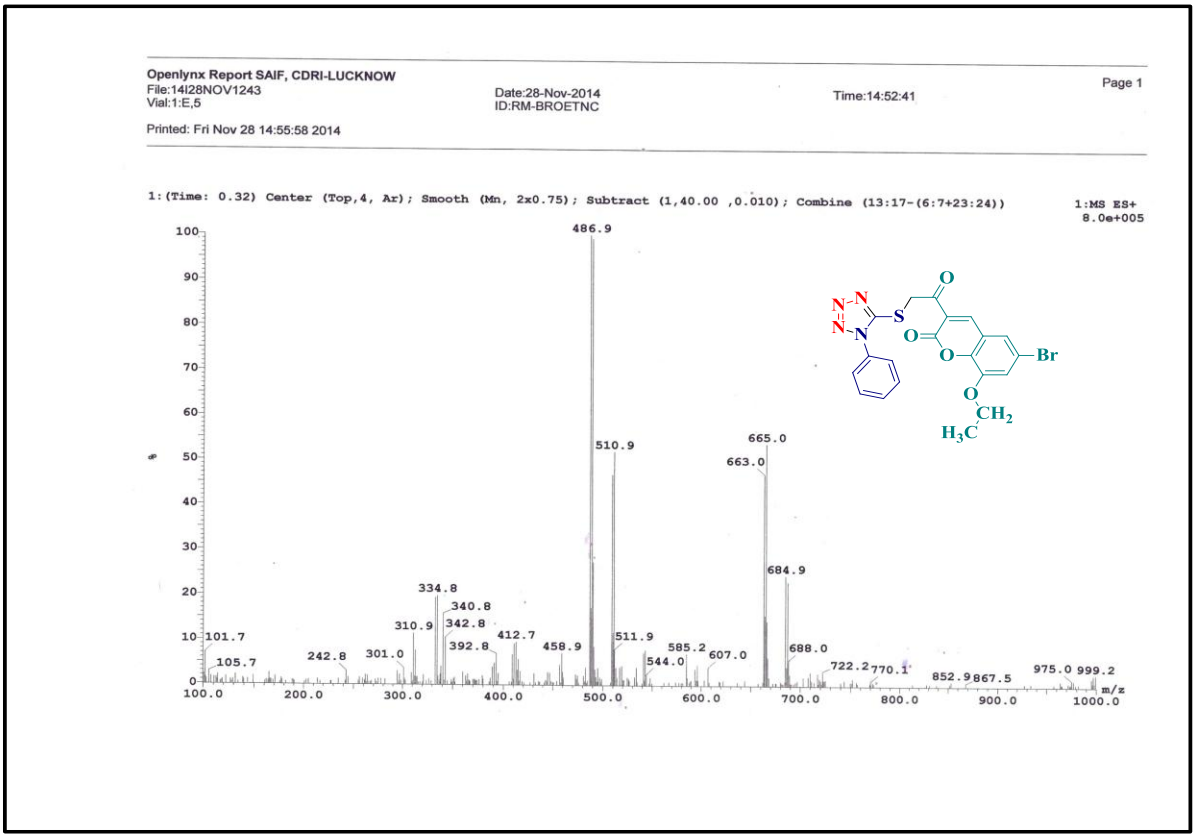


Fig. Mass spectrum of compound 31l

CHAPTER-VII

A FACILE ONE POT MULTICOMPONENT SYNTHESIS OF 2,4 AND 2,3,4-SUBSTITUTED THIAZOLES AND THEIR BIOLOGICAL APPLICATIONS

INTRODUCTION:

Development of novel therapeutic agents having nitrogen and sulphur as hetero atoms in the five member ring containing heterocycles has received tremendous importance in the field of medicinal chemistry. A five member heterocyclic compound with two hetero atoms may have two positional isomers. Nitrogen and sulphur containing five member cyclic system exhibits two isomeric forms (**Fig.1**) i.e. 1, 2-thiazole (isothiazole) and 1,3-thiazole (thiazole). Among these two isomers, 1,3-thiazole is a well studied heterocyclic compound with diversified applications. Thiazoles are important class of five member heterocyclic compounds having sulphur and nitrogen hetero atoms at 1 and 3 positions in the cyclic ring system. Thiazole exhibits various resonance forms by the delocalization of sulphur lone pair of electrons in the ring system (**Fig.2**). 1, 3- thiazoles are considered as a cyclic analogue of thiosemicarbazone[1]. Thiazoles are important building core units and these are ubiquitously found in many natural and synthetic organic molecules. Among the important heterocyclic compounds, thiazoles are considered crucial therapeutic compounds in medicinal chemistry. Thiazole was first synthesized by German chemist Hantzsch[2] by the condensation reaction of thioamides with α-halo carbonyl compounds.

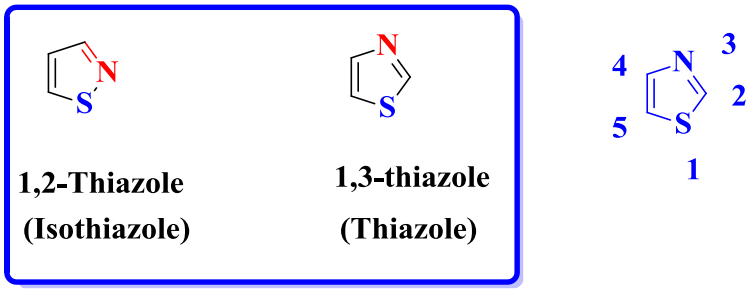


Fig. 1 Isomeric forms of thiazoles

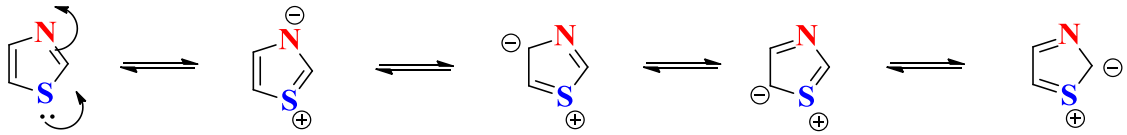


Fig.2 Various resonance forms of thiazole.

A large variety of therapeutic agents contain thiazoles as core pharmacophore unit and some of the drug molecules having thiazole as core unit as shown in **Fig.3**.

Thiazole skeleton was found to possess a large variety of therapeutic and material applications such as antibacterial[3-5], antifungal[6-8], antiviral[9-11], anti inflammation[12-14], anti hypertension[15], anticancer[16-18], herbicidal[19-21], antifilarial[22], estrogen receptor[23], schizophrenia[24], liquid crystals[25,26], photoluminiscent[27], molecular switches[28] and sensors[29]. In addition to the biological applications, thiazoles are also used as an important synthetic intermediate in numerous organic compounds. Due to the wide applications of thiazoles in the field of medicine, agriculture and materials, considerable attention was given by the scientific community across the globe. The development of pharmacologically active thiazole compounds through simple, efficient, catalytic free and greener protocol is a major issue for the medicinal chemists.

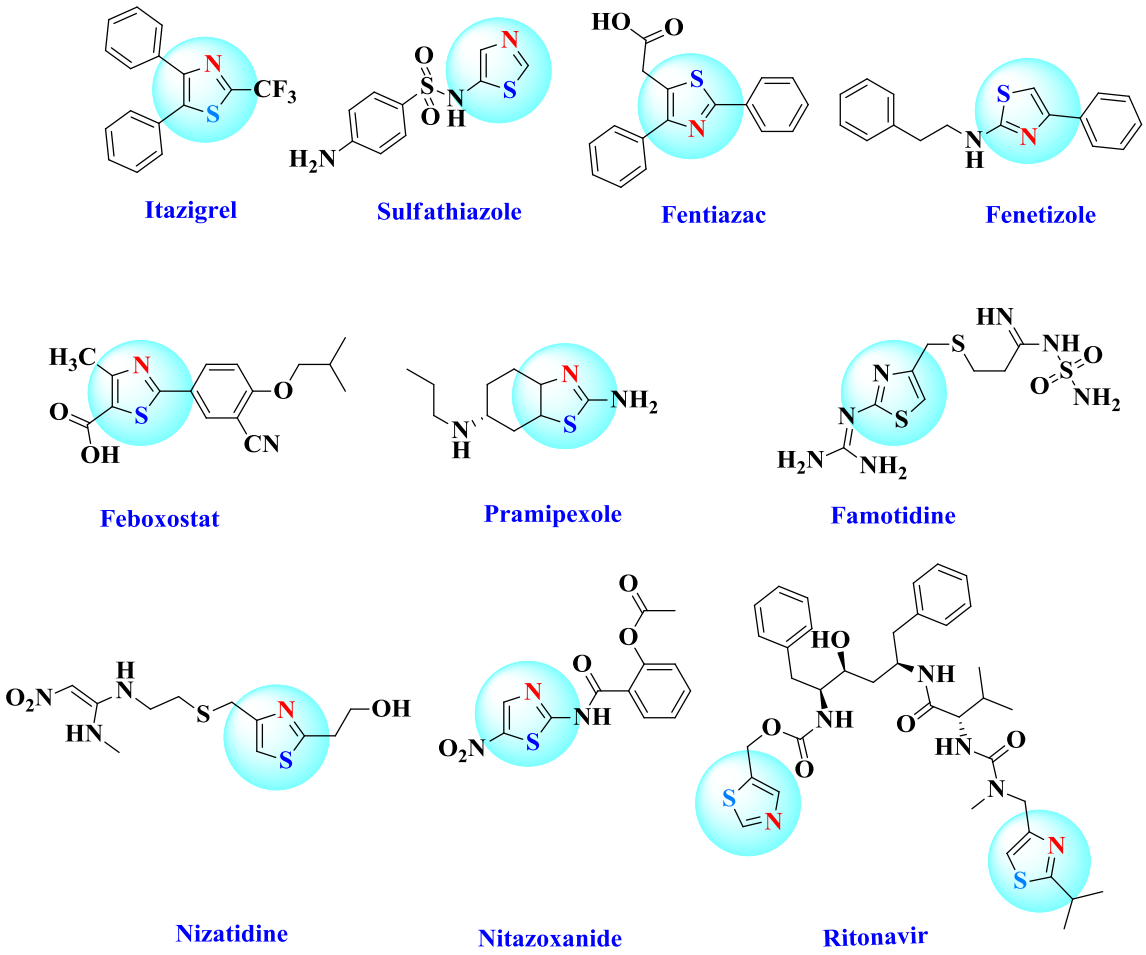
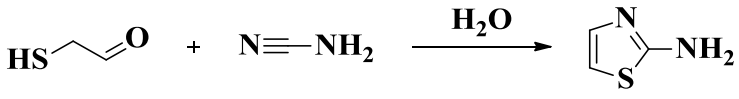


Fig. 3 Thiazoles as important therapeutic agents.

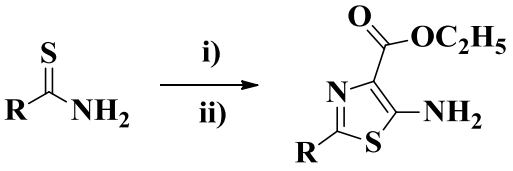
Here, is a brief review of literature for the synthesis of thiazoles.

Powner *et al.* [30] described an efficient synthesis of 2-aminothiazole **3** starting from β-mercapto-acetaldehyde **1** and cyanamide **2** in presence of water under neutral pH condition.



1 **2** **3**

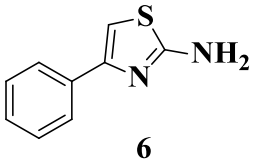
Cheng *et al.* [31] reported synthesis of 2-substituted ethyl 5-amino thiazole-4-carboxylates **5** through Strecker's synthesis. Aliphatic and aromatic thioamides **4** were treated with ethyl glyoxalete to give the 1,2-addition hemiaminal intermediate, which further treated with acetyl chloride in presence of pyridine to give the corresponding imine. The intermediate imine was treated with aqueous sodium cyanide to give the Strecker's amino-thiazoles in good yield with three reaction steps.



4 **5** **R = Ph, Ethyl, isopropyl**

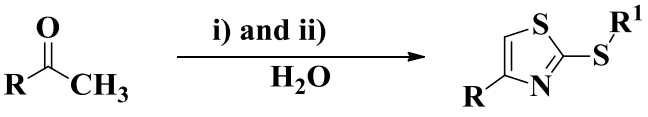
i) = $CHOCOOC_2H_5$ / Py ii) NaCN / CH_3CN -H_2O

Facchinetti *et al.* [32] developed an efficient, simple, fast and eco-friendly synthesis of 2, 4 – substituted thiazoles **6** under solvent free reaction condition with excellent yields. The final product was obtained in a shorter reaction time by easy reaction work up.



6

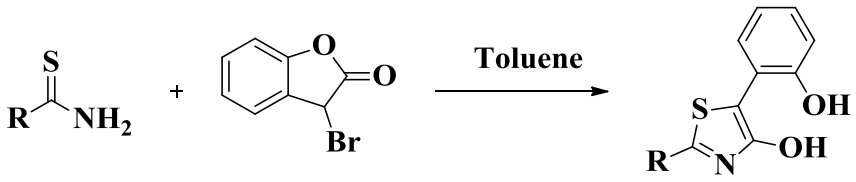
Ziyaei Halimehjani *et al.* [33] developed a simple, efficient one pot two step procedure for the synthesis of 2,4-disubstituted thiazoles **8** starting from acetophenone **7.** Initially acetophenone was converted into phenacyl iodide by the reaction of I_2 / Cuo in presence of ethanol under reflux condition. In the second step the phenacyl iodide was reacted with *S*-alkyldithiocarbamate to give the 2,4-disubstituted thiazole with high yield.



7 **8** **R = Ph, R^1 = Aryl / Alkyl**

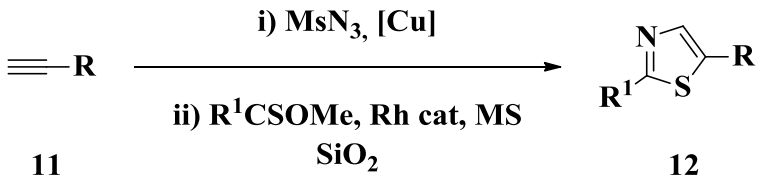
i) = I_2/ CuO / Ethanol ii) = Dithiocarbamate

Kammel *et al.* [34] synthesized 2-aryl-4-hydroxy-5(2'-hydroxyphenyl) thiazoles (**10**) by the reaction of substituted thioamide (**4**) with 3-bromo-1-benzofuran-2(3*H*)-one (**9**) and pyridine in presence of toluene at reflux temperature.



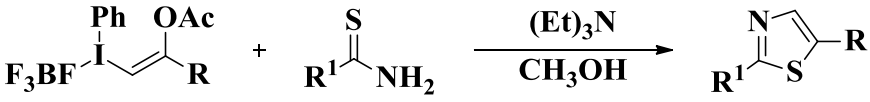
4 **9** **10**

Miura *et al.* [35] developed a sequential one pot synthetic procedure for the synthesis of 2, 5-substituted thiazoles (**12**) starting from terminal alkynes (**11**), sulfonyl azides and thiono esters with excellent yields. The copper (I) catalyzed cycloaddition reaction of alkynes with sulfonyl azides generates 1-sulfonyl triazole, which further reaction with thionoester in presence of rhodium (II) catalyst followed by aromatization gives the target compound.



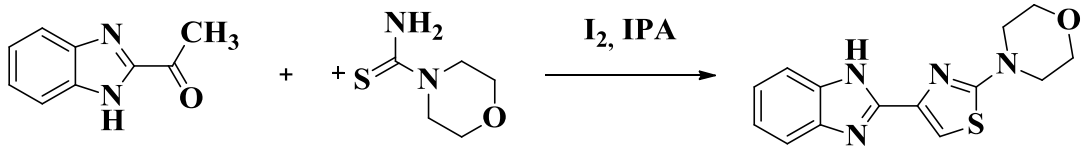
11 **12**

Ochiai *et al*.[36] reported the synthesis of 2,4-disubstituted thiazoles (**15**) by the reaction of (2-acetoxy-1-alkenyl)phenyl-λ^3-iodanes (**13**) with thioamides (**14**) in presence of triethylamine with excellent yields.



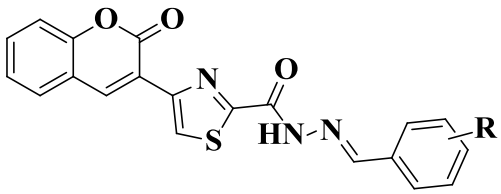
13 **14** **15**

Tayade[37] described the synthesis of 4-4(1*H*-benzo[*d*]imidazol-2-yl) thiazol-2yl) morpholine (**18**) by the reaction of 2-acetyl benzimidazole (**16**) with morpholine-4-carbothioamide (**17**) and iodine in presence of isopropyl alcohol under reflux condition with shorter reaction time and good yields.



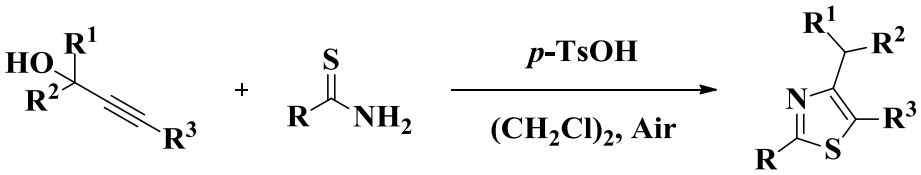
16 17 18

Wang *et al.* [38] synthesized various *N*'-benzylidene-4-(2-oxo-2*H*-chromen-3-yl)thiazole-2-carbohydrazides (**19**) by the reaction of 4-(2-oxo-2*H*-chromen-3-yl)thiazole-2-carbohydrazide with various substituted benzaldehydes in presence of ethanol. The title compounds were obtained in good yields.



19

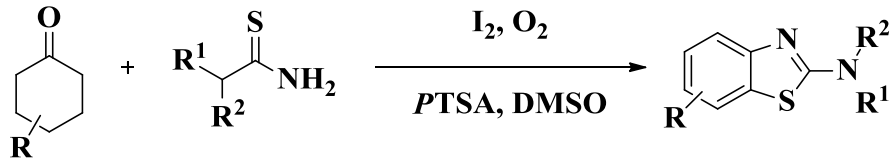
Zang *et al.* [39] developed an efficient synthetic method for the synthesis of di and tri substituted thiazoles (**21**). The target compounds were synthesized by *p*-TsOH catalyzed reaction of trisubstituted propargylic alcohols (**20**) with thioamides (**4**) at high temperatures in good yields.



20 4 21

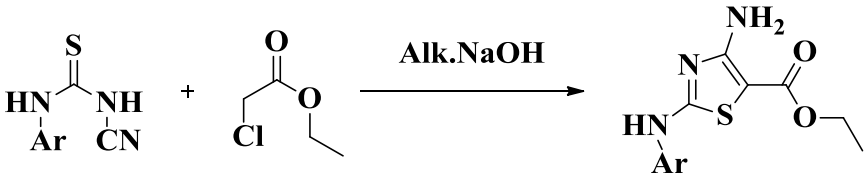
R^1, R^2 = alkyl, aryl, heteroaryl
R^3 = H, alkyl, aryl, heteroaryl
R^4 = alkyl and aryl

Zhao *et al.* [40] developed a method for the synthesis of 2-aminobenzothiazoles (**24**) starting from cyclohexanone (**22**) and substituted thiourea (**23**) through iodine catalyzed oxidative cyclization followed by dehydrogenation. The title compounds were obtained in satisfactory yields.



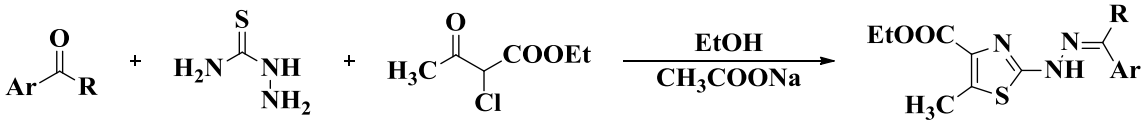
22 23 24

Zhu *et al.* [41] synthesized ethyl 4-amino-2-(phenylamino)thiazole-5-carboxylate (**27**) by the reaction of 25 with ethyl 2-chloroacetate (26) in presence of alkaline NaOH solution at ambient temperature with good yields.



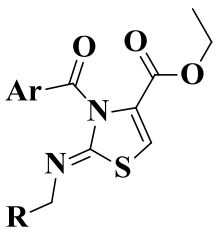
25 26 27

Xiabing *et al.* [42] developed an efficient one pot multicomponent reaction for environmentally benign and clean synthesis of thiazoles (**31**). The thiazole compounds were prepared by sodium acetate catalyzed three component reaction of thiosemicarbazide (**28**) with aldehydes / ketones (**29**) followed by ethyl 2-chloro-3-oxobutanoate (**30**) in presence of ethanol with excellent yields.



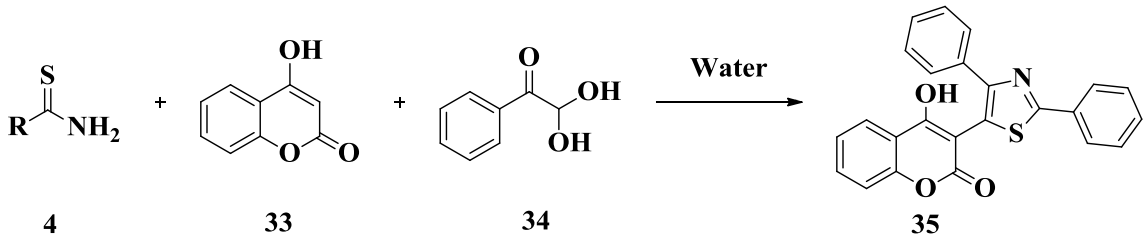
28 29 30 31

Iravani *et al.* [43] reported green, efficient solvent free four component synthesis of *N*-acyl-2-alkylimino-2,3-dihydrothiazole-4-carboxylate (**32**) heterocyclic compounds. The title compounds were obtained from the reaction of acid chloride, ammonium thiocyanate, alkyl amine and ethyl bromopyruvate under mild reaction conditions.



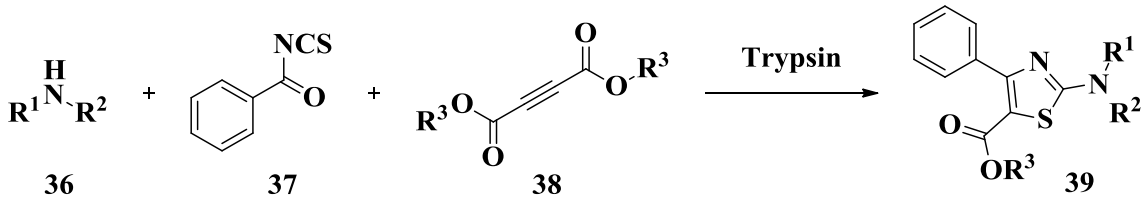
32

Karamthulla *et al.*[44] synthesized trisubstituted thiazoles (**35**) through multicomponent reaction approach. The desired compounds were obtained by the domino reaction of thioamide (**4**), 4-hydroxy coumarin (**33**) and 2,2-dihydroxy-1-phenylethanone (**34**) under green reaction condition with excellent yields.



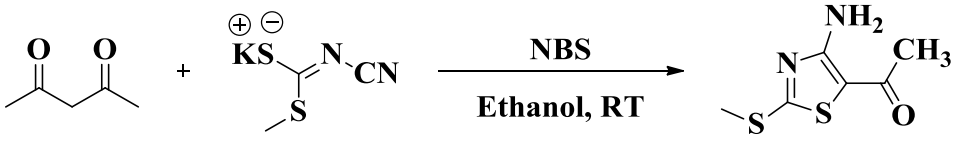
4 **33** **34** **35**

Zheng *et al.*[45] reported an efficient chemo enzymatic synthesis thiazole derivatives via multicomponent reaction approach. The target molecules were synthesized by trypsin catalysed one pot multicomponent reaction of secondary amine (**36**), benzoyl iosthiocyanate (**37**) and dialkyl acetylenedicarboxylate (**38**) with excellent yields.



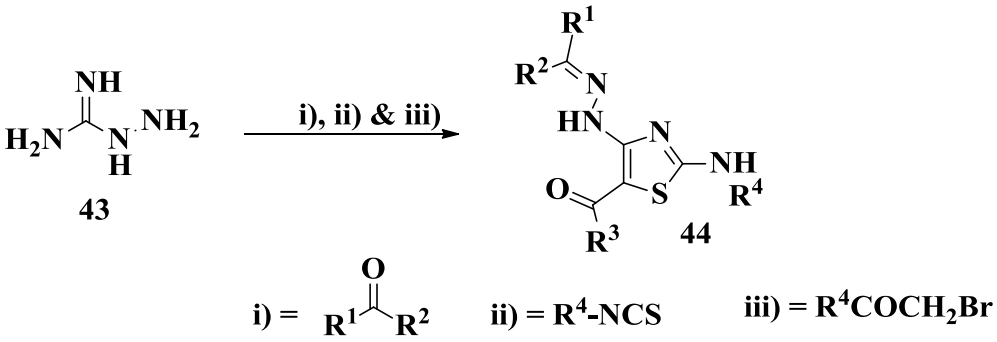
36 **37** **38** **39**

Luo *et al.* [46] developed *N*-bromo succinimide catalysed sequential one pot synthesis of 1-(4-amino-2-(methylthio)thiazol-5-yl)ethanone (**42**). The target compound was synthesized by the reaction of acetylacetone (**40**) and potassium salt of mercaptonitrile (**41**) under mild reaction conditions with good yields.



40 **41** **42**

S. Titus and Sreejaleksmi[47] reported a facile one pot synthesis of 4-hydarzino thiazoles (**44**) via four component method. The target molecules were synthesized via sequential reaction of aminoguanidine (**43**) with carbonyl compounds followed by isothiocyantes and α-halo carbonyl compounds with good yields.



The present chapter deals a facile one pot multicomponent synthesis of 3-(1-(2-(4'-methyl-2'-phenyl-[4,5'-bithiazol]-2-yl) hydrazono) ethyl)-2*H*-chromen-2-one derivatives and their antiviral activity (**Part-A**) and synthesis and antiviral activity of *N*-(3-(2-(1*H*-indol-3-yl)ethyl)-4'-methyl-2'-phenyl-[4,5'-bithiazol]-2(3*H*)-ylidene)aniline derivatives (**Part-B**), a facile one pot pseudo five component reaction of 3,3''-(ethane-1,2-diyl)bis(4'-methyl-2'-phenyl-[4,5'-bithiazole]-3(3*h*)-yl-2(3*h*)-ylidene)) dianiline derivatives and their antiviral activity (**Part-C**).

CHAPTER-VII
PART-A

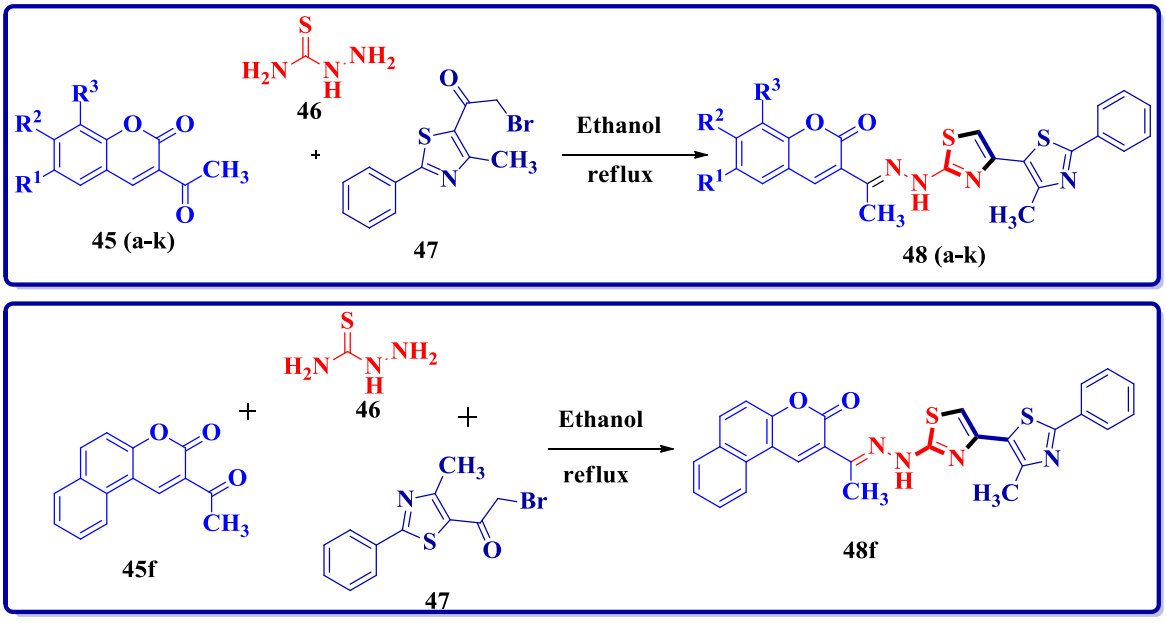
A FACILE ONE POT MULTICOMPONENT SYNTHESIS OF 3-(1-(2-(4'-METHYL-2'-PHENYL-[4,5'-BITHIAZOL]-2-YL) HYDRAZONO)ETHYL)-2*H*-CHROMEN-2-ONE DERIVATIVES AND THEIR ANTIVIRAL ACTIVITY

PART-A

PRESENT WORK:

In view of the biological significance of substituted coumarins and thiazoles, there is a desirable need to develop an efficient, simple reaction method to synthesize these heterocyclic compounds. Based on the literature search, there is no work done on the synthesis of 3-(1-(2-(4'-methyl-2'-phenyl-[4,5'-bithiazol]-2-yl) hydrazono) ethyl)-2*H*-chromen-2-one heterocyclic compounds via an efficient one pot multicomponent reaction method. The present reaction involves in the formation of Hantzsch- thiazole scaffold followed by Schiff's base formation. In the present study we described the synthesis and antiviral activity of 3-(1-(2-(4'-methyl-2'-phenyl-[4,5'-bithiazol]-2-yl) hydrazono) ethyl)-2*H*-chromen-2-one derivatives.

The title compounds were synthesized via one pot multicomponent approach by the reaction of 2-bromo-1-(4-methyl-2-phenylthiazol-5-yl)ethanone with thiosemicarbazide followed by the reaction of various substituted 3-acetylcoumarins in presence of absolute ethanol under reflux condition in a shorter reaction time with excellent yields (**Scheme-1**).



Scheme: 1 A facile one pot synthesis of 3-(1-(2-(4'-methyl-2'-phenyl-[4,5'-bithiazol]-2-yl) hydrazono) ethyl)-2*H*-chromen-2-one derivatives 48 (a-l).

Table.1 A facile one pot synthesis of 3-(1-(2-(4'-methyl-2'-phenyl-[4,5'-bithiazol]-2-yl) hydrazono) ethyl)-2*H*-chromen-2-one derivatives 48 (a-l).

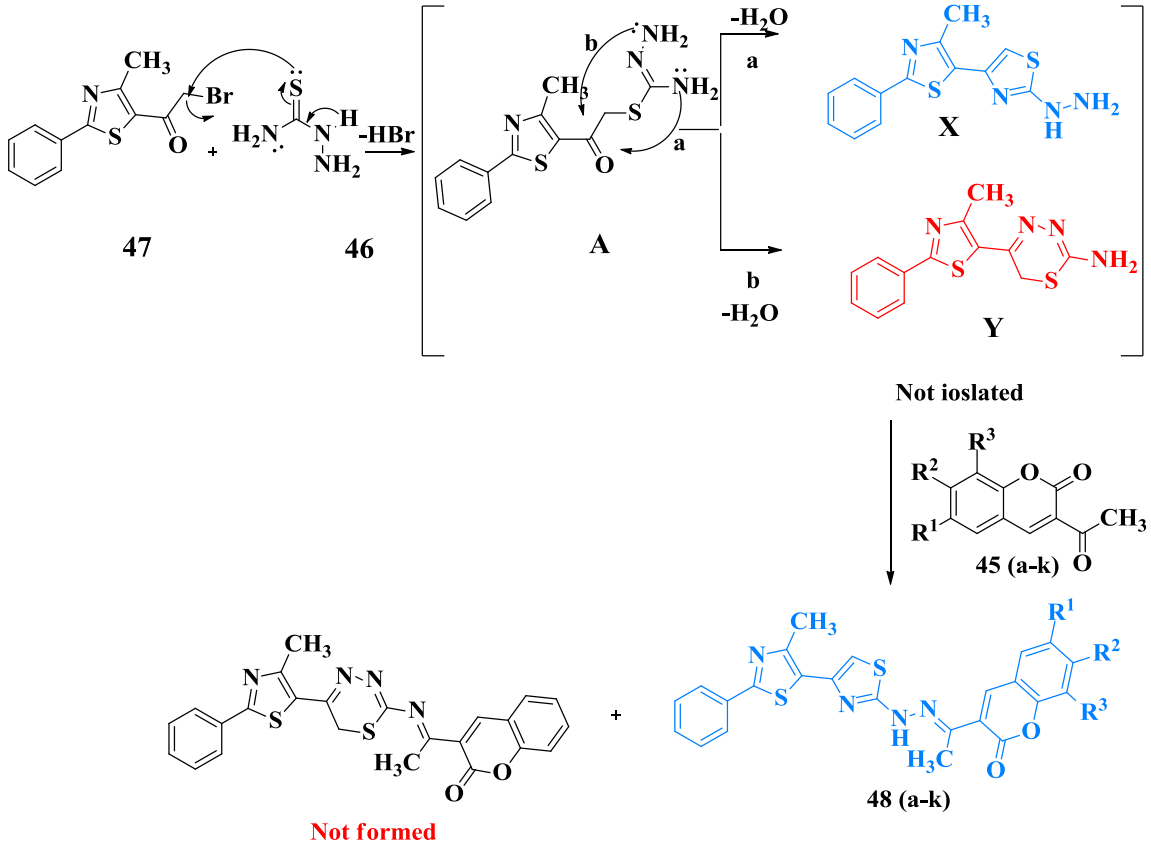
S. No.	Product [a]	R^1	R^2	R^3	Time (h)	Yield (%) [b]	m.p. (°C)
1	**48a**	H	H	H	3	85	202 – 204
2	**48b**	Cl	H	H	3.5	90	186 – 188
3	**48c**	Cl	H	Cl	4	90	174 – 176
4	**48d**	Br	H	H	4	92	230 - 232
5	**48e**	Br	H	Br	4	94	224 – 226
6	**48g**	H	H	OCH_3	3	86	167 – 169
7	**48h**	Br	H	OCH_3	4	90	158 – 160
8	**48i**	H	H	OC_2H_5	4	88	188 – 190
9	**48j**	Br	H	OC_2H_5	4.5	92	175 – 177
10	**48k**	H	OH	-CH3	4	85	210 - 212
11	**48l**	NO_2	H	H	4.5	89	275- 277

[a] Reaction Condition: 2 -bromo-1-(4-methyl-2-phenylthiazol-5-yl)ethanone **47** (1 eq), thiosemicabazide **46** (1 eq) and substituted 3-acetyl coumarins **45a-k** (1 eq) in ethanol at 60 °C,
[b] Isolated yields without recrystallization.

Mechanism:

The plausible reaction mechanism for the synthesis of the target compounds **48(a-l)** has been proposed by the following reaction pathway. In the first step 2 -bromo-1-(4-methyl-2-phenylthiazol-5-yl)ethanone **47** reacts with thiosemicarbazide **46** and forms 2-(4-methyl-2-phenylthiazol-5-yl)-2-oxoethyl carbamohydrazonothioate (**A**). Cyclization of **A** may form two intermediates i.e. 2-hydrazinyl-4'-methyl-2'-phenyl-4,5'-bithiazole **X** via **path a** and 5-(4-methyl-2-phenylthiazol-5-yl)-6H-1,3,4-thiadiazin-2-amine **Y** via **path b**. The intermediate **X** further reacts with substituted 3-acetyl coumarins **45 a-k** and forms exclusively single product (**48a-l**).

The formation of single product was further confirmed by physical and analytical spectral data. The TLC in chloroform: methanol (10:1) mobile phase shows single spot. The IR spectrum of the title compound exhibited characteristic –NH peak at 3155 – 3349 cm^{-1}, the ^1H NMR of the compound shows broad singlet at 11.53 – 11.64 ppm corresponding to –NH proton, the peak at 7.16 – 7.19 ppm corresponds to thiazole-H.



Scheme-2: Plausible synthetic reaction mechanism of 3-(1-(2-(4'-methyl-2'-phenyl-[4,5'-bithiazol]-2-yl) hydrazono) ethyl)-2*H*-chromen-2-one derivatives.

All the synthesized 3-(1-(2-(4'-methyl-2'-phenyl-[4,5'-bithiazol]-2-yl) hydrazono) ethyl)-2*H*-chromen-2-one derivatives were screened against a wide range of human viral strains in different types of cell culture medium as described in the chapter-II (Reference 65-67). The antiviral activity data clearly indicate that the synthesized compounds were selectively inhibiting the influenza viral strains and the compounds were shown poor antiviral activity for remaining viral strains. The compound **48i** is exhibited excellent activity of EC_{50} of 5.06, 1.06, 4.1, 3.23, 8.95 and 5.75 µM against *Influenza A* and *Influenza B* viral strains and is more potent when compared to standard drugs Ribavirin, Amantadine and Rimantadine. The compound **48a** is potent against *Influenza A/H1N1A/Ned/378/05* and *Influenza A/H3N2 A/HK/7/87* with EC_{50} of 2.7, 4.23, 7.66 and 3.83 µM concentration. The antiviral activity data is shown in **Table 2- 6**.

Table. 2 Cytotoxicity and antiviral activity in: MDCK cell cultures

Compound	Cytotoxicity		Antiviral EC_{50}[c]					
	CC_{50}[a]	Minimum cytotoxic concentration[b]	*Influenza A/H1N1 A/Ned/378/05*		*Influenza A/H3N2 A/HK/7/87*		*Influenza B B/Ned/537/05*	
			visual CPE score	MTS	visual CPE score	MTS	visual CPE score	MTS
48a	>100	100	**2.7**	**4.23**	**7.66**	**3.83**	>100	>100
48b	>100	20	>100	>100	>100	>100	>100	>100
48c	>100	≥20	>100	>100	>100	>100	>100	>100
48d	>100	≥20	>100	>100	>100	>100	>100	>100
48e	>100	100	>100	>100	>100	>100	>100	>100
48f	>100	100	>100	>100	>100	>100	>100	>100
48g	24.9	4	>100	>100	>100	>100	>100	>100
48h	46.5	20	>100	>100	>100	>100	>100	>100
48i	>100	100	**5.06**	**1.06**	**4.1**	**3.23**	**8.95**	**5.75**
48j	>100	100	>100	>100	>100	>100	>100	>100
48k	>100	100	>100	>100	>100	>100	>100	>100
48l	>100	100	>100	>100	>100	>100	>100	>100
Zanamivir	>100	>100	0.4	0.1	0.8	0.1	0.5	0.4
Ribavirin	>100	100	8.9	4.4	4.0	1.0	8.9	8.4
Amantadine	>100	>100	100	>100	0.2	0.1	>100	>100
Rimantadine	>200	>200	>200	>200	0.02	0.01	>200	>200

[a]50% Cytotoxic concentration, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay.

[b]Minimum compound concentration that causes a microscopically detectable alteration of normal cell morphology.

[c]50% Effective concentration, or concentration producing 50% inhibition of virus-induced cytopathic effect, as determined by visual scoring of the CPE, or by measuring the cell viability with the colorimetric formazan-based MTS assay.

MDCK cells: Madin Darby canine kidney cells

Table. 3 Cytotoxicity and antiviral activity in: HEL cell cultures

Compound	Minimum cytotoxic concentration[a] (μM)	EC_{50}[b] (μM)					
		Herpes simplex virus-1 (KOS)	*Herpes simplex virus-2 (G)*	*Herpes simplex virus-1 TK-KOS ACVr*	*Vaccinia virus*	*Adeno virus-2*	*Human Coronavirus (229E)*
48a	>100	>100	>100	>100	>100	>100	>100
48b	20	>100	>100	>100	>100	>100	>100
48c	20	>100	>100	>100	>100	>100	>100
48d	20	>100	>100	>100	>100	>100	>100
48e	20	>100	>100	>100	>100	>100	>100
48f	≥20	>100	>100	>100	>100	>100	>100
48g	20	>100	>100	>100	>100	>100	>100
48h	>100	>100	>100	>100	>100	>100	>100
48i	≥20	>100	>100	>100	>100	>100	>100
48j	20	>100	>100	>100	>100	>100	>100
48k	>100	>100	>100	>100	>100	>100	>100
48l	>100	>100	>100	>100	>100	>100	>100
Brivudin	>250	0.008	112	10	10	-	-
Cidofovir	>250	4.5	2	3.4	22	29	-
Acyclovir	>250	0.7	0.2	85	>250	-	-
Ganciclovir	>250	0.16	0.06	4	>100	-	-
Zalcitabine	>250	-	-	-	-	19	-
Alovudine	>250	-	-	-	-	50	-
UDA	>100	-	-	-	-	-	1.8
Ribavirin	>250	-	-	-	-	-	112

[a] Required to cause a microscopically detectable alteration of normal cell morphology.
[b] Required to reduce virus-induced cytopathogenicity by 50 %.

Table. 4 Cytotoxicity and antiviral activity in: HeLa cell cultures

Compound	Minimum cytotoxic concentration[a]	EC50[b]		
		Vesicular stomatitis virus	*Coxsackie virus B4*	*Respiratory syncytial virus*
48a	100	>100	>100	>100
48b	20	>100	>100	>100
48c	20	>100	>100	>100
48d	20	>100	>100	>100
48e	≥20	>100	>100	>100
48f	20	>100	>100	>100
48g	100	>100	>100	>100
48h	100	>100	>100	>100
48i	20	>100	>100	>100
48j	100	>100	>100	>100

48k	100	>100	>100	>100
48l	100	>100	>100	>100
DS-10.000	>100	0.4	6.8	0.4
Ribavirin	>250	25	50	10

[a]Required to cause a microscopically detectable alteration of normal cell morphology.
[b]Required to reduce virus-induced cytopathogenicity by 50 %.

Table. 5 Cytotoxicity and antiviral activity in: Vero cell cultures

Compound	Minimum cytotoxic conc.[a] (µM)	EC_{50}[b] (µM)					
		Para influenza-3 virus	*Reovirus-1*	*Sindbis virus*	*Coxsackie virus B4*	*Punta Toro virus*	*Yellow Fever virus*
48a	≥20	>100	>100	>100	>100	>100	>100
48b	20	>100	>100	>100	>100	>100	>100
48c	20	>100	>100	>100	>100	>100	>100
48d	20	>100	>100	>100	>100	>100	>100
48e	≥20	>100	>100	>100	>100	>100	>100
48f	≥4	>100	>100	>100	>100	>100	>100
48g	≥4	>100	>100	>100	>100	>100	>100
48h	100	>100	>100	>100	>100	>100	>100
48i	20	>100	>100	>100	>100	>100	>100
48j	20	>100	>100	>100	>100	>100	>100
48k	20	>100	>100	>100	>100	>100	>100
48l	≥20	>100	>100	>100	>100	>100	>100
DS.10.000	>100	>100	>100	100	45	2.3	0.8
Ribavirin	>250	146	>250	>250	>250	125	>250
Mycophenolic acid	≥100	0.4	0.5	20	45	4.0	0.8

[a]Required to cause a microscopically detectable alteration of normal cell morphology.
[b]Required to reduce virus-induced cytopathogenicity by 50 %.

Table. 6 Cytotoxicity and antiviral activity in: CRFK cell cultures

Compound	CC_{50} (µM)[a]	EC_{50} (µM)[b]	
		Feline Corona Virus (FIPV)	*Feline Herpes Virus*
48a	55.5	>100	>100
48b	11.4	>100	>100
48c	36.1	>100	>100
48d	35.1	>100	>100
48e	52.1	>100	>100
48f	11.6	>100	>100
48g	32.0	>100	>100

48h	>100	>100	>100
48i	>100	>100	>100
48j	>100	>100	>100
48k	>100	>100	>100
48l	>100	>100	>100
HHA	>100	9.4	2.3
UDA	>100	5.7	2.0
Ganciclovir	>100	>100	3.4

[a]50% Cytotoxic concentration, as determined by measuring the cell viability with the colorimetric formazanb50%
[b]Effective concentration, or concentration producing 50% inhibition of virus-induced cytopathic effect, as
CRFK cells: Crandell-Rees Feline Kidney cells.

From the antiviral activity results, it is evident that the presence of ethoxy functional group at 8th position on coumarin exhibited excellent activity against *Influenza A* and *Influenza B* viral strains. Whereas the presence of electron releasing groups (OCH_3) and electron withdrawing groups (Cl and Bromine) at 6th and 8th position of coumarin did not exhibited any antiviral activity against tested viral strains.

In summary, we have developed an efficient one pot three component reaction method for the synthesis of 3-(1-(2-(4'-methyl-2'-phenyl-[4,5'-bithiazol]-2-yl) hydrazono) ethyl)-2*H*-chromen-2-one derivatives and evaluated for their antiviral activity against different human viral strains. The method applied for the synthesis has several advantages such as shorter reaction time, mild reaction condition, easy workup and higher yields. The antiviral activity data clearly exhibiting that the target compounds were selectively inhibiting the influenza viral strains.

EXPERIMENTAL

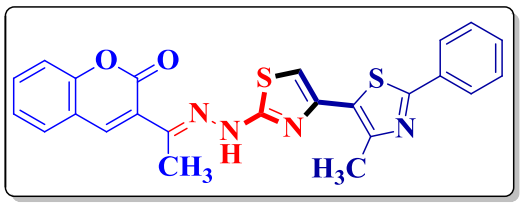
A facile one pot synthesis of 3-(1-(2-(4'-methyl-2'-phenyl-[4,5'-bithiazol]-2-yl) hydrazono) ethyl)-2*H*-chromen-2-one derivatives 48 (a-l):

An equivalent reaction mixture of substituted 3-acetyl coumrins **45a-l** (1.0 mmol), thiosemicarbazide **46** (0.091 g, 1.0 mmol) and 2-bromo-1-(4-methyl-2-phenylthiazol-5-yl)ethanone **47** (0.296 g, 1.0 mmol) were taken in a round bottom flask having absolute ethanol (5 mL) as a solvent. The reaction mixture was stirred at room temperature for about 10 minutes and finally the temperature of the reaction was raised to 60 °C. The temperature of the reaction was maintained for about 3-5 h. After completion of the

reaction (monitored through TLC), the reaction mixture was cooled to room temperature and the isolated product was filtered, washed with ethanol and dried. The product was recrytallized from ethanol solvent.

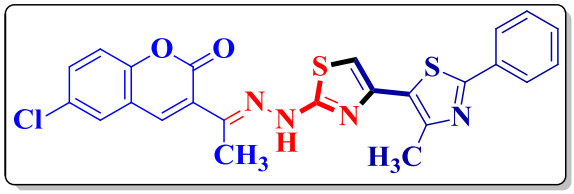
SPECTRAL DATA:

3-(1-(2-(4'-Methyl-2'-phenyl-[4,5'-bithiazol]-2-yl)hydrazono)ethyl)-2*H*-chromen-2-one (48a):



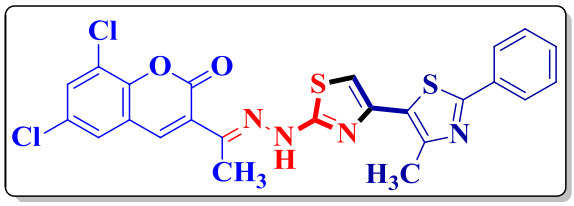
Red solid; **FTIR (KBr, υ_{max}, cm^{-1}):** 3351 (-NH), 1723 (lactone-C=O), 1606 (-C=N); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 2.29 (s, 3H, $-CH_3$), 2.63 (s, 3H, $-CH_3$), 7.16 (s, 1H, Ar-H), 7.28 (d, *J* = 6 Hz, s, 1H, Ar-H), 7.40 – 7.51 (m, 4H, Ar-H), 7.62 – 7.68 (m, 1H, Ar-H), 7.87 (d, *J* = 7.6 Hz, 1H, Ar-H), 7.93 (t, *J* = 6.4 Hz, 2H, Ar-H), 8.18 (s, 1H, C_4-H of coumarin), 11.53 (bs, 1H, -NH); **^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ** 16.28, 17.34, 106.12, 115.96, 118.87, 124.75, 125.82, 125.97, 126.43, 128.01, 129.12, 129.21, 130.08, 132.26, 132.99, 140.81, 142.56, 145.58, 148.65, 152.08, 153.29, 159.11, 162.92, 169.18; **ESI m/z:** 459.0930 $[M+H]^+$; Anal.Calcd for $C_{24}H_{18}N_4O_2S_2$: C, 62.86, H, 3.96, N, 12.22%; Found: C, 62.82, H, 3.99, N, 12.25%.

6-Chloro-3-(1-(2-(4'-methyl-2'-phenyl-[4,5'-bithiazol]-2-yl)hydrazono)ethyl)-2*H*-chromen-2-one (48b):



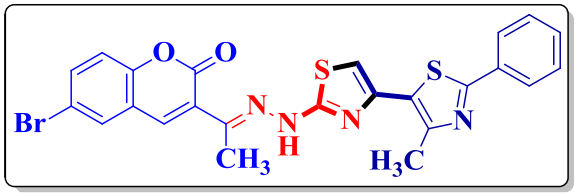
Yellow solid; **FTIR (KBr, υ_{max} cm^{-1}):** 3412 (-NH), 1705 (lactone-C=O), 1603 (-C=N); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 2.28 (s, 3H, $-CH_3$), 2.63 (s, 3H, $-CH_3$), 7.18 (s, 1H, Ar-H), 7.47 – 7.51 (m, 4H, Ar-H), 7.66 – 7.69 (m, 1H, Ar-H), 7.91 – 7.94 (m, 2H, Ar-H), 8.02 (d, *J* = 2.4Hz, 1H, Ar-H), 8.15 (s, 1H, C_4-H of coumarin), 11.57 (bs, 1H, -NH); **^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ** 16.21, 17.36, 106.23, 117.97, 120.35, 125.82, 126.07, 128.09, 128.44, 129.24, 139.51, 145.29, 151.93, 158.71, 162.96, 169.15; **HR-MS (ESI m/z):** Exact Mass calculated for $C_{24}H_{18}ClN_4O_2S_2$ $[M+H]^+$: 493.0560, Found: 493.0555 $[M+H]^+$; Anal. Calcd for $C_{24}H_{17}ClN_4O_2S_2$: C, 58.47, H, 3.48, N, 11.36%; Found: C, 58.51, H, 3.43, N, 11.39%.

6,8-Dichloro-3-(1-(2-(4'-methyl-2'-phenyl-[4,5'-bithiazol]-2-yl)hydrazono)ethyl)-2*H*-chromen-2-one (48c):



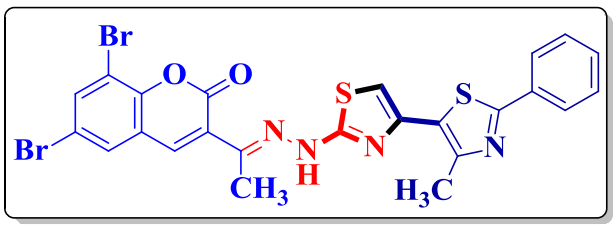
Yellow solid; **FTIR (KBr, υ_{max} cm^{-1}):** 3412 (-NH), 1734 (lactone-C=O), 1603 (-C=N); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 2.28 (s, 3H, -CH_3), 2.63 (s, 3H, -CH_3), 7.18 (s, 1H, Ar-H), 7.47 – 7.52 (m, 2H, Ar-H), 7.66 – 7.68 (m, 1H, Ar-H), 7.71 (s, 1H, Ar-H), 7.93 (d, *J* = 7.6 Hz, 1H, Ar-H), 8.04 (t, *J* = 5.6 Hz, 1H, Ar-H), 8.15 (s, 1H, Ar-H), 8.61 (s, 1H, C_4-H of coumarin), 11.57 (bs, 1H, -NH); Anal. Calcd for $C_{24}H_{16}Cl_2N_4O_2S_2$: C, 54.65, H, 3.06, N, 10.62%; Found: C, 54.60, H, 3.03, N, 10.67%.

6-Bromo-3-(1-(2-(4'-methyl-2'-phenyl-[4,5'-bithiazol]-2-yl)hydrazono)ethyl)-2*H*-chromen-2-one (48d):



Red solid; **FTIR (KBr, υ_{max} cm^{-1}):** 3404 (-NH), 1731 (lactone-C=O), 1602 (-C=N); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 2.27 (s, 3H, -CH_3), 2.63 (s, 3H, -CH_3), 7.18 (s, 1H, Ar-H), 7.41 – 7.49 (m, 1H, Ar-H), 7.51 (d, *J* = 5.6 Hz, 3H, Ar-H), 7.77 – 7.80 (m, 1H, Ar-H), 7.93 (d, *J* = 8 Hz, 2H, Ar-H), 8.15 (s, 2H, Ar-H & C_4-H of coumarin), 11.57 (bs, 1H, -NH); **^{13}C NMR (100 MHz, DMSO-d_6, ppm):** δ 16.21, 17.36, 106.25, 109.01, 116.27, 118.41, 120.86, 126.07, 129.31, 132.53, 136.59, 139.44, 145.64, 152.45, 158.66, 162.92, 166.45; **HR-MS (ESI m/z):** Exact Mass calculated for $C_{24}H_{18}BrN_4O_2S_2$ $[M+H]^+$: 537.0055, Found: 537.0051$[M+H]^+$; Anal. Calcd for $C_{24}H_{17}BrN_4O_2S_2$: C, 53.63, H, 3.19, N, 10.42%; Found: C, 53.66, H, 3.22, N, 10.38%.

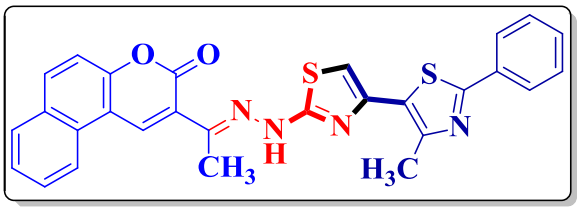
6,8-Dibromo-3-(1-(2-(4'-methyl-2'-phenyl-[4,5'-bithiazol]-2-yl)hydrazono)ethyl)-2*H*-chromen-2-one (48e):



Red solid; **FTIR (KBr, υ_{max} cm^{-1}):** 3340 (-NH), 1738 (lactone-C=O), 1568 (-C=N); **^{1}H NMR (400 MHz,**

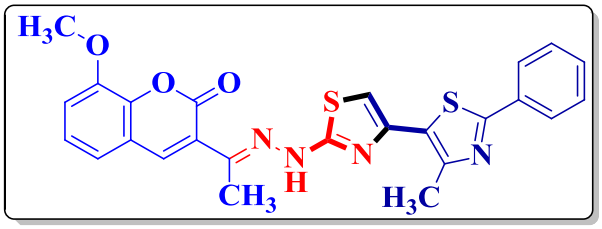
DMSO-d_6, δ ppm): 2.28 (s, 3H, -CH_3), 2.63 (s, 3H, -CH_3), 7.19 (s, 1H, Ar-H), 7.47 – 7.53 (m, 2H, Ar-H), 7.93 (t, *J* = 6.4 Hz, 2H, Ar-H), 8.14 – 8.18 (m, 2H, Ar-H), 8.26 (t, *J* = 2.4 Hz, 1H, Ar-H), 8.58 (s, 1H, C4-H of coumarin), 11.61 (bs, 1H, -NH); **^{13}C NMR (100 MHz, DMSO-d_6, ppm):** δ 16.11, 17.37, 106.30, 116.30, 125.98, 129.32, 132.33, 138.38, 139.05, 142.64, 144.19, 148.77, 151.32, 157.99, 162.93, 166.43; Anal. Calcd for $C_{24}H_{16}Br_2N_4O_2S_2$: C, 46.77, H, 2.62, N, 9.09%; Found: C, 46.72, H, 2.60, N, 9.15%.

2-(1-(2-(4'-Methyl-2'-phenyl-[4,5'-bithiazol]-2-yl)hydrazono)ethyl)-3*H*-benzo[*f*] chromen-3-one (48f):



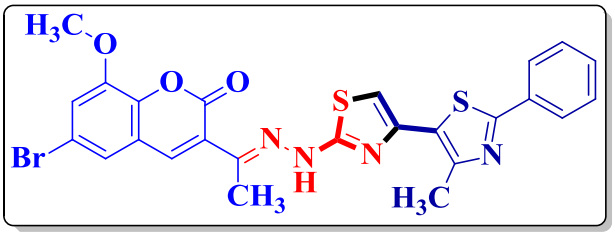
Red solid; **FTIR (KBr, υ_{max} cm^{-1}):** 3406 (-NH), 1715 (lactone-C=O), 1566 (-C=N); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 2.31 (s, 3H, -CH_3), 2.66 (s, 3H, -CH_3), 7.49 (s, 2H, Ar-H), 7.64 (t, *J* = 3.6 Hz, 2H, Ar-H), 7.78 (d, *J* = 8 Hz, 2H, Ar-H), 7.92 (s, 1H, Ar-H), 8.07 – 8.12 (m, 2H, Ar-H), 8.17 – 8.24 (m, 1H, Ar-H), 8.65 – 8.75 (m, 1H, Ar-H), 8.92 (s, 1H, Ar-H), 9.11 (s, 1H, C_4-H of coumarin), 11.63 (bs, 1H, -NH); Anal. Calcd for $C_{28}H_{20}N_4O_2S_2$: C, 66.12, H, 3.96, N, 11.02%; Found: C, 66.16, H, 3.90; N, 11.05%.

8-Methoxy-3-(1-(2-(4'-methyl-2'-phenyl-[4,5'-bithiazol]-2-yl)hydrazono)ethyl)-2*H*-chromen-2-one (48g):



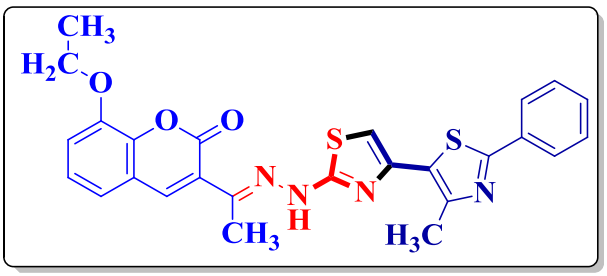
Yellow solid; **FTIR (KBr, υ_{max} cm^{-1}):** 3399 (-NH), 1713 (lactone-C=O), 1608 (-C=N), 1275 (-O-CH_3); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 2.28 (s, 3H, -CH_3), 2.63 (s, 3H, -CH_3), 3.93 (s, 3H, -OCH_3), 7.16 (s, 1H, Ar-H), 7.32 (t, *J* = 4 Hz, 2H, Ar-H), 7.40 – 7.42 (m, 1H, Ar-H), 7.47 – 7.52 (m, 3H, Ar-H), 7.91 – 7.94 (m, 2H, Ar-H), 8.14 (s, 1H, C_4-H of coumarin), 11.54 (bs, 1H, -NH); **^{13}C NMR (100 MHz, DMSO-d_6, ppm):** δ 16.24, 17.33, 56.12, 106.12, 114.48, 119.42, 120.22, 124.68, 125.81, 126.04, 126.52, 127.99, 129.21, 130.08, 132.97, 141.00, 141.30, 142.54, 145.52, 146.27, 148.67, 152.05, 158.82, 162.91, 169.16; Anal. Calcd for $C_{25}H_{20}N_4O_3S_2$: C, 61.46, H, 4.13, N, 11.47%; Found: C, 61.42, H, 4.16, N, 11.50%.

6-Bromo-8-methoxy-3-(1-(2-(4'-methyl-2'-phenyl-[4,5'-bithiazol]-2-yl)hydrazono) ethyl)-2*H*-chromen-2-one (48h):



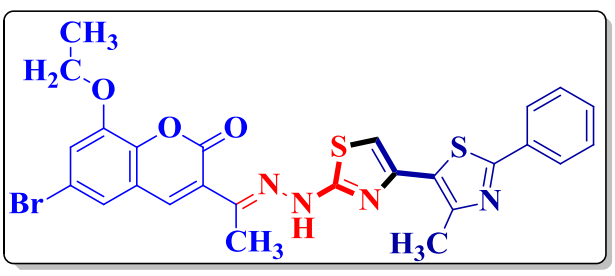
Red solid; **FTIR (KBr, υ_{max} cm^{-1}):** 3081 (-NH), 1721 (lactone-C=O), 1604 (-C=N), 1236 (-OCH_3); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 2.27 (s, 3H, -CH_3), 2.63 (s, 3H, -CH_3), 3.95 (s, 3H, -OCH_3), 7.18 (s, 1H, Ar-H), 7.48 – 7.51 (s, 4H, Ar-H), 7.70 (d, *J* = 2 Hz, 1H, Ar-H), 7.91 – 7.94 (m, 2H, Ar-H), 8.11 (s, 1H, C_4-H of coumarin), 11.57 (bs, 1H, -NH); Anal. Calcd for $C_{25}H_{19}BrN_4O_3S_2$: C, 52.91, H, 3.37, N, 9.87%; Found: C, 52.95, H, 3.35, N, 9.85%.

8-Ethoxy-3-(1-(2-(4'-methyl-2'-phenyl-[4,5'-bithiazol]-2-yl)hydrazono)ethyl)-2*H*-chromen-2-one (48i):



Yellow solid; **FTIR (KBr, υ_{max} cm^{-1}):** 3404 (-NH), 1718 (lactone-C=O), 1607 (-C=N), 1278 (-OCH_2); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 1.41 (t, *J* = 6.8 Hz, 3H, -CH_2CH_3), 2.28 (s, 3H, -CH_3), 2.63 (s, 3H, -CH_3), 4.17 – 4.22 (m, 2H, -OCH_2), 7.16 (s, 1H, Ar-H), 7.31 (t, *J* = 6.8 Hz, 2H, Ar-H), 7.39 – 7.41 (m, 1H, Ar-H), 7.49 (t, *J* = 5.6 Hz, 3H, Ar-H), 7.93 (t, *J* = 6 Hz, 2H, Ar-H), 8.14 (s, 1H, C_4-H of coumarin), 11.54 (bs, 1H, -NH); **^{13}C NMR (100 MHz, DMSO-d_6, ppm):** δ 14.59, 16.27, 17.35, 64.42, 106.12, 115.37, 119.53, 120.29, 124.69, 125.82, 126.51, 129.23, 130.09, 132.99, 141.06, 145.50, 158.91, 162.90, 169.17; Anal. Calcd for $C_{26}H_{22}N_4O_3S_2$: C, 62.13, H, 4.41, N, 11.15%; Found: C, 62.15, H, 4.45, N, 11.10%.

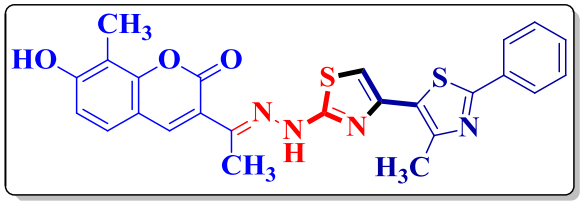
6-Bromo-8-ethoxy-3-(1-(2-(4'-methyl-2'-phenyl-[4,5'-bithiazol]-2-yl)hydrazono) ethyl)-2*H*-chromen-2-one (48j):



Red solid; **FTIR (KBr, υ_{max} cm^{-1}):** 3342 (-NH), 1713 (Lactone-C=O), 1604 (-C=N), 1265 (-C-O-C); **^{1}H NMR (400 MHz, DMSO-d_6, δ**

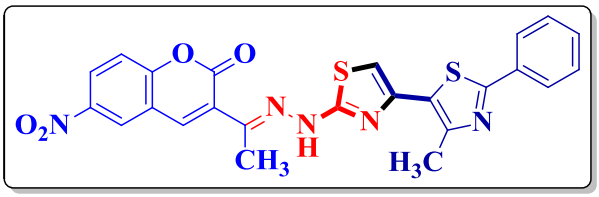
ppm): 1.40 (t, *J* = 6.8 Hz, 3H, -CH_2CH_3), 2.27 (s, 3H, -CH_3), 2.63 (s, 3H, -CH_3), 4.19 – 4.24 (m, 2H, -OCH_2), 7.18 (s, 1H, Ar-H), 7.46 – 7.50 (m, 4H, Ar-H), 7.69 (d, *J* = 2 Hz, 1H, Ar-H), 7.91 – 7.94 (m, 2H, Ar-H), 8.10 (s, 1H, C_4-H of coumarin), 11.57 (bs, 1H, -NH); Anal. Calcd for $C_{26}H_{21}BrN_4O_3S_2$: C, 53.70, H, 3.64, N, 9.63%; Found: C, 53.67, H, 3.69, N, 9.60%.

7-Hydroxy-8-methyl-3-(1-(2-(4'-methyl-2'-phenyl-[4,5'-bithiazol]-2-yl) hydrazono) ethyl)-2*H*-chromen-2-one (48k):



Yellow solid; **FTIR (KBr, υ_{max} cm^{-1}):** 3183 (-OH), 1676 (lactone-C=O), 1605 (-C=N); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 2.18 (s, 3H, Ar-CH_3), 2.27 (s, 3H, -CH_3), 2.63 (s, 3H, -CH_3), 6.89 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.13 (s, 1H, Ar-H), 7.49 – 7.54 (m, 4H, Ar-H), 7.93 (d, *J* = 8 Hz, 2H, Ar-H), 8.04 (s, 1H, C_4-H of coumarin), 10.65 (bs, 1H, -OH), 11.43 (bs, 1H, -NH); **^{13}C NMR (100 MHz, DMSO-d_6, ppm):** δ 7.85, 16.37, 17.37, 105.91, 110.44, 111.34, 112.58, 125.81, 127.35, 129.32, 141.89, 148.69, 153.36, 159.62, 162.86, 169.31; **MS (ESI m/z):** 488.98 $[M+H]^+$; Anal. Calcd for $C_{25}H_{20}N_4O_3S_2$: C, 61.46, H, 4.13, N, 11.47%; Found: C, 61.49, H, 4.08, N, 11.51%.

3-(1-(2-(4'-Methyl-2'-phenyl-[4,5'-bithiazol]-2-yl)hydrazono)ethyl)-6-nitro-2*H*-chromen-2-one (48l)



Red solid; **IR (KBr, υ_{max} cm^{-1}):** 3342 (-NH), 1713 (Lactone-C=O), 1604 (-C=N), 1357 (-N-O); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 2.29 (s, 3H, -CH_3), 2.63 (s, 3H, -CH_3), 7.20 (s, 1H, Ar-H), 7.46 – 7.51 (m, 4H, Ar-H), 7.66 (d, *J* = 9.2 Hz, 1H, Ar-H), 7.93 (t, *J* = 6 Hz, 1H, Ar-H), 8.38 (s, 1H, Ar-H), 8.42 – 8.45 (m, 1H, Ar-H), 8.91 (s, 1H, C_4-H of coumarin), 11.63 (bs, 1H, -NH); Anal. Calcd for $C_{24}H_{17}N_5O_4S_2$: C, 57.24, H, 3.40, N, 13.91%; Found: C, 57.20, H, 3.45, N, 13.93%.

Antiviral activity experiment of 3-(1-(2-(4'-methyl-2'-phenyl-[4,5'-bithiazol]-2-yl) hydrazono) ethyl)-2*H*-chromen-2-one derivatives 48 (a-l):

The antiviral activity of the newly synthesized compounds was determined in different cell types as described in chapter –II (Reference 65 - 67). (i) HEL cell culture was used for viruses (*Herpes simplex virus type 1, Herpes simplex virus type 2, Herpes simplex virus type 1 TK- ACV*^{r}, *Vaccinia virus, Adeno virus-2* or *Vesicular stomatitis virus*), (ii) Vero cells were used for the virus (*Coxsackie virus B4, Sindbis virus, Parainfluenza virus 3, Punta Toro virus* or *Reovirus*), (iii) HeLa cells were used for the virus (*Coxsackie virus B4, Vesicular stomatitis virus* or *Respiratory syncytial virus*), (iv) MDCK cells were used for the virus (*Influenza A/H1N1 A/Ned/378/05, Influenza A/H3N2 A/HK/7/87* or *Influenza B B/Ned/537/05*) (v) CRFK cells for the virus (*Feline herpes virus* or *Feline corona virus*). After incubation at 37°C the virus-induced cytopathicity was determined by measuring the cell viability with the colorimetric formazan-based MTS assay.

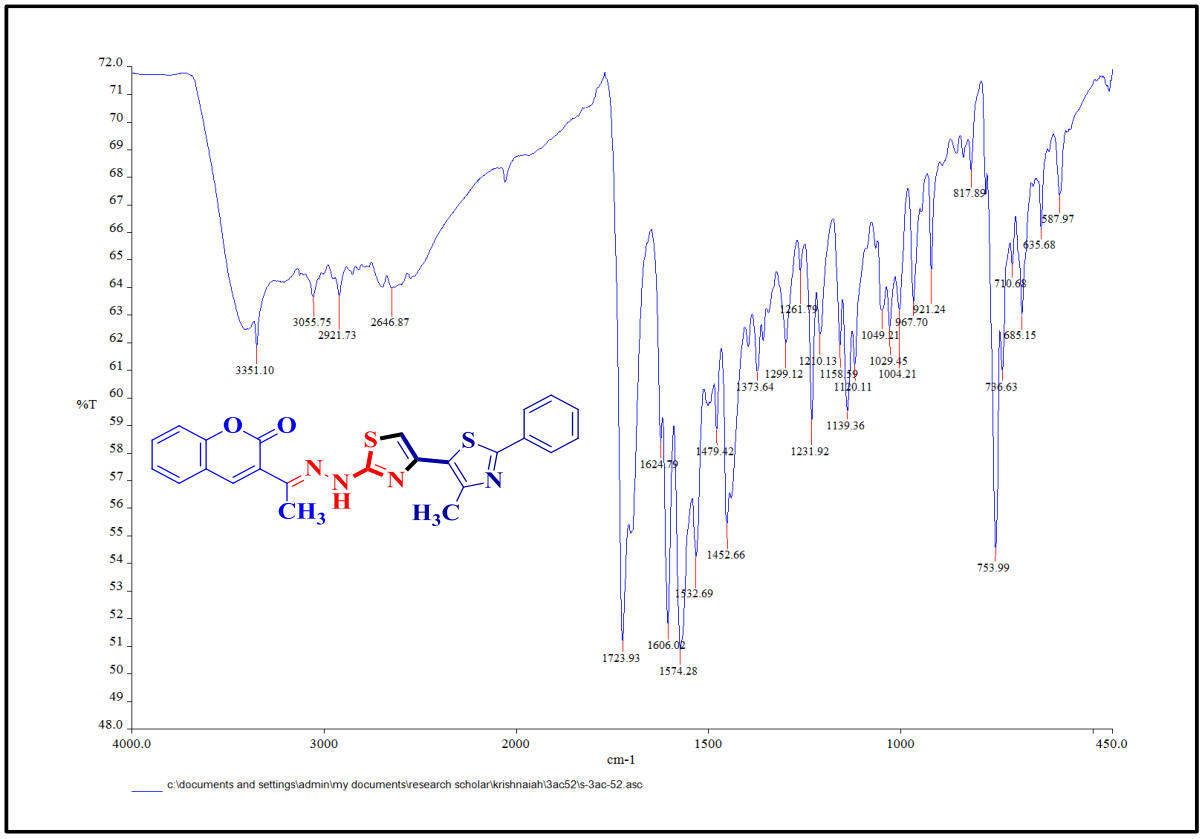


Fig: IR Spectrum of compound **48a**

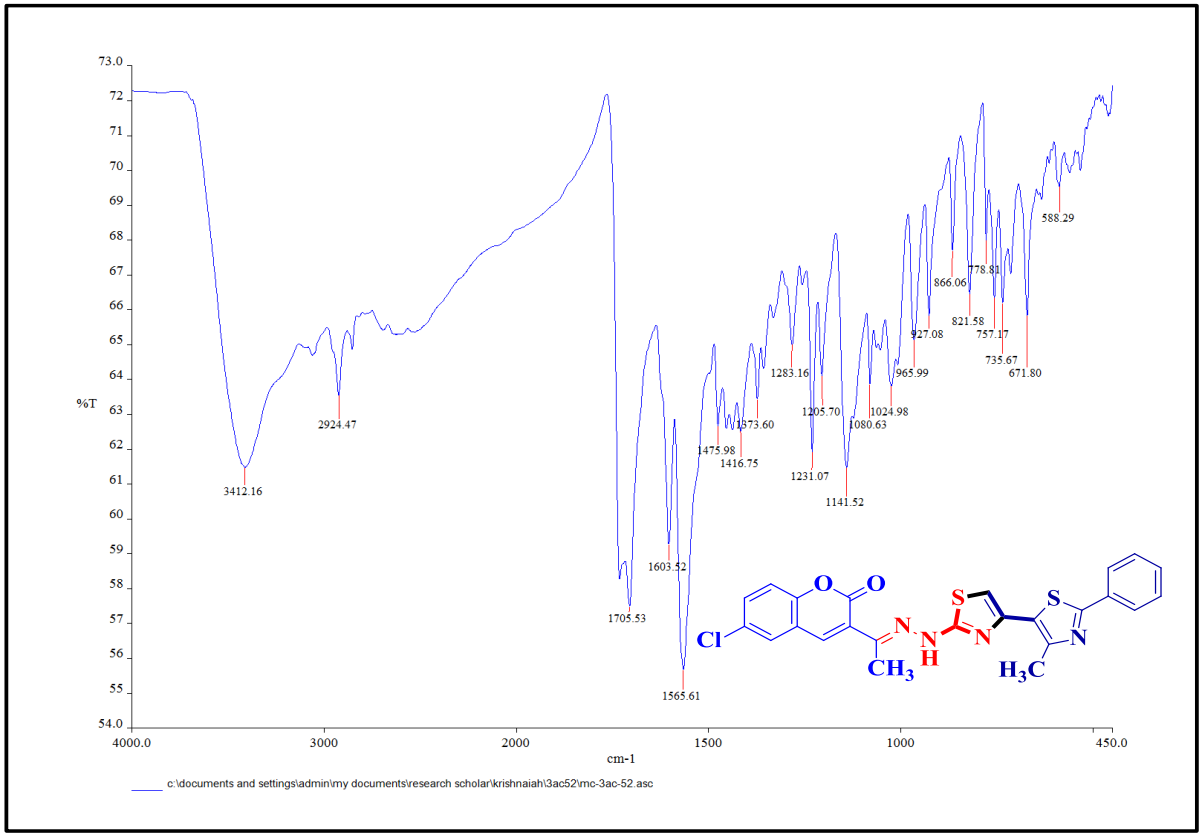


Fig: IR Spectrum of compound **48b**

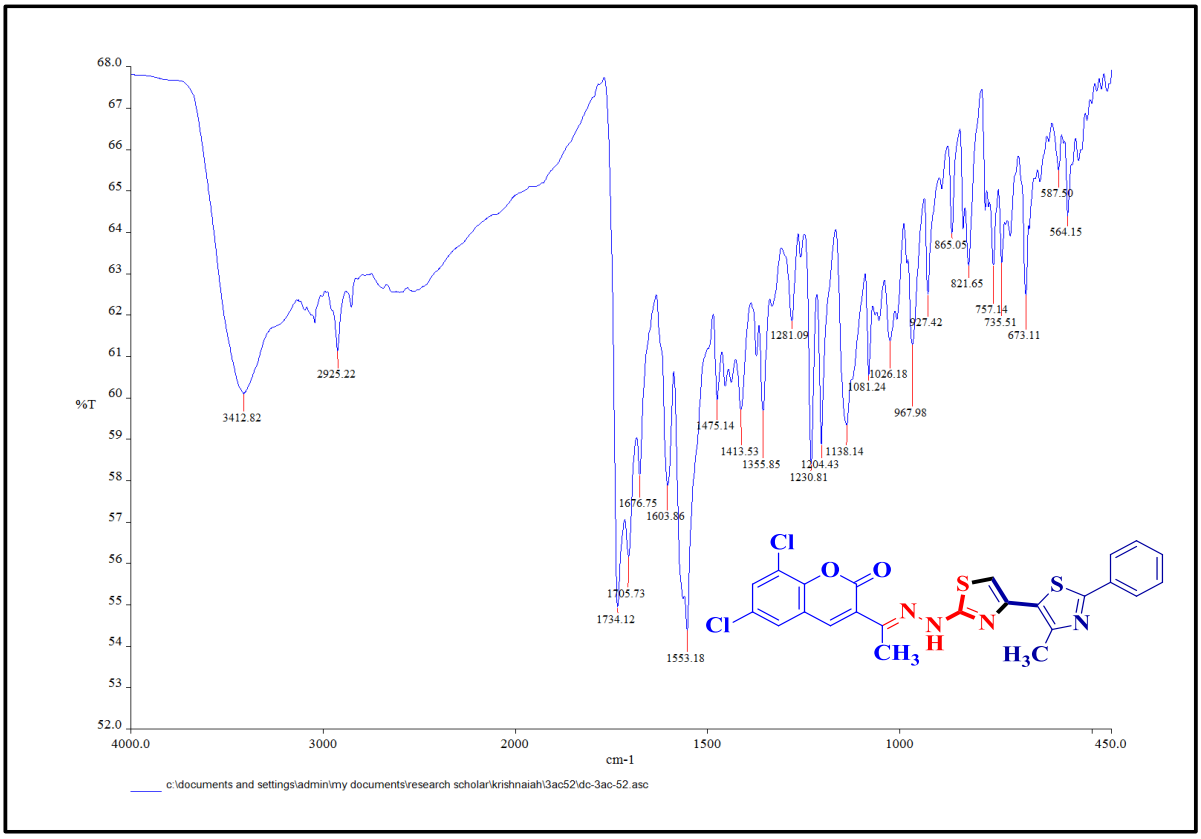


Fig: IR Spectrum of compound **48c**

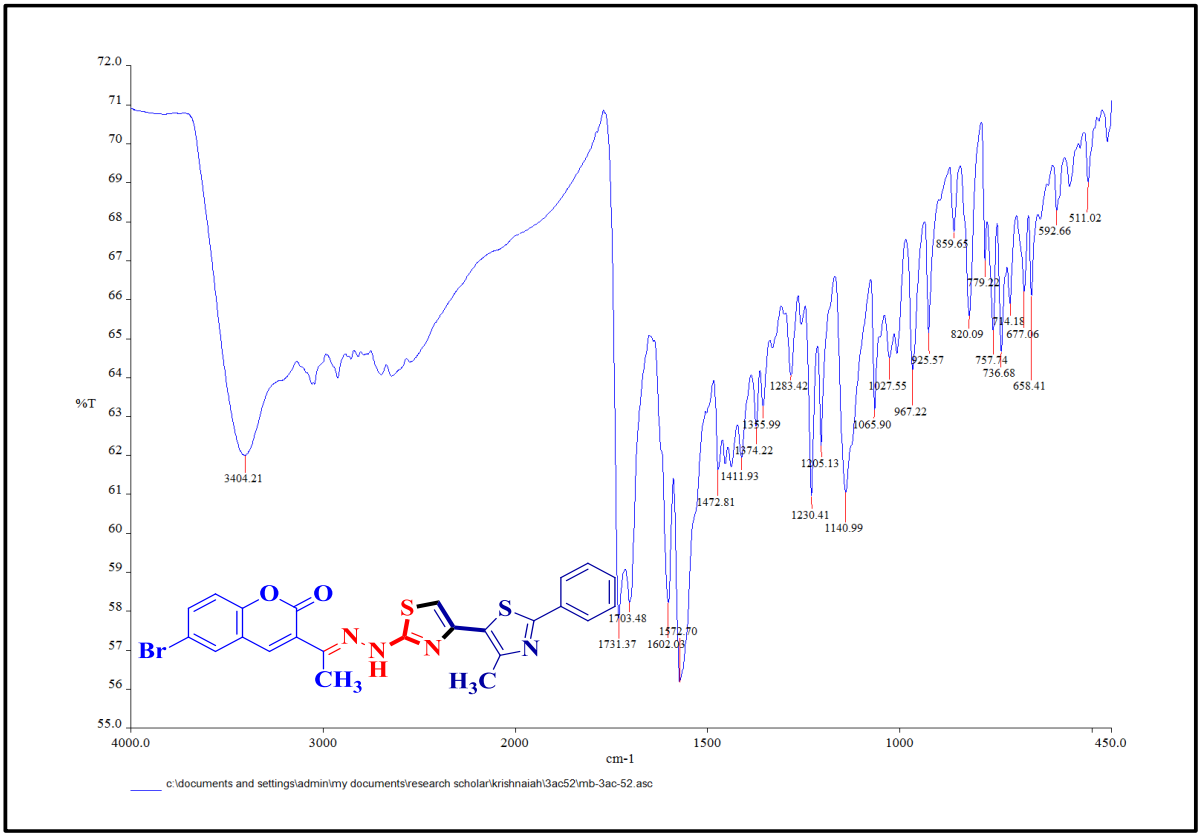


Fig: IR Spectrum of compound **48d**

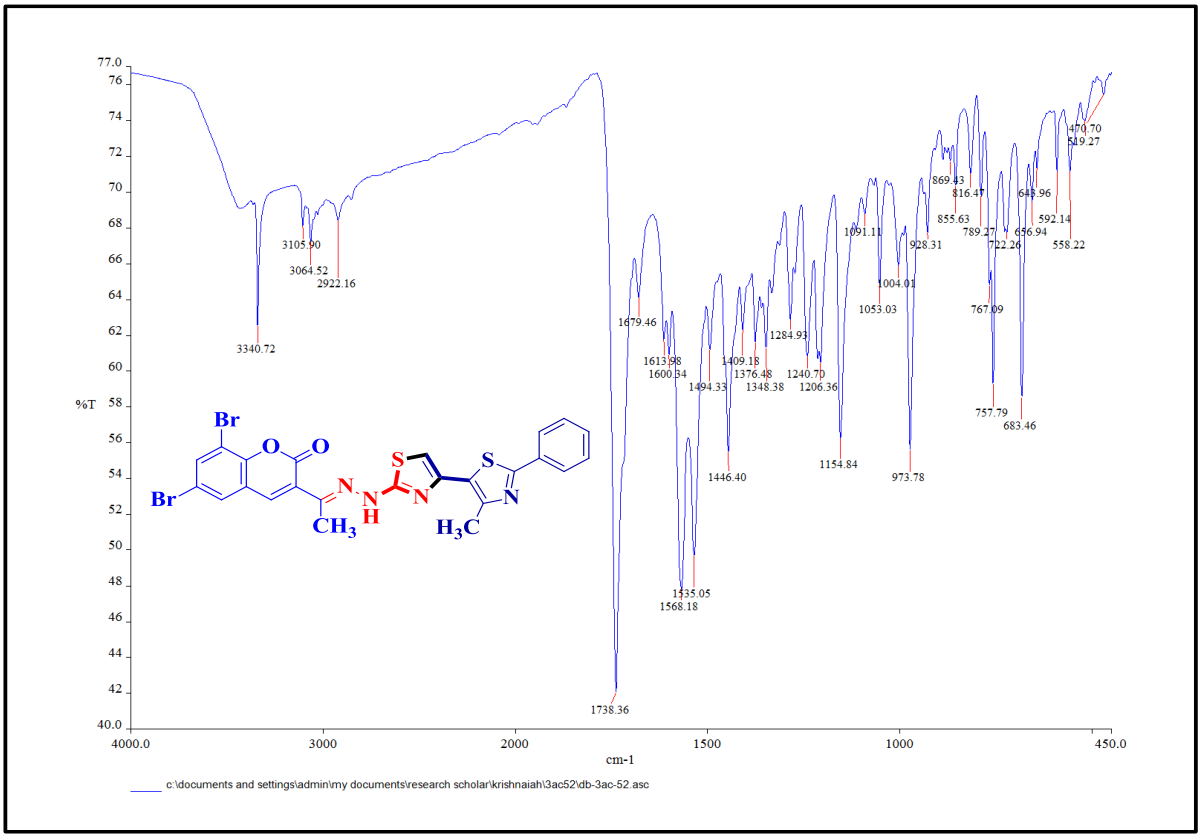


Fig: IR Spectrum of compound **48e**

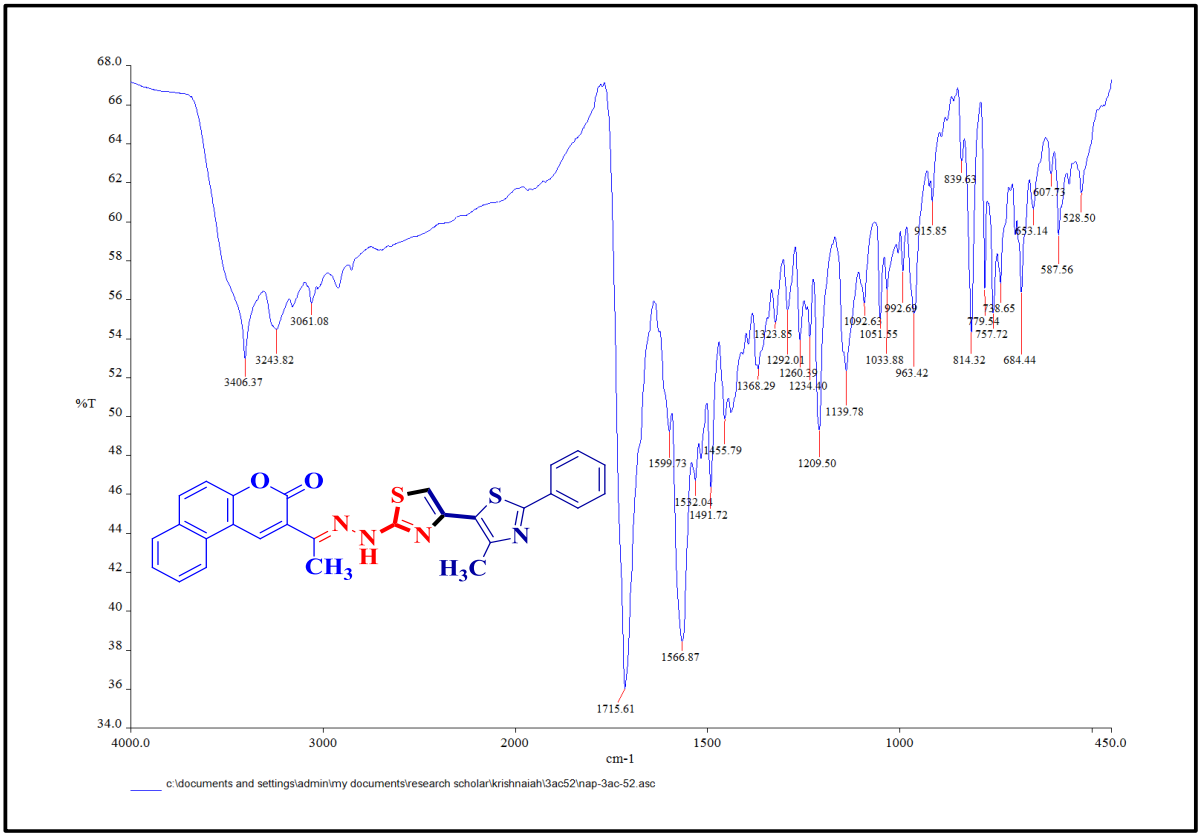


Fig: IR Spectrum of compound **48f**

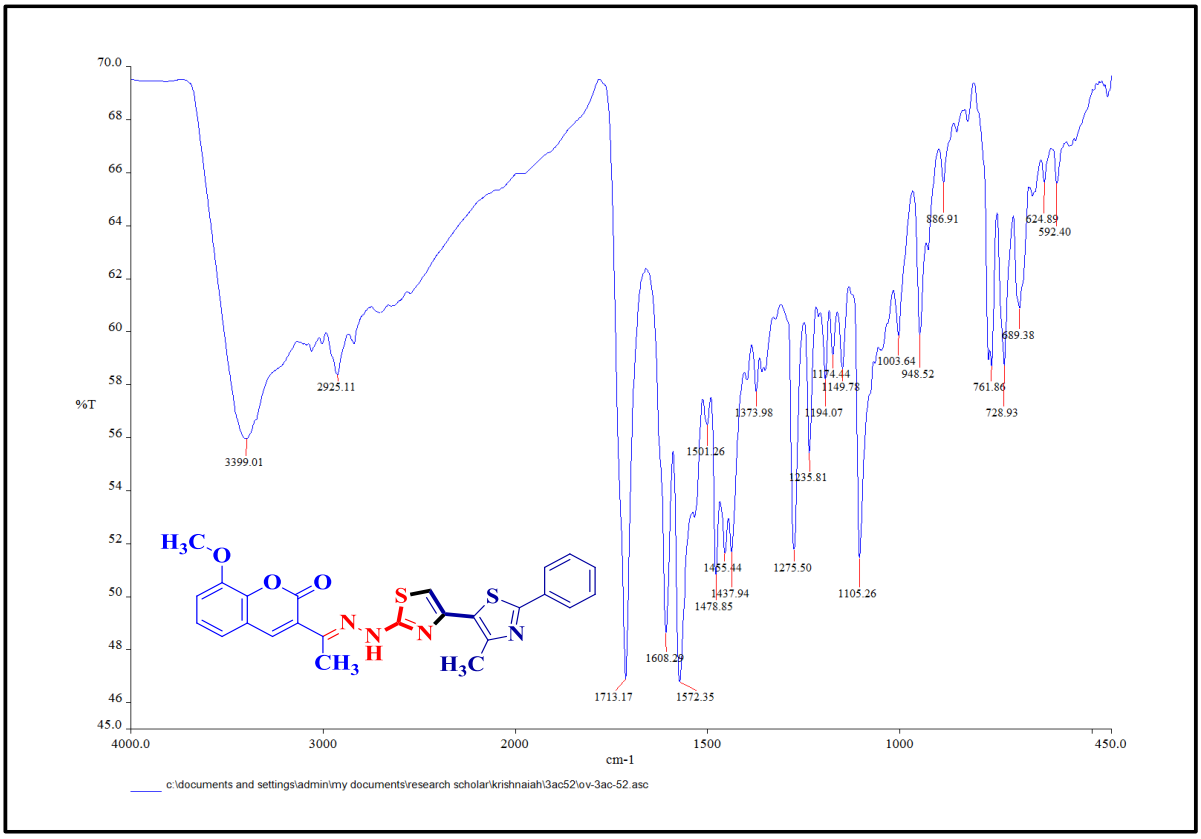


Fig: IR Spectrum of compound **48g**

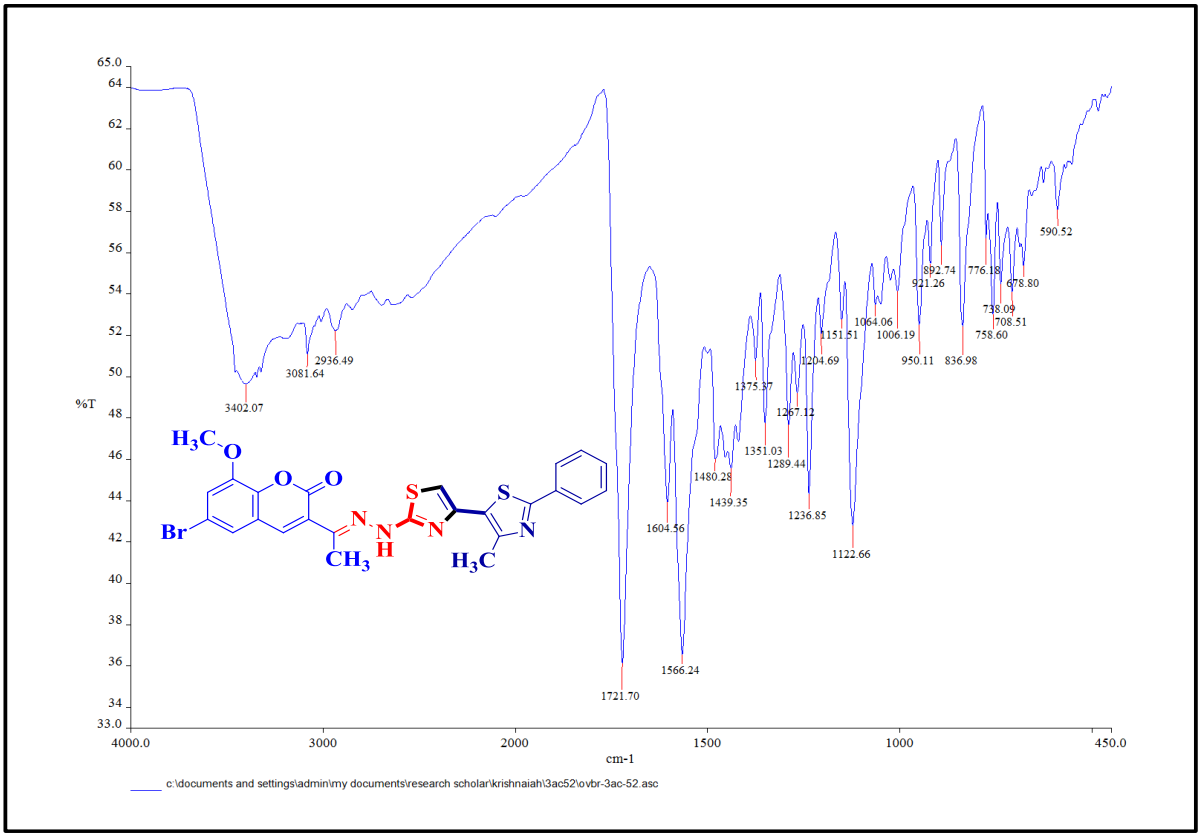


Fig: IR Spectrum of compound **48h**

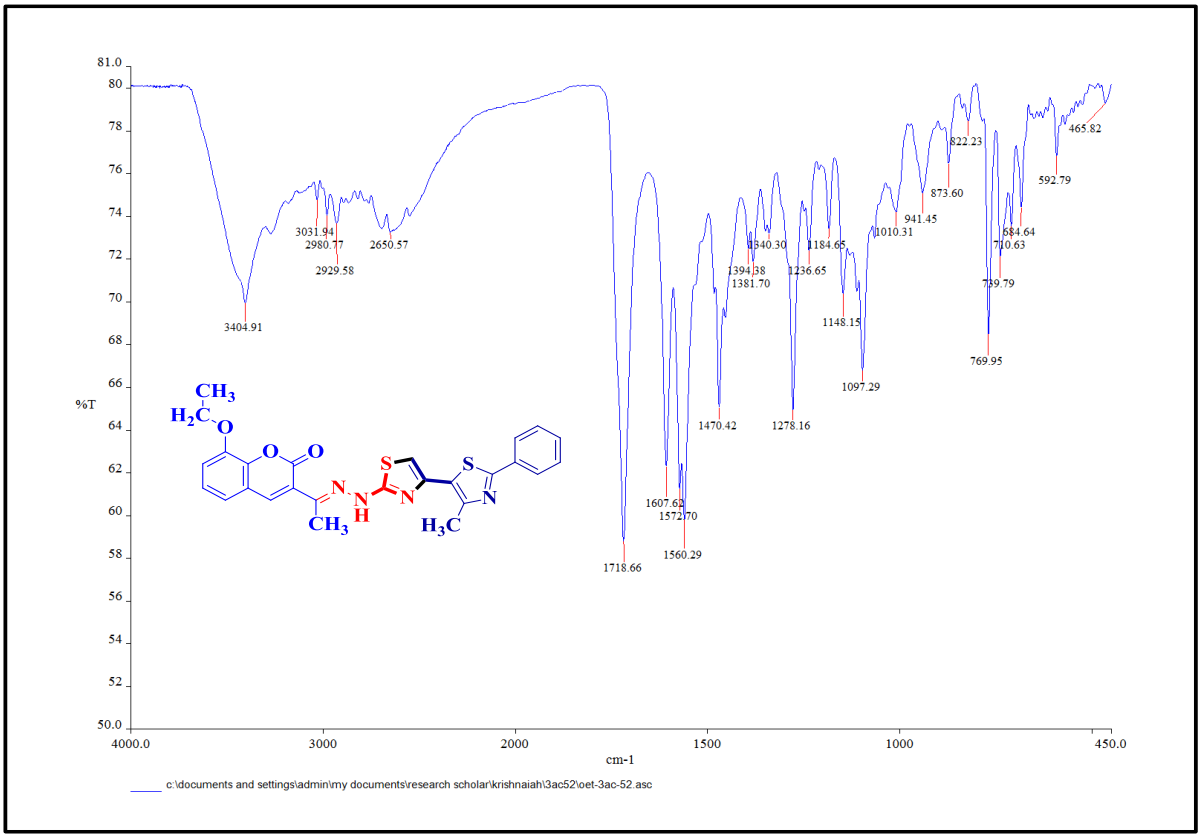


Fig: IR Spectrum of compound **48i**

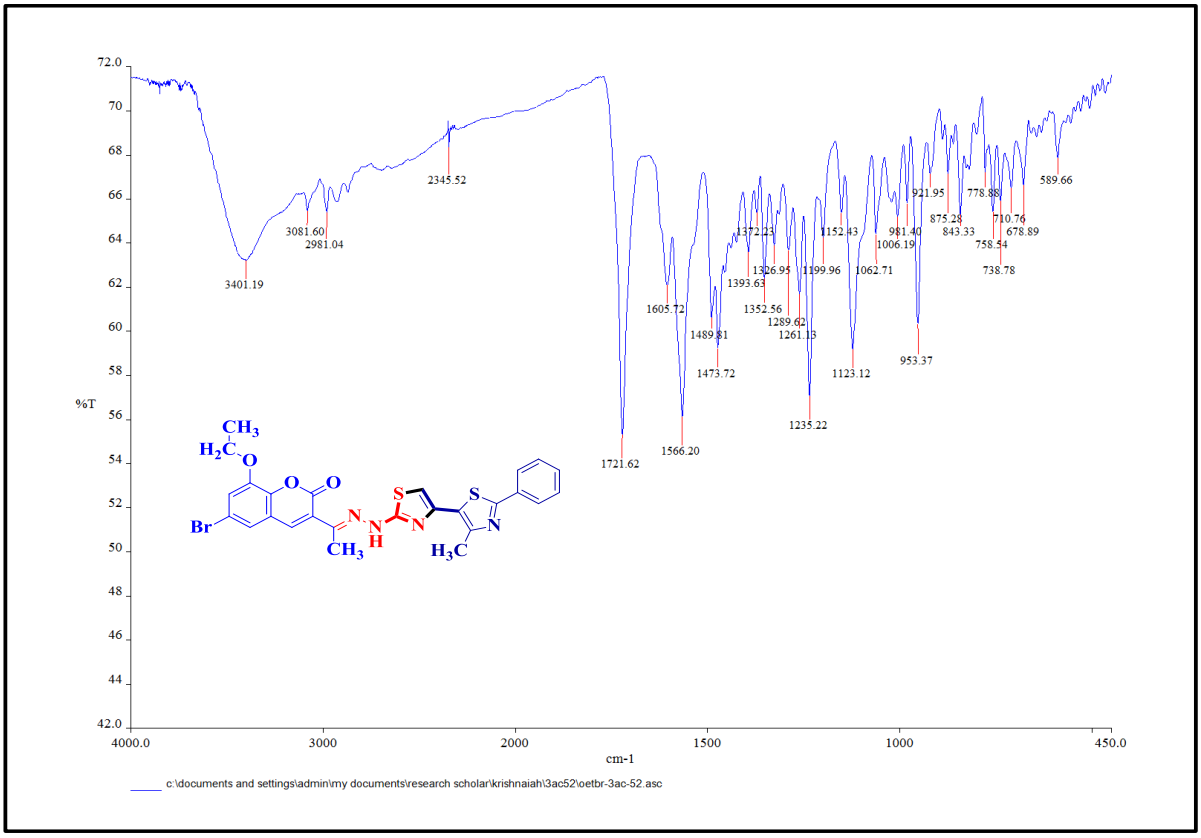


Fig: IR Spectrum of compound **48j**

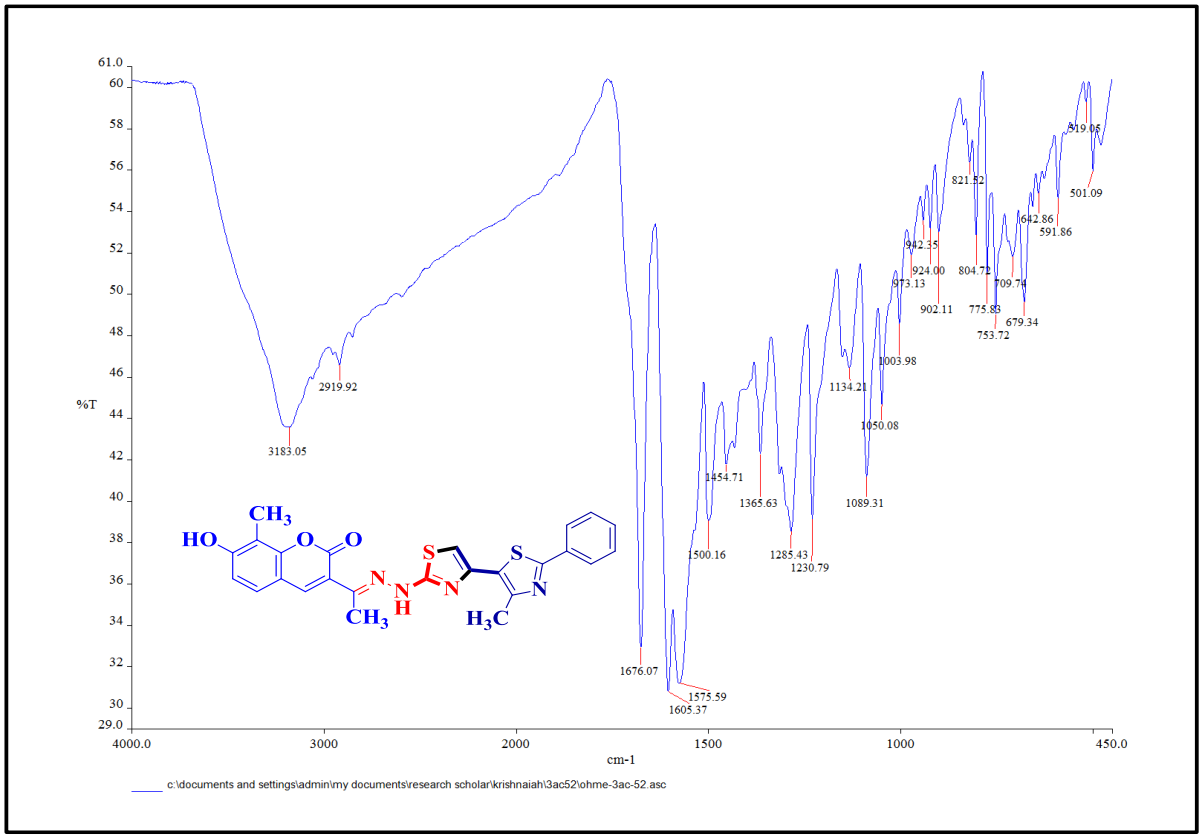


Fig: IR Spectrum of compound **48k**

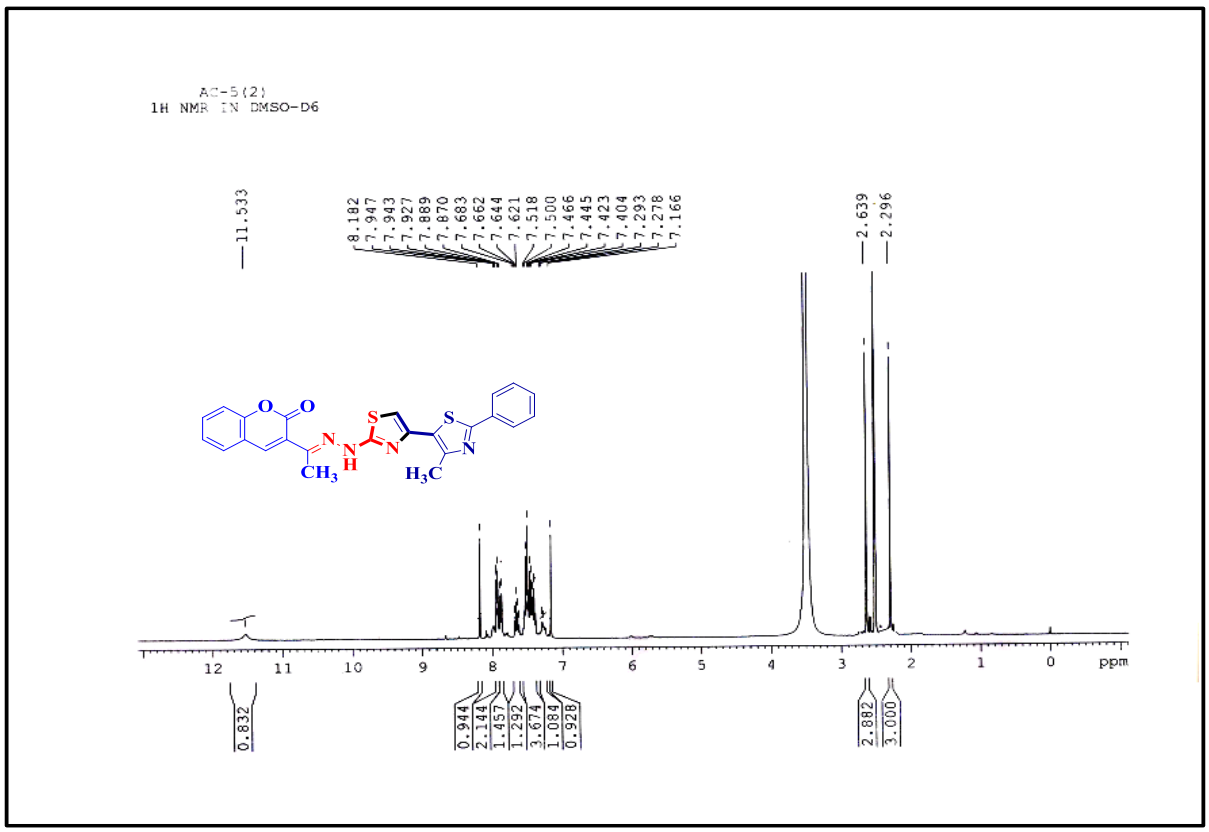


Fig: ^{1}H NMR Spectrum of compound **48a**

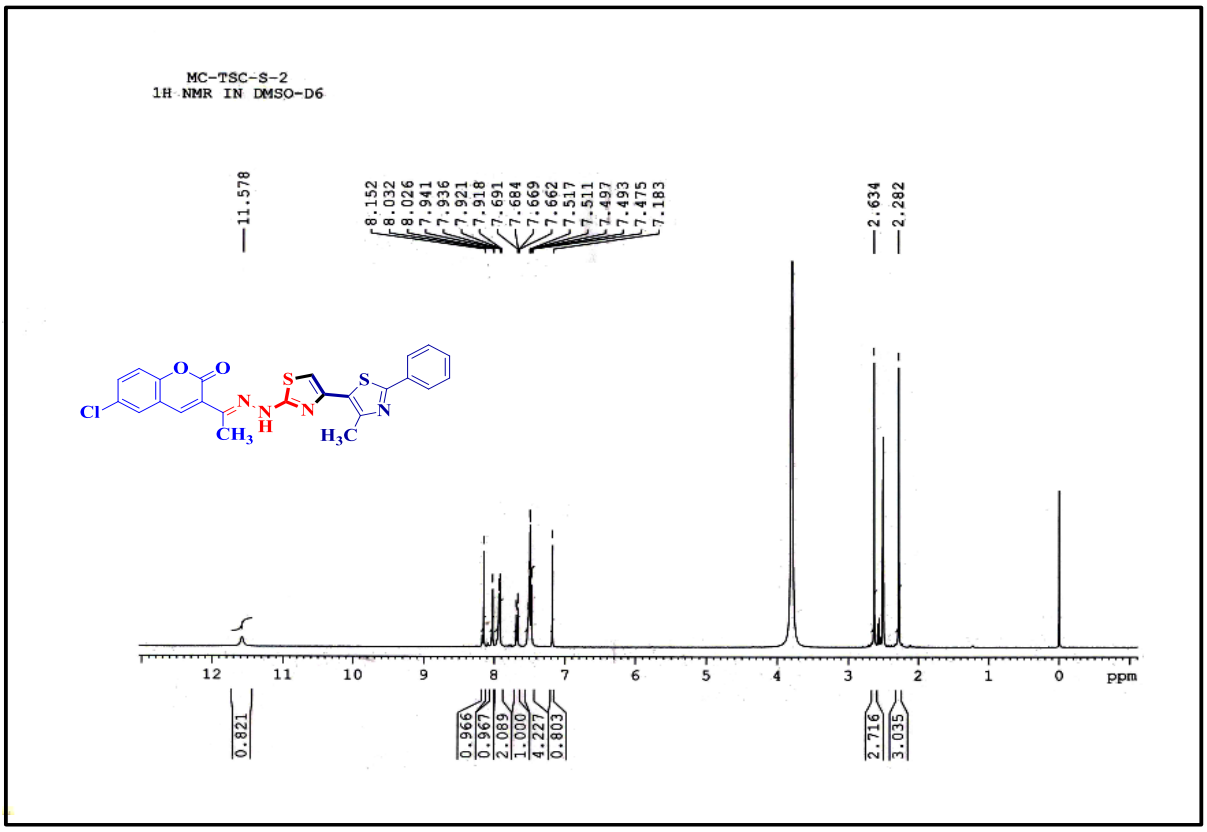


Fig: ^{1}H NMR Spectrum of compound **48b**

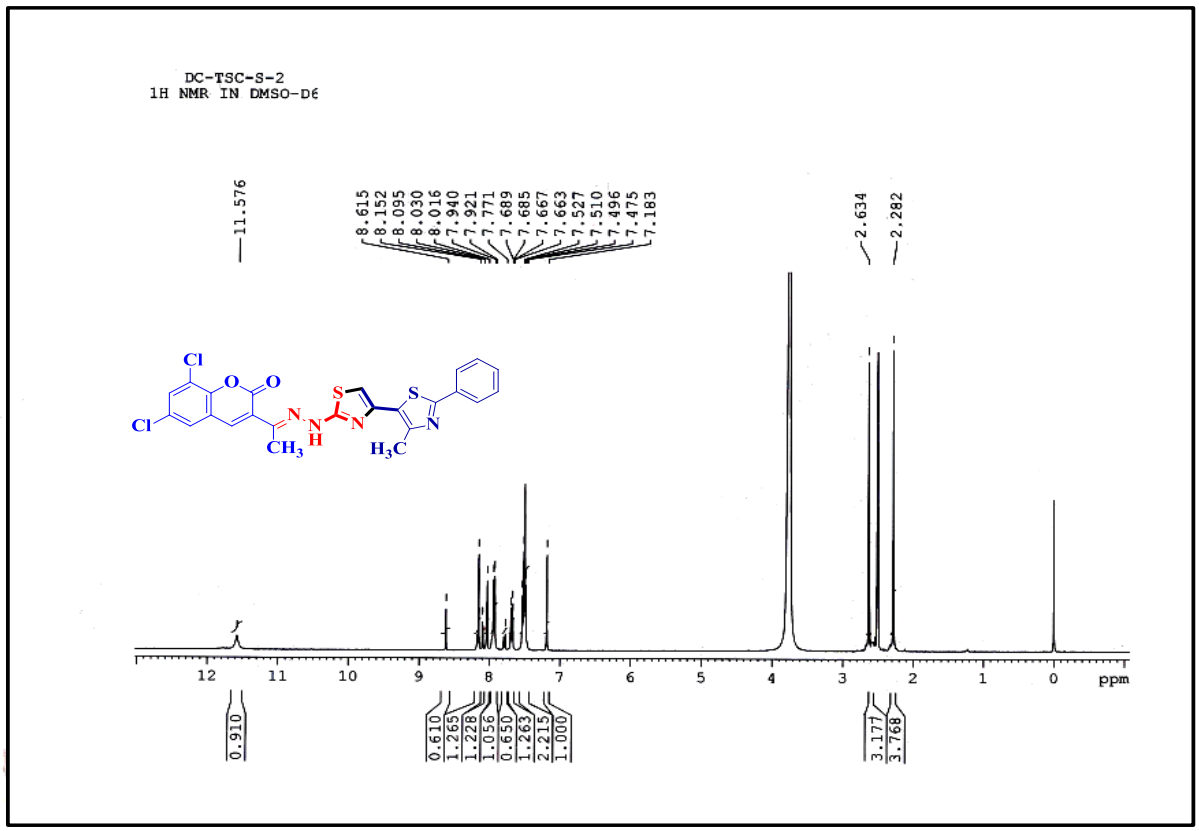


Fig: ^{1}H NMR Spectrum of compound **48c**

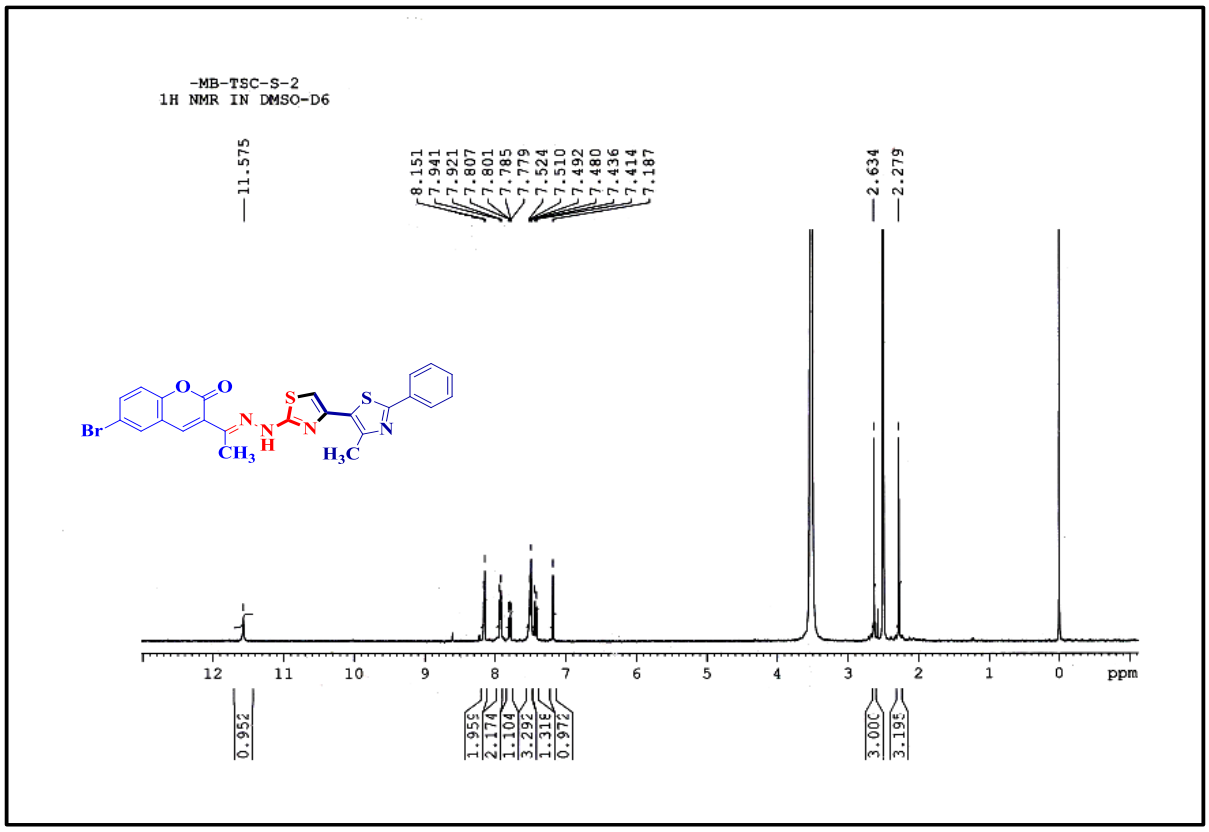


Fig: ^{1}H NMR Spectrum of compound **48d**

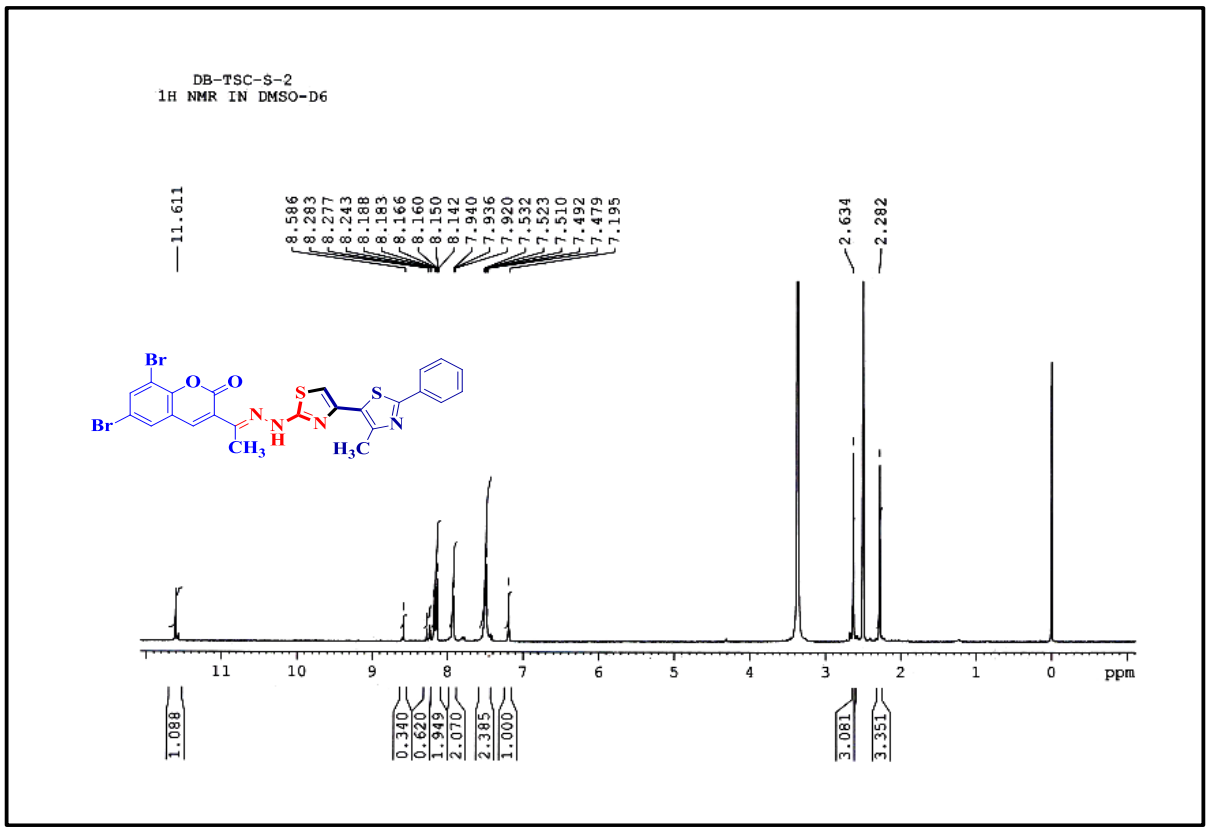


Fig: ^{1}H NMR Spectrum of compound **48e**

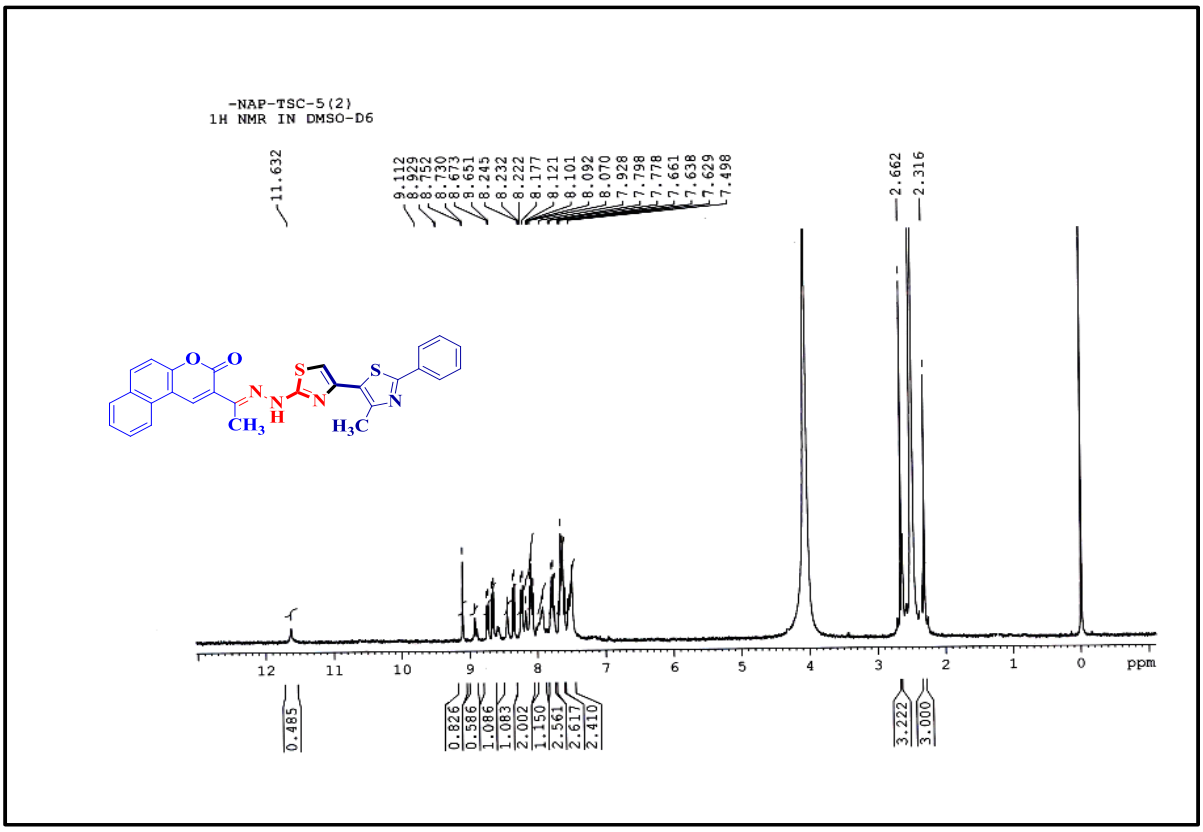


Fig: ^{1}H NMR Spectrum of compound **48f**

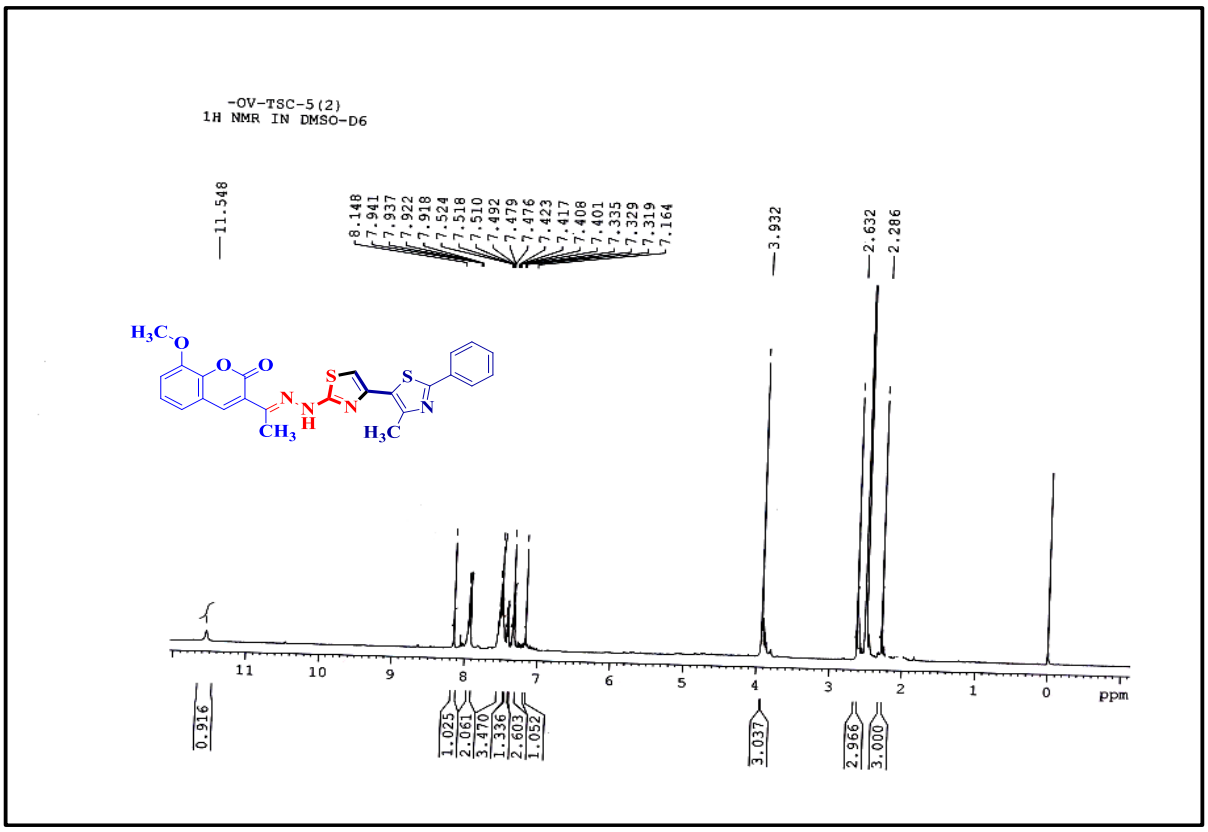


Fig: ^{1}H NMR Spectrum of compound **48g**

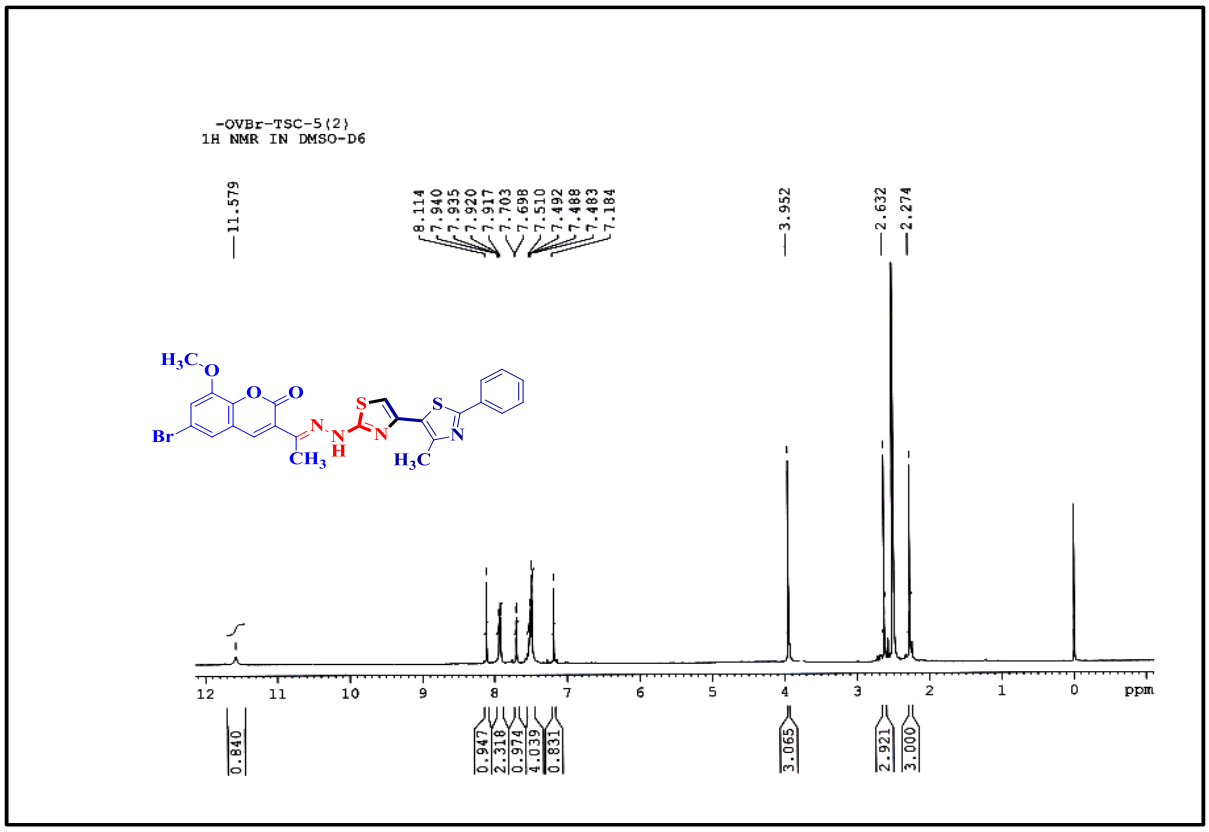


Fig: ^{1}H NMR Spectrum of compound **48h**

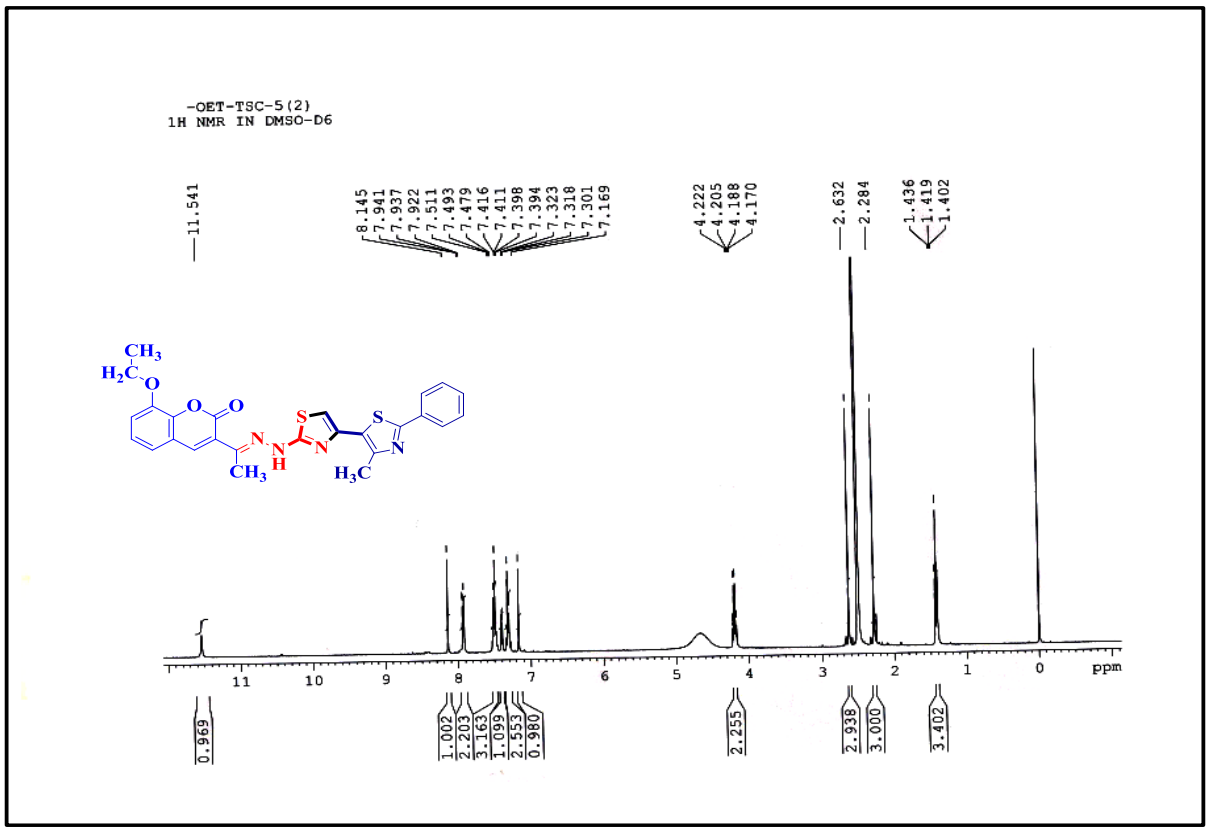


Fig: ^{1}H NMR Spectrum of compound **48i**

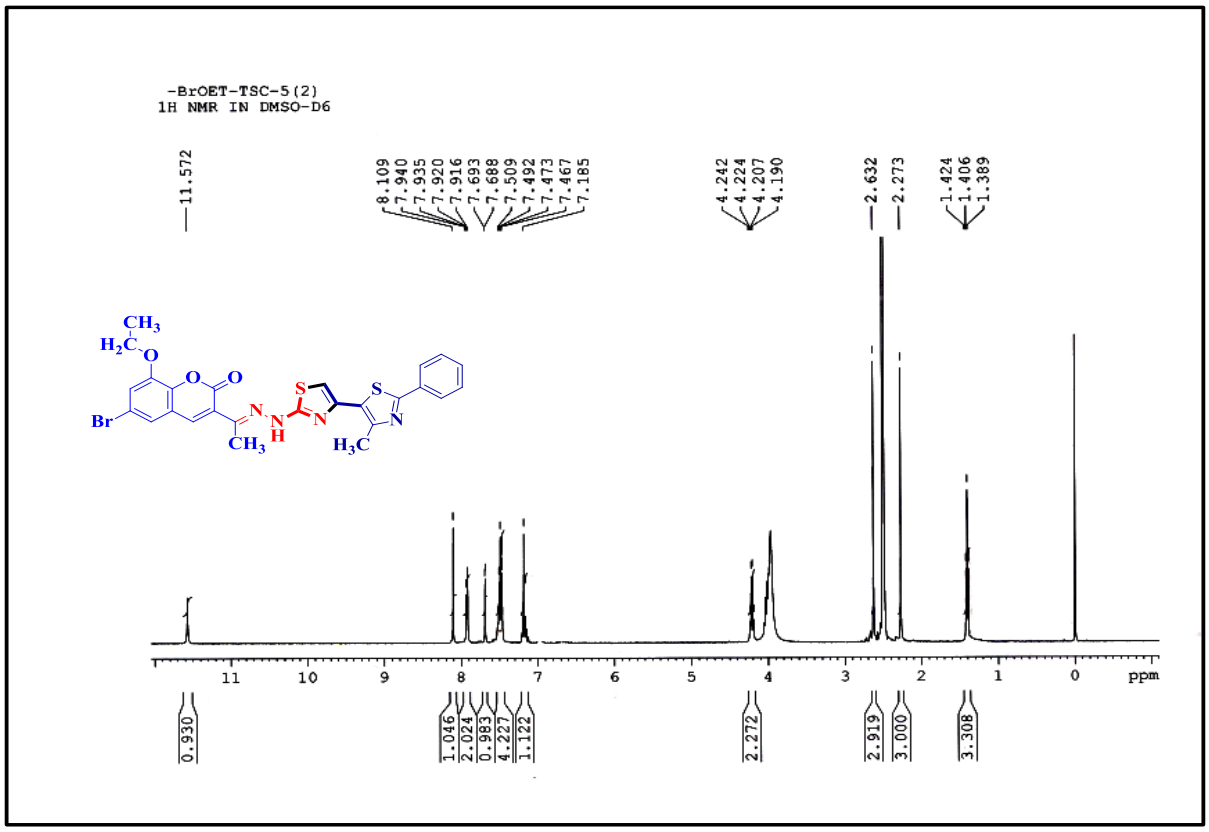


Fig: ^{1}H NMR Spectrum of compound **48j**

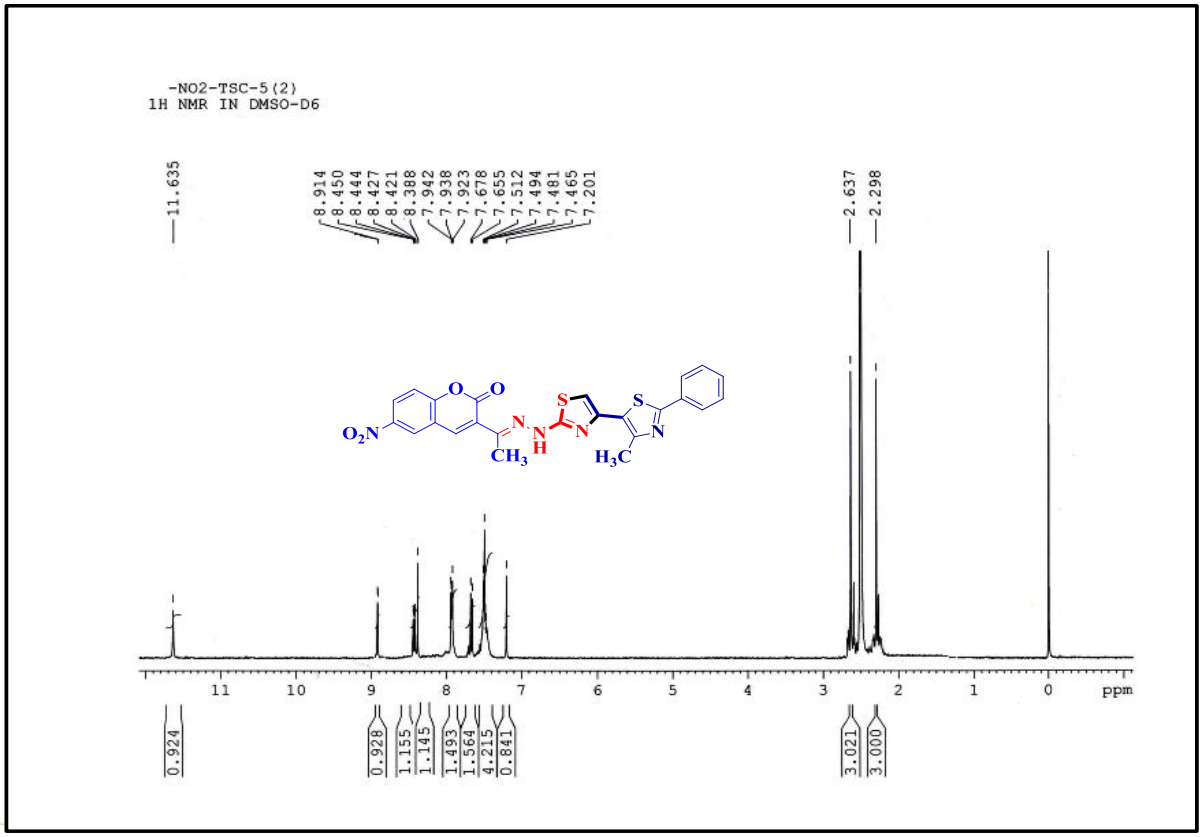


Fig: ^{1}H NMR Spectrum of compound **48l**

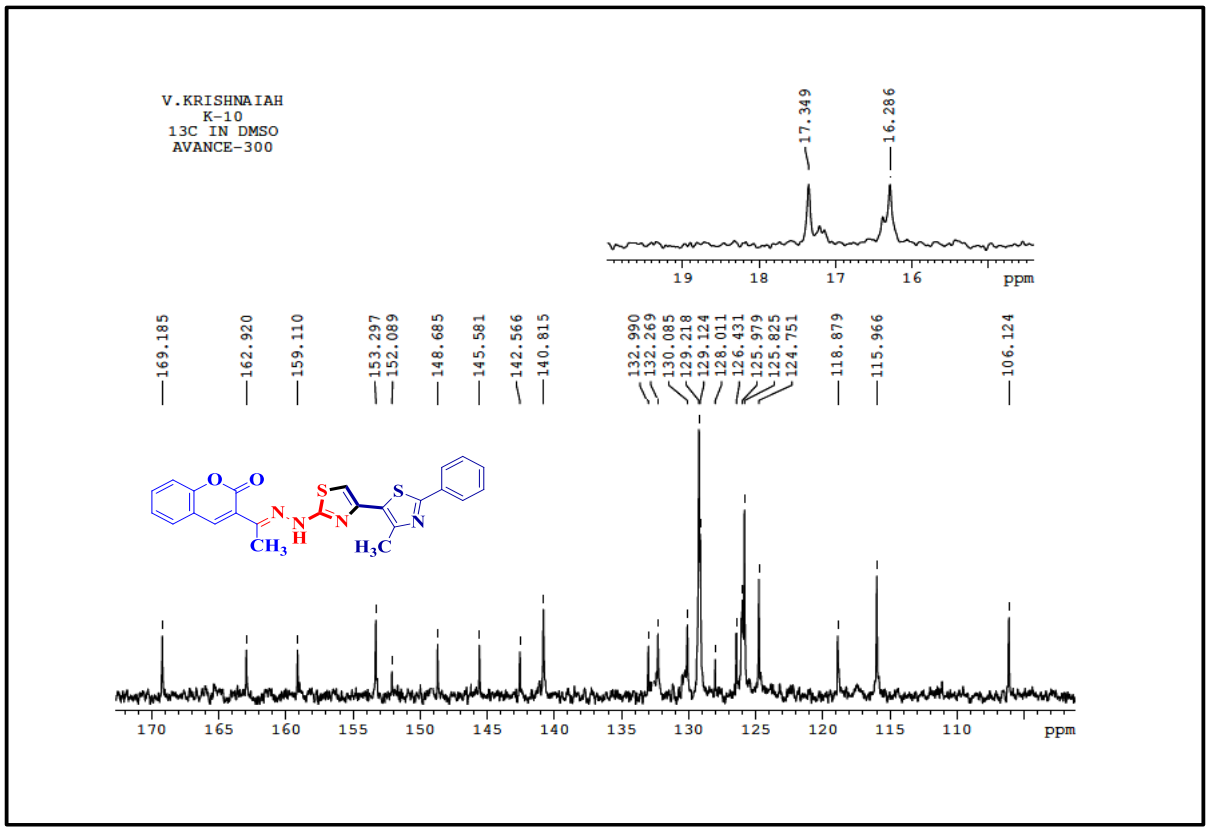


Fig: ^{13}C NMR Spectrum of compound **48a**

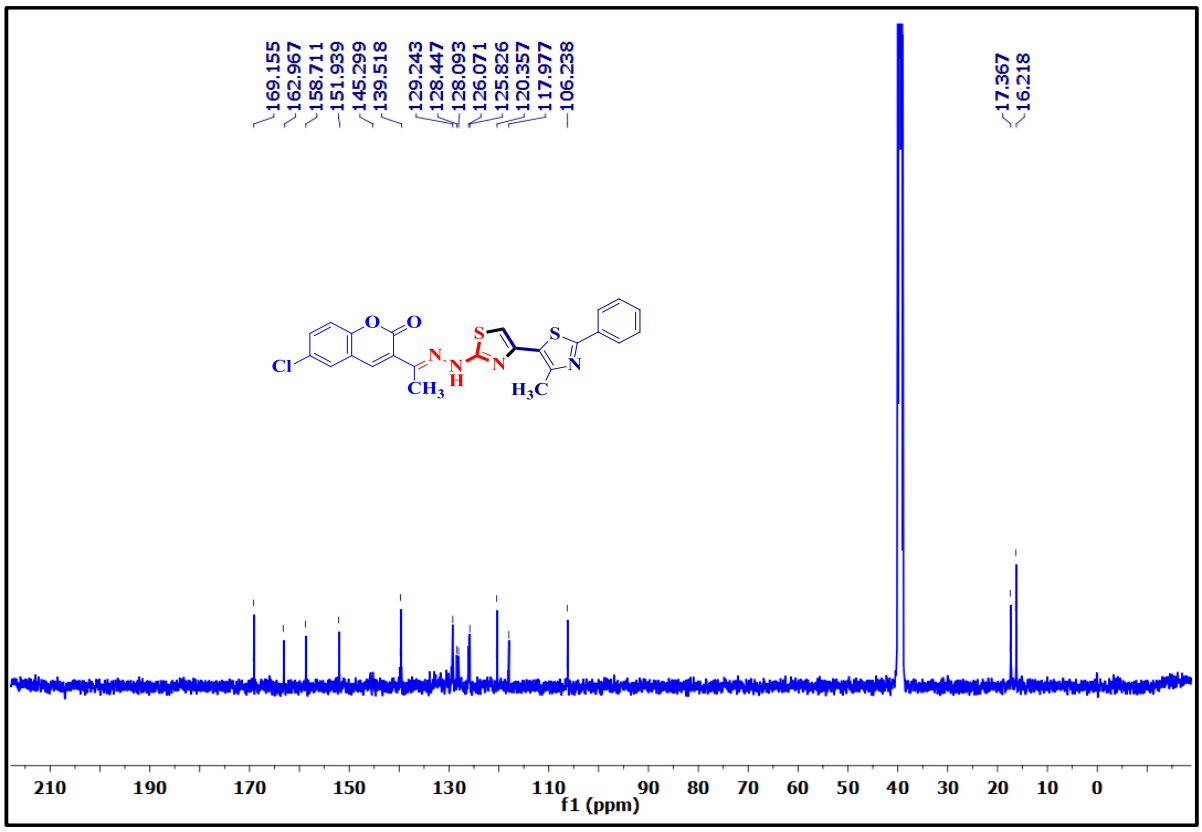


Fig: ^{13}C NMR Spectrum of compound **48b**

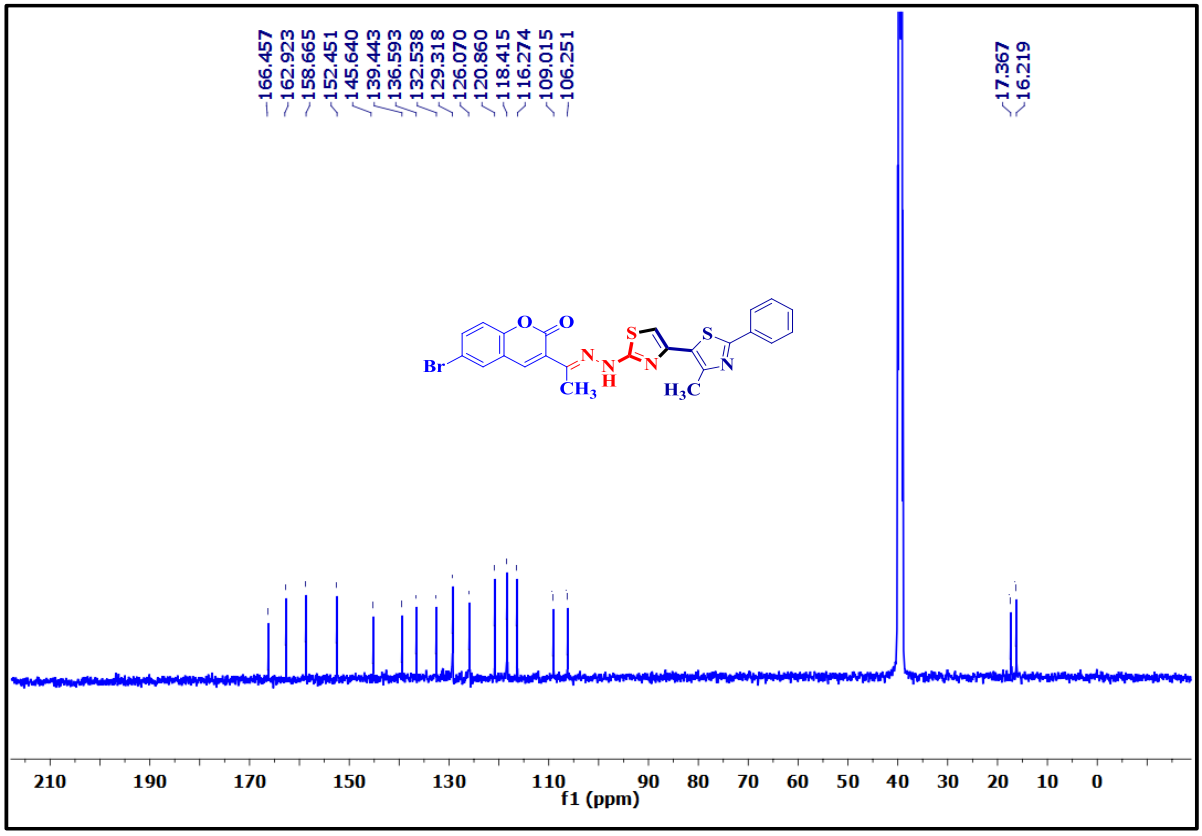


Fig: ^{13}C NMR Spectrum of compound **48d**

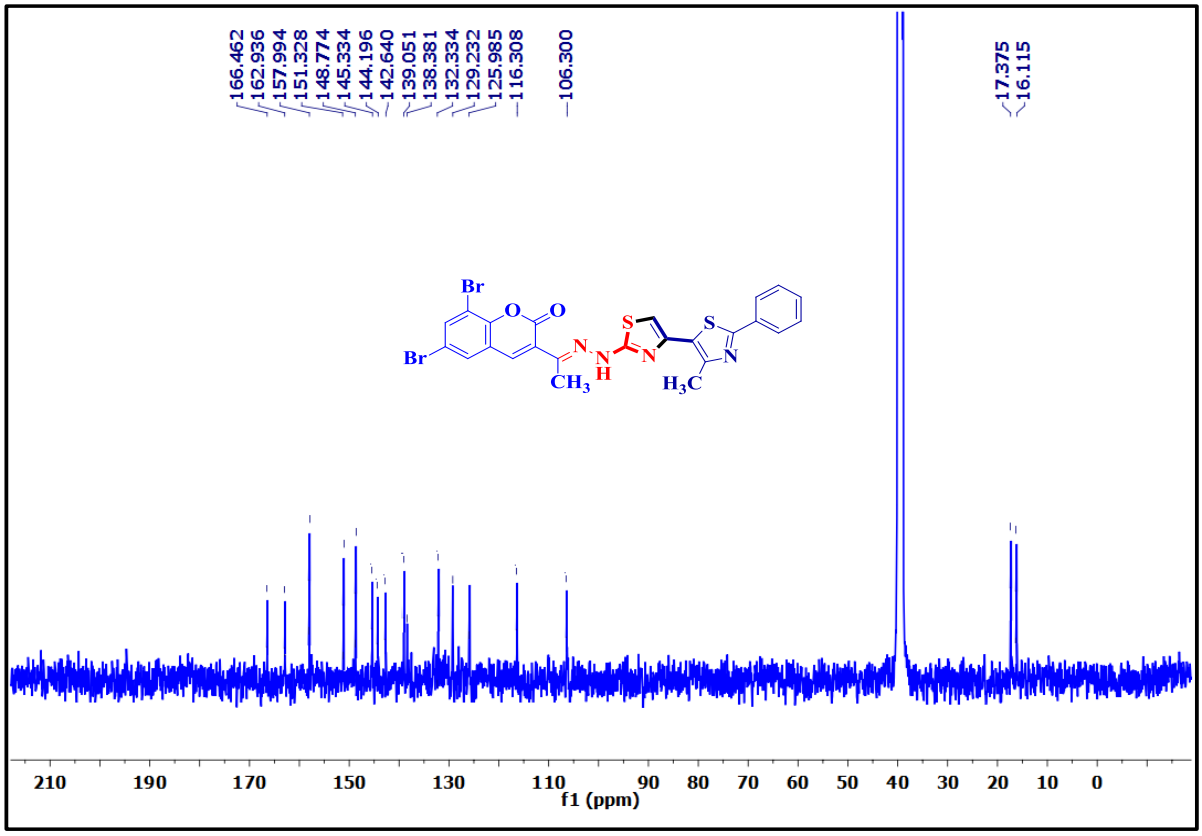


Fig: ^{13}C NMR Spectrum of compound **48e**

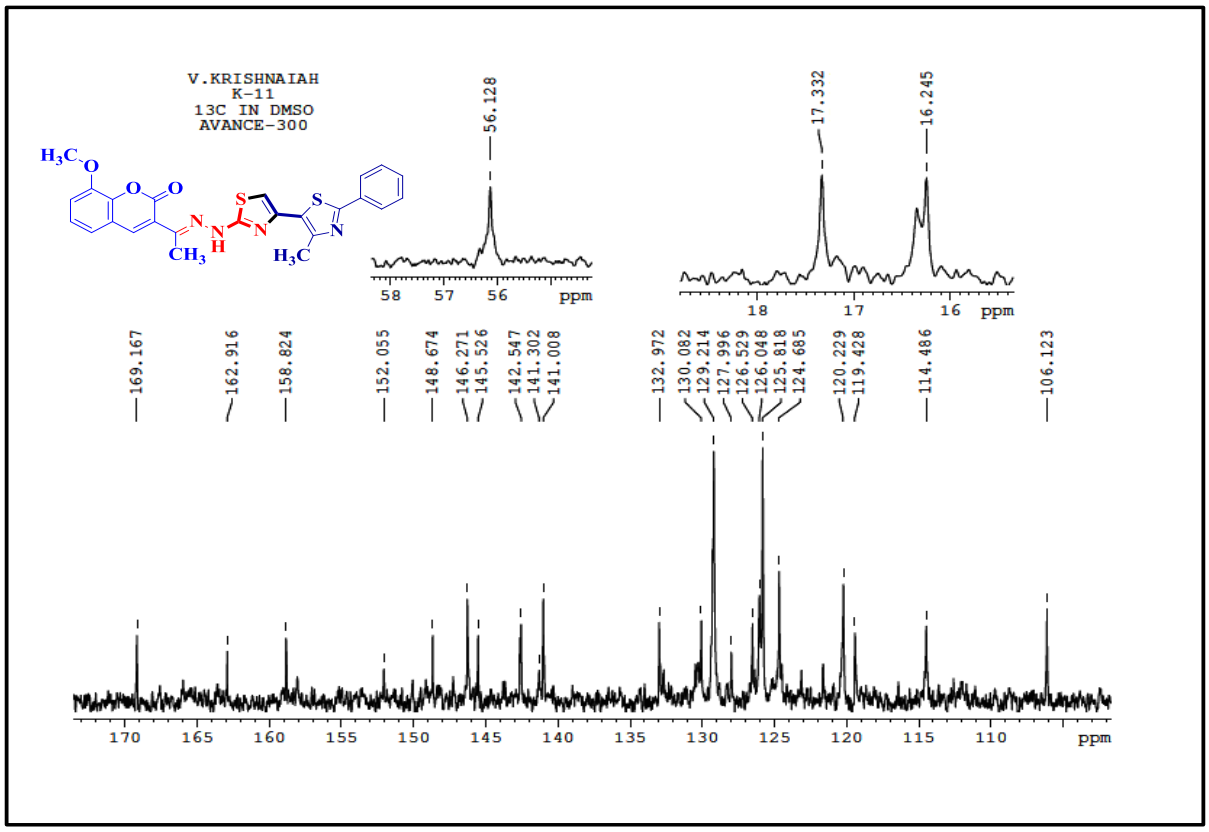


Fig: ^{13}C NMR Spectrum of compound **48g**

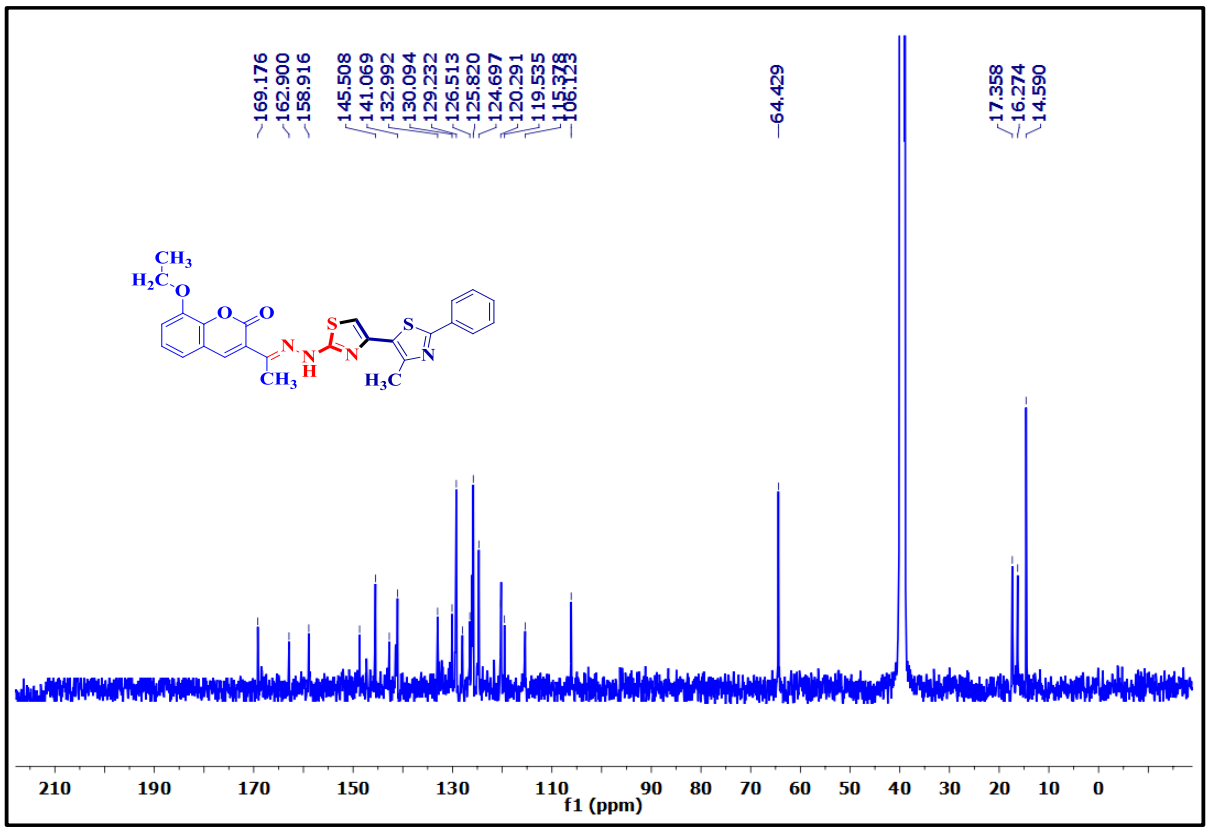


Fig: ^{13}C NMR Spectrum of compound **48i**

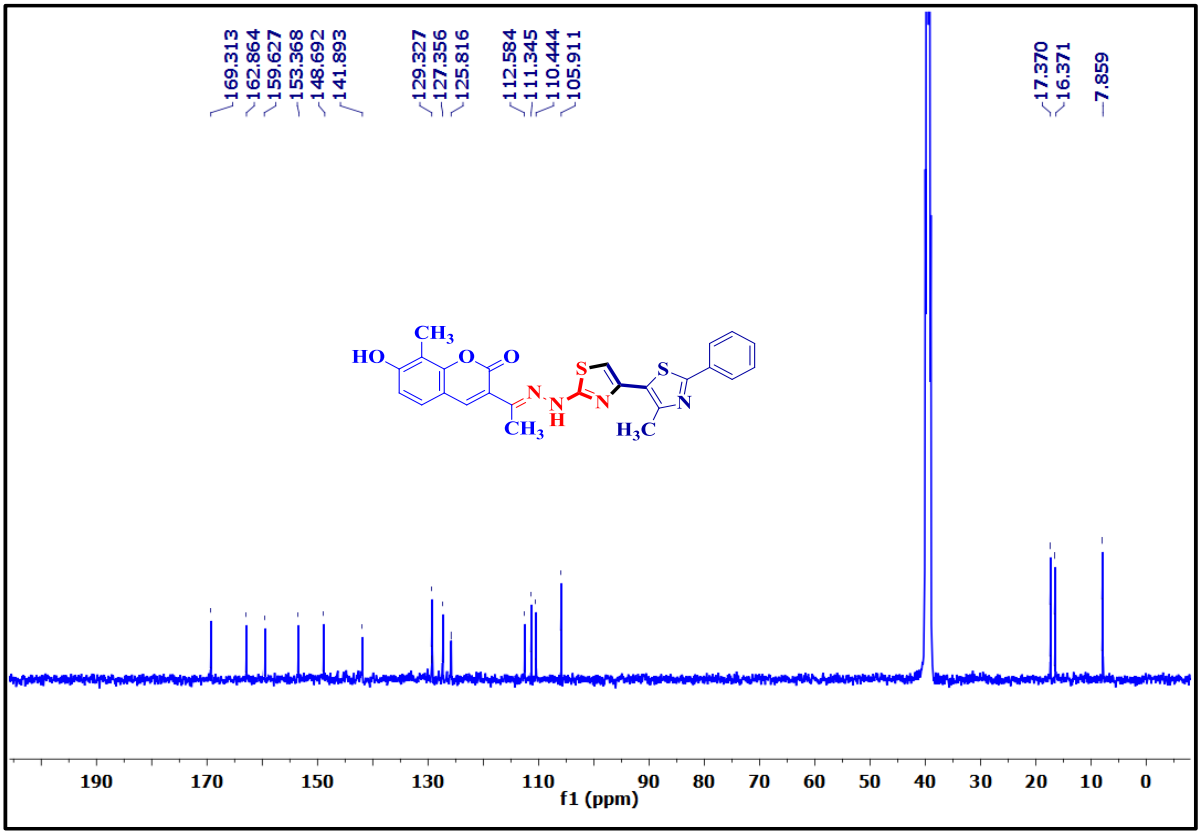


Fig: ^{13}C NMR Spectrum of compound **48k**

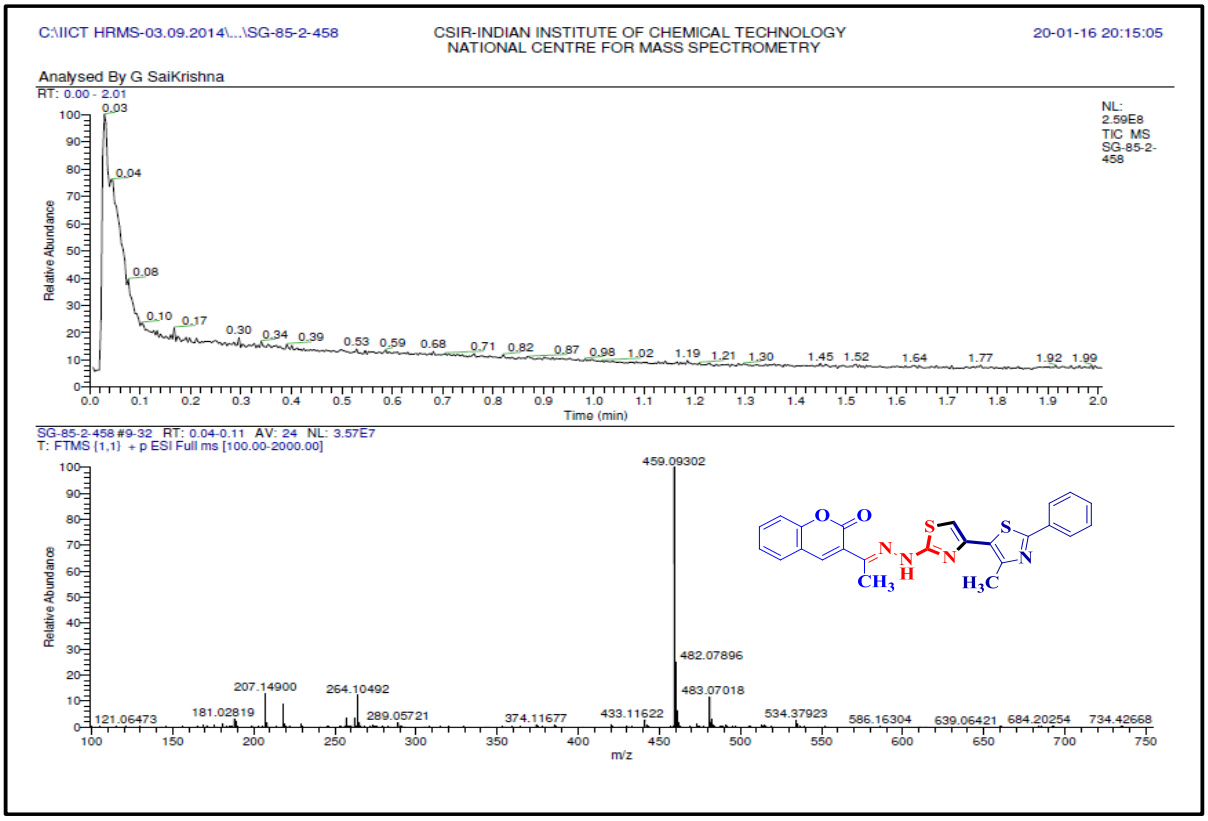


Fig: Mass Spectrum of compound **48a**

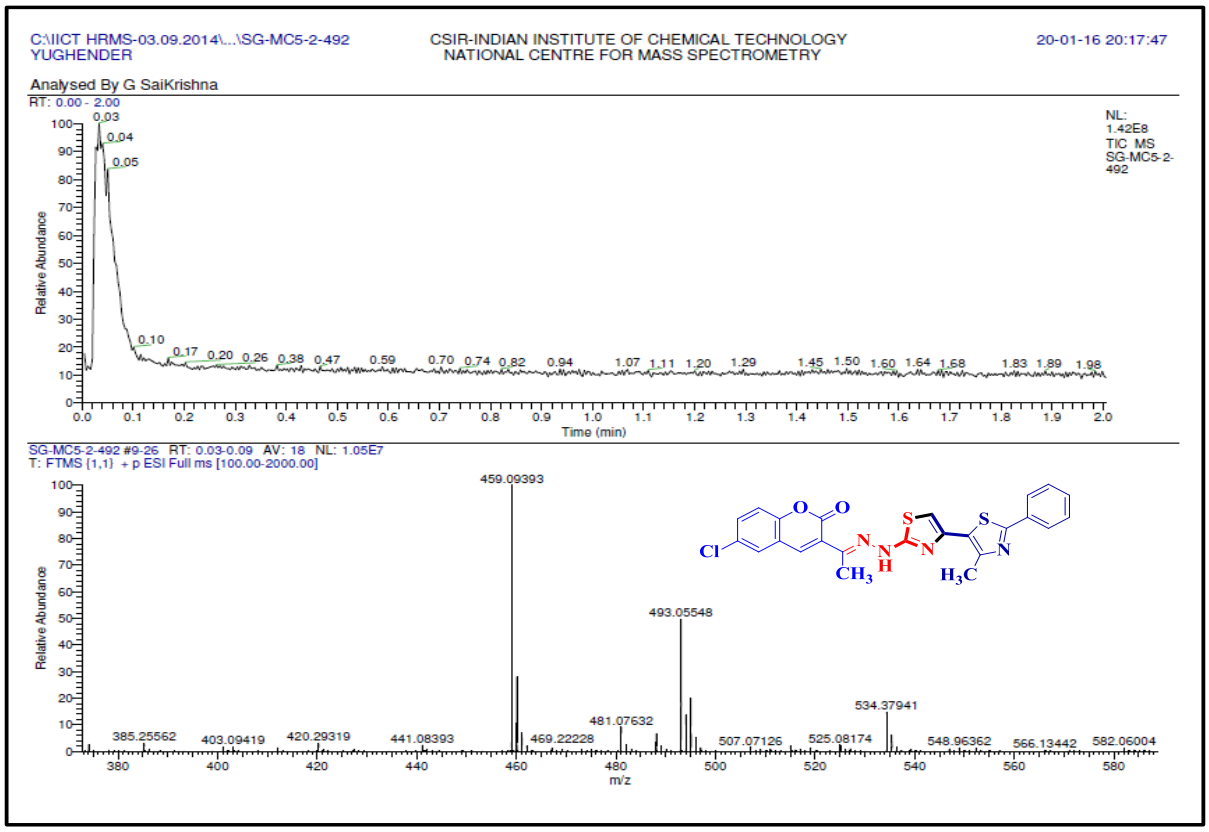


Fig: Mass Spectrum of compound **48b**

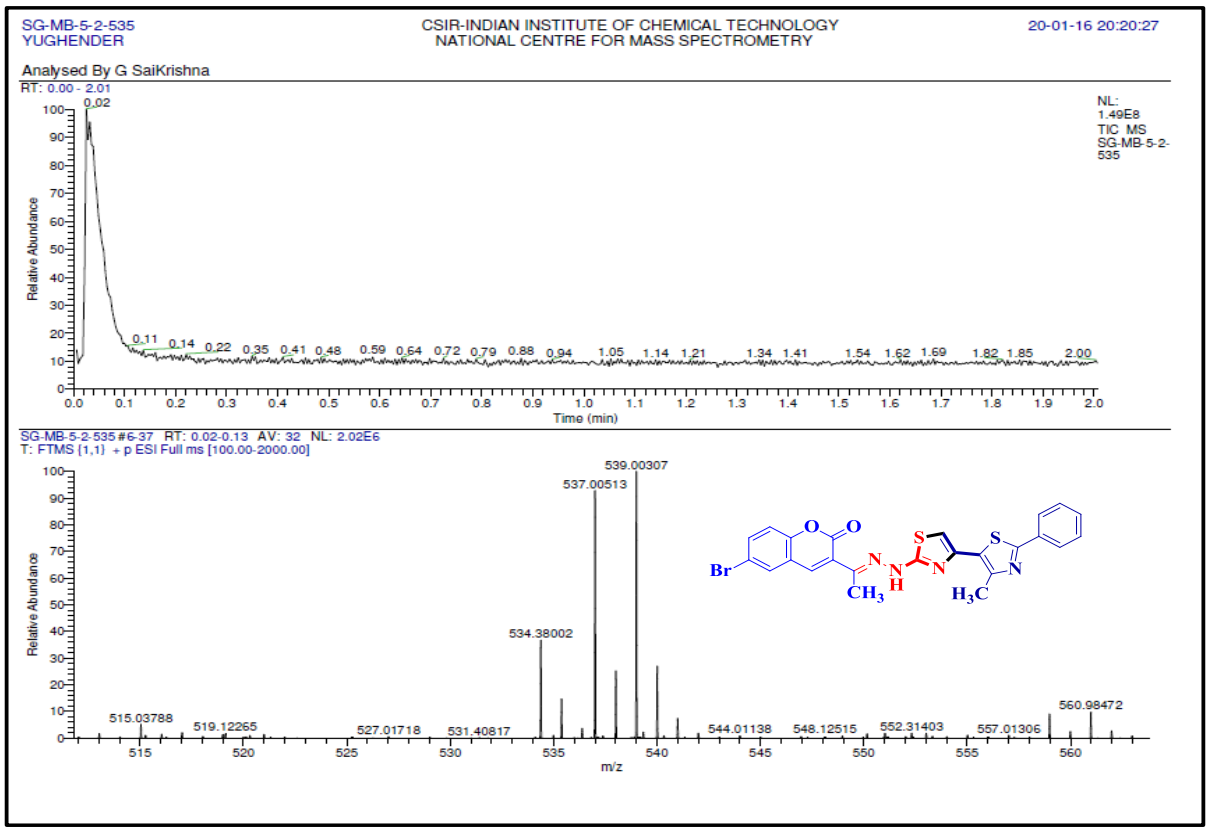


Fig: Mass Spectrum of compound **48d**

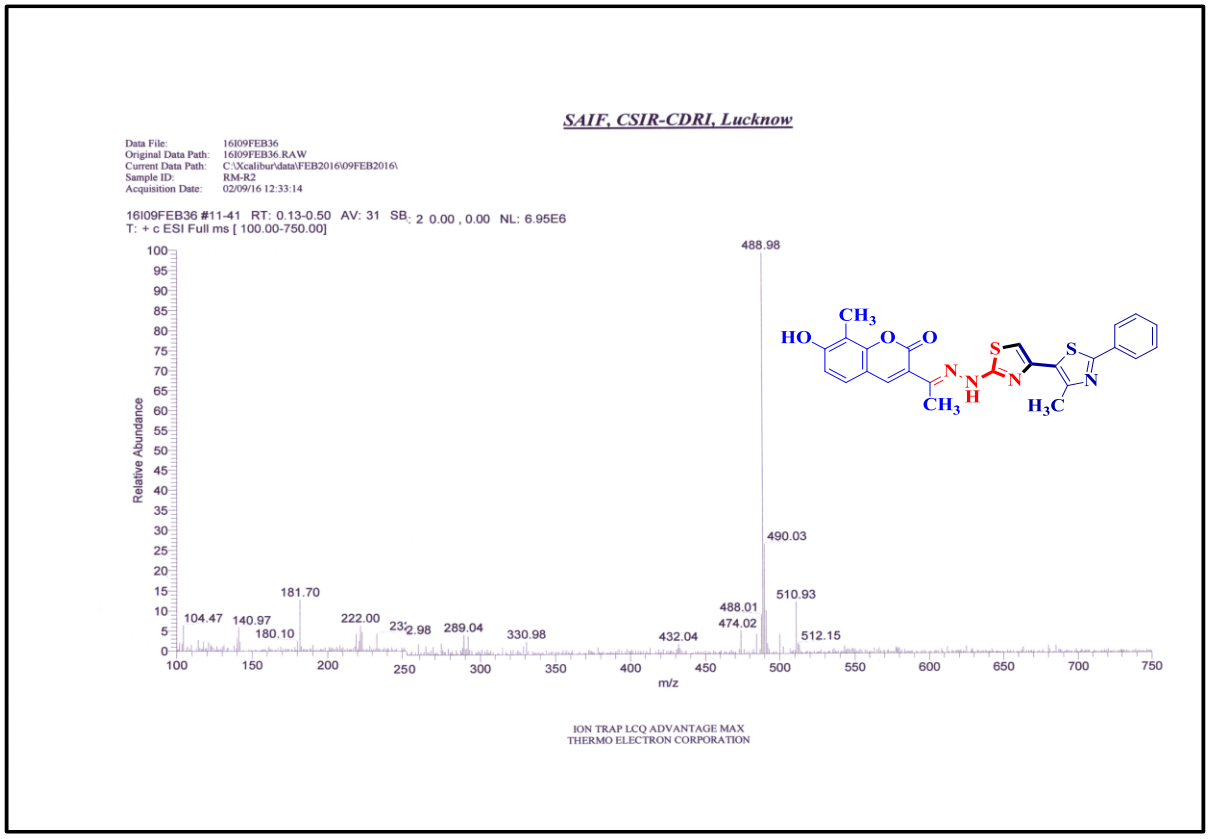


Fig: Mass Spectrum of compound **48k**

CHAPTER-VI
PART-B

ONE POT MULTICOMPONENT SYNTHESIS OF *N*-(3-(2-(1*H*-INDOL-3-YL)ETHYL)-4'-METHYL-2'-PHENYL-[4,5'-BITHIAZOL]-2(3*H*)-YLIDENE)ANILINE DERIVATIVES AND THEIR ANTIVIRAL ACTIVITY

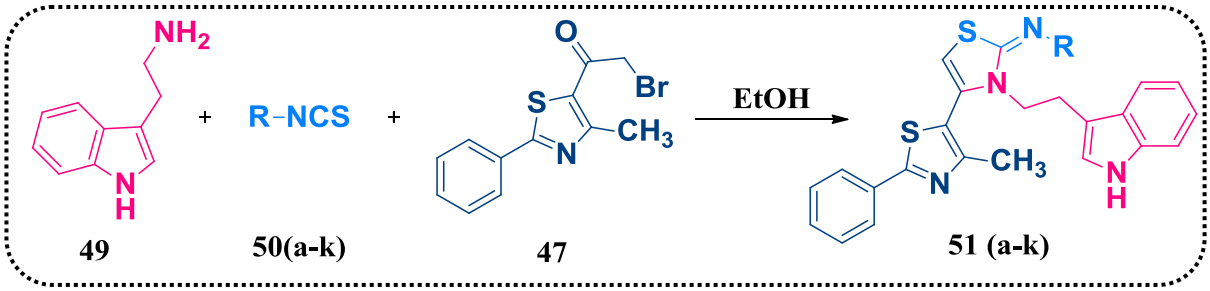
PART-B

PRESENT WORK:

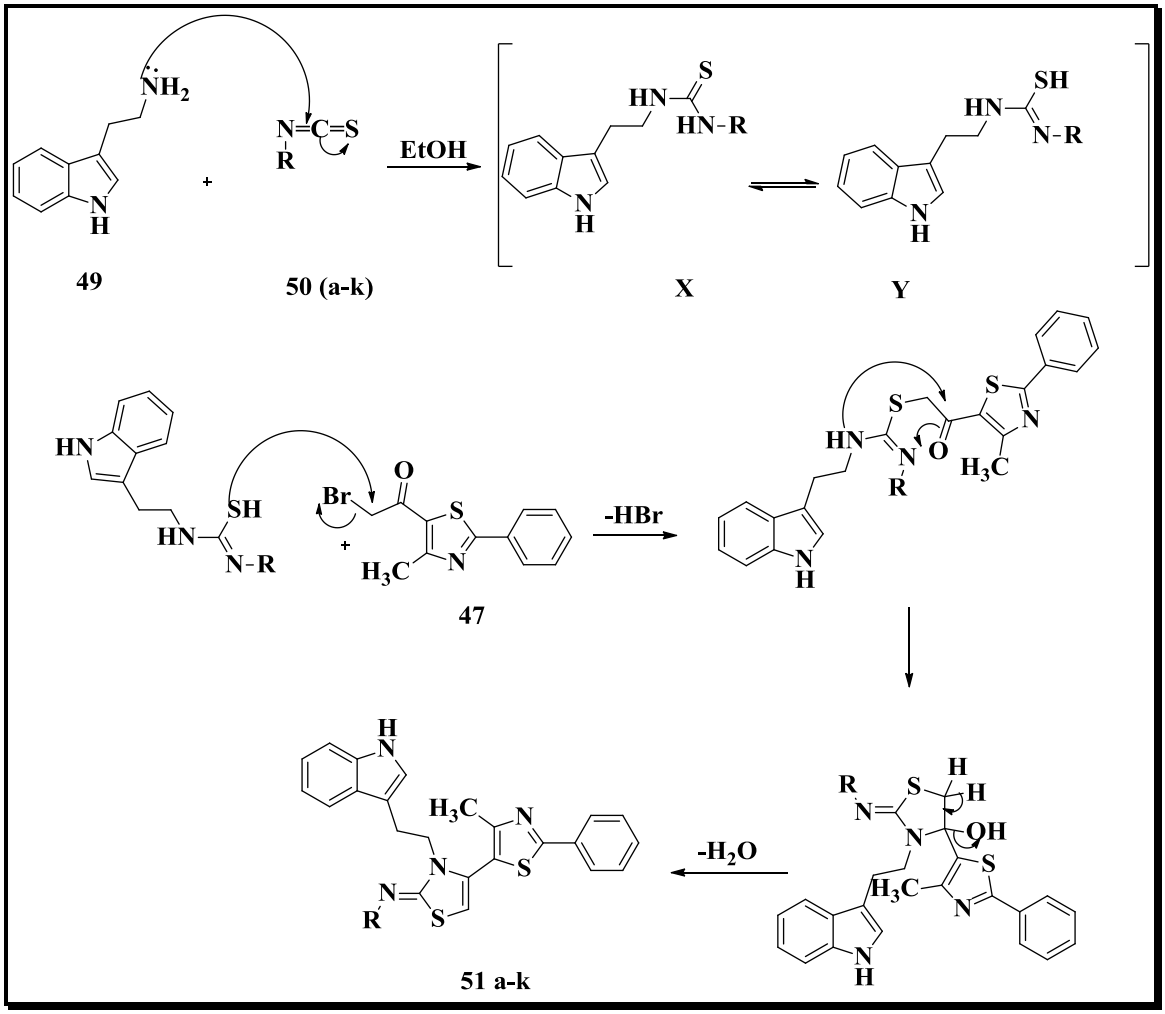
Various alkyl/ aryl isothiocyantes **50 (a-k)**, tryptamine **49** and 2-bromo-1-(4-methyl-2-phenylthiazol-5-yl)ethanone **47** were procured from commercial source and used without any further purification.

The literature survey revealed that thiazoles, tryptamines and isothiocyantes are pharmacologically active compounds. Based on these observations we made an attempt to incorporate all these heterocyclic systems into part of molecule which would lead to the new type of biologically significant active molecules. As per the literature reviews, the methods applied for the synthesis of imino thiazoles involves step wise reaction methods by using different types of catalysts, harsh reaction conditions, and longer reaction times. However, the step wise reaction methods have their own limitation in terms of efficiency, economy and environmental problems. Consequently, there is huge demand to develop an efficient method to synthesize these iminothiazolines through easy reaction path, lower reaction time and minimization of catalysts.

The present chapter describes an efficient one pot three component synthesis 2,3,4-subtituted thiazoles by the condensation reaction of tryptamine **49** with aryl isothiocyanates **50(a-j)** followed by 2-bromo-1-(4-methyl-2-phenylthiazol-5-yl)ethanone **47** in presence of absolute ethanol (**Scheme-2**). The advantages of the present reaction are single operation, no catalyst, easy reaction work up, shorter reaction time and excellent yields (**Table 7**).



Scheme-3: An efficient one pot multicomponent synthesis of 2,3,4-substituted thiazoline derivatives.



Scheme-4: Plausible reaction mechanism for the formation of imino thiazoles

The mechanism for the synthesis of the title compounds can be explained as shown in scheme 4. In the initial step the nucleophilic amine group of tryptamine **49** will reacts with electrophilic carbon centre of aryl isothiocyanate **50 (a-j)** and forms an unsymmetrical thiourea intermediate. The thiourea intermediate exists in two tautomeric forms **X** and **Y**. In the next step the thiol nucleophile reacts with the easy leaving bromine atom of 2-bromo-1-(4-methyl-2-phenylthiazol-5-yl) ethanone **47** and forms S-alkylated intermediate. This intermediate further undergoes intramolecular cyclocondensation to give the target compound.

All the newly synthesized compounds were screened against various viral species in different types of cell culture medium as described in the preceding chapter. The antiviral activity experiment was carried out in triplicate and the average values were taken for report. The antiviral activity data was shown in **Table 8-12**.

Table. 7 An efficient one pot synthesis of imino thiazoline derivatives

S. No.	Product	R	Reaction Time (h)	Yield (%)	M.P. (°C)
1	**51a**	Isopropyl	3	79	166 – 168
2	**51b**	Phenyl	2.5	85	190 – 192
3	**51c**	Benzyl	3	88	187 – 189
4	**51d**	*p*-tolyl	3.5	89	145 – 147
5	**51e**	4-Methoxyphenyl	3	89	185 – 187
6	**51f**	4-Cyano phenyl	4	90	206 – 208
7	**51g**	4-Fluoro phenyl	4	91	192 – 194
8	**51h**	3-Chloro phenyl	3.5	92	224 – 226
9	**51i**	4-Chloro phenyl	4	91	212 – 214
10	**51j**	4-Nitro phenyl	4	92	236 – 238

The antiviral activity reveals that the newly synthesized imino thiazoline derivatives exhibited promising antiviral activity against tested viral species. The compounds **51g**, **51f** and **51i** exhibited excellent activity in HEL cell culture medium against *Herpes simplex virus-1* (KOS), *Herpes simplex virus-2* (G), *Herpes simplex virus-1*TK-KOS ACVr, *Vaccina virus*, *Adeno Virus*, *Human Corona Virus* (229E), whereas remaining compounds are inactive against these viruses (**Table 8**).

Table.8 Cytotoxicity and antiviral activity in HEL cell cultures

Compound	Minimum cytotoxic concentration[a] (μM)	EC_{50}[b] (μM)					
		Herpes simplex virus-1 (KOS)	Herpes simplex virus-2 (G)	Herpes simplex virus-1 TK-KOS ACVr	Vaccinia virus	Adeno virus-2	Human Coronavirus (229E)
51a	20	>100	>100	>100	>100	>100	>100
51b	20	>100	>100	>100	>100	>100	>100
51c	20	>100	>100	>100	>100	>100	>100
51d	20	>100	>100	>100	>100	>100	>100
51e	≥20	>100	>100	>100	>100	>100	>100
51f	>100	**11.5**	**10**	**15.7**	**10**	**30**	**15**
51g	>100	**8.9**	**6.5**	**10**	**12.6**	**11**	**16**
51h	>100	>100	>100	>100	>100	>100	>100
51i	>100	**13**	**10**	**25**	**20**	**15**	**20**
51j	>100	>100	>100	>100	>100	>100	>100
Brivudin	>250	0.008	112	10	10	-	-
Cidofovir	>250	4.5	2	3.4	22	29	-
Acyclovir	>250	0.7	0.2	85	>250	-	-
Ganciclovir	>250	0.16	0.06	4	>100	-	-
Zalcitabine	>250	-	-	-	-	19	-
Alovudine	>250	-	-	-	-	50	-

UDA	>100	-	-	-	-	-	1.8
Ribavirin	>250	-	-	-	-	-	112

[a] Required to cause a microscopically detectable alteration of normal cell morphology.
[b] Required to reduce virus-induced cytopathogenicity by 50 %.

The compounds **51 a-k** were tested for their antiviral activity in HeLa cell culture medium against *Vesicular stomatitis virus*, *Coxsackie virus B4* and *Respiratory syncytial virus*. The compounds did not exhibit any activity against the tested viral species (**Table 9**).

Table.9 Cytotoxicity and antiviral activity in HeLa cell cultures

Compound	Minimum	EC50[b]		
	cytotoxic concentration[a]	Vesicular stomatitis virus	Coxsackie virus B4	Respiratory syncytial virus
51a	20	>100	>100	>100
51b	20	>100	>100	>100
51c	20	>100	>100	>100
51d	≥20	>100	>100	>100
51e	20	>100	>100	>100
51f	100	>100	>100	>100
51g	100	>100	>100	>100
51h	100	>100	>100	>100
51i	100	>100	>100	>100
51j	100	>100	>100	>100
DS-10.000	>100	0.4	6.8	0.4
Ribavirin	>250	25	50	10

[a]Required to cause a microscopically detectable alteration of normal cell morphology.
[b]Required to reduce virus-induced cytopathogenicity by 50 %.

The newly synthesized compounds were evaluated for their antiviral activity against *Parainfluenza 3-virus*, *Reovirus*, *sindis virus*, *Coxsackie virus*, *Puntatoro virus* and *Yellow fever virus* in Vero cell culture medium. The compound **51g** exhibited good antiviral activity against all the six viral strains, compound **51f** is exhibited good antiviral activity against *Parainfluenza 3*-virus and yellow fever virus, where as the compound **51i** shown promising activity against *Parainfluenza 3-virus*, *Reovirus*, *Puntatoro virus* and *Yellow fever virus* (**Table.10**). The remaining compounds shown poor activity against the viral species.

Table. 10 Cytotoxicity and antiviral activity in: Vero cell cultures

Compound	Minimum cytotoxic conc.[a] (µM)	EC_{50}[b] (µM)					
		Para influenza-3 virus	Reovirus-1	Sindbis virus	Coxsackie virus B4	Punta Toro virus	Yellow Fever virus
51a	20	>100	>100	>100	>100	>100	>100
51b	20	>100	>100	>100	>100	>100	>100
51c	20	>100	>100	>100	>100	>100	>100
51d	≥20	>100	>100	>100	>100	>100	>100
51e	≥100	>100	>100	>100	>100	>100	>100
51f	≥4	**25**	>100	>100	>100	>100	**20**
51g	100	**10**	**8.9**	**14.6**	**10**	**20**	**12.6**
51h	100	>100	>100	>100	>100	>100	>100
51i	100	**25**	**11.6**	>100	>100	**20**	**15**
51j	100	>100	>100	>100	>100	>100	>100
DS.10.000	>100	>100	>100	100	45	2.3	0.8
Ribavirin	>250	146	>250	>250	>250	125	>250
Mycophenolic acid	≥100	0.4	0.5	20	45	4.0	0.8

[a]Required to cause a microscopically detectable alteration of normal cell morphology.
[b]Required to reduce virus-induced cytopathogenicity by 50 %.

In CRFK cell culture medium the compounds were screened against feline corona virus (FIPV) and feline herpes virus. From the activity results, none of the compounds exhibited activity against the tested viral species (**Table 11**).

Similarly the compounds **51(a-k)** were tested for antiviral activity in MDCK cell culture medium against *Influenza AH1N1* A/Ned/378/05, *Influenza* AH3N2A/HK/7/87 and *Influenza B* B/Ned/537/05. The compounds **51f, 51g, 51h** and **51i** shown promising activity against tested *Influenza* viral species (**Table 12**).

Table. 11 Cytotoxicity and antiviral activity in: CRFK cell cultures

Compound	CC_{50} (µM)[a]	EC_{50} (µM)[b]	
		Feline Corona Virus (FIPV)	Feline Herpes Virus
51a	11.4	>100	>100
51b	36.1	>100	>100
51c	35.1	>100	>100
51d	52.1	>100	>100
51e	11.6	>100	>100
51f	32.0	>100	>100
51g	>100	>100	>100
51h	>100	>100	>100

51i	>100	>100	>100
51j	>100	>100	>100
HHA	>100	9.4	2.3
UDA	>100	5.7	2.0
Ganciclovir	>100	>100	3.4

[a]50% Cytotoxic concentration, as determined by measuring the cell viability with the colorimetric formazan 50%
[b]Effective concentration, or concentration producing 50% inhibition of virus-induced cytopathic effect, as
CRFK cells: Crandell-Rees Feline Kidney cells.

Table. 12 Cytotoxicity and antiviral activity in: **MDCK cell cultures**

Compound	Cytotoxicity		Antiviral EC50[c]					
	CC_{50}[a]	Minimum cytotoxic concentration[b]	Influenza A/H1N1 A/Ned/378/05		Influenza A/H3N2 A/HK/7/87		Influenza B B/Ned/537/05	
			visual CPE score	MTS	visual CPE score	MTS	visual CPE score	MTS
51a	>100	20	>100	>100	>100	>100	>100	>100
51b	>100	≥20	>100	>100	>100	>100	>100	>100
51c	>100	≥20	>100	>100	>100	>100	>100	>100
51d	>100	100	>100	>100	>100	>100	>100	>100
51e	24.9	4	>100	>100	>100	>100	>100	>100
51f	>100	100	**10.20**	**5.46**	**12.56**	**8.02**	**6.25**	**3.85**
51g	>100	100	**15.26**	**8.25**	**10.26**	**5.75**	**20.56**	**9.25**
51h	>100	100	**25.08**	**15.23**	**30**	**16.25**	**25**	**11.75**
51i	>100	>100	**8.96**	**10.25**	**12.30**	**9.25**	**15.23**	**10.26**
51j	>100	100	>100	>100	>100	>100	>100	>100
Zanamivir	>100	>100	0.4	0.1	0.8	0.1	0.5	0.4
Ribavirin	>100	100	8.9	4.4	4.0	1.0	8.9	8.4
Amantadine	>100	>100	100	>100	0.2	0.1	>100	>100
Rimantadine	>200	>200	>200	>200	0.02	0.01	>200	>200

[a]50% Cytotoxic concentration, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay.

[b]Minimum compound concentration that causes a microscopically detectable alteration of normal cell morphology.

[c]50% Effective concentration, or concentration producing 50% inhibition of virus-induced cytopathic effect, as determined by visual scoring of the CPE, or by measuring the cell viability with the colorimetric formazan-based MTS assay.

MDCK cells: Madin Darby canine kidney cells

From the antiviral activity results, it is clear that the presence of substituted phenyl isothiocyantes(4-CN, 4-F, 4-Cl and 3-Cl) on thiazole ring are crucial for antiviral activity. The presence of electron withdrawing groups at 4th position of phenyl ring is important for antiviral activity.

In conclusion, we have developed an efficient one pot multicomponent synthesis of imino thiazoline derivative through a mild reaction conditions, easy work up, without any catalyst and high yields and also evaluated for their antiviral activity against different human viral strains.

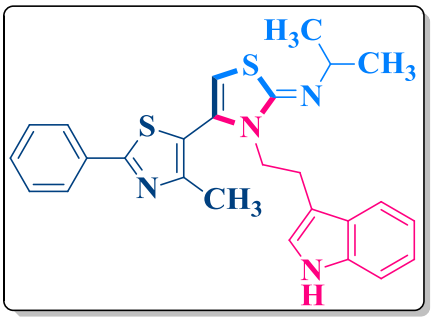
EXPERIMENTAL:

Genearal procedure for the synthesis of 2,3,4-substituted thiazoline derivatives (51a-j)

To the magnetically stirred reaction mixture of tryptamine **49** (0.160 g, 1.0 mmol) and alkyl / aryl isothiocyantaes **50a-j** (1.0 mmol) in presence of absolute ethanol (5 mL) at room temperature, was added 2-bromo-1-(4-methyl-2-phenylthiazol-5-yl)ethanone **47** (0.296 g, 1.0 mmol). The reaction mixture was stirred at room temperature for about 10 minutes and kept at 60 °C for about 2-4 h. The progress of the reaction of was monitored through TLC by using ethyl acetate and hexane in 1:2 ratio. After completion of the reaction, the reaction temperature was decreased to room temperature. The isolated solid product was filtered washed with ethanol and dried. The product was recrystallized from ethanol.

SPECTRAL DATA:

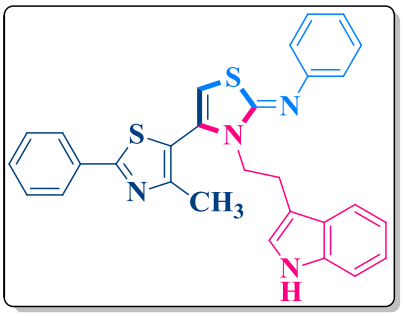
***N*-(3-(2-(1*H*-Indol-3-yl)ethyl)-4'-methyl-2'-phenyl-[4,5'-bithiazol]-2(3*H*)-ylidene) propan-2-amine (51a)**



Yellow solid; **FTIR (KBr, υ_{max} cm^{-1}):** 3197 (-NH), 1602 (-C=N); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 1.48 (d, *J* = 6.4 Hz, 6H, -CH_3), 2.79 (s, 3H, thiazole-CH_3), 3.15 (t, *J* = 6.8 Hz, 2H, -NCH_2), 3.46 – 3.55 (m, 1H, -CH), 4.80 (t, *J* = 6.4 Hz, 2H, -NCH_2), 6.63 (s, 1H, Ar-H), 6.79 (t, *J* = 7.2 Hz, 1H, Ar-H), 6.94 (d, *J* = 7.6 Hz, 1H, Ar-H), 7.01 (t, *J* = 7.2 Hz, 1H, Ar-H), 7.31 – 7.35 (m, 1H, Ar-H), 7.47 (t, *J* = 7.2 Hz, 2H, Ar-H), 7.82 (d, *J*

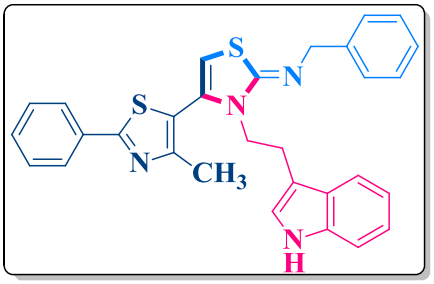
= 8 Hz, 1H, Ar-H), 7.98 (d, *J* = 8 Hz, 2H, Ar-H), 8.64 (s, 1H, Ar-H), 10.57 (bs, 1H, -NH); Anal. Calcd for $C_{26}H_{26}N_4S_2$: C, 68.09; H, 5.71; N, 12.22%; Found: C, 68.05; H, 5.69; N, 12.27%.

_N_-(3-(2-(1*H*-Indol-3-yl)ethyl)-4'-methyl-2'-phenyl-[4,5'-bithiazol]-2(3*H*)-ylidene) aniline (51b)



Yellow solid; **FTIR (KBr, υ_{max} cm^{-1}):** 3247 (-NH), 1597 (-C=N); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 2.39 (s, 3H, thiazole-CH_3), 3.29 (t, *J* = 8 Hz, 2H, -NCH_2), 5.07 (t, *J* = 8Hz, 2H, -NCH_2), 6.62 (s, 1H, Ar-H), 6.90 (t, *J* = 8 Hz, 1H, Ar-H), 7.06 – 7.13 (m, 2H, Ar-H), 7.21 (d, *J* = 8 Hz, 2H, Ar-H), 7.32 – 7.40 (m, 4H, Ar-H), 7.50 (d, *J* = 8 Hz, 3H, Ar-H), 7.80 (d, *J* = 8 Hz, 2H, Ar-H), 8.26 (s, 1H, Ar-H), 12.49 (bs, 1H, -NH); **^{13}C NMR (100 MHz, DMSO-d_6, ppm):** δ 15.77, 22.81, 47.62, 108.91, 111.53, 117.30, 118.37, 121.05, 123.89, 124.44, 126.22, 126.87, 129.31, 130.31, 130.99, 131.30, 132.21, 136.18, 155.67, 168.33; **HR-MS (ESI) m/z:** Calculated for $C_{29}H_{25}N_4S_2$ [M + H]$^+$:493.1521, Found: 493.1526; Anal. Calcd for $C_{29}H_{24}N_4S_2$: C, 70.70; H, 4.91; N, 11.37%; Found: C, 70.75; H, 4.87; N, 11.39%.

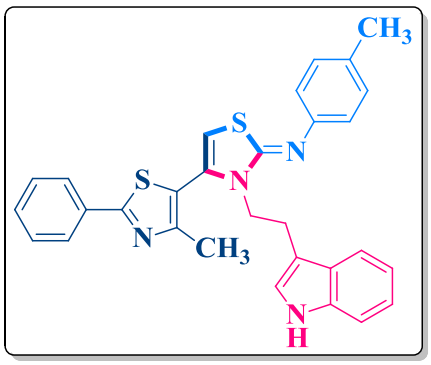
_N_-(3-(2-(1*H*-Indol-3-yl)ethyl)-4'-methyl-2'-phenyl-[4,5'-bithiazol]-2(3*H*)-ylidene)-1-phenylmethanamine (51c)



Yellow solid; **FTIR (KBr, υ_{max} cm^{-1}):** 3206 (-NH), 1593 (-C=N); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 2.17 (s, 3H, thiazole-H), 3.06 (t, *J* = 6.4 Hz, 2H, -NCH_2), 4.26 (t, *J* = Hz, 2H, -NCH_2), 4.73 (s, 2H, Ar-CH_2), 6.60 (t, *J* = 7.2 Hz, 1H, Ar-H), 6.89 – 6.98 (m, 3H, Ar-H), 7.23 (s, 1H, Ar-H), 7.31 (d, *J* = 8Hz, 1H, Ar-H), 7.41 (d, *J* = 6.8 Hz, 1H, Ar-H), 7.45 – 7.54 (m, 7H, Ar-H), 7.80 – 7.83 (m, 2H, Ar-H), 10.66 (bs, 1H, -NH); **^{13}C NMR (100 MHz, DMSO-d_6, ppm):** δ 15.66, 22.34, 47.50, 50.01, 108.65, 109.75, 111.50, 116.62, 117.18, 118.33, 121.05,123.76, 126.18, 126.77, 127.95, 128.21, 128.83, 129.30, 130.97, 131.74, 132.18, 134.79, 136.10, 155.72, 167.84, 168.42; **HR-MS (ESI) m/z**: Calculated for $C_{30}H_{27}N_4S_2$ [M +

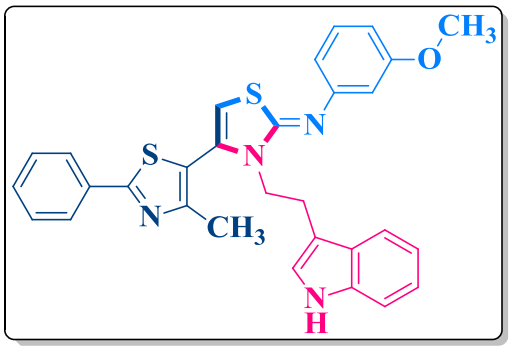
H]$^+$:507.1677, Found:507.1681; Anal. Calcd for $C_{30}H_{26}N_4S_2$: C, 71.11; H, 5.17; N, 11.06%; Found: C, 71.08; H, 5.21; N, 11.03%.

_N_-(3-(2-(1_H_-Indol-3-yl)ethyl)-4'-methyl-2'-phenyl-[4,5'-bithiazol]-2(3_H_)-ylidene)-4-methylaniline (51d)



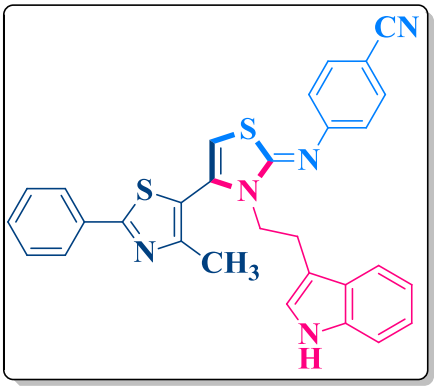
Yellow solid; **FTIR (KBr, υ_{max} cm^{-1}):** 3180 (-NH), 1585 (-C=N); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 2.29 (s, 3H, thiazole-CH_3), 2.38 (s, 3H, Ar-CH_3), 3.12 (t, *J* = 6.8 Hz, 2H, -NCH_2), 4.29 (s, 2H, -NCH_2), 6.68 (t, *J* = 7.6 Hz, 1H, Ar-H), 6.96 (t, *J* = 8 Hz, 1H, Ar-H), 7.05 (d, *J* = 7.6 Hz, 1H, Ar-H), 7.13 (s, 1H, Ar-H), 7.31 – 7.38 (m, 5H, Ar-H), 7.54 (d, *J* = 5.2 Hz, 3H, Ar-H), 7.85 – 7.87 (m, 3H, Ar-H), 10.94 (bs, 1H, -NH); **^{13}C NMR (100 MHz, DMSO-d_6, ppm):** δ 15.77, 20.70, 22.81, 47.71, 108.86, 111.53, 117.28, 118.37, 121.05, 123.96, 124.59, 126.22, 129.32, 130.76, 131.00, 131.39, 132.21, 136.17, 155.72, 168.36; **HR-MS (ESI m/z):** Calculated for $C_{30}H_{27}N_4S_2$ [M + H]$^+$:516.1677, Found: 516.1681; Anal.Calcd for $C_{30}H_{26}N_4S_2$: C, 71.11; H, 5.17; N, 11.06%; Found: C, 71.15; H, 5.15; N, 11.02%.

_N_-(3-(2-(1_H_-Indol-3-yl)ethyl)-4'-methyl-2'-phenyl-[4,5'-bithiazol]-2(3_H_)-ylidene)-3-methoxyaniline (51e)



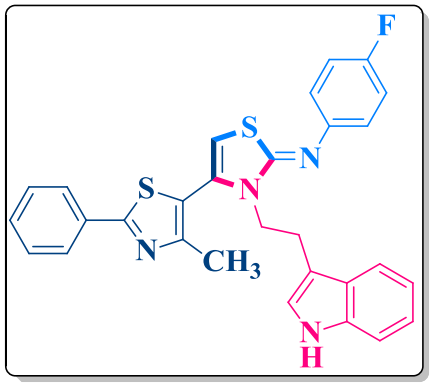
Yellow solid; **FTIR (KBr, υ_{max} cm^{-1}):** 3244 (-NH), 1604 (-C=N), 1268 (-OCH_3); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 2.35 (s, 3H, thiazole-CH_3), 3.27 (t, *J* = 6.8 Hz, 2H, -NCH_2), 3.79 (s, 3H, -OCH_3), 5.04 (t, *J* = 6.4 Hz, 2H, -NCH_2), 6.62 (s, 1H, Ar-H), 6.74 (d, *J* = 7.6 Hz, 1H, Ar-H), 6.82 – 6.89 (m, 2H, Ar-H), 6.96 (s, 1H. Ar-H), 7.07 (t, *J* = 8.4 Hz, 2H, Ar-H), 7.22 (d, *J* = 8 Hz, 1H, Ar-H), 7.32 (d, *J* = 8.4Hz, 1H, Ar-H), 7.42 (s, 1H, Ar-H), 7.48 (d, *J* = 6.8 Hz, 2H, Ar-H), 7.88 (d, *J* = 8 Hz, 2H, Ar-H), 8.45 (s, 1H, Ar-H), 12.41 (bs, 1H, -NH); Anal. Calcd for $C_{30}H_{26}N_4OS_2$: C, 68.94; H, 5.01; N, 10.72%; Found: C, 68.98; H, 4.96; N, 10.74%.

4-((3-(2-(1_H_-Indol-3-yl)ethyl)-4'-methyl-2'-phenyl-[4,5'-bithiazol]-2(3_H_)-ylidene) amino)benzonitrile (51f)



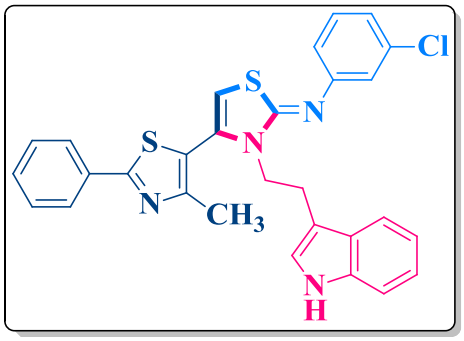
Yellow solid; **FTIR (KBr, υ_{max} cm^{-1}):** 3358 (-NH), 2223 (-CN), 1591 (-C=N); **^{1}H NMR (400 MHz, DMSO-*d*$_6$, δ ppm):** 2.28 (s, 3H, thiazole -CH_3), 3.10 (t, *J* = 6.8 Hz, 2H, -NCH_2), 4.10 (t, *J* = 6.8 Hz, 2H, -NCH_2), 6.70 (t, *J* = 7.2 Hz, 1H, Ar-H), 6.82 (s, 1H, Ar-H), 6.97 (t, *J* = 7.6 Hz, 1H, Ar-H), 7.08 (s, 1H, Ar-H), 7.14 (d, *J* = 8 Hz, 1H, Ar-H), 7.32 (t, *J* = 8.8 Hz, 3H, Ar-H), 7.53 (t, *J* = 3.2 Hz, 3H, Ar-H), 7.86 (t, *J* = 5.6 Hz, 4H, Ar-H); 10.88 (bs, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-*d*$_6$, ppm): δ 15.75, 23.01, 46.78, 105.92, 109.70, 111.47, 117.55, 118.29, 119.09, 120.98, 122.72, 123.47, 126.17, 127.01, 129.29, 129.93, 130.86, 132.32, 134.04, 136.23, 154.83, 167.70; **HR-MS (ESI m/z)** calculated for $C_{30}H_{24}N_5S_2$ $[M + H]^+$:518.1473, Found: 518.1481; Anal. Calcd for $C_{30}H_{23}N_5S_2$: C, 69.60; H, 4.48; N, 13.53%; Found: C, 69.57; H, 4.52; N, 13.50%.

***N*-(3-(2-(1*H*-Indol-3-yl)ethyl)-4'-methyl-2'-phenyl-[4,5'-bithiazol]-2(3*H*)-ylidene)-4-fluoroaniline (51g)**



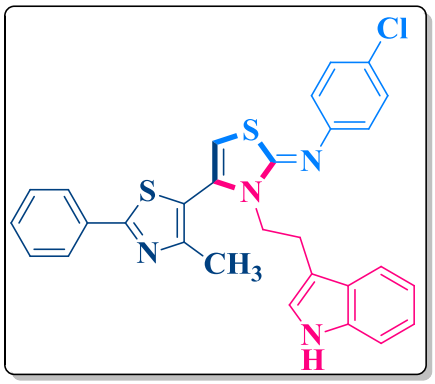
Yellow solid; **FTIR (KBr, υ_{max} cm^{-1}):** 3055 (-NH), 1575 (-C=N), 1091 (C-F); **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm):** 2.29 (s, 3H, thiazole -CH_3), 3.11 (t, *J* = 6.8 Hz, 2H, -NCH_2), 4.20 (s, 2H, -NCH_2), 6.68 (t, *J* = 7.2 Hz, 1H, Ar-H), 6.96 (t, *J* = 7.2 Hz, 1H,Ar-H), 7.08 – 7.14 (m, 2H, Ar-H), 7.34 (t, *J* = 8.4 Hz, 5H, Ar-H), 7.53 – 7.54 (m, 3H, Ar-H), 7.84 – 7.87 (m, 3H, Ar-H), 10.91 (bs, 1H, -NH); **^{13}C NMR (100 MHz, DMSO-*d*$_6$, ppm):** δ 15.77, 22.80, 47.53, 108.95, 111.51, 117.05, 117.32, 118.36, 121.05, 123.88, 126.21, 126.87, 129.30, 130.98, 132.21, 136.17, 155.60, 168.28; **HR-MS (ESI m/z):** Calculated for $C_{29}H_{24}FN_4S_2$ $[M + H]^+$:511.1426, Found: 511.1430; Anal. Calcd for $C_{29}H_{23}FN_4S_2$: C, 68.21; H, 4.54; N, 10.97%; Found: C, 68.25; H, 4.50; N, 10.95%.

***N*-(3-(2-(1*H*-Indol-3-yl)ethyl)-4'-methyl-2'-phenyl-[4,5'-bithiazol]-2(3*H*)-ylidene)-3-chloroaniline (51h)**



Yellow solid; **FTIR (KBr, υ_{max} cm^{-1}):** 3200 (-NH), 1588 (-C=N), 745 (-C-Cl); **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm):** 2.44 (s, 3H, thiazole-CH_3), 3.25 (t, *J* = 4 Hz, 2H, -NCH_2), 5.08 (t, *J* = 8 Hz, 2H, -NCH_2), 6.71 (s, 1H, Ar-H), 6.86 (s, 1H, Ar-H), 6.94 (t, *J* = 8Hz, 1H, Ar-H), 7.08 (d, *J* = 8 Hz, 1H, Ar-H), 7.14 (t, *J* = 12 Hz, 1H, Ar-H), 7.20 (d, *J* = 8Hz, 1H, Ar-H), 7.35 (d, *J* = 8Hz, 2H, Ar-H), 7.51 (d, *J* = 8Hz, 4H, Ar-H), 7.93 (d, *J* = 8Hz, 2H, Ar-H), 8.31 (s, 1H,Ar-H), 12.49 (bs, 1H, -NH); **^{13}C NMR (100 MHz, DMSO-d_6, ppm):** δ 15.79, 22.95, 47.28, 109.23, 111.51, 117.43, 118.35, 121.06,122.23, 123.81, 126.22, 122.94, 129.32, 130.96, 131.75, 132.26, 134.08, 136.21, 155.35, 168.08;Anal. Calcd for $C_{29}H_{23}ClN_4S_2$: C, 66.08; H, 4.40; N, 10.63%; Found: C, 66.05; H, 4.37; N, 10.68%.

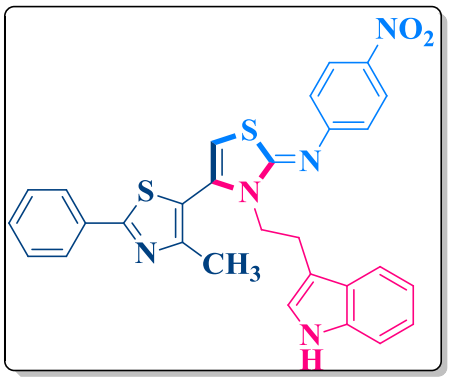
(3-(2-(1*H*-Indol-3-yl)ethyl)-4'-methyl-2'-phenyl-[4,5'-bithiazol]-2(3*H*)-ylidene)-4-chloroaniline (51i)



Yellow solid; **FTIR (KBr, υ_{max} cm^{-1}):** 3218 (-NH), 1597 (-C=N), 743 (-C-Cl); **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm):** 2.28 (s, 3H, thiazole -CH_3), 3.10 (t, *J* = 6.8 Hz, 2H, -NCH_2), 4.15 (s, 2H, -NCH_2), 6.69 (t, *J* = 7.6 Hz, 1H, Ar-H), 6.96 (t, *J* = 7.6 Hz, 1H, Ar-H), 7.10 (s, 2H, Ar-H), 7.31 (d, *J* = 8 Hz, 4H, Ar-H), 7.52 – 7.54 (m, 5H, Ar-H), 7.84 – 7.87 (m, 2H, Ar-H), 10.90 (bs, 1H, -NH); **MS (ESI-m/z):** 527 $[M+H]^+$; Anal. Calcd for $C_{29}H_{23}ClN_4S_2$: C, 66.08; H, 4.40; N, 10.63%; Found: C, 66.12; H, 4.42; N, 10.58%.

***N*-(3-(2-(1*H*-Indol-3-yl)ethyl)-4'-methyl-2'-phenyl-[4,5'-bithiazol]-2(3*H*)-ylidene)-4-nitroaniline (51j)**

Red solid; **FTIR (KBr, υ_{max} cm^{-1}):** 3366 (-NH), 1566 (-C=N), 1521& 1341 (NO_2); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 2.28 (s, 3H, thiazole -CH_3), 3.10 (t, *J* = 7.2 Hz, 2H, -NCH_2), 4.10 (t, *J* = 7.2 Hz, 2H, -NCH_2), 6.72 (t, *J* = 7.2 Hz, 1H, Ar-H), 6.81 (s, 1H, Ar-H), 6.97 (t, *J* = 7.6 Hz, 1H, Ar-H), 7.07 (s, 1H, Ar-H), 7.17 (d, *J* = 8 Hz, 1H, Ar-H),



7.32 (t, *J* = 9.6 Hz, 3H, Ar-H), 7.53 (d, *J* = 6.4 Hz, 3H, Ar-H), 7.86 (d, *J* = 7.2 Hz, 2H, Ar-H), 8.26 (d, *J* = 8.8 Hz, 2H, Ar-H), 10.87 (bs, 1H, -NH); **^{13}C NMR (100 MHz, DMSO-d_6, ppm):** δ 16.01, 18.54, 51.63, 111.28, 115.54, 116.77, 117.64, 120.07, 121.64, 121.91, 123.27, 125.62, 126.38, 127.47, 129.48, 131.03, 131.28, 132.17, 133.75, 144.71, 149.26, 156.68, 168.32, 169.20; Anal. Calcd for $C_{29}H_{23}N_5O_2S_2$: C, 64.78; H, 4.31; N, 13.03%; Found: C, 64.74; H, 4.36; N, 13.01%.

Antiviral activity experiment of trisubstituted thiazoline derivatives (51a-j):

The antiviral activity of the newly synthesized compounds was determined by using different cell culture mediums as reported in chapter-II (Reference 65 - 67). (i) HEL cell culture was used for viruses (*Herpes simplex virus type 1*, *Herpes simplex virus type 2*, *Herpes simplex virus type 1 TK- ACV^r*, *Vaccinia virus*, *Adeno virus-2* or *Vesicular stomatitis virus*), (ii) Vero cells were used for the virus (*Coxsackie virus B4*, *Sindbis virus*, *Parainfluenza virus 3*, *Punta Toro virus* or *Reovirus*), (iii) HeLa cells were used for the virus (*Coxsackie virus B4*, *Vesicular stomatitis virus* or *Respiratory syncytial virus*), (iv) MDCK cells were used for the virus (*Influenza A/H1N1 A/Ned/378/05*, *Influenza A/H3N2 A/HK/7/87* or *Influenza B B/Ned/537/05*) (v) CRFK cells for the virus (*Feline herpes virus* or *Feline corona virus*). After incubation at 37°C the virus-induced cytopathicity was determined by measuring the cell viability with the colorimetric formazan-based MTS assay.

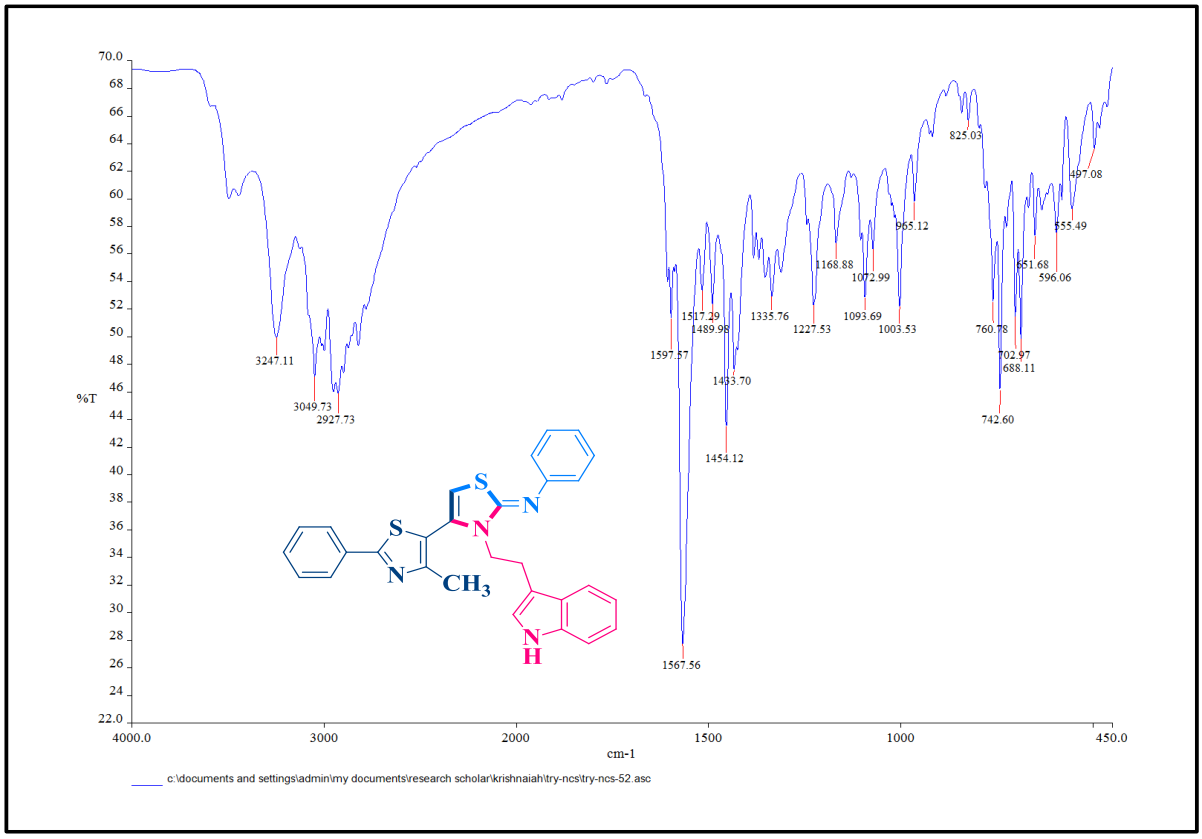


Fig: IR Spectrum of compound **51b**

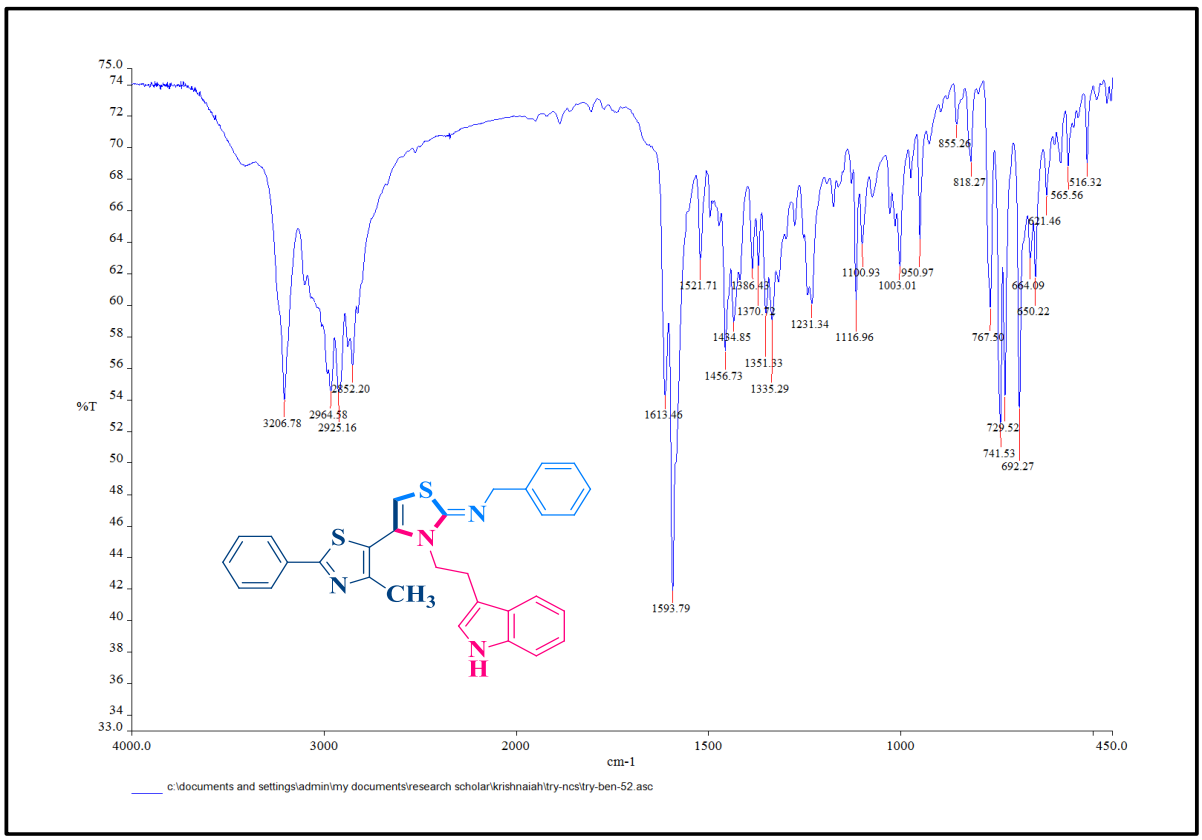


Fig: IR Spectrum of compound **51c**

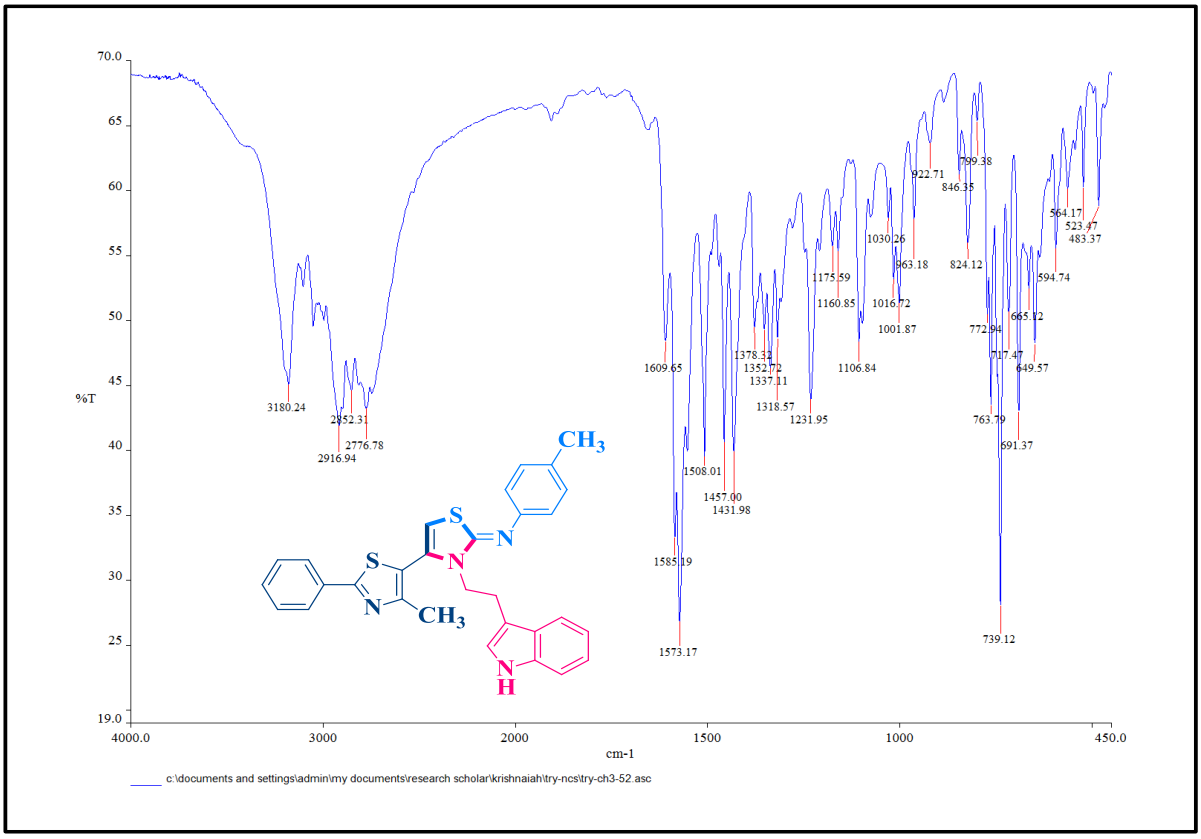


Fig: IR Spectrum of compound **51d**

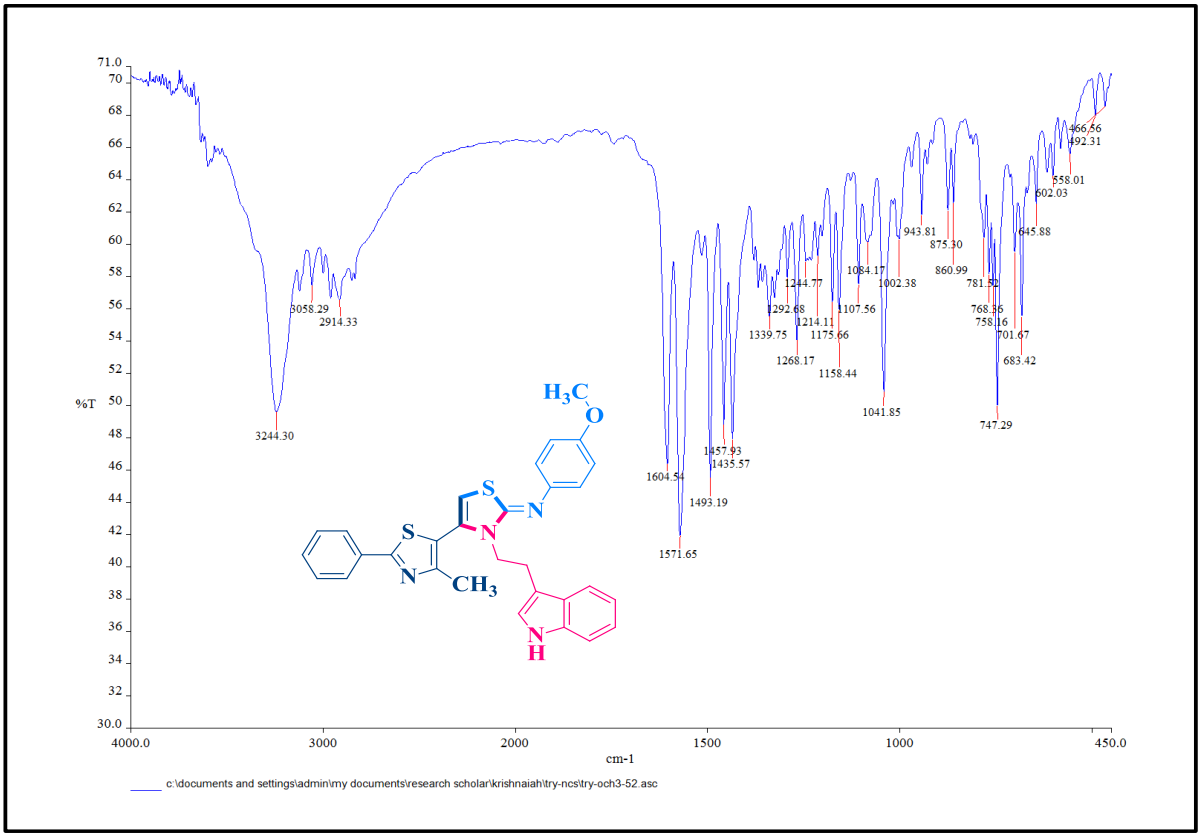


Fig: IR Spectrum of compound **51e**

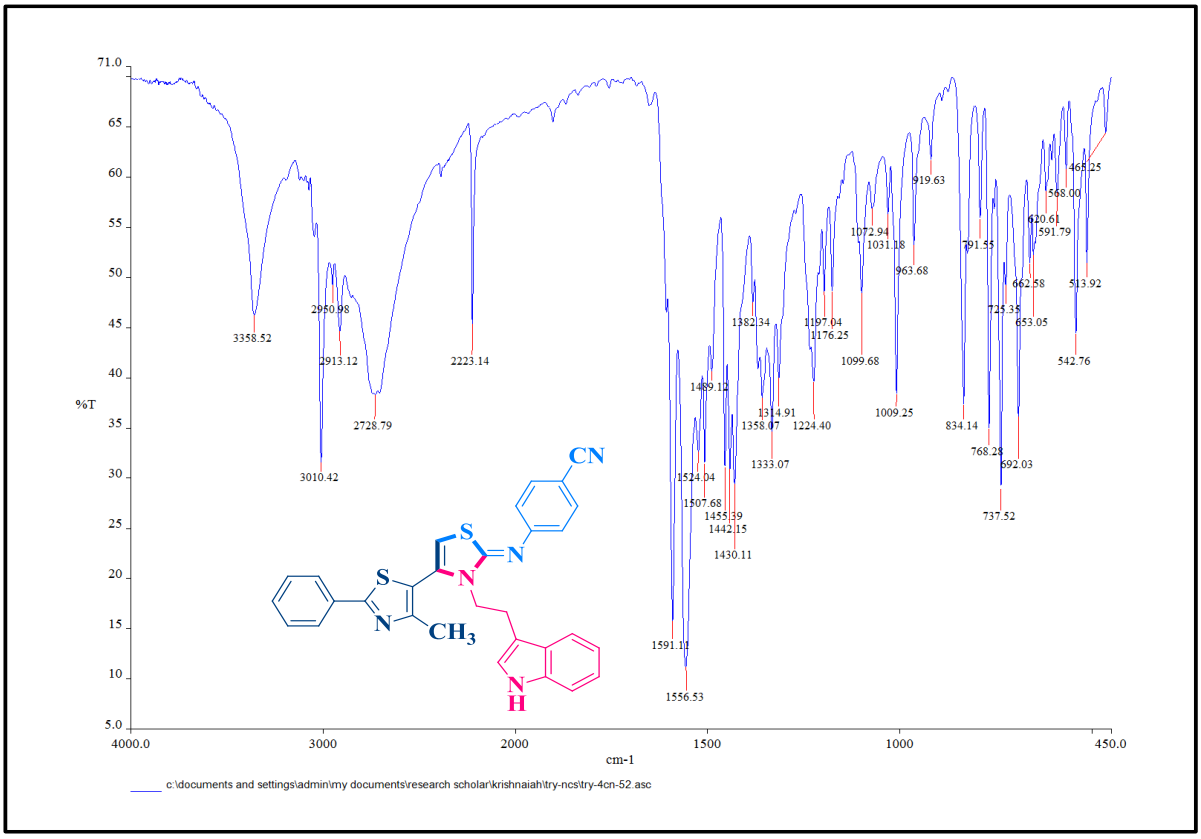


Fig: IR Spectrum of compound **51f**

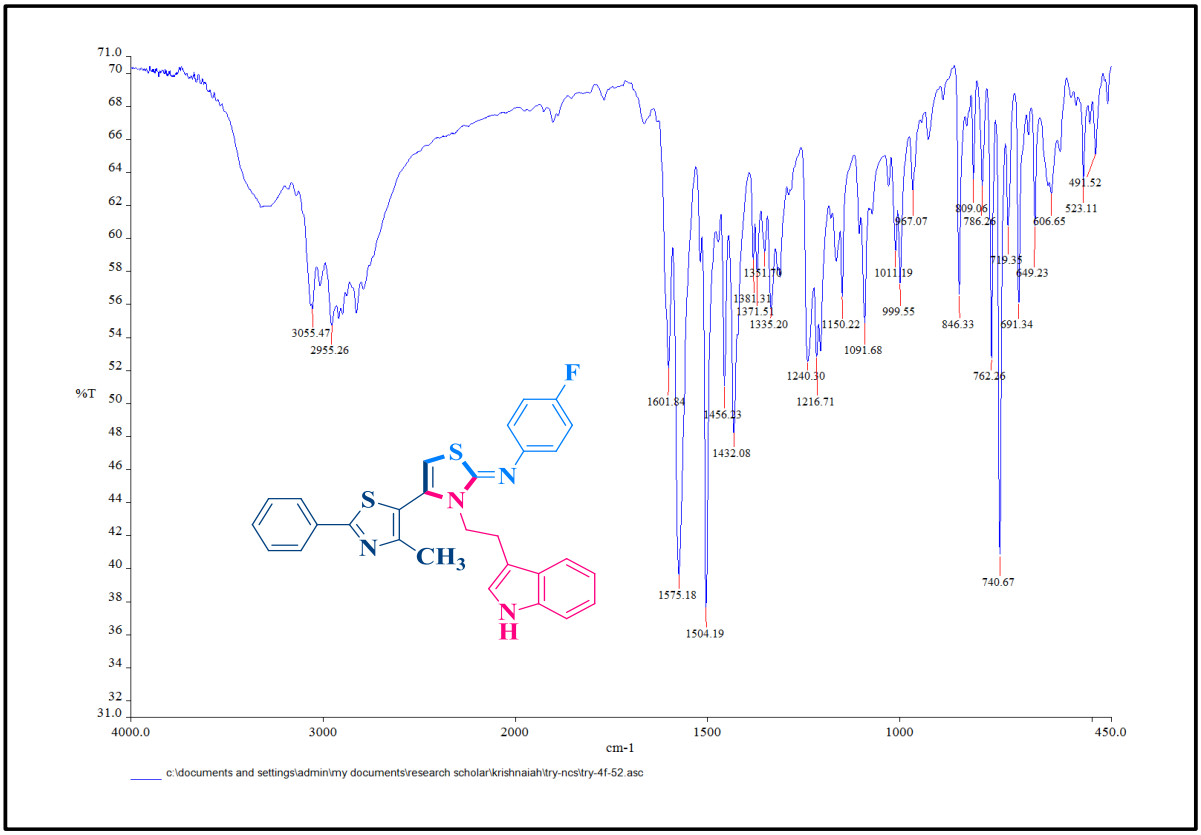


Fig: IR Spectrum of compound **51g**

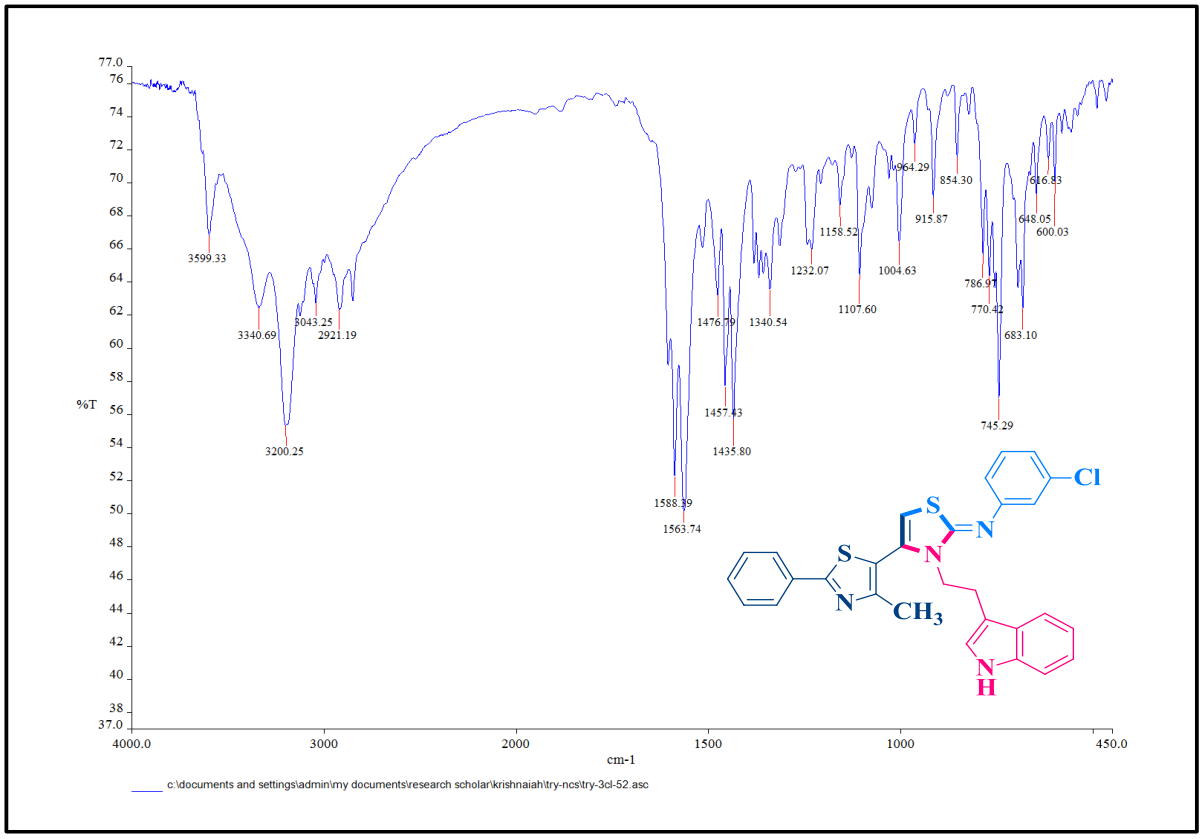


Fig: IR Spectrum of compound **51h**

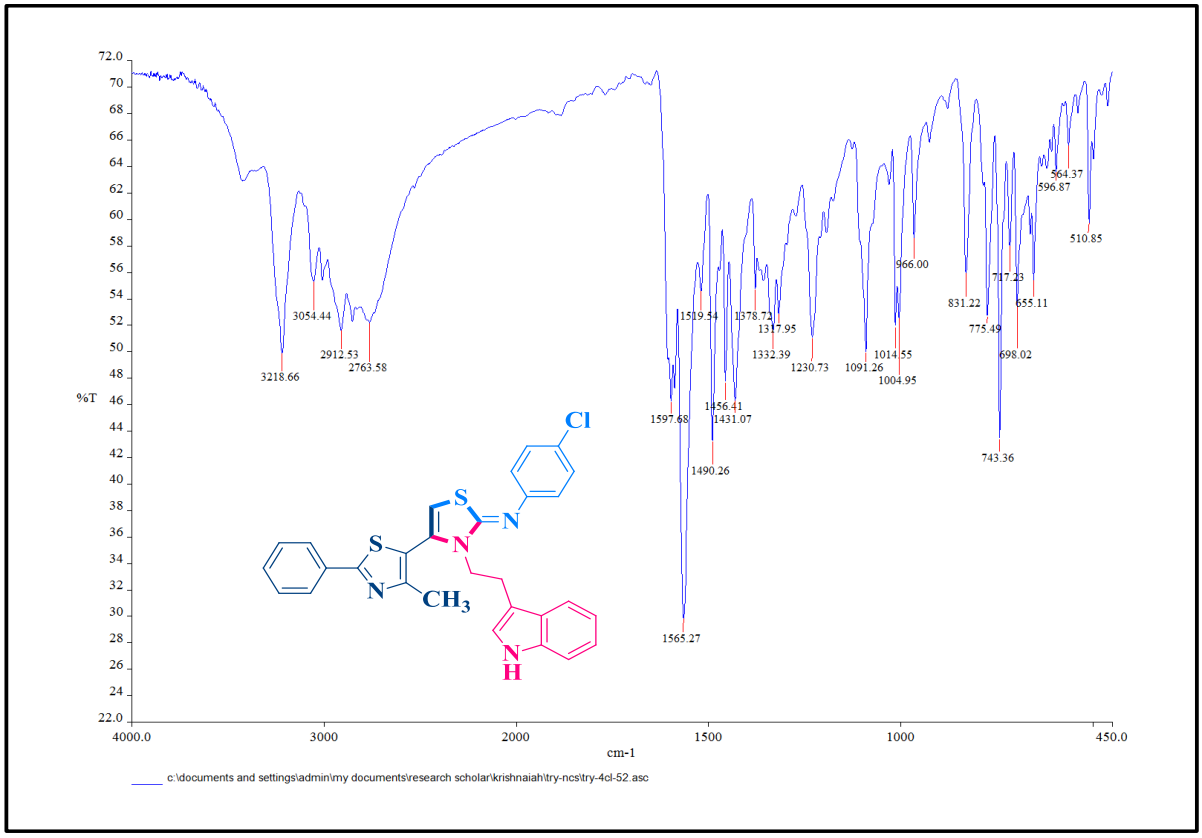


Fig: IR Spectrum of compound**51i**

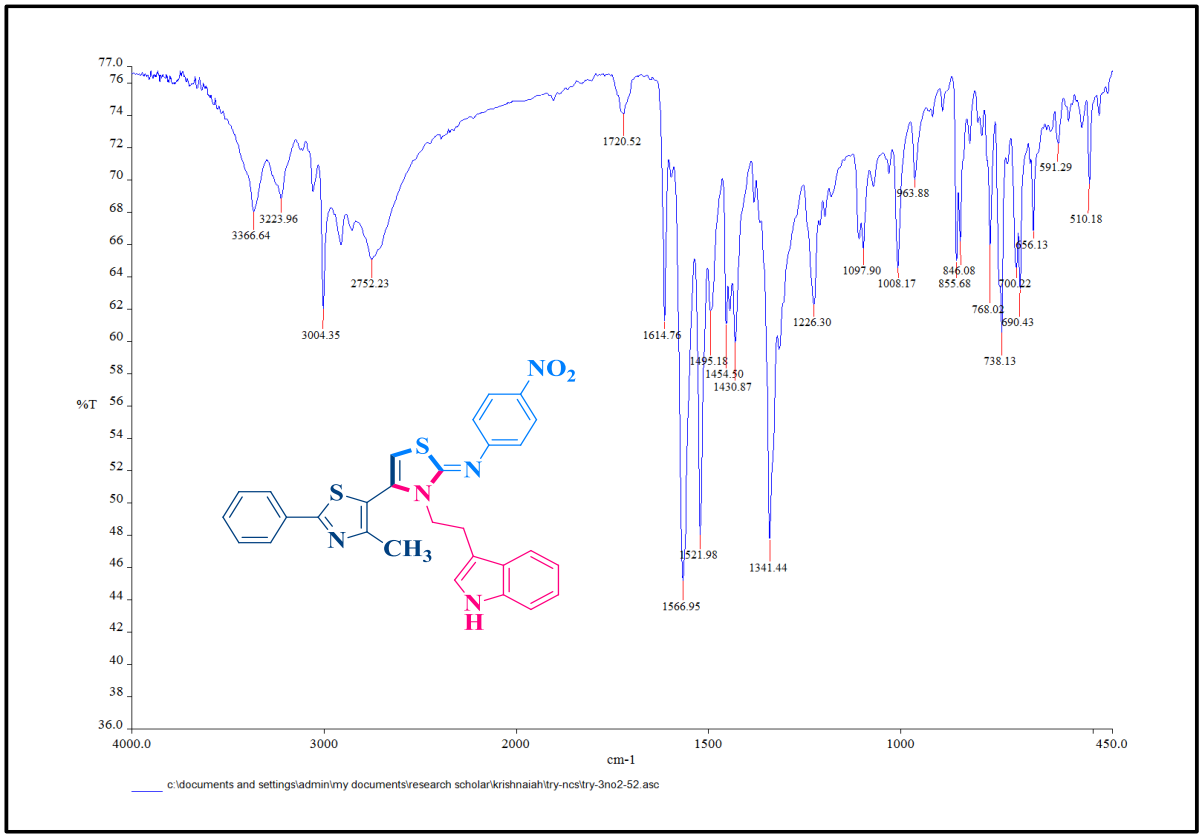


Fig: IR Spectrum of compound **51j**

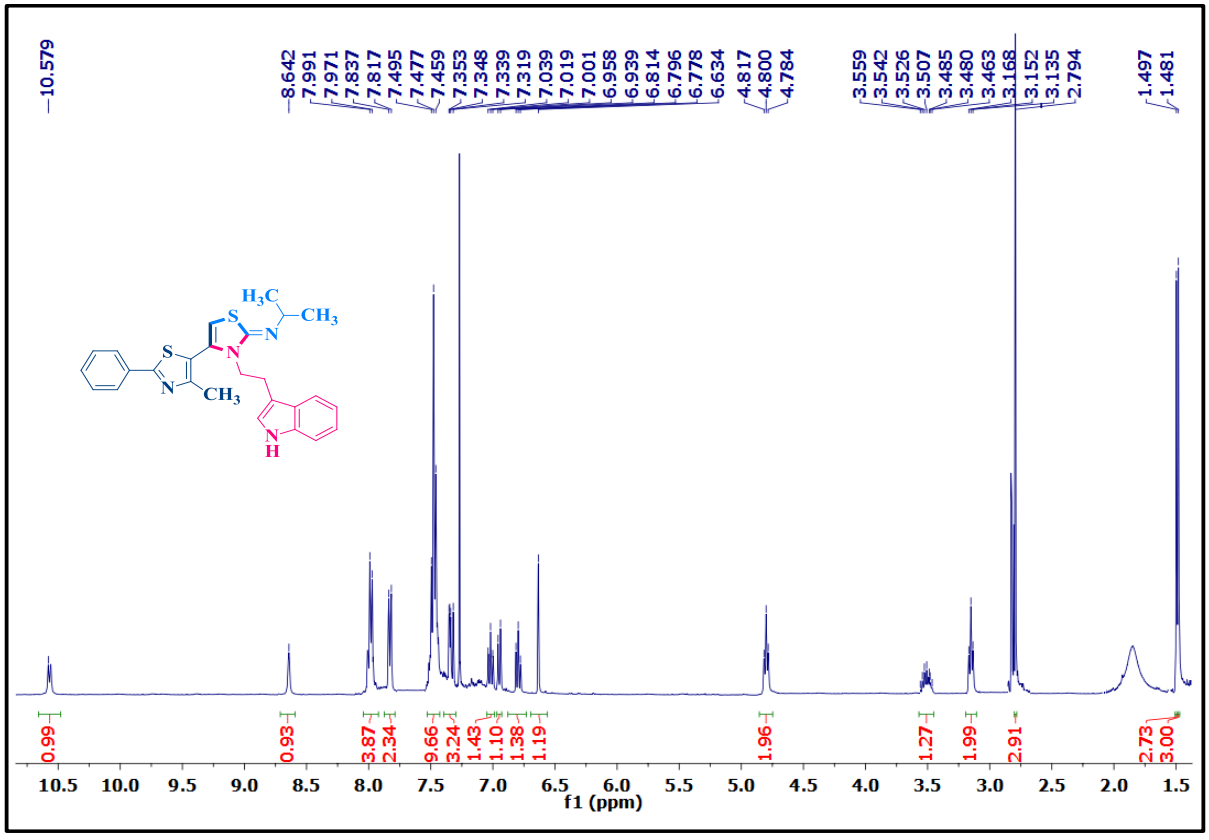


Fig: ^{1}H NMR Spectrum of compound **51a**

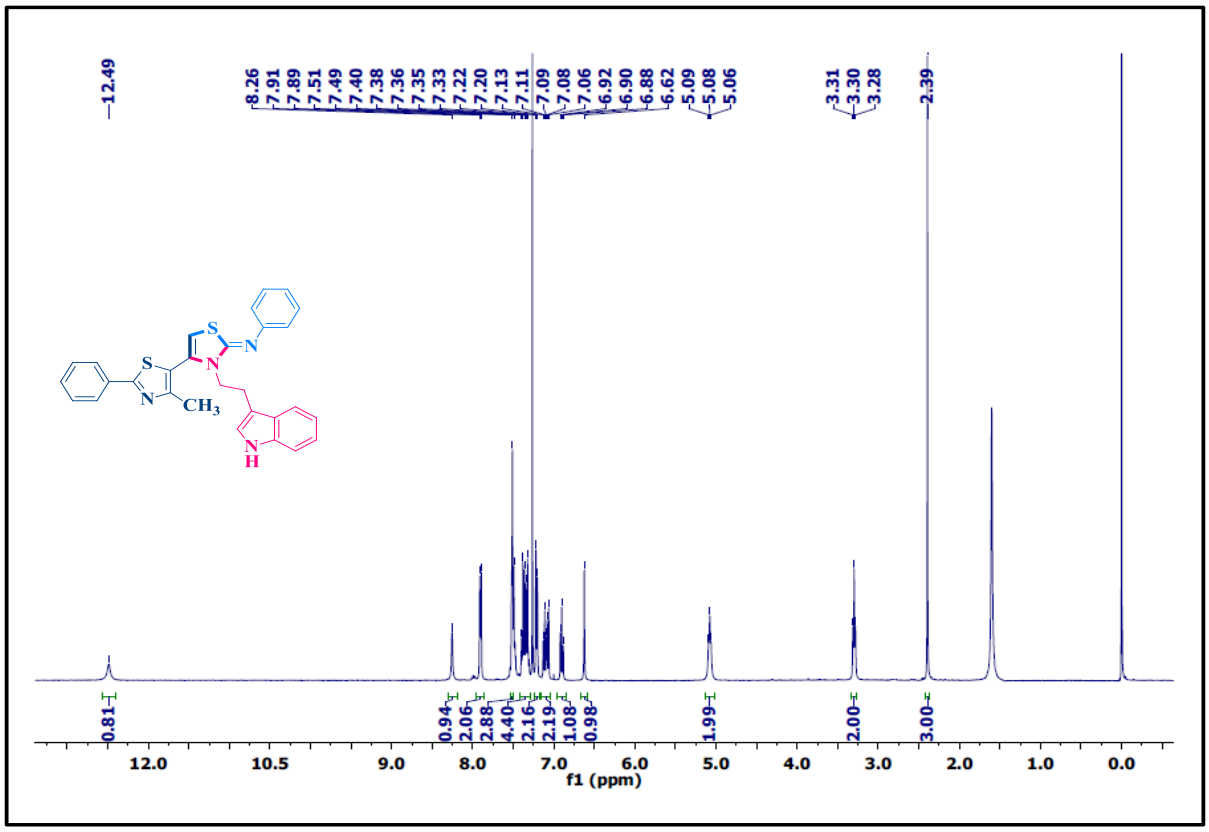


Fig: ^{1}H NMR Spectrum of compound **51b**

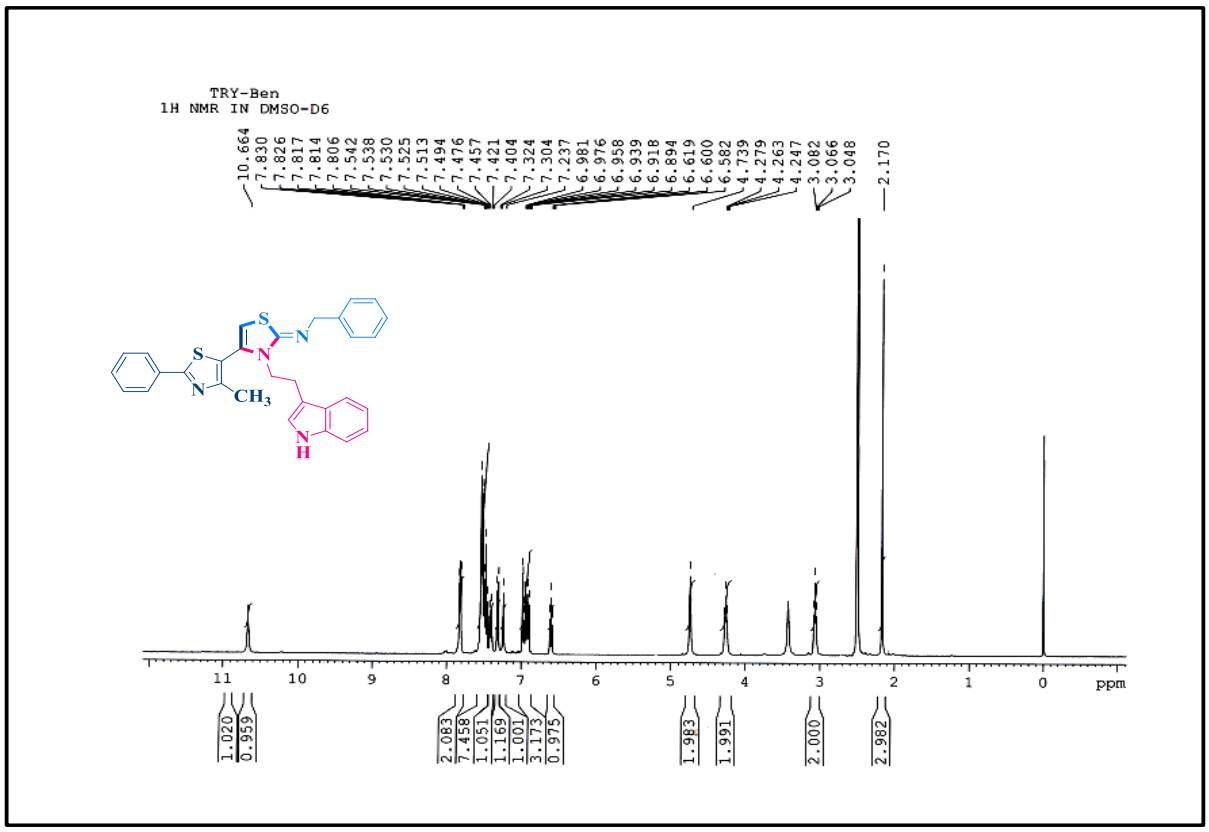


Fig: ^{1}H NMR Spectrum of compound **51c**

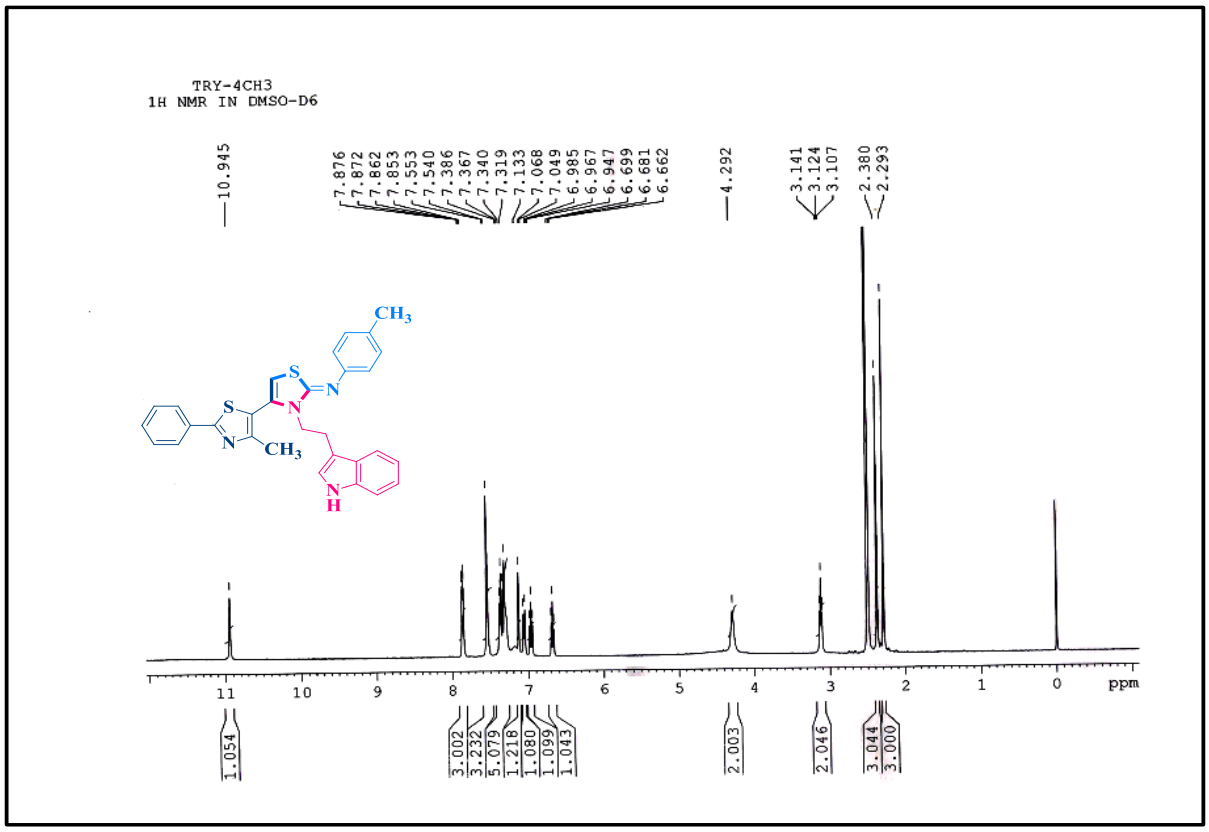


Fig: ^{1}H NMR Spectrum of compound **51d**

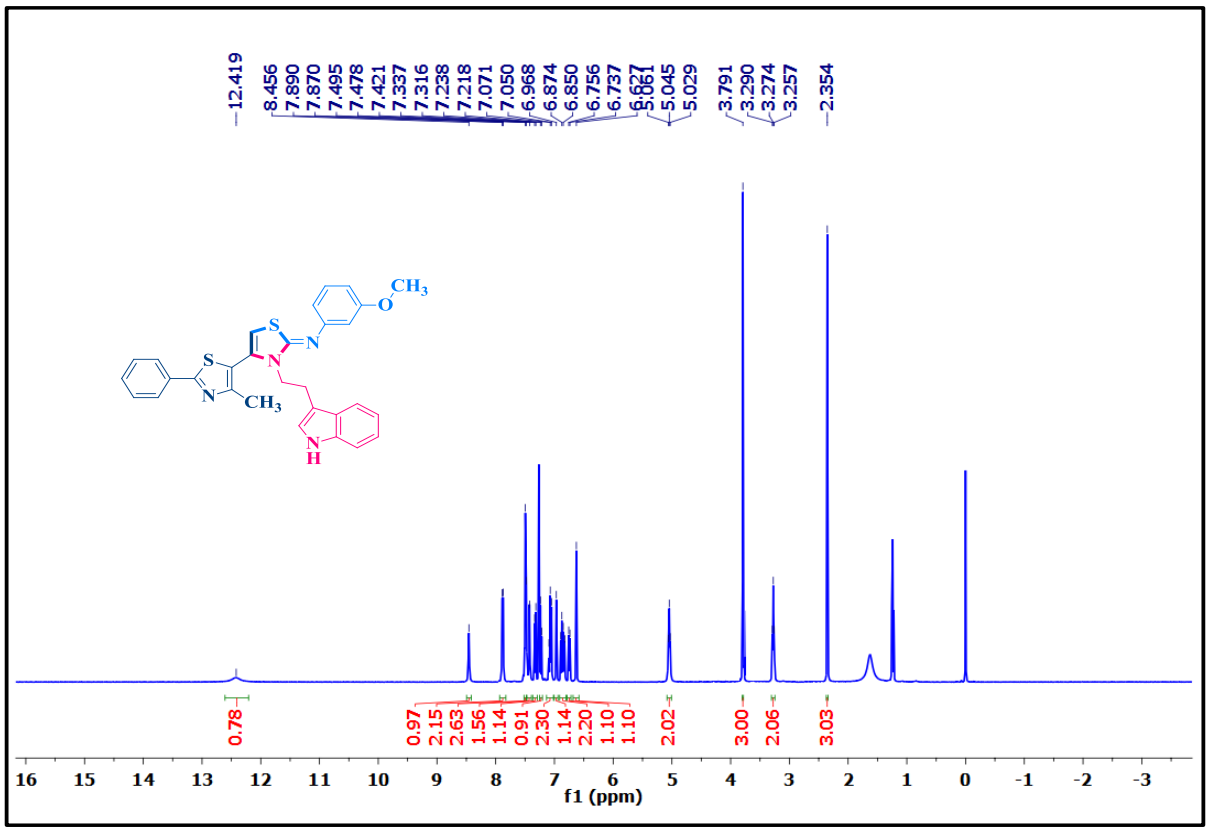


Fig: ^{1}H NMR Spectrum of compound **51e**

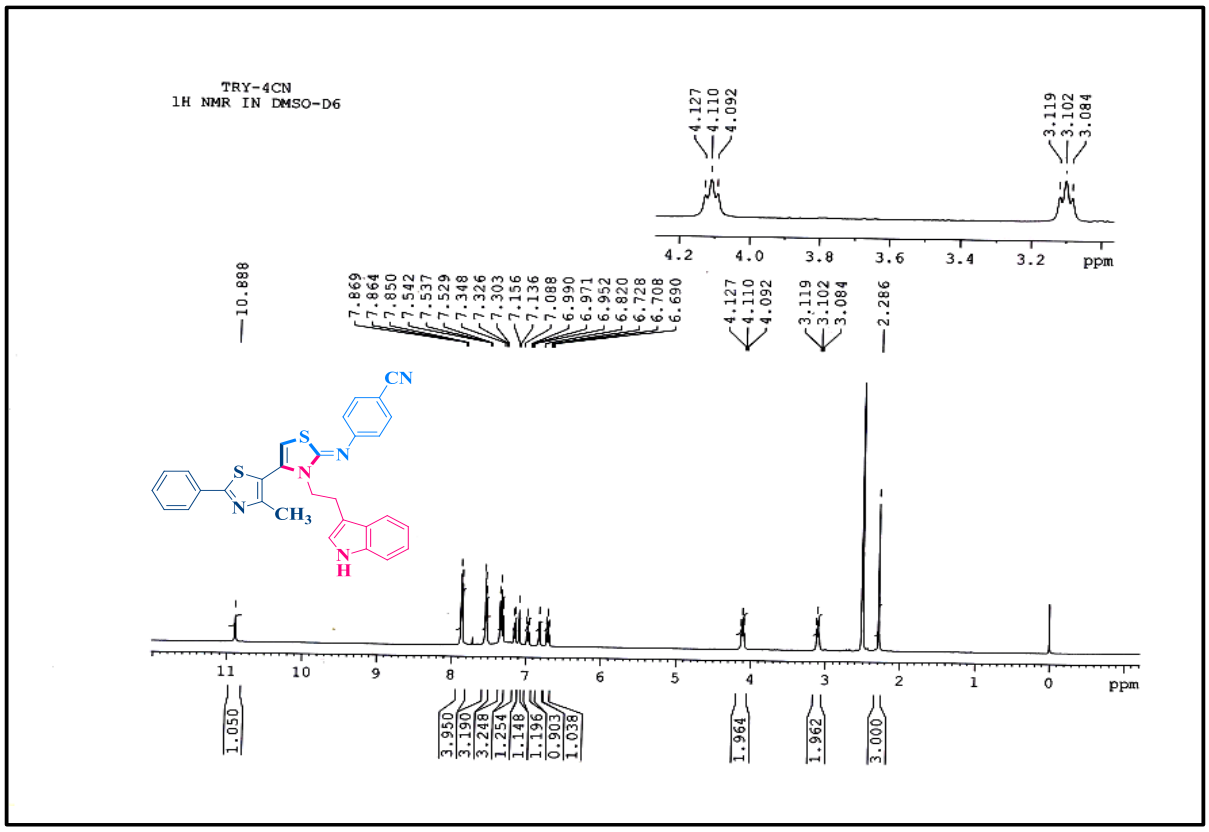


Fig: ^{1}H NMR Spectrum of compound **51f**

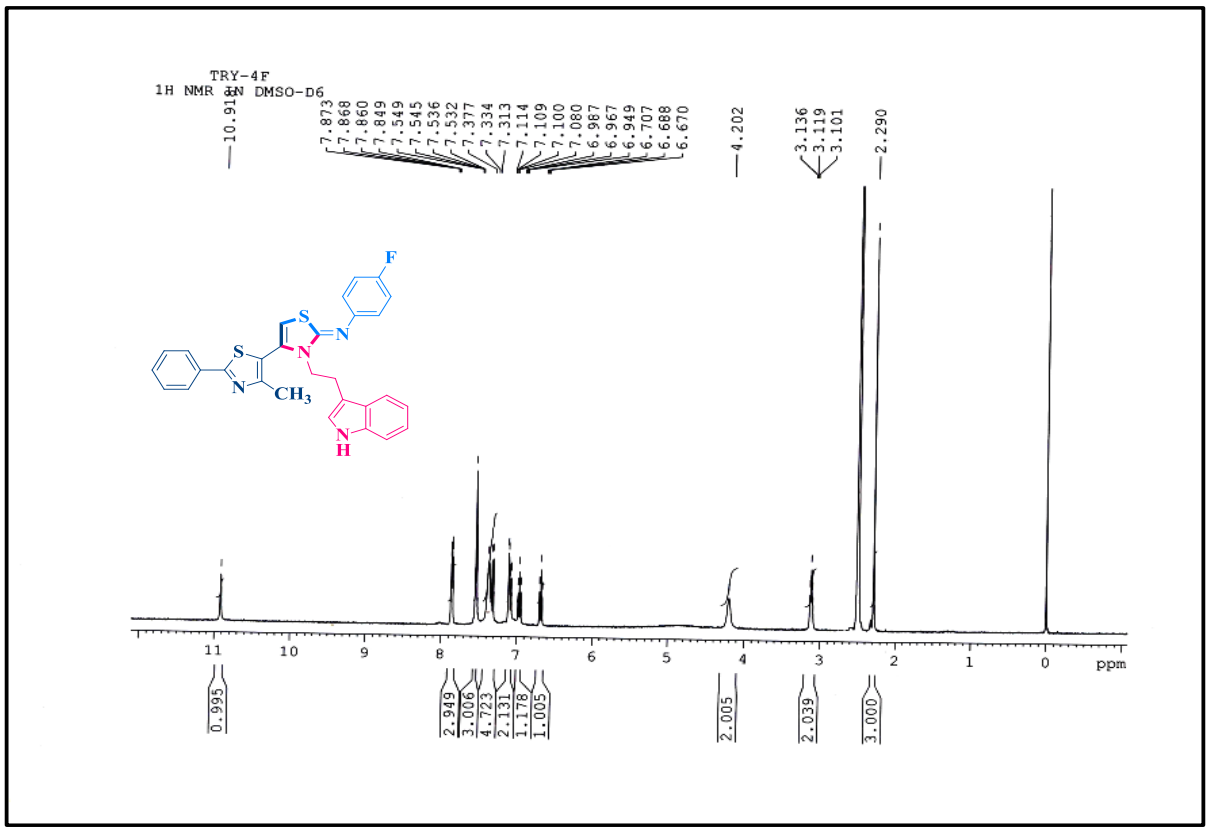


Fig: ^{1}H NMR Spectrum of compound **51g**

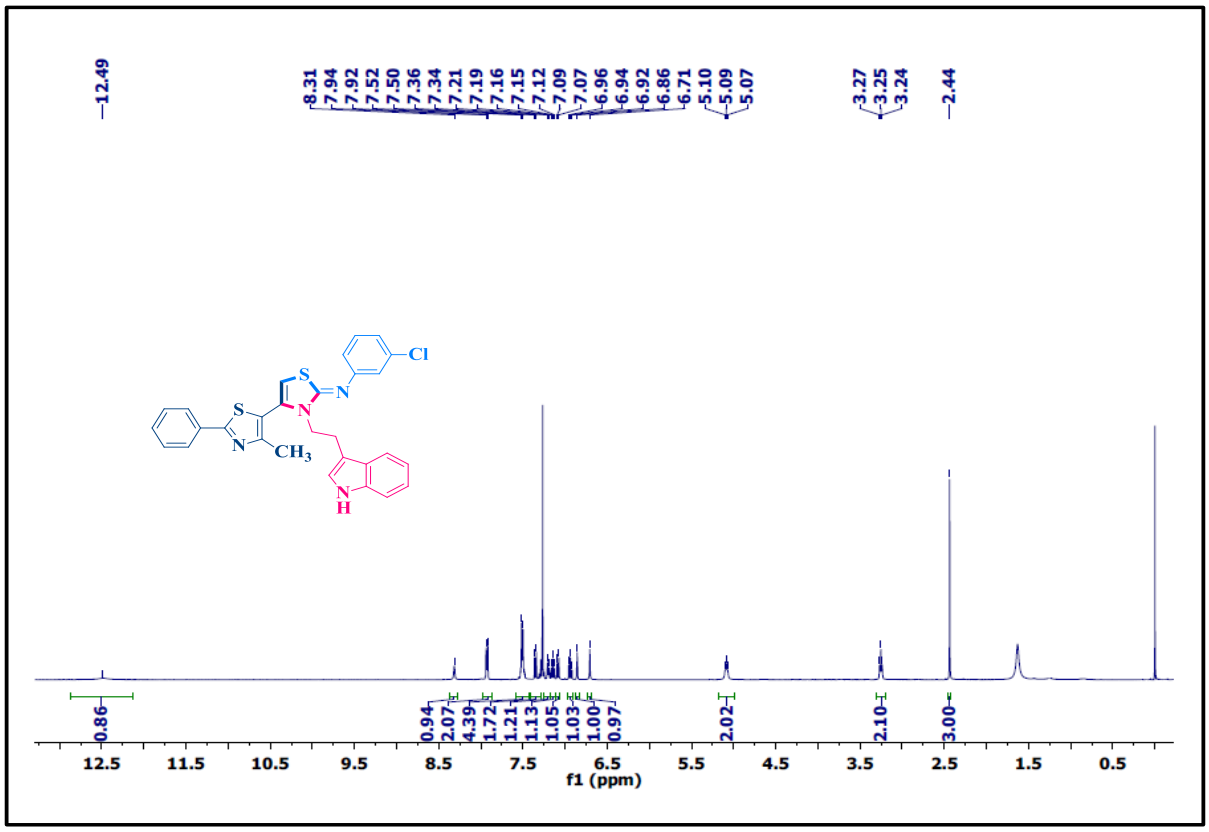


Fig: ^{1}H NMR Spectrum of compound **51h**

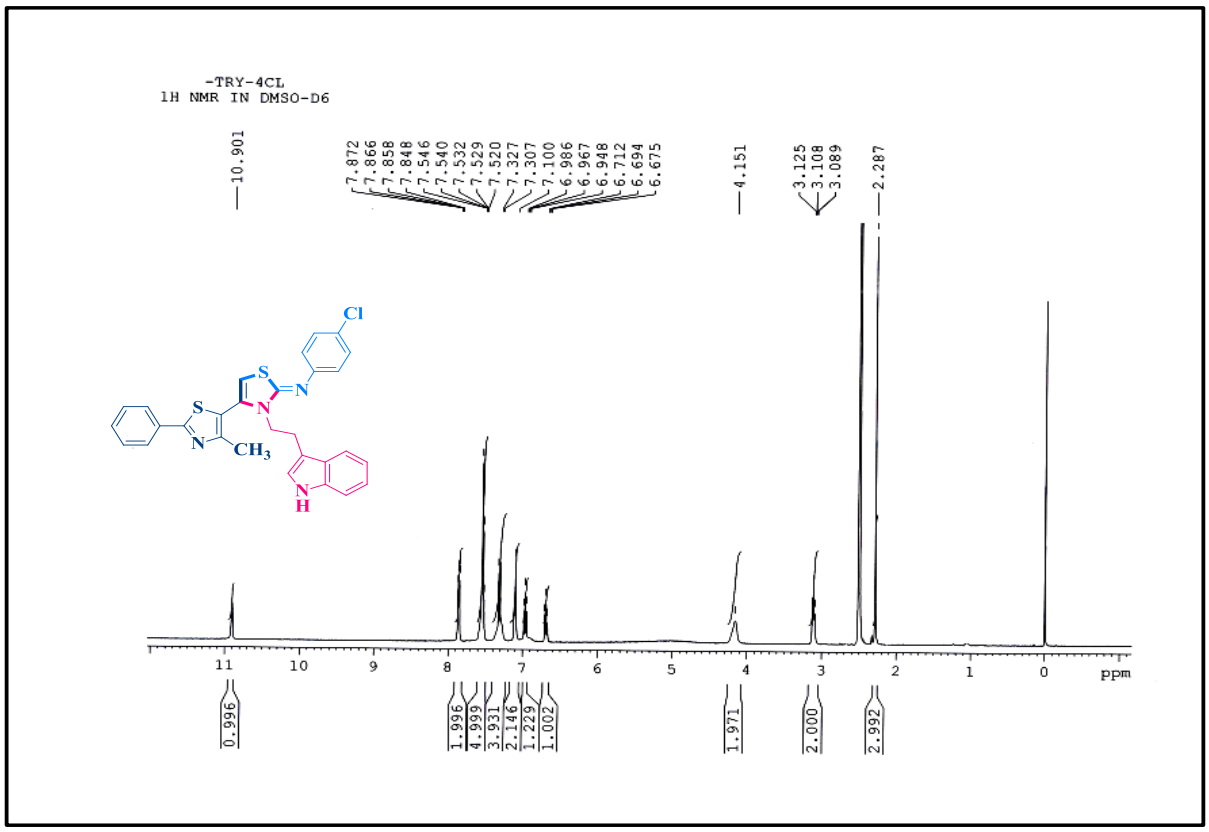


Fig: ^{1}H NMR Spectrum of compound **51i**

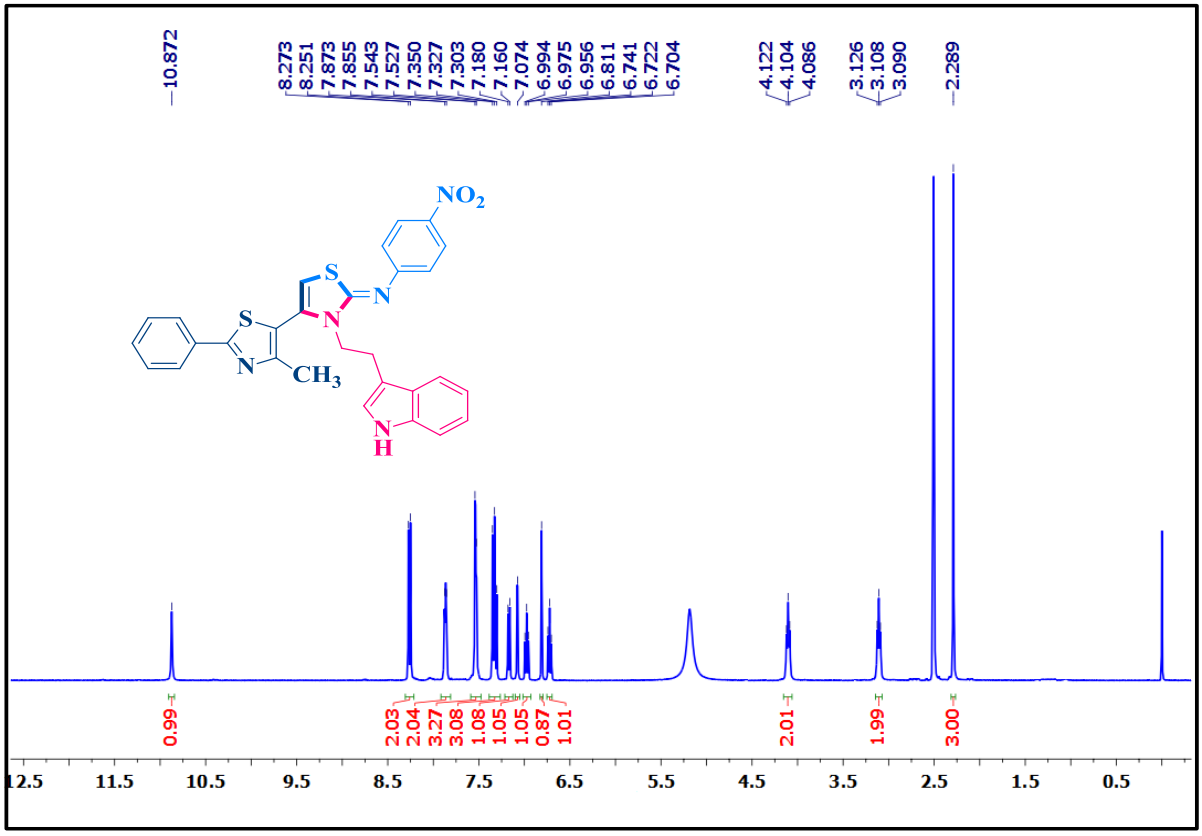


Fig: ^{1}H NMR Spectrum of compound **51j**

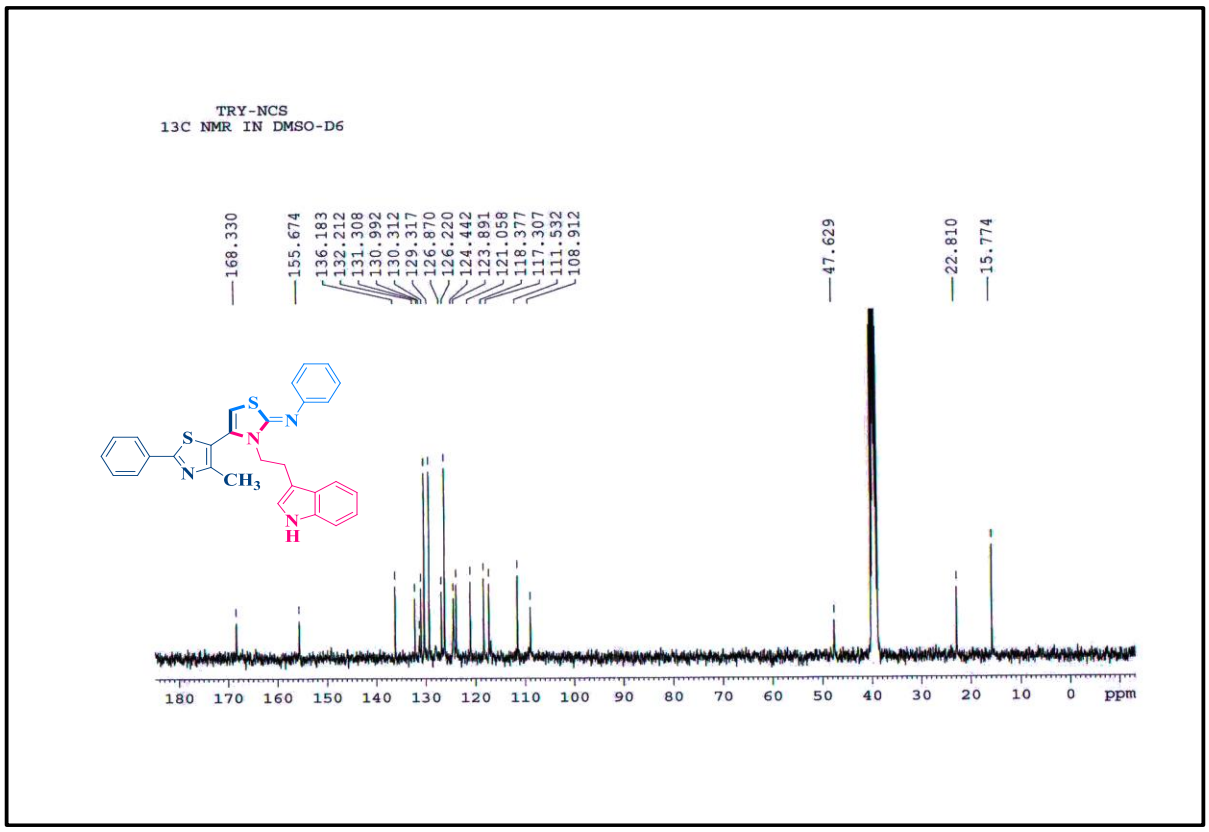


Fig: ^{13}C NMR Spectrum of compound **51b**

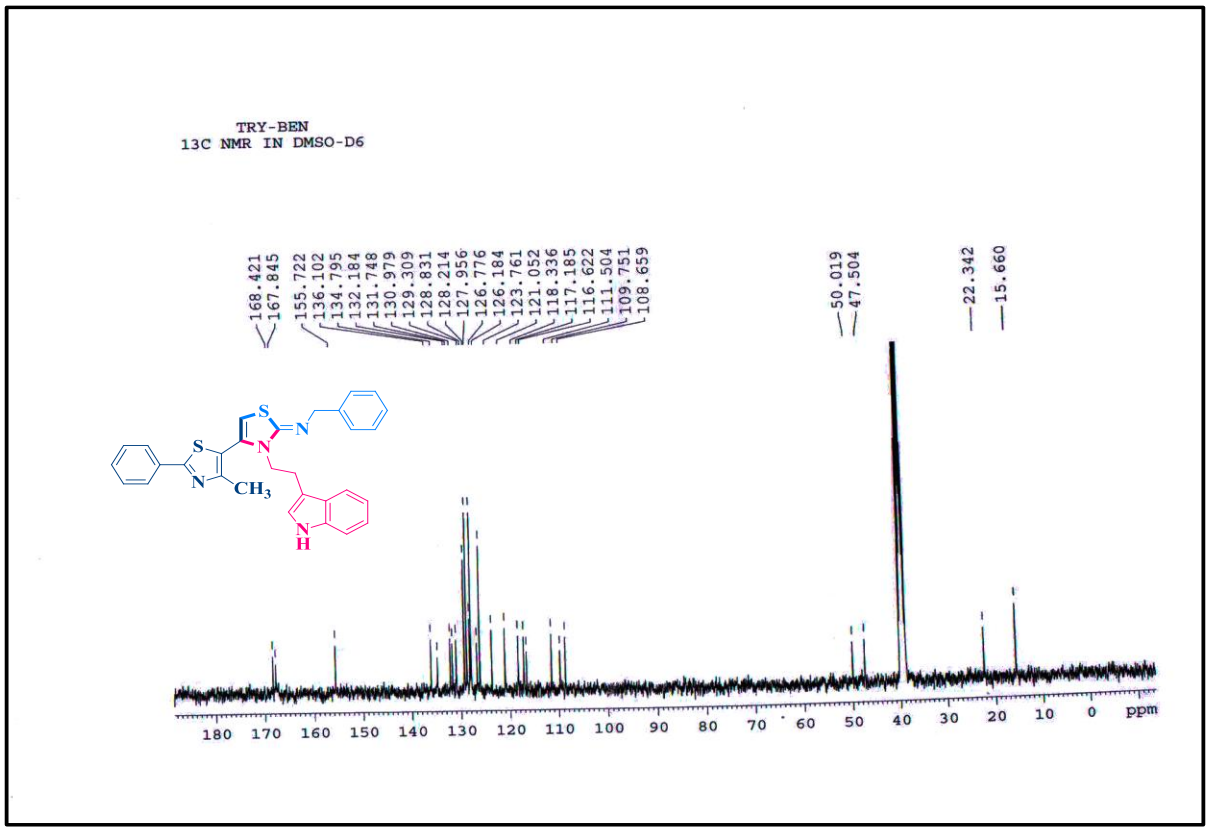


Fig: ^{13}C NMR Spectrum of compound **51c**

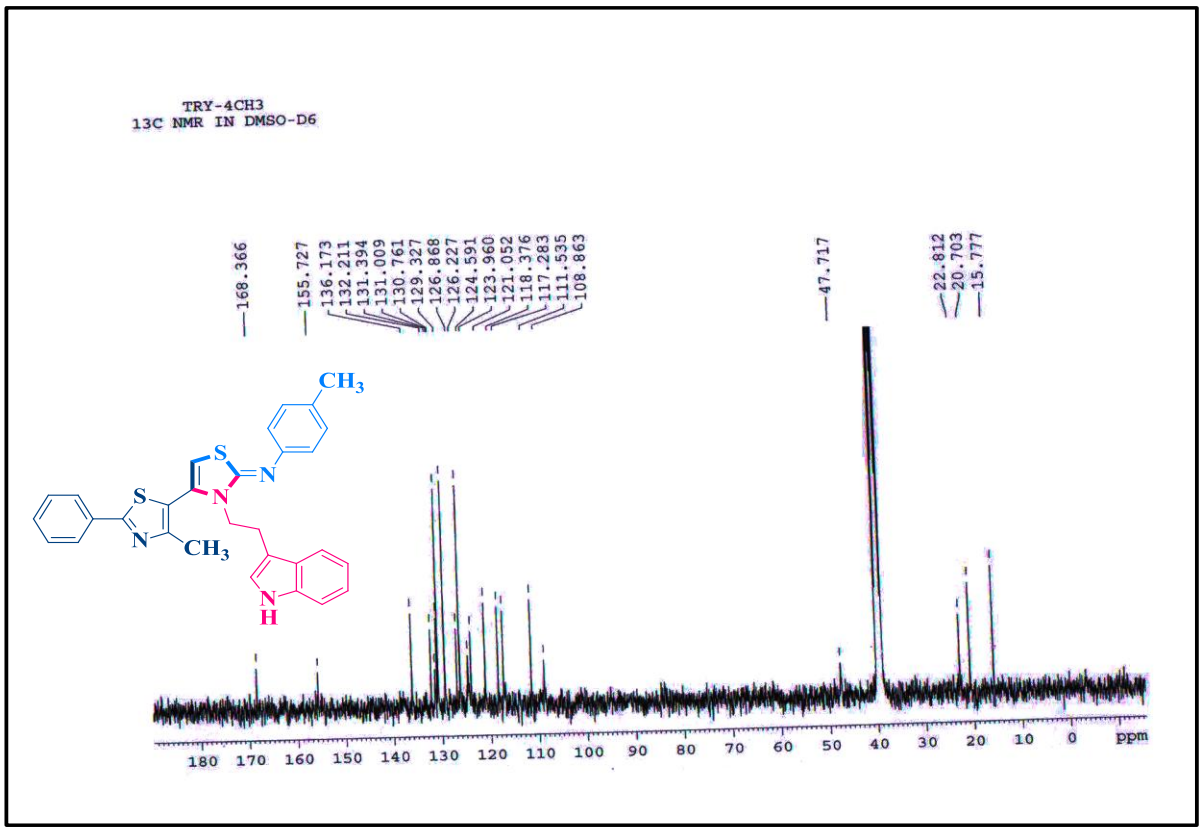


Fig: ^{13}C NMR Spectrum of compound **51d**

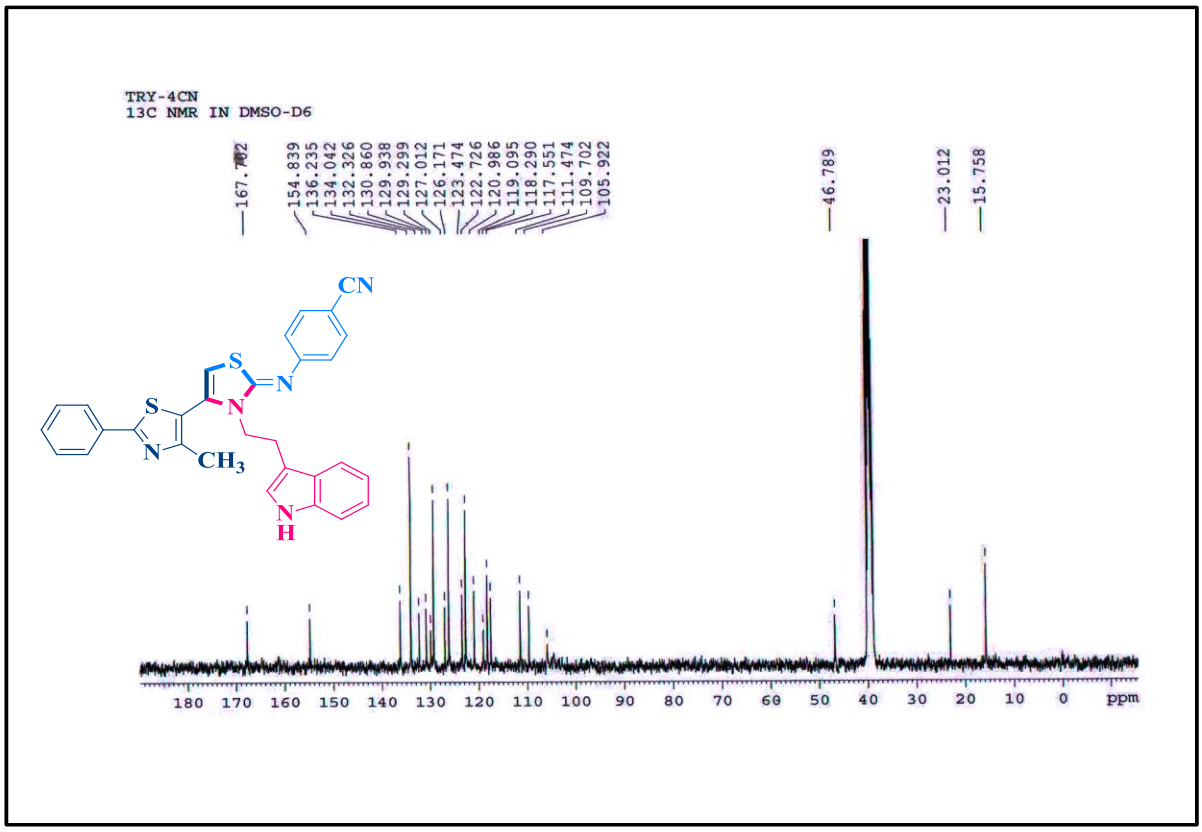


Fig: ^{13}C NMR Spectrum of compound **51f**

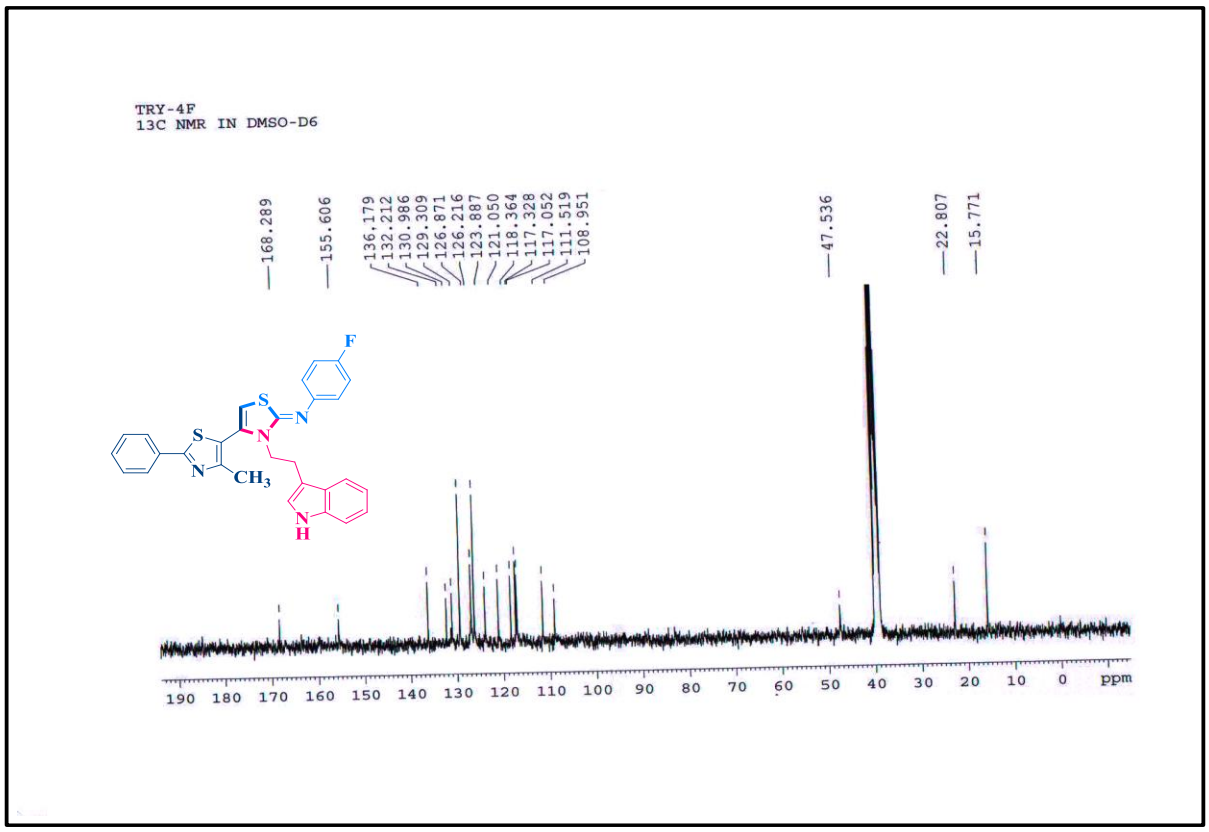


Fig: ^{13}C NMR Spectrum of compound **51g**

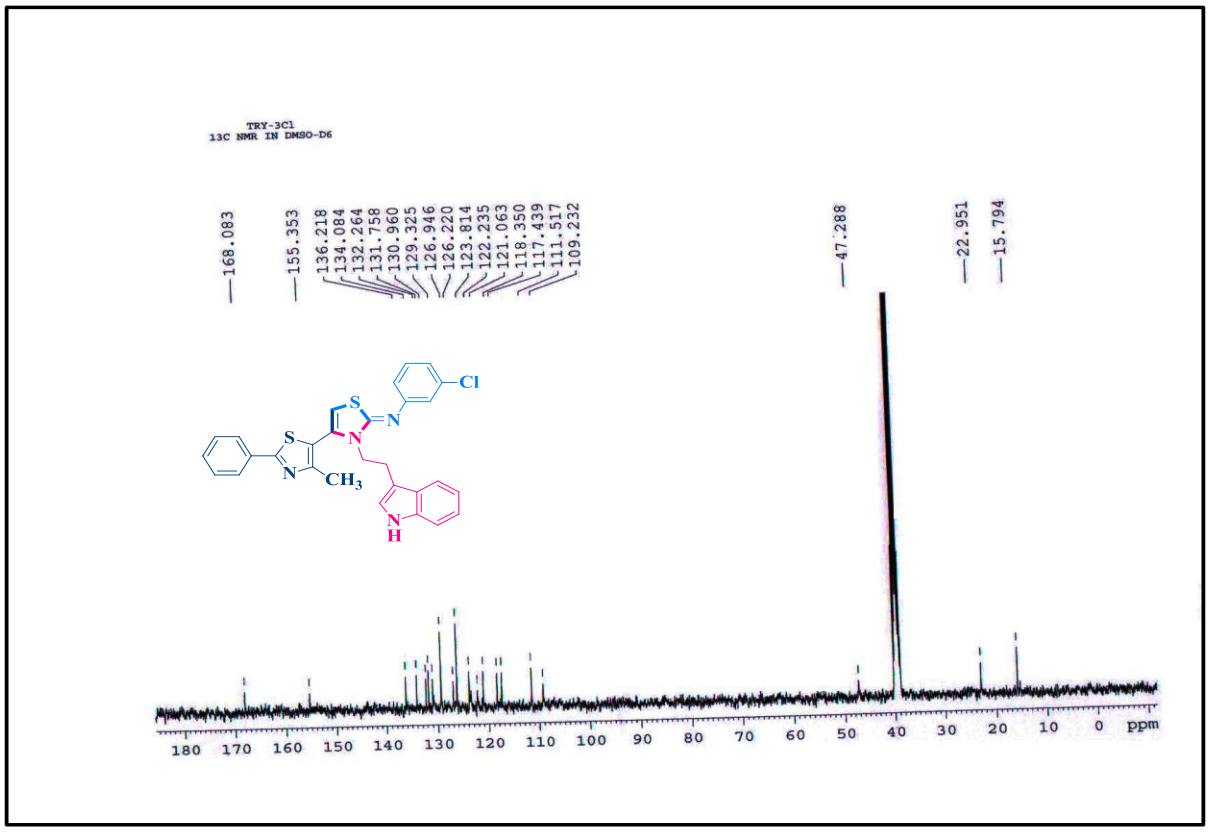


Fig: ^{13}C NMR Spectrum of compound **51h**

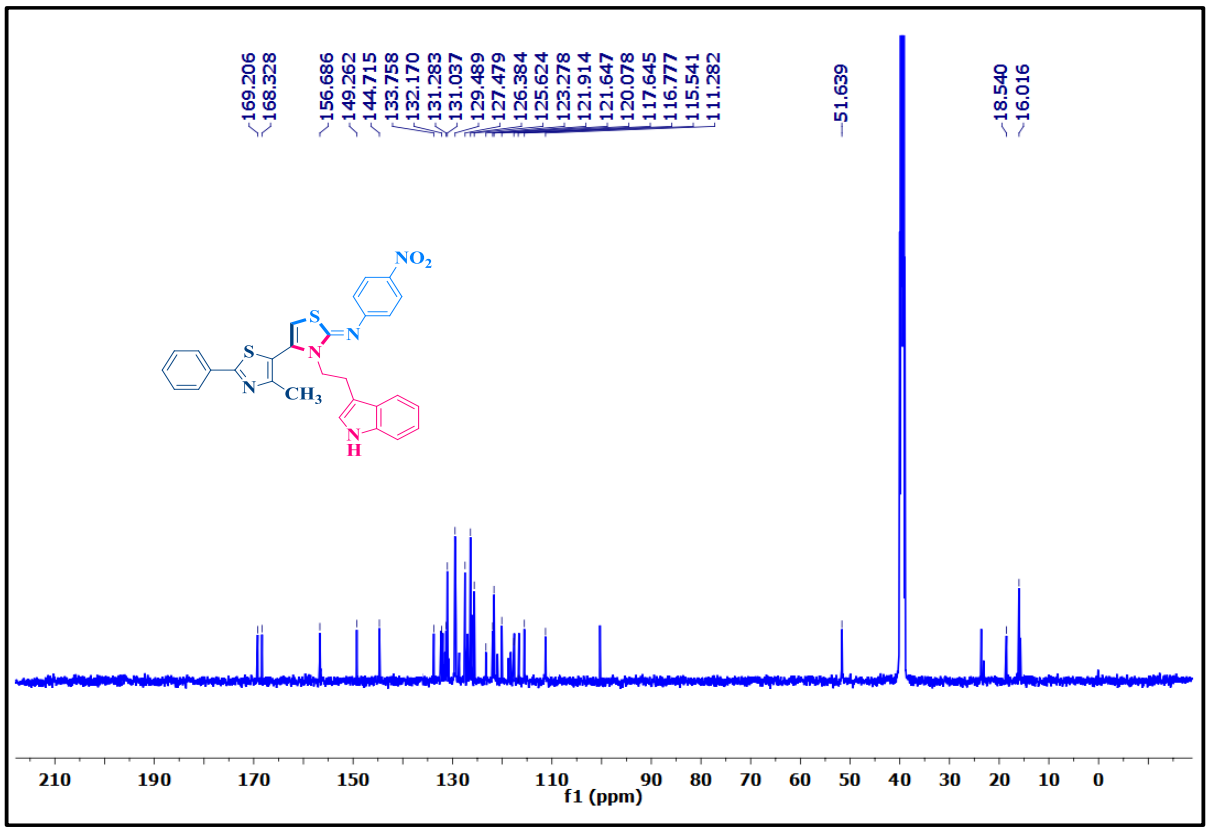


Fig: ^{13}C NMR Spectrum of compound **51j**

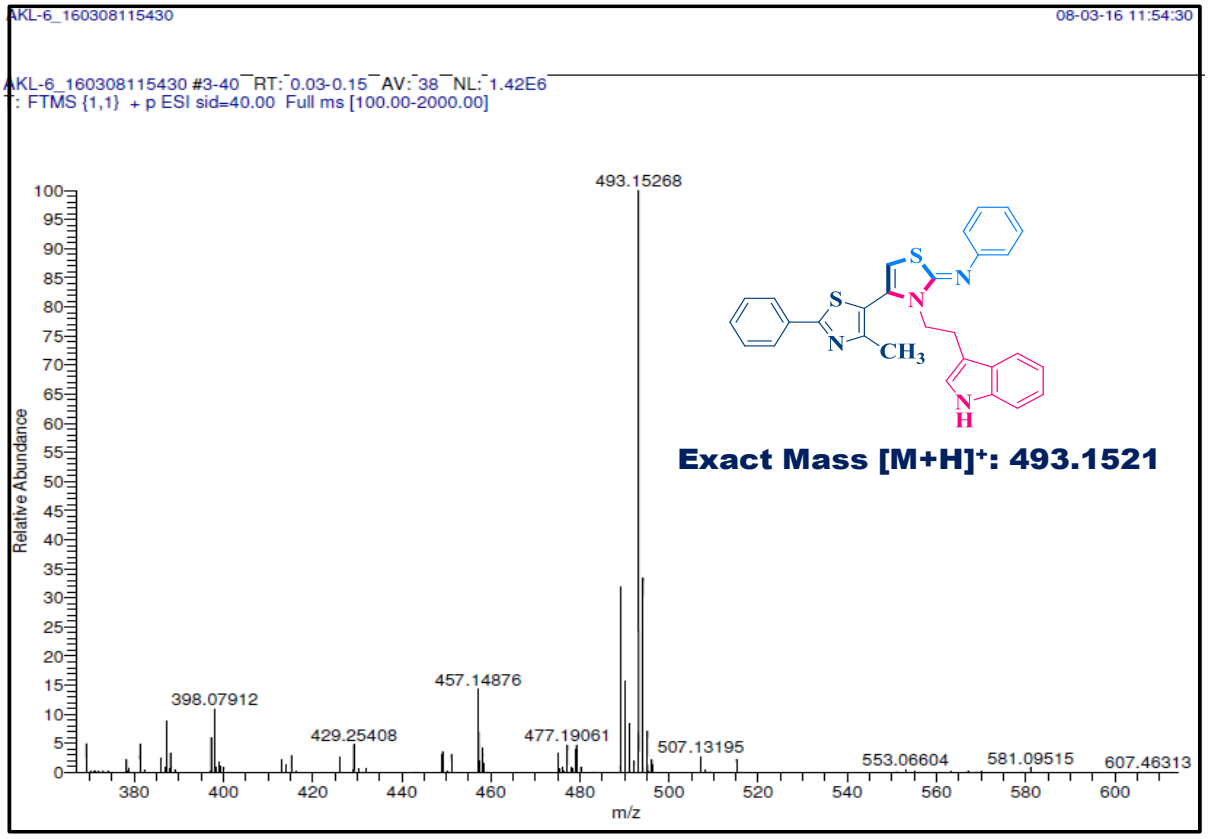


Fig: Mass Spectrum of compound **51b**

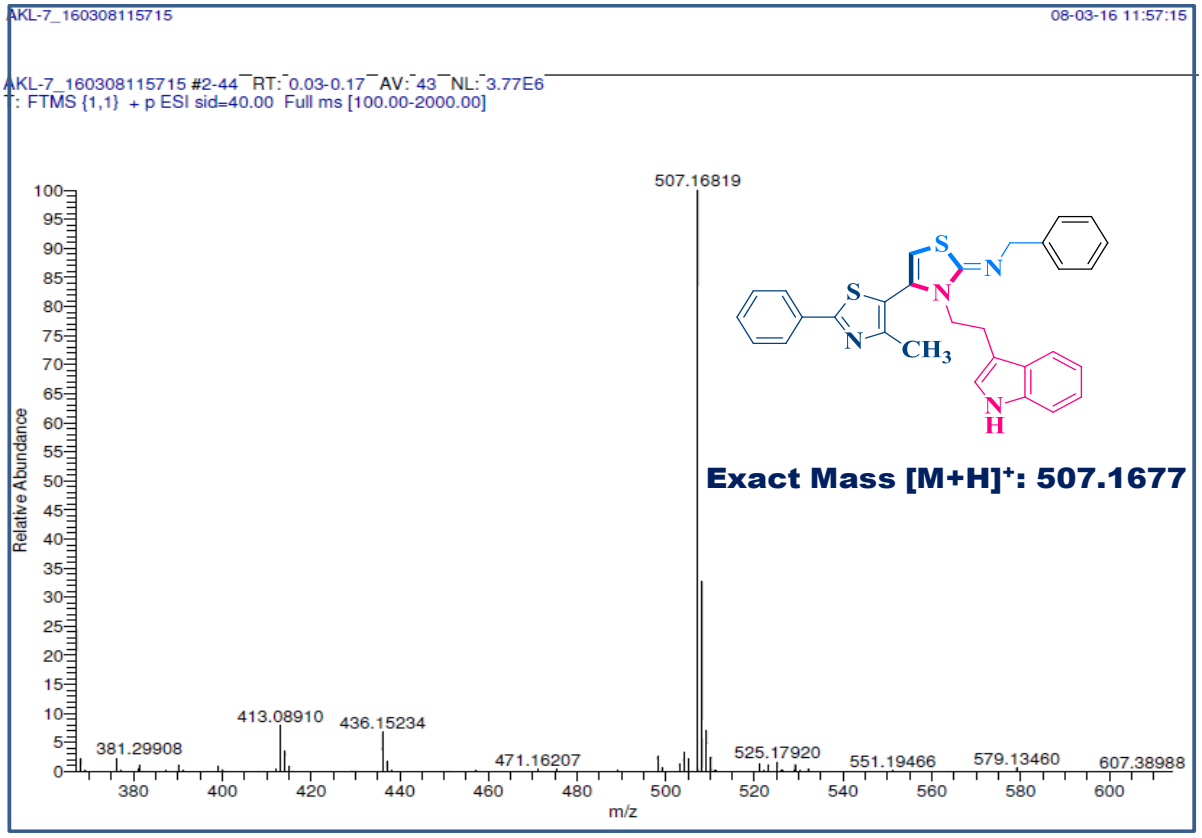


Fig: Mass Spectrum of compound **51c**

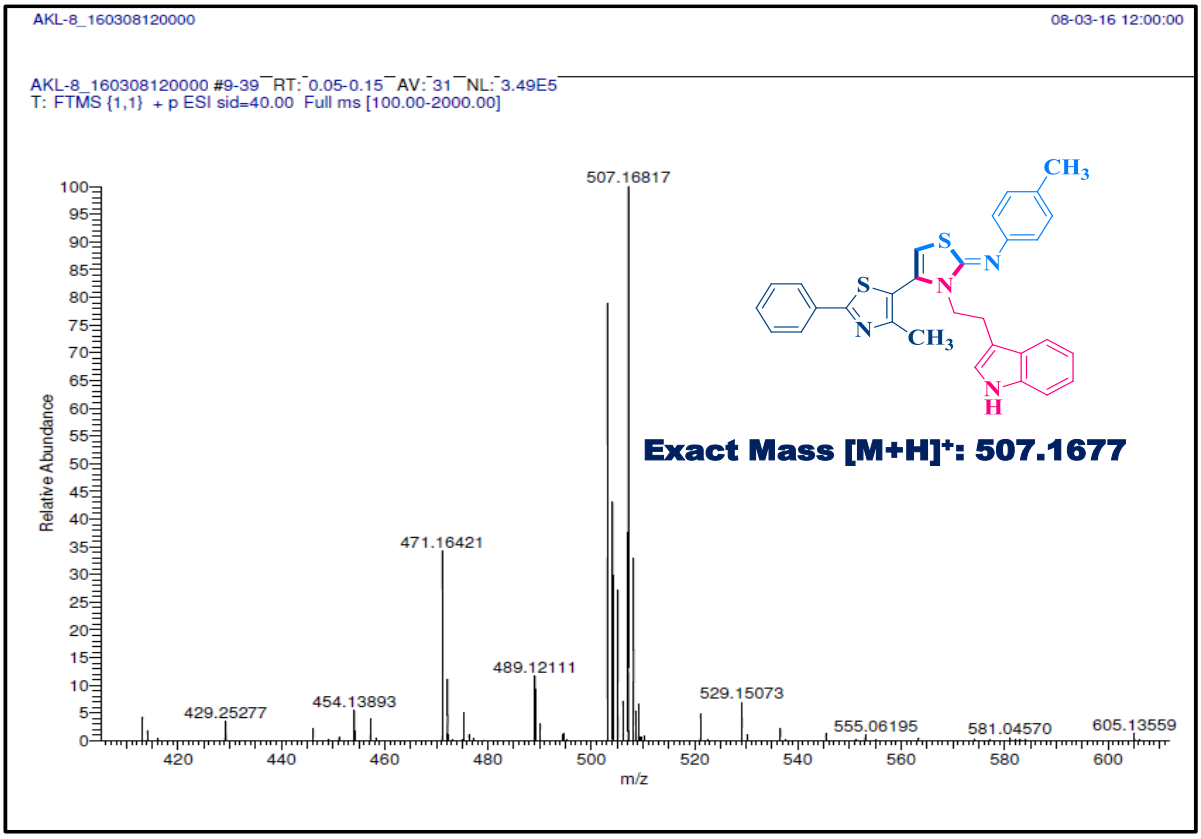


Fig: Mass Spectrum of compound **51d**

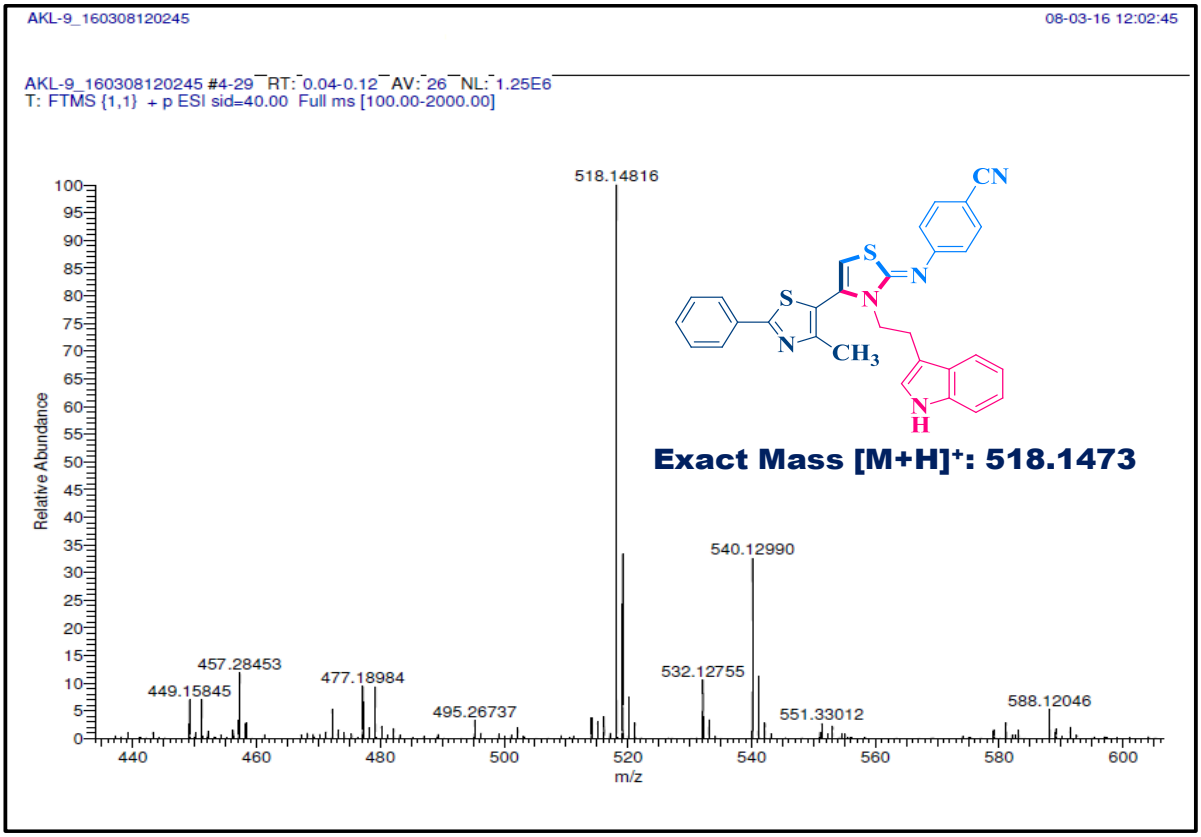


Fig: Mass Spectrum of compound **51f**

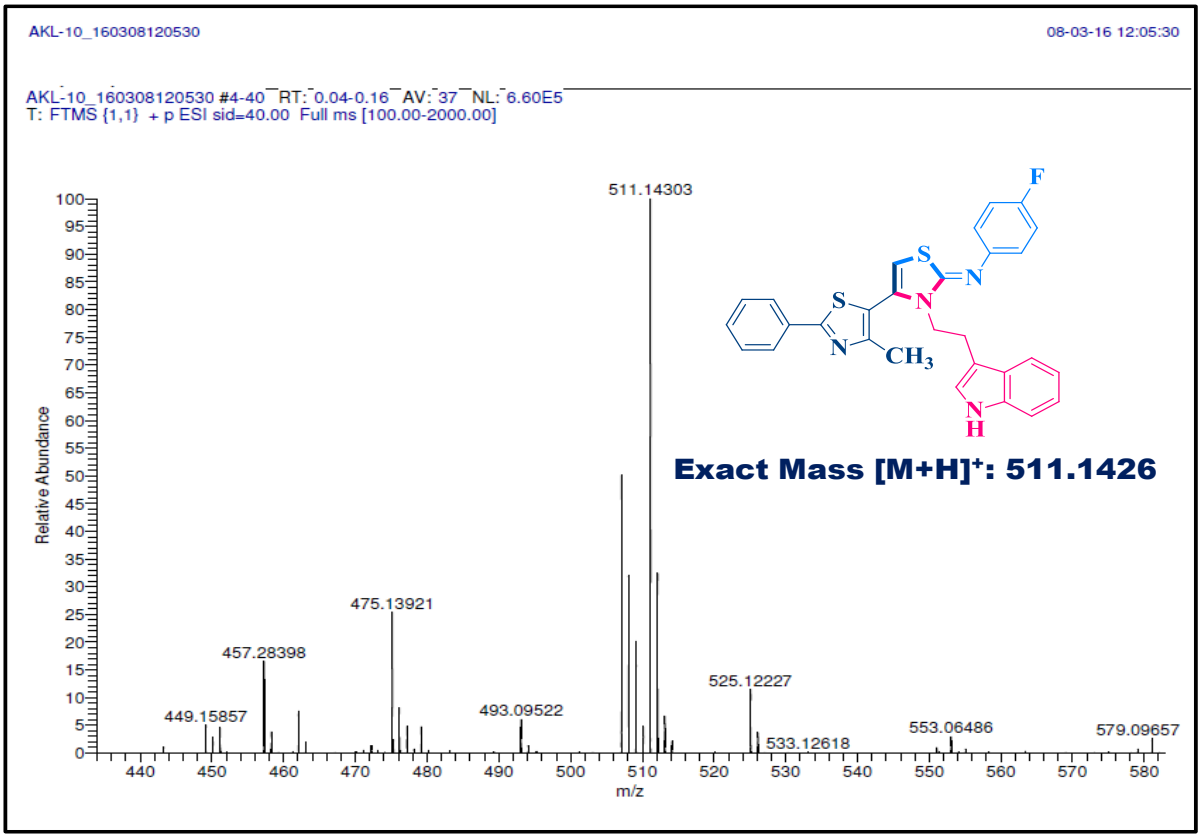


Fig: Mass Spectrum of compound **51g**

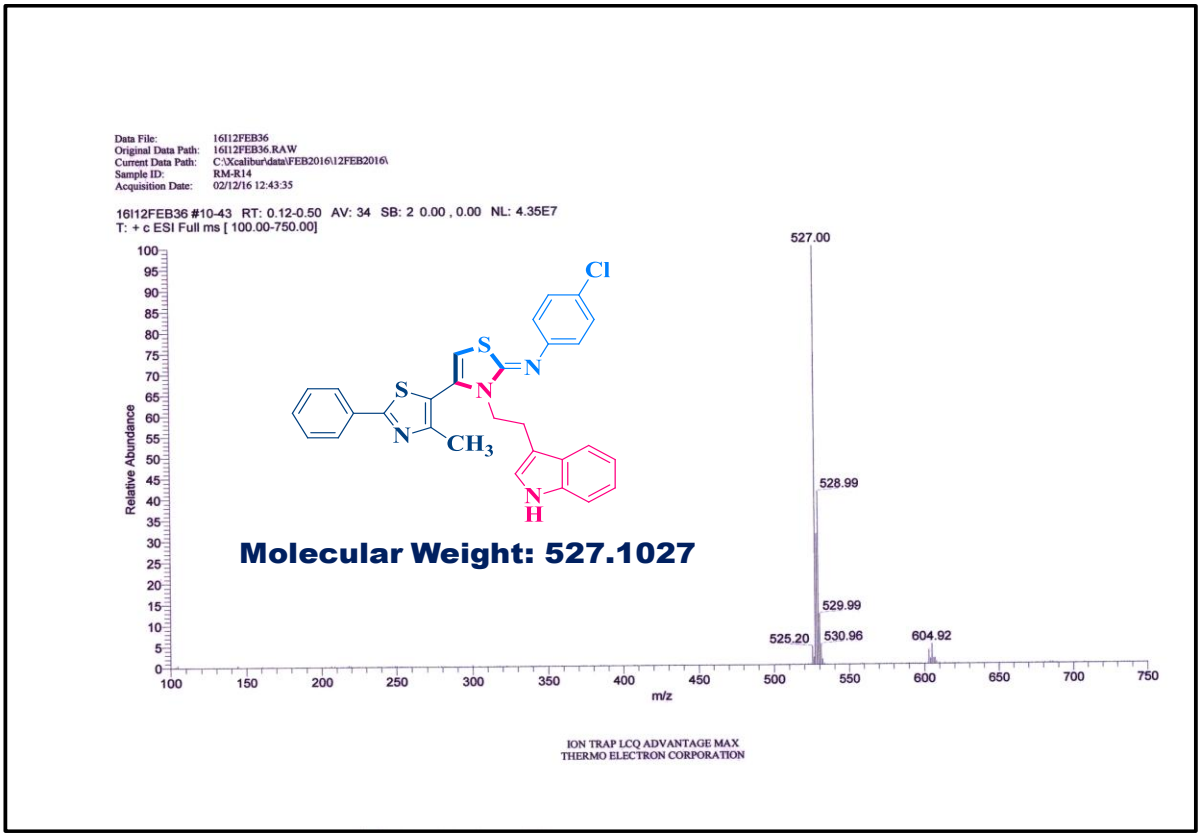


Fig: Mass Spectrum of compound **51i**

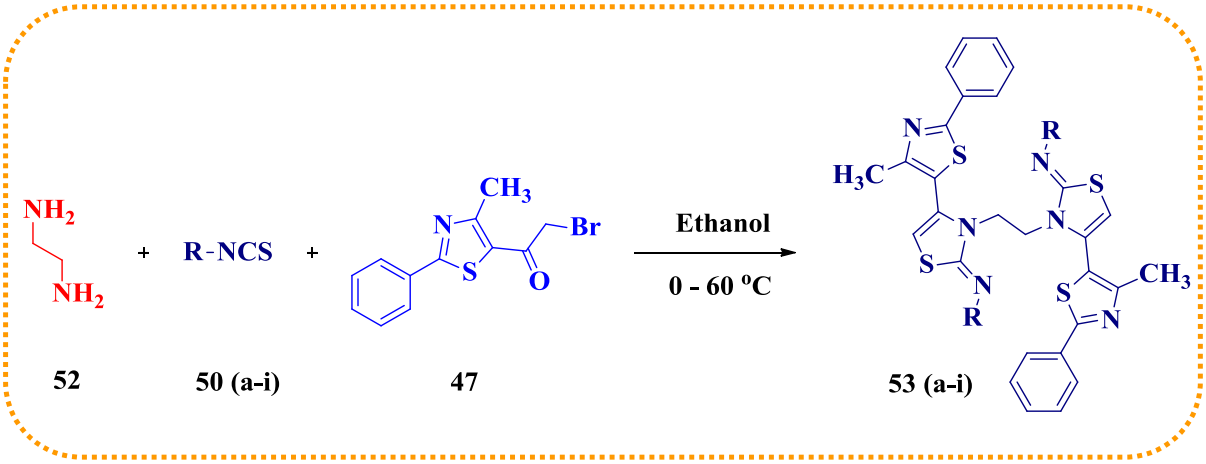
CHAPTER-VII
PART-C

A FACILE ONE POT PSEUDO FIVE COMPONENT REACTION OF 3,3''-(ETHANE-1,2-DIYL)BIS(4'-METHYL-2'-PHENYL-[4,5'-BITHIAZOLE]-3(3*H*)-YL-2(3*H*)-YLIDENE)) DIANILINE DERIVATIVES AND THEIR ANTIVIRAL ACTIVITY

PART-C

PRESENT WORK:

As described in the introduction part of the present chapter, the methods available for the synthesis of thiazoles and the applications of thiazoles, there is a necessary to develop the efficient synthetic methods with simple, environmentally benign, catalyst free and simple reaction work up. The present work describes an efficient one pot pseudo five component reaction of 3,3''-(ethane-1,2-diyl)bis(4'-methyl-2'-phenyl-[4,5'-bithiazole]-3(3*H*)-yl-2(3*H*)-ylidene))dianiline derivatives via a multicomponent reaction method with simple reaction conditions. The target compounds were synthesized by the reaction of ethylenediamine with two equivalents of alkyl/arylisothiocyanates at 0-5 °C followed by the addition of two equivalents of 2-bromo-1-(4-methyl-2-phenylthiazol-5-yl)ethanone in the presence of ethanol as a solvent under reflux conditions as shown in **Scheme 5**.The present method used for the synthesis of 3,3''-(ethane-1,2-diyl)bis(4'-methyl-2'-phenyl-[4,5'-bithiazole]-3(3*H*)-yl-2(3*H*)-ylidene))di aniline derivatives provide good yields with a shorter reaction time (**Table 13**).

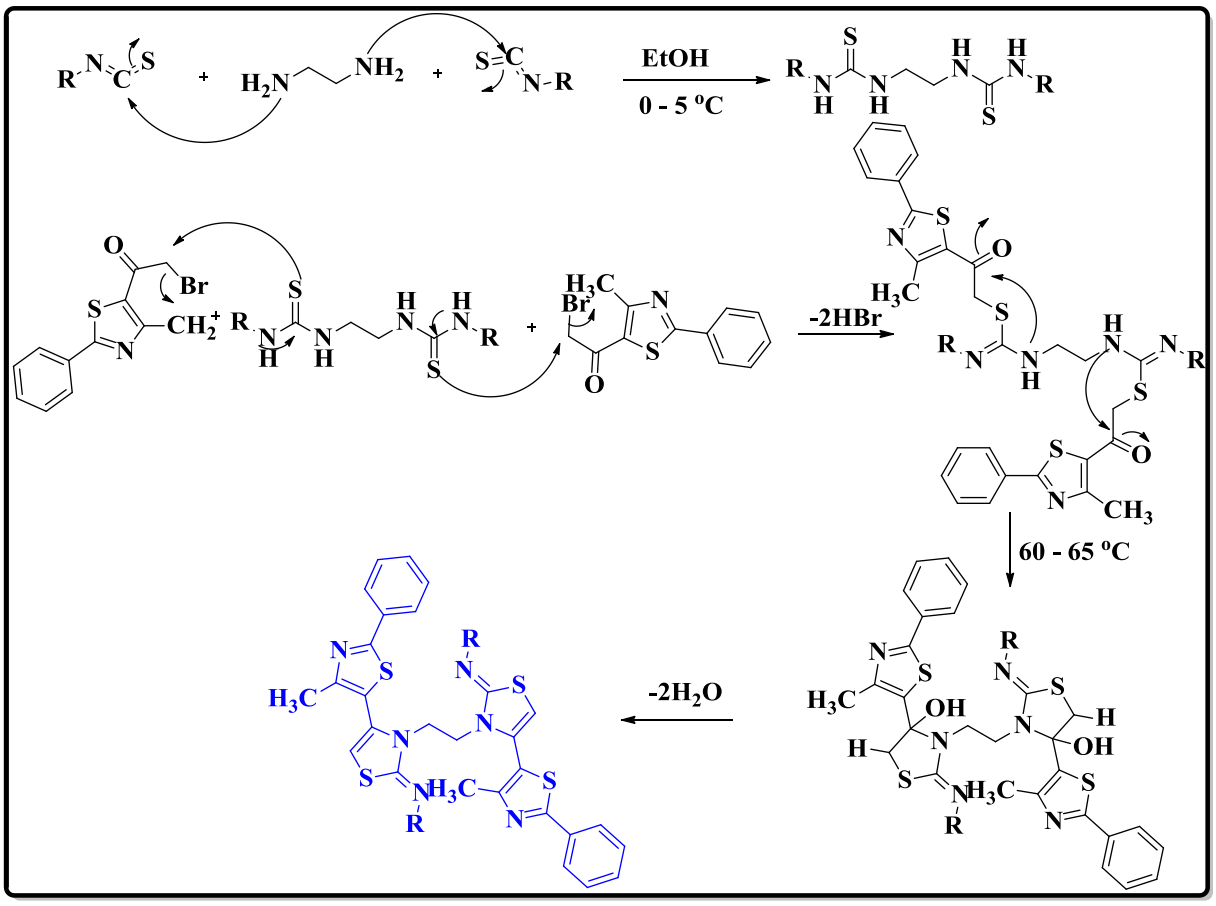


Scheme.5 An efficient one pot pseudo five component reaction of 3,3''-(ethane-1,2-diyl)bis(4'-methyl-2'-phenyl-[4,5'-bithiazole]-3(3*H*)-yl-2(3*H*)-ylidene))dianiline derivatives

Table.13 One pot pseudo five component reaction of 3,3''-(ethane-1,2-diyl)bis(4'-methyl-2'-phenyl-[4,5'-bithiazole]-3(3*H*)-yl-2(3*H*)-ylidene))dianiline derivatives

S.No	Product[a]	R	Reaction Time (h)	Yield (%)[b]	M.pt.
1	**53a**	*Iso*-propyl	1.5	80	125 – 127
2	**53b**	Phenyl	2	88	198 – 200
3	**53c**	Benzyl	2	82	222 – 224
4	**53d**	*p*-Tolyl	1.5	86	258 – 260
5	**53e**	4-Methoxylphenyl	1.5	82	186 – 188
6	**53f**	4-Cyanophenyl	2	90	274 – 276
7	**53g**	4-Fluorophenyl	2.5	81	165 – 167
8	**53h**	3-Chlorophenyl	2.5	85	216 – 218
9	**53i**	4-Nitrophenyl	3	88	287 – 289

[a] Reaction Condition: Ethylenediamine **52** (1 mmol), alkyl/arylisothiocyanate **50 (a-i)** (2 mmol) and 2 -bromo-1-(4-methyl-2-phenylthiazol-5-yl)ethanone **47** (2 mmol) in ethanol at 0- 60 °C, [b] Isolated yields without recrystallization.



Scheme 6. Plausible reaction mechanism for the synthesis of 3,3''-(ethane-1,2-diyl)bis(4'-methyl-2'-phenyl-[4,5'-bithiazole]-3(3*H*)-yl-2(3*H*)-ylidene))dianiline derivatives

Mechanism:

The plausible reaction mechanism for the synthesis of 3,3"-(ethane-1,2-diyl)bis(4'-methyl-2'-phenyl-[4,5'-bithiazole]-3(3*H*)-yl-2(3*H*)-ylidene))dianiline derivatives was shown in **Scheme 6.** In the initial step the nucleophilic amino functional group of ethylenediamine readily reacts with two equivalents of alkyl / arylisothiocyante and forms the intermediate 1,1'-(ethane-1,2-diyl) bis(3-alkyl/arylthiourea). The resulted intermediate undergoes cyclization reaction with 2 -bromo-1-(4-methyl-2-phenylthiazol-5-yl)ethanone and forms the target product by the elimination of hydrogen bromide and water molecules.

All the newly synthesized compounds were well characterized by their physical and analytical spectral data.

All the newly synthesized 3,3"-(ethane-1,2-diyl)bis(4'-methyl-2'-phenyl-[4,5'-bithiazole]-3(3*H*)-yl-2(3*H*)-ylidene))dianiline derivatives (**53a-i**) were screened against nineteen human viral species as described in chapter-II (Reference 65 -67). The antiviral activity experiment was carried out in triplicate and the average values were taken for report. The antiviral activity data was shown in **Table 14-18**.

The antiviral activity reveals that the newly synthesized 3,3"-(ethane-1,2-diyl)bis(4'-methyl-2'-phenyl-[4,5'-bithiazole]-3(3*H*)-yl-2(3*H*)-ylidene))dianiline derivatives (**53a-i**) exhibited promising antiviral activity against tested viral species. Brivudin, Cidofovir, Acyclovir and Ganciclovir were used as reference drugs. The compounds **53e**, **53f** and **53g** exhibited good activity in HEL cell culture medium against *Herpes simplex virus-1* (KOS) and *Herpes simplex virus-2* (G), similarly the compound 53g also exhibited antiviral activity against Herpes simplex virus-1TK-KOS ACVr, Adeno Virus and Human corona viruses. Whereas remaining compounds are inactive against these viral species (**Table 14**).

The compounds **53 a-i** were tested for their antiviral activity in HeLa cell culture medium against *Vesicular stomatitis virus*, *Coxsackie virus B4* and *Respiratory syncytial virus* by using Dextran sulphate (DS-10.000) and Ribavirin as standard drugs. Compounds **53e**, **53f**, **53g** and **53h** exhibited excellent activity against *Vesicular stomatitis* virus and *Coxsackie* virus B4 when compared to the standard antiviral drug

Ribavirin. The remaining compounds did not exhibit any activity against the tested viral species (**Table 15**).

Table.14 Cytotoxicity and antiviral activity in HEL cell cultures

Compound	Minimum cytotoxic concentration[a] (μM)	EC_{50}[b] (μM)					
		Herpes simplex virus-1 (KOS)	*Herpes simplex* virus-2 (G)	*Herpes simplex* virus-1 TK-KOS ACVr	*Vaccinia* virus	*Adeno* virus-2	*Human Corona* virus (229E)
53a	20	>100	>100	>100	>100	>100	>100
53b	20	>100	>100	>100	>100	>100	>100
53c	>100	>100	>100	>100	>100	>100	>100
53d	>100	>100	>100	>100	>100	>100	>100
53e	>100	**6.8**	**13.5**	>100	>100	>100	>100
53f	>100	**9.5**	**11**	>100	>100	>100	**12**
53g	>100	**10.2**	**8.5**	**10**	**25**	**20**	**8**
53h	>100	>100	>100	>100	>100	>100	>100
53i	>100	>100	>100	>100	>100	>100	>100
Brivudin	>250	0.008	112	10	10	-	-
Cidofovir	>250	4.5	2	3.4	22	29	-
Acyclovir	>250	0.7	0.2	85	>250	-	-
Ganciclovir	>250	0.16	0.06	4	>100	-	-
Zalcitabine	>250	-	-	-	-	19	-
Alovudine	>250	-	-	-	-	50	-
UDA	>100	-	-	-	-	-	1.8
Ribavirin	>250	-	-	-	-	-	112

[a] Required to cause a microscopically detectable alteration of normal cell morphology.
[b] Required to reduce virus-induced cytopathogenicity by 50 %.

Table.15 Cytotoxicity and antiviral activity in HeLa cell cultures

Compound	Minimum cytotoxic concentration[a]	EC50[b]		
		Vesicular stomatitis virus	*Coxsackie* virus B4	*Respiratory syncytial* virus
53a	100	>100	>100	>100
53b	20	>100	>100	>100
53c	100	>100	>100	>100
53d	≥100	56	45	>100
53e	≥100	**22**	**25**	45
53f	>100	**12**	**8.5**	25
53g	>100	**15**	**22**	**12**
53h	>100	**25**	**18.5**	35
53i	100	>100	>100	>100
DS-10.000	>100	0.4	6.8	0.4
Ribavirin	>250	25	50	10

[a]Required to cause a microscopically detectable alteration of normal cell morphology.
[b]Required to reduce virus-induced cytopathogenicity by 50 %.

The compounds **53a-i** were evaluated for their antiviral activity against *Parainfluenza 3-virus, Reovirus, sindis virus, Coxsackie virus, Puntatoro virus* and *Yellow fever virus* in Vero cell culture medium. DS.10.000, Ribavirin and Mycophenolic acid were used as reference antiviral drugs. The newly synthesized compounds did not exhibit any potency against the tested viral species (**Table 16**).

Table 16 Cytotoxicity and antiviral activity in: Vero cell cultures

Compound	Minimum cytotoxic conc.[a] (μM)	EC_{50}[b] (μM)					
		Para influenza- 3 virus	*Reo* virus-1	*Sindbis* virus	*Coxsackie* virus B4	*Punta Toro* virus	*Yellow Fever* virus
53a	20	>100	>100	>100	>100	>100	>100
53b	20	>100	>100	>100	>100	>100	>100
53c	20	>100	>100	>100	>100	>100	>100
53d	≥20	>100	>100	>100	>100	>100	>100
53e	≥100	>100	>100	>100	>100	>100	>100
53f	≥4	>100	>100	>100	>100	>100	>100
53g	100	55	34	>100	>100	>100	>100
53h	100	>100	>100	>100	>100	>100	>100
53i	100	>100	>100	>100	>100	>100	>100
DS.10.000	>100	>100	>100	100	45	2.3	0.8
Ribavirin	>250	146	>250	>250	>250	125	>250
Mycophenolic acid	≥100	0.4	0.5	20	45	4.0	0.8

[a]Required to cause a microscopically detectable alteration of normal cell morphology.
[b]Required to reduce virus-induced cytopathogenicity by 50 %.

The compounds **53 (a-i)** were evaluated for their antiviral activity against *Feline corona* virus (FIPV) and *Feline herpes* virus in CRFK cell culture medium using HHA, UDA and Ganciclovir as reference drugs. The antiviral activity results show that the compounds 53f and 53g exhibited moderate activity against *Feline corona* virus (FIPV) and *Feline herpes* virus (**Table 17**).Whereas the remaining compounds did not exhibit any antiviral activity.

Table. 17 Cytotoxicity and antiviral activity in: CRFK cell cultures

Compound	CC_{50}	EC_{50} (μM)[b]

	(μM) [a]	*Feline Corona* Virus (FIPV)	*Feline Herpes* Virus
53a	>100	>100	>100
53b	>100	>100	>100
53c	>100	>100	>100
53d	>100	>100	>100
53e	>100	>100	>100
53f	>100	**18.5**	**22**
53g	>100	**12**	**11.89**
53h	>100	>100	>100
53i	>100	>100	>100
HHA	>100	9.4	2.3
UDA	>100	5.7	2.0
Ganciclovir	>100	>100	3.4

[a]50% Cytotoxic concentration, as determined by measuring the cell viability with the colorimetric formazan 50%
[b]Effective concentration, or concentration producing 50% inhibition of virus-induced cytopathic effect, as
CRFK cells: Crandell-Rees Feline Kidney cells.

Similarly the compounds **53(a-i)** were tested for antiviral activity in MDCK cell culture medium against *Influenza AH1N1* A/Ned/378/05, *Influenza* AH3N2A/HK/7/87 and *Influenza B* B/Ned/537/05 by using Zanamivir, Ribavirin, Amantadine and Rimantadine as antiviral standard drugs. The compounds **51f** and **51g** shown promising activity against tested *Influenza* viral species (**Table 18**).

Table. 18 Cytotoxicity and antiviral activity in: **MDCK cell cultures**

Compound	Cytotoxicity		Antiviral EC50[c]					
	CC_{50}[a]	Minimum cytotoxic concentration[b]	*Influenza* A/H1N1 A/Ned/378/05		*Influenza* A/H3N2 A/HK/7/87		*Influenza* B B/Ned/537/05	
			visual CPE score	MTS	visual CPE score	MTS	visual CPE score	MTS
53a	>100	20	>100	>100	>100	>100	>100	>100
53b	>100	≥20	>100	>100	>100	>100	>100	>100
53c	>100	≥20	>100	>100	>100	>100	>100	>100
53d	>100	100	>100	>100	>100	>100	>100	>100
53e	>100	100	>100	>100	>100	>100	>100	>100
53f	>100	>100	**8.5**	**10**	**15.25**	**10.5**	**9.5**	**8.5**
53g	>100	>100	**15.5**	**9.25**	**7.5**	**9.5**	**22.25**	**15.75**
53h	>100	>100	>100	>100	>100	>100	>100	>100

53i	>100	>100	>100	>100	>100	>100	>100	>100
Zanamivir	>100	>100	0.4	0.1	0.8	0.1	0.5	0.4
Ribavirin	>100	100	8.9	4.4	4.0	1.0	8.9	8.4
Amantadine	>100	>100	100	>100	0.2	0.1	>100	>100
Rimantadine	>200	>200	>200	>200	0.02	0.01	>200	>200

[a]50% Cytotoxic concentration, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay.

[b]Minimum compound concentration that causes a microscopically detectable alteration of normal cell morphology.

[c]50% Effective concentration, or concentration producing 50% inhibition of virus-induced cytopathic effect, as determined by visual scoring of the CPE, or by measuring the cell viability with the colorimetric formazan-based MTS assay.

MDCK cells: Madin Darby canine kidney cells

The structure activity relationship studies of newly synthesized 3,3"-(ethane-1,2-diyl) bis(4'-methyl-2'-phenyl-[4,5'-bithiazole]-3(3*H*)-yl-2(3*H*)-ylidene))dianiline derivatives (**53a-i**) clearly exhibited that the presences of electron withdrawing functional groups like 4-CN, 4-F and 3-Cl are crucial for antiviral activity and also the electron releasing group like $–OCH_3$ is important for potential antiviral activity.

In conclusion, we have developed an efficient one pot pseudo five component reaction for the synthesis of 3,3"-(ethane-1,2-diyl)bis(4'-methyl-2'-phenyl-[4,5'-bithiazole]-3(3*H*)-yl-2(3*H*)-ylidene))dianiline derivatives (**53a-i**) through a simple reaction conditions, easy work up, without any catalyst and in good yields. they also exhibited good antiviral activity.

EXPERIMENTAL:

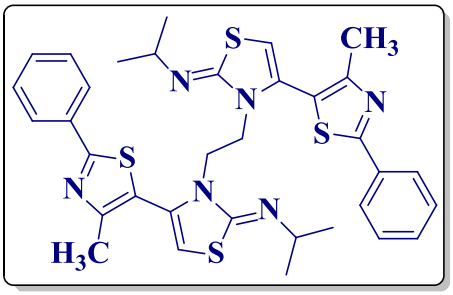
General procedure for the synthesis of 3,3''-(ethane-1,2-diyl)bis(4'-methyl-2'-phenyl-[4,5'-bithiazole]-3(3*H*)-yl-2(3*H*)-ylidene))dianiline derivatives:

To an ice cold ethanolic solution of ethylenediamine (0.060g, 1.0 mmol), alkyl / aryl isothiocyanate (2.0 mmol) was added slowly. The reaction mixture was stirred at 0- 5 °C for about 15 minutes and 2-bromo-1-(4-methyl-2-phenylthiazol-5-yl)ethanone (0.592 g, 2.0 mmol) was added. The temperature of the reaction mixture was raised to 60 – 65 °C and maintained for about 1 – 3 h. After completion of the reaction (monitored through TLC) the reaction temperature was cooled to room temperature, the separated solid was

filtered, washed with ethanol and dried. The product was recrystallized from ethanol solvent.

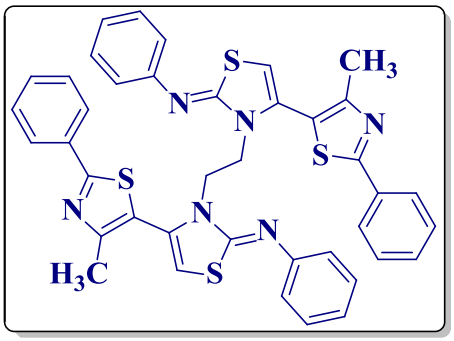
SPECTRAL DATA:

(*N,N'Z,N,N'Z*)-*N,N'*-(3,3''-(ethane-1,2-diyl)bis(4'-methyl-2'-phenyl-[4,5'-bithiazole]-3(3*H*)-yl-2(3H)-ylidene))bis(propan-2-amine):



Yellow solid; **FTIR (KBr, υ_{max} cm^{-1}): ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 1.14 (d, *J* = 6.4 Hz, 12H, $CH(CH_3)_2$), 2.70 (s, 6H, thiazole-CH_3), 3.05 (s, 2H, -CH), 4.06 (s, 4H, -NCH_2), 7.54 (t, *J* = 7.2 Hz, 6H, Ar-H), 7.92 – 8.03 (m, 6H, Ar-H); **^{13}C NMR (100 MHz, DMSO-d_6, ppm):** δ 18.77, 36.79, 41.98, 67.94, 127.07, 129.89, 132.06, 132.46, 159.99, 169.33; Anal. Calcd for $C_{34}H_{36}N_6S_4$: C, 62.16; H, 5.52; N, 12.79; S, 19.52%; Found: C, 62.12; H, 5.58; N, 12.83, S, 19.48%.

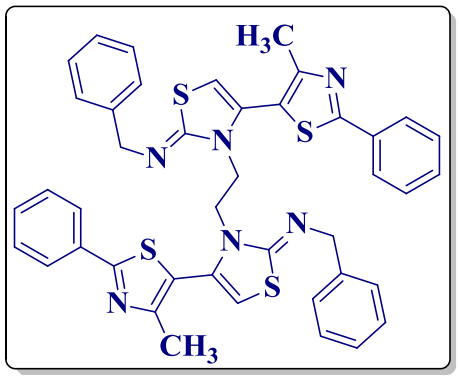
***(N,N'Z,N,N'Z)*-*N,N'*-(3,3''-(ethane-1,2-diyl)bis(4'-methyl-2'-phenyl-[4,5'-bithiazole] 3(3*H*)-yl-2(3*H*)-ylidene))dianiline:**



Yellow solid; **FTIR (KBr, ν_{max}, cm^{-1}): ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 2.38 (s, 6H, thiazole-CH_3), 4.27 (s, 4H, -NCH_2), 6.82 (d, *J* = 7.6 Hz, 6H, Ar-H), 7.00 (t, *J* = 6.8 Hz, 2H, Ar-H), 7.09 (t, *J* = 7.2 Hz, 4H, Ar-H), 7.49 – 7.55 (m, 6H, Ar-H), 7.89 (d, *J* = 6.8 Hz, 4H, Ar-H); Anal. Calcd for $C_{40}H_{32}N_6S_4$: C, 66.27, H, 4.45, N, 11.59, S, 17.69%; Found: C, 66.23, H, 4.43, N, 11.62, S, 17.71%.

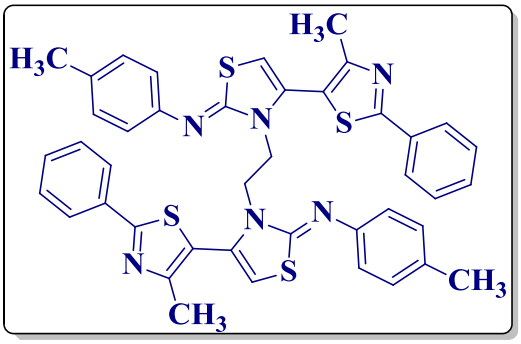
(*N,N'Z,N,N'Z*)-*N,N'*-(3,3''-(ethane-1,2-diyl)bis(4'-methyl-2'-phenyl-[4,5'-bithiazole]-3(3*H*)-yl-2(3*H*)-ylidene))bis(1-phenylmethanamine):

Yellow solid; **FTIR (KBr, ν_{max}, cm^{-1}): ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 2.36 (s, 6H, -CH_3), 4.13 (s, 4H, -NCH_2), 4.94 (s, 4H, Ph-CH_2), 7.02 (t, *J* = 5.2 Hz, 6H, Ar-H), 7.31 (t, *J* = 7.6 Hz, 3H, Ar-H), 7.54 (d, *J* = 7.6 Hz, 5H, Ar-H), 7.84 (s, 4H, Ar-H), 7.92



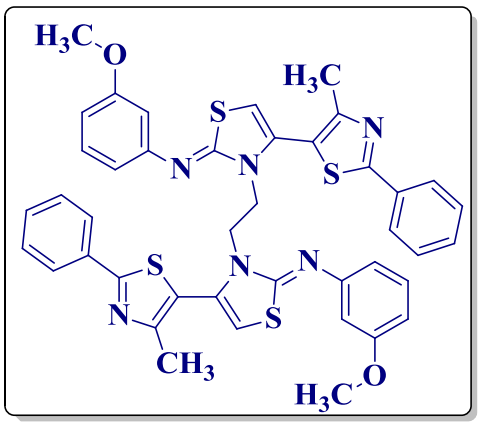
(s, 4H, Ar-H); Anal. Calcd for $C_{42}H_{36}N_6S_4$: C, 66.99, H, 4.82, N, 11.16, S, 17.03%; Found: C, 67.02, H, 4.86, N, 11.12, S, 16.99%.

(*N*,*N*'*Z*,*N*,*N*'*Z*)-*N*,*N*'-(3,3''-(ethane-1,2-diyl)bis(4'-methyl-2'-phenyl-[4,5'-bithiazole]-3(3*H*)-yl-2(3*H*)-ylidene))bis(4-methylaniline):



Yellow solid; **FTIR (KBr, ν_{max}, cm^{-1}): ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 2.22 (s, 6H, -CH_3), 2.39 (s, 6H, -CH_3), 4.35 (s, 4H, -NCH_2), 6.85 (d, *J* = 7.6 Hz, 4H, Ar-H), 6.99 (t, *J* = 8 Hz, 6H, Ar-H), 7.49 – 7.58 (m, 6H, Ar-H), 7.87 (d, *J* = 6.8 Hz, 4H, Ar-H); **^{13}C NMR (100 MHz, DMSO-d_6, ppm):** δ 16.56, 21.03, 44.33, 117.42, 123.59, 126.73, 129.85, 130.41, 130.69, 131.59, 132.48, 156.09, 168.98; Anal. Calcd for $C_{42}H_{36}N_6S_4$: C, 66.99, H, 4.82, N, 11.16, S, 17.03%; Found: C, 66.96, H, 4.86, N, 11.19, S, 16.98 %.

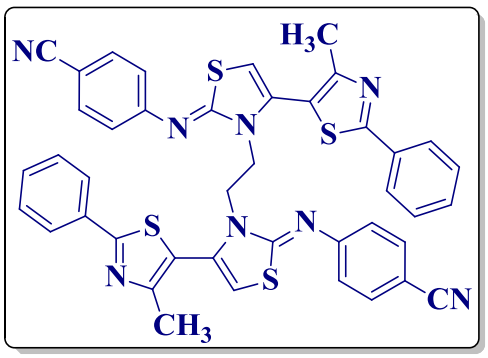
(N,N'Z,N,N'Z)-N,N'-(3,3''-(ethane-1,2-diyl)bis(4'-methyl-2'-phenyl-[4,5'-bithiazole]-3(3H)-yl-2(3H)-ylidene))bis(3-methoxyaniline):



Yellow solid; **FTIR (KBr, ν_{max}, cm^{-1}): ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 2.38 (s, 6H, -CH_3), 3.64 (s, 6H, -OCH_3), 4.31 (s, 4H, -NCH_2), 6.52 (d, *J* = 9.6 Hz, 4H, Ar-H), 6.67 (d, *J* = 8 Hz, 2H, Ar-H), 6.93 (s, 2H, Ar-H), 7.07 (t, *J* = 8 Hz, 2H, Ar-H), 7.48 (t, *J* = 6.8 Hz, 4H, Ar-H), 7.53 (t, *J* = 7.2 Hz, 2H, Ar-H), 7.84 (d, *J* = 7.2 Hz, 4H, Ar-H); **^{13}C NMR (100 MHz, DMSO-d_6, ppm):** δ 16.45, 43.99, 55.49, 108.67, 111.64, 114.88,

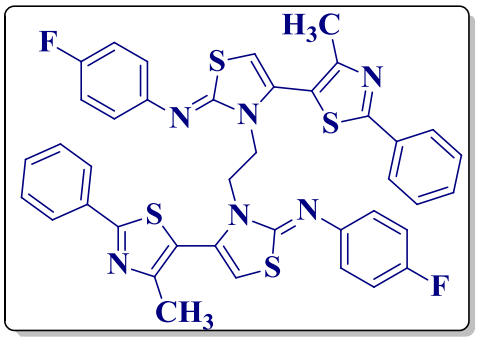
118.07, 126.64, 129.77, 130.88, 131.44, 132.59, 155.71, 160.75, 168.71; Anal. Calcd for $C_{42}H_{36}N_6O_2S_4$: C, 64.26, H, 4.62, N, 10.71, S, 16.34%. Found: C, 64.30, H, 4.65, N, 10.68, S, 16.30%.

4,4'-((1*Z*,1'*Z*)-(3,3''-(ethane-1,2-diyl)bis(4'-methyl-2'-phenyl-[4,5'-bithiazole]-3(3*H*)-yl-2(3*H*)-ylidene))bis(azanylylidene))dibenzonitrile:



Yellow solid; **FTIR (KBr, ν_{max}, cm^{-1}): ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 2.42 (s, 6H, $-CH_3$), 4.01 (t, *J* = 6 Hz, 4H, $-NCH_2$), 6.77 (s, 1H, Ar-H), 7.25 (d, *J* = 8.8 Hz, 3H, Ar-H), 7.56 (s, 4H, Ar-H), 7.82 (d, *J* = 8.8 Hz, 7H, Ar-H), 7.97 – 8.01 (m, 5H, Ar-H); Anal. Calcd for $C_{42}H_{30}N_8S_4$: C, 65.09, H, 3.90, N, 14.46, S, 16.55%; Found: C, 65.04, H, 3.93, N, 14.49, S, 16.52%.

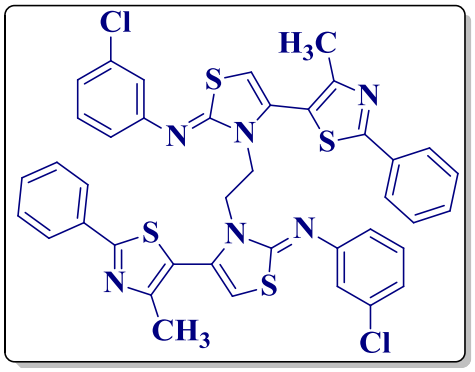
(*N*,*N*'*Z*,*N*,*N*'*Z*)-*N*,*N*'-(3,3''-(ethane-1,2-diyl)bis(4'-methyl-2'-phenyl-[4,5'-bithiazole]-3(3*H*)-yl-2(3*H*)-ylidene))bis(4-fluoroaniline):



Yellow solid; **FTIR (KBr, ν_{max}, cm^{-1}): ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 2.37 (s, 6H, $-CH_3$), 4.25 (s, 4H, $-NCH_2$), 6.84 (t, *J* = 6.8 Hz, 5H, Ar-H), 6.90 (t, *J* = 8.8 Hz, 5H, Ar-H), 7.48 – 7.55 (m, 6H, Ar-H), 7.87 (d, *J* = 6.8 Hz, 4H, Ar-H); **^{13}C NMR (100 MHz, DMSO-d_6, ppm):** δ 16.45, 43.56, 116.50, 116.72, 118.38, 122.80, 124.46, 126.63, 129.79, 131.43, 132.63, 155.48, 158.30, 168.48; Anal. Calcd for $C_{40}H_{30}F_2N_6S_4$: C, 63.13, H, 3.97, N, 11.04, S, 16.85%. Found: C, 63.10, H, 4.01, N, 11.01, S, 16.89%.

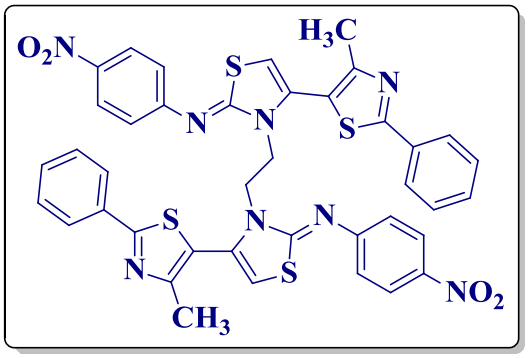
(*N*,*N*'*Z*,*N*,*N*'*Z*)-*N*,*N*'-(3,3''-(ethane-1,2-diyl)bis(4'-methyl-2'-phenyl-[4,5'-bithiazole]-3(3*H*)-yl-2(3*H*)-ylidene))bis(3-chloroaniline)

Yellow solid; **FTIR (KBr, ν_{max}, cm^{-1}): ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 2.37 (s, 6H, $-CH_3$), 4.24 (s, 4H, $-NCH_2$), 6.79 (t, *J* = 8 Hz, 4H, Ar-H), 6.89 (s, 2H, Ar-H), 7.03 (d, *J* = 8 Hz, 2H, Ar-H), 7.10 (t, *J* = 8 Hz, 2H, Ar-H), 7.46 – 7.52 (m, 6H, Ar-H),



7.85 (d, *J* = 7.2 Hz, 4H, Ar-H); **^{13}C NMR (100 MHz, DMSO-d_6, ppm):** δ 16.44, 43.72, 118.42, 121.09, 122.39, 124.67, 126.61, 129.77, 131.39, 131.47, 132.60, 134.25, 155.42, 162.42, 168.48; Anal. Calcd for $C_{40}H_{30}Cl_2N_6S_4$: C, 60.52, H, 3.81, N, 10.59, S, 16.16%. Found: C, 60.55, H, 3.77, N, 10.63, S, 16.14%.

(*N*,*N*'*Z*,*N*,*N*'*Z*)-*N*,*N*'-(3,3''-(ethane-1,2-diyl)bis(4'-methyl-2'-phenyl-[4,5'-bithiazole]-3(3*H*)-yl-2(3*H*)-ylidene))bis(4-nitroaniline):



Red solid; **FTIR (KBr, ν_{max}, cm^{-1}): ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 2.36 (s, 6H, -CH_3), 4.16 (s, 4H, -N-CH_2), 6.82 (s, 1H, Ar-H), 7.32 (d, *J* = 8.8 Hz, 4H, Ar-H), 7.72 (d, *J* = 8.8 Hz, 3H, Ar-H), 7.80 (d, *J* = 6.8 Hz, 4H, Ar-H), 8.04 (d, *J* = 8.8 Hz, 4H, Ar-H), 8.23 (d, *J* = 8.8 Hz, 4H, Ar-H); Anal. Calcd for $C_{40}H_{30}N_8O_4S_4$: C, 58.95, H, 3.71, N, 13.75, S, 15.74%. Found: C, 58.99, H, 3.68, N, 13.78, S, 15.70%.

Antiviral activity experiment of trisubstituted thiazoline derivatives 53 a-i:

The antiviral activity of the newly synthesized compounds was determined by using different cell culture mediums as reported in chapter-II (Reference 65 -67). (i) HEL cell culture was used for viruses (*Herpes simplex virus type 1, Herpes simplex virus type 2, Herpes simplex virus type 1 TK- ACV^r, Vaccinia virus, Adeno virus-2* or *Vesicular stomatitis virus*), (ii) Vero cells were used for the virus (*Coxsackie virus B4, Sindbis virus, Parainfluenza virus 3, Punta Toro virus* or *Reovirus*), (iii) HeLa cells were used for the virus (*Coxsackie virus B4, Vesicular stomatitis virus* or *Respiratory syncytial virus*), (iv) MDCK cells were used for the virus (*Influenza A/H1N1 A/Ned/378/05, Influenza A/H3N2 A/HK/7/87* or *Influenza B B/Ned/537/05*) (v) CRFK cells for the virus (*Feline herpes virus* or *Feline corona virus*). After incubation at 37°C the virus-induced

cytopathicity was determined by measuring the cell viability with the colorimetric formazan-based MTS assay.

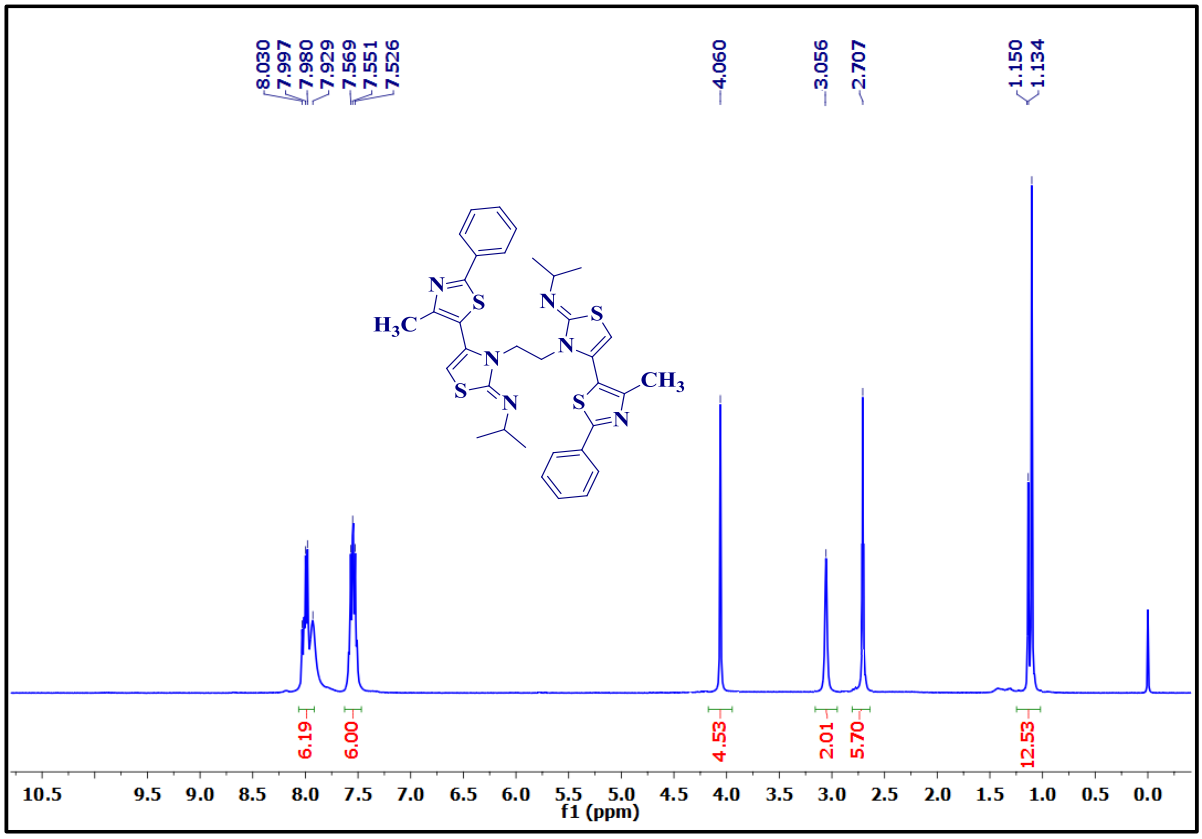


Fig: ^{1}H NMR Spectrum of compound **53a**

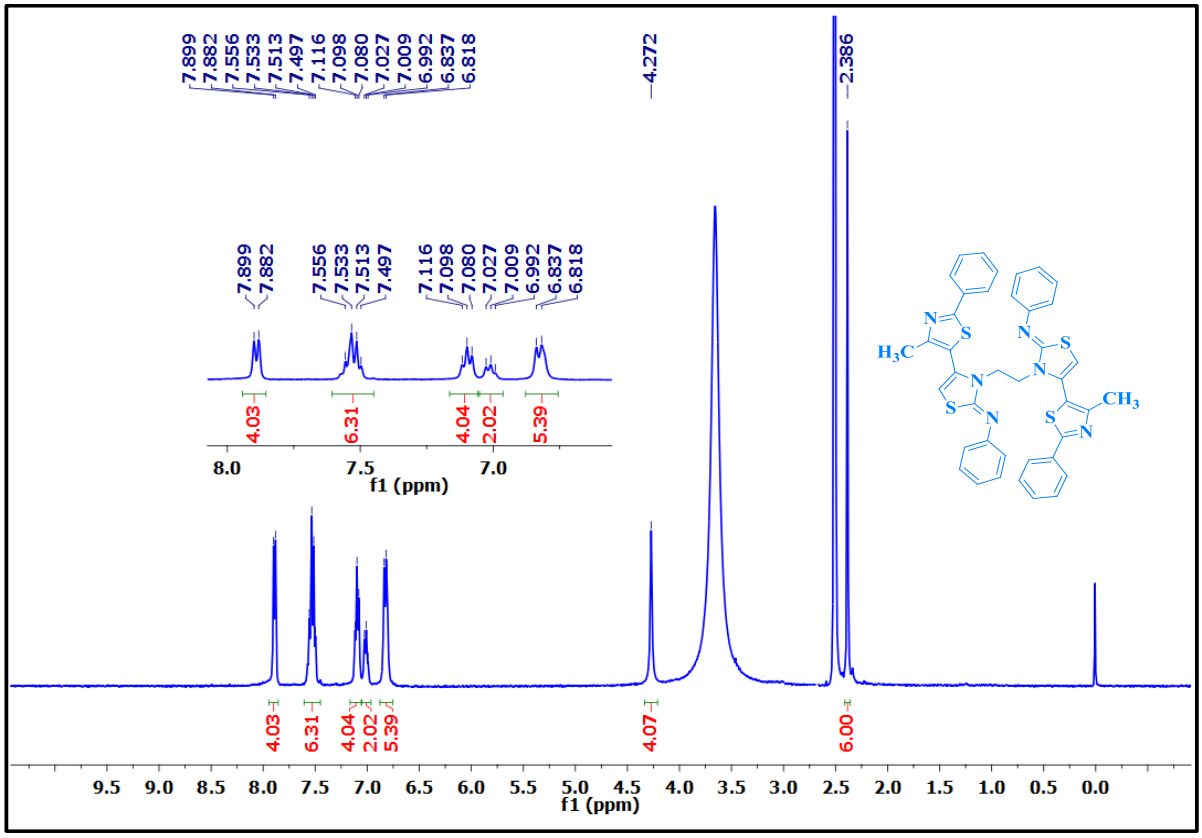


Fig: ^{1}H NMR Spectrum of compound **53b**

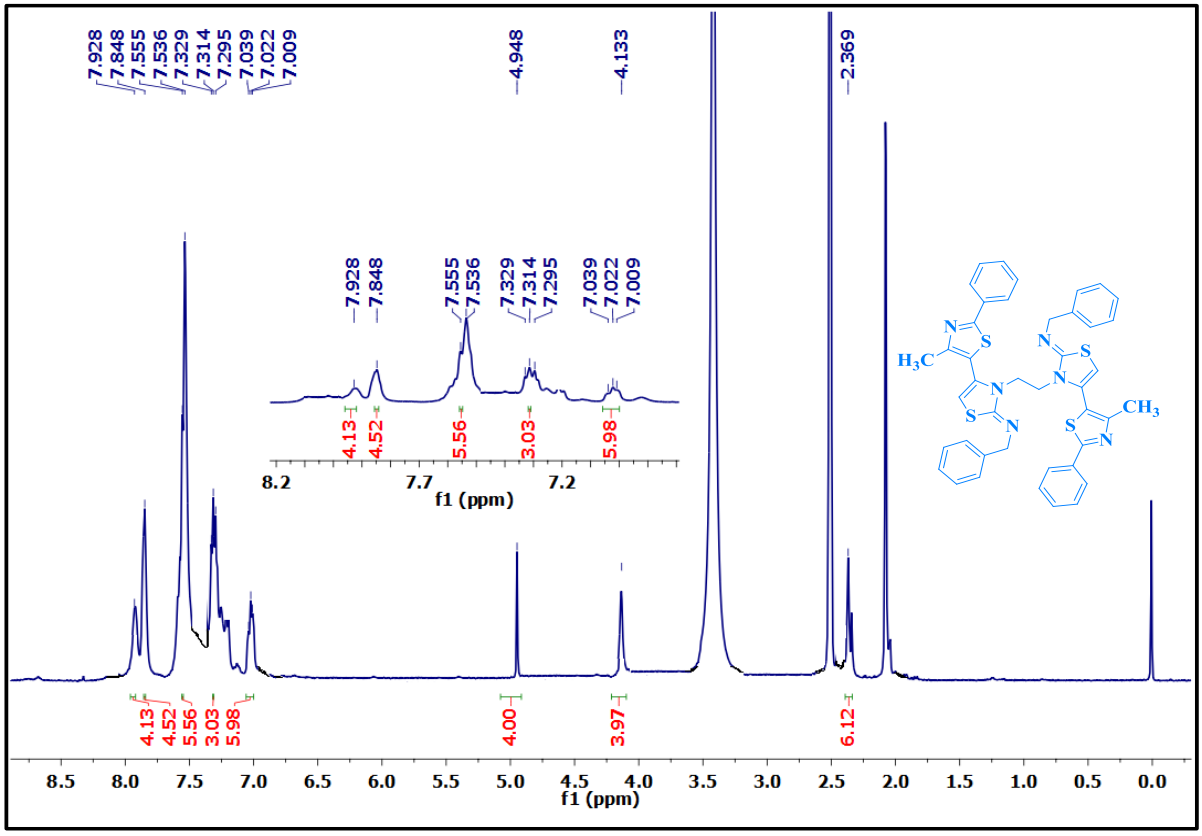


Fig: [1]H NMR Spectrum of compound **53c**

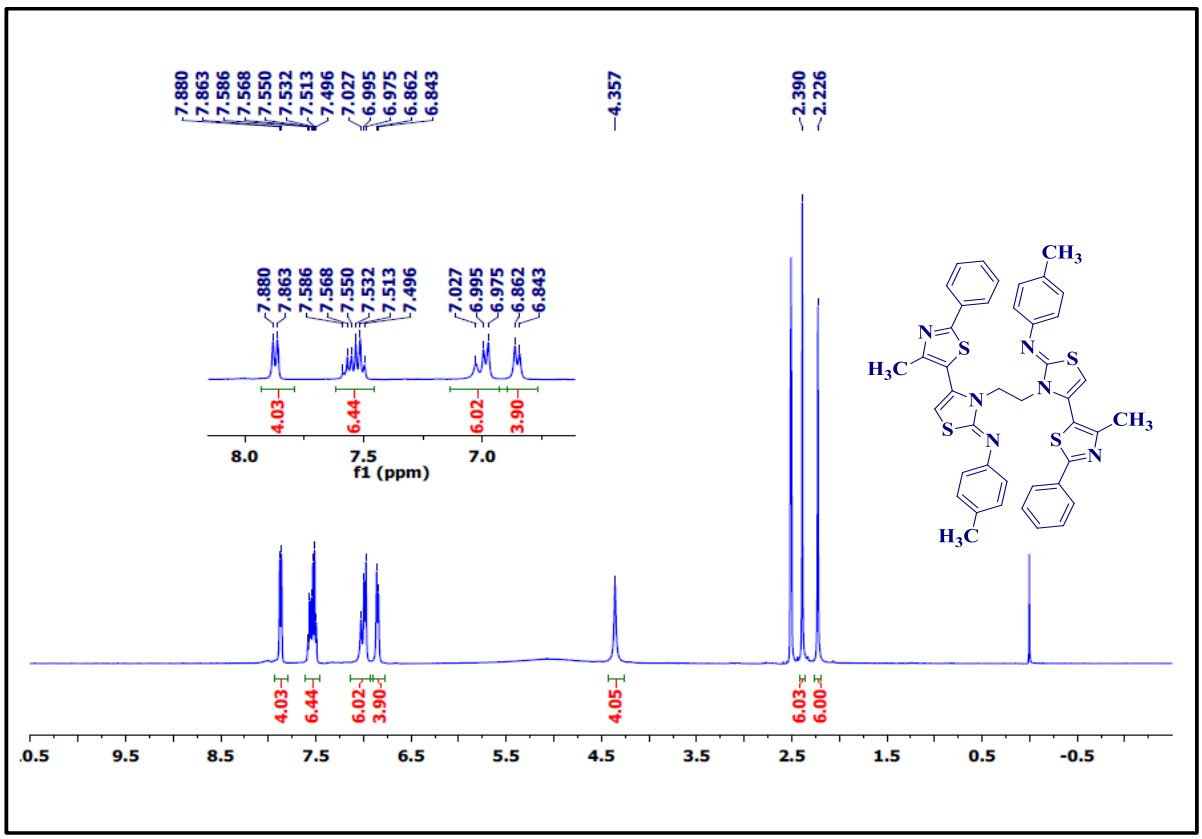


Fig: [1]H NMR Spectrum of compound **53d**

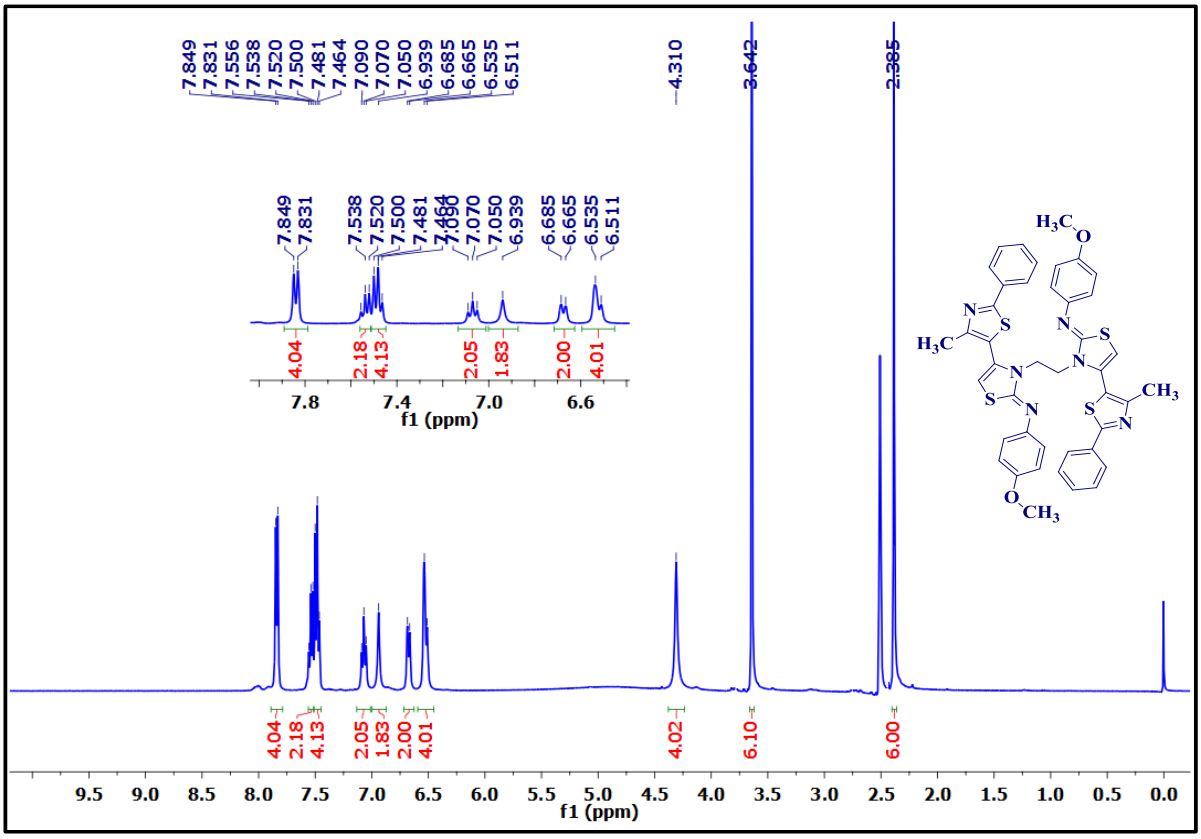


Fig: ^{1}H NMR Spectrum of compound **53e**

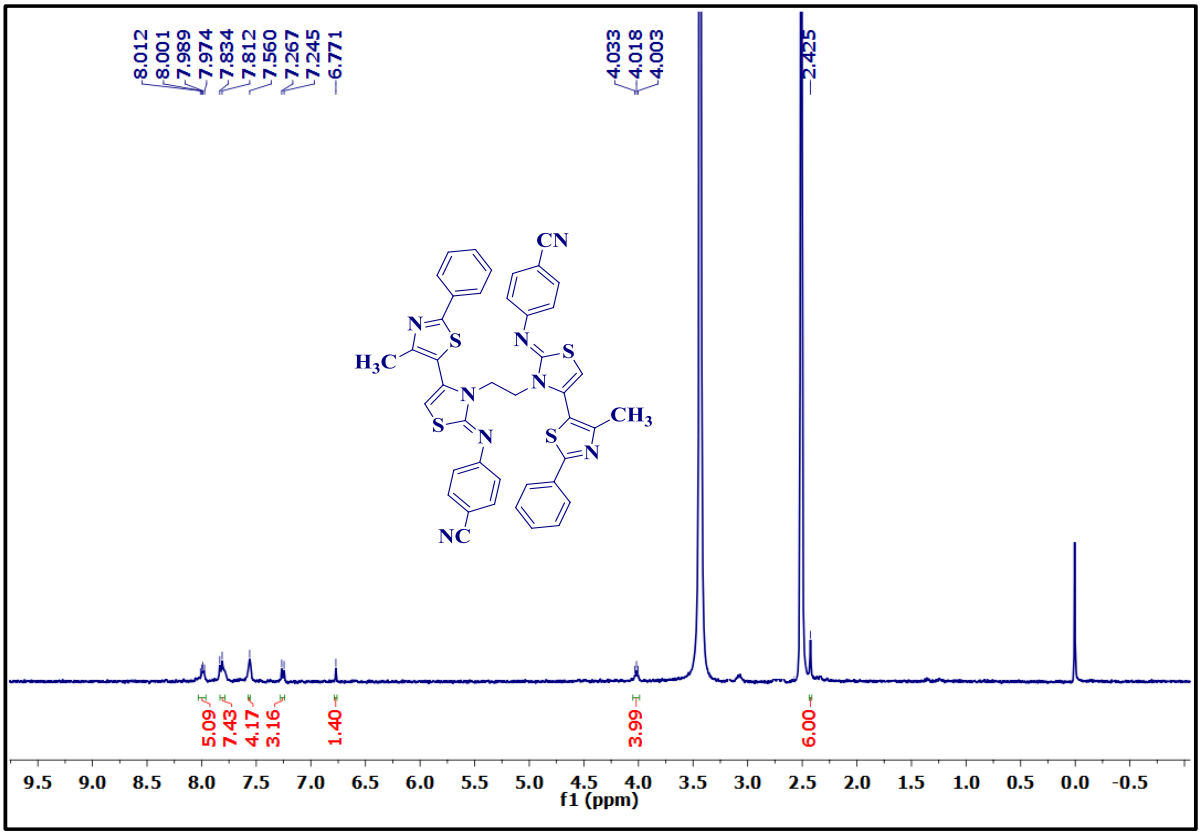


Fig: ^{1}H NMR Spectrum of compound **53f**

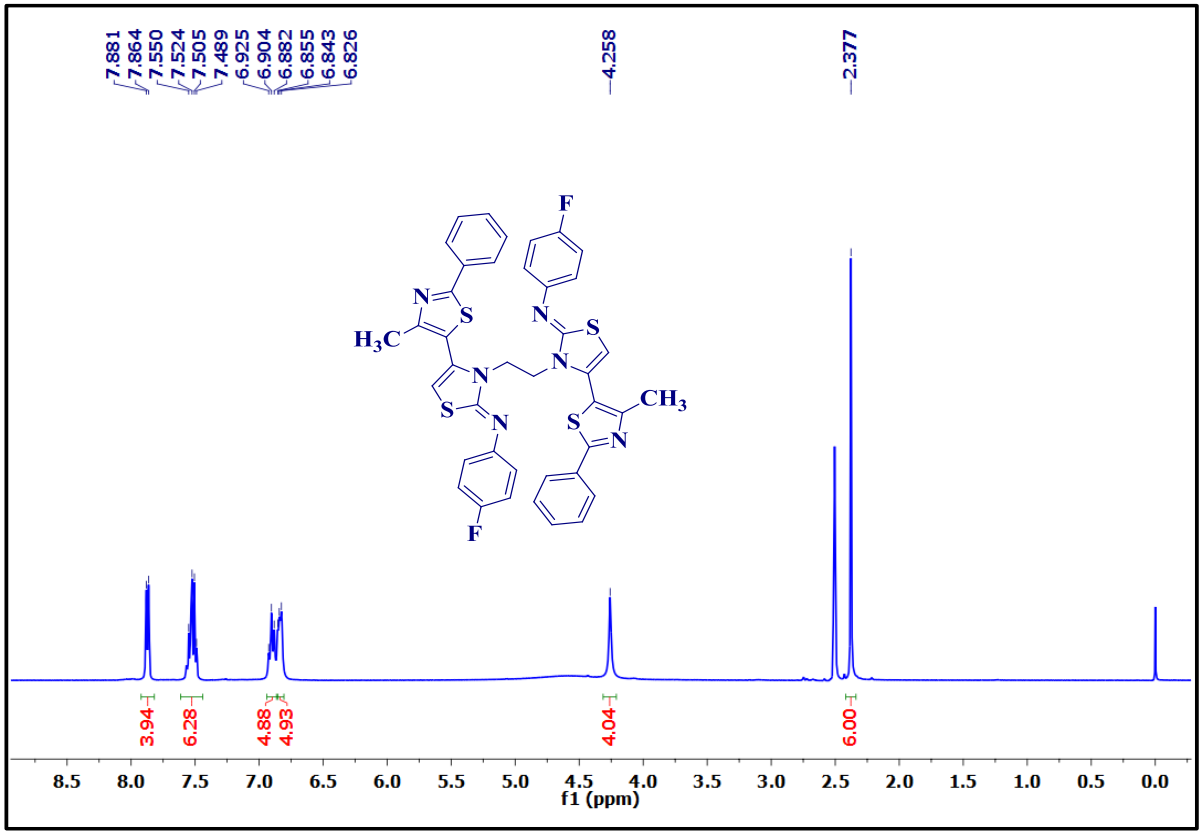


Fig: ^{1}H NMR Spectrum of compound **53g**

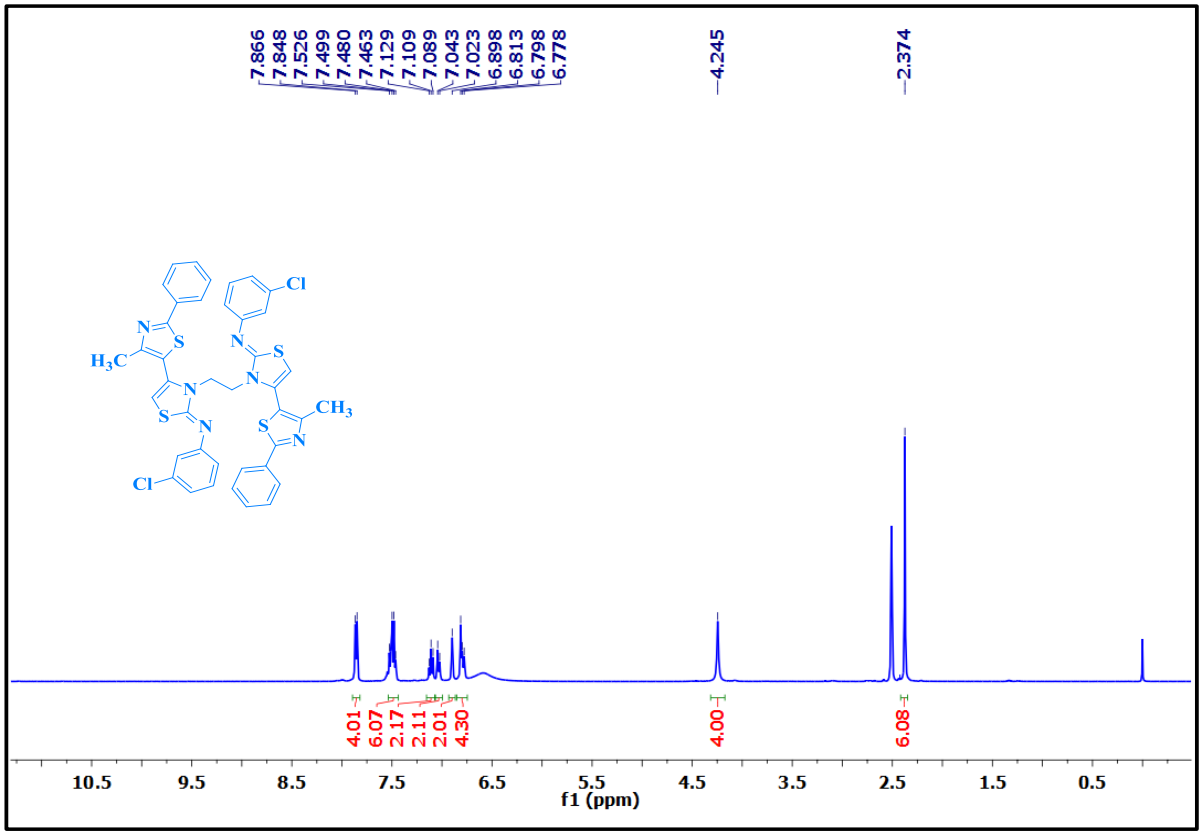


Fig: ^{1}H NMR Spectrum of compound **53h**

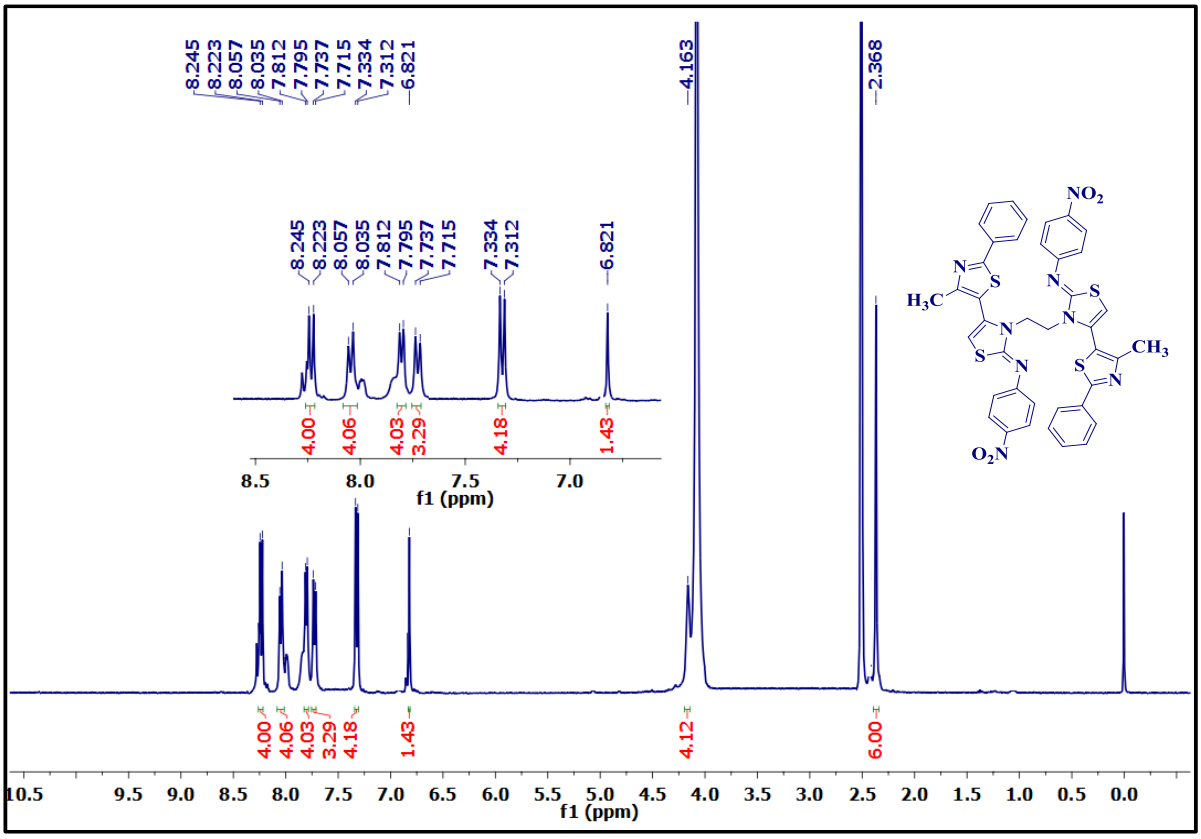


Fig: ^{1}H NMR Spectrum of compound **53i**

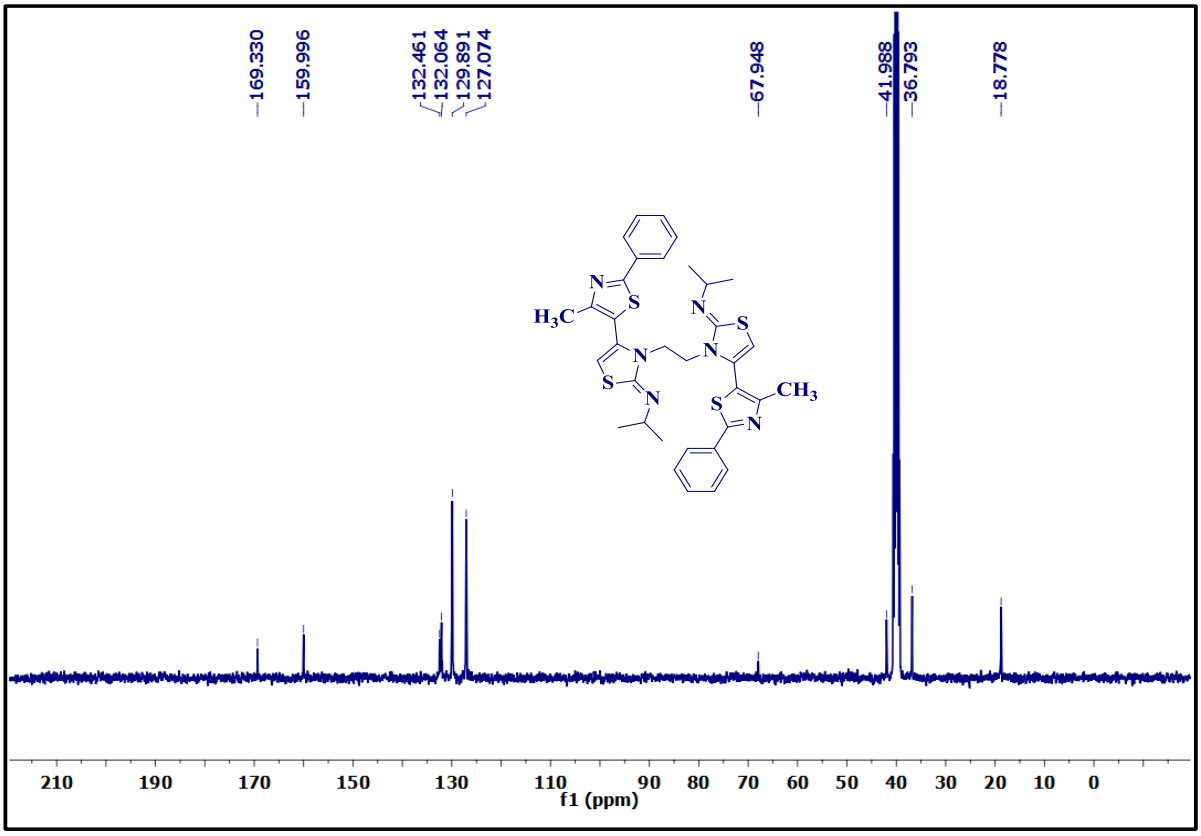


Fig: ^{13}C NMR Spectrum of compound **53a**

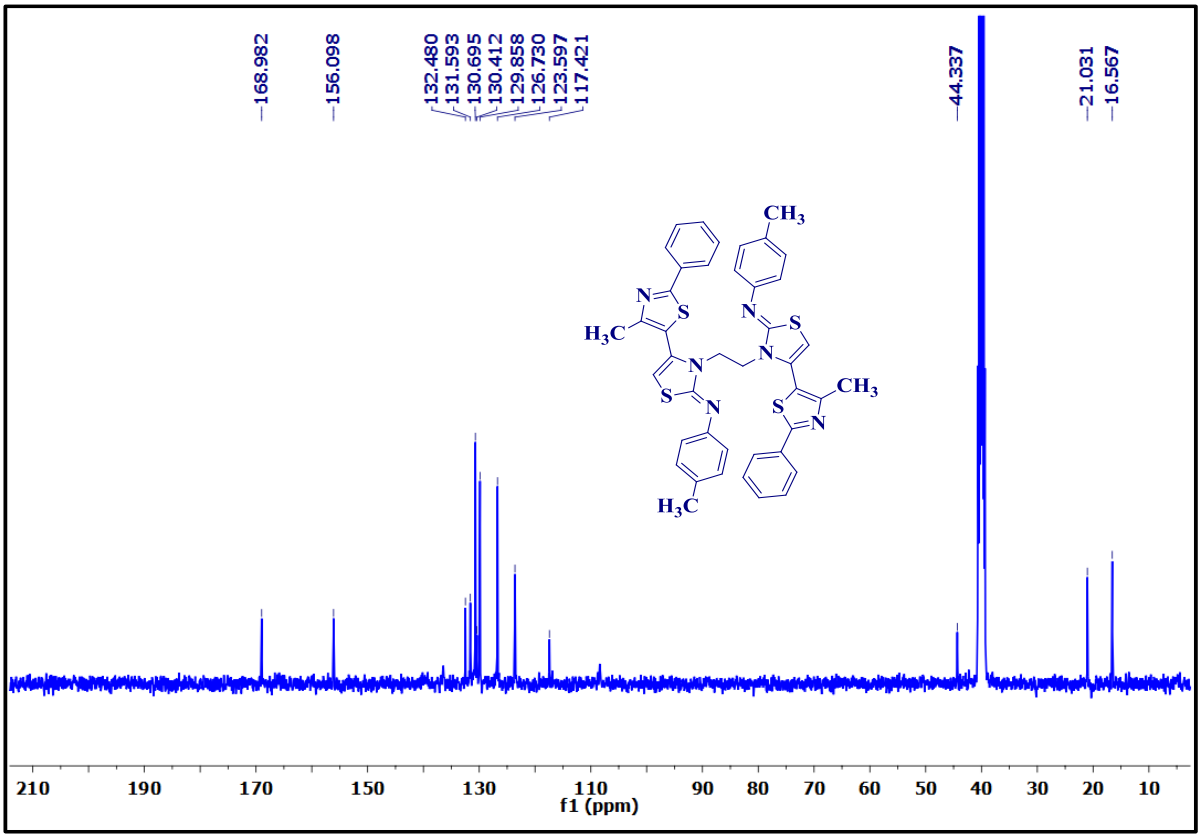


Fig: ^{13}C NMR Spectrum of compound **53d**

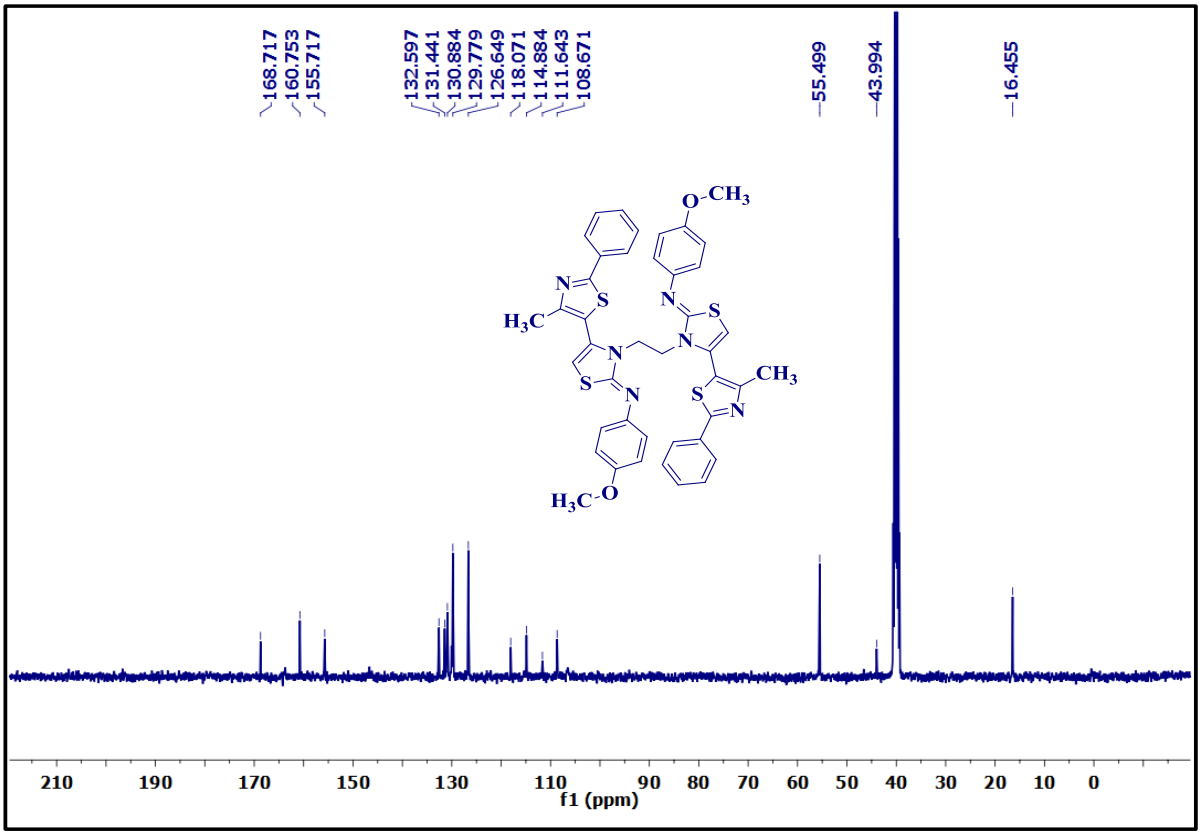


Fig: ^{13}C NMR Spectrum of compound **53e**

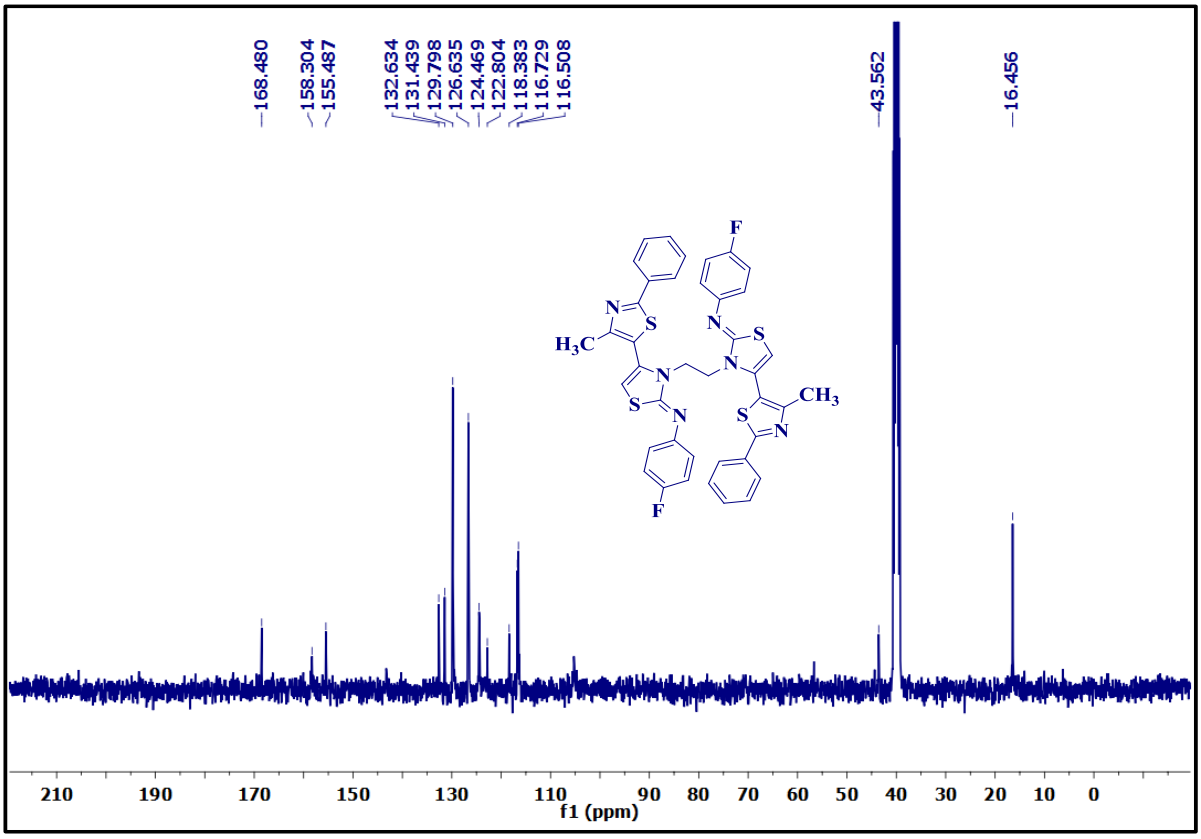


Fig: ^{13}C NMR Spectrum of compound **53g**

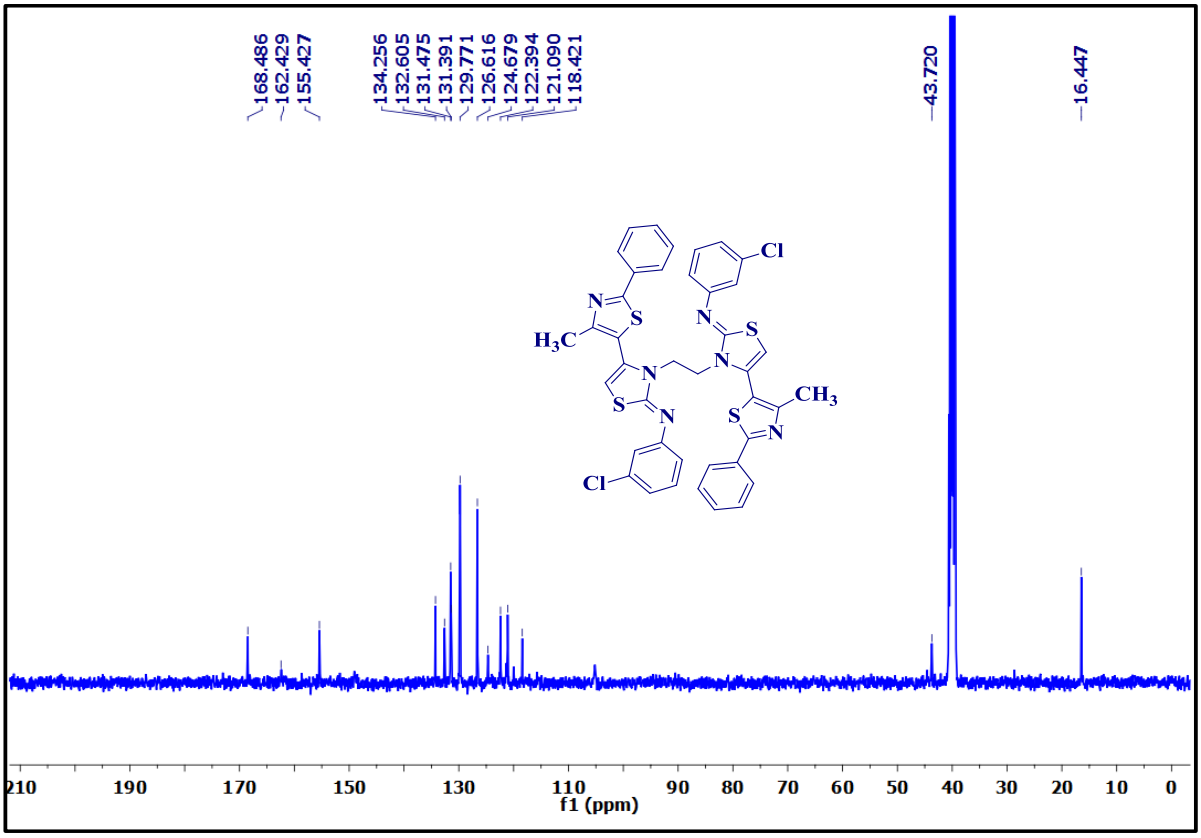


Fig: ^{13}C NMR Spectrum of compound **53h**

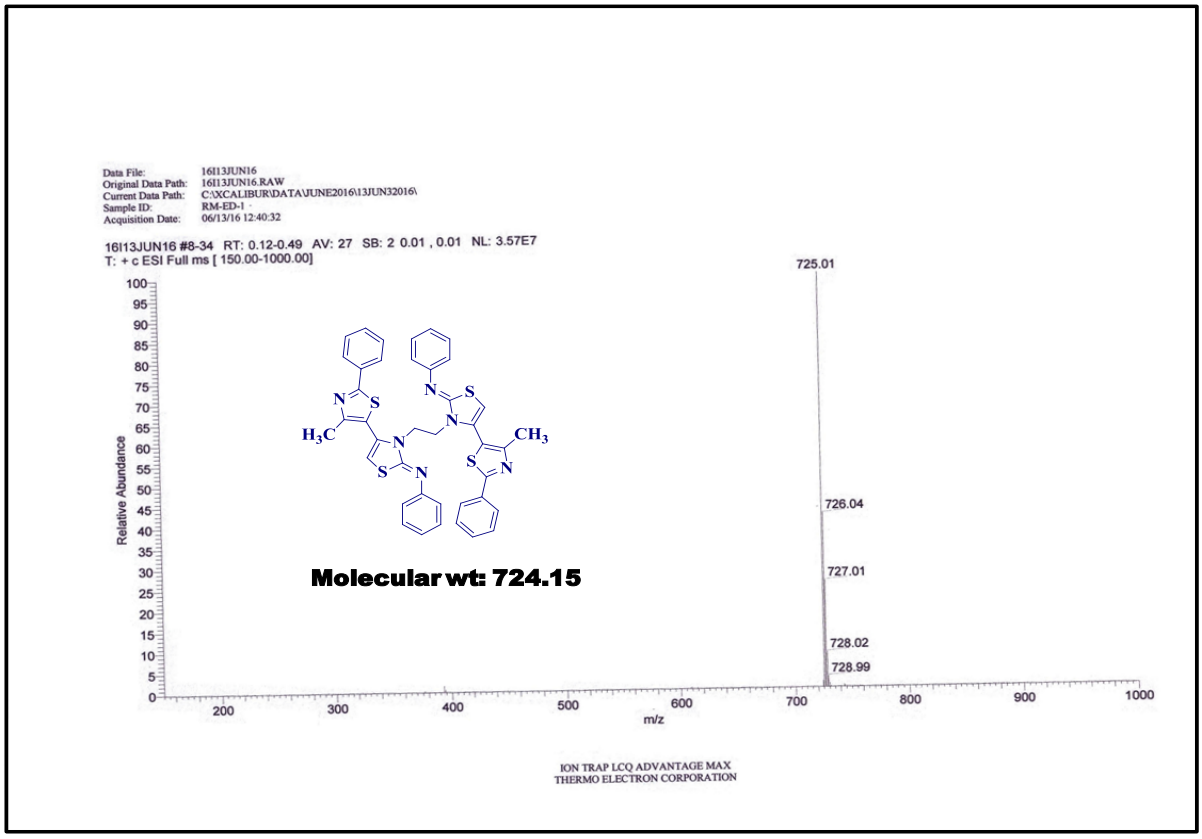


Fig: Mass spectrum of compound **53a**

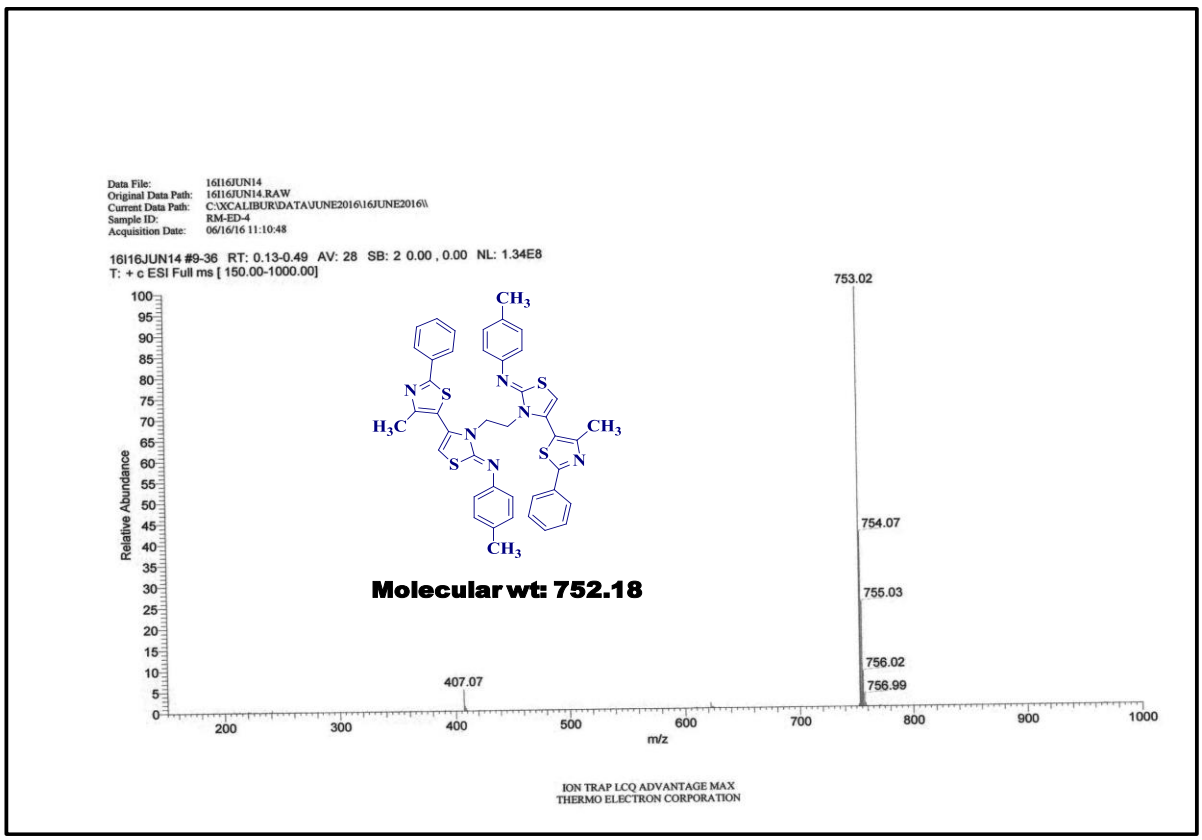


Fig: Mass spectrum of compound **53**

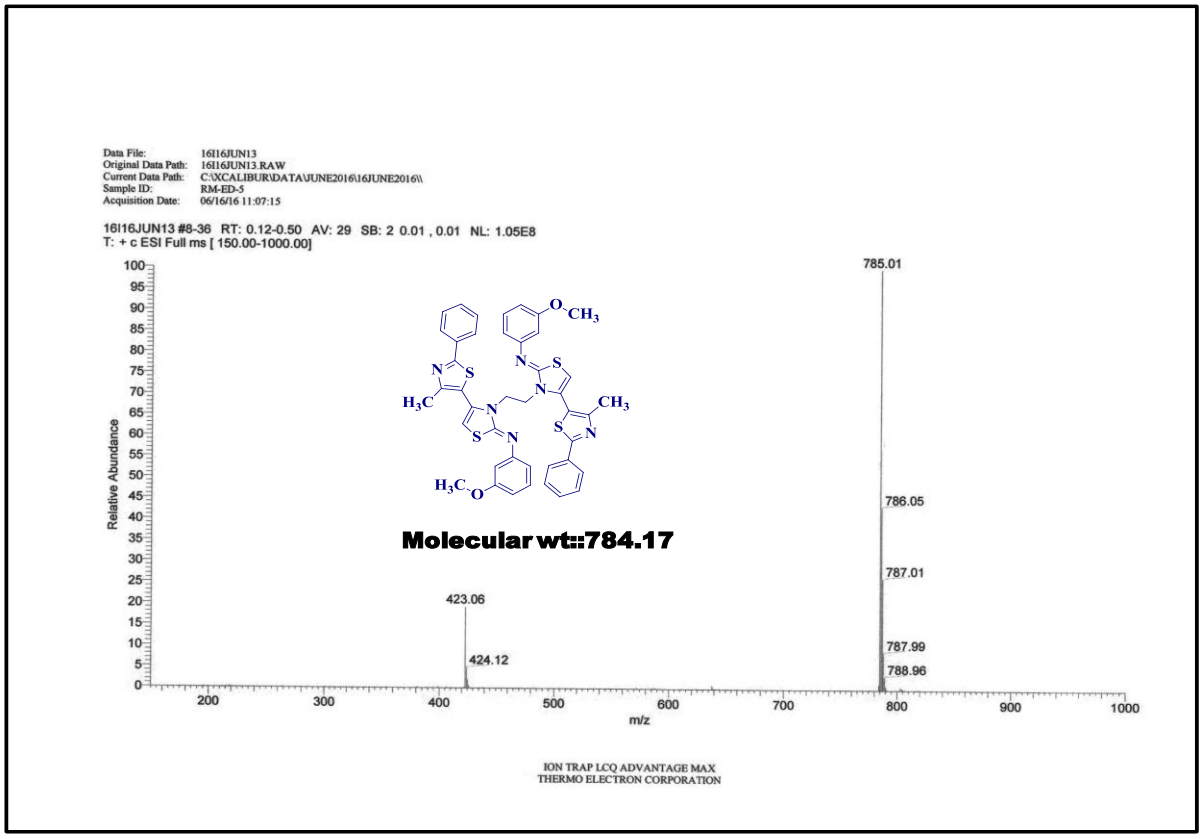


Fig: Mass spectrum of compound **53**

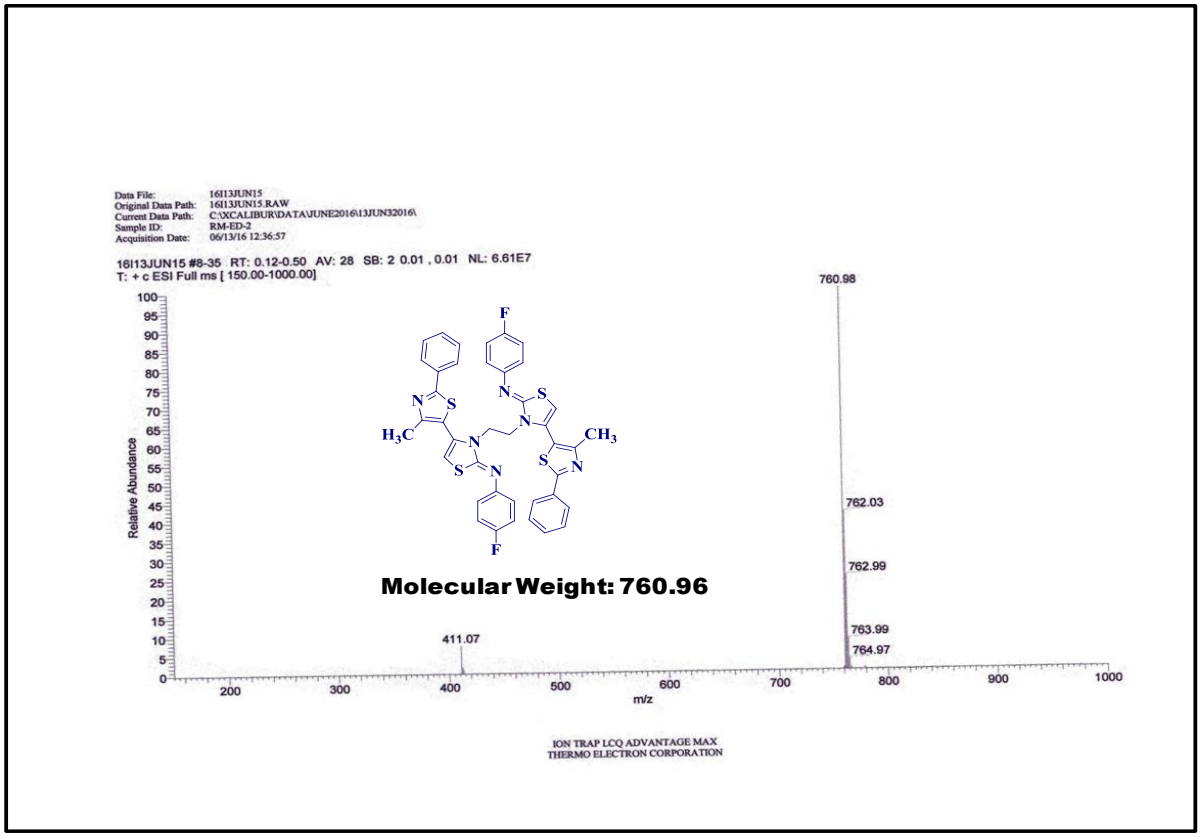


Fig: Mass spectrum of compound **53**

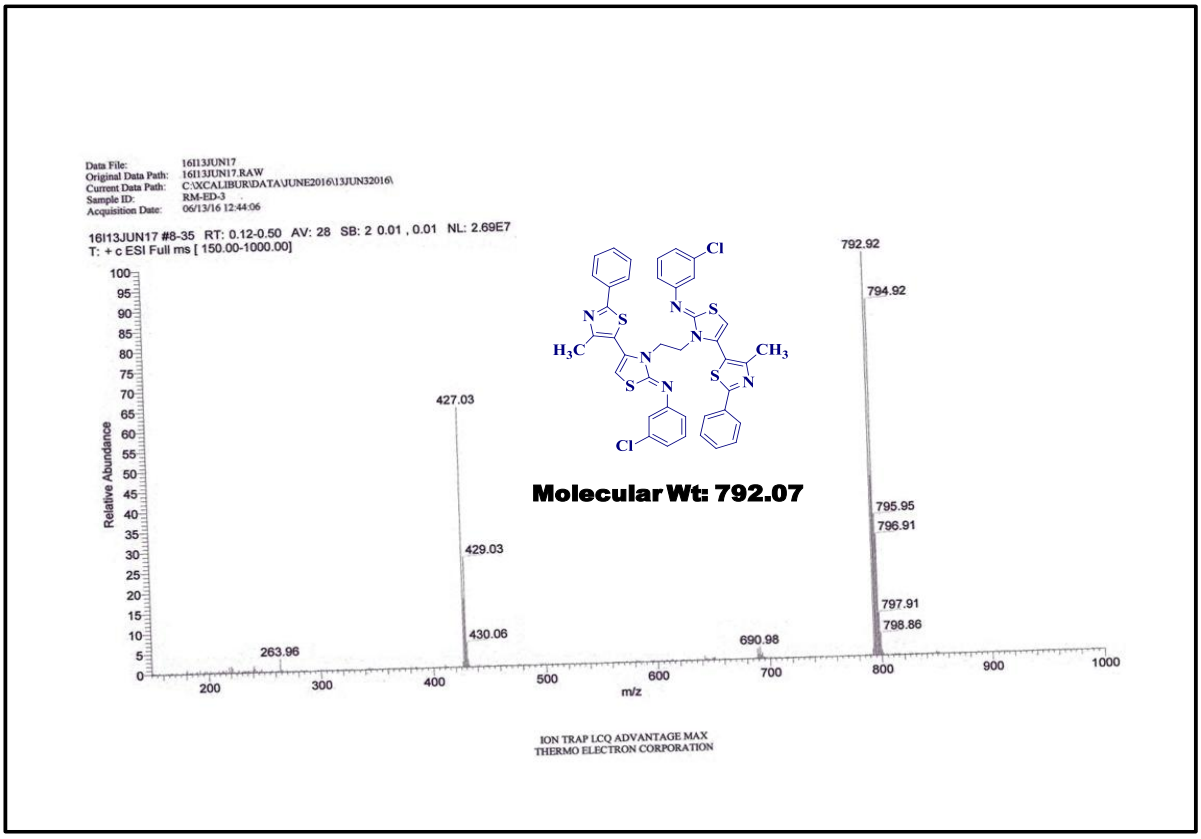


Fig: Mass spectrum of compound **53**

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CHAPTER-VIII

A FACILE SYNTHESIS OF 2,5-SUBSTITUTED 1,3,4-THIADIAZINES AND THEIR BIOLOGICAL APPLICATIONS

INTRODUCTION:

Chemistry of heterocyclic compounds is considered the important area in medicinal chemistry. Heterocyclic compounds with nitrogen and sulphur atoms in five membered and six membered ring systems are exhibiting variety of biological, material, electron applications and have received significant importance in organic chemistry. Among these nitrogen and sulphur heterocyclic compounds, thiadiazines have received promising importance in medicinal chemistry.

Thiadiazine is a six membered heterocyclic ring system possessing two nitrogen atoms and one sulphur atom. Thiadiazines will exist in various isomeric forms i.e. 1,2,4-thiadiazine (**1**), 1,2,6-thiadiazine (**2**), 1,3,4-thiadiazine (**3**) and 1,3,5-thiadiazines (**4**) (**Fig. 1**). Among these isomeric thiadiazine heterocyclic systems, 1,3,4-thiadiazine is a well studied isomeric system and have huge applications in the field of bio chemical, pharmacology, materials, photography, dyes and agriculture.

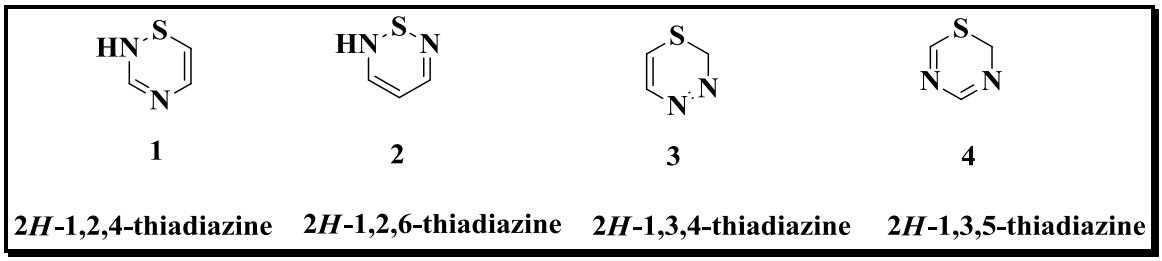


Fig 1. Isomeric structures of thiadiazine heterocyclic system

In 1,3,4-thiadiazine heterocyclic system sulphur and two nitrogen atoms are present at first, third and fourth positions. 1,3,4-thiadiazine exhibits three isomeric structures i.e. 2*H*- 1,3,4-thiadiazine, 4*H*- 1,3,4-thiadiazine and 6*H*- 1,3,4-thiadiazine (**Fig. 2**). The most common synthetic method for the synthesis of 1,3,4-thiadiazine heterocyclic system was the condensation reaction of α- haloketones compounds with thiosemicarbazide in acidic reaction condition[1]. Substituted 1,3,4- thiadiazine derivatives represents the most active class of compounds and are known to exhibit variety of therapeutic applications such as antimicrobial[2,3], anticancer[4], anti-inflammatory[5], cardiotonic[6,7], antistress[8] and metalloproteinase inhibition[9] applications. Fused 1,3,4-thiadiazines with triazoles heterocyclic compounds exhibit broad spectrum of biological applications[10-13].

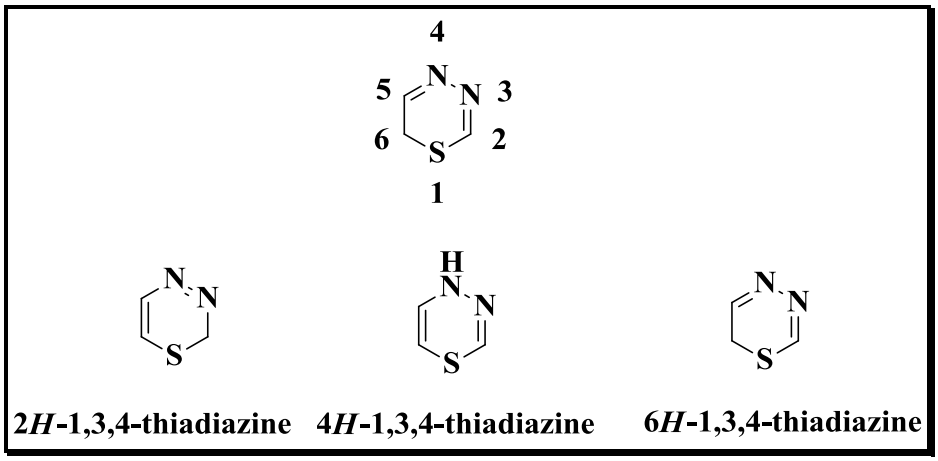
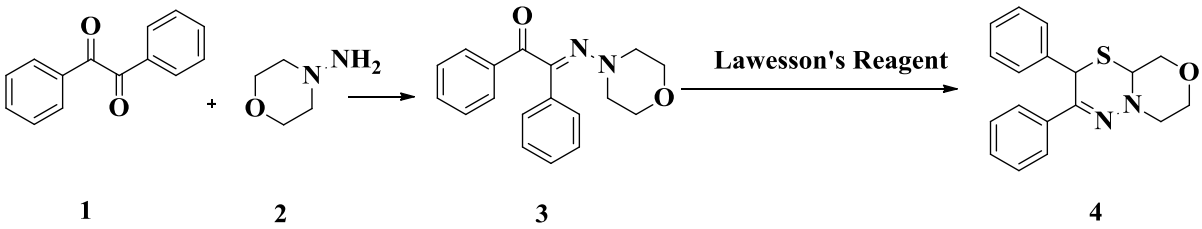


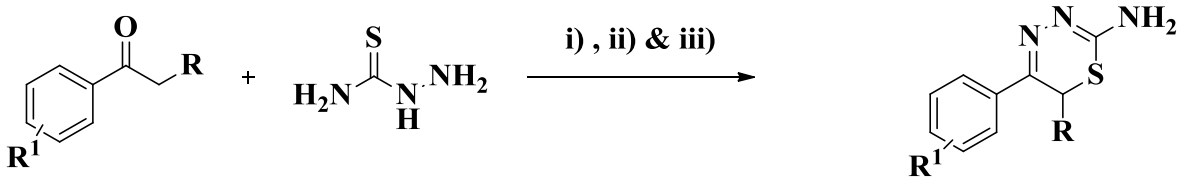
Fig. 2 Isomeric structures of 1,3,4-thiadiazine heterocyclic system

The following is a brief review of literature on synthetic methods on substituted 1,3,4-thiadiazine derivatives.

Charrier[14] developed an efficient synthetic for the synthesis of 1,3,4-thiadiazines (**4**). The target compound was synthesized by the reaction of benzil (**1**) with *N*- amino morpholine followed by intra molecular cyclization with Lawesson's reagent.



A versatile one pot three step procedure was reported by Hartmann [15] *et al.* by direct transformation of aromatic aryl ketones **5** in to 5-aryl-6*H*-1,3,4-thiadiazine 2-amines **7**. Initially various aryl ketones were brominated by using *N*-bromosuccinimide and *p*-toluenesulphonic acid in presence of acetonitrile. In the second step the brominated aryl ketones were treated with thiosemicarbazide **6** to get the uncyclized intermediate. In the third step the uncyclized intermediate was treated with potassium hydroxide to get the desired product with moderate to excellent yields.



5 6 7

R = H, Me, Ph

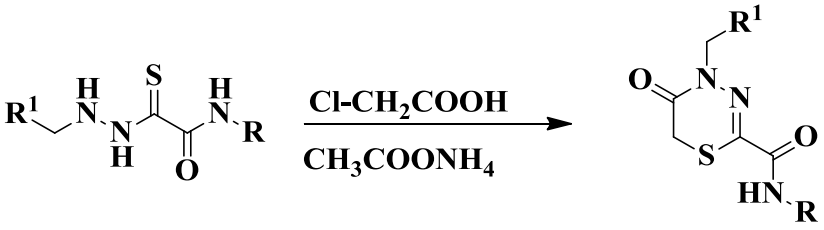
R^1 = H, OCH3, 2-Cl, 4-Cl,

i) = NBS, TsOH, MeCN, reflux

ii) = Thiosemicarbazide

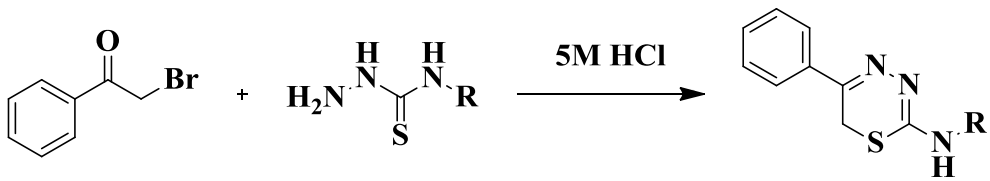
iii) = KOH, reflux

Zigangirova [16] *et al.* synthesized 2,4-substituted thiadiazines **9** with good yields by the reaction of 2-(2-benzylhydrazinyl)-*N*-aryl-2-thioxoacetamide **8** with chloroacetic acid in presence of ammonium acetate. The method applied for the synthesis was simple and gave the compounds with shorter reaction times.



8 9

An efficient method was developed by Pfeiffer [17] *et al.* to synthesize *N*-alkyl-5-phenyl-6*H*-1,3,4-thiadiazin-2- amine **12** derivatives. The target molecules were synthesized by the reaction of α-bromoacetophenone **10** with 4-methyl and 4-ethylthiosemicarbazide **11** in presence of 5M hydrochloric acid solution.

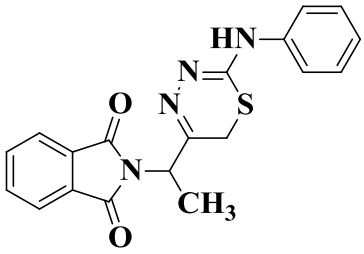


10 11 12

R = CH_3, C_2H_5

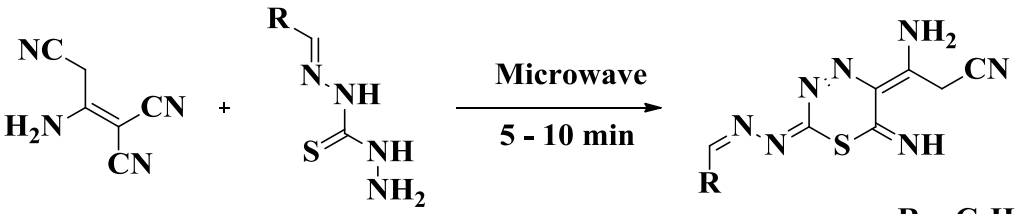
Yang [18] *et al.* reported the synthesis of 2-(1-(2-(phenylamino)-6*H*-1,3,4-thiadiazin-5-yl) ethyl) isoindoline-1,3-dione derivatives **13** in a step wise reaction method. The title compound was prepared starting from condensation reaction of isobenzofuran-1,3-dione

with amino acids followed by bromination and cyclization with thiosemicarbazide in moderate to good yields.



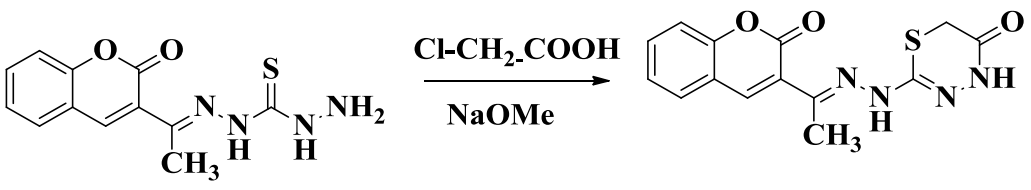
13

6-Imino-1,3,4-thiadiazene derivatives **16** were prepared by Elkanzi [19] *et al.* by the microwave reaction using 2-aminoprop-1-ene-1,1,3-tricarbonitrile **14** with thiocarbohydrazones **15** as starting materials. The target molecules were obtained in a shorter reaction time with simple reaction work up and in an excellent yield.



14 **15** **16**

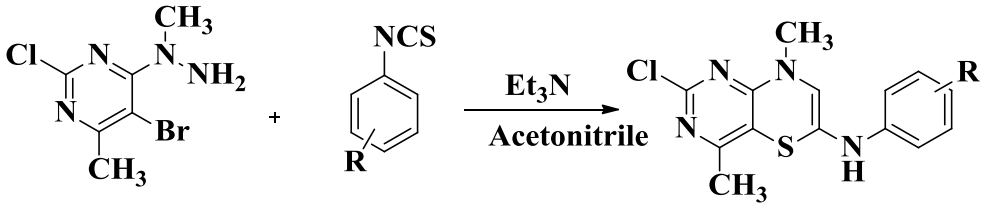
Abdel-Aziem[20] described the synthesis of 2-(2-(1-(2-oxo-2*H*-chromen-3-yl)ethylidene) hydrazinyl)-4*H*-1,3,4-thiadiazin-5(6*H*)-one **18** by the reaction of *N'*-(1-(2-oxo-2*H*-chromen-3-yl)ethylidene)hydrazinecarbothiohydrazide **17** with chloroacetic acid in presence of ethanol having sodium methoxide under reflux condition. The target compounds were obtained with in a shorter reaction time with good yields.



17 **18**

One pot synthesis of 2-anilino-pyrimido [4,5-*e*][1,3,4]-thiadiazine derivatives **21** were reported by M. Bakavoli [21] *et al.* by the reaction of 5-bromo-2-chloro-4-methyl-6-(1-

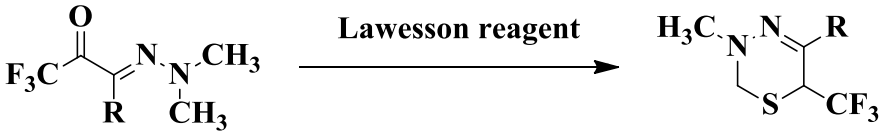
methylhydrazinyl)pyrimidine **19** with various substituted arylisothiocyantes **20** in presence of acetonitrile under reflux condition using triethylamine as a catalyst.



19 **20** **21**

R = H, 3-Cl, 4-F, 4-Cl, 4-Br

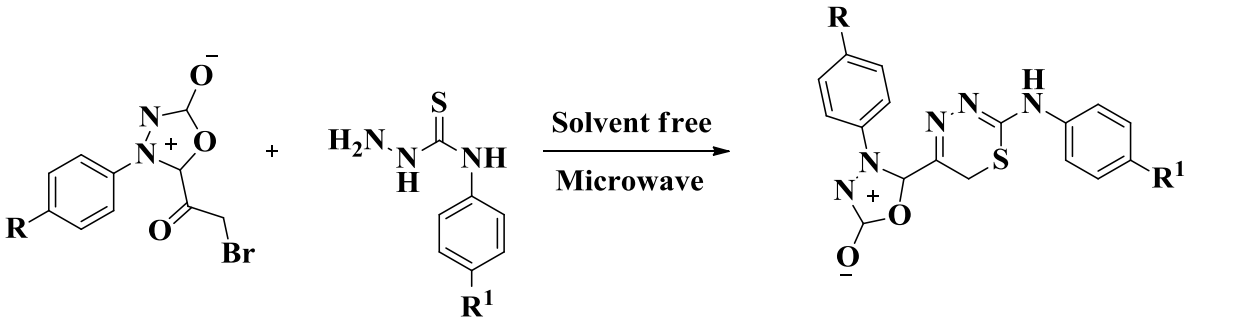
Y. Kamitori[22] *et al.* developed an efficient synthetic method for the synthesis of 6-trifluoromethyl-3,6-dihydro-2*H*-[1,3,4]thiadiazine derivatives **23** starting from 3-dimethydrazono-1,1,1-trifluoro-2-alkanones **22** with Lawesson reagent.



22 **23**

R = H, Ph

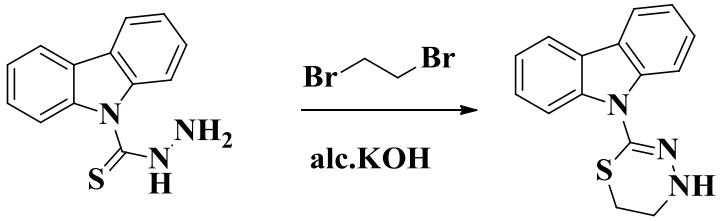
Solvent free and environmentally benign synthetic method was developed by Hegde[23] *et al.* to synthesize the 2-arylamino-5-(3'-arylsydnone-4-yl)-1,3,4-thiadiazine **26** derivatives under microwave reaction condition. The target compounds were obtained in a shorter reaction time with good yields by the reaction of 3-aryl-4-bromoacetylsydnone **24** with aryl thiosemicarbazide **25**.



24 **25** **26**

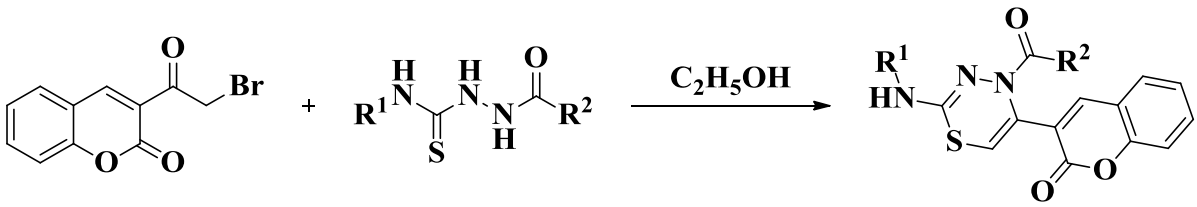
R = H, CH_3, -OCH_3 **R^1 = H, CH_3, -OCH_3, -Cl**

Abdel-Rahman[24] reported the synthesis of 2-(9*H*-carbazol-9-yl)-5,6-dihydro-4*H*-1,3,4-thiadiazine **28** in presence of alcaholic potassium hydroxide solution by the reaction of 9*H*-carbazole-9-carbothiohydrazide 27 and 1,2-dibromoethane with good yields.



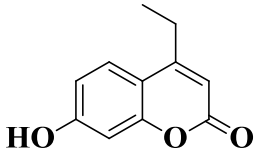
27 28

A series of trisubstituted 1,3,4- thiadiazine derivatives **31** were synthesized by Cacic [25] *et al.* starting from the reaction of 3-(2-bromoacetyl)coumarin **29** with 1,4- disubstituted thiosemicabazides **30** in presence of ethanol under reflux conditions. The title compounds were obtained in moderate to good yields with shorter reaction time.

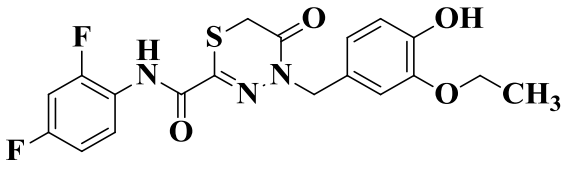


29 30 31

$R^1 = CH_3, C_2H_5, Ph$ $R^2 =$



Nesterenko[26] *et al.* described the synthesis of *N*-(2,4-difluorophenyl)-4-(3-ethoxy-4-hydroxybenzyl)-5-oxo-5,6-dihydro-4*H*-1,3,4-thiadiazine-2-carboxamide **32** and studied the *in vivo* antibacterial activity application against salmonella bacteria infections.



32

CHAPTER-VIII
PART-A

SYNTHESIS OF 3-(2-MORPHOLINO-6*H*-1,3,4-THIADIAZIN-5-YL)-2*H*-CHROMEN-2-ONE DERIVATIVES AND THEIR ANTIMICROBIAL ACTIVITIES

PART-A

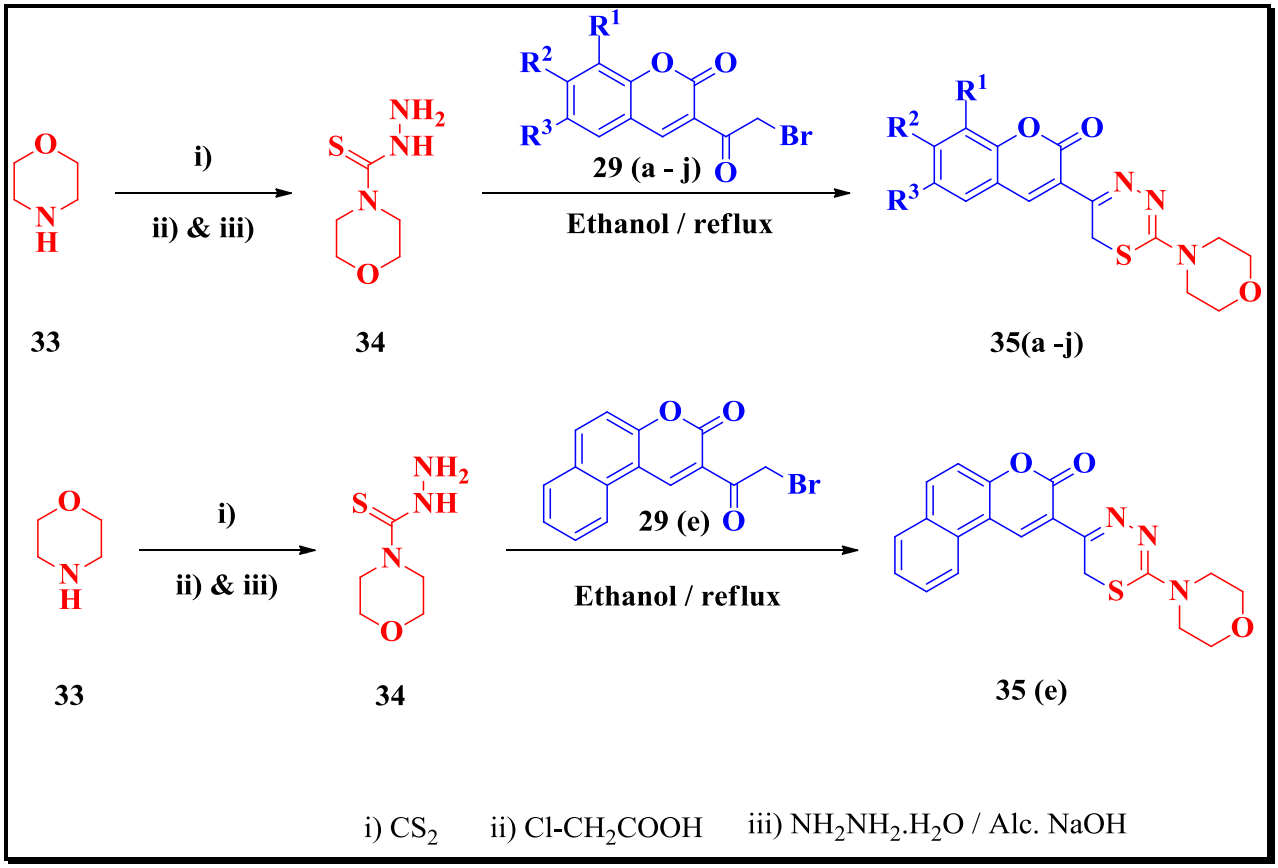
STARTING MATERIALS:

The reactants used for the synthesis of 3-(2-morpholino-6*H*-1, 3, 4-thiadiazin-5-yl)-2*H*-chromen-2-one derivatives (**35 a-j**) were prepared by using literature procedures. Morpholine-4-carbothiohydrazide[27] was prepared in three steps by using morpholine as a starting material. Various substituted 3-(2-bromoacetyl)coumarins were prepared according to the literature procedure as described in the earlier chapter starting from various substituted salicylaldehydes. Morpholine, carbon disulphide, chloroacetic acid and hydrazine hydrate were procured from commercial sources and used as such without any further purification.

Many biologically active compounds possess morpholine as one of the substituent in the heterocyclic system. In view of the synthetic, biochemical and therapeutic importance of 2, 5- disubstituted 1, 3, 4-thiadines, there is a huge demand to develop the simple, efficient and environmentally benign synthetic methods across the globe. The present chapter deals with the synthesis and antimicrobial activity evaluation of 3-(2-morpholino-6*H*-1, 3, 4-thiadiazin-5-yl)-2*H*-chromen-2-one derivatives.

The title compounds were synthesized in two steps by using morpholine as a starting material (**Scheme 1**). In the first step morpholine (**33**) was converted into morpholine-4-carbothiohydrazide (**34**) by the reaction with carbon disulphide, chloroacetic acid followed by refluxing with hydrazine hydrate in presence of alcoholic NaOH solution. The isolated morpholine-4-carbothio hydrazide intermediate was separated. The final step consists of the reaction of morpholine-4-carbothiohydrazide with substituted 3-(2-bromo acetyl) coumarins **29(a-j)** in presence of ethanol under reflux condition to generate the target compounds. The method was applied for the synthesis of target compounds, which gave very good yields with simple reaction conditions, easy work up and shorter reaction time (**Table 1**).

The newly synthesized compounds were well characterized by physical and analytical (IR, ^{1}H NMR, ^{13}C NMR and Mass) spectral data. The NMR and mass spectral data is consistent with the assigned structures of the title compounds.



Scheme 1: An efficient synthesis of 3-(2-morpholino-6*H*-1, 3, 4-thiadiazin-5-yl)-2*H*-chromen-2-one derivatives (**35 a-j**)

Table 1: An efficient synthesis of 3-(2-morpholino-6*H*-1, 3, 4-thiadiazin-5-yl)-2*H*-chromen-2-one derivatives

S. No	Compound	R^1	R^2	R^3	Yield (%)
1	**35a**	H	H	H	83
2	**35b**	H	H	Cl	85
3	**35c**	H	H	Br	88
4	**35d**	Br	H	Br	90
5	**35f**	OCH_3	H	H	82
6	**35g**	OCH_3	H	Br	86
7	**35h**	OC_2H_5	H	H	81
8	**35i**	OC_2H_5	H	Br	87
9	**35j**	CH_3	OH	H	80

The title compounds were screened against various human viral species i.e. *Feline Corona* Virus (FIPV) and *Feline Herpes* Virus, *Influenza* A/H1N1 A/Ned/378/05, *Influenza* A/H3N2 A/HK/7/87, *Influenza B* B/Ned/537/05, *Vesicular stomatitis* virus, *Coxsackie virus* B4, *Respiratory syncytial* virus, *Herpes simplex* virus-1 (KOS), *Herpes simplex* virus-2 (G), *Herpes simplex* virus-1 TK- KOS ACVr, *Vaccinia* virus, *Adeno*

virus-2, *Human Corona* virus (229E), *Para influenza*-3 virus, *Reo* virus, *Sindbis* virus, *Coxsackie* virus, *Puntatoro* virus and *Yellow fever* virus using different cell culture mediums (Crandell-Rees Feline Kidney cells (CRFK Cells), Madin Darby canine kidney cells (MDCK cells), HeLa Cells, HEL cells, Vero Cell culture medium).

The antiviral activity experiment was done in triplicate and the average values are taken for representing the EC_{50} Values and the antiviral activity results were shown in **Table 2-6**. From the antiviral activity results the compound **35f** exhibited moderate activity against *Feline Herpes* Virus at EC_{50} value of 38.95 µM concentration and the remaining compounds did not exhibited any activity against *Feline Corona* virus and *Feline Herpes* virus in CRFK cell culture medium (**Table 2**).

Table 2: Cytotoxicity and antiviral activity in: CRFK cell cultures

Compound	CC_{50} (µM) [a]	EC_{50} (µM)[b]	
		Feline Corona Virus (FIPV)	*Feline Herpes* Virus
35a	>100	>100	>100
35b	>100	>100	>100
35c	48.6	>100	>100
35d	55.0	>100	>100
35e	8.6	>100	>100
35f	>100	>100	**38.95**
35g	61.1	>100	>100
35h	57.7	>100	>100
35i	53.0	>100	>100
35j	44.7	>100	>100
HHA	>100	9.4	2.3
UDA	>100	5.7	2.0
Ganciclovir	>100	>100	3.4

[a]50% Cytotoxic concentration, as determined by measuring the cell viability with the colorimetric formazanb50%
[b]Effective concentration, or concentration producing 50% inhibition of virus-induced cytopathic effect, as
CRFK cells: Crandell-Rees Feline Kidney cells.

The title compounds were screened against *Influenza* A/H1N1 A/Ned/378/05, *Influenza* A/H3N2 A/HK/7/87 and *Influenza B* B/Ned/537/05 in MDCK cell culture medium (**Table.3**). The compound **35e** exhibited excellent activity against *Influenza* A/H1N1 A/Ned/378/05 at 5.2 µM (CPE score), 0.8 µM (MTS assay). The compound **35e** is more potent than the antiviral standard drugs like Ribavirin, Amantadine and Remantadine.

Table 3. Cytotoxicity and antiviral activity in: **MDCK cell cultures**

Compound	Cytotoxicity		Antiviral EC50[c]					
	CC_{50}[a]	Minimum cytotoxic concentration[b]	*Influenza* A/H1N1 A/Ned/378/05		*Influenza* A/H3N2 A/HK/7/87		*Influenza* B B/Ned/537/05	
			visual CPE score	MTS	visual CPE score	MTS	visual CPE score	MTS
35a	>100	100	>100	>100	>100	>100	>100	>100
35b	39.0	100	>100	>100	>100	>100	>100	>100
35c	45.6	100	>100	>100	>100	>100	>100	>100
35d	43.8	100	>100	>100	>100	>100	>100	>100
35e	>100	>100	**5.2**	**0.83**	>100	>100	>100	>100
35f	>100	100	>100	>100	>100	>100	>100	>100
35g	85.9	100	>100	>100	>100	>100	>100	>100
35h	>100	100	>100	>100	>100	>100	>100	>100
35i	62.2	100	>100	>100	>100	>100	>100	>100
35j	45.3	≥20	>100	>100	>100	>100	>100	>100
Zanamivir	>100	>100	0.4	0.1	0.8	0.1	0.5	0.4
Ribavirin	>100	100	8.9	4.4	4.0	1.0	8.9	8.4
Amantadine	>100	>100	100	>100	0.2	0.1	>100	>100
Rimantadine	>200	>200	>200	>200	0.02	0.01	>200	>200

[a]50% Cytotoxic concentration, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay.
[b]Minimum compound concentration that causes a microscopically detectable alteration of normal cell morphology.
[c]50% Effective concentration, or concentration producing 50% inhibition of virus-induced cytopathic effect, as determined by visual scoring of the CPE, or by measuring the cell viability with the colorimetric formazan-based MTS assay.
MDCK cells: Madin Darby canine kidney cells

The target compounds were screened against *Herpes simplex* virus-1 (KOS), *Herpes simplex* virus-2 (G), *Herpes simplex* virus-1 TK- KOS ACVr, *Vaccinia* virus, *Adeno* virus-2, *Human Corona* virus (229E) strains using HEL cell culture medium and Brividine, Cidofovir, Acyclovir, Ganciclovir, Zalcitabine, Alovudine, UDA and Ribavirin as reference drugs. The compounds 35a-j did not exhibit any antiviral activity against the tested viral species (**Table 4**).

Table 4. Cytotoxicity and antiviral activity in: HEL cell cultures

Compound	Minimum cytotoxic concentration[a] (μM)	EC_{50}[b] (μM)					
		Herpes simplex virus-1 (KOS)	*Herpes simplex* virus-2 (G)	*Herpes simplex* virus-1 TK-KOS ACVr	*Vaccinia* virus	*Adeno* virus-2	*Human Corona* virus (229E)
35a	>100	>100	>100	>100	>100	>100	>100
35b	100	>100	>100	>100	>100	>100	>100
35c	100	>100	>100	>100	>100	>100	>100
35d	100	>100	>100	>100	>100	>100	>100
35e	>100	>100	>100	>100	>100	>100	>100
35f	>100	>100	>100	>100	>100	>100	>100
35g	≥100	>100	>100	>100	>100	>100	>100
35h	≥100	>100	>100	>100	>100	>100	>100
35i	≥20	>100	>100	>100	>100	>100	>100
35j	20	>100	>100	>100	>100	>100	>100
Brivudin	>250	0.008	112	10	10	-	-
Cidofovir	>250	4.5	2	3.4	22	29	-
Acyclovir	>250	0.7	0.2	85	>250	-	-
Ganciclovir	>250	0.16	0.06	4	>100	-	-
Zalcitabine	>250	-	-	-	-	19	-
Alovudine	>250	-	-	-	-	50	-
UDA	>100	-	-	-	-	-	1.8
Ribavirin	>250	-	-	-	-	-	112

[a] Required to cause a microscopically detectable alteration of normal cell morphology.
[b] Required to reduce virus-induced cytopathogenicity by 50 %.

The target molecules were tested for their antiviral activity against *Vesicular stomatitis* virus, *Coxsackie virus* B4 and *Respiratory syncytial* virus in HeLa cell culture medium using Dextron Sulphate (DS-10.000) and Ribavirin as standard drugs. None of the compounds exhibited any activity against the tested viral species (**Table 5**).

Table 5. Cytotoxicity and antiviral activity in: **HeLa cell cultures**

Compound	Minimum cytotoxic concentration[a]	EC50[b]		
		Vesicular stomatitis virus	*Coxsackie* virus B4	*Respiratory syncytial* virus
35a	≥100	>100	>100	>100
35b	100	>100	>100	>100
35c	20	>100	>100	>100
35d	100	>100	>100	>100
35e	>100	>100	>100	>100

35f	≥100	>100	>100	>100
35g	100	>100	>100	>100
35h	≥100	>100	>100	>100
35i	20	>100	>100	>100
35j	20	>100	>100	>100
DS-10.000	>100	0.4	6.8	0.4
Ribavirin	>250	25	50	10

[a]Required to cause a microscopically detectable alteration of normal cell morphology.
[b]Required to reduce virus-induced cytopathogenicity by 50 %.

Similarly the title compounds **35 a-j** were evaluated for antiviral activity against *Para influenza*-3 virus, *Reo* virus, *Sindbis* virus, *Coxsackie* virus, *Punta Toro* virus and *Yellow fever* virus using Dextron sulphate, Ribavirin, Mycophenolic acid as antiviral reference drugs in Vero cell culture medium. The tested compounds did not exhibit any potency against the viral species (**Table 6**).

Table 6. Cytotoxicity and antiviral activity in: Vero cell cultures

Compound	Minimum cytotoxic conc.[a] (μM)	EC_{50}[b] (μM)					
		Para influenza-3 virus	*Reo*virus-1	*Sindbis* virus	*Coxsackie* virus B4	*Punta Toro* virus	*Yellow Fever* virus
35a	≥100	>100	>100	>100	>100	>100	>100
35b	≥100	>100	>100	>100	>100	>100	>100
35c	100	>100	>100	>100	>100	>100	>100
35d	100	>100	>100	>100	>100	>100	>100
35e	100	>100	>100	>100	>100	>100	>100
35f	>100	>100	>100	>100	>100	>100	>100
35g	≥20	>100	>100	>100	>100	>100	>100
35h	≥100	>100	>100	>100	>100	>100	>100
35i	100	>100	>100	>100	>100	>100	>100
35j	20	>100	>100	>100	>100	>100	>100
DS.10.000	>100	>100	>100	100	45	2.3	0.8
Ribavirin	>250	146	>250	>250	>250	125	>250
Mycophenolic acid	≥100	0.4	0.5	20	45	4.0	0.8

[a]Required to cause a microscopically detectable alteration of normal cell morphology.
[b]Required to reduce virus-induced cytopathogenicity by 50 %.

In summary, we have developed an efficient method for the synthesis of 3-(2-Morpholino-6*H*-1, 3, 4-thiadiazin-5-yl)-2*H*-chromen-2-one derivatives through a simple reaction conditions using readily available starting materials with good yields. The title

compounds were selectively inhibiting the Feline Herpes Virus and *Influenza* A/H1N1 A/Ned/378/05 viral growth. The compound **35f** is an excellent antiviral agent when compared to the standard antiviral drugs like Ribavirin, Amantadine and Remantadine.

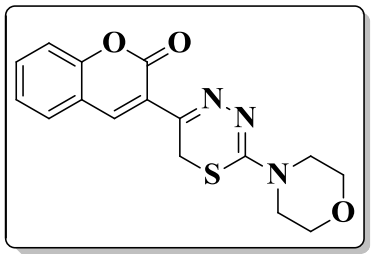
EXPERIMENTAL:

3-(2-Morpholino-6*H*-1, 3, 4-thiadiazin-5-yl)-2*H*-chromen-2-one (35a):

An equivalent mixture of morpholine-4-carbothiohydrazide (0.161 g, 1.0 mmol) and substituted 3-(2-bromoacetyl)-2*H*-chromen-2-ones (1.0 mmol) were taken in round bottom flask containing ethanol solvent (5 mL). The reaction mixture was heated to 60 – 65 °C for about 3 - 4 h. After completion of the reaction (by monitored TLC) the reaction mixture was cooled to room temperature and the isolated product was filtered. The product was recrystallized from methanol.

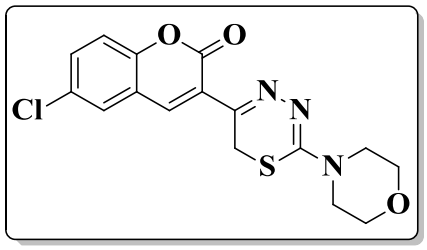
SPECTRAL DATA:

3-(2-Morpholino-6*H*-1, 3, 4-thiadiazin-5-yl)-2*H*-chromen-2-one (35a):



Red solid; m.p.: 188 - 190 °C; **FTIR (KBr, υ_{max}, cm^{-1}):** 1713 (lactone –C=O); 1606 (-C=N); 1276 (-C-O-C); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 3.79(d, *J* = Hz, 4H, -N-CH_2), 3.86 (s, 4H, -OCH_2), 4.16 (s, 2H, -SCH_2), 7.46 (t, *J* = 7.6 Hz, 1H, Ar-H), 7.53 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.73 – 7.77 (m, 1H, Ar-H), 7.94 (d, *J* = 7.6 Hz, 1H, Ar-H), 8.50 (s, 1H, C_4-H of coumarin); **^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ** 23.68, 49.26, 65.14, 116.21, 118.50, 122.04, 125.12, 129.79, 133.64, 143.60, 149.08, 153.79, 158.71, 160.90; **MS (ESI m/z):** 330 $[M+H]^+$; Anal.Calcd for $C_{16}H_{15}N_3O_3S$: C, 58.34; H, 4.59; N, 12.76%; Found: C, 58.40; H, 4.55; N, 12.73 %.

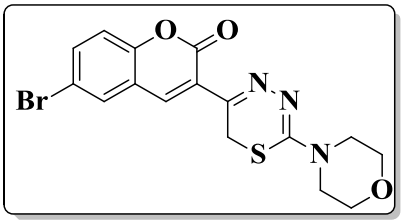
6-Chloro-3-(2-morpholino-6*H*-1, 3, 4-thiadiazin-5-yl)-2*H*-chromen-2-one (35b):



Red solid; m.p.: 157 - 159 °C; **FTIR (KBr, υ_{max}, cm^{-1}):** 1717 (Lactone-C=O); 1605 (-C=N); 1244 (-C-O-C); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 3.60 (d, *J* = Hz, 4H, -NCH_2), 3.77 (d, *J* = Hz, 4H, -OCH_2), 4.13 (s, 2H, -SCH_2); 7.55 (d, *J* = 8.8 Hz, 1H, Ar-H), 7.75 – 7.78 (m, 1H, Ar-H), 8.07 (d, *J* = 2.4 Hz, 1H, Ar-H), 8.41 (s, 1H, C_4-H of

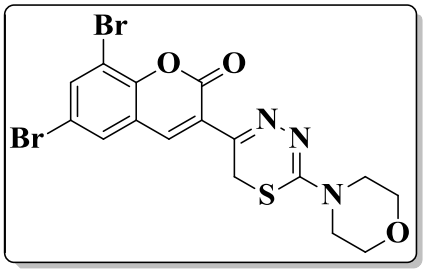
coumarin); **^{13}C NMR (100 MHz, DMSO-d_6, δ ppm):** 23.67, 49.38, 65.11, 118.33, 119.91, 123.24, 128.66, 129.50, 133.06, 133.83, 142.19, 145.68, 148.97, 152.46, 158.37, 161.30; **MS (ESI m/z):** 364 $[M+H]^+$; Anal. Calcd for $C_{16}H_{14}ClN_3O_3S$: C, 52.82; H, 3.88; N, 11.55%; Found: C, 52.78; H, 3.92; N, 11.58%.

6-Bromo-3-(2-morpholino-6*H*-1, 3, 4-thiadiazin-5-yl)-2*H*-chromen-2-one (35c):



Red solid; m.p.: 178 - 180 °C; **FTIR (KBr, υ_{max}, cm^{-1}):** 1719 (Lactone –C=O); 1580 (-C=N); 1242 (-C-O-C); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 3.77 (d, *J* = 4.8Hz, 4H, -NCH_2), 3.81 (d, *J* = 4.8Hz, 4H, -OCH_2); 4.08 (s, 2H, -SCH_2); 7.48 (d, *J* = 8.8Hz, 1H, Ar-H), 7.85 – 7.88 (m, 1H, Ar-H), 8.19 (d, *J* = 2.4 Hz, 1H, Ar-H), 8.40 (s, 1H, C_4-H of coumarin); **^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ** 23.59, 49.22, 65.11, 116.59, 118.54, 120.40, 123.27, 131.60, 135.76, 141.98, 148.76, 152.84, 158.33; **MS(ESI m/z):** 410 $[M+2H]^+$; Anal. Calcd for $C_{16}H_{14}BrN_3O_3S$ (408.26): C, 47.07; H, 3.46; N, 10.29%; Found: C, 47.13; H, 3.41; N, 10.31%.

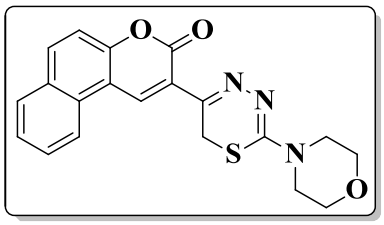
6, 8-Dibromo-3-(2-morpholino-6*H*-1, 3, 4-thiadiazin-5-yl)-2*H*-chromen-2-one (35d):



Red solid; yield: (0.438 g, 90%); mp: 169 - 171 °C; **IR (KBr, υ_{max}, cm^{-1}):** 1722 (Lactone-C=O); 1599 (-C=N), 1243 (-C-O-C); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 3.76(d, *J* = 4.8 Hz, 4H, -NCH_2), 3.81(d, *J* = 4.8 Hz, -OCH_2), 4.02(s, 2H, -SCH_2), 8.19 (t, *J* = 2.4 Hz, 2H, Ar-H), 8.38 (s, 1H, C_4-H of coumarin); **^{13}C NMR (100 MHz, DMSO-d_6, δ ppm):** 23.65; 49.35; 65.10; 116.63; 118.58; 120.40; 123.21; 131.64; 135.82; 142.08; 148.93; 152.87; 158.34; **MS (ESI m/z):** 488 $[M+H]^+$; Anal. Calcd for $C_{16}H_{13}Br_2N_3O_3S$: C, 39.45; H, 2.69; N, 8.63%; Found: C, 39.48; H, 2.72; N, 8.58%.

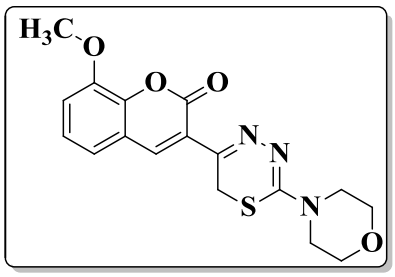
2-(2-Morpholino-6*H*-1, 3, 4-thiadiazin-5-yl)-3*H*-benzo[*f*]chromen-3-one (35e):

Red solid; m.p.: 238 - 240 °C; **FTIR (KBr, υ_{max}, cm^{-1}):** 1712 (Lactone-C=O); 1597 (-C=N); 1275 (-C-O-C); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 3.80 (d, *J* = 4.4 Hz, 4H, -NCH_2), 3.88 (d, *J* = 4.4 Hz, 4H, -OCH_2), 4.26 (s, 2H, -SCH_2), 7.66 – 7.70 (m, 2H, Ar-H), 7.80 (t, *J* = 7.6 Hz, 1H, Ar-H), 8.12 (d, *J* = 8.4 Hz, 1H, Ar-H), 8.33 (d, *J* = 9.2Hz,



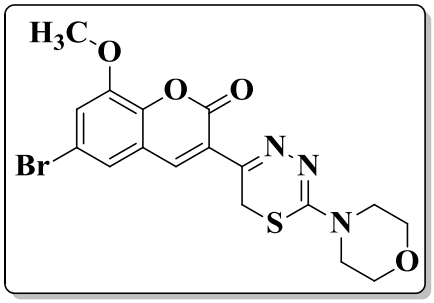
1H, Ar-H), 8.62 (d, *J* = 8.4 Hz, 1H, Ar-H), 9.24 (s, 1H, C_4-H of coumarin); **^{13}C NMR (100 MHz, DMSO-d_6, δ ppm):** 23.86; 49.44; 65.19; 112.78; 116.53; 120.51; 122.28; 126.58; 129.00; 129.13; 130.02; 135.38; 139.91; 149.41; 154.27; 158.06; 161.67; **MS (ESI m/z):** 380 $[M+H]^+$; Anal. Calcd for $C_{20}H_{17}N_3O_3S$: C, 63.31; H, 4.52; N, 11.07%; Found: C, 63.28; H, 4.59; N, 11.05%.

8-Methoxy-3-(2-morpholino-6*H*-1, 3, 4-thiadiazin-5-yl)-2*H*-chromen-2-one (35f):



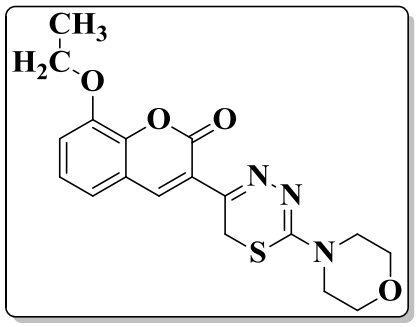
Red solid; m.p.: 164 -166 °C; **FTIR (KBr, υ_{max}, cm^{-1}):** 1707 (Lactone –C=O); 1608 (-C=N); 1275 (-C-O-C); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 3.76 (d, *J* = 4.8 Hz, 4H, -NCH_2), 3.81 (d, *J* = 4.8 Hz, 4H, -OCH_2), 3.94 (s, 3H, -OCH_3), 4.09 (s, 2H, -SCH_2), 7.34 – 7.46 (m, 3H, Ar-H), 8.45 (s, 1H, C_4-H of coumarin); **^{13}C NMR (100 MHz, DMSO-d_6, δ ppm):** 23.61, 49.15, 56.23, 65.17, 115.72, 119.08, 119.53, 120.75, 122.26, 125.07, 143.09, 143.76, 146.27, 146.38, 158.45; **MS (ESI m/z):** 360 $[M+H]^+$; Anal. Calcd for $C_{17}H_{17}N_3O_4S$ (359.39): C, 56.81; H, 4.77; N, 11.69%; Found: C, 56.85; H, 4.72; N, 11.72%.

6-Bromo-8-methoxy-3-(2-morpholino-6*H*-1, 3, 4-thiadiazin-5-yl)-2*H*-chromen-2-one (35g):



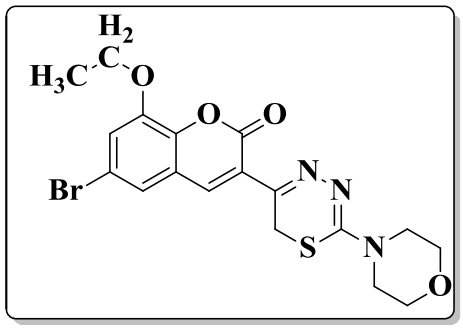
Red solid; m.p.: 155-157 °C; **IR (KBr, υ_{max}, cm^{-1}):** 1706 (Lactone C=O); 1603 (-C=N); 1276 (-C-O-C); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): MS (ESI m/z):** 440 $[M+2H]^+$; Anal. Calcd for $C_{17}H_{16}BrN_3O_4S$ (438.29): C, 46.59; H, 3.68; N, 9.59%; Found: C, 46.62; H, 3.61; N, 9.63%.

8-Ethoxy-3-(2-morpholino-6*H*-1, 3, 4-thiadiazin-5-yl)-2*H*-chromen-2-one (35h):



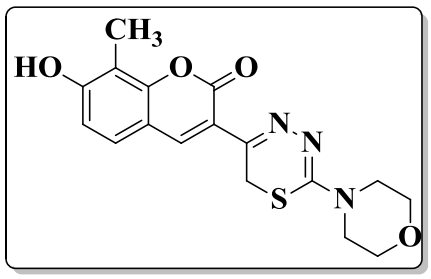
Red solid; m.p.: 210 - 212 °C; **FTIR (KBr, υ_{max}, cm^{-1}):** 1704 (Lactone –C=O); 1608 (-C=N); 1281 (-C-O-C); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 1.43 (t, *J* = 6.8 Hz, 3H, $-CH_3$), 3.79 (d, *J* = 4Hz, 4H, $-NCH_2$), 3.86 (d, *J* = 3.6 Hz, 4H, $-OCH_2$), 4.17 (s, 2H, $-SCH_2$), 4.20 – 4.25 (m, 2H, $-OCH_2$), 7.36 (t, *J* = 7.6 Hz, 1H, Ar-H), 7.42 -7.47 (m, 2H, Ar-H), 8.47 (s, 1H, C_4-H of coumarin); **MS (ESI m/z):** 374 $[M+H]^+$; Anal. Calcd for $C_{18}H_{19}N_3O_4S$ (373.42): C, 57.89; H, 5.13; N, 11.25%; Found: C, 57.83; H, 5.16; N, 11.29%.

6-Bromo-8-ethoxy-3-(2-morpholino-6*H*-1, 3, 4-thiadiazin-5-yl)-2*H*-chromen-2-one (35i):



Red solid; yield: (0.393 g, 87%); mp: 196 - 198 °C; **FTIR (KBr, υ_{max}, cm^{-1}):** 1733 (Lactone-C=O); 1599 (-C=N); 1273 (-C-O-C); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): MS (ESI m/z):** 452 $[M]^+$, 454 $[M+2H]^+$; Anal. Calcd for $C_{18}H_{18}BrN_3O_4S$ (452.32): C, 47.80; H, 4.01; N, 9.29%**;** Found: C, 47.84; H, 3.98; N, 9.32%.

7-Hydroxy-8-methyl-3-(2-morpholino-6*H*-1,3,4-thiadiazin-5-yl)-2*H*-chromen-2-one (35j):



Red solid; m.p.: 229 - 231 °C; **FTIR (KBr, υ_{max}, cm^{-1}):** 3452 (-OH); 1703 (Lactone – C=O); 1610 (-C=N); 1256 (C-O-C); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 2.18 (s, 3H, $-CH_3$), 3.78 (d, *J* = 4 Hz, 4H, $-NCH_2$), 3.84 (d, *J* = 3.6 Hz, 4H, $-OCH_2$), 4.21

(s, 2H, -SCH_2), 6.94 (d, *J* = 8.8 Hz, 1H, Ar-H), 7.60 (d, *J* = 8.4 Hz, 1H, Ar-H), 8.40 (s, 1H, C_4-H of coumarin), 11.00 (s, 1H, -OH); **MS (ESI m/z):** 360.08 $[M+H]^+$; Anal. Calcd for $C_{17}H_{17}N_3O_4S$ (359.39): C, 56.81; H, 4.77; N, 11.69%; Found: C, 56.84; H, 4.80; N, 11.65%.

Antiviral activity experiment of 3-(2-Morpholino-6*H*-1, 3, 4-thiadiazin-5-yl)-2*H*-chromen-2-one derivatives 35 a-j:

The antiviral activity of the newly synthesized compounds was determined as reported in chapter-II (Reference 65 - 67). (i) HEL cell culture medium was used for viruses (*Herpes simplex virus type 1, Herpes simplex virus type 2, Herpes simplex virus type 1 TK- ACV*^{r}*, Vaccinia virus, Adeno virus-2* or *Vesicular stomatitis virus*), (ii) Vero cells were used for the virus (*Coxsackie virus B4, Sindbis virus, Parainfluenza virus 3, Punta Toro virus* or *Reovirus*), (iii) HeLa cells were used for the virus (*Coxsackie virus B4, Vesicular stomatitis virus* or *Respiratory syncytial virus*), (iv) MDCK cells were used for the virus (*Influenza A/H1N1 A/Ned/378/05, Influenza A/H3N2 A/HK/7/87* or *Influenza B B/Ned/537/05*) (v) CRFK cells for the virus (*Feline herpes virus* or *Feline corona virus*). After incubation at 37°C the virus-induced cytopathicity was determined by measuring the cell viability with the colorimetric formazan-based MTS assay.

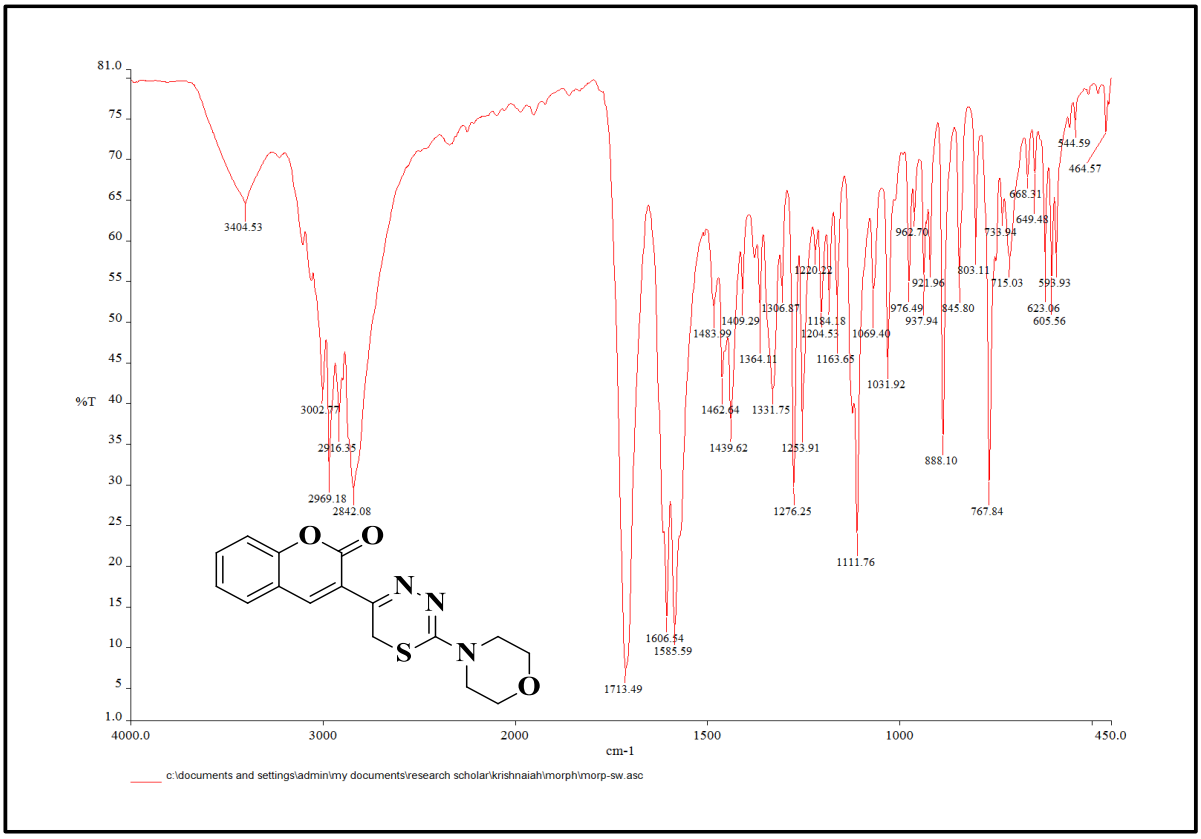


Fig: IR Spectrum of Compound **35a**

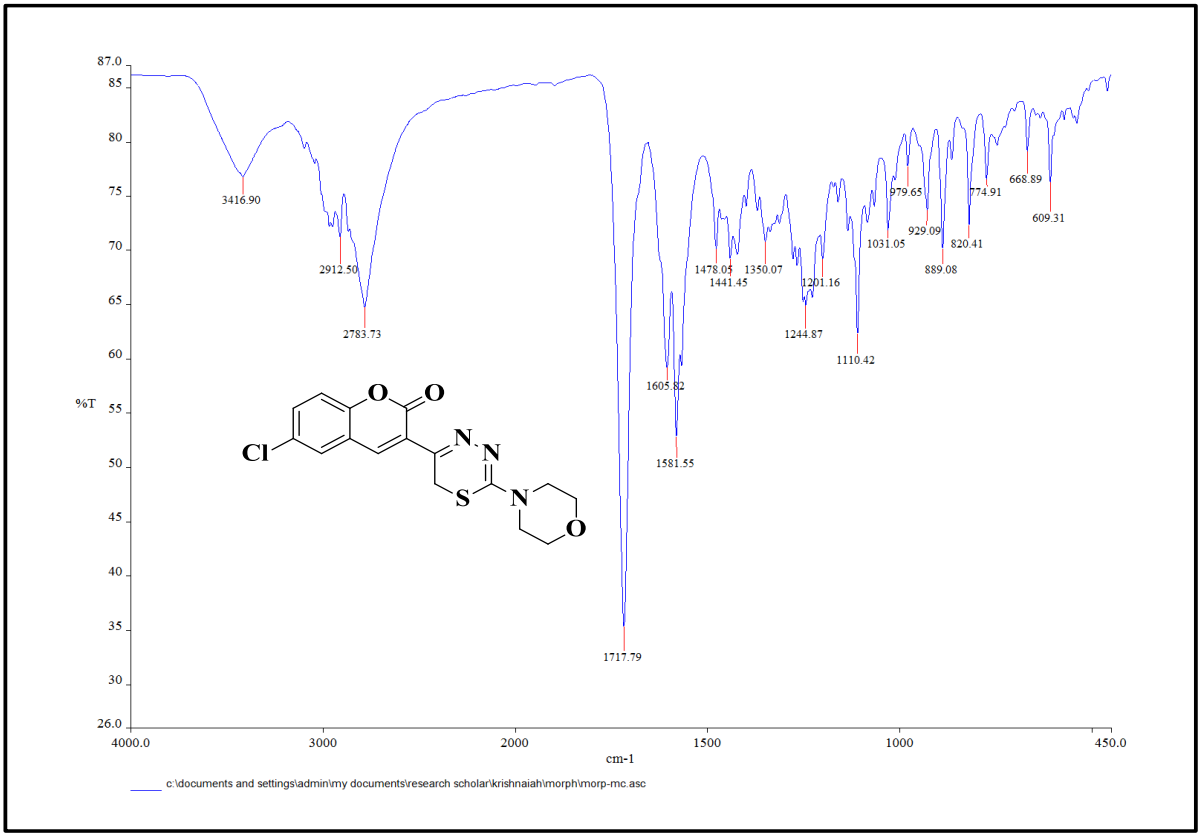


Fig: IR Spectrum of Compound **35b**

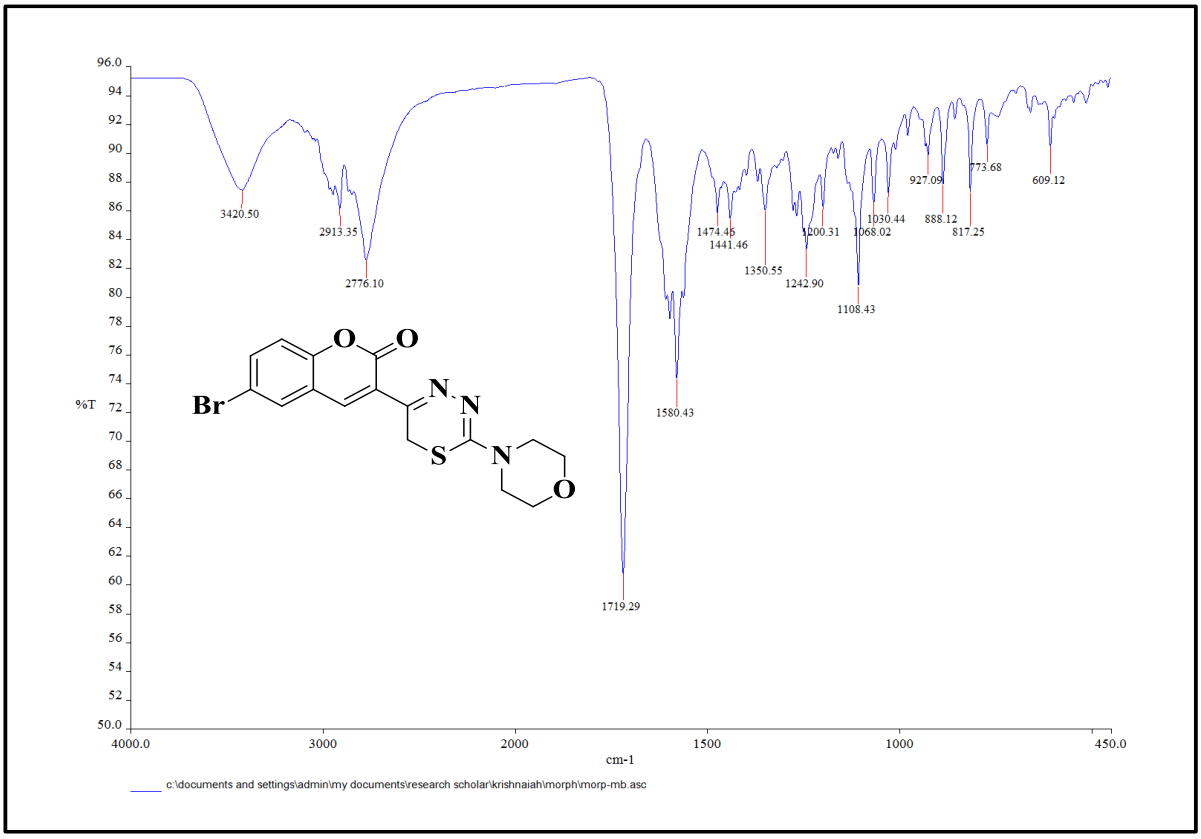


Fig: IR Spectrum of Compound **35c**

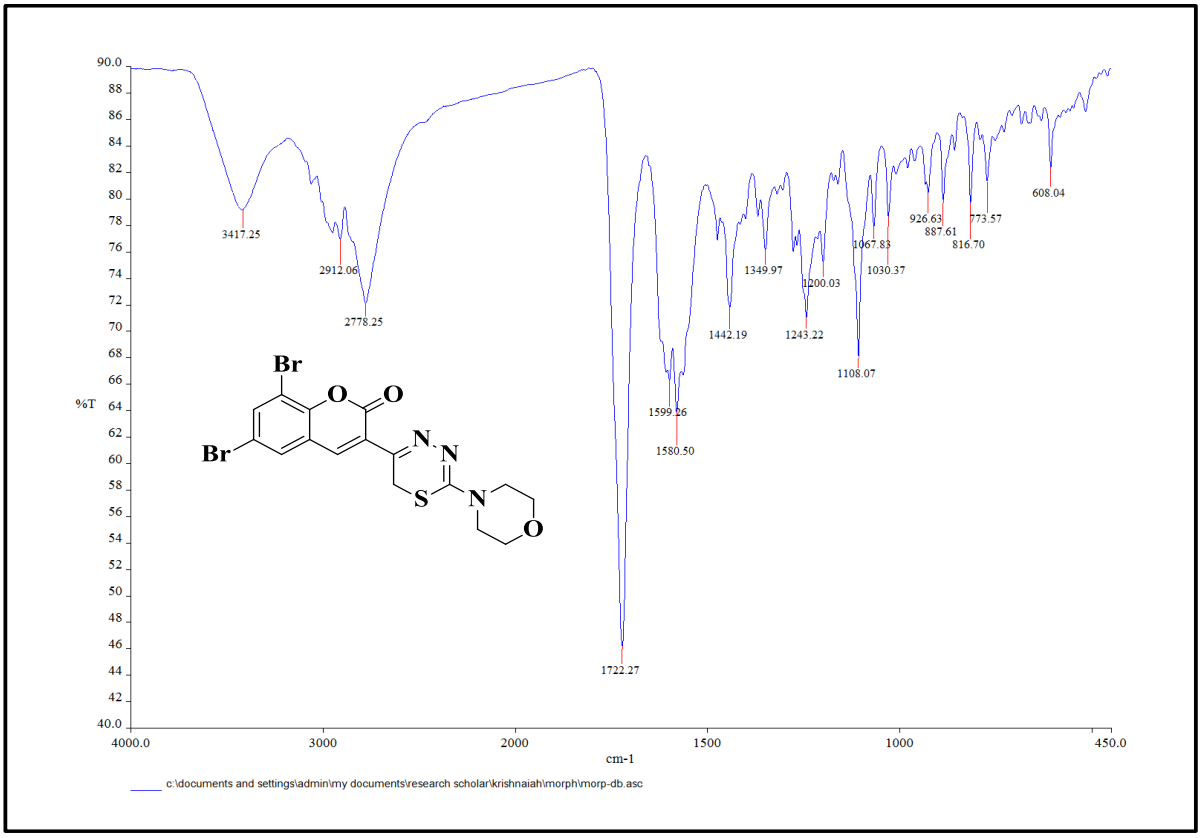


Fig: IR Spectrum of Compound **35d**

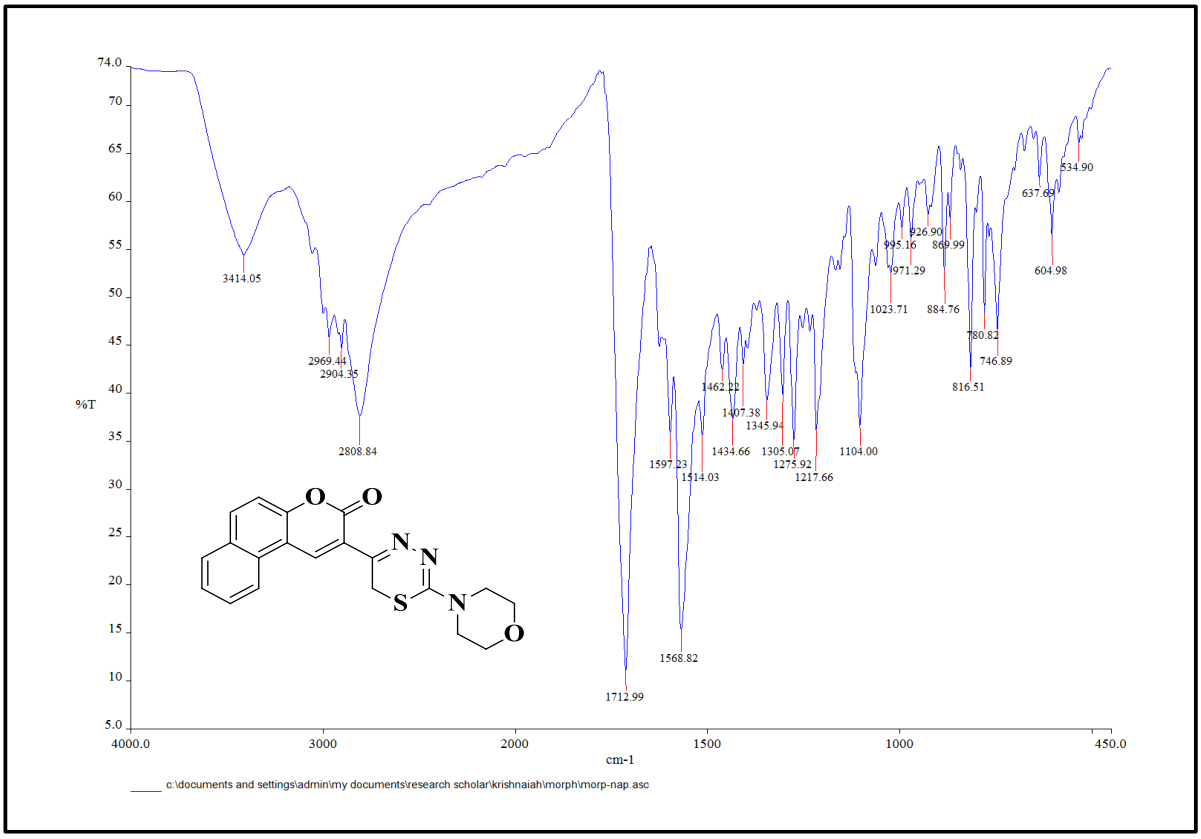


Fig: IR Spectrum of Compound **35e**

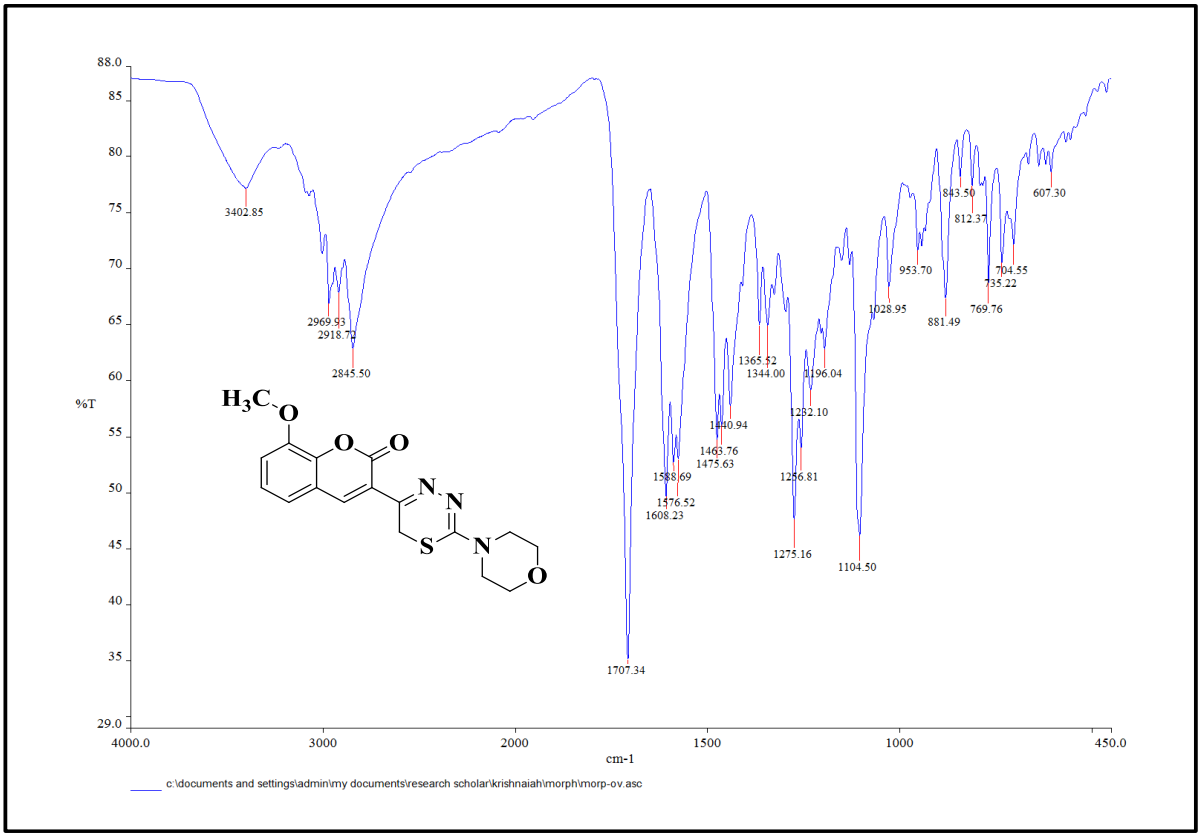


Fig: IR Spectrum of Compound **35f**

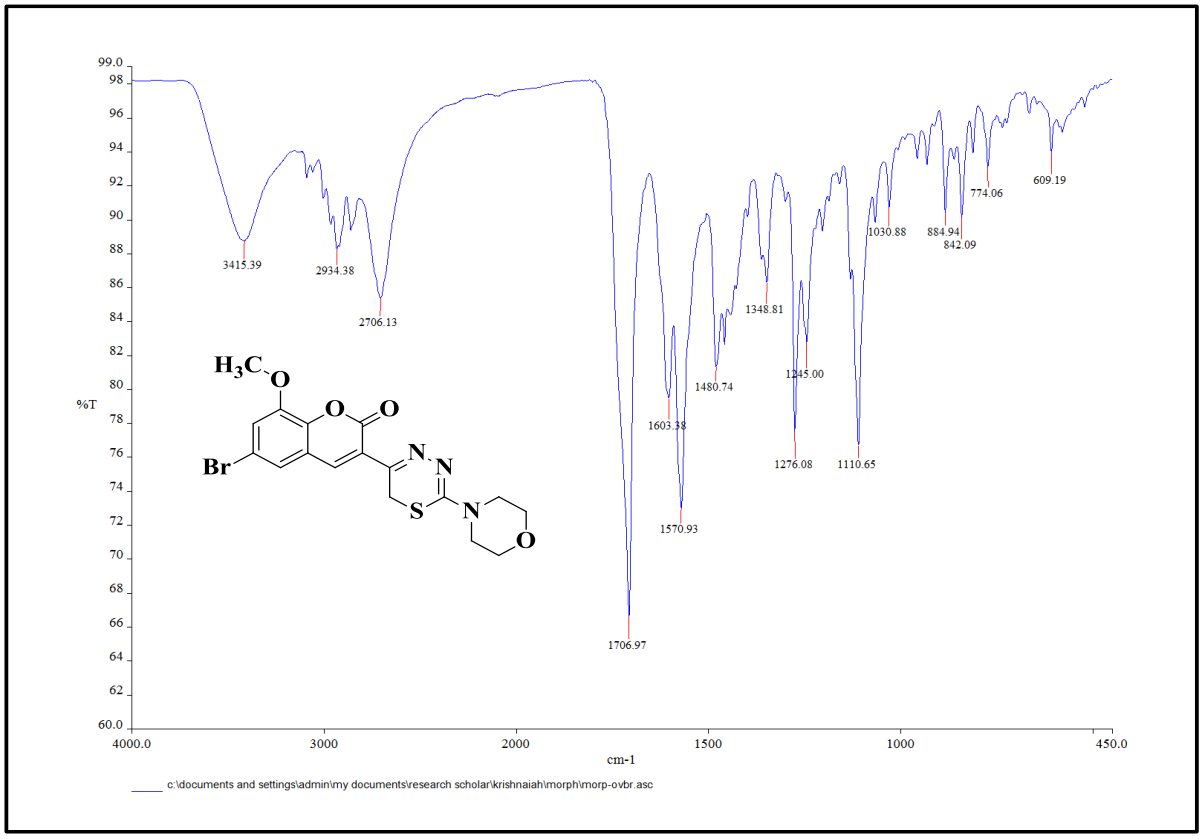


Fig: IR Spectrum of Compound **35g**

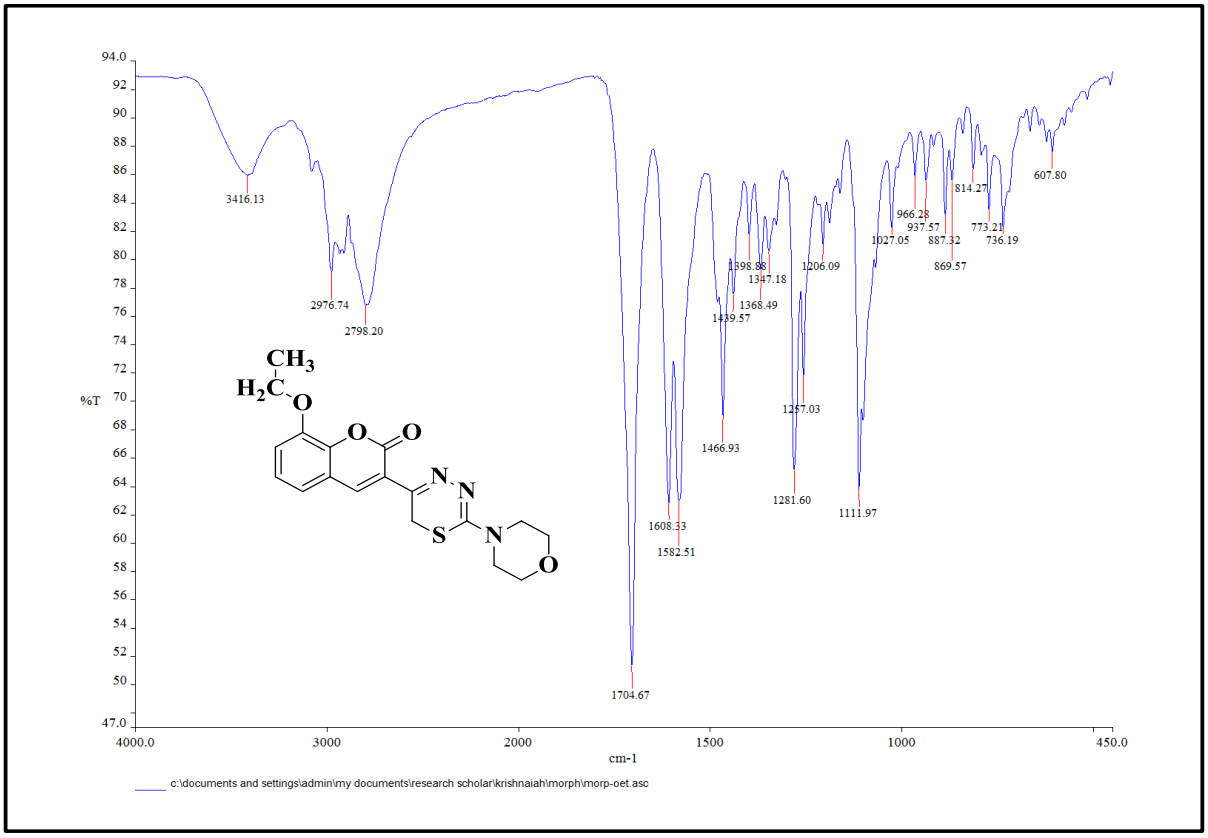


Fig: IR Spectrum of Compound **35h**

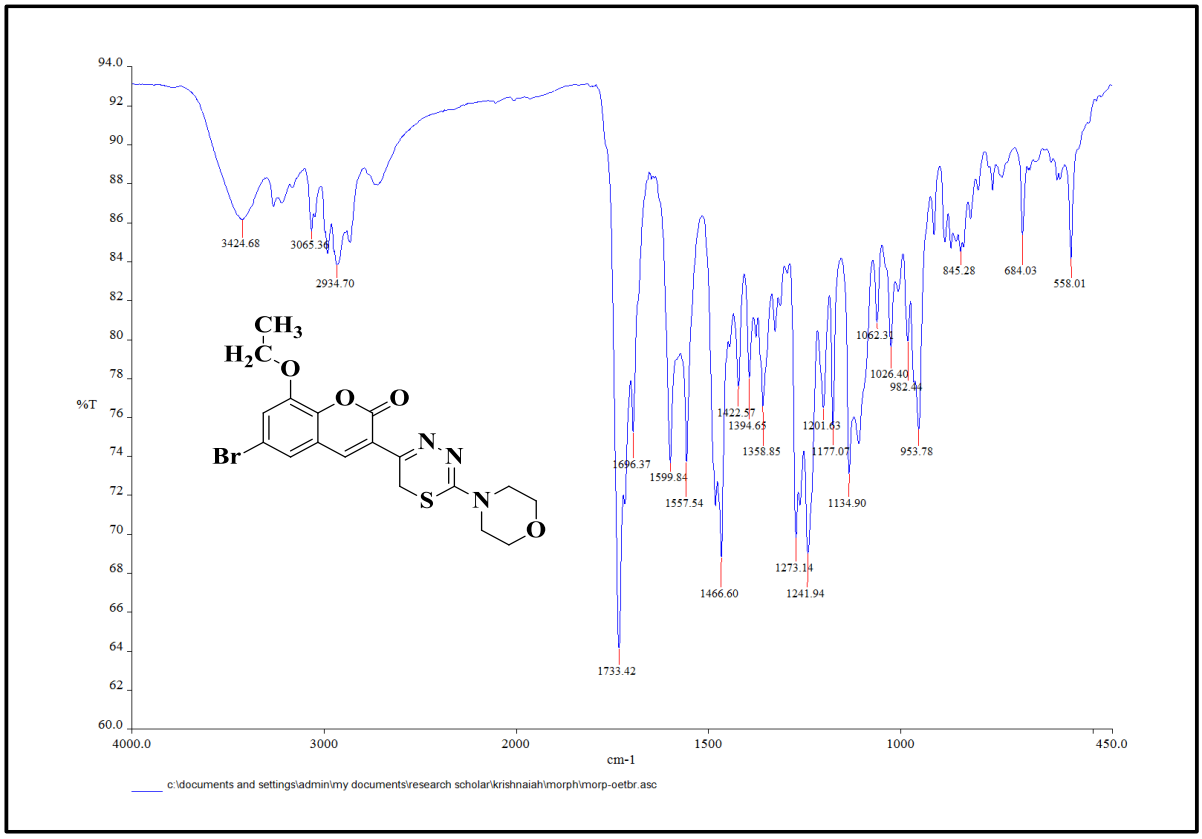


Fig: IR Spectrum of Compound **35i**

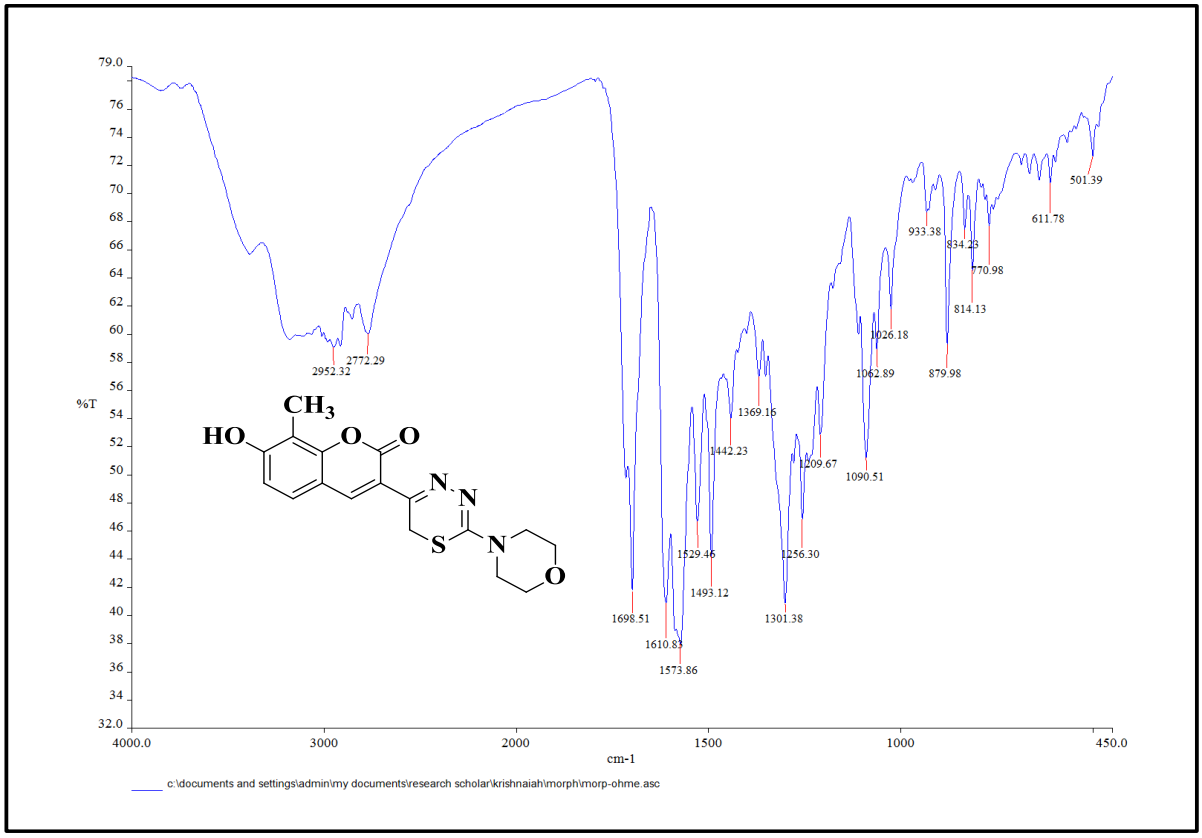


Fig: IR Spectrum of Compound **35j**

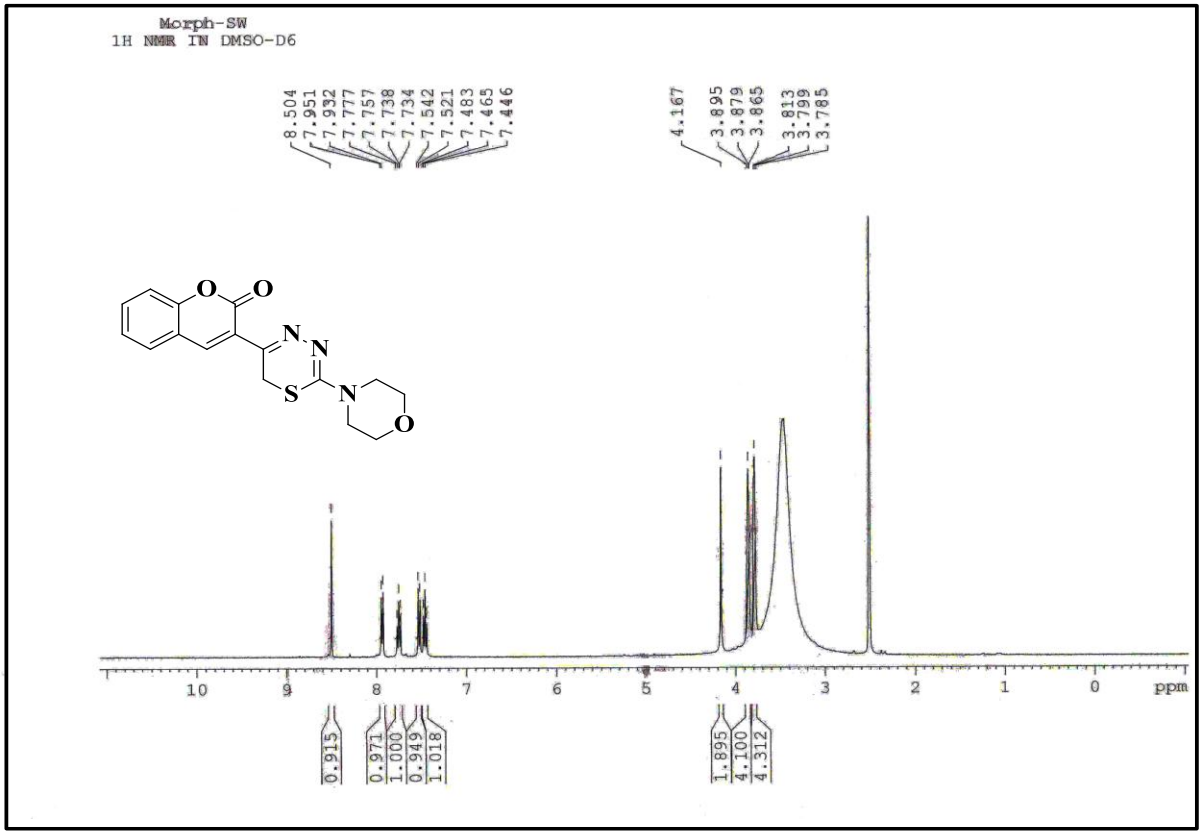


Fig: ^{1}H NMR Spectrum of Compound **35a**

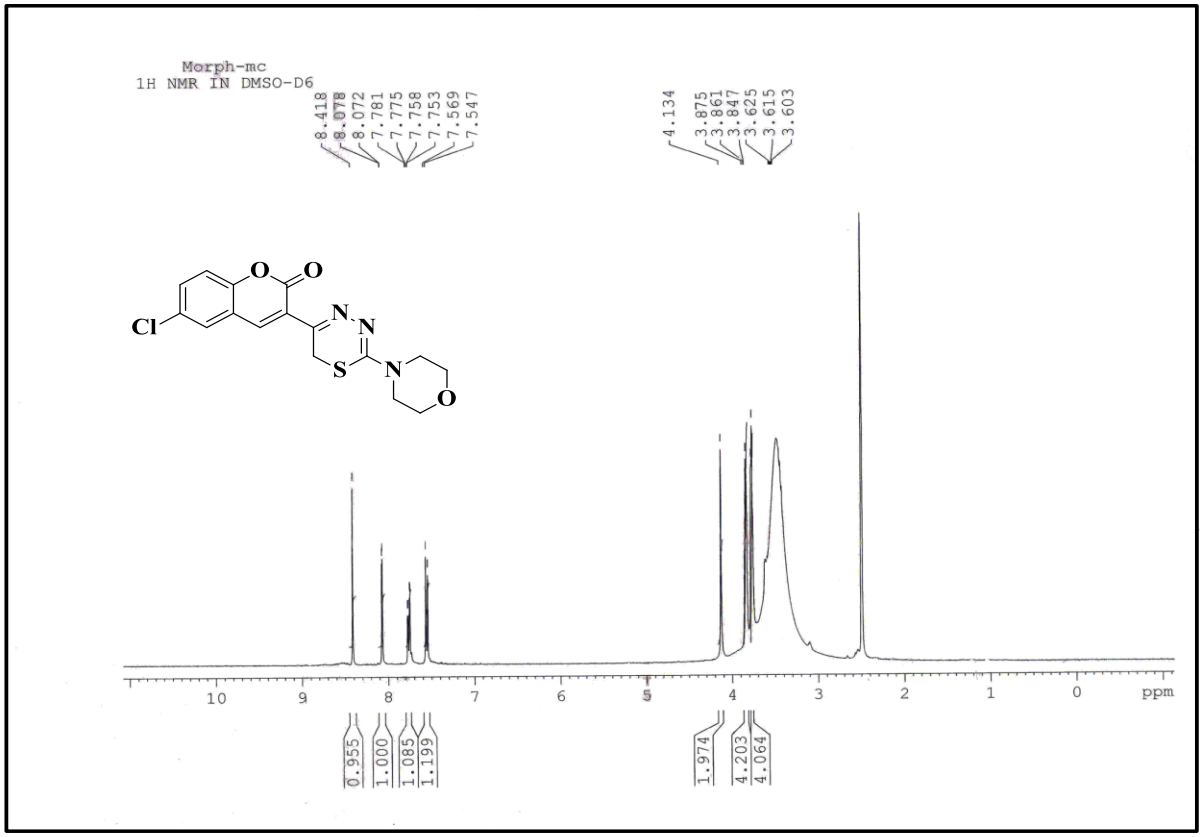


Fig: ^{1}H NMR Spectrum of Compound **35b**

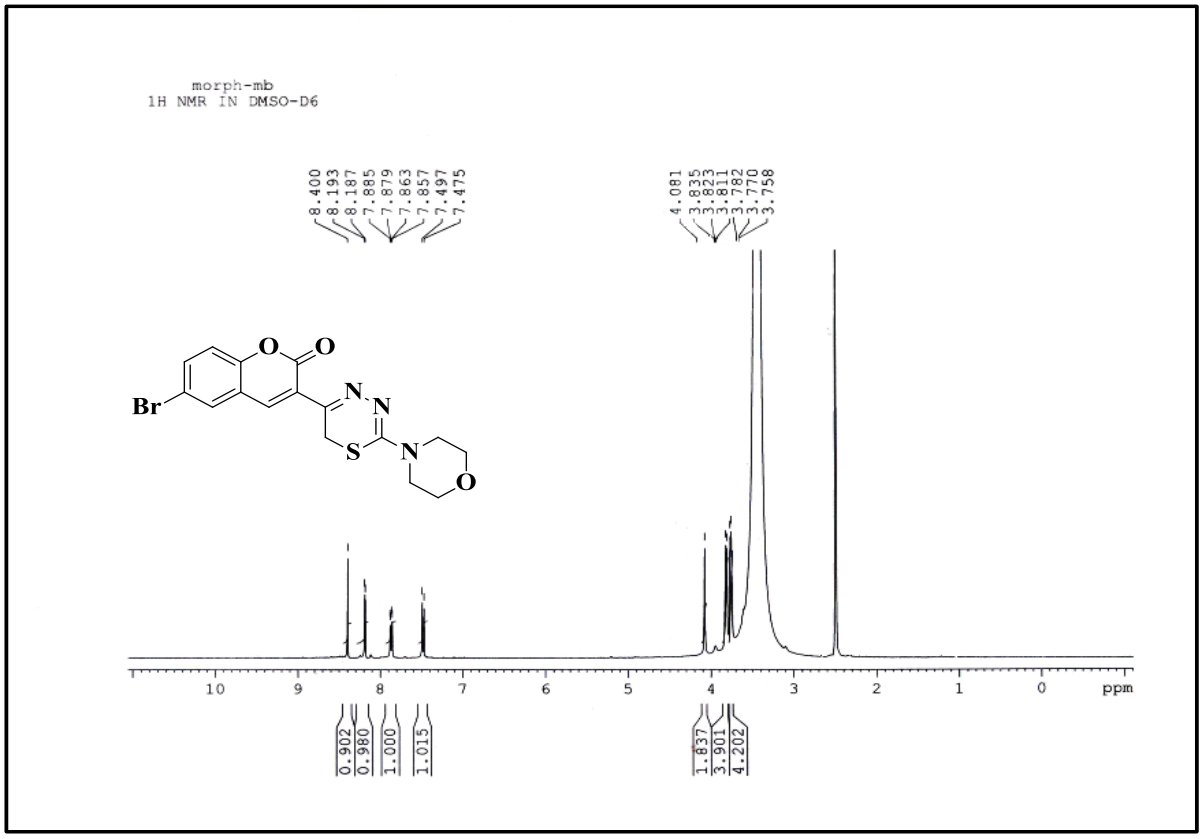


Fig: ^{1}H NMR Spectrum of Compound **35c**

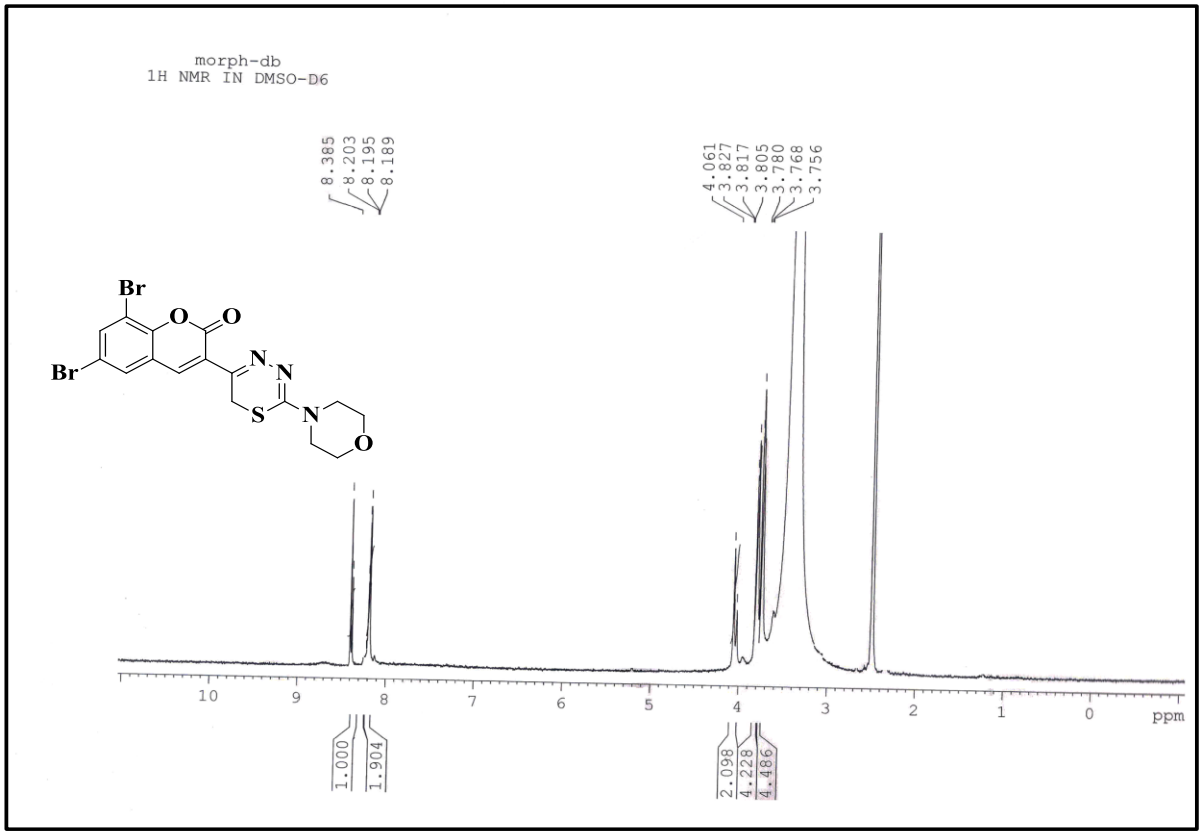


Fig: ^{1}H NMR Spectrum of Compound **35d**

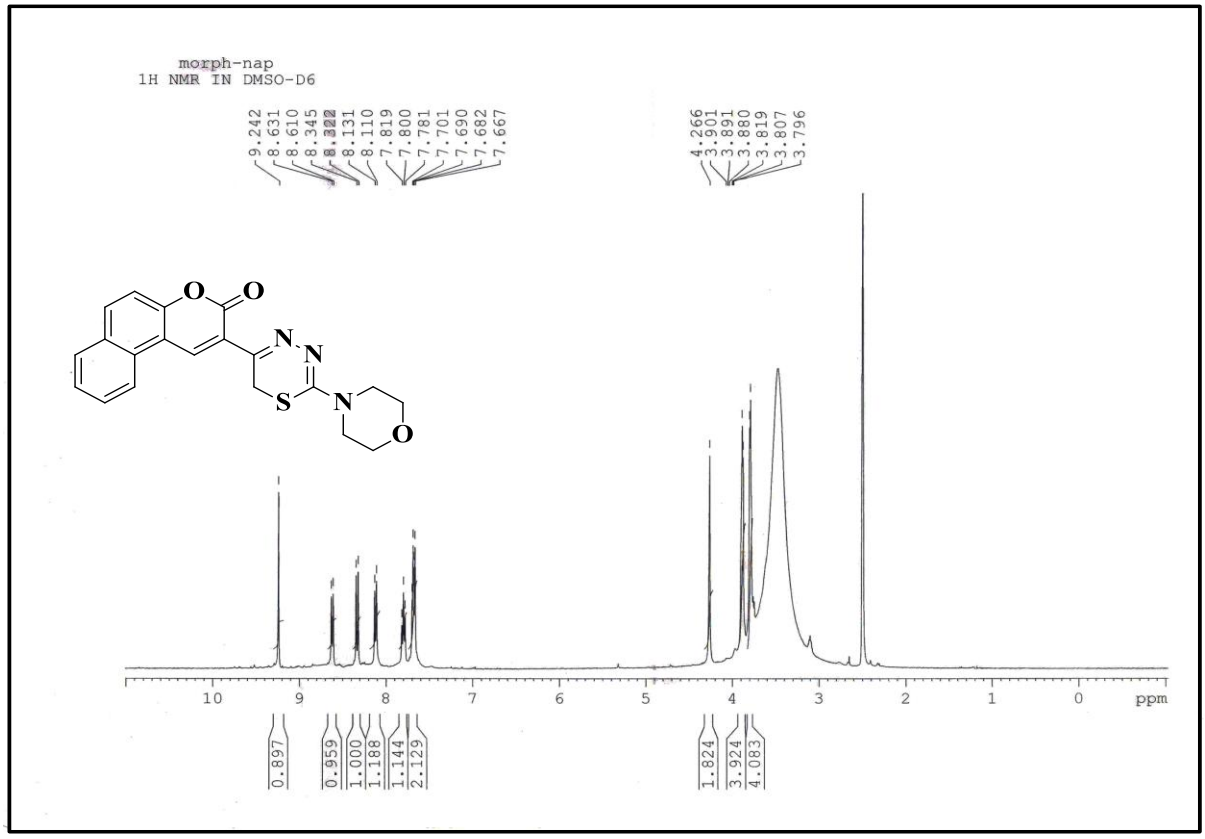


Fig: ^{1}H NMR Spectrum of Compound **35e**

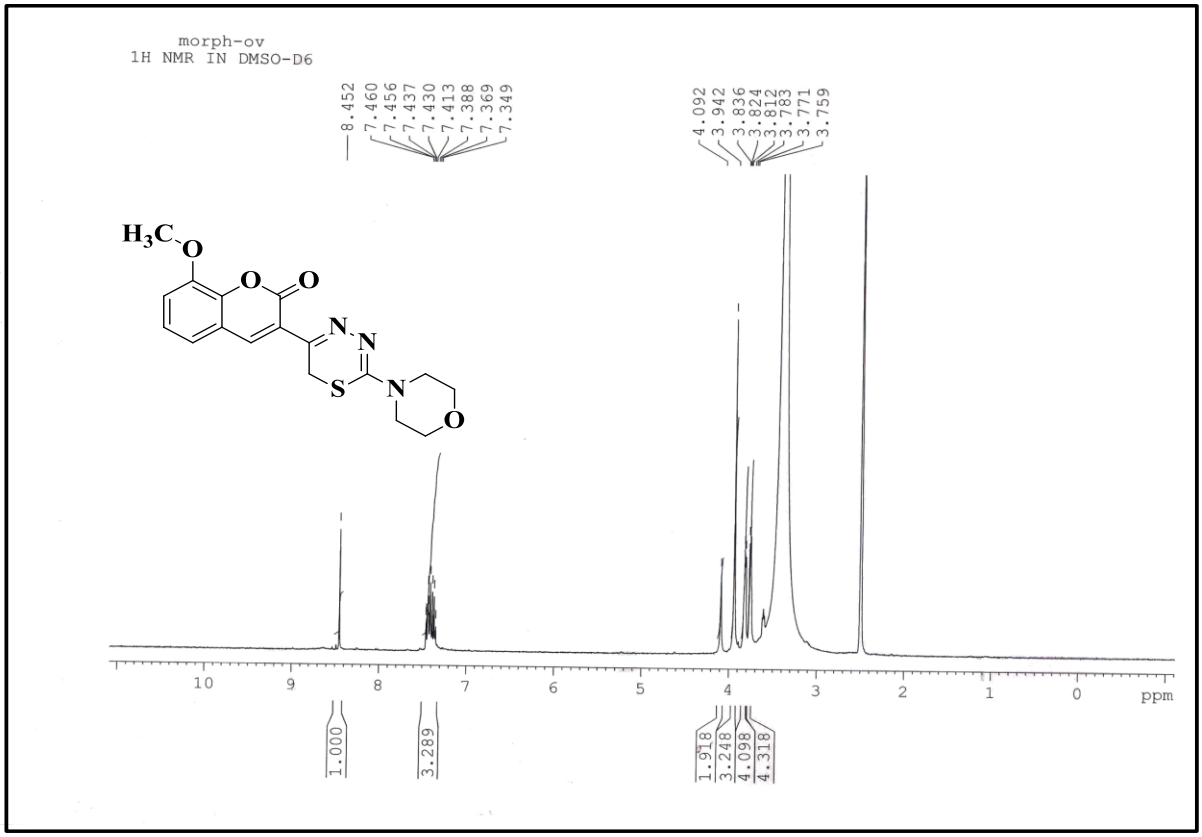


Fig: ^{1}H NMR Spectrum of Compound **35f**

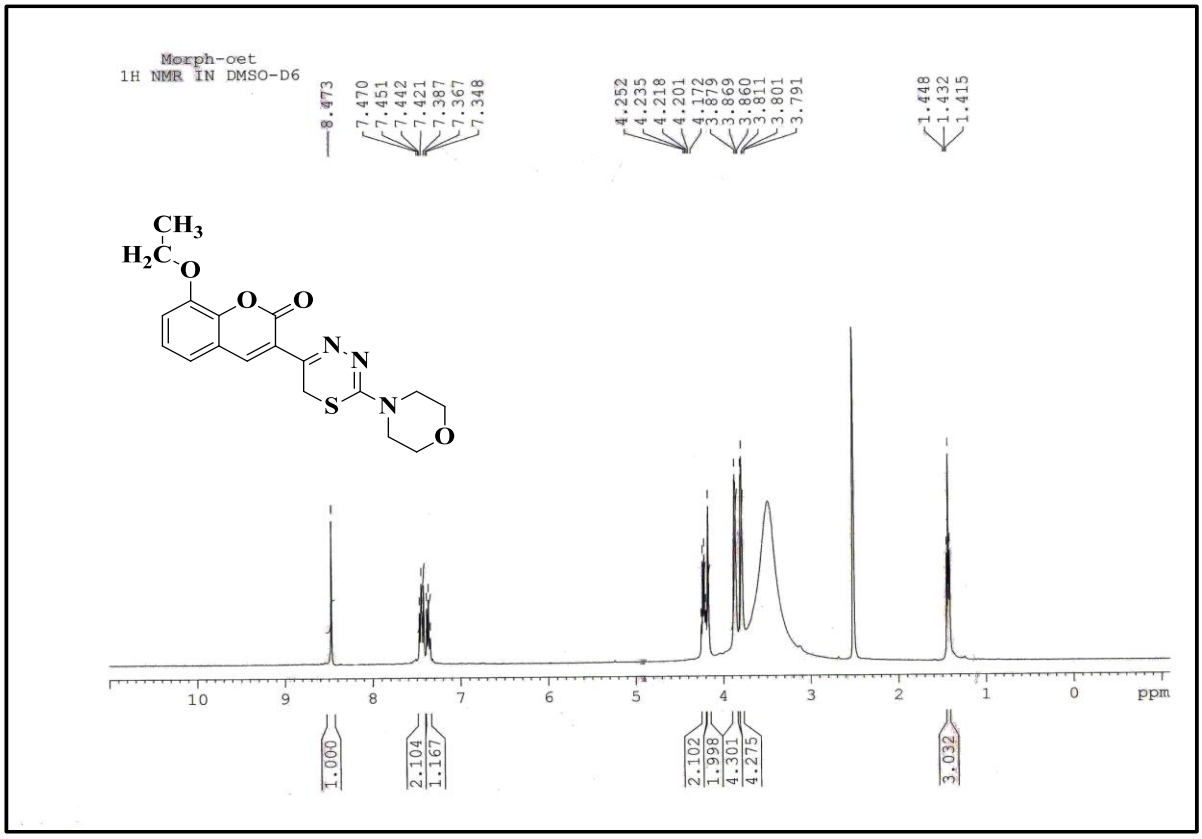


Fig: ^{1}H NMR Spectrum of Compound **35h**

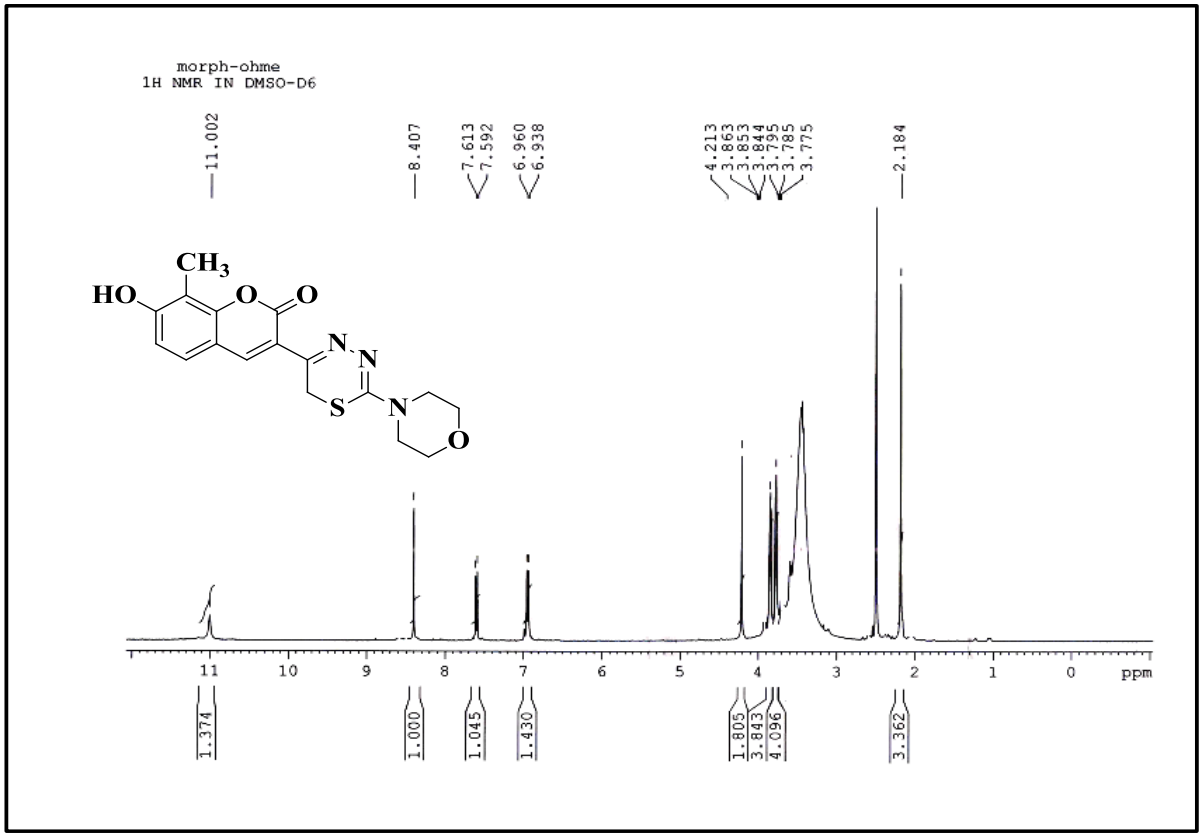


Fig: ^{1}H NMR Spectrum of Compound **35j**

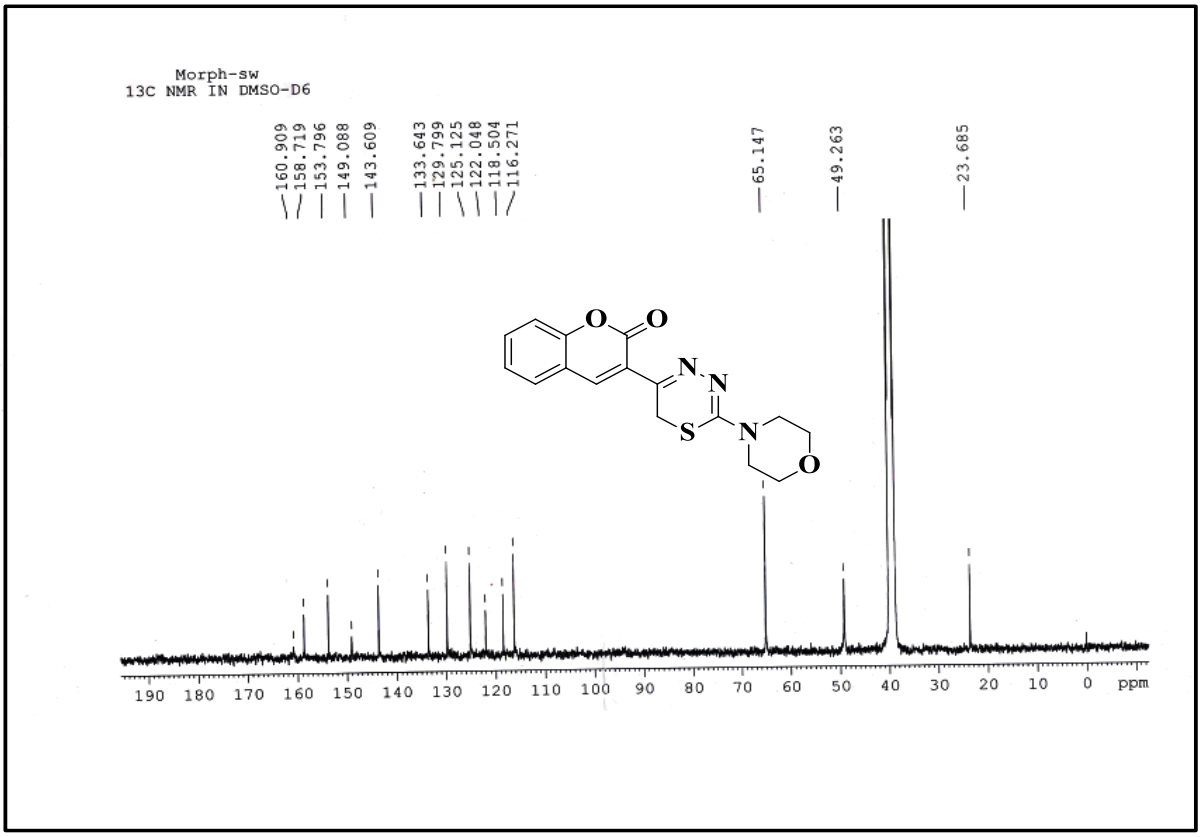


Fig: ^{13}C NMR Spectrum of Compound **35a**

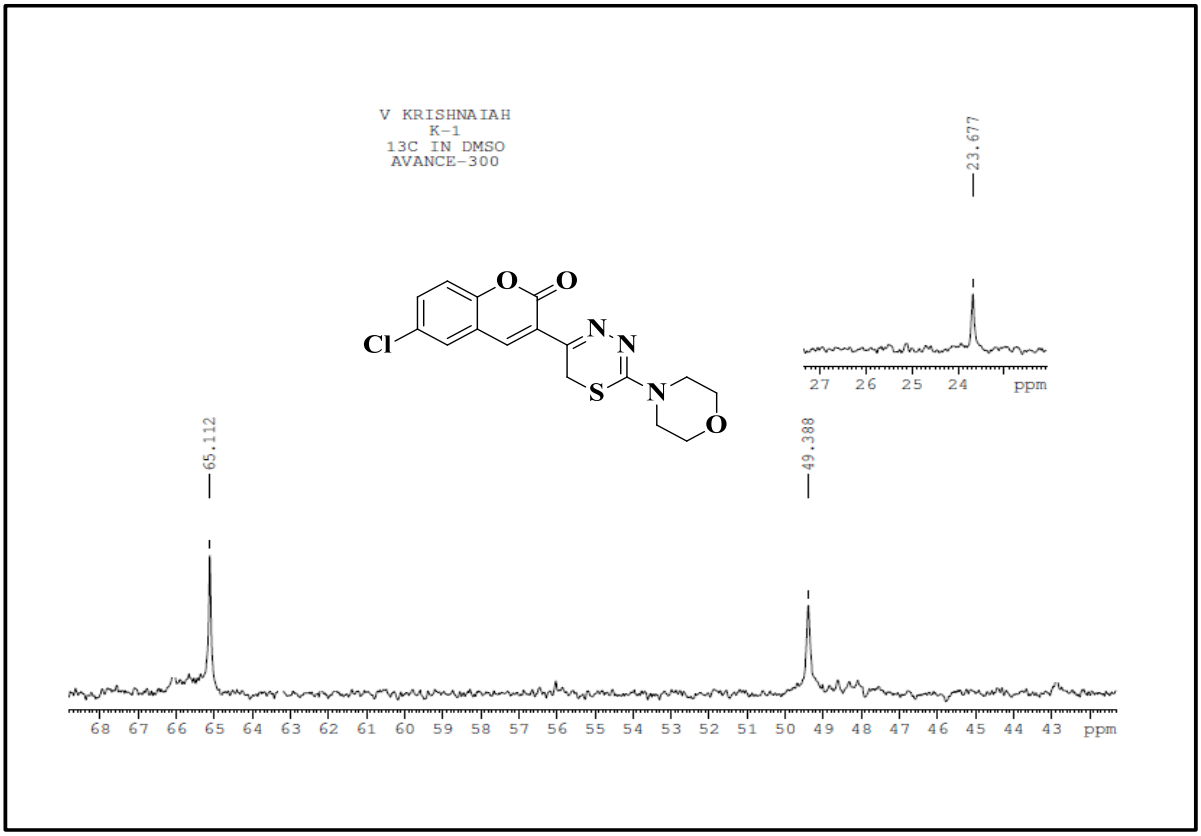


Fig: ^{13}C NMR Spectrum of Compound **35b**

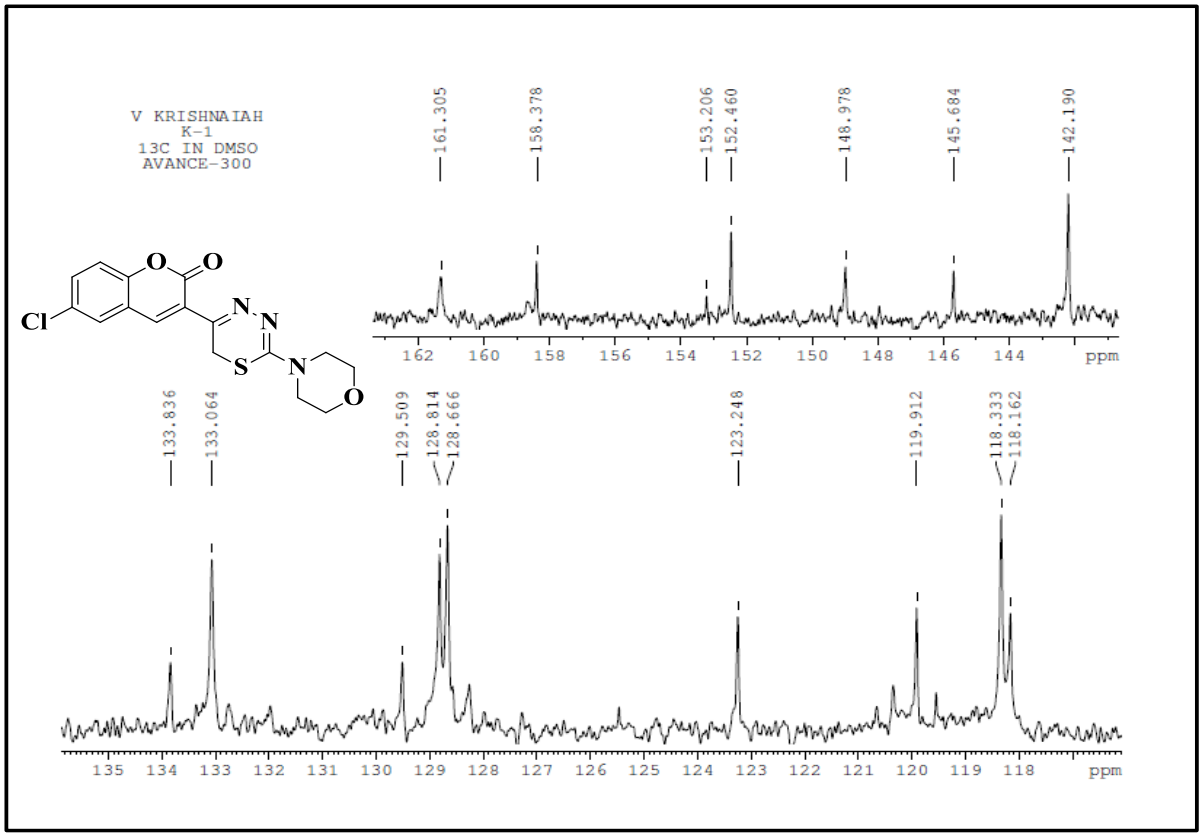


Fig: ^{13}C NMR Spectrum of Compound **35b**

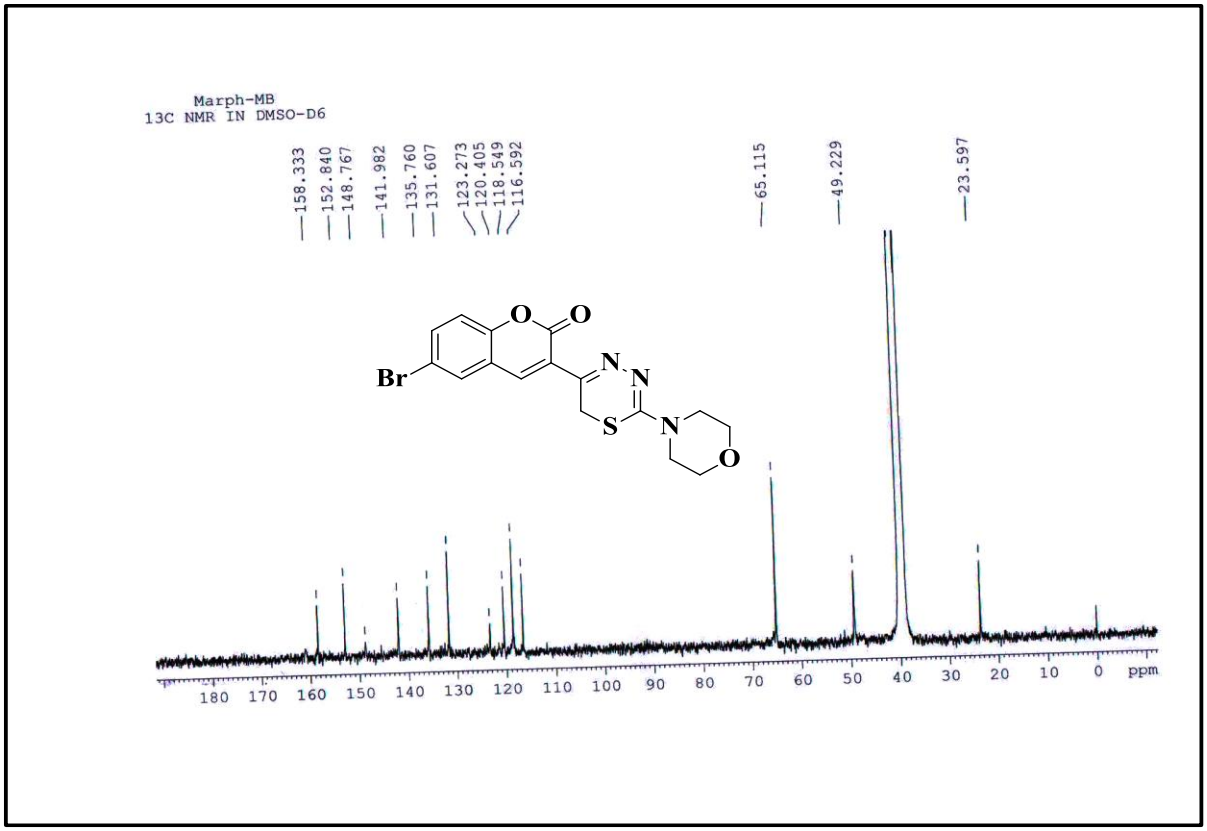


Fig: ^{13}C NMR Spectrum of Compound **35c**

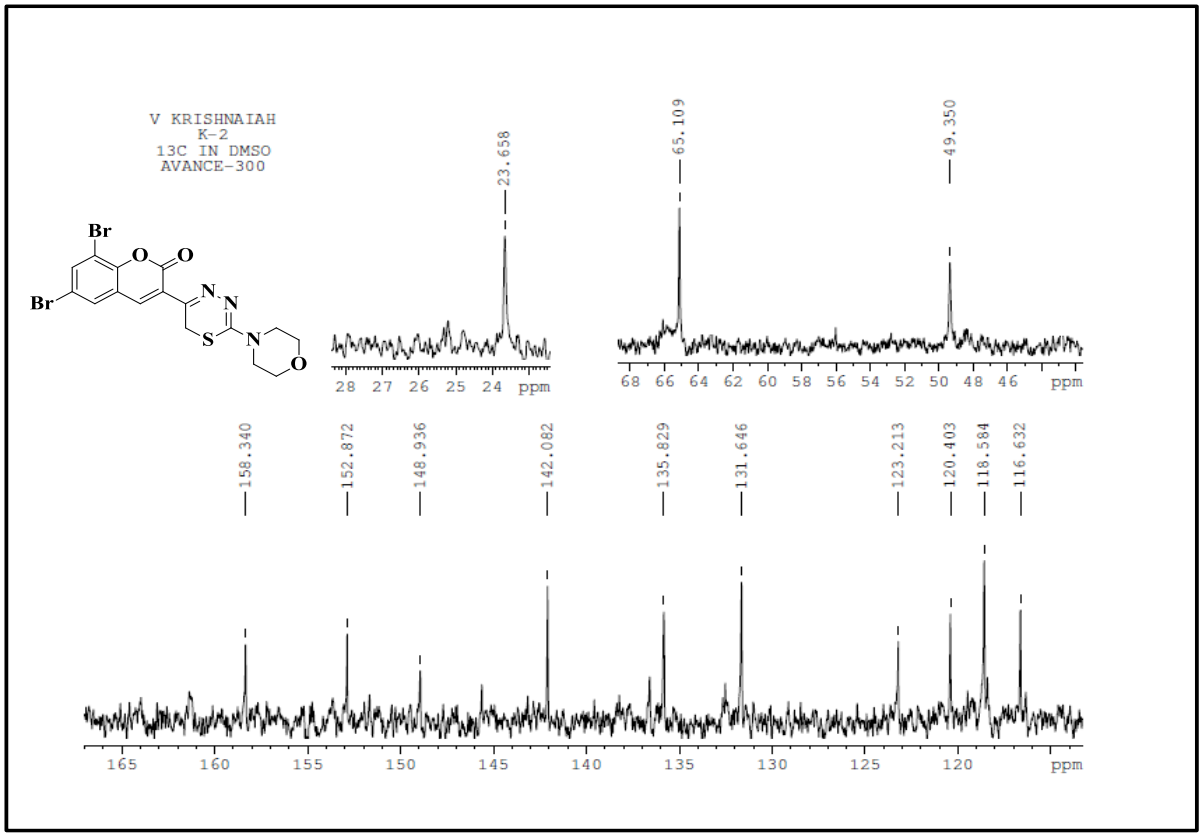


Fig: ^{13}C NMR Spectrum of Compound **35d**

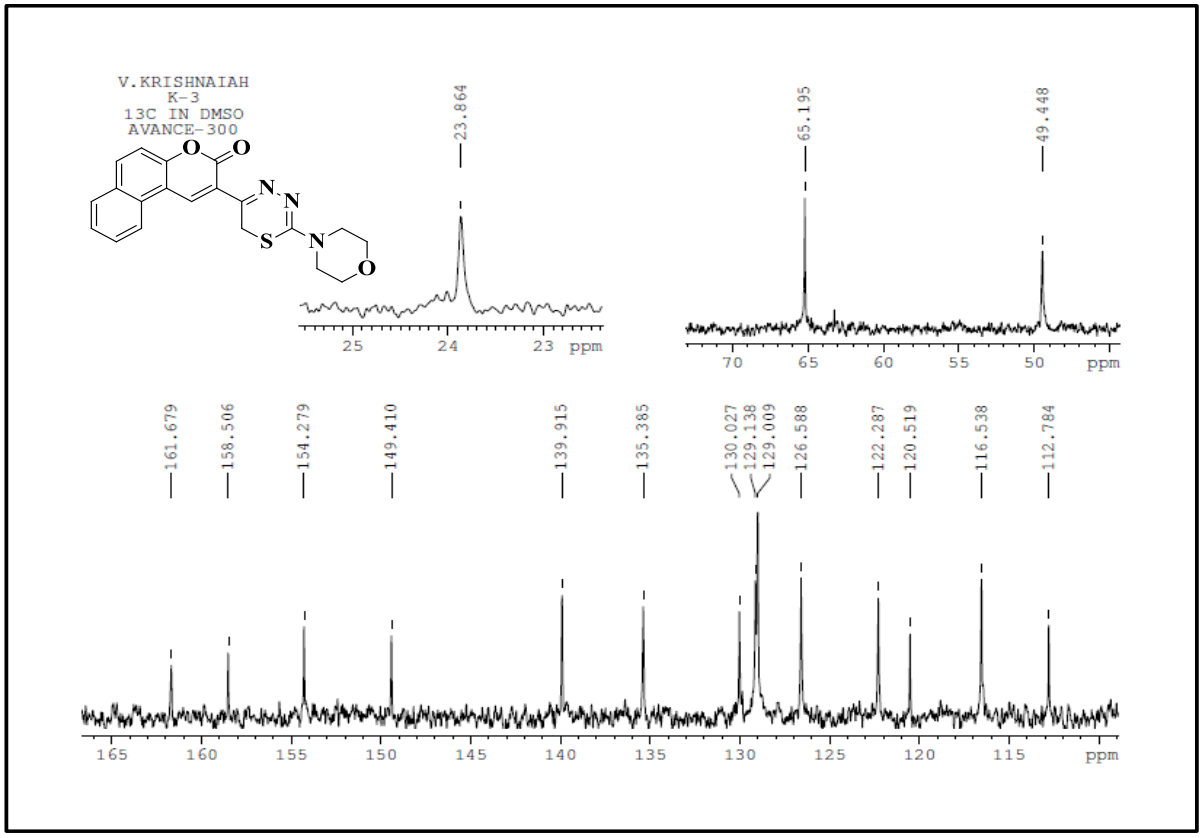


Fig: ^{13}C NMR Spectrum of Compound **35e**

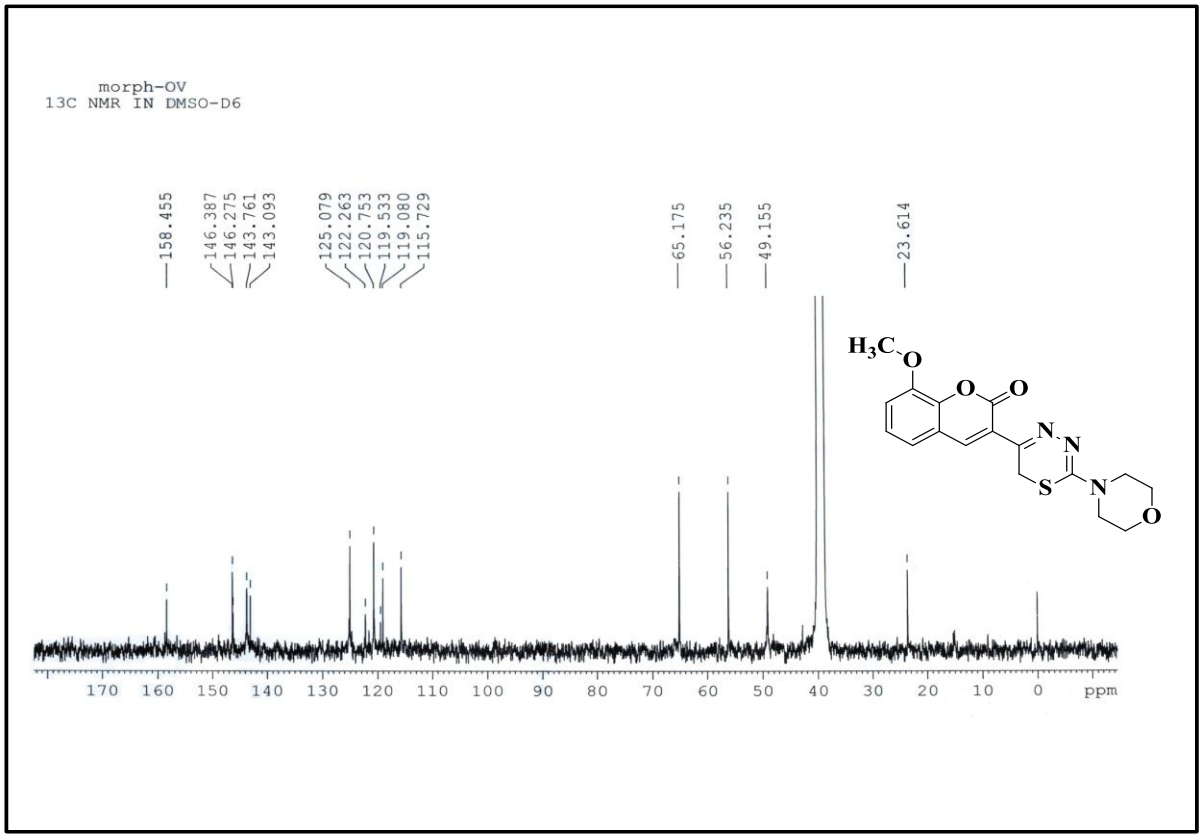


Fig: ^{13}C NMR Spectrum of Compound **35f**

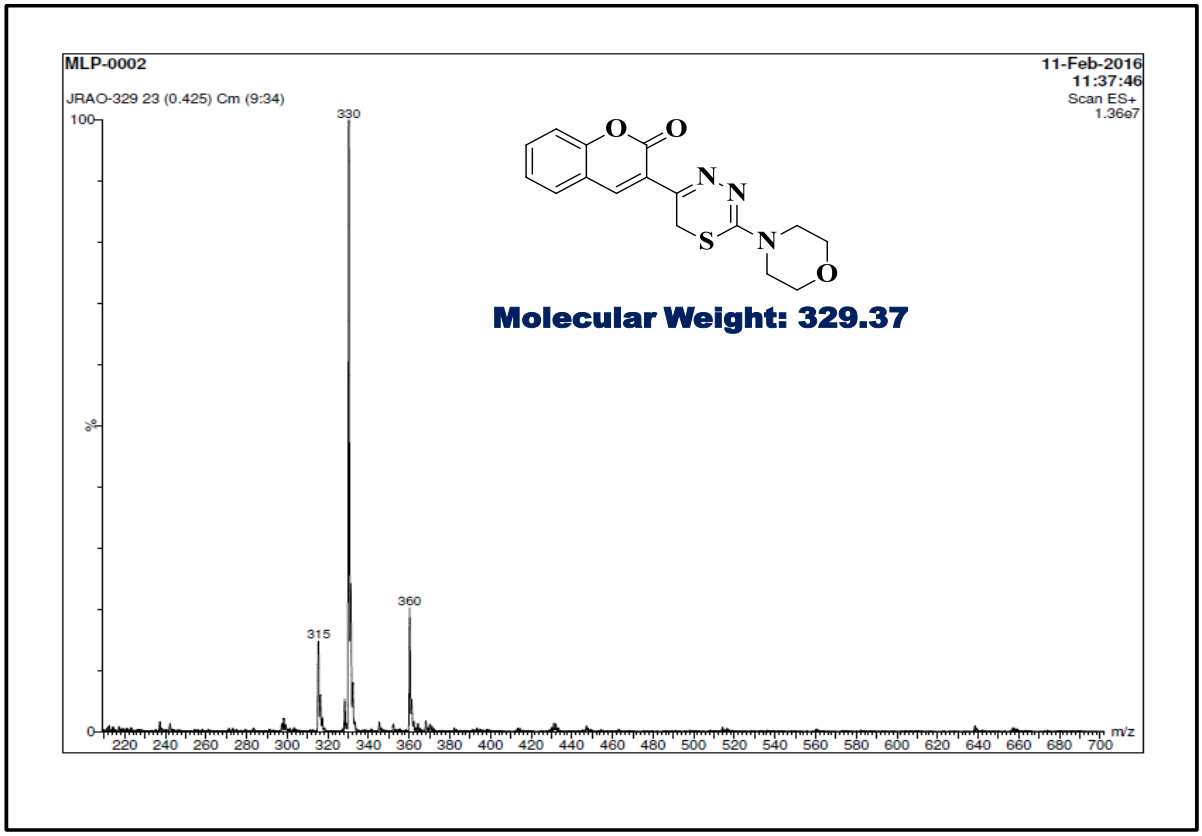


Fig: Mass Spectrum of Compound **35a**

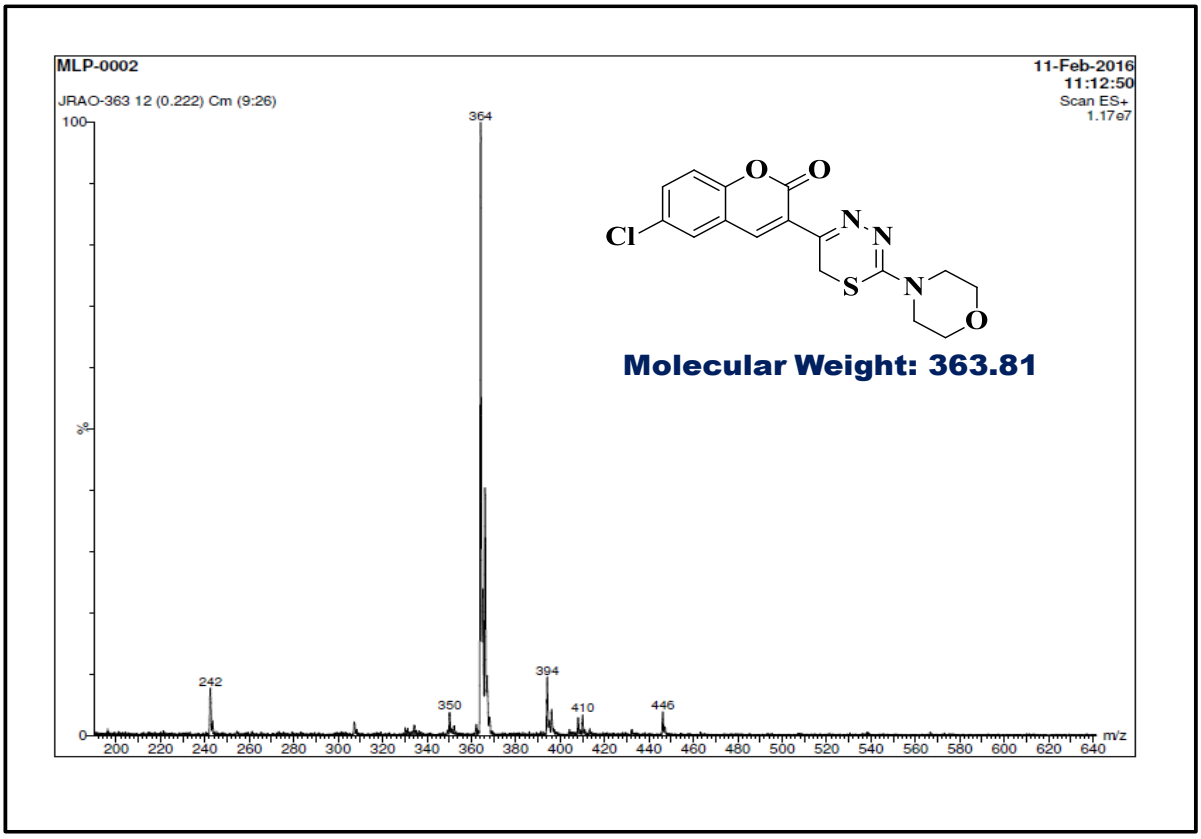


Fig: Mass Spectrum of Compound **35b**

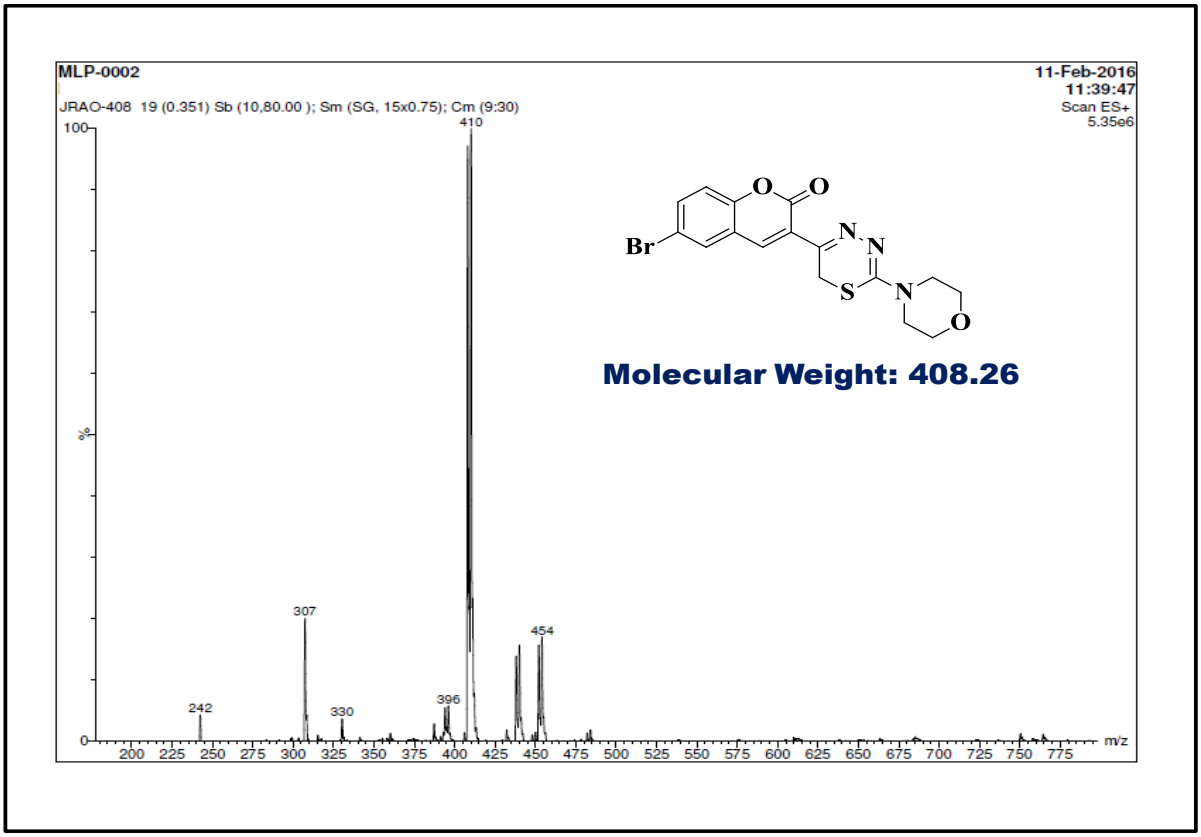


Fig: Mass Spectrum of Compound **35c**

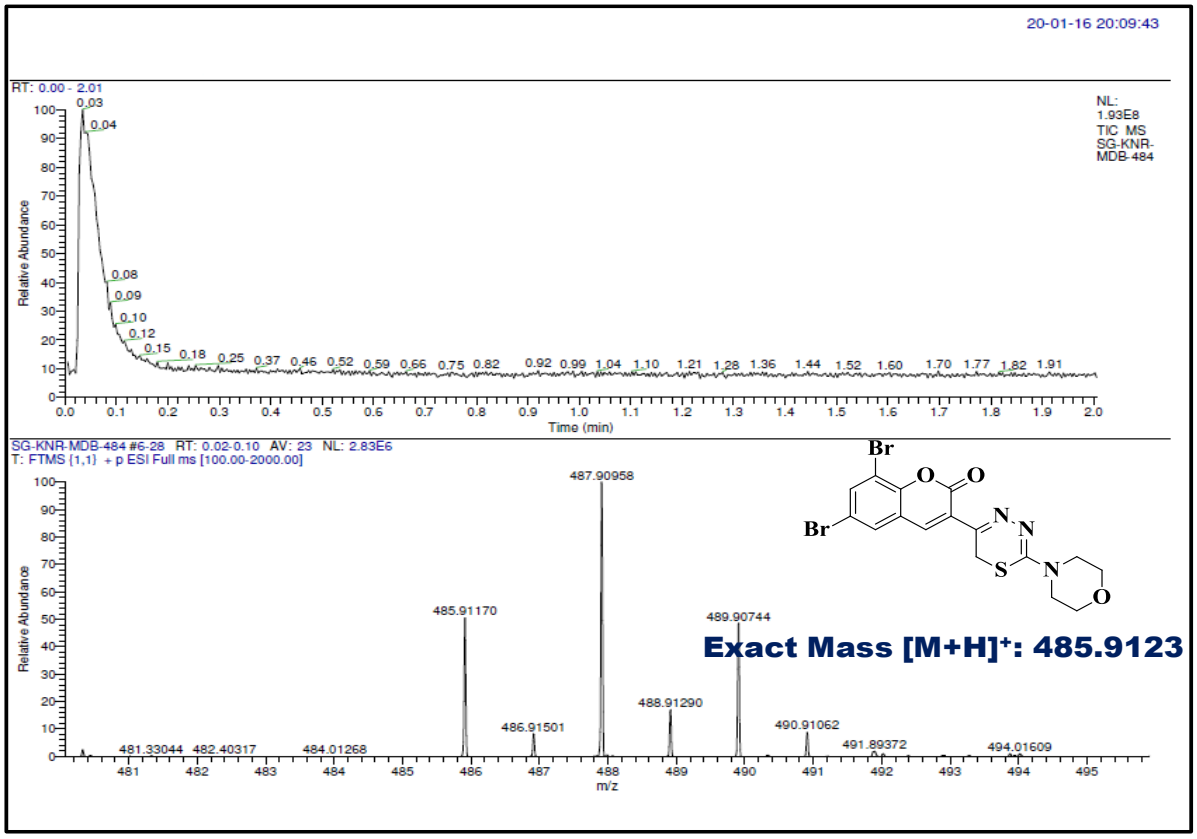


Fig: Mass Spectrum of Compound **35d**

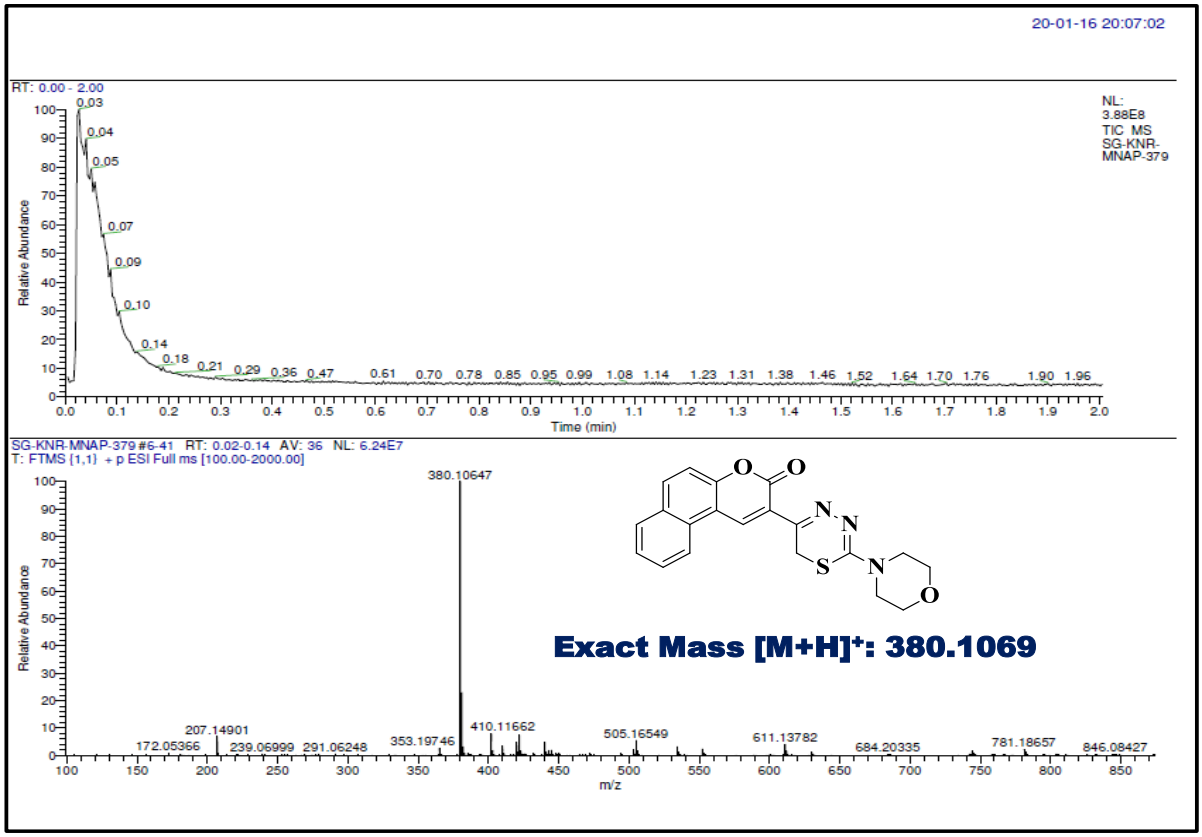


Fig: Mass Spectrum of Compound **35e**

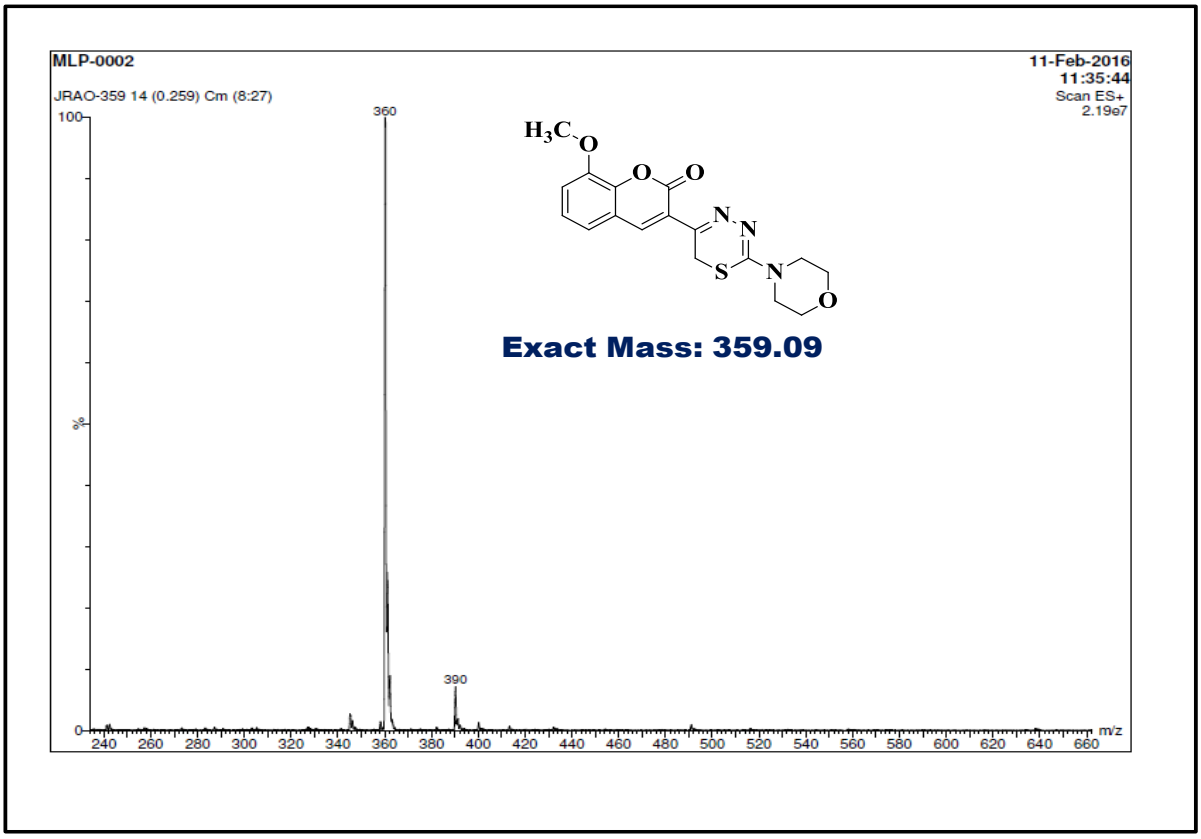


Fig: Mass Spectrum of Compound **35f**

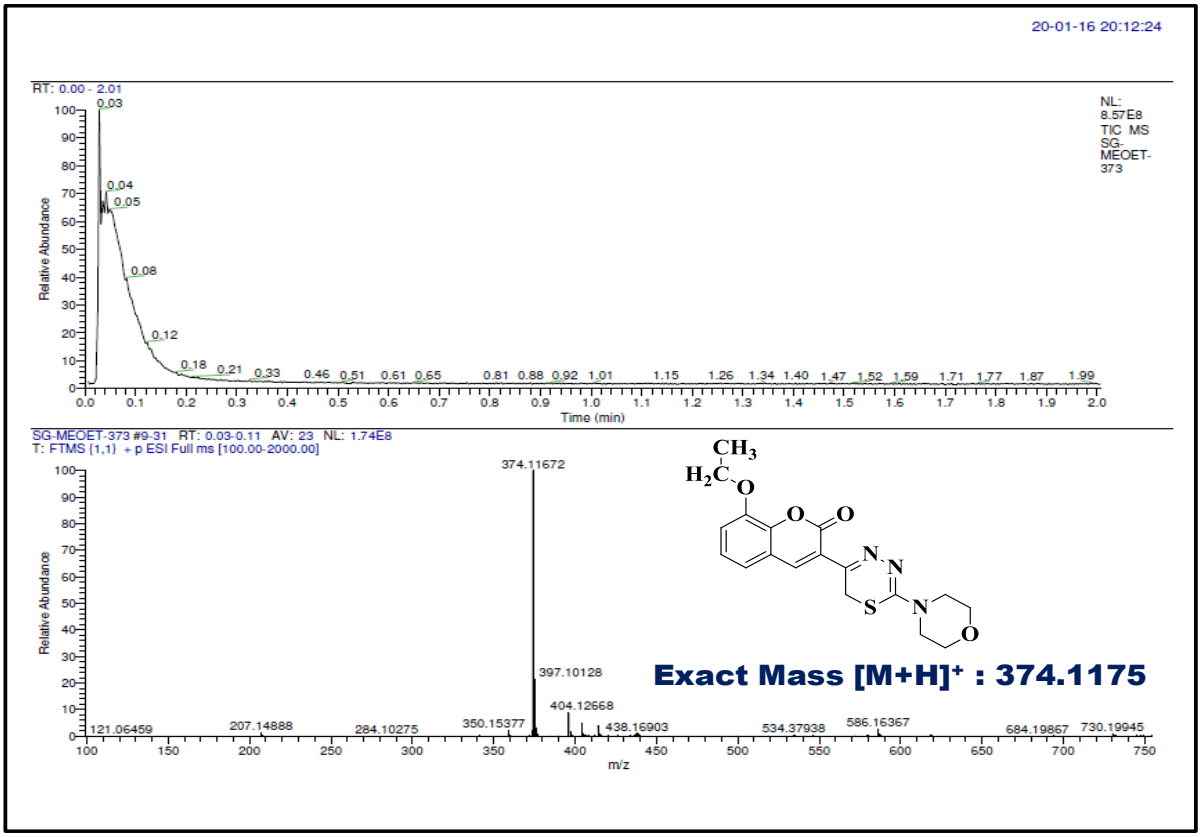


Fig: Mass Spectrum of Compound **35h**

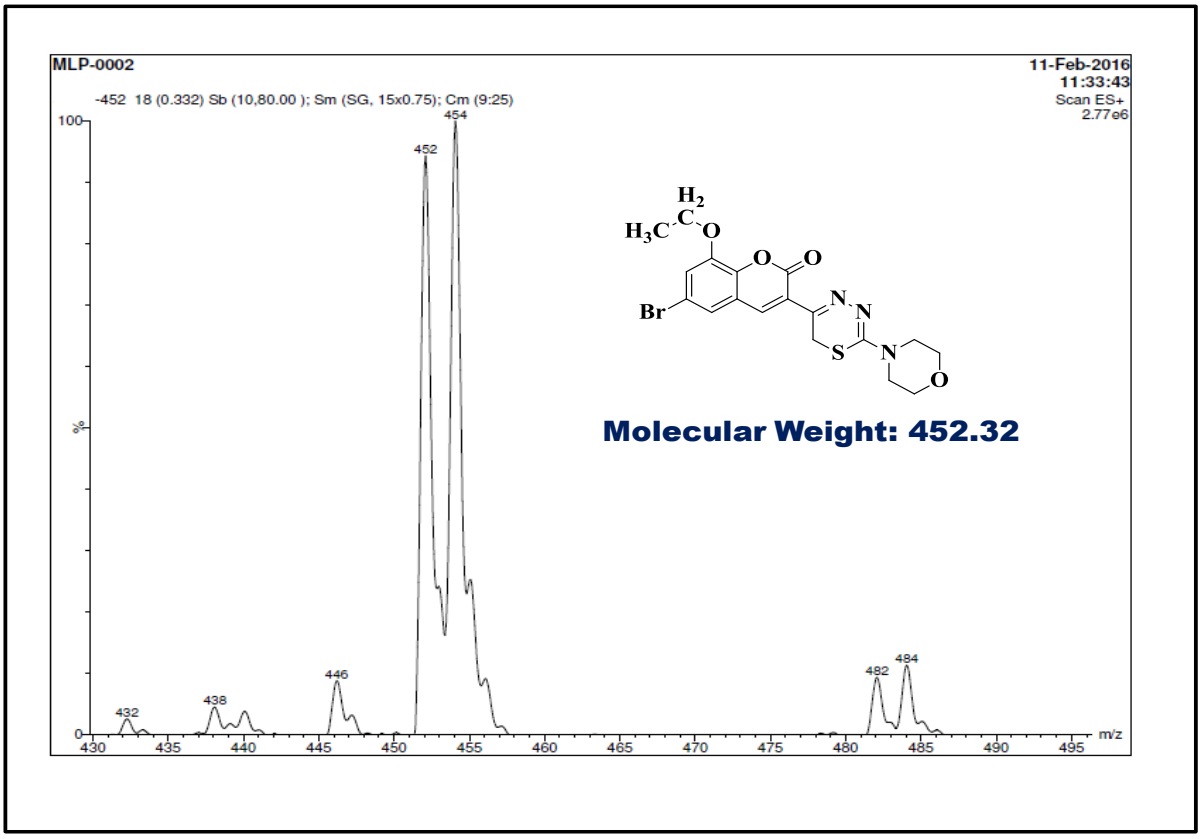


Fig: Mass Spectrum of Compound **35i**

CHAPTER-VIII
PART-B

SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF 2, 5 AND 2,3,5 SUBSTITUTED 3,6-DIHYDRO 1,3,4-THIADIAZINES

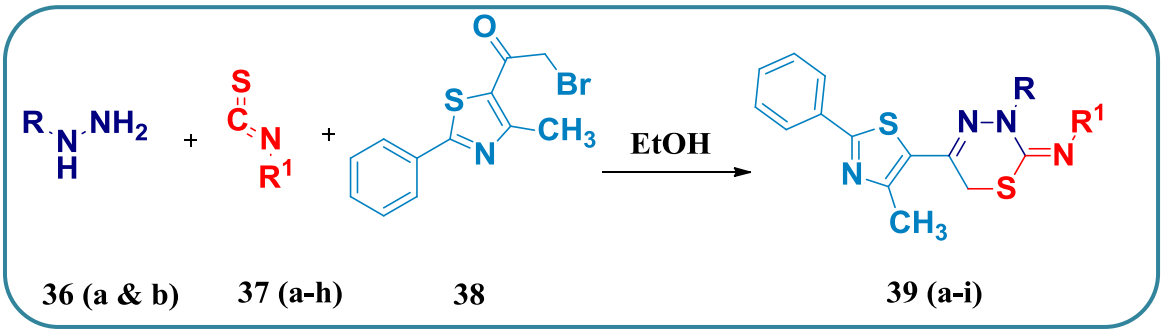
PART-B

STARTING MATERIALS

To synthesize the 2,5-di substituted and 2,3,5-tri substituted 1,3,4-thiadiazines, hydrazine hydrate, phenyl hydrazine, substituted aryl isothiocyanets and 2-bromo-1-(4-methyl-2-phenylthiazol-5-yl)ethanone were used as starting reactants. All the starting materials were procured from commercial source and they were used further without any purification.

In view of the importance of the synthesis and biological applications of 1,3,4-thiadiazines and thiazoles, there is a desirable need to develop the heterocyclic compounds having 1,3,4-thiazdiazines through efficient one pot multicomponent reaction approach. Hence, we have undertaken the present work for the synthesis of new thiadiazinyl heterocyclic compounds and evaluation of their antimicrobial activity.

The present chapter describes the synthesis of 2,5 and 2,3,5 tri substituted 1,3,4-thiadiazines starting from the reaction of hydrazine hydrate / phenyl hydrazine with alkyl / aryl isothiocyantes followed by 2-bromo-1-(4-methyl-2-phenylthiazol-5-yl)ethanone in presence of ethanol under reflux condition (**Scheme 2**). The method which is used for the synthesis of title compounds afford the target compounds with reasonable good yields (**Table 7**). The plausible mechanism for the synthesis of 2,5 and 2,3,5 substituted 1,3,4-thiadiazines 39 a-k derivatives was shown in **Scheme-3**. Reaction of hydrazine hydrate with alkyl / aryl isothiocyante generates the intermediate *N*-alkyl / aryl hydrazinecarbothioamide. This intermediate further reacts with 2-bromo-1-(4-methyl-2-phenylthiazol-5-yl)ethanone in presence of ethanol and undergoes cyclization to form the title product as shown in the mechanism.



Scheme.1: A facile one pot multicomponent synthesis of 2,5 and 2,3,5 substituted 1,3,4-thiadiazines

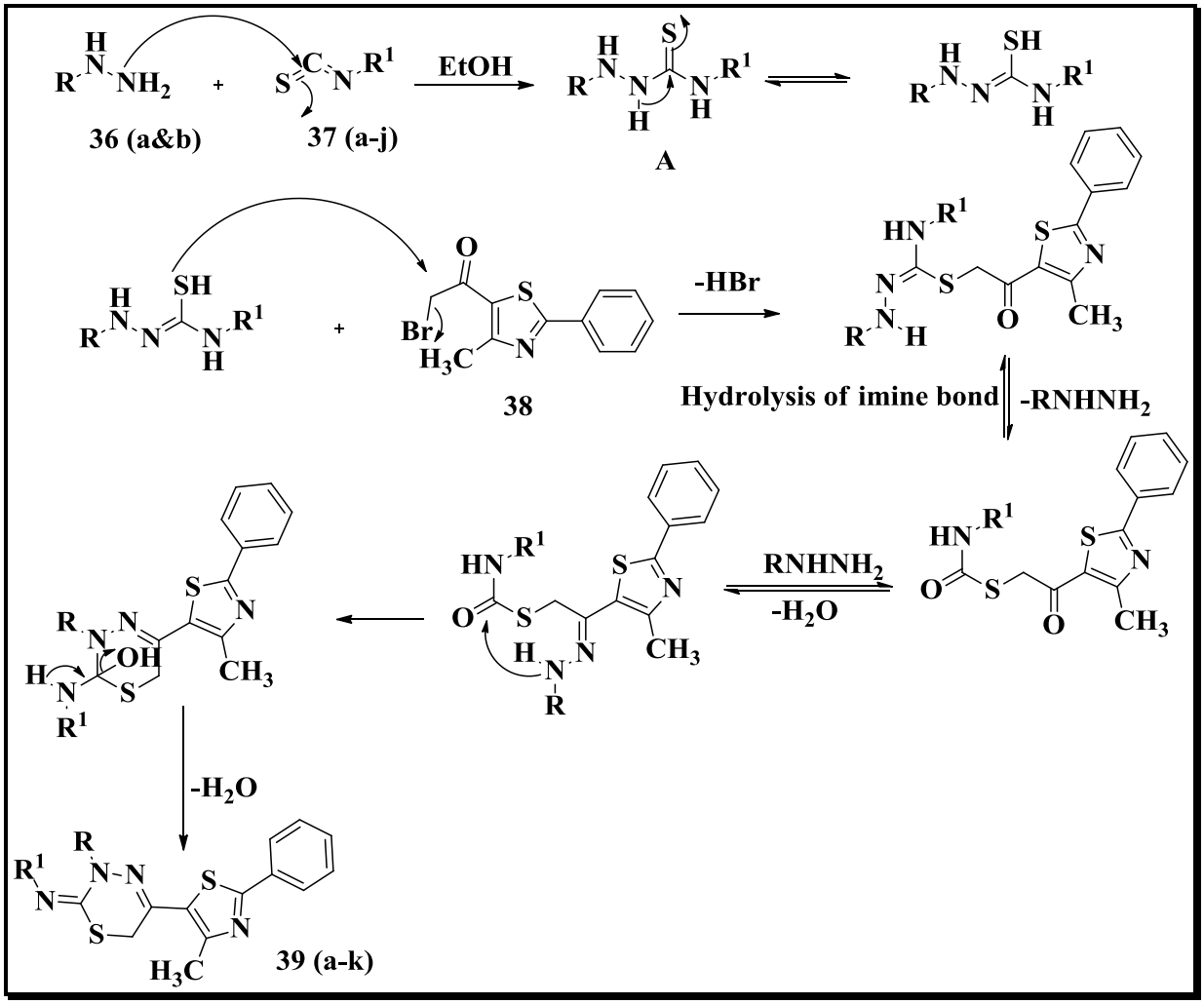
Table: 7 A facile one pot multicomponent synthesis of 2,5 and 2,3,5 substituted 1,3,4-thiadiazines **39 a-k.**

S.No.	Compound [a]	R	R^1	Reaction time (h)	Yield (%) [b]
1	**39a**	H	Allyl	2	82
2	**39b**	H	Isopropyl	1.5	85
3	**39c**	H	Phenyl	3	85
4	**39d**	H	Benzyl	2.5	86
5	**39e**	H	*p*-Tolyl	3	84
6	**39f**	H	3-methoxyphenyl	2.5	89
7	**39g**	H	4-Cyanophenyl	2	86
8	**39h**	H	4-Fluorophenyl	3	88
9	**39i**	H	3-Chlorophenyl	3	89
10	**39j**	H	4-Nitrophenyl	3.5	88
11	**39k**	Ph	Benzyl	3	90

[a]**Reaction condition:** Hydrazine hydrate / phenyl hydrazine **36** (1.0 mmol), alkyl / aryl isothiocyantes **37a-i** (1.0 mmol) and 2-bromo-1-(4-methyl-2-phenylthiazol-5-yl)ethanone **38** (1.0 mmol) in presence of ethanol at 60 °C. [b] Isolated yields.

All the newly synthesized 1,3,4-thiadiazines were well characterized by physical and analytical spectral data like IR, ^{1}H NMR, ^{13}C NMR and Mass spectra. The analytical and spectral data was consistent with the assigned structures of the title compounds.

Mechanism:



Scheme.3: Plausible reaction mechanism for the synthesis of 2,5 and 2,3,5 substituted 1,3,4-thiadiazines

The newly synthesized compounds were evaluated for their antiviral activity using standard MTS protocol as described in chapter-II (Reference 65 - 67) using **HeLa** cell culture medium for *Vesicular stomatitis* virus, *Coxsackie virus* B4 and *Respiratory syncytial* virus, Crandell-Rees Feline Kidney (**CRFK**) cell culture medium for *Feline Corona* Virus (FIPV) and *Feline Herpes* Virus, **Vero cell** culture medium for *Para influenza*-3 virus, *Reo* virus, *Sindbis* virus, *Coxsackie* virus, *Puntatoro* virus and *Yellow fever* virus, Madin Darby canine kidney (**MDCK**) cell culture medium for *Influenza* A/H1N1 A/Ned/378/05, *Influenza* A/H3N2 A/HK/7/87 and *Influenza B* B/Ned/537/05 and **HEL** cell culture for *Herpes simplex* virus-1 (KOS), *Herpes simplex* virus-2 (G), *Herpes simplex* virus-1 TK- KOS ACVr, *Vaccinia* virus, *Adeno* virus-2 and *Human Corona* virus (229E).

The antiviral activity experiments were carried out in triplicate and the average values were taken for representing the EC_{50} values (**Table 8-12**). The title compounds were evaluated for antiviral activity in HeLa cell culture medium against *Vesicular stomatitis* virus, *Coxsackie virus* B4 and *Respiratory syncytial* virus using Dextron sulphate and Ribavirin as standard drugs. None of the compounds exhibited antiviral activity against the tested viral species (**Table 8**).

Table. 8 Cytotoxicity and antiviral activity in: HeLa cell cultures

Compound	Minimum cytotoxic concentration[a]	EC50[b] *Vesicular stomatitis* virus	*Coxsackie* virus B4	*Respiratory syncytial* virus
39a	≥100	>100	>100	>100
39b	100	>100	>100	>100
39c	20	>100	>100	>100
39d	100	>100	>100	>100
39e	>100	>100	>100	>100
39f	≥100	>100	>100	>100
39g	100	>100	>100	>100
39h	≥100	>100	>100	>100
39i	20	>100	>100	>100
39j	20	>100	>100	>100
39k	≥100	>100	>100	>100
DS-10.000	>100	0.4	6.8	0.4
Ribavirin	>250	25	50	10

[a]Required to cause a microscopically detectable alteration of normal cell morphology.
[b]Required to reduce virus-induced cytopathogenicity by 50 %.

2,5-Di substituted and 2,3,5-tri substituted 1,3,4-thiadiazine derivatives were screened for antiviral activity against the *Feline Corona* virus (FIPV) and *Feline Herpes* Virus in CRFK cell culture medium using HHA, UDA and Ganciclovir as reference drugs. The compound **39g** exhibited excellent activity against *Feline Corona* virus (FIPV) and *Feline Herpes* Virus at the concentrations (EC_{50}) of **1.9** and **2.4** µM. The compound 4g is more potent than the standard drugs HHA, UDA and Ganciclovir. Similarly the compounds **39h, 39f, 39i** and **39e** also exhibited excellent potency against the tested viral species (**Table 9**).

Table. 9 Cytotoxicity and antiviral activity in: CRFK cell cultures

Compound	CC_{50} (μM) [a]	EC_{50} (μM)[b]	
		Feline Corona Virus (FIPV)	*Feline Herpes* Virus
39a	≥100	>100	>100
39b	>100	>100	>100
39c	20	>100	>100
39d	>100	>100	>100
39e	>100	**25.2**	**15.5**
39f	≥100	**8.5**	**10.0**
39g	≥100	**1.9**	**2.4**
39h	≥100	**3.3**	**9.6**
39i	≥100	**11.5**	**8.9**
39j	≥100	>100	>100
39k	>100	>100	>100
HHA	>100	9.4	2.3
UDA	>100	5.7	2.0
Ganciclovir	>100	>100	3.4

[a]50% Cytotoxic concentration, as determined by measuring the cell viability with the colorimetric formazanb50%
[b]Effective concentration, or concentration producing 50% inhibition of virus-induced cytopathic effect, as
CRFK cells: Crandell-Rees Feline Kidney cells.

The compounds **39 a-k** were tested for antiviral activity in Vero cell culture medium against *Para influenza*-3 virus, *Reo* virus, *Sindbis* virus, *Coxsackie* virus, *Puntatoro* virus and *Yellow fever* virus. Dextron sulphate (DS-10.000), Ribavirin and Mycophenolic acid were used as standard drugs. The compound **39 g** shown potency against *Para influenza*-3 virus, *Reo* virus, *Puntatoro* virus and *Yellow fever* virus at **8.3**, **15.9**, **20.1** and **18.9** μM concentrations. The compound **39f** exhibited moderate activity against *Para influenza*-3 virus at **24.6** μM concentration. The remaining compounds did not exhibit any antiviral activity against the tested viral species (**Table 10**).

Table. 10 Cytotoxicity and antiviral activity in: Vero cell cultures

Compound	Minimum cytotoxic conc.[a] (μM)	EC_{50}[b] (μM)					
		Para influenza-3 virus	*Reo*virus-1	*Sindbis* virus	*Coxsackie* virus B4	*Punta Toro* virus	*Yellow Fever* virus
39a	≥100	>100	>100	>100	>100	>100	>100
39b	≥100	>100	>100	>100	>100	>100	>100
39c	≥20	>100	>100	>100	>100	>100	>100
39d	≥20	>100	>100	>100	>100	>100	>100

39e	≥100	>100	>100	>100	>100	>100	>100
39f	>100	**24.6**	51.4	>100	>100	>100	>100
39g	>100	**8.3**	**15.9**	32.4	>100	**20.1**	**18.9**
39h	≥100	>100	>100	>100	>100	>100	>100
39i	100	>100	>100	>100	>100	>100	>100
39j	≥100	>100	>100	>100	>100	>100	>100
39k	≥20	>100	>100	>100	>100	>100	>100
DS.10.000	>100	>100	>100	100	45	2.3	0.8
Ribavirin	>250	146	>250	>250	>250	125	>250
Mycopheno lic acid	≥100	0.4	0.5	20	45	4.0	0.8

[a]Required to cause a microscopically detectable alteration of normal cell morphology.
[b]Required to reduce virus-induced cytopathogenicity by 50 %.

The target compounds **39 a-k** were evaluated against *Influenza* A/H1N1 A/Ned/378/05, *Influenza* A/H3N2 A/HK/7/87 and *Influenza B* B/Ned/537/05 viruses using Zanamivir, Ribavirin, Amantaidine and Rimantadine as reference drugs. The compound **39g** has shown excellent antiviral activity against the tested viruses with visual CPE scores of **1.5**, **2.8** and **3.7** µM concentrations and MTS scores **6.4**, **1.9** and **2.1** µM concentrations. Similarly the compounds **39h**, **39i 39e** and **39f** exhibited promising antiviral activity against the viruses. The remaining compounds did not exhibit any antiviral activity (**Table 11**).

Table. 11Cytotoxicity and antiviral activity in: MDCK cell cultures

	Cytotoxicity		Antiviral EC50[c]					
	CC_{50}[a]	Minimum cytotoxic concentration[b]	*Influenza A/H1N1* A/Ned/378/05		*Influenza A/H3N2* A/HK/7/87		*Influenza B* B/Ned/537/05	
Compound			visual CPE score	MTS	visual CPE score	MTS	visual CPE score	MTS
39a	>100	100	>100	>100	>100	>100	>100	>100
39b	>100	100	>100	>100	>100	>100	>100	>100
39c	>100	100	>100	>100	>100	>100	>100	>100
39d	>100	100	>100	>100	>100	>100	>100	>100
39e	>100	>100	**15.6**	**9.8**	**22.9**	**15.8**	36.2	48.9
39f	>100	100	**25.9**	**16.4**	**12.2**	**7.5**	**14.4**	**22.8**
39g	>100	100	**1.5**	**6.4**	**2.8**	**1.9**	**3.7**	**2.1**
39h	>100	100	**10.1**	**6.4**	**15.4**	**9.6**	**21.2**	**16.1**
39i	100	100	**18.4**	**11.3**	**10.5**	**6.4**	**15.9**	**13.6**
39j	100	≥20	>100	>100	>100	>100	>100	>100

39k	>100	100	>100	>100	>100	>100	>100	>100
Zanamivir	>100	>100	0.4	0.1	0.8	0.1	0.5	0.4
Ribavirin	>100	100	8.9	4.4	4.0	1.0	8.9	8.4
Amantadine	>100	>100	100	>100	0.2	0.1	>100	>100
Rimantadine	>200	>200	>200	>200	0.02	0.01	>200	>200

[a]50% Cytotoxic concentration, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay.
[b]Minimum compound concentration that causes a microscopically detectable alteration of normal cell morphology.
[c]50% Effective concentration, or concentration producing 50% inhibition of virus-induced cytopathic effect, as determined by visual scoring of the CPE, or by measuring the cell viability with the colorimetric formazan-based MTS assay.
MDCK cells: Madin Darby canine kidney cells

2,5-Di substituted and 2,3,5 – tri substituted 1,3,4-thiadiazines were tested for antiviral activity against *Herpes simplex* virus-1 (KOS), *Herpes simplex* virus-2 (G), *Herpes simplex* virus-1 TK- KOS ACVr, *Vaccinia* virus, *Adeno* virus-2 and *Human Corona* virus (229E) in HEL cell culture medium. Among the tested compounds, compound **39g** exhibited good activity against *Herpes simplex* virus-2 (G), *Herpes simplex* virus-1 (KOS) and *Human Corona* virus (229E) with EC50 values of **19.4**, **22.1** and **25.6** µM concentrations (**Table 12**).

Table.12 Cytotoxicity and antiviral activity in: HEL cell cultures

Compound	Minimum cytotoxic concentration[a] (µM)	EC_{50}[b] (µM)					
		Herpes simplex virus- 1 (KOS)	*Herpes simplex virus-* 2 (G)	*Herpes simplex virus-* 1 TK-KOS ACVr	*Vaccinia virus*	*Adeno virus-* 2	*Human Coronavirus* (229E)
39a	>100	>100	>100	>100	>100	>100	>100
39b	>100	>100	>100	>100	>100	>100	>100
39c	>100	>100	>100	>100	>100	>100	>100
39d	100	>100	>100	>100	>100	>100	>100
39e	>100	>100	>100	>100	>100	>100	>100
39f	>100	>100	>100	>100	>100	>100	>100
39g	≥100	**22.1**	**19.4**	32.6	48.2	34.8	**25.6**
39h	≥100	>100	>100	>100	>100	>100	>100
39i	≥20	>100	>100	>100	>100	>100	>100
39j	>100	>100	>100	>100	>100	>100	>100
39k	100	>100	>100	>100	>100	>100	>100
Brivudin	>250	0.008	112	10	10	-	-
Cidofovir	>250	4.5	2	3.4	22	29	-
Acyclovir	>250	0.7	0.2	85	>250	-	-

Ganciclovir	>250	0.16	0.06	4	>100	-	-
Zalcitabine	>250	-	-	-	-	19	-
Alovudine	>250	-	-	-	-	50	-
UDA	>100	-	-	-	-	-	1.8
Ribavirin	>250	-	-	-	-	-	112

[a] Required to cause a microscopically detectable alteration of normal cell morphology.
[b] Required to reduce virus-induced cytopathogenicity by 50 %.

In summary from the above antiviral activity results of the title compounds it has been observed that when electron withdrawing groups such as –CN, F and Cl exhibited excellent antiviral activity. Whereas the compounds having electron releasing groups (OCH_3 and CH_3) exhibited moderate activity.

In summary, we have developed an efficient one pot multicomponent reaction method for the synthesis of substituted 1,3,4-thiadiazine derivatives. The method adapted for the synthesis of title compounds provide simple, shorter reaction time, easy work up with moderate to good yields. All the newly synthesized compounds were screened against broad panel of human viruses and the compounds 4g, 4h, 4i, 4e and 4f exhibited good antiviral activity against influenza viral species.

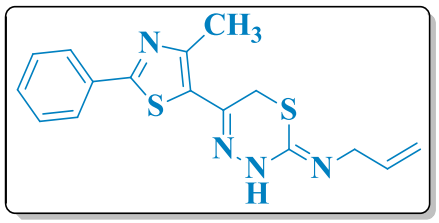
EXPERIMENTAL:

General procedure for the synthesis of 2,5 and 2,3,5 substituted 1,3,4-thiadiazines:

Hydrazine hydrate / phenylhydrazine (1.0 mmol), alkyl / aryl isothiocyante **37a** (1.0 mmol) and 2-bromo-1-(4-methyl-2-phenylthiazol-5-yl)ethanone **38** (0.296 g, 1.0 mmol) were taken in a round bottom flask containing ethanol solvent (5 mL). The reaction mixture was stirred at room temperature for about 10 minutes and after that at refluxing temperature for 2-4 h. The progress of the reaction was monitored by TLC by using hexane/ethyl acetate in 2:1 ratio. After completion of the reaction, the temperature of the reaction mixture was cooled to room temperature. The solid separated was filtered, washed with chilled ethanol and dried. The product was recrystallized from ethanol.

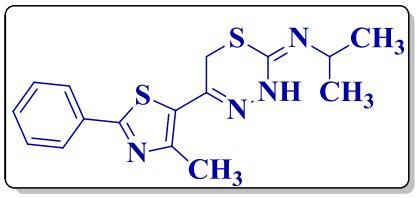
SPECTRAL DATA:

***N*-(5-(4-Methyl-2-phenylthiazol-5-yl)-3,6-dihydro-2*H*-1,3,4-thiadiazin-2-ylidene) prop-2-en-1-amine 39a:**



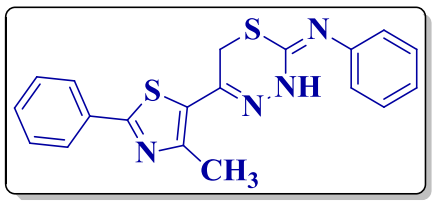
Yellow solid; m.p.: 135 – 137 °C; **FTIR (KBr, υ_{max} cm^{-1}):** 3127 (-NH), 1604 (-C=N); **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm):** 2.72 (s, 3H, -CH_3), 3.97 (s, 2H, -SCH_2), 4.11 (d, *J* = 4Hz, 2H, CH_2), 5.35 – 5.45 (m, 2H, =CH_2), 5.84 – 5.93 (m, 1H, =CH), 7.45 (d, *J* = 4 Hz, 3H, Ar-H), 7.91 (d, *J* = 8 Hz, 2H, Ar-H), 11.26 (bs, 1H, -NH); **HR-MS ESI m/z):** Exact mass calculated for $C_{16}H_{17}N_4S_2$ $[M+H]^+$: 329.0895, Found: 329.0891; Anal. Calcd. for $C_{16}H_{16}N_4S_2$: C, 58.51; H, 4.91; N, 17.06%; Found: C, 58.56; H, 4.93; N, 16.99%.

N-(5-(4-Methyl-2-phenylthiazol-5-yl)-3,6-dihydro-2*H*-1,3,4-thiadiazin-2-ylidene) propan-2-amine 39b:



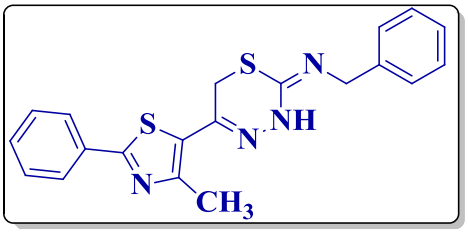
Yellow solid; m.p.: 128 – 130 °C; **FTIR (KBr, υ_{max} cm^{-1}):** 3168 (-NH), 1607 (-C=N); **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm):** 1.46 (d, *J* = 4 Hz, 6H, -CH_3), 2.72 (s, 3H, -CH_3), 2.80 (s, 1H, -CH), 3.91 (s, 2H, -SCH_2), 7.44 -7.48 (m, 5H, Ar-H), 13.11 (bs, 1H, -NH); **HR-MS ESI m/z):** Exact mass calculated for $C_{16}H_{19}N_4S_2$ $[M+H]^+$: 331.1051, Found: 331.1057; Anal. Calcd for $C_{16}H_{18}N_4S_2$: C, 58.15, H, 5.49, N, 16.95%; Found: C, 58.11, H, 5.52; N, 16.99%.

N-(5-(4-Methyl-2-phenylthiazol-5-yl)-3,6-dihydro-2*H*-1,3,4-thiadiazin-2-ylidene)aniline 39c:



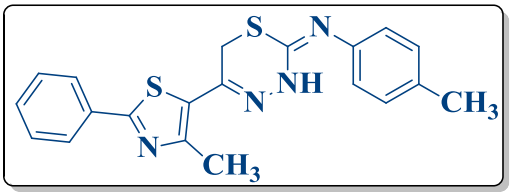
Yellow solid; m.p.: 162 - 163 °C; **FTIR (KBr, υ_{max} cm^{-1}):** 3134 (-NH), 1604 (-C=N); **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm):** 2.72 (s, 3H, -CH_3), 3.80 (s, 2H, -SCH_2), 7.19 (d, *J* = 8Hz, 2H, Ar-H), 7.30 (t, *J* = 8 Hz, 1H, Ar-H), 7.40 (bs, 1H, -NH), 7.42 (s, 1H, Ar-H) 7.44 – 7.48 (m, 4H, Ar-H), 7.93 -7.96 (m, 2H, Ar-H); **^{13}C NMR (100 MHz, DMSO-d_6, ppm):** δ 18.06, 24.20, 122.94, 125.40, 126.21, 128.16, 129.13, 129.34, 130.91, 132.36, 140.42, 144.09, 153.25, 166.06; **MS (ESI m/z):** 365 $[M+H]^+$; Anal. Calcd for $C_{19}H_{16}N_4S_2$ (364.48): C, 62.61; H, 4.42; N, 15.37%; Found: C, 62.58; H, 4.46; N, 15.34%.

N-(5-(4-Methyl-2-phenylthiazol-5-yl)-3,6-dihydro-2*H*-1,3,4-thiadiazin-2-ylidene)-1-phenylmethanamine 39d:



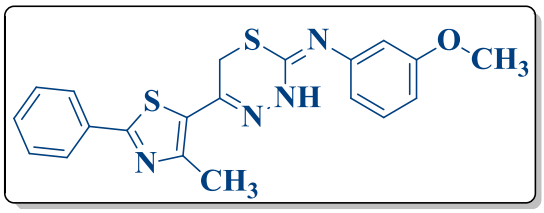
Yellow solid; m.p.: 155 -157 °C; **FTIR (KBr, υ_{max} cm^{-1}):** 3064 (-NH), 1596 (-NH); **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm):** 2.71 (s, 3H, -CH_3), 3.86 (s, 2H, -SCH_2), 4.66 (s, 2H, -$PhCH_2$), 7.36 – 7.40 (m, 4H, Ar-H), 7.44 – 7.47 (m, 4H, Ar-H), 7.91 – 7.94 (m, 2H, Ar-H), 11.92 (bs, 1H, -NH); **HR-MS ESI m/z):** Exact mass calculated for $C_{20}H_{19}N_4S_2$ $[M+H]^+$: 379.1051, Found: 379.1057; Anal. Calcd for $C_{20}H_{18}N_4S_2$: C, 63.46, H, 4.79, N, 14.80%; Found: C, 63.52, H, 4.75; N, 14.76%.

4-Methyl-*N*-(5-(4-methyl-2-phenylthiazol-5-yl)-3,6-dihydro-2*H*-1,3,4-thiadiazin-2-ylidene)aniline 39e:



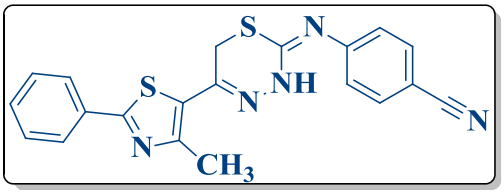
Yellow solid; m.p.: 174 - 176 °C; **FTIR (KBr, υ_{max} cm^{-1}):** 3146 (-NH), 1599 (-C=N); **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm):** 2.40 (s, 3H, Ar-CH_3), 2.74 (s, 3H, -CH_3), 3.87 (s, 2H, SCH_2), 7.20 (d, *J* = 8 Hz, 2H, Ar-H), 7.25 (s, 1H, Ar-H), 7.27 (s, 1H, Ar-H), 7.47 (d, *J* = 8 Hz, 3H, Ar-H), 7.94 (d, *J* = 8 Hz, 2H, Ar-H), 13.27 (bs, 1H, -NH); **HR-MS ESI m/z):** Exact mass calculated for $C_{20}H_{19}N_4S_2$ $[M+H]^+$: 379.1051, Found: 379.1053; Anal. Calcd for $C_{20}H_{18}N_4S_2$: C, 63.46, H, 4.79, N, 14.80%; Found: C, 63.42, H, 4.76, N, 14.85%.

3-Methoxy-*N*-(5-(4-methyl-2-phenylthiazol-5-yl)-3,6-dihydro-2*H*-1,3,4-thiadiazin-2-ylidene)aniline 39f:



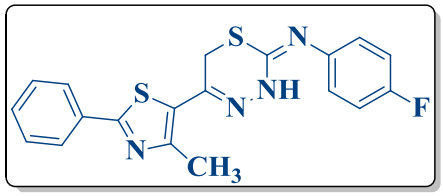
Yellow solid; m.p.: 167 - 169 °C; **FTIR (KBr, υ_{max} cm^{-1}):** 3167 (-NH), 1594 (-C=N), 1271 (-OCH_3); **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm):** 2.72 (s, 3H, -CH_3), 3.82 (s, 3H, -OCH_3), 3.90 (s, 2H, -SCH_2), 6.84 (d, *J* = 6.8 Hz, 2H, Ar-H), 6.90 (d, *J* = 7.6 Hz, 1H, Ar-H), 7.33 (t, *J* = 8.4 Hz, 1H, Ar-H), 7.45 (d, *J* = 6 Hz, 3H, Ar-H), 7.92 (d, *J* = 7.6 Hz, 2H, Ar-H), 9.49 (bs, 1H, -NH); Anal. Calcd for $C_{20}H_{18}N_4OS_2$: C, 60.89; H, 4.60; N 14.20%; Found: C, 60.93; H, 4.57; N, 14.22%.

4-((5-(4-Methyl-2-phenylthiazol-5-yl)-3,6-dihydro-2*H*-1,3,4-thiadiazin-2-ylidene) amino)benzonitrile 39g:



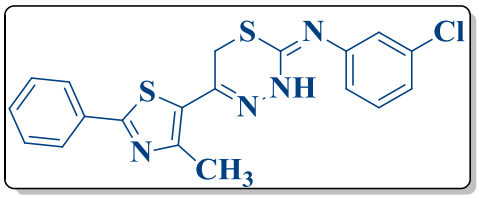
Yellow solid; m.p.: 189 - 191 °C; **FTIR (KBr, υ_{max} cm^{-1}):** 3189 (-NH), 2223 (-CN), 1607 (-C=N); **^{1}H NMR (400 MHz, DMSO-*d*$_6$, δ ppm):** 2.65 (s, 3H, -CH_3), 4.02 (s, 2H, -SCH_2), 7.52 (t, *J* = 3.6 Hz, 5H, Ar-H), 7.77 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.94 – 7.97 (m, 2H, Ar-H), 11.21 (bs, 1H, -NH); **^{13}C NMR (100 MHz, DMSO-*d*$_6$, ppm):** δ 18.01, 23.97, 104.77, 119.19, 121.27, 126.17, 128.91, 129.32, 130.79, 132.48, 133.21, 152.65, 165.76; **HR-MS ESI m/z):** Exact mass calculated for $C_{20}H_{15}N_5S_2$ $[M]^+$: 389.0769, Found: 389.0772; Anal. Calcd for $C_{20}H_{15}N_5S_2$: C, 61.67; H, 3.88; N, 17.98%; Found: C, 61.71; H, 3.86; N, 17.95%.

4-Fluoro-*N*-(5-(4-methyl-2-phenylthiazol-5-yl)-3,6-dihydro-2*H*-1,3,4-thiadiazin-2-ylidene)aniline 39h:



Yellow solid; m.p.: 201 - 203 °C; **FTIR (KBr, υ_{max} cm^{-1}):** 3156 (-NH), 1602 (-C=N); **^{1}H NMR (400 MHz, DMSO-*d*$_6$, δ ppm):** 2.74 (s, 3H, -CH_3), 3.91 (s, 2H, -SCH_2), 7.17 (t, *J* = 8 Hz, 2H, Ar-H), 7.33 – 7.36 (m, 2H, Ar-H), 7.47 (d, *J* = 4H, 3H, Ar-H), 7.93 – 7.96 (m, 2H, Ar-H), 13.19 (bs, 1H, -NH); **^{13}C NMR (100 MHz, DMSO-*d*$_6$, ppm):** δ 18.21, 24.28, 116.33, 118.95, 126.06, 126.32, 127.54, 129.42, 131.11, 132.27, 135.22, 137.13, 145.12, 154.14, 155.95, 157.45, 159.16, 161.52, 166.59; **HR-MS ESI m/z):** Exact mass calculated for $C_{19}H_{16}FN_4S_2$ $[M+H]^+$: 383.0800, Found: 383.0801; Anal. Calcd for $C_{19}H_{15}FN_4S_2$: C, 59.66, H, 3.95, N, 14.65%; Found: C, 59.70; H, 3.98, N, 14.59%.

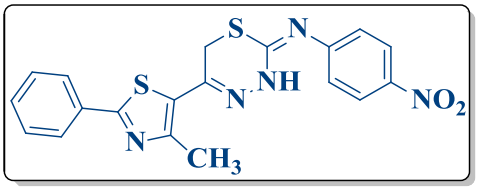
3-Chloro-*N*-(5-(4-methyl-2-phenylthiazol-5-yl)-3,6-dihydro-2*H*-1,3,4-thiadiazin-2-ylidene)aniline 39i:



Yellow solid; m.p.: 212 - 214 °C; **FTIR (KBr, υ_{max} cm^{-1}):** 3131 (-NH), 1611 (-C=N), 780 (-C-Cl); **^{1}H NMR (400 MHz, DMSO-*d*$_6$, δ ppm):** 2.69 (s, 3H, -CH_3), 3.76 (s, 2H, -SCH_2), 6.88 (d, *J* = 4Hz, 1H, Ar-H), 7.04 (s, 1H, Ar-H), 7.09 – 7.10 (dd, *J* =4 Hz, 1H, Ar-H), 7.11 – 7.12 (dd, *J* = 4 Hz, 1H, Ar-H), 7.45 (t, *J* = 4 Hz, 3H, Ar-H), 7.92 -7.95 (dd, *J* = 4 Hz, 2H, Ar-H), 9.04 (bs, 1H, Ar-H); **^{13}C NMR (100 MHz, DMSO-*d*$_6$, ppm):** δ 17.88, 23.78, 126.08, 129.28, 130.27, 130.64, 132.57, 132.98, 151.95, 161.08, 162.30; **HR-MS ESI m/z):** Exact mass calculated for $C_{19}H_{16}ClN_4S_2$ $[M+H]^+$: 399.0505, Found: 399.0504;

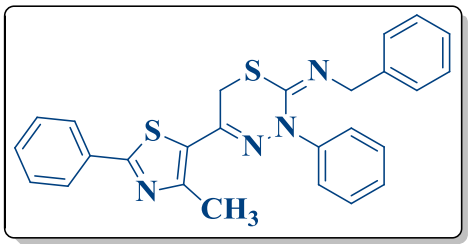
Anal. Calcd for $C_{29}H_{15}ClN_4S_2$: C, 57.20; H, 3.79; N, 14.04%; Found: C, 57.25; H, 3.76; N, 14.00%.

N-(5-(4-methyl-2-phenylthiazol-5-yl)-3,6-dihydro-2H-1,3,4-thiadiazin-2-ylidene)-4-nitroaniline 39j:



Red solid; m.p.: 242 - 244 °C; **FTIR (KBr, υ_{max} cm^{-1}):** 3154 (-NH), 1601 (-C=N), 1505 (N-O), 1342 (-N-O); **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm):** 2.66 (s, 3H, $-CH_3$), 4.02 (s, 2H, $-SCH_2$), 7.52 (t, *J* = 2.8 Hz, 4H, Ar-H), 7.79 (d, *J* = 9.2 Hz, 2H, Ar-H), 8.23 (t, *J* = 9.2 Hz, 3H, Ar-H), 10.87 (bs, 1H, -NH); Anal. Calcd for $C_{19}H_{15}N_5O_2S_2$: C, 55.73; H, 3.69; N, 17.10%; Found: C, 55.69; H, 3.72; N, 17.12%.

***N*-(5-(4-Methyl-2-phenylthiazol-5-yl)-3-phenyl-3,6-dihydro-2*H*-1,3,4-thiadiazin-2-ylidene)-1-phenylmethanamine 39k:**



Yellow solid; m.p.: 213 - 215 °C; **FTIR (KBr, υ_{max} cm^{-1}):** 2918 (-C-H), 1597 (-C=N); **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):** 2.69 (s, 3H, $-CH_3$), 4.43 (s, 2H, $-SCH_2$), 4.93 (s, 2H, $PhCH_2$), 7.29 – 7.35 (m, 3H, Ar-H), 7.46 (t, *J* = 7.6 Hz, 5H, Ar-H), 7.55(t, *J* = 7.2 Hz, 1H, Ar-H), 7.62 (t, *J* = 7.6 Hz, 2H, Ar-H), 7.80 (d, *J* = 7.2 Hz, 2H, Ar-H), 7.92 (d, *J* = 8 Hz, 2H, Ar-H); Anal. Calcd for $C_{26}H_{22}N_4S_2$ (454.64): C, 68.69; H, 4.88; N, 12.32%; Found: C, 68.65; H, 4.90; N, 12.35 %

Antiviral activity experiment of 2,5-di substituted and 2,3,5-tri substituted 1,3,4-thiadiazines derivatives 39 a-k:

The antiviral activity of the newly synthesized compounds was determined by using different cell culture mediums as reported in chapter-II (Reference 65 - 67). (i) HEL cell culture was used for viruses (*Herpes simplex virus type 1, Herpes simplex virus type 2, Herpes simplex virus type 1 TK- ACV^r, Vaccinia virus, Adeno virus-2* or *Vesicular stomatitis virus*), (ii) Vero cells were used for the virus (*Coxsackie virus B4, Sindbis virus, Parainfluenza virus 3, Punta Toro virus* or *Reovirus*), (iii) HeLa cells were used for the virus (*Coxsackie virus B4, Vesicular stomatitis virus* or *Respiratory syncytial virus*), (iv) MDCK cells were used for the virus (*Influenza A/H1N1 A/Ned/378/05,*

Influenza A/H3N2 A/HK/7/87 or *Influenza B B/Ned/537/05*) (v) CRFK cells for the virus (*Feline herpes virus* or *Feline corona virus*). After incubation at 37°C the virus-induced cytopathicity was determined by measuring the cell viability with the colorimetric formazan-based MTS assay.

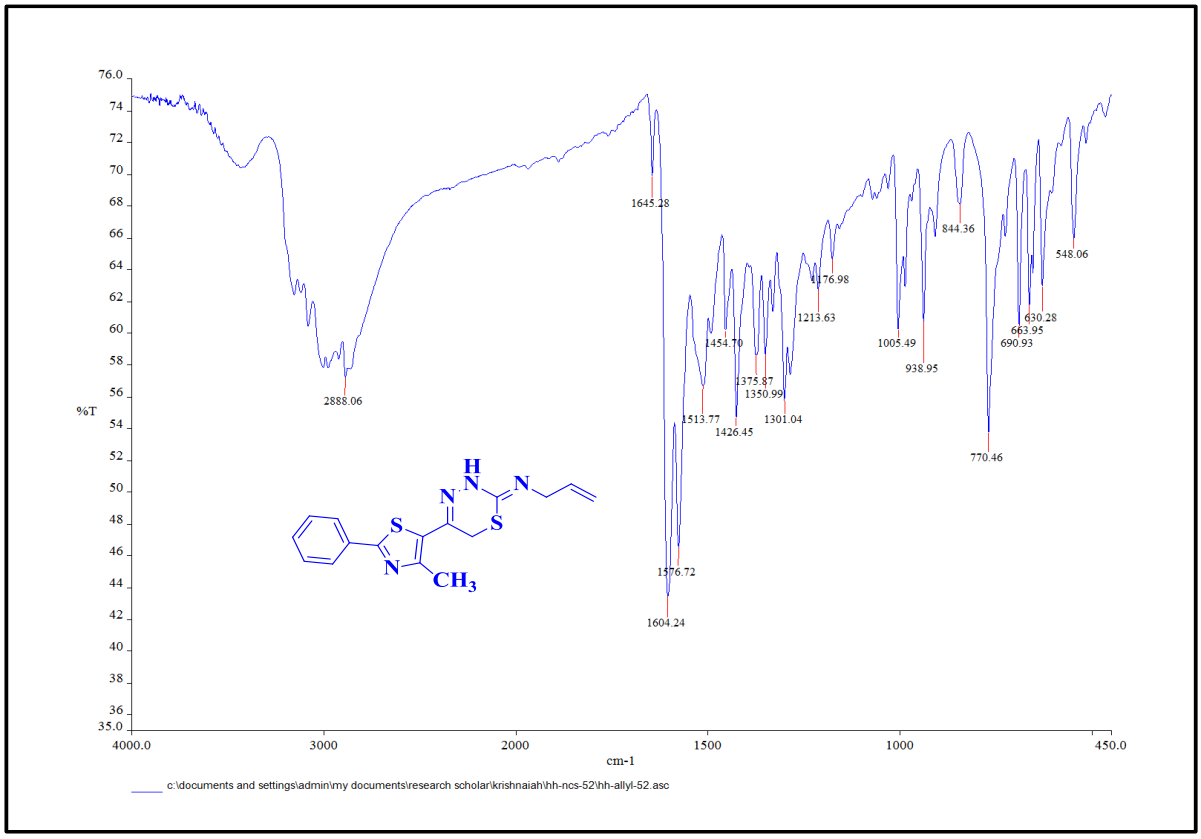


Fig: IR Spectrum of compound **39a**

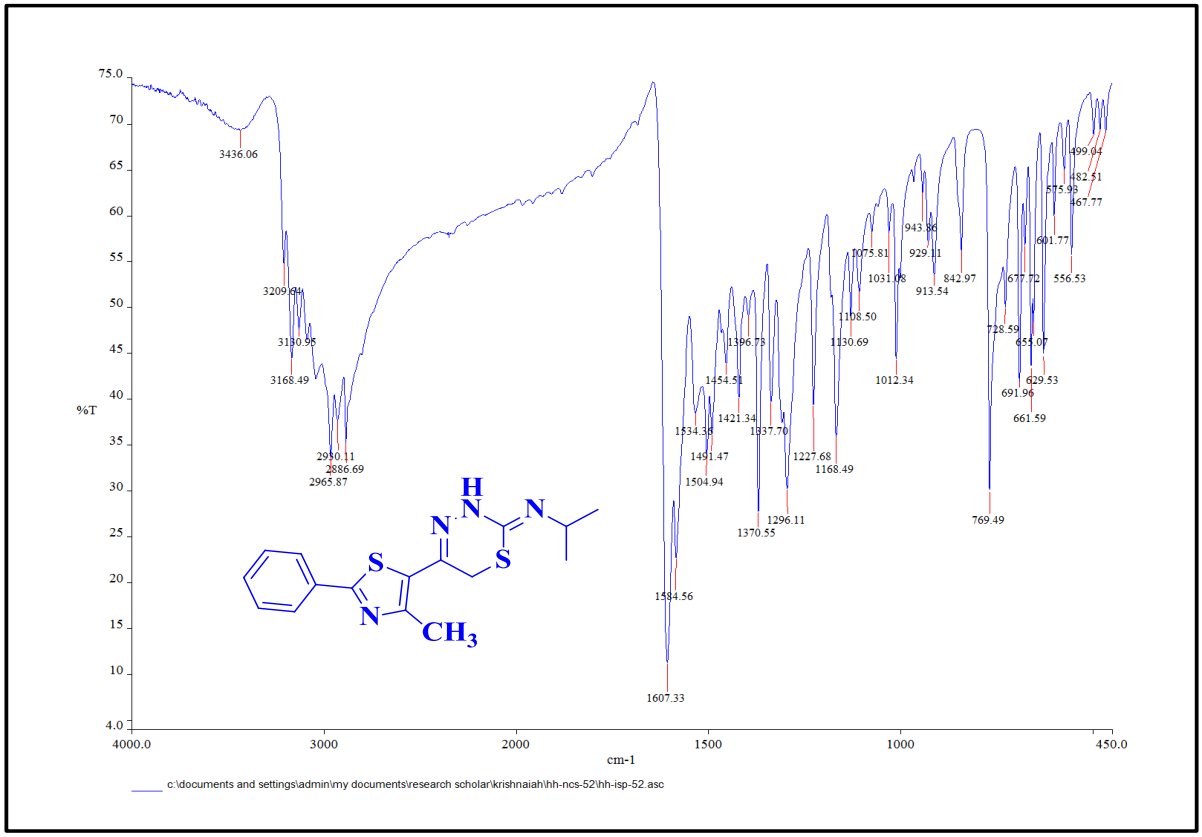


Fig: IR Spectrum of compound **39b**

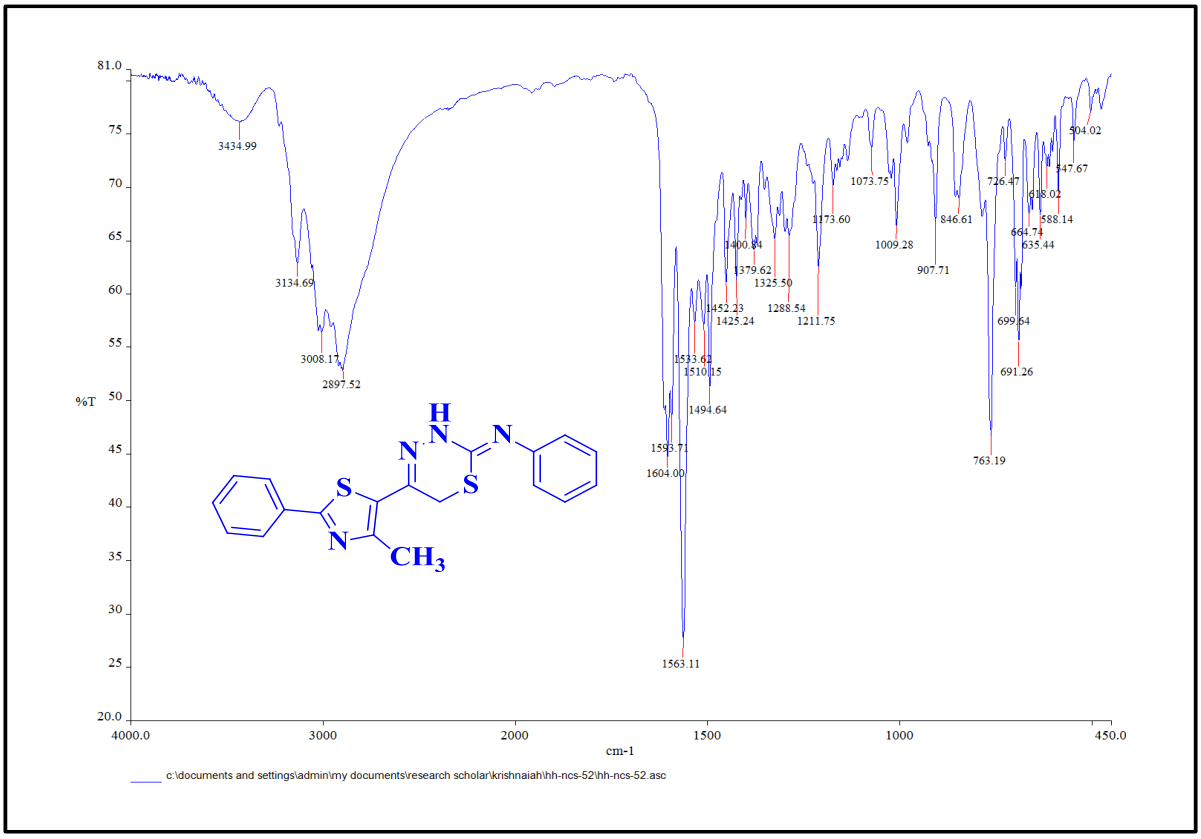


Fig: IR Spectrum of compound **39c**

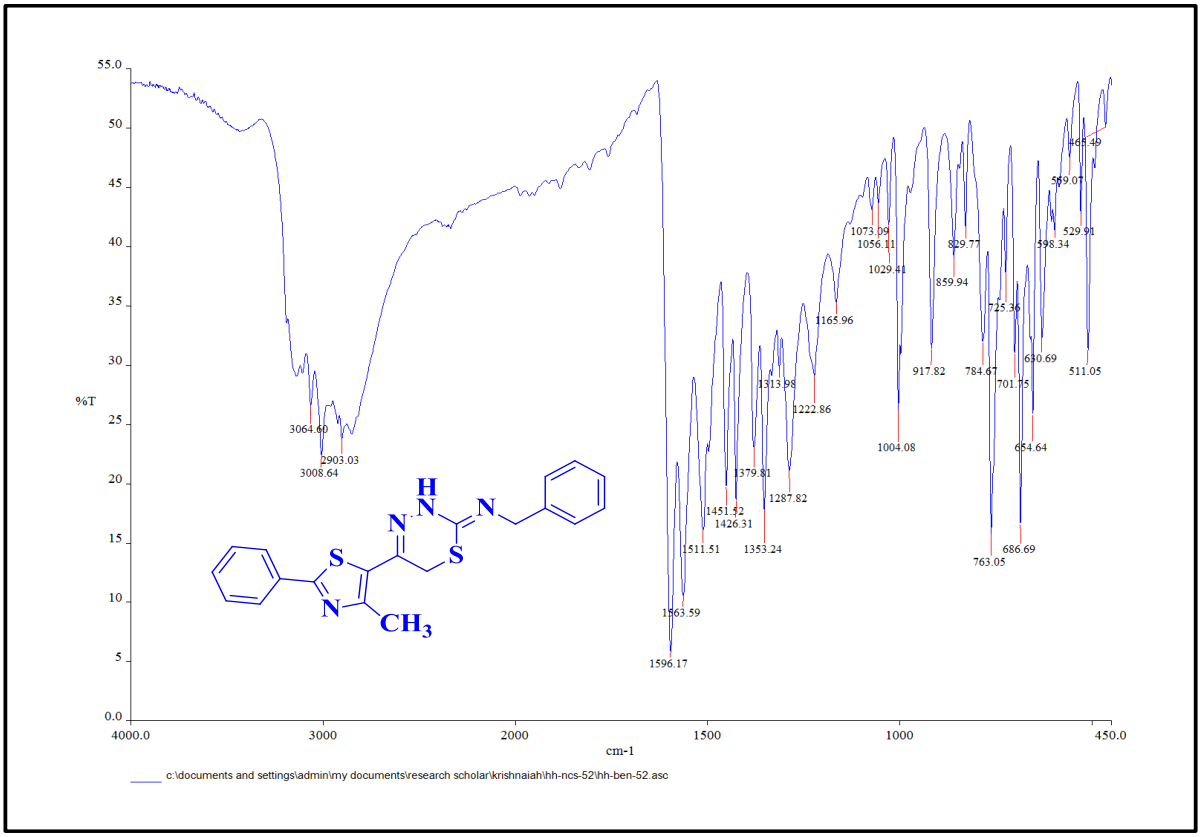


Fig: IR Spectrum of compound **39d**

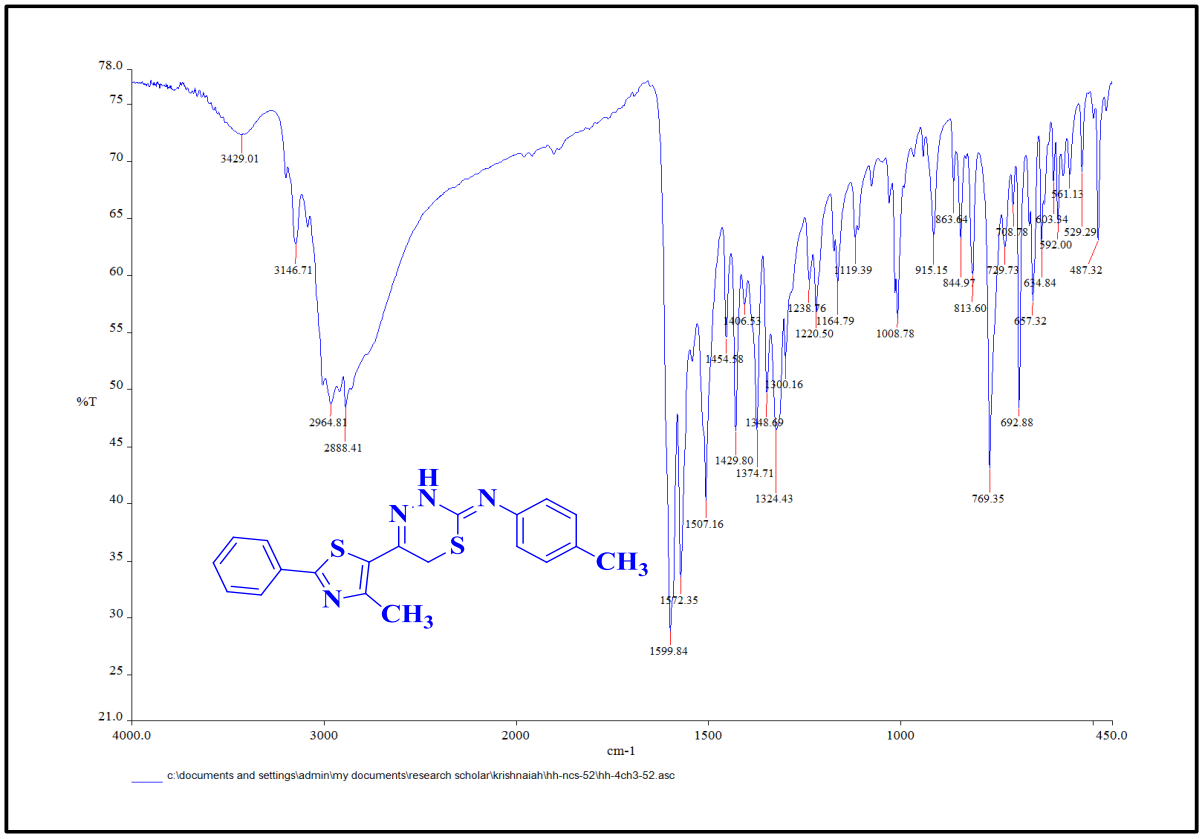


Fig: IR Spectrum of compound **39e**

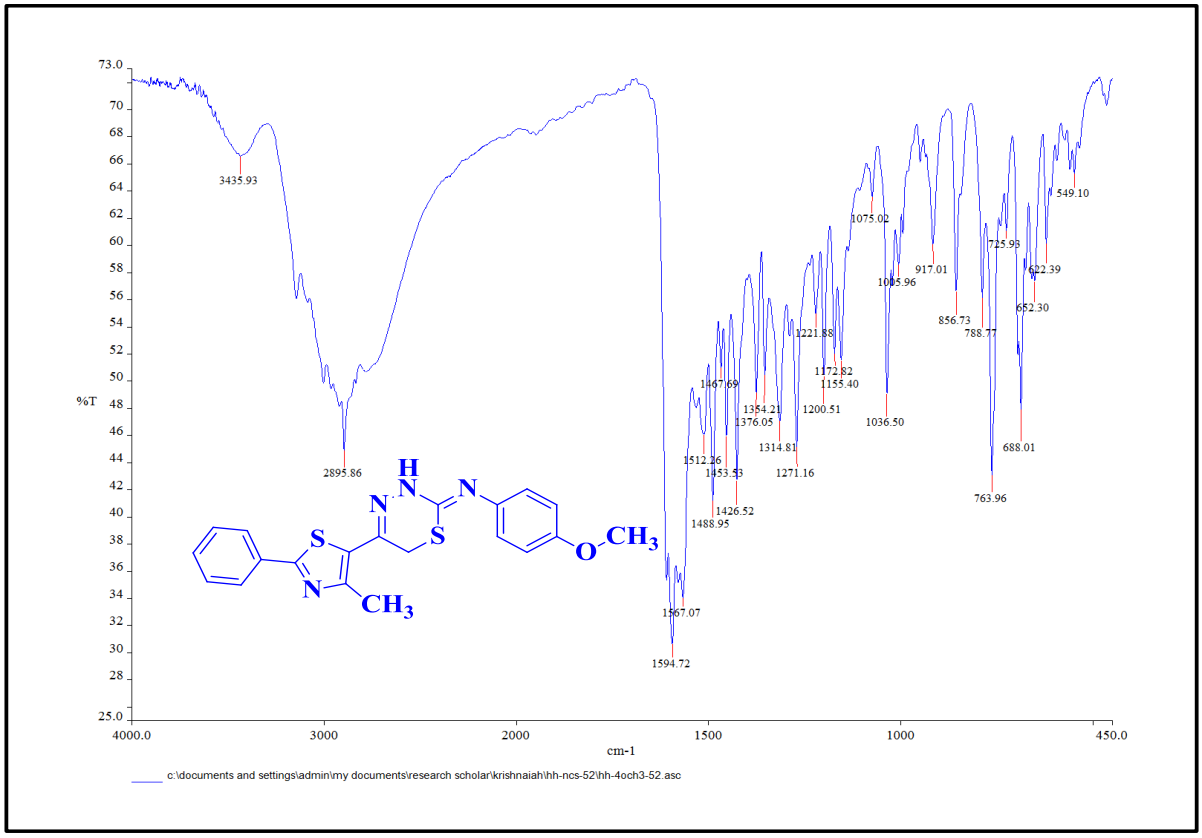


Fig: IR Spectrum of compound **39f**

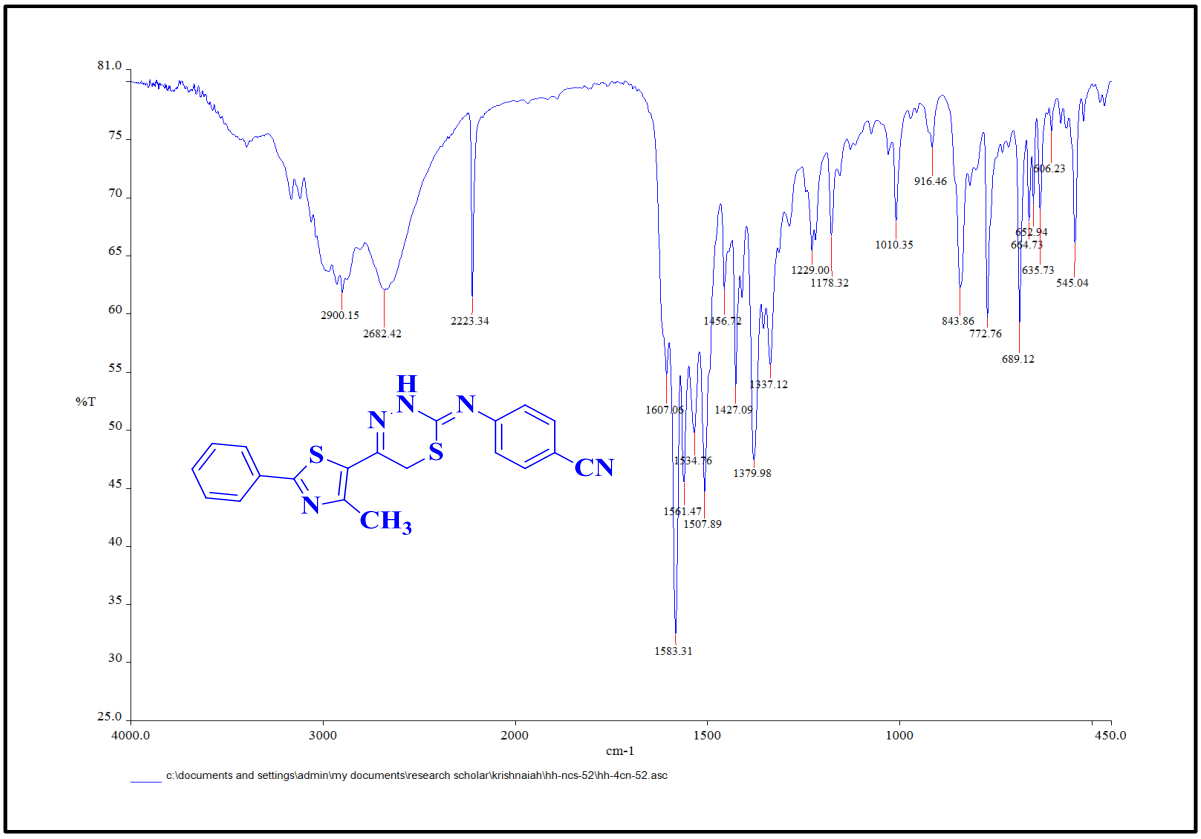


Fig: IR Spectrum of compound **39g**

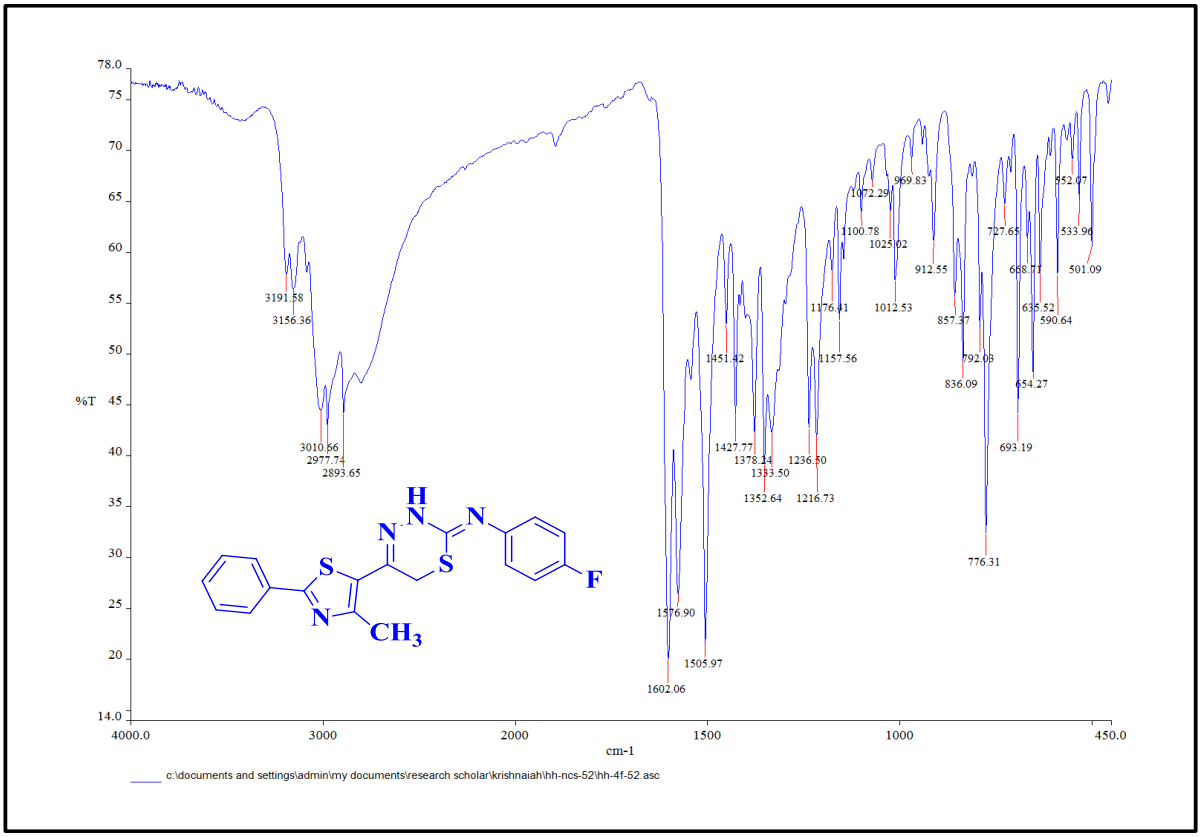


Fig: IR Spectrum of compound **39h**

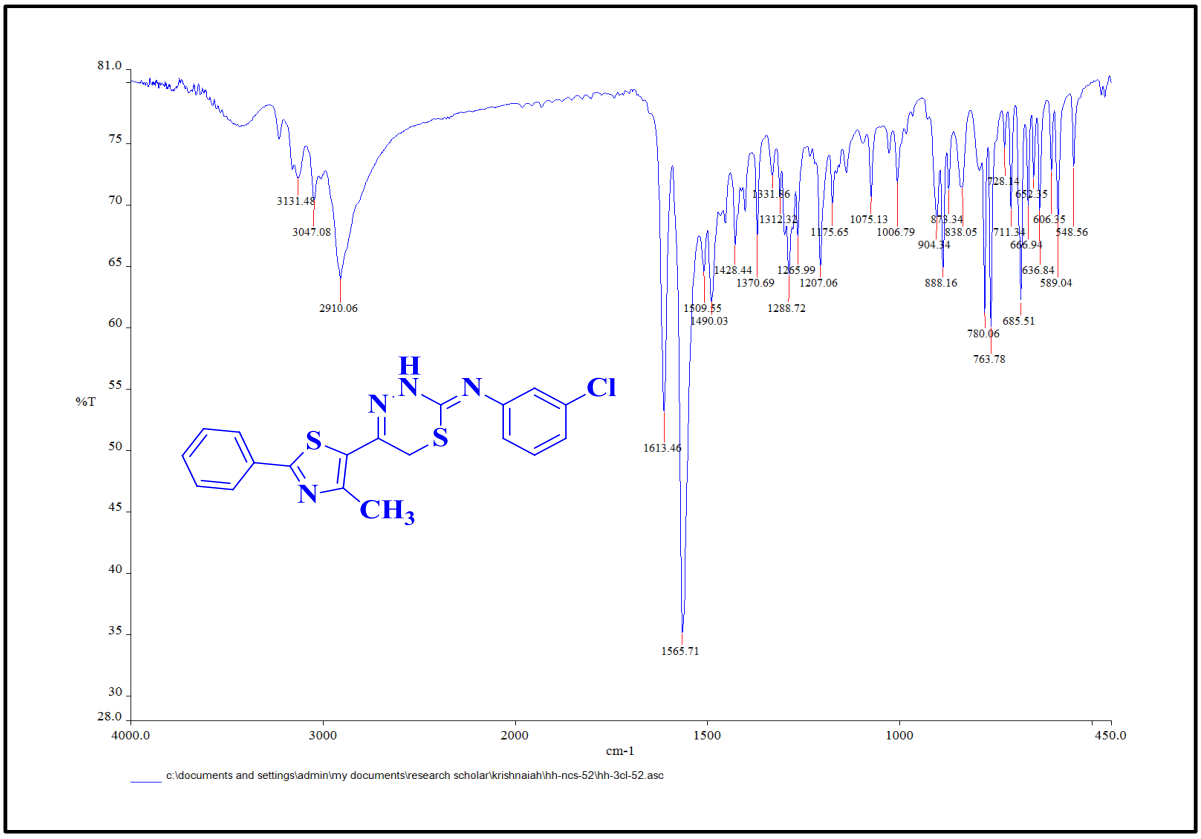


Fig: IR Spectrum of compound **39i**

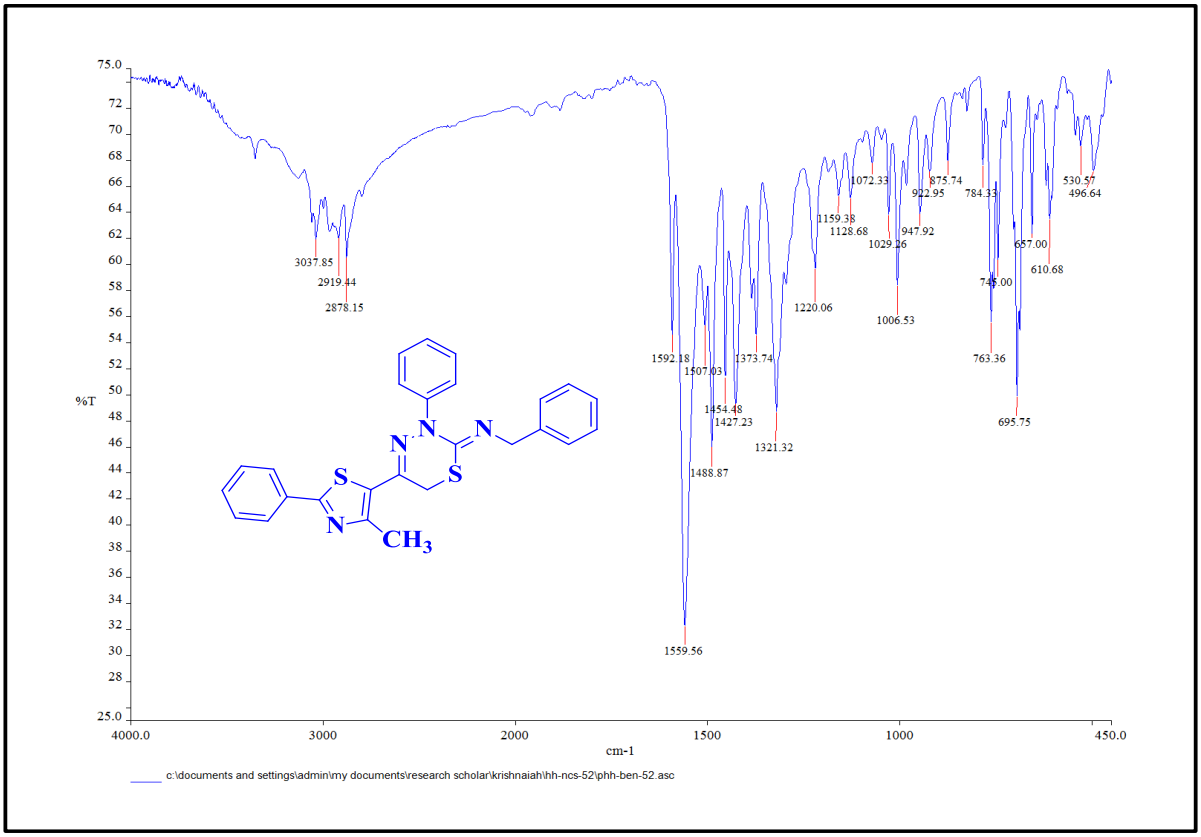


Fig: IR Spectrum of compound **39k**

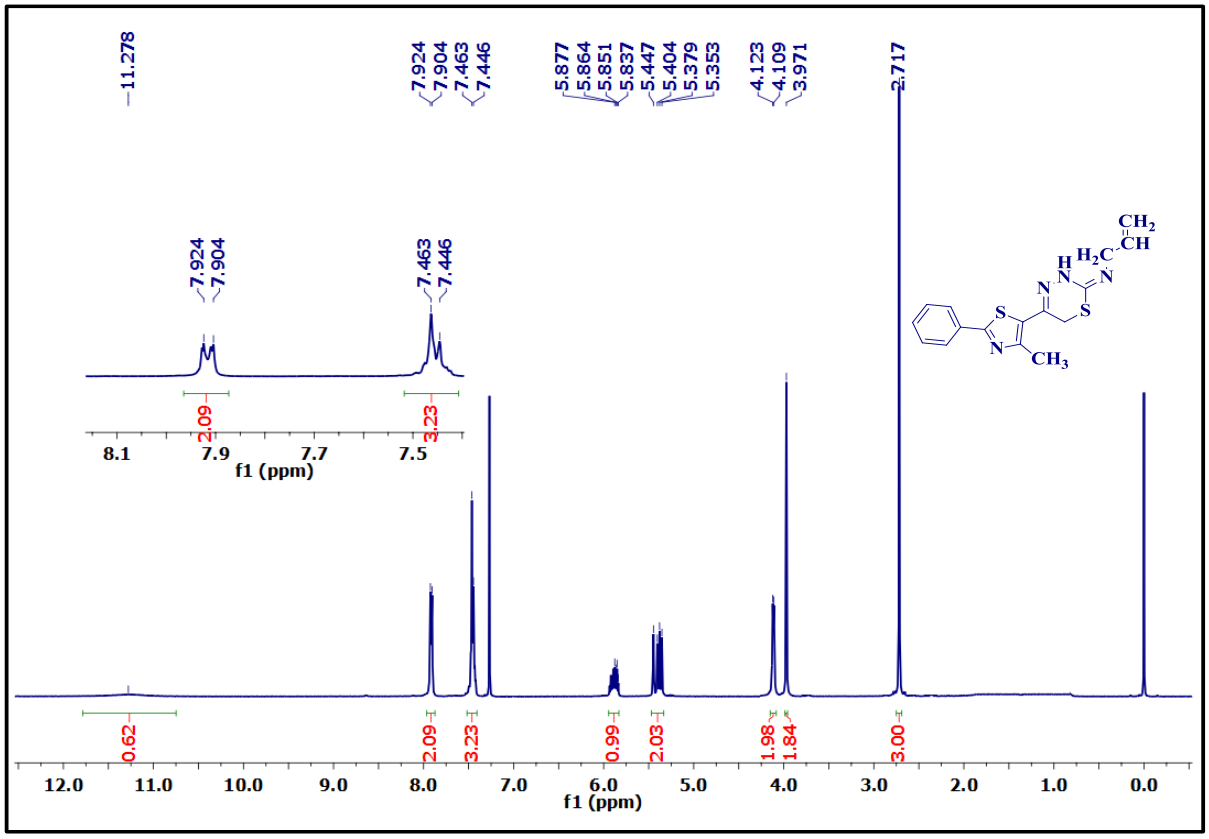


Fig: [1]H NMR Spectrum of compound **39a**

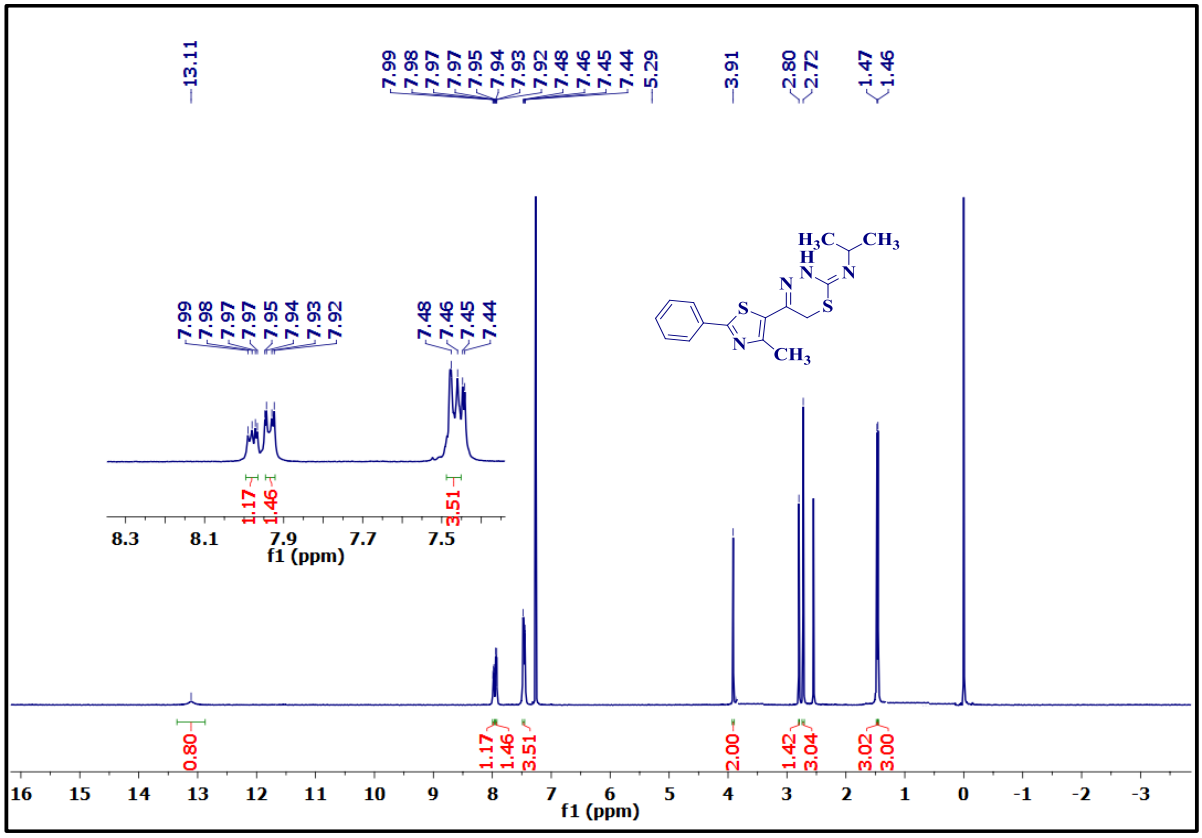


Fig: [1]H NMR Spectrum of compound **39b**

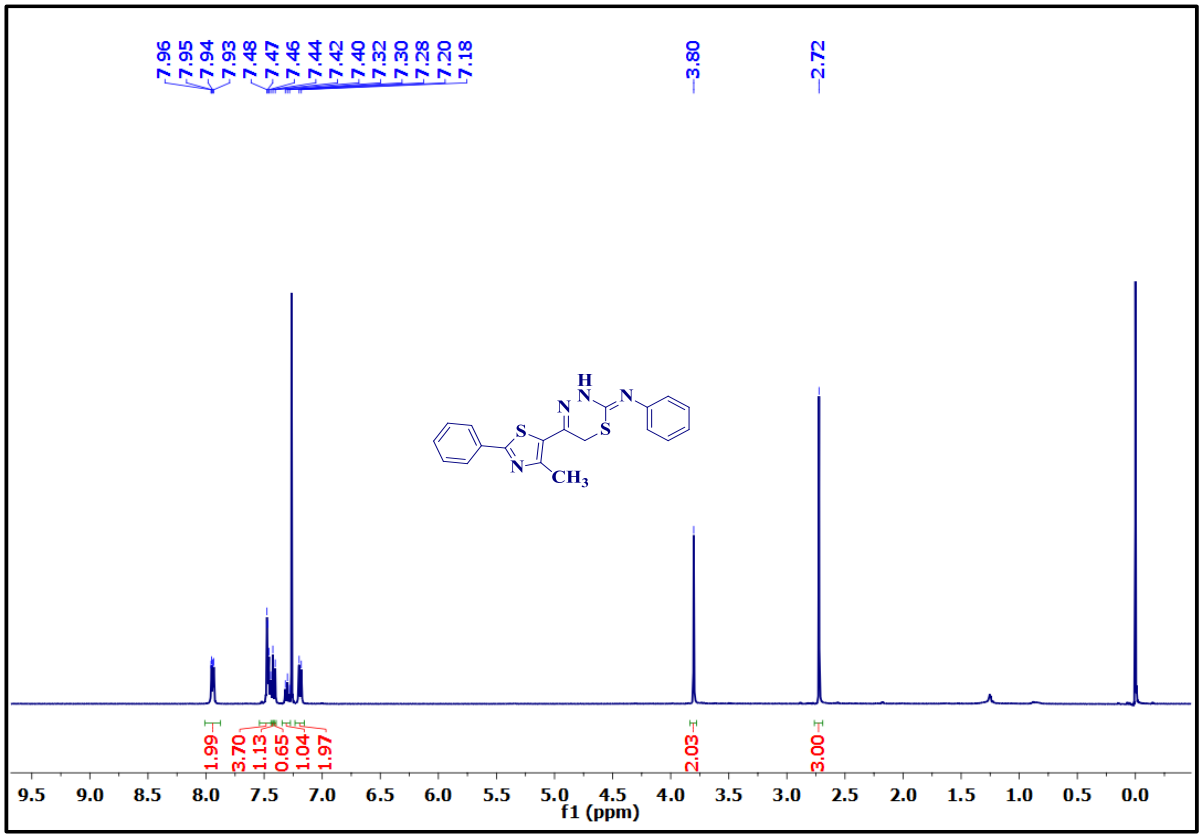


Fig: ^{1}H NMR Spectrum of compound **39c**

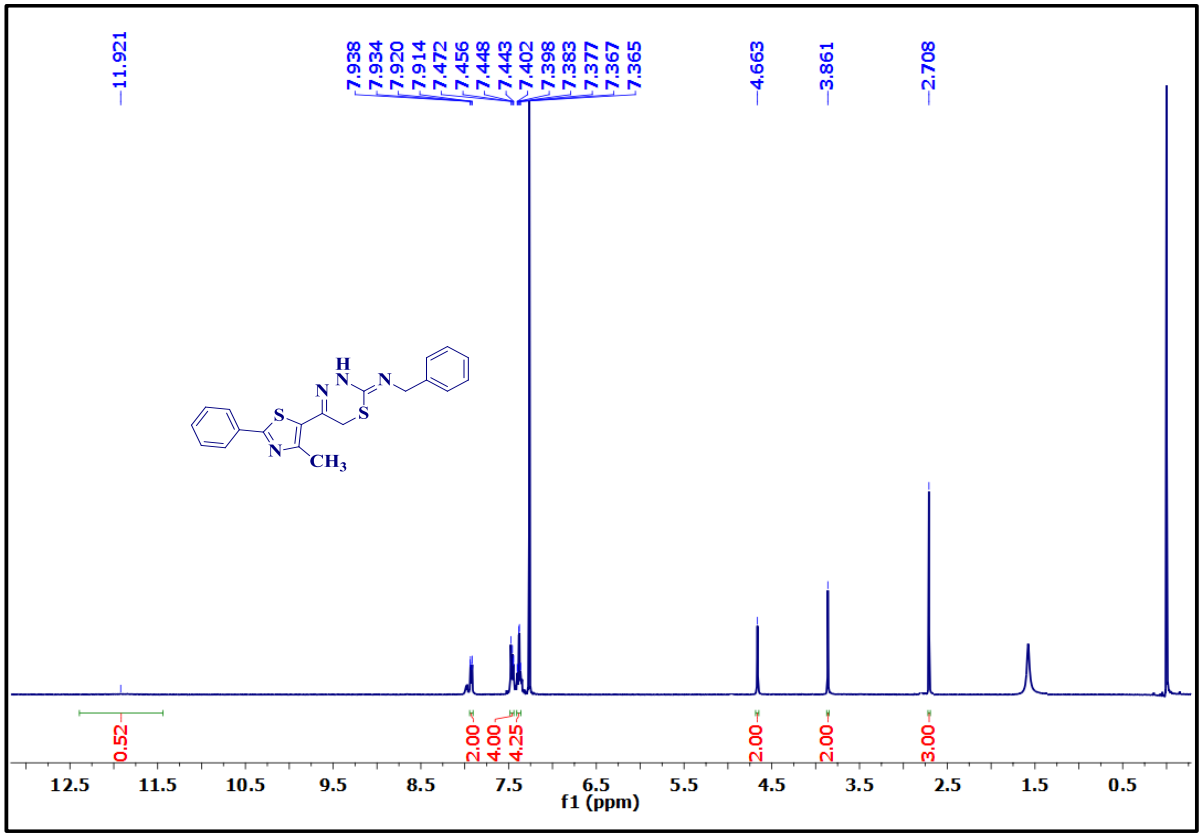


Fig: ^{1}H NMR Spectrum of compound **39d**

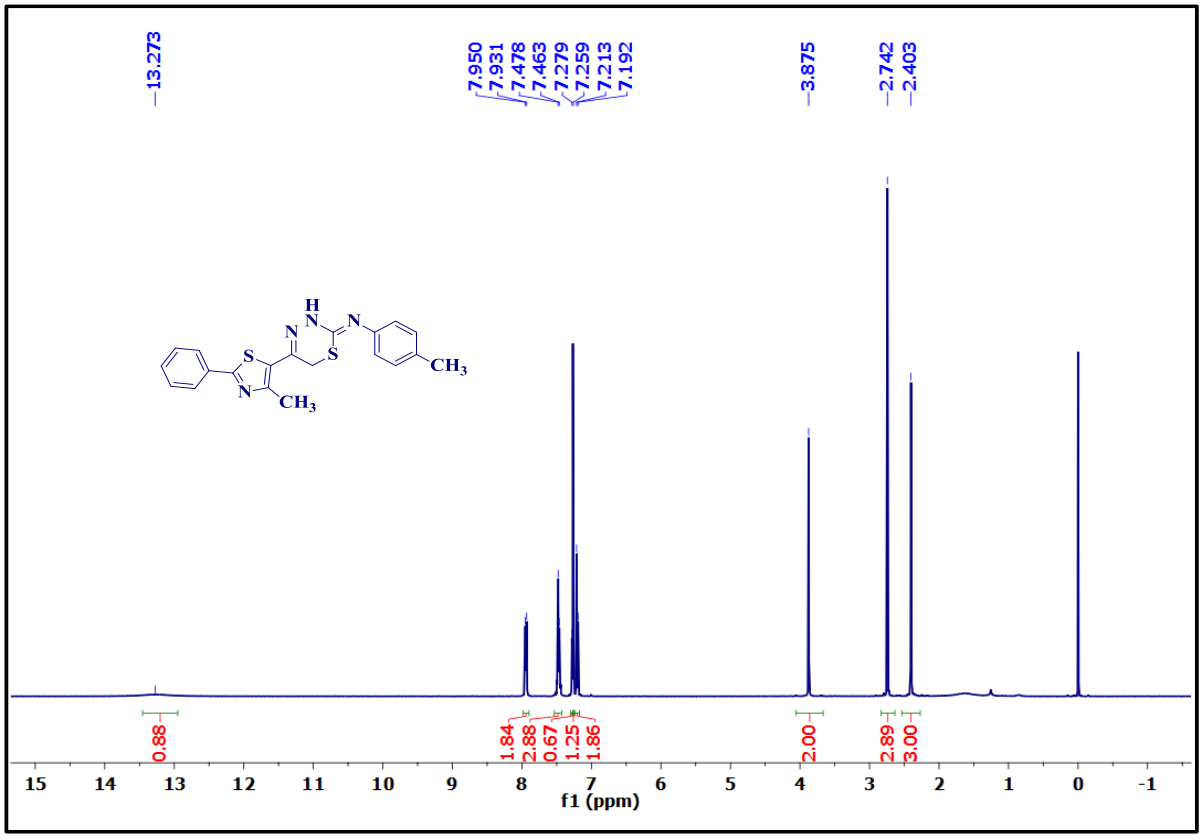


Fig: ^{1}H NMR Spectrum of compound **39e**

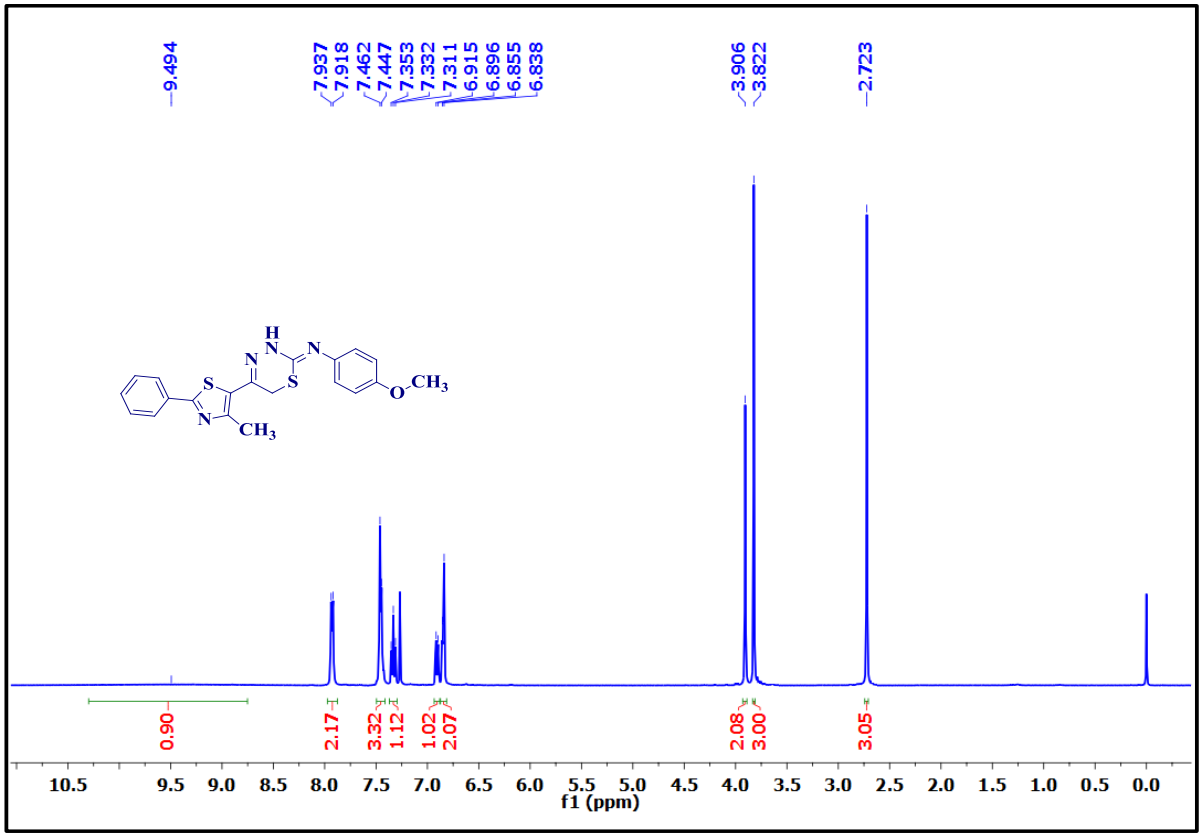


Fig: ^{1}H NMR Spectrum of compound **39f**

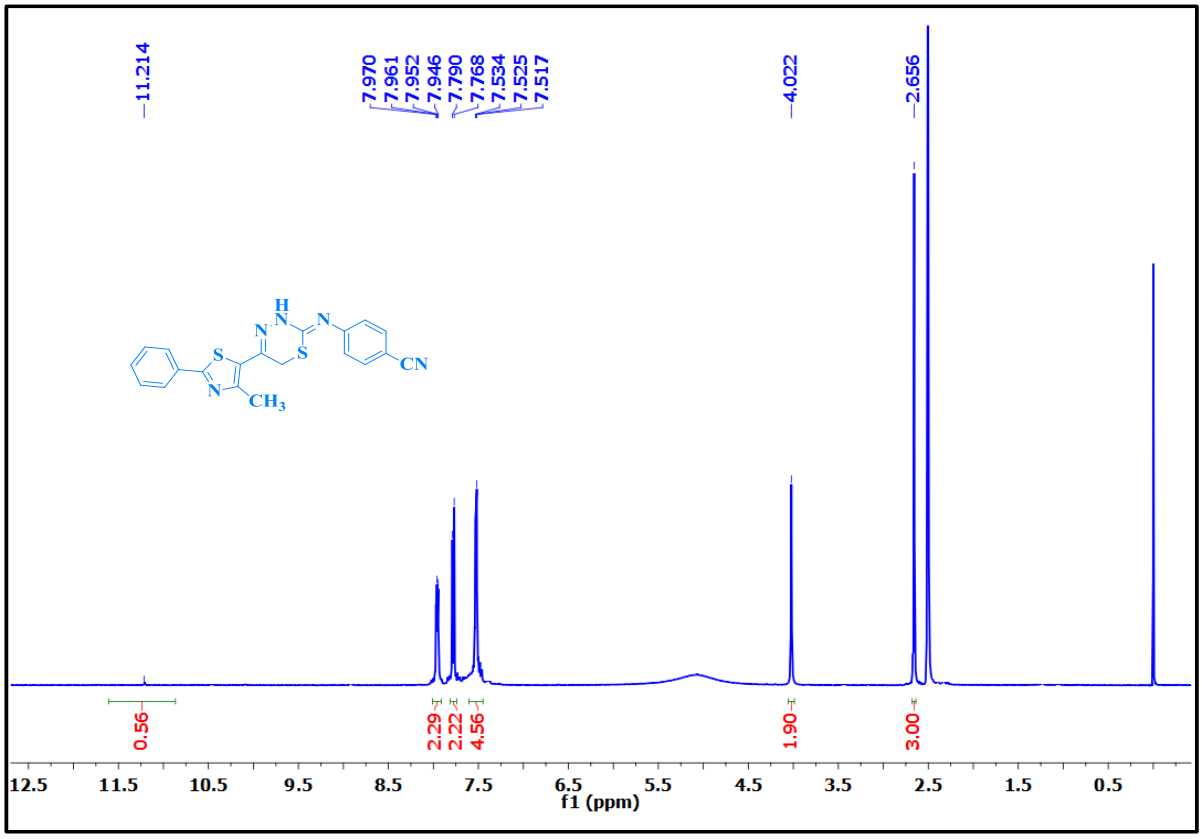


Fig: ^{1}H NMR Spectrum of compound **39g**

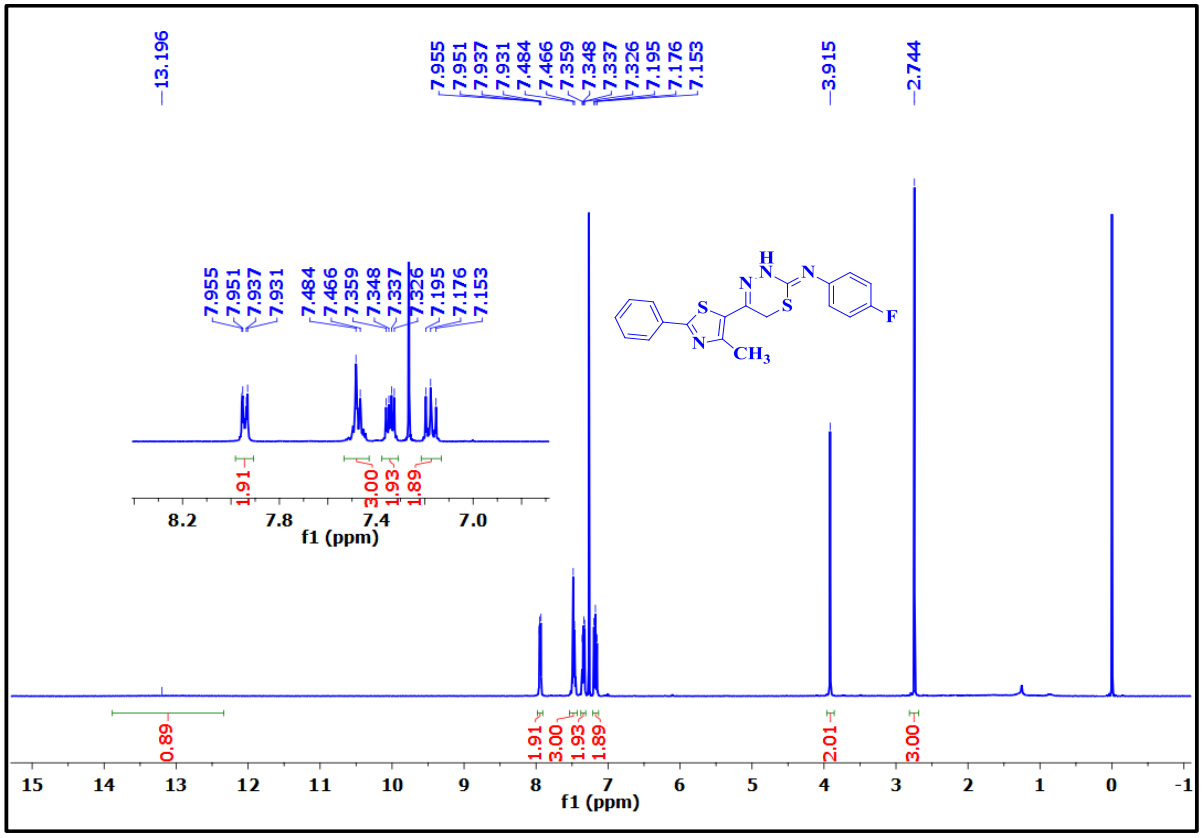


Fig: ^{1}H NMR Spectrum of compound **39h**

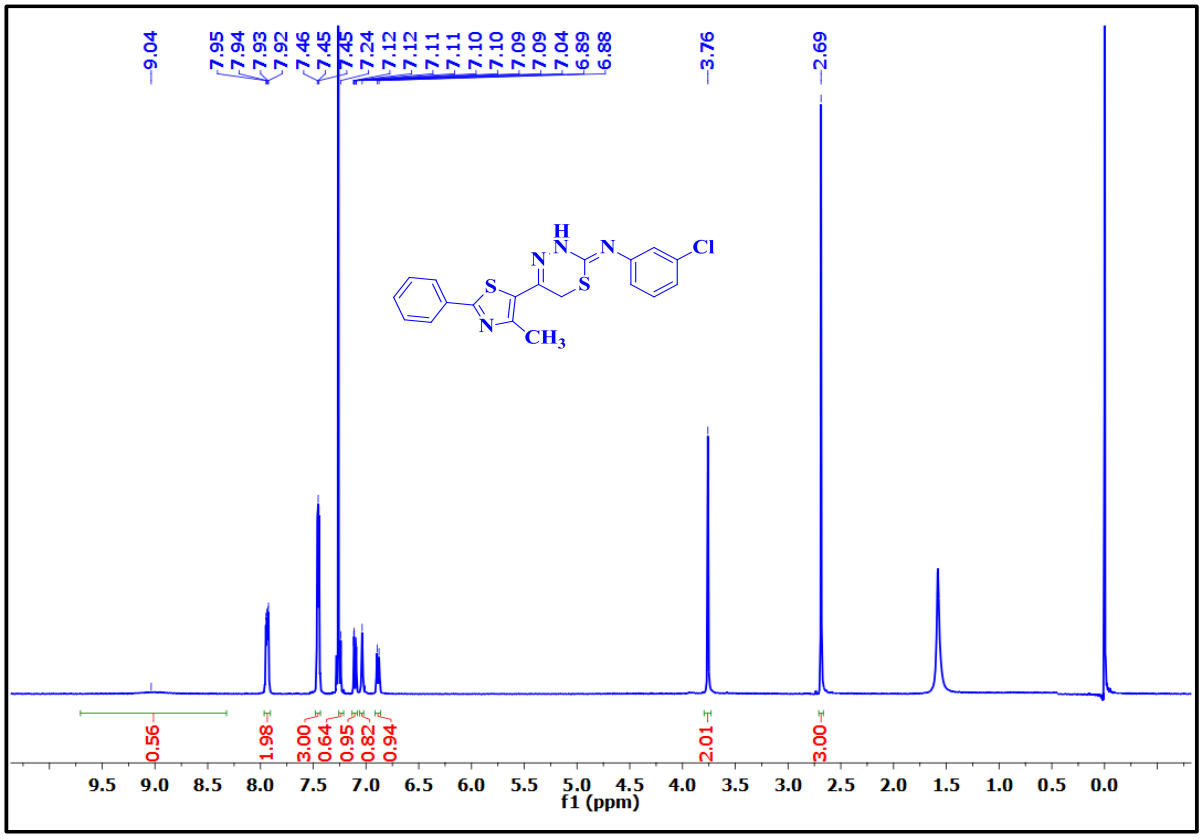


Fig: [1]H NMR Spectrum of compound **39i**

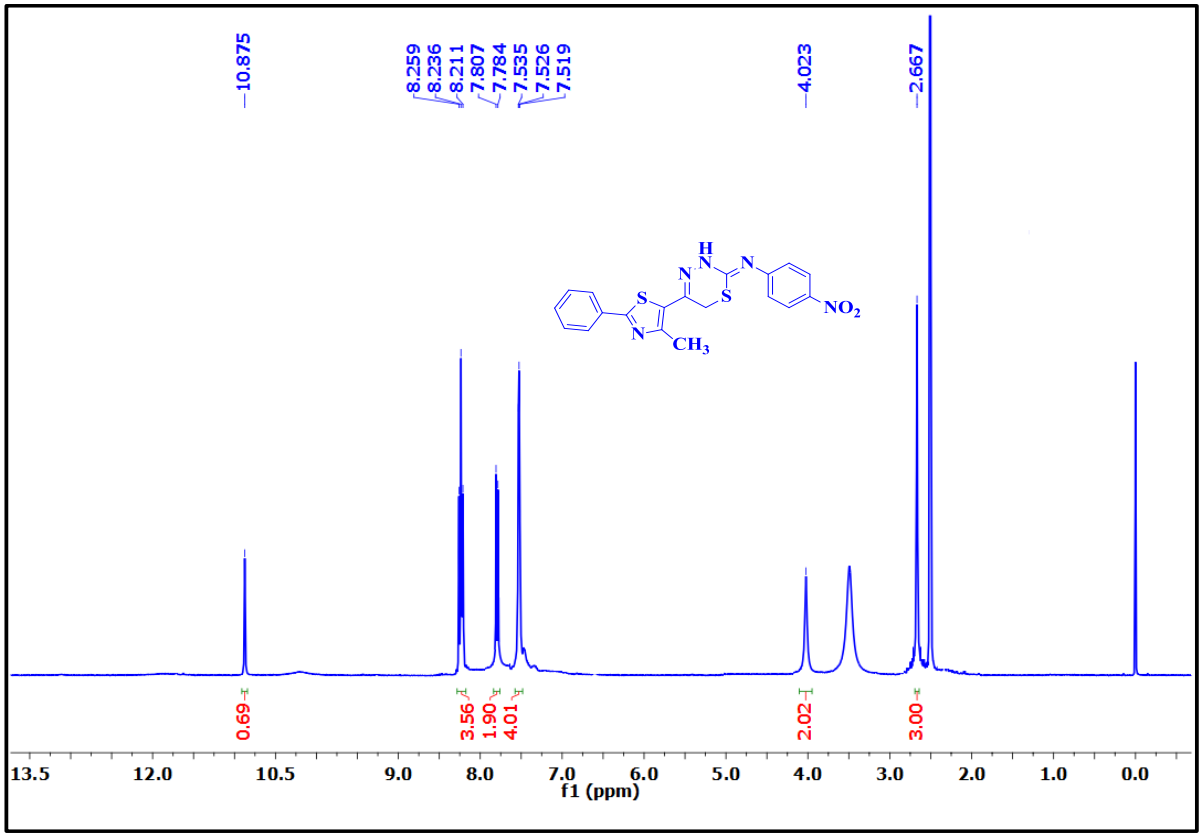


Fig: [1]H NMR Spectrum of compound **39j**

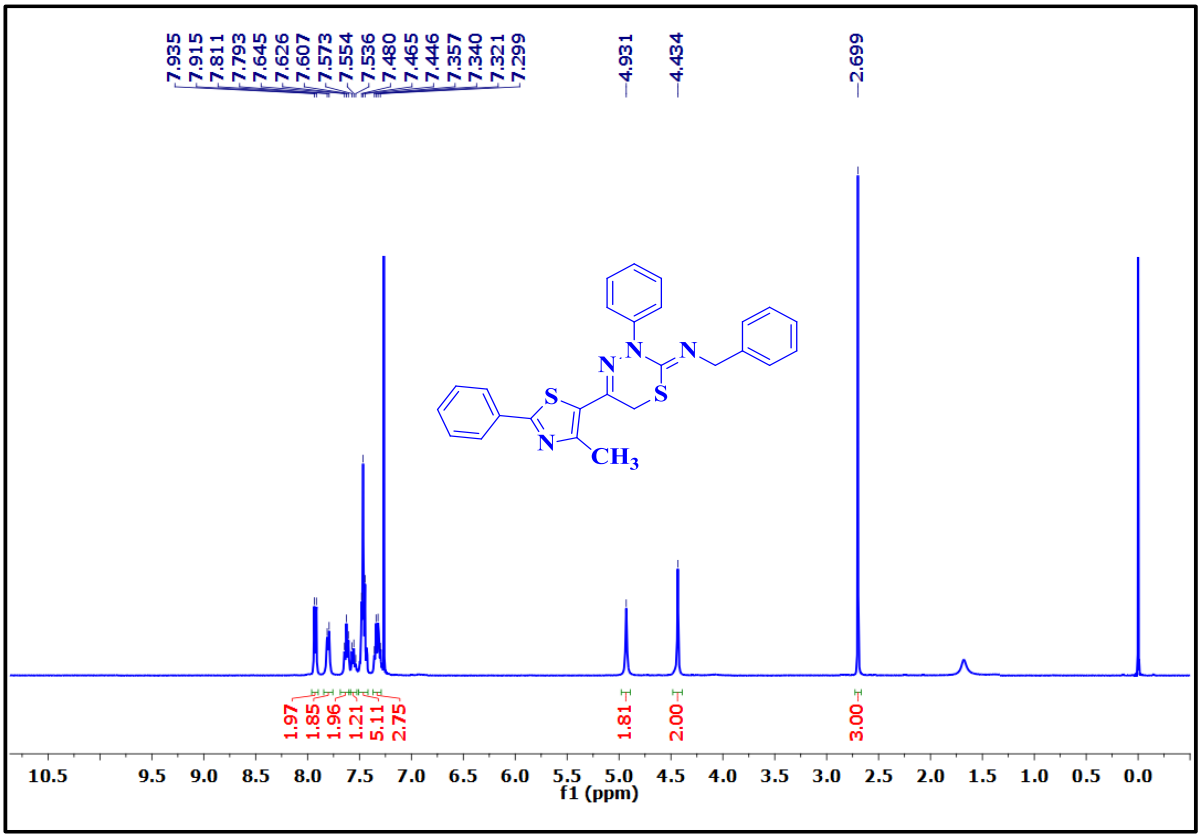


Fig: ^{1}H NMR Spectrum of compound **39k**

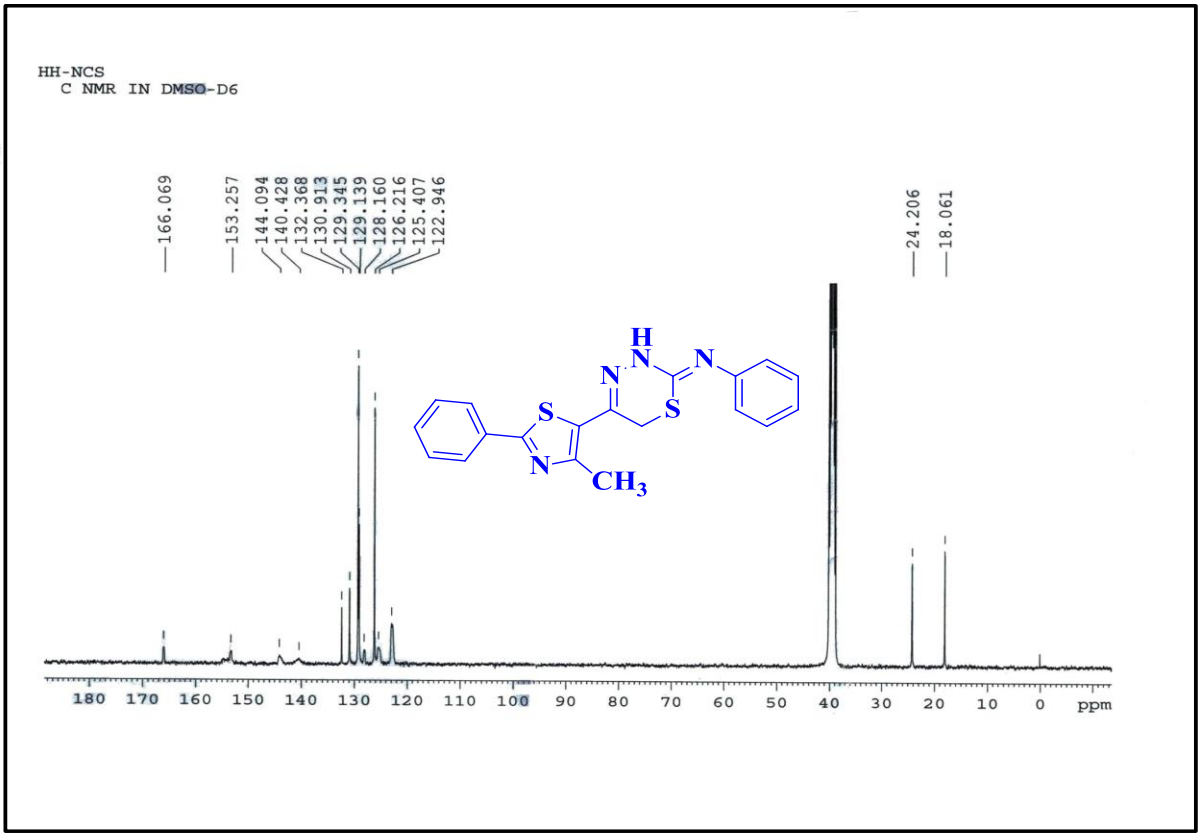


Fig: ^{13}C NMR Spectrum of compound **39c**

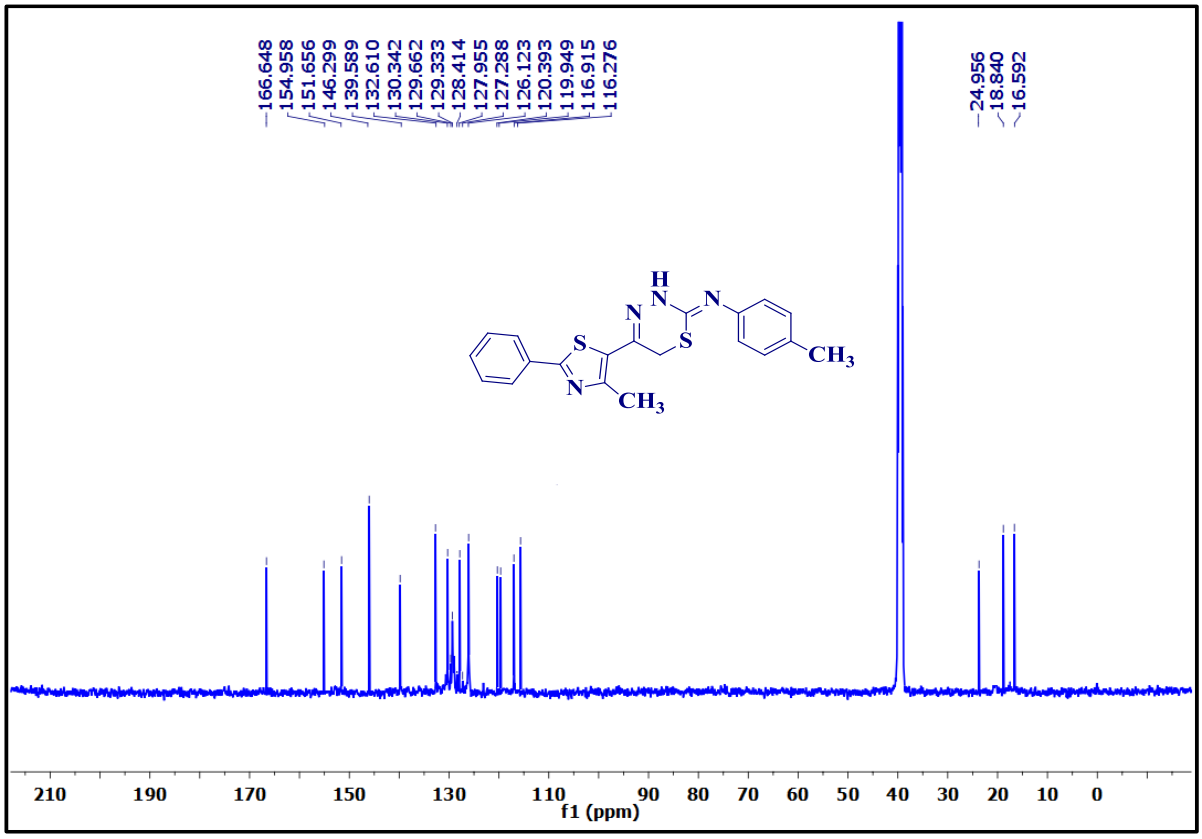


Fig: ^{13}C NMR Spectrum of compound **39e**

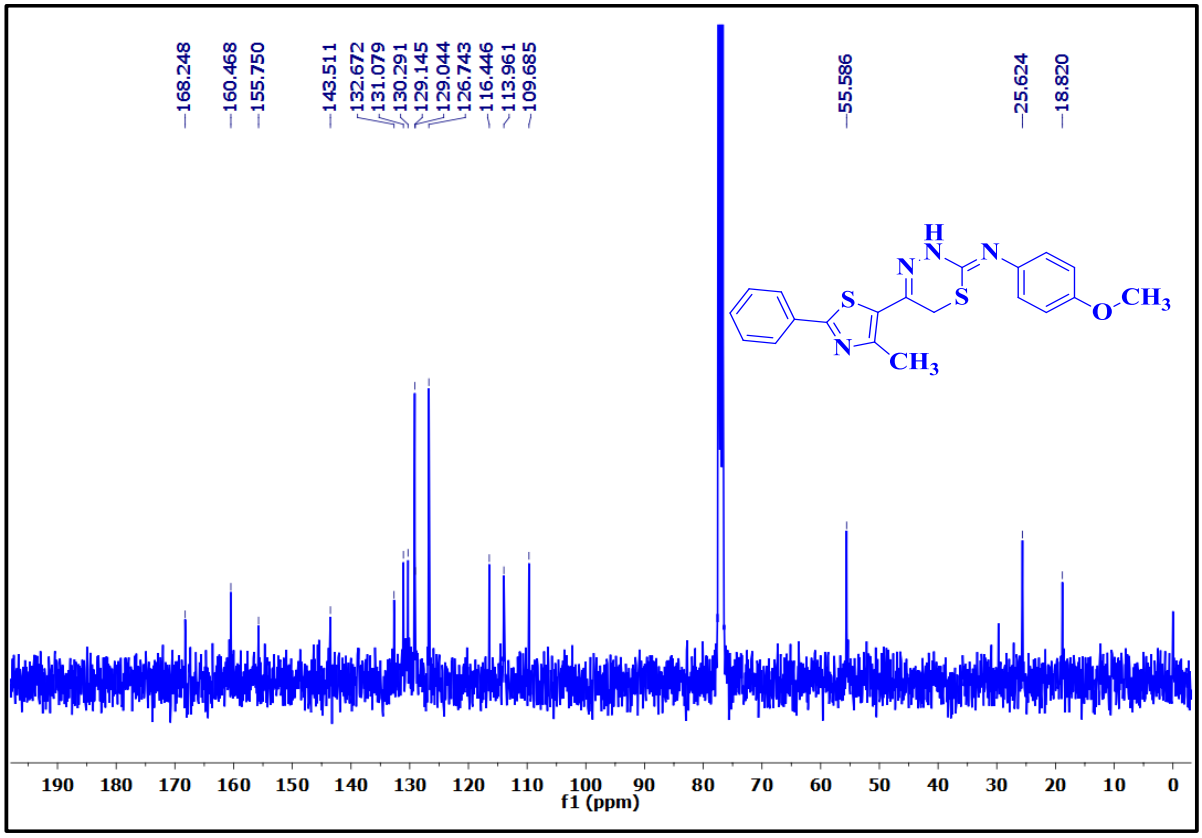


Fig: ^{13}C NMR Spectrum of compound **39f**

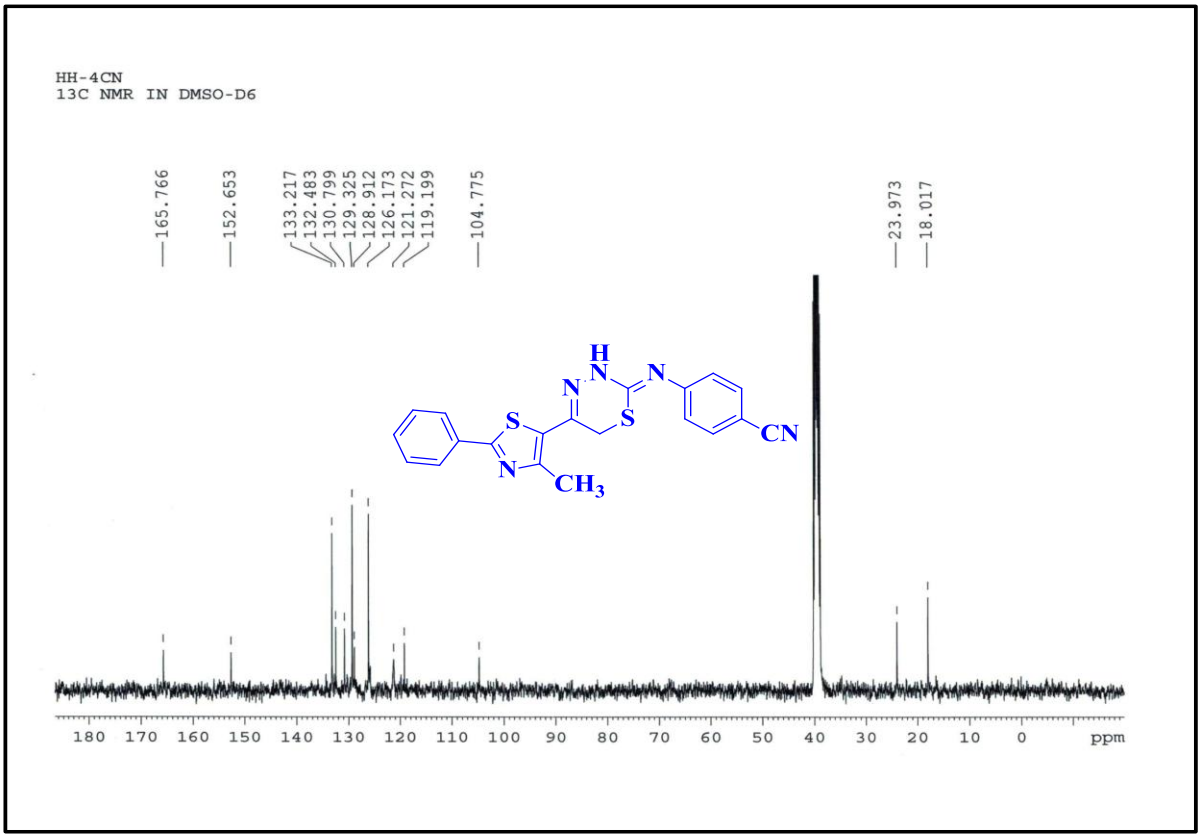


Fig: ^{13}C NMR Spectrum of compound **39g**

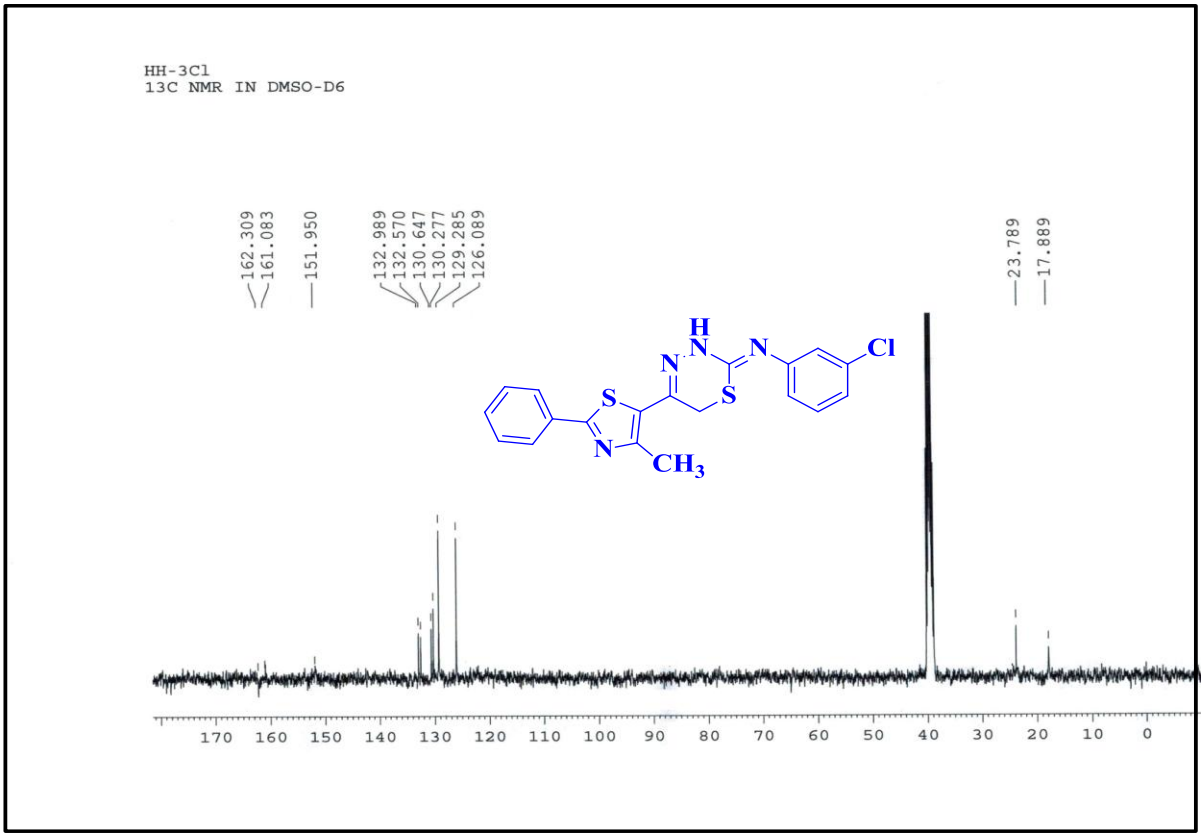


Fig: ^{13}C NMR Spectrum of compound **39i**

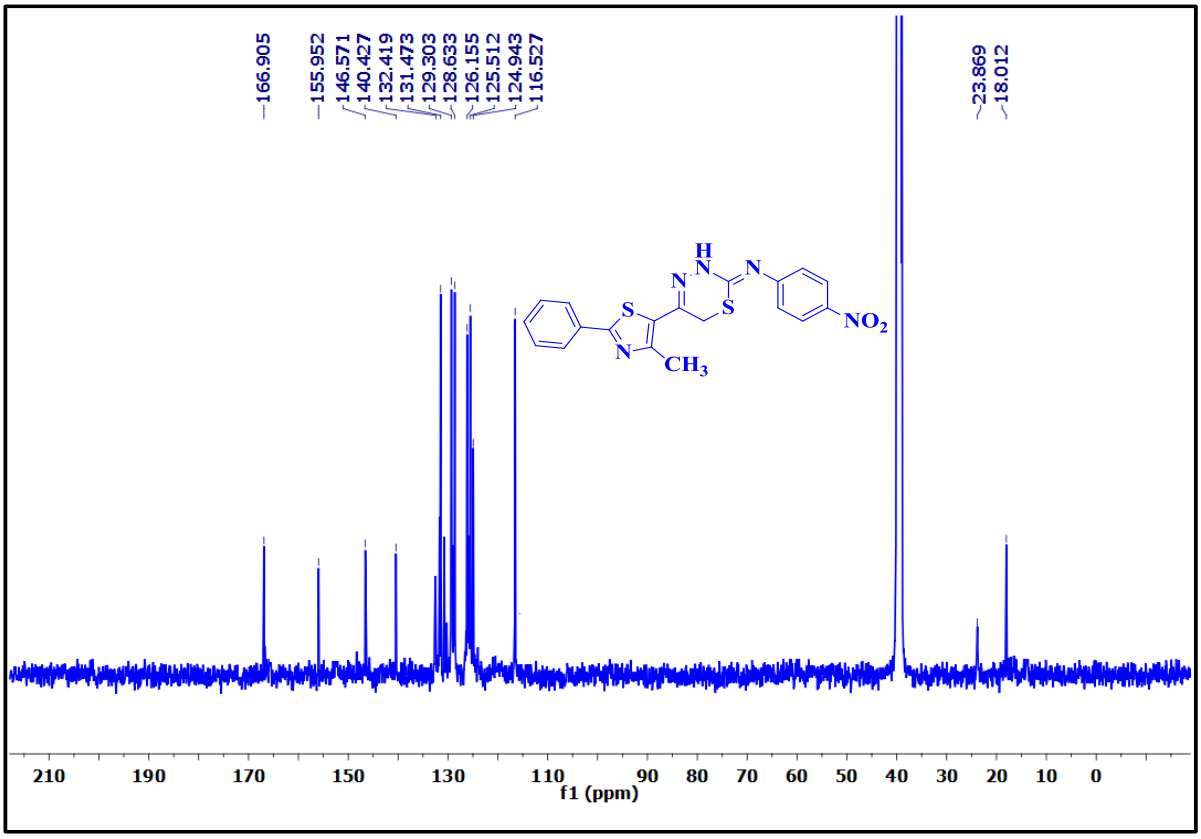


Fig: ^{13}C NMR Spectrum of compound **39j**

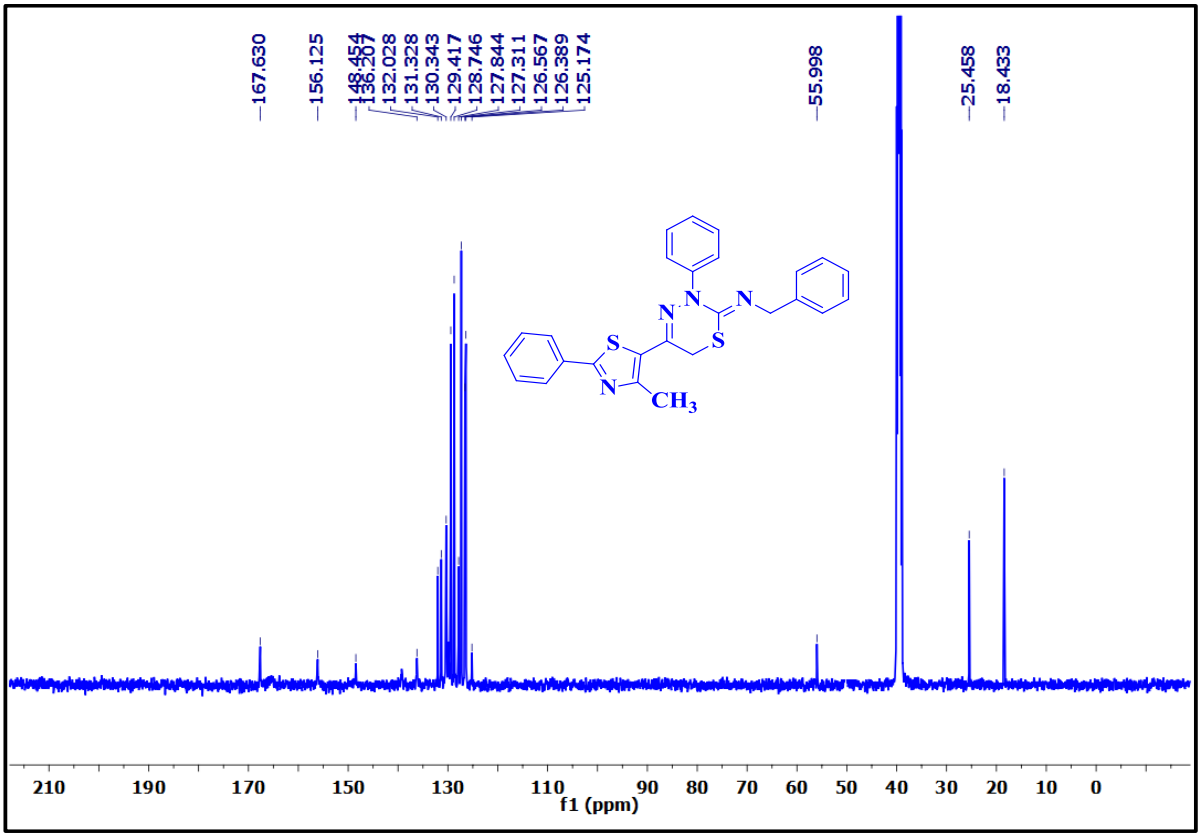


Fig: ^{13}C NMR Spectrum of compound **39k**

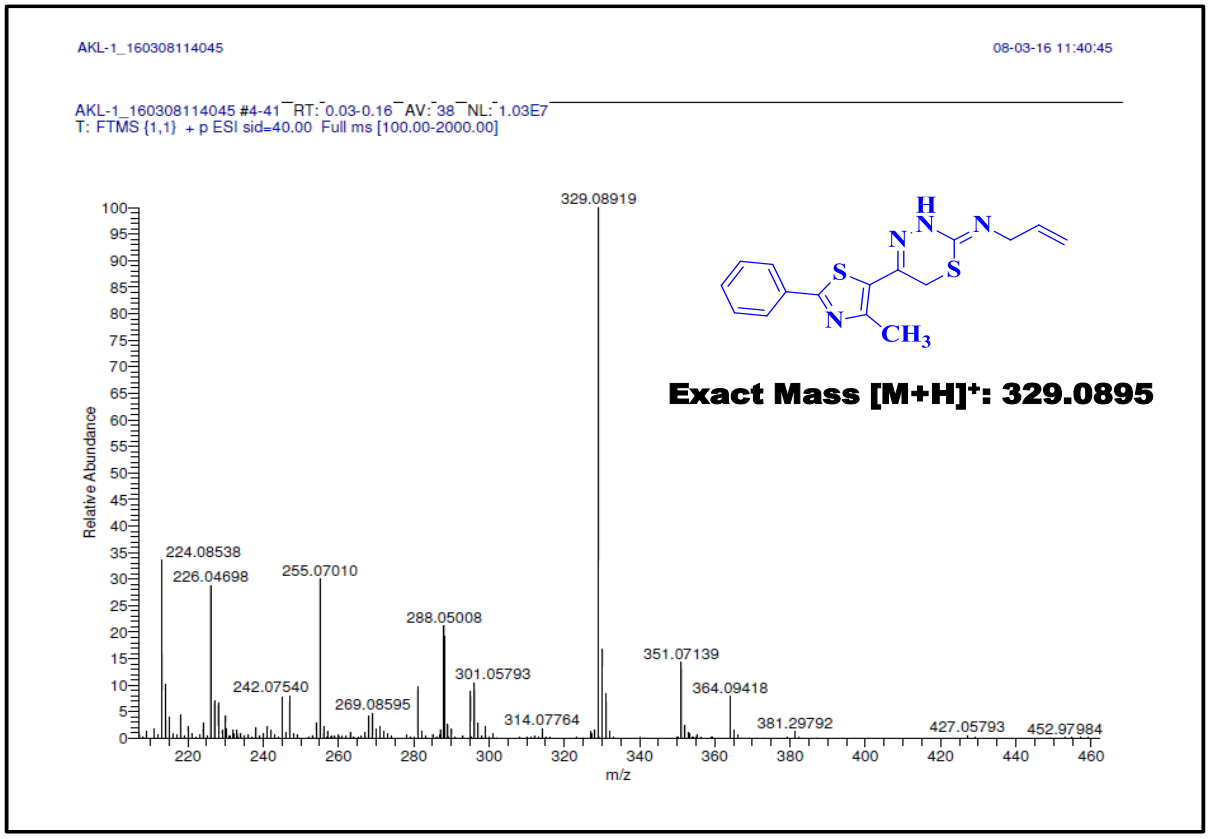


Fig: Mass Spectrum of compound **39a**

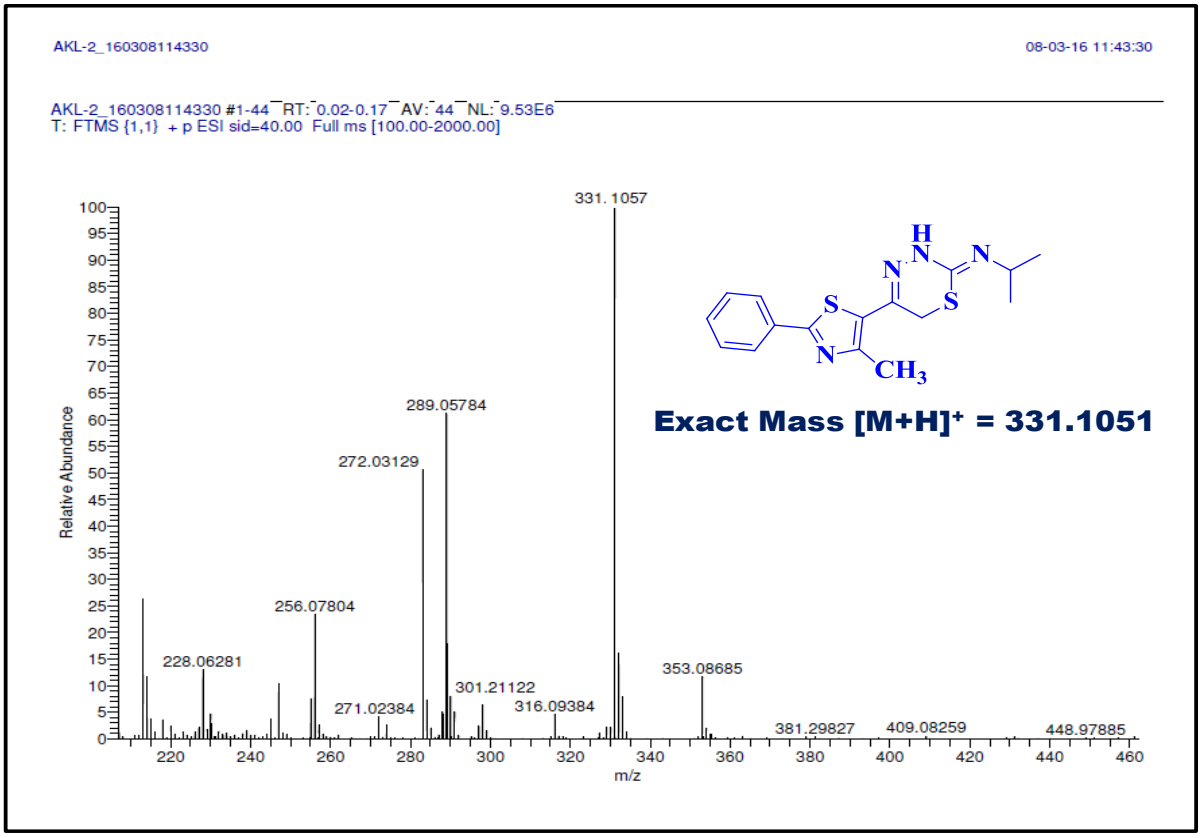


Fig: Mass Spectrum of compound **39b**

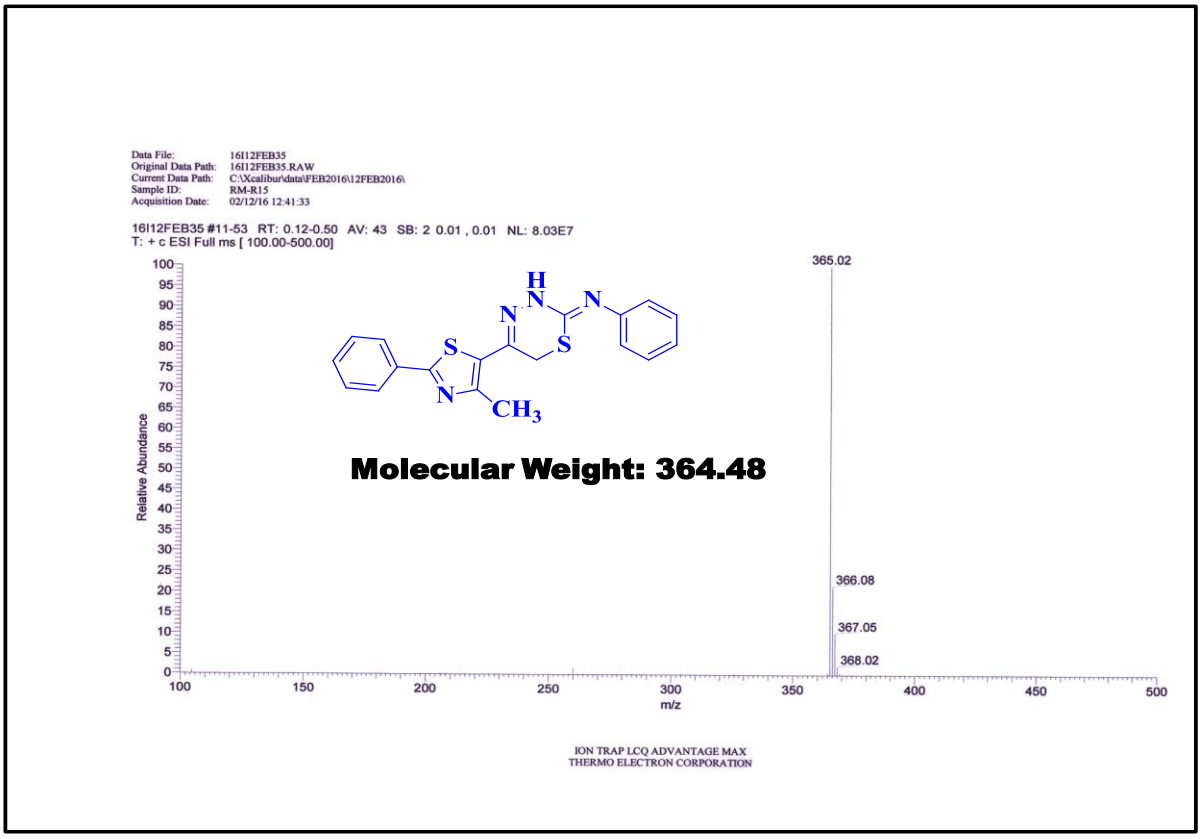


Fig: Mass Spectrum of compound **39c**

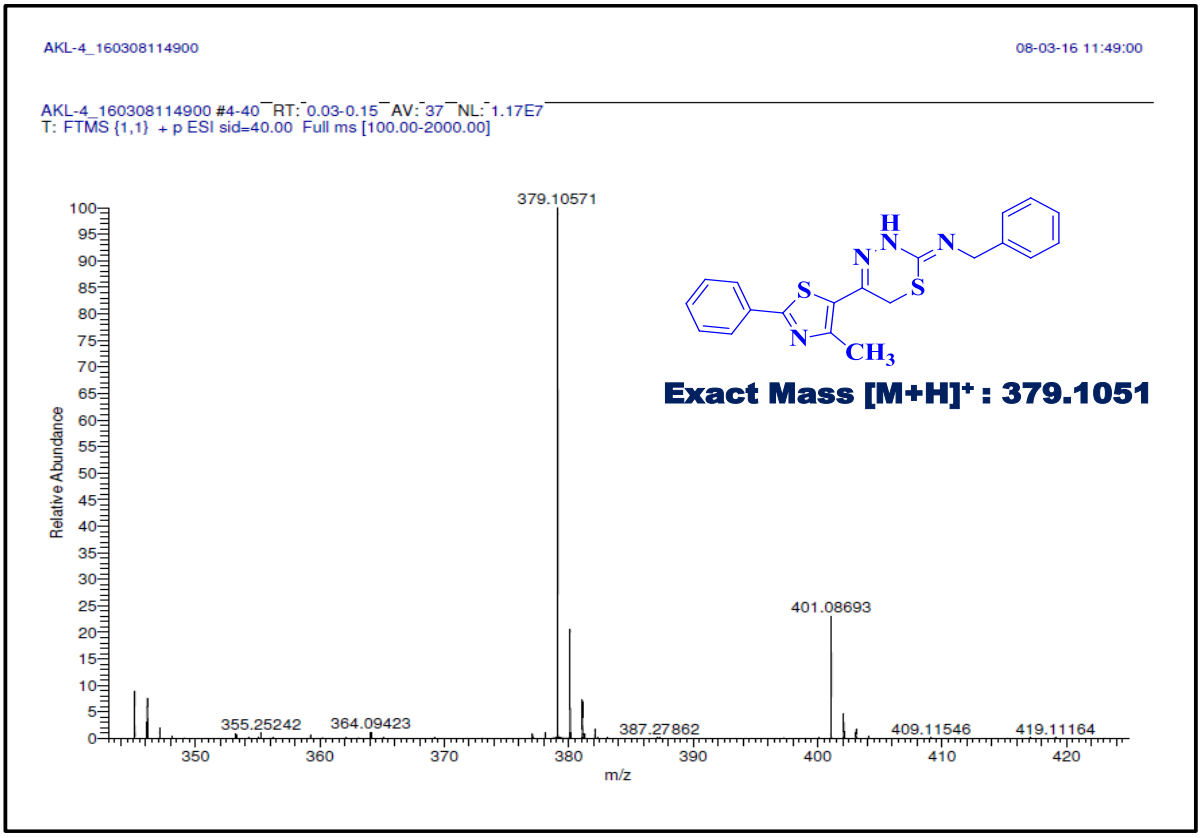


Fig: Mass Spectrum of compound **39d**

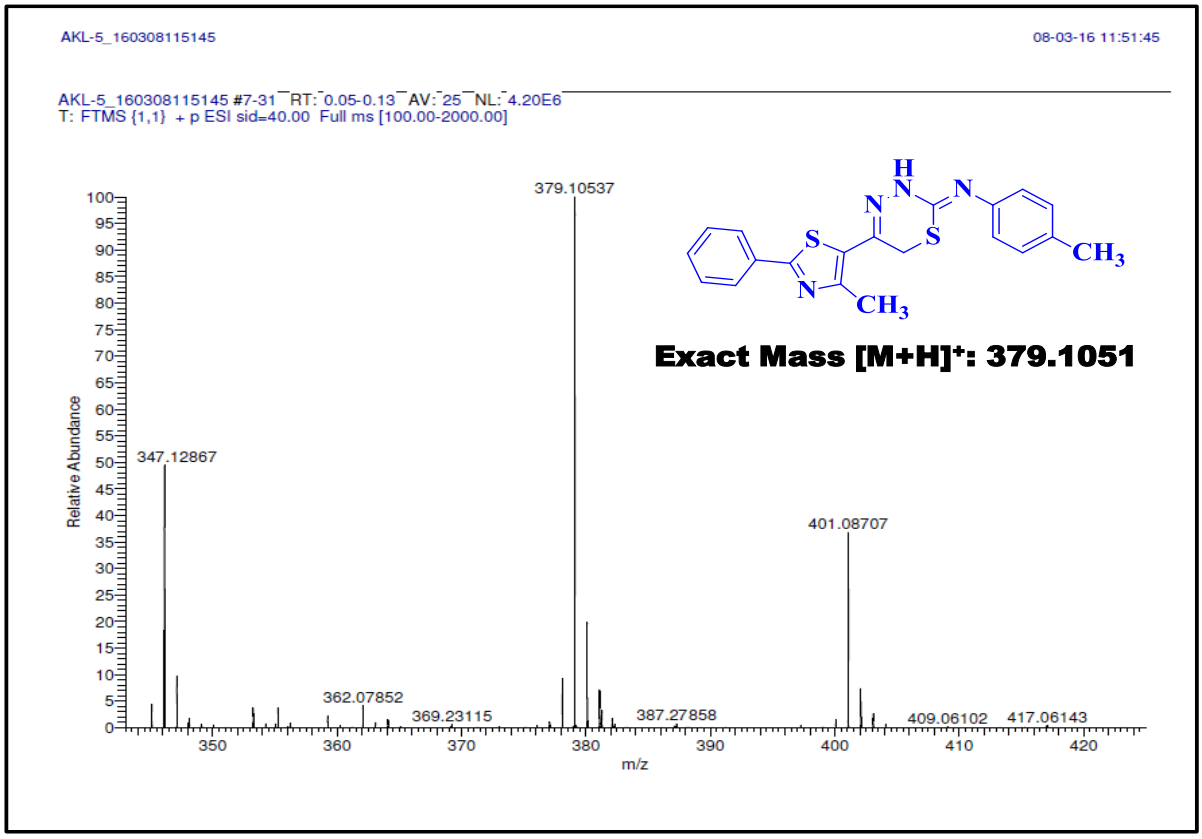


Fig: Mass Spectrum of compound **39e**

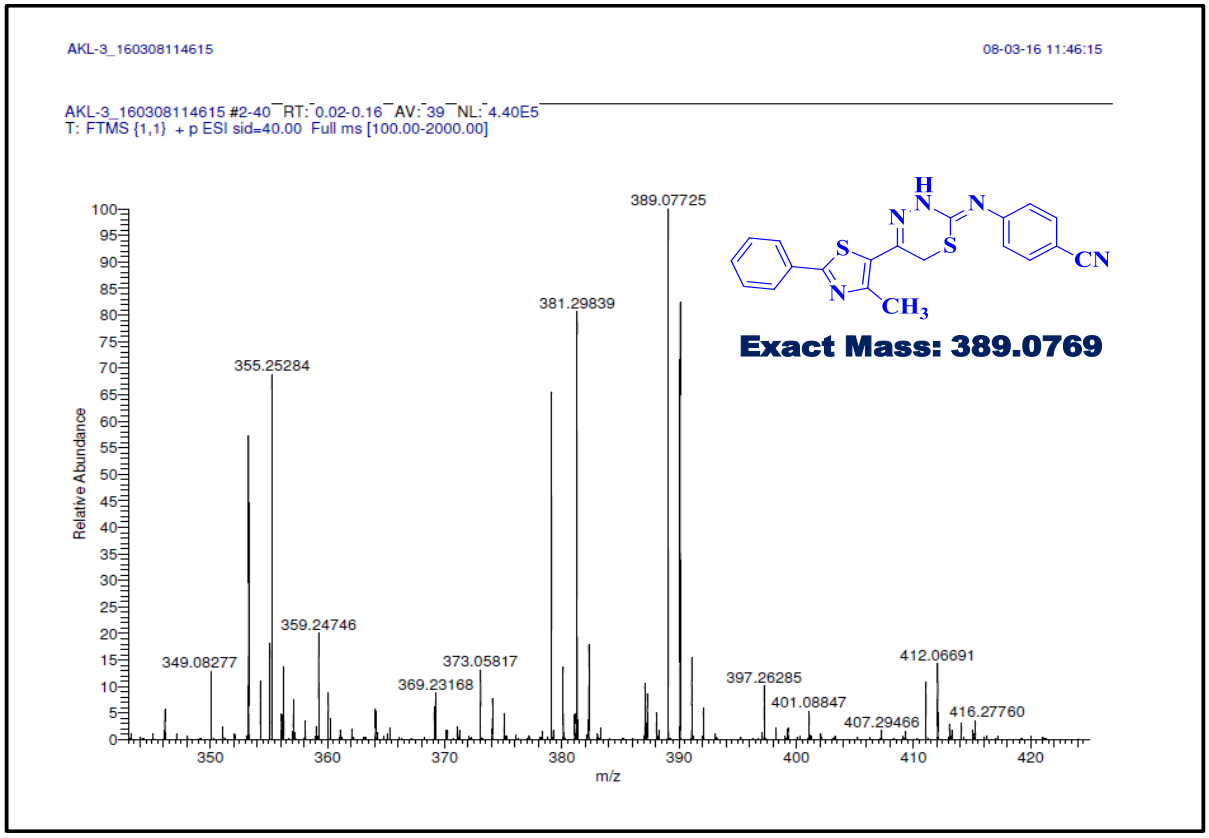


Fig: Mass Spectrum of compound **39g**

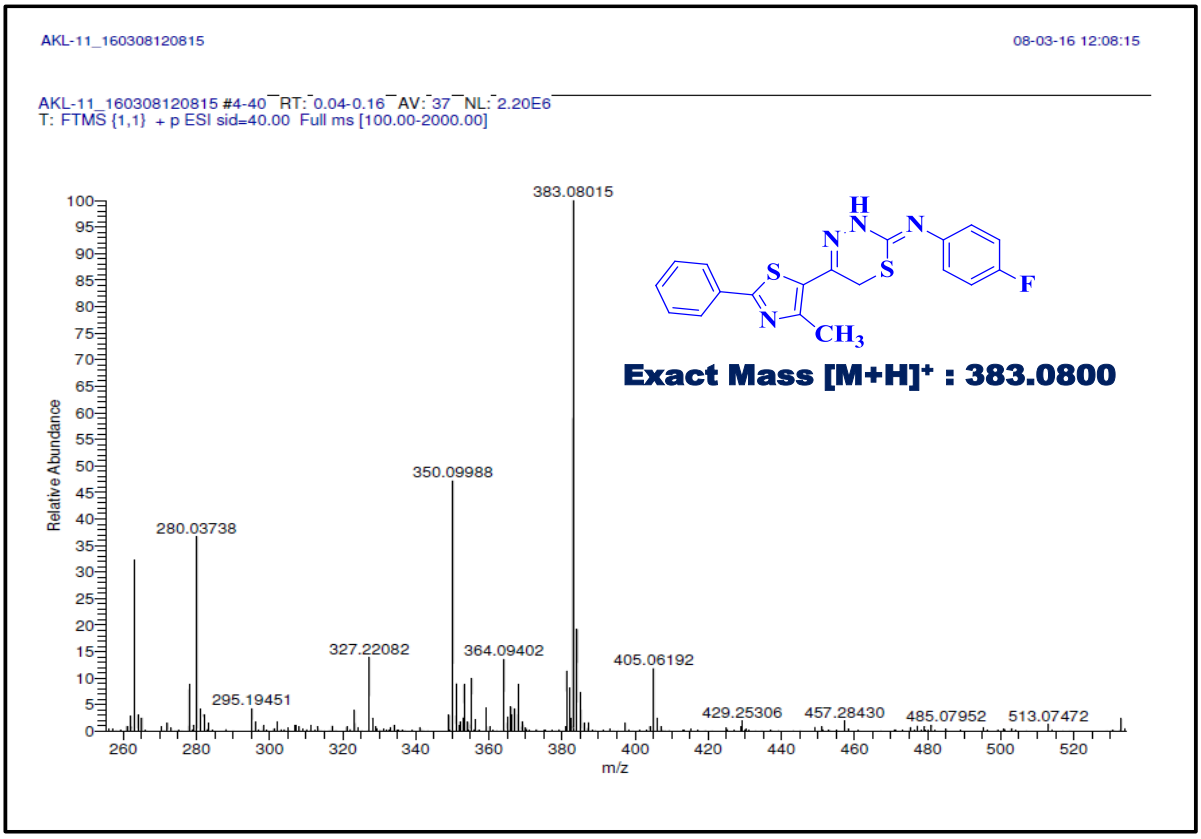


Fig: Mass Spectrum of compound **39h**

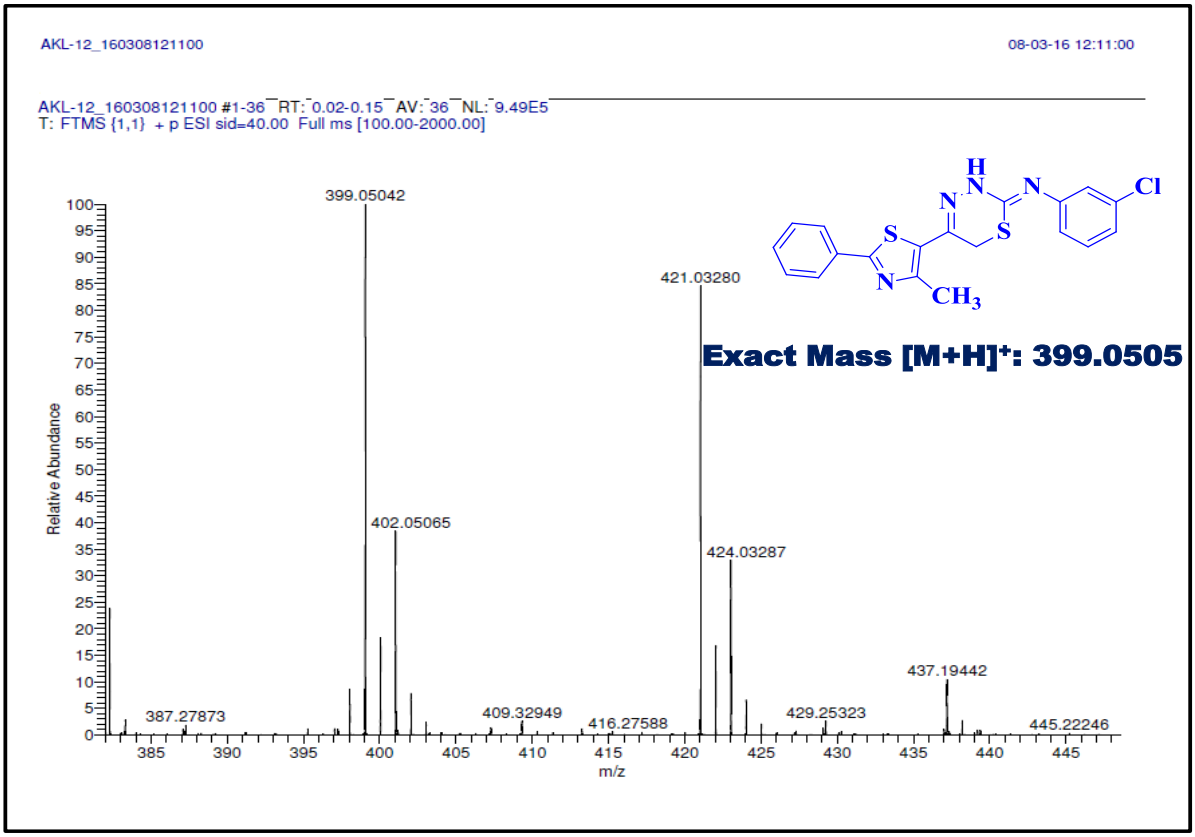


Fig: Mass Spectrum of compound **39i**

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