SYNTHESIS OF NEW NITROGEN AND SULPHUR HETEROCYCLIC COMPOUNDS USING MULTICOMPONENT APPROACH AND STUDIES ON THEIR BIOLOGICAL ACTIVITY

THESIS SUBMITTED TO

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DOCTOR OF PHILOSOPHY

IN CHEMISTRY

BY

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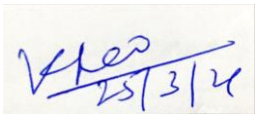
CERTIFICATE

This is to certify that the research work presented in this thesis entitled **"Synthesis of new nitrogen and sulphur heterocyclic compounds using multicomponent approach and studies on their biological activity"** submitted by **Mr. Mamidala Srikanth** for the award of the degree of **Doctor of Philosophy in Chemistry**, National Institute of Technology, Warangal (Telangana), under my guidance and supervision. This work has not been submitted earlier either in part or in full for any degree or diploma to this or any other university.



(**Prof. V. Rajeswar Rao)**

DECLARATION

I hereby declare that the research work presented in this thesis entitled **"Synthesis of new nitrogen and sulphur heterocyclic compounds using multicomponent approach and studies on their biological activity"** has been carried out by me under the supervision of **Prof. V. Rajeswar Rao**, Department of Chemistry, National Institute of Technology-Warangal. I declare that this work is original and has not been submitted in part or full, for any degree or diploma to this or any other university.

Date: 26-03-2021

Place: Warangal

(Srikanth Mamidala)

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(Srikanth Mamidala)

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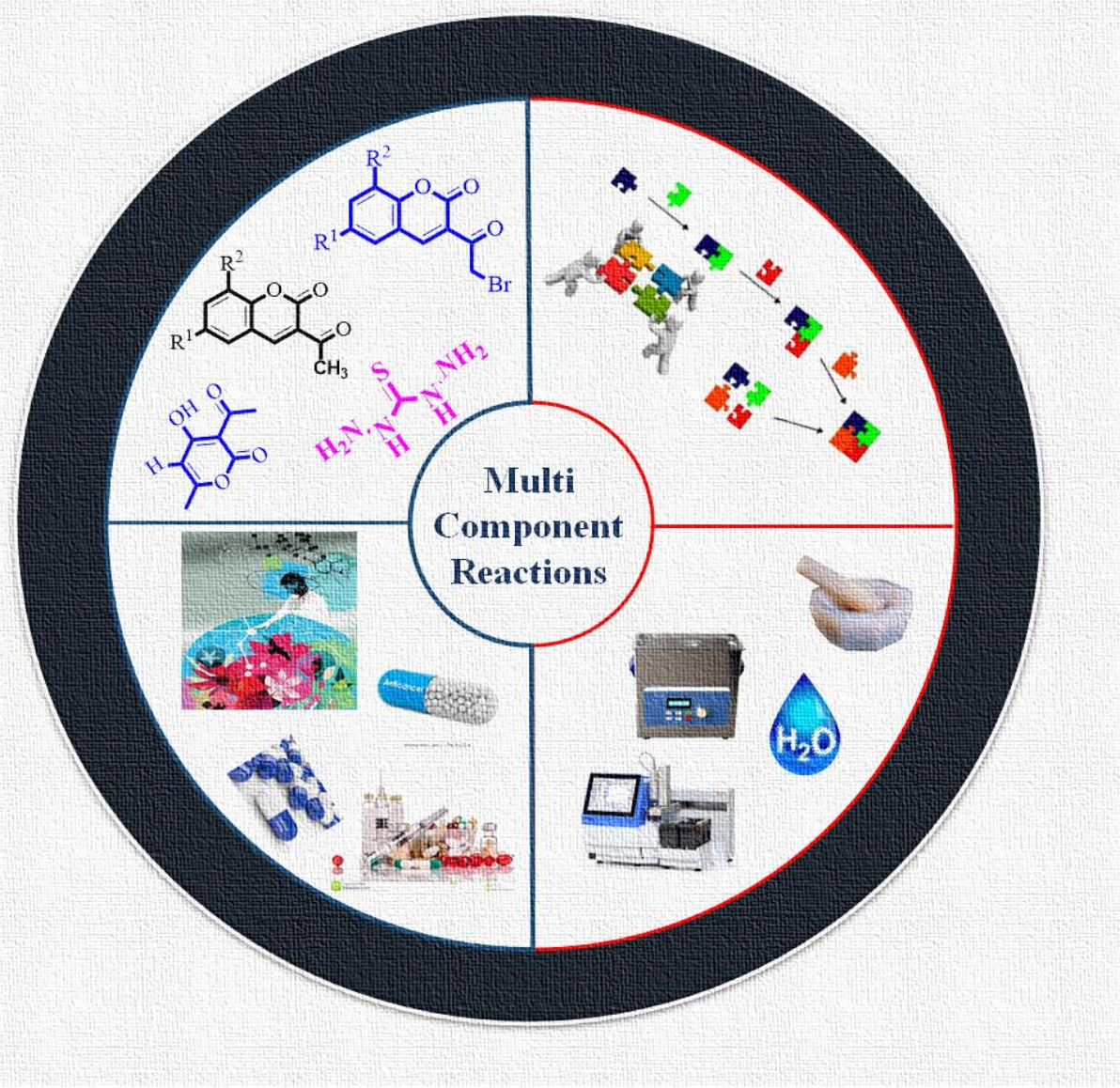


CHAPTER-I

1. Introduction:

The main theme of green chemistry[1,2] is to minimize the production of toxic and hazardous substances during the synthesis and designing synthetic protocols in the direction of low risk to nature. This insists the need of exploring green and novel perspectives towards the synthesis of pharmacologically active polyheterocyclic analogues, which are yet confronting in modern drug exploration and evolution programs. This can be executed through proper choice of safer chemicals in designing renewable raw materials, atom economic procedures with less number of chemical steps, usage of green solvents and development of simple workup and purification techniques[3].

1.1. Multi component reactions:

Multi component reactions[4–6] are eco-friendly methodologies. They are powerful synthetic organic reactions in which readily available starting materials having three or more components are amalgamated in a step to fabricate products that assimilate substantial fragments of all the reactants. Multi component reactions provide a fascinating strategy to accomplish structurally divergent derivatives of chemical and medicinal interest. The particular arrangement of different bonds in a single viable step provides a challenge, a profound perspective for enhancing atom economy, eco-friendly, comparatively mild reaction procedures, faster reaction rates and lower reaction times with high yields. Nowadays MCRs have been widely used in the area of organic synthesis[7], medicinal chemistry[8–10], natural product synthesis[11,12], polymer chemistry[13–17], agro chemistry[18,19], and combinatorial chemistry[20,21].

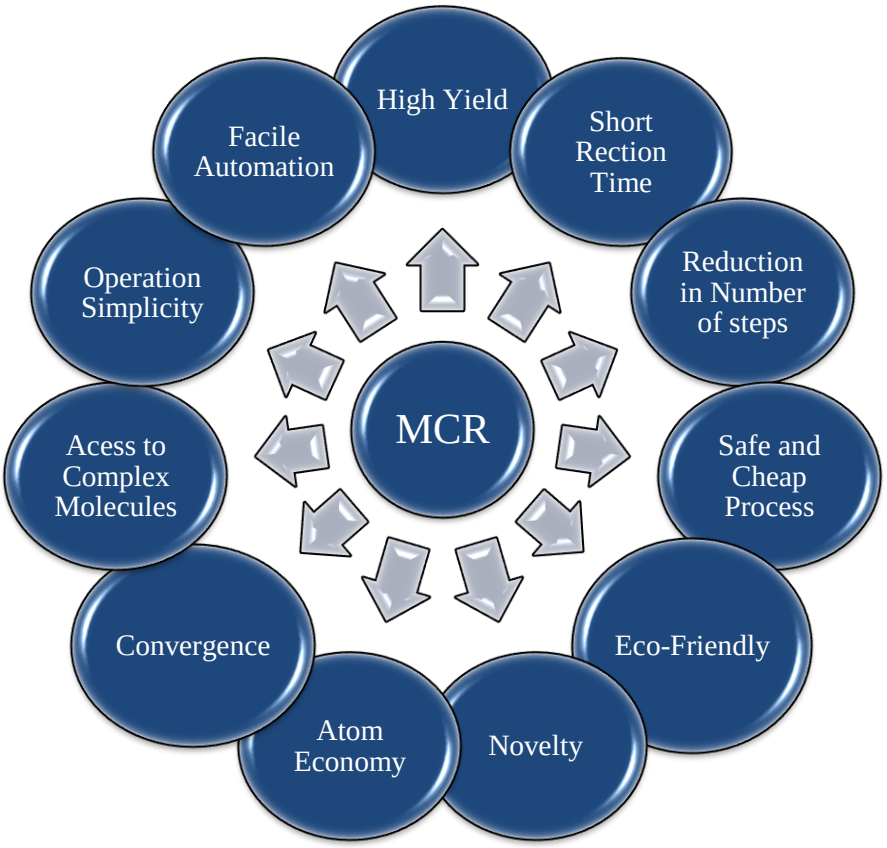
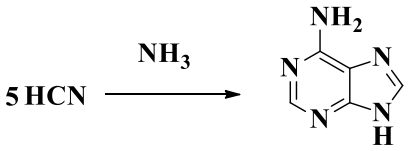


Figure 1.1

1.1.1. History of multi component reactions:

Multi component reaction itself occurs in nature in the evolution procedure[22]. RNA and DNA purine bases i.e., adenines are formed by multi component reaction approach *via* condensation reaction of HCN, which is catalyzed ammonia (Scheme 1.1).



Scheme 1.1

The following reactions are some examples of the principal multi component reactions based on named organic reactions.

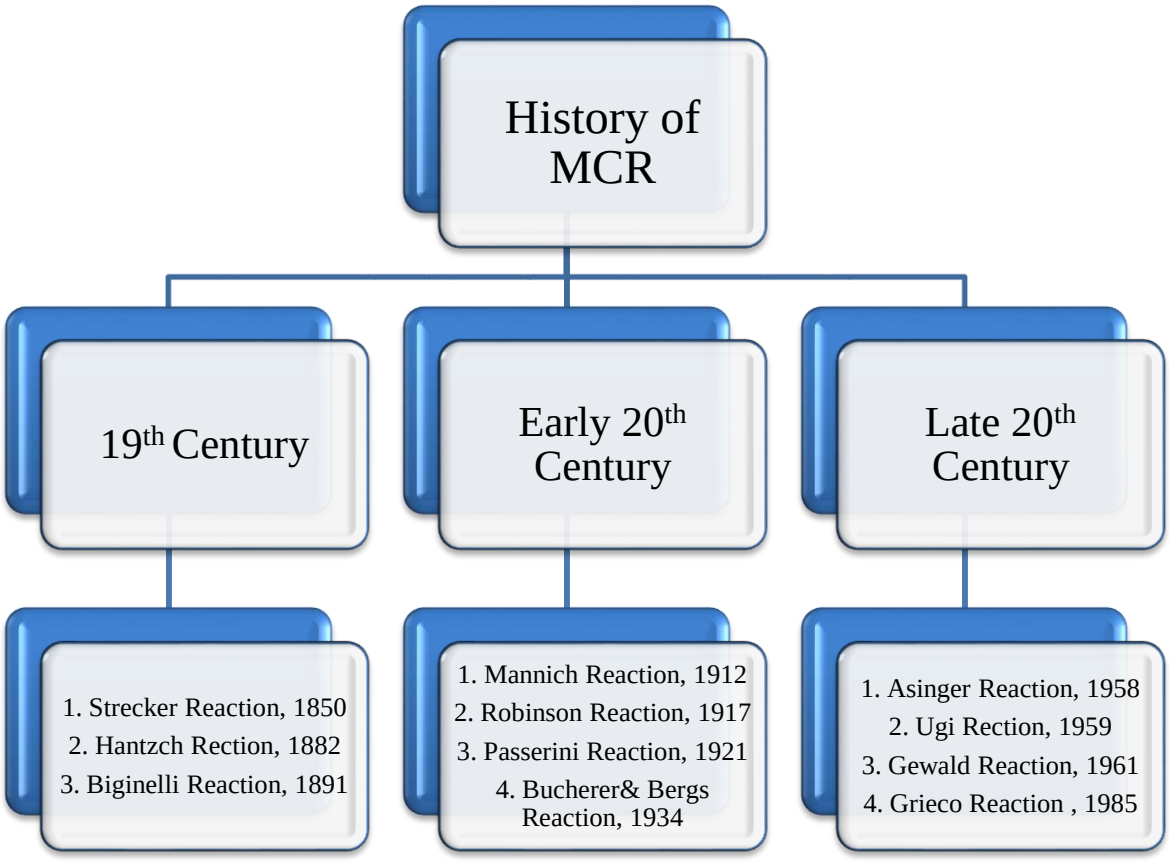


Figure 1.2

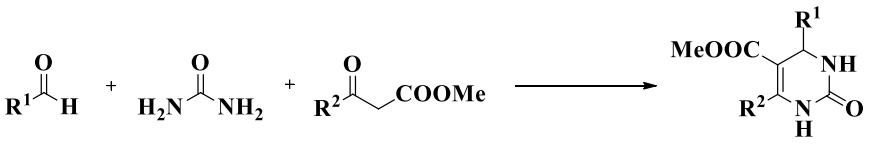
Strecker's synthesis:

The first report on multi component reactions was disclosed by Strecker[23] in 1850. Strecker synthesized α – amino nitriles *via* a one-pot three component reaction by using ammonia, aldehyde, hydrogen cyanide as starting materials (Scheme 1.2).

Scheme 1.2

Biginelli reaction:

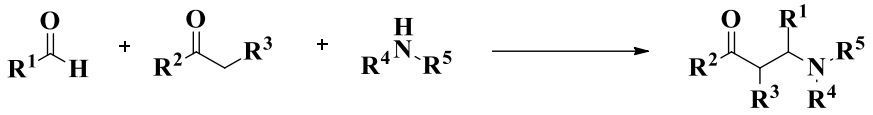
An Italian chemist, P. Biginelli[24] established a multi component reaction for the synthesis of dihydropyrimidines. the dihydropyrimidines analogues were reported by three component reaction of urea, aldehydes and β–keto esters in presence of ethanol under reflux condition under acid catalyst (Scheme 1.3).



Scheme 1.3

Mannich reaction:

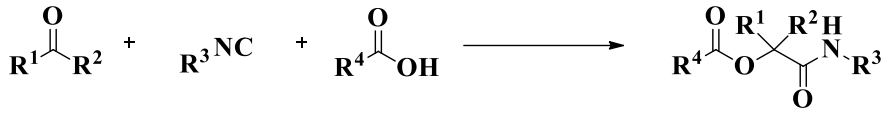
Carl Mannich[25] established a multi component condensation reaction of aldehyde, amine with carbonyl compounds having active methylene group to generate the corresponding analogues (Scheme 1.4).



Scheme 1.4

Passerini reaction:

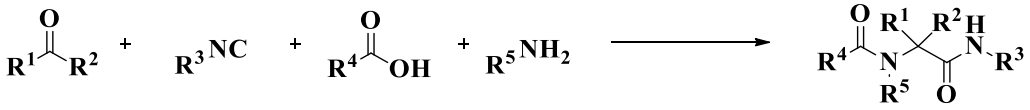
Passerini[26] established first isocyanides based one pot multi component reaction. He reported the synthesis of α-Acyloxy carbamides by reaction of isocyanide, carbonyl compound and carboxylic acid (Scheme 1.5).



Scheme 1.5

Ugi reaction:

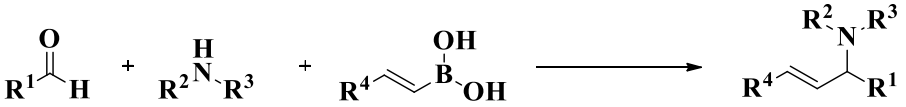
Ivar Karl Ugi[27] established four component reactions. Ugi reported the synthesis of α-acylamino amides by condensation reaction of carboxylic acids, isonitriles, ketones and amines (Scheme 1.6). This reaction is enormously described and applied in modern organic synthesis.



Scheme 1.6

Petasis reaction:

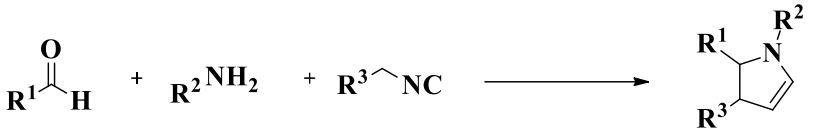
Petasis[28] established one of the three component reaction. Petasis synthesized substituted amines compounds by the condensation of carbonyl compounds amines and vinyl or aryl boronic acids to form substituted amines (Scheme 1.7).



Scheme 1.7

Orru reaction:

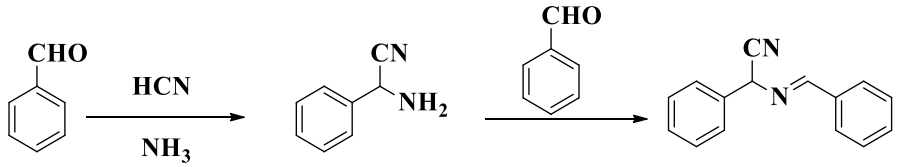
Orru[29] developed one of the most important multi component reactions to synthesize 2-imidazolines by the condensation reaction of amine, an aldehyde and an α-acidic isocyanide (Scheme 1.8).



Scheme 1.8

Laurent and Gerhardt multicomponent reaction:

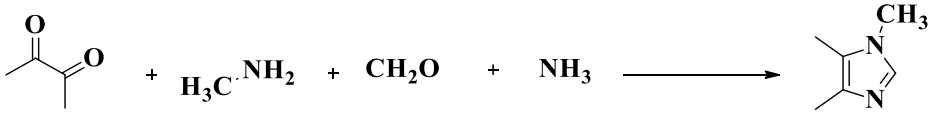
Laurent and Gerhardt[30] disclosed the multi component reaction of "benzoyl azotid". In which benzaldehyde in the presence of hydrogen cyanide and ammonia produced amino benzyl cyanide. Further, the reaction mixture was added by the another mole of benzaldehyde to produce the anils of benzyl cyanide i.e. "benzoyl azotid" (Scheme 1.9).



Scheme 1.9

Radziszewski imidazole synthesis:

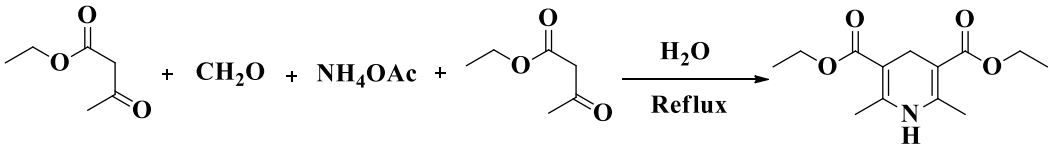
Four component one pot reaction was established to produce substituted imidazoles by Radziszewski[31]. In this reaction, the starting materials 1,2-dicarbonyl compounds, formaldehyde, primary amine and ammonia were used to produce the substituted imidazoles (Scheme 1.10).



Scheme 1.10

Hantzsch dihydropyridine synthesis:

Hantzsch[32] established a four component reaction for the synthesis of substituted 1,4-dihydropyridines. Cyclo condensation reaction among ethylacetoacetate, ammonium acetate and aldehyde gave dihydropyridines (Scheme 1.11).

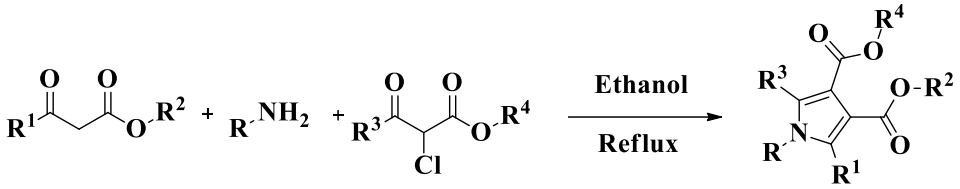


Scheme 1.11

Hantzsch pyrrole synthesis:

A one pot three component reaction was established by Hantzsch[33] for the synthesis of

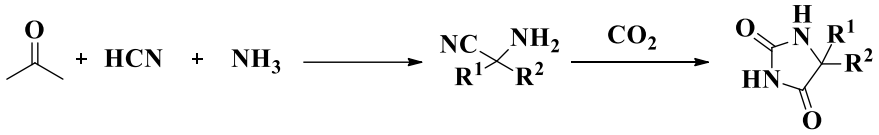
pyrroles. In this reaction the synthesis of pyrroles are reported by reaction of β–keto esters with α-halo β – keto esters and primary amines (Scheme 1.12).



Scheme 1.12

Bucherer Berg's hydantoin synthesis:

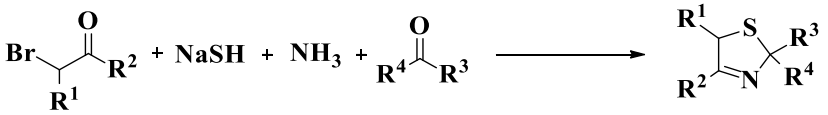
Bucherer and Bergs[34] developed a four component reaction approach for the synthesis of hydantoin. This reaction comprises the generation of hydantoin *via* cyclo condensation of carbonyl compounds, hydrogen cyanide, ammonia and carbon dioxide (Scheme 1.13).



Scheme 1.13

Asinger reaction:

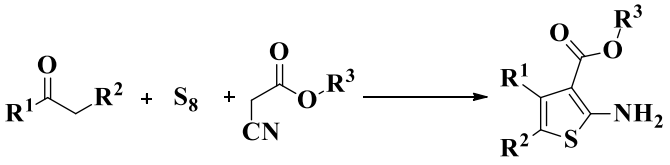
Asinger[35] established the synthesis of thiazolines by using a multicomponent reaction approach. Here, thiazolines are formed from the reaction of alpha halogenated carbonyl compounds, ammonia and sodium hydrosulphide (Scheme 1.14).



Scheme 1.14

Gewald reaction:

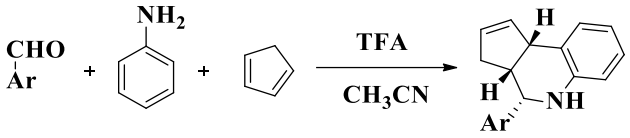
Karl Gewald[36] established an efficient three component reaction approach for the synthesis of poly substituted 2- amino thiophenes. These compounds were synthesized by reaction of α-methylene carbonyl compounds, elemental sulphur, α-cyano esters in presence of base (Scheme 1.15).



Scheme 1.15

Grieco synthesis (three component) of tetrahydro quinolines:

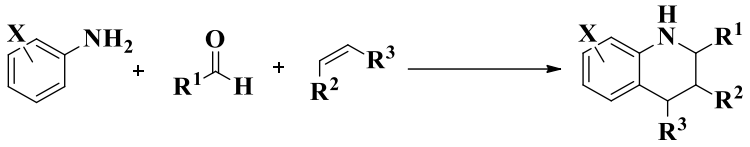
Grieco[37] diclosed the synthesis of tetrahydro quinolines via a three component reaction. These compounds were generated by cyclo addition reaction of amines, aldehydes and cyclopentadiene in presence of trifluroacetic acid (Scheme 1.16).



Scheme 1.16

Povarov three component reaction:

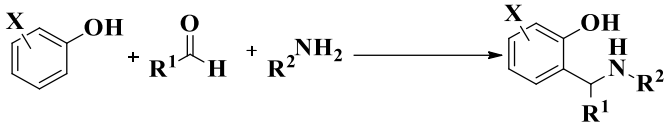
Povarov[38] disclosed a three component reaction for the synthesis of quinolone. At first aniline and benzaldehyde undergoes condensation reaction, the intermediate reacts with lewis acid like boron trifluoride and then it is reacted with aromatic ring to form target compound (Scheme 1.17).



Scheme 1.17

Betti three component reaction:

Betti[39] reported the three component reaction for the synthesis of α-aminobenzylphenols. In which primary aromatic amines and aldehydes form imines, the intermediate react with phenol to form target compounds (Scheme 1.18).



Scheme 1.18

1.1.2. Classifications of multi component reactions:

Multi component reactions can be classified in to several types as follows

1. Based on the reactants involved in the synthesis they are classified as three component reactions, four component reactions, five component reactions, etc.

2. Based on the functional groups present on the reactants as following.

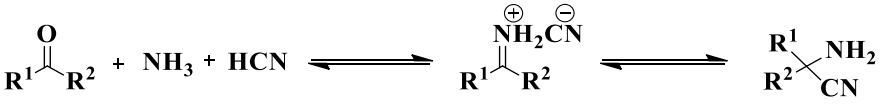
i. Imine based multi component reactions

ii. Isocyanide based multi component reactions

Several multi component reactions were known to be pertained to the first type of classification. In this type of reactions, the products containing imine functional group were produced by the condensation of carbonyl functional group compounds with amines. Whereas, isocyanide is the starting material for the reactions involved in isocyanide based multi component reactions.

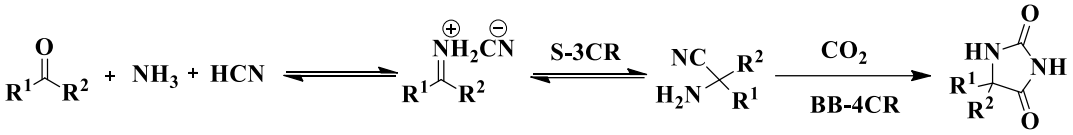
3. Based on the reaction kinetic paths i.e. reversible or irreversible, based on reactivity and isolability of the products.

Type-I: In this type, starting materials used for the reaction, reaction intermediates and the final products are in equilibrium with each other. These reactions are thermodynamically controlled reaction because the yield and isolability of the reaction depends on the thermodynamics of the reaction (Scheme 1.19).



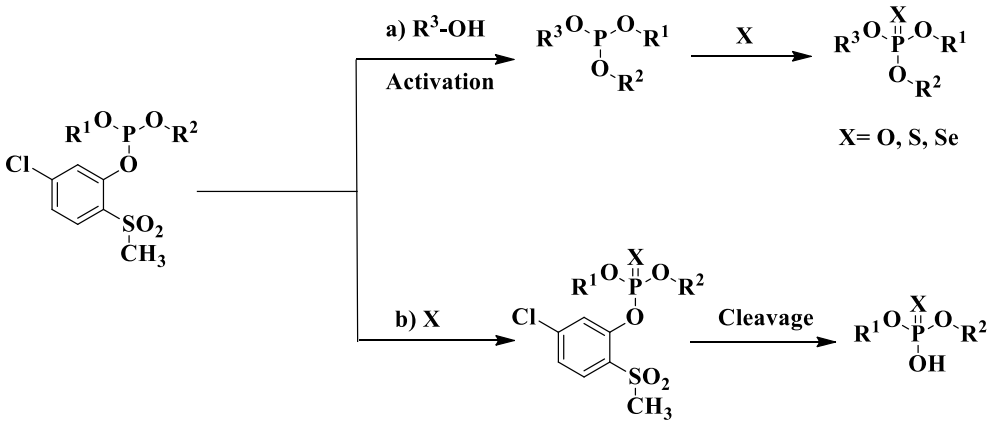
Scheme 1.19

Type-II: In these reactions, equilibrium exists among substrates and reaction intermediates. The reaction is terminated by irreversible step among the intermediate and the product (Scheme 1.20).



Scheme 1.20

Type-III: In these types of reactions one product is formed by all sub reactions involving irreversible steps (Scheme 1.21).



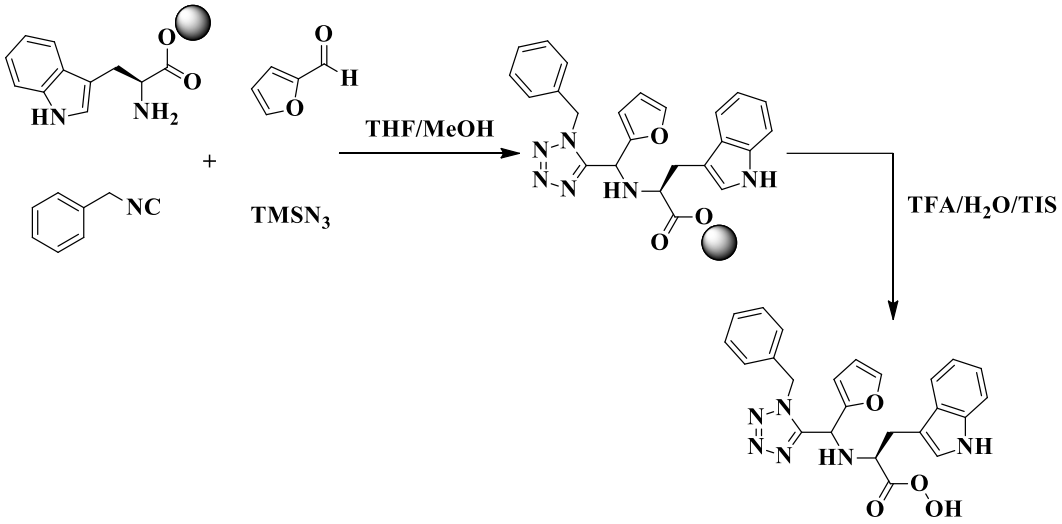
Scheme 1.21

1.1.3. Different approaches in MCRs:

There are different approaches developed other than normal conventional methods during the course of time to minimize the time, solve purification problems, improve yields, develop easier work up techniques etc.

Solid Phase MCRs:

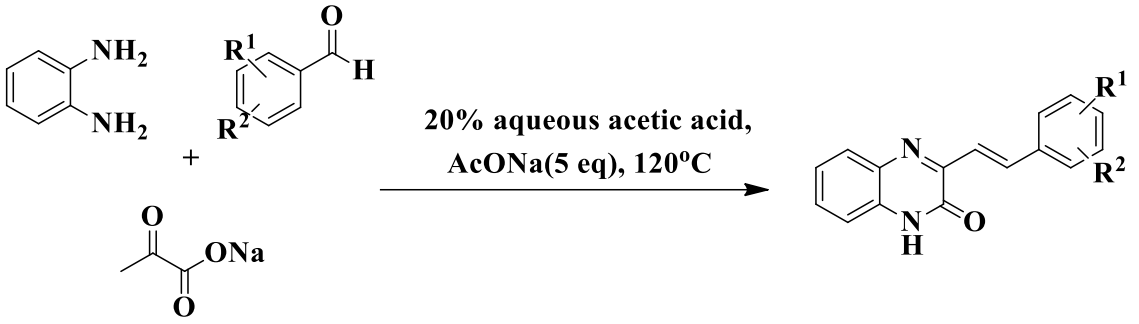
Multi component reactions like Ugi reaction and Biginelli condensation reactions are primarly useful for the generation of different chemical libraries on the solid phase. Four component condensation reactions have been reported recently by Yanira Mendez[40] for the synthesis of small ring heterocycles by solid phase synthesis. For instance, Ugi-azide-4CR one pot reaction of an amine, aldehyde, followed by the addition of isocyanide and a trimethylsilyl azide provides a tetrazole-peptidomimetics (Scheme 1.22). It can serve as inhibitors of the *Escherichia coli* M1-aminopeptidase.



Scheme 1.22

Aqueous medium MCRs:

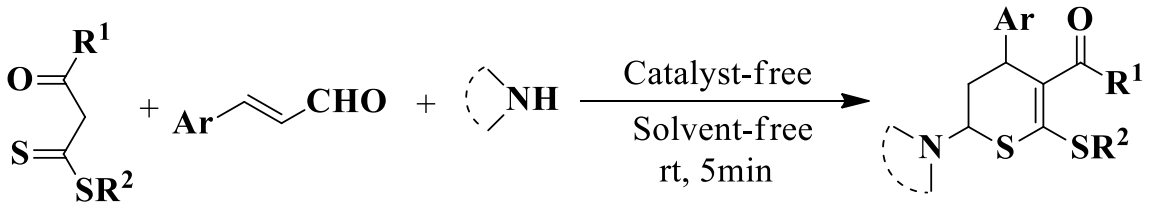
F. G. Menezes[41] *et al.* developed an efficient synthetic method for the synthesis of SQXO derivatives and it is described in a sequential reaction (aqueous mediated multi component reaction) of *o*-phenylenediamine, aldehydes and sodium pyruvate in 20% aqueous acetic acid containing sodium acetate provided the target products in good yields (Scheme 1.23).



Scheme 1.23

Solvent free MCRs:

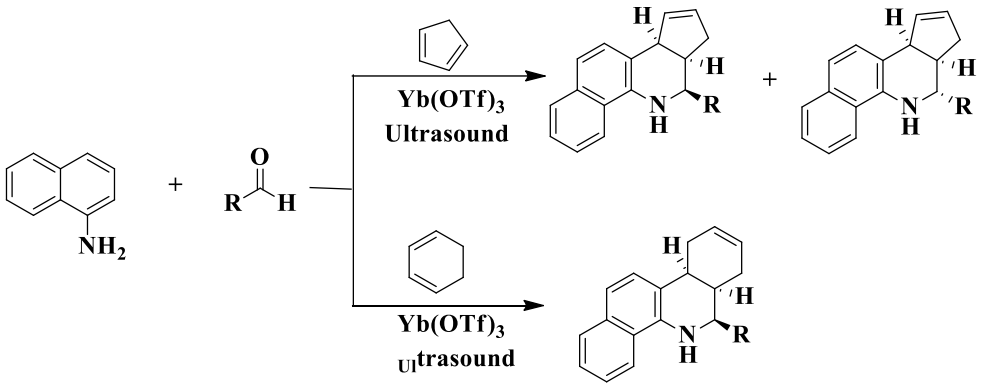
M. S. Singh[42] group developed a highly regioselective method to synthesize the 5,6-dihydro-4*H*-thiopyrans through one pot three component domino coupling of *α*,*β*-unsaturated aldehydes, *β*-oxodithioesters and cyclic aliphatic secondary amines at room temperature under solvent-free and catalyst-free conditions in good yields (Scheme 1.24).



Scheme 1.24

Ultrasonic MCRs:

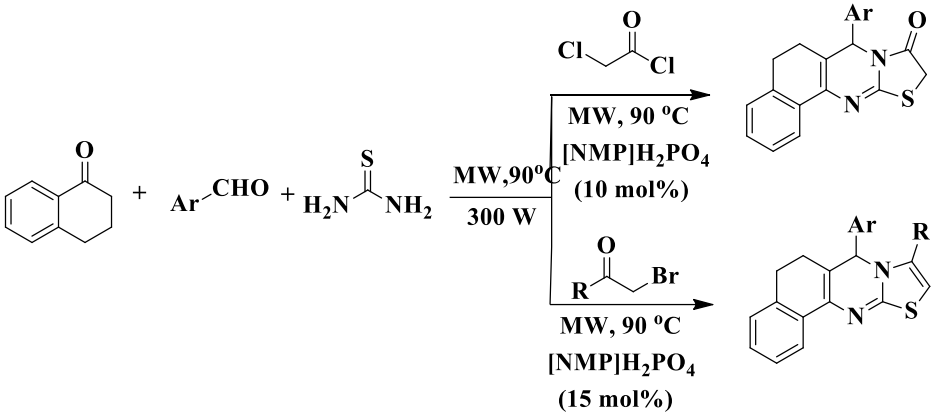
E. Pelit[43] and co-worker developed an efficient approach for the synthesis of hexahydrophenathridines and tetrahydroquinolines through a three component one pot aza-Diels-Alder (Povarov) reaction of 1-naphthylamine, aromatic aldehydes and cyclic dienes using Ytterbium (III) trifluoromethanesulfonate ($Yb(OTf)_3$) as a catalyst under ultrasonic conditions (Scheme 1.25).



Scheme 1.25

Microwave MCRs:

B. Rajitha[44] *et al*. synthesized a new series of microwave assisted one pot, two step reaction thiazolo[2,3-b]quinazoline and thiazolo[2,3-b]quinazolinone analogues in quantitative yield in the presence of acidic task-specific ionic liquid [NMP]H_2PO_4 (Scheme 1.26).



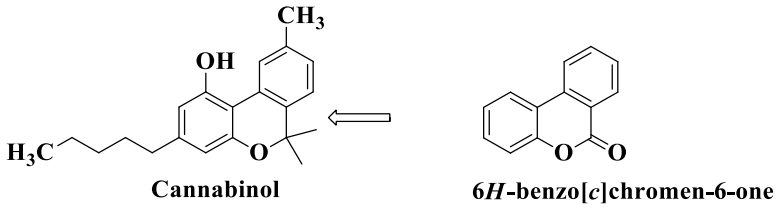
Scheme 1.26

1.1.4. Applications of multi component reactions:

Applications in natural product synthesis:

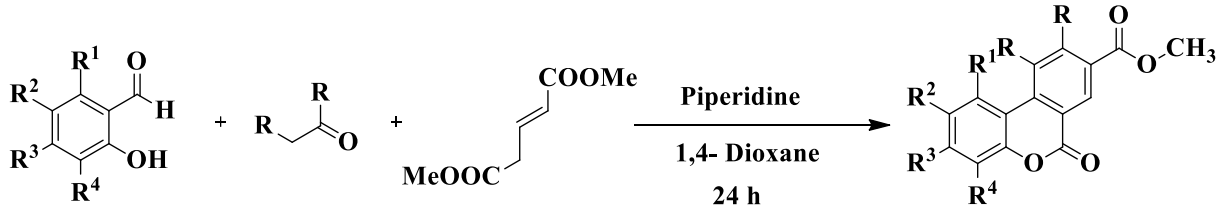
Different naturally occurred products were widely used in medicinal chemistry. Because of their limited availability, it is important to synthesis these natural products with quantitative. In this regard, multi component reactions play a vital role in the synthesis of natural products. The following are some instances of the role of MCRs in the synthesis of natural products.

The cannabinols are the natural products that are used as CNS (central nervous system) G-protein agonists. In their synthesis, 6*H*-dibenzo[*b*,*d*]pyranone is recognized as one of the intermediates. G. J. Bodwell[45] and co-worker established an efficient multi component reaction approach for the synthesis of 6*H*-dibenzo[*b*,*d*]pyranone (Scheme 1.27).



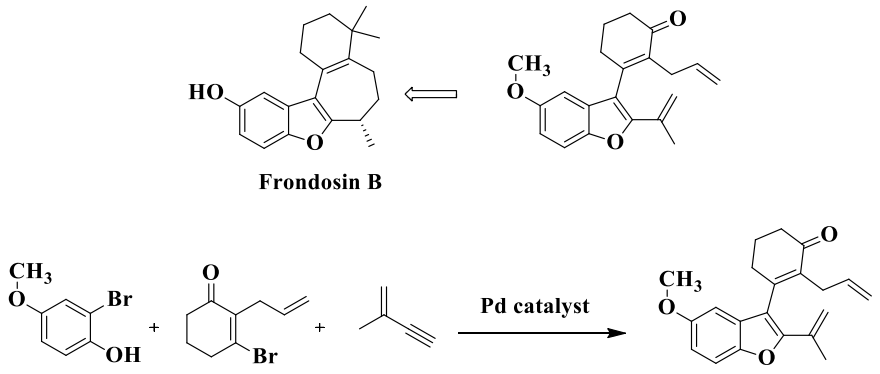
Cannabinol

6*H*-benzo[*c*]chromen-6-one



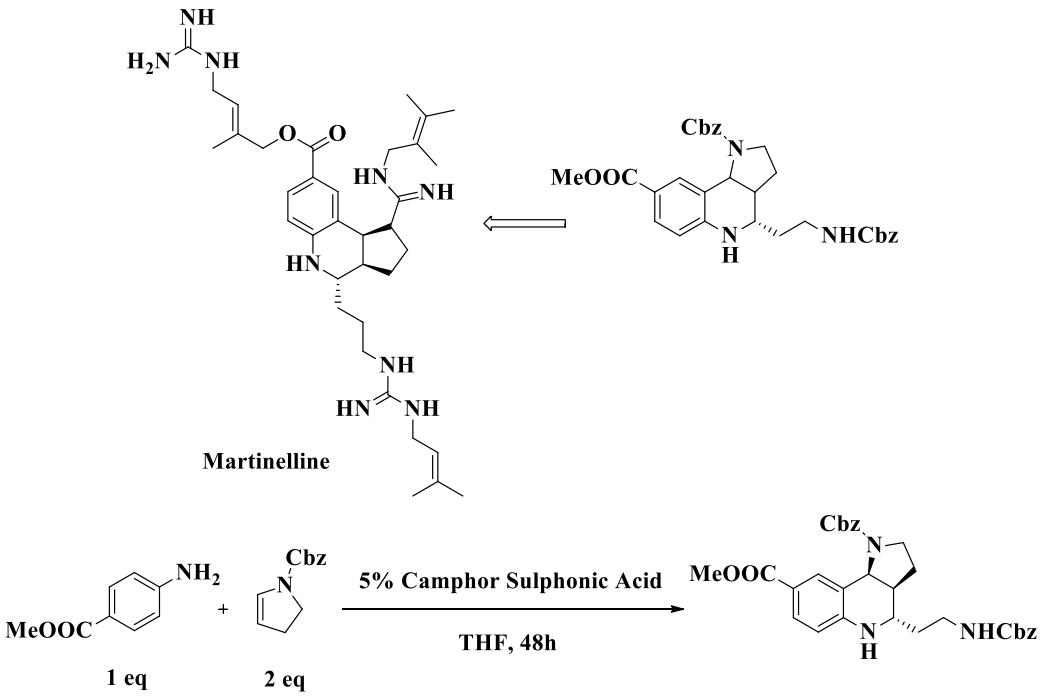
Scheme 1.27

Dysidea frondosa is a marine sponge; from this marine sponge marine sesquiterpenoid Frondosin B is isolated. It behaves as an interleukin-8 receptor antagonist. On account of its medicinal significance, most of the natural product chemists have been motivated to synthesize the Frondosin B. J. H. Chaplin and B. L. Flynn[46] established a multi component reaction approach by using substituted alkyne, 2-bromophenol and bromoenone by palladium catalysis to produce the benzofuran analogue (Scheme 1.28).



Scheme 1.28

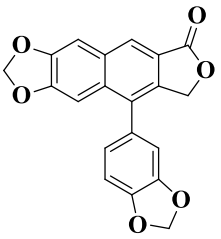
Martinelline is isolated from *Martinella iquitosensis* vine roots and used as a natural nonpeptidic bradykinin B2 receptor antagonist. The alkaloid comprises hexahydropyrrolo[3,2-*c*]quinoline as prime moiety with three pendant isoprenyl-derived guanidine structures. D. A. Powell[47] and co-worker established multi component Povarov approach to synthesize Martinelline precursor (Scheme 1.29).



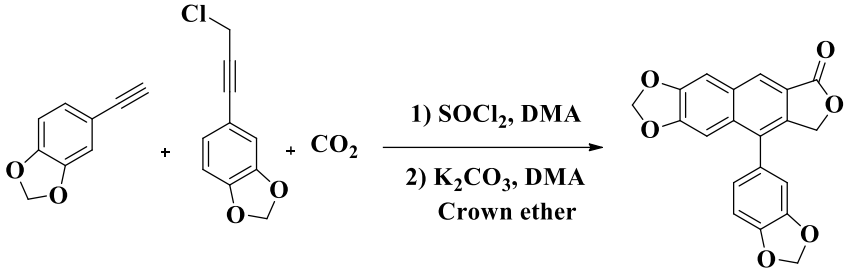
Scheme 1.29

Justicidine E is a naturally obtaining Lignan type of arylnaphthalene lactone ring moieties. P. T.

Anastas[48] and his coworkers established a one pot multi component reaction for Justicidine E. which was synthesized from 5-(3-chloroprop-1-yn-1-yl)benzo[*d*][1,3]dioxole, 5-ethynylbenzo[*d*][1,3]dioxole and carbon dioxide (Scheme 1.30).



Justicidin E



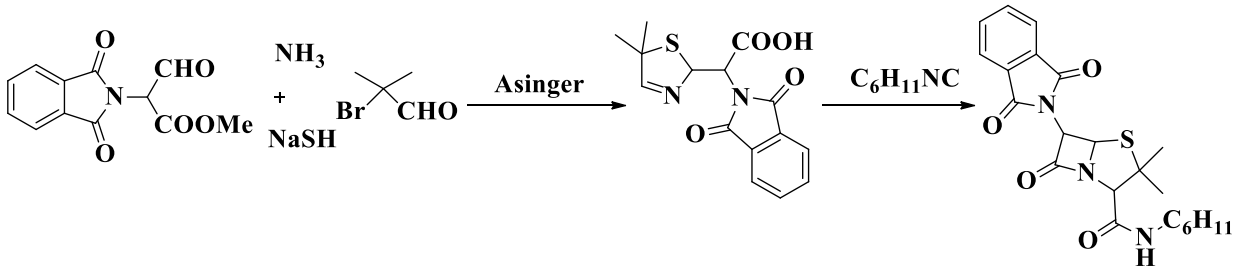
Scheme 1.30

Multi component reaction applications in the synthesis of drugs:

Multi component reactions are considered as a powerful modern synthetic organic tool, because they used for the synthesis of library of compounds and drug like moieties. For example, they are efficient protocols to synthesize well known drugs such as pencillin analogue, crixivan, bicalutamide and (S)-clopidogrel etc.

Synthesis of Pencillin analogues *via* Asinger and Ugi multi component reaction:

Ugi established a multi component reaction for the synthesis of pencillin[49] analogues by coupling Asinger and Ugi reaction (Scheme 1.31).

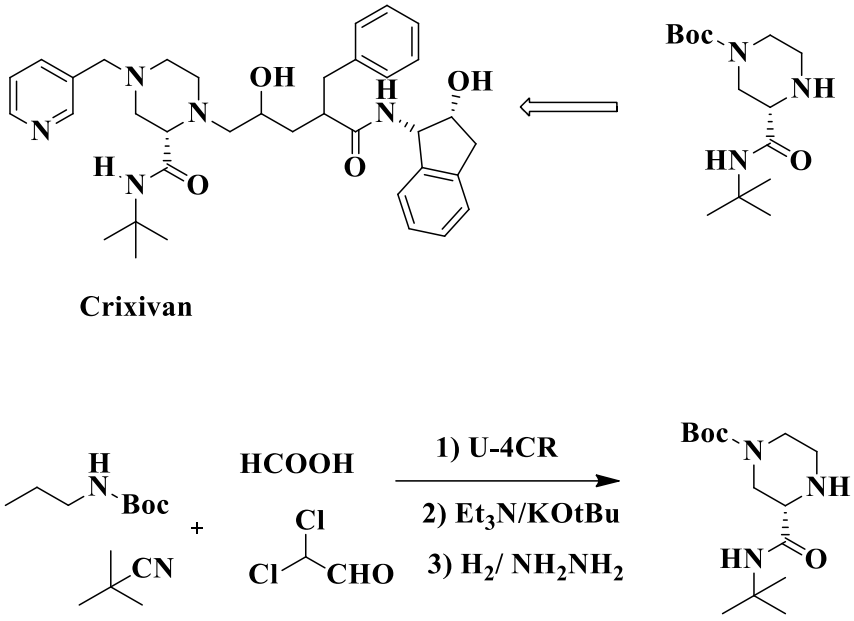


Scheme 1.31

Synthesis of HIV-protease inhibitor crixivan intermediate (piperazine intermediate):

K. Rossen[50] *et al.* established an efficient synthetic approach for the synthesis of crixivan intermediate. This piperizine intermediate was synthesized from *tert*-butyl propylcarbamate,

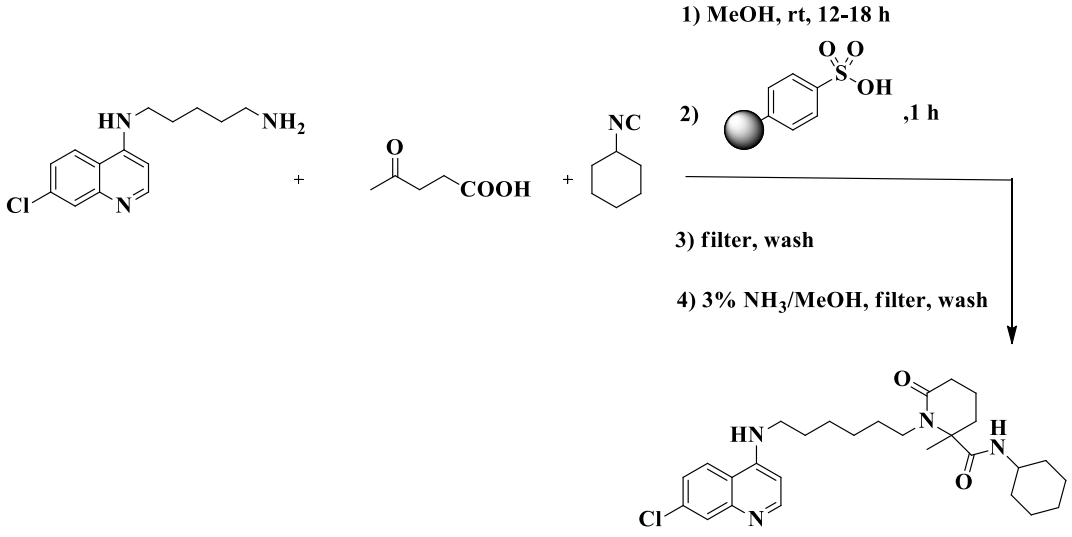
tert-butylisocyanide, dichloroacetaldehyde and formicacid by Ugi multi component reaction (Scheme 1.32).



Scheme 1.32

Synthesis of anti-malarial drug *via* Ugi multi component reaction:

K. Chibale[51] *et al.* developed an efficient synthetic method for the synthesis of anti-malarial drug (lactams). The lactam was synthesized using diamines, 4-acetylbutyric acid and cyclohexylisocyanide using Ugi multi component reaction (Scheme 1.33).

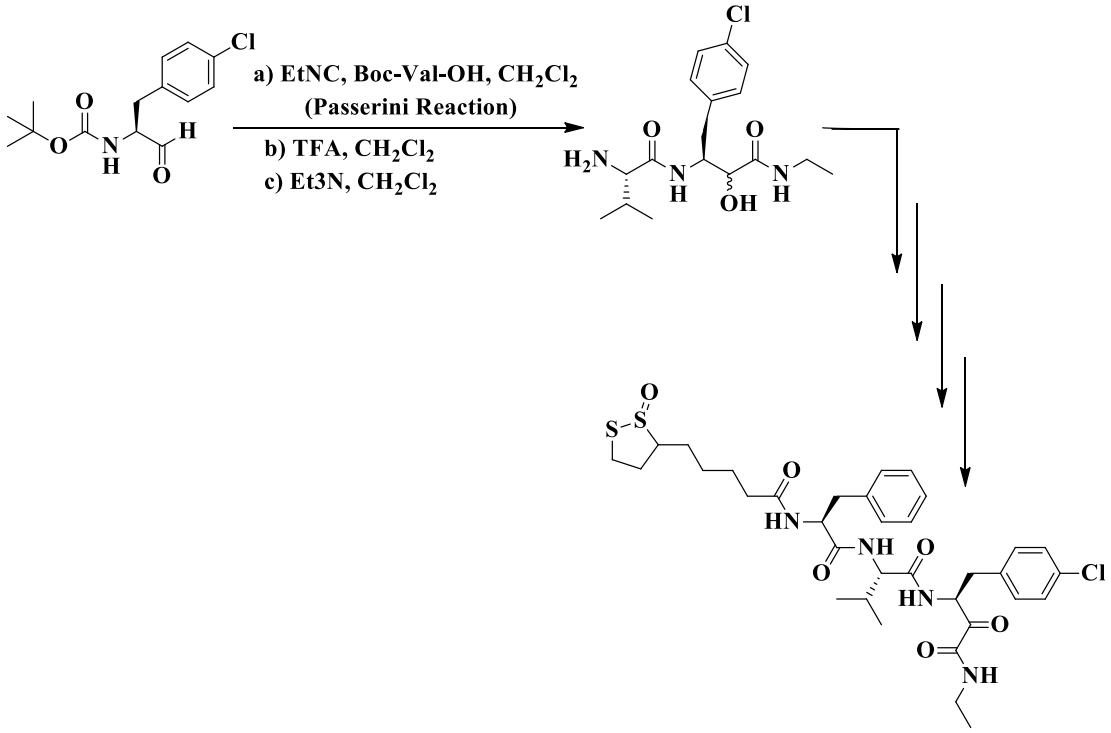


Scheme 1.33

Synthesis of enzyme inhibitor (calpain) *via* Passerini multi component reaction:

An efficient synthetic method for the synthesis of enzyme inhibitor (calpain) intermediate i.e. dipeptide moiety. The dipeptide moiety was synthesized using Boc protected amino aldehyde, an isonitrile and a suitably protected amino acid using Passerini multicomponent reaction[52]

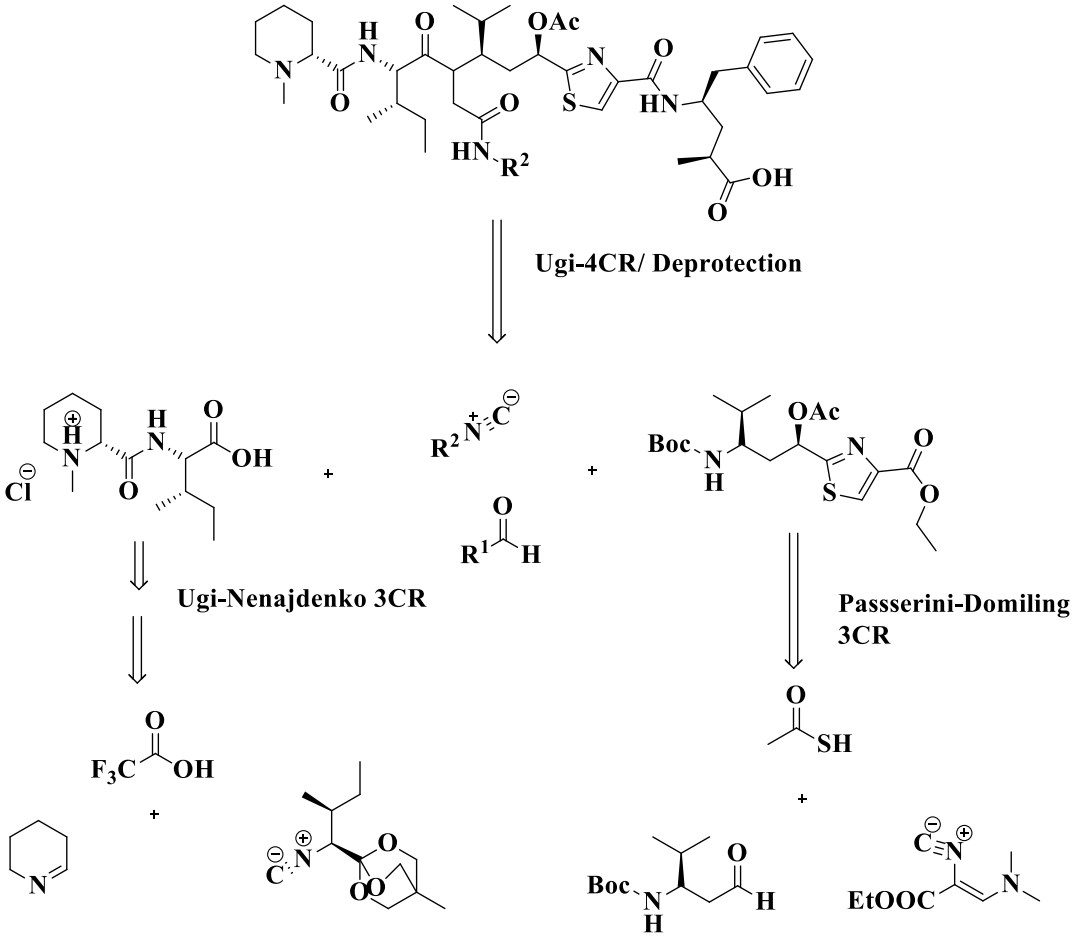
(Scheme 1.34).



Scheme 1.34

Synthesis of anti-cancer drug *via* multiple multi component reaction:

An efficient method for the synthesis of a new generation of highly cytotoxic tubulysin analogues (tubugis) was deeloped. The tubulysin moieties are between the most potent artificial anti-cancer agents ever invented and manifest the first example of a target oriented synthesis method using multiple multi component reactions[53]. In this Passerine-Domiling, Ugi-Nenajdenko 3 component reactions and Ugi 4 component reaction were used (Scheme 1.35).



Scheme 1.35

1.2. Coumarins:

Coumarin is a significant natural product. Moreover, synthetic heterocyclic compound also, which is oxygen heterocyclic system of benzopyran-2-one. Coumarins are the most abundant metabolites found in extracts of many plant families, such as Euphorbiaceae, Rutaceae, Orchidaceae, Asteraceae[54,55] and microorganisms by various extraction methods. Coumarin was first isolated by Vogel[56] from Tonka beans. They were firstly synthesized by Henry perkin in 1868. Chemically, coumarins can be synthesized by Pechmann[57], Reformatsky[58,59], Knoevenagel[60,61], Perkin[62], Hoesch[63], Claisen[64,65] and Wittig[66] cyclization reaction approaches.

1

5,6-benzo-alpha-pyrone

Coumarin

2

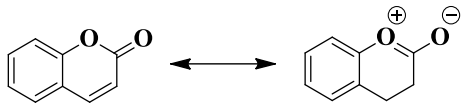
Benzo-.gamma.-pyrone

Chromone

Figure 1.3

1.2.1. General characteristics of coumarins:

It is always ambiguous regarding the aromatic nature of heterocyclic ring of due to its dual chemical reaction nature i.e. it manifests both aromatic and aliphatic characteristic properties. In this moiety, the lactone carbonyl (-O-C=O) group donates lone pair of electrons to the ring moiety to generate the aromatic system of 10 π-electrons (Figure 1.4).



3

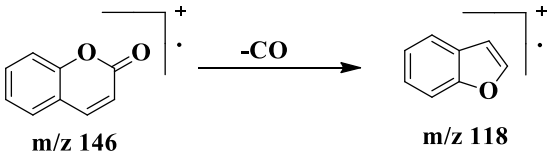
Figure 1.4

From the UV spectrum[67,68] of coumarins, it has been observed that they exhibited characteristic absorption values. The results showed that the 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 $M^{-1}cm^{-1}$) and 309 nm (37,449 $M^{-1}cm^{-1}$) respectively.

The IR spectrum[69] of coumarin manifests characteristic stretching frequency for lactone carbonyl at 1705 cm^{-1}, C=C stretching frequency at 1608 and 1450 cm^{-1}.

Dharmatti [70] *et al.* described proton NMR spectrum of coumarin. The ^1H NMR displays characteristic resonance peaks for C3-H and C4-H at 6.45 and 7.80 ppm respectively.

Barnes and Occolowitz[71] described the electron impact ionization mass spectra of coumarin. The molecular ion peak, fragmentation peaks display the transformation of coumarin to benzofuran (Figure 1.5).



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

4

Figure 1.5

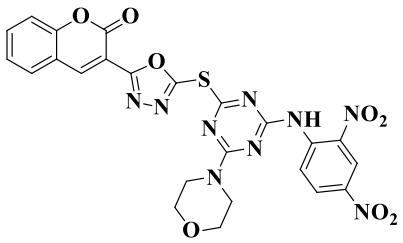
1.2.2. Applications of 3-heteryl coumarins:

Nowadays, natural and synthetic 3-substituted coumarin analogues have drawn notable attention of scientific community on account of their extensive applications in the field of optoelectronics, therapeutics, agrochemicals, material science and dyes. Furthermore, different 3-substituted coumarin analogues act as privileged heterocyclic analogues with diverse biologicalactivities like antitumor, antioxidant, antimicrobial, anti-coagulant, anti-tubercular carbonic anhydrase

inhibitors and MAO-B inhibitor activities. Hence, development of novel or new synthetic and semi synthetic coumarin heterocyclic derivatives with widespread applications is essential. The concept of molecular hybridization is a useful approach to develop heterocyclic compounds and their analogues with potential medicinal values.

Antibacterial activity of 3-substituted coumarins:

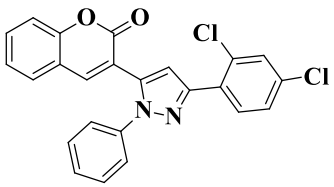
A novel series of 3-substituted coumarinyl triazine (**5**) analogues were synthesized by R. P. Modh[72] *et al*. by using coumarin-3-carboxylic acid as starting material and described their antimicrobial activity. These compounds manifested promising antibacterial activity against Gram positive (*Bacillus cereus* and *S. aureus*) and Gram negative bacteria (*S. typhi* and *E.coli*).



5

Figure 1.6

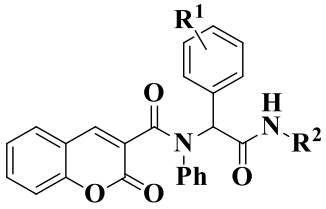
P. Aragade[73] *et al.* synthesized a new series of 3-[3-(substituted phenyl)-1-phenyl-1*H*-pyrazol-5-yl]-2*H*chromen-2-one analogues (**6**) and described their antibacterial activity against Gram positive bacteria *Bacillus subtilis*, *Staphylococcus aureus* and Gram negative bacteria *Pseudomonas, aeruginosa* and *Escherichia coli.* Compound **6** showed remarkable anti-bacterial activity against all the tested bacteria.



6

Figure 1.7

S. Kumar[74] *et al*. synthesized a series of coumarin based alpha-acyl amino amides **7** *via* a one pot Knoevenagel-Ugi five component sequential reaction and described anti-bacterial activity for Gram-positive and Gram-negative strains. Compound **7** exhibits a good anti-bacterial activity.

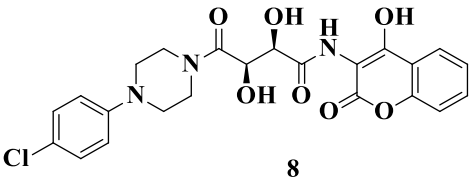


7

Figure 1.8

Antifungal activity of 3-substituted coumarins:

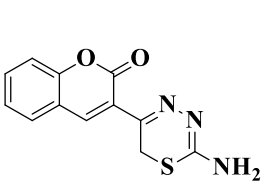
A series of novel 3-substituted amino-4-hydroxycoumarin analogues were synthesized by Z. Ge[75] *et al*. and described their antifungal activity against *Cryptococcus neoformans.* Compound **8** is potential for the treatment of fungal infections.



8

Figure 1.9

A new series coumarinyl 1,3,4-thiadiazine analogues were synthesized by M. Cacic[76] *et al*. utilizing 3- (2-bromoacetyl)coumarins and thiosemicarbazide as starting materials. These prepared moieties were screened for antifungal property against *F.verticillioides, A. ochraceus, F.graminearum and A. flavus. The m*olecules **9** and **10** showed potential antifungal activity.



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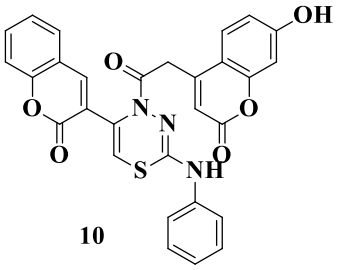
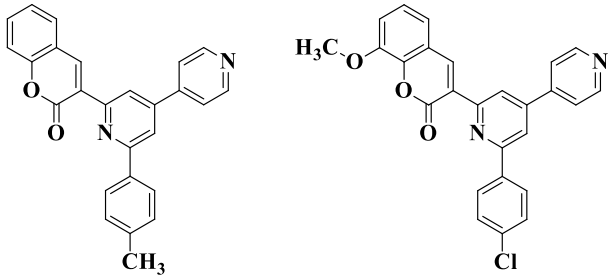


10

Figure 1.10

H. B. Lad[77] *et al*. synthesized a new series of 3-bipyridyl substituted coumarin derivatives and described antifungal activity of against *Aspergillus niger* and *Candida albicans* using Greseofulvin as antifungal standard drug. The molecules **11** and **12** were claimed as equipotent antifungal active molecules when compared to Greseofulvin standard drug.

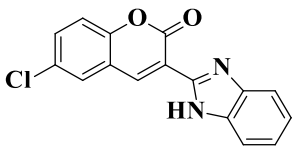


11 **12**

Figure 1.11

Anti-inflammatory activity of 3-substituted coumarins:

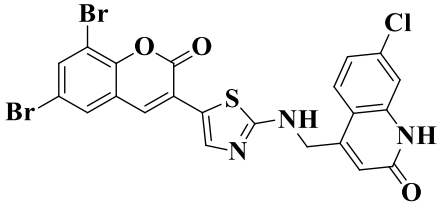
R. K. Arora[78] *et al*. synthesized a novel 2-coumarinyl benzimidazole analogues starting from the reaction of coumarin-3-carboxylic acid and *o*-phenylenediamine. Moreover, these molecules were screened for anti- inflammatory activity and results showed that the compound **13** has exhibited excellent anti-inflammatory activity.



13

Figure 1.12

A new series of thiazolyl coumarin heterocyclic derivatives are synthesized by R. G. Kalkhambkar[79] *et al*. starting from 3-(2-bromoacetyl)coumarins and described their anti-inflammatory properties. Molecule **14** exhibited good activity.

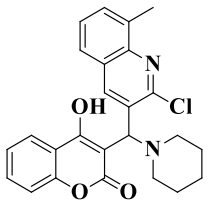


14

Figure 1.13

Antioxidant activity of 3-substituted coumarins:

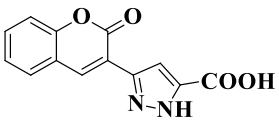
Z. Zaheer[80] *et al.* synthesized a series of novel 3-substituted-4-hydroxycoumarin derivatives from the reaction of 4-hydroxycoumarin and studied antioxidant properties. Molecule **15** has shown remarkable antioxidant activity as compared with the standard drug.



15

Figure 1.14

M. Salem[81] *et al.* synthesized 3-(2-oxo-2*H*- chromen-3-yl)-1*H*-pyrazole-5-carboxylic acid **16**, 5-(2-oxo-2*H*-chromen-3-yl)-2,3-dihydroisoxazole-3-carboxylic acid **17** starting from 4-oxo-4-(2-oxo-2*H*-chromen-3-yl)but-2-enoic acid, hydrazine hydrate or hydroxylamine hydrochloride. These derivatives were tested for their antioxidant activity and they showed excellent activity.



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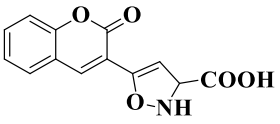
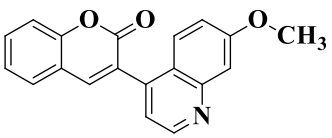


17

Figure 1.15

W. S. Hamama[82] *et al.* synthesized 3-(7-methoxyquinolin-4-yl)-2*H*-chromen-2-one 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 compounds were tested for their antioxidant activity. The ascorbic acid was used as reference drug. Compound **18** manifested moderate antioxidant activity.

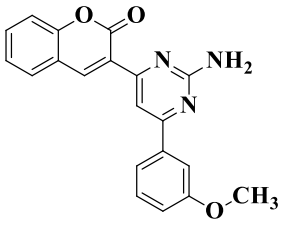


18

Figure 1.16

Analgesic activity of 3-substituted coumarins:

J. K. Gupta[83] *et al.* synthesized 2-amino-4-(coumarin-3-yl)-6-aryl pyrimidine analogues from 3-acetyl coumarin. These synthesized derivatives were tested for *in vivo* analgesic property. The diclophenac sodium was used as reference drug. Compound **19** manifested potential activity.

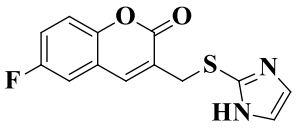


19

Figure 1.17

Anti-viral activity of 3-substituted coumarins:

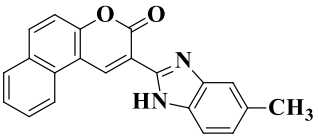
M. Z. Hassan[84] *et al*. synthesized a new series of 3-imidazolylthio methyl-coumarin derivatives and described for their anti-hepatitis C virus activity. In which Compound **20** has manifested excellent antiviral activity.



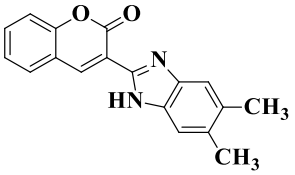
20

Figure 1.18

S.C. Tsay[85] *et al*. synthesized a novel series of benzimidazolyl – coumarin derivatives starting from substituted coumarin-3-carboxylate and phenylenediamine in presence of *ortho*-phosphoric acid. These derivatives were evaluated for their anti- HCV (Hepatitis-C-virus) activity and the compounds **21** and **22** manifested excellent activity.



21

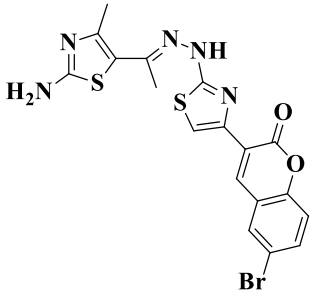


22

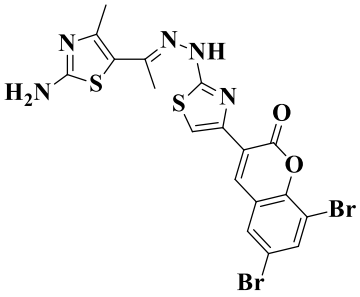
Figure 1.19

Anti-malarial activity of 3-substituted coumarins:

K. Sujatha[86] *et al.* synthesized a series of new thiazolyl hydrazonothiazolamine derivatives starting from 3-(2-bromoacetyl)coumarins. These title compounds were evaluated for *in vitro* anti-malarial properties. Compound **23, 24** exhibited potential activity against malaria.



23

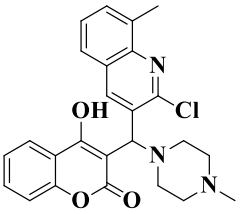


24

Figure 1.20

Antileishmanial activity of 3-substituted coumarins:

A series of novel 3-substituted amino-4-hydroxycoumarin derivatives were synthesized by Z. Zaheer[80] *et al*. and studied their antileishmanial activity against Leishmania donovani promastigotes. Compound **25** exhibited antileishmanial activity when compared with standard antileishmanial agents pentamidine and miltefosine.

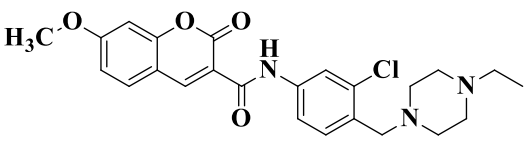


25

Figure 1.21

Acetylcolinesterase inhibitor activity of 3-substituted coumarins:

A new 2-oxo-N-(4-(2-(piperazin-1-yl)ethyl)phenyl)-2H-chromene-3-carboxamide analogues were synthesized by D. Yao[87] *et al.* by using 3-chloro-4-((4-ethylpiperazin-1-yl)methyl)aniline with 2-oxo-2H-chromene-3-carbonyl chloride and described acetylcholine esterase inhibition. Compound **26** manifested remarkable AChE inhibition activity. Here, Huperzine A was the standard drug.

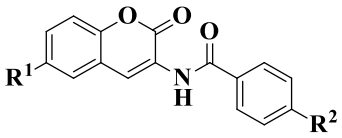


26

Figure 1.22

D. Vina[88] *et al*. synthesized a new series of *N*-(2-oxo-2*H*-chromen-3-yl)benzamide analogues **27** from 3-aminocoumarin and substituted acid chlorides in dichloromethane and in presence of pyridine as base. These derivatives were screened for acetylcholine esterase inhibition activity

and the moieties manifested moderate AChE activity.



27

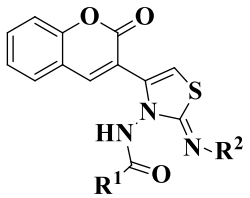
R^1= H, CH_3

R^2= H, CH_3, -OCH_3, Cl, NO_2

Figure 1.23

Glucosidase inhibitor activity of 3-substituted coumarins:

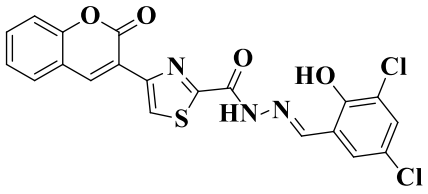
A series of new 3-thiazolylcoumarins **28** were synthesized *via* a one pot approach by U. Salar[89] *et al.* starting from 3-(2- bromoacetyl)coumarins and 2-benzoyl-N-arylhydrazinecarbothioamide in ethanol. These analogues were evaluated for *in vitro* α-glucosidase inhibitory activity and the compounds manifested excellent inhibitory activity.



28

Figure 1.24

A series of new thiazolyl coumarin analogues synthesized by G. Wang[90] *et al* starting from ethyl 4-(2-oxo-2*H*-chromen-3-yl)thiazole-2-carboxylate with substituted aldehydes and hydrazine hydrate. These analogues were screened for their α- glucosidase inhibitory activity. The results unveiled that, most of the compounds manifested potential inhibition activity and compound **29** exhibited remarkable inhibitory activity among all the synthesized compounds.

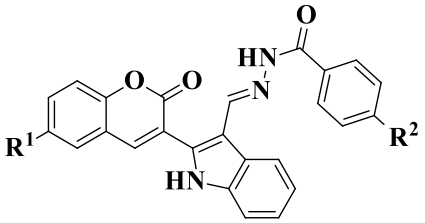


29

Figure 1.25

Anti-cancer activity of 3-substituted coumarins:

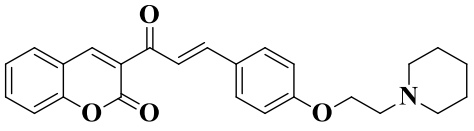
A series of novel *N*'-((2-(2-oxo-2*H*-chromen-3-yl)-1*H*-indol-3-yl)methylene)benzohydrazide **30** analogues were reported by P. R. Kamath[91] *et al*. and described the anticancer property. These derivatives were evaluated against human breast adenocarcinoma cells (MCF-7). The molecules manifested potential for breast cancer chemotherapy with apoptosis properties.



30

Figure 1.26

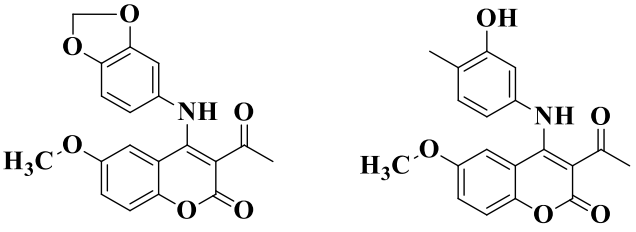
A series of novel substituted coumarin-chalcone hybrids were synthesized by S. N. Mokale[92] *et al*. from 3-acetyl coumarin and who studied their anticancer activity against MCF-7, MDA-MB-435 breast cancer cell lines. Compound **31** exhibited good anticancer activity.



31

Figure 1.27

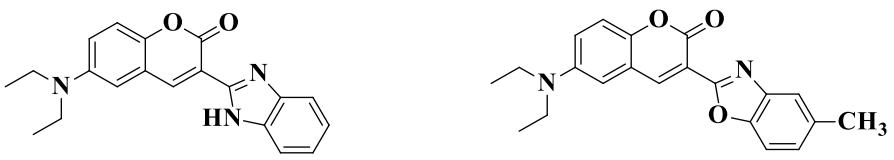
H. Xiang[93] *et al.* synthesized 3-substituted 4-anilino-coumarin derivatives and studied *in vitro* anti-proliferative properties against MCF-7, HepG2, HCT116 and Panc-1 cancer cell lines. Compound **32**, **33** exhibited good anti-proliferative activity.



32 **33**

Figure 1.28

M. Ataeefard and F. Nourmohammadian[94] established coumarin dyes as fluorescent ink for digital printing. Benzimidazolyl / benzoxazoly coumarin analogues (**34** and **35**) were comprised into polystyrene-acrylic acid and described the emission and reflectance properties of fluorescent laser printing ink.



34 **35**

Figure 1.29

1.2.3. Preparation of coumarins:

For the synthesis of coumarins various approaches are available in the literature. Coumarins can be synthesized by Perkin reaction, Claisen rearrangement, Pechmann condensation, and Knoevenagel condensation.

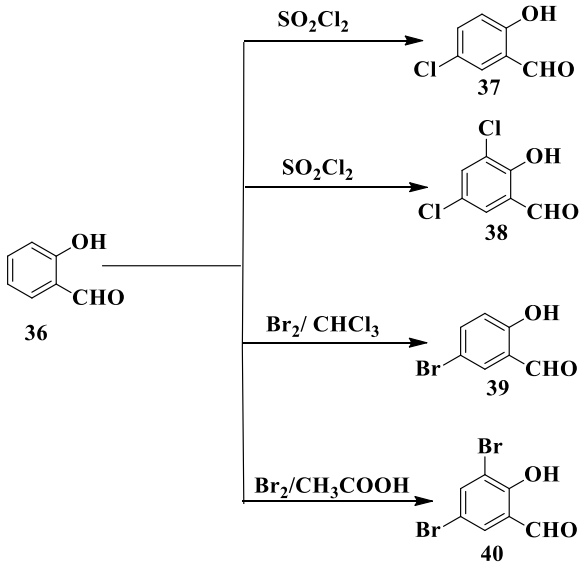
Preparation of various substituted 3-(2-bromoacetyl)coumarins:

In the present study, 3-acetylcoumarins and 3-(2-bromoacetyl) coumarins were used as starting materials for the synthesis of various heterocyclic compounds. For the preparation of varios 3-(2-bromoacetyl) coumarins involves in three steps.

i. Preparation of salicylaldehydes.
i. Preparation of 3-acetyl-2H-1-benzopyran-2-ones.
ii. Bromination of 3-acetyl-2H-1-benzopyran-2-ones.

Step 1: Preparation of substituted salicylaldehydes:

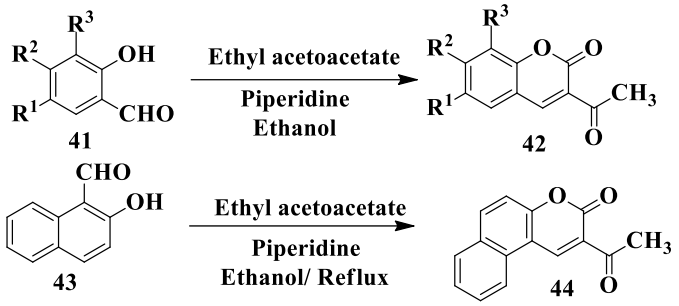
The required substituted salicylaldehydes have been prepared by following different procedures. The halo salicylaldehydes have been prepared by halogenation. 5-Chloro salicylaldehyde was prepared by reacting salicylaldehyde with sulfuryl chloride[95]. 3,5-Dichlorosalicylaldehyde was prepared by treating salicylaldehyde with 2 equivalents of sulfuryl chloride. Bromination of salicylaldehyde in chloroform gave the 5-isomer as an exclusive product[96], while dibromosalicylaldehyde is formed as the sole products when the bromination is carried out in acetic acid medium with two molar proportions of bromine[97] (Scheme 1.36). Similarly, the *o*-hydroxy aldehydes like *o*-vanillin and 2-hydroxy-1-naphthaldehyde were purchased from commercial sources and used as such.



Scheme 1.36

Step 2: Preparation of 3-acetyl-2*H*-1-benzopyran-2-ones:

Ethyl acetoacetate is condensed with substituted salicylaldehydes[98,99] in alcohol in the presence of piperidine. The reaction has been carried out at reflux temperature and the 3-acetyl-2*H*-1-benzopyran-2-ones were separated as shining dense crystals from the clear solution at the end of reflux. Various substituted salicylaldehydes have been used for this purpose (Scheme 1.37).



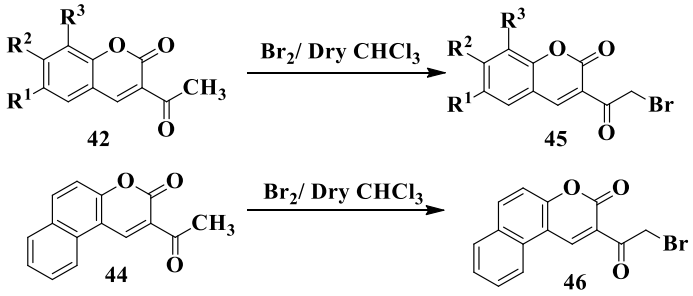
41 42 43 44

R^1= H, R^2= H, R^3= H
R^1= Cl, R^2= H, R^3= H
R^1= Cl, R^2= H, R^3= Cl
R^1= Br, R^2= H, R^3= H
R^1= Br, R^2= H, R^3= Br
R^1= H, R^2= H, R^3= OCH_3
R^1= Br, R^2= H, R^3= OCH_3

Scheme 1.37

Step 3: **Bromination of 3-acetyl-2*H*-1-benzopyran-2-ones:**

3-(2-Bromoacetyl)-2*H*-chromen-2-ones[100–102] were prepared by using equal amounts 3-acetyl coumarins and bromine in presence of dry chloroform under heating condition (Scheme 1.38).



42 45 44 46

R^1= H, R^2= H, R^3= H
R^1= Cl, R^2= H, R^3= H
R^1= Cl, R^2= H, R^3= Cl
R^1= Br, R^2= H, R^3= H
R^1= Br, R^2= H, R^3= Br
R^1= H, R^2= H, R^3= OCH_3
R^1= Br, R^2= H, R^3= OCH_3

Scheme 1.38

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

Present work:

From the preceding review, it is evident that multi component reactions have drawn special attention on account of the advent of high-throughput screening techniques that enabled rapid identification of potential new medicines among large collections of organic compounds. This

required the development of new methods to synthesize the organic compounds. The approaches that would provide rapid access to high-efficient compound libraries came into high demand. Multi component reactions ideally suited for this new demand concept, and creates more interest in the earlier developed reactions and in the invention of similar or even fundamentally new ones. The modern society also sets stringent environmental requirements for any industrial production process including the production of chemicals and pharmaceuticals. Multi-stage syntheses are generally associated with the loss of matter during isolation and purification of intermediates and usually require considerable amounts of solvents, which implicates waste disposal issues. Conversely, multi-component reactions, which give rise to numerous chemical bonds in a one-pot procedure, appear to be much more ecologically sound.

This research work is aimed at developing efficient synthetic approaches to preparation the new heterocyclic systems by the use of multi component reaction (MCR) method and describes on biological activity applications.

Aims and objectives of the research work:

1.To establish an environmentally benign, an efficient, facile methods for the synthesis of biologically potent molecules.

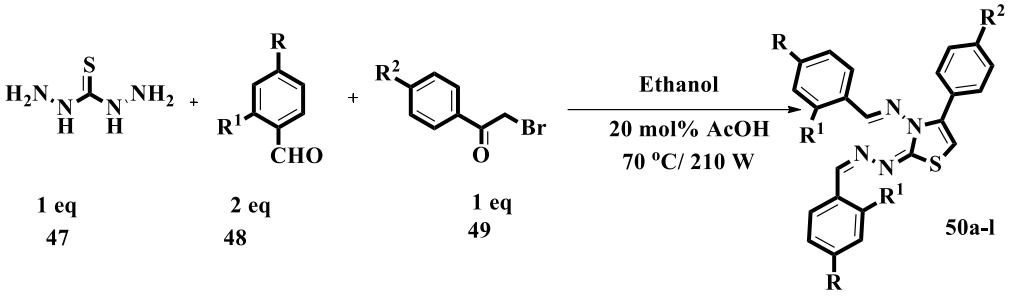
2.To establish the structures of newly synthesized compounds by analytical & spectral methods.

3.Screening of biological activities of the newly synthesized heterocyclic compounds.

The present research work briefly covers the synthesis of sulphur and nitrogen heterocyclic compounds and their biological activity studies. The target heterocyclic compounds were synthesized by taking readily available starting materials like substituted 3-(2-bromoacetyl)coumarins, 3-acetyl coumarins, thiocarobohydrazide, thiosemicarbazide, Phenacyl bromides, 1-Boc-3-cyano-4-piperidone and 1-Boc-3-cyano-4-pyrrolidone.

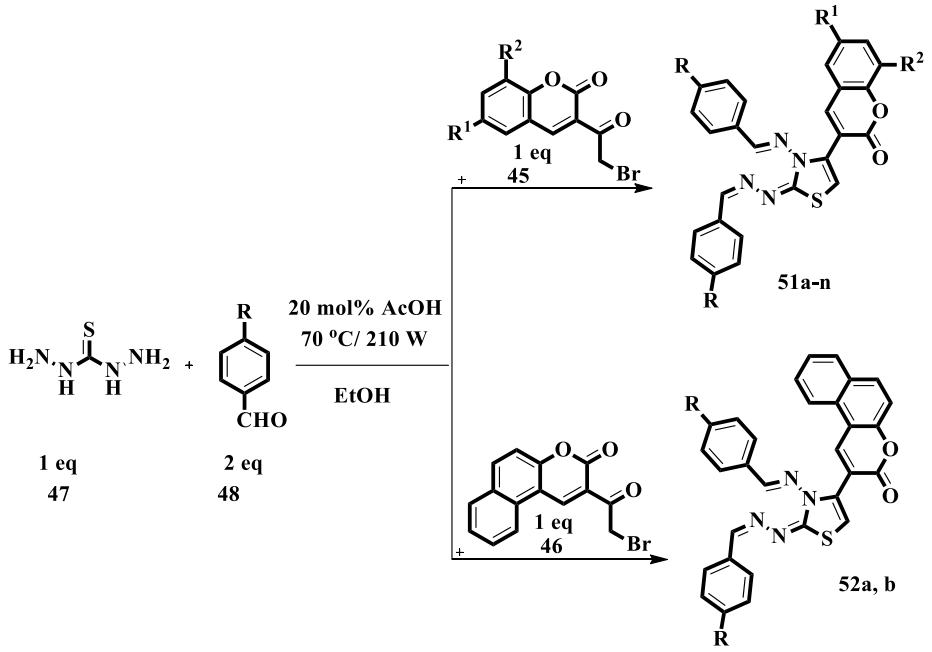
Chapter-I deals with introduction to multi component reactions (history and their applications in the synthesis of various biologically valuable heterocyclic systems) and synthesis and biological activity applications of 3-substituted coumarins.

Chapter-II deals with microwave irradiated one pot, pseudo four component synthesis of new thiazoles (Scheme 1.39) and microwave irradiated one pot, pseudo four component synthesis of a new series of hybrid coumarin based thiazoles (Scheme 1.40).



50a-R=Cl, R^1=H, R^2=H
50b-R=OH, R^1=OH, R^2=OCH_3
50c-R=Cl, R^1=H, R^2=OCH_3
50d-R=Br, R^1=H, R^2=OCH_3
50e-R=Cl, R^1=H, R^2=F
50f-R=Br, R^1=H, R^2=F
50g-R=Cl, R^1=H, R^2=Cl
50h-R=Br, R^1=H, R^2=Cl
50i-R=Cl, R^1=H, R^2=Br
50j-R=Br, R^1=H, R^2=Br
50k-R=Cl, R^1=H, R^2=NO_2
50l-R=Br, R^1=H, R^2=NO_2

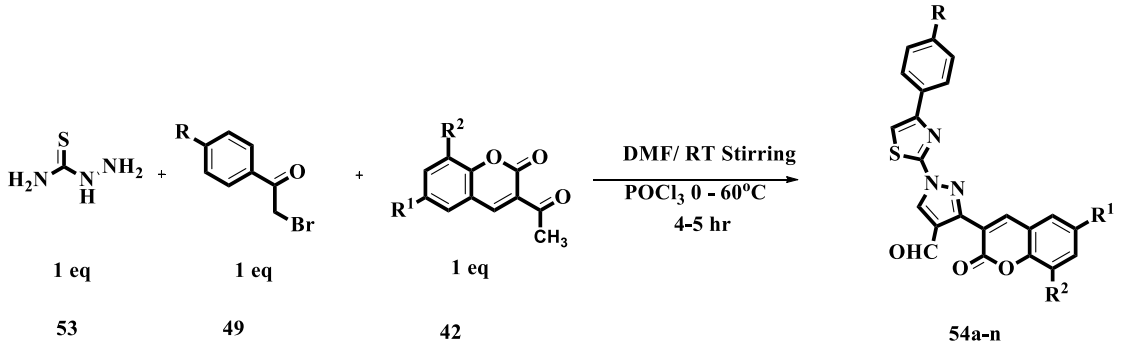
Scheme 1.39



51a- R= Cl, R^1= H, R^2= H
51b- R= Br, R^1= H, R^2= H
51c- R= Cl, R^1= Cl, R^2= H
51d- R=Br, R^1= Cl, R^2= H
51e- R= Cl, R^1= Cl, R^2= Cl
51f- R= Br, R^1= Cl, R^2= Cl
51g- R= Cl, R^1= Br, R^2= H
51h- R= Br, R^1= Br, R^2= H
51i- R= Cl, R^1= Br, R^2= Br
51j- R= Br,R^1= Br, R^2= Br
51k- R= Cl, R^1= H, R^2= OCH_3
51l- R= Br,R^1= H, R^2= OCH_3
51m- R= Cl, R^1= Br, R^2= OCH_3
51n- R= Br,R^1= Br, R^2= OCH_3

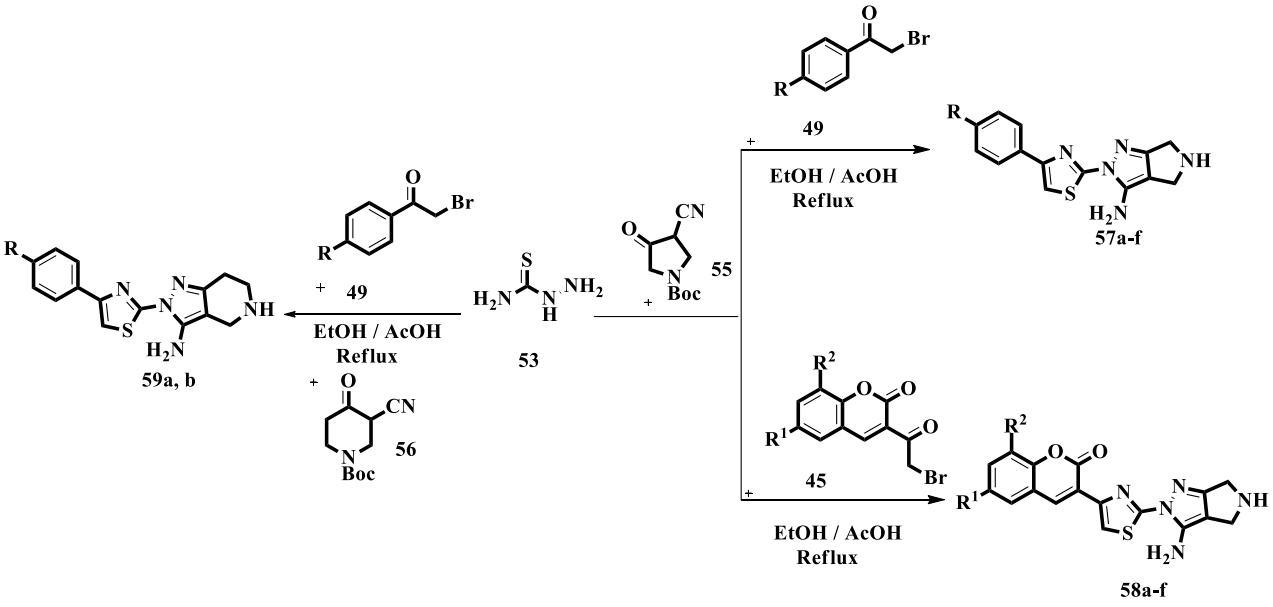
Scheme 1.40

Chapter-III describes with a facile one-pot three component synthesis of new thiazolyl pyrazole carbaldehydes (Scheme 1.41), a facile one-pot three component synthesis of new thiazolyl pyrazoles (Scheme 1.42) and microwave-assisted synthesis of new pyrazolylthiazoles *via* multi component approach (Scheme 1.43).



54a, 54h- R^1= H, R^2= H
54b, 54i- R^1= Cl, R^2= H
54c, 54j- R^1= Cl, R^2= Cl
54d, 54k- R^1= Br, R^2= H
54e, 54l- R^1= Br, R^2= Br
54f, 54m- R^1= H, R^2= OCH_3
54g, 54n- R^1= Br, R^2= OCH_3

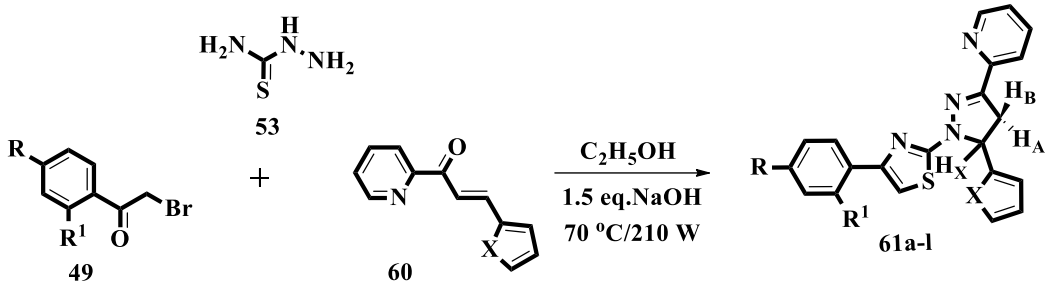
Scheme 1.41



57a-R=H
57b, 59a-R=CH_3
57c, 59b=R-OCH_3
57d-R=F
57e-R=Br
57f-R=NO_2

58a- R^1= H, R^2= H
58b- R^1= Cl, R^2= H
58c- R^1= Br, R^2= H
58d- R^1= Br, R^2= Br
58e- R^1= H, R^2= OCH_3
58f- R^1= Br, R^2= OCH_3

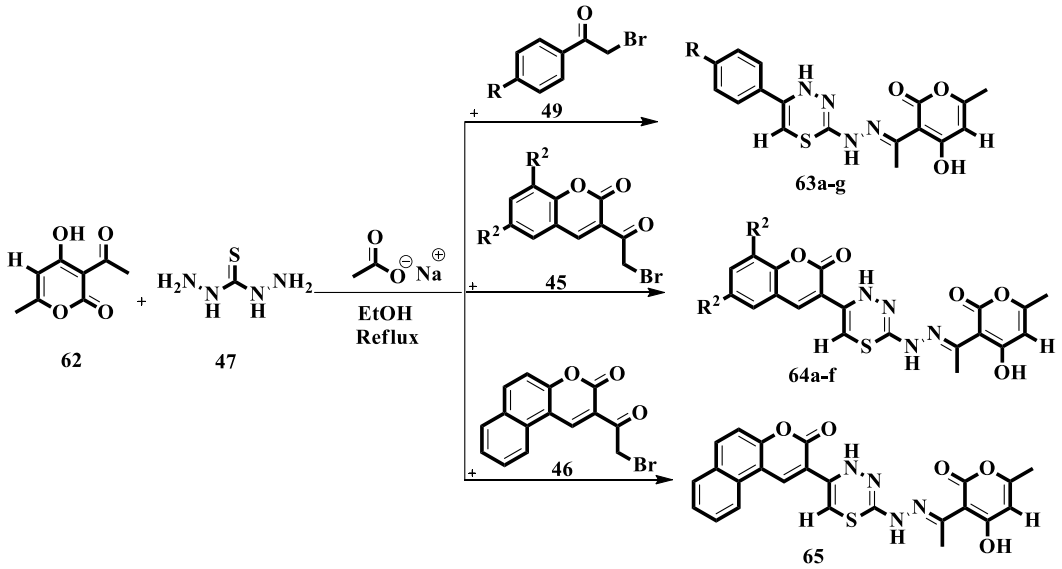
Scheme 1.42



61a, 61i- R= H, R^1= H
61b- R= H, R^1= H
61c- R= H, R^1= H
61d- R= H, R^1= H
61e, 61j- R= H, R^1= H
61f, 61k- R= H, R^1= H
61g, 61l- R= H, R^1= H
61h- R= H, R^1= H
61a-h- X= O
61i-l- X= S

Scheme 1.43

Chapter-IV describes with one-pot, three component synthesis of new 1,3,4 thiadiazines (Scheme 1.44).



63a- R= H
63b- R= CH_3
63c- R= OCH_3
63d- R= F
63e- R= Cl
63f- R= NO_2
63g- R= Ph

64a- R^1= H, R^2= H
64b- R^1= Cl, R^2= H
64c- R^1= Br, R^2= H
64d- R^1= Br, R^2= Br
64e- R^1= H, R^2= OCH_3
64f- R^1= Br, R^2= OCH_3

Scheme 1.44

Chapter-V deals with the evaluation of biological activity of the synthesized compounds.

References:

[1] P. T. Anastas, M. M. Kirchhoff, *Acc. Chem. Res.* **2002**, *35*, 686–694.

[2] B. A. de Marco, B. S. Rechelo, E. G. Tótoli, A. C. Kogawa, H. R. N. Salgado, *Saudi Pharm. J.* **2019**, *27*, 1–8.

[3] M. Kaljurand, M. Koel, *Crit. Rev. Anal. Chem.* **2011**, *41*, 2–20.

[4] H. Bienaymé, C. Hulme, G. Oddon, P. Schmitt, *Chem. - A Eur. J.* **2000**, *6*, 3321–3329.

[5] A. Dömling, W. Wang, K. Wang, *Chem. Rev.* **2012**, *112*, 3083–3135.

[6] R. C. Cioc, E. Ruijter, R. V. A. Orru, *Green Chem.* **2014**, *16*, 2958–2975.

[7] Y. Gu, *Green Chem.* **2012**, *14*, 2091.

[8] E. Ruijter, R. V. A. Orru, *Drug Discov. Today Technol.* **2013**, *10*, e15–e20.

[9] C. Hulme, V. Gore, *Curr. Med. Chem.* **2003**, *10*, 51–80.

[10] I. Akritopoulou-Zanze, *Curr. Opin. Chem. Biol.* **2008**, *12*, 324–331.

[11] A. Ulaczyk-Lesanko, D. G. Hall, *Curr. Opin. Chem. Biol.* **2005**, *9*, 266–276.

[12] B. B. Touré, D. G. Hall, *Chem. Rev.* **2009**, *109*, 4439–4486.

[13] R. Kakuchi, *Angew. Chemie Int. Ed.* **2014**, *53*, 46–48.

[14] Z. Zhang, Z.-B. Tan, C.-Y. Hong, D.-C. Wu, Y.-Z. You, *Polym. Chem.* **2016**, *7*, 1468–1474.

[15] R. Dong, Q. Chen, X. Cai, Q. Zhang, Z. Liu, *Polym. Chem.* **2020**, *11*, 5200–5206.

[16] J. Zhang, M. Zhang, F.-S. Du, Z.-C. Li, *Macromolecules* **2016**, *49*, 2592–2600.

[17] L. Yang, Z. Zhang, B. Cheng, Y. You, D. Wu, C. Hong, *Sci. China Chem.* **2015**, *58*, 1734–1740.

[18] T. W. Jabusch, R. S. Tjeerdema, *J. Agric. Food Chem.* **2006**, *54*, 5962–5967.

[19] M. A. Gonzalez, D. B. Gorman, C. T. Hamilton, G. A. Roth, *Org. Process Res. Dev.* **2008**, *12*, 301–303.

[20] R. V. Sumesh, M. Muthu, A. I. Almansour, R. Suresh Kumar, N. Arumugam, S. Athimoolam, E. A. Jeya Yasmi Prabha, R. R. Kumar, *ACS Comb. Sci.* **2016**, *18*, 262–270.

[21] Y.-S. Kim, S. H. Kwak, Y.-D. Gong, *ACS Comb. Sci.* **2015**, *17*, 365–373.

[22] S. L. Miller, *Science (80-.).* **1953**, *117*, 528–529.

[23] A. Strecker, *Ann. der Chemie und Pharm.* **1850**, *75*, 27–45.

[24] P. Biginelli, *Berichte der Dtsch. Chem. Gesellschaft* **1891**, *24*, 1317–1319.

[25] C. Mannich, W. Krösche, *Arch. Pharm. (Weinheim).* **1912**, *250*, 647–667.

[26] M. Passerini, *Gazz. Chim. Ital.* **1921**, *51*, 126–129.

[27] I. Ugi, R. Meyr, *Angew. Chemie* **1958**, *70*, 702–703.

[28] N. A. Petasis, I. Akritopoulou, *Tetrahedron Lett.* **1993**, *34*, 583–586.

[29] R. S. Bon, C. Hong, M. J. Bouma, R. F. Schmitz, F. J. J. de Kanter, M. Lutz, A. L. Spek, R. V. A. Orru, *Org. Lett.* **2003**, *5*, 3759–3762.

[30] A. Laurent, Gerhardt, *Ann. der Pharm.* **1838**, *28*, 265–269.

[31] B. Radziszewski, *Berichte der Dtsch. Chem. Gesellschaft* **1882**, *15*, 1493–1496.

[32] A. Hantzsch, *Justus Liebig's Ann. der Chemie* **1882**, *215*, 1–82.

[33] A. Hantzsch, *Berichte der Dtsch. Chem. Gesellschaft* **1890**, *23*, 1474–1476.

[34] H. T. Bucherer, W. Steiner, *J. fuer Prakt. Chemie* **1934**, *140*, 291–316.

[35] F. Asinger, *Angew. Chemie* **1956**, *68*, 413–413.

[36] K. Gewald, E. Schinke, H. Böttcher, *Chem. Ber.* **1966**, *99*, 94–100.

[37] S. D. Larsen, P. A. Grieco, *J. Am. Chem. Soc.* **1985**, *107*, 1768–1769.

[38] L. S. Povarov, *Russ. Chem. Rev.* **1967**, *36*, 656–670.

[39] C. Cardellicchio, M. A. M. Capozzi, F. Naso, *Tetrahedron: Asymmetry* **2010**, *21*, 507–517.

[40] Y. Méndez, G. De Armas, I. Pérez, T. Rojas, M. E. Valdés-Tresanco, M. Izquierdo, M. Alonso del Rivero, Y. M. Álvarez-Ginarte, P. A. Valiente, C. Soto, et al., *Eur. J. Med. Chem.* **2019**, *163*, 481–499.

[41] E. P. da Costa, S. E. Coelho, A. H. de Oliveira, R. M. Araújo, L. N. Cavalcanti, J. B. Domingos, F. G. Menezes, *Tetrahedron Lett.* **2018**, *59*, 3961–3964.

[42] S. Koley, S. Chowdhury, T. Chanda, B. J. Ramulu, M. S. Singh, *Tetrahedron* **2013**, *69*, 8013–8018.

[43] E. Pelit, Z. Turgut, *Ultrason. Sonochem.* **2014**, *21*, 1600–1607.

[44] G. Ramesh, R. Gali, R. Velpula, B. Rajitha, *Res. Chem. Intermed.* **2016**, *42*, 3863–3873.

[45] P. R. Nandaluru, G. J. Bodwell, *Org. Lett.* **2012**, *14*, 310–313.

[46] J. H. Chaplin, B. L. Flynn, *Chem. Commun.* **2001**, *1*, 1594–1595.

[47] D. A. Powell, R. A. Batey, *Org. Lett.* **2002**, *4*, 2913–2916.

[48] P. Foley, N. Eghbali, P. T. Anastas, *J. Nat. Prod.* **2010**, *73*, 811–813.

[49] I. Ugi, E. Wischhöfer, *Chem. Ber.* **1962**, *95*, 136–140.

[50] K. Rossen, P. J. Pye, L. M. DiMichele, R. P. Volante, P. J. Reider, *Tetrahedron Lett.* **1998**, *39*, 6823–6826.

[51] C. C. Musonda, J. Gut, P. J. Rosenthal, V. Yardley, R. C. Carvalho de Souza, K. Chibale, *Bioorg. Med. Chem.* **2006**, *14*, 5605–5615.

[52] E. Ramiro, *Guix d'infantil, núm.60* **2011**, 33–36.

[53] P. Slobbe, E. Ruijter, R. V. A. Orru, *Medchemcomm* **2012**, *3*, 1189.

[54] Z. E. Nazari, M. Iranshahi, *Phyther. Res.* **2010**, n/a-n/a.

[55] S. Rosselli, A. M. Maggio, N. Faraone, V. Spadaro, S. L. Morris-Natschke, K. F. Bastow, K.-H. Lee, M. Bruno, *Nat. Prod. Commun.* **2009**, *4*, 1934578X0900401.

[56] A. Vogel, *Ann. der Phys. und der Phys. Chemie* **1820**, *64*, 161–166.

[57] H. Von Pechmann, C. Duisberg, *Berichte der Dtsch. Chem. Gesellschaft* **1883**, *16*, 2119–2128.

[58] Y. Fall, C. Terán, M. Teijeira, L. Santana, E. Uriarte, *Synthesis (Stuttg).* **2000**, *2000*, 643–645.

[59] S. Reformatsky, *Berichte der Dtsch. Chem. Gesellschaft* **1887**, *20*, 1210–1211.

[60] E. Knoevenagel, *Berichte der Dtsch. Chem. Gesellschaft* **1898**, *31*, 730–737.

[61] H. Valizadeh, S. Vaghefi, *Synth. Commun.* **2009**, *39*, 1666–1678.

[62] W. H. Perkin, *J. Chem. Soc.* **1875**, *28*, 10–15.

[63] K. Hoesch, *Berichte der Dtsch. Chem. Gesellschaft* **1915**, *48*, 1122–1133.

[64] L. Claisen, A. Claparède, *Berichte der Dtsch. Chem. Gesellschaft* **1881**, *14*, 2460–2468.

[65] N. Cairns, L. M. Harwood, D. P. Astles, *J. Chem. Soc., Perkin Trans. 1* **1994**, 3101–3107.

[66] G. Wittig, W. Haag, *Chem. Ber.* **1955**, *88*, 1654–1666.

[67] R. H. Goodwin, B. M. Pollock, *Am. J. Bot.* **1954**, *41*, 516.

[68] K. Sen, P. Bagchi, *J. Org. Chem.* **1959**, *24*, 316–319.

[69] K.-H. Lee, T. O. Soine, *J. Pharm. Sci.* **1969**, *58*, 681–683.

[70] Y. P. V. S. S. Dharmatti, G. Govil, C. R. Kanekar, C. L. Khetrapal, *Proc. Indian Acad. Sci.* **1962**, 71–85.

[71] C. Barnes, J. Occolowitz, *Aust. J. Chem.* **1964**, *17*, 975.

[72] R. P. Modh, A. C. Patel, K. H. Chikhalia, *Heterocycl. Commun.* **2013**, *19*, 343–349.

[73] P. Aragade, R. V. Narayanan, V. Maddi, P. Patil, P. Shinde, M. Agrawal, *J. Enzyme Inhib. Med. Chem.* **2012**, *27*, 849–853.

[74] S. Kumar, K. Mukesh, K. Harjai, V. Singh, *Tetrahedron Lett.* **2019**, *60*, 8–12.

[75] Z. Ge, Q. Ji, C. Chen, Q. Liao, H. Wu, X. Liu, Y. Huang, L. Yuan, F. Liao, *J. Enzyme Inhib. Med. Chem.* **2016**, *31*, 219–228.

[76] M. Čačić, V. Pavić, M. Molnar, B. Šarkanj, E. Has-Schön, *Molecules* **2014**, *19*, 1163–1177.

[77] H. B. Lad, R. R. Giri, D. I. Brahmbhatt, *Chinese Chem. Lett.* **2013**, *24*, 227–229.

[78] R. K. Arora, N. Kaur, Y. Bansal, G. Bansal, *Acta Pharm. Sin. B* **2014**, *4*, 368–375.

[79] R. G. Kalkhambkar, G. M. Kulkarni, H. Shivkumar, R. N. Rao, *Eur. J. Med. Chem.* **2007**, *42*, 1272–1276.

[80] Z. Zaheer, F. A. K. Khan, J. N. Sangshetti, R. H. Patil, *Chinese Chem. Lett.* **2016**, *27*, 287–294.

[81] M. Salem, M. Marzouk, A. El-Kazak, *Molecules* **2016**, *21*, 249.

[82] W. S. Hamama, M. A. Berghot, E. A. Baz, M. A. Gouda, *Arch. Pharm. (Weinheim).* **2011**, *344*, 710–718.

[83] J. K. Gupta, P. K. Sharma, R. Dudhe, A. Chaudhary, A. Singh, P. K. Verma, S. C. Mondal, R. K. Yadav, S. Kashyap, *Med. Chem. Res.* **2012**, *21*, 1625–1632.

[84] M. Z. Hassan, H. Osman, M. A. Ali, M. J. Ahsan, *Eur. J. Med. Chem.* **2016**, *123*, 236–255.

[85] S.-C. Tsay, J. R. Hwu, R. Singha, W.-C. Huang, Y. H. Chang, M.-H. Hsu, F. Shieh, C.-C. Lin, K. C. Hwang, J.-C. Horng, et al., *Eur. J. Med. Chem.* **2013**, *63*, 290–298.

[86] K. Sujatha, N. B. Ommi, A. Mudiraj, P. P. Babu, R. R. Vedula, *Arch. Pharm. (Weinheim).* **2019**, *352*, 1900079.

[87] D. Yao, J. Wang, G. Wang, Y. Jiang, L. Shang, Y. Zhao, J. Huang, S. Yang, J. Wang, Y. Yu, *Bioorg. Chem.* **2016**, *68*, 112–123.

[88] D. Viña, M. J. Matos, M. Yáñez, L. Santana, E. Uriarte, *Med. Chem. Commun.* **2012**, *3*, 213–218.

[89] U. Salar, M. Taha, K. M. Khan, N. H. Ismail, S. Imran, S. Perveen, S. Gul, A. Wadood, *Eur. J. Med. Chem.* **2016**, *122*, 196–204.

[90] G. Wang, D. He, X. Li, J. Li, Z. Peng, *Bioorg. Chem.* **2016**, *65*, 167–174.

[91] P. R. Kamath, D. Sunil, A. A. Ajees, K. S. R. Pai, S. Biswas, *Eur. J. Med. Chem.* **2016**, *120*, 134–147.

[92] S. N. Mokale, A. Begum, N. S. Sakle, V. R. Shelke, S. A. Bhavale, *Biomed. Pharmacother.* **2017**, *89*, 966–972.

[93] G. Luo, M. Muyaba, W. Lyu, Z. Tang, R. Zhao, Q. Xu, Q. You, H. Xiang, *Bioorg. Med. Chem. Lett.* **2017**, *27*, 867–874.

[94] M. Ataeefard, F. Nourmohammadian, *J. Lumin.* **2015**, *167*, 254–260.

[95] T. H. Durrans, *J. Chem. Soc., Trans.* **1923**, *123*, 1424–1429.

[96] L. C. Raiford, L. K. Tanzer, *J. Org. Chem.* **1941**, *06*, 722–731.

[97] C. M. Brewster, *J. Am. Chem. Soc.* **1924**, *46*, 2463–2468.

[98] R. Aggarwal, S. Kumar, P. Kaushik, D. Kaushik, G. K. Gupta, *Eur. J. Med. Chem.* **2013**, *62*, 508–514.

[99] A. Arshad, H. Osman, M. C. Bagley, C. K. Lam, S. Mohamad, A. S. M. Zahariluddin, *Eur. J. Med. Chem.* **2011**, *46*, 3788–3794.

[100] C. F. Koelsch, *J. Am. Chem. Soc.* **1950**, *72*, 2993–2995.

[101] N. Guravaiah, V. R. Rao, *Phosphorus. Sulfur. Silicon Relat. Elem.* **2010**, *185*, 361–367.

[102] P. V. Kumar, V. R. Rao, *Indian J. Chem.* **2005**, *44*, 2120–2125.

CHAPTER-II

Microwave irradiated one-pot, pseudo four component synthesis of new thiazoles

and

microwave irradiated one-pot, pseudo four component synthesis of a new series of hybrid coumarin based thiazoles

CHAPTER-II

2.1. Introduction:

Compounds with sulphur and nitrogen as hetero atoms in the five-member ring has received stupendous significance in the field of medicinal chemistry. A five-member heterocyclic compound with two hetero atoms may have two positional isomers. Sulphur and nitrogen having five-member ring system shows two isomeric forms (Figure 2.1). Those are 1,3-thiazole (thiazole) and 1, 2-thiazole (isothiazole). Out of these two isomers, 1,3-Thiazole is having variety of applications. Thiazole is a major class of five-member heterocyclic compound containing sulphur and nitrogen hetero atoms at 1 and 3 positions in the cyclic ring system. Thiazole manifests different resonance forms by delocalization of sulphur lone pair of electrons in the ring system (Figure 2.2). 1, 3- thiazoles are contemplated as a cyclic scaffold of thiosemicarbazone[1]. Thiazole is a principal core unit and it is omnipresent found in many natural and synthetic organic compounds. Out of the prime heterocyclic compounds, thiazoles are known to be pivotal biological active compound in medicinal chemistry. In 1887, Hantzsch[2] and co-workers identified and established molecular structure of thiazole, starting from thioamides, α-halo carbonyl compounds.

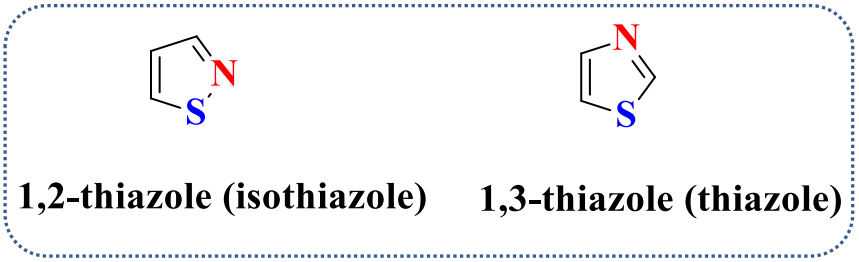


Figure 2.1: Isomeric forms of thiazoles

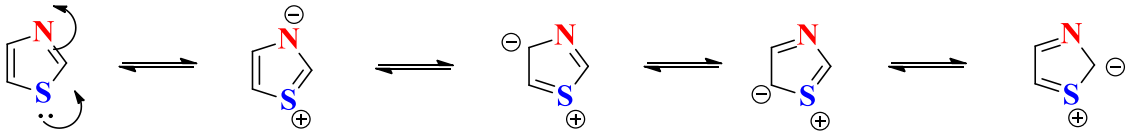


Figure 2.2: different resonance forms of thiazole.

A wide variety of biological active agents having thiazole as a core pharmacophore unit and

several drug molecules containing thiazole as a core unit as exhibited in Figure 2.3.

Thiazole ring is present in various marine or mundane products[3–5], which has biological activity. Thiazole moiety has attracted a profound interest due to their antiviral[6–9], anti-bacterial[10–15], anti-microbial[16–18], anti-inflammatory[19–21], antineoplastic[22,23] properties. On the other hand, meticulous literature study has shown that thiazole ring system has held a special place in the synthesis and design of new biological active molecules with phenomenal treatment of antimycobacterial[24], HIV infections[25], antihelminthic agent[26], allergies[27] and also treatment of hypertension[28], FabH inhibitors[29], CNS active agents[30], as novel inhibitors of bacterial DNA gyrase B[31,32], molecular switches[33], liquid crystals[34,35], sensors[36], photoluminiscent[37]. On account of wide range of applications and significance has attracted across the world on the chemistry of thiazole based compounds.

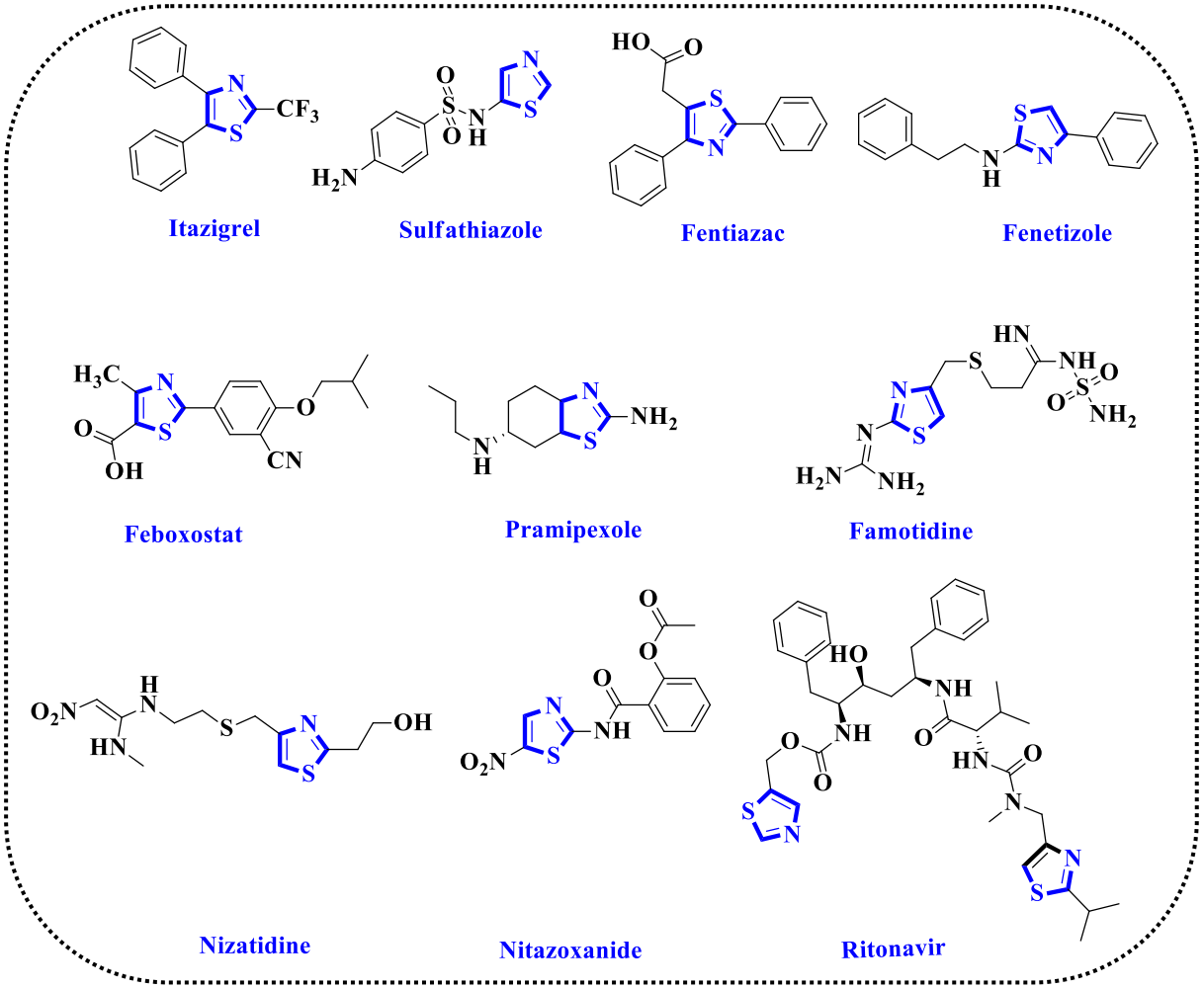
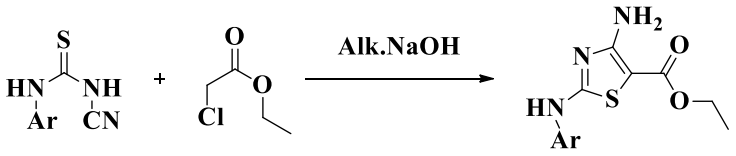


Figure 2.3: Thiazoles as major biological active agents.

The following are compact review of related literature for the synthesis of thiazoles.

L. Han[38] and co-workers synthesized a new series of ethyl 4-amino-2-(phenylamino)thiazole-

5-carboxylates from appropriate thiourea with ethyl 2-chloroacetate in the presence of NaOH (Scheme 2.1). Moreover, they are evaluated for biological activity against human dihydroorotate dehydrogenase, in which compound **1, 2, 3** (Figure 2.4) showed excellent acitivity against human dihydroorotate dehydrogenase (HsDHODH).



Scheme 2.1

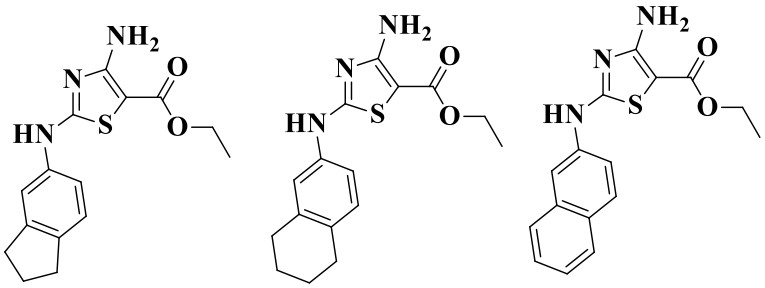
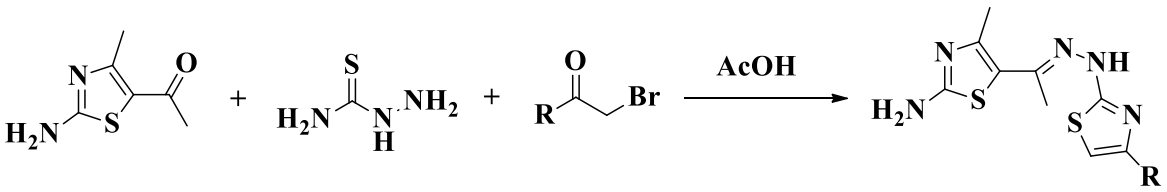
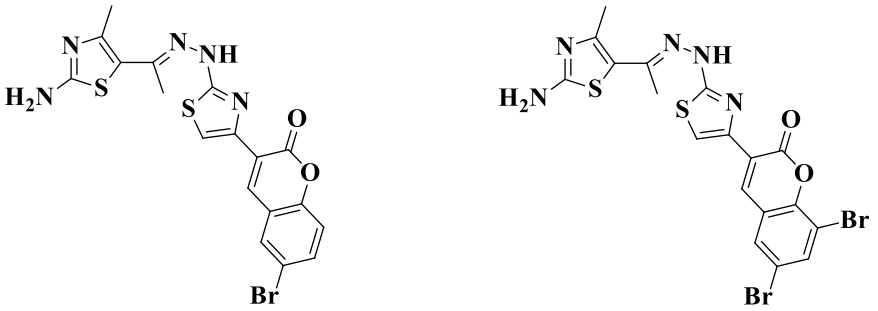


Figure 2.4

R. R. Vedula[39] *et al.* synthesized a series of novel 1,3,4-thiadiazinyl hydrazonothiazolamine derivatives via a multi component reaction approach. For this reactions starting materials are 2-amino-4-methyl-5-acetylthiazole, thiosemicarbazide, 3-(2-bromoacetyl)-2*H*-chromen-2-ones or phenacyl bromides (Scheme 2.2). The title compounds were screened for *in vitro* anti-malarial properties. The Compounds **4, 5** (Figure 2.5) exhibited potential activity against malaria.



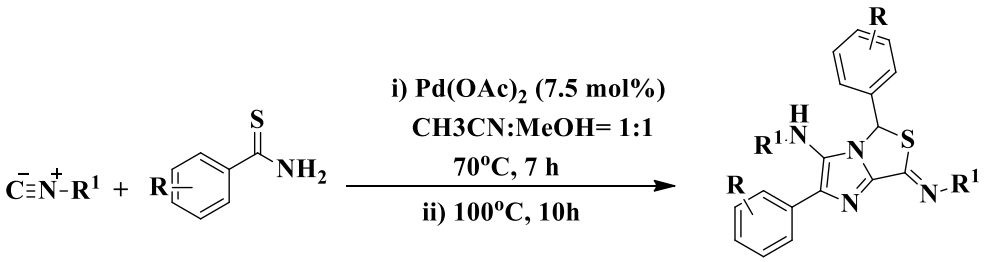
Scheme 2.2



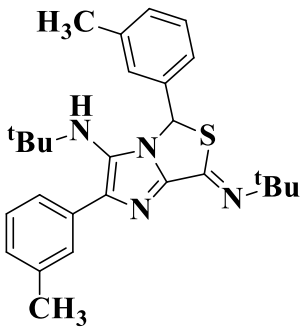
4 **5**

Figure 2.5

H. Wang[40] *et al.* synthesized a series of imidazo[1,2-c]thiazoles *via* Pd-catalyzed cascade bicyclization starting from isonitriles, thioamides (Scheme 2.3) and evaluated anti-tumor activity against cancer lines. Furthermore, compound **6** (Figure 2.6) exhibited good anti-tumor activity against HepG2.



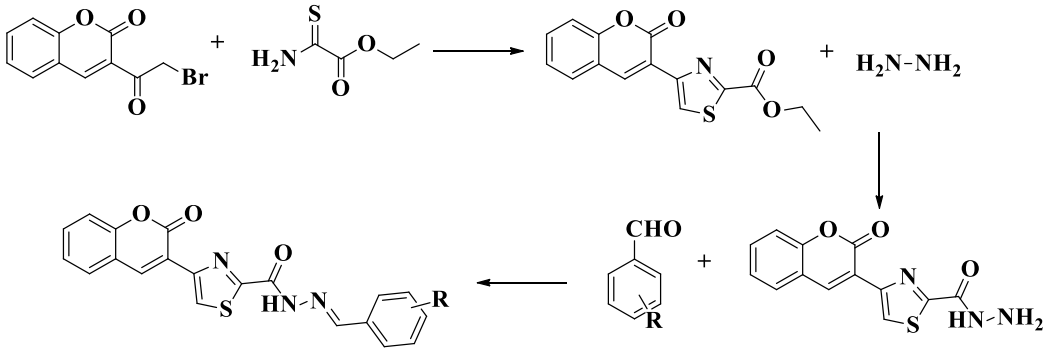
Scheme 2.3



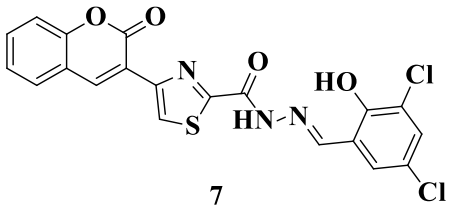
6

Figure 2.6

G. Wang[41] *et al.* synthesized a series of novel *N*'-benzylidene-4-(2-oxo-2*H*-chromen-3-yl)thiazole-2-carbohydrazide derivatives using different starting materials such as 3-(2-bromoacetyl)coumarin, ethyl thiooxamate, hydrazine, substituted benzaldehydes(Scheme 2.4). Moreover, the title compounds were evaluated against α-glucosidase inhibitory activity. In which compound **7** (Figure 2.7) showed excellent activity.



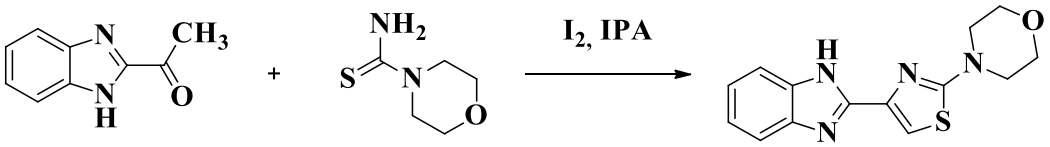
Scheme 2.4



7

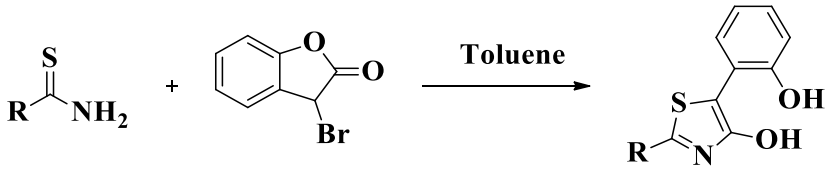
Figure 2.7

R. P. Tayade and N. Sekar[42] reported the synthesis of 4-4(1*H*-benzo[*d*]imidazol-2-yl) thiazol-2yl) morpholine starting from 2-acetyl benzimidazole, morpholine-4-carbothioamide and iodine in the presence of isopropyl alcohol under reflux condition with less time and excellent yields (Scheme 2.5).



Scheme 2.5

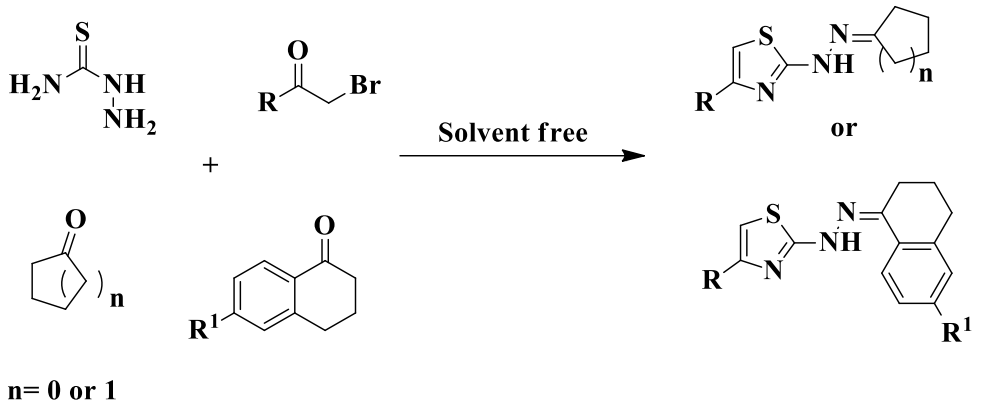
J. Hanusek[43] and co-workers synthesized a series of 2-aryl-4-hydroxy-5(2'-hydroxyphenyl) thiazole derivatives starting from substituted thioamides, 3-bromo-1-benzofuran-2(3*H*)-one and pyridine in presence of toluene under reflux conditions. Furthermore, the title compounds showed good fluorescence properties under neutral and alkaline atmosphere (Scheme 2.6).



Scheme 2.6

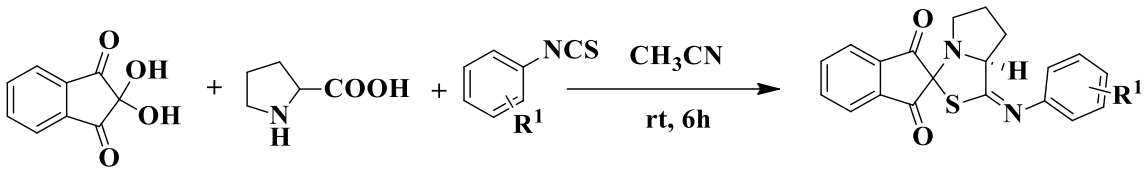
K. Sujatha and R. R. Vedula[44] synthesized a series of 2,4-disubstituted thiazoles *via* a multicomponent approach. The title compounds synthesized using thiosemicarbazide, 3-(2-bromoacetyl)-2*H*-chromen-2-ones or phenacyl bromides and cyclic ketones under solvent free

conditions with good yields (Scheme 2.7).



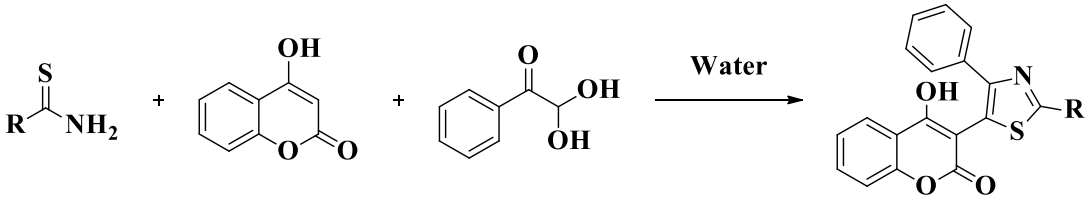
Scheme 2.7

P. R. Likhar[45] *et al.* synthesized a new series of spiro fused thiazole analogues *via* a one pot three component reaction. These title compounds were synthesized using readily available starting materials i.e. ninhydrin with proline and substituted isothiocyanates *via* (3+2) a dipolar cycloaddition with excellent yields (Scheme 2.8).



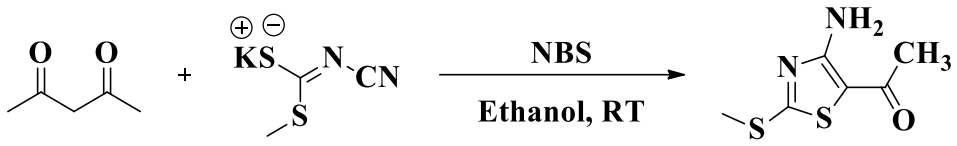
Scheme 2.8

L. H. Choudhury[46] and co-workers synthesized a series of novel trisubstituted 1,3-thiazole analogues *via* a microwave irradiated multi component reaction method. The title compounds were synthesized using readily available starting materials i.e. thioamide with 4-hydroxy coumarin and 2,2-dihydroxy-1-phenylethanone under catalyst free reaction condition with good yields (Scheme 2.9).



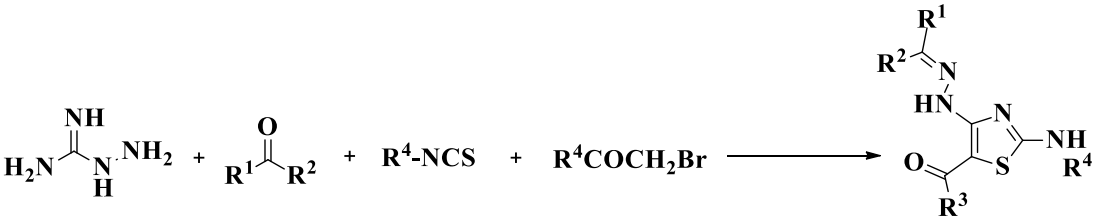
Scheme 2.9

R. Li[47] *et al.* established *N*-bromo succinimide catalysed a Thorpe–Ziegler cyclization one pot synthesis of 1-(4-amino-2-(methylthio)thiazol-5-yl)ethanone. The title compound synthesized using readily available starting materials i.e. acetylacetone, potassium salt of mercaptonitrile under mild reaction conditions with excellent yields (Scheme 2.10).



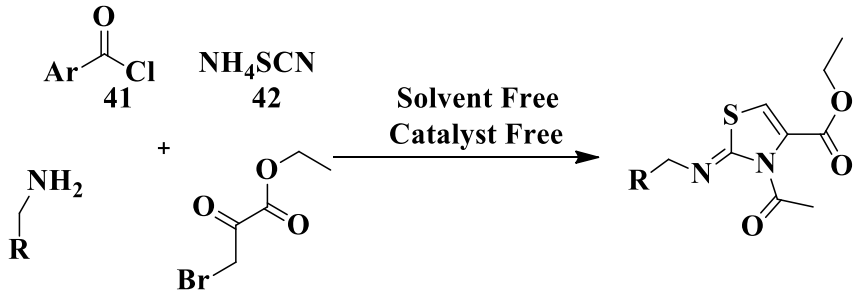
Scheme 2.10

S. Titus and K. G. Sreejalekshmi [48] synthesized a series of 4-hydarzinothiazole derivatives *via* a one pot four component reaction approach. The title compounds were synthesized using aminoguanidine, carbonyl compounds, isothiocyantes and α-halo carbonyl compounds with excellent yields (Scheme 2.11).



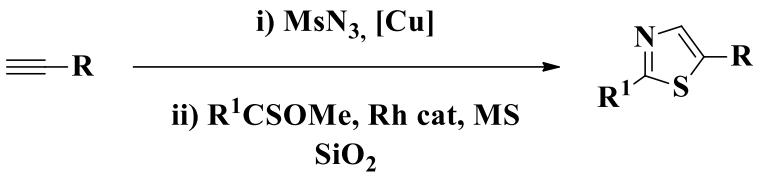
Scheme 2.11

M. Keshavarz[49] *et al.* developed a series of new *N*-acyl-2-alkylimino-2,3-dihydrothiazole-4-carboxylate derivatives *via* a green and solvent free four component synthesis.. The target molecules were synthesized from the reaction of acid chloride, ammonium thiocyanate, alkyl amine and ethyl bromopyruvate under mild reaction conditions with good yields (Scheme 2.12).



Scheme 2.12

T. Miura[50] *et al.* synthesized a series of new 2, 5-substituted thiazole analogues *via* a sequential multi component approach. The title compounds were synthesized from the reaction of terminal alkynes with sulfonyl azides and thiono esters. The copper (I) catalyzed cycloaddition reaction of alkynes with sulfonyl azides generates 1-sulfonyl triazole, which further it is reaction with thionoester in presence of rhodium (II) followed by aromatization gives the title compound with good yields (Scheme 2.13).



Scheme 2.13

M. de Souza[51] *et al.* synthesized a series of new 2, 4 – substituted thiazole (Figure 2.8) analogues *via* Hantzsch cyclization by a fast, eco-friendly and solvent-free reaction conditions with good yields.

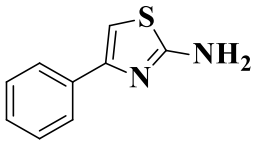
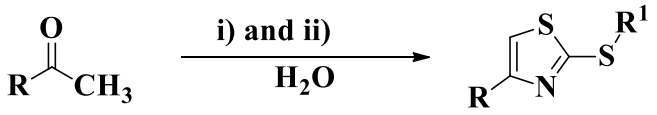


Figure 2.8

A. Ziyaei Halimehjani[52] *et al.* synthesized a series of new 2,4-disubstituted thiazoles *via* a simple, eco-friendly and one pot two-step process. The title compounds were synthesized using acetophenone and dithiocarbamates with excellent yields (Scheme 2.14).

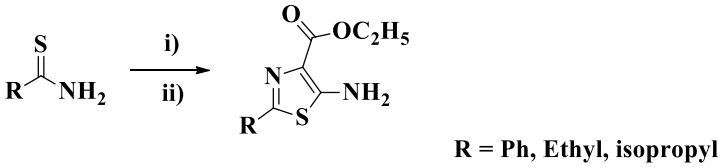


R = Ph, R^1 = Aryl / Alkyl

i) = I_2/ CuO / Ethanol ii) = Dithiocarbamate

Scheme 2.14

A. McClory[53] *et al.* synthesized a new series of 2-substituted ethyl 5-amino thiazole-4-carboxylate analogues *via* Strecker's cyclisation. Aliphatic and aromatic thioamides undergo 1,2-addition with ethyl glyoxalete to give hemiaminal intermediate, which is further treated with acetyl chloride in presence of pyridine to give the parallel imine. The intermediate imine was treated with aqueous sodium cyanide to give the Strecker's amino-thiazoles in excellent yields (Scheme 2.15).

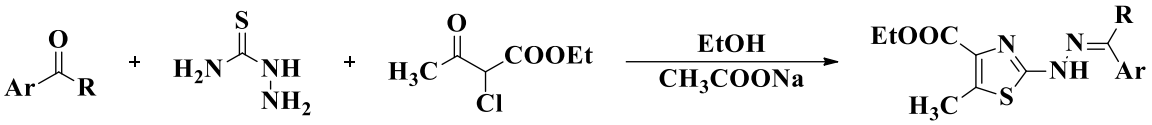


R = Ph, Ethyl, isopropyl

i) =EtO_2CHO / Py ii) NaCN / CH_3CN -H_2O

Scheme 2.15

K. Ablajan[54] *et al.* synthesized a series of new thiazoles *via* a multi component reaction. The title compounds were synthesized from starting materials like aldehydes / ketones with thiosemicarbazide and ethyl 2-chloro-3-oxobutanoate, sodium acetate as a catalyst in the presence of ethanol under reflux conditions. Furthermore, it is an eco-friendly and clean synthesis (Scheme 2.16).

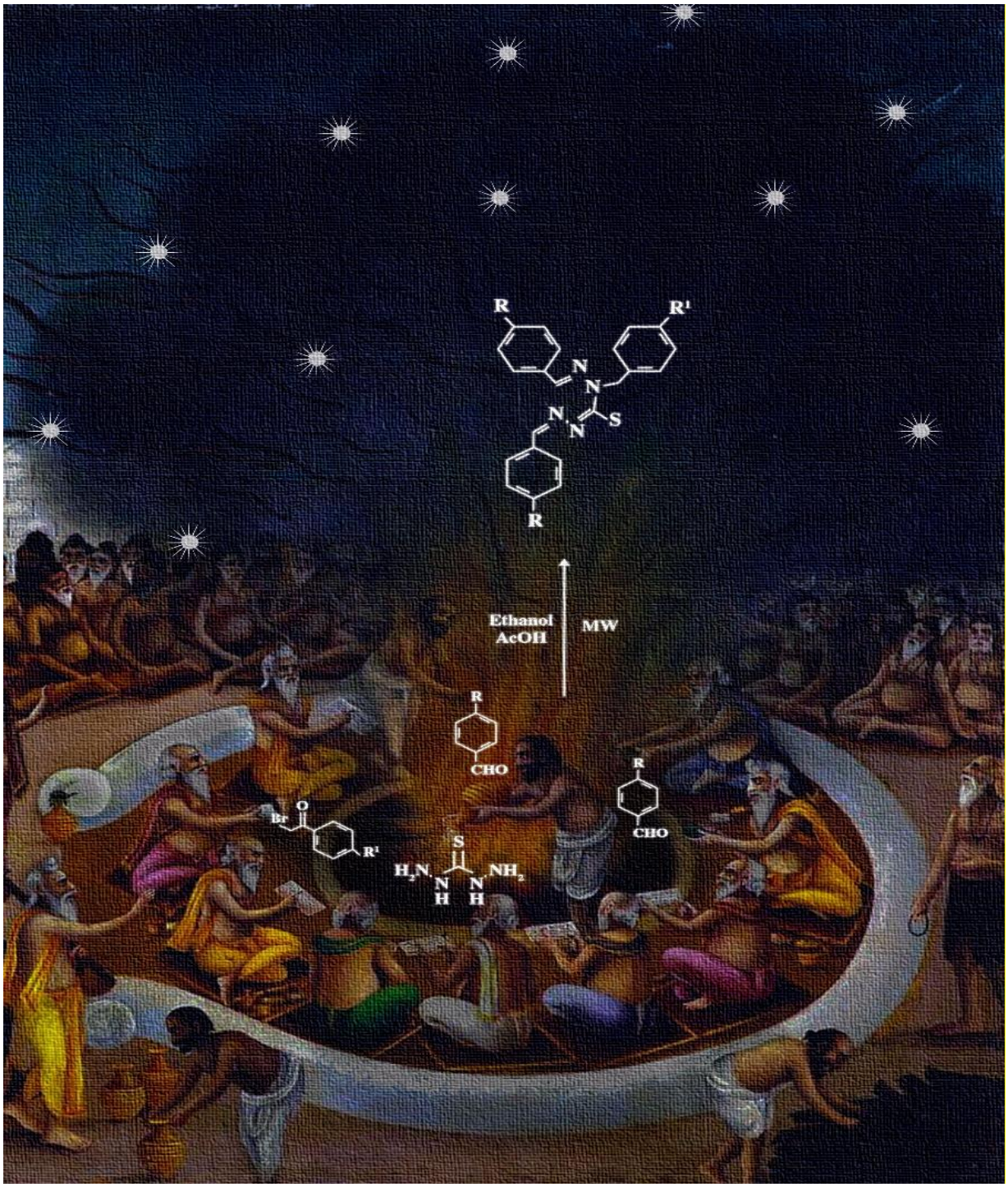


Scheme 2.16

The present chapter deals with microwave irradiated one pot, pseudo four component synthesis of a new thiazoles (**Part-A**) and microwave irradiated one pot, pseudo four component synthesis of a new series of hybrid coumarin based thiazoles (**Part-B**).

CHAPTER-II
SECTION-A

Microwave irradiated one-pot, pseudo four component synthesis of new thiazoles



CHAPTER-II
SECTION-A

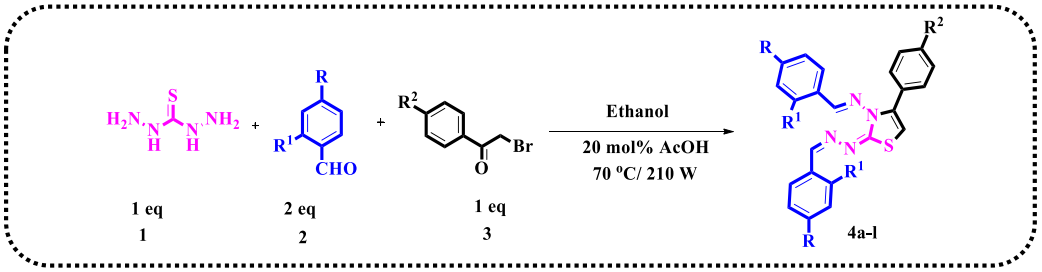
2A. Present Work:

2A.1. Starting Materials:

In this present chapter, the synthesis of new thiazole hybrids was described. The starting materials required for the synthesis of the target compounds are thiocarbohydrazide, substituted aldehydes and substituted 2-bromoacetophenones. Thiocarbohydrazide was prepared by using hydrazine hydrate and carbon disulfide[55,56]. Aldehydes and substituted 2-bromoacetophenones were procured from commercial sources.

2A.2. Synthesis of Thiazoles:

The synthesis of target thiazole analogues were carried out as outlined in Scheme 2A.1. The title substituted thiazoles (**4a-l**) were synthesized by a reaction of thiocarbohydrazide (**1**), aldehyde (**2**) and phenacyl bromides (**3**) (1:2:1) in ethanol in the presence of catalytic amount of acetic acid under microwave irradiation with good yields.

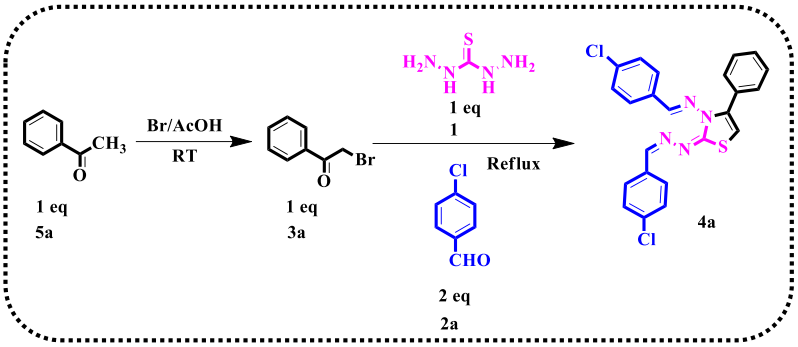


Scheme 2A.1: Synthesis of thiazoles; Reagents and conditions: EtOH, AcOH, microwave irradiation at 70°C and 210 W.

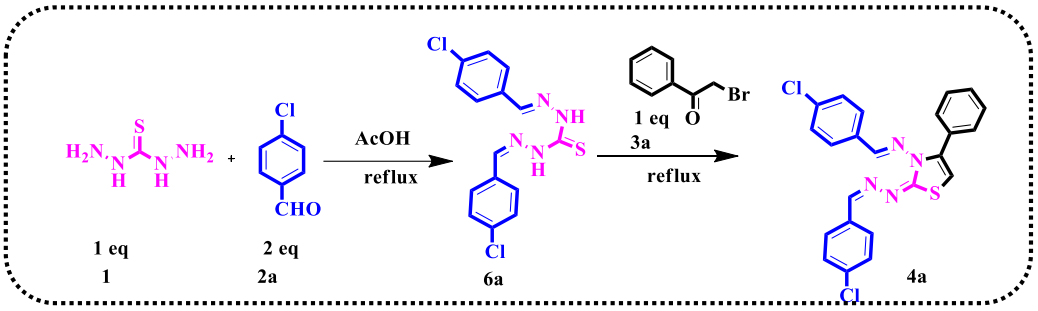
2A.3. Results and discussion:

At first, the synthesis of thiazole moiety was carried out simultaneously in two reactions scheme 2A.2 and scheme 2A.3. Since the yields were poor (22% and 26% respectively) in both the cases, optimization for better yield of the desired product was done in conventional and microwave methods. In conventional method, reaction was carried out in one-pot multicomponent approach using thiocarbohydrazide (**1**), aldehyde (**2a**), phenacyl bromide (**3a**) as starting materials in different solvents like methanol, ethanol, dimethyl sulfoxide, dimethylformamide and acetonitrile (Table 2A.1, entries 1–5). The initial screening results indicated that ethanol (Table 2A.1, entry 5) was best among the tested solvents in terms of time and yield. Additional optimization of the reaction involved usage of a catalyst like acetic acid, hydrochloric and sulfuric acid. Their percentages (Table 2A.1, entries 6–10) were tested in ethanol and it has been noticed

that acetic acid (20 mol %) (Table 2A.1, entry 9) was a suitable catalyst which gives more yields. However, beyond 60^{o}C (reflux) there is no further improvisation of the product (Table 2A.1, entry 11). Finally, highest conversion rate and lesser time with an yield of 72% obtained at 60 ^{o}C (Table 2A.1, entry 9).



Scheme 2A.2: Synthesis of thiazole hybrids route-1.



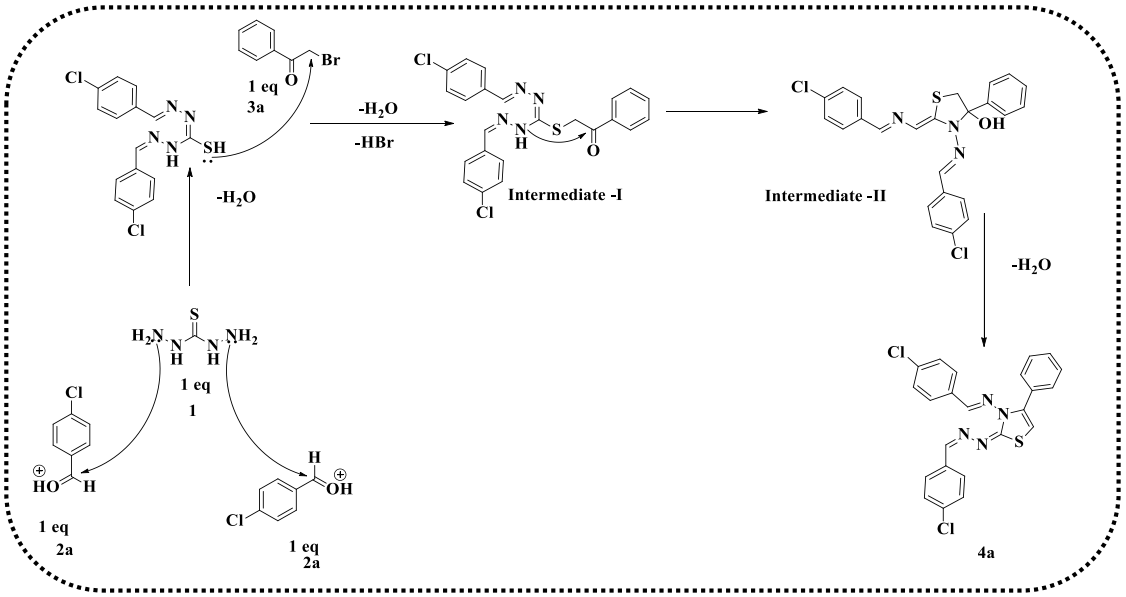
Scheme 2A.3: Synthesis of thiazole hybrids route-2.

Microwave irradiation method was employed to confirm the reaction feasibility by choosing thiocarbohydrazide (**1)**, aldehyde (**2a)** and phenacyl bromide (**3a**) as starting materials in the above-mentioned solvents (Table 2A.1, entries 1–5). Out of the solvents screened, results shown (Table 2A.1, entry 5), implies ethanol as the best solvent in terms of time and yield. Optimization of the reaction in terms of catalyst like acetic acid, hydrochloric acid and sulfuric acid (Table 2A.1, entries 6–10) in ethanol was also carried out. Results indicate that 20 mol% of acetic acid (Table 2A.1, entry 9) gave good yields and was considered for further reactions. An efficient green protocol with microwave assisted reaction temperature and power has been tested (Table 2A.1, entries 10–11). On the other hand, no additional improvisation of the product was observed beyond (temperature/power). Subsequently, 70/210 (Table 2A.1, entry 11) gave 88 % yield with highest conversion rate and lower time. To conclude, high yield at less reaction time was observed in ethanol at 70 ^{0}C with 20 mol% acetic acid as catalyst under microwave irradiation (210W).

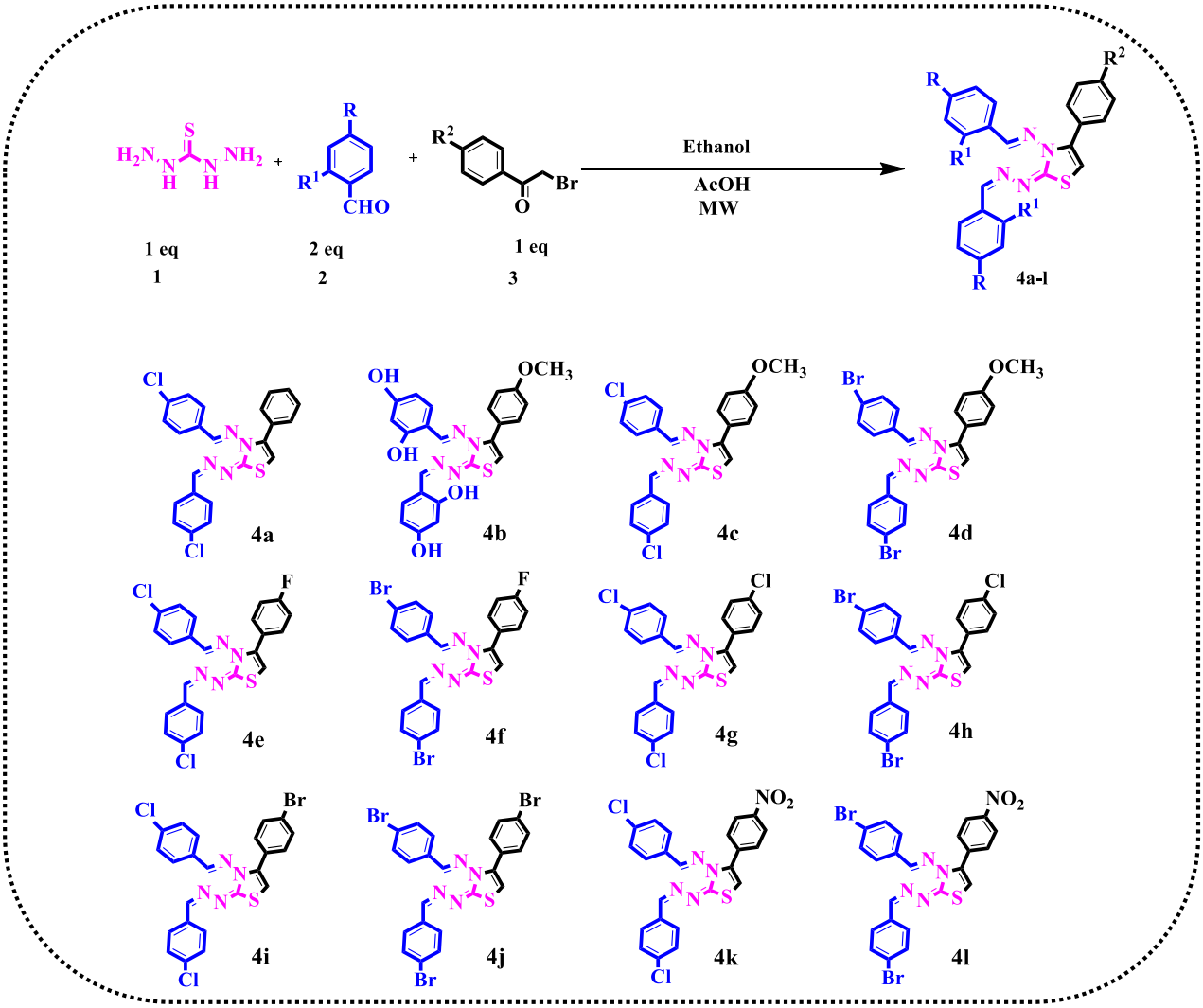
Table 2A.1: Optimizing the reactions conditions under conventional and microwave conditions for the synthesis of thiazoles.

Entry	Solvent	Catalyst (mol%)	Conventional			Microwave		
			Temperature (ºC)	Time(h)	Yield(%)	Temperature (ºC/W)	Time(min)	Yield[a](%)
1	MeOH	-	60	14	22	70/140	24	28
2	EtOH	-	60	10	28	70/140	20	34
3	DMSO	-	60	16	25	70/140	28	22
4	DMF	-	60	15	23	70/140	26	18
5	CH_3CN	-	60	18	19	70/140	30	20
6	EtOH	CH_3COOH(10)	60	13	42	70/140	18	48
7	EtOH	HCl(10)	60	12	33	70/140	15	40
8	EtOH	H_2SO_4(10)	60	10	31	70/140	16	35
9	EtOH	CH_3COOH(20)	60	2	72	70/140	9	76
10	EtOH	CH_3COOH(30)	60	2	52	70/140	11	55
11	**EtOH**	**CH_3COOH(20)**	reflux	2	58	**70/210**	**6**	**88**
12	EtOH	CH_3COOH(20)	-	-	-	70/240	8	64

A plausible mechanism of the formation of compound **4a** is illustrated in Scheme 2A.4. In this reaction at first 2 equivalents of aromatic aldehydes with thiocarbohydrazide by acid catalyzed condensation to form bis-thiocarbohydrazone. Afterwards, the SN^2-type attack of bis-thiocarbohydrazone sulfur on the C–Br of phenacyl bromide gives intermediate-I, Subsequent attack of NH nitrogen on the carbonyl of the 2-bromo-1-phenylethanone part leads to the formation of intermediate-II, which further undergoes acid-catalyzed dehydration to form thiazole **4a**.



Scheme 2A.4: A plausible mechanism for the one-pot three-component formation of thiazole compound **4a**.



Scheme 2A.5: Synthesis of thiazole hybrids (**4**) Reagents and conditions: EtOH, AcOH Cat., MW, 4-6 min. Structures of all the synthesized compounds were in coincidence with their spectral and analytical data.

Table 2A.2: Different substitutions of thiazole hybrids (**4a-l**), time, [a]isolated yield.

Entry	Product	R	R^1	R^2	Time(min)	Yield (%)[a]
1	**4a**	Cl	H	H	6	88
2	**4b**	OH	OH	OCH_3	5	86
3	**4c**	Cl	H	OCH_3	4	91
4	**4d**	Br	H	OCH_3	5	89
5	**4e**	Cl	H	F	5	82
6	**4f**	Br	H	F	5	85
7	**4g**	Cl	H	Cl	5	92
8	**4h**	Br	H	Cl	6	86
9	**4i**	Cl	H	Br	5	89
10	**4j**	Br	H	Br	6	83
11	**4k**	Cl	H	NO_2	4	92
12	**4l**	Br	H	NO_2	5	85

The newly synthesized compounds structures were confirmed by their physical and analytical data. The NMR spectra of the compounds have shown characteristic thiazole ring proton in the

range of δ 6.15-7.09 ppm, while methine protons are in the range of δ 8.38-10.27 ppm. The ^{13}C NMR spectra have shown signals in the range of 161.33-168.69 ppm corresponding to the thiazole C_2 respectively. The mass spectra of all the synthesized compounds exhibited molecular ion peek representing their molecular formula.

The U.V absorption spectrum of **4b** exhibited two peaks at 280 and 395 nm that are characteristic bands to thiazole ring (Figure 2A.1A). In fluorescence spectroscopy, the thiazole motif exhibited fluorescence (Figure 2A.1A dotted line) at 500 nm when excited at 383 nm. Figure 2A.1B) represents the histogram of particles size distribution obtained by the DLS analysis for **4b**. The particle sizes show a narrow range of distribution and proved an average diameter of 100 ± 5 nm for **4b** and presented a positive average zeta potential of 8.92 mV.

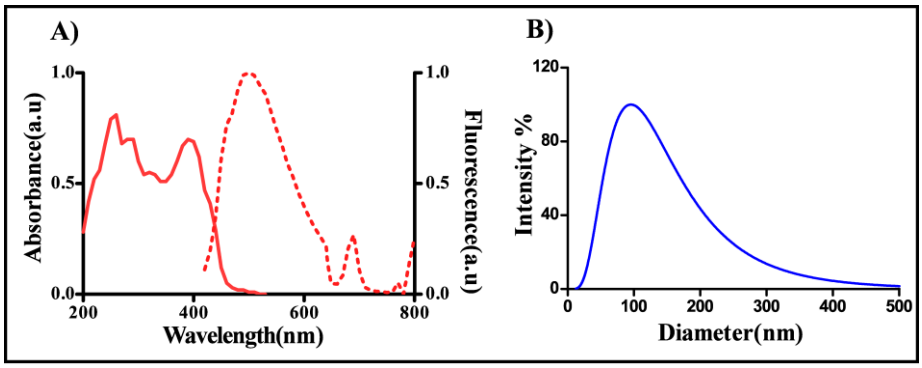


Figure 2A.1: A) UV-Visible and fluorescence spectra of **4b**, **2A.1:** B) DLS analysis of **4b**.

2A.4. Conclusion:

In summary, we have developed a potential green protocol for the synthesis of new thiazole derivatives by the microwave-assisted MCR approach.

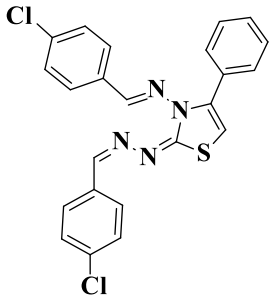
2A.5. Experimental:

General procedure for the synthesis of **N-(benzylidene)-2-(benzylidene)hydrazono)-4-phenylthiazol-3(2*H*)-amine (4a- 4l)**

A mixture of aldehyde (2 m mol), thiocarbohydrazide (1 m mol) and phenacyl bromides (1 m mol) in catalytic amount of acetic acid (20 mol%) and ethanol as a solvent was placed in a 10 mL pressurized vial and subjected to MW irradiation (mono-mode, CEM Discover microwave synthesis system at 210 W) at a temperature of 70°C for about 4–6 min; progress of the reaction is monitored by thin layer chromatography. After the completion of the reaction, product is filtered and isolated, washed with ethanol, dried and recrystallized from ethanol.

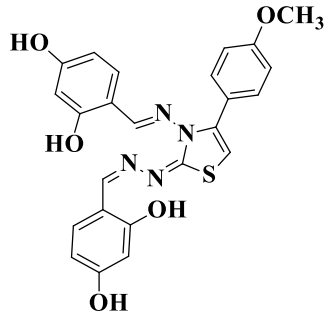
2A.6. Spectral Data:

N-(4-chlorobenzylidene)-2-((4-chlorobenzylidene)hydrazono)-4-phenylthiazol-3(2*H*)-amine (4a):



Yellow solid; yield: 88%; mp: 149-151ºC; IR (KBr) cm^{-1}: 1604 (C=N); ^{1}H NMR (400MHz, $CDCl_3$, ppm): δ 6.17 (s, 1H, thiazole-H), 7.32 (d, J = 8.4 Hz, 2H), 7.38 (d, J = 8.4 Hz, 2H), 7.43 (t, J = 2.8 Hz, 3H), 7.53-7.55 (m, 4H), 7.72 (d, J = 8.4 Hz, 2H), 8.40 (s, 1H, NCH), 10.23 (s, 1H, NCH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 100.03, 128.04, 128.76, 128.82, 128.96, 129.26, 131.40, 133.57, 133.68, 135.79, 136.19, 141.37, 151.94, 152.88, 166.46 ppm; Mass (ESI-HRMS) (m/z): 451.0552 $[M+H]^+$; Anal. Calcd. For $C_{23}H_{16}Cl_2N_4S$: C, 61.20; H, 3.57; N, 12.41%. Found: C, 61.24; H, 3.53; N, 12.45%.

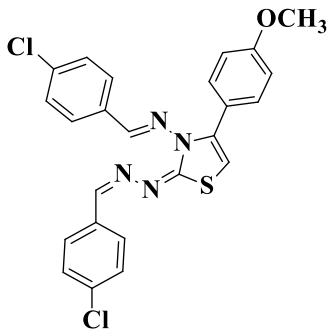
4-(3-(2,4-dihydroxybenzylidene)amino)-4-(4-methoxyphenyl)thiazol-2(3*H*)-ylidene)hydrazono)methyl)benzene-1,3-diol (4b):



Brown solid; yield: 86%; mp: 165-167ºC; IR (KBr) cm^{-1}: 1603 (C=N); ^{1}H NMR (400MHz, DMSO-d_6, ppm): δ 3.77 (s, 3H, OCH_3), 6.34 (s, 1H, thiazole-H), 6.38 (s, 4H), 6.92 (s, 1H), 6.99 (d, J = 8.8 Hz, 2H), 7.06 -7.09 (m, 1H), 7.42 – 7.48 (m, 4H), 7.59 (d, J = 9.2 Hz, 1H), 7.65 (d, J = 8.4 Hz, 1H), 8.64 (s, 1H, NCH), 9.24 (s, 1H, NCH)); ^{13}C NMR (100MHz, DMSO-d_6): δ 54.52, 89.94, 102.62, 102.84, 102.96, 108.66, 109.18, 109.86, 115.58, 118.32, 134.88, 162.29, 163.28,165.65 ppm; Mass (ESI-HRMS) (m/z): 477.1227 $[M+H]^+$; Anal. Calcd. For $C_{24}H_{20}N_4O_5S$: C, 60.49; H, 4.23; N, 11.76%. Found: C, 60.44; H, 4.27; N, 11.72%.

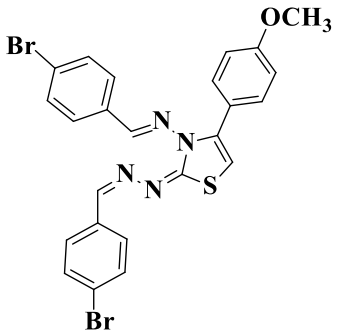
N-(4-chlorobenzylidene)-2-((4-chlorobenzylidene)hydrazono)-4-(4-methoxyphenyl)thiazol-

3(2*H*)-amine (4c):



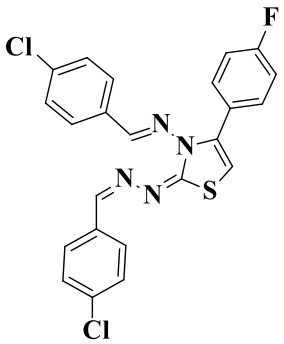
Yellow solid; yield: 91%; mp: 145-147ºC; IR (KBr) cm^{-1}: 1608 (C=N); ^{1}H NMR (400MHz, DMSO-d_6, ppm): δ 3.81 (s, 3H, OCH_3), 6.64 (s, 1H, thiazole-H), 7.02 (d, J = 8.8 Hz, 2H), 7.50-7.55 (m, 6H), 7.66 (d, J = 8.8 Hz, 2H), 7.79 (d, J = 8.4 Hz, 2H), 8.53 (s, 1H, NCH), 10.11 (s, 1H, NCH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 55.70, 114.02, 128.28, 129.38, 129.45, 129.58, 129.85, 130.23, 130.84, 134.04, 134.61, 135.06, 153.43, 153.97, 160.05, 166.08 ppm; Mass (ESI-HRMS) (m/z): 481.0587 $[M+H]^+$; Anal. Calcd. For $C_{24}H_{18}C_{l2}N_4OS$: C, 59.88; H, 3.77; N, 11.64%. Found: C, 59.85; H, 3.73; N, 11.69%.

N-(4-bromobenzylidene)-2-((4-bromobenzylidene)hydrazono)-4-(4-methoxyphenyl)thiazol-3(2*H*)-amine (4d):



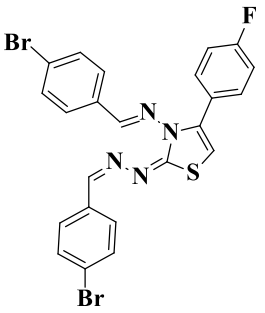
Yellow solid; yield: 89%; mp: 148-150ºC; IR (KBr) cm^{-1}: 1606 (C=N); ^{1}H NMR (400MHz, $CDCl_3$, ppm): δ 3.87 (s, 3H, OCH_3), 6.09 (s, 1H, thiazole-H), 6.94 (d, J = 8.8 Hz, 2H), 7.45-7.48 (m, 6H), 7.53 (d, J = 8.4 Hz, 2H), 7.64 (d, J = 8.4 Hz, 2H), 8.37 (s, 1H, NCH), 10.19 (s, 1H, NCH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 55.38, 98.90, 113.44, 123.82, 124.15, 124.61, 129.00, 129.03, 130.64, 131.89, 131.92, 134.01, 134.13, 141.11, 152.11, 152.84, 159.98, 166.53 ppm; Mass (ESI-HRMS) (m/z): 568.9677 $[M+H]^+$; Anal. Calcd. For $C_{24}H_{18}Br_2N_4OS$: C, 50.54; H, 3.18; N, 9.82%. Found: C, 50.50; H, 3.14; N, 9.87%.

N-(4-chlorobenzylidene)-2-((4-chlorobenzylidene)hydrazono)-4-(4-fluorophenyl)thiazol-3(2*H*)-amine (4e):



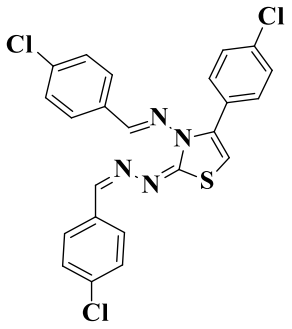
Yellow solid; yield: 82%; mp: 155-157 ºC; IR (KBr) cm^{-1}: 1606 (C=N); ^{1}H NMR (400MHz, $CDCl_3$, ppm): δ 6.15 (s, 1H, thiazole-H), 7.13 (t, J = 8.4 Hz, 2H), 7.34 (d, J = 8.4 Hz, 2H), 7.38 (d, J = 8.4 Hz, 2H), 7.45-7.53 (m, 4H), 7.72 (d, J = 8.4 Hz, 2H), 8.40 (s, 1H, NCH), 10.24 (s, 1H, NCH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 99.97, 115.02, 115.24, 128.70, 128.84, 128.97, 129.03, 131.13, 131.21, 133.56, 135.88, 136.32, 140.35, 151.98, 153.05,166.27 ppm; Mass (ESI-HRMS) (m/z): 468.0464 $[M+]^+$; Anal. Calcd. For $C_{23}H_{15}Cl_{l2}FN_4S$: C, 58.86; H, 3.22; N, 11.94%. Found: C, 58.82; H, 3.27; N, 11.90%.

N-(4-bromobenzylidene)-2-((4-bromobenzylidene)hydrazono)-4-(4-fluorophenyl)thiazol-3(2*H*)-amine (4f):



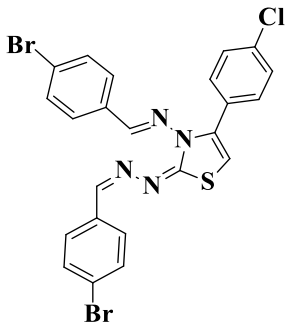
Yellow solid; yield: 85%; mp: 156-159ºC; IR (KBr) cm^{-1}: 1605 (C=N); ^{1}H NMR (400MHz, DMSO-d_6, ppm): δ 6.77 (s, 1H, thiazole-H), 7.32 (t, J = 8.8 Hz, 2H), 7.57 (d, J = 8.8 Hz, 2H), 7.62-7.68 (m, 6H), 7.73 (d, J = 8.8 Hz, 2H), 8.53 (s, 1H, NCH), 10.12 (s, 1H, NCH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 111.00, 115.82, 116.03, 127.94, 128.01, 128.56, 129.65, 131.73, 132.29, 132.80, 133.13, 134.60, 140.53, 149.04, 153.88, 158.20, 166.85 ppm; Mass (ESI-HRMS) (m/z): 556.9496 $[M+H]^+$; Anal. Calcd. For $C_{23}H_{15}Br_2FN_4S$: C, 49.48; H, 2.71; N, 10.04%. Found: C, 49.42; H, 2.75; N, 9.97%.

N-(4-chlorobenzylidene)-2-((4-chlorobenzylidene)hydrazono)-4-(4-chlorophenyl)thiazol-3(2*H*)-amine (4g):



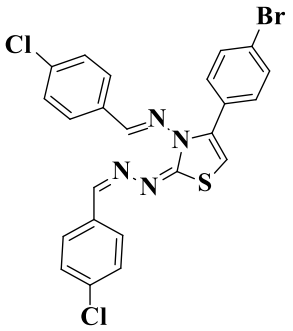
Yellow solid; yield: 92%; mp: 150-152°C; IR (KBr) cm^{-1}: 1610 (C=N); ^{1}H NMR (400MHz, $CDCl_3$, ppm): δ 6.17 (s, 1H, thiazole-H), 7.35 (d, J = 8.4 Hz, 2H), 7.39 (t, J = 8.4 Hz, 4H), 7.48 (d, J = 8.4 Hz, 2H), 7.53 (d, J = 8.4 Hz, 2H), 7.72 (d, J = 8.4 Hz, 2H), 8.40 (s, 1H, NCH), 10.24 (s, 1H, NCH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 100.48, 128.32, 128.72, 128.84, 128.98, 129.83, 130.48, 133.47, 134.77, 135.90, 136.37, 140.24, 152.07, 153.14,166.24 ppm; Mass (ESI-HRMS) (m/z): 484.0096 $[M+]^+$; Anal. Calcd. For $C_{23}H_{15}Cl_3N_4S$: C, 56.86; H, 3.11; N, 11.53%. Found: C, 56.82; H, 3.15; N, 11.58%.

N-(4-bromobenzylidene)-2-((4-bromobenzylidene)hydrazono)-4-(4-chlorophenyl)thiazol-3(2*H*)-amine (4h):



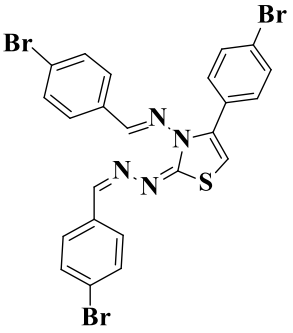
Yellow solid; yield: 86%; mp: 160-162°C; IR (KBr) cm^{-1}: 1611 (C=N); ^{1}H NMR (400MHz, $CDCl_3$, ppm): δ 6.18 (s, 1H, thiazole-H), 7.40 (d, J = 8.4 Hz, 2H), 7.45-7.50 (m, 6H), 7.54 (d, J = 8.4 Hz, 2H), 7.65 (d, J = 8.4 Hz, 2H), 8.38 (s, 1H, NCH), 10.23 (s, 1H, NCH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 100.52, 128.32, 128.93, 129.07, 130.49, 131.92, 132.01, 133.89, 134.80, 140.24, 152.12, 153.24, 166.59 ppm; Mass (ESI-HRMS) (m/z): 572.9086 $[M+H]^+$; Anal. Calcd. For $C_{23}H_{15}Br_2ClN_4S$: C, 48.07; H, 2.63; N, 9.75%. Found: C, 47.97; H, 2.67; N, 9.71%.

4-(4-Bromophenyl)-N-(4-chlorobenzylidene)-2-((4-chlorobenzylidene)hydrazono)thiazol-3(2*H*)-amine (4i):



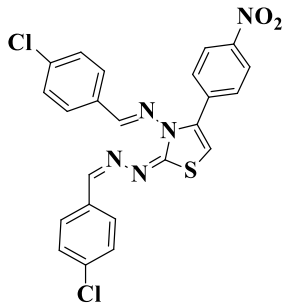
Yellow solid; yield: 89%; mp: 143-145^{o}C; IR (KBr) cm^{-1}: 1609 (C=N); ^{1}H NMR (400MHz, DMSO-d$_{6,}$ ppm): δ 6.83 (s, 1H, thiazole-H), 7.55 (d, J = 8.4 Hz, 6H), 7.67 (t, J = 8.4 Hz, 4H), 7.80 (d, J = 8.8 Hz, 2H), 8.55 (s, 1H, NCH), 10.13 (s, 1H, NCH); ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 105.21, 110.65, 118.52, 121.02, 128.01, 128.33, 129.39, 129.85, 130.23, 131.66, 132.01, 134.61, 138.96, 139.85, 140.54, 148.81, 155.40, 168.69 ppm; Mass (ESI-HRMS) (m/z): 528.9613 [M+H]$^{+}$; Anal. Calcd. For $C_{23}H_{15}BrCl_2N_4S$: C, 52.10; H, 2.85; N, 10.57%. Found: C, 52.14; H, 2.81; N, 10.53%.

N-(4-bromobenzylidene)-2-((4-bromobenzylidene)hydrazono)-4-(4-bromophenyl)thiazol-3(2*H*)-amine (4j):



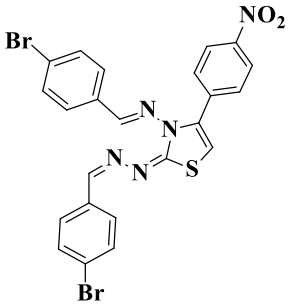
Orange solid; yield: 83%; mp: 153-155^{o}C; IR (KBr) cm^{-1}: 1610 (C=N); ^{1}H NMR (400MHz, DMSO-d$_{6,}$ ppm): δ 6.84 (s, 1H, thiazole-H), 7.54 (d, J = 8.4 Hz, 2H), 7.58 (d, J = 8.4 Hz, 2H), 7.67-7.69 (m, 6H), 7.73 (d, J = 8.8 Hz, 2H), 8.53 (s, 1H, NCH), 10.12 (s, 1H, NCH); ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 105.24, 110.99, 118.64, 128.01, 128.58, 131.72, 132.02, 132.29, 132.79, 133.13, 134.11, 134.60, 145.64, 149.90, 154.92, 168.67 ppm; Mass (ESI-HRMS) (m/z): 616.8655 [M+H]$^{+}$; Anal. Calcd. For $C_{23}H_{15}Br_3N_4S$: C, 44.62; H, 2.44; N, 9.05%. Found: C, 44.66; H, 2.40; N, 8.96%.

N-(4-chlorobenzylidene)-2-((4-chlorobenzylidene)hydrazono)-4-(4-nitrophenyl)thiazol-3(2*H*)-amine (4k):

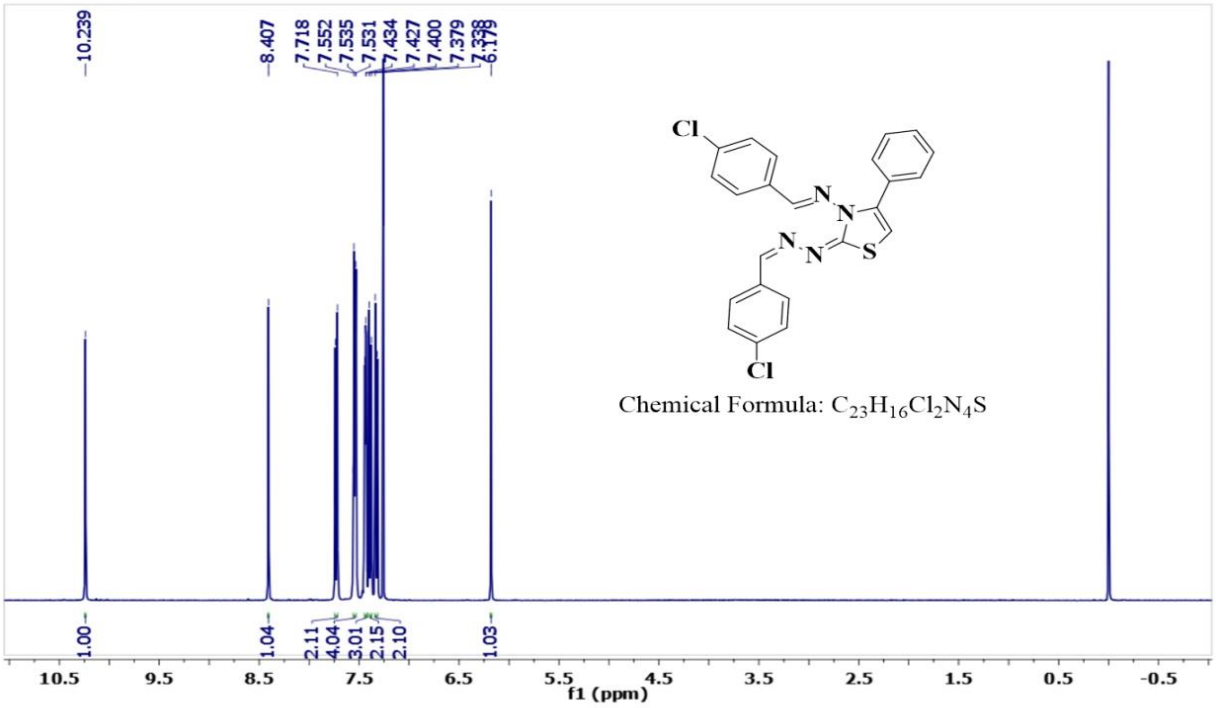


Yellow solid, yield: 92%; mp: 216-218ºC; IR (KBr) cm^{-1}: 1611 (C=N); ^{1}H NMR (400MHz, $CDCl_3$, ppm): δ 6.37 (s, 1H, thiazole-H), 7.35 -7.41 (m, 4H), 7.52 (d, J = 8 Hz, 2H), 7.73 (d, J = 7.6 Hz, 4H), 8.30 (d, J = 9.2 Hz, 2H), 8.42 (s, 1H, NCH), 10.27 (s, 1H, NCH), ^{13}C NMR (100 MHz, $CDCl_3$) δ 103.3, 123.40, 128.72, 128.93, 129.03, 129.18, 129.75, 133.15, 133.26, 136.14, 136.69, 137.46, 139.27, 147.62, 152.46, 153.73, 165.84 ppm. Mass (ESI-HRMS) (m/z): 495.0474 $[M+]^+$; Anal. Calcd. For $C_{23}H_{15}Cl_2N_5O_2S$: C, 55.65; H, 3.05; N, 14.11%. Found: C, 55.69; H, 3.02; N, 14.15%.

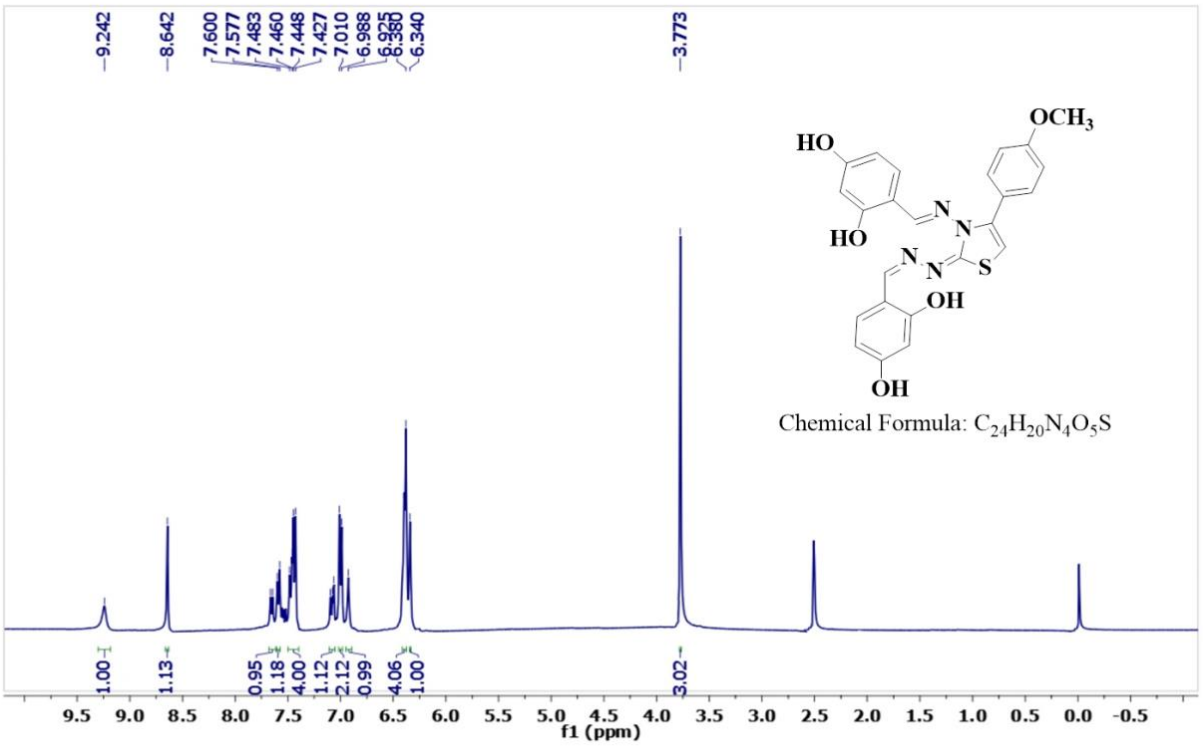
N-(4-bromobenzylidene)-2-((4-bromobenzylidene)hydrazono)-4-(4-nitrophenyl)thiazol-3(2*H*)-amine (4l):



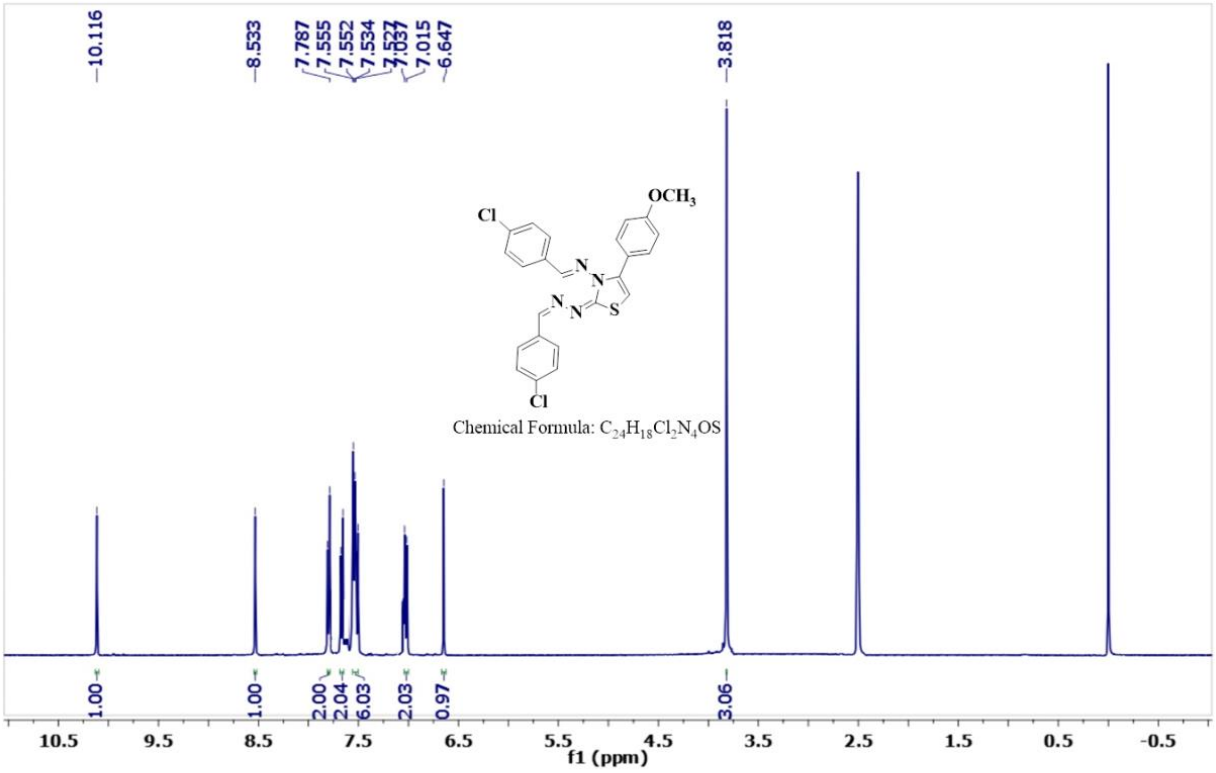
Orange solid; yield: 85%; mp: 231-233ºC; IR (KBr) cm^{-1}: 1609 (C=N); ^{1}H NMR (400MHz, DMSO-d_6, ppm): δ 7.09 (s, 1H, thiazole-H), 7.59 (d, J = 8.4 Hz, 2H), 7.67 -7.70 (m, 4H), 7.74 (d, J = 9.2 Hz, 2H), 7.89 (d, J = 9.2 Hz, 2H), 8.32 (d, J = 9.2 Hz, 2H), 8.56 (s, 1H, NCH), 10.14 (s, 1H, NCH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 109.23, 122.95, 124.59, 126.82, 128.63, 132.30, 133.13, 134.60, 141.01, 146.70, 151.06, 157.88, 159.46, 161.33 ppm; Mass (ESI-HRMS) (m/z): 583.9412 $[M+H]^+$; Anal. Calcd. For $C_{23}H_{15}Br_2N_5O_2S$: C, 47.20; H, 2.58; N, 11.97%. Found: C, 47.17; H, 2.61; N, 11.95%.



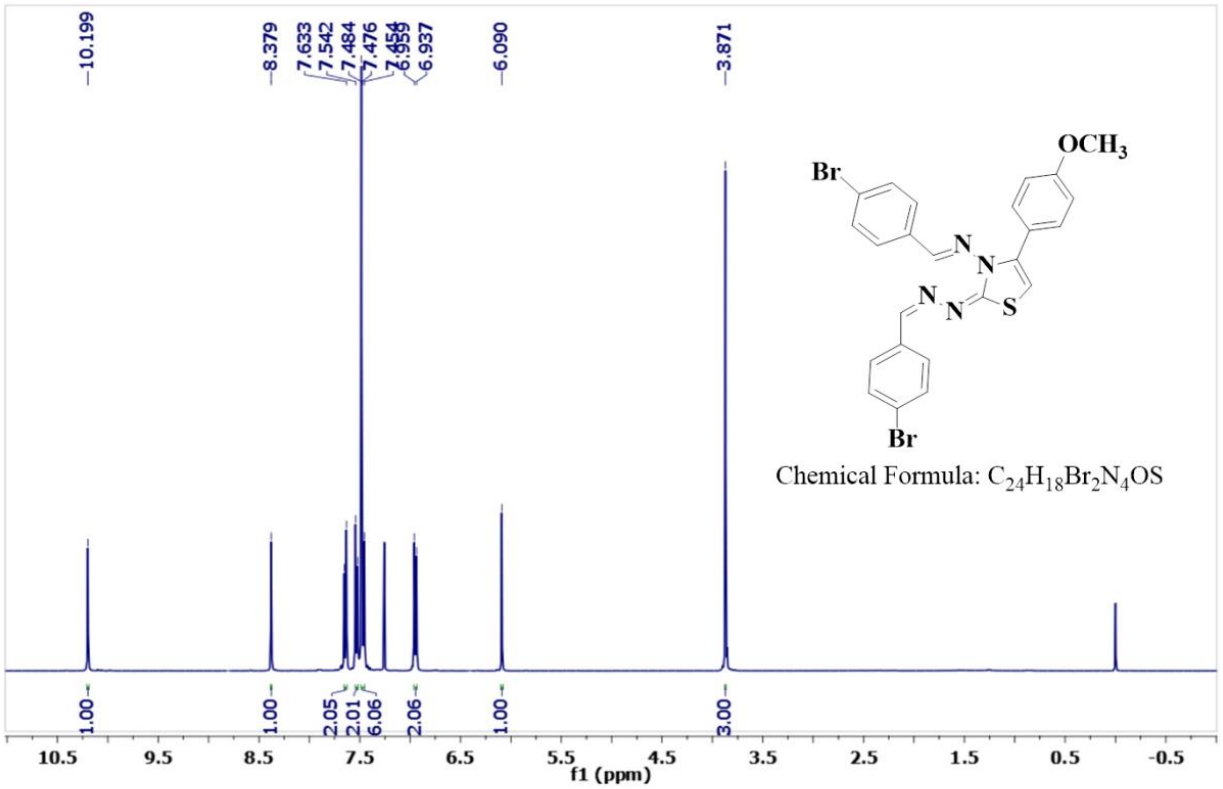
^{1}H NMR spectrum of compound **4a** (400 MHz, $CDCl_3$)



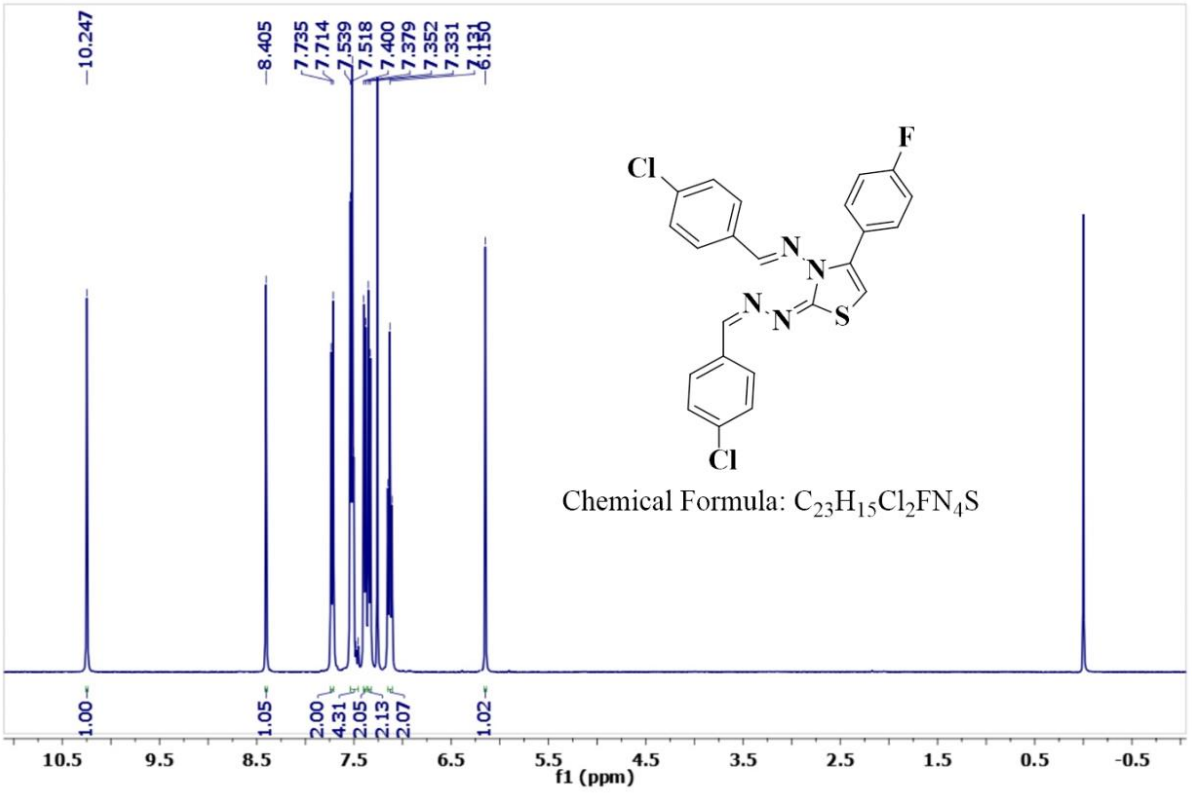
^{1}H NMR spectrum of compound **4b** (400 MHz, DMSO-d_6)



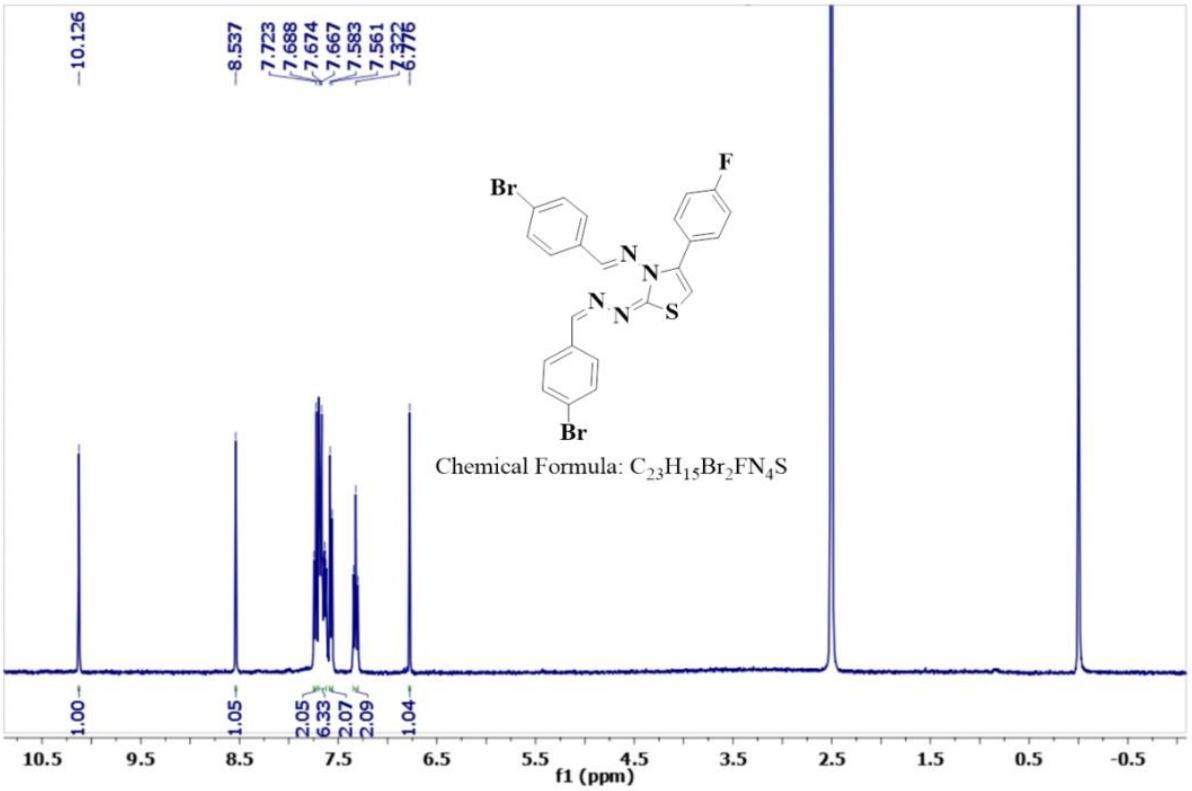
^{1}H NMR spectrum of compound **4c** (400 MHz, DMSO-d_6)



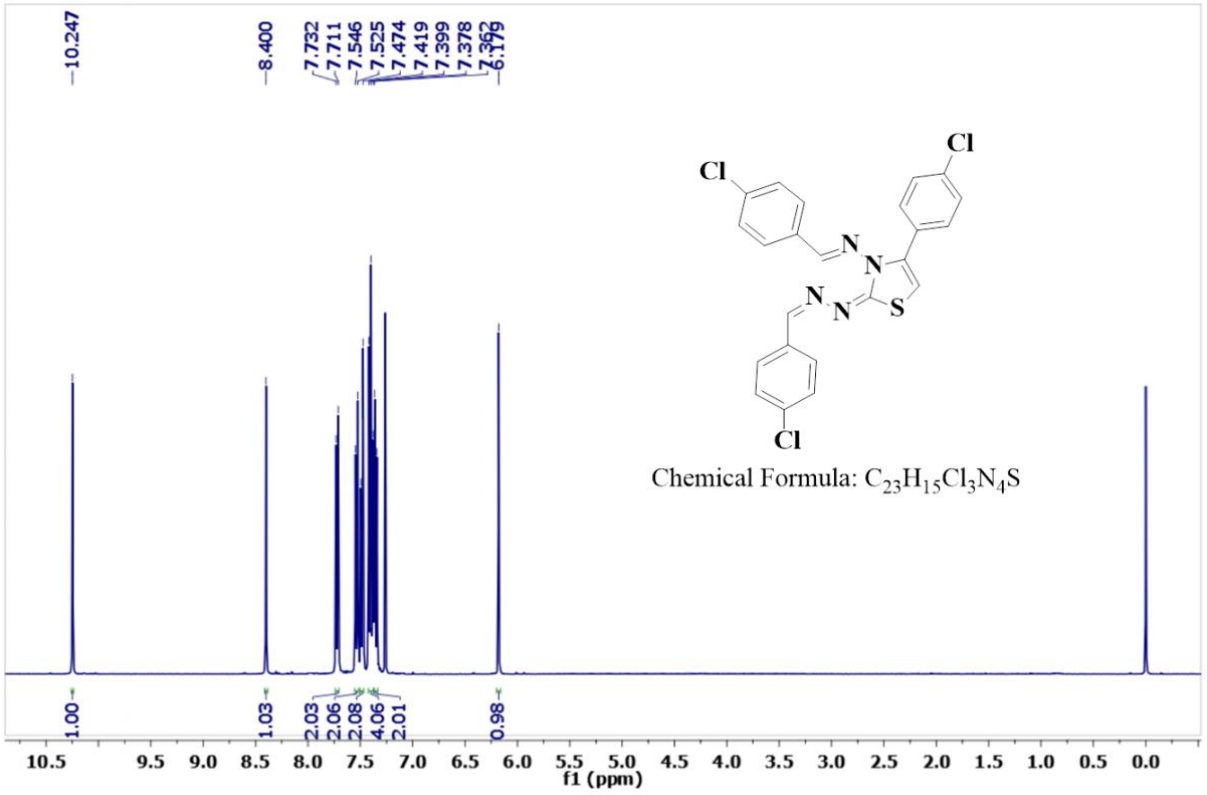
^{1}H NMR spectrum of compound **4d** (400 MHz, $CDCl_3$)



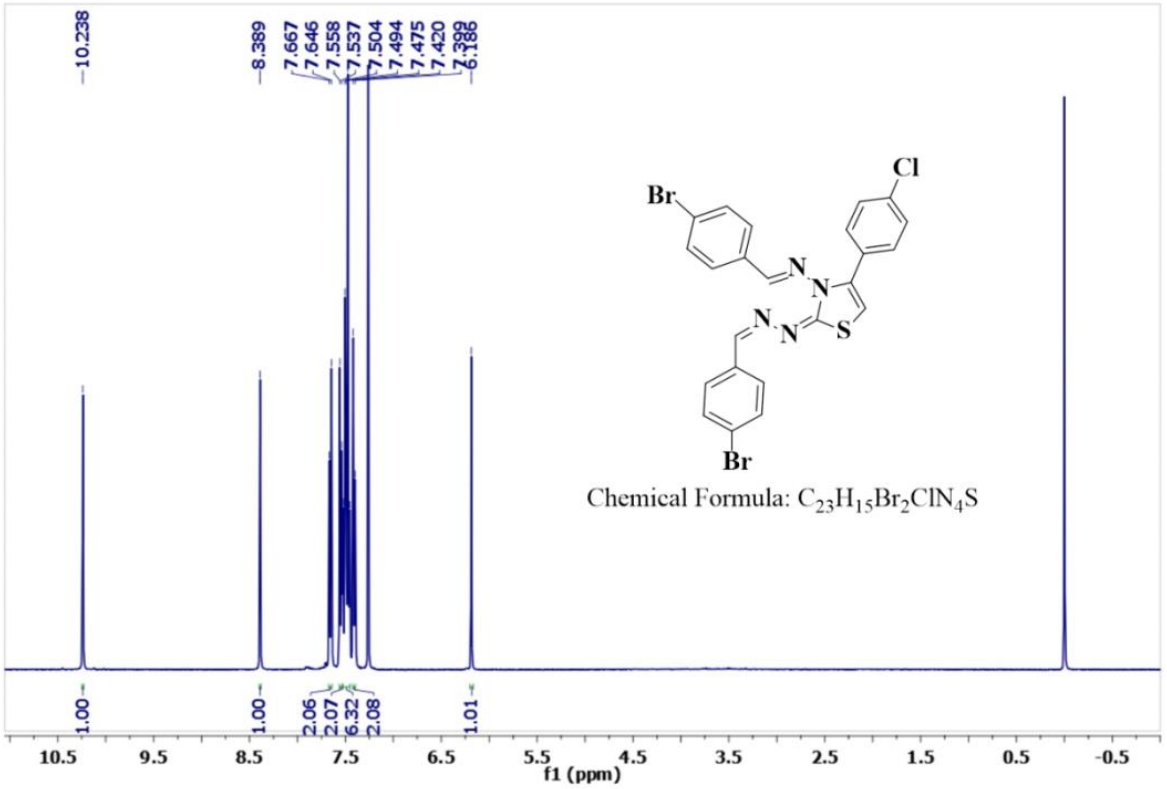
^{1}H NMR spectrum of compound **4e** (400 MHz, $CDCl_3$)



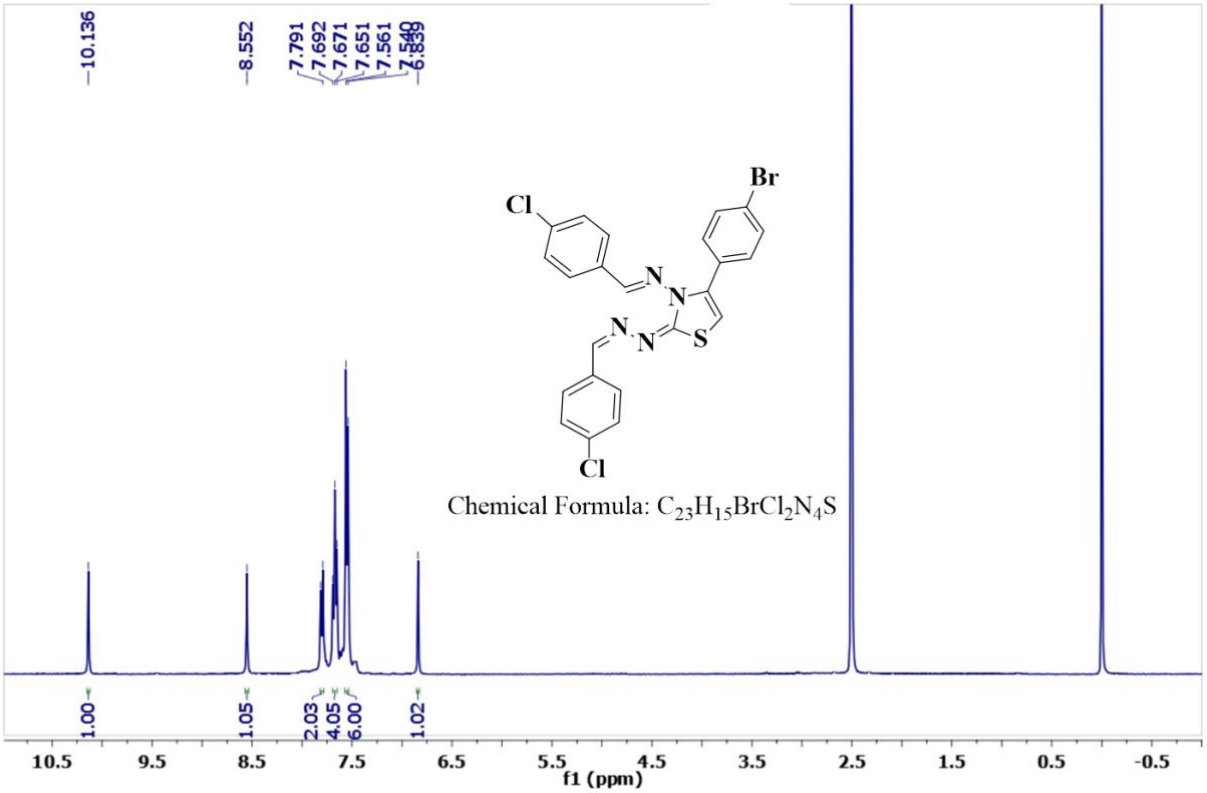
^{1}H NMR spectrum of compound **4f** (400 MHz, DMSO-d_6)



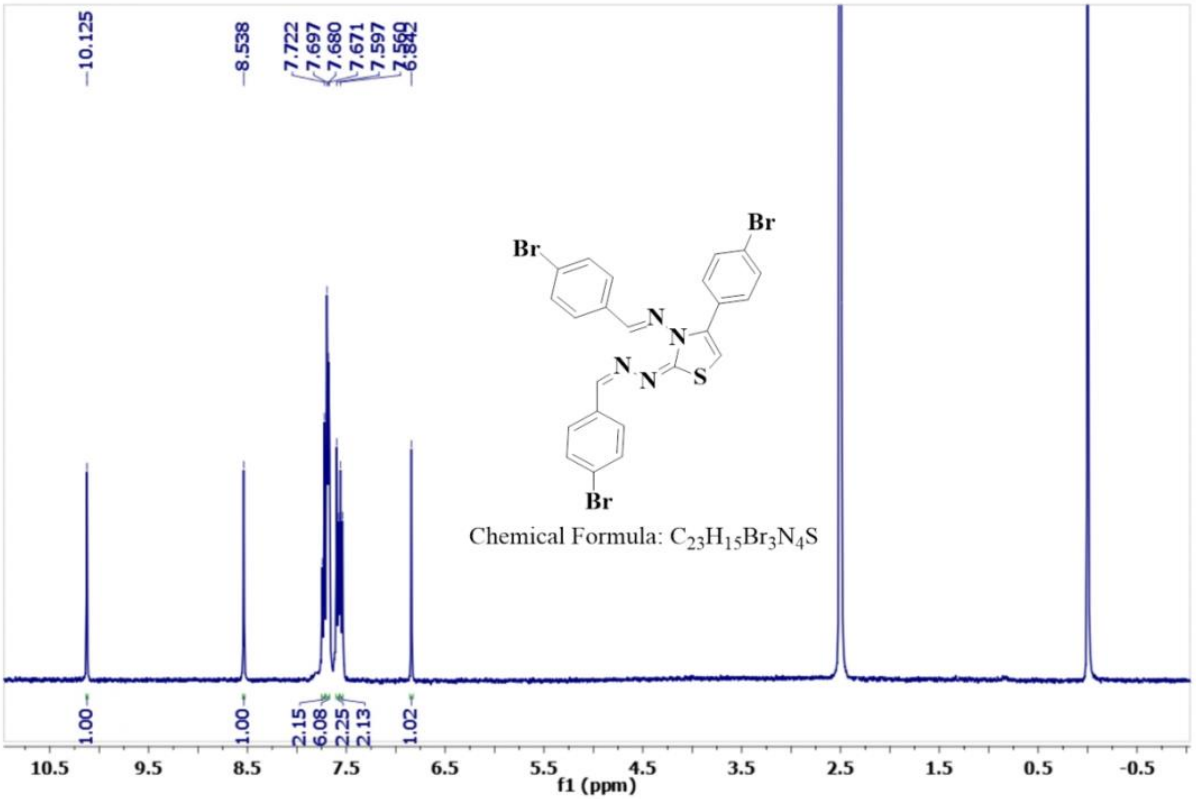
^{1}H NMR spectrum of compound **4g** (400 MHz, $CDCl_3$)



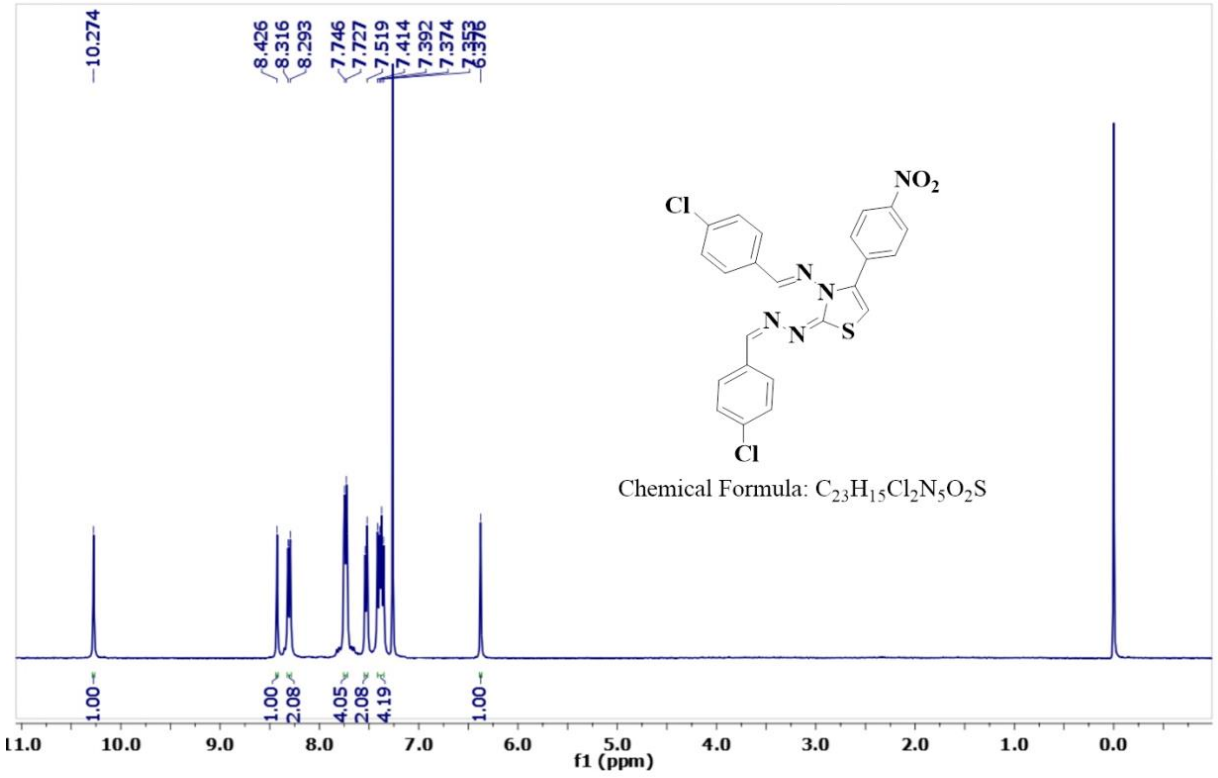
^{1}H NMR spectrum of compound **4h** (400 MHz, $CDCl_3$)



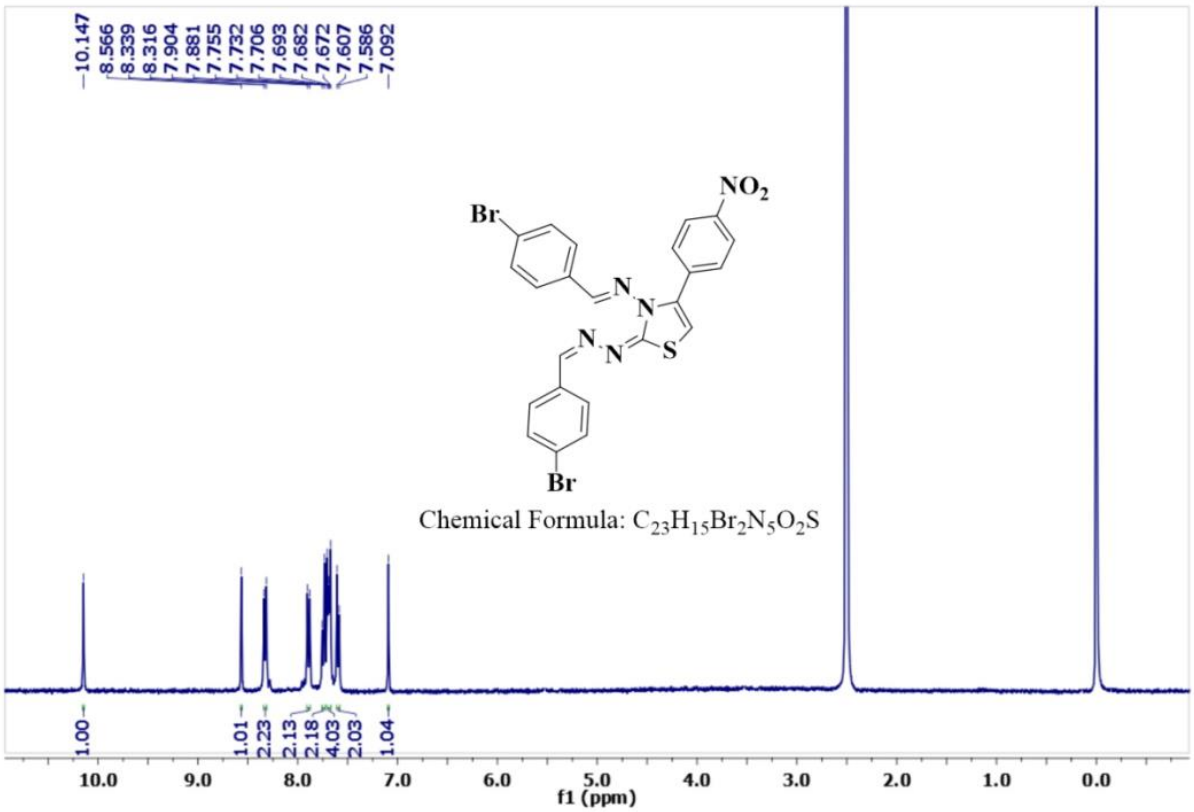
^{1}H NMR spectrum of compound **4i** (400 MHz, DMSO-d_6)



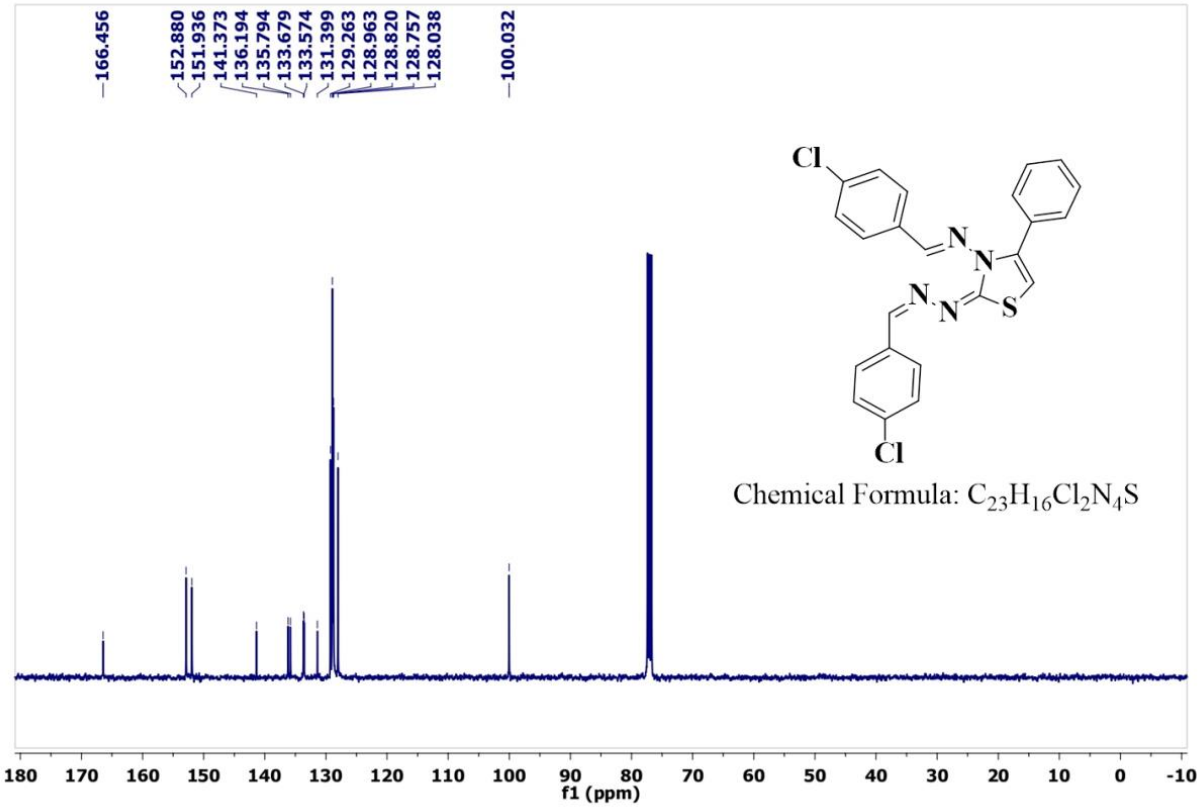
^{1}H NMR spectrum of compound **4j** (400 MHz, DMSO-d_6)



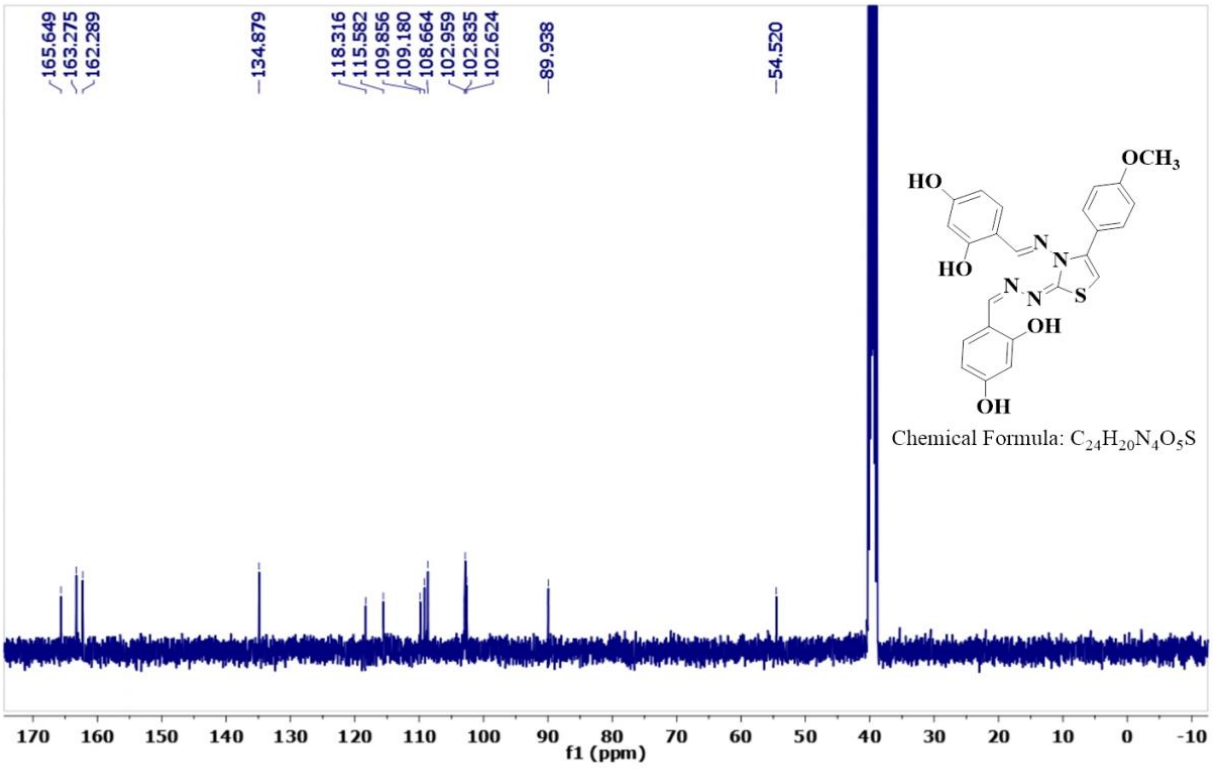
^{1}H NMR spectrum of compound **4k** (400 MHz, $CDCl_3$)



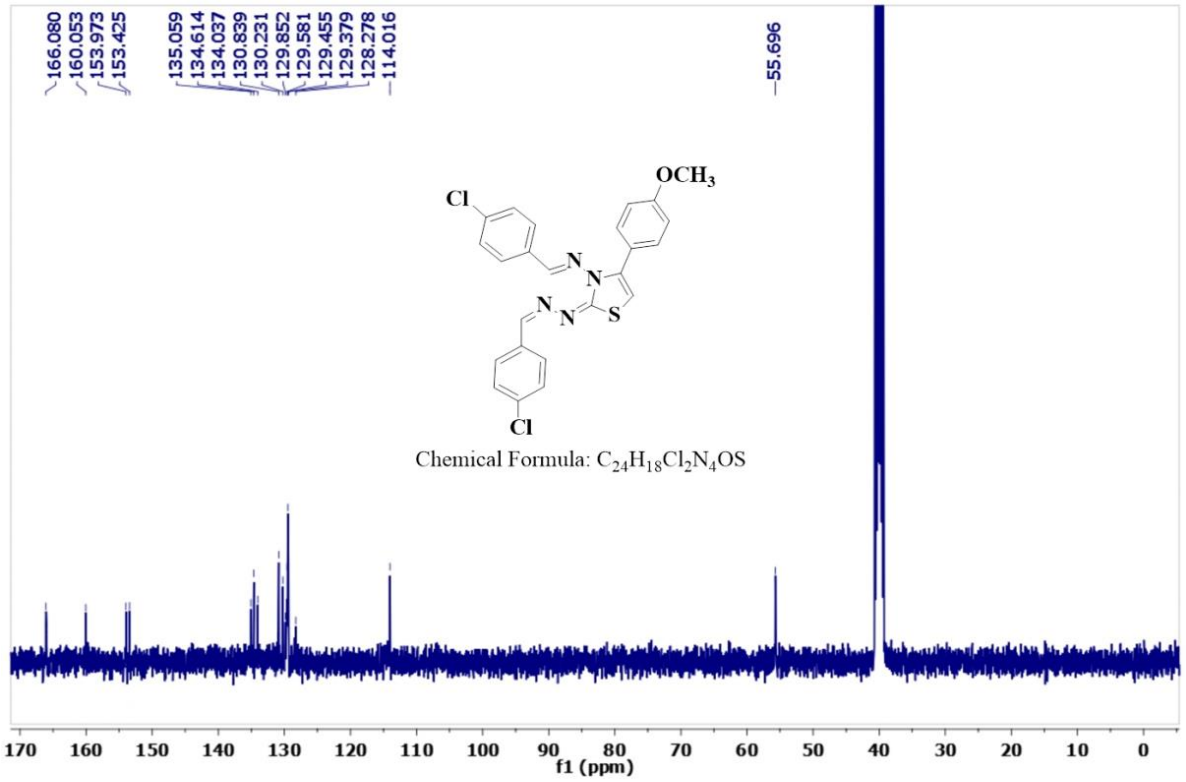
^{1}H NMR spectrum of compound **4l** (400 MHz, DMSO-d_6)



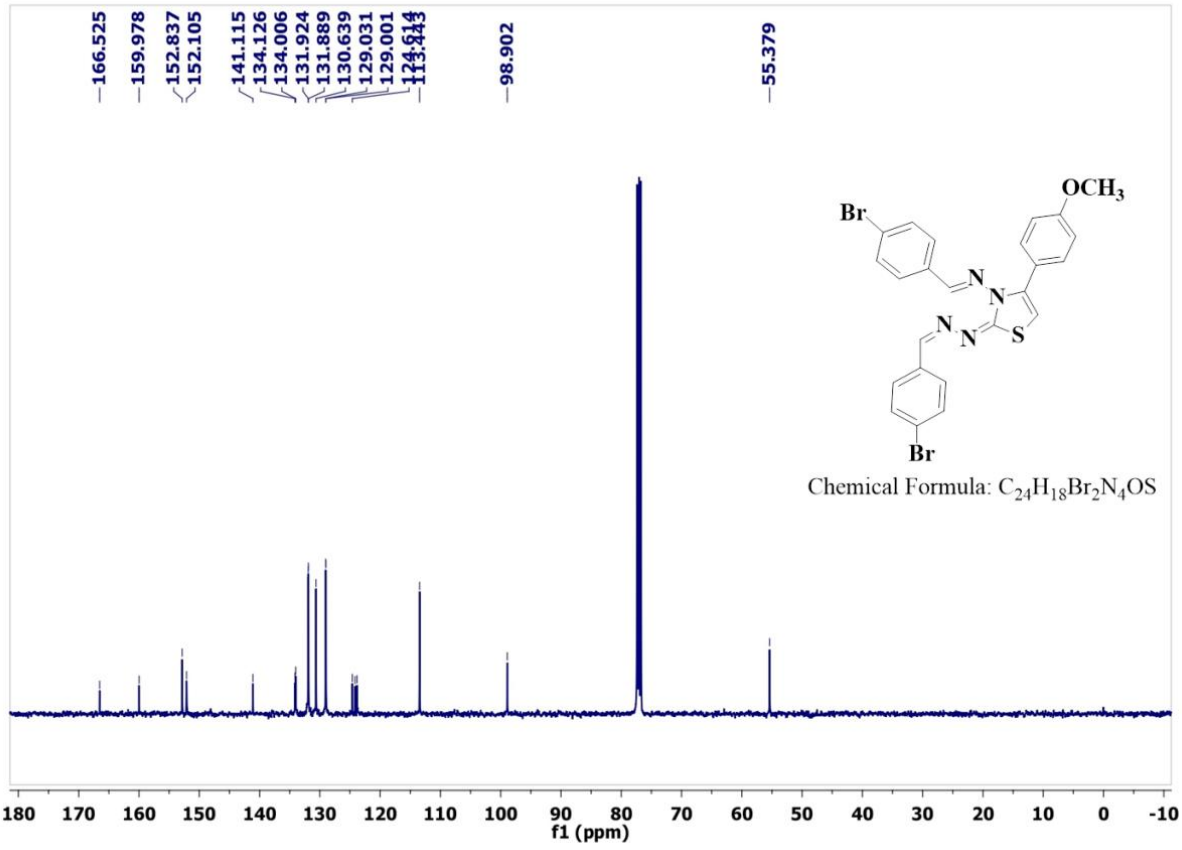
^{13}C NMR spectrum of compound **4a** (100 MHz, $CDCl_3$)



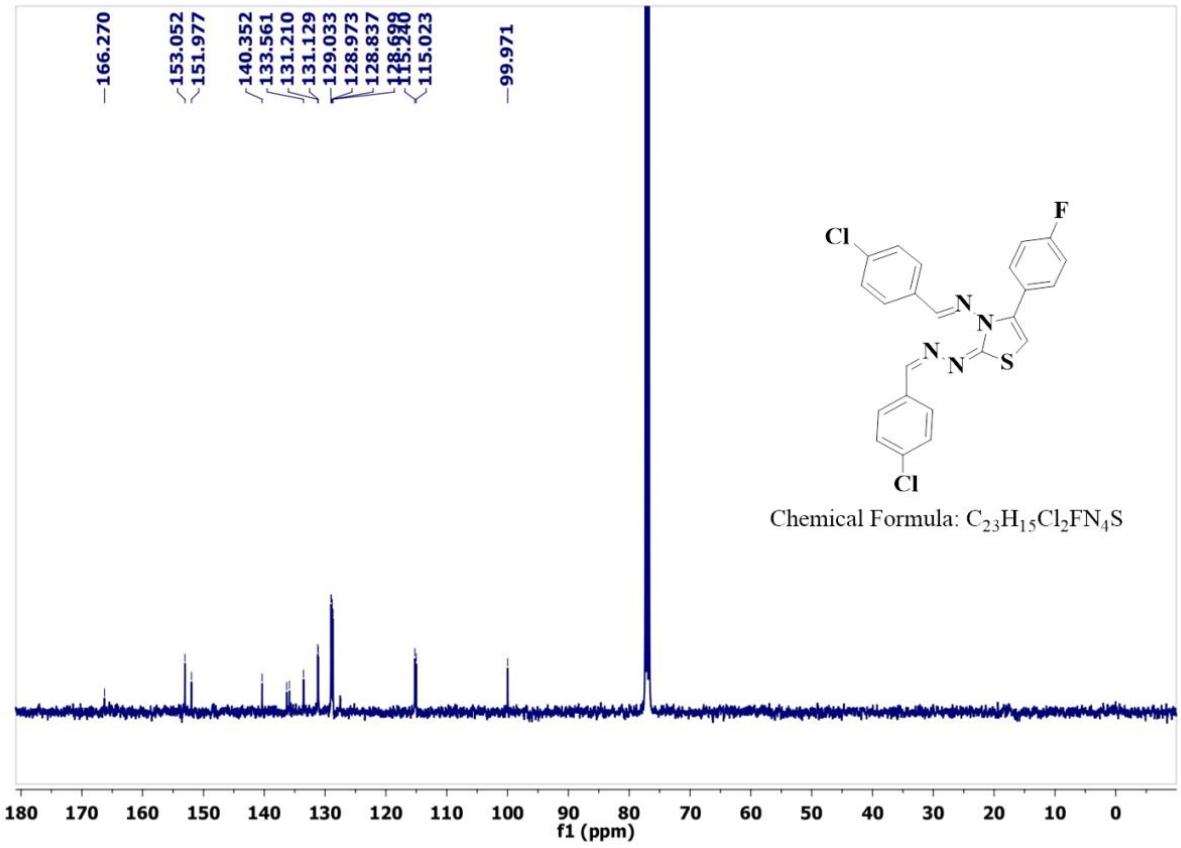
^{13}C NMR spectrum of compound **4b** (100 MHz, DMSO-d_6)



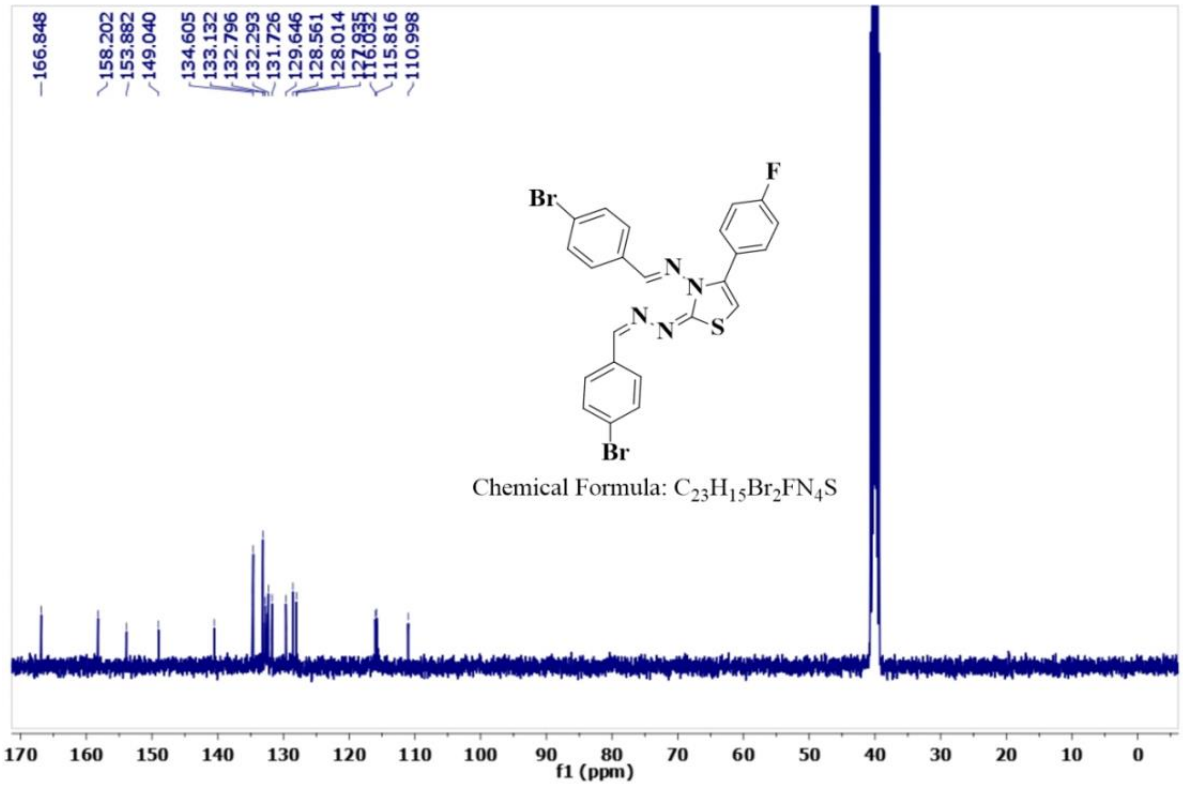
^{13}C NMR spectrum of compound **4c** (100 MHz, DMSO-d_6)



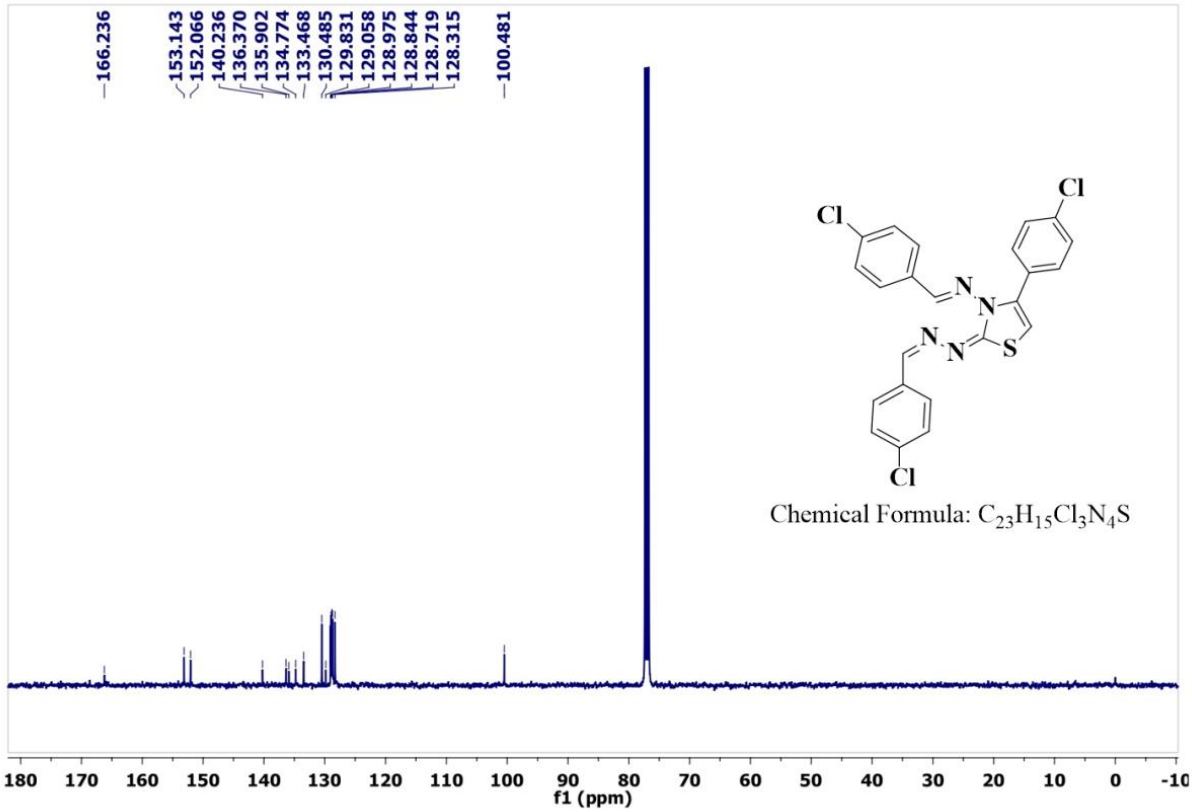
^{13}C NMR spectrum of compound **4d** (100 MHz, $CDCl_3$)



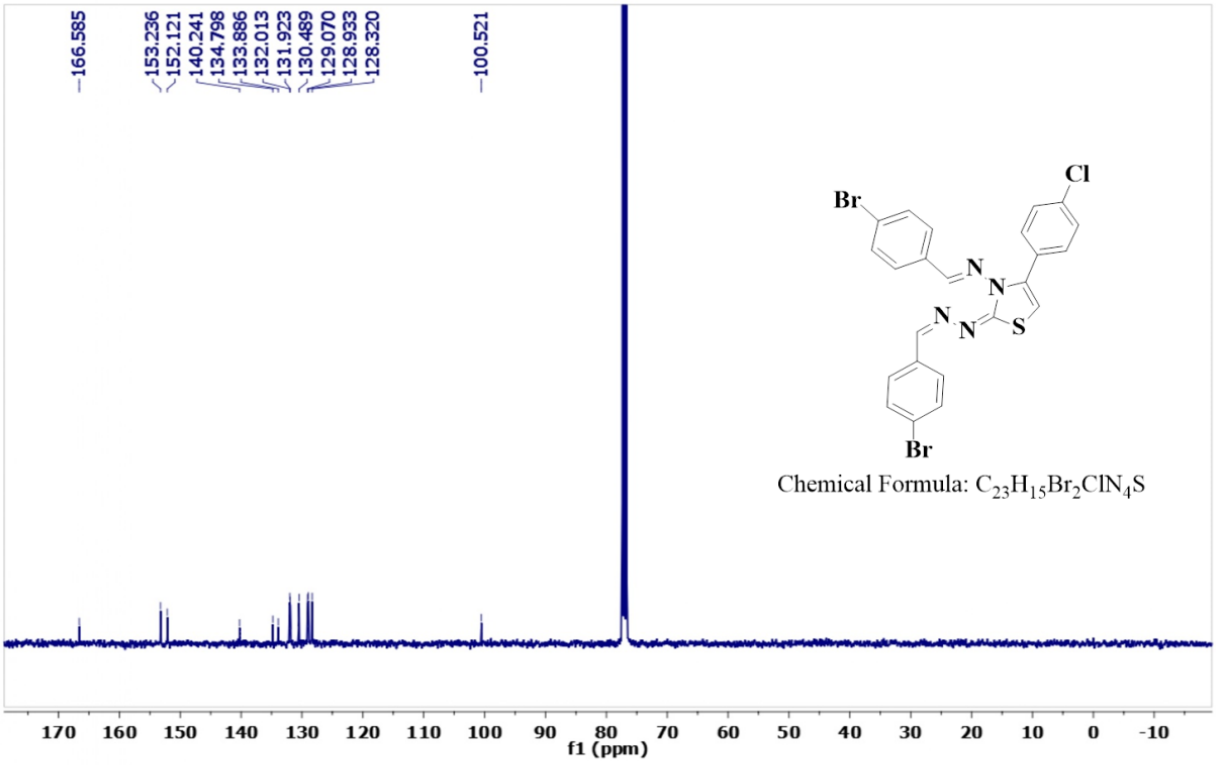
^{13}C NMR spectrum of compound **4e** (100 MHz, $CDCl_3$)



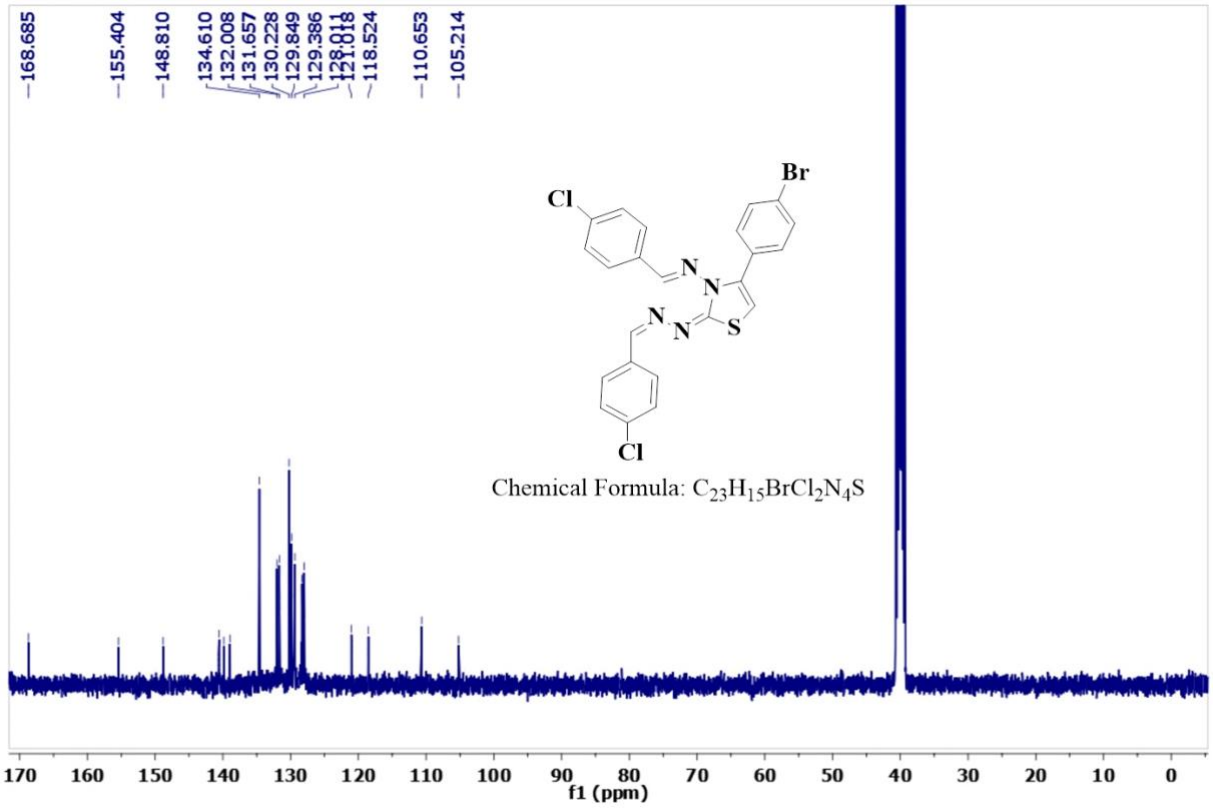
^{13}C NMR spectrum of compound **4f** (100 MHz, DMSO-d_6)



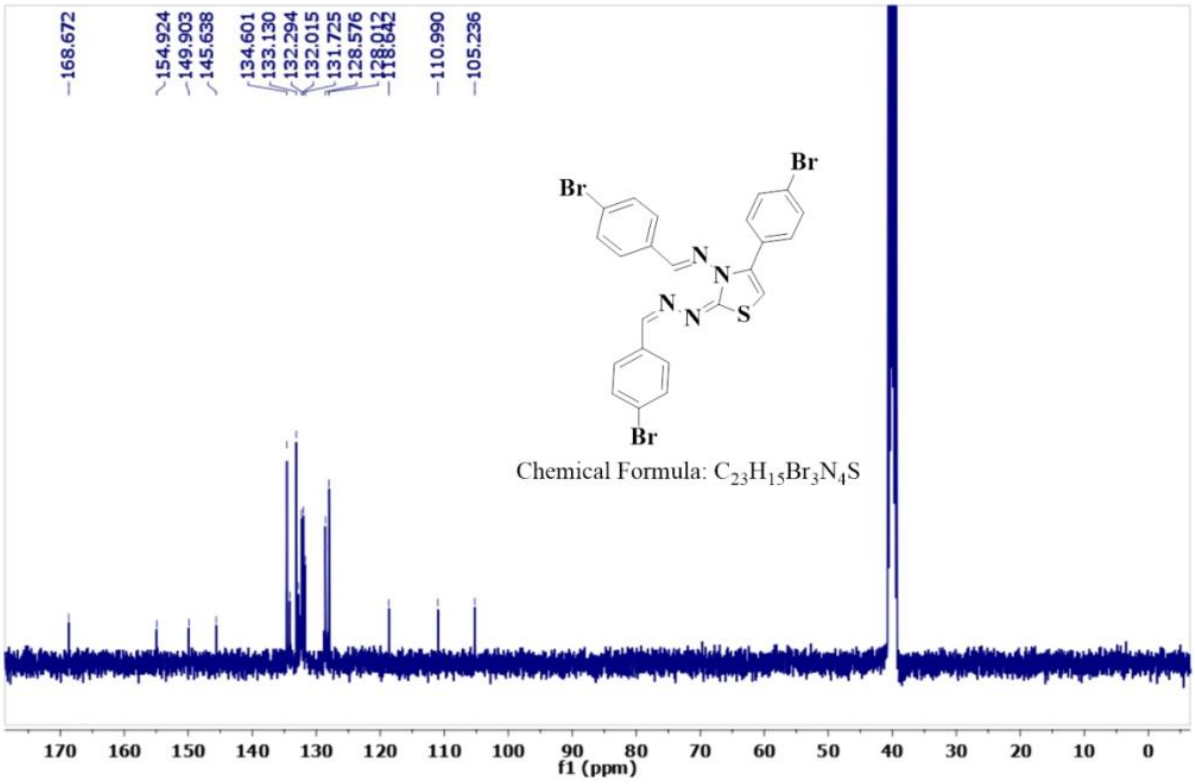
^{13}C NMR spectrum of compound **4g** (100 MHz, $CDCl_3$)



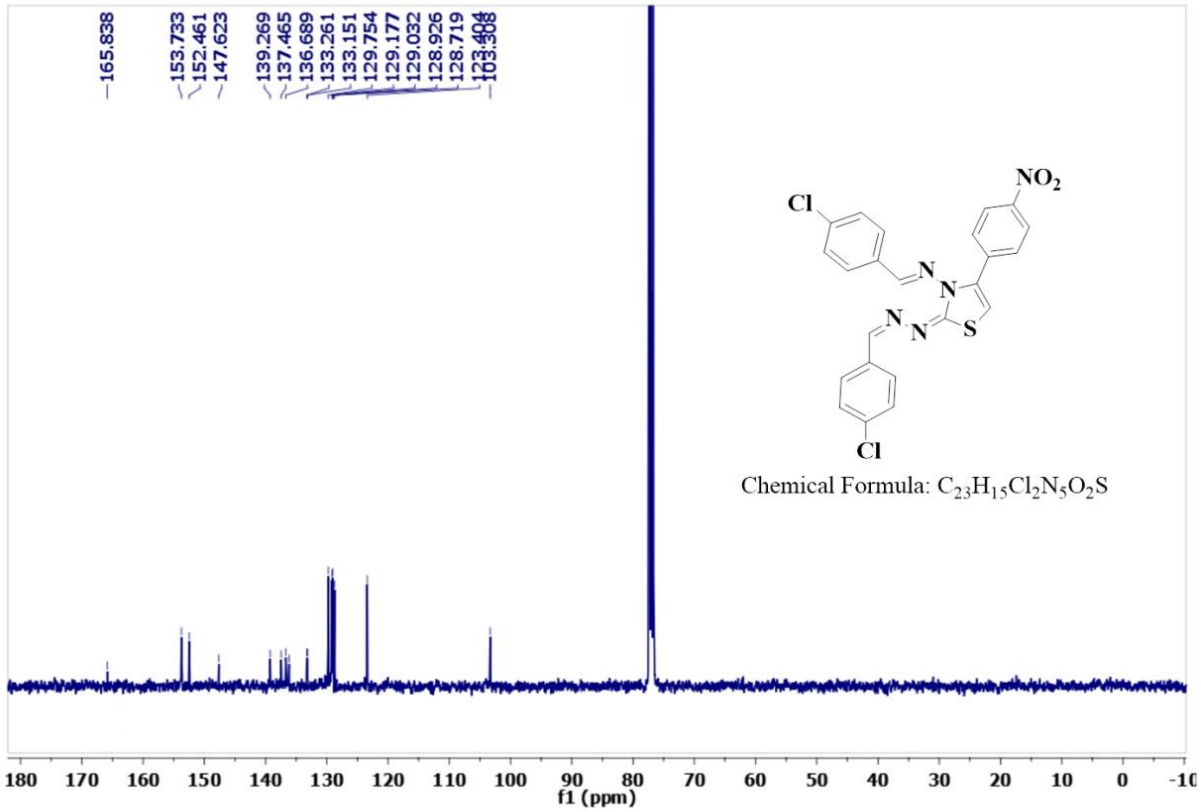
^{13}C NMR spectrum of compound **4h** (100 MHz, $CDCl_3$)



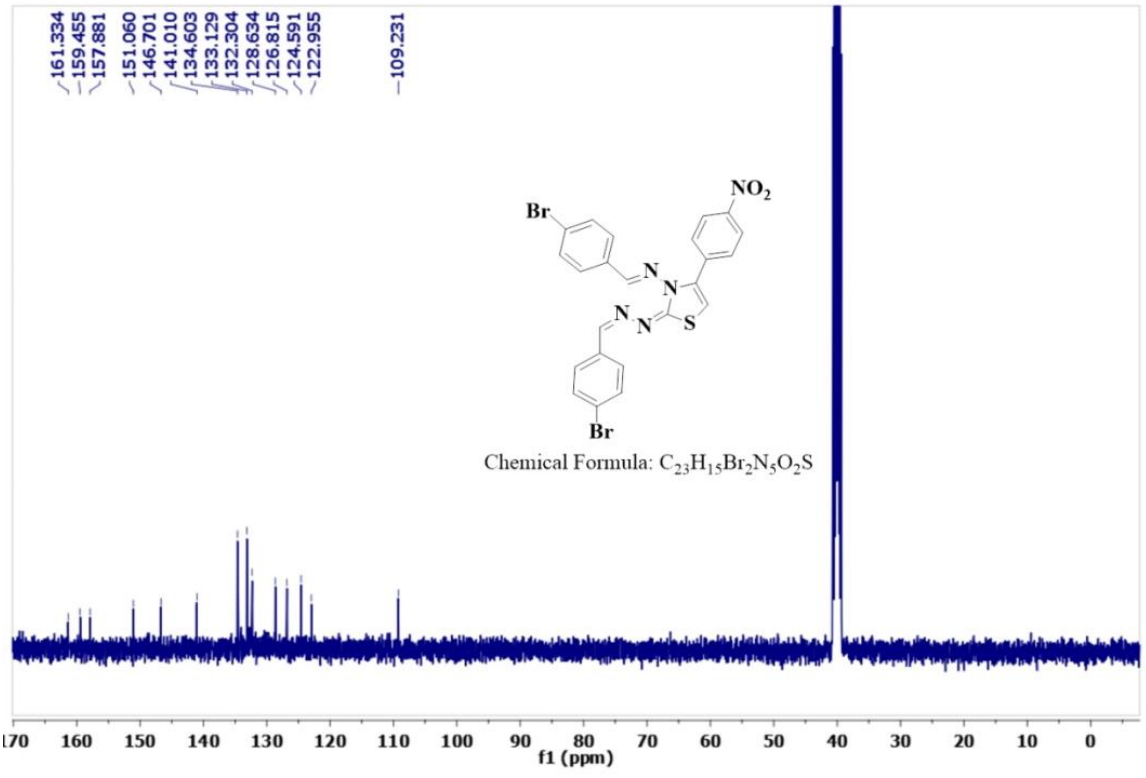
^{13}C NMR spectrum of compound **4i** (100 MHz, DMSO-d_6)



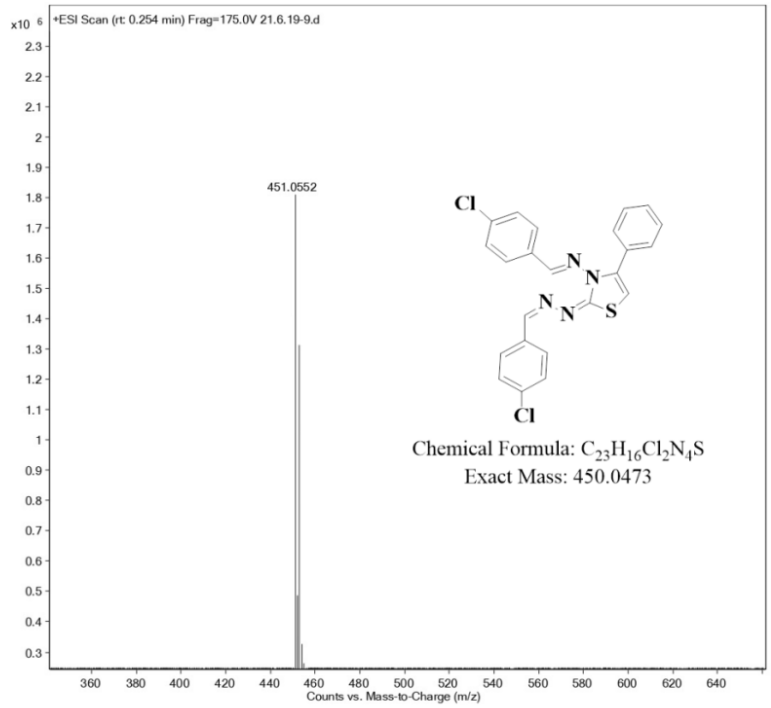
^{13}C NMR spectrum of compound **4j** (100 MHz, DMSO-d_6)



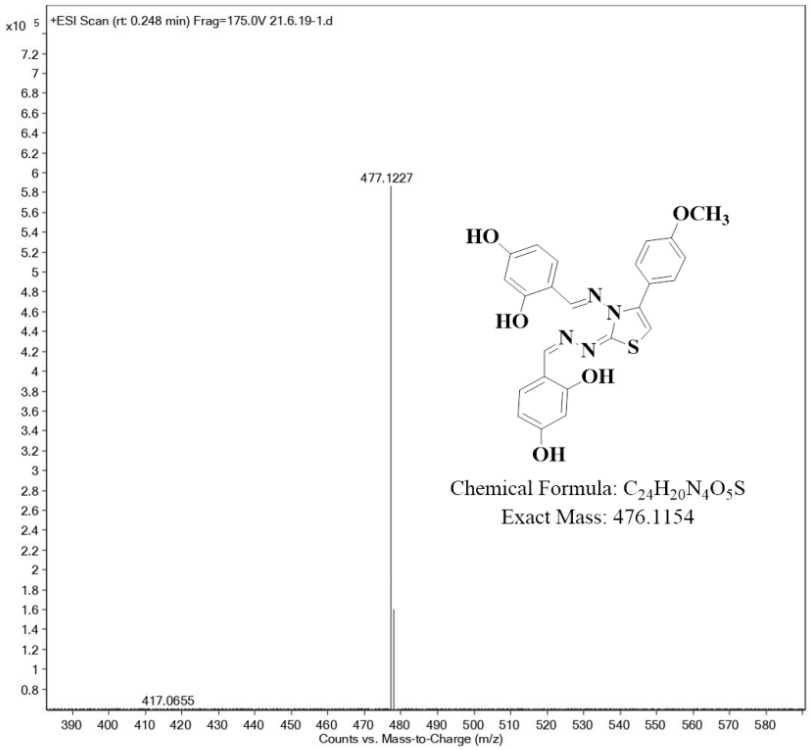
^{13}C NMR spectrum of compound **4k** (100 MHz, $CDCl_3$)



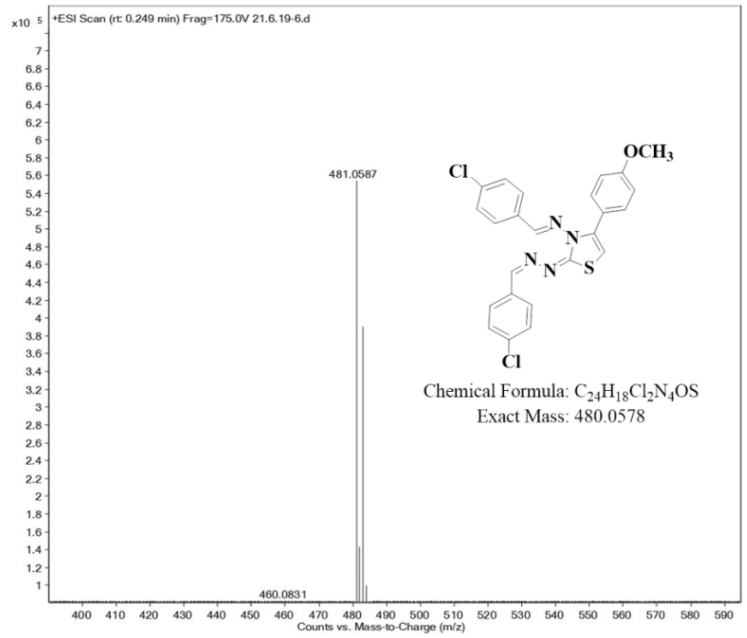
^{13}C NMR spectrum of compound **4l** (100 MHz, DMSO-d_6)



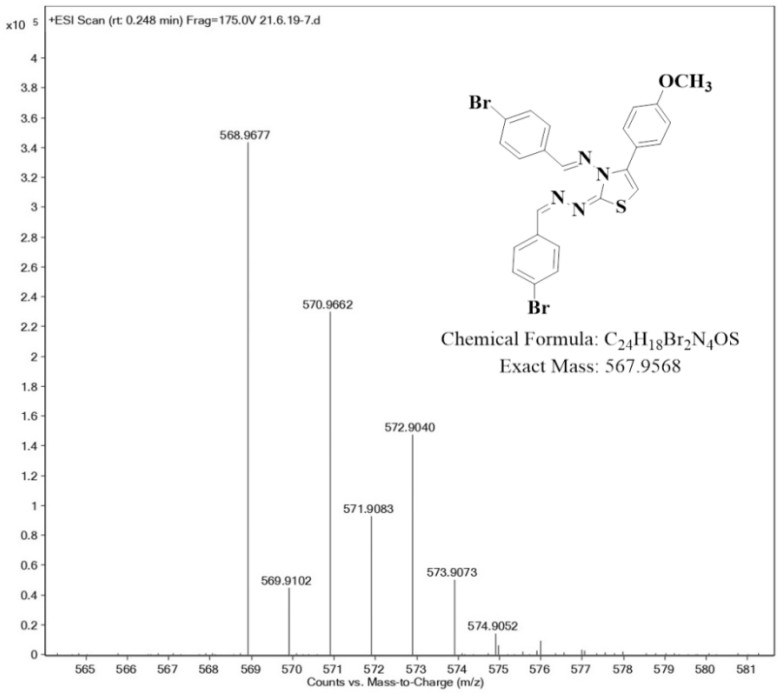
Mass spectrum of compound **4a**



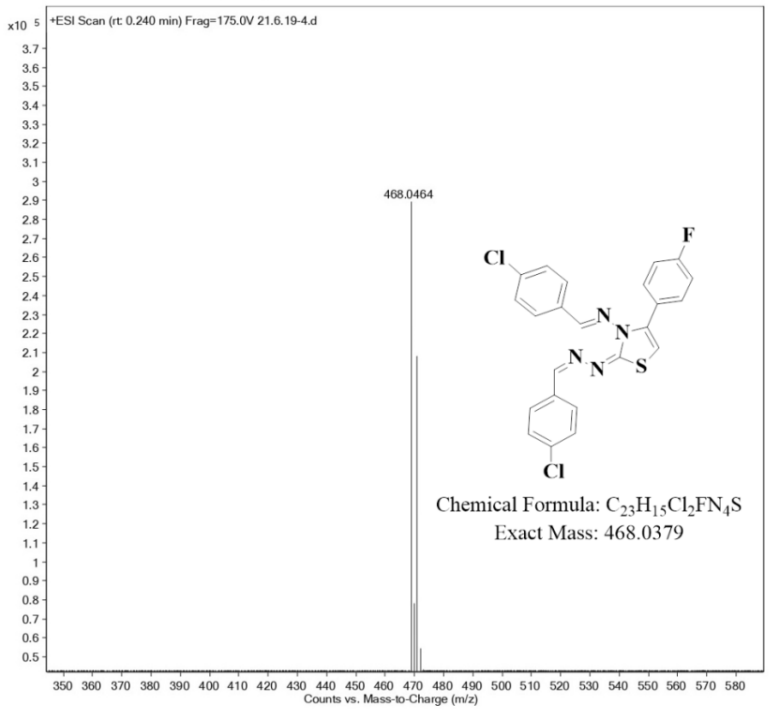
Mass spectrum of compound **4b**



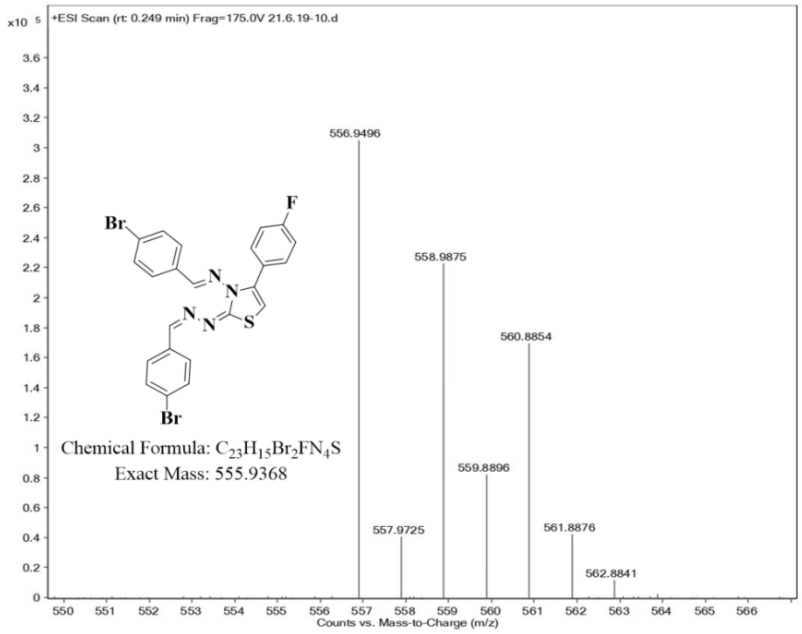
Mass spectrum of compound **4c**



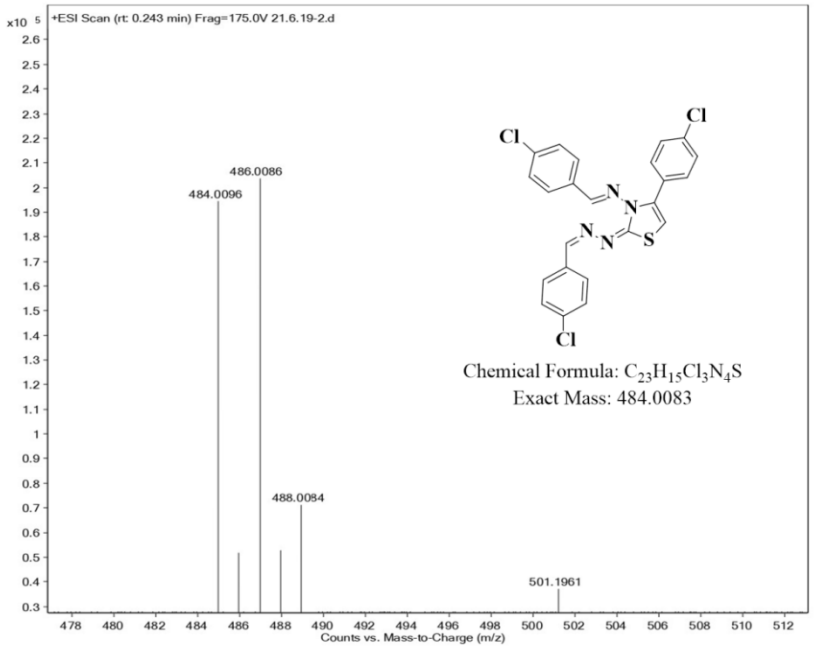
Mass spectrum of compound **4d**



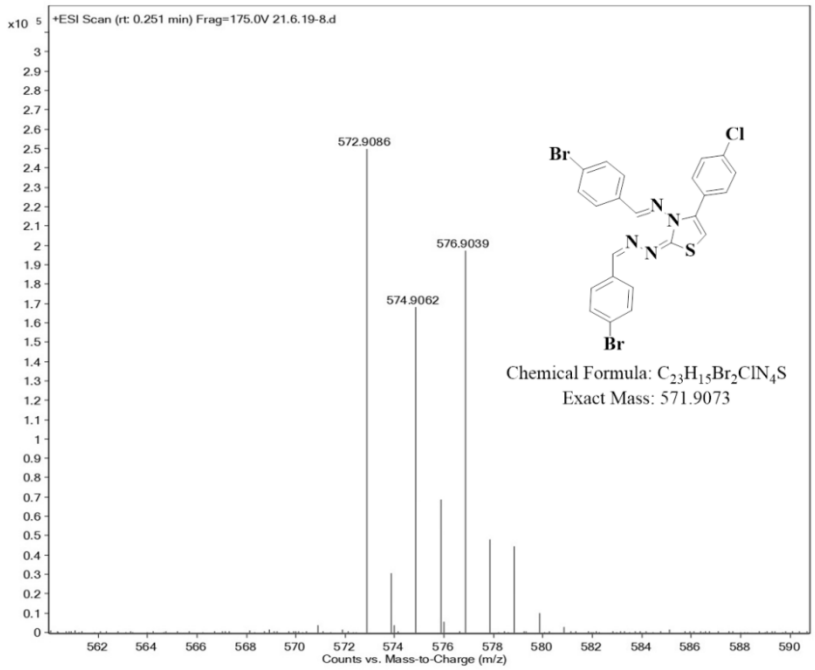
Mass spectrum of compound **4e**



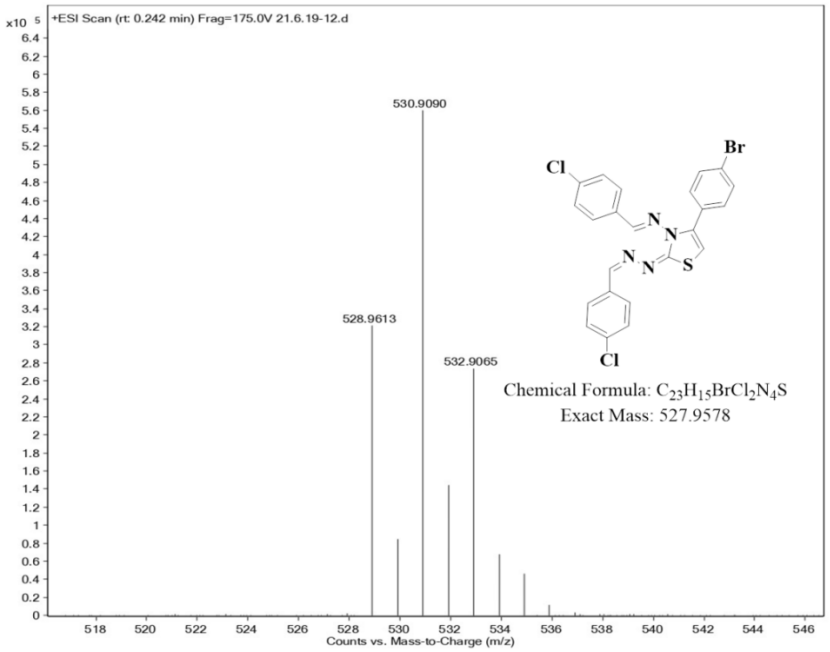
Mass spectrum of compound **4f**



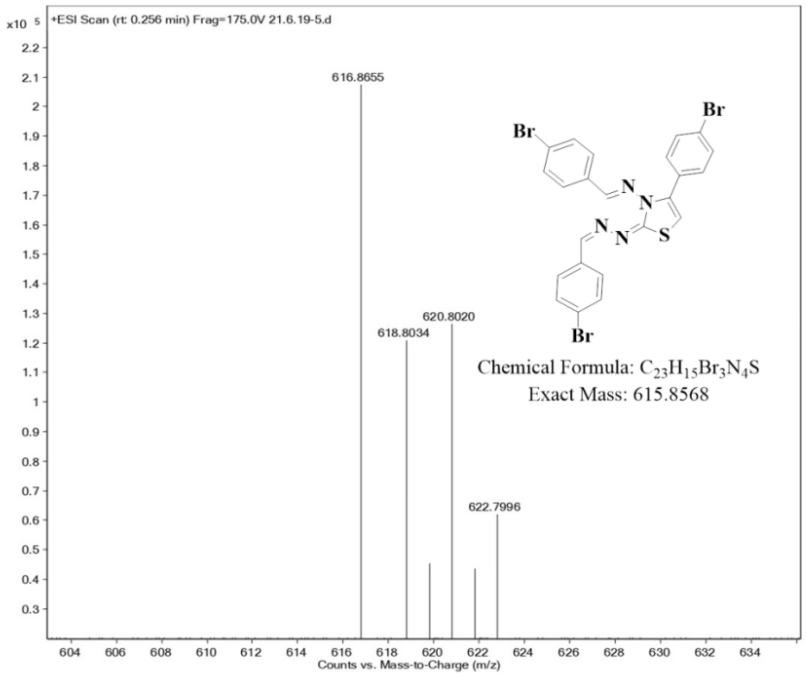
Mass spectrum of compound **4g**



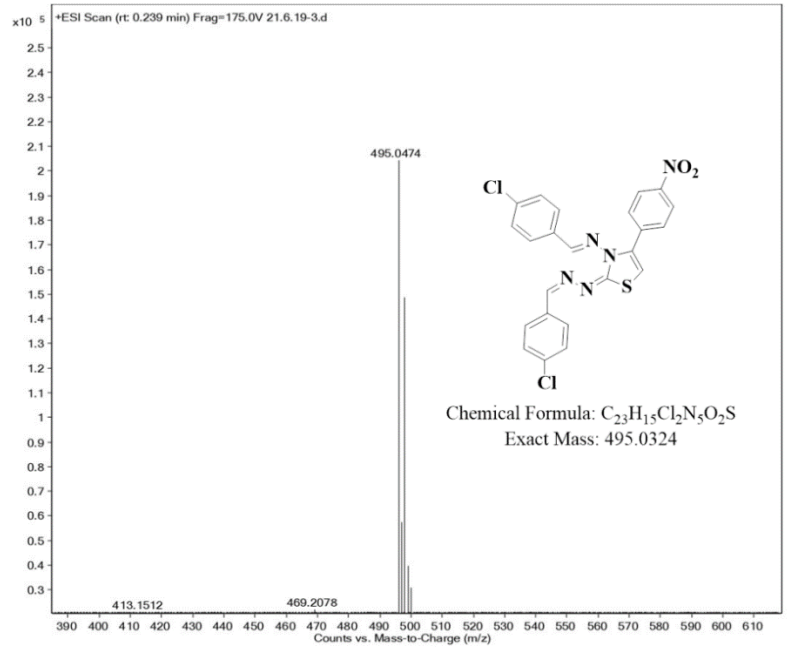
Mass spectrum of compound **4h**



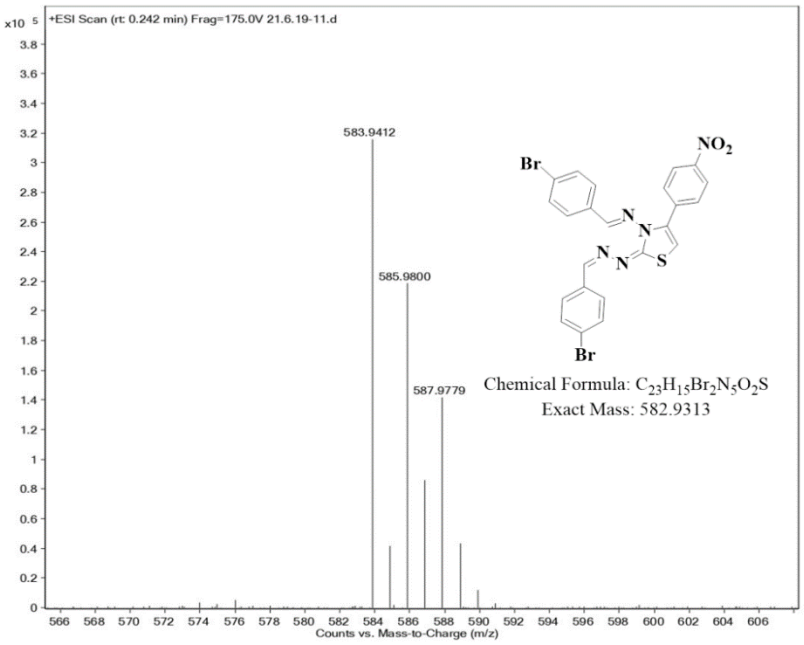
Mass spectrum of compound **4i**



Mass spectrum of compound **4j**



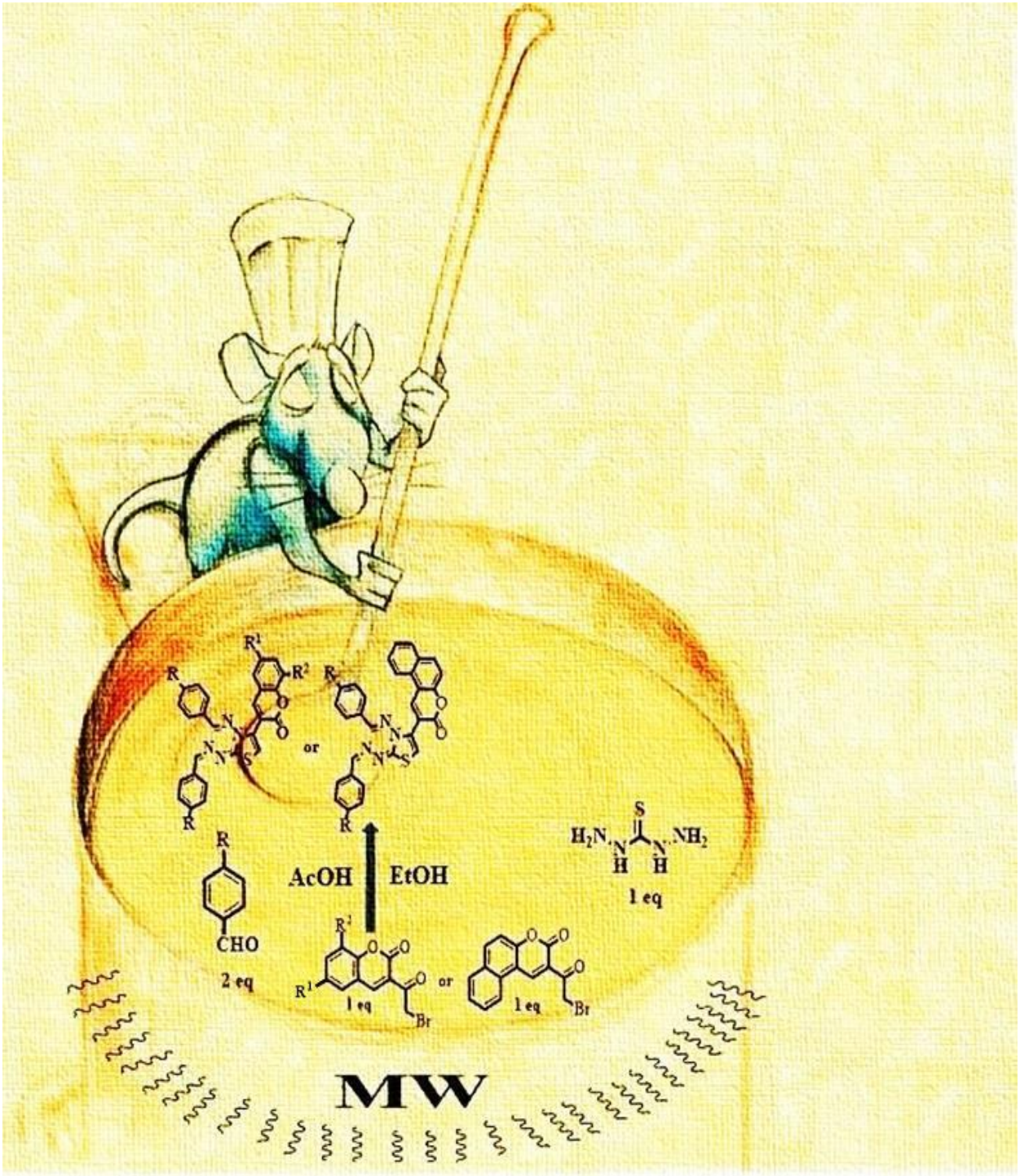
Mass spectrum of compound **4k**



Mass spectrum of compound **4l**

CHAPTER-II
SECTION-B

Microwave irradiated one-pot, pseudo four component synthesis of a new series of hybrid coumarin based thiazoles



CHAPTER-II
SECTION-B

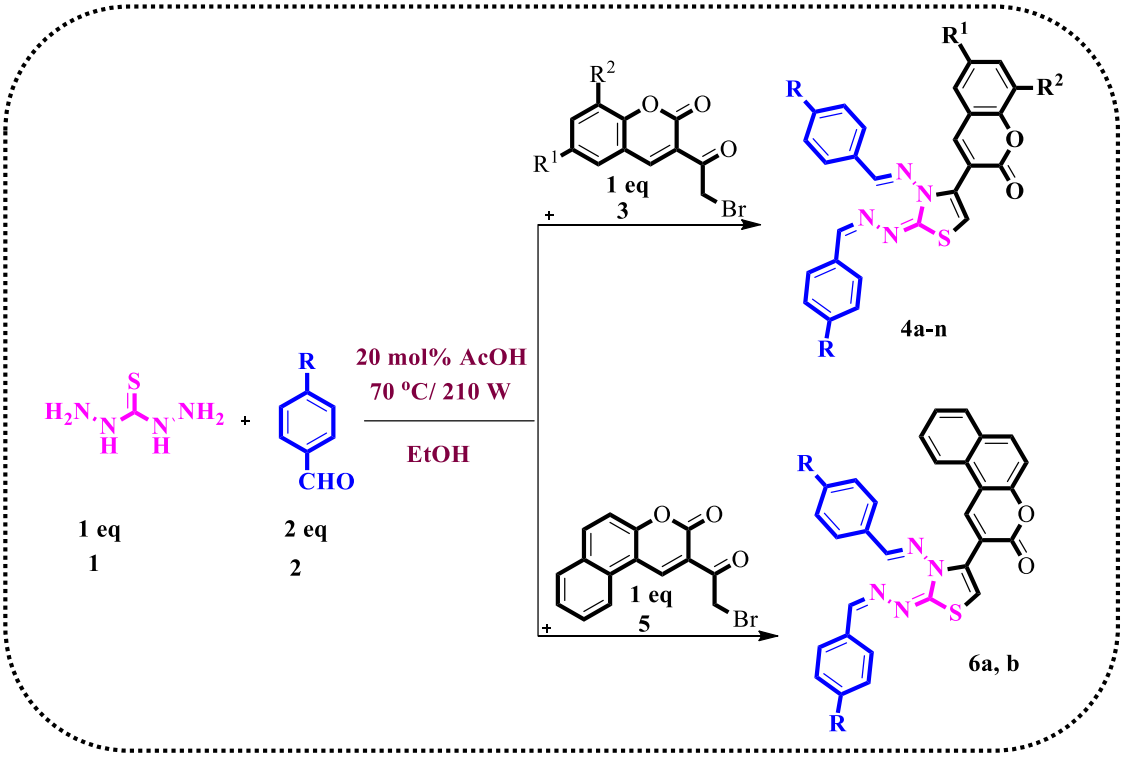
2B. Present work:

2B.1. Starting materials:

The present chapter describes the synthesis of new coumarin based thiazole hybrids. The starting materials required for the synthesis of the title compounds are thiocarbohydrazide, substituted aldehydes and substituted 3-(2-bromoacetyl) coumarins. Thiocarbohydrazide was prepared by using hydrazine hydrate and carbon disulfide[55,56]. Aldehydes were procured from commercial sources.

2B.2. Synthesis of coumarin based thiazoles:

The synthesis of target coumarin based thiazole analogues were carried out as outlined in scheme 2B.1. The title coumarin based thiazoles (**4a-n**; **6a**, **b**) were synthesized by a combination of thiocarbohydrazide (**1**), aldehyde (**2**) and substituted 3-(2-bromoacetyl) coumarins (**3, 5**) (1:2:1) in ethanol in the presence of catalytic amount of acetic acid under microwave irradiation with good yields.



Scheme 2B.1: Synthesis of coumarin based thiazoles; Reagents and conditions: EtOH, AcOH, microwave irradiation at 70°C and 210 W.

2B.3. Results and discussion:

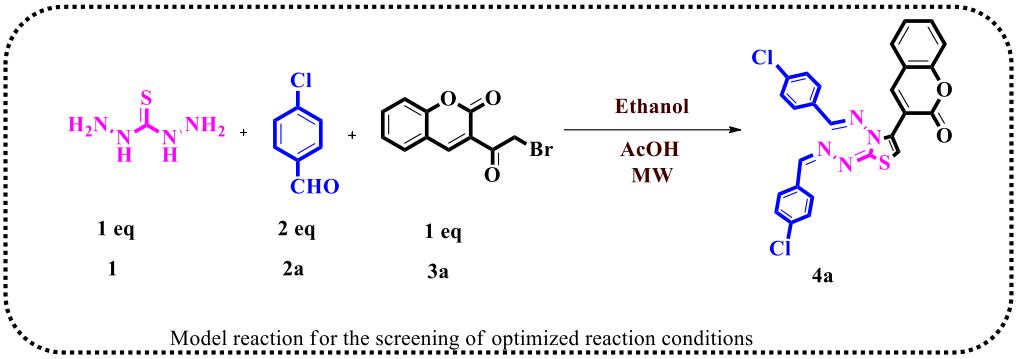
At first in conventional method, reaction was carried out in one-pot MCR approach using

thiocarbohydrazide (**1)**, aldehyde (**2a)**, 3-(2-bromoacetyl) coumarin (**3a**) as a starting materials in methanol. Afterwards for the optimization of this reaction we employed both in conventional and microwave method. In the beginning different solvents like methanol, ethanol, dimethyl sulfoxide, dimethylformamide and acetonitrile (Table 2B.1, entries 1–5) were used for the screening of solvents in both methods, initially it was concluded that ethanol (Table 2B.1, entry 2) was best among the tested solvents in terms of yields. When we continue this reaction to get more yields, it has been identified that ethanol (Table 2B.1, entry 7) was best out of the tested solvents with reference to time and yield in conventional and microwave irradiation methods.

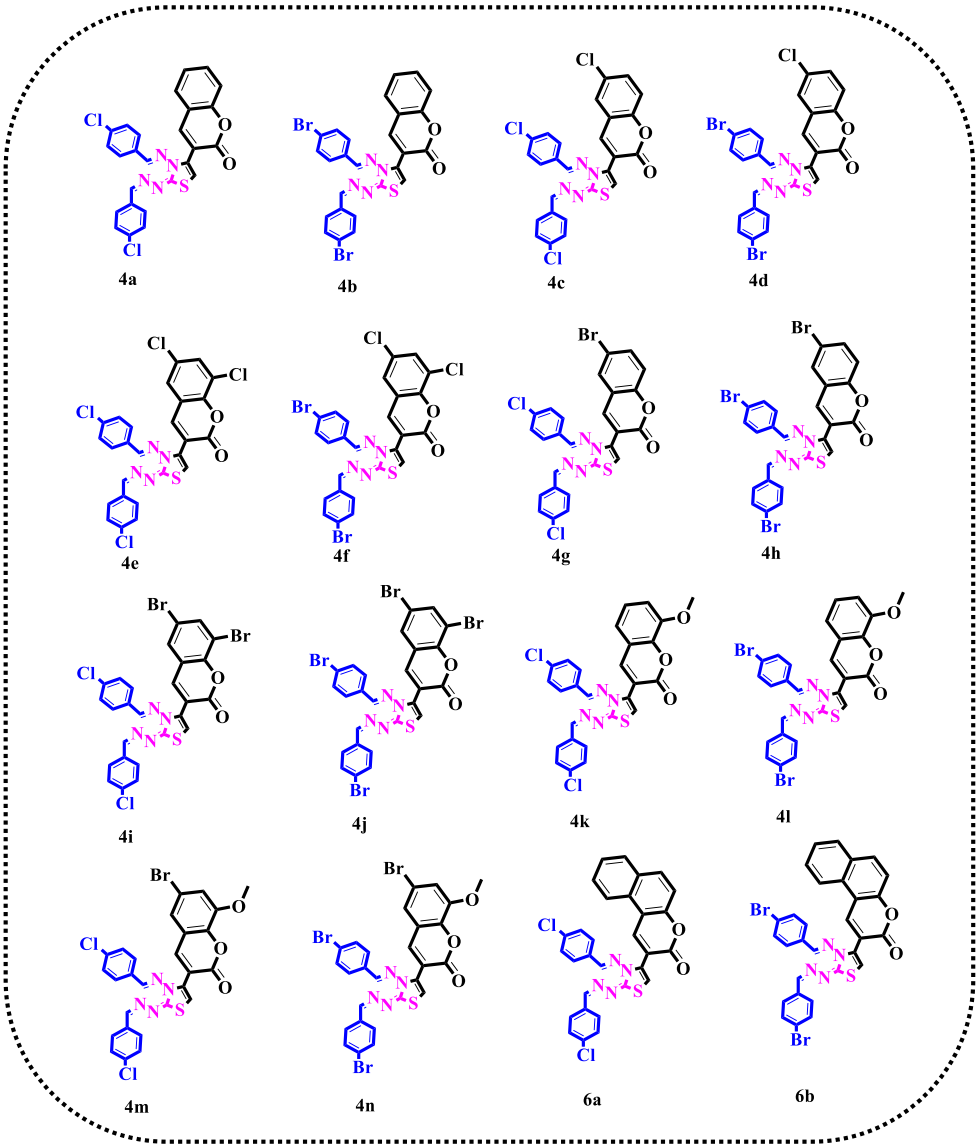
Furthermore, optimization of the reaction was carried out by using catalyst like acetic acid, hydrochloric and sulfuric acid (Table 2B.1, entries 11–13) were tested in ethanol and it has been identified that acetic acid (Table 2B.1, entry 11) was a suitable catalyst which gave more yields in conventional and microwave method. Then acetic acid percentages (Table 2B.1, entries 11, 14, 15) were tested in ethanol and it has been noticed that acetic acid (20 mol %) (Table 2B.1, entry 14) was a suitable catalyst which gave more yields in both methods. Nonetheless, beyond 60^{o}C (reflux) there is no further improvisation of the product (Table 2B.1, entry 16) in conventional method. On the other hand, in microwave irradiation approach for the improvisation of this reaction, optimized in terms of temperature and power (Table 2B.1, entries 16–18) results confirmed that 70/210 (Table 2B.1, entry 17) gave 90 % yield with highest conversion rate and lower time. To conclude, high yield at less reaction time was observed in ethanol at 70 ^{0}C with 20 mol% acetic acid as a catalyst under microwave irradiation (210 W).

Table 2B.1: Optimizing the reactions conditions under conventional and microwave conditions for the synthesis of coumarin based thiazoles.

Entry	Solvent	Catalyst (mol%)	Conventional			Microwave		
			Temperature (ºC)	Time(h)	Yield(%)	Temperature (ºC/W)	Time(min)	Yield[a](%)
1	MeOH	-	60	12	14	70/140	15	15
2	EtOH	-	60	12	21	70/140	15	21
3	DMSO	-	60	12	14	70/140	15	10
4	DMF	-	60	12	13	70/140	15	9
5	CH_3CN	-	60	12	10	70/140	15	7
6	MeOH	-	60	16	20	70/140	25	26
7	EtOH	-	60	14	26	70/140	22	32
8	DMSO	-	60	18	23	70/140	32	21
9	DMF	-	60	17	21	70/140	28	17
10	CH_3CN	-	60	20	17	70/140	35	18
11	EtOH	CH_3COOH(10)	60	3	40	70/140	10	45
12	EtOH	HCl(10)	60	3	34	70/140	10	41
13	EtOH	H_2SO_4(10)	60	3	32	70/140	10	33
14	EtOH	CH_3COOH(20)	60	3	70	70/140	10	73
15	EtOH	CH_3COOH(30)	60	3	48	70/140	10	53
16	EtOH	CH_3COOH(20)	reflux	3	54	70/140	7	52
17	**EtOH**	**CH_3COOH(20)**	-	-	-	**70/210**	**7**	**90**
18	EtOH	CH_3COOH(20)	-	-	-	70/240	7	68



Scheme 2B.2: Synthesis of coumarin based thiazole hybrid (**4a**), reagents and conditions: EtOH, AcOH Cat., MW, 7 min.

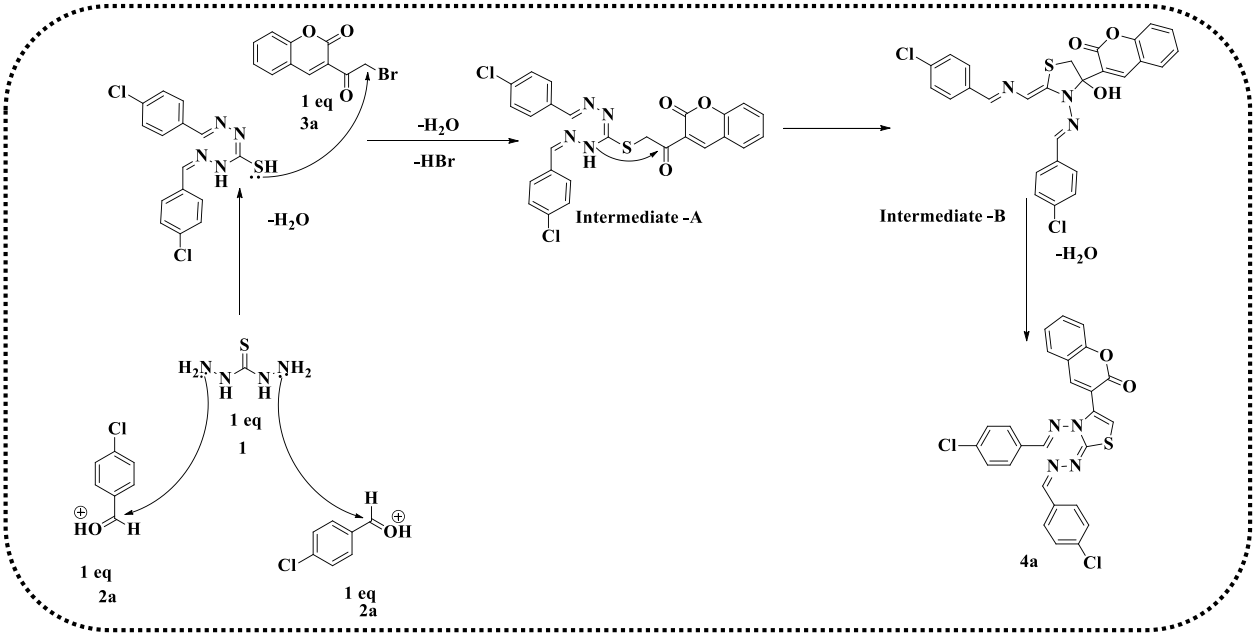


Scheme 2B.3: Synthesis of coumarin based thiazoles hybrids (**4a-n; 6a, b**) reagents and conditions: EtOH, AcOH Cat., MW, 5-8 min.

Table 2B.2: Different substituted coumarin based thiazole hybrids (**4a-n, 6a-b**), time, [a]isolated yield.

Entry	Product	R	R^1	R^2	R^3	Time(min)	Yield (%)[a]
1	**4a**	Cl	H	H	H	7	90
2	**4b**	Br	H	H	H	8	89
3	**4c**	Cl	H	Cl	H	6	93
4	**4d**	Br	H	Cl	H	7	91
5	**4e**	Cl	H	Cl	Cl	8	89
6	**4f**	Br	H	Cl	Cl	7	90
7	**4g**	Cl	H	Br	H	5	93
8	**4h**	Br	H	Br	H	6	88
9	**4i**	Cl	H	Br	Br	6	93
10	**4j**	Br	H	Br	Br	8	92
11	**4k**	Cl	H	H	OCH_3	5	90
12	**4l**	Br	H	H	OCH_3	6	89
13	**4m**	Cl	H	Br	OCH_3	6	88
14	**4n**	Br	H	Br	OCH_3	6	91
15	**6a**	Cl	H	-	-	7	92
16	**6b**	Br	H	-	-	8	89

Here a plausible mechanism for the formation of target compound **4a** is represented in scheme 2B.4. Initially 2 eq of aldehydes react with one eq of thiocarbohydrazide in presence of acetic acid to yield bis-thiocarbohydrazone. Subsequently, the SN^2-type attack of bis-thiocarbohydrazone sulfur on the C–Br of 3-(2-bromoacetyl) coumarin gives intermediate-A, afterwards attack of NH nitrogen on the carbonyl of the 3-(2-bromoacetyl)-2H-chromen-2-one leads to the formation of Intermediate-B, which further undergoes acid-catalyzed dehydration to form title coumarin based thiazole **4a**.



Scheme 2B.4: A plausible mechanism for the one-pot, three-component formation of compound **4a**.

All the newly synthesized compounds structures were confirmed by their analytical and spectral data. The ^{1}H NMR spectra of the compounds have shown a characteristic thiazole ring proton in the range of δ 6.62-7.08 ppm. Methine protons from the range of δ 8.37-10.25 ppm. The ^{13}C NMR spectra have shown signals from the range of 162.46-169.07 ppm corresponding to the thiazole C_2 respectively. In the mass spectra of all the synthesized compounds have shown molecular ion peeks corresponding to their molecular formula and which is in agreement with their chemical structure.

2B.4. Conclusion:

In summary, we have developed a potential green protocol for the synthesis of new coumarin based thiazole analogues by the microwave-assisted MCR approach.

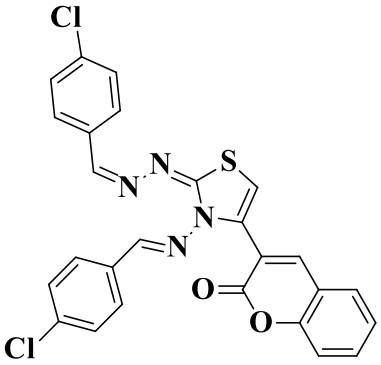
2B.5. Experimental:

General procedure for the Synthesis of **3-(-3-(-(4-benzylidene)amino)-2-(-(4-benzylidene)hydrazono)-2,3-dihydrothiazol-4-yl)-2*H*-chromen-2-one (4a-n**; **6a**, **b**)

A mixture of thiocarbohydrazide (1 m mol), aldehyde (2 m mol), 3-(2-bromoacetyl) coumarins (1 m mol) and catalytic amount of acetic acid (20 mol%) in ethanol was placed in a 10 mL pressurized vial and subjected to MW irradiation (mono-mode, CEM Discover microwave synthesis system at 210 W) at a temperature of 70^{o}C for about 5–8 min; progress of the reaction is monitored by thin layer chromatography. After completion of the reaction, the product was filtered and isolated, washed with ethanol, dried and recrystallized from ethanol.

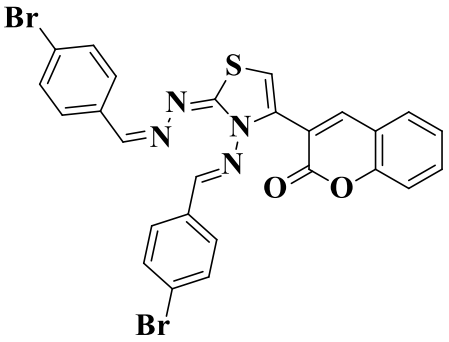
2B.6. Spectral data:

3-(-3-(-(4-Chlorobenzylidene)amino)-2-(-(4-chlorobenzylidene)hydrazono)-2,3-dihydrothiazol-4-yl)-2*H*-chromen-2-one (4a):



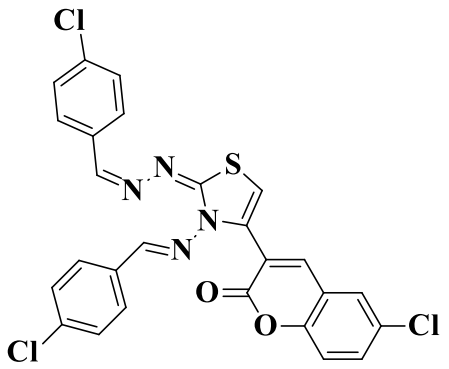
Yellow solid; yield 90%; mp: 206-208ºC; IR (KBr) cm^{-1}: 1601 (C=N); ^1H NMR (400MHz, $CDCl_3$, ppm): δ 6.6 (s, 1H, Thiazole-H), 7.31-7.41 (m, 6H), 7.55 (d, J = 8.4 Hz, 3H), 7.61 (t, J = 8 Hz, 1H), 7.72 (d, J = 8.4 Hz, 2H), 7.98 (s, 1H), 8.39 (s, 1H, NCH), 10.23 (s, 1H, NCH).^{13}C NMR (100MHz, $CDCl_3$) δ 104.41, 116.82, 118.75, 124.85, 128.30, 128.71, 128.87, 128.96, 129.03, 132.37, 133.39, 135.04, 136.33, 142.51, 152.09, 153.33, 153.86, 159.02, 165.34 ppm; Mass (ESI-HRMS) (*m/z*): 519.20 $[M+H]^+$; Anal. Calcd. For $C_{26}H_{16}Cl_2N_4O_2S$: C, 60.12; H, 3.10; N, 10.79%. Found: C, 60.15; H, 3.14; N, 10.75%.

3-(-3-(-(4-Bromobenzylidene)amino)-2-(-(4-bromobenzylidene)hydrazono)-2,3-dihydrothiazol-4-yl)-2*H*-chromen-2-one (4b):



Yellow solid; yield 89%; mp: 216-218ºC; IR (KBr) cm^{-1}: 1610 (C=N); ^1H NMR (400MHz, DMSO-d_6, ppm): δ 6.97 (s, 1H, Thiazole-H), 7.63 (s, 2H), 7.73 (t, J = 6.4 Hz, 4H), 7.82 (s, 2H), 8.04 (s, 1H), 8.31 (s, 2H), 8.37 (s, 1H, NCH), 8.54 (s, 2H), 10.13 (s, 1H, NCH).^{13}C NMR (100 MHz, DMSO-d_6) δ 104.50, 114.66, 119.64, 128.63, 129.32, 129.51, 132.32, 132.57, 133.14, 134.61, 135.36, 135.70, 138.57, 146.22, 154.04, 154.56, 156.72, 160.07, 168.52 ppm; Mass (ESI-HRMS) (*m/z*): 606.94 $[M+H]^+$; Anal. Calcd. For $C_{26}H_{16}Br_2N_4O_2S$: C, 51.34; H, 2.65; N, 9.21%. Found: C, 51.30; H, 2.69; N, 9.17%.

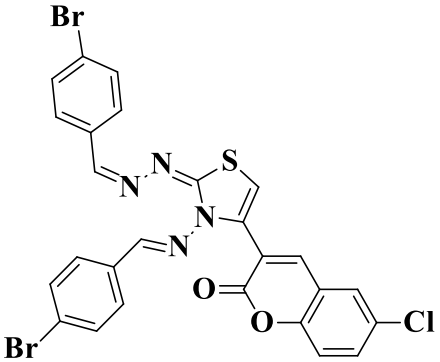
6-Chloro-3-(-3-(-(4-chlorobenzylidene)amino)-2-(-(4-chlorobenzylidene)hydrazono)-2,3-dihydrothiazol-4-yl)-2*H*-chromen-2-one (4c):



Yellow solid; yield 93%; mp: 203-205ºC; IR (KBr) cm^{-1}: 1610 (C=N); ^1H NMR (400MHz,

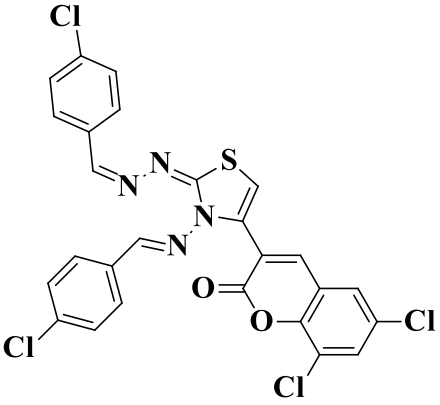
DMSO-d_6, ppm): δ 6.98 (s, 1H, Thiazole-H), 7.49-7.58 (m, 4H), 7.63 (d, *J* = 8 Hz, 2H), 7.68 (d, *J* = 8.2 Hz, 2H), 7.74 (d, *J* = 8.8 Hz, 1H), 7.81 (d, *J* = 8.8 Hz, 2H), 8.32 (s, 1H), 8.56 (s, 1H, NCH), 10.14 (s, 1H, NCH).^{13}C NMR (100 MHz, DMSO-d_6) δ 103.57, 112.13, 118.32, 121.09, 128.19, 128.38, 128.99, 129.40, 130.23, 134.61, 138.96, 144.21, 151.35, 153.83, 158.81, 168.12 ppm; Mass (ESI-HRMS) (*m/z*): 551.99 $[M+]^+$; Anal. Calcd.For : $C_{26}H_{15}Cl_3N_4O_2S$: C, 56.38; H, 2.73; N, 10.12%. Found: C, 56.35; H, 2.77; N, 10.16%.

3-(-3-(-(4-Bromobenzylidene)amino)-2-(-(4-bromobenzylidene)hydrazono)-2,3-dihydrothiazol-4-yl)-6-chloro-2*H*-chromen-2-one (4d):



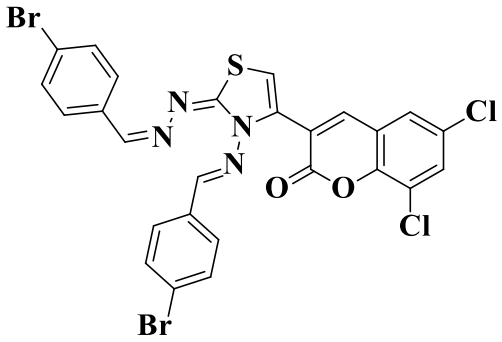
Yellow solid; yield 91%; mp: 236-238ºC; IR (KBr) cm^{-1}: 1605 (C=N); ^{1}H NMR (400MHz, DMSO-d_6, ppm): δ 6.99 (s, 1H, Thiazole-H), 7.56-7.59 (m, 4H), 7.71-7.74 (m, 4H), 7.83 (s, 2H), 7.96 (s, 1H), 8.33 (s, 1H), 8.57 (s, 1H, NCH), 10.14 (s, 1H, NCH).^{13}C NMR (100 MHz, DMSO-d_6) δ 106.78, 112.16, 118.34, 118.65, 121.11, 128.63, 131.73, 132.31, 132.80, 133.13, 134.60, 135.60, 137.32, 142.87, 145.25, 151.37, 154.67, 158.82, 168.12 ppm; Mass (ESI-HRMS) (*m/z*): 642.8431 $[M+]^+$; Anal. Calcd. For $C_{26}H_{15}Br_2ClN_4O_2S$: C, 48.58; H, 2.35; N, 8.72%. Found: C, 48.55; H, 2.39; N, 8.67%.

6,8-Dichloro-3-(-3-(-(4-chlorobenzylidene)amino)-2-(-(4-chlorobenzylidene)hydrazono)-2,3-dihydrothiazol-4-yl)-2*H*-chromen-2-one (4e):



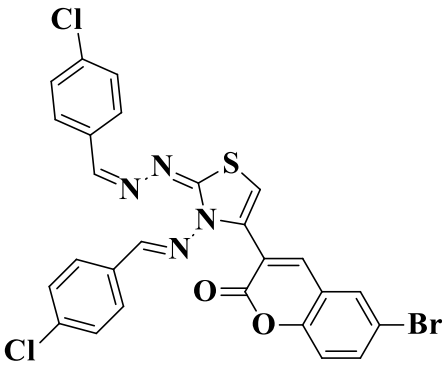
Yellow solid; yield 89%; mp: 258-260ºC; IR (KBr) cm^{-1}: 1610 (C=N); ^{1}H NMR (400MHz, DMSO-d_6, ppm): δ 6.63 (s, 1H, Thiazole-H), 7.35-7.4 (m, 6H), 7.55-7.6(m, 4H), 7.9 (s, 1H), 8.39 (s, 1H, NCH), 10.22 (s, 1H, NCH).^{13}CNMR (100 MHz, DMSO-d_6) δ 105.25, 112.08, 118.26, 121.01, 121.81, 128.08, 128.31, 129.01, 129.35, 129.82, 130.21, 131.65, 133.61, 134.21, 134.55, 137.25, 138.97, 140.90, 144.14, 151.27, 158.80, 168.05 ppm; Mass (ESI-HRMS) (*m/z*): 585.9692 $[M+]^+$; Anal. Calcd. For $C_{26}H_{14}Cl_4N_4O_2S$: C, 53.08; H, 2.40; N, 9.52%. Found: C, 53.12; H, 2.44; N, 9.56%.

3-(-3-(-(4-Bromobenzylidene)amino)-2-(-(4-bromobenzylidene)hydrazono)-2,3-dihydrothiazol-4-yl)-6,8-dhloro-2*H*-chromen-2-one (4f):



Yellow solid; yield 90%; mp: 247-249ºC; IR (KBr) cm^{-1}: 1613 (C=N); ^{1}H NMR (400MHz, DMSO-d_6, ppm): δ 6.99 (s, 1H, Thiazole-H), 7.55-7.59 (m, 4H), 7.71-7.74 (m, 4H), 8.05 (s, 1H), 8.33 (s, 1H), 8.49 (s, 1H), 8.56 (s, 1H, NCH), 10.14 (s, 1H, NCH).^{13}C NMR (100 MHz, DMSO-d_6) δ 105.19, 117.48, 118.34, 121.13, 123.94, 128.64, 131.73, 132.31, 133.14, 134.61, 143.06, 147.81, 151.06, 153.82, 161.65, 165.98 ppm; Mass (ESI-HRMS) (*m/z*): 674.6793 $[M+H]^+$; Anal. Calcd. For $C_{26}H_{14}Br_2Cl_2N_4O_2S$: C, 46.11; H, 2.08; N, 8.27%. Found: C, 46.16; H, 2.15; N, 8.33%.

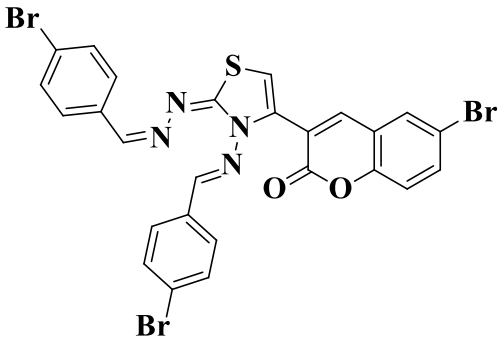
6-Bromo-3-(-3-(4-chlorobenzylidene)amino)-2-(-(4-chlorobenzylidene)hydrazono)-2,3-dihydrothiazol-4-yl)-2*H*-chromen-2-one (4g):



Yellow solid; yield 93%; mp: 243-245ºC; IR (KBr) cm^{-1}: 1610 (C=N); ^{1}H NMR (400MHz, DMSO-d_6, ppm): δ 6.62 (s, 1H, Thiazole-H), 7.32-7.39(m, 7H), 7.67-7.72 (m, 4H), 7.89 (s, 1H),

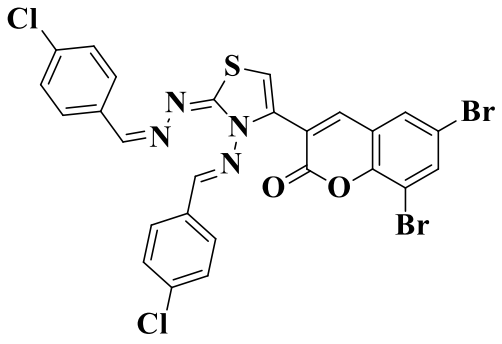
8.39 (s, 1H, NCH), 10.22 (s, 1H, NCH).^{13}C NMR (100 MHz, DMSO-d_6) δ 105.11, 116.96, 119.13, 120.70, 120.92, 128.37, 129.33, 129.48, 129.60, 129.85, 130.23, 133.85, 134.61, 144.26, 153.07, 158.31, 161.17, 168.11 ppm; Mass (ESI-HRMS) (*m/z*): 598.8955 $[M+]^+$; Anal. Calcd.For : $C_{26}H_{15}BrCl_2N_4O_2S$: C, 52.19; H, 2.53; N, 9.36%. Found: C, 52.14; H, 2.58; N, 9.31%.

6-Bromo-3--3-(-(4-bromobenzylidene)amino)-2-(-(4-bromobenzylidene)hydrazono)-2,3-dihydrothiazol-4-yl)-2*H*-chromen-2-one (4h):



Yellow solid; yield 89%; mp: 205-207ºC; IR (KBr) cm^{-1}: 1605 (C=N); ^{1}H NMR (400MHz, DMSO-d_6, ppm): δ 6.62 (s, 1H, Thiazole-H), 7.30 (d, *J* = 9.6 Hz, 1H), 7.48 (d, *J* = 4.8 Hz, 2H), 7.53 (t, *J* = 8.2 Hz, 4H), 7.65 (t, *J*= 8 Hz, 4H), 7.8 (s, 1H), 8.37 (s, 1H, NCH), 10.20(s, 1H, NCH).^{13}C NMR (100 MHz, DMSO-d_6) δ 108.39, 116.96, 118.58, 120.92, 128.37, 129.33, 129.38, 129.60, 138.96, 140.62, 144.26, 152.56, 153.07, 154.17, 164.95, 165.57 ppm; Mass (ESI-MS) (*m/z*): 684.85 $[M+H]^+$; Anal. Calcd. For $C_{26}H_{15}Br_3N_4O_2S$: C, 45.44; H, 2.20; N, 8.15%. Found: C, 45.40; H, 2.25; N, 8.19%.

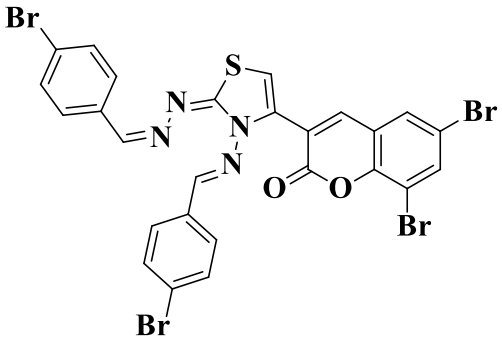
6,8-Dibromo-3-(-3-(-(4-chlorobenzylidene)amino)-2-(-(4-chlorobenzylidene)hydrazono)-2,3-dihydrothiazol-4-yl)-2*H*-chromen-2-one (4i):



Orange solid; yield 93%; mp: 226-228ºC; IR (KBr) cm^{-1}: 1608 (C=N); ^{1}H NMR (400MHz, DMSO-d_6, ppm): δ 6.69 (s, 1H, Thiazole-H), 7.35-7.37 (m, 5H), 7.55-7.6(m, 5H), 7.9 (s, 1H), 8.38 (s, 1H, NCH), 10.21(s, 1H, NCH).^{13}C NMR (100 MHz, DMSO-d_6) δ 103.30, 115.13, 118.54, 125.92, 129.32, 129.81, 130.20, 130.38, 131.65, 133.17, 134.52, 139.00, 145.18, 152.76, 158.07,

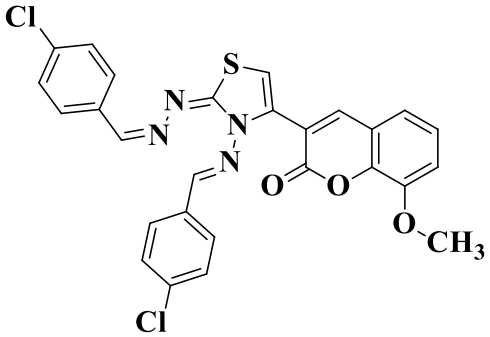
162.84, 165.14 ppm; Mass (ESI-HRMS) (*m/z*): 678.1966 $[M+H]^+$; Anal. Calcd. For $C_{26}H_{14}Br_2Cl_2N_4O_2S$: C, 46.11; H, 2.08; N, 8.27%. Found: C, 46.16; H, 2.14; N, 8.24%.

6,8-Dibromo-3-(-3-(-(4-bromobenzylidene)amino)-2-(-(4-bromobenzylidene)hydrazono)-2,3-dihydrothiazol-4-yl)-2*H*-chromen-2-one (4j):



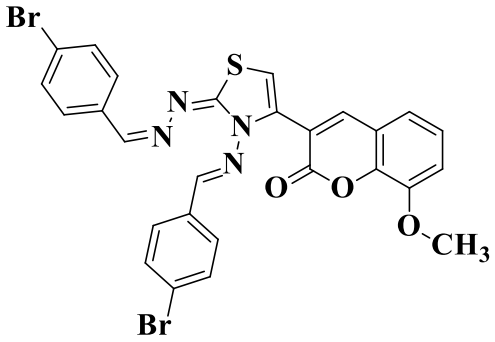
Brown solid; yield 92%; mp: 182-184^{o}C; IR (KBr) cm^{-1}: 1607 (C=N); ^{1}H NMR (400MHz, DMSO-d_6, ppm): δ 6.98 (s, 1H, Thiazole-H), 7.69-7.76 (m, 4H), 8.08-8.17 (m, 6H), 8.27 (s, 1H), 8.41 (s, 1H, NCH), 10.12 (s, 1H, NCH).^{13}C NMR (100 MHz, DMSO-d_6) δ 107.65, 112.78, 116.87, 128.61, 129.58, 130.81, 132.28, 133.13, 134.60, 136.79, 137.28, 138.60, 148.59, 149.73, 154.37, 162.65, 168.17 ppm; Mass (ESI-HRMS) (*m/z*): 762.7592 $[M+H]^+$; Anal. Calcd. For $C_{26}H_{14}Br_4N_4O_2S$: C, 40.76; H, 1.84; N, 7.31%. Found: C, 40.71; H, 1.89; N, 7.35%.

3-(-3-(-(4-Chlorobenzylidene)amino)-2-(-(4-chlorobenzylidene)hydrazono)-2,3-dihydrothiazol-4-yl)-8-methoxy-2*H*-chromen-2-one (4k):



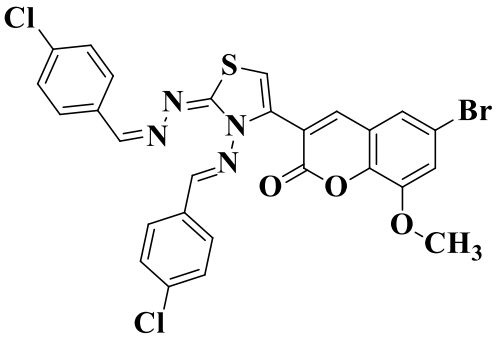
Yellow solid; yield 90%; mp: 204-206^{o}C; IR (KBr) cm^{-1}: 1610 (C=N); ^{1}H NMR (400MHz, DMSO-d_6, ppm): δ 3.96 (s, 3H), 6.98 (s, 1H, Thiazole-H), 7.53 (s, 1H), 7.55 (s, 1H), 7.57 (s, 1H), 7.64 (d, J = 8.4 Hz, 2H), 7.70 (d, J = 8 Hz, 2H), 7.82 (d, J = 8.8 Hz, 3H), 7.91 (d, J = 8.4 Hz, 1H), 8.36 (s, 1H), 8.57 (s, 1H, NCH), 10.15 (s, 1H, NCH).^{13}CNMR (100 MHz, DMSO-d_6) δ 56.55, 103.63, 111.35, 114.37, 120.45, 125.12, 128.36, 129.40, 129.48, 130.23, 134.62, 138.83, 142.06, 146.74, 153.08, 155.08, 158.94, 168.07 ppm; Mass (ESI-HRMS) (*m/z*): 549.0545 $[M+H]^+$; Anal. Calcd. For $C_{27}H_{18}Cl_2N_4O_3S$: C, 59.02; H, 3.30; N, 10.20%. Found: C, 59.08; H, 3.35; N, 10.25%.

3-(-3-(-(4-Bromobenzylidene)amino)-2-(-(4-bromobenzylidene)hydrazono)-2,3-dihydrothiazol-4-yl)-8-methoxy-2*H*-chromen-2-one (4l):



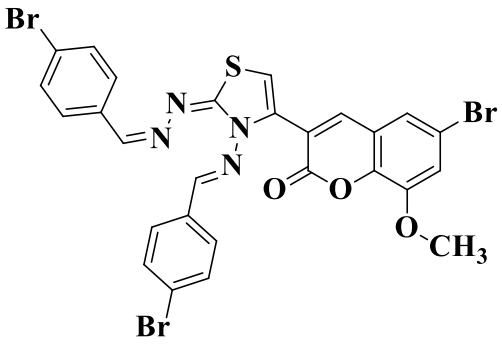
Yellow solid; yield 89%; mp: 194-196ºC; IR (KBr) cm^{-1}: 1609 (C=N); ^{1}H NMR (400MHz, DMSO-d_6, ppm): δ 3.95 (s, 3H), 6.97 (s, 1H, Thiazole-H), 7.55 (d, J = 8.4 Hz, 2H), 7.63-7.67 (m, 4H), 7.69-7.75 (m, 4H), 7.81 (s, 1H), 8.35 (s, 1H), 8.55 (s, 1H, NCH), 10.13 (s, 1H, NCH).^{13}C NMR (100 MHz, DMSO-d_6) δ 56.56, 102.35, 115.98, 118.65, 125.14, 127.53, 128.62, 132.31, 133.13, 133.79, 134.61, 137.10, 144.42, 151.26, 151.68, 157.37, 162.46 ppm; Mass (ESI-HRMS) (*m/z*): 636.92 $[M+H]^+$; Anal. Calcd. For $C_{27}H_{18}Br_2N_4O_3S$: C, 50.80; H, 2.84; N, 8.78%. Found: C, 50.75; H, 2.80; N, 8.73%.

6-Bromo-3-(-3-(-(4-chlorobenzylidene)amino)-2-(-(4-chlorobenzylidene)hydrazono)-2,3-dihydrothiazol-4-yl)-8-methoxy-2*H*-chromen-2-one (4m):



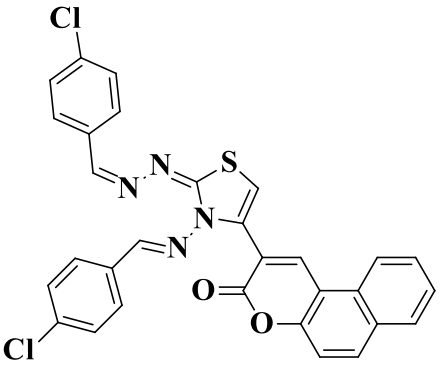
Olive Green solid; yield 88%; mp: 248-250ºC; IR (KBr) cm^{-1}: 1608 (C=N); ^{1}H NMR (400MHz, DMSO-d_6, ppm): δ 3.97 (s, 3H), 6.98 (s, 1H, Thiazole-H), 7.50-7.55 (m, 6H), 7.76-7.83 (m, 4H), 8.07 (s, 1H), 8.44 (s, 1H, NCH), 10.15 (s, 1H, NCH).^{13}C NMR (100 MHz, DMSO-d_6) δ 108.84, 116.96, 117.80, 125.12, 127.91, 128.37, 130.24, 134.21, 134.63, 136.42, 137.43, 143.11, 144.19, 146.71, 157.38, 158.44, 161.15, 165.24 ppm; Mass (ESI-HRMS) (*m/z*): 628.4876 $[M+]^+$; Anal. Calcd. For $C_{27}H_{17}BrCl_2N_4O_3S$: C, 51.61; H, 2.73; N, 8.92%. Found: C, 51.66; H, 2.68; N, 8.96%.

6-Bromo-3-(-3-(-(4-bromobenzylidene)amino)-2-(-(4-bromobenzylidene)hydrazono)-2,3-dihydrothiazol-4-yl)-8-methoxy-2*H*-chromen-2-one (4n):



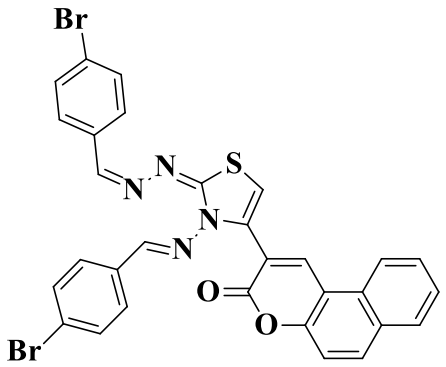
Olive Green solid; yield 91%; mp: 246-248ºC; IR (KBr) cm^{-1}: 1604 (C=N); ^{1}H NMR (400MHz, DMSO-d_6, ppm): δ 3.95 (s, 3H), 6.97 (s, 1H, Thiazole-H), 7.61-7.66 (m, 6H), 7.72-7.78 (m, 4H), 8.03 (s, 1H), 8.42 (s, 1H, NCH), 10.12 (s, 1H, NCH).^{13}C NMR (100 MHz, DMSO-d_6) δ 102.37, 111.00, 116.70, 122.30, 126.27, 128.61, 130.48, 131.05, 131.73, 132.30, 132.80, 133.13, 134.61, 147.59, 150.10, 151.90, 157.94, 166.26 ppm; Mass (ESI-HRMS) (*m/z*): 718.4491 $[M+H]^+$; Anal. Calcd. For $C_{27}H_{17}Br_3N_4O_3S$: C, 45.21; H, 2.39; N, 7.81%. Found: C, 45.26; H, 2.44; N, 7.86%.

2-(-3-(-(4-Chlorobenzylidene)amino)-2-(-(4-chlorobenzylidene)hydrazono)-2,3-dihydrothiazol-4-yl)-3*H*-benzo[f]chromen-3-one (6a):

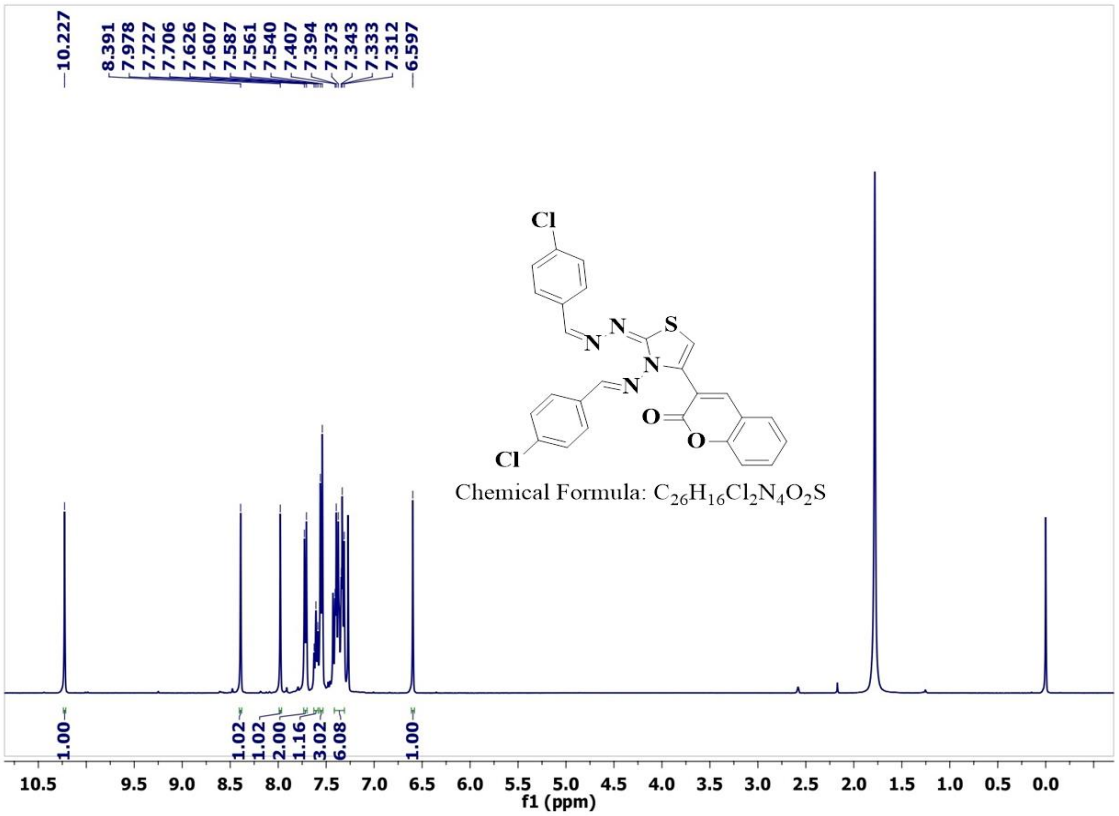


Brown solid; yield 92%; mp: 187-189ºC; ^{1}H NMR (400MHz, $CDCl_3$+ DMSO-d_6, ppm): δ 6.86 (s, 1H, Thiazole-H), 7.32 (d, *J* = 8.4 Hz, 2H), 7.43 (d, *J* = 8 Hz, 2H), 7.55 (s, 1H), 7.60 (s, 1H), 7.77 (d, *J* = 8 Hz, 2H), 7.90 (s, 4H), 8.15 (d, *J* = 9.2 Hz, 1H),8.42 (d, *J* = 8.8 Hz, 1H), 8.46 (s, 1H), 8.98 (s, 1H, NCH), 10.25(s, 1H, NCH).^{13}C NMR (100 MHz, $CDCl_3$+ DMSO-d_6) δ 109.05, 124.64, 125.06, 125.35, 126.06, 126.89, 127.68, 129.56, 129.69, 129.81, 131.47, 141.17, 146.75, 149.20, 158.70, 169.07 ppm; IR (KBr) cm^{-1}: 1609 (C=N); Mass (ESI-HRMS) (*m/z*): 569.0050 $[M+H]^+$; Anal. Calcd. For $C_{30}H_{18}Cl_2N_4O_2S$: C, 63.27; H, 3.19; N, 9.84%. Found: C, 63.23; H, 3.15; N, 9.79%.

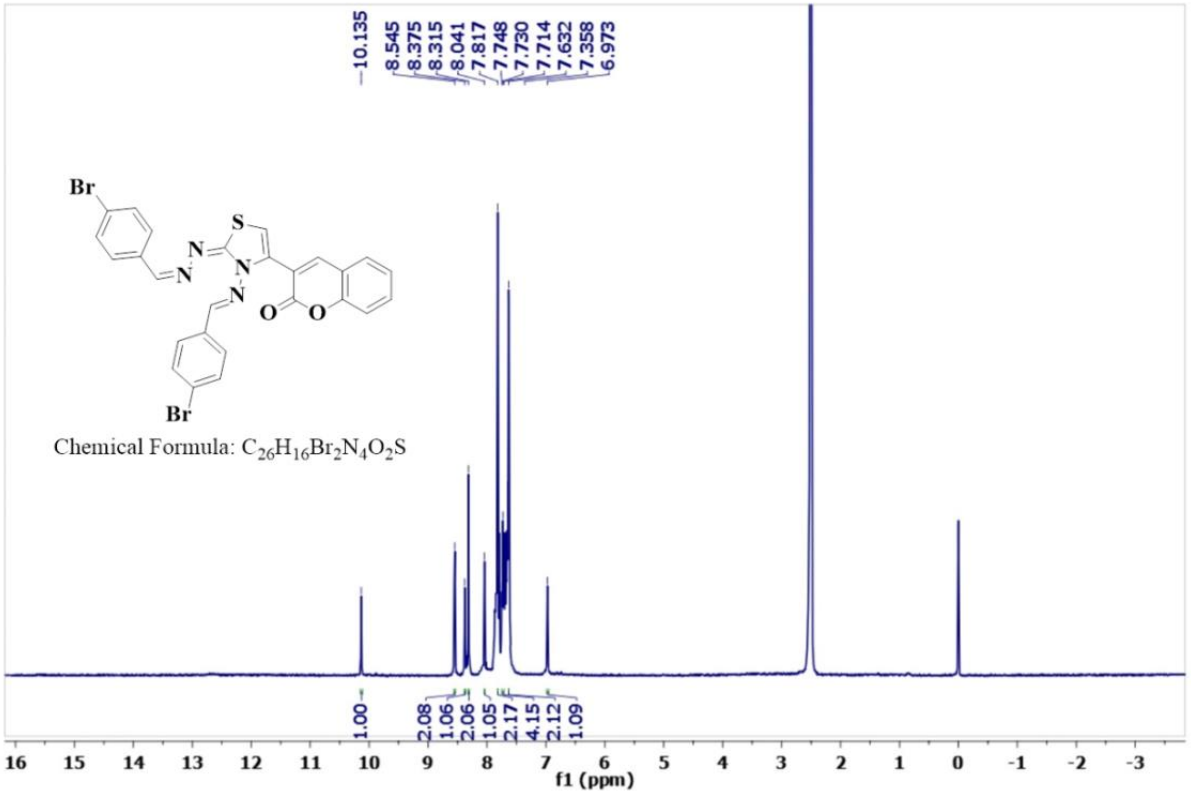
2-(-3-(-(4-Bromobenzylidene)amino)-2-(-(4-bromobenzylidene)hydrazono)-2,3-dihydrothiazol-4-yl)-3*H*-benzo[f]chromen-3-one (6b):



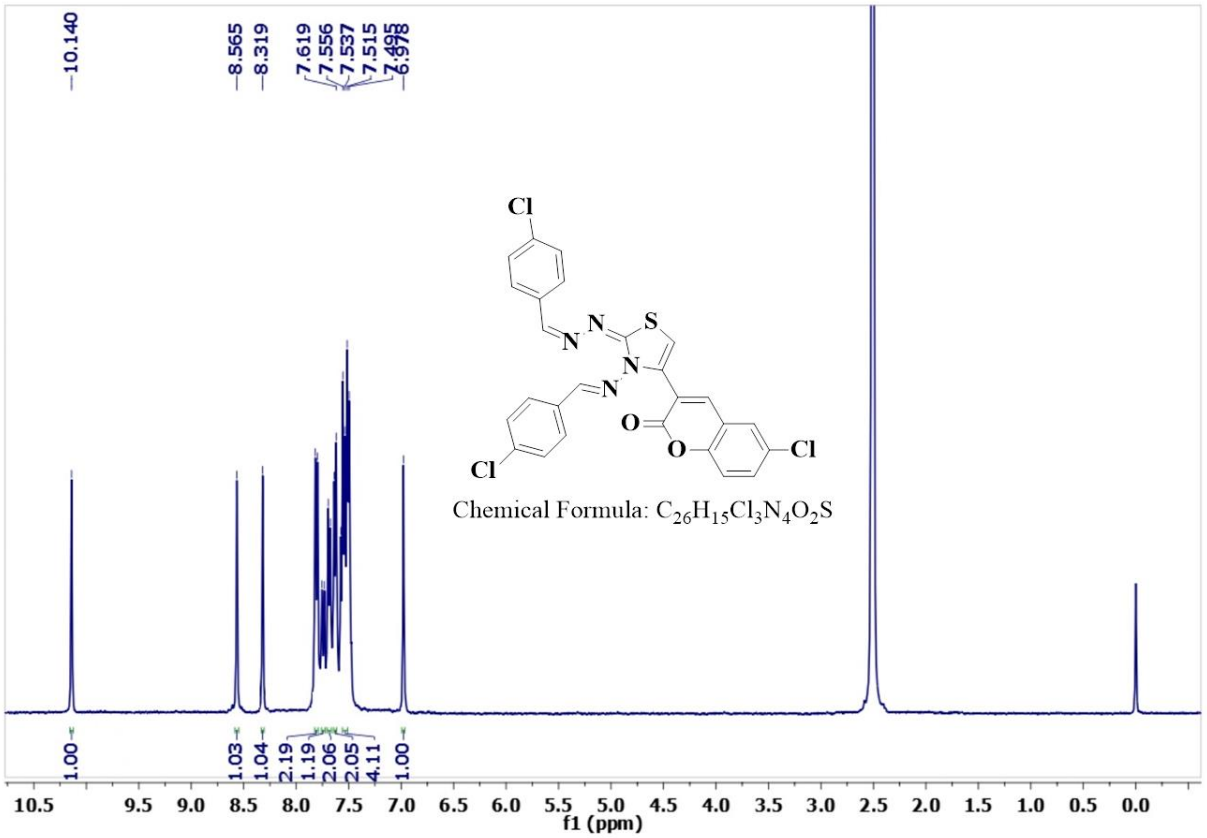
Yellow solid; yield 89%; mp: 254-256ºC; ^{1}H NMR (400MHz, DMSO-$d_{6,}$ ppm): δ 7.08 (s, 1H, Thiazole-H), 7.56-7.63 (m, 6H), 7.83 (s, 2H), 7.85 (s, 2H), 8.12 (d, J = 8 Hz, 2H), 8.23 (d, J = 8.8 Hz, 2H), 8.58 (s, 1H), 9.22 (s, 1H, NCH), 10.17(s, 1H, NCH).^{13}C NMR (100 MHz, DMSO-d_6) δ 109.72, 124.50, 124.88, 125.35, 126.33, 127.29, 128.48, 129.03, 129.69, 131.12, 138.70, 141.09, 146.37, 160.64, 168.28 ppm; IR (KBr) cm^{-1}: 1604 (C=N); Mass (ESI-HRMS) (*m/z*): 658.4496 [M+]$^{+}$; Anal. Calcd. For $C_{30}H_{18}Br_2N_4O_2S$: C, 54.73; H, 2.76; N, 8.51%. Found: C, 54.70; H, 2.71; N, 8.56%.



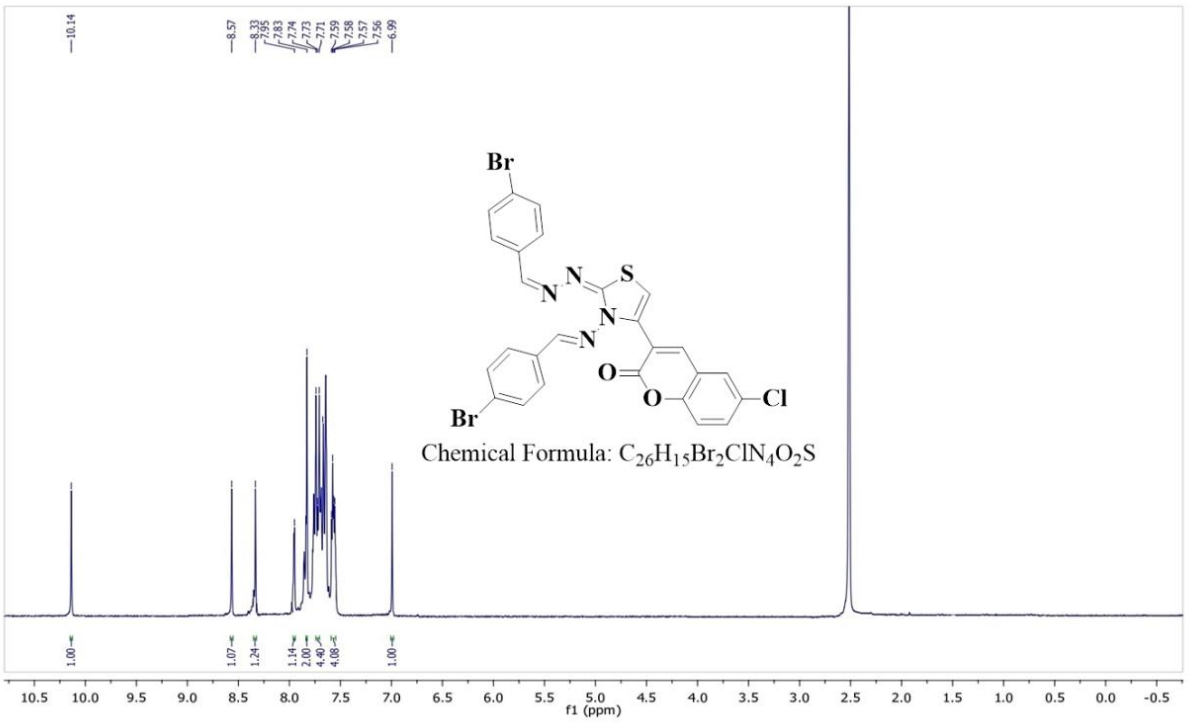
^{1}H NMR spectrum of compound **4a** (400 MHz, $CDCl_3$)



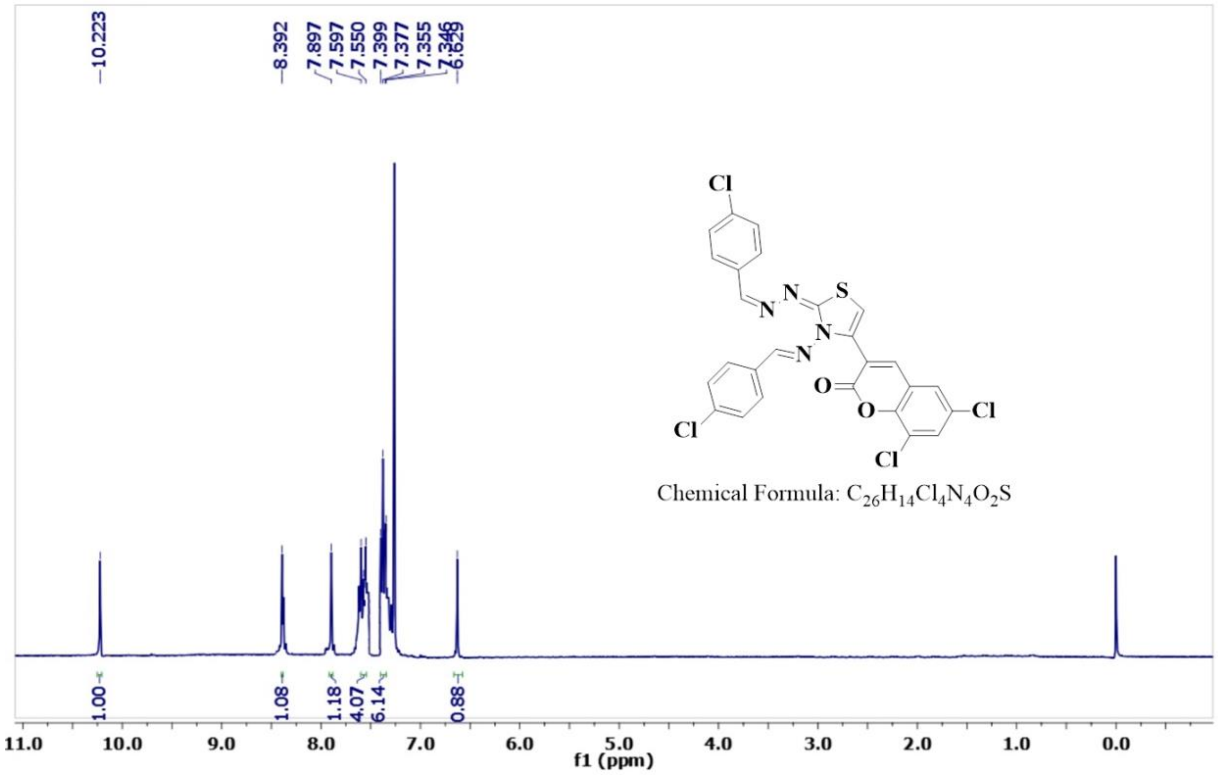
^{1}H NMR spectrum of compound **4b** (400 MHz, DMSO-d_6)



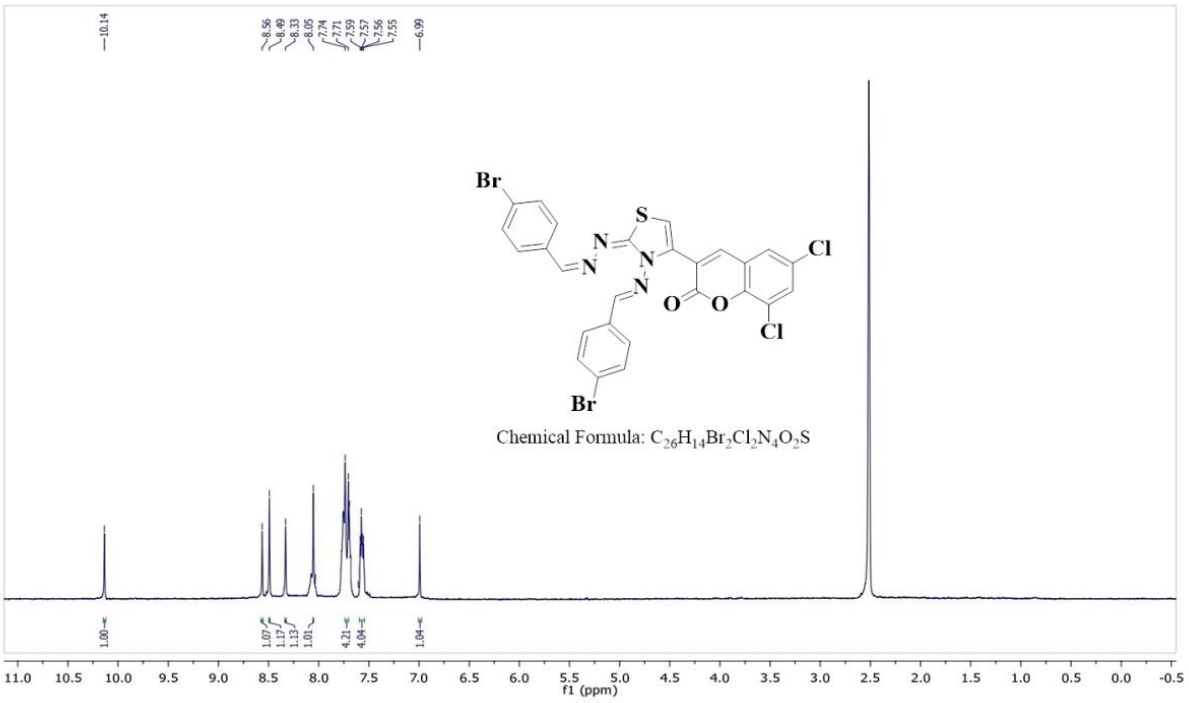
^{1}H NMR spectrum of compound **4c** (400 MHz, DMSO-d_6)



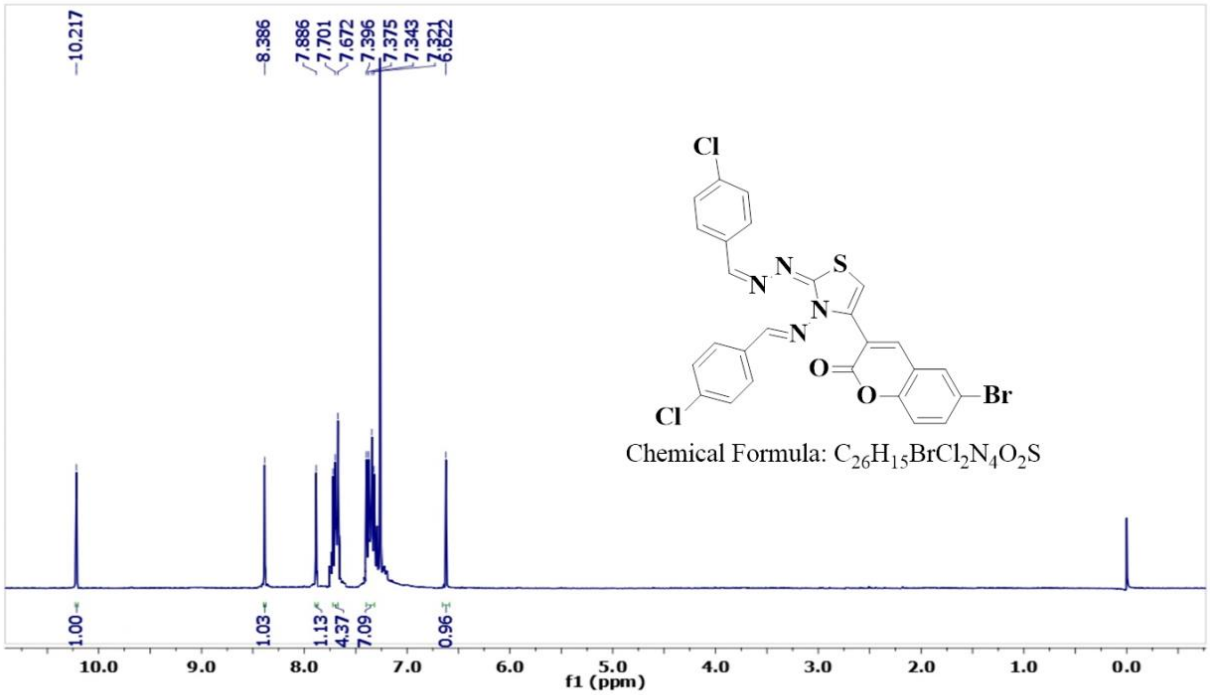
^{1}H NMR spectrum of compound **4d** (400 MHz, DMSO-d_6)



^{1}H NMR spectrum of compound **4e** (400 MHz, DMSO-d_6)

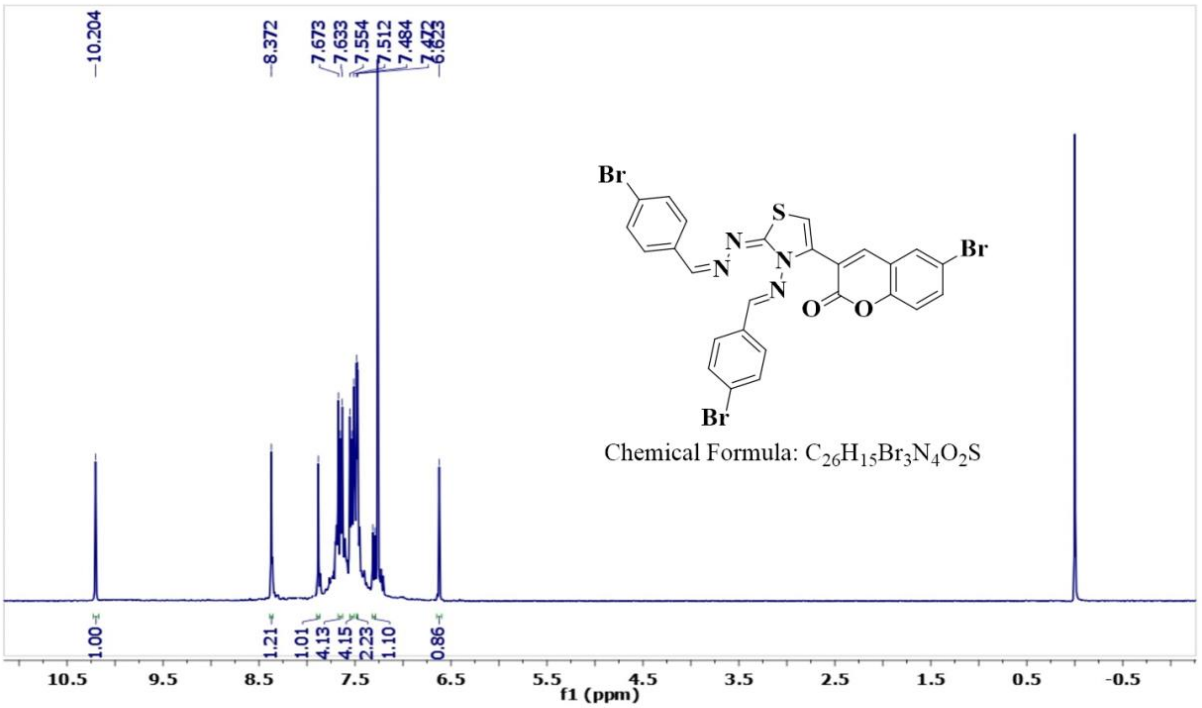


^{1}H NMR spectrum of compound **4f** (400 MHz, DMSO-d_6)



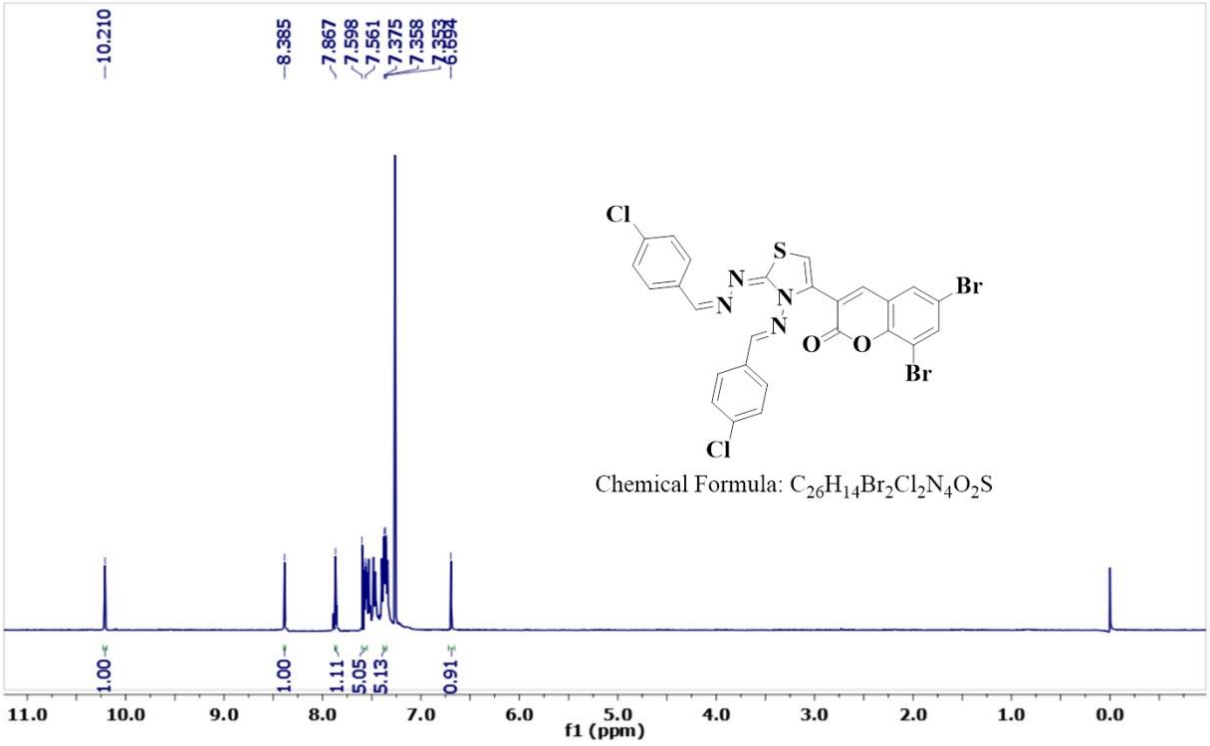
Chemical Formula: $C_{26}H_{15}BrCl_2N_4O_2S$

^{1}H NMR spectrum of compound **4g** (400 MHz, DMSO-d_6)

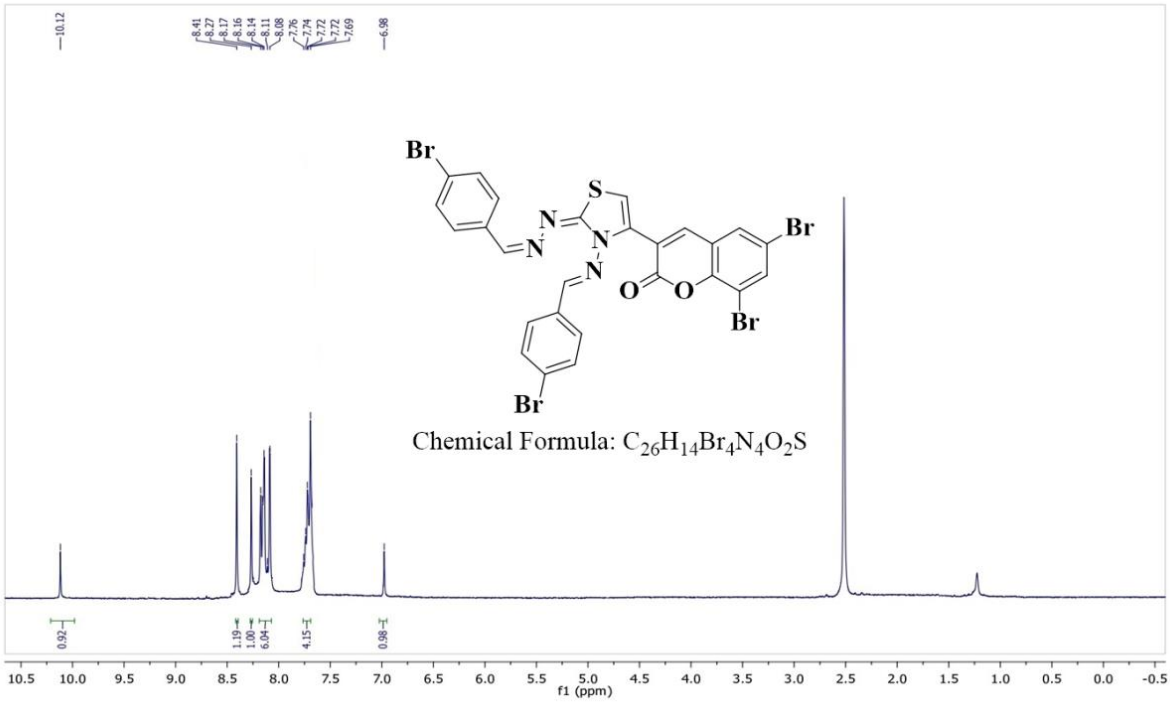


Chemical Formula: $C_{26}H_{15}Br_3N_4O_2S$

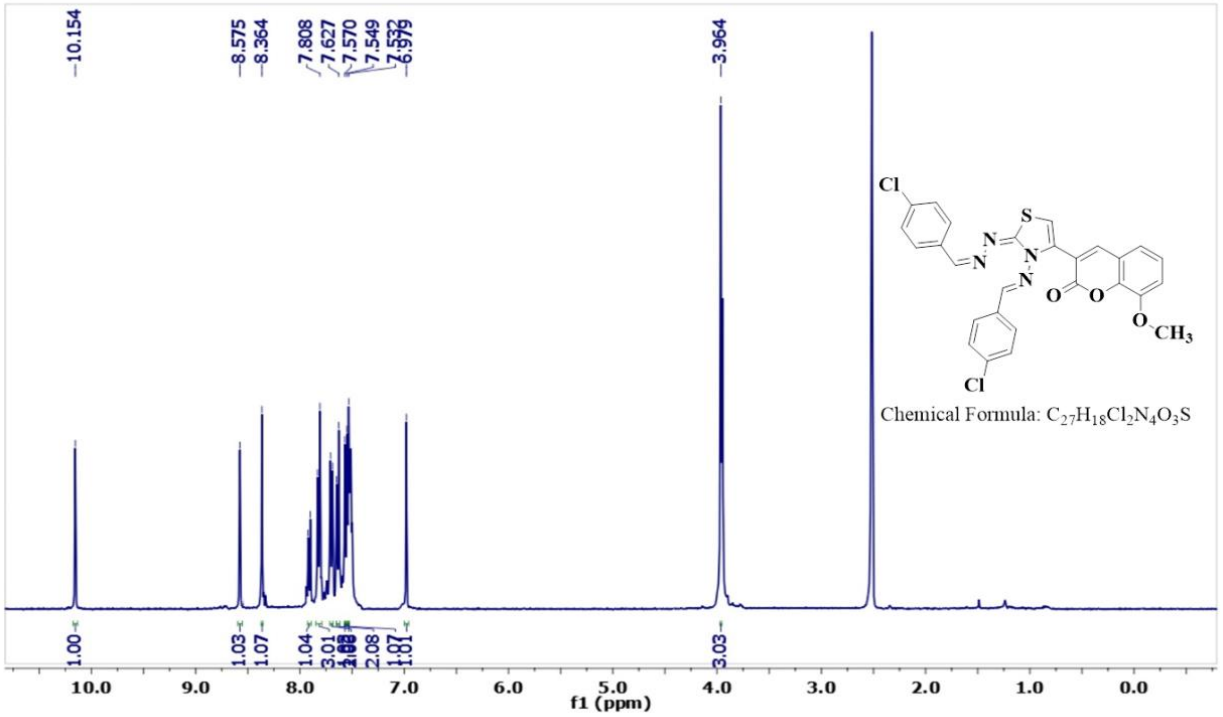
^{1}H NMR spectrum of compound **4h** (400 MHz, DMSO-d_6)



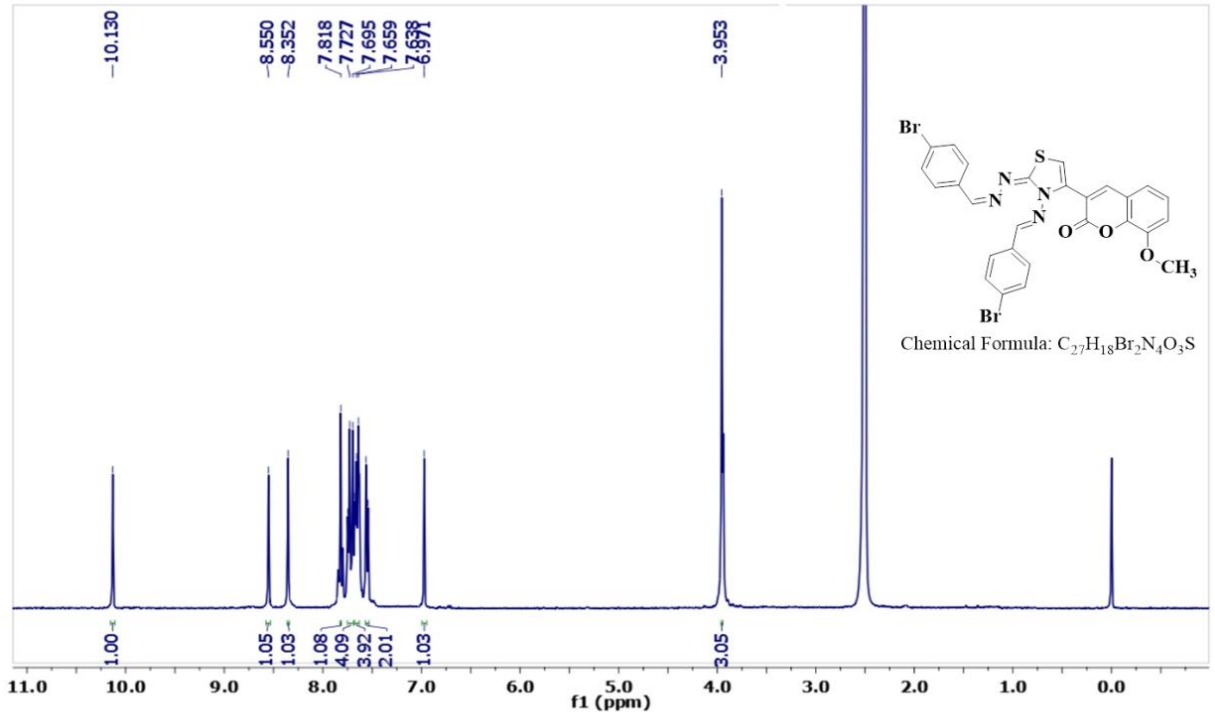
^{1}H NMR spectrum of compound **4i** (400 MHz, DMSO-d_6)



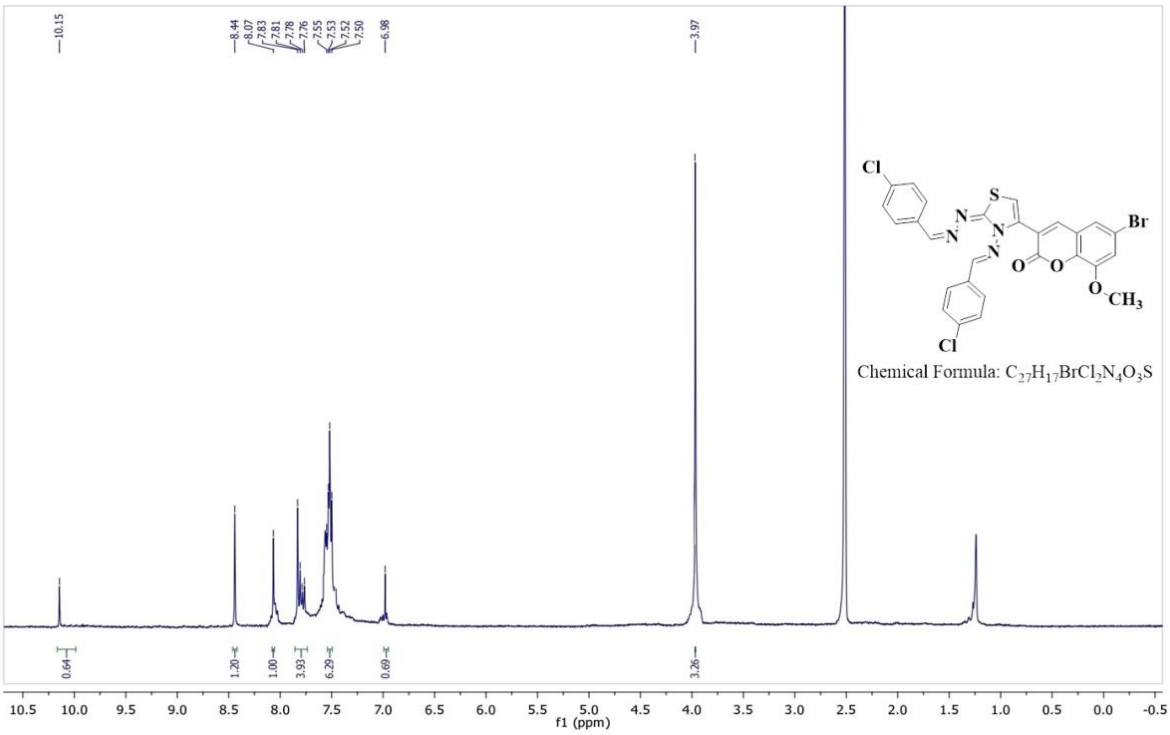
^{1}H NMR spectrum of compound **4j** (400 MHz, DMSO-d_6)



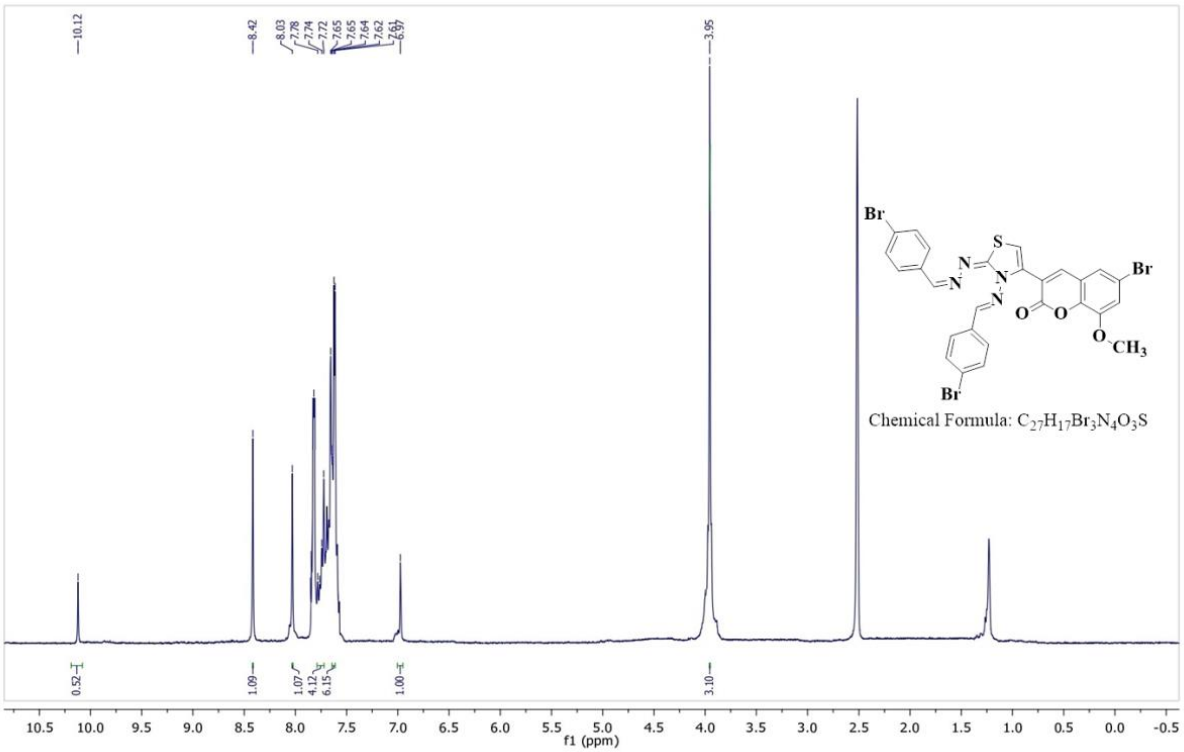
^{1}H NMR spectrum of compound **4k** (400 MHz, DMSO-d_6)



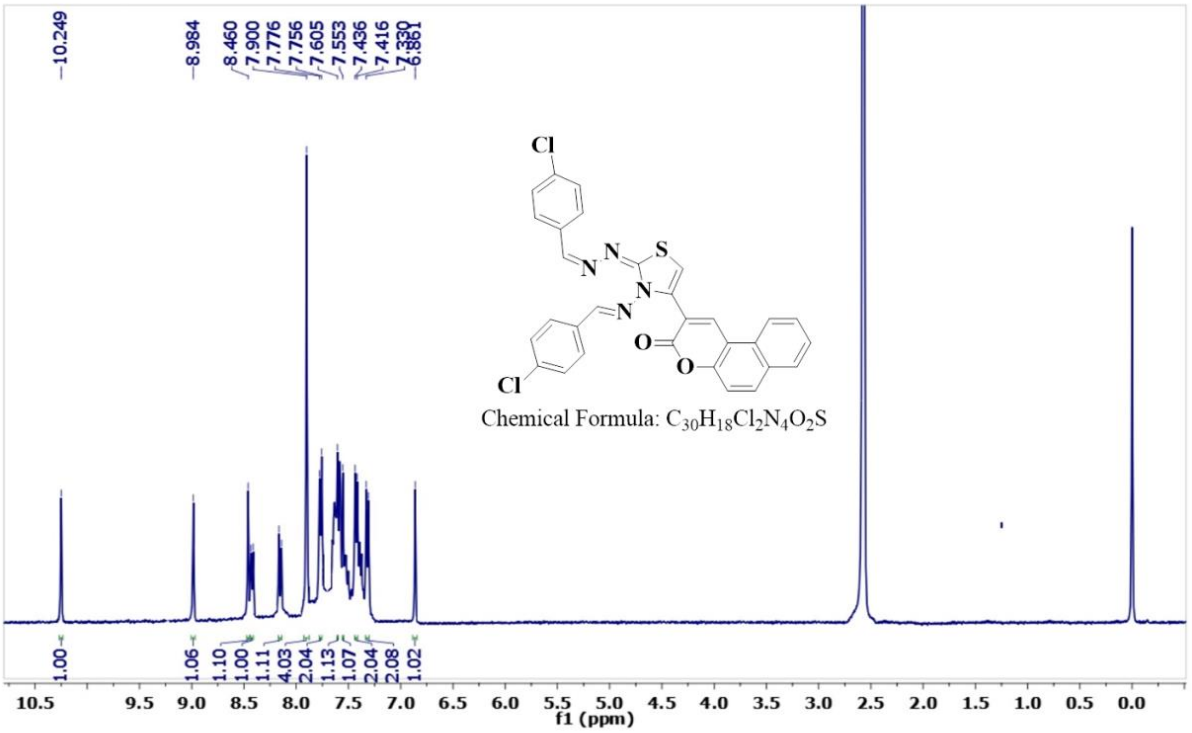
^{1}H NMR spectrum of compound **4l** (400 MHz, DMSO-d_6)



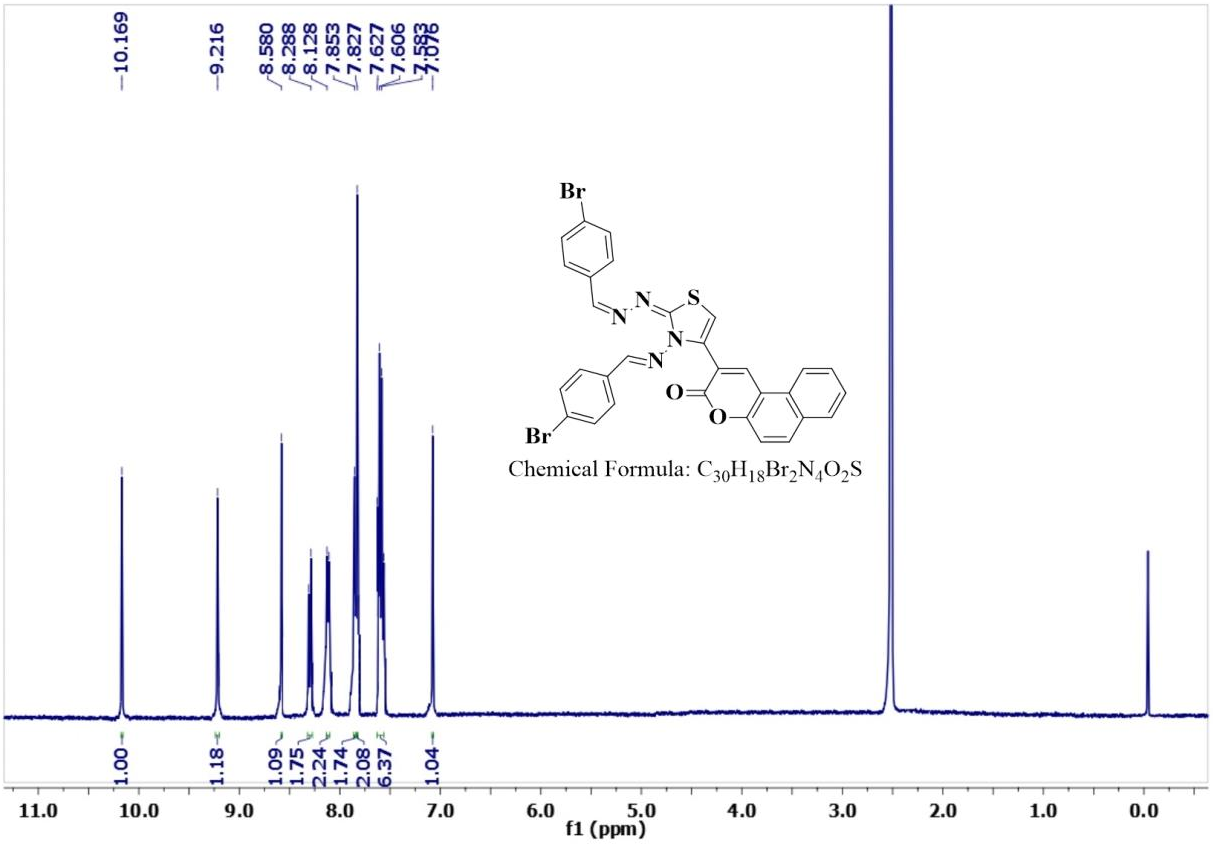
^{1}H NMR spectrum of compound **4m** (400 MHz, DMSO-d_6)



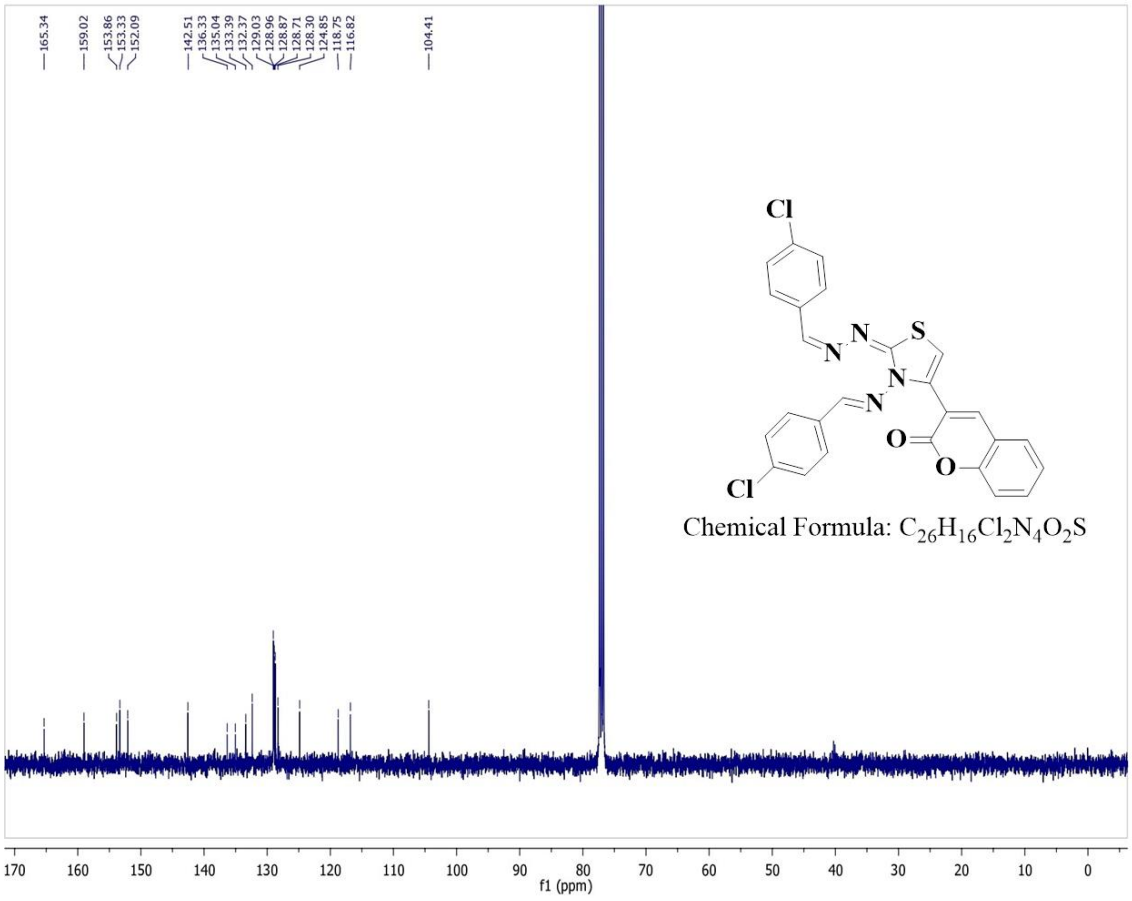
^{1}H NMR spectrum of compound **4n** (400 MHz, DMSO-d_6)



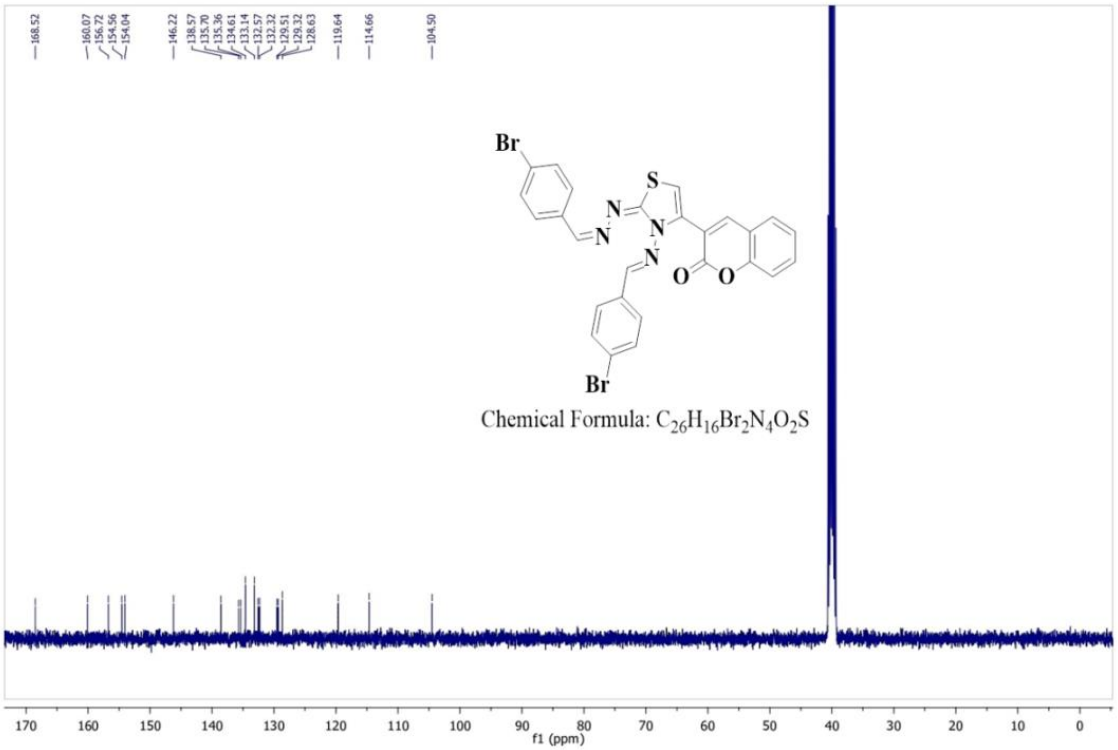
^{1}H NMR spectrum of compound **6a** (400 MHz, $CDCl_3$+DMSO-d_6)



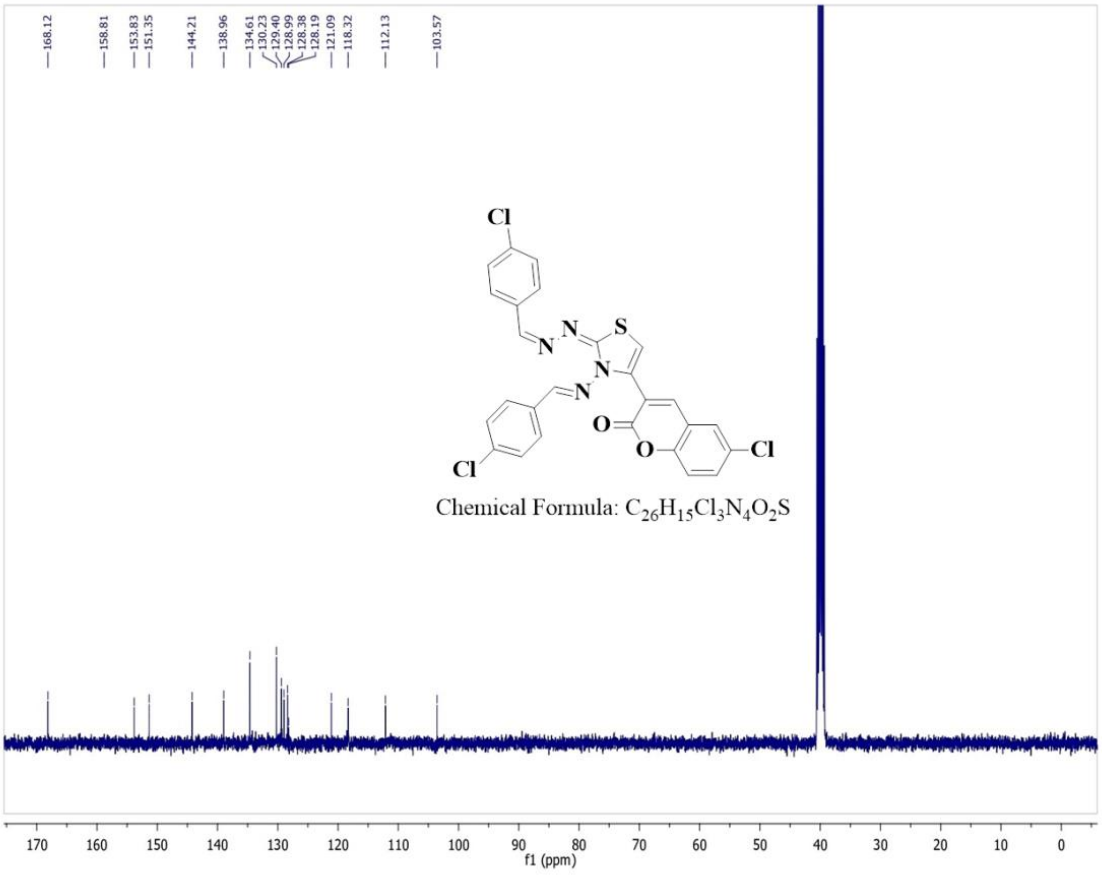
^{1}H NMR spectrum of compound **6b** (400 MHz, DMSO-d_6)



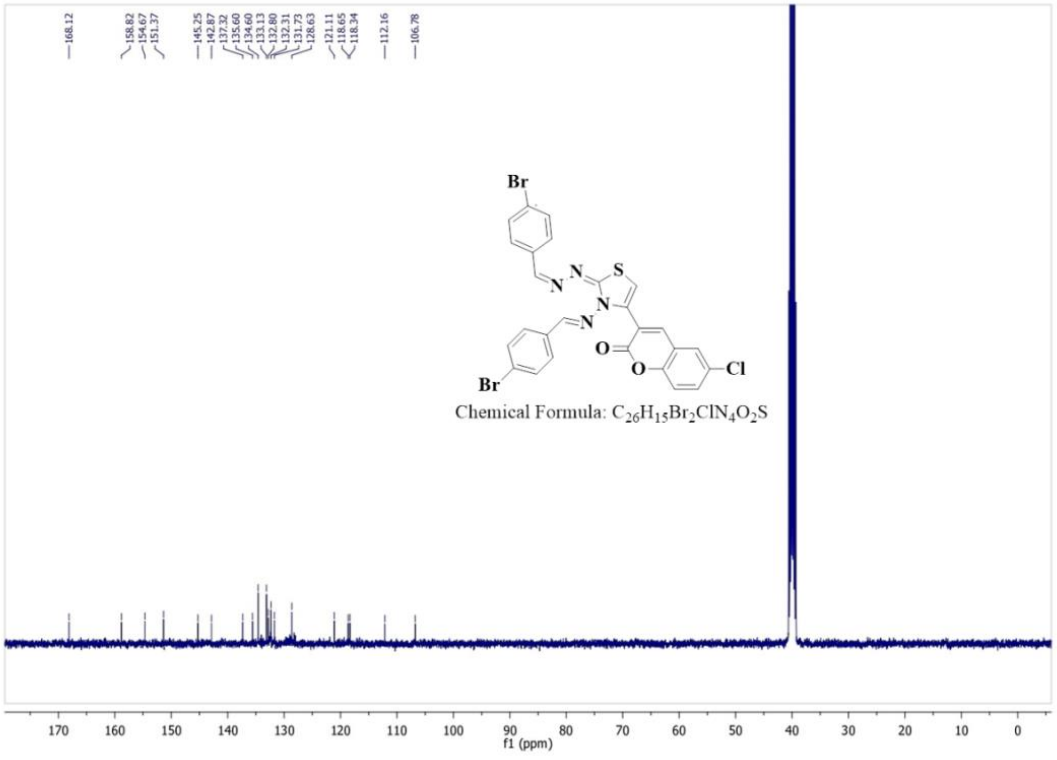
^{13}C NMR spectrum of compound 4**a** (100 MHz, $CDCl_3$)



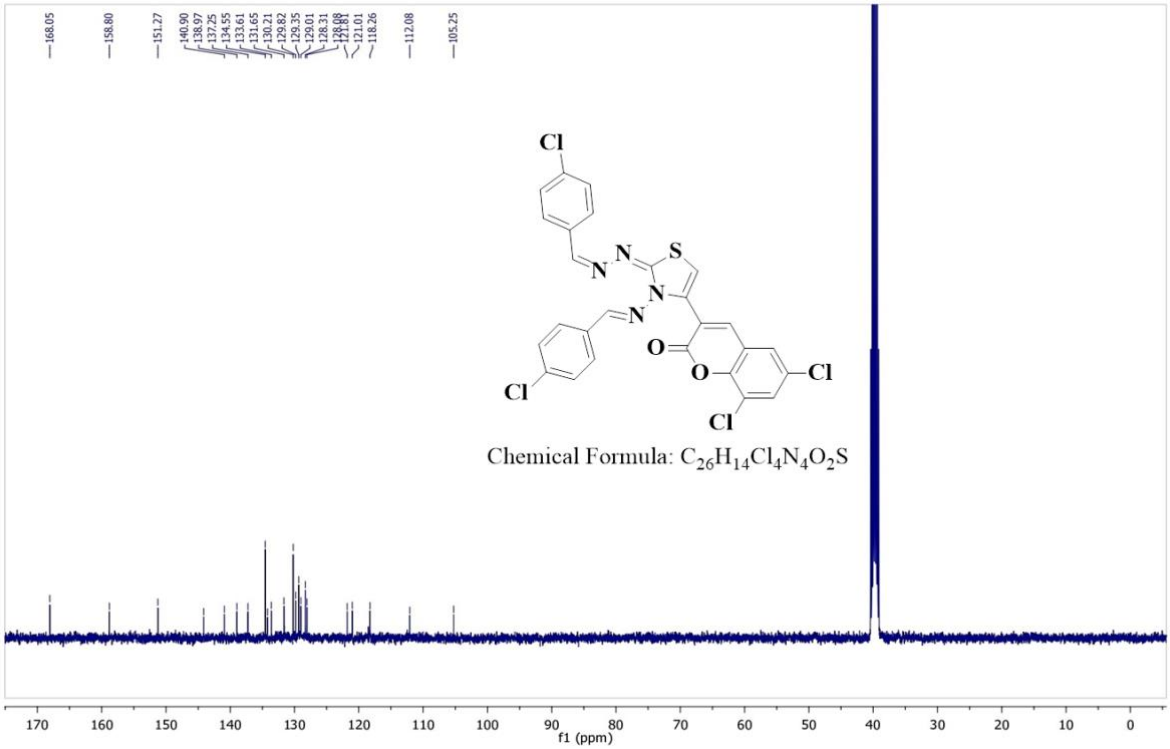
^{13}C NMR spectrum of compound **4b** (100 MHz, DMSO-d_6)



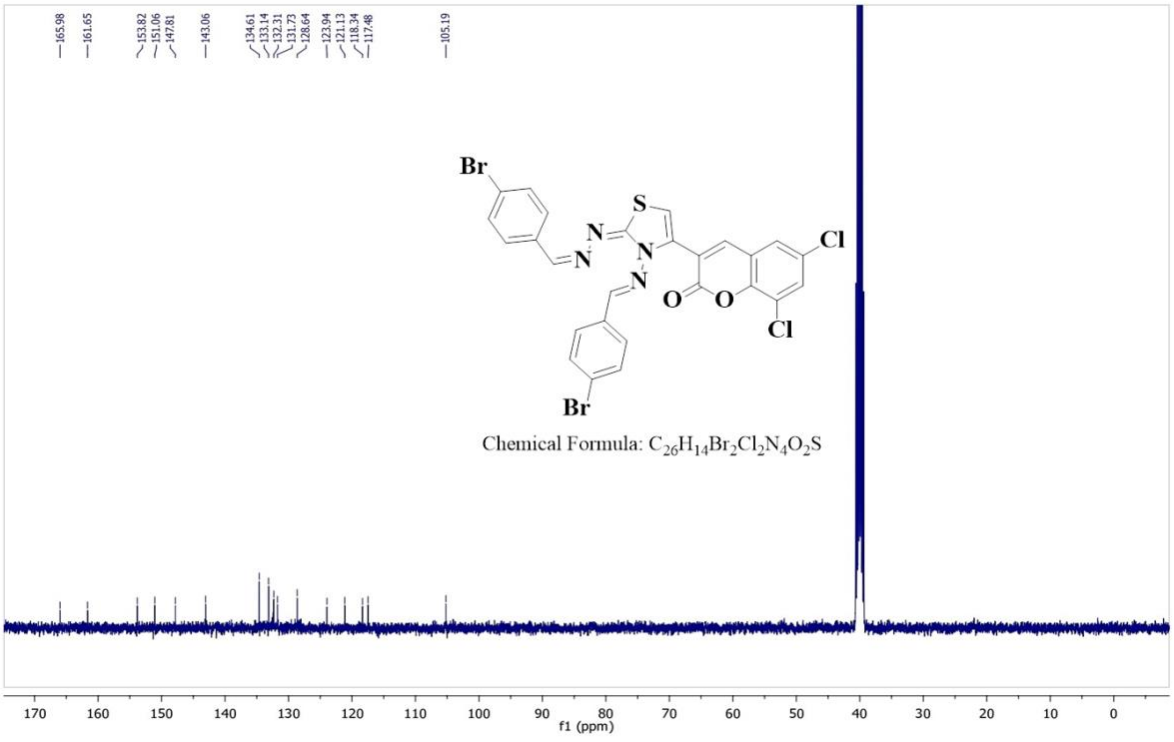
^{13}C NMR spectrum of compound **4c** (100 MHz, DMSO-d_6)



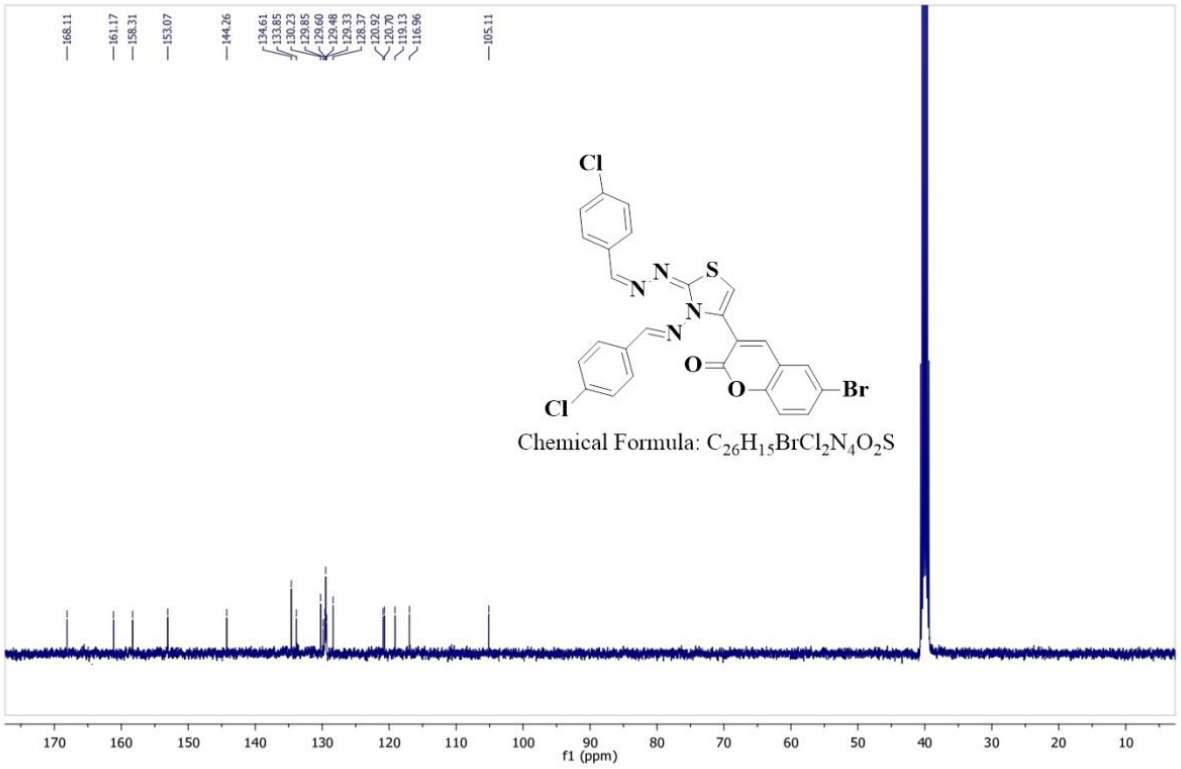
^{13}C NMR spectrum of compound **4d** (100 MHz, DMSO-d_6)



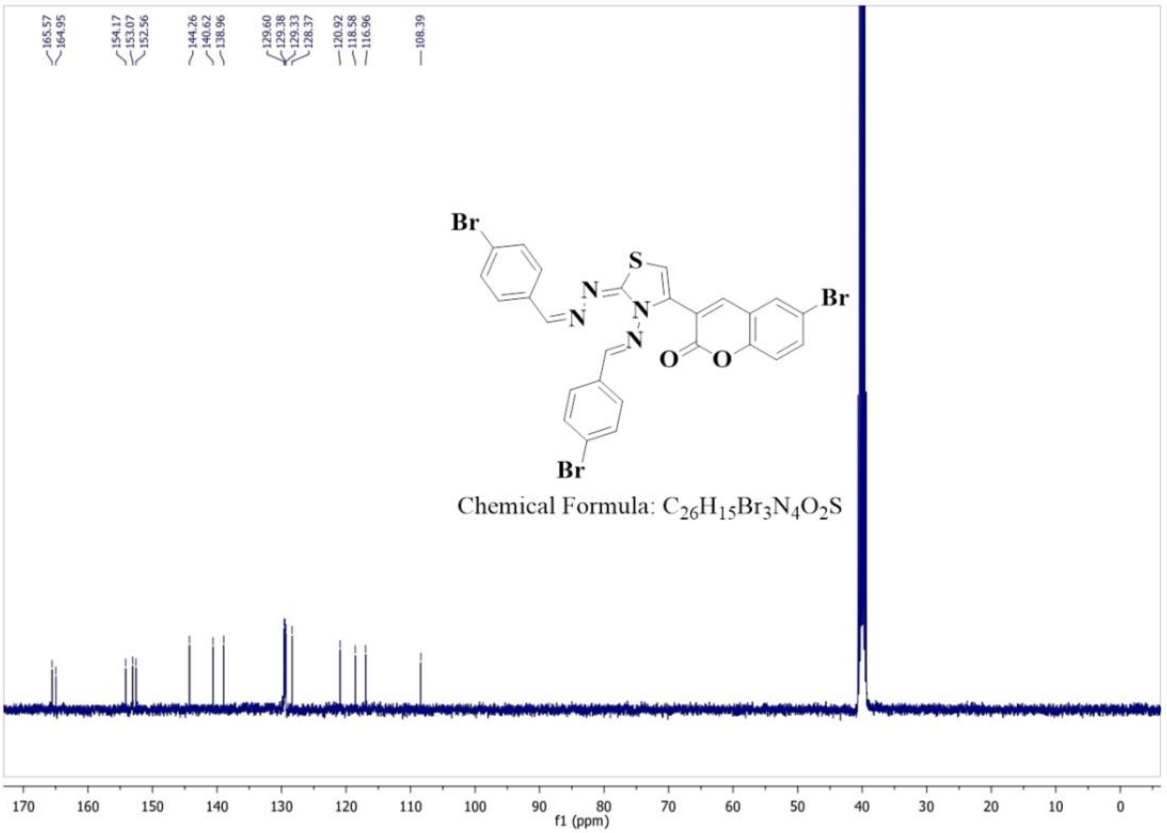
^{13}C NMR spectrum of compound **4e** (100 MHz, DMSO-d_6)



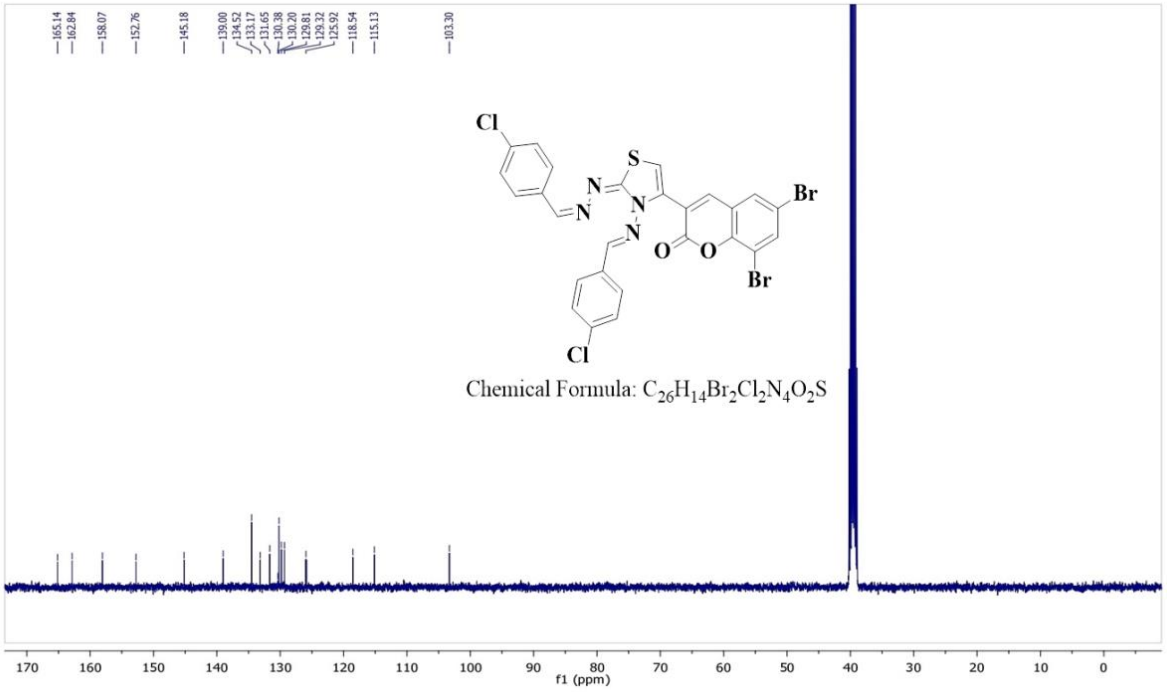
^{13}C NMR spectrum of compound **4f** (100 MHz, DMSO-d_6)



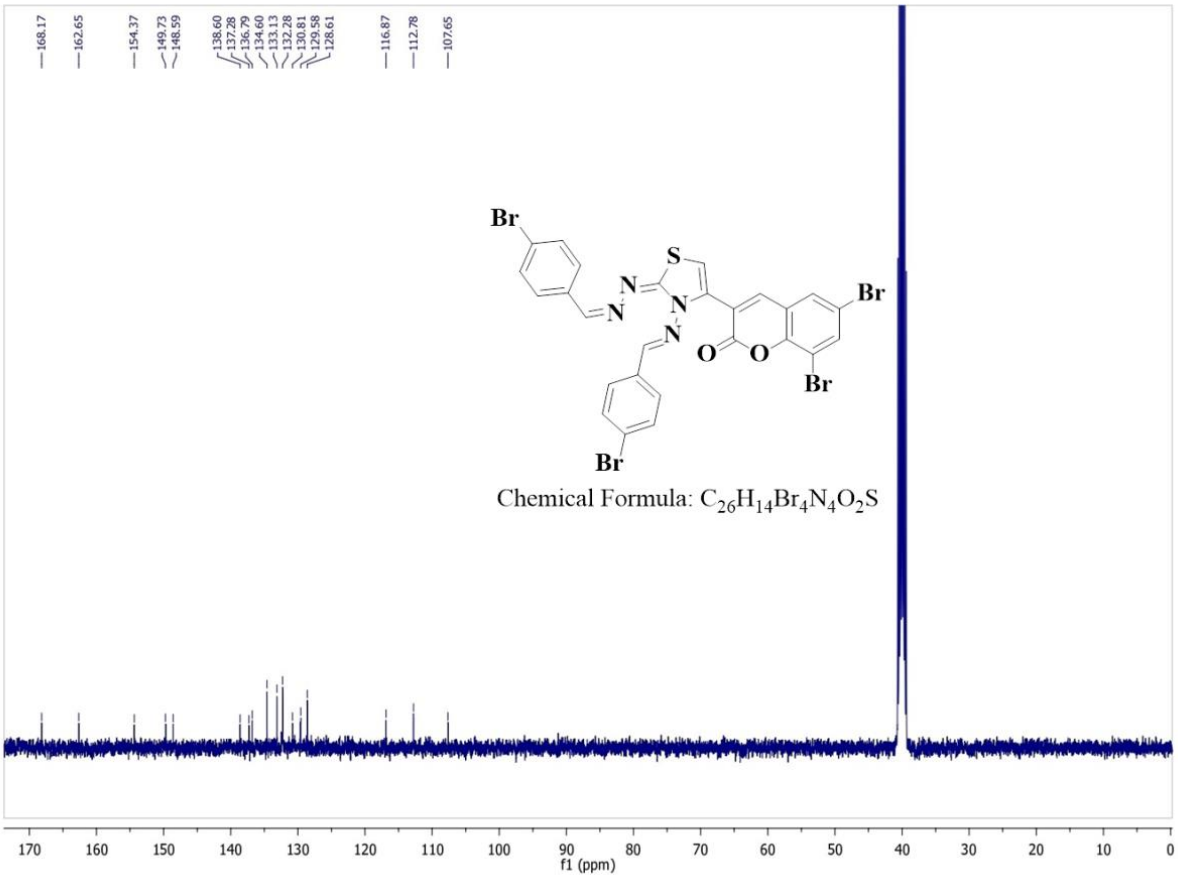
^{13}C NMR spectrum of compound **4g** (100 MHz, DMSO-d_6)



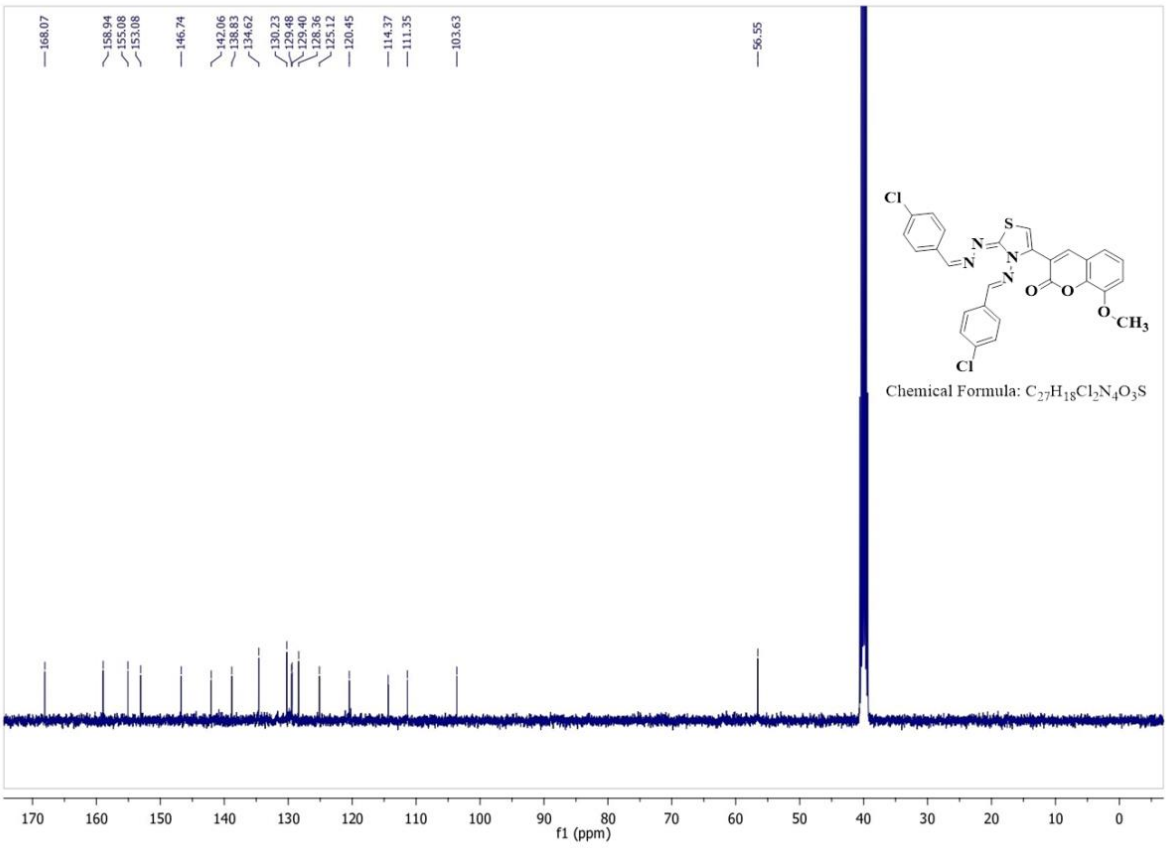
^{13}C NMR spectrum of compound **4h** (100 MHz, DMSO-d_6)



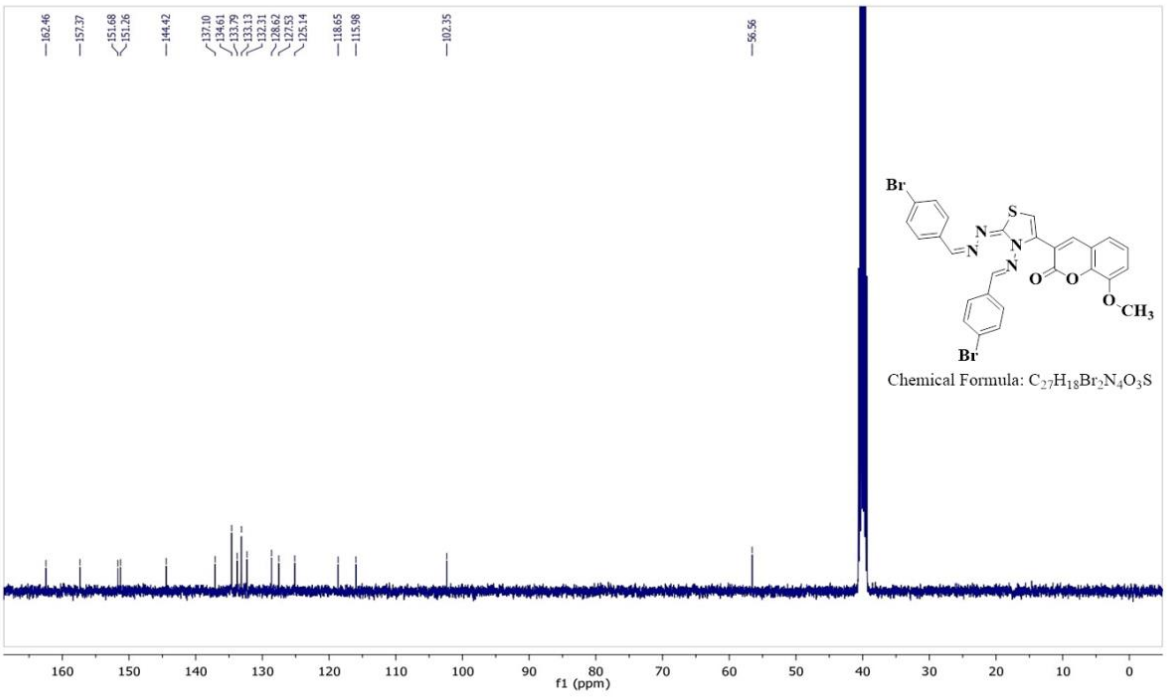
^{13}C NMR spectrum of compound **4i** (100 MHz, DMSO-d_6)



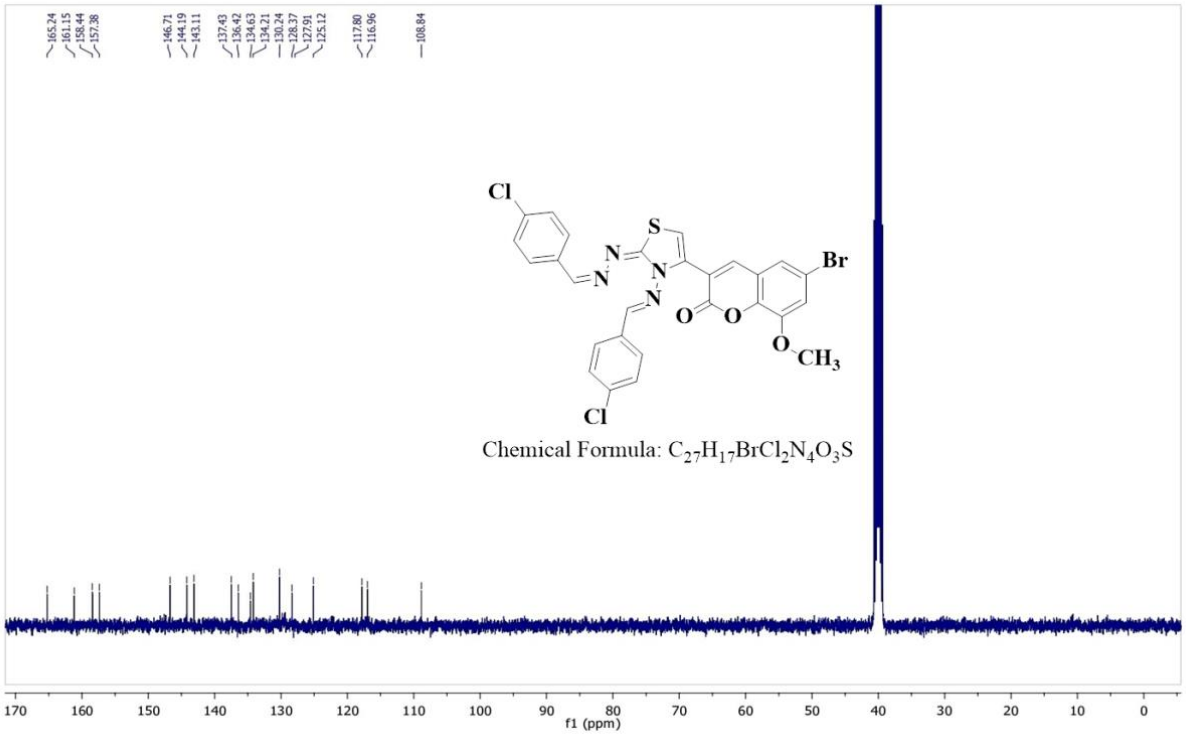
^{13}C NMR spectrum of compound **4j** (100 MHz, DMSO-d_6)



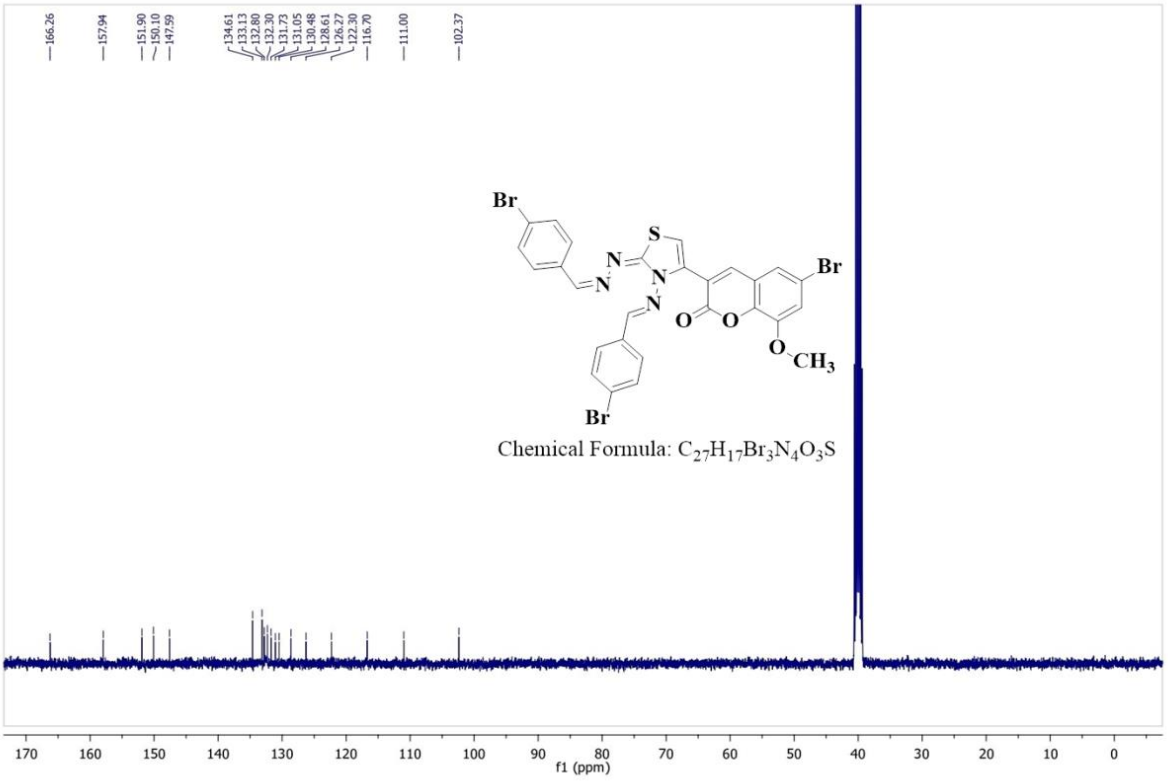
^{13}C NMR spectrum of compound **4k** (100 MHz, DMSO-d_6)



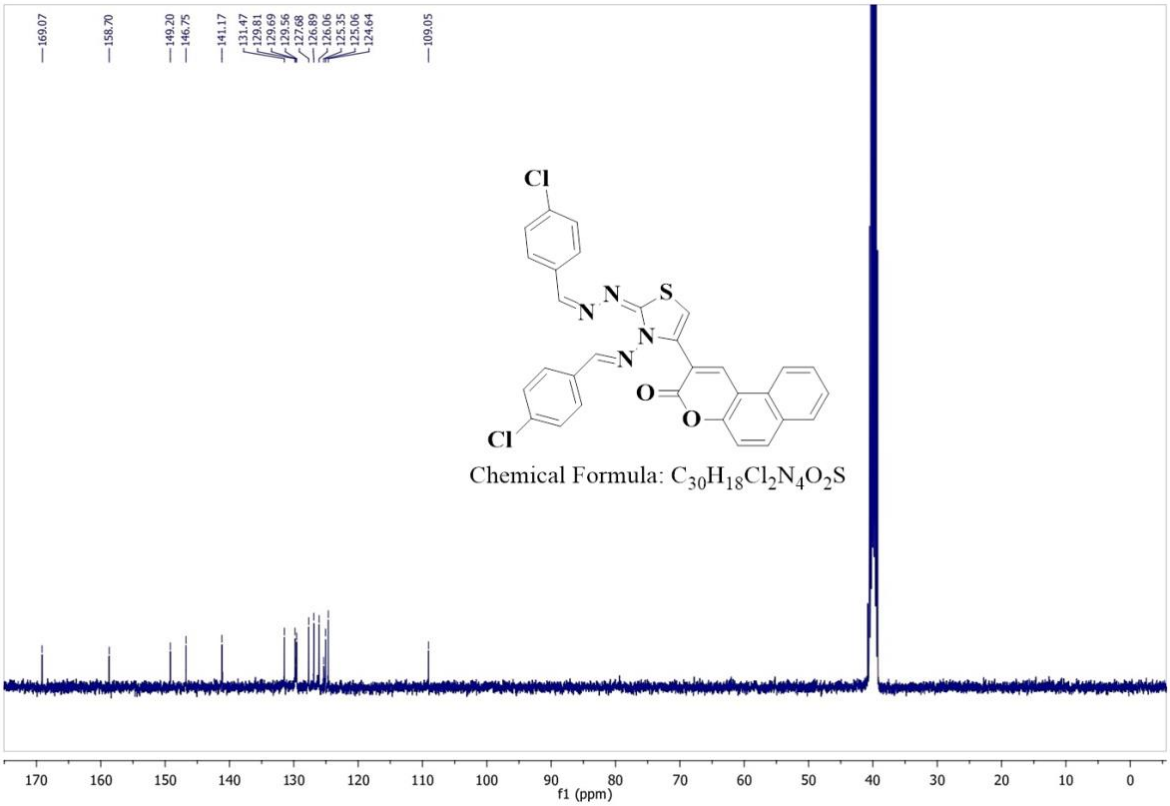
^{13}C NMR spectrum of compound **4l** (100 MHz, DMSO-d_6)



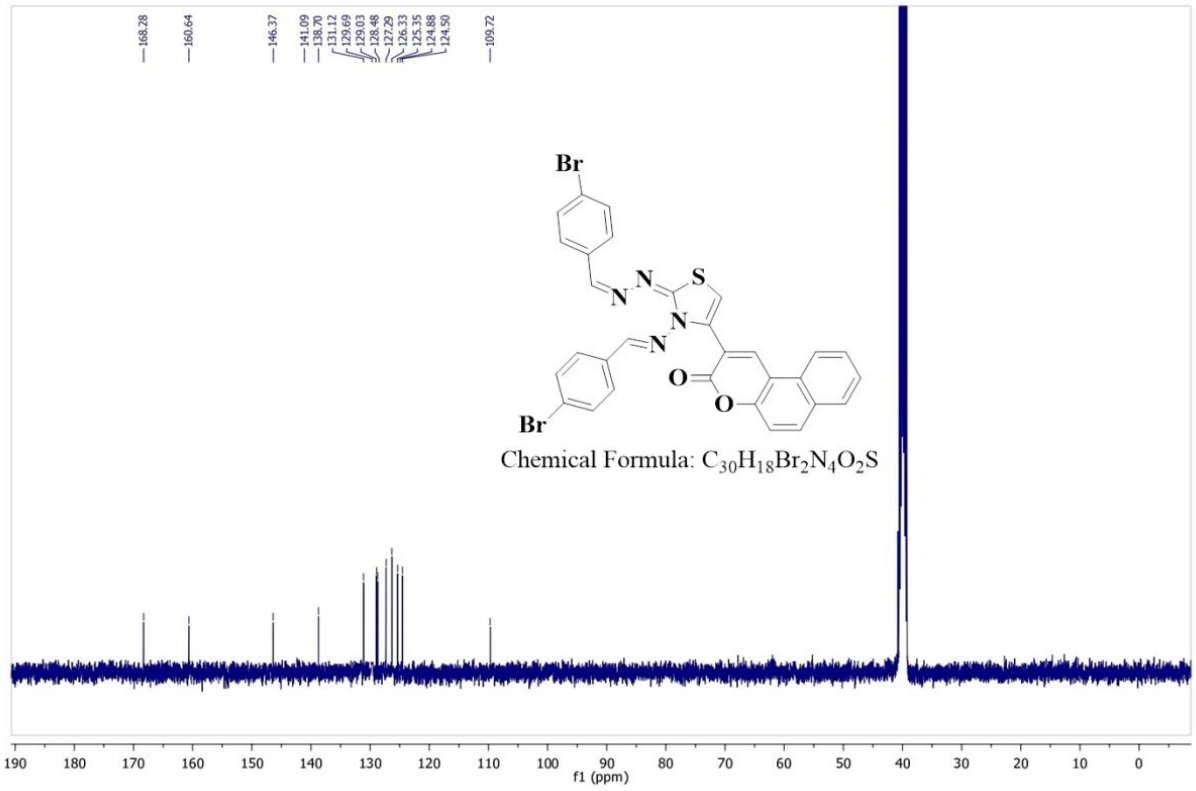
^{13}C NMR spectrum of compound **4m** (100 MHz, DMSO-d_6)



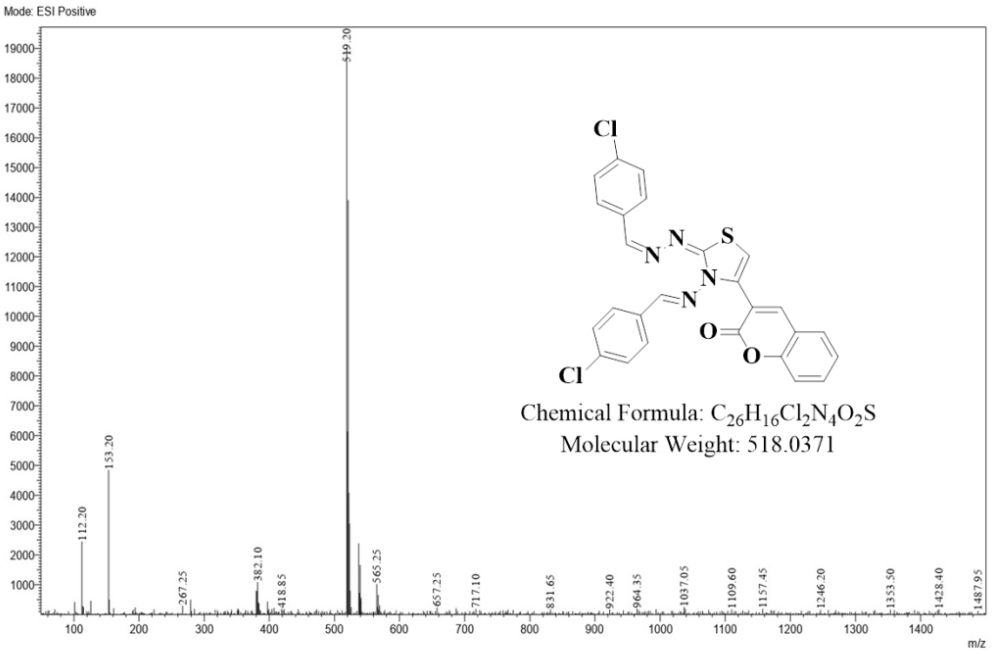
^{13}C NMR spectrum of compound **4n** (100 MHz, DMSO-d_6)



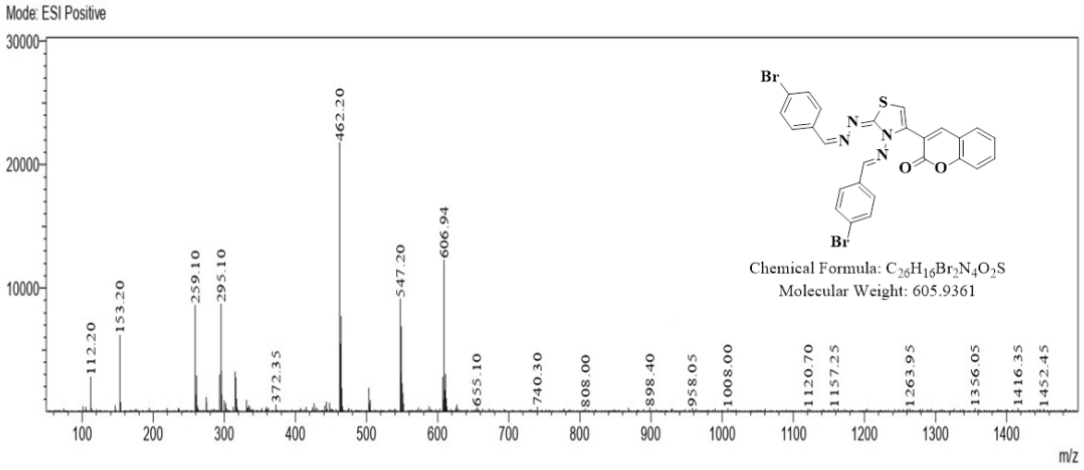
^{13}C NMR spectrum of compound **6a** (100 MHz, $CDCl_3$+DMSO-d_6)



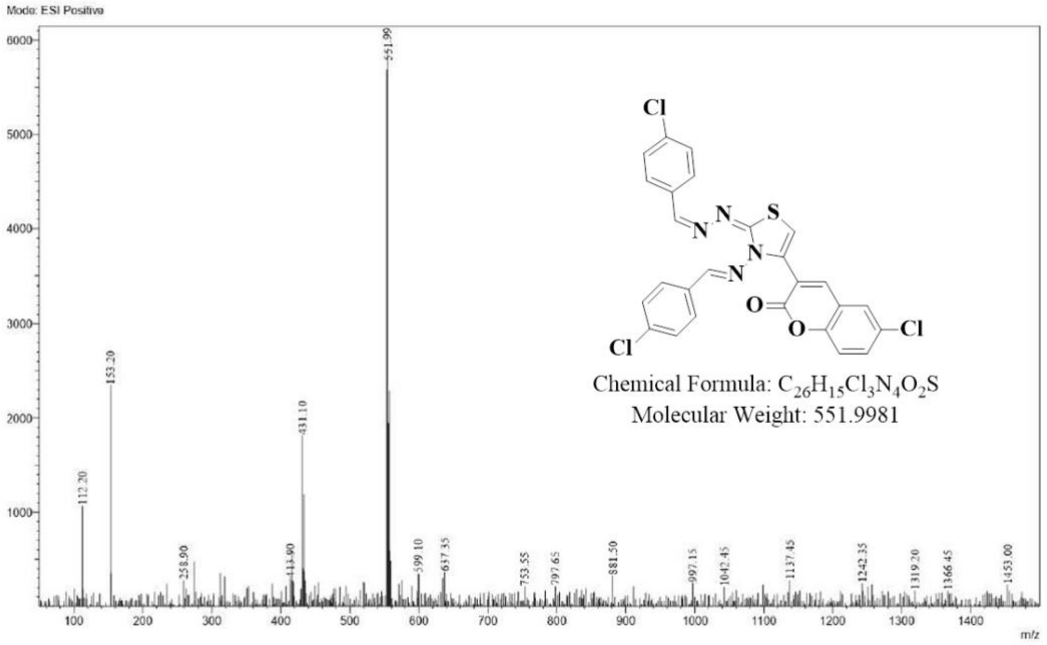
^{13}C NMR spectrum of compound **6b** (100 MHz, DMSO-d_6)



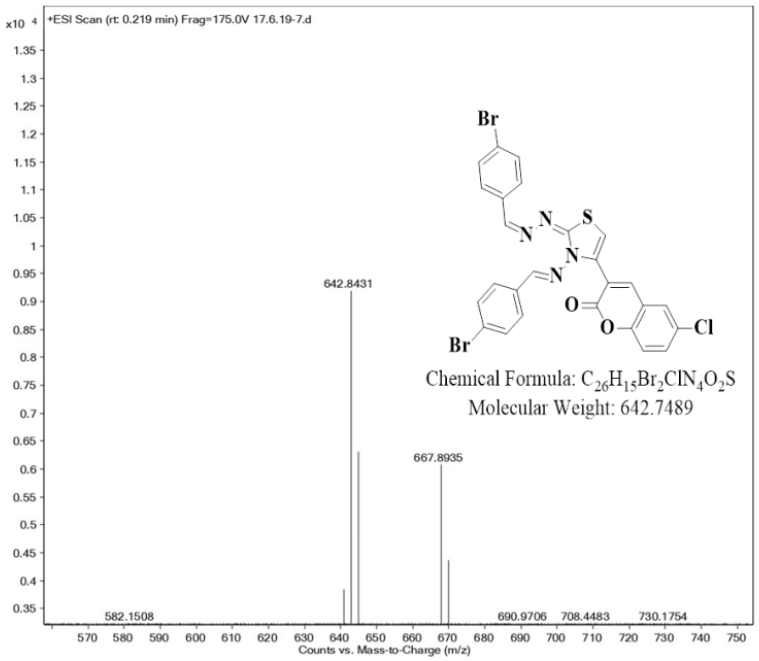
Mass spectrum of compound **4a**



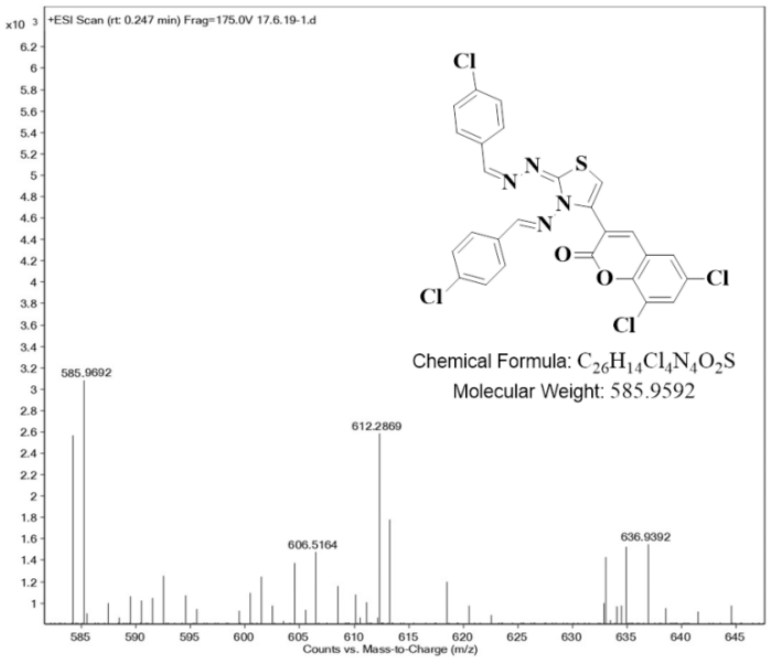
Mass spectrum of compound **4b**



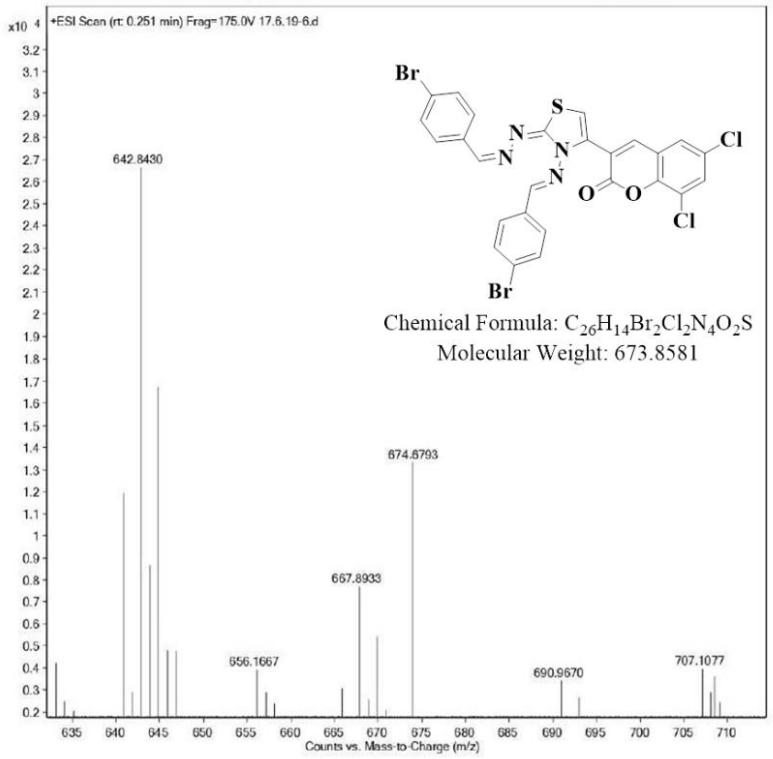
Mass spectrum of compound **4c**



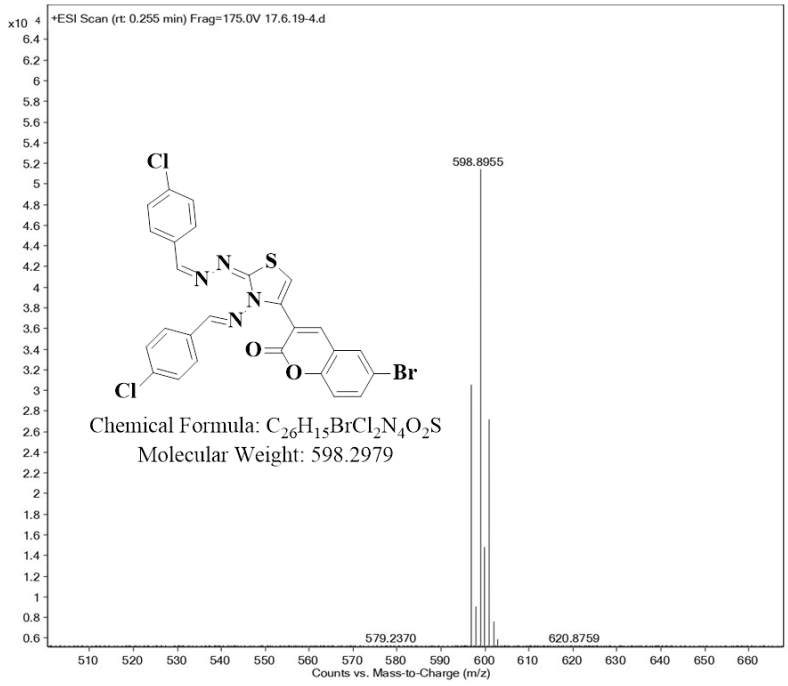
Mass spectrum of compound **4d**



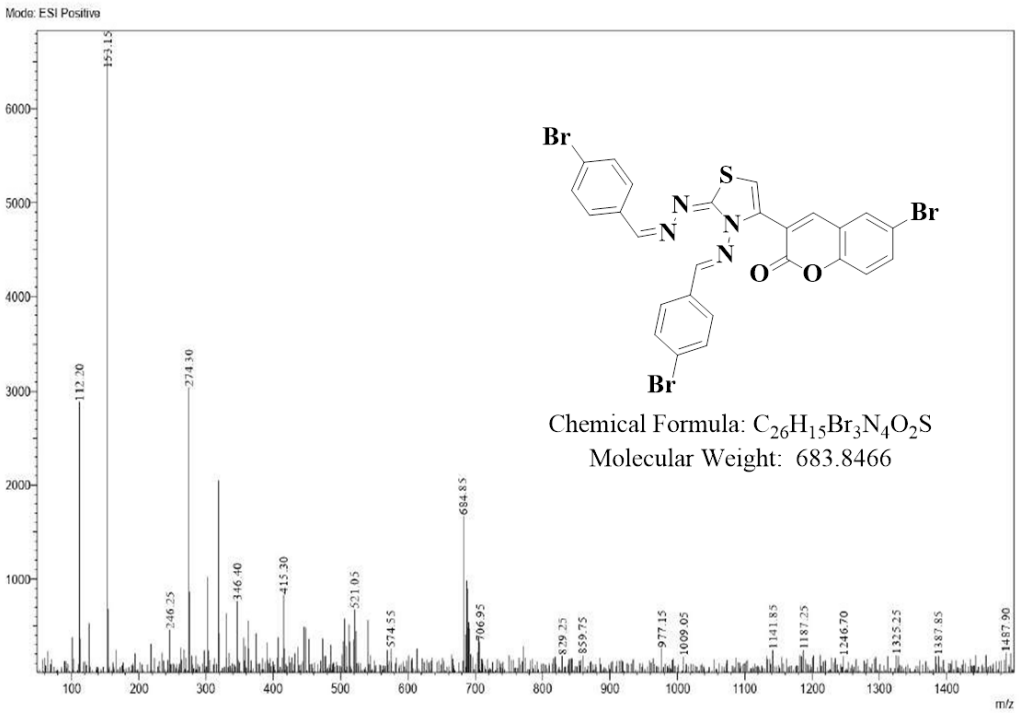
Mass spectrum of compound **4e**



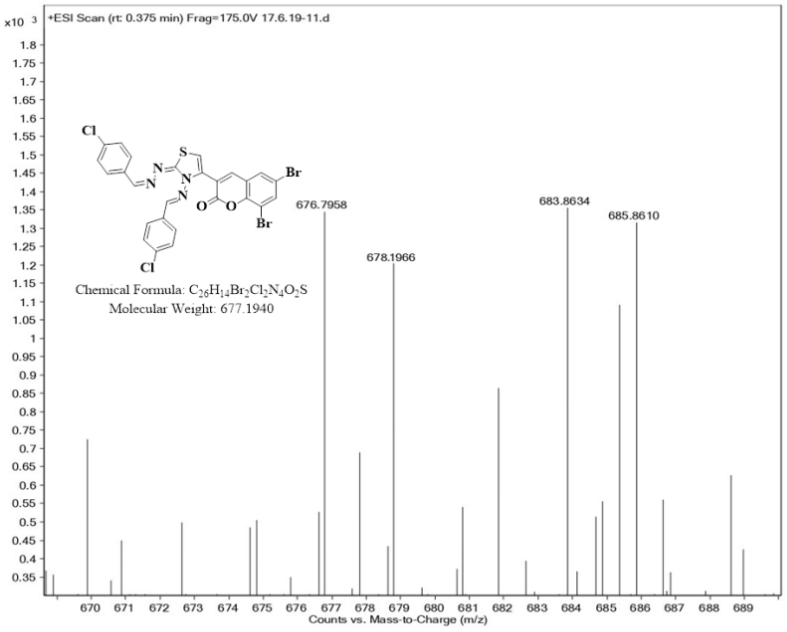
Mass spectrum of compound **4f**



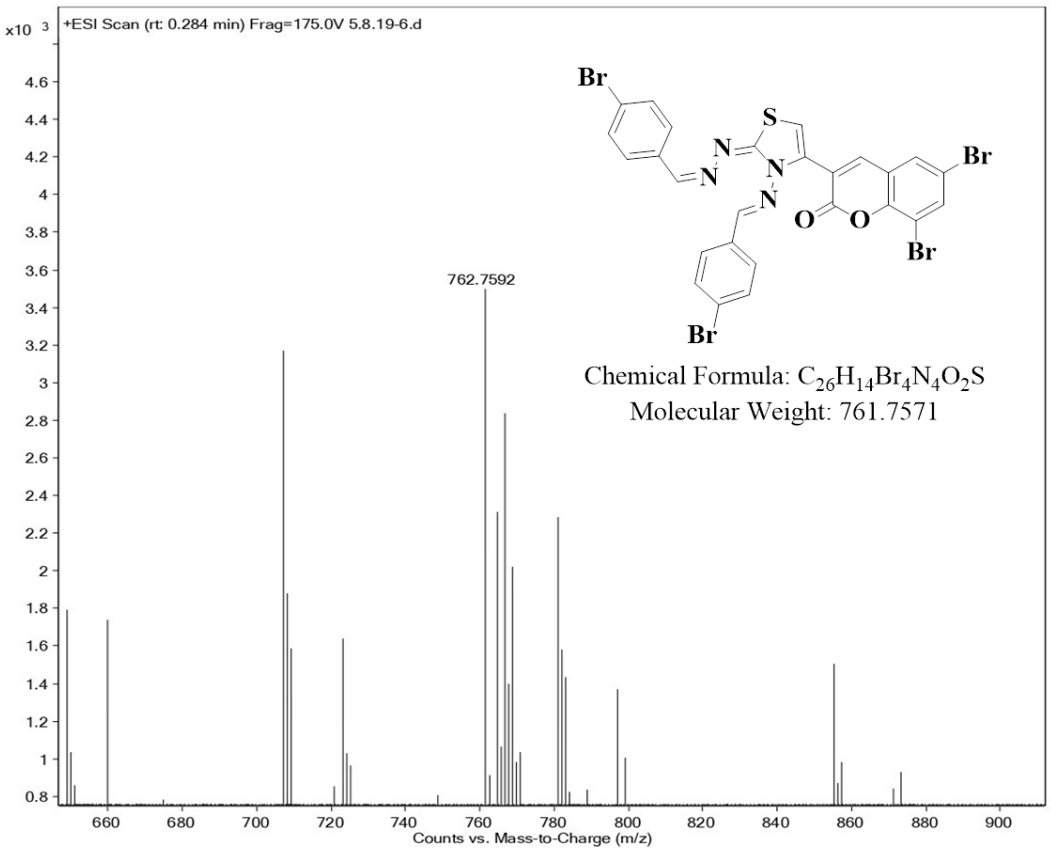
Mass spectrum of compound **4g**



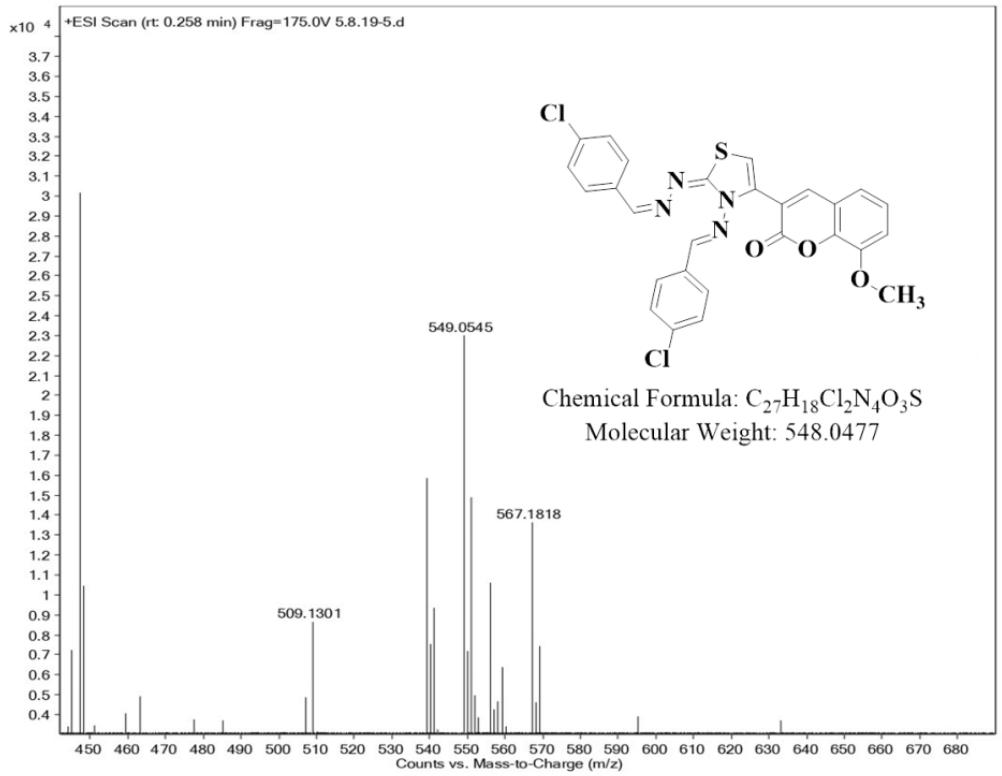
Mass spectrum of compound **4h**



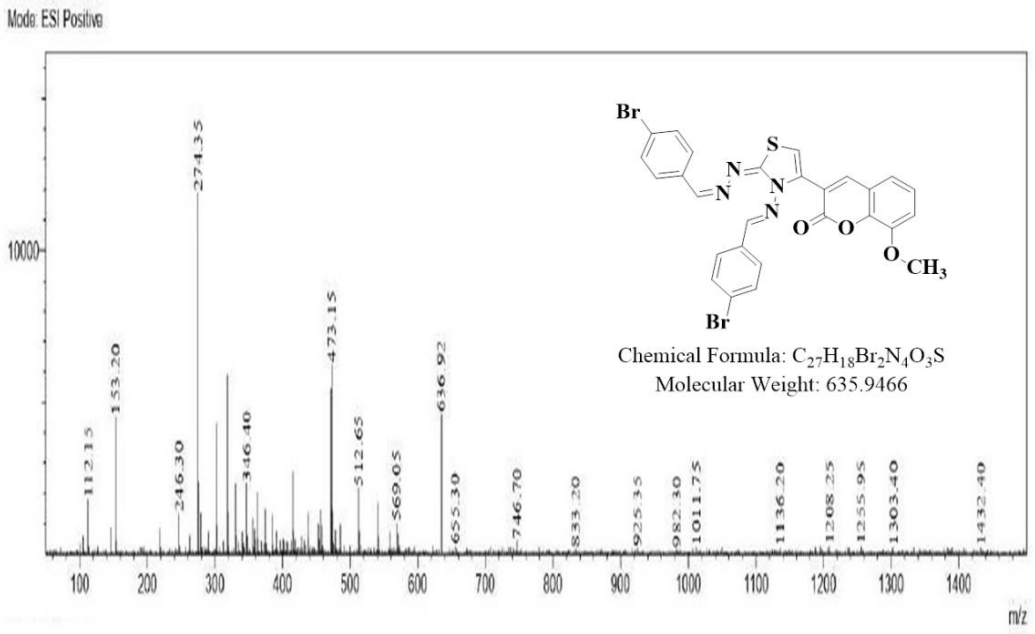
Mass spectrum of compound **4i**



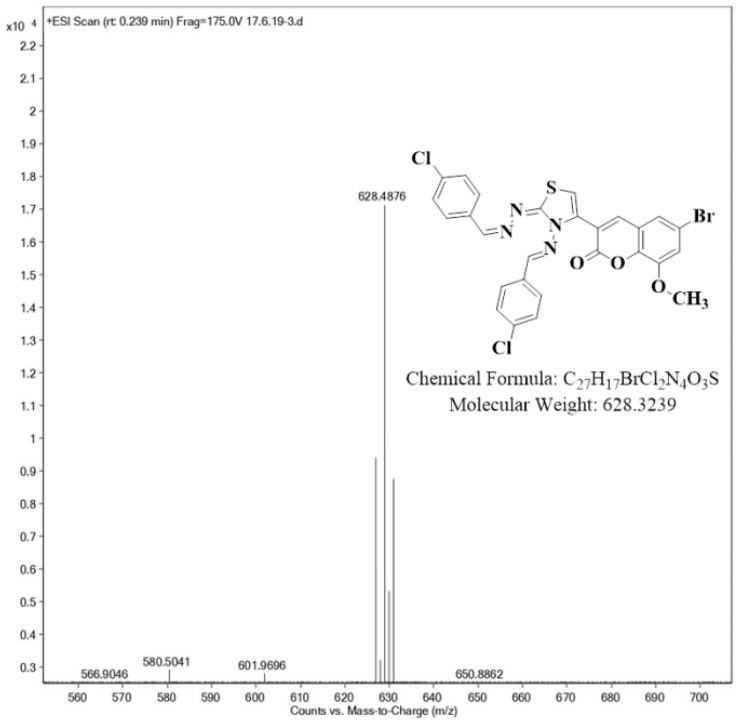
Mass spectrum of compound **4j**



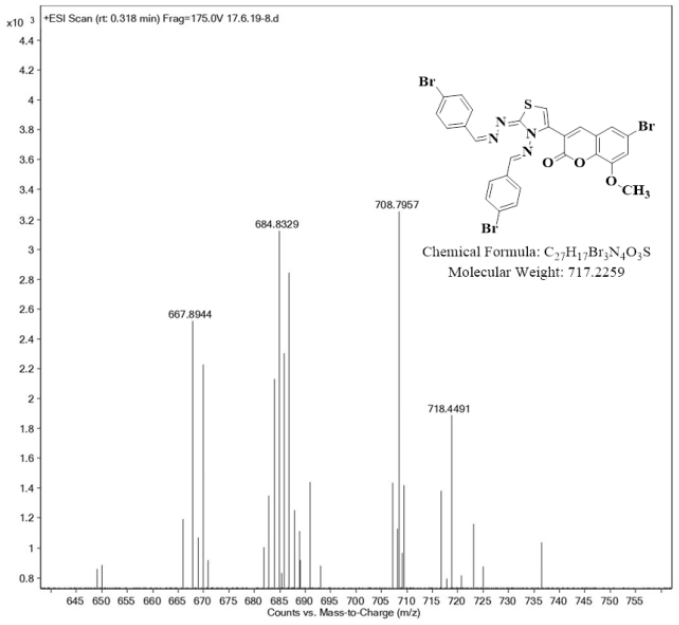
Mass spectrum of compound **4k**



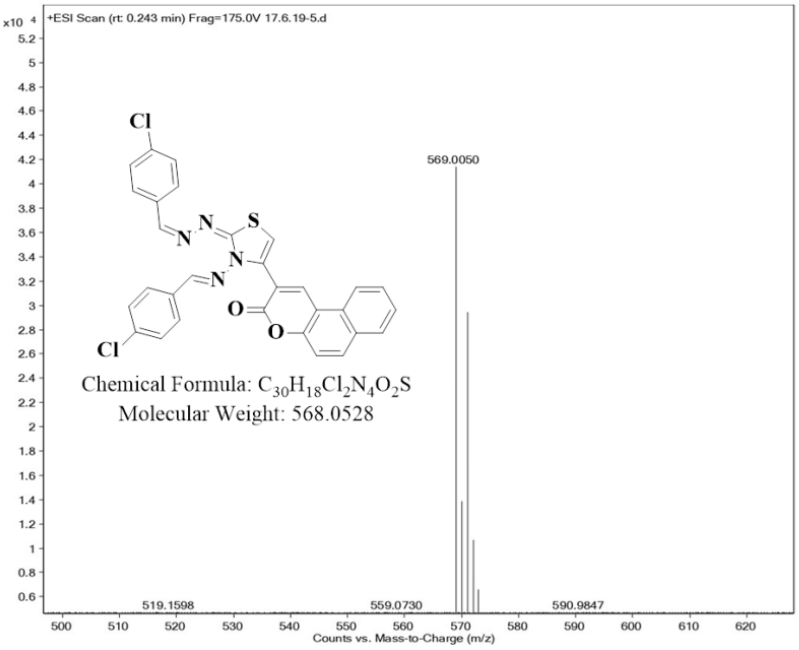
Mass spectrum of compound **4l**



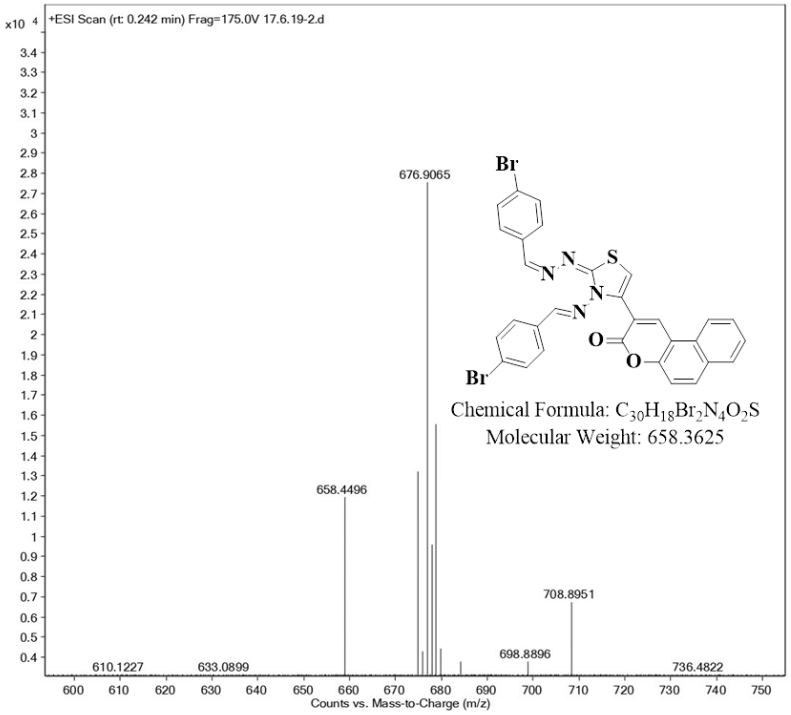
Mass spectrum of compound **4m**



Mass spectrum of compound **4n**



Mass spectrum of compound **6a**



Mass spectrum of compound **6b**

References:

[1] P. A. T. De Moraes Gomes, M. De Oliveira Barbosa, E. Farias Santiago, M. V. De Oliveira Cardoso, N. T. Capistrano Costa, M. Z. Hernandes, D. R. M. Moreira, A. C. Da Silva, T. A. R. Dos Santos, V. R. A. Pereira, et al., *Eur. J. Med. Chem.* **2016**, DOI 10.1016/j.ejmech.2016.05.050.

[2] A. Hantzsch, J. H. Weber, *Berichte der Dtsch. Chem. Gesellschaft* **1887**, *20*, 3118–3132.

[3] J. W. Blunt, A. R. Carroll, B. R. Copp, R. A. Davis, R. A. Keyzers, M. R. Prinsep, *Nat. Prod. Rep.* **2018**, *35*, 8–53.

[4] S.-J. Li, X. Zhang, X.-H. Wang, C.-Q. Zhao, *Eur. J. Med. Chem.* **2018**, *156*, 316–343.

[5] R. T. Williamson, N. Sitachitta, W. H. Gerwick, *Tetrahedron Lett.* **1999**, *40*, 5175–5178.

[6] J. S. Barradas, M. I. Errea, N. B. D'Accorso, C. S. Sepúlveda, E. B. Damonte, *Eur. J. Med. Chem.* **2011**, *46*, 259–264.

[7] Z. Xu, M. Ba, H. Zhou, Y. Cao, C. Tang, Y. Yang, R. He, Y. Liang, X. Zhang, Z. Li, et al., *Eur. J. Med. Chem.* **2014**, *85*, 27–42.

[8] R. Meleddu, S. Distinto, A. Corona, G. Bianco, V. Cannas, F. Esposito, A. Artese, S. Alcaro, P. Matyus, D. Bogdan, et al., *Eur. J. Med. Chem.* **2015**, *93*, 452–460.

[9] S. S. Jadav, S. Kaptein, A. Timiri, T. De Burghgraeve, V. N. Badavath, R. Ganesan, B. N. Sinha, J. Neyts, P. Leyssen, V. Jayaprakash, *Bioorg. Med. Chem. Lett.* **2015**, *25*, 1747–1752.

[10] S. Belveren, H. Ali Dondas, M. Ülger, S. Poyraz, E. García-Mingüens, M. Ferrándiz-Saperas, J. M. Sansano, *Tetrahedron* **2017**, *73*, 6718–6727.

[11] M. Zhou, C. Ning, R. Liu, Y. He, N. Yu, *Bioorg. Med. Chem. Lett.* **2013**, *23*, 3200–3203.

[12] J.-R. Li, D.-D. Li, R.-R. Wang, J. Sun, J.-J. Dong, Q.-R. Du, F. Fang, W.-M. Zhang, H.-L. Zhu, *Eur. J. Med. Chem.* **2014**, *75*, 438–447.

[13] H. Mohammad, P. V. N. Reddy, D. Monteleone, A. S. Mayhoub, M. Cushman, G. K. Hammac, M. N. Seleem, *PLoS One* **2015**, *10*, e0130385.

[14] A. M. Ghatole, K. R. Lanjewar, M. K. Gaidhane, K. M. Hatzade, *Spectrochim. Acta Part A Mol. Biomol. Spectrosc.* **2015**, *151*, 515–524.

[15] M. P. Tantak, J. Wang, R. P. Singh, A. Kumar, K. Shah, D. Kumar, *Bioorg. Med. Chem. Lett.* **2015**, *25*, 4225–4231.

[16] V. Padmavathi, C. Prema kumari, B. C. Venkatesh, A. Padmaja, *Eur. J. Med. Chem.* **2011**, *46*, 5317–5326.

[17] C. D. Pawar, A. P. Sarkate, K. S. Karnik, S. S. Bahekar, D. N. Pansare, R. N. Shelke, C. S. Jawale, D. B. Shinde, *Bioorg. Med. Chem. Lett.* **2016**, *26*, 3525–3528.

[18] G. M. Reddy, J. R. Garcia, V. H. Reddy, A. M. de Andrade, A. Camilo, R. A. Pontes Ribeiro, S. R. de Lazaro, *Eur. J. Med. Chem.* **2016**, *123*, 508–513.

[19] R. M. Mohareb, M. Y. Zaki, N. S. Abbas, *Steroids* **2015**, *98*, 80–91.

[20] G. A. El-Achkar, M. Jouni, M. F. Mrad, T. Hirz, N. El Hachem, A. Khalaf, S. Hammoud, H. Fayyad-Kazan, A. A. Eid, B. Badran, et al., *Eur. J. Pharmacol.* **2015**, *750*, 66–73.

[21] N. Chandak, P. Kumar, P. Kaushik, P. Varshney, C. Sharma, D. Kaushik, S. Jain, K. R. Aneja, P. K. Sharma, *J. Enzyme Inhib. Med. Chem.* **2014**, *29*, 476–484.

[22] V. Gupta, V. Kant, *Sci. Int.* **2013**, *1*, 253–260.

[23] M. Popsavin, S. Spaić, M. Svirčev, V. Kojić, G. Bogdanović, V. Popsavin, *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4123–4127.

[24] Y. K. Abhale, A. V. Sasane, A. P. Chavan, S. H. Shekh, K. K. Deshmukh, S. Bhansali, L. Nawale, D. Sarkar, P. C. Mhaske, *Eur. J. Med. Chem.* **2017**, *132*, 333–340.

[25] Y.-T. Lai, T. Wang, S. O'Dell, M. K. Louder, A. Schön, C. S. F. Cheung, G.-Y. Chuang, A. Druz, B. Lin, K. McKee, et al., *Nat. Commun.* **2019**, *10*, 47.

[26] R. O. Dillman, K. P. Ryan, J. B. Dillman, D. L. Shawler, R. Maguire, *Mol. Biother.* **1992**, *4*, 10–4.

[27] A. Rouf, C. Tanyeli, *Eur. J. Med. Chem.* **2015**, *97*, 911–27.

[28] W. C. Patt, H. W. Hamilton, M. D. Taylor, M. J. Ryan, D. G. Taylor, C. J. C. Connolly, A. M. Doherty, S. R. Klutchko, I. Sircar, *J. Med. Chem.* **1992**, *35*, 2562–2572.

[29] P.-C. Lv, K.-R. Wang, Y. Yang, W.-J. Mao, J. Chen, J. Xiong, H.-L. Zhu, *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6750–6754.

[30] C. B. Mishra, S. Kumari, M. Tiwari, *Eur. J. Med. Chem.* **2015**, *92*, 1–34.

[31] M. Brvar, A. Perdih, M. Oblak, L. P. Mašič, T. Solmajer, *Bioorg. Med. Chem. Lett.* **2010**, *20*, 958–962.

[32] T. Khan, K. Sankhe, V. Suvarna, A. Sherje, K. Patel, B. Dravyakar, *Biomed. Pharmacother.* **2018**, *103*, 923–938.

[33] Y. Wu, Y. Xie, Q. Zhang, H. Tian, W. Zhu, A. D. Q. Li, *Angew. Chemie Int. Ed.* **2014**, *53*, 2090–2094.

[34] A. A. Kiryanov, P. Sampson, A. J. Seed, *J. Org. Chem.* **2001**, *66*, 7925–7929.

[35] A. Mori, A. Sekiguchi, K. Masui, T. Shimada, M. Horie, K. Osakada, M. Kawamoto, T. Ikeda, *J. Am. Chem. Soc.* **2003**, *125*, 1700–1701.

[36] B.-Y. Kim, H.-S. Kim, A. Helal, *Sensors Actuators B Chem.* **2015**, *206*, 430–434.

[37] H. Leopold, A. Tronnier, G. Wagenblast, I. Münster, T. Strassner, *Organometallics* **2016**, *35*, 959–971.

[38] J. Zhu, L. Han, Y. Diao, X. Ren, M. Xu, L. Xu, S. Li, Q. Li, D. Dong, J. Huang, et al., *J. Med. Chem.* **2015**, *58*, 1123–1139.

[39] K. Sujatha, N. B. Ommi, A. Mudiraj, P. P. Babu, R. R. Vedula, *Arch. Pharm. (Weinheim).* **2019**, *352*, 1900079.

[40] X. Peng, F. Qin, M. Xu, S. Zhu, Y. Pan, H. Tang, X. Meng, H. Wang, *Org. Biomol. Chem.* **2019**, *17*, 8403–8407.

[41] G. Wang, D. He, X. Li, J. Li, Z. Peng, *Bioorg. Chem.* **2016**, *65*, 167–174.

[42] R. P. Tayade, N. Sekar, *Dye. Pigment.* **2016**, *128*, 111–123.

[43] R. Kammel, D. Tarabová, O. Machalický, M. Nepraš, B. Frumarová, J. Hanusek, *Dye. Pigment.* **2016**, *128*, 101–110.

[44] K. Sujatha, R. R. Vedula, *Synth. Commun.* **2018**, *48*, 302–308.

[45] P. R. Mali, N. B. Khomane, B. Sridhar, H. M. Meshram, P. R. Likhar, *New J. Chem.* **2018**, *42*, 13819–13827.

[46] S. Karamthulla, S. Pal, M. N. Khan, L. H. Choudhury, *RSC Adv.* **2014**, *4*, 37889–37899.

[47] L. Luo, L. Meng, Q. Sun, Z. Ge, R. Li, *Tetrahedron Lett.* **2014**, *55*, 259–263.

[48] S. Titus, K. G. Sreejalekshmi, *Tetrahedron Lett.* **2014**, *55*, 5465–5467.

[49] N. Iravani, M. Keshavarz, T. Haghnegahdar, M. Salehi, *J. Chinese Chem. Soc.* **2014**, *61*, 1259–1264.

[50] T. Miura, Y. Funakoshi, Y. Fujimoto, J. Nakahashi, M. Murakami, *Org. Lett.* **2015**, *17*, 2454–2457.

[51] V. Facchinetti, M. Avellar, A. Nery, C. Gomes, T. Vasconcelos, M. de Souza, *Synthesis (Stuttg).* **2015**, *48*, 437–440.

[52] A. Ziyaei Halimehjani, L. Hasani, M. Ali Alaei, M. R. Saidi, *Tetrahedron Lett.* **2016**, *57*, 883–886.

[53] K. Cheng, A. McClory, W. Walker, J. Xu, H. Zhang, R. Angelaud, F. Gosselin, *Tetrahedron Lett.* **2016**, *57*, 1736–1738.

[54] M. Xiabing, K. Ablajan, M. Obul, M. Seydimemet, R. Ruzi, L. Wenbo, *Tetrahedron* **2016**, *72*, 2349–2353.

[55] L. F. Audrieth, E. S. Scott, P. S. Kippur, *J. Org. Chem.* **1954**, *19*, 733–741.

[56] J. R. Reid, N. D. Heindel, *J. Heterocycl. Chem.* **1976**, *13*, 925–926.

CHAPTER-III

A facile one-pot three component synthesis of new thiazolyl pyrazole carbaldehydes

and

a facile one-pot three component synthesis of new thiazolyl pyrazoles

and

microwave-assisted synthesis of new pyrazolothiazoles *via* multicomponent approach

CHAPTER-III

3.1. Introduction:

Nowadays medicinal chemists fascinated to develop an efficient therapeutic agents mainly heterocyclic compounds with different biological applications. Heterocyclic compounds having more than one hetero atom with diversified functional units are considering greater utility in pharmaceutical chemistry. Out of the heterocyclic compounds, pyrazoles are considered as an effective therapeutic agent with diversified pharmaceutical, agrochemical, material applications. Pyrazoles are an important aromatic five membered diazole heterocyclic molecule containing two nitrogen atoms adjacent position in a cyclic ring. Consistent focus has been made by the organic chemists to synthesize the pyrazoles or *N*- substituted pyrazoles.

Furthermore, *N*- substituted pyrazoles with aryl or heteryl substituents were found to be the numerous effective medicinal properties. Thiazoles are the most promising compounds having different applications. Combination of thiazoles and pyrazoles would expect to exhibit intensified therapeutic applications. Synthesis of substituted thiazolyl pyrazoles captivated more attention for scientific community because of their combined medicinal applications.

Moreover, Pyrazole and thiazole rings are quotidian motifs personifying an interest in heterocyclic compounds manifesting in a broad range of pharmacological activities such as anticancer[1–4], antimicrobial[5–8], antitubercular[9,10], anti-inflammatory[11–13] etc. Some of the pharmacological compounds containing pyrazole and thiazole scaffolds were shown in Figure 3.1, In view of the aforementioned importance of pyrazole, thiazole rings[14–16], we have made an attempt to synthesize thiazolyl pyrazoles, hoping that the combination of these two rings in one molecule may exhibit and enhance good biological activity.

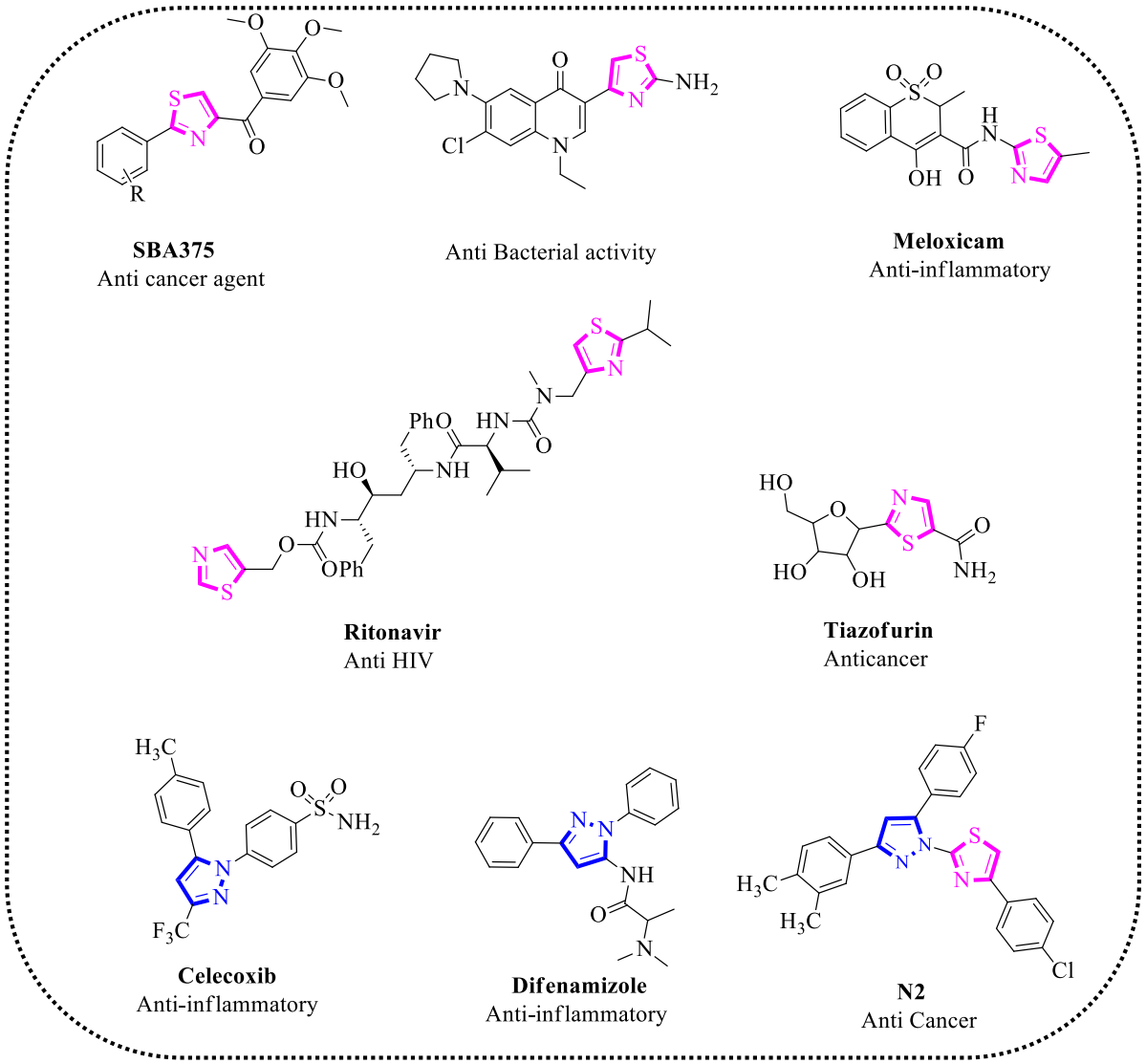
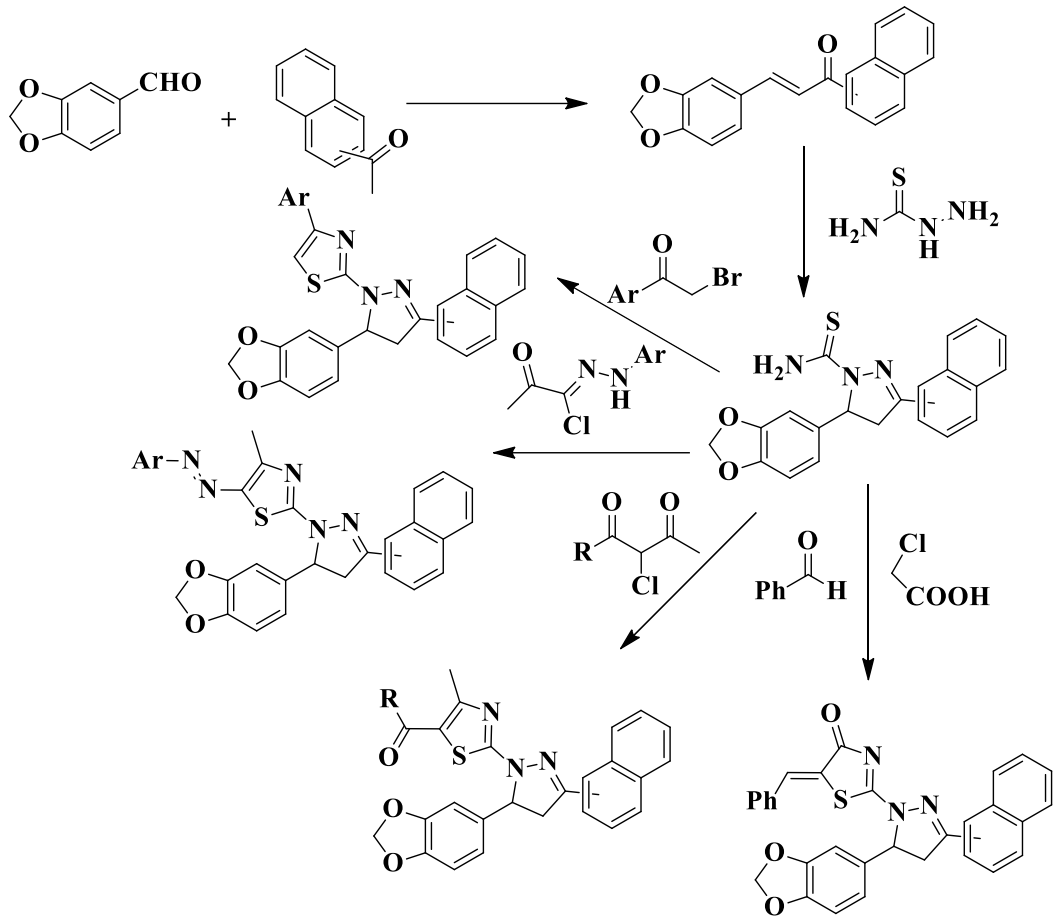


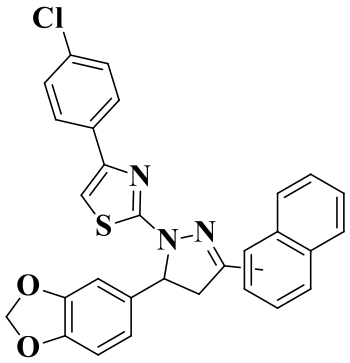
Figure 3.1: Some of the biologically potent synthetic compounds bearing thiazole and pyrazole motifs.

The following is a brief literature review on the synthesis of pyrazolyl thiazoles and thiazolyl pyrazoles.

A. Aboelnaga[17] *et al.* synthesized a new series of thiazolyl pyrazoline linked to benzo[1,3]dioxole analogues. These title compounds were evaluated against antiproliferative, anti fngal and anti-microbial activity. Out of these thiazolyl pyrazoline derivatives compound **1** exhibited antifungal and anti-proliferative activity.



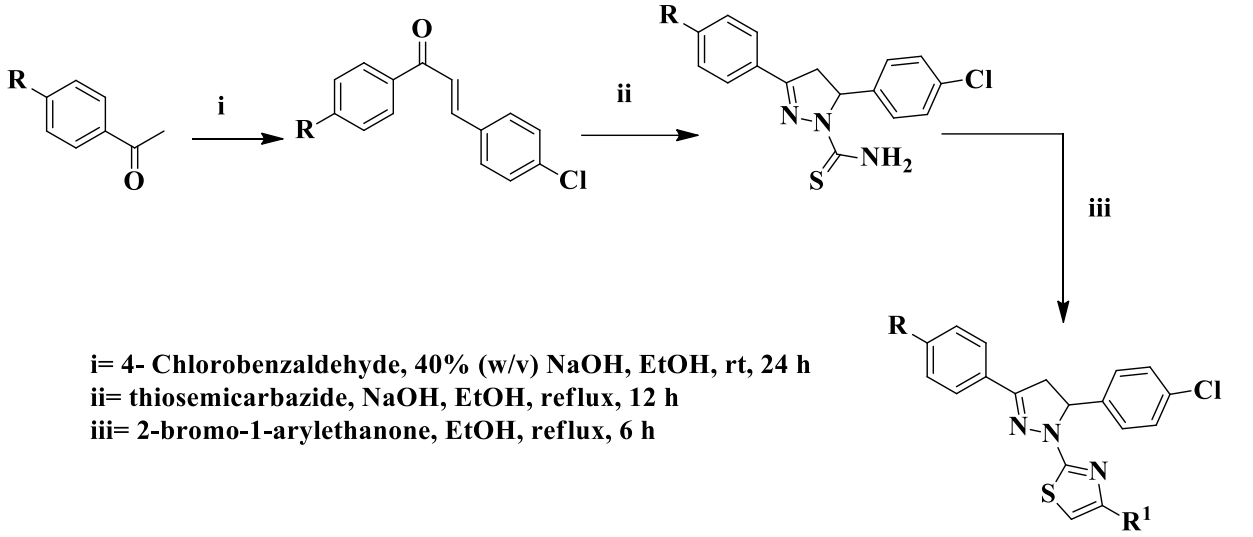
Scheme 3.1



1

Figure 3.2

M. D. Altıntop[18] *et.al* synthesized a new series of thiazolyl-pyrazoline analogues starting from 4[l]-morpholino or piperidinoacetophenone, 4-chlorobenzaldehyde and 2-bromo-1-arylethanone (Scheme 3.2). Moreover, these title compounds were evaluated against anti-cancer activity on A549, MCF-7 and A375 human cancer lines. Furthermore, among these title compounds **2**, **3** and **4** exhibited epidermal growth factor receptor (EGFR) and HER2 inhibitor characteristics.



Scheme 3.2

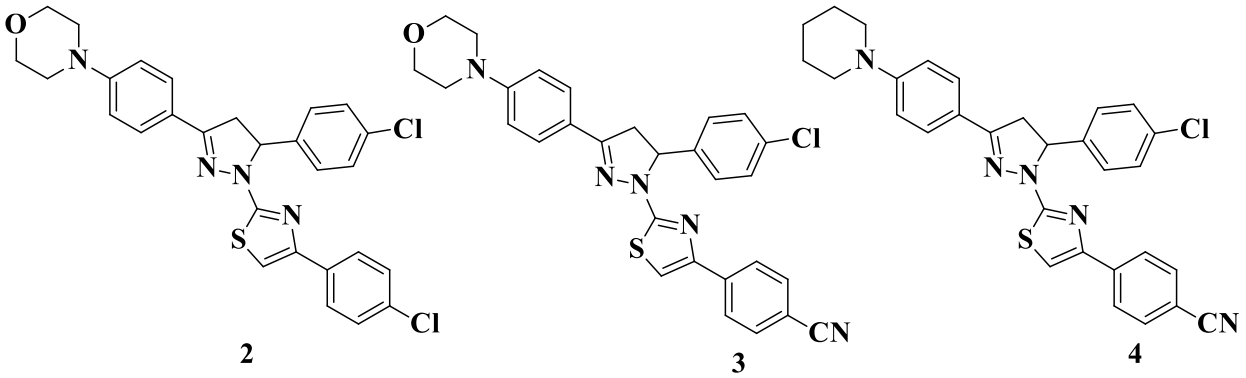
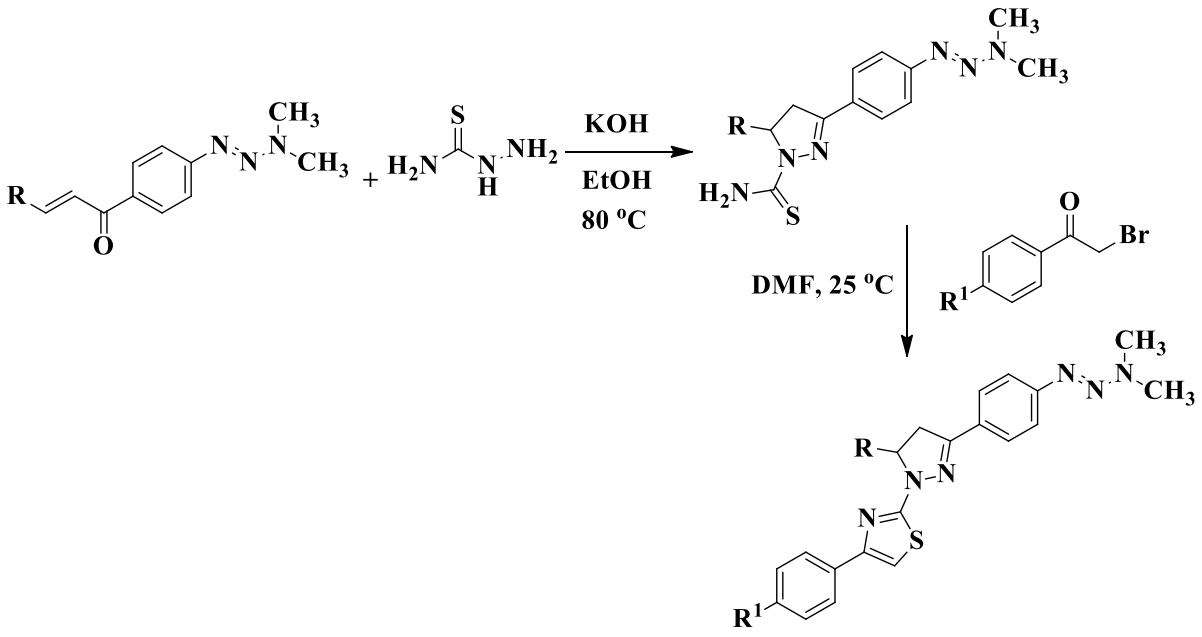
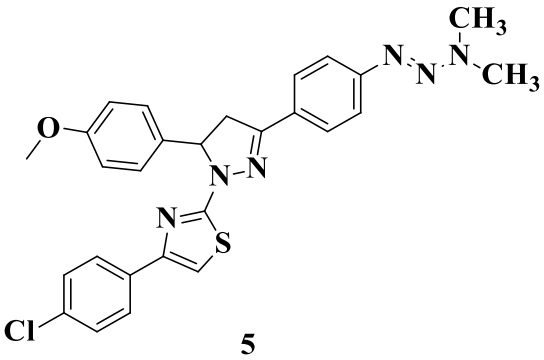


Figure 3.3

P. Ravula[19] and co-workers synthesized a new series of dimethyl triazene incorporated thiazolyl pyrazolines derivatives (Scheme 3.3) and evaluated against anticancer activity on MCF-7 and HT-29 human cancer cell lines. Out of these title compounds **5** exhibited good activity.



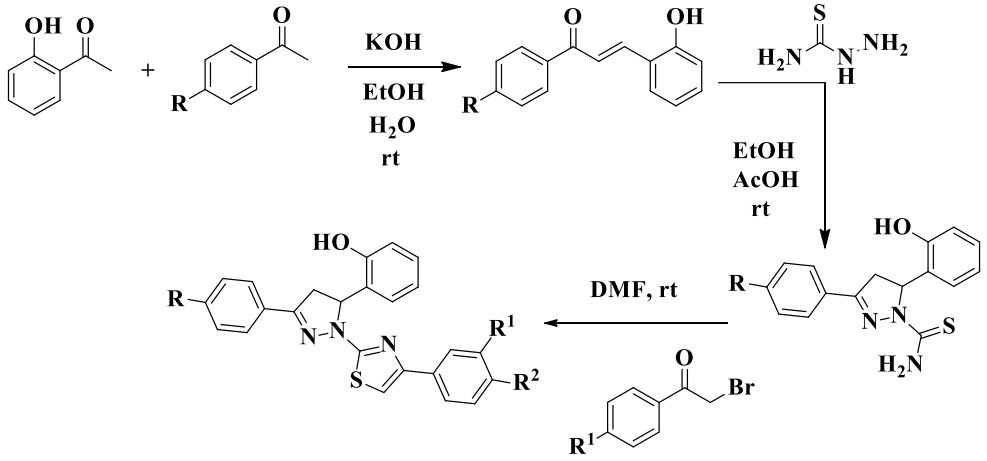
Scheme 3.3



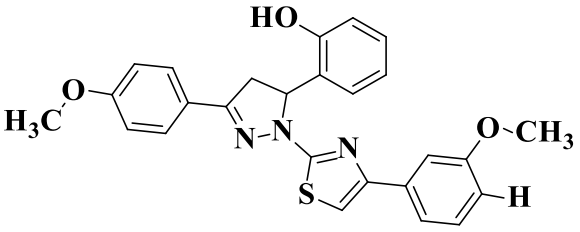
5

Figure 3.4

Y.-D. Lu[20] *et al.* synthesized a new series of dihydro pyrazolothiazole analogues (Scheme 3.4). Moreover, these compounds were tested for their anticancer activity against MCF-7, HeLa and HepG2 cancer cell lines. They were also screened for MMP-2 inhibitory activity also. Out of these title compounds **6** exhibited potent activity.



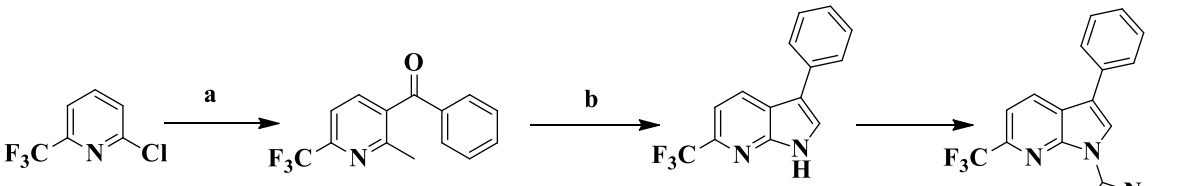
Scheme 3.4



6

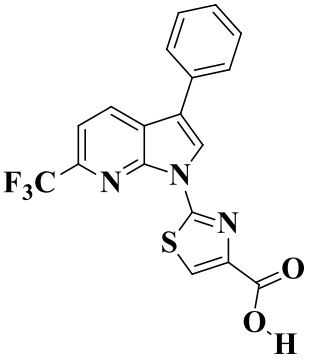
Figure 3.5

M. Atobe[21] *et al.* synthesized a new series pyrazolothiazole analogues (Scheme 3.5). Furthermore, the title compounds were evaluated against EP1 antagonistic activity. Among these title compounds compound **7** exhibited excellent potent activity.



a= LDA,THF, -78 ^{o}C
b= $(NH_2)_2.H_2O$, EtOH, 70 ^{o}C
c=ethyl 2-bromothiazole-4-carboxylate

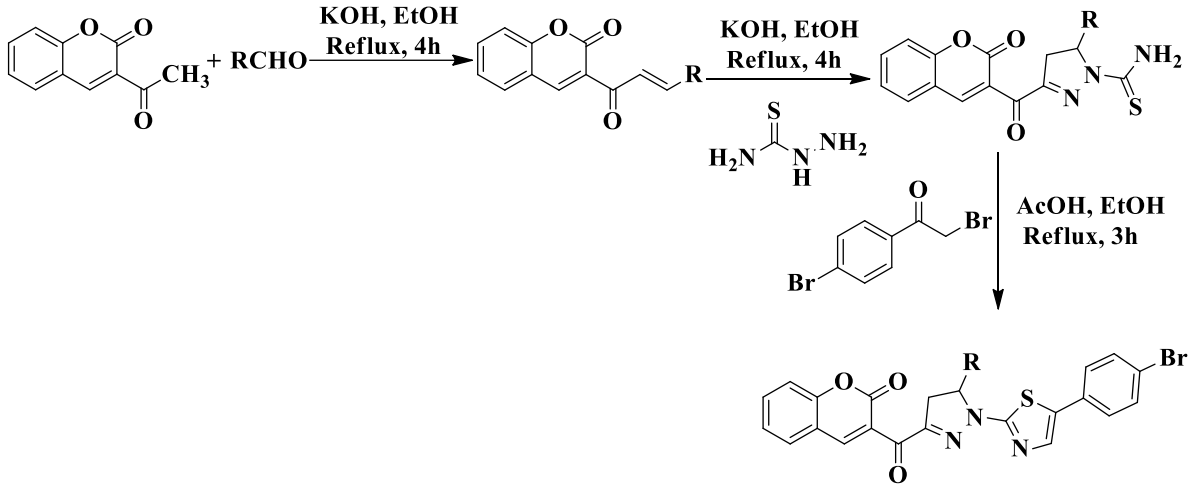
Scheme 3.5



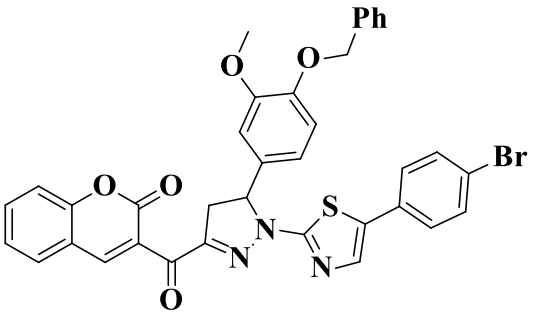
7

Figure 3.6

A. Saeed[22] *et al.* synthesized a new series of coumarinyl-pyrazolinyl substituted thiazole analogues starting from 3-acetyl coumarin, aldehyde and substituted phenacyl bromide (Scheme 3.6). Furthermore, the title compounds were evaluated against mushroom tyrosinase inhibitory activity. Among these title compounds **8** exhibited potent activity.



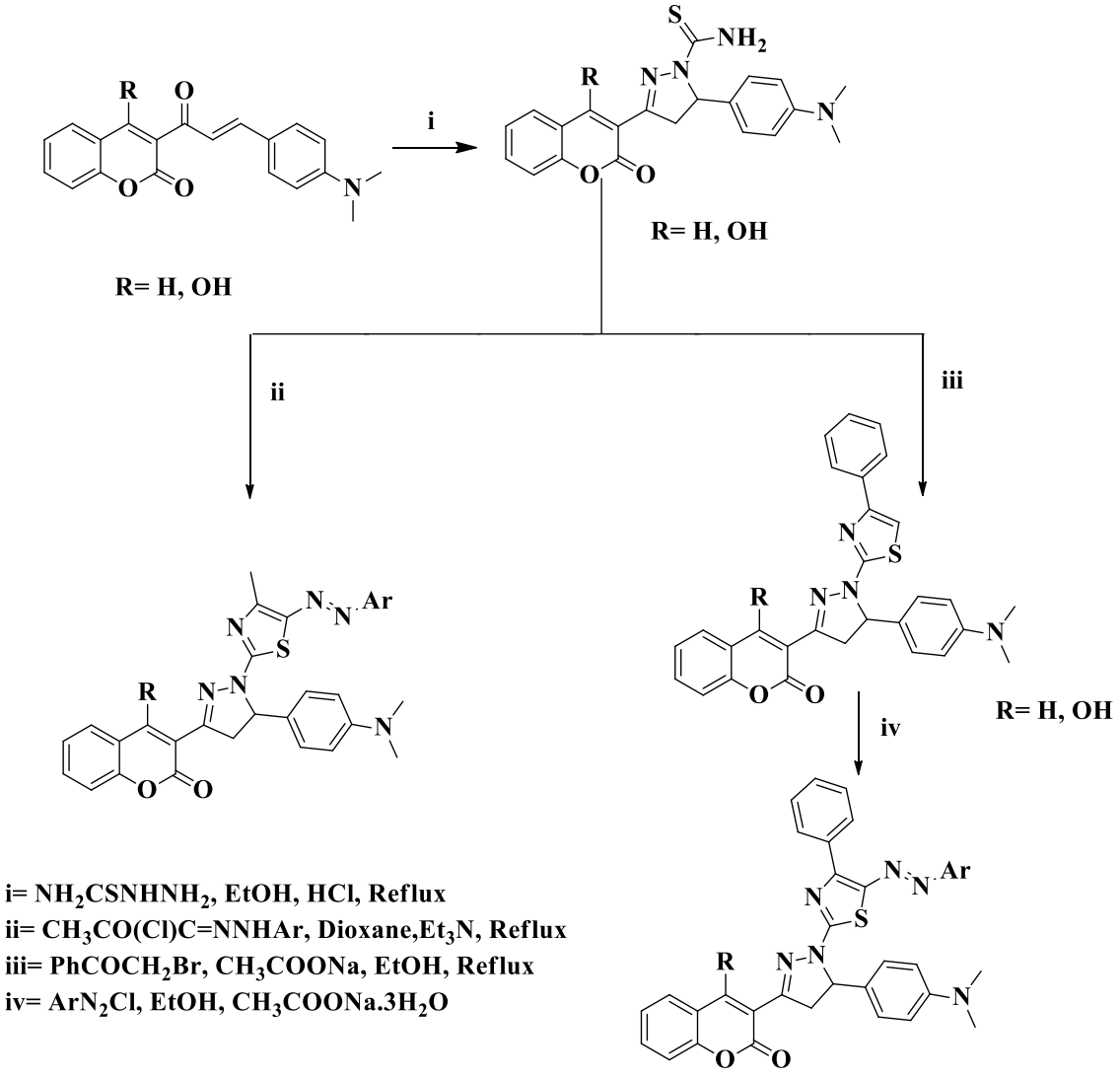
Scheme 3.6



8

Figure 3.7

R. Z. Batran[23] *et al.* synthesized a new series of thiazolylpyrazolyl coumarin analogues (Scheme 3.7). Moreover, these compounds were tested for their anticancer activity against prostate PC3, lung A549, liver HepG2, breast MCF-7 human cancer cell lines. Among these title compounds **9**, **10**, **11** and **12** exhibited excellent activity.



Scheme 3.7

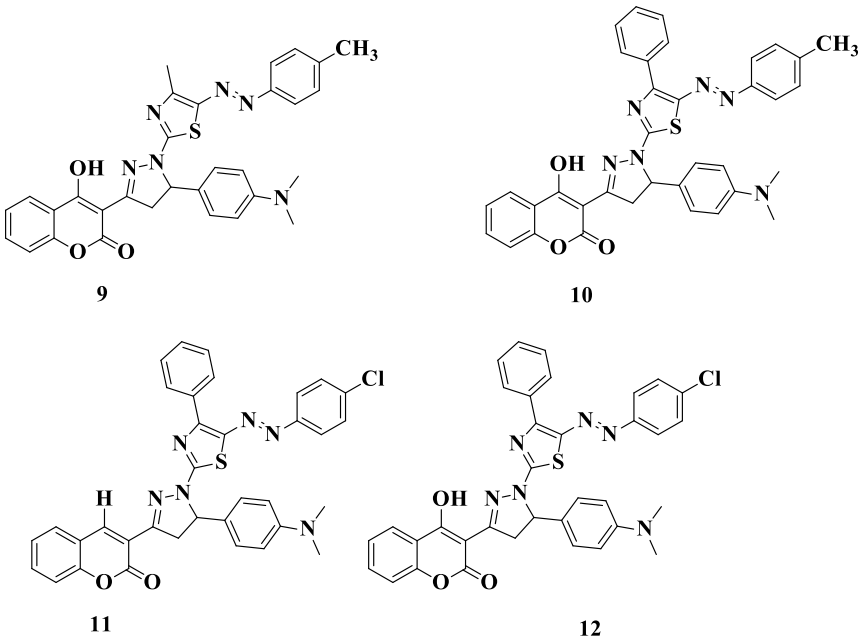
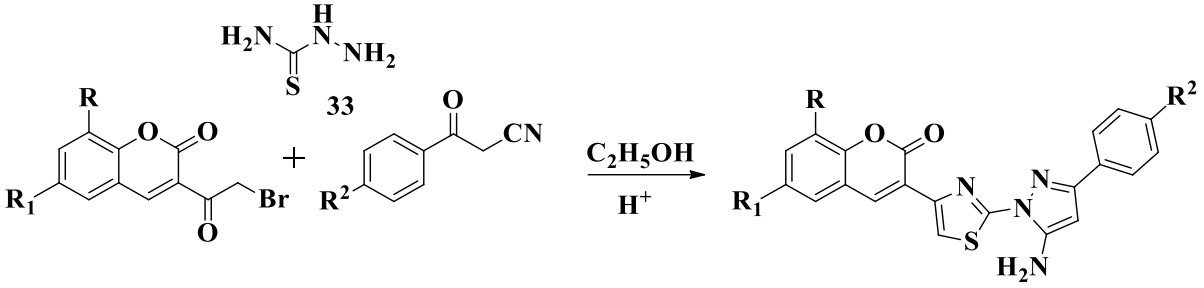
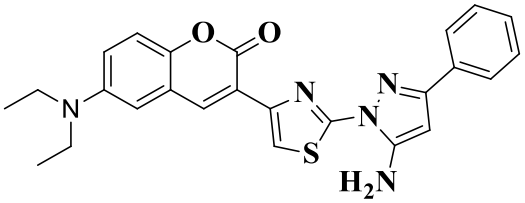


Figure 3.8

R. R. Vedula[24] *et al.* synthesized a new series of 3-(2-(5 amino-3-aryl-1*H*-pyrazol-1-yl)thiazol-4-yl)-2*H*-chromen-2-one derivatives *via* a one pot three component reaction starting from 3-(2-bromoacetyl)coumarins, thiosemicarbazide and substituted benzoylacetonitriles (Scheme 3.8). Furthermore, these compounds were evaluated against anticancer activity on human cancer cell lines HeLa, DU-145, MCF-7, CEM, and L1210. Out of these prazolyl thiazole compounds, 6-diethylamino substituted derivative **13** manifested excellent activity.



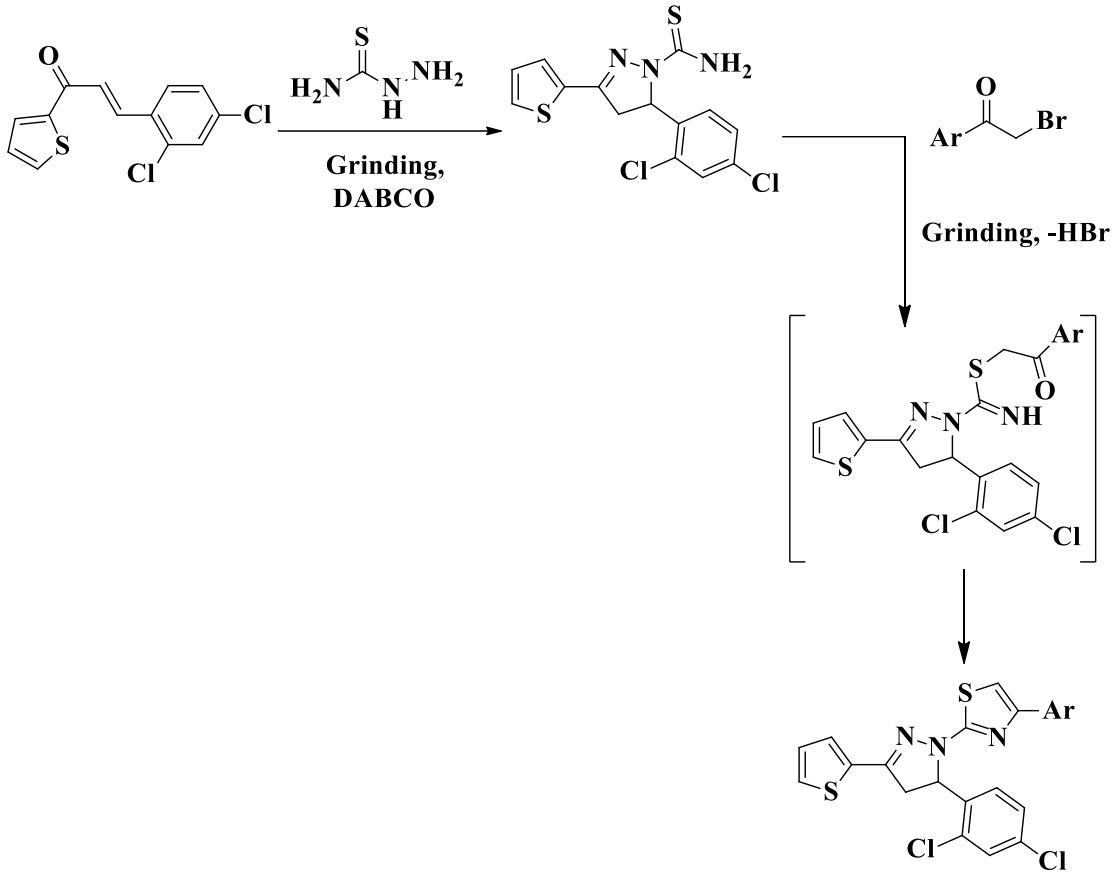
Scheme 3.8



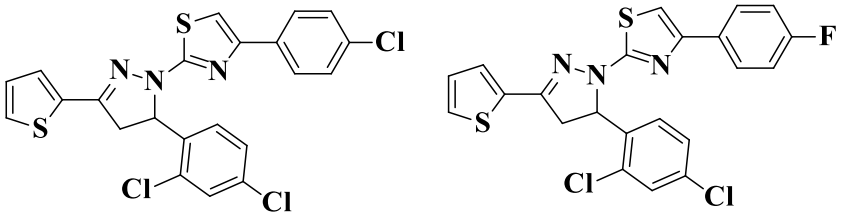
13

Figure 3.9

M. Edrees[25] *et al.* synthesized a new series of pyrazolylthiazole analogues *via* an environmental friendly catalyst by solvent-drop grinding approach starting from alpha-haloketones, pyrazole-1-carbothioamide and DABCO (Scheme 3.9). Furthermore, these compounds were evaluated against anticancer activity on liver carcinoma HepG2 cell line. Among these compounds **14** and **15** exhibited excellent activity.



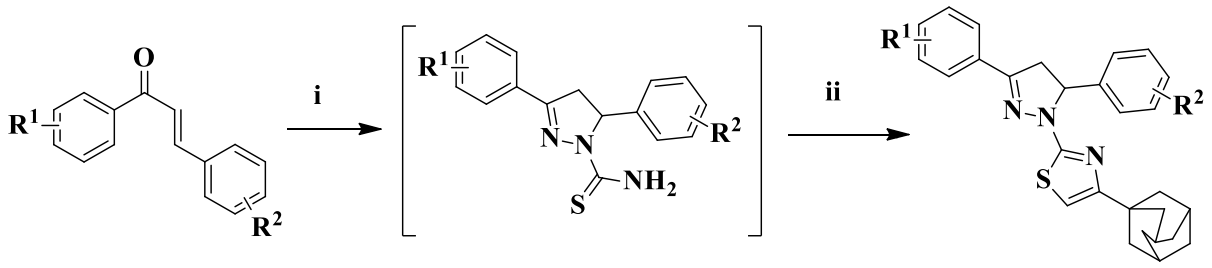
Scheme 3.9



14 **15**

Figure 3.10

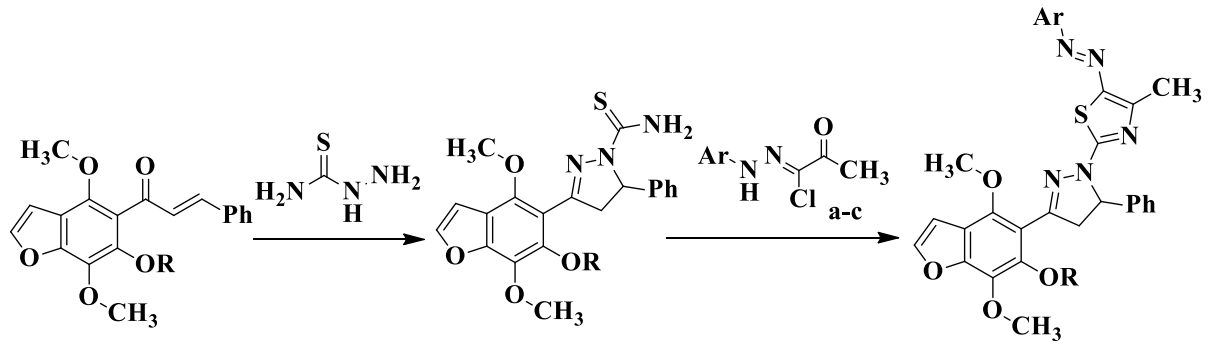
Anusha Sebastian[26] *et al.* synthesized a new series of adamantanyl-based thiadiazolyl pyrazole derivatives starting from chalcones, thiosemicarbazide and 1-butyl-3-methylimidazolium tetrafluoroborate under catalytic amount of piperidine; by this dihydropyrazole intermediate was formed. Thereafter, for this intermediate 2-(adamantan-1-yl)- acetylbromide was added and target compound generated (Scheme 3.10). Moreover, these title compounds were evaluated against epidermal growth factor receptor (EGFR) with excellent activity.



i= [BMIM][BF_4], Thiosemicarbazide,Piperidine, 80 ^oC, 4h
ii= 2-(adamantan-1-yl)acetylbromide, 80 ^oC, 2h

Scheme 3.10

S. M. Gomha[27] *et al.* synthesized a new series of 1,3-thiazolyl pyrazolyl benzofuran analogues starting from chalcones, thiosemicarbazide, 3-(6-hydroxy-4,7-dimethoxybenzofuran-5-yl)-5-phenyl-4, 5-dihydro-1*H*-pyrazole-1-carbothioamide and substituted hydrazonoyl chlorides (Scheme 3.11). Moreover, these title compounds were evaluated for their anti-cancer activity against MCF-7 breast cancer cell line, manifested promising activity.



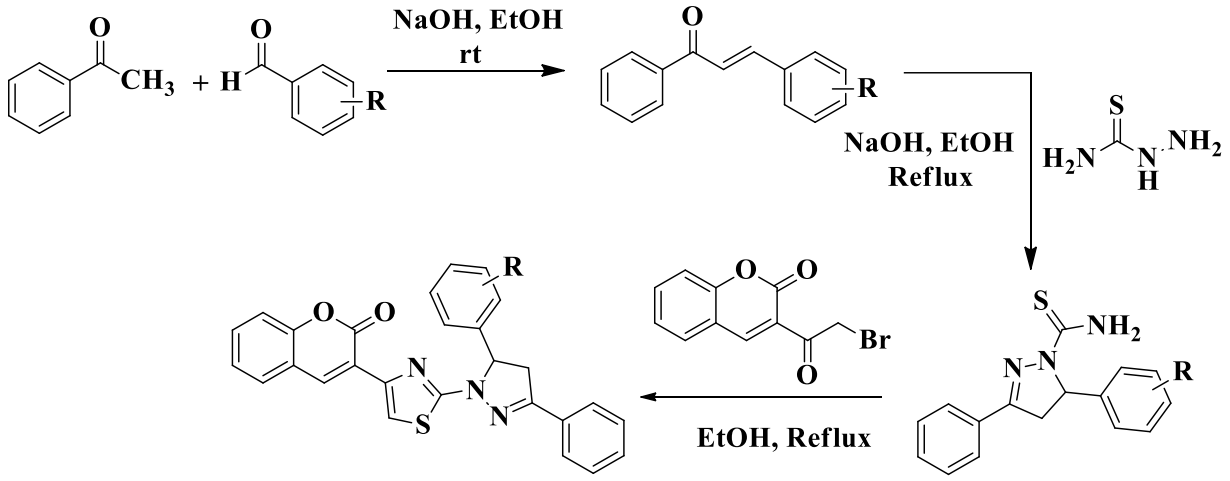
a, R= H; b, R= Me

a, R= H; b, R= Me

a, Ar= C_6H_5
b, Ar= 4-$CH_3C_6H_4$
c, Ar= 4-ClC_6H_4

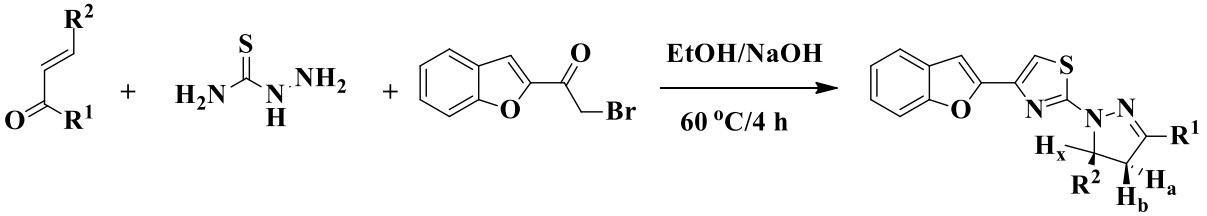
Scheme 3.11

S. Hameed[28] *et al.* synthesized a new series of coumarin based pyrazolylthiazole analogues (Scheme 3.12). Moreover, these title compounds were evaluated against acetyl cholinesterase activity and exhibited promising activity.



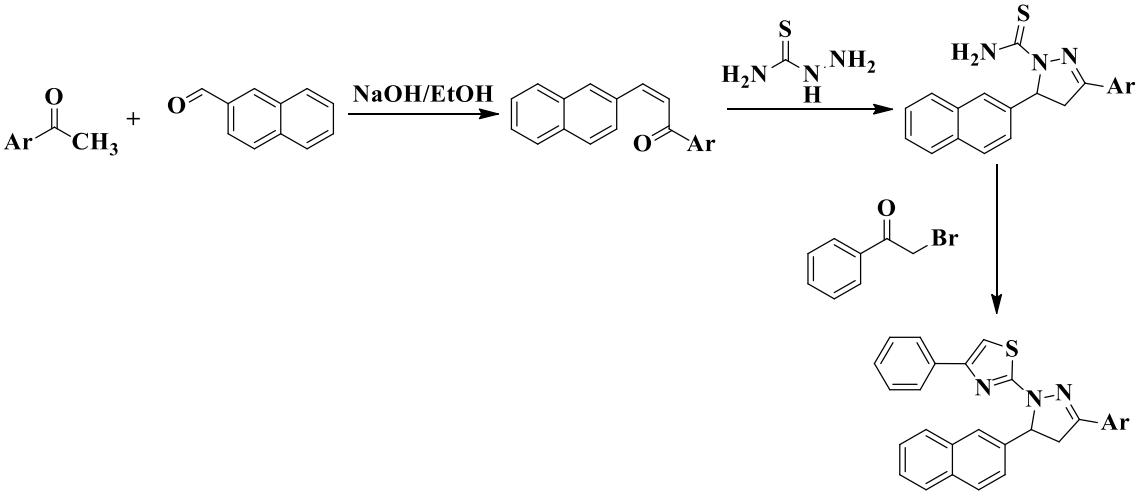
Scheme 3.12

R. R. Vedula[29] *et al.* synthesized a new series of substituted 4,5-dihydro-1*H*-pyrazol-1yl)thiazole analogues *via* a one pot three component reaction. These title compounds were synthesized by using a readily starting available materials thiosemicarbazide, aryl/hetryl chalcones and 1-(benzofuran-2-yl)-2-bromoethan-1-one with good yields (Scheme 3.13).



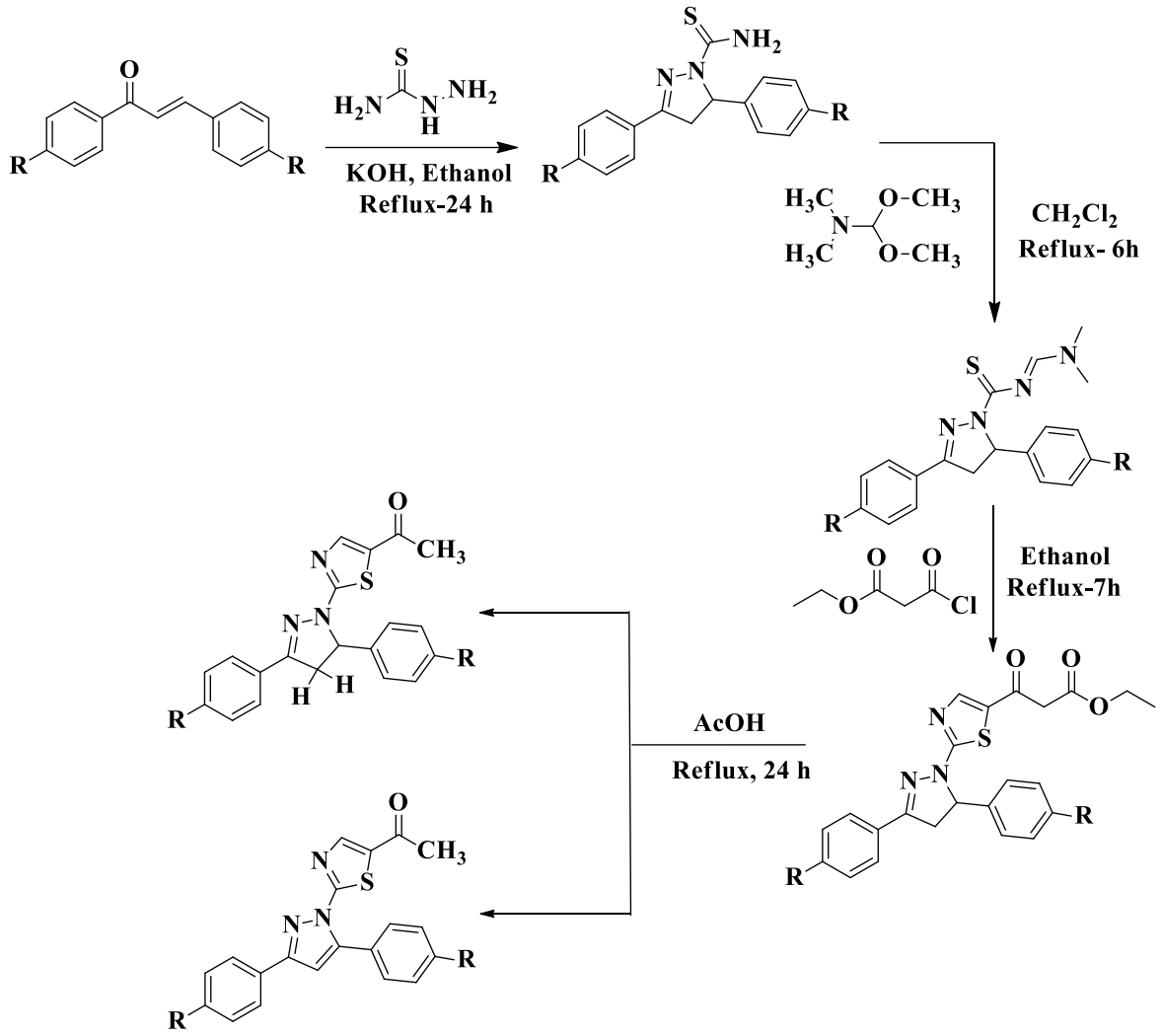
Scheme 3.13

R. S. Gouhar[30] *et al.* synthesized 2-(5-(naphthalen-2-yl)-3-(5-((3(trifluoromethyl)phenyl)diazenyl)benzofuran-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)-4-phenylthiazole derivative starting from naphthalene chalcone derivative, thiosemicarbazide and phenacyl bromides with good yields (Scheme 3.14).



Scheme 3.14

M. Gümüş[31] *et al*. synthesized a new series of β-Ketoester and ethanone analogues *via* a one pot synthesis. These title compounds were synthesized using readily available compounds such as chalcones, thiosemicarbazide, dimethyl formamide dimethyl acetal and ethyl 4-chloro-3-oxo butanoate with good yields (Scheme 3.15).



Scheme 3.15

The present chapter deals with facile, one-pot three component synthesis of a new thiazolyl pyrazole carbaldehydes (**Part-A**), a new thiazolyl pyrazoles (**Part-B**) and microwave-assisted synthesis of new pyrazolothiazoles *via* multi component approach (**Part-C**).

CHAPTER-III
SECTION-A

A facile one-pot three component synthesis of new thiazolyl pyrazole carbaldehydes

CHAPTER-III
SECTION-A

3A. Present work:

3A.1. Starting materials:

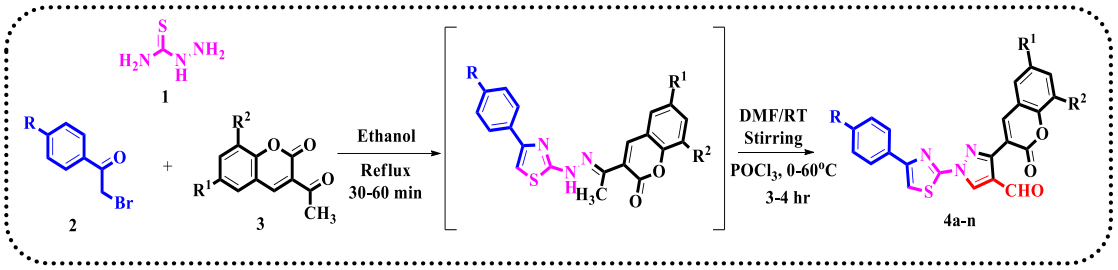
A facile and efficient one pot multi component approach for the synthesis of coumarin substituted thiazolyl pyrazole carbaldehyde analogues was described in the present chapter.

The substituted 3-(2-bromoacetyl)coumarins were prepared *via* bromination of substituted 3-acetyl coumarins. According to the literature procedure, which was mentioned in the previous chapter. Thiosemicarbazide, various acetophenones, DMF and $POCl_3$ (Phosphorous oxychloride) were purchased commercially.

Due to the vital role of coumarin, pyrazole and thiazole rings in the field of medicinal chemistry, we become interested to synthesize a new structural unit which consists of all three moieties.

3A.2. Synthesis of thiazolyl pyrazole carbaldehydes:

Titled compounds(**4a-n**) (Figure 3A.1) were achieved by performing sequential Hantzch thiazole synthesis and Vilsmeier–Haack formylation in one-pot by without isolating the intermediate. For the formation of titled compounds is illustrated in Scheme 3A.1



Scheme 3A.1: Synthesis of thiazolyl pyrazole carbaldehydes

3A.3. Results and discussion:

To perceive the viability of the reaction was performed in one-pot MCR method by using thiosemicarbazide (**1**), simple phenacyl bromide (**2a**) and 3-acetylcoumarin (**3a**) in presence of EtOH. In this process thiosemicarbazide (**1**) reacts with phenacyl bromide (**2a**) and form the Hanztsch thiazole product. Additionally reaction with 3-acetylcoumarin (**3a**) leads to the formation of an intermediate 3-(1-(2-(4-phenylthiazol-2-yl)hydrazono)ethyl)-2H-chromen-2-one. To this intermediate (without isolation), Vilsmeyer-Hack reagent is added to get the title thiazolyl pyrazole carbaldehyde compound (Scheme 3A.2).

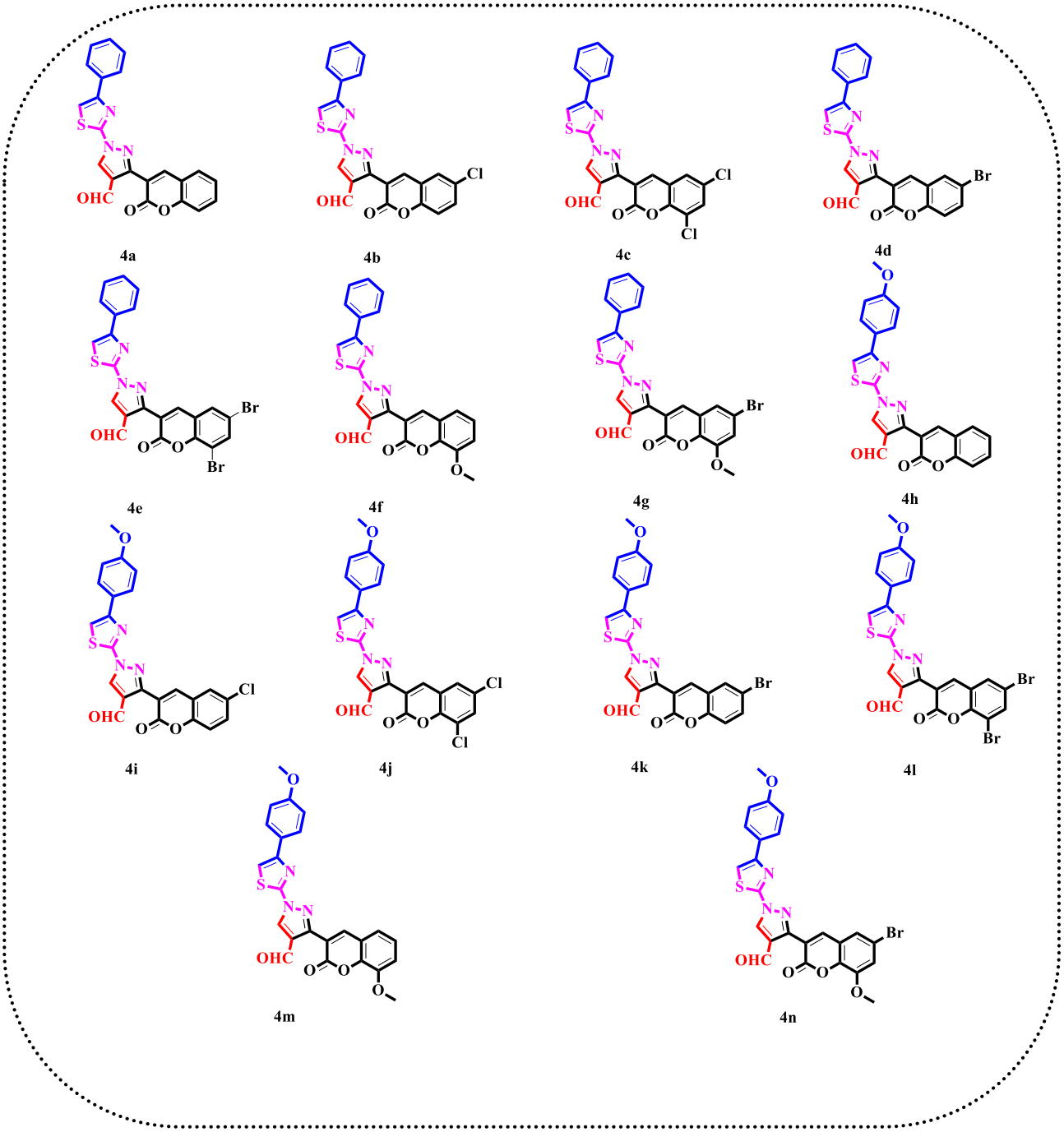
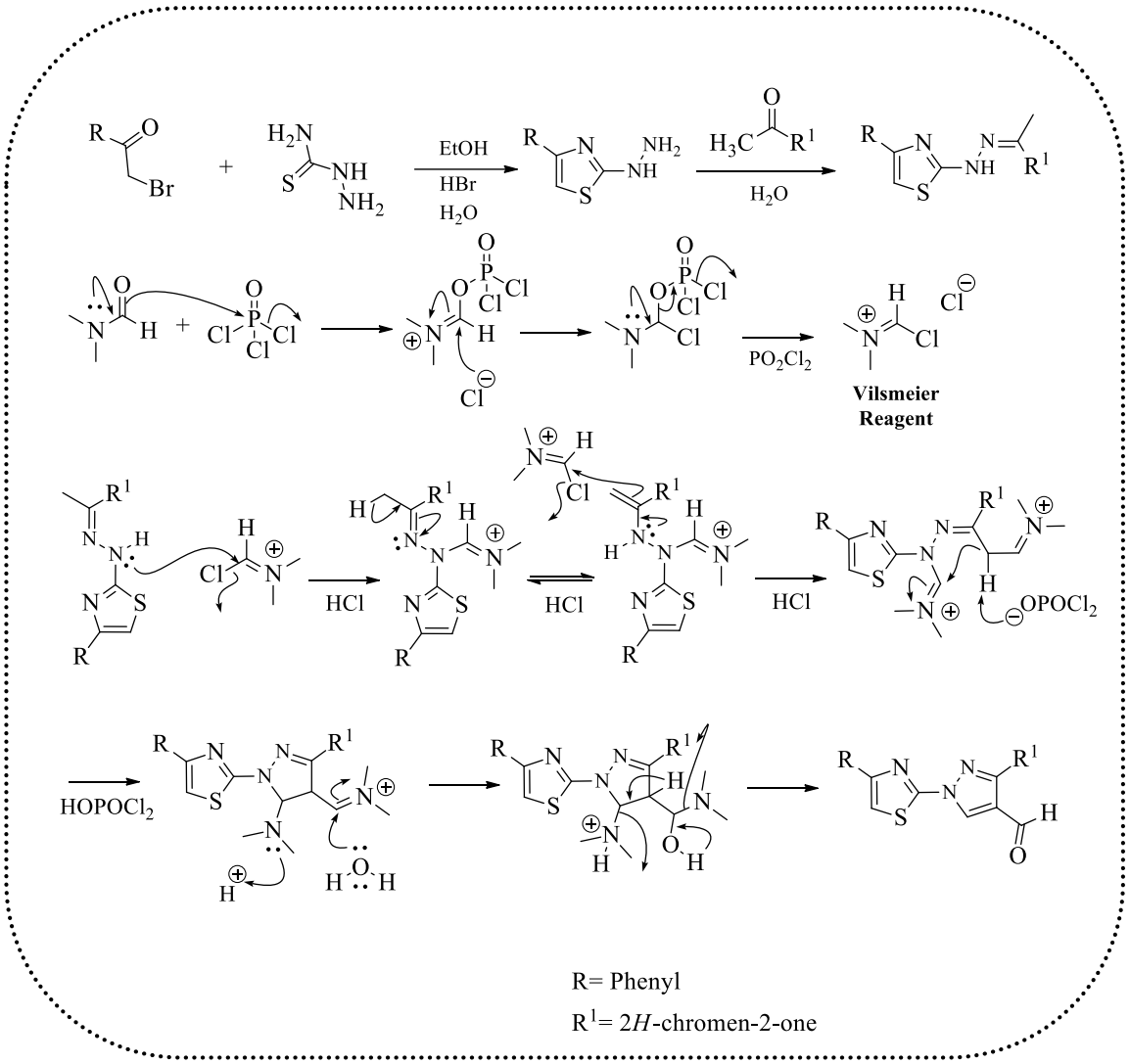


Figure 3A.1: Synthesis of thiazolyl pyrazole carbaldehyde hybrids (**4a-n**).



Scheme 3A.2. Proposed mechanism for the synthesis of titled compound (**4a**)

Table 3A.1: Different substituted thiazolyl pyrazole carbaldehyde hybrids (**4a-n**), time, [a]isolated yield.

Entry	Product	R	R^1	R^2	Time(h)	Yield (%)[a]
1	**4a**	H	H	H	4	88
2	**4b**	H	Cl	H	4.5	85
3	**4c**	H	Cl	Cl	4.5	90
4	**4d**	H	Br	H	5	88
5	**4e**	H	Br	Br	5	92
6	**4f**	H	H	OCH_3	4	89
7	**4g**	H	Br	OCH_3	4.5	87
8	**4h**	OCH_3	H	H	4	91
9	**4i**	OCH_3	Cl	H	4.5	86
10	**4j**	OCH_3	Cl	Cl	5	90
11	**4k**	OCH_3	Br	H	5	92
12	**4l**	OCH_3	Br	Br	5	91
13	**4m**	OCH_3	H	OCH_3	4.5	89
14	**4n**	OCH_3	Br	OCH_3	4.5	90

The newly synthesized compound structures (**4a-n)** were confirmed on the basis of their analytical data. The IR spectra of the compounds have shown characteristic bands for C=N in the range of 1599- 1607 cm^{-1}, CHO in the range of 1676- 1688 cm^{-1} and C=O of lactone in the range of 1711-1738 cm^{-1}. The ^{1}H-NMR spectra of the compounds have shown the characteristic pyrazole ring proton in the range of δ 8.21- 8.73 ppm, thiazole ring proton in the range of δ 9.07- 9.42 ppm and aldehyde proton in the range of 9.98- 10.05 ppm. The ^{13}C-NMR spectra have shown for the aldehyde carbon from the range of 185.20- 185.89 ppm. The mass spectra of all the synthesized compounds have shown the molecular peaks confirming their molecular formulae and structure.

3A.4. Conclusion:

In summary, we have developed a potential protocol for the synthesis of new thiazolyl pyrazole carbaldehyde derivatives by the VMH multi component reaction approach.

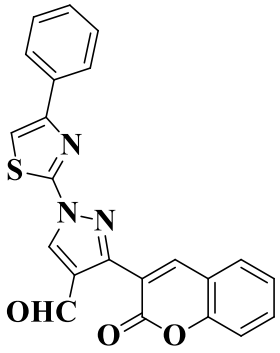
3A.5. Experimental section:

General procedure for the synthesis of 3-(2-oxo-2*H*-chromen-3-yl)-1-(4-arylthiazol-2-yl)-1*H*-pyrazole-4-carbaldehydes (4a-n):

Title compounds **(4a-n)** were synthesized by one-pot by a sequential Hantzch thiazole synthesis and Vilsmeier–Haack reaction. First, to a stirred equimolar mixture of *α*-haloketone **2** (1 mM) and aminothiourea **1** (1 mM) in an ethanol, was added substituted 3-acetylcoumarins **3** (1 mM) after confirming the disappearance of starting materials by TLC and continued the stirring under refluxing temperature for 30−60 min. After the completion of reaction (ensured by TLC), solvent from the reaction mixture was extracted under vacuum. To the above round-bottomed flask, an ice-cold, stirred solution of DMF (3 mL) and $POCl_3$ (3.5 mM) was added and allowed to stir at 0−5˚C for 15 min followed by at room temperature for another 15 min. After, that the reaction mixture was stirred at 55−60˚C for 3−4 h. After ensuring the completion of the reaction, the reaction mixture was poured into a beaker containing crushed ice or ice-cold water. Filtered the precipitated solid, washed several times with ice-cold water to wipe-off the acid remains from the crude product, dried and crystallized from ethanol to afford the target compounds thiazolyl pyrazole carbaldehydes **(4a-n)**.

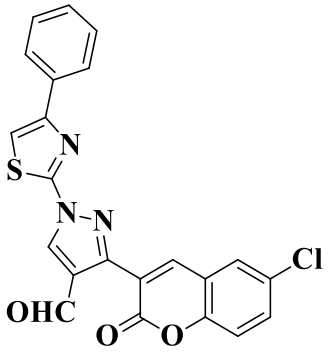
3A.6. Spectral data:

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



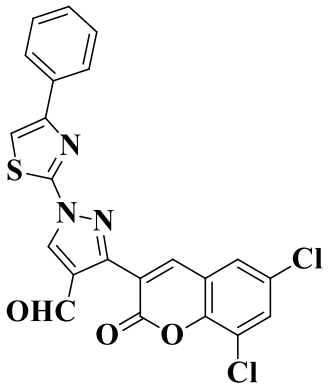
Brown solid; yield 88%; mp: 143–145^{o}C; IR (KBr) cm^{-1}: 1607 (C=N), 1685 (-CHO), 1722 (-C=O); ^{1}H NMR (400MHz, $CDCl_3$ + DMSO-d_6, ppm): δ 7.39 – 7.41 (m, 2H, Ar-H), 7.44 – 7.48 (m, 3H, Ar-H), 7.53 (s, 1H, Ar-H), 7.66 (t, 1H, *J* = 8 Hz, Ar-H), 7.71 (d, 1H, *J* = 8.0 Hz, Ar-H), 7.95(d, 2H, *J* = 7.2 Hz, Ar-H), 8.26 (s, 1H, C4-H of pyrazole), 9.07 (s, 1H, C5-H of thiazole), 10.05(s, 1H, -CHO; ^{13}C NMR (125 MHz, DMSO-d_6) δ 113.39, 116.71, 119.00, 119.55, 124.34, 125.44, 126.41, 129.34, 129.70, 133.28, 133.61, 143.96, 148.85, 152.23, 154.03, 159.27, 159.41, 185.82 ppm; Mass (ESI-HRMS) (*m/z*): 399.0738 $[M]^+$; Anal. Calcd. For $C_{22}H_{13}N_3O_3S$: C, 66.15; H, 3.28; N, 10.52%. Found: C, 66.19; H, 3.23; N, 10.48%.

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



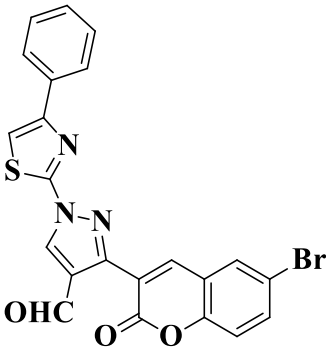
Yellow solid; yield 85%; mp: 167–169^{o}C; IR (KBr) cm^{-1}: 1604 (C=N), 1680 (-CHO), 1716 (-C=O); ^{1}H NMR (400MHz, $CDCl_3$ + DMSO-d_6, ppm): δ 7.46 (t, 2H, *J* = 8.0 Hz, Ar-H), 7.56 (s, 1H, Ar-H), 7.59 (s, 1H, Ar-H), 7.73 (s, 1H, Ar-H), 7.8 (s, 1H, Ar-H), 7.95(d, 2H, *J* = 7.2 Hz, Ar-H), 8.22 (s, 1H, C4-H of coumarin), 8.60 (s, 1H, C4-H of pyrazole), 9.09 (s, 1H, C5-H of thiazole), 10.04(s, 1H, -CHO); ^{13}C NMR (125 MHz, DMSO-d_6) δ 113.48, 118.50, 120.14, 122.89, 126.42, 129.04, 129.27, 129.43, 134.23, 143.72, 144.82, 152.31, 157.18, 185.83 ppm; Mass (ESI-HRMS) (*m/z*): 433.0386 $[M]^+$; Anal. Calcd. For $C_{22}H_{12}ClN_3O_3S$: C, 60.90; H, 2.79; Cl, 8.17; N, 9.69%. Found: C, 60.93; H, 2.74; N, 9.73%.

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



Yellow solid; yield 90%; mp: 173–175°C; IR (KBr) cm^{-1}: 1604 (C=N), 1686 (-CHO), 1716 (-C=O); ^{1}H NMR (400MHz, $CDCl_3$ + DMSO-d_6, ppm): δ 7.41 (s, 1H, Ar-H), 7.46 (d, 2H, J = 8.0 Hz, Ar-H), 7.70 (s, 1H, Ar-H), 7.76 (s, 1H, Ar-H), 7.94 (d, 2H, J = 7.2 Hz, Ar-H), 8.19 (s, 1H, C4-H of coumarin), 8.56 (s, 1H, C4-H of pyrazole), 9.08 (s, 1H, C5-H of thiazole), 10.05 (s, 1H, -CHO); ^{13}C NMR (125 MHz, DMSO-d_6) δ 113.50, 118.51, 118.77, 120.16, 122.92, 126.43, 129.04, 129.26, 129.43, 134.23, 142.68, 143.74, 144.81, 152.32, 157.21, 185.86 ppm; Mass (ESI-HRMS) (*m/z*): 466.9929 $[M]^+$; Anal. Calcd. For $C_{22}H_{11}Cl_2N_3O_3S$: C, 56.42; H, 2.37; N, 8.97%. Found: C, 56.38; H, 2.41; N, 8.94%.

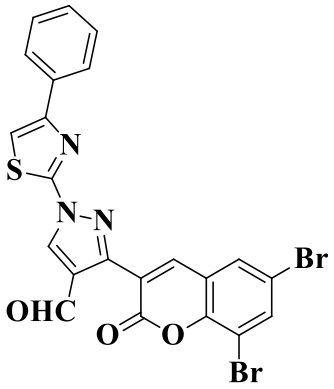
3-(6-Bromo-2-oxo-2*H*-chromen-3-yl)-1-(4-phenylthiazol-2-yl)-1*H*-pyrazole-4-carbaldehyde (4d):



Yellow solid; yield 88%; mp: 145–147°C; IR (KBr) cm^{-1}: 1600 (C=N), 1682 (-CHO), 1722 (-C=O); ^{1}H NMR (400MHz, $CDCl_3$ + DMSO-d_6, ppm): δ 7.33 (d, 2H, J = 8.8 Hz, Ar-H), 7.46 (d, 2H, J = 7.2 Hz, Ar-H), 7.95 (t, 3H, J = 7.2 Hz, Ar-H), 8.00 (s, 1H, Ar-H), 8.25 (s, 1H, C4-H of coumarin), 8.65 (s, 1H, C4-H of pyrazole), 9.12 (s, 1H, C5-H of thiazole), 10.03 (s, 1H, -CHO); ^{13}C NMR (125 MHz, DMSO-d_6) δ 113.33, 117.09, 118.68, 119.01, 120.57, 122.77, 126.40, 129.01,

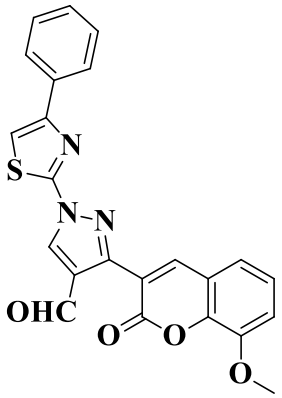
129.27, 131.62, 132.44, 136.98, 142.60, 143.70, 144.79, 152.70, 157.07, 185.64 ppm; Mass (ESI-HRMS) (*m/z*): 476.9788 $[M]^+$; Anal. Calcd. For $C_{22}H_{12}BrN_3O_3S$: C, 55.24; H, 2.53; N, 8.78%. Found: C, 55.19; H, 2.56; N, 8.74%.

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



Yellow solid; yield 92%; mp: 195–197°C; IR (KBr) cm^{-1}: 1599 (C=N), 1681 (-CHO), 1738 (-C=O); ^1H NMR (400MHz, DMSO-d_6, ppm): δ 7.42 (d, 1H, *J* = 7.6 Hz, Ar-H), 7.51 (t, 2H, *J* = 7.2 Hz, Ar-H), 8.04 (d, 2H, *J* = 7.2 Hz, Ar-H), 8.11 (s, 1H, C4-H of coumarin), 8.19 (s, 1H, Ar-H), 8.25 (s, 1H, Ar-H), 8.41 (s, 1H, C4-H of pyrazole), 9.40 (s, 1H, C5-H of thiazole), 10.00 (s, 1H, -CHO); ^{13}C NMR (125 MHz, DMSO-d_6) δ 113.71, 116.41, 126.45, 129.11, 131.08, 134.05, 139.51, 149.79, 150.71, 153.45, 153.74, 159.01, 159.76, 160.99, 185.07 ppm; Mass (ESI-HRMS) (*m/z*): 554.8885 $[M]^+$; Anal. Calcd. For $C_{22}H_{11}Br_2N_3O_3S$: C, 47.42; H, 1.99; N, 7.54%. Found: C, 47.47; H, 1.95; N, 7.57%.

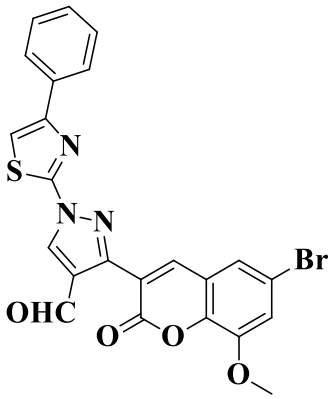
3-(8-Methoxy-2-oxo-2*H*-chromen-3-yl)-1-(4-phenylthiazol-2-yl)-1*H*-pyrazole-4-carbaldehyde (4f):



Brown solid; yield 89%; mp: 159–161°C; IR (KBr) cm^{-1}: 1600 (C=N), 1682 (-CHO), 1711 (-

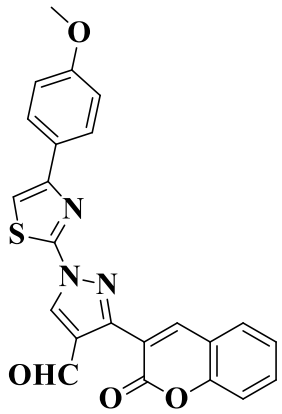
C=O); ^{1}H NMR (400MHz, DMSO-$d_{6,}$ ppm): δ 3.96 (s, 3H, OCH_3) 7.37- 7.45 (m, 4H, Ar-H), 7.50 (t, 2H, *J* = 7.6 Hz, Ar-H), 8.04 (d, 2H, *J* = 7.6 Hz, Ar-H), 8.09 (s, 1H, C4-H of coumarin), 8.42 (s, 1H, C4-H of pyrazole), 9.37 (s, 1H, C5-H of thiazole), 9.98 (s, 1H, -CHO); ^{13}C NMR (125 MHz, DMSO-d_6) δ 56.65, 113.47, 120.77, 125.49, 126.44, 129.16, 129.41, 133.48, 133.63, 137.94, 143.33, 144.24, 146.89, 148.87, 152.25, 159.17, 185.90 ppm; Mass (ESI-HRMS) (*m/z*): 430.0868 $[M+H]^+$; Anal. Calcd. For $C_{23}H_{15}N_3O_4S$: C, 64.33; H, 3.52; N, 9.78%. Found: C, 64.29; H, 3.56; N, 9.82%.

3-(6-Bromo-8-methoxy-2-oxo-2*H*-chromen-3-yl)-1-(4-phenylthiazol-2-yl)-1*H*-pyrazole-4-carbaldehyde (4g):



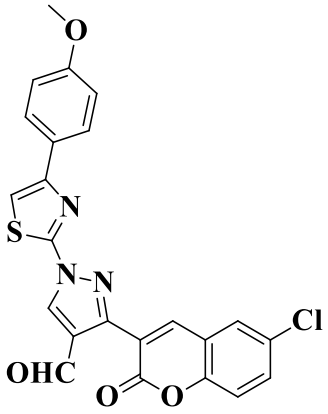
Brown solid; yield 87%; mp: 243–245^{o}C; IR (KBr) cm^{-1}: 1602 (C=N), 1676 (-CHO), 1730 (-C=O); ^{1}H NMR (400MHz, $CDCl_3$ + DMSO-$d_{6,}$ ppm): δ 4.02 (s, 3H, OCH_3) 7.35- 7.40 (m, 3H, Ar-H), 7.45 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.66 (s, 1H, Ar-H), 7.96 (d, 2H, *J* = 7.6 Hz, Ar-H), 8.21 (s, 1H, C4-H of pyrazole), 9.13 (s, 1H, C5-H of thiazole), 10.02 (s, 1H, -CHO); ^{13}C NMR (125 MHz, DMSO-d_6) δ 57.10, 113.22, 116.87, 118.00, 122.60, 126.38, 128.94, 129.11, 129.20, 130.19, 142.83, 147.76, 148.40, 152.31, 158.58, 159.17, 185.52 ppm; Mass (ESI-HRMS) (*m/z*): 506.9885 $[M]^+$; Anal. Calcd. For $C_{23}H_{14}BrN_3O_4S$: C, 54.34; H, 2.78; N, 8.27%. Found: C, 54.39; H, 2.74; N, 8.32%.

1-(4-(4-Methoxyphenyl)thiazol-2-yl)-3-(2-oxo-2*H*-chromen-3-yl)-1*H*-pyrazole-4-carbaldehyde (4h):



Yellow solid; yield 91%; mp: 229–231°C; IR (KBr) cm^{-1}: 1607 (C=N), 1681 (-CHO), 1725 (-C=O); ^{1}H NMR (400MHz, DMSO-d_6, ppm): δ 3.82 (s, 3H, OCH_3), 7.05 (d, 1H, *J* = 8.2 Hz, Ar-H), 7.15 (d, 1H, J = 8.0 Hz, Ar-H), 7.45 (d, 2H, *J* = 7.6 Hz, Ar-H), 7.52 (d, 1H, *J* = 8.0 Hz, Ar-H), 7.92 – 7.98 (m, 4H, Ar-H), 8.45 (s, 1H, C4-H of pyrazole), 9.36 (s, 1H, C5-H of thiazole), 9.98 (s, 1H, -CHO); ^{13}C NMR (125 MHz, DMSO-d_6) δ 57.23, 114.71, 114.97, 118.05, 122.67, 123.41, 127.91, 129.42, 132.77, 133.37, 147.83, 153.91, 158.65, 160.06, 185.88 ppm; Mass (ESI-HRMS) (*m/z*): 430.0884 $[M+H]^+$; Anal. Calcd. For $C_{23}H_{15}N_3O_4S$: C, 64.33; H, 3.52; N, 9.78%. Found: C, 64.39; H, 3.56; N, 9.82%.

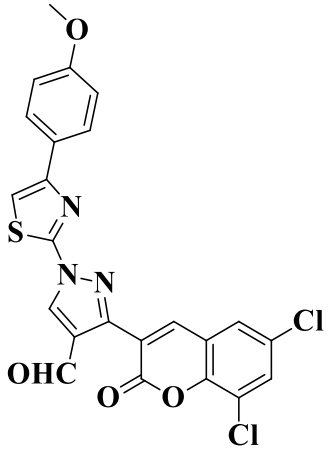
1-(4-(4-Methoxyphenyl)thiazol-2-yl)-3-(2-oxo-2*H*-chromen-3-yl)-1*H*-pyrazole-4-carbaldehyde (4i):



Yellow solid; yield 86%; mp: 226–228°C; IR (KBr) cm^{-1}: 1607 (C=N), 1687 (-CHO), 1731 (-C=O); ^{1}H NMR (400MHz, DMSO-d_6, ppm): δ 3.83 (s, 3H, OCH_3), 7.05 (d, 1H, *J* = 9.2 Hz, Ar-H), 7.16 (d, 1H, J = 8.8 Hz, Ar-H), 7.75 – 7.78 (m, 3H, Ar-H), 7.96 (s, 1H, Ar-H), 8.04 – 8.06 (m, 2H, Ar-H), 8.42 (s, 1H, C4-H of pyrazole), 9.38 (s, 1H, C5-H of thiazole), 9.98 (s, 1H, -CHO); ^{13}C NMR (125 MHz, DMSO-d_6) δ 55.72, 114.72, 118.77, 121.39, 125.77, 127.92, 129.00, 129.69, 132.19, 137.69, 140.10, 152.89, 153.54, 162.61, 163.52, 185.89 ppm; Mass (ESI-HRMS) (*m/z*):

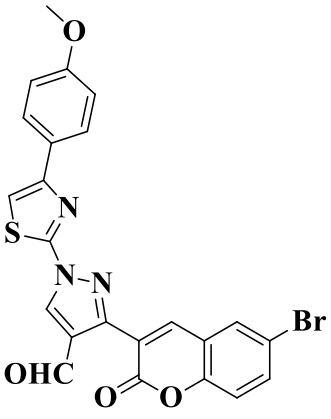
481.0499 $[M+NH_4]^+$; Anal. Calcd. For $C_{23}H_{14}ClN_3O_4S$: C, 59.55; H, 3.04; N, 9.06%. Found: C, 59.59; H, 3.09; N, 9.10%.

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



Yellow solid; yield 90%; mp: 240–242ºC; IR (KBr) cm^{-1}: 1606 (C=N), 1688 (-CHO), 1719 (-C=O); ^1H NMR (400MHz, DMSO-d_6, ppm): δ 3.86 (s, 3H, OCH_3), 6.98 (d, 2H, J = 8.8 Hz, Ar-H), 7.53 (s, 1H, Ar-H), 7.72 (d, 1H, J = 8 Hz, Ar-H), 7.98- 8.01 (m, 3H, Ar-H), 8.28 (s, 1H, C4-H of pyrazole), 9.16 (s, 1H, C5-H of thiazole), 10.03 (s, 1H, -CHO); ^{13}C NMR (125 MHz, DMSO-d_6) δ 55.89, 112.61, 114.05, 122.97, 125.61, 127.09, 129.14, 130.03, 136.53, 146.55, 152.33, 155.14, 159.57, 159.87, 161.41, 185.82 ppm; Mass (ESI-HRMS) (*m/z*): 497.0131 $[M]^+$; Anal. Calcd. For $C_{23}H_{13}C_{l2}N_3O_4S$: C, 55.43; H, 2.63; N, 8.43%. Found: C, 55.47; H, 2.58; N, 8.48%.

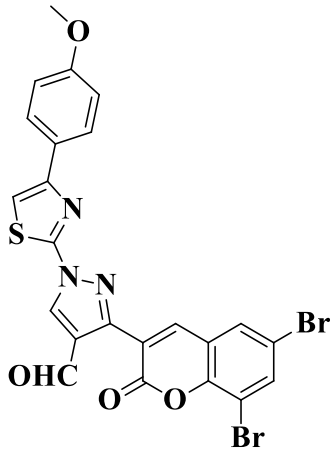
3-(6-Bromo-2-oxo-2*H*-chromen-3-yl)-1-(4-(4-methoxyphenyl)thiazol-2-yl)-1*H*-pyrazole-4-carbaldehyde (4k):



Yellow solid; yield 92%; mp: 232–234ºC; IR (KBr) cm^{-1}: 1605 (C=N), 1688 (-CHO), 1718 (-

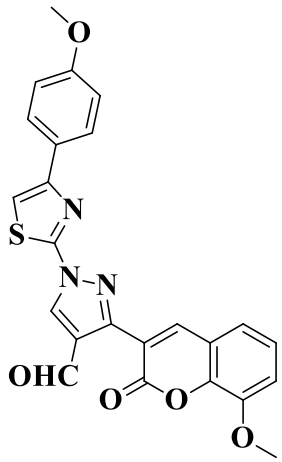
C=O); ^{1}H NMR (400MHz, $CDCl_3$ + DMSO-d_6, ppm): δ 3.86 (s, 3H, OCH_3), 6.98 (d, 1H, *J* = 8.8 Hz, Ar-H), 7.42 (t, 3H, *J* = 8.0 Hz, Ar-H), 7.53 (d, 1H, *J* = 6.8 Hz, Ar-H), 7.66- 7.68 (m, 2H, Ar-H), 8.30 (s, 1H, C4-H of coumarin), 8.73 (s, 1H, C4-H of pyrazole), 9.16 (s, 1H, C5-H of thiazole), 10.01 (s, 1H, -CHO); ^{13}C NMR (125 MHz, DMSO-d_6) δ 55.70, 111.37, 114.71, 119.05, 120.87, 124.74, 127.90, 131.63, 133.50, 135.73, 142.62, 152.20, 155.91, 160.05, 185.89 ppm; Mass (ESI-HRMS) (*m/z*): 506.9883 $[M]^+$; Anal. Calcd. For $C_{23}H_{14}BrN_3O_4S$: C, 54.34; H, 2.78; N, 8.27%. Found: C, 54.30; H, 2.81; N, 8.31%.

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



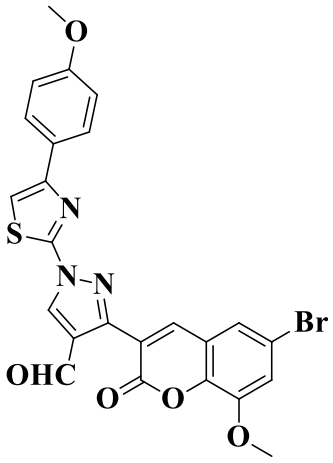
Brown solid; yield 91%; mp: 161–163^{o}C; IR (KBr) cm^{-1}: 1603 (C=N), 1686 (-CHO), 1730 (-C=O); ^{1}H NMR (400MHz, DMSO-d_6, ppm): δ 3.82 (s, 3H, OCH_3), 7.04 (d, 1H, *J* = 8.8 Hz, Ar-H), 7.14 (d, 1H, *J* = 8.8 Hz, Ar-H), 7.85 – 7.90 (m, 2H, Ar-H), 7.95 (t, 3H, *J* = 8.4 Hz, Ar-H), 8.39 (s, 1H, C4-H of pyrazole), 9.36 (s, 1H, C5-H of thiazole), 9.99 (s, 1H, -CHO); ^{13}C NMR (125 MHz, DMSO-d_6) δ 55.72, 114.73, 117.11, 120.93, 126.87, 129.47, 129.65, 133.46, 137.57, 139.02, 140.32, 156.23, 158.32, 163.12, 163.54, 185.20 ppm; Mass (ESI-HRMS) (*m/z*): 584.8990 $[M]^+$; Anal. Calcd. For $C_{23}H_{13}Br_2N_3O_4S$: C, 47.04; H, 2.23; N, 7.16%. Found: C, 47.13; H, 2.20; N, 7.20%.

3-(8-Methoxy-2-oxo-2*H*-chromen-3-yl)-1-(4-(4-methoxyphenyl)thiazol-2-yl)-1*H*-pyrazole-4-carbaldehyde (4m):



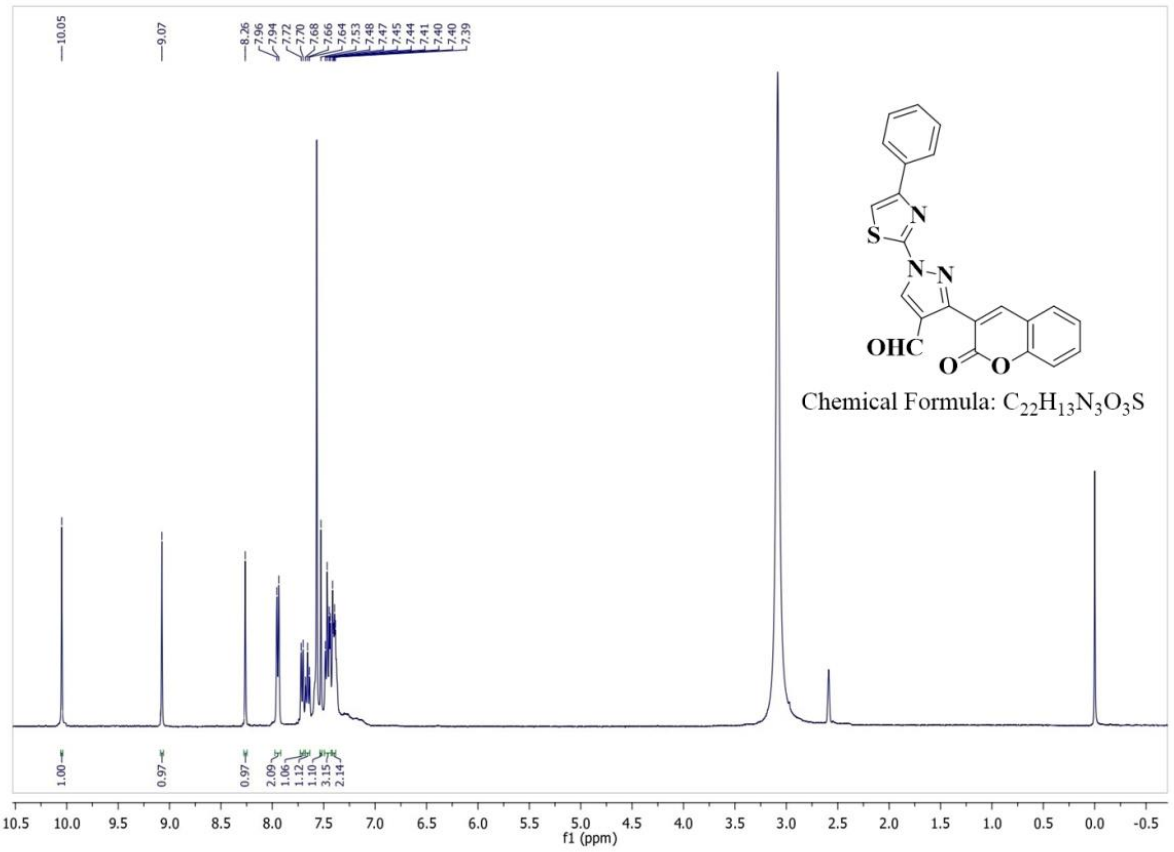
Yellow solid; yield 89%; mp: 237–239ºC; IR (KBr) cm^{-1}: 1603 (C=N), 1681 (-CHO), 1713 (-C=O); ^{1}H NMR (400MHz, DMSO-d_6, ppm): δ 3.82 (s, 3H, OCH_3), 3.96 (s, 3H, OCH_3) 7.05 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.37- 7.45 (m, 3H, Ar-H), 7.92 (s, 1H, Ar-H), 7.97 (d, 2H, *J* = 7.6 Hz, Ar-H), 8.42 (s, 1H, C4-H of pyrazole), 9.36 (s, 1H, C5-H of thiazole), 9.98 (s, 1H, -CHO); ^{13}C NMR (125 MHz, DMSO-d_6) δ 55.70, 60.32, 111.31, 114.72, 116.75, 124.30, 125.51, 126.44, 127.90, 129.72, 133.50, 144.02, 148.82, 152.19, 160.03, 160.46, 185.89 ppm; Mass (ESI-HRMS) (*m/z*): 460.0976 $[M+H]^+$; Anal. Calcd. For $C_{24}H_{17}N_3O_5S$: C, 62.74; H, 3.73; N, 9.15%. Found: C, 62.70; H, 3.76; N, 9.11%.

3-(6-Bromo-8-methoxy-2-oxo-2*H*-chromen-3-yl)-1-(4-(4-methoxyphenyl)thiazol-2-yl)-1*H*-pyrazole-4-carbaldehyde (4n):

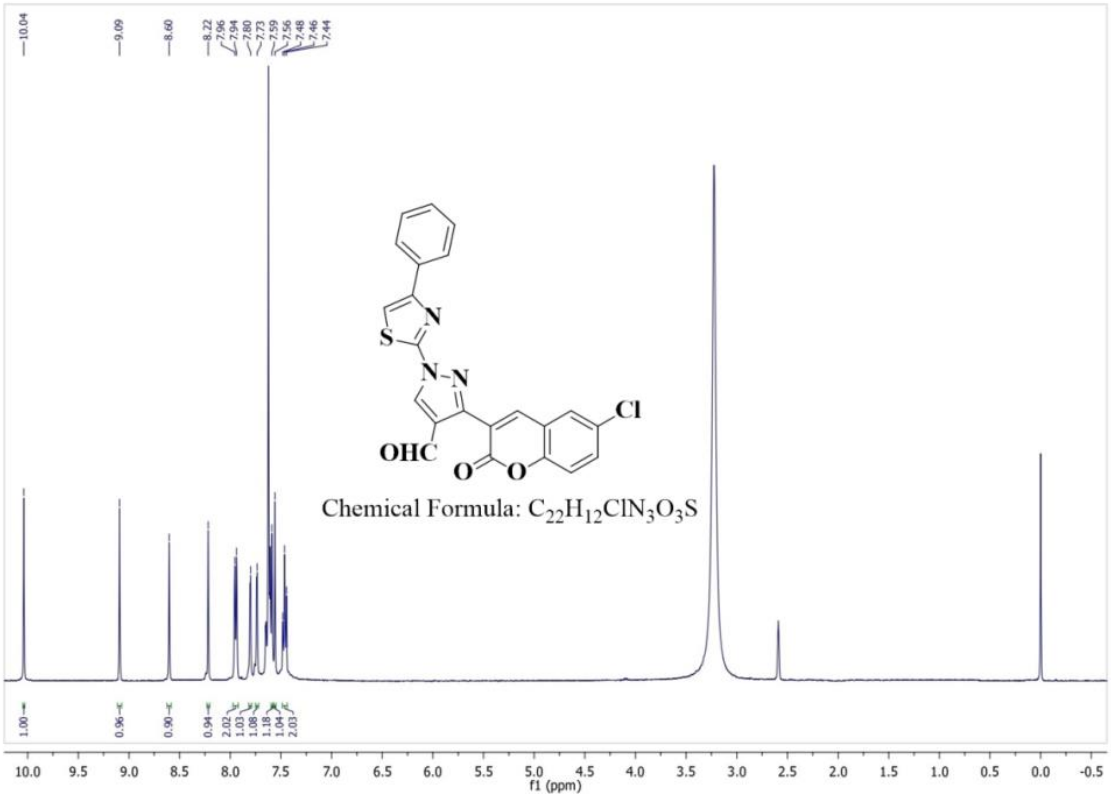


Olive green solid; yield 90%; mp: 186–188ºC; IR (KBr) cm^{-1}: 1600 (C=N), 1688 (-CHO), 1730 (-C=O); ^{1}H NMR (400MHz, DMSO-d_6, ppm): δ 3.87 (s, 3H, OCH_3), 3.99 (s, 3H, OCH_3), 7.05 (d, 1H, *J* = 8.8 Hz, Ar-H), 7.15 (d, 1H, *J* = 8.8 Hz, Ar-H), 7.56 – 7.58 (m, 1H, Ar-H), 7.70 – 7.72 (m, 1H, Ar-H), 7.93 - 7.98 (m, 3H, Ar-H), 8.39 (s, 1H, C4-H of pyrazole), 9.42 (s, 1H, C5-H of thiazole), 10.00 (s, 1H, -CHO); ^{13}C NMR (125 MHz, DMSO-d_6) δ 55.72, 56.67, 111.36, 114.71,

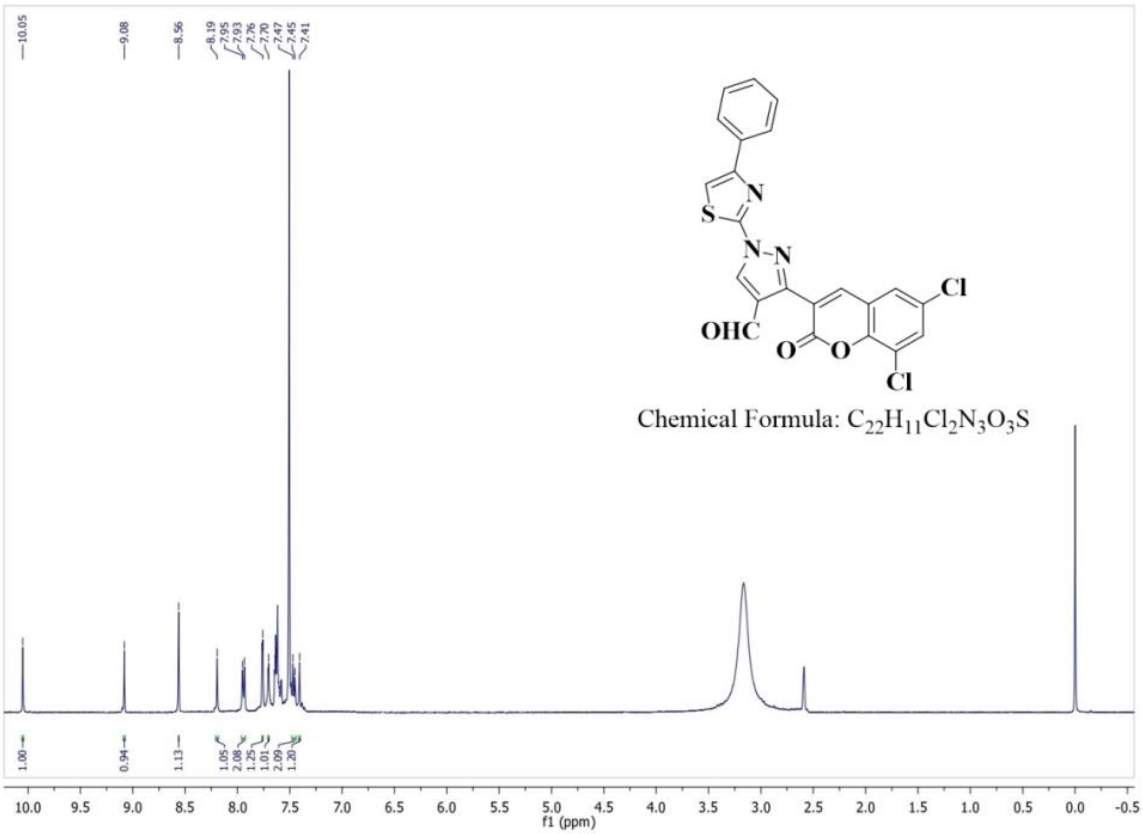
115.58, 119.65, 120.79, 124.34, 125.40, 127.91, 133.25, 143.39, 144.18, 146.91, 148.90, 152.20, 159.14, 160.06, 185.88 ppm; Mass (ESI-HRMS) (*m/z*): 536.9995 $[M]^+$; Anal. Calcd. For $C_{24}H_{16}BrN_3O_5S$: C, 53.54; H, 3.00; N, 7.81%. Found: C, 53.59; H, 2.95; N, 7.77%.



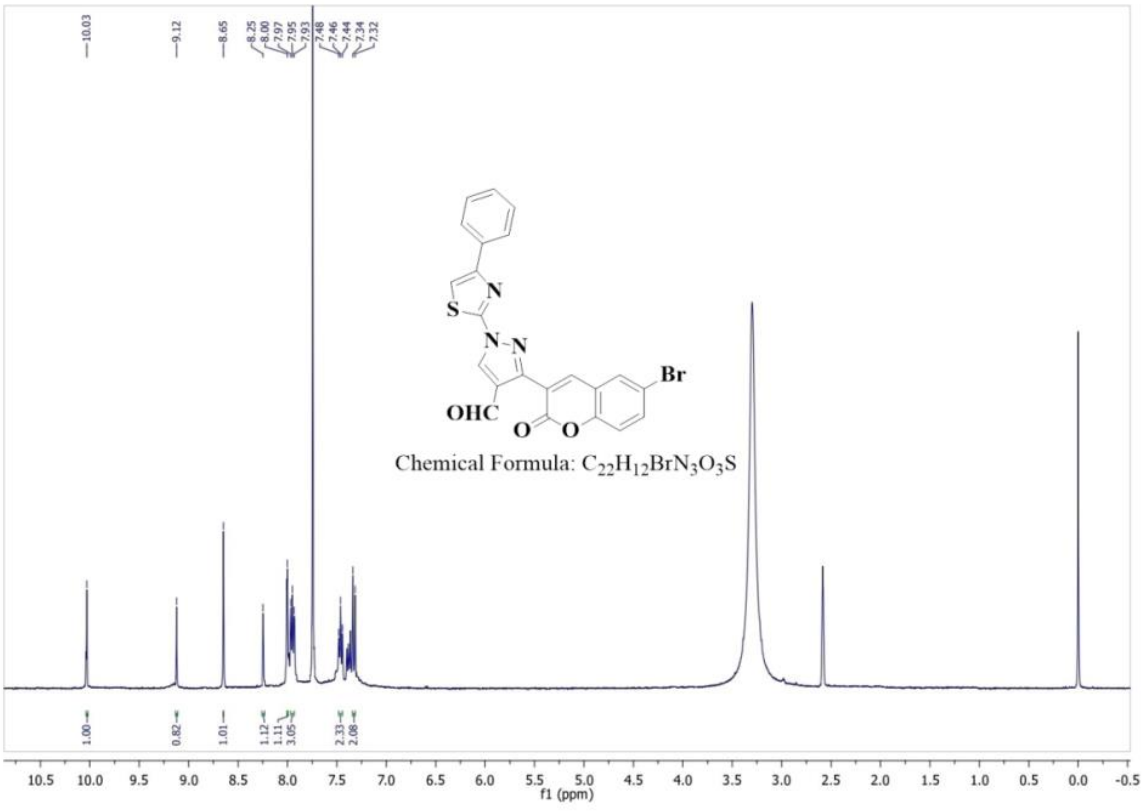
^{1}H NMR spectrum of compound **4a** (400 MHz, $CDCl_3$+DMSO-d_6)



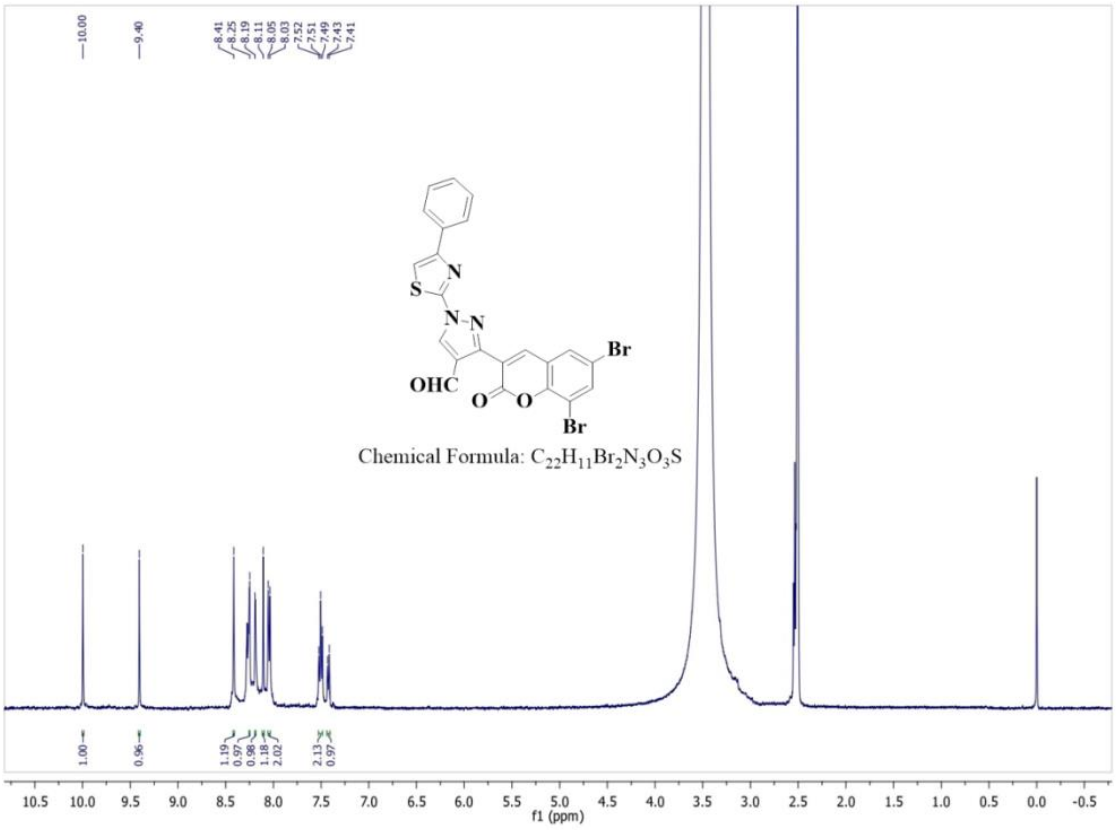
^{1}H NMR spectrum of compound **4b** (400 MHz, $CDCl_3$+DMSO-d_6)



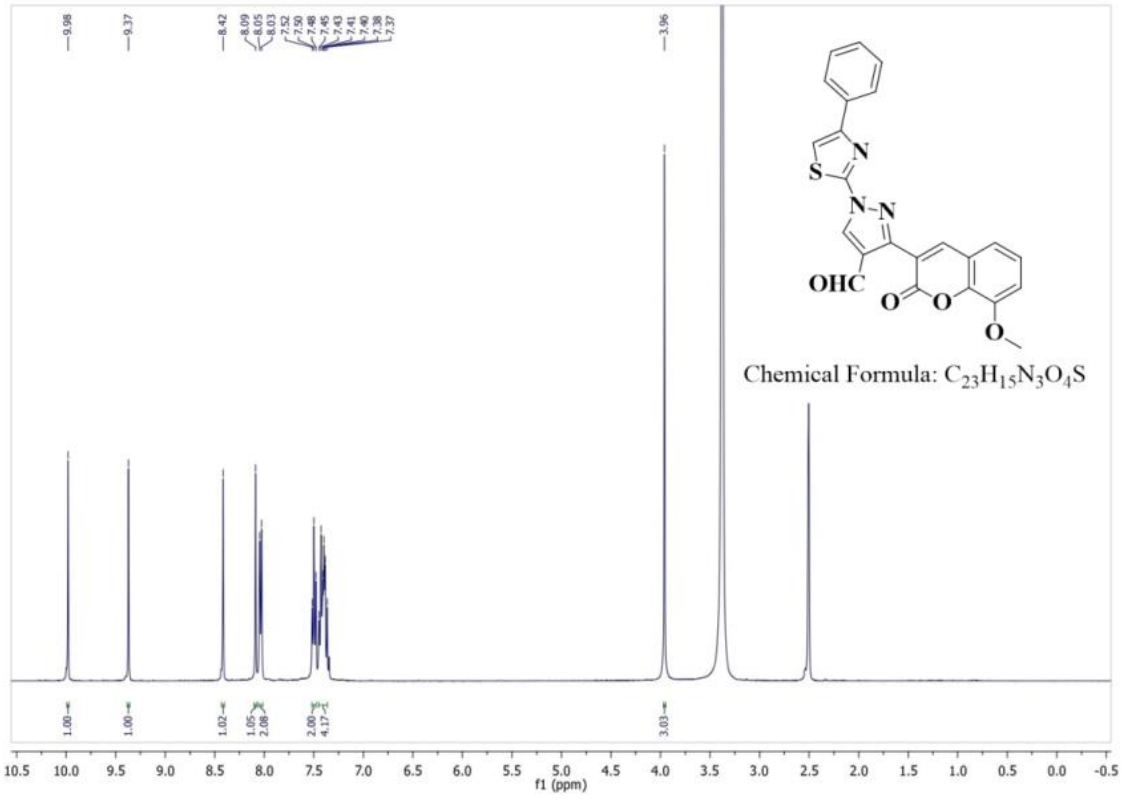
^{1}H NMR spectrum of compound **4c** (400 MHz, $CDCl_3$+DMSO-d_6)



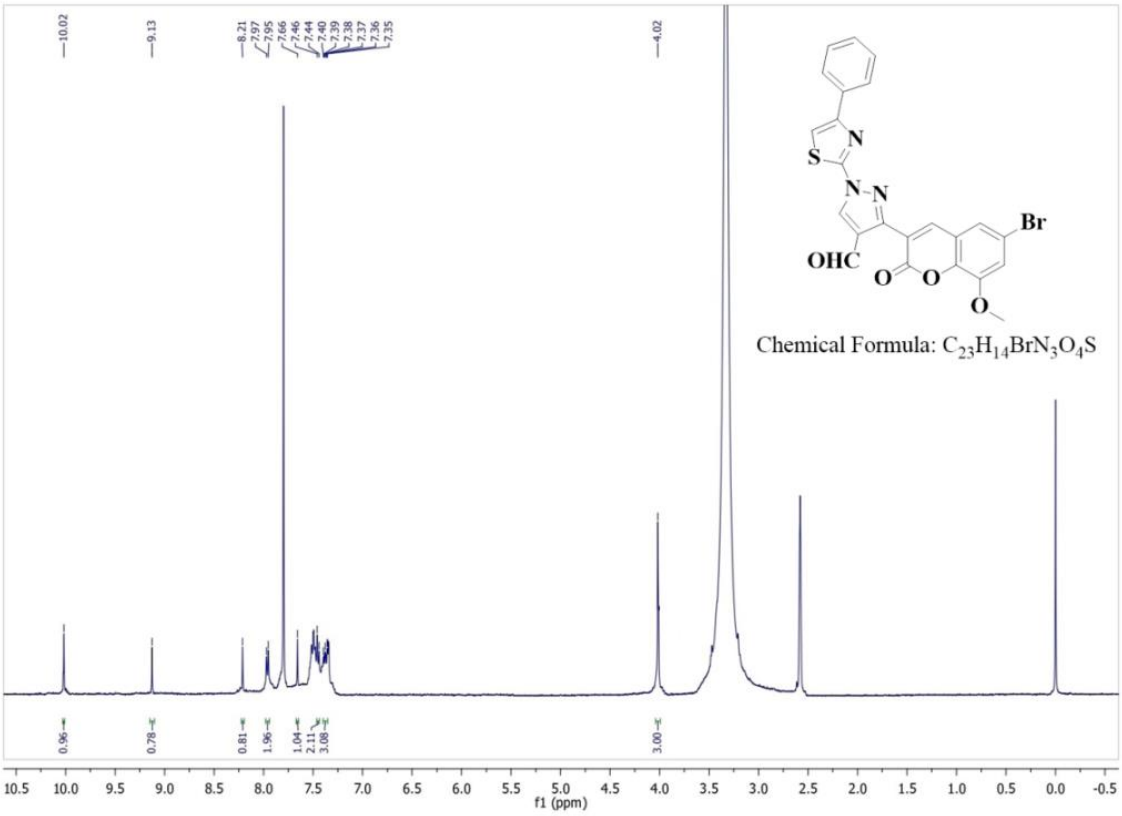
^{1}H NMR spectrum of compound **4d** (400 MHz, $CDCl_3$+DMSO-d_6)



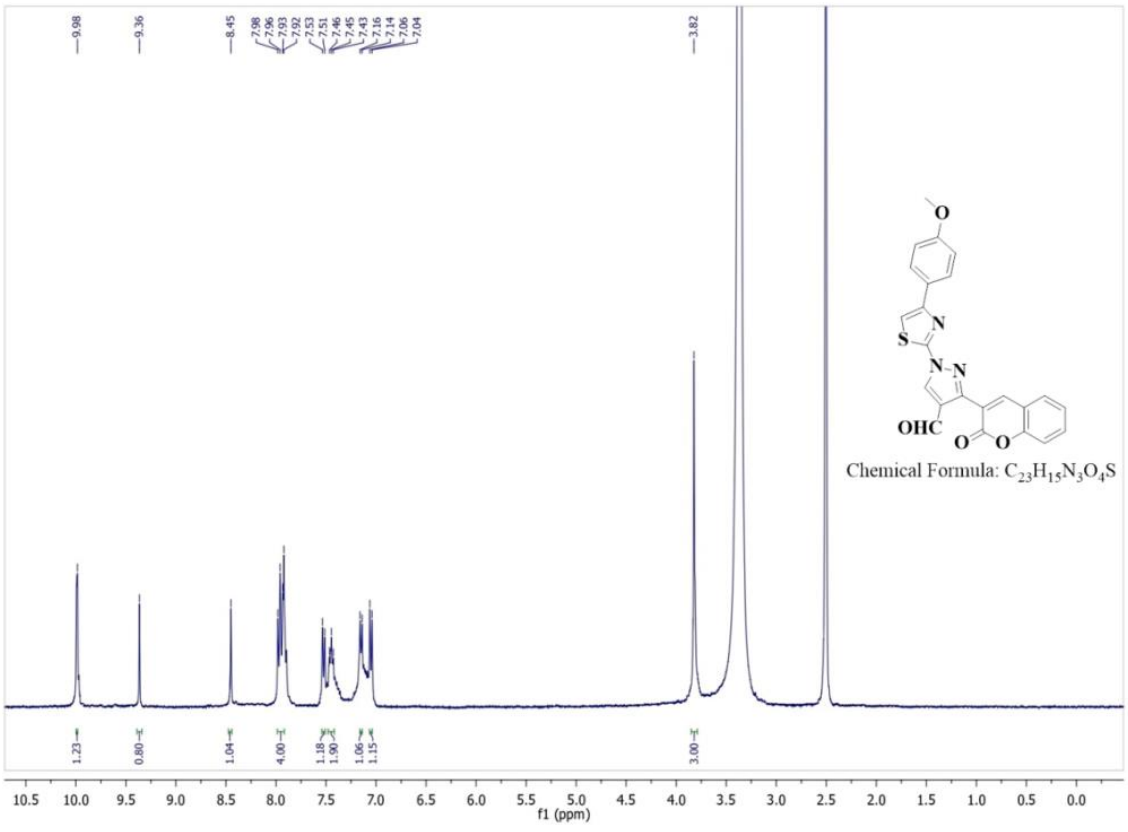
^{1}H NMR spectrum of compound **4e** (400 MHz, DMSO-d_6)



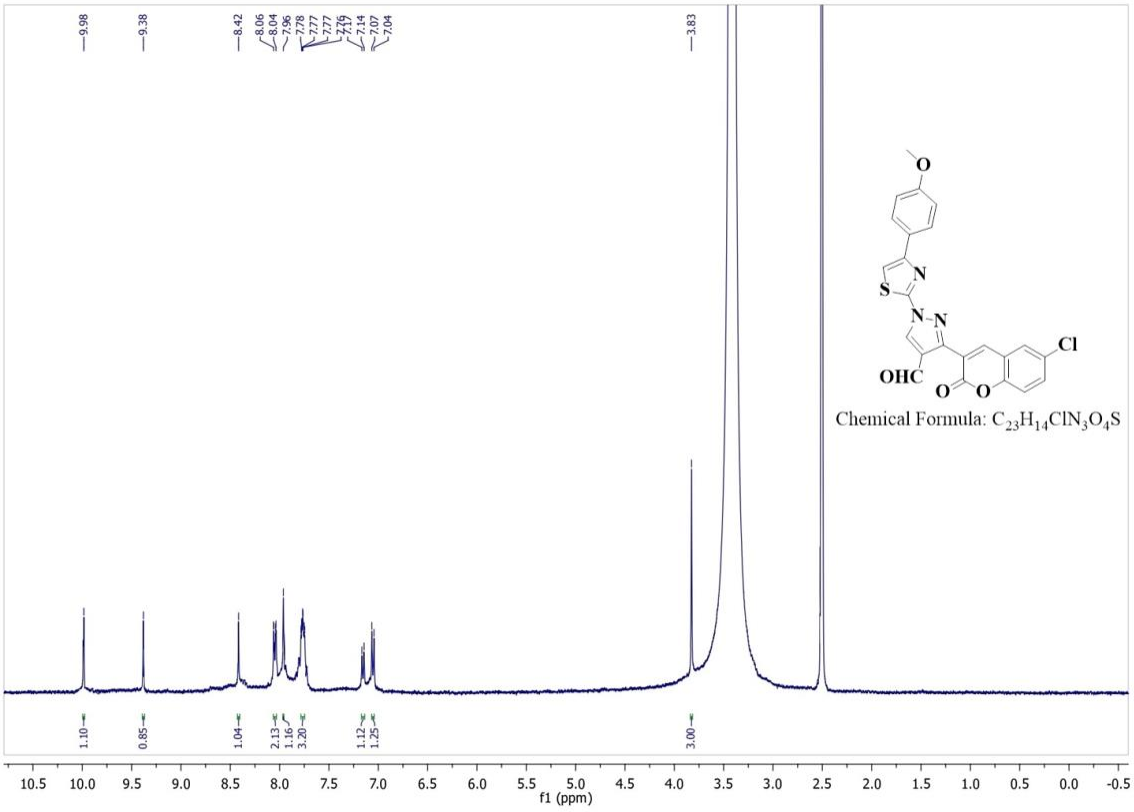
^{1}H NMR spectrum of compound **4f** (400 MHz, DMSO-d_6)



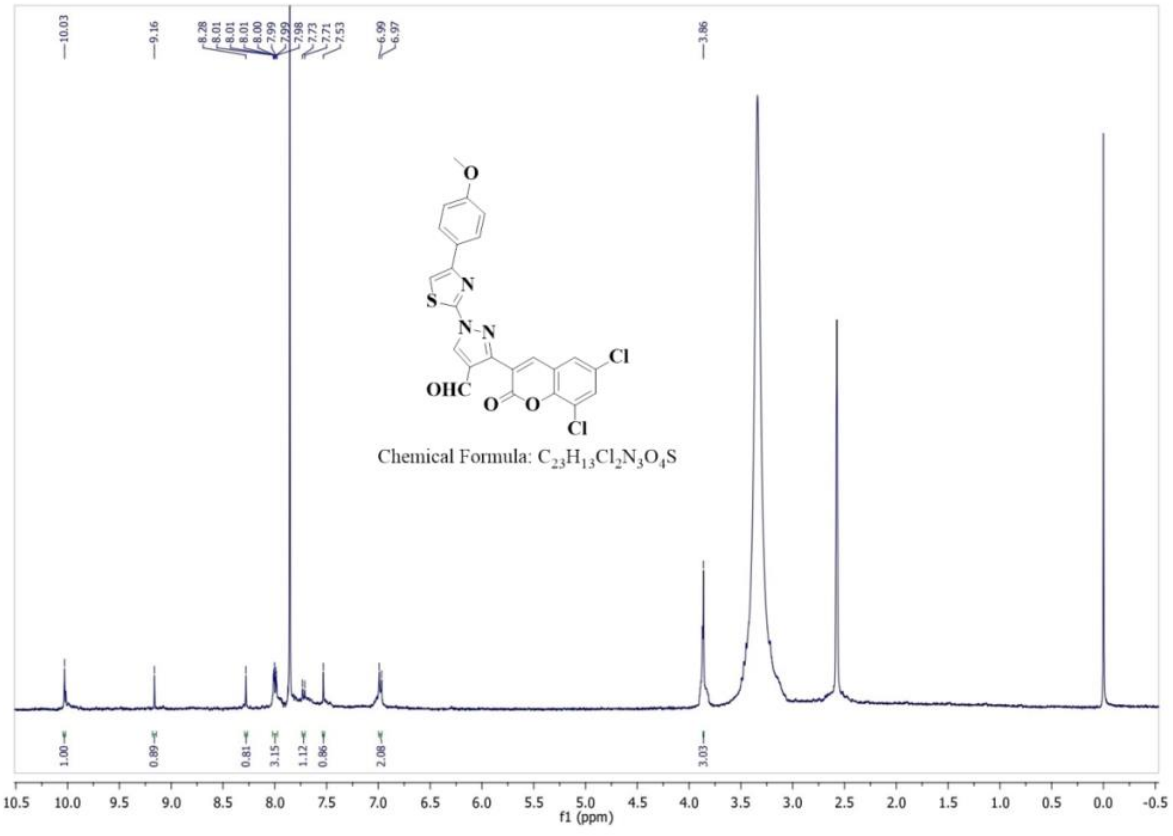
^{1}H NMR spectrum of compound **4g** (400 MHz, $CDCl_3$+DMSO-d_6)



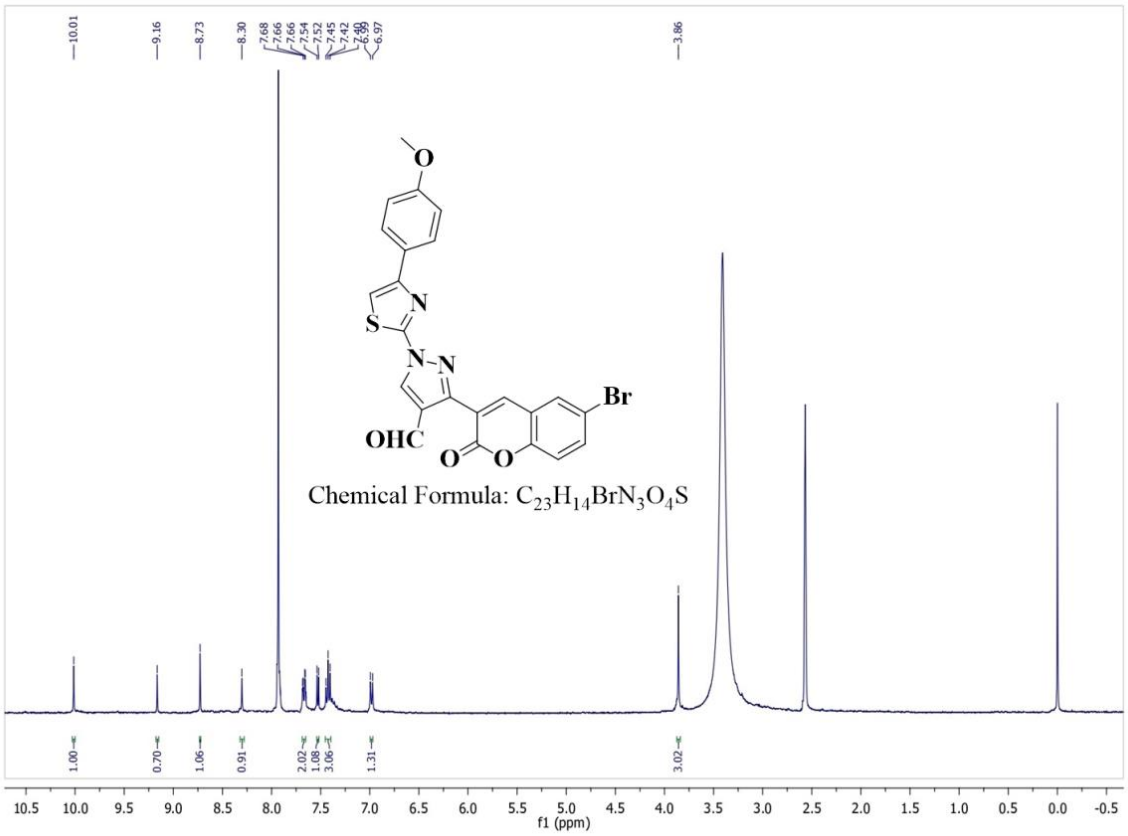
^{1}H NMR spectrum of compound **4h** (400 MHz, DMSO-d_6)



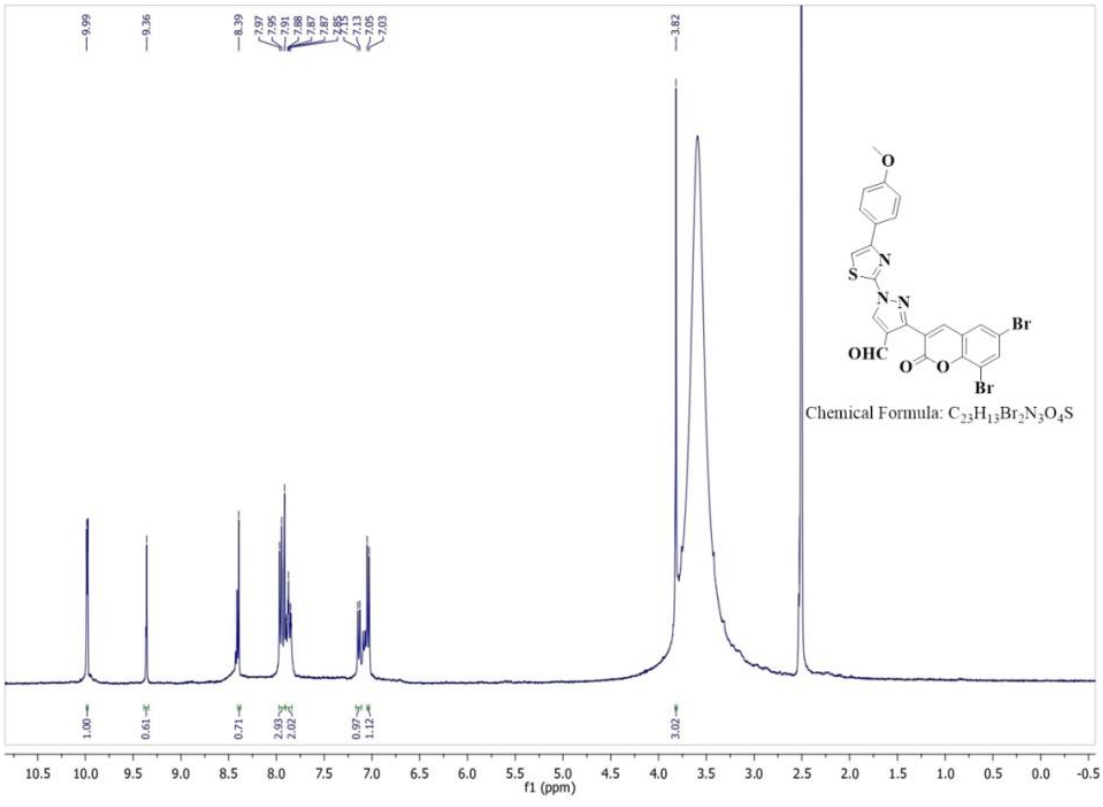
^{1}H NMR spectrum of compound **4i** (400 MHz, DMSO-d_6)



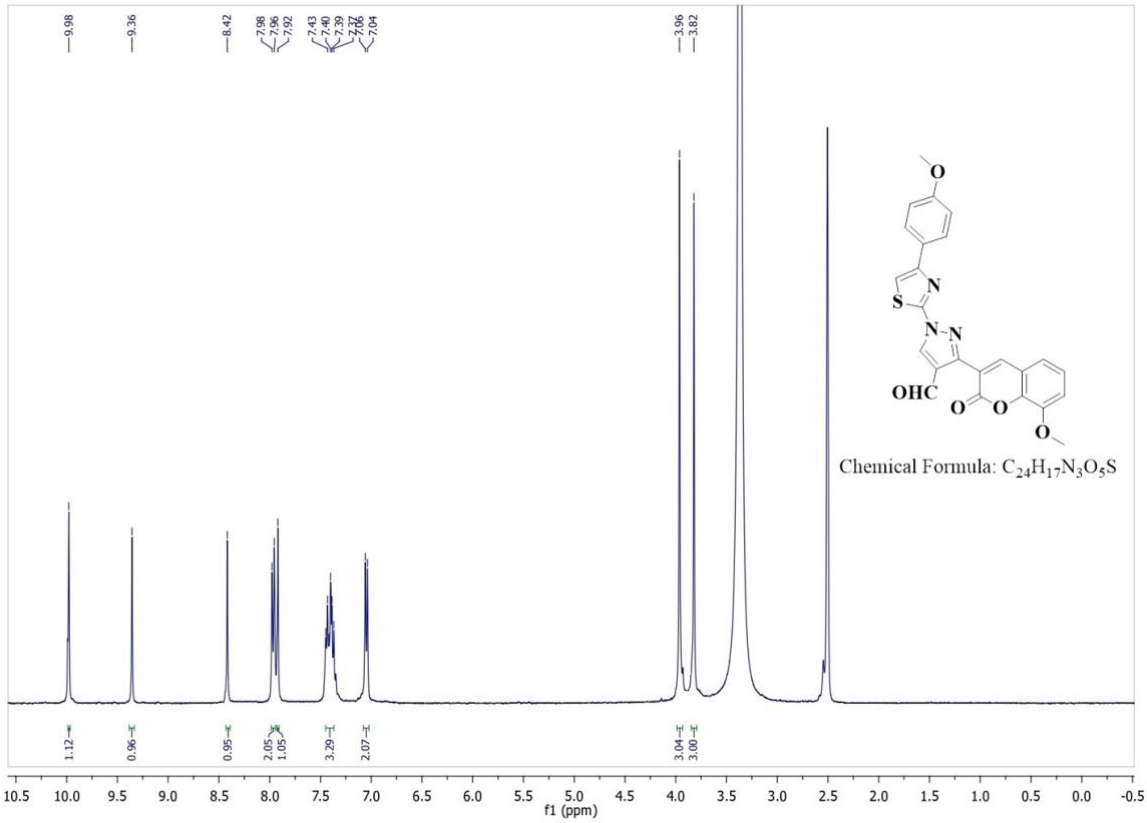
^{1}H NMR spectrum of compound **4j** (400 MHz, DMSO-d_6)



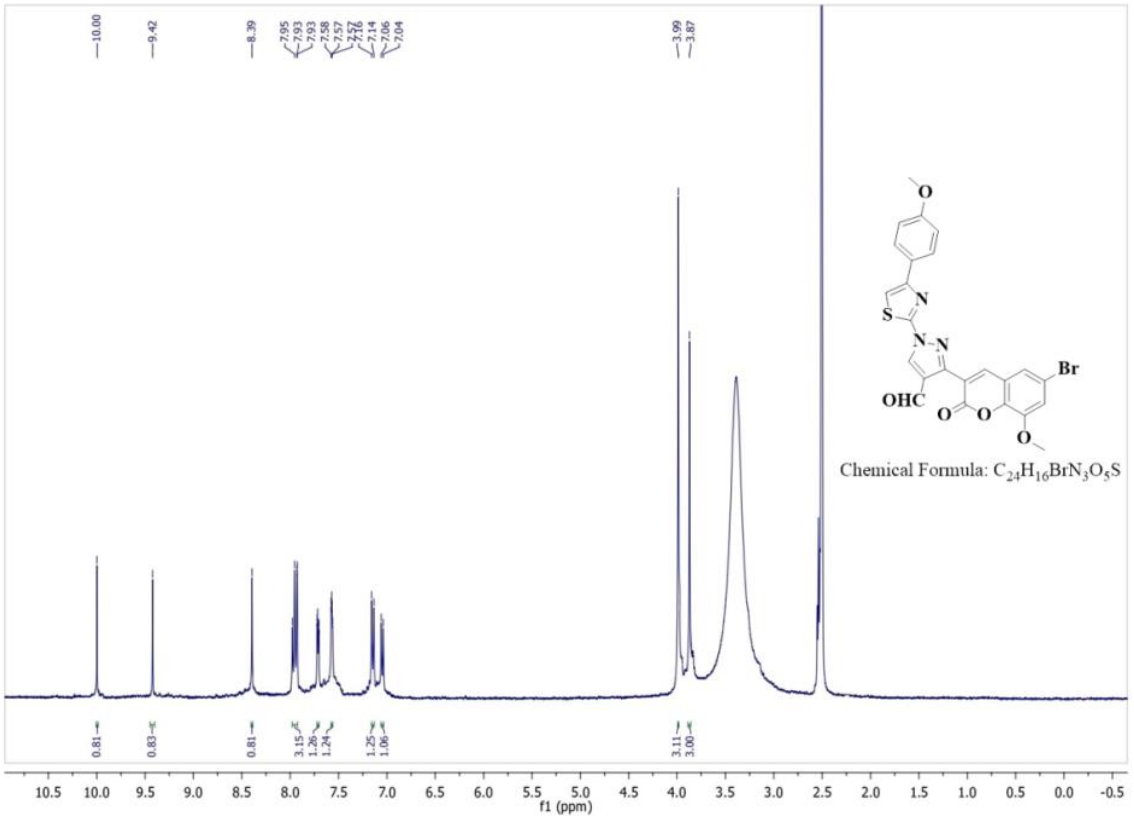
^{1}H NMR spectrum of compound **4k** (400 MHz, $CDCl_3$+DMSO-d_6)



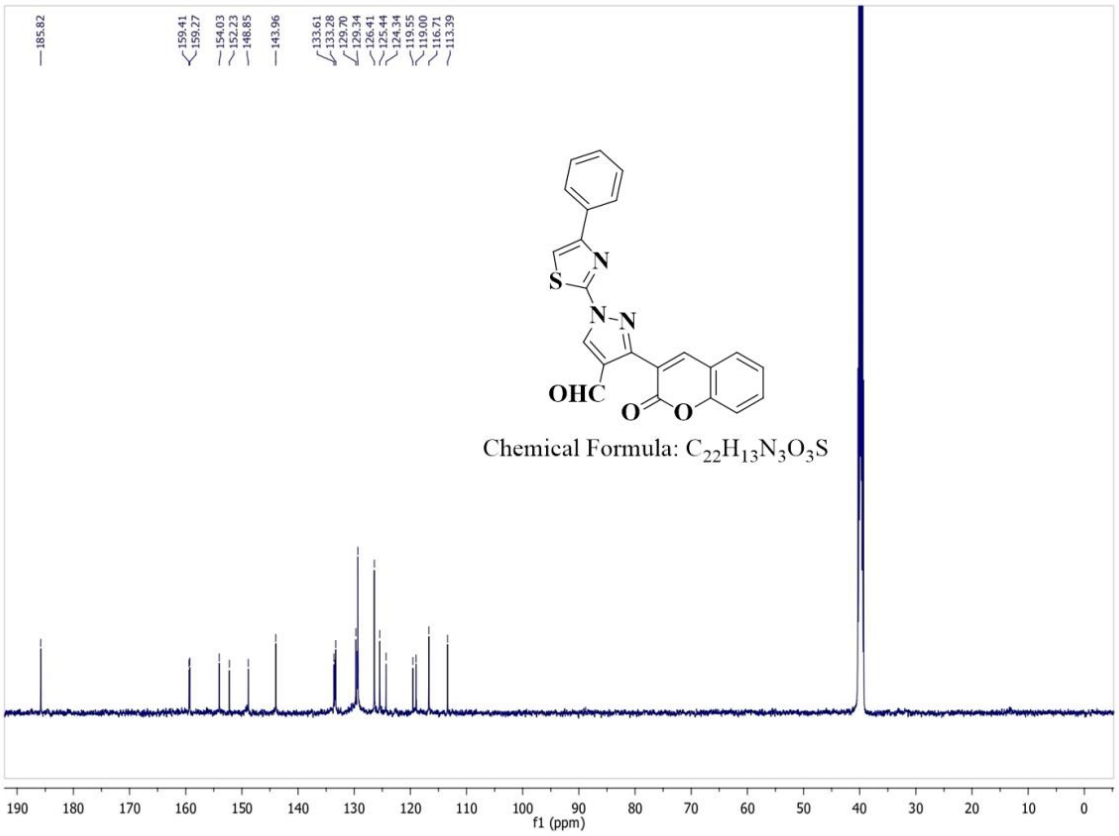
^{1}H NMR spectrum of compound **4l** (400 MHz, DMSO-d_6)



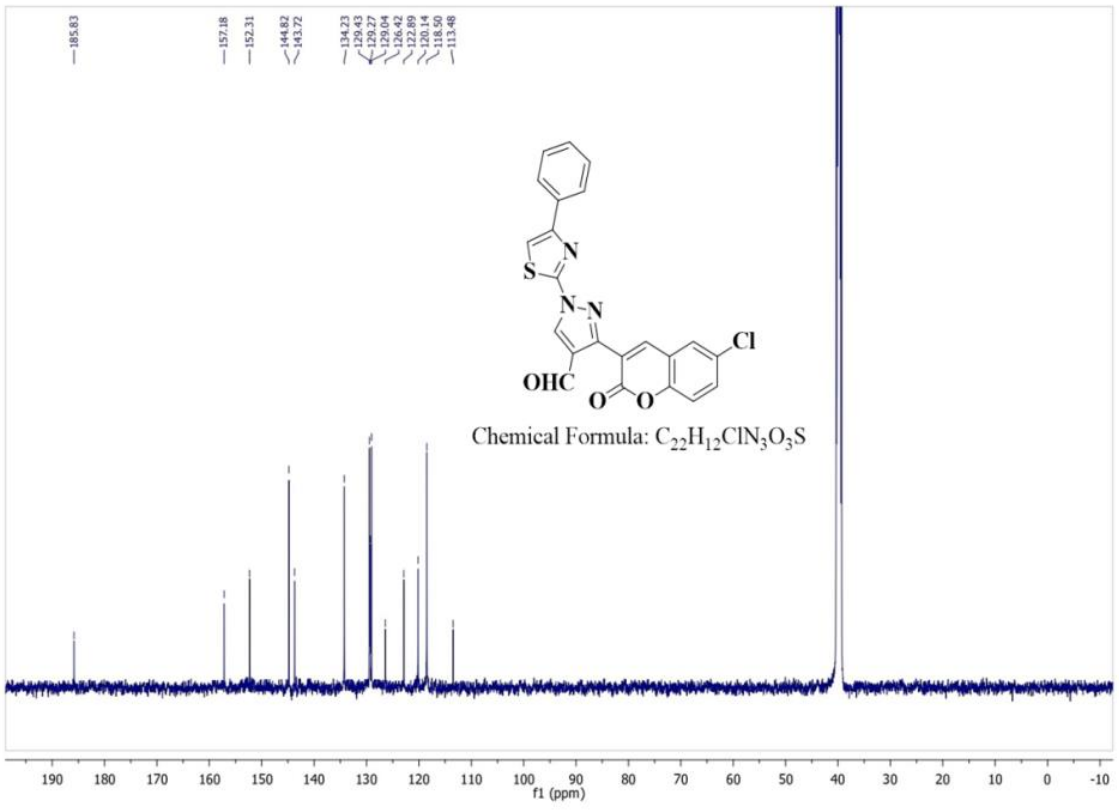
^{1}H NMR spectrum of compound **4m** (400 MHz, DMSO-d_6)



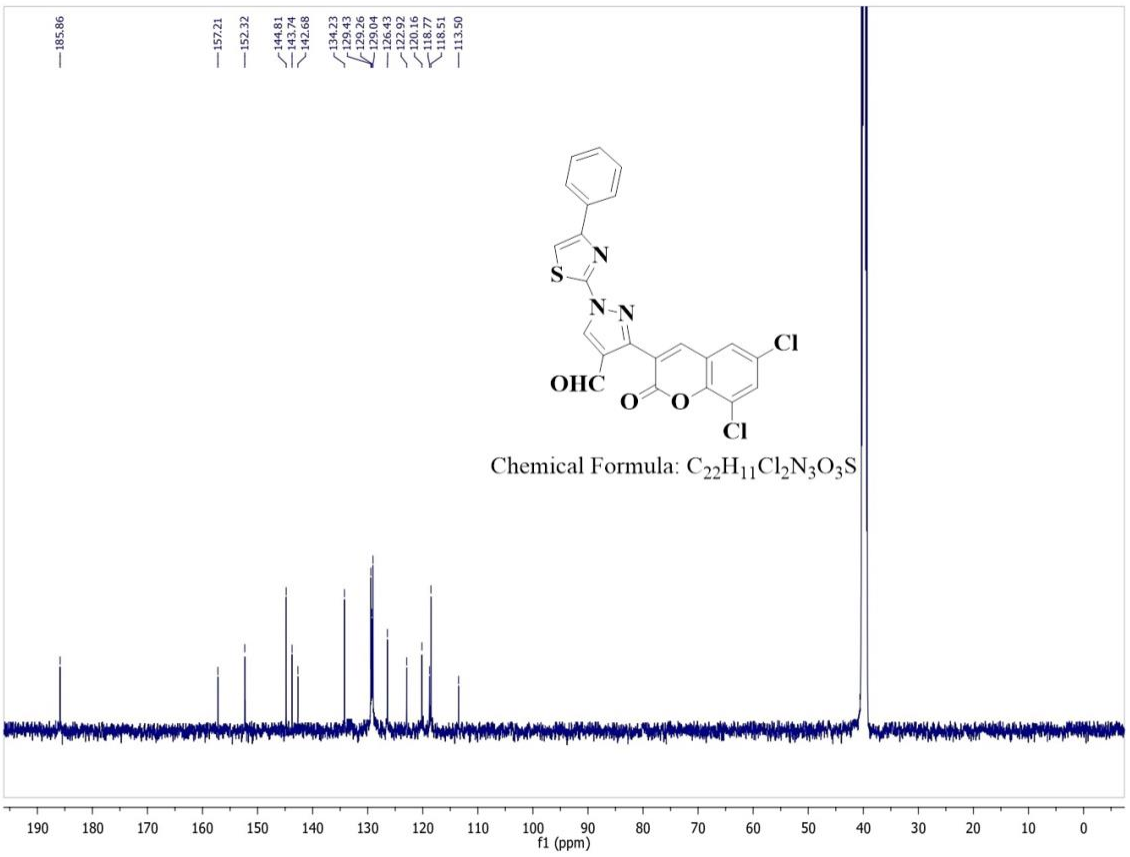
^{1}H NMR spectrum of compound **4n** (400 MHz, DMSO-d_6)



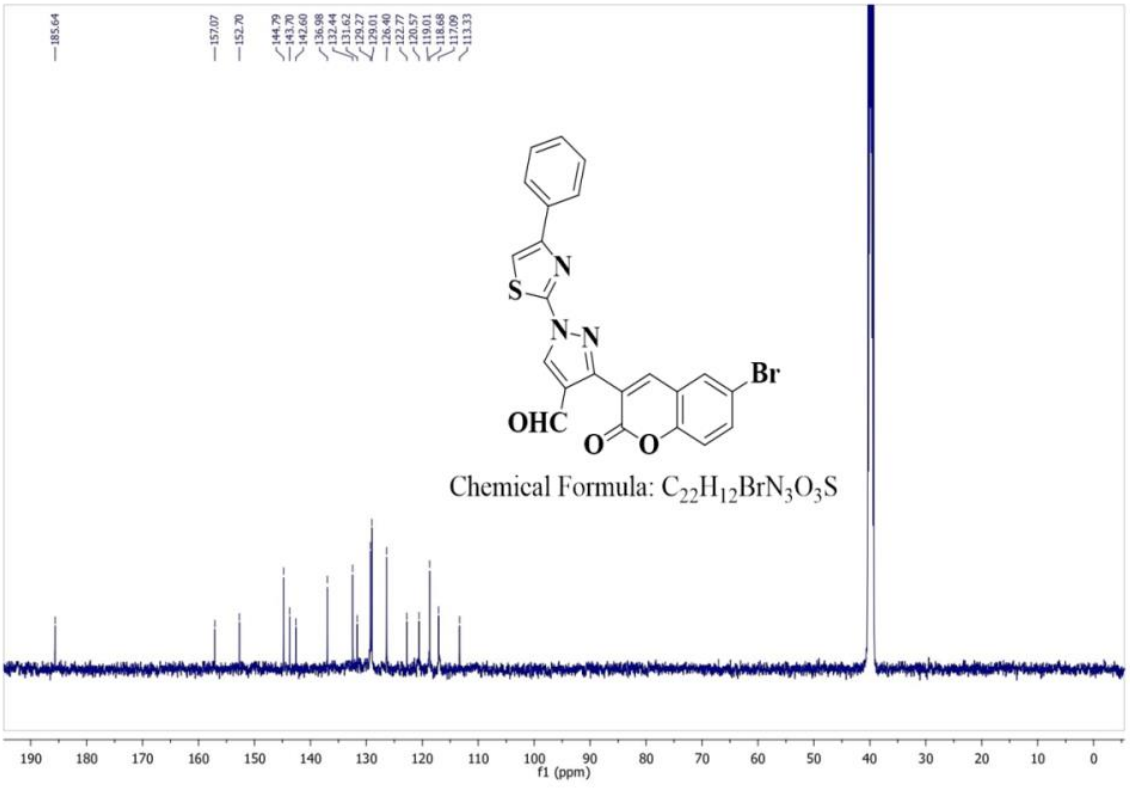
^{13}C NMR spectrum of compound **4a** (125 MHz, DMSO-d_6)



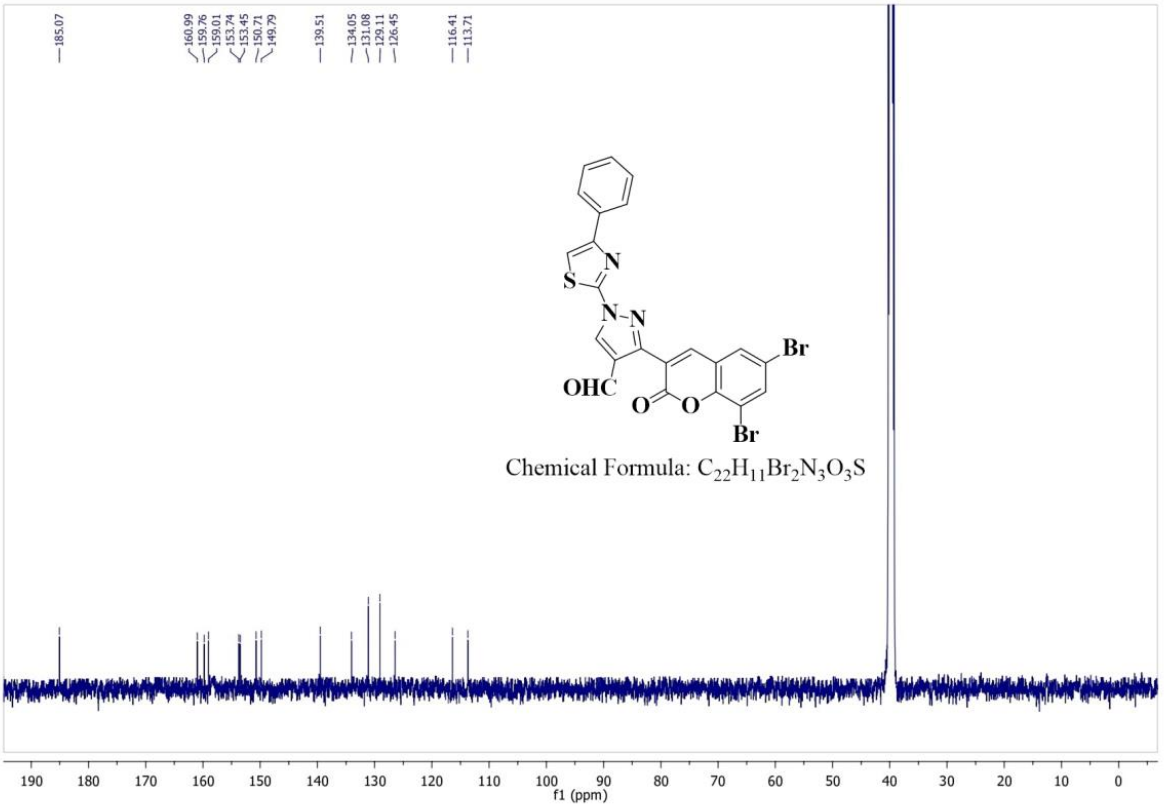
^{13}C NMR spectrum of compound **4b** (125 MHz, DMSO-d_6)



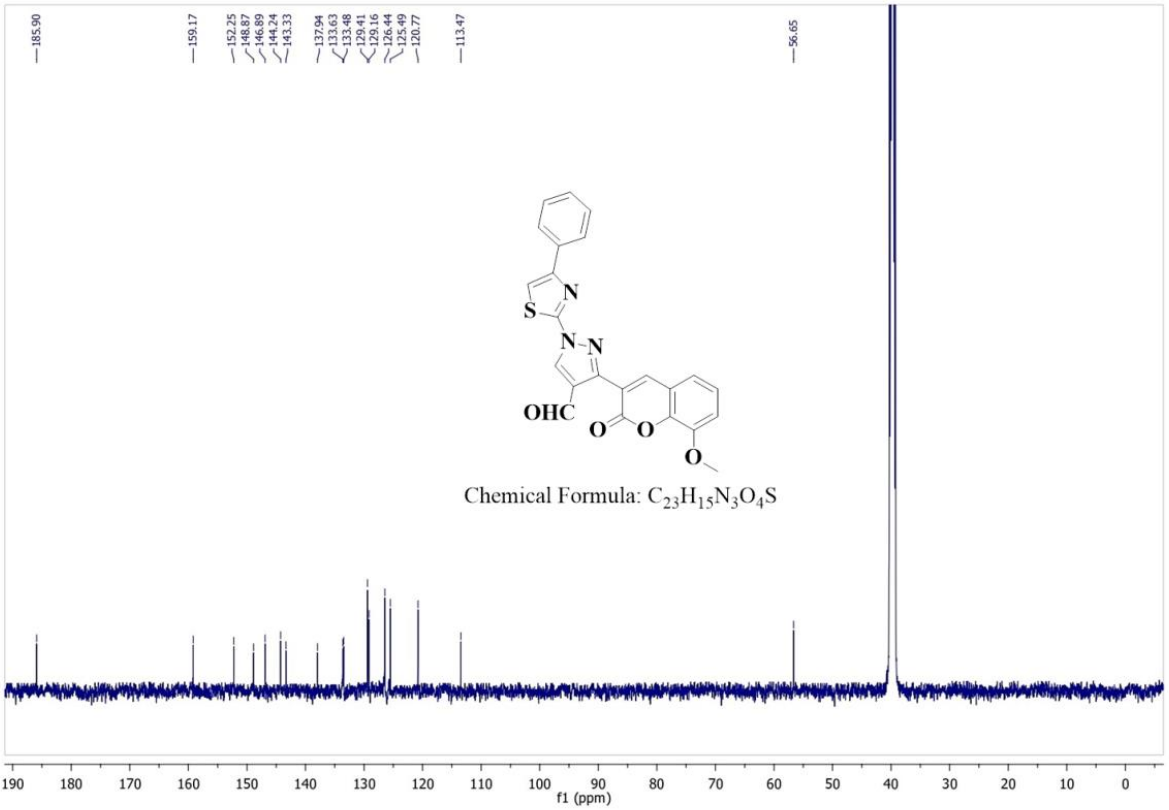
^{13}C NMR spectrum of compound **4c** (125 MHz, DMSO-d_6)



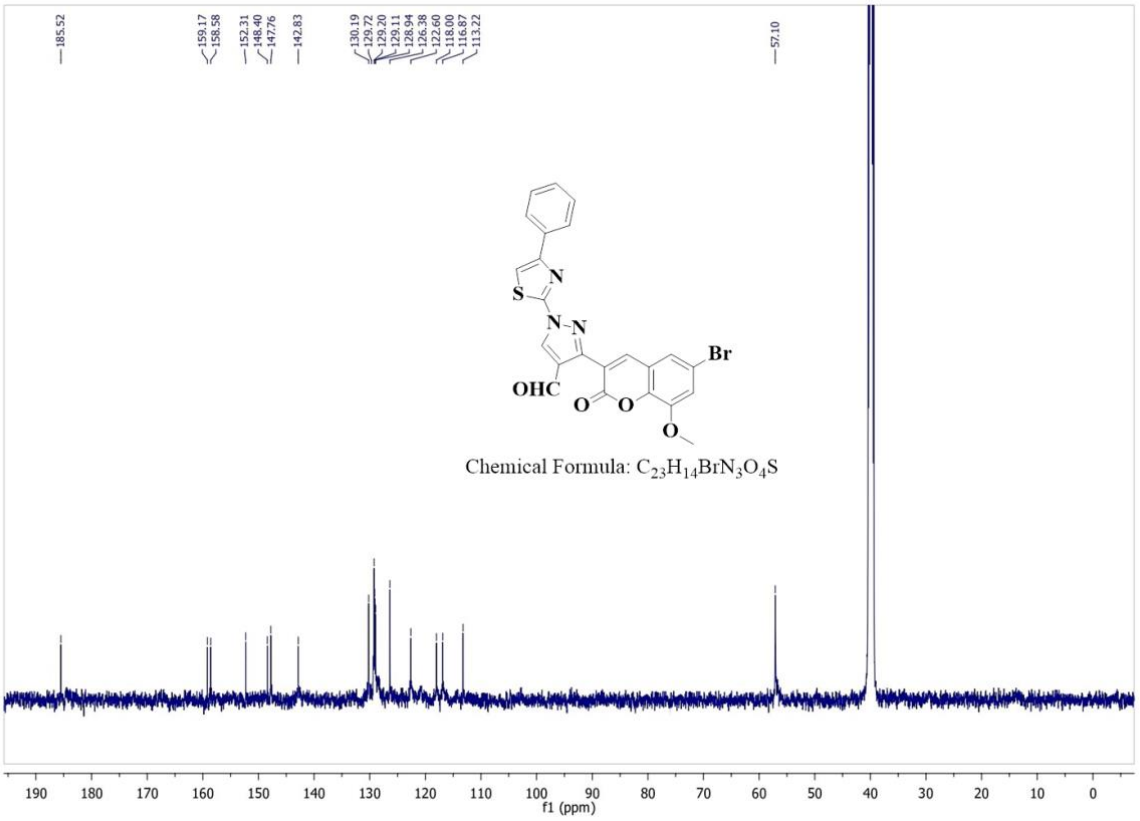
^{13}C NMR spectrum of compound **4d** (125 MHz, DMSO-d_6)



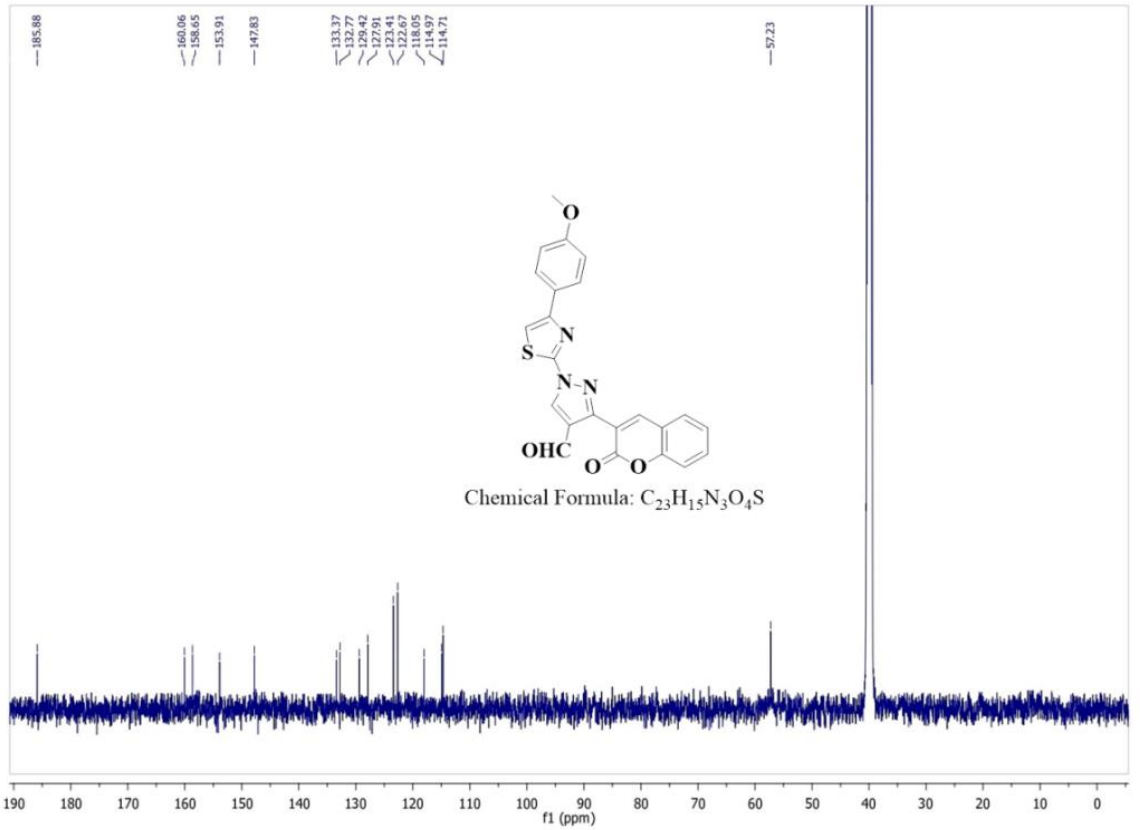
^{13}C NMR spectrum of compound **4e** (125 MHz, DMSO-d_6)



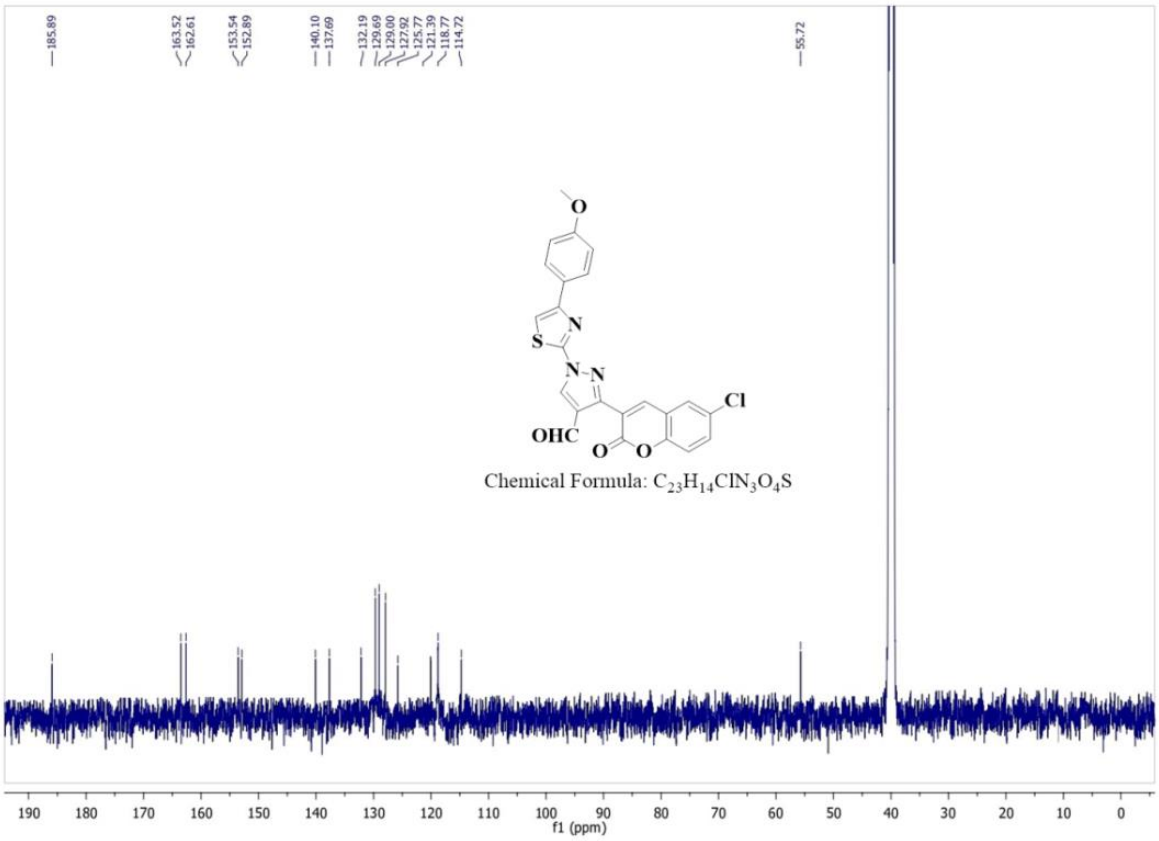
^{13}C NMR spectrum of compound **4f** (125 MHz, DMSO-d_6)



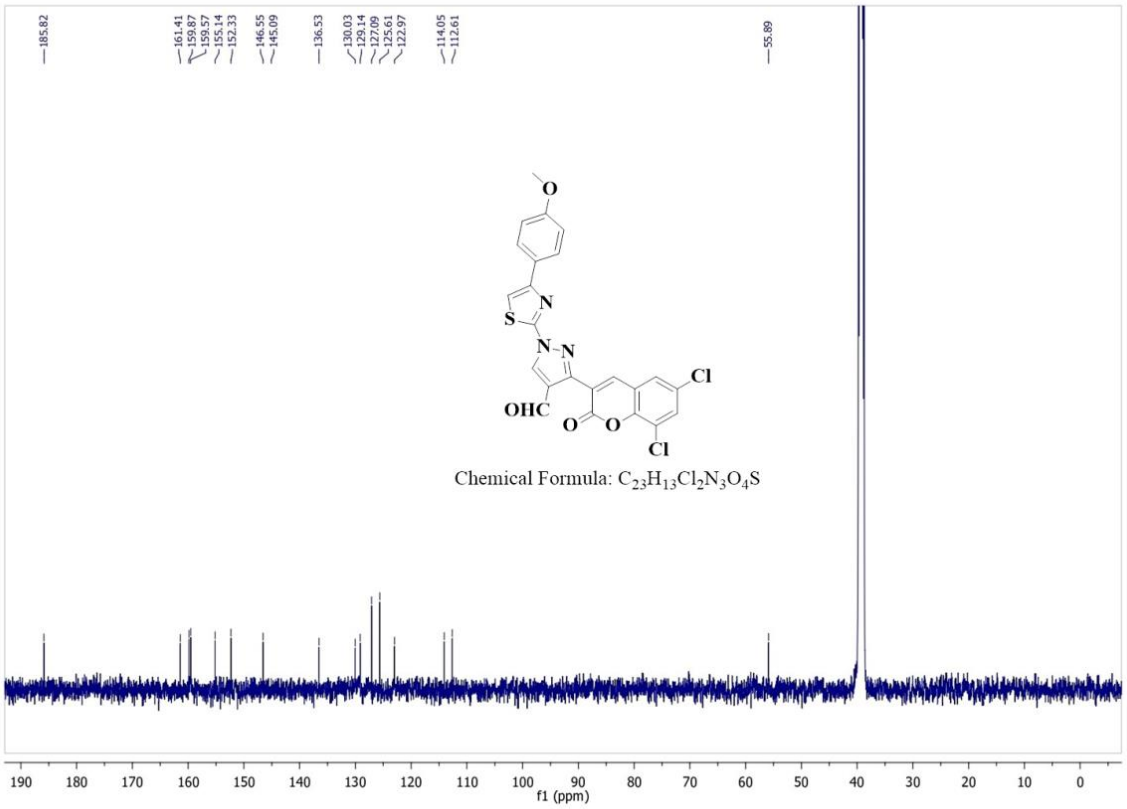
^{13}C NMR spectrum of compound **4g** (125 MHz, DMSO-d_6)



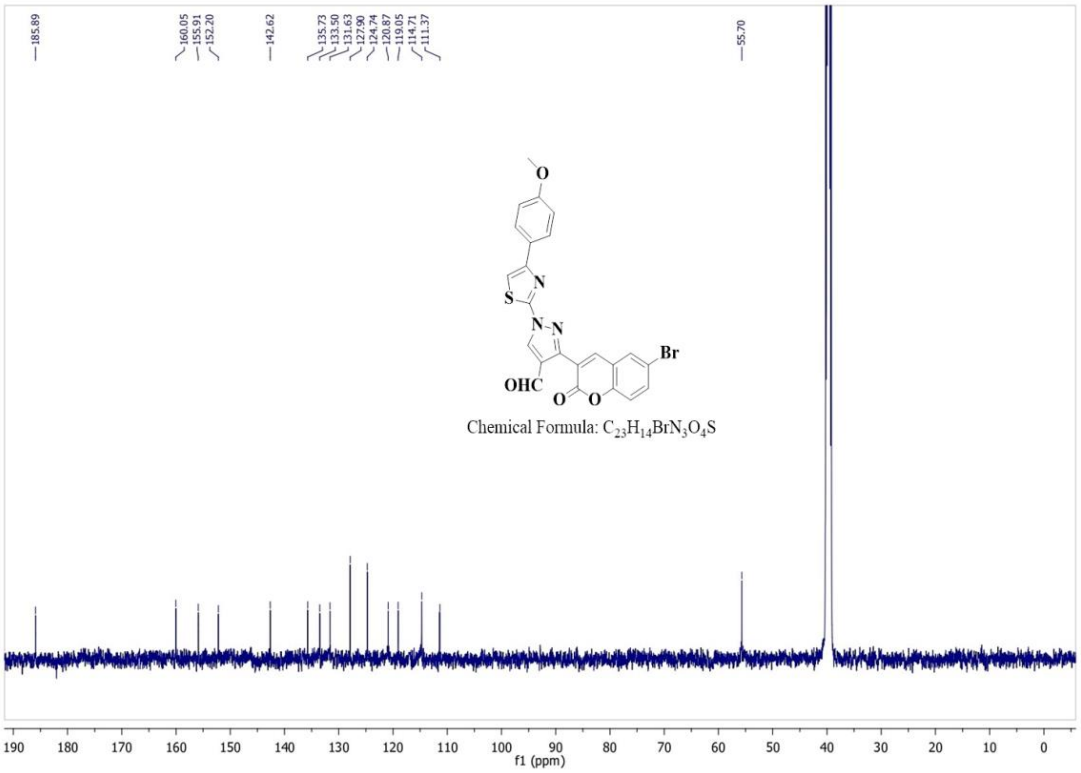
^{13}C NMR spectrum of compound **4h** (125 MHz, DMSO-d_6)



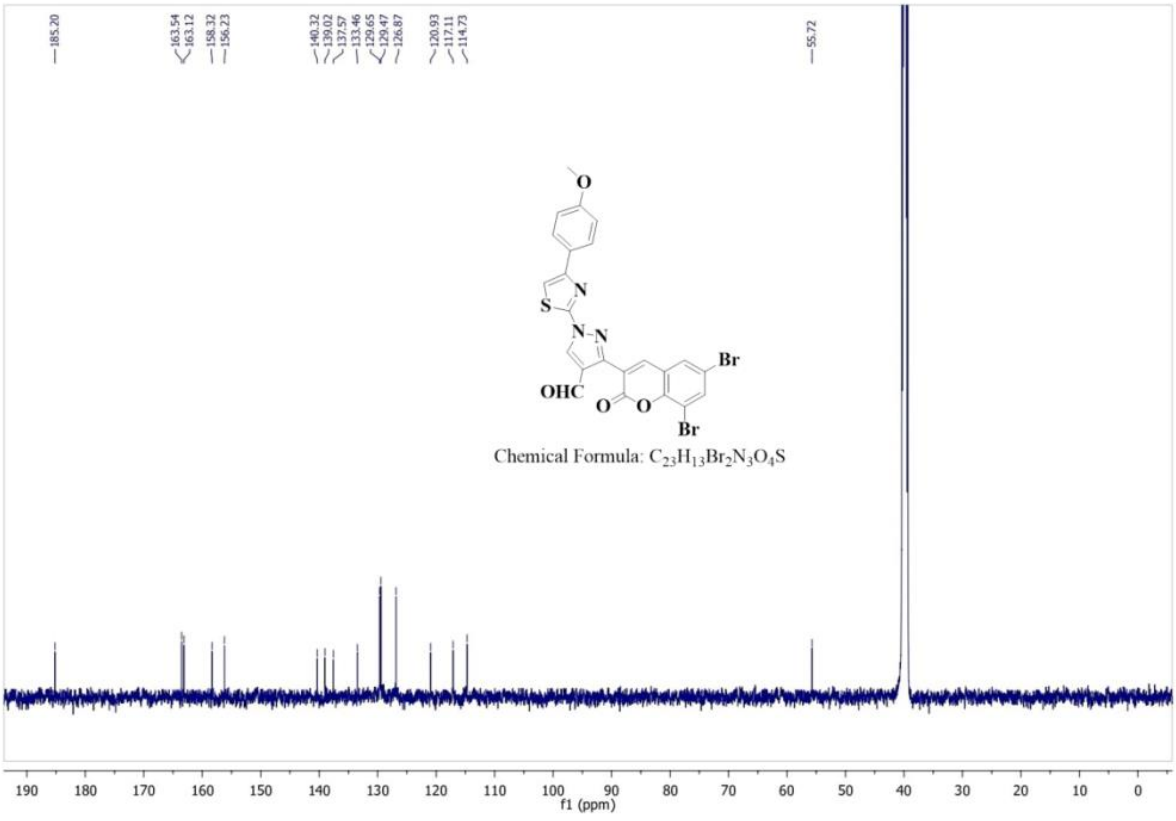
^{13}C NMR spectrum of compound **4i** (125 MHz, DMSO-d_6)



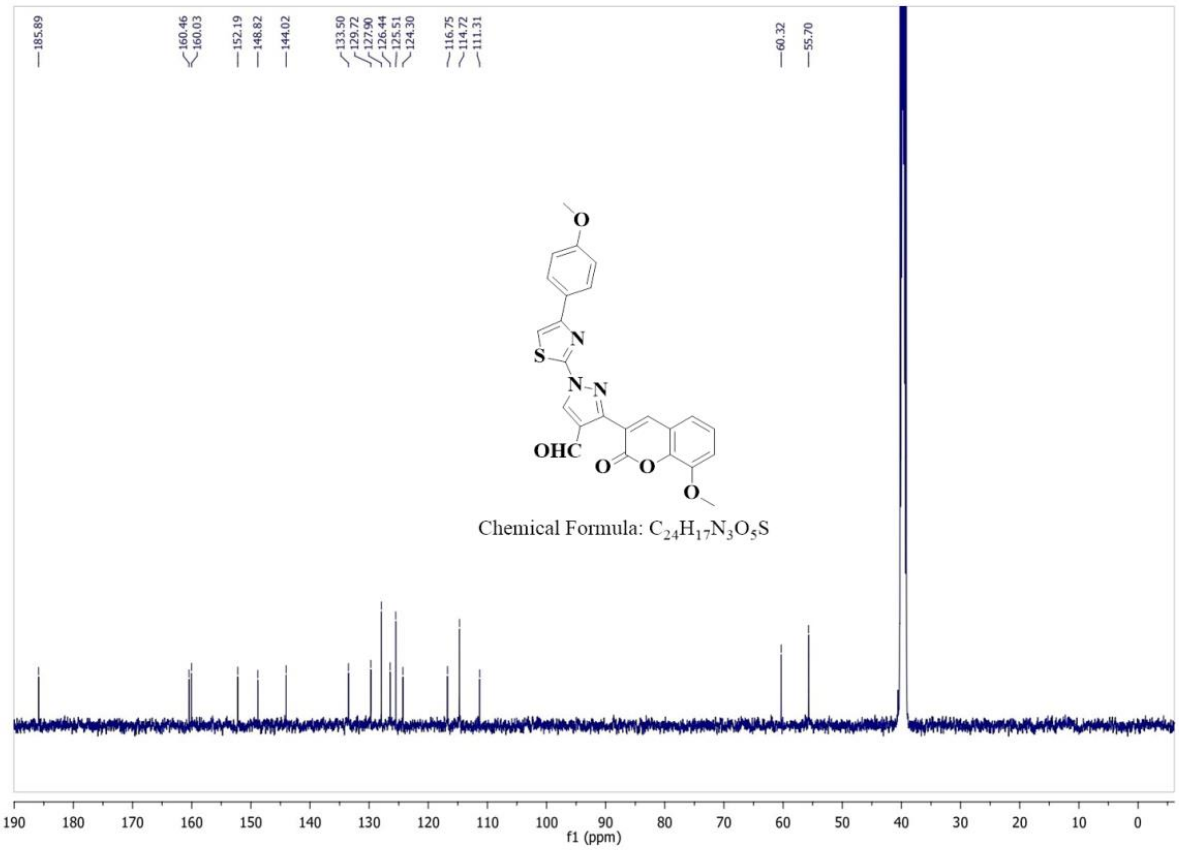
^{13}C NMR spectrum of compound **4j** (125 MHz, DMSO-d_6)



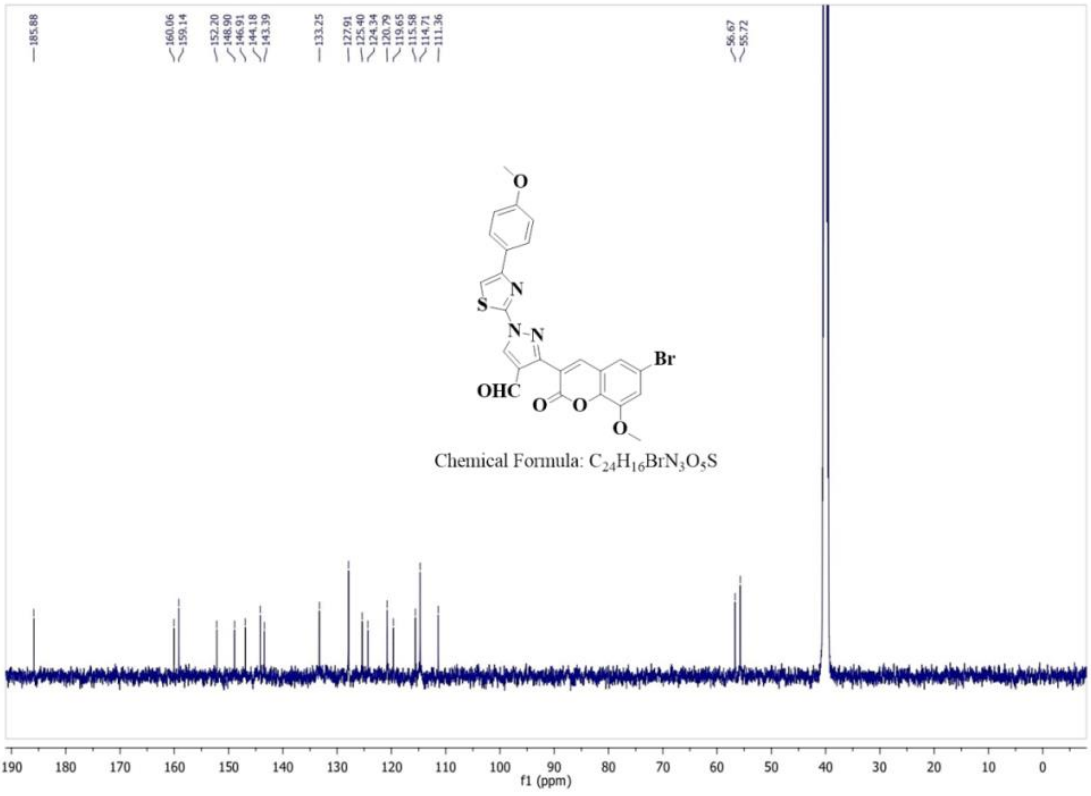
^{13}C NMR spectrum of compound **4k** (125 MHz, DMSO-d_6)



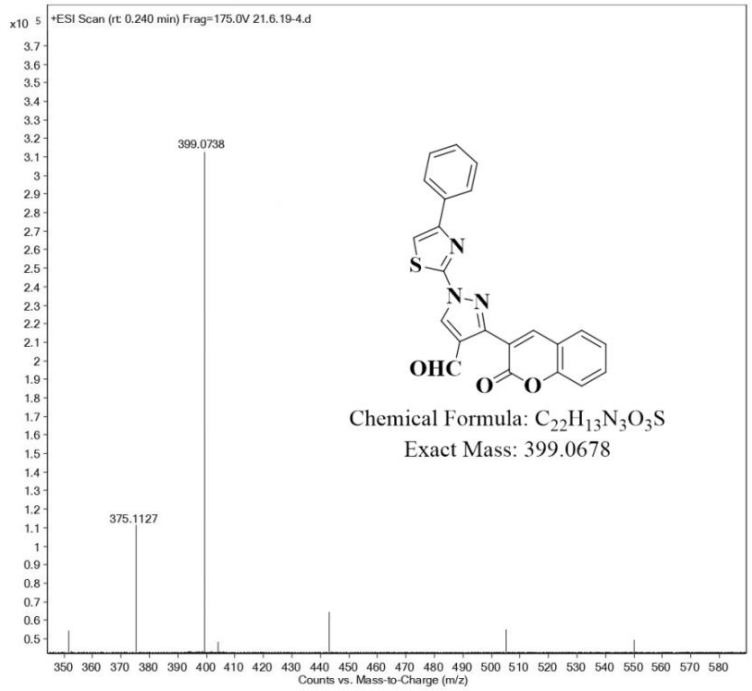
^{13}C NMR spectrum of compound **4l** (125 MHz, DMSO-d_6)



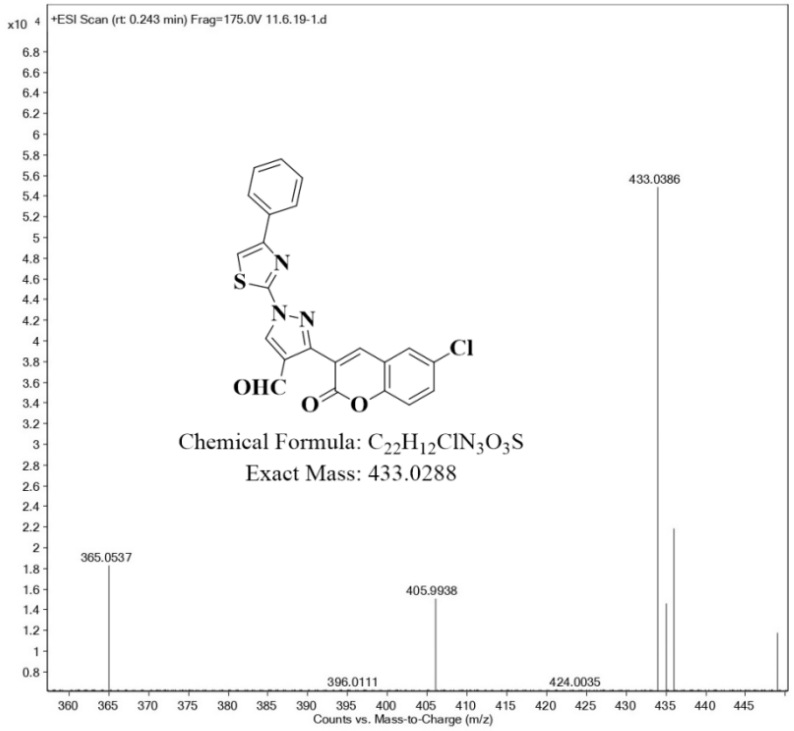
^{13}C NMR spectrum of compound **4m** (125 MHz, DMSO-d_6)



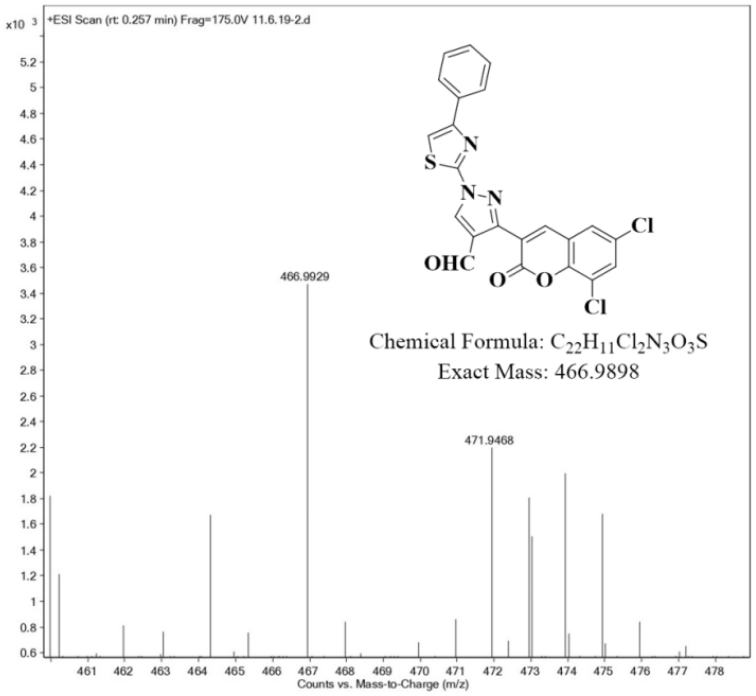
^{13}C NMR spectrum of compound **4n** (125 MHz, DMSO-d_6)



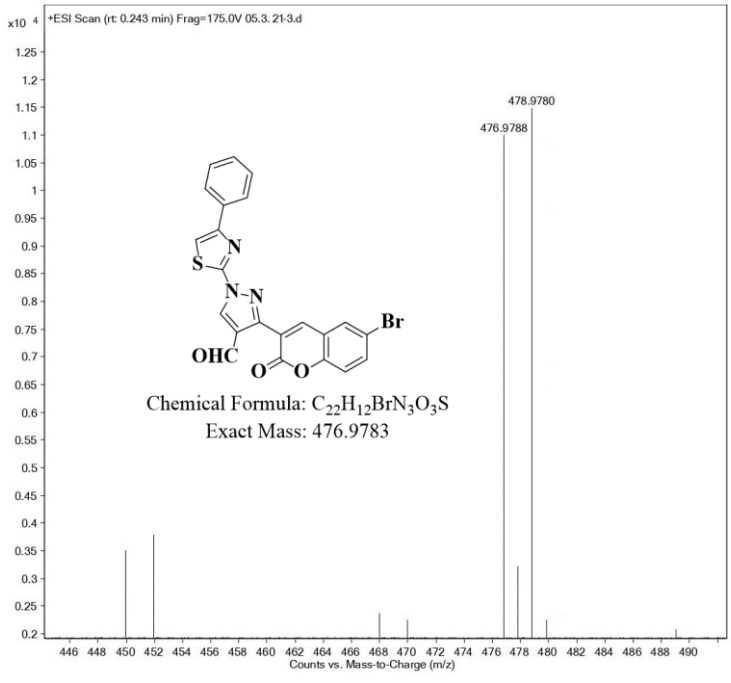
Mass spectrum of compound **4a**



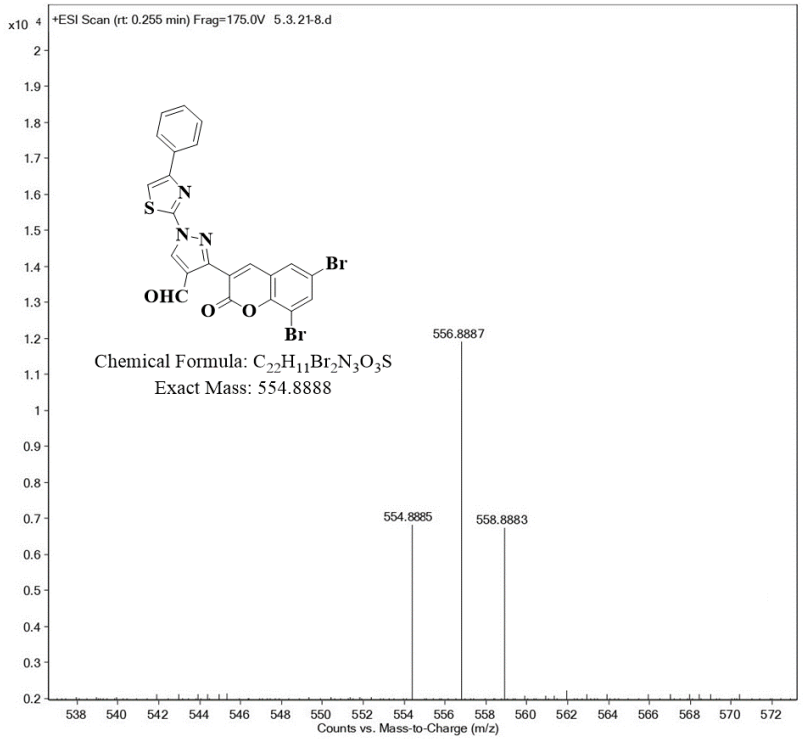
Mass spectrum of compound **4b**



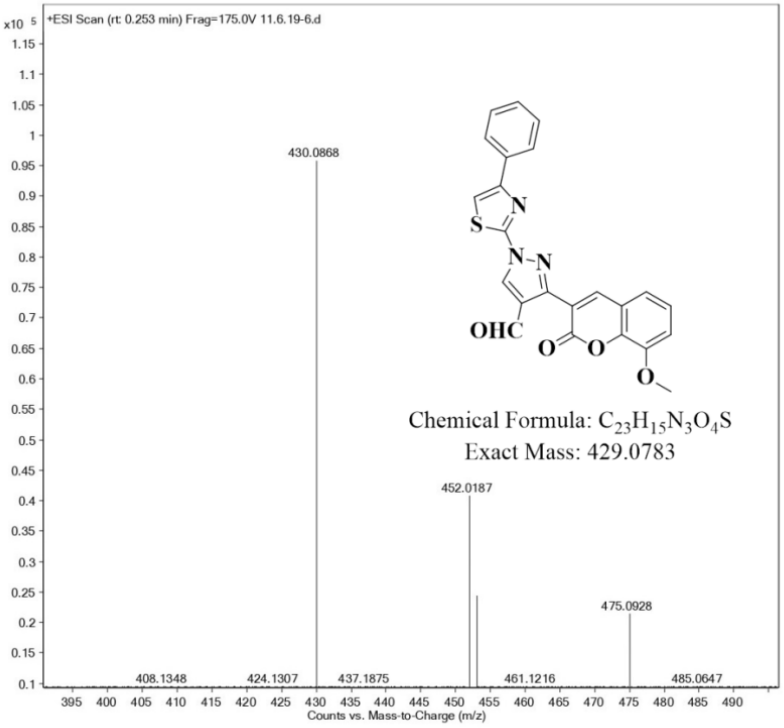
Mass spectrum of compound **4c**



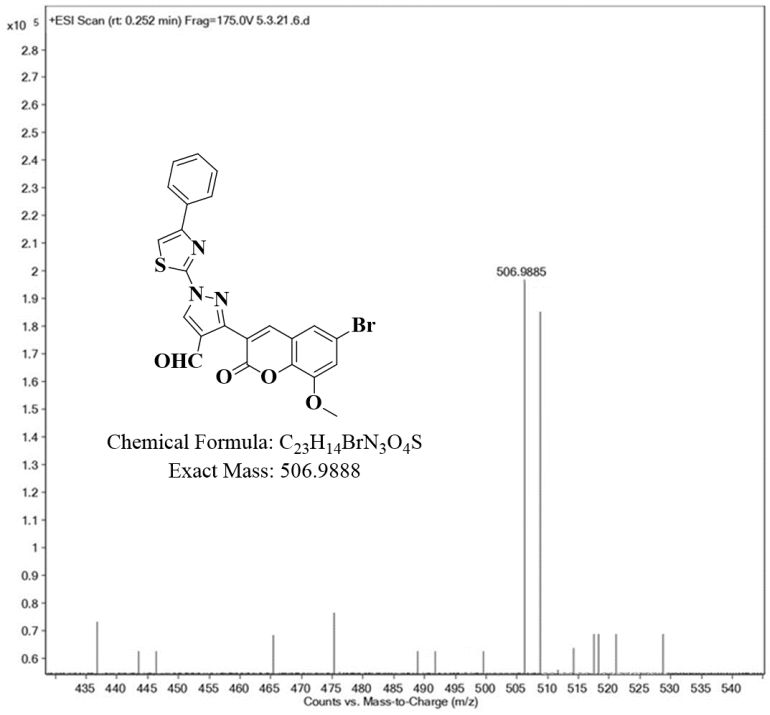
Mass spectrum of compound **4d**



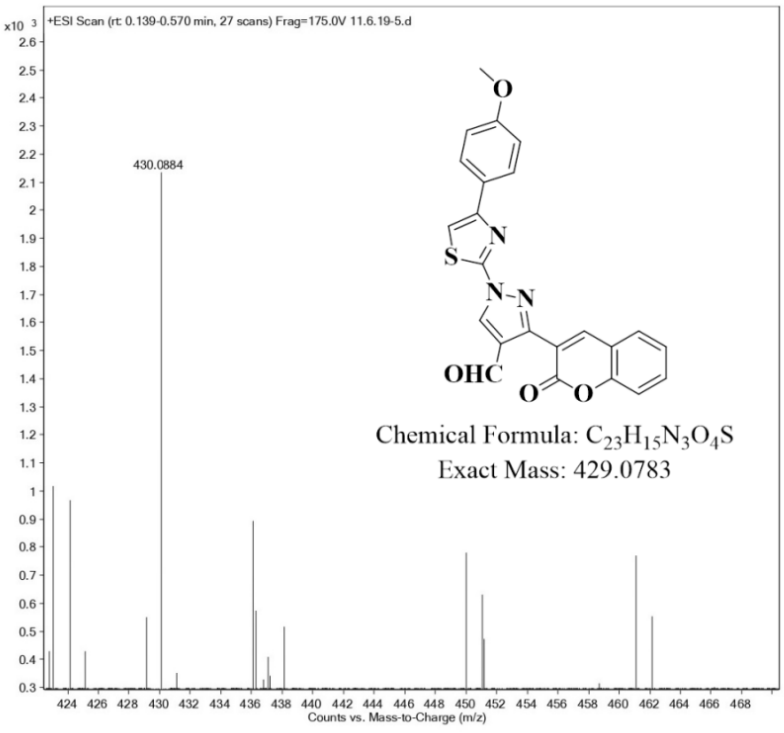
Mass spectrum of compound **4e**



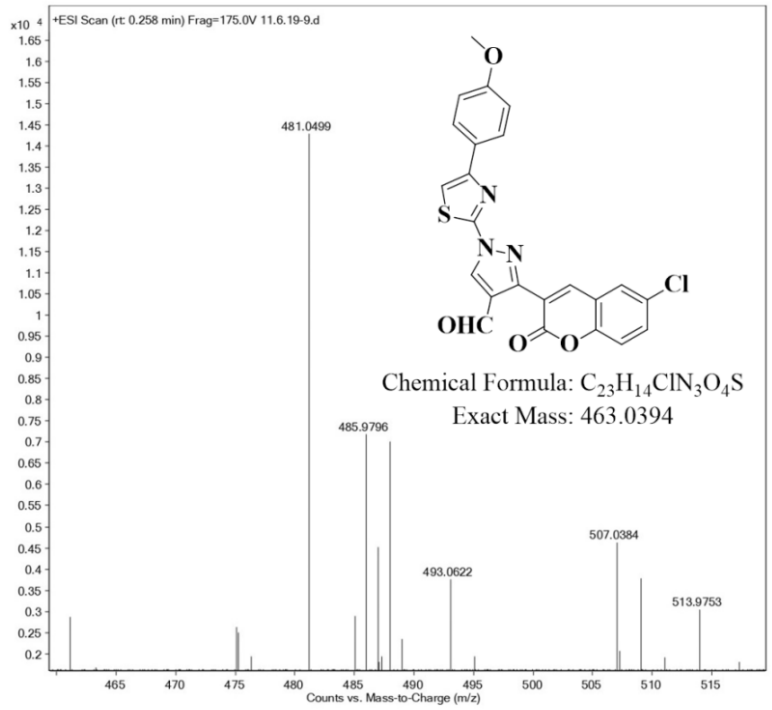
Mass spectrum of compound **4f**



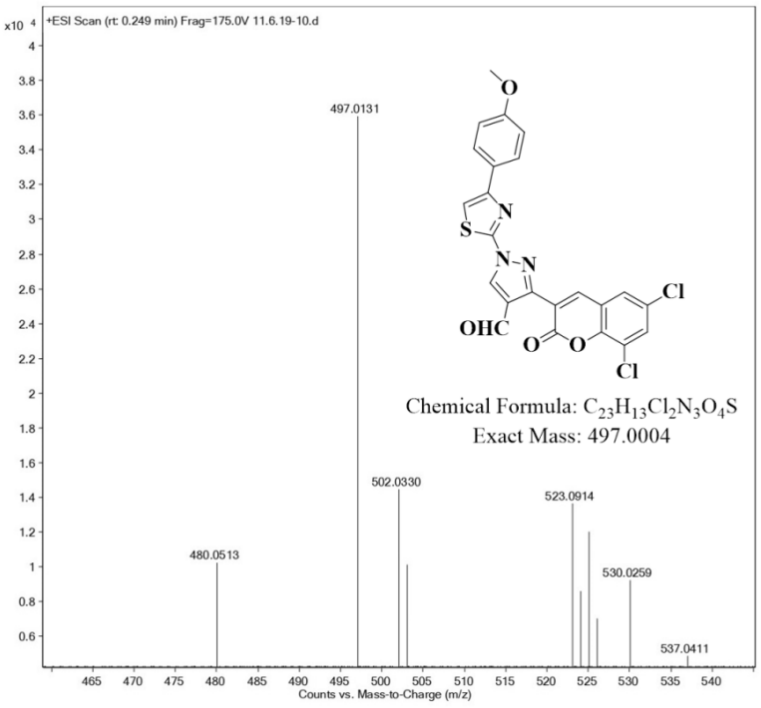
Mass spectrum of compound **4g**



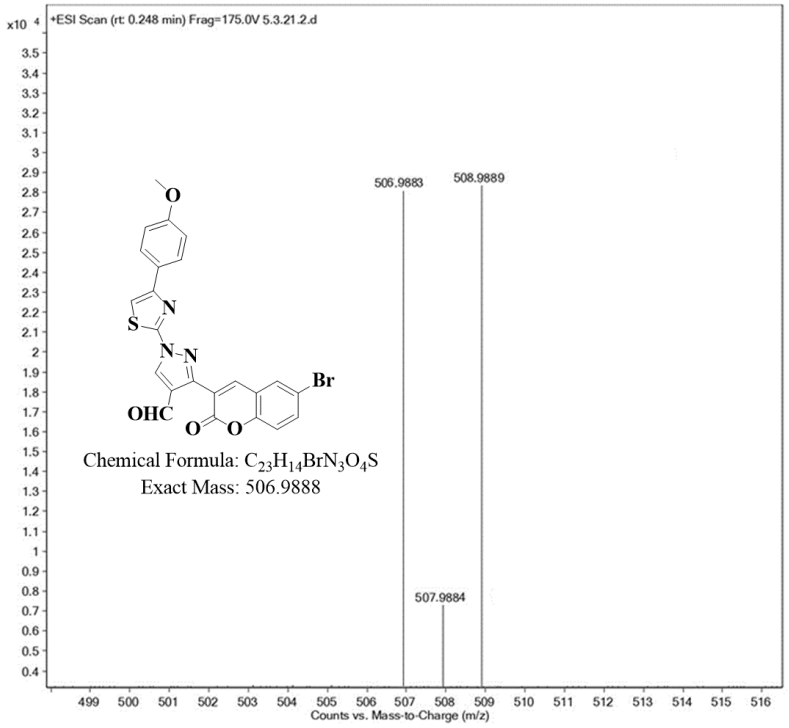
Mass spectrum of compound **4h**



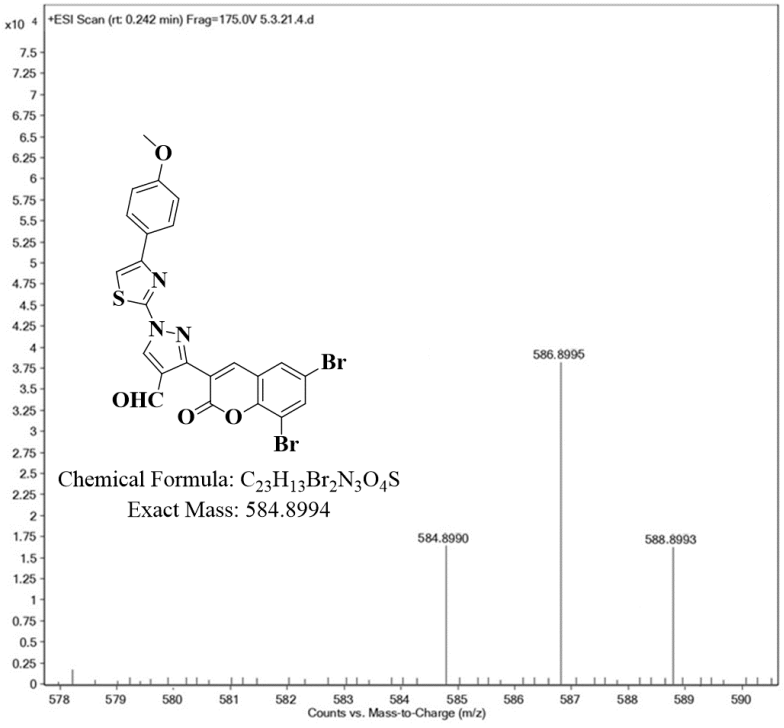
Mass spectrum of compound **4i**



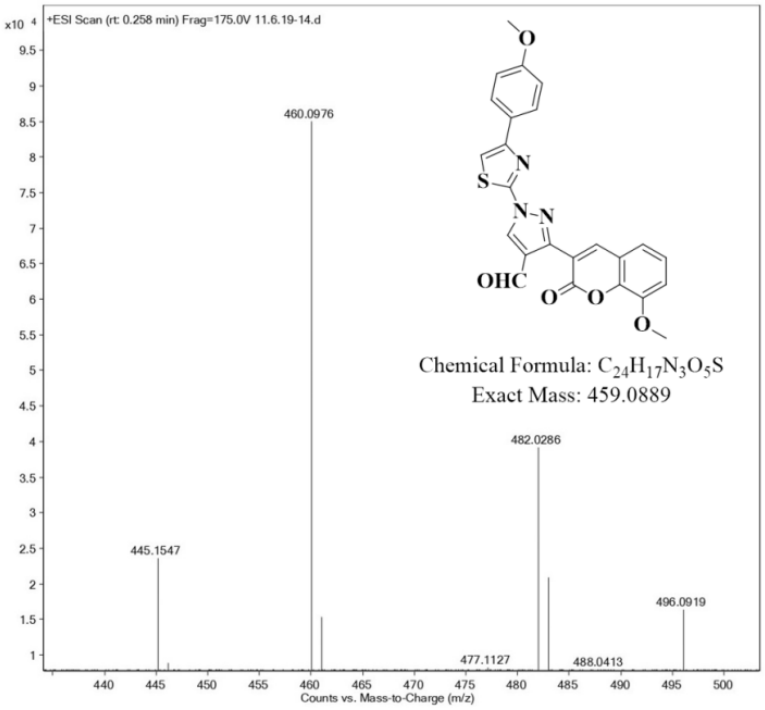
Mass spectrum of compound **4j**



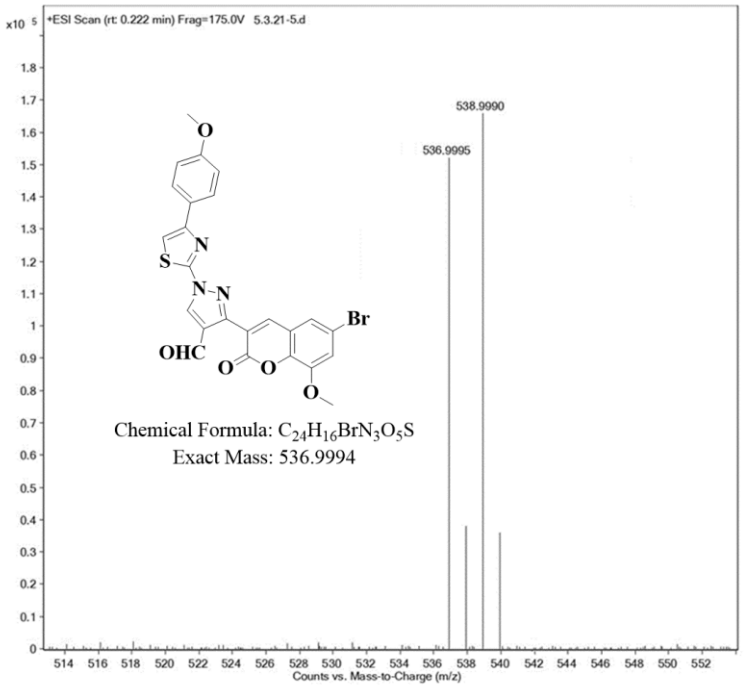
Mass spectrum of compound **4k**



Mass spectrum of compound **4l**



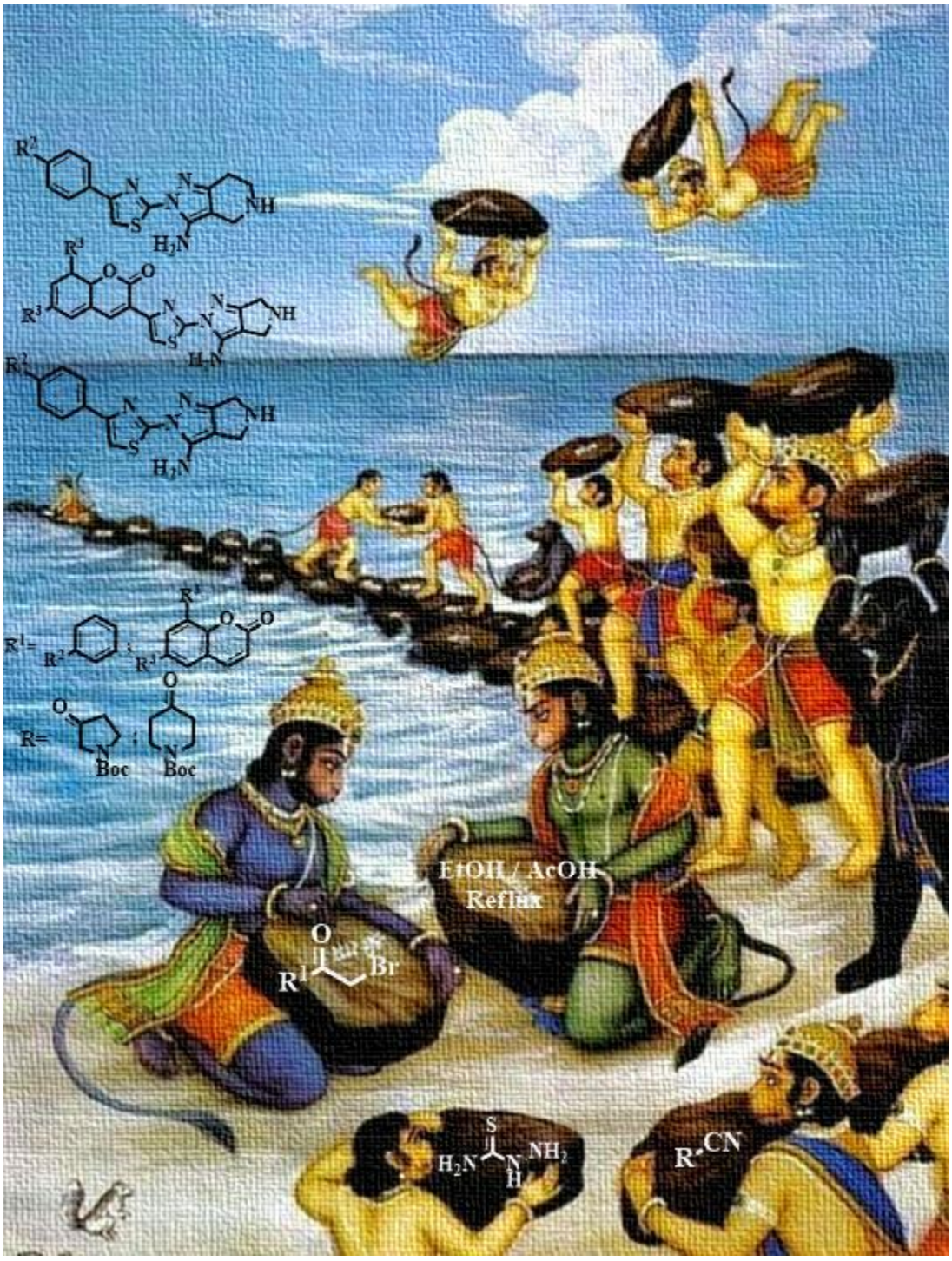
Mass spectrum of compound **4m**



Mass spectrum of compound **4n**

CHAPTER-III
SECTION-B

A facile one-pot three component synthesis of new thiazolyl pyrazoles



CHAPTER-III
SECTION-B

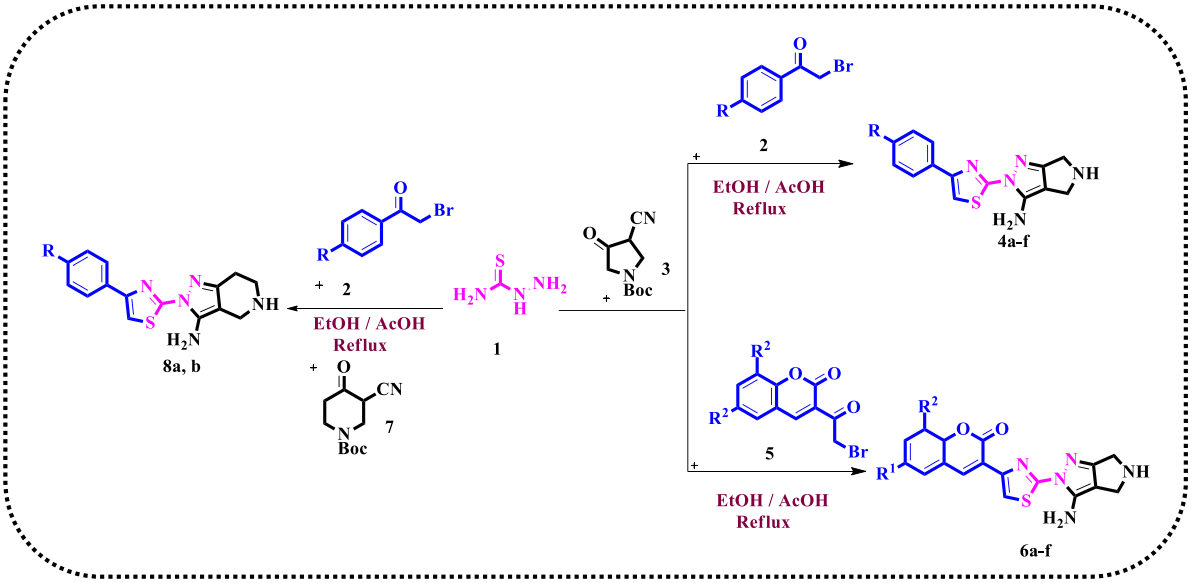
3B. Present work:

3B.1. Starting materials:

In this chapter the synthesis of 2-(4-arylthiazol-2-yl)-2,4,5,6-tetrahydropyrrolo[3,4-c]pyrazol-3-amines, 3-(2-(3-amino-5,6-dihydropyrrolo[3,4-c]pyrazol-2(4*H*)-yl)thiazol-4-yl)-2*H*-chromen-2-ones and 2-(4-(arylthiazol-2-yl)-4,5,6,7-tetrahydro-2*H*-pyrazolo[4,3-c]pyridin-3-amines *via* an efficient and facile one pot multicomponent reaction approach was described. In this regarad, substituted 3-(2-bromoacetyl) coumarins, phenacyl bromides, 1-Boc-3-cyano-4-pyrrolidone and 1-Boc-3-cyano-4-piperidone were used as the starting materials required for the synthesis of the target compounds. Except substituted 3-(2-bromoacetyl) coumarins, remaining all starting materials procured from commercial sources.

3B.2. Synthesis of thiazolyl pyrazoles:

The title compounds (**4a-f**, **6a-f** and **8a, b**) were obtained by reaction an equimolar ratio of thiosemicarbazide (**1**), substituted phenacyl bromides (**2**) or substituted 3-(2-bromoacetyl) coumarins (**5**) with 1-Boc-3-cyano-4-pyrrolidone (**3**) or 1-Boc-3-cyano-4-piperidone (**7**) and catalytic amount of acetic acid and ethanol as a solvent under reflux conditions with good yields. The general schematic representation of reaction is outlined in Scheme 3B.1

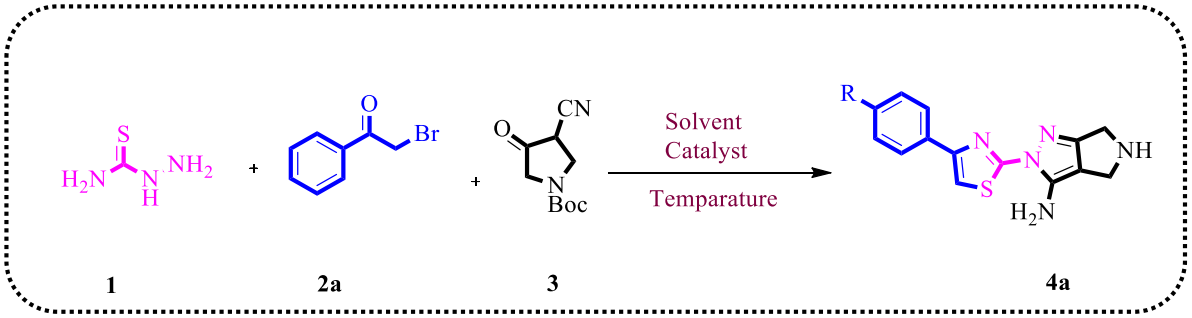


Scheme 3B.1: Synthesis of thiazolyl pyrazoles; Reagents and conditions: EtOH, AcOH, under reflux conditions.

3B.3. Results and discussion:

At first the test reaction was carried out *via* one- pot MCR approach using thiosemicarbazide (**1**),

simple phenacyl bromide (**2a**), 1-Boc-3-cyano-4-pyrrolidone (**3**) in MeOH as a solvent, then for the optimization of the reaction on screening of different solvents were used (Scheme 3B.2). Variety of solvents such as MeOH, EtOH, DMSO, DMF, CH_3CN (Table 3B.2, entries 1–5) were used, concluded that EtOH (Table 3B.2, entry 2) was best out of the screened solvents in terms of yield. Nonetheless, for the improvisation of yield it has been continued (Table 3B.2, entries 6–10). From the screening it has been observed that EtOH (Table 3B.2, entry 7) was best out of the tested solvents in terms of yield and time. Moreover, for the optimization of reaction, we tested various catalysts such as CH_3COOH, HCl and H_2SO_4 as 5 mol% (Table 3B.2, entries 11–13) were used, concluded that CH_3COOH was best among the tested catalysts in terms of yield. Subsequently, we screened catalyst load to know the best optimizing conditions (Table 3B.2, entries 11, 14, 15, 16, 17), from this we concluded that 30 mol% CH_3COOH was the best between different catalyst loads (Table 3B.2, entry 16). Simultaneously for the improvisation of the reaction we screened based on temperature (Table 3B.2, entries 16, 18), end of the optimization 30 mol% of CH_3COOH as a catalyst, ethanol as a solvent under reflux conditions (Table 3B.2, entry 18) has manifested best results in terms of yield and time.



Scheme 3B.2: Test reaction for the Synthesis of thiazolyl pyrazoles.

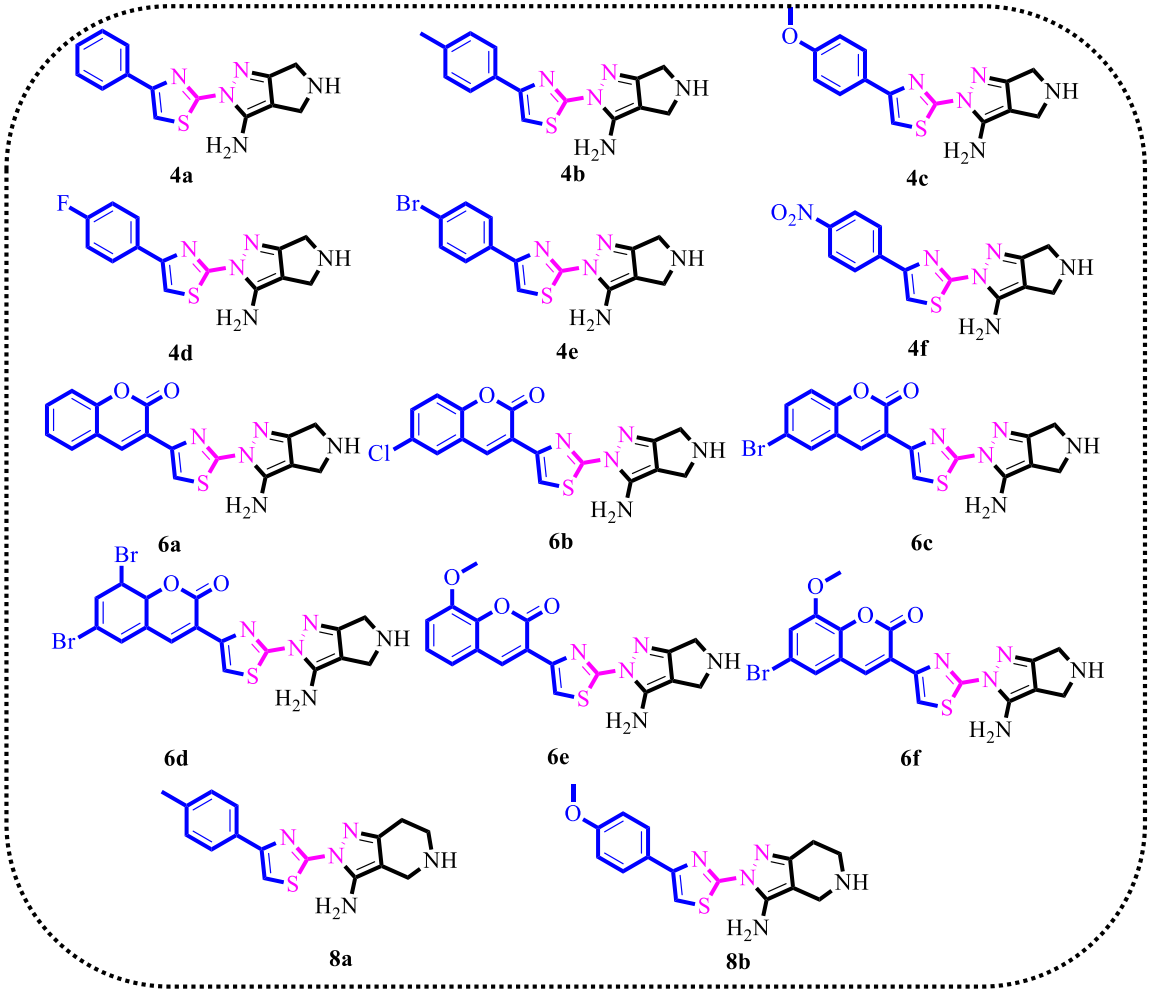


Figure 3B.1 thiazolyl pyrazole hybrids (**4a-f, 6a-f** and **8a-b**).

Table 3B.1 Different substituted thiazolyl pyrazole hybrids (**4a-f, 6a-f** and **8a-b**), time, [a]isolated yield.

Entry	Product	R	R^1	R^2	Time(h)	Yield (%)[a]
1	**4a**	H	-	-	5	83
2	**4b**	CH_3	-	-	5	84
3	**13c**	OCH_3	-	-	6	88
4	**4d**	F	-	-	5	84
5	**4e**	Br	-	-	6	87
6	**4f**	NO_2	-	-	6	88
7	**6a**	-	H	H	5	91
8	**6b**	-	Cl	H	6	89
9	**6c**	-	Br	H	5	90
10	**6d**	-	Br	Br	5	91
11	**6e**	-	H	OCH_3	6	89
12	**6f**	-	Br	OCH_3	5	92
13	**8a**	CH_3	-	-	6	87
14	**8b**	OCH_3	-	-	5	85

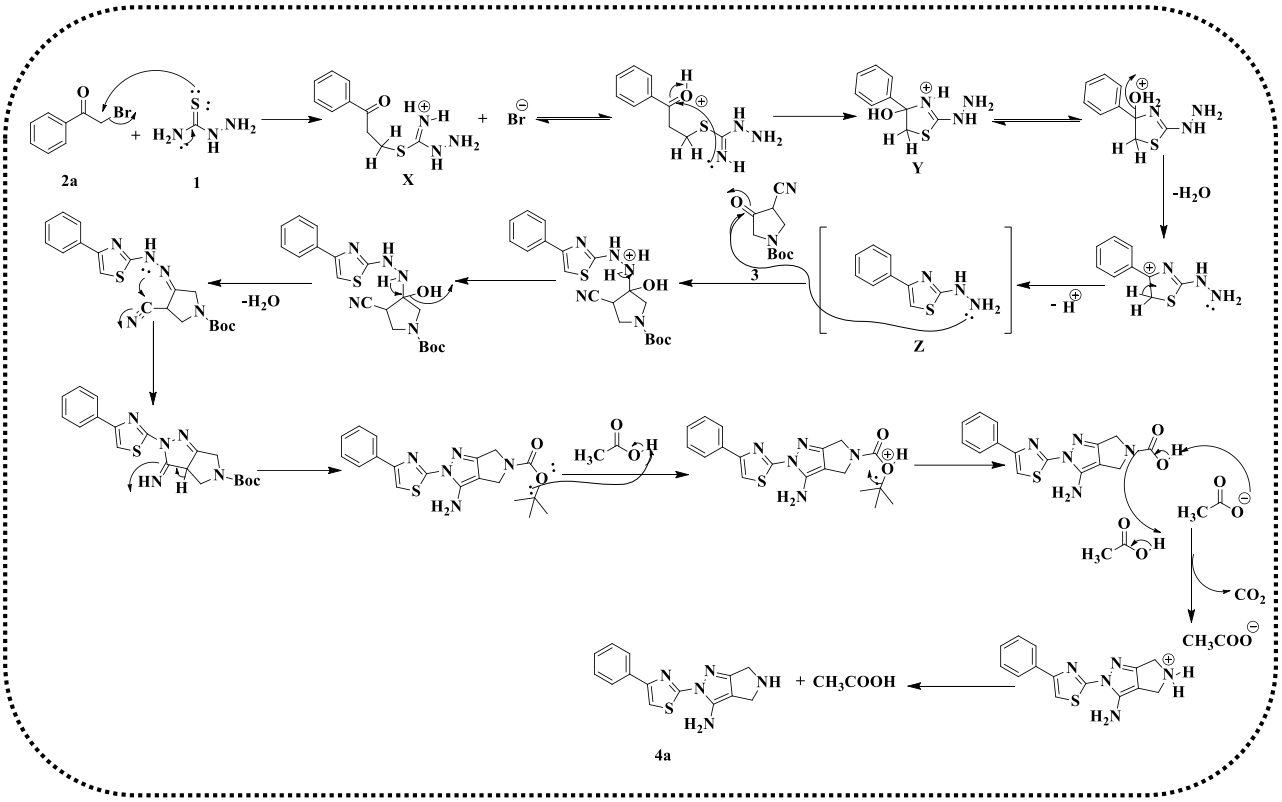
Table 3B.2 Optimizing the reactions conditions for the synthesis of thiazolyl pyrazoles.

S. No.	Solvent	Catalyst (mol%)	Temperature (ºC)	Time (h)	Yield (%) [a]
1	MeOH	-	60	9	19
2	EtOH	-	60	9	26
3	DMSO	-	60	9	18
4	DMF	-	60	9	17
5	CH_3CN	-	60	9	15
6	MeOH	-	60	13	25
7	EtOH	-	60	12	31
8	DMSO	-	60	15	28
9	DMF	-	60	14	26
10	CH_3CN	-	60	16	27
11	EtOH	CH_3COOH(5)	60	6	49
12	EtOH	HCl(5)	60	6	38
13	EtOH	H_2SO_4(5)	60	6	34
14	EtOH	CH_3COOH(10)	60	6	72
15	EtOH	CH_3COOH(20)	60	6	58
16	EtOH	CH_3COOH(30)	60	6	67
17	EtOH	CH_3COOH(40)	60	6	64
18	**EtOH**	**CH_3COOH(30)**	**reflux**	**5**	**83**

[a] Isolated yields

From the below mechanism, it is clear that the substitution of the alpha bromine atom of the phenacyl bromide by the Sulphur atom of the thiosemicarbazide (thioamide part) to give an open chain alpha thio ketone (**X**) which under trans protonation proceeds to yield 4-hydroxy-Δ^2-thiazoline (**Y**) in a protic solvent or a thiazole (**Z**) by acid catalyzed dehydration of the intermediate thiazoline in protic solvents.

The tertiary butyl carbamate becomes protonated. Loss of the tertiary butyl cation results on a carbamic acid. Decarboxylation of the carbamic acid results in the free secondary amine. Protonation of the secondary amine under acidic condition provide the heterocyclic ring as shown below in the salt and further it gives final compound **4a**.



Scheme 3B.3: A plausible mechanism for the synthesis of **4a** *via* one-pot three component reaction.

The synthesized compound structures (**4a-f**, **6a-f** and **8a-b**) were established by their analytical data. The IR of synthesized compounds (**4a-f**, **6a-f** and **8a-b**) revealed the presence of imine (C=N), amino (-NH_2) functional groups in the region of 1619–1602 and 3432–3329 cm^{-1} respectively. In the ^{1}H-NMR spectrum for the **4(a-f)** compounds showed characteristic peaks for the NH and NH_2 protons in the range of δ 9.52- 9.79 and 6.86- 7.96 ppm respectively. Furthermore, for the **6a-f** and **8a-b** compounds showed characteristic peaks for the NH and NH_2 protons in the range of δ 6.95- 7.33 and 9.07- 9.63 ppm respectively. Moreover, these compounds have exhibited characteristic thiazole ring proton in the range of δ 7.20- 9.01 ppm. In addition, in ^{13}C-NMR spectra they have shown peaks from the range of 158.71- 166.27 ppm corresponding to the thiazole C_2 carbon. Mass spectra of all these synthesized compounds have exhibited molecular ion (M+) peak indicating their chemical formula, which their molecular structure.

3B.4. Conclusion:

In summary, we have developed a potential green protocol for the synthesis of new thiazolyl pyrazole derivatives by the multi component reaction approach.

3B.5. Experimental:

General procedure for the synthesis of **2-(4-phenylthiazol-2-yl)-2,4,5,6-tetrahydropyrrolo[3,4-c]pyrazol-3-amine**

A mixture of thiosemicarbazide (1 m mol), substituted phenacyl bromides (1 m mol), 1-Boc-3-cyano-4-pyrrolidone (1 m mol) and catalytic amount of acetic acid, ethanol was placed in a round bottom flask and refluxed for about 5-6 h. The progress of the reaction was monitored by thin layer chromatography. After the completion of the reaction, the reaction mixture was cooled to room temperature and the products were filtered and isolated. Further, these produced were washed with ethanol, dried and recrystallized from ethanol.

General procedure for the synthesis of **3-(2-(3-amino-5,6-dihydropyrrolo[3,4-c]pyrazol-2(4*H*)-yl)thiazol-4-yl)-2*H*-chromen-2-one**

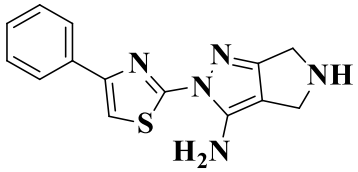
A mixture of thiosemicarbazide (1 m mol), substituted 3-(2-bromoacetyl) coumarins (1 m mol), 1-Boc-3-cyano-4-pyrrolidone (1 m mol) and catalytic amount of acetic acid and ethanol was placed in a round bottom flask and refluxed for about 5-6 h. The progress of the reaction was monitored by thin layer chromatography. After completion of the reaction, the reaction mixture was cooled to room temperature and the product was filtered and isolated, washed with ethanol, dried and recrystallized from ethanol.

General procedure for the synthesis of **2-(4-phenylthiazol-2-yl)-4,5,6,7-tetrahydro-2*H*-pyrazolo[4,3-c]pyridin-3-amine**

A mixture of thiosemicarbazide (1 m mol), substituted phenacyl bromides (1 m mol), 1-Boc-3-cyano-4-piperidone (1 m mol) and catalytic amount of acetic acid and ethanol was placed in a round bottom flask and refluxed for about 5-6 h. The progress of the reaction was monitored by thin layer chromatography. After the completion of the reaction, the reaction mixture was cooled to room temperature and the product was filtered and isolated, washed with ethanol, dried and recrystallized from ethanol.

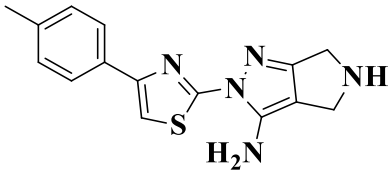
3B.6. Spectral data:

2-(4-Phenylthiazol-2-yl)-2,4,5,6-tetrahydropyrrolo[3,4-c]pyrazol-3-amine (4a):



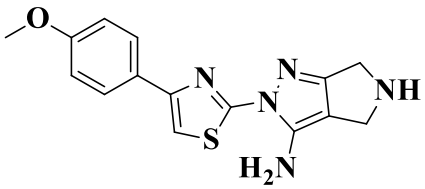
Orange solid; yield 83%; mp: >300ºC; IR (KBr) cm^{-1}: 1612 (C=N), 3410 (NH_2); ^1H NMR (400MHz, $CDCl_3$ + DMSO-d_6, ppm): δ 4.06 (s, 2H, CH_2), 4.19 (s, 2H, CH_2), 7.36 (s, 2H, -NH_2), 7.45 (t, J = 6.4 Hz, 3H, Ar-H), 7.77 (s, 1H, Ar-H), 7.97 (d, J = 8 Hz, 2H, Ar-H), 9.52 (s, 1H, NH). ^{13}C NMR (125 MHz, $CDCl_3$ + DMSO-d_6) δ 28.56, 43.76, 87.10, 109.50, 126.27, 128.78, 129.28, 133.73, 151.45, 152.98, 156.45, 159.48 ppm; Mass (ESI-HRMS) (*m/z*): 284.0961 $[M+H]^+$; Anal. Calcd. For $C_{14}H_{13}N_5S$: C, 59.34; H, 4.62; N, 24.72%. Found: C, 59.30; H, 4.66; N, 24.75%.

2-(4-(P-tolyl)thiazol-2-yl)-2,4,5,6-tetrahydropyrrolo[3,4-c]pyrazol-3-amine (4b):



Brown solid; yield 84%; mp: >300ºC; IR (KBr) cm^{-1}: 1614 (C=N), 3412 (NH_2); ^1H NMR (400MHz, $CDCl_3$ + DMSO-d_6, ppm): δ 2.37 (s, 3H, CH_3), 4.24 (s, 2H, CH_2), 4.28 (s, 2H, CH_2), 7.12 (s, 2H, -NH2) 7.20 (s, 1H, Ar-H), 7.23 - 7.27 (m, 2H, Ar-H), 7.79 (d, J = 8.8 Hz, 2H, Ar-H), 9.79 (s, 1H, NH). ^{13}C NMR (125 MHz, $CDCl_3$ + DMSO-d_6) δ 22.38, 28.59, 44.45, 98.51, 113.43, 124.36, 127.15, 128.72, 139.90, 147.05, 154.08, 157.38, 163.02 ppm; Mass (ESI-HRMS) (*m/z*): 298.1308 $[M+H]^+$; Anal. Calcd. For $C_{15}H_{15}N_5S$: C, 60.58; H, 5.08; N, 23.55%. Found: C, 60.54; H, 5.14; N, 23.51%.

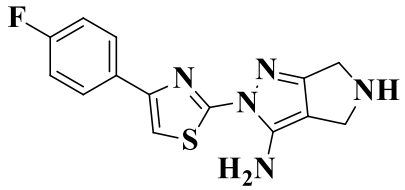
2-(4-(4-Methoxyphenyl)thiazol-2-yl)-2,4,5,6-tetrahydropyrrolo[3,4-c]pyrazol-3-amine (4c):



Brown solid; yield 88%; mp: >300ºC; IR (KBr) cm^{-1}: 1613 (C=N), 3400 (NH_2); ^1H NMR (400MHz, DMSO-d_6, ppm): δ 3.80 (s, 3H, OCH_3), 4.18 (s, 2H, CH_2), 4.27 (s, 2H, CH_2), 6.86 (s, 2H, NH_2), 7.11 (s, 2H, Ar-H), 7.65 (s, 1H, Ar-H), 7.92 (t, J = 8.4 Hz, 2H, Ar-H), 9.57 (s, 1H, NH). ^{13}C NMR (125 MHz, DMSO-d_6) δ 28.44, 31.69, 43.79, 55.66, 107.49, 114.61, 123.93, 127.82,

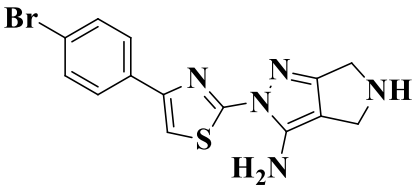
142.79, 151.35, 154.49, 157.23, 162.20 ppm; Mass (ESI-HRMS) (*m/z*): 314.1048 $[M+H]^+$; Anal. Calcd. For $C_{15}H_{15}N_5OS$: C, 57.49; H, 4.82; N, 22.35%. Found: C, 57.46; H, 4.85; N, 22.39%.

2-(4-(4-Fluorophenyl)thiazol-2-yl)-2,4,5,6-tetrahydropyrrolo[3,4-c]pyrazol-3-amine (4d):



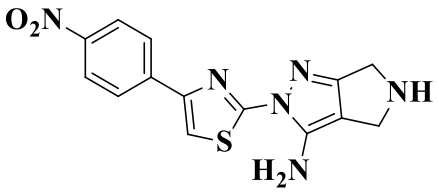
Saddle Brown solid; yield 84%; mp: >300ºC; IR (KBr) cm^{-1}: 1611 (C=N), 3421 (NH_2); ^1H NMR (400MHz, $CDCl_3$ + DMSO-d_6, ppm): δ 4.30 (s, 4H, 2 CH_2), 7.15 -7.19 (m, 3H, Ar-H, -NH_2), 7.50 (s, 1H, Ar-H), 7.92 -7.97 (m, 3H, Ar-H), 9.72 (s, 1H, NH). ^{13}C NMR (125 MHz, $CDCl_3$ + DMSO-d_6) δ 28.43, 43.79, 97.05, 108.35, 117.73, 126.16, 129.70, 138.08, 142.81, 151.30, 156.95, 163.22 ppm; Mass (ESI-HRMS) (*m/z*): 302.0867 $[M+H]^+$; Anal. Calcd. For $C_{14}H_{12}FN_5S$: C, 55.80; H, 4.01; N, 23.24%. Found: C, 55.84; H, 4.05; N, 23.20%.

2-(4-(4-Bromophenyl)thiazol-2-yl)-2,4,5,6-tetrahydropyrrolo[3,4-c]pyrazol-3-amine (4e):



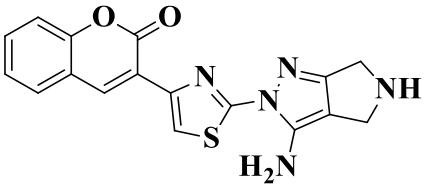
Saddle Brown solid; yield 87%; mp: >300ºC; IR (KBr) cm^{-1}: 1607 (C=N), 3430 (NH_2); ^1H NMR (400MHz, $CDCl_3$ + DMSO-d_6, ppm): δ 4.29 (s, 2H, CH_2), 4.30 (s, 2H, CH_2), 7.56 -7.60 (m, 3H, Ar-H, -NH_2), 7.65 (s, 1H, Ar-H), 7.82 -7.89 (m, 3H, Ar-H), 9.73 (s, 1H, NH). ^{13}C NMR (125 MHz, $CDCl_3$ + DMSO-d_6) δ 28.58, 43.81, 99.61, 108.52, 115.97, 130.51, 142.28, 142.83, 150.34, 154.10, 157.01, 162.72 ppm; Mass (ESI-HRMS) (*m/z*): 361.9916 $[M+H]^+$; Anal. Calcd. For $C_{14}H_{12}BrN_5S$: C, 46.42; H, 3.34; N, 19.33%. Found: C, 46.46; H, 3.38; N, 19.30%.

2-(4-(4-Nitrophenyl)thiazol-2-yl)-2,4,5,6-tetrahydropyrrolo[3,4-c]pyrazol-3-amine (4f):



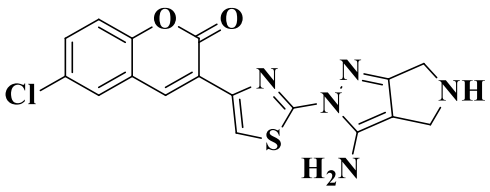
Gold solid; yield 88%; mp: >300°C; IR (KBr) cm^{-1}: 1602 (C=N), 3418 (NH_2); ^1H NMR (400MHz, $CDCl_3$ + DMSO-d_6, ppm): δ 4.31 (s, 4H, 2 CH_2), 7.96 (s, 2H, -NH_2), 8.21 (s, 1H, Ar-H), 8.25 (s, 2H, Ar-H), 8.35 (d, J = 8.4 Hz, 2H, Ar-H), 9.73 (s, 1H, NH). ^{13}C NMR (125 MHz, $CDCl_3$ + DMSO-d_6) δ 28.56, 44.41, 98.38, 109.69, 121.88, 128.05, 131.93, 142.85, 150.25, 154.18, 156.82, 162.44 ppm; Mass (ESI-HRMS) (*m/z*): 329.0854 $[M+H]^+$; Anal. Calcd. For $C_{14}H_{12}N_6O_2S$: C, 51.21; H, 3.68; N, 25.59%. Found: C, 51.25; H, 3.64; N, 25.54%.

3-(2-(3-Amino-5,6-dihydropyrrolo[3,4-c]pyrazol-2(4*H*)-yl)thiazol-4-yl)-2*H*-chromen-2-one (6a):



Moccasin solid; yield 91%; mp: >300°C; IR (KBr) cm^{-1}: 1605 (C=N), 3421 (NH_2); ^1H NMR (400MHz, DMSO-d_6, ppm): δ 4.21 (s, 2H, CH_2), 4.28 (s, 2H, CH_2), 6.96 (s, 1H, NH), 7.41 -7.46 (m, 2H, Ar-H), 7.63 -7.68 (m, 1H, Ar-H), 7.94 (d, J = 7.6 Hz, 1H, Ar-H), 8.17 (s, 1H, Ar-H), 9.01 (s, 1H, Ar-H), 9.62 (s, 2H, -NH_2). ^{13}C NMR (125 MHz, DMSO-d_6) δ 28.55, 43.74, 98.44, 115.88, 116.39, 125.36, 129.55, 132.74, 140.43, 142.83, 152.88, 157.65, 159.34, 162.01 ppm; Mass (ESI-HRMS) (*m/z*): 352.0810 $[M+H]^+$; Anal. Calcd. For $C_{17}H_{13}N_5O_2S$: C, 58.11; H, 3.73; N, 19.93%. Found: C, 58.15; H, 3.77; N, 19.97%.

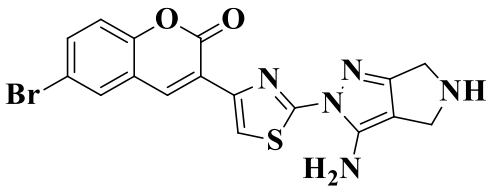
3-(2-(3-Amino-5,6-dihydropyrrolo[3,4-c]pyrazol-2(4*H*)-yl)thiazol-4-yl)-6-chloro-2*H*-chromen-2-one (6b):



Gold solid; yield 89%; mp: >300°C; IR (KBr) cm^{-1}: 1608 (C=N), 3398 (NH_2); ^1H NMR (400MHz, $CDCl_3$ + DMSO-d_6, ppm): δ 4.22 (s, 2H, CH_2), 4.28 (s, 2H, CH_2), 7.11 (s, 1H, NH), 7.83 (s, 1H, Ar-H), 7.95 (s, 1H, Ar-H), 8.03 (s, 1H, Ar-H), 8.18 (s, 1H, Ar-H), 8.95 (s, 1H, Ar-H), 9.63 (s, 2H, -NH_2). ^{13}C NMR (125 MHz, $CDCl_3$ + DMSO-d_6) δ 22.12, 28.45, 43.83, 87.52, 98.46, 116.37, 118.30, 121.05, 128.35, 129.03, 131.89, 138.97, 142.85, 144.60, 151.54, 158.71 ppm; Mass (ESI-

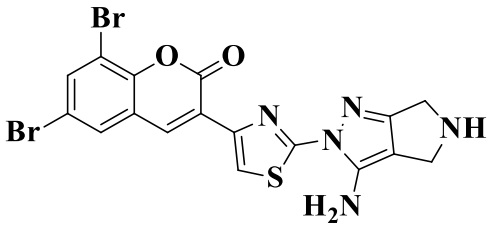
HRMS) (*m/z*): 385.0493 $[M+H]^+$; Anal. Calcd. For $C_{17}H_{12}ClN_5O_2S$: C, 52.92; H, 3.13; N, 18.15%. Found: C, 52.96; H, 3.16; N, 18.18%.

3-(2-(3-Amino-5,6-dihydropyrrolo[3,4-c]pyrazol-2(4*H*)-yl)thiazol-4-yl)-6-bromo-2*H*-chromen-2-one (6c):



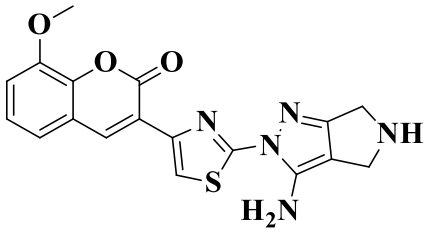
Gold solid; yield 90%; mp: >300ºC; IR (KBr) cm^{-1}: 1603 (C=N), 3419 (NH_2); ^1H NMR (400MHz, $CDCl_3$ + DMSO-d_6, ppm): δ 4.11 (s, 2H, CH_2), 4.34 (s, 2H, CH_2), 7.14 (s, 1H, NH), 7.65 (t, J = 9.2 Hz, 2H, Ar-H), 7.86 (d, J = 7.2 Hz, 2H, Ar-H), 8.03 (s, 1H, Ar-H), 9.44 (s, 2H, -NH_2). ^{13}C NMR (125 MHz, $CDCl_3$ + DMSO-d_6) δ 23.60, 29.45, 47.59, 85.41, 94.53, 100.67, 116.93, 118.58, 121.52, 131.31, 134.88, 139.80, 151.93, 158.65, 159.82 ppm; Mass (ESI-HRMS) (*m/z*): 429.9945 $[M+H]^+$; Anal. Calcd. For $C_{17}H_{12}BrN_5O_2S$: C, 47.45; H, 2.81; N, 16.28%. Found: C, 47.49; H, 2.85; N, 16.24%.

3-(2-(3-Amino-5,6-dihydropyrrolo[3,4-c]pyrazol-2(4*H*)-yl)thiazol-4-yl)-6,8-dibromo-2*H*-chromen-2-one (6d):



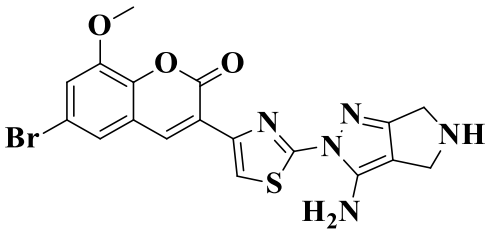
Gold solid; yield 91%; mp: >300ºC; IR (KBr) cm^{-1}: 1619 (C=N), 3421 (NH_2); ^1H NMR (400MHz, $CDCl_3$ + DMSO-d_6, ppm): δ 4.12 (s, 2H, CH_2), 4.36 (s, 2H, CH_2), 7.28 (s, 1H, NH), 7.89 (s, 1H, Ar-H), 7.96 (s, 1H, Ar-H), 8.01 (s, 1H, Ar-H), 8.37 (s, 1H, Ar-H), 9.42 (s, 2H, -NH_2). ^{13}C NMR (125 MHz, $CDCl_3$ + DMSO-d_6) δ 24.56, 28.60, 43.80, 87.29, 98.47, 130.97, 136.55, 138.51, 139.99, 142.83, 153.51, 157.58, 158.04, 166.27 ppm; Mass (ESI-HRMS) (*m/z*): 507.9507 $[M+H]^+$; Anal. Calcd. For $C_{17}H_{11}Br_2N_5O_2S$: C, 40.10; H, 2.18; N, 13.75%. Found: C, 40.14; H, 2.14; N, 13.76%.

3-(2-(3-Amino-5,6-dihydropyrrolo[3,4-c]pyrazol-2(4*H*)-yl)thiazol-4-yl)-8-methoxy-2*H*-chromen-2-one (6e):



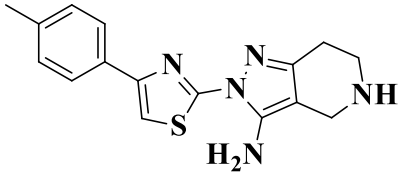
Golden solid; yield 89%; mp: 242-244^{o}C; IR (KBr) cm^{-1}: 1609 (C=N), 3432 (NH_2); ^{1}H NMR (400MHz, DMSO-d_6, ppm): δ 3.94 (s, 3H, OCH_3), 4.18 (s, 2H, CH_2), 4.28 (s, 2H, CH_2), 7.33 (s, 1H, NH), 7.39 -7.43 (m, 2H, Ar-H), 8.17 (s, 1H, Ar-H), 8.47 (s, 1H, Ar-H), 8.98(s, 1H, Ar-H), 9.62 (s, 2H, -NH_2). ^{13}C NMR (125 MHz, DMSO-d_6) δ 25.77, 31.37, 43.69, 56.46, 98.42, 114.64, 115.88, 120.01, 120.66, 125.31, 129.45, 140.53, 142.07, 144.71, 146.57, 157.36, 161.94 ppm; Mass (ESI-HRMS) (*m/z*): 382.0888 $[M+H]^+$; Anal. Calcd. For $C_{18}H_{15}N_5O_3S$: C, 56.68; H, 3.96; N, 18.36%. Found: C, 56.64; H, 3.94; N, 18.32%.

3-(2-(3-Amino-5,6-dihydropyrrolo[3,4-c]pyrazol-2(4*H*)-yl)thiazol-4-yl)-6-bromo-8-methoxy-2*H*-chromen-2-one (6f):



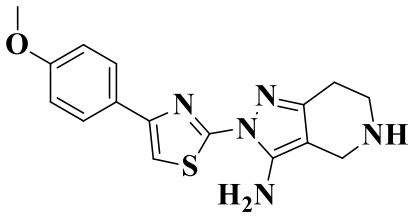
Golden solid; yield 92%; mp: 252-254^{o}C; IR (KBr) cm^{-1}: 1612 (C=N), 3411 (NH_2); ^{1}H NMR (400MHz, $CDCl_3$ + DMSO-d_6, ppm): δ 3.85 (s, 3H, OCH_3), 4.15 (s, 2H, CH_2), 4.34 (s, 2H, CH_2), 6.95 (s, 1H, NH), 6.97 (s, 1H, Ar-H), 7.18 (s, 1H, Ar-H), 7.78 (d, J = 9.2 Hz, 2H, Ar-H), 9.41 (s, 2H, -NH_2). ^{13}C NMR (125 MHz, $CDCl_3$ + DMSO-d_6) δ 20.84, 28.54, 41.61, 55.62, 90.16, 107.34, 114.56, 126.70, 127.71, 144.48, 147.92, 151.40, 159.75, 162.18 ppm; Mass (ESI-HRMS) (*m/z*): 500.0107 $[M+H]^+$; Anal. Calcd. For $C_{18}H_{14}BrN_5O_3S$: C, 46.97; H, 3.07; N, 15.21%. Found: C, 46.95; H, 3.14; N, 15.25%.

2-(4-(P-tolyl)thiazol-2-yl)-4,5,6,7-tetrahydro-2*H*-pyrazolo[4,3-c]pyridin-3-amine (8a):

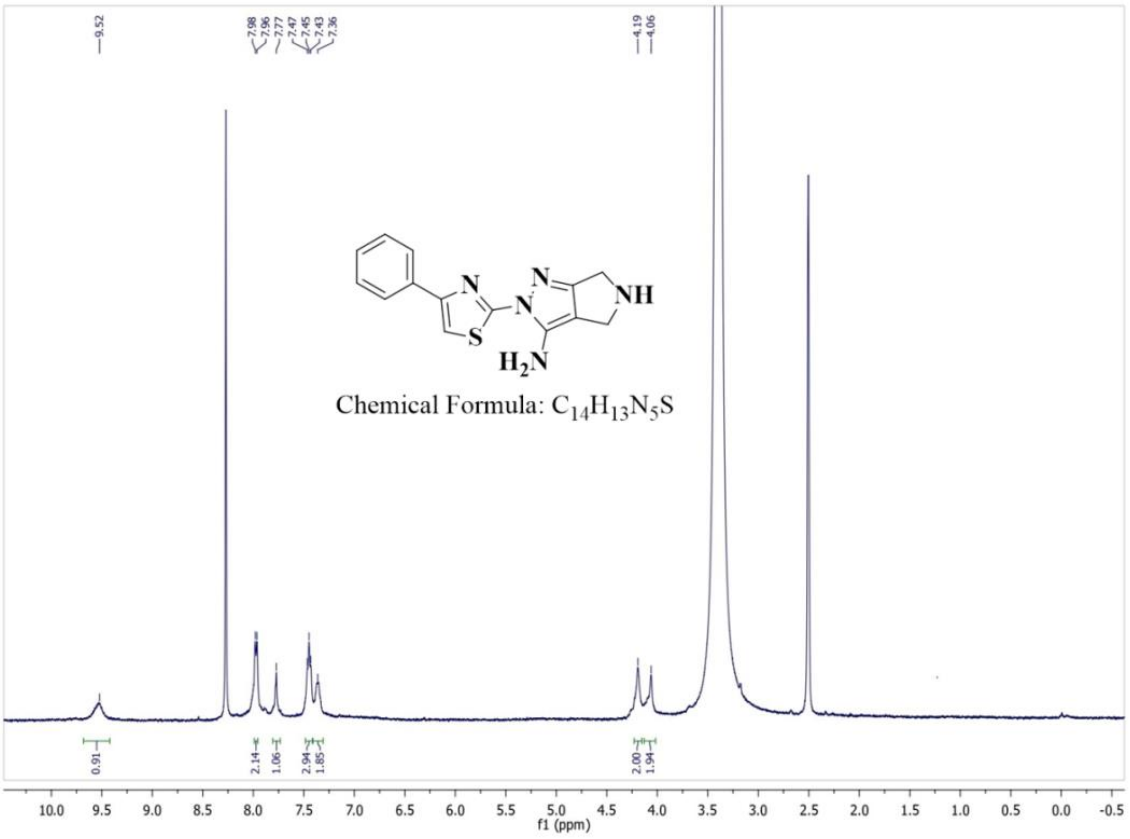


White solid; yield 87%; mp: 285-287°C; IR (KBr) cm^{-1}: 1610 (C=N), 3329 (NH_2); ^1H NMR (400MHz, $CDCl_3$ + DMSO-d_6, ppm): δ 2.38 (s, 3H, CH_3), 2.93 (t, J = 6 Hz, 2H, CH_2), 3.42 (d, J = 4.8 Hz, 2H, CH_2), 4.1 (s, 2H, CH_2), 6.97 (s, 1H, NH), 7.23 (d, J = 7.6 Hz, 2H, Ar-H), 7.43 (s, 1H), 7.77 (d, J = 8 Hz, 2H, Ar-H), 9.13 (s, 2H, -NH2). ^{13}C NMR (125 MHz, $CDCl_3$ + DMSO-d_6) δ 20.85, 21.32, 41.79, 89.91, 107.82, 126.02, 129.59, 131.15, 138.04, 144.56, 147.51, 151.73, 162.17 ppm; Mass (ESI-HRMS) (*m/z*): 312.1274 $[M+H]^+$; Anal. Calcd. For $C_{16}H_{17}N_5S$: C, 61.71; H, 5.50; N, 22.49%. Found: C, 61.67; H, 5.54; N, 22.45%.

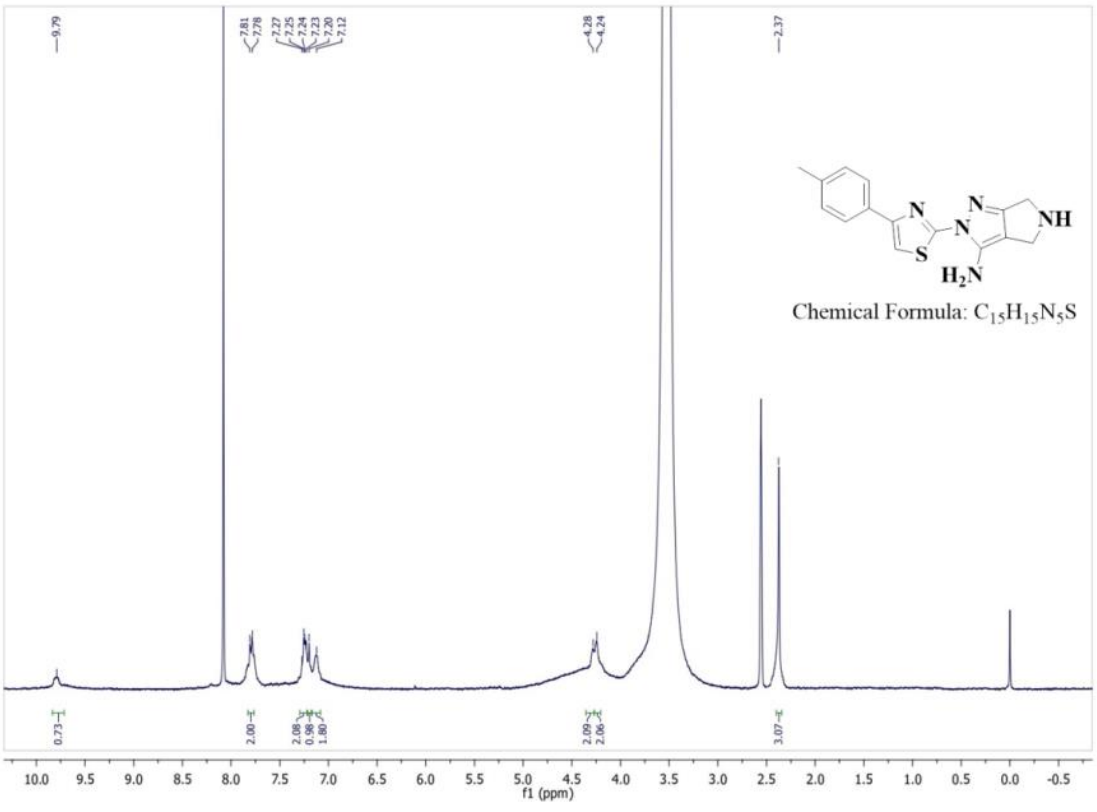
2-(4-(4-Methoxyphenyl)thiazol-2-yl)-4,5,6,7-tetrahydro-2*H*-pyrazolo[4,3-c]pyridin-3-amine (8b):



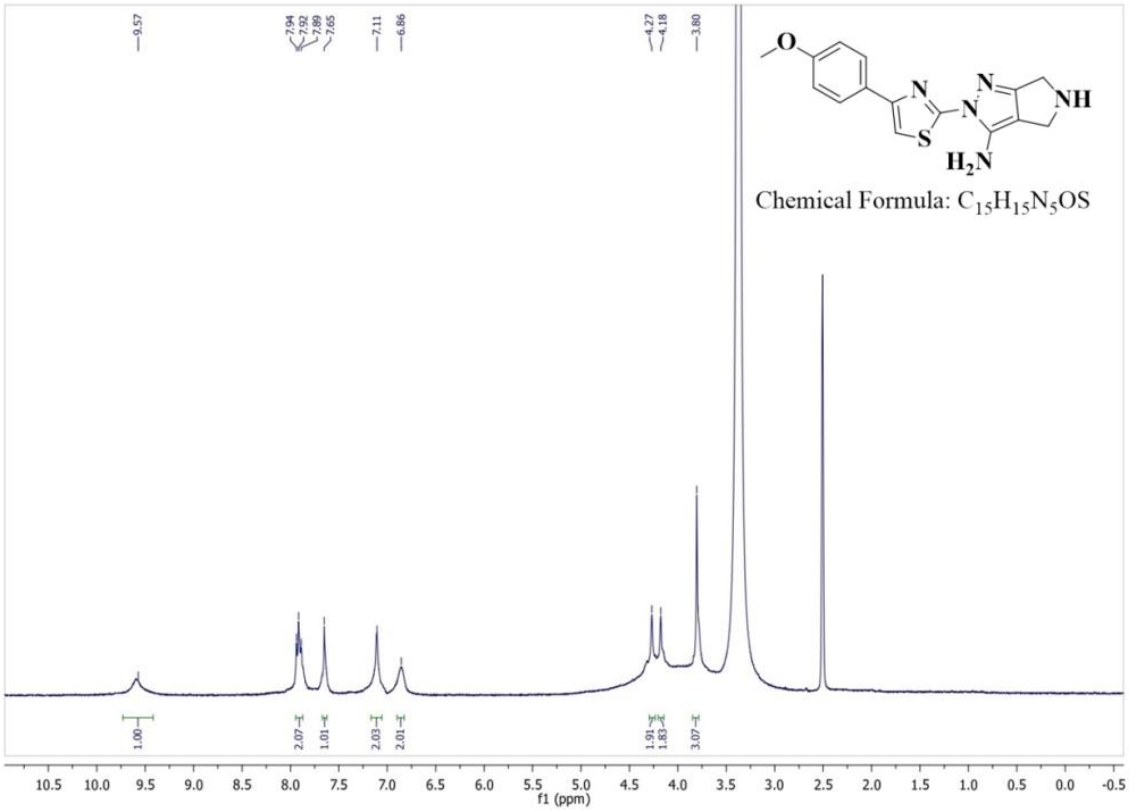
White solid; yield 85%; mp: 265-267°C; IR (KBr) cm^{-1}: 1614 (C=N), 3403 (NH_2); ^1H NMR (400MHz, $CDCl_3$ + DMSO-d_6, ppm): δ 2.92 (t, J = 6 Hz, 2H, CH_2), 3.43 (d, J = 4.8 Hz, 2H, CH_2), 3.84 (s, 3H, OCH_3), 4.09 (s, 2H, CH_2), 6.95 (s, 1H, NH), 7.17 (d, J = 7.6 Hz, 2H, Ar-H), 7.37 (s, 1H, Ar-H), 7.83 (d, J = 8.8 Hz, 2H, Ar-H), 9.07 (s, 2H, -NH_2). ^{13}C NMR (125 MHz, $CDCl_3$ + DMSO-d_6) δ 20.85, 41.79, 55.46, 89.92, 106.74, 114.34, 126.69, 127.50, 144.54, 147.51, 151.50, 159.70, 162.14 ppm; Mass (ESI-HRMS) (*m/z*): 328.1286 $[M+H]^+$; Anal. Calcd. For $C_{16}H_{17}N_5OS$: C, 58.70; H, 5.23; N, 21.39%. Found: C, 58.64; H, 5.20; N, 21.35%.



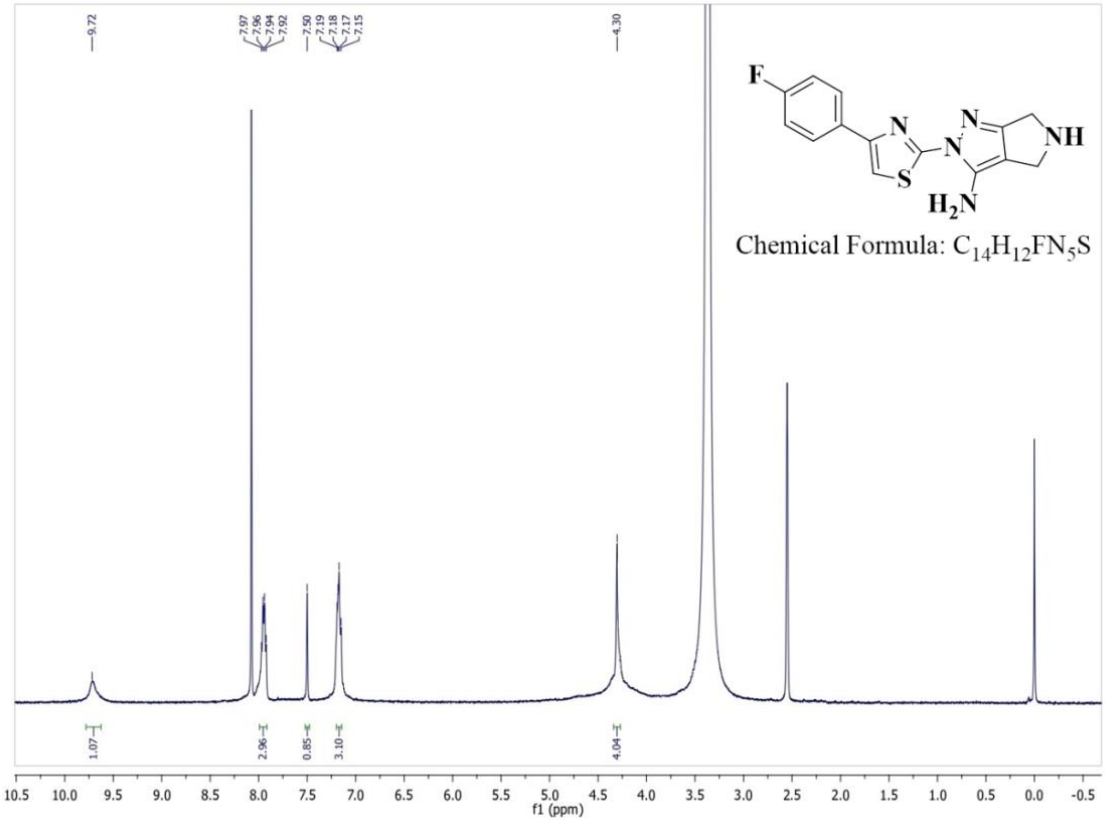
^{1}H NMR spectrum of compound **4a** (400 MHz, $CDCl_3$+DMSO-d_6)



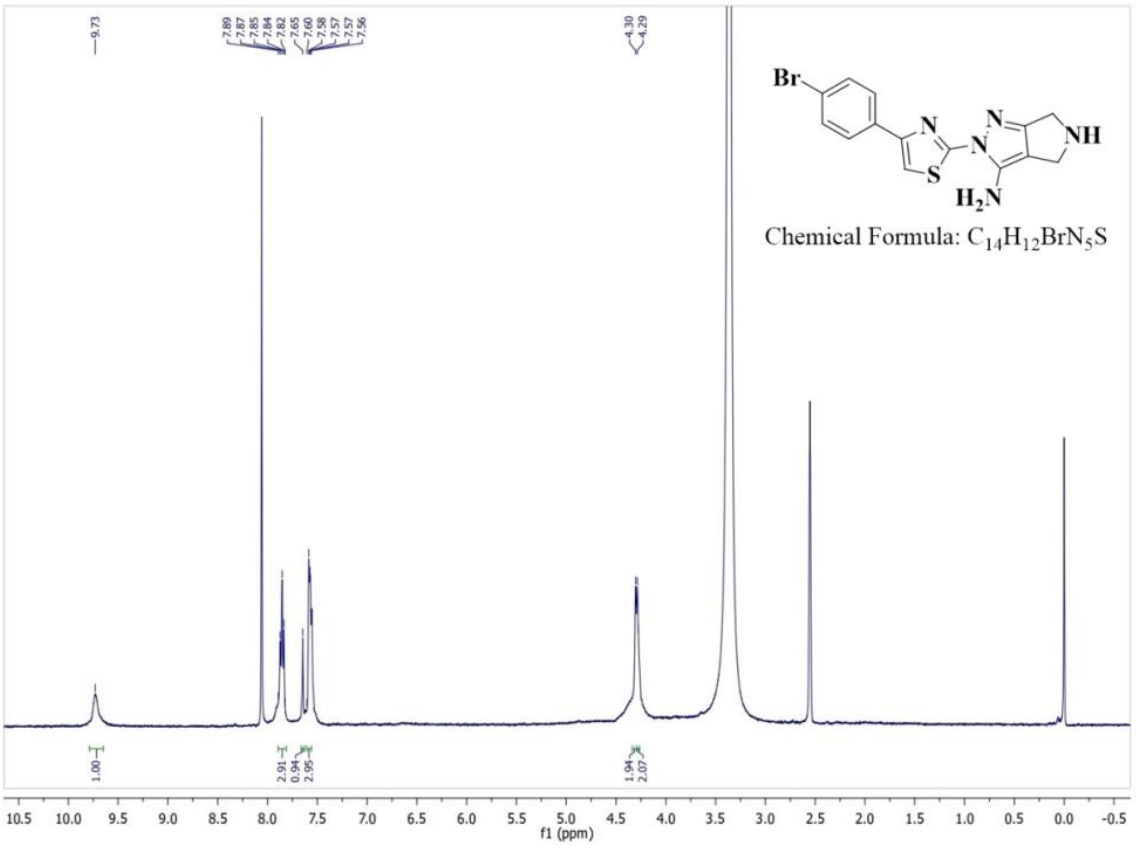
^{1}H NMR spectrum of compound **4b** (400 MHz, $CDCl_3$+DMSO-d_6)



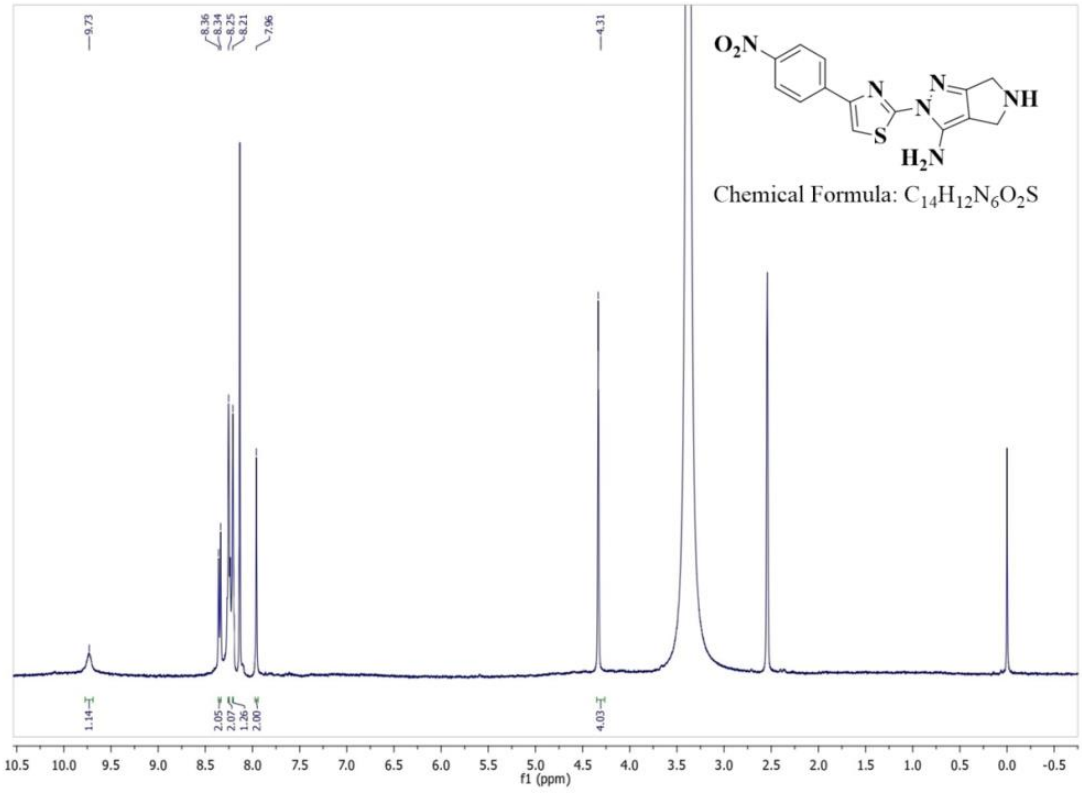
^{1}H NMR spectrum of compound **4c** (400 MHz, DMSO-d_6)



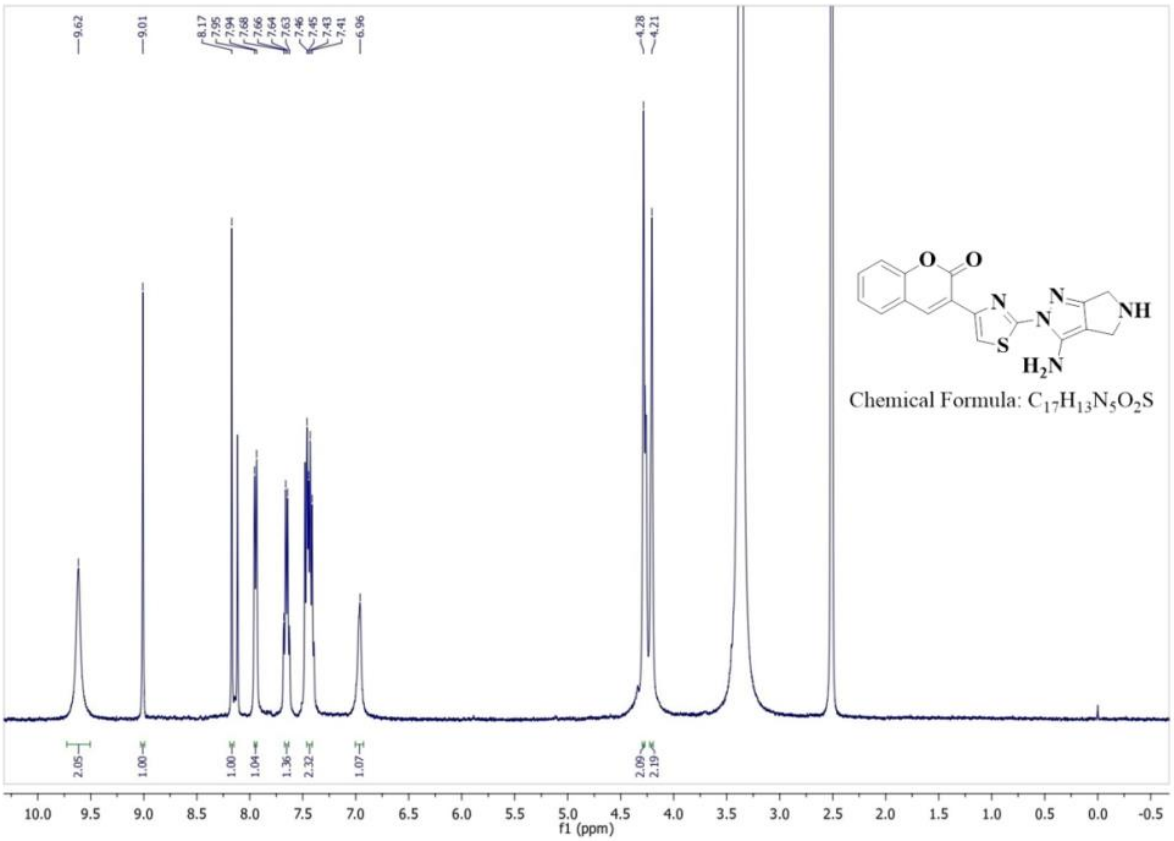
^{1}H NMR spectrum of compound **4d** (400 MHz, $CDCl_3$+DMSO-d_6)



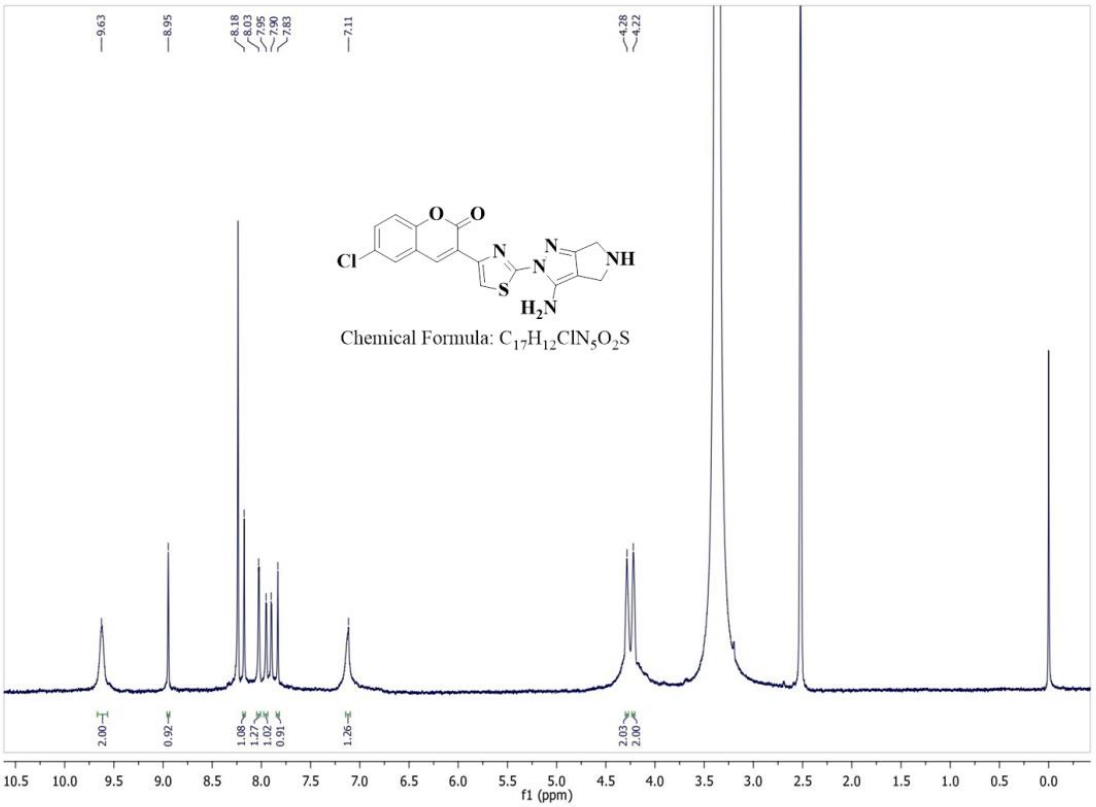
^{1}H NMR spectrum of compound **4e** (400 MHz, $CDCl_3$+DMSO-d_6)



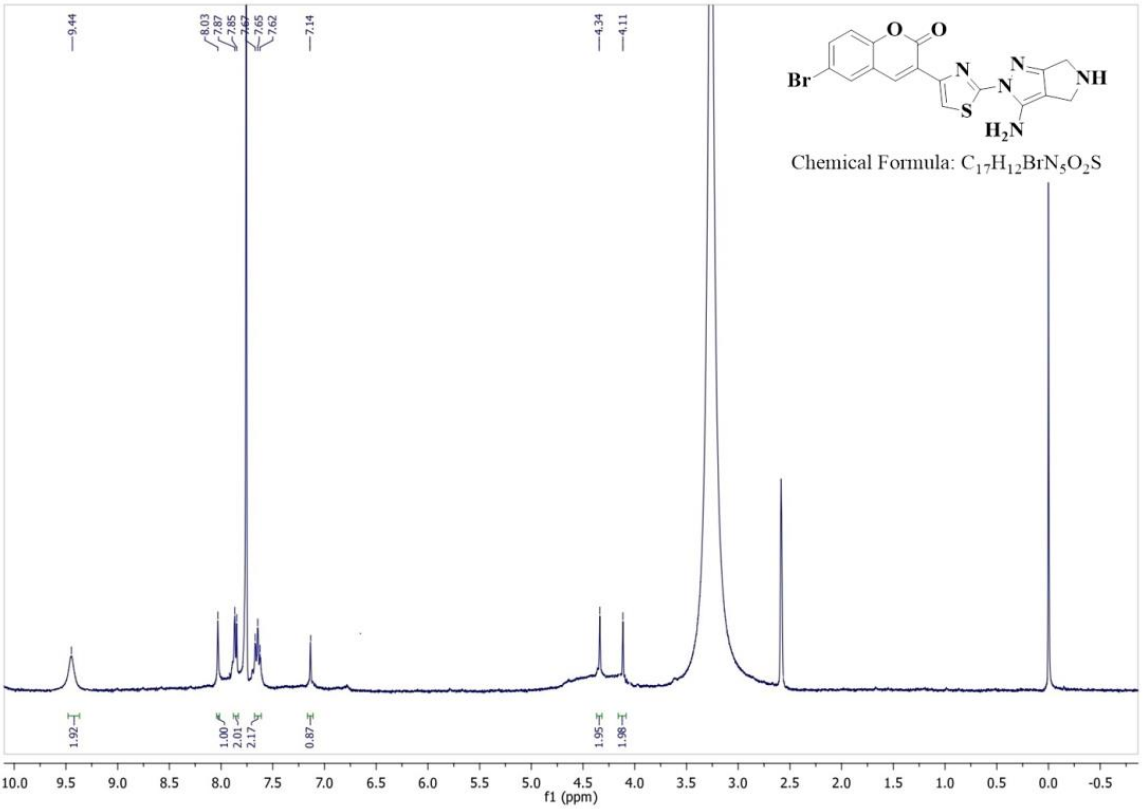
^{1}H NMR spectrum of compound **4f** (400 MHz, $CDCl_3$+DMSO-d_6)



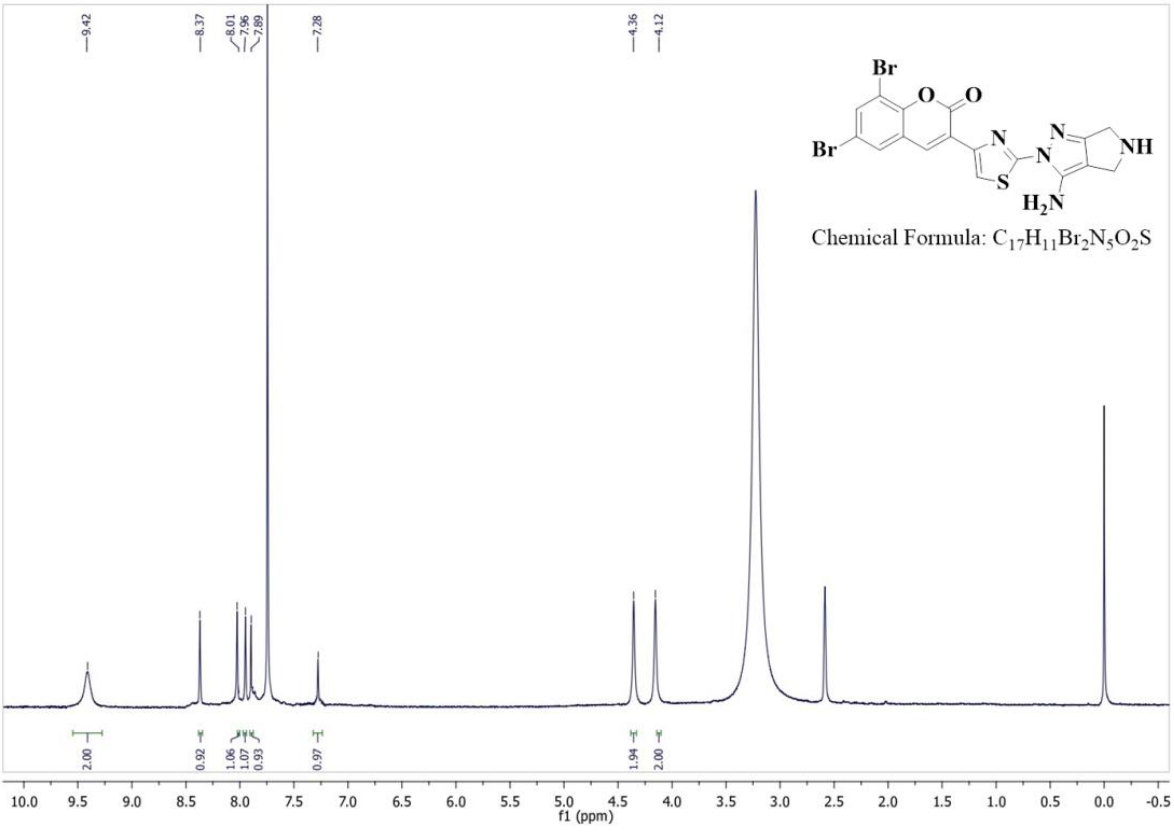
^{1}H NMR spectrum of compound **6a** (400 MHz, DMSO-d_6)



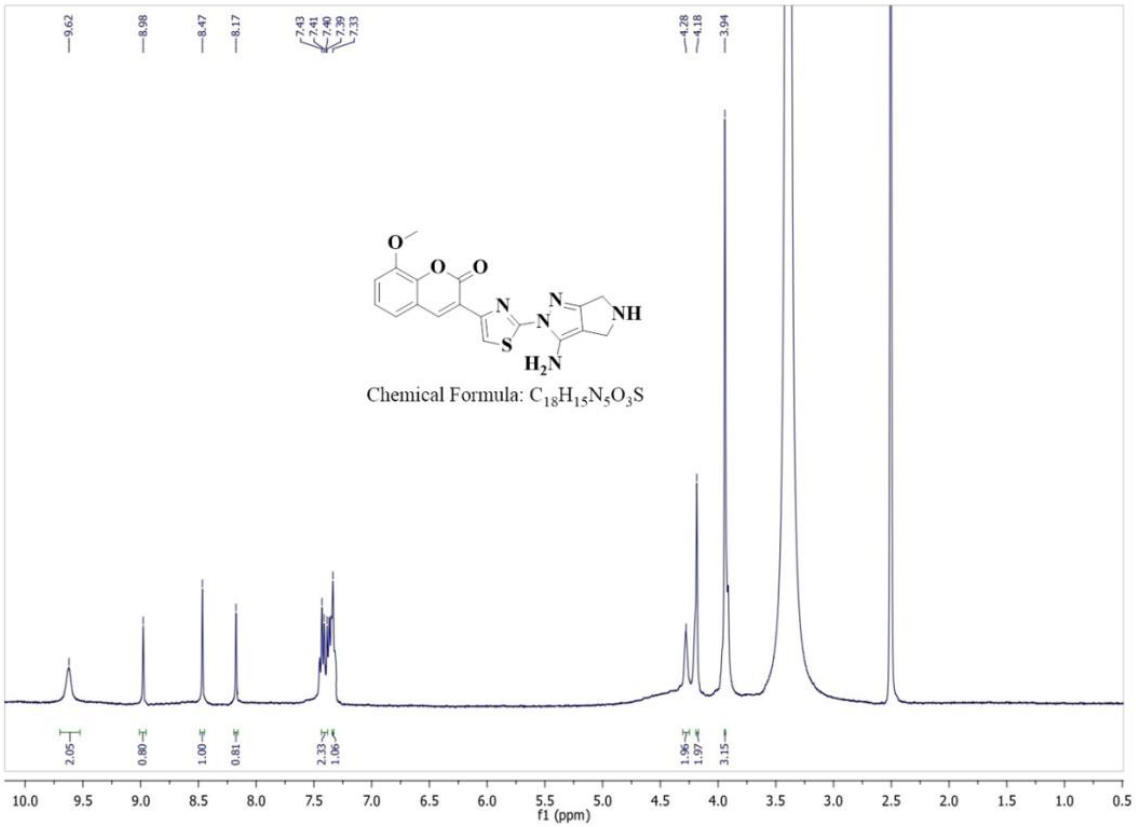
^{1}H NMR spectrum of compound **6b** (400 MHz, $CDCl_3$+DMSO-d_6)



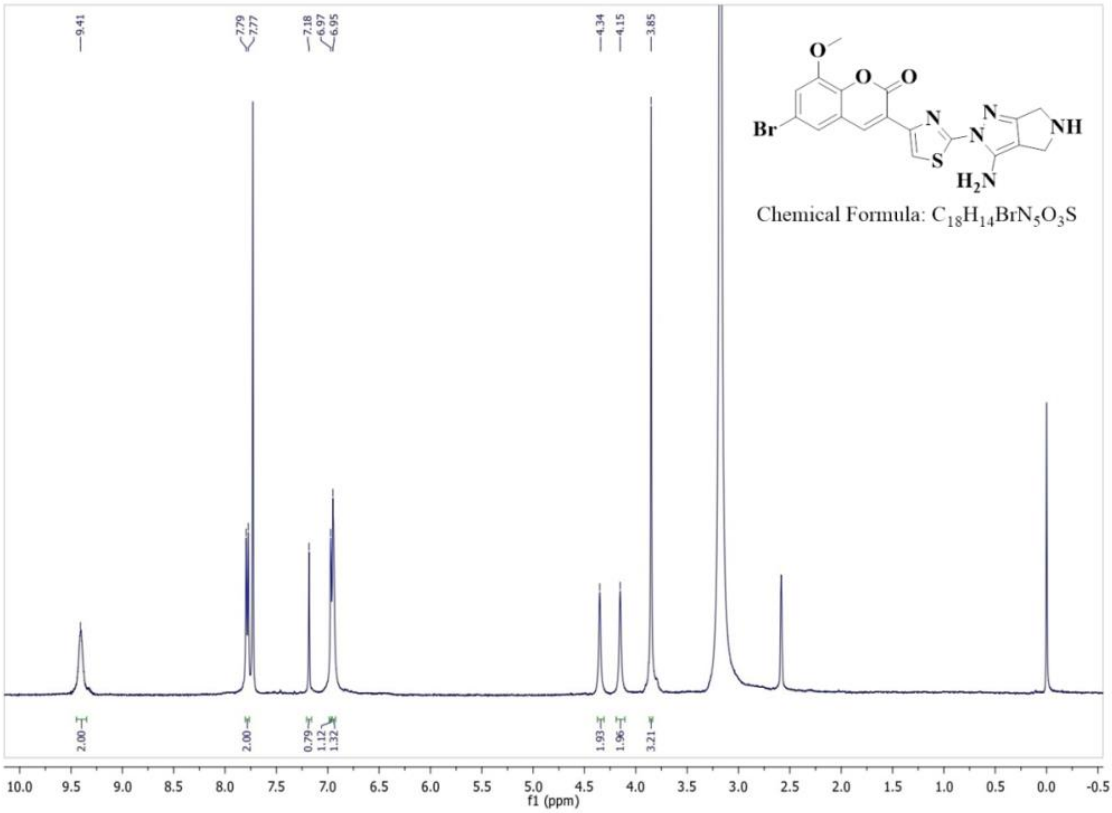
^{1}H NMR spectrum of compound **6c** (400 MHz, $CDCl_3$+DMSO-d_6)



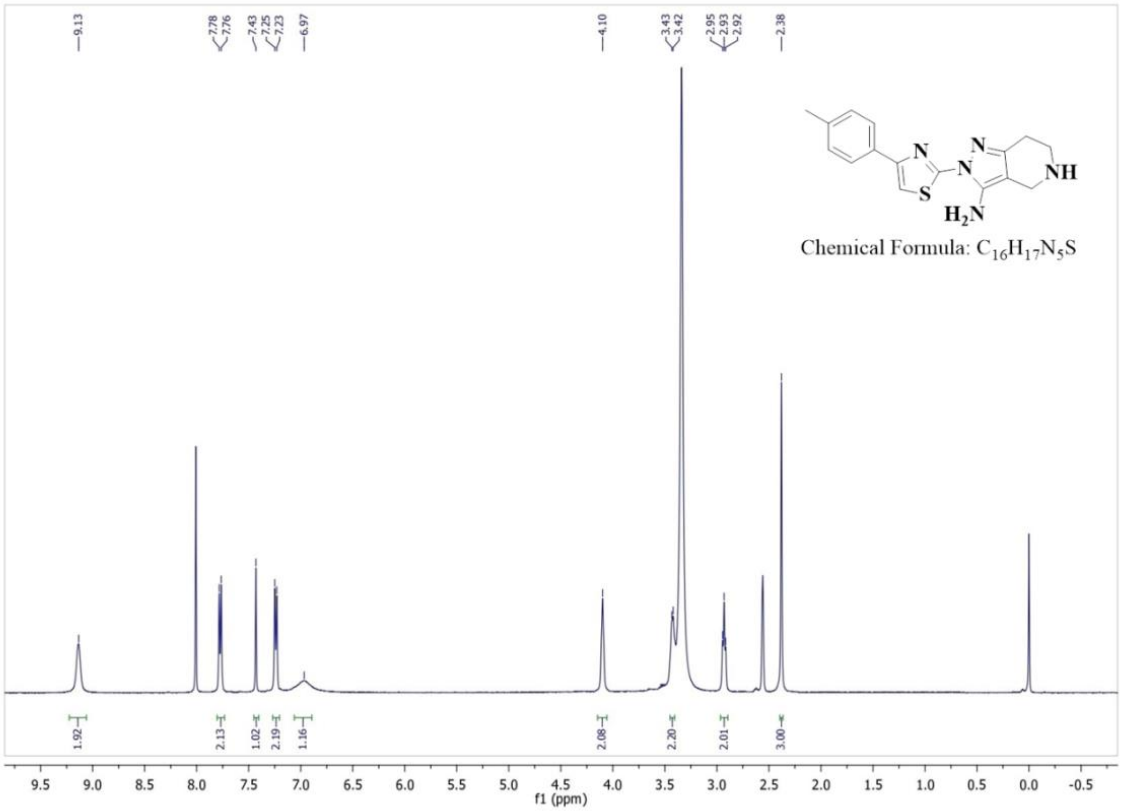
^{1}H NMR spectrum of compound **6d** (400 MHz, $CDCl_3$+DMSO-d_6)



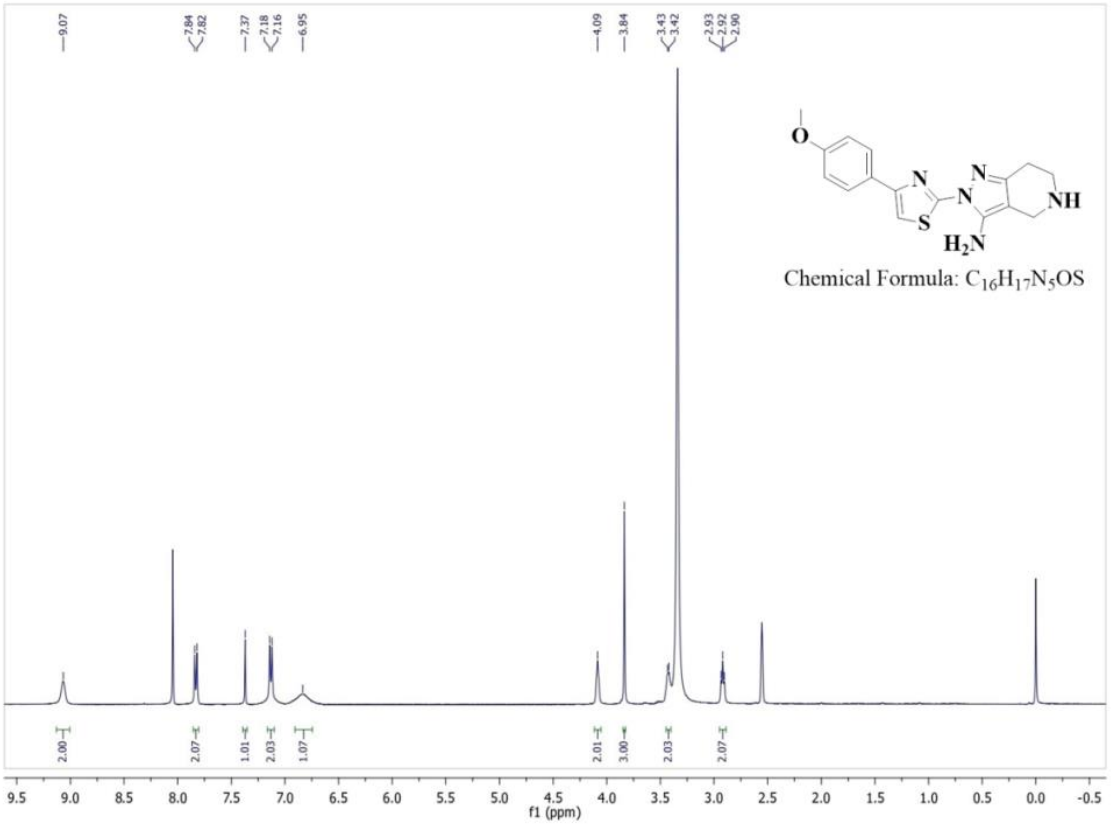
^{1}H NMR spectrum of compound **6e** (400 MHz, DMSO-d_6)



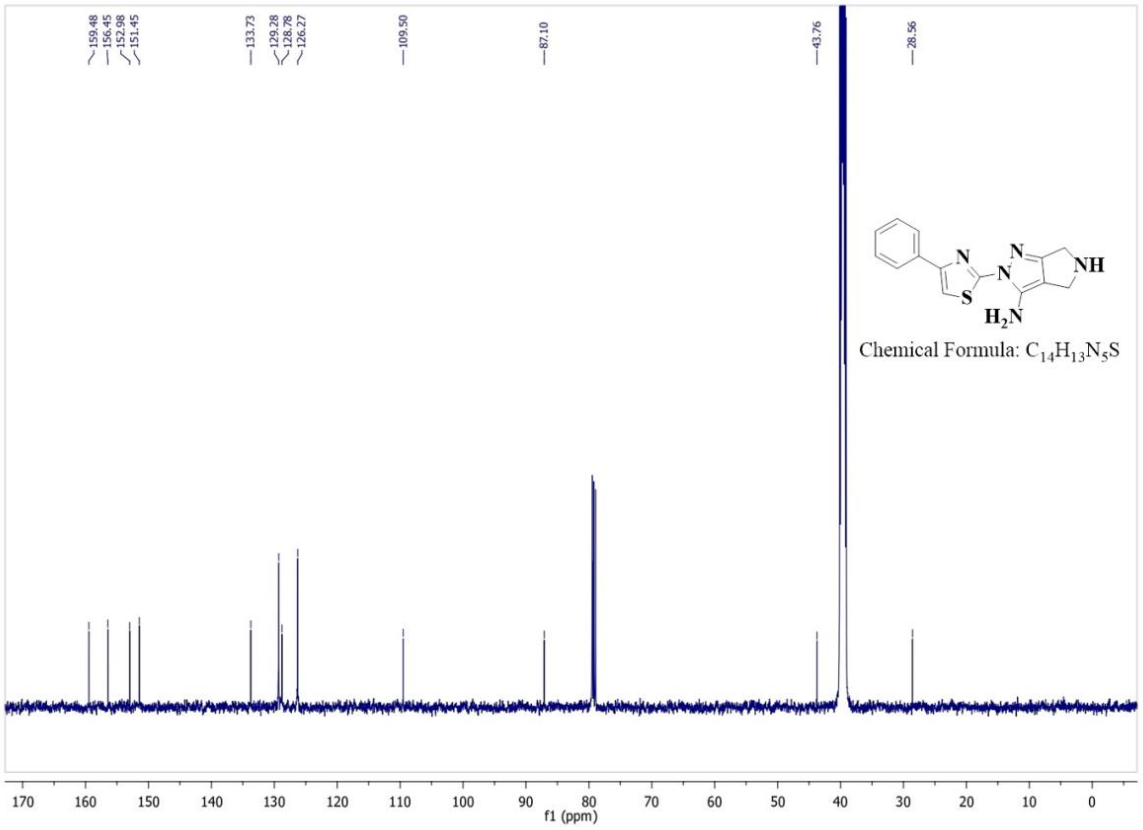
^{1}H NMR spectrum of compound **6f** (400 MHz, $CDCl_3$+DMSO-d_6)



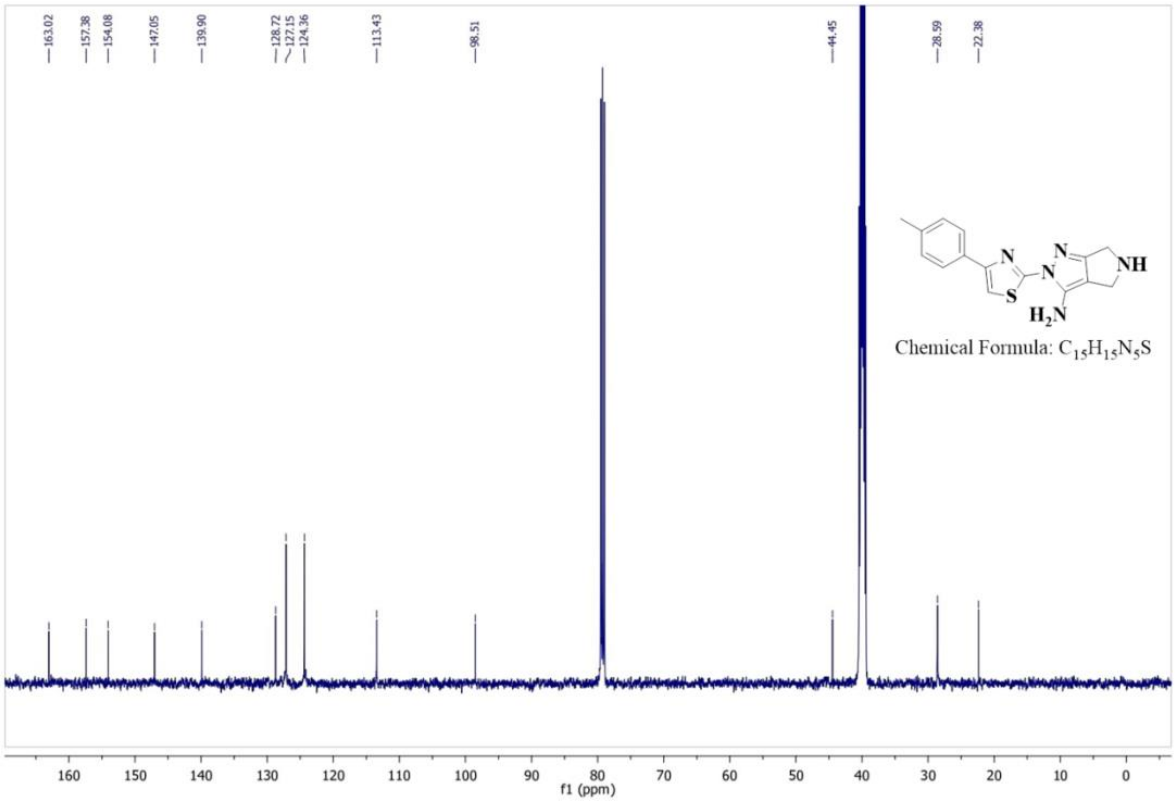
^{1}H NMR spectrum of compound **8a** (400 MHz, $CDCl_3$+DMSO-d_6)



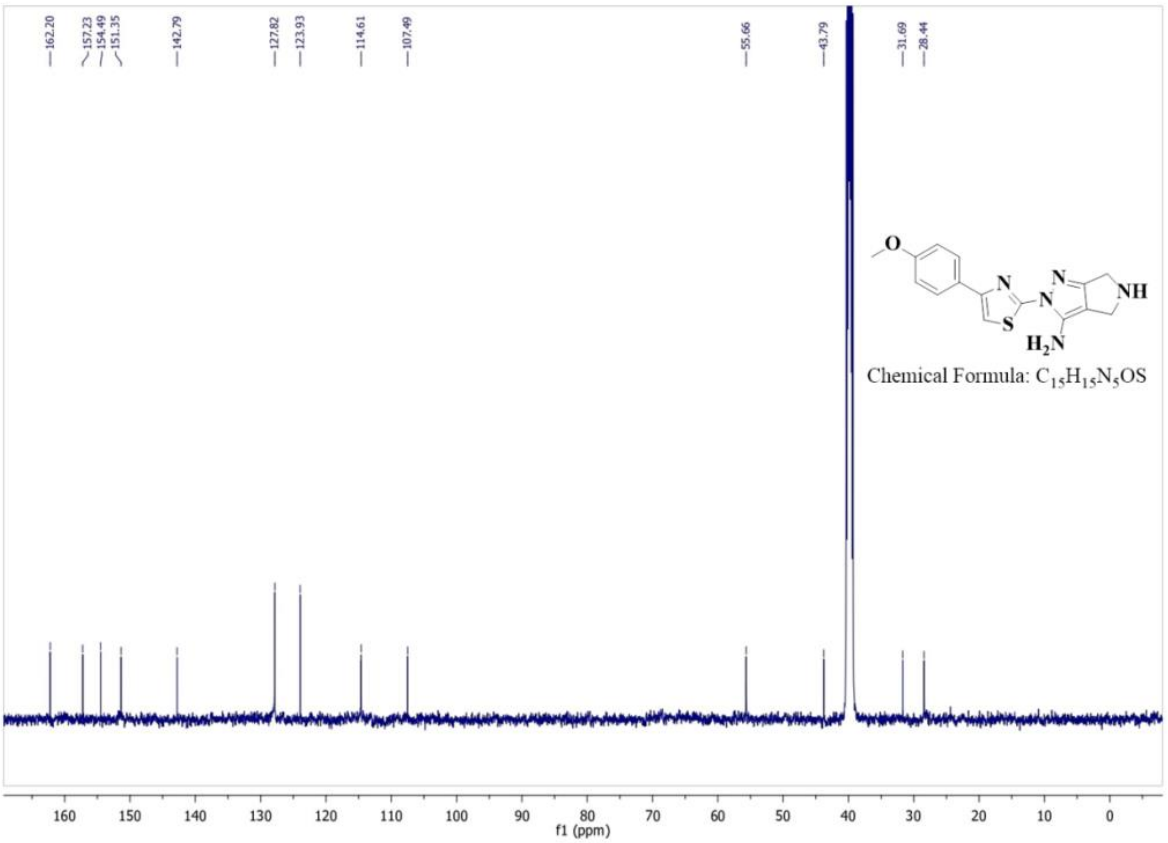
^{1}H NMR spectrum of compound **8b** (400 MHz, $CDCl_3$+DMSO-d_6)



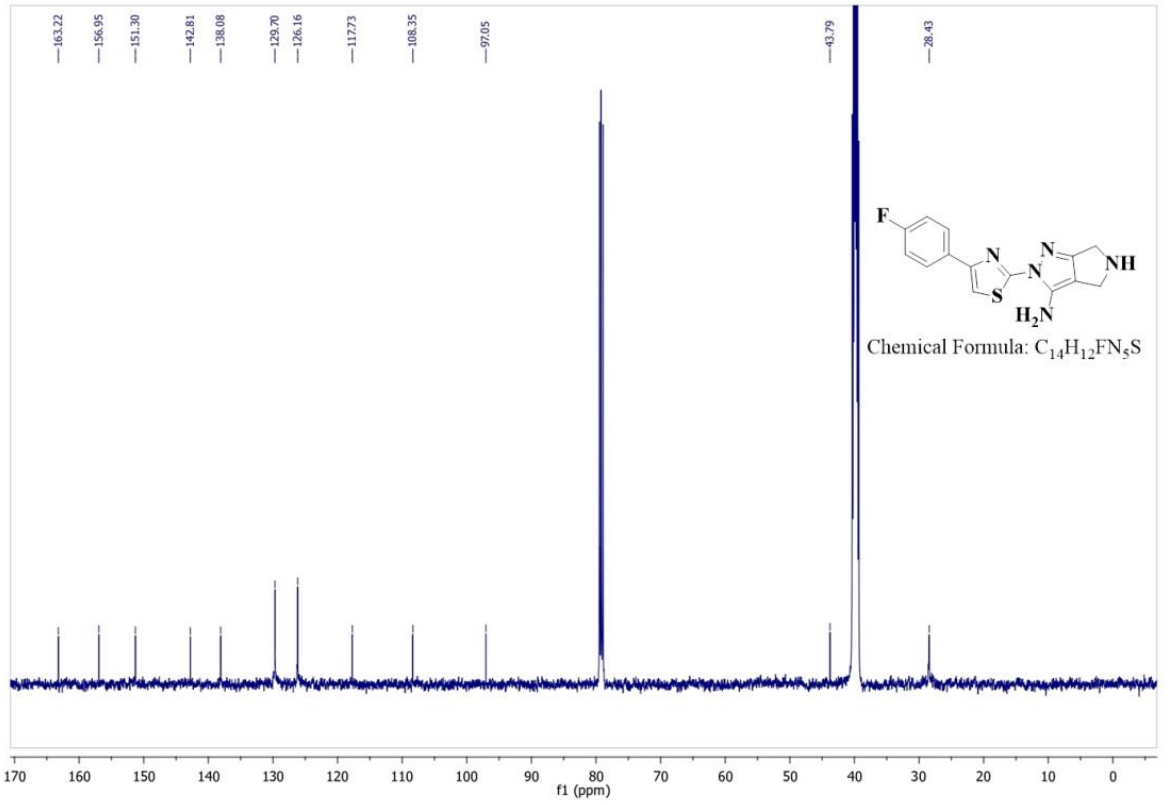
^{13}C NMR spectrum of compound **4a** (125 MHz, $CDCl_3$+DMSO-d_6)



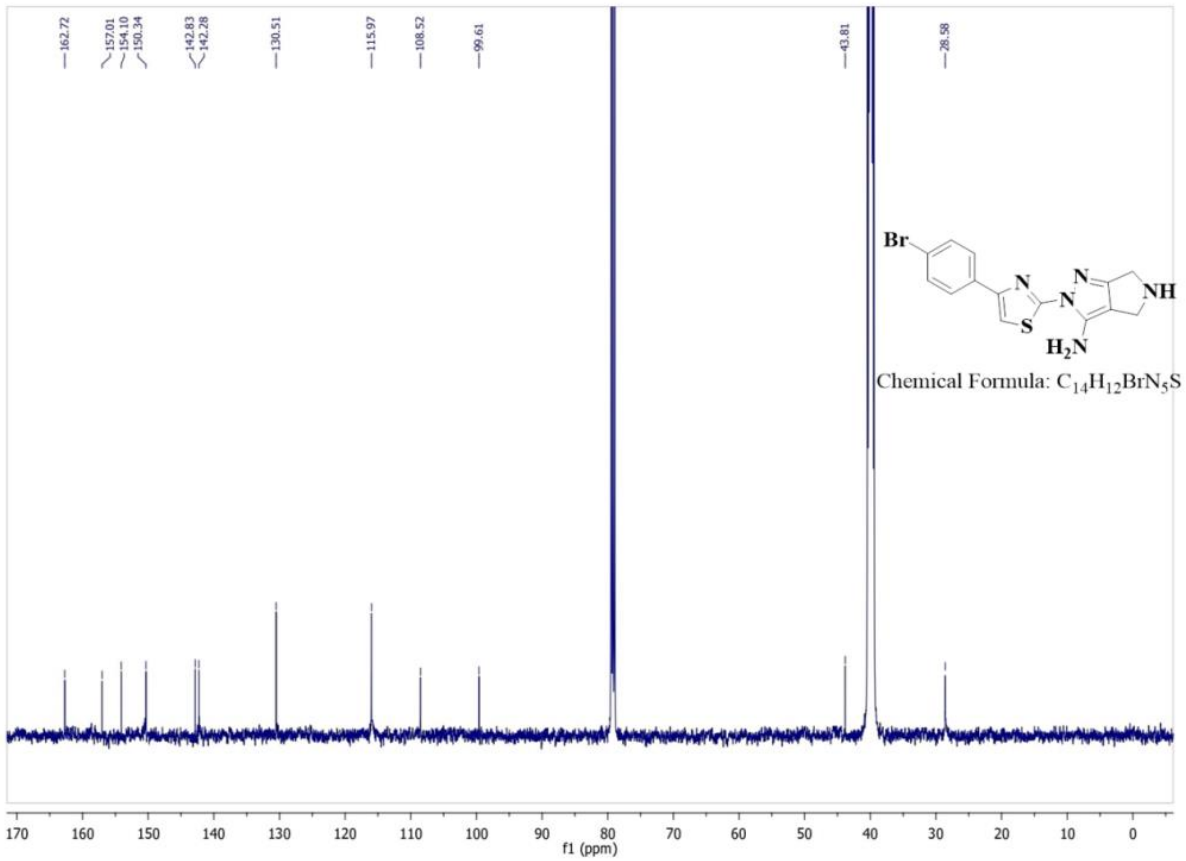
^{13}C NMR spectrum of compound **4b** (125 MHz, $CDCl_3$+DMSO-d_6)



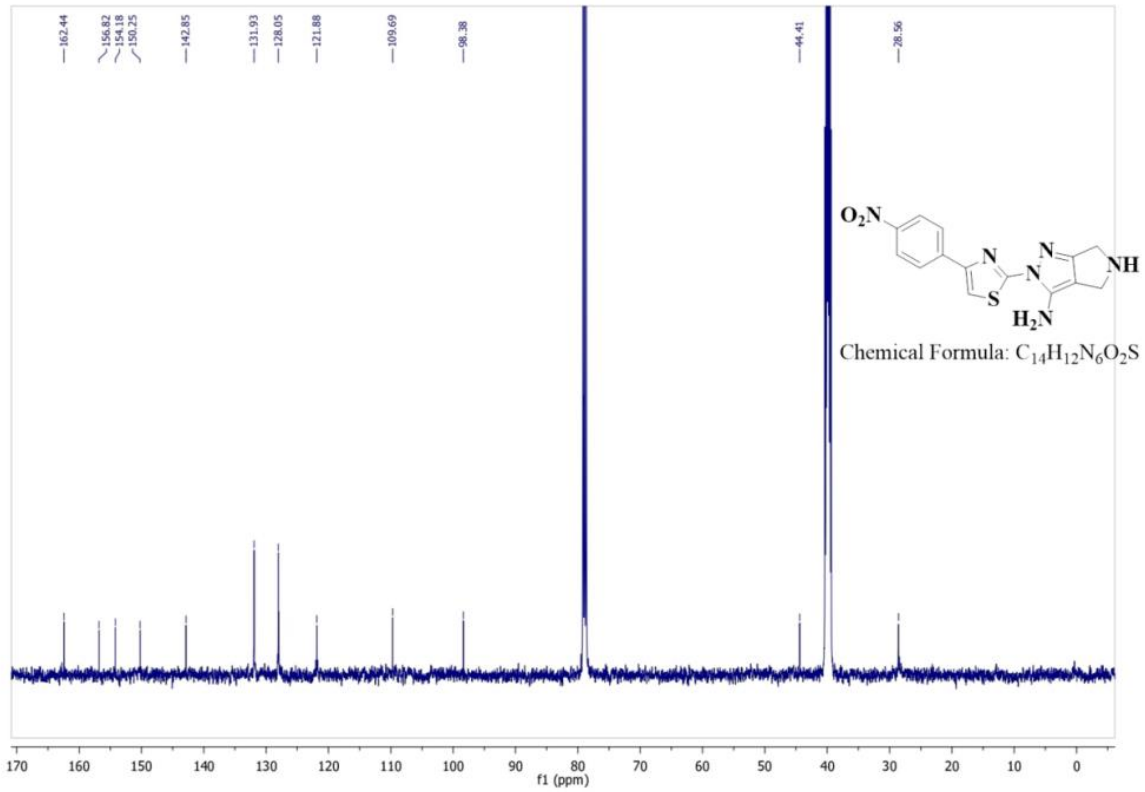
^{13}C NMR spectrum of compound **4c** (125 MHz, DMSO-d_6)



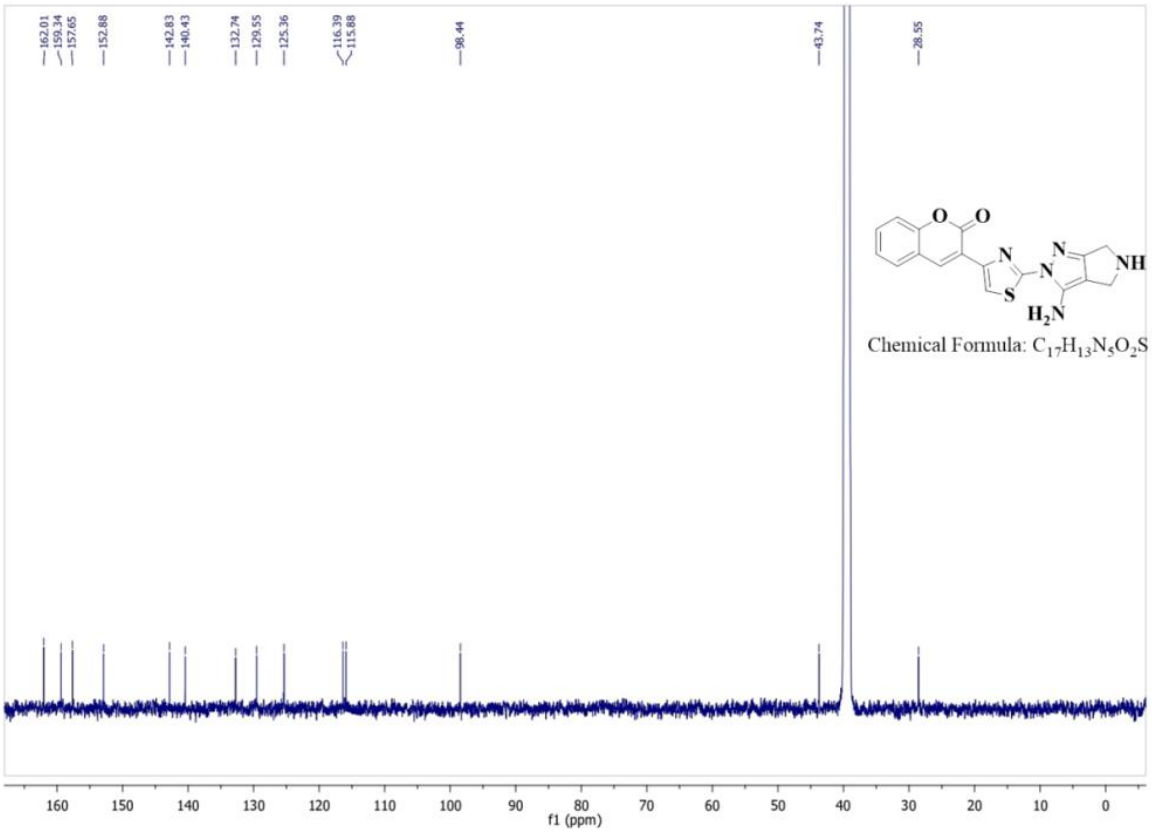
^{13}C NMR spectrum of compound **4d** (125 MHz, $CDCl_3$+DMSO-d_6)



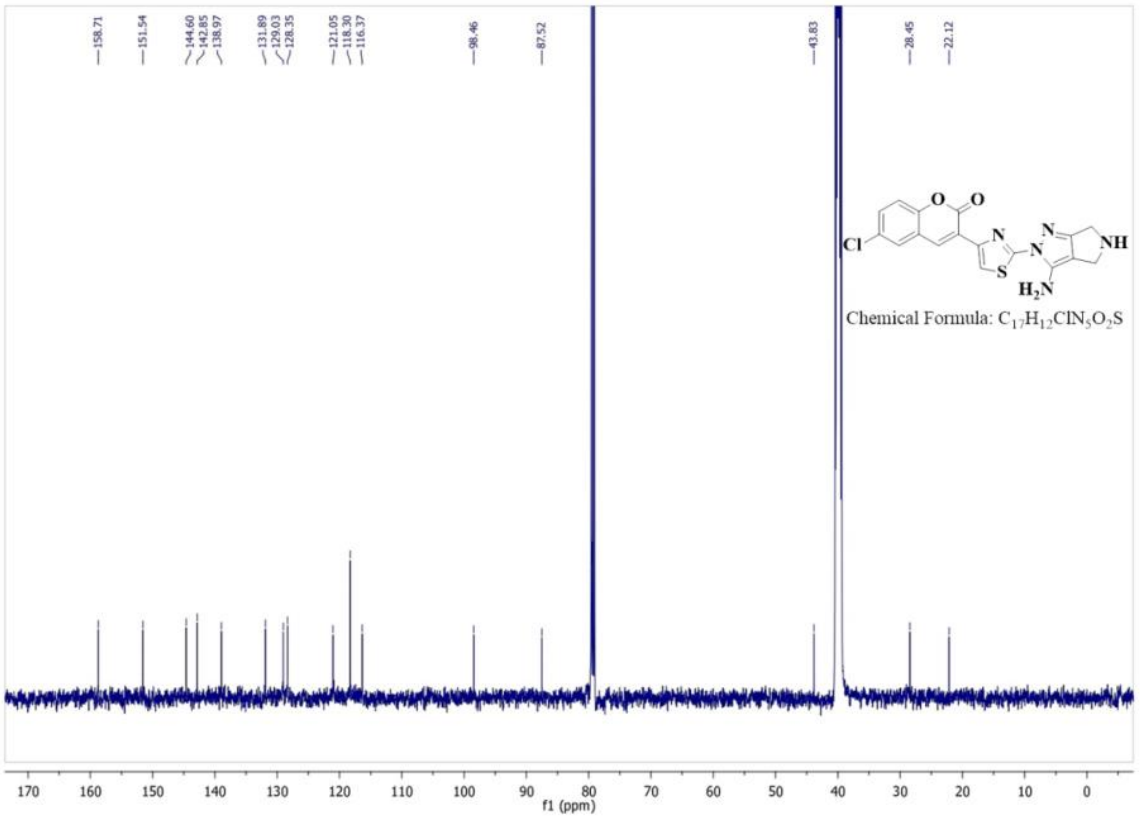
^{13}C NMR spectrum of compound **4e** (125 MHz, $CDCl_3$+DMSO-d_6)



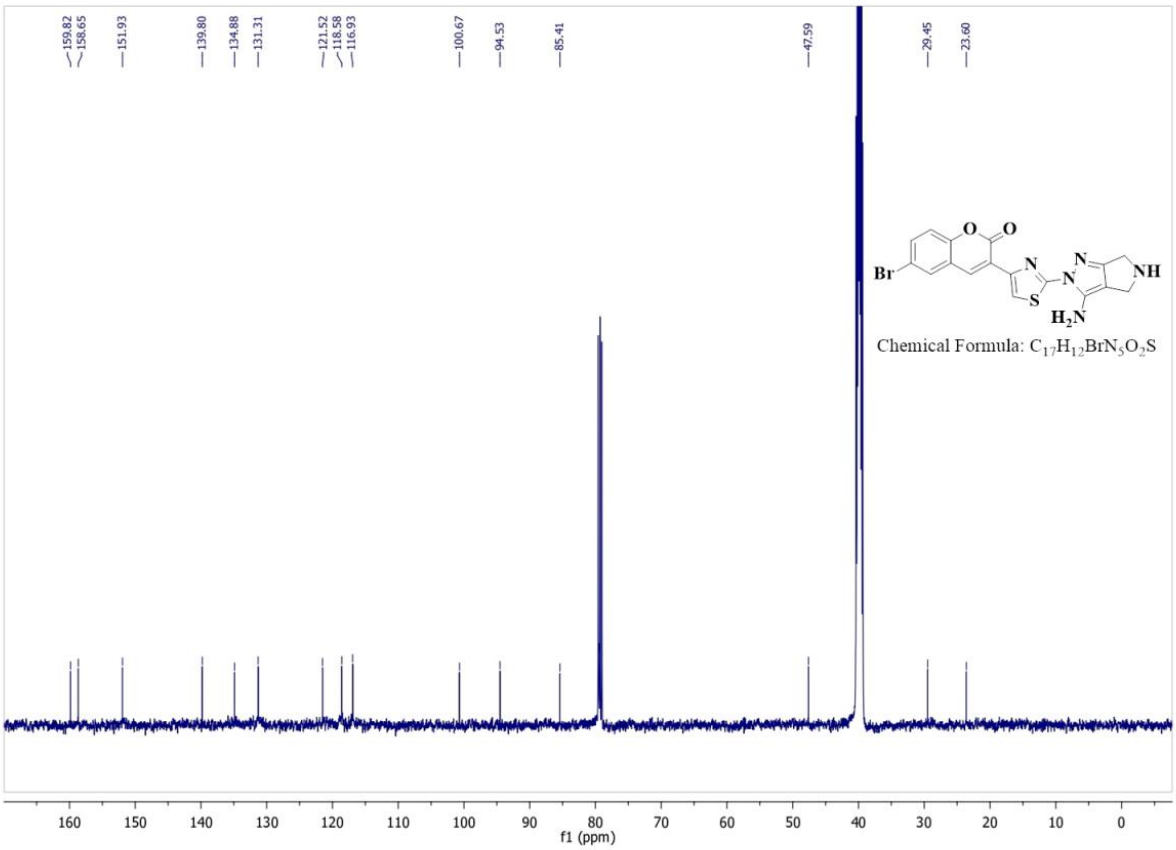
^{13}C NMR spectrum of compound **4f** (125 MHz, $CDCl_3$+DMSO-d_6)



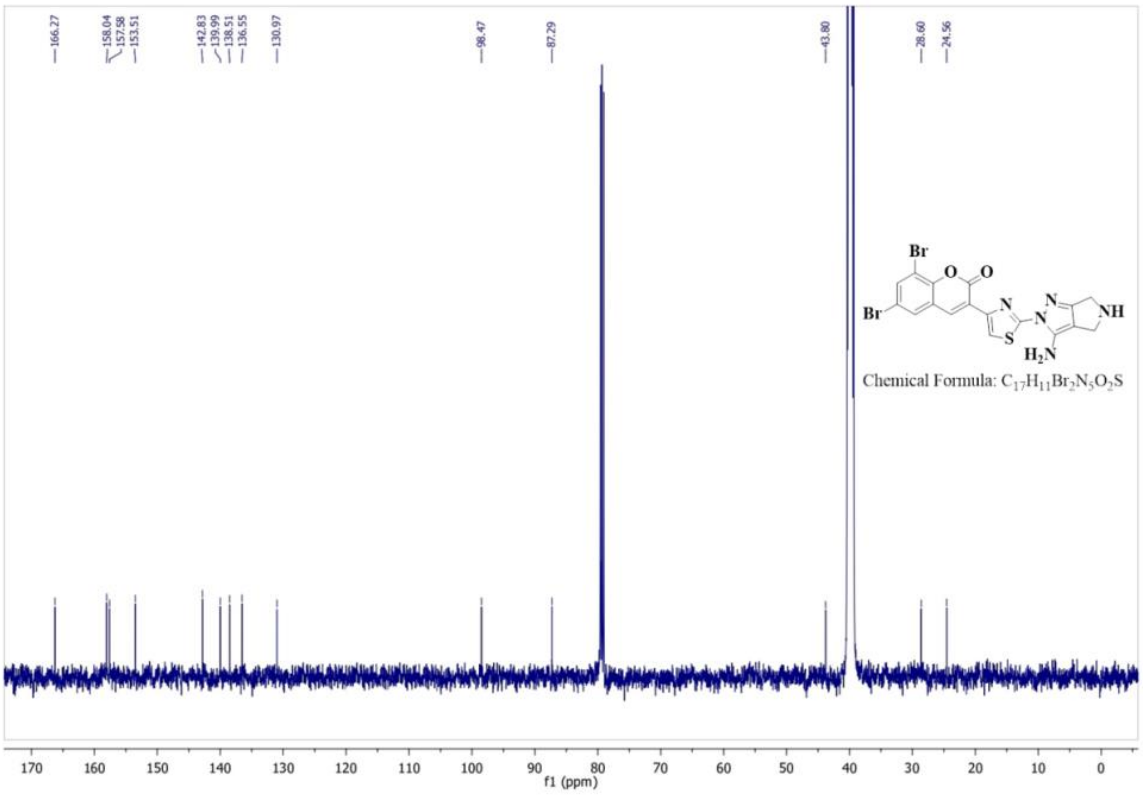
^{13}C NMR spectrum of compound **6a** (125 MHz, DMSO-d_6)



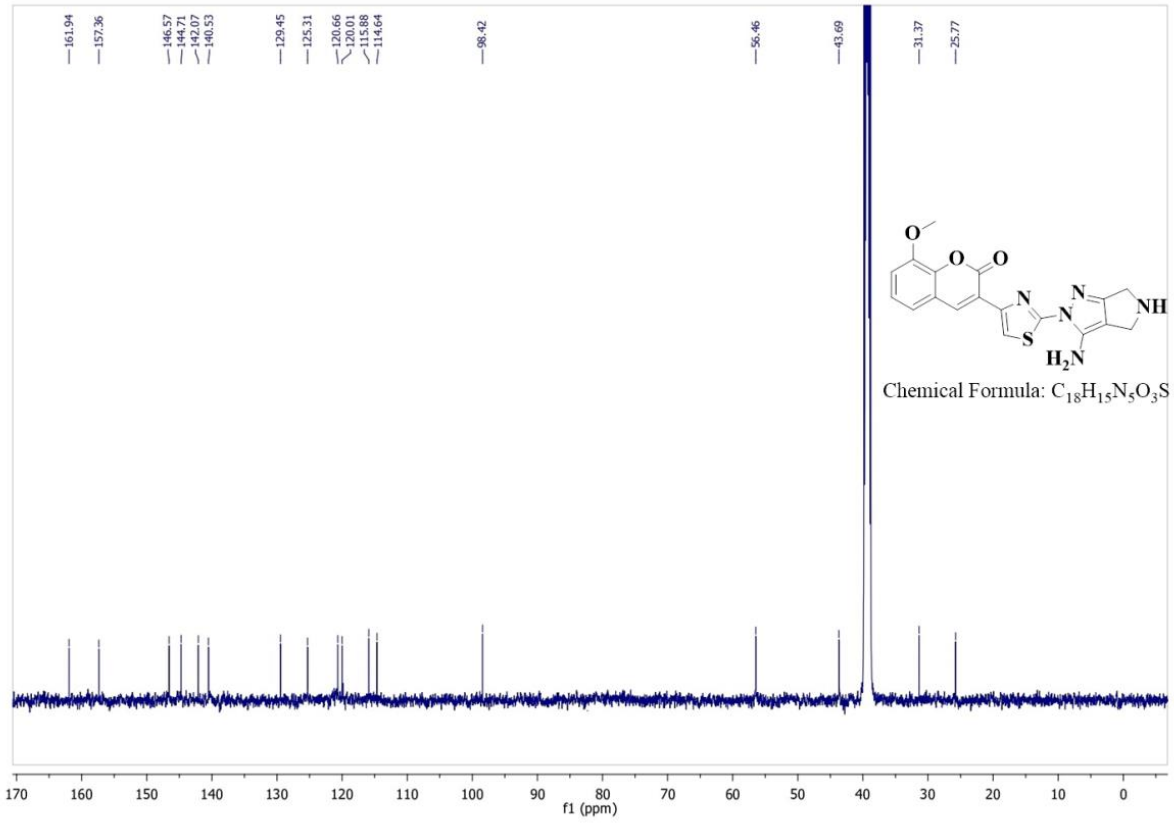
^{13}C NMR spectrum of compound **6b** (125 MHz, $CDCl_3$+DMSO-d_6)



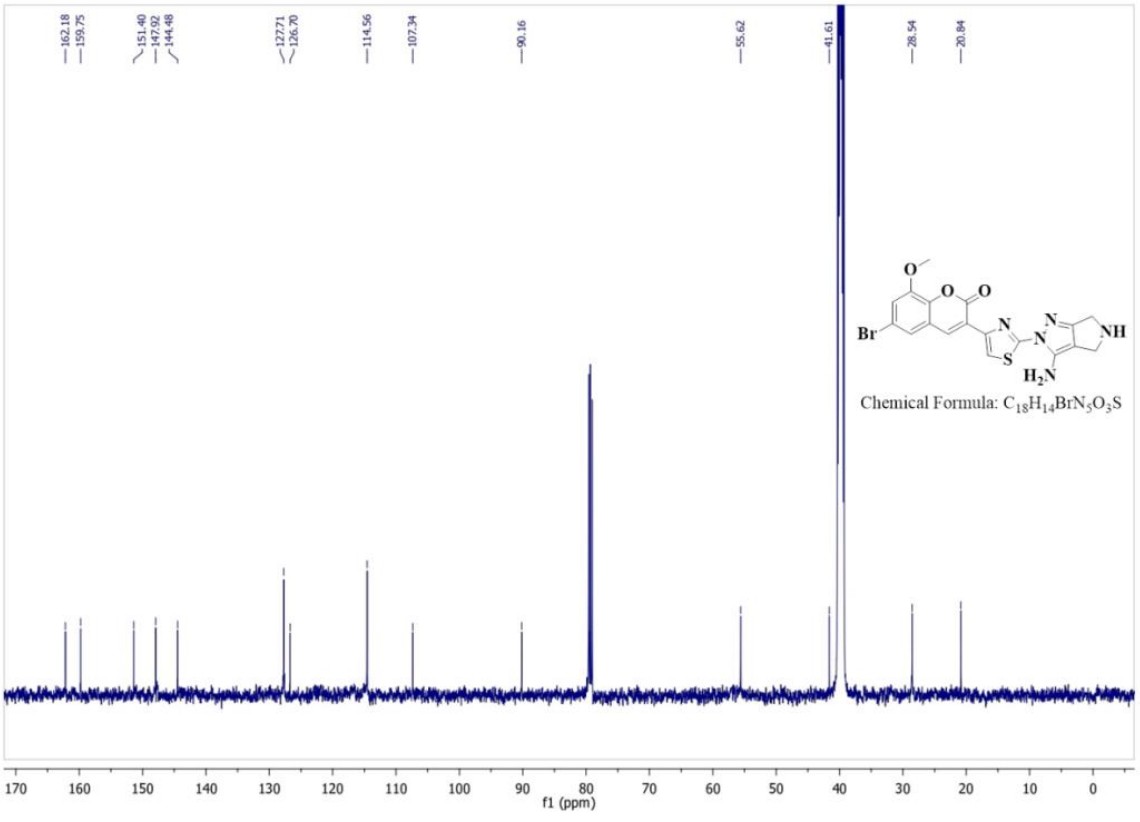
^{13}C NMR spectrum of compound **6c** (125 MHz, $CDCl_3$+DMSO-d_6)



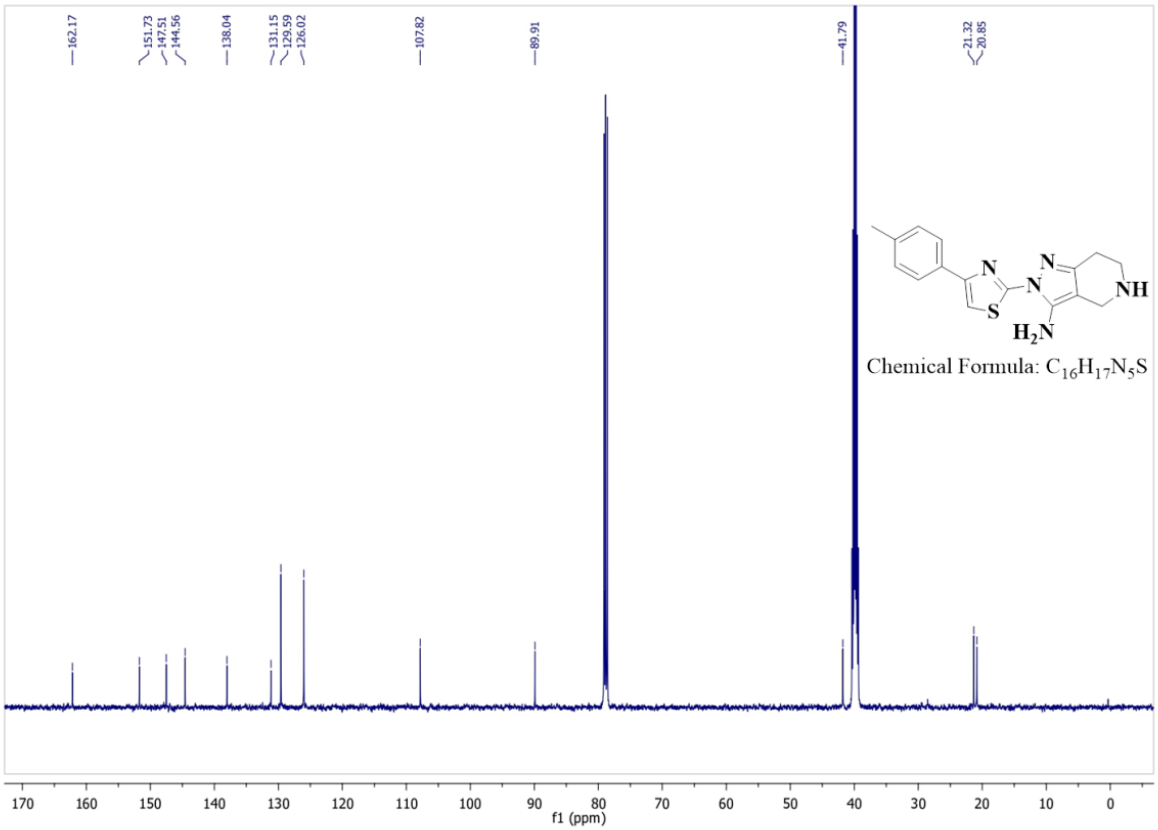
^{13}C NMR spectrum of compound **6d** (125 MHz, $CDCl_3$+DMSO-d_6)



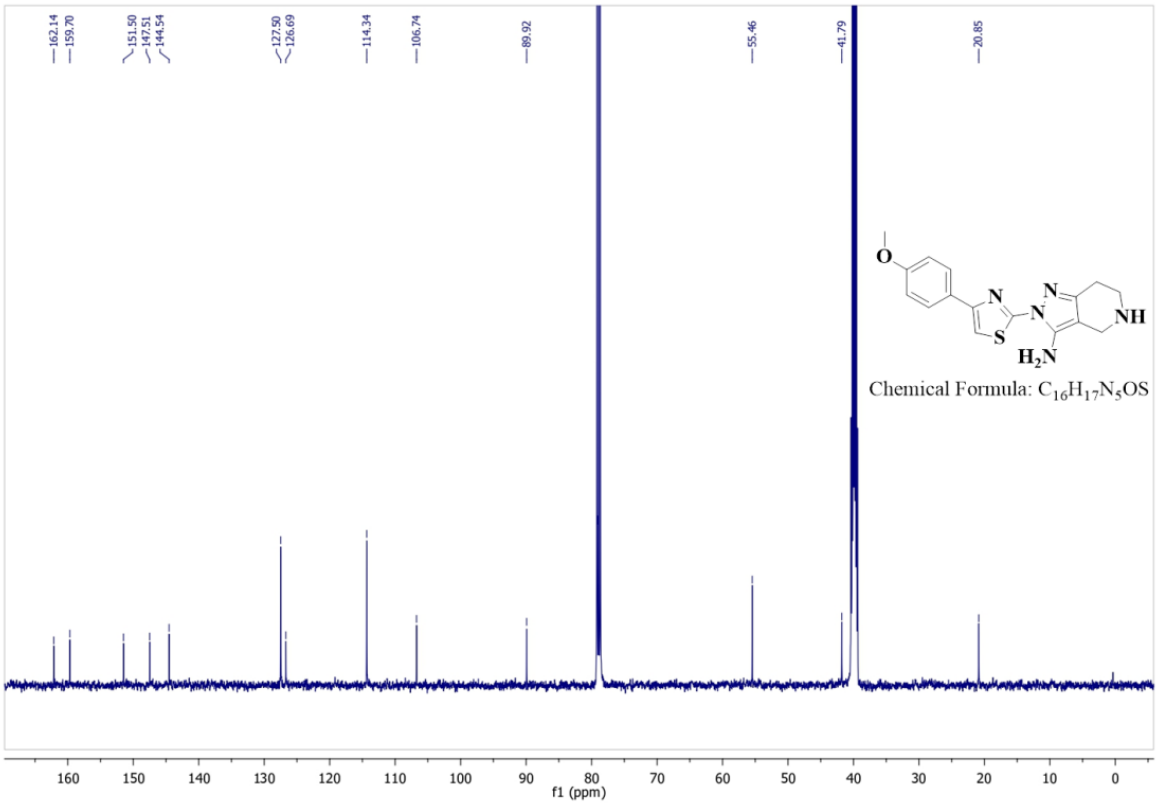
^{13}C NMR spectrum of compound **6e** (125 MHz, DMSO-d_6)



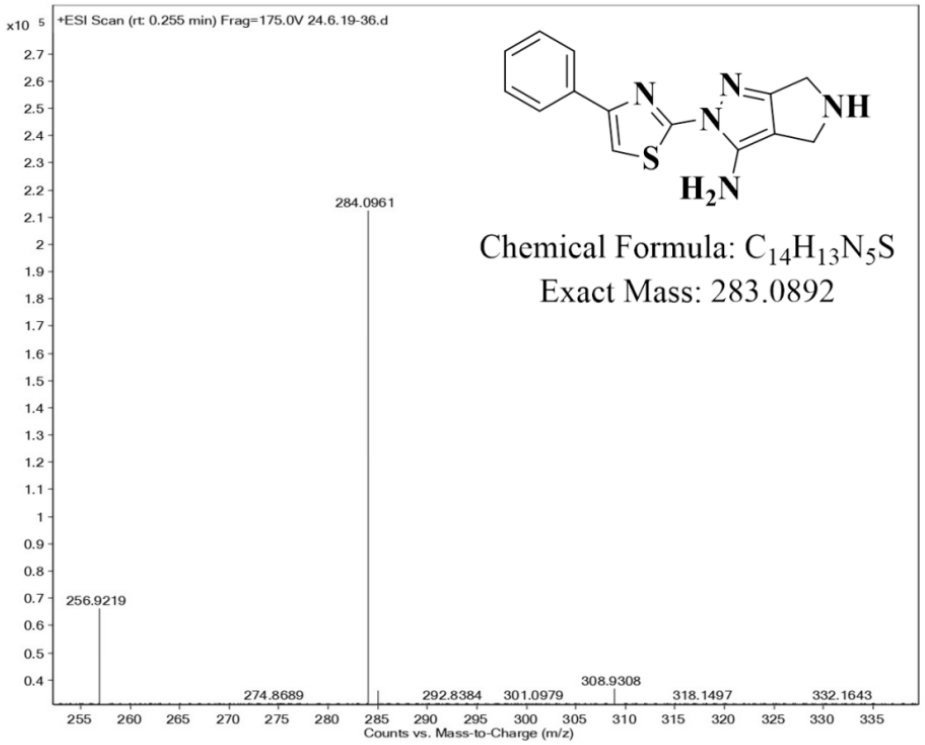
^{13}C NMR spectrum of compound **6f** (125 MHz, $CDCl_3$+DMSO-d_6)



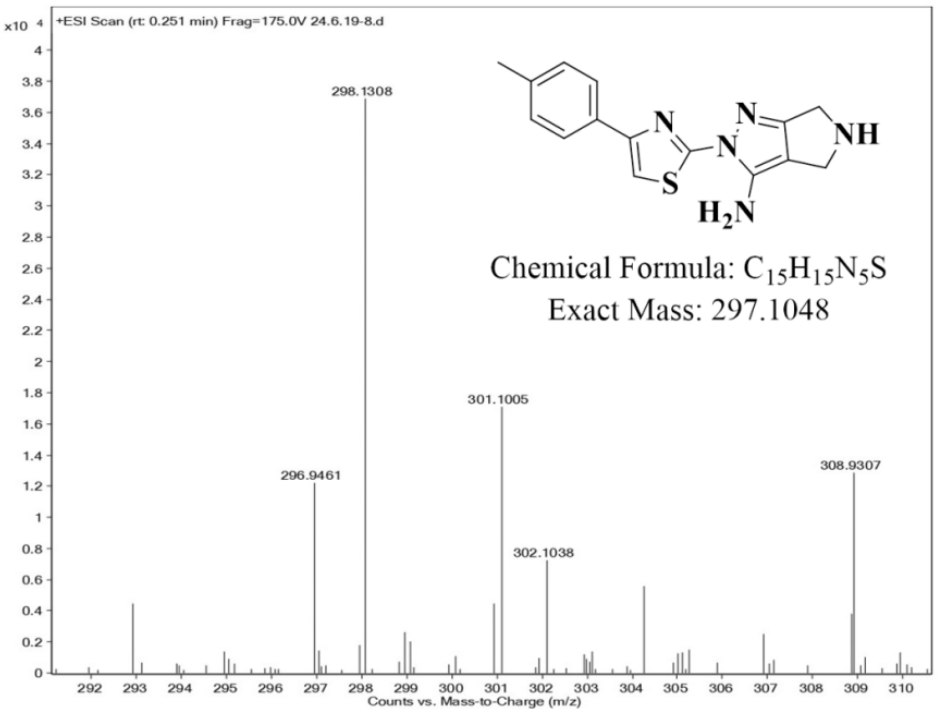
^{13}C NMR spectrum of compound **8a** (125 MHz, $CDCl_3$+DMSO-d_6)



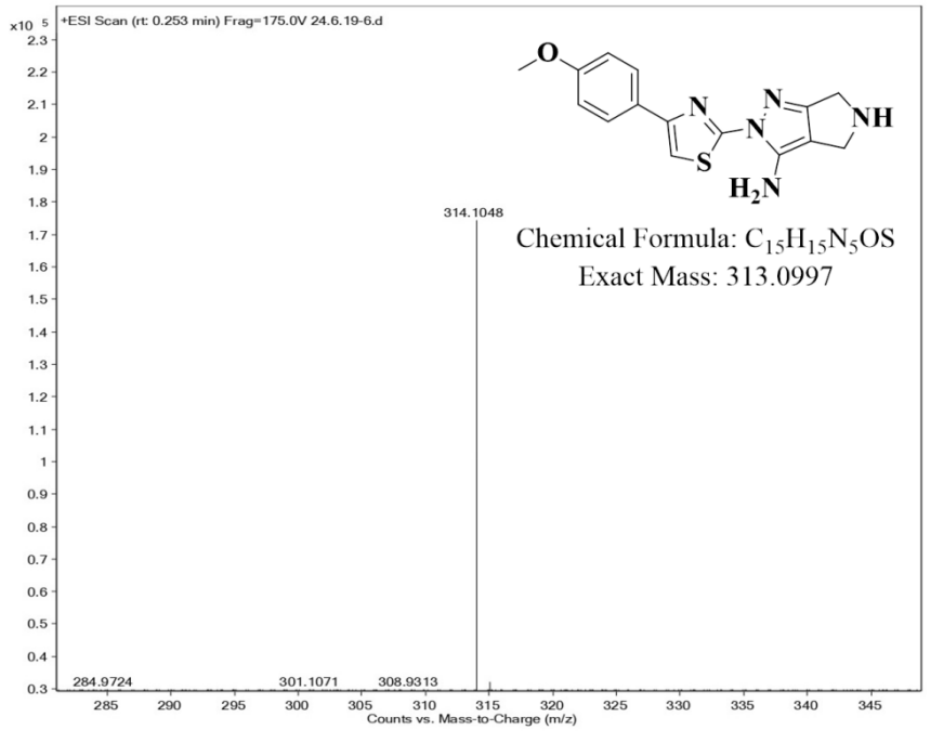
^{13}C NMR spectrum of compound **8b** (125 MHz, $CDCl_3$+DMSO-d_6)



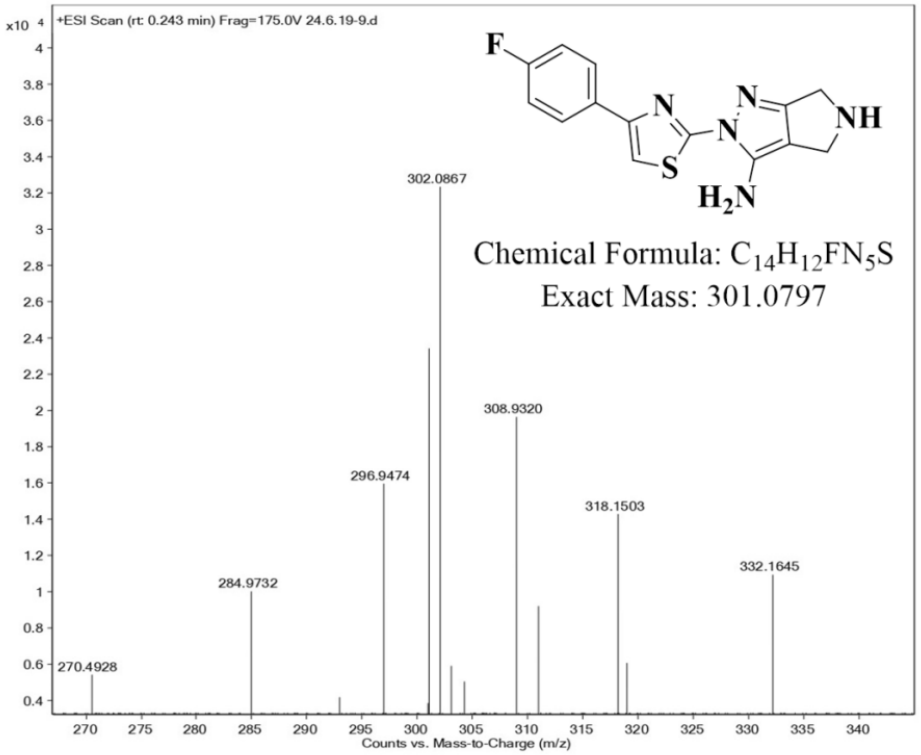
Mass spectrum of compound **4a**



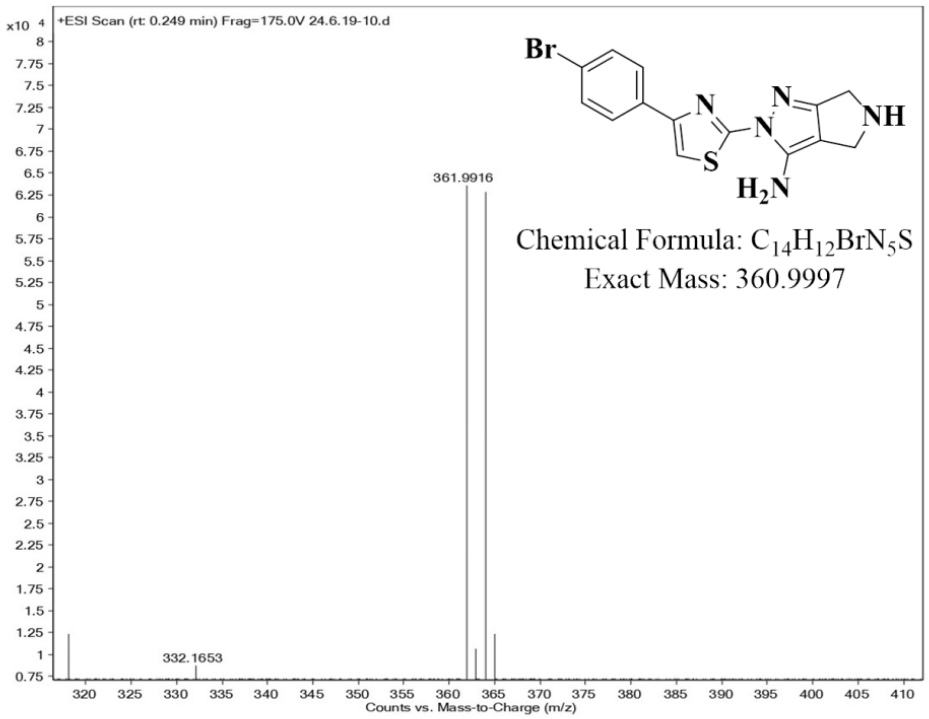
Mass spectrum of compound **4b**



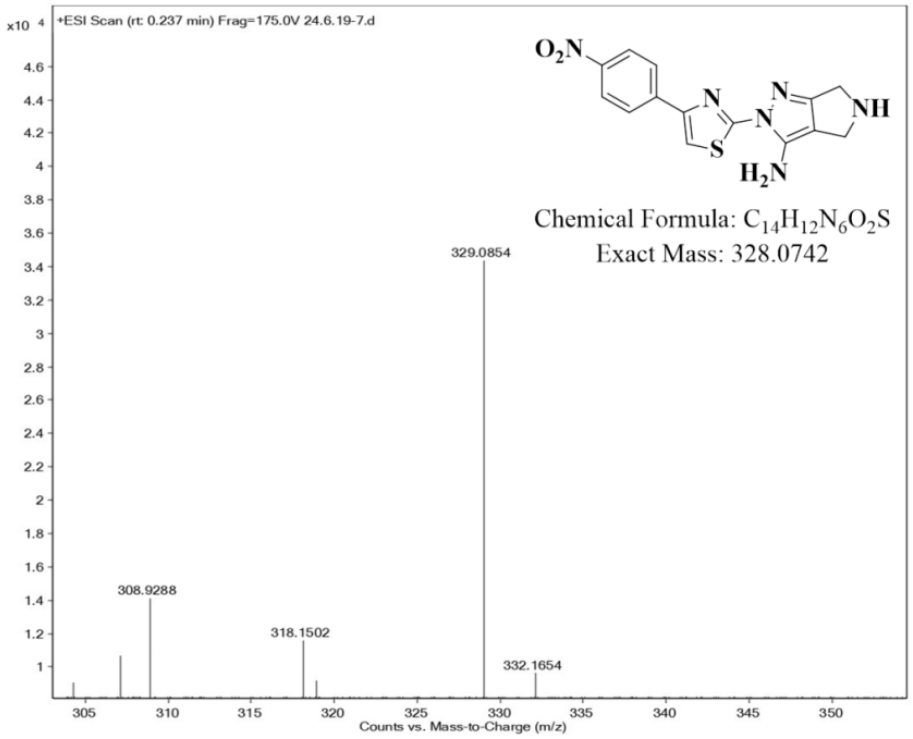
Mass spectrum of compound **4c**



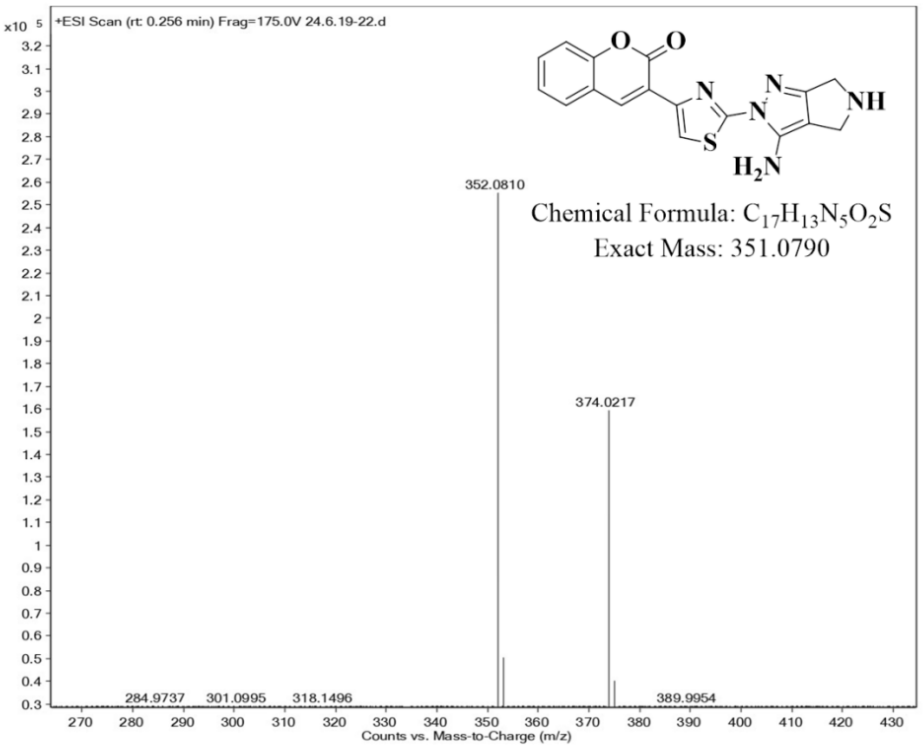
Mass spectrum of compound **4d**



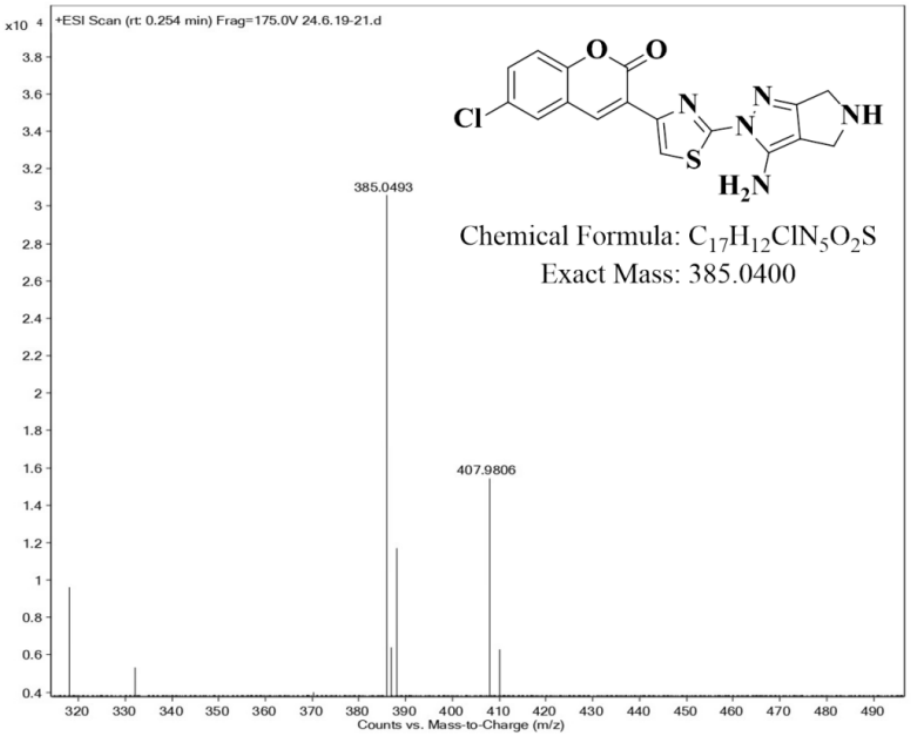
Mass spectrum of compound **4e**



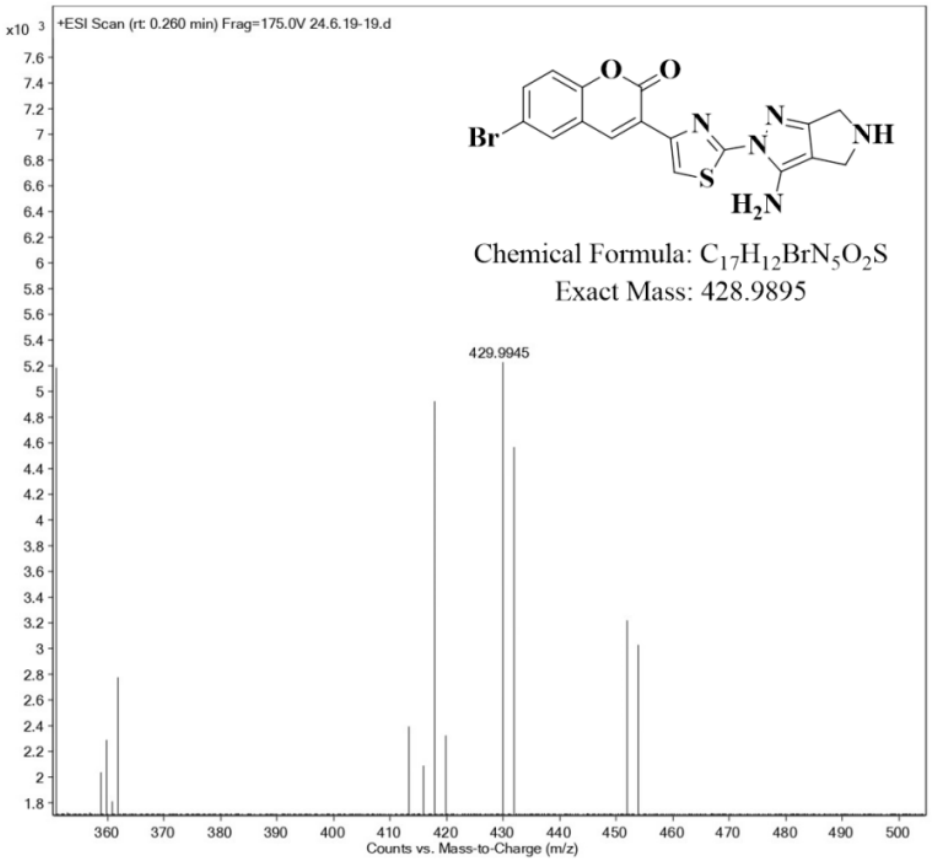
Mass spectrum of compound **4f**



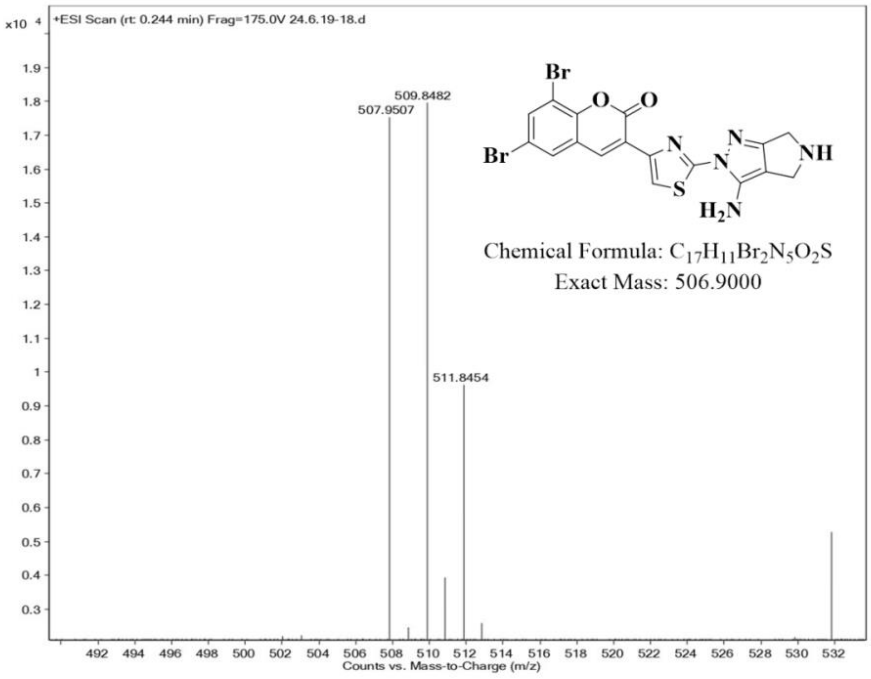
Mass spectrum of compound **6a**



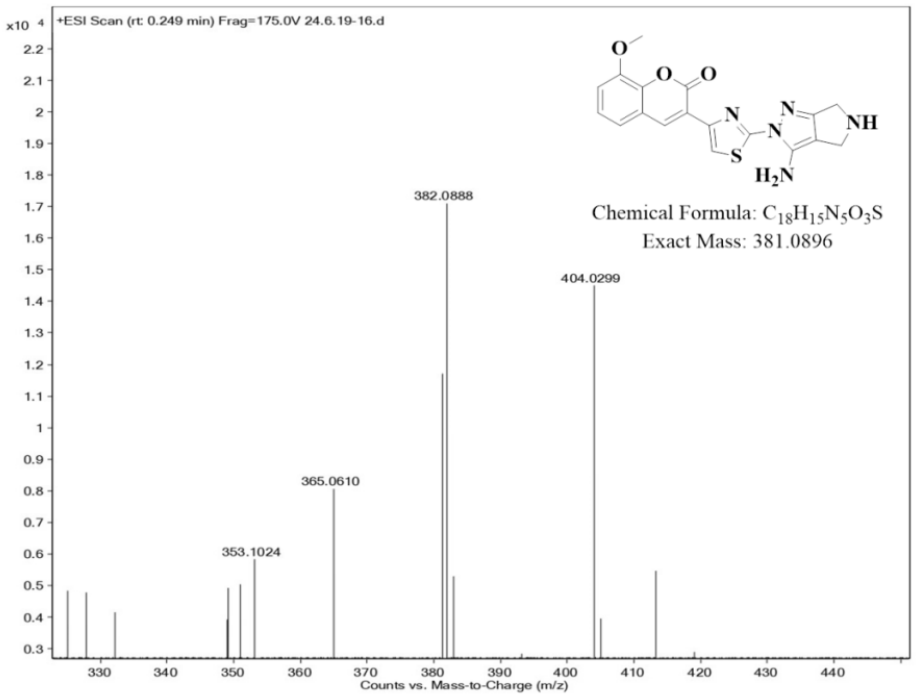
Mass spectrum of compound **6b**



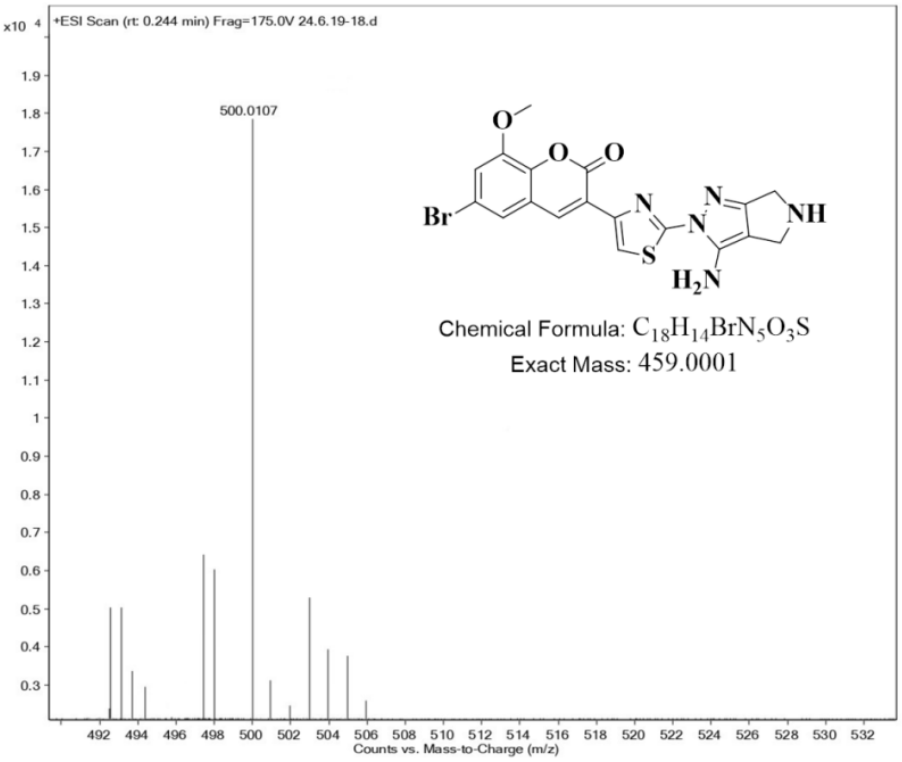
Mass spectrum of compound **6c**



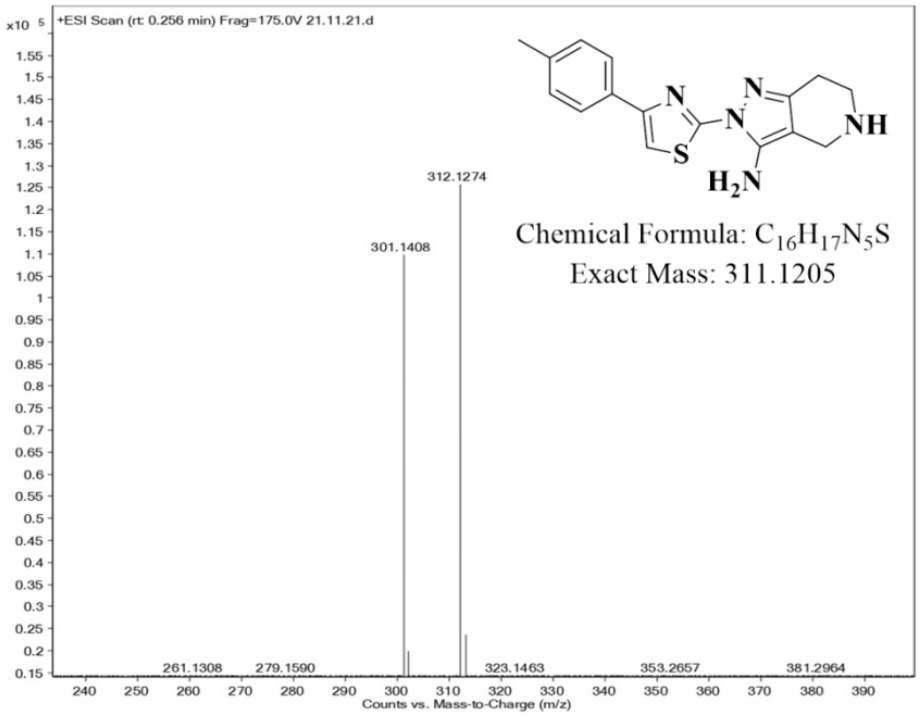
Mass spectrum of compound **6d**



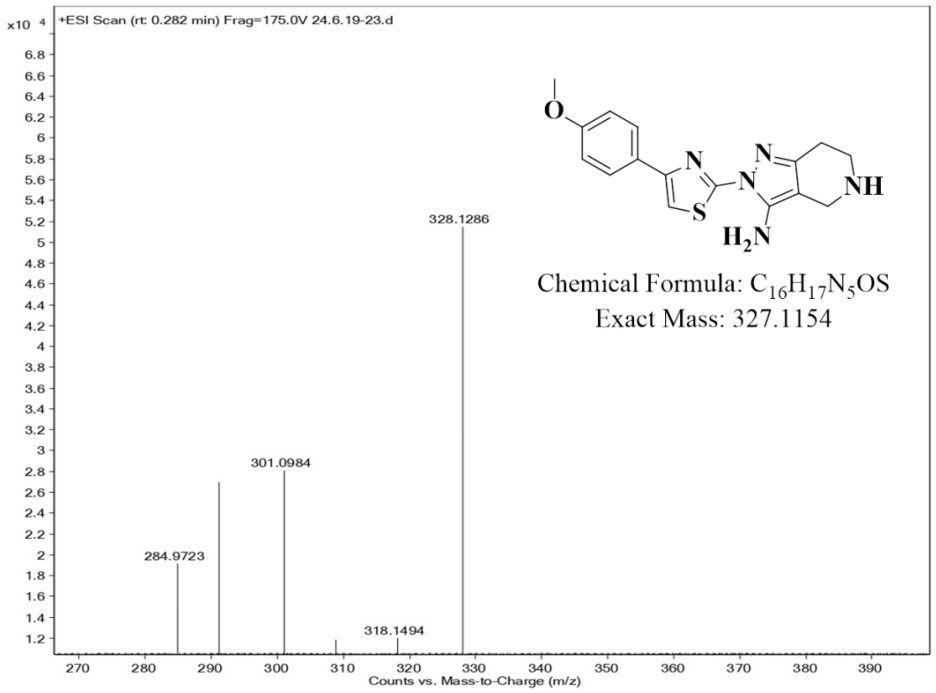
Mass spectrum of compound **6e**



Mass spectrum of compound **6f**



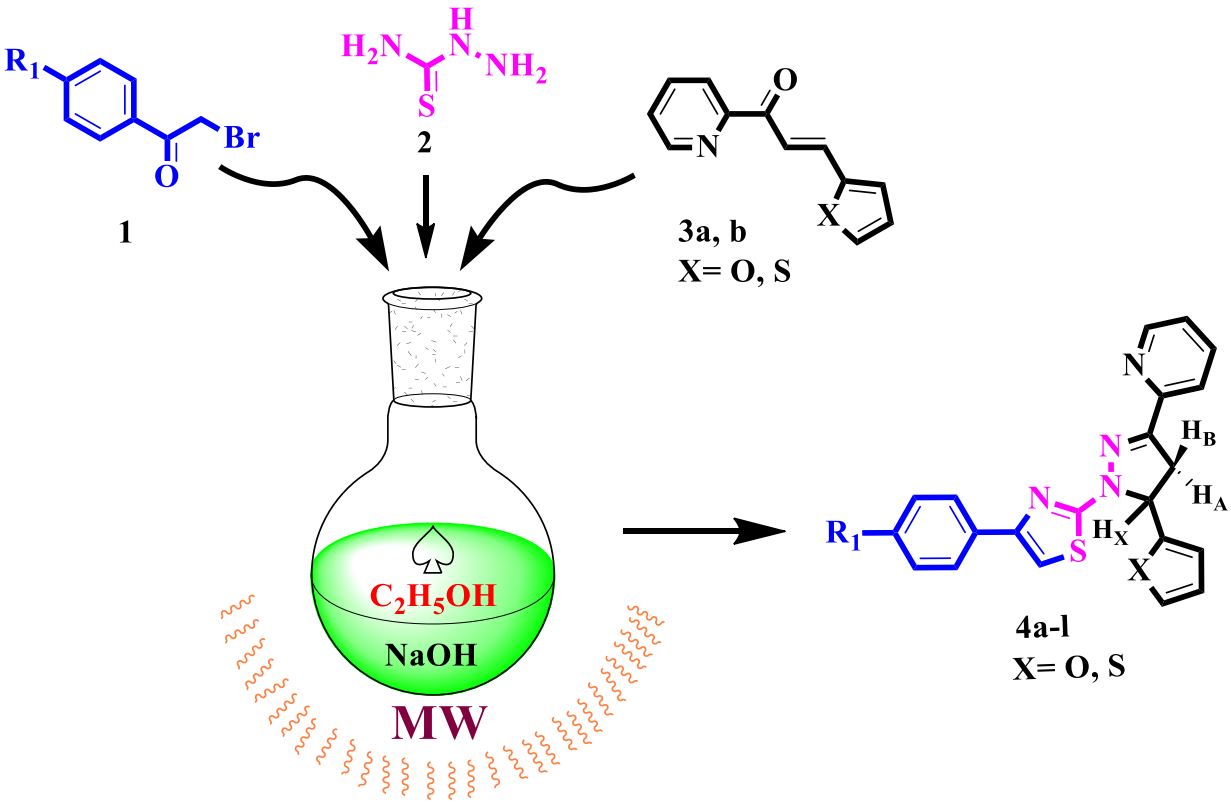
Mass spectrum of compound **8a**



Mass spectrum of compound **8b**

CHAPTER-III
SECTION-C

Microwave-assisted synthesis of new pyrazolothiazoles *via* multicomponent approach



CHAPTER-III
SECTION-C

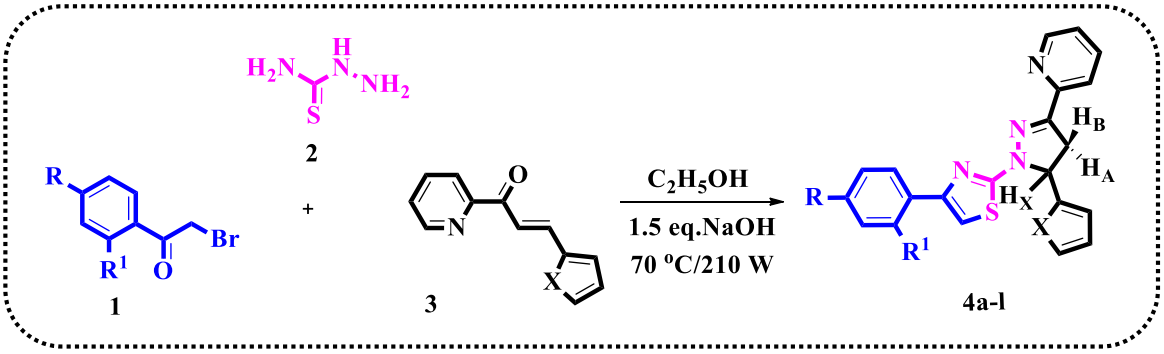
3C. Present work:

3C.1. Starting materials:

The present part describes the synthesis and biological activity evaluation of new pyrazolothiazole hybrids. The starting materials required for the synthesis of the title compounds are thiosemicarbazide, 3-(furan-2-yl)-1-(pyridin-2-yl)prop-2-en-1-one and 1-(pyridin-2-yl)-3-(thiophen-2-yl)prop-2-en-1-one with substituted 2-bromoacetophenones. Thiosemicarbazide, 2-bromoacetophenones were procured from commercial sources.

3C.2. Synthesis of pyrazolothiazoles:

Dihydropyrazoles are considered prime pharmacophores in many medicinal applications. In this 2-(5-(furan-2-yl)-3-(pyridin-2-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-phenylthiazoles (**4a-l**) were synthesized in presence of alcoholic NaOH solution starting from the reaction of substituted 2-bromoacetophenones (**1**) with thiosemicarbazide and (**2**) 3-(furan-2-yl)-1-(pyridin-2-yl)prop-2-en-1-one (**3a**) or 1-(pyridin-2-yl)-3-(thiophen-2-yl)prop-2-en-1-one (**3b**) under microwave irradiation (Scheme 3C.1 and 3C.2) with satisfactory yields (Table 3C.1).



Scheme 3C.1 Synthesis of pyrazolothiazoles; Reagents and conditions: EtOH, 1.5 eq NaOH, microwave irradiation at 70 ^{o}C and 210 W.

3C.3. Results and discussion:

Initially, in order to find out optimistic reaction conditions (in terms of suitable solvent, temperature and base) under conventional method, a test reaction was carried out in one-pot MCR approach by taking equimolar mixture of phenacyl bromide **1a**, thiosemicarbazide **2** and chalcone **3a** as starting materials in different solvents like MeOH, EtOH, DMSO, DMF and MeCN (Table 3C.1, entries 1–5) at different temperatures. From the primary screening results, it was observed that at room temperature, there is no transformation of reactants to products and also found that

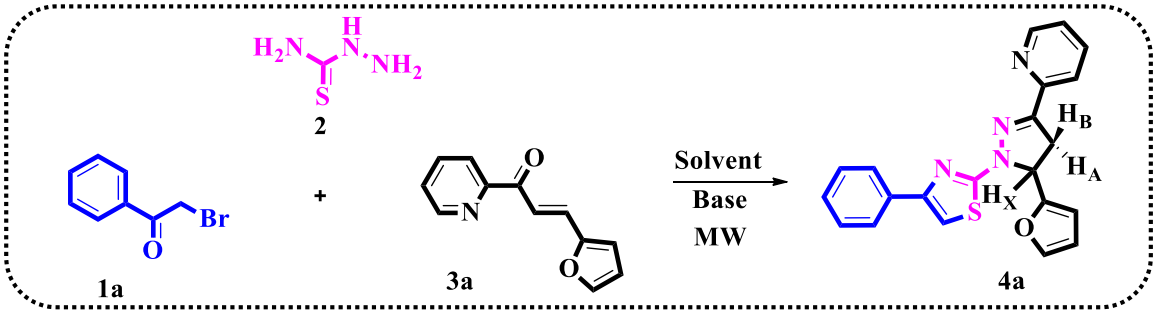
ethanol at 60 ^{o}C (Table 3C.1, entry 5) as the best solvent among the tested solvents in terms of yield and time. Further optimized the reaction in terms of base, screened. Some bases like K_2CO_3, Na_2CO_3, Et_3N, KOH and NaOH (Table 3C.1, entries 6–10) in an ethanolic solvent. It was observed from the test reactions that NaOH (Table 3C.1, entry 10) is the best among the tested bases, which has given optimum yields. In addition, further optimization of the reaction was carried out by the gradual increase of NaOH concentration from 0.5 to 2 (eq) (Table 3C.1, entries 10–13) and found the best conversion rate at NaOH concentration of 1.5 eq (Table 3C.1, entry 12). However, there is no further improvisation of the product yield (Table 3C.1, entry 14) beyond 60 ^{o}C rise in reaction temperature. Finally, lower time and highest conversion rate with the yield of 82% obtained at 60 ^{o}C.

In the same way, to know the feasibility of the reaction, the same model reaction as above was performed under microwave irradiation in different solvents and bases like as above (Table 3C.1, entries 1–10). And from the results, it was observed that the maximum transformation of reactants occurs in EtOH in the 1.5 eq of NaOH catalytic medium (Table 3C.1, entry 12). Further, temperature and power of microwave irradiation at which maximum yields would obtain were also tested (Table 3C.1, entries 10–13). It was found that lower time and highest conversion rate with the yield of 95% obtained at 70 ^{o}C/210 W (Table 3C.1, entry 14) and beyond the 70 ^{o}C/210 W. We observed there is no further enhancement in the yield of the product (Table 3C.1, entry 15). Finally, high yields of the desired product at shorter reaction time has been observed in ethanol at 70 ^{0}C in presence of NaOH (1.5 eq) under microwave (210 W).

Table 3C.1 Optimizing the reactions conditions under conventional and microwave conditions for the synthesis of pyrazolothiazoles ([a]Isolated yields).

Entry	**Solvent**	**Base (eq)**	**Conventional**			**Microwave**		
			Temperature (ºC)	**Time(h)**	**Yield(%)**	**Temperature (ºC/W)**	**Time(min)**	**Yield[a](%)**
1	MeOH	-	60	16	18	70/140	22	24
2	EtOH	-	60	12	22	70/140	16	33
3	DMSO	-	60	18	16	70/140	24	19
4	DMF	-	60	17	14	70/140	24	11
5	CH_3CN	-	60	20	12	70/140	29	16
6	EtOH	K_2CO_3(0.5)	60	14	30	70/140	18	39
7	EtOH	Na_2CO_3(0.5)	60	16	28	70/140	21	35
8	EtOH	Et_3N(0.5)	60	17	21	70/140	23	32
9	EtOH	KOH(0.5)	60	10	32	70/140	16	40
10	EtOH	NaOH(0.5)	60	10	41	70/140	14	48
11	EtOH	NaOH(1)	60	8	46	70/140	12	53
12	EtOH	NaOH(1.5)	60	4	82	70/140	9	65
13	EtOH	NaOH(2)	60	4	39	70/140	10	46
14	**EtOH**	**NaOH(1.5)**	reflux	4	52	**70/210**	**5**	**95**
15	EtOH	NaOH(1.5)	-	-	-	70/230	11	60

Utilizing the above-optimized conditions, the scope and efficiency of the the protocol was explored; whereby the target compounds (**4a-l**) were synthesized in excellent yields. In this substituted phenacyl bromide reacts with thiosemicarbazide to give Hantzsch thiazole product having hydrazino at the 2nd position of thiazole, this on further reaction with chalcones lead to the formation of final products.



Scheme 3C.2 Model reaction for the screening of optimized reaction conditions.

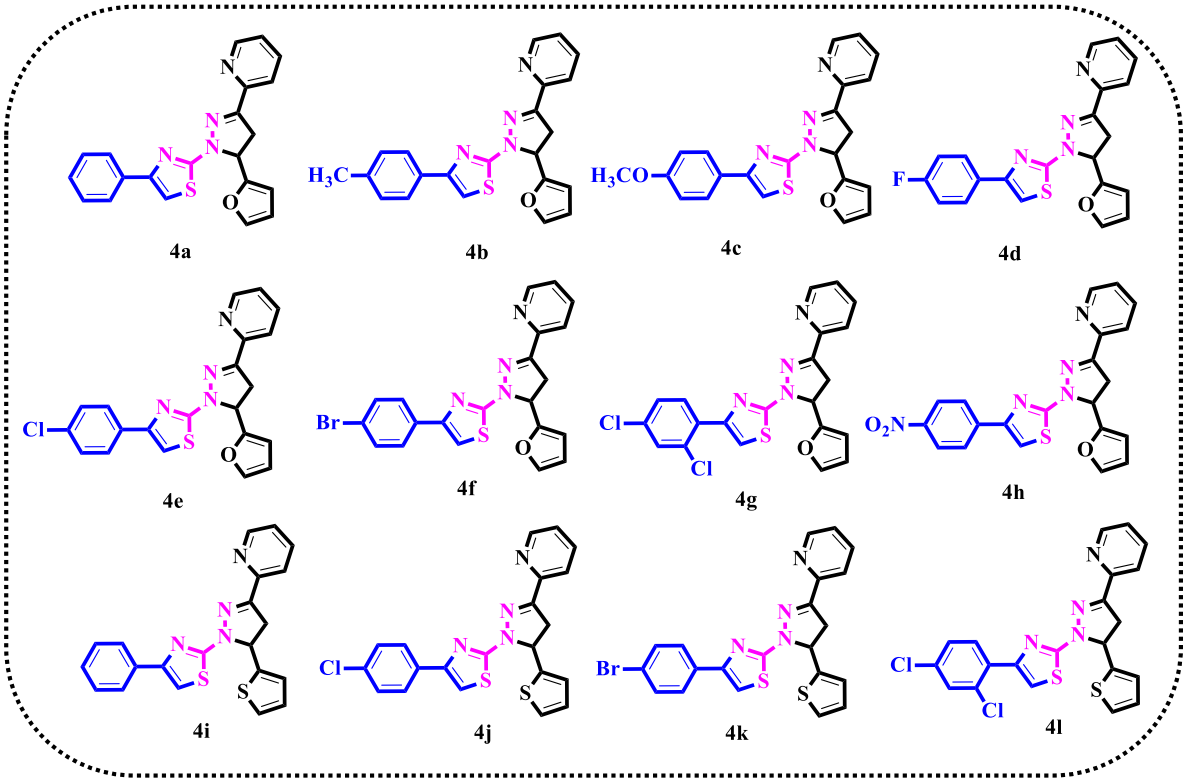


Figure 3C.1 pyrazolothiazoles hybrids (**4a-l**).

Table 3C.2: Different substitutions of pyrazolothiazole hybrids (**4a-l**), time, [a]isolated yield.

Entry	Product	R	R^1	X	Time(min)	Yield (%)[a]
1	**4a**	H	H	O	5	95
2	**4b**	CH3	H	O	6	93
3	**4c**	OCH3	H	O	6	92
4	**4d**	F	H	O	5	91
5	**4e**	Cl	H	O	6	88
6	**4f**	Br	H	O	5	96
7	**4g**	Cl	Cl	O	6	88
8	**4h**	NO2	H	O	5	90
9	**4i**	H	H	S	6	86
10	**4j**	Cl	H	S	5	81
11	**4k**	Br	H	S	6	87
12	**4l**	Cl	Cl	S	6	92

All the newly synthesized compounds structures (**4a-l**) were determined by their analytical and spectral data. In the ^{1}H-NMR spectra of all the compounds it was observed characteristic peaks of ABX pattern which corresponds to the 4th and 5th position protons of pyrazole ring, which exhibiting three double doublets (dd) ranging from δ 3.35-3.90 ppm (corresponds to pyrazole-H_A), δ 3.90-4.10 ppm (corresponds to pyrazole-H_B) and δ 5.67-5.99 ppm (corresponds to pyrazole-H_X). The thiazole 5th position proton in all the derivatives was observed as a singlet (s) from the range of δ 6.44 to 7.69 ppm. In the ^{13}C-NMR spectra of compounds (**4a-l**) it was observed two peaks in the range of δ 34.19 to 43.92 ppm, δ 52.52 to 62.78 ppm those correspond to pyrazole C_4 and C_5 respectively. And, thiazole C_2 appeared at the downfield region from the range of δ 163.27-167.40 ppm. Mass spectra of all the synthesized compounds have shown molecular ion (M^+) peak indicating their molecular formula.

3C.4. Conclusion:

In summary, we have developed a potential green protocol for the synthesis of new pyrazolothiazole derivatives by microwave assisted multi component reaction.

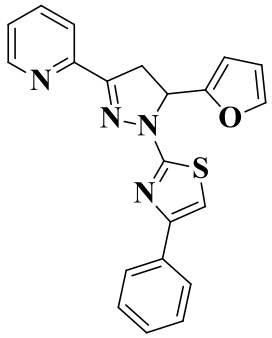
3C.5. Experimental:

General procedure for the Synthesis of **2-(5-(furan-2-yl)-3-(pyridin-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)-4-phenylthiazoles.**

An equimolar mixture of substituted phenacyl bromide (**1**), thiosemicarbazide (**2**), heteryl chalcone (**3**) and 1.5 eq of NaOH and ethanol as a solvent are placed in a 10 mL pressurized vial and subjected to MW irradiation (mono-mode, CEM Discover microwave synthesis system at 210 W) at a temperature of 70ºC for about 5–6 min; progress of the reaction was monitored by thin layer chromatography. After completion of the reaction, product was filtered and isolated, washed with ethanol, dried and recrystallized from ethanol.

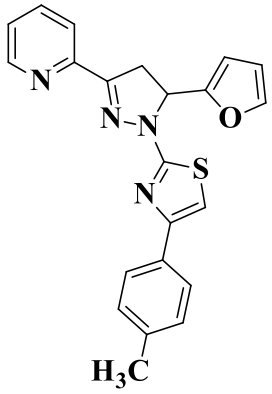
3C.6. Spectral data:

2-(5-(Furan-2-yl)-3-(pyridin-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)-4-phenylthiazole (4a):



Orange solid; yield 95%; mp: 148-150 ^{o}C; IR (KBr) cm^{-1}: 1605 (-C=N); ^{1}H NMR (400MHz, $CDCl_3$, ppm): δ 3.88 (dd, J = 18.4 Hz, 7.2 Hz, 1H, Pyrazole-H_A), 3.96 (dd, J = 14.4 Hz, 12 Hz, 1H, Pyrazole-H_B), 5.90 (dd, J = 8 Hz, 6.8 Hz, 1H, Pyrazole-H_X), 6.37 (dd, J = 3.2 Hz, 1.6 Hz, 1H, Ar-H), 6.59 (d, J = 2.4 Hz, 1H, Ar-H), 6.90 (s, 1H, Thiazole-H), 7.30-7.42 (m, 5H, Ar-H), 7.81-7.83 (m, 3H, Ar-H), 8.21 (d, J = 7.6 Hz, 1H, Ar-H), 8.66 (d, J = 4.4 Hz, 1H, Ar-H); ^{13}C NMR (100MHz, $CDCl_3$): δ 34.19, 53.73, 99.28, 104.31, 104.92, 105.77, 116.76, , 119.14, 121.20, 122.91, 123.79, 137.53, 144.63, 146.87, 158.71, 163.27 ppm; Mass (MS-ESI) (*m/z*): 373 $[M+H]^+$; Anal. Calcd. For $C_{21}H_{16}N_4OS$: C, 67.72; H, 4.33; N, 15.04%. Found: C, 67.76; H, 4.38; N, 15.12%.

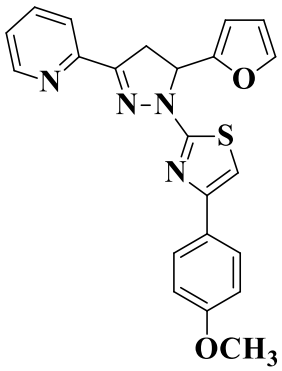
2-(5-(Furan-2-yl)-3-(pyridin-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)-4-(p-tolyl)thiazole (4b):



Orange solid; yield 93%; mp: 199-201 ^{o}C; IR (KBr) cm^{-1}: 1610 (-C=N); ^{1}H NMR (400MHz, $CDCl_3$, ppm): δ 2.32 (s, 3H, -CH_3), 3.87 (dd, J = 18 Hz, 6.8 Hz, 1H, Pyrazole-H_A), 3.98 (dd, J = 18.4 Hz, 12 Hz, 1H, Pyrazole-H_B), 5.99 (dd, J = 12 Hz, 6.4 Hz, 1H, Pyrazole-H_X), 6.29 (d, J = 2 Hz, 1H, Ar-H), 6.59 (d, J = 2.8 Hz, 1H, Ar-H), 6.80 (s, 1H, Thiazole-H), 7.15 (d, J = 8 Hz, 2H, Ar-H) 7.28 (s, 1H, Ar-H), 7.42 (t, J = 6.4 Hz, 1H, Ar-H), 7.64 (d, J = 8 Hz, 2H, Ar-H), 7.90 (t, J = 7.6 Hz, 1H, Ar-H), 8.21 (d, J = 8 Hz, 1H, Ar-H), 8.66 (d, J = 4.4 Hz, 1H, Ar-H); ^{13}C NMR (100MHz, $CDCl_3$): δ 26.09, 39.85, 62.59, 113.86, 114.02, 115.41, 129.12, 130.57, 132.80, 133.61,

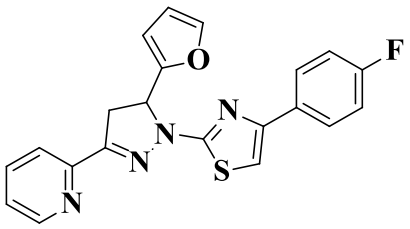
134.03, 142.65, 147.14, 154.39, 156.09, 156.23, 157.97, 167.40 ppm; Mass (MS-ESI) (*m/z*): 387 $[M+H]^+$; Anal. Calcd. For $C_{22}H_{18}N_4OS$: C, 68.37; H, 4.69; N, 14.50%. Found: C, 68.39; H, 4.6; N, 14.54%.

2-(5-(Furan-2-yl)-3-(pyridin-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)-4-(4-methoxyphenyl)thiazole (4c):



Brown solid; yield 92%; mp: 169-171 ^oC; IR (KBr) cm^{-1}: 1606 (-C=N); ^1H NMR (400MHz, $CDCl_3$, ppm): δ 3.82 (dd, J = 18.4 Hz, 7.2 Hz, 1H, Pyrazole-H_A), 3.85 (s, 3H, -OCH_3), 3.90 (dd, J = 18.4 Hz, 12 Hz, 1H, Pyrazole-H_B), 5.84 (dd, J = 11.6 Hz, 7.2 Hz, 1H, Pyrazole-H_X), 6.36 (dd, J = 2.8 Hz, 1.6 Hz, 1H, Ar-H), 6.56 (d, J = 4.8 Hz, 1H, Ar-H), 6.74 (s, 1H, Thiazole-H), 6.93 (d, J = 8.8 Hz, 2H, Ar-H), 7.30-7.32 (m, 1H, Ar-H), 7.36 (s, 1H, Ar-H), 7.76 (t, J = 7.2 Hz, 3H, Ar-H), 8.17 (d, J = 8 Hz, 1H, Ar-H), 8.63 (d, J = 4.8 Hz, 1H, Ar-H); ^{13}C NMR (100MHz, $CDCl_3$): δ 38.96, 55.33, 58.45, 102.14, 108.99, 110.50, 113.92, 121.45, 123.85, 127.24, 136.62, 142.25, 148.84, 151.25, 152.69, 159.27, 164.47 ppm; Mass (MS-ESI) (*m/z*): 403 $[M+1]^+$; Anal. Calcd. For $C_{22}H_{18}N_4O_2S$: C, 65.65; H, 4.51; N, 13.92%. Found: C, 65.69; H, 4.55; N, 13.99%.

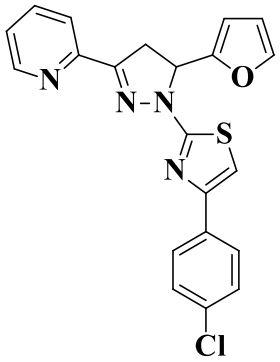
4-(4-Fluorophenyl)-2-(5-(furan-2-yl)-3-(pyridin-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)thiazole (4d):



Orange solid; yield 91%; mp: 192-194^oC; IR (KBr) cm^{-1}: 1608 (-C=N); ^1H NMR (400MHz, DMSO-d_6, ppm): δ 3.49 (dd, J = 17.6 Hz, 7.2 Hz, 1H, Pyrazole-H_A), 3.97 (dd, J = 18.4 Hz, 12 Hz, 1H, Pyrazole-H_B), 5.87 (dd, J = 12 Hz, 6.4 Hz, 1H, Pyrazole-H_X), 6.44 (s, 1H, Thiazole-H), 6.61

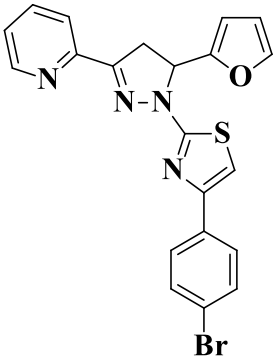
(d, *J* = 3.2 Hz, 1H, Ar-H), 7.21-7.33 (m, 2H, Ar-H), 7.39 (s, 1H, Ar-H), 7.52 (t, *J* = 6.8 Hz, 1H, Ar-H), 7.60 (s, 1H, Ar-H), 7.86-7.89 (m, 2H, Ar-H), 7.99 (t, *J* =7.2 Hz, 1H, Ar-H), 8.06 (d, *J* = 8 Hz, 1H, Ar-H), 8.68 (d, *J* = 4.4 Hz, 1H, Ar-H); ^{13}C NMR (100MHz, DMSO-d_6): δ 42.38, 58.34, 105.24, 109.34, 111.13, 115.82, 116.04 , 121.68, 125.14, 128.10, 131.49, 138.27, 143.30, 149.47, 149.66, 149.98, 152.23, 153.53, 164.35 ppm; Mass (MS-ESI) (*m/z*): 391 $[M+H]^+$; Anal. Calcd. For $C_{21}H_{15}FN_4OS$: C, 64.60; H, 3.87; N, 14.35%. Found: C, 64.65; H, 3.89; N, 14.30%.

4-(4-Chlorophenyl)-2-(5-(furan-2-yl)-3-(pyridin-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)thiazole (4e):



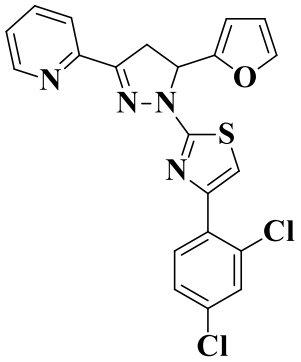
Orange solid; yield 88%; mp: 163-165ºC; IR (KBr) cm^{-1}: 1610 (-C=N); ^{1}H NMR (400MHz, DMSO-d_6, ppm): δ 3.59 (dd, *J* = 18 Hz, 7.2 Hz, 1H, Pyrazole-H_A), 3.97 (dd, *J* = 18 Hz, 12.4 Hz, 1H, Pyrazole-H_B), 5.87 (dd, *J* = 12 Hz, 6.8 Hz, 1H, Pyrazole-H_X), 6.44 (s, 1H, Thiazole-H), 6.60 (d, *J* = 2.8 Hz, 1H, Ar-H), 7.45 (s, 1H, Ar-H), 7.48 (d, *J* = 8.4 Hz, 2H, Ar-H), 7.52 (d, *J* = 6 Hz, 1H, Ar-H), 7.60 (s, 1H, Ar-H), 7.86 (d, *J* = 8.4 Hz, 2H, Ar-H), 7.97 (t, *J* =8 Hz, 1H, Ar-H), 8.06 (d, *J* = 7.6 Hz, 1H, Ar-H), 8.67 (d, *J* = 4.4 Hz, 1H, Ar-H); ^{13}C NMR (100MHz, DMSO-d_6): δ 43.92, 58.30, 106.28, 109.35, 111.13, 121.62, 125.12, 127.77, 129.11, 132.56, 133.72, 138.10, 143.32, 149.79, 152.21, 153.81, 164.40 ppm; Mass (MS-ESI) (*m/z*): 407 $[M+H]^+$; Anal. Calcd. For $C_{21}H_{15}ClN_4OS$: C, 61.99; H, 3.72; N, 13.77%. Found: C, 61.92; H, 3.76; N, 13.72%.

4-(4-Bromophenyl)-2-(5-(furan-2-yl)-3-(pyridin-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)thiazole (4f):



Orange solid; yield 96%; mp: 204-206 ^{o}C; IR (KBr) cm^{-1}: 1589 (-C=N); ^{1}H NMR (400MHz, $CDCl_3$, ppm): δ 3.90 (dd, J = 18 Hz, 7.2 Hz, 1H, Pyrazole-H_A), 4.01 (dd, J = 18.4 Hz, 12 Hz, 1H, Pyrazole-H_B), 5.89 (dd, J = 12 Hz, 6.4 Hz, 1H, Pyrazole-H_X), 6.36 (dd, J = 3.2 Hz, 2 Hz, 1H, Ar-H), 6.56 (d, J = 3.2 Hz, 1H, Ar-H), 6.90 (s, 1H, Thiazole-H), 7.35 (s, 1H, Ar-H), 7.41 (t, J = 6.4 Hz, 1H, Ar-H), 7.51 (d, J = 8.4 Hz, 2H, Ar-H), 7.67 (d, J = 8.4 Hz, 2H, Ar-H), 7.89 (t, J = 7.6 Hz, 1H, Ar-H), 8.24 (d, J = 8 Hz, 1H), 8.69 (d, J = 4.8 Hz, 1H); ^{13}C NMR (100MHz, $CDCl_3$): δ 35.19, 52.52, 99.09, 103.21, 104.70, 118.45, 121.61, 123.85, 125.30, 125.65, 127.78. 131.92, 136.45, 144.21, 145.53, 146.05, 156.89, 158.37, 165.49 ppm; Mass (MS-ESI) (*m/z*): 451.35 $[M+]^{+}$; Anal. Calcd. For $C_{21}H_{15}BrN_4OS$: C, 55.88; H, 3.35; N, 12.41%. Found : C, 55.83; H, 3.39; N, 12.46%.

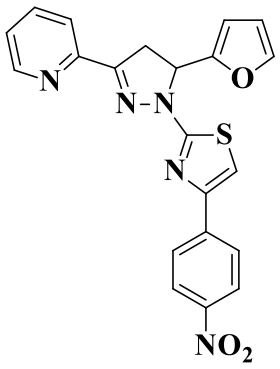
4-(2,4-Dichlorophenyl)-2-(5-(furan-2-yl)-3-(pyridin-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)thiazole (4g):



Light green solid; yield 88%; mp: 146-148 ^{o}C; IR (KBr) cm^{-1}: 1612 (-C=N); ^{1}H NMR (400MHz, $CDCl_3$, ppm): δ 3.81 (dd, J = 18.4 Hz, 7.2 Hz, 1H, Pyrazole-H_A), 3.91 (dd, J = 18.4 Hz, 12 Hz, 1H, Pyrazole-H_B), 5.79 (dd, J = 12 Hz, 6.8 Hz, 1H, Pyrazole-H_X), 6.36 (dd, J = 2.8 Hz, 2 Hz, 1H, Ar-H), 6.49 (d, J = 3.2 Hz, 1H, Ar-H), 7.29 (s, 1H, Thiazole-H), 7.32-7.30 (m, 2H, Ar-H), 7.36 (s, 1H, Ar-H), 7.44 (d, J = 2 Hz, 1H, Ar-H), 7.77 (t, J = 7.6 Hz, 1H, Ar-H), 7.86 (d, J = 8.8 Hz, 1H, Ar-H), 8.17 (d, J = 8 Hz, 1H, Ar-H), 8.63 (d, J = 4.8 Hz, 1H, Ar-H); ^{13}C NMR (100MHz, $CDCl_3$): δ 39.24, 58.39, 108.74, 109.95, 110.46, 121.47, 123.95, 127.15, 130.12, 131.92, 132.13,

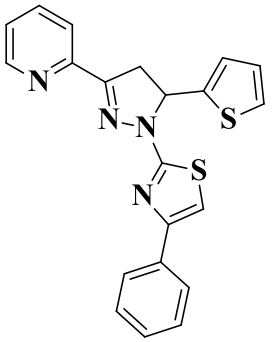
132.17, 133.25, 136.67, 142.32, 146.85, 148.88, 151.78, 163.50 ppm; Mass (MS-ESI) (*m/z*): 441.15 $[M+]^+$; Anal. Calcd. For $C_{21}H_{14}Cl_2N_4OS$: C, 57.15; H, 3.20; N, 12.69%. Found: C, 57.19; H, 3.24; N, 12.74%.

2-(5-(Furan-2-yl)-3-(pyridin-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)-4-(4-nitrophenyl)thiazole (4h):



Yellow solid; yield 90%; mp: 212-214^{o}C; IR (KBr) cm^{-1}: 1595 (-C=N); ^{1}H NMR (400MHz, DMSO-d_6, ppm): δ 3.62 (dd, J = 18.4 Hz, 6.4 Hz, 1H, Pyrazole-H_A), 3.99 (dd, J = 18.4 Hz, 12 Hz, 1H, Pyrazole-H_B), 5.88 (dd, J = 12 Hz, 6.4 Hz, 1H, Pyrazole-H_X), 6.45 (s, 1H,Thiazole-H), 6.65 (d, J = 2.8 Hz, 1H, Ar-H), 7.50 (t, J =5.6Hz, 2H, Ar-H), 7.62 (d, J = 4.8 Hz, 1H, Ar-H), 7.78 (s, 1H, Ar-H), 7.96 (t, J =7.2 Hz, 1H, Ar-H), 8.04-8.11 (m, 3H, Ar-H), , 8.30 (dd, J = 23.2 Hz, 8.8 Hz, 1H, Ar-H), 8.67 (d, J = 4.4 Hz, 1H, Ar-H); ^{13}C NMR (100MHz, DMSO-d_6): δ 42.93, 58.26, 109.52, 110.25, 111.17, 121.55, 124.59, 125.15, 126.90, 129.13, 137.84, 140.84, 143.38, 146.80, 149.82, 152.06, 154.41, 164.64 ppm; Mass (MS-ESI) (*m/z*): 418 $[M+H]^+$; Anal. Calcd. For $C_{21}H_{15}N_5O_3S$: C, 60.42; H, 3.62; N, 16.78%. Found: C, 60.46; H, 3.66; N, 16.73%

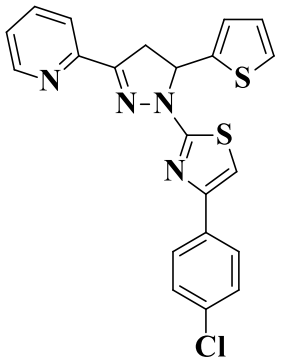
4-Phenyl-2-(3-(pyridin-2-yl)-5-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)thiazole (4i):



Pale yellow solid; yield 86%; mp: 154-156^{o}C; IR (KBr) cm^{-1}: 1618 (-C=N); ^{1}H NMR (400MHz, $CDCl_3$, ppm): δ 3.35 (dd, J = 17.2 Hz, 7.2 Hz, 1H, Pyrazole-H_A), 3.98 (dd, J = 17.2 Hz, 12 Hz,

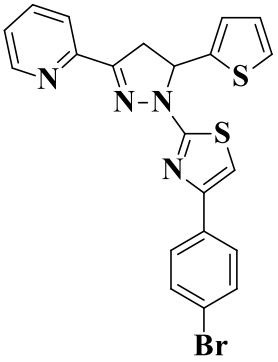
1H, Pyrazole-H_B), 5.71 (dd, *J* = 12 Hz, 7.2 Hz, 1H, Pyrazole-H_X), 6.86 (s, 1H, Thiazole-H), 7.10 (t, *J* = 4.4 Hz, 2 Hz, 1H, Ar-H), 7.21-7.26 (m, 2H, Ar-H), 7.32 (d, *J* = 8 Hz, 3H, Ar-H), 7.45 (d, *J* = 4.8 Hz, 1H, Ar-H), 7.65 (d, *J* = 8.4 Hz, 2H, Ar-H), 7.76 (d, *J* = 7.6 Hz, 1H, Ar-H), 8.57 (d, *J* = 4.4 Hz, 1H, Ar-H), 8.80 (s, 1H, Ar-H); ^{13}C NMR (100MHz, $CDCl_3$): δ 43.82, 62.58, 104.08, 124.30, 125.78, 127.65, 127.69, 128.15, 128.54, 128.61, 134.49, 134.62, 135.79, 137.91, 147.41, 147.64, 151.50, 164.73 ppm; Mass (MS-ESI) (*m/z*): 389 $[M+H]^+$; Anal. Calcd. For $C_{21}H_{16}N_4S_2$: C, 70.86; H, 4.31; N, 8.55%. Found: C, 70.82; H, 4.35; N, 8.51%.

4-(4-Chlorophenyl)-2-(3-(pyridin-2-yl)-5-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)thiazole (4j):



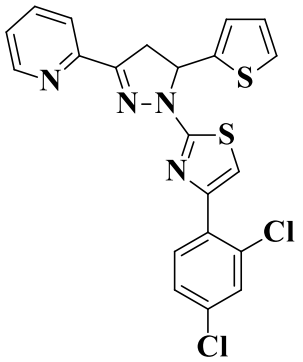
Pale yellow solid; yield 81%; mp: 203-205^{o}C; IR (KBr) cm^{-1}: 1602 (-C=N); ^{1}H NMR (400MHz, DMSO-d_6, ppm): δ 3.52 (dd, *J* = 18 Hz, 7.2 Hz, 1H, Pyrazole-H_A), 4.10 (dd, *J* = 18 Hz, 11.6 Hz, 1H, Pyrazole-H_B), 5.74 (dd, *J* = 12 Hz, 7.2 Hz, 1H, Pyrazole-H_X), 7.17-7.21 (m, 1H, Ar-H), 7.34 (d, *J* = 8.8 Hz, 1H, Ar-H), 7.40 (s, 1H, Thiazole-H) 7.41-7.43 (m, 2H, Ar-H), 7.69 (s, 1H, Ar-H), 7.71 (s, 1H, Ar-H), 7.77 (s, 1H, Ar-H), 7.85-7.88 (m, 1H, Ar-H), 8.04 (d, *J* =8.8 Hz, 1H, Ar-H), 8.53 (dd, *J* = 4.8 Hz, 1.6 Hz, 1H, Ar-H), 8.74 (d, *J* = 2 Hz, 1H, Ar-H); ^{13}C NMR (100MHz, DMSO-d_6): δ 43.79, 62.75, 106.04, 106.56, 127.60, 129.04, 129.04, 129.98, 131.84, 132.51, 132.66, 133.61, 133.78, 134.16, 149.61, 149.84, 150.07, 164.86 ppm; Mass (MS-ESI) (*m/z*): 423 $[M+H]^+$; Anal. Calcd. For $C_{21}H_{15}ClN_4S_2$: C, 59.63; H, 3.57; N, 13.25%. Found: C, 59.68; H, 3.61; N, 13.20%.

4-(4-Bromophenyl)-2-(3-(pyridin-2-yl)-5-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)thiazole (4k):



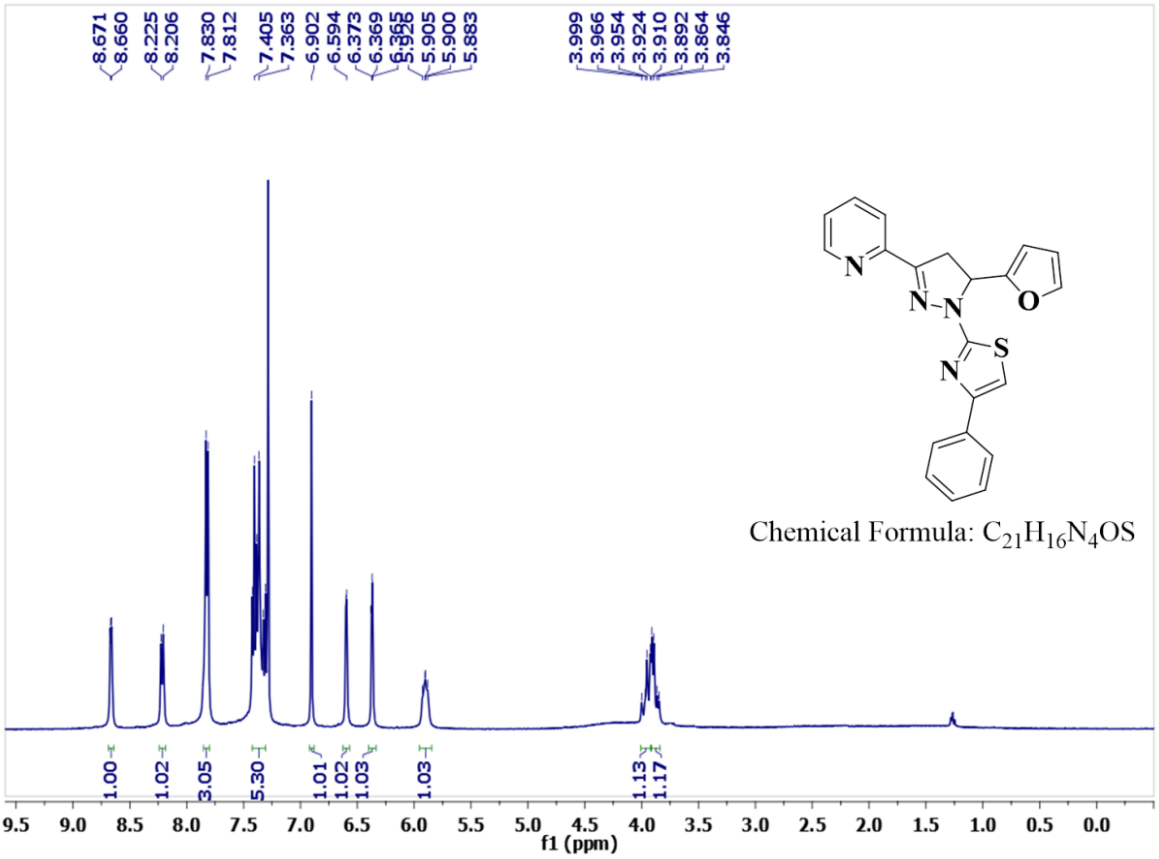
Pale yellow solid; yield 87%; mp: 209-211°C; IR (KBr) cm^{-1}: 1582 (-C=N); ^1H NMR (400MHz, DMSO-d_6, ppm): δ 3.51 (dd, J = 17.6 Hz, 7.2 Hz, 1H, Pyrazole-H_A), 4.10 (dd, J = 17.6 Hz, 12 Hz, 1H, Pyrazole-H_B), 5.74 (dd, J = 12 Hz, 7.2 Hz, 1H, Pyrazole-H_X), 7.19 (dd, J = 4.8 Hz, 3.6 Hz, 1H, Ar-H), 7.41-7.45 (m, 2H, Ar-H), 7.47 (dd, J = 3.6 Hz, 0.9 Hz, 1H, Ar-H), 7.53 (s, 1H, Thiazole-H), 7.55 (s, 1H, Ar-H), 7.62 (s, 1H, Ar-H), 7.64 (s, 1H, Ar-H), 7.76 (dd, J = 5.2 Hz, 0.8 Hz, 1H, Ar-H), 7.83-7.86 (m, 1H, Ar-H), 8.52 (dd, J = 4.8 Hz, 1.6 Hz, 1H, Ar-H), 8.73 (d, J = 2 Hz, 1H, Ar-H); ^{13}C NMR (100MHz, DMSO-d_6): δ 43.83, 62.78, 106.08, 121.11, 124.54, 127.90, 128.59, 129.96, 130.50, 131.94, 133.98, 134.19, 149.66, 149.82, 164.83 ppm; Mass (MS-ESI) (*m/z*): 468 $[M+H]^+$; Anal. Calcd. For $C_{21}H_{15}BrN_4S_2$: C, 53.96; H, 3.23; N, 11.99%. Found: C, 53.91; H, 3.27; N, 11.93%.

4-(2,4-Dichlorophenyl)-2-(3-(pyridin-2-yl)-5-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)thiazole (4l):

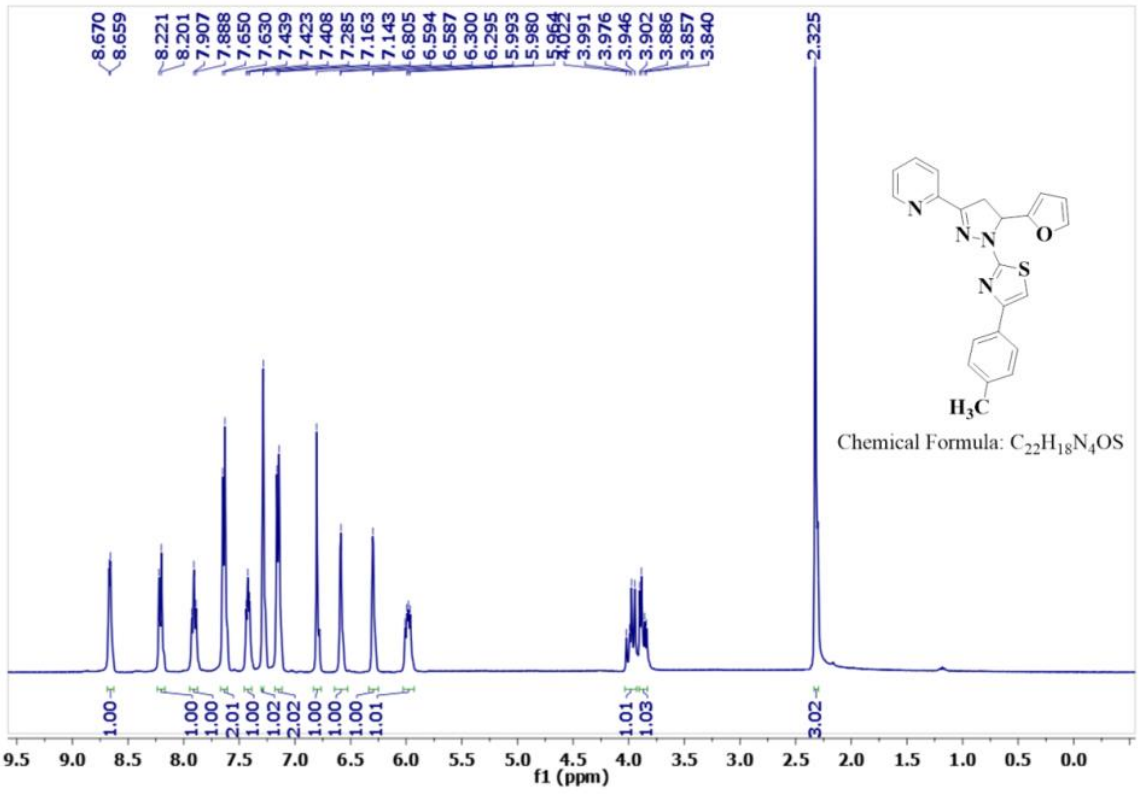


Pale yellow solid; yield 92%; mp: 176-178°C; IR (KBr) cm^{-1}: 1617 (C=N); ^1H NMR (400MHz, $CDCl_3$, ppm): δ 3.36 (dd, J = 17.2 Hz, 7.2 Hz, 1H, Pyrazole-H_A), 3.98 (dd, J = 17.2 Hz, 12 Hz, 1H, Pyrazole-H_B), 5.67 (dd, J = 12 Hz, 7.2 Hz, 1H, Pyrazole-H_X), 7.10 (dd, J = 4.8 Hz, 4 Hz, 1H, Ar-H), 7.2-7.25 (m, 2H, Ar-H), 7.28 (s, 1H, Thiazole-H), 7.31 (dd, J = 8 Hz, 4.8 Hz, 1H, Ar-H), 7.39 (d, J = 2 Hz, 1H, Ar-H), 7.46 (d, J = 4.8 Hz, 1H, Ar-H), 7.57 (d, J = 8.4 Hz, 1H, Ar-H), 7.71 (d, J = 8 Hz, 1H, Ar-H), 8.57 (d, J = 4.4 Hz, 1H, Ar-H), 8.75 (s, 1H, Ar-H); ^{13}C NMR (100MHz,

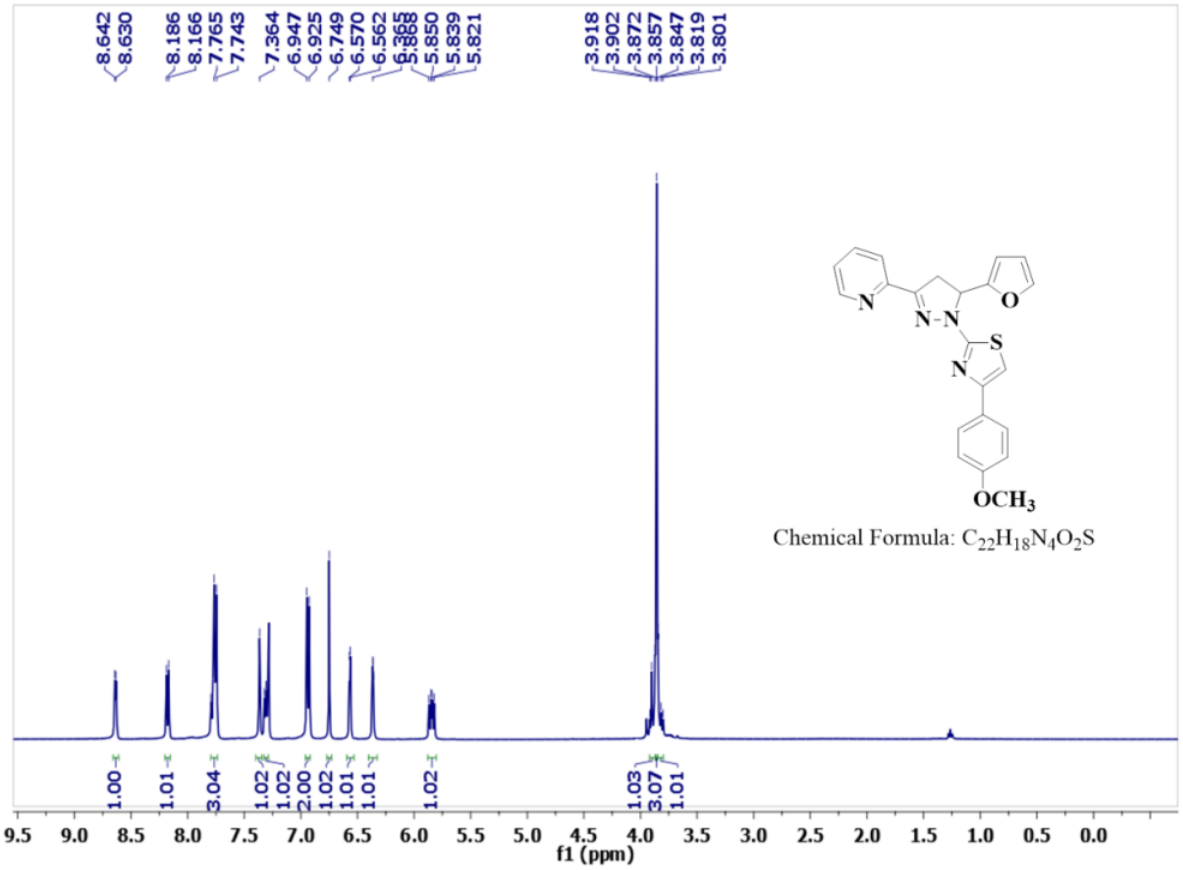
$CDCl_3$): δ 43.92, 62.55, 109.95, 124.08, 127.15, 127.69, 128.18, 128.69, 130.10, 131.70, 131.88, 132.11, 134.44, 135.04, 146.82, 147.52, 147.89, 148.30, 163.65 ppm; Mass (MS-ESI) (*m/z*): 457.40 $[M+]^+$; Anal. Calcd. For $C_{21}H_{14}Cl_2N_4S_2$: C, 55.14; H, 3.09; N, 12.25%. Found: C, 55.19; H, 3.12; N, 12.29%.



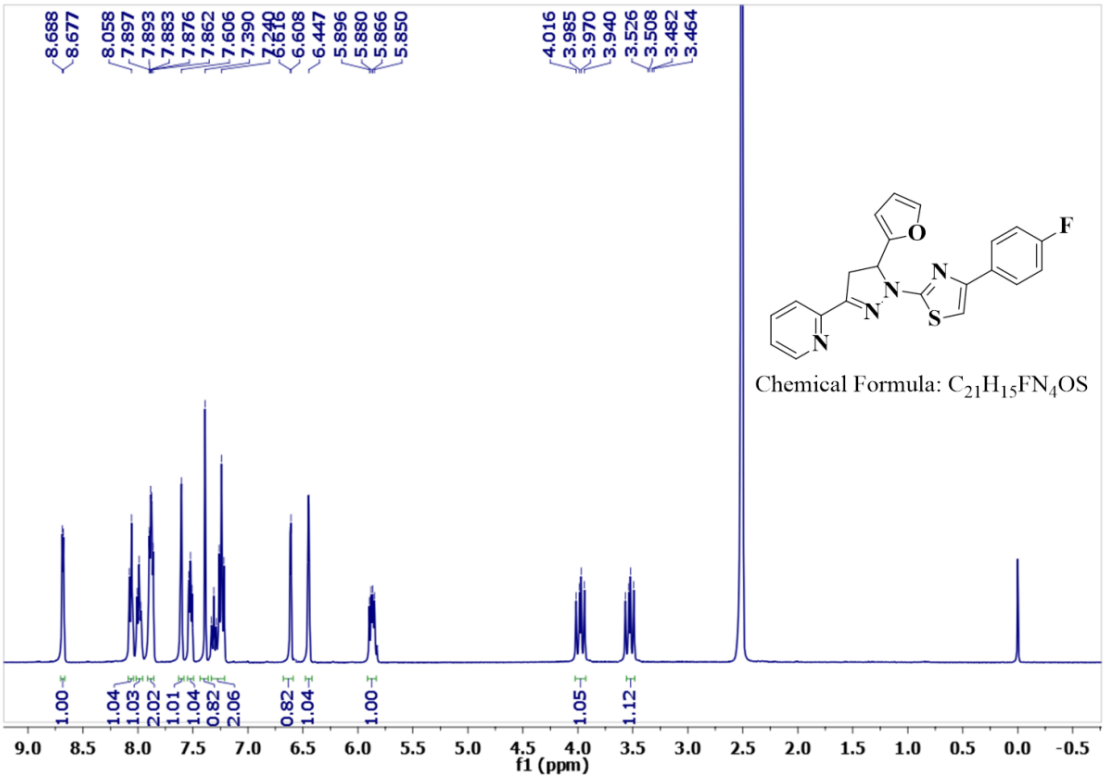
^{1}H NMR spectrum of compound **4a** (400 MHz, $CDCl_3$)



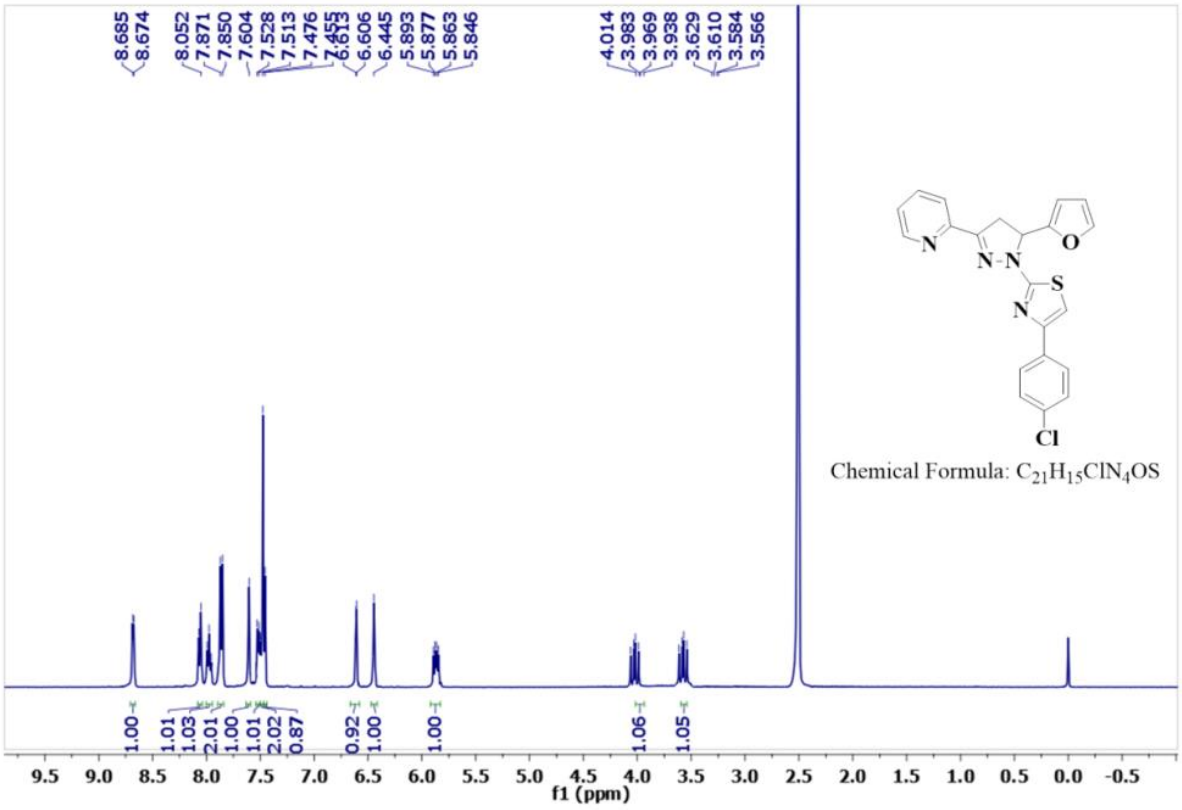
^{1}H NMR spectrum of compound **4b** (400 MHz, $CDCl_3$)



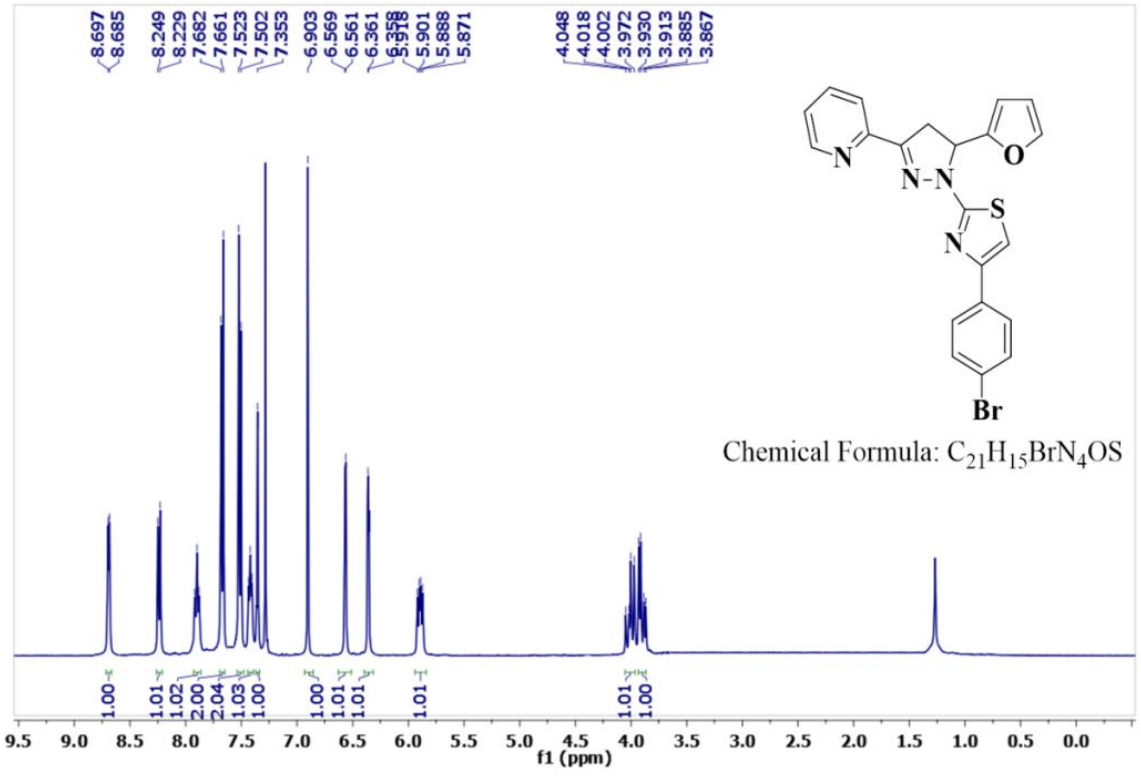
^{1}H NMR spectrum of compound **4c** (400 MHz, $CDCl_3$)



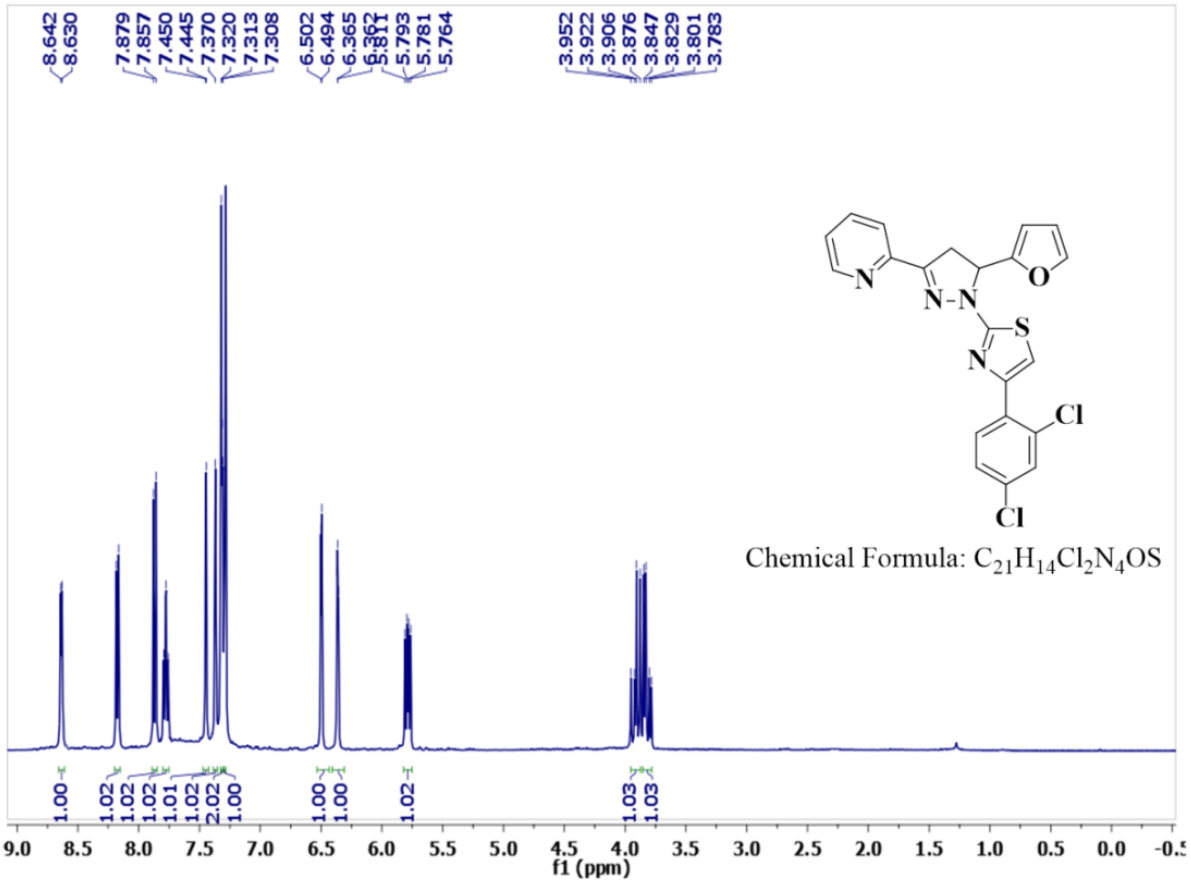
^{1}H NMR spectrum of compound **4d** (400 MHz, DMSO-d_6)



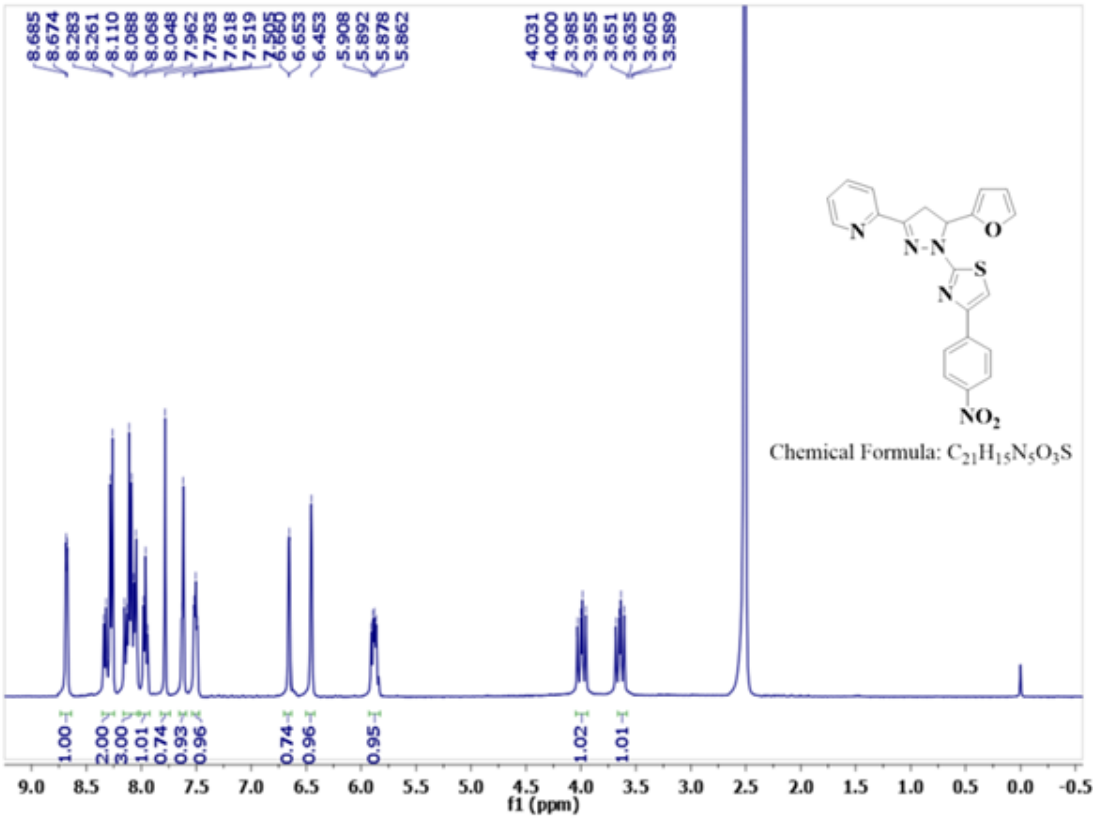
^{1}H NMR spectrum of compound **4e** (400 MHz, DMSO-d_6)



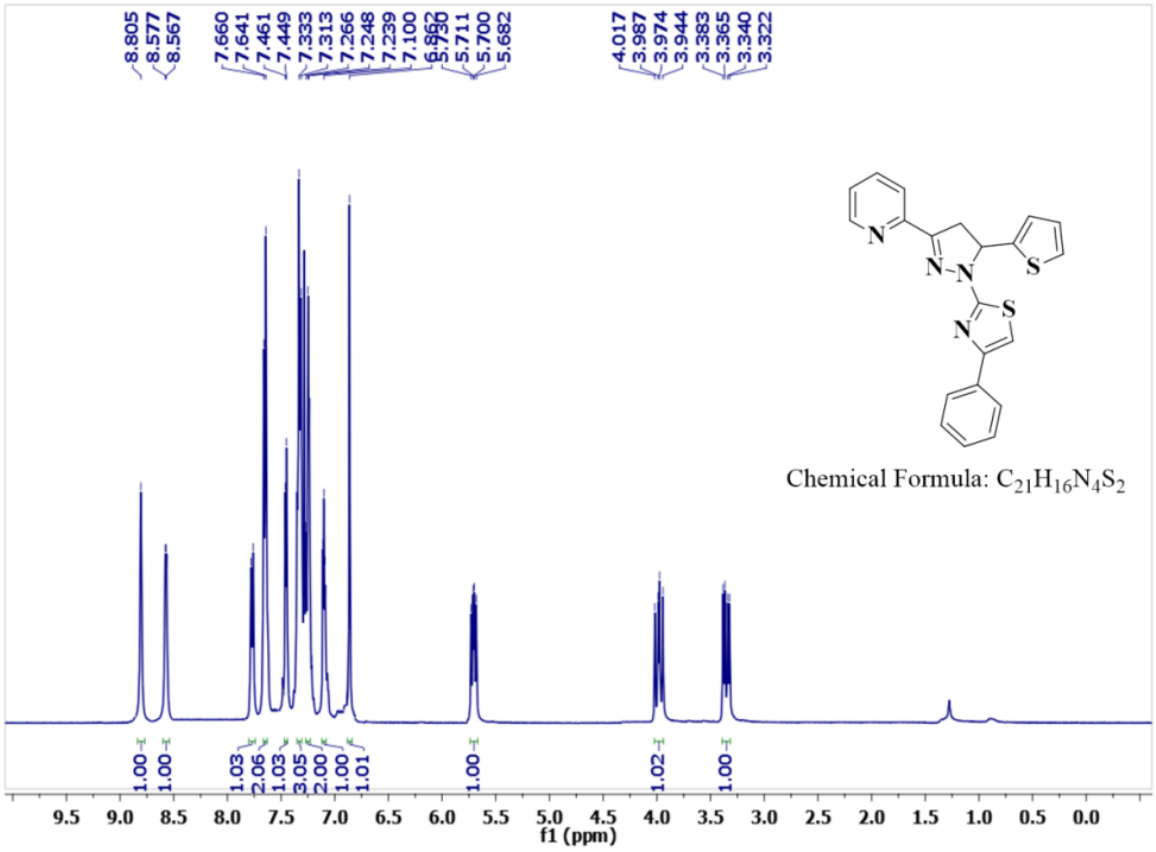
^{1}H NMR spectrum of compound **4f** (400 MHz, $CDCl_3$)



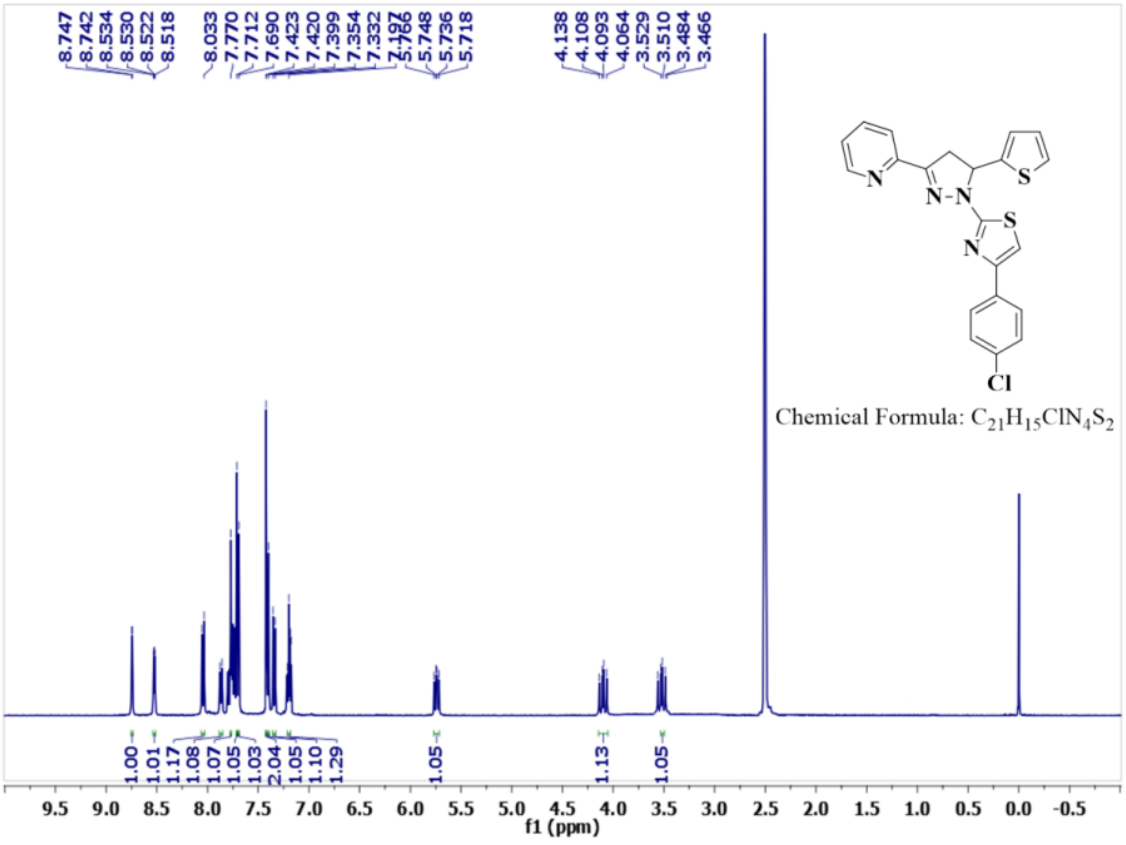
^{1}H NMR spectrum of compound **4g** (400 MHz, $CDCl_3$)



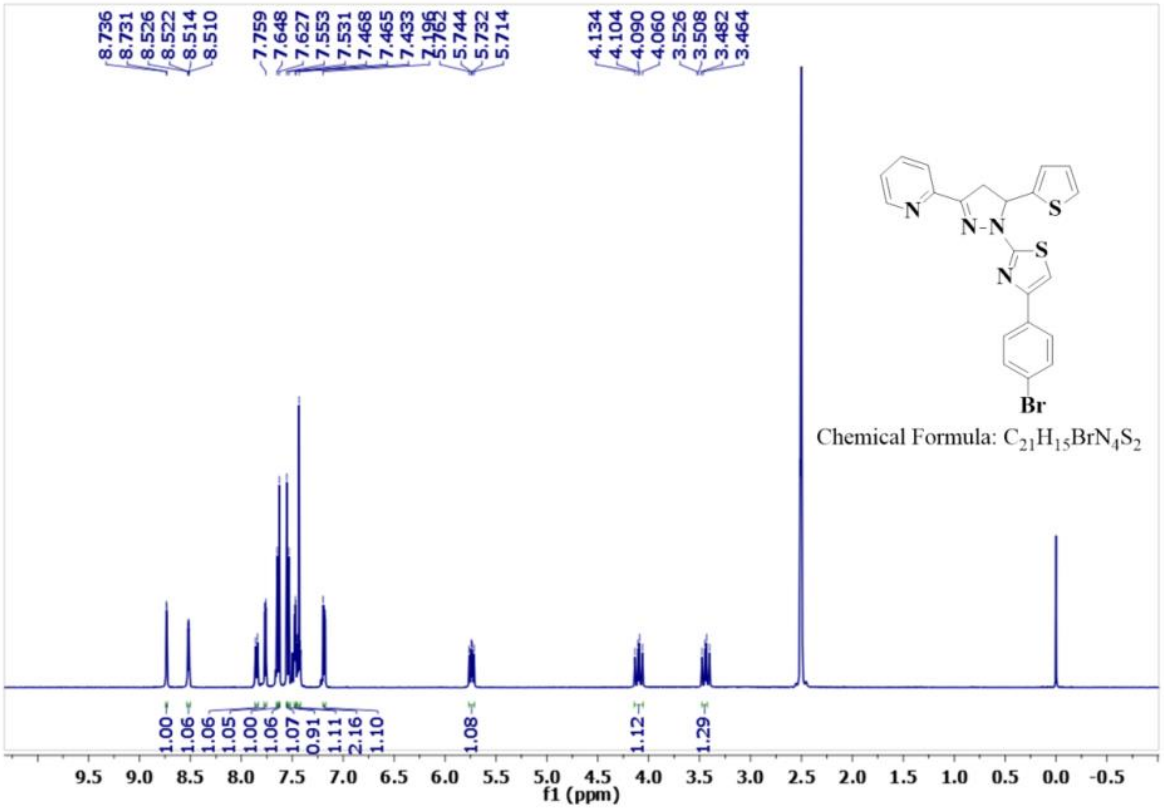
^{1}H NMR spectrum of compound **4h** (400 MHz, $CDCl_3$)



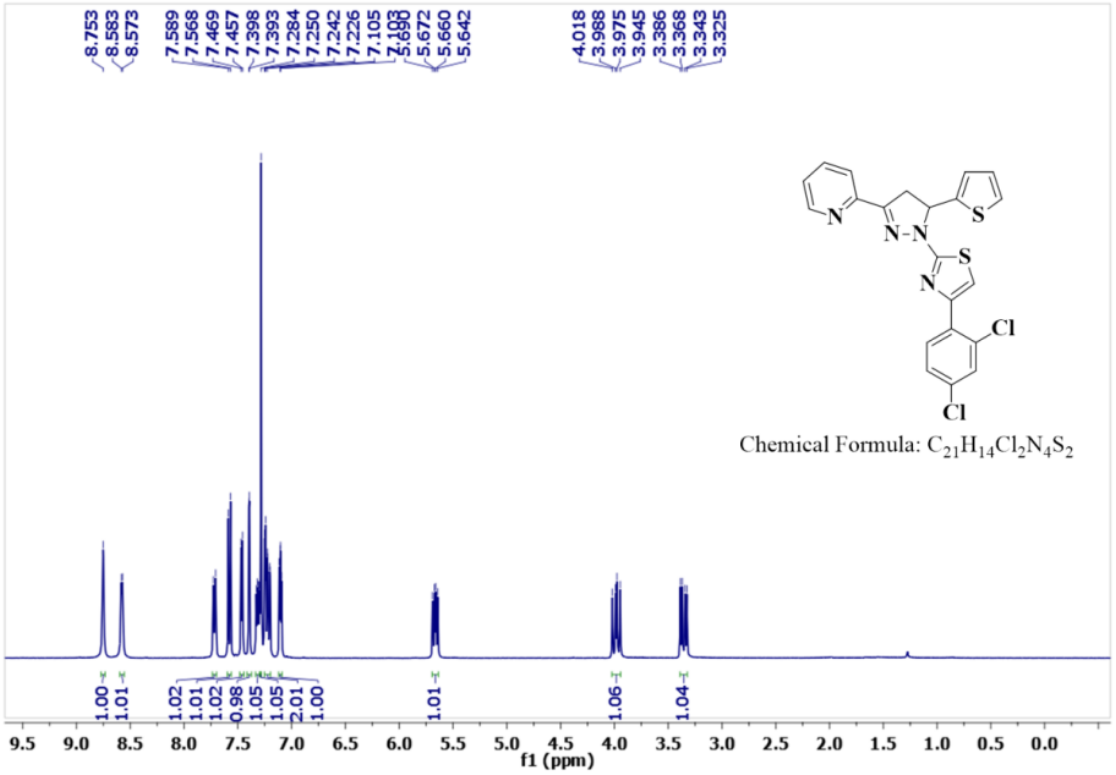
^{1}H NMR spectrum of compound **4i** (400 MHz, $CDCl_3$)



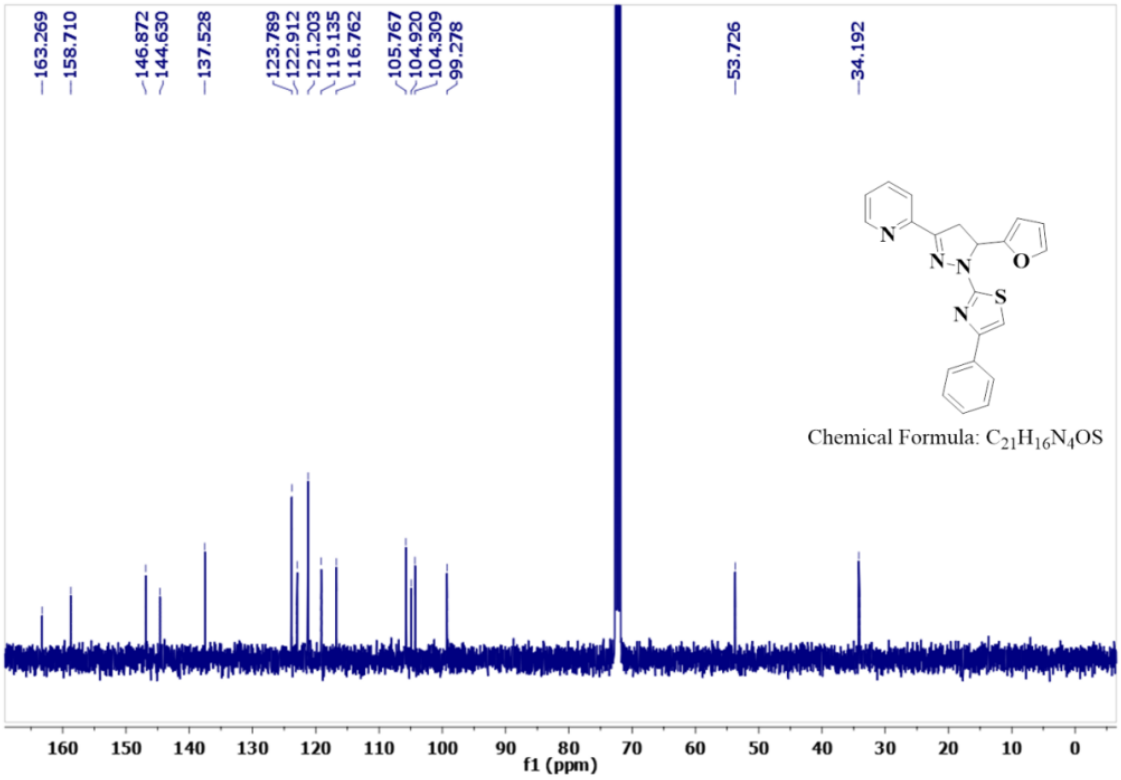
^{1}H NMR spectrum of compound **4j** (400 MHz, DMSO-d_6)



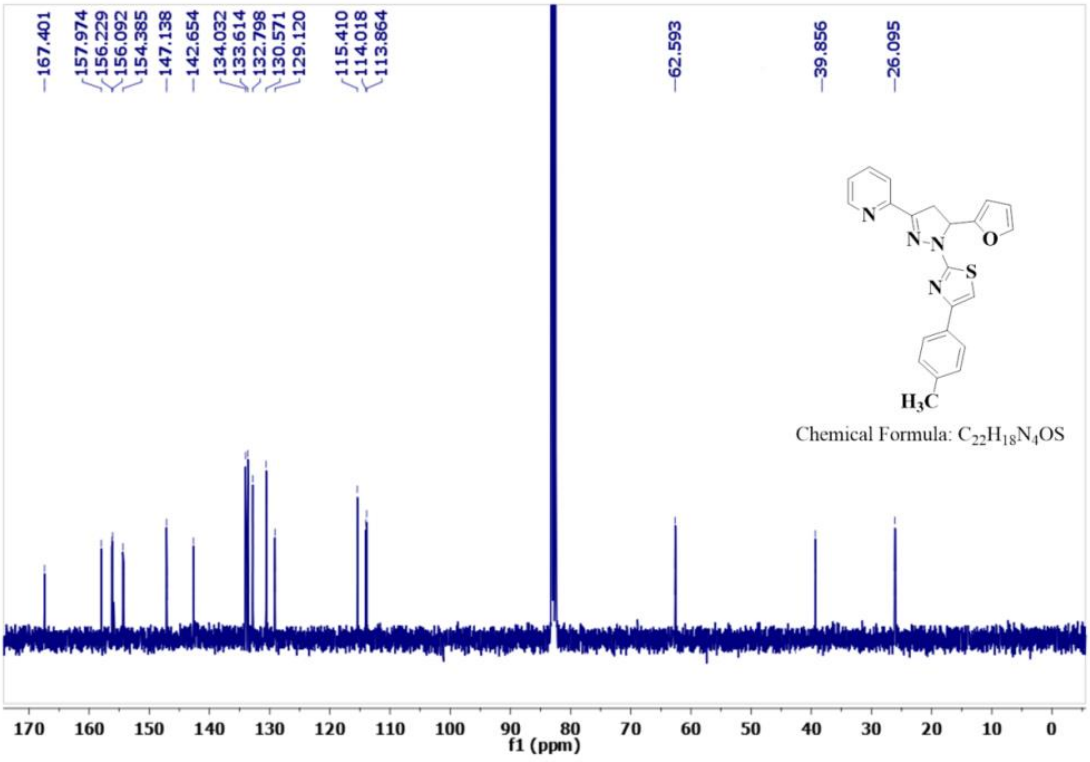
^{1}H NMR spectrum of compound **4k** (400 MHz, DMSO-d_6)



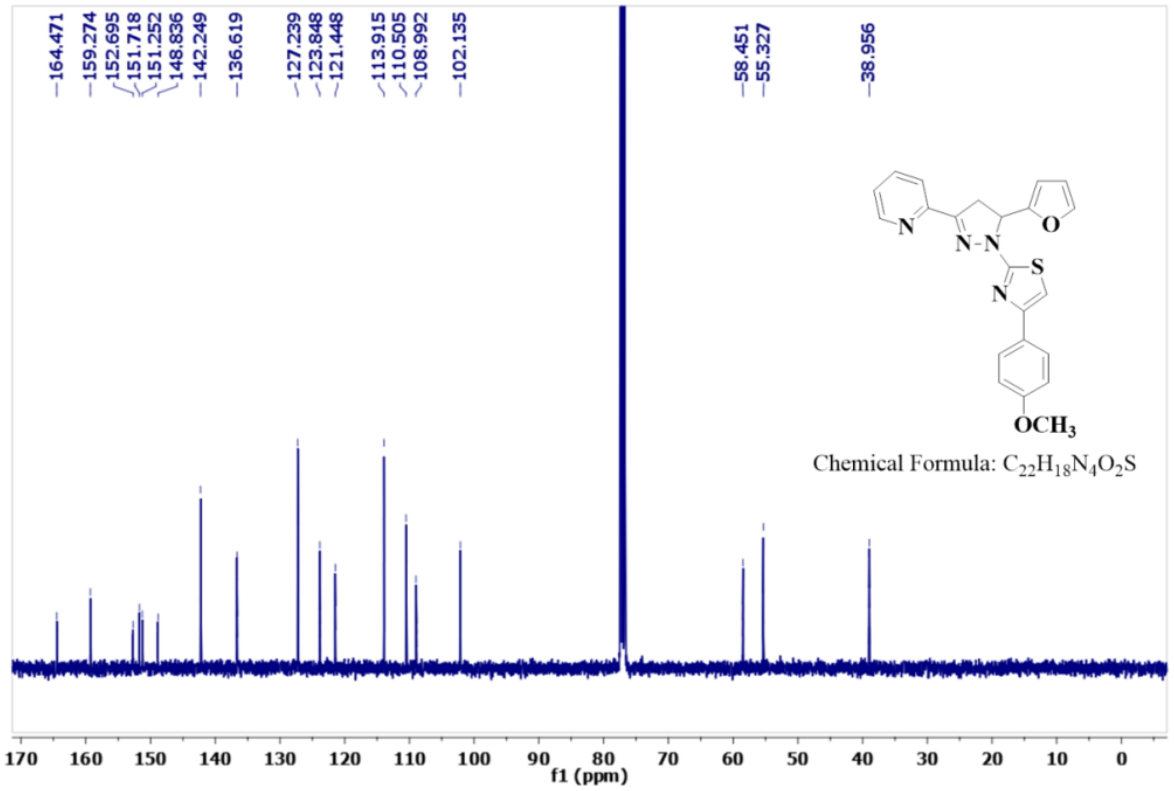
^{1}H NMR spectrum of compound **4l** (400 MHz, $CDCl_3$)



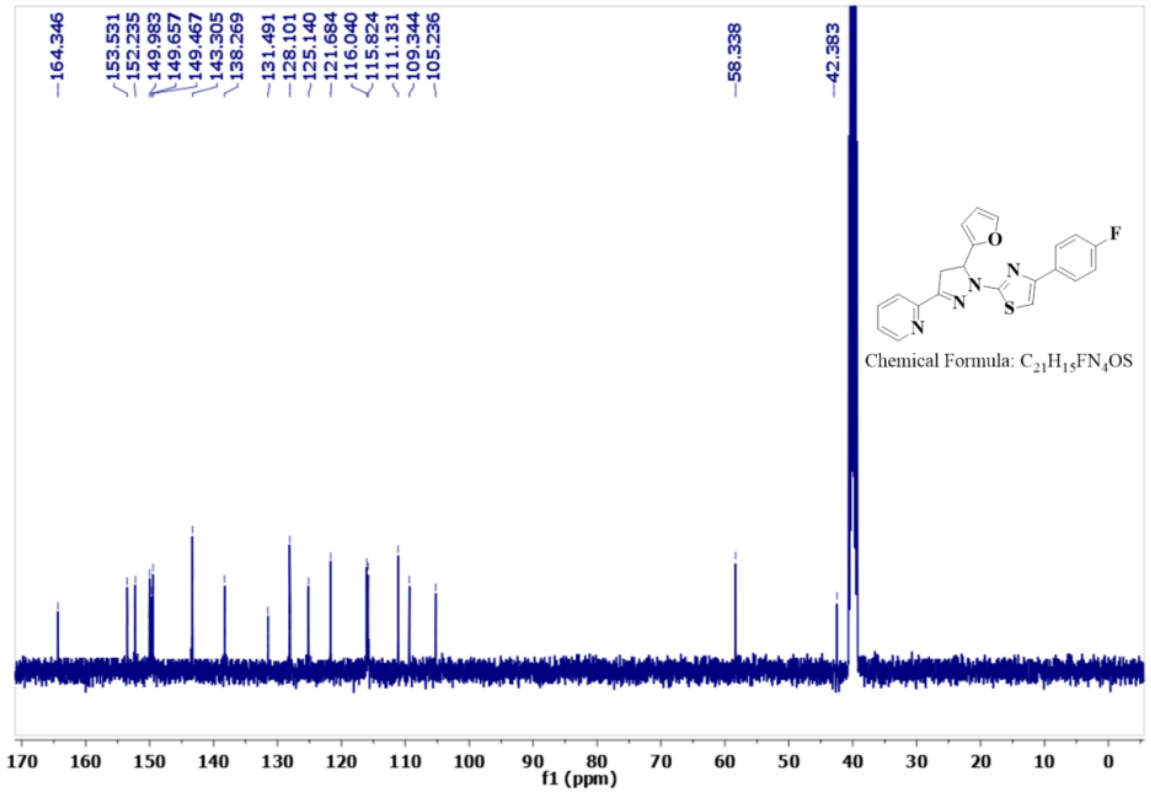
^{13}C NMR spectrum of compound 4**a** (100 MHz, $CDCl_3$)



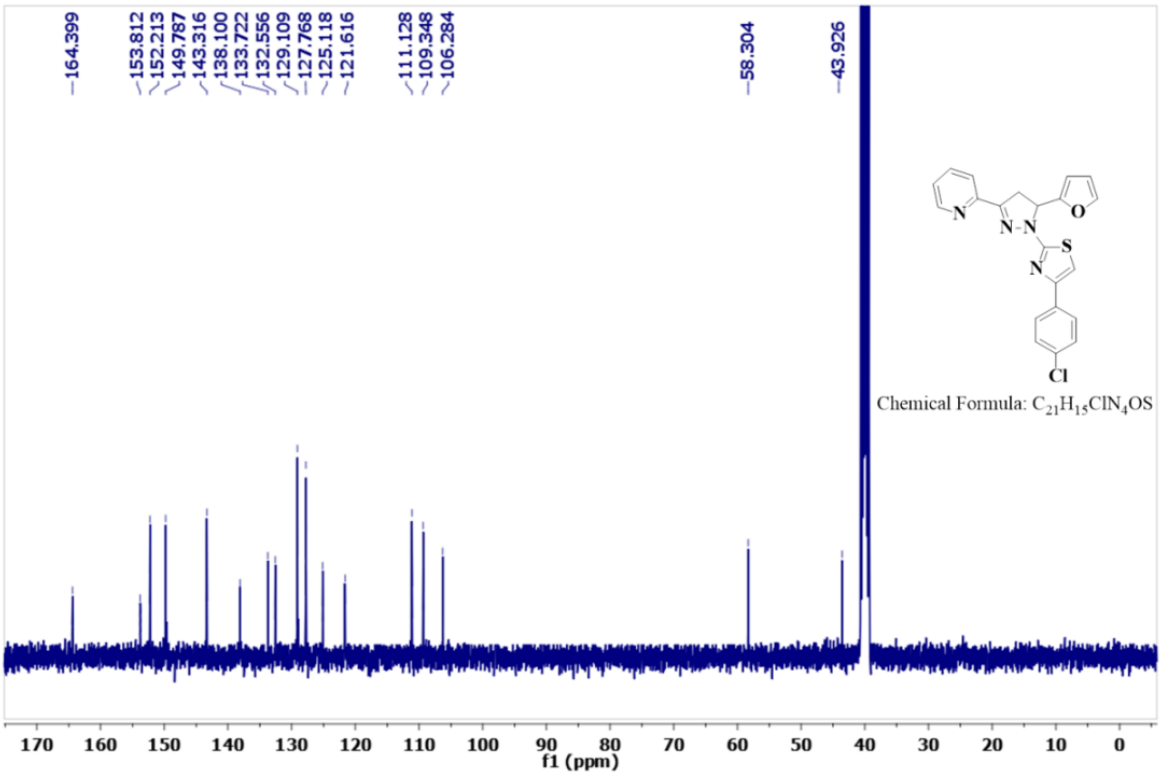
^{13}C NMR spectrum of compound **4b** (100 MHz, $CDCl_3$)



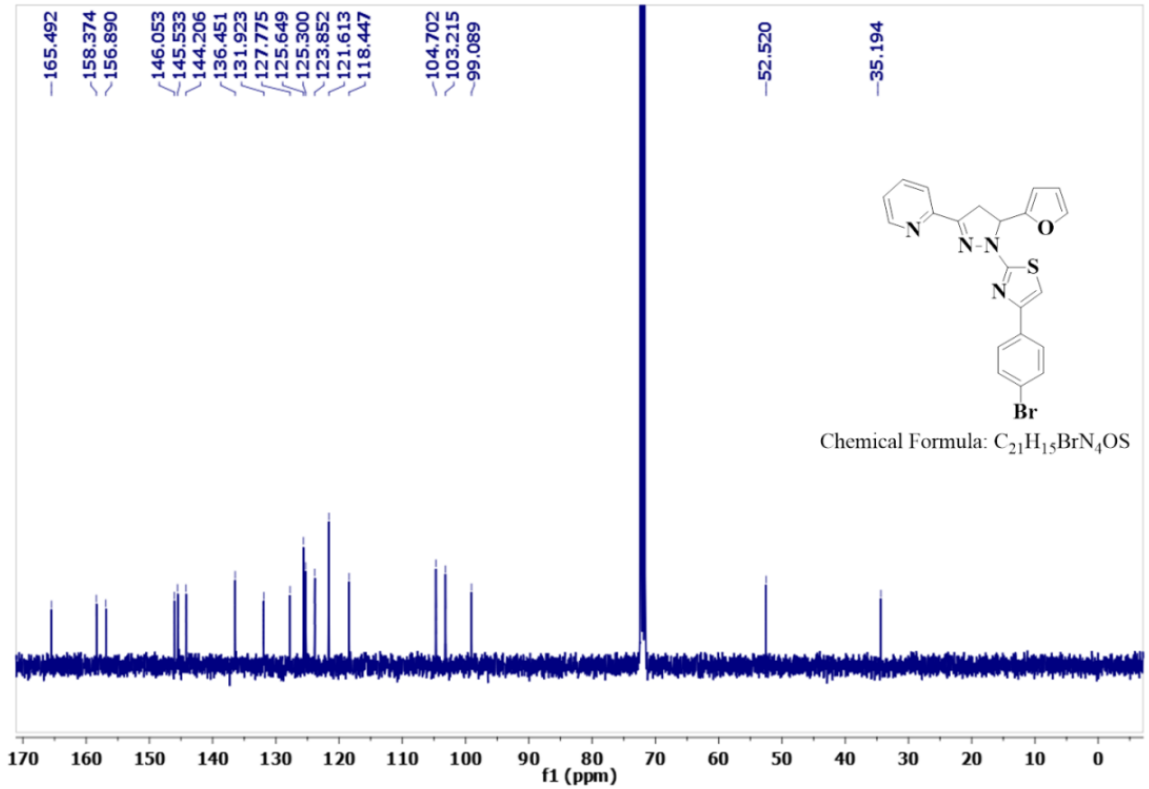
^{13}C NMR spectrum of compound **4c** (100 MHz, $CDCl_3$)



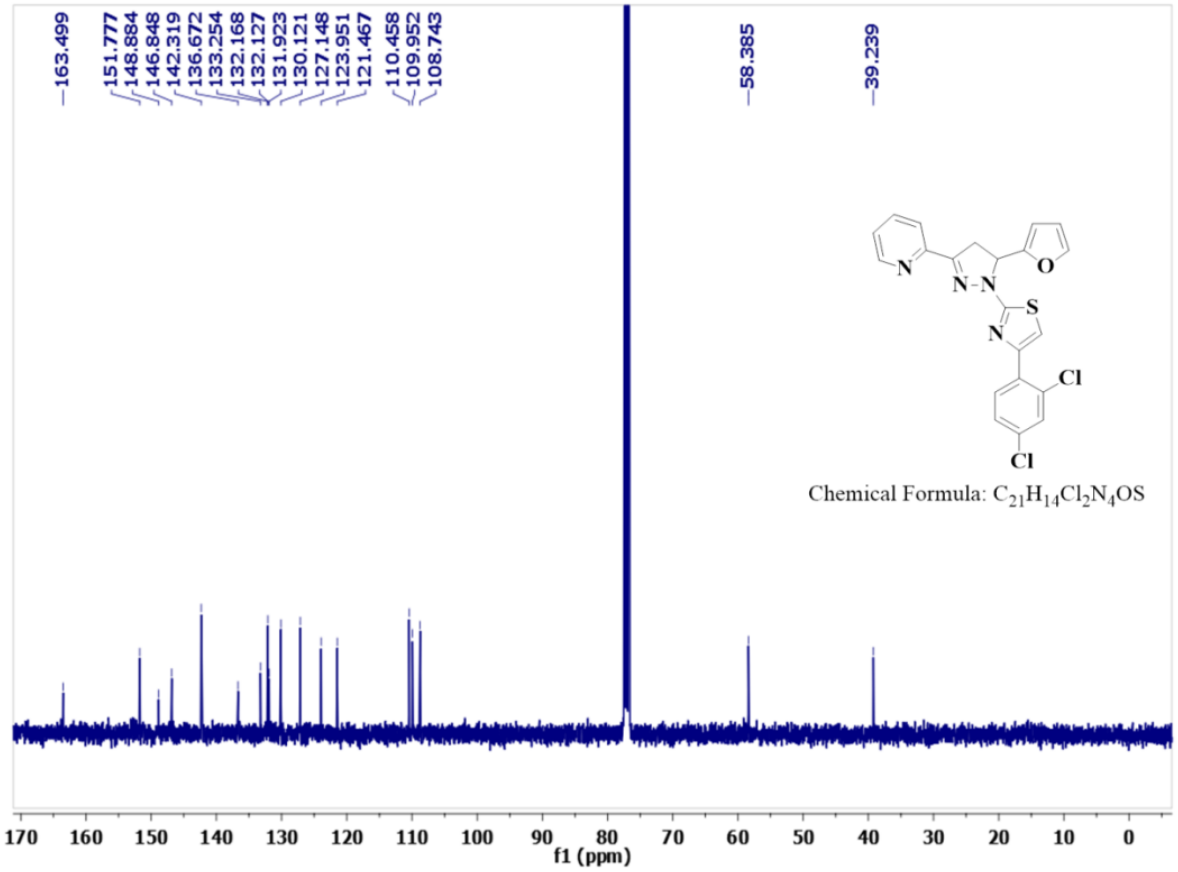
^{13}C NMR spectrum of compound **4d** (100 MHz, DMSO-d_6)



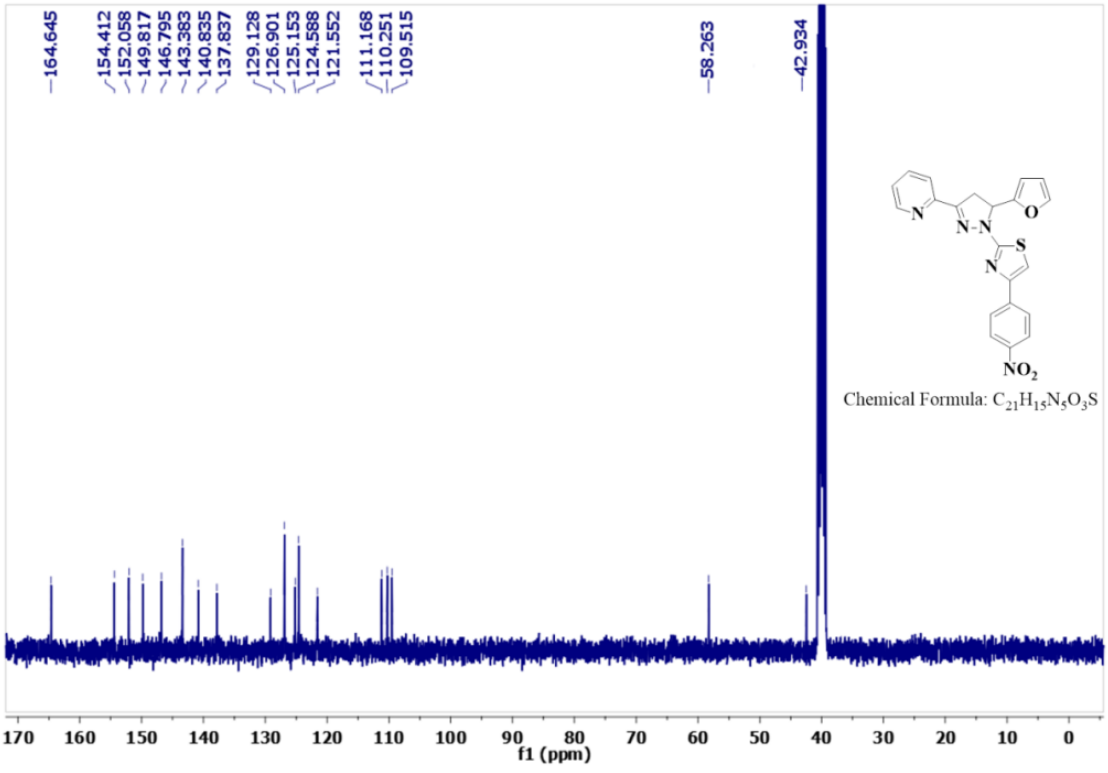
^{13}C NMR spectrum of compound **4e** (100 MHz, DMSO-d_6)



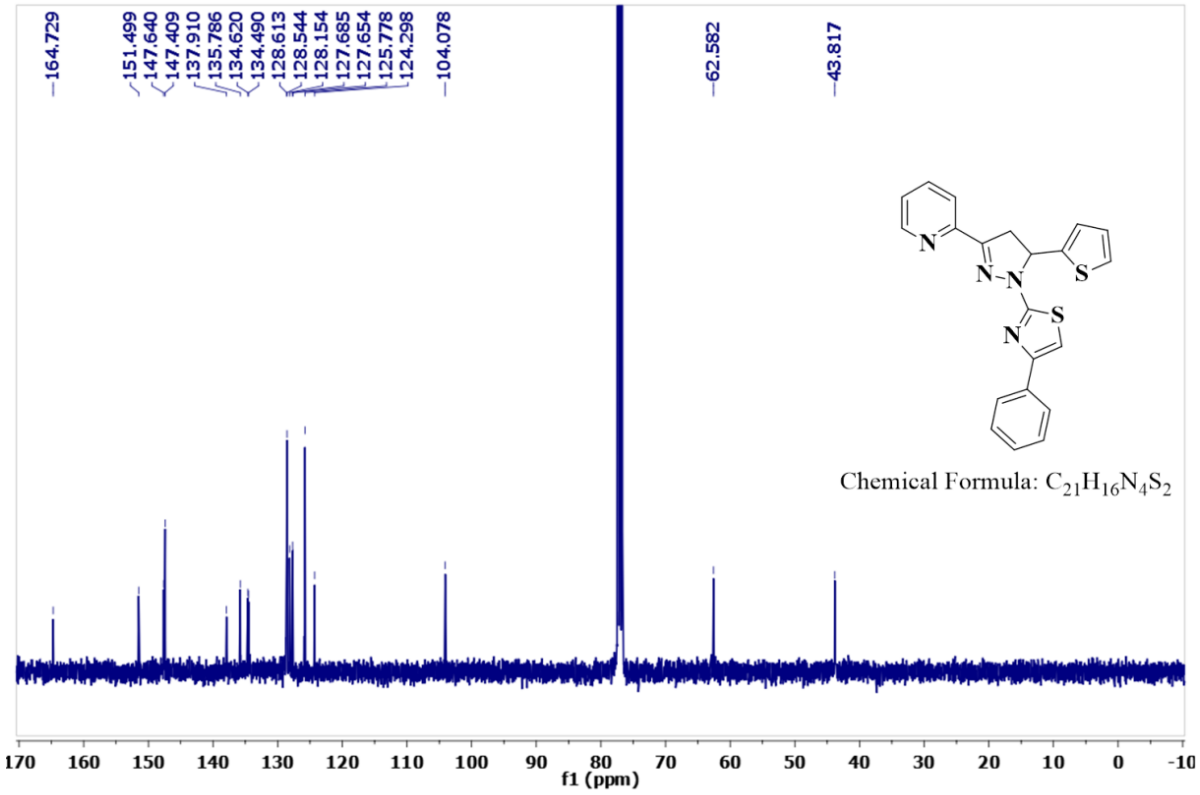
^{13}C NMR spectrum of compound **4f** (100 MHz, $CDCl_3$)



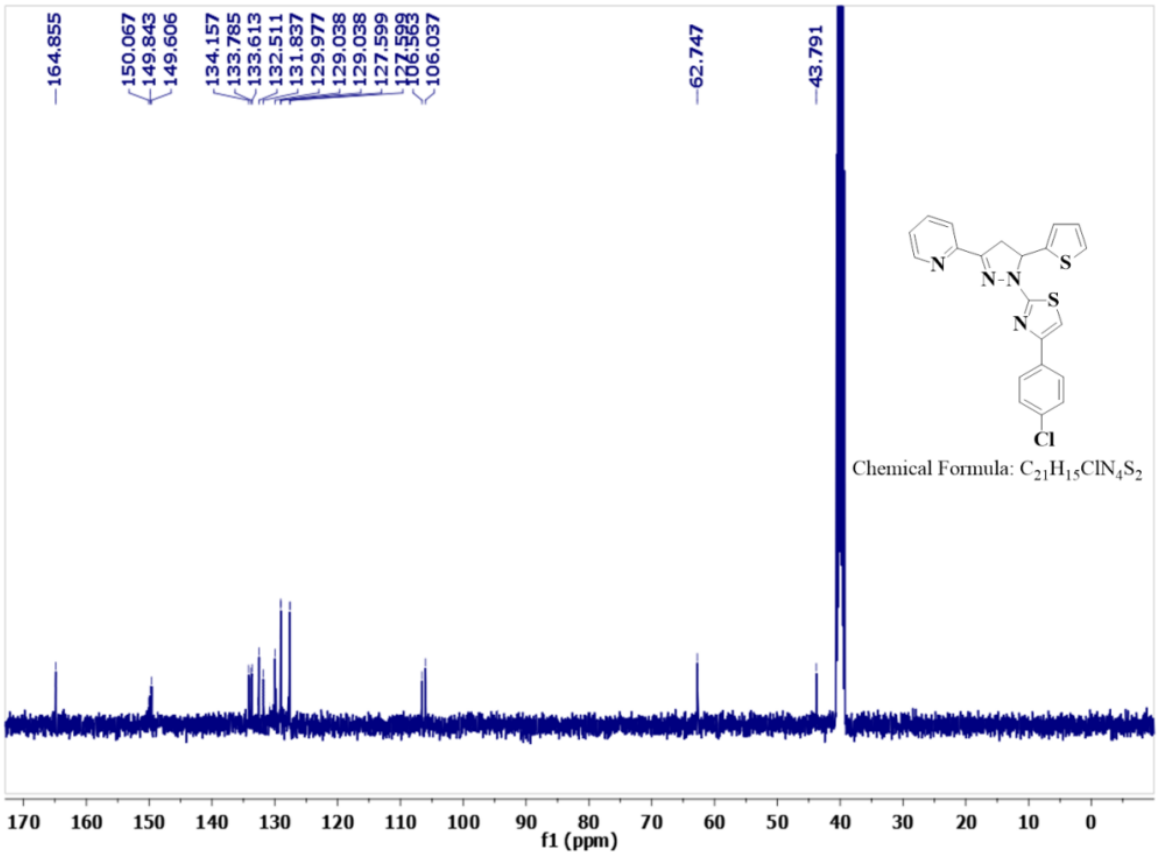
^{13}C NMR spectrum of compound **4g** (100 MHz, $CDCl_3$)



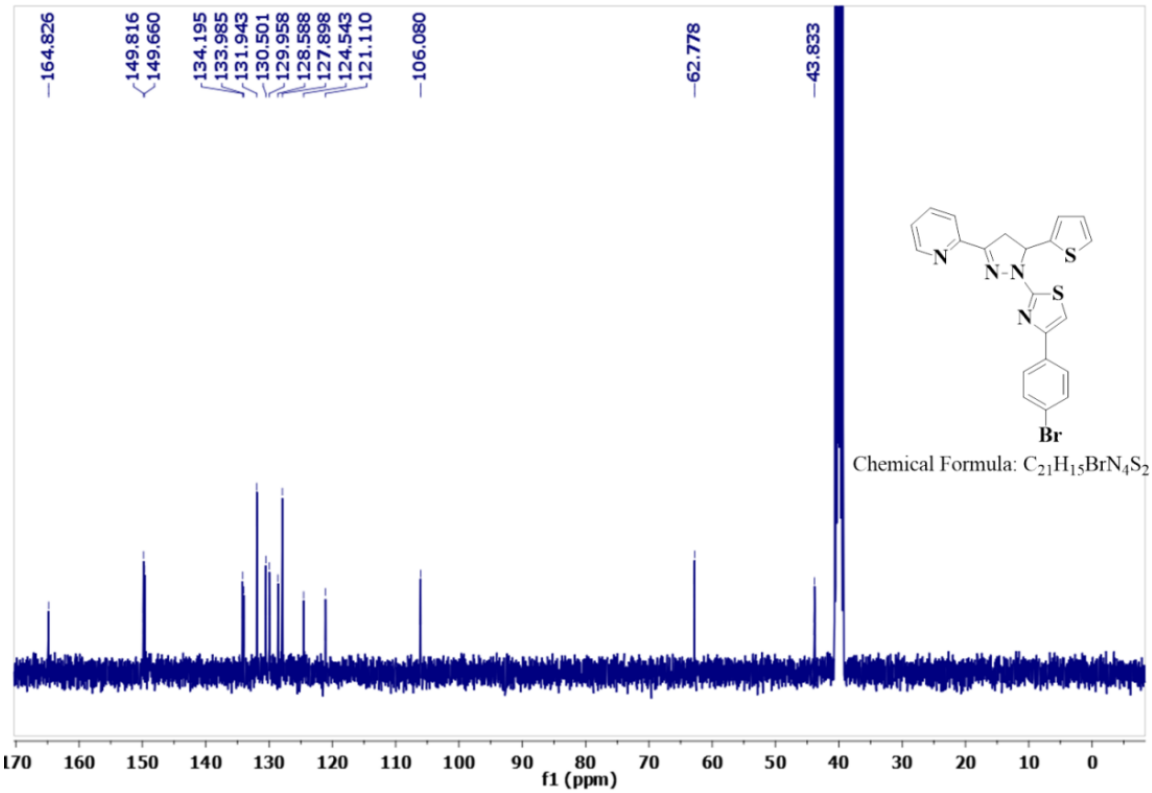
^{13}C NMR spectrum of compound **4h** (100 MHz, DMSO-d_6)



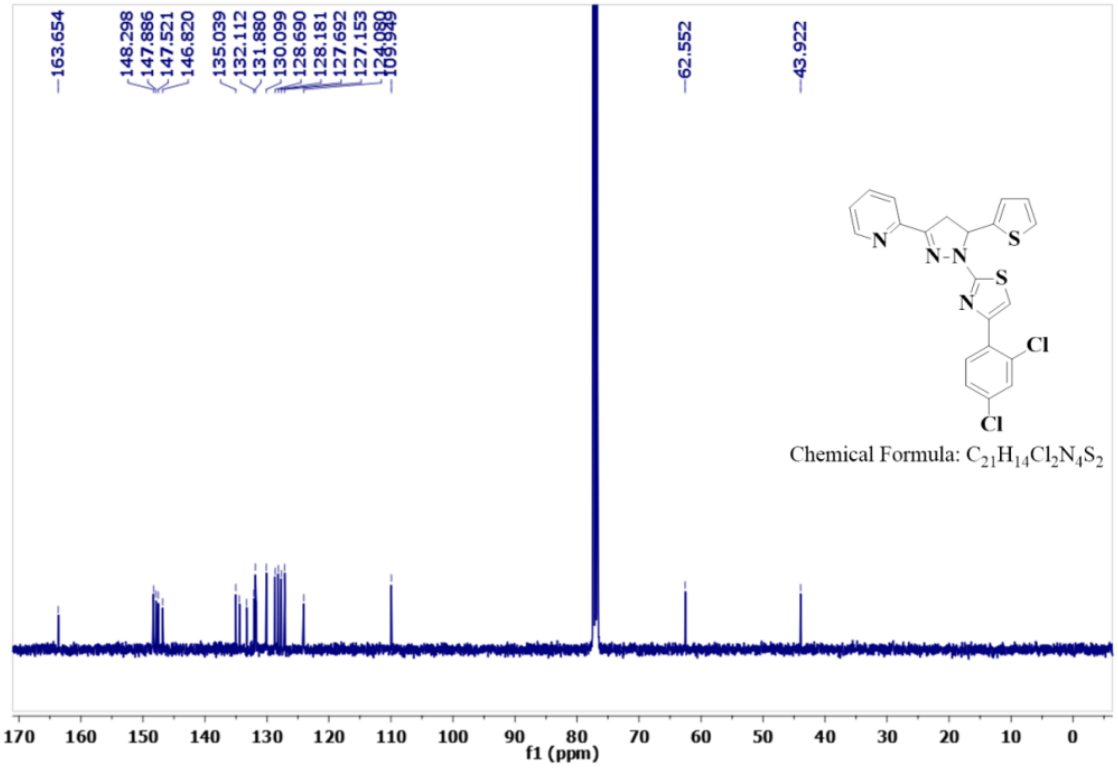
^{13}C NMR spectrum of compound **4i** (100 MHz, $CDCl_3$)



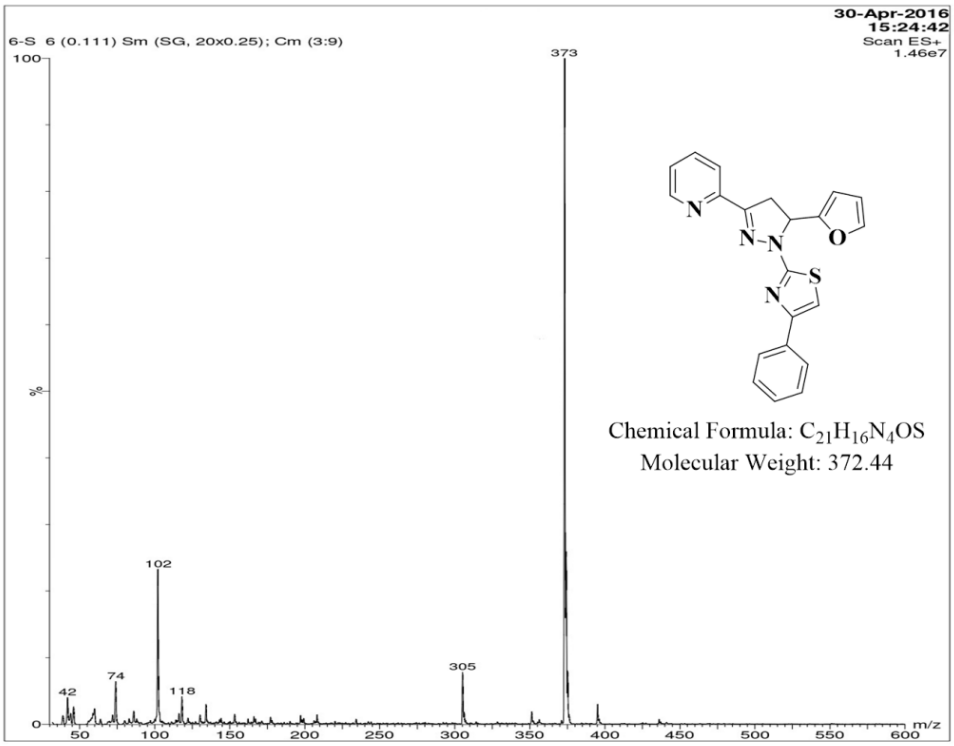
^{13}C NMR spectrum of compound **4j** (100 MHz, DMSO-d_6)



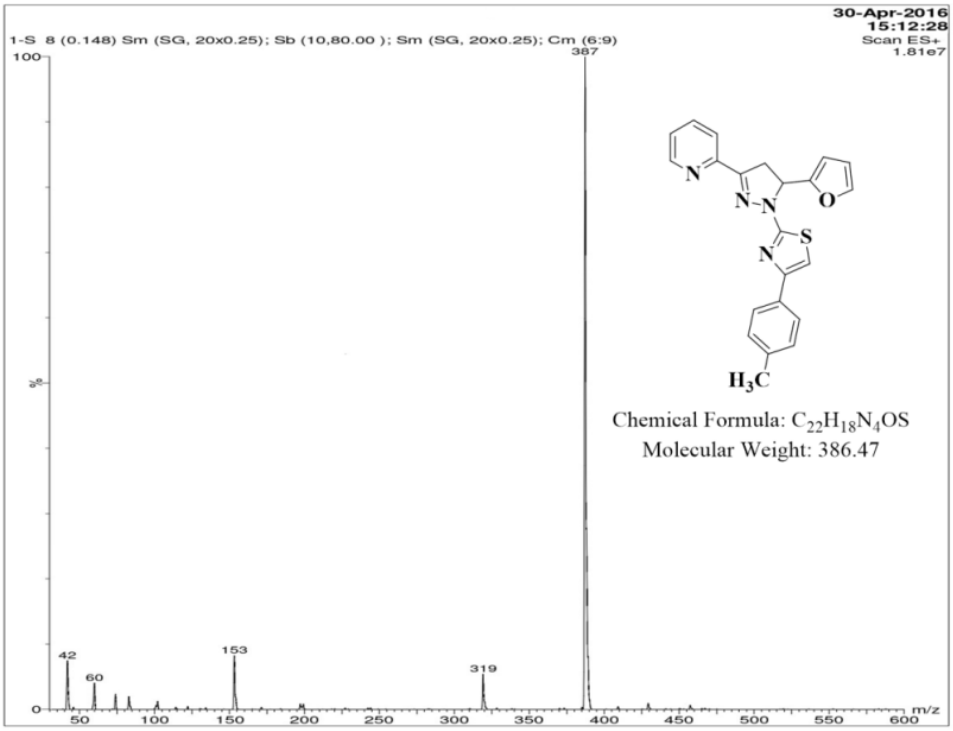
^{13}C NMR spectrum of compound **4k** (100 MHz, DMSO-d_6)



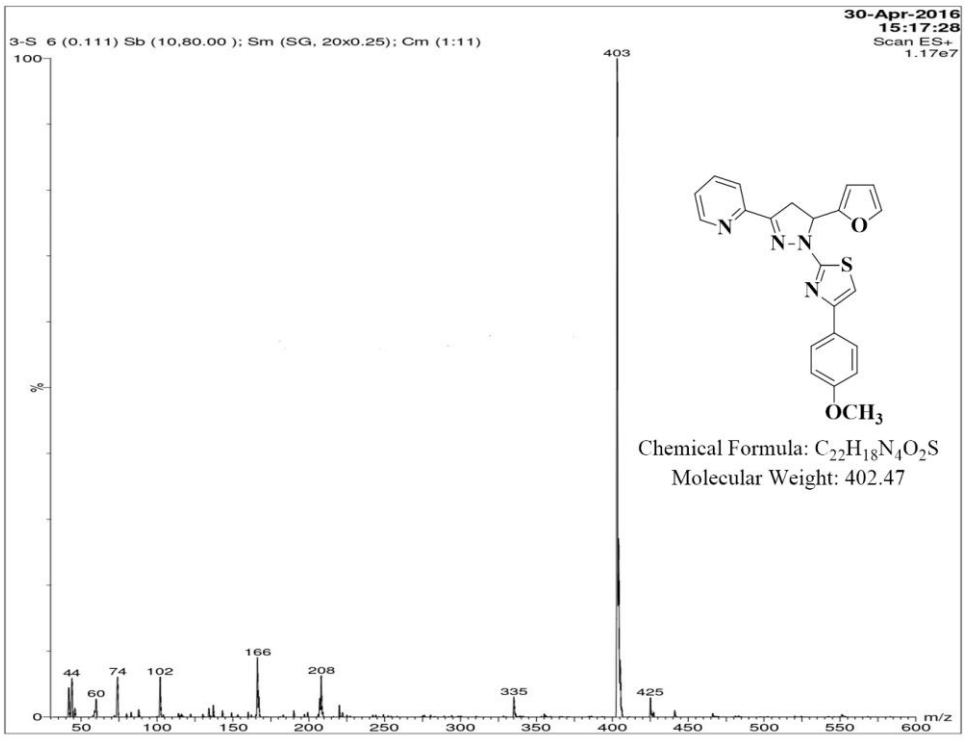
^{13}C NMR spectrum of compound **4l** (100 MHz, $CDCl_3$)



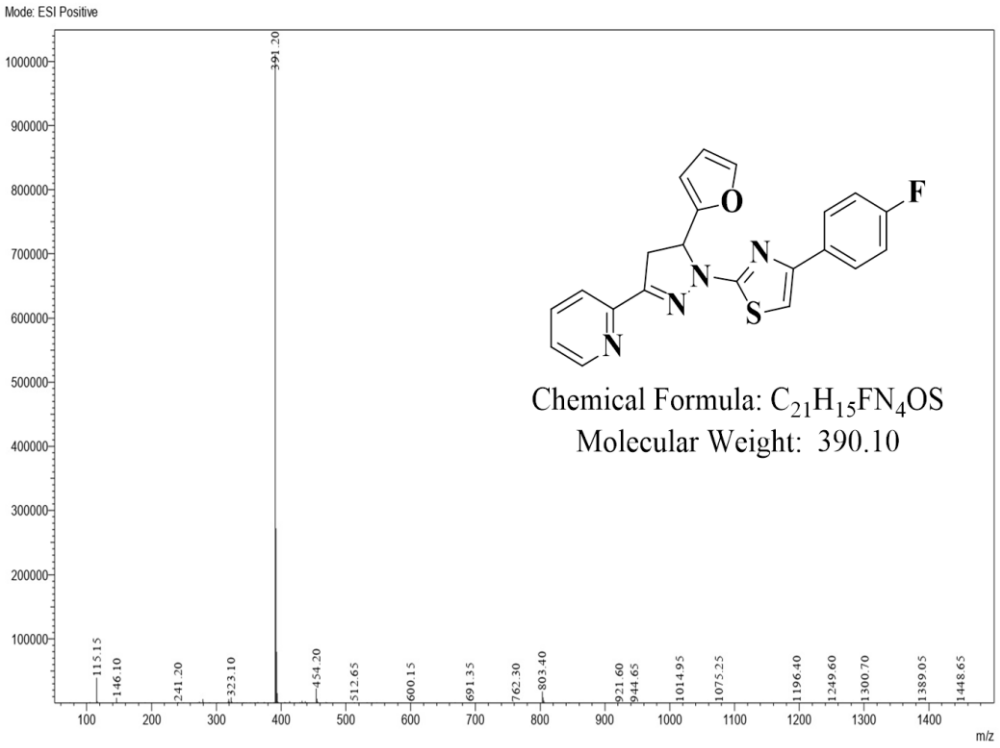
Mass spectrum of compound **4a**



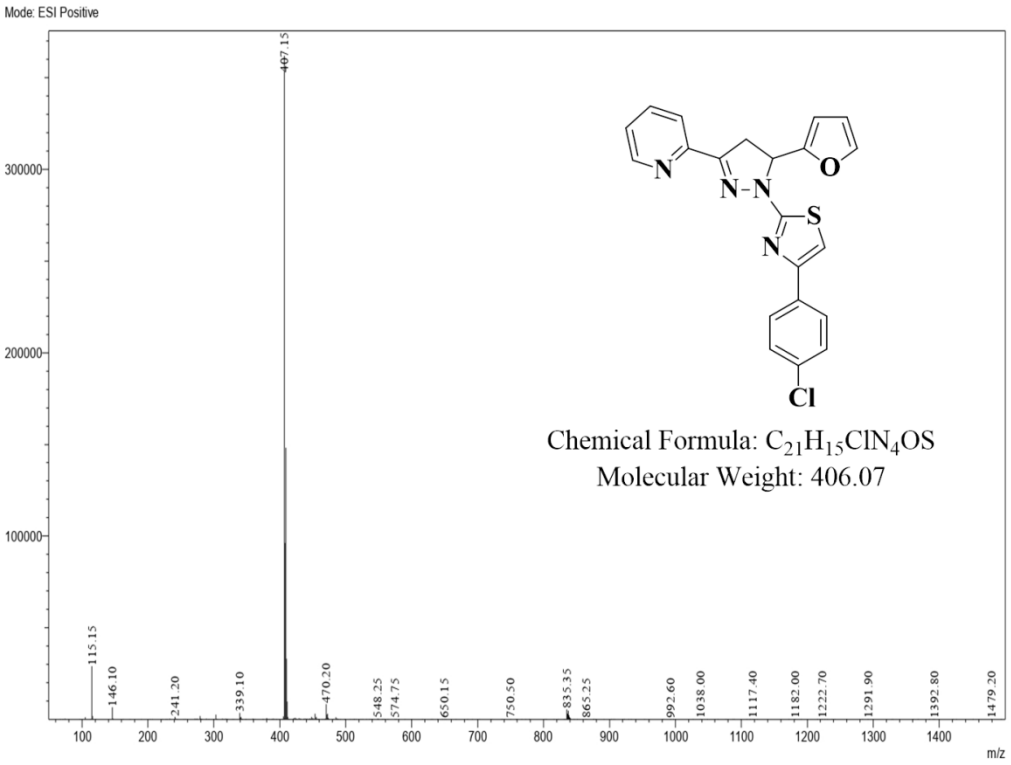
Mass spectrum of compound **4b**



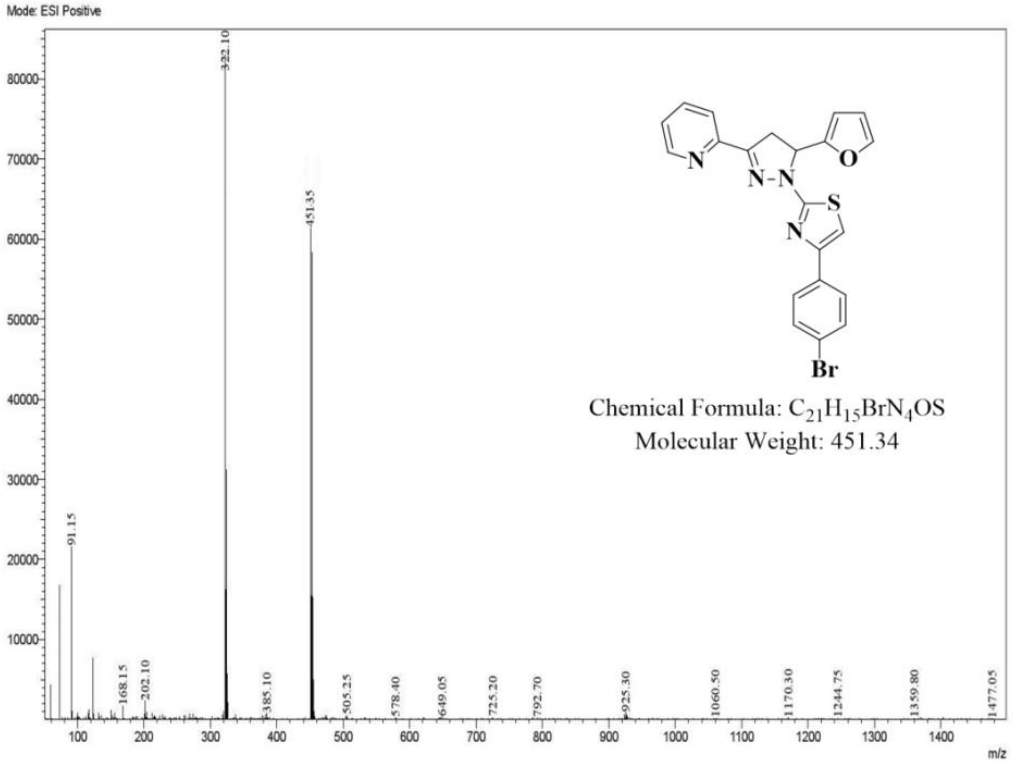
Mass spectrum of compound **4c**



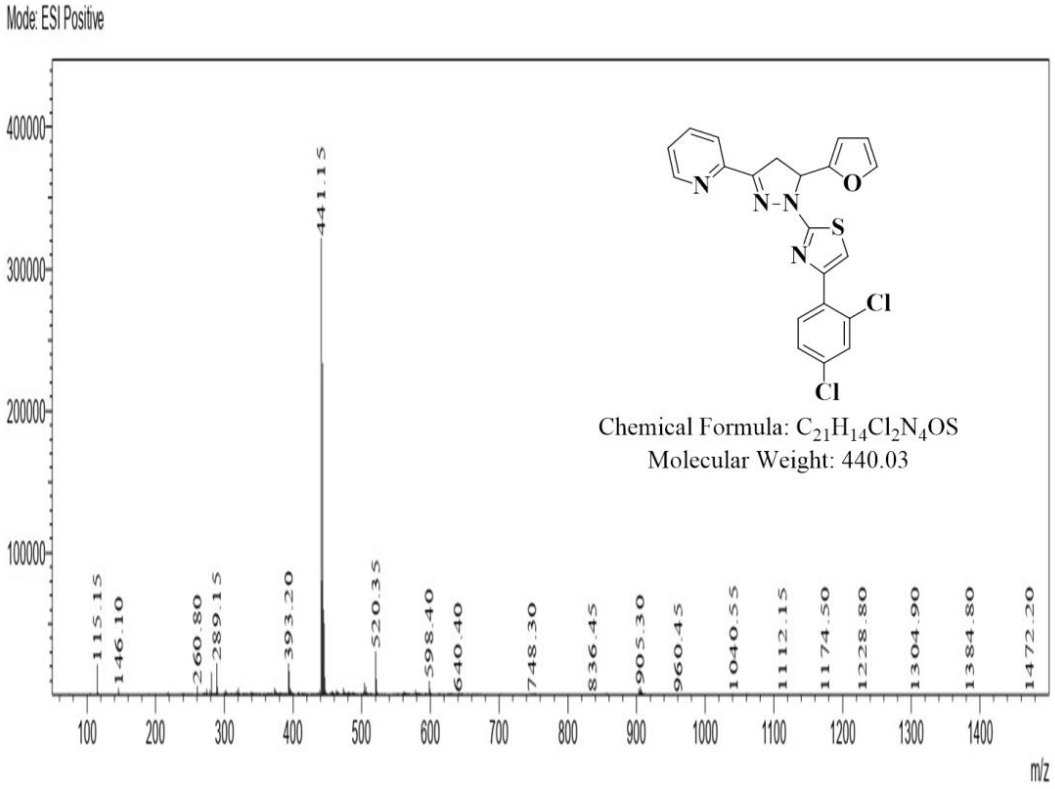
Mass spectrum of compound **4d**



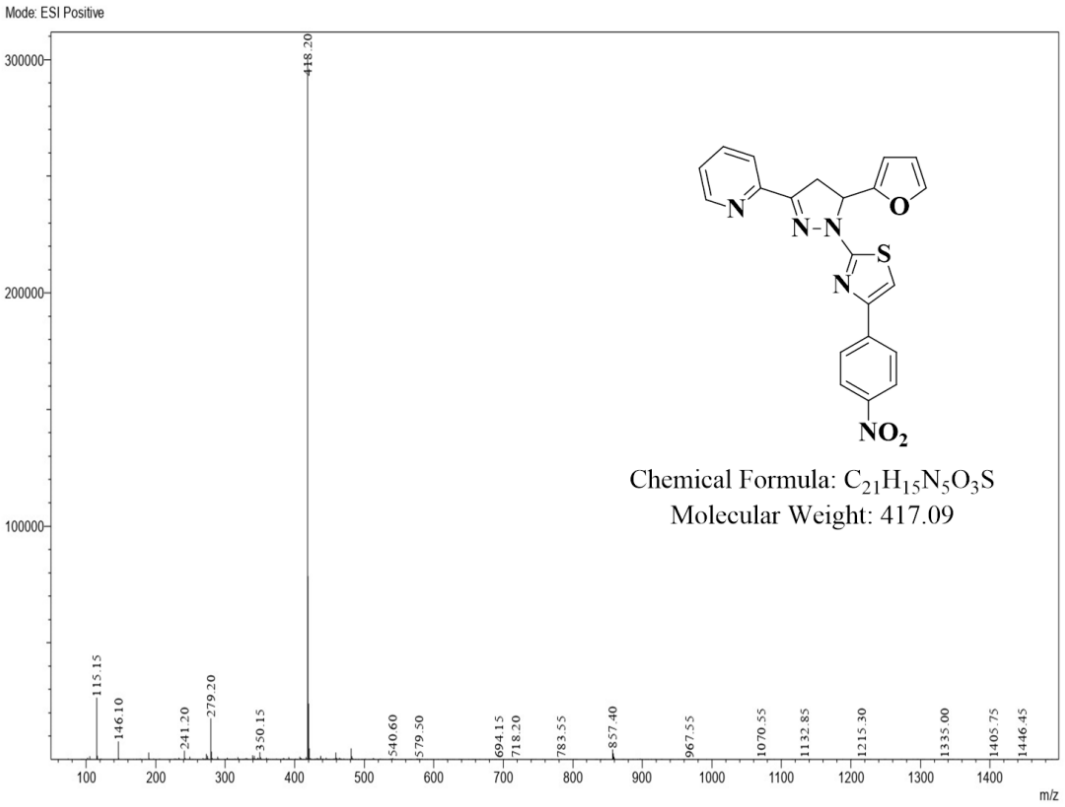
Mass spectrum of compound **4e**



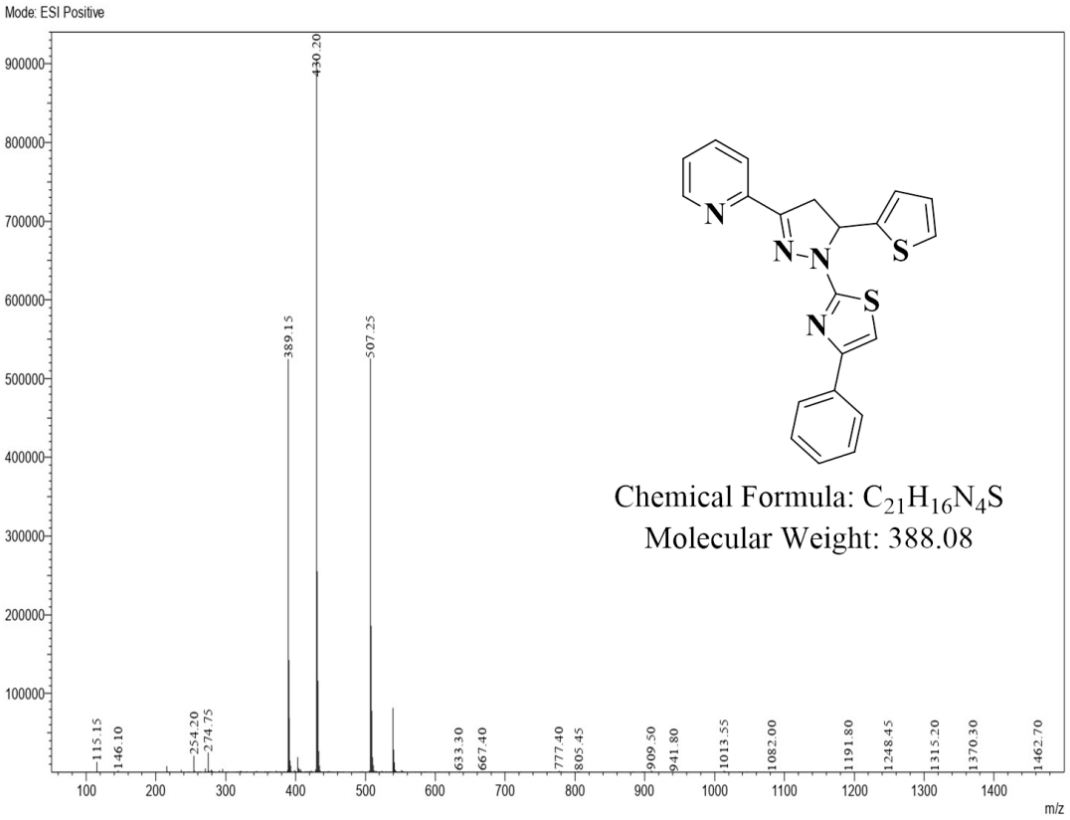
Mass spectrum of compound **4f**



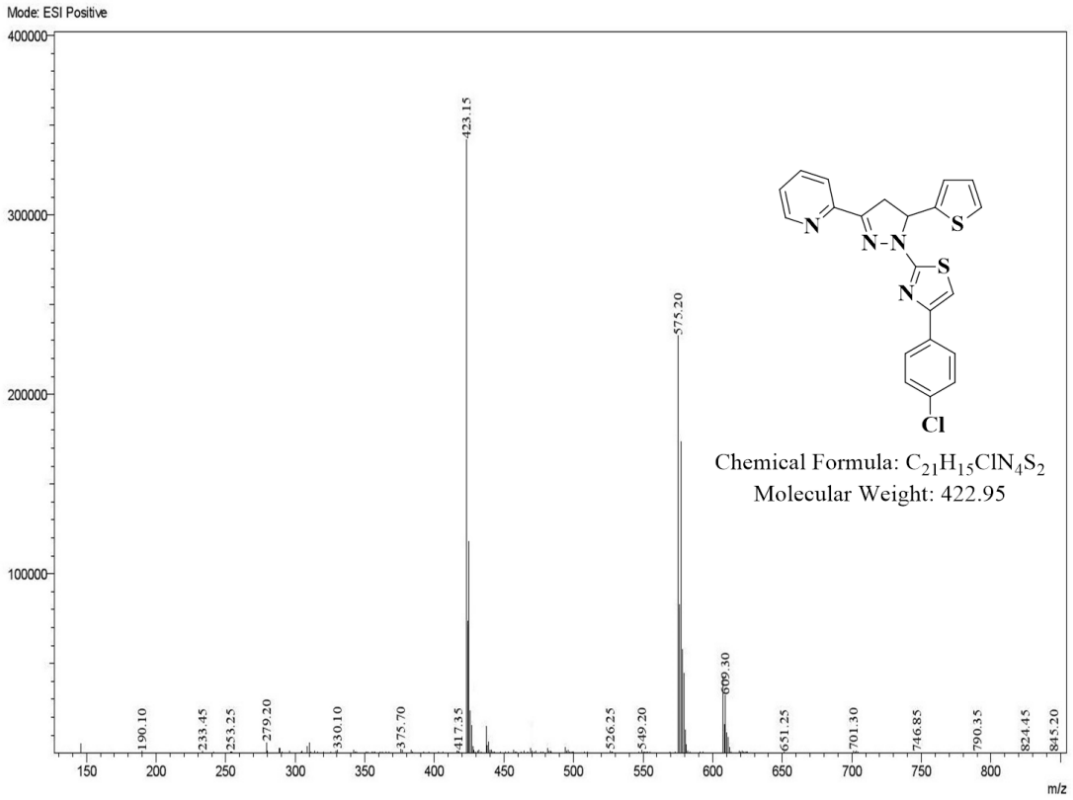
Mass spectrum of compound **4g**



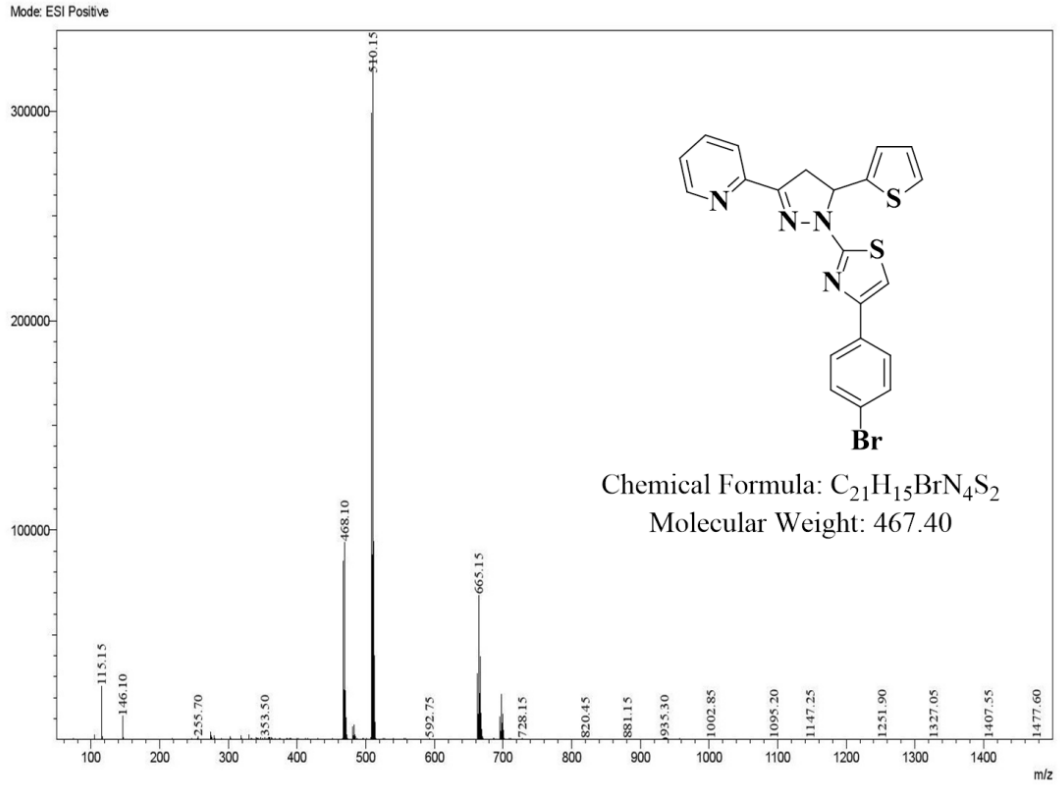
Mass spectrum of compound **4h**



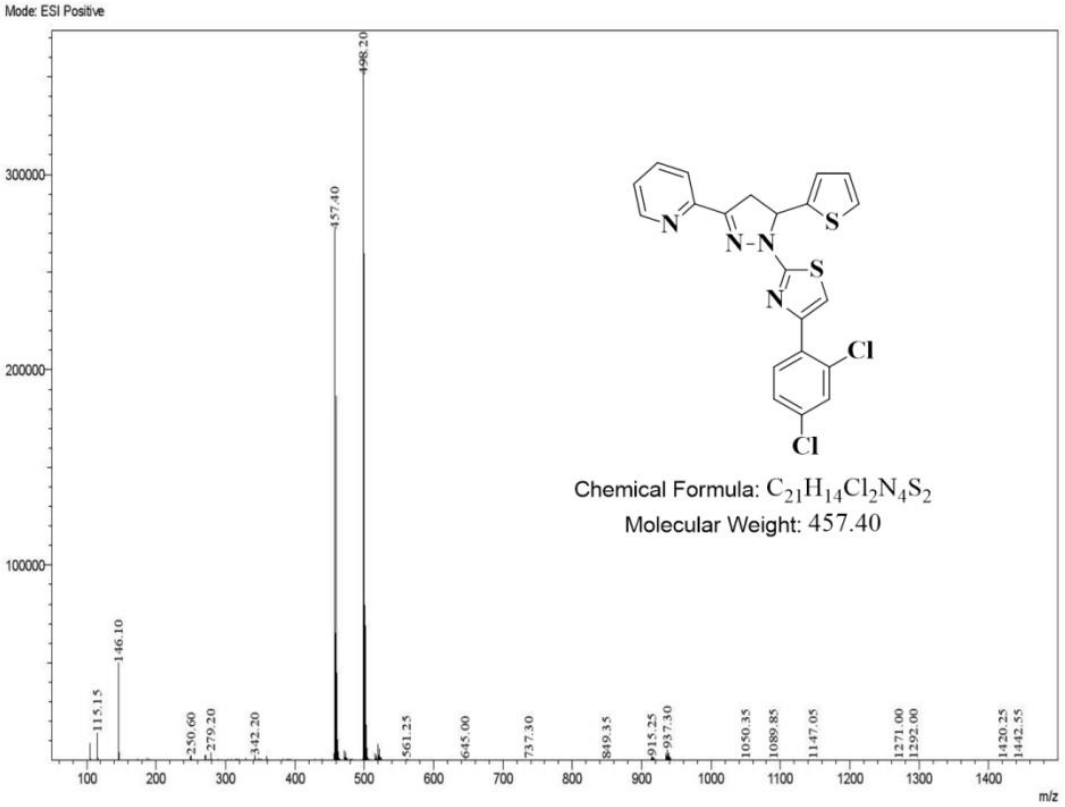
Mass spectrum of compound **4i**



Mass spectrum of compound **4j**



Mass spectrum of compound **4k**



Mass spectrum of compound **4l**

References:

[1] G. Mahler, G. Serra, S. Dematteis, J. Saldaña, L. Domínguez, E. Manta, *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1309–1311.

[2] S. M. Riyadh, T. A. Farghaly, M. A. Abdallah, M. M. Abdalla, M. R. Abd El-Aziz, *Eur. J. Med. Chem.* **2010**, *45*, 1042–1050.

[3] R. F. George, E. M. Samir, M. N. Abdelhamed, H. A. Abdel-Aziz, S. E. S. Abbas, *Bioorg. Chem.* **2019**, *83*, 186–197.

[4] K. Vaarla, R. K. Kesharwani, K. Santosh, R. R. Vedula, S. Kotamraju, M. K. Toopurani, *Bioorg. Med. Chem. Lett.* **2015**, *25*, 5797–5803.

[5] R. V. Ragavan, V. Vijayakumar, N. S. Kumari, *Eur. J. Med. Chem.* **2010**, *45*, 1173–1180.

[6] A. M. Vijesh, A. M. Isloor, V. Prabhu, S. Ahmad, S. Malladi, *Eur. J. Med. Chem.* **2010**, *45*, 5460–5464.

[7] S. Ningaiah, U. K. Bhadraiah, S. D. Doddaramappa, S. Keshavamurthy, C. Javarasetty, *Bioorg. Med. Chem. Lett.* **2014**, *24*, 245–248.

[8] D. M. Masoud, R. A. Azzam, F. Hamdy, A. A. I. Mekawey, H. A. Abdel-Aziz, *J. Heterocycl. Chem.* **2019**, *56*, 3030–3041.

[9] Z. Xu, C. Gao, Q.-C. Ren, X.-F. Song, L.-S. Feng, Z.-S. Lv, *Eur. J. Med. Chem.* **2017**, *139*, 429–440.

[10] A. Ayati, S. Emami, A. Asadipour, A. Shafiee, A. Foroumadi, *Eur. J. Med. Chem.* **2015**, *97*, 699–718.

[11] R. Bashir, S. Ovais, S. Yaseen, H. Hamid, M. S. Alam, M. Samim, S. Singh, K. Javed, *Bioorg. Med. Chem. Lett.* **2011**, *21*, 4301–4305.

[12] S. Viveka, Dinesha, P. Shama, G. K. Nagaraja, S. Ballav, S. Kerkar, *Eur. J. Med. Chem.* **2015**, *101*, 442–451.

[13] A. Bekhit, *Eur. J. Med. Chem.* **2003**, *38*, 27–36.

[14] M. F. Khan, M. M. Alam, G. Verma, W. Akhtar, M. Akhter, M. Shaquiquzzaman, *Eur. J. Med. Chem.* **2016**, *120*, 170–201.

[15] J. V. Faria, P. F. Vegi, A. G. C. Miguita, M. S. dos Santos, N. Boechat, A. M. R. Bernardino, *Bioorg. Med. Chem.* **2017**, *25*, 5891–5903.

[16] K. M. Dawood, T. M. A. Eldebss, H. S. A. El-Zahabi, M. H. Yousef, P. Metz, *Eur. J. Med. Chem.* **2013**, *70*, 740–749.

[17] E. Mansour, A. Aboelnaga, E. M. Nassar, S. I. Elewa, *Synth. Commun.* **2020**, *50*, 368–379.

[18] B. Sever, M. D. Altıntop, M. O. Radwan, A. Özdemir, M. Otsuka, M. Fujita, H. I. Ciftci, *Eur. J. Med. Chem.* **2019**, *182*, 111648.

[19] P. Ravula, H. B. Vamaraju, M. Paturi, S. Bodige, K. C. Gulipalli, J. N. Narendra Sharath Chandra, *J. Heterocycl. Chem.* **2018**, *55*, 1313–1323.

[20] Z.-C. Wang, F.-Q. Shen, M.-R. Yang, L.-X. You, L.-Z. Chen, H.-L. Zhu, Y.-D. Lu, F.-L. Kong, M.-H. Wang, *Bioorg. Med. Chem. Lett.* **2018**, *28*, 3816–3821.

[21] M. Atobe, K. Naganuma, M. Kawanishi, T. Hayashi, H. Suzuki, M. Nishida, H. Arai, *Bioorg. Med. Chem. Lett.* **2018**, *28*, 2408–2412.

[22] A. Saeed, P. A. Mahesar, P. A. Channar, Q. Abbas, F. A. Larik, M. Hassan, H. Raza, S.-Y. Seo, *Bioorg. Chem.* **2017**, *74*, 187–196.

[23] T. K. Mohamed, R. Z. Batran, S. A. Elseginy, M. M. Ali, A. E. Mahmoud, *Bioorg. Chem.* **2019**, *85*, 253–273.

[24] K. Vaarla, S. Karnewar, D. Panuganti, S. R. Peddi, R. R. Vedula, V. Manga, S. Kotamraju, *ChemistrySelect* **2019**, *4*, 4324–4330.

[25] M. Edrees, S. Melha, A. Saad, N. Kheder, S. Gomha, Z. Muhammad, *Molecules* **2018**, *23*, 2970.

[26] A. Sebastian, V. Pandey, C. D. Mohan, Y. T. Chia, S. Rangappa, J. Mathai, C. P. Baburajeev, S. Paricharak, L. H. Mervin, K. C. Bulusu, et al., *ACS Omega* **2016**, *1*, 1412–1424.

[27] S. M. Gomha, A. O. Abdelhamid, N. A. Abdelrehem, S. M. Kandeel, *J. Heterocycl. Chem.* **2018**, *55*, 995–1001.

[28] M. Madni, M. N. Ahmed, S. Hameed, S. W. Ali Shah, U. Rashid, K. Ayub, M. N. Tahir, T. Mahmood, *J. Mol. Struct.* **2018**, *1168*, 175–186.

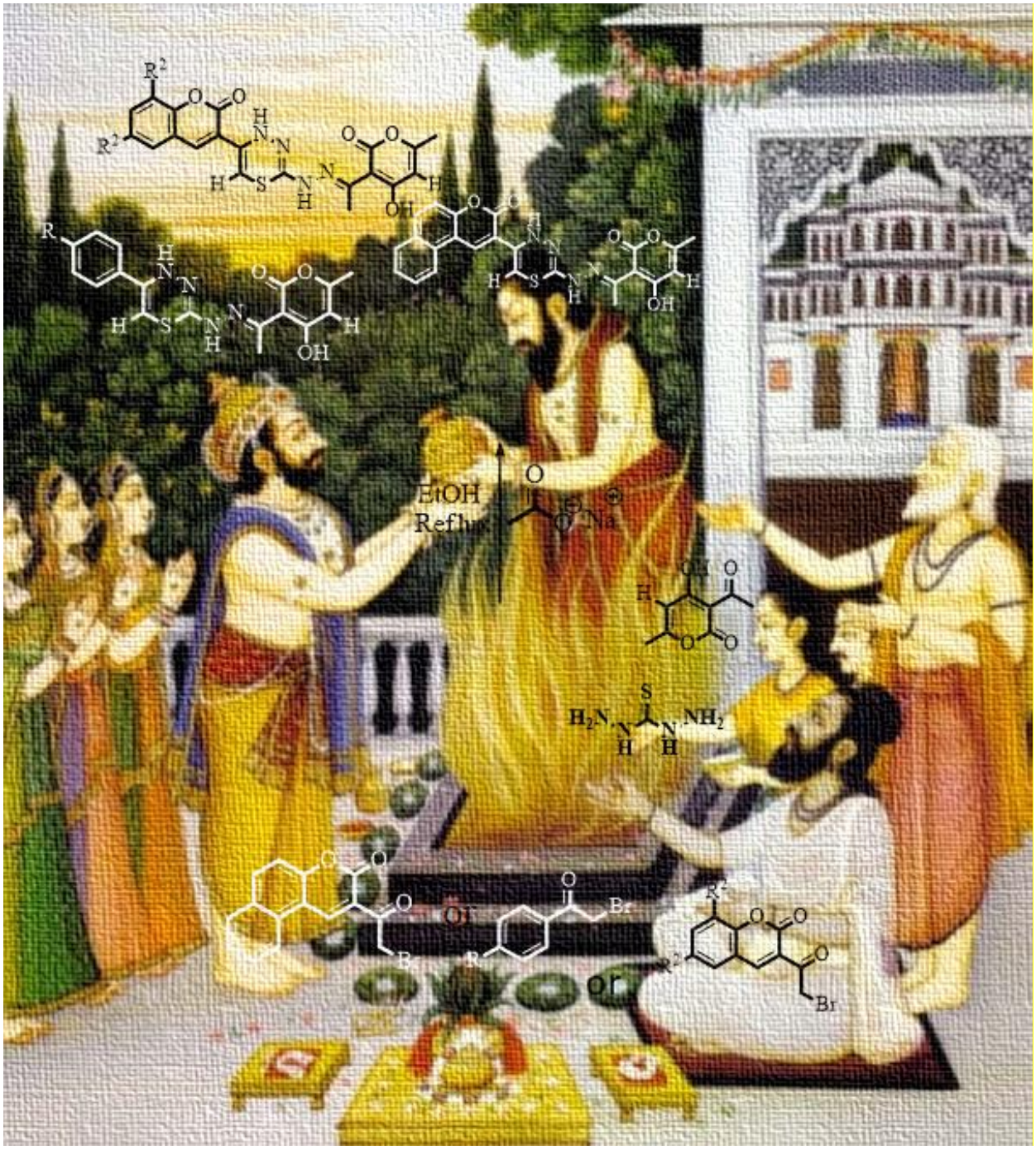
[29] V. Arandkar, K. Vaarla, R. R. Vedula, *Synth. Commun.* **2018**, *48*, 1285–1290.

[30] R. S. Gouhar, E. F. Ewies, M. F. El-Shehry, M. N. F. Shaheen, E.-M. M. E. Ibrahim, *J. Heterocycl. Chem.* **2018**, *55*, 2368–2380.

[31] M. Gümüş, A. Dişli, M. Yakan, S. Yiğitcan, İ. Koca, *Org. Commun.* **2018**, *11*, 98–110.

CHAPTER-IV

One-pot, three component synthesis of a new series of 1,3,4-thiadiazines



CHAPTER-IV

4.1. Introduction:

Heterocyclic chemistry is considered as an important field in medicinal chemistry. In heterocyclic chemistry; the compound which has nitrogen and sulphur atoms in five membered and six membered ring systems are manifesting different biological and material applications and have received a pivotal role in organic chemistry. Out of these nitrogen and sulphur heterocyclic compounds, thiadiazine derivatives are a prominent significance in medicinal chemistry.

Thiadiazine molecule is a six membered heterocyclic ring system having one sulphur atom and two nitrogen atoms. Thiadiazines will exist in different isomeric forms, those are 1,2,4-thiadiazine (**A**), 1,2,6-thiadiazine (**B**), 1,3,4-thiadiazine (**C**) and 1,3,5-thiadiazines (**D**) (Figure 4.1). Out of these isomeric forms, 1,3,4-thiadiazine is a well described isomeric system and have variety of applications in the field of pharmacology, bio chemical, materials, dyes and agriculture and photography.

Figure 4.1 Isomeric forms of thiadiazines.

In 1,3,4-thiadiazine heterocyclic system sulphur and two nitrogen atoms are present at first, third and fourth positions. 1,3,4-thiadiazine has three isomeric structures i.e. 2*H*- 1,3,4-thiadiazine, 4*H*- 1,3,4-thiadiazine and 6*H*- 1,3,4-thiadiazine (Figure 4.2). The most common method for the synthesis of 1,3,4-thiadiazine heterocyclic system was the condensation reaction of α-haloketones with thiosemicarbazide in acidic reaction condition[1].

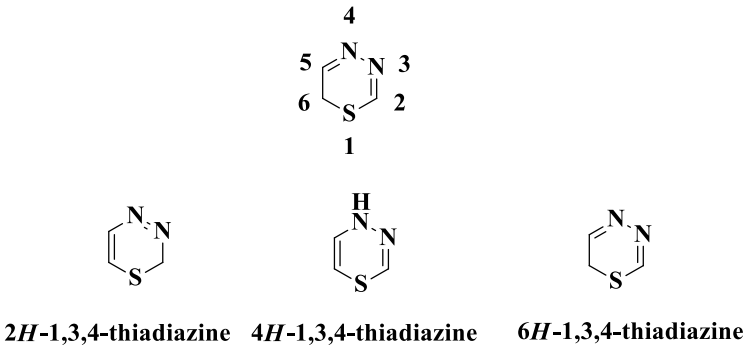


Figure 4.2 Isomeric forms of 1,3,4-thiadiazines.

Furthermore, substituted 1,3,4-thiadiazine and their derivatives are a prominent class of medicinally relevant compounds on account of their anti-inflammatory[2], antimicrobial[3], antistress[4], anticancer[5], metalloproteinase inhibition[6], STAT3 inhibitor[7], cyclic AMP phosphodiesterase inhibitor[8], hepatitis C virus polymerase inhibitor[9], cholinesterase inhibitor[10], cyclindependent kinase inhibitor[11] and PDE4 inhibitor[12]. Subsequently coumarin based derivatives are one of the prime class of biologically active compounds due to their antioxidant[13,14], anti-inflammatory[15], antimicrobial[16,17], antiviral[18], antituberculosis[19], anticancer[20,21], anticoagulant[22], anticholinesterase[23,24], antidepressant agent[25] (Figure 4.3).

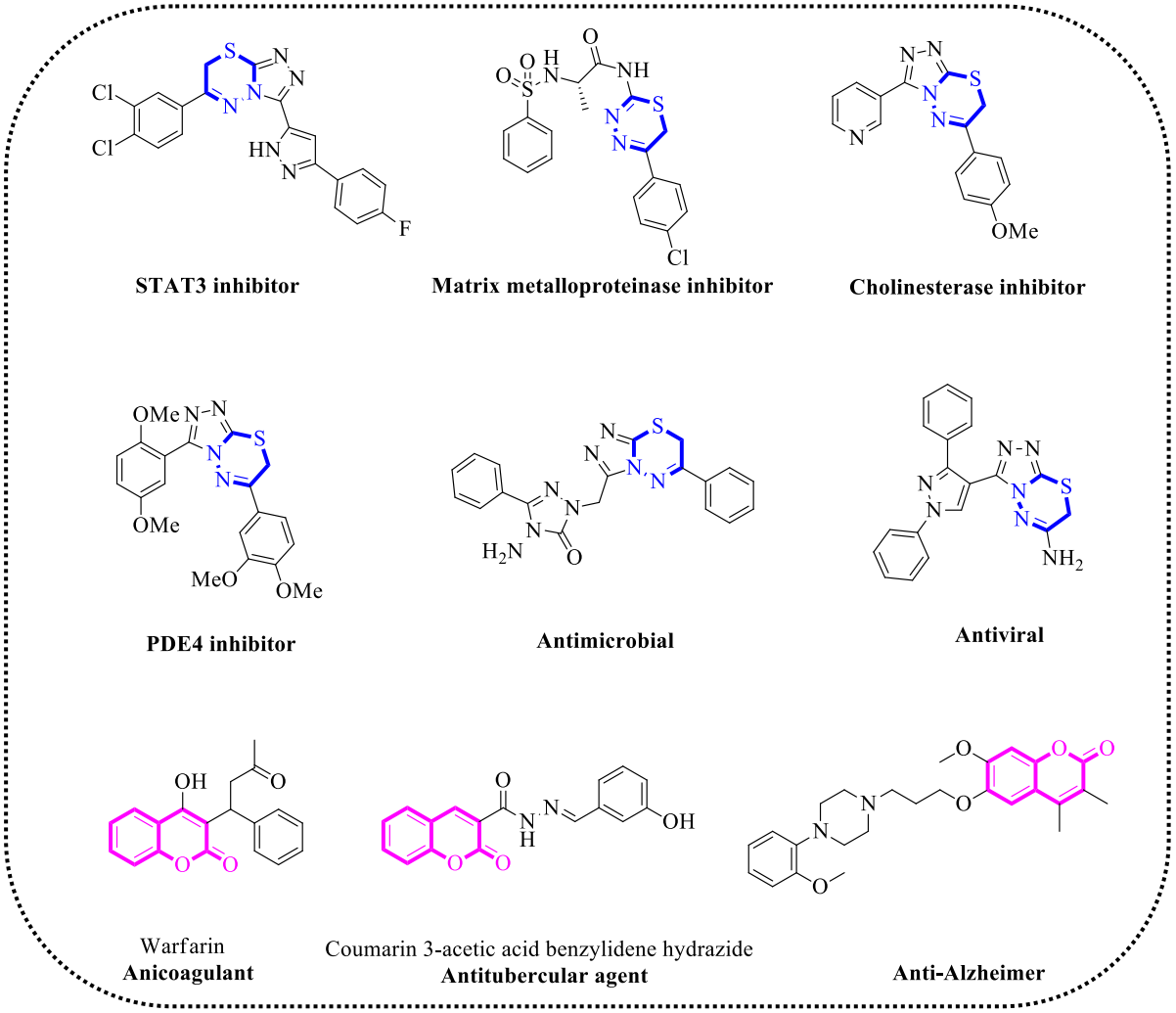
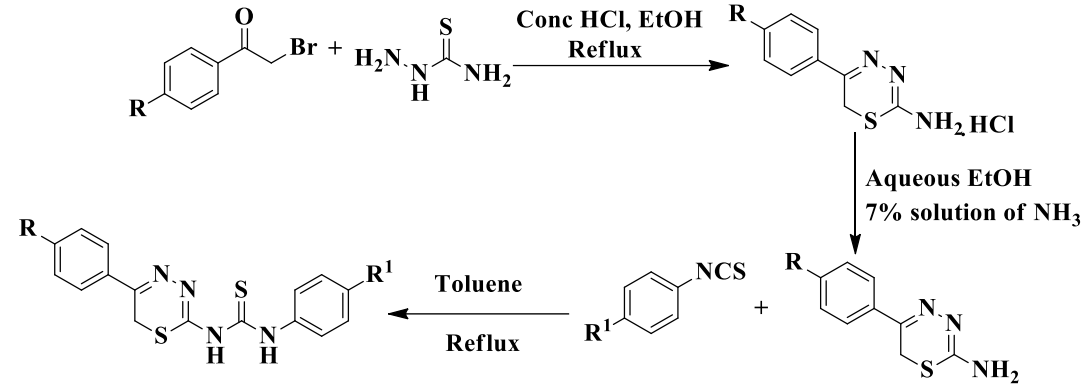


Figure 4.3 Some of the biological active important derivatives of thiadiazines, coumarins.

The following is a compact review of literature on substituted 1,3,4-thiadiazine analogues.

Heba Abdelrasheed Allama[26] *et al.* synthesized a new series of 1,3,4-thiadiazine-thiourea analogues from α-bromoketones, thiosemicarbazide and substituted phenyl isothiocyanate (Scheme 4.1). Furthermore, these title compounds were evaluated *in vitro* cytotoxic activity against Non-Small Cell Lung Cancer. In which compounds **1**, **2**, **3** (Figure 4.4) exhibited good anticancer activity.



Scheme 4.1

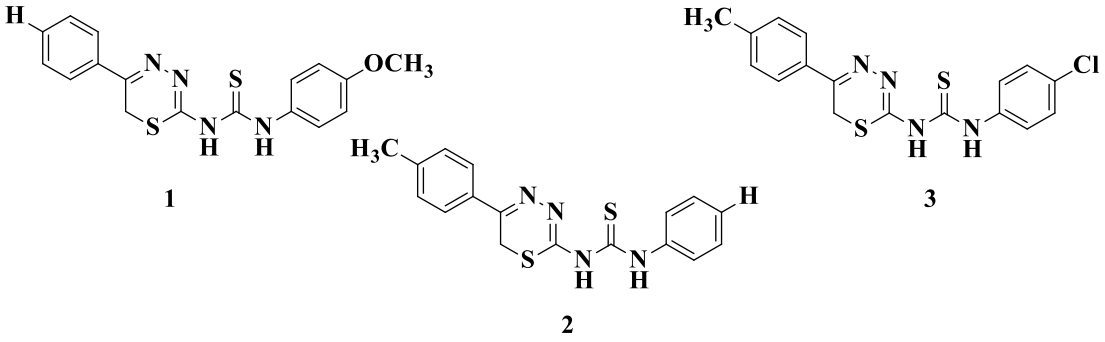
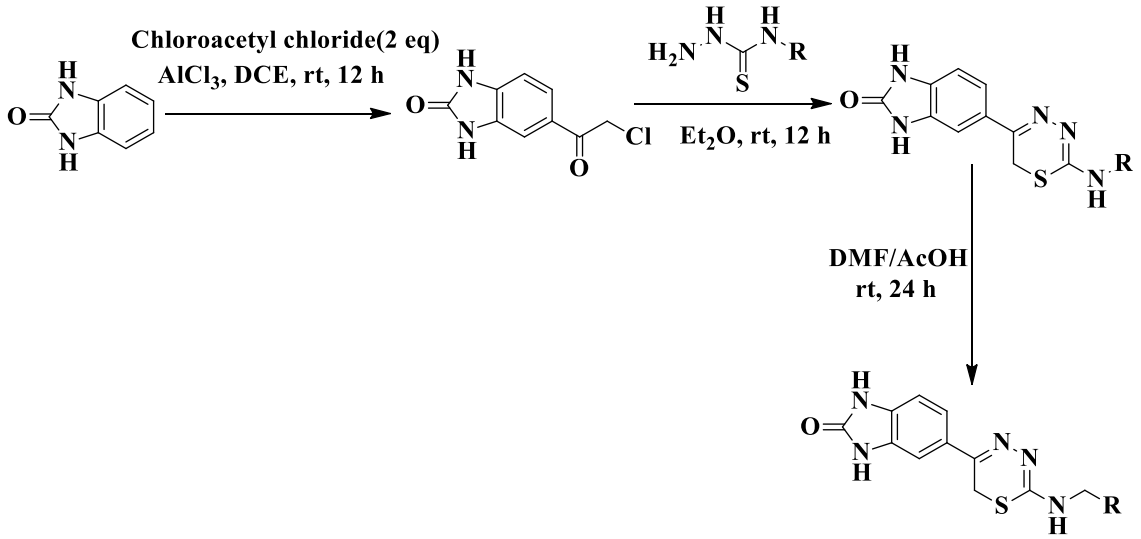


Figure 4.4

DeVita[27] *et al.* synthesized a new series of 1,3,4 thiadiazine derivatives using starting materials such as 2-hydroxybenzimidazole, chloroacetyl chloride and substituted thiocarbohydrazides (Scheme 4.2). In addition, these title compounds were evaluated for the Dual-Specificity Tyrosine Phosphorylation-Regulated Kinase (DYRK1A) inhibitors, from the results **4**, **5** (Figure 4.5) manifested excellent activity.



Scheme 4.2

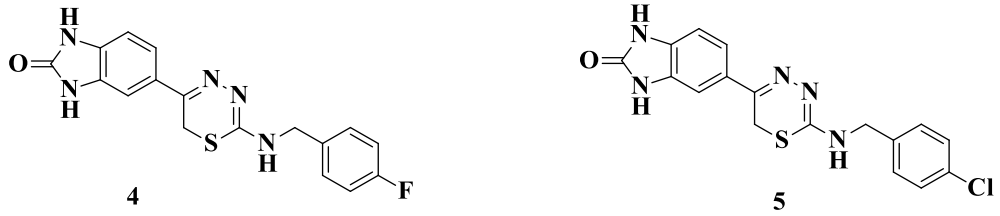
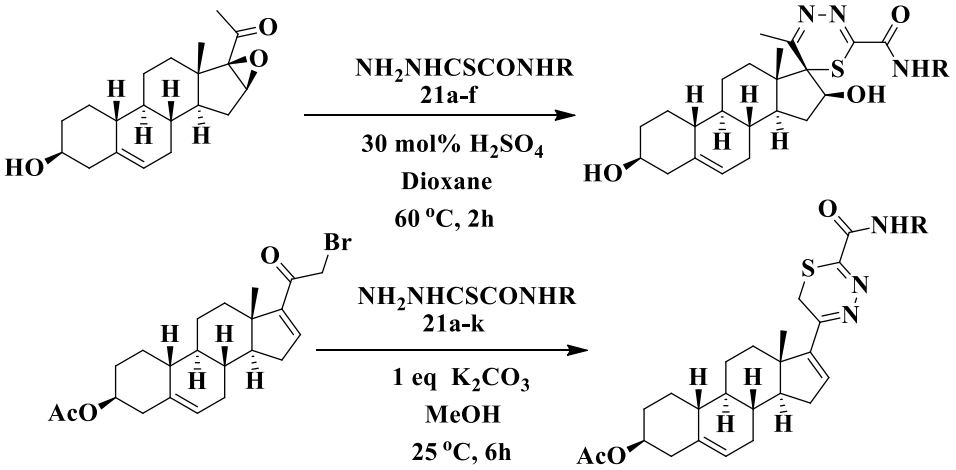


Figure 4.5

Volkova[28] *et al.* synthesized a new series of steroidal 1,3,4-thiadiazine analogues from 16*β*,17*β*-epoxypregnenolone, 21-bromopregna-5,16-dien-20-one and oxamic acid thiohydrazides (Scheme 4.3). Moreover, these title compounds were evaluated against human androgen receptor-positive prostate cancer cell line 22Rv1. Out of these compounds **6**, **7** (Figure 4.6) exhibits excellent activity compared to antiandrogen bicalutamide (standard drug).



Scheme 4.3

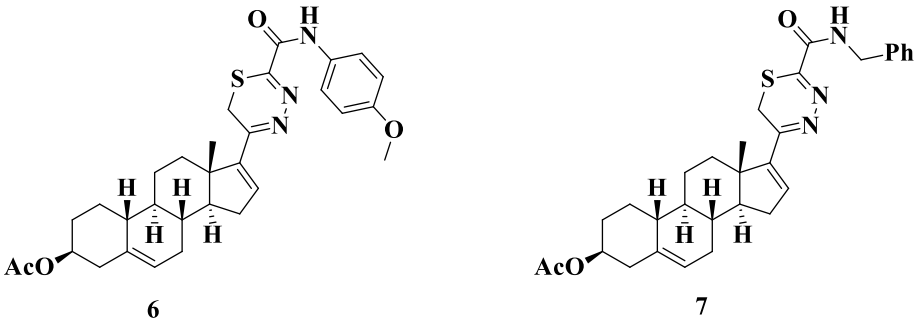
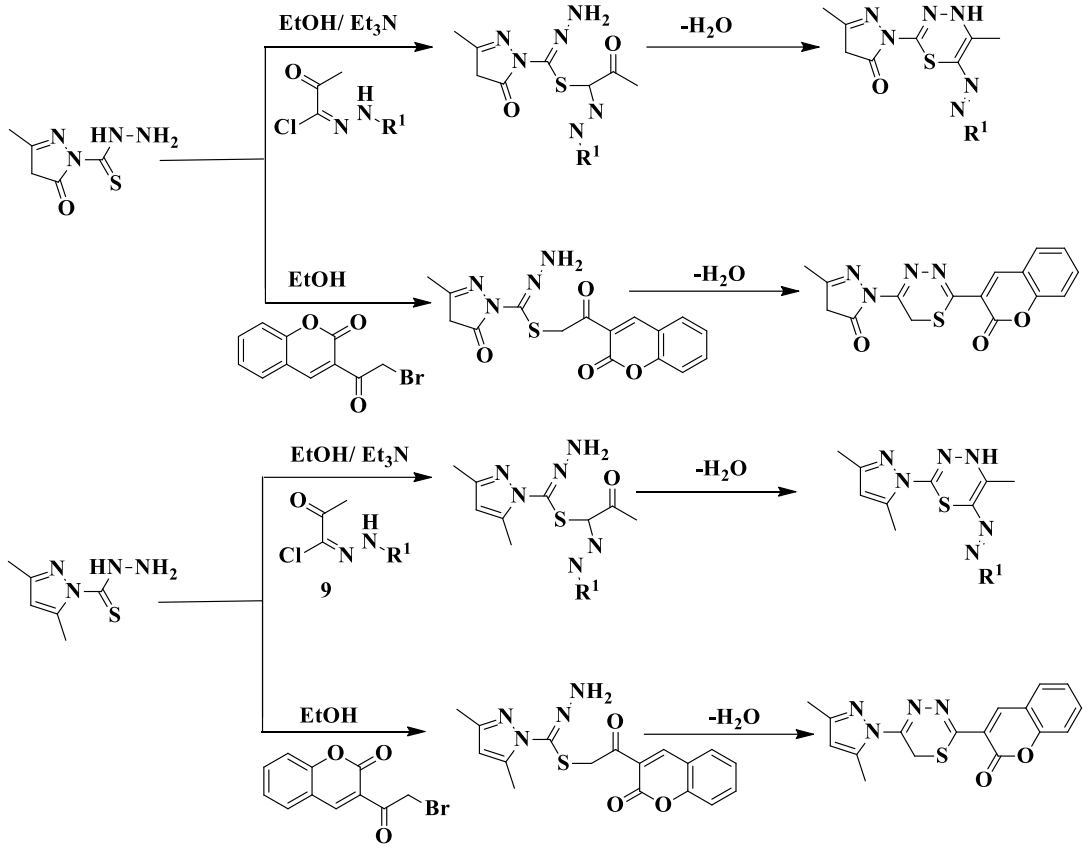


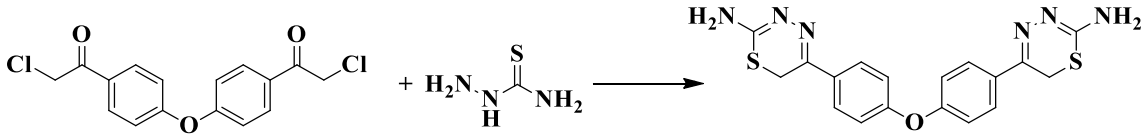
Figure 4.6

Ibrahim Ali M. Radini[29] synthesized a series of new pyrazolyl 1,3,4-thiadiazine derivatives using pyrazole-1-carbothiohydrazide, 2-oxo-N[l]-arylpropanehydrazonoyl chloride, 3-bromoacetylcoumarin(Scheme 4.4). Moreover, these title compounds evaluated anti-microbial activity, exhibited moderate to good activity.



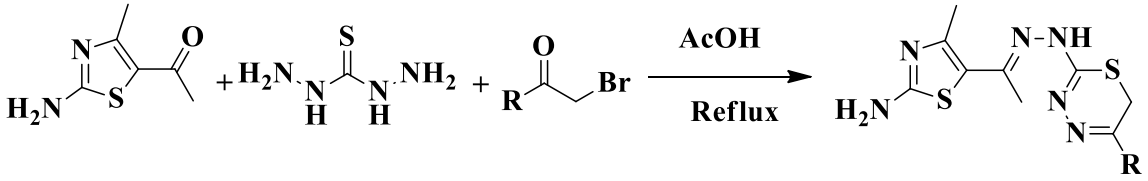
Scheme 4.4

Ahmet Burak Sarıguney[30] *et al.* synthesized a new series of thidiazine analogues from chloroacetophenone, substituted thiosemicarbazides (Scheme 4.5). Furthermore, these title compounds exhibited anti-microbial activity against *Bacillus cereus*, *Salmonella typhimurium*, *Staphylococcus aureus* and *Escherichia coli* Gram positive bacteria.



Scheme 4.5

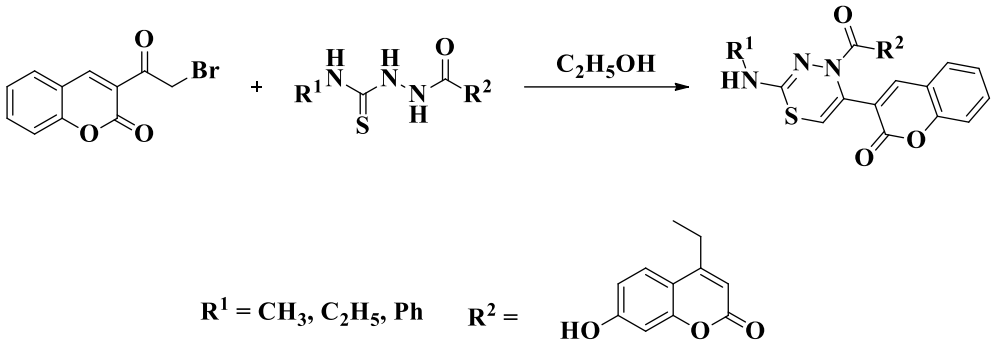
Rajeswar Rao[31] *et al.* synthesized a series of new 1,3,4-thiadiazinyl hydrazonothiazolamine analogues starting from 2-amino-4-methyl-5-acetylthiazole, thiocarbohydrazide and substituted 3-(2-bromoacetyl)coumarins or phenacyl bromides (Scheme 4.6). These compounds were evaluated for their *in vitro* anti-malarial properties and exhibited good antimalarial activity.



Scheme 4.6

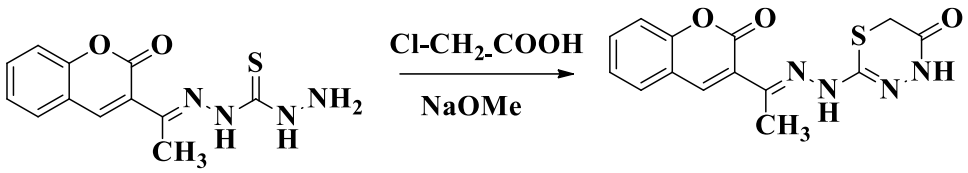
M. Cacic[32] *et al.* synthesized a new series of trisubstituted 1,3,4- thiadiazine analogues using 3-

(2-bromoacetyl)coumarins with substituted thiosemicarbazides (Scheme 4.7). Furthermore, these title compounds were evaluated for their antifungal, antioxidant activities and exhibited good activity against *A. ochraceus, F. verticillioides*.



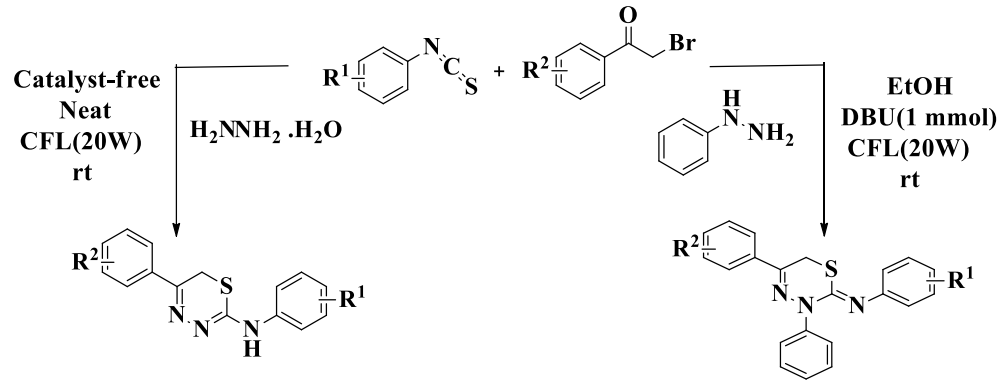
Scheme 4.7

Anhar Abdel-Aziem[33] synthesized a new series of 1,3,4-thiadiazines starting from *N'*-(1-(2-oxo-2*H*-chromen-3-yl)ethylidene)hydrazinecarbothiohydrazide and chloroacetic acid (Scheme 4.8). Moreover, these title compounds showed good antimicrobial activity.



Scheme 4.8

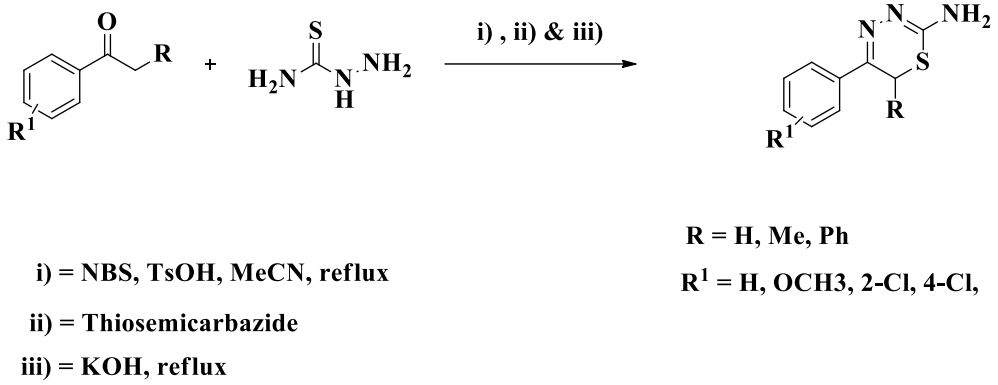
Jagdamba Singh[34] and co-workers synthesized a series of new 1,3,4-thiadiazine derivatives via a photochemical and solvent free reaction. These title compounds were synthesized using arylisothiocyanate, α-bromoketones and hydrazines under CFL with good yields (Scheme 4.9).



Scheme 4.9

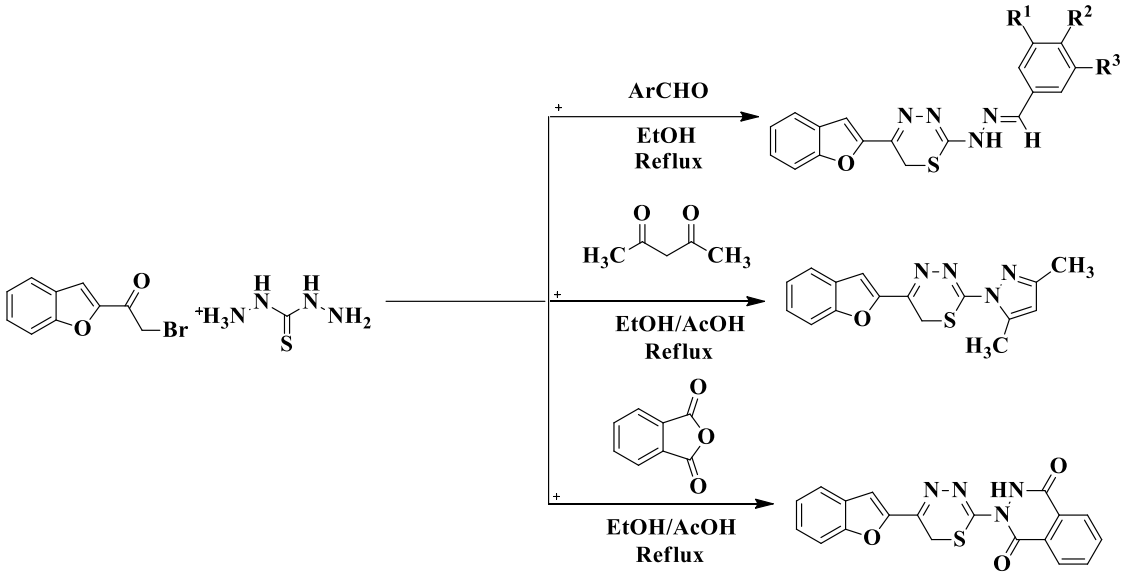
Fredrik Lehmann[35] *et al.* synthesized a series of 6*H*-1,3,4-thiadiazine analogues starting from readily available aromatic aryl ketones *via* a one pot three step synthesis. At first the bromination of aryl ketones using *N*-bromosuccinimide, *p*-toluenesulphonic acid gave bromo aryl ketones. After thiosemicarbazide reacts with brominated aryl ketones form uncyclised

product. Furthermore, in third step uncyclised product reacts with KOH to get the title compounds with good yields (Scheme 4.10).



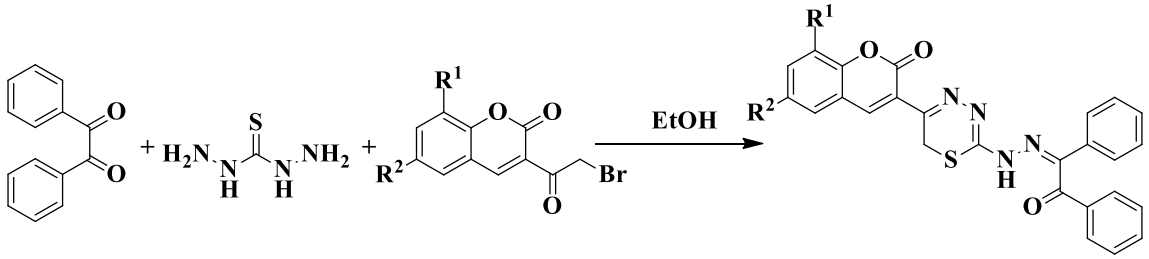
Scheme 4.10

Rajeswar Rao[36] *et al.* synthesized a new series of 2,5-disubstituted-1,3,4-thidiazine analogues *via* a one pot three component reaction. These title compounds were synthesized from readily available starting materials like 2-(2-bromoacetyl)benzofuran, thiocarbohydrazide with different aromatic aldehydes, acetylacetone and pthalic anhydride with excellent yields (Scheme 4.11).



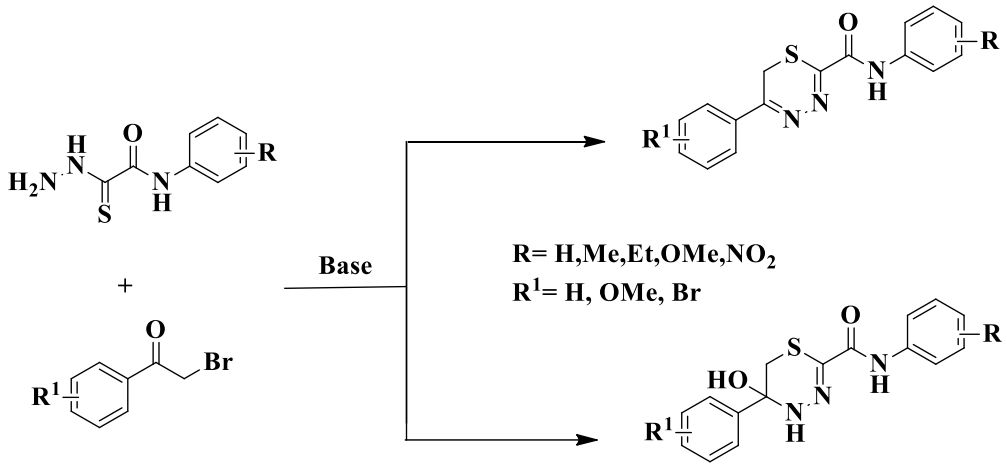
Scheme 4.11

Rajeswar Rao[37] *et al.* synthesized a new series of 2-oxo-1,2-diphenylethylidene hydrazinyl thiadiazinyl-2*H*-chromen-2-one analogues *via* a one pot three component reaction starting from benzyl, thiocarobohydrazide, substituted 3-(2-bromoacetyl)-2*H*-chromen-2-one derivatives with good yields (Scheme 4.12).



Scheme 4.12

A. Volkova[38] *et al*. synthesized a new series of 2-carboxamide-substituted 1,3,4-thiadiazines and 5,6-dihydro-4*H*-1,3,4-thiadiazin-5-ols starting from oxamic acid, thiohydrazides and phenacyl bromides with good yields (Scheme 4.13).



Scheme 4.13

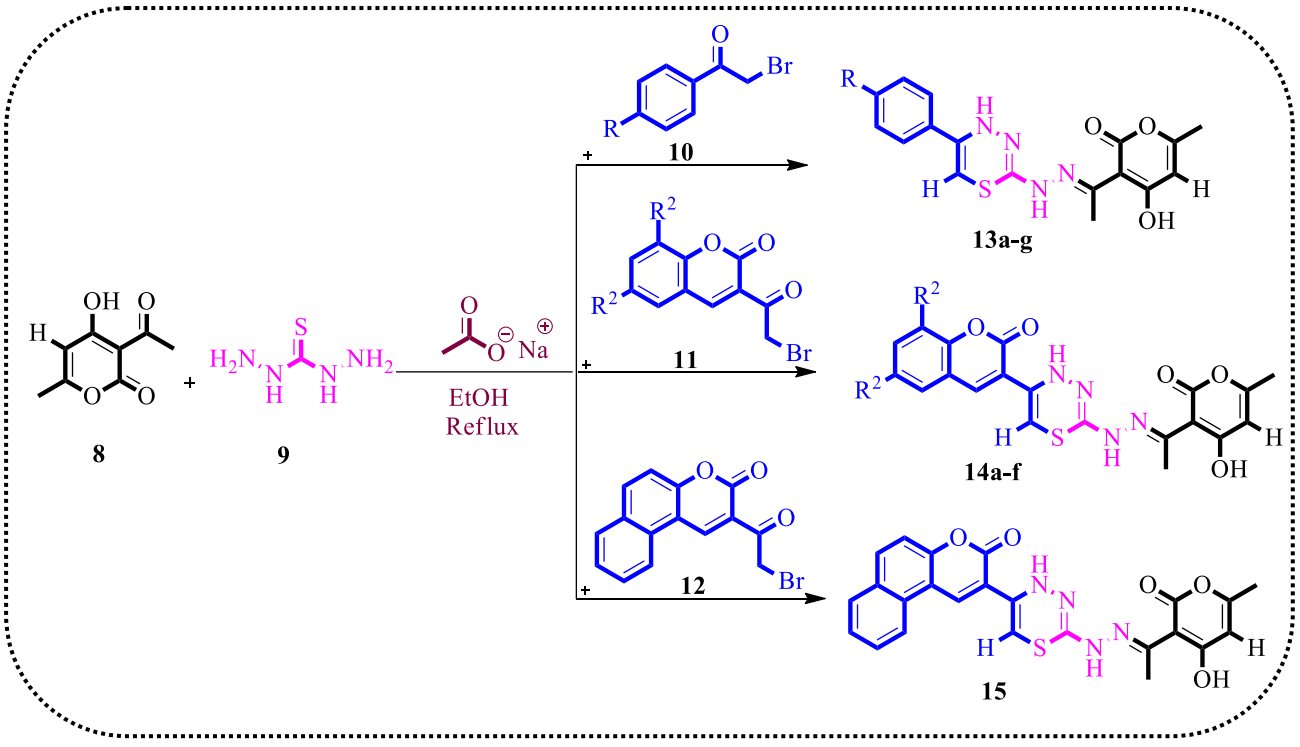
4.2. Present work:

4.2.1. Starting materials:

In the present chapter, the synthesis of new 1,3,4 thiadiazin hybrids was described dehydroacetic acid, thiocarbohydrazide, phenacyl bromides and substituted 3-(2-bromoacetyl) coumarins were used as the starting materials required for the synthesis of the title compounds. Dehydroacetic acid, thiocarbohydrazide, phenacyl bromides were procured from commercial sources. as per the literature described in chapter-1, substituted 3-(2-bromoacetyl) coumarins were prepared.

4.2.2. Synthesis of 1,3,4 thiadiazines:

The title compounds (**13a-g**, **14a-f** and **15**) were obtained by the reaction an equimolar ratio of dehydroacetic acid (**8**), thiocarbohydrazide (**9**) and phenacyl bromides (**10a-g**) or substituted 3-(2-bromoacetyl) coumarins (**11a-f, 12**), along with catalytic amount of sodium acetate in ethanol under reflux with good yields. The general schematic representation is outlined in scheme 4.14.

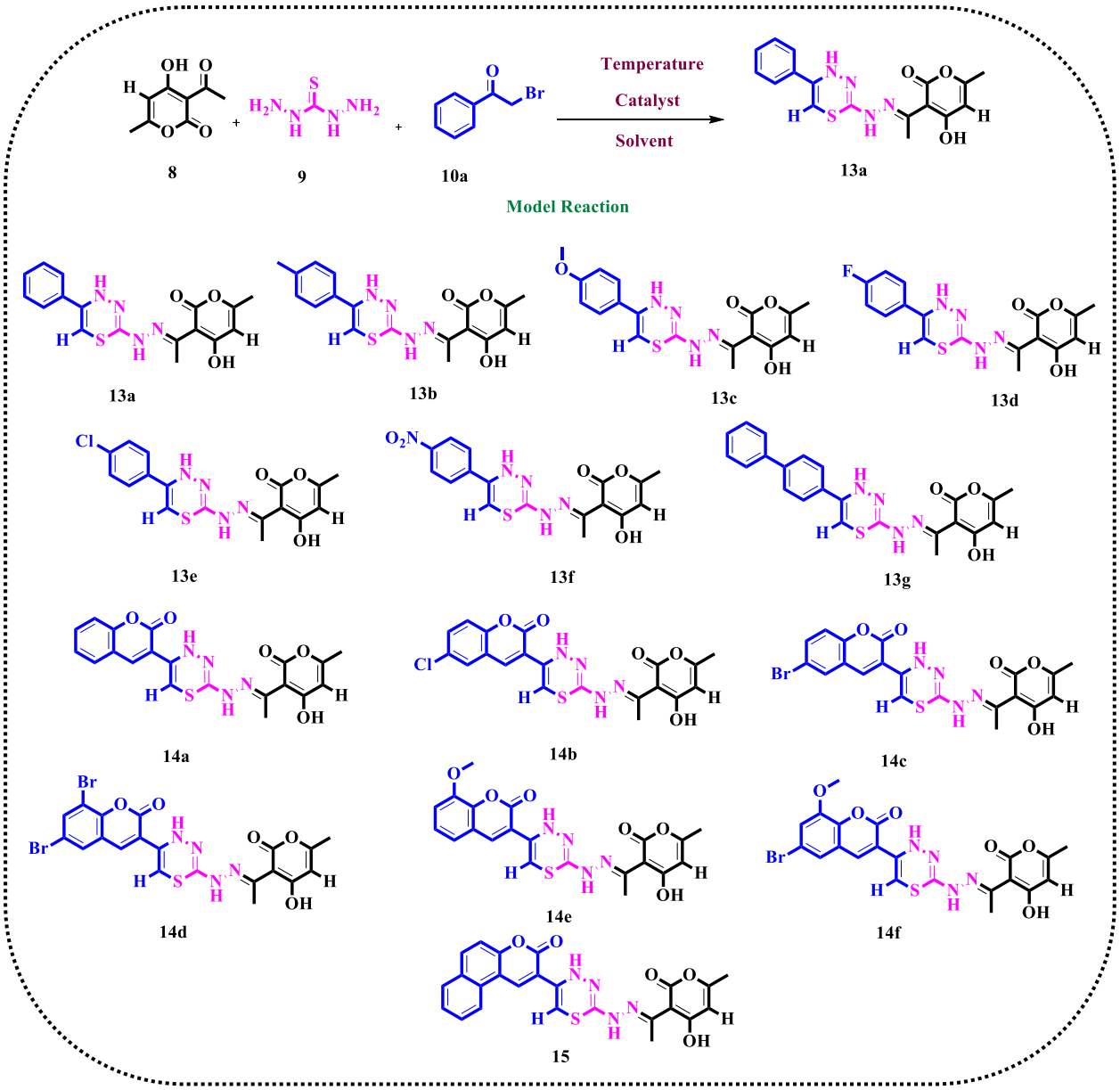


Scheme 4.14: Synthesis of 1,3,4-thiadiazine *via* multi component reaction.

4.2.3. Results and discussion:

At first the test reaction was carried out by using a one- pot MCR approach with dehydroacetic acid (**8**), thiocarbohydrazide (**9**) and substituted phenacyl bromides (**10a**) in EtOH, then for the optimization of this reaction was done by screening of solvents (Scheme 4.15). Variety of solvents such as EtOH, DMSO, DMF, CH_3CN (Table 4.2, entries 1–5) were used. From this it was concluded that EtOH (Table 4.2, entry 1) was best solvent among the tested solvents in

terms of yields. Moreover, for the improving the yields we continued these test reactions. It has been manifested that EtOH (Table 4.2, entry 6) was best among the screened solvents in terms of time and yield. Furthermore, for the optimization of reaction, we screened different catalysts (Scheme 4.15) like NaOH, KOH, Na_2CO_3, $NaHCO_3$, Piperidine, Et_3N and CH_3COONa (Table 4.2, entries 11–18). After the screening of catalyst, sodium acetate (Table 4.2, entry 18) has shown good results in terms of yields, and also it is an environmental friendly catalyst, high yield, low cost simple procedure to get the title compounds.



Scheme 4.15: Optimization of 1,3,4-thiadiazine synthesis, hybrids, time and isolated yields.

Table 4.1: Different substitutions of 1,3,4-thiadiazine hybrids (**13a-g**, **14a-f** and **15**), time, [a]isolated yield.

Entry	Product	R	R^1	R^2	Time(h)	Yield (%)[a]
1	**13a**	H	-	-	5	91
2	**13b**	CH_3	-	-	6	90
3	**13c**	OCH_3	-	-	6	92
4	**13d**	F	-	-	6	92
5	**13e**	Cl	-	-	5	89
6	**13f**	NO_2	-	-	5	91
7	**13g**	Ph	-	-	5	84
8	**14a**	-	H	H	5	93
9	**14b**	-	Cl	H	5	89
10	**14c**	-	Br	H	5	93
11	**14d**	-	Br	Br	5	92
12	**14e**	-	H	OCH_3	5	88
13	**14f**	-	Br	OCH_3	5	94
14	**15**	-	-	-	6	91

Table 4.2: Optimization of the solvent and catalyst for the synthesis of **13a** (1,3,4-thiadiazine) *via* three-component reaction.

S. No.	Solvent	Catalyst	Temperature (ºC)	Time (h)	Yield (%) [a]
1	EtOH	-	60	10	29
2	MeOH	-	60	10	22
3	DMSO	-	60	10	21
4	DMF	-	60	10	20
5	CH_3CN	-	60	10	18
6	EtOH	-	60	13	34
7	MeOH	-	60	14	28
8	DMSO	-	60	16	31
9	DMF	-	60	15	29
10	CH_3CN	-	60	17	30
11	EtOH	NaOH	60	5	51
12	EtOH	KOH	60	5	40
13	EtOH	Na_2CO_3	60	5	37
14	EtOH	$NaHCO_3$	60	5	32
15	EtOH	Piperdine	60	5	59
17	EtOH	Et_3N	60	5	60
18	**EtOH**	**CH_3COONa**	**60**	**5**	**84**

[a] Isolated yields.

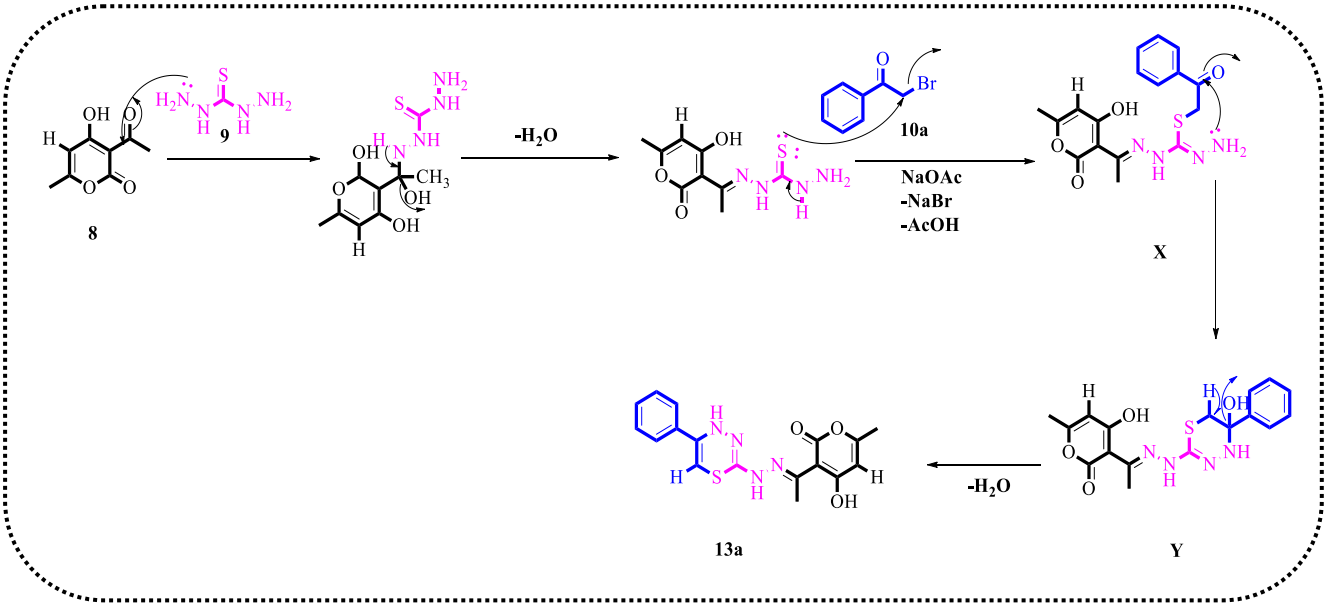
Nonetheless, for the optimization of reaction we screened the temperature, and loads of the catalyst (Scheme 4.15). The reaction was carried out at different temperatures (Table 4.3, entries 1– 3). By screening of temperature under reflux conditions we got best results in terms of yield, then subsequently reaction was carried out with different sodium acetate mol percentages (Table 4.3, entries 4– 6). Hence high yield at less reaction time was observed in ethanol under reflux conditions with 30 mol% sodium acetate as a catalyst.

Table 4.3: Optimization of reaction temperature and time for the synthesis of **13a** (1,3,4-thiadiazine) *via* three-component reaction.

S. No.	Solvent	CH_3COONa (mol%)	Temperature (°C)	Time (h)	Yield (%) [a]
1	EtOH	30	40	5	75
2	EtOH	30	60	5	84
3	**EtOH**	**30**	**Reflux**	**5**	**91**
4	EtOH	10	Reflux	5	42
5	EtOH	20	Reflux	5	62
6	EtOH	40	Reflux	5	86

[a] Isolated yields.

A plausible mechanism for the formation of thiadiazin hydrazone (**13a)** is portrayed in scheme 4.16. At first dehydroacetic acid (**8**) is treated with thiocarbohydrazide (**9**) to form thiocarbazone, this intermediate further reacts with simple phenacyl bromide (**10a**) to form title compound **13a.**



Scheme 4.16: A plausible mechanism for the synthesis of **13a** *via* a one-pot three-component reaction.

The synthesized compound structures (**13a-g**, **14a-f** and **15**) were established by their physical and analytical data. The IR spectra of synthesized compounds (**13a-g, 14a-f** and **15**) exhibited the presence of imine (C=N), lactone carbonyl (C=O), amine (NH) and hydroxyl (OH) functional group appears in the region of 1650-1668 cm^{-1}, 1699-1706 cm^{-1}, 3206-3275 cm^{-1} and 3389-3425 cm^{-1} respectively. In the ^{1}H-NMR spectra showed characteristic peaks for the 2 NH protons in the range of δ 5.01- 5.68, 5.66- 5.81 ppm respectively, thiadiazin proton in the range of δ 5.78- 5.89 ppm. And pyran proton appears in the range of δ 6.13- 6.77 ppm and also pyran

attached proton appears in the range of δ 11.41- 11.92 ppm. Furthermore, in the ^{13}C-NMR spectra exhibited characteristic peaks for the thiadiazin C-5 carbon in the range of δ 152.56-64.74 ppm, whereas pyran C-2 carbon in the range of δ 162.71- 166.68 ppm and pyran C4 carbon in the range of δ 180.67- 183.89 ppm. For example, the ^{1}H-NMR of compound **13a** exhibited characteristic peaks of 2 methyl protons appears as singlets at δ 2.15, 2.75 ppm respectively, peak at δ 5.44 ppm appears as singlet, it represents 2 NH protons, whereas 1,3,4 thiadiazin proton appears as singlet at δ 5.89 ppm, peak at δ 6.46 ppm appears as singlet, it represents pyran proton and pyran OH proton appeared as singlet at δ 11.77 ppm. while the aromatic protons appeared at δ 7.43–7.64 ppm. In ^{13}C NMR of compound **13a** manifested 2 methyl carbons appears at δ 16.93, 19.84 ppm respectively, the thiadiazin C-2 carbon appears at δ 142.35 ppm, thiadiazin C-5 carbon appears at δ 162.64 ppm and pyran adjacent C=N carbon appears at δ 162.72 ppm and also pyran C-2 carbon (carbonyl) appears at δ 163.19 ppm whereas pyran ring C-6 carbon appears at δ 164.40 ppm. And the most down field peak is due to pyran ring C-4 carbon at δ 180.67 ppm. The remaining sp^2 hybridized carbons appeared from 105.46 to 130.46 ppm. In the mass spectra of all the synthesized compounds have shown molecular ions corresponding to their molecular formula, which confirmed its chemical structure.

4.2.4. Conclusion:

In summary, we have developed a potential protocol for the synthesis of new 1,3,4 thiadiazine derivatives by a one-pot MCR approach.

4.2.5. Experimental:

General procedure for the synthesis of **4-hydroxy-6-methyl-3-(1-(2-(5-aryl-4*H*-1,3,4-thiadiazin-2-yl)hydrazono)ethyl)-2*H*-pyran-2-ones** (**13a- 13g**):

A mixture of dehydroacetic acid (1 m mol), thiocarbohydrazide (1 m mol), substituted phenacyl bromides (1 m mol) along with catalytic amount of sodium acetate (30 mol%) and ethanol was taken in a round bottom flask. Then reaction mixture was refluxed for 5 h. Progress of the reaction was monitored by thin layer chromatography. After completion of the reaction, the product was filtered and separated, washed with ethanol, dried and recrystallized from ethanol.

General procedure for the synthesis of **3-(2-(2-(1-(4-hydroxy-6-methyl-2-oxo-2*H*-pyran-3-yl)ethylidene)hydrazinyl)-4*H*-1,3,4-thiadiazin-5-yl)-2*H*-chromen-2-ones (14a- 14f):**

A mixture of dehydroacetic acid (1 m mol), thiocarbohydrazide (1 m mol), substituted 3-(2-bromoacetyl) coumarins (1 m mol) and catalytic amount of sodium acetate (30 mol%) in ethanol was refluxed in a round bottom flask for about 5 h; progress of the reaction is monitored by thin layer chromatography. After completion of the reaction, the product was filtered and isolated,

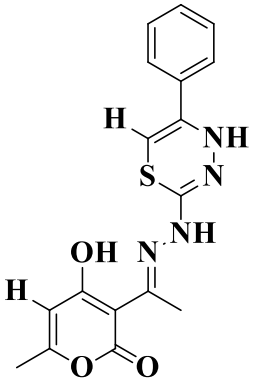
washed with ethanol, dried and recrystallized from ethanol.

General procedure for the synthesis of **2-(2-(2-(1-(4-hydroxy-6-methyl-2-oxo-2*H*-pyran-3-yl)ethylidene)hydrazinyl)-4*H*-1,3,4-thiadiazin-5-yl)-3*H*-benzo[f]chromen-3-one (15):**

A mixture of dehydroacetic acid (1 m mol), thiocarbohydrazide (1 m mol), 2-(2-bromoacetyl)-3H-benzo[f]chromen-3-one (1 m mol) and catalytic amount of sodium acetate (30 mol%) in ethanol was placed in a round bottom flask under reflux conditions for about 6 h; the progress of the reaction was monitored by thin layer chromatography. After the completion of the reaction, product was filtered and isolated, washed with ethanol, dried and recrystallized from ethanol.

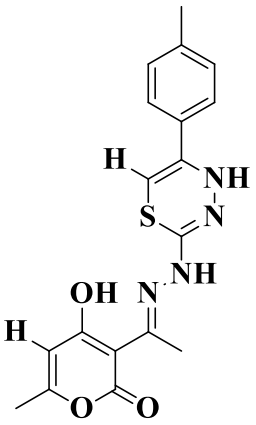
4.2.6. Spectral data:

4-Hydroxy-6-methyl-3-(1-(2-(5-phenyl-4*H*-1,3,4-thiadiazin-2-yl)hydrazono)ethyl)-2*H*-pyran-2-one (**13a**):



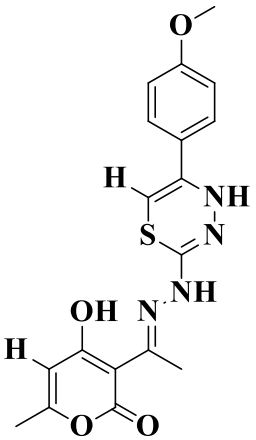
Yellow solid; yield 91%; mp: 195-197^{o}C; IR (KBr) cm^{-1}: 1661 (C=N), 1701 (lactone C=O), 3206 (NH), 3425 (-OH); ^{1}H NMR (400MHz, DMSO-d$_{6,}$ ppm): δ 2.15 (s, 3H, CH_3), 2.75 (s, 3H, CH_3), 5.44 (s, 2H, 2NH), 5.89 (s, 1H, thiadiazin-H), 6.46 (s, 1H, pyran-H), 7.43-7.44 (m, 3H), 7.62-7.64 (m, 2H), 11.77 (s, 1H, OH). ^{13}C NMR (100 MHz, $CDCl_3$ + DMSO-d$_6$) δ 16.93, 19.84, 94.77, 94.99, 105.46, 128.41, 129.24, 130.46, 142.35, 162.64, 162.72, 163.19, 164.40, 180.67 ppm; Mass (ESI-HRMS) (*m/z*): 357.0951 $[M+H]^+$; Anal. Calcd. For $C_{17}H_{16}N_4O_3S$: C, 57.29; H, 4.52; N, 15.72 %. Found: C, 57.33; H, 4.56; N, 15.75 %.

4-Hydroxy-6-methyl-3-(1-(2-(5-(p-tolyl)-4*H*-1,3,4-thiadiazin-2-yl)hydrazono)ethyl)-2*H*-pyran-2-one (13b):



Yellow solid; yield 90%; mp: 177-179^{o}C; IR (KBr) cm^{-1}: 1659 (C=N), 1700 (lactone C=O), 3200 (NH), 3389 (-OH); ^{1}H NMR (400MHz, $CDCl_3$ + DMSO-d_6, ppm): δ 2.10 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 2.72 (s, 3H, CH_3), 5.17 (s, 1H, NH), 5.66 (s, 1H, NH), 5.78 (s, 1H, thiadiazin-H), 6.19 (s, 1H, pyran-H), 7.52 (d, J = 8 Hz, 2H), 7.68 (d, J = 8 Hz, 2H), 11.49 (s, 1H, OH). ^{13}C NMR (125 MHz, DMSO-d_6) δ 17.04, 19.75, 21.33, 95.17, 101.46, 105.53, 113.43, 126.39, 129.84, 132.09, 140.24, 147.3, 156.65, 162.74, 163.40, 166.30, 181.21 ppm; Mass (ESI-HRMS) (*m/z*): 371.1205 $[M+H]^{+}$; Anal. Calcd. For $C_{18}H_{18}N_4O_3S$: C, 58.36; H, 4.90; N, 15.12 %. Found: C, 58.40; H, 4.85; N, 15.16 %.

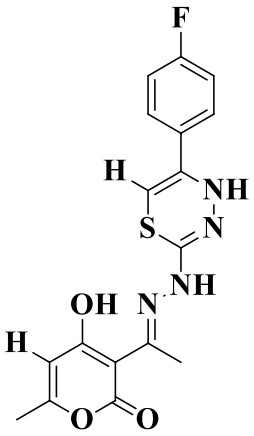
4-Hydroxy-3-(1-(2-(5-(4-methoxyphenyl)-4*H*-1,3,4-thiadiazin-2-yl)hydrazono)ethyl)-6-methyl-2*H*-pyran-2-one (13c):



Yellow solid; mp: 164-166^{o}C; yield 92%; IR (KBr) cm^{-1}: 1661 (C=N), 1704 (lactone C=O), 3220 (NH), 3390 (-OH); ^{1}H NMR (400MHz, DMSO-d_6, ppm): δ 2.10 (s, 3H, CH_3), 2.72 (s, 3H, CH_3), 3.80 (s, 3H, OCH_3), 5.67 (s, 1H, NH), 5.77 (s, 1H, NH), 5.80 (s, 1H, thiadiazin-H), 6.13 (s, 1H, pyran-H), 7.56 (d, J = 8 Hz, 2H), 7.75 (d, J = 8 Hz, 2H), 11.41 (s, 1H, OH). ^{13}C NMR (125 MHz, DMSO-d_6) δ 17.02, 19.73, 55.76, 95.13, 105.61, 114.61, 128.08, 130.71, 147.36, 156.84, 161.16, 162.93, 164.40, 166.20, 181.31 ppm; Mass (ESI-HRMS) (*m/z*): 387.1154

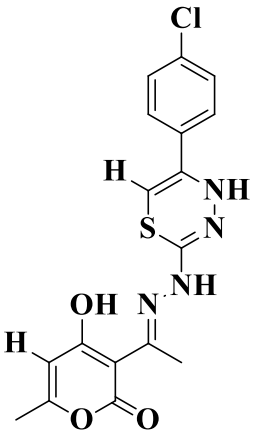
$[M+H]^+$; Anal. Calcd. For $C_{18}H_{18}N_4O_4S$: C, 55.95; H, 4.70; N, 14.50 %. Found: C, 55.93; H, 4.68; N, 14.53 %.

3-(1-(2-(5-(4-Fluorophenyl)-4*H*-1,3,4-thiadiazin-2-yl)hydrazono)ethyl)-4-hydroxy-6-methyl-2*H*-pyran-2-one (13d):



Olive Green solid; yield 92%; mp: 185-187ºC; IR (KBr) cm^{-1}: 1659 (C=N), 1701 (lactone C=O), 3275 (NH), 3420 (-OH); ^{1}H NMR (400MHz, $CDCl_3$ + DMSO-d_6, ppm): δ 2.16 (s, 3H, CH_3), 2.73 (s, 3H, CH_3), 5.14 (s, 1H, NH), 5.78 (s, 1H, NH), 5.81 (s, 1H, thiadiazin-H), 6.21 (s, 1H, pyran-H), 7.64 (d, J = 8.4 Hz, 2H), 7.82 (d, J = 8.4 Hz, 2H), 11.50 (s, 1H, OH). ^{13}C NMR (125 MHz, DMSO-d_6) δ 17.05, 19.74, 105.50, 113.38, 115.65, 116.33, 128.84, 131.62, 146.27, 154.33, 156.35, 163.54, 164.60, 166.51, 181.23 ppm; Mass (ESI-HRMS) (*m/z*): 375.0949 $[M+H]^+$; Anal. Calcd. For $C_{17}H_{15}FN_4O_3S$: C, 54.54; H, 4.04; N, 14.96 %. Found: C, 54.50; H, 4.09; N,14.92 %.

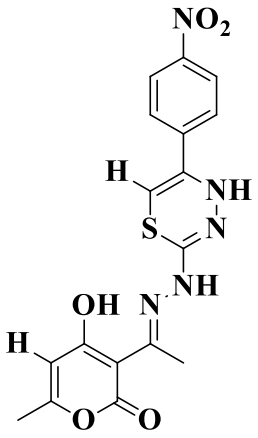
3-(1-(2-(5-(4-Chlorophenyl)-4*H*-1,3,4-thiadiazin-2-yl)hydrazono)ethyl)-4-hydroxy-6-methyl-2*H*-pyran-2-one (13e):



Yellow solid; yield 89%; mp: 188-190ºC; IR (KBr) cm^{-1}: 1656 (C=N), 1700 (lactone C=O), 3248 (NH), 3412 (-OH); ^{1}H NMR (400MHz, $CDCl_3$ + DMSO-d_6, ppm): δ 2.10 (s, 3H, CH_3),

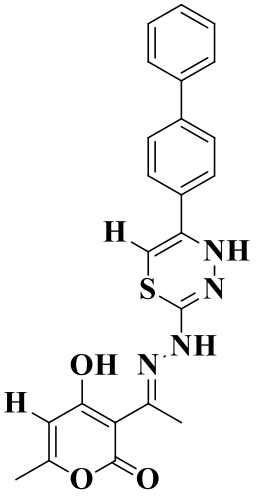
2.71 (s, 3H, CH_3), 5.29 (s, 1H, NH), 5.68 (s, 1H, NH), 5.80 (s, 1H, thiadiazin-H), 6.36 (s, 1H, pyran-H), 7.66 (d, *J* = 8.4 Hz, 2H), 7.81 (d, *J* = 8.4 Hz, 2H), 11.69 (s, 1H, OH). ^{13}C NMR (125 MHz, DMSO-d_6) δ 17.05, 19.71, 95.20, 105.52, 113.29, 128.17, 128.63, 129.04, 129.28, 131.01, 145.83, 156.05, 163.61, 164.74, 166.68, 166.83, 181.32 ppm; Mass (ESI-HRMS) (*m/z*): 391.0654 $[M+H]^+$; Anal. Calcd. For $C_{17}H_{15}ClN_4O_3S$: C, 52.24; H, 3.87; N, 14.33 %. Found: C, 52.28; H, 3.83; N, 14.36 %.

4-Hydroxy-6-methyl-3-(1-(2-(5-(4-nitrophenyl)-4*H*-1,3,4-thiadiazin-2-yl)hydrazono)ethyl)-2*H*-pyran-2-one (13f):



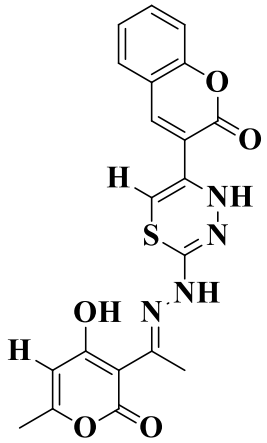
Orange solid; yield 91%; mp: 192-194^{o}C; IR (KBr) cm^{-1}: 1668 (C=N), 1704 (lactone C=O), 3216 (NH), 3410 (-OH); ^{1}H NMR (400MHz, $CDCl_3$ + DMSO-d_6, ppm): δ 2.11 (s, 3H, CH_3), 2.77 (s, 3H, CH_3), 5.67 (s, 1H, NH), 5.77 (s, 1H, NH), 5.84 (s, 1H, thiadiazin-H), 6.39 (s, 1H, pyran-H), 7.91 (d, *J* = 8.8 Hz, 2H), 8.00 (d, *J* = 8.8 Hz, 2H), 11.64 (s, 1H, OH). ^{13}C NMR (125 MHz, DMSO-d_6) δ 17.07, 19.74, 95.34, 105.36, 113.35, 124.38, 127.41, 141.02, 143.87, 148.11, 155.02, 163.71, 167.02, 181.14 ppm; Mass (ESI-HRMS) (*m/z*): 402.0890 $[M+H]^+$; Anal. Calcd. For $C_{17}H_{15}N_5O_5S$: C, 50.87; H, 3.77; N, 17.45 %. Found: C, 50.83; H, 3.73; N, 17.41 %.

3-(1-(2-(5-([1,1'-Biphenyl]-4-yl)-4*H*-1,3,4-thiadiazin-2-yl)hydrazono)ethyl)-4-hydroxy-6-methyl-2*H*-pyran-2-one (13g):



Yellow solid; yield 84%; mp: 151-153^{o}C; IR (KBr) cm^{-1}: 1662 (C=N), 1690 (lactone C=O), 3199 (NH), 3395 (-OH); ^{1}H NMR (400MHz, $CDCl_3$ + DMSO-d_6, ppm): δ 2.17 (s, 3H, CH_3), 2.75 (s, 3H, CH_3), 5.13 (s, 1H, NH), 5.78 (s, 1H, NH), 5.81 (s, 1H, thiadiazin-H), 6.25 (s, 1H, pyran-H), 7.63- 7.67 (m, 6H), 7.88 (d, J = 8 Hz, 3H), 11.48 (s, 1H, OH). ^{13}C NMR (125 MHz, DMSO-d_6) δ 17.06, 19.75, 95.22, 105.51, 127.11, 129.54, 133.82, 139.54, 146.65, 156.35, 163.46, 166.43, 181.22 ppm; Mass (ESI-HRMS) (*m/z*): 433.1326 $[M+H]^{+}$; Anal. Calcd. For $C_{23}H_{20}N_4O_3S$: C, 63.87; H, 4.66; N, 12.95 %. Found: C, 63.83H, 4.61; N, 12.92 %.

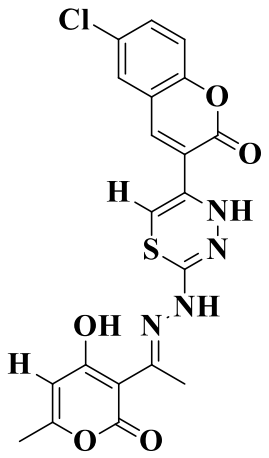
3-(2-(2-(1-(4-Hydroxy-6-methyl-2-oxo-2*H*-pyran-3-yl)ethylidene)hydrazinyl)-4*H*-1,3,4-thiadiazin-5-yl)-2*H*-chromen-2-one (14a):



Orange solid; yield 93%; mp: 202-204^{o}C; IR (KBr) cm^{-1}: 1653 (C=N), 1700 (lactone C=O), 3246 (NH), 3408 (-OH); ^{1}H NMR (400MHz, $CDCl_3$ + DMSO-d_6, ppm): δ 2.17 (s, 3H, CH_3), 2.76 (s, 3H, CH_3), 5.01 (s, 1H, NH), 5.78 (s, 1H, NH), 5.82 (s, 1H, thiadiazin-H), 6.55 (s, 1H, pyran-H), 7.61 (d, J = 7.6 Hz, 2H), 7.66 (d, J = 7.6 Hz, 2H), 8.25 (s, 1H), 11.64 (s, 1H, OH). ^{13}C NMR (125 MHz, DMSO-d_6) δ 17.07, 19.74, 95.24, 105.44, 113.35, 116.59, 125.53, 129.90, 133.44, 142.27, 153.93, 159.77, 163.67, 164.87, 166.85, 183.89 ppm; Mass (ESI-HRMS) (*m/z*):

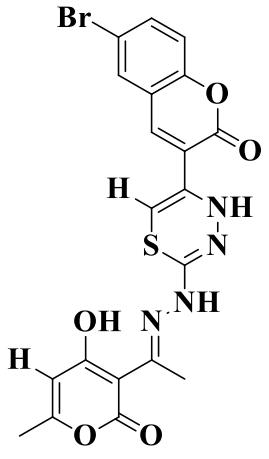
425.0989 $[M+H]^+$; Anal. Calcd. For $C_{20}H_{16}N_4O_5S$: C, 56.60; H, 3.80; N, 13.20 %. Found: C, 56.65; H, 3.76; N, 13.24%.

6-Chloro-3-(2-(2-(1-(4-hydroxy-6-methyl-2-oxo-2*H*-pyran-3-yl)ethylidene)hydrazinyl)-4*H*-1,3,4-thiadiazin-5-yl)-2*H*-chromen-2-one (14b):



Yellow solid; yield 89%; mp: 220-222°C; IR (KBr) cm^{-1}: 1656 (C=N), 1700 (lactone C=O), 3212 (NH), 3413 (-OH); ^{1}H NMR (400MHz, $CDCl_3$ + DMSO-d_6, ppm): δ 2.10 (s, 3H, CH_3), 2.74 (s, 3H, CH_3), 5.11 (s, 1H, NH), 5.68 (s, 1H, NH), 5.79 (s, 1H, thiadiazin-H), 6.64 (s, 1H, pyran-H), 7.38 (d, J = 5.2 Hz, 3H), 8.23 (s, 1H), 11.81 (s, 1H, OH). ^{13}C NMR (125 MHz, DMSO-d_6) δ 17.07, 19.76, 101.45, 105.41, 113.40, 118.60, 124.85, 128.71, 129.17, 129.48, 132.77, 140.78, 144.19, 152.56, 163.64, 166.88, 180.95 ppm; Mass (ESI-HRMS) (*m/z*): 459.0530 $[M+H]^+$; Anal. Calcd. For $C_{20}H_{15}ClN_4O_5S$: C, 52.35; H, 3.29; N, 12.21 %. Found: C, 52.30; H, 3.33; N, 12.25 %.

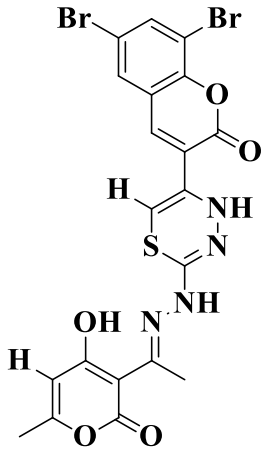
6-Bromo-3-(2-(2-(1-(4-hydroxy-6-methyl-2-oxo-2*H*-pyran-3-yl)ethylidene)hydrazinyl)-4*H*-1,3,4-thiadiazin-5-yl)-2*H*-chromen-2-one (14c):



Yellow solid; yield 93%; mp: 211-213°C; IR (KBr) cm^{-1}: 1655 (C=N), 1699 (lactone C=O), 3209 (NH), 3419 (-OH); ^{1}H NMR (400MHz, $CDCl_3$ + DMSO-d_6, ppm): δ 2.11 (s, 3H, CH_3),

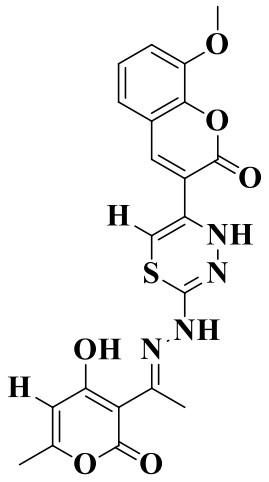
2.76 (s, 3H, CH_3), 5.68 (s, 1H, NH), 5.78 (s, 1H, NH), 5.82 (s, 1H, thiadiazin-H), 6.58 (s, 1H, pyran-H), 7.30 (d, *J* = 8.8 Hz, 3H), 8.18 (s, 1H), 11.65 (s, 1H, OH). ^{13}C NMR (125 MHz, DMSO-d_6) δ 17.07, 19.77, 95.30, 101.46, 105.40, 116.98, 118.86, 121.13, 128.38, 131.71, 140.67, 156.27, 159.24, 162.71, 166.85, 181.13 ppm; Mass (ESI-HRMS) (*m/z*): 503.0077 $[M+H]^+$; Anal. Calcd. For $C_{20}H_{15}BrN_4O_5S$: C, 47.73; H, 3.00; N, 11.13 %. Found: C, 47.77; H, 2.97; N, 11.16 %.

6,8-Dibromo-3-(2-(2-(1-(4-hydroxy-6-methyl-2-oxo-2*H*-pyran-3-yl)ethylidene)hydrazinyl)-4*H*-1,3,4-thiadiazin-5-yl)-2*H*-chromen-2-one (14d):



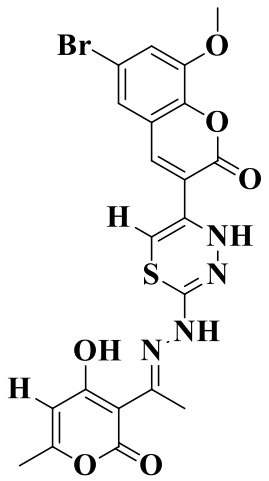
Orange solid; mp: 218-220^{o}C; yield 92%; IR (KBr) cm^{-1}: 1651 (C=N), 1706 (lactone C=O), 3273 (NH), 3414 (-OH); ^{1}H NMR (400MHz, $CDCl_3$ + DMSO-d_6, ppm): δ 2.17 (s, 3H, CH_3), 2.73 (s, 3H, CH_3), 5.15 (s, 1H, NH), 5.79 (s, 1H, NH), 5.83 (s, 1H, thiadiazin-H), 6.70 (s, 1H, pyran-H), 7.96 (d, *J* = 2 Hz, 2H), 8.23 (s, 1H), 11.89 (s, 1H, OH). ^{13}C NMR (125 MHz, DMSO-d_6) δ 17.07, 19.79, 95.32, 105.37, 110.51, 117.02, 122.14, 125.47, 131.37, 140.26, 149.83, 156.05, 158.57, 163.55, 166.89, 181.08 ppm; Mass (ESI-HRMS) (*m/z*): 583.0247 $[M+H]^+$; Anal. Calcd. For $C_{20}H_{14}Br_2N_4O_5S$: C, 41.26; H, 2.42; N, 9.62 %. Found: C, 41.29; H, 2.45; N, 9.67 %.

3-(2-(2-(1-(4-Hydroxy-6-methyl-2-oxo-2*H*-pyran-3-yl)ethylidene)hydrazinyl)-4*H*-1,3,4-thiadiazin-5-yl)-8-methoxy-2*H*-chromen-2-one (14e):



Yellow solid; yield 88%; mp: 168-170^{o}C; IR (KBr) cm^{-1}: 1652 (C=N), 1711 (lactone C=O), 3208 (NH), 3429 (-OH); ^{1}H NMR (400MHz, $CDCl_3$ + DMSO-d_6, ppm): δ 2.16 (s, 3H, CH_3), 2.74 (s, 3H, CH_3), 3.98 (s, 3H, OCH_3), 5.08 (s, 1H, NH), 5.78 (s, 1H, NH), 5.81 (s, 1H, thiadiazin-H), 6.61 (s, 1H, pyran-H), 7.23- 7.29 (m, 3H), 8.22 (s, 1H), 11.74 (s, 1H, OH). ^{13}C NMR (125 MHz, DMSO-d_6) δ 17.06, 19.77, 56.61, 98.74, 101.45, 113.45, 115.56, 120.54, 120.93, 125.43, 142.40, 146.79, 156.47, 159.45, 161.06, 163.56, 166.75, 180.66 ppm; Mass (ESI-HRMS) (*m/z*): 455.1024 $[M+H]^+$; Anal. Calcd. For $C_{21}H_{18}N_4O_6S$: C, 55.50; H, 3.99; N, 12.33 %. Found: C, 55.55; H, 3.96; N, 12.37 %.

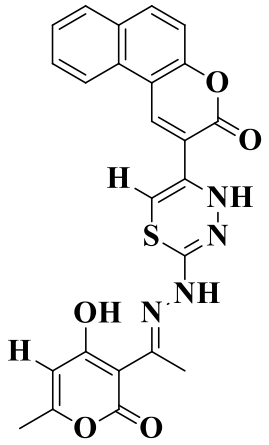
6-Bromo-3-(2-(2-(1-(4-hydroxy-6-methyl-2-oxo-2*H*-pyran-3-yl)ethylidene)hydrazinyl)-4*H*-1,3,4-thiadiazin-5-yl)-8-methoxy-2*H*-chromen-2-one (14f):



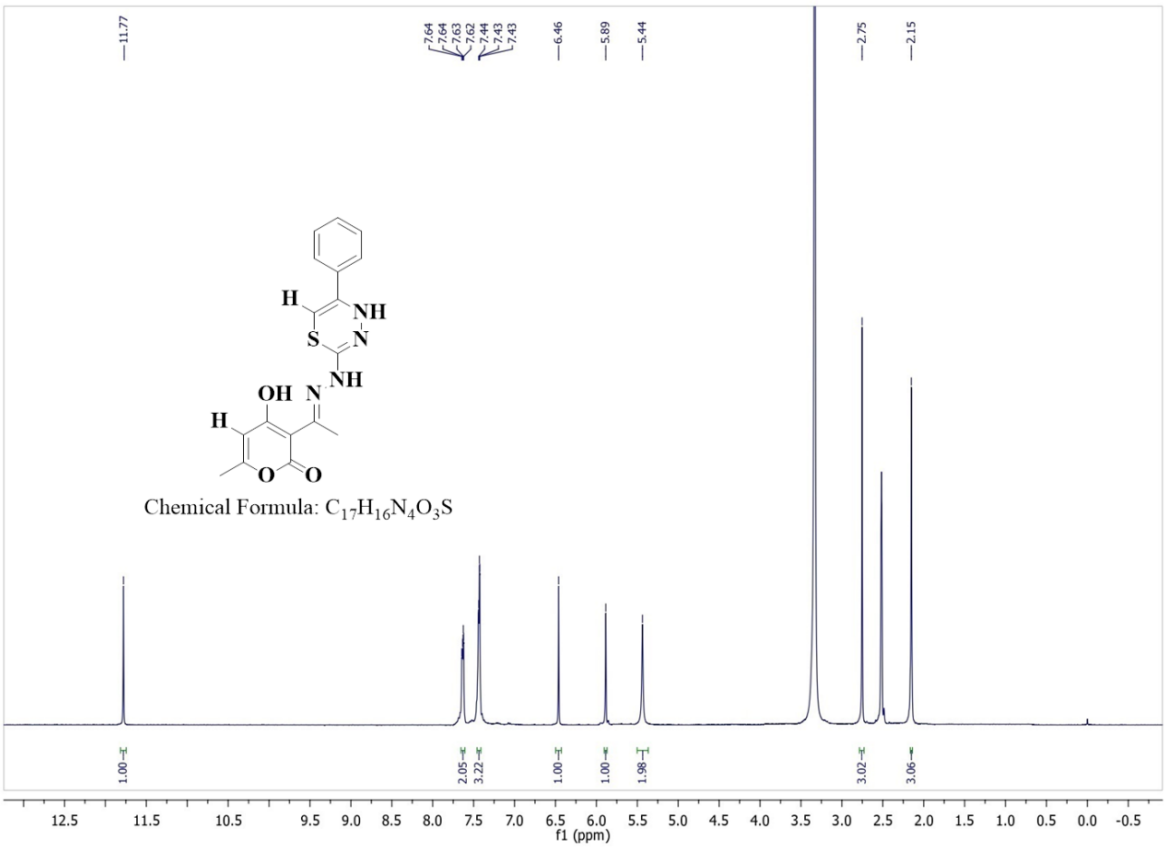
Yellow solid; yield 94%; mp: 186-188^{o}C; IR (KBr) cm^{-1}: 1650 (C=N), 1703 (lactone C=O), 3205 (NH), 3420 (-OH); ^{1}H NMR (400MHz, $CDCl_3$ + DMSO-d_6, ppm): δ 2.16 (s, 3H, CH_3), 2.70 (s, 3H, CH_3), 3.81 (s, 3H, OCH_3), 5.23 (s, 1H, NH), 5.81 (s, 1H, NH), 5.84 (s, 1H, thiadiazin-H), 6.55 (s, pyran-H, 1H), 7.38 (d, J = 2 Hz, 1H), 7.54 (d, J = 2 Hz, 1H), 8.22 (s, 1H), 11.92 (s, 1H, OH). ^{13}C NMR (125 MHz, DMSO-d_6) δ 17.05, 19.79, 57.09, 89.23, 101.46,

113.27, 116.88, 121.76, 122.70, 140.95, 143.86, 147.67, 147.76, 156.13, 158.89, 163.42, 166.82, 181.11 ppm; Mass (ESI-HRMS) (*m/z*): 533.0151 $[M+H]^+$; Anal. Calcd. For $C_{21}H_{17}BrN_4O_6S$: C, 47.29; H, 3.21; N, 10.50 %. Found: C, 47.25; H, 3.25; N, 10.55 %.

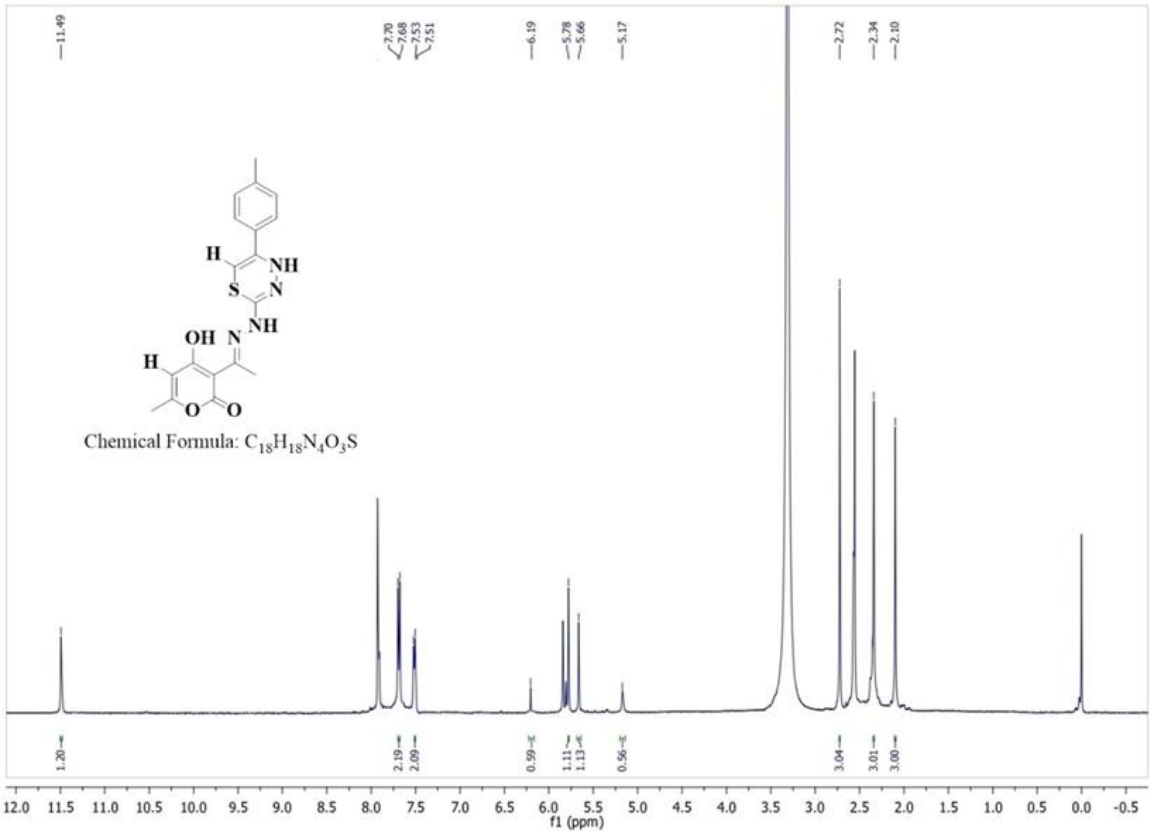
2-(2-(2-(1-(4-Hydroxy-6-methyl-2-oxo-2*H*-pyran-3-yl)ethylidene)hydrazinyl)-4*H*-1,3,4-thiadiazin-5-yl)-3*H*-benzo[f]chromen-3-one (15):



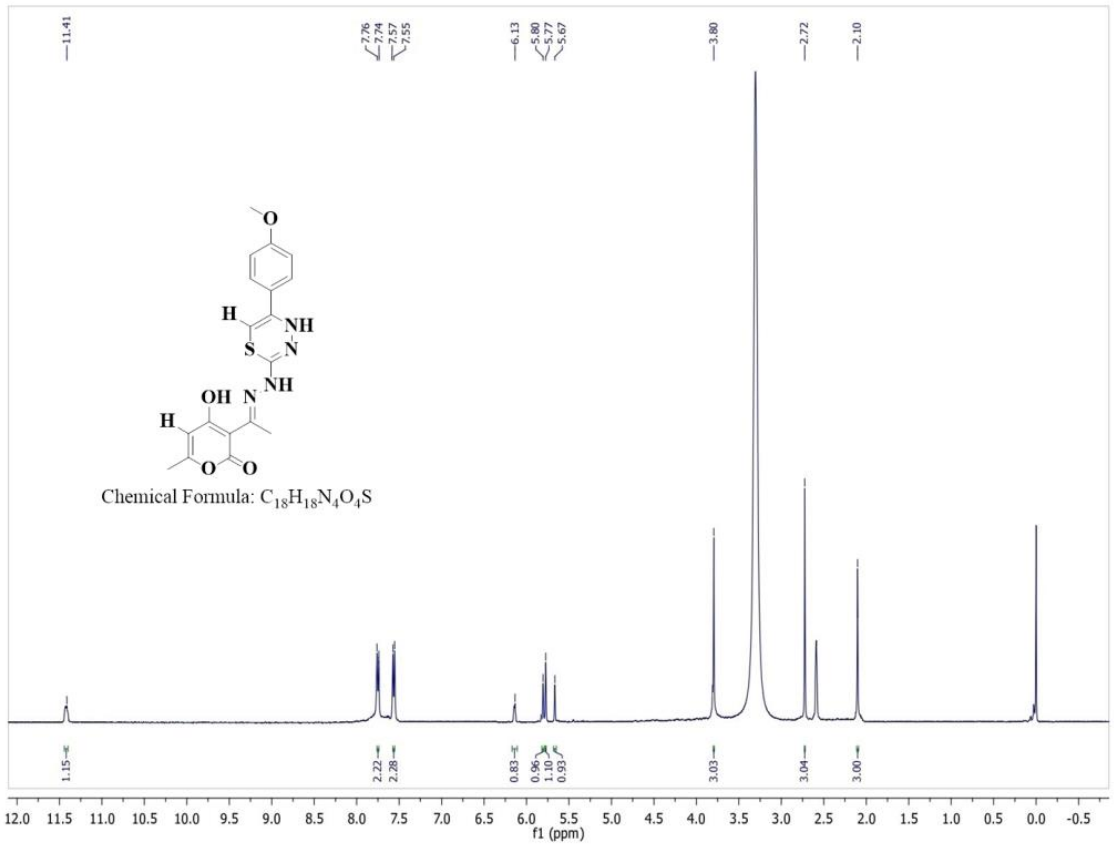
Orange solid; yield 91%; mp: 206-208^oC; IR (KBr) cm^{-1}: 1656 (C=N), 1706 (lactone C=O), 3258 (NH), 3413 (-OH); ^1H NMR (400MHz, $CDCl_3$ + DMSO-d_6, ppm): δ 2.16 (s, 3H, CH_3), 2.75 (s, 3H, CH_3), 5.63 (s, 1H, NH), 5.79 (s, 1H, NH), 5.82 (s, 1H, thiadiazin-H), 6.77 (s, 1H, pyran-H), 7.50- 7.52 (m, 2H), 7.62 (t, J = 7.6 Hz, 3H), 7.98 (d, J = 8.0 Hz, 2H), 11.81 (s, 1H, OH). ^{13}C NMR (125 MHz, DMSO-d_6) δ 17.07, 19.76, 101.45, 113.41, 116.89, 126.88, 129.23, 129.44, 130.45, 134.81, 138.04, 154.10, 159.63, 160.47, 163.54, 164.14, 166.75, 181.19 ppm; Mass (ESI-HRMS) (*m/z*): 475.1095 $[M+H]^+$; Anal. Calcd. For $C_{24}H_{18}N_4O_5S$: C, 60.75; H, 3.82; N, 11.81%. Found: C, 60.72; H, 3.86; N, 11.85%.



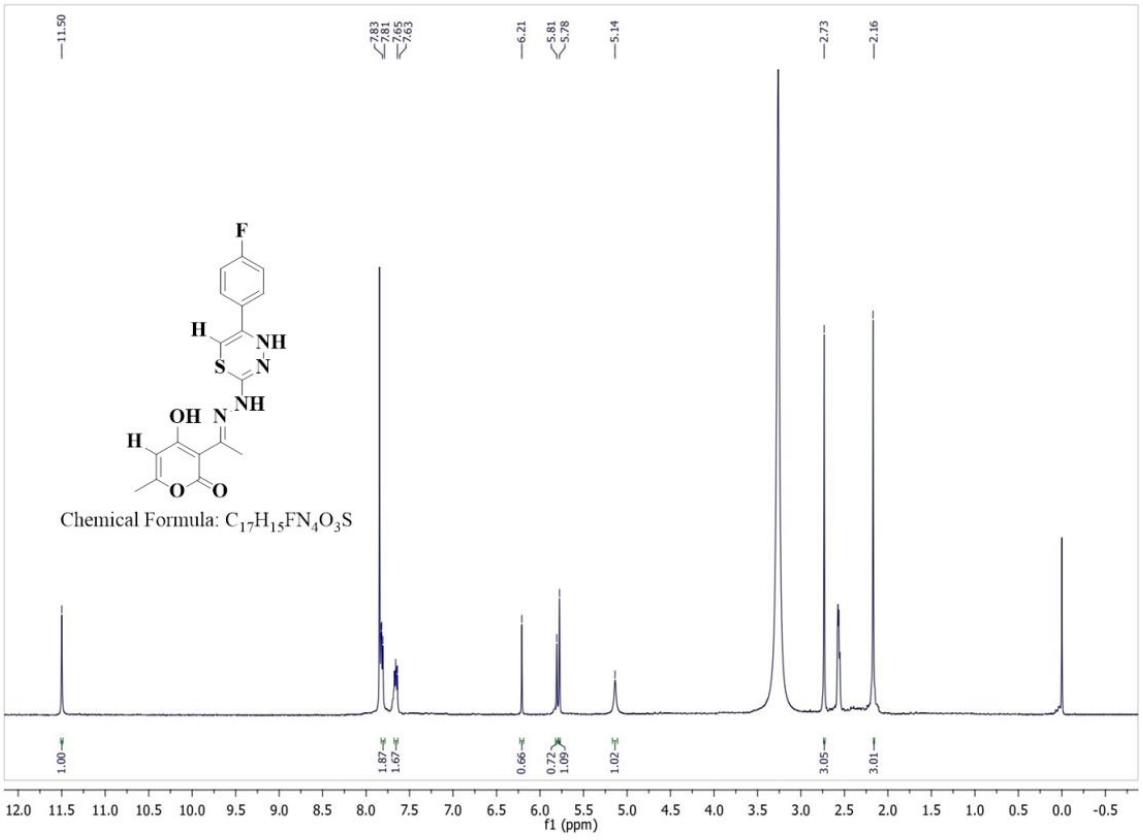
^{1}H NMR spectrum of compound **13a** (400 MHz, DMSO-d_6)



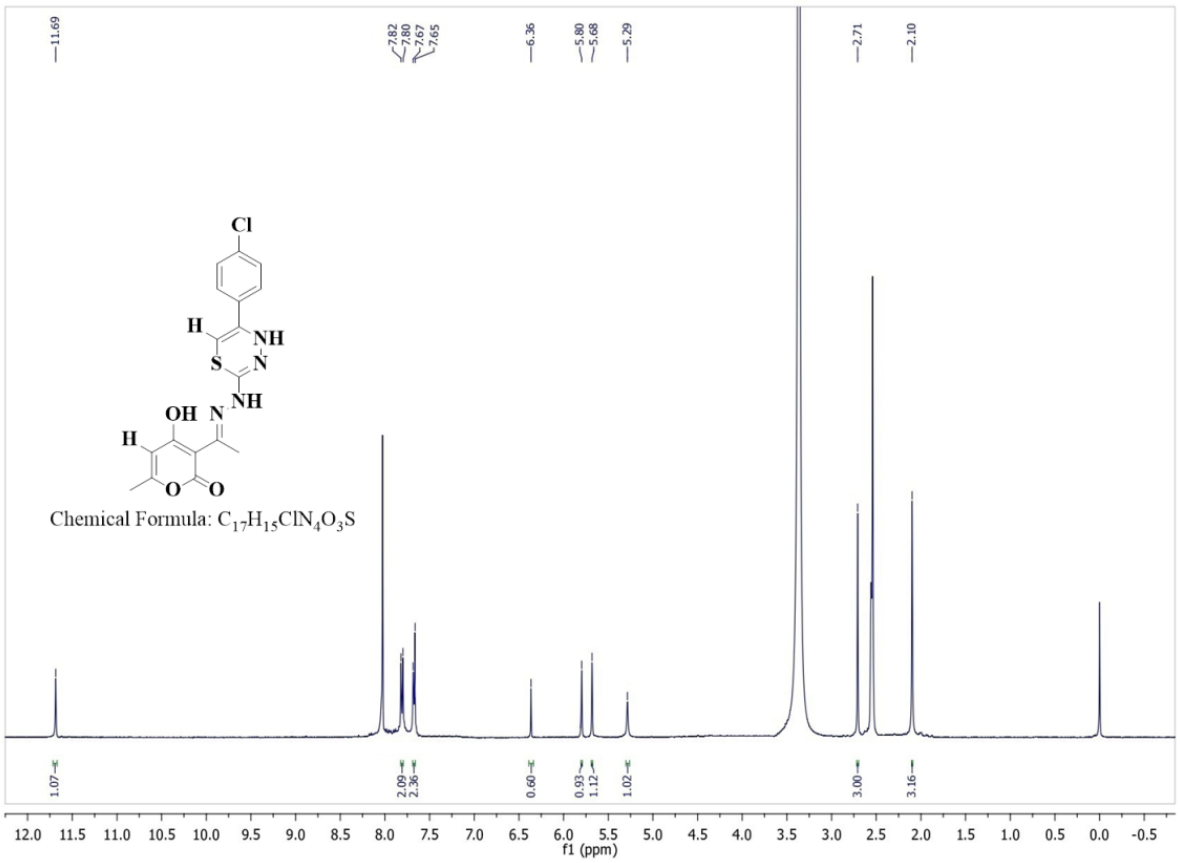
^{1}H NMR spectrum of compound **13b** (400 MHz, $CDCl_3$+DMSO-d_6)



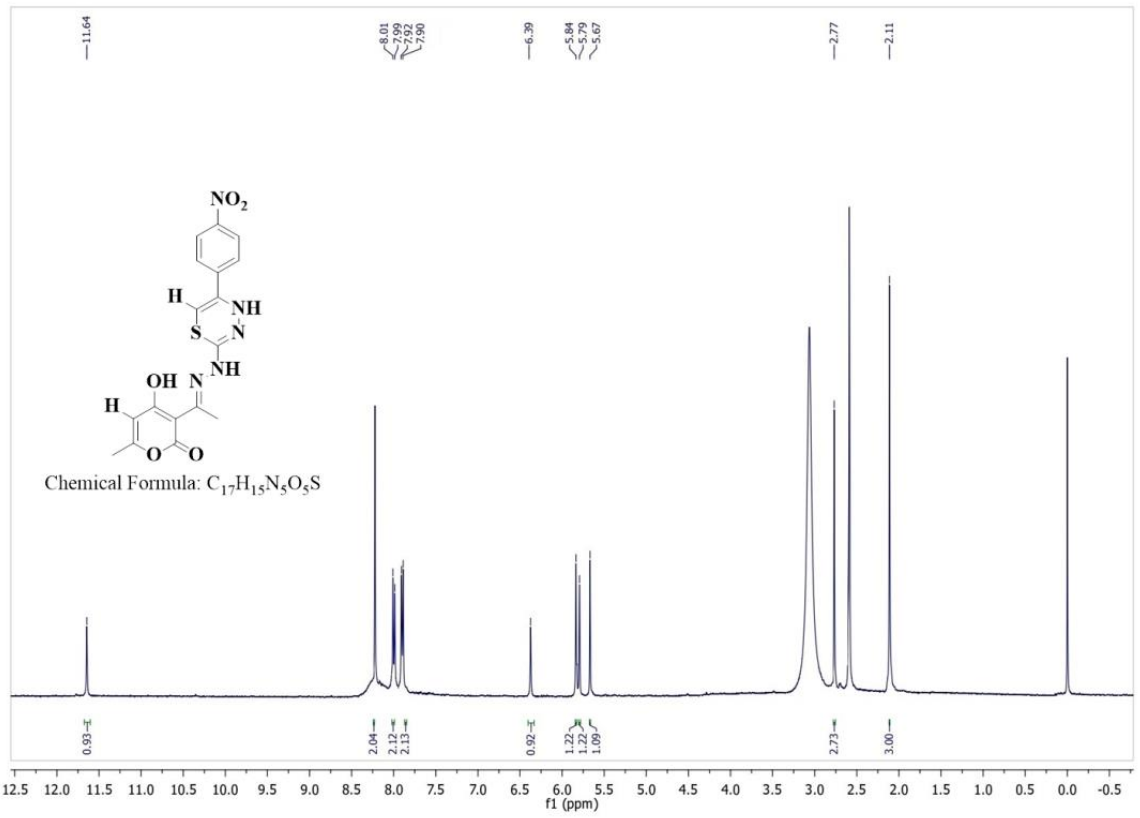
^{1}H NMR spectrum of compound **13c** (400 MHz, DMSO-d_6)



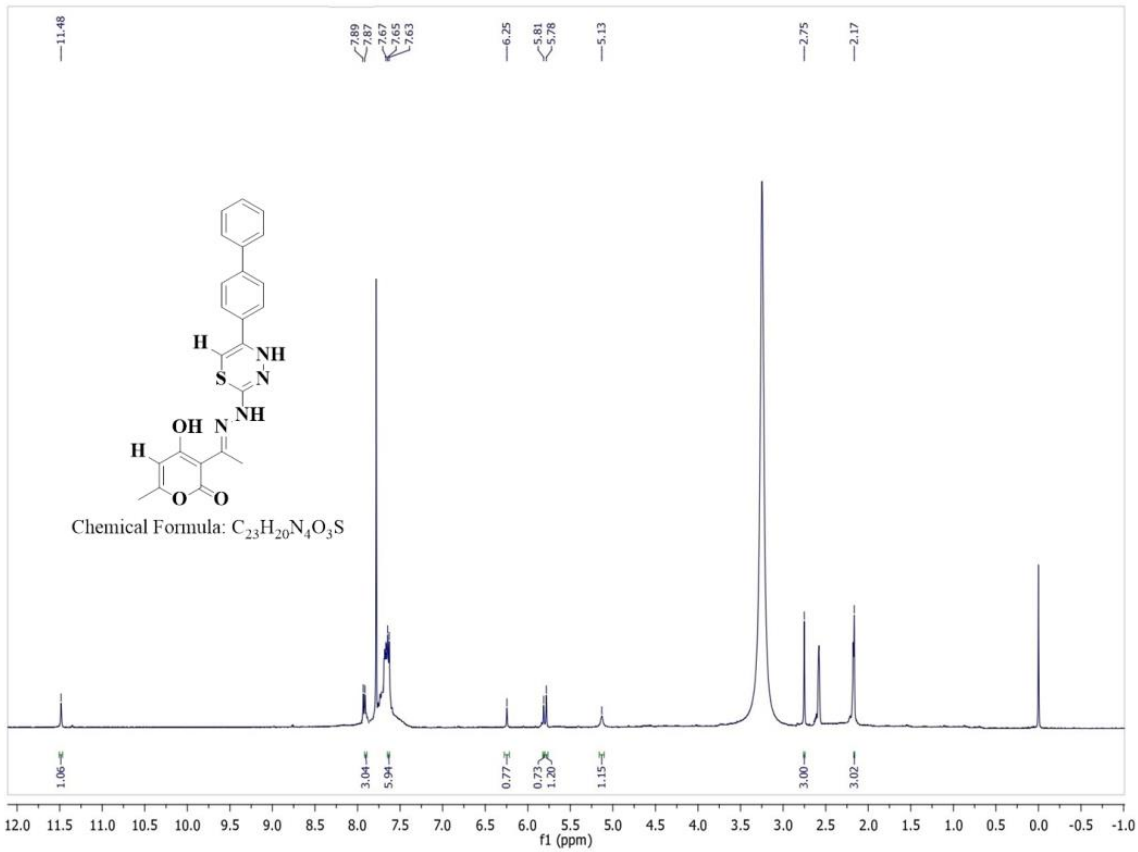
^{1}H NMR spectrum of compound **13d** (400 MHz, $CDCl_3$+DMSO-d_6)



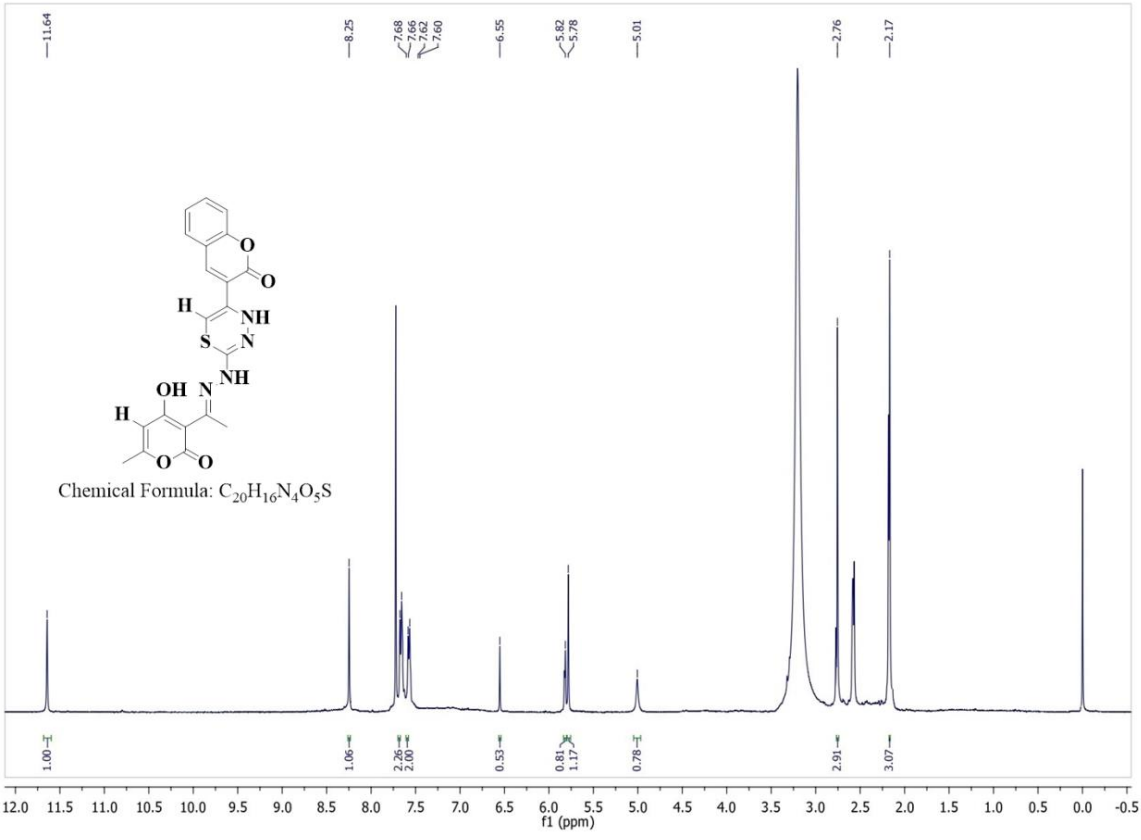
^{1}H NMR spectrum of compound **13e** (400 MHz, $CDCl_3$+DMSO-d_6)



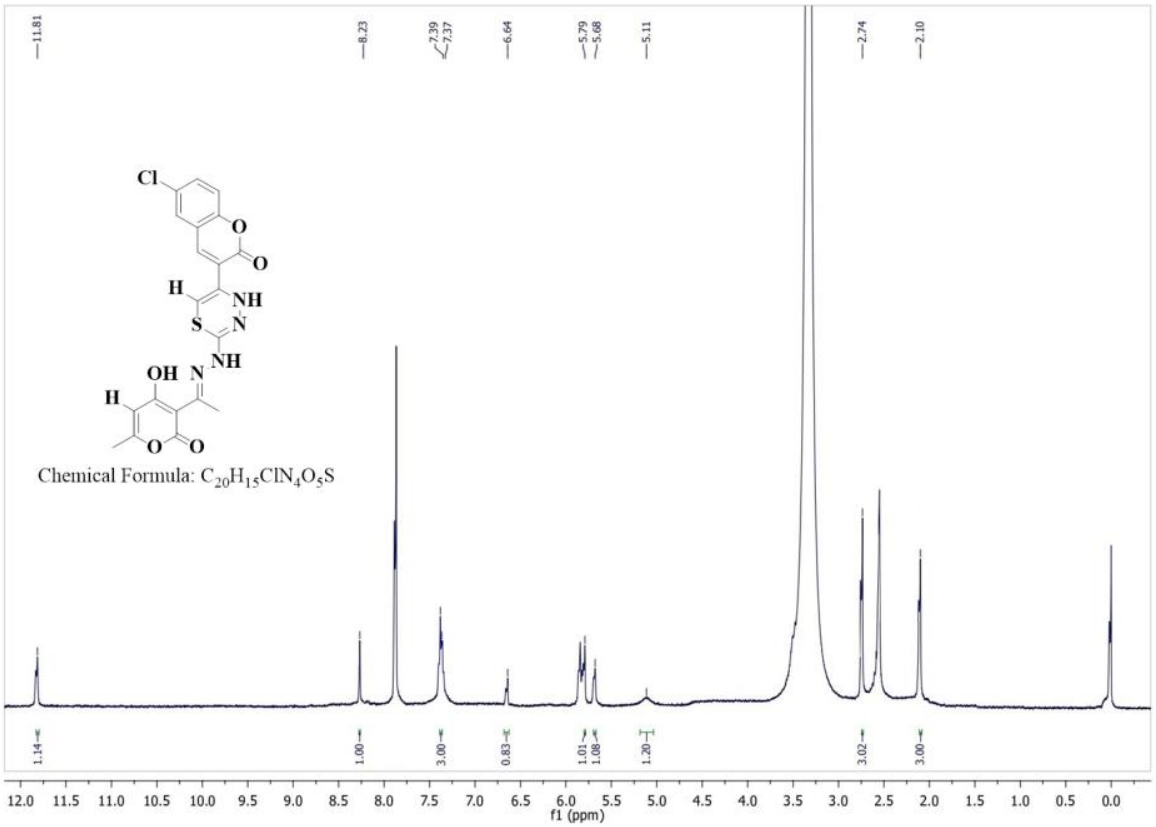
^{1}H NMR spectrum of compound **13f** (400 MHz, $CDCl_3$+DMSO-d_6)



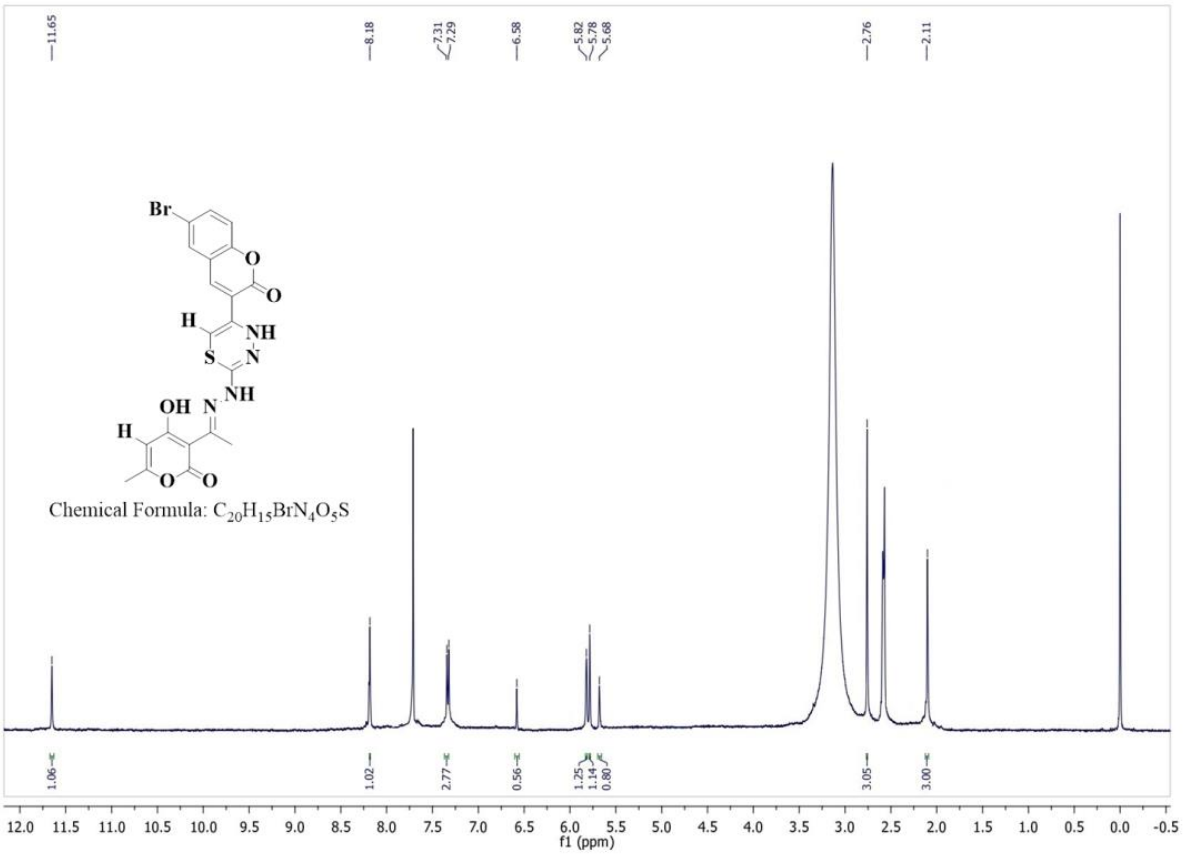
^{1}H NMR spectrum of compound **13g** (400 MHz, $CDCl_3$+DMSO-d_6)



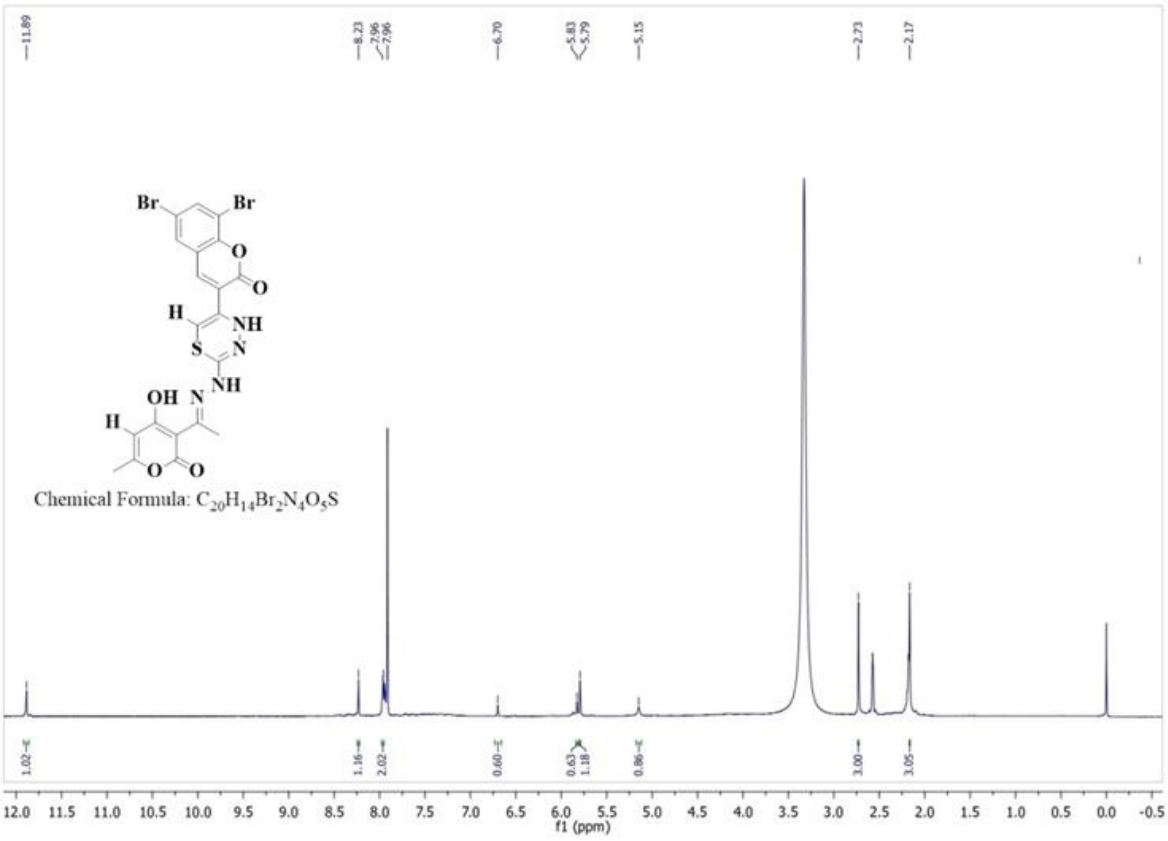
^{1}H NMR spectrum of compound **14a** (400 MHz, $CDCl_3$+DMSO-d_6)



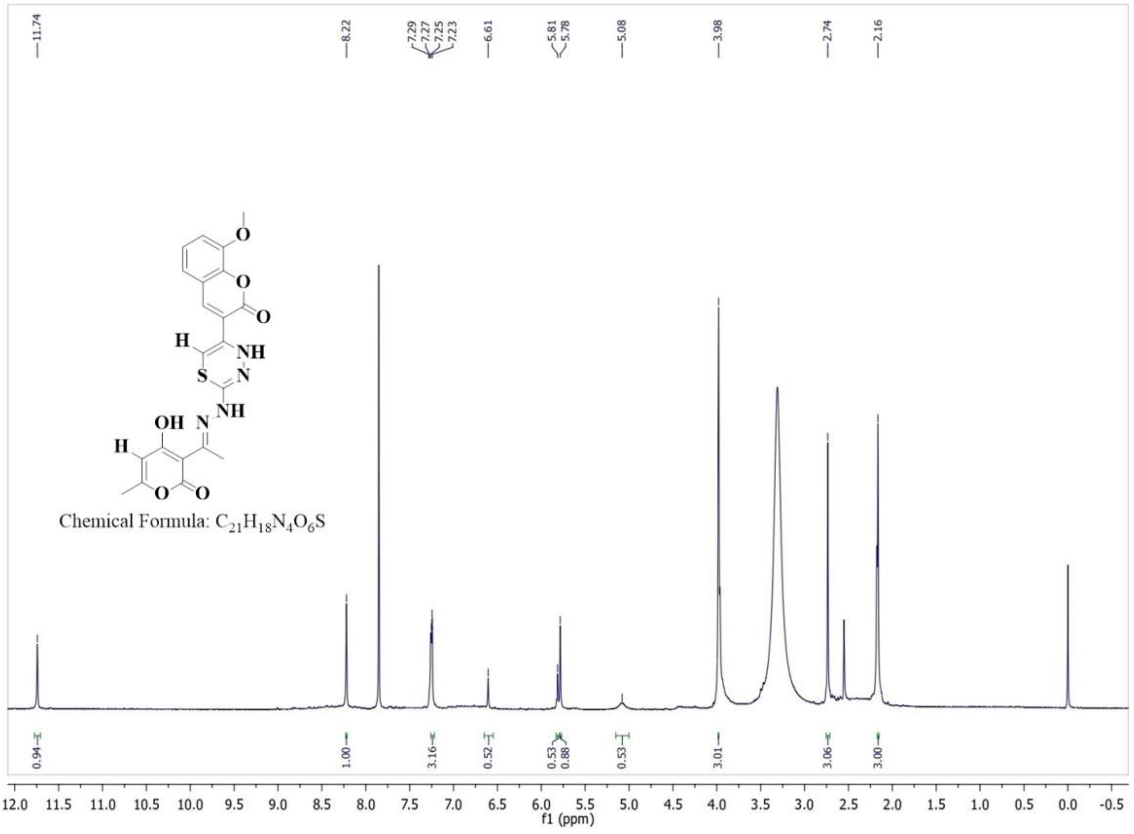
^{1}H NMR spectrum of compound **14b** (400 MHz, $CDCl_3$+DMSO-d_6)



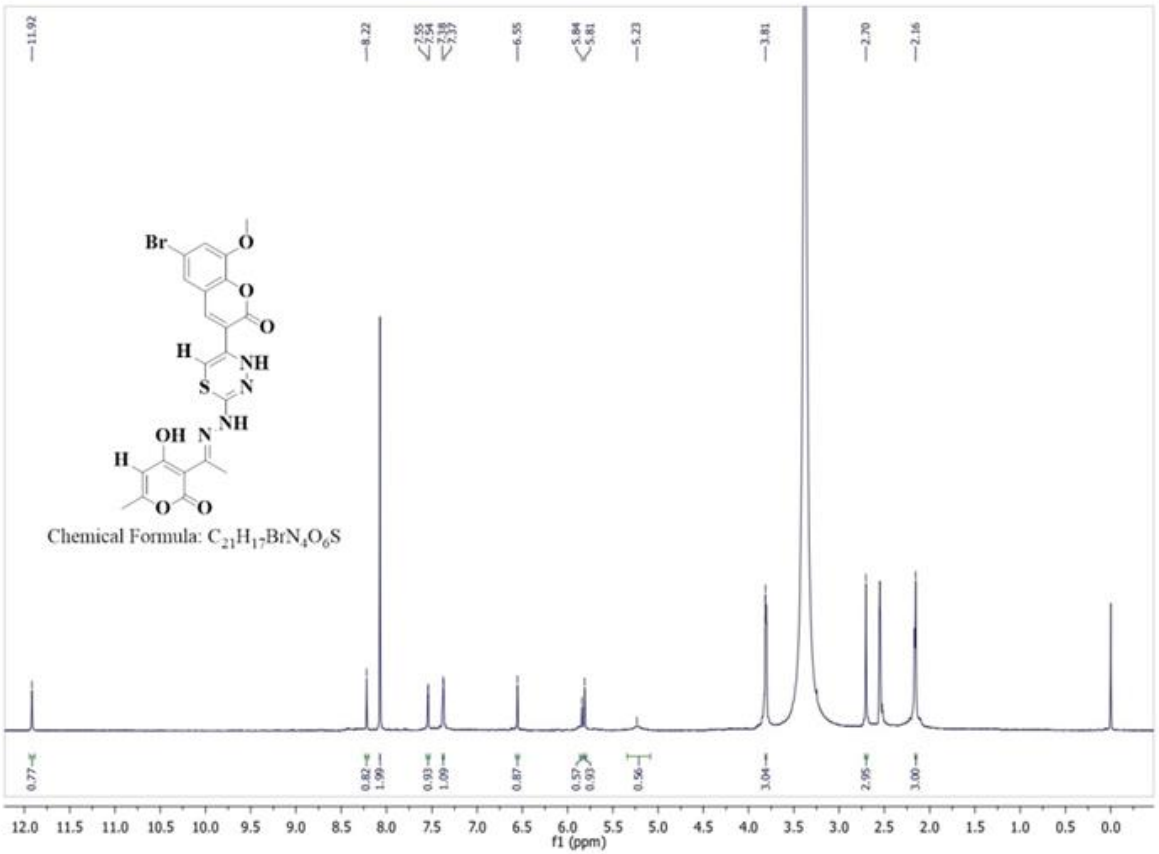
^{1}H NMR spectrum of compound **14c** (400 MHz, $CDCl_3$+DMSO-d_6)



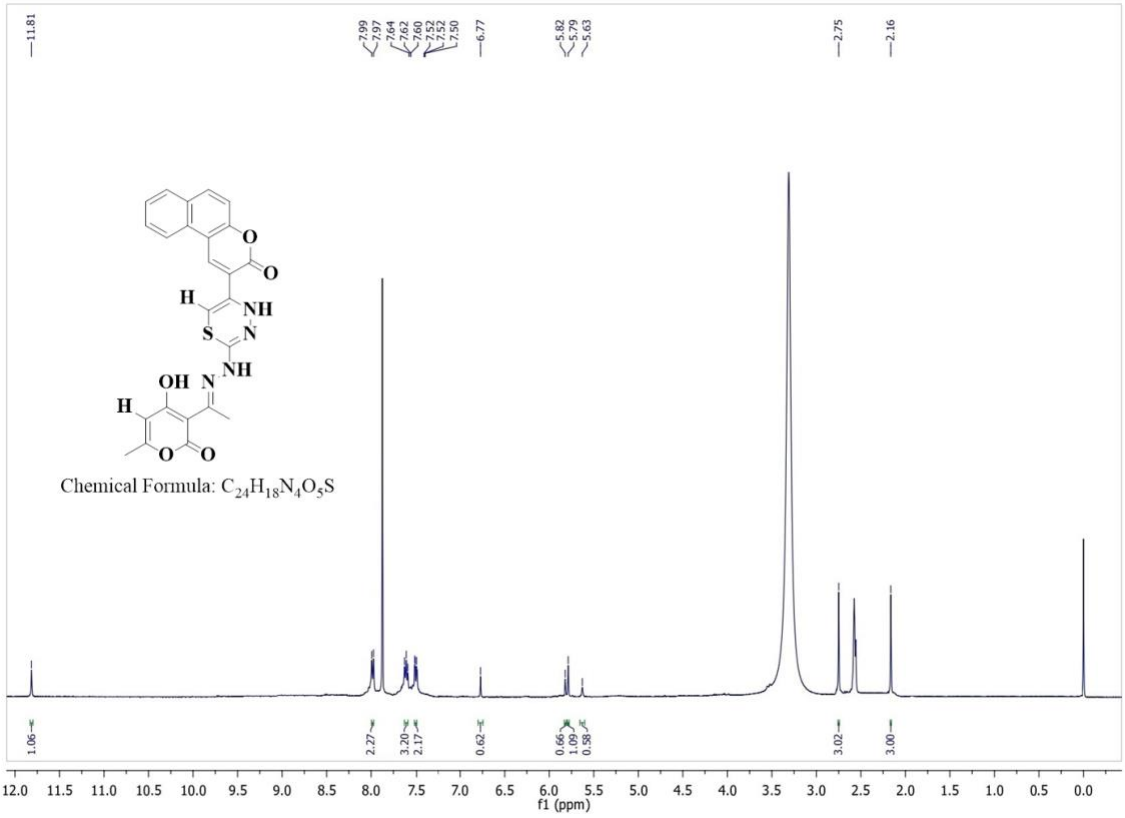
^{1}H NMR spectrum of compound **14d** (400 MHz, $CDCl_3$+DMSO-d_6)



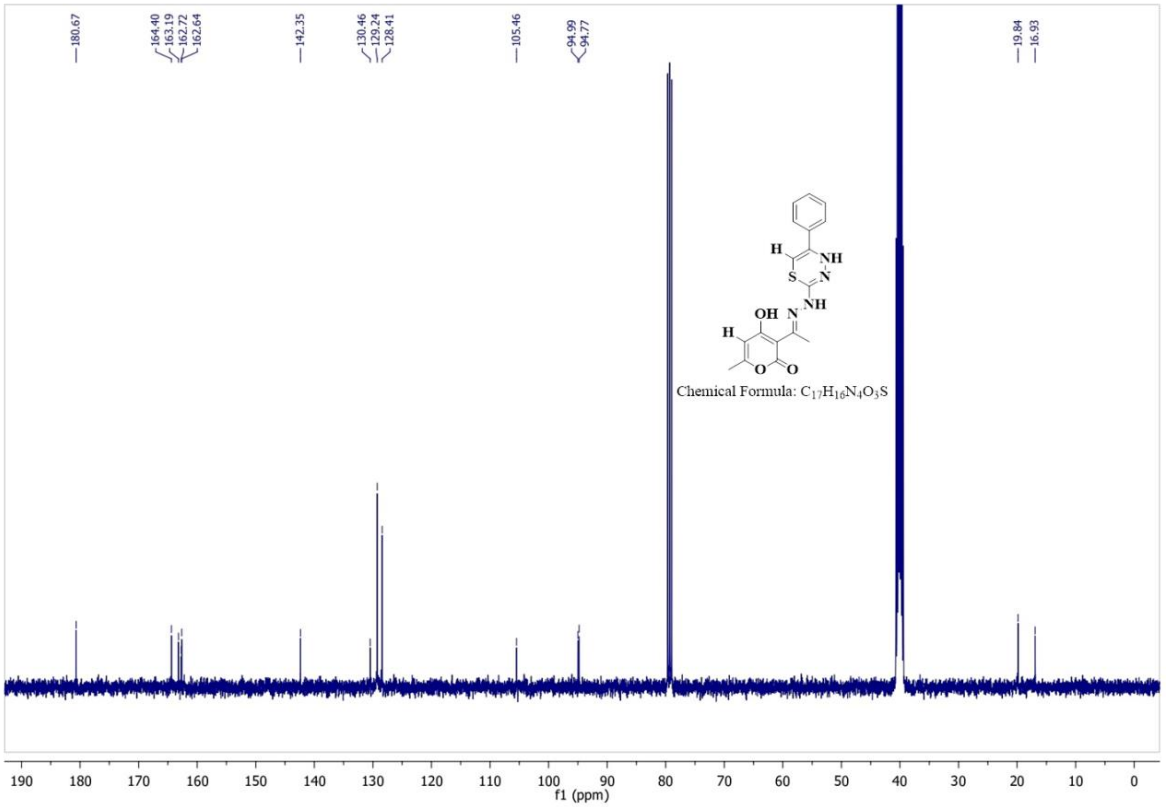
^{1}H NMR spectrum of compound **14e** (400 MHz, $CDCl_3$+DMSO-d_6)



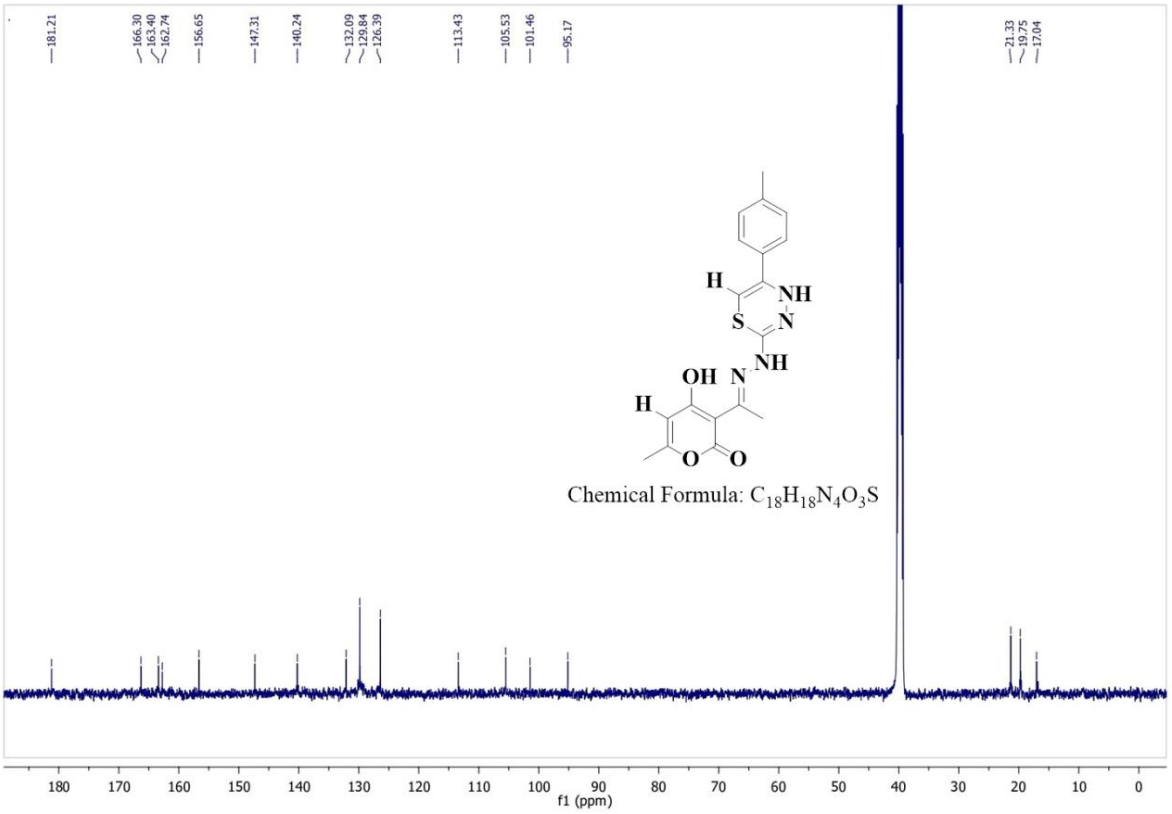
^{1}H NMR spectrum of compound **14f** (400 MHz, $CDCl_3$+DMSO-d_6)



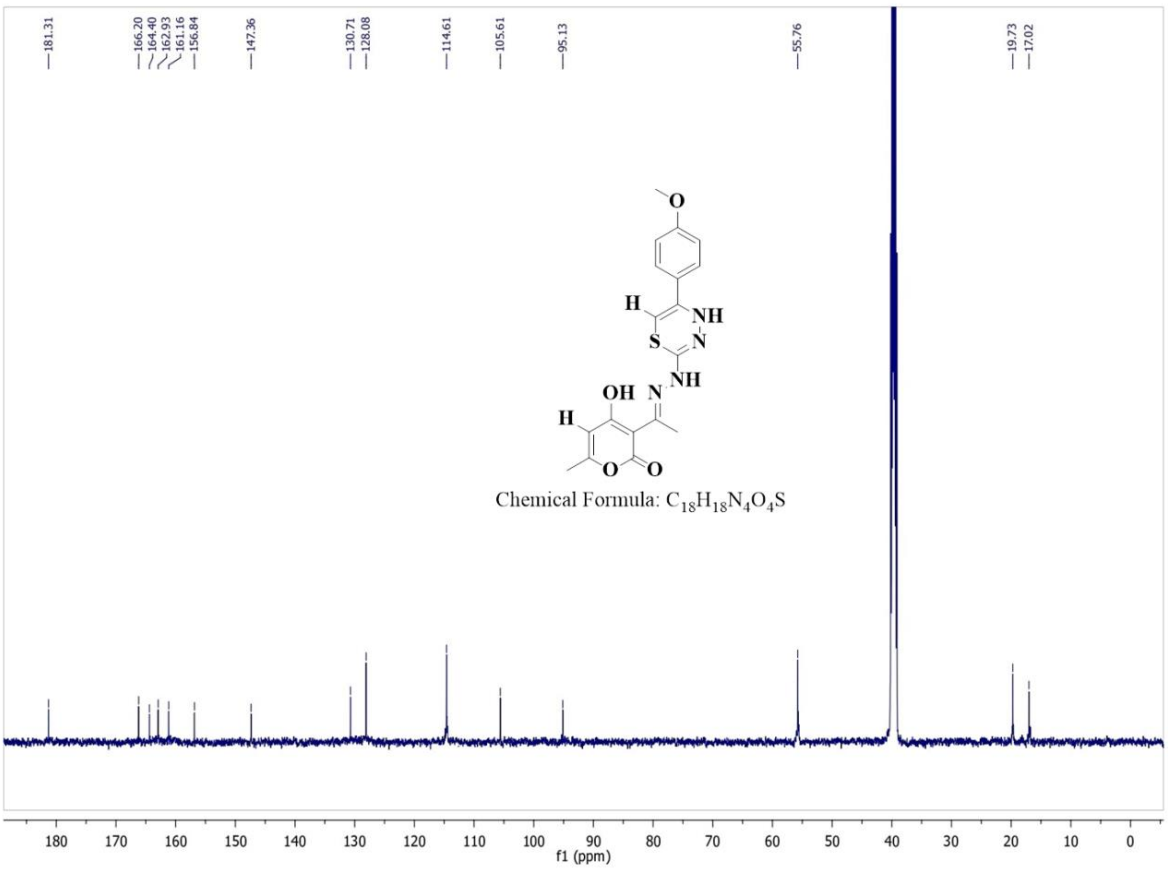
^{1}H NMR spectrum of compound **15** (400 MHz, $CDCl_3$+DMSO-d_6)



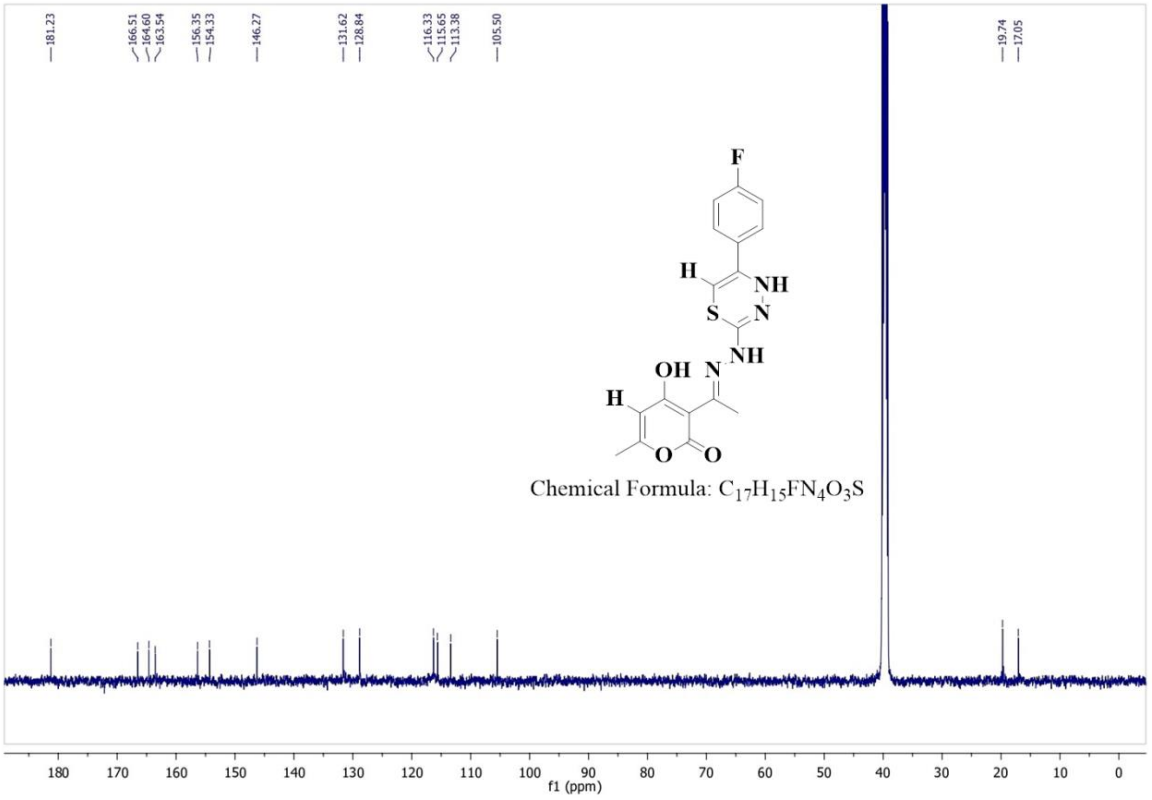
^{13}C NMR spectrum of compound **13a** (100 MHz, $CDCl_3$+DMSO-d_6)



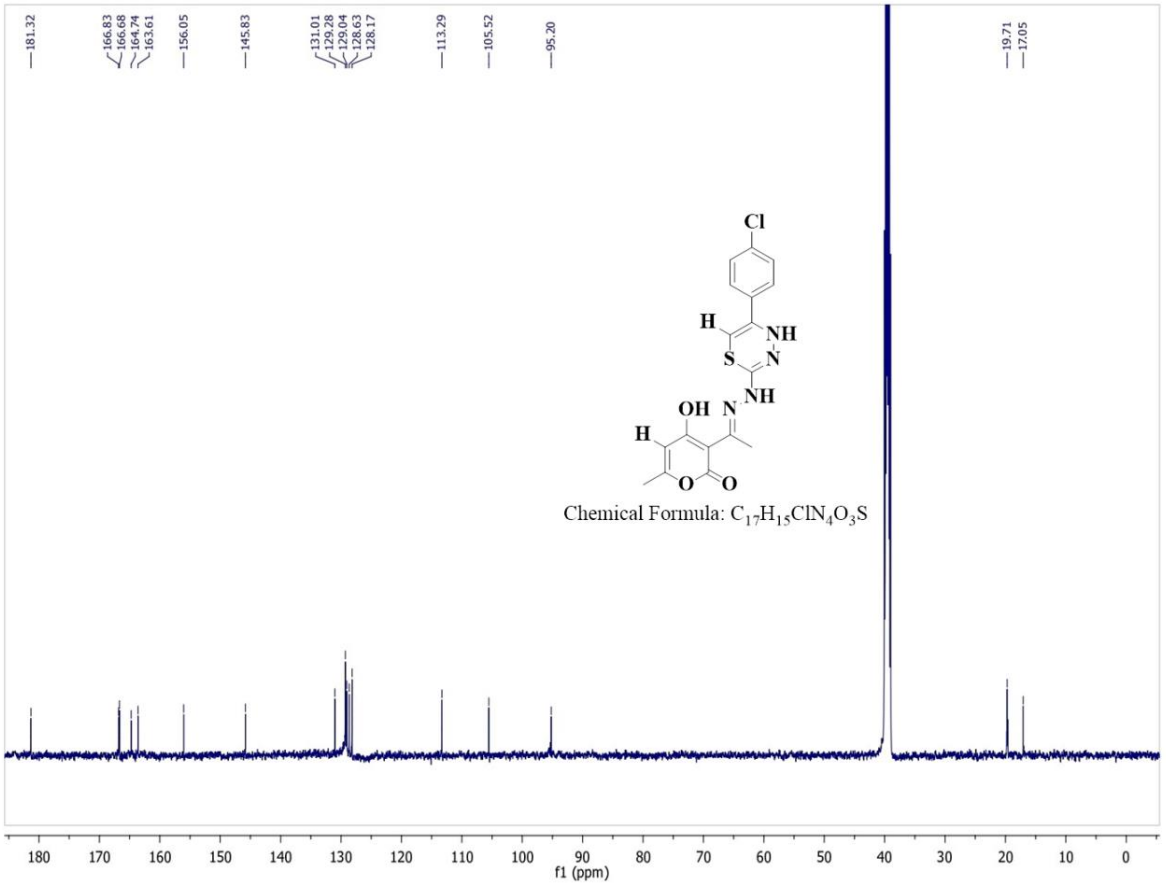
^{13}C NMR spectrum of compound **13b** (125 MHz, DMSO-d_6)



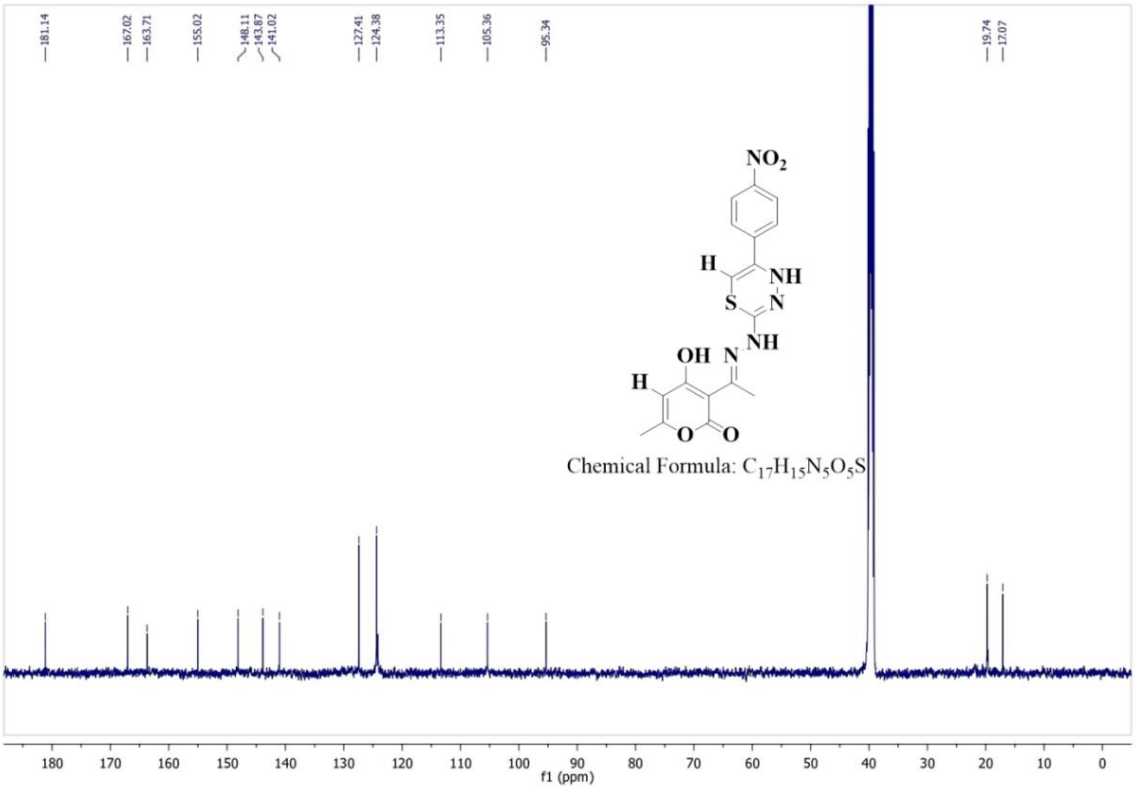
^{13}C NMR spectrum of compound **13c** (125 MHz, DMSO-d_6)



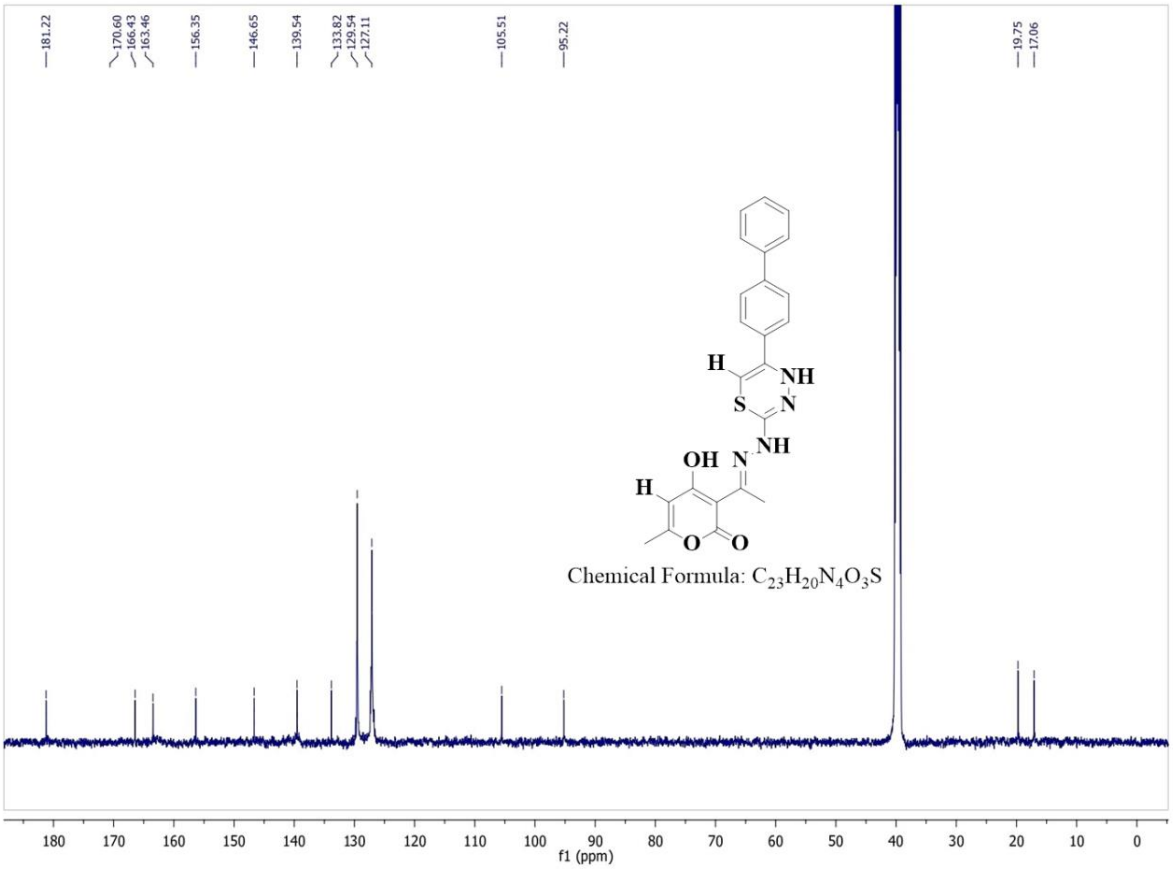
^{13}C NMR spectrum of compound **13d** (125 MHz, DMSO-d_6)



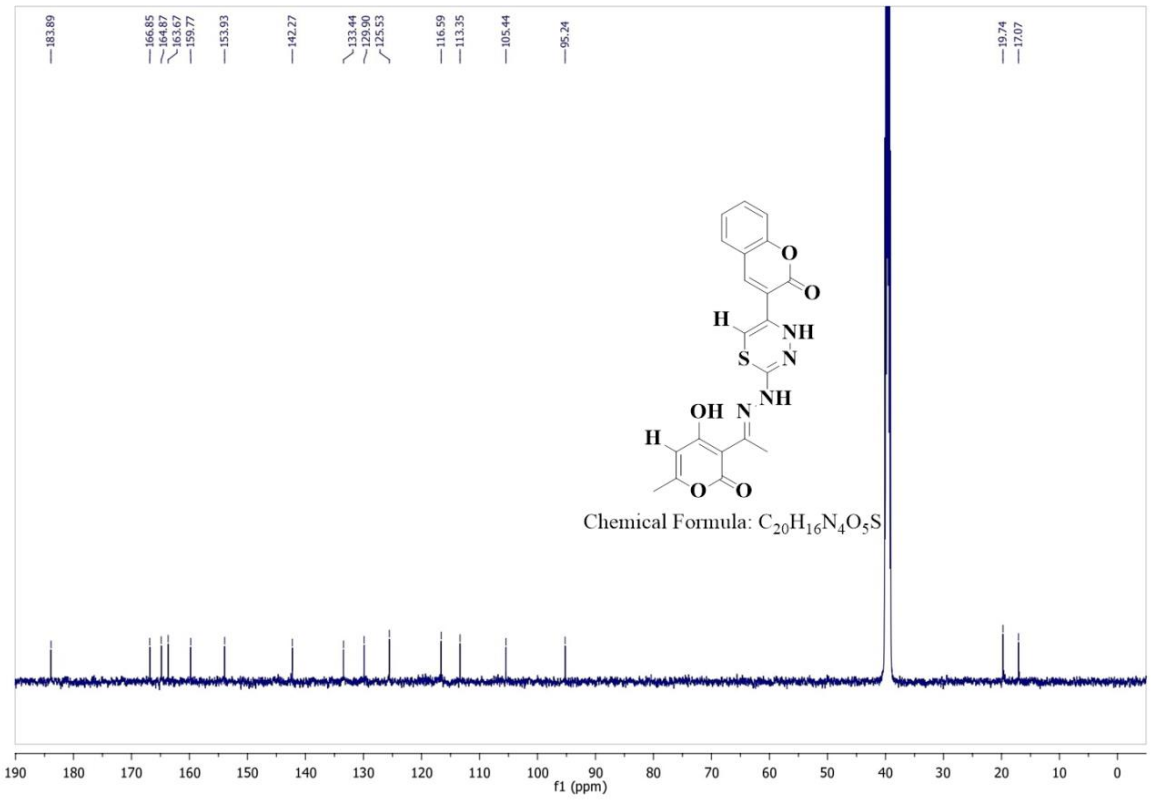
^{13}C NMR spectrum of compound **13e** (125 MHz, DMSO-d_6)



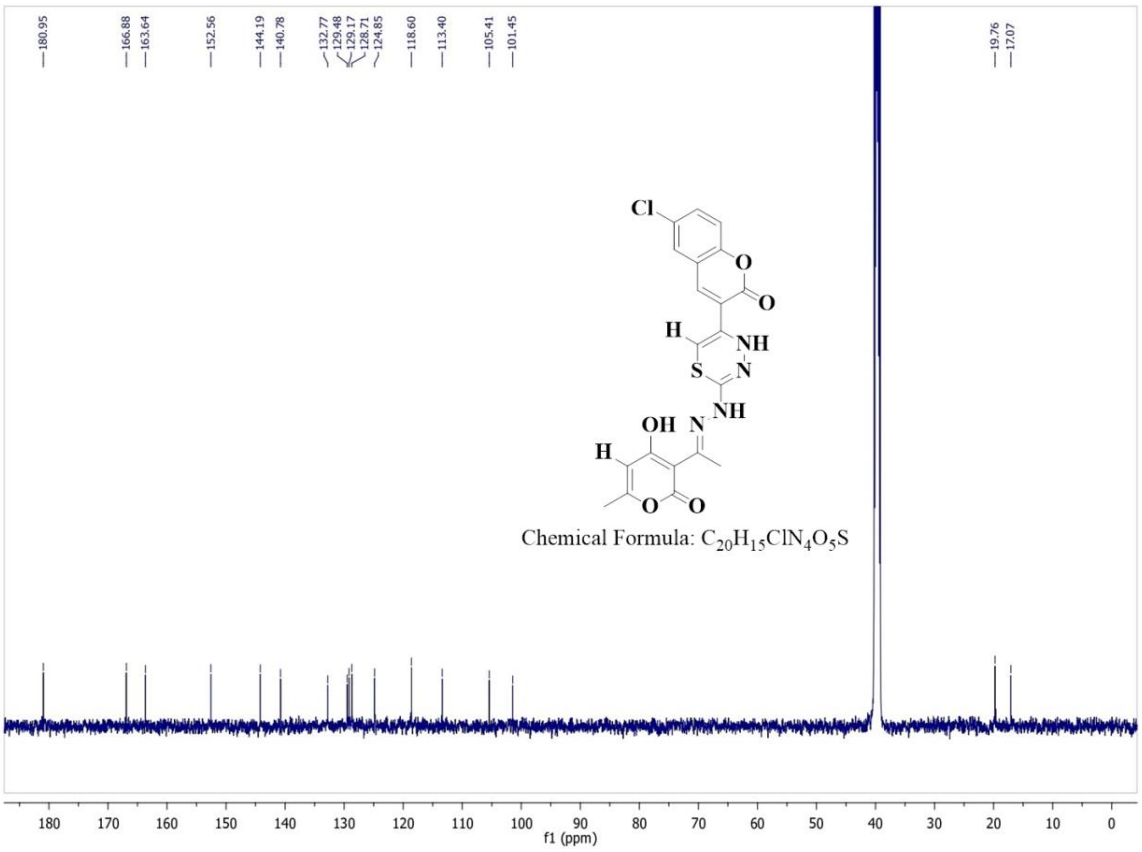
^{13}C NMR spectrum of compound **13f** (125 MHz, DMSO-d_6)



^{13}C NMR spectrum of compound **13g** (125 MHz, DMSO-d_6)



^{13}C NMR spectrum of compound5 **14a** (125 MHz, DMSO-d_6)



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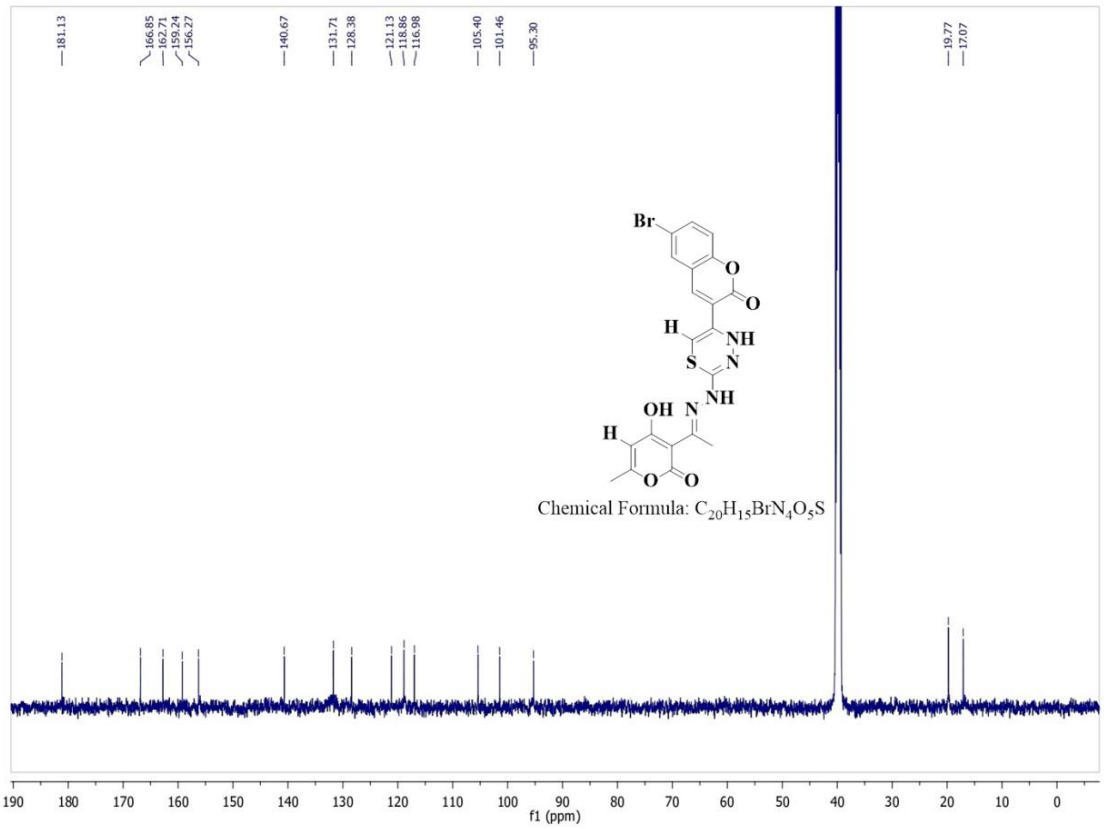

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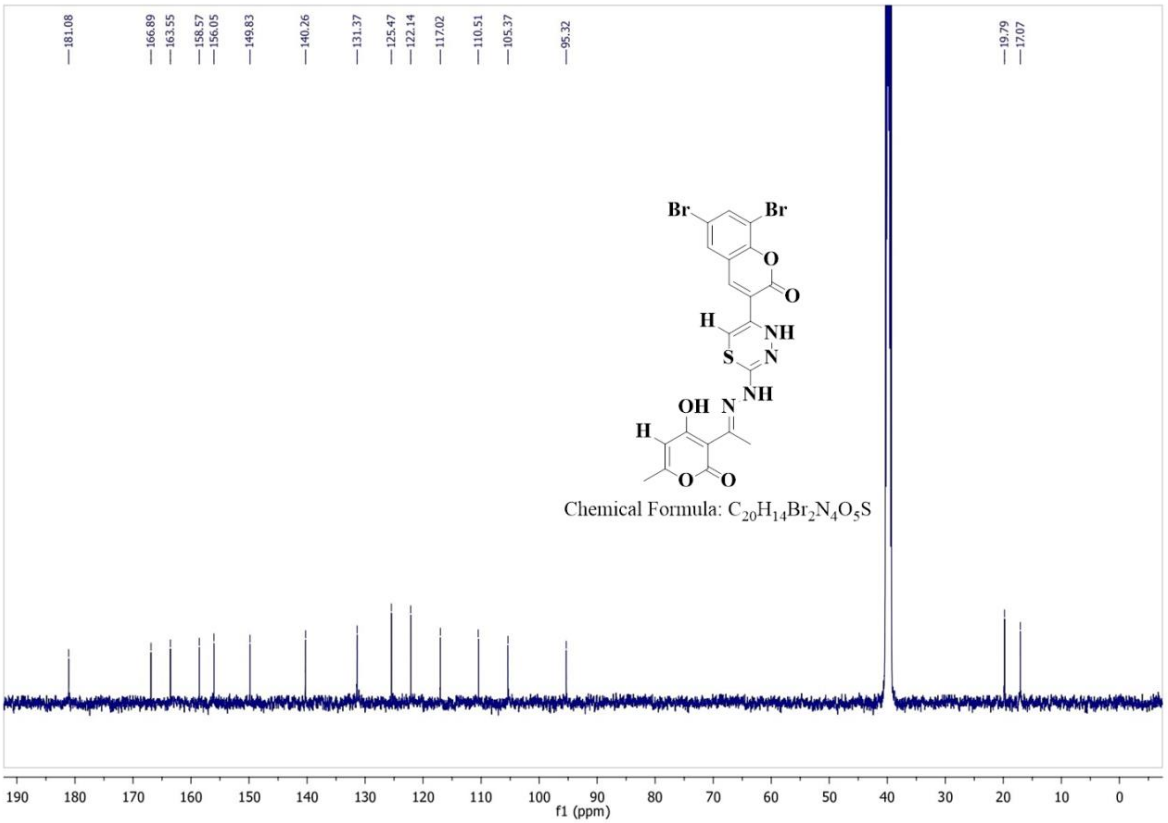

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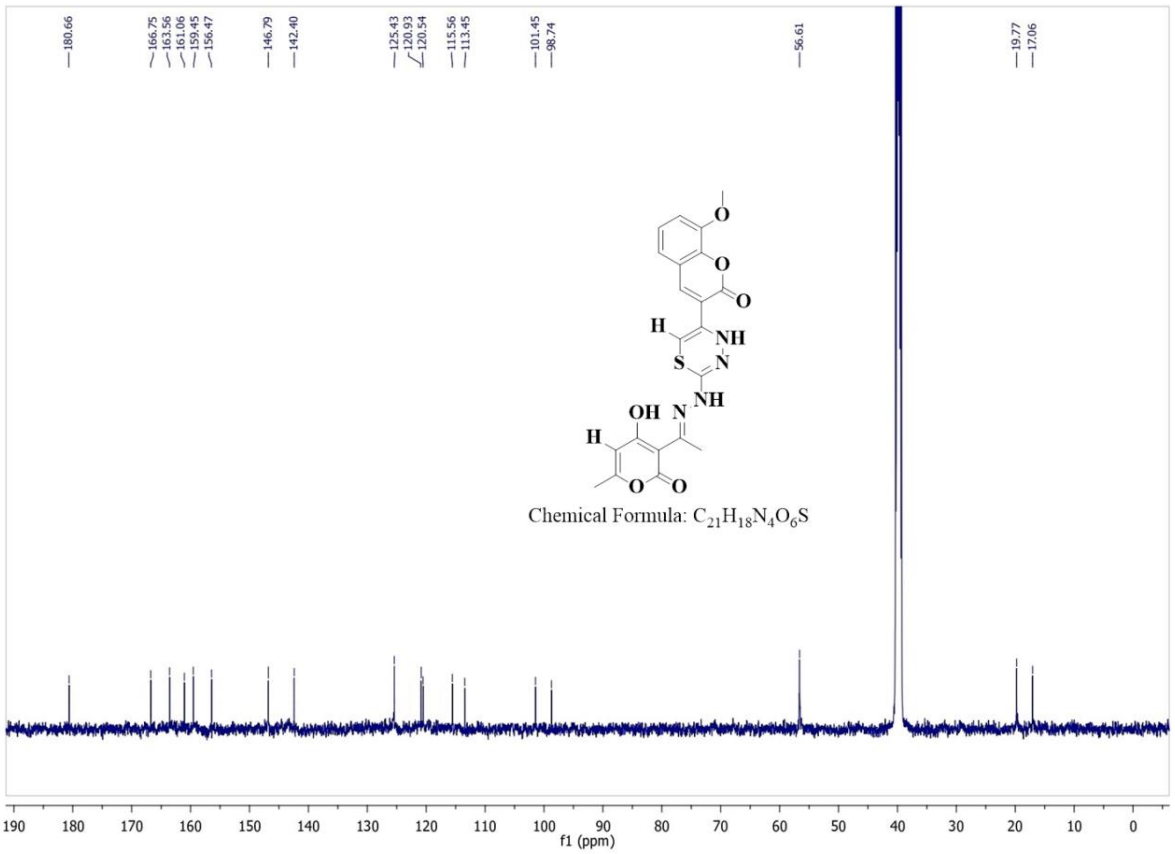

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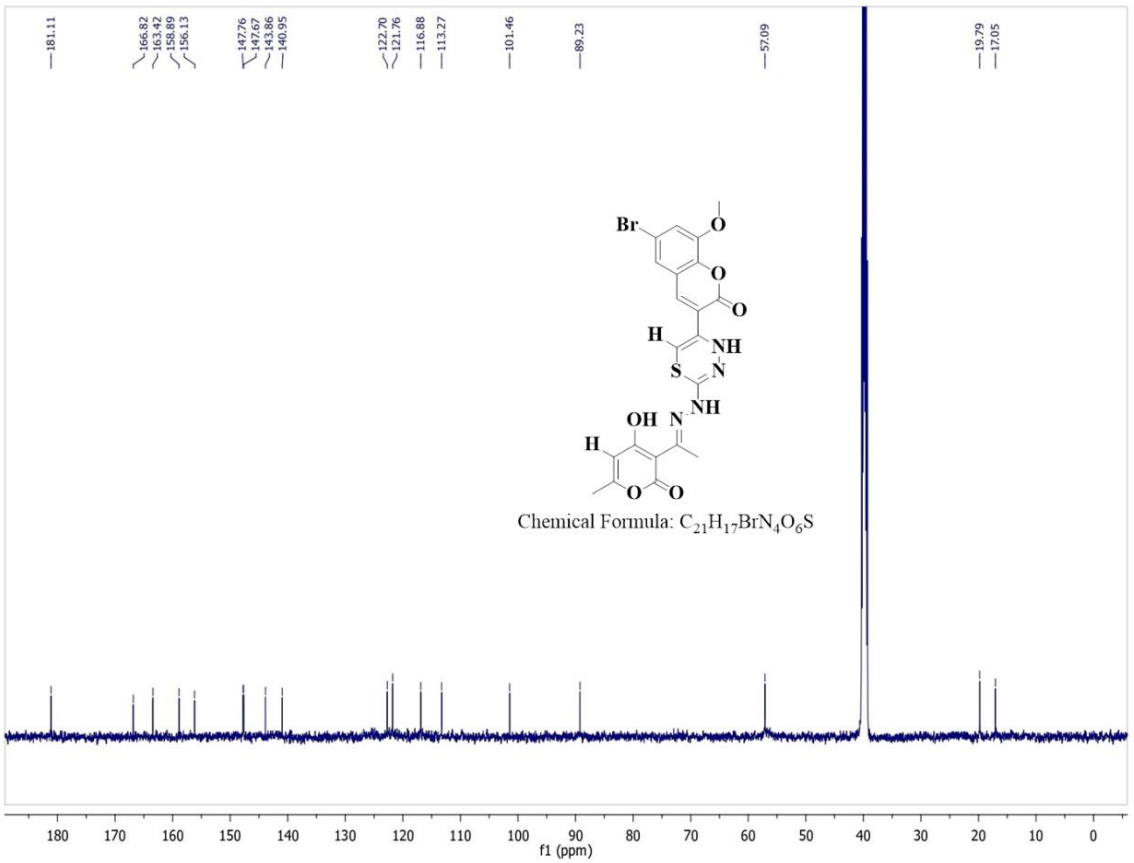

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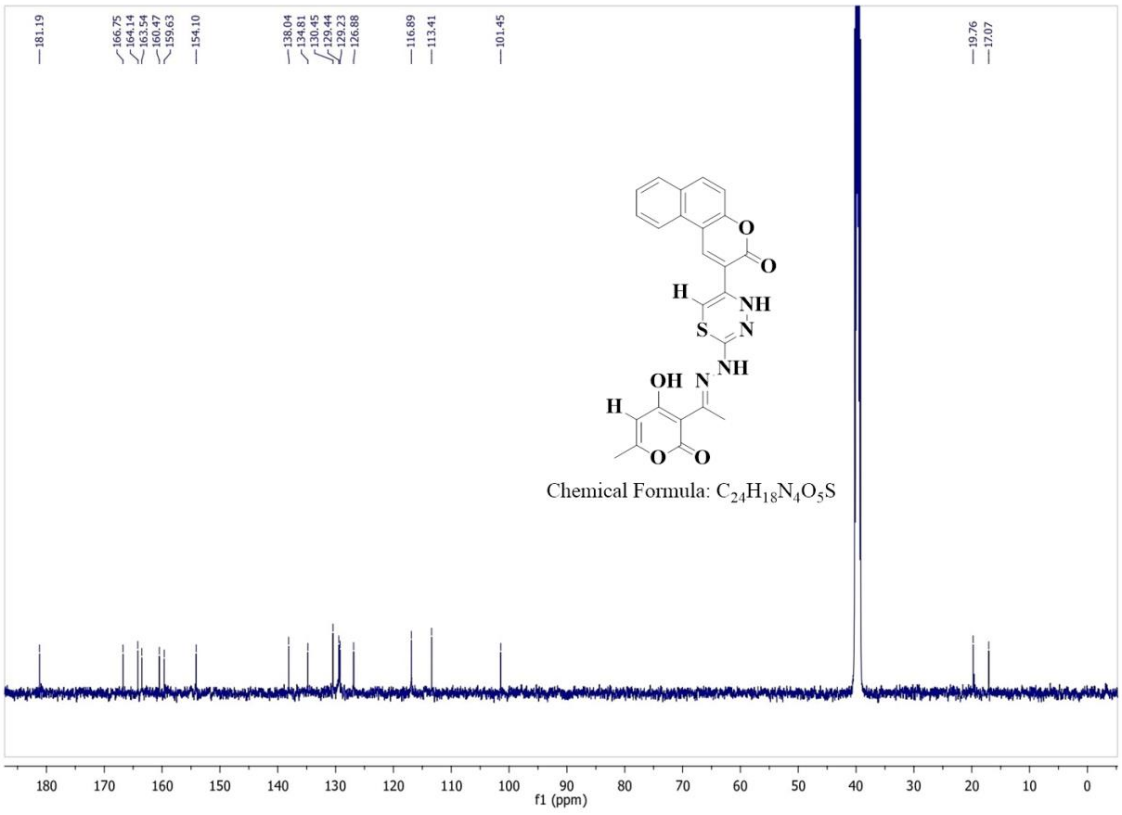
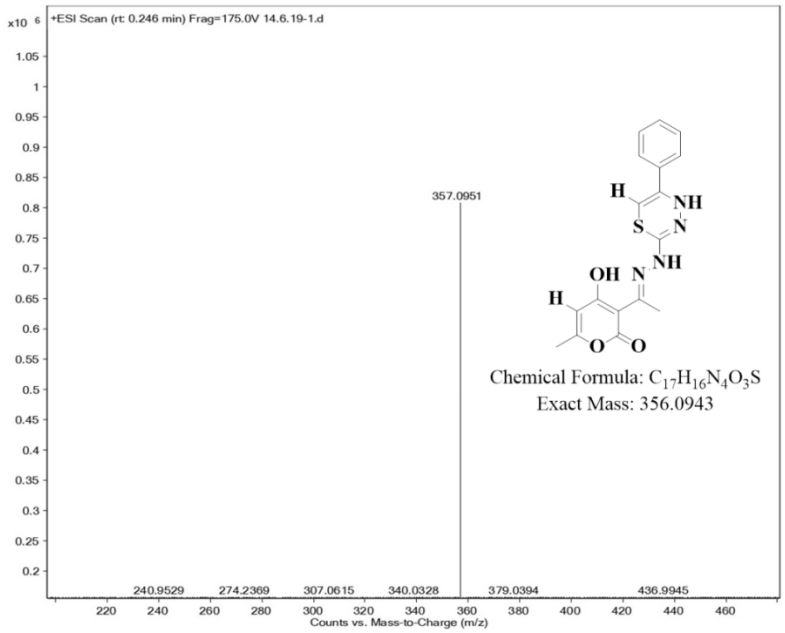
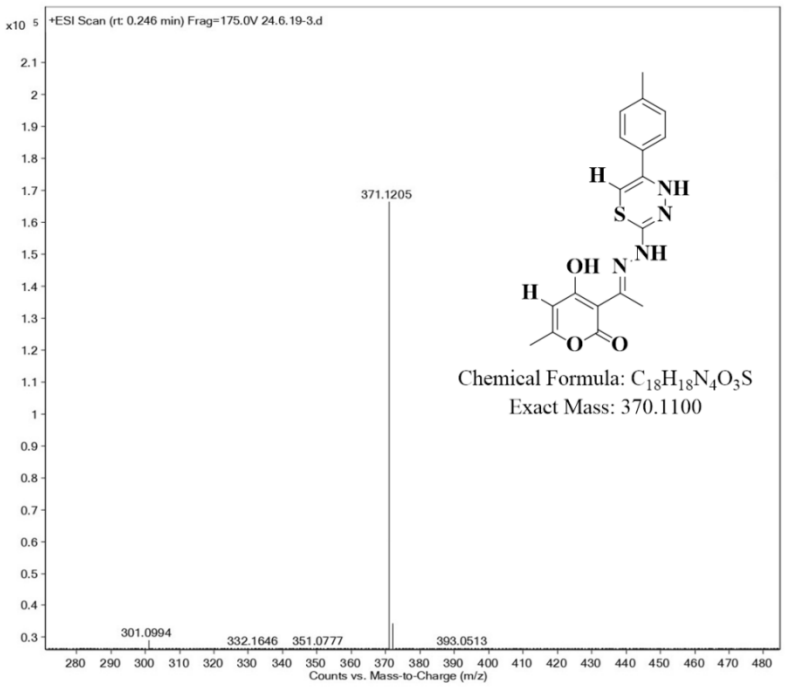


^{13}C NMR spectrum of compound **15** (125 MHz, DMSO-d_6)



Mass spectrum of compound **13a**



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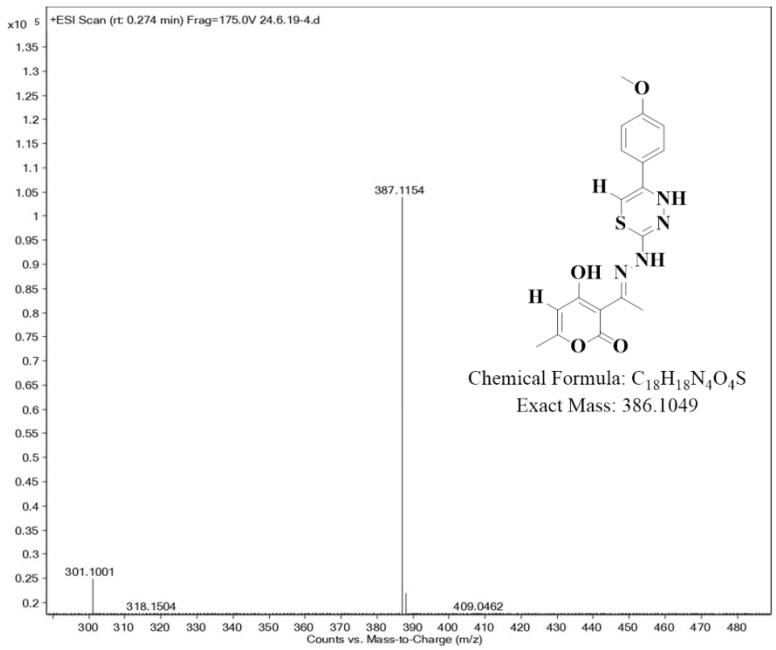

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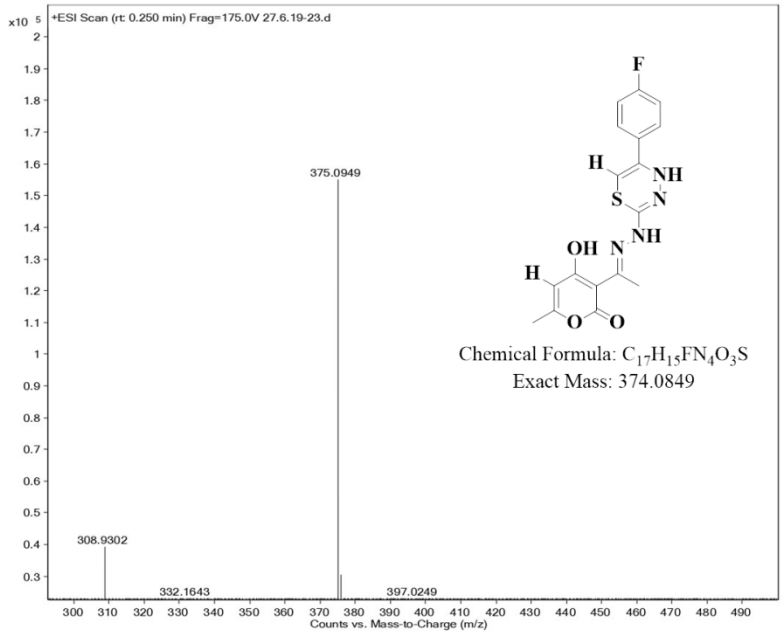
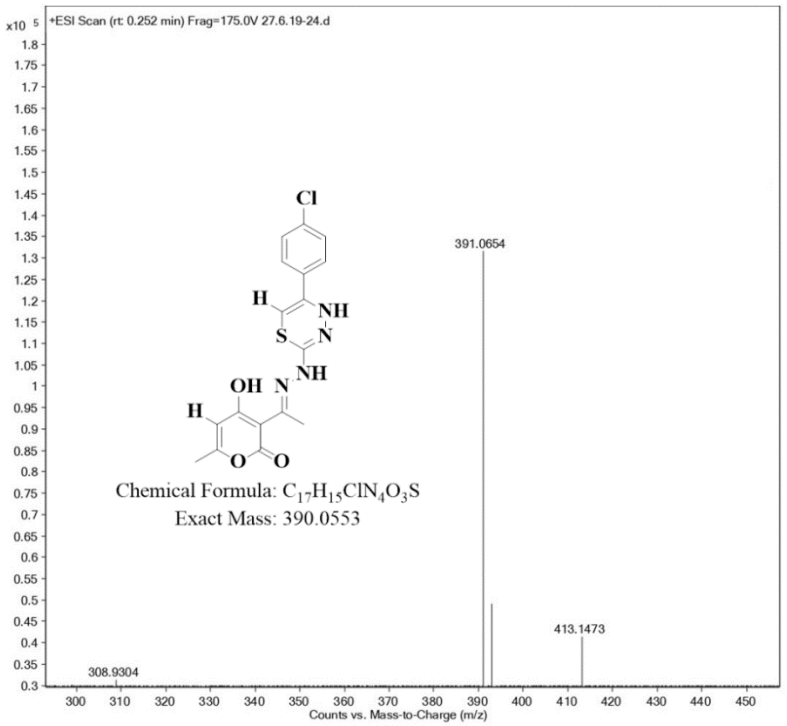


Mass spectrum of compound **13d**



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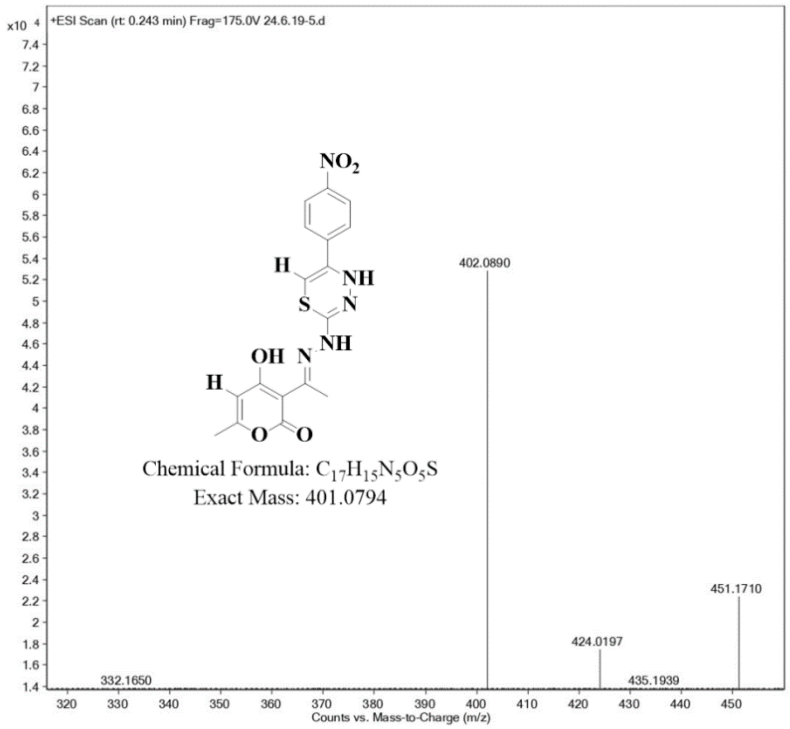
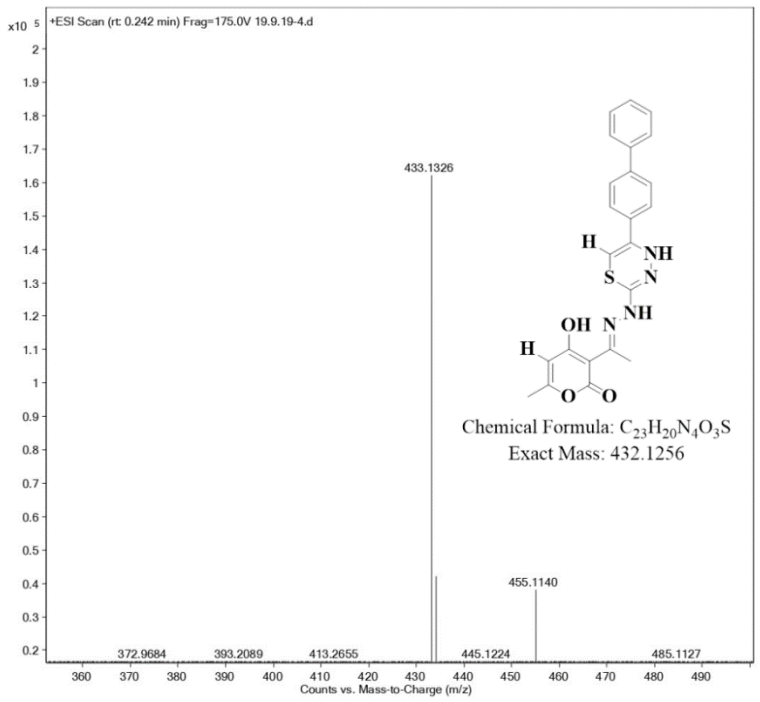
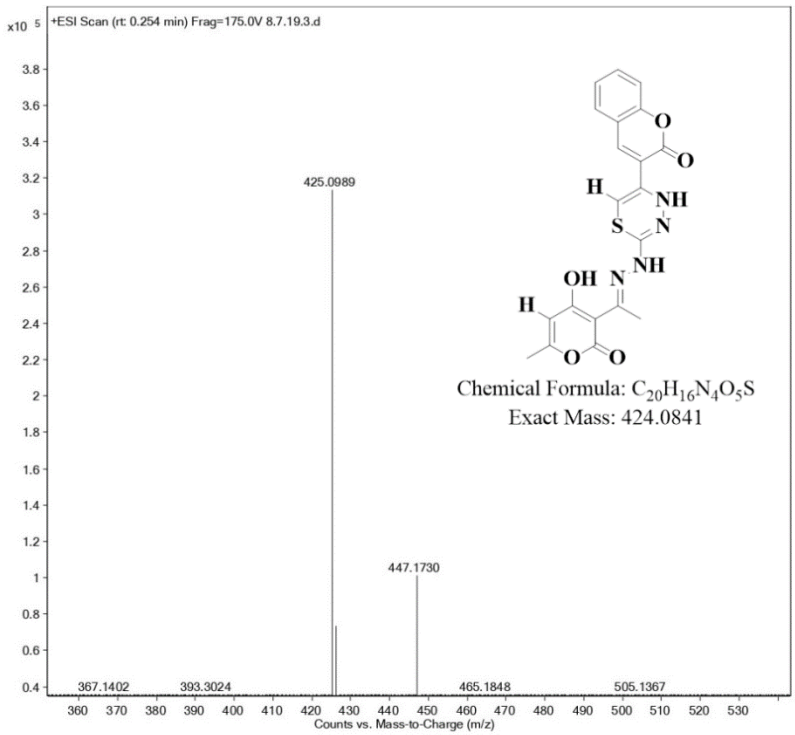


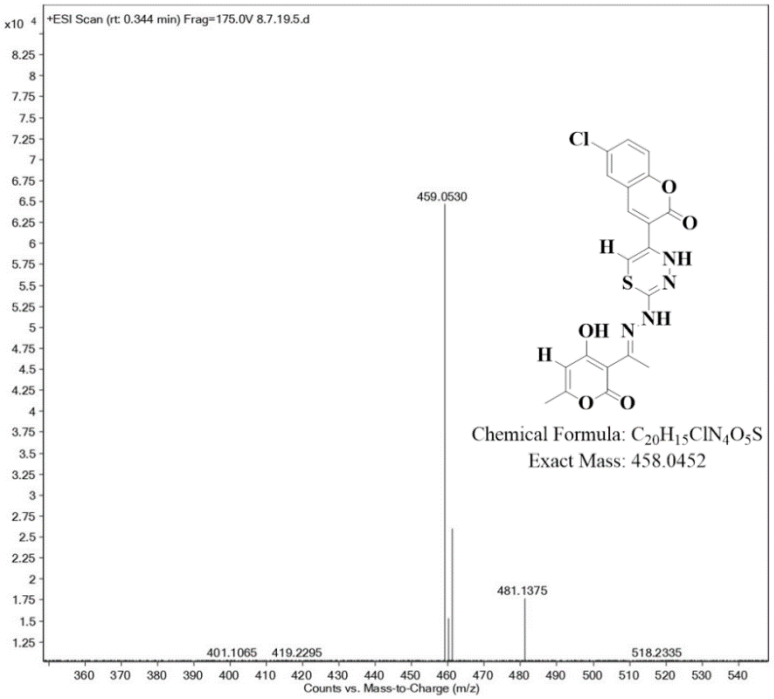
Mass spectrum of compound **13f**



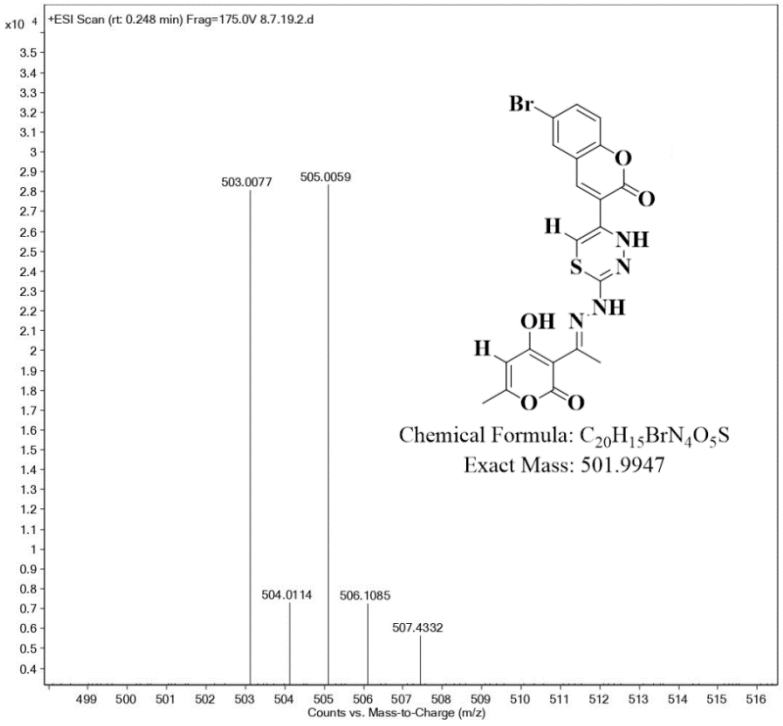
Mass spectrum of compound **13g**



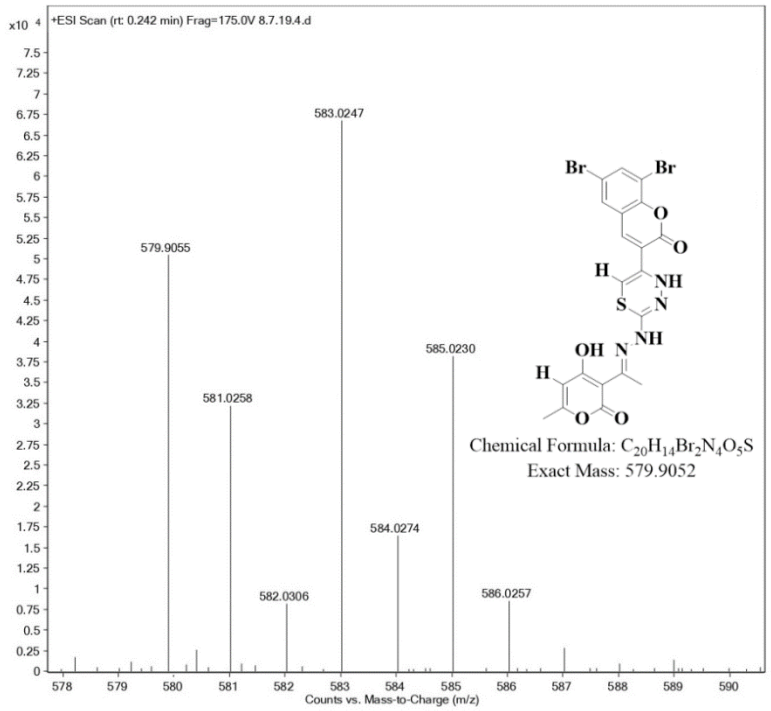
Mass spectrum of compound **14a**



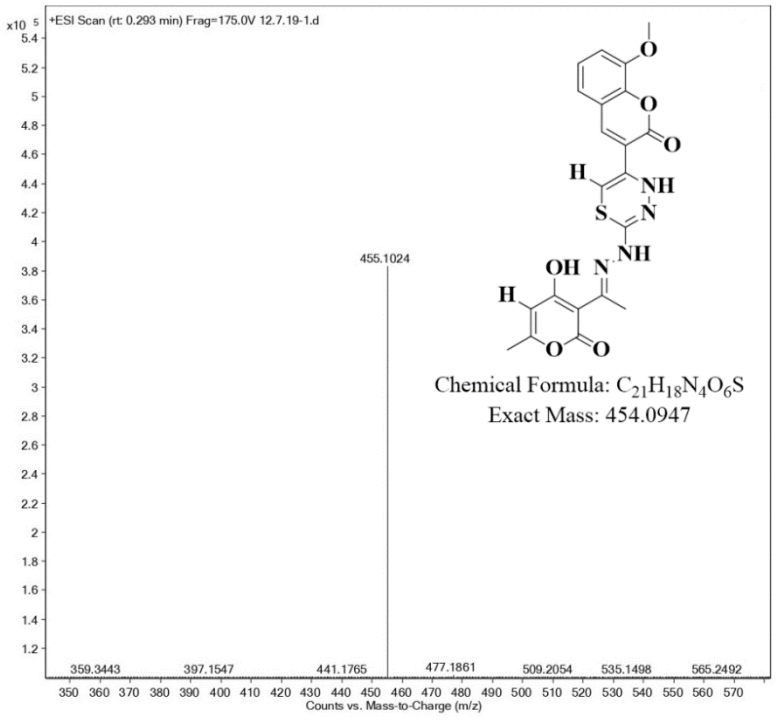
Mass spectrum of compound **14b**



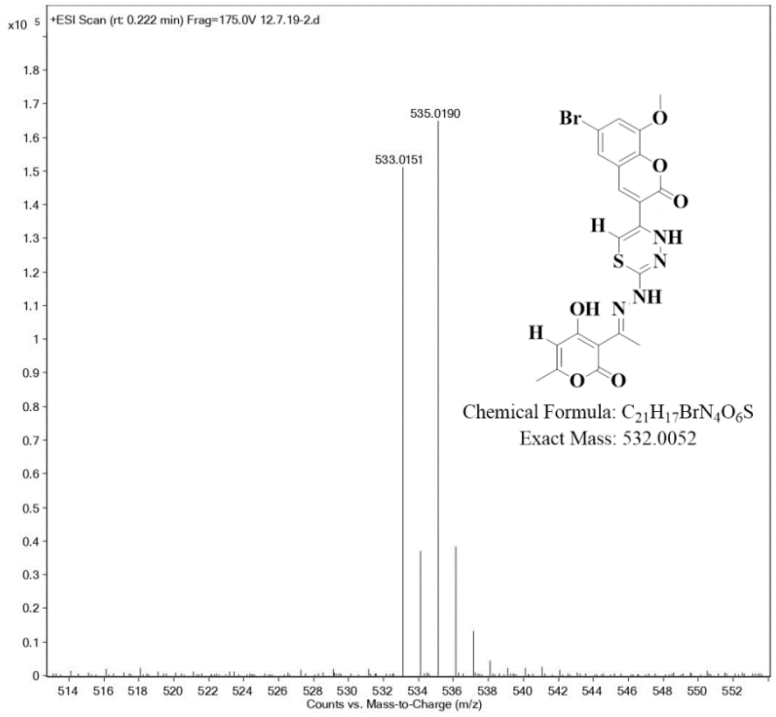
Mass spectrum of compound **14c**



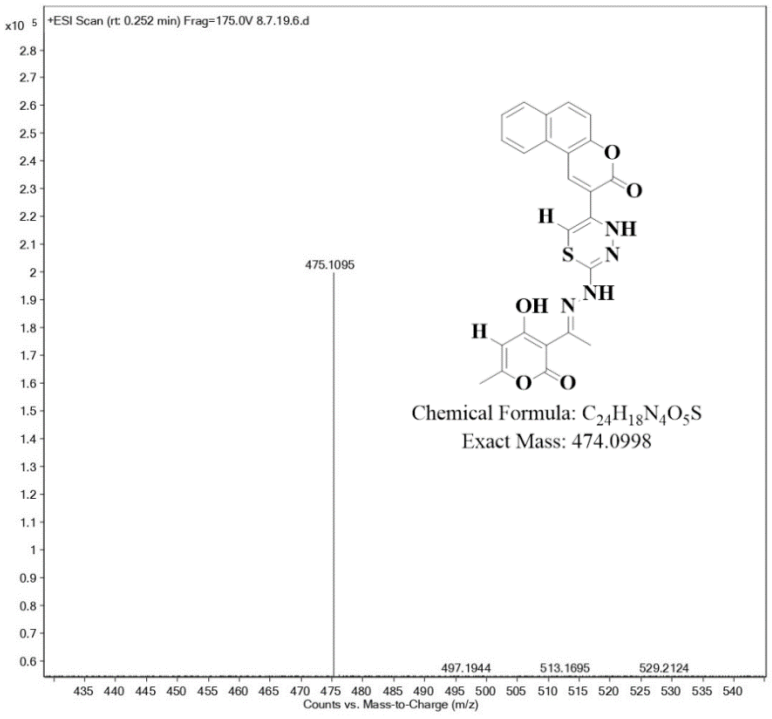
Mass spectrum of compound **14d**



Mass spectrum of compound **14e**



Mass spectrum of compound **14f**



Mass spectrum of compound **15**

References:

[1] Y. Tomita, S. Kabashima, T. Okawara, T. Yamasaki, M. Furukawa, *J. Heterocycl. Chem.* **1990**, *27*, 707–710.

[2] P. Sarapultsev, O. Chupakhin, A. Sarapultsev, M. Rantsev, L. Sidorova, S. Medvedeva, I. Danilova, *Int. J. Exp. Pathol.* **2012**, *93*, 18–23.

[3] K. Shubakar, K. B. Umesha, N. Srikantamurthy, J. Chethan, *Bulg. Chem. Commun.* **2013**, *45*, 274–279.

[4] A. Sarapultsev, O. Chupakhin, P. Sarapultsev, L. Sidorova, T. Tseitler, *Pharmaceuticals* **2016**, *9*, 27.

[5] R. Miao, J. Wei, M. Lv, Y. Cai, Y. Du, X. Hui, Q. Wang, *Eur. J. Med. Chem.* **2011**, *46*, 5000–5009.

[6] J. Schröder, A. Henke, H. Wenzel, H. Brandstetter, H. G. Stammler, A. Stammler, W. D. Pfeiffer, H. Tschesche, *J. Med. Chem.* **2001**, *44*, 3231–3243.

[7] M. G. LaPorte, Z. Wang, R. Colombo, A. Garzan, V. A. Peshkov, M. Liang, P. A. Johnston, M. E. Schurdak, M. Sen, D. P. Camarco, et al., *Bioorg. Med. Chem. Lett.* **2016**, *26*, 3581–3585.

[8] M. C. Forest, P. Lahouratate, M. Martin, G. Nadler, M. J. Quiniou, R. G. Zimmermann, *J. Med. Chem.* **1992**, *35*, 163–172.

[9] A. N. Shaw, R. Tedesco, R. Bambal, D. Chai, N. O. Concha, M. G. Darcy, D. Dhanak, K. J. Duffy, D. M. Fitch, A. Gates, et al., *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4350–4353.

[10] I. Khan, A. Ibrar, S. Zaib, S. Ahmad, N. Furtmann, S. Hameed, J. Simpson, J. Bajorath, J. Iqbal, *Bioorg. Med. Chem.* **2014**, *22*, 6163–6173.

[11] M. R. Shiradkar, M. B. Padhalingappa, S. Bhetalabhotala, K. C. Akula, D. A. Tupe, R. R. Pinninti, S. Thummanagoti, *Bioorg. Med. Chem.* **2007**, *15*, 6397–6406.

[12] A. P. Skoumbourdis, C. A. LeClair, E. Stefan, A. G. Turjanski, W. Maguire, S. A. Titus, R. Huang, D. S. Auld, J. Inglese, C. P. Austin, et al., *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3686–3692.

[13] I. Kostova, S. Bhatia, P. Grigorov, S. Balkansky, V. S. Parmar, A. K. Prasad, L. Saso, *Curr. Med. Chem.* **2011**, *18*, 3929–3951.

[14] M. Khoobi, A. Foroumadi, S. Emami, M. Safavi, G. Dehghan, B. H. Alizadeh, A. Ramazani, S. K. Ardestani, A. Shafiee, *Chem. Biol. Drug Des.* **2011**, *78*, 580–586.

[15] Y. Bansal, P. Sethi, G. Bansal, *Med. Chem. Res.* **2013**, *22*, 3049–3060.

[16] S.-F. Wang, Y. Yin, F. Qiao, X. Wu, S. Sha, L. Zhang, H.-L. Zhu, *Bioorg. Med. Chem.* **2014**, *22*, 2409–2415.

[17] M. Joao Matos, S. Vazquez-Rodriguez, L. Santana, E. Uriarte, C. Fuentes-Edfuf, Y. Santos, A. Munoz-Crego, *Med. Chem. (Los. Angeles).* **2012**, *8*, 1140–1145.

[18] Y. Shikishima, Y. Takaishi, G. Honda, M. Ito, Y. Takeda, O. K. Kodzhimatov, O. Ashurmetov, K.-H. Lee, *Chem. Pharm. Bull. (Tokyo).* **2001**, *49*, 877–880.

[19] A. Manvar, A. Bavishi, A. Radadiya, J. Patel, V. Vora, N. Dodia, K. Rawal, A. Shah, *Bioorg. Med. Chem. Lett.* **2011**, *21*, 4728–4731.

[20] M. E. Riveiro, A. Moglioni, R. Vazquez, N. Gomez, G. Facorro, L. Piehl, E. R. de Celis, C. Shayo, C. Davio, *Bioorg. Med. Chem.* **2008**, *16*, 2665–2675.

[21] M. Basanagouda, V. B. Jambagi, N. N. Barigidad, S. S. Laxmeshwar, V. Devaru, Narayanachar, *Eur. J. Med. Chem.* **2014**, *74*, 225–233.

[22] M. Watzka, C. Geisen, C. G. Bevans, K. Sittinger, G. Spohen, S. Rost, E. Seifried, C. R. Muller, J. Oldenburg, *J. Thromb. Haemost.* **2011**, *9*, 109–118.

[23] A. Asadipour, M. Alipour, M. Jafari, M. Khoobi, S. Emami, H. Nadri, A. Sakhteman, A. Moradi, V. Sheibani, F. Homayouni Moghadam, et al., *Eur. J. Med. Chem.* **2013**, *70*, 623–630.

[24] M. Alipour, M. Khoobi, A. Moradi, H. Nadri, F. Homayouni Moghadam, S. Emami, Z. Hasanpour, A. Foroumadi, A. Shafiee, *Eur. J. Med. Chem.* **2014**, *82*, 536–544.

[25] K. V. Sashidhara, A. Kumar, M. Chatterjee, K. B. Rao, S. Singh, A. K. Verma, G. Palit, *Bioorg. Med. Chem. Lett.* **2011**, *21*, 1937–1941.

[26] F. A. F. Ragab, S. A. Abdel-Aziz, M. Kamel, A. M. A. Ouf, H. A. Allam, *Bioorg. Chem.* **2019**, *93*, 103323.

[27] K. Kumar, P. Man-Un Ung, P. Wang, H. Wang, H. Li, M. K. Andrews, A. F. Stewart, A. Schlessinger, R. J. DeVita, *Eur. J. Med. Chem.* **2018**, *157*, 1005–1016.

[28] A. S. Komendantova, A. M. Scherbakov, A. V. Komkov, V. V. Chertkova, A. O. Gudovanniy, E. I. Chernoburova, D. V. Sorokin, Y. U. Dzichenka, V. Z. Shirinian, Y. A. Volkova, et al., *Bioorg. Chem.* **2019**, *91*, 103142.

[29] I. Radini, *Molecules* **2018**, *23*, 2092.

[30] A. B. Sarıgüney, E. Kocabaş, F. Erci, E. Torlak, A. Coşkun, *J. Heterocycl. Chem.* **2018**, *55*, 2107–2110.

[31] K. Sujatha, N. B. Ommi, A. Mudiraj, P. P. Babu, R. R. Vedula, *Arch. Pharm. (Weinheim).* **2019**, *352*, 1900079.

[32] M. Čačić, V. Pavić, M. Molnar, B. Šarkanj, E. Has-Schön, *Molecules* **2014**, *19*, 1163–1177.

[33] A. Abdel-Aziem, *J. Heterocycl. Chem.* **2015**, *52*, 251–259.

[34] A. Mishra, P. Rai, J. Singh, J. Singh, *ChemistrySelect* **2018**, *3*, 8408–8414.

[35] R. Hartmann, J. Malmström, G. Sibley, F. Lehmann, *Synlett* **2016**, *27*, 864–867.

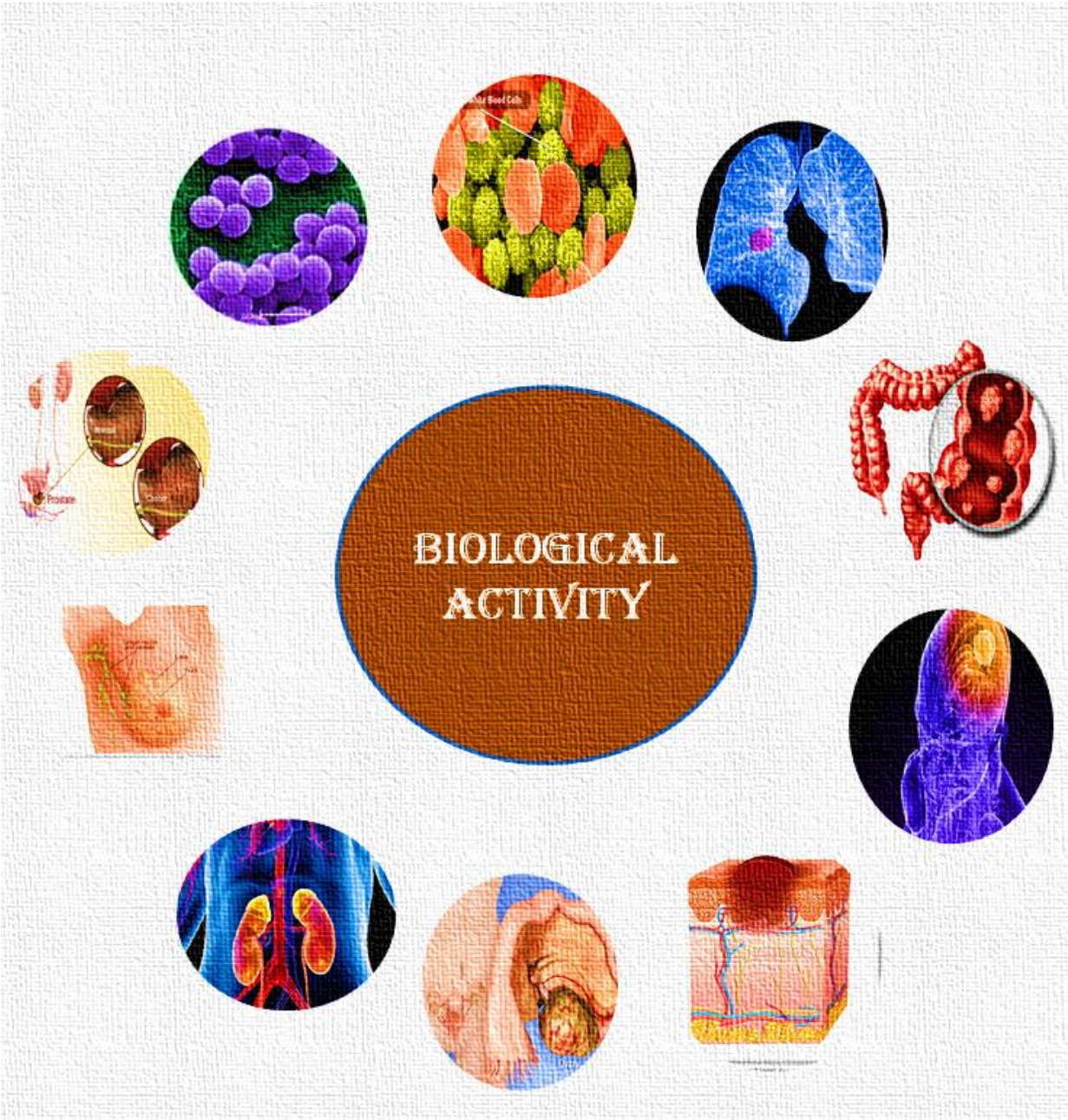
[36] R. K. Ramagiri, K. Vaarla, R. R. Vedula, *Synth. Commun.* **2016**, *46*, 1499–1506.

[37] S. Pavurala, K. Vaarla, R. R. Vedula, *Phosphorus. Sulfur. Silicon Relat. Elem.* **2017**, *192*, 69–72.

[38] A. S. Komendantova, K. A. Ivanova, K. V. Lyssenko, Y. A. Volkova, I. V. Zavarzin, *Chem. Heterocycl. Compd.* **2019**, *55*, 665–671.

CHAPTER-V

Biological evaluation of synthesized hetero cyclic compounds



CHAPTER-V

5.1. Introduction:

In pharmacology, biological activity or pharmacological activity describes the beneficial or adverse effects of a drug on living matter. Among the various properties of chemical compounds, biological activity plays a crucial role since it indicates uses of the compounds in medical applications. However, chemical compounds may show some adverse and toxic effects which may prevent their use in medical practice.

Moreover, the medicinal application of a newly synthesized organic compound can be evaluated by subjecting the compound to a variety of biological screening tests under different conditions. The final results of a series of interlinked chemical reactions or the observed manifestations of an interference with delicately balanced system of interdependent chemical and physical processes is called biological activity. The interference should eradicate or destroy the parasites responsible for the infection, without damaging the tissues of the host. Such substances are suitable as drugs. The relationships of biological activity and chemical constitution in a number of compounds exhibiting different types of activities have been extensively studied. The biological activity of a molecule depends on various factors. Among these, the pharmacophore determining the type of physiological activity, position of substituents, optimum carbon skeleton, membrane permeability, lipid solubility, stereo chemical configuration etc., of the molecule play not only an important role in determining the activity but also the species selectivity.

Furthermore, among the wide variety of clinical applications heterocyclic compounds have a remarkable active role as anti-microbial[1–8] , anti-tubercular[9–11], anti-viral[12,13], antitumor[14–18], anti-HIV[19–24], anti-inflammatory and antioxidant[25–29], anti-Alzheimer[30,31], anti-tubercular, AMP phosphodiesterase inhibitor[32], Hepatitis C virus polymerase inhibitor[33], anticoagulants[34–36], treatment of hypertension[37–41], allergies[42–45], Schizophrenia[46,47], and inhibitors of bacterial DNA gyrase B[48,49], etc. Some of the hetero cyclic biological active molecules are shown in Figure 5.1.

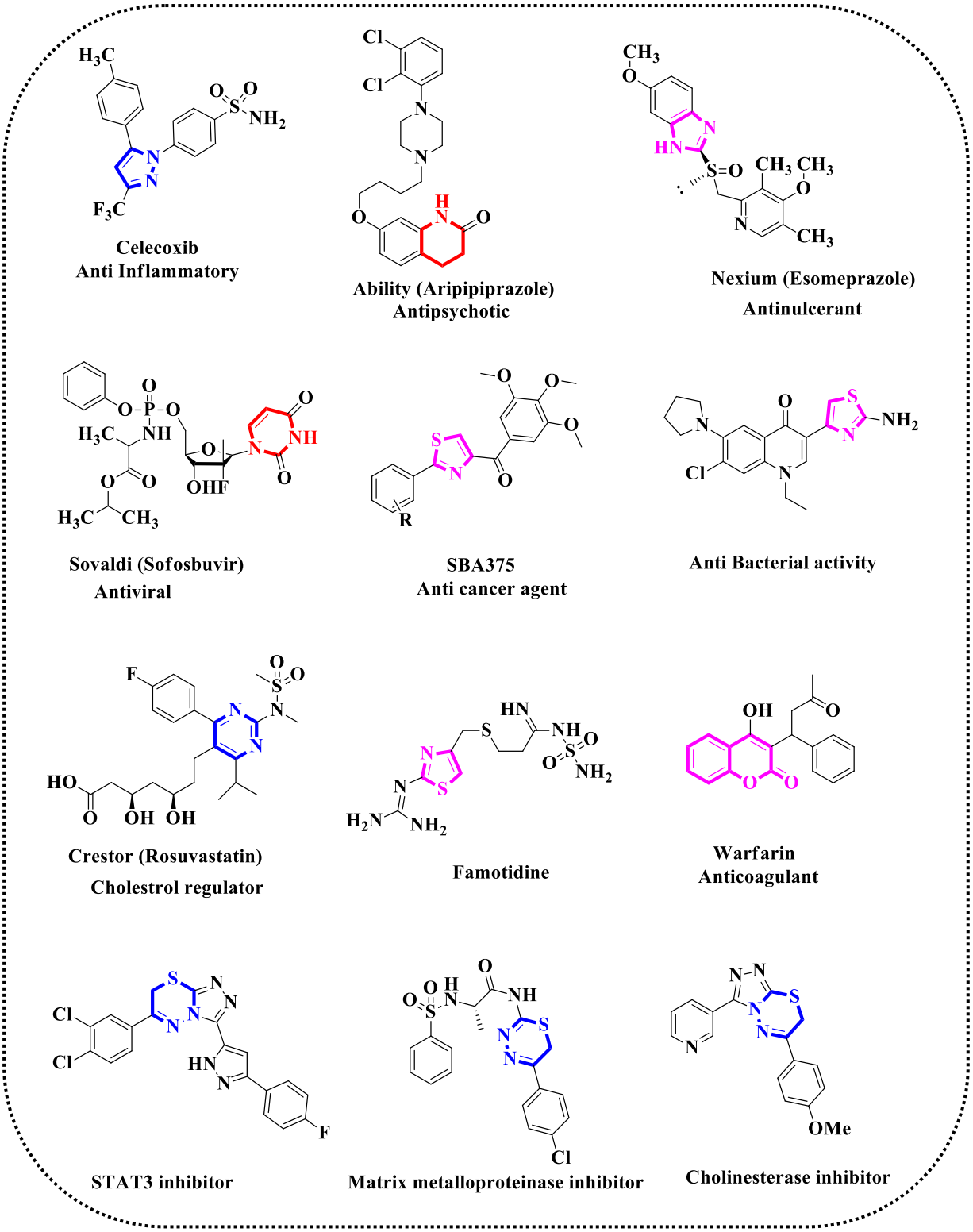


Figure 5.1: Biological active compounds having thiazoles, thiazolyl pyrazoles, 1,3,4 thiadiazines.

5.1.1. Molecular docking studies:

The major application of the molecular docking studies is to design the compounds *in silico* and targeted against proteins (macromolecules), the binding modes of these compounds (ligands) with active site of a target protein. Thus the compounds which are strongly bound to a protein

were treated as lead molecules. In *in vitro* experiments, drugs are discovered by a chance in a trial-and-error method by using high-throughput screening of a large number of compounds against a given target. This process is a time consuming and highly expensive. Whereas, if the 3D structure of the compound is known, then the molecular docking is a useful tool in the identification of drug candidates by a virtual screening of compound databases. The energetically more favorable ligand conformation is suitable for the docking. In general, low energy scores represent the better protein-ligand bindings[50]. The most commonly used heuristic search algorithms that have been applied to molecular docking are simulated annealing, tabu search and evolutionary algorithm[51–53].The molecular docking studies presented in the thesis were carried out by using AutoDock Tools (ADT) version 1.5.6 and AutoDock version 4.2.5.1 docking program and GLIDE 5.6[54] of schrodinger suite 2010.

5.1.2. Biological activity:

As per the literature, different synthesized compounds which is having heterocyclic moiety showing biological properties. Hence, it is reasonable to presume that some of the synthesized compounds would also be active against some tumour cells and micro-organisms. Therefore, in the present study, all the synthesized compounds have been tested for their biological activities such as anticancer, antimicrobial activities.

Indeed from the past so many years forth, the prime cause of death because of infections[55–57], which is caused by only microorganisms and also microorganisms resistance to multiple antifungal and antibacterial agents have become a major issue[58,59]. The birth of multidrug resistant microbes in various bacterial strains is an alarming element[60,61]. Hence evolution and discovery of novel antibiotics are compulsory to eradicate the growth of MDR pathogens. Nonetheless, as per IARC, cancer is the second most leading cause of death globally and according to GLOBOCAN 2018, by 2040 estimated incidences would increase to 29.5 million. In spite of this expanding antitumor agents evolved to date, breast cancer is a major type of cancer observed compare to other cancers in woman affecting approximately 2.1 million people every year. Survey regarding the mortality of breast cancer shows that 0.62 million women died of breast cancer in 2018[62]. Inadequacy of selectivity and inability to multi-drug resistance are main hurdles to tumor treatment. Consequently, full efforts have been devised for the discovery of new potent and selective compounds that cease tumor proliferation, which eradicate malignancy and breast cancer severity.

Cancer is a disease manifested by uncontrolled proliferation of abnormal cells. The etiology of cancer is not fully understood but multitude factors participate in disease onset[63]. Both

endogenous and exogenous stimuli can initiate cancer. Endogenous factors viz., genetic mutations (inherited / developed) and exogenous factors (exposure to UV, smoking, obesity etc.) may act in synergy or in sequence to promote disease susceptibility[64,65]. Lung, blood, prostate, colorectal, cervical, lymph node, breast, thyroid and liver cancers are the most common types of cancer in human[66,67]. External or internal stimulus can lead to mutation which when left unaddressed by the cell may lead to their abnormal growth. Cancer breaks the homeostasis between cell division and death triggering uncontrolled cell proliferation. Instinct cellular mechanism has the ability to repair DNA breaks and mutagenic areas to save the cell from abnormalities. But sometimes activation of proto-oncogenes and suppression of tumor suppressor genes triggers autonomous cell behavior by passing cell death[68].

In general, cells multiply and die in an ordered fashion whereas in cancer cells grow abnormally by expressing proteins that inhibit cell apoptosis[69,70]. The resulting cellular mass deposition is called a tumor which can be benign or malignant. A tumor is called benign when the cells are restricted to a proper region and cannot be spread to various organs in the body. In contrast to benign tumors, malignant tumors are cancerous and can propagate from one part to other through blood or lymph. A cancer is named by the type of tissue effected and a tumor sometimes travels in the blood stream and travels to other organ and this process is known as metastasis[71]. Cancer can be treated when identified earlier. Research helped to identify the root cause of many cancers and still many questions have to be answered as this is a multi-factorial disease involving multiple pathways and proteins. Screening helps in preventing many cancers like breast, cervix, lung, prostrate, etc. About 30-50 % of cancers can be cured by avoiding risk factors and employing medical strategies[72].

Common types of cancer treatments include hormone therapy, chemotherapy, radiation therapy, targeted therapy and immune therapy[73]. All the above treatments provide better relief and successful but have many side effects depending upon and the type and treatment adopted. These treatment strategies are limited by cytotoxic effects on neighboring normal tissues. Radiation therapy is more effective in some tissues but not recommended frequently as it sometimes disrupt bone marrow and other underlying mechanisms[74,75].

***In vitro* tests for anticancer activity:**

Studies MTT assay and trypanblue exclusion assay are the proliferation tests, by which the rate of proliferation and rate of growth inhibition by the drug is measured. Acridine orange staining and giemsa staining are morphological tests that measure the morphological changes produced by the drug action like membrane blebbing, nuclear condensation etc. These morphological

changes ultimately lead to the death of the cells through apoptosis. DNA laddering is usually carried out for continuing the apoptotic effect of the drug on cancer cells. MTT assay is a standard colourimetric assay, which is used to measure cell growth by measuring the color changes. It is generally useful in the determination of the cytotoxicity of potential biologically active agents and other toxic materials.

Indeed from the past so many years forth, the second prime cause of death because of infections[55–57], which is caused by only microorganisms and also microorganisms resistance to multiple antifungal and antibacterial agents have become a major issue[58,59]. The birth of multidrug resistant microbes in various bacterial strains is an alarming element[60,61]. Hence evolution and discovery of novel antibiotics are compulsory to eradicate the growth of MDR pathogens. In which, *staphylococcus aureus* is a Gram positive bacteria, it is connected with hospital infections, which causes pneumonia, skin infections, bacteremia and also endocarditis[76,77]. As per the Liu *et al*[78]., *S.aureus* may become unsusceptible to lincomycins, methicillin, tetracyclines, pencillins, vancomycin and rifampicin**.**

5.2. Present work:

5.2.1. Anti-cancer activity of thiazole compounds:

Here, out of the twelve compounds (**1a-l**) based on computational studies10 compounds were selected for the screening of their biological activity and the cytotoxic effect of these compounds on 9 different sets (i.e., non-small cell lung, leukemia, Melanoma, Colon, CNS, Prostrate, renal, Ovarian and breast cancers) of cell lines has been determined.

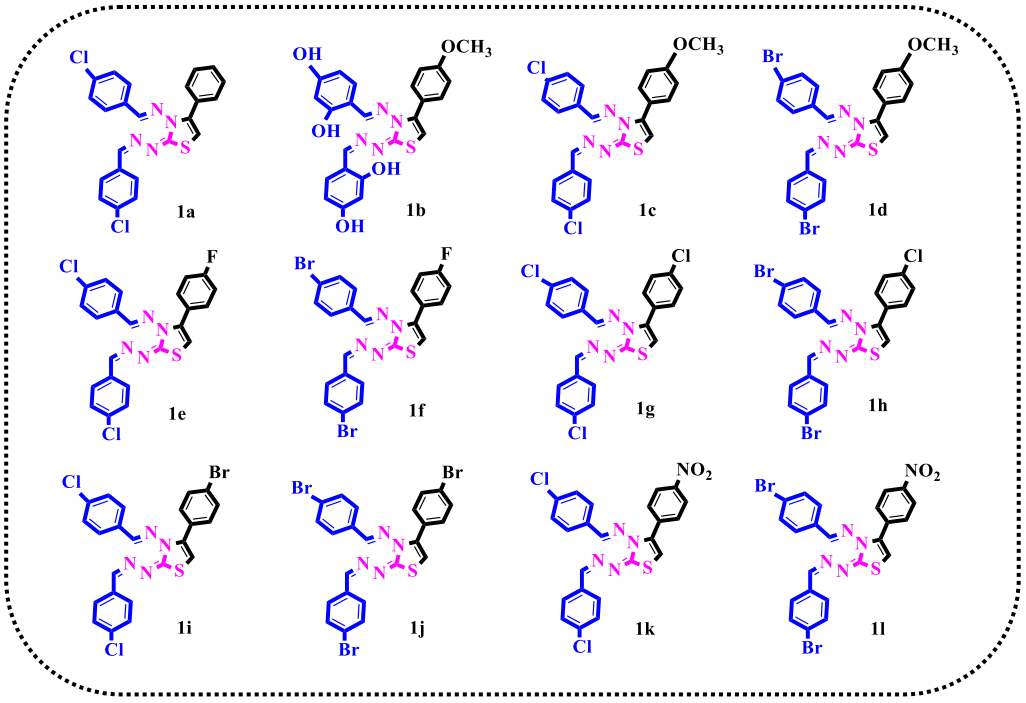


Figure 5.2: thiazole hybrids (**1a-l**).

5.2.1.1. Experimental:

Biological activity:

Cytotoxic effects of the synthesized thiazole derivatives were determined by using MTT [3-(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide] assay. 120 µL aliquots of a cell suspension (50,000 cells ml/1) in 96-well micro plates were incubated at 37 °C and 10% CO_2 and allowed to grow for 48 h. Then 60 µL of serial dilutions of the compounds were added. After 24 h of incubation at 37 °C and 10% CO_2, 20 µl MTT in phosphate buffered saline (PBS) were added to a final MTT concentration of 0.5 mg/ml. After 2 h the precipitate of formazan crystals was centrifuged and the supernatant discarded. The precipitate was washed with 100 µl PBS and dissolved in 100 ml isopropanol containing 0.4% hydrochloric acid. The resulting colour was quantified at 590 nm using an ELISA plate reader. For 1-dose 60 cell testing, For 5-dose 60 cell testing[79] were determined using NCI standard protocols. The IC50 values were determined from the dose–response curves as the concentrations of compounds, which resulted in 50% of the absorbance of untreated control cells.

Molecular docking protocol:

In silico docking studies are useful tools to assess the binding affinity of the ligand-protein receptor. The synthesized compounds were subjected to molecular docking by using the AutoDock Tools (ADT) version 1.5.6 and AutoDock version 4.2.5.1 docking program. The 3D-structures of all the synthesized compounds were prepared by using chem3D pro 12.0 software. The optimized 3D structures were saved in .pdb format. The crystal structures of the proteins with PDB ID 3OG7, 2AE4, 1SA0, 1M17, 1T2F and 1QH4 were extracted from the protein data bank[80–85](http://www.rcsb.org/pdb). The bound ligand and water molecules in protein were removed by using Discovery Studio Visualizer version 4.0 to prepare the protein. Nonpolar hydrogens were merged and gasteiger charges were added to the protein. The grid file was saved in. gpf format. The grid box having dimensions 80 x 80 x 80 $Å^3$ was created around the protein with spacing 0.3750 Å. The genetic algorithm was carried out with the population size and the maximum number of evaluations were 150 and 25,00,000, respectively. The docking output file was saved as Lamarckian Ga (4.2) in .dpf format. The ligand-protein complex binding sites were visualized by Discovery Studio Visualizer version 4.0. The interactions between the ligands and target protein were represented as follows

- Hydrogen bonding interactions
- Electrostatic and other interactions
- Hydrophobic interactions

5.2.1.2. Result and discussions:

Biological activity:

The growth percentages of the cells were represented in Table 5.1**.** Among 10 compounds **1a – 1j**, all the compounds except **1b** have less or no effect on the growth percentage of the cells. Dose response curves have been shown figure 5.3 and 5.4. Whereas the compound **1b** has significant effect on growth of 17 cell lines with at least two cell lines from each set. A negative value in the growth percentage table indicates the stimulation or cell proliferation by the compound.

Table 5.1: Growth percentages of all cell lines.

Panell/Cell Line	Growth Percent									
	4a	**4b**	**4c**	**4e**	**4g**	**4h**	**4i**	**4j**	**4k**	**4l**
Leukemia										
CCRF-CEM	79.73	2.63	74.4	99.14	96.99	104.01	101.41	94.77	101.04	99.65
HL-60(TB)	92.77	-32.59	75.78	66.58	84.47	88.59	72.16	90.75	53.62	94.77
K-562	106.77	-4.98	84.59	87.87			87.49		84.23	
MOLT-4	83.33	-35.3	77.15	83.44	91.74	102.46	84.51	91.41	94.29	94.01
RPMI-8226	97.74	-25.94	98.27	99.74	105.94	100.81	102.01	98.17	96.03	106.2
SR	88.38	6.76	72.73	81.33	93	92.47	88.82	93.19	88.01	85.61
Non-Small Cell Lung Cancer										
A549/ATCC	91.22	-21.51	53.76	65.64	75.69	84.48	76.07	81.05	73.77	80.72
EKVX	90.25	7.59	83.82	94.78	108.92	93.69	83.08	94.76	87.99	110.53
HOP-62	100.7	-38.52	78.66	69.48	106.67	99.43	73.34	97.4	89.21	94.44
HOP-92	160.31	2.31	106.19	112.06	105.64	96.42	109.7	96.85	128.08	99.99
NCI-H226	111.5	-30.54	80.69	95.74	110.79	107.69	99.18	110.05	95.79	107.2
NCI-H23	96.03	-28.54	81.97	76.89	94.37	95.09	82.24	93.97	77.46	96.79
NCI-H322M	104.43	5.37	101.55	103.53	95.69	98.15	104.74	100.19	98.1	89.98
NCI-H460	108.43	-10.48	87.46	99.76	97.89	104.33	99.54	103.19	94.32	103.84
NCI-H522	72.37	-35.62	64.42	62.72	68.74	82.43	47.61	89.7	57.9	62.63
ColonCancer										
COLO 205	115.9	-81.08	111.37	106.25	113.2	113.45	114.25	116.04	106.35	108.53
HCC-2998	96.77	4.8	85.01	91.52	103.55	102.02	100.7	101.63	91.6	106.79
HCT-116	98	-52.78	67.99	103.13	89.97	98.02	88.74	87.44	76.04	91.26
HCT-15	99.29	-53.13	84.25	106.6	111.24	105.67	88.55	105.75	89.18	110.3
HT 29	70.6	-29.02	45.36	69.8	76.52	91.89	62.73	106.74	63.63	77.4
KM 12	105.41	-50.98	101.81	102.03	104.64	103.44	103.54	102.58	95.39	108.66
SW-620	110.94	-51.25	96.2	103.5	99.86	101.48	102.44	99.28	102.78	102.58

Panell/Cell Line	Growth Percent									
	4a	**4b**	**4c**	**4e**	**4g**	**4h**	**4i**	**4j**	**4k**	**4l**
CNS Cancer										
SF-268	108.62	4.27	88.47	91.37	97.83	102.56	91.52	109.37	84.25	100.9
SF-295	95.82	-24.35	70.45	94.67	108.53	111.63	78.77	99.5	77.65	115.4
SF-539	99.87	-33.12	86.95	94.19	96.15	103.59	89.4	104.99	70.66	102.81
SNB-19	109.9	5.62	75.55	92.51	90.01	100.03	88.24	94.03	74.69	98.17
SNB-75	95.12	29.65	76.87	75.16	91.62	98.24	78.56	97.83	70.86	109.84
U251	105.5	-35.75	76.59	67.92	97.57	84.86	78.53	94.37	74.92	103.38
Melanoma										
LOX IMVI	100.72	-35.97	83.45	95.8	109.41	104.09	96.82	106.72	96.3	102.98
MALME-3M	109.38	-22.51	99.38	102.56	100.27	110.53	91.79	109.86	110.1	105.3
M14	104.63	-19.04	90.39	103.44	102.12		96.17		99.41	
MDA-MB-435	102.08	-32.8	98.51	107.98	103.41	101.44	105.14	106.97	104.04	112.41
SK-MEL-2	99.99	-28.45	85.5	89.86	91.39	104.64	78.38	106.53	86.69	86.34
SK-MEL-28	101.18	-22.88	87.447	108.55	106.47	111.13	99.9	111.06	99.61	104.69
SK-MEL-5	105.89	-38.59	99.07	102.98	105.61	101.83	99.01	103.25	97.68	105.18
UACC-257	88.27	-3.6	65.46	71.2	77.86	90.99	75.46	87.6	73.67	80.73
UACC-62	101.44	-36.3	81.55	91.17	98.69	96.84	91.42	97.4	88.93	98.22
Ovarian Cancer										
IGROV1	109.25	-17.22	9.41	104.95	103.21	109.55	99.86	106.03	96.51	101.98
OVCAR-3	113.11	-63.52	100.86	96.23	114.34	112.47	111.12	116.31	107.28	106.77
OVCAR-4	105.52	-7.88	102.12	121.83	114.12	114.72	103.84	120.22	74.08	112.84
OVCAR-5	104.53	10.56	93.1	116.75	105.37	109.31	99.39	108.4	97.25	107.82
OVCAR-8	101.47	-17.08	67.35	67.82	99.53	102.49	66.96	99.88	81.78	98.29
NCI/ADR-RES	103.71	-12.42	83.34	95.45	109.44	106.35	100.94	107.68	92.42	104.92
SK-OV-3	89.85	-30.78	77.84	70.77	102.46	93.54	62.26	95.19	87.53	95.11

Panell/Cell Line	Growth Percent									
	4a	**4b**	**4c**	**4e**	**4g**	**4h**	**4i**	**4j**	**4k**	**4l**
Renal Cancer										
786-0	100.13	6.51	67.05	78.91	98.12	100.35	75.48	95.91	76.97	100.91
A498	117.04	-6.11	104.18	92.63	92.12	105.91	99	101.72	90.79	108.31
ACHN	102.71	-34.28	88.61	106.67	105.75	110.14	105.14	112.2	104.03	107.77
RXF 393	121.22	-35.57	97.74	96.41	108.08	105.61	75.06	107.28	81.04	106.97
SN 12C	110.91	4.01	95.55	108.65	102.13	99.79	104.54	102.69	103.2	103.87
TK-10	114.64	-31.28	69.09	83.21	92.48	108.78	75.49	102.61	83.25	102.17
UO-31	98.25	-65	87.54	109.38	95.65	100.11	98.69	92.76	106.47	94.08
Prostate Cancer										
PC-3	101.21	-67.26	87.54	98.33	102.1	101.83	101.96	99.4	102.87	100.37
DU-145	112.38	4.25	107.21	101.79	105.36	103.8	107.77	112.59	95.12	102.03
Breast Cancer										
MCF7	89.38	-11.53	85.86	93.97	93.55	97.84	84.77	96.78	85.58	106.12
MDA-MB-231/ATCC	97.25	1.96	97.88	99.57	101.36	96.69	94.69	92.27	88.44	93.85
HS 578T	112.01	24.12	81.88	84.32	96.5	93.88	88.42	103.23	80.47	101.16
BT-549	104.09	-10.4	74.74	98.33	106.58	107.11	89.92	108.61	78.23	108.99
T-47D	90.09	-36.83	74.65	89.35	96.11	94.76	67.69	95.11	68.78	103.14
MDA-MB-468	126.57	-23.26	103.02	106.5	114.56	109.23	98.47	111.69	84.08	115.25

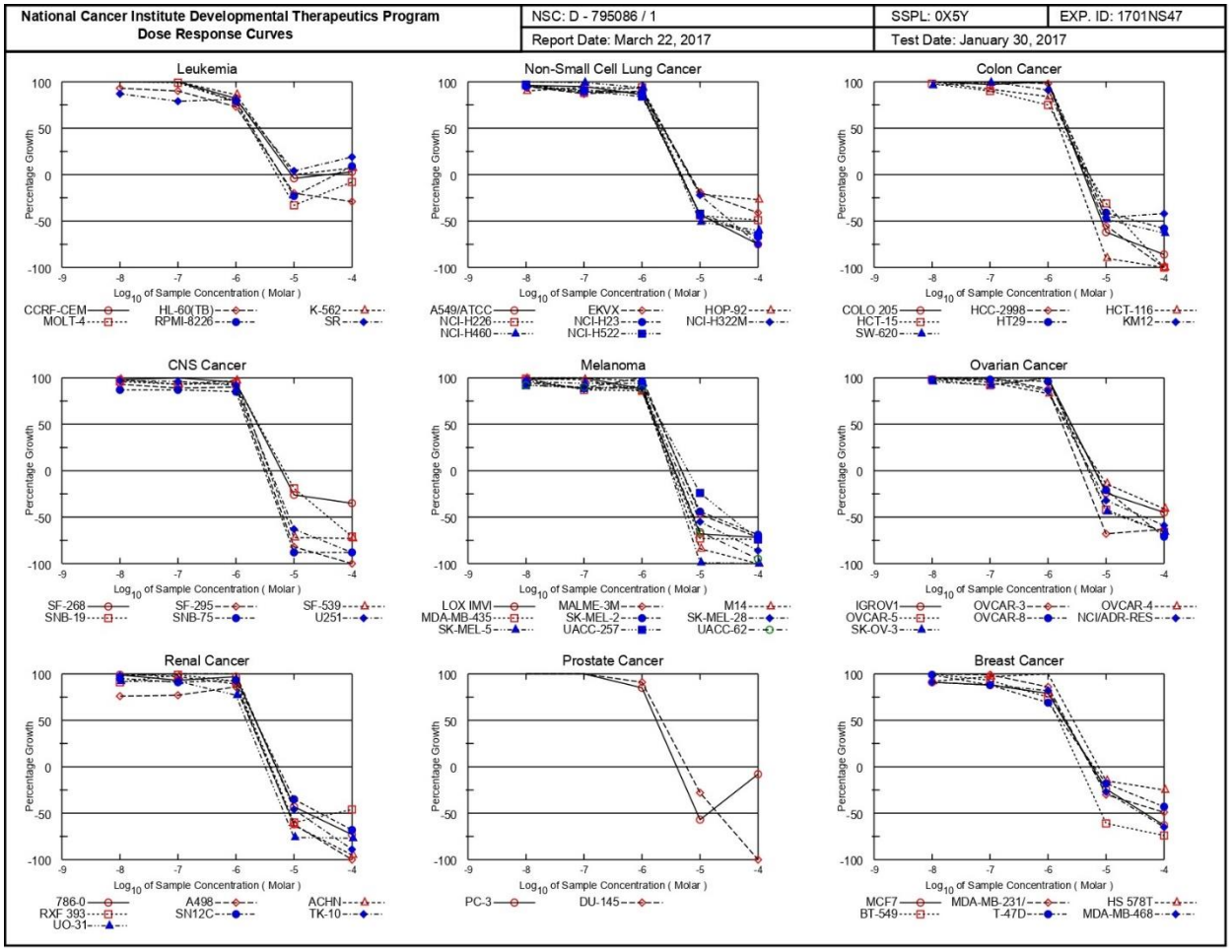


Figure 5.3: Developmental therapeutics program dose response individual cancer curves of compound **1b**.

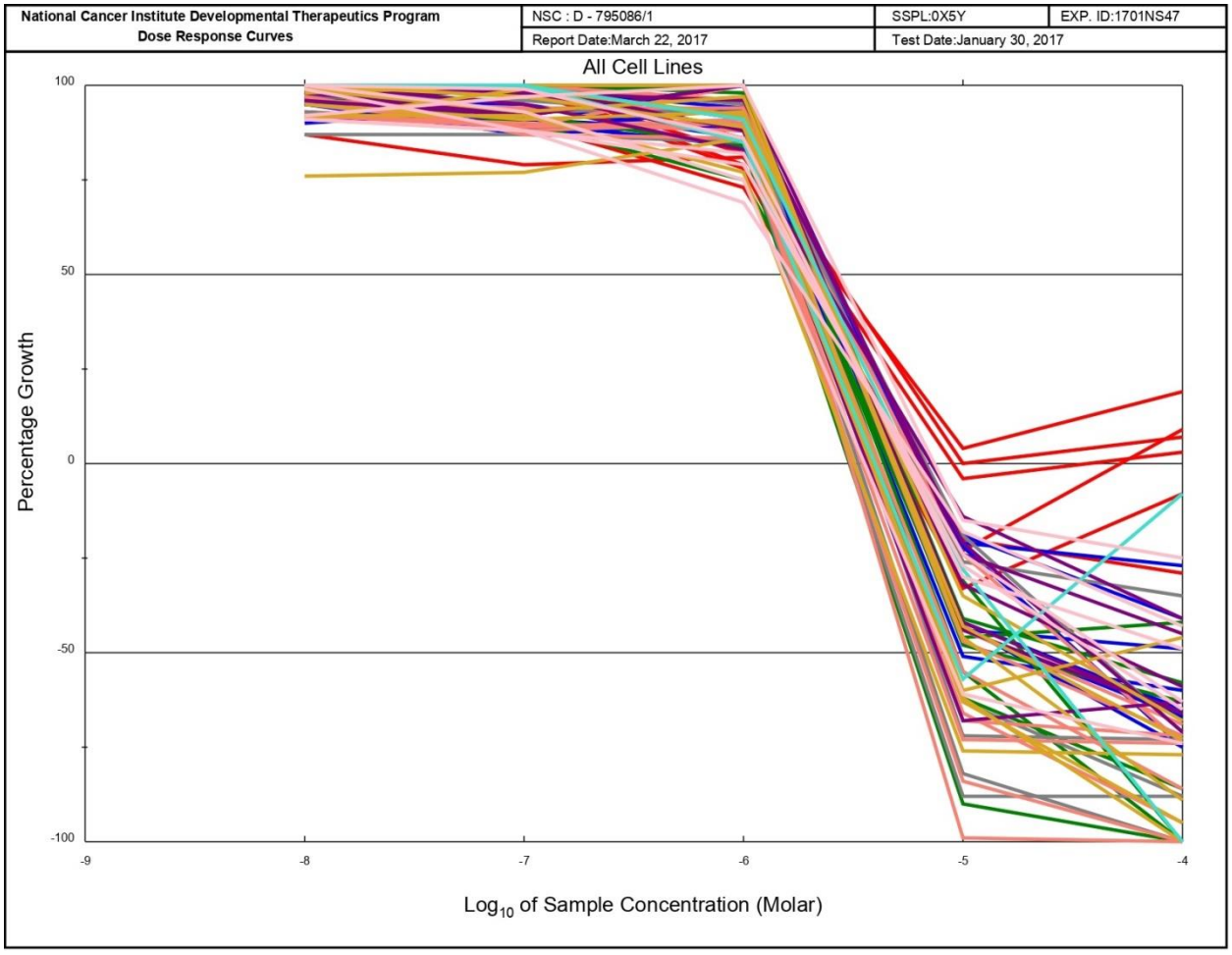


Figure 5.4: Developmental therapeutics program for all cell lines dose response curves of compound **1b**.

The IC50 values of **1b** compound alone were evaluated as the remaining compounds exhibited unsubstantial toxic effects on the cells. Compounds with negative values have less effect on cell death and were omitted. The IC50 values generated from MTT assay were tabulated in table 5.2. To our observation, the compound **1b** exerted highest cytotoxicity against SK-MEL-5 (Melanoma cell line). Amongst all the cell line sets it has to be noted that the compound has no effect on leukemia cell lines with IC50 values >100 μM. Amongst non-small cell lung cancer set, IC50 values were in the range of 9 – 34 μM, Out of these non-small cell lung cancer cell lines good activity against NCI-H460 and moderate activity against A549/ATCC, NCI-H23 and NCI-H522 cell lines. In the case of colon cancer, IC50 values were in the range of 5 - ~34 μM with highest effect against HCT-116 and moderate activity observed against COLO 205, HCC-2998, SW-620 cell lines. In the set of CNS cancer cell lines, the compound **1b** exhibited good activity against SNB-75, SF-295, 539 and U251 whereas moderate activity was observed on SNB-19. In melanoma cluster of cell lines, the IC50 values fell in the range of 5.58-17.6 μM.

highest cytotoxic effect was observed against SK-MEL-5 and moderate to lowest activity was noticed against MDA-MB-435, M14, LOX IMVI, SK-MEL-2, 5 MALME-3M, UACC-62 and UACC-257 respectively. The IC50 values of ovarian cancer cell lines observed in the range of 18- 45 μM. Highest cytotoxicity was exhibited against OVCAR-3. Least cytotoxicity was observed on IGROVI, OVCAR-4 and the compound **1b** has moderate effect on all the remaining cell lines in the group. Range of cytotoxicity on renal cancer cell lines exerted by compound **1b** was in the limit of 6-28 μM. Moderate inhibition of cell growth was observed in the case of prostate cancer cell line DU-145 and no such effect on PC-3 has been noticed. The IC50 values of breast cancer cell lines observed in the range of 8.37- >100 μM. Highest cytotoxicity was exhibited against BT-549. Least cytotoxicity was observed on T-47D, HS 578T.

Table 5.2: IC50 values (in μM) of cancer cell lines.

Leukemia (μM)					
CCRF-CEM	**HL-60(TB)**	**K-562**	**MOLT-4**	**RPMI-8226**	**SR**
>100	>100	>100	>100	>100	>100

Non-Small Cell Lung Cancer (μM)							
A549/ATCC	**EKVX**	**HOP-92**	**NCI-H226**	**NCI-H23**	**NCI-H322M**	**NCI-H460**	**NCI-H522**
16.3	>100	>100	>100	17.9	33.9	9.79	22.8

Colon Cancer (μM)						
COLO 205	**HCC-2998**	**HCT-116**	**HCT-15**	**HT29**	**KM12**	**SW-620**
8.43	9.30	5.89	18.9	34.1	>100	14.2

CNS Cancer (μM)					
SF-268	**SF-295**	**SF-539**	**SNB-19**	**SNB-75**	**U251**
>100	6.48	7.38	39.6	6	14.2

Melanoma (μM)								
LOXIMVI	**MALME-3M**	**M14**	**MDA-MB-435**	**SK-MEL-2**	**SK-MEL-28**	**SK-MEL-5**	**UACC-257**	**UACC-62**
7.68	12.8	6.31	7.17	17.6	9.17	5.58	33.2	7.88

Ovarian Cancer (μM)						
IGROV1	**OVCAR-3**	**OVCAR-4**	**OVCAR-5**	**OVCAR-8**	**NCI/ADR-RES**	**SK-OV-3**
>100	7.7	>100	21.8	37.8	45.1	18.2

Renal Cancer (μM)						
786-0	**A498**	**ACHN**	**RXF 393**	**SN 12C**	**TK-10**	**UO-31**
17.3	8.33	8.21	>100	28.7	12.3	6.79

Prostate Cancer (μM)	
PC-3	**DU-145**
>100	20.3

Breast Cancer (μM)					
MCF7	**MDA-MB-231/ATCC**	**HS 578T**	**BT-549**	**T-47D**	**MDA-MB-468**
40.65	50	>100	8.37	>100	41.2

Pure compound cytotoxicity criteria indicate the compounds with IC50 values < 4μg/ml or 10 μM were considered to be highly toxic to the cells. As per these criteria the compound can be considered as effective against NCI-H460, HCC-2998, HCT-116, SF 295, 539, SNB-75, LOX IMVI, M14, MDA-MB 435, SK-MEL 5, 28, OVCAR-3, UACC-62, A498, ACHN, UO-31 and BT-549. The cell lines with IC50 values lesser than 10 μM were tabulated in table 5.3. Amongst 10 compounds synthesized, **1b** exhibited substantial yet remarkable cytotoxicity. This compound can be a promising novel chemotherapeutic agent as it has the capability to act against many carcinogenic complications.

Table 5.3: IC50 values < 10 μM of the compound **1b.**

Cell Line	**IC50 value**
NCI-H460	9.79
HCC-2998	9.3
HCT-116	5.89
SF-295	6.48
SF-539	7.38
SNB-75	6
LOX IMVI	7.68
M14	6.31
MDA-MB-435	7.17
SK-MEL-28	9.17
SK-MEL-5	5.58
UACC-62	7.88
OVCAR-3	7.7
A498	8.33
ACHN	8.21
UO-31	6.79
BT-549	8.37

Molecular docking studies:

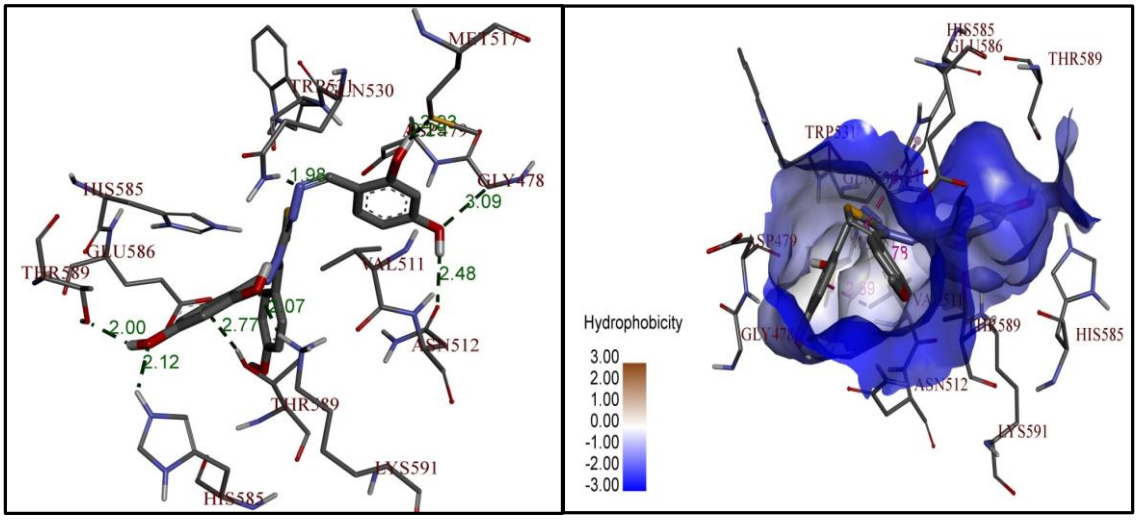
In human cancers, B-RAF is the most frequently mutated protein kinase. The oncogenic mutations of BARF are common in melanoma, which are dependent on the RAF/MEK/ERK pathway. Hence, the inhibition of B-RAF kinase activity will reduce the melanoma cancer[80]. Cephalosporin acylase mutants (PDB ID: 2AE4) are most common in human cancers. Cephalosporin acylase pertained to N-terminal nucleophile hydrolase family. The sequential primary and secondary autoproteolytic reactions activated these mutants and release the pro segment[81]. Microtubles, the cytoskeletal polymers of tublin, were involved in many cellular functions. The dynamic stability of the tublulins is controlled by various compounds. Hence, Tubulin Stathmin-Like Domain Complex (PDB: 1SA0) is an attractive target for to found the potential of the new drug like compounds[82]. The EGFR (epidermal growth factor receptor) kinase (PDB ID: 1M17) is highly expressed in colon, breast cancer, small lung cancer cells and renal[83]. The lactate production levels were elevated increased glucolytic activity, which was increased the increased glucose uptake in tumor cells. The tumor cells contained the lactate dehydrogenase-5 (LDH-5) that regulated the lactate release. LDH-5 plays eminent in tumor conservation in most human cancers. Hence, LDH-5 (PDB ID: 1T2F) is the best significant target for the compounds with improved anticancer activity[84]. Creatine kinase (CK) enzyme (PDB ID: 1QH4) exists in tissues and represents the family of guanidine kinases. The muscle and brain cells/tissues that are excitable, exhibited the highly fluctuating consumption of ATP. This ATP is regenerated from phosphocreatine by CK enzyme. CK is overexpressed in a several solid tumors and also causes many age related and neurodegenerative diseases[85]. Hence, all these aforementioned proteins are the one of the best attractive targets for the several compounds.

The molecular docking results showed potent activity against all the tested proteins. Among all of them, the compound **1b** exhibited more potential towards the proteins 2AE4, 1M17 and 1QH4 with binding energies -9.83, -9.26 and -9.54 kcal/mol respectively. The docking results were shown in table 5.4. The interactions between the target compound **1b** with tested protein were explained as follows.

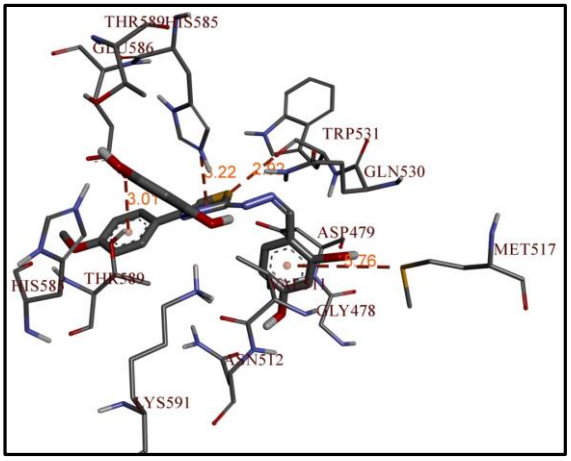
Table 5.4: Molecular docking results of the compound **1b** with the various target proteins.

PDB ID	Molecular docking results of the compound 5b with the various target proteins 3OG7, 2AE4, 1SA0, 1M17, 1T2F and 1QH4			
	Binding energy (kcal)	**No. of hydrogen bonds**	**Amino acid residues involved in the hydrogen bonding**	**Bond lengths of hydrogen bonds (A°)**
3OG7	-8.73	9	GLN530, THR589, ASP497, ASN512, MET517, LYS591, HIS585, GLY478, THR589	1.98, 2.00, 2.07, 2.12 2.29, 2.48, 2.77, 2.93, 3.09
2AE4	-9.83	6	HIS231, ASN327, ASN329, PRO220	2.66 , 1.92, 2.10, 1.74, 3.04, 2.77
1SA0	-7.92	6	VAL153, ASN258, PHE351, GLU71, GLU254	1.72, 1.73, 1.86, 2.43, 3.16, 3.16
1M17	-9.26	5	THR766, ASP831, LYS721	3.02, 1.87, 2.30, 2.54, 2.70
1T2F	-8.02	3	GLN73, GLY41, SER38	1.90, 2.11, 3.24
1QH4	-9.54	5	GLU232, CYS283, GLY73, SER199	1.93, 2.16, 2.47, 3.40, 3.58

The target compound **1b** exhibited 9 hydrogen bonds with the protein 3OG7. One hydrogen bond was formed in between –N- atom of the hydrazone with the amino acid residue GLN530 (1.98 A°). Four hydrogen bonds were observed in between the hydrogen atoms of hydroxy groups of resorcinol ring with the amino acid residues THR589, ASP497, ASN512, and MET517 with bond lengths 2.00, 2.29, 2.48 and 2.93 A° respectively. The three oxygen atoms of the two resorcinol groups exhibited three hydrogen bonds with the amino acid residues LYS591, HIS585 and GLY478 with bond lengths 2.07, 2.12 and 3.09 A° respectively. One hydrogen bond was observed in between the resorcinol ring and the amino acid residue THR589 (2.77 A°). The thiazole ring and the resorcinol ring interact with the amino acid residues VAL511 and HIS585 through hydrophobic interactions. There exist two electrostatic interactions between the thiazole ring with amino acid residue HIS585 (pi-cation type) and resorcinol ring with amino acid residue GLU586 (pi-anion type). There are some other interactions i.e., sulphur-X type interaction between the sulphur atom of the thiazole ring with amino acid residue TPR531 and pi-sulphur interaction between the resorcinol ring with the amino acid residue MET517. The hydrogen bonding interactions, hydrophobic interactions, electrostatic and other interactions were shown in figure 5.5.



a) Hydrogen bonding interactions b) Hydrophobic interactions.

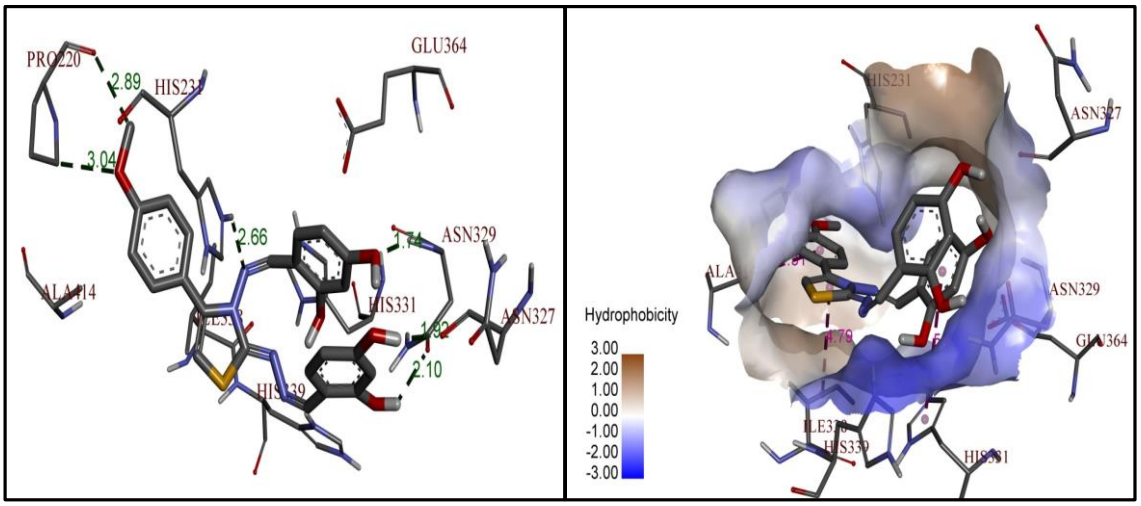


c) Electrostatic and other interactions.

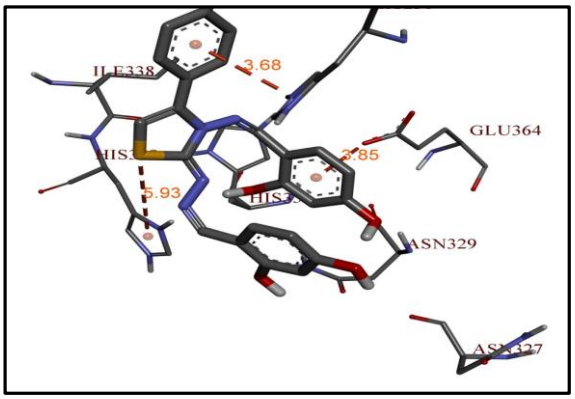
Figure 5.5: The best docking poses of the compound **1b** with the protein 3OG7.

The compound **1b** was exhibited six hydrogen bonds with the protein 2AE4. One hydrogen bond was formed in between –N- atom of the hydrazone with the amino acid residue HIS231 (2.66 A°). Two hydrogen bonds were observed in between the hydrogen atoms of hydroxy groups of resorcinol ring with the amino acid residues ASN327 and ASN329 with bond lengths 1.92 and 2.10 A° respectively. The one oxygen atoms of the resorcinol ring exhibited one hydrogen bond with the amino acid residue ASN329 (1.74 A°). Two hydrogen bonds were observed in between the oxygen and hydrogen atoms of the methoxy group and the amino acid residue PRO220 with bond lengths 3.04 and 2.77 A° respectively. The resorcinol ring interacts with the amino acid residue HIS331 (Pi-stacked), the thiazole ring interacts with the amino acid residue ILE338 (pi-alkyl) and the phenyl ring interact with the amino acid residues ALA414 (pi-alkyl) through hydrophobic interactions. There exist two electrostatic interactions i.e., in between the thiazole ring with amino acid residue HIS231 (pi-cation type) and resorcinol ring

with amino acid residue GLU364 (pi-anion type). There was a pi-sulphur type interaction between the sulphur atom of the thiazole ring with amino acid residue HIS331. The hydrogen bonding interactions, hydrophobic interactions, electrostatic and other interactions were shown in figure 5.6.



a) Hydrogen bonding interactions b) Hydrophobic interactions.

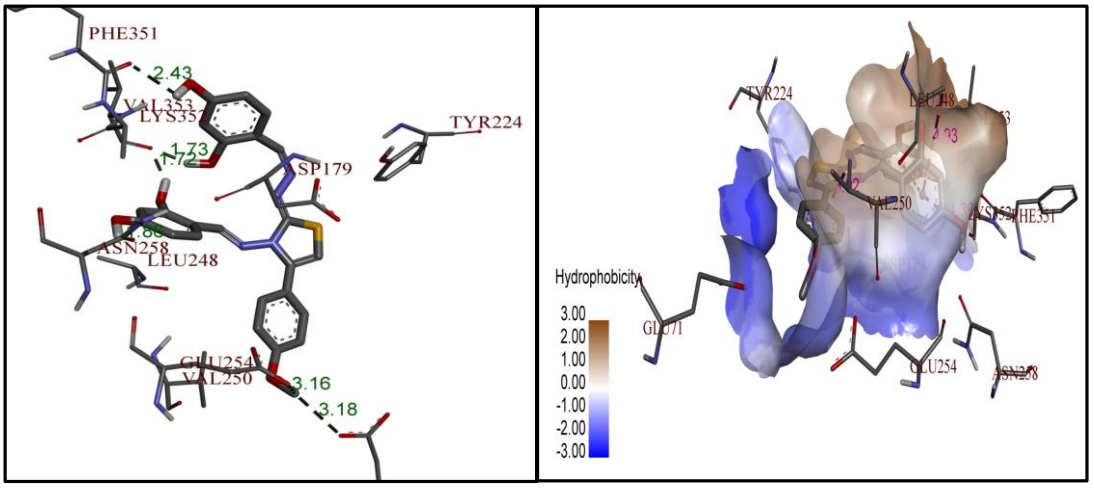


c) Electrostatic and other interactions.

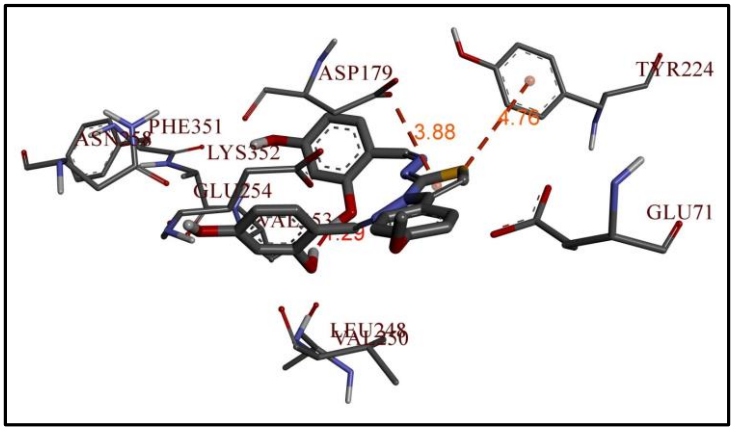
Figure 5.6: The best docking poses of the compound **1b** with the protein 2AE4.

The target compound **1b** exhibited six hydrogen bonds with the protein 1SA0. Four hydrogen bonds were formed in between four –H atoms of the hydroxy groups of resorcinol ring with the amino acid residuesVAL153, ASN258, and PHE351 with bond lengths 1.72, 1.73, 1.86, 2.43 A° respectively. Two hydrogen bonds were observed in between the hydrogen atoms of methoxy group of phenyl ring with the amino acid residues GLU71 and GLU254 with bond length 3.16 A°. The resorcinol ring interacts with the amino acid residues LEU248 (Pi-alkyl) and LYS352 (pi-alkyl), the phenyl ring interacts with the amino acid residue VAL250 (pi-sigma) and through hydrophobic interactions. There was an electrostatic interaction between the thiazole ring with amino acid residue ASP179 (pi-anion type). There was a pi-sulphur type interaction between the sulphur atom of the thiazole ring with amino acid residue TYR224. The hydrogen bonding

interactions, hydrophobic interactions, electrostatic and other interactions were shown in figure 5.7.



a) Hydrogen bonding interactions b) Hydrophobic interactions.

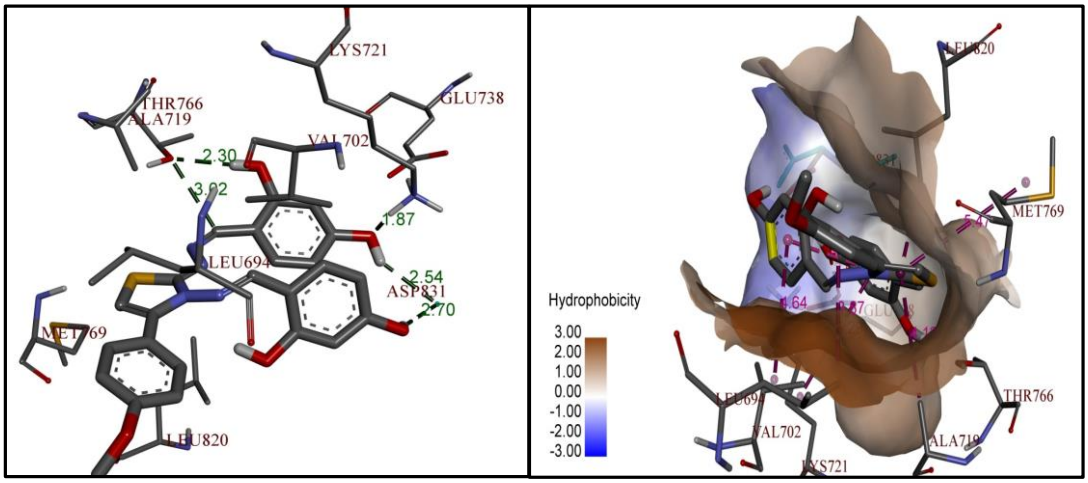


c) Electrostatic and other interactions.

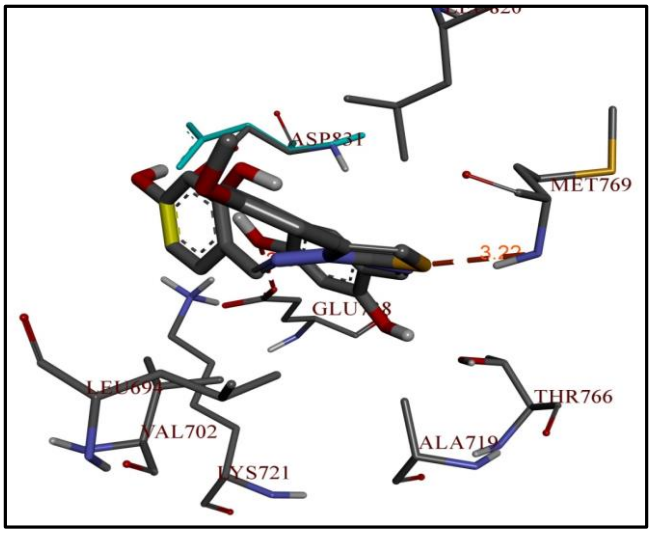
Figure 5.7: The best docking poses of the compound **1b** with the protein 1SA0.

The target compound **1b** exhibited 5 hydrogen bonds with the protein 1M17. Three hydrogen bonds were formed in between –3- atoms of the resorcinol ring with the amino acid residues THR766 and ASP831 with bond lengths 2.30, 2.54 and 2.70 A° respectively. One hydrogen bond was observed in between the oxygen atom of hydroxy group of resorcinol ring with the amino acid residues LYS721 (1.87 A°). The hydrogen atom of the carbon in imine group exhibited one hydrogen bond with the amino acid residue THR766 (3.02 A°). The thiazole ring interact with the amino acid residues LEU820, MET769 and ALA719, the two resorcinol rings interact with the amino acid residues VAL720 and LYS721 separately and the phenyl ring interact with the amino acid residue LEU694 through hydrophobic interactions. There was sulphur-X type interaction between the sulphur atom of the thiazole ring with amino acid residue MET769. The hydrogen bonding interactions, hydrophobic interactions, electrostatic and other

interactions were shown in figure 5.8.



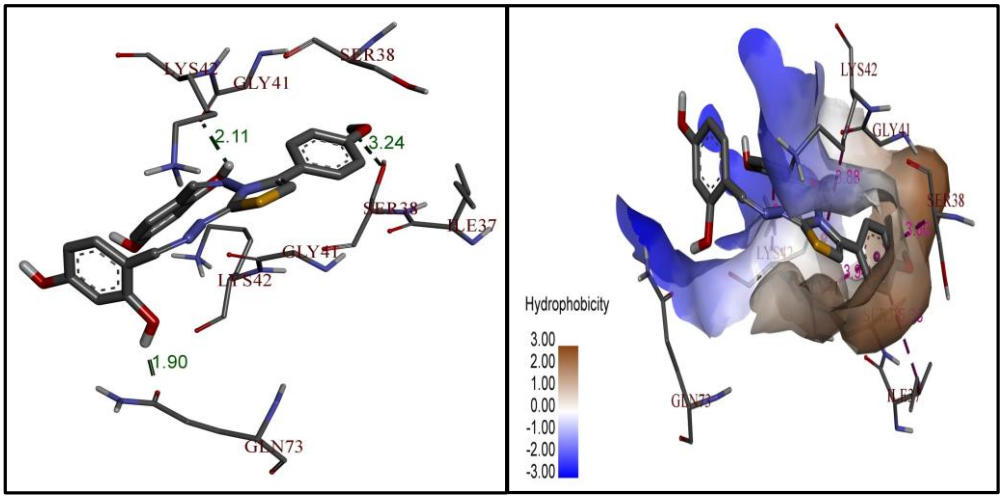
a) Hydrogen bonding interactions b) Hydrophobic interactions.



c) Electrostatic and other interactions.

Figure 5.8: The best docking poses of the compound **1b** with the protein 1M17.

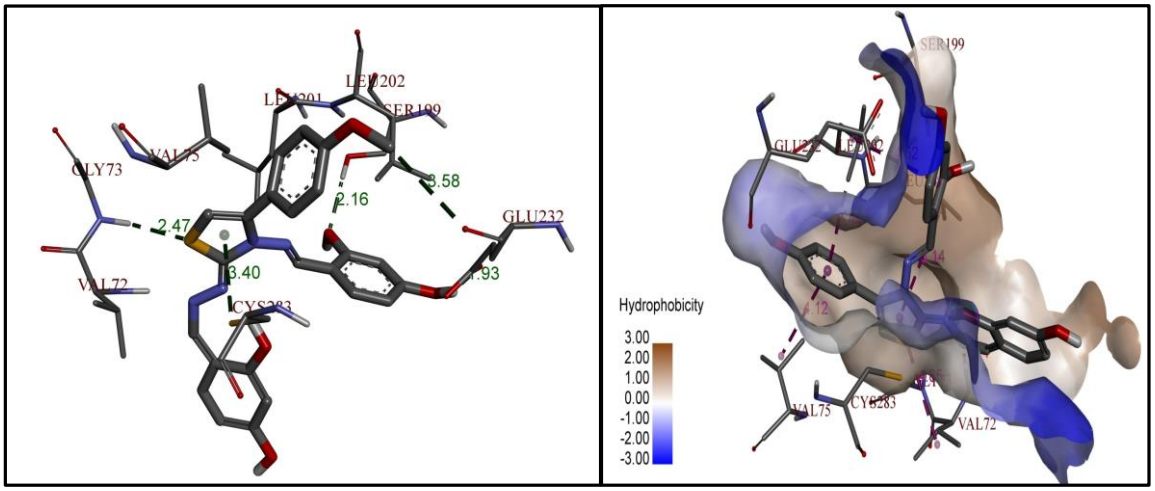
The target compound **1b** exhibited three hydrogen bonds with the protein 1T2F. Two hydrogen bonds were formed in between –H- atom of the hydroxy groups of each resorcinol ring with the amino acid residues GLN73 and GLY41 with bond lengths 1.90 and 2.11 A° respectively. One hydrogen bond was observed in between the hydrogen atom of methoxy groups of phenyl ring with the amino acid residue SER38 (3.24 A°). The two resorcinol rings interact with the amino acid residues SER38 (pi-sigma), GLY41 (pi-sigma), ILE37 (pi-alkyl) and LYS42 (pi-sigma) and the thiazole ring interacts with the amino acid residue LYS42 (pi-alkyl) through hydrophobic interactions. There were no electrostatic and other interactions exist in between the compound and protein. The hydrogen bonding interactions and hydrophobic interactions were shown in figure 5.9.



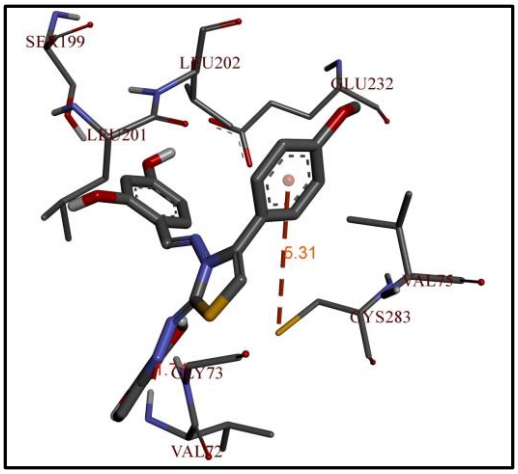
a) Hydrogen bonding interactions b) Hydrophobic interactions.

Figure 5.9: The best docking poses of the compound **1b** with the protein 1T2F.

The target compound **1b** exhibited five hydrogen bonds with the protein 1QH4. One hydrogen bond was formed in between –H- atom of the resorcinol ring with the amino acid residue GLU232 (1.93 A°). Two hydrogen bonds were observed in between the thiazole ring with the amino acid residues GLY73 and CYS283 with bond lengths 2.47 and 3.40 A° respectively. The oxygen atoms of the resorcinol ring exhibited one hydrogen bond with the amino acid residue SER199 (2.16 A°). One hydrogen bond was observed in between the hydrogen of the methoxy group and the amino acid residue GLU232 (3.58 A°). The resorcinol ring interacts with the amino acid residue LEU202 (pi-alkyl), the thiazole ring interact with the amino acid residues VAL72 (pi-alkyl) and LEU201 (pi-alkyl) and the phenyl ring interact with the amino acid residues LEU202 (pi-sigma) and VAL75 (pi-alkyl) through hydrophobic interactions. There was a pi-sulphur type interaction in between the phenyl ring with amino acid residue CYS283. The hydrogen bonding interactions, hydrophobic interactions, electrostatic and other interactions were shown in figure 5.10.



a) Hydrogen bonding interactions b) Hydrophobic interactions.



c) Electrostatic and other interactions.

Figure 5.10: The best docking poses of the compound **1b** with the protein 1QH4.

The docking analysis revealed that compound **1b** which displayed good activity showed good dock score of -6.195 kcal/mol among newly synthesized thiazole derivatives. It showed three hydrogen bond interactions with Ile 17, Thr 19 and Tyr 88 (Figure 5.11). The OH groups in compound **1b** were responsible for the formation of three hydrogen bonds. These compounds showed hydrogen bond interactions with Lys 40 and Asp 150, salt bridges with Lys 40 and π-cationic interactions with Lys 134 apart from interactions shown by compound **1b**.

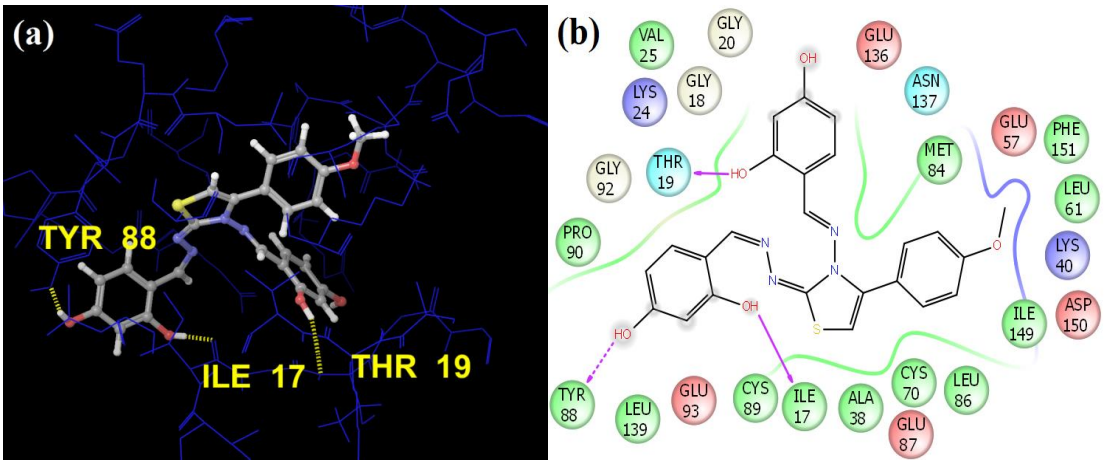


Figure 5.11: Dock pose (a) and Ligand interaction (b) diagram of compound **1b** showing hydrogen bond interactions with Ile 17, Thr 19 and Tyr 88

Structure activity relationship:

In order to explore and understand the structure activity relationship (SAR) of new thiazole derivatives, the synthesized compounds were screened against cancer cell lines. The new thiazole derivatives were synthesized by incorporating various electron donating groups (methoxy and hydroxyl) and electron withdrawing groups (Fluorine, Chlorine, Bromine and Nitro) at R, R_1 and R_2 positions. Among the newly synthesized compounds, compound 4b which is having hydroxyl group at R and R_1 positions and methoxy group at R_2 position displayed potent activity against all cancer cell lines (Table 5.5). Compounds **1h**, **1j** and **1l** possessing bromine at 'R' position showed comparatively low activity against all cancer cell lines when compared with remaining compounds in the series. It indicates that presence of bulky electron withdrawing groups at 'R' position may not be beneficial for activity instead small electron withdrawing groups may favour the activity. Compounds which possessed electron withdrawing groups at R, R_1 and R_2 positions could not show any significant effect on activity. Therefore, it was evident from the anti-cancer data that electron donating groups at R, R_1 and R_2 positions will be beneficial for activity.

All the compounds were screened for their *in vitro* cancer activity and the results revealed that the compound **1b** is the lead compound. **1b** exhibited potent activity against CNS, Ovarian, Colon, Renal, Breast, Non-Small Cell Lung and Melanoma cancer. The molecular docking studies were also well correlate with these results. The molecular docking results showed that the compound **1b** exhibited least binding energies -9.83, -9.26 and -9.54 kcal/mol with the proteins 2AE4, 1M17 and 1QH4 respectively. Moreover, it can be judged from molecular docking studies that the presence of –OH groups and the thiazole ring in **1b** can be favorable for anti-cancer activity. Furthermore, *in vivo* studies for this compound **1b** have to be carried out.

5.2.2. Antibacterial activity of coumarin based thiazoles:

In vitro antibacterial activity of newly synthesized coumarin based thiazole compounds were evaluated against human pathogen of Gram positive organism *S. aureus.*

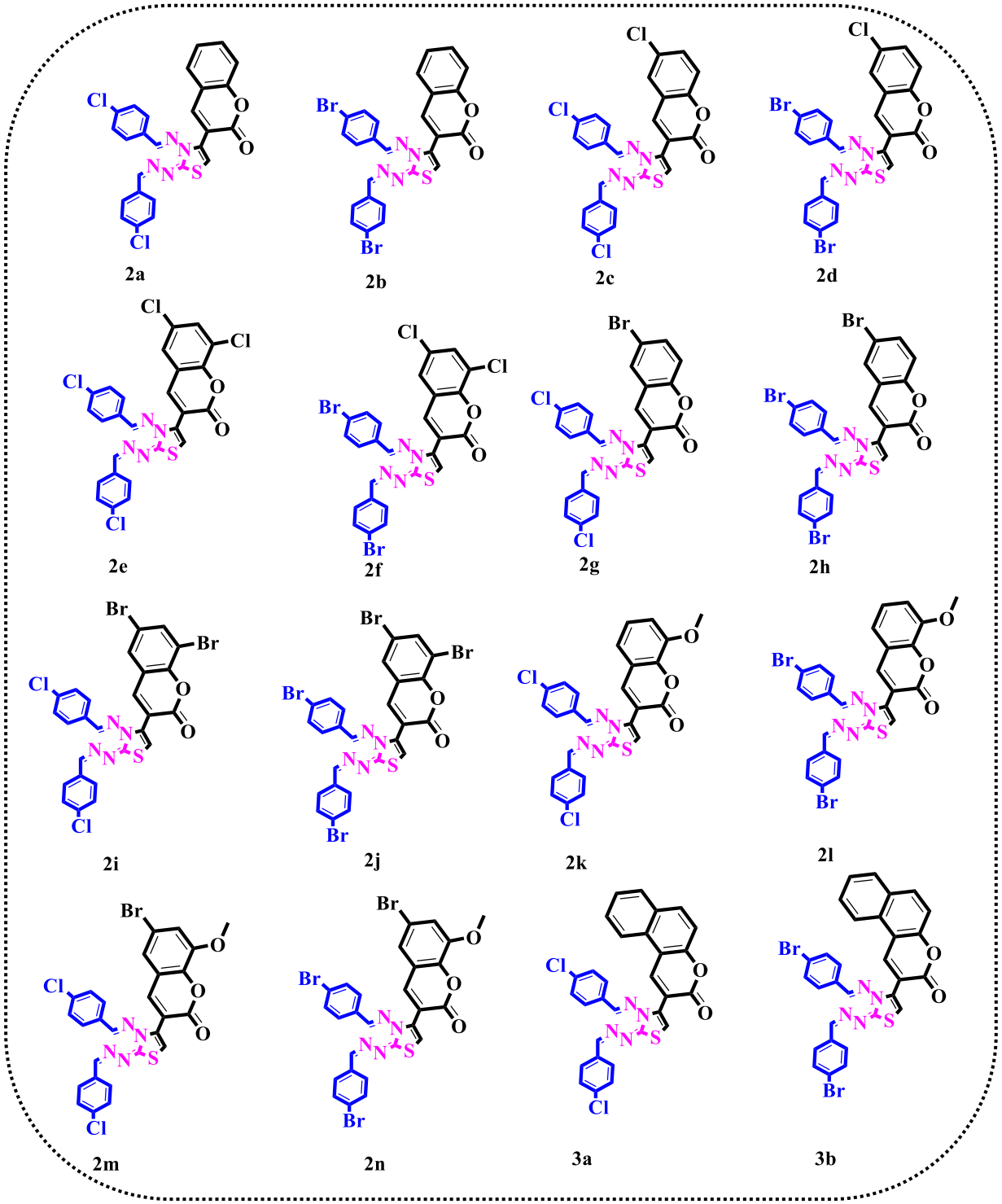


Figure 5.12: coumarin based thiazole hybrids (**2a-n; 3a-b**).

5.2.2.1. Experimental:

Biological activity:

In vitro antibacterial activity was evaluated against human pathogen of Gram positive organism

S. aureus by micro dilution method. The microorganism was grown in Muller-Hinton broth after cultivation for 16–18 h at 37 °C, a bacterium was harvested and their density was determined by measuring OD at A_{600}. MIC of the compounds was determined by agar dilution method. Suspension of microorganism was prepared to contain approximately (1×10^4- 2×10^4 CFU/mL) and applied to the plates with serially diluted compounds (dissolved in DMSO) to be tested and the reference drug (Novobiocin) and incubated at 37 °C overnight. Minimum inhibitory concentration was considered to be the lowest concentration that completely inhibited the growth of microorganism on the plates. After 24 h and MIC values were determined.

Molecular docking protocol:

Docking of coumarin based thiazoles was performed using GLIDE 5.6 of schrodinger suite 2010 against the catalytic domain of topoisomerase IV enzyme in complex with novobiocin of *S. aureus*. The synthesized molecules were built using maestro build panel and geometrically refined using LigPrep module of the schrodinger. All possible states of these derivatives were generated at a pH range of 7 ± 2. The low energy conformers were then retained for docking. The protein structure which was downloaded from protein data bank (PDB id: 4URN) was prepared by protein preparation wizard. The crystallographic water molecules were removed, hydrogen's and missing side chains were added to the protein. Bond orders were assigned to the protein and subjected to optimization. Finally, it was minimized to a root mean square deviation (RMSD) value of 0.30, a default in the protein minimization wizard by applying OPLS 2005 force field. Receptor grid was then generated by separating the co-crystallized ligand from the active site of the protein. During the grid generation the vanderwaals radii was fixed to 0.9 Å (5) while the partial atomic charge was set to 0.25. Finally, following the default Glide settings docking were carried out using extra precision mode of Glide module. The output of docking studies i.e. dock scores and interactions of each ligand with the receptor was intercepted from the pose viewer file.

5.2.2.2. Result and discussions:

Biological activity:

The *in vitro* antibacterial activities of the synthesized coumarin based thiazoles were carried out against a Gram positive bacterium *Staphylococcus aureus* (ATCC-12600). The MICs of standard (Novobiocin) drug, tested compounds(**2a-n** and **3a-b**) were measured using a well-defined and standardized broth micro-dilution technique[86].The minimum concentration of anti-bacterial agents needed to inhibit the bacterial growth is called minimum inhibitory concentration (MIC). The results were summarized in Table 5.5. When we compare with the

standard (Novobiocin) drug (MIC value 2 μg/ml) compound **2e** showed significant antibacterial influence on the *S. aureus* with Minimum Inhibitory Concentration value 3 μg/ml. And also compounds **2f, 2h, 2n,** and **3b** exhibit moderate activity against *S. aureus* with MIC values 6, 5, 6 and 5 μg/ml respectively.

Table 5.5: The antibacterial activity of the target coumarin based thiazoles.

Entry	**Compound**	**MIC(**μg/ml) *S.aureus*
1	**2a**	9
2	**2b**	18
3	**2c**	14
4	**2d**	12
5	**2e**	3
6	**2f**	6
7	**2g**	14
8	**2h**	5
9	**2i**	>64
10	**2j**	8
11	**2k**	22
12	**2l**	16
13	**2m**	>64
14	**2n**	6
15	**3a**	>64
16	**3b**	5
17	**Std**	2

Std: Novobiocin

Molecular docking:

To understand the molecular interaction between the topoisomerase and coumarin based thiazoles derivatives, molecular docking studies were carried out at the catalytic domain of topoisomerase of *S. aureus.* Topoisomerase IV which is known to an efficient DNA decatenase is crucial for separation of daughter chromosomes during DNA replication. Coumarin antibiotics such as novobiocin and chlorobiocin act upon bacteria by targeting these topoisomerase IV[87][88] Maxwell A *et al*., has systematically evaluated novclobiocins (coumarin analogs) against

topoisomerase IV and found that these compounds are extremely effective against topoisomerase IV from his decatenation experiments[87]. In present study novobiocin which was used as standard in the pharmacological assay is co-crystallized ligand in the protein crystal structure (PDB: 4URN). Further the pharmacophore moiety in the synthesized compounds is coumarin itself. Hence the crystal structure of *S. aureus* topoisomerase IV complexed with novobiocin (PDB: 4URN) was chosen for docking studies.

Protein-ligand interactions between novobiocin (standard) and topoisomerase showed three hydrogen bond interactions (two with Arg 138 and one with Lys 36), one π-cationic interaction with Lys 36 and one salt bridge with Arg 79 (Figure 5.14(a)), dock score of Novobiocin was found to be -7.356 kcal/mol. Compound **2e** which exhibited magnificent anti-bacterial activity showed high dock score of -6.342 kcal/mol. It showed a hydrogen bond interaction with Arg 138 (Figure 5.14(b) and Figure 5.15). Almost all the synthesized compounds showed hydrogen bond interactions with either Arg 138 (or) Lys 36 as that of standard novobiocin except compound **2b** and **2c**. Compound **2b** formed π-π stacking interactions with Arg 138 whereas interactions were absent in case of compound **2c**. The synthesized compounds exhibited dock score in the range of -3.170 to -6.342 kcal/mol. The dock scores are presented in the table 5.6. The carbonyl group was responsible for the formation of hydrogen bond interactions in the newly synthesized compounds, a characteristic feature resembling with that of standard novobiocin.A regression analysis was also carried out between activity values (pMIC) and dock scores which gave a correlation coefficient of 0.81 indicating a sterling correlation topoisomerase binding (dock scores) and anti-bacterial activity. Figure 5.13 depicts the scatter plot of pMIC versus dock scores.

Table 5.6: Dock scores and pMIC values of newly synthesized coumarin based thiazoles.

Compound	Dock score (kcal/mol)	pMIC
2a	-4.977	5.046
2b	-4.131	4.745
2c	-4.019	4.854
2d	-4.349	4.921
2e	-6.342	5.523
2f	-3.995	5.222
2g	-4.257	4.854
2h	-5.970	5.301
2j	-4.977	5.097
2k	-3.710	4.658
2l	-3.749	4.796
2n	-4.897	5.222
3b	-5.160	5.301
Standard	-7.356	5.699

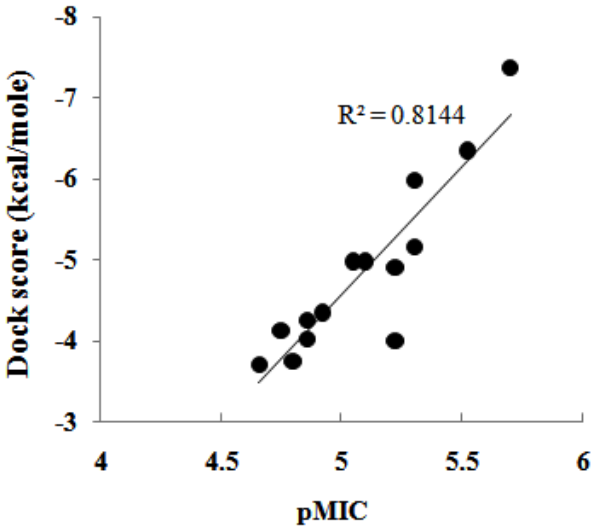


Figure 5.13: Scatter plot of pMIC versus dock score.

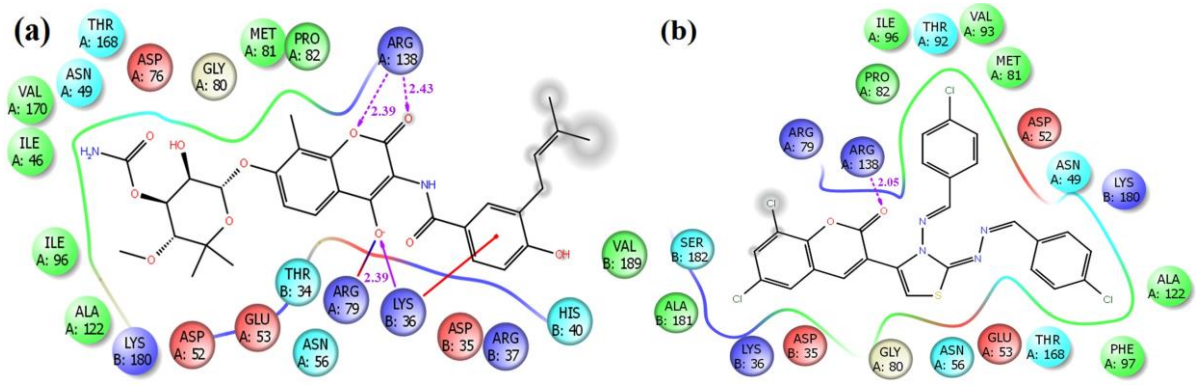


Figure 5.14: Ligand interaction diagrams (**a, b**) of standard novobiocin and synthesized compound **2e** showing hydrogen bond interactions with Arg 138 and Lys 36 (pink lines) along with distances in Å, salt bridge with Arg 79 and π-cationic interactions with Lys 36.

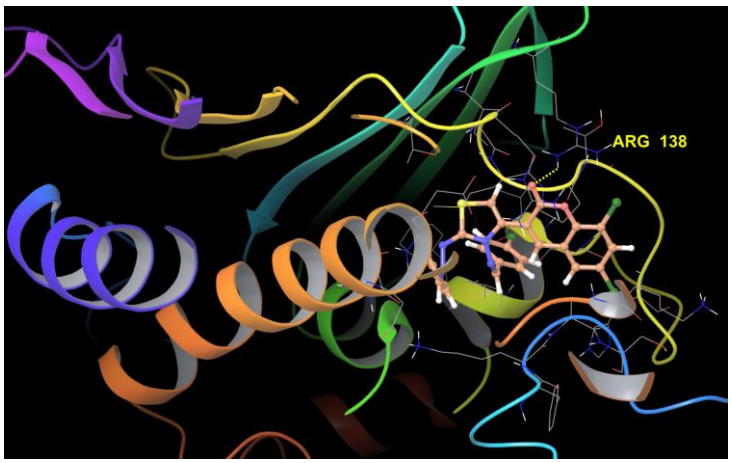


Figure 5.15: Ribbon structure of topoisomerase with compound **2e** at the active site showing hydrogen bond interaction with Arg 138.

All the coumarin based thiazole compounds were screened for their *in vitro* anti-bacterial activity and the results revealed that the compound **2e** exhibited remarkable activity against *S. aureus.* Furthermore, it was established from docking studies that interactions with Arg 138 (or) Lys 36 in coumarin based thiazoles might play a pivotal role in binding of inhibitors to topoisomerase.

5.2.3. Anti-cancer activity of thiazolyl pyrazole carbaldehyde compounds:

The newly synthesized thiazolyl pyrazole carbaldehyde compounds were evaluated for their biological activity and the cytotoxic effect on HeLa, MCF 7, A549 cell lines has been determined.

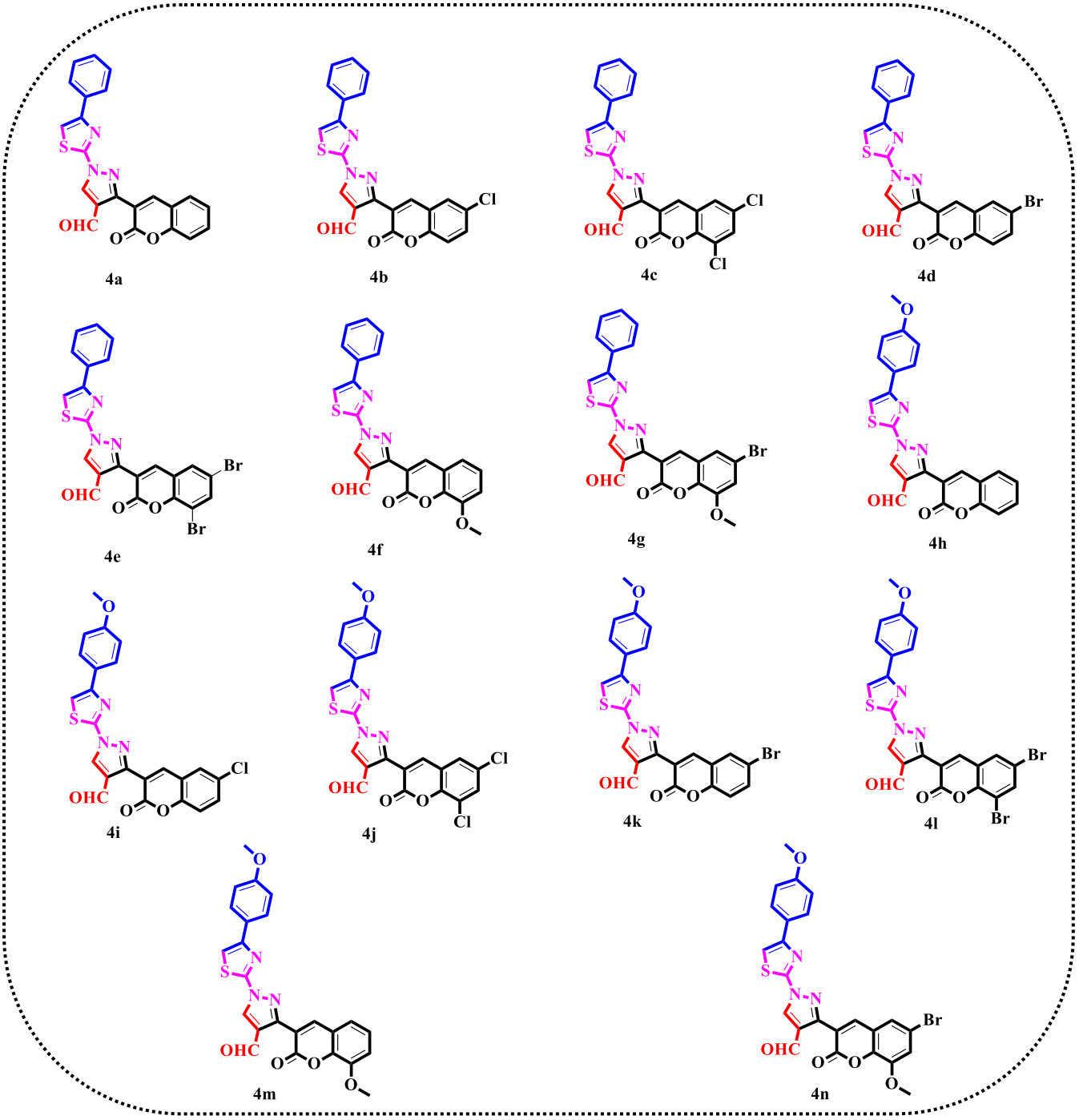


Figure 5.16: Thiazolyl pyrazole carbaldehyde hybrids (**4a-n**)

5.2.3.1. Experimental:

Biological activity:

The cell lines were cultured routinely in Dulbecco's modified Eagle's High-glucose medium (DMEM) supplemented with 10% (v/v) Fetal bovine serum (FBS), 1% L-Glutamine and 100U/ml penicillin/streptomycin and were maintained at 37 °C in a humidified atmosphere containing 5% CO2.

Cytotoxicity of different concentrations of synthesized thiazolyl pyrazole carbaldehydes were evaluated by methyl thiazolyltetrazolium (MTT) assay on cell lines. These cells were seeded in

culture flasks with DMEM medium along with 10% FBS and incubated for 24 h at 37°C in 5% CO_2 and 95% humidity to attain a cell growth of 80% confluency. Prior to the experiment, the cells were trypsinzed and transferred to a 96-well cell culture plate at a density of 1 $\times 10^4$ cells/well. The cells were incubated with different concentrations of synthesized thiazolyl pyrazole carbaldehydes (1–50 µg/mL) for 48 h followed by the addition of 100 µL MTT (0.5 mg/mL) and incubated for 3 h at 37 °C. Formazan crystals formed were dissolved with the addition 100 µL of dimethyl sulfoxide (DMSO) per well. An incubation period of 15 min at 37 °C was provided. The absorbance was measured at 570 nm using a plate reader (Enspire multimode plate reader (Perkin Elmer, USA). The cells treated with media were used as negative control.

Molecular docking:

Molecular docking simulations of newly synthesized thiazolyl pyrazole carbaldehyde derivatives was carried out using a procedure reported by peddi[89] *et al*. The protein was prepared by default parameters using protein preparation module available in schrodinger suite 2010. A grid was created around the colchicine binding site of β-tubulin with vander Waals scaling for non-polar atoms as 0.9. Finally, the coumarin derivatives which were built using maestro build panel and prepared by Ligprep module were docked into the active site by employing extra precision (XP) docking mode[90].

5.2.3.2. Result and discussions:

Biological activity:

The anti-cancer activity of the synthesized thiazolyl pyrazole carbaldehyde derivatives were tested against different human cancer cell lines such as cervical cancer (HeLa), breast cancer (MCF7) and adenocarcinoma (A549) cell lines by using nocadazole as a positive control. The cellular viability in the absence and presence of the synthesized thiazolyl pyrazole carbaldehyde compounds were determined using MTT assay. The IC_{50} values obtained were summarized in table 5.7. In the context of HeLa cell lines, compound **4m** (IC_{50} = 9.05 µM) exhibited significant antiproliferative when compared to the other synthesized compounds. Whereas all other synthesized compounds manifested moderate to low activity against HeLa. Compound **4m** against MCF7 cell line have shown good activity with IC_{50} value 7.12 µM. and **4a** against MCF7 exhibited moderate activity with IC_{50} value of 15.46 µM, remaining all other synthesized thiazolyl pyrazole carbaldehyde compounds showed low activity against MCF7. Compound **4m** exhibited good activity with IC_{50} value 6.34 µM against the cell line A549. And also, the compound **4a** against the A549 cell line have shown modest inhibiting activity with IC_{50} value

11.64 µM. whereas all other synthesized compounds showed less anti-cancer activity on the A549 cell line.

Table 5.7: IC_{50} values in µM for the compounds **4a–n** against human cancer cell lines through Cell Viability (MTT) Assay.[a]

Compound	HeLa	MCF7	A549
4a	28.63 ± 0.07	15.46 ± 0.05	11.64 ± 0.01
4b	39.70 ± 0.03	31.36 ± 0.04	21.26 ± 0.18
4c	60.19 ± 0.04	69.73 ± 0.02	48.34 ± 0.04
4d	68.17 ± 0.03	58.06 ± 0.03	50.08 ± 0.07
4e	78.45 ± 0.08	64.18 ± 0.07	52.63 ± 0.06
4f	53.71 ± 0.06	49.27 ± 0.02	43.07 ± 0.03
4g	>100	>100	88.91 ± 0.05
4h	42.51 ± 0.02	39.96 ± 0.04	28.93 ± 0.01
4i	79.96 ± 0.05	74.43 ± 0.02	68.71 ± 0.15
4j	83.58 ± 0.07	65.61 ± 0.04	53.53 ± 0.04
4k	45.52 ± 0.04	33.15 ± 0.07	26.50 ± 0.03
4l	91.32 ± 0.05	78.72 ± 0.05	64.56 ± 0.04
4m	9.05 ± 0.04	7.12 ± 0.04	6.34 ± 0.06
4n	41.62 ± 0.02	32.75 ± 0.07	26.38 ± 0.04
Nocodazole[b]	1.785± 0.07	1.578±0.005	1.809±003

ADME/T analysis:

Drug-likeness is a qualitative parameter that assesses the chances of oral bioavailability of a chemical compound. The physicochemical, pharmacokinetic, pharmacodynamic properties and ADME profile (absorption, distribution, metabolism and excretion) of the synthesized compounds (**4a-n**) were predicted (ADME) using SwissADME (Table 5.8). A chemical species to become a drug candidate, it should not violate more than one of the following parameters: octanol-water partition coefficient (XLogP3 ≤ 5), molecular weight (MW ≤ 500 $gmol^{-1}$), number of hydrogen bond acceptors (n-NO ≤ 10) and number of hydrogen bond donors (n-NHOH ≤ 5)[91]. According to the rule of 5 (RO5), when H-bond donors and acceptors are >5 and >10 respectively results in the poor absorption and permeability of a drug molecule [92]. Though the ligands **4e**, **4g**, **4k**, **4l** and **4n** has one violation each in molecular weight (exceeding 500

$gmol^{-1}$), all the synthesized compounds (**4a**-**n**) are within the criteria for the oral bioavailability. Based on the following parameters like lipophilicity (XLogP3), molecular weight, polarity (TPSA < 200; higher is the value, higher is the polarity)[93], solubility (Log S(ESOL <6)), SP^3 carbon fraction (Fraction Csp3 < 1) and flexibility (no. of rotatable bonds < 9), molecules can be categorized in to four bioavailability classes i.e., 11%, 17%, 55% (acceptable score) and 85% [94,95]. From the predictions, it was observed that all the compounds have good bioavailability with a 55% score. Further, the synthetic accessibility of the molecules (**4a**-**n**) assessed and the results suggesting the simple synthetic routes as tabulated in table 5.8.

Table 5.8: Drug-likeness, bioavailability and synthetic accessibility of compounds by SwissADME.

S.No.	MW	XLOGP3	n-NO	n-NHOH	Molar Refractivity	Lipinski violations	TPSA (Å^2)	Log S (ESOL)	Fraction Csp3	No. of Rotatable Bonds	Bioavailability Score	Synthetic accessibility
RO5	≤500	≤5	≤10	≤5	40-130	≤1	<`200	–	–	–	–	–
4a	399.42	4.04	5	0	111.54	0	106.23	–5.26	0.00	4	0.55	3.61
4b	433.87	4.67	5	0	116.55	0	106.23	–5.85	0.00	4	0.55	3.59
4c	468.31	5.29	5	0	121.56	0	106.23	–6.43	0.00	4	0.55	3.64
4d	478.32	4.73	5	0	119.24	0	106.23	–6.16	0.00	4	0.55	3.61
4e	557.21	5.42	5	0	126.94	1	106.23	–7.07	0.00	4	0.55	3.60
4f	429.45	4.01	6	0	118.03	0	115.46	–5.32	0.04	5	0.55	3.75
4g	508.34	4.70	6	0	125.73	1	115.46	–6.22	0.04	5	0.55	3.76
4h	429.45	4.01	6	0	118.03	0	115.46	–5.32	0.04	5	0.55	3.71
4i	463.89	4.64	6	0	123.04	0	115.46	–5.91	0.04	5	0.55	3.69
4j	498.34	5.27	6	0	128.05	0	115.46	–6.50	0.04	5	0.55	3.73
4k	508.34	4.70	6	0	125.73	1	115.46	–6.22	0.04	5	0.55	3.71
4l	587.24	5.39	6	0	133.43	1	115.46	–7.13	0.04	5	0.55	3.70
4m	459.47	3.98	7	0	124.52	0	124.69	–5.38	0.08	6	0.55	3.89
4n	538.37	4.67	7	0	132.22	1	124.69	–6.29	0.08	6	0.55	3.91

MW: molecular weight; **XLOGP3**: logarithm of partition coefficient of a chemical compound between lipid (n-octanol) and aqueous phases; **n-NO**: number of hydrogen bond acceptors; **n-NHOH**: number of hydrogen bond donors; **TPSA**: topological polar surface area; **LogS**: logarithm of aqueous solubility of a compound; **Fraction Csp3**: the ratio of SP^3 hybridized carbons over the total carbon count of the molecule; **RO5**: Lipinski's or Pfizer's rule of five.

The key property that greatly influences the druggability of a molecule is the absorption which in turn depends on its aqueous and non-aqueous solubility. As we know, to deliver the active pharmaceutical ingredient, the aqueous solubility of a drug is very important to show its bio-activity. Higher the aqueous solubility, higher will be the activity and it can be measured in terms of consensus Log S scale (mean predicted aq. Solubility). Drug molecules can be categorized based on their LogS values as follows: If Log S values are <–10, the compound is poorly soluble; <–6, moderately soluble; <–4, soluble; <–2, very soluble and < 0 highly soluble in an aqueous medium. Results (Table 5.9), suggesting that among the synthesized compounds only **4a**, **4b**, **4f**, **4h**, **4i** and **4m** were moderately soluble in water and remaining were poorly soluble. Non-aqueous solubility of the drug molecule is measured in terms of consensus Log $P_{o/w}$ (mean predicted lipophilicity). More negative Log $P_{o/w}$ value indicates higher solubility. But, from the results, it is evident that none of the molecules was soluble in non-aqueous medium.

Table 5.9: Predicted absorption parameters of compounds.

S.No.	Compound	Consensus Log $P_{o/w}$	Consensus Log S	Solubility Class
1	**4a**	3.90	–5.26	Moderately soluble
2	**4b**	4.42	–5.85	Moderately soluble
3	**4c**	4.92	–6.43	Poorly soluble
4	**4d**	4.51	–6.16	Poorly soluble
5	**4e**	5.10	–7.07	Poorly soluble
6	**4f**	3.95	–5.32	Moderately soluble
7	**4g**	4.54	–6.22	Poorly soluble
8	**4h**	3.91	–5.32	Moderately soluble
9	**4i**	4.43	–5.91	Moderately soluble
10	**4j**	4.93	–6.50	Poorly soluble
11	**4k**	4.56	–6.22	Poorly soluble
12	**4l**	5.10	–7.13	Poorly soluble
13	**4m**	3.95	–5.38	Moderately soluble
14	**4n**	4.50	–6.29	Poorly soluble

After, the absorption of the drug molecule, it has to cross-over several membrane barriers like gastrointestinal tract (GIT) and blood-brain barrier (BBB) etc [96]. All the synthesized compounds (**4a-n**) were predicted for their GI-absorption and brain penetration using the Brain Or IntestinaL EstimateD permeation method (BOILED-Egg) [97]. Based on the lipophilicity and polarity of a molecule, BOILED-Egg model predicts their distribution parameters and displays the results in the form of boiled egg in which white and yellow regions indicate passive

gastrointestinal absorption and passive brain permeation. From the results (Table 5.10), none of the compounds has BBB permeant means metabolites of them have no harmful effects on the brain and bloodstream. Except, the compounds **4a**, **4b**, **4d**, **4f** and **4h**, the reaming compounds have low GI-absorption. Also, form the table it was observed that the compounds **4f**, **4h**, **4k** and **4l** were least skin permeant (Log Kp, more negative means less skin permeant).

Table 5.10: Predicted distribution parameters of compounds.

S.No.	Compound		GI absorption	BBB permeant	Log Kp Skin permeant (cm/s)
1	**4a**		High	No	–5.87
2	**4b**		High	No	–5.63
3	**4c**		Low	No	–5.40
4	**4d**		High	No	–5.86
5	**4e**		Low	No	–5.85
6	**4f**		High	No	–6.07
7	**4g**		Low	No	–6.06
8	**4h**		High	No	–6.07
9	**4i**		Low	No	–5.84
10	**4j**		Low	No	–5.60
11	**4k**		Low	No	–6.06
12	**4l**		Low	No	–6.06
13	**4m**		Low	No	–5.63
14	**4n**		Low	No	–5.63

In vitro toxicity calculations were performed by using AdmetSAR and are illustrated in table 5.11. It was observed from the results that all the synthesized compounds (**4a-n**) exhibited positive result for blood-brain barrier (BBB) suggesting that the compounds could pass through the BBB. It was evident from the results that all the compounds are non-carcinogenic and exhibited Category-III acute toxicity which means they are relatively safe for the oral administration. All the compounds (**4a-n**) are found to interrupt the absorption, permeability and retention of chemical species by inhibiting the P-glycoprotein and showed highest human intestinal absorption values ranging from +0.9672 to +0.9926, among them **4d** and **4e** exhibited higher values. All the compounds exhibited weak inhibitory towards human ether-a-go-go-related gene (Herg) that encodes IKr potassium channel which leads to long QT syndrome (LQTS). Finally, rat acute toxicity values suggesting higher lethal dose (LD_{50}) values for the compounds.

Table 5.11: Drug-likeness, bioavailability and synthetic accessibility of compounds by SwissADME.

Compound	Human intestinal absorption	Blood-brain barrier	P-glycoprotein inhibitor	Human ether – a-go-go related gene inhibition	Carcinogen	Acute oral toxicity	Rat acute toxicity LD50 (mol/kg)
4a	+0.9910	+0.9270	NI (0.8614)	WI (0.8376)	NC (0.8846)	III	2.1648
4b	+0.9926	+0.8655	NI (0.8861)	WI (0.8305)	NC (0.8358)	III	2.1573
4c	+0.9926	+0.8655	NI (0.8861)	WI (0.8305)	NC (0.8358)	III	2.1573
4d	+0.9893	+0.8658	NI (0.8357)	WI (0.8376)	NC (0.8840)	III	2.2205
4e	+0.9893	+0.8658	NI (0.8357)	WI (0.8840)	NC (0.8589)	III	2.2205
4f	+0.9788	+0.7468	NI (0.6718)	WI (0.9419)	NC (0.8963)	III	2.3003
4g	+0.9753	+0.6523	NI (0.6158)	WI (0.9469)	NC (0.8710)	III	2.3348
4h	+0.9820	+0.7605	NI (0.7502)	WI (0.8886)	NC (0.8898)	III	2.2613
4i	+0.9855	+0.6726	NI (0.8614)	WI (0.8491)	NC (0.8405)	III	2.2326
4j	+0.9855	+0.6726	NI (0.7502)	WI (0.8491)	NC (0.8405)	III	2.2326
4k	+0.9790	+0.6665	NI (0.6490)	WI (0.8976)	NC (0.8630)	III	2.2956
4l	+0.9790	+0.6665	NI (0.6490)	WI (0.8976)	NC (0.8630)	III	2.2956
4m	+0.9718	+0.7044	NI (0.6442)	WI (0.9566)	NC (0.8844)	III	2.3578
4n	+0.9672	+0.6112	NI (0.5744)	WI (0.9604)	NC (0.8557)	III	2.3780

NI-Non-inhibitor; **WI**-Weak inhibitor; **NC**-Non-carcinogen

Xenobiotic metabolism is biotransformation of pharmacological substances in the body utilizing enzyme-mediated hydrolysis, oxidation and reduction and it has an important role in drug-bioavailability. Cytochrome P450 (CYP450) is the largest clade of proteins (CYP1A2, CYP2C19, CYP2C9, CYP2D6 and CYP3A4) which usually involves in the oxidation of xenobiotics. Permeability glycoprotein 1 (P-gp) is a cell-membrane protein which is also known as multidrug resistance protein 1 (MDR1) acts as an ATP-dependent efflux pump (pumps toxic or foreign substances out of the cell) through the cell membranes and CYP450-enzymes. Hence, predicting the intractability of synthesized compounds (**4a-n**) with CYP450-enzymes and P-gp protein could give us a better understanding of whether they are the substrates or inhibitors or both. From the table 5.12, it was observed that none of the ligands (**4a-n**) was the substrates of P-gp means they will pump out of the cell. Except for **4a**, all the ligands were the non-inhibitors of CYP1A2. All the compounds are acting as inhibitors and non-inhibitors of CYP450 isoforms CYP2C9 and CYP2D6 respectively. In case of CYP2C19, except **4a**, **4b** and **4d** all are acting as non-inhibitors. Finally, the compounds **4f**, **4h**, **4m** and **4n** acting as inhibitors of CYP3A4.

Table 5.12: Predicted metabolism parameters of compounds.

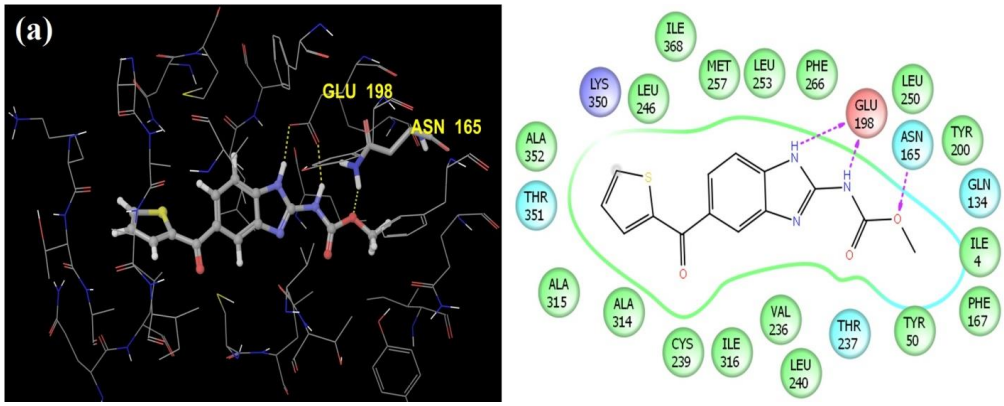
Compound	P-gp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor
4a	No	Yes	Yes	Yes	No	No
4b	No	No	Yes	Yes	No	No
4c	No	No	No	Yes	No	No
4d	No	No	Yes	Yes	No	No
4e	No	No	No	Yes	No	No
4f	No	No	No	Yes	No	Yes
4g	No	No	No	Yes	No	No
4h	No	No	No	Yes	No	Yes
4i	No	No	No	Yes	No	No
4j	No	No	No	Yes	No	No
4k	No	No	No	Yes	No	No
4l	No	No	No	Yes	No	No
4m	No	No	No	Yes	No	Yes
4n	No	No	No	Yes	No	Yes

Molecular docking:

To rationalize our experimental findings and explore the molecular interactions between the synthesized coumarin derivatives and β-tubulin molecular docking studies were performed. Numerous coumarin analogues were systematically investigated for their anticancer potential based on their mechanism of action and SAR studies[98–100]. Recent studies have also shown

that coumarin analogues (4-(3-hydroxy-4-methoxyphenyl)-2H-chromen-2-ones) act as good tubulin inhibitors by binding to the colchicine site[101]. Compounds like 7-diethyl amino-3(2'-benzoxazolyl)-coumarin[102] was reported as microtubule inhibitors with potent antimitotic activity in multidrug resistant cancer cells. Nocodazole is a potent β-tubulin inhibitor which disrupts microtubule assembly/disassembly dynamics, thereby prevents mitosis and instigates apoptosis in tumor cells. The standard used in this study was nocodazole and the co-crystallized ligand in 5CA1 protein is also nocodazole. Therefore, this particular protein was selected for performing docking studies.

The X-ray crystal structure of β-tubulin was retrieved from the protein data bank (PDB id: 5CA1). GLIDE 5.6[54] was used for docking the newly synthesized coumarin derivatives into 5CA1 active site. The standard nocodazole showed three hydrogen bond interactions with Asn 165 and Glu 198 (Figure 5.17(a)) at the colchicine binding site of β-tubulin with a dock score of -6.869 kcal/mol. The newly synthesized coumarin derivatives showed hydrogen bond interactions with Asn 165, Cys 239, Gln 245 and Thr 351 and π-cationic interactions with Lys 350. The dock scores of these derivatives ranged from -4.376 to -6.054 kcal/mol. (Table 5.13). Compound **4m** which showed relatively good dock score in the series showed hydrogen bond interaction with Asn 165 (Figure 5.17(b)) with dock score of -6.054 kcal/mol. The dock scores of newly synthesized coumarin derivatives corroborated well with the experimental anti-proliferation studies.



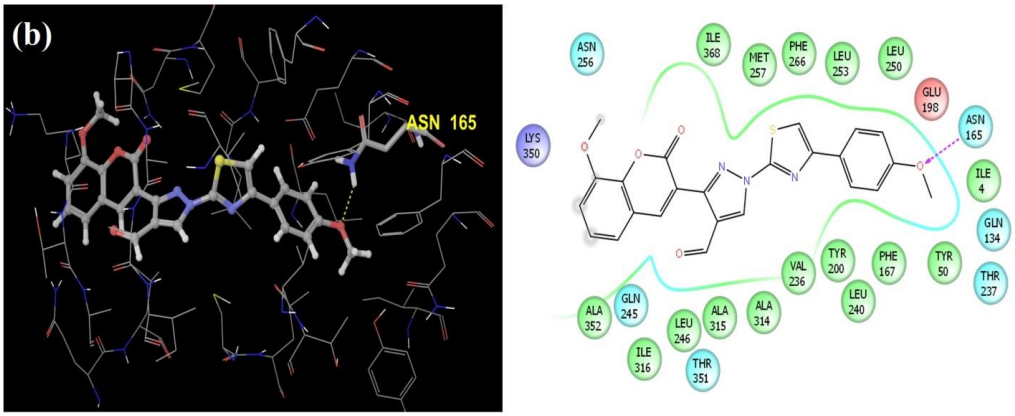


Figure 5.17: Dock pose overlay and Ligand interaction diagram of (a) nocodazole and (b) compound **4m** at the colchicine binding site of β-tubulin.

Table 5.13: Dock scores of newly synthesized coumarin derivatives.

Entry	Compound	Dock score (kcal/mol)
1	**4a**	-5.808
2	**4b**	-5.599
3	**4c**	-5.014
4	**4d**	-5.099
5	**4e**	-4.951
6	**4f**	-5.363
7	**4g**	-4.917
8	**4h**	-5.408
9	**4i**	-4.930
10	**4j**	-4.376
11	**4k**	-5.537
12	**4l**	-4.749
13	**4m**	**-6.054**
14	**4n**	-5.598
15	**Nocodazole**	-6.869

All the thiazolyl pyrazole carbaldehyde compounds were tested for their *in vitro* anti-cancer activity and the results revealed that the compound **4m** exhibited remarkable activity against A549, MCF7 and HeLa cancer lines. From docking studies, it can be presumed that the newly synthesized thiazolyl pyrazole carbaldehyde derivatives bind to the colchicine binding site of β-tubulin and can act as promising anti-cancer agents.

5.2.4. Anti-cancer activity of thiazolyl pyrazole compounds:

The newly synthesized thiazolyl pyrazole compounds were evaluated biological activity and the cytotoxic effect on HeLa, A549, MDA-MB-231 cell lines has been determined.

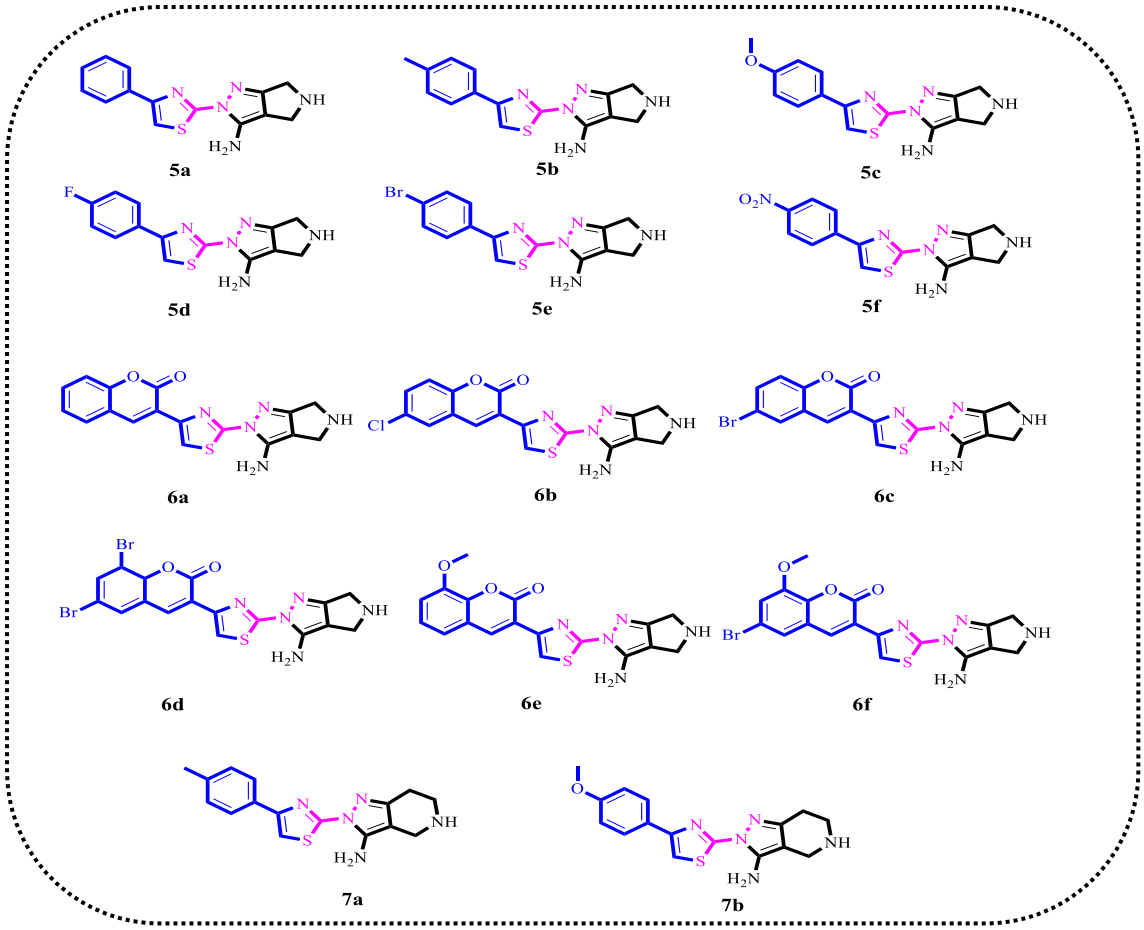


Figure 5.18: Thiazolyl pyrazole carbaldehyde hybrids (**5a-f, 6a-f** and **7a-b**).

5.2.4.1. Experimental:

Biological activity:

Cytotoxic effects of the synthesized thiazolyl pyrazole derivatives were determined by using MTT [3-(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide] (Sigma) assay. 120 µl aliquots of a cell suspension (50,000 cells ml/1) in 96-well micro plates were incubated at 37 °C and 10% CO_2 and allowed to grow for 48 h. Then 60 µl of serial dilutions of the compounds were added. After 24 h of incubation at 37 °C and 10% CO_2, 20 µl MTT in phosphate buffered saline (PBS) were added to a final MTT concentration of 0.5 mg/ml. After 2 h the precipitate of formazan crystals was centrifuged and the supernatant discarded. The precipitate was washed with 100 µl PBS and dissolved in 100 ml isopropanol containing 0.4% hydrochloric acid. The resulting colour was quantified at 590 nm using an ELISA plate

reader. All investi- gations were carried out in two parallel experiments. The IC50 values were determined from the dose–response curves as the concentrations of compounds, which resulted in 50% of the absorbance of untreated control cells.

Molecular docking protocol:

The docking simulations of the newly synthesized thiazolyl pyrazole derivatives was performed with GLIDE 5.6[54] available in the Schrodinger suite 2010. The protein which was retrieved from the RCSB protein data bank (PDB ID: 4YJ2)[103] was processed, optimized and minimized using protein preparation wizard employing OPLS 2005 force field. The Vander Waals scaling for non-polar atoms was set to 0.9 and a grid was generated at the colchicine binding site of β-tubulin. The synthesized thiazolyl pyrazole derivatives were then sketched using maestro build panel and prepared by Ligprep module in the Schrodinger 2010. Finally, the prepared compounds were docked into the grid generated around the active site using extra precision (XP) docking mode[90].

5.2.4.2. Results and discussion:

Biological activity:

The anti-cancer activity of the synthesized thiazolyl pyrazole derivatives were screened against different human cancer cell lines such as cervical cancer (HeLa), adenocarcinoma (A549) and breast cancer (MCF7) cell lines by using combretastatin A-4 as a positive control. The cellular viability in the presence and absence of the synthesized thiazolyl pyrazole derivatives were determined using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. The IC_{50} values obtained were summarized in table 5.14. In the context of HeLa cell lines, compounds **7b** (IC_{50} = 3.60 µM) and **7a** (IC_{50} = 4.61 µM) exhibited good antiproliferative activity when compared to the other synthesized compounds. And compound **5d** (IC_{50} = 8.17 µM) showed significant activity on HeLa cell lines, whereas all other synthesized compounds manifested moderate to low activity against HeLa. Compound **7b** (IC_{50} = 4.17 µM) and **7a** (IC_{50} = 5.29 µM) have shown good activity against A549 cell line furthermore, **5d** (IC_{50} = 9.06 µM) and **5b** (IC_{50} = 9.64 µM) manifested modest activity against A549 cell line. While other synthesized thiazolyl pyrazole compounds have shown low activity against A549 cell line. Compound **7b** and **7a** exhibited good activity with IC_{50} values 3.94 µM, 4.92 µM against the cell line MDA-MB-231. And also, the compound **5d** against the MDA-MB-231 cell line have shown modest inhibiting activity with IC_{50} value

9.63 µM. whereas all other synthesized compounds showed less anti-cancer activity on the MDA-MB-231 cell line.

Table 5.14: IC_{50} values in µM for the compounds **(5a-f, 6a-f** and **7a-b)** against human cancer cell lines through Cell Viability (MTT) Assay.[a]

Entry	Compounds	HeLa	A549	MDA-MB-231
1	**5a**	34.06 ± 0.01	29.64 ± 0.07	41.36 ± 0.03
2	**5b**	10.18 ± 0.06	**9.64 ± 0.03**	11.36 ± 0.01
3	**5c**	28.17 ± 0.02	36.54 ± 0.04	40.81 ± 0.15
4	**5d**	**8.17 ± 0.03**	**9.06 ± 0.02**	**9.63 ± 0.07**
5	**5e**	24.38 ± 0.02	31.79 ± 0.08	18.63 ± 0.04
6	**5f**	12.32 ± 0.18	18.87 ± 0.03	16.07 ± 0.02
7	**6a**	53.13 ± 0.03	46.50 ± 0.02	61.75 ± 0.04
8	**6b**	58.91 ± 0.01	49.97 ± 0.04	63.23 ± 0.03
9	**6c**	49.86 ± 0.07	51.73 ± 0.01	56.63 ± 0.04
10	**6d**	76.21 ± 0.05	68.47 ± 0.01	71.42 ± 0.04
11	**6e**	54.72 ± 0.01	44.07 ± 0.03	42.09 ± 0.02
12	**6f**	65.13 ± 0.01	57.50 ± 0.07	68.91 ± 0.03
13	**7a**	**4.61 ± 0.03**	**5.29 ± 0.05**	**4.92 ± 0.02**
14	**7b**	**3.60 ± 0.05**	**4.17 ± 0.07**	**3.94 ± 0.05**
15	**CA-4**	0.009 ± 0.03	0.038 ± 0.03	0.028 ± 0.04

Absorption, distribution, metabolism, excretion (ADME) properties

To become a successful drug candidate, besides efficacy at low concentrations and with low toxicity, also the molecules should possess an appropriate pharmacokinetic profile (ADME properties) which decides the availability of drug molecule in active form until the desired effect takes place. Thus, ADME profiles of new drug candidates are very important and their initial assessment through *in silico* makes drug discovery and development process simple in terms of cost and time. Also, *in silico* prediction of ADME lowers the risk of failure of drug election at final stages of clinical trials. The pharmacokinetic parameters of synthesized molecules were predicted *in silico* using SwissADME[104] and pkCSM[105]. ADME profiles of

synthesized compounds were illustrated in Table 5.15 and 5.16.

Based on the solubility measurement and intestinal permeability, evaluated drug absorption. The predicted aqueous solubility (logS) of the compounds is given as the logarithm of molar concentration, which reflects the solubility of compounds in aqueous medium at 25°C. From the Table 5.16, it was observed that, the solubility of synthesized compounds ranging from –2.393 to –3.903, which clearly reflecting their moderate solubility in water due to the presence of lipophilic groups. As we know, most of the drugs from an orally administered solution are primarily absorbed through the small intestine due to its large surface area and permeability of membranes than those in stomach. Hence, predicted percentage of drug that can be absorbed through the small intestine (human). Interestingly, all the synthesized compounds exhibited high intestinal absorption in the range of 88–95% which is similar to that of Combretastatin A-4 (94%). To predict the absorption of orally administered drugs in terms of Caco-2 permeability values used an *in vitro* model of the human intestinal mucosa. In general, higher the logarithm of the apparent permeability coefficient (log Papp $>8\times10^{-6}$ cm/s) higher will be the Caco-2 permeability. From the results, it was predicted that all the compounds except **5a** and **6d-f** have shown high Caco-2 permeability in the range of 0.948 to 1.244.

Further, the distribution parameters of the synthesized compounds were assessed based on volume of distribution (VDss), fraction unbound and blood-brain barrier permeability (BBB perm.). Log VDss >0.45 and <–0.15 indicates higher distribution of drug molecule in tissue than in plasma and vice-versa respectively. From the Table 5.16, it was observed that all the compounds with high VDss values ranging between 0.494 to 0.811 are predicted to have higher distribution in plasma than the drug Combretastatin A-4 (–0.072). For a given drug molecule, its efficacy depends up on degree to which it binds to blood proteins. If more it binds to blood proteins lesser it will be traverse or diffuse through the membranes. The results indicating that, all the screened compounds were free to diffuse. After, the absorption of the drug molecule, it has to cross-over several membrane barriers like gastrointestinal tract (GIT) and blood-brain barrier (BBB) etc. All the synthesized compounds (**5a-f**, **6a-f**, **7a** and **7b**) were predicted for their brain penetration using both SwissADME and pkCSM. From the results (Table-5.16), except the standard drug Combretastatin A-4 (CA–4), all the compounds are BBB non-permeant. Also, all the compounds were interacting with cytochrome P450 either as substrates or as inhibitors. The total clearance of drugs including both hepatic and renal clearance can be measured by the proportionality constant CLtot and was measured for

our synthesized compounds and presented in Table 5.16. It was observed form the results that, the derivatives **5a-f**, **7a** and **7b** have shown high CLtot ranging between 0.941–1.048 logml/min/kg while the derivatives **6a-f** displayed moderate CLtot ranging between 0.487–0.87 logml/min/kg

Finally, from the Table 5.15 and 5.16, it can be concluded that all the synthesized compounds except **6d** have displaying appropriate pharmacokinetic parameters and were obeying the Lipinski's "Rule of Five" and Veber Rule and hence they have drug-likeness and can be considered as probable lead compounds for the development of anticancer drugs.

Table 5.15: **Pharmacokinetic analysis of the compounds by the Molinspiration server**

S.No.		**Lipinski's Rule of 5 (RO5)**						**Veber Rule**	
		Log P	**Mol. Wt**	**H-bond donor**	**H-bond acceptor**	**No. of violations**		**TPSA (Å^2)**	**No. of rotatable bonds**
		≤5	**≤500**	**≤5**	**≤10**	**≤1**		**≤140**	**≤10**
5a		1.78	283.36	3	5	0		68.77	2
5b		2.23	297.39	3	5	0		68.77	2
5c		1.84	313.39	3	6	0		78.00	3
5d		1.94	301.35	3	5	0		68.77	2
5e		2.59	362.26	3	5	0		68.77	2
5f		1.74	328.36	3	8	0		114.59	3
6a		1.78	351.39	3	7	0		98.98	2
6b		2.44	385.84	3	7	0		98.98	2
6c		2.57	430.29	3	7	0		98.98	2
6d		3.31	509.18	3	7	1		98.98	2
6e		1.79	381.42	3	8	0		108.21	3
6f		2.55	460.31	3	8	0		108.21	3
7a		2.63	311.41	3	5	0		68.77	2
7b		2.24	327.41	3	6	0		78.00	3
CA-4		3.47	316.35	1	5	0		57.16	6

Log P: logarithm of partition coefficient of a chemical compound between lipid (n-octanol) and aqueous phases; **Mol. Wt.**: molecular weight; **H-donor**: number of hydrogen bond donors; **H-acceptor**: number of hydrogen bond acceptors; **TPSA**: topological polar surface area.

Table 5.16: **Calculation of ADME properties of the compounds using pkCSM and SwissADME**

S.No		Absorption			Distribution				Metabolism	Excretion Total clearance Log(ml/min/kg)
		Log S (log mol/L)	Caco-2 perm. (log Papp in 10^{-6} cm/s)	Int. abs. (% Absorbed)	VDss (log L/kg)	Fract. Unb (Fu)	BBB perm. (log BB)	BBB pred.		
5a		–2.393	0.854	93.251	0.685	0.329	–0.524	No	CYP3A4 substrate, CYP1A2 inhibitor	0.969
5b		–2.645	0.974	93.261	0.764	0.36	–0.538	No	CYP3A4 substrate, CYP1A2 inhibitor	0.975
5c		–2.645	1.222	94.279	0.777	0.343	–0.758	No	CYP3A4 substrate, CYP1A2 inhibitor	1.048
5d		–2.639	1.175	92.87	0.706	0.361	–0.746	No	CYP3A4 substrate, CYP1A2 inhibitor	0.975
5e		–3.033	0.948	91.735	0.742	0.339	–0.722	No	CYP3A4 substrate, CYP1A2 inhibitor	0.943
5f		–2.583	1.073	88.929	0.646	0.248	–1.041	No	CYP3A4 substrate, CYP1A2 inhibitor	0.756
6a		–2.852	1.129	91.618	0.74	0.305	–0.981	No	CYP3A4 substrate, CYP1A2, CYP3A4 inhibitor	0.858
6b		–3.109	1.005	92.797	0.784	0.31	–1.162	No	CYP3A4 substrate, CYP1A2, CYP3A4 inhibitor	0.812
6c		–3.154	1.007	92.533	0.802	0.307	–1.17	No	CYP3A4 substrate, CYP1A2, CYP3A4 inhibitor	0.765
6d		–3.903	–0.02	95.609	0.513	0.38	–1.333	No	CYP3A4 substrate, CYP1A2, CYP3A4 inhibitor	0.487
6e		–3.277	0.193	92.226	0.494	0.365	–1.196	No	CYP3A4 substrate, CYP1A2, CYP3A4 inhibitor	0.87
6f		–3.513	0.106	93.173	0.519	0.377	–1.382	No	CYP3A4 substrate, CYP1A2, CYP2C19, CYP3A4 inhibitor	0.707
7a		–2.737	0.989	93.665	0.799	0.352	–0.524	No	CYP3A4 substrate, CYP1A2 inhibitor	0.941
7b		–2.72	1.244	94.683	0.811	0.335	–0.744	No	CYP3A4 substrate, CYP1A2 inhibitor	1.014
CA–4		–5.215	1.321	94.659	–0.072	0.036	0.187	Yes	CYP3A4 substrate, CYP1A2, CYP2C19, CYP2C9, CYP3A4 inhibitor	0.213

Molecular docking:

Combretastatin A-4 is a well-known tubulin polymerization inhibitor which binds to colchicine binding site of β-tubulin. The standard used in this study was combretastatin A-4 therefore crystal structure of tubulin i.e. 4YJ2 was chosen for docking studies. To understand and explore the plausible binding modes and mode of action, the synthesized thiazolyl pyrazole derivatives were docked into the colchicine binding site of β-tubulin. The standard combretastatin A-4 showed three hydrogen bond interactions, two with Glh 200 and one with Leu 255 (Figure 5.19 (a) and (b)). at colchicine binding site of β-tubulin. It showed a dock score of -8.452 kcal/mol (Table 5.17). The best active molecule among the series i.e. **7b** showed two hydrogen bond interactions with Gln 136 and Glh 200 (Figure 5.19 (c) and (d)) with a dock score of -8.791 kcal/mol. The dock scores of remaining compounds ranged from -2.481 to-8.777 kcal/mol (Table 5.17). These compounds displayed hydrogen bond interactions with Gln 136, Cys 241, Leu 255, Glh 200, Tyr 202 and val 238. The dock scores of these newly synthesized thiazolyl pyrazole derivatives well concurred with the experimental studies. According to the structure activity relationship the presence of pipyridyl moiety in the compounds **7a** and **7b** was responsible for the potential anti-cancer activity of these compounds.

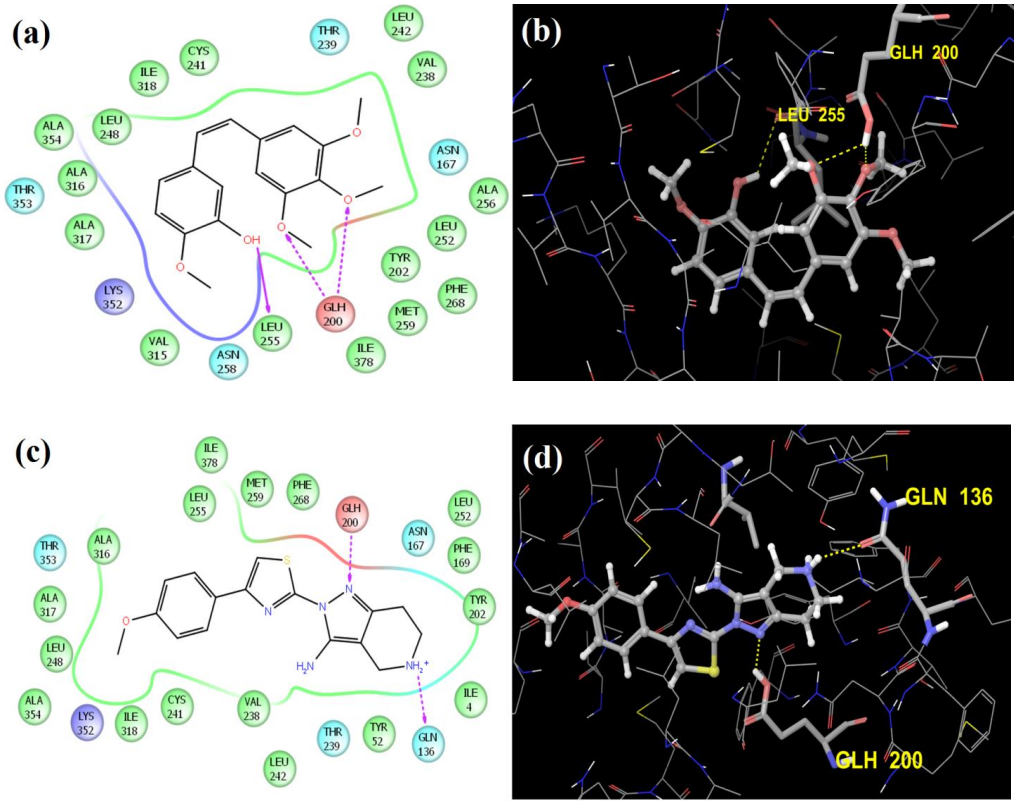


Figure 5.19: Ligand interaction diagram and docked pose of Combretastatin A-4 ((a) and (b)) and compound **7b** ((c) and (d)) respectively at the colchicine binding site of β-tubulin.

Table 5.17: Docking simulation results of newly synthesized thiazolyl pyrazole derivatives.

Entry	Compound	Dock score (kcal/mol)
1	**5a**	-6.593
2	**5b**	-7.997
3	**5c**	-6.474
4	**5d**	-8.152
5	**5e**	-7.115
6	**5f**	-7.766
7	**6a**	-3.814
8	**6b**	-3.544
9	**6c**	-3.840
10	**6d**	-2.481
11	**6e**	-3.984
12	**6f**	-3.163
13	**7a**	-8.777
14	**7b**	**-8.791**
15	**Combretastatin A-4**	-8.452

All the thiazolyl pyrazole compounds were screened for their *in vitro* anti-cancer activity and the results revealed that the compound **7b** and **7a** exhibited good activity against HeLa, A549 and MDA-MB-231 cancer lines. And the in vitro anti-proliferative studies were further supported by docking simulations.

5.2.5. Anti-cancer activity of pyrazolothiazole compounds:

The newly synthesized pyrazolothiazole compounds were evaluated anti-cancer activity on A549, HeLa, DU-145 and SK-N-SH cell lines has been determined.

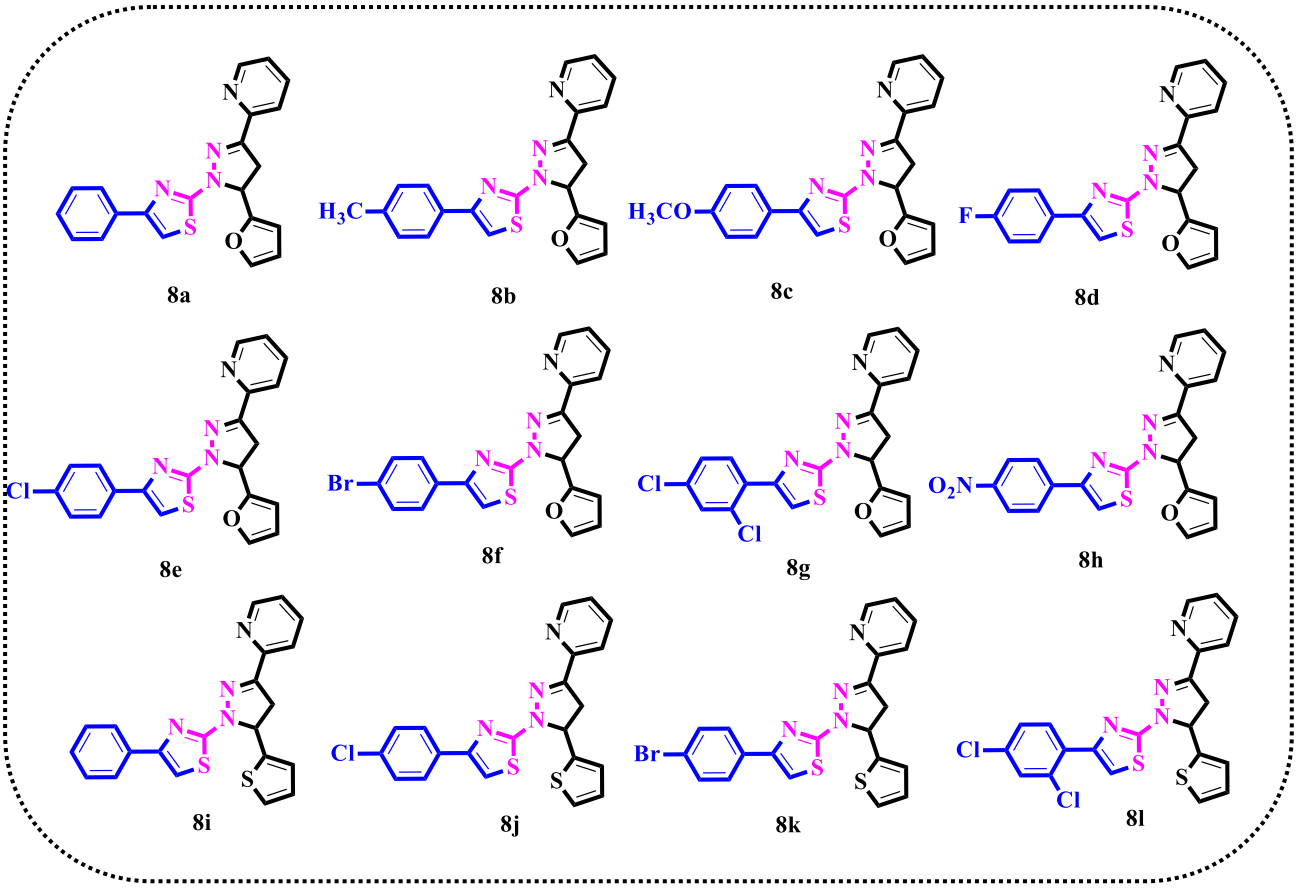


Figure 5.20: Thiazolyl pyrazole carbaldehyde hybrids (**8a-l**).

5.2.5.1. Experimental:

Biological activity:

MTT [3-(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide] (Sigma) was used to measure the metabolic activity of cells (alive cells are able to reduce MTT to a violet coloured formazan product which can be quantified spectro photo metrically). Briefly, 120 µl aliquots of a cell suspension (50,000 cells ml/1) in 96-well micro plates were incubated at 37 °C and 10% CO_2 and allowed to grow for two days. Then 60 µl of serial dilutions of the compounds were added. After 24 h of incubation at 37 °C and 10% CO_2, 20 µl MTT in phosphate buffered saline (PBS) were added to a final MTT concentration of 0.5 mg/ml. After 2 h the precipitate of formazan crystals was centrifuged and the supernatant discarded. The precipitate was washed with 100 µl PBS and dissolved in 100 ml isopropanol containing 0.4% hydrochloric acid. The resulting colour was quantified at 590 nm using an ELISA plate reader. All investi- gations were carried out in two parallel experiments. The IC50 values were determined from the dose–response curves as the concentrations of compounds, which resulted in 50% of the absorbance of untreated control cells.

5.2.5.2. Result and discussions:

Biological activity:

The anti-proliferative potency of the synthesized compounds was screened against various human cancer cell lines *viz.*, adenocarcinoma (A549), cervical cancer (HeLa), human neuroblastoma (SK-N-SH) and human prostate cancer (DU-145) cell lines by using doxorubicin (DOX) as a positive control. The IC_{50} values, *i.e.*, the cellular viability in the presence and absence of the synthesized compounds were determined using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. The IC_{50} values obtained were summarized in table 5.18. In the context of HeLa cell lines, compound **8h** (IC_{50} = 9.97 µM) have shown considerable antiproliferative when compared to the other synthesized compounds. After **8h**, the compounds **8j**, **8i** and **8c** have shown good activity with IC_{50} values 15.61, 18.47, 19.78 µM respectively. Whereas all other compounds exhibited moderate to low activity against HeLa. Compound **8g** with 2, 4-di-chloro phenyl substitution against DU-145 cell line and **8f** with *p*-bromo phenyl substitution against A549 have shown moderate activity with IC_{50} values of 22.51 and 28.91 µM respectively. The derivative bearing *p*-nitro phenyl substitution i.e., **8h** showed modest activity with IC_{50} values 28.91 and 23.42 µM against the cell lines A549 and SK-N-SH. Also, the compound **8b** against the SK-N-SH cell line showed moderate inhibiting activity with IC_{50} value 22.44 µM. The cytotoxicity assay of the compounds on the DU145 cell line indicates that none of the compounds except **8g**, **8h** and **8j** have shown modest activity and rest of the compounds showed less or no activity.

Structure activity relationship:

The structure-activity relationship forms the basis of numerous correlations that can be established from the derived data of the synthesized compounds. This data also supports the nature of the cytotoxic activity not only based on the steric properties but also the electronic properties of the aromatic ring and substituted groups. The novelty of the heterocyclic framework and the presence of the various substitutions might be a reason for the activity of the compounds. Out of 12 compounds synthesized, compound **8h** exhibit strongest against HeLa cell line, whereas moderate activity against the other tested cell lines. The negative inductive effect (-ve I-effect) of $-NO_2$ group in **8h** supposed to be a contribution to the antiproliferative activity. Majority of the compounds did not possess a selective resistance with the cell lines chosen. But the antiproliferative activity of **8h**, **8j**, **8b** and **8f** is anticipated to be the effect of the groups that are attached to the backbone. The strategic development of these kinds of schemes

and new molecular hybrids find a way in unfolding novel therapies for the betterment of mankind.

Table 5.18: IC_{50} values in µM for the compounds **8a–l** against human cancer cell lines through Cell Viability (MTT) Assay.[a]

Entry	**Compounds**	**A549**	**HeLa**	**DU-145**	**SK-N-SH**
1	**8a**	46.18 ± 0.04	53.64 ± 0.18	58.36 ± 0.11	41.02 ± 0.05
2	**8b**	50.70 ± 0.07	45.07 ± 0.02	64.13 ± 0.12	22.44 ± 0.04
3	**8c**	60.17 ± 0.04	19.78 ± 0.03	40.37 ± 0.06	60.35 ± 0.16
4	**8d**	43.17 ± 0.03	42.06 ± 0.02	40.03 ± 0.01	42.57 ± 0.05
5	**8e**	64.38 ± 0.08	44.79 ± 0.02	62.63 ± 0.08	52.57 ± 0.11
6	**8f**	22.51 ± 0.02	51.87 ± 0.03	68.07 ± 0.03	42.68 ± 0.15
7	**8g**	45.13 ± 0.04	35.50 ± 0.02	28.91 ± 0.03	31.23 ± 0.10
8	**8h**	28.91 ± 0.03	**9.97 ± 0.05**	30.92 ± 0.02	23.42 ± 0.10
9	**8i**	49.92 ± 0.07	18.47 ± 0.01	48.93 ± 0.02	58.13 ±0.15
10	**8j**	31.52 ± 0.02	15.61 ± 0.06	29.53 ± 0.01	44.33 ± 0.04
11	**8k**	58.72 ± 0.04	48.07 ± 0.05	65.50 ± 0.15	66.28 ± 0.04
12	**8l**	60.60 ± 0.05	59.17 ± 0.04	55.94 ± 0.01	60 ± 0.18
13	**Doxorubicin**	2.76 ± 0.16	2.27 ± 0.09	3.52 ± 0.24	3.32 ± 0.09

All the compounds were screened for their *in vitro* antiproliferative activity and the results revealed that the compound **8h** exhibited significant activity against HeLa cell line.

5.2.6. Anti-cancer activity of 1,3,4 thiadiazine compounds:

The newly synthesized pyrazolothiazole compounds were evaluated anti-cancer activity has been determined.

5.2.6.1. Experimental:

Biological activity:

Cytotoxicity (MTT) assay: GL261 and CHO cells (~ 5000 cells/well) were plated in 96-well plate and cultured for 24 h. The cells were incubated with Compounds and DMSO vehicle control in DMEM containing 10% added fetal bovine serum (FBS) for 24 h. MTT reagent (5 mg/ mL stock concentration in PBS) was added to the cells and incubated for 4 h. Thereafter, 50

µL DMSO:Methanol mixture (1:1, v/v) was added, mixture nutated for 5 min and the absorbances of the solutions were measured at 570 nm. The percent cell viabilities were assessed using the relation:

% Cell Viability = [A570(treated cells)-background/A570 (untreated cells)- background] × 100.

Molecular docking protocol:

The crystal structure of STAT3 with PDB id: 6NJS[106] was obtained from RCSB protein data bank. GLDE 5.6[54] module in the Schrodinger suite 2010 was used for molecular docking simulations. The protein was prepared using default parameters with the protein preparation wizard in maestro. A grid was created around the active site of STAT3 by selecting co-crystallized ligand with vander Waals scaling for non-polar atoms as 0.9. The synthesized thiadiazine derivatives were sketched using maestro build panel and prepared by using Ligprep application. Finally, the low energy conformers of the ligands were docked into the active site by employing extra precision (XP) docking mode[90].

5.2.6.2. Result and discussions:

Biological activity:

Further, to examine cytotoxic efficacies of above-mentioned synthesized molecules in GL261 and CHO cells, we performed conventional cell viability assays using MTT reagent. The increasing concentrations (2.5-100 µM) of among synthesized molecules, 6A and 6F showed significant cytotoxicity in GL261 (Figure 5.21) and CHO cells (Figure 5.22) at 24 h. Compound 6A and 6F showed more cytotoxicity in cancerous GL261 cells compared to normal CHO cells (Figure 5.21 & 5.22).

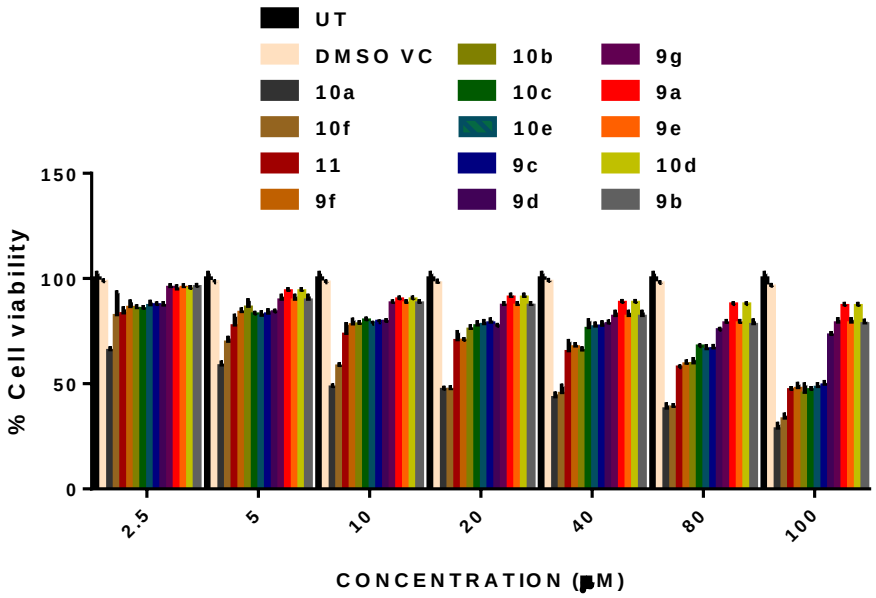


Figure 5.21: Percentage cell viability profiles of CHO cells incubated for 24 h with: Compounds **10a, 10f, 11, 9f, 10b, 10c, 10e, 9c, 9d, 9g, 9a, 9e, 10d, 9b** and DMSO vehicle control (**DMSO VC**).

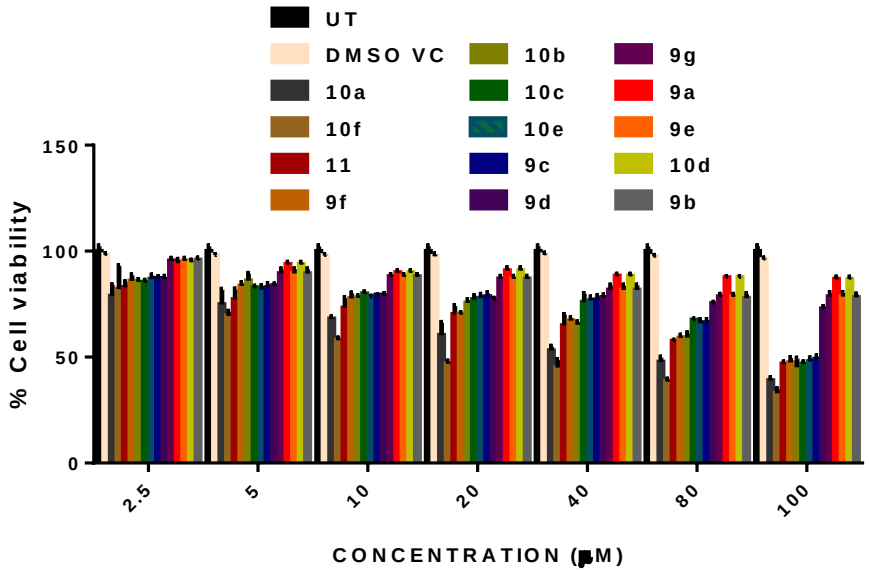


Figure 5.22: Percentage cell viability profiles of CHO cells incubated for 24 h with: Compounds **10a, 10f, 11, 9f, 10b, 10c, 10e, 9c, 9d, 9g, 9a, 9e, 10d, 9b** and DMSO vehicle control (**DMSO VC**).

The remaining synthesized molecules (except 1**0a** and **10f**) showed IC50 of >100 µM in GL261 and CHO cells. The compound **10a** showed nearly comparable cytotoxicity (IC_{50} of 7.1 µM) with the commercially available WP1066 (STAT3 inhibitor, IC_{50} of 4.91 µM) in GL261 cells[107] According to the table 5.19, it clearly indicated that the cell killing properties of **10a** is more in cancerous cells compared to non-cancer cells. Therefore, low doses of compound **10a** is efficiently kill cancerous cells.

Table 5.19: IC_{50} values in µM for the compounds **10a** and **10f** against human cancer cell line through Cell Viability (MTT) Assay.[a]

Entry	Compound	IC_{50} (GL261 cells) µM	IC_{50} (CHO cells) µM
1	**10a**	**7.1 ± 1.3**	21 ± 2.53
2	**10f**	15 ± 1.48	20 ± 2.47

Molecular docking:

WP 1066 displayed potent cancer cell cytotoxicity against various cancers and demonstrated therapeutic in vivo efficacy against leukemia[108], squamous cell cancer[109], gliomas[110–112],

renal cell cancer[113], melanoma[114–116], breast cancer[117] and non-small cell lung cancers[118]. It is under clinical trials (NCT01904123) and effectively blocks the transcriptional activity of STAT3. In present study WP1066 was used as standard. Further recent studies have shown that thiadiazine can act as selective inhibitors of STAT3[119]. Therefore, 6NJS was selected for carrying out docking studies.

In order to gain more insights into the interactions, the synthesized thiadiazine derivatives were docked into the active site of STAT3. The compounds showed hydrogen bond interactions with Glu 638, Tyr 640, Tyrb 657, Lys 658, Met 660, Gln 644 and Gly 656, and π-cationic interactions with Lys 658 with dock scores ranging from -0.129 to -1.647 kcal/mol (Table 5.20). The standard WP 1066 showed only one hydrogen bond interaction with Tyr 640 (Figure 5.23 (a) and (b)) with a dock score of -1.371 kcal/mol. The best active compounds **10a** and **10f** showed two and three hydrogen bond interactions respectively with Tyr 657, Lys 658 (Figure 5.23 (c) and (d)) and Glu 638, Tyr 640 and Lys 658 with dock scores of -1.647 and -1.470 kcal/mol respectively. Additionally, compound **10f** showed π-cationic interaction with Lys 658 apart from hydrogen bond interactions. It is evident that compounds **10a** and **10f** were found to be potent and had high dock scores against STAT3.

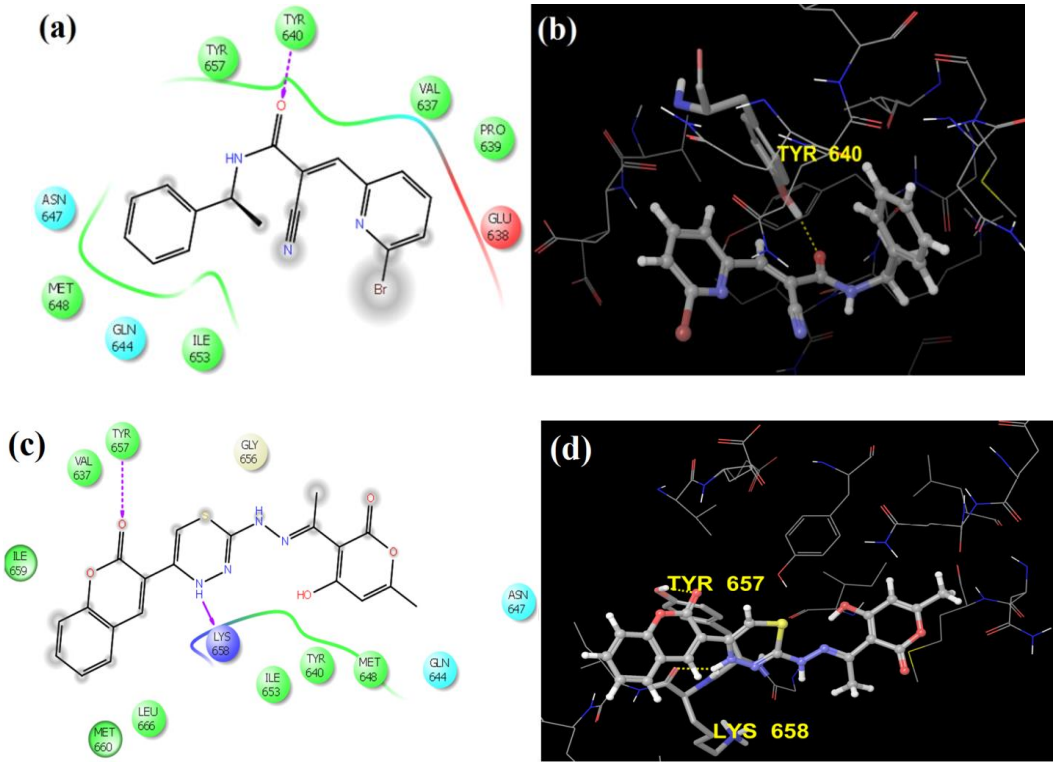


Figure 5.23: 2D Ligand interaction diagram and 3D docked pose of WP 1066 ((a) and (b)) and compound **10a** ((c) and (d)) respectively with the active site of STAT3.

Table 5.20: Docking results of newly synthesized thiadiazine derivatives.

Entry	Compound	Dock score (kcal/mol)
1	**9a**	-0.611
2	**9b**	-0.129
3	**9c**	-1.178
4	**9d**	-1.010
5	**9e**	-0.569
6	**9f**	-1.276
7	**9g**	-0.860
8	**10a**	**-1.647**
9	**10b**	-1.256
10	**10c**	-1.206
11	**10d**	-0.462
12	**10e**	-1.195
13	**10f**	-1.470
14	**11**	-1.366
15	**WP 1066**	-1.371

According to the structure activity relationship, the presence of coumarin moiety in the compound **10a** was responsible for the potential anti-cancer activity of these compounds. While in the other compounds i.e., the compounds **10b-f** and **11** also contain the coumarin moiety in their core structures they are not much potent as **10f**. since, there are substitutions on the coumarin moiety, the sterical hindrance plays vital role in their moderate activity.

All the compounds were screened for their *in vitro* anti-cancer activity and the results revealed that the compound **10a** manifested significant activity against GL261 cells. Moreover, the binding patterns of all synthesized thiadiazines with STAT3 was explored through molecular docking simulations. The study revealed that compounds 6a and 6f has high dock scores with favourable orientation within the STAT3 binding site.

5.3. Conclusion:

In summary, among all the tested compounds (**1a-l, 2a-n, 3a-b, 4a-n, 5a-f, 6a-f, 7a-b, 8a-l, 9a-g, 10a-f and 11**), compound **1b, 4m, 7b, 7a, 8h, 10a** exhibited good anticancer activity and also compound **2e** showed good antimicrobial activity.

References:

[1] J. Ramírez, L. Svetaz, J. Quiroga, R. Abonia, M. Raimondi, S. Zacchino, B. Insuasty, *Eur. J. Med. Chem.* **2015**, *92*, 866–875.

[2] S. Ningaiah, U. K. Bhadraiah, S. D. Doddaramappa, S. Keshavamurthy, C. Javarasetty, *Bioorg. Med. Chem. Lett.* **2014**, *24*, 245–248.

[3] A. M. Vijesh, A. M. Isloor, V. Prabhu, S. Ahmad, S. Malladi, *Eur. J. Med. Chem.* **2010**, *45*, 5460–5464.

[4] A. Tanitame, Y. Oyamada, K. Ofuji, M. Fujimoto, N. Iwai, Y. Hiyama, K. Suzuki, H. Ito, H. Terauchi, M. Kawasaki, et al., *J. Med. Chem.* **2004**, *47*, 3693–3696.

[5] A. Manvar, A. Bavishi, A. Radadiya, J. Patel, V. Vora, N. Dodia, K. Rawal, A. Shah, *Bioorg. Med. Chem. Lett.* **2011**, *21*, 4728–4731.

[6] D. A. Ostrov, J. A. Hernández Prada, P. E. Corsino, K. A. Finton, N. Le, T. C. Rowe, *Antimicrob. Agents Chemother.* **2007**, *51*, 3688–3698.

[7] M. Joao Matos, S. Vazquez-Rodriguez, L. Santana, E. Uriarte, C. Fuentes-Edfuf, Y. Santos, A. Munoz-Crego, *Med. Chem. (Los. Angeles).* **2012**, *8*, 1140–1145.

[8] Y. Shi, C.-H. Zhou, *Bioorg. Med. Chem. Lett.* **2011**, *21*, 956–960.

[9] P. Shah, T. M. Dhameliya, R. Bansal, M. Nautiyal, D. N. Kommi, P. S. Jadhavar, J. P. Sridevi, P. Yogeeswari, D. Sriram, A. K. Chakraborti, *Med. Chem. Commun.* **2014**, *5*, 1489–1495.

[10] B. P. Mallikarjuna, B. S. Sastry, G. V. Suresh Kumar, Y. Rajendraprasad, S. M. Chandrashekar, K. Sathisha, *Eur. J. Med. Chem.* **2009**, *44*, 4739–4746.

[11] Z. Xu, C. Gao, Q.-C. Ren, X.-F. Song, L.-S. Feng, Z.-S. Lv, *Eur. J. Med. Chem.* **2017**, *139*, 429–440.

[12] A. E. Rashad, M. I. Hegab, R. E. Abdel-Megeid, N. Fathalla, F. M. E. Abdel-Megeid, *Eur. J. Med. Chem.* **2009**, *44*, 3285–3292.

[13] R. K. Rawal, R. Tripathi, S. B. Katti, C. Pannecouque, E. De Clercq, *Eur. J. Med. Chem.* **2008**, *43*, 2800–2806.

[14] M. Popsavin, S. Spaić, M. Svirčev, V. Kojić, G. Bogdanović, V. Popsavin, *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4123–4127.

[15] G. Nitulescu, C. Draghici, O. Olaru, *Int. J. Mol. Sci.* **2013**, *14*, 21805–21818.

[16] H. Kumar, D. Saini, S. Jain, N. Jain, *Eur. J. Med. Chem.* **2013**, *70*, 248–258.

[17] M. E. Riveiro, A. Moglioni, R. Vazquez, N. Gomez, G. Facorro, L. Piehl, E. R. de Celis, C. Shayo, C. Davio, *Bioorg. Med. Chem.* **2008**, *16*, 2665–2675.

[18] M. Basanagouda, V. B. Jambagi, N. N. Barigidad, S. S. Laxmeshwar, V. Devaru,

Narayanachar, *Eur. J. Med. Chem.* **2014**, *74*, 225–233.

[19] Y. Kashman, K. R. Gustafson, R. W. Fuller, J. H. Cardellina, J. B. McMahon, M. J. Currens, R. W. Buckheit, S. H. Hughes, G. M. Cragg, M. R. Boyd, *J. Med. Chem.* **1992**, *35*, 2735–2743.

[20] Y. Shikishima, Y. Takaishi, G. Honda, M. Ito, Y. Takeda, O. K. Kodzhimatov, O. Ashurmetov, K.-H. Lee, *Chem. Pharm. Bull. (Tokyo).* **2001**, *49*, 877–880.

[21] C. M. Yeung, L. L. Klein, C. A. Flentge, J. T. Randolph, C. Zhao, M. Sun, T. Dekhtyar, V. S. Stoll, D. J. Kempf, *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2275–2278.

[22] B. Das, V. Saidi Reddy, R. Ramu, *J. Mol. Catal. A Chem.* **2006**, *252*, 235–237.

[23] A. K. Ghosh, D. D. Anderson, *Future Med. Chem.* **2011**, *3*, 1181–1197.

[24] Y.-T. Lai, T. Wang, S. O'Dell, M. K. Louder, A. Schön, C. S. F. Cheung, G.-Y. Chuang, A. Druz, B. Lin, K. McKee, et al., *Nat. Commun.* **2019**, *10*, 47.

[25] R. G. Kalkhambkar, G. M. Kulkarni, H. Shivkumar, R. N. Rao, *Eur. J. Med. Chem.* **2007**, *42*, 1272–1276.

[26] M. A. Chowdhury, K. R. A. Abdellatif, Y. Dong, D. Das, M. R. Suresh, E. E. Knaus, *J. Med. Chem.* **2009**, *52*, 1525–1529.

[27] S. Viveka, Dinesha, P. Shama, G. K. Nagaraja, S. Ballav, S. Kerkar, *Eur. J. Med. Chem.* **2015**, *101*, 442–451.

[28] Y. Bansal, P. Sethi, G. Bansal, *Med. Chem. Res.* **2013**, *22*, 3049–3060.

[29] K. Fylaktakidou, D. Hadjipavlou-Litina, K. Litinas, D. Nicolaides, *Curr. Pharm. Des.* **2004**, *10*, 3813–3833.

[30] P. Anand, B. Singh, N. Singh, *Bioorg. Med. Chem.* **2012**, *20*, 1175–1180.

[31] L. Piazzi, A. Cavalli, F. Colizzi, F. Belluti, M. Bartolini, F. Mancini, M. Recanatini, V. Andrisano, A. Rampa, *Bioorg. Med. Chem. Lett.* **2008**, *18*, 423–426.

[32] M. C. Forest, P. Lahouratate, M. Martin, G. Nadler, M. J. Quiniou, R. G. Zimmermann, *J. Med. Chem.* **1992**, *35*, 163–172.

[33] A. N. Shaw, R. Tedesco, R. Bambal, D. Chai, N. O. Concha, M. G. Darcy, D. Dhanak, K. J. Duffy, D. M. Fitch, A. Gates, et al., *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4350–4353.

[34] M. Manjunatha, V. H. Naik, A. D. Kulkarni, S. A. Patil, *J. Coord. Chem.* **2011**, *64*, 4264–4275.

[35] M. Watzka, C. Geisen, C. G. Bevans, K. Sittinger, G. Spohn, S. Rost, E. Seifried, C. R. Muller, J. Oldenburg, *J. Thromb. Haemost.* **2011**, *9*, 109–118.

[36] S. Weigt, N. Huebler, R. Strecker, T. Braunbeck, T. H. Broschard, *Reprod. Toxicol.* **2012**, *33*, 133–141.

[37] W. C. Patt, H. W. Hamilton, M. D. Taylor, M. J. Ryan, D. G. Taylor, C. J. C. Connolly, A. M. Doherty, S. R. Klutchko, I. Sircar, *J. Med. Chem.* **1992**, *35*, 2562–2572.

[38] C. Abstracts, A. Haragun, *United States Patent (19) AfC*, **1999**.

[39] I. G. E. Gore, N. Branch, T. Roman, E. Orange, K. Wayne, R. A. Gore, *United States Patent (19)*, **1998**.

[40] P. E. G. Daus, A. E. M. Kapner, *Nir*, **1986**.

[41] T. Baier, G. Dupeux, S. Herbert, S. Hardt, D. Quéré, *Phys. Rev. E* **2013**, *87*, 021001.

[42] H. N. Karade, B. N. Acharya, M. Sathe, M. P. Kaushik, *Med. Chem. Res.* **2008**, *17*, 19–29.

[43] A. Rouf, C. Tanyeli, *Eur. J. Med. Chem.* **2015**, *97*, 911–27.

[44] M. Narender, M. S. Reddy, R. Sridhar, Y. V. D. Nageswar, K. R. Rao, *Tetrahedron Lett.* **2005**, *46*, 5953–5955.

[45] K. D. Hargrave, F. K. Hess, J. T. Oliver, *J. Med. Chem.* **1983**, *26*, 1158–1163.

[46] P. R. Verhoest, D. S. Chapin, M. Corman, K. Fonseca, J. F. Harms, X. Hou, E. S. Marr, F. S. Menniti, F. Nelson, R. O'Connor, et al., *J. Med. Chem.* **2009**, *52*, 5188–5196.

[47] J. C. Jaen, L. D. Wise, B. W. Caprathe, H. Tecle, S. Bergmeier, C. C. Humblet, T. G. Heffner, L. T. Meltzer, T. A. Pugsley, *J. Med. Chem.* **1990**, *33*, 311–317.

[48] M. Brvar, A. Perdih, M. Oblak, L. P. Mašič, T. Solmajer, *Bioorg. Med. Chem. Lett.* **2010**, *20*, 958–962.

[49] T. Khan, K. Sankhe, V. Suvarna, A. Sherje, K. Patel, B. Dravyakar, *Biomed. Pharmacother.* **2018**, *103*, 923–938.

[50] R. Thomsen, M. H. Christensen, *J. Med. Chem.* **2006**, *49*, 3315–3321.

[51] D. S. Goodsell, A. J. Olson, *Proteins Struct. Funct. Genet.* **1990**, *8*, 195–202.

[52] G. Jones, P. Willett, R. C. Glen, A. R. Leach, R. Taylor, *J. Mol. Biol.* **1997**, *267*, 727–748.

[53] G. M. Morris, D. S. Goodsell, R. S. Halliday, R. Huey, W. E. Hart, R. K. Belew, A. J. Olson, *J. Comput. Chem.* **1998**, *19*, 1639–1662.

[54] Schrödinger Llc New York Ny, *Glid. Schrödinger LLC NY* **2011**.

[55] N. Hoettecke, S. Rotzoll, U. Albrecht, M. Lalk, C. Fischer, P. Langer, *Bioorg. Med. Chem.* **2008**, *16*, 10319–10325.

[56] L. Kouegnigan Rerambiah, J.-C. Ndong, P. Mbakob Mengue Massoua, S. Medzegue, M. Elisee-Ndam, A. Mintsa-Ndong, J. F. Djoba Siawaya, *Int. J. Infect. Dis.* **2014**, *29*, 48–53.

[57] B. Ryan-Payseur, Z. Ali, D. Huang, C. Y. Chen, L. Yan, R. C. Wang, W. E. Collins, Y. Wang, Z. W. Chen, *J. Infect. Dis.* **2011**, *204*, 1450–62.

[58] P. Aragade, R. V. Narayanan, V. Maddi, P. Patil, P. Shinde, M. Agrawal, *J. Enzyme Inhib. Med. Chem.* **2012**, *27*, 849–853.

[59] V. Varshney, N. N. Mishra, P. K. Shukla, D. P. Sahu, *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3573–3576.

[60] B. Spellberg, R. Guidos, D. Gilbert, J. Bradley, H. W. Boucher, W. M. Scheld, J. G. Bartlett, J. Edwards, *Clin. Infect. Dis.* **2008**, *46*, 155–164.

[61] T. Felicetti, R. Cannalire, D. Pietrella, G. Latacz, A. Lubelska, G. Manfroni, M. L. Barreca, S. Massari, O. Tabarrini, K. Kieć-Kononowicz, et al., *J. Med. Chem.* **2018**, *61*, 7827–7848.

[62] J. Ferlay, M. Colombet, I. Soerjomataram, C. Mathers, D. M. Parkin, M. Piñeros, A. Znaor, F. Bray, *Int. J. Cancer* **2019**, *144*, 1941–1953.

[63] S. H. Hassanpour, M. Dehghani, *J. Cancer Res. Pract.* **2017**, *4*, 127–129.

[64] N. Parsa, *Iran. J. Public Health* **2012**, *41*, 1–9.

[65] A. Lewandowska, M. Rudzki, S. Rudzki, T. Lewandowski, B. Laskowska, *Ann. Agric. Environ. Med.* **2019**, *26*, 1–7.

[66] M. C. White, D. M. Holman, J. E. Boehm, L. A. Peipins, M. Grossman, S. Jane Henley, *Am. J. Prev. Med.* **2014**, *46*, S7–S15.

[67] W. T. Johnston, F. Erdmann, R. Newton, E. Steliarova-Foucher, J. Schüz, E. Roman, *Cancer Epidemiol.* **2020**, 101662.

[68] C. B. Blackadar, *World J. Clin. Oncol.* **2016**, *7*, 54.

[69] R. S. Y. Wong, *J. Exp. Clin. Cancer Res.* **2011**, DOI 10.1186/1756-9966-30-87.

[70] A. Letai, *Annu. Rev. Cancer Biol.* **2017**, *1*, 275–294.

[71] T. N. Seyfried, L. C. Huysentruyt, *Crit. Rev. Oncog.* **2013**, DOI 10.1615/CritRevOncog.v18.i1-2.40.

[72] A. C. Society, *CA. Cancer J. Clin.* **2020**, 1–76.

[73] M. Vanneman, G. Dranoff, *Nat. Rev. Cancer* **2012**, DOI 10.1038/nrc3237.

[74] V. Schirrmacher, *Int. J. Oncol.* **2019**, *54*, 407–419.

[75] C. Regnard, M. Kindlen, C. Regnard, M. Kindlen, in *Support. Palliat. Care Cancer*, **2019**.

[76] R. M. Klevens, *JAMA* **2007**, *298*, 1763.

[77] T. J. Foster, J. A. Geoghegan, V. K. Ganesh, M. Höök, *Nat. Rev. Microbiol.* **2014**, *12*, 49–62.

[78] S. J. Liu, S. W. N. Ueng, E. C. Chan, S. S. Lin, C. H. Tsai, F. C. Wei, C. H. Shih, *J. Biomed. Mater. Res.* **1999**, *48*, 613–620.

[79] R. H. Shoemaker, *Nat. Rev. Cancer* **2006**, *6*, 813–823.

[80] G. Bollag, P. Hirth, J. Tsai, J. Zhang, P. N. Ibrahim, H. Cho, W. Spevak, C. Zhang, Y. Zhang, G. Habets, et al., *Nature* **2010**, *467*, 596–599.

[81] J. K. Kim, I. S. Yang, H. J. Shin, K. J. Cho, E. K. Ryu, S. H. Kim, S. S. Park, K. H. Kim, *Proc. Natl. Acad. Sci.* **2006**, *103*, 1732–1737.

[82] R. B. G. Ravelli, B. Gigant, P. A. Curmi, I. Jourdain, S. Lachkar, A. Sobel, M. Knossow, *Nature* **2004**, *428*, 198–202.

[83] I. A. Al-Suwaidan, A. A.-M. Abdel-Aziz, T. Z. Shawer, R. R. Ayyad, A. M. Alanazi, A. M. El-Morsy, M. A. Mohamed, N. I. Abdel-Aziz, M. A. A. El-Sayed, A. S. El-Azab, *J. Enzyme Inhib. Med. Chem.* **2016**, *31*, 78–89.

[84] T. A. Farghaly, N. El-Metwaly, A. M. Al-Soliemy, H. A. Katouah, Z. A. Muhammad, R. Sabour, *Polycycl. Aromat. Compd.* **2019**, *6638*, 1–17.

[85] M. Eder, U. Schlattner, T. Wallimann, A. Becker, W. Kabsch, K. Fritz-Wolf, *Protein Sci.* **2008**, *8*, 2258–2269.

[86] E. Pitta, E. Tsolaki, A. Geronikaki, J. Petrović, J. Glamočlija, M. Soković, E. Crespan, G. Maga, S. S. Bhunia, A. K. Saxena, *Medchemcomm* **2015**, *6*, 319–326.

[87] R. H. Flatman, A. Eustaquio, S.-M. Li, L. Heide, A. Maxwell, *Antimicrob. Agents Chemother.* **2006**, *50*, 1136–42.

[88] J. Lu, S. Patel, N. Sharma, S. M. Soisson, R. Kishii, M. Takei, Y. Fukuda, K. J. Lumb, S. B. Singh, *ACS Chem. Biol.* **2014**, *9*, 2023–2031.

[89] S. R. Peddi, S. K. Sivan, V. Manga, *J. Biomol. Struct. Dyn.* **2018**, *36*, 486–503.

[90] R. A. Friesner, J. L. Banks, R. B. Murphy, T. A. Halgren, J. J. Klicic, D. T. Mainz, M. P. Repasky, E. H. Knoll, M. Shelley, J. K. Perry, et al., *J. Med. Chem.* **2004**, *47*, 1739–1749.

[91] P. Ertl, B. Rohde, P. Selzer, *J. Med. Chem.* **2000**, *43*, 3714–3717.

[92] C. A. Lipinski, F. Lombardo, B. W. Dominy, P. J. Feeney, *Adv. Drug Deliv. Rev.* **1997**, *23*, 3–25.

[93] M. M. Matin, S. C. Bhattacharjee, P. Chakraborty, M. S. Alam, *Carbohydr. Res.* **2019**, *485*, 107812.

[94] S. Alam, F. Khan, *Sci. Rep.* **2018**, *8*, 5524.

[95] K. swamy G, G. Ramesh, P. K, S. Banu, R. B, H. J. Preritha, B. S. Rajeshwari, M. Ravikumar, P. Raghuram Shetty, D. B. Aruna Kumar, et al., *Chem. Data Collect.* **2020**, *28*, 100452.

[96] C. M. Nisha, A. Kumar, A. Vimal, B. M. Bai, D. Pal, A. Kumar, *J. Mol. Graph. Model.* **2016**, *65*, 100–107.

[97] A. Daina, V. Zoete, *ChemMedChem* **2016**, DOI 10.1002/cmdc.201600182.

[98] A. Thakur, R. Singla, V. Jaitak, *Eur. J. Med. Chem.* **2015**, *101*, 476–495.

[99] S. Fortin, L. Wei, E. Moreau, J. Lacroix, M.-F. Côté, E. Petitclerc, L. P. Kotra, R. C.-Gaudreault, *J. Med. Chem.* **2011**, *54*, 4559–4580.

[100] D.-J. Hwang, J. Wang, W. Li, D. D. Miller, *ACS Med. Chem. Lett.* **2015**, *6*, 993–997.

[101] S. Combes, P. Barbier, S. Douillard, A. McLeer-Florin, V. Bourgarel-Rey, J.-T. Pierson, A. Y. Fedorov, J.-P. Finet, J. Boutonnat, V. Peyrot, *J. Med. Chem.* **2011**, *54*, 3153–3162.

[102] S.-N. Kim, N. H. Kim, Y. S. Park, H. Kim, S. Lee, Q. Wang, Y. K. Kim, *Biochem. Pharmacol.* **2009**, *77*, 1773–1779.

[103] D. E. McNamara, S. Senese, T. O. Yeates, J. Z. Torres, *Protein Sci.* **2015**, *24*, 1164–1172.

[104] A. Daina, O. Michielin, V. Zoete, *Sci. Rep.* **2017**, *7*, 42717.

[105] D. E. V. Pires, T. L. Blundell, D. B. Ascher, *J. Med. Chem.* **2015**, *58*, 4066–4072.

[106] L. Bai, H. Zhou, R. Xu, Y. Zhao, K. Chinnaswamy, D. McEachern, J. Chen, C.-Y. Yang, Z. Liu, M. Wang, et al., *Cancer Cell* **2019**, *36*, 498-511.e17.

[107] M. Ott, C. Kassab, A. Marisetty, Y. Hashimoto, J. Wei, D. Zamler, J.-S. Leu, K.-H. Tomaszowski, A. Sabbagh, D. Fang, et al., *Clin. Cancer Res.* **2020**, *26*, 4983–4994.

[108] A. Ferrajoli, S. Faderl, Q. Van, P. Koch, D. Harris, Z. Liu, I. Hazan-Halevy, Y. Wang, H. M. Kantarjian, W. Priebe, et al., *Cancer Res.* **2007**, *67*, 11291–11299.

[109] X. ZHOU, Y. REN, A. LIU, L. HAN, K. ZHANG, S. LI, P. LI, P. LI, C. KANG, X. WANG, et al., *Oncol. Rep.* **2014**, *31*, 2173–2180.

[110] T. A. Doucette, L.-Y. Kong, Y. Yang, S. D. Ferguson, J. Yang, J. Wei, W. Qiao, G. N. Fuller, K. P. Bhat, K. Aldape, et al., *Neuro. Oncol.* **2012**, *14*, 1136–1145.

[111] A. Iwamaru, S. Szymanski, E. Iwado, H. Aoki, T. Yokoyama, I. Fokt, K. Hess, C. Conrad, T. Madden, R. Sawaya, et al., *Oncogene* **2007**, *26*, 2435–2444.

[112] L.-Y. Kong, A. S. Wu, T. Doucette, J. Wei, W. Priebe, G. N. Fuller, W. Qiao, R. Sawaya, G. Rao, A. B. Heimberger, *Clin. Cancer Res.* **2010**, *16*, 5722–5733.

[113] A. Horiguchi, T. Asano, K. Kuroda, A. Sato, J. Asakuma, K. Ito, M. Hayakawa, M. Sumitomo, T. Asano, *Br. J. Cancer* **2010**, *102*, 1592–1599.

[114] L.-Y. Kong, M. K. Abou-Ghazal, J. Wei, A. Chakraborty, W. Sun, W. Qiao, G. N. Fuller, I. Fokt, E. A. Grimm, R. J. Schmittling, et al., *Clin. Cancer Res.* **2008**, *14*, 5759–5768.

[115] L.-Y. Kong, J. Wei, A. K. Sharma, J. Barr, M. K. Abou-Ghazal, I. Fokt, J. Weinberg, G. Rao, E. Grimm, W. Priebe, et al., *Cancer Immunol. Immunother.* **2009**, *58*, 1023–1032.

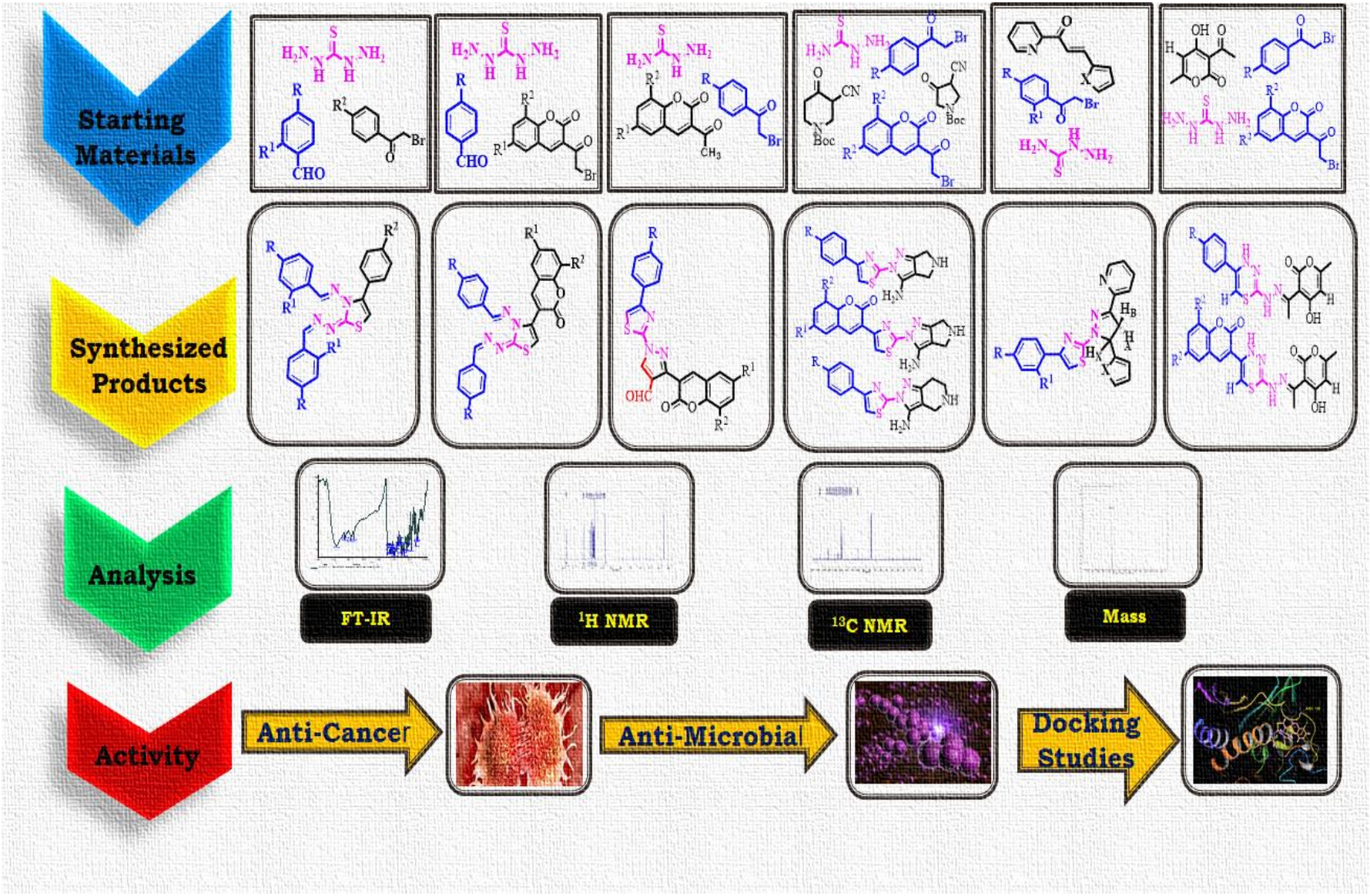
[116] M. A. Hatiboglu, L.-Y. Kong, J. Wei, Y. Wang, K. A. McEnery, G. N. Fuller, W. Qiao, M. A. Davies, W. Priebe, A. B. Heimberger, *Int. J. Cancer* **2012**, *131*, 8–17.

[117] H.-T. Lee, J. Xue, P.-C. Chou, A. Zhou, P. Yang, C. A. Conrad, K. D. Aldape, W. Priebe, C. Patterson, R. Sawaya, et al., *Oncotarget* **2015**, *6*, 10016–10029.

[118] H.-C. Chiu, D.-L. Chou, C.-T. Huang, W.-H. Lin, T.-W. Lien, K.-J. Yen, J. T. A. Hsu, *Biochem. Pharmacol.* **2011**, *81*, 1263–1270.

[119] M. G. LaPorte, Z. Wang, R. Colombo, A. Garzan, V. A. Peshkov, M. Liang, P. A. Johnston, M. E. Schurdak, M. Sen, D. P. Camarco, et al., *Bioorg. Med. Chem. Lett.* **2016**, *26*, 3581–3585.

SUMMARY


Starting Materials
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^{1}H NMR
^{13}C NMR
Mass
Activity
Anti-Cancer
Anti-Microbial
Docking Studies

The thesis entitled **"Synthesis of new nitrogen and sulphur heterocyclic compounds using multicomponent approach and studies on their biological activity"** consists of five chapters, out of which Chapter-I describes about multicomponent reactions and their applications in the synthesis of biological active compounds. The chapters II, III and IV describe the synthesis of nitrogen and sulphur heterocyclic compounds. The V chapter describes the evaluation of biological activity of the synthesized compounds.

CHAPTER-I

A micro review on multicomponent condensation reactions and their utility in the synthesis of biologically active compounds

This chapter describes about the history and importance of multicomponent reactions (MCRs) and 3-substituted coumarins. In the present study the main theme of green chemistry is utilized to synthesize some new heterocyclic compounds. The main advantage of green chemistry[1] is to minimize the production of toxic and hazardous substances during the synthetic protocols in the direction of low risk to nature. This insists the need of exploring green and novel perspectives towards the synthesis of pharmacologically active heterocyclic analogues, which are yet confronting in modern drug exploration and evolution programs. This can be executed through proper choice of safer chemicals in designing renewable raw materials, atom economic procedures with less number of chemical steps, usage of green solvents and development of simple workup and purification techniques[2]. Moreover, there has been an emerging demand for the development of eco-friendly procedures, by way of illustration of multicomponent reactions[3], aqueous medium reactions[4], solid phase synthesis[5], solvent-free reactions[6], ultrasonication[7], and microwave irradiation[8], etc., have been exercised in the pharmaceutical and organic chemistry.

Nevertheless, out of these methods MCR approach furnishes a captivating approach to achieve structurally different scaffolds of organic and pharmaceutical interest. The quick formation of complex compounds from simple substances is a big obstacle in drug discovery and modern organic chemistry[9–11]. For the fabrication of complex compounds, multicomponent reactions indicate a robust chemical tool by reason of lower reaction time, lesser protecting group strategies, mild reaction conditions furthermore step and atom economy[12]. In recent years, the discovery of novel protocols using multicomponent strategy has become an increasingly active area of organic synthesis[13], medicinal chemistry[14], natural product synthesis[15], polymer chemistry[16], agro chemistry[17], and combinatorial chemistry[18].Thus, we have selected the development of new methodologies by applying multicomponent strategy as our research

program.

Objectives of the present work are mentioned and outlines of the work carried out in the present investigations are given.

1. To establish facile, an efficient, and environmentally benign methods for the synthesis of potent biologically active molecules.

2. To establish the structures of newly synthesized compounds by analytical and spectral methods.

3.Screening of the biological activities of the newly synthesized compounds.

The target compounds were synthesized by using readily available starting materials like substituted 3-acetyl coumarins[19,20], 3-(2-bromoacetyl)coumarins[21–23], thiosemicarbazide, thiocarobohydrazide[24,25], Phenacyl bromides, 1-Boc-3-cyano-4-pyrrolidone, 1-Boc-3-cyano-4-piperidone and dehydroacetic acid.

CHAPTER-II

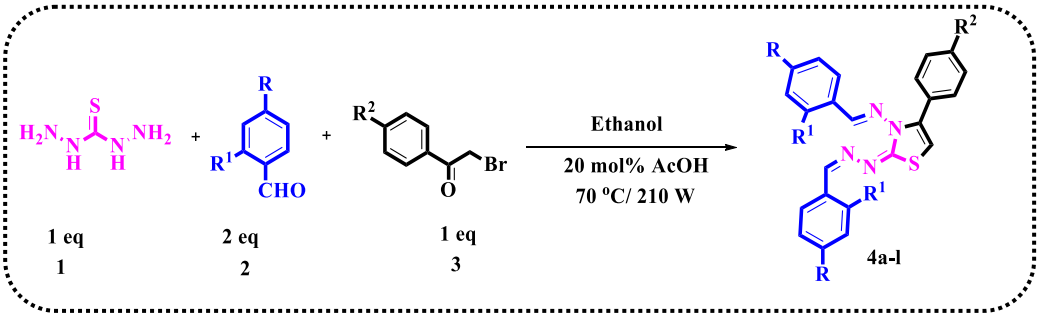
This chapter is divided into two sections.

Microwave irradiated one-pot, pseudo four component synthesis of new thiazoles and microwave irradiated one-pot, pseudo four component synthesis of a new series of hybrid coumarin based thiazoles.

Section-A:

Microwave irradiated one-pot, pseudo four component synthesis of new thiazoles.

The synthesis of target thiazole analogues were carried out as outlined in scheme 1. The title thiazoles (**4a-l**) were synthesized by using thiocarbohydrazide (**1**), aldehydes (**2**) and phenacyl bromides (**3**) (1:2:1) in ethanol in the presence of catalytic amount of acetic acid under microwave irradiation with good yields.



Scheme 1: Synthesis of thiazoles.

For the optimization of reaction, we screened the solvent, temperature, loads of the catalyst and microwave irradiation. The reaction was carried out in different solvents, with temperature,

catalyst mol percentages and different microwave conditions. Hence high yield at less reaction time was observed in ethanol at 70 ^{0}C with 20 mol% acetic acid as catalyst under microwave irradiation (210W).

Table 1: Different substitutions of thiazole hybrids (**4a-l**), time, [a]isolated yield.

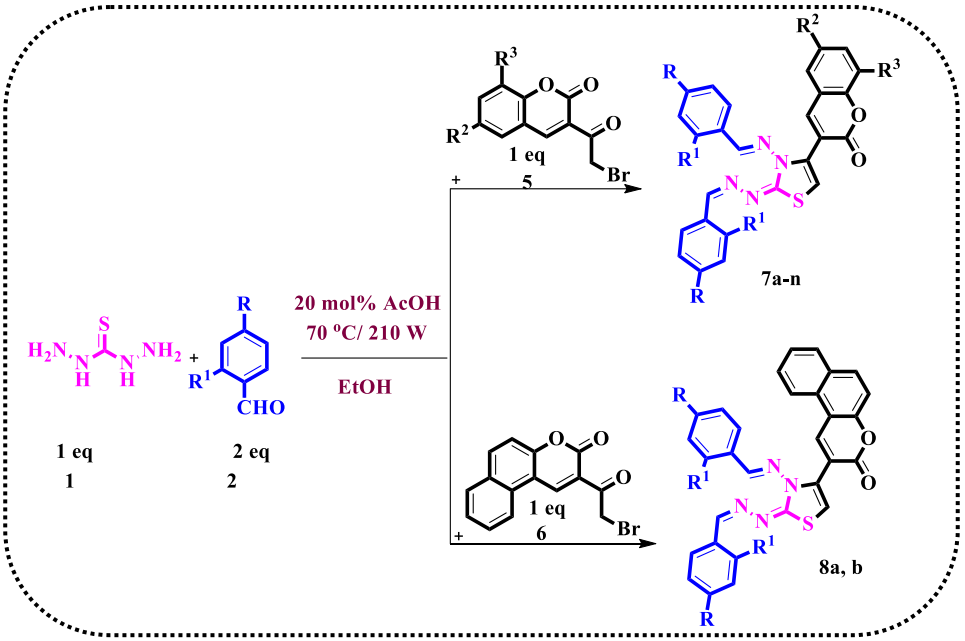
Entry	Product	R	R^1	R^2	Time(min)	Yield (%)[a]
1	**4a**	Cl	H	H	6	88
2	**4b**	OH	OH	OCH_3	5	86
3	**4c**	Cl	H	OCH_3	4	91
4	**4d**	Br	H	OCH_3	5	89
5	**4e**	Cl	H	F	5	82
6	**4f**	Br	H	F	5	85
7	**4g**	Cl	H	Cl	5	92
8	**4h**	Br	H	Cl	6	86
9	**4i**	Cl	H	Br	5	89
10	**4j**	Br	H	Br	6	83
11	**4k**	Cl	H	NO_2	4	92
12	**4l**	Br	H	NO_2	5	85

All the synthesized compounds (**4a-l**) were confirmed by analytical and spectral studies. In conclusion, we have developed a potential green protocol for the synthesis of new thiazole derivatives by the microwave-assisted MCR approach.

Section-B:

Microwave irradiated one-pot, pseudo four component synthesis of a new series of hybrid coumarin based thiazoles.

The synthesis of target coumarin based thiazole analogues were carried out as outlined in scheme 2. The title coumarin based thiazoles (**7a-n**; **8a-b**) were synthesized by a combination of thiocarbohydrazide (**1**), aldehydes (**2**) and substituted 3-(2-bromoacetyl) coumarins (**5, 6**) (1:2:1) in ethanol in the presence of catalytic amount of acetic acid under microwave irradiation with good yields.



Scheme 2: Synthesis of coumarin based thiazoles.

For the optimization of reaction, we screened the solvent, temperature, loads of the catalyst and microwave irradiation. The reaction was carried out in different solvents, with temperature, catalyst mol percentages and different microwave conditions. Hence high yield at less reaction time was observed in ethanol at 70 ^{0}C with 20 mol% acetic acid as catalyst under microwave irradiation (210W).

Table 2: Synthesis of coumarin based thiazole hybrids (**7a-n** and **8a-b**), time, [a]isolated yield.

Entry	Product	R	R^1	R^2	R^3	Time(min)	Yield (%)[a]
13	**7a**	Cl	H	H	H	7	90
14	**7b**	Br	H	H	H	8	89
15	**7c**	Cl	H	Cl	H	6	93
16	**7d**	Br	H	Cl	H	7	91
17	**7e**	Cl	H	Cl	Cl	8	89
18	**7f**	Br	H	Cl	Cl	7	90
19	**7g**	Cl	H	Br	H	5	93
20	**7h**	Br	H	Br	H	6	88
21	**7i**	Cl	H	Br	Br	6	93
22	**7j**	Br	H	Br	Br	8	92
23	**7k**	Cl	H	H	OCH_3	5	90
24	**7l**	Br	H	H	OCH_3	6	89
25	**7m**	Cl	H	Br	OCH_3	6	88
26	**7n**	Br	H	Br	OCH_3	6	91
27	**8a**	Cl	H	-	-	7	92
28	**8b**	Br	H	-	-	8	89

All the synthesized compounds (**7a-n, 8a, b**) were confirmed by their analytical and spectral studies. In conclusion, we have developed a potential green protocol for the synthesis of new

coumarin based thiazole analogues by the microwave-assisted MCR approach.

CHAPTER-III

This chapter includes a short review of related literature on the synthesis and importance of thiazolyl pyrazoles.

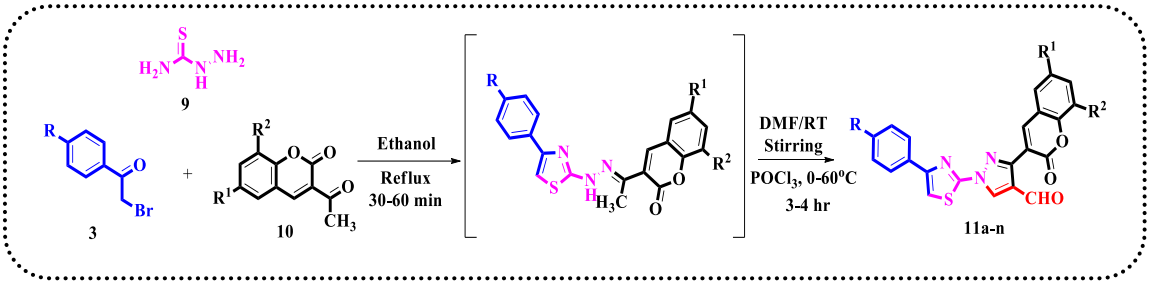
This chapter is divided into three sections.

A facile one-pot three component synthesis of new thiazolyl pyrazole carbaldehydes and a facile one-pot three component synthesis of new thiazolyl pyrazoles and microwave – assisted synthesis of new pyrazolylthiazoles *via* multicomponent approach.

Section-A:

A facile one-pot three component synthesis of new thiazolyl pyrazole carbaldehydes.

Titled compounds(**4a-n**) were achieved by performing sequential Hantzch thiazole synthesis and Vilsmeier–Haack formylation in one-pot by without isolating the intermediate. For the formation of titled compounds is illustrated in scheme 3.



Scheme 3: Synthesis of thiazolyl pyrazole carbaldehydes.

Table 3: Synthesis of coumarin based thiazolyl pyrazole carbaldehydes (**11a-n**), time, [a]isolated yield.

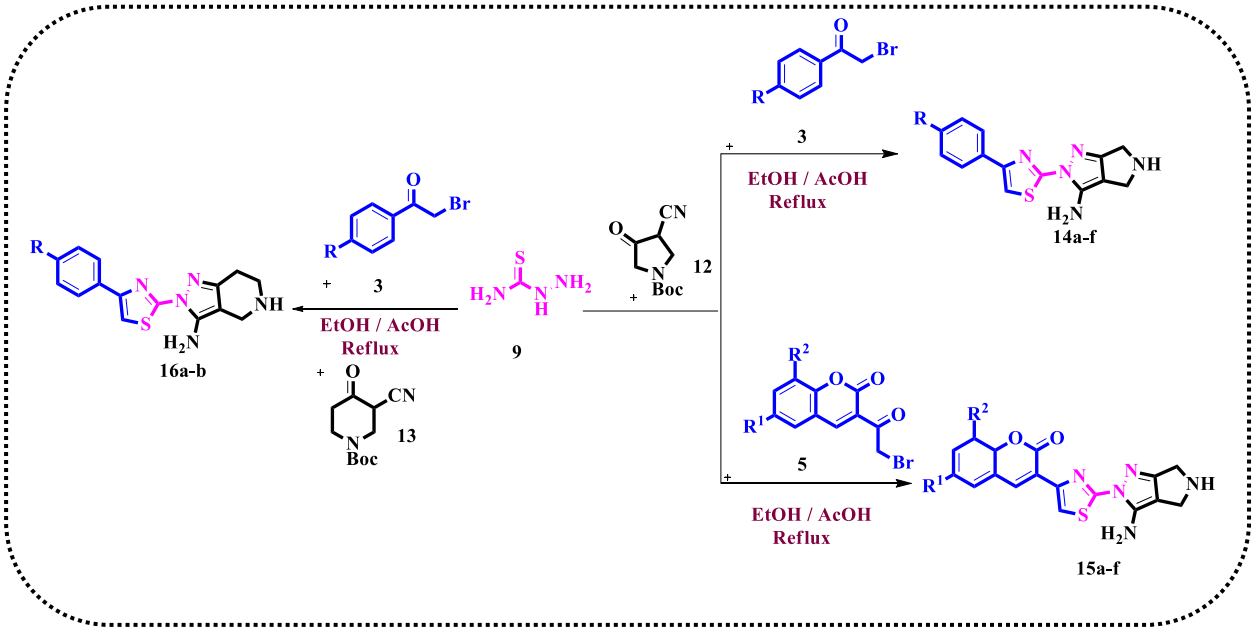
Entry	Product	R	R^1	R^2	Time(h)	Yield (%)[a]
29	**11a**	H	H	H	4	88
30	**11b**	H	Cl	H	4.5	85
31	**11c**	H	Cl	Cl	4.5	90
32	**11d**	H	Br	H	5	88
33	**11e**	H	Br	Br	5	92
34	**11f**	H	H	OCH_3	4	89
35	**11g**	H	Br	OCH_3	4.5	87
36	**11h**	OCH_3	H	H	4	91
37	**11i**	OCH_3	Cl	H	4.5	86
38	**11j**	OCH_3	Cl	Cl	5	90
39	**11k**	OCH_3	Br	H	5	92
40	**11l**	OCH_3	Br	Br	5	91
41	**11m**	OCH_3	H	OCH_3	4.5	89
42	**11n**	OCH_3	Br	OCH_3	4.5	90

All the synthesized compounds (**11a-n**) were confirmed by analytical and spectral studies. In summary, we have developed a potential green protocol for the synthesis of new thiazolyl pyrazole carbaldehyde derivatives by the VMHR multi component reaction approach.

Section-B:

Facile one-pot three component synthesis of new thiazolyl pyrazoles.

The title compounds (**14a-f**, **15a-f** and **16a-b**) were synthesized by reaction an equimolar ratio of thiosemicarbazide (**9**), substituted phenacyl bromides (**3**) or substituted 3-(2-bromoacetyl) coumarins (**5**) with 1-Boc-3-cyano-4-pyrrolidone (**12**) or 1-Boc-3-cyano-4-piperidone (**13**) and catalytic amount of acetic acid and ethanol as solvent under reflux conditions with good yields. The reaction and its conditions were outlined in scheme 4.



Scheme 4: Synthesis of thiazolyl pyrazoles

For the optimization of reaction, we screened the solvents, temperature, and loads of the catalyst. By screening of solvents under ethanol as a solvent, we got best results in terms of yield. Then subsequently reaction was carried out with different temperature and different acetic acid mol percentages. Hence 30 mol% of CH_3COOH as a catalyst, ethanol as a solvent under reflux conditions has manifested best results in terms of yield and time.

Table 4: Synthesis of thiazolyl pyrazole hybrids (1**4a-f, 15a-f** and **16a-b**), time, [a]isolated yield.

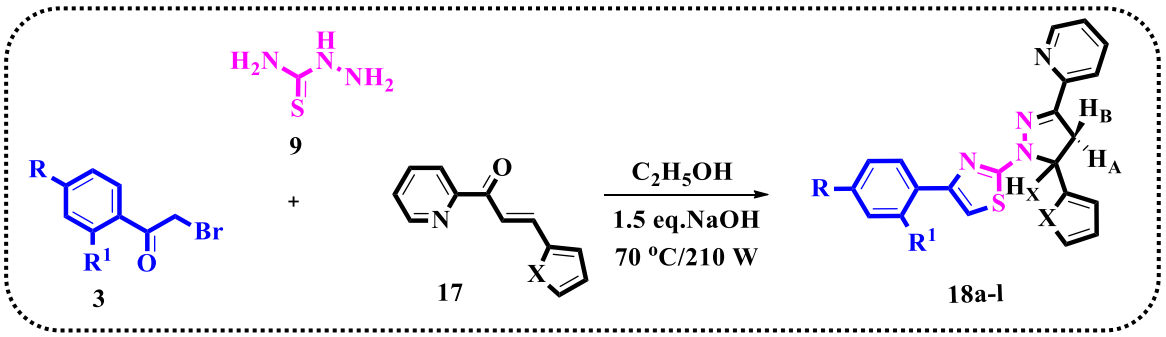
Entry	Product	R	R^1	R^2	Time(h)	Yield (%)[a]
43	**14a**	H	-	-	5	83
44	**14b**	CH_3	-	-	5	84
45	**14c**	OCH_3	-	-	6	88
46	**14d**	F	-	-	5	84
47	**14e**	Br	-	-	6	87
48	**14f**	NO_2	-	-	6	88
49	**15a**	-	H	H	5	91
50	**15b**	-	Cl	H	6	89
51	**15c**	-	Br	H	5	90
52	**15d**	-	Br	Br	5	91
53	**15e**	-	H	OCH_3	6	89
54	**155f**	-	Br	OCH_3	5	92
55	**16a**	CH_3	-	-	6	87
56	**16b**	OCH_3	-	-	5	85

All the synthesized compounds (**14a-f, 15a-f** and **16a-b**) were confirmed by their analytical and spectral studies. In summary, we have developed a potential green protocol for the synthesis of new thiazolyl pyrazole derivatives by a multi component reaction approach.

Section-C:

Microwave–assisted synthesis of new pyrazolylthiazoles *via* multicomponent approach.

Dihydropyrazoles are considered prime pharmacophores in many medicinal applications. In this 2-(5-(furan-2-yl)-3-(pyridin-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)-4-phenylthiazoles (**18a-l**) were synthesized starting from the reaction of substituted 2-bromoacetophenones (**3**) with thiosemicarbazide (**9**) and 3-(furan-2-yl)-1-(pyridin-2-yl)prop-2-en-1-one **17a** or 1-(pyridin-2-yl)-3-(thiophen-2-yl)prop-2-en-1-one **17b** under microwave irradiation in presence of alcoholic sodium hydroxide. The general schematic representation of reaction is outlined in scheme 5.



Scheme 5: Synthesis of pyrazolylthiazoles.

For the optimization of reaction, we screened the solvent, temperature, loads of the base and

microwave irradiation. By screening of solvents under ethanol as a solvent, we got best results in terms of yield. Then subsequently reaction was carried out with different temperature, bases and different microwave conditions. Hence high yields of the desired product at shorter reaction time has been observed in ethanol at 70 ^{0}C in presence of NaOH (1.5 eq) under microwave (210 W).

Table 5: Synthesis of pyrazolylthiazole hybrids (**18a-l**), time, [a]isolated yield.

Entry	Product	R	R^1	X	Time(min)	Yield (%)[a]
57	**18a**	H	H	O	5	95
58	**18b**	CH3	H	O	6	93
59	**18c**	OCH3	H	O	6	92
60	**18d**	F	H	O	5	91
61	**18e**	Cl	H	O	6	88
62	**18f**	Br	H	O	5	96
63	**18g**	Cl	Cl	O	6	88
64	**18h**	NO2	H	O	5	90
65	**18i**	H	H	S	6	86
66	**18j**	Cl	H	S	5	81
67	**18k**	Br	H	S	6	87
68	**18l**	Cl	Cl	S	6	92

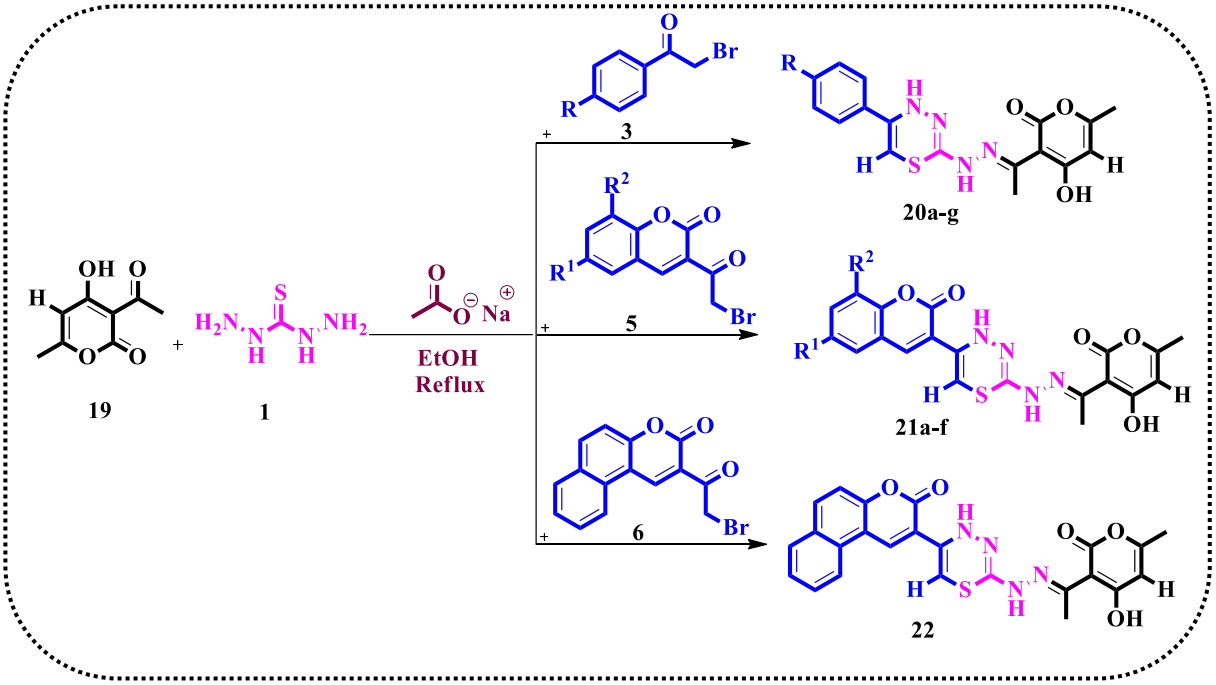
All these target compounds (**18a-l**) were confirmed by their spectral and analytical studies. In summary, we have developed a potential green protocol for the synthesis of new pyrazolylthiazole derivatives by microwave assisted multi component reaction.

CHAPTER-IV

One-pot, three component synthesis of a new series of 1,3,4 thiadiazines

This chapter includes a short review of related literature on the synthesis and importance of 1,3,4-thiadiazines.

The title compounds (**20a-g**, **21a-f** and **22**) were obtained by the reaction of an equimolar ratio of dehydroacetic acid (**19**), thiocarbohydrazide (**1**) and phenacyl bromides (**3**) or substituted 3-(2-bromoacetyl) coumarins (**5, 6**), along with sodium acetate in a catalytic amount and ethanol under reflux conditions with good yields. The general schematic representation is outlined in scheme 6.



Scheme 6: Synthesis of 1,3,4-thiadiazines *via* multi component reaction.

For the optimization of reaction, we screened the solvents, temperature, and loads of the catalyst. By screening of solvents under ethanol as a solvent, we got best results in terms of yield. Then subsequently reaction was carried out with different temperature and different sodium acetate mol percentages. Hence high yield at less reaction time was observed in ethanol under reflux conditions with 30 mol% sodium acetate as a catalyst.

Table 6: Synthesis of 1,3,4-thiadiazine hybrids (**20a-g, 21a-f** and **22**), time, [a]isolated yield.

Entry	Product	R	R^1	R^2	Time(h)	Yield (%)[a]
69	**20a**	H	-	-	5	91
70	**20b**	CH_3	-	-	6	90
71	**20c**	OCH_3	-	-	6	92
72	**20d**	F	-	-	6	92
73	**20e**	Cl	-	-	5	89
74	**20f**	NO_2	-	-	5	91
75	**20g**	Ph	-	-	5	84
76	**21a**	-	H	H	5	93
77	**21b**	-	Cl	H	5	89
78	**21c**	-	Br	H	5	93
79	**21d**	-	Br	Br	5	92
80	**21e**	-	H	OCH_3	5	88
81	**21f**	-	Br	OCH_3	5	94
82	**22**	-	-	-	6	91

All the synthesized compounds (**20a-g**, **21a-f** and **22**) were confirmed by analytical and spectral studies. In summary, we have developed a potential green protocol for the synthesis of new

1,3,4 thiadiazine derivatives by a one pot MCR approach.

CHAPTER-V

Biological activity evaluation of synthesized hetero cyclic compounds.

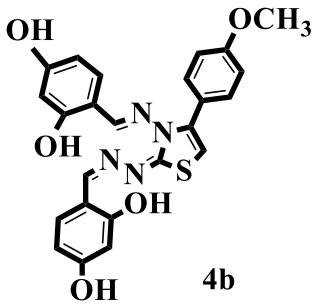
This chapter includes a short review of related literature on the importance of biological heterocyclic compounds.

Anticancer activity of synthesized thiazole derivatives

Biological activity:

Here, out of the twelve compounds (**4a-l**) based on computational studies10 compounds were selected for the screening of their biological activity and the cytotoxic effect of these compounds on 9 different sets (i.e., non-small cell lung, leukemia, CNS, Prostrate, renal, Colon, Ovarian, Melanoma and breast cancers) of cell lines has been determined. Among 10 compounds **4a – 4j**, all the compounds except **4b** have less or no effect on the growth percentage of the cells. Whereas the compound **4b** has significant effect on growth of 17 cell lines with at least two cell lines from each set.

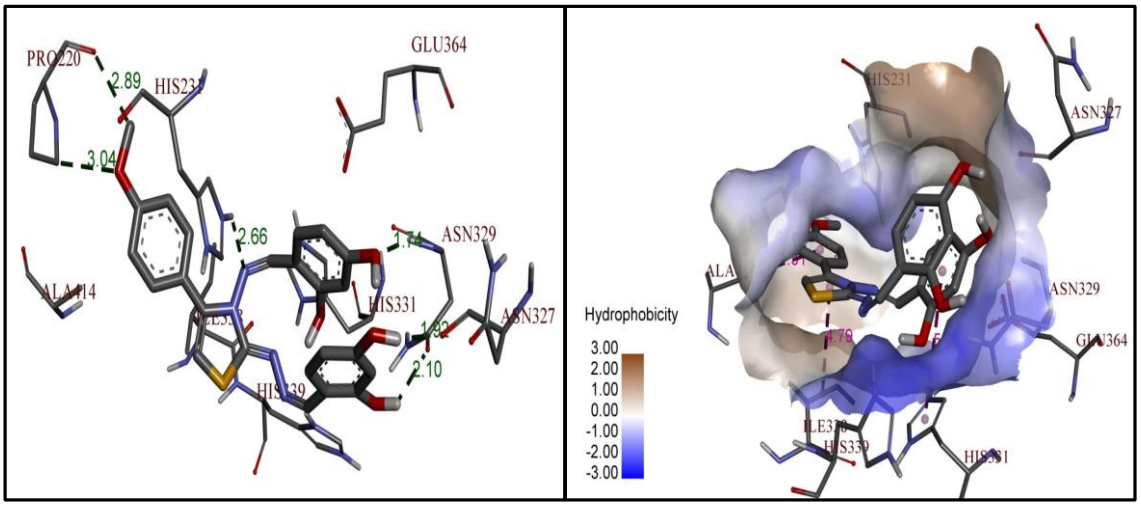
Amongst all the cell line sets it has to be noted that the compound has no effect on leukemia cell lines with IC_{50} values >100 μM. Amongst non-small cell lung cancer set, IC_{50} values were in the range of 9 – 34 μM, Out of these non-small cell lung cancer cell lines good activity against NCI-H460 and moderate activity against A549/ATCC, NCI-H23 and NCI-H522 cell lines. In the case of colon cancer, IC_{50} values were in the range of 5 - ~34 μM with highest effect against HCT-116 and moderate activity observed against COLO 205, HCC-2998, SW-620 cell lines. In the set of CNS cancer cell lines, the compound **4b** exhibited good activity against SNB-75, SF-295, 539 and U251 whereas moderate activity was observed on SNB-19. In melanoma cluster of cell lines, the IC_{50} values fell in the range of 5.58-17.6 μM. highest cytotoxic effect was observed against SK-MEL-5 and moderate to lowest activity was noticed against MDA-MB-435, M14, LOX IMVI, SK-MEL-2, 5 MALME-3M, UACC-62 and UACC-257 respectively. The IC_{50} values of ovarian cancer cell lines observed in the range of 18- 45 μM. Highest cytotoxicity was exhibited against OVCAR-3. Least cytotoxicity was observed on IGROVI, OVCAR-4 and the compound **4b** has moderate effect on all the remaining cell lines in the group. Range of cytotoxicity on renal cancer cell lines exerted by compound **4b** was in the limit of 6-28 μM. Moderate inhibition of cell growth was observed in the case of prostate cancer cell line DU-145 and no such effect on PC-3 has been noticed. The IC_{50} values of breast cancer cell lines observed in the range of 8.37- >100 μM. Highest cytotoxicity was exhibited against BT-549. Least cytotoxicity was observed on T-47D, HS 578T.



4b

Molecular docking:

The molecular docking studies were also well correlate with these results. The molecular docking results showed that the compound **4b** exhibited least binding energies -9.83, -9.26 and -9.54 kcal/mol with the proteins 2AE4, 1M17 and 1QH4 respectively. Moreover, it can be judged from molecular docking studies that the presence of –OH groups and the thiazole ring in **4b** can be favorable for anti-cancer activity. Furthermore, *in vivo* studies for this compound **4b** have to be carried out.



a) Hydrogen bonding interactions b) Hydrophobic interactions.

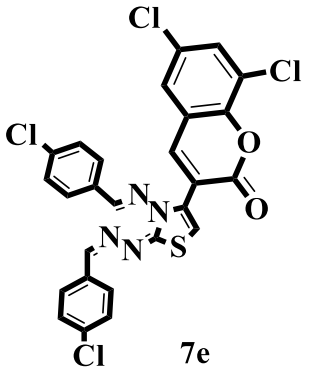
Figure 1: The best docking poses of the compound **4b** with the protein 2AE4.

Antibacterial activity of synthesized coumarin based thiazole derivatives

Biological activity:

The *in vitro* antibacterial activities of the synthesized coumarin based thiazoles (**7a-n** and **8a-b**) were carried out against a Gram positive bacterium *Staphylococcus aureus* (ATCC-12600). The MICs of standard (Novobiocin) drug, tested compounds(**7a-n** and **8a-b**) were measured using a well-defined and standardized broth micro-dilution technique[26].The minimum concentration of anti-bacterial agents needed to inhibit the bacterial growth is called minimum inhibitory

concentration (MIC). When we compare with the standard (Novobiocin) drug (MIC value 2 µg/ml) compound **7e** showed significant antibacterial influence on the *S. aureus* with Minimum Inhibitory Concentration value 3 µg/ml. And also compounds **7f, 7h, 7n,** and **8b** exhibit moderate activity against *S. aureus* with MIC values 6, 5, 6 and 5 µg/ml respectively.



7e

Molecular docking:

Protein-ligand interactions between novobiocin (standard) and topoisomerase showed three hydrogen bond interactions (two with Arg 138 and one with Lys 36), dock score of Novobiocin was found to be -7.356 kcal/mol. Compound **7e** which exhibited magnificent anti-bacterial activity showed high dock score of -6.342 kcal/mol. It showed a hydrogen bond interaction with Arg 138. Almost all the synthesized compounds showed hydrogen bond interactions with either Arg 138 (or) Lys 36 as that of standard novobiocin. The carbonyl group was responsible for the formation of hydrogen bond interactions in the newly synthesized compounds.

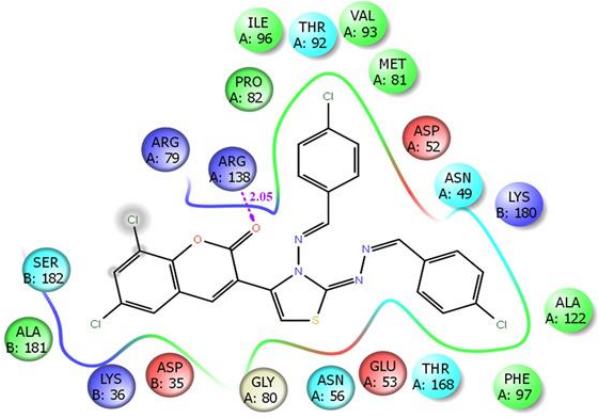
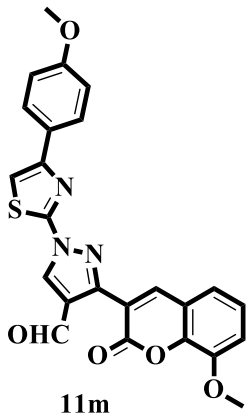


Figure 2: Ligand interaction diagrams synthesized compound **7e** showing hydrogen bond interactions with Arg 138 and Lys 36 (pink lines) along with distances in Å, salt bridge with Arg 79 and π-cationic interactions with Lys 36.

Anticancer activity of synthesized thiazolyl pyrazole carbaldehyde derivatives

Biological activity:

The anti-cancer activity of the synthesized thiazolyl pyrazole carbaldehyde derivatives(**11a-n**) were tested against different human cancer cell lines such as cervical cancer (HeLa), breast cancer (MCF7) and adenocarcinoma (A549) cell lines by using nocadazole as a positive control. The cellular viability in the absence and presence of the synthesized thiazolyl pyrazole carbaldehyde compounds were determined using MTT assay. In the context of HeLa cell lines, compound **11m** (IC_{50} = 9.05 µM) exhibited significant antiproliferative when compared to the other synthesized compounds. Moreover, compound **11m** against MCF7 cell line have shown good activity with IC_{50} value 7.12 µM. and **11a** against MCF7 exhibited moderate activity with IC_{50} value of 15.46 µM, Furthermore, compound **11m** exhibited good activity with IC_{50} value 6.34 µM against the cell line A549. And also, the compound **11a** against the A549 cell line have shown modest inhibiting activity with IC_{50} value 11.64 µM. whereas all other synthesized compounds showed less anti-cancer activity on the A549 cell line.



11m

Molecular docking:

The X-ray crystal structure of β-tubulin was retrieved from the protein data bank (PDB id: 5CA1). it was used for docking the newly synthesized coumarin derivatives into 5CA1 active site. The standard nocodazole showed three hydrogen bond interactions with Asn 165 and Glu 198 at the colchicine binding site of β-tubulin with a dock score of -6.869 kcal/mol. The newly synthesized coumarin derivatives showed hydrogen bond interactions with Asn 165, Cys 239, Gln 245 and Thr 351 and π-cationic interactions with Lys 350. The dock scores of these derivatives ranged from -4.376 to -6.054 kcal/mol. Compound **11m** which showed relatively good dock score in the series showed hydrogen bond interaction with Asn 165 with dock score of -6.054 kcal/mol. The dock scores of newly synthesized coumarin derivatives corroborated well with the experimental anti-proliferation studies.

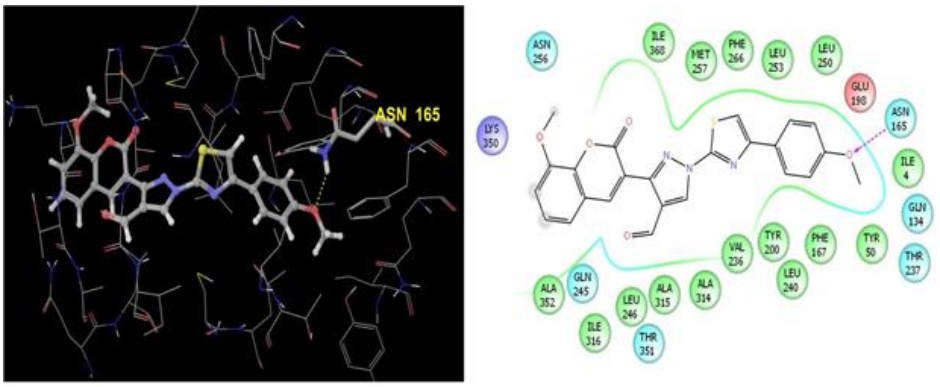
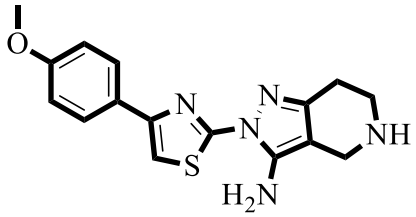


Figure 3: Dock pose overlay and Ligand interaction diagram of compound **11m** at the colchicine binding site of β-tubulin.

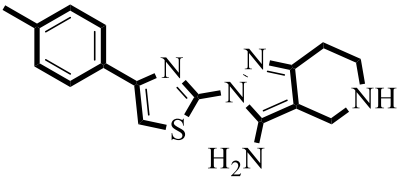
Anticancer activity of synthesized thiazolyl pyrazole derivatives

Biological activity:

The anti-cancer activity of the synthesized thiazolyl pyrazole derivatives (**14a-f, 15a-f** and **16a-b)** were screened against different human cancer cell lines such as cervical cancer (HeLa), adenocarcinoma (A549) and breast cancer (MCF7) cell lines by using combretastatin A-4 as a positive control. The cellular viability in the presence and absence of the synthesized thiazolyl pyrazole derivatives were determined using MTT assay. In the context of HeLa cell lines, compounds **16b** (IC_{50} = 3.60 µM) and **16a** (IC_{50} = 4.61 µM) exhibited good antiproliferative activity when compared to the other synthesized compounds. And compound **5d** (IC_{50} = 8.17 µM) showed significant activity on HeLa cell lines. Moreover, Compound **7b** (IC_{50} = 4.17 µM) and **7a** (IC_{50} = 5.29 µM) have shown good activity against A549 cell line furthermore, **5d** (IC_{50} = 9.06 µM) and **5b** (IC_{50} = 9.64 µM) manifested modest activity against A549 cell line. And also, Compound **7b** and **7a** exhibited good activity with IC_{50} values 3.94 µM, 4.92 µM against the cell line MDA-MB-231. Furthermore, the compound **5d** against the MDA-MB-231 cell line have shown modest inhibiting activity with IC_{50} value 9.63 µM.



16b



16a

Molecular docking:

Combretastatin A-4 is a well-known tubulin polymerization inhibitor which binds to colchicine

binding site of β-tubulin. The standard used in this study was combretastatin A-4 therefore crystal structure of tubulin i.e. 4YJ2 was chosen for docking studies. To understand and explore the plausible binding modes and mode of action, the synthesized thiazolyl pyrazole derivatives (**14a-f, 15a-f** and **16a-b)** were docked into the colchicine binding site of β-tubulin. The standard combretastatin A-4 showed three hydrogen bond interactions, two with Glh 200 and one with Leu 255 at colchicine binding site of β-tubulin. It showed a dock score of -8.452 kcal/mol. The best active molecule among the series i.e. **16b** showed two hydrogen bond interactions with Gln 136 and Glh 200 (Figure 5.19 (c) and (d)) with a dock score of -8.791 kcal/mol. The dock scores of remaining compounds ranged from -2.481 to-8.777 kcal/mol. These compounds displayed hydrogen bond interactions with Gln 136, Cys 241, Leu 255, Glh 200, Tyr 202 and val 238. The dock scores of these newly synthesized thiazolyl pyrazole derivatives well concurred with the experimental studies.

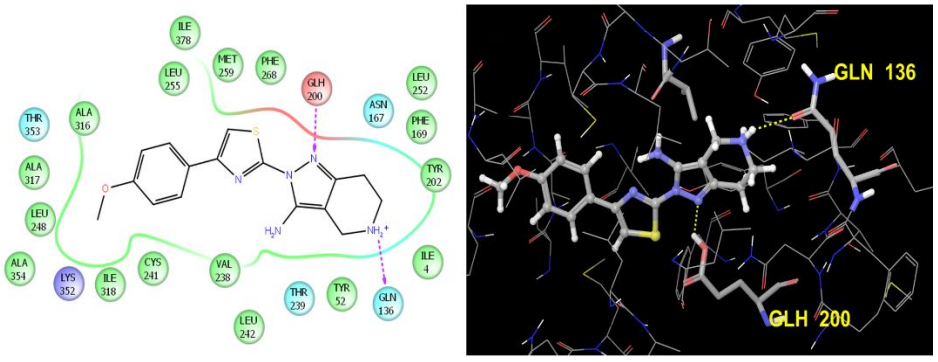


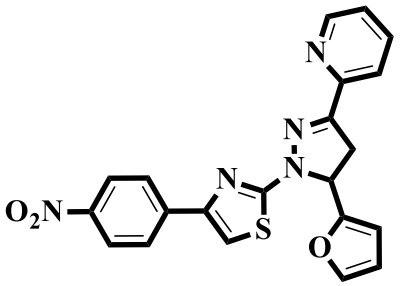
Figure 4: Ligand interaction diagram and docked pose of compound **16b** at the colchicine binding site of β-tubulin.

Anticancer activity of synthesized pyrazolylthiazole derivatives

Biological activity:

The anti-proliferative potency of the synthesized compounds (**18a-l**) was screened against various human cancer cell lines *viz.*, adenocarcinoma (A549), cervical cancer (HeLa), human neuroblastoma (SK-N-SH) and human prostate cancer (DU-145) cell lines by using doxorubicin (DOX) as a positive control. The IC_{50} values, *i.e.*, the cellular viability in the presence and absence of the synthesized compounds were determined using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. In the context of HeLa cell lines, compound **18h** (IC_{50} = 9.97 µM) have shown considerable antiproliferative when compared to the other synthesized compounds. After **8h**, the compounds **8j**, **8i** and **8c** have shown modest activity with

IC_{50} values 15.61, 18.47, 19.78 µM respectively. From the *in vitro* results, it was found that the compound **18h** has potent activity against HeLa cell line with IC_{50} value 9.97 µM.

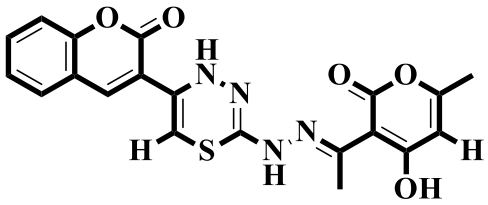


18h

Anticancer activity of synthesized 1,3,4 thiadiazine derivatives

Biological activity:

All the newly-synthesized compounds (**20a-g, 21a-f, 22**) were evaluated for their *in vitro* anticancer activity. The compound **21a** showed nearly comparable cytotoxicity (IC_{50} of 7.1 µM) with the commercially available WP1066 (STAT3 inhibitor, IC_{50} of 4.91 µM) in GL261 cells.



21a

Molecular docking:

In order to gain more insights into the interactions, the synthesized thiadiazine derivatives (**20a-g, 21a-f, 22**) were docked into the active site of STAT3. The compounds showed hydrogen bond interactions with Glu 638, Tyr 640, Tyrb 657, Lys 658, Met 660, Gln 644 and Gly 656, and π-cationic interactions with Lys 658 with dock scores ranging from -0.129 to -1.647 kcal/mol. The standard WP 1066 showed only one hydrogen bond interaction with Tyr 640 with a dock score of -1.371 kcal/mol. The best active compounds **21a** and **21f** showed two and three hydrogen bond interactions respectively with Tyr 657, Lys 658 and Glu 638, Tyr 640 and Lys 658 with dock scores of -1.647 and -1.470 kcal/mol respectively. Additionally, compound **21f** showed π-cationic interaction with Lys 658 apart from hydrogen bond interactions. It is evident that compounds **21a** and **21f** were found to be potent and had high dock scores against STAT3.

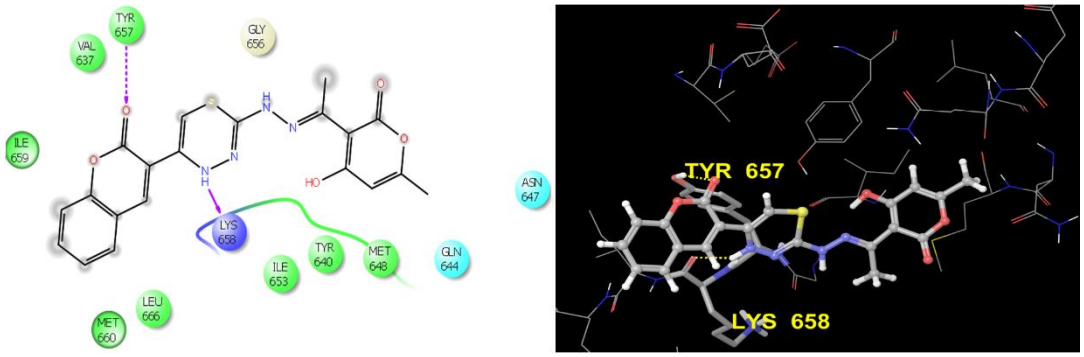


Figure 5: 2D Ligand interaction diagram and 3D docked pose of compound **21a** with the active site of STAT3.

conclusion:

In this research work, we developed a potential protocol for the synthesis of thiazoles, coumarin based thiazoles, thiazolyl pyrazole carbaldehydes, thiazolyl pyrazoles, pyrazole thiazoles and 1,3,4-thiadiazines by multicomponent reaction approach. Moreover, we synthesized 82 compounds, were confirmed by their analytical and spectral studies. Furthermore, these synthesized compounds were screened for their biological activity. Among all the tested compounds (**4a-l, 7a-n, 8a-b, 11a-n, 14a-f, 15a-f, 16a-b, 18a-l, 20a-g, 21a-f and 22**), compound **4b, 11m, 16b, 16a, 18h, 21a** exhibited good anticancer activity and also compound **7e** showed good antimicrobial activity.

References:

[1] B. A. de Marco, B. S. Rechelo, E. G. Tótoli, A. C. Kogawa, H. R. N. Salgado, *Saudi Pharm. J.* **2019**, *27*, 1–8.

[2] M. Kaljurand, M. Koel, *Crit. Rev. Anal. Chem.* **2011**, *41*, 2–20.

[3] R. C. Cioc, E. Ruijter, R. V. A. Orru, *Green Chem.* **2014**, *16*, 2958–2975.

[4] E. P. da Costa, S. E. Coelho, A. H. de Oliveira, R. M. Araújo, L. N. Cavalcanti, J. B. Domingos, F. G. Menezes, *Tetrahedron Lett.* **2018**, *59*, 3961–3964.

[5] Y. Méndez, G. De Armas, I. Pérez, T. Rojas, M. E. Valdés-Tresanco, M. Izquierdo, M. Alonso del Rivero, Y. M. Álvarez-Ginarte, P. A. Valiente, C. Soto, et al., *Eur. J. Med. Chem.* **2019**, *163*, 481–499.

[6] P. Sun, D. Yang, W. Wei, X. Sun, W. Zhang, H. Zhang, Y. Wang, H. Wang, *Tetrahedron* **2017**, *73*, 2022–2029.

[7] A. Shaabani, S. E. Hooshmand, *Ultrason. Sonochem.* **2018**, *40*, 84–90.

[8] M. B. Gawande, V. D. B. Bonifácio, R. Luque, P. S. Branco, R. S. Varma, *ChemSusChem* **2014**, *7*, 24–44.

[9] H. M. L. Davies, E. J. Sorensen, *Chem. Soc. Rev.* **2009**, *38*, 2981.

[10] A. Fürstner, *Chem. Soc. Rev.* **2009**, *38*, 3208.

[11] T. Hudlicky, J. W. Reed, *Chem. Soc. Rev.* **2009**, *38*, 3117.

[12] S. Brauch, S. S. van Berkel, B. Westermann, *Chem. Soc. Rev.* **2013**, *42*, 4948.

[13] Y. Gu, *Green Chem.* **2012**, *14*, 2091.

[14] E. Ruijter, R. V. A. Orru, *Drug Discov. Today Technol.* **2013**, *10*, e15–e20.

[15] A. Ulaczyk-Lesanko, D. G. Hall, *Curr. Opin. Chem. Biol.* **2005**, *9*, 266–276.

[16] Y.-H. Jin, L. Cai, Z.-S. Cheng, H. Cheng, T. Deng, Y.-P. Fan, C. Fang, D. Huang, L.-Q. Huang, Q. Huang, et al., *Mil. Med. Res.* **2020**, *7*, 4.

[17] T. W. Jabusch, R. S. Tjeerdema, *J. Agric. Food Chem.* **2006**, *54*, 5962–5967.

[18] R. V. Sumesh, M. Muthu, A. I. Almansour, R. Suresh Kumar, N. Arumugam, S. Athimoolam, E. A. Jeya Yasmi Prabha, R. R. Kumar, *ACS Comb. Sci.* **2016**, *18*, 262–270.

[19] R. Aggarwal, S. Kumar, P. Kaushik, D. Kaushik, G. K. Gupta, *Eur. J. Med. Chem.* **2013**, *62*, 508–514.

[20] A. Arshad, H. Osman, M. C. Bagley, C. K. Lam, S. Mohamad, A. S. M. Zahariluddin, *Eur. J. Med. Chem.* **2011**, *46*, 3788–3794.

[21] C. F. Koelsch, *J. Am. Chem. Soc.* **1950**, *72*, 2993–2995.

[22] N. Guravaiah, V. R. Rao, *Phosphorus. Sulfur. Silicon Relat. Elem.* **2010**, *185*, 361–367.

[23] P. V. Kumar, V. R. Rao, *Indian J. Chem.* **2005**, *44*, 2120–2125.

[24] L. F. Audrieth, E. S. Scott, P. S. Kippur, *J. Org. Chem.* **1954**, *19*, 733–741.

[25] J. R. Reid, N. D. Heindel, *J. Heterocycl. Chem.* **1976**, *13*, 925–926.

[26] E. Pitta, E. Tsolaki, A. Geronikaki, J. Petrović, J. Glamočlija, M. Soković, E. Crespan, G. Maga, S. S. Bhunia, A. K. Saxena, *Medchemcomm* **2015**, *6*, 319–326.

List of publications

1. A facile one-pot, three component synthesis of a new series of 1,3,4-thiadiazines: anticancer evaluation and molecular docking studies.
   S Mamidala, Venugopal Vangala, Saikiran Reddy Peddi, Chedupaka Raju, Vijjulatha Manga, Rajeswar Rao Vedula (2021).
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3. Design and synthesis of new thiazoles by microwave-assisted method: Evaluation as an anti-breast cancer agents and molecular docking studies.
   S Mamidala, VS Mudigunda, SR Peddi, KK Bokara, V Manga, RR Vedula (2020).
   Synthetic Communications, 50 (16), 2488-2501.
4. Microwave-assisted synthesis and biological evaluation of some new pyrazolothiazoles via a multicomponent approach.
   S Mamidala, RK Aravilli, K Vaarla, RR Vedula (2019).
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6. One-Pot, Multi-Component Synthesis of Substituted 2-(6-Phenyl-7*H*-[1,2,4]Triazolo[3,4-*b*][1,3,4]Thiadiazin-3-yl)-2,3-Dihydrophthalazine-1,4-Diones.
   PC Jilloju, **M Srikanth**, SV Kumar, RR Vedula (2020).
   Polycyclic Aromatic Compounds, 1-12.

7. Metal-catalyzed routes to dibenzodiazepines (DBDAs) and structural analogues: recent advances journal of materials chemistry.
   X Aniban, **S Mamidala**, AJ Burke (2018).
   European Journal of Organic Chemistry 2018 (47), 6743-6753.

Presentation & participation in symposia

1. International Conference on Advances in Chemical Sciences and Technologies (ACST 2019) held on 23-25th September 2019, organized by **NIT Warangal**.
2. International Symposium on Synthesis and Catalysis (ISy SyCat)-2017 held on 5-8th September 2017, organized by university of **Evora, PORTUGAL**.
3. 6th international symposium on "Current Trends in Drug Discovery and Research" held on 25-28th February 2016, organised by **CSIR-CDRI, Lucknow**.
4. 7th IEEE International Conference on Technology for Education (T4E) on technology for education held on 10-12th December 2015, organized by **NIT Warangal**.
5. Medicinal Chemistry Conference-cum-workshop (MEDCHEM-2015) on Anti-Diabetic Drug Discovery and Development held on 29-30th October 2015, organized by **IIT Madras**.
6. National Conference on Emerging Trends in Instrumental Methods of Chemical Analysis (ETIMCA- 2019) 30-31st January, 2019, organized by **NIT Warangal**.
7. Five days **GIAN** programme on "Process Intensification" held on 07-11th January 2019, organised by National Institute of Technology Warangal department of Chemical Engineering.
8. National Conference on Recent Advances in Organic Synthesis 29th June, 2016, organized by **NIT Warangal**.
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A facile one-pot, three component synthesis of a new series of 1,3,4-thiadiazines: Anticancer evaluation and molecular docking studies

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ABSTRACT

A series of new 1,3,4-thiadiazines were synthesized by the conventional method of dehydroacetic acid, thiocarbohydrazide and substituted phenacyl bromides or substituted 3-(2-bromoacetyl) coumarins. Structures of all the synthesized compounds were confirmed by spectral (^{1}H &^{13}C NMR, FTIR, Mass) and analytical data. The target compounds were screened for their in vitro anticancer activity, From the in vitro anticancer results, it was found that the compound 6a has shown significant activity with the standard. Furthermore, the synthesized 1,3,4-thiadiazines were inflicted to molecular docking simulations for gaining insights into their mechanism of action and possible mode of binding against STAT3. The docking results were consistent with the experimental data.

1. Introduction

Green chemistry is described by Environmental Protection Agency as adapt of chemical procedures and products that minimize or eradicate the use or generation of hazardous chemicals. The Green chemistry or sustainable chemistry has initiated to save the environment, in which E factor, process mass intensity and atom economy in the chemical procedures are the major focusing areas [1,2].

Moreover, in the green chemistry process for instance Multi Component Reaction [3–5] (MCR), aqueous medium reactions [6], solvent free reactions [7,8], ultra-sonication reactions [9,10], solid phase synthesis [11,12], microwave irradiation [13,14], photochemical synthesis [15] etc. among these green chemistry processes multi component reaction has gained preference on top of conventional and multi-step reaction. Furthermore, it is a powerful chemical tool for the synthesis of complex molecules, in this reaction without isolation of intermediates, minimization of cost, time and waste and also diversified, high atom economy process. In consequence it is a powerful robust tool to synthesize the biological active compounds in pharmaceutical industry [16,17]. Subsequently multi component reaction process is extremely effective to synthesize a wide variety of heterocyclic compounds [18,19].

Among this hetero cyclic compounds 1,3,4-thiadiazines and their derivatives are a prominent class of medicinally relevant compounds on account of their anticancer [20], anti-viral [21], antimicrobial [22], antifungal [23], antibacterial [24], antioxidant [25]. Moreover, thiadiazines and its analogues manifest inhibitory activities like STAT3 inhibitor [26], matrix metalloproteinase inhibitor [27], cyclic AMP phosphodiesterase inhibitor [28], Hepatitis C virus polymerase inhibitor [29], cholinesterase inhibitor [30], cyclindependent kinase inhibitor [31], PDE4 inhibitor [32]. Subsequently coumarin based derivatives are one of the prime class of biologically active compounds due to their antioxidant [33,34], anti-inflammatory [35], antimicrobial [36–38], antiviral [39], antituberculosis [40], anticancer [41,42], anticoagulant [43], anticholinesterase [44,45], antidepressant agent [46] (Fig. 1).

Considering the enormous medicinal and biological importance of 1,3,4-thiadiazines and coumarin motifs and in continu-

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Microwave irradiated one pot, three component synthesis of a new series of hybrid coumarin based thiazoles: Antibacterial evaluation and molecular docking studies

Srikanth Mamidala[a], Sudhir Reddy Peddi[b], R Kowshik Aravilli[c], Parameshwara Chary Jilloju[a], Vijjulatha Manga[b], Rajeswar Rao Vedula[a,*]

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ABSTRACT

A series of new coumarin based thiazoles were synthesized by the microwave irradiation of thiocarbohydrazide, aldehydes and 3-(2-bromoacetyl) coumarins. Structures of all the synthesized compounds were confirmed by spectral (^{1}H &^{13}C NMR, FTIR, Mass) and analytical data. The target compounds were screened for their *in vitro* cytotoxic activity against a Gram positive spheroid firmicute. From the *in vitro* results, it was found that the compound **4e** has bordering on activity with the standard. Furthermore docking studies were also done on these hybrids which endorsed well with the *in vitro* results.

1. Introduction

Indeed from the past so many years forth, the second prime cause of death because of infections [1–3], which is caused by only microorganisms and also microorganisms resistance to multiple antifungal and antibacterial agents have become a major issue [4,5]. The birth of multidrug resistant microbes in various bacterial strains is an alarming element [6,7]. Hence evolution and discovery of novel antibiotics are compulsory to eradicate the growth of MDR pathogens. *Staphylococcus aureus* is a Gram positive bacteria, it is connected with hospital infections, which causes pneumonia, skin infections, bacteremia and also endocarditis [8,9]. As per the Liu et al., S.aureus may become unsusceptible to lincomycins, methicillin, tetracyclines, pencillins, vancomycin and rifampicin [10].

Furthermore, natural products [11] have a vital impact on medicinal chemistry, drug discovery. Coumarins are some of the most abundant metabolites found in extracts of many plant families, such as Euphorbiaceae, Rutaceae, Orchidaceae, and Asteraceae [12,13]. Coumarin and its analogues having exceptional biological activity, these are reported for promising anti-bacterial [14], antifungal [15] and also modulators of antibiotic resistance [16]. And also thiazole ring is available in different natural products [17–19]. Thiazole ring has stimulated a great deal of attraction due to their antineoplastic [20], anti-microbial [21–23], antiviral [24], antimycobacterial [25], HIV infections [26], antihelminthic agent [27], allergies [28] and also treatment of hypertension [29], FabH inhibitors [30], CNS active agents [31] at the same time, diligent literature survey is showing that, thiazole ring associated with coumarin has phenomenal biological activity. Some of the biological imperative compounds containing coumarin, thiazole and also hybrid coumarin and thiazole scaffolds [15,32] were shown Fig. 1.

In continuation of our earlier work on thiazoles [33,34], herein we would like to communicate a green approach for medicinally important coumarin based thiazole compounds. In green chemistry [35,36] environment congenial methodologies for instance multicomponent reaction approach [37–39], aqueous medium reactions [40,41], solid phase synthesis [42,43], solvent free reactions [44–46], ultra sonication [47,48], microwave irradiation [49,50] and so forth. Among these methodologies microwave irradiation and

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Design and synthesis of new thiazoles by microwave-assisted method: Evaluation as an anti-breast cancer agents and molecular docking studies

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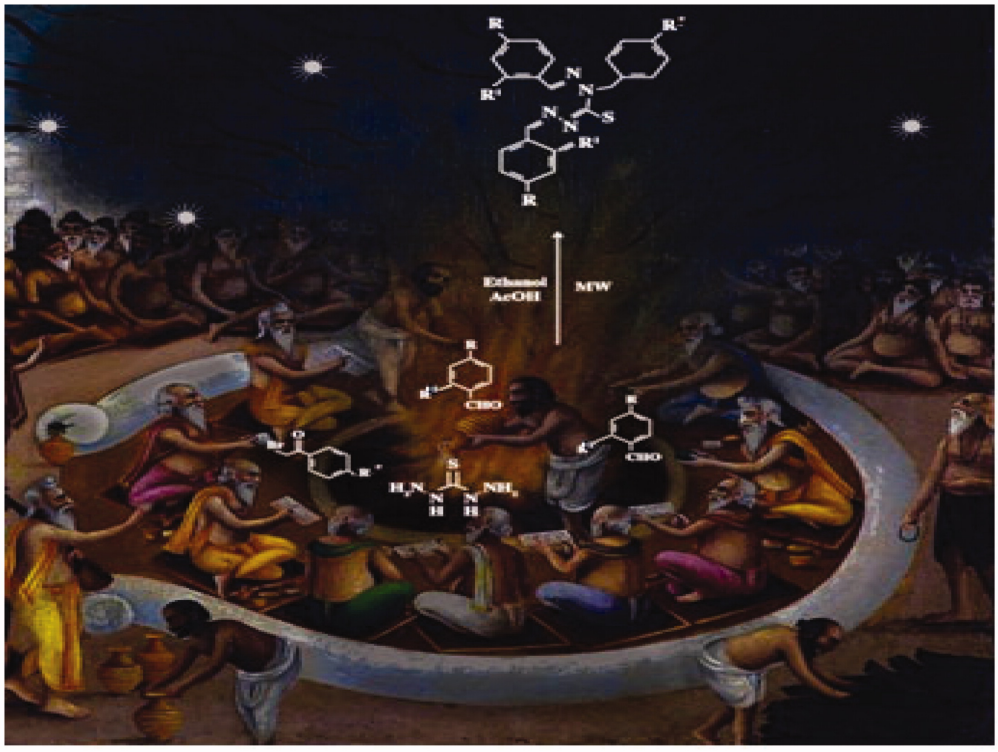
ABSTRACT

A series of novel thiazole analogues were designed and synthesized by the microwave-assisted multicomponent reaction of thiocarbohydrazide, aldehydes with substituted phenacyl bromides. Structures of all the synthesized compounds were in good agreement with their spectral (^{1}H and ^{13}C NMR, FTIR, Mass) and analytical data. The target thiazole compounds were screened for their *in vitro* cytotoxic activity by testing against MCF-7, MDA-MB-231/ATCC, HS 578 T, BT-549, T-47D and MDA-MB-468 Breast cancer cell lines. From the *in vitro* results, it was found that the compound **4b** has potent activity against MDA-MB-231/ATCC cell line. Docking studies were also done on these compounds to probe the possible binding site interactions which corroborated well with the *in vitro* results.



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Medicinal Chemistry & Drug Discovery

Microwave-Assisted Synthesis and Biological Evaluation of Some New Pyrazolothiazoles via a Multicomponent Approach

Srikanth Mamidala,[a] R Kowshik Aravilli,[b] Krishnaiah Vaarla,[a] and Rajeswar Rao Vedula*[a]

A series of new pyrazolothiazole hybrids were designed and synthesized by the microwave-assisted multicomponent reaction of thiosemicarbazide, 3-(furan-2-yl)-1-(pyridin-2-yl)prop-2-en-1-one and 1-(pyridin-2-yl)-3-(thiophen-2-yl)prop-2-en-1-one with substituted 2-bromoacetophenones. Structures of all the synthesized compounds were in good agreement with their spectral (FTIR, ^{1}H & ^{13}CNMR, Mass) and analytical data (CHN analysis). The target compounds were screened for their *in vitro* cytotoxic activity by testing against human adenocarcinoma (A549), cervical cancer (HeLa), human neuroblastoma (SK–N-SH) and human prostate cancer (DU-145) cell lines. From the *in vitro* results, it was found that the compound derived from *p*-NO_2 phenyl (**4h**) has potent activity against HeLa cell line with IC_{50} value 9.97 ± 0.05 μM. Also, the compounds having *p*-OCH_3 phenyl (**4c**), phenyl (**4i**), and *p*-Cl phenyl (**4j**) substitutions exhibited significant activity against HeLa cell line with IC_{50} values 19.78 ± 0.03 μM, 18.47 ± 0.01 μM and 15.61 ± 0.06 μM respectively.

The main theme of green chemistry[1,2] is to minimize the production of toxic and hazardous substances during the synthesis and designing synthetic protocols in the direction of low risk to nature. This insists the need of exploring green and novel perspectives towards the synthesis of pharmacologically active polyheterocyclic analogues, which are yet confronting in modern drug exploration and evolution programs. This can be executed through proper choice of safer chemicals in designing renewable raw materials, atom economic procedures with less number of chemical steps, usage of green solvents and development of simple workup and purification techniques.[3]

Moreover, eco-friendly methodologies such as aqueous medium reactions,[4,5] solvent-free reactions,[6–8] solid phase synthesis,[9,10] multi-component reaction approach,[11–14] microwave irradiation[15–17] and ultrasonication,[18–23] etc., have been employing in the organic, medicinal chemistry. However, among the aforementioned green approaches, microwave irradiation has been prioritized as one of the vital substitutes to traditional synthesis. It has been attained great attention from the scientific community, because of its some attracting properties like uniform heat dissipation, fewer reaction times, low energy utilization, higher atom economy thereby minimal byproduct production.

Furthermore, multicomponent reactions provide a fascinating strategy to accomplish structurally divergent analogues of chemical and medicinal interest. The particular arrangement of various bonds in a single viable step provides a challenge and also a profound perspective for enhancing atom economy, comparatively mild reaction procedures, faster reaction rates and lower reaction times with high yields.

Pyrazole[24] and thiazole[25] rings are quotidian motifs personifying an interest in heterocyclic compounds manifesting in a broad range of pharmacological activities such as anticancer,[26,27] antimicrobial,[28–30] antitubercular,[31,32] anti-inflammatory[33–35] etc. Some of the pharmacological important compounds containing pyrazole and thiazole scaffolds in their molecular frame shown were shown in figure 1, In view of the aforementioned importance of pyrazole, thiazole rings,[36–38] we have concentrated on the sketch of a new structural unit that

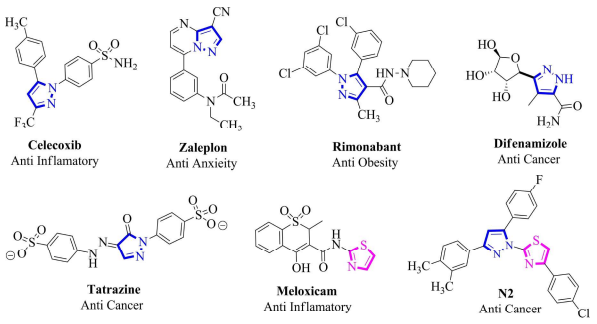

Figure 1. Some of the biologically potent synthetic compounds bearing pyrazole and thiazole motifs.

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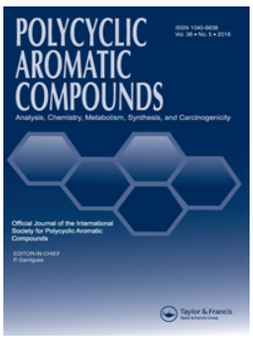

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One-Pot, Multi-Component Synthesis of Substituted 2-(6-Phenyl-7*H*-[1,2,4]Triazolo[3,4-b][1,3,4]Thiadiazin-3-yl)-2,3-Dihydrophthalazine-1,4-Diones

Parameshwara Chary Jilloju, Mamidala Srikanth, Sthalam Vinay Kumar & Rajeswar Rao Vedula

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Microreview

Routes to Dibenzodiazepines

Metal-Catalyzed Routes to Dibenzodiazepines (DBDAs) and Structural Analogues: Recent Advances

Xaiza Aniban,[a] Srikanth Mamidala,[a,b] and Anthony J. Burke*[a,c]

Abstract: It is common knowledge that the use of transition metals as catalysts has greatly revolutionized various coupling procedures to access heterocyclic compounds of significant industrial interest. Dibenzodiazepines (DBDAs) are a particularly important group of heterocyclic compounds, with considerable pharmaceutical applications. In this review, we look at some of the catalytic methods that have been developed during the last 10 years for the synthesis of these targets. Palladium catalysts have been frequently used for these transformations, and particularly for the Buchwald–Hartwig reaction which has been a key reaction in a number of synthetic pathways. Copper has also been frequently used, including some other metals like iron and molybdenum, but to a lesser extent. In most cases, the examples chosen are for the synthesis of DBDAs with interesting medicinal properties and will be of interest to medicinal chemists. It should also be mentioned that due to the structural characteristics of these compounds the potential for diversification – principally for functional group incorporation – is immense. Emerging and facilitating technologies have also been employed for the synthesis of these molecules and are reviewed here.

1.0 Introduction

Many compounds of pharmacological importance contain at least one nitrogen atom.[1] It is well known that nitrogen containing heterocyclic compounds show broad spectrum biological activities, and are present in many pharmaceutical compositions. Within this category, benzodiazepines are particularly attractive because of their broad biological activity spectrum, which range from: anthelmintic, antimicrobial, anticonvulsant, anti-cancer, antipyretic, anti-inflammatory, antianxiety and *anti*-depressive properties.[2–6]

Dibenzodiazepines are a subclass of this family, consisting of a diazepine unit fused with two benzene rings (Figure 1). These compounds have demonstrated antianaphyclactic, antipsychotic and anxiolytic properties.[7–9] Clozapine, a 1,4-dibenzodiazepine is used for treating schizophrenia (Figure 2).[10] Dibenzepine, which is in fact a dibenzodiazepinone derivative, is an antidepressant.[11] Atypical antipsychotics such as olanzapine are also widely used in the treatment of schizophrenia. As a close derivative of clozapine, olanzapine, has beneficial effects on the positive, negative and, to a lesser extent, the cognitive symptoms of schizophrenia in many patients.[12–14]

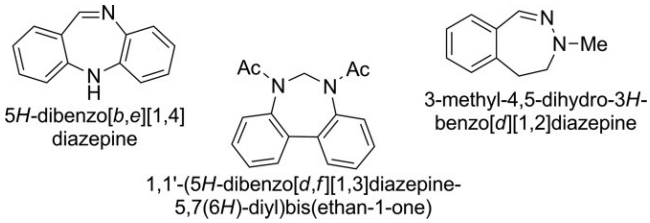

Figure 1. 1,2-, 1,3- and 1,4-dibenzodiazepine variants.

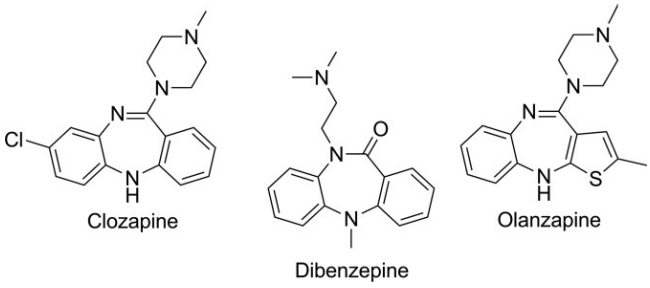

Figure 2. Examples of medicinally relevant DBDAs.

In 1964 the group of Schmutz reported the first synthesis of dibenzodiazepines and their derivatives.[15] Traditionally, dibenzodiazepines and their structural analogues are obtained using classical chemistry via lactam[16] or amide[17] intermediates and then the heterocyclic scaffold is subsequently functionalized to introduce substituents.

However, this generally requires harsh conditions and purification after every step.

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