

**MCR APPROACH FOR THE SYNTHESIS OF NEW
HETEROCYCLIC COMPOUNDS FROM 4-AMINO-5-
HYDRAZINYL-4*H*-1,2,4-TRIAZOLE-3-THIOL**

**THESIS SUBMITTED TO
NATIONAL INSTITUTE OF TECHNOLOGY-WARANGAL
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DOCTOR OF PHILOSOPHY IN CHEMISTRY**

BY

J. PARAMESHWARA CHARY

ROLL NO. 715089

**RESEARCH SUPERVISOR
PROF. V. RAJESWAR RAO**



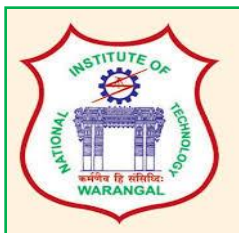
**DEPARTMENT OF CHEMISTRY
NATIONAL INSTITUTE OF TECHNOLOGY-WARANGAL
WARANGAL, TELANGANA STATE, INDIA**

506004

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Prof. V. Rajeswar Rao

E-mail: vrajesw@nitw.ac.in



**Professor, Dean R &C,
Department of Chemistry,
National Institute of Technology,
Warangal-506004, (T.S.),
India.**

CERTIFICATE

This is to certify that the thesis entitled “**MCR Approach for the synthesis of new heterocyclic compounds from 4-amino-5-hydrazinyl-4H-1,2,4-triazole-3-thiol**” submitted by **J. Parameshwara Chary** for the award of the degree of **Doctor of Philosophy in Chemistry**, National Institute of Technology, Warangal (T.S.), under my guidance and supervision. This work has not been submitted earlier either in part or in full for any degree or diploma of any other University.

(Prof. V. Rajeswar Rao)

DECLARATION

I hereby declare that the matter embodied in this thesis entitled “***MCR APPROACH FOR THE SYNTHESIS OF NEW HETEROCYCLIC COMPOUNDS FROM 4-AMINO-5-HYDRAZINYL-4H-1,2,4-TRIAZOLE-3-THIOL***” is based entirely on the results of the investigations and research work carried out by me under the supervision of **Prof. V. Rajeswar Rao**, Department of Chemistry, National Institute of Technology-Warangal. I declare that this is original and has not been submitted in part or full, for any degree or diploma to this or any other University.

(J. PARAMESHWARA CHARY)

Place: Warangal

Date: March 2021

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(Parameshwara Chary Jilloju)

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RESEARCH PUBLICATIONS

CHAPTER-I

A MICRO REVIEW ON MULTI-COMPONENT REACTIONS AND THEIR APPLICATIONS.

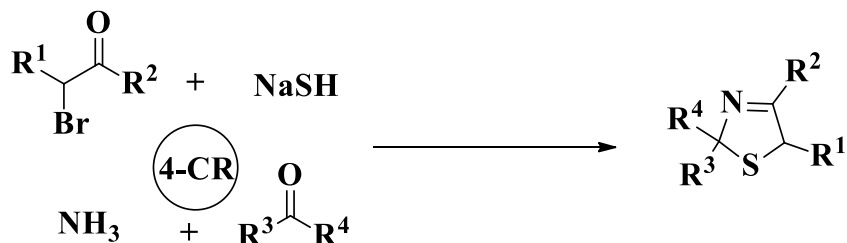
1. INTRODUCTION:

Multi-component condensation reactions (MCRs) are the chemical reactions in which three or more components come together in a single reaction vessel to form a product that retains the majority of the atoms of all the reactants. The MCRs provide rapid access to molecular diversity by combining several reactants into functionalized molecules. Hence, MCRs paid great attention to generating structurally diverse chemical molecules of the drug like heterocyclic compounds. The MCRs have been known for over 150 years with the Strecker synthesis of α -amino cyanides in 1850 from which α -amino acids could be derived. Some important achievements have been taken into account of the discovery of the Biginelli condensation of ethyl acetoacetate, benzaldehyde, and Urea, Mannich reaction, an amino alkylation of acidic proton placed next to carbonyl functional group with formaldehyde and ammonia or primary or secondary amine; Passerini reaction, which involve an isocyanide, an aldehyde (or ketone) and a carboxylic acid to form an α -acyloxy amide, culminating in 1959 when Ugi published the most versatile MCR based on the reactivity of isocyanides. The reagents employed may be different molecules or they may be different functional groups of the same reagent. Devising such types of MCRs that achieve the formation of multiple bonds in a single operation is one of the major challenges in modern organic synthesis. These processes avoid time consuming and costly purification processes, as well as protection and deprotection steps, they are more environmentally benign, atom economy, speed, diversity, efficiency, and environmental amiability are some of the key features of this class of reactions. Most of the biologically active compounds have been effectively synthesized through multi-component reactions¹⁻³³. In view of the growing importance for the generation of large heterocyclic compound libraries, the development of the new and synthetically valuable protocol for multi-component reactions vestiges a dispute for both academia and industrial researchers.

1.1. Some Important Multi-Component Reactions:

1.1.1. Asinger Reaction:

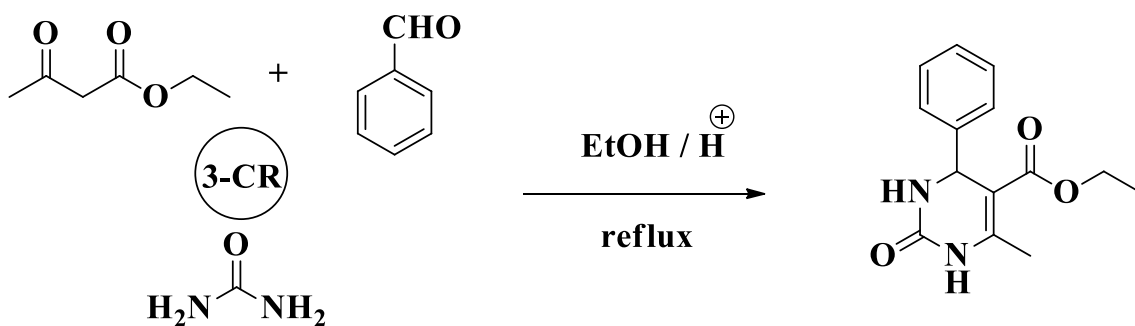
Friedrich Asinger³⁴ reported a four-component reaction. An α -halogenated carbonyl compound reacts with sodium hydrosulphide and forms a thiol *in-situ*. The thiol reacts with another carbonyl compound and ammonia to give thiazolines (**Scheme-1**).



Scheme-1

1.1.2. Biginelli Reaction:

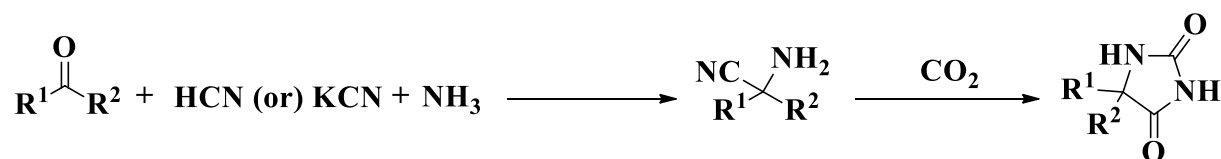
Pietro Biginelli was an Italian chemist. He developed a methodology for multi-component condensation reaction in which ethyl acetoacetate (EAA), aryl aldehydes, and urea were reacted in the acidic medium in ethanol to afford the corresponding heterocyclic compounds 3,4-dihydropyrimidinones^{35,36} shown in the **Scheme-2**.



Scheme-2

1.1.3. Bucherer-Bergs Reaction:

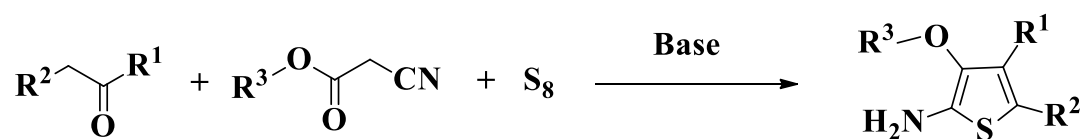
Bucherer and Bergs³⁷ developed a four-component reaction for the synthesis of hydantoins. In this reaction, the carbonyl compounds, potassium or hydrogen cyanide, ammonia, and carbon dioxide were cyclocondensed to produce the hydantoins (**Scheme-3**).



Scheme-3

1.1.4. Gewald Reaction:

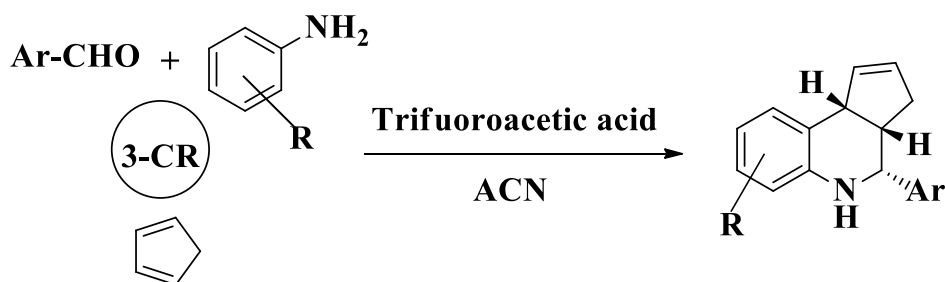
Karl Gewald³⁸ used a three-component reaction for the synthesis of polysubstituted 2-amino thiophenes. The desired compound was produced by the condensation of α -methylene ketones or aldehydes, α -cyano ester with elemental sulfur (S) in presence of a base (**Scheme-4**).



Scheme-4

1.1.5. Grieco three-component condensation:

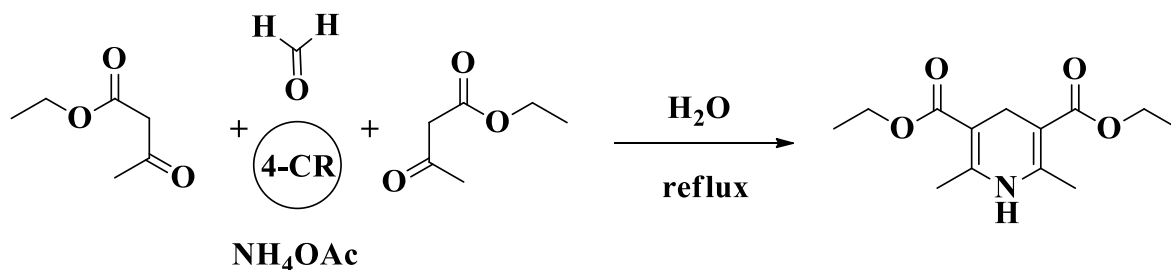
Paul A. Grieco^{39,40} described a three-component cyclocondensation reaction for the synthesis of nitrogen-containing six-membered heterocyclic compounds. In the condensation reaction, aldehydes and arylamines were cyclized with cyclopentadiene in presence of trifluoroacetic acid (TFA) to give the desire tetrahydroquinolines (**Scheme-5**).



Scheme-5

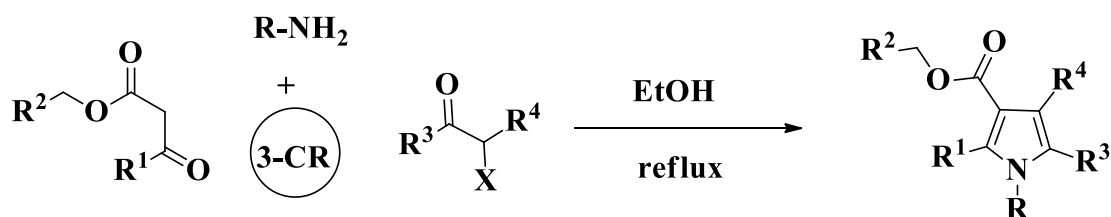
1.1.6. Hantzsch Dihydropyridine (Pyridine) Synthesis:

Arthur Rudolf Hantzsch⁴¹ used a four-component reaction for the synthesis of substituted dihydropyridines. In this method, the cyclocondensation reaction occurred between aldehyde (formaldehyde), two equivalents of active methylene compound (ethyl acetoacetate), and ammonium acetate to produce the substituted dihydropyridine (**Scheme-6**).



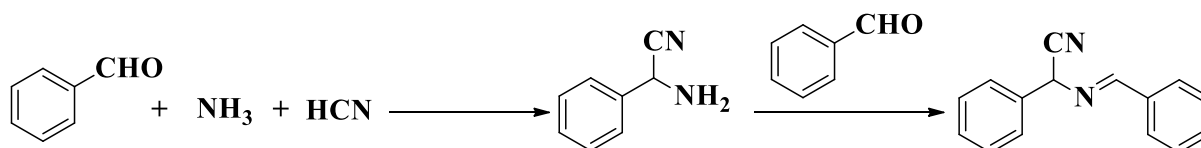
1.1.7. Hantzsch Pyrrole Synthesis:

Arthur R. Hantzsch⁴² developed a three-component reaction for the synthesis of pyrroles. Ethyl acetoacetate (β -keto ester) was condensed with α -halo β -ketones and primary (1°) amines to give pyrrole derivatives (**Scheme-7**).



1.1.8. Laurent and Gerhardt MCR:

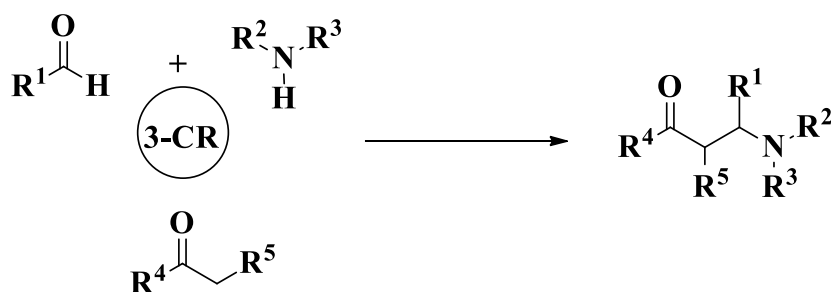
Laurent and C. F. Gerhardt⁴³ developed the multi-component reaction for the synthesis of “Benzoyl Azotid”. Benzaldehyde containing bitter almond oil reacted with ammonia and methanenitrile (HCN) to give α -amino benzyl cyanide and addition of another mole of bitter almond oil to form the Schiff base of benzyl cyanide (Benzoyl Azotid) (**Scheme-8**).



Scheme-8

1.1.9. Mannich Reaction:

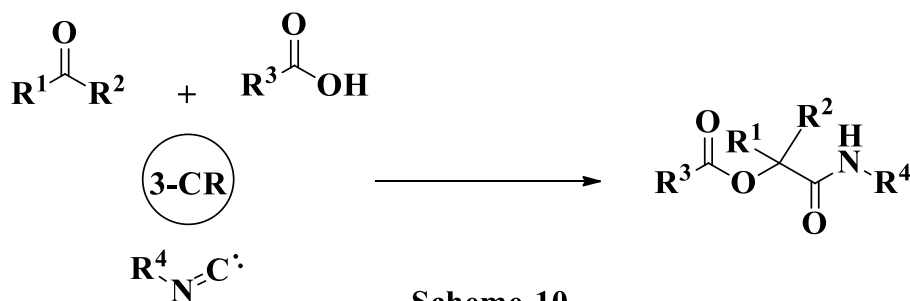
Carl Mannich⁴⁴ established C—C and C—N bond formation in a one-pot, three-component reaction. Aldehyde and amine were condensed with α -methylene carbonyl compounds to produce the β -amino carbonyl compounds (**Scheme-9**).



Scheme-9

1.1.10. Passerini Reaction:

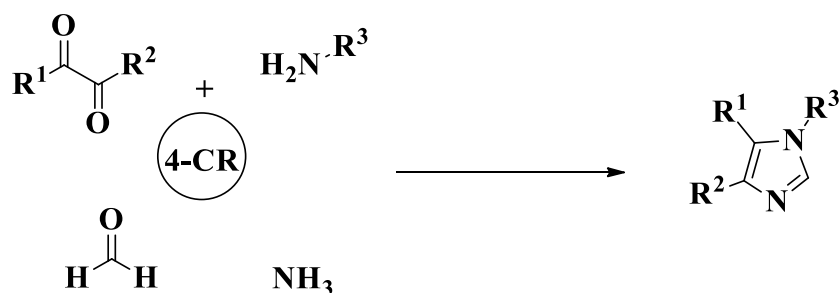
Mario Passerini^{45,46} introduced the first isocyanide based multi-component reaction. The reaction between carbonyl compounds, carboxylic acids, and isocyanides to form C—C and C—O bonds of α -acyloxy amide via a one-pot, three-component reaction (**Scheme-10**).



Scheme-10

1.1.11. Radziszewski Imidazole Synthesis:

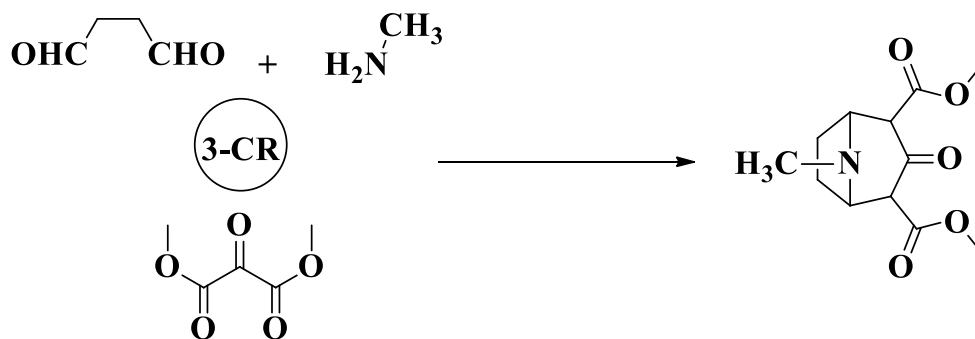
Radziszewski⁴⁷ introduced a one-pot, four-component reaction for the synthesis of substituted imidazoles. The reaction involves 1,2-dicarbonyl compounds, formaldehyde, primary (1°) amine, and ammonia to generate the corresponding imidazole derivatives (**Scheme-11**).



Scheme-11

1.1.12. Robinson's Synthesis:

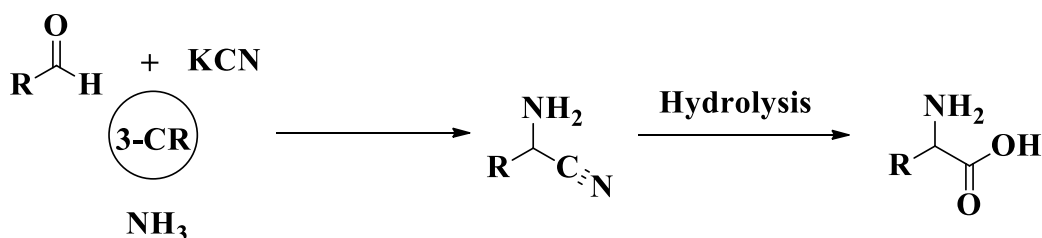
Robert Robinson⁴⁸ used the Mannich reaction extensively in the synthesis of tropinone via a one-pot, multi-component reaction. Tropinone alkaloid was synthesized using a double Mannich reaction of butanedial, aminomethane, and dimethyl-1,3-acetodicarboxylate (**Scheme-12**).



Scheme-12

1.1.13. Strecker Synthesis of amino acids:

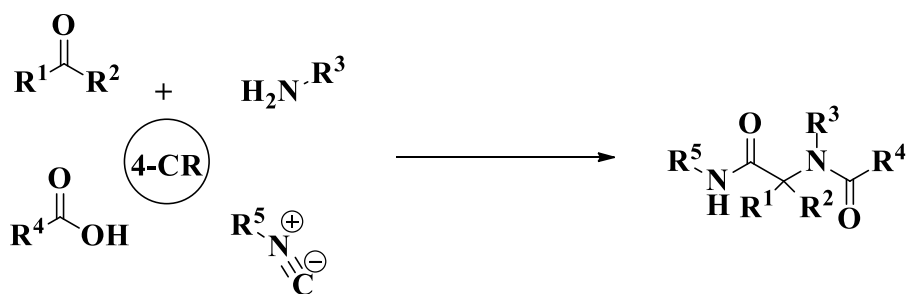
Adolph Strecker⁴⁹ reported the first multi-component reaction in 1950. Amino acids were synthesized using aldehydes, potassium cyanide, and ammonia followed by hydrolysis of α -amino nitrile *via* a one-pot, three-component reaction (**Scheme-13**).



Scheme-13

1.1.14. Ugi Reaction:

Ivar Karl Ugi⁵⁰ reported a one-pot multi-component reaction for the synthesis of α -acylaminoamides. The condensation of carbonyl compounds, amines with carboxylic acids and carbylamines to generate the bis-amide *via* a one-pot, four-component reaction (**Scheme-14**).



Scheme-14

2. Types of multi-component reactions

Multi-component reactions can be described based on the functional groups of the reactants participating in the reaction as follows.

- i) Isocyanide based MCRs
- ii) Imine based MCRs

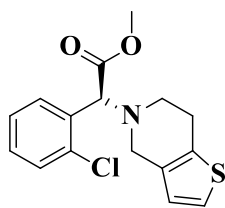
Isocyanides are used as starting materials in the isocyanide based multi-component reactions. While amines are used as starting materials in imine based MCRs, most of the multi-component reactions belong to imine based type of multi-component reactions.

On the other multi-component reactions can be classified based on the number of reactants contributed in the reaction to produce the designed compound as pseudo-component, three-component, four-component, five-component reactions, etc...

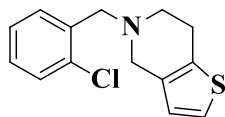
3. Applications of multi-component reactions:

3.1. Applications in generating drugs through MCRs:

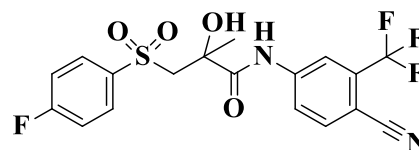
Multi-component reactions are useful to synthesize the well known drugs like (S)-clopidogrel, ticlopidine, and bicalutamide^{51,52} via a three-component Ugi reaction and Passerini reaction.



(S)-clopidogrel

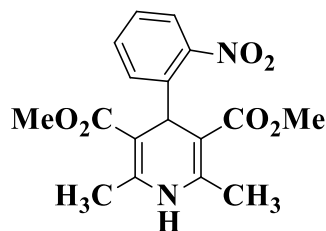


Ticlopidine



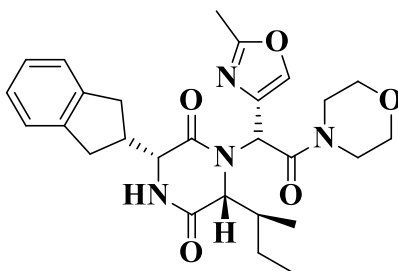
Bicalutamide

Harjit⁵³ *et al.* reported the synthesis of Nifedipine drug and its derivatives via a one-pot multi-component reaction. Nifedipine is used to reduce high blood pressure.



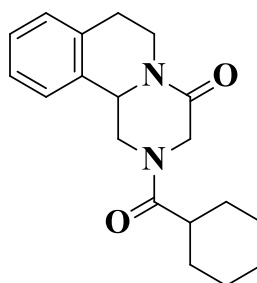
Nifedipine

John⁵⁴ *et al.* described the one-pot, multi-component synthesis of Retosiban drug and its derivatives.



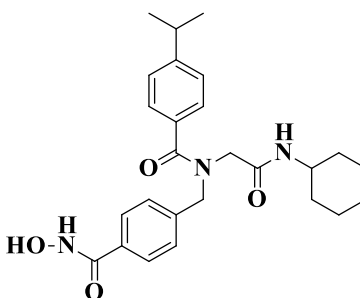
Retosiban

Haiping⁵⁵ et al. used Ugi four-component reaction for the synthesis of Schistosomiasis drug Praziquantel with good yields.



Praziquantel

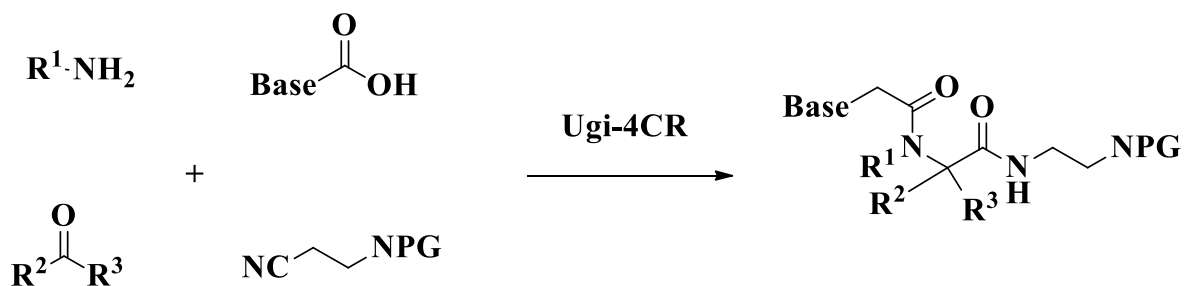
Daniela⁵⁶ et al. reported the one-pot, multi-component synthesis of *N*-(2-(cyclohexylamino)-2-oxoethyl)-*N*-(4-(hydroxycarbamoyl)benzyl)-4-isopropylbenzamide and tested for anti-malarial activity.



N-(2-(cyclohexylamino)-2-oxoethyl)-*N*-(4-(hydroxycarbamoyl)benzyl)-4-isopropylbenzamide

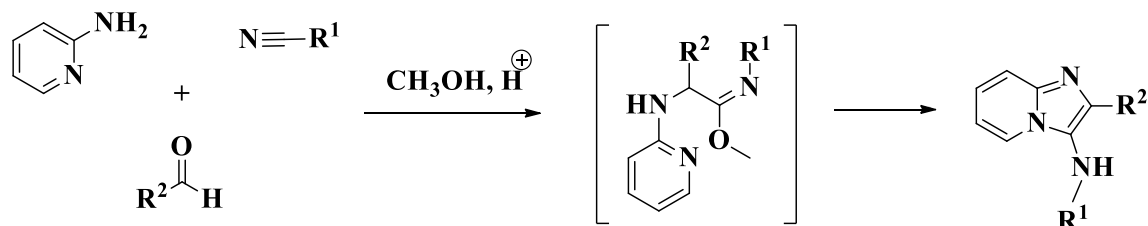
3.2. Applications in combinatorial chemistry:

Alexander⁵⁷ et al. developed a novel method to highly versatile monomeric Peptide Nucleic Acid (PNA) building blocks by Ugi four-component reaction as shown in the **Scheme-15**.



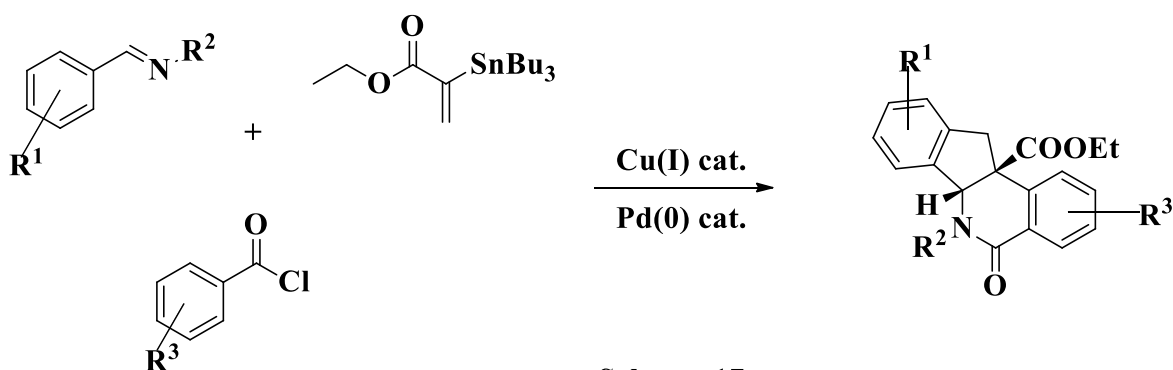
Scheme-15

Mironov⁵⁸ *et al.* developed the combinatorial method for the synthesis of imidazo[1,2-*a*]pyridines by using Ugi three-component reaction (Scheme-16).



Scheme-16

Sarvesh⁵⁹ *et al.* reported the synthesis of combinatorial libraries of dihydroindeno[1,2-*c*]isoquinolines through a multi-component reaction using Cu/Pd-catalysts (Scheme-17).

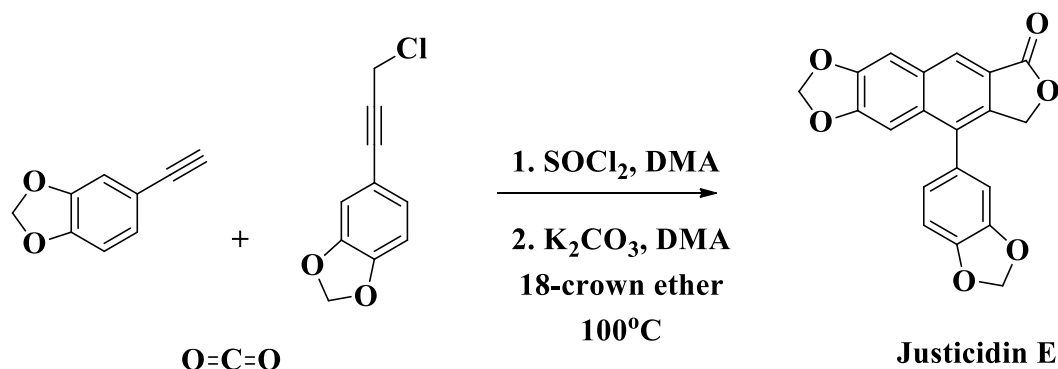


Scheme-17

3.3. Applications in the synthesis of natural products:

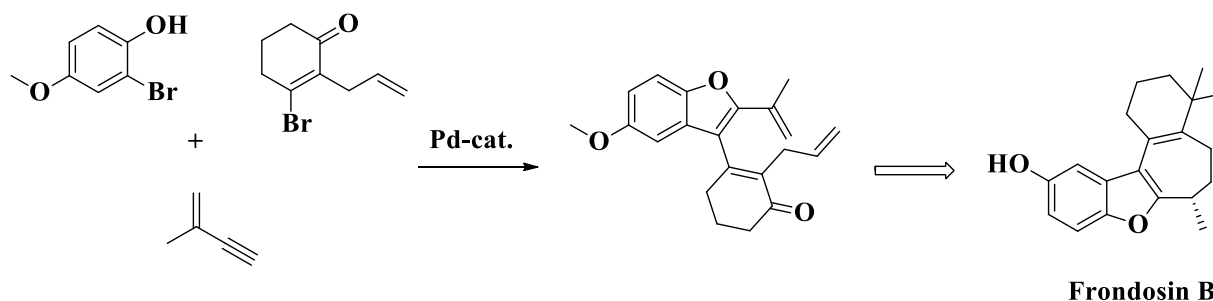
Various natural products were isolated and used in therapeutics. Some of the natural products were synthesized in the laboratory to improve the quantity and quality of natural products using multi-component reactions.

Patrick⁶⁰ et al. reported the synthesis of Justicidin E using 5-(3-chloroprop-1-yn-1-yl)benzo[d][1,3]dioxole, 5-ethynylbenzo[d][1,3]dioxole and carbon dioxide *via* a one-pot, multi-component reaction (**Scheme-18**).



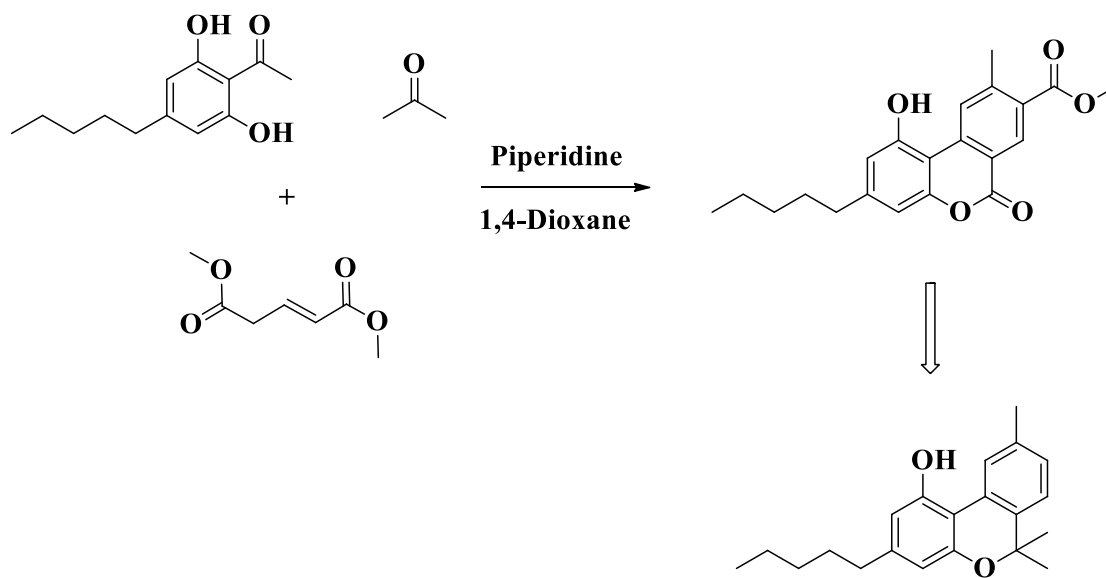
Scheme-18

Jason⁶¹ and co-worker described the synthesis of 2-allyl-3-(5-methoxy-2-(prop-1-en-2-yl)benzofuran-3-yl)cyclohex-2-enone from the reaction of 2-bromo-4-methoxyphenol, 2-allyl-3-bromocyclohex-2-enone and 2-methylbut-1-en-3-yne using Pd-catalyst through multi-component reaction pathway (**Scheme-19**). Further, it was converted into Frondosin B.



Scheme-19

Penchal⁶² and co-worker reported an efficient multi-component strategy for the synthesis of methyl 1-hydroxy-9-methyl-6-oxo-3-pentyl-6*H*-benzo[*c*]chromene-8-carboxylate from the reaction of 1-(2,6-dihydroxy-4-pentylphenyl)ethanone, propan-2-one, and dimethyl pent-2-enedioate in presence of piperidine and 1,4-dioxane (**Scheme-20**). It was used as an intermediate for the Cannabinol.



Scheme-20

Cannabinol

4. PRESENT WORK:

Based on the literature work on MCRs, we have been inspired to synthesize various N and S heterocyclic compounds by this methodology. In addition to this many, triazoles were associated with good biological activity. Prompted by these observations in the present study, we have synthesized new heterocyclic systems starting from a triazole derivative 4-amino-5-hydrazinyl-4*H*-1,2,4-triazole-3-thiol.

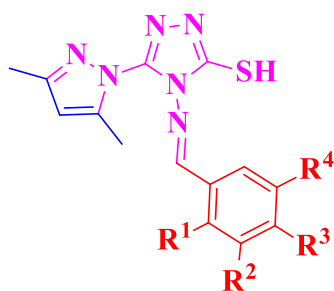
5. AIMS AND OBJECTIVES:

- To develop facile, efficient, eco-friendly synthetic methods for the preparation of different heterocyclic motifs.
- To evaluate the newly synthesized compounds for their biological and molecular docking studies.
- To illustrate the structures of the newly synthesized compounds by their analytical and spectral methods.

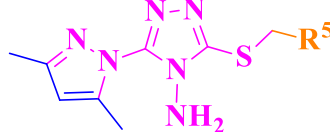
Our current research work explains the synthesis of nitrogen and sulfur heterocyclic compounds. These heterocyclic derivatives were synthesized utilizing the easily available starting materials such as 4-amino-5-hydrazinyl-4*H*-1,2,4-triazole-3-thiol, substituted phenacyl bromides, acetylacetone, various aromatic aldehydes, alkyl/aralkyl halides, different phthalic anhydrides, and substituted benzoic acids. The present research work is divided into seven chapters.

Chapter-I deals with a micro review on multi-component reactions and their utility in the synthesis of biologically active compounds.

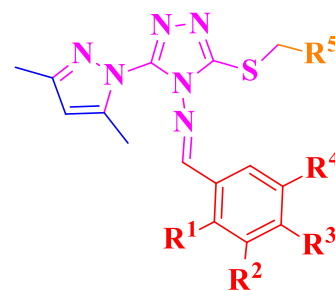
Chapter-II is divided into three parts. **Part-A** deals with the one-pot multi-component synthesis of substituted benzylideneamino-3,5-dimethyl-1*H*-Pyrazoles, **Part-B** explains the synthesis of various aralkyl/alkyl thio-3,5-dimethyl-1*H*-pyrazolyl-4*H*-1,2,4-triazol-4-amines *via* a multi-component cascade reaction and their molecular docking studies. **Part-C** describes the one-pot, four-component synthesis of substituted (*E*)-*N*-benzylidene-3-(benzylthio)-5-(3,5-dimethyl-1*H*-pyrazol-1-yl)-4*H*-1,2,4-triazol-4-amines and their DNA binding and molecular interactions.



Part-A

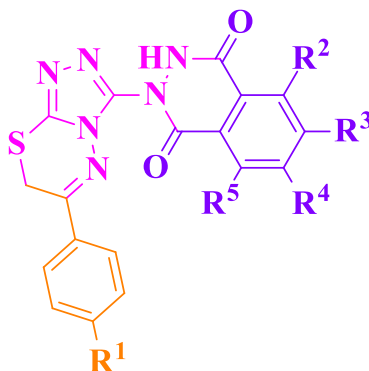


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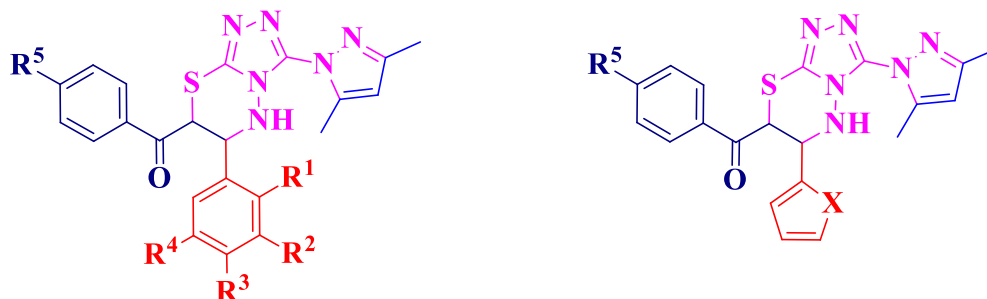


Part-C

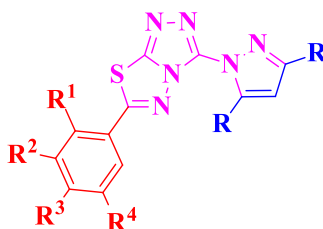
Chapter-III describes the one-pot, multi-component synthesis of 2-(6-phenyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)-2,3-dihydrophthalazine-1,4-dione derivatives.



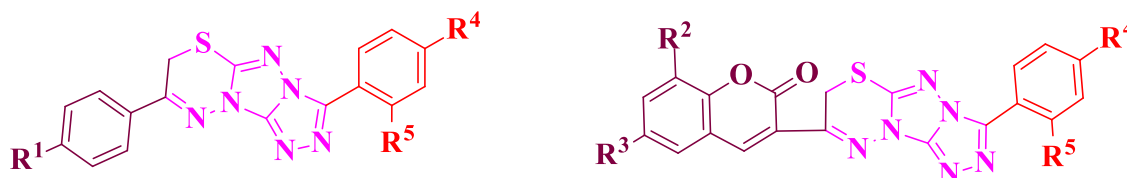
Chapter-IV illustrates the discovery of 3-(1*H*-pyrazol-1-yl)-6,7-dihydro-5*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine derivatives with promising anti-coronavirus and anti-tumoral activity.



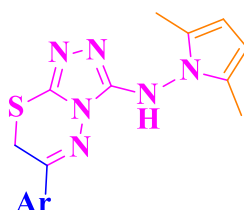
Chapter-V portrays an efficient one-pot synthesis of 6-phenyl-3-(1*H*-pyrazol-1-yl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole derivatives and their antimicrobial evaluation and molecular docking studies.



Chapter-VI depicts the novel one-pot, three component synthesis of 7*H*-[1,2,4]triazolo[4',3':1,5][1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine derivatives.



Chapter-VII delineates the an efficient one-pot three component synthesis of *N*-(2,5-dimethyl-1*H*-pyrrol-1-yl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-amine derivative.



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CHAPTER-II

A FACILE ONE-POT, MULTI-COMPONENT CASCADE SYNTHESIS OF

- **BENZYLIDENEAMINO-3,5-DIMETHYL-1*H*-PYRAZOLE DERIVATIVES**
- **VARIOUS ARALKYL/ALKYL THIO-3,5-DIMETHYL-1*H*-PYRAZOLYL-4*H*-1,2,4-TRIAZOL-4-AMINE AND THEIR DOCKING STUDIES**
- **(*E*)-*N*-BENZYLIDENE-3-(BENZYLTHIO)-5-(3,5-DIMETHYL-1*H*-PYRAZOL-1-YL)-4*H*-1,2,4-TRIAZOL-4-AMINES AND THEIR DNA BINDING AND MOLECULAR DOCKING STUDIES.**

INTRODUCTION:

Pyrazoles are the interesting and most significant heterocyclic compounds with two nitrogen atoms at adjacent position. In these two nitrogen atoms, one can behave as the weak base and the other one is neutral. Most of the pyrazoles are aromatic five-membered heterocyclic rings. Numerous pyrazole derivatives play a versatile role in drug discovery due to the vast range of their remarkable bioactivities such as anticancer¹⁻³, anti-inflammatory^{4,5}, antiviral^{6,7}, anti-microbial^{8,9}, anti-tumor^{10,11}, anti-diabetic¹², anti-fungal^{13,14}, fungicidal¹⁵, anti-malarial¹⁶, and anti-leishmanial¹⁷ activities (**Fig. 1**).

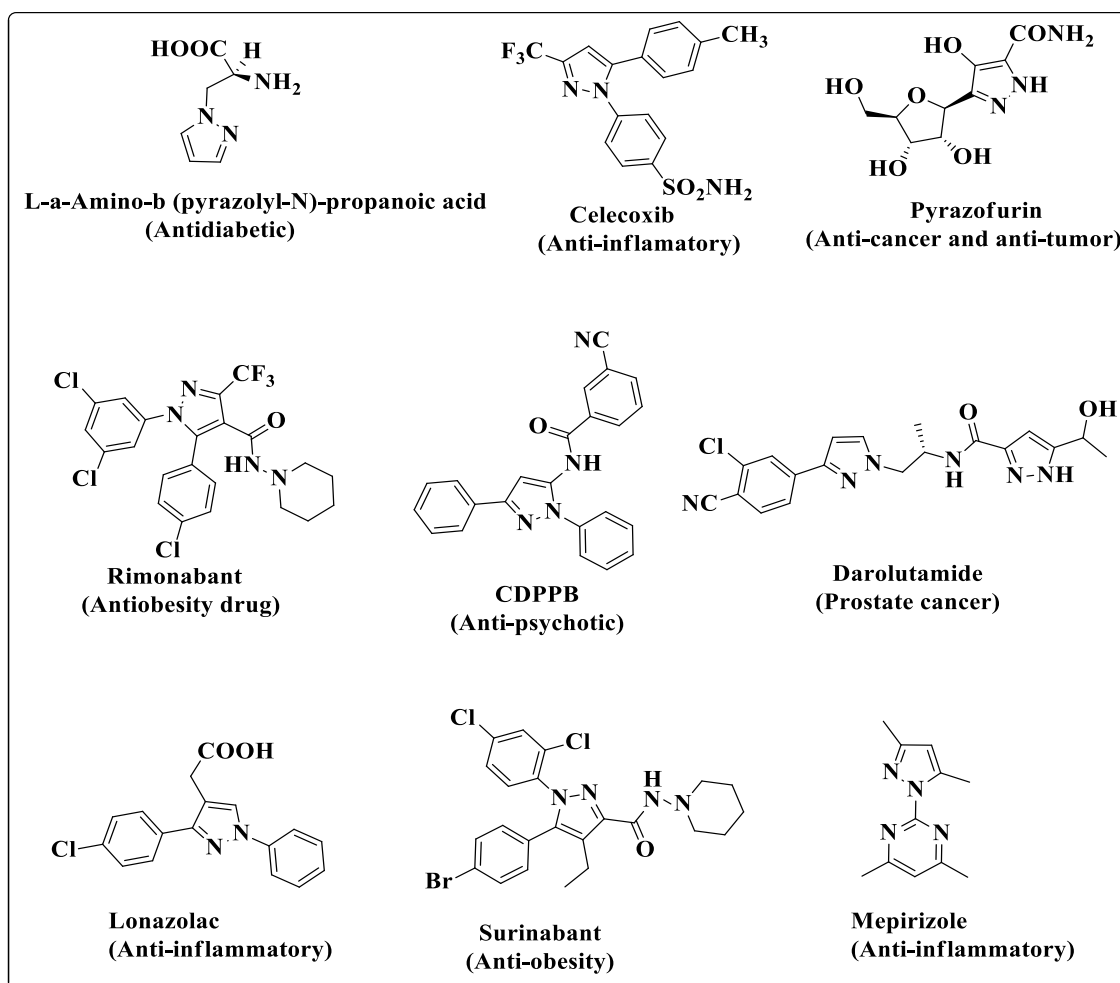
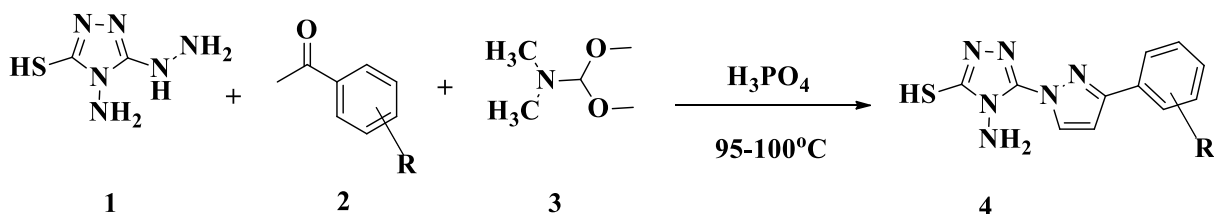


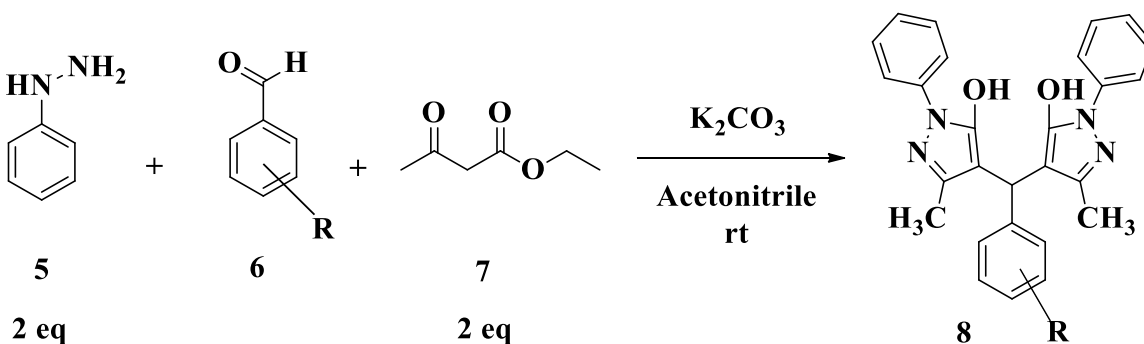
Fig. 1: Some of the drugs containing pyrazole ring as core moiety.

Literature survey shows that there are several synthetic procedures available for the preparation of the pyrazoles. The following are some of the literature reports on the pyrazoles.

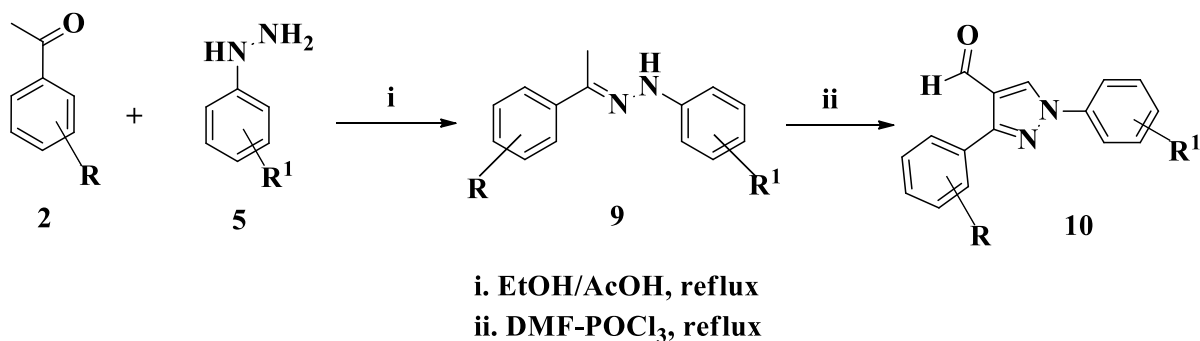
Amer and Moustafa¹⁸ synthesized the 4-amino-5-(1*H*-pyrazol-1-yl)-4*H*-1,2,4-triazole-3-thiol (**4**) derivatives by the reaction of 4-amino-5-hydrazinyl-4*H*-1,2,4-triazole-3-thiol (**1**), substituted acetophenones (**2**) and 1,1-dimethoxy-*N,N*-dimethylmethanamine (**3**) in H₃PO₄ at 95-100°C.



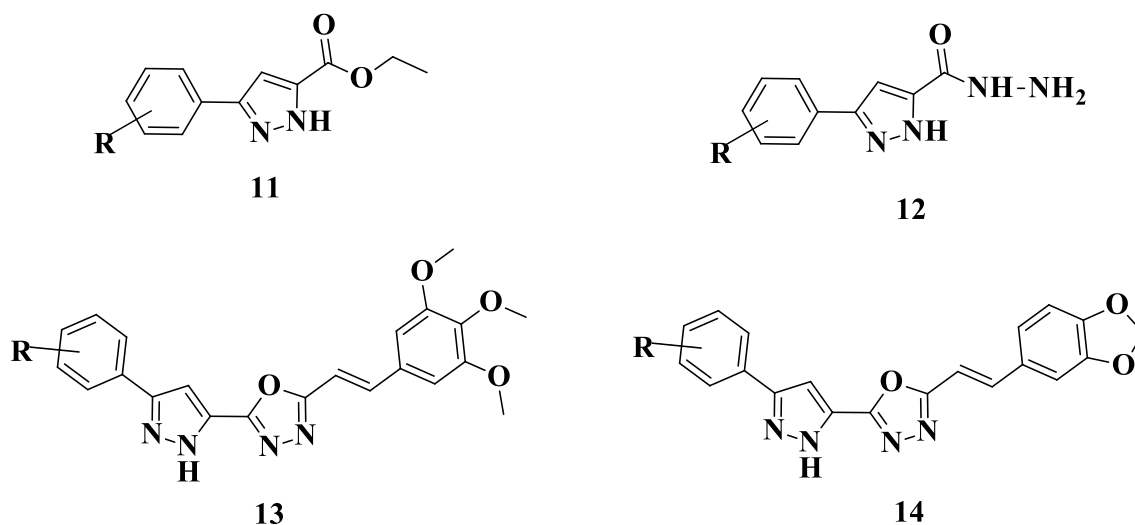
Banerjee¹⁹ *et al.* reported the synthesis of 4,4'-(phenylmethylene)bis(3-methyl-1-phenyl-1*H*-pyrazol-5-ol) (**8**) derivatives by using phenylhydrazine (2 eq) (**5**), various benzaldehydes (1 eq) (**6**) and ethyl acetoacetate (2 eq) (**7**) *via* a one-pot, pseudo multi-component reaction.



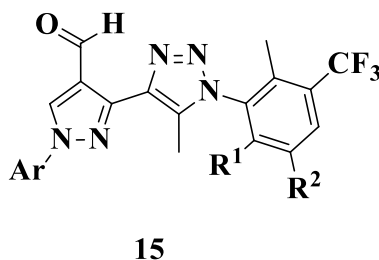
Assali²⁰ *et al.* reported the synthesis of substituted 1,3-diphenyl-1*H*-pyrazole-4-carbaldehydes (**10**) using the Vilsmeier-Haack reagent and these derivatives were screened for their COX-1 and COX-2 inhibitory activity with good results.



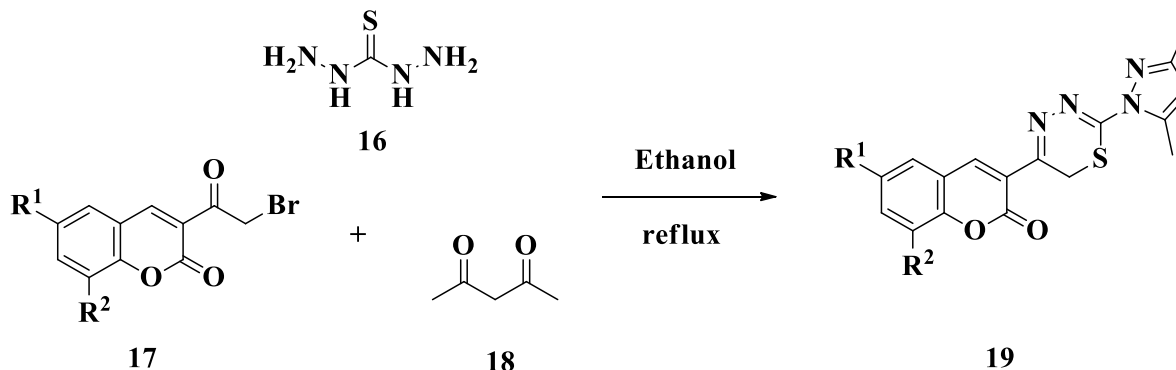
Kamal²¹ *et al.* described the synthesis of various pyrazole compounds **11**, **12**, **13**, and **14**. These derivatives were monitored for their anti-proliferative activity with moderate results.



Bhat²² *et al.* reported the stepwise synthesis of 3-(5-methyl-1*H*-1,2,3-triazol-4-yl)-1*H*-pyrazole-4-carbaldehyde (**15**) derivatives through the Vilsmeier-Haack reaction. Among the final compounds, some of the pyrazole molecules exhibited potent anti-microbial activity.



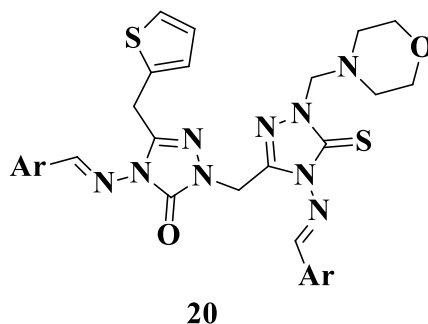
Pyrazolo-thiadiazine (**19**) derivatives were synthesized by **Pavurala²³ et al.** via a one-pot three-component by the reaction of thiocarbohydrazide (**16**), different 3-(2-bromoacetyl)-2H-chromen-2-one (**17**) and acetylacetone (**18**) in presence of ethanol in 70-85% yields.



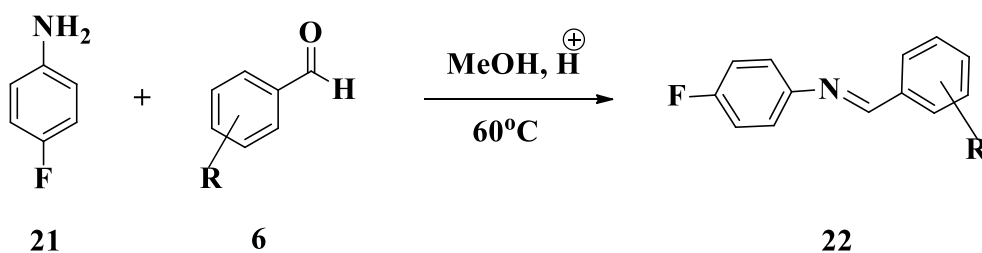
Schiff bases play a significant role in synthetic organic chemistry. Condensation of carbonyl compounds (aldehydes or ketones) with primary amines produces Schiff bases. They have an imine or an azomethine ($-\text{C}=\text{N}-$) functional group. In the formation of metal complexes Schiff bases act as ligands^{24,25}. Molecules with Schiff base are exhibiting a wide range of applications such as biological, catalysts, dyes, and stabilizers²⁶. Many compounds having Schiff bases are reported to display broad range of biological activities such as anti-microbial^{27,28}, anti-proliferative²⁹⁻³¹, anti-oxidant^{32,33}, anti-inflammatory³⁴, cytotoxic³⁶, anti-viral³⁷, anti-tubercular^{38,39}, anti-fungal⁴⁰, anti-HIV^{41,42}, anti-candida⁴³, anti-dyslipidemic⁴⁴, anti-glycation⁴⁵, herbicidal⁴⁶ and insecticidal⁴⁷ activities.

The following are literature reports on the synthesis of Schiff bases and their biological applications.

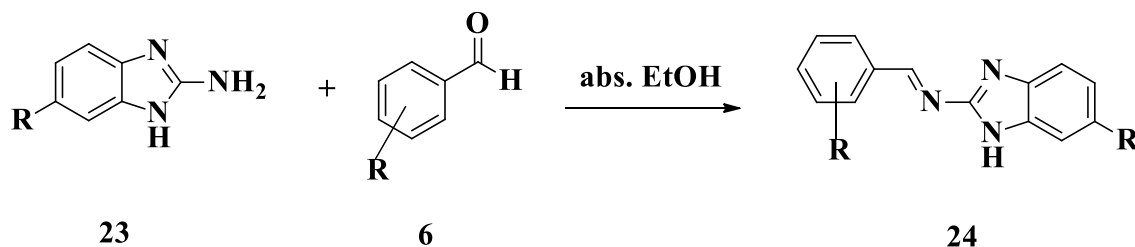
Unver⁴⁸ et al. described the stepwise synthesis of triazole containing Schiff bases (**20**) with good yields. Some of their compounds exhibited good anti-microbial and anti-oxidant activities.



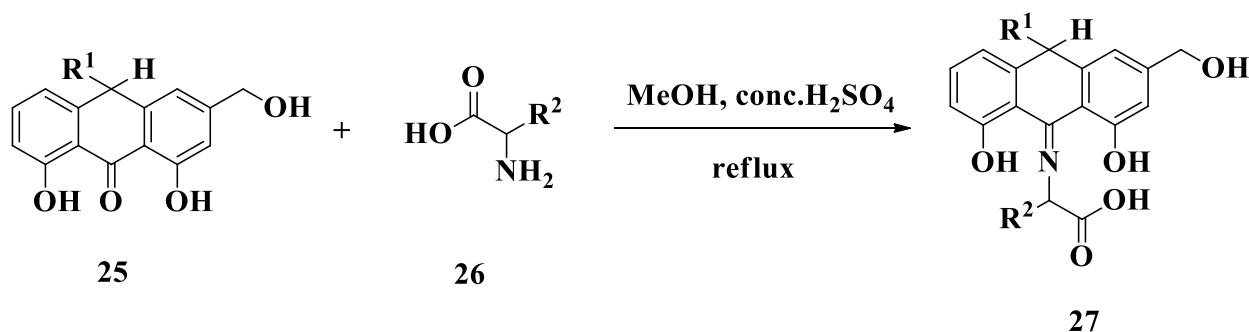
Santhosh⁴⁹ *et al.* conducted the reaction between 4-fluoroaniline (**21**) and substituted benzaldehydes (**6**) in the methanol and few drops of acetic acid at 60°C to generate the benzylidene-4-fluoroaniline (**22**) derivatives. The target compounds were screened for their anti-biocide activity.



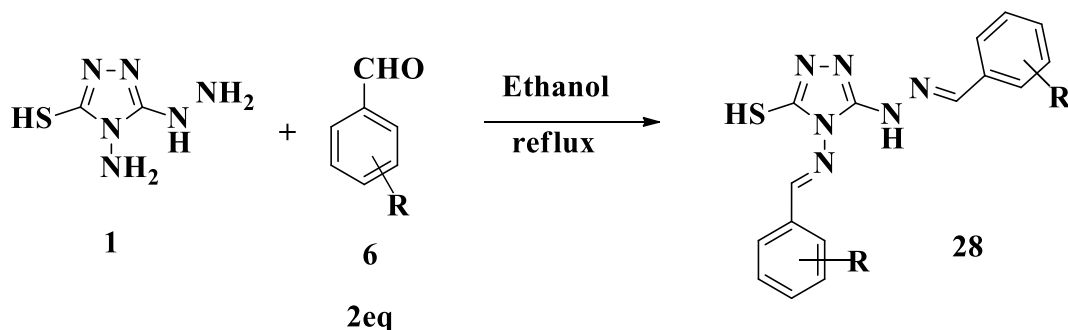
Benzylidene-1*H*-benzo[*d*]imidazol-2-amine (**24**) derivatives were reported by **Hranjec**⁵⁰ *et al.* using different 1*H*-benzo[*d*]imidazol-2-amines (**23**) and aromatic aldehydes (**6**) in absolute ethanol under reflux conditions. Some of the final compounds have displayed promising anti-proliferative activity.



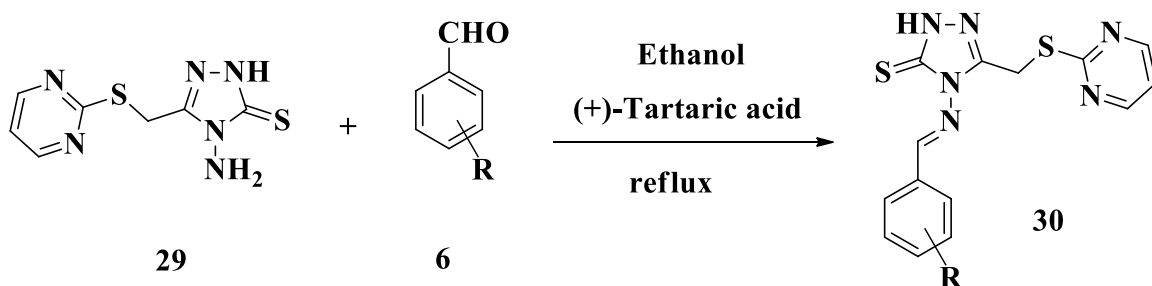
Matharasi⁵¹ et al. described the synthesis of glycones (**27**) by the reaction of aloin (**25**) with various amino acids (**26**) in the presence of methanol and catalytic amounts of conc. H₂SO₄ under the reflux condition.



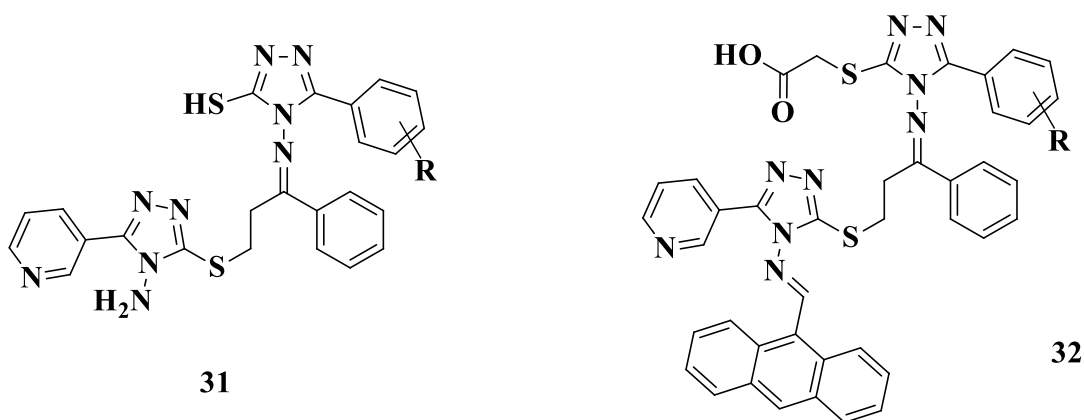
Compound **1** was treated with two equivalents of aromatic benzaldehydes (**6**) at reflux temperature in ethanol to produce the triazole Schiff base derivatives (**28**). This was published by **Yu⁵² et al.**



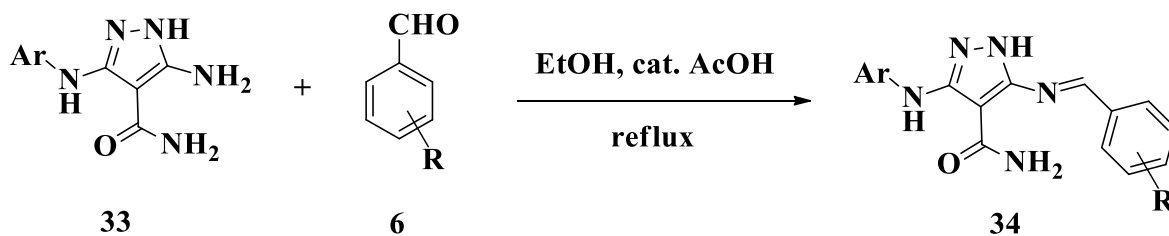
Sim⁵³ et al. carried out the reaction between 4-amino-3-((pyrimidin-2-ylthio)methyl)-1H-1,2,4-triazole-5(4H)-thione (**29**) and substituted aromatic aldehydes (**6**) in the presence of ethanol and (+)-Tartaric acid catalyst under reflux condition to produce the (*E*)-4-(benzylideneamino)-3-((pyrimidin-2-ylthio)methyl)-1H-1,2,4-triazole-5(4H)-thione (**30**) analogues. Among these few compounds manifested their anti-bacterial activity.



Hu⁵⁴ *et al.* designed stepwise synthesis of the various triazole Schiff bases **31** and **32**. The target compounds were evaluated for their anti-tumor activity with good results.

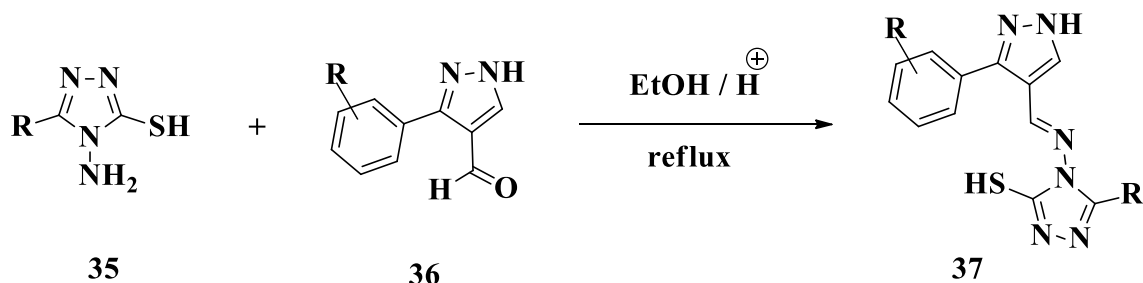


5-Amino-3-(phenylamino)-1*H*-pyrazole-4-carboxamides (**33**) react with various arylaldehydes (**6**) in ethanol and cat. AcOH resulted in the formation of the Schiff bases (**34**) with good yields. This was reported by **Hassan**⁵⁵ *et al.* and these compounds were having good anti-bacterial activity.

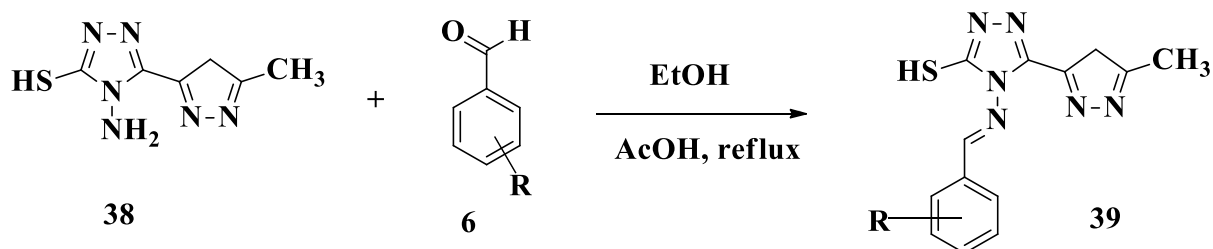


Malladi⁵⁶ *et al.* synthesized the pyrazole containing Schiff bases (**37**) using 4-amino-4*H*-1,2,4-triazole-3-thiols (**35**) and substituted 3-phenyl-1*H*-pyrazole-4-carbaldehydes (**36**) in the

acidified ethanolic solution under reflux conditions. Further, its derivatives were screened for their anti-bacterial activity.



Pillai⁵⁷ *et al.* carried out the reaction between 4-amino-5-(5-methyl-4*H*-pyrazol-3-yl)-4*H*-1,2,4-triazole-3-thiol (**38**) and substituted aromatic benzaldehydes (**6**) at reflux condition in ethanol containing a catalytic amount of acetic acid to generate (*E*)-4-(benzylideneamino)-5-(5-methyl-4*H*-pyrazol-3-yl)-4*H*-1,2,4-triazole-3-thiol (**39**) derivatives in good yields.



Organosulfur compounds are important in organic synthesis. Compounds with C—S—C bond play a significant role in organic chemistry⁵⁸⁻⁶¹. Sulfur atom behaves as the bridge to form alkyl, aryl, aryl alkyl, heteryl, and heteryl alkyl thioethers. These are beneficial and important compounds in different branches such as materials, agriculture, industry, pharmaceutical, medicine, heterocyclic chemistry, and biological processes⁶²⁻⁷¹. In the field of medicine, organosulfur compounds are broadly used for the treatment of different types of diseases such as Alzheimer's, cancer, tuberculosis, and Parkinson⁷²⁻⁷⁵ (**Fig. 2**).

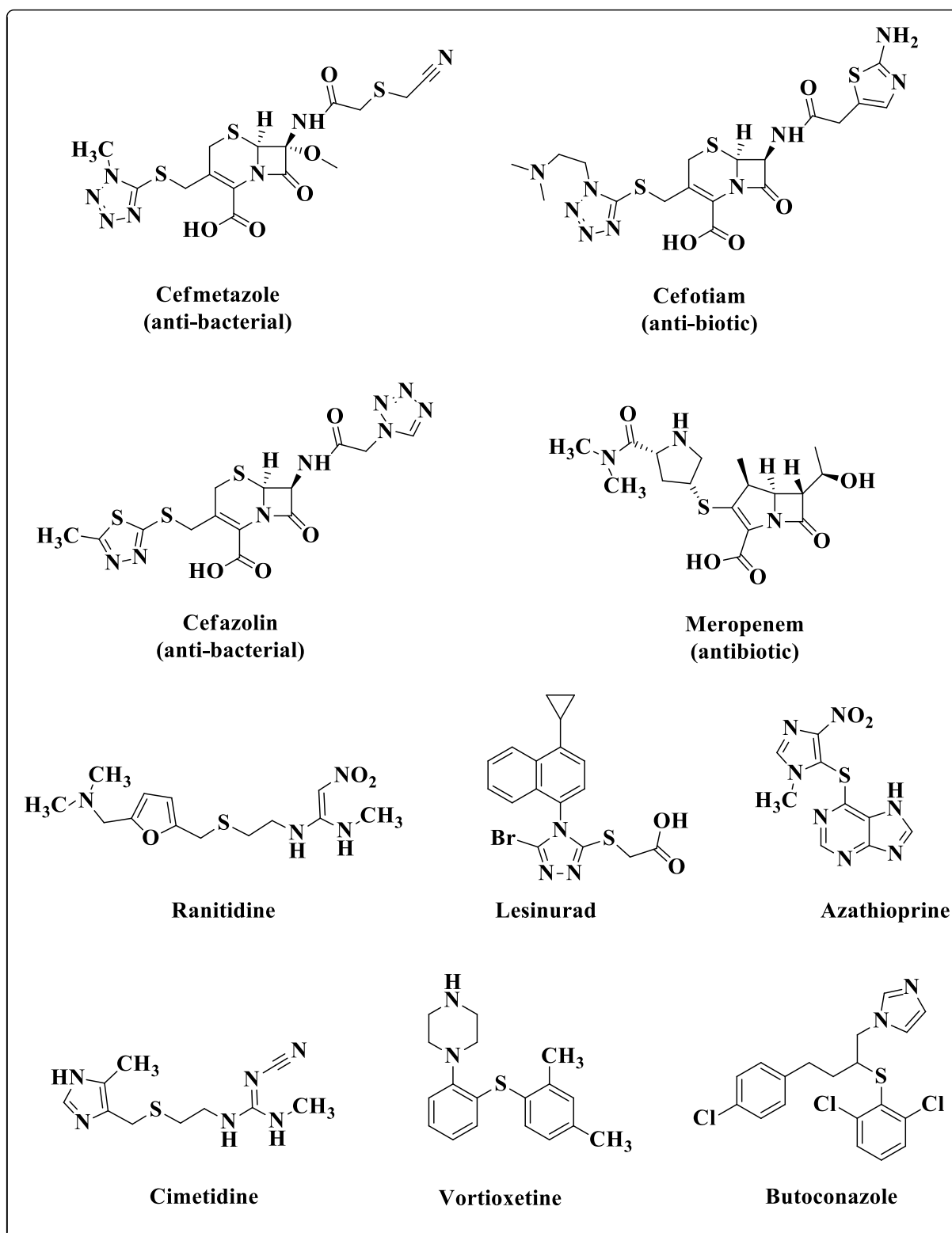
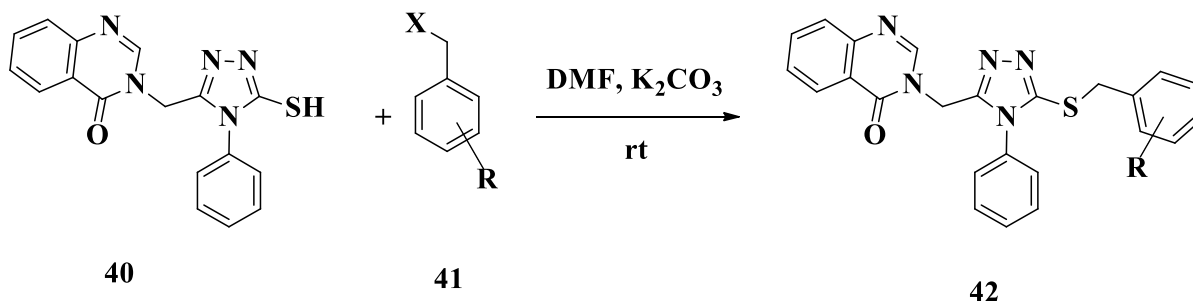


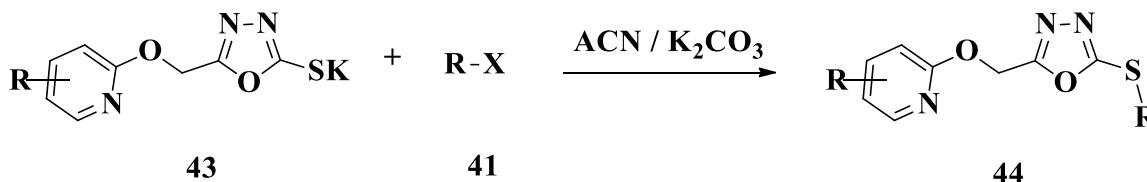
Fig. 2: Some of the US-FDA approved drugs having sulfur atom as thioether linkage.

The following paragraphs indicate a few literature reports on the synthesis of thioethers.

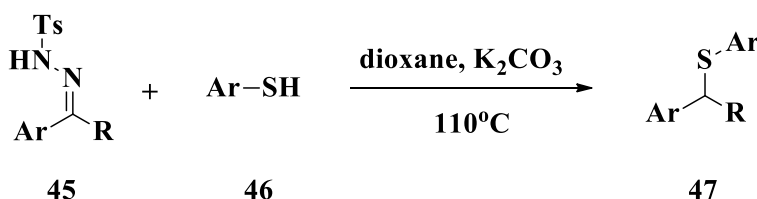
Yan⁷⁶ et al reported the synthesis of 3-((5-(benzylthio)-4-phenyl-4*H*-1,2,4-triazol-3-yl)methyl)quinazolin-4(3*H*)-ones (**42**) from the reaction of various benzyl halides (**41**) with 3-((5-mercapto-4-phenyl-4*H*-1,2,4-triazol-3-yl)methyl)quinazolin-4(3*H*)-one (**40**) in dimethylformamide (DMF) and K₂CO₃ at room temperature.



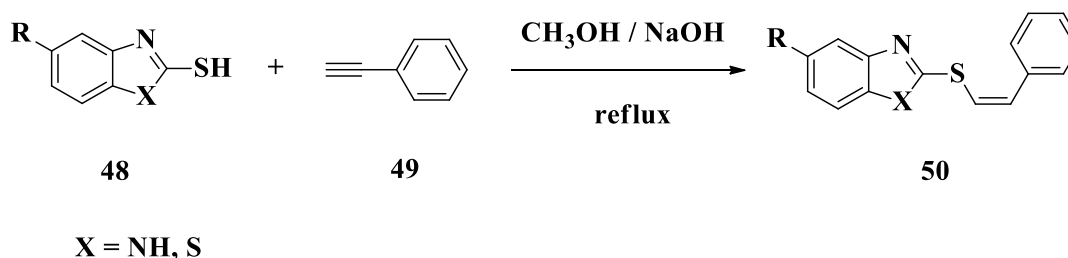
Chen⁷⁷ et al. described the synthesis and nematocidal activity of thioethers **44**. These compounds (**44**) were synthesized by the reaction of potassium 5-((pyridin-2-yloxy)methyl)-1,3,4-oxadiazole-2-thiolate (**43**) with various alkyl or benzyl halides (**41**) in presence of acetonitrile (ACN) and potassium carbonate.



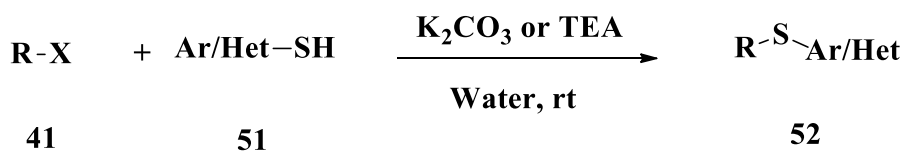
Ding⁷⁸ et al. reported the synthesis of various thioethers (**47**) by the reaction of different tosylhydrazones (**45**) with substituted benzenethiols (**46**) at 110°C temperature in dioxane and K₂CO₃.



A mixture of 2-mercaptobenzimidazoles/thiazoles (**48**) and phenylacetylene (**49**) in presence of basified methanol with NaOH under reflux condition generated the styrylthiobenzimidazoles/thiazoles (**50**) in 78-92% yields. This work was published by **Guravaiah and Rao**⁷⁹.



Azizi⁸⁰ *et al.* reported the green synthesis of thioether (**52**) derivatives from the reaction of alkyl/aralkyl halides (**41**) and substituted benzenethiols/heterylthiols (**51**) in the green solvent water containing potassium carbonate or triethylamine at room temperature in high yields.

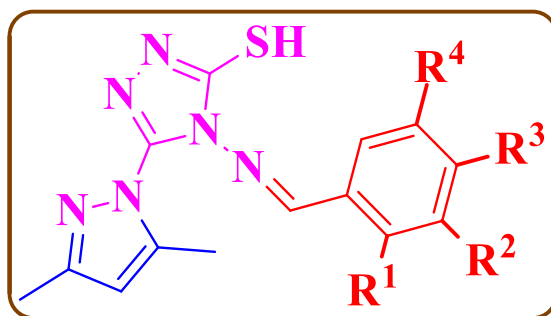


Taking into consideration of all the above synthetic approaches and biological applications of pyrazoles, Schiff's bases, and thioethers, we have incorporated all these motifs in our final molecules. Chapter-II has been classified into three parts, Part-A containing a facile one-pot three-component synthesis of benzylideneamino-3,5-dimethyl-1*H*-pyrazoles. Part-B describes the one-pot, multi-component cascade reaction for the synthesis of various aralkyl/alkyl thio-3,5-dimethyl-1*H*-pyrazolyl-4*H*-1,2,4-triazol-4-amine and their docking studies. Part-C explains the four-component, one-pot synthesis of (*E*)-*N*-benzylidene-3-(benzylthio)-5-(3,5-dimethyl-1*H*-pyrazol-1-yl)-4*H*-1,2,4-triazol-4-amines and their DNA binding and molecular docking studies.

CHAPTER-II

PART-A

A FACILE ONE-POT THREE-COMPONENT SYNTHESIS OF BENZYLIDENEAMINO-3,5-DIMETHYL-1*H*-PYRAZOLES.

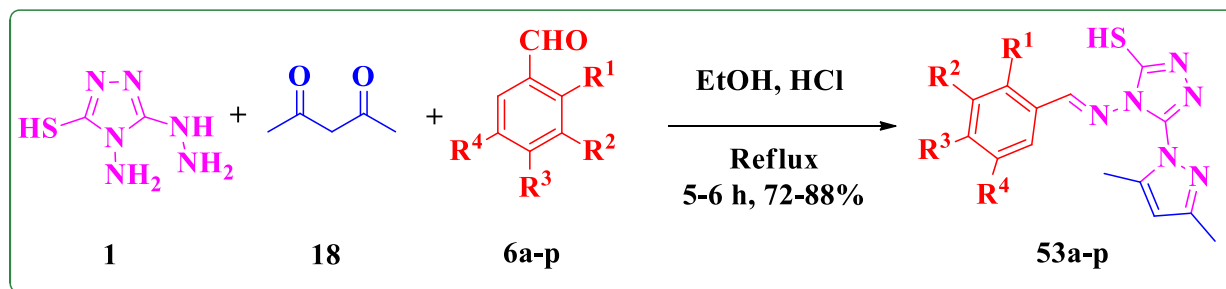


PART-APRESENT WORK

This part deals with the synthesis of benzylideneamino-3,5-dimethyl-1*H*-pyrazoles *via* a facile one-pot three-component reaction.

Because of various biological activities associated with triazoles, pyrazoles, and Schiff bases, our present study is aimed at the development of new methodologies for the synthesis of pyrazoles having both triazole and Schiff bases^{81,82}. In continuation of our earlier work on MCRs^{83,84}, we have developed a one-pot multicomponent reaction for the synthesis of title compounds aiming that these derivatives may have good biological activities.

The reaction of 4-amino-5-hydrazino-4*H*-[1,2,4]triazole-3-thiol (**1**), acetylacetone (**18**), and aromatic aldehydes (**6**) in absolute ethanol under reflux gave only 12% of the expected product. When the same reaction is carried out in presence of two drops of concentrated HCl and absolute ethanol yielded the corresponding product is 86% after recrystallization. Additionally, when the same reaction is examined by using various solvents produced lower yields of the products when compared to ethanol solvent using an acid catalyst. Hence, the optimized condition for the heterocyclization and condensation is the catalytic amount of HCl in absolute ethanol under reflux condition (**Table-1**).



Scheme-1: One-pot synthesis of substituted benzylideneamino-3,5-dimethyl-1*H*-pyrazole **53a-p**.

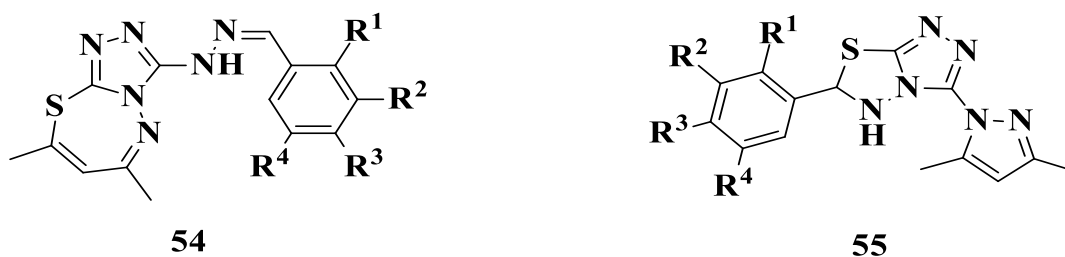
Table-1: Optimization of the reaction conditions.

entry	solvent	acid	Yield (%) of 53a
1	EtOH	—	12
2	EtOH	HCl	86
3	MeOH	HCl	50
4	CH ₂ Cl ₂	HCl	15
5	H ₂ O	HCl	0

***Reaction conditions:** **1** (1 mmol), **18** (1 mmol), **6** (1 mmol), and two drops of con. HCl in 5 mL of solvent.

The reaction between **1**, acetylacetone and aromatic aldehydes is expected to give **54** or **55** or both depending on the mode of cyclization. In the present investigation, the reaction proceeds in such a way that only the hydrazino part of **1** underwent condensation during heterocyclization followed by the second condensation of the amino group of **1** with aromatic aldehyde lead to the formation of final products benzylideneamino-3,5-dimethyl-1*H*-pyrazoles (**Scheme-2**) (only one product based on TLC). The yields of products obtained were 72-88% (**Scheme-1**). From the above optimization studies, we found that the solvent and reaction conditions have played a crucial role in the selective hetero cyclization reaction.

The alternative products i.e. **54** and **55** (**Fig. 3**) can be rejected based on spectral studies (IR, ¹H-, ¹³C-NMR and mass). The specialty of the condensation is that simultaneously pyrazole ring and C=N of Schiff base were formed at a time. No mixture of products is detected (by TLC).

**Fig. 3:** Alternate possible products.

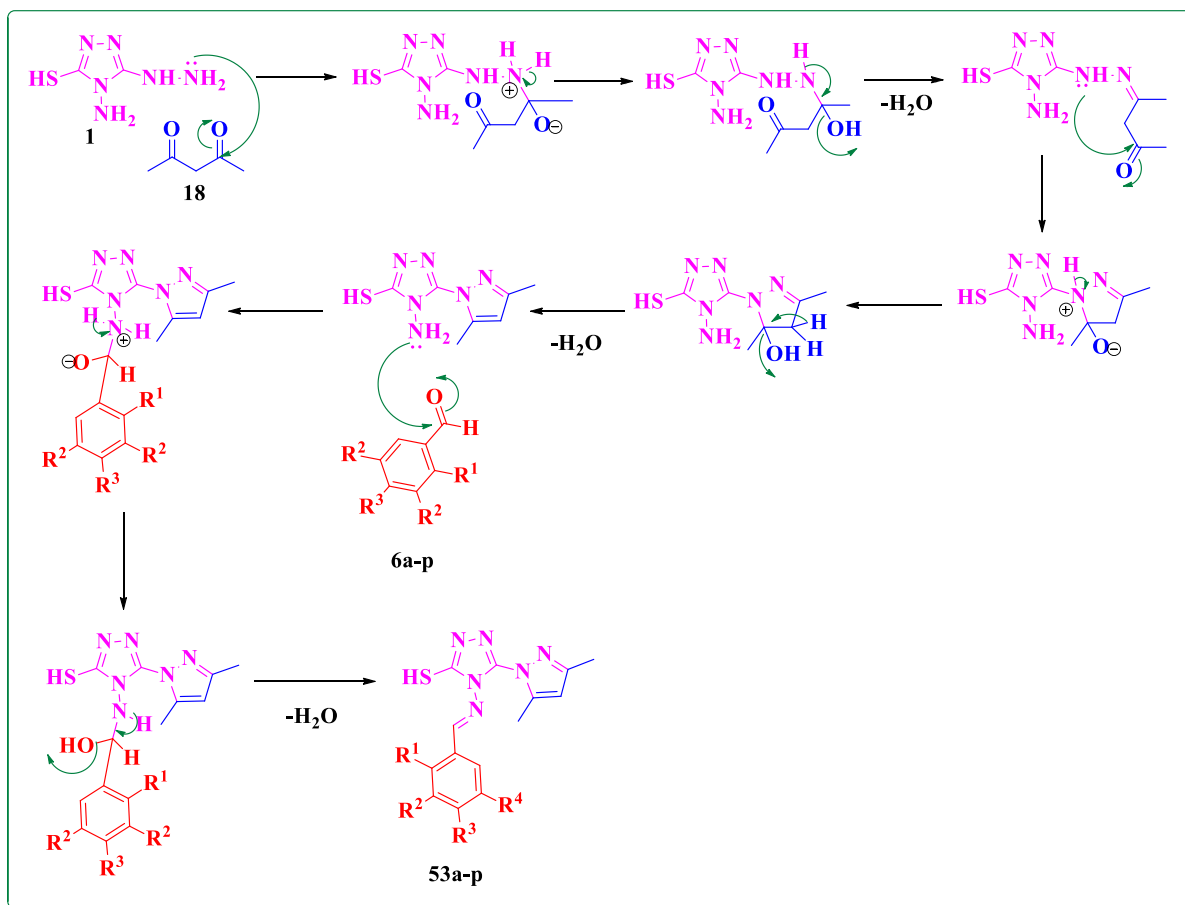
The structures of the title compounds were confirmed by spectral data. The FT-IR spectrum of compound **53a** showed prominent peaks at 2733 cm^{-1} and 1603 cm^{-1} for thiol and $\text{C}=\text{N}$ groups respectively. The ^1H -NMR spectrum of compound **53a** showed characteristic singlets for two methyl groups of pyrazole δ 2.29, 2.33, and singlet for methyl group on benzene ring at δ 2.42. The pyrazole proton appears as a singlet at δ 6.05. The Schiff base proton and thiol proton appear at δ 9.89 and δ 11.91 respectively. The ^{13}C -NMR of **53a** showed the peaks at δ 11.3, 13.6 for $-\text{CH}_3$ of pyrazole, and $-\text{CH}_3$ of benzene ring appeared at δ 21.8. The δ 107.9 and 129.9 corresponds to pyrazole and Schiff's base carbons.

With the one-pot reaction conditions in hand, the relative reactivity of various substituted benzaldehydes was investigated. Initially, the reaction of 3-nitrobenzaldehyde (**6b**) with **1** and **18** reacted smoothly to produce corresponding Schiff's base derivative **53b** in 85% yield (**Table-2**). The independent reactions of 2-hydroxybenzaldehyde (**6c**) and 2-hydroxy-3-methoxy benzaldehyde (**6d**) were furnished well and the expected products (**53c** and **53d**) were obtained in 80% and 75% yields, respectively. Furthermore, 2-chloro benzaldehyde (**6e**) reacted well in the standard conditions to give **53e** in 78% yield. Electron-donating groups at various positions of benzaldehydes (**6f-m**) were utilized in the present conditions, to give the corresponding heterocyclic products in good yields (**53f-m**; 75-88%). Similarly, 4-chlorobenzaldehyde (**6n**) and 4-hydroxybenzaldehyde (**6o**) were treated with **1** and **18** to furnish the expected products in 72% (**53n**) and 80% (**53o**) yields, respectively in good yields. Finally, the reaction of 4-nitrobenzaldehyde (**6p**) in the one-pot conditions progressed well to deliver the compound **53p** in 72% yield. The one-pot reaction of aromatic aldehydes containing electron-withdrawing groups proceeded slowly when compared to the electron-donating aromatic aldehydes.

In general, the time required for the formation of products **53a-p** depends on the type and nature of the substituent present in the aromatic aldehyde. When an electron-withdrawing group like $-\text{NO}_2$ is present in the aldehyde meta-position time required is less when compared to the similar type of substituent at the para position. On the other hand when electron-donating groups are present in the benzene ring of aldehyde the reaction requires more time.

Table-2: Benzylideneamino-3,5-dimethyl-1*H*-pyrazole derivatives (**53a-p**).

Product	R ¹	R ²	R ³	R ⁴	Time (hrs)	Yield (%)
53a	H	H	CH ₃	H	5.20	86
53b	H	NO ₂	H	H	5.00	85
53c	OH	H	H	H	5.30	80
53d	OH	OCH ₃	H	H	5.40	75
53e	Cl	H	H	H	5.50	78
53f	OH	H	N(C ₂ H ₅) ₂	H	5.50	80
53g	H	OCH ₃	OCH ₃	H	5.40	82
53h	OCH ₃	OCH ₃	H	H	5.40	85
53i	OCH ₃	H	OCH ₃	H	5.40	81
53j	H	OCH ₃	OCH ₃	OCH ₃	6.00	78
53k	H	OCH ₃	H	H	5.40	75
53l	OH	OCH ₂ CH ₃	H	H	5.50	88
53m	H	H	OCH ₃	H	5.30	78
53n	H	H	Cl	H	5.50	72
53o	H	H	OH	H	5.40	80
53p	H	H	NO ₂	H	5.30	72



Scheme-2: A plausible mechanism for the synthesis of benzylideneamino-3,5-dimethyl-1H-pyrazoles (**53a-p**).

In conclusion, a facile, one-pot three-component reaction for the synthesis of benzylideneamino-3,5-dimethyl-1H-pyrazoles (**53a-p**) has been achieved via a multi-component approach using readily available starting materials. This method provides various advantages, such as the good of yields, neat reaction conditions, easy workup, and purification. The synthesized heterocyclic compounds may be beneficial for drug discovery.

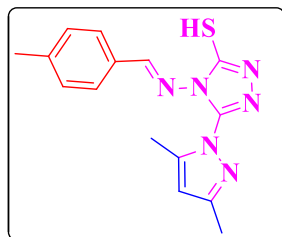
EXPERIMENTAL:

General procedure for the synthesis of benzylideneamino-3,5-dimethyl-1H-pyrazole derivatives (53a-p).

An equimolar mixture of 4-amino-5-hydrazino-4H-[1,2,4] triazole-3-thiol (1 mmol), pentane-2,4-dione (1 mmol), and appropriate aromatic aldehyde (1 mmol) was taken in 5 mL of dry ethanol, with two drops of conc. HCl. The reaction mixture was refluxed for 5-6 h by monitoring TLC ($\text{CHCl}_3:\text{CH}_3\text{OH} = 95:5$), after completion of the reaction, the mixture was allowed to cool and the solid separated was filtered. The crude product was recrystallized from 8 mL ethanol.

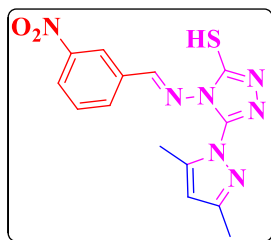
SPECTRAL DATA:

5-(3,5-Dimethyl-1H-pyrazol-1-yl)-4-((4-methylbenzylidene)amino)-4H-1,2,4-triazole-3-thiol (53a):



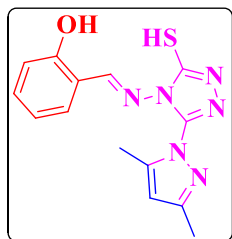
Brown color solid (0.268g, 86%); m.p.: 200-202°C; **FT-IR (KBr, $\nu_{\text{max}}/\text{cm}^{-1}$):** 2733 (-SH), 1603 (-C=N); **$^1\text{H-NMR}$ (400 MHz, CDCl_3 , δ ppm):** 2.29 (s, 3H, - CH_3), 2.42 (s, 3H, - CH_3), 2.33 (s, 3H, - CH_3), 6.06 (s, 1H, -CH- of pyrazole ring), 7.25 (d, 2H, $J=7.6$ Hz, Ar-H), 7.66 (d, 2H, $J=7.6$ Hz, Ar-H), 9.89 (s, 1H, -N=CH-), 11.91 (s, 1H, -SH); **$^{13}\text{C-NMR}$ (100 MHz, CDCl_3 , δ ppm):** 11.3, 13.6, 21.8, 107.9, 129.0, 129.4, 129.6, 129.7, 129.9, 143.5, 143.7, 152.7, 164.4; **ESI-MS (m/z):** 311 $[\text{M-H}]^+$; Analytical calculated formulae $\text{C}_{15}\text{H}_{16}\text{N}_6\text{S}$: C, 57.67; H, 5.16; N, 26.90; S, 10.26. Found: C, 57.63; H, 5.12; N, 26.85; S, 10.21.

5-(3,5-Dimethyl-1H-pyrazol-1-yl)-4-((3-nitrobenzylidene)amino)-4H-1,2,4-triazole-3-thiol (53b):



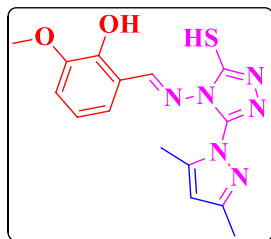
Yellow color solid (0.291g, 85%); m.p.: 212-214°C; **FT-IR (KBr, $\nu_{\max}/\text{cm}^{-1}$):** 2756 (-SH), 1601 (-C=N); **$^1\text{H-NMR}$ (400 MHz, CDCl_3 , δ ppm):** 2.21 (s, 3H, -CH₃), 2.25 (s, 3H, -CH₃), 6.01 (s, 1H, -CH- of pyrazole ring), 7.56 (t, 1H, $J=8.0$ Hz, Ar-H), 7.96 (d, 1H, $J=7.6$ Hz, Ar-H), 8.26 (d, 1H, $J=8.0$ Hz, Ar-H), 8.50 (s, 1H, Ar-H), 10.51 (s, 1H, -N=CH-), 13.65 (s, 1H, -SH); **$^{13}\text{C-NMR}$ (100 MHz, $\text{CDCl}_3+\text{DMSO-}d_6$, δ ppm):** 11.2, 13.5, 108.0, 123.1, 126.4, 130.0, 134.3, 134.3, 143.3, 143.8, 148.6, 152.8, 158.8, 163.2; **ESI-MS (m/z):** 342 [M-H]⁺; Analytical calculated formulae C₁₄H₁₃N₇O₂S: C, 48.97; H, 3.82; N, 28.55; S, 9.34. Found: C, 48.94; H, 3.87; N, 28.51; S, 9.31.

2-(((3-(3,5-Dimethyl-1H-pyrazol-1-yl)-5-mercapto-4H-1,2,4-triazol-4-yl)imino)methyl)phenol (53c):



Brown color solid (0.251g, 80%); m.p.: 184-186°C; **FT-IR (KBr, $\nu_{\max}/\text{cm}^{-1}$):** 2777 (-SH), 1603 (-C=N); **$^1\text{H-NMR}$ (400 MHz, $\text{DMSO-}d_6$, δ ppm):** 2.15 (s, 3H, -CH₃), 2.27 (s, 3H, -CH₃), 6.17 (s, 1H, -CH- of pyrazole ring), 6.91 (t, 1H, $J=7.6$ Hz, Ar-H), 6.98 (d, 1H, $J=8.4$ Hz, Ar-H), 7.43 (t, 1H, $J=8.0$ Hz, Ar-H), 7.62 (d, 1H, $J=8.0$ Hz, Ar-H), 9.88 (s, 1H, -OH), 10.50 (s, 1H, -N=CH-), 14.40 (s, 1H, -SH); **$^{13}\text{C-NMR}$ (100 MHz, $\text{DMSO-}d_6$, δ ppm):** 11.2, 13.7, 108.2, 117.2, 118.2, 120.1, 128.4, 135.1, 142.9, 143.4, 152.0, 159.0, 162.9, 163.1; **ESI-MS (m/z):** 313 [M-H]⁺; Analytical calculated formulae C₁₄H₁₄N₆OS: C, 53.49; H, 4.49; N, 26.73; S, 10.20. Found: C, 53.45; H, 4.46; N, 26.77; S, 10.24.

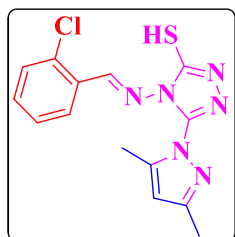
2-(((3-(3,5-Dimethyl-1H-pyrazol-1-yl)-5-mercapto-4H-1,2,4-triazol-4-yl)imino)methyl)-6-methoxyphenol (53d):



Grey color solid (0.258g, 75%); m.p.: 186-188°C; **FT-IR (KBr, $\nu_{\max}/\text{cm}^{-1}$):** 2769 (-SH), 1597 (-C=N); **$^1\text{H-NMR}$ (400 MHz, $\text{CDCl}_3+\text{DMSO-}d_6$, δ ppm):** 2.28 (s, 3H, -CH₃), 2.33 (s, 3H, -CH₃), 3.89 (s, 3H, -CH₃), 6.06 (s, 1H, -CH- of pyrazole ring), 6.90 (t, 1H, $J=8.0$ Hz, Ar-H), 7.04 (t, 2H, $J=6.4$ Hz, Ar-H), 9.98 (s, 1H, -OH), 10.35 (s, 1H, -N=CH-), 14.13 (s, 1H, -SH); **$^{13}\text{C-NMR}$ (100 MHz, $\text{CDCl}_3+\text{DMSO-}d_6$, δ ppm):** 11.4, 13.6, 56.2, 108.2, 115.9, 116.8, 119.5, 123.8, 142.3, 143.4, 148.3, 149.2, 152.8, 163.1, 163.2; **ESI-MS**

(*m/z*): 345 [M+H]⁺; Analytical calculated formulae C₁₅H₁₆N₆O₂S: C, 52.31; H, 4.68; N, 24.40; S, 9.31. Found: C, 52.37; H, 4.73; N, 24.35; S, 9.35.

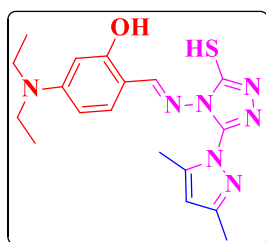
4-((2-Chlorobenzylidene)amino)-5-(3,5-dimethyl-1*H*-pyrazol-1-yl)-4*H*-1,2,4-triazole-3-thiol (53e):



Cream color solid (0.258g, 78%); m.p.: 202-204°C; **FT-IR (KBr, $\nu_{\max}/\text{cm}^{-1}$):** 2742 (-SH), 1618 (-C=N); **¹H-NMR (400 MHz, CDCl₃+DMSO-*d*₆, δ ppm):** 2.26 (s, 3H, -CH₃), 2.30 (s, 3H, -CH₃), 6.07 (s, 1H, -CH- of pyrazole ring), 7.30-7.34 (m, 1H, Ar-H), 7.46 (d, 2H, *J*=4.0 Hz, Ar-H), 7.90 (d, 1H, *J*=7.6 Hz, Ar-H), 10.65 (s, 1H, -N=CH-), 14.68 (s, 1H, -SH); **¹³C-NMR**

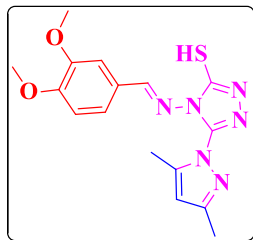
(100 MHz, CDCl₃+DMSO-*d*₆, δ ppm): 11.2, 13.6, 107.7, 127.3, 127.8, 130.1, 130.2, 133.4, 136.3, 143.1, 143.5, 152.3, 158.4, 163.2; **ESI-MS (*m/z*):** 331 [M-H]⁺; Analytical calculated formulae C₁₄H₁₃ClN₆S: C, 50.52; H, 3.94; Cl, 10.65; N, 25.25; S, 9.63. Found: C, 50.56; H, 3.90; Cl, 10.68; N, 25.21; S, 9.68.

5-(Diethylamino)-2-(((3-(3,5-dimethyl-1*H*-pyrazol-1-yl)-5-mercapto-4*H*-1,2,4-triazol-4-yl)imino)methyl)phenol (53f):



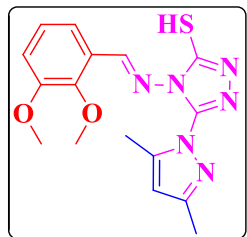
Dark Brown color solid (0.295g, 80%); m.p.: 172-174°C; **FT-IR (KBr, $\nu_{\max}/\text{cm}^{-1}$):** 2731 (-SH), 1631 (-C=N); **¹H-NMR (400 MHz, CDCl₃+DMSO-*d*₆, δ ppm):** 1.19 (t, 6H, *J*=6.8 Hz, -CH₃), 2.26 (s, 3H, -CH₃), 2.28 (s, 3H, -CH₃), 3.36-3.41 (m, 4H, -N-CH₂-), 6.03 (s, 1H, -CH- of pyrazole ring), 6.11 (s, 1H, Ar-H), 6.25 (d, 1H, *J*=8.8 Hz, Ar-H), 7.10 (d, 1H, *J*=8.8 Hz, Ar-H), 9.65 (s, 1H, -OH), 10.24 (s, 1H, -N=CH-); **¹³C-NMR (100 MHz, CDCl₃+DMSO-*d*₆, δ ppm):** 11.2, 12.6, 13.6, 44.6, 97.7, 104.4, 105.0, 107.8, 134.7, 142.3, 143.3, 152.5, 152.6, 161.8, 163.1, 164.7; **ESI-MS (*m/z*):** 386 [M+H]⁺; Analytical calculated formulae C₁₈H₂₃N₇OS: C, 56.08; H, 6.01; N, 25.43; S, 8.32. Found: C, 56.12; H, 6.12; N, 25.49; S, 8.37.

4-((3,4-Dimethoxybenzylidene)amino)-5-(3,5-dimethyl-1H-pyrazol-1-yl)-4H-1,2,4-triazole-3-thiol (53g):



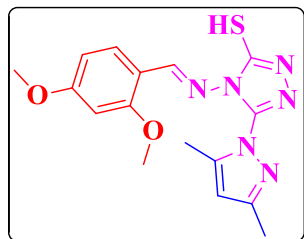
Light yellow color solid (0.293g, 82%); m.p.: 215-217°C; **FT-IR (KBr, $\nu_{\max}/\text{cm}^{-1}$):** 2736 (-SH), 1610 (-C=N); **$^1\text{H-NMR}$ (400 MHz, CDCl_3 +DMSO- d_6 , δ ppm):** 2.16 (s, 3H, -CH₃), 2.22 (s, 3H, -CH₃), 3.78 (s, 3H, -OCH₃), 3.85 (s, 3H, -OCH₃), 5.98 (s, 1H, -CH- of pyrazole ring), 6.87 (d, 1H, J =8.0 Hz, Ar-H), 7.20 (d, 1H, J =8.4 Hz, Ar-H), 7.63 (s, 1H, Ar-H), 9.71 (s, 1H, -N=CH-), 14.04 (s, 1H, -SH); **$^{13}\text{C-NMR}$ (100 MHz, CDCl_3 +DMSO- d_6 , δ ppm):** 11.3, 13.6, 55.8, 56.1, 107.6, 109.0, 110.9, 124.9, 124.9, 143.0, 143.5, 149.3, 152.0, 153.1, 163.1, 163.3; **ESI-MS (m/z):** 359 $[\text{M}+\text{H}]^+$; Analytical calculated formulae C₁₆H₁₈N₆O₂S: C, 53.62; H, 5.06; N, 23.45; S, 8.95. Found: C, 53.68; H, 4.95; N, 23.49; S, 8.91.

4-((2,3-Dimethoxybenzylidene)amino)-5-(3,5-dimethyl-1H-pyrazol-1-yl)-4H-1,2,4-triazole-3-thiol (53h):



Light yellow color solid (0.304g, 85%); m.p.: 180-182°C; **FT-IR (KBr, $\nu_{\max}/\text{cm}^{-1}$):** 2742 (-SH), 1614 (-C=N); **$^1\text{H-NMR}$ (400 MHz, CDCl_3 , δ ppm):** 2.29 (s, 3H, -CH₃), 2.33 (s, 3H, -CH₃), 3.91 (s, 3H, -OCH₃), 3.96 (s, 3H, -OCH₃), 6.05 (s, 1H, -CH- of pyrazole ring), 7.08 (m, 2H, Ar-H), 7.47 (d, 1H, J =7.2 Hz, Ar-H), 10.20 (s, 1H, -N=CH-), 11.66 (s, 1H, -SH); **$^{13}\text{C-NMR}$ (100 MHz, CDCl_3 , δ ppm):** 11.3, 13.6, 55.9, 62.3, 107.9, 116.3, 118.5, 124.3, 126.1, 143.5, 143.6, 150.7, 152.7, 152.9, 161.0, 163.5; **ESI-MS (m/z):** 359 $[\text{M}+\text{H}]^+$; Analytical calculated formulae C₁₆H₁₈N₆O₂S: C, 53.62; H, 5.06; N, 23.45; S, 8.95. Found: C, 53.68; H, 4.95; N, 23.49; S, 8.91.

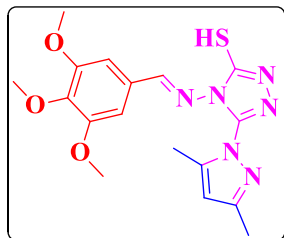
4-((2,4-Dimethoxybenzylidene)amino)-5-(3,5-dimethyl-1H-pyrazol-1-yl)-4H-1,2,4-triazole-3-thiol (53i):



Yellow color solid (0.302g, 78%); m.p.: 188-200°C; **FT-IR (KBr, $\nu_{\max}/\text{cm}^{-1}$):** 2737 (-SH), 1605 (-C=N); **$^1\text{H-NMR}$ (400 MHz, , DMSO- d_6 , δ ppm):** 2.22 (s, 3H, -CH₃), 2.27 (s, 3H, -CH₃), 3.86 (s, 3H, -OCH₃), 3.88 (s, 3H, -OCH₃), 6.04 (s, 1H, -CH- of pyrazole ring), 6.49 (s, 1H, Ar-H), 6.53 (d, 1H, J =8.8 Hz, Ar-H), 7.79 (d, 1H, J =10.4 Hz, Ar-H), 9.82 (s, 1H, -N=CH-), 14.09 (s, 1H, -SH); **$^{13}\text{C-NMR}$ (100 MHz, CDCl_3 +DMSO- d_6 , δ ppm):** 11.2, 13.6, 55.7, 55.9, 97.9, 106.7, 107.6, 113.4, 128.6, 133.0,

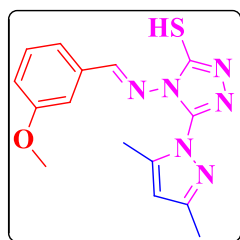
143.3, 151.9, 160.8, 161.4, 163.2, 165.2; **ESI-MS (m/z):** 359 $[M+H]^+$; Analytical calculated formulae $C_{16}H_{18}N_6O_2S$: C, 53.62; H, 5.06; N, 23.45; S, 8.95. Found: C, 53.68; H, 4.95; N, 23.49; S, 8.91.

5-(3,5-Dimethyl-1H-pyrazol-1-yl)-4-((3,4,5-trimethoxybenzylidene)amino)-4H-1,2,4-triazole-3-thiol (53j):



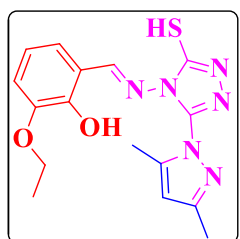
Golden yellow color solid (0.289g, 81%); m.p.: 174-176°C; **FT-IR (KBr, $\nu_{\max}/\text{cm}^{-1}$):** 2746 (-SH), 1610 (-C=N); **$^1\text{H-NMR}$ (400 MHz, CDCl_3 , δ ppm):** 2.28 (s, 3H, -CH₃), 2.32 (s, 3H, -CH₃), 3.87 (s, 6H, -OCH₃), 3.91 (s, 3H, -OCH₃), 6.05 (s, 1H, -CH- of pyrazole ring), 6.99 (s, 2H, Hz, Ar-H), 9.88 (s, 1H, -N=CH-), 11.68 (s, 1H, -SH); **$^{13}\text{C-NMR}$ (100 MHz, CDCl_3 , δ ppm):** 11.4, 13.6, 56.2, 61.0, 106.1, 107.9, 127.3, 142.0, 143.5, 143.8, 152.7, 153.4, 163.3, 163.6; **ESI-MS (m/z):** 389 $[M+H]^+$; Analytical calculated formulae $C_{17}H_{20}N_6O_3S$: C, 52.56; H, 5.19; N, 21.64; S, 8.25. Found: C, 52.51; H, 5.16; N, 21.60; S, 8.29.

5-(3,5-Dimethyl-1H-pyrazol-1-yl)-4-((3-methoxybenzylidene)amino)-4H-1,2,4-triazole-3-thiol (53k):



Golden yellow color solid (0.246g, 75%); m.p.: 179-181°C; **FT-IR (KBr, $\nu_{\max}/\text{cm}^{-1}$):** 2740 (-SH), 1607 (-C=N); **$^1\text{H-NMR}$ (400 MHz, CDCl_3 , δ ppm):** 2.28 (s, 3H, -CH₃), 2.31 (s, 3H, -CH₃), 3.82 (s, 3H, -OCH₃), 6.04 (s, 1H, -CH- of pyrazole ring), 7.06 (d, 1H, $J=7.2$ Hz, Ar-H), 7.29 (s, 1H, Ar-H), 7.34 (m, 3H, Ar-H), 9.99 (s, 1H, -N=CH-), 11.74 (s, 1H, -SH); **$^{13}\text{C-NMR}$ (100 MHz, $\text{CDCl}_3+\text{DMSO}-d_6$, δ ppm):** 11.3, 13.6, 55.3, 107.7, 112.3, 119.0, 122.2, 129.8, 133.7, 143.3, 143.6, 152.4, 159.8, 162.7, 163.2; **ESI-MS (m/z):** 329 $[M+H]^+$; Analytical calculated formulae $C_{15}H_{16}N_6OS$: C, 54.86; H, 4.91; N, 25.59; S, 9.76. Found: C, 54.81; H, 4.97; N, 25.54; S, 9.71.

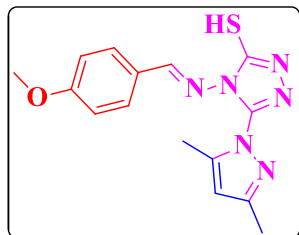
2-(((3-(3,5-Dimethyl-1H-pyrazol-1-yl)-5-mercapto-4H-1,2,4-triazol-4-yl)imino)methyl)-6-ethoxyphenol (53l):



Grey color solid (0.315g, 88%); m.p.: 172-174°C; **FT-IR (KBr, $\nu_{\max}/\text{cm}^{-1}$):** 2733 (-SH), 1603 (-C=N); **$^1\text{H-NMR}$ (400 MHz, CDCl_3 , δ ppm):** 1.46 (t, 3H, $J=6.8$ Hz, -CH₃), 2.30 (s, 3H, -CH₃), 2.33 (s, 3H, -CH₃), 4.12 (m, 2H, Hz, -OCH₂-), 6.05 (s, 1H, -CH- of pyrazole ring), 6.88 (t, 1H, $J=8.0$ Hz, Ar-H), 7.02 (m, 2H, Ar-H), 9.83 (s, 1H, -OH), 10.25 (s, 1H, -N=CH-),

11.69 (s, 1H, -SH); $^{13}\text{C-NMR}$ (100 MHz, $\text{CDCl}_3+\text{DMSO}-d_6$, δ ppm): 11.4, 13.6, 14.8, 64.9, 108.3, 116.7, 117.7, 119.4, 124.6, 142.5, 143.4, 147.6, 149.7, 153.1, 163.2, 163.9; **ESI-MS** (m/z): 359 $[\text{M}+\text{H}]^+$; Analytical calculated formulae $\text{C}_{16}\text{H}_{18}\text{N}_6\text{O}_2\text{S}$: C, 53.62; H, 5.06; N, 23.45; S, 8.95. Found: C, 53.68; H, 5.14; N, 23.40; S, 8.99.

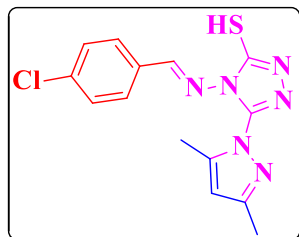
5-(3,5-Dimethyl-1H-pyrazol-1-yl)-4-((4-methoxybenzylidene)amino)-4H-1,2,4-triazole-3-thiol (53m):



White color solid (0.255g, 78%); m.p.: 182-184°C; **FT-IR** (KBr, $\nu_{\text{max}}/\text{cm}^{-1}$): 2729 (-SH), 1603 (-C=N); $^1\text{H-NMR}$ (400 MHz, CDCl_3 , δ ppm): 2.26 (s, 3H, -CH₃), 2.31 (s, 3H, -CH₃), 3.86 (s, 3H, -OCH₃), 6.03 (s, 1H, -CH- of pyrazole ring), 6.93 (d, 2H, $J=8.0$ Hz, Ar-H), 7.71 (d, 1H, $J=8.4$ Hz, Ar-H), 9.76 (s, 1H, -N=CH-), 11.47 (s, 1H, -

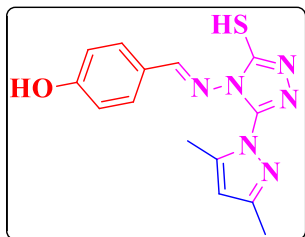
SH); $^{13}\text{C-NMR}$ (100 MHz, CDCl_3 , δ ppm): 11.3, 13.6, 55.5, 107.9, 114.4, 124.6, 131.0, 143.6, 152.7, 163.4, 163.4, 164.3; **ESI-MS** (m/z): 327 $[\text{M}-\text{H}]^+$; Analytical calculated formulae $\text{C}_{15}\text{H}_{16}\text{N}_6\text{OS}$: C, 54.86; H, 4.91; N, 25.59; S, 9.76. Found: C, 54.81; H, 4.97; N, 25.54; S, 9.71.

4-((4-Chlorobenzylidene)amino)-5-(3,5-dimethyl-1H-pyrazol-1-yl)-4H-1,2,4-triazole-3-thiol (53n):



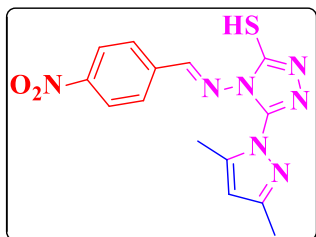
White color solid (0.239g, 72%); m.p.: 207-209°C; **FT-IR** (KBr, $\nu_{\text{max}}/\text{cm}^{-1}$): 2741 (-SH), 1619 (-C=N); $^1\text{H-NMR}$ (400 MHz, CDCl_3 , δ ppm): 2.28 (s, 3H, -CH₃), 2.32 (s, 3H, -CH₃), 6.06 (s, 1H, -CH- of pyrazole ring), 7.30 (d, 1H, $J=6.4$ Hz, Ar-H), 7.44 (d, 2H, $J=4.8$ Hz, Ar-H), 7.92 (d, 1H, $J=7.6$ Hz, Ar-H), 10.46 (s, 1H, -N=CH-), 10.59 (s, 1H, -SH); $^{13}\text{C-NMR}$ (100 MHz, $\text{CDCl}_3+\text{DMSO}-d_6$, δ ppm): 11.2, 13.6, 107.7, 127.1, 127.8, 130.0, 133.3, 136.3, 143.5, 152.3, 158.5, 163.2; **ESI-MS** (m/z): 333 $[\text{M}+\text{H}]^+$; Analytical calculated formulae $\text{C}_{14}\text{H}_{13}\text{ClN}_6\text{S}$: C, 50.52; H, 3.94; Cl, 10.65; N, 25.25; S, 9.63. Found: C, 50.56; H, 3.90; Cl, 10.68; N, 25.21; S, 9.68.

4-(((3-(3,5-Dimethyl-1H-pyrazol-1-yl)-5-mercapto-4H-1,2,4-triazol-4-yl)imino)methyl)phenol (53o):



White color solid (0.251g, 80%); m.p.: 195-197°C; **FT-IR (KBr, $\nu_{\max}/\text{cm}^{-1}$):** 2725 (-SH), 1605 (-C=N); **$^1\text{H-NMR}$ (400 MHz, $\text{CDCl}_3+\text{DMSO}-d_6$, δ ppm):** 2.14 (s, 3H, -CH₃), 2.19 (s, 3H, -CH₃), 5.92 (s, 1H, -CH- of pyrazole ring), 6.48 (d, 1H, $J=8.0$ Hz, Ar-H), 6.86 (d, 1H, $J=8.0$ Hz, Ar-H), 7.49 (d, 1H, $J=8.0$ Hz, Ar-H), 7.65 (d, 1H, $J=8.0$ Hz, Ar-H), 9.55 (s, 1H, -OH), 9.72 (s, 1H, -N=CH-), 13.84 (s, 1H, -SH); **$^{13}\text{C-NMR}$ (100 MHz, $\text{CDCl}_3+\text{DMSO}-d_6$, δ ppm):** 11.2, 13.6, 107.5, 116.0, 123.25, 130.9, 132.2, 143.4, 152.1, 161.9, 163.0, 164.3; **ESI-MS (m/z):** 315 $[\text{M}+\text{H}]^+$; Analytical calculated formulae $\text{C}_{14}\text{H}_{14}\text{N}_6\text{OS}$: C, 53.49; H, 4.49; N, 26.73; S, 10.20. Found: C, 53.45; H, 4.46; N, 26.77; S, 10.24.

5-(3,5-Dimethyl-1H-pyrazol-1-yl)-4-((4-nitrobenzylidene)amino)-4H-1,2,4-triazole-3-thiol (53p):



Yellow color solid (0.251g, 80%); m.p.: 217-219°C; **FT-IR (KBr, $\nu_{\max}/\text{cm}^{-1}$):** 2755 (-SH), 1625 (-C=N); **$^1\text{H-NMR}$ (400 MHz, CDCl_3 , δ ppm):** 2.28 (s, 3H, -CH₃), 2.34 (s, 3H, -CH₃), 6.09 (s, 1H, -CH- of pyrazole ring), 7.90 (d, 2H, $J=8.8$ Hz, Ar-H), 8.29 (d, 2H, $J=8.8$ Hz, Ar-H), 10.53 (s, 2H, 1H of -N=CH- and 1H of -SH); **$^{13}\text{C-NMR}$ (100 MHz, CDCl_3 , δ ppm):** 11.1, 13.6, 108.3, 124.1, 124.3, 129.5, 130.5, 138.1, 143.7, 153.0, 158.8, 162.0; **ESI-MS (m/z):** 344 $[\text{M}+\text{H}]^+$; Analytical calculated formulae $\text{C}_{14}\text{H}_{13}\text{N}_7\text{O}_2\text{S}$: C, 48.97; H, 3.82; N, 28.55; S, 9.34. Found: C, 48.50; H, 3.93; N, 28.51; S, 9.38.

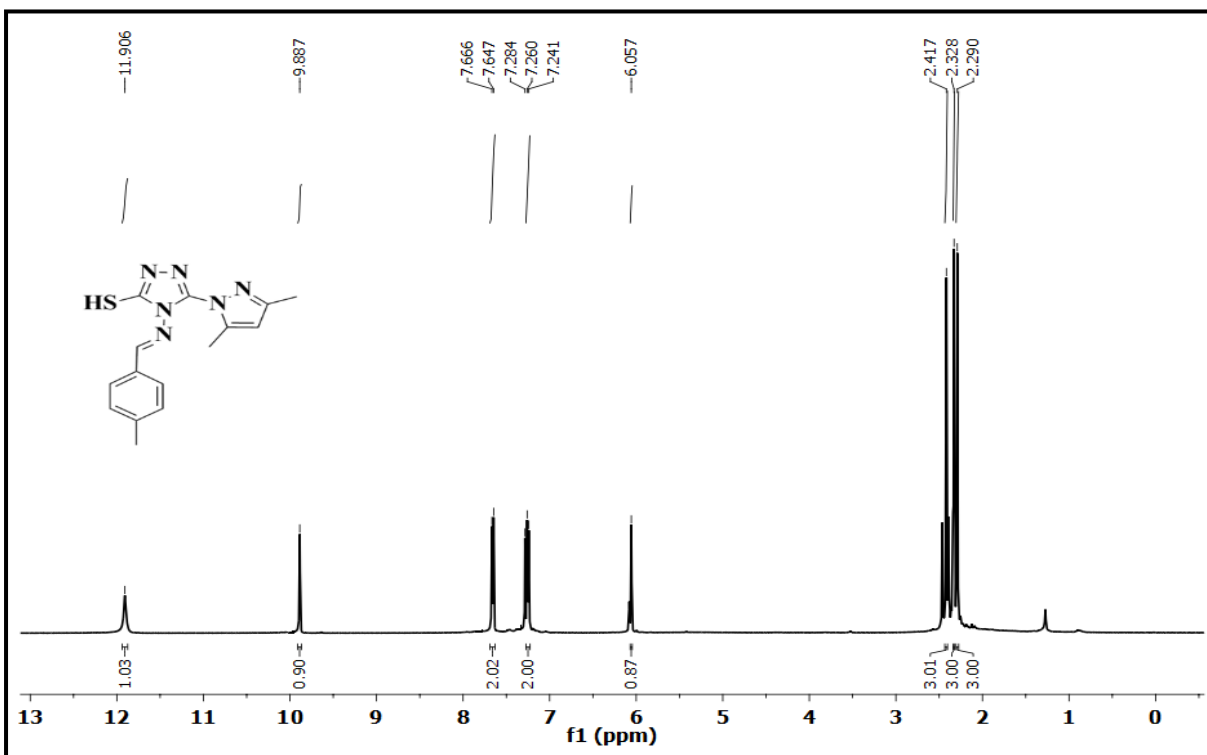
SPECTRA

Fig: ¹H-NMR Spectrum of Compound 53a (400 MHz, CDCl₃)

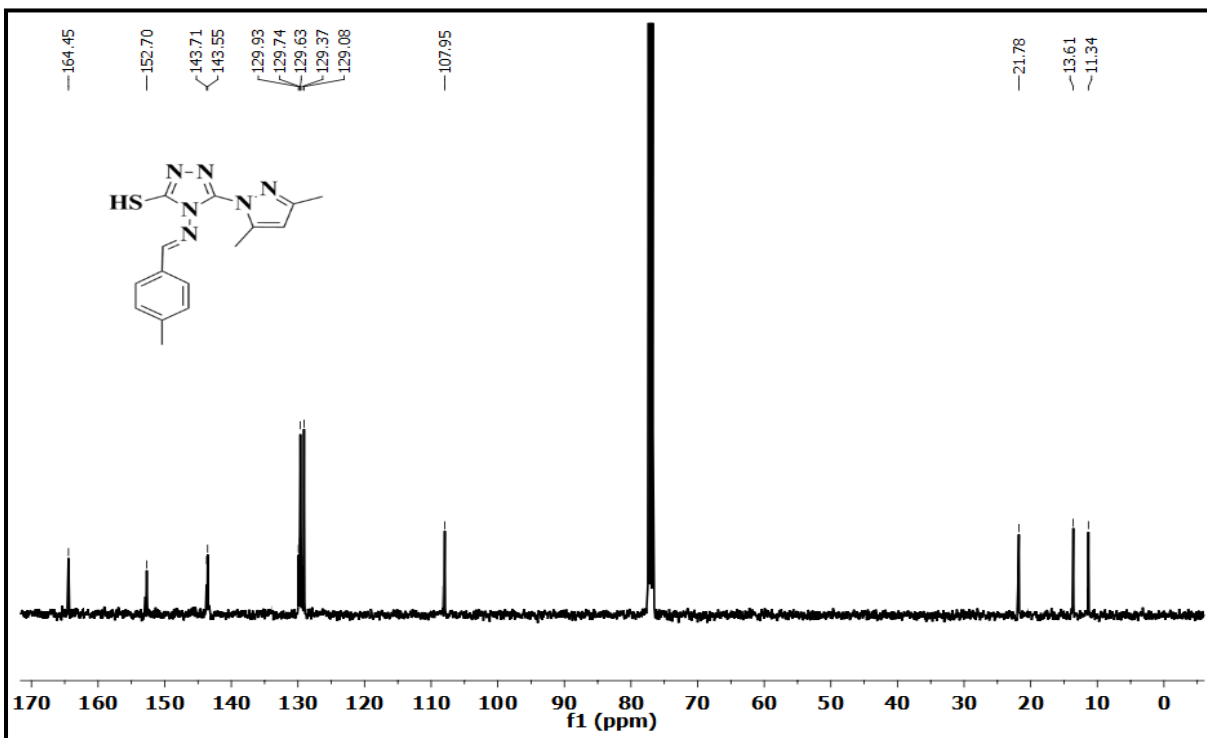


Fig: ¹³C-NMR Spectrum of Compound 53a (100 MHz, CDCl₃)

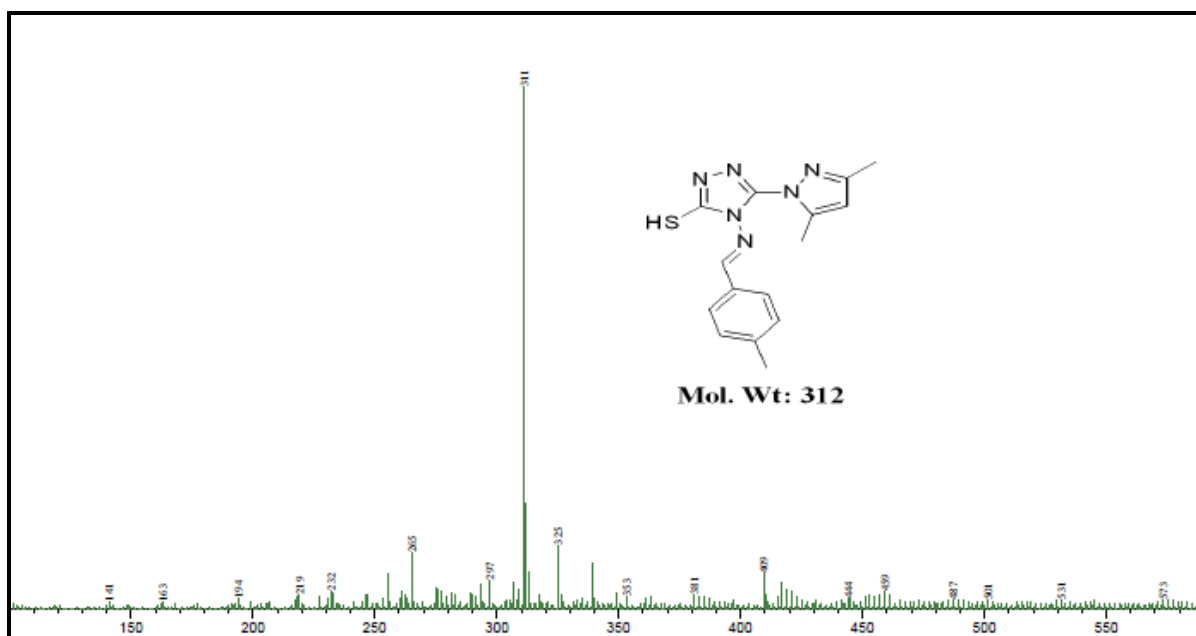


Fig: Mass Spectrum of Compound 53a

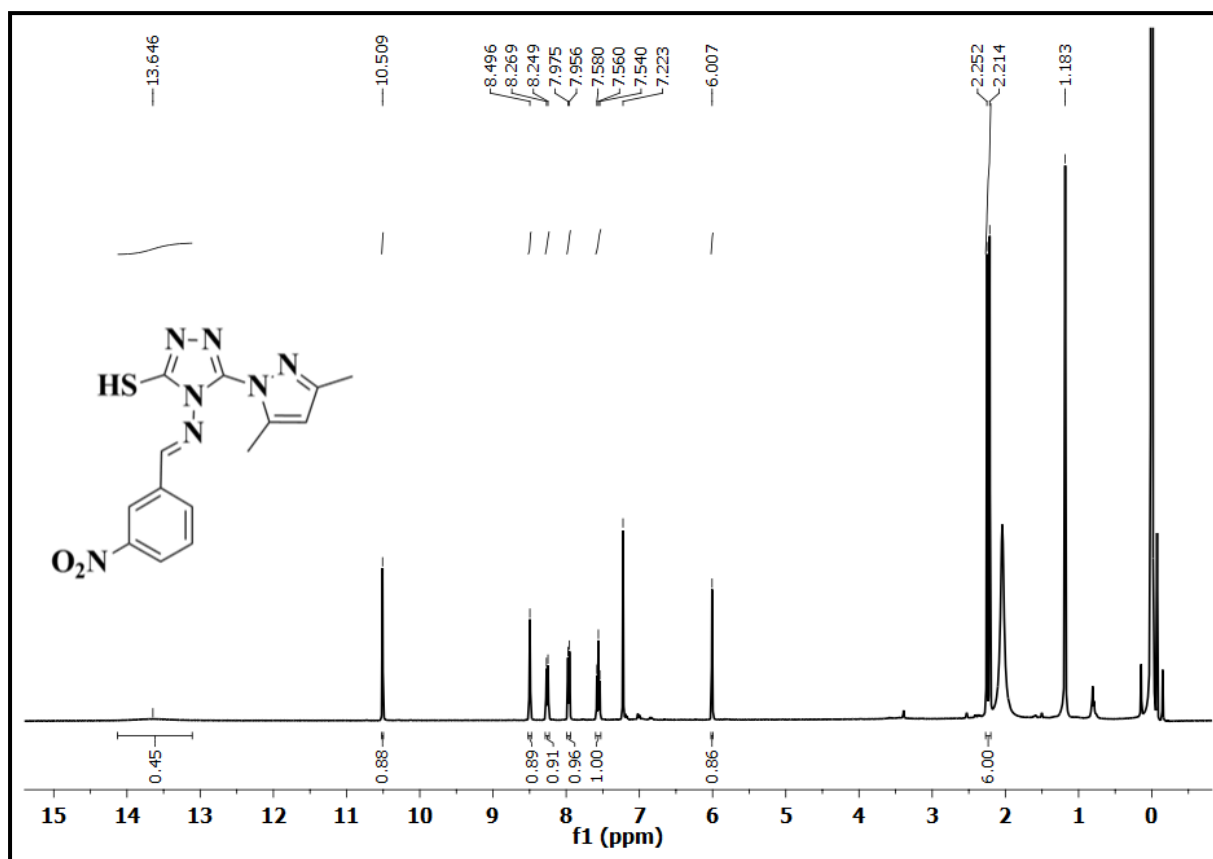


Fig: ¹H-NMR Spectrum of Compound 53b (400 MHz, CDCl₃)

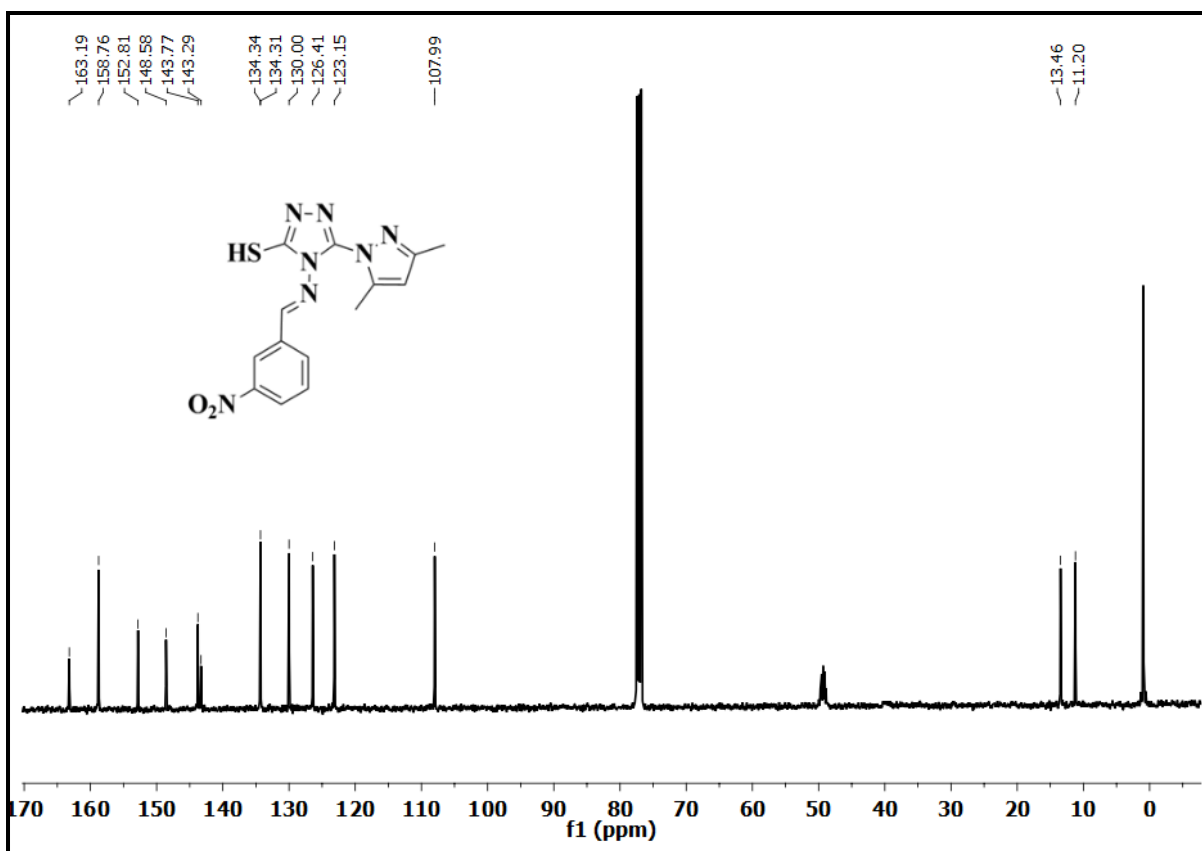


Fig: ¹³C-NMR Spectrum of Compound **53b** (100 MHz, CDCl₃+DMSO-*d*₆)

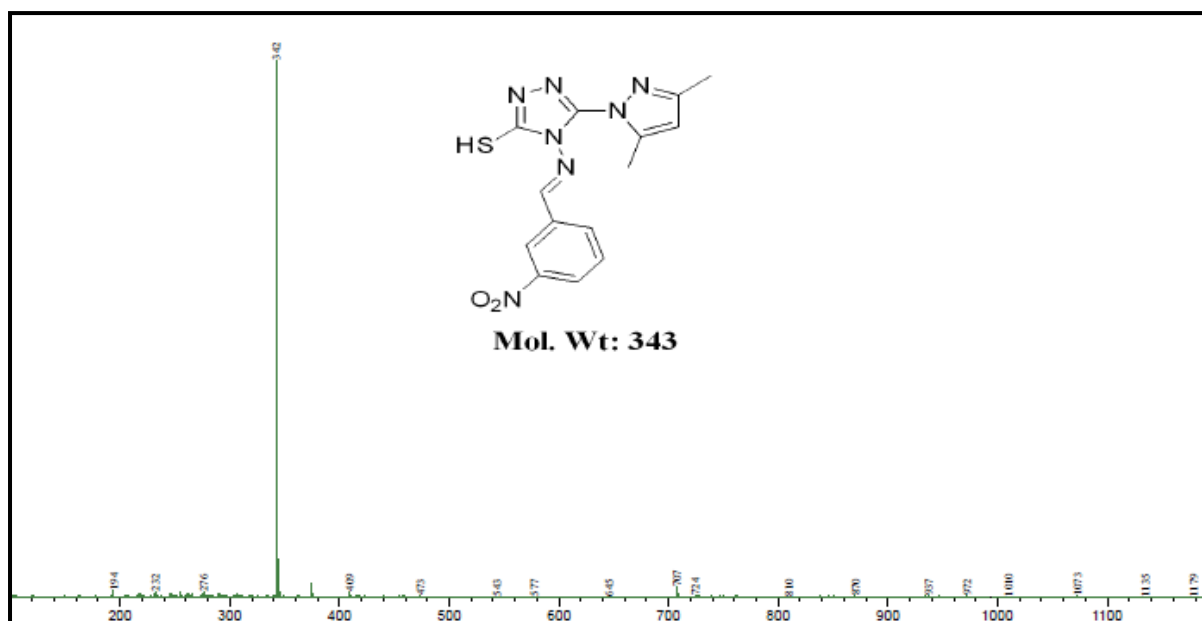


Fig: Mass Spectrum of Compound **53b**

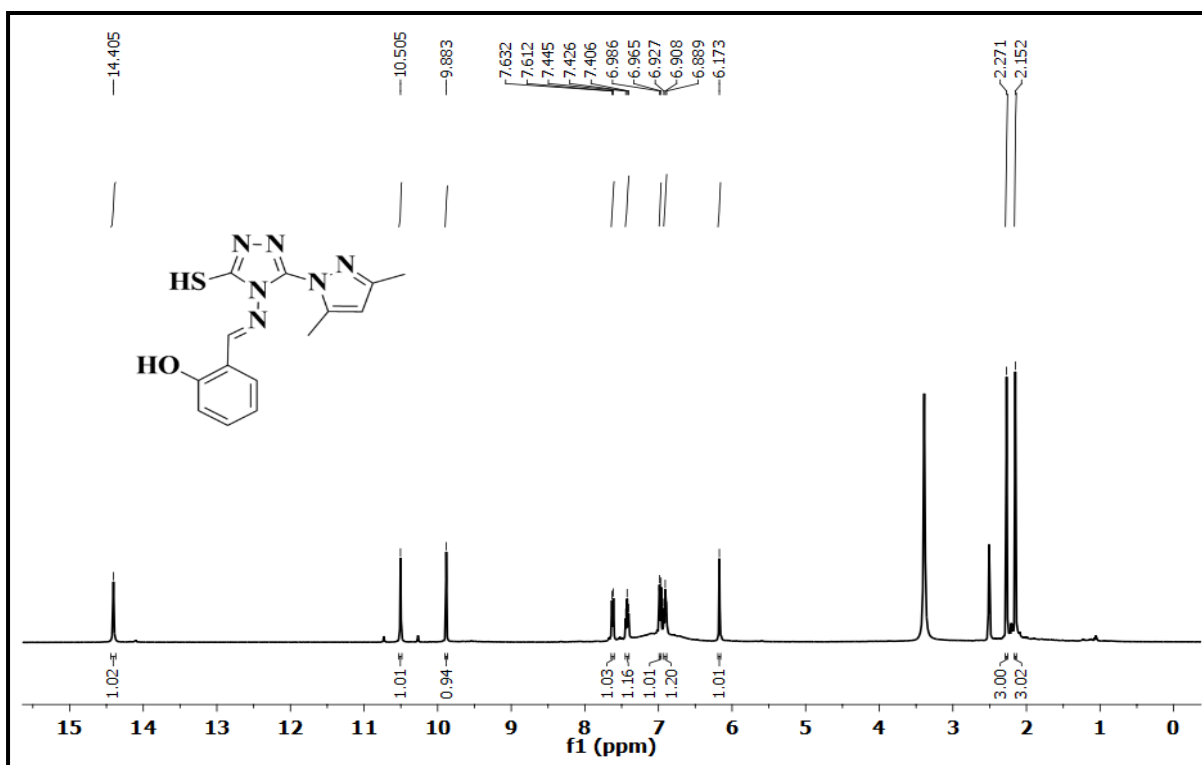


Fig: ¹H-NMR Spectrum of Compound 53c (400 MHz, DMSO-*d*₆)

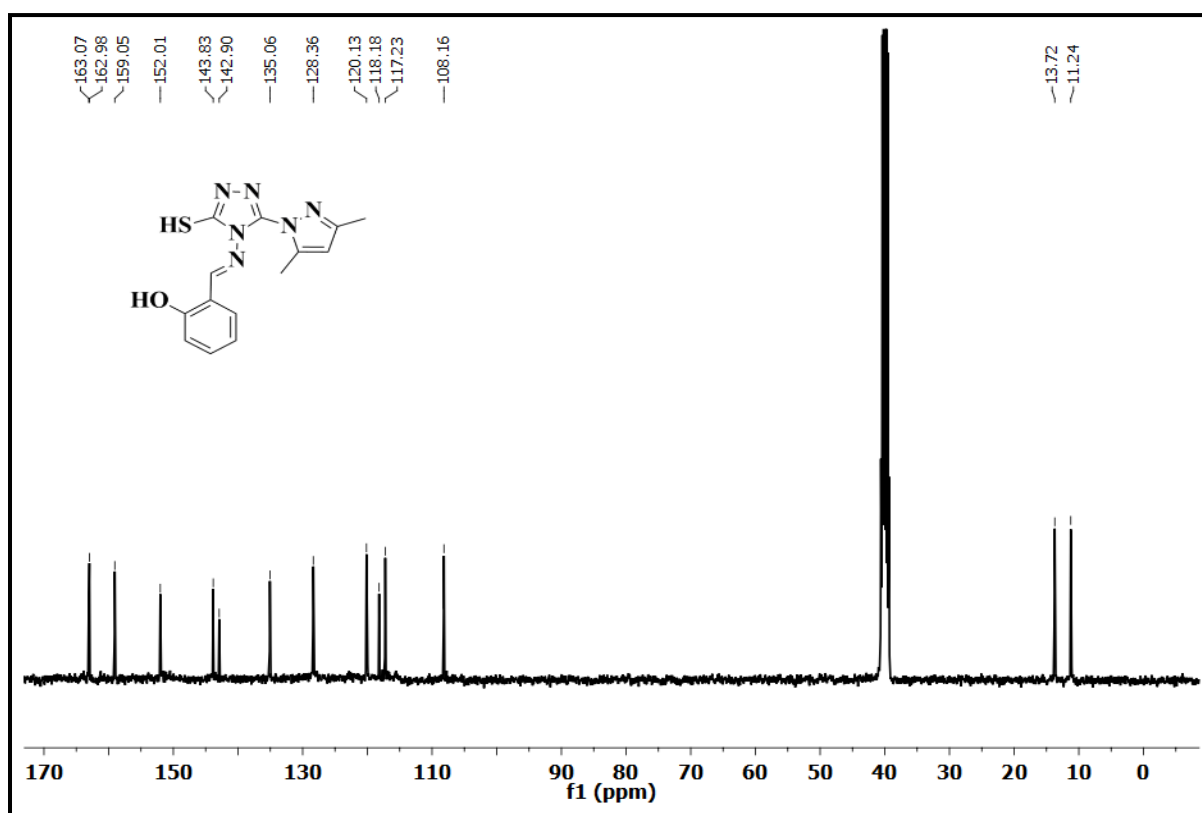


Fig: ¹³C-NMR Spectrum of Compound 53c (100 MHz, DMSO-*d*₆)

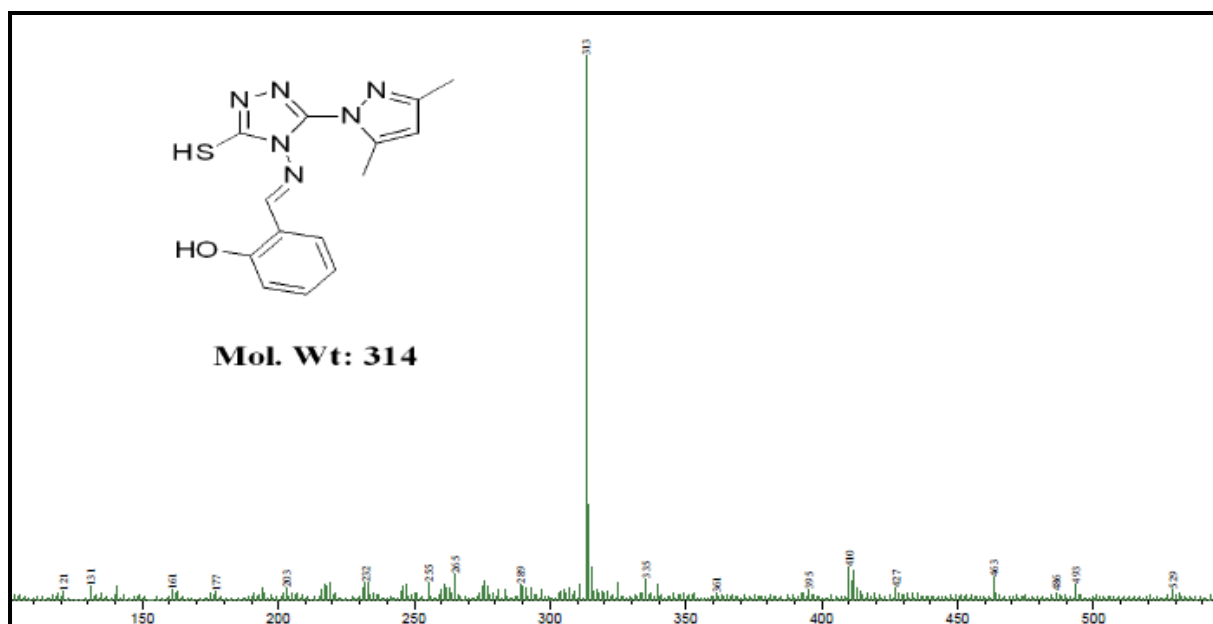


Fig: Mass Spectrum of Compound 53c

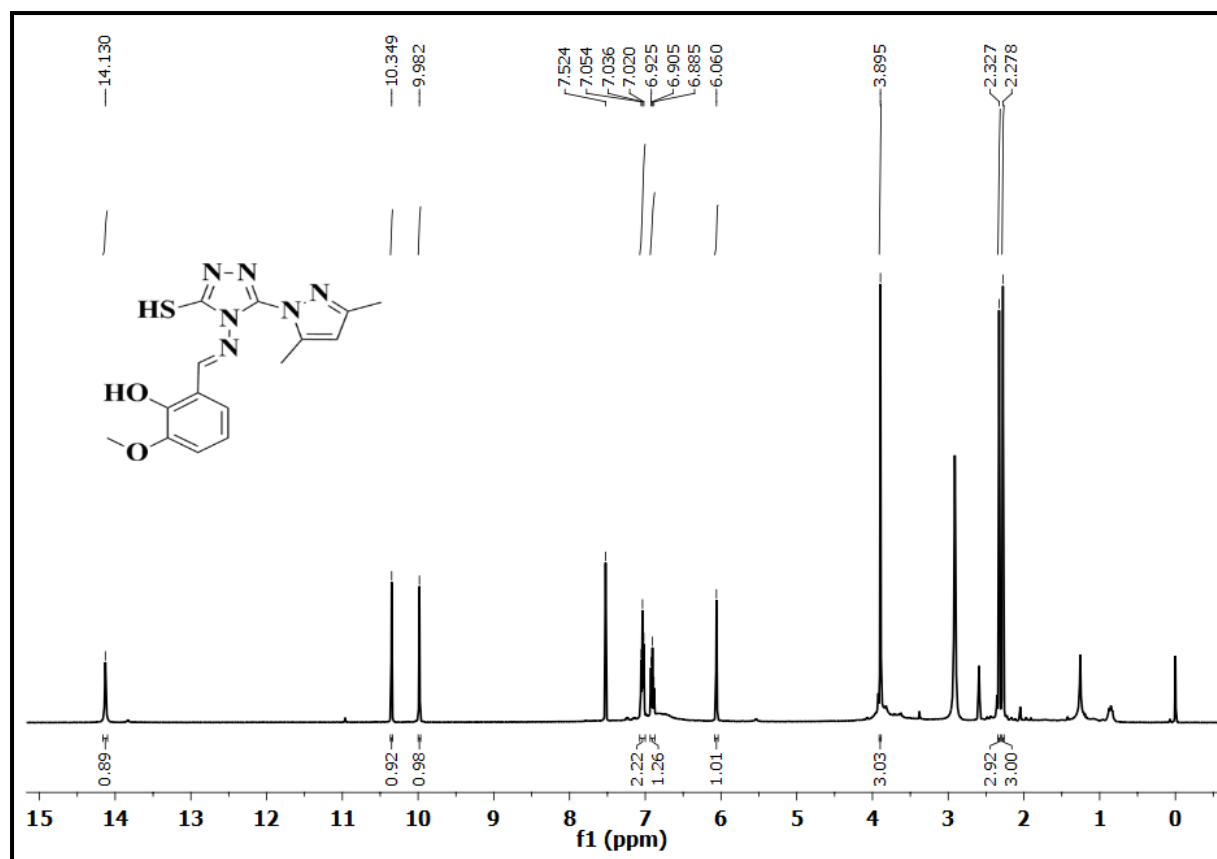


Fig: ¹H-NMR Spectrum of Compound 53d (400 MHz, CDCl₃+DMSO-d₆)

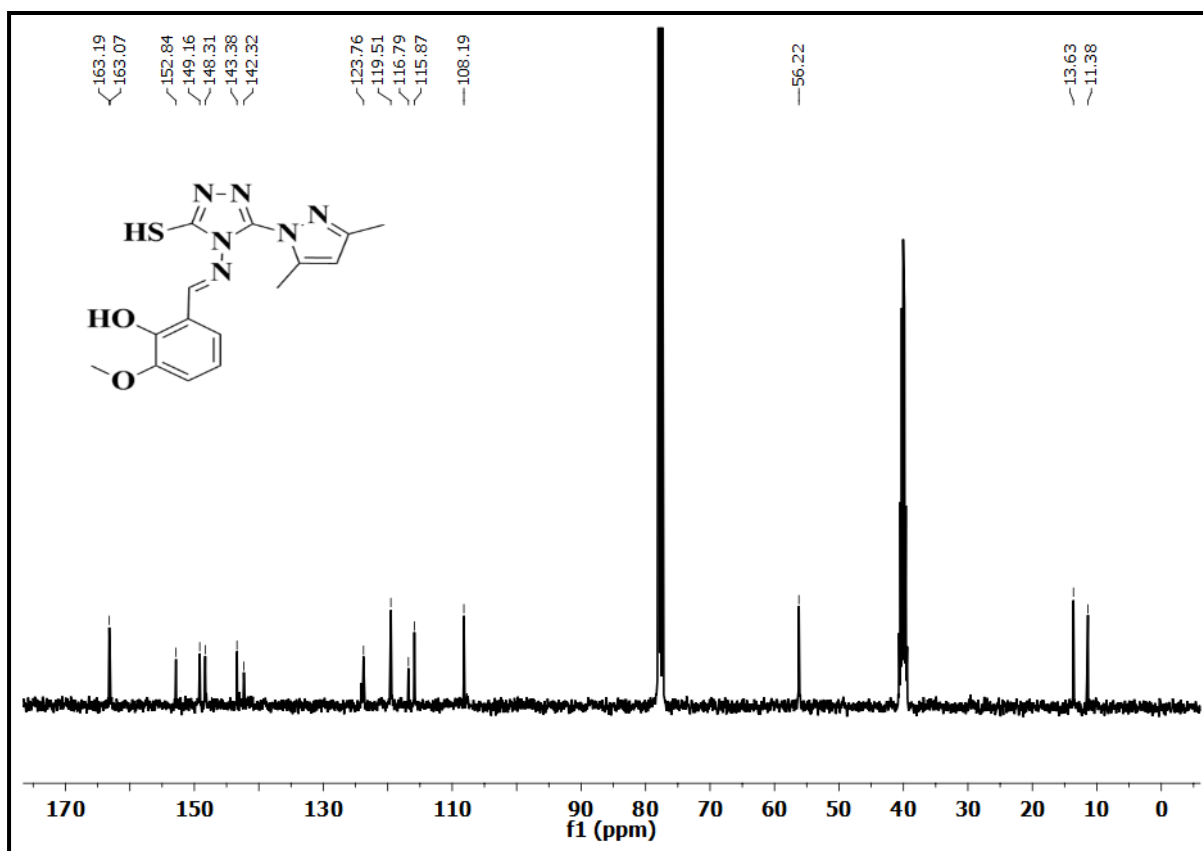


Fig: ¹³C-NMR Spectrum of Compound **53d** (100 MHz, CDCl₃+DMSO-*d*₆)

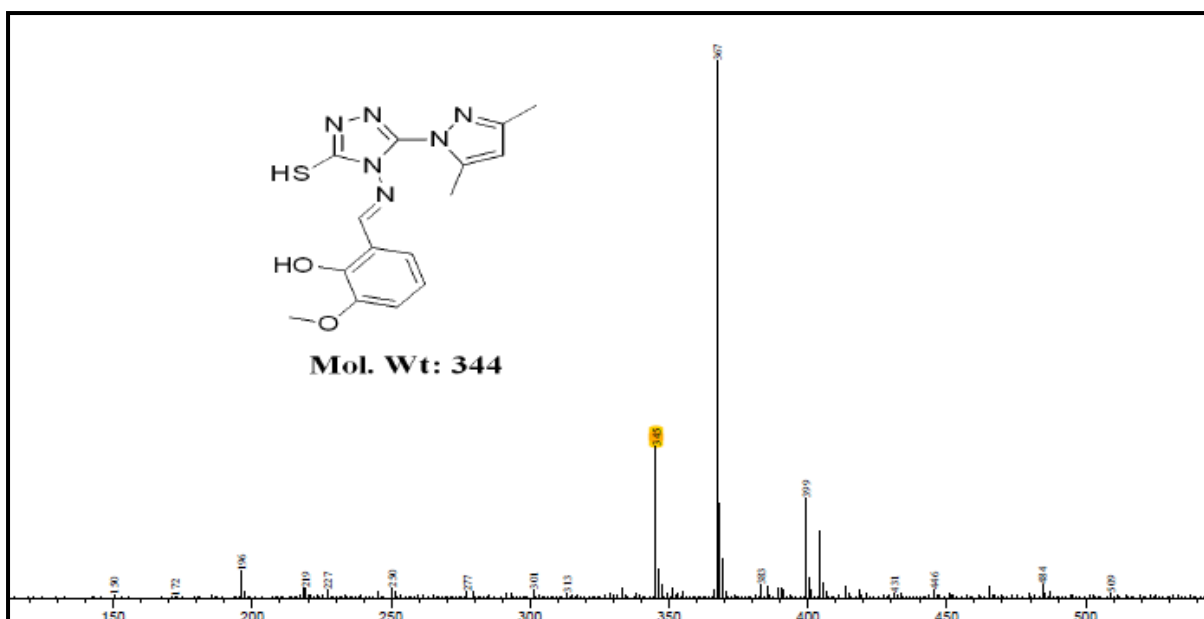


Fig: Mass Spectrum of Compound **53d**

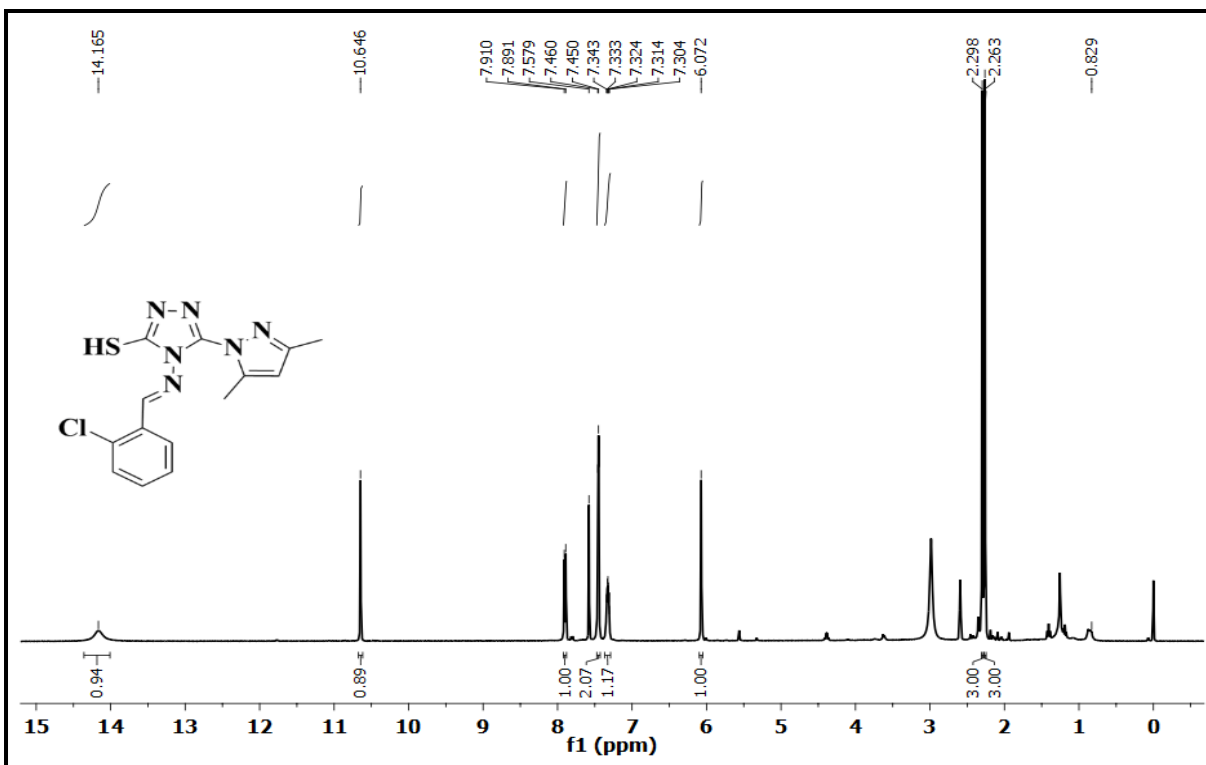


Fig: ¹H-NMR Spectrum of Compound 53e (400 MHz, CDCl₃+DMSO-*d*₆)

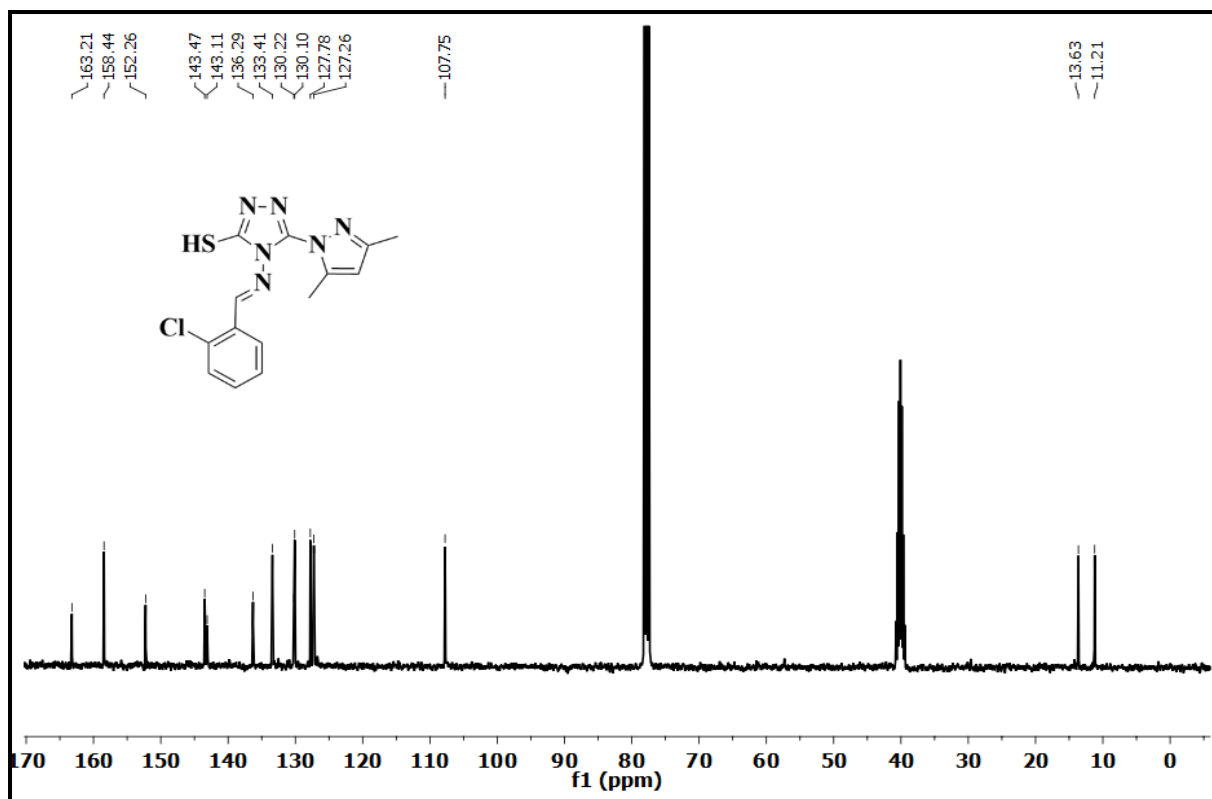


Fig: ¹³C-NMR Spectrum of Compound 53e (100 MHz, CDCl₃+DMSO-*d*₆)

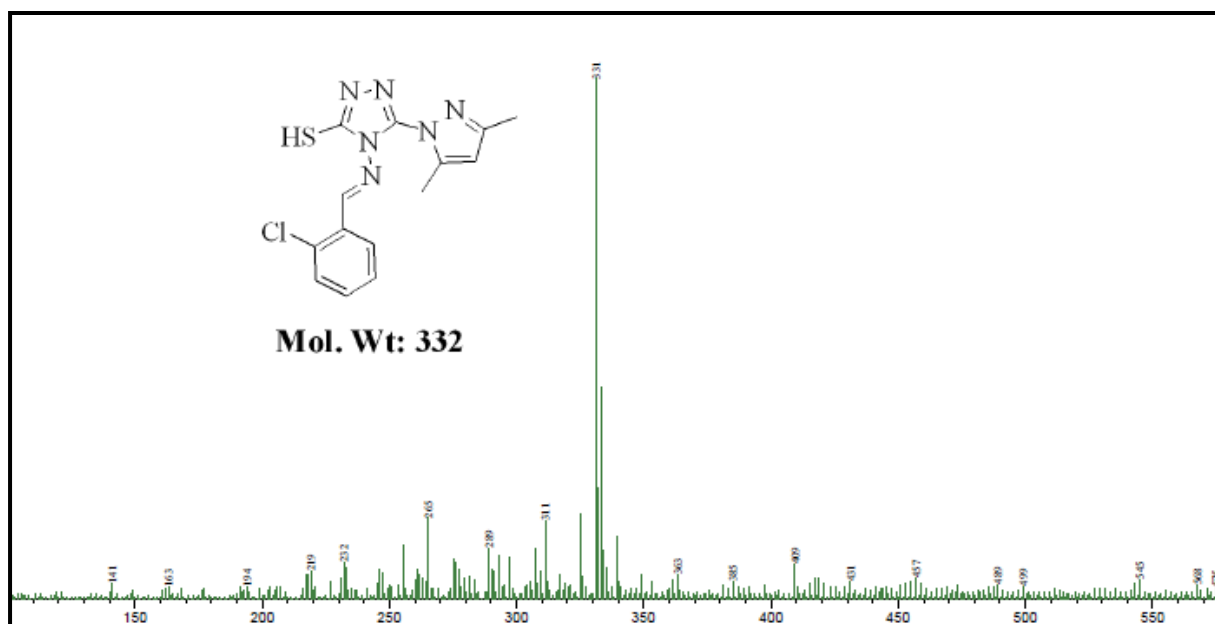


Fig: Mass Spectrum of Compound 53e

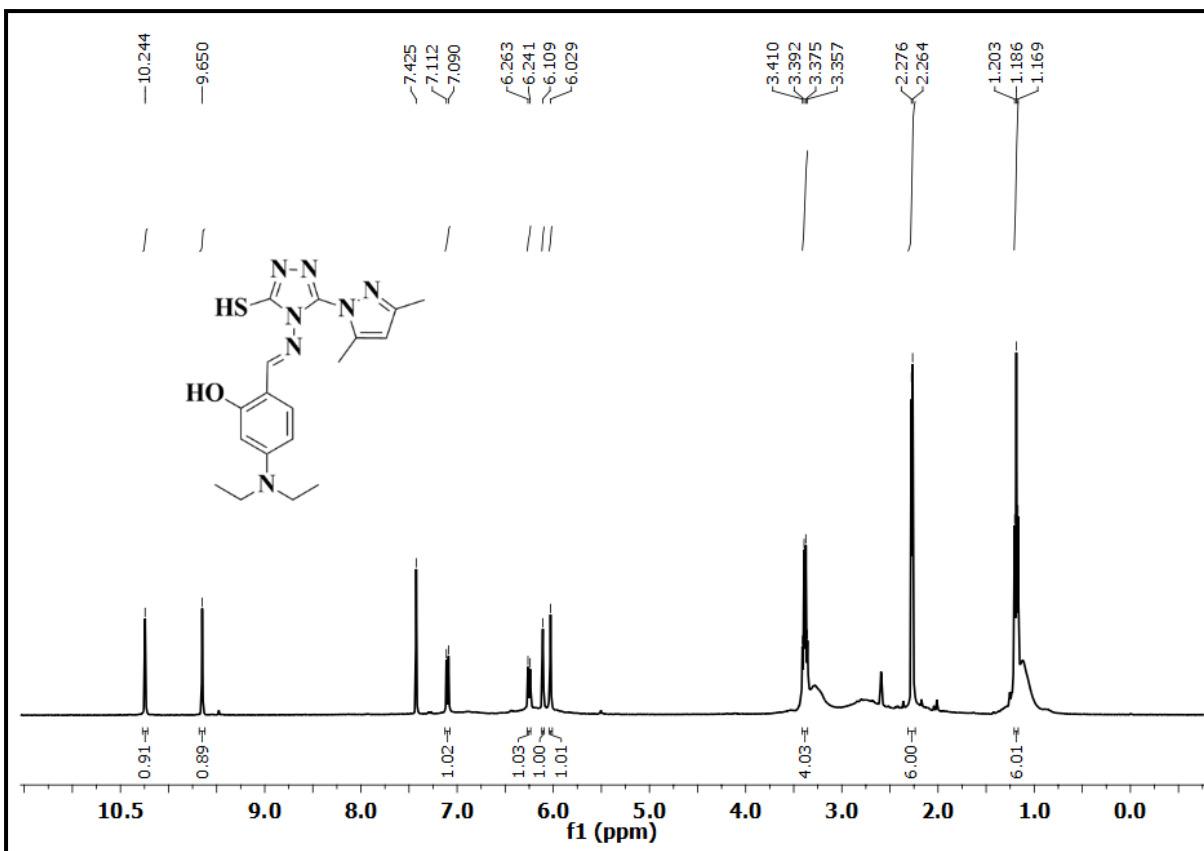


Fig: ¹H-NMR Spectrum of Compound 53f (400 MHz, CDCl₃+DMSO-d₆)

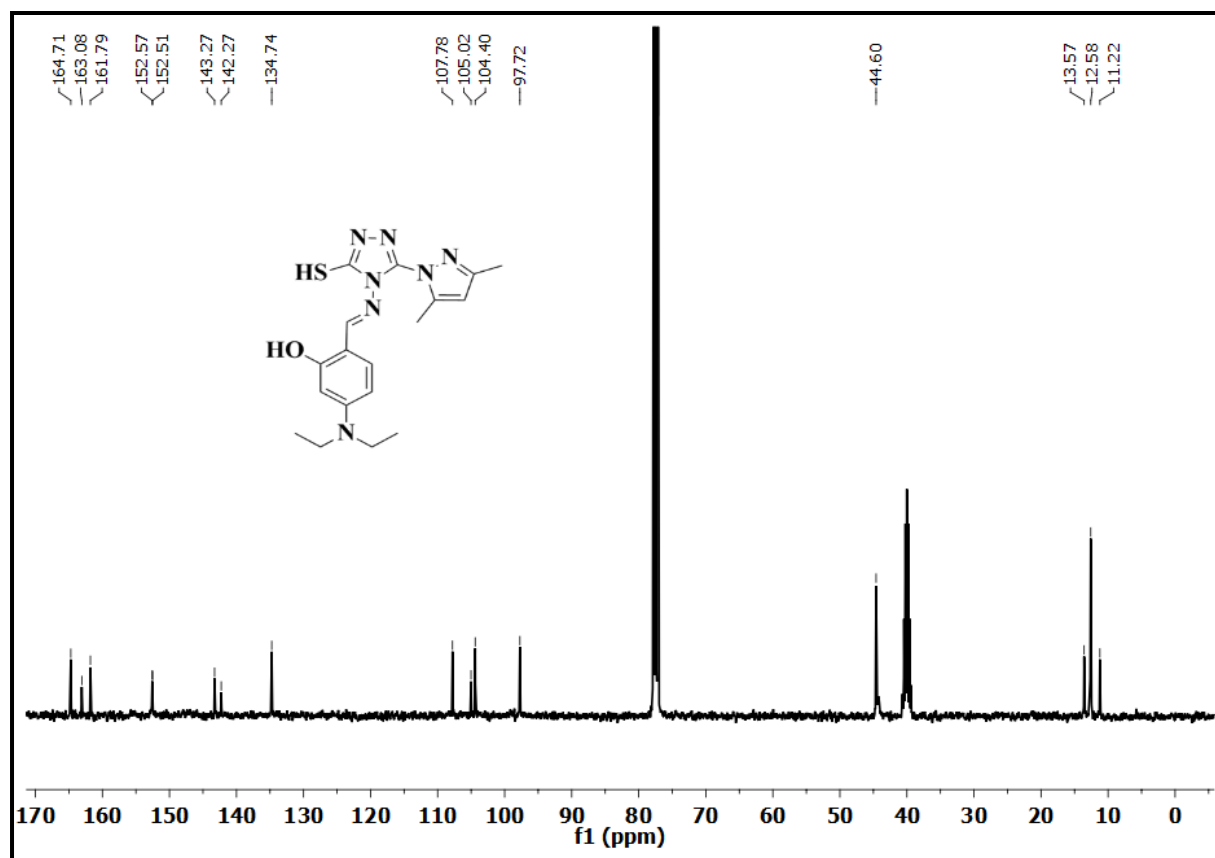


Fig: ¹³C-NMR Spectrum of Compound 53f (100 MHz, CDCl₃+DMSO-*d*₆)

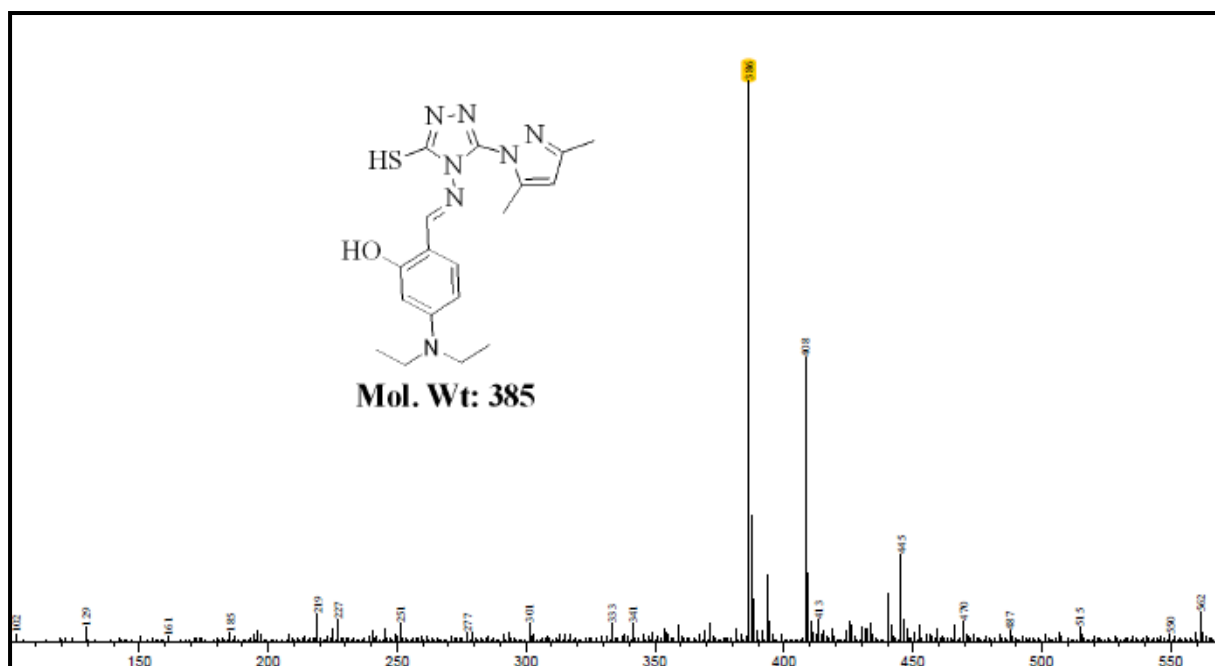


Fig: Mass Spectrum of Compound 53f

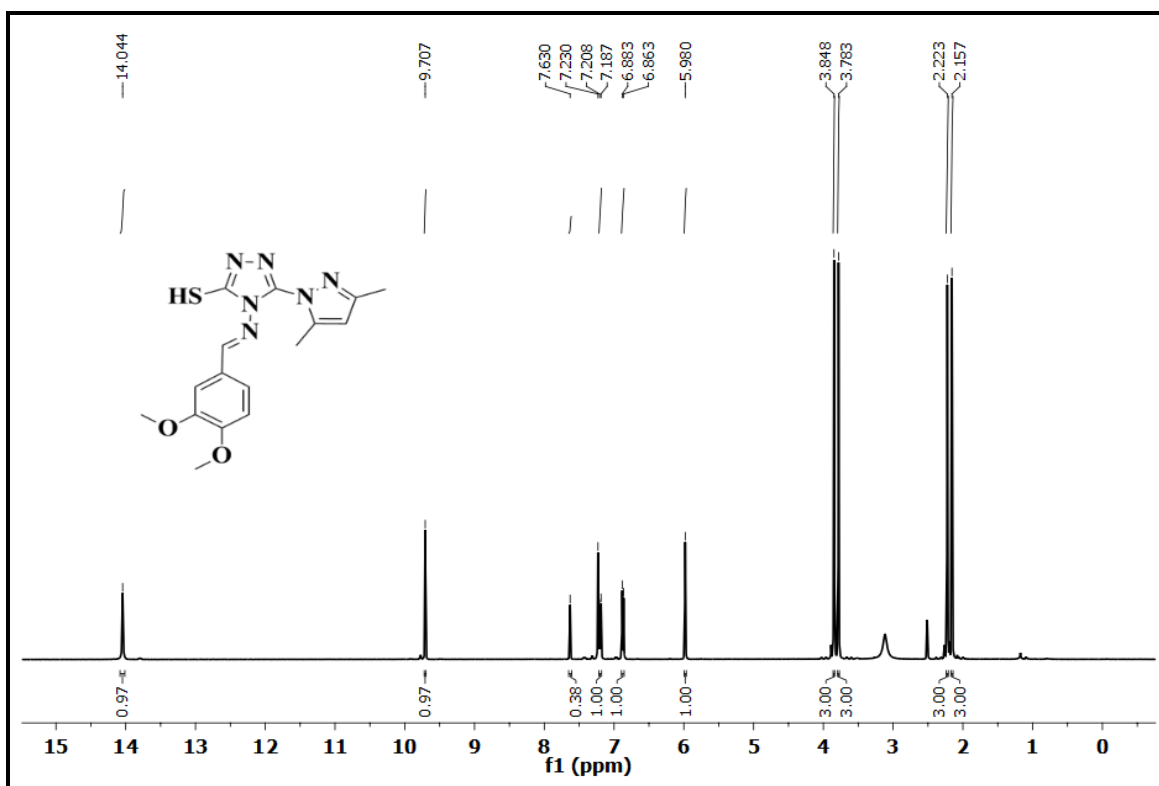


Fig: ¹H-NMR Spectrum of Compound 53g (400 MHz, CDCl₃+DMSO-*d*₆)

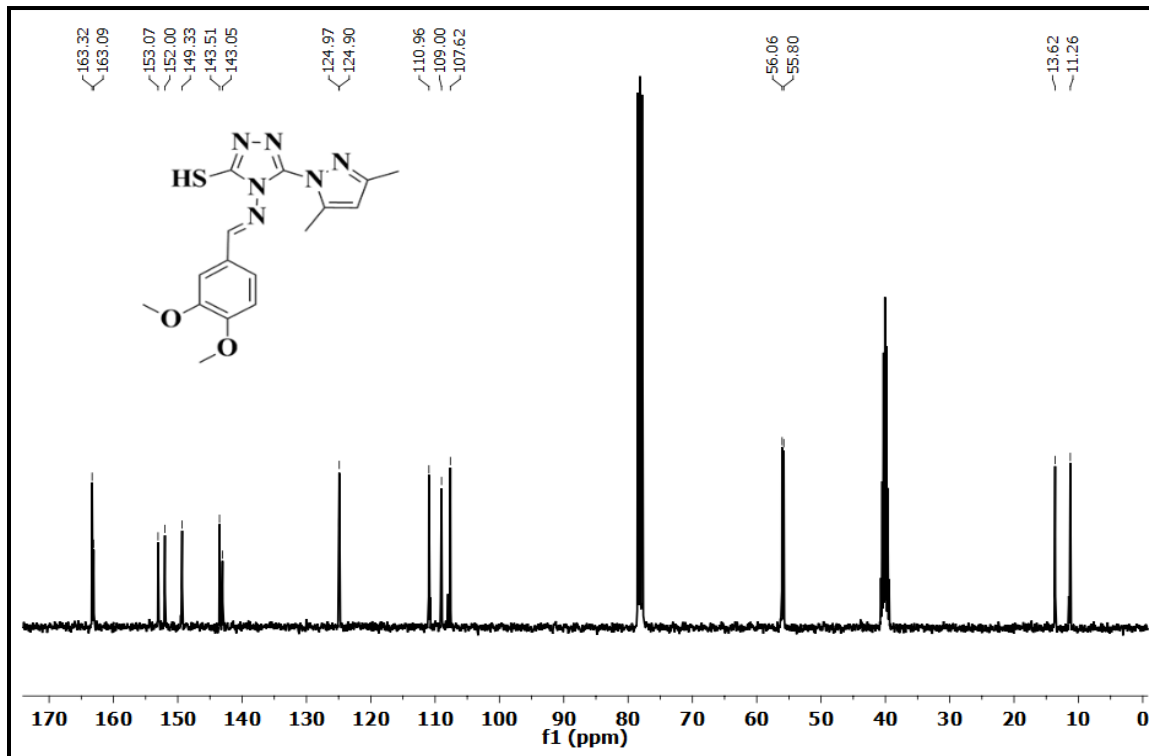


Fig: ¹³C-NMR Spectrum of Compound 53g (100 MHz, CDCl₃+DMSO-*d*₆)

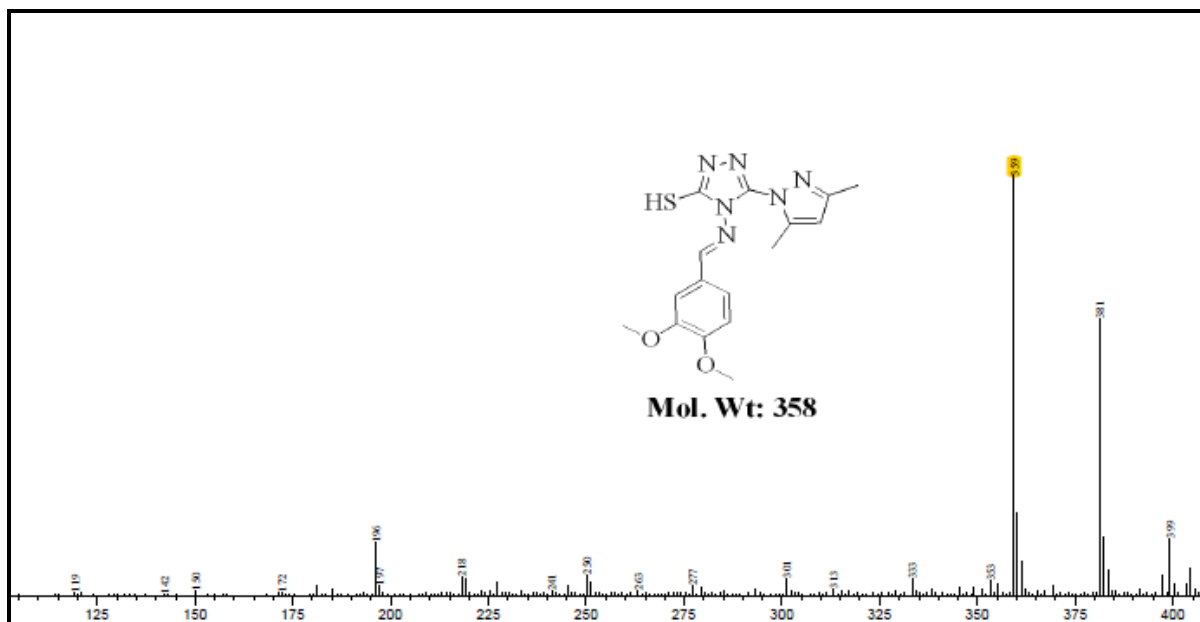


Fig: Mass Spectrum of Compound 53g

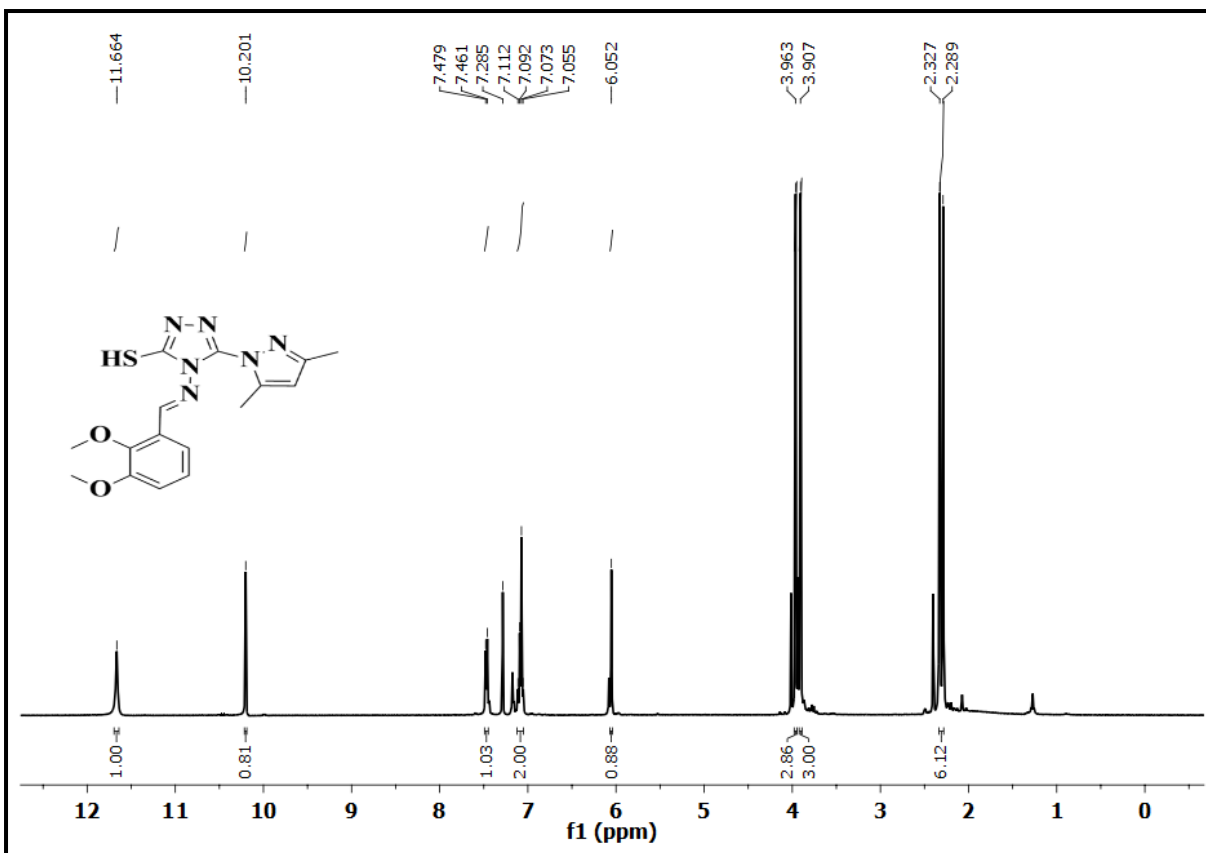


Fig: ¹H-NMR Spectrum of Compound 53h (400 MHz, CDCl₃)

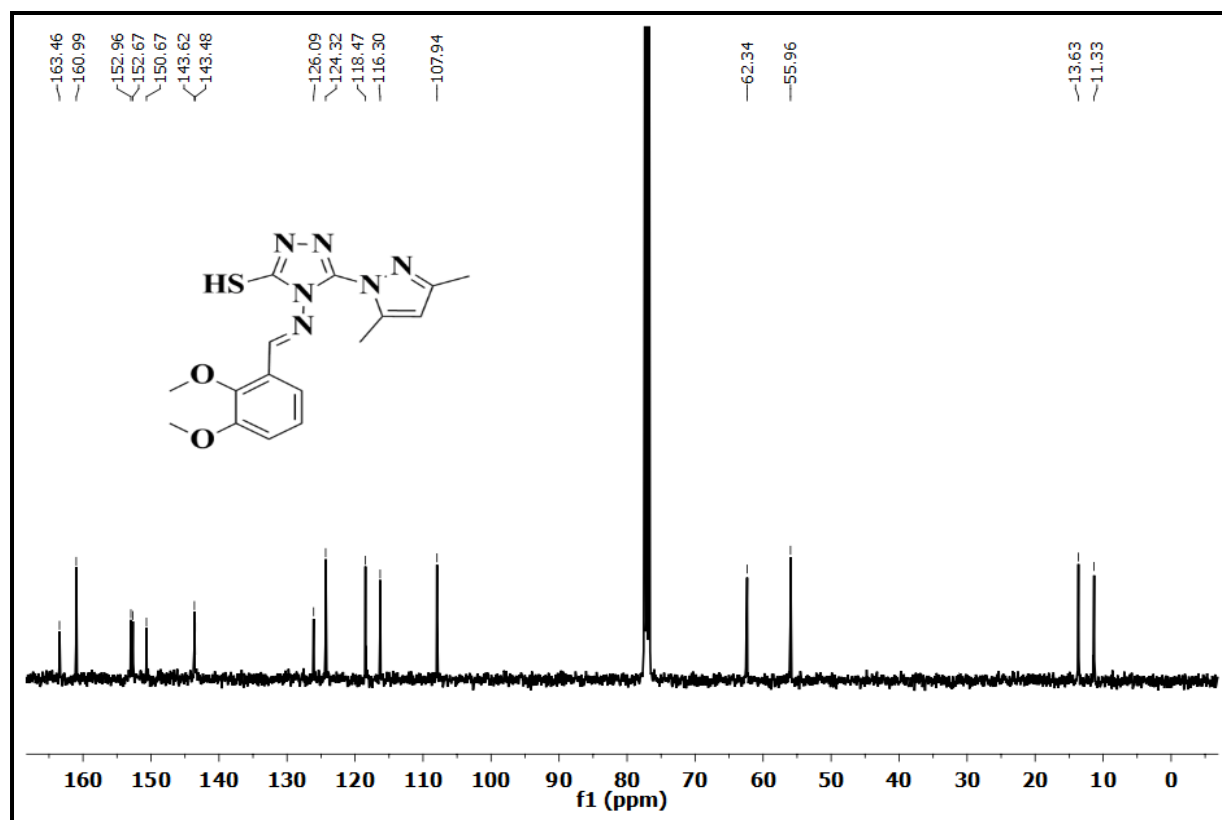


Fig: ¹³C-NMR Spectrum of Compound **53h** (100 MHz, CDCl₃)

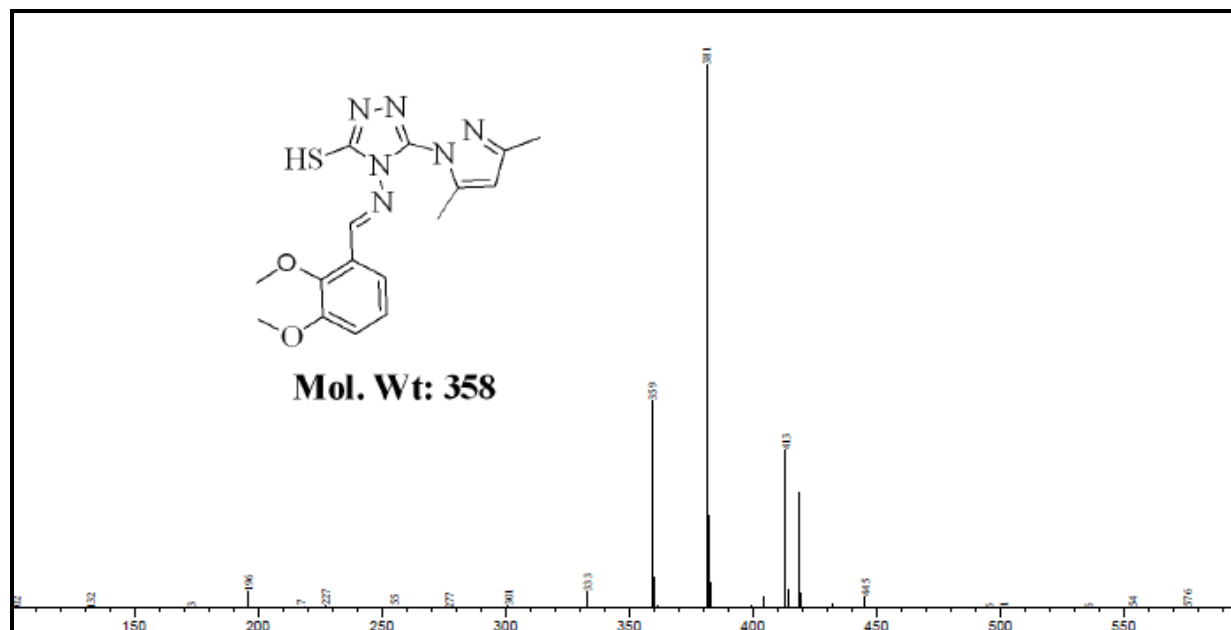


Fig: Mass Spectrum of Compound **53h**

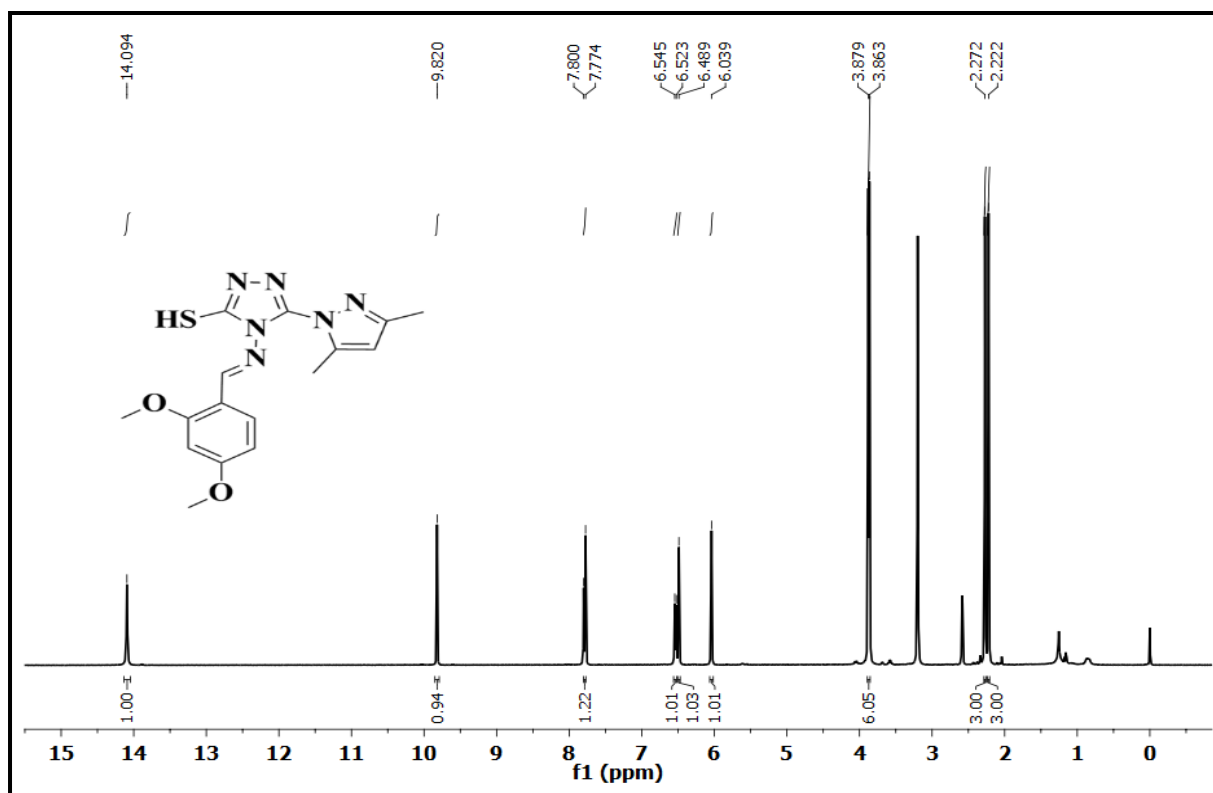


Fig: ¹H-NMR Spectrum of Compound 53i (400 MHz, DMSO-*d*₆)

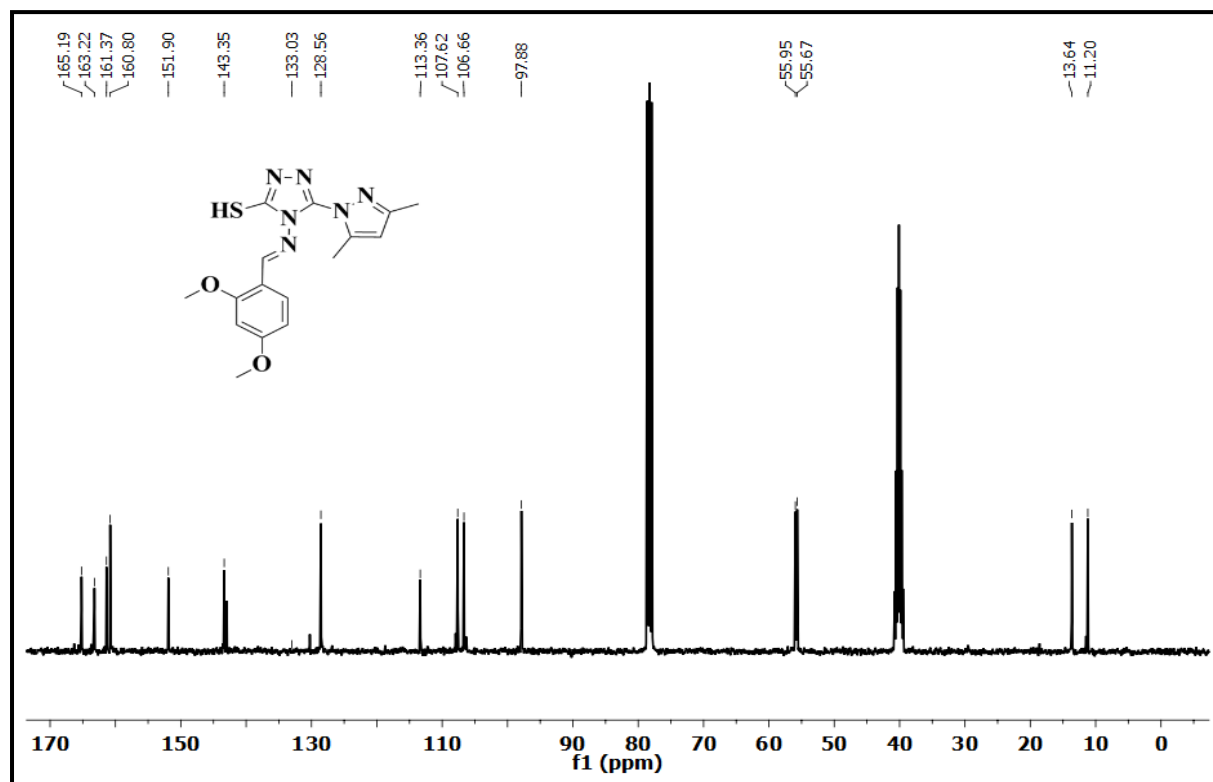


Fig: ¹³C-NMR Spectrum of Compound 53i (100 MHz, CDCl₃+DMSO-*d*₆)

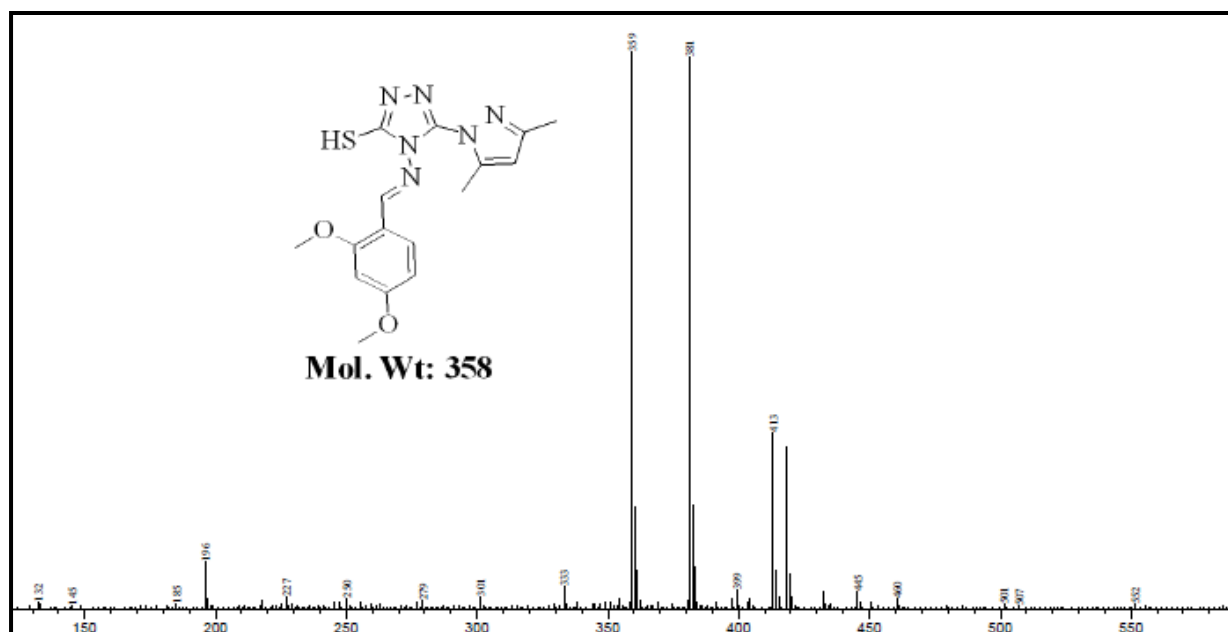


Fig: Mass Spectrum of Compound 53j

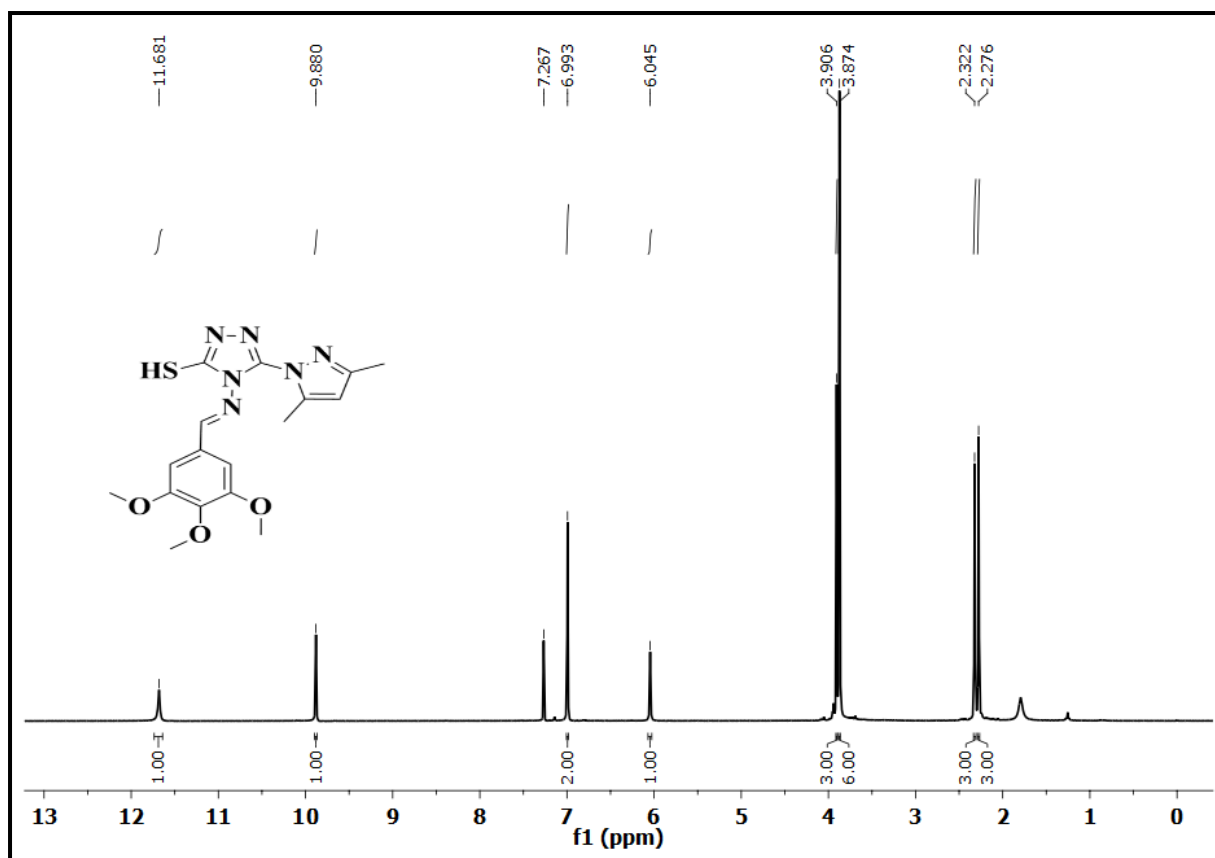


Fig: ¹H-NMR Spectrum of Compound 53j (400 MHz, CDCl₃)

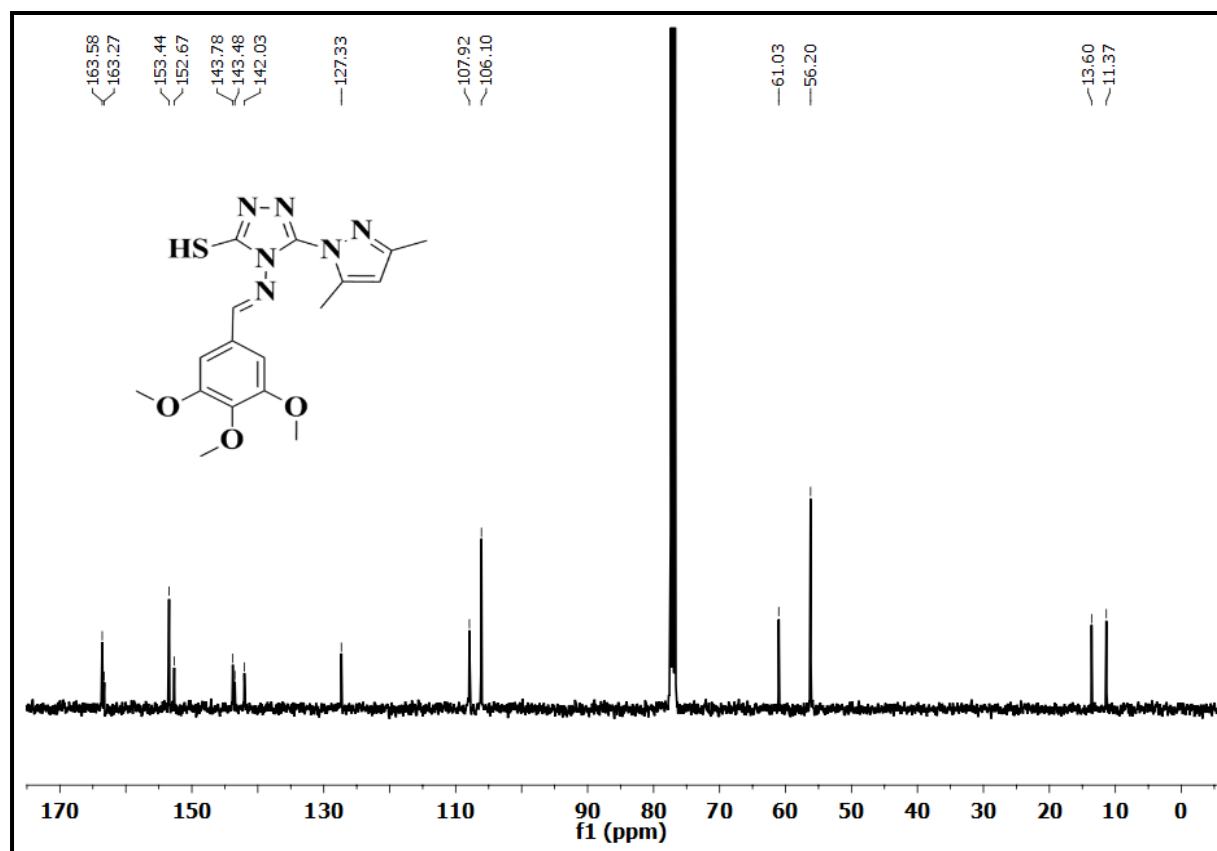


Fig: ¹³C-NMR Spectrum of Compound **53j** (400 MHz, CDCl₃)

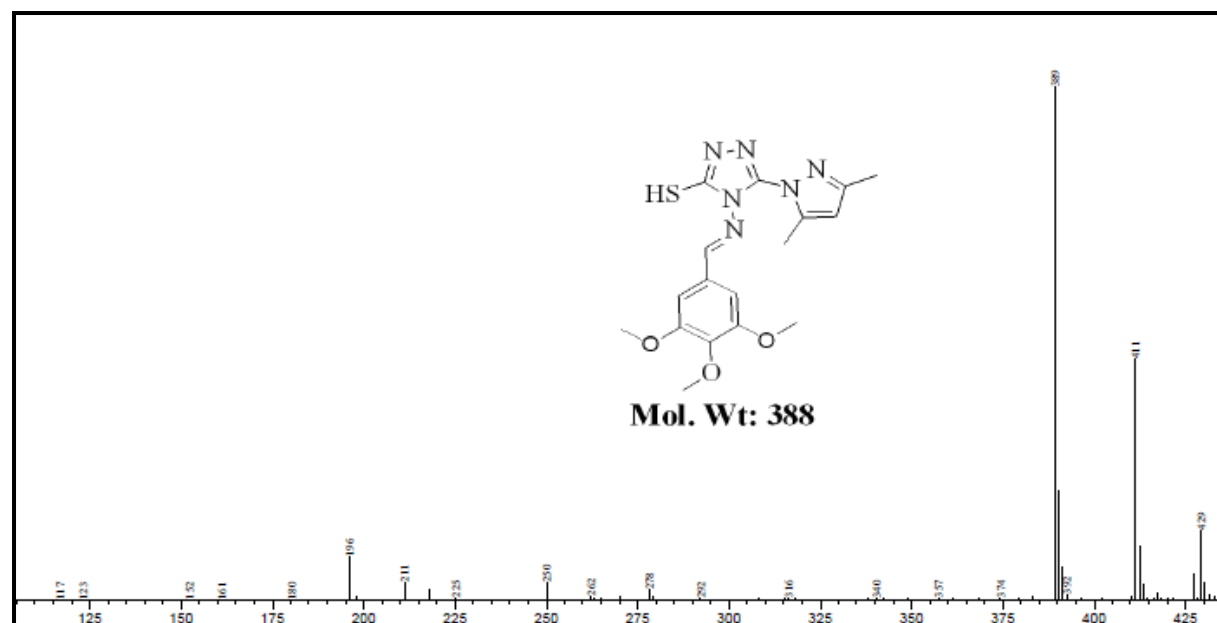


Fig: Mass Spectrum of Compound **53j**

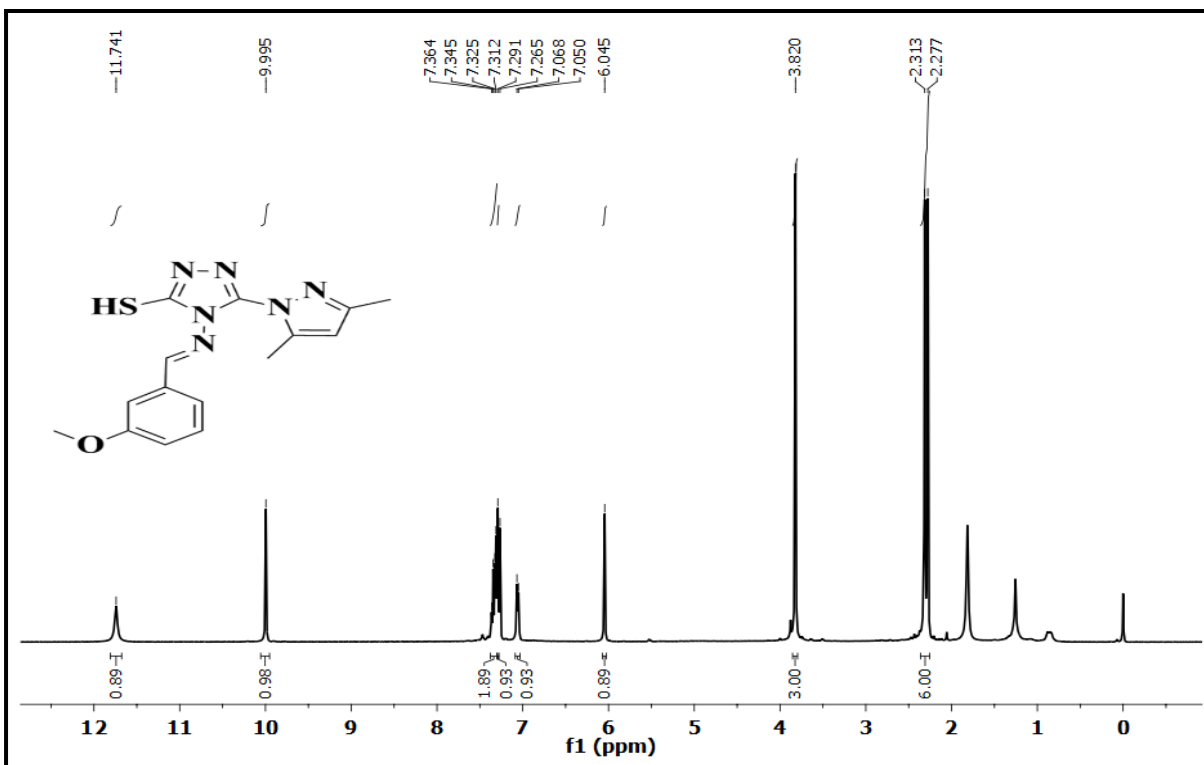


Fig: ¹H-NMR Spectrum of Compound **53k** (400 MHz, CDCl₃)

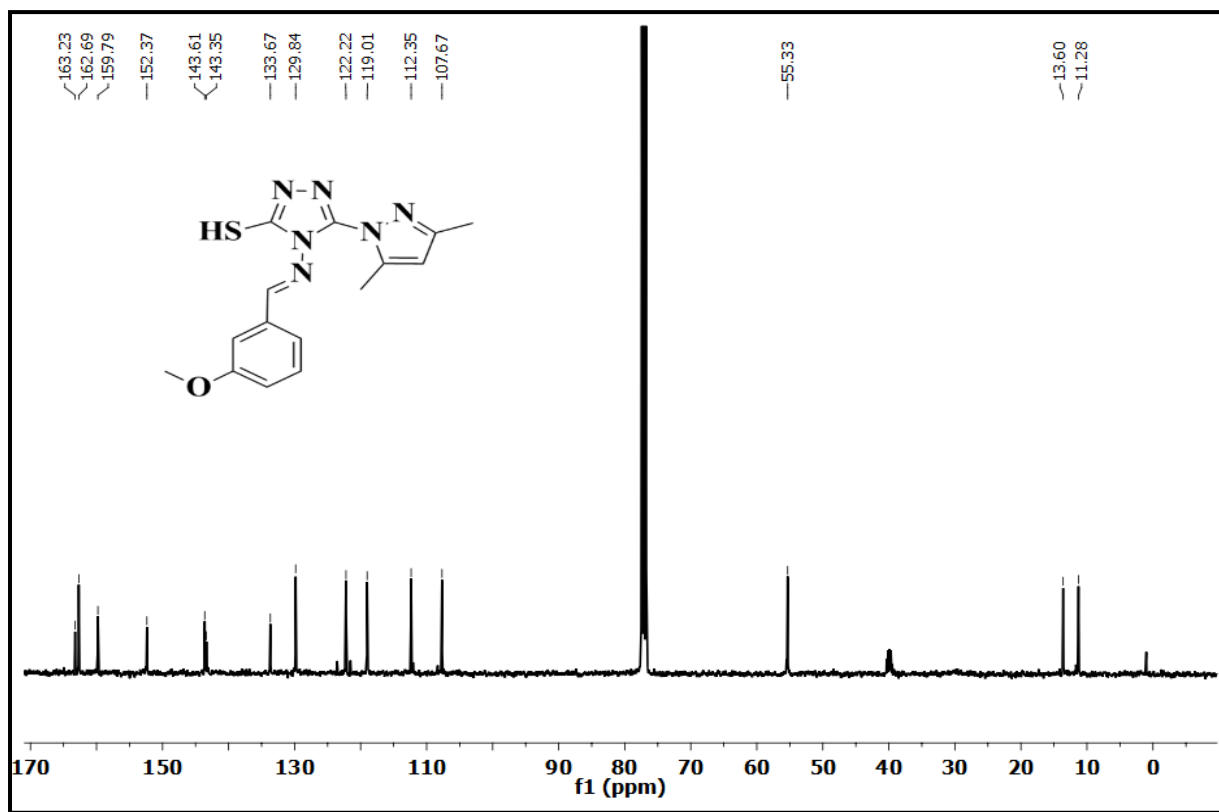


Fig: ¹³C-NMR Spectrum of Compound **53k** (100 MHz, CDCl₃+DMSO-*d*₆)

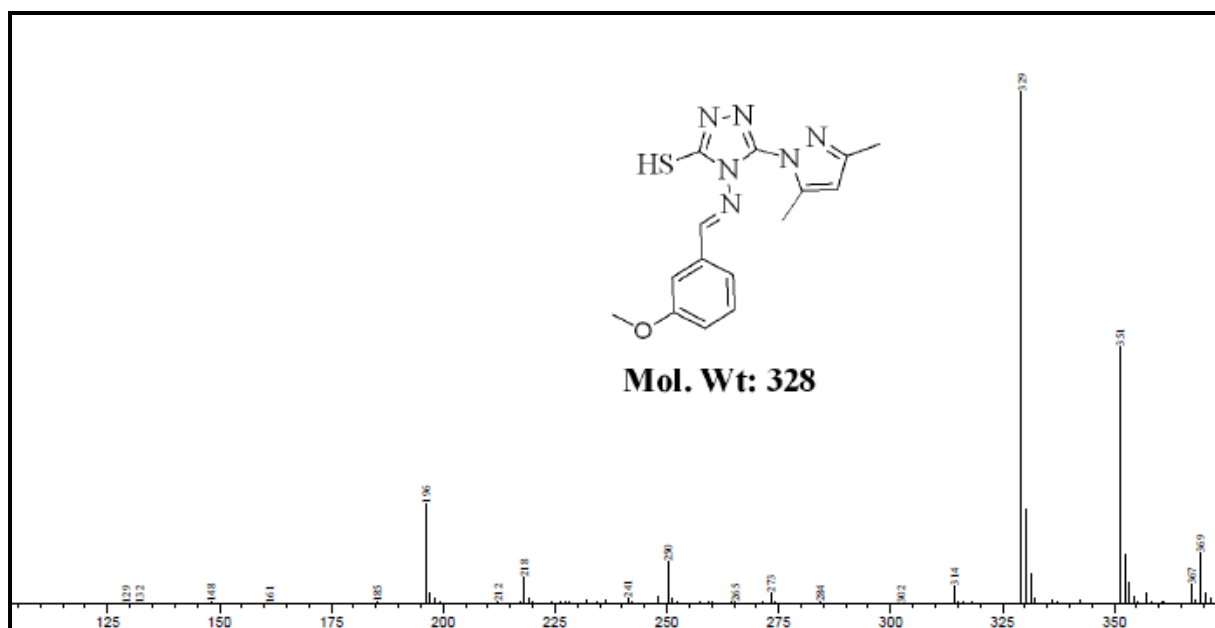


Fig: Mass Spectrum of Compound 53k

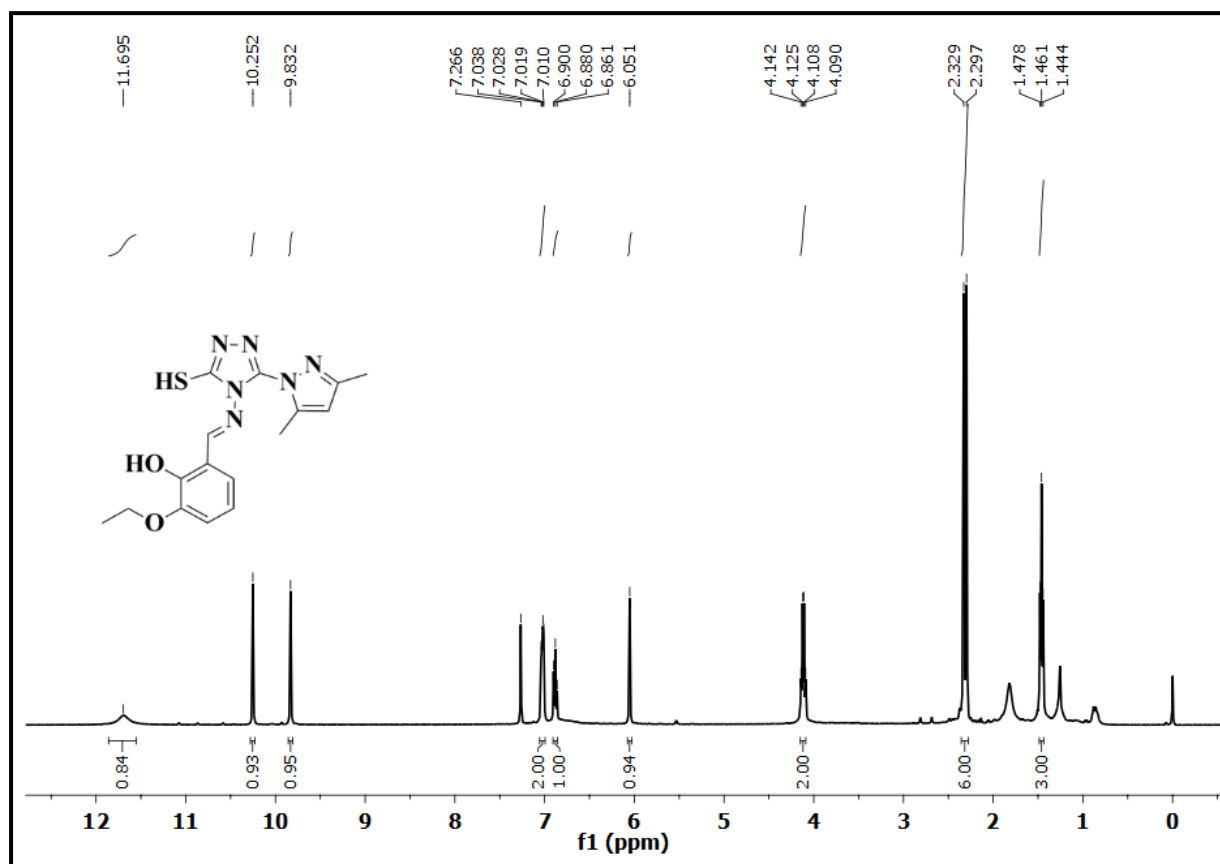


Fig: ¹H-NMR Spectrum of Compound 53l (400 MHz, CDCl₃)

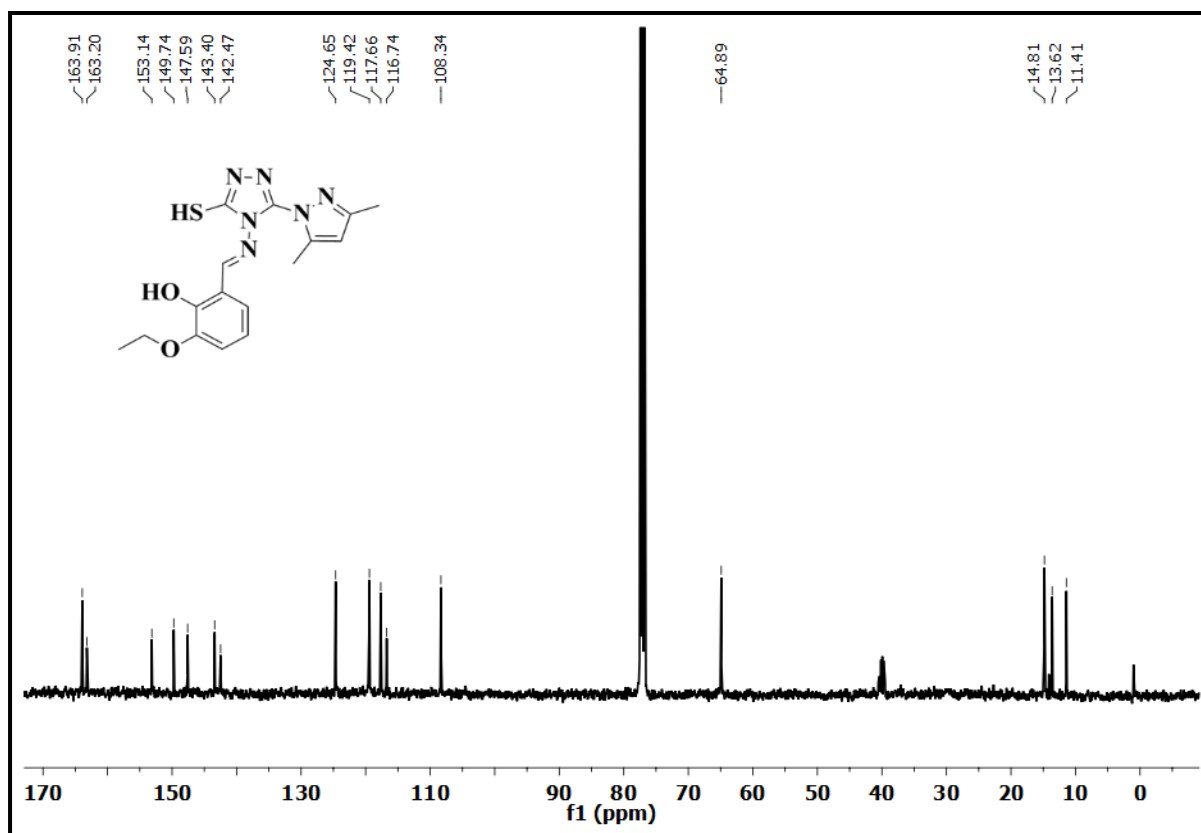


Fig: ¹³C-NMR Spectrum of Compound 53I (100 MHz, CDCl₃+DMSO-*d*₆)

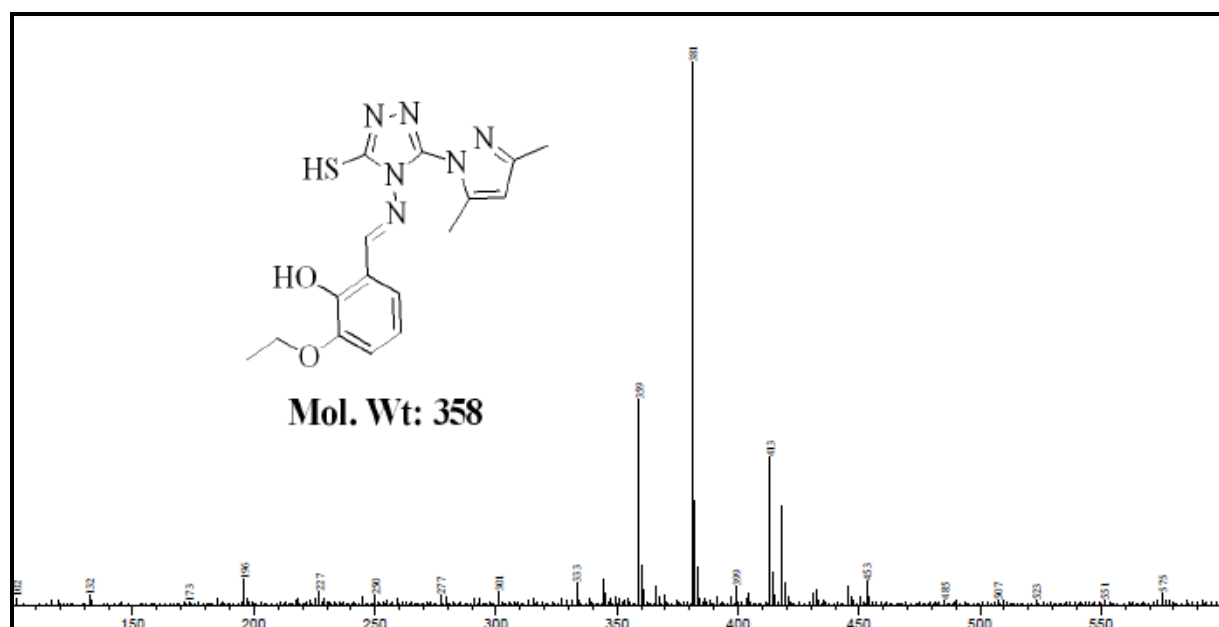


Fig: Mass Spectrum of Compound 53I

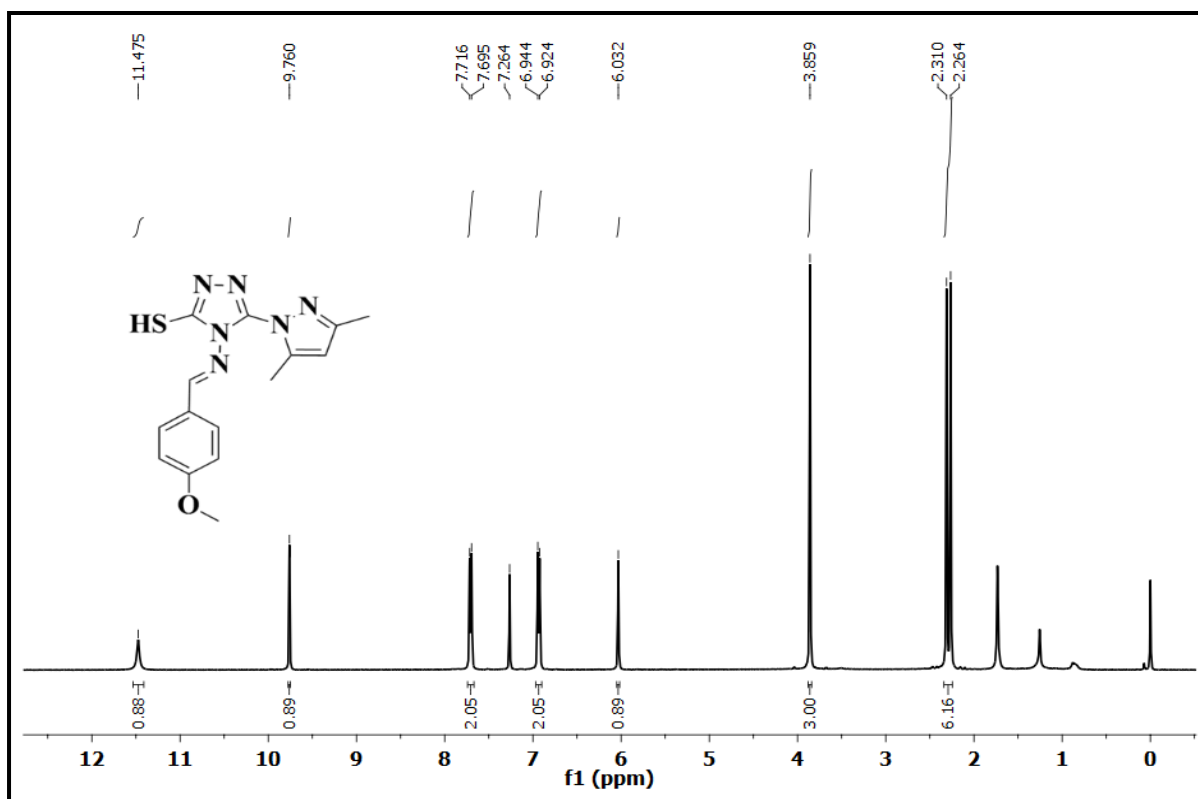


Fig: ¹H-NMR Spectrum of Compound **53m** (400 MHz, CDCl₃)

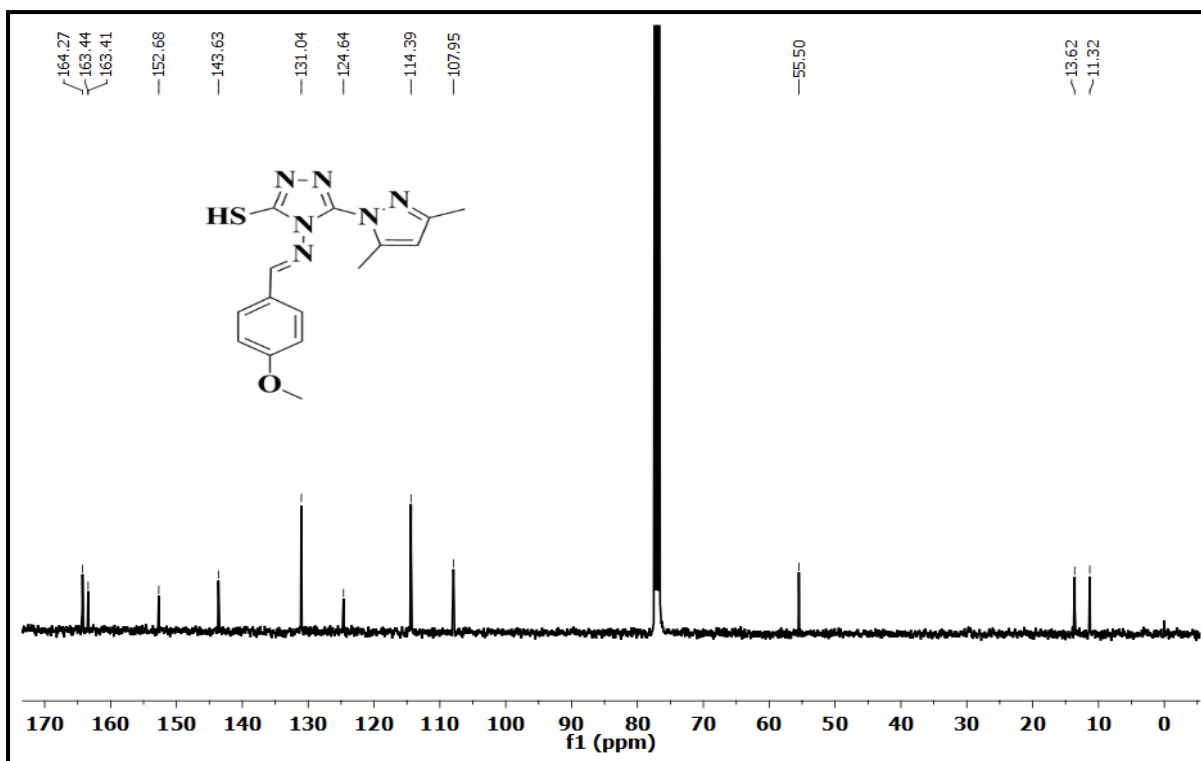


Fig: ¹³C-NMR Spectrum of Compound **53m** (100 MHz, CDCl₃)

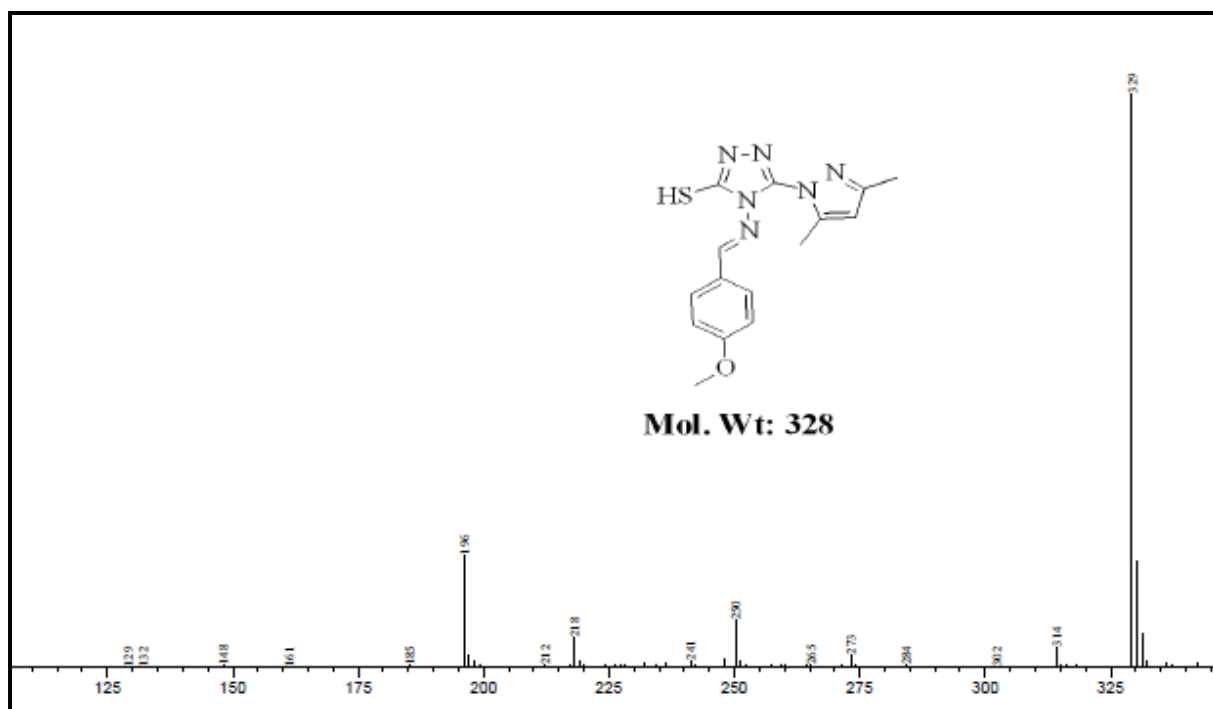


Fig: Mass Spectrum of Compound 53m

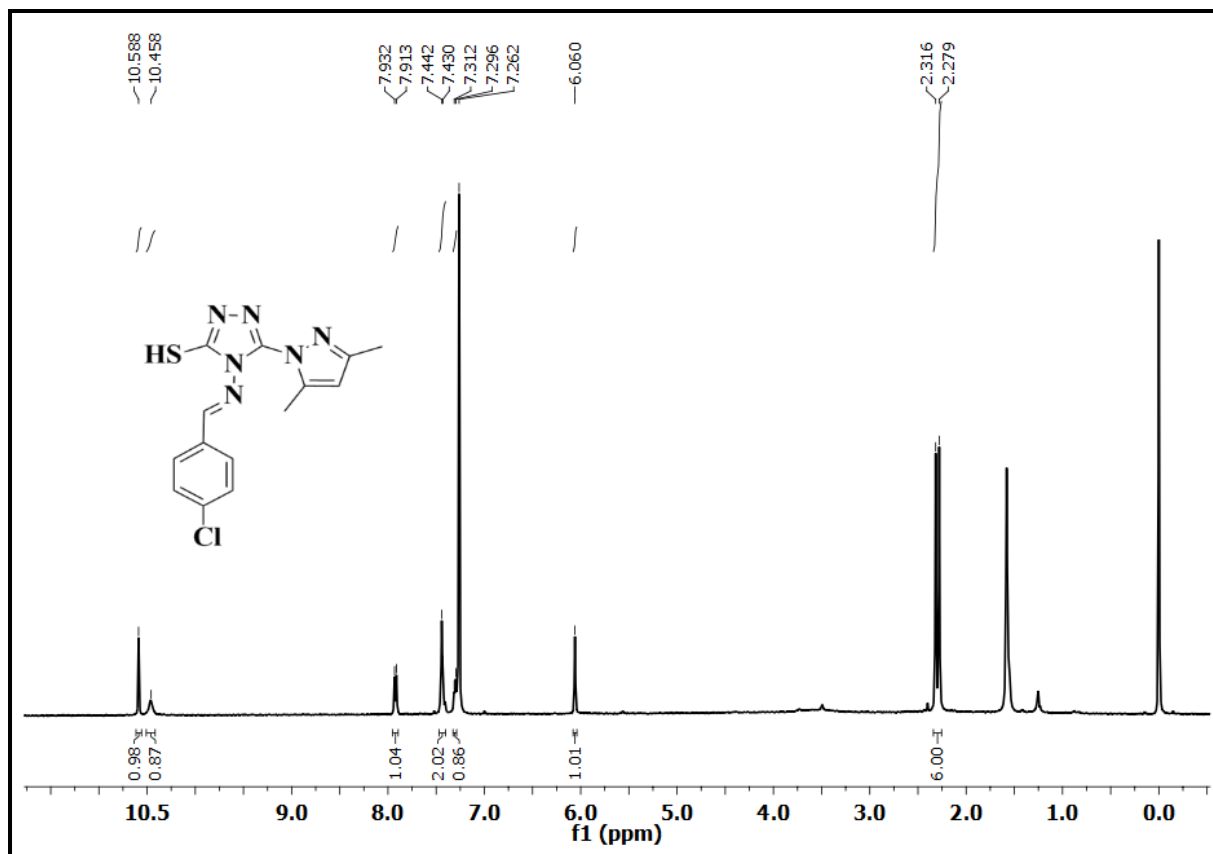


Fig: ¹H-NMR Spectrum of Compound 53n (400 MHz, CDCl₃)

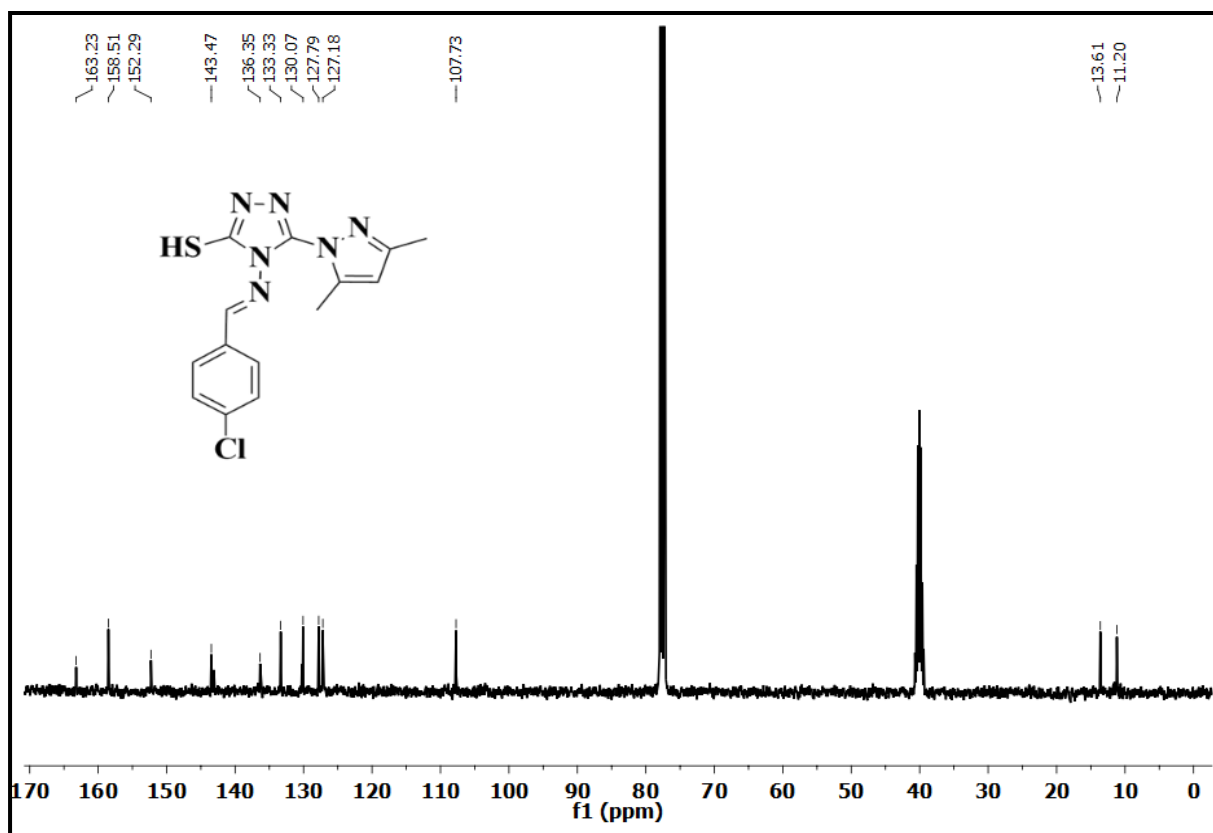


Fig: ¹³C-NMR Spectrum of Compound **53n** (100 MHz, CDCl₃+DMSO-*d*₆)

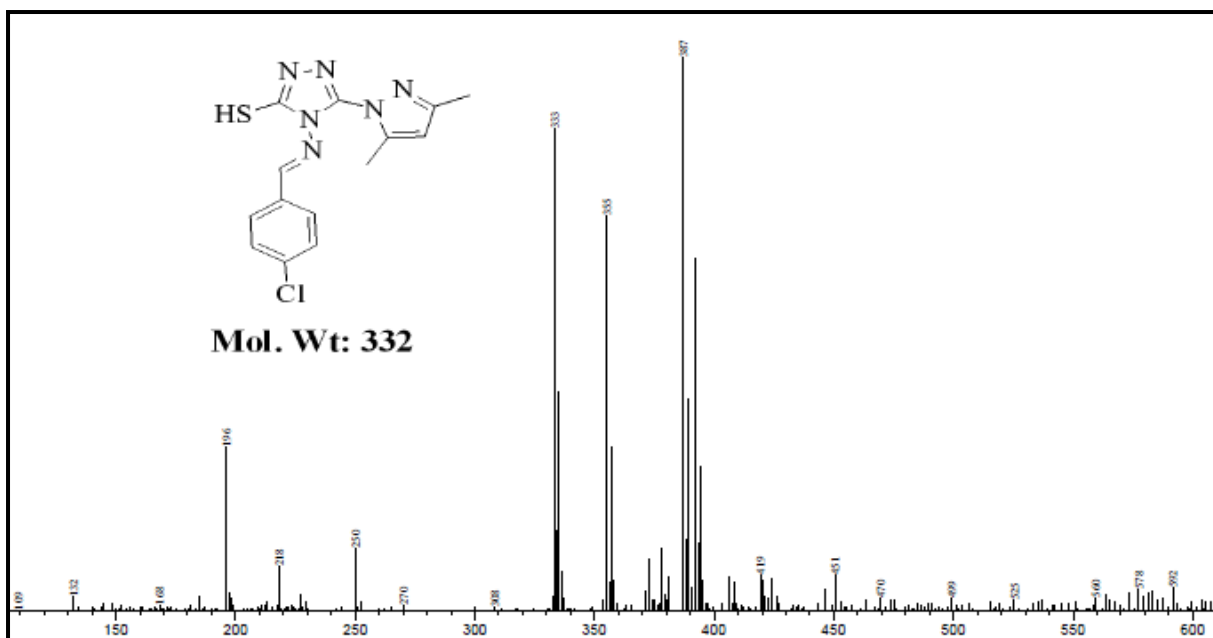


Fig: Mass Spectrum of Compound **53n**

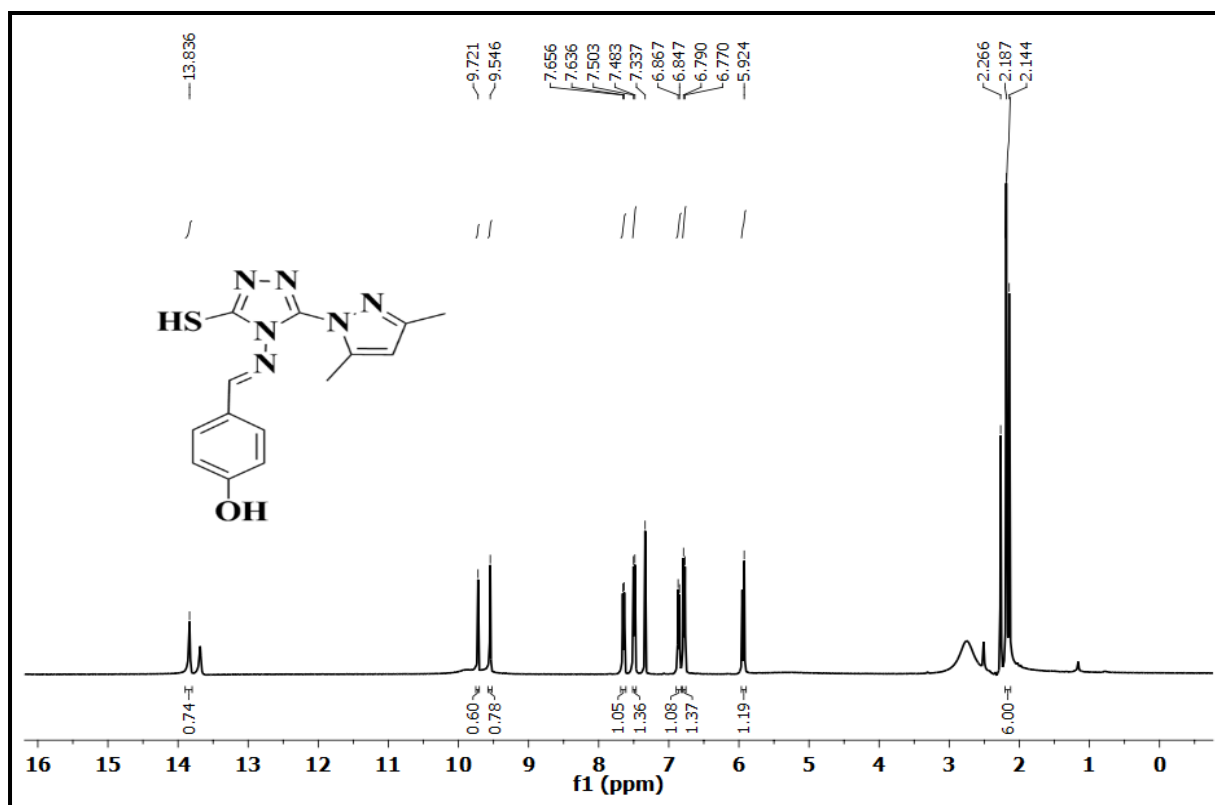


Fig: ¹H-NMR Spectrum of Compound **53o** (400 MHz, CDCl₃+DMSO-*d*₆)

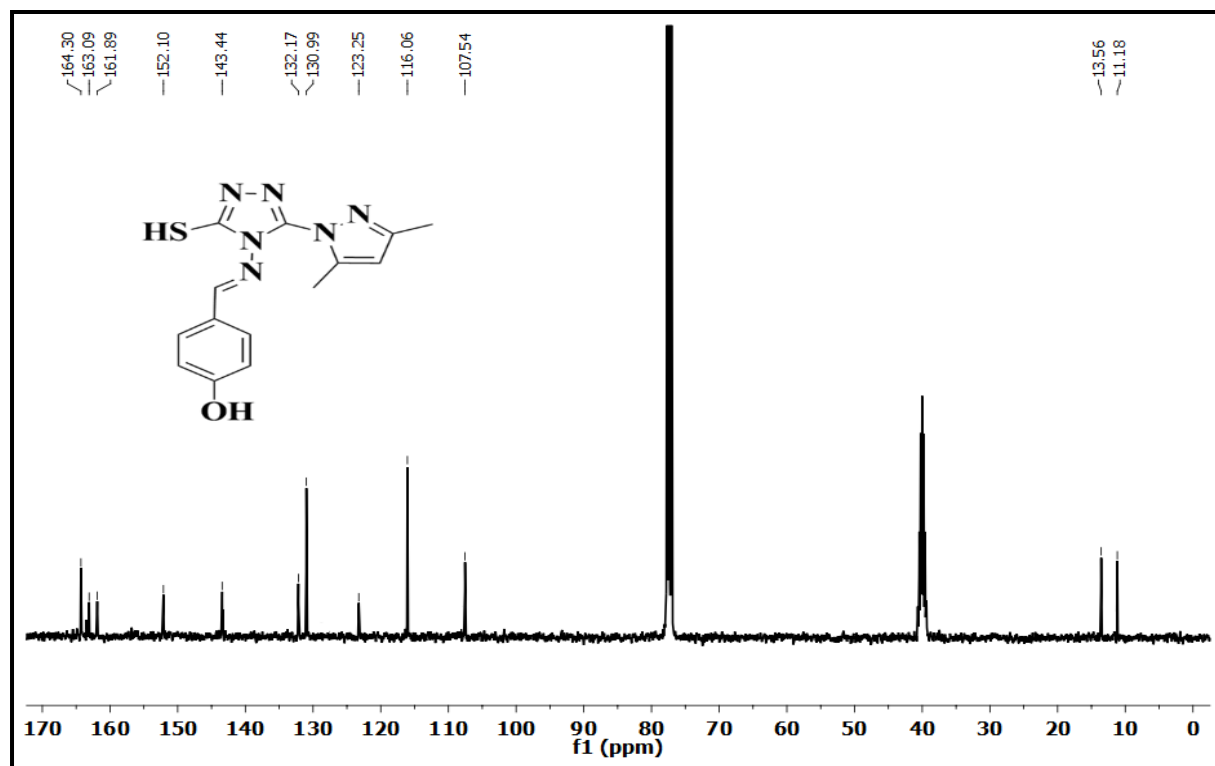


Fig: ¹³C-NMR Spectrum of Compound **53o** (100 MHz, CDCl₃+DMSO-*d*₆)

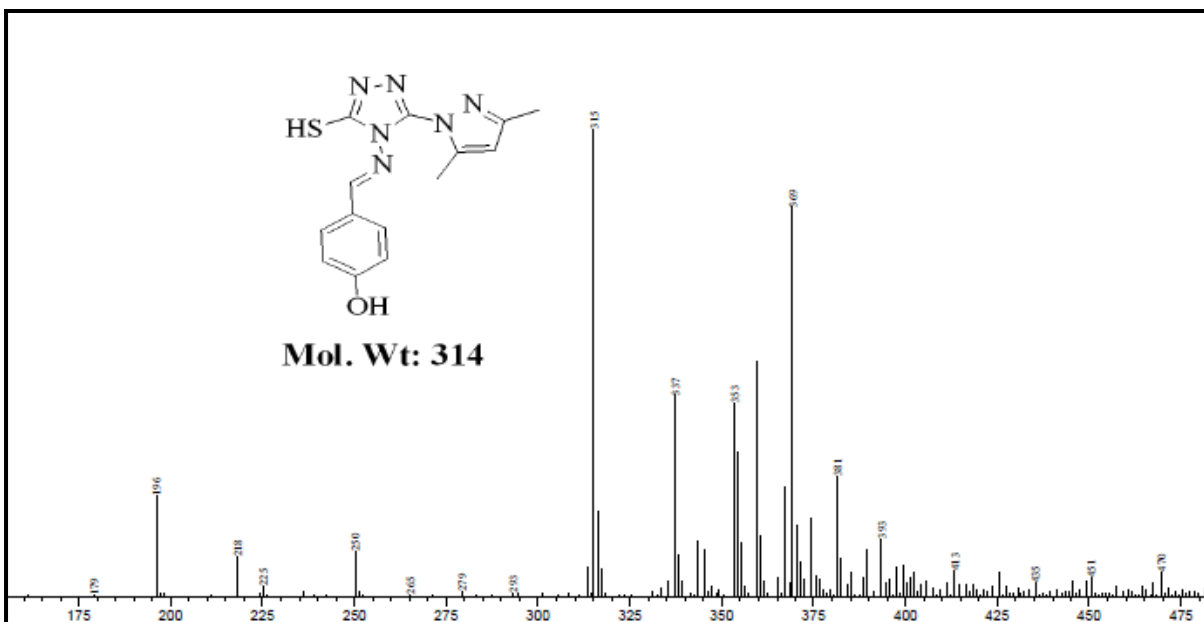


Fig: Mass Spectrum of Compound 53o

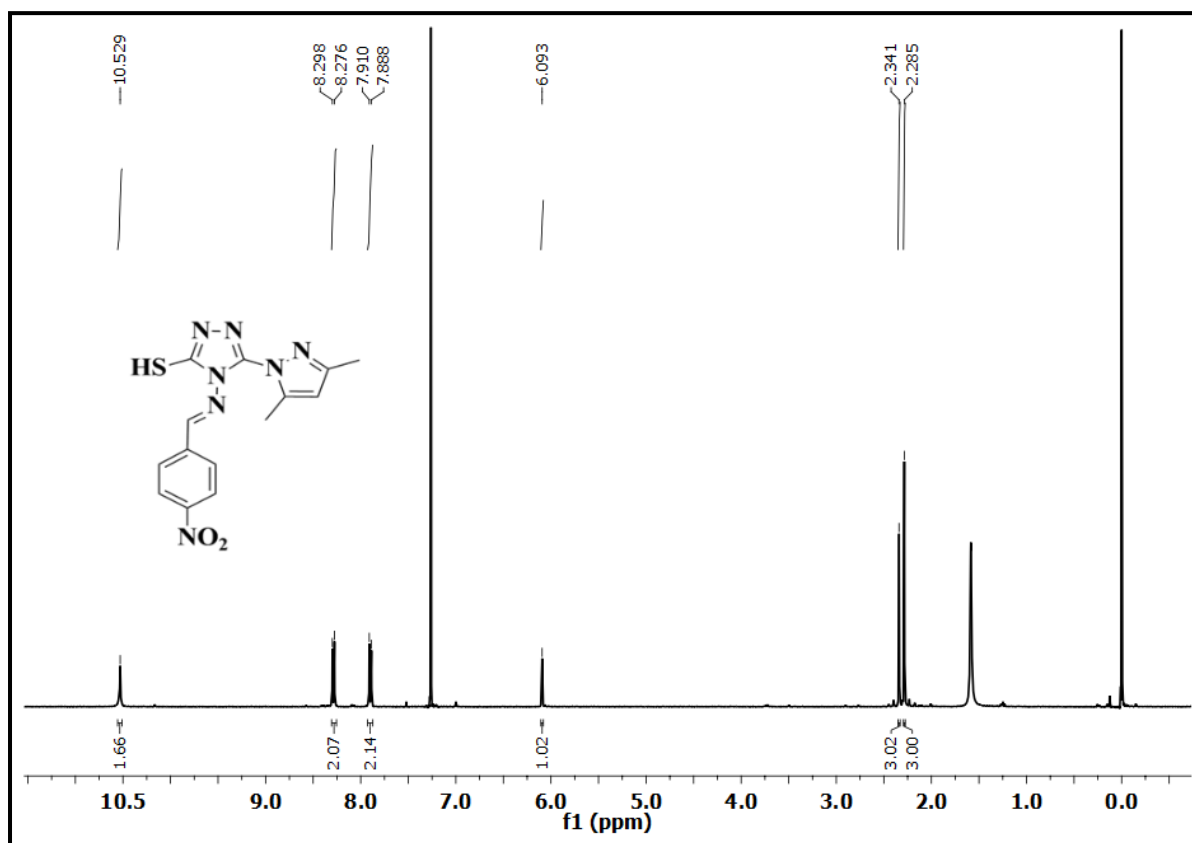


Fig: ¹H-NMR Spectrum of Compound 53p (400 MHz, CDCl₃)

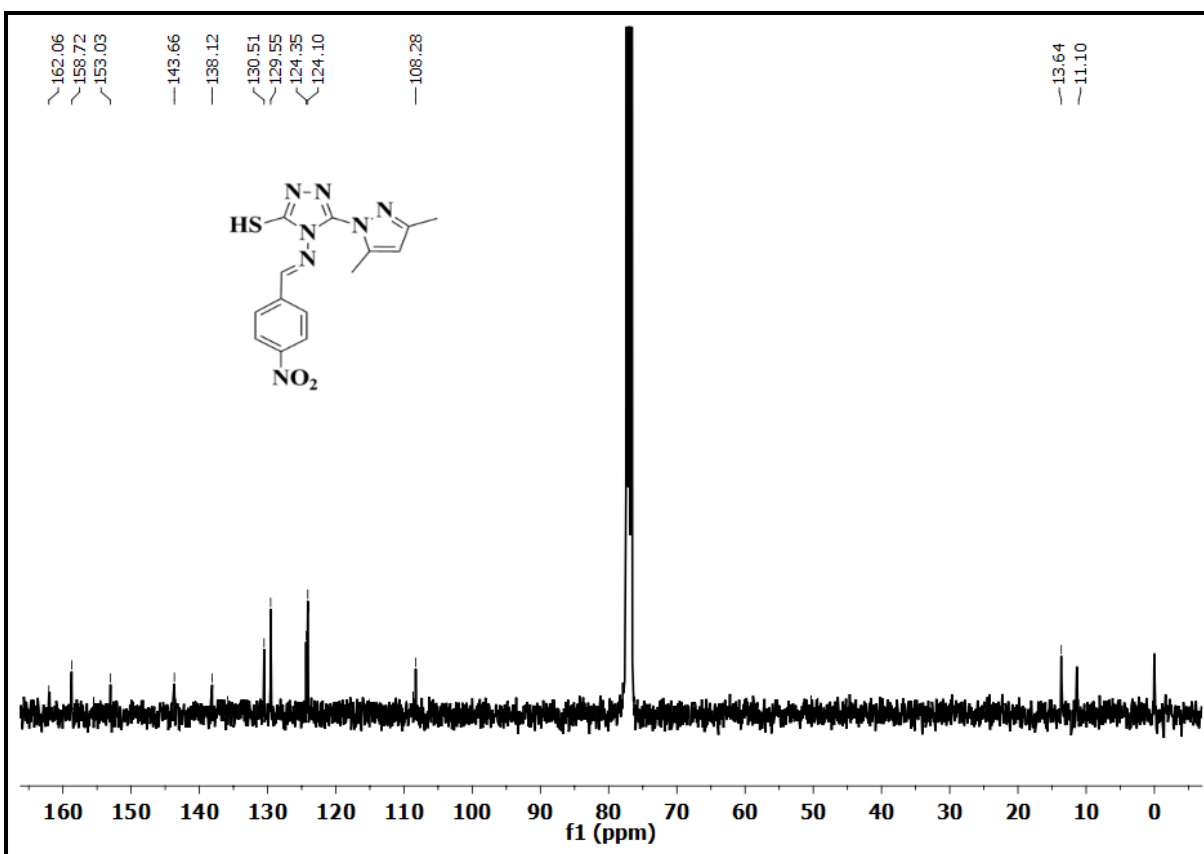


Fig: ¹³C-NMR Spectrum of Compound **53p** (400 MHz, CDCl₃)

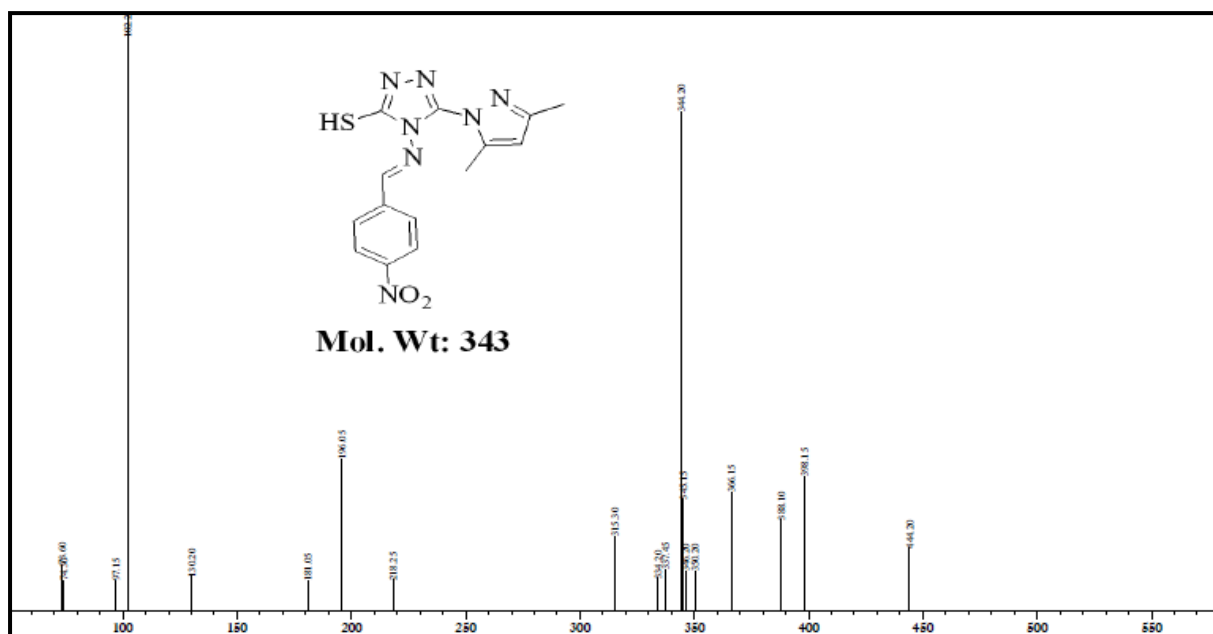
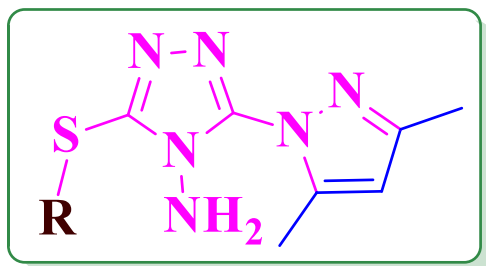


Fig: Mass Spectrum of Compound **53p**

CHAPTER-II

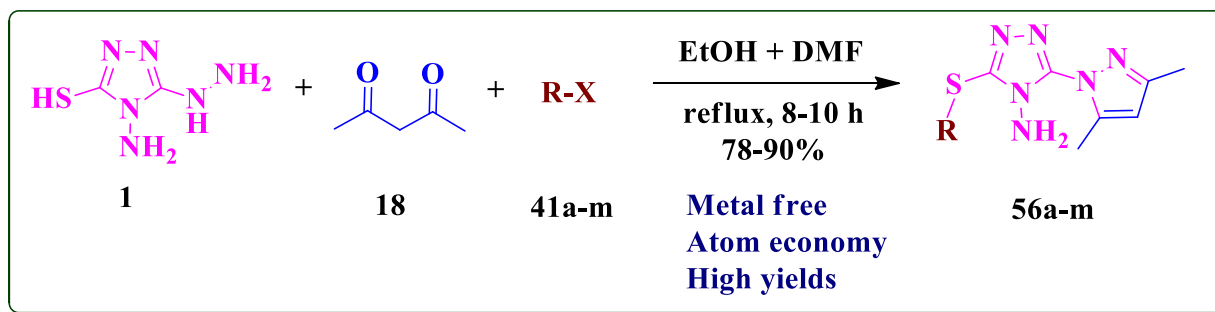
PART-B

ONE-POT, MULTI-COMPONENT CASCADE REACTION FOR THE
SYNTHESIS OF VARIOUS ARALKYL/ALKYL THIO-3,5-DIMETHYL-
1*H*-PYRAZOLYL-4*H*-1,2,4-TRIAZOL-4-AMINES AND THEIR DOCKING
STUDIES



PRESENT WORK

In view of the importance of triazoles, pyrazoles and thioethers we would like to incorporate all these units in a single heterocyclic system. So that the resulting molecule may exhibit good biological activity. In continuation of earlier work on multi-component reactions^{85,86} in the present work we are reporting the one-pot, multi-component cascade reaction for the synthesis of various aralkyl/alkyl thio-3,5-dimethyl-1*H*-pyrazolyl-4*H*-1,2,4-triazol-4-amines (**Scheme-3**) and their docking studies.



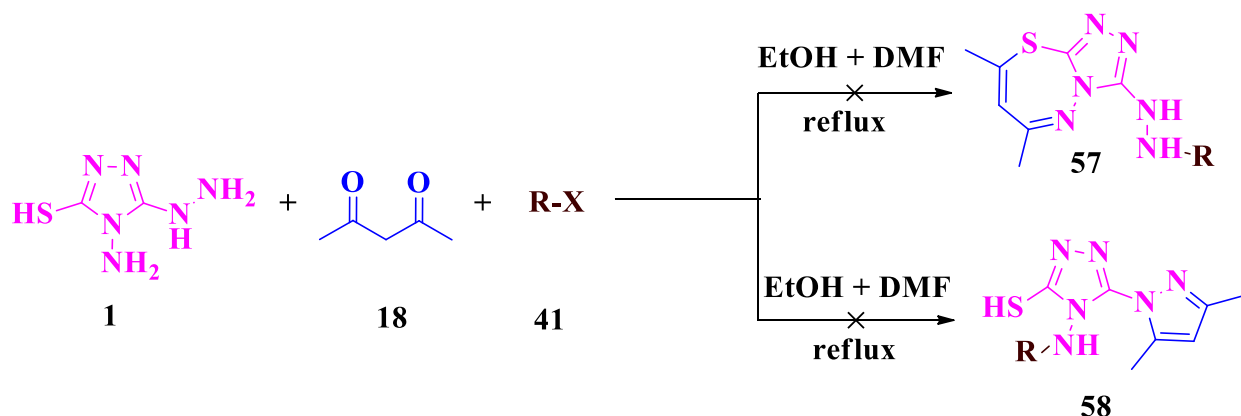
For the optimization of reaction conditions, the reaction was performed with 4-amino-5-hydrazino-4*H*-[1,2,4]triazole-3-thiol (**1**), acetylacetone (**18**), and aralkyl/alkyl halides (**41**) in the water under reflux condition, and no product was formed. On the other hand, when the same reaction was carried out in a mixture of an equal amount of dry ethanol and DMF (1:1) under reflux produced the final compound in 90% yield. Additionally, when the same reaction was examined by using EtOH/Et₃N or MeOH/Et₃N produced lower yields of the product when compared to a mixture of dry ethanol and DMF. The optimized conditions for the hetero cyclization and alkylation are a mixture of dry ethanol and DMF under reflux conditions (**Table-3**).

Table-3: Optimization conditions for the reaction **56a**.

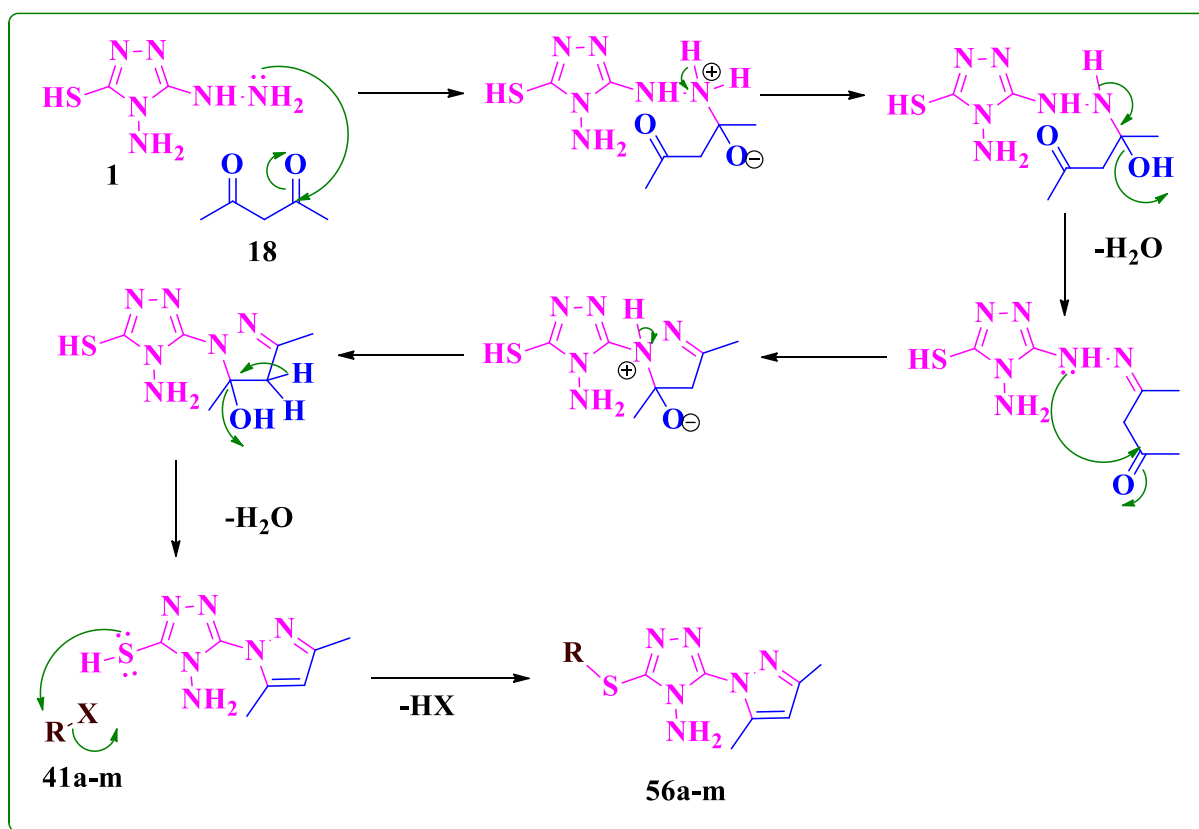
Entry	Solvent	Base	Yield (%) of 56a
1	H ₂ O	—	0
2	EtOH	DMF	90
3	EtOH	Et ₃ N	40
4	MeOH	Et ₃ N	25

***Reaction conditions:** **1** (1 mmol), **18** (1 mmol), **44** (1 mmol) and base in the solvents.

When the reaction is carried out between **1**, **18**, and **41** there is a probability of formation of a mixture of products like **56**, **57**, or **58** or all of them. But in our investigation, only one product (via TLC) formation is observed i.e. **56** only. The alternative products such as **57** and **58** from the reaction can be rejected on the basis of spectral studies (**Scheme-4**).

**Scheme-4:** Alternative possible products of the reaction of **18**, **41** with **1**.

In the present process the reaction proceeds in such a way that only the hydrazino part of compound **1** undergoes condensation with **18** during heterocyclization followed by aralkylation/alkylation of the -SH group of compound **1** that lead to the formation of target title compounds. The formation of S-aralkylated/S-alkylated products can be explained due to the high nucleophilicity of the thiol group compared to the amino group (**Scheme-5**). The yields of products obtained were 78-90%.



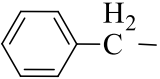
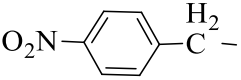
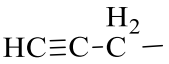
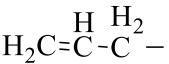
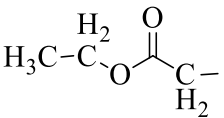
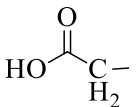
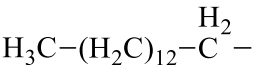
Scheme-5: Plausible mechanism for the synthesis of various aralkyl/alkyl thio-3,5-dimethyl-1H-pyrazolyl-4H-1,2,4-triazol-4-amine (**56a-m**).

The structures of the products were confirmed by their analytical and spectral studies. For example in the FT-IR spectrum of compound **56a** displayed a peak at 3323 cm^{-1} ($-\text{NH}_2$) and 1584 cm^{-1} ($-\text{C}=\text{C}-$). In the $^1\text{H-NMR}$ (CDCl_3) spectrum of compound **56a** gave characteristic singlets for two $-\text{CH}_3$ groups of pyrazole at δ 2.26, 2.53, a singlet for $-\text{SCH}_2-$ at δ 4.46, $-\text{NH}_2$ two protons showed as a singlet at δ 5.22 and the pyrazole ring proton appeared as a singlet at δ 6.02. The $^{13}\text{C-NMR}$ spectrum of compound **56a** displayed the peaks at δ 12.3 and 13.6 for two methyl carbons of pyrazole. The benzylic ($-\text{S}-\text{CH}_2-$) carbon appeared at δ 35.9 and pyrazole carbon showed at δ 108.1. In the mass spectrum, the compound **56a** appeared at m/z 301 $[\text{M}+\text{H}]^+$.

In the present one-pot, multi-component cascade reaction conditions the relative reactivity of various aralkyl and alkyl halides were examined. When the benzyl bromide (**41a**) and 4-nitrobenzyl bromide (**41b**) were reacted with **1** and **18** they easily produced the corresponding thioether derivatives (**56a** and **56b**) in 90% and 88% yields, respectively (**Table-4**). Similarly, when the allyl halides such as Propargyl bromide (**41c**) and allyl bromide (**41d**)

were used the expected products (**56c** and **56d**) were formed in 86% and 82% yields, accordingly. When the reaction was carried out with bromoethyl acetate (**41e**) and chloroacetic acid (**41f**) the expected products (**56e** and **56f**) were obtained in 80% and 84% yields, correspondingly. Furthermore, various alkyl halides (**41g-m**) were also utilized in the present reaction conditions, to give corresponding derivatives in good yields (**56g-m**; 82-78%). From this investigation, we have observed that the time required for the formation of the products **56a-m** depends on the type of aralkyl and alkyl halides. When compared to the alkyl halides the aralkyl halides require less time and gave high yields of products (**Table-4**).

Table-4: Synthesis of various aralkyl/alkyl thio-3,5-dimethyl-1*H*-pyrazolyl-4*H*-1,2,4-triazol-4-amines (**56a-m**).

Products	R-	M.F.	M.Wt.	Time (h)	Yield (%)
56a		C ₁₄ H ₁₆ N ₆ S	300	8.00	90
56b		C ₁₄ H ₁₅ N ₇ O ₂ S	345	8.30	88
56c		C ₁₀ H ₁₂ N ₆ S	248	8.20	86
56d		C ₁₀ H ₁₄ N ₆ S	250	8.40	82
56e		C ₁₁ H ₁₆ N ₆ O ₂ S	296	8.50	80
56f		C ₉ H ₁₂ N ₆ O ₂ S	268	9.00	84
56g		C ₂₁ H ₃₈ N ₆ S	406	9.20	82

56h	$\text{H}_3\text{C}-(\text{CH}_2)_{10}-\overset{\text{H}_2}{\text{C}}-$	$\text{C}_{19}\text{H}_{34}\text{N}_6\text{S}$	378	9.20	83
56i	$\text{H}_3\text{C}-(\text{H}_2\text{C})_8-\overset{\text{H}_2}{\text{C}}-$	$\text{C}_{17}\text{H}_{30}\text{N}_6\text{S}$	350	9.30	81
56j	$\text{H}_3\text{C}-(\text{H}_2\text{C})_6-\overset{\text{H}_2}{\text{C}}-$	$\text{C}_{15}\text{H}_{26}\text{N}_6\text{S}$	322	9.30	85
56k	$\text{H}_3\text{C}-(\text{H}_2\text{C})_5-\overset{\text{H}_2}{\text{C}}-$	$\text{C}_{14}\text{H}_{24}\text{N}_6\text{S}$	308	9.40	88
56l	$\text{H}_3\text{C}-(\text{H}_2\text{C})_4-\overset{\text{H}_2}{\text{C}}-$	$\text{C}_{13}\text{H}_{22}\text{N}_6\text{S}$	294	9.40	84
56m	$\text{H}_3\text{C}-(\text{H}_2\text{C})_3-\overset{\text{H}_2}{\text{C}}-$	$\text{C}_{12}\text{H}_{20}\text{N}_6\text{S}$	280	9.50	78

Molecular Modeling Studies:

The molecular docking tool is helpful to investigate and to gain a profound insight into the mode of binding interactions of each ligand molecules (**56a-m**) with receptor structure. All chemical structures were drawn by using ChemDraw Ultra 12.0 and 2D structures drawn and converted to mol2 format by using Open Babel GUI version 2.3.2 (OpenBableGUI; Chris Morley, USA), Molecular energy was minimized using the Energy Minimization module of Discovery Studio version 4.1 (Accelrys Inc., San Diego, CA, USA) under the chemistry at Harvard Macromolecular Mechanics (CHARMM) force field.

The three-dimensional structure of Tubulin protein was retrieved from Protein Data Bank (PDB ID: 1SAO)⁸⁷. Molecular docking studies for the synthesized compounds of **56a-m** were performed. The structure preparation and correction protein were performed using Discovery Studio 4.1 suite. The target protein file was prepared by removing the structural water molecule, heteroatoms, and co-factors by leaving only the residues associated with protein by using Accelrys Discovery Studio 4.1 (ADS) tool was used to prepare target protein file addition of polar hydrogen's to the macromolecule, an essential step to correct the calculation of partial charge by keeping all other values as default. The molecular docking was performed using the

Ligand Fit module and obtained results were scrutinized based on the highest dock score and number of H-bonds by SS Viewer⁸⁸. The docking studies revealed that all the synthesized molecules exhibited excellent binding energies towards the receptor active sites.

Results:

We have carried out the docking studies and found that the binding site of the receptor makes the synthesized compounds easy to fit into the binding site and it gives supporting evidence in the results. Microtubules are cytoskeletal polymers of Tubulin involved in numerous cellular functions. Their dynamic instability is controlled by several compounds and proteins, including Colchicine. Molecular docking results are identified the best-interacted ligands were scrutinized on the best ligand binding poses were identified using the low binding energy, high docking score, and the number of H-bonding, hydrophobic interactions at the receptor site. **Table-5** represents the docking score and binding energies of (56a-m).

Docking analysis of most excellent active products in the active site of 1SAO receptor of Tubulin Colchicine discovered that the hydrophobic interactions are significant for the activity. All these compounds showed good binding interactions with the receptor. The most potent compounds of **56e**, **56f**, **56g**, and **56j** showed maximum dock score good H-bond interactions with the receptor, and these compounds are tabularized in **Table-5**. Compounds **56e**, **56f**, **56g**, and **56j** show the superior docking score in both open (PDB-1SAO) and closed conformations of the protein which confirms its high binding affinity. Besides, the high affinity of compounds can be further testified by their optimal potential energy, columbic, and Van der Waals forces, interaction profile as well as excellent hydrogen bonding efficiency in the active site of the protein. The docking images of the best compounds of **56e**, **56f**, **56g**, and **56j** were shown in **Fig.4** receptor structure (Amino acids). Are shown in wireframe representation and ligand structure as shown in sticks representation and H-bonds are visualized by dotted lines.

Table-5: The docking interactions of **56a-m** compounds with the active site of TUBULIN-COLCHICINE (1SAO).

Ligand	<u>Interacting Residues</u>		Distance (Å)	Docking Energy(Kcal/mol)
	Ligand	Receptor		
56a	NH	OD1-ASP98	2.69	-88.2354

	NH	OD2-ASP69	2.86	
	NH	OE2-GLU71	2.97	
56b	NH	OG-SER140	2.74	-86.8753
56c	NH	OG-SER140	2.72	-88.1245
56d	NH	OD1-ASN228	2.60	-88.4578
56e	NH	OG-SER140	2.55	-89.2546
56f	O	NH-THR145	2.60	-88.1124
	O	NH-GLY146	2.95	
56g	NH	O-SER178	2.59	-89.9887
	NH	O-VAL177	2.97	
56h	NH	O-SER178	2.75	-79.2456
	NH	OE2-GLU183	2.78	
56i	NH	OG-SER140	2.69	-80.2013
56j	NH	O-SER178	2.60	-79.2451
	NH	O-VAL177	2.72	
56k	NH	OG-SER140	2.60	-79.2456
56l	NH	OD1-ASN228	2.77	-79.2345
56m	NOT GOOD INTERACTIONS	—	—	—

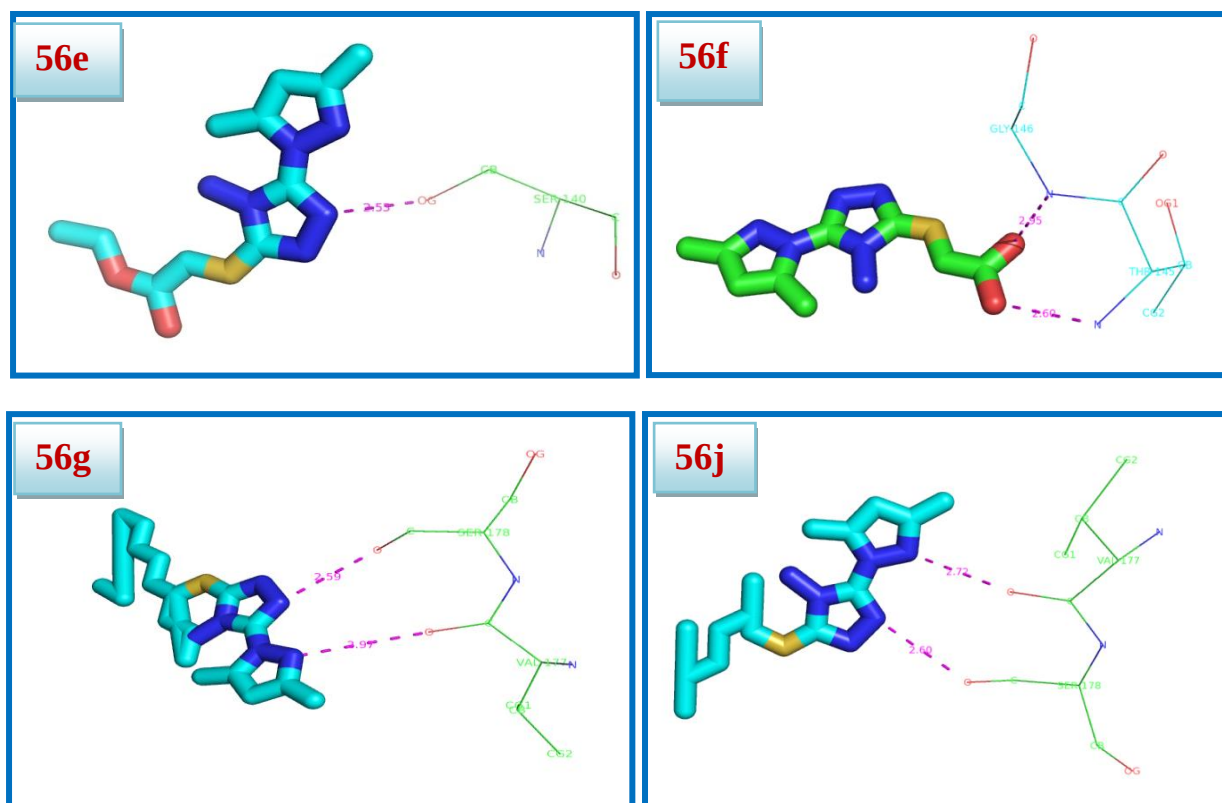


Fig. 4: H-bonding interactions between amino acid residues at the active site of the TUBULIN and compounds **56e**, **56f**, **56g**, and **56j**.

In summary, a facile and simple procedure for the synthesis of various aralkyl/alkyl thio-3,5-dimethyl-1*H*-pyrazolyl-4*H*-1,2,4-triazol-4-amines **56a-m** has been achieved via a multi-component approach using readily available chemicals. The attractive and notable features of this approach are that good yields, neat reaction conditions, easy purification of product, metal-free, atom economy, and avoiding toxic catalyst. The synthesized compounds could be useful for medicinal chemistry applications. The biological activity of these compounds is in progress.

EXPERIMENTAL:

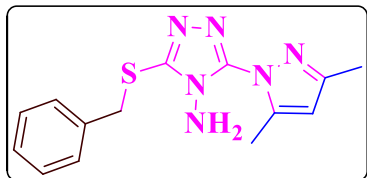
General procedure for the one-pot, multi-component cascade reaction for the synthesis of various aralkyl/alkyl thio-3,5-dimethyl-1*H*-pyrazolyl-4*H*-1,2,4-triazol-4-amine (56a-m):

A mixture of 4-amino-5-hydrazino-4*H*-[1,2,4]triazole-3-thiol (0.001mol) and pentane-2,4-dione (0.001mol) was refluxed for 3 h in dry ethanol (2 mL) in a 25 mL round bottom flask. Then to the reaction mixture appropriate aralkyl/alkyl halide (0.001mol) and DMF (2 mL) were

added. The reaction mixture was refluxed for 5-7 h at 80-90°C by monitoring TLC. After completion of the reaction, the resulting mixture was cooled, the separated solid was filtered and recrystallized from 6-8 mL ethanol.

SPECTRAL DATA

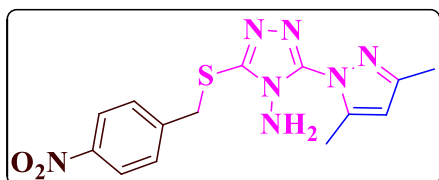
3-(Benzylthio)-5-(3,5-dimethyl-1H-pyrazol-1-yl)-4H-1,2,4-triazol-4-amine (56a):



White color solid (0.270g, 90%); m.p.: 140-142°C; **IR (KBr, $\nu_{\max}/\text{cm}^{-1}$):** 3323 (-NH₂); **¹H-NMR (400 MHz, CDCl₃, δ ppm):** 2.26 (s, 3H, -CH₃), 2.53 (s, 3H, -CH₃), 4.46 (s, 2H, -SCH₂-), 5.22 (s, 2H, -NH₂), 6.02 (s, 1H, -CH- of pyrazole ring), 7.26-7.34

(m, 3H, Ar-H), 7.43 (d, 2H, $J=7.6$ Hz, Ar-H); **¹³C-NMR (100 MHz, CDCl₃, δ ppm):** 12.34, 13.56, 35.89, 108.11, 127.74, 128.68, 129.18, 136.75, 143.20, 147.66, 151.31, 151.83; **ESI-MS (m/z):** 301 [M+H]⁺; Analytical calculated formulae C₁₄H₁₆N₆S; C, 55.98; H, 5.37; N, 27.98; S, 10.67; Found: C, 55.95; H, 5.40; N, 27.93; S, 10.71.

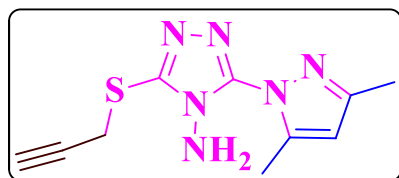
3-(3,5-Dimethyl-1H-pyrazol-1-yl)-5-((4-nitrobenzyl)thio)-4H-1,2,4-triazol-4-amine (56b):



White color solid (0.303g, 88%); m.p.: 168-170°C; **IR (KBr, $\nu_{\max}/\text{cm}^{-1}$):** 3312 (-NH₂); **¹H-NMR (400 MHz, DMSO-*d*₆, δ ppm):** 2.24 (s, 3H, -CH₃), 2.35 (s, 3H, -CH₃), 4.56 (s, 2H, -SCH₂-), 5.92 (s, 2H, -NH₂), 6.10 (s, 1H, -CH-

of pyrazole ring), 7.73 (d, 2H, $J=8.4$ Hz, Ar-H), 8.16 (d, 2H, $J=8.8$ Hz, Ar-H); **¹³C-NMR (100 MHz, DMSO-*d*₆, δ ppm):** 11.6, 13.8, 33.9, 107.8, 123.9, 130.8, 143.1, 146.4, 147.1, 149.2, 151.1, 151.9; **ESI-MS (m/z):** 346 [M+H]⁺; Analytical calculated formulae C₁₄H₁₅N₇O₂S; C, 48.69; H, 4.38; N, 28.39; S, 9.28; Found: C, 48.65; H, 4.33; N, 28.42; S, 9.25.

3-(3,5-Dimethyl-1H-pyrazol-1-yl)-5-(prop-2-yn-1-ylthio)-4H-1,2,4-triazol-4-amine (56c):

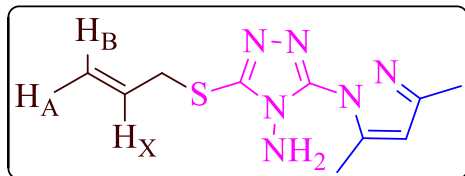


White color solid (0.213g, 86%); m.p.: 165-167°C; **IR (KBr, $\nu_{\max}/\text{cm}^{-1}$):** 3434 (-NH₂); **¹H-NMR (400 MHz, CDCl₃, δ ppm):** 1.26 (s, 1H, -C≡C-H), 2.29 (s, 3H, -CH₃), 2.56 (s, 3H, -CH₃), 4.02 (s, 2H, -SCH₂-), 5.44 (s, 2H, -NH₂), 6.05 (s, 1H, -

CH- of pyrazole ring); **¹³C-NMR (100 MHz, CDCl₃+DMSO-*d*₆, δ ppm):** 12.1, 13.6, 19.7, 72.6, 78.7, 107.9, 142.9, 148.4, 150.4, 151.7; **ESI-MS (m/z):** 249 [M+H]⁺; Analytical calculated

formulae $C_{10}H_{12}N_6S$; C, 48.37; H, 4.87; N, 33.85; S, 12.91; Found: C, 48.34; H, 4.84; N, 33.89; S, 12.95.

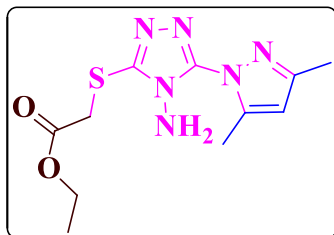
3-(Allylthio)-5-(3,5-dimethyl-1H-pyrazol-1-yl)-4H-1,2,4-triazol-4-amine (56d):



White color solid (0.205g, 82%); m.p.: 93-95°C; **IR** (KBr, $\nu_{\max}/\text{cm}^{-1}$): 3314 ($-\text{NH}_2$); **$^1\text{H-NMR}$ (400 MHz, CDCl_3 , δ ppm)**: 2.29 (s, 3H, $-\text{CH}_3$), 2.55 (s, 3H, $-\text{CH}_3$), 3.91 (d, 2H, $J=7.2$ Hz, $-\text{SCH}_2-$), 5.19 (d, 1H, $J=H_X$ H_A ,

$J=10.0$ Hz, H_A of allyl group), 5.35 (d, 1H, $J=H_X$ H_B , $J=17.6$ Hz, H_B of allyl group), 5.38 (s, 2H, $-\text{NH}_2$), 5.98-6.08 (m, 1H, $=\text{CH}_X-$), 6.04 (s, 1H, $-\text{CH}-$ of pyrazole ring); **$^{13}\text{C-NMR}$ (100 MHz, CDCl_3 , δ ppm)**: 12.3, 13.6, 34.1, 108.1, 119.0, 132.7, 143.2, 147.6, 151.2, 151.8; **ESI-MS (m/z)**: 251 $[\text{M}+\text{H}]^+$; Analytical calculated formulae $C_{10}H_{14}N_6S$; C, 47.98; H, 5.64; N, 33.57; S, 12.81; Found: C, 47.95; H, 5.60; N, 33.60; S, 12.85.

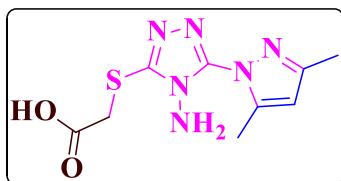
Ethyl-2-((4-amino-5-(3,5-dimethyl-1H-pyrazol-1-yl)-4H-1,2,4-triazol-3-yl)thio)acetate (56e):



White color solid (0.236g, 80%); m.p.: 100-102°C; **IR** (KBr, $\nu_{\max}/\text{cm}^{-1}$): 3304 ($-\text{NH}_2$), 1733 ($-\text{C}=\text{O}$); **$^1\text{H-NMR}$ (400 MHz, CDCl_3 , δ ppm)**: 1.30 (t, 3H, $J=7.2$ Hz, $-\text{CH}_3$), 2.29 (s, 3H, $-\text{CH}_3$), 2.54 (s, 3H, $-\text{CH}_3$), 4.05 (s, 2H, $-\text{SCH}_2-$), 4.21-4.26 (m, 2H, $-\text{CH}_2\text{O}-$), 5.49 (s, 2H, $-\text{NH}_2$) 6.04 (s, 1H, $-\text{CH}-$ of pyrazole ring);

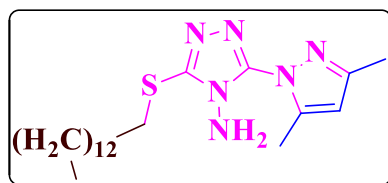
$^{13}\text{C-NMR}$ (100 MHz, CDCl_3 , δ ppm): 12.3, 13.6, 14.1, 33.6, 62.1, 108.1, 143.2, 147.9, 150.4, 151.9, 158.6; **ESI-MS (m/z)**: 297 $[\text{M}+\text{H}]^+$; Analytical calculated formulae $C_{11}H_{16}N_6O_2S$; C, 44.58; H, 5.44; N, 28.36; S, 10.82; Found: C, 44.62; H, 5.47; N, 28.39; S, 10.87.

2-((4-Amino-5-(3,5-dimethyl-1H-pyrazol-1-yl)-4H-1,2,4-triazol-3-yl)thio)acetic acid (56f):

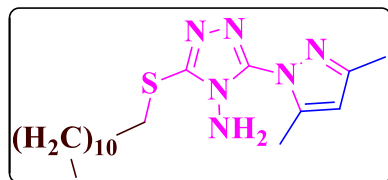


White color solid (0.225g, 84%); m.p.: 202-204°C; **IR** (KBr, $\nu_{\max}/\text{cm}^{-1}$): 3293 ($-\text{NH}_2$), 1746 ($-\text{C}=\text{O}$); **$^1\text{H-NMR}$ (400 MHz, $\text{DMSO}-d_6$, δ ppm)**: 2.21 (s, 3H, $-\text{CH}_3$), 2.25 (s, $-\text{NH}_2$), 2.34 (s, 3H, $-\text{CH}_3$), 3.93 (s, 2H, $-\text{SCH}_2-$), 6.03 (s, 1H, $-\text{CH}-$ of pyrazole

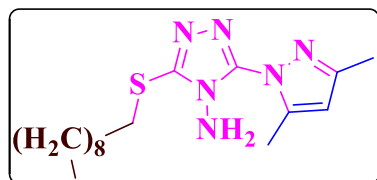
ring), 7.93 (s, 1H, $-\text{OH}$); **$^{13}\text{C-NMR}$ (100 MHz, $\text{CDCl}_3+\text{DMSO}-d_6$, δ ppm)**: 11.6, 13.8, 33.3, 107.7, 143.1, 149.0, 151.1, 152.4, 170.0; **ESI-MS (m/z)**: 269 $[\text{M}+\text{H}]^+$; Analytical calculated formulae $C_9H_{12}N_6O_2S$; C, 40.29; H, 4.51; N, 31.32; S, 11.95; Found: C, 40.33; H, 4.55; N, 31.35; S, 11.99.

3-(3,5-Dimethyl-1H-pyrazol-1-yl)-5-(tetradecylthio)-4H-1,2,4-triazol-4-amine (56g):

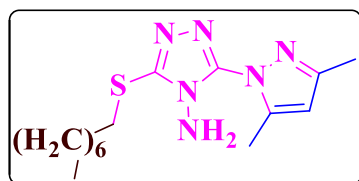
White color solid (0.332g, 82%); m.p.: 96-98°C; **IR (KBr, $\nu_{\max}/\text{cm}^{-1}$):** 3340 (-NH₂); **¹H-NMR (400 MHz, CDCl₃, δ ppm):** 0.88 (t, 3H, $J=7.2$ Hz, -CH₃), 1.26 (unresolved m, 20H, -(CH₂)₁₀-), 1.42-1.49 (m, 2H, -CH₂-), 1.77-1.84 (m, 2H, -CH₂-), 2.28 (s, 3H, -CH₃), 2.54 (s, 3H, -CH₃), 3.26 (t, 2H, $J=7.2$ Hz, -SCH₂-), 5.32 (s, 2H, -NH₂), 6.03 (s, 1H, -CH- of pyrazole ring); **¹³C-NMR (100 MHz, DMSO-*d*₆, δ ppm):** 11.6, 13.8, 22.6, 28.5, 29.0, 29.2, 29.4, 29.5, 30.9, 31.8, 107.6, 143.0, 148.9, 151.0, 152.9; **ESI-MS (m/z):** 407 [M+H]⁺; Analytical calculated formulae C₂₁H₃₈N₆S; C, 62.03; H, 9.42; N, 20.67; S, 7.89; Found: C, 61.97; H, 9.38; N, 20.62; S, 7.93.

3-(3,5-Dimethyl-1H-pyrazol-1-yl)-5-(dodecylthio)-4H-1,2,4-triazol-4-amine (56h):

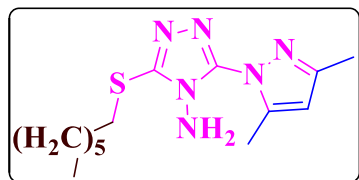
White color solid (0.313g, 83%); m.p.: 73-75°C; **IR (KBr, $\nu_{\max}/\text{cm}^{-1}$):** 3340 (-NH₂); **¹H-NMR (400 MHz, CDCl₃, δ ppm):** 0.89 (t, 3H, $J=6.4$ Hz, -CH₃), 1.29 (unresolved m, 16H, -(CH₂)₈-), 1.45-1.47 (m, 2H, -CH₂-), 1.78-1.85 (m, 2H, -CH₂-), 2.26 (s, 3H, -CH₃), 2.56 (s, 3H, -CH₃), 3.29 (t, 2H, $J=7.2$ Hz, -SCH₂-), 5.27 (s, 2H, -NH₂), 6.04 (s, 1H, -CH- of pyrazole ring); **¹³C-NMR (100 MHz, DMSO-*d*₆, δ ppm):** 11.5, 13.8, 14.4, 22.6, 28.5, 29.0, 29.1, 29.4, 29.6, 30.9, 31.8, 107.7, 143.1, 148.9, 151.0, 152.9; **ESI-MS (m/z):** 351 [M+H]⁺; Analytical calculated formulae C₁₉H₃₄N₆S; C, 60.28; H, 9.05; N, 22.20; S, 8.47; Found: C, 60.23; H, 9.11; N, 22.25; S, 8.43.

3-(Decylthio)-5-(3,5-dimethyl-1H-pyrazol-1-yl)-4H-1,2,4-triazol-4-amine (56i):

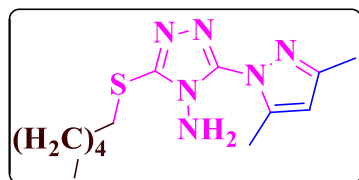
White color solid (0.283g, 81%); m.p.: 78-80°C; **IR (KBr, $\nu_{\max}/\text{cm}^{-1}$):** 3341 (-NH₂); **¹H-NMR (400 MHz, CDCl₃, δ ppm):** 0.89 (t, 3H, $J=6.8$ Hz, -CH₃), 1.28 (unresolved m, 12H, -(CH₂)₆-), 1.45-1.47 (m, 2H, -CH₂-), 1.78-1.85 (m, 2H, -CH₂-), 2.29 (s, 3H, -CH₃), 2.55 (s, 3H, -CH₃), 3.27 (t, 2H, $J=7.2$ Hz, -SCH₂-), 5.35 (s, 2H, -NH₂), 6.04 (s, 1H, -CH- of pyrazole ring); **¹³C-NMR (100 MHz, CDCl₃, δ ppm):** 12.3, 13.6, 14.1, 22.7, 28.8, 29.1, 29.3, 29.4, 29.5, 31.2, 31.9, 108.1, 143.2, 147.6, 151.8, 152.2; **ESI-MS (m/z):** 351 [M+H]⁺; Analytical calculated formulae C₁₇H₃₀N₆S; C, 58.25; H, 8.63; N, 23.98; S, 9.15; Found: C, 58.20; H, 8.68; N, 23.95; S, 9.19.

3-(3,5-Dimethyl-1H-pyrazol-1-yl)-5-(octylthio)-4H-1,2,4-triazol-4-amine (56j):

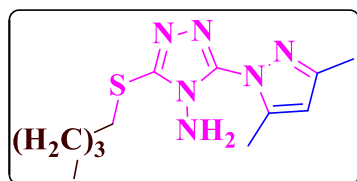
White color solid (0.278g, 85%); m.p.: 95-97°C; **IR (KBr, $\nu_{\max}/\text{cm}^{-1}$):** 3325 (-NH₂); **¹H-NMR (400 MHz, CDCl₃, δ ppm):** 0.88 (t, 3H, $J=7.2$ Hz, -CH₃), 1.27 (unresolved m, 8H, -(CH₂)₄-), 1.42-1.49 (m, 2H, -CH₂-), 1.76-1.84 (m, 2H, -CH₂-), 2.27 (s, 3H, -CH₃), 2.53 (s, 3H, -CH₃), 3.25 (t, 2H, $J=7.6$ Hz, -SCH₂-), 5.34 (s, 2H, -NH₂), 6.02 (s, 1H, -CH- of pyrazole ring); **¹³C-NMR (100 MHz, CDCl₃, δ ppm):** 12.4, 13.6, 14.1, 22.6, 28.8, 29.1, 29.2, 29.5, 31.3, 31.8, 108.1, 143.2, 147.5, 151.8, 152.2; **ESI-MS (m/z):** 323 [M+H]⁺; Analytical calculated formulae C₁₅H₂₆N₆S; C, 55.87; H, 8.13; N, 26.06; S, 9.94; Found: C, 55.83; H, 8.10; N, 26.12; S, 9.90.

3-(3,5-Dimethyl-1H-pyrazol-1-yl)-5-(heptylthio)-4H-1,2,4-triazol-4-amine (56k):

White color solid (0.283g, 90%); m.p.: 90-92°C; **IR (KBr, $\nu_{\max}/\text{cm}^{-1}$):** 3296 (-NH₂); **¹H-NMR (400 MHz, CDCl₃, δ ppm):** 0.88 (t, 3H, $J=7.2$ Hz, -CH₃), 1.29-1.35 (m, 6H, -(CH₂)₃-), 1.42-1.49 (m, 2H, -CH₂-), 1.77-1.84 (m, 2H, -CH₂-), 2.27 (s, 3H, -CH₃), 2.53 (s, 3H, -CH₃), 3.25 (t, 2H, $J=7.6$ Hz, -SCH₂-), 5.33 (s, 2H, -NH₂), 6.02 (s, 1H, -CH- of pyrazole ring); **¹³C-NMR (100 MHz, CDCl₃, δ ppm):** 12.4, 13.6, 14.1, 22.6, 28.7, 28.8, 29.5, 31.3, 31.7, 108.1, 143.2, 147.5, 151.8, 152.2; **ESI-MS (m/z):** 309 [M+H]⁺; Analytical calculated formulae C₁₄H₂₄N₆S; C, 54.52; H, 7.84; N, 27.25; S, 10.40; Found: C, 54.55; H, 7.80; N, 27.29; S, 10.46.

3-(3,5-Dimethyl-1H-pyrazol-1-yl)-5-(hexylthio)-4H-1,2,4-triazol-4-amine (56l):

White color solid (0.283g, 90%); m.p.: 143-145°C; **IR (KBr, $\nu_{\max}/\text{cm}^{-1}$):** 3341 (-NH₂); **¹H-NMR (400 MHz, CDCl₃+DMSO-*d*₆, δ ppm):** 0.80 (t, 3H, $J=6.0$ Hz, -CH₃), 1.22-1.24 (m, 4H, -(CH₂)₂-), 1.36-1.39 (m, 2H, -CH₂-), 1.71 (t, 2H, $J=8.8$ Hz, -CH₂-), 2.19 (s, 3H, -CH₃), 2.28 (s, 3H, -CH₃), 3.24 (t, 2H, $J=7.2$ Hz, -SCH₂-), 4.26 (s, 2H, -NH₂), 5.96 (s, 1H, -CH- of pyrazole ring); **¹³C-NMR (100 MHz, CDCl₃+DMSO-*d*₆, δ ppm):** 11.1, 11.5, 13.7, 14.3, 29.5, 30.9, 31.2, 34.9, 107.8, 143.6, 144.8, 145.7, 151.5, 167.3; **ESI-MS (m/z):** 295 [M+H]⁺; Analytical calculated formulae C₁₃H₂₂N₆S; C, 53.03; H, 7.53; N, 28.54; S, 10.89; Found: C, 52.97; H, 7.50; N, 28.57; S, 10.83.

3-(3,5-Dimethyl-1H-pyrazol-1-yl)-5-(pentylthio)-4H-1,2,4-triazol-4-amine (56m):

White color solid (0.218g, 78%); m.p.: 126-128°C; **IR (KBr, $\nu_{\text{max}}/\text{cm}^{-1}$):** 3341 (-NH₂); **¹H-NMR (400 MHz, CDCl₃+DMSO-*d*₆, δ ppm):** 0.82 (t, 3H, *J*=6.4 Hz, -CH₃), 1.25-1.29 (m, 2H, -(CH₂)-), 1.33-1.38 (m, 2H, -(CH₂)-), 1.68-1.73 (m, 2H, -CH₂-), 2.19 (s, 3H, -CH₃), 2.27 (s, 3H, -CH₃), 3.21 (t, 2H, *J*=6.8 Hz, -SCH₂-), 4.02 (s, 2H, -NH₂), 5.96 (s, 1H, -CH- of pyrazole ring); **¹³C-NMR (100 MHz, CDCl₃+DMSO-*d*₆, δ ppm):** 11.5, 13.8, 14.3, 22.1, 29.2, 30.7, 30.9, 107.8, 143.3, 148.9, 151.1, 151.4; **ESI-MS (*m/z*):** 281 [M+H]⁺; Analytical calculated formulae C₁₂H₂₀N₆S; C, 51.40; H, 7.19; N, 29.97; S, 11.44; Found: C, 51.44; H, 7.15; N, 29.94; S, 11.40.

SPECTRA

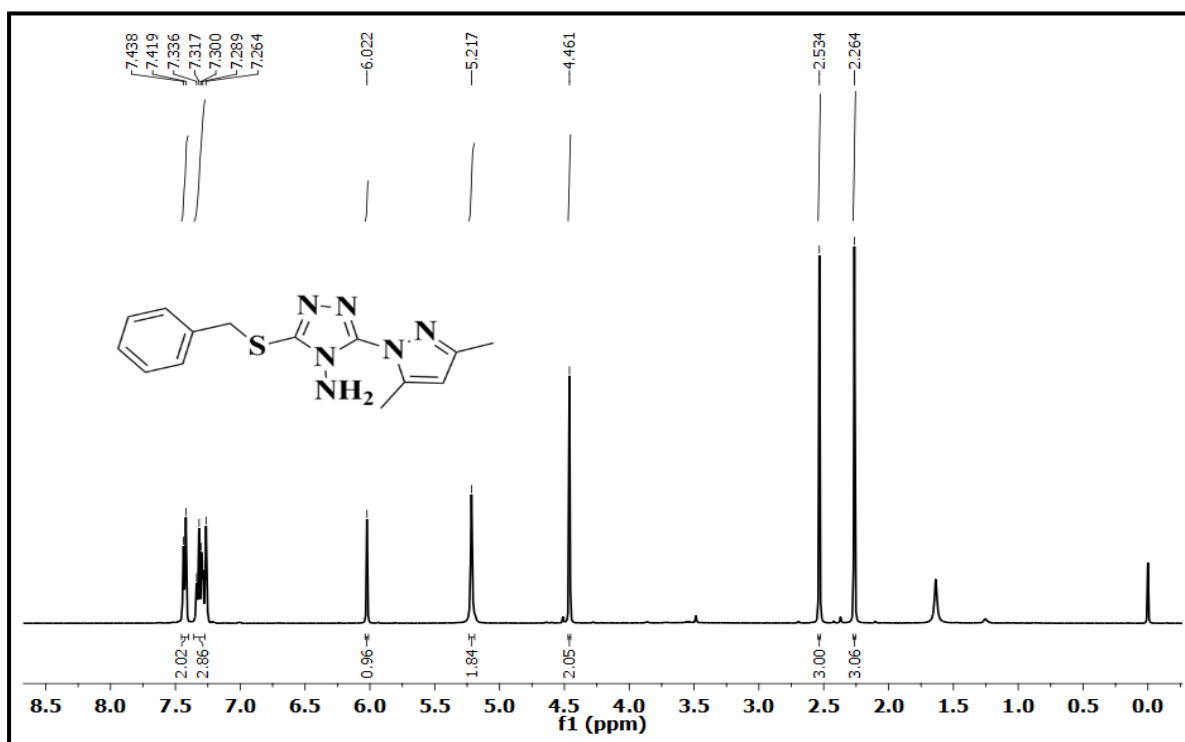


Fig: ¹H-NMR Spectrum of Compound **56a** (400 MHz, CDCl₃)

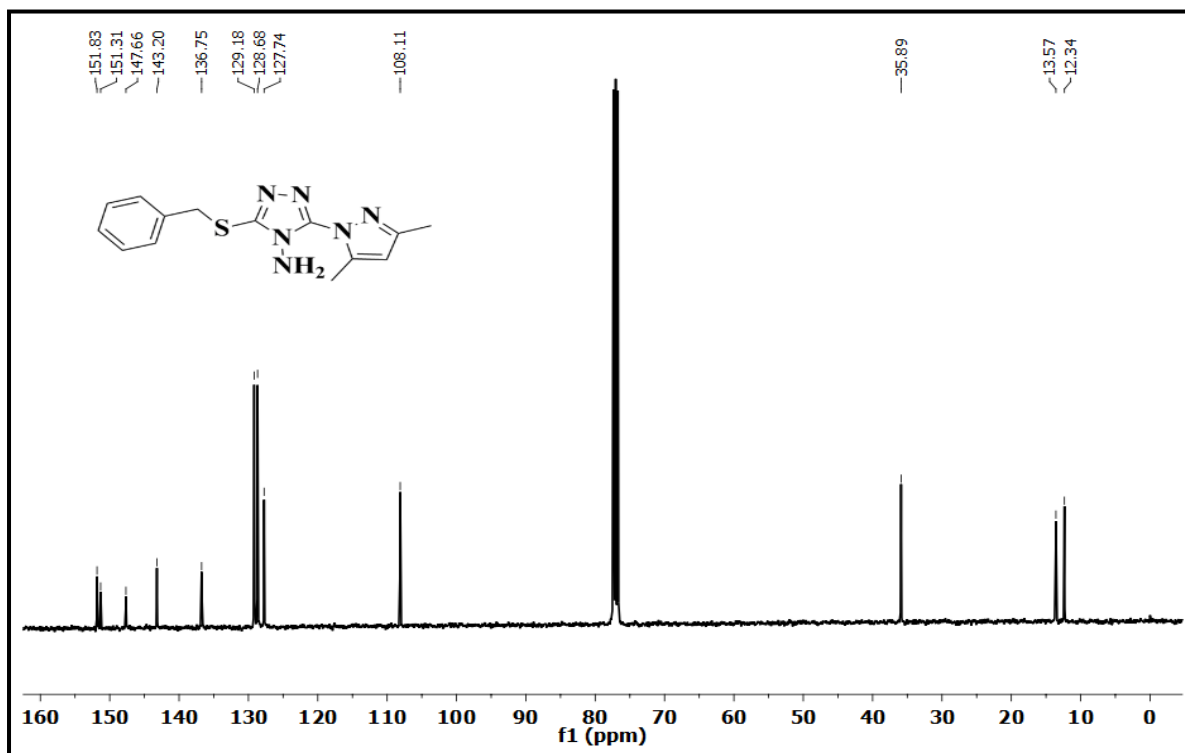


Fig: ¹³C-NMR Spectrum of Compound **56a** (100 MHz, CDCl₃)

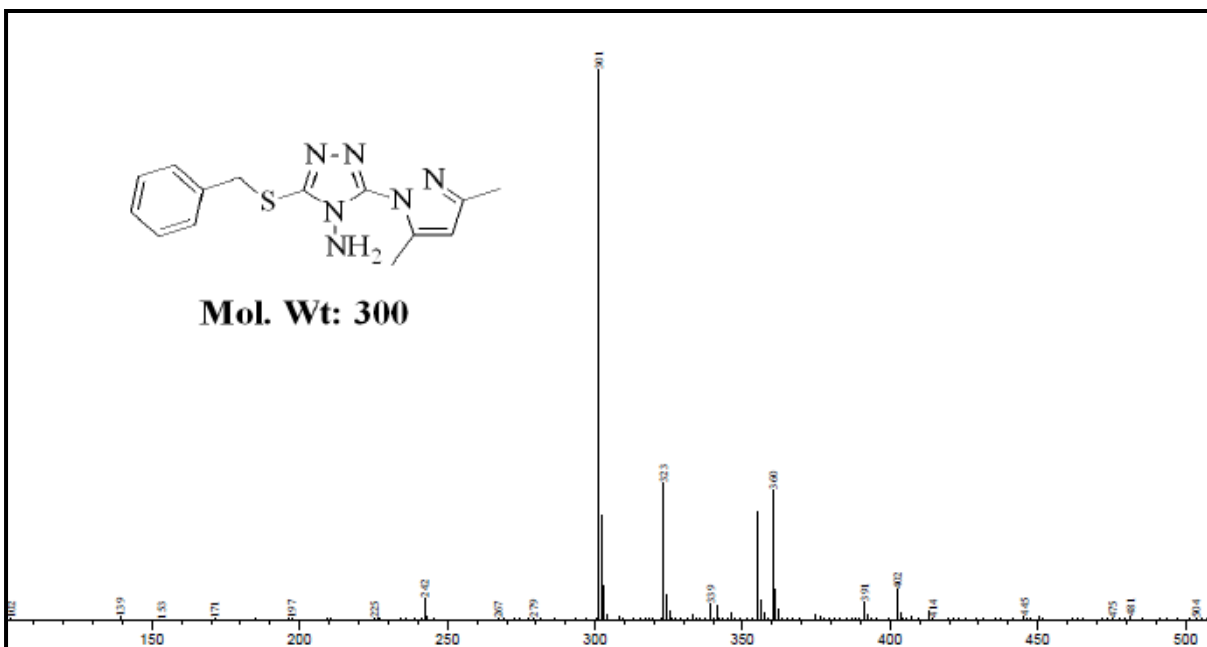


Fig: Mass Spectrum of Compound 56a

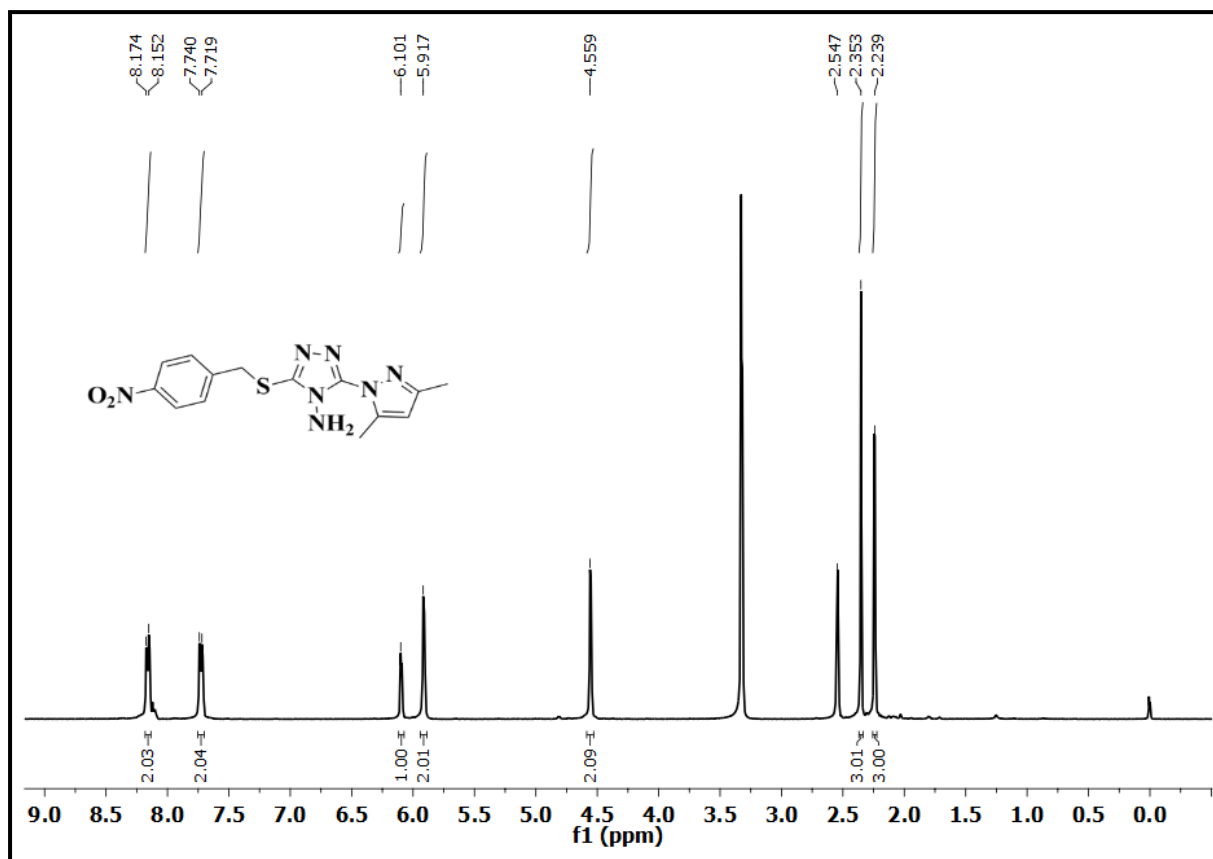


Fig: ¹H-NMR Spectrum of Compound 56b (400 MHz, DMSO-*d*₆)

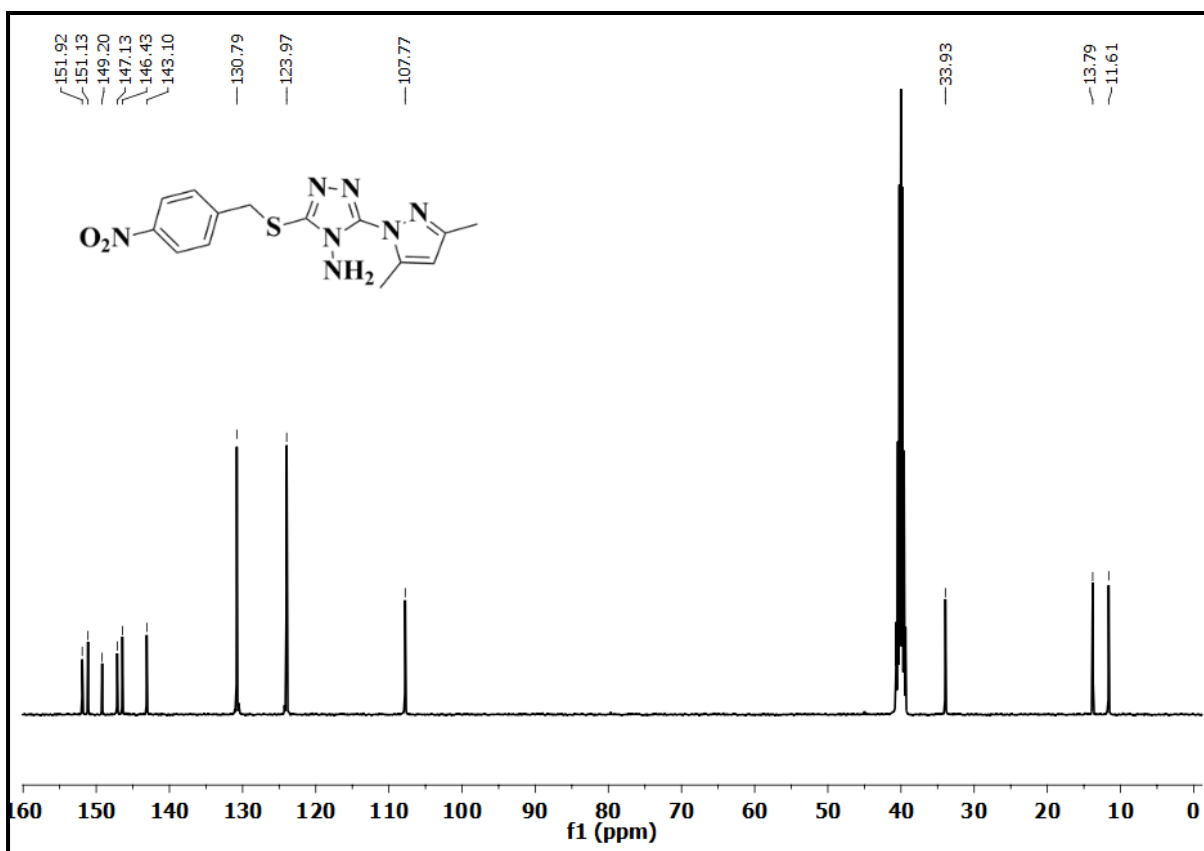


Fig: ¹³C-NMR Spectrum of Compound **56b** (100 MHz, DMSO-*d*₆)

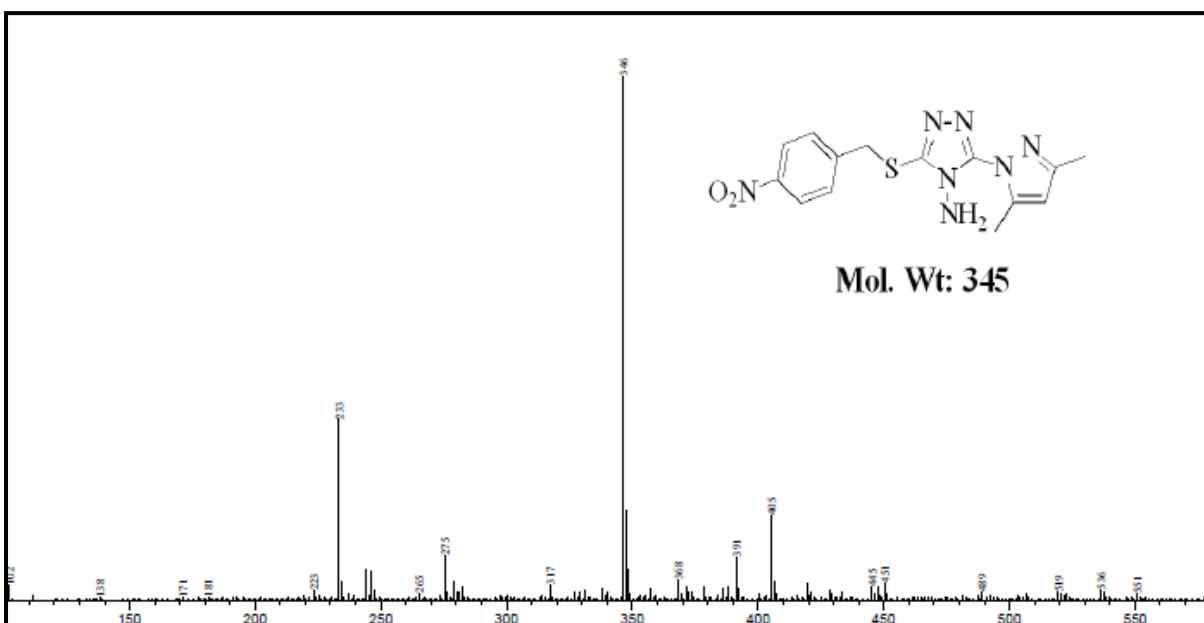


Fig: Mass Spectrum of Compound **56b**

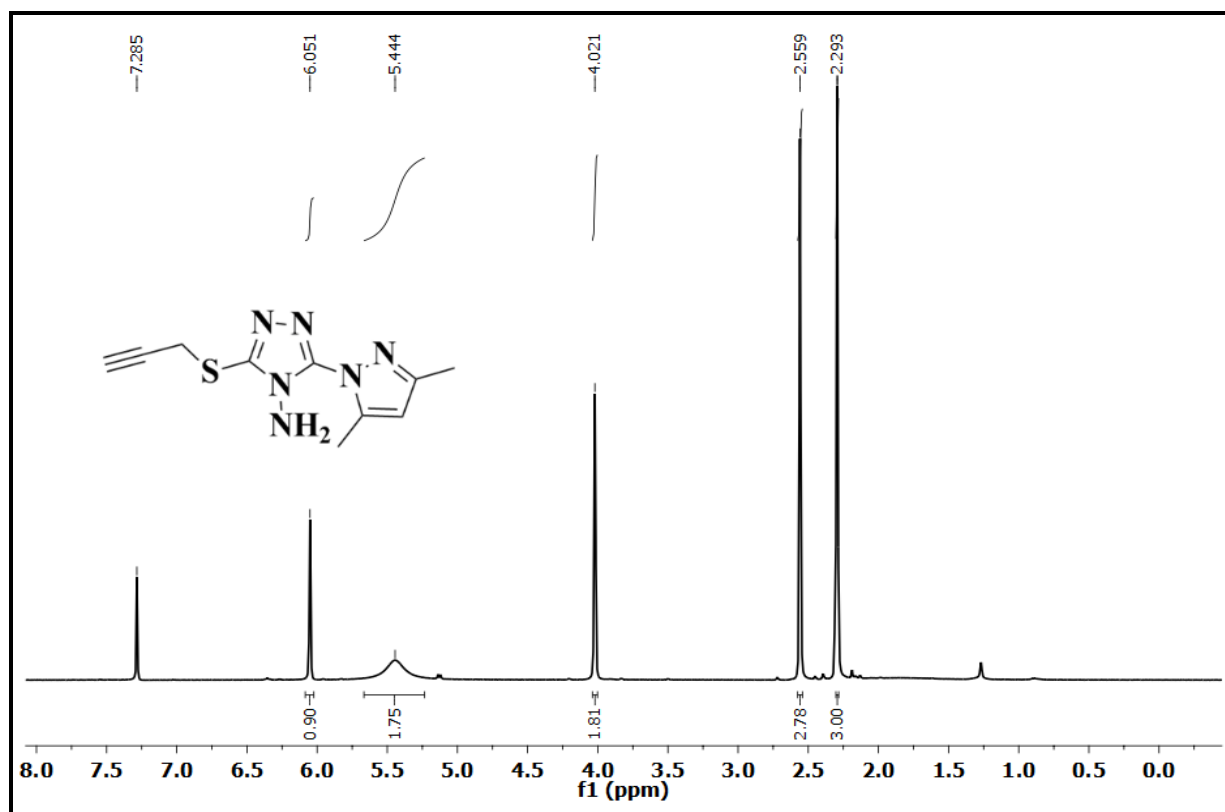


Fig: ¹H-NMR Spectrum of Compound 56c (400 MHz, CDCl₃)

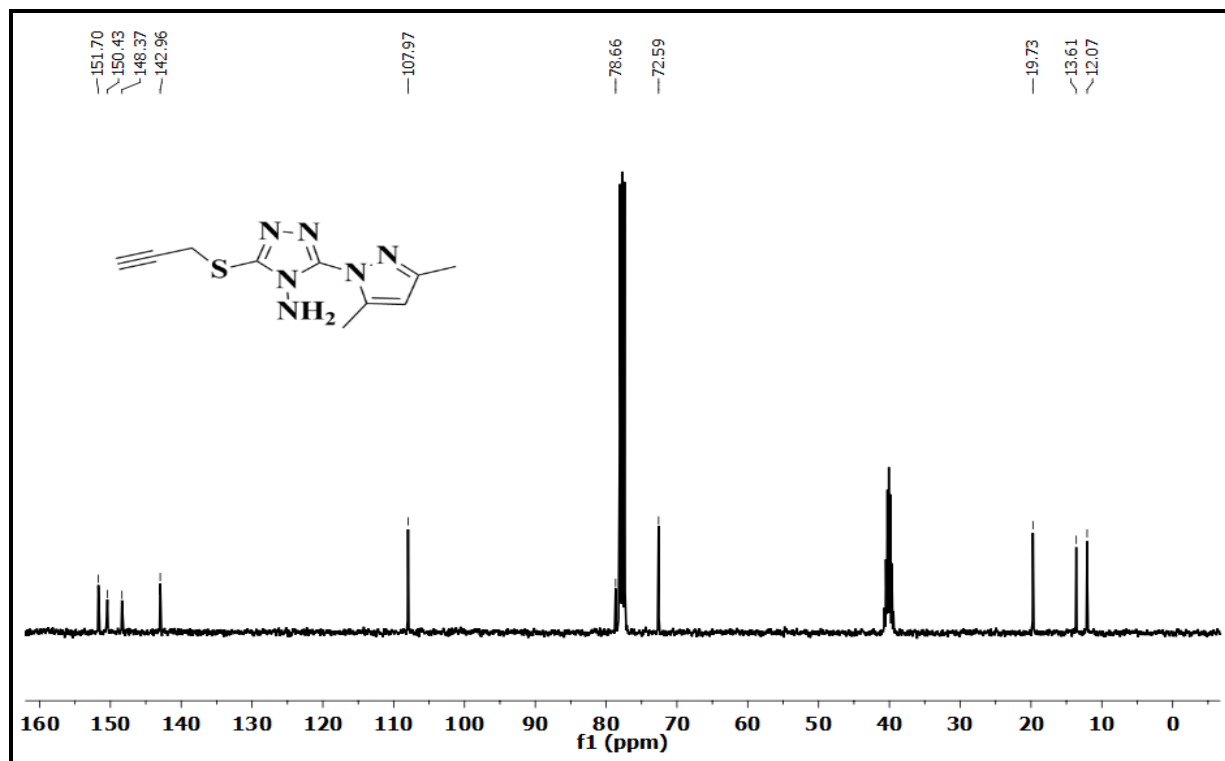


Fig: ¹³C-NMR Spectrum of Compound 56c (100 MHz, CDCl₃+DMSO-*d*₆)

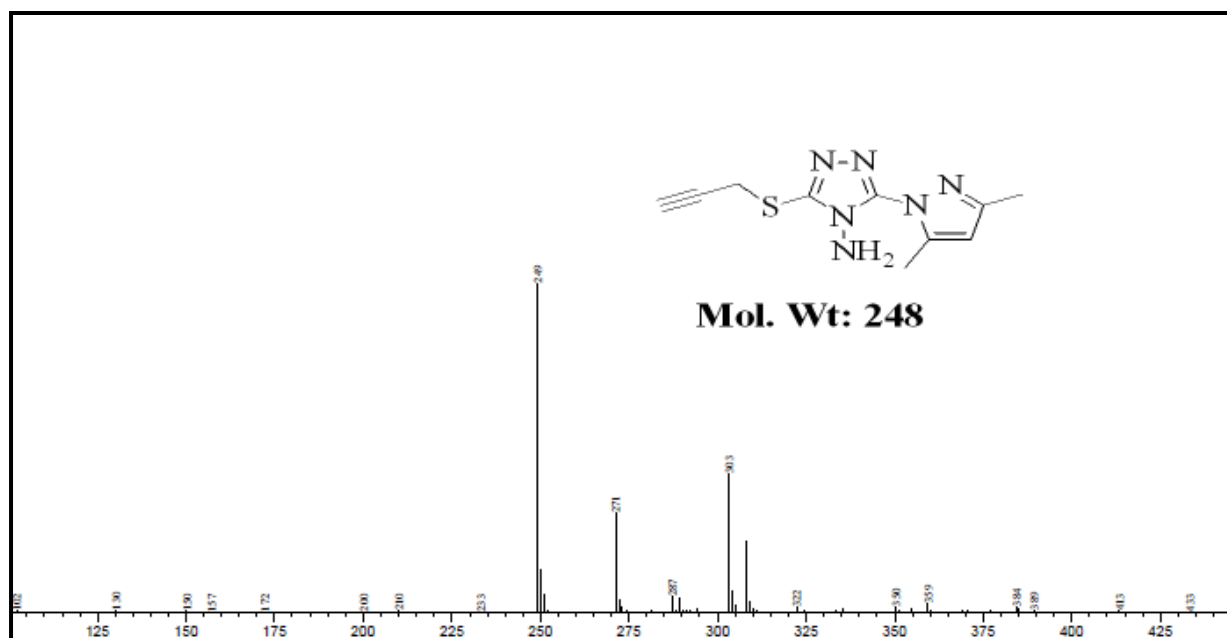


Fig: Mass Spectrum of Compound 56c

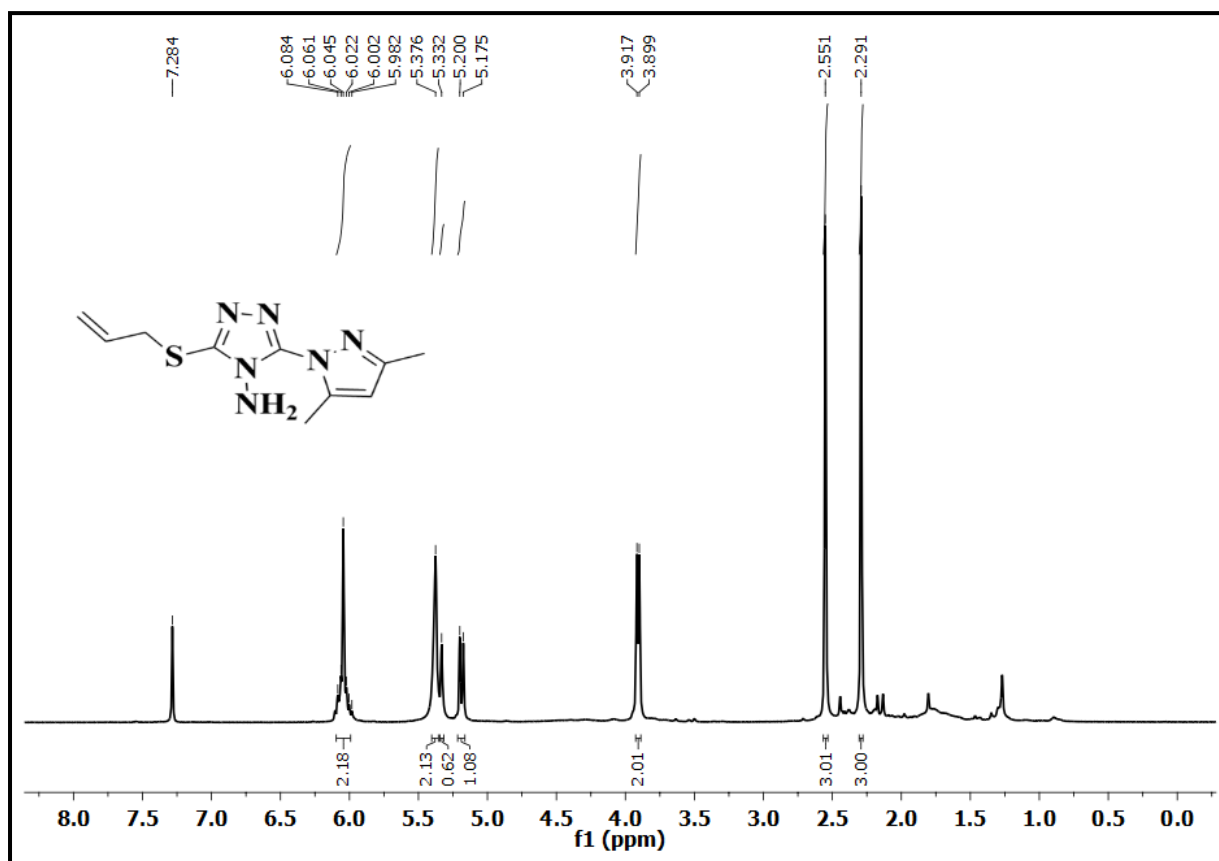


Fig: ¹H-NMR Spectrum of Compound 56d (400 MHz, CDCl₃)

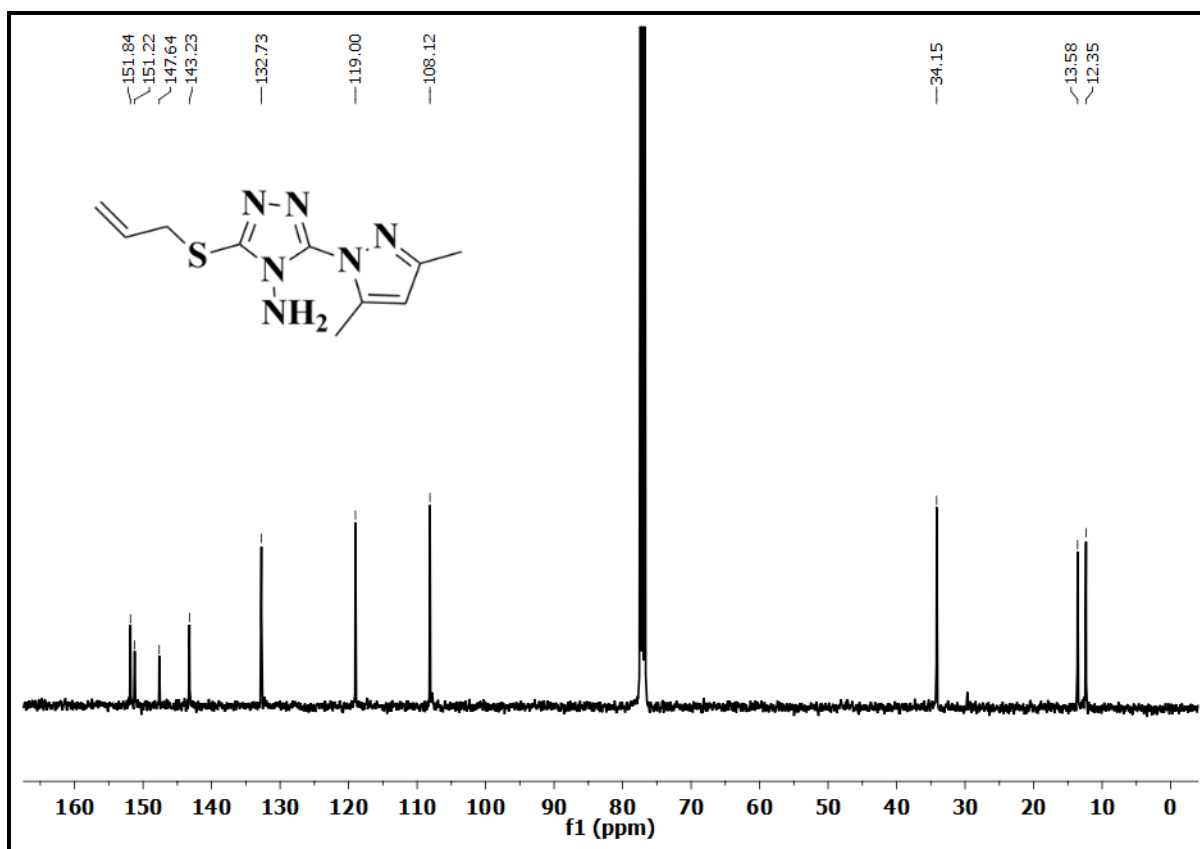


Fig: ¹³C-NMR Spectrum of Compound **56d** (100 MHz, CDCl₃)

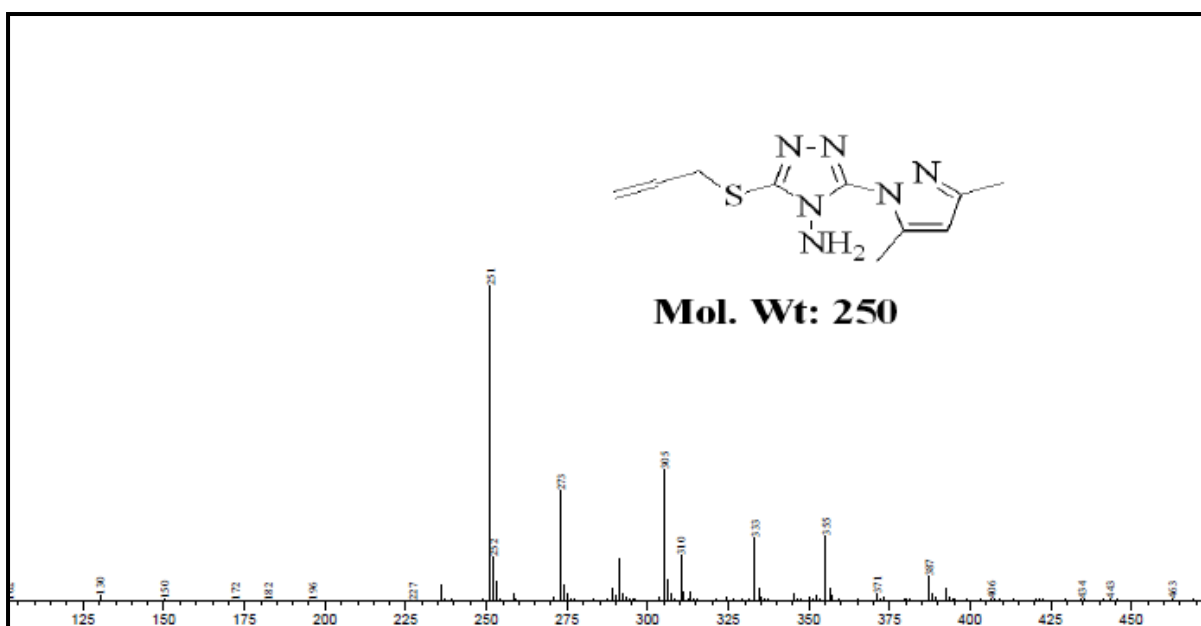


Fig: Mass Spectrum of Compound **56d**

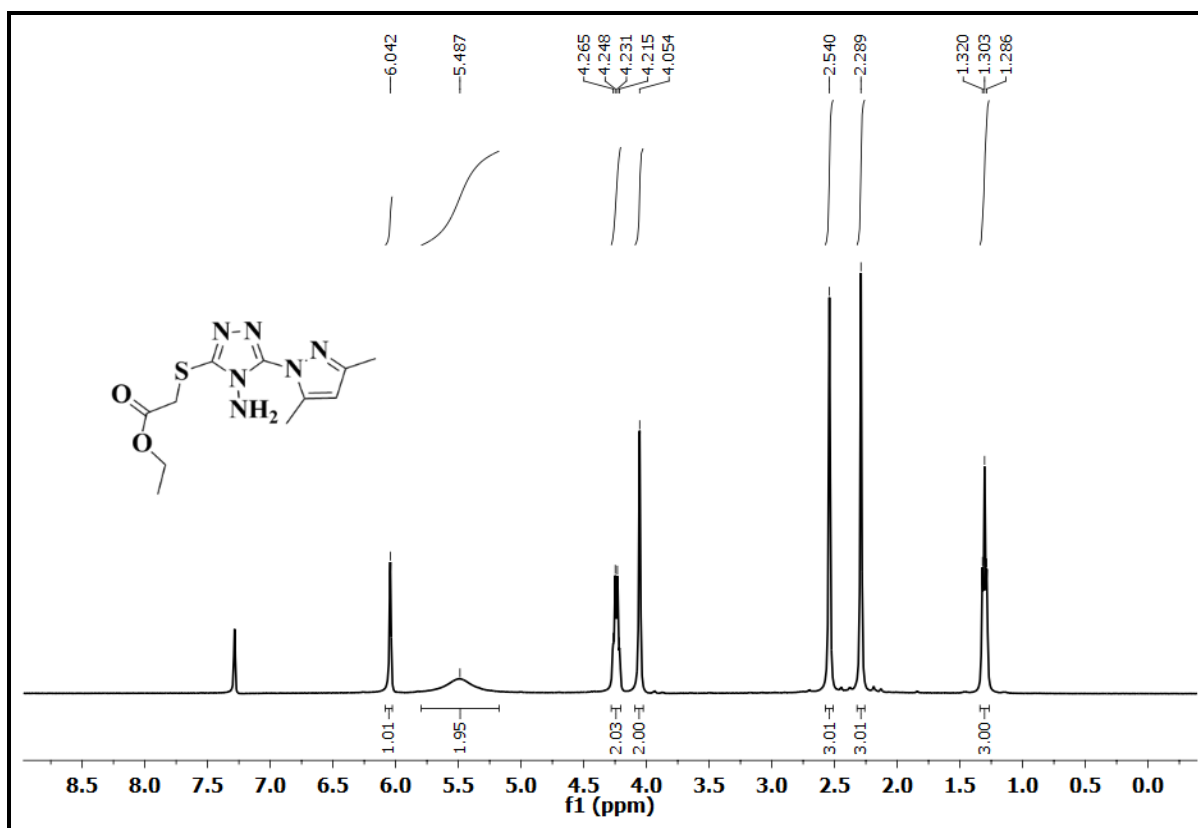


Fig: ¹H-NMR Spectrum of Compound 56e (400 MHz, CDCl₃)

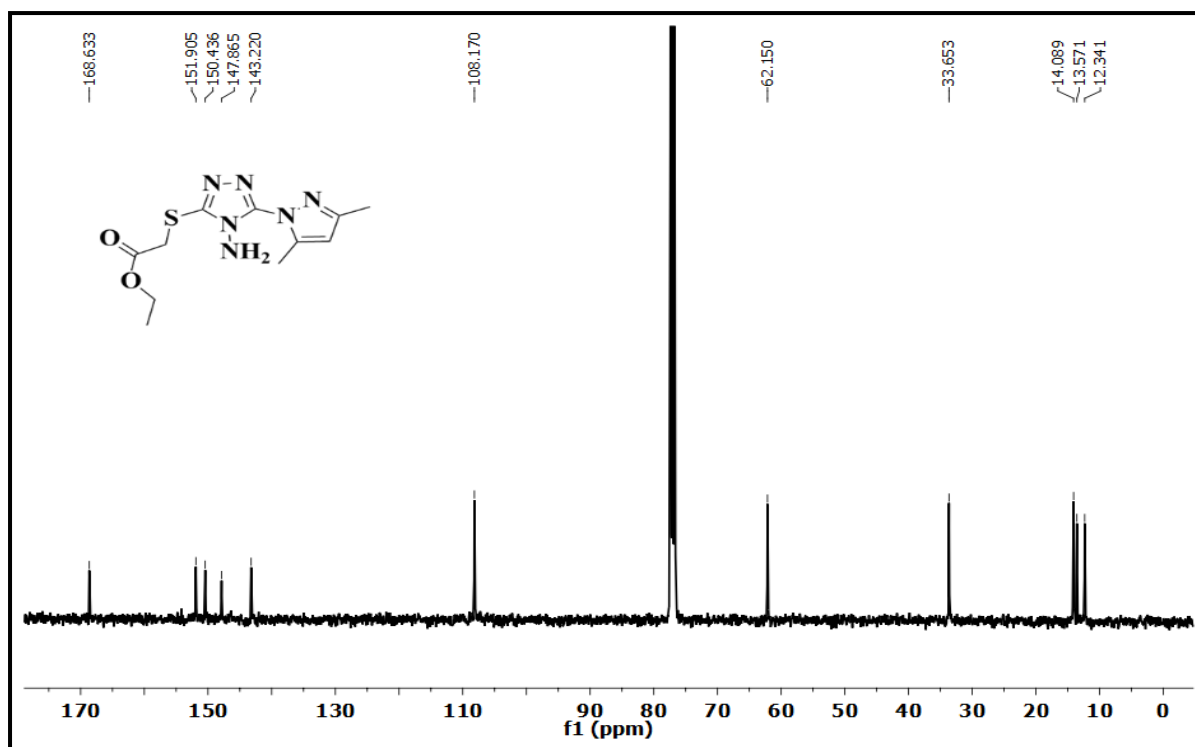


Fig: ¹³C-NMR Spectrum of Compound 56e (100 MHz, CDCl₃)

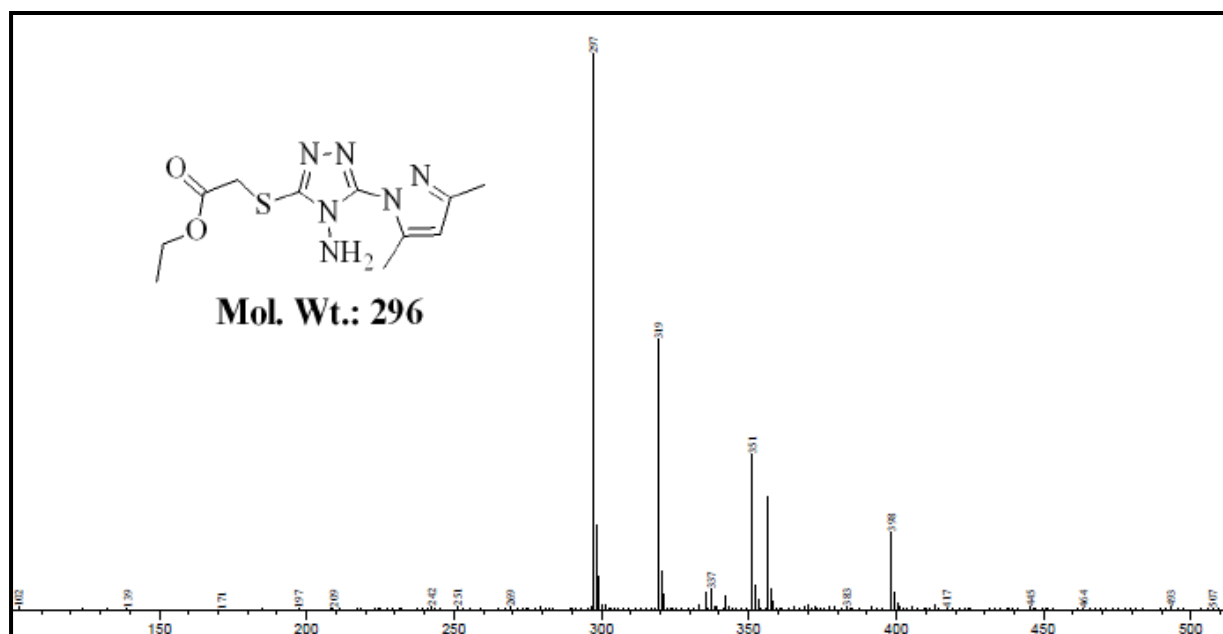


Fig: Mass Spectrum of Compound 56e

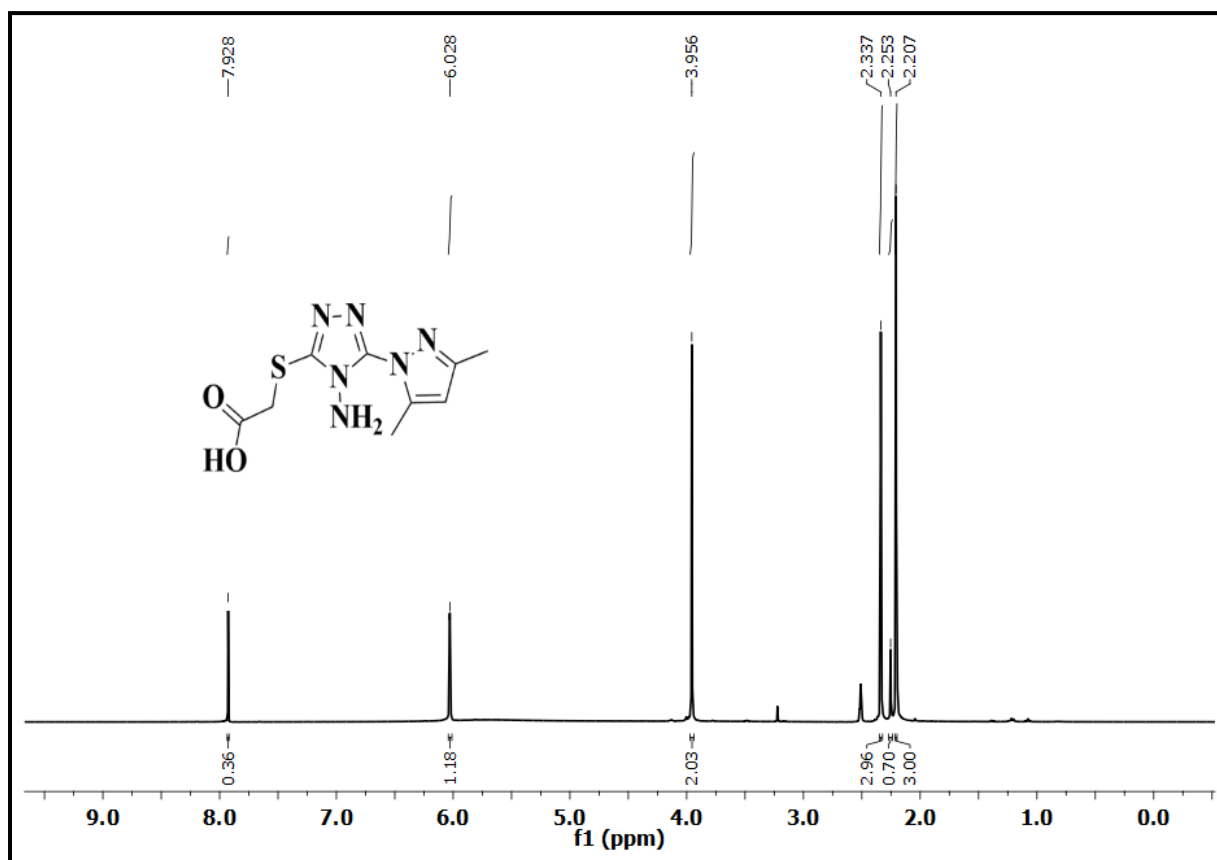


Fig: ¹H-NMR Spectrum of Compound 56f (400 MHz, DMSO-d₆)

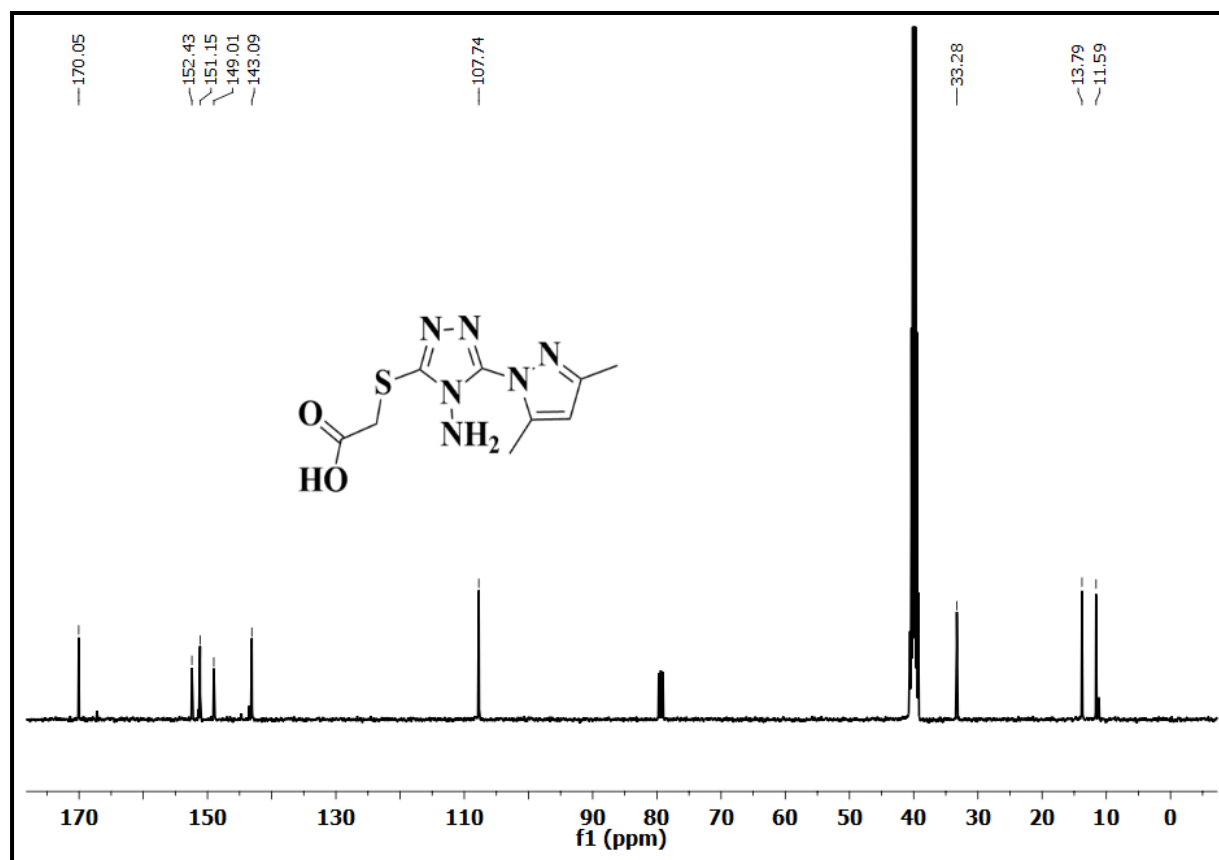


Fig: ¹³C-NMR Spectrum of Compound **56f** (100 MHz, CDCl₃+DMSO-*d*₆)

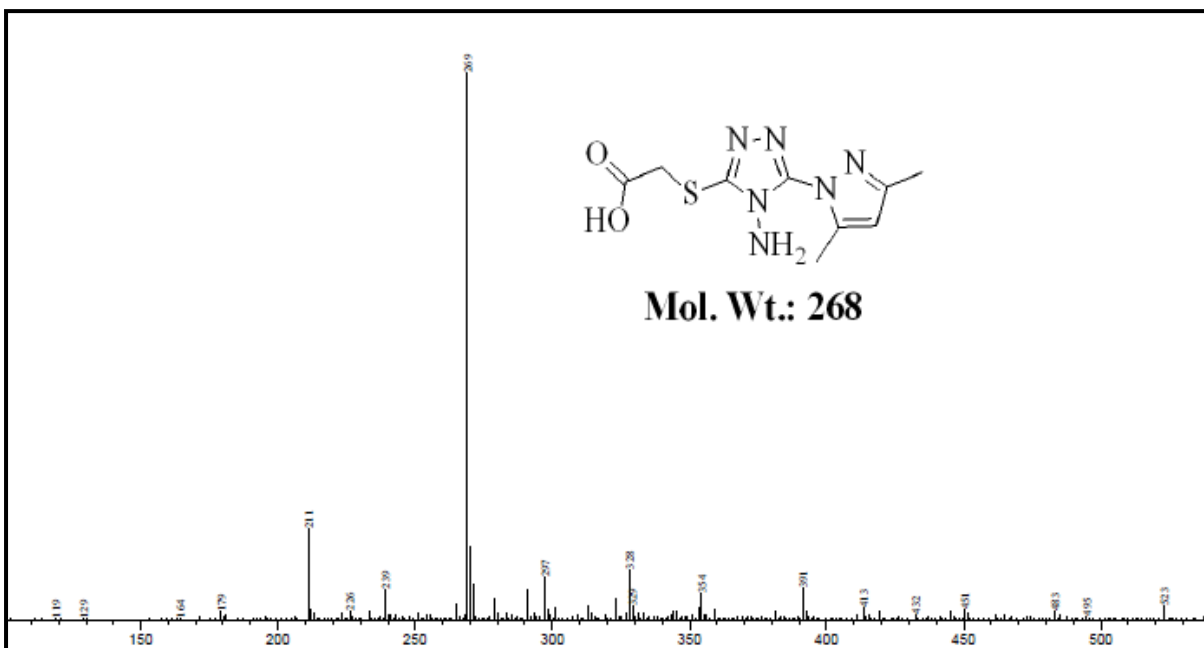


Fig: Mass Spectrum of Compound **56f**

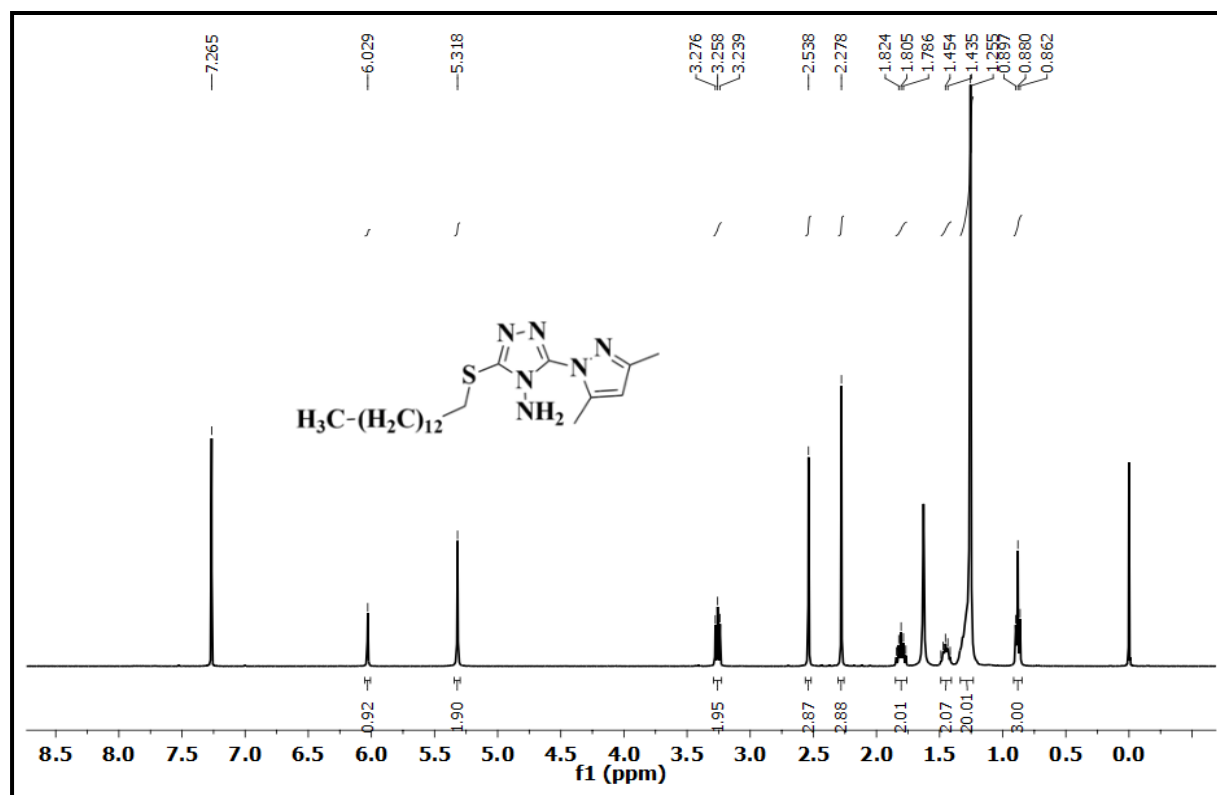


Fig: ¹H-NMR Spectrum of Compound 56g (400 MHz, CDCl₃)

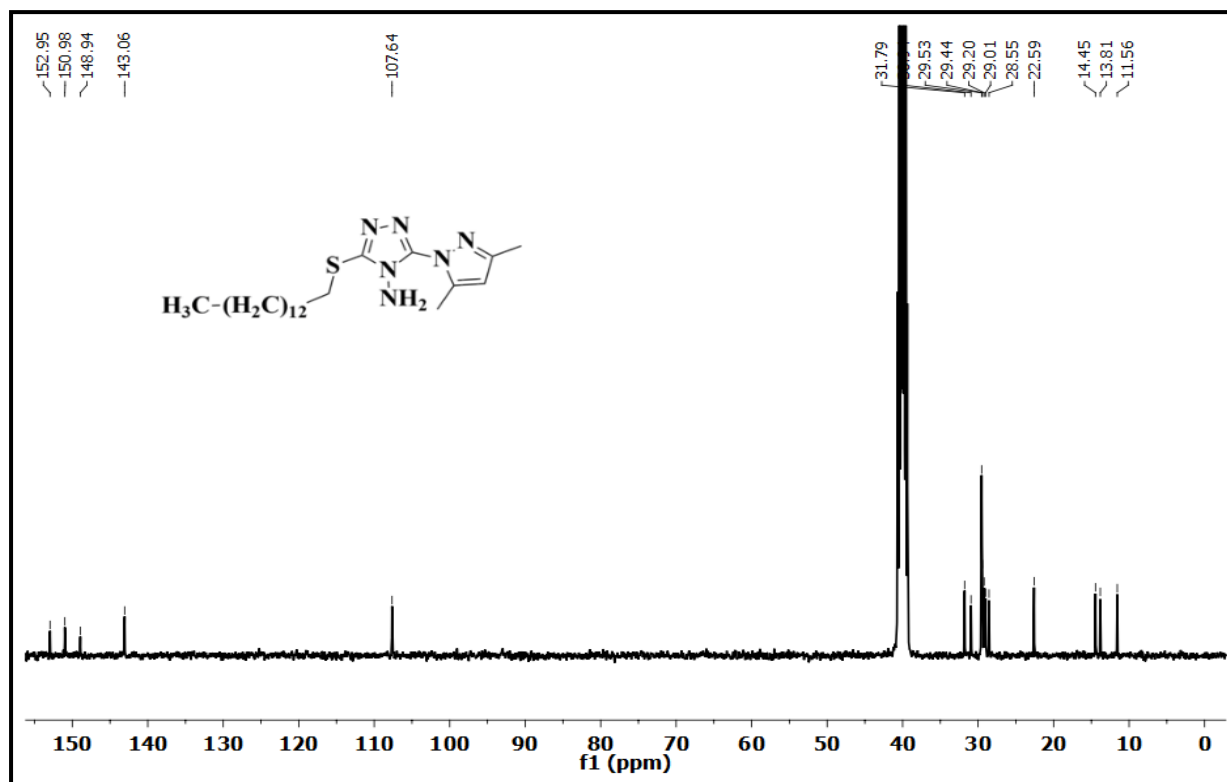


Fig: ¹³C-NMR Spectrum of Compound 56g (100 MHz, DMSO-*d*₆)

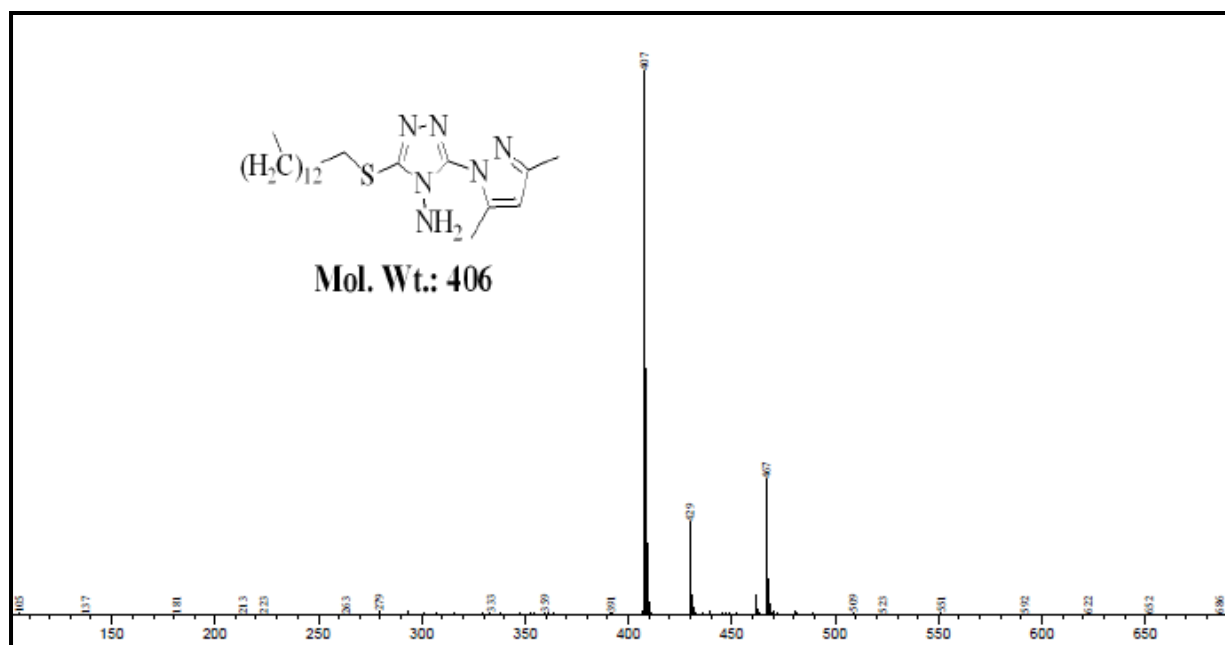


Fig: Mass Spectrum of Compound 56g

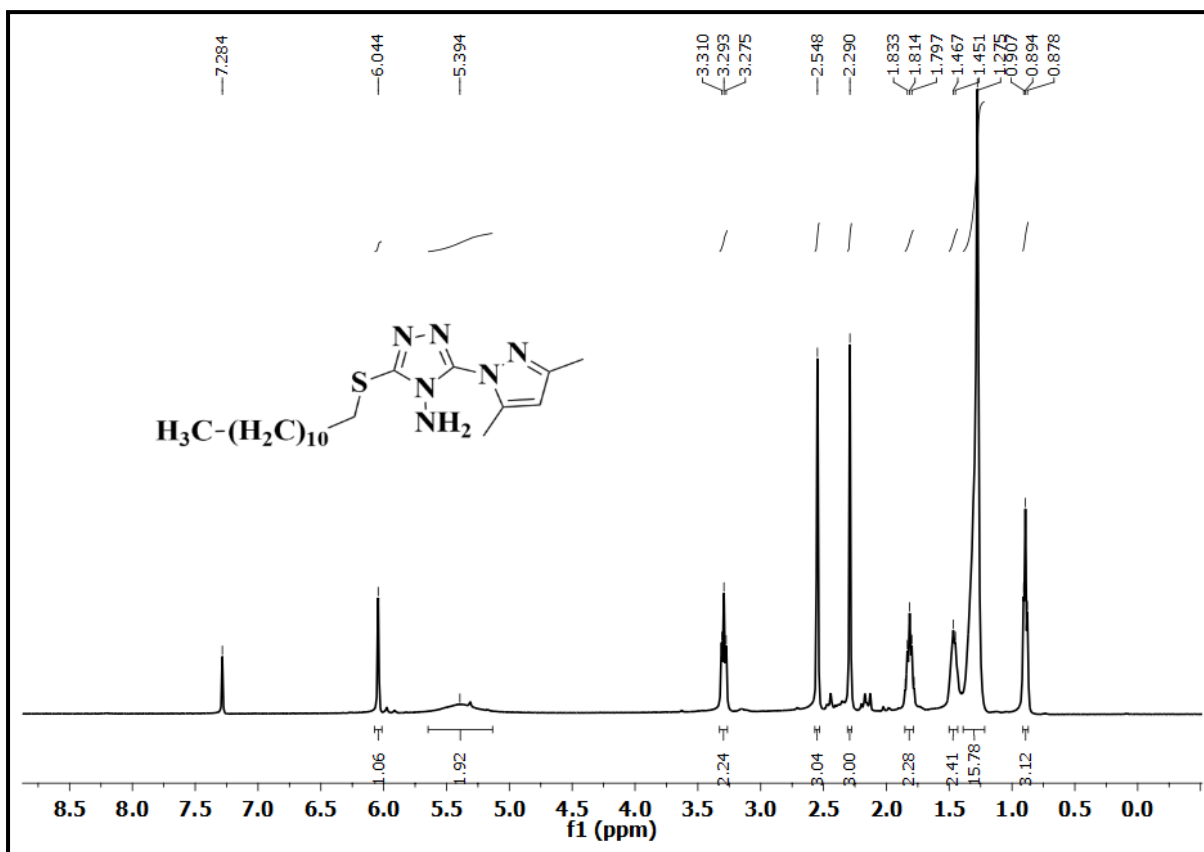


Fig: 1H -NMR Spectrum of Compound 56h (400 MHz, $CDCl_3$)

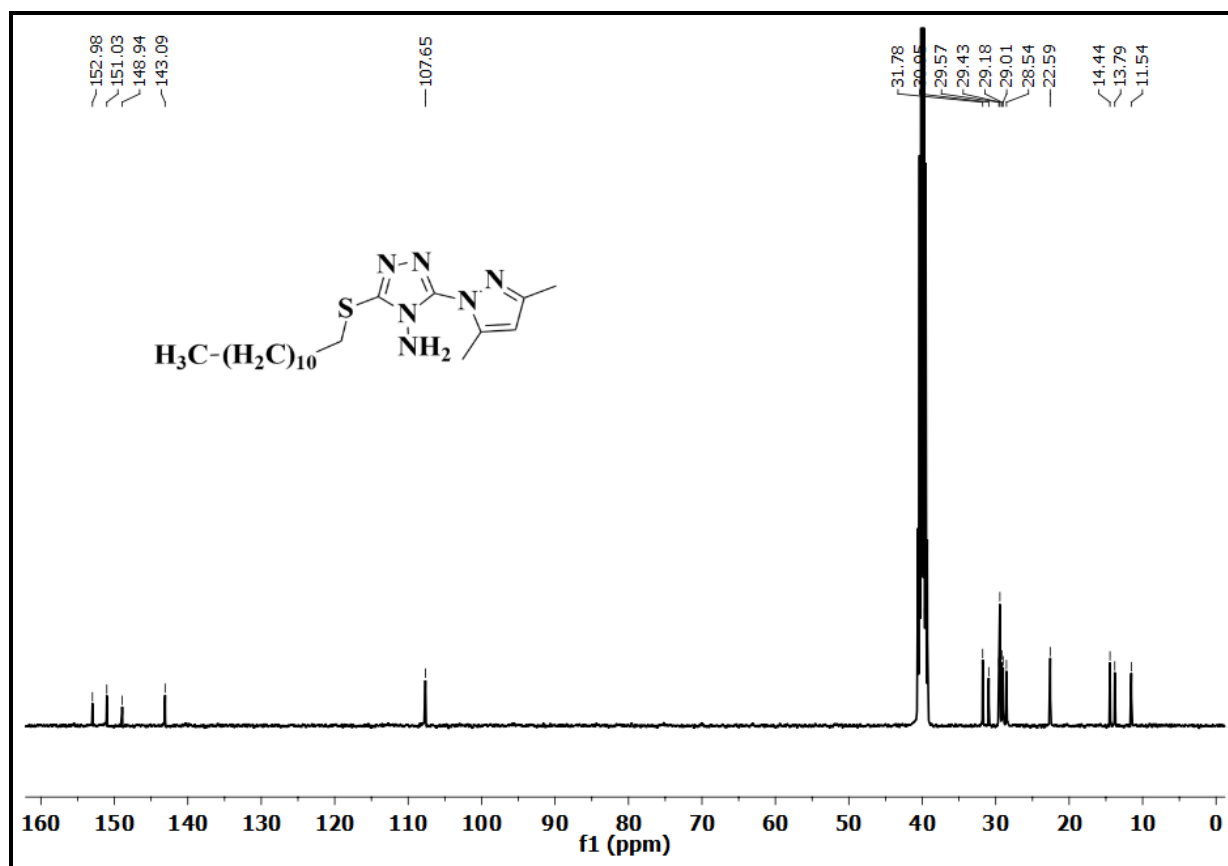


Fig: ^{13}C -NMR Spectrum of Compound **56h** (100 MHz, $\text{DMSO}-d_6$)

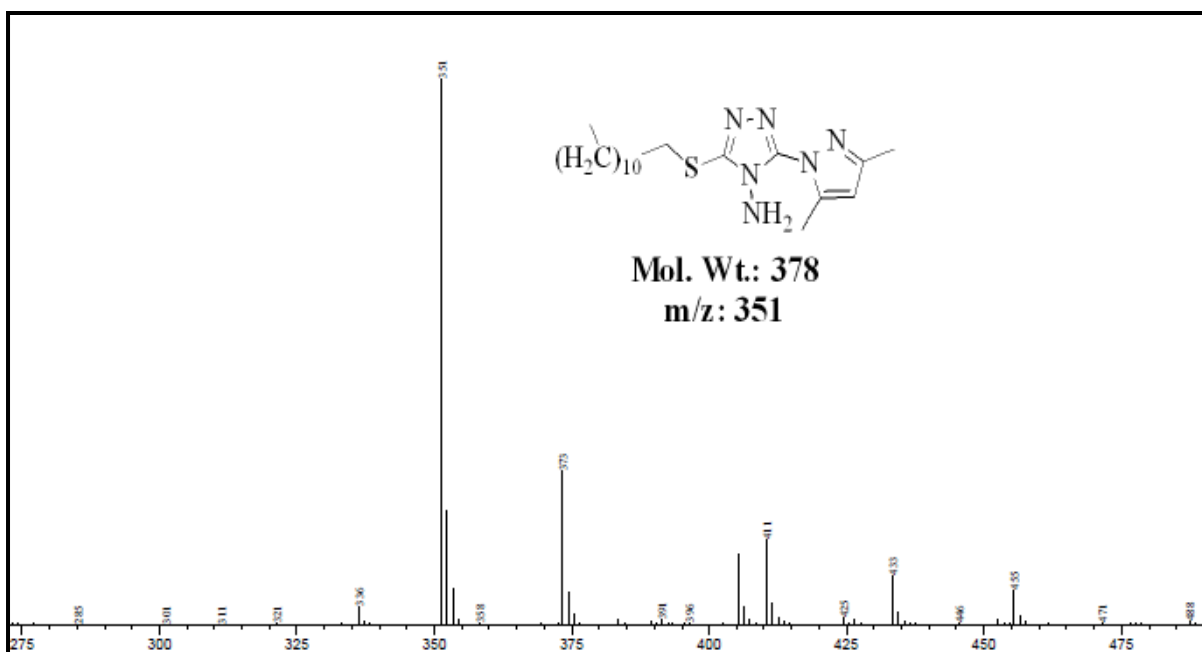


Fig: Mass Spectrum of Compound **56h**

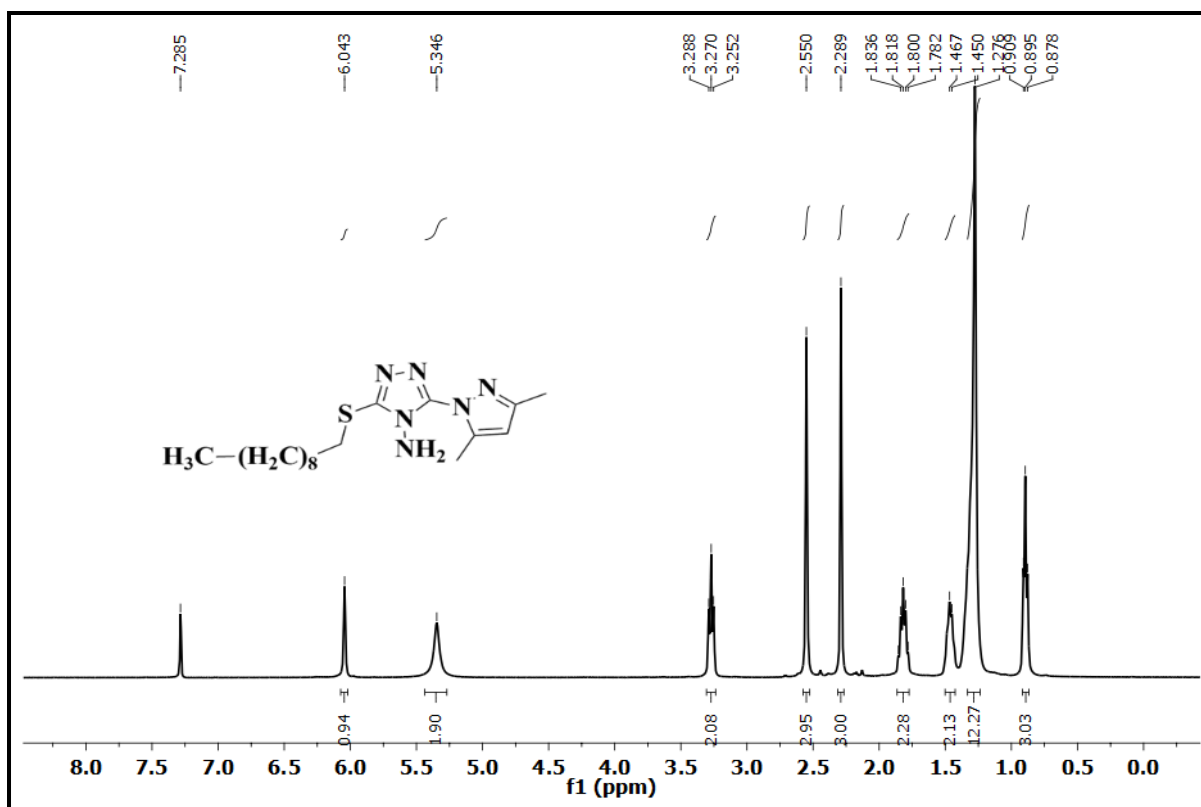


Fig: ¹H-NMR Spectrum of Compound **56i** (400 MHz, CDCl₃)

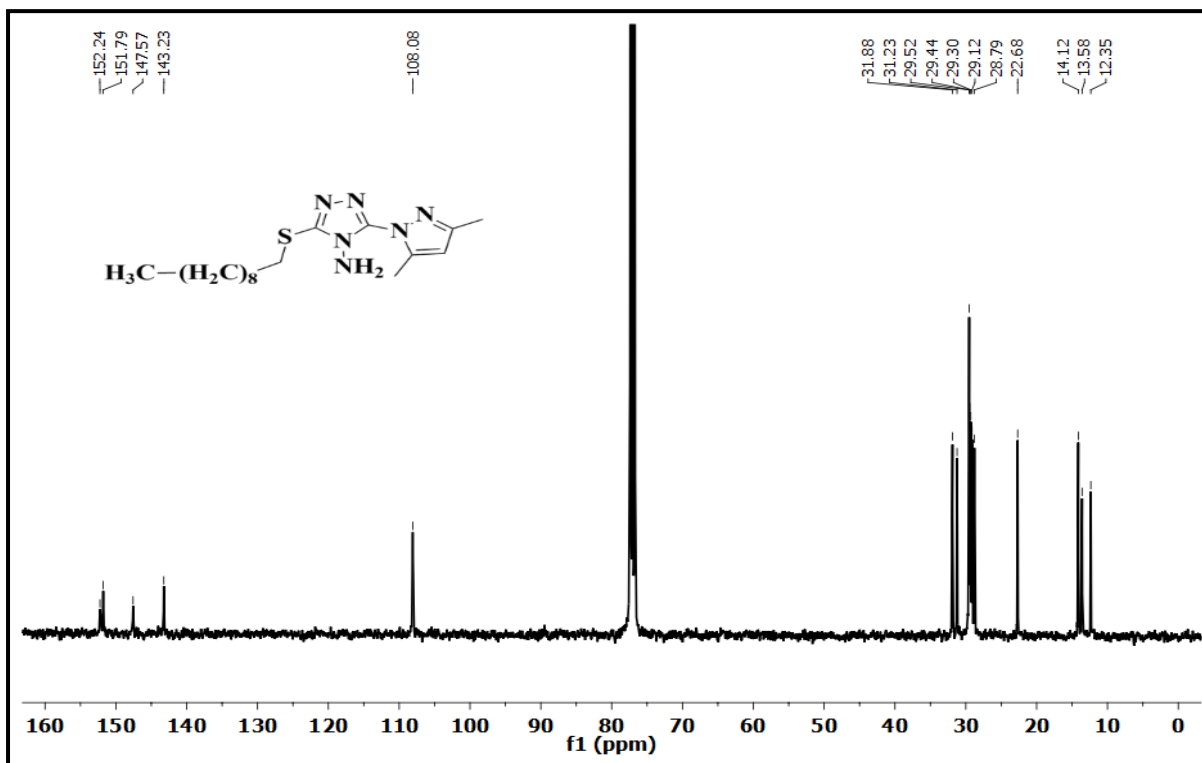


Fig: ¹³C-NMR Spectrum of Compound **56i** (100 MHz, CDCl₃)

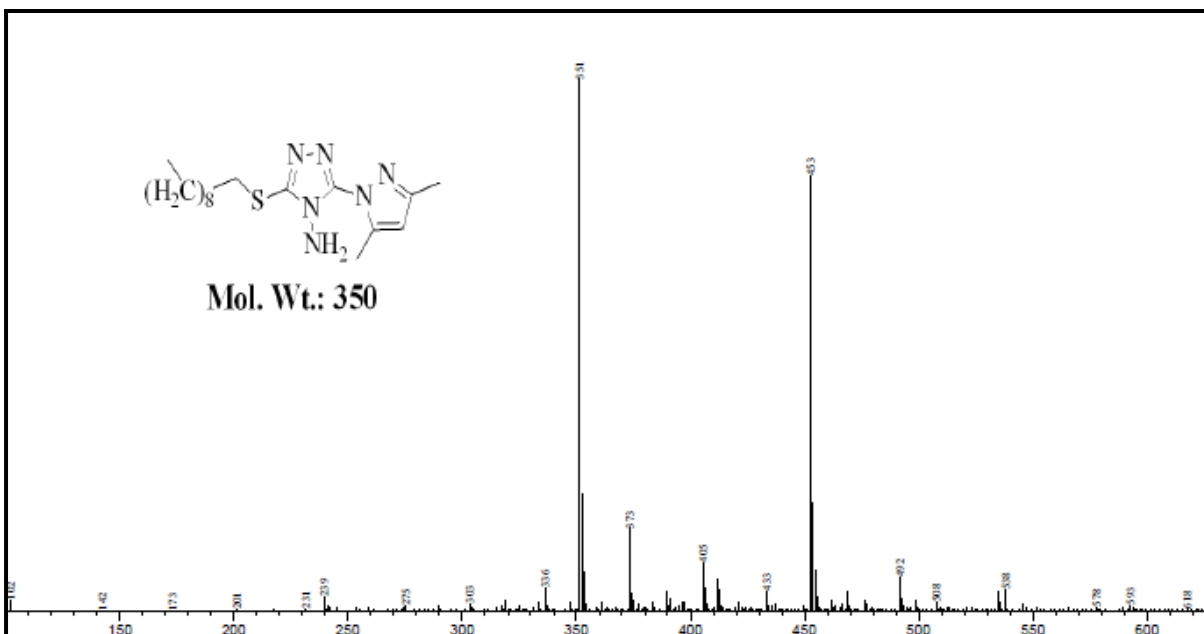


Fig: Mass Spectrum of Compound 56i

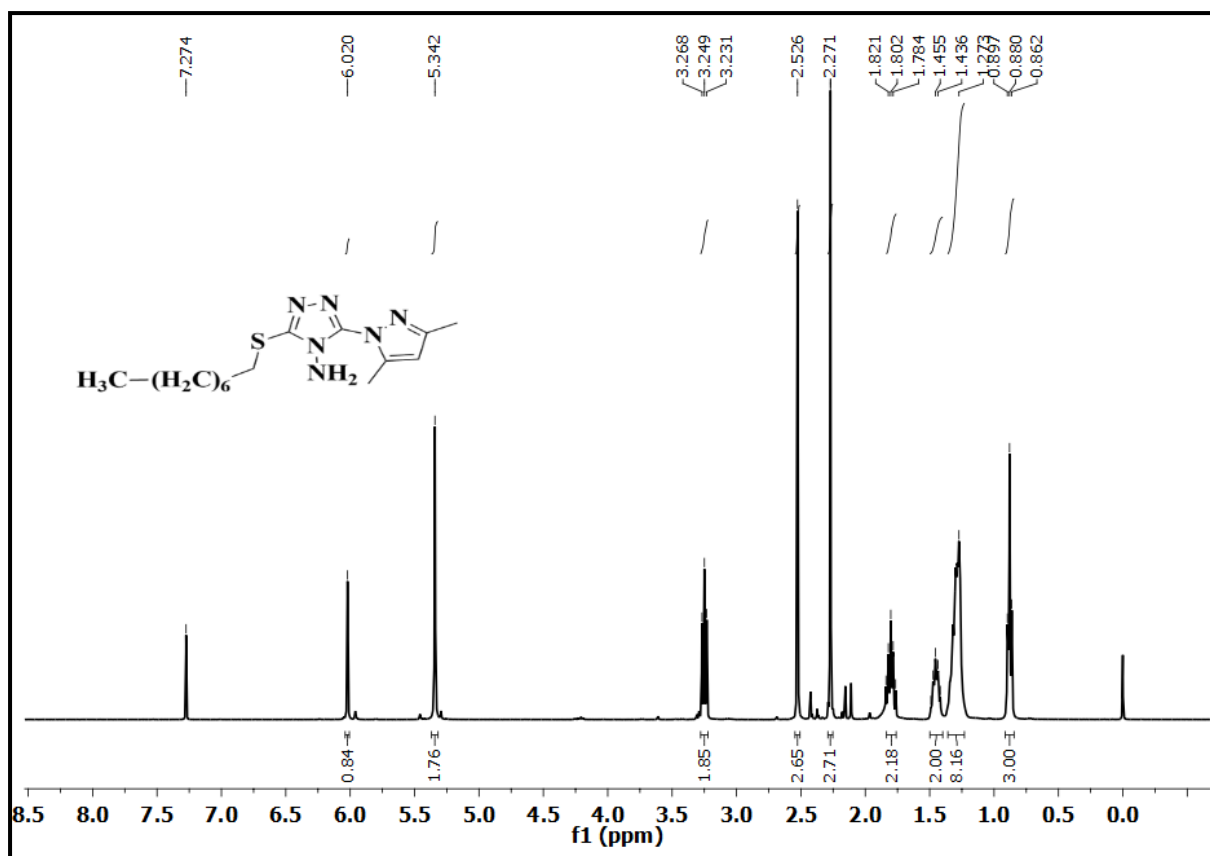


Fig: ¹H-NMR Spectrum of Compound 56j (400 MHz, CDCl₃)

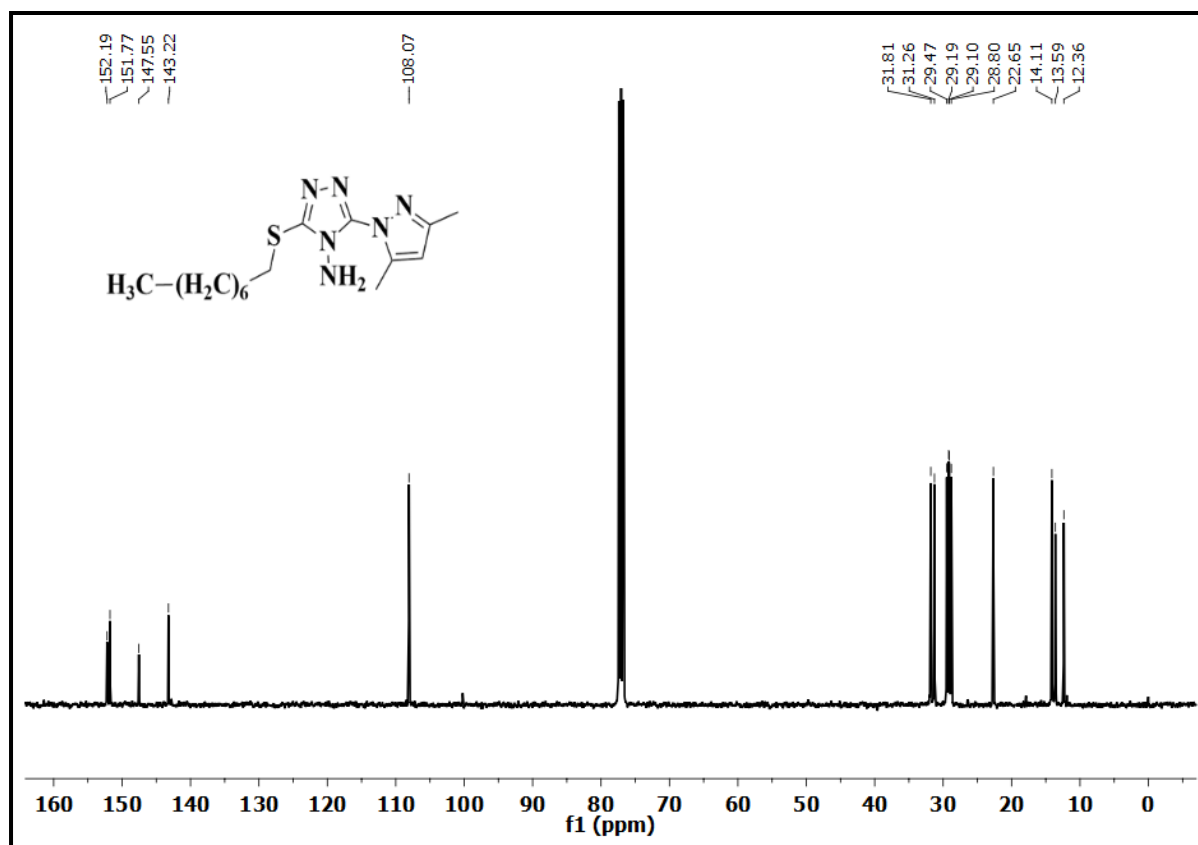


Fig: ¹³C-NMR Spectrum of Compound **56j** (100 MHz, CDCl₃)

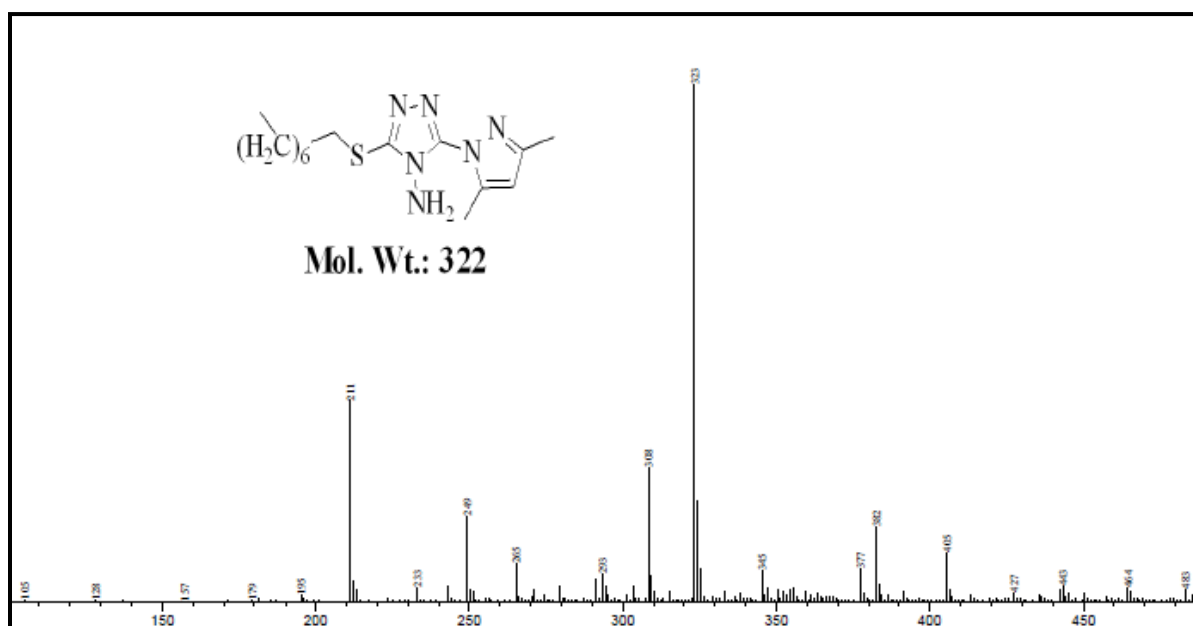


Fig: Mass Spectrum of Compound **56j**

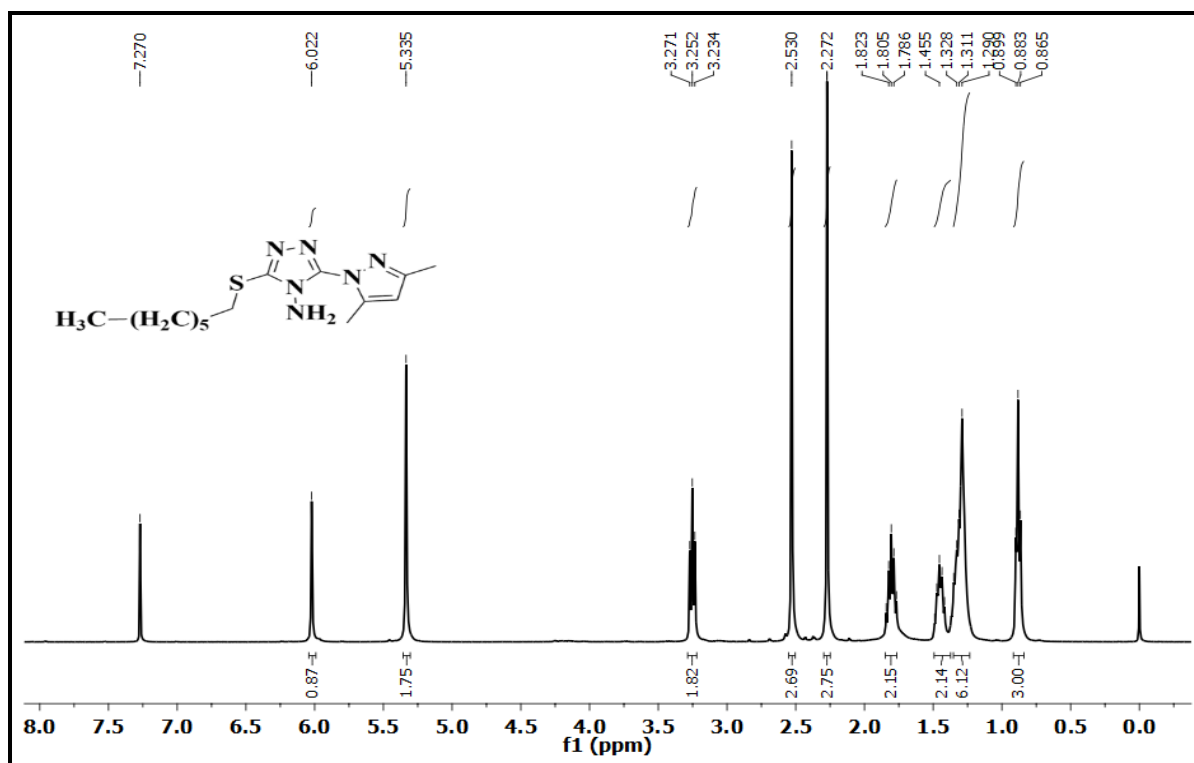


Fig: ¹H-NMR Spectrum of Compound **56k** (400 MHz, CDCl₃)

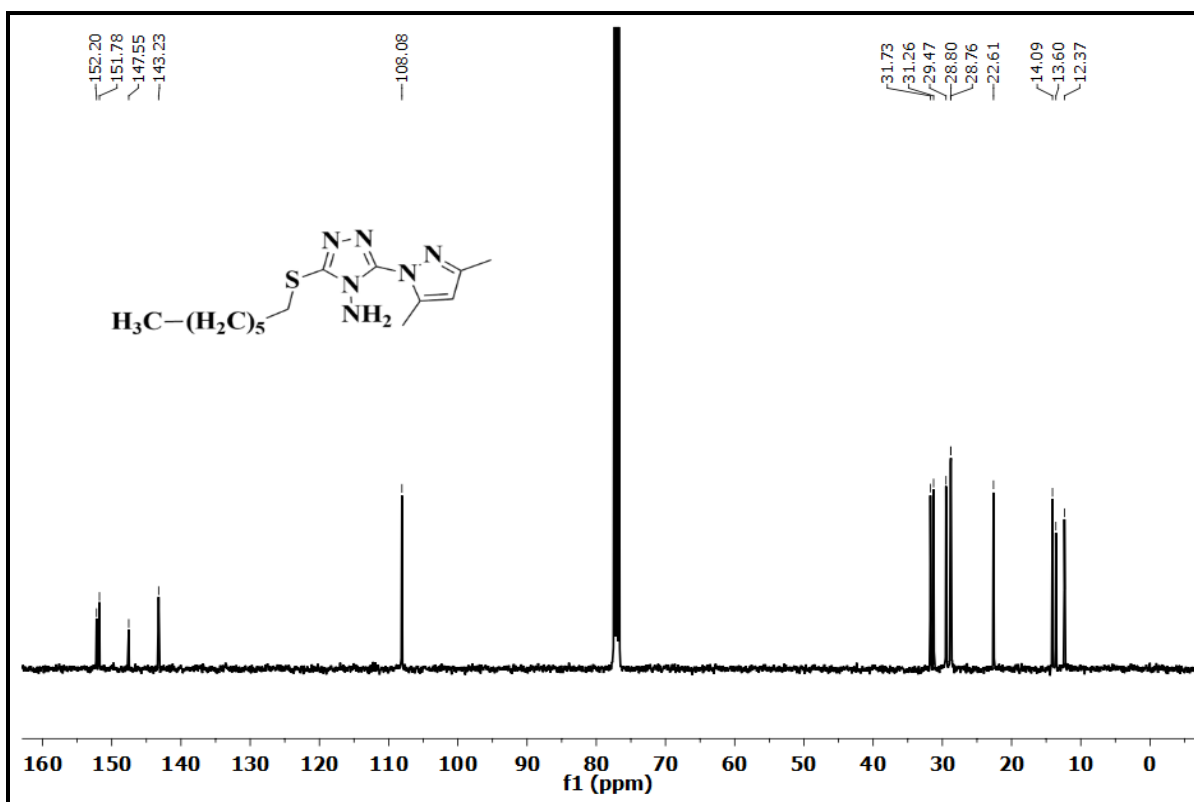


Fig: ¹³C-NMR Spectrum of Compound **56k** (100 MHz, CDCl₃)

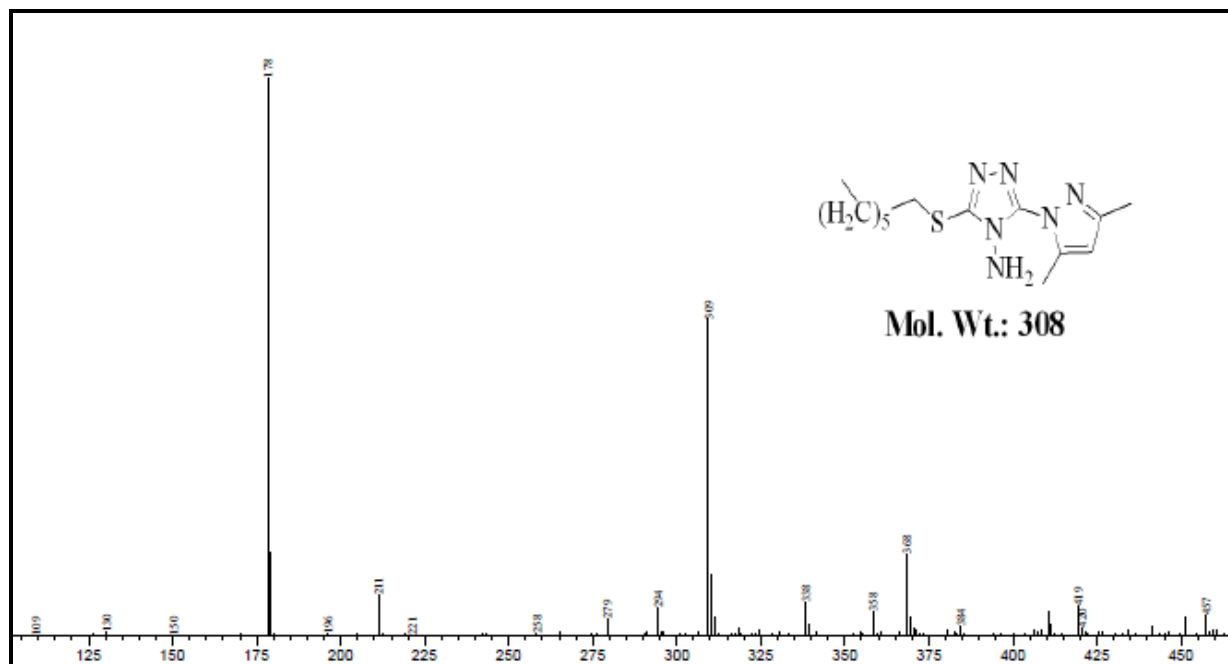


Fig: Mass Spectrum of Compound 56k

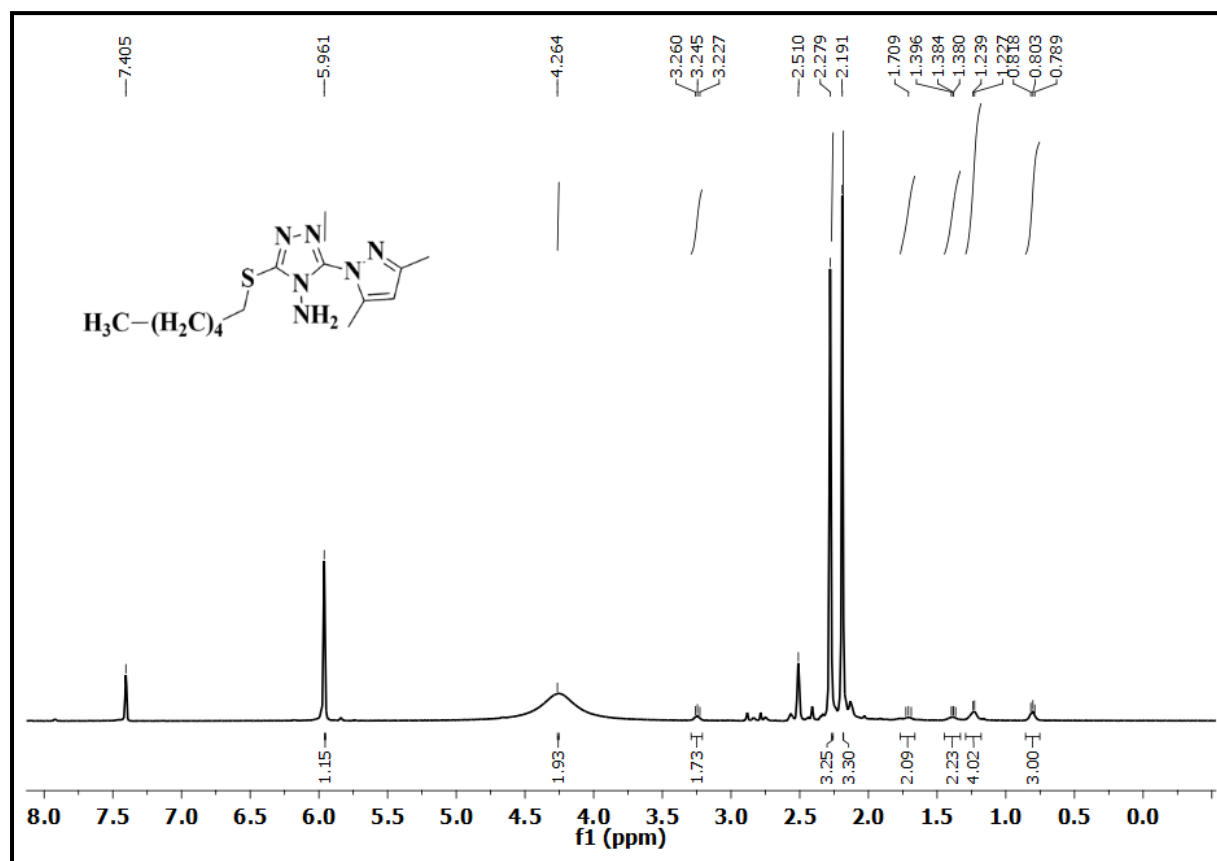


Fig: ¹H-NMR Spectrum of Compound 56l (400 MHz, CDCl₃+DMSO-d₆)

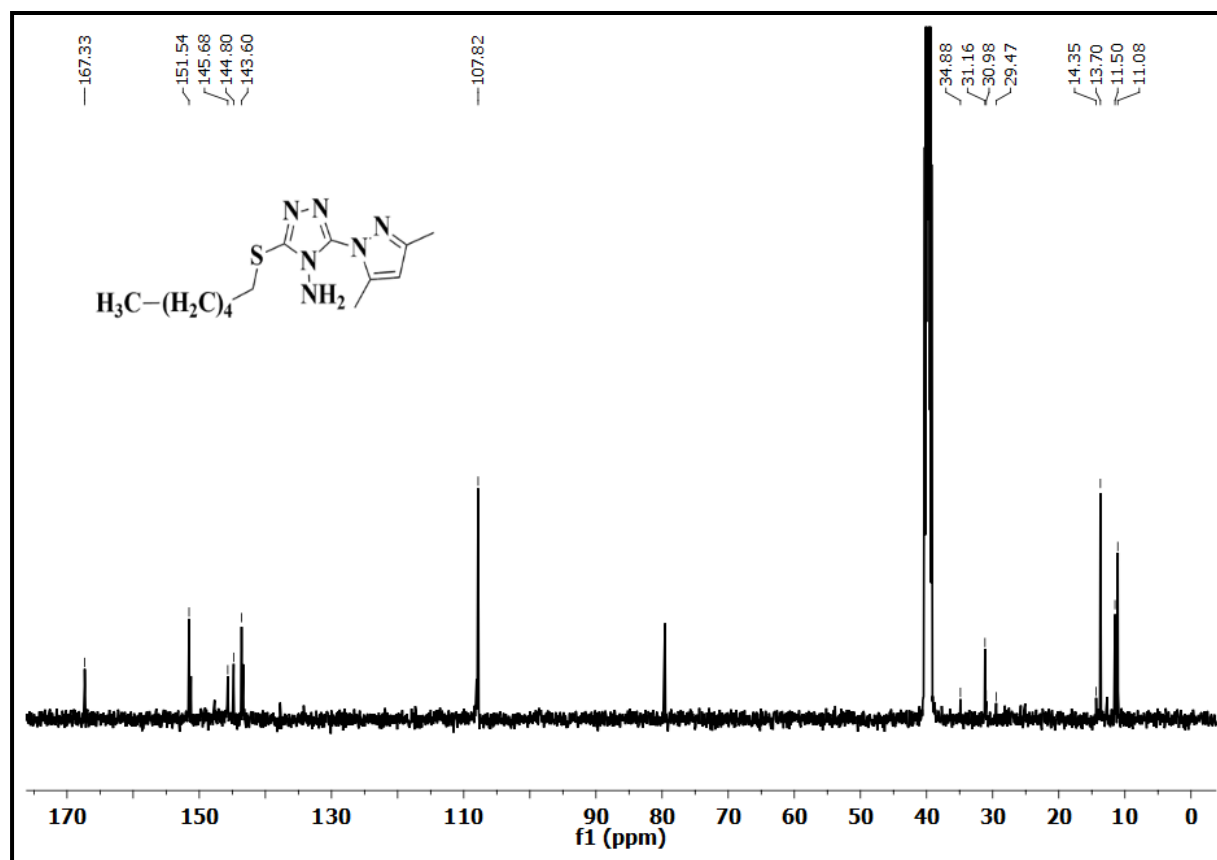


Fig: ¹³C-NMR Spectrum of Compound **56l** (100 MHz, CDCl₃+DMSO-*d*₆)

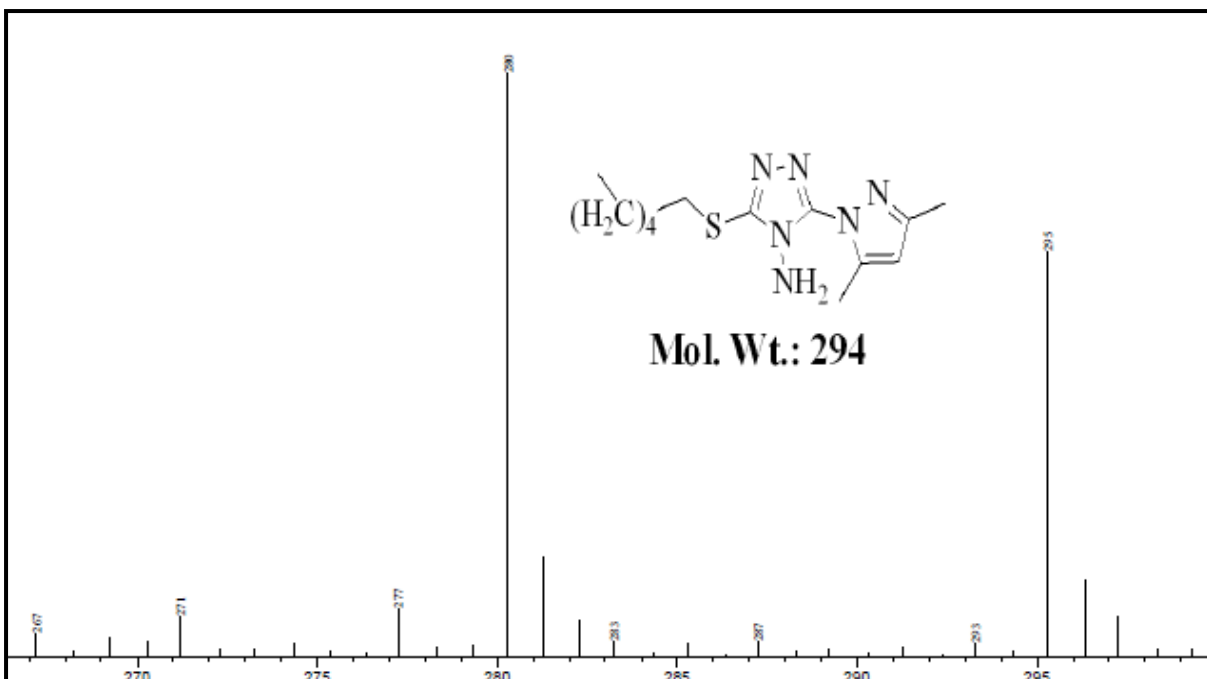


Fig: Mass Spectrum of Compound **56l**

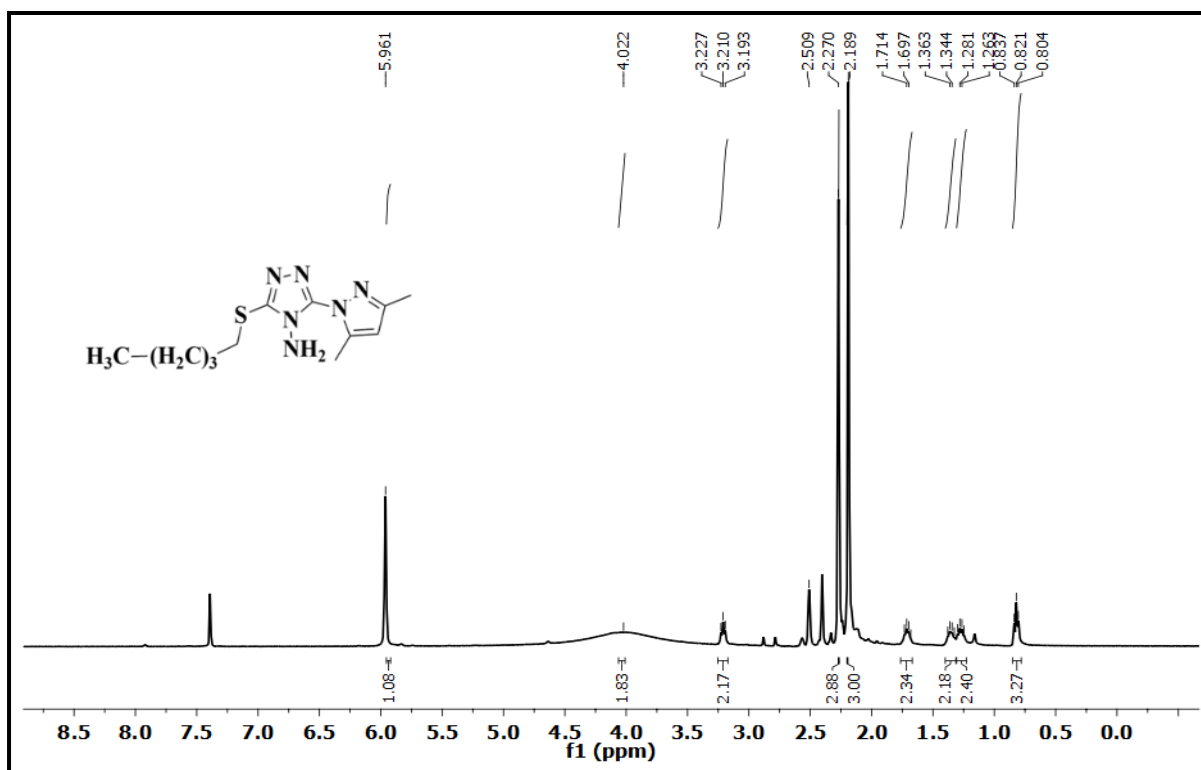


Fig: ¹H-NMR Spectrum of Compound **56m** (400 MHz, CDCl₃+DMSO-*d*₆)

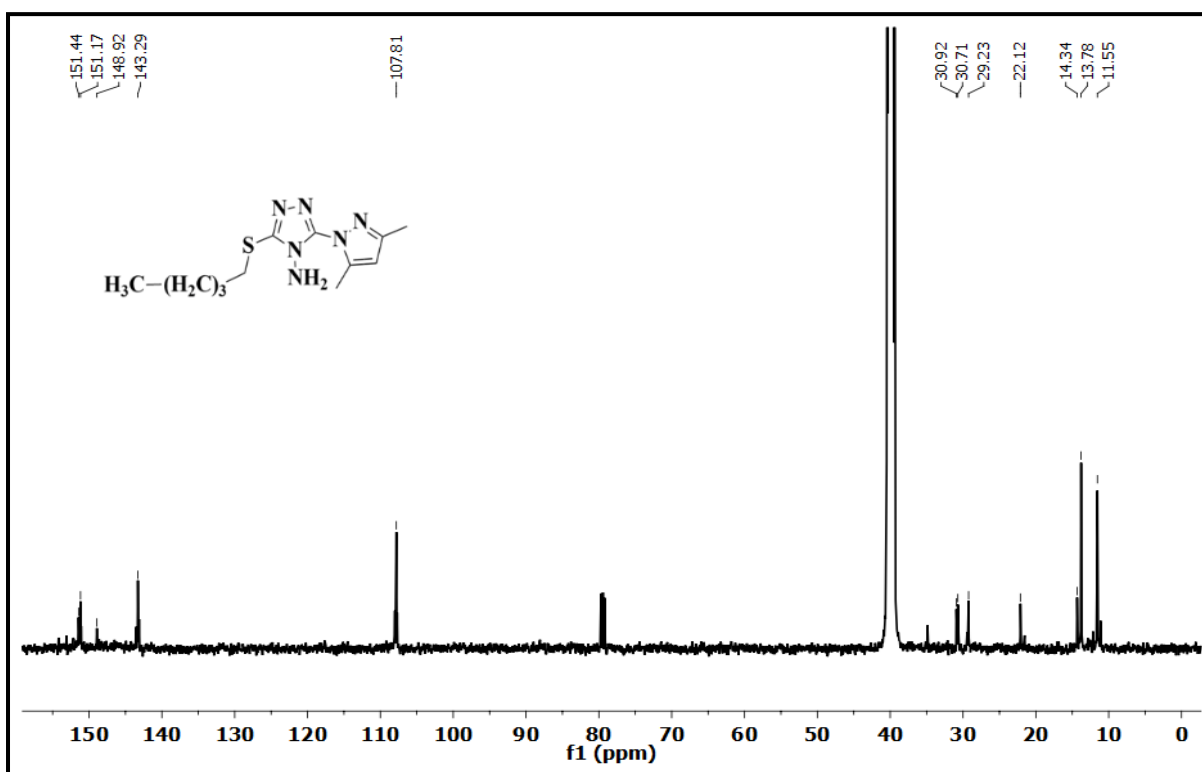


Fig: ¹³C-NMR Spectrum of Compound **56m** (100 MHz, CDCl₃+DMSO-*d*₆)

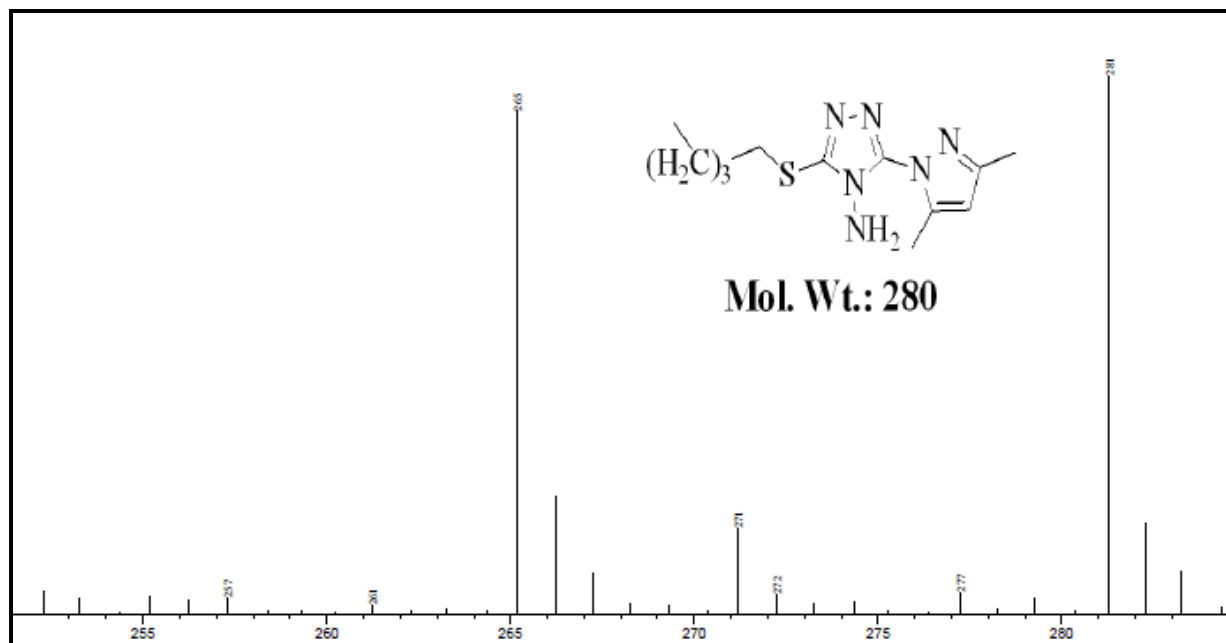
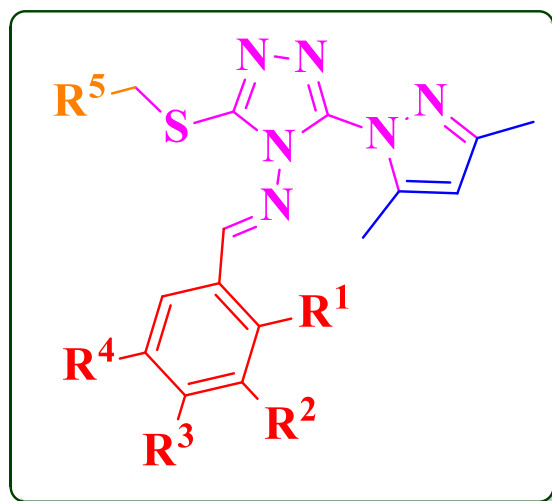


Fig: Mass Spectrum of Compound **56m**

CHAPTER-II

PART-C

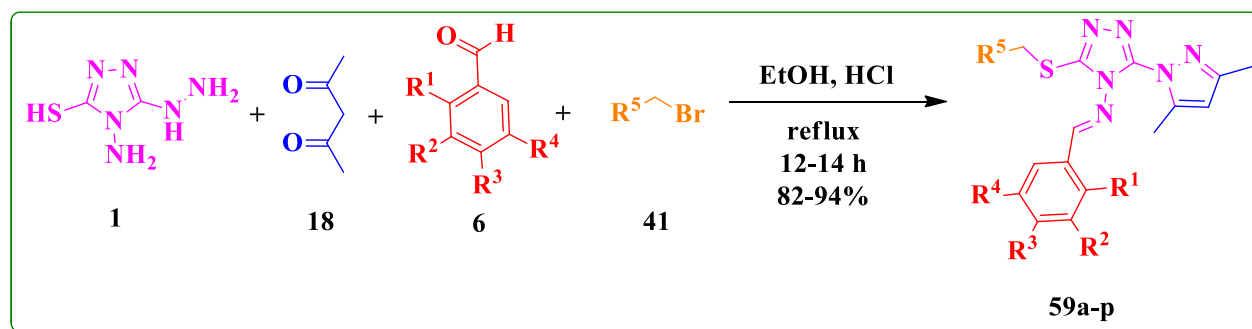
FOUR-COMPONENT, ONE-POT SYNTHESIS OF (*E*)-*N*-BENZYLIDENE-3-(BENZYLTHIO)-5-(3,5-DIMETHYL-1*H*-PYRAZOL-1-YL)-4*H*-1,2,4-TRIAZOL-4-AMINES AND THEIR DNA BINDING AND MOLECULAR DOCKING STUDIES



PART-CPRESENT WORK

The current part explains the synthesis of (*E*)-*N*-benzylidene-3-(benzylthio)-5-(3,5-dimethyl-1*H*-pyrazol-1-yl)-4*H*-1,2,4-triazol-4-amines *via* a one-pot four-component reaction, their DNA binding, and docking interactions.

In view of the synthetic, pharmaceutical, and biological importance of triazoles, pyrazoles, Schiff bases, and thioethers, we became interested to develop a novel one-pot four-component condensation strategy in producing triazole substituted pyrazole having Schiff base and thioether moiety in a single scaffold. The target compounds (**59a–p**) were synthesized by the reaction of 4-amino-5-hydrazinyl-4*H*-1,2,4-triazole-3-thiol (**1**) with pentane-2,4-dione (**18**), substituted aromatic aldehydes (**6**), and aralkyl/allyl/propargyl bromides (**41**) *via* a one-pot, four-component condensation as shown in the **Scheme-6**.



Scheme-6: Method 1, A one-pot, four-component synthesis of (*E*)-*N*-benzylidene-3-(benzylthio)-5-(3,5-dimethyl-1*H*-pyrazol-1-yl)-4*H*-1,2,4-triazol-4-amines (**59a–p**).

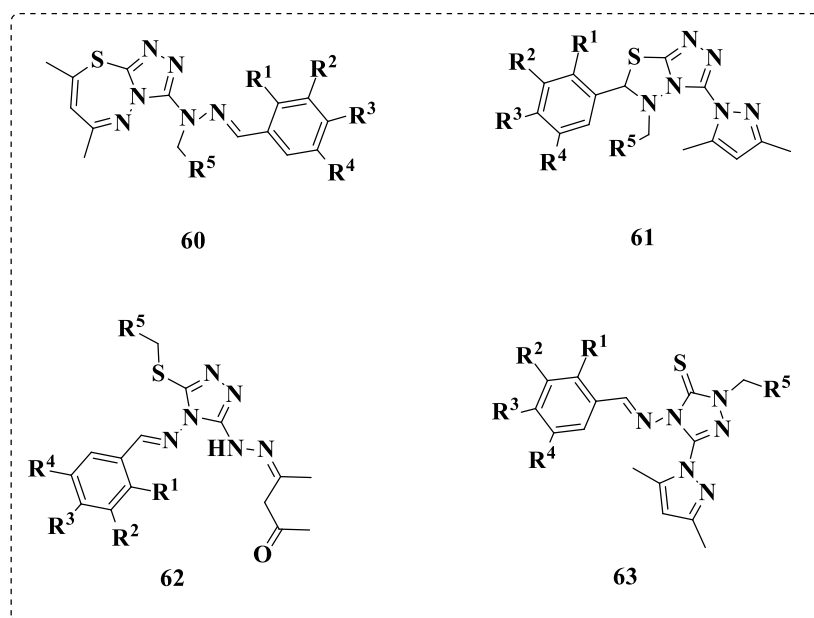
For the synthesis of title compounds, the reaction conditions were optimized. Initially, 4-amino-5-hydrazinyl-4*H*-1,2,4-triazole-3-thiol (**1**) was treated with pentane-2,4-dione (**18**), substituted aromatic aldehydes **6**, and aralkyl/propargyl/allyl bromides (**41**) in presence of solvent and also the catalytic amount of HCl under reflux conditions. Finally, the best reaction condition was EtOH (3 mL) and two drops of HCl under reflux. The yield of the product **59a** was 90% (entry 6, **Table-6**).

Table-6: Screening of reaction conditions for the synthesis of compound **59a**.

Entry	Solvent	Acid	Yield (%) of 59a
1	H ₂ O	HCl	0
2	DCM	HCl	10
3	MeOH	—	30
4	MeOH	HCl	45
5	EtOH	—	55
6	EtOH	HCl	90

***Reaction Conditions:** **1** (1mmol), **18** (1mmol), **6** (1mmol) and **41**(1mmol) in EtOH (3 mL), two drops of HCl under reflux.

When the reaction was operated between **1**, **18**, **6**, and **41** there is a possibility of the formation of a mixture of products such as **59**, **60**, **61**, **62**, **63**, or all of them depending on the reaction. But in the current investigation, we have been ended up with only one product **59** (By TLC, **Scheme-6**). The other possible products like **60**, **61**, **62**, and **63** can be denied based on spectral evidence (**Fig. 5**).

**Fig. 5:** Other possible products of reaction between **1**, **18**, **6** and **41**.

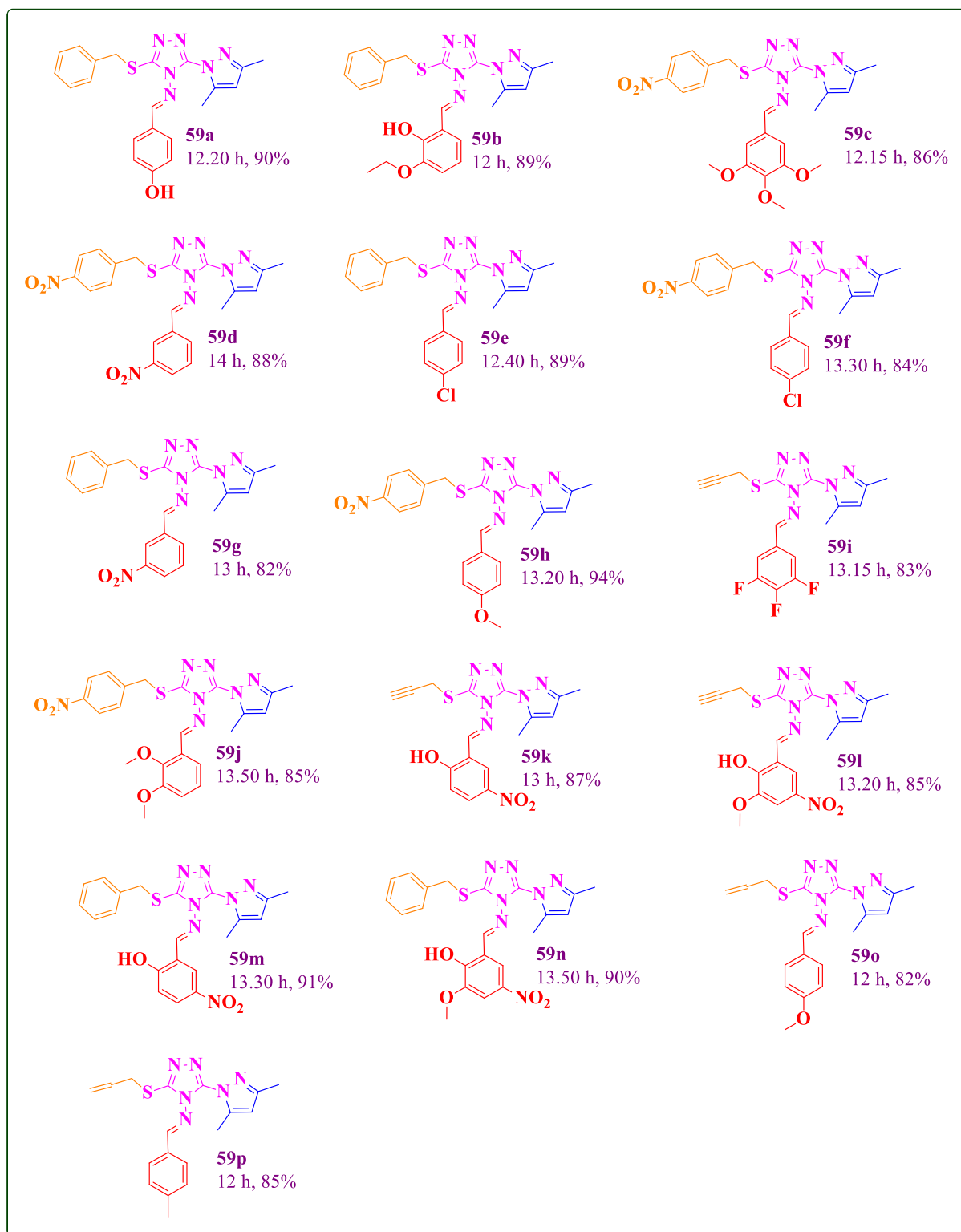
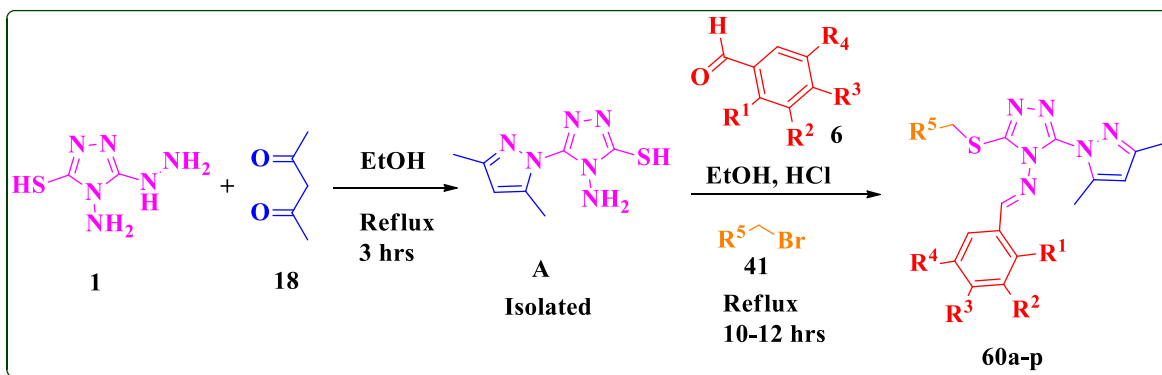


Fig. 6: Synthesis of *(E)*-*N*-benzylidene-3-(benzylthio)-5-(3,5-dimethyl-1*H*-pyrazol-1-yl)-4*H*-1,2,4-triazol-4-amines (59a-p). All reactions were carried out in 1 mmol scale.

These initial observations inspired us to extend this procedure to other substrates. Surprisingly, different aldehydes, allyl, propargyl, and benzyl halides participated in this reaction. The good reaction condition to synthesize target compounds **59a-p** was EtOH (3mL), two drops of HCl under reflux. Further, the time taken for the synthesis of **59a-p** was 12-14 h, with yields ranging from 82-94% after recrystallization as shown in **Fig. 6**.

In another way also the compounds **59a-p** can be synthesized by a three-component condensation process. In this method first we have reacted **1** with acetylacetone to give 4-amino-5-(3,5-dimethyl-1*H*-pyrazol-1-yl)-4*H*-1,2,4-triazole-3-thiol (**A**) (**Fig. 7**). Then followed by reaction with aromatic aldehydes and aralkyl/propargyl/allyl halides in ethanol and a few drops of conc. HCl. In this reaction, one N=C and one C-S bond are formed synchronously (**Scheme-7**). Compared to one-pot four-component condensation this three-component reaction yields are less (65%) and hence we had carried out four-component synthesis (**Scheme-6, Method-1**).



Scheme-7: Method 2, Alternative three-component synthesis of compounds **59a-p** by isolating **A**.

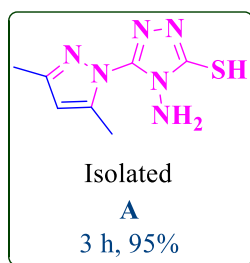
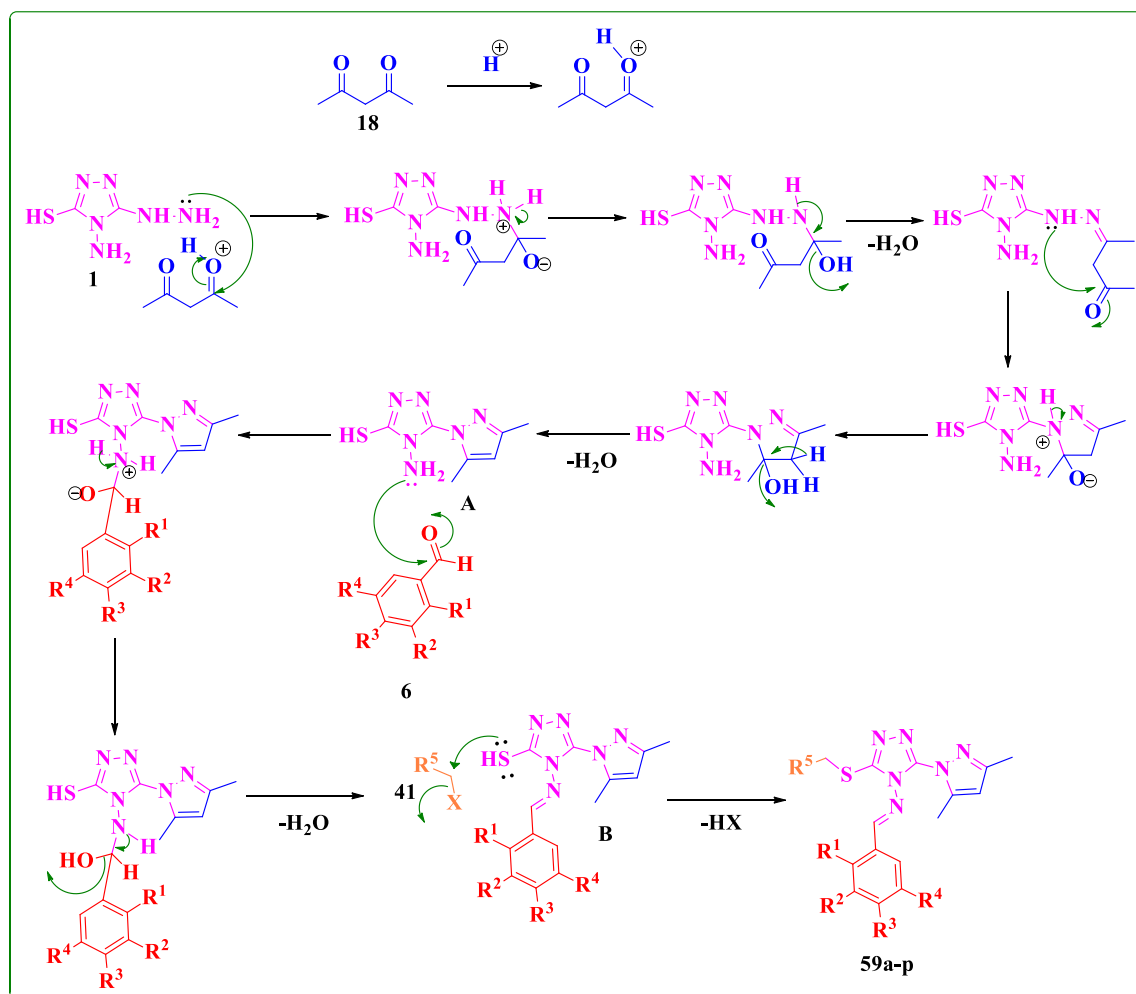


Fig. 7: Isolated intermediate 4-amino-5-(3,5-dimethyl-1*H*-pyrazol-1-yl)-4*H*-1,2,4-triazole-3-thiol (**A**)

Mechanistically the reaction is believed to proceed first through the initial formation of dimethyl pyrazole intermediate **A** by the attack of --NH--NH_2 group of 4-amino-5-hydrazinyl-4*H*-1,2,4-triazole-3-thiol (**1**) on pentane-2,4-dione (**18**). This is further confirmed by the isolation of intermediate **A** and establishing its structure by spectra. Later on the N-amino group of reactive intermediate **A** reacts with various aromatic aldehydes **6** to give second intermediate Schiff's bases **B**. Finally, the added aralkyl/allyl/propargyl bromide (**41**) reacts with the thiol group of in-situ formed **B** to yield final products as presented in **Scheme-8**. The uniqueness of this four-component reaction is that at a time many bonds like two C=N , one C=N , and one C-S are formed contemporaneously. Another interesting observation of the reaction is that there is selective heterocyclization and selective S-alkylation over N-alkylation. The observed selective S-alkylation was due to the high nucleophilicity of S-H over the N-H group of **B** (**Scheme-8**).



Scheme-8: Plausible reaction pathway for **59a-p**.

The structures of the title compounds were confirmed by their analytical and spectral data. In the FT-IR spectrum, compound **59a** displayed peaks at 3410 cm^{-1} ($-\text{OH}$), 2922 cm^{-1} ($-\text{CH}_2-$), and 1600 cm^{-1} ($-\text{C}=\text{N}-$). The ^1H -NMR ($\text{CDCl}_3+\text{DMSO}-d_6$) of the compound **59a** showed two characteristic singlets at δ 2.22 and 2.23 for the two $-\text{CH}_3$ groups of pyrazole, one singlet at δ 4.50 for $-\text{S}-\text{CH}_2-$. The pyrazole proton appeared as a singlet at δ 6.03. The $-\text{OH}$ and azomethine protons appeared as a singlet at δ 7.86 and δ 9.91 respectively. The remaining aromatic protons appeared at δ 6.87 - 7.49. The ^{13}C -NMR ($\text{CDCl}_3+\text{DMSO}-d_6$) spectrum of the compound **59a** displayed peaks at δ 13.9 and δ 18.5 for the two methyl carbons of pyrazole. The $-\text{S}-\text{CH}_2-$ carbon appeared at δ 40.9 and pyrazole ring carbon appeared at δ 112.5. The peak at δ 154.8 is due to azomethine ($-\text{CH}=\text{N}-$) carbon. In the mass spectrum compound **59a** gave a peak at m/z 405 due to $[\text{M}+\text{H}]^+$.

Isolated intermediate 4-amino-5-(3,5-dimethyl-1*H*-pyrazol-1-yl)-4*H*-1,2,4-triazole-3-thiol (**A**) structure was confirmed by its analytical and spectral analysis. Intermediate **A** showed peaks at 3370 and 2740 for $-\text{NH}_2$ and $-\text{SH}$ respectively in the FT-IR spectrum. In the ^1H -NMR ($\text{CDCl}_3+\text{DMSO}-d_6$) spectrum compound **A** displayed a singlet for pyrazole proton peak at δ 5.99. The amino ($-\text{NH}_2$) and thiol ($-\text{SH}$) group protons of compound **A** showed as two singlets at δ 5.3 and 13.79 respectively. The ^{13}C -NMR ($\text{CDCl}_3+\text{DMSO}-d_6$) spectrum of intermediate **A** showed a peak at δ 108.1 is due to the pyrazole carbon. In the mass spectrum (ESI) the intermediate **A** gave a peak at m/z 211($\text{M}+\text{H}$).

DNA Binding Studies:

The UV absorption spectral analysis was carried out to recognize the DNA binding properties of the newly synthesized compounds (**59a-p**). The compounds with $32\mu\text{M}$ constant concentration were used for titration against the different concentrations of $10\mu\text{L}$ CT-DNA ($0-9\mu\text{M}$) was used for the UV absorption experimental study. The Emission spectral or fluorescence spectral studies were done with the CT-DNA intercalation Ethidium bromide (EB). The fluorescence spectra of new compounds were recorded with the titration of CT-DN, Ethidium bromide at constant concentrations of $10\mu\text{M}$, $12\mu\text{M}$ against different concentrations of compounds ($10\mu\text{L}$) ranges from $0-50\mu\text{M}$.

In the present investigation the absorption spectra of compounds **59b**, **59e**, **59g**, **59h**, and **59j** shown single broadband at 291, 280, 292, 305, and 298 nm respectively and **59n** having two bands at 352 nm and 420 nm. All the compounds exhibited hypochromism (decrease intensity) at their respective bands with increasing concentration of CT-DNA without blue and red color shift at the absorption maxima (**Fig. 8**). Hence, there may be an intercalative mode of binding present between compounds and CT-DNA. Calculation of intrinsic binding constant K_b from the ratio of the slope to y intercept in plots of $[DNA]/[\epsilon_a - \epsilon_f]$ versus $[DNA]^{89}$ gave the binding strengths for the compounds **59b**, **59e**, **59g**, **59h**, **59j** and **59n** with CT-DNA (**Table-7**).

Table-7: Intrinsic Binding constant (K_b) for the **59b**, **59e**, **59g**, **59h**, **59j**, and **59n** with CT-DNA

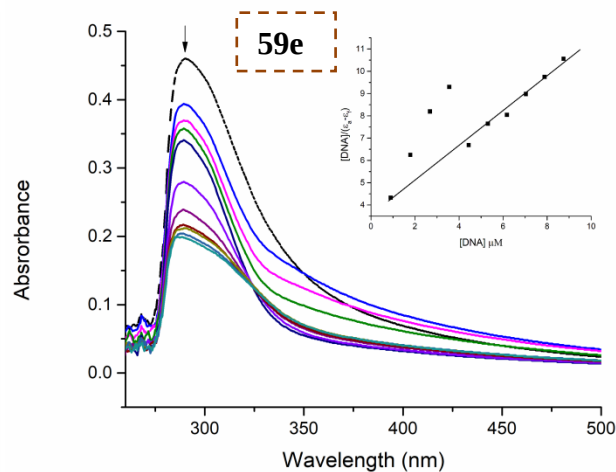
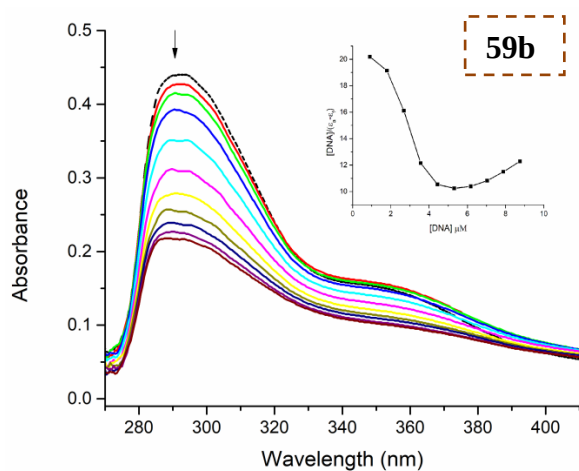
Compound	K_b
59b	$0.598 \times 10^5 M^{-1}$
59e	$1.08 \times 10^5 M^{-1}$
59g	$2.20 \times 10^5 M^{-1}$
59h	$1.51 \times 10^5 M^{-1}$
59j	$0.75 \times 10^5 M^{-1}$
59n	$1.33 \times 10^5 M^{-1}$

The emission spectral studies revealed more information for the binding nature of the newly synthesized compounds with CT-DNA. When the compounds **59b**, **59e**, **59g**, **59h**, **59j**, and **59n** were titrated against EB bounded CT-DNA, the concentration of EB and CT-DNA was constant and the concentrations of the compounds were increased then the emission bands of the compounds were recorded at 591 nm (**Fig. 9**). While increasing the concentrations of the compounds the intensity of the bands decreased which is hypochromism. However, further the plot I_0/I Vs $[Q]$ was used to calculate the Stern-Volmer quenching constant K_{sv} . K_{app} is apparent DNA binding constant calculated using $K_{EB}[EB] = K_{app}[\text{Compound}]$. Both constants K_{sv} and K_{app}

values were shown in **Table-8**. Hence, the above two investigations proposed that the compounds bind with the CT-DNA through the intercalative mode of binding⁹⁰⁻⁹².

Table-8: Stern–Volmer quenching constant K_{sv} and apparent DNA binding constant K_{app} .

Compound	K_{sv}	K_{app}
59b	$3.59 \times 10^3 \text{ M}^{-1}$	$4.36 \times 10^5 \text{ M}^{-1}$
59e	$6.19 \times 10^3 \text{ M}^{-1}$	$7.54 \times 10^5 \text{ M}^{-1}$
59g	$5.16 \times 10^3 \text{ M}^{-1}$	$6.33 \times 10^5 \text{ M}^{-1}$
59h	$6.19 \times 10^3 \text{ M}^{-1}$	$7.53 \times 10^5 \text{ M}^{-1}$
59j	$7.37 \times 10^3 \text{ M}^{-1}$	$8.90 \times 10^5 \text{ M}^{-1}$
59n	$8.23 \times 10^3 \text{ M}^{-1}$	$9.98 \times 10^5 \text{ M}^{-1}$



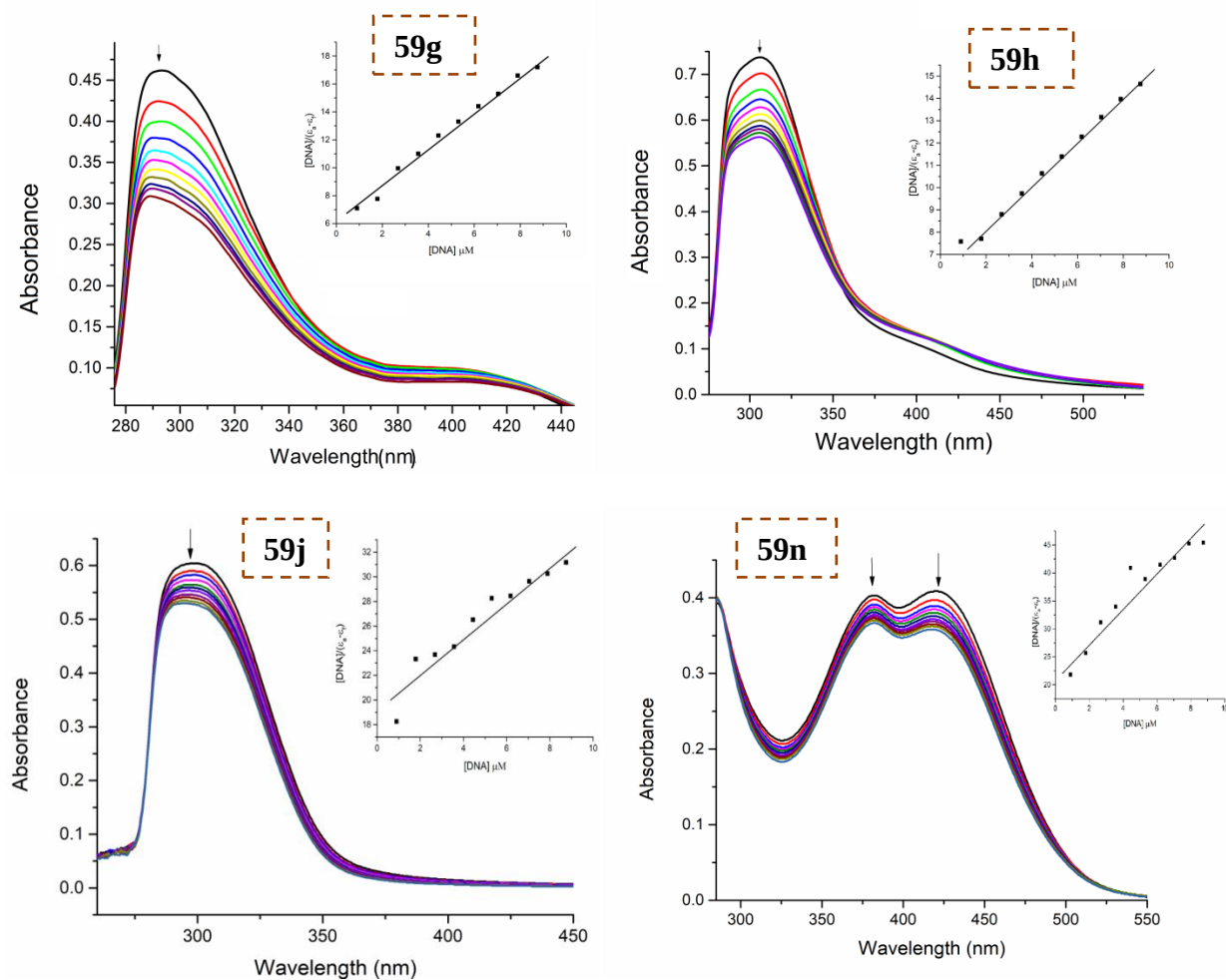
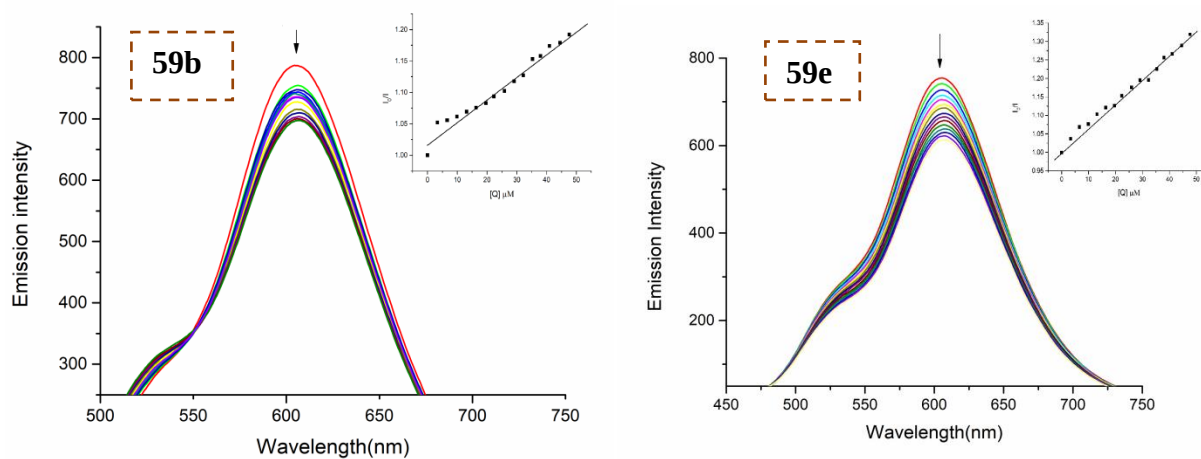


Fig. 8: Absorption spectra of compounds 59b, 59e, 59g, 59h, 59j, and 59n



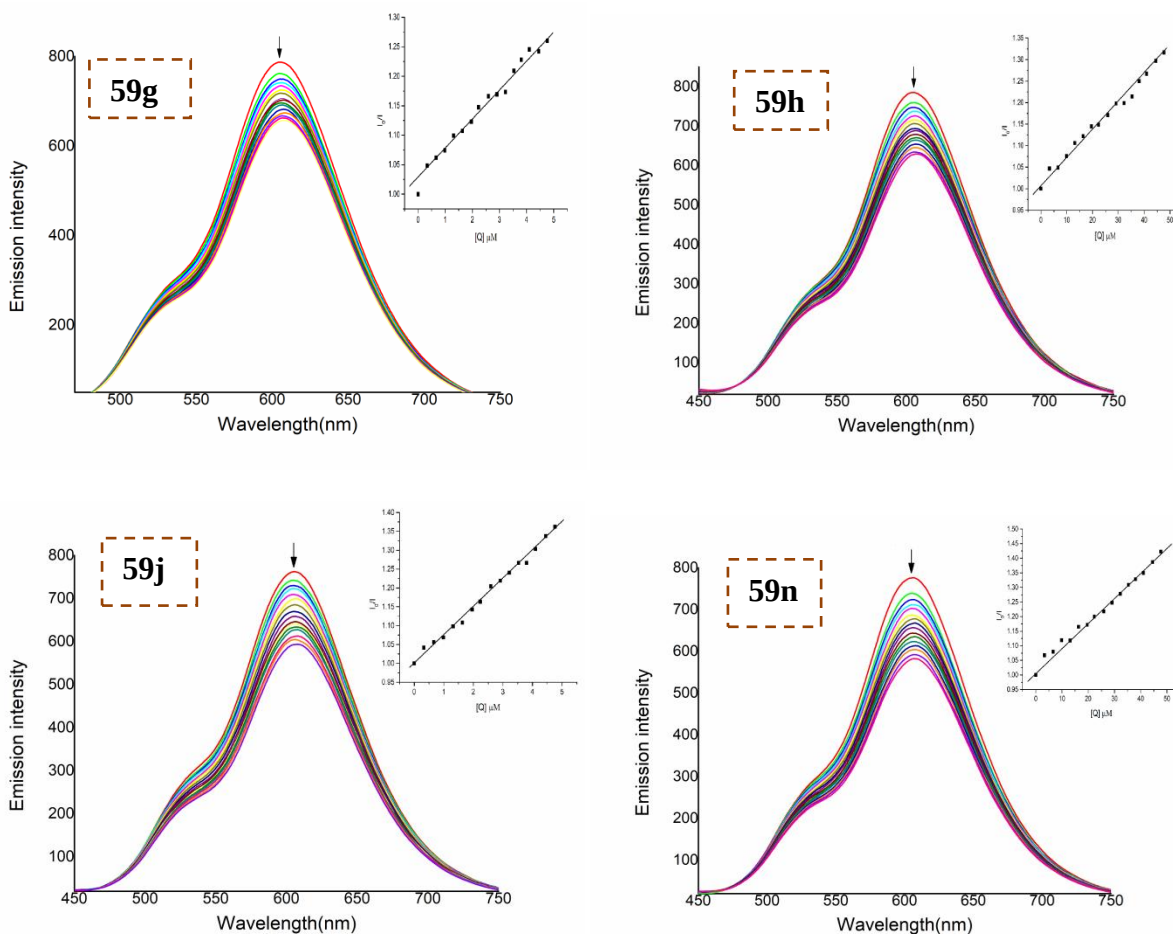


Fig. 9: Emission spectra of compounds **59b**, **59e**, **59g**, **59h**, **59j**, and **59n**

Molecular Docking Studies:

Molecular docking is widely used in molecular structural biology to know the interaction between two molecules (receptor and ligand). All the synthesized chemical (ligand) compounds of **59a–p** were drawn 2D models using Chemdraw software and converted into 3D structures using Open Babel GUI version 2.3.2 (OpenBableGUI; Chris Morley, USA). Molecular energy was minimized using the Energy Minimization module of Maestro Tool (Schrodinger software) under the Chemistry at Harvard Macromolecular Mechanics (CHARMM) force field. The three-dimensional structure of EGFR–kinase in complex with compounds was retrieved from Protein Data Bank (PDB ID: 6s9b.pdb)⁹³. The structure preparation and correction of protein were performed using the protein preparation suite. The target protein file was prepared by removing the structural water molecule, heteroatom's, and co-factors by leaving only the residues associated with protein by using protein preparation suite (wizard) tool was used to prepare

target protein file addition of polar hydrogen's to the macromolecule, an essential step to correct the calculation of partial charge by keeping all other values as default. Further grid was prepared and used for molecular docking was performed using Glide docking module and obtained results were scrutinized based on highest dock score and number of H-bonds by visualizing in Pymol.

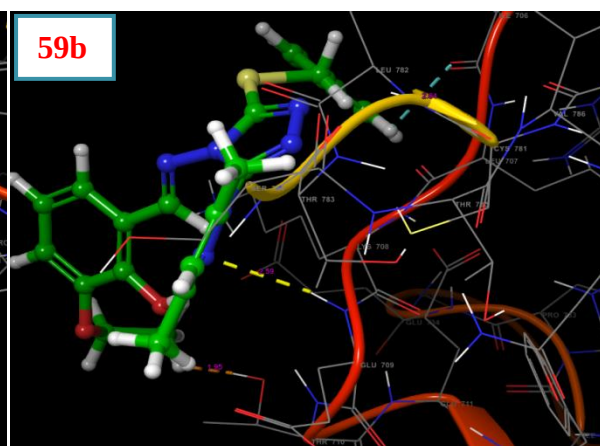
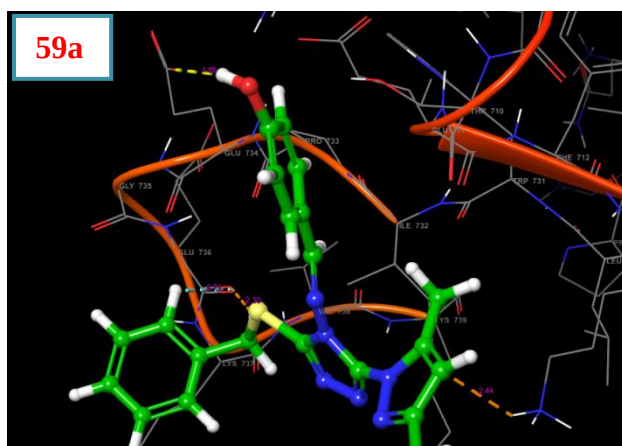
Results:

The docking studies revealed that all the synthesized molecules exhibited excellent binding energies towards the receptor active sites. Molecular docking results were identified basis on the ideal interacted ligands were scrutinized based on the greatest ligand binding poses were identified using the low binding energy, high docking score, and the number of H-bonding, hydrophobic interactions at receptor site i.e., **59a**, **59b**, **59c**, **59h**, **59j**, and **59n** (**Fig. 10**). **Table-9** represents the docking score, Hydrogen bond distance, and interacting atoms. All the compounds were found to be buried.

Table-9: Molecular interactions of **59a–p** with receptor **6S9B**.

Ligand	Receptor Interaction Atoms	Ligand Interaction Atoms	H-bond Distance (Å)	Docking score (Kcal/mol)
59a	Glu 734-O	H	1.86	-0.572
	Glu 736-O	S	2.30	
59b	Thr 710-H	H	1.95	-0.623
	Glu 709-H	N	2.59	
59c	Thr 710-H	O	2.07	-1.332
	Lys 713-H	O	1.92	
59d		No bond		-0.735
59e	Lys 713-H	N	2.20	-0.56
59f	Thr 710-O	H	2.30	-2.22
59g	Lys 739-H	O	2.20	-0.293
59h	Thr 710-H	O	2.08	-0.945
59i	Thr 710-O	H	2.34	-1.41

59j	Leu 782-H	S	2.29	-2.166
	Lys 739-H	O	2.02	
	Lys 739-H	O	2.20	
	Val 738-H	H	2.01	
59k	Glu 709-H	N	2.39	-1.136
	Lys 708-H	O	2.08	
59l	Lys 739-H	H	2.41	-2.691
	Lys 713-H	O	2.15	
59m	Glu 709-H	S	2.25	-0.601
59n	Lys 713-H	O	2.07	-2.854
	Lys 713-N	O	2.94	
	Lys 739-H	N	2.33	
59o	Glu 709-H	S	2.25	-1.078
59p	Thr 710-O	H	2.27	0.012
	Glu 736-O	S	2.30	



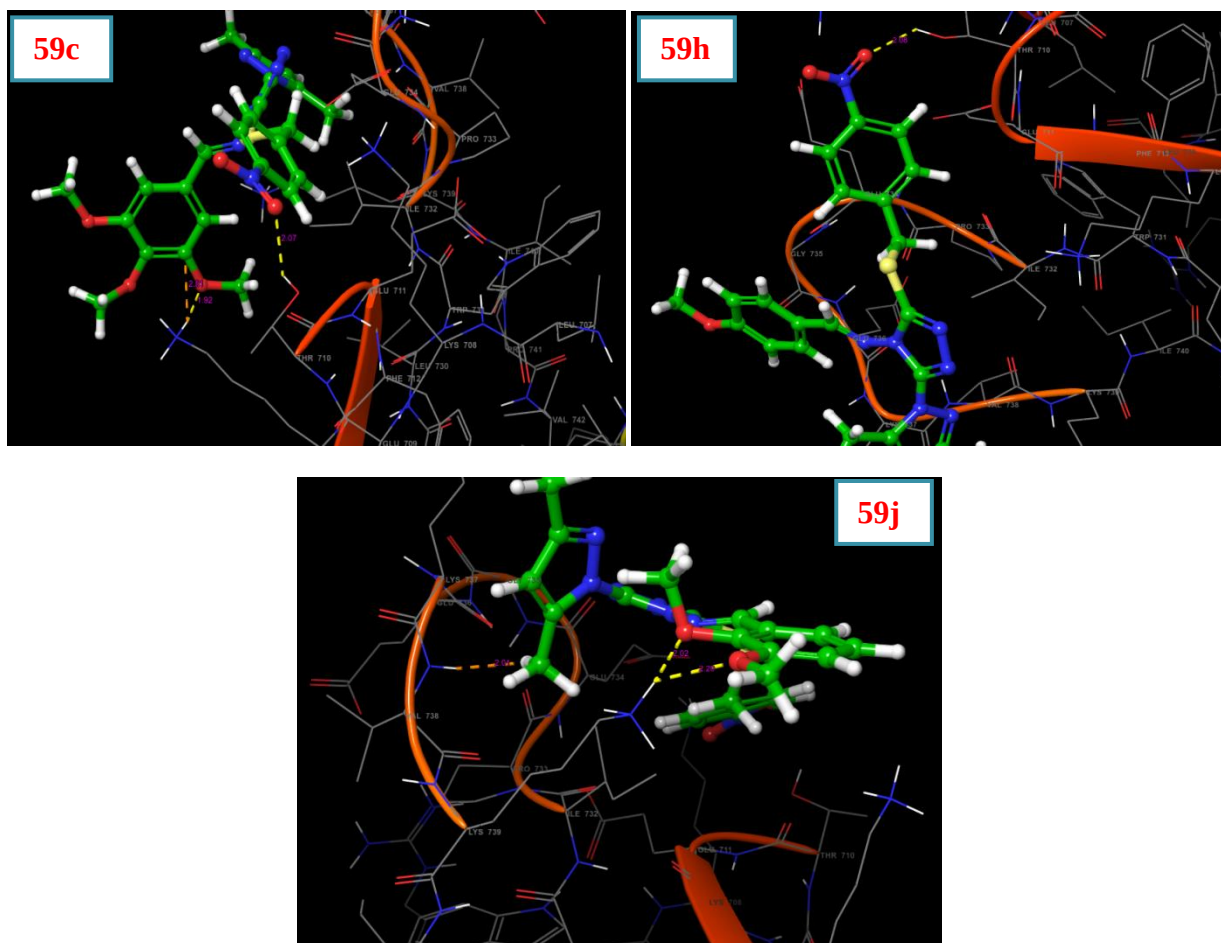


Fig. 10: Molecular interactions of the Receptor with ligands **59a**, **59b**, **59c**, **59h**, and **59j**.

In summary, (*E*)-*N*-benzylidene-3-(benzylthio)-5-(3,5-dimethyl-1*H*-pyrazol-1-yl)-4*H*-1,2,4-triazol-4-amines (**59a-p**) were synthesized in good yields via a one-pot, four-component reaction by readily available starting materials, and no column chromatography is needed for the purification of the product. This method is operationally simple. Further, DNA binding studies were also carried out by using UV-visible and fluorescence experiments. Furthermore, molecular docking studies were confirmed the binding interactions with the EGFR receptor. Hence, the newly synthesized compounds may be useful in the drug discovery process.

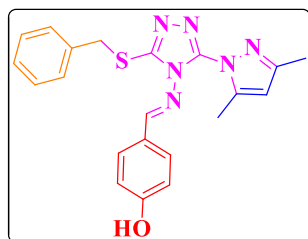
EXPERIMENTAL:**General procedure for the synthesis of (*E*)-*N*-benzylidene-3-(benzylthio)-5-(3,5-dimethyl-1*H*-pyrazol-1-yl)-4*H*-1,2,4-triazol-4-amines (59a–p) involving four-component reaction.**

A mixture of 4-amino-5-hydrazino-4*H*-[1,2,4] triazole-3-thiol (**1**) (0.001 mol) and pentane-2,4-dione (**18**) (0.001 mol) was refluxed for 3 h in ethanol (3 mL). Then to this reaction mixture, appropriate aromatic aldehyde (**6**) (0.001 mol) and two drops of conc. HCl was added and refluxed for 4-5 h. Then appropriate aralkyl/propargyl/allyl bromide (**41**) (0.001 mol) were added to the reaction mixture and refluxed for 5-6 h, by monitoring TLC (CHCl₃+CH₃OH 5:5). After completion of the reaction, the reaction mixture was allowed to cool, then diluted with water and the solid separated was filtered. The compound was recrystallized from 5-6 mL ethanol.

Alternative procedure for the synthesis 59a-p via a three-component condensation reaction by isolating 4-amino-5-(3,5-dimethyl-1*H*-pyrazol-1-yl)-4*H*-1,2,4-triazole-3-thiol (A).

A mixture of 4-amino-5-hydrazino-4*H*-[1,2,4]-triazole-3-thiol (**1**) (0.001 mol) and pentane-2,4-dione (**18**) (0.001 mol) was refluxed for 3 h in ethanol (3 mL). The reaction mixture was cooled and solid formed was filtered and recrystallized from ethanol (5 mL).

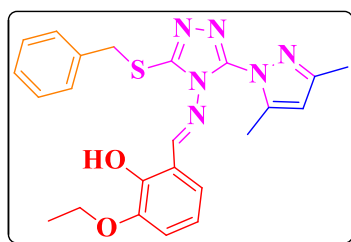
A mixture of 4-amino-5-(3,5-dimethyl-1*H*-pyrazol-1-yl)-4*H*-1,2,4-triazole-3-thiol (**A**) (0.001 mol) and requisite aromatic aldehyde (**6**) (0.001 mol) was taken in ethanol (5 ml) containing 2 drops of conc. HCl and aralkyl/propargyl/allylbromide (**41**) (0.001 mol) were added and refluxed for 10-12 h. After completing the reaction (vide TLC), the reaction mixture was cooled, solid produced was filtered and recrystallized from ethanol (5-6 mL) (yield 95%; mp: 182-184°C).

SPECTRAL DATA:**(*E*)-4-(((3-(Benzylthio)-5-(3,5-dimethyl-1*H*-pyrazol-1-yl)-4*H*-1,2,4-triazol-4-yl)imino)methyl)phenol (59a):**

Brick red color solid (0.363g, 90%); m.p.: 187-189°C; **FT-IR (KBr, $\nu_{\text{max}}/\text{cm}^{-1}$):** 3410 (-OH), 2922 (-CH₂-), 1590 (-C=N-); **¹H-NMR (400 MHz, CDCl₃+DMSO-*d*₆, δ ppm):** 2.22 (s, 3H, -CH₃), 2.23 (s, 3H, -CH₃), 4.50 (s, 2H, -CH₂-), 6.03 (s, 1H, -CH- of pyrazole ring), 6.87

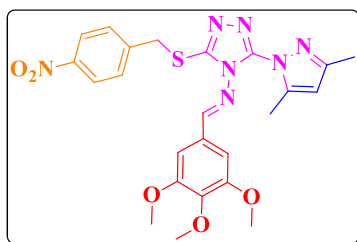
(d, 2H, $J=8.0$ Hz, Ar-H) 7.26-7.32 (m, 3H, Ar-H), 7.41 (d, 2H, $J=7.2$ Hz, Ar-H), 7.49 (d, 2H, $J=8.0$ Hz, Ar-H), 7.86 (s, 1H, -OH), 9.91 (s, 1H, -N=CH-); $^{13}\text{C-NMR}$ (100 MHz, $\text{CDCl}_3+\text{DMSO}-d_6$, δ ppm): 15.9, 18.3, 40.9, 112.5, 121.0, 127.4, 132.5, 133.4, 133.9, 135.9, 141.0, 148.3, 154.8, 156.4, 156.9, 167.3, 169.2; **ESI-MS** (m/z): 405 $[\text{M}+\text{H}]^+$; Analytical calculated formulae $\text{C}_{21}\text{H}_{20}\text{N}_6\text{OS}$: C, 62.36; H, 4.98; N, 20.78; S, 7.93; Found: C, 62.31; H, 4.95; N, 20.76; S, 7.90.

(E)-2-(((3-(Benzylthio)-5-(3,5-dimethyl-1H-pyrazol-1-yl)-4H-1,2,4-triazol-4-yl)imino)methyl)-6-ethoxyphenol (59b):



Cream color solid (0.398g, 89%); m.p.: 172-174°C; **FT-IR** (KBr, $\nu_{\text{max}}/\text{cm}^{-1}$): 3405 (-OH), 2928 (-CH₂-), 1610 (C=N); $^1\text{H-NMR}$ (400 MHz, CDCl_3 , δ ppm): 1.49 (t, 3H, $J=6.8$ Hz, -CH₃), 2.26 (s, 3H, -CH₃), 2.31 (s, 3H, -CH₃), 4.14 (m, 2H, -CH₂-), 4.55 (s, 2H, -SCH₂-), 6.04 (s, 1H, -CH- of pyrazole ring), 6.83 (dd, 1H, $J=9.2$ Hz, $J=7.6$ Hz, Ar-H), 6.88 (t, 1H, $J=8.0$ Hz, Ar-H), 7.03 (dd, 1H, $J=8.8$ Hz, $J=7.6$ Hz, Ar-H), 7.32 (m, 3H, Ar-H), 7.42 (dd, 2H, $J=9.2$ Hz, $J=7.6$ Hz, Ar-H), 8.29 (s, 1H, -OH), 9.70 (s, 1H, -N=CH-); $^{13}\text{C-NMR}$ (100 MHz, CDCl_3 , δ ppm): 11.4, 13.6, 14.8, 36.8, 64.8, 108.1, 116.4, 117.4, 119.8, 123.6, 127.9, 128.8, 129.2, 136.0, 143.9, 144.4, 147.7, 149.5, 149.8, 152.6, 165.1; **ESI-MS** (m/z): 449 $[\text{M}+\text{H}]^+$; Analytical calculated formulae $\text{C}_{23}\text{H}_{24}\text{N}_6\text{O}_2\text{S}$: C, 61.59; H, 5.39; N, 18.74; S, 7.15; Found: C, 61.55; H, 5.42; N, 18.70; S, 7.18.

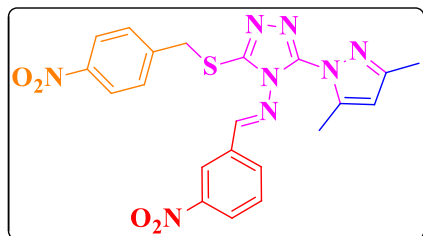
(E)-3-(3,5-Dimethyl-1H-pyrazol-1-yl)-5-(((4-nitrobenzyl)thio)-N-(3,4,5-trimethoxybenzylidene)-4H-1,2,4-triazol-4-amine (59c):



Pale yellow color solid (0.452g, 86%); m.p.: 156-158°C; **FT-IR** (KBr, $\nu_{\text{max}}/\text{cm}^{-1}$): 2924 (-CH₂-), 1605 (C=N); $^1\text{H-NMR}$ (400 MHz, CDCl_3 , δ ppm): 2.24 (s, 3H, -CH₃), 2.27 (s, 3H, -CH₃), 3.88 (s, 6H, -CH₃), 3.91 (s, 3H, -CH₃), 4.60 (s, 2H, -SCH₂-), 6.03 (s, 1H, -CH- of pyrazole ring), 6.87 (s, 2H, Ar-H), 7.65 (d, 2H, $J=8.8$ Hz, Ar-H), 8.03 (s, 1H, -N=CH-), 8.17 (d, 2H, $J=8.8$ Hz, Ar-H); $^{13}\text{C-NMR}$ (100 MHz, CDCl_3 , δ ppm): 11.4, 13.6, 34.8, 56.3, 61.1, 106.2, 108.0, 123.9, 126.7, 130.2, 142.6, 143.9, 144.4, 144.5, 147.4, 149.7, 152.4, 153.6, 164.2; **ESI-MS** (m/z): 524 $[\text{M}+\text{H}]^+$; Analytical

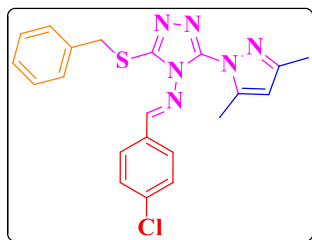
calculated formulae $C_{24}H_{25}N_7O_5S$: C, 55.06; H, 4.81; N, 18.73; S, 6.12; Found: C, 55.14; H, 4.85; N, 18.76; S, 6.15.

(E)-3-(3,5-Dimethyl-1H-pyrazol-1-yl)-5-((4-nitrobenzyl)thio)-N-(3-nitrobenzylidene)-4H-1,2,4-triazol-4-amine (59d):



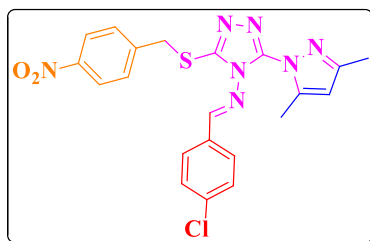
White color solid (0.420g, 88%); m.p.: 170-172°C; **FT-IR (KBr, $\nu_{\max}/\text{cm}^{-1}$):** 2924 ($-\text{CH}_2-$), 1605 ($\text{C}=\text{N}$); **$^1\text{H-NMR}$ (400 MHz, CDCl_3 , δ ppm):** 2.27 (s, 3H, $-\text{CH}_3$), 2.32 (s, 3H, $-\text{CH}_3$), 4.60 (s, 2H, $-\text{SCH}_2-$), 6.09 (s, 1H, $-\text{CH}-$ of pyrazole ring), 7.68 (m, 4H, Ar-H), 7.98 (d, 1H, $J=6.0$ Hz, Ar-H), 8.19 (d, 2H, $J=6.8$ Hz, Ar-H), 8.37 (d, 1H, $J=6.8$ Hz, Ar-H), 8.47 (s, 1H, $-\text{N}=\text{CH}-$); **$^{13}\text{C-NMR}$ (100 MHz, CDCl_3 , δ ppm):** 11.5, 13.6, 34.5, 108.5, 123.4, 123.8, 123.9, 127.1, 130.2, 134.0, 144.2, 144.3, 144.4, 148.7, 149.1, 152.0, 153.0, 153.6, 159.1; **ESI-MS (m/z):** 479 $[\text{M}+\text{H}]^+$; Analytical calculated formulae $C_{21}H_{18}N_8O_4S$: C, 52.71; H, 3.79; N, 23.42; S, 6.70; Found: C, 52.74; H, 3.76; N, 23.45; S, 6.74.

(E)-3-(Benzylthio)-N-(4-chlorobenzylidene)-5-(3,5-dimethyl-1H-pyrazol-1-yl)-4H-1,2,4-triazol-4-amine (59e):



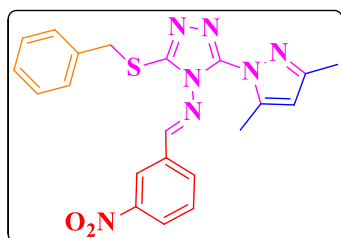
White color solid (0.375, 89%); m.p.: 132-134°C; **FT-IR (KBr, $\nu_{\max}/\text{cm}^{-1}$):** 2923 ($-\text{CH}_2-$), 1597 ($\text{C}=\text{N}$); **$^1\text{H-NMR}$ (400 MHz, CDCl_3 , δ ppm):** 2.25 (s, 3H, $-\text{CH}_3$), 2.27 (s, 3H, $-\text{CH}_3$), 4.56 (s, 2H, $-\text{SCH}_2-$), 6.03 (s, 1H, $-\text{CH}-$ of pyrazole ring), 7.31 (m, 3H, Ar-H), 7.40 (d, 2H, $J=8.8$ Hz, Ar-H), 7.44 (d, 2H, $J=8.0$ Hz, Ar-H), 7.58 (d, 2H, $J=8.8$ Hz, Ar-H), 7.99 (s, 1H, $-\text{N}=\text{CH}-$); **$^{13}\text{C-NMR}$ (100 MHz, CDCl_3 , δ ppm):** 11.3, 13.6, 36.1, 108.0, 127.9, 128.8, 129.2, 129.3, 129.4, 130.0, 130.3, 130.4, 136.9, 144.0, 144.2, 152.5, 161.6; **ESI-MS (m/z):** 423 $[\text{M}+\text{H}]^+$; Analytical calculated formulae $C_{21}H_{19}\text{ClN}_6\text{S}$: C, 59.64; H, 4.53; N, 19.87; S, 7.58; Found: C, 59.68; H, 4.56; N, 19.90; S, 7.61.

(E)-N-(4-Chlorobenzylidene)-3-(3,5-dimethyl-1H-pyrazol-1-yl)-5-((4-nitrobenzyl)thio)-4H-1,2,4-triazol-4-amine (59f):



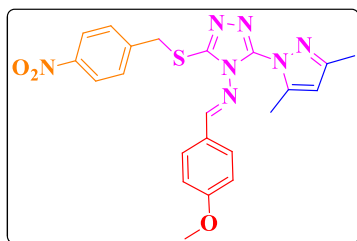
White color solid (0.394g, 84%); m.p.: 155-157°C; **FT-IR (KBr, $\nu_{\max}/\text{cm}^{-1}$):** 2929 (-CH₂-), 1610 (C=N); **¹H-NMR (400 MHz, CDCl₃, δ ppm):** 2.24 (s, 3H, -CH₃), 2.27 (s, 3H, -CH₃), 4.61 (s, 2H, -SCH₂-), 6.04 (s, 1H, -CH- of pyrazole ring), 7.42 (d, 2H, $J=8.4$ Hz, Ar-H), 7.58 (d, 2H, $J=8.4$ Hz, Ar-H), 7.66 (d, 2H, $J=8.8$ Hz, Ar-H), 8.01 (s, 1H, -N=CH-), 8.17 (d, 2H, $J=8.8$ Hz, Ar-H); **¹³C-NMR (100 MHz, CDCl₃, δ ppm):** 11.4, 13.6, 36.2, 108.3, 123.5, 126.9, 127.9, 128.8, 129.3, 130.2, 133.9, 136.1, 144.2, 148.6, 151.5, 153.8, 159.3; **ESI-MS (m/z):** 466 [M-H]⁺; Analytical calculated formulae C₂₁H₁₈ClN₇O₂S: C, 53.90; H, 3.88; N, 20.95; S, 6.85; Found: C, 53.94; H, 3.85; N, 20.97; S, 6.88.

(E)-3-(Benzylthio)-5-(3,5-dimethyl-1H-pyrazol-1-yl)-N-(3-nitrobenzylidene)-4H-1,2,4-triazol-4-amine (59g):



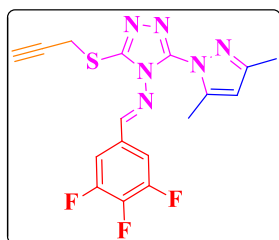
Pale yellow color solid (0.355g, 82%); m.p.: 168-170°C; **FT-IR (KBr, $\nu_{\max}/\text{cm}^{-1}$):** 2925 (-CH₂-), 1597 (C=N); **¹H-NMR (400 MHz, CDCl₃, δ ppm):** 2.31 (s, 3H, -CH₃), 2.31 (s, 3H, -CH₃), 4.57 (s, 2H, -SCH₂-), 6.07 (s, 1H, -CH- of pyrazole ring), 7.32 (m, 3H, Ar-H), 7.44 (d, 2H, $J=5.6$ Hz, Ar-H), 7.64 (t, 1H, $J=6.4$ Hz, Ar-H), 8.00 (d, 1H, $J=6.0$ Hz, Ar-H), 8.13 (s, 1H, Ar-H), 8.35 (d, 1H, $J=7.6$ Hz, Ar-H), 8.43 (s, 1H, -N=CH-); **¹³C-NMR (100 MHz, CDCl₃, δ ppm):** 11.4, 13.6, 34.6, 108.2, 123.9, 128.0, 128.1, 129.1, 129.5, 130.0, 130.2, 139.4, 144.0, 144.2, 144.3, 147.4, 150.5, 152.7, 161.6; **ESI-MS (m/z):** 434 [M+H]⁺; Analytical calculated formulae C₂₁H₁₉N₇O₂S: C, 58.19; H, 4.42; N, 22.62; S, 7.40; Found: C, 58.22; H, 4.46; N, 22.67; S, 7.44.

(E)-3-(3,5-Dimethyl-1H-pyrazol-1-yl)-N-(4-methoxybenzylidene)-5-((4-nitrobenzyl)thio)-4H-1,2,4-triazol-4-amine (59h):



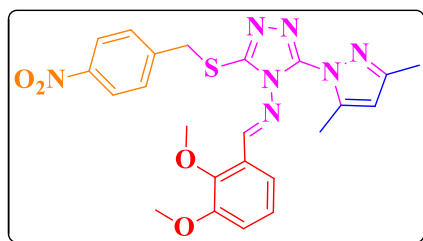
Pale yellow color solid (0.435g, 94%); m.p.: 135-137°C; **FT-IR** (KBr, $\nu_{\max}/\text{cm}^{-1}$): 2921 (-CH₂-), 1607 (C=N); **¹H-NMR** (400 MHz, CDCl₃, δ ppm): 2.24 (s, 6H, -CH₃), 3.86 (s, 3H, -CH₃), 4.59 (s, 2H, -SCH₂-), 6.01 (s, 1H, -CH- of pyrazole ring), 6.93 (d, 2H, $J=6.8$ Hz, Ar-H), 7.59 (d, 2H, $J=6.8$ Hz, Ar-H), 7.64 (d, 2H, $J=6.8$ Hz, Ar-H), 7.98 (s, 1H, -N=CH-), 8.16 (d, 2H, $J=6.8$ Hz, Ar-H); **¹³C-NMR** (100 MHz, CDCl₃, δ ppm): 11.3, 13.6, 34.8, 55.6, 107.9, 114.6, 123.9, 124.2, 130.0, 130.2, 130.9, 143.8, 144.5, 147.4, 149.8, 152.4, 163.6, 163.7; **ESI-MS** (m/z): 464 [M+H]⁺; Analytical calculated formulae C₂₂H₂₁N₇O₃S: C, 57.01; H, 4.57; N, 21.15; S, 6.92; Found: C, 57.14; H, 4.54; N, 21.19; S, 6.95.

(E)-3-(3,5-Dimethyl-1H-pyrazol-1-yl)-5-(prop-2-yn-1-ylthio)-N-(3,4,5-trifluorobenzylidene)-4H-1,2,4-triazol-4-amine (59i):



White color solid (0.323g, 83%); m.p.: 131-133°C; **FT-IR** (KBr, $\nu_{\max}/\text{cm}^{-1}$): 3308 ($\equiv\text{C}-\text{H}$), 2925 (-CH₂-), 1619 (C=N); **¹H-NMR** (400 MHz, CDCl₃+DMSO-*d*₆, δ ppm): 2.25 (s, 3H, -CH₃), 2.28 (s, 3H, -CH₃), 4.08 (s, 2H, -CH₂-), 4.37 (s, 1H, $\equiv\text{C}-\text{H}$), 6.11 (s, 1H, -CH- of pyrazole ring), 6.93 (t, 2H, $J=8.8$ Hz, Ar-H) 8.28 (s, 1H, -N=CH-); **¹³C-NMR** (100 MHz, CDCl₃, δ ppm): 11.3, 13.6, 19.8, 72.6, 78.0, 101.6, 108.3, 143.8, 144.2, 152.7, 152.8, 153.0, 153.4, 163.4, 163.9; **ESI-MS** (m/z): 391 [M+H]⁺; Analytical calculated formulae C₁₇H₁₃F₃N₆S: C, 52.30; H, 3.36; N, 21.53; S, 8.21; Found: C, 52.34; H, 3.32; N, 21.50; S, 8.19.

(E)-N-(2,3-Dimethoxybenzylidene)-3-(3,5-dimethyl-1H-pyrazol-1-yl)-5-((4-nitrobenzyl)thio)-4H-1,2,4-triazol-4-amine (59j):



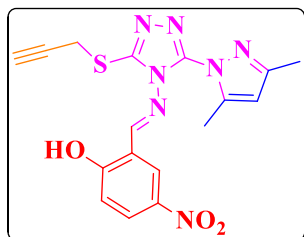
Pale yellow color solid (0.419g, 85%); m.p.: 143-145°C;

FT-IR (KBr, $\nu_{\text{max}}/\text{cm}^{-1}$): 2923 (-CH₂-), 1604 (C=N);

¹H-NMR (400 MHz, CDCl₃, δ ppm): 2.25 (s, 3H, -CH₃), 2.26 (s, 3H, -CH₃), 3.75 (s, 3H, -OCH₃), 3.87 (s, 3H, -OCH₃), 4.60 (s, 2H, -SCH₂-), 6.04 (s, 1H, -CH- of pyrazole ring),

7.04 (dd, 1H, $J=7.6$ Hz, $J=6.4$ Hz Ar-H), 7.09 (t, 1H, $J=6.4$ Hz, Ar-H), 7.53 (d, 1H, $J=6.4$ Hz, Ar-H), 7.66 (d, 2H, $J=6.8$ Hz, Ar-H), 8.17 (d, 2H, $J=6.8$ Hz, Ar-H), 8.39 (s, 1H, -N=CH-); **¹³C-NMR (100 MHz, CDCl₃, δ ppm):** 11.2, 13.7, 34.6, 55.9, 61.8, 108.0, 116.5, 118.2, 123.9, 124.4, 125.6, 130.2, 143.6, 144.2, 144.5, 147.4, 150.4, 150.7, 152.7, 152.8, 158.3; **ESI-MS (m/z):** 494 [M+H]⁺; Analytical calculated formulae C₂₃H₂₃N₇O₄S: C, 55.97; H, 4.70; N, 19.87; S, 6.50; Found: C, 55.95; H, 4.74; N, 19.91; S, 6.47.

(E)-2-(((3-(3,5-Dimethyl-1H-pyrazol-1-yl)-5-(prop-2-yn-1-ylthio)-4H-1,2,4-triazol-4-yl)imino)methyl)-4-nitrophenol (59k):

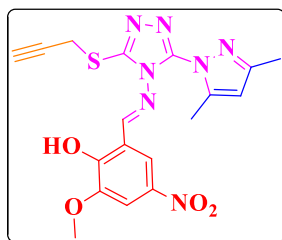


Pale green color solid (0.345g, 87%); m.p.: 168-170°C; **FT-IR (KBr, $\nu_{\text{max}}/\text{cm}^{-1}$):** 3422 (-OH), 3292 ($\equiv$ -H), 2969 (-CH₂-), 1615 (C=N);

¹H-NMR (400 MHz, DMSO-*d*₆, δ ppm): 2.25 (s, 3H, -CH₃), 2.31 (s, 3H, -CH₃), 2.59 (s, 1H, $\equiv$ -H), 4.10 (s, 2H, -SCH₂-), 6.09 (s, 1H, -CH- of pyrazole ring), 7.08 (d, 2H, $J=9.2$ Hz, Ar-H), 7.64 (s, 1H, -

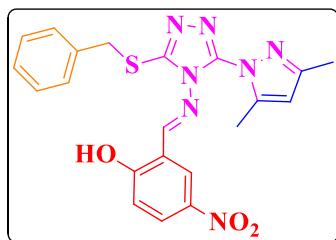
OH), 8.20 (d, 2H, $J=9.6$ Hz, Ar-H), 8.56 (s, 1H, Ar-H), 8.66 (s, 1H, -N=CH-); **¹³C-NMR (100 MHz, CDCl₃+DMSO-*d*₆, δ ppm):** 11.3, 13.6, 20.4, 73.2, 78.2, 108.2, 117.5, 118.3, 124.2, 129.1, 143.4, 143.7, 144.5, 149.4, 152.6, 158.8, 164.1; **ESI-MS (m/z):** 398 [M+H]⁺; Analytical calculated formulae C₁₇H₁₅N₇O₃S: C, 51.38; H, 3.80; N, 24.67; S, 8.07; Found: C, 51.34; H, 3.83; N, 24.71; S, 7.94.

(E)-2-(((3-(3,5-Dimethyl-1H-pyrazol-1-yl)-5-(prop-2-yn-1-ylthio)-4H-1,2,4-triazol-4-yl)imino)methyl)-6-methoxy-4-nitrophenol (59l):



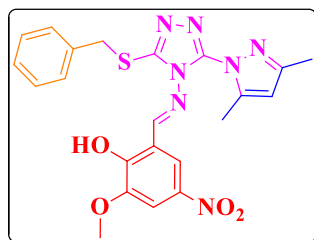
Green color solid (0.362g, 85%); m.p.: 175-177°C; **FT-IR (KBr, $\nu_{\max}/\text{cm}^{-1}$):** 3401 (-OH), 3369 ($\equiv\text{C-H}$), 2940 (-CH₂-), 1611 (C=N); **¹H-NMR (400 MHz, DMSO-*d*₆, δ ppm):** 2.24 (s, 3H, -CH₃), 2.33 (s, 3H, -CH₃), 2.58 (s, 1H, $\equiv\text{C-H}$), 3.99 (s, 3H, -OCH₃), 4.09 (s, 2H, -SCH₂-), 6.08 (s, 1H, -CH- of pyrazole ring), 7.72 (s, 1H, -OH), 7.78 (s, 1H, Ar-H), 8.34 (s, 1H, Ar-H), 8.56 (s, 1H, -N=CH-); **¹³C-NMR (100 MHz, CDCl₃+ DMSO-*d*₆, δ ppm):** 11.3, 13.6, 20.4, 56.7, 73.2, 78.1, 108.3, 108.8, 116.1, 117.4, 139.8, 143.6, 144.5, 145.4, 148.6, 149.3, 152.7, 159.8; **ESI-MS (*m/z*):** 428 [M+H]⁺; Analytical calculated formulae C₁₈H₁₇N₇O₄S: C, 50.58; H, 4.01; N, 22.94; S, 7.50; Found: C, 50.62; H, 3.96; N, 22.90; S, 7.46.

(E)-2-(((3-(Benzylthio)-5-(3,5-dimethyl-1H-pyrazol-1-yl)-4H-1,2,4-triazol-4-yl)imino)methyl)-4-nitrophenol (59m):



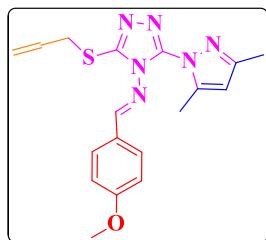
Yellow color solid (0.408g, 91%); m.p.: 149-151°C; **FT-IR (KBr, $\nu_{\max}/\text{cm}^{-1}$):** 3425 (-OH), 2964 (-CH₂-), 1614 (C=N); **¹H-NMR (400 MHz, CDCl₃, δ ppm):** 2.28 (s, 3H, -CH₃), 2.43 (s, 3H, -CH₃), 4.56 (s, 2H, -SCH₂-), 6.08 (s, 1H, -CH- of pyrazole ring), 7.11 (d, 1H, *J*=9.2 Hz, Ar-H), 7.31(m, 3H, Ar-H), 7.39 (d, 2H, *J*=6.8 Hz, Ar-H), 8.13 (s, 1H, -OH), 7.28 (d, 1H, *J*=6.8 Hz, Ar-H), 8.45 (s, 1H, Ar-H), 11.00 (s, 1H, -N=CH-); **¹³C-NMR (100 MHz, CDCl₃, δ ppm):** 11.9, 13.5, 37.9, 108.7, 115.9, 118.9, 128.2, 128.8, 128.9, 129.2, 129.3, 135.7, 140.6, 144.2, 145.0, 148.1, 153.1, 161.5, 164.1; **ESI-MS (*m/z*):** 448 [M-H]⁺; Analytical calculated formulae C₂₁H₁₉N₇O₃S: C, 56.11; H, 4.26; N, 21.81; S, 7.13; Found: C, 56.15; H, 4.22; N, 21.85; S, 7.16.

(E)-2-(((3-(Benzylthio)-5-(3,5-dimethyl-1H-pyrazol-1-yl)-4H-1,2,4-triazol-4-yl)imino)methyl)-6-methoxy-4-nitrophenol (59n):



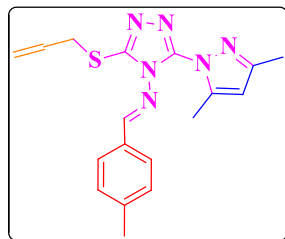
Yellow color solid (0.431g, 90%); m.p.: 173-175°C; **FT-IR (KBr, $\nu_{\max}/\text{cm}^{-1}$):** 3402 (-OH), 2939 (-CH₂-), 1609 (C=N); **¹H-NMR (400 MHz, CDCl₃, δ ppm):** 2.27 (s, 3H, -CH₃), 2.29 (s, 3H, -CH₃), 4.00 (s, 3H, -OCH₃), 4.54 (s, 2H, -SCH₂-), 6.07 (s, 1H, -CH- of pyrazole ring), 7.30 (m, 3H, Ar-H), 7.38 (d, 2H, $J=6.4$ Hz, Ar-H), 7.84 (s, 2H, Ar-H), 8.39 (s, 1H, -OH), 10.74 (s, 1H, -N=CH-); **¹³C-NMR (100 MHz, CDCl₃, δ ppm):** 11.7, 13.6, 37.5, 56.7, 108.6, 109.5, 115.4, 119.4, 128.1, 128.8, 129.2, 135.8, 140.5, 144.1, 144.6, 148.8, 149.0, 153.1, 154.4, 161.5; **ESI-MS (m/z):** 478 [M-H]⁺; Analytical calculated formulae C₂₂H₂₁N₇O₄S: C, 55.11; H, 4.41; N, 20.45; S, 6.69; Found: C, 55.15; H, 4.46; N, 20.49; S, 6.65.

(E)-3-(Allylthio)-5-(3,5-dimethyl-1H-pyrazol-1-yl)-N-(4-methoxybenzylidene)-4H-1,2,4-triazol-4-amine (59o):



White color solid (0.301g, 82%); m.p.: 142-144°C; **FT-IR (KBr, $\nu_{\max}/\text{cm}^{-1}$):** 3062 (=C-H), 2918 (-CH₂-), 1603 (C=N); **¹H-NMR (400 MHz, CDCl₃, δ ppm):** 2.29 (s, 3H, -CH₃), 2.42 (s, 3H, -CH₃), 2.33 (s, 3H, -CH₃), 6.06 (s, 1H, -CH- of pyrazole ring), 7.25 (d, 2H, $J=8$ Hz, Ar-H), 7.66 (d, 2H, $J=8$ Hz, Ar-H), 9.89 (s, 1H, -N=CH-), 11.91 (s, 1H, -SH); **¹³C-NMR (100 MHz, CDCl₃, δ ppm):** 11.34, 13.61, 21.78, 107.95, 129.08, 129.37, 129.63, 129.74, 129.93, 143.55, 143.71, 152.70, 164.45; **ESI-MS (m/z):** 469 [M+H]⁺; Analytical calculated formulae C₁₈H₂₀N₆OS: C, 58.68; H, 5.47; N, 22.81; S, 8.70; Found: C, 58.73; H, 5.43; N, 22.85; S, 8.74.

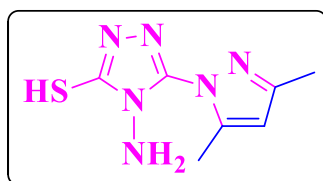
(E)-3-(Allylthio)-5-(3,5-dimethyl-1H-pyrazol-1-yl)-N-(4-methylbenzylidene)-4H-1,2,4-triazol-4-amine (59p):



Cream color solid (0.299g, 85%); m.p.: 147-149°C; **FT-IR (KBr, $\nu_{\max}/\text{cm}^{-1}$):** 3074 (=C-H), 2918 (-CH₂-), 1603 (C=N); **¹H-NMR (400 MHz, CDCl₃+DMSO-*d*₆, δ ppm):** 2.26 (s, 3H, -CH₃), 2.31 (s, 3H, -CH₃), 2.40 (s, 3H, -CH₃), 3.97 (d, 2H, $J=7.2$ Hz, -SCH₂-), 5.19 (d, 1H,

$J=10.0$ Hz, $-C=C-H$), 5.35 (d, 1H, $J=11.6$ Hz, $-C=C-H$), 6.03 (s, 1H, $-CH-$ of pyrazole ring), 6.05 (m, 1H, $-C=C-H$), 7.23 (d, 2H, $J=7.6$ Hz, Ar-H), 7.64 (d, 2H, $J=8.0$ Hz, Ar-H), 9.89 (s, 1H, $-N=CH-$); $^{13}\text{C-NMR}$ (100 MHz, CDCl_3 , δ ppm): 11.3, 13.6, 21.8, 34.5, 107.9, 119.3, 129.1, 129.6, 129.8, 132.4, 143.5, 143.7, 152.7, 163.4, 164.0, 164.3; **ESI-MS** (m/z): 353 $[\text{M}+\text{H}]^+$; Analytical calculated formulae $\text{C}_{18}\text{H}_{20}\text{N}_6\text{S}$: C, 61.34; H, 5.72; N, 23.84; S, 9.10; Found: C, 61.38; H, 5.75; N, 23.80; S, 9.14.

4-Amino-5-(3,5-dimethyl-1H-pyrazol-1-yl)-4H-1,2,4-triazole-3-thiol (A):



White solid; yield (0.197g, 94%); m.p.: 182-184°C; **FT-IR** (KBr, $\nu_{\text{max}}/\text{cm}^{-1}$): 3370 ($-\text{NH}_2$), 2740 ($-\text{SH}$); $^1\text{H-NMR}$ (400 MHz, $\text{CDCl}_3+\text{DMSO}-d_6$, δ ppm): 2.19 (s, 3H, $-\text{CH}_3$); 2.26 (s, 3H, $-\text{CH}_3$); 5.53 (s, 2H, $-\text{NH}_2$); 5.99 (s, 1H, $-CH-$ of pyrazole ring); 13.79 (s, 1H, $-\text{SH}$); $^{13}\text{C-NMR}$ (100 MHz, $\text{CDCl}_3+\text{DMSO}-d_6$, δ ppm): 11.5; 13.6; 108.1; 143.0; 143.2; 152.1; 165.2; **ESI-MS** (m/z): 211 $[\text{M}+\text{H}]^+$. Analytical calculated formulae $\text{C}_7\text{H}_{10}\text{N}_6\text{S}$: C, 39.99; H, 4.79; N, 39.97; S, 15.25. Found: C, 40.04; H, 4.86; N, 40.02; S, 15.20%.

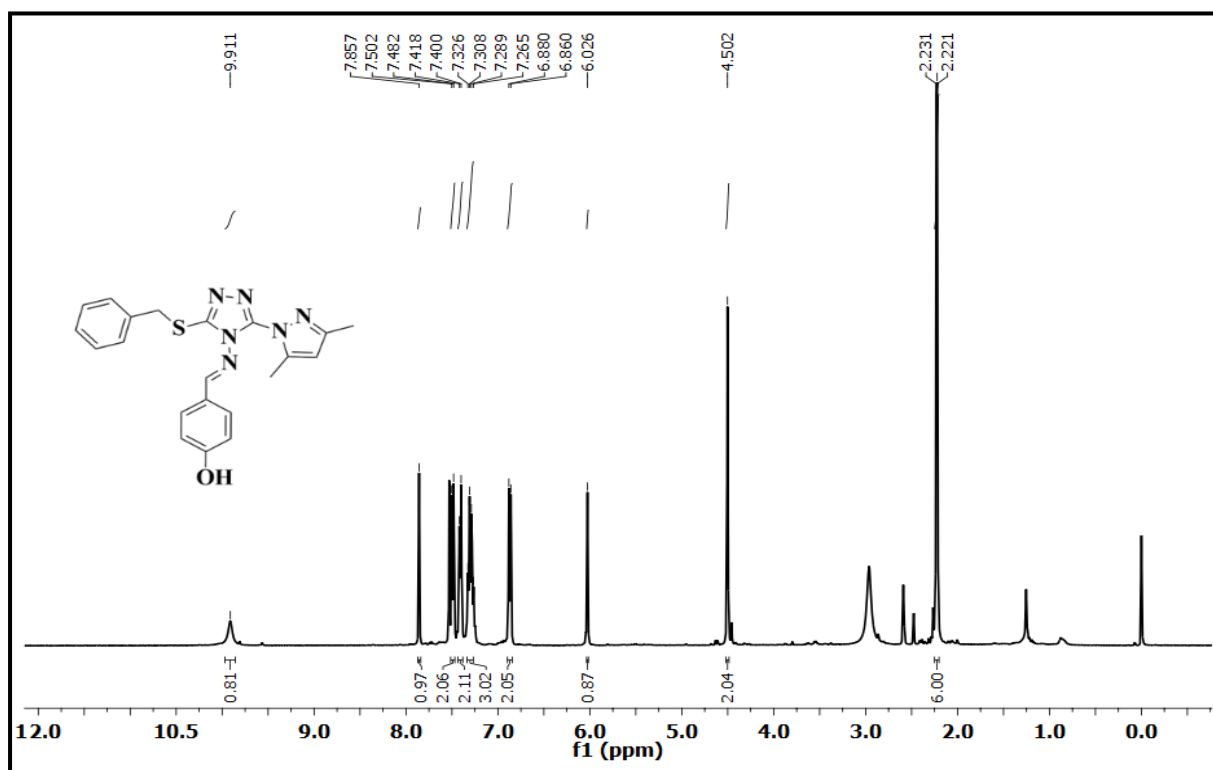
SPECTRA

Fig: ¹H-NMR Spectrum of Compound 59a (400 MHz, CDCl₃+DMSO-*d*₆)

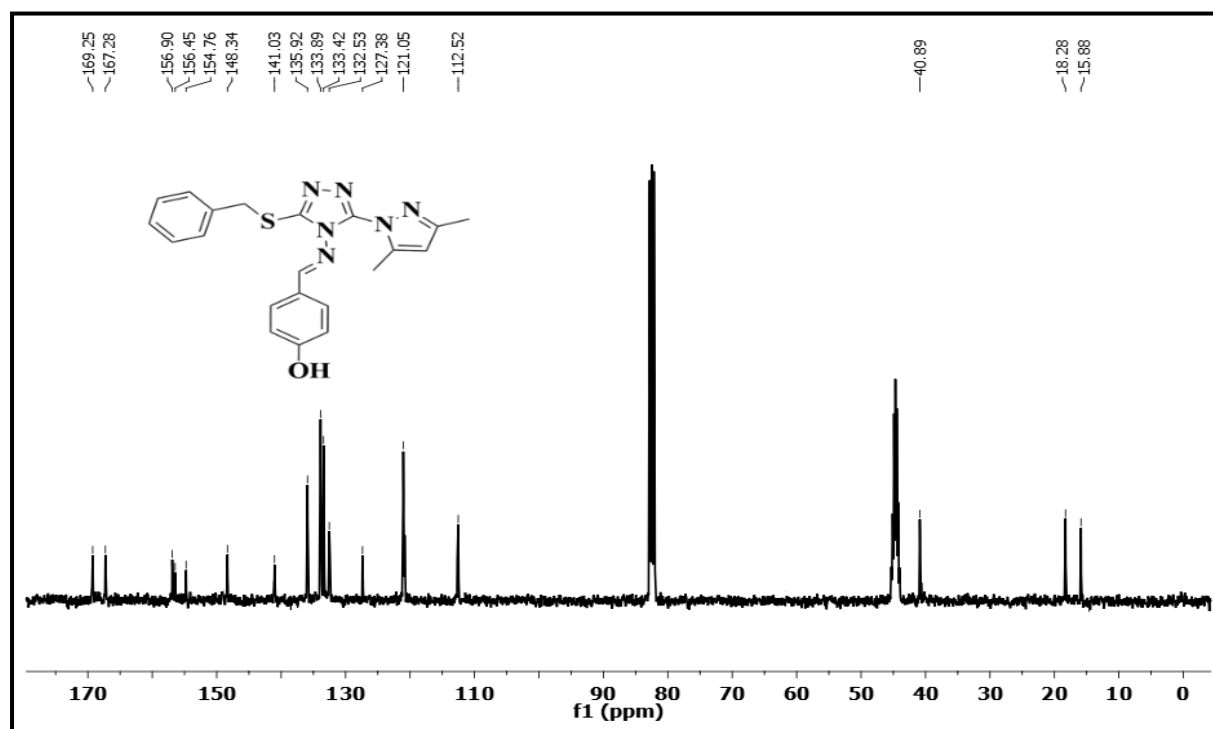


Fig: ¹³C-NMR Spectrum of Compound 59a (100 MHz, CDCl₃+DMSO-*d*₆)

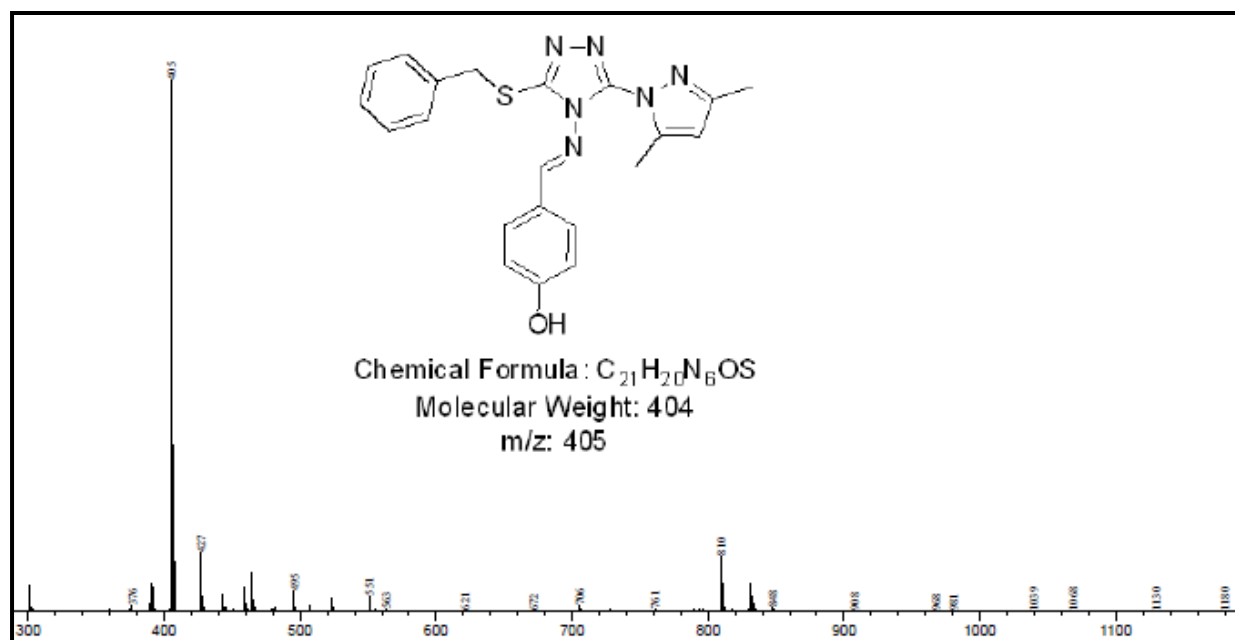
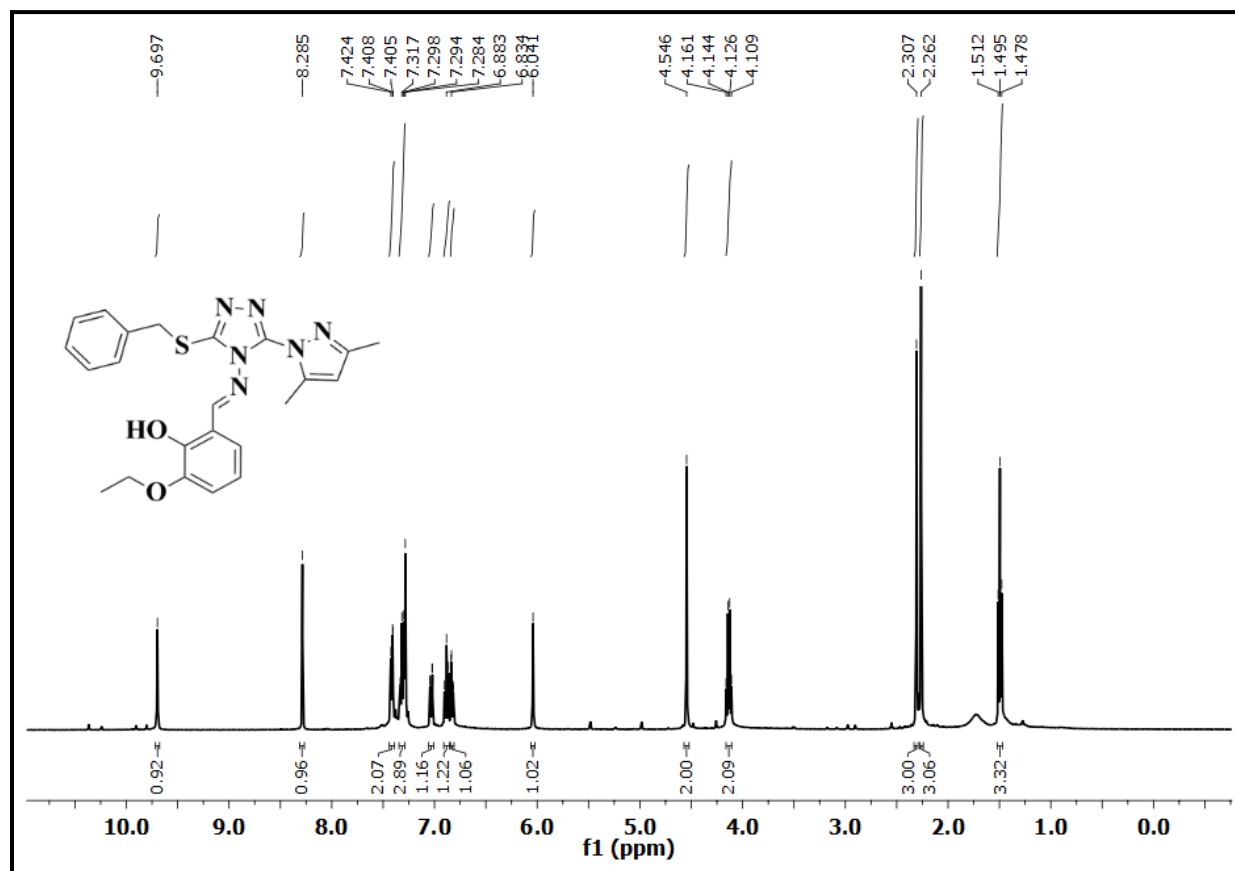


Fig: Mass Spectrum of Compound 59a

Fig: 1H -NMR Spectrum of Compound 59b (400 MHz, $CDCl_3$)

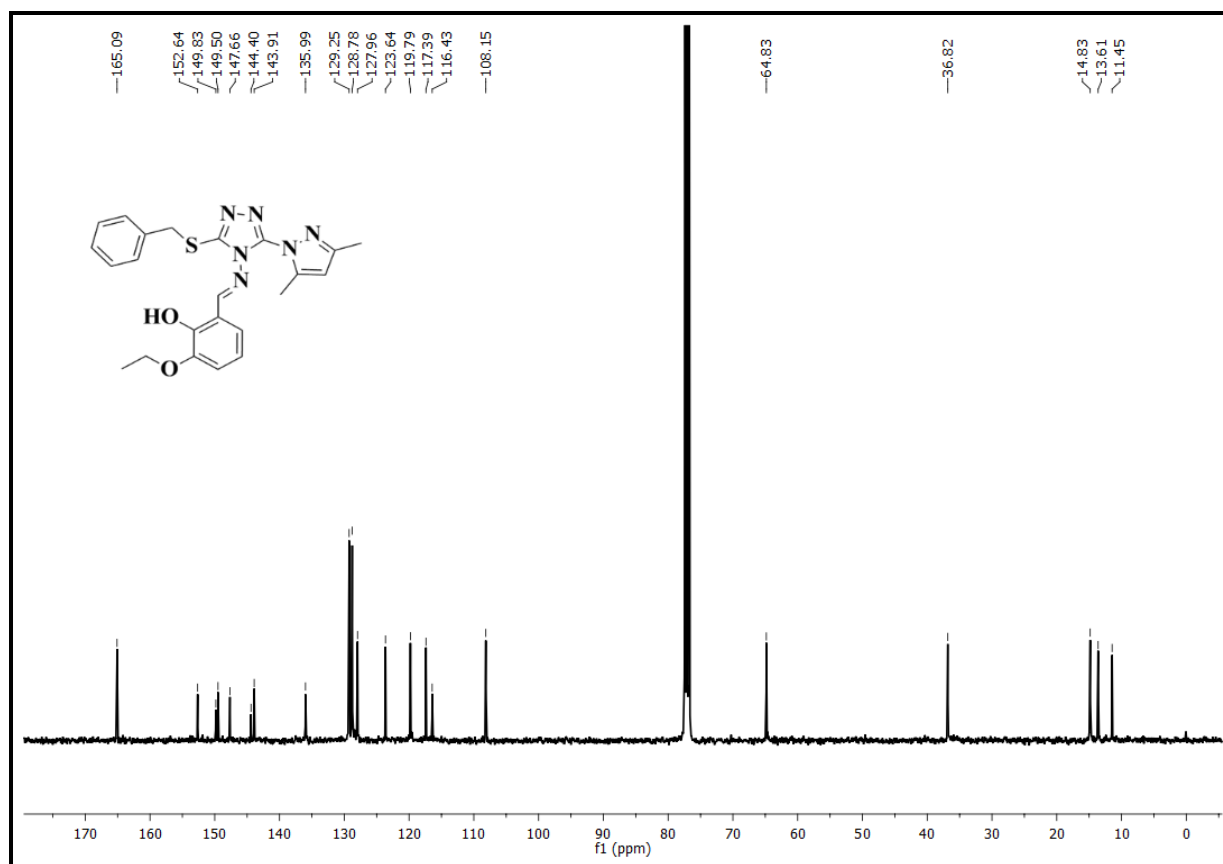


Fig: ^{13}C -NMR Spectrum of Compound **59b** (100 MHz, CDCl_3)

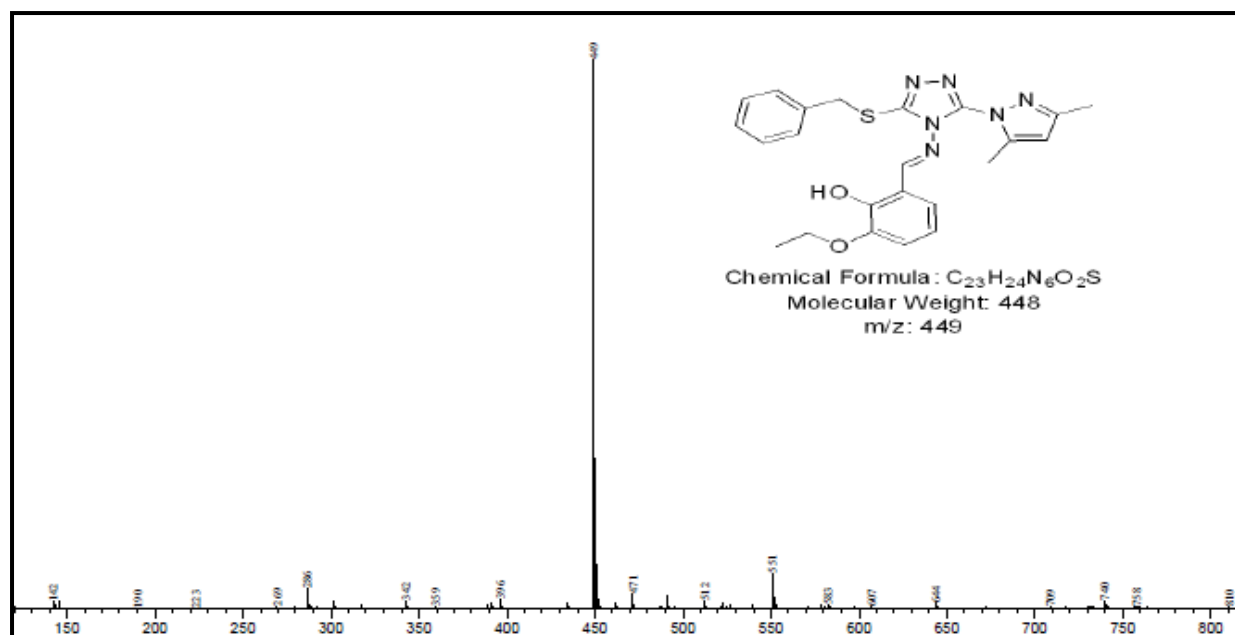


Fig: Mass Spectrum of Compound **59b**

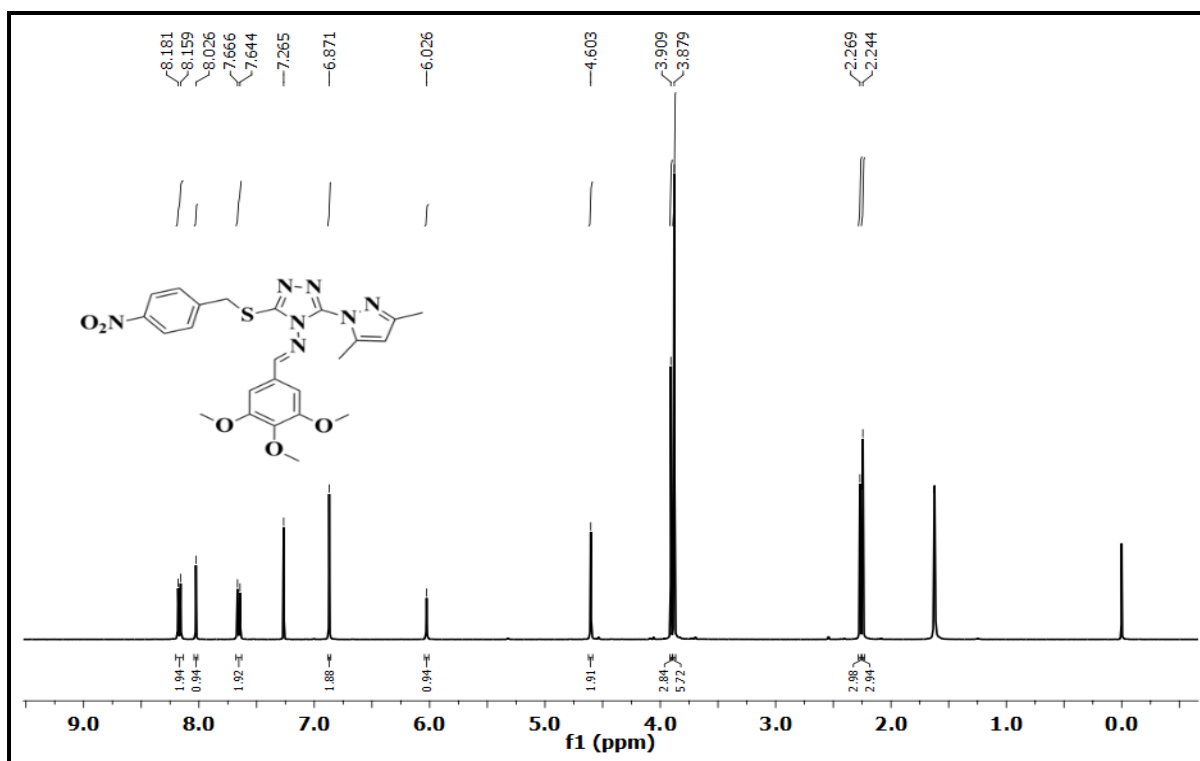


Fig: ¹H-NMR Spectrum of Compound 59c (400 MHz, CDCl₃)

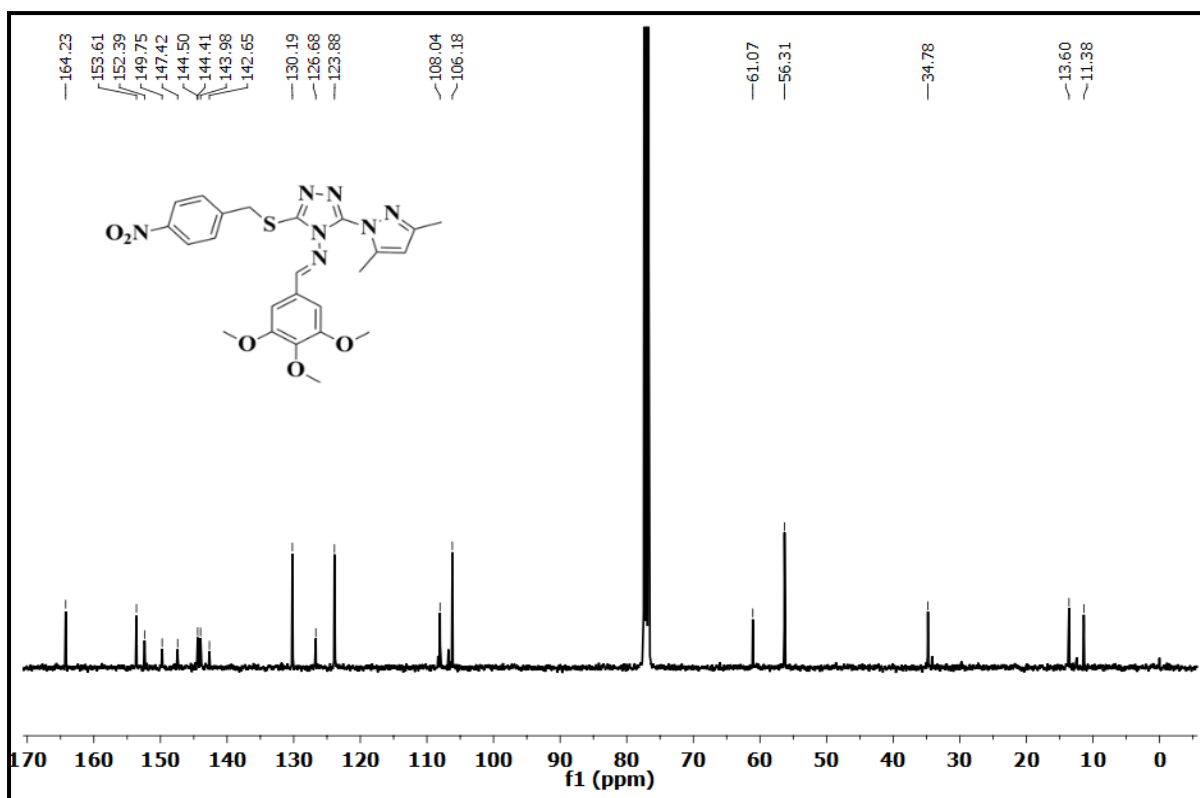


Fig: ¹³C-NMR Spectrum of Compound 59c (100 MHz, CDCl₃)

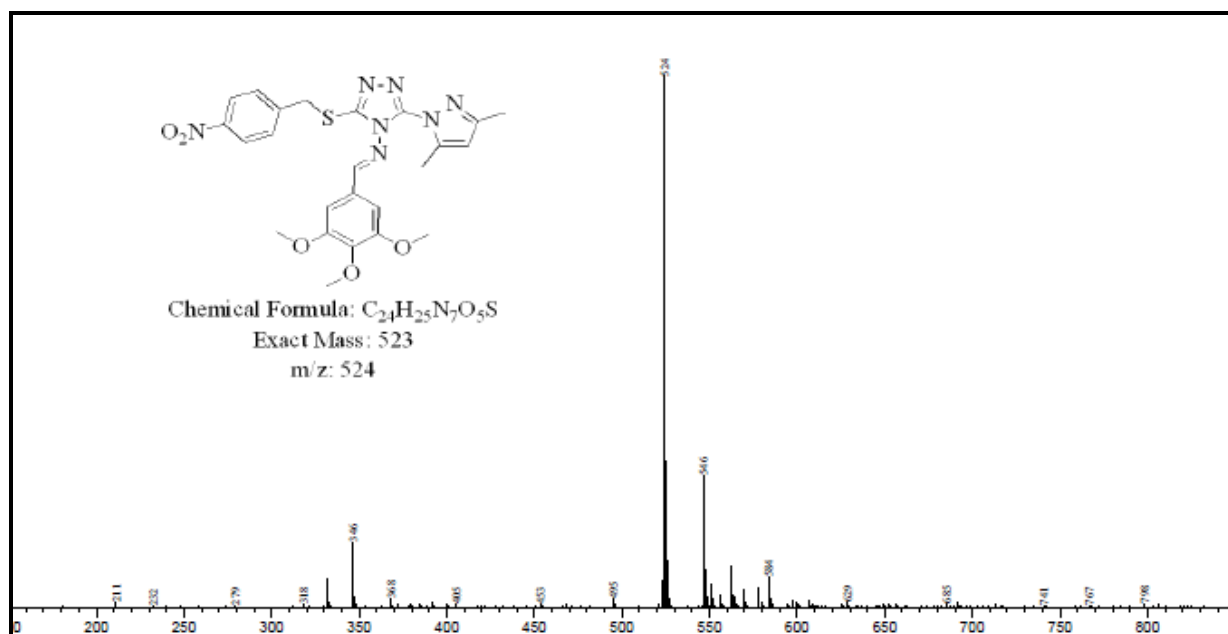


Fig: Mass Spectrum of Compound 59c

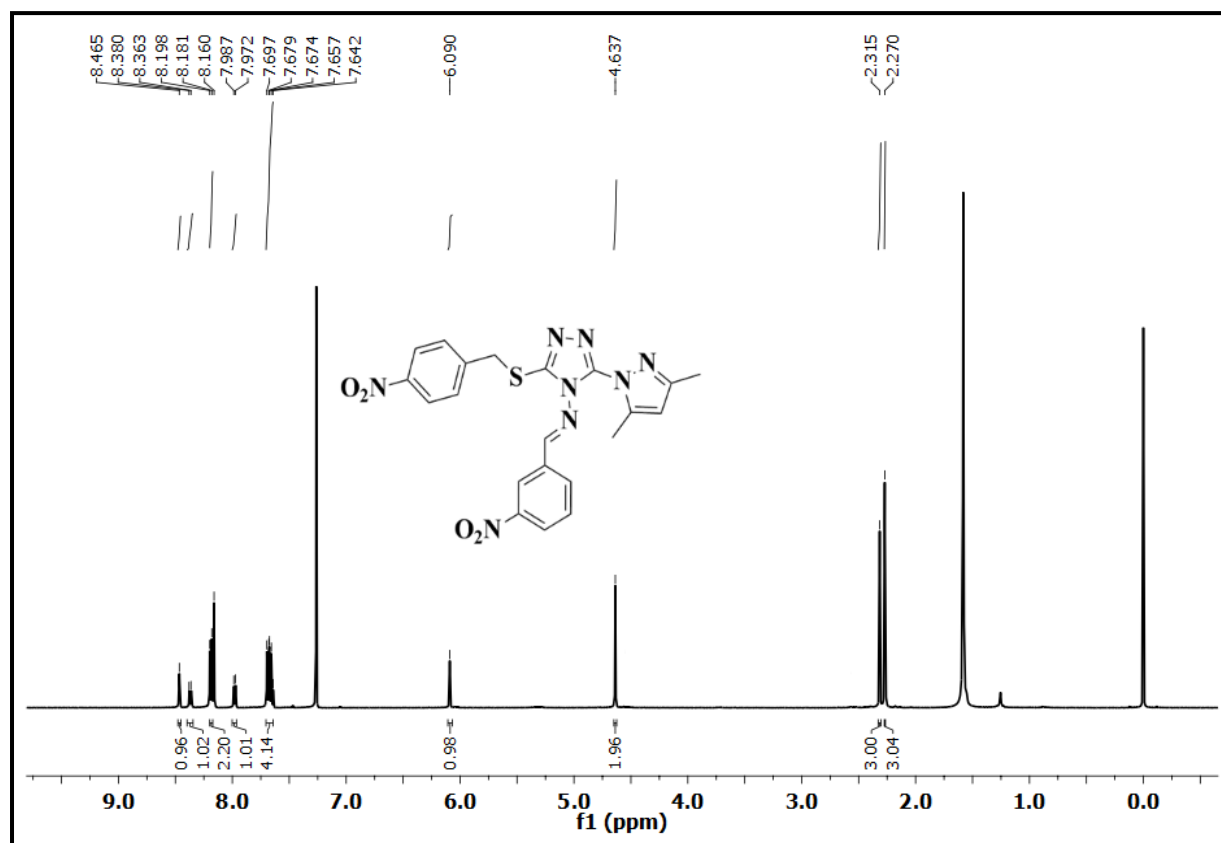


Fig: 1H -NMR Spectrum of Compound 59d (400 MHz, $CDCl_3 + DMSO-d_6$)

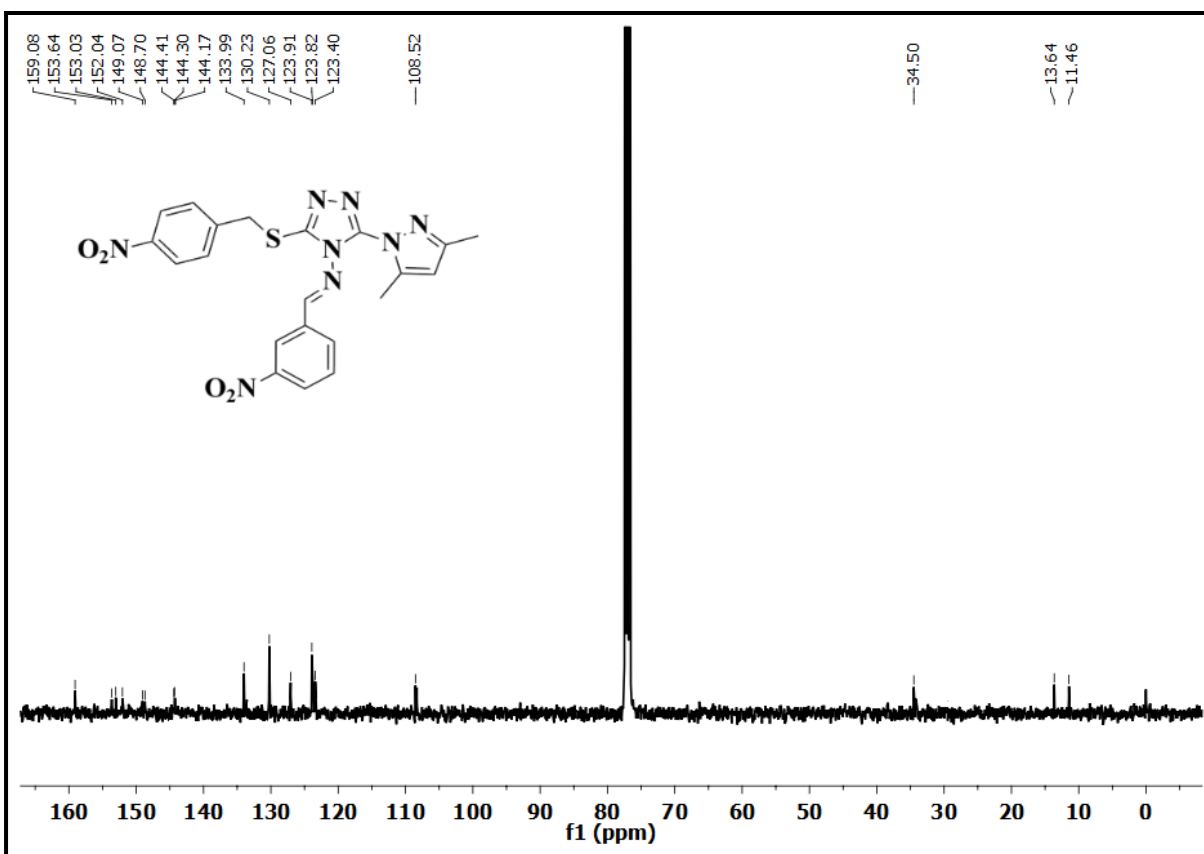


Fig: ^{13}C -NMR Spectrum of Compound **59d** (100 MHz, CDCl_3)

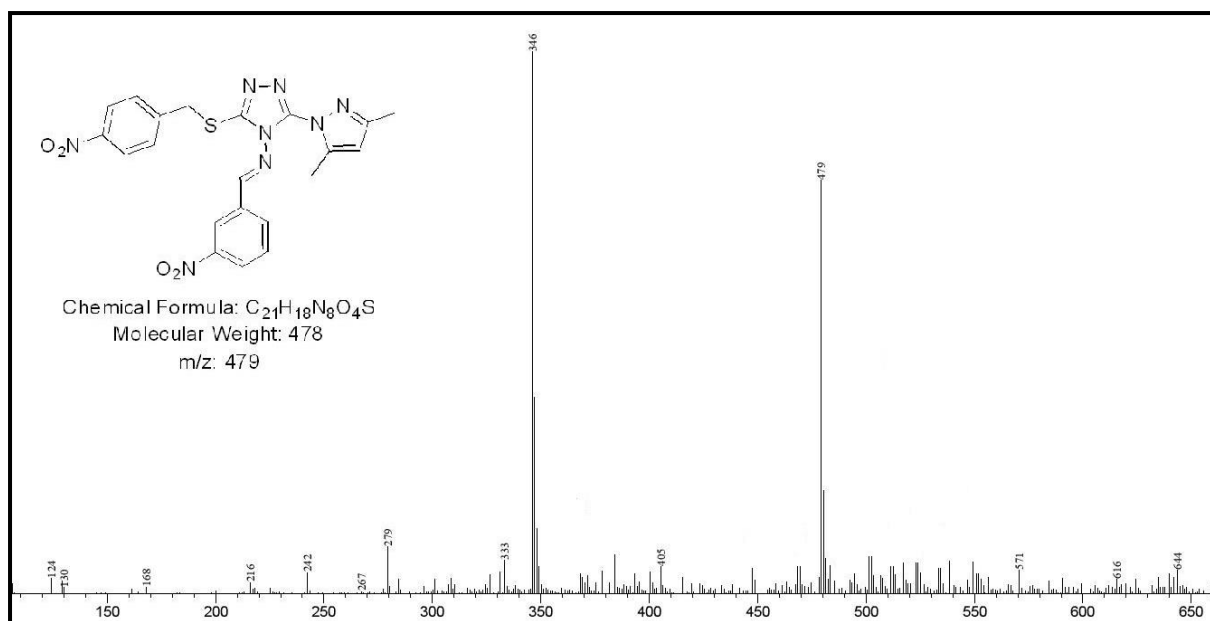


Fig: Mass Spectrum of Compound **59d**

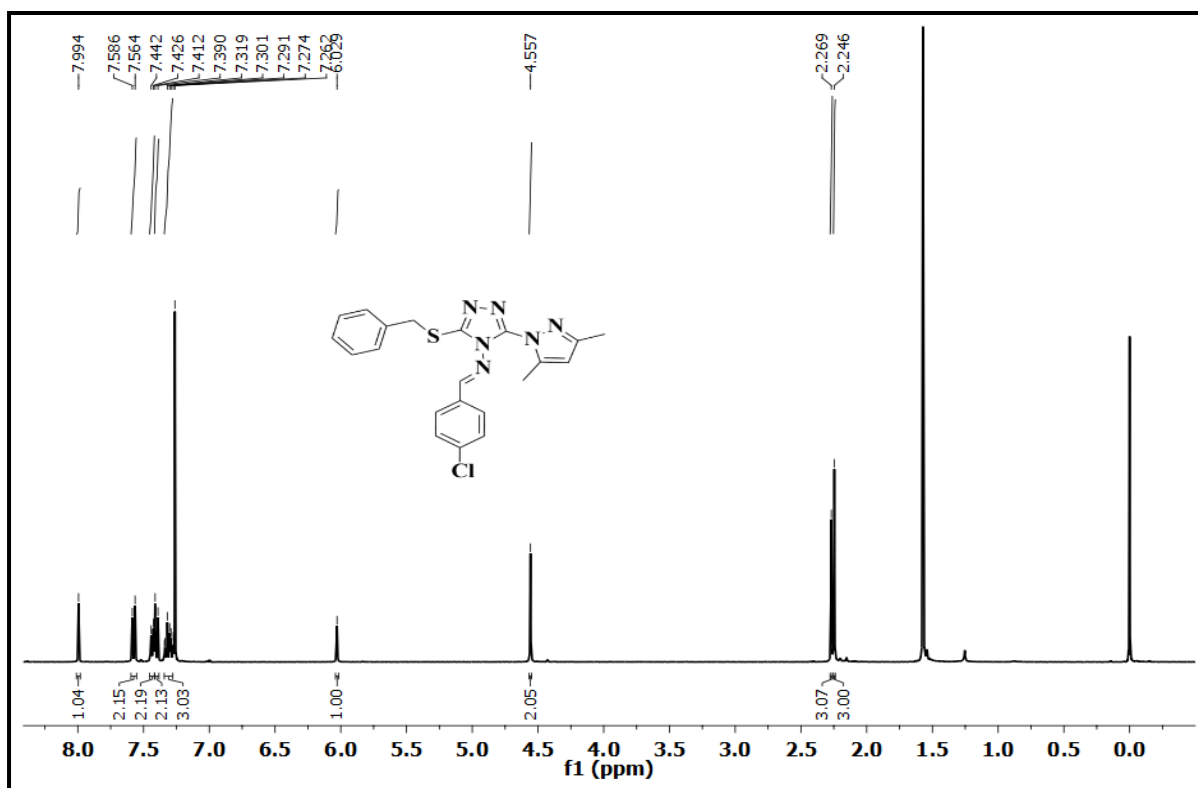


Fig: ¹H-NMR Spectrum of Compound 59e (400 MHz, CDCl₃)

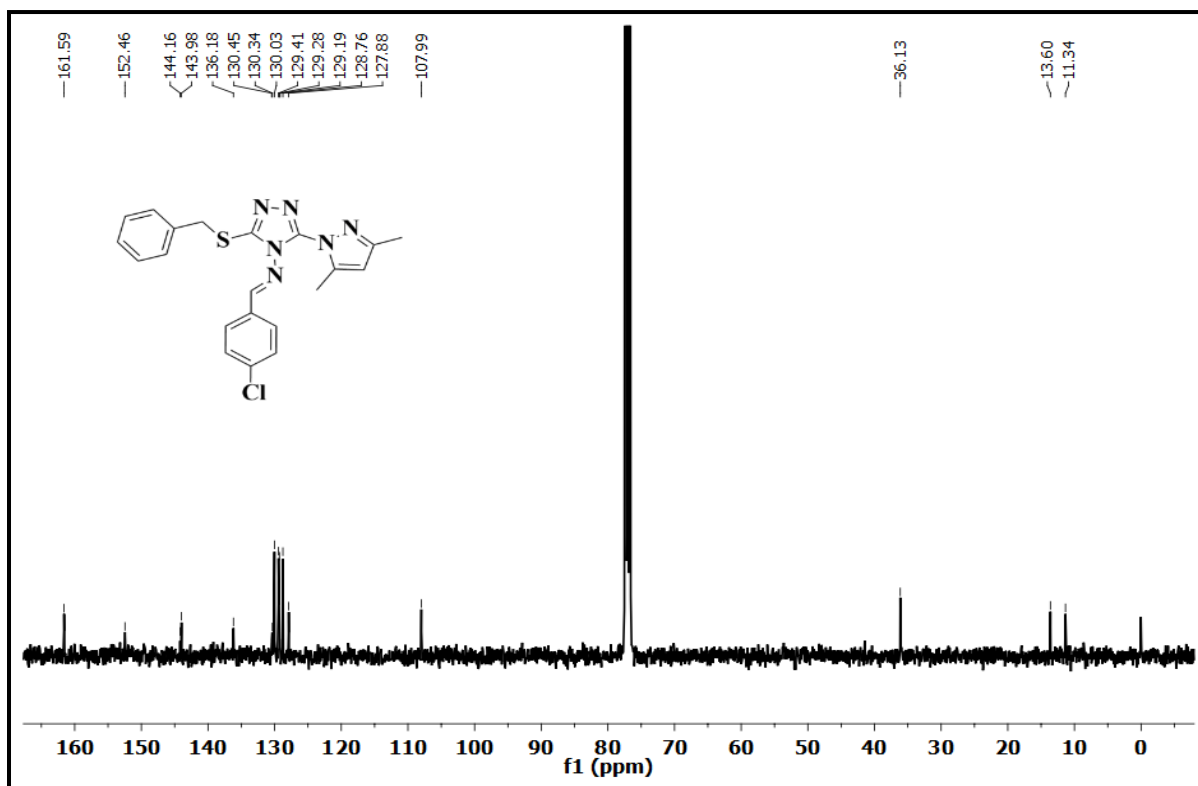


Fig: ¹³C-NMR Spectrum of Compound 59e (100 MHz, CDCl₃)

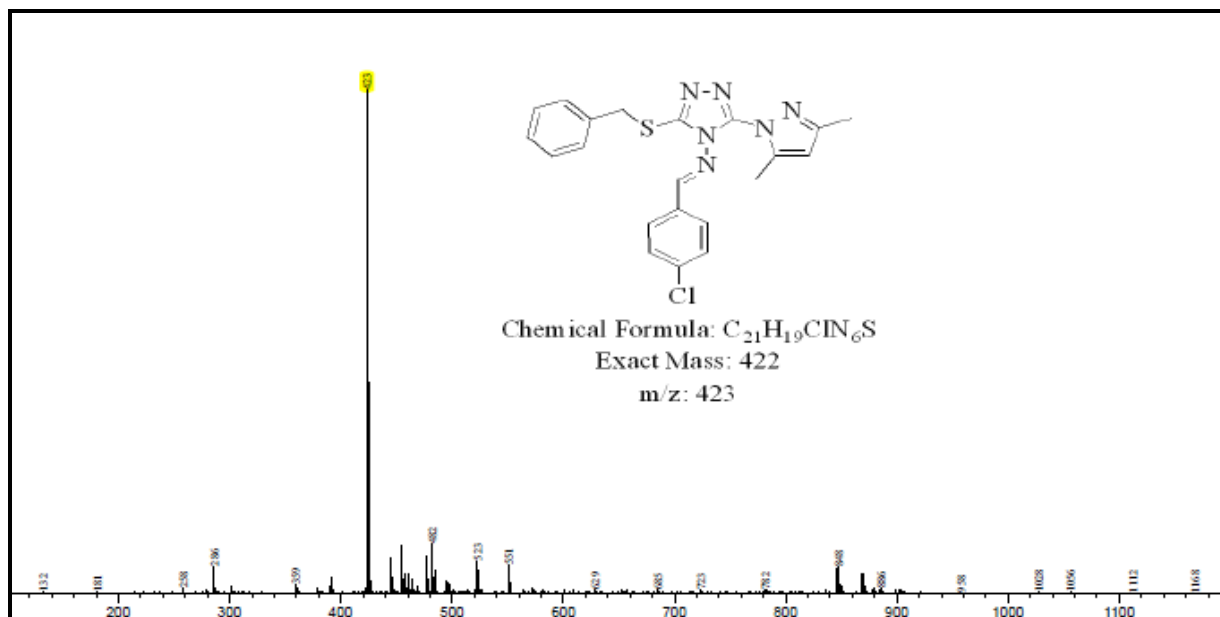


Fig: Mass Spectrum of Compound 59e

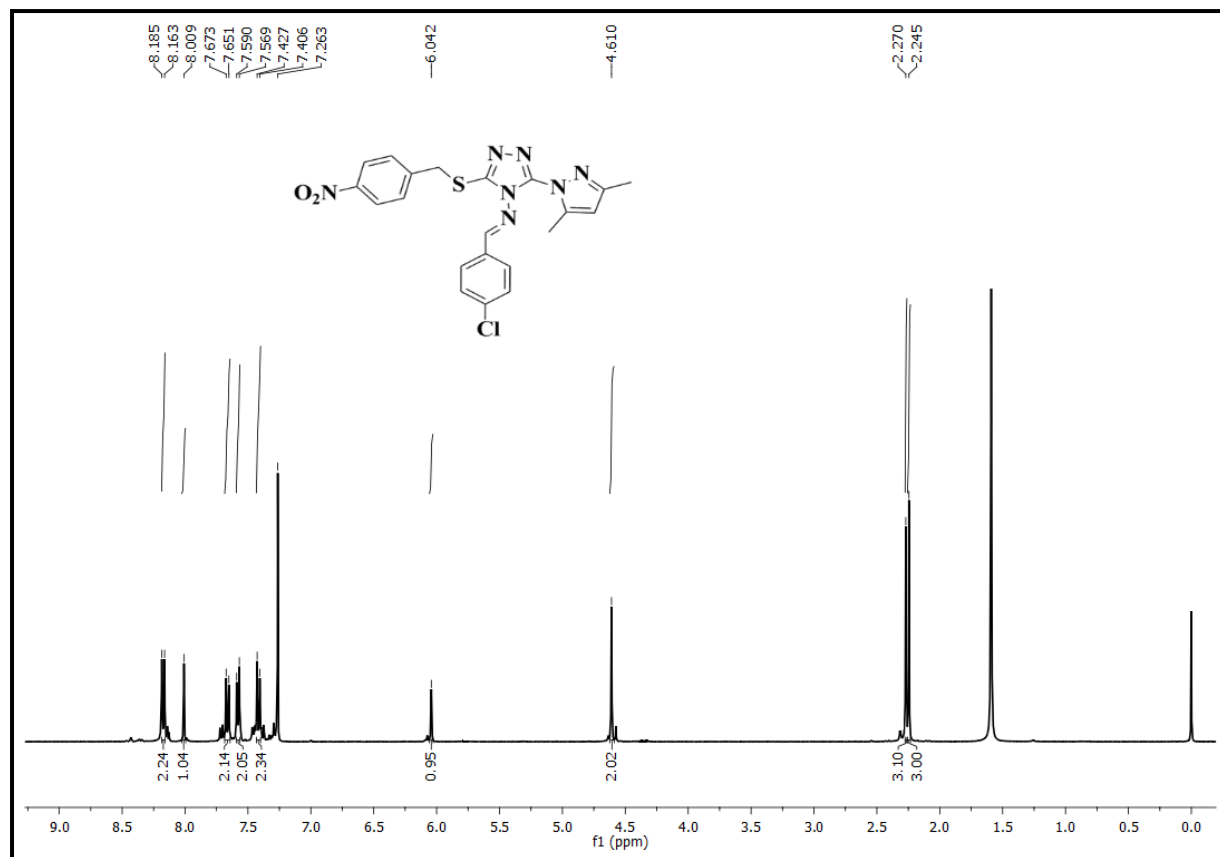


Fig: 1H -NMR Spectrum of Compound 59f (400 MHz, $CDCl_3$)

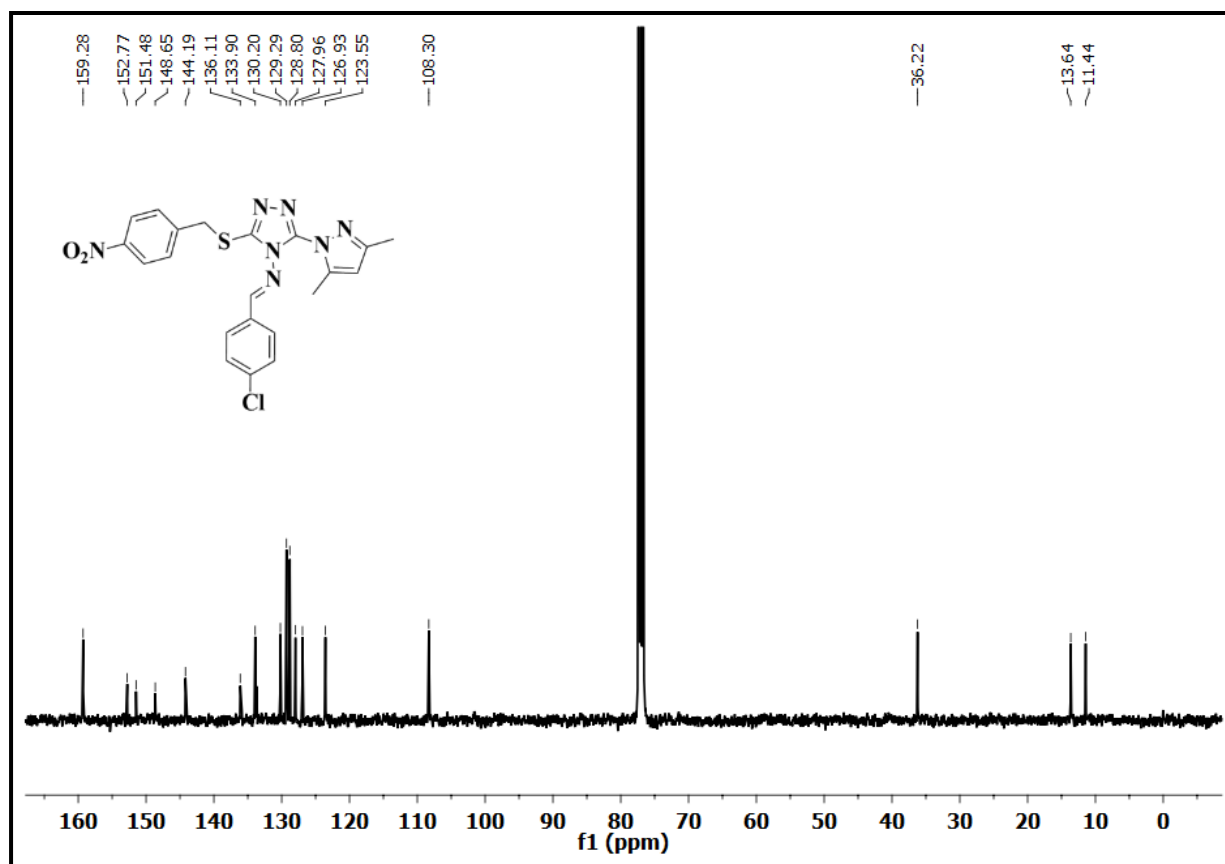


Fig: ^{13}C -NMR Spectrum of Compound **59f** (100 MHz, CDCl_3)

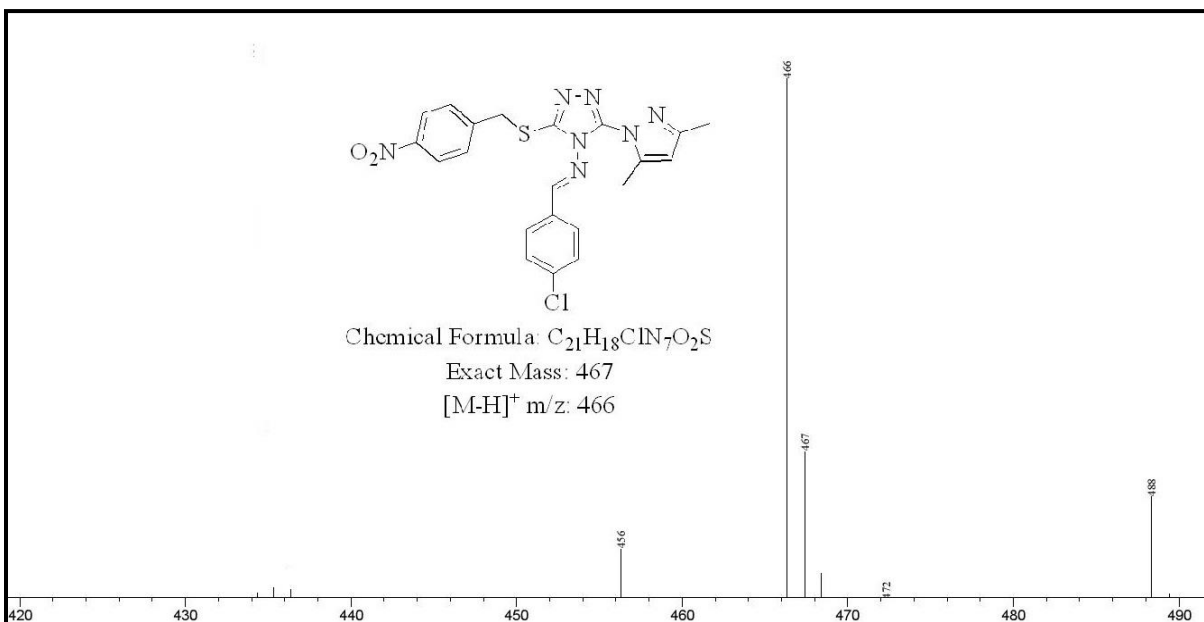


Fig: Mass Spectrum of Compound **59f**

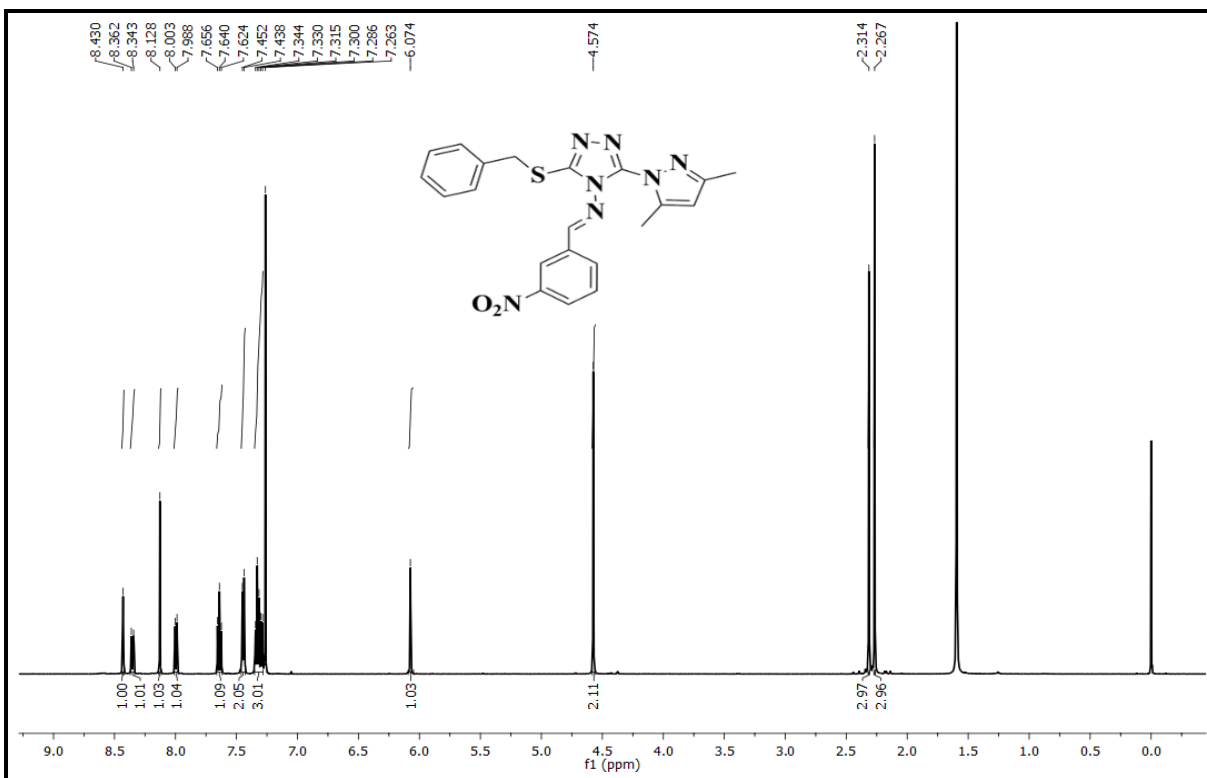


Fig: ¹H-NMR Spectrum of Compound **59g** (400 MHz, CDCl₃)

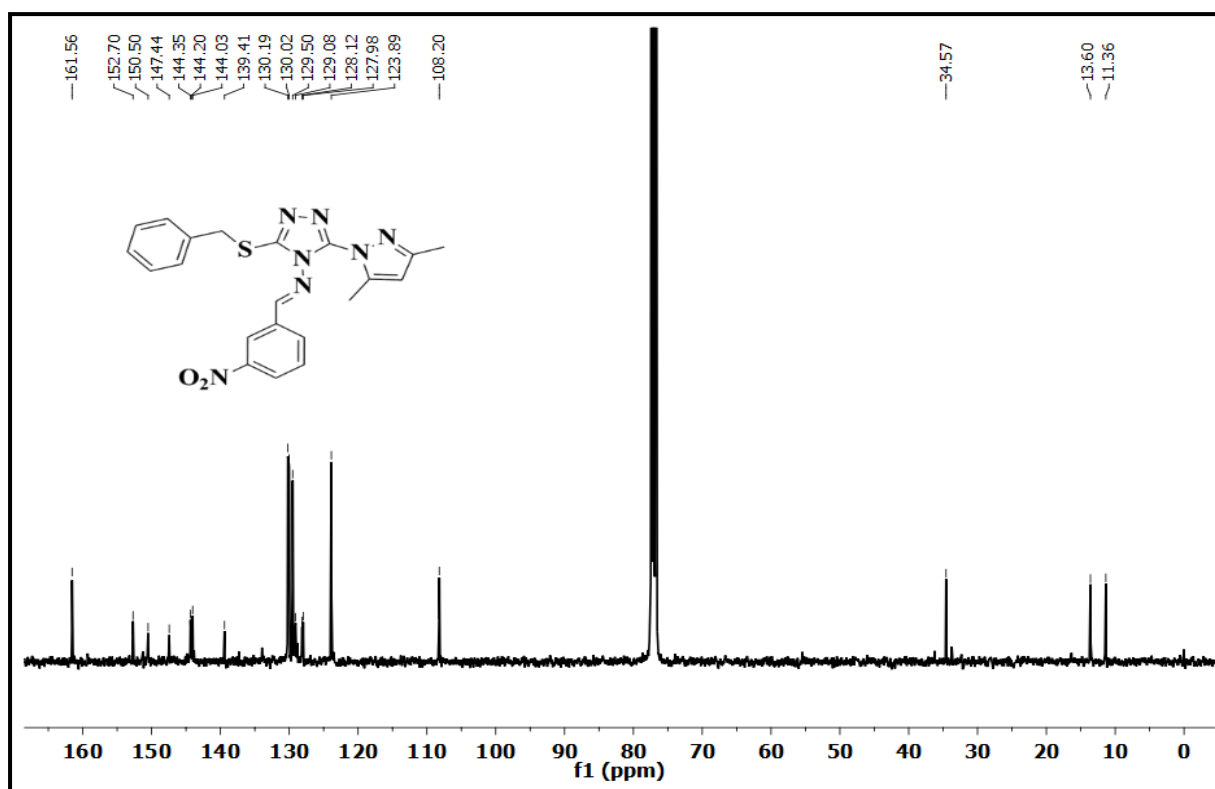


Fig: ¹³C-NMR Spectrum of Compound **59g** (100 MHz, CDCl₃)

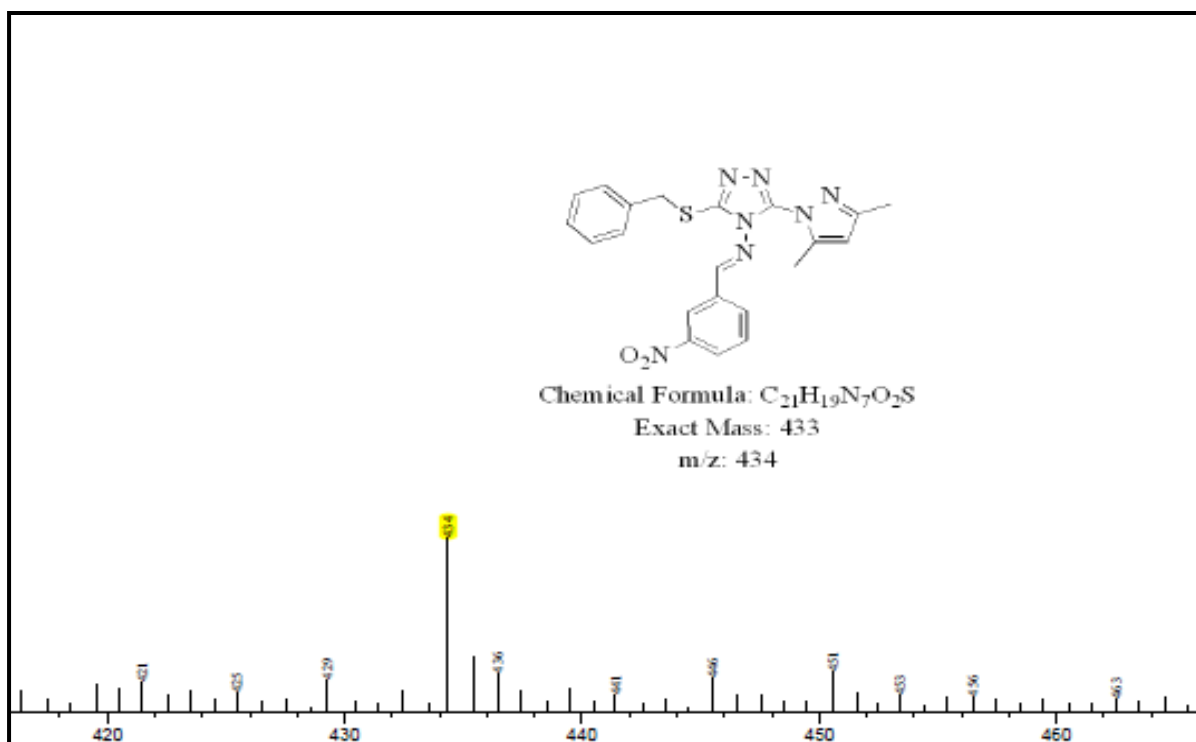


Fig: Mass Spectrum of Compound 59g

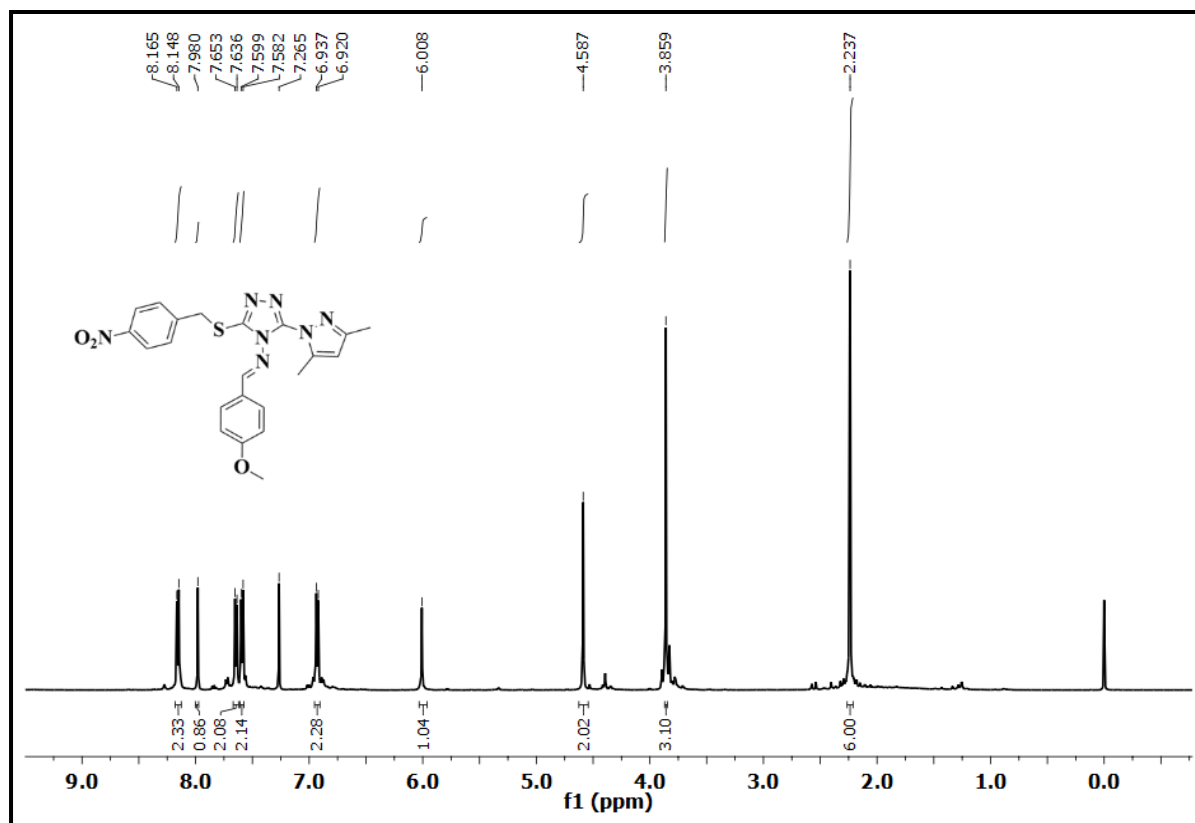


Fig: 1H -NMR Spectrum of Compound 59h (400 MHz, $CDCl_3$)

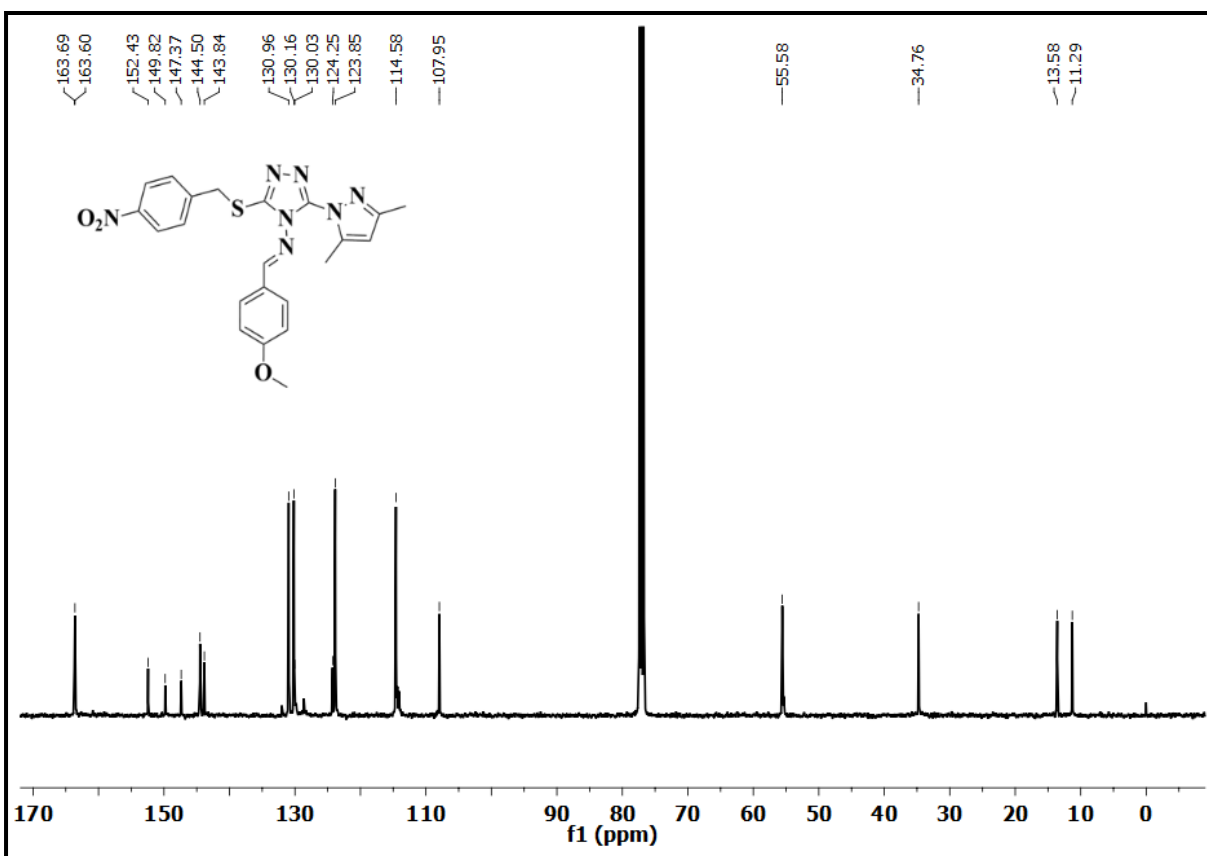


Fig: ^{13}C -NMR Spectrum of Compound **59h** (100 MHz, CDCl_3)

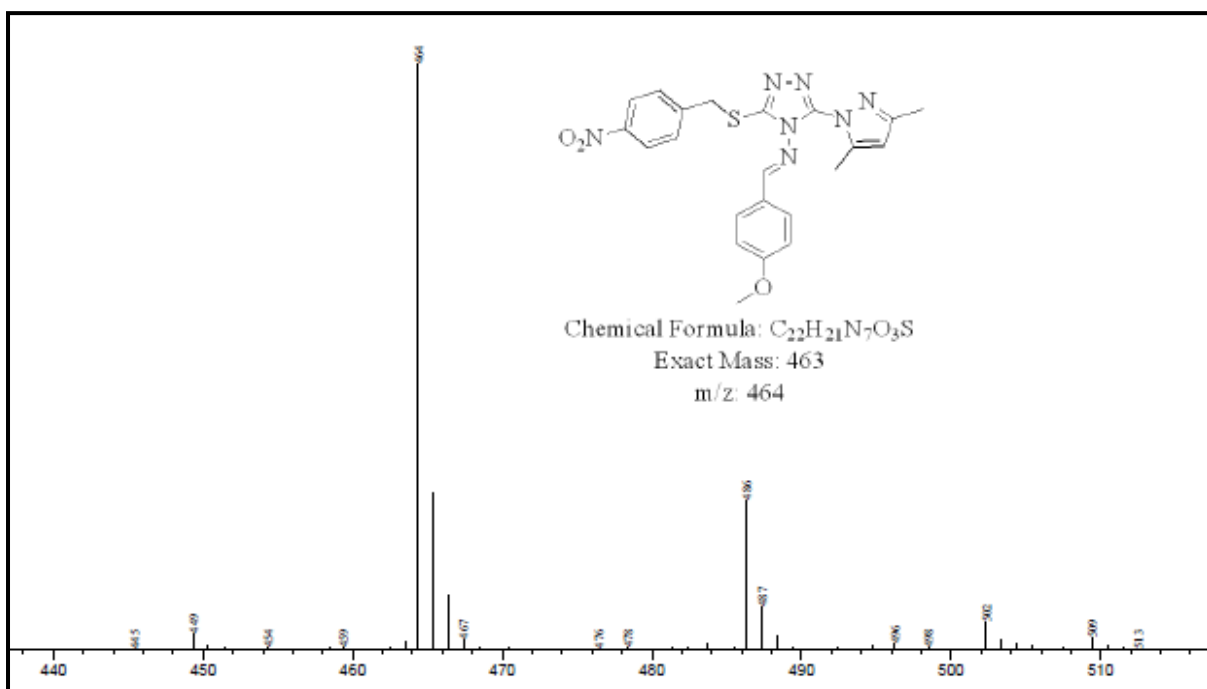


Fig: Mass Spectrum of Compound **59h**

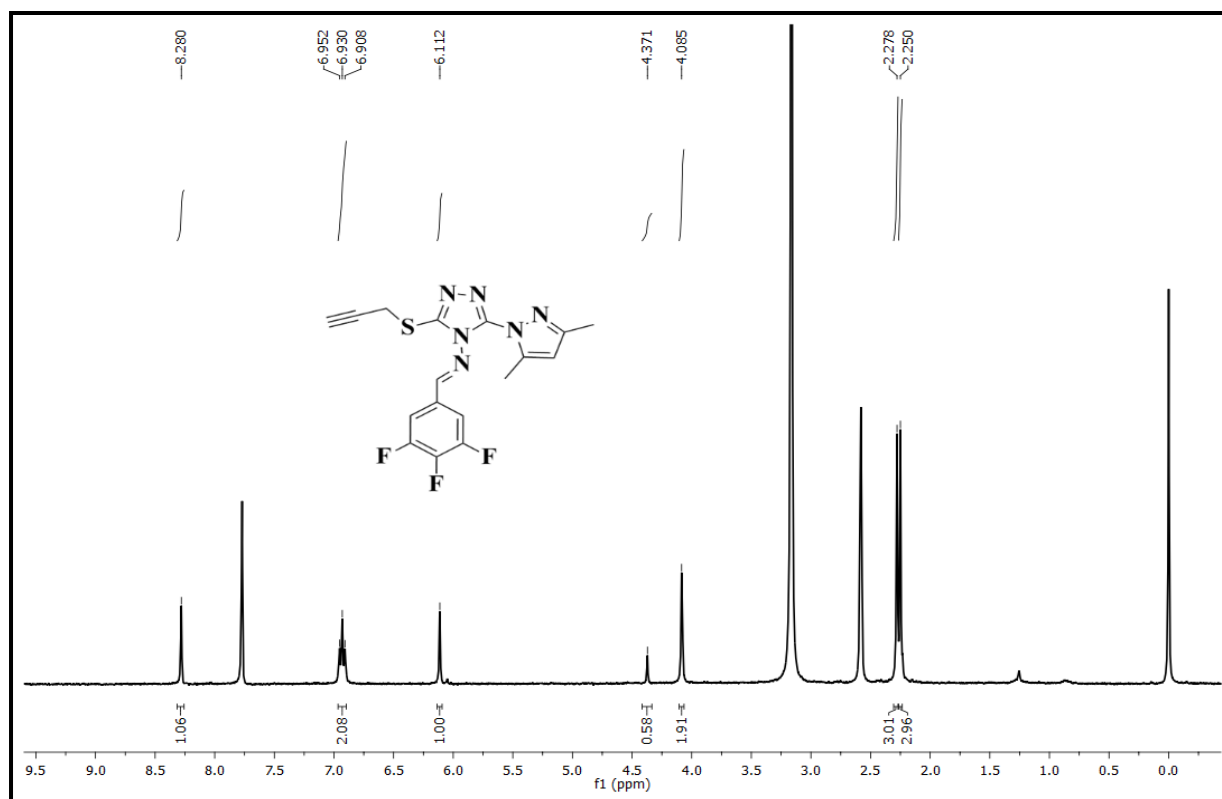


Fig: ¹H-NMR Spectrum of Compound **59i** (400 MHz, CDCl₃+DMSO-*d*₆)

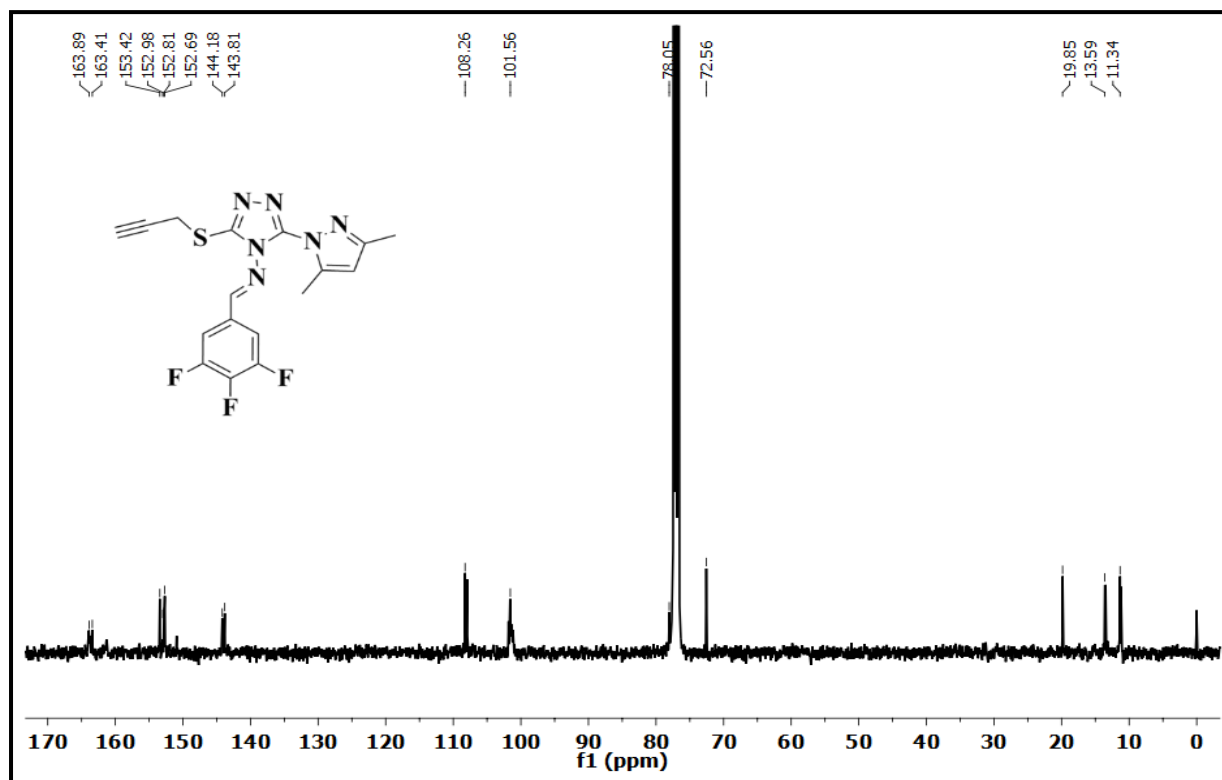


Fig: ¹³C-NMR Spectrum of Compound **59i** (100 MHz, CDCl₃)

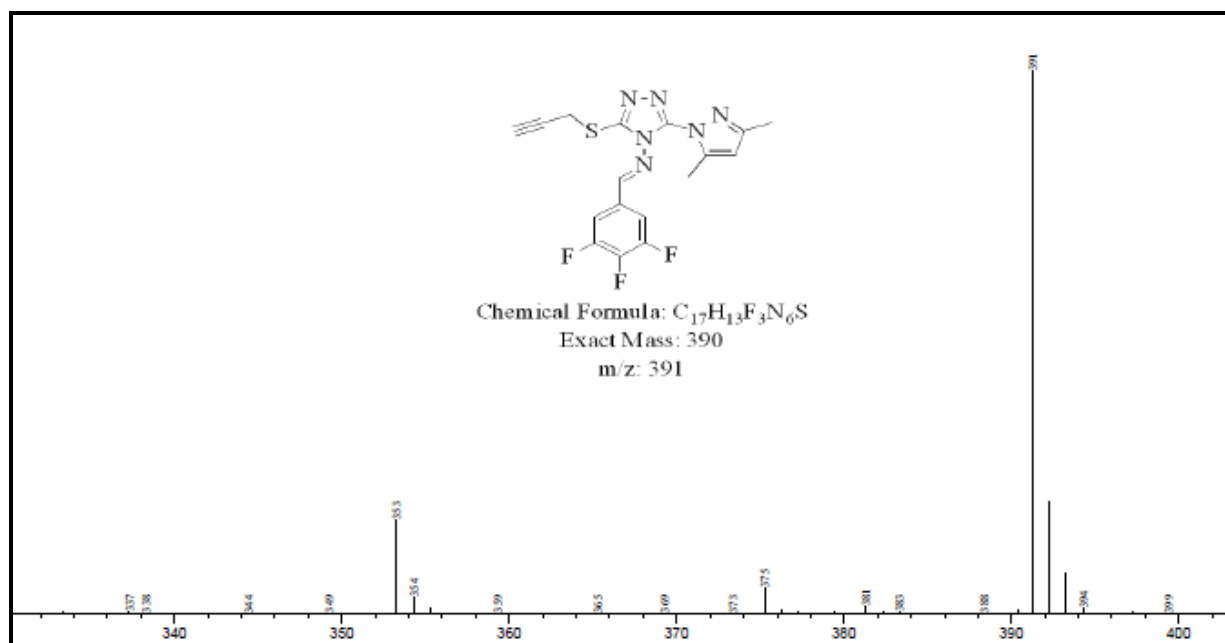


Fig: Mass Spectrum of Compound **59i**

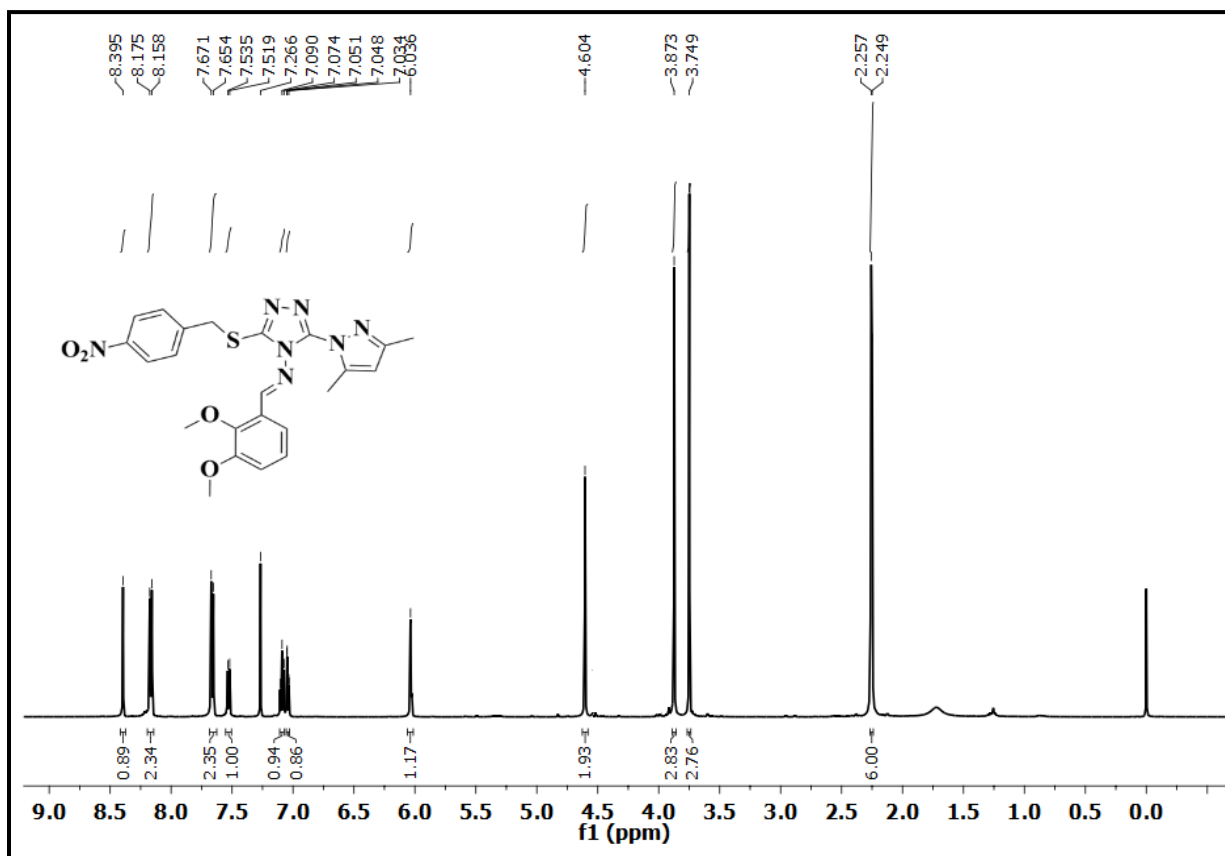


Fig: ^1H -NMR Spectrum of Compound **59j** (400 MHz, CDCl_3)

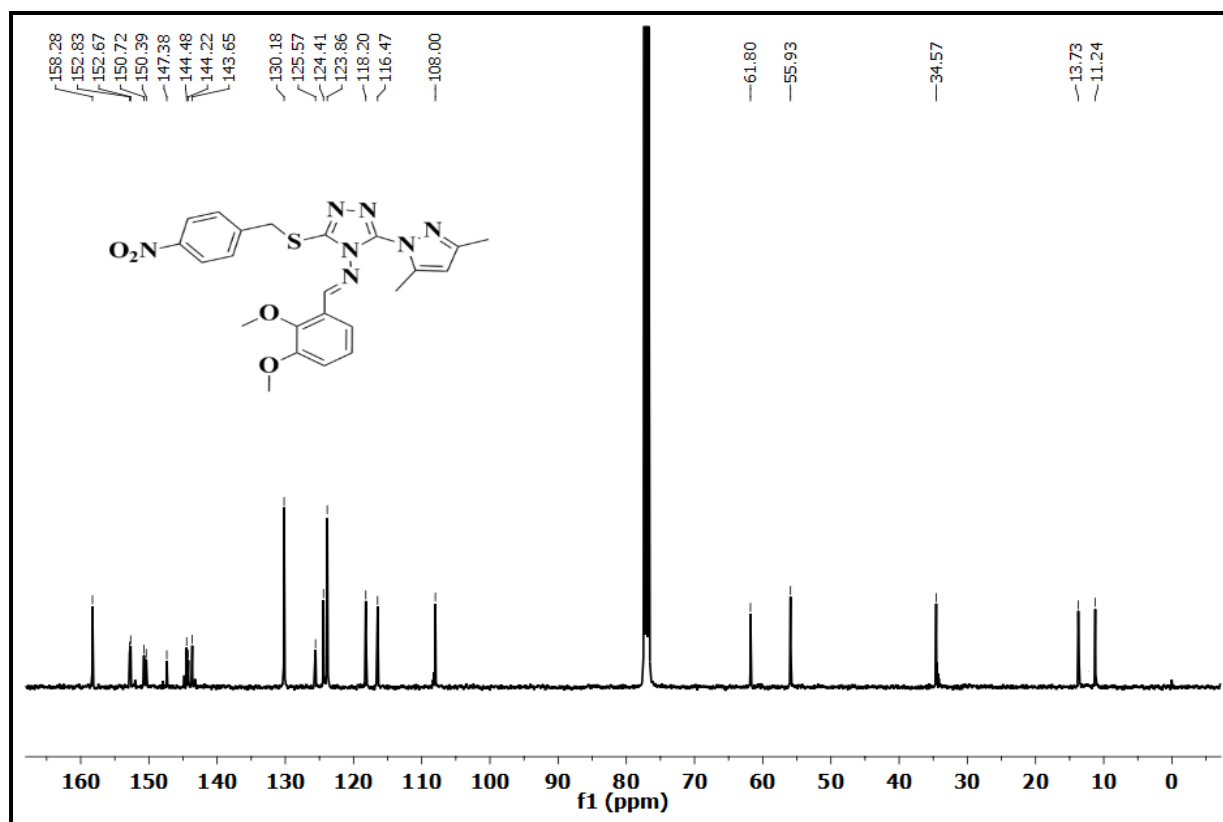


Fig: ¹³C-NMR Spectrum of Compound **59j** (100 MHz, CDCl₃)

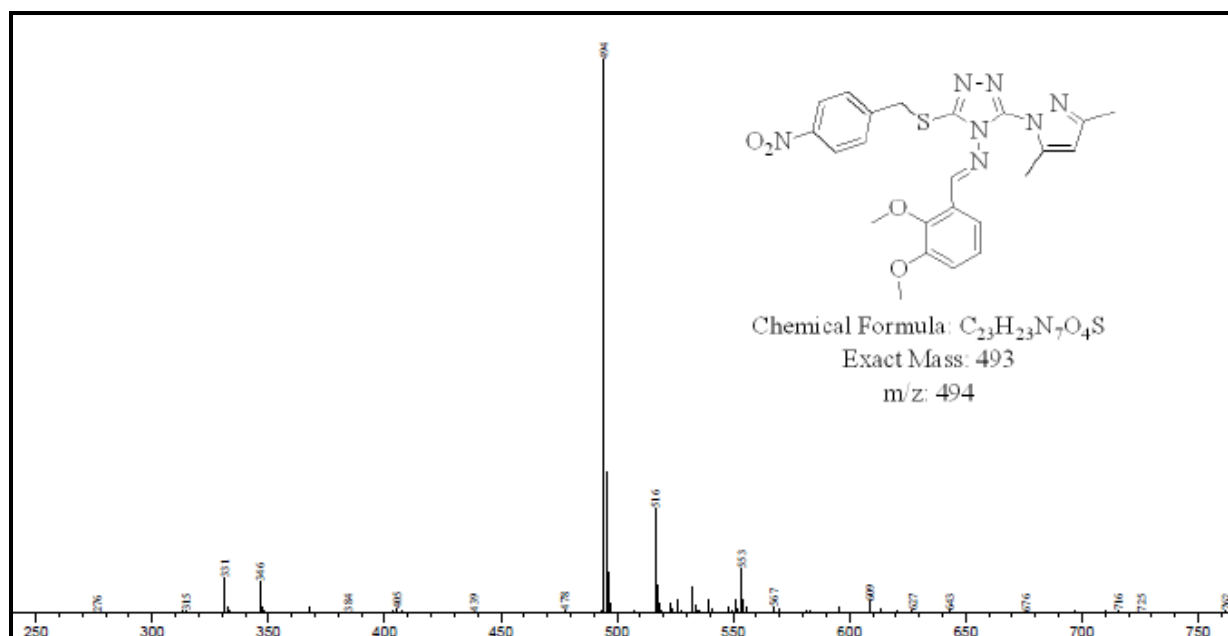


Fig: Mass Spectrum of Compound **59j**

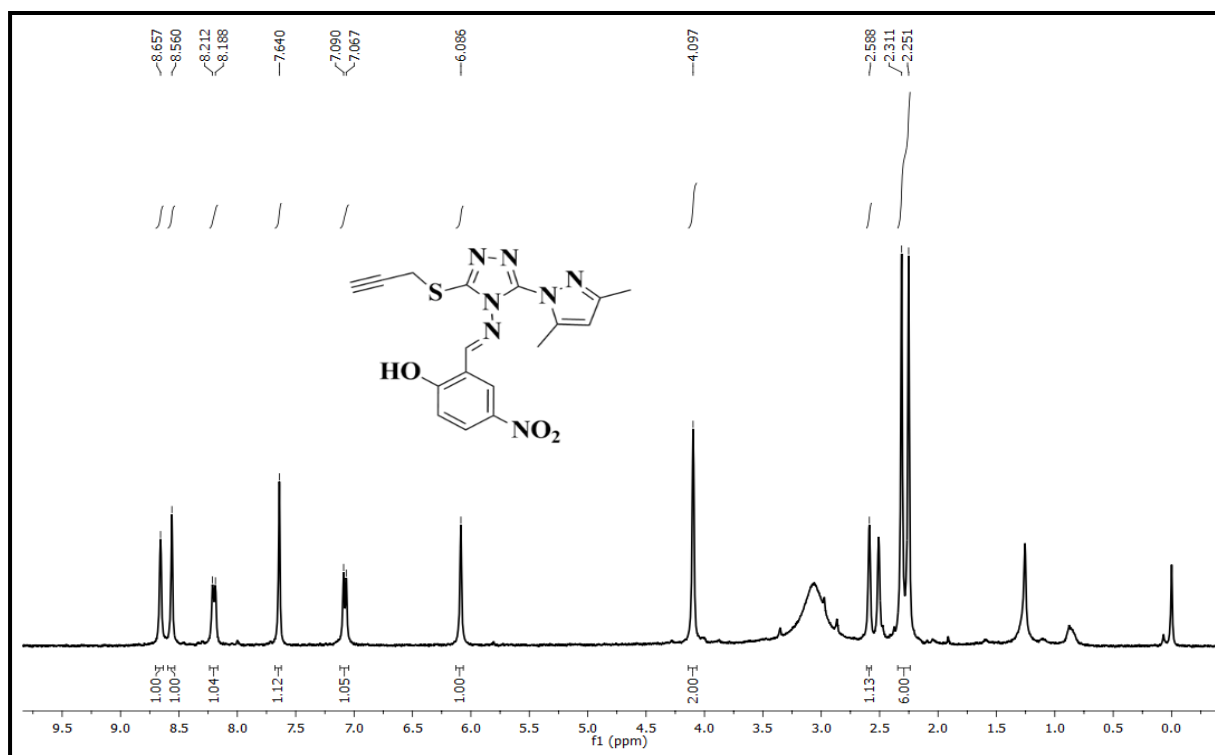


Fig: ¹H-NMR Spectrum of Compound 59k (400 MHz, DMSO-*d*₆)

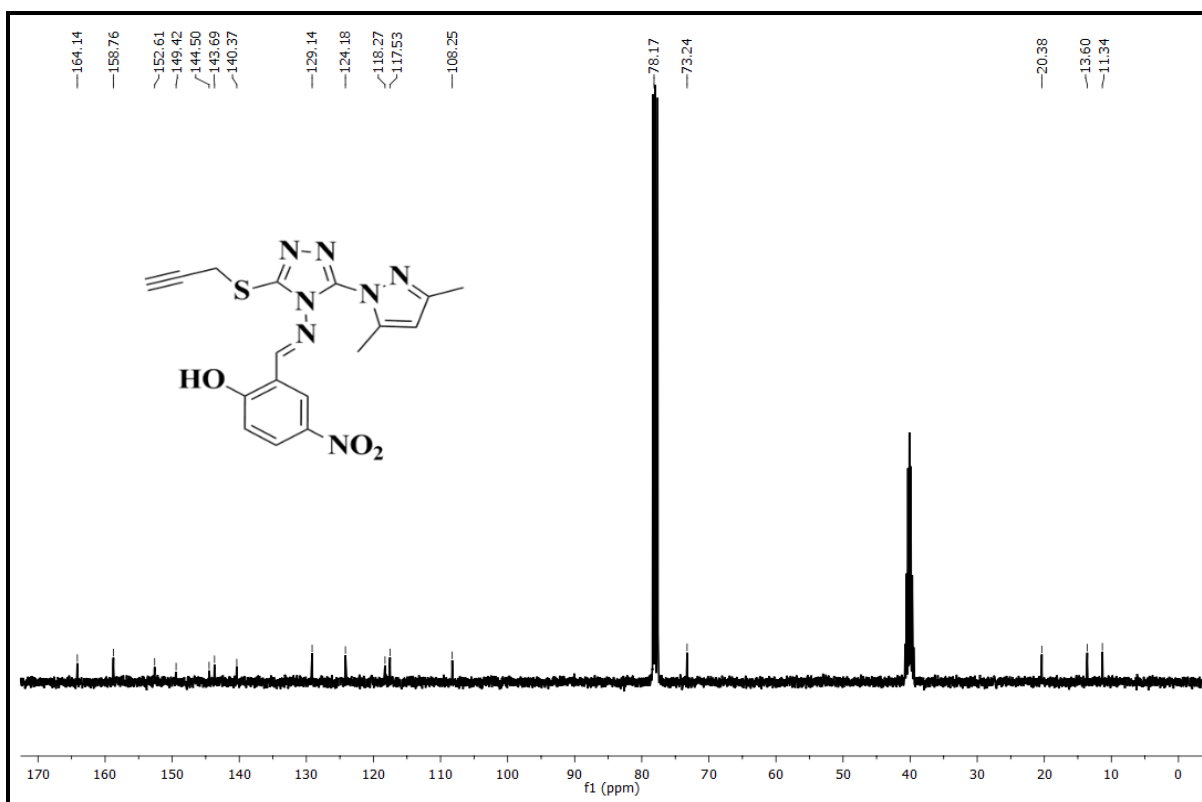


Fig: ¹³C-NMR Spectrum of Compound 59k (100 MHz, CDCl₃+DMSO-*d*₆)

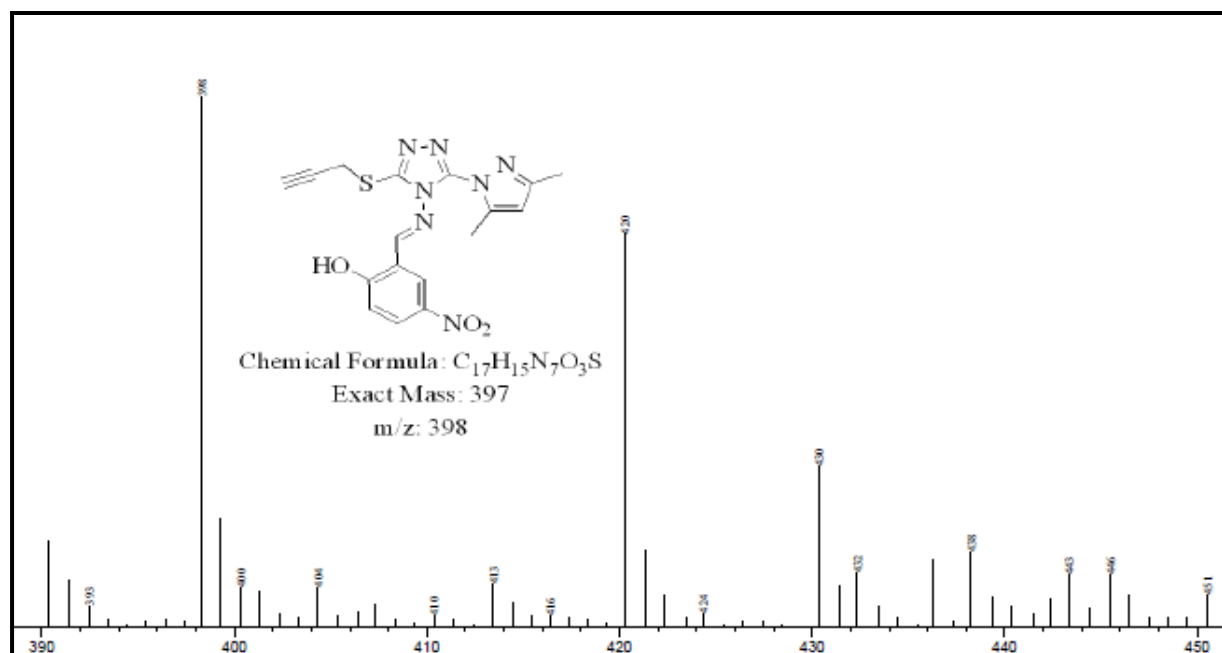


Fig: Mass Spectrum of Compound 59k

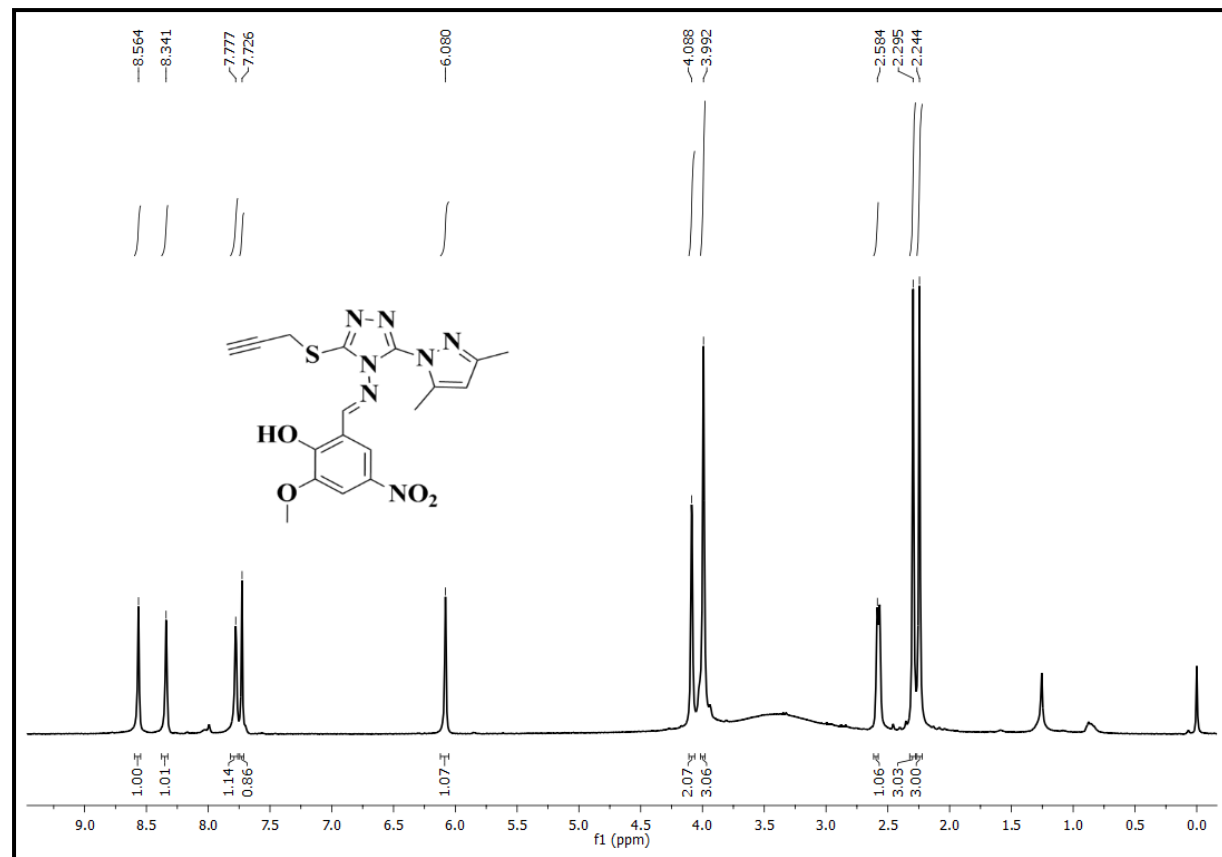


Fig: ^1H -NMR Spectrum of Compound 59l (400 MHz, $\text{DMSO}-d_6$)

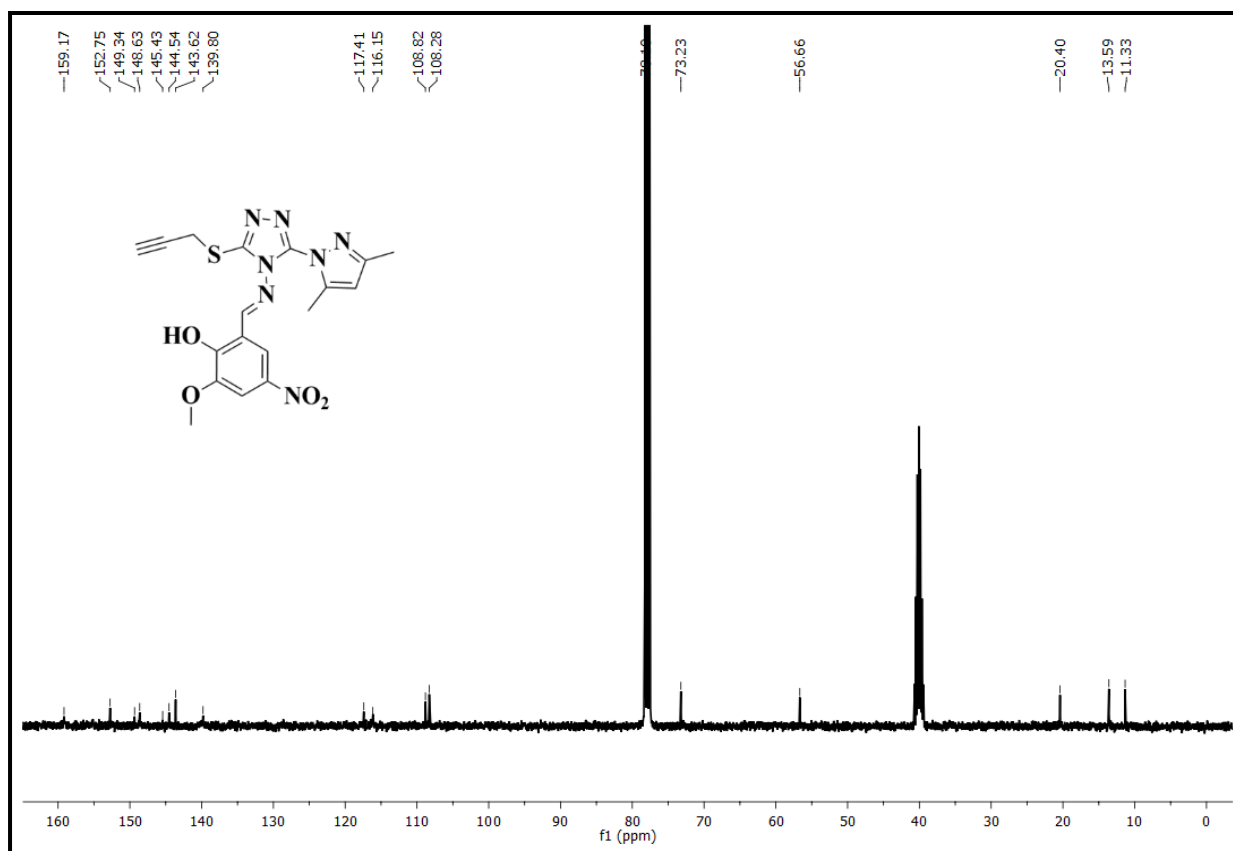


Fig: ^{13}C -NMR Spectrum of Compound **59I** (100 MHz, $\text{CDCl}_3 + \text{DMSO}-d_6$)

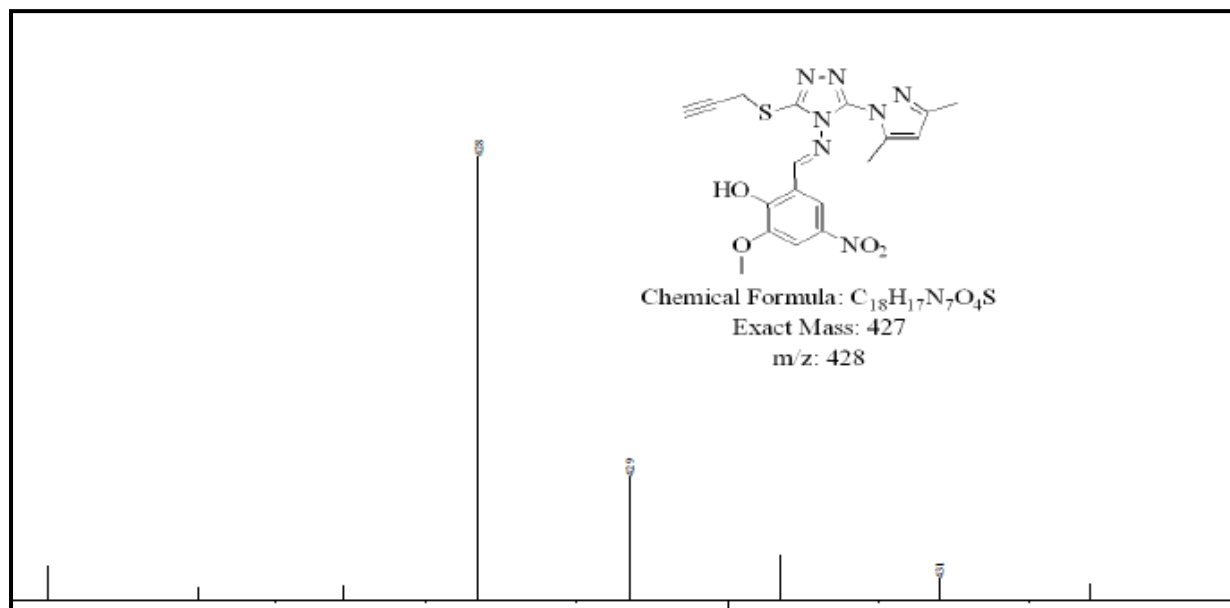


Fig: Mass Spectrum of Compound **59I**

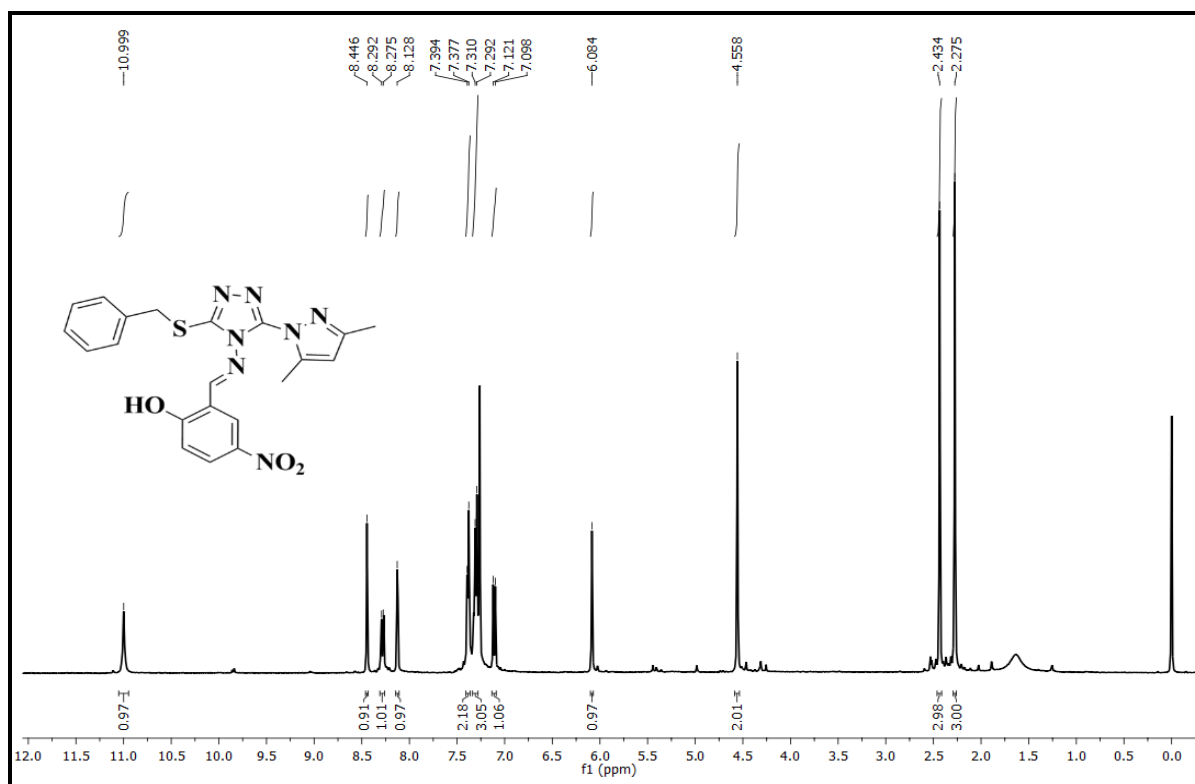


Fig: ¹H-NMR Spectrum of Compound 59m (400 MHz, CDCl₃)

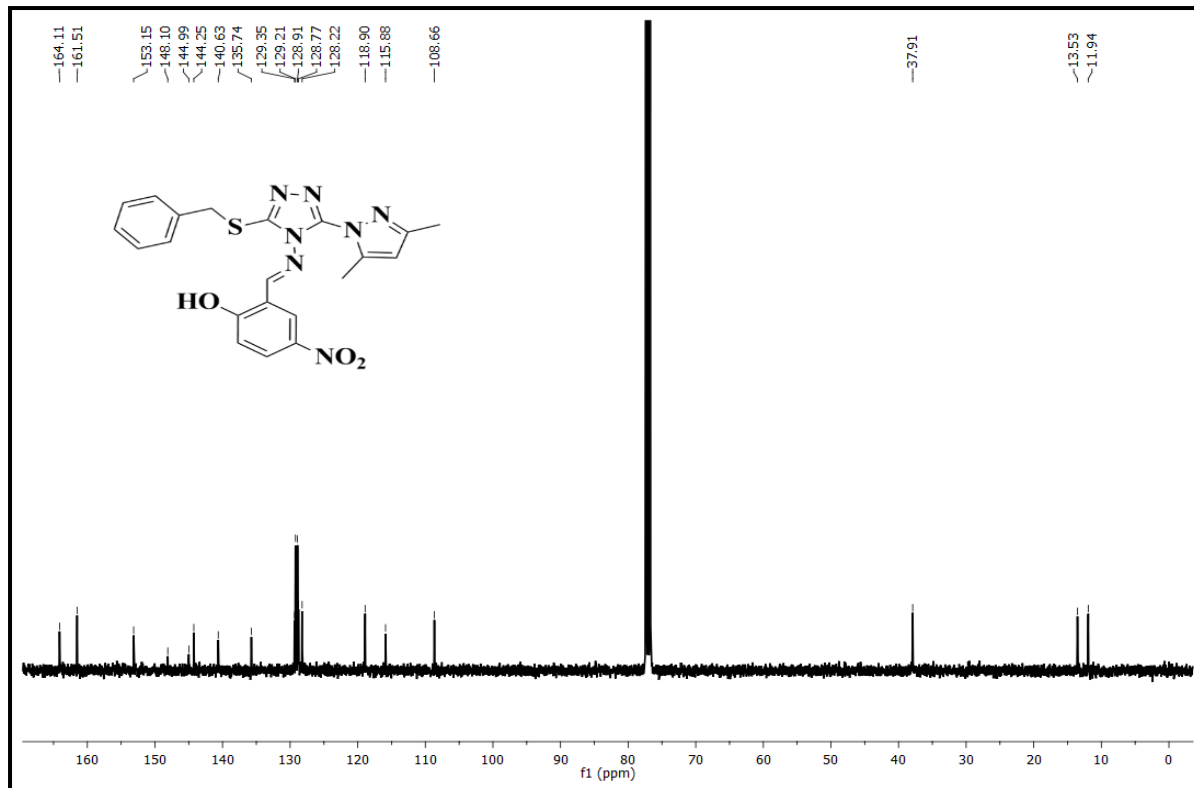


Fig: ¹³C-NMR Spectrum of Compound 59m (100 MHz, CDCl₃)

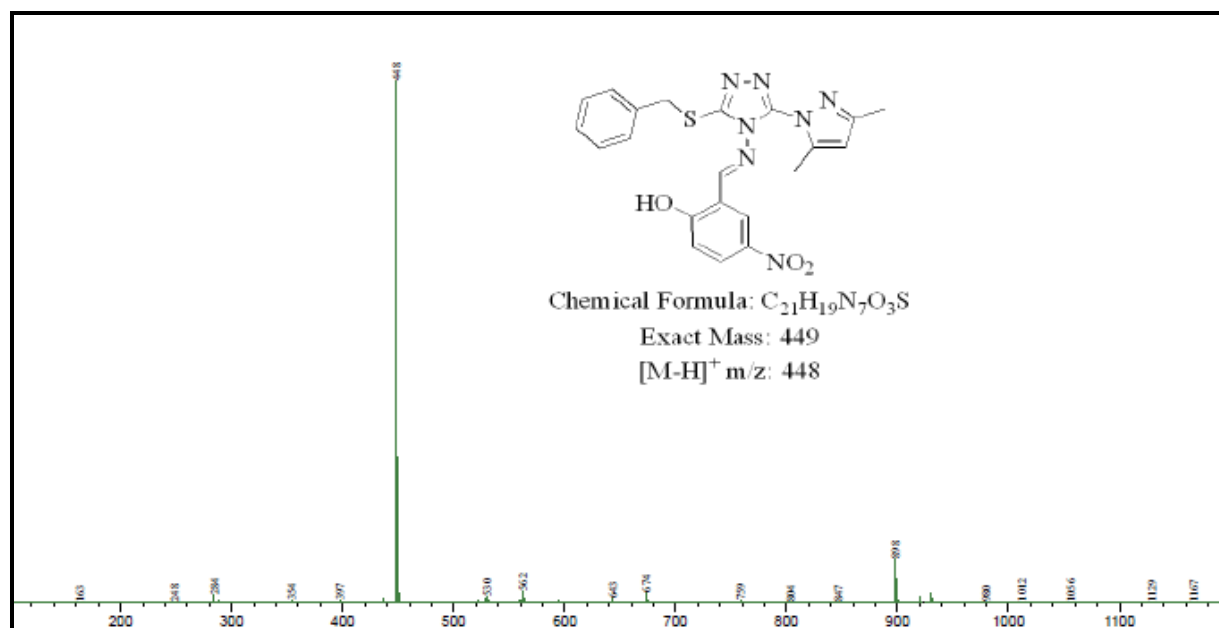


Fig: Mass Spectrum of Compound 59m

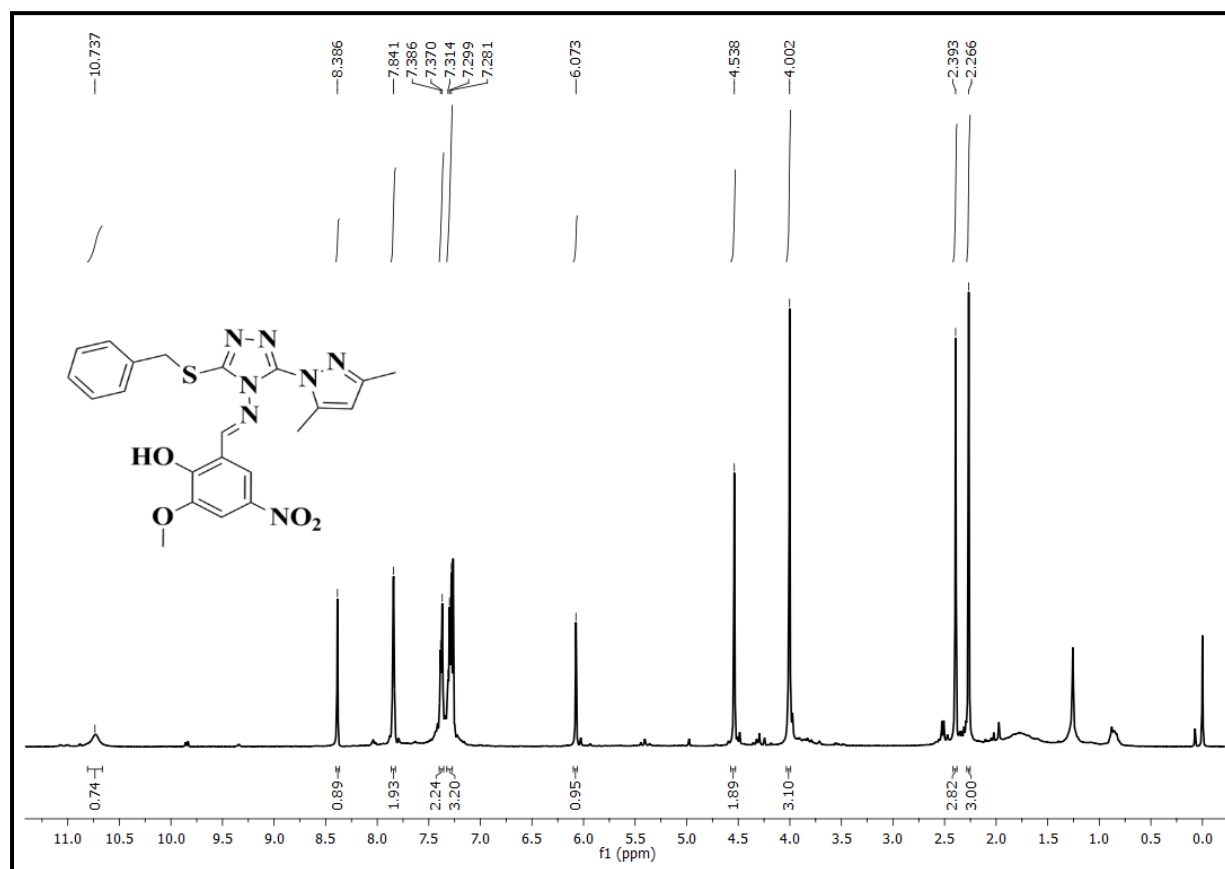


Fig: ¹H-NMR Spectrum of Compound 59n (400 MHz, CDCl₃)

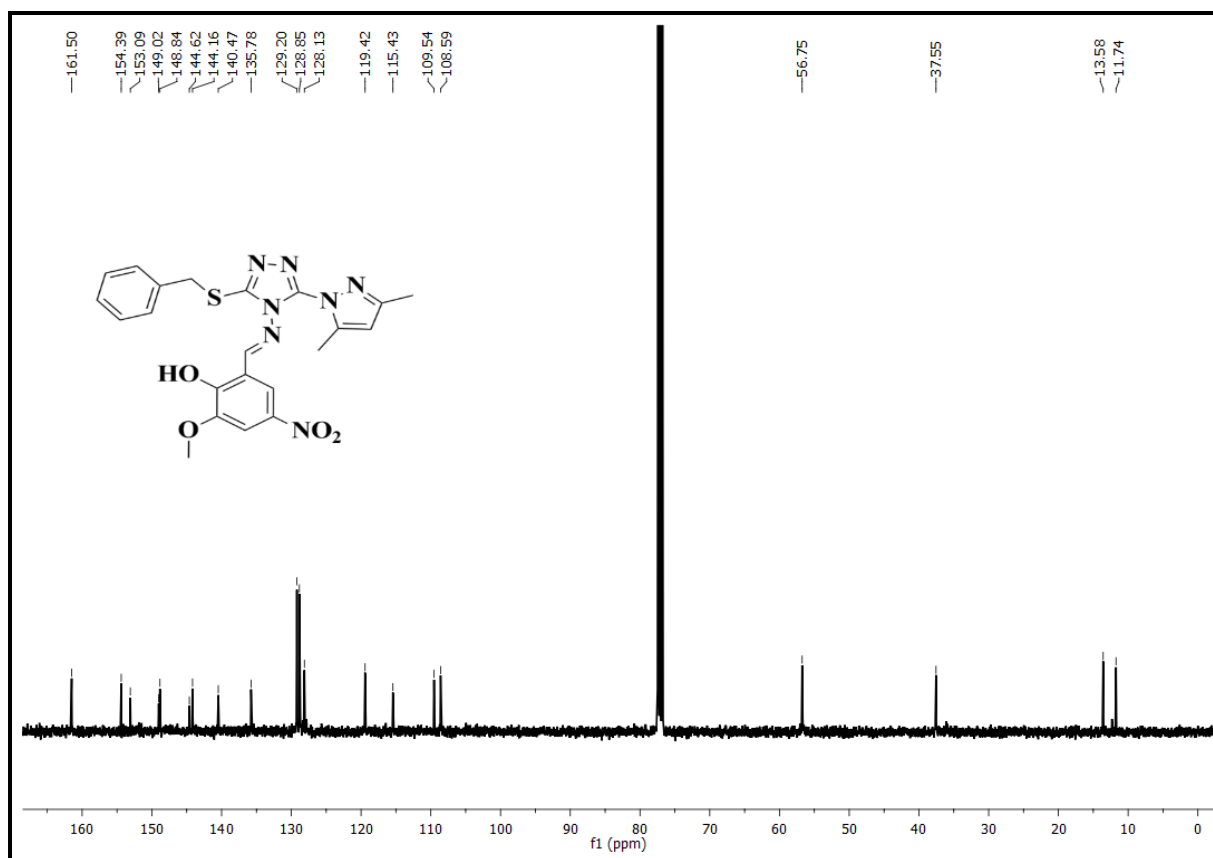


Fig: ¹³C-NMR Spectrum of Compound 59n (100 MHz, CDCl₃)

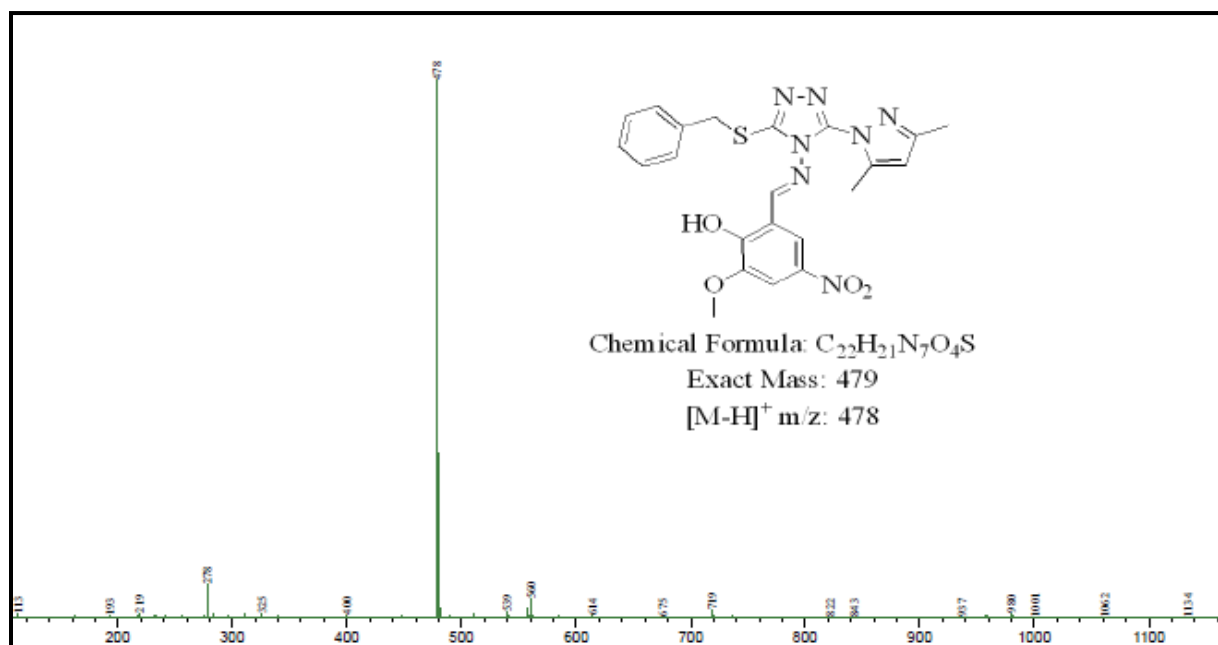


Fig: Mass Spectrum of Compound 59n

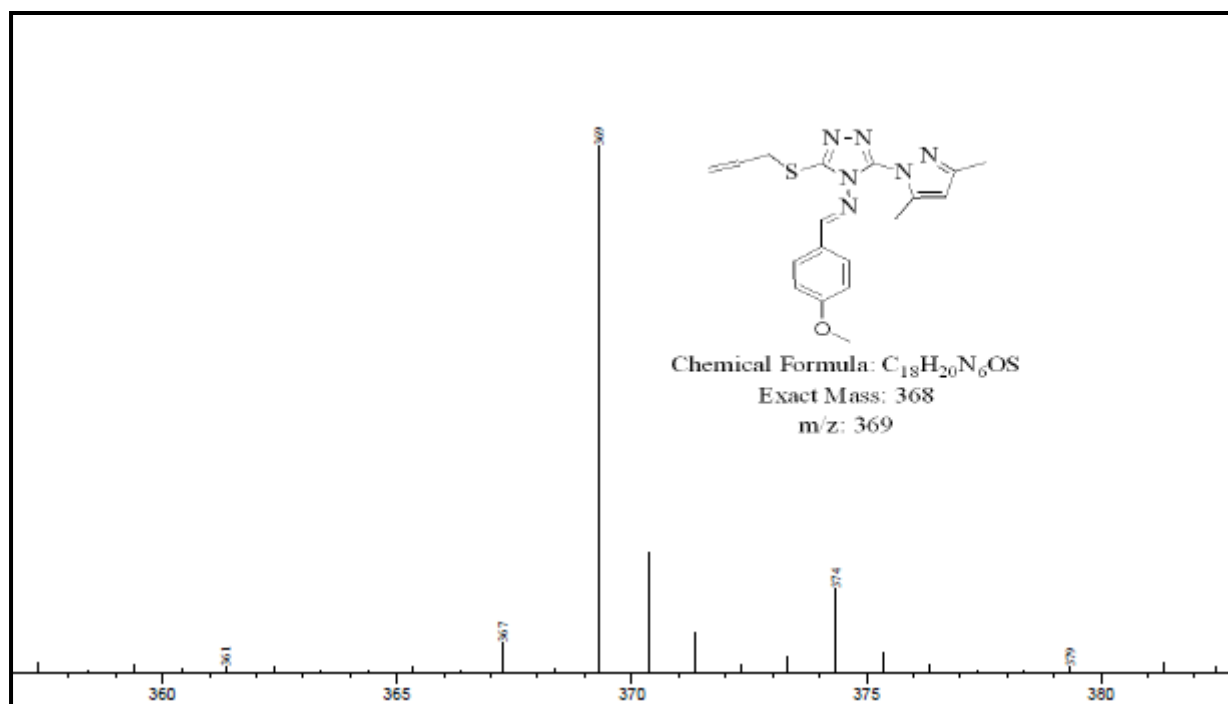


Fig: Mass Spectrum of Compound **59o**

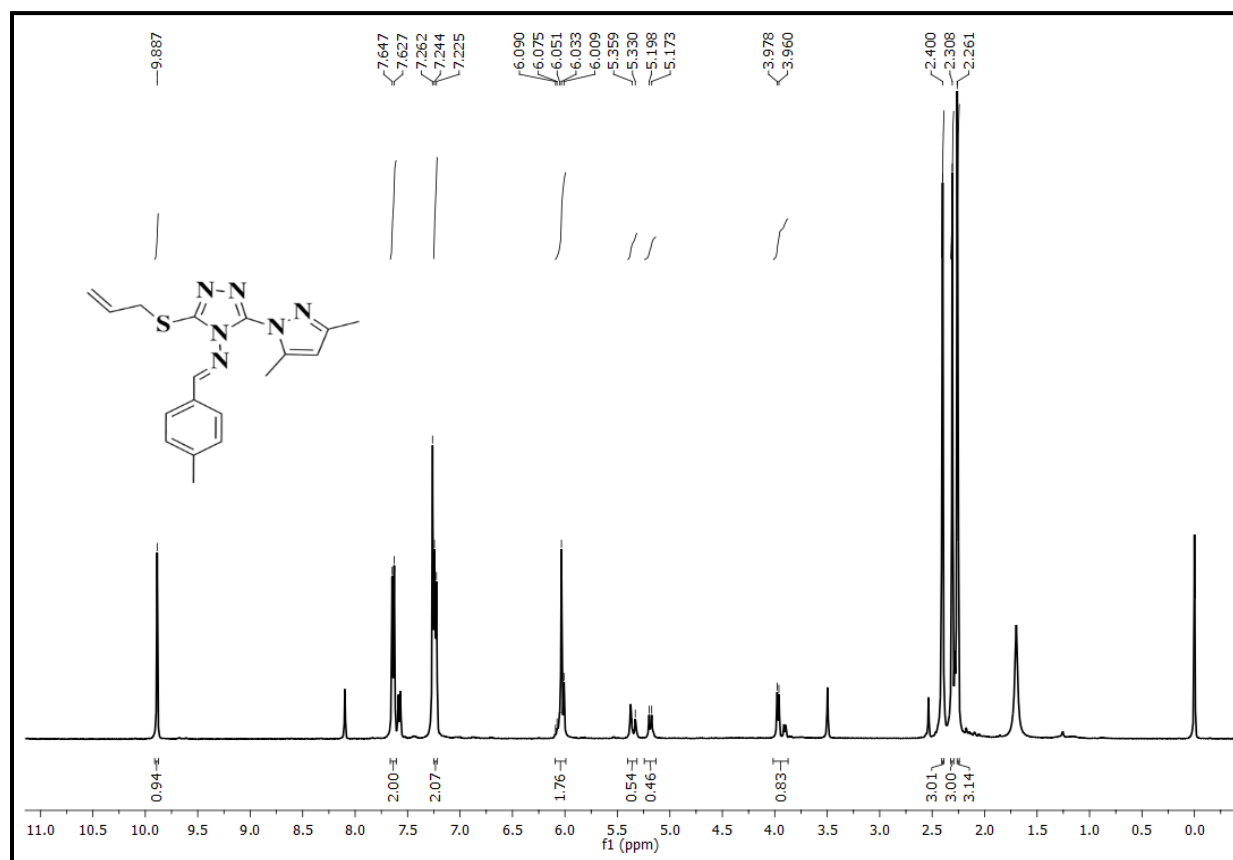


Fig: 1H -NMR Spectrum of Compound **59p** (400 MHz, $CDCl_3 + DMSO-d_6$)

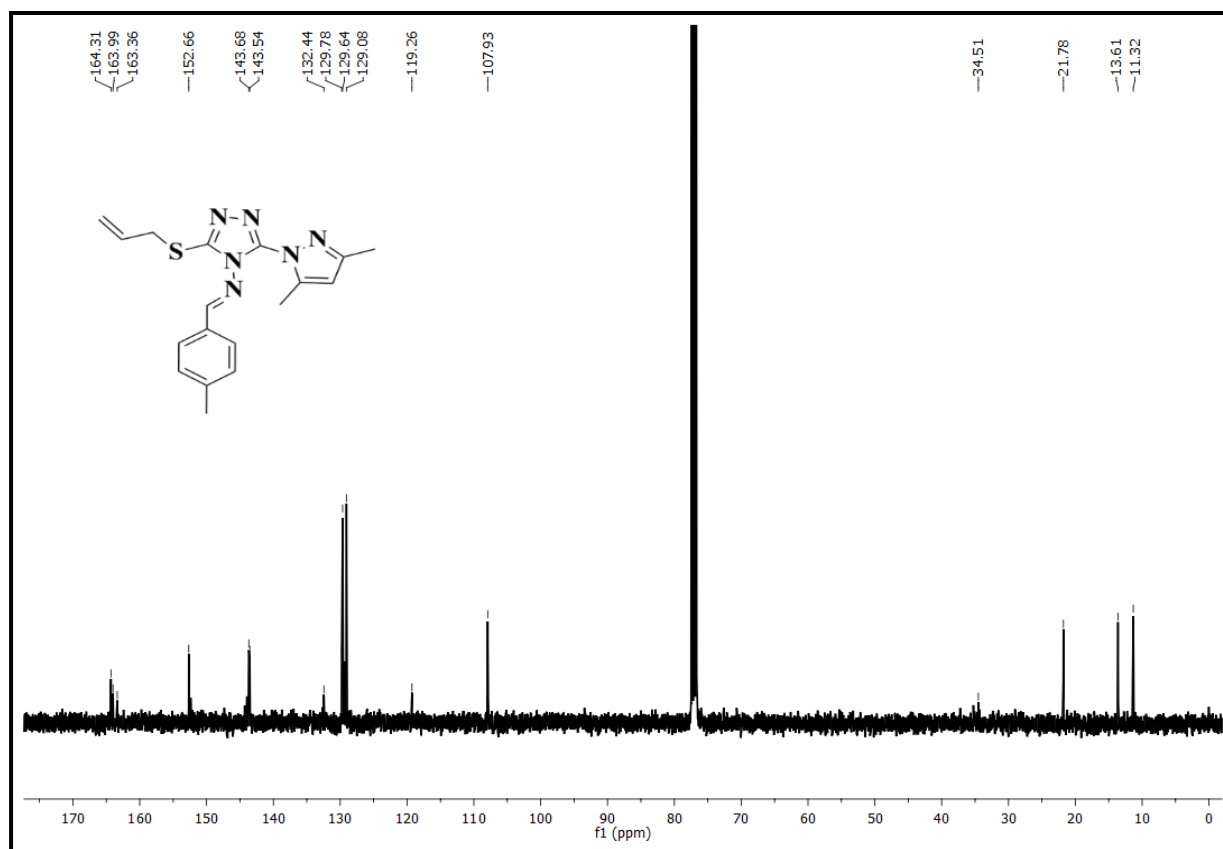


Fig: ^{13}C -NMR Spectrum of Compound **59p** (100 MHz, CDCl_3)

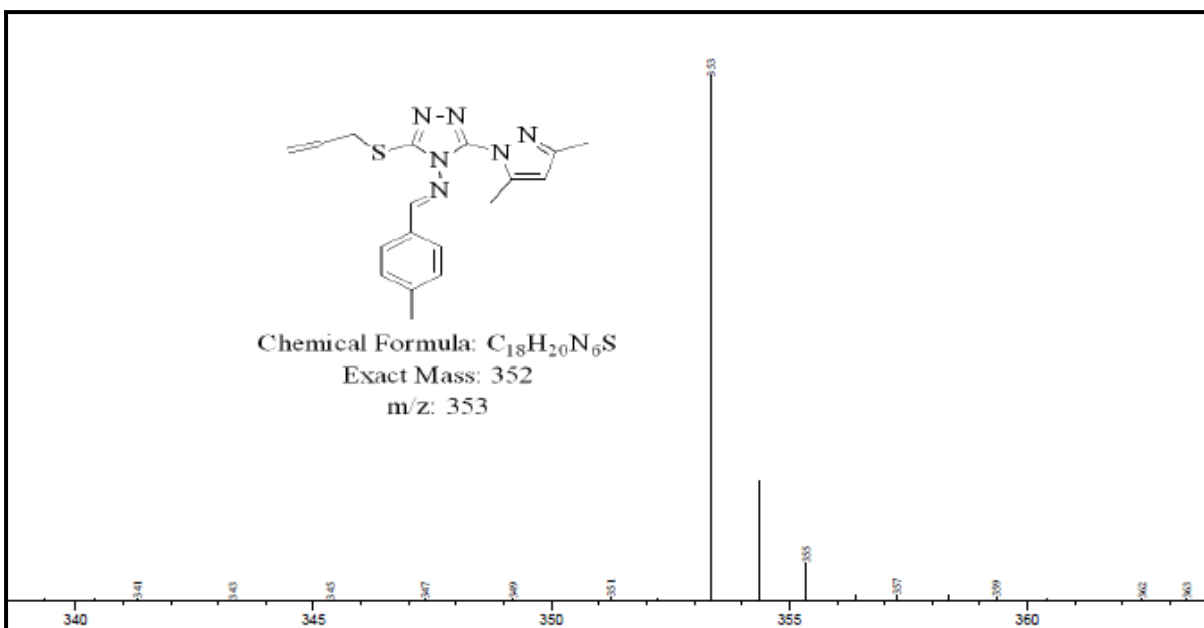


Fig: Mass Spectrum of Compound **59p**

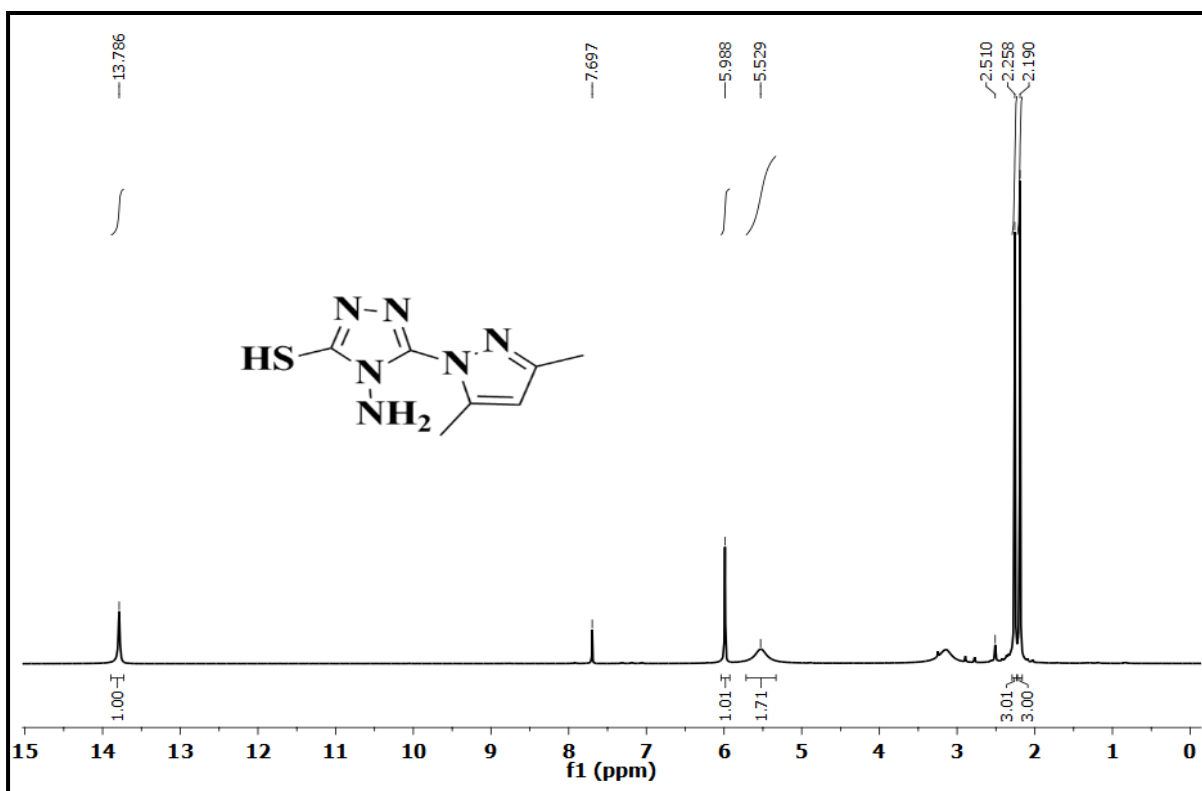


Fig: ¹H-NMR Spectrum of Compound A (400 MHz, CDCl₃+DMSO-*d*₆)

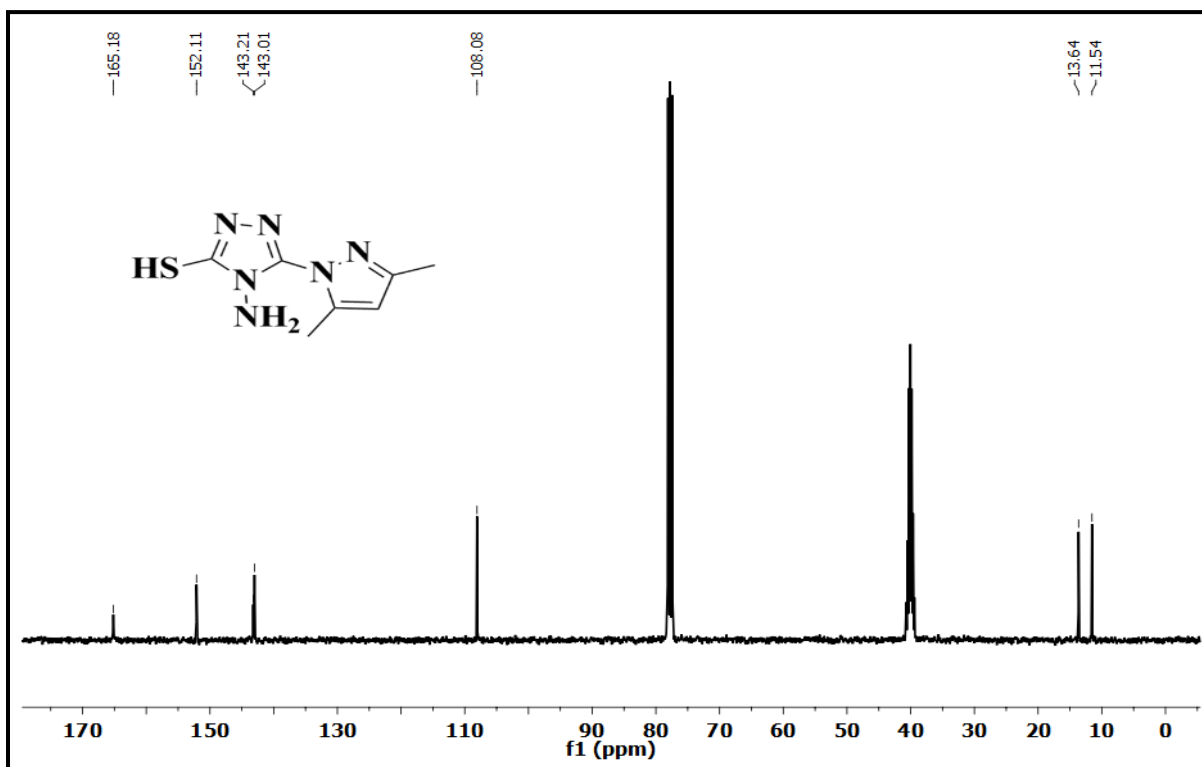


Fig: ¹³C-NMR Spectrum of Compound A (100 MHz, CDCl₃+DMSO-*d*₆)

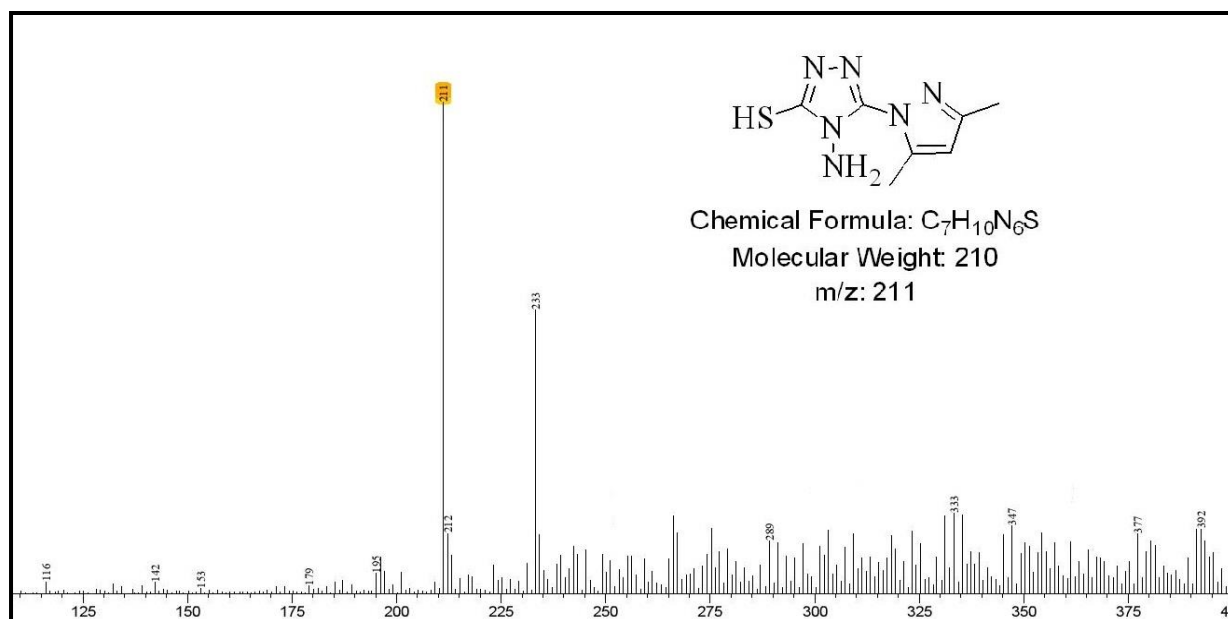


Fig: Mass Spectrum of Compound A

References:

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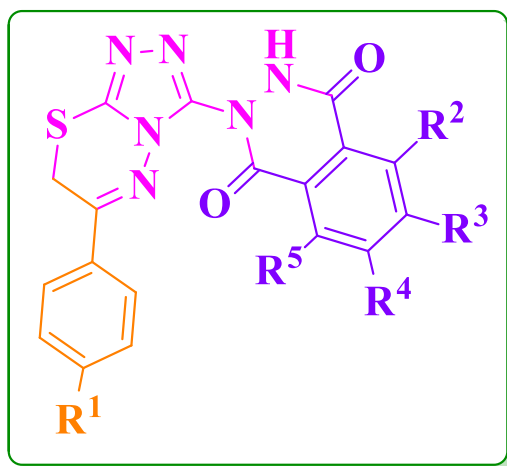
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CHAPTER-III

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-DIHYDROPTHALAZINE-1,4-DIONES



INTRODUCTION:

Phthalazine is a six-membered heterocyclic ring fused with benzene having two nitrogen atoms placed at adjacent positions in the ring. It belongs to the nitrogen heterocyclic compounds category. Sometimes phthalazine is also called as benzo-orthodiazine or benzopyridazine (**Fig. 1**). Most of the phthalazine derivatives exhibit a wide range of biological activities such as anti-diabetic¹, anti-cancer²⁻⁴, anti-fungal⁵, anti-microbial^{6,7}, anti-tumor⁸⁻¹⁰, cytotoxicity^{11,12}, anti-inflammatory^{13,14}, anti-convulsant^{15,16}, vasorelaxant¹⁷⁻¹⁹, anti-bacterial²⁰, vasodilator²¹ anti-thrombotic²², anti-hypertensive²³ activities. In the current method, we have reported the formation of dihydro-1,4-phthalazinedione ring.

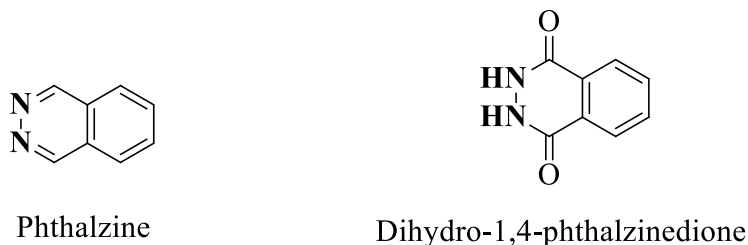
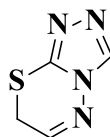


Fig. 1: Structures of phthalazine and dihydro phthalazine dione.

When thiadiazine ring is fused with 1,2,4-triazole that results in the formation of 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine derivatives. Most promising biological and pharmaceutical applications have been reported on various thiadiazine derivatives such as anti-cancer²⁴⁻²⁶, anti-microbial^{27,28}, anti-bacterial²⁹, analgesic/anti-inflammatory³⁰, anti-viral³¹, anti-fungal³², anti-oxidative³³, molluscicidal³⁴, anti-HIV/anti-tumor³⁵ and anti-leishmanial³⁶ activities (**Fig. 2**).



[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine

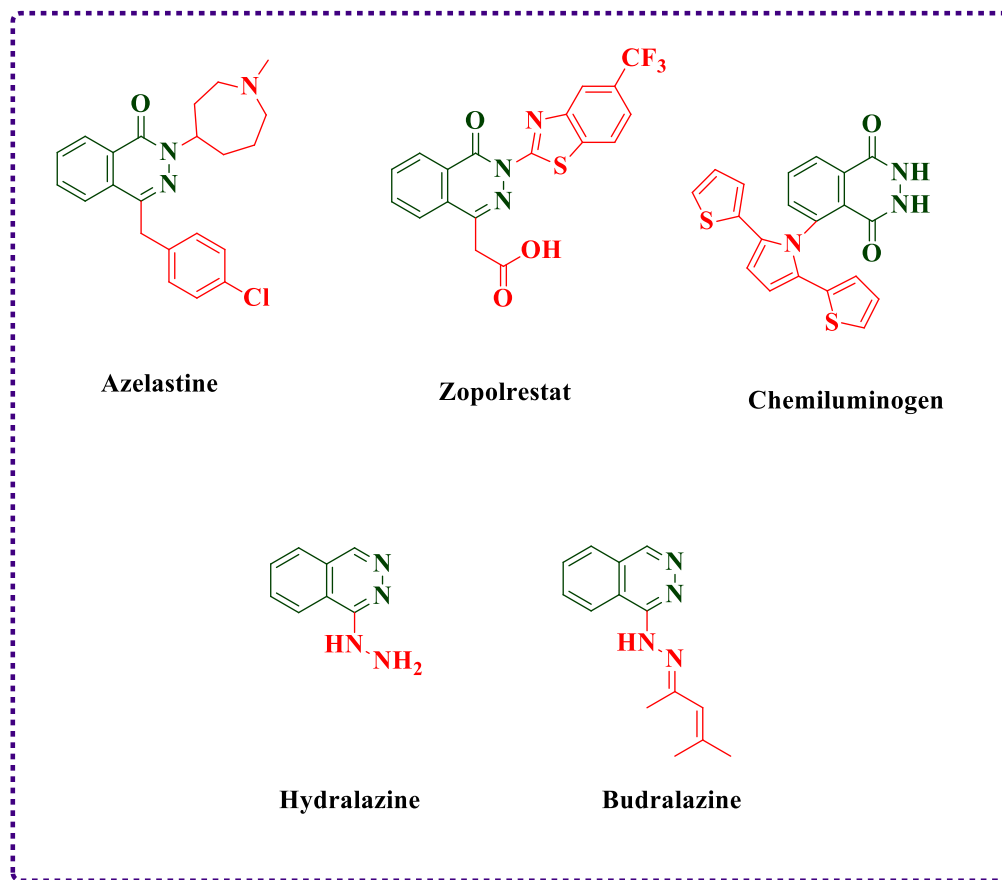
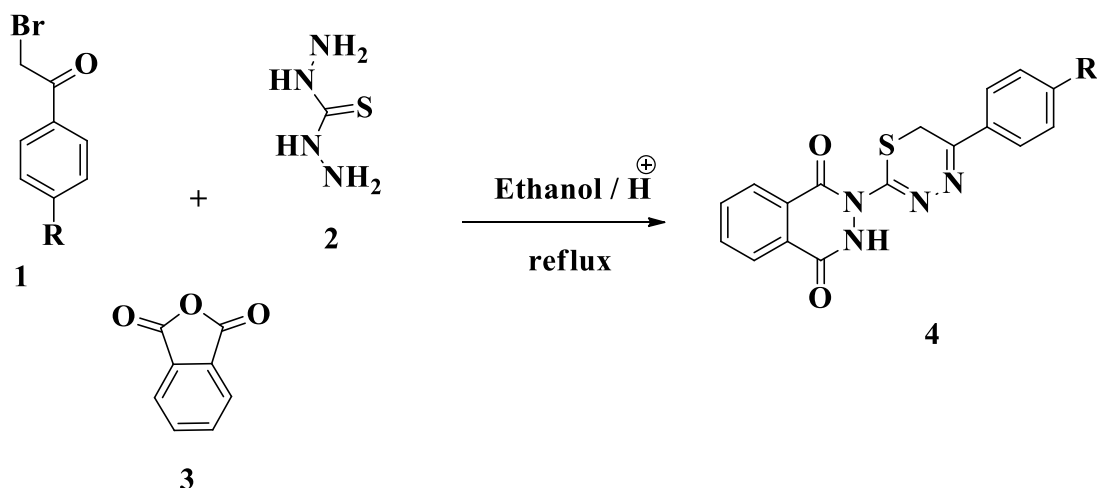


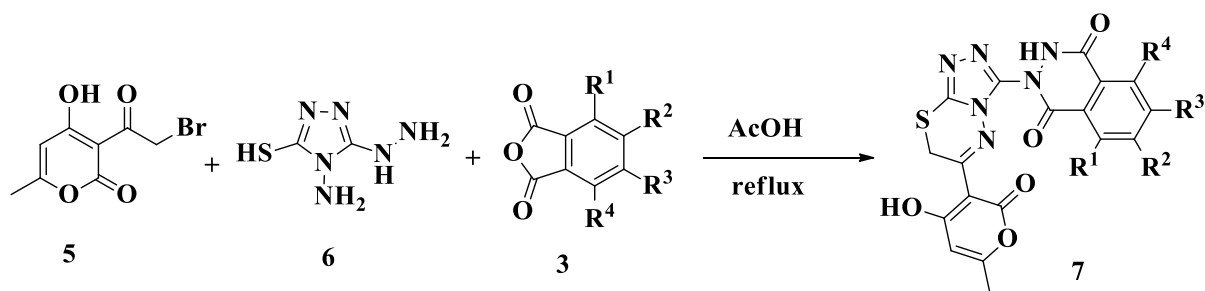
Fig. 2: Some of the drugs having phthalazine moiety.

The following are some of the brief literature surveys on the synthesis of phthalazines and thiadiazines.

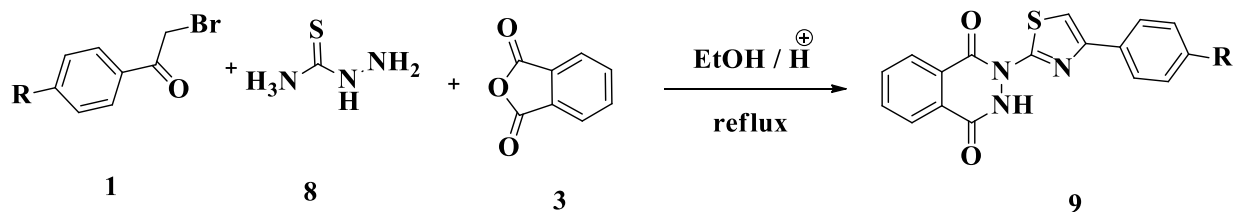
Chunduru³⁷ and co-worker synthesized various 1,3,5-thiadiazinyl phthalazine-1,4-diones (**4**) via a one-pot, multi-component reaction by treating substituted phenacyl bromides (**1**), thiocarbohydrazide (**2**), and phthalic anhydride (**3**) in presence of acidified ethanol under reflux condition. The target compounds were formed with good yields.



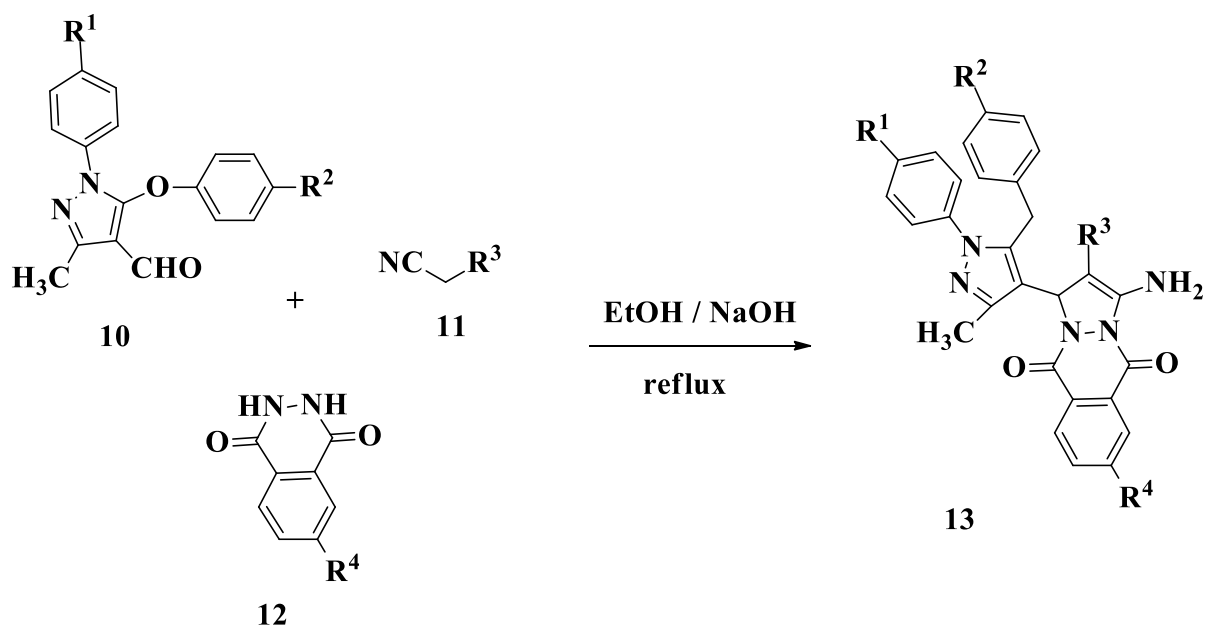
Thirupaiah³⁸ and co-worker reported a one-pot multi-component reaction for the synthesis of 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)-2,3-dihydrophthalazine-1,4-dione (7) analogs from the reaction of 3-(2-bromoacetyl)-4-hydroxy-6-methyl-2*H*-pyran-2-one (5), purpald (6), and substituted phthalic anhydride (3) in presence of acetic acid under reflux condition with good yields.



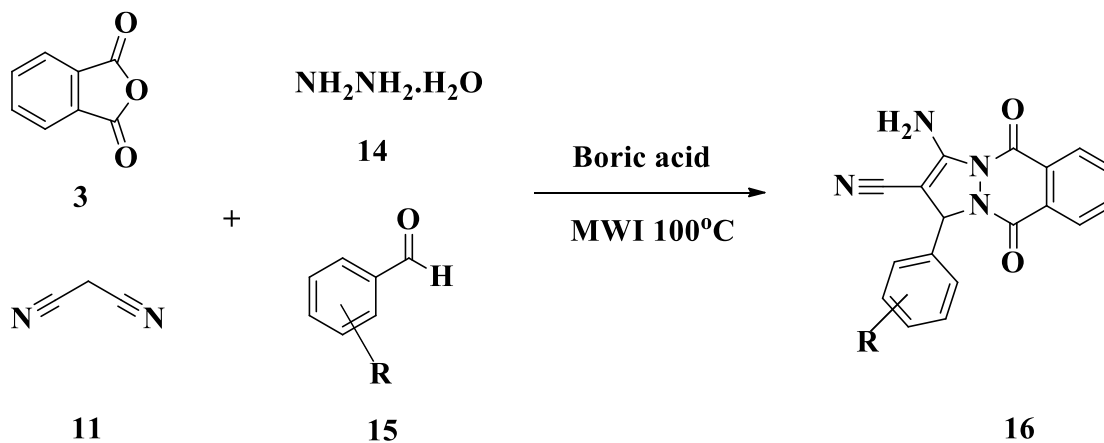
Chunduru³⁹ and co-worker reported the synthesis of 2-(4-phenylthiazol-2-yl)-2,3-dihydrophthalazine-1,4-dione (9) derivatives by the condensation of 2-bromo-1-phenylethanone (1), hydrazine carbothioamide (8), and isobenzofuran-1,3-dione (3) in the presence of acidified ethanol in a single reaction vessel with high yields.



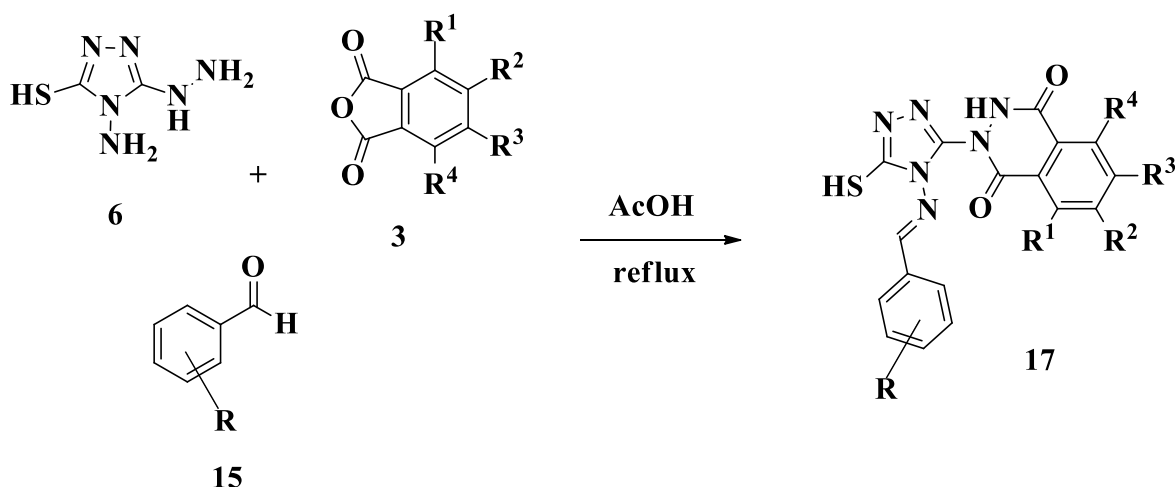
Sangani⁴⁰ et al. conducted a reaction between 1*H*-pyrazole-4-carbaldehydes (**10**), propanedinitrile/ethyl cyanoacetate (**11**), and 2,3-dihydrophthalazine-1,4-diones (**12**) in the presence of absolute ethanol having NaOH under reflux condition to generated the derivatives of 1*H*-pyrazolo[1,2-*b*]phthalazine-5,10-diones (**13**). These compounds exhibited good anti-microbial, anti-tuberculosis, and anti-oxidant activities.



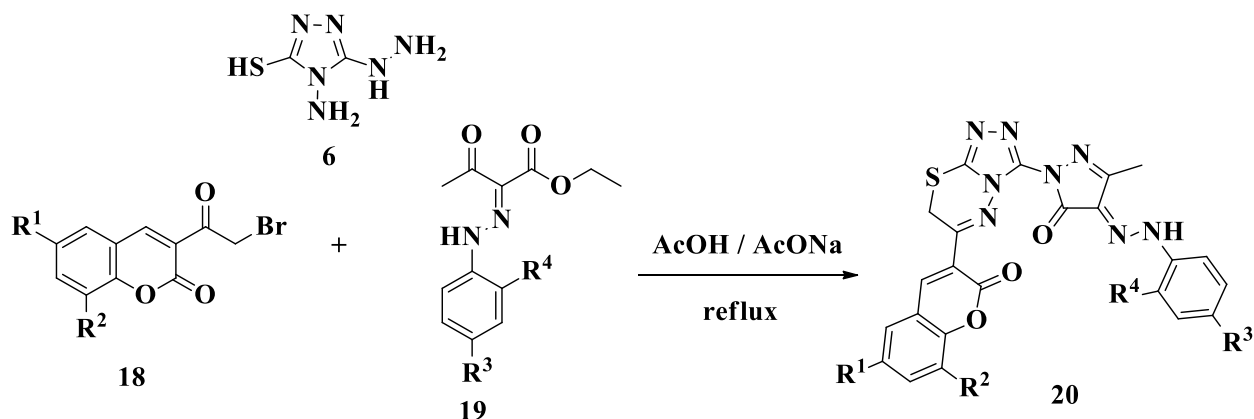
Kolsepatil⁴¹ et al. prepared analogs of pyrazole dihydro phthalazine dione (**16**) using phthalic anhydride (**3**), hydrazine hydrate (**14**), malononitrile (**11**), and substituted benzaldehydes (**15**) adding boric acid under the microwave irradiation in a one-pot.



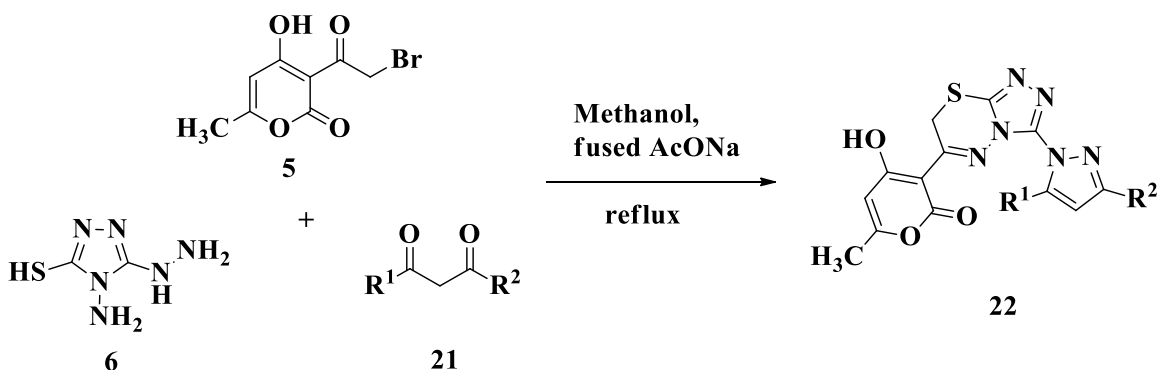
Sujatha and Rao⁴² performed a one-pot multi-component reaction for the synthesis of benzylideneamino dihydrophthalazine-1,4-dione (**17**) derivatives by condensation of 4-amino-5-hydrazinyl-4*H*-1,2,4-triazole-3-thiol (**6**), different phthalic anhydrides (**3**), and various aromatic aldehydes (**15**) in a round bottom flask containing acetic acid under reflux condition with good yields.



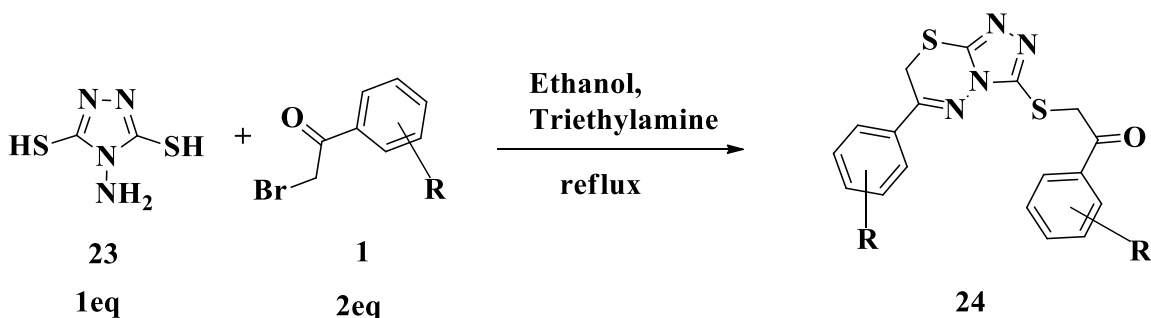
Some of the [1,2,4]-triazolo[3,4-*b*][1,3]thiazine (**20**) derivatives were synthesized by using substituted 3-(2-bromoacetyl)-2*H*-chromen-2-ones (**18**), 4-amino-5-hydrazinyl-4*H*-1,2,4-triazole-3-thiol (**6**), and different (*E*)-ethyl-3-oxo-2-(2-phenylhydrazono)butanoates (**19**) in presence of AcOH/AcONa through a one-pot reaction with high yields by **Aychiluhim and Rao**⁴³.



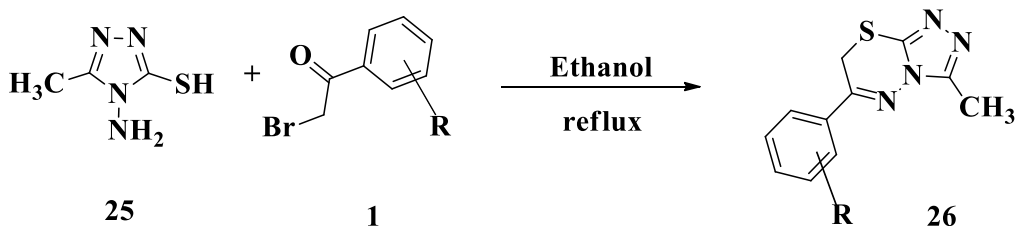
Penta⁴⁴ et al. synthesized different pyrazolyl[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines (**22**) by the cyclo condensation of 3-(2-bromoacetyl)-4-hydroxy-6-methyl-2*H*-pyran-2-one (**5**), purpald (**6**) and acetylacetone/ethyl acetoacetate (**21**) in presence of MeOH and fused AcONa *via* a one-pot synthesis. The final derivatives were produced with good yields. These compounds were shown good anti-microbial and anti-nematicidal activities.



4-Amino-4*H*-1,2,4-triazole-3,5-dithiol (**23**) and two equivalents of various 2-bromo-1-phenylethanone (**1**) were refluxed in presence of ethanol/TEA to synthesize the triazolothiadiazines (**24**) *via* a facile, one-pot synthesis by **Arandkar and Vedula⁴⁵**. These compounds were screened for their anti-cancer activity with moderate results.



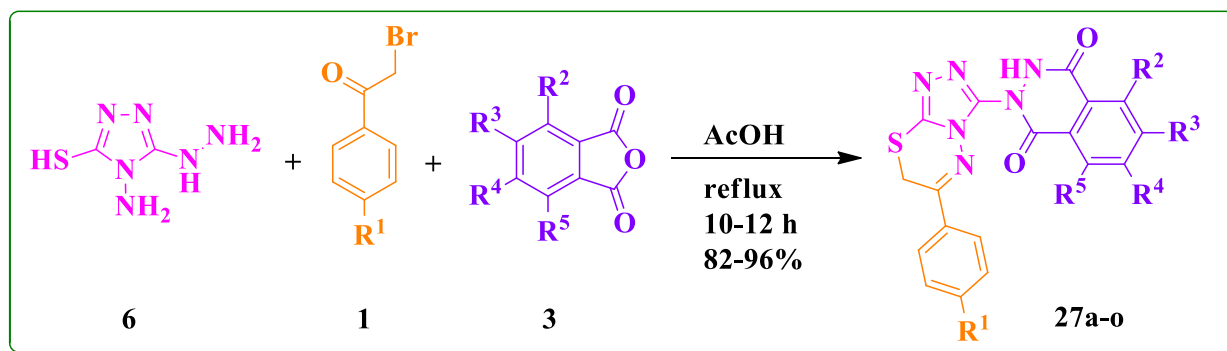
Prakash⁴⁶ and co-worker synthesized 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine (**26**) derivatives using substituted 2-bromo-1-phenylethanone (**1**) and 4-amino-5-methyl-4*H*-1,2,4-triazole-3-thiol (**25**) in presence of ethanol under reflux condition.



In the present chapter we have synthesized [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)-2,3-dihydrophthalazine-1,4-dione derivatives *via* a one-pot, three-component reaction. Both the dihydrophthalazine-1,4-dione and thiadiazine rings were formed simultaneously in a single reaction vessel by the cyclocondensation reaction of 4-amino-5-hydrazino-4*H*-[1,2,4]triazole-3-thiol, substituted phenacyl bromides and different phthalic anhydrides in presence of acetic acid under reflux conditions.

PRESENT WORK:

Novel hybrid compounds containing two or three biological motifs were expected to possess good biological activities. Based on the literature review we have designed a new hybrid template containing phthalazine and [1,2,4]triazole[3,4-*b*][1,3,4]thiadiazine units in a single molecular structure with the hope that the new compounds may exhibit good biological activities. 4-Amino-5-hydrazino-4*H*-[1,2,4]triazole-3-thiol (**6**) was used as the precursor for the synthesis of fused heterocyclic compounds^{47,48}. Herein we are reporting 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-dihydro phthalazine-1,4-diones (**27a-o**) (**Scheme 1**).



Scheme-1: Method-I, Synthesis of 2-(6-phenyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)-2,3-dihydrophthalazine-1,4-dione (**27a-o**) derivatives.

Initially, we have screened the reaction conditions using 4-amino-5-hydrazino-4*H*-[1,2,4]triazole-3-thiol (**6**), 2-bromo-1-(4-methoxyphenyl)ethanone (**1**) and isobenzofuran-1,3-dione (phthalic anhydride) (**3**). The reaction was conducted in the water under reflux conditions where no product was observed even after 10 hours. When the same reaction was operated in the methanol only a 10% yield of the product was formed. On the other hand, when the same reaction was carried out in ethanol under reflux condition the yield of the product was 20%. Finally, when the reaction was operated in the AcOH under reflux condition generated the designed compound **27a** in 86% yield. Hence, the 2-(6-phenyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)-2,3-dihydrophthalazine-1,4-diones (**27a-o**) were synthesized in acetic acid under reflux condition (**Method-I, Table-1**).

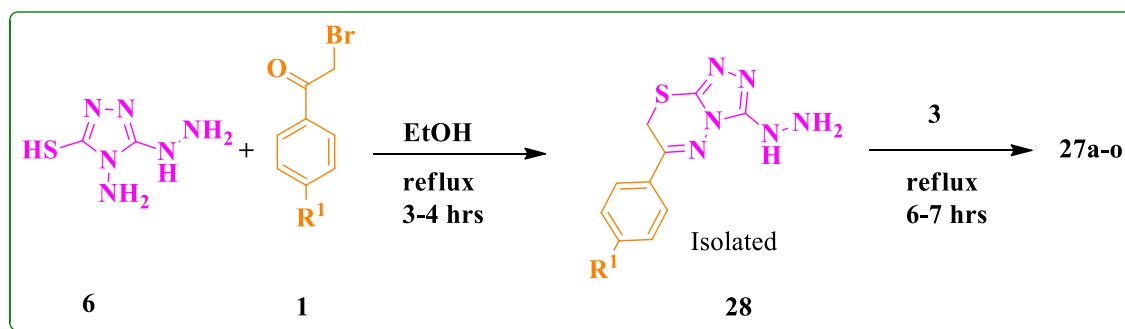
Table-1: Optimization of reaction conditions for the synthesis of **27a**.

Entry	Solvent	Yield (%) of 27a
1	H ₂ O	0
2	MeOH	10
3	EtOH	20
4	AcOH	86

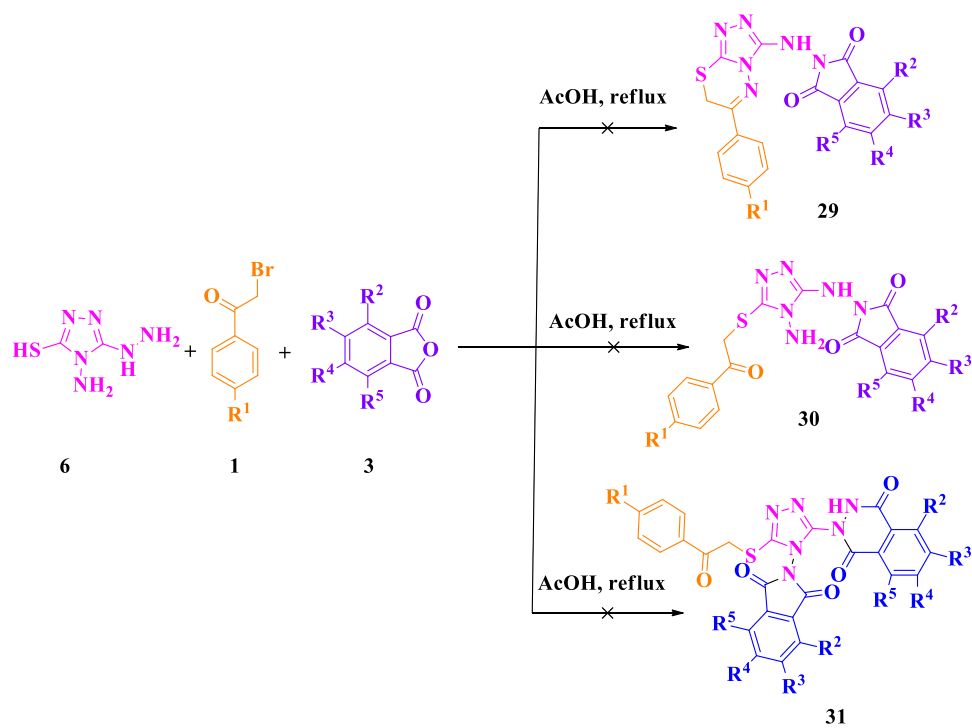
***Reaction conditions:** **6** (1 mmol), **1** (1 mmol), and **3** (1 mmol) in the solvents.

The target compounds (**27a-o**) can also be synthesized through an alternative method involving condensation of 4-amino-5-hydrazino-4*H*-[1,2,4]triazole-3-thiol (**6**) with substituted 2-bromo-1-phenylethanone (**1**) to produce the respective intermediates 3-hydrazinyl-6-phenyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines (**28**). These on further reaction with one equivalent of phthalic anhydride (**3**) under reflux in ethanol produced the compounds (**27a-o**) through a two-step process (**Method-II**). The yields of the products are 60%. The title derivatives (**27a-o**) obtained both the ways were found to be identical by examining their mixed melting point measurement, co-TLC, and IR spectra. The formation of **27a-o** is displayed in **Scheme-2**. In the present investigation, **Method-I** was preferred over **Method-II**, because of the high yields of the products in **Method-I**.

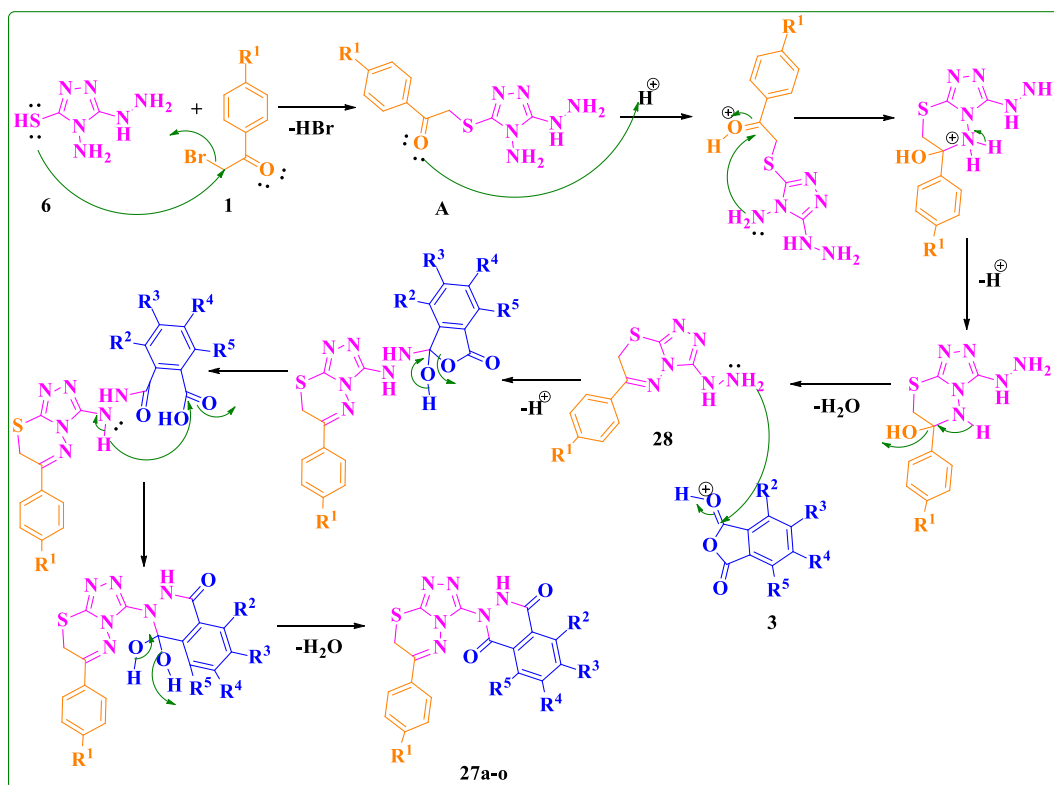
When the reaction was treated between **6**, **1**, and **3** in acetic acid there is a probability of formation of **29** or **30** or **31** or all based on the mode of cyclization. But in the present condition, only one product **27** (vide TLC) was produced. The alternative products (**29**, **30**, and **31**) were rejected on the evidence of their spectral data (**Scheme-3**).



Scheme-2: Method-II, Alternative route for the synthesis of **27a-o**



Scheme-3: Alternative possible products by a multi-component reaction between **6**, **1**, and **3**.



Scheme-4: Plausible reaction mechanism for the synthesis of compounds **27a-o**.

Table-2: 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 (**27a-o**).

Product	R ¹	R ²	R ³	R ⁴	R ⁵	Time (h)	Yield (%)
27a	OCH ₃	H	H	H	H	11.00	86
27b	CH ₃	H	H	H	H	10.30	85
27c	NO ₂	H	H	H	H	11.30	82
27d	Br	H	H	H	H	11.15	90
27e	Cl	H	H	H	H	11.00	86
27f	F	H	H	H	H	11.00	82
27g	CH ₃	H	NO ₂	H	H	11.45	88
27h	F	H	NO ₂	H	H	12.00	84
27i	H	Br	Br	Br	Br	10.20	95
27j	OCH ₃	Br	Br	Br	Br	10.45	92
27k	CH ₃	Br	Br	Br	Br	10.45	89
27l	F	Br	Br	Br	Br	10.30	91
27m	Cl	Br	Br	Br	Br	11.00	96
27n	Br	Br	Br	Br	Br	10.15	94
27o	H	H	H	H	H	10.00	88

In the present one-pot, multi-component reaction condition, substituted 2-bromo-1-phenylethanone (**1**) and substituted isobenzofuran-1,3-dione (**3**) were treated with 4-amino-5-hydrazino-4*H*-[1,2,4]triazole-3-thiol (**6**) to produce the 2-(6-phenyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)-2,3-dihydrophthalazine-1,4-diones. The yields of **27a-o** were 86-88% respectively (**Table-2**). In the current process, the reaction progresses in such a route that the -

SH and -NH₂ functional groups of the compound **6** undergoes cyclization initially with **1** to form thiadiazine ring. Finally, the hydrazino part of the compound condenses with **3** leading to the formation of the phthalazinediones.

The structures of all the newly synthesized derivatives were confirmed by their analytical and spectral data. In the I.R. spectrum compound **27a** displayed peaks at 3451 cm⁻¹ for -NH-, 1790 and 1736 cm⁻¹ for cyclic -C=O. In the ¹H-NMR (CDCl₃) spectrum of compound **27a** gave characteristic singlet for -CH₂- of thiadiazine two protons at δ 4.38 and the -NH- one proton appeared at δ 10.10, -OCH₃ protons appeared as a singlet at δ 3.85. The remaining eight aromatic protons appeared in between δ 7.00-8.00. The ¹³C-NMR spectrum of compound **27a** displayed the peak at δ 23.4 for the thiadiazine methylene carbon, the methyl carbon of -OCH₃ appeared at δ 56.0 and the remaining sp² carbons appeared in the aromatic region at δ 114.0-166.0. The compound **27a** gave a molecular ion peak at *m/z* 407 [M+H]⁺ in the mass spectrum.

In conclusion, we have synthesized 2-(6-phenyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)-2,3-dihydrophthalazine-1,4-diones **27a-o** via a one-pot, multi-component reaction using readily available starting materials. This approach is very simple to operate, it involves clean and simple reaction conditions with high yields. The advantage of this method is that the reaction was carried out without using metals and toxic catalysts and the products were obtained in a single step with the high atom-economy.

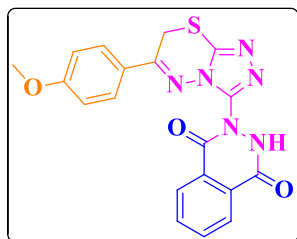
EXPERIMENTAL:

General procedure for the 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-dione via a one-pot multi-component reaction (27a-o)

An equimolar mixture of 4-amino-5-hydrazino-4*H*-[1,2,4]triazole-3-thiol (**6**) (0.001 mol), 2-bromo-1-phenylethanones (**1**) (0.001 mol) and isobenzofuran-1,3-diones (**3**) (0.001 mol) was taken in acetic acid (5 mL) and then refluxed for 10-12 h. The progress of the reaction was monitored by TLC and after the completion of the reaction, the mixture was cooled, solid separated was filtered, and recrystallized from 5-6 mL ethanol to give the title compounds in good yields.

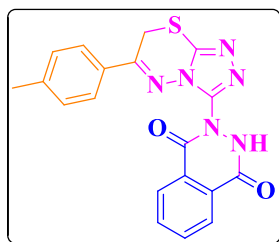
SPECTRAL DATA:

2-(6-(4-Methoxyphenyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)-2,3-dihydrophthalazine-1,4-dione (27a):



White solid (0.349g, 86%); m.p.: 244-246°C; **IR (KBr, $\nu_{\max}/\text{cm}^{-1}$):** 3451 (-NH-), 1790 (-C=O), 1736 (-C=O); **$^1\text{H-NMR}$ (400 MHz, DMSO- d_6 , δ ppm):** 3.85 (s, 3H, -OMe), 4.38 (s, 2H, -CH₂- of thiadiazine), 7.06 (d, 2H, $J=8.8$ Hz, Ar-H), 7.96 (d, 2H, $J=8.4$ Hz, Ar-H), 7.98-8.00 (m, 4H, Ar-H), 10.10 (s, 1H, -NH-); **$^{13}\text{C-NMR}$ (100 MHz, CDCl₃+DMSO- d_6 , δ ppm):** 23.5, 56.0, 114.8, 124.3, 125.8, 129.8, 135.9, 138.4, 151.6, 154.6, 162.6, 166.0; **ESI-MS (m/z):** 407 [M-H]⁺; Analytical calculated formulae C₁₉H₁₄N₆O₃S; C, 56.15; H, 3.47; N, 20.68; S, 7.89; Found: C, 56.18; H, 3.43; N, 20.72; S, 7.85.

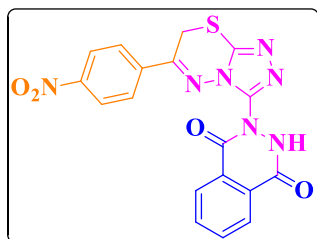
2-(6-(p-Tolyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)-2,3-dihydrophthalazine-1,4-dione (27b):



White solid (0.331g, 85%); m.p.: 250-252°C; **IR (KBr, $\nu_{\max}/\text{cm}^{-1}$):** 3435 (-NH-), 1790 (-C=O), 1736 (-C=O); **$^1\text{H-NMR}$ (400 MHz, DMSO- d_6 , δ ppm):** 2.42 (s, 3H, -CH₃), 4.16 (s, 2H, -CH₂- of thiadiazine), 7.27 (d, 2H, $J=8.0$ Hz, Ar-H), 7.77 (s, 1H, -NH-), 7.83-7.87 (m, 4H, Ar-H), 7.92 (d, 2H, $J=8.4$ Hz, Ar-H); **$^{13}\text{C-NMR}$ (100**

MHz, CDCl₃+DMSO- d_6 , δ ppm): 21.5, 23.3, 124.3, 127.8, 129.9, 130.0, 130.8, 135.9, 138.5, 142.7, 151.7, 155.0, 159.3, 166.0; **ESI-MS (m/z):** 391 [M+H]⁺; Analytical calculated formulae C₁₉H₁₄N₆O₂S; C, 58.45; H, 3.61; N, 21.53; S, 8.21; Found: C, 58.41; H, 3.65; N, 21.58; S, 8.25.

2-(6-(4-Nitrophenyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)-2,3-dihydrophthalazine-1,4-dione (27c):

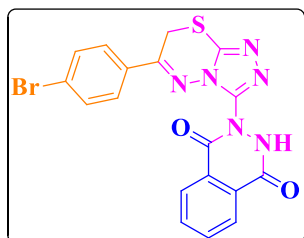


Yellow solid (0.346g, 82%); m.p.: 233-235°C; **IR (KBr, $\nu_{\max}$, cm^{-1}):** 3434 (-NH-), 1793(-C=O), 1736 (-C=O); **$^1\text{H-NMR}$ (400 MHz, DMSO- d_6 , δ ppm):** 4.42 (s, 2H, -CH₂- of thiadiazine), 7.90-7.92 (m, 4H, Ar-H), 8.11 (s, 1H, -NH-), 8.27 (d, 2H, $J=8.4$ Hz, Ar-H), 8.31 (d, 2H, $J=8.4$ Hz, Ar-H). **$^{13}\text{C-NMR}$ (100 MHz, CDCl₃+ DMSO- d_6 ,**

δ ppm): 23.5, 124.2, 124.3, 129.2, 129.9, 135.7, 138.1, 139.7, 149.9, 151.9, 153.2, 165.9. **ESI-**

MS (*m/z*): 422 [M+H]⁺; Analytical calculated formulae C₁₈H₁₁N₇O₄S; C, 51.30; H, 2.63; N, 23.27; S, 7.61: Found: C, 51.35; H, 2.67; N, 23.22; S, 7.66.

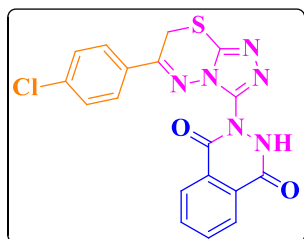
2-(6-(4-Bromophenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)-2,3-dihydro phthalazine-1,4-dione (27d):



White solid (0.410g, 90%); m.p.: 248-250°C; **IR (KBr, $\nu_{\max}$, cm⁻¹):** 3432 (-NH-), 1792 (-C=O), 1732 (-C=O); **¹H-NMR (400 MHz, DMSO-*d*₆, δ ppm):** 4.43 (s, 2H, -CH₂- of thiadiazine), 7.76 (d, 2H, *J*=8.0 Hz, Ar-H), 7.95-8.01 (m, 6H, Ar-H), 10.20(s, 1H, -NH-); **¹³C-NMR (100 MHz, CDCl₃+DMSO-*d*₆, δ ppm):** 23.3, 124.3, 126.2,

129.8, 129.9, 132.4, 132.9, 135.9, 138.2, 151.7, 154.2, 165.9. **ESI-MS (*m/z*):** 457 [M+2]⁺; Analytical calculated formulae C₁₈H₁₁BrN₆O₂S; C, 47.48; H, 2.44; N, 18.46; S, 7.04: Found: C, 47.44; H, 2.49; N, 18.40; S, 7.12.

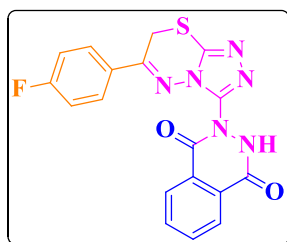
2-(6-(4-Chlorophenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)-2,3-dihydro phthalazine-1,4-dione (27e):



White solid (0.352g, 86%); m.p.: 239-241°C; **IR (KBr, $\nu_{\max}$, cm⁻¹):** 3435 (-NH-), 1790 (-C=O), 1734 (-C=O); **¹H-NMR (400 MHz, DMSO-*d*₆, δ ppm):** 4.34 (s, 2H, -CH₂- of thiadiazine), 7.51 (d, 2H, *J*=8.8 Hz, Ar-H), 7.89-7.96 (m, 4H, Ar-H), 8.03 (d, 2H, *J*=8.4 Hz, Ar-H), 8.10 (s, 1H, -NH-); **¹³C-NMR (100 MHz, DMSO-*d*₆, δ ppm):**

23.2, 129.6, 129.8, 129.9, 131.9, 137.8, 139.5, 139.9, 151.2, 151.4, 155.4, 170.8, 173.4; **ESI-MS (*m/z*):** 411 [M+H]⁺; Analytical calculated formulae C₁₈H₁₁ClN₆O₂S; C, 52.62; H, 2.70; N, 20.46; S, 7.80: Found: C, 52.68; H, 2.75; N, 20.42; S, 7.84.

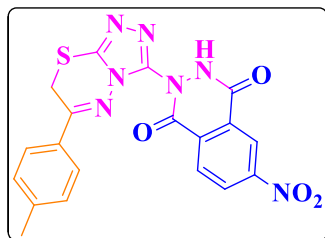
2-(6-(4-Fluorophenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)-2,3-dihydro phthalazine-1,4-dione (27f):



White solid (0.323g, 82%); m.p.: 243-245°C; **IR (KBr, $\nu_{\max}$, cm⁻¹):** 3446 (-NH-), 1790 (-C=O), 1735 (-C=O); **¹H-NMR (400 MHz, DMSO-*d*₆, δ ppm):** 4.39 (s, 2H, -CH₂- of thiadiazine), 7.73 (t, 2H, *J*=8.0 Hz, Ar-H), 7.95-7.97 (m, 4H, Ar-H), 8.07 (t, 2H, *J*=6.0 Hz, Ar-H), 8.24 (s, 1H, -NH-); **¹³C-NMR (100 MHz, CDCl₃+ DMSO-*d*₆, δ**

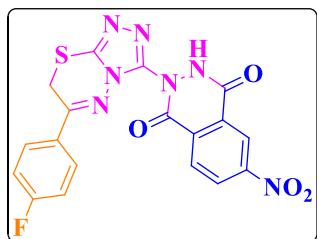
ppm): 23.5, 116.4, 116.6, 124.3, 130.5, 130.6, 135.8, 151.7, 154.0, 160.5, 163.7, 165.9, 169.0; **ESI-MS (*m/z*):** 395 [M+H]⁺; Analytical calculated formulae C₁₈H₁₁FN₆O₂S; C, 54.82; H, 2.81; N, 21.31; S, 8.13; Found: C, 54.87; H, 2.85; N, 21.36; S, 8.16.

6-Nitro-2-(6-(*p*-tolyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)-2,3-dihydro phthalazine-1,4-dione (27g):



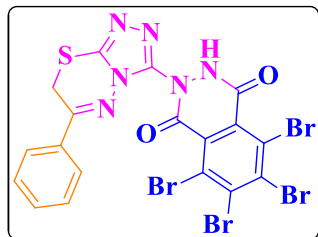
White solid (0.382g, 88%); m.p.: 216-218°C; **IR (KBr, $\nu_{\max}$, cm⁻¹):** 3403 (-NH-), 1800 (-C=O), 1745 (-C=O); **¹H-NMR (400 MHz, DMSO-*d*₆, δ ppm):** 2.39 (s, 3H, CH₃), 4.41 (s, 2H, -CH₂- of thiadiazine), 7.35 (d, 2H, *J*=7.6 Hz, Ar-H), 7.90 (d, 2H, *J*=7.6 Hz, Ar-H), 8.18 (t, 1H, *J*=7.2 Hz, Ar-H), 8.31 (d, 1H, *J*=7.6 Hz, Ar-H), 8.42 (d, 1H, *J*=7.6 Hz, Ar-H), 10.33 (s, 1H, -NH-); **¹³C-NMR (100 MHz, DMSO-*d*₆, δ ppm):** 21.5, 23.3, 121.5, 127.8, 128.0, 128.2, 130.0, 130.9, 137.7, 137.8, 138.6, 142.6, 142.7, 144.9, 155.1, 161.5, 163.9; **ESI-MS (*m/z*):** 434 [M-H]⁺; Analytical calculated formulae C₁₉H₁₃N₇O₄S; C, 52.41; H, 3.01; N, 22.52; S, 7.36; Found: C, 52.46; H, 3.12; N, 22.56; S, 7.40.

2-(6-(4-Fluorophenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)-6-nitro-2,3-dihydro phthalazine-1,4-dione (27h):



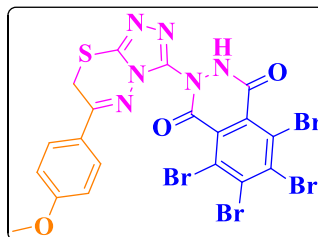
White solid (0.370g, 84%); m.p.: 225-227°C; **IR (KBr, $\nu_{\max}$, cm⁻¹):** 3389 (-NH-), 1800 (-C=O), 1746 (-C=O); **¹H-NMR (400 MHz, DMSO-*d*₆, δ ppm):** 4.16 (s, 2H, -CH₂- of thiadiazine), 7.16 (t, 2H, *J*=8.4 Hz, Ar-H), 7.44 (t, 1H, *J*=8.0 Hz, Ar-H), 7.88 (s, 1H, -NH-), 7.93 (d, 2H, *J*=8.0 Hz, Ar-H), 8.09 (dd, 2H, *J*=8.8 Hz, *J*=5.2 Hz, Ar-H); **¹³C-NMR (100 MHz, DMSO-*d*₆, δ ppm):** 23.2, 116.3, 116.5, 127.2, 130.5, 130.7, 130.8, 134.0, 138.0, 152.9, 153.7, 159.1, 161.0, 162.6, 165.9, 167.1; **ESI-MS (*m/z*):** 440 [M-H]⁺; Analytical calculated formulae C₁₈H₁₀FN₇O₄S; C, 49.20; H, 2.29; N, 22.31; S, 7.30; Found: C, 49.25; H, 2.31; N, 22.35; S, 7.34.

5,6,7,8-Tetrabromo-2-(6-phenyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)-2,3-dihydro phthalazine-1,4-dione (27i):



White solid (0.656g, 95%); m.p.: 251-253°C; **IR (KBr, $\nu_{\max}$, cm^{-1}):** 3429 (-NH-), 1783 (-C=O), 1742 (-C=O); **$^1\text{H-NMR}$ (400 MHz, $\text{DMSO-}d_6$, δ ppm):** 3.17 (s, 1H, -NH-), 4.43(s, 2H, -CH₂- of thiadiazine), 7.53 (t, 2H, $J=7.2$ Hz, Ar-H), 7.60 (t, 1H, $J=7.2$ Hz, Ar-H), 7.97 (d, 2H, $J=7.6$ Hz, Ar-H); **$^{13}\text{C-NMR}$ (100 MHz, $\text{DMSO-}d_6$, δ ppm):** 23.5, 121.7, 127.9, 128.2, 129.4, 129.5, 129.7, 132.4, 137.9, 153.4, 154.2, 155.4, 162.0; **ESI-MS (m/z):** 692 $[\text{M}+\text{H}]^+$; Analytical calculated formulae $\text{C}_{18}\text{H}_8\text{Br}_4\text{N}_6\text{O}_2\text{S}$; C, 31.24; H, 1.17; N, 12.14; S, 4.63; Found: C, 31.27; H, 1.14; N, 12.12; S, 4.60.

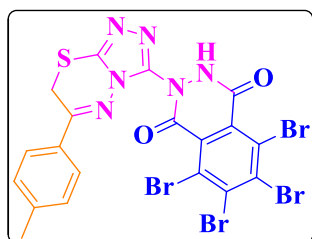
5,6,7,8-Tetrabromo-2-(6-(4-methoxyphenyl)-7H-[1,2,4]thiadiazolo[3,4-b][1,3,4]thiadiazin-3-yl)-2,3-dihydrophthalazine-1,4-dione (27j):



White solid (0.664g, 92%); m.p.: 245-247°C; **IR (KBr, $\nu_{\max}$, cm^{-1}):** 3345 (-NH-), 1804 (-C=O), 1744 (-C=O); **$^1\text{H-NMR}$ (400 MHz, $\text{DMSO-}d_6$, δ ppm):** 4.39 (s, 3H, -OCH₃), 4.85 (s, 2H, -CH₂- of thiadiazine), 7.06 (s, 1H, -NH-), 7.93 (d, 2H, $J=8.0$ Hz, Ar-H), 8.00 (d, 2H, $J=8.0$ Hz, Ar-H); **$^{13}\text{C-NMR}$ (100 MHz, $\text{DMSO-}d_6$, δ ppm):**

31.1, 56.0, 114.7, 121.7, 129.5, 129.7, 135.1, 137.9, 143.5, 145.5, 154.9, 162.1, 162.7, 166.4; **ESI-MS (m/z):** 723 $[\text{M}+\text{H}]^+$; Analytical calculated formulae $\text{C}_{19}\text{H}_{10}\text{Br}_4\text{N}_6\text{O}_3\text{S}$; C, 31.61; H, 1.40; N, 11.64; S, 4.44; Found: C, 31.615; H, 1.44; N, 11.60; S, 4.40.

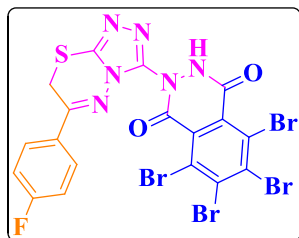
5,6,7,8-Tetrabromo-2-(6-(*p*-tolyl)-7H-[1,2,4]thiadiazolo[3,4-b][1,3,4]thiadiazin-3-yl)-2,3-dihydrophthalazine-1,4-dione (27k):



White solid (0.628g, 89%); m.p.: 252-254°C; **IR (KBr, $\nu_{\max}$, cm^{-1}):** 3422 (-NH-), 1783 (-C=O), 1745 (-C=O); **$^1\text{H-NMR}$ (400 MHz, $\text{DMSO-}d_6$, δ ppm):** 2.39 (s, 3H, -CH₃), 4.39 (s, 2H, -CH₂- of thiadiazine), 7.33 (d, 2H, $J=8.0$ Hz, Ar-H), 7.84 (d, 2H, $J=8.0$ Hz, Ar-H), 8.31 (s, 1H, -NH-); **$^{13}\text{C-NMR}$ (100 MHz, $\text{DMSO-}d_6$, δ**

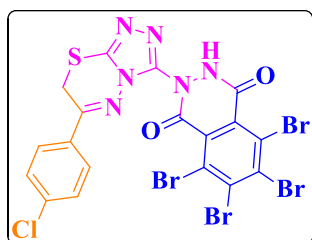
ppm): 23.7, 29.2, 125.9, 126.1, 129.7, 137.9, 139.7, 146.7, 147.0, 151.0, 156.6, 160.4, 161.3, 165.1; **ESI-MS (m/z):** 706; Analytical calculated formulae $\text{C}_{19}\text{H}_{10}\text{Br}_4\text{N}_6\text{O}_2\text{S}$; C, 32.32; H, 1.43; N, 11.90; S, 4.54; Found: C, 32.35; H, 1.46; N, 11.94; S, 4.58.

5,6,7,8-Tetrabromo-2-(6-(4-fluorophenyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)-2,3-dihydrophthalazine-1,4-dione (27l):



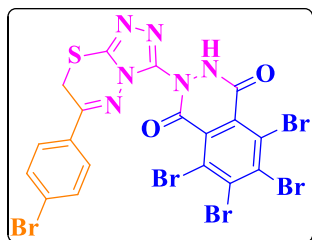
White solid (0.645g, 91%); m.p.: 257-259°C; **IR (KBr, $\nu_{\max}$, cm^{-1}):** 3411 (-NH-), 1784 (-C=O), 1742 (-C=O); **$^1\text{H-NMR}$ (400 MHz, $\text{DMSO-}d_6$, δ ppm):** 4.23 (s, 2H, -CH₂- of thiadiazine), 7.21 (t, 2H, $J=8.4$ Hz, Ar-H), 7.85 (s, 1H, -NH-), 8.03 (dd, 2H, $J=8.8$ Hz, $J=5.2$ Hz, Ar-H); **$^{13}\text{C-NMR}$ (100 MHz, $\text{DMSO-}d_6$, δ ppm):** 23.5, 116.6, 130.5, 130.6, 131.1, 137.8, 139.8, 140.0, 154.6, 154.9, 157.6, 160.2, 163.5; **ESI-MS (m/z):** 710 $[\text{M}+\text{H}]^+$; Analytical calculated formulae $\text{C}_{18}\text{H}_7\text{Br}_4\text{FN}_6\text{O}_2\text{S}$; C, 30.45; H, 0.99; N, 11.84; S, 4.52: Found: C, 30.49; H, 0.94; N, 11.87; S, 4.56.

5,6,7,8-Tetrabromo-2-(6-(4-chlorophenyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)-2,3-dihydrophthalazine-1,4-dione (27m):



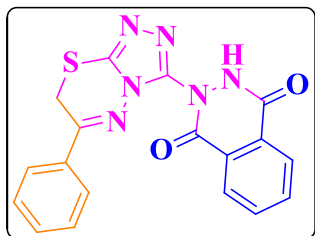
White solid (0.699g, 96%); m.p.: 286-288°C; **IR (KBr, $\nu_{\max}$, cm^{-1}):** 3416 (-NH-), 1783 (-C=O), 1744 (-C=O); **$^1\text{H-NMR}$ (400 MHz, $\text{DMSO-}d_6$, δ ppm):** 3.46 (s, 1H, -NH-), 4.40 (s, 2H, -CH₂- of thiadiazine), 7.59 (d, 2H, $J=8.4$ Hz, Ar-H), 7.99 (d, 2H, $J=8.4$ Hz, Ar-H); **$^{13}\text{C-NMR}$ (100 MHz, $\text{DMSO-}d_6$, δ ppm):** 23.4, 121.7, 129.4, 129.5, 129.7, 132.6, 137.2, 137.8, 138.5, 144.0, 151.5, 154.2, 162.0; **ESI-MS (m/z):** 727 $[\text{M}+\text{H}]^+$; Analytical calculated formulae $\text{C}_{18}\text{H}_7\text{Br}_4\text{ClN}_6\text{O}_2\text{S}$; C, 29.76; H, 0.97; N, 11.57; S, 4.41: Found: C, 29.71; H, 0.93; N, 11.53; S, 4.46.

5,6,7,8-Tetrabromo-2-(6-(4-bromophenyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)-2,3-dihydrophthalazine-1,4-dione (27n):



White solid (0.738g, 94%); m.p.: 292-294°C; **IR (KBr, $\nu_{\max}$, cm^{-1}):** 3426 (-NH-), 1783 (-C=O), 1746 (-C=O); **$^1\text{H-NMR}$ (400 MHz, $\text{DMSO-}d_6$, δ ppm):** 4.41 (s, 2H, -CH₂- of thiadiazine), 7.71 (d, 2H, $J=8.8$ Hz, Ar-H), 7.98 (d, 2H, $J=8.8$ Hz, Ar-H), 8.18 (s, 1H, -NH-); **$^{13}\text{C-NMR}$ (100 MHz, $\text{CDCl}_3+\text{DMSO-}d_6$, δ ppm):** 23.3, 121.7, 129.4, 129.8, 132.4, 132.9, 137.8, 147.1, 147.4, 151.5, 154.5, 160.5, 161.9; **ESI-MS (m/z):** 768 $[\text{M}-2]^+$; Analytical calculated formulae $\text{C}_{18}\text{H}_7\text{Br}_5\text{N}_6\text{O}_2\text{S}$; C, 28.05; H, 0.92; N, 10.90; S, 4.16: Found: C, 28.12; H, 0.98; N, 10.95; S, 4.12.

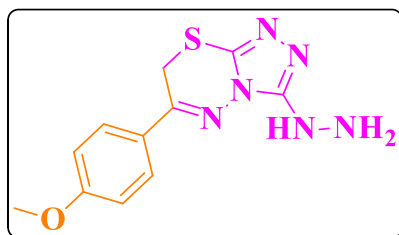
2-(6-Phenyl-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine-3-yl)-2,3-dihydrophthalazine-1,4-dione (27o):



White solid (0.330g, 88%); m.p.: 247-249°C; **IR (KBr, $\nu_{\max}$, cm^{-1}):** 3440 (-NH-), 1789 (-C=O), 1739 (-C=O); **$^1\text{H-NMR}$ (400 MHz, DMSO- d_6 , δ ppm):** 4.44 (s, 2H, -CH₂- of thiadiazine), 7.53 (t, 2H, $J=7.2$ Hz, Ar-H), 7.60 (t, 2H, $J=7.2$ Hz, Ar-H), 7.98-8.00 (m, 5H, Ar-H), 10.16 (s, 1H, -NH-); **$^{13}\text{C-NMR}$ (100 MHz, CDCl₃+DMSO- d_6 , δ ppm):** 23.5, 124.3, 127.9, 129.3, 129.8, 132.3, 133.8, 135.9, 138.2, 151.6, 155.0, 165.9; **ESI-MS (m/z):** 377 [M+H]⁺; Analytical calculated formulae C₁₈H₁₂N₆O₂S; C, 57.44; H, 3.21; N, 22.33; S, 8.52; Found: C, 57.48; H, 3.25; N, 22.38; S, 8.56.

3-Hydrazinyl-6-(4-methoxyphenyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (28):

An equimolar mixture of 4-amino-5-hydrazino-4H-[1,2,4]triazole-3-thiol (**6**) (0.001 mol) and 2-bromo-1-(4-methoxyphenyl)ethanone (**1**) (0.001 mol) was taken in ethanol (3 mL) and then refluxed for 4 h. After completion of the reaction, the mixture cooled to room temperature. The solid separated was filtered and recrystallized from ethanol.



Yellow solid (0.226g, 82%); m.p.: 192-194°C; **IR (KBr, $\nu_{\max}$, cm^{-1}):** 3460 (-NH₂), 3451 (-NH-), 1605 (-C=N-); **$^1\text{H-NMR}$ (400 MHz, CDCl₃+DMSO- d_6 , δ ppm):** 3.31 (s, 1H, -NH-), 3.90 (s, 3H, -OMe), 4.31 (s, 2H, -CH₂- of thiadiazine), 6.97 (s, 2H, -NH₂), 7.03 (d, 2H, $J=8.8$ Hz, Ar-H), 8.06 (d, 2H, $J=9.2$ Hz, Ar-H); **$^{13}\text{C-NMR}$ (100 MHz, CDCl₃+DMSO- d_6 , δ ppm):** 38.2, 55.7, 114.2, 125.9, 129.3, 131.2, 161.4, 161.5, 168.1; **ESI-MS (m/z):** 276; Analytical calculated formulae C₁₁H₁₂N₆OS; C, 47.81; H, 4.38; N, 30.41; S, 11.60; Found: C, 47.85; H, 4.33; N, 30.45; S, 11.67.

Conversion of compound 28 into 27

A mixture of compound **28** (0.001 mol) and phthalic anhydride (**3**) (0.001 mol) in 3 mL acetic acid refluxed for 6-7 h, cooled and solid separated was filtered and washed with water and recrystallized from ethanol.

SPECTRA

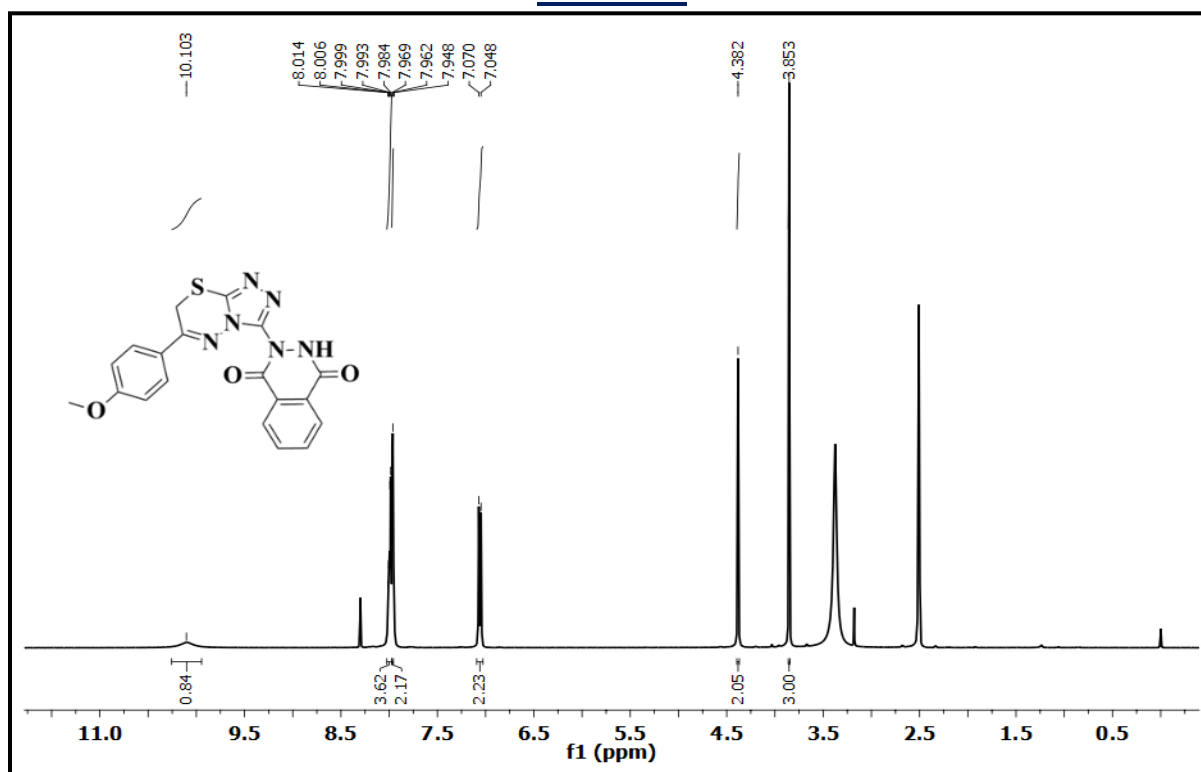


Fig: ¹H-NMR Spectrum of Compound 27a (400 MHz, DMSO-*d*₆)

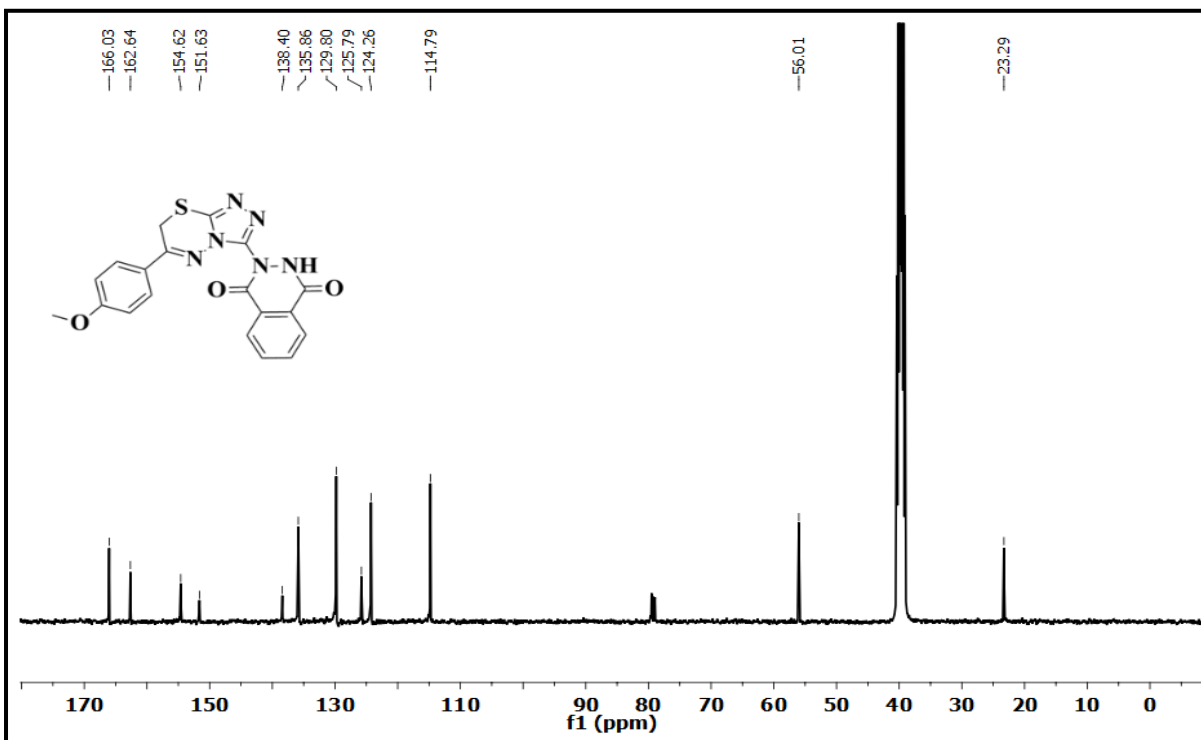


Fig: ¹³C-NMR Spectrum of Compound 27a (100 MHz, CDCl₃+DMSO-*d*₆)

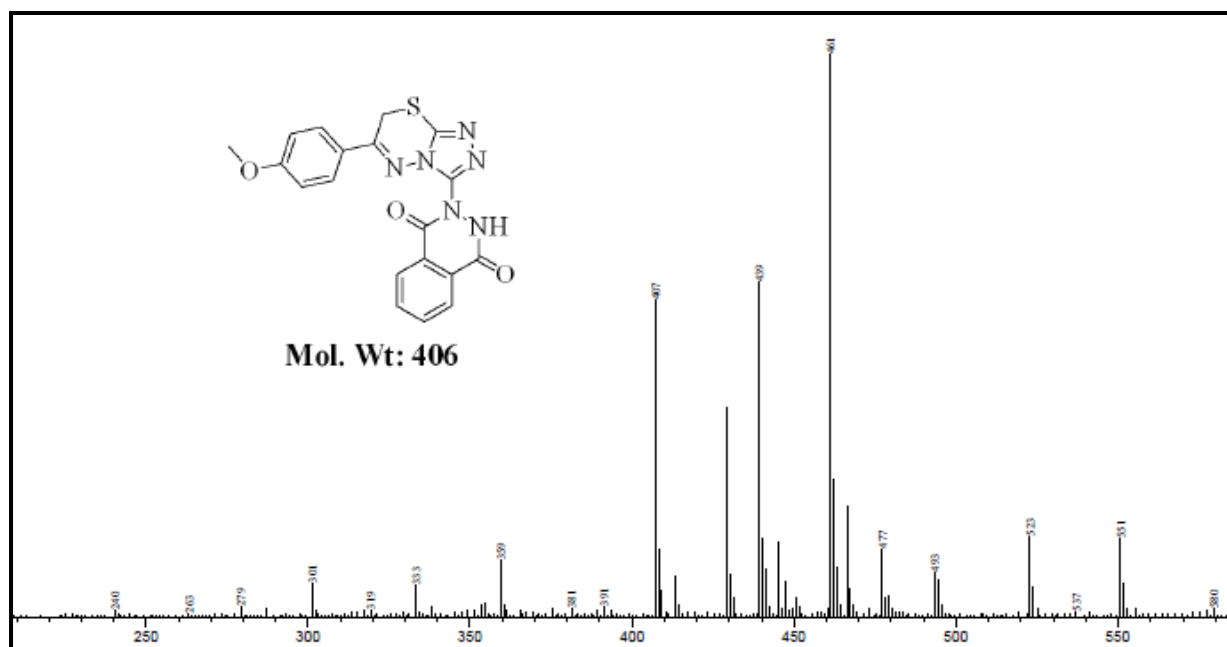


Fig: Mass Spectrum of Compound 27a

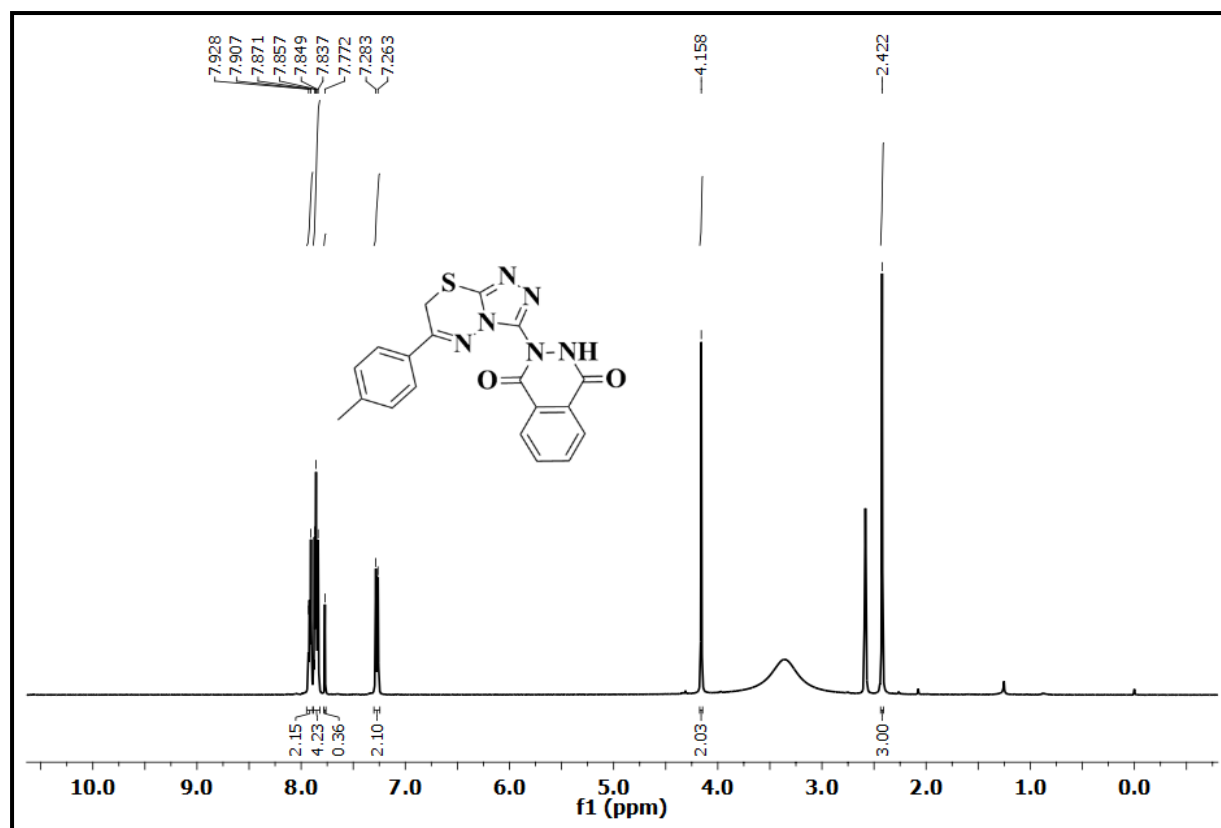


Fig: ^1H -NMR Spectrum of Compound 27b (400 MHz, $\text{DMSO}-d_6$)

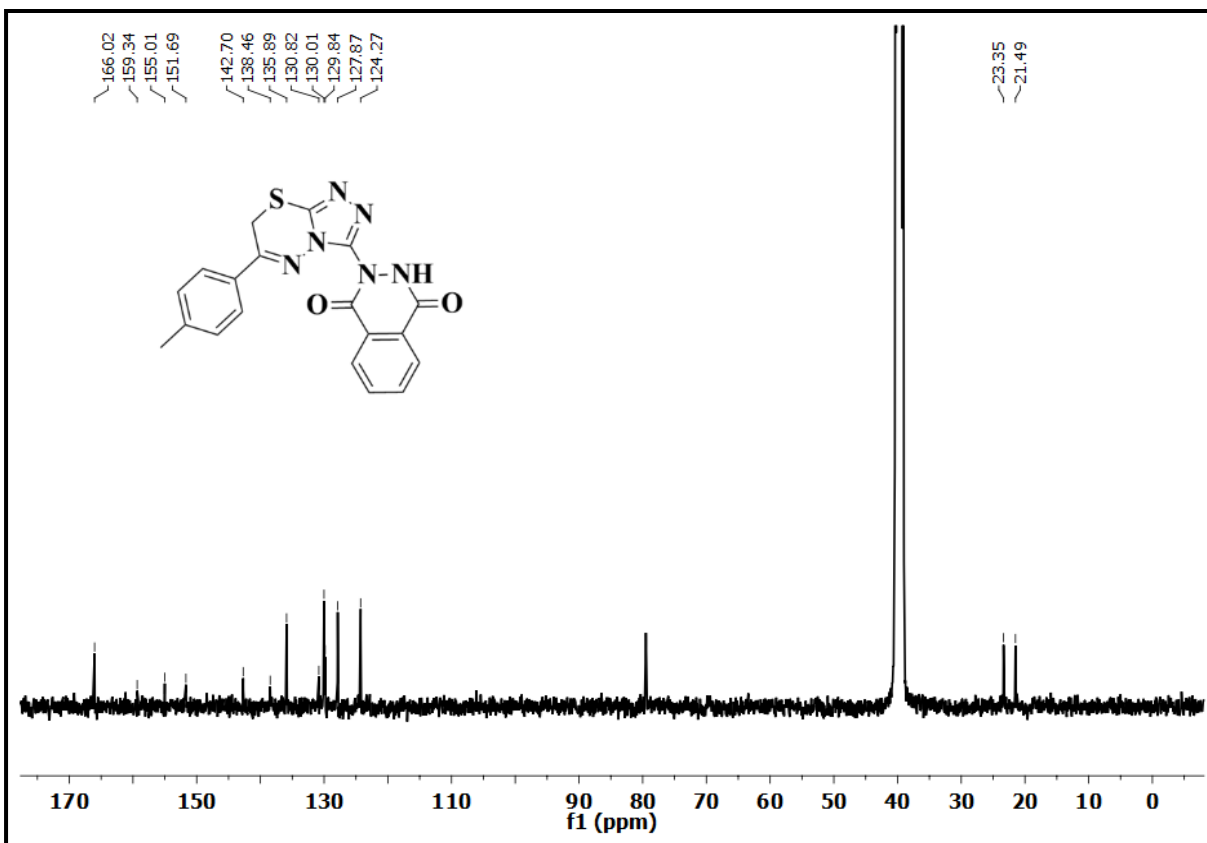


Fig: ¹³C-NMR Spectrum of Compound 27b (100 MHz, CDCl₃+DMSO-*d*₆)

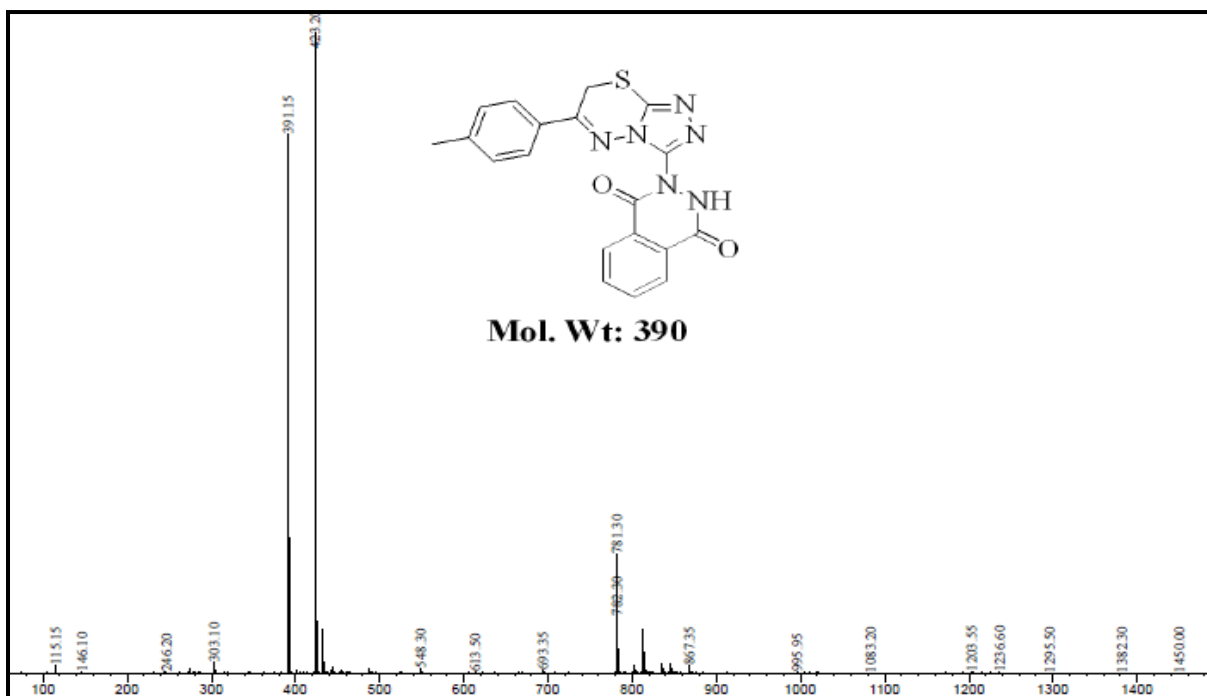


Fig: Mass Spectrum of Compound 27b

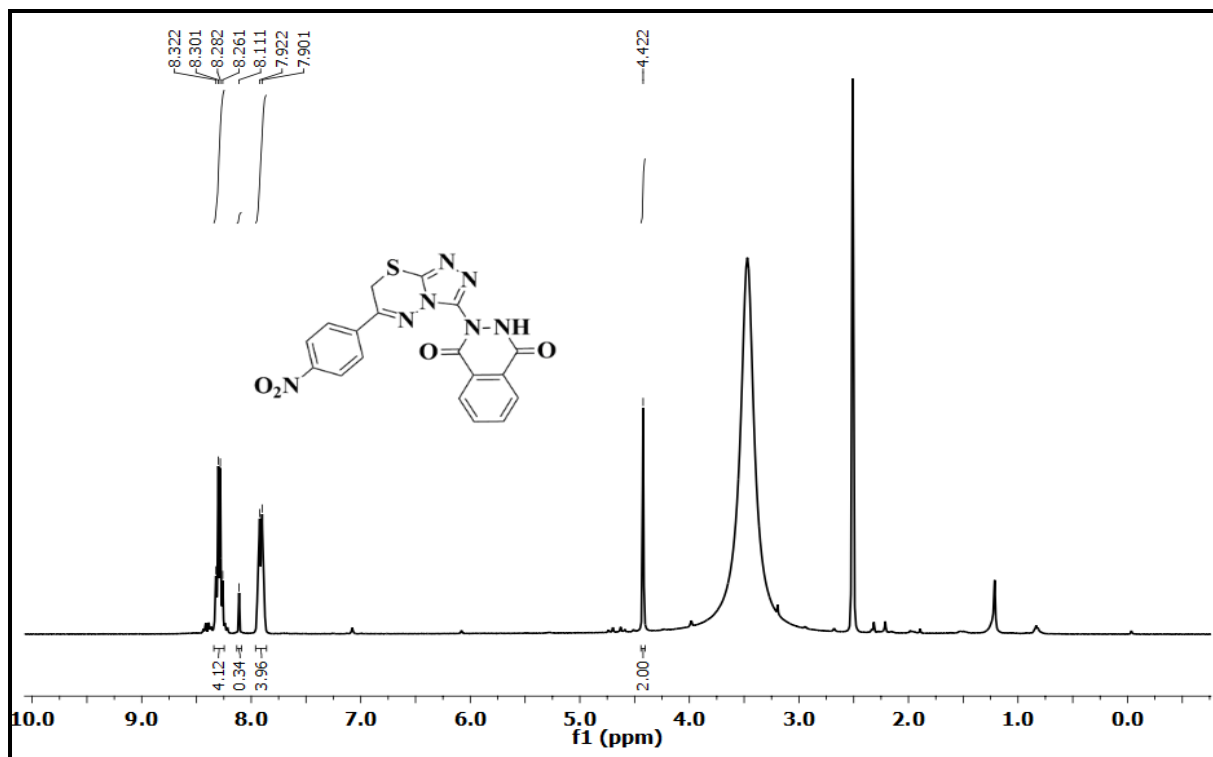


Fig: ¹H-NMR Spectrum of Compound 27c (400 MHz, DMSO-*d*₆)

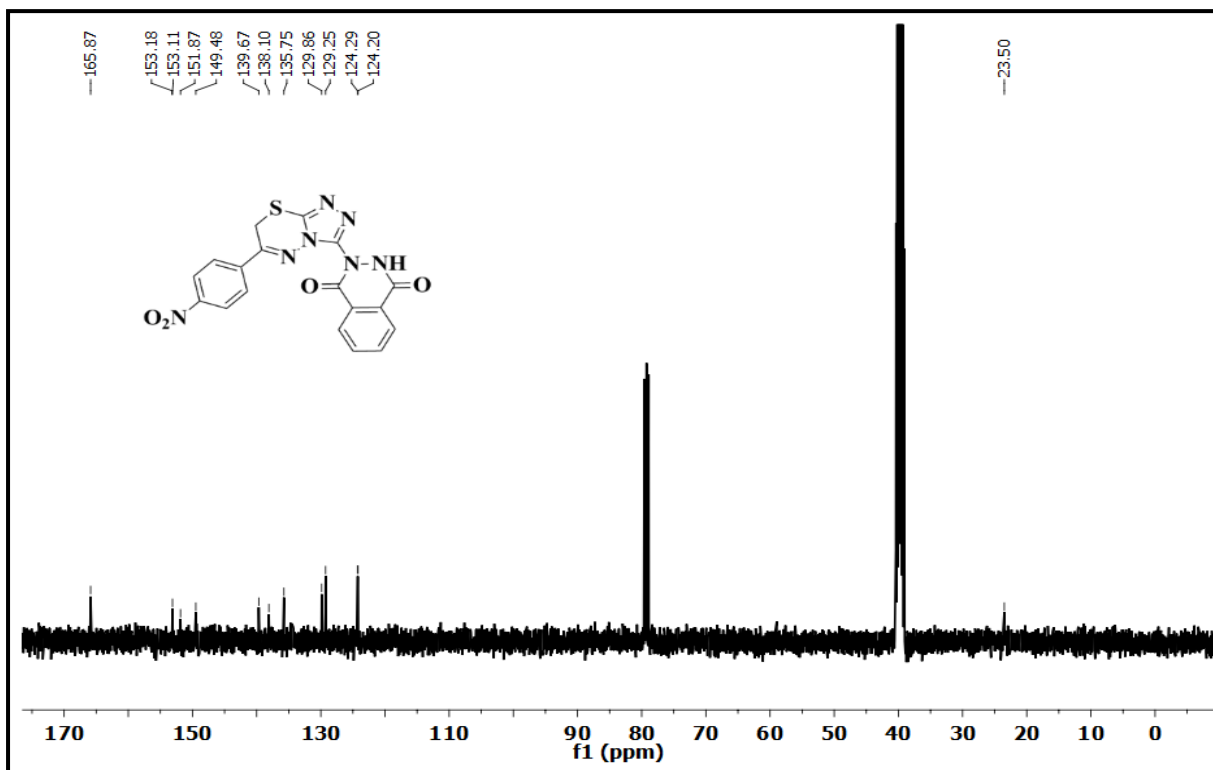


Fig: ¹³C-NMR Spectrum of Compound 27c (100 MHz, CDCl₃+ DMSO-*d*₆)

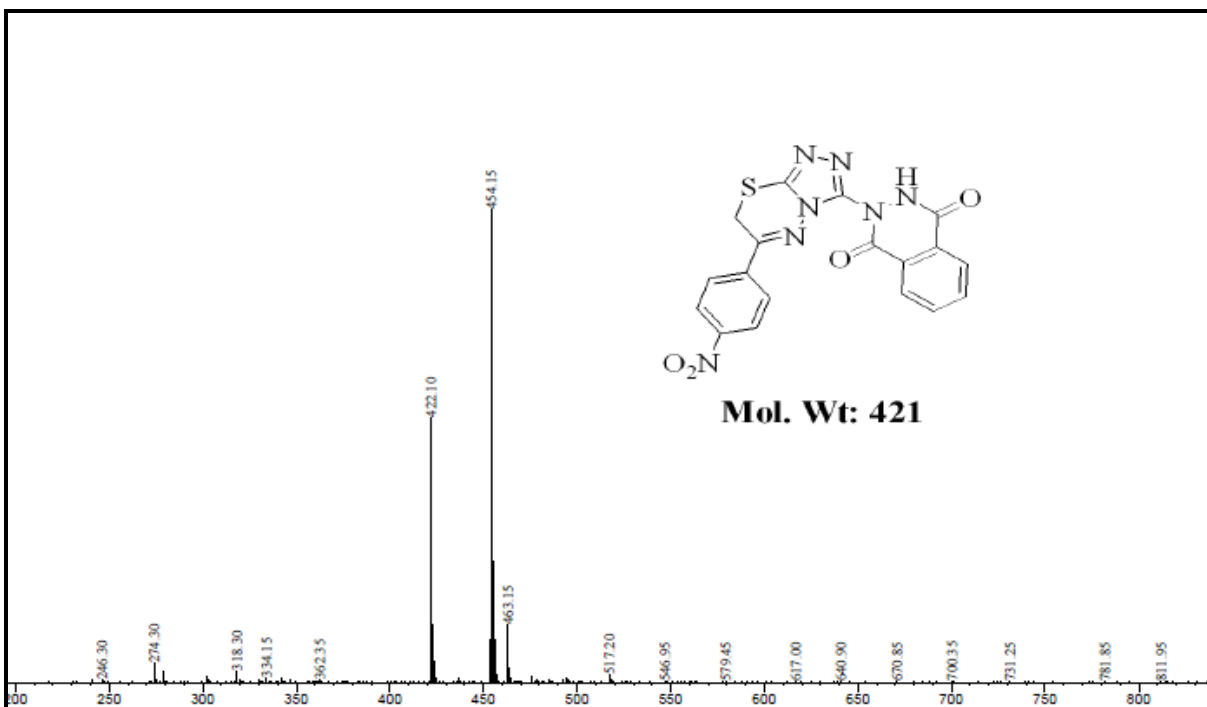


Fig: Mass Spectrum of Compound 27c

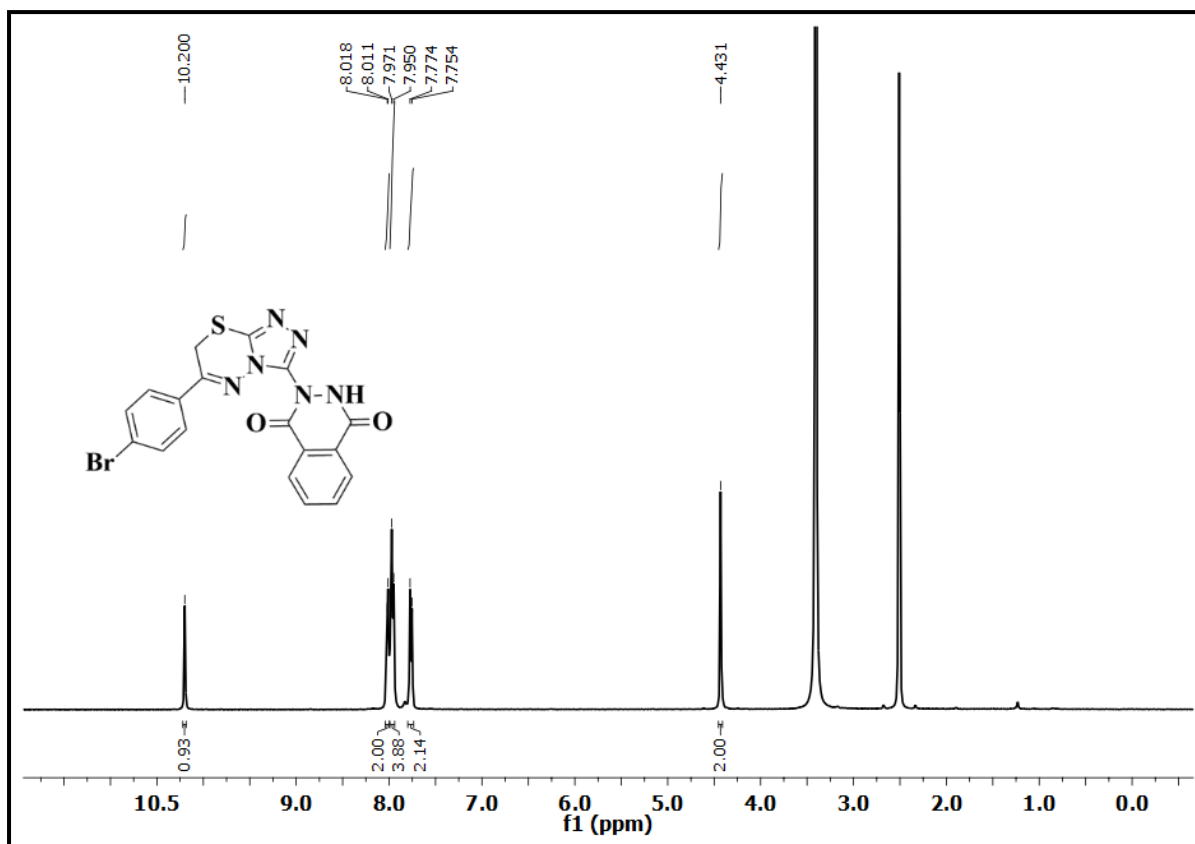


Fig: ¹H-NMR Spectrum of Compound 27d (400 MHz, DMSO-*d*₆)

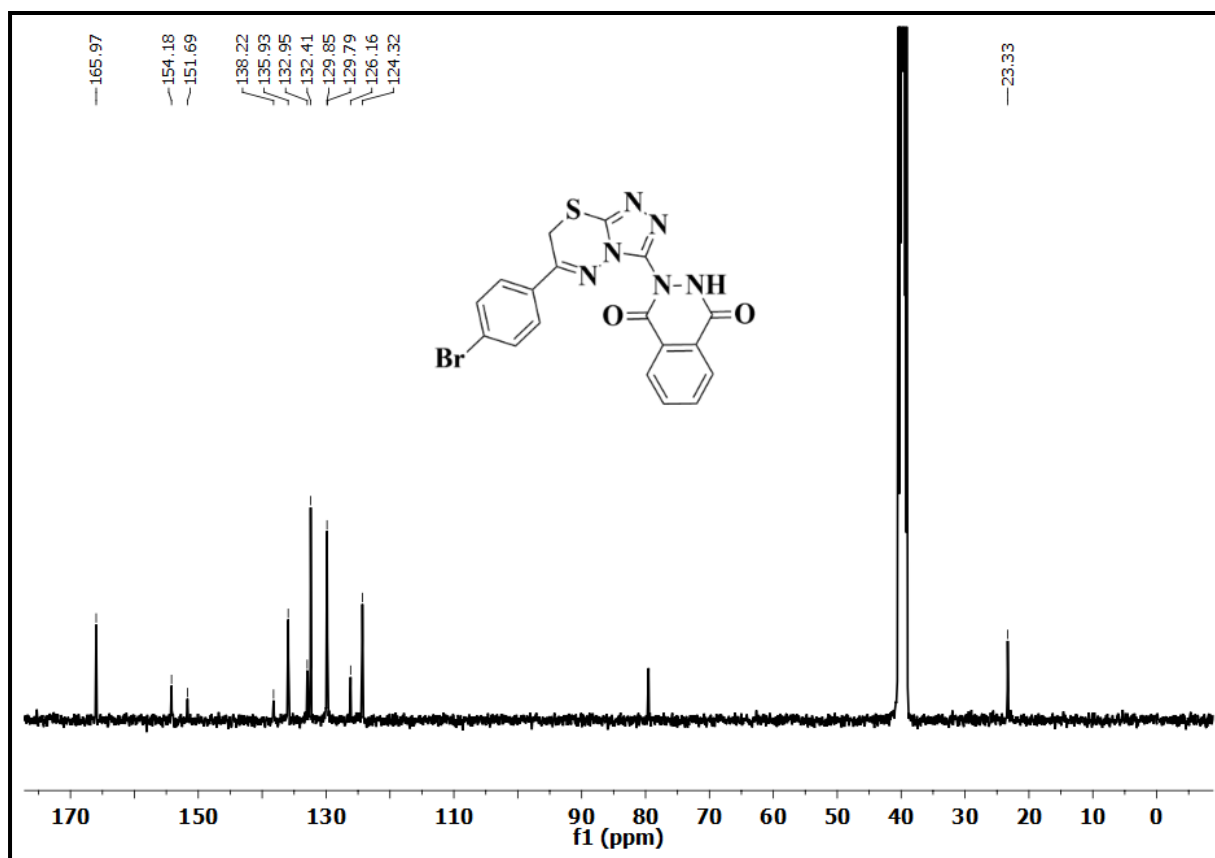


Fig: ¹³C-NMR Spectrum of Compound 27d (100 MHz, CDCl₃+ DMSO-*d*₆)

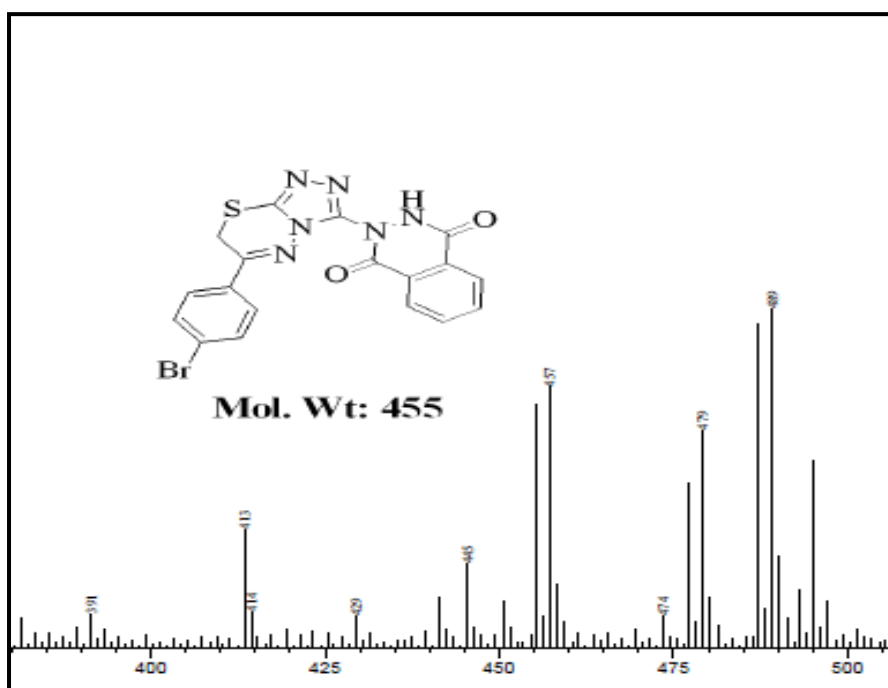


Fig: Mass Spectrum of Compound 27d

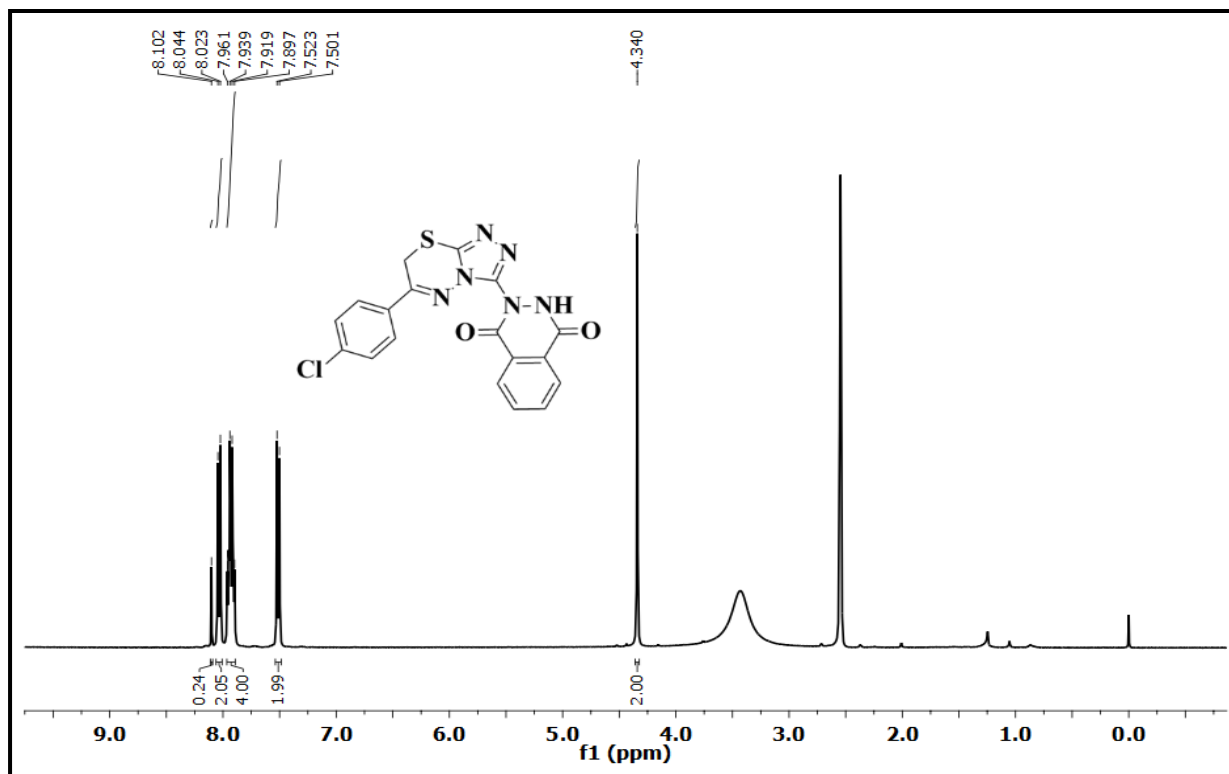


Fig: ¹H-NMR Spectrum of Compound 27e (400 MHz, DMSO-*d*₆)

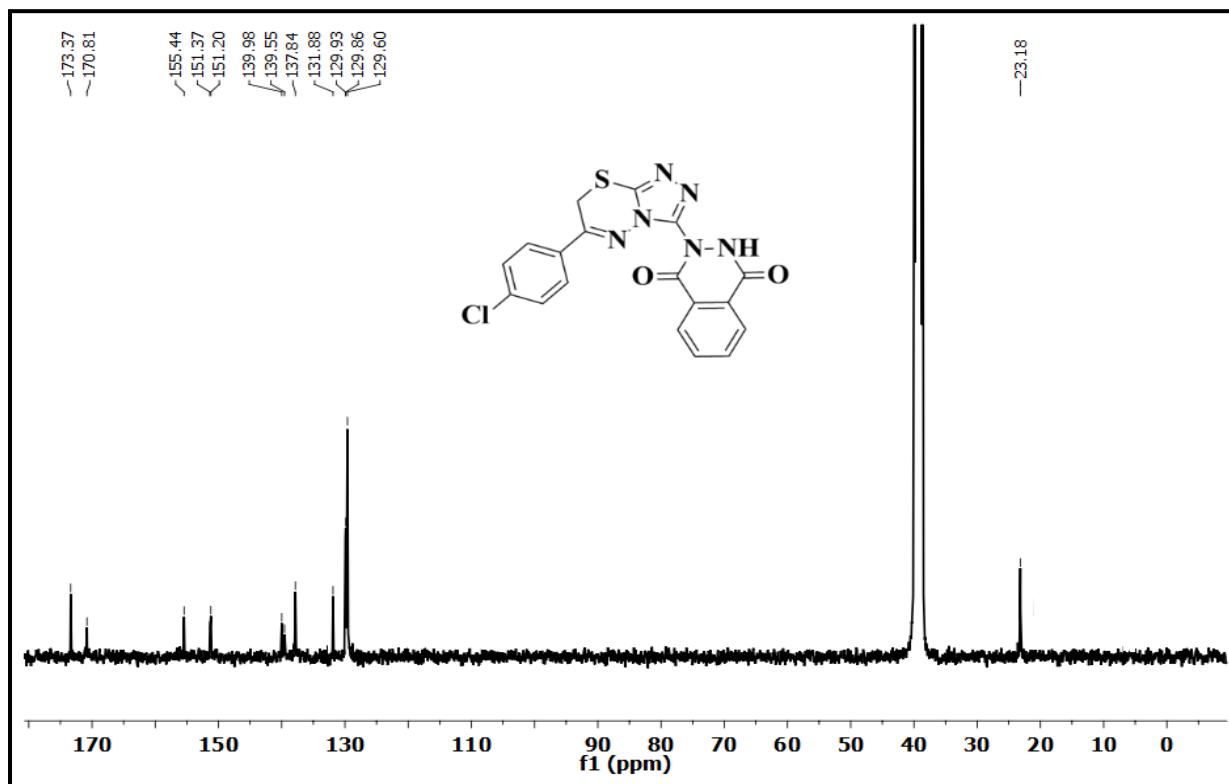


Fig: ¹³C-NMR Spectrum of Compound 27e (100 MHz, DMSO-*d*₆)

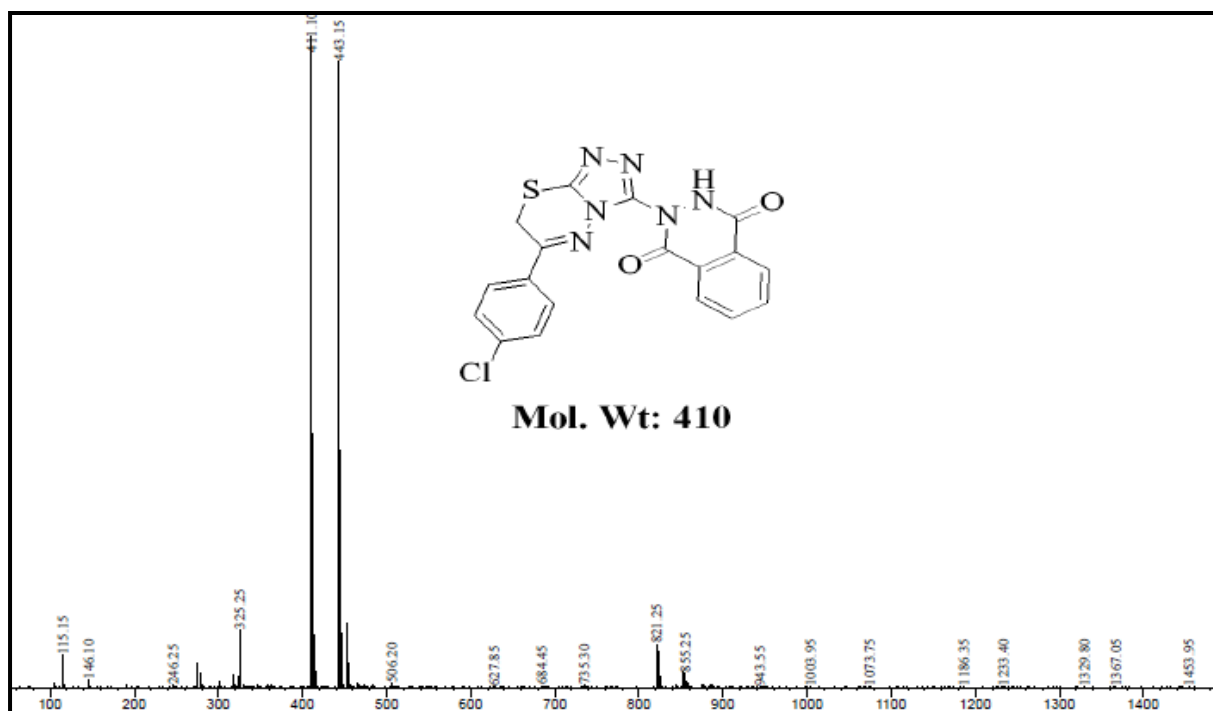


Fig: Mass Spectrum of Compound **27e**

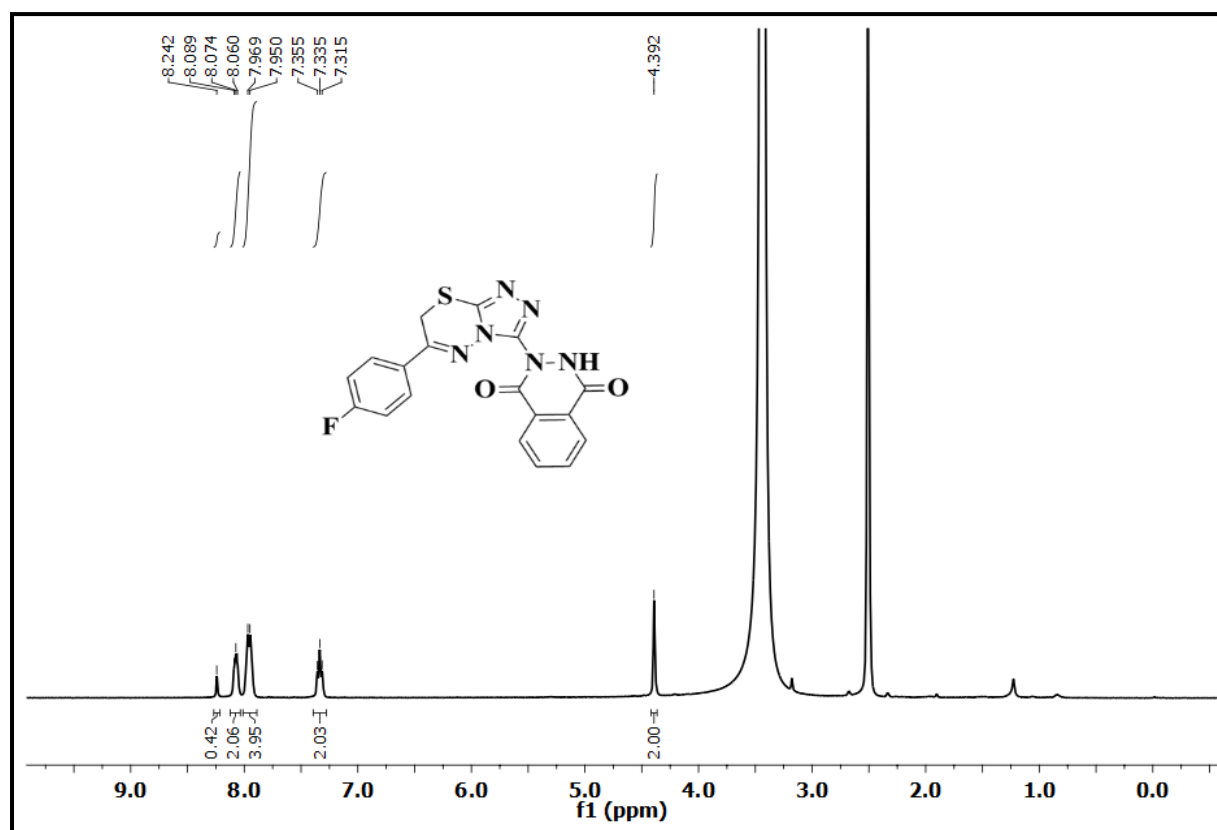


Fig: ^1H -NMR Spectrum of Compound **27f** (400 MHz, $\text{DMSO-}d_6$)

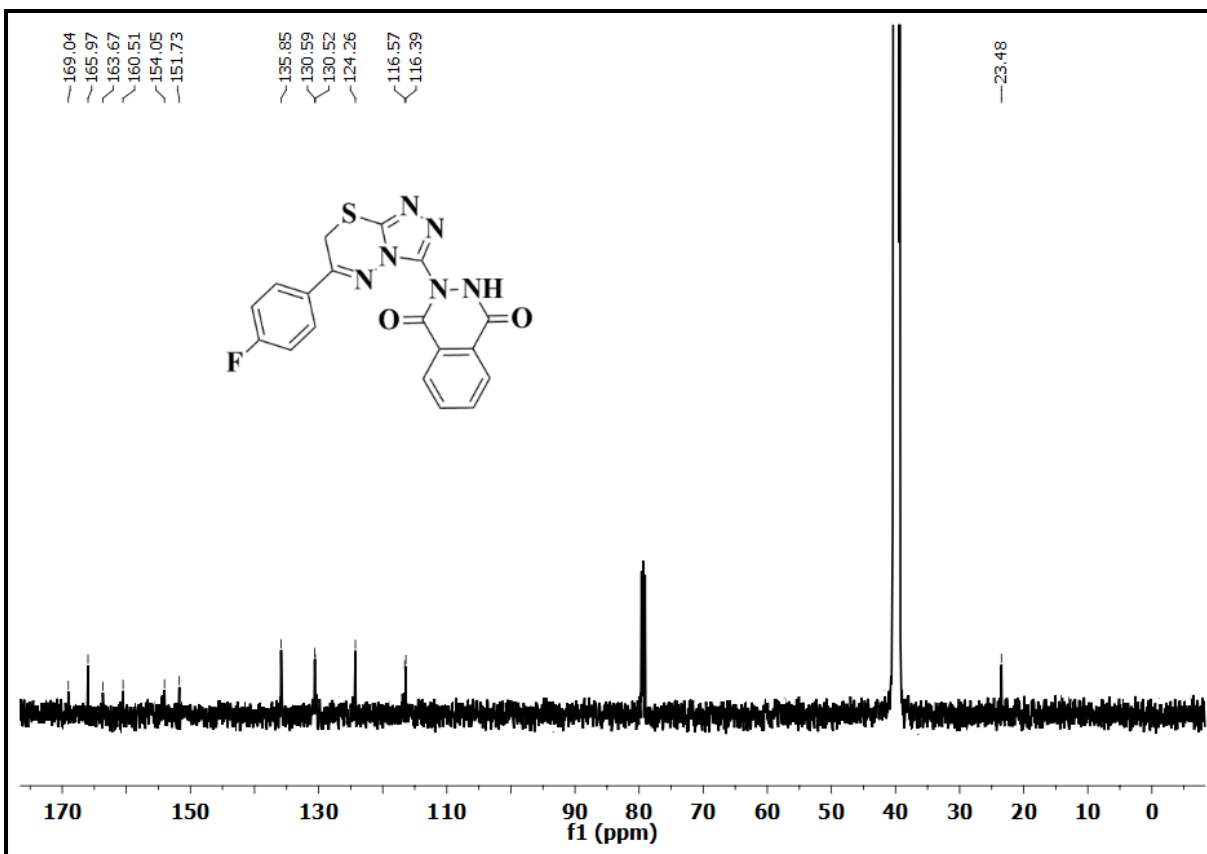


Fig: ¹³C-NMR Spectrum of Compound 27f (100 MHz, CDCl₃+ DMSO-*d*₆)

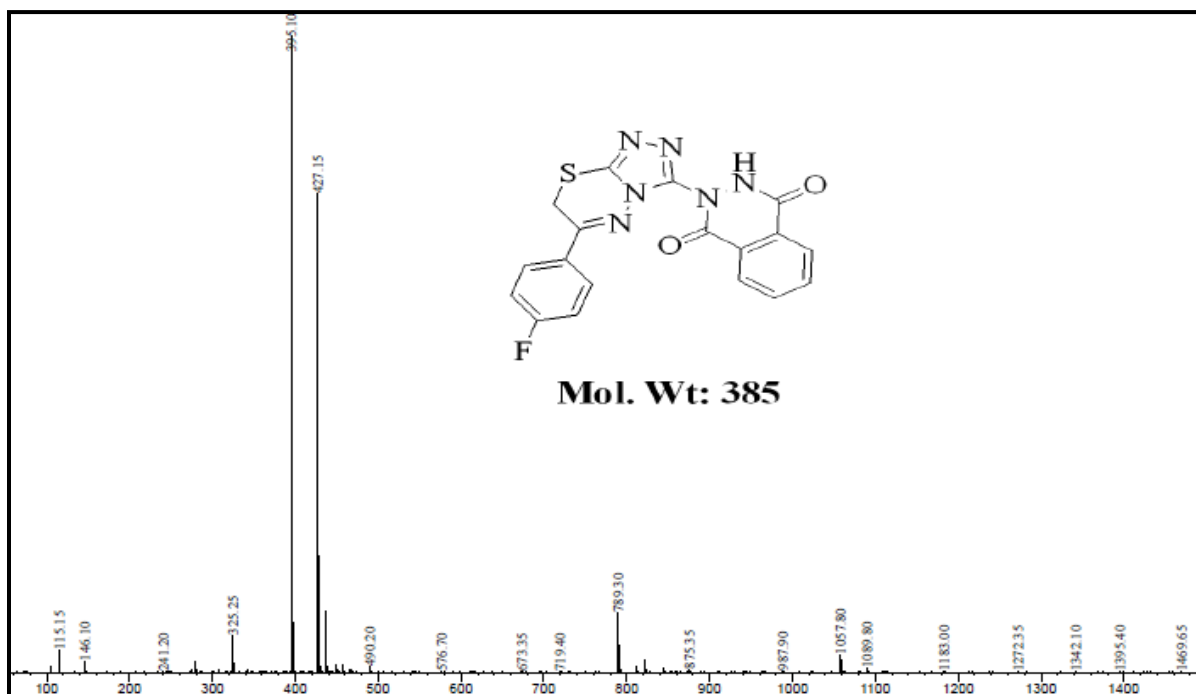


Fig: Mass Spectrum of Compound 27f

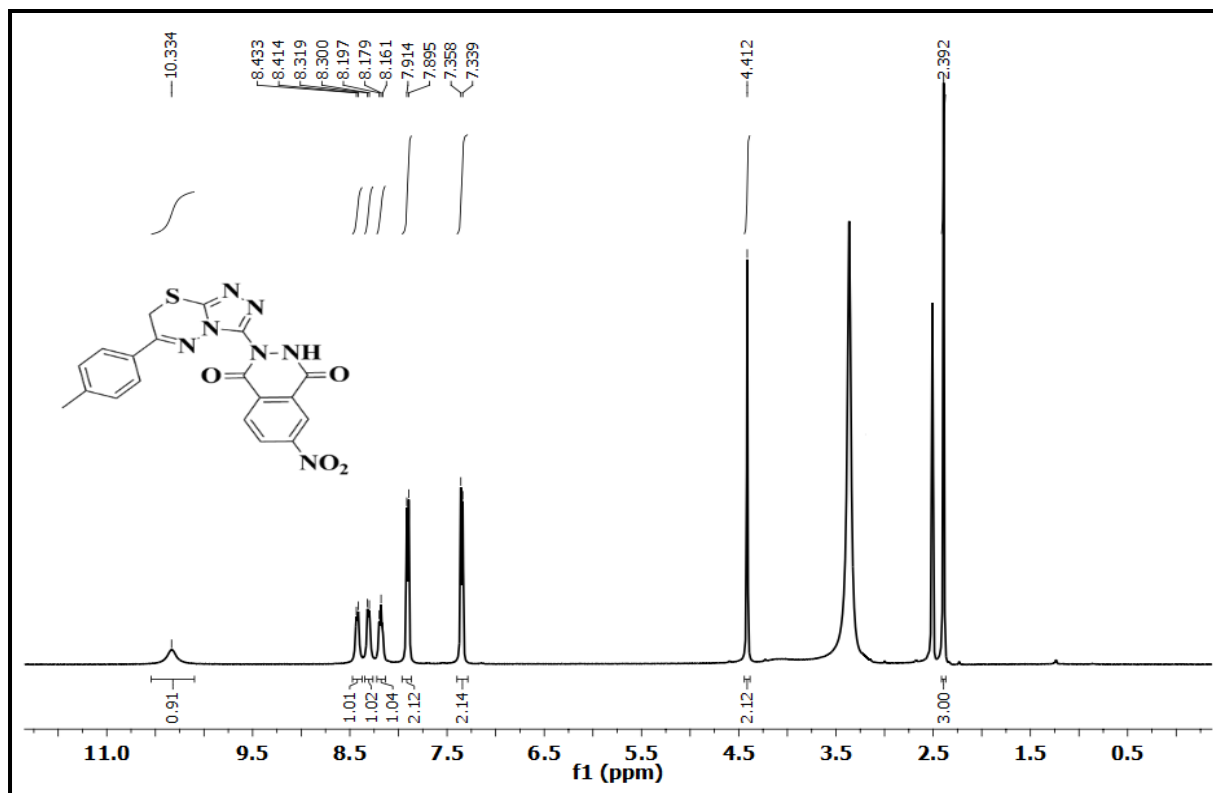


Fig: ¹H-NMR Spectrum of Compound **27g** (400 MHz, DMSO-*d*₆)

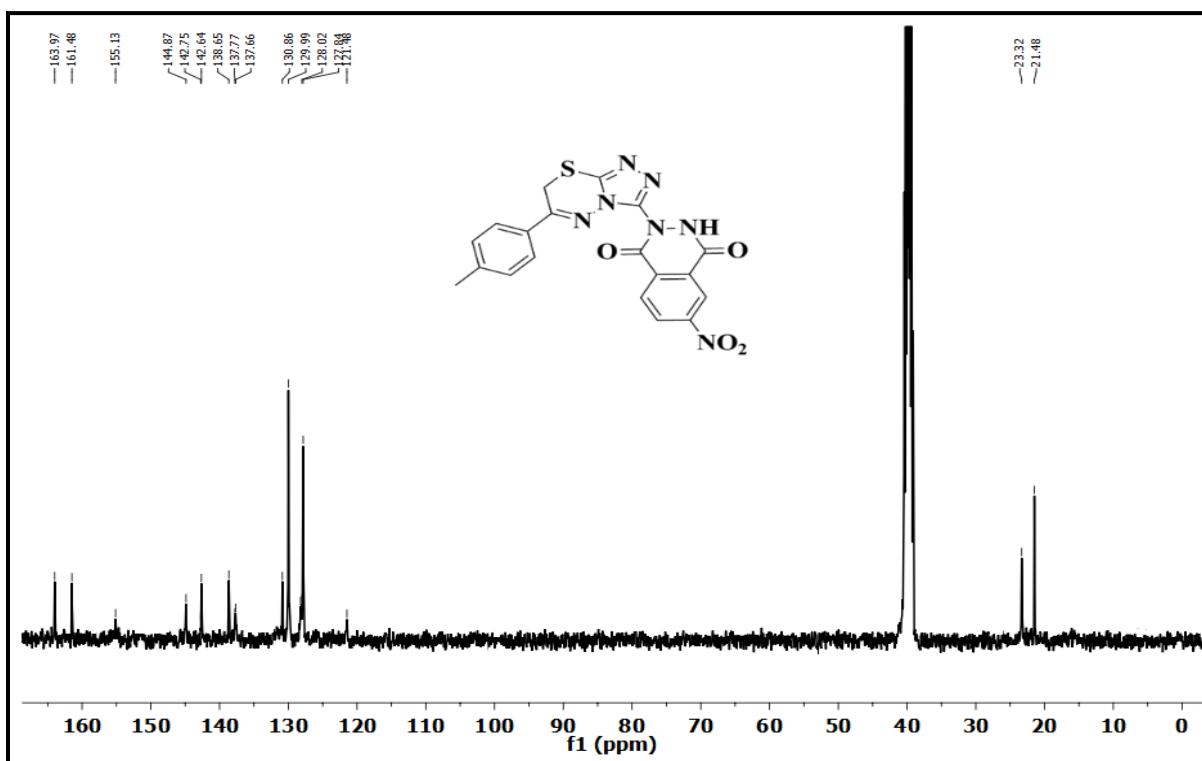


Fig: ¹³C-NMR Spectrum of Compound **27g** (100 MHz, DMSO-*d*₆)

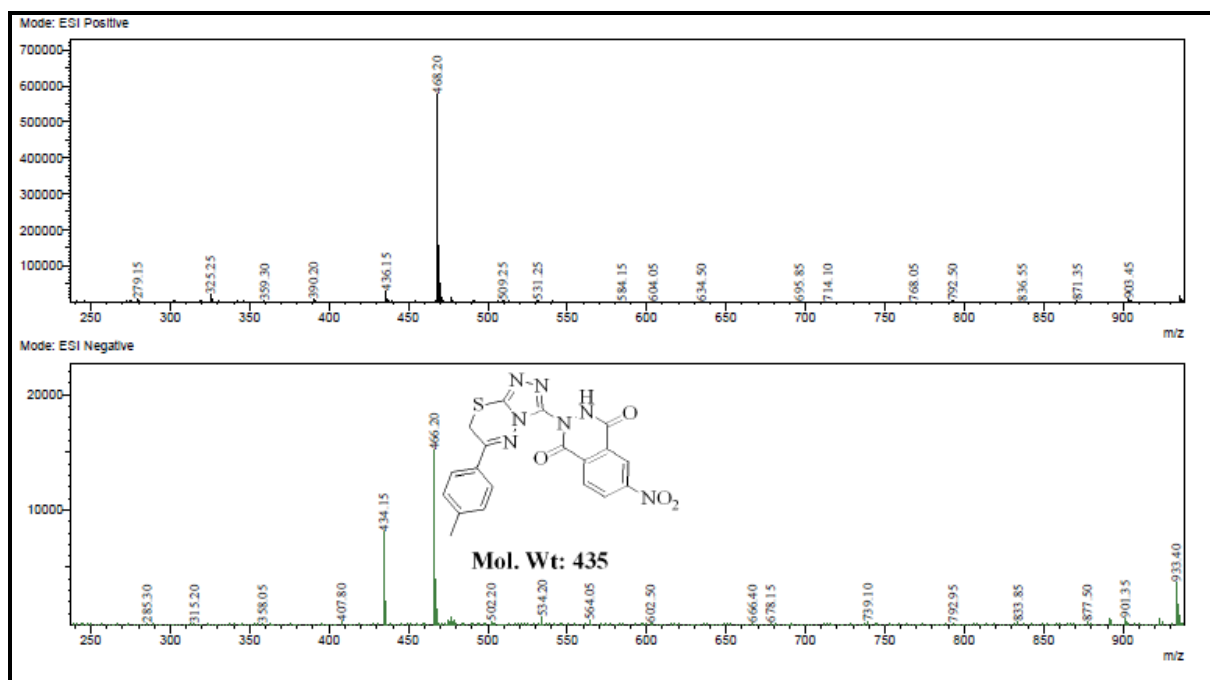


Fig: Mass Spectrum of Compound 27g

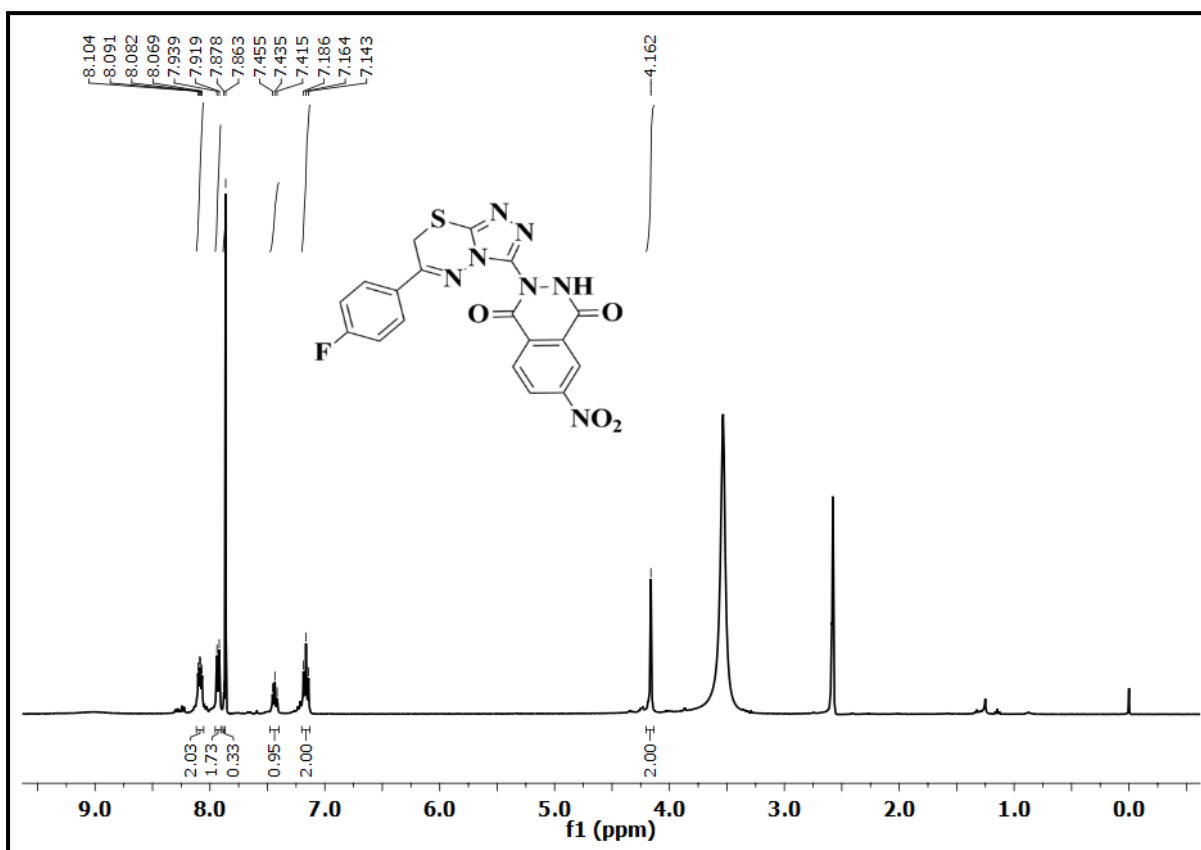


Fig: ¹H-NMR Spectrum of Compound 27h (400 MHz, DMSO-d₆)

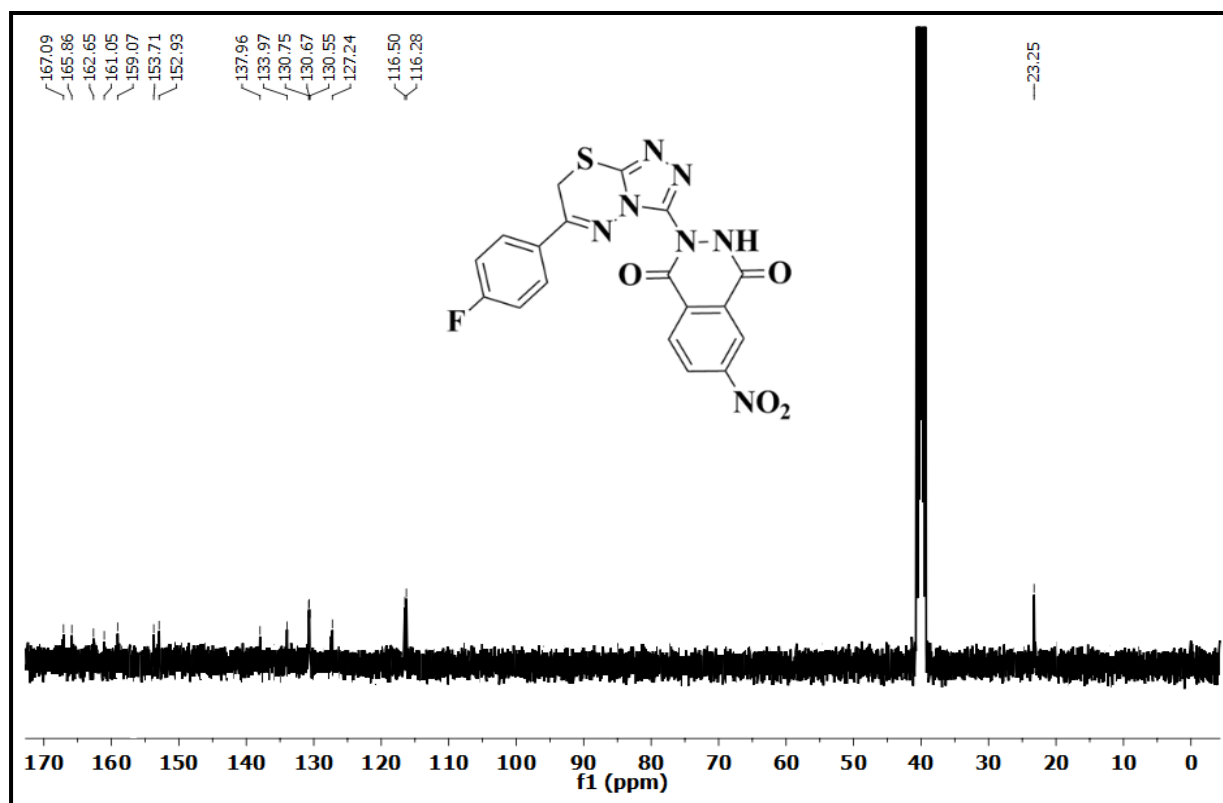


Fig: ¹³C-NMR Spectrum of Compound **27h** (100 MHz, DMSO-*d*₆)

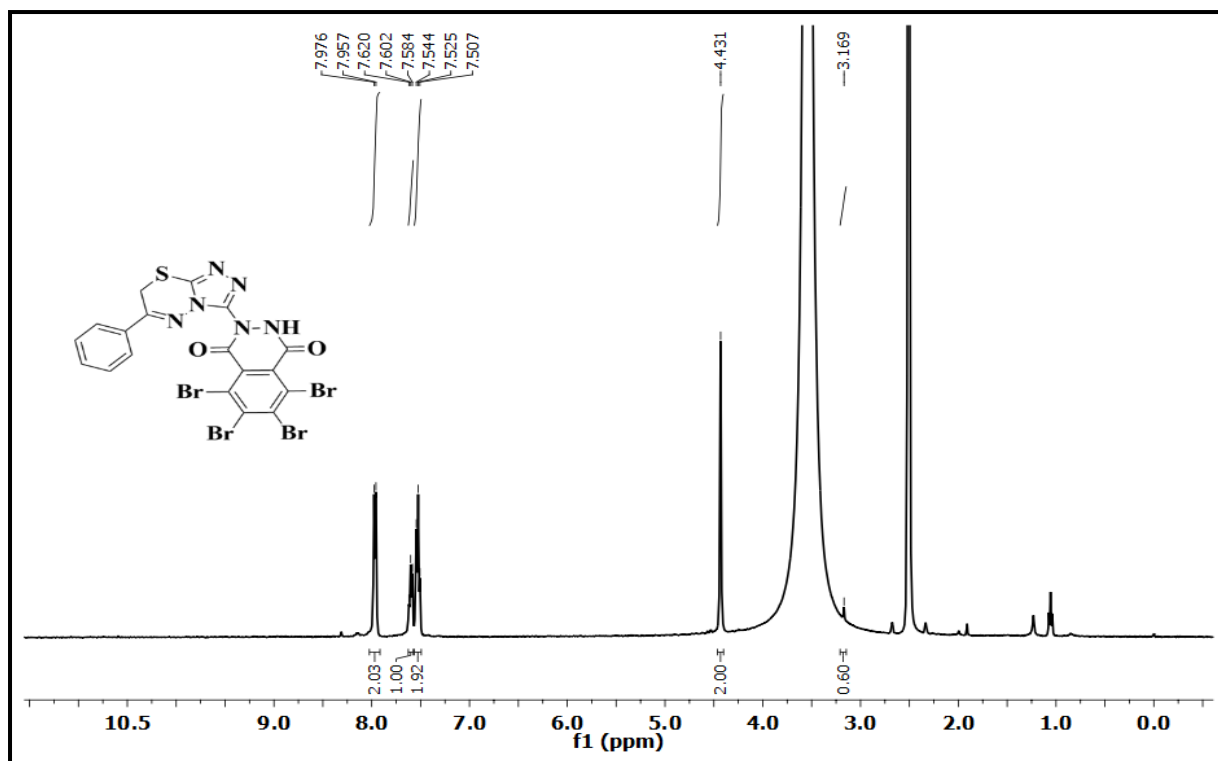


Fig: ¹H-NMR Spectrum of Compound **27i** (400 MHz, DMSO-*d*₆)

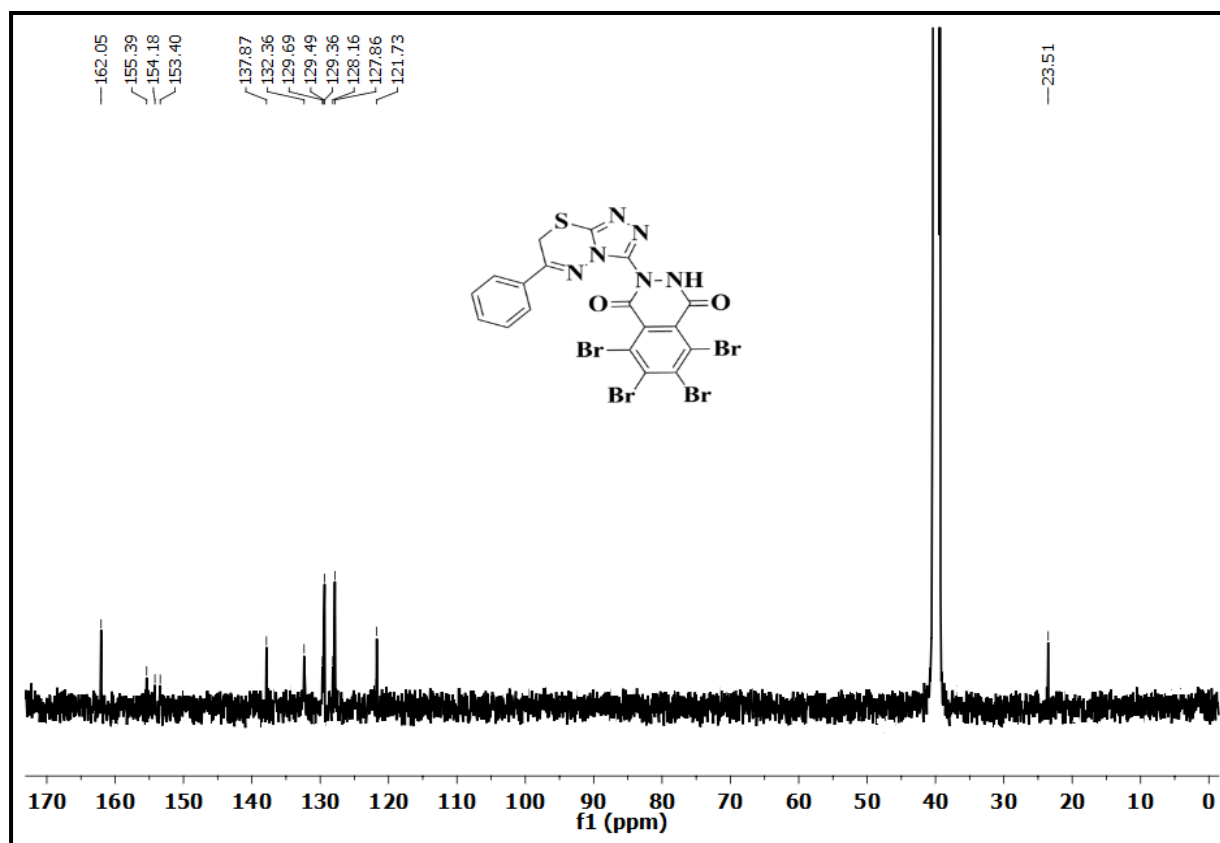


Fig: ¹³C-NMR Spectrum of Compound 27i (100 MHz, DMSO-*d*₆)

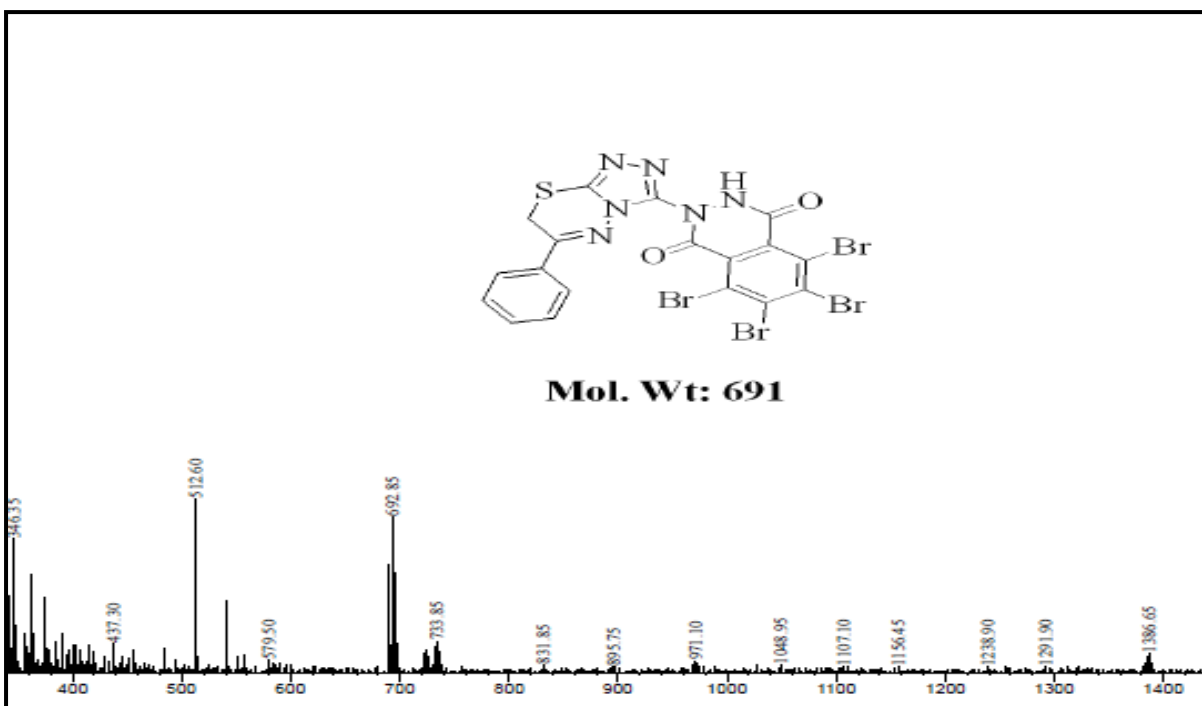


Fig: Mass Spectrum of Compound 27i

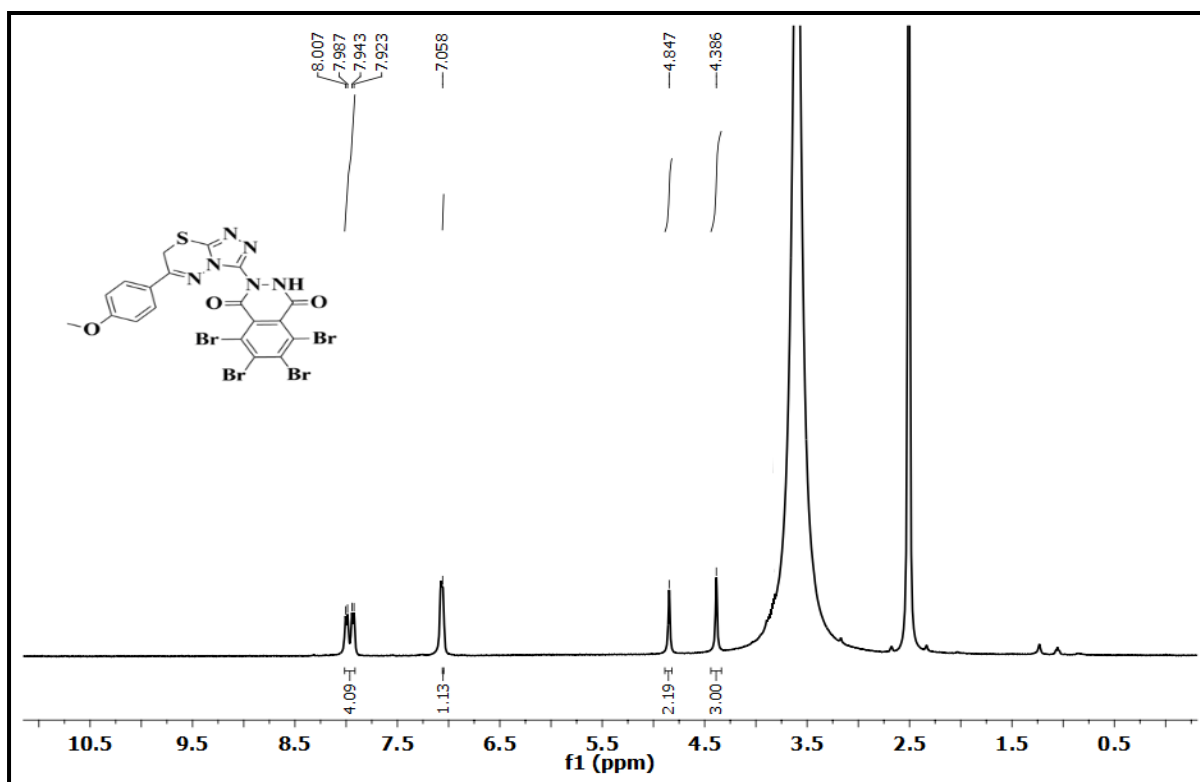


Fig: ¹H-NMR Spectrum of Compound **27j** (400 MHz, DMSO-*d*₆)

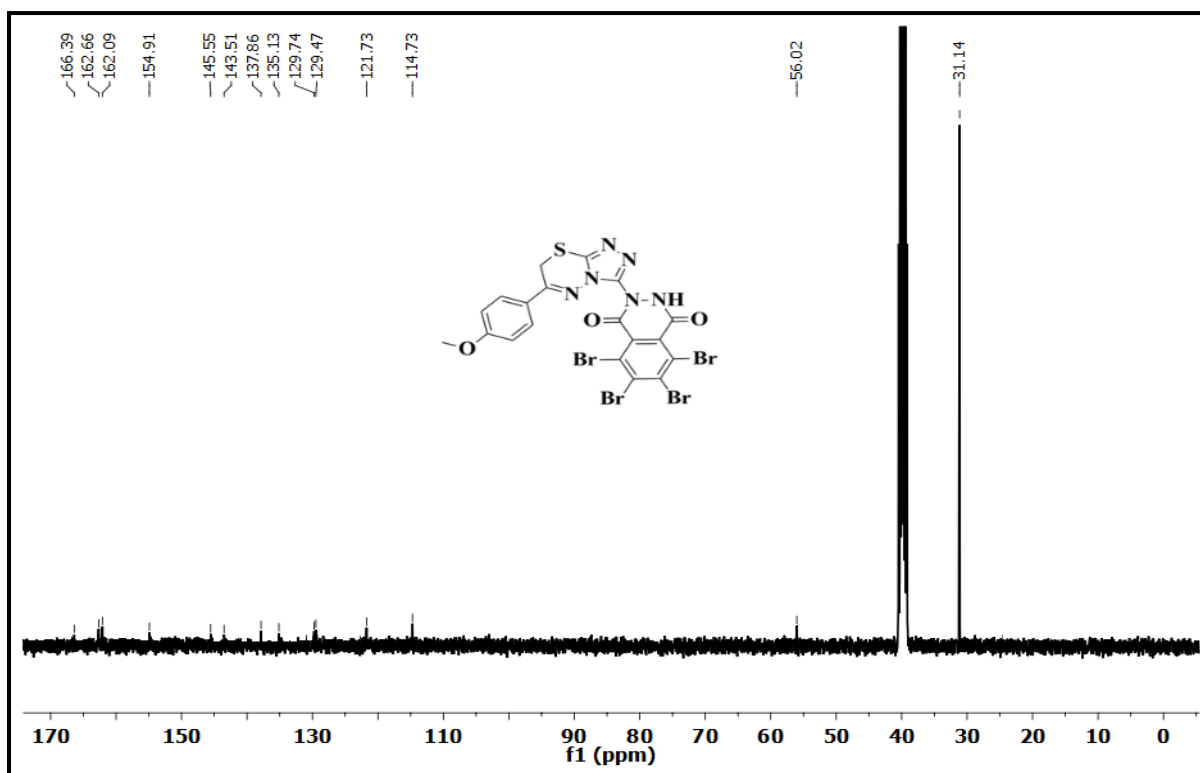


Fig: ¹³C-NMR Spectrum of Compound **27j** (100 MHz, DMSO-*d*₆)

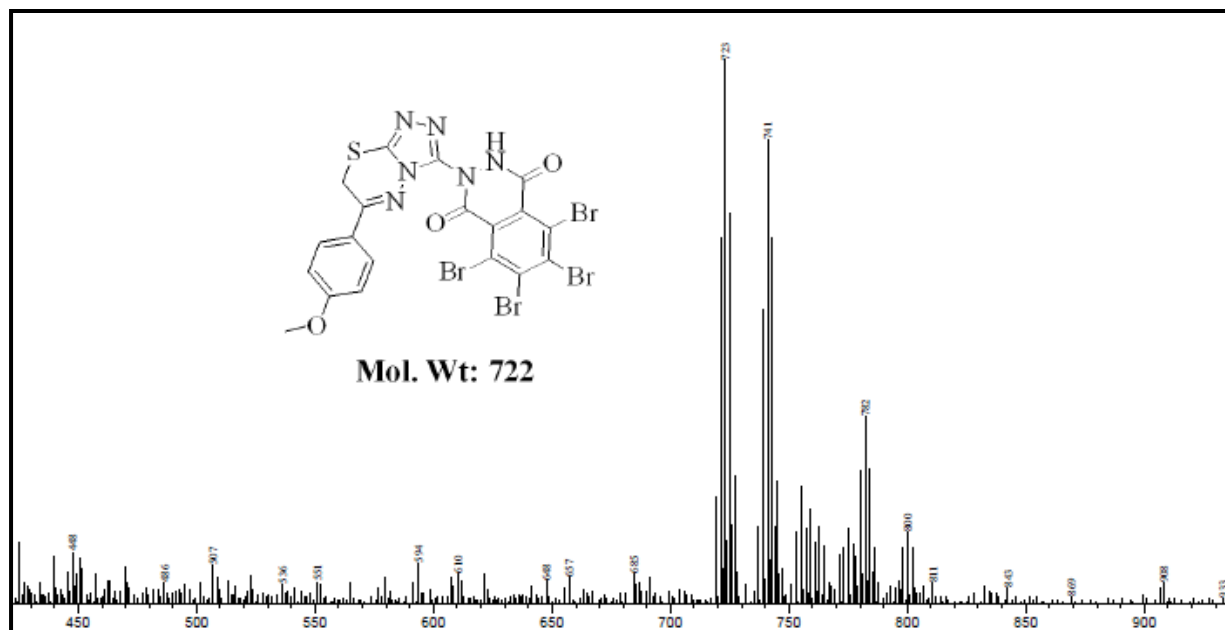


Fig: Mass Spectrum of Compound 27j

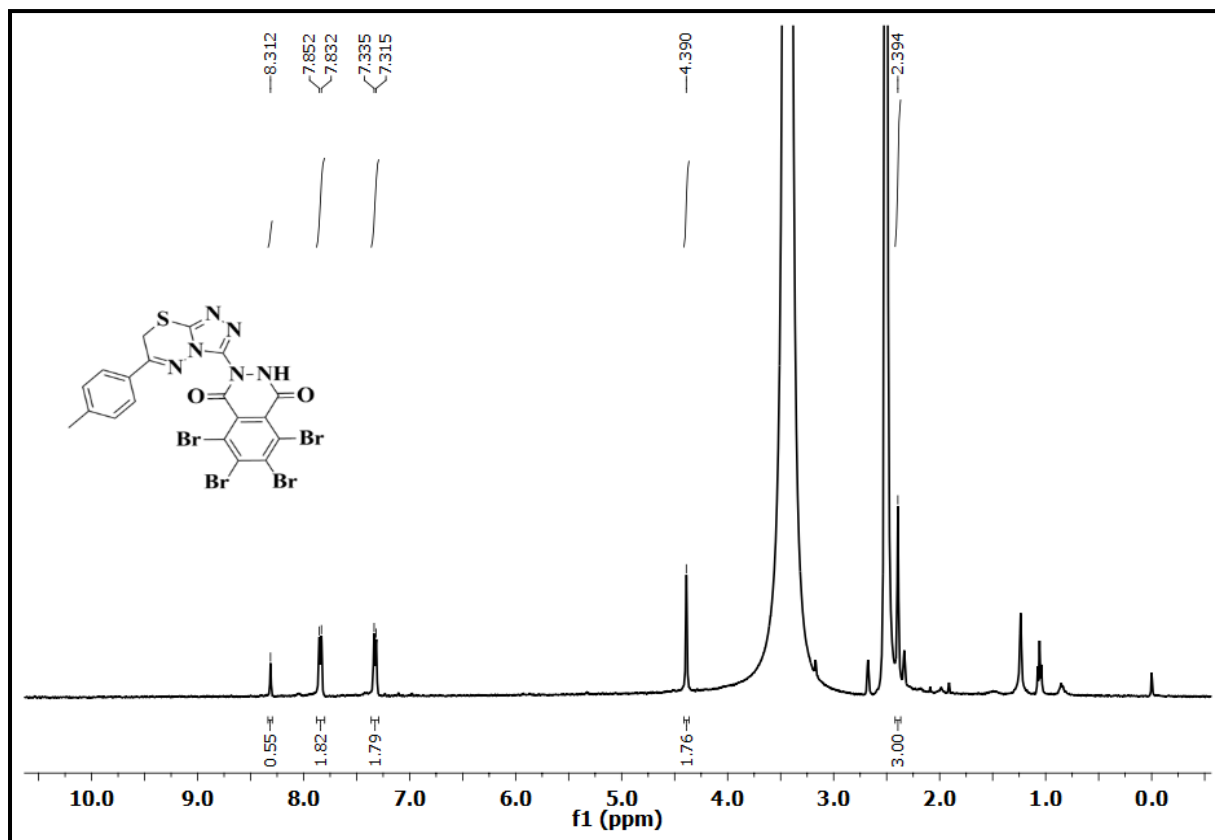


Fig: ^1H -NMR Spectrum of Compound 27k (400 MHz, $\text{DMSO}-d_6$)

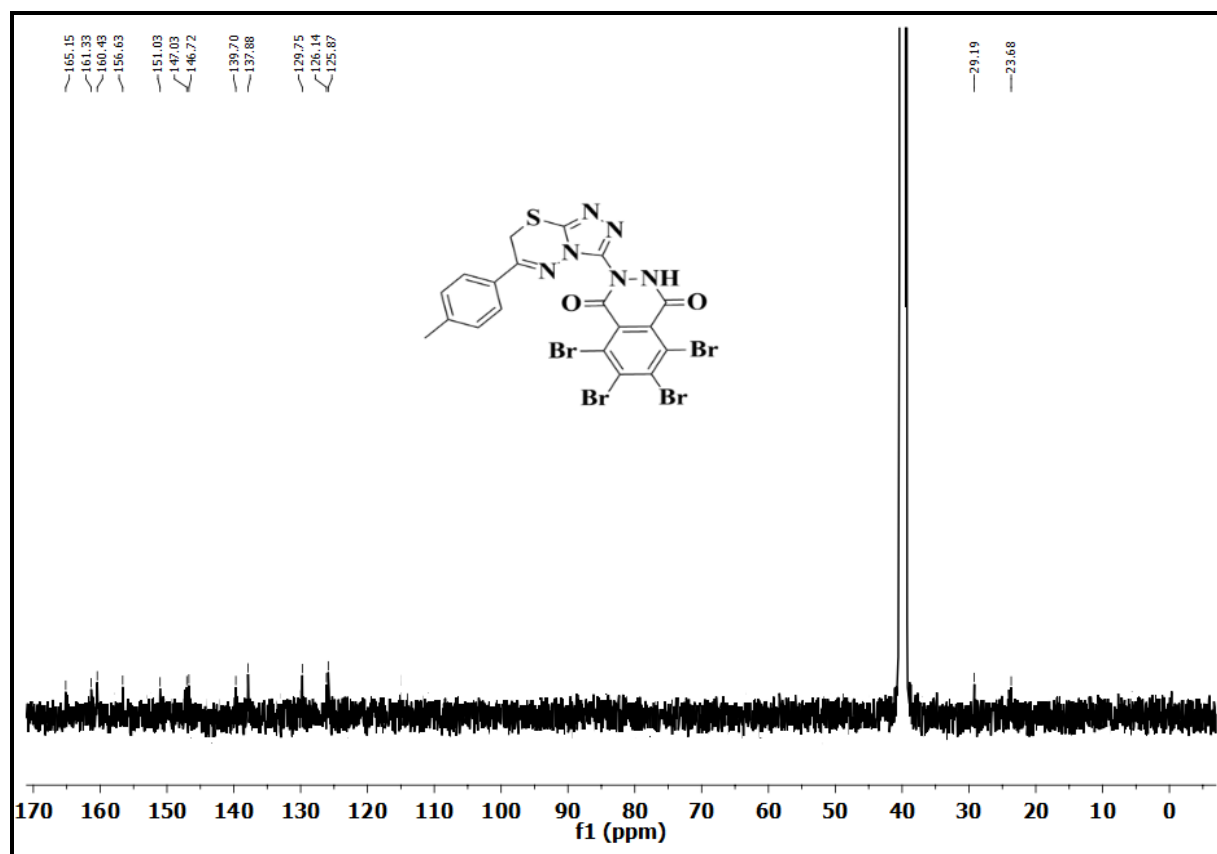


Fig: ¹³C-NMR Spectrum of Compound 27k (100 MHz, DMSO-*d*₆)

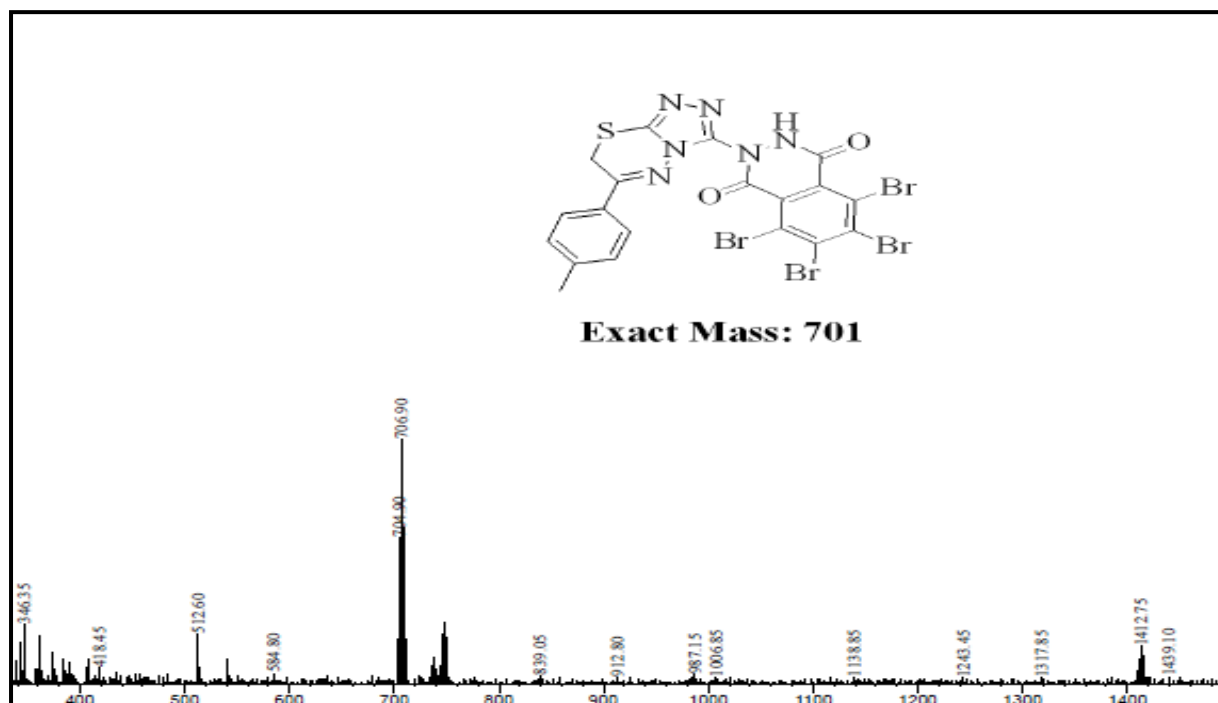


Fig: Mass Spectrum of Compound 27k

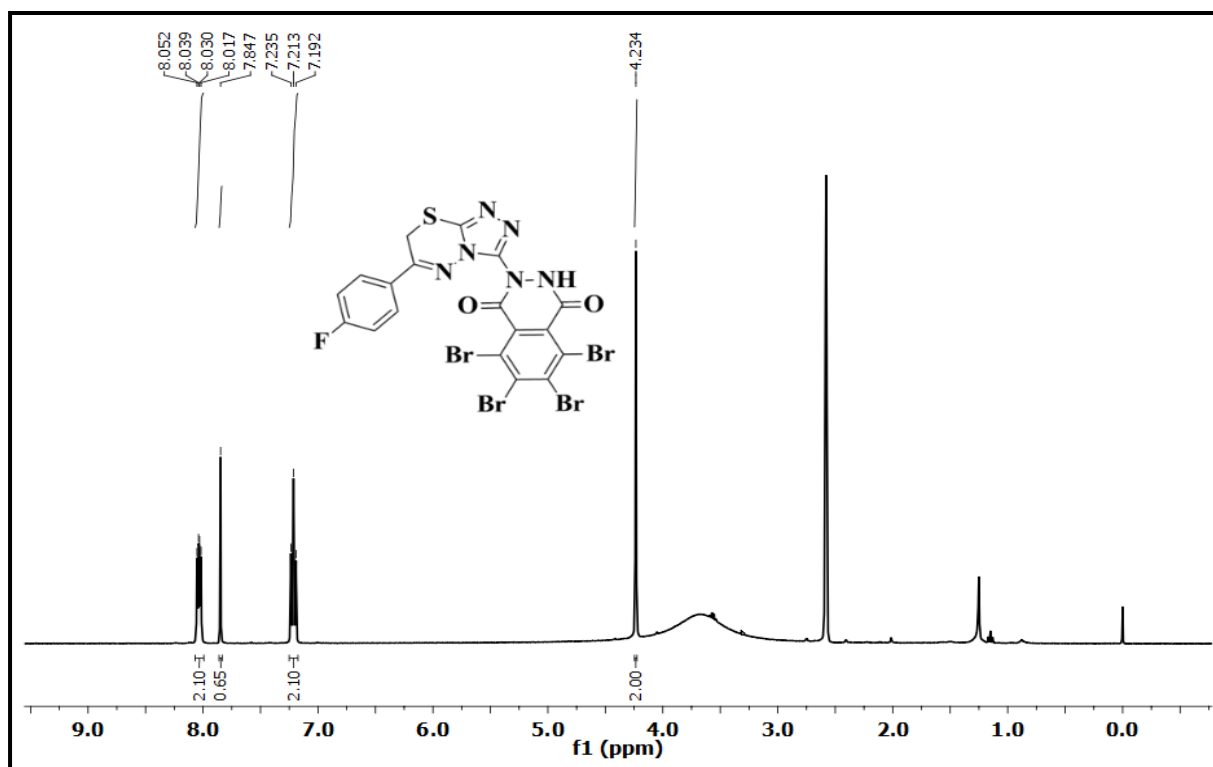


Fig: ¹H-NMR Spectrum of Compound 27I (400 MHz, DMSO-*d*₆)

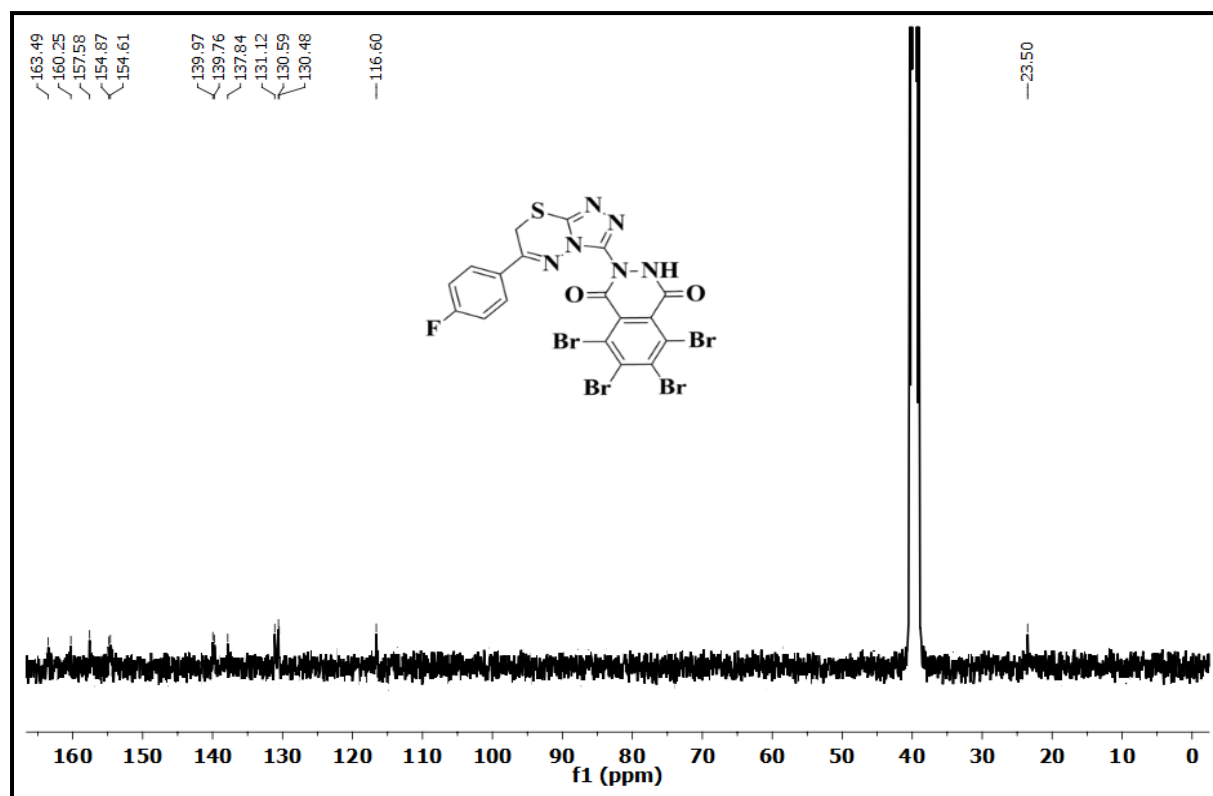


Fig: ¹³C-NMR Spectrum of Compound 27I (100 MHz, DMSO-*d*₆)

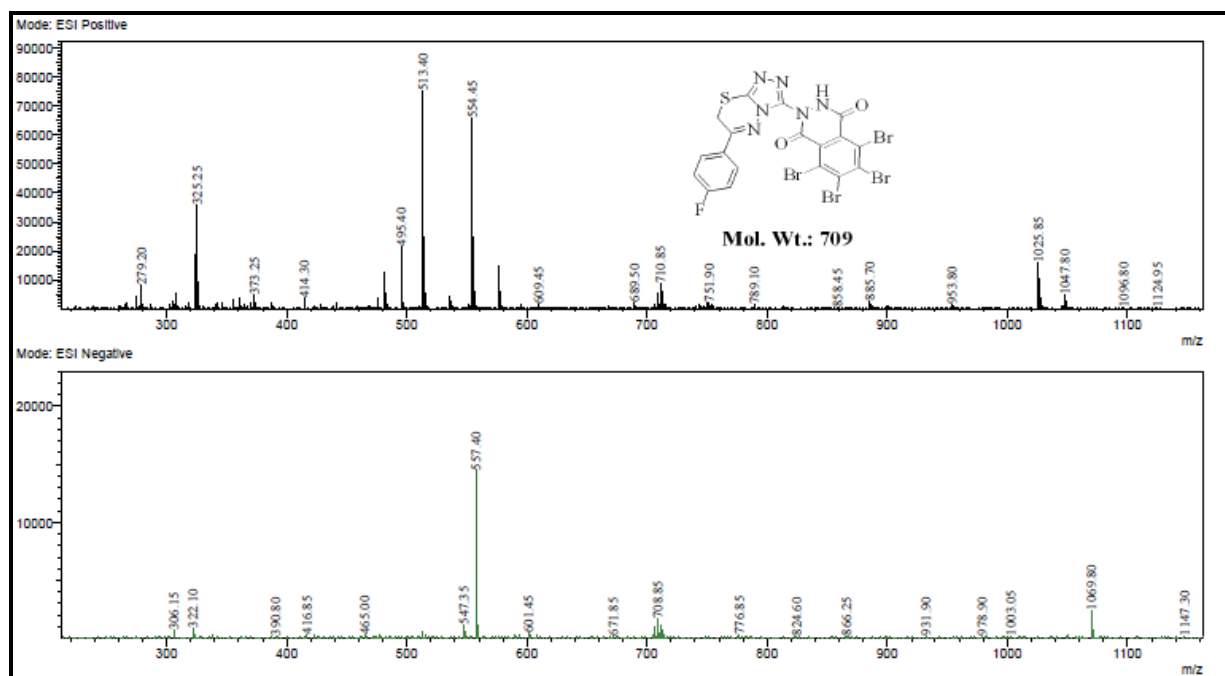


Fig: Mass Spectrum of Compound 27l

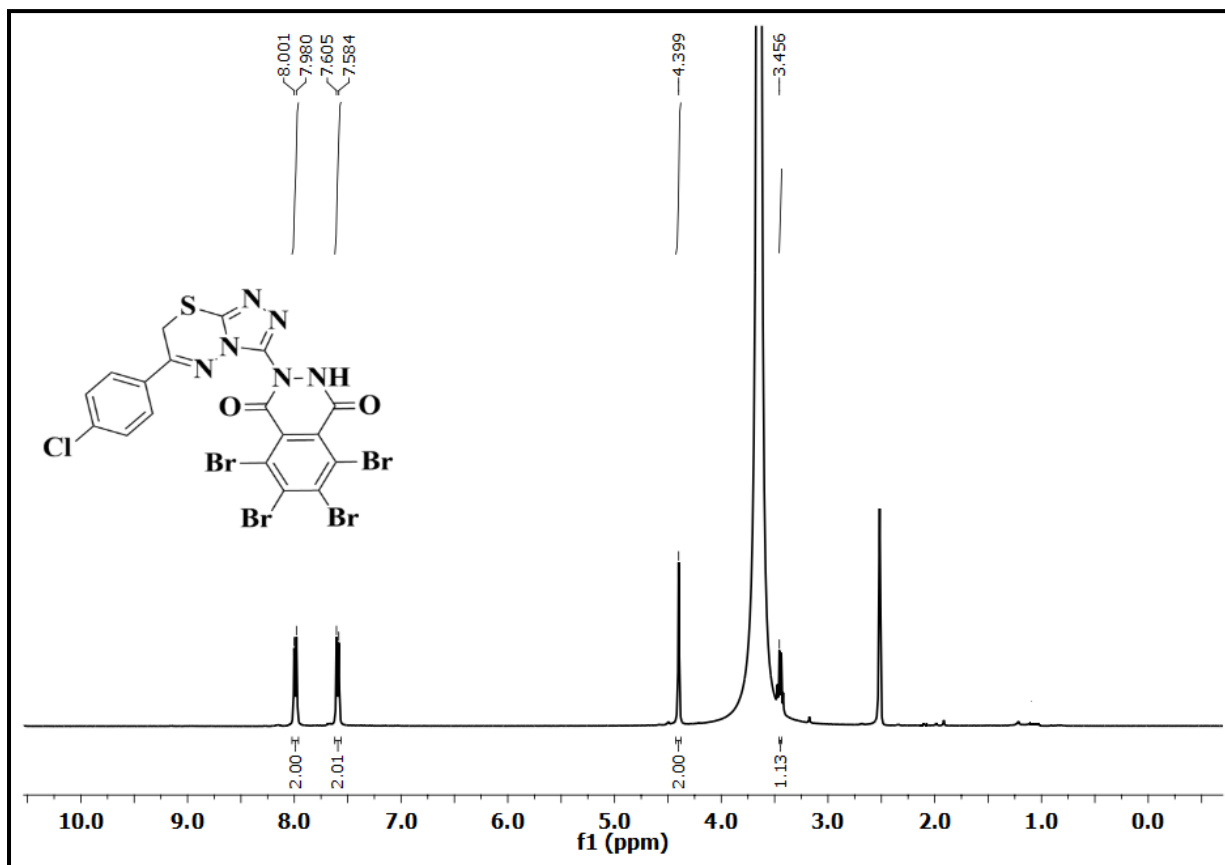


Fig: ¹H-NMR Spectrum of Compound 27m (400 MHz, DMSO-d₆)

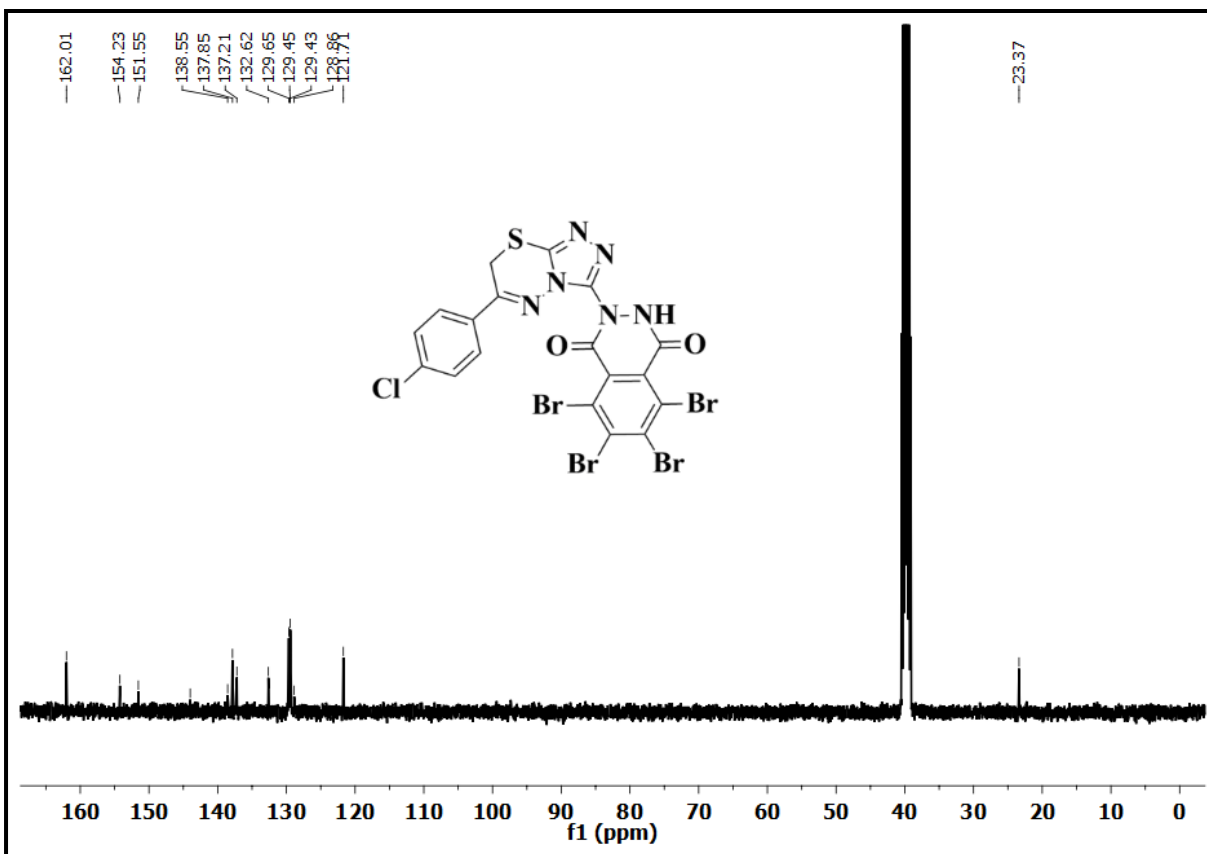


Fig: ¹³C-NMR Spectrum of Compound 27m (100 MHz, DMSO-*d*₆)

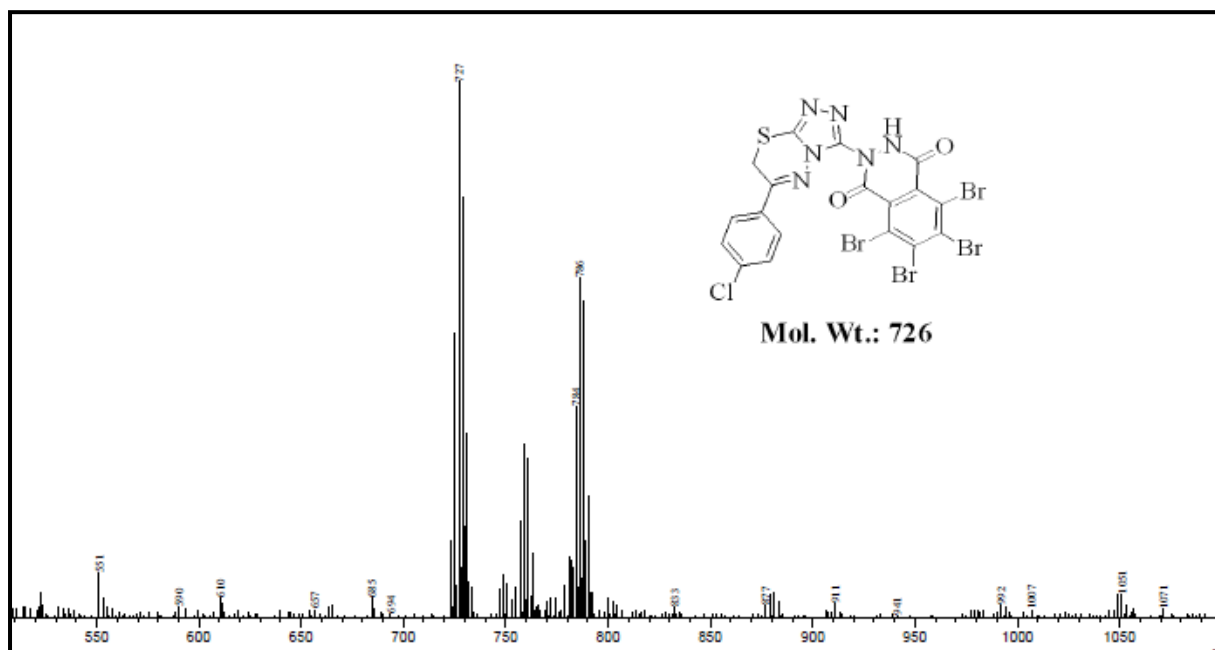


Fig: Mass Spectrum of Compound 27m

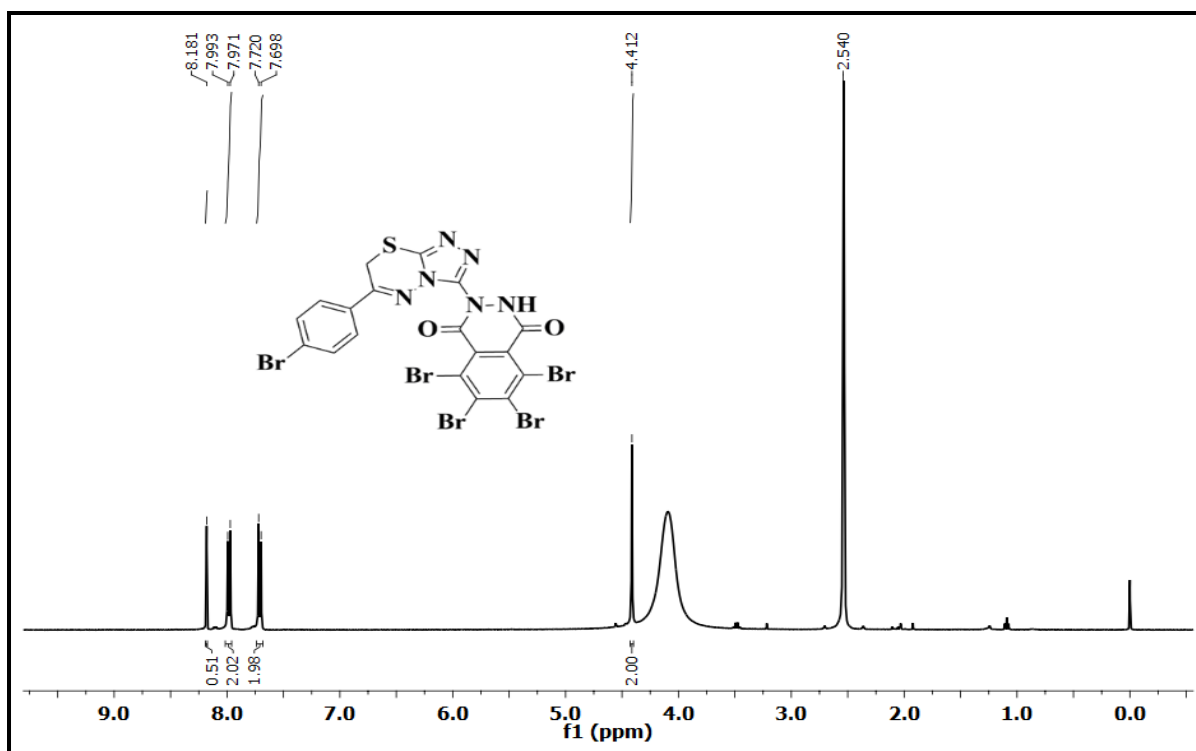


Fig: ¹H-NMR Spectrum of Compound 27n (400 MHz, DMSO-*d*₆)

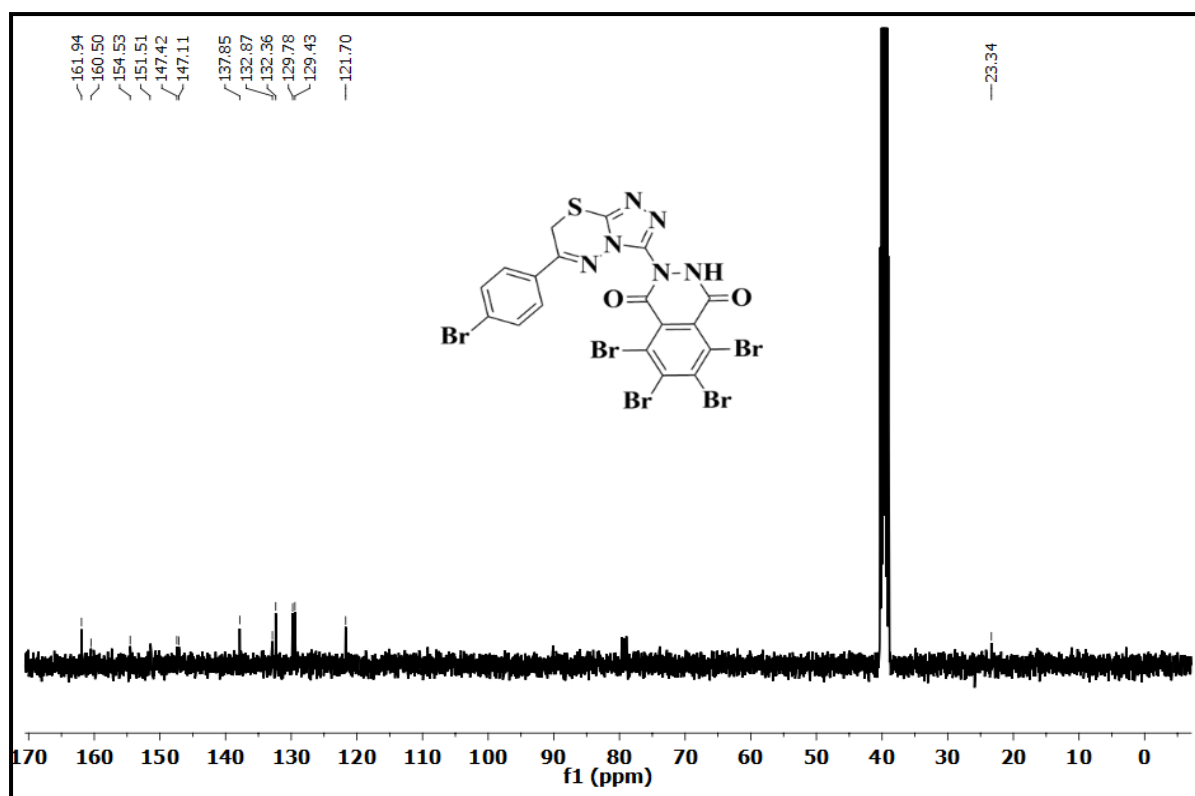


Fig: ¹³C-NMR Spectrum of Compound 27n (100 MHz, CDCl₃+DMSO-*d*₆)

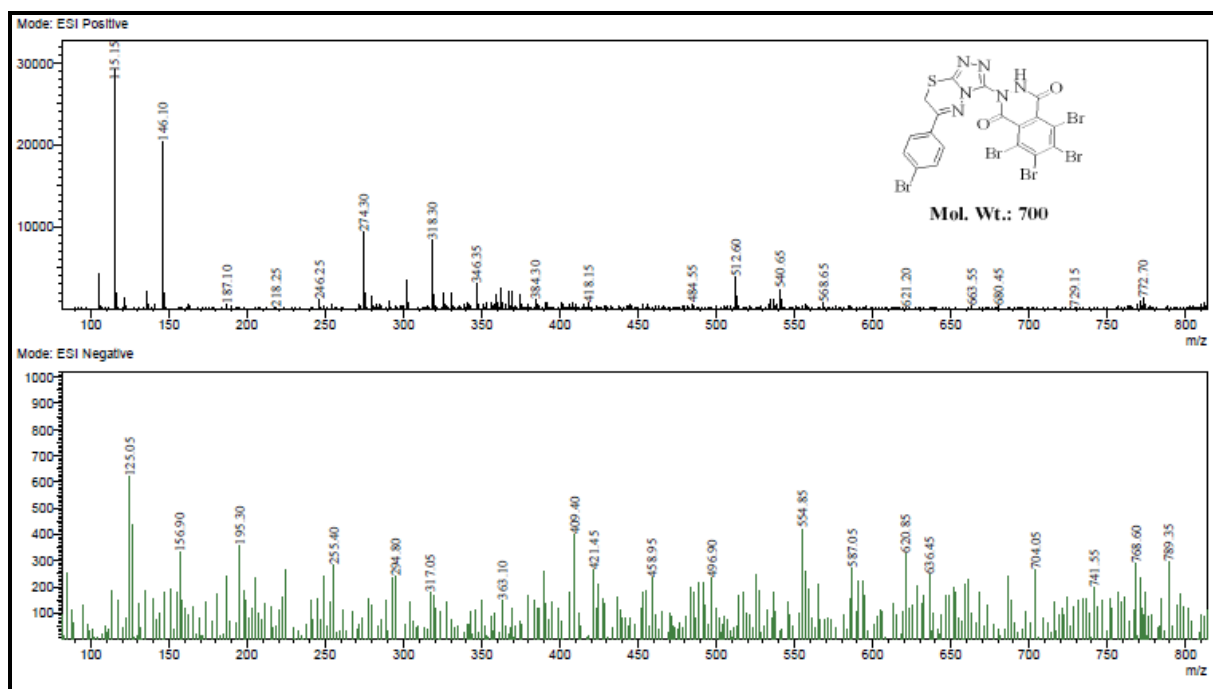


Fig: Mass Spectrum of Compound 27n

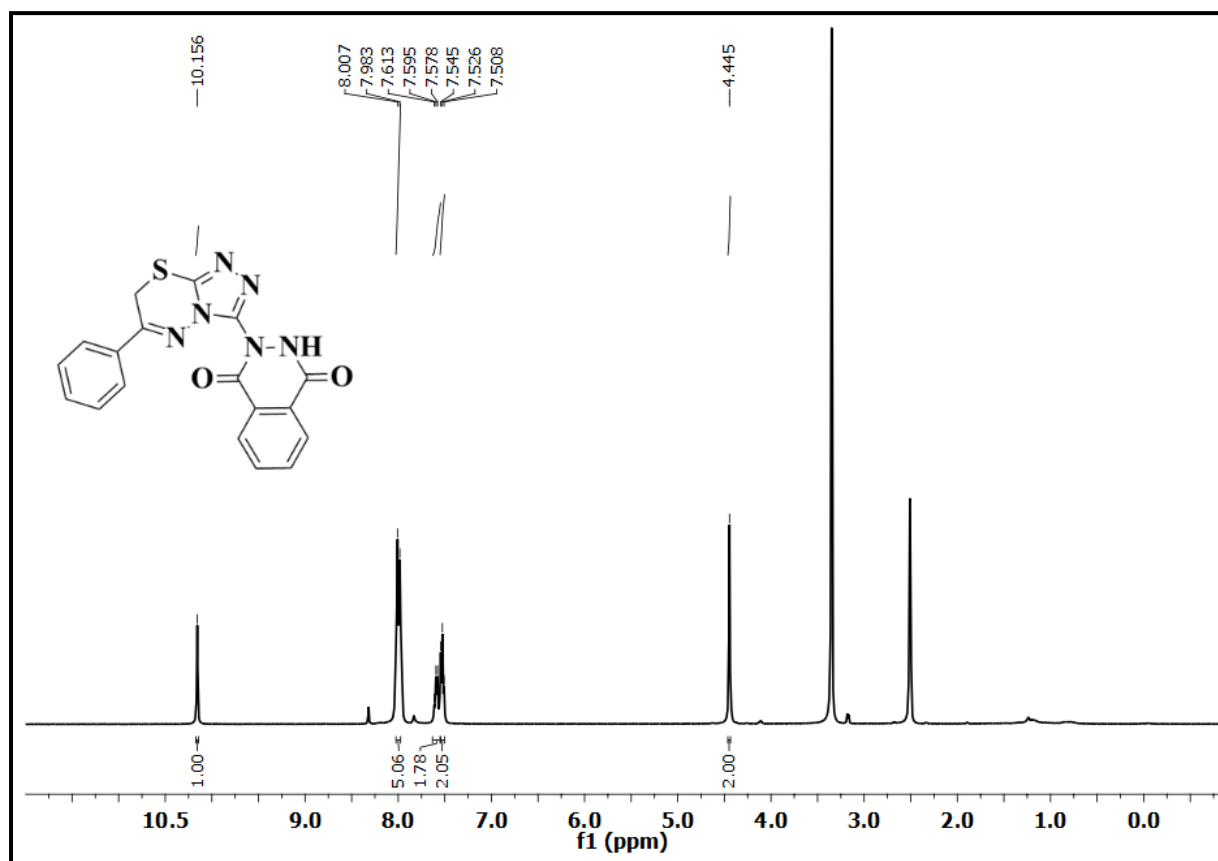


Fig: ¹H-NMR Spectrum of Compound 27o (400 MHz, DMSO-d₆)

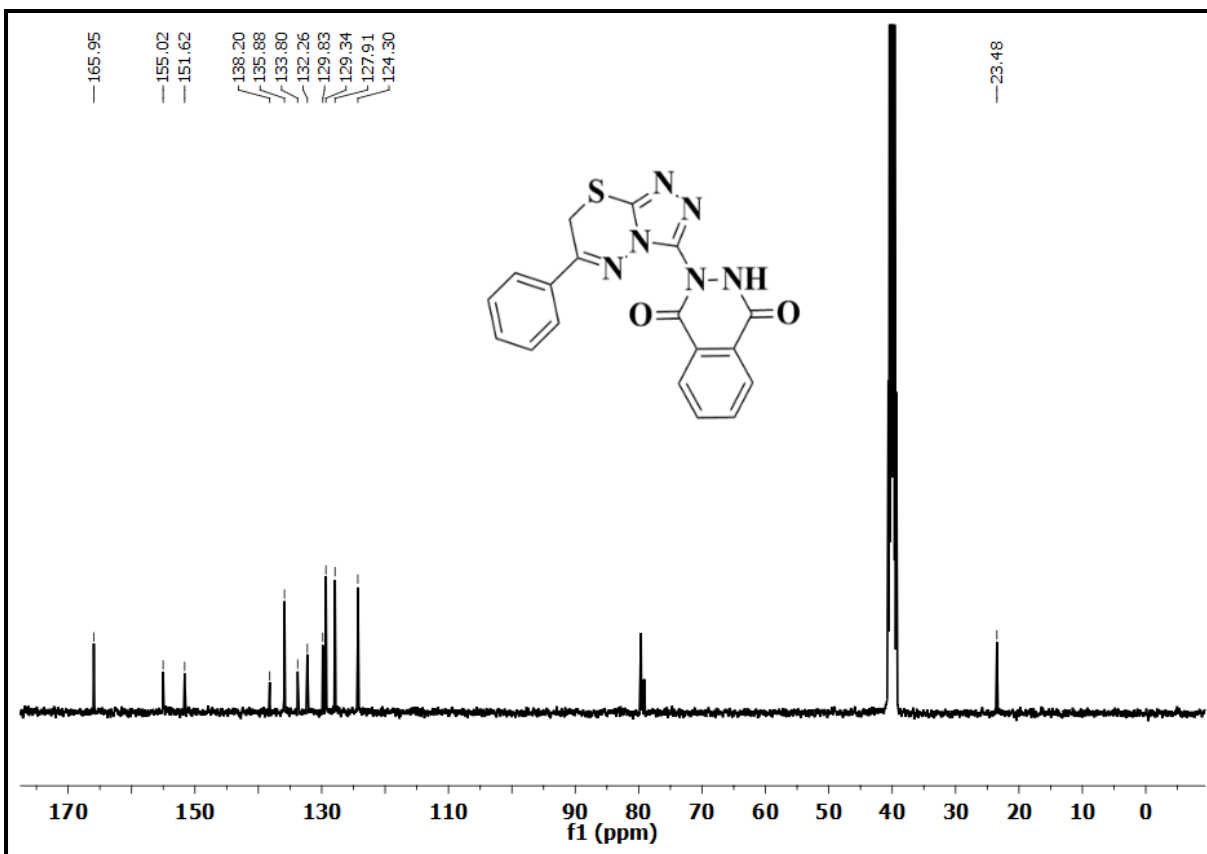


Fig: ¹³C-NMR Spectrum of Compound 27o (100 MHz, CDCl₃+DMSO-*d*₆)

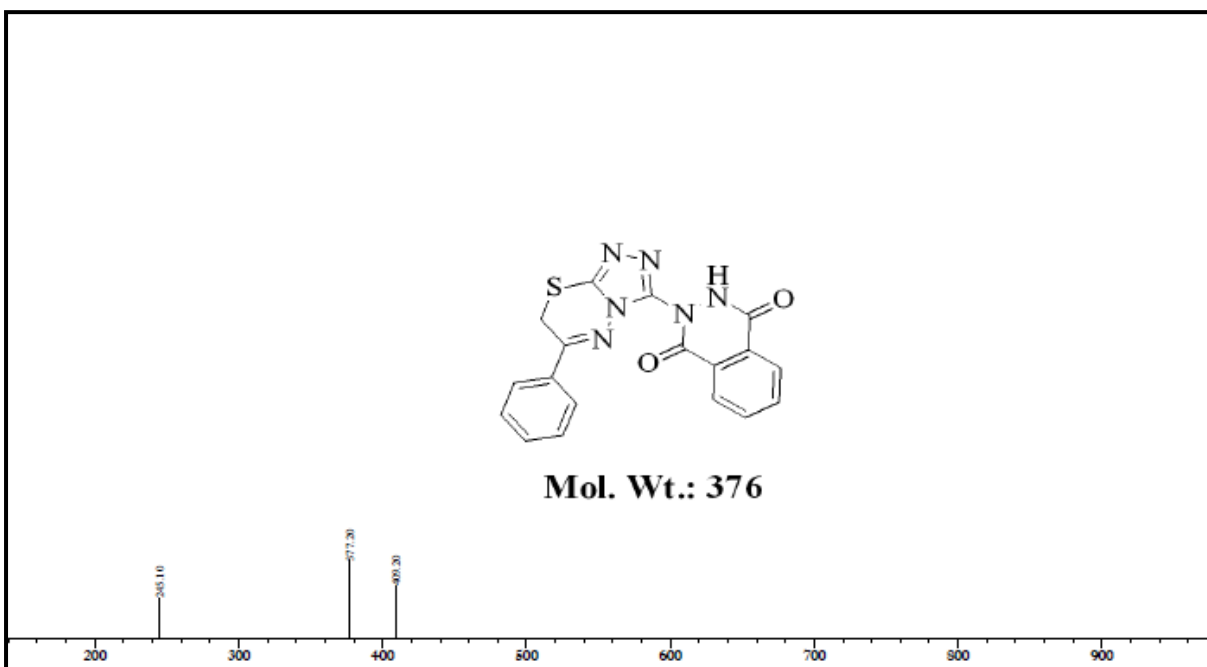


Fig: Mass Spectrum of Compound 27o

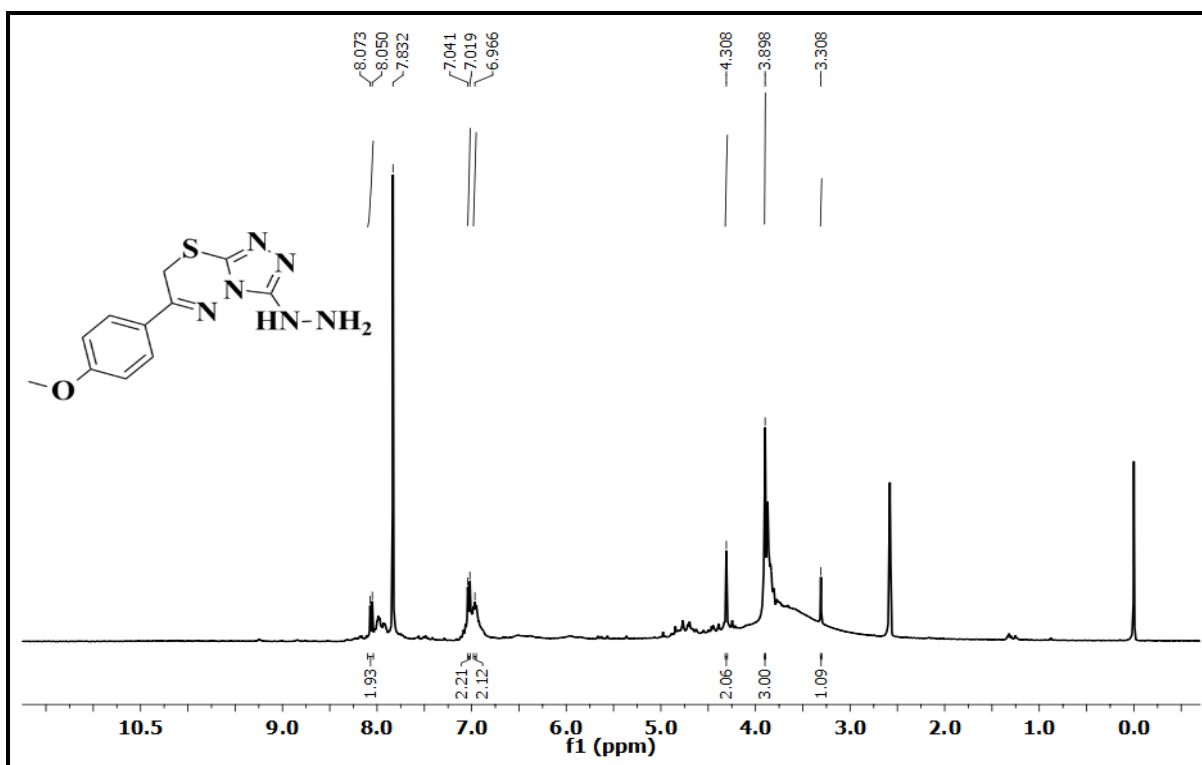


Fig: ¹H-NMR Spectrum of Compound **28** (400 MHz, CDCl₃+DMSO-*d*₆)

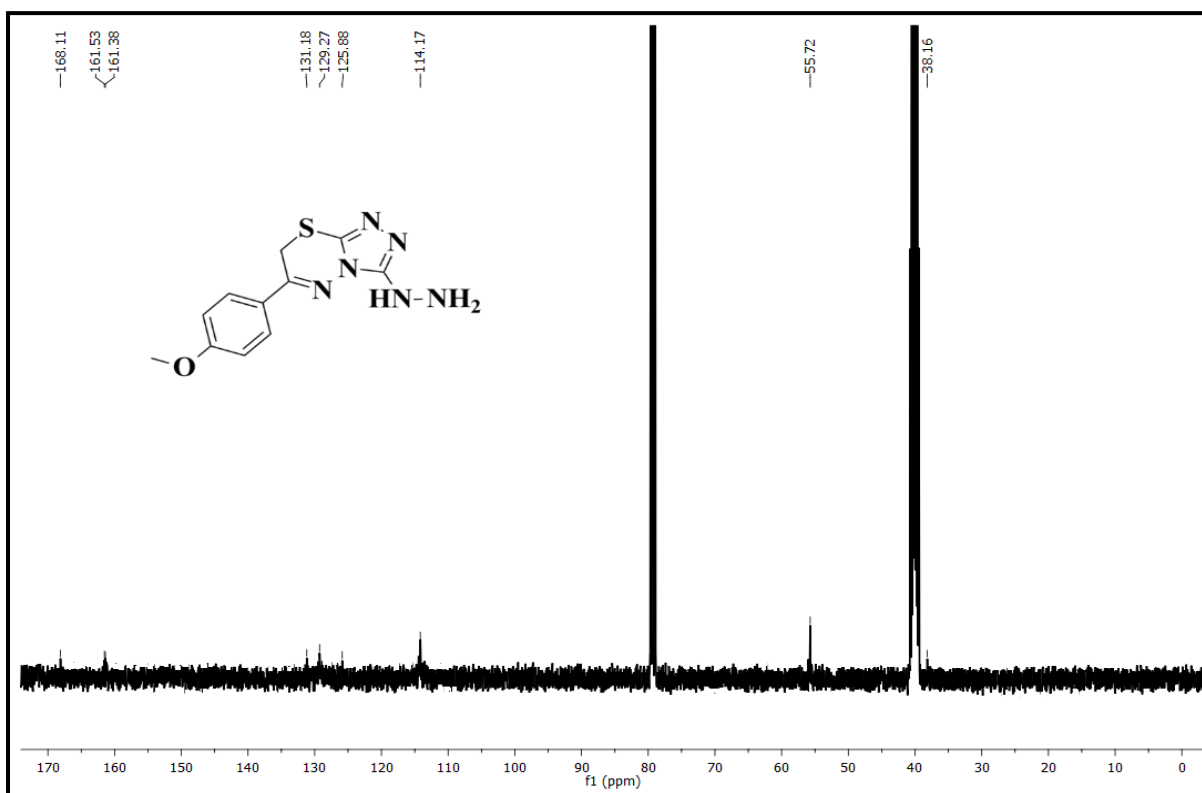


Fig: ¹³C-NMR Spectrum of Compound **28** (100 MHz, CDCl₃+DMSO-*d*₆)

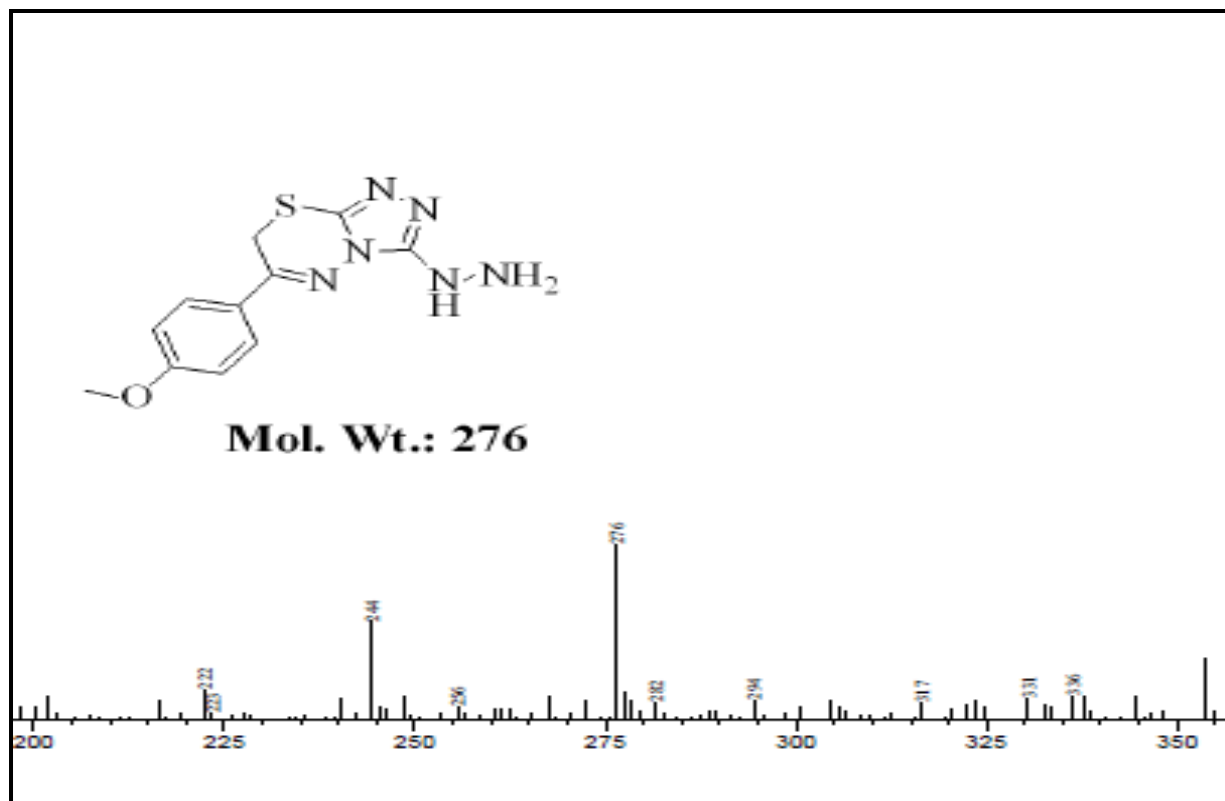


Fig: Mass Spectrum of Compound 28

References:

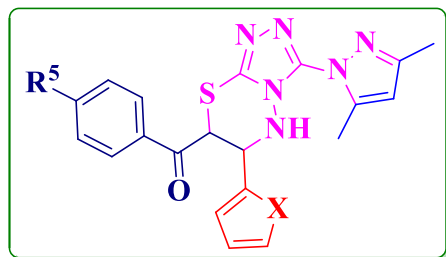
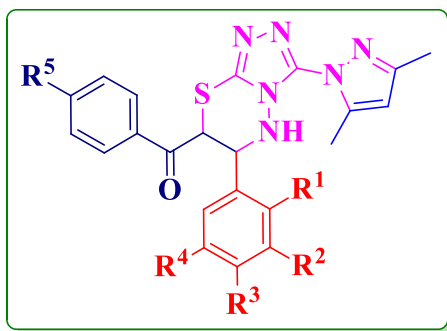
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CHAPTER-IV

DISCOVERY OF 3-(1*H*-PYRAZOL-1-YL)-6,7-DIHYDRO-5*H*- [1,2,4]TRIAZOLO[3,4-*b*][1,3,4]THIADIAZINE DERIVATIVES WITH PROMISING ANTICORONAVIRUS AND ANTITUMORAL ACTIVITY



INTRODUCTION:

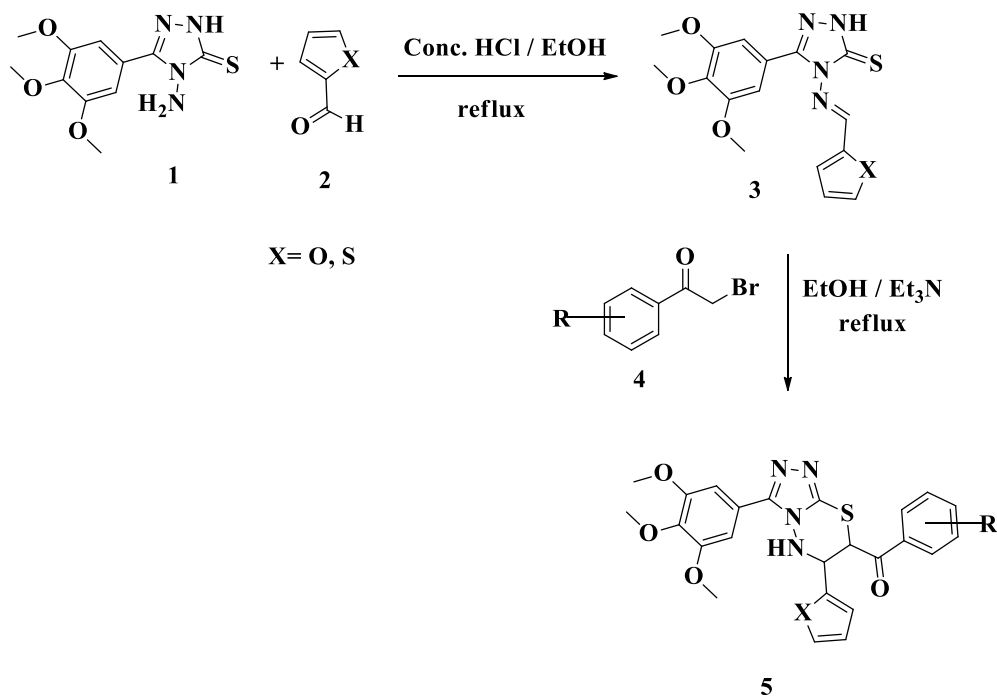
Sulfur-containing heterocyclic compounds exhibit extensive biological applications. 1,2,4-Triazoles and thiadiazines are themselves showing good biological activities such as anti-microbial, anti-bacterial, anti-viral, anti-inflammatory, anti-oxidant, anti-cancer, anti-analgesic, anti-tumor, anti-tubercular, anti-candidal, and cytotoxic activities¹⁻¹⁸. In the light of the above literature and abundance of bio-potentials of triazole and thiadiazines, we designed dihydro[1,2,4]triazole[3,4-*b*]thiadiazines hoping that these structural frameworks may be useful for the discovery of new molecules with good biological activity.



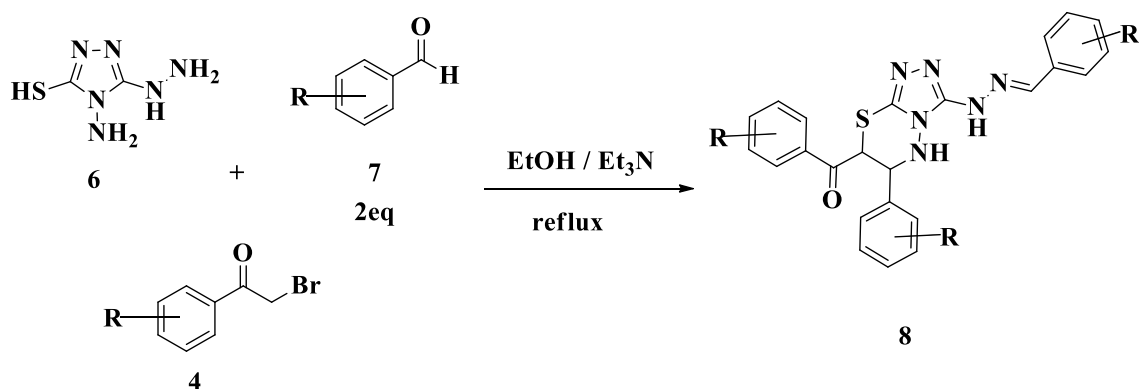
Fig. 1: General structures of thiadiazines.

Sporadic reports are available in the literature on the synthesis of dihydro[1,2,4]triazole[3,4-*b*]thiadiazines.

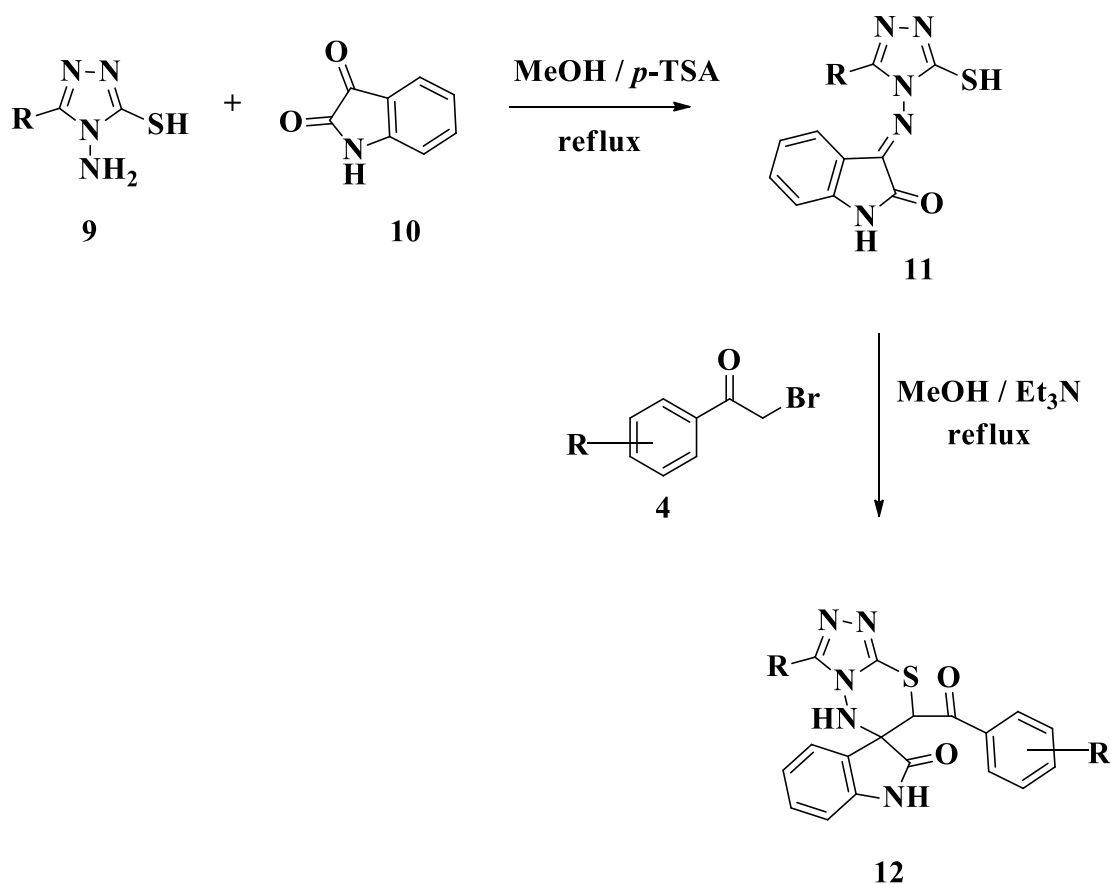
Zhang¹⁹ et al. described the novel synthesis of different dihydro[1,2,4]triazole[3,4-*b*]thiadiazines (**5**) containing furan and thiophene nucleus through a stepwise synthetic route. Initially, 4-amino-3-(3,4,5-trimethoxyphenyl)-1*H*-1,2,4-triazole-5(4*H*)-thione (**1**) was condensed with furan or thiophene (**2**) in the ethanol containing a catalytic amount of conc. HCl to obtain the triazole Schiff bases (**3**) bearing furan or thiophene ring. In the next step, these compounds **3** were treated with substituted phenacyl bromides (**4**) in the presence of ethanol having triethylamine under reflux condition to generate the target dihydro[1,2,4]triazole[3,4-*b*]thiadiazines (**5**).



Sujatha and Vedula²⁰ reported the one-pot multi-component synthesis of substituted dihydro[1,2,4]triazolo[3,4-*b*]thiadiazines containing Schiff bases (**8**) from the reaction of 4-amino-5-hydrazinyl-4H-1,2,4-triazole-3-thiol (**6**), substituted aromatic aldehydes (**7**) and different phenacyl bromides (**4**) in absolute ethanol having the catalytic amount of triethylamine at reflux temperature.



Liangun²¹ et al. synthesized the substituted dihydrospiro[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine indolinones (**12**) from two steps synthesis. In the first step different 4-amino-4*H*-1,2,4-triazole-3-thiols (**9**) were treated with indoline-2,3-dione (**10**) in the presence of methanol having *p*-TSA as the catalyst to produce the compounds (**11**) at reflux temperature. Then substituted phenacyl bromides (**4**) were reacted with compounds (**11**) in methanol containing a catalytic amount of triethylamine (TEA) under reflux condition to generate the target derivatives (**12**) in the second step. The final molecules were subjected to their anti-cancer activity with good results.



Pyrazole is one of the versatile heterocyclic compounds exhibiting a wide range of applications in the field of pharmaceutical, agrochemical, and therapeutic medicines. Pyrazole is a five-member heterocyclic molecule with two nitrogen atoms positioned at 1,2 or adjacent. Some of the living organisms are found with pyrazole-based natural products^{22,23}. Most of the pyrazole derivatives play a significant role in biological applications such as anti-HIV, anti-malarial, antioxidant, anti-inflammatory, anti-microbial, anti-tumor, anti-pyretic, anti-analgesic, ant-tubercular, anti-leishmanial, and anti-cancer activities²⁴⁻³⁸ (**Fig.2**).

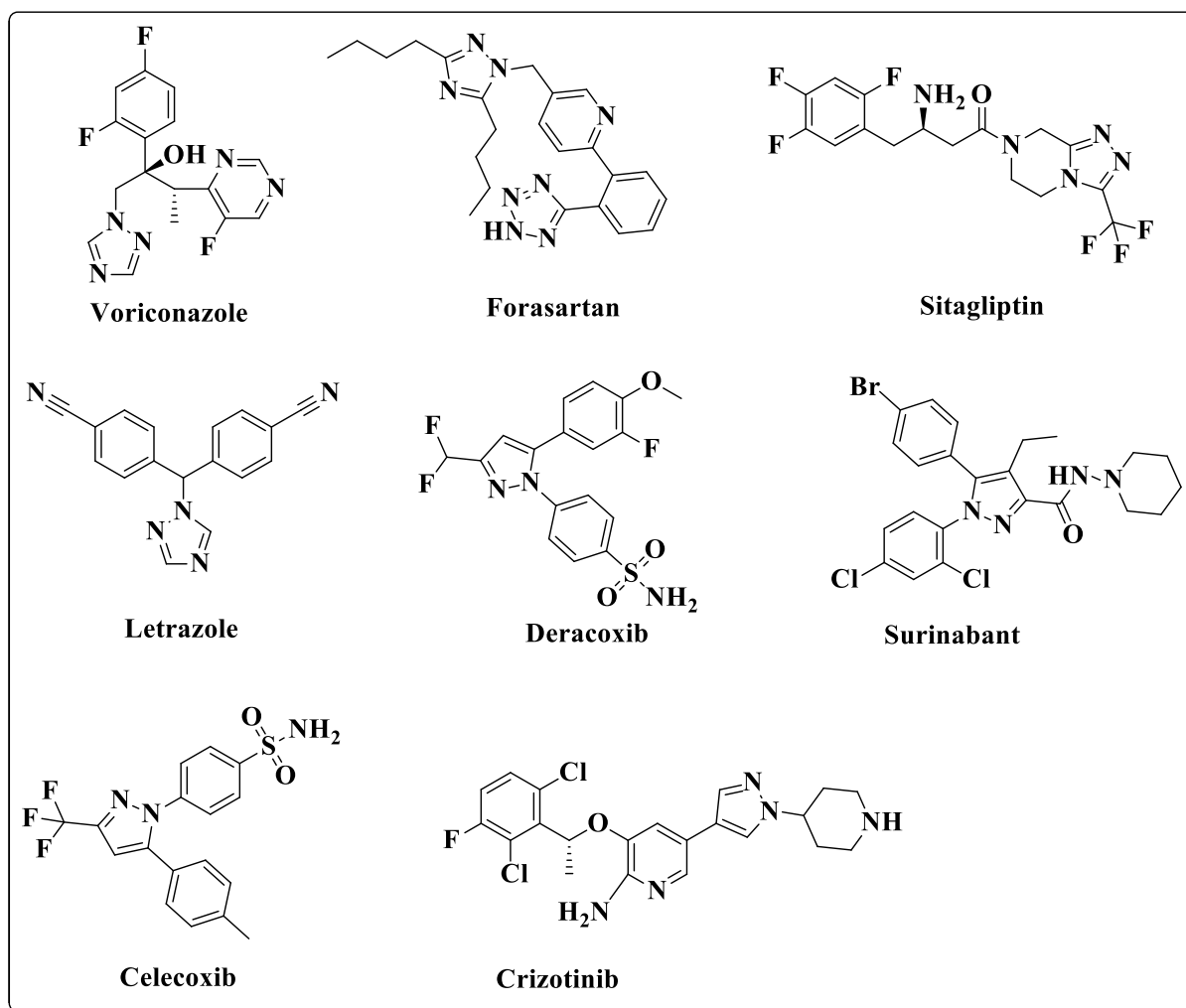
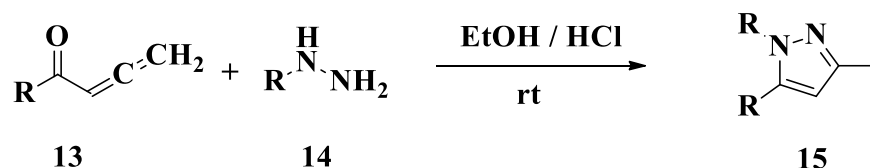


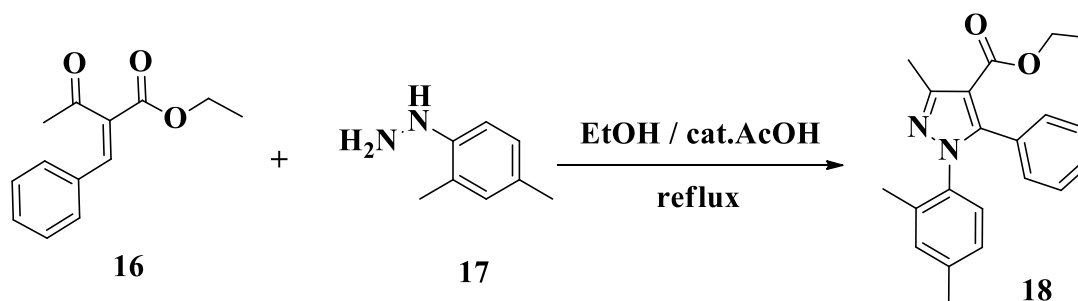
Fig. 2: Marketed drugs based on 1,2,4-triazole and pyrazole scaffold.

There are many reported methods available for the synthesis of pyrazole derivatives. Some of the literature reports are presented as below.

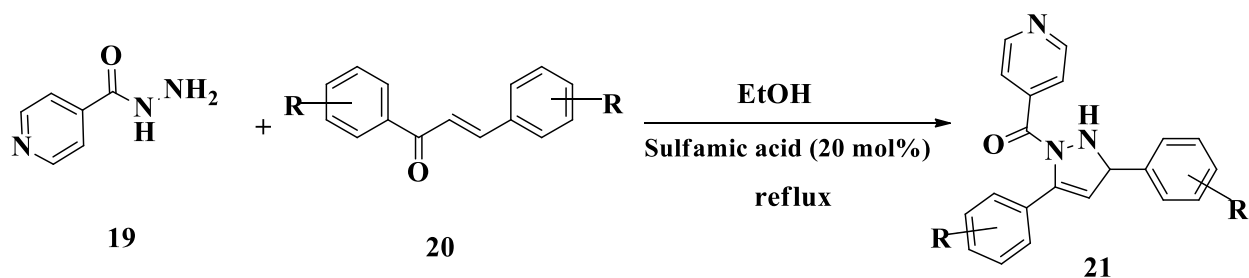
Sarkara and Sahoo³⁹ described the synthesis of pyrazole derivatives (**15**) from the reaction of different dienones (**13**) with various hydrazines (**14**) in the acid-catalyzed ethanolic solvent at room temperature.



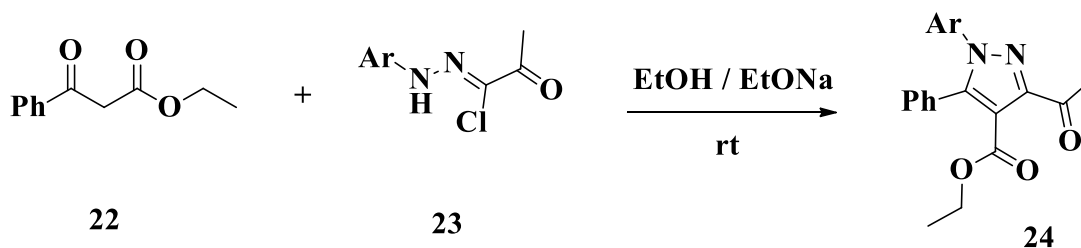
Naveen⁴⁰ *et al.* reported recently the synthesis, anti-oxidant activity, and crystal structure of ethyl 1-(2,4-dimethylphenyl)-3-methyl-5-phenyl-1*H*-pyrazole-4-carboxylate (**18**) by refluxing (*E*)-ethyl 2-benzylidene-3-oxobutanoate (**16**) with (2,4-dimethylphenyl)hydrazine (**17**) in the presence of ethanol bearing acetic acid as catalyst.



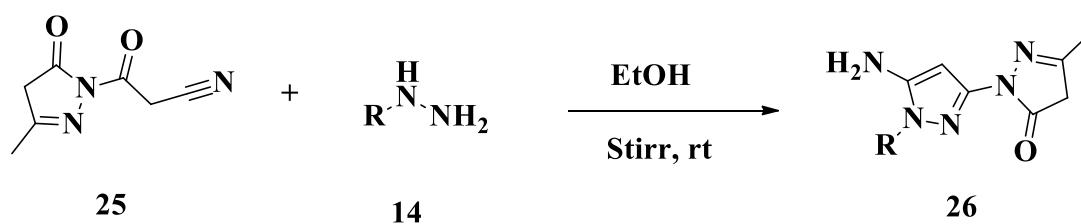
Bhirud⁴¹ *et al.* conducted the reaction between the isonicotinohydrazide (**19**) and various chalcones (**20**) using sulfamic acid as catalyst in the absolute ethanol at reflux condition to give substituted (3,5-diphenyl-2,3-dihydro-1*H*-pyrazol-1-yl)(pyridin-4-yl)methanones (**21**). These compounds were evaluated for their pharmacological activities.



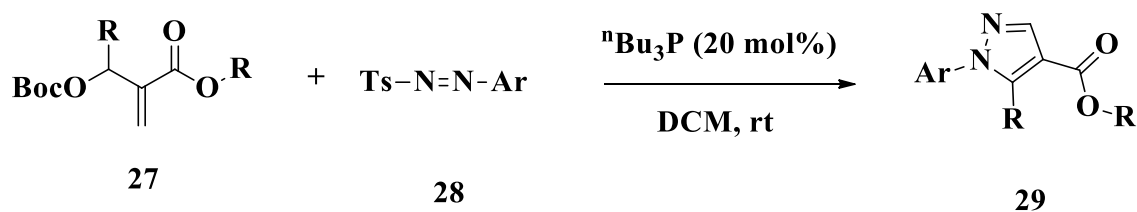
Abdelhamida⁴² and co-worker synthesized the different pyrazole derivatives (**24**) by the reaction of various β -ketoesters (**22**) with oxopropanehydrazonoyl chlorides (**23**) at room temperature in EtOH having sodium acetate.



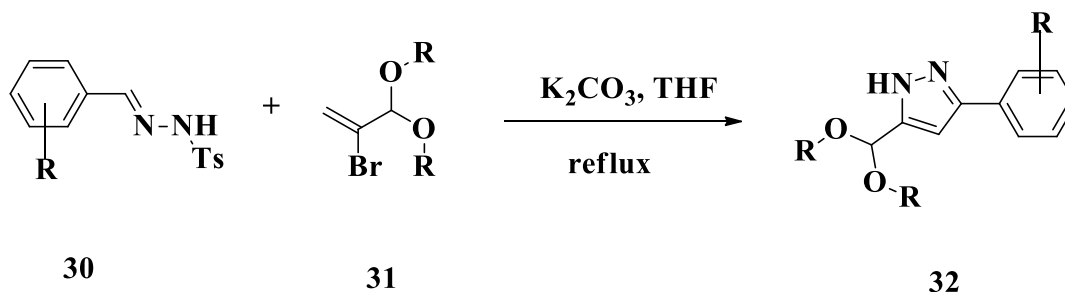
Toche⁴³ et al. described the green synthesis of pyrazole (**26**) derivatives from the reaction of 3-(3-methyl-5-oxo-4,5-dihydro-1H-pyrazol-1-yl)-3-oxopropanenitrile (**25**) with hydrazines (**14**) in ethanol solvent at room temperature in good yields.



Zhang⁴⁴ et al. developed the cyclocondensation reaction between allylic carbonates (**27**) and arylazosulfones (**28**) using $^n\text{Bu}_3\text{P}$ catalyst in the presence of dichloromethane at room temperature to yield the pyrazoles (**29**).



Westermeyer⁴⁵ *et al.* reported the regioselective synthesis of different pyrazole derivatives (32) from the reaction tosylhydrazones (30) with various bromovinyl acetals in tetrahydrofuran (THF) containing potassium carbonate at reflux condition.

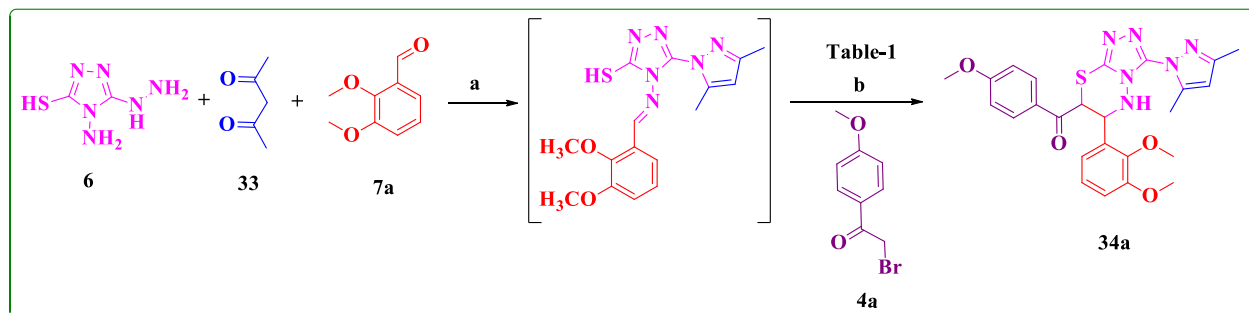


PRESENT WORK:

Novel hybrid molecules containing three biological active scaffolds were expected to possess good anti-viral and anti-cancer activities. Based on the literature survey, we designed a new hybrid template having triazole, thiadiazine, and pyrazole in search of promising anti-viral and anti-cancer activities. In the present chapter, we have developed pyrazolyl-dihydro[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine core heterocyclic compounds *via* a one-pot, multi-component reaction.

The synthesis of 3-(1*H*-pyrazol-1-yl)-6,7-dihydro-5*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine derivatives was carried out by a one-pot four-component reaction. In order to optimize the chemistry, a model reaction using 2-amino-5-hydrazinyl-4*H*-1,2,4-triazole-3-thiol (6), acetylacetone (33), 2,3-dimethoxybenzaldehyde (7a) and 4-methoxyphenacyl bromide (4a) as starting materials (**Scheme-1**). The first step of the reaction was carried out in ethanol as solvent at reflux temperature, in the presence of a catalytic amount of HCl yielding the intermediate 5-(3,5-dimethyl-1*H*-pyrazol-1-yl)-4-((4-methoxybenzylidene)amino)-4*H*-1,2,4-triazole-3-thiol⁴⁶. The intermediate was not isolated instead of it 4-methoxyphenacyl bromide (4a) was added to this reaction mixture. In order to drive the ring closure and to form the thiadiazine moiety, various reaction conditions were explored (**Table-1**). On running this reaction, either at room temperature or reflux temperature failed to yield the desired product. Upon the addition of an organic base (such as pyridine, piperidine, or triethylamine), the desired

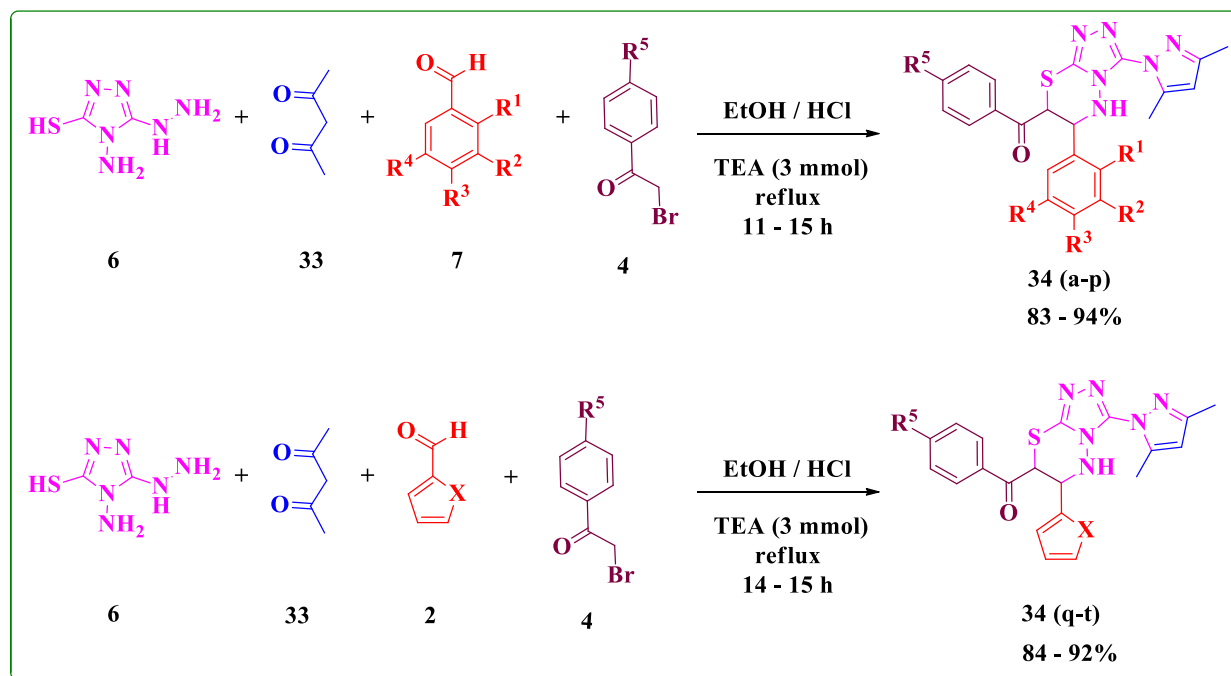
product was formed. Using triethylamine and carrying the reaction at reflux temperature (entry 4) resulted in the formation of **34a** in excellent yield.



Scheme-1: Model reaction. **Reaction conditions:** a) **6** (1 mmol), **33** (1 mmol), **7a** (1 mmol), EtOH, HCl; b) **4a** (1 mmol), TEA (3 mmol), EtOH, reflux.

Table-1: Screening of the base catalyst.

Entry	Solvent	Base	Yield (%) of 34a
1	EtOH	---	0
2	EtOH	Pyridine	55
3	EtOH	Piperidine	48
4	EtOH	Triethylamine	92



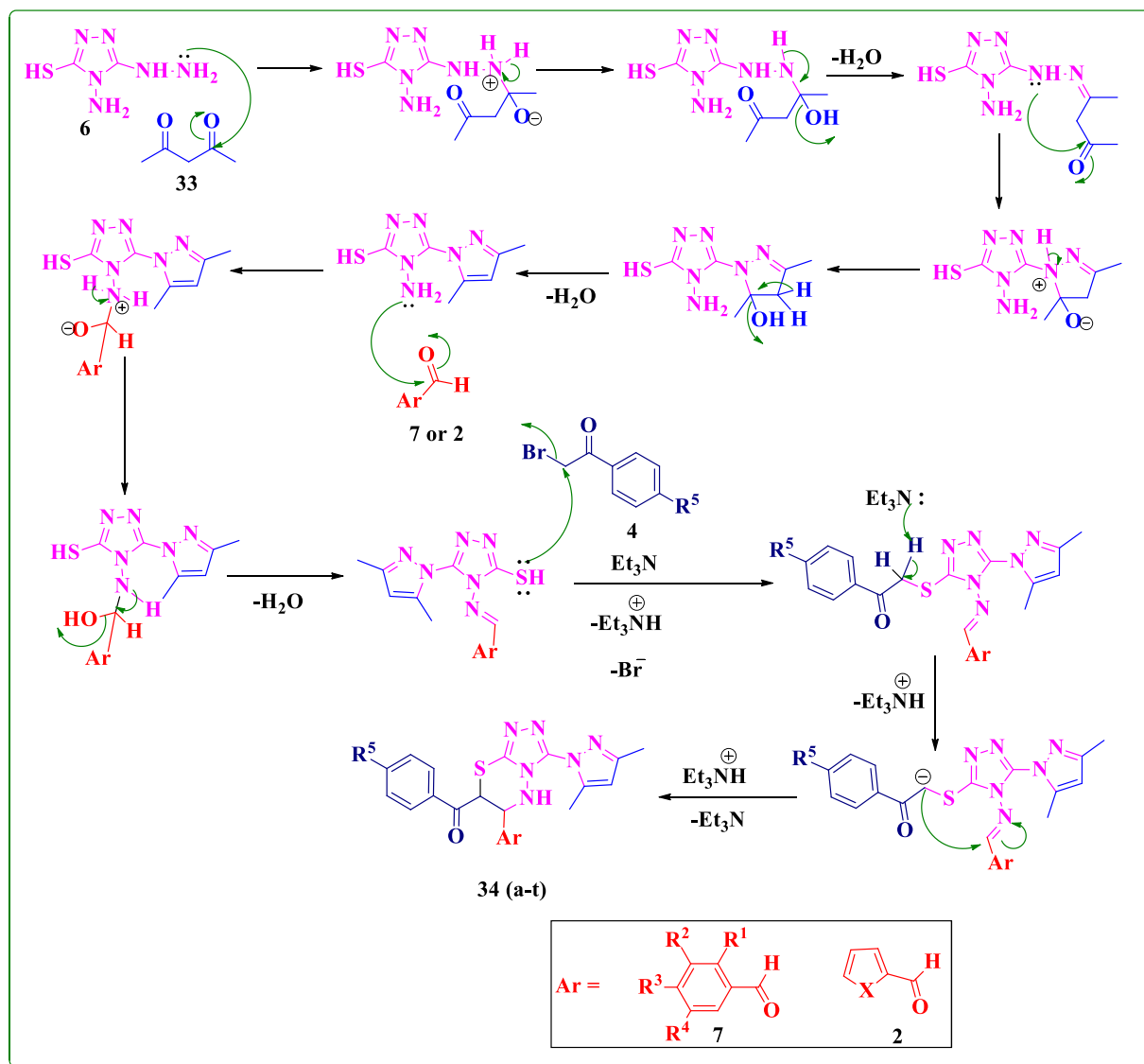
Scheme-2: One-pot, four-component synthesis of pyrazolyl-dihydro[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine derivatives **34a-t**.

Using this methodology, a series of compounds were prepared using various substituted benzaldehydes/heteryl aldehydes and phenacyl bromides (**Scheme-2**). This approach is simple and affords the desired products in 83 to 94% yields (**Table-2**).

In the present investigation, pyrazole and dihydrothiadiazine skeletons were developed using a one-pot, four-component reaction. Initially, the hydrazino functional group of compound **6** underwent cyclo condensation with acetylacetone **33** to form a pyrazole ring. Then an appropriate amount of different aldehydes and substituted phenacyl bromides **4** were reacted with amine ($-\text{NH}_2$) and thiol ($-\text{SH}$) groups of compound **6** respectively by using triethylamine to establish the dihydrothiadiazines (**Scheme-3**).

Table-2: 3-(1*H*-pyrazol-1-yl)-6,7-dihydro-5*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine derivatives 34a-t.

Product	R ¹	R ²	R ³	R ⁴	R ⁵	X	Time (h)	Yield (%)
34a	OCH ₃	OCH ₃	H	H	OCH ₃	—	11.30	92
34b	H	H	CH ₃	H	CH ₃	—	11.00	91
34c	H	H	NO ₂	H	NO ₂	—	14.30	83
34d	H	OCH ₃	H	H	Cl	—	12.00	86
34e	Br	H	H	H	F	—	12.30	90
34f	Br	H	H	H	CH ₃	—	11.50	92
34g	H	H	Cl	H	Br	—	13.00	90
34h	H	H	Cl	H	CH ₃	—	12.00	93
34i	H	OCH ₃	OH	OCH ₃	H	—	11.40	89
34j	H	F	F	F	CH ₃	—	14.00	92
34k	H	F	F	F	H	—	13.30	90
34l	H	OCH ₃	OCH ₃	OCH ₃	F	—	13.15	88
34m	H	OCH ₃	OCH ₃	OCH ₃	H	—	11.40	94
34n	Cl	H	H	H	OCH ₃	—	14.15	86
34o	OCH ₃	H	OCH ₃	H	OCH ₃	—	12.00	94
34p	OCH ₃	H	OCH ₃	H	NO ₂	—	14.00	92
34q	—	—	—	—	OCH ₃	O	14.30	87
34r	—	—	—	—	CH ₃	O	14.15	85
34s	—	—	—	—	NO ₂	S	15.00	89
34t	—	—	—	—	NO ₂	O	14.50	92



Scheme-3: Plausible reaction mechanism for the synthesis of compounds **34a-t**.

The structures of all the final products were confirmed by their spectral data. The FT-IR spectrum of product **34b** showed a characteristic stretching band at 1681 cm^{-1} corresponding to the C=O functional group, whereas the NH group appeared at 3135 cm^{-1} . The $^1\text{H-NMR}$ spectrum of compound **34b** showed characteristic peaks, such as two singlets at 2.21 and 2.95 ppm, arising from the two methyl groups on the pyrazole ring. Another two singlets appeared at 2.37 and 2.43 ppm that were assigned to the methyl groups on both phenyl moieties. The two CH protons of the dihydrothiadiazine skeleton were visible as two doublets at 5.05 and 5.25 ($J=5.2\text{ Hz}$) ppm, respectively. The proton of the pyrazole ring showed a singlet at 6.00 ppm,

whereas the–NH–proton appeared at 7.42 ppm. The remaining aromatic protons appeared in the region of 7.11–7.80 ppm. The ^{13}C -NMR spectrum of compound **34b** showed peaks at 11.9 and 13.6 ppm for the two methyl groups on the pyrazole ring and 21.1 and 21.8 ppm for the two methyl groups on the phenyl moiety. The characteristic carbons of the dihydrothiadiazine skeleton appeared at 44.2 and 59.3 ppm, respectively. The pyrazole carbon displayed a peak at 107.8 ppm, whereas the carbonyl peak appeared as the most downfield signal at 193.7 ppm. The remaining aromatic carbons appeared in the range of 127.3 to 151.8 ppm. Mass spectral analysis of compound **5b** showed a molecular mass ion m/z at 445.

X-ray Crystallography:

To confirm the structure, the crystalline material of compound **34h** was isolated, and single-crystal X-ray diffraction data were obtained. The compound crystallizes in a monoclinic $P2_1/n$ space group. The molecular structure of **34h** in ORTEP representation was shown in **Fig. 3**.

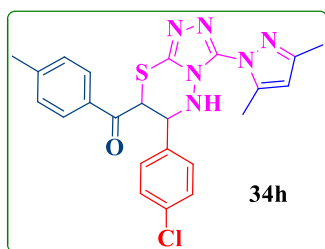
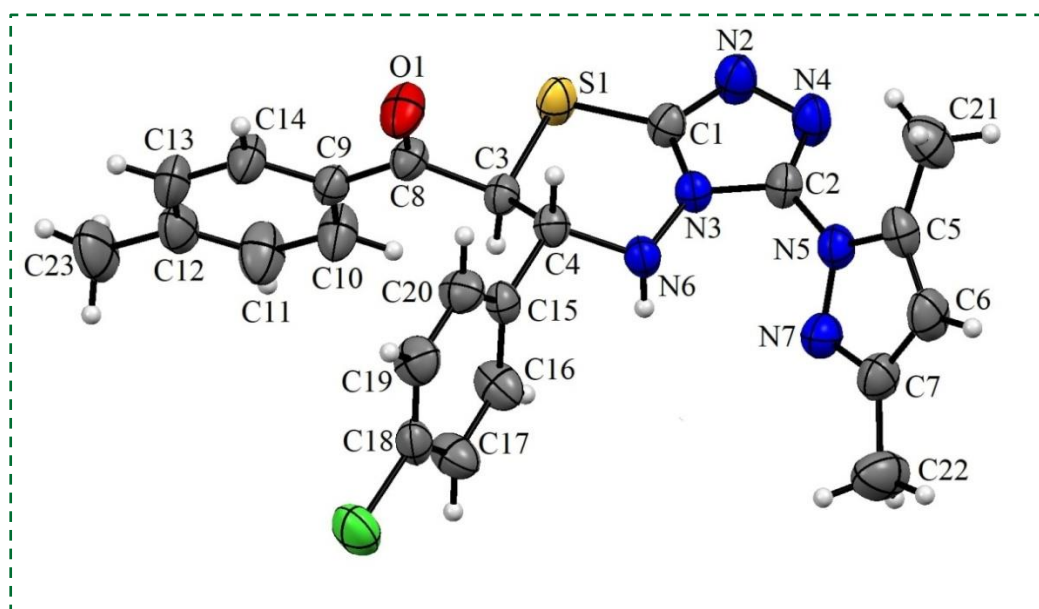


Fig. 3: ORTEP representation of compound **34h**.

Compound **34h** has a 4-methylbenzoyl group and 4-chlorophenyl group on two adjacent chiral centers of six-membered dihydrothiadiazine ring. The dihydrothiadiazine moiety is fused with a triazole ring, which is further connected to a pyrazole ring through a C-N single bond. The phenyl rings of 4-methylbenzoyl and 4-chlorophenyl groups are almost perpendicular (79.92° and 82.28° respectively) to the mean plane of the fused six- and five-membered rings. The pyrazole ring attached to triazole is making an angle of 55.59° with the mean plane of the fused six- and five-membered rings. The bond distances and angles are consistent with the structure derived from NMR data. The centrosymmetric space group ($P2_1/n$) indicates that the material is a racemic mixture (**Table-4**). The unit cell contains two pairs of enantiomers and is connected through non-covalent interactions.

Non-covalent intermolecular interactions, such as hydrogen bonding, play an essential role in the binding of drugs to their targets, such as DNA or proteins. In this context, the possibility of the presence of non-covalent interactions in the solid-state structure of compound **34h** was explored. As a result, we were able to identify one N–H ... N hydrogen bonding, one C–H ... O interaction, and one C–H ... N interaction (**Fig. 4**). The interactions and corresponding symmetry transformations are listed in **Table-3**.

Table-3: Hydrogen bonding interactions

S. No	D-H...A	H...A (Å)	D...A (Å)	D-H...A (°)
1	N(6)-H(6) ... N(2) ⁱ	2.50	3.1298	130
2	C(4)-H(4) ... N(4) ⁱ	2.38	3.2637	150
3	C(19)-H(19) ... O(1) ⁱⁱ	2.46	3.3727	160

Symmetry transformations used: (i) $\frac{1}{2}-x, \frac{1}{2}+y, \frac{1}{2}+z$; (ii) $-\frac{1}{2}-x, \frac{1}{2}+y, \frac{1}{2}-z$;

Table-4: Important crystallographic data for compound **34h**.

Compound	34h
Chemical formula	C ₂₃ H ₂₁ Cl N ₆ O S
Formula weight	464.97
Crystal system	Monoclinic
Space group	<i>P</i> 2 ₁ /n
<i>a</i> (Å)	14.2063(18)
<i>b</i> (Å)	8.4877(11)
<i>c</i> (Å)	20.022(3)
α (°)	90
β (°)	107.576(5)
γ (°)	90
<i>V</i> (Å ³)	2301.6(5)
<i>Z</i>	4
ρ (g cm ⁻³)	1.342
μ (mm ⁻¹)	0.285
Reflections collected	34696
Reflections unique	4077
Reflections [<i>I</i> ≥ 2σ(<i>I</i>)]	4077
Parameters	289
<i>R</i> 1, <i>wR</i> 2 [<i>I</i> ≥ 2σ(<i>I</i>)]	0.0471, 0.1330
<i>R</i> 1, <i>wR</i> 2 [all data]	0.0537, 0.1387
GOF on <i>F</i> ²	1.136
Max./Min. Δρ (e Å ⁻³)	0.323 / -0.710

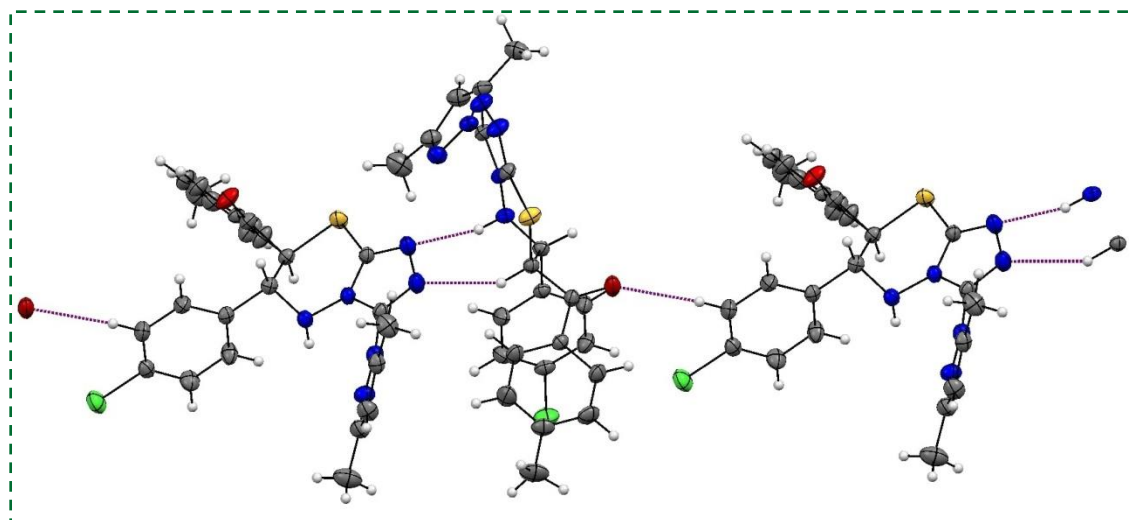


Fig. 4: Intermolecular H-bonding interactions of compound **34h** in the crystal lattice.

Antiviral screening

The synthesized compounds (**34a-t**) were subjected to a broad antiviral screening. At a concentration of 100 μM , no selective antiviral activity was observed for the following viruses: influenza A (H1N1 and H3N2) and influenza B virus (in MDCK cells), respiratory syncytial virus (in hep2 cells), yellow fever virus (in Huh7 cells), herpes simplex virus type 1 and 2 (in HEL cells). However, several derivatives do show antiviral activity against the human coronavirus 229E (hCoV229E) in HEL cells (**Table-5**). Especially compounds **34b** and **34f** display promising activity with EC_{50} values of 4.7 and 3.2 μM , respectively. Besides, both derivatives lack cytotoxicity for the HEL cells giving rise to favorable selectivity indexes.

Table-5: Antiviral evaluation of compounds **34a-t** against hCoV-229E. CC_{50} : 50% cytotoxic concentration, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay. EC_{50} : 50% effective concentration or concentration producing 50% inhibition of virus-induced cytopathic effect, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay. UDA: plant lectin Urticadioica agglutinin.

Compound	Conc. unit	hCoV229E (HEL cells)	
		CC_{50}	EC_{50}
34b	μM	81.5	4.7 $\pm$ 0.5 (*)

34c	μM	>100	24.4
34e	μM	>100	>100
34f	μM	>100	3.2 $\pm$ 1.8 (*)
34g	μM	>100	>100
34h	μM	>100	38.0
345j	μM	23.8	>100
34k	μM	>100	>100
34m	μM	>100	95.7
34n	μM	>100	>100
34p	μM	>100	>100
34q	μM	<0.8	>100
34r	μM	>100	>100
34s	μM	>100	>100
34t	μM	>100	>100
UDA	$\mu\text{g/ml}$	>100	2.1

(*) Mean value of three independent experiments $\pm$ SEM.

Antitumoral screening

To investigate their anticancer potential, compounds **34a-t** were tested *in vitro* for their antiproliferative properties, using a real-time IncuCyte proliferation assay against an array of solid and hematological cancers including LN-229 (glioblastoma), Capan-1 (pancreatic adenocarcinoma), HCT-116 (colorectal carcinoma), NCI-H460 (lung carcinoma), DND-41 (acute lymphoblastic leukemia), HL-60 (acute myeloid leukemia), K-562 (chronic myeloid leukemia) and Z-138 (non-Hodgkin lymphoma) cell lines. Docetaxel (a microtubule depolymerization inhibitor) and staurosporine (STS, a pan-kinase inhibitor) were used as positive

controls. From this screening campaign, two derivatives (compounds **34j** and **34q**) emerged that showed low μM activity against the different cell lines (**Table-6**).

Table-6: Antitumoral evaluation of compounds from **34a-t**. IC₅₀: 50% inhibitory concentration, LN-229: glioblastoma, Capan-1: pancreatic adenocarcinoma, HCT-116: colorectal carcinoma, NCI-H460: lung carcinoma, DND-41: acute lymphoblastic leukemia, HL-60: acute myeloid leukemia, K-562: chronic myeloid leukemia, Z-138: non-Hodgkin lymphoma, STS: staurosporine.

Compd	IC ₅₀ (μM)							
	LN-229	Capan-1	HCT-116	NCI-H460	DND-41	HL-60	K-562	Z-138
34b	47.1	57.5	67.8	>100	39.3	50.9	10.4	48.4
34c	>100	>100	>100	>100	>100	>100	>100	>100
34e	>100	>100	>100	>100	>100	>100	>100	>100
34f	>100	>100	>100	>100	>100	>100	>100	>100
34g	>100	>100	>100	>100	>100	>100	>100	>100
34h	>100	>100	>100	>100	>100	>100	>100	>100
34j*	2.7 $\pm$ 0.2	2.3 $\pm$ 0.2	2.5 $\pm$ 0.09	56.0	2.4 $\pm$ 0.4	13.0 $\pm$ 2.8	3.4 $\pm$ 0.2	1.9 $\pm$ 0.03
34k	>100	>100	>100	>100	>100	>100	>100	>100
34m	>100	>100	>100	>100	>100	>100	>100	>100
34n	68.3	63.1	>100	>100	91.7	71.2	53.2	53.9
34p	>100	>100	>100	>100	>100	>100	>100	>100
34q*	0.7 $\pm$ 0.09	1.1 $\pm$ 0.7	1.0 $\pm$ 0.4	2.5 $\pm$ 0.2	0.6 $\pm$ 0.2	2.0 $\pm$ 0.4	2.3 $\pm$ 1.6	0.4 $\pm$ 0.005
34r	43.9	54.3	69.0	47.0	70.2	54.0	23.5	50.2
34s	>100	>100	>100	>100	>100	>100	>100	>100
34t	62.7	>100	>100	>100	>100	74.5	79.3	49.5
Docetaxel*	0.0087 $\pm$ 0.0004	0.0042 $\pm$ 0.0021	0.0009 $\pm$ 0.0008	0.0038 $\pm$ 0.0029	0.0033 $\pm$ 0.0014	0.0023 $\pm$ 0.0003	0.0037 $\pm$ 0.0003	0.0011 $\pm$ 0.0008
STS*	0.0229 $\pm$ 0.0021	0.0007 $\pm$ 0.0002	0.0004 $\pm$ 0.0001	0.00010 $\pm$ 0.0000	0.0015 $\pm$ 0.0004	0.0043 $\pm$ 0.0022	0.0074 $\pm$ 0.0017	0.0224 $\pm$ 0.0074

(*) Mean value of two independent experiments $\pm$ SEM.

Because of the promising anti-tumoral profile of compounds **34j** and **34q**, their apoptogenic potential in non-cancerous peripheral blood mononuclear cells (PBMCs) was determined as counter screening. The activation of the executioner caspases-3 and -7 normally precedes the manifestation of apoptosis as massive DNA fragmentation. Therefore, the caspase-3/7 Green reagent was added to the PBMCs, which are also treated with different concentrations of compounds **34j** and **34q**. When activated caspase 3 or 7 are intracellularly present, they will cleave the Caspase-3/7 Green Reagent at the DEVD motif. This results in the release of a DNA binding dye that fluorescently labels nuclear DNA of apoptotic cells. In addition, in order to distinguish dead cells from live cells, a propidium iodide (PI) staining was carried out. As can be derived from **Fig. 5**, only very high concentrations of compounds **34j** and **34q** (100 μ M) give rise to a small increase in the number of apoptotic and dead cells. Overall, these data indicate that compounds **34j** and **34q** did not inhibit the viability of normal PBMCs and demonstrate selectivity towards cancer cells over normal cells (**Fig. 5**).

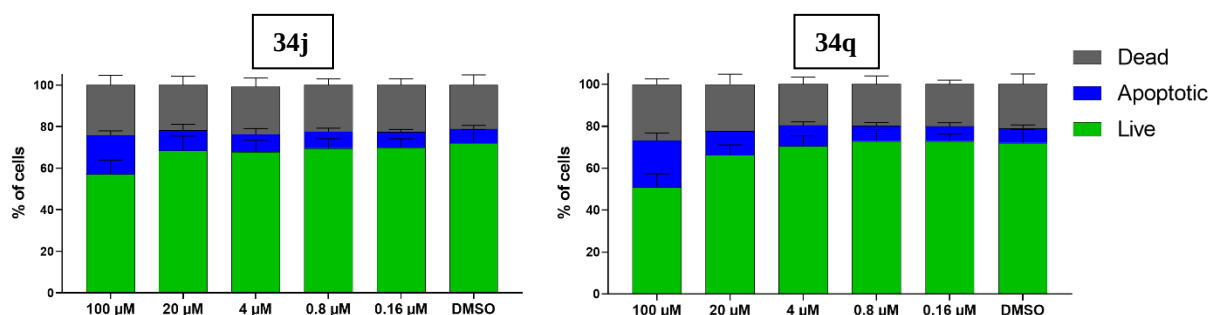


Fig. 5: Analysis of apoptosis induction by compounds **34j** (left) and **34q** (right) in PBMC originating from two healthy donors. Apoptosis was determined by staining with IncuCyte® Caspase 3/7 Green Reagent, whereas dead cells were identified by propidium staining, followed by live cell monitoring. The percentages of cells with different staining patterns after 72 hours are shown (mean $\pm$ standard error bars).

Despite their promising antitumoral profile, the exact molecular target of compounds **34j** and **34q** remained elusive. In order to assess whether they interact with tubulin, an immune fluorescence analysis of tubulin in HEp-2 cells treated for 3 hours with compounds **34j** and **34q** was performed and compared to DMSO (vehicle control) and vincristine (a known tubulin polymerization inhibitor, used as positive control). It can be observed that both compounds **34j** and **34q** inhibit the polymerization of tubulin in a dose-dependent manner (**Fig. 6**).

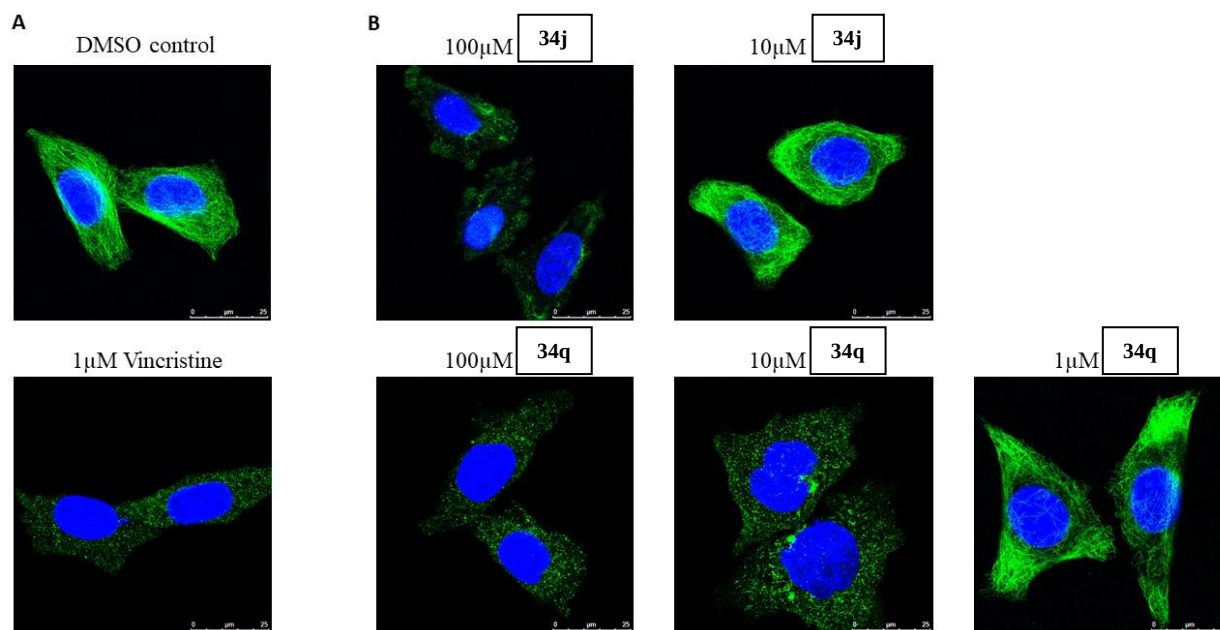


Fig. 6: Immunofluorescence staining of alpha-tubulin in HEp-2 cells treated for 3 hours with indicated compounds. (A) Representative images of normal alpha-tubulin after treatment with DMSO (top) or typical phenotype after treatment with vincristine (bottom), (B) treatment with compounds **34j** and **34q**. Green: alpha-tubulin, blue: DAPI. Scale bar: 25 μm

In conclusion, the synthesis of a new series of 3-(1*H*-pyrazol-1-yl)-6,7-dihydro-5*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine derivatives was carried out in excellent yields via a one-pot, four-component method using readily available starting materials. The reaction proceeds in such a way with a high atom economy, leading to the formation of one C=N, two C–N, one C–C, and one C–S bonds in a single operation, giving multi-annulated products. All the final compounds were tested for their antiviral and antitumoral activity. It was demonstrated that subtle structural modifications on the phenyl moieties allowed to tune the biological properties of the compounds. Among the newly synthesized compounds, a number of derivatives show promising antiviral activity against the hCoV-229E, whereas other derivatives exhibited cytotoxicity in various cancer cell lines. In addition, it was demonstrated that the antitumoral activity of these compounds is caused by the inhibition of tubulin polymerization.

EXPERIMENTAL:

General procedure for the synthesis of (3-(3,5-dimethyl-1*H*-pyrazol-1-yl)-6-phenyl-6,7-dihydro-5*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-7-yl)(phenyl)methanone derivatives (34a-t).

4-Amino-5-hydrazino-4*H*-[1,2,4] triazole-3-thiol **6** (0.001 mol), acetylacetone **33** (0.001 mol), and appropriate aromatic aldehydes/heterocyclic aldehyde **7** or **2** (0.001 mol) were taken into the round bottom flask having 5 mL of dry ethanol, and a catalytic amount of conc. HCl and then refluxed for 5-7 h. To the reaction mixture substituted phenacyl bromides **4** (0.001 mol) and triethylamine (TEA) (0.003 mol) were added. Then the reaction was continued under the reflux condition for 6-8 h by monitoring TLC (CHCl₃:CH₃OH=95:5). The reaction mixture was cooled to room temperature, diluted with water and the solid separated was filtered. The final product was recrystallized from 6-8 mL ethanol.

X-ray Crystallography

The diffraction data was collected on Bruker APEX2 single-crystal X-ray diffractometer equipped with a CCD area detector system, graphite monochromator, and a Mo-K_α fine focus sealed tube ($\lambda = 0.71073 \text{ \AA}$). Bruker SAINT PLUS⁴⁷ was used for data reduction, SHELXT-2014⁴⁸ was used for structure solution and SHELXL-2018⁴⁹ was used for full-matrix least-squares refinement. Mercury 3.3 was used for molecular graphics⁵⁰. All non-hydrogen atoms were refined using anisotropic thermal parameters. All hydrogen atoms bound to carbons were positioned geometrically and refined using a riding model.

Antiviral assay

The antiviral evaluation of the compounds against hCoV-229E was performed by seeding HEL 299 cells (ATCC CCL-137; human lung fibroblast) into 384-well dishes. After 24 h at 37°C, serial dilutions of the compounds were added to the cells before infection with hCoV-229E at 30 CCID₅₀ (50% cell culture infective doses) per well. At 7 days post-infection, the virus-induced cytopathogenic effect was measured colorimetrically by the formazan-based 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium (MTS) cell viability assay (Cell Titer 96 Aqueous One Solution Cell Proliferation Assay from Promega, Madison, WI), and the antiviral activity was expressed as the 50% effective concentration (EC₅₀). In parallel, the 50% cytotoxic concentration (CC₅₀) was derived from mock-infected cells.

Antitumoral assays

Cancer cell lines LN-229, Capan-1, HCT-116, NCI-H460, HL-60, K-562 and Z-138 were acquired from the American Type Culture Collection (ATCC, Manassas, VA, USA) and the DND-41 cell line was purchased from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ Leibniz-Institut, Germany). All cell lines were cultured as recommended by the suppliers. Adherent cell lines LN-229, Capan-1, HCT-116, and NCI-H460 were seeded at a density between 500 and 1500 cells per well, in 384-well tissue culture plates (Greiner). After overnight incubation, cells were treated with different concentrations of the test compounds. Suspension cell lines HL-60, K-562, Z-138, and DND-41 were seeded at densities ranging from 2500 to 5500 cells per well in 384-well culture plates containing the test compounds at the same concentration points. The plates were incubated and monitored at 37°C for 72 h in an IncuCyte® (Essen BioScience Inc., Sartorius) for real-time imaging of cell proliferation. Brightfield images were taken every 3 h, with one field imaged per well under 10x magnification. Cell growth was then quantified based on the percent cellular confluence as analyzed by the IncuCyte® image analysis software and used to calculate CC₅₀ values by linear interpolation.

Apoptosis induction assay

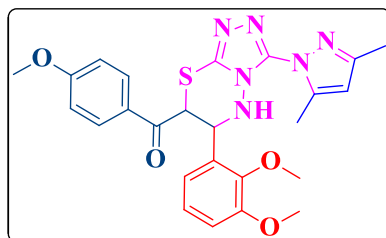
Buffy coat preparations from healthy donors were obtained from the Blood Transfusion Center in Leuven, Belgium. Peripheral blood mononuclear cells (PBMC) were isolated by density gradient centrifugation over Lymphoprep (d=1.077 g/ml) (Nycomed, Oslo, Norway) and cultured in a cell culture medium (DMEM/F12, Gibco Life Technologies, USA) containing 8% FBS. PBMC were seeded at 28000 cells per well in 384-well, black-walled, clear-bottomed tissue culture plates containing the test compounds at five different concentrations ranging from 100 to 0.16 µM. Propidium iodide was added at a final concentration of 1 µg/ml, and IncuCyte® Caspase 3/7 Green Reagent was added as recommended by the supplier. The plates were incubated and monitored at 37°C for 72 hours in the IncuCyte®. Images were taken every 3 hours in the brightfield and the green and red fluorescence channels, with one field imaged per well under 10x magnification. Quantification of the fluorescent signal after 72 hours in both channels using the IncuCyte® image analysis software allowed calculating the percentage of live, dead, and apoptotic cells. All compounds were tested in two independent experiments, implying PBMC originated from two different donors.

Tubulin immunofluorescence staining

Human cervix carcinoma HEP-2 cells were seeded at 15000c/well in 8-well chamber slides (Ibidi). After overnight incubation, they were treated with compound or carrier (DMSO) for 3h and then fixed with 4% paraformaldehyde (PFA), washed, and permeabilized. Further treatment was performed according to standard immune fluorescence procedures and cell nuclei were counterstained with DAPI. Employed antibodies are mouse anti-alpha tubulin (sc-5286, Santa Cruz Biotechnology) and secondary goat anti-mouse IgG conjugated to Alexa Fluor® 488 (A11001, Invitrogen). Images were taken with a Leica TCS SP5 confocal microscope employing a HCX PL APO 63x (NA 1.2)/water immersion objective.

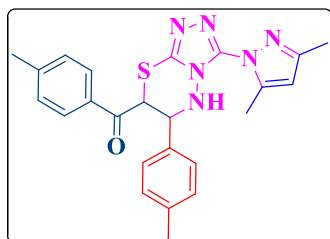
SPECTRAL DATA:

(6-(2,3-Dimethoxyphenyl)-3-(3,5-dimethyl-1H-pyrazol-1-yl)-6,7-dihydro-5H-[1,2,4]triazole [3,4-b][1,3,4]thiadiazin-7-yl)(4-methoxyphenyl)methanone (34a):



Light yellow color solid (0.465 g, 92%); m.p.: 192-194°C; **IR (KBr, $\nu_{\max}/\text{cm}^{-1}$):** 3211 (-NH-), 1668 (-C=O); **$^1\text{H-NMR}$ (400 MHz, CDCl_3 , δ ppm):** 2.24 (s, 3H, -CH₃), 2.40 (s, 3H, -CH₃), 3.87 (s, 6H, -OCH₃), 3.90 (s, 3H, -OCH₃), 5.28 (unresolved doublet, 2H, -CH-), 6.00 (s, 1H, -CH- of pyrazole ring), 6.68 (d, 1H, $J=7.6$ Hz, Ar-H), 6.88 (d, 1H, $J=8.0$ Hz, Ar-H), 6.94 (s, 1H, -NH-), 6.97 (d, 2H, $J=8.4$ Hz, Ar-H), 7.11 (d, 1H, $J=8.0$ Hz, Ar-H), 7.94 (d, 2H, $J=8.8$ Hz, Ar-H); **$^{13}\text{C-NMR}$ (100 MHz, CDCl_3 , δ ppm):** 11.7, 13.7, 42.6, 54.9, 55.7, 55.8, 61.0, 107.7, 112.9, 114.3, 119.7, 124.4, 127.4, 129.4, 130.3, 130.8, 131.2, 142.9, 146.1, 151.8, 152.6, 164.6, 193.2; **ESI-MS (m/z):** 507 [M+H]⁺; Analytical calculated formulae C₂₅H₂₆N₆O₄S: C, 59.27; H, 5.17; N, 16.59; S, 6.33; Found: C, 59.22; H, 5.22; N, 16.53; S, 6.30.

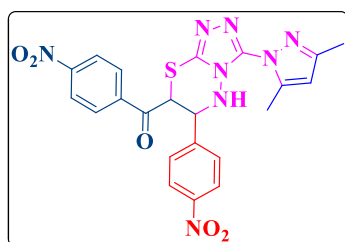
(3-(3,5-Dimethyl-1H-pyrazol-1-yl)-6-(*p*-tolyl)-6,7-dihydro-5H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-7-yl)(*p*-tolyl)methanone (34b):



White solid (0.408 g, 91%); m.p.: 194-196°C; **IR (KBr, $\nu_{\max}/\text{cm}^{-1}$):** 3135 (-NH-), 1681 (-C=O); **$^1\text{H-NMR}$ (400 MHz, CDCl_3 , δ ppm):** 2.22 (s, 3H, -CH₃), 2.30 (s, 3H, -CH₃), 2.38 (s, 3H, -CH₃), 2.43 (s, 3H, -CH₃), 5.05 (unresolved doublet, 1H, -CH-), 5.25 (d, 1H, $J=5.2$ Hz, -CH-), 6.00 (s, 1H, -CH- of pyrazole ring), 7.11 (d,

2H, $J=8.0$ Hz, Ar-H), 7.29 (d, 4H, $J=7.2$ Hz, Ar-H), 7.42 (s, 1H, -NH-), 7.80 (d, 2H, $J=8.0$ Hz, Ar-H); **$^{13}\text{C-NMR}$ (100 MHz, CDCl_3 , δ ppm):** 11.9, 13.6, 21.1, 21.8, 44.2, 59.3, 107.8, 127.3, 128.8, 129.7, 129.8, 132.0, 132.7, 138.8, 141.3, 143.1, 145.6, 145.7, 151.8, 193.7; **ESI-MS (m/z):** 445 $[\text{M}+\text{H}]^+$; Analytical calculated formulae $\text{C}_{24}\text{H}_{24}\text{N}_6\text{OS}$: C, 64.84; H, 5.44; N, 18.90; S, 7.21; Found: C, 64.89; H, 5.40; N, 18.85; S, 7.18.

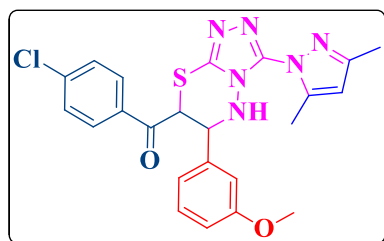
(3-(3,5-Dimethyl-1H-pyrazol-1-yl)-6-(4-nitrophenyl)-6,7-dihydro-5H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-7-yl)(4-nitrophenyl)methanone (34c):



Yellow solid (0.419 g, 83%); m.p.: 242-244°C; **IR (KBr, $\nu_{\text{max}}/\text{cm}^{-1}$):** 3302 (-NH-), 1641 (-C=O); **$^1\text{H-NMR}$ (400 MHz, CDCl_3 , δ ppm):** 2.16 (s, 3H, -CH₃), 2.44 (s, 3H, -CH₃), 5.05 (d, 1H, $J=6.0$ Hz, -CH-), 5.20 (d, 1H, $J=6.0$ Hz, -CH-), 6.00 (s, 1H, -CH- of pyrazole ring), 7.30 (d, 2H, $J=8.4$ Hz, Ar-H), 7.38 (d, 2H, $J=8.4$

Hz, Ar-H), 7.65 (d, 2H, $J=8.1$ Hz, Ar-H), 7.73 (s, 1H, -NH-), 7.77 (d, 2H, $J=8.4$ Hz, Ar-H); **$^{13}\text{C-NMR}$ (100 MHz, CDCl_3 , δ ppm):** 12.0, 13.5, 44.1, 59.1, 107.9, 128.9, 129.0, 129.2, 129.9, 131.9, 134.3, 134.9, 141.0, 143.1, 145.5, 146.0, 151.8, 193.5; **ESI-MS (m/z):** 507 $[\text{M}+\text{H}]^+$; Analytical calculated formulae $\text{C}_{22}\text{H}_{18}\text{N}_8\text{O}_5\text{S}$: C, 52.17; H, 3.58; N, 22.12; S, 6.33; Found: C, 52.23; H, 3.54; N, 22.17; S, 6.30.

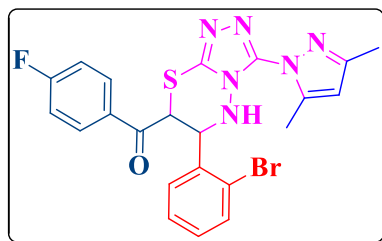
(4-Chlorophenyl)(3-(3,5-dimethyl-1H-pyrazol-1-yl)-6-(3-methoxyphenyl)-6,7-dihydro-5H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-7-yl)methanone (34d):



Cream color solid (0.412 g, 86%); m.p.: 188-190°C; **IR (KBr, $\nu_{\text{max}}/\text{cm}^{-1}$):** 3241 (-NH-), 1672 (-C=O); **$^1\text{H-NMR}$ (400 MHz, CDCl_3 , δ ppm):** 2.19 (s, 3H, -CH₃), 2.41 (s, 3H, -CH₃), 3.74 (s, 3H, -OCH₃), 5.04 (t, 1H, $J=4.0$ Hz, -CH-), 5.25 (d, 1H, $J=4.4$ Hz, -CH-), 6.00 (s, 1H, -CH- of pyrazole ring), 6.83 (d, 1H,

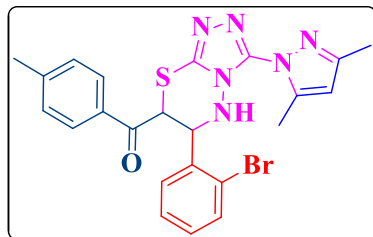
$J=6.8$ Hz, Ar-H), 6.96 (s, 1H, Ar-H), 6.98 (d, 1H, $J=6.4$ Hz, Ar-H), 7.23 (t, 1H, $J=6.4$ Hz, Ar-H), 7.47 (d, 2H, $J=6.4$ Hz, Ar-H), 7.58 (s, 1H, -NH-), 7.84 (d, 2H, $J=6.8$ Hz, Ar-H); **$^{13}\text{C-NMR}$ (100 MHz, CDCl_3 , δ ppm):** 12.0, 13.6, 44.9, 55.3, 59.8, 107.9, 113.3, 114.4, 119.4, 129.5, 130.1, 130.2, 132.9, 137.1, 140.9, 141.2, 143.2, 145.5, 151.8, 160.0, 192.9; **ESI-MS (m/z):** 481 $[\text{M}+\text{H}]^+$; Analytical calculated formulae $\text{C}_{23}\text{H}_{21}\text{ClN}_6\text{O}_2\text{S}$: C, 57.44; H, 4.40; N, 17.47; S, 6.67; Found: C, 57.48; H, 4.45; N, 17.42; S, 6.62.

(6-(2-Bromophenyl)-3-(3,5-dimethyl-1H-pyrazol-1-yl)-6,7-dihydro-5H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-7-yl)(4-fluorophenyl)methanone (34e):



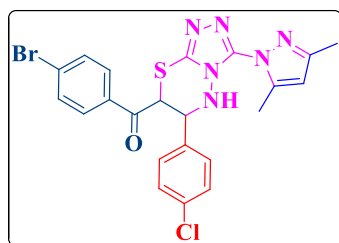
Golden yellow color solid (0.461 g, 90%); m.p.: 195-197°C; **IR** (KBr, $\nu_{\max}/\text{cm}^{-1}$): 3138 (-NH-), 1692 (-C=O); **$^1\text{H-NMR}$** (400 MHz, CDCl_3 , δ ppm): 2.15 (s, 3H, -CH₃), 2.48 (s, 3H, -CH₃), 5.22 (d, 1H, $J=4.0$ Hz, -CH-), 5.50 (t, 1H, $J=4.8$ Hz, -CH-), 5.99 (s, 1H, -CH- of pyrazole ring), 7.17 (s, 1H, -NH-), 7.20 (d, 2H, $J=8.4$ Hz, Ar-H), 7.24 (d, 2H, $J=2.0$ Hz, Ar-H), 7.59 (d, 1H, $J=7.6$ Hz, Ar-H), 7.78 (d, 1H, $J=5.6$ Hz, Ar-H), 8.01 (dd, 2H, $J=8.8$ Hz, $J=5.2$ Hz, Ar-H); **$^{13}\text{C-NMR}$** (100 MHz, CDCl_3 , δ ppm): 11.9, 13.3, 43.1, 58.3, 107.8, 116.3, 116.5, 123.2, 128.1, 128.6, 130.2, 131.7, 131.8, 133.4, 136.4, 140.0, 142.9, 145.5, 151.7, 166.2, 167.8, 192.5; **ESI-MS** (m/z): 515 $[\text{M}+2]^+$; Analytical calculated formulae $\text{C}_{22}\text{H}_{18}\text{BrFN}_6\text{OS}$: C, 51.47; H, 3.53; N, 16.37; S, 6.25; Found: C, 51.42; H, 3.57; N, 16.32; S, 6.20.

(6-(2-Bromophenyl)-3-(3,5-dimethyl-1H-pyrazol-1-yl)-6,7-dihydro-5H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-7-yl)(p-tolyl)methanone (34f):



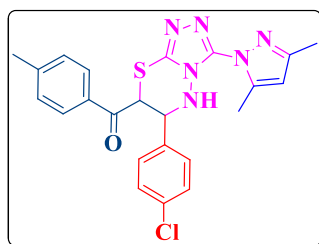
Lemon yellow color solid (0.468 g, 92%); m.p.: 201-203°C; **IR** (KBr, $\nu_{\max}/\text{cm}^{-1}$): 3148 (-NH-), 1673 (-C=O); **$^1\text{H-NMR}$** (400 MHz, CDCl_3 , δ ppm): 2.20 (s, 3H, -CH₃), 2.44 (s, 3H, -CH₃), 2.46 (s, 3H, -CH₃), 5.22 (d, 1H, $J=4.0$ Hz, -CH-), 5.49 (t, 1H, $J=4.8$ Hz, -CH-), 6.00 (s, 1H, -CH- of pyrazole ring), 7.19 (t, 2H, $J=8.0$ Hz, Ar-H), 7.23 (s, 1H, -NH-), 7.31 (d, 2H, $J=8.0$ Hz, Ar-H), 7.58 (d, 1H, $J=7.6$ Hz, Ar-H), 7.65 (d, 1H, $J=5.2$ Hz, Ar-H), 7.86 (d, 2H, $J=8.0$ Hz, Ar-H); **$^{13}\text{C-NMR}$** (100 MHz, CDCl_3 , δ ppm): 11.9, 13.5, 21.8, 42.9, 57.8, 107.8, 123.0, 128.1, 128.6, 128.9, 129.9, 130.2, 131.7, 133.4, 136.6, 140.1, 142.9, 145.6, 145.7, 151.8, 193.7; **ESI-MS** (m/z): 511 $[\text{M}+2]^+$; Analytical calculated formulae $\text{C}_{23}\text{H}_{21}\text{BrN}_6\text{OS}$: C, 54.23; H, 4.16; N, 16.50; S, 6.29; Found: C, 54.28; H, 4.21; N, 16.44; S, 6.33.

(4-Bromophenyl)(6-(4-chlorophenyl)-3-(3,5-dimethyl-1H-pyrazol-1-yl)-6,7-dihydro-5H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-7-yl)methanone (34g):



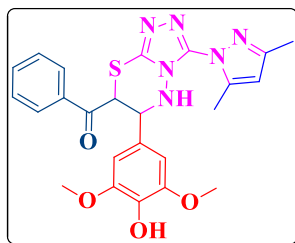
White solid (0.476 g, 90%); m.p.: 205-207°C; **IR (KBr, $\nu_{\text{max}}/\text{cm}^{-1}$):** 3291 (-NH-), 1688 (-C=O); **$^1\text{H-NMR}$ (400 MHz, CDCl_3 , δ ppm):** 2.16 (s, 3H, -CH₃), 2.44 (s, 3H, -CH₃), 5.05 (dd, 1H, $J=6.0$ Hz, $J=3.6$ Hz, -CH-), 5.2 (d, 1H, $J=6.0$ Hz, -CH-), 6.00 (s, 1H, -CH- of pyrazole ring), 7.30 (d, 2H, $J=8.4$ Hz, Ar-H), 7.38 (d, 2H, $J=8.4$ Hz, Ar-H), 7.65 (d, 2H, $J=8.1$ Hz, Ar-H), 7.72 (s, 1H, -NH-), 7.77 (d, 2H, $J=8.4$ Hz, Ar-H); **$^{13}\text{C-NMR}$ (100 MHz, CDCl_3 , δ ppm):** 12.0, 13.5, 44.9, 59.5, 107.9, 129.0, 129.3, 132.1, 130.2, 132.6, 133.2, 134.2, 135.1, 140.6, 143.1, 145.3, 151.8, 192.8; **ESI-MS (m/z):** 531 $[\text{M}+2]^+$; Analytical calculated formulae $\text{C}_{22}\text{H}_{18}\text{BrClN}_6\text{OS}$: C, 49.87; H, 3.42; N, 15.86; S, 6.05; Found: C, 49.84; H, 3.48; N, 15.83; S, 6.12.

(6-(4-Chlorophenyl)-3-(3,5-dimethyl-1H-pyrazol-1-yl)-6,7-dihydro-5H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-7-yl)(p-tolyl)methanone (34h):



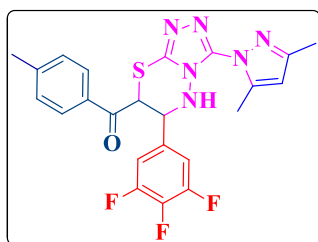
White solid (0.431 g, 93%); m.p.: 214-216°C; **IR (KBr, $\nu_{\text{max}}/\text{cm}^{-1}$):** 3219 (-NH-), 1675 (-C=O); **$^1\text{H-NMR}$ (400 MHz, $\text{CDCl}_3+\text{DMSO}-d_6$, δ ppm):** 2.25 (s, 3H, -CH₃), 2.31 (s, 3H, -CH₃), 2.44 (s, 3H, -CH₃), 3.21 (s, 1H, -NH-), 4.99 (unresolved singlet, 1H, -CH-), 5.69 (d, 1H, $J=5.2$ Hz, -CH-), 6.06 (s, 1H, -CH- of pyrazole ring), 7.19 (d, 1H, $J=7.2$ Hz, Ar-H), 7.28 (d, 1H, $J=6.4$ Hz, Ar-H), 7.33 (d, 2H, $J=7.6$ Hz, Ar-H), 7.47 (s, 2H, Ar-H), 7.90 (s, 2H, Ar-H); **$^{13}\text{C-NMR}$ (100 MHz, $\text{CDCl}_3+\text{DMSO}-d_6$, δ ppm):** 11.4, 13.7, 21.8, 42.5, 58.2, 107.6, 128.9, 129.1, 129.3, 129.8, 132.2, 133.9, 135.2, 141.8, 142.8, 145.6, 146.6, 151.4, 194.3; **ESI-MS (m/z):** 465 $[\text{M}+\text{H}]^+$; Analytical calculated formulae $\text{C}_{23}\text{H}_{21}\text{ClN}_6\text{OS}$: C, 59.41; H, 4.55; N, 18.07; S, 6.90; Found: C, 59.45; H, 4.51; N, 18.10; S, 6.85.

(3-(3,5-Dimethyl-1*H*-pyrazol-1-yl)-6-(4-hydroxy-3,5-dimethoxyphenyl)-6,7-dihydro-5*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-7-yl)(phenyl)methanone (34i):



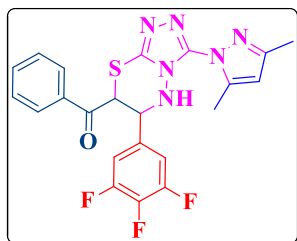
Green solid (0.437 g, 89%); m.p.: 196-198°C; **IR (KBr, $\nu_{\max}/\text{cm}^{-1}$):** 3435 (-OH), 3134 (-NH-), 1653 (-C=O); **$^1\text{H-NMR}$ (400 MHz, $\text{CDCl}_3+\text{DMSO}-d_6$, δ ppm):** 2.21 (s, 3H, -CH₃), 2.25 (s, 3H, -CH₃), 3.71 (s, 6H, -OCH₃), 4.80 (t, 1H, $J=6.8$ Hz, -CH-), 5.86 (d, 1H, $J=6.0$ Hz, -CH-), 6.11 (s, 1H, -CH- of pyrazole ring), 6.35 (s, 1H, -OH), 6.77 (s, 2H, Ar-H), 7.07 (s, 1H, -NH-), 7.54 (t, 2H, $J=8.0$ Hz, Ar-H), 7.67 (t, 1H, $J=7.2$ Hz, Ar-H), 8.00 (d, 2H, $J=7.2$ Hz, Ar-H); **$^{13}\text{C-NMR}$ (100 MHz, CDCl_3 , δ ppm):** 11.9, 13.5, 45.4, 56.2, 60.8, 104.8, 107.8, 128.7, 129.1, 131.0, 134.5, 134.7, 138.4, 141.5, 143.2, 145.4, 151.7, 153.5, 194.1; **ESI-MS (m/z):** 493 $[\text{M}+\text{H}]^+$; Analytical calculated formulae $\text{C}_{24}\text{H}_{24}\text{N}_6\text{O}_4\text{S}$: C, 58.52; H, 4.91; N, 17.06; S, 6.51; Found: C, 58.57; H, 4.94; N, 17.10; S, 6.47.

(3-(3,5-Dimethyl-1*H*-pyrazol-1-yl)-6-(3,4,5-trifluorophenyl)-6,7-dihydro-5*H*-[1,2,4]triazole [3,4-*b*][1,3,4]thiadiazin-7-yl)(*p*-tolyl)methanone (34j):



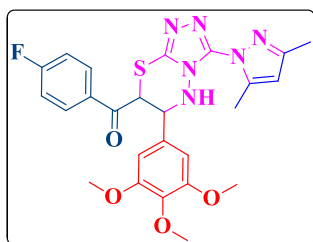
White solid (0.445 g, 92%); m.p.: 215-217°C; **IR (KBr, $\nu_{\max}/\text{cm}^{-1}$):** 3219 (-NH-), 1674 (-C=O); **$^1\text{H-NMR}$ (400 MHz, CDCl_3 , δ ppm):** 2.19 (s, 3H, -CH₃), 2.43 (s, 3H, -CH₃), 2.50 (s, 3H, -CH₃), 3.48 (s, 1H, -NH-), 5.035 (t, 1H, $J=8.0$ Hz, -CH-), 5.48 (d, 1H, $J=8.0$ Hz, -CH-), 6.00 (s, 1H, -CH- of pyrazole ring), 6.67 (t, 2H, $J=8.4$ Hz, Ar-H), 7.30 (d, 2H, $J=8.4$ Hz, Ar-H), 7.82 (d, 2H, $J=8.0$ Hz, Ar-H); **$^{13}\text{C-NMR}$ (100 MHz, CDCl_3 , δ ppm):** 11.9, 13.5, 21.8, 44.1, 53.1, 101.3, 107.9, 127.5, 128.9, 129.9, 129.8, 131.6, 141.3, 143.2, 145.6, 146.1, 151.8, 160.0, 192.2; **ESI-MS (m/z):** 485 $[\text{M}+\text{H}]^+$; Analytical calculated formulae $\text{C}_{23}\text{H}_{19}\text{F}_3\text{N}_6\text{OS}$: C, 57.02; H, 3.95; N, 17.35; S, 6.62; Found: C, 57.17; H, 3.99; N, 17.39; S, 6.62.

(3-(3,5-Dimethyl-1*H*-pyrazol-1-yl)-6-(3,4,5-trifluorophenyl)-6,7-dihydro-5*H*-[1,2,4]triazole [3,4-*b*][1,3,4]thiadiazin-7-yl)(phenyl)methanone (34k):



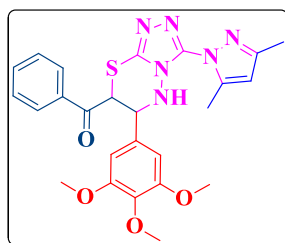
Light yellow color solid (0.423 g, 90%); m.p.: 182-184°C; **IR (KBr, $\nu_{\max}/\text{cm}^{-1}$):** 3207 (-NH-), 1681 (-C=O); **$^1\text{H-NMR}$ (400 MHz, CDCl_3 , δ ppm):** 2.15 (s, 3H, -CH₃), 2.49 (s, 3H, -CH₃), 5.34 (t, 1H, $J=8.0$ Hz, -CH-), 5.56 (d, 1H, $J=8.4$ Hz, -CH-), 5.99 (s, 1H, -CH- of pyrazole ring), 6.67 (t, 2H, $J=8.4$ Hz, Ar-H), 7.15 (s, 1H, -NH-), 7.51 (t, 2H, $J=7.6$ Hz, Ar-H), 7.64 (t, 1H, $J=7.2$ Hz, Ar-H), 7.94 (d, 2H, $J=7.6$ Hz, Ar-H); **$^{13}\text{C-NMR}$ (100 MHz, CDCl_3 , δ ppm):** 11.9, 13.4, 44.3, 53.2, 101.3, 107.9, 127.5, 128.9, 129.1, 129.2, 130.4, 134.8, 134.9, 141.2, 143.2, 145.6, 151.8, 192.7; **ESI-MS (m/z):** 471 $[\text{M}+\text{H}]^+$; Analytical calculated formulae $\text{C}_{22}\text{H}_{17}\text{F}_3\text{N}_6\text{OS}$: C, 56.16; H, 3.64; N, 17.86; S, 6.82; Found: C, 56.12; H, 3.60; N, 17.90; S, 6.87.

(3-(3,5-Dimethyl-1*H*-pyrazol-1-yl)-6-(3,4,5-trimethoxyphenyl)-6,7-dihydro-5*H*-[1,2,4]triazole[3,4-*b*][1,3,4]thiadiazin-7-yl)(4-fluorophenyl)methanone (34l):



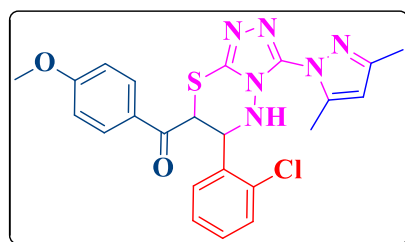
White solid (0.461 g, 88%); m.p.: 196-198°C; **IR (KBr, $\nu_{\max}/\text{cm}^{-1}$):** 3175 (-NH-), 1665 (-C=O); **$^1\text{H-NMR}$ (400 MHz, $\text{CDCl}_3 + \text{DMSO-}d_6$, δ ppm):** 2.18 (s, 3H, -CH₃), 2.22 (s, 3H, -CH₃), 3.69 (s, 3H, -OCH₃), 3.70 (s, 3H, -OCH₃), 3.87 (s, 3H, -OCH₃), 4.45 (t, 1H, $J=6.8$ Hz, -CH-), 5.83 (d, 1H, $J=6.4$ Hz, -CH-), 6.15 (s, 1H, -CH- of pyrazole ring), 6.87 (s, 1H, Ar-H), 6.97 (s, 1H, Ar-H), 7.07 (s, 1H, -NH-), 7.12 (d, 2H, $J=7.2$ Hz, Ar-H), 8.01 (d, 2H, $J=8.4$ Hz, Ar-H); **$^{13}\text{C-NMR}$ (100 MHz, CDCl_3 , δ ppm):** 11.7, 13.7, 42.5, 54.9, 55.7, 55.8, 61.0, 107.7, 112.9, 114.3, 119.7, 124.4, 127.4, 129.4, 131.2, 142.9, 151.7, 152.6, 164.6, 193.2; **ESI-MS (m/z):** 525 $[\text{M}+\text{H}]^+$; Analytical calculated formulae $\text{C}_{25}\text{H}_{25}\text{FN}_6\text{O}_4\text{S}$: C, 57.24; H, 4.80; N, 16.02; S, 6.11; Found: C, 57.20; H, 4.85; N, 16.17; S, 6.15.

(3-(3,5-Dimethyl-1*H*-pyrazol-1-yl)-6-(3,4,5-trimethoxyphenyl)-6,7-dihydro-5*H*-[1,2,4]triazole[3,4-*b*][1,3,4]thiadiazin-7-yl)(phenyl)methanone (34m):



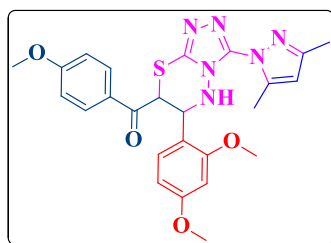
Golden yellow color solid (0.475 g, 94%); m.p.: 192-194°C; **IR (KBr, $\nu_{\text{max}}/\text{cm}^{-1}$):** 3129 (-NH-), 1680 (-C=O); **$^1\text{H-NMR}$ (400 MHz, CDCl_3 , δ ppm):** 2.19 (s, 3H, -CH₃), 2.39 (s, 3H, -CH₃), 3.48 (s, 6H, -OCH₃), 3.78 (s, 3H, -OCH₃), 5.00 (t, 1H, $J=5.2$ Hz, -CH-), 5.31 (d, 1H, $J=5.6$ Hz, -CH-), 6.00 (s, 1H, -CH- of pyrazole ring), 6.66 (s, 2H, Ar-H), 7.50 (t, 2H, $J=7.6$ Hz, Ar-H), 7.59 (s, 1H, -NH-), 7.63 (t, 1H, $J=7.6$ Hz, Ar-H), 7.90 (d, 2H, $J=7.6$ Hz, Ar-H); **$^{13}\text{C-NMR}$ (100 MHz, CDCl_3 , δ ppm):** 11.9, 13.5, 45.4, 56.2, 60.6, 60.8, 104.8, 107.8, 128.7, 129.1, 131.0, 134.5, 134.7, 138.4, 141.5, 143.2, 145.4, 151.7, 153.5, 194.1; **ESI-MS (m/z):** 507 $[\text{M}+\text{H}]^+$; Analytical calculated formulae $\text{C}_{25}\text{H}_{26}\text{N}_6\text{O}_4\text{S}$: C, 59.27; H, 5.17; N, 16.59; S, 6.33; Found: C, 59.24; H, 5.20; N, 16.54; S, 6.38.

(6-(2-Chlorophenyl)-3-(3,5-dimethyl-1*H*-pyrazol-1-yl)-6,7-dihydro-5*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-7-yl)(4-methoxyphenyl)methanone (34n):



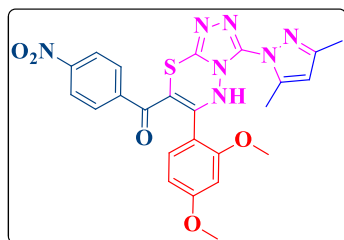
White solid (0.412 g, 86%); m.p.: 198-200°C; **IR (KBr, $\nu_{\text{max}}/\text{cm}^{-1}$):** 3143 (-NH-), 1671 (-C=O); **$^1\text{H-NMR}$ (400 MHz, CDCl_3 , δ ppm):** 2.21 (s, 3H, -CH₃), 2.45 (s, 3H, -CH₃), 3.90 (s, 3H, -OCH₃), 5.18 (d, 1H, $J=4.0$ Hz, -CH-), 5.50 (t, 1H, $J=4.4$ Hz, -CH-), 6.00 (s, 1H, -CH- of pyrazole ring), 6.98 (d, 2H, $J=8.8$ Hz, Ar-H), 7.16-7.23 (m, 2H, Ar-H), 7.29 (s, 1H, -NH-), 7.40 (d, 1H, $J=8.0$ Hz, Ar-H), 7.60 (d, 1H, $J=4.8$ Hz, Ar-H), 7.94 (d, 2H, $J=8.4$ Hz, Ar-H); **$^{13}\text{C-NMR}$ (100 MHz, CDCl_3 , δ ppm):** 12.0, 13.5, 21.8, 44.1, 59.1, 107.9, 128.9, 129.0, 129.2, 129.9, 131.9, 134.3, 134.9, 141.0, 143.1, 145.5, 145.9, 151.8, 193.5; **ESI-MS (m/z):** 481 $[\text{M}+\text{H}]^+$; Analytical calculated formulae $\text{C}_{23}\text{H}_{21}\text{ClN}_6\text{O}_2\text{S}$: C, 57.44; H, 4.40; N, 17.47; S, 6.67; Found: C, 57.40; H, 4.45; N, 17.1; S, 6.62.

(6-(2,4-Dimethoxyphenyl)-3-(3,5-dimethyl-1H-pyrazol-1-yl)-6,7-dihydro-5H-[1,2,4]triazole [3,4-b][1,3,4]thiadiazin-7-yl)(4-methoxyphenyl)methanone (34o):



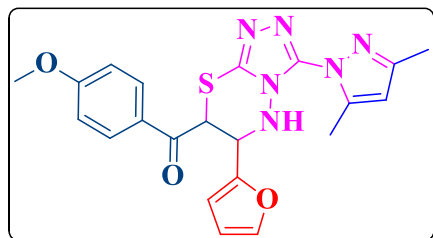
Yellow solid (0.475 g, 94%); m.p.: 150-152°C; **IR (KBr, $\nu_{\text{max}}/\text{cm}^{-1}$):** 3210 (-NH-), 1670 (-C=O); **$^1\text{H-NMR}$ (400 MHz, $\text{DMSO-}d_6$, δ ppm):** 2.19 (s, 3H, -CH₃), 2.22 (s, 3H, -CH₃), 3.69 (s, 3H, -OCH₃), 3.70 (s, 3H, -OCH₃), 3.87 (s, 3H, -OCH₃), 4.45 (t, 1H, $J=6.8$ Hz, -CH-), 5.83 (d, 1H, $J=6.4$ Hz, -CH-), 6.16 (s, 1H, -CH- of pyrazole ring), 6.86 (d, 1H, $J=8.4$ Hz, Ar-H), 6.98 (d, 1H, $J=8.4$ Hz, Ar-H), 7.07 (s, 1H, -NH-), 7.10 (d, 2H, $J=8.0$ Hz, Ar-H), 7.12 (s, 1H, Ar-H), 8.01 (d, 2H, $J=8.4$ Hz, Ar-H); **$^{13}\text{C-NMR}$ (100 MHz, CDCl_3 , δ ppm):** 11.7, 13.7, 42.6, 54.9, 55.7, 55.8, 61.0, 107.7, 112.9, 114.3, 119.7, 124.4, 127.4, 129.4, 130.3, 130.8, 131.2, 142.9, 146.1, 151.8, 152.6, 164.6, 193.2; **ESI-MS (m/z):** 507 $[\text{M}+\text{H}]^+$; Analytical calculated formulae $\text{C}_{25}\text{H}_{26}\text{N}_6\text{O}_4\text{S}$: C, 59.27; H, 5.17; N, 16.59; S, 6.33; Found: C, 59.24; H, 5.14; N, 16.63; S, 6.30.

(6-(2,4-dimethoxyphenyl)-3-(3,5-dimethyl-1H-pyrazol-1-yl)-5H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-7-yl)(4-nitrophenyl)methanone (34p).



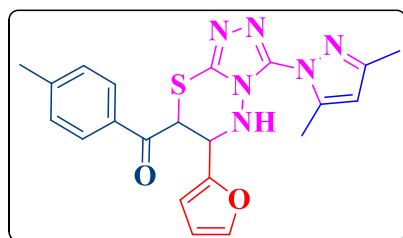
Yellow solid (0.456 g, 88%); m.p.: 236-238°C; **IR (KBr, $\nu_{\text{max}}/\text{cm}^{-1}$):** 3135 (-NH-), 1681 (-C=O); **$^1\text{H-NMR}$ (400 MHz, CDCl_3 , δ ppm):** 2.35 (s, 3H, -CH₃), 2.42 (s, 3H, -CH₃), 3.79 (s, 3H, -OCH₃), 3.88 (s, 3H, -OCH₃), 6.08 (s, 1H, -CH- of pyrazole ring), 6.48 (s, 1H, Ar-H), 6.62 (dd, 1H, $J=8.4$ Hz, $J=6.4$ Hz, Ar-H), 7.27 (s, 1H, -NH-), 7.43 (d, 1H, $J=8.8$ Hz, Ar-H), 7.95 (d, 2H, $J=8.8$ Hz, Ar-H), 8.31 (d, 2H, $J=8.8$ Hz, Ar-H); **$^{13}\text{C-NMR}$ (100 MHz, CDCl_3 , δ ppm):** 11.7, 13.7, 55.6, 55.7, 98.5, 104.7, 108.1, 112.9, 114.6, 123.9, 131.0, 131.2, 138.3, 141.2, 143.6, 146.5, 149.4, 152.4, 16.2, 159.1, 163.3, 196.0; Analytical calculated formulae $\text{C}_{24}\text{H}_{21}\text{N}_7\text{O}_5\text{S}$: C, 55.48; H, 4.07; N, 18.87; S, 6.17; Found: C, 55.43; H, 4.02; N, 18.92; S, 6.21.

(3-(3,5-Dimethyl-1*H*-pyrazol-1-yl)-6-(furan-2-yl)-6,7-dihydro-5*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-7-yl)(4-methoxyphenyl)methanone (34q):



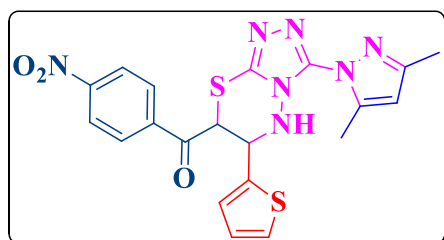
Brown solid (0.379 g, 87%); m.p.: 152-154°C; **IR (KBr, $\nu_{\text{max}}/\text{cm}^{-1}$):** 3195 (-NH-), 1672 (-C=O); **$^1\text{H-NMR}$ (400 MHz, CDCl_3 , δ ppm):** 2.30 (s, 3H, -CH₃), 2.34 (s, 3H, -CH₃), 3.92 (s, 3H, -OCH₃), 5.23 (unresolved singlet, 2H, -CH- and =CH), 6.03 (s, 1H, -CH- of pyrazole ring), 6.30 (s, 1H, -CH-), 6.32 (s, 1H, =CH), 7.02 (d, 3H, $J=8.8$ Hz, Ar-H), 7.35 (s, 1H, -NH-), 7.94 (d, 2H, $J=8.4$ Hz, Ar-H); **ESI-MS (m/z):** 437 [$\text{M}+\text{H}$]⁺; Analytical calculated formulae C₂₁H₂₀N₆O₃S: C, 57.79; H, 4.62; N, 19.25; S, 7.35; Found: C, 57.83; H, 4.66; N, 19.21; S, 7.31.

(3-(3,5-Dimethyl-1*H*-pyrazol-1-yl)-6-(furan-2-yl)-6,7-dihydro-5*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-7-yl)(*p*-tolyl)methanone (34r):



Brown solid (0.357 g, 85%); m.p.: 149-151°C; **IR (KBr, $\nu_{\text{max}}/\text{cm}^{-1}$):** 3204 (-NH-), 1666 (-C=O); **$^1\text{H-NMR}$ (400 MHz, CDCl_3 , δ ppm):** 2.30 (s, 3H, -CH₃), 2.34 (s, 3H, -CH₃), 2.46 (s, 3H, -CH₃), 5.26 (unresolved singlet, 2H, -CH-), 6.03 (s, 1H, -CH- of pyrazole ring), 6.31 (d, 2H, $J=8.0$ Hz, Ar-H), 7.03 (s, 1H, -NH-), 7.35 (d, 3H, $J=8.8$ Hz, Ar-H), 7.85 (d, 2H, $J=8.0$ Hz, Ar-H); **$^{13}\text{C-NMR}$ (100 MHz, CDCl_3 , δ ppm):** 11.6, 13.7, 21.9, 39.8, 53.0, 107.7, 109.2, 111.0, 128.9, 130.0, 131.6, 140.6, 142.7, 143.1, 146.1, 146.7, 148.3, 152.0, 194.4; **ESI-MS (m/z):** 421 [$\text{M}+\text{H}$]⁺; Analytical calculated formulae C₂₁H₂₀N₆O₂S: C, 59.98; H, 4.79; N, 19.99; S, 7.63; Found: C, 59.94; H, 4.7; N, 19.94; S, 7.68.

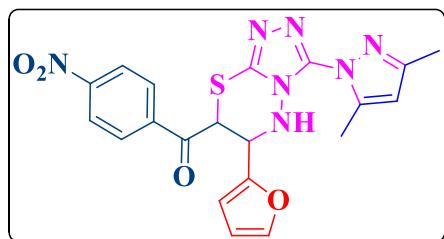
(3-(3,5-Dimethyl-1*H*-pyrazol-1-yl)-6-(thiophen-2-yl)-6,7-dihydro-5*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-7-yl)(4-nitrophenyl)methanone (34s):



Golden color solid (0.415 g, 89%); m.p.: 204-206°C; **IR (KBr, $\nu_{\text{max}}/\text{cm}^{-1}$):** 3281(-NH-), 1696 (-C=O); **$^1\text{H-NMR}$ (400 MHz, $\text{CDCl}_3+\text{DMSO-}d_6$, δ ppm):** 2.26 (s, 3H, -CH₃), 2.27 (s, 3H, -CH₃), 5.47 (t, 1H, $J=3.6$ Hz, -CH-), 5.88 (d, 1H, $J=3.2$ Hz, -CH-), 6.10 (s, 1H, -CH- of pyrazole ring), 6.97 (t, 1H, $J=4.4$ Hz, Ar-H), 7.17 (d, 1H, $J=4.4$ Hz, Ar-H), 7.31 (d, 1H, $J=4.8$ Hz, Ar-H), 7.35 (s, 1H, -NH-), 8.37 (s, 4H, Ar-H); **$^{13}\text{C-NMR}$ (100 MHz, $\text{CDCl}_3+\text{DMSO-}d_6$, δ**

ppm): 11.4, 13.8, 42.9, 53.4, 107.5, 124.1, 126.3, 126.5, 127.4, 129.3, 130.8, 139.9, 142.9, 146.9, 149.6, 150.7, 151.3, 194.5; **ESI-MS (*m/z*):** 468 [M+H]⁺; Analytical calculated formulae C₂₀H₁₇N₇O₃S: C, 51.38; H, 3.67; N, 20.97; S, 13.72; Found: C, 51.34; H, 3.62; N, 20.94; S, 13.76.

(3-(3,5-Dimethyl-1*H*-pyrazol-1-yl)-6-(furan-2-yl)-6,7-dihydro-5*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-7-yl)(4-nitrophenyl)methanone (34t):



White solid (0.414 g, 92%); m.p.: 201-203°C; **IR (KBr, $\nu_{\text{max}}/\text{cm}^{-1}$):** 3278 (-NH-), 1698 (-C=O); **¹H-NMR (400 MHz, CDCl₃+DMSO-*d*₆, δ ppm):** 2.25 (s, 3H, -CH₃), 2.27 (s, 3H, -CH₃), 5.28-5.32 (m, 1H, -CH-), 5.78 (d, 1H, *J*=4.0 Hz, -CH-), 6.08 (s, 1H, -CH- of pyrazole ring), 6.45 (s, 1H, -NH-), 7.14 (d, 1H, *J*=4.0 Hz, Ar-H), 7.48 (d, 1H, *J*=4.0 Hz, Ar-H), 7.85 (t, 1H, *J*=8.4 Hz, Ar-H), 8.29 (d, 2H *J*=8.4 Hz, Ar-H), 8.33 (d, 2H, *J*=7.2 Hz, Ar-H); **¹³C-NMR (100 MHz, CDCl₃+DMSO-*d*₆, δ ppm):** 11.2, 13.8, 49.8, 52.5, 107.5, 109.2, 111.0, 124.1, 124.3, 130.4, 130.6, 142.9, 143.0, 148.1, 149.1, 150.7 151.3, 194.0; **ESI-MS (*m/z*):** 452 [M+H]⁺; Analytical calculated formulae C₂₀H₁₇N₇O₄S: C, 53.21; H, 3.80; N, 21.72; S, 7.10; Found: C, 53.25; H, 3.85; N, 21.7; S, 7.15.

SPECTRA

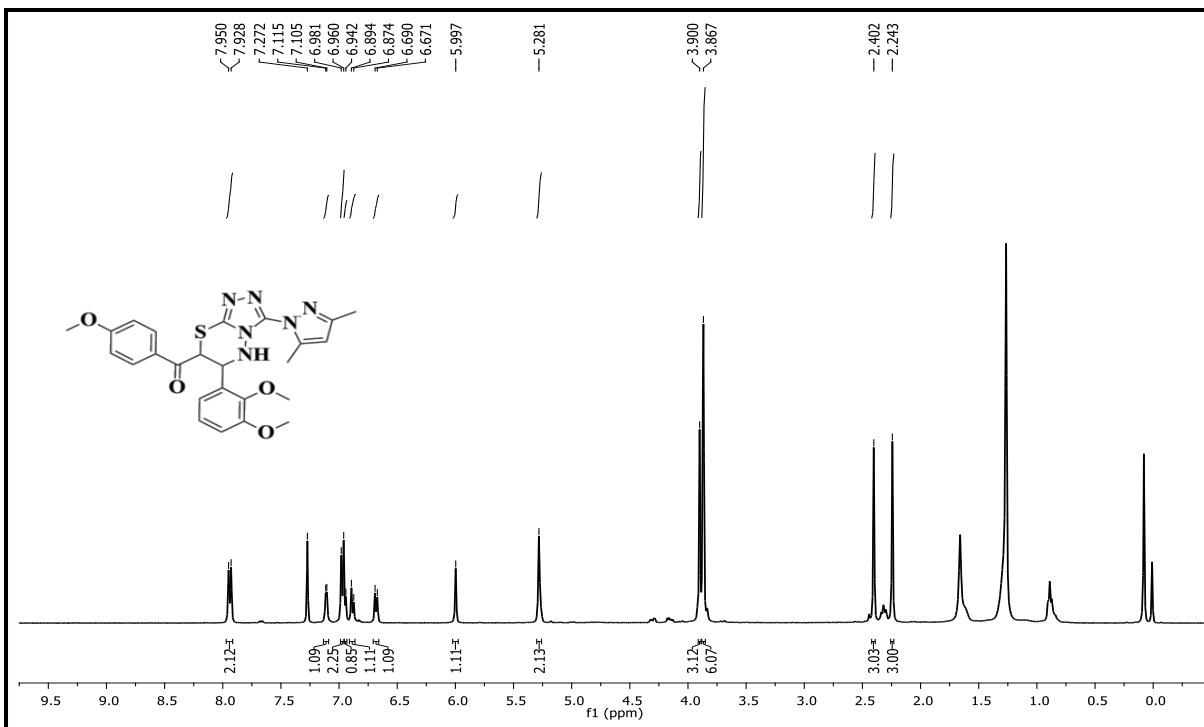


Fig: ¹H-NMR Spectrum of Compound 34a (400 MHz, CDCl₃)

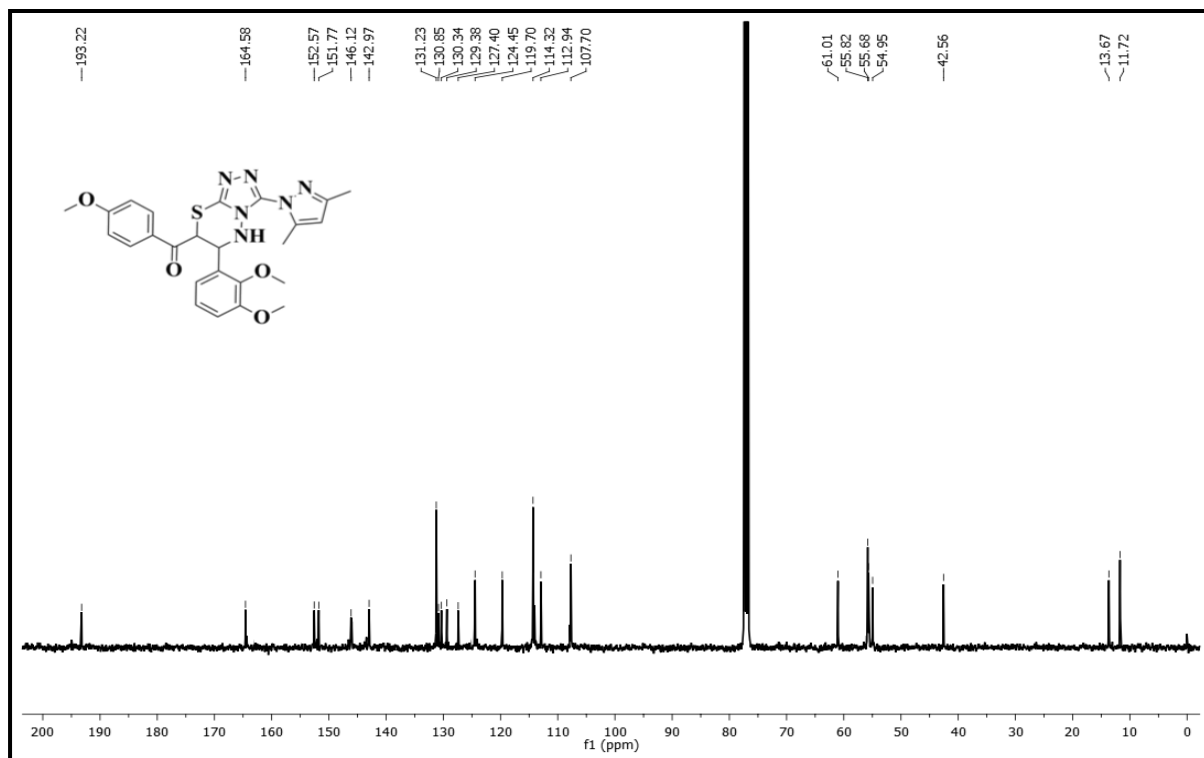


Fig: ¹³C-NMR Spectrum of Compound 34a (100 MHz, CDCl₃)

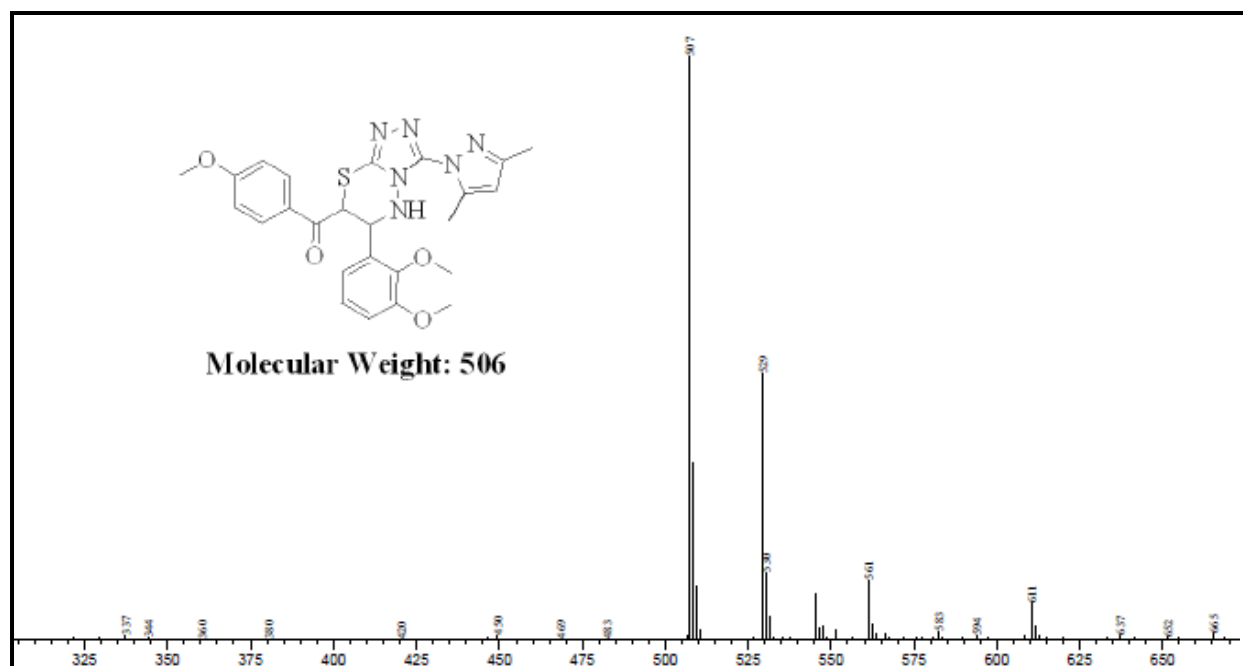


Fig: Mass Spectrum of Compound **34a**

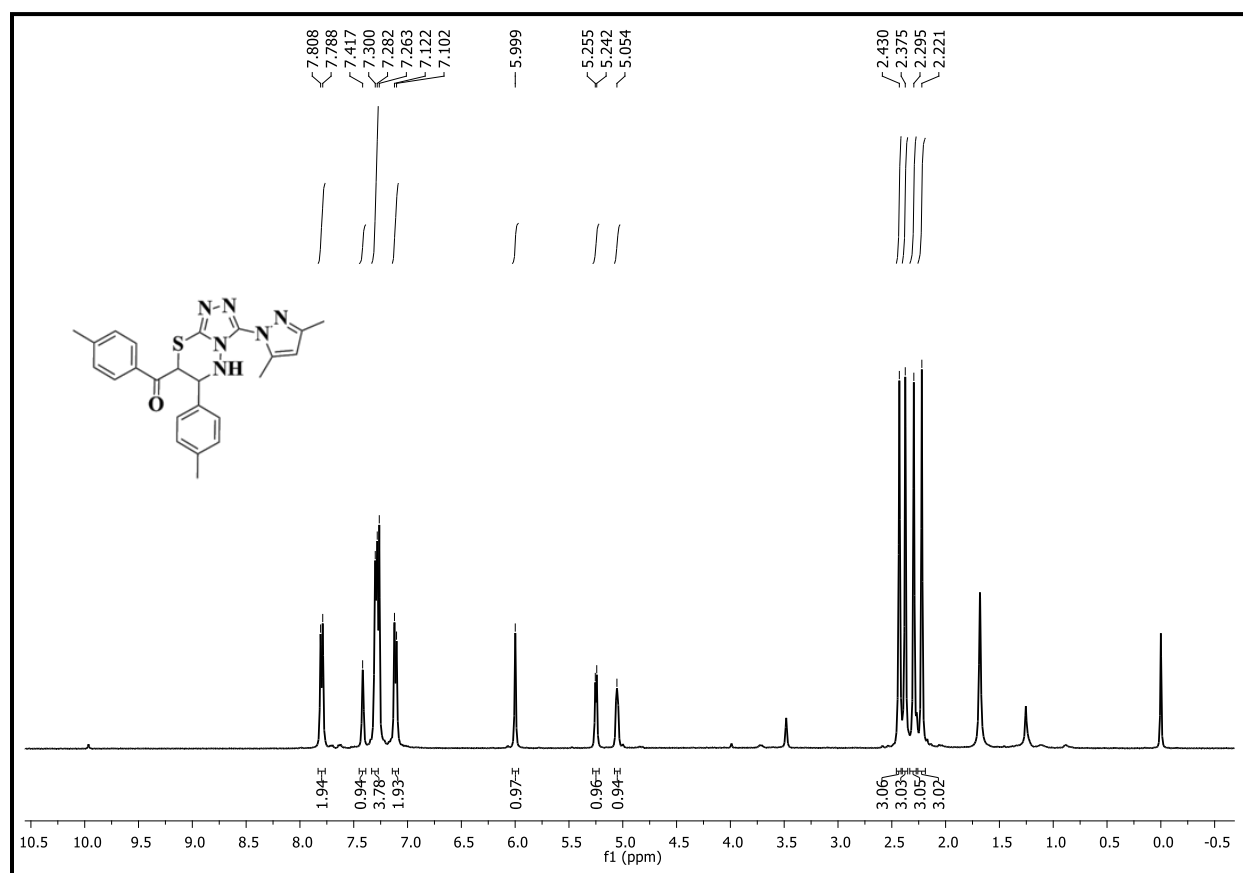


Fig: ^1H -NMR Spectrum of Compound **34b** (400 MHz, CDCl_3)

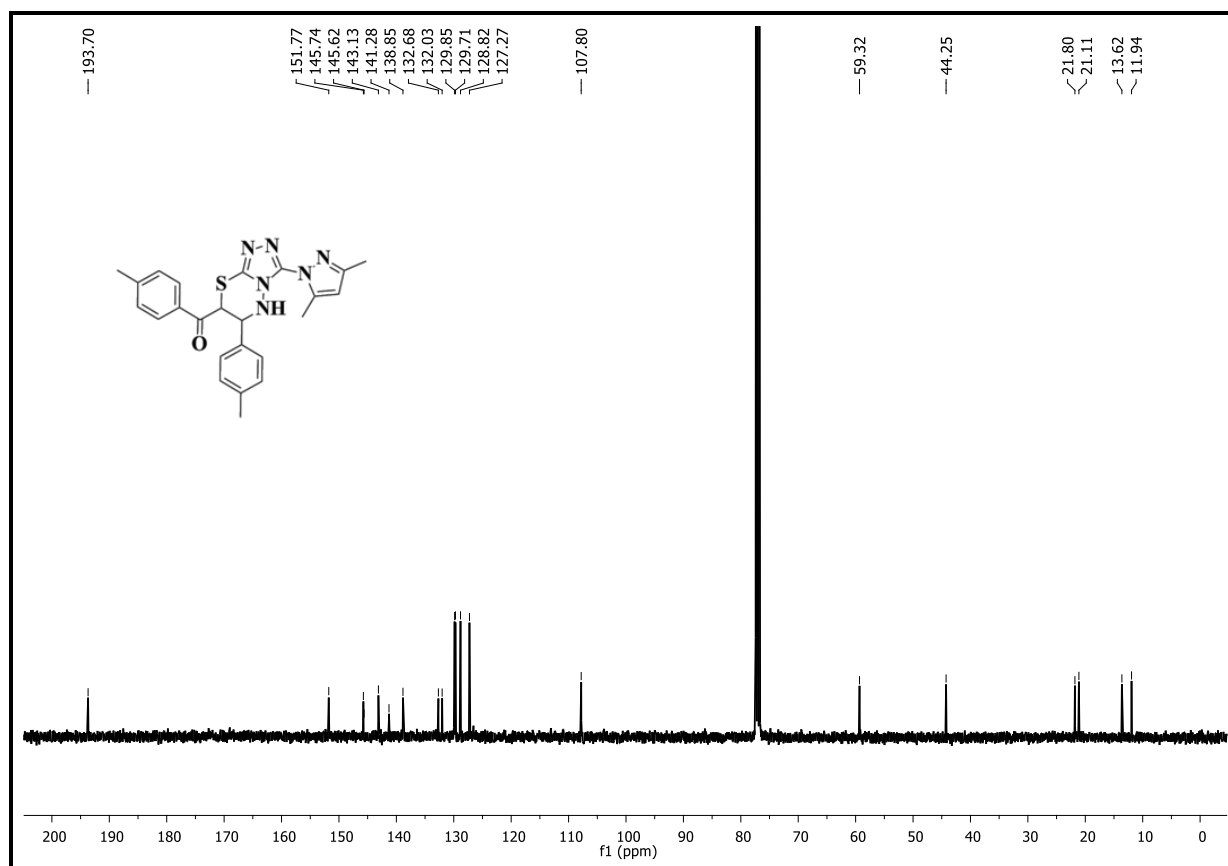


Fig: ¹³C-NMR Spectrum of Compound **34b** (100 MHz, CDCl₃)

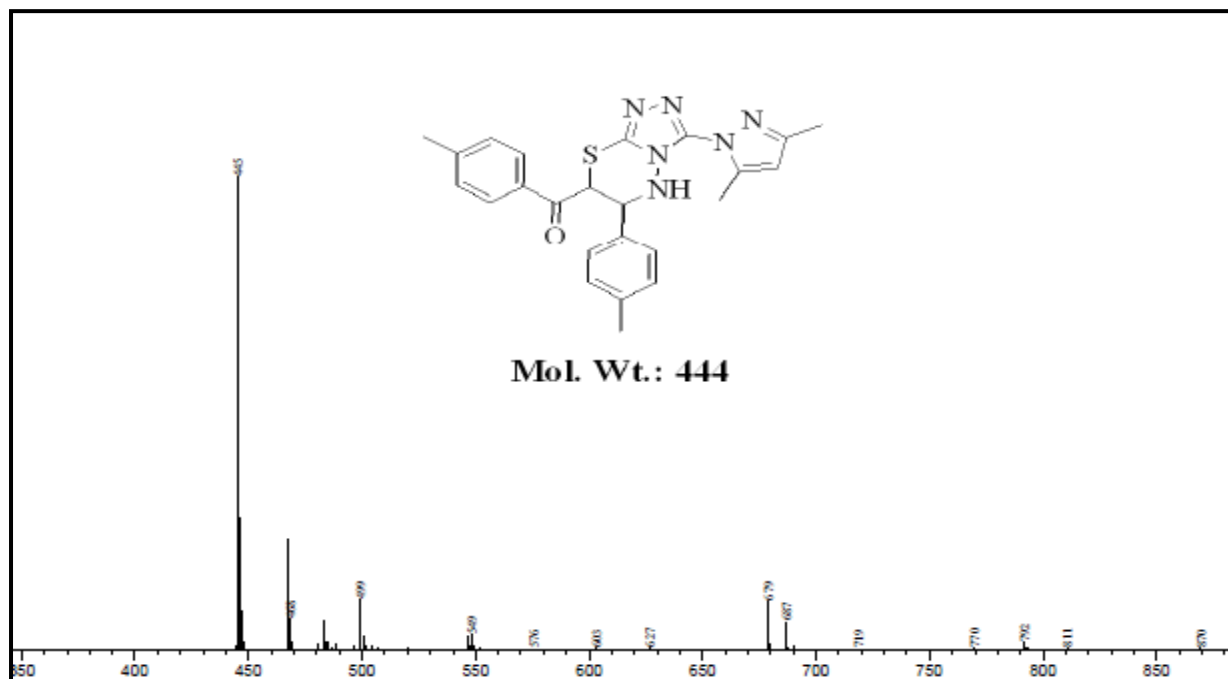


Fig: Mass Spectrum of Compound **34b**

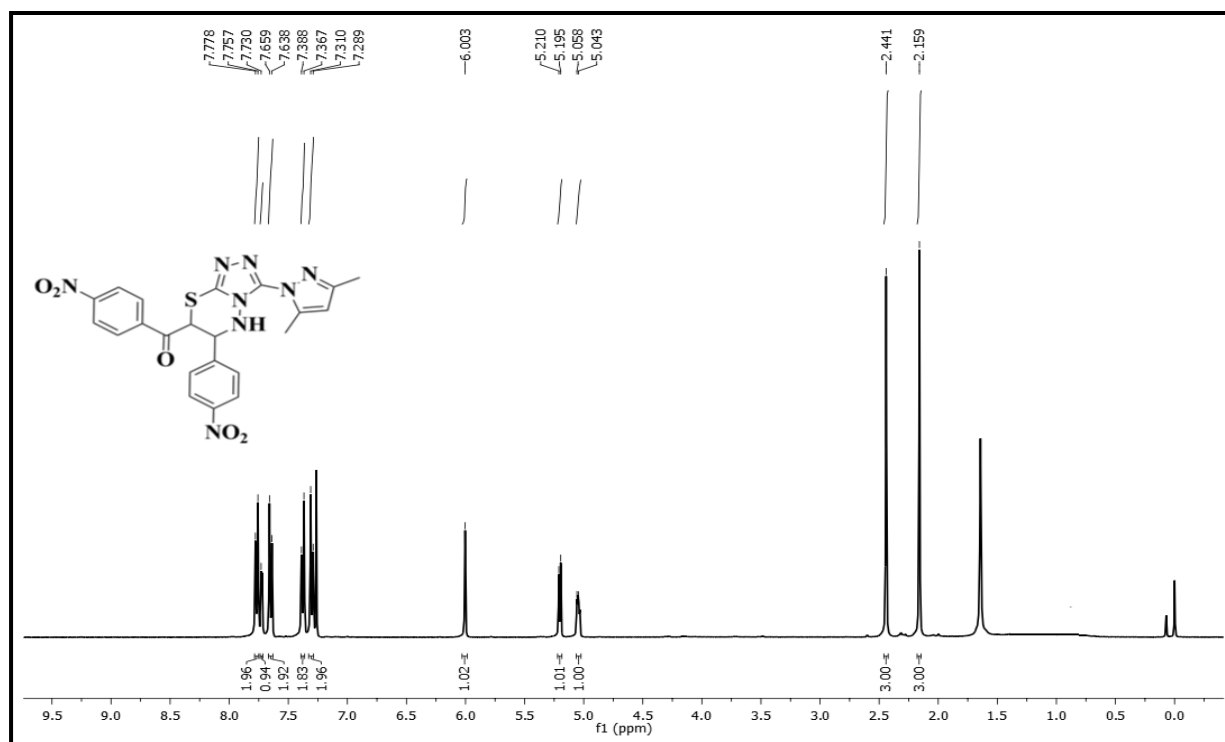


Fig: ¹H-NMR Spectrum of Compound **34c** (400 MHz, CDCl₃)

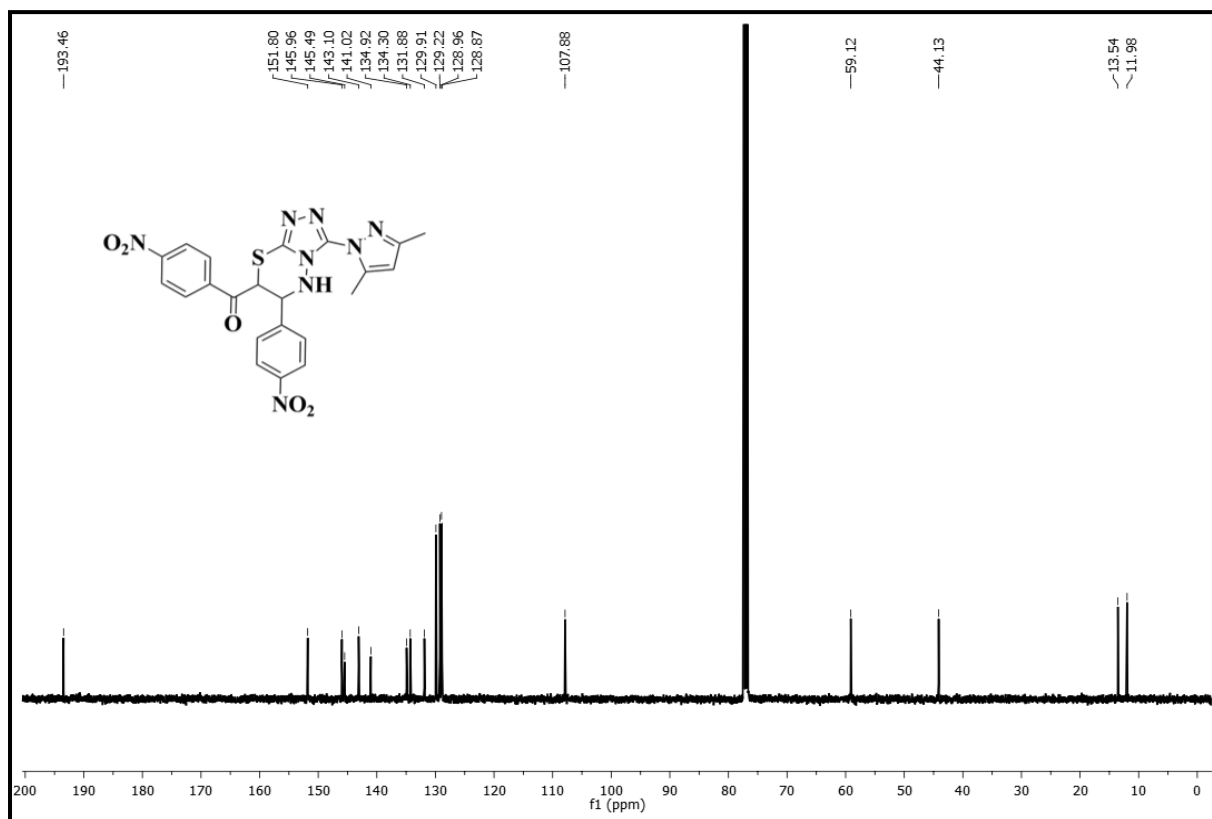


Fig: ¹³C-NMR Spectrum of Compound **34c** (100 MHz, CDCl₃)

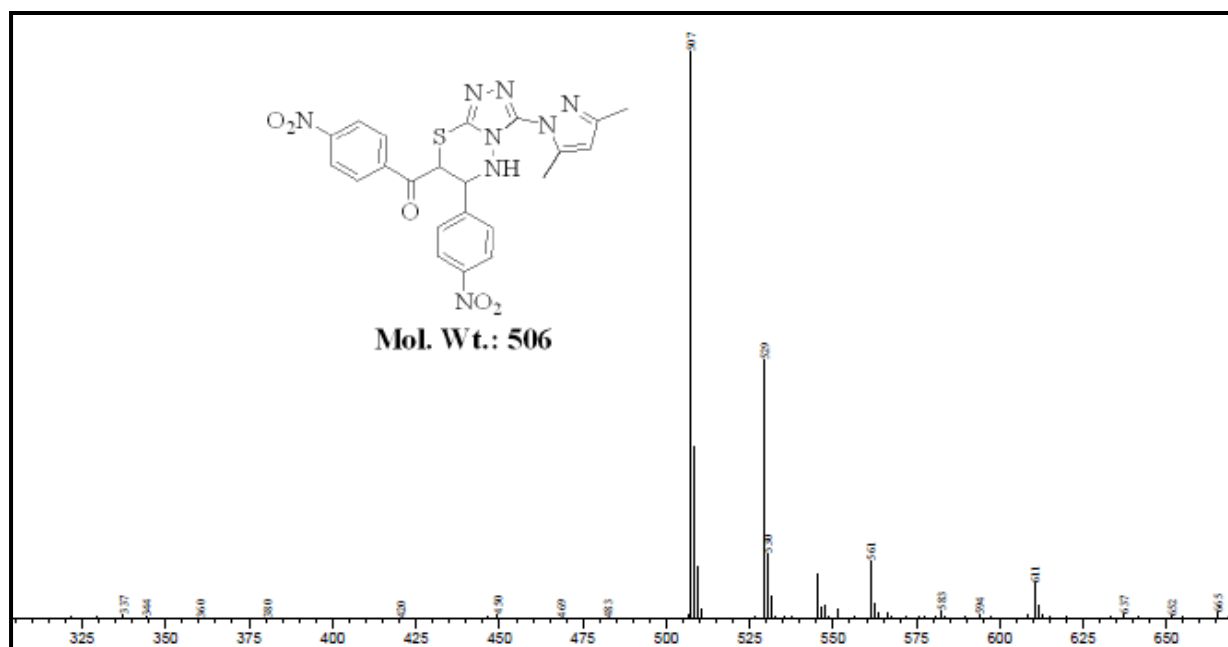


Fig: Mass Spectrum of Compound **34c**

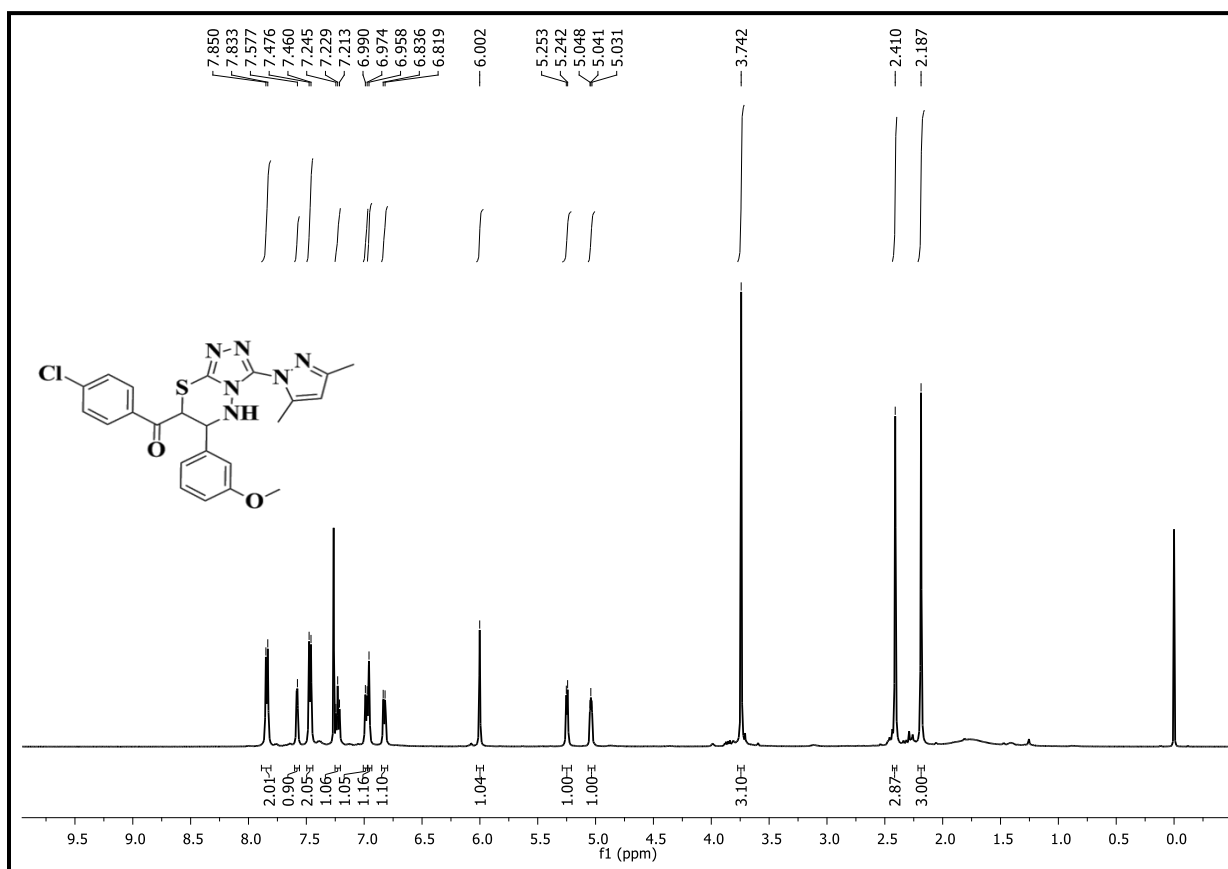


Fig: ¹H-NMR Spectrum of Compound **34d** (400 MHz, CDCl₃)

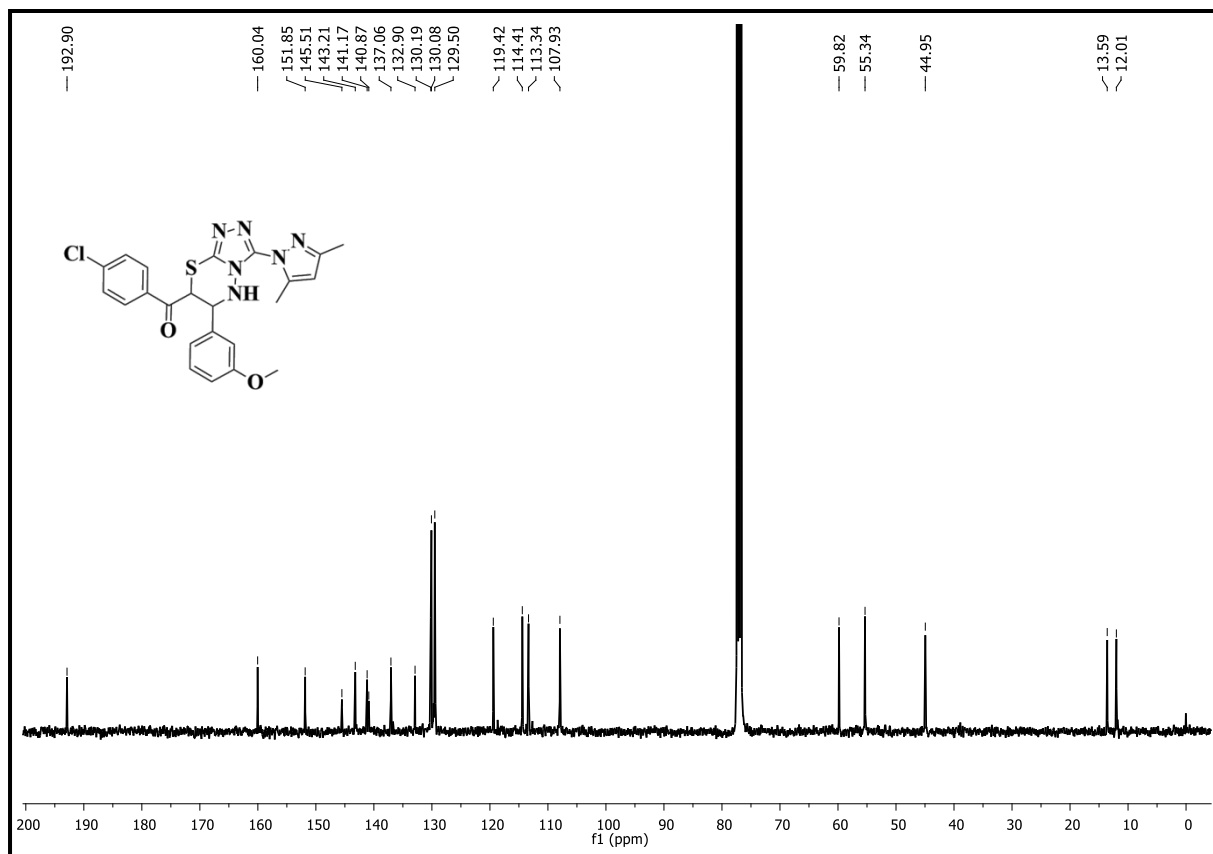


Fig: ¹³C-NMR Spectrum of Compound **34d** (100 MHz, CDCl₃)

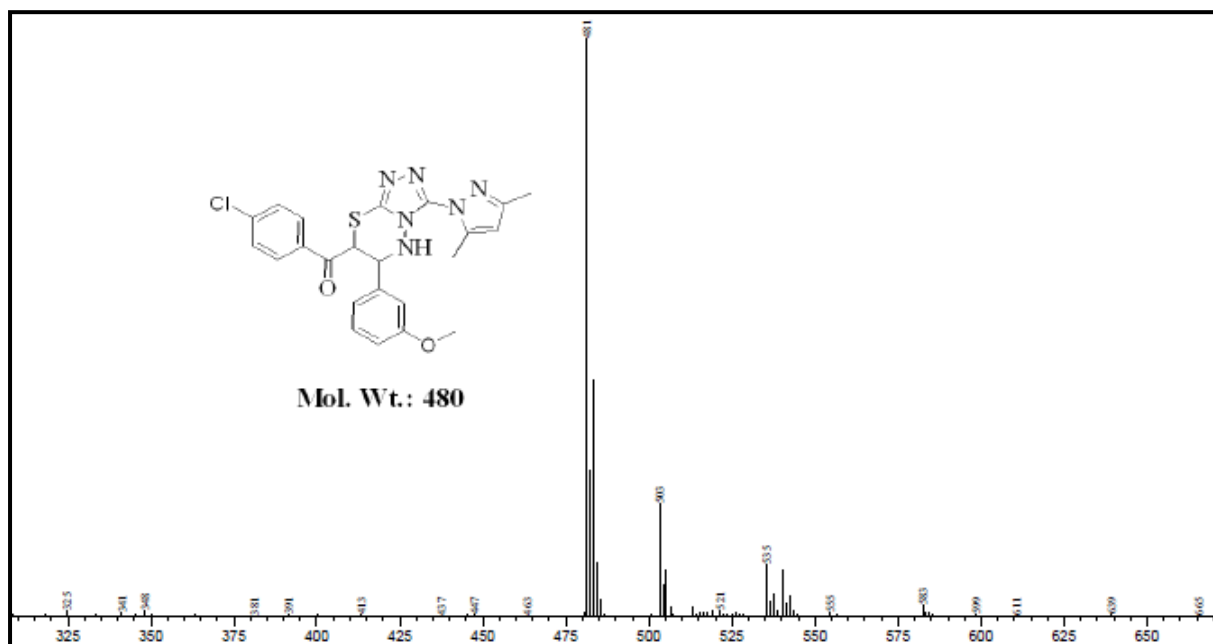


Fig: Mass Spectrum of Compound **34d**

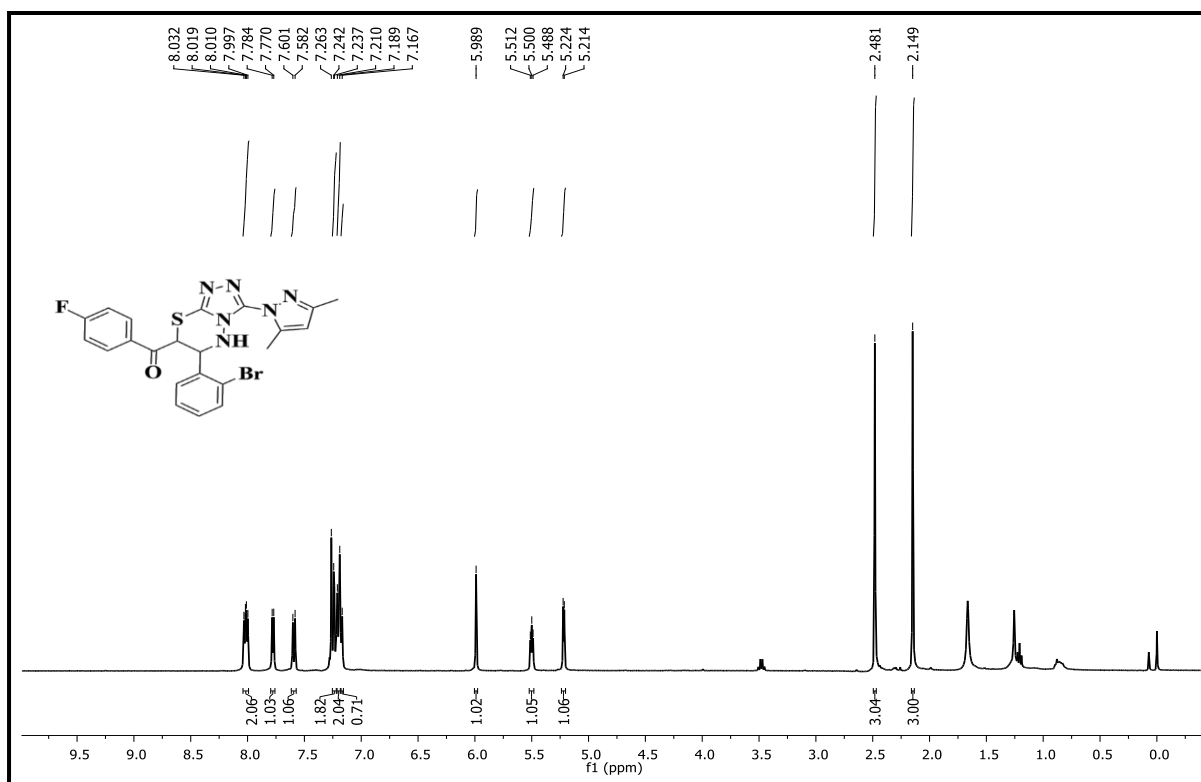


Fig: ¹H-NMR Spectrum of Compound 34e (400 MHz, CDCl₃)

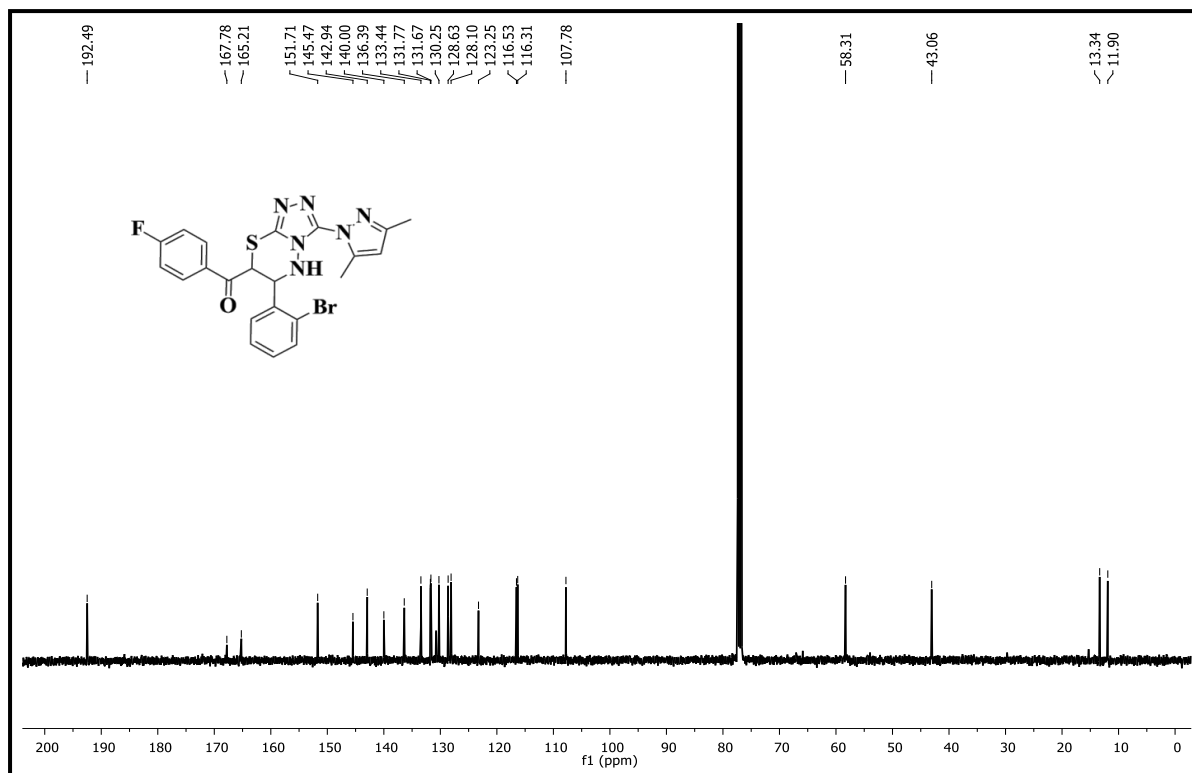


Fig: ¹³C-NMR Spectrum of Compound 34e (100 MHz, CDCl₃)

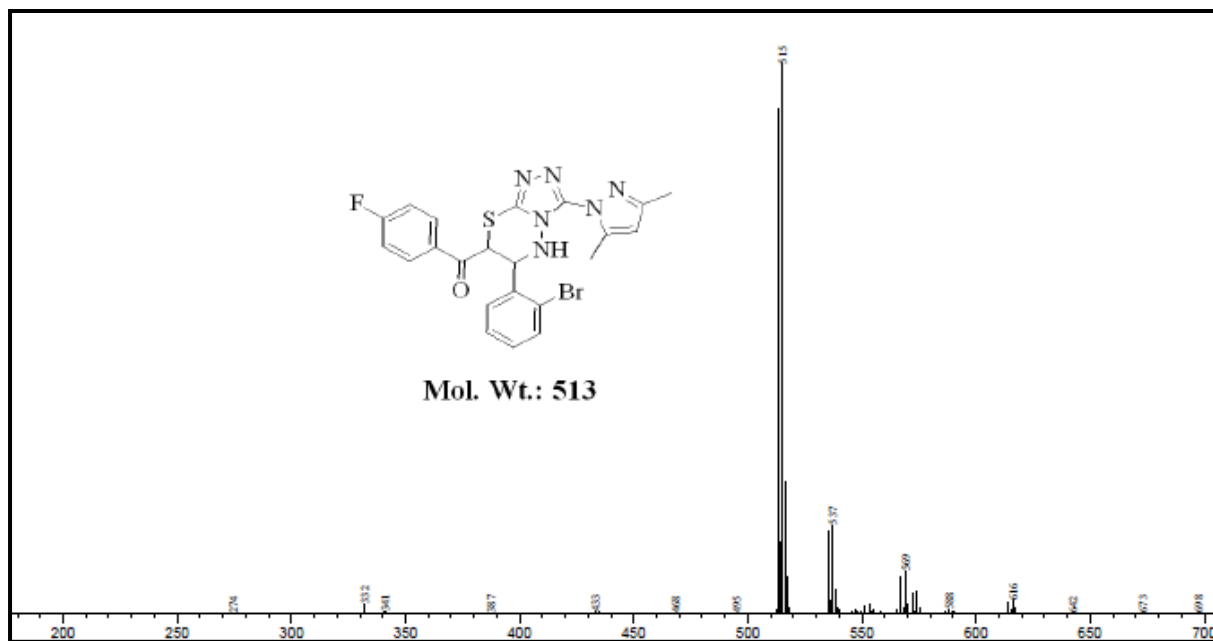


Fig: Mass Spectrum of Compound 34e

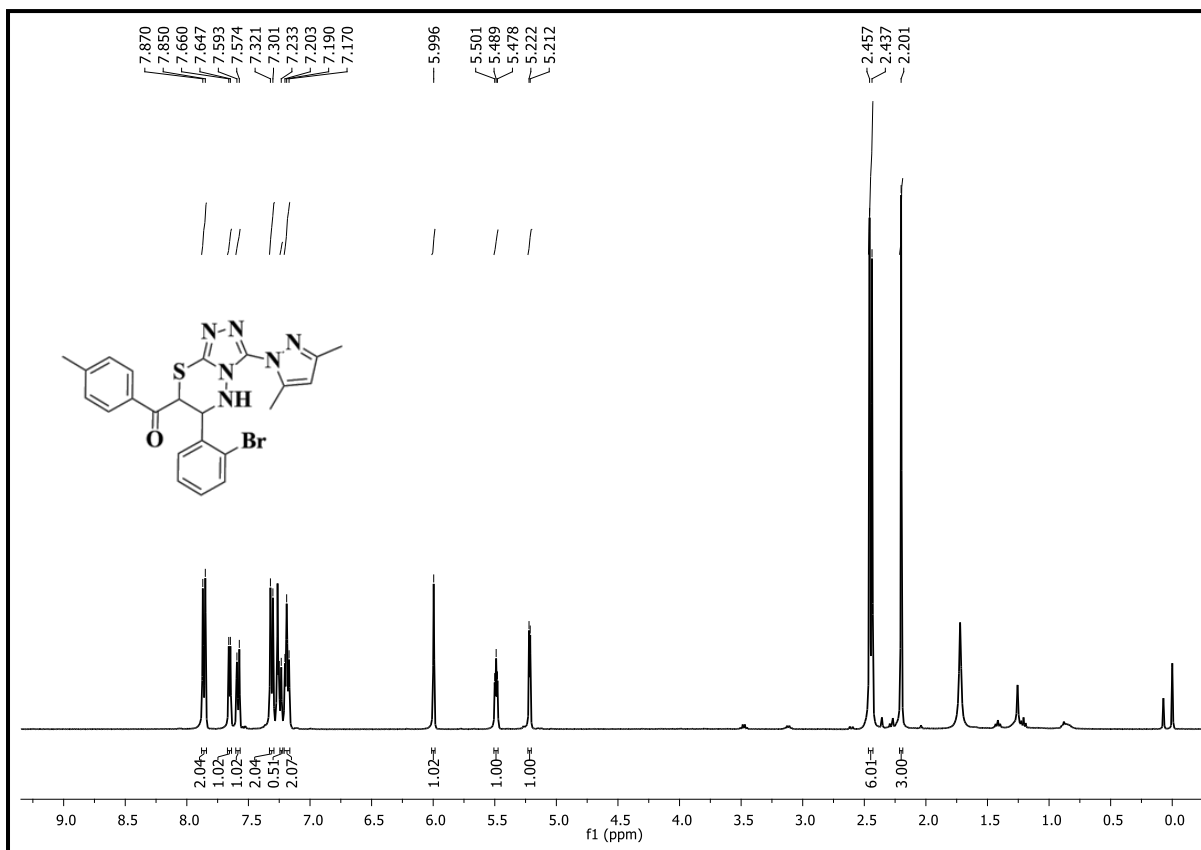


Fig: ^1H -NMR Spectrum of Compound 34f (400 MHz, CDCl_3)

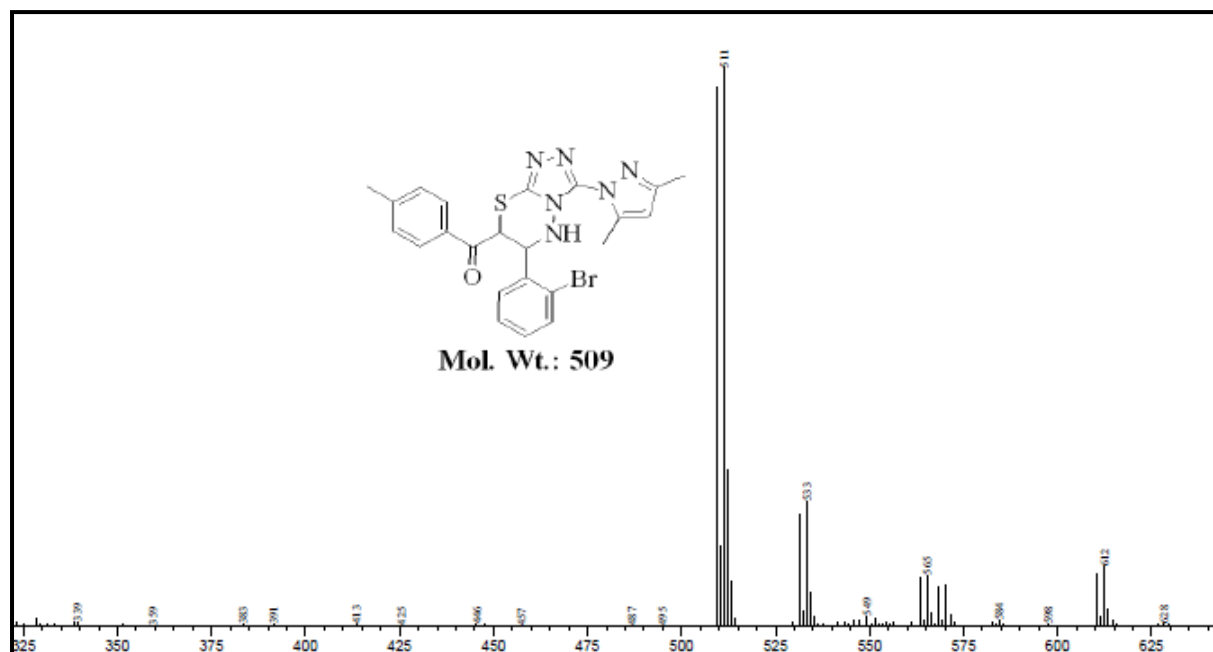


Fig: Mass Spectrum of Compound **34f**

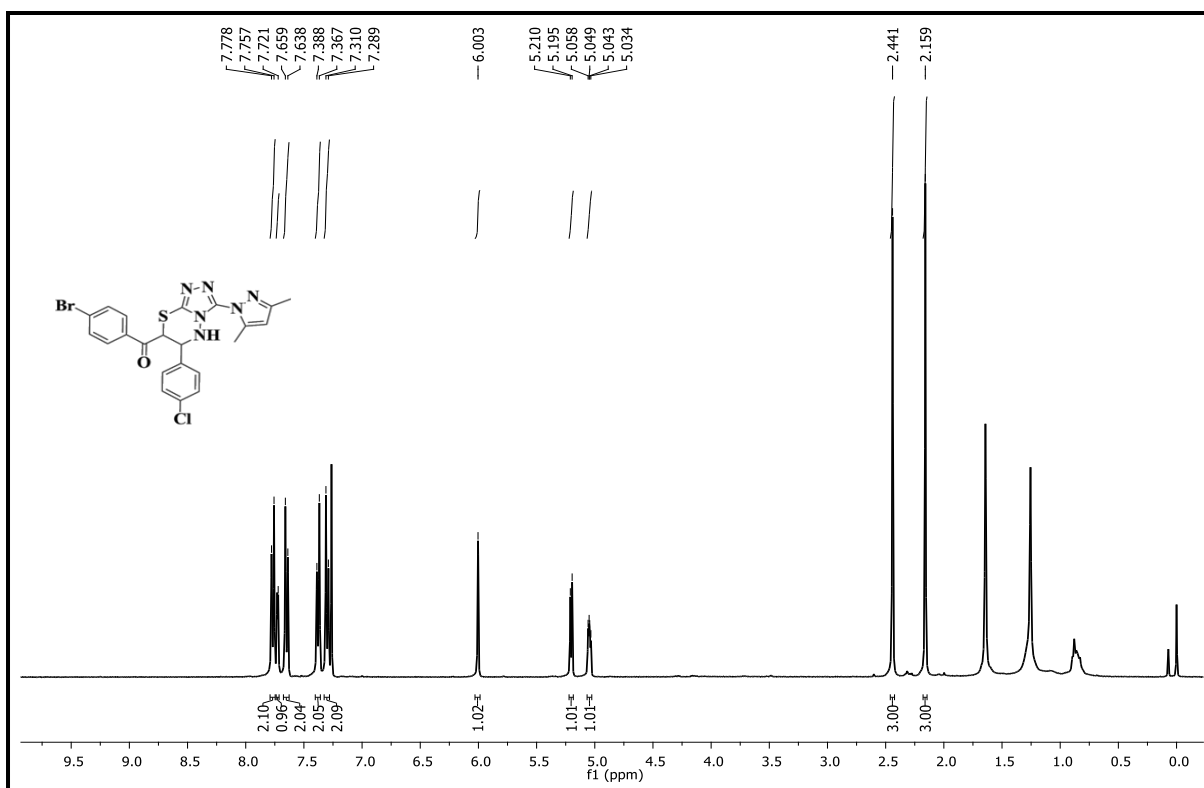


Fig: ¹H-NMR Spectrum of Compound 34g (400 MHz, CDCl₃)

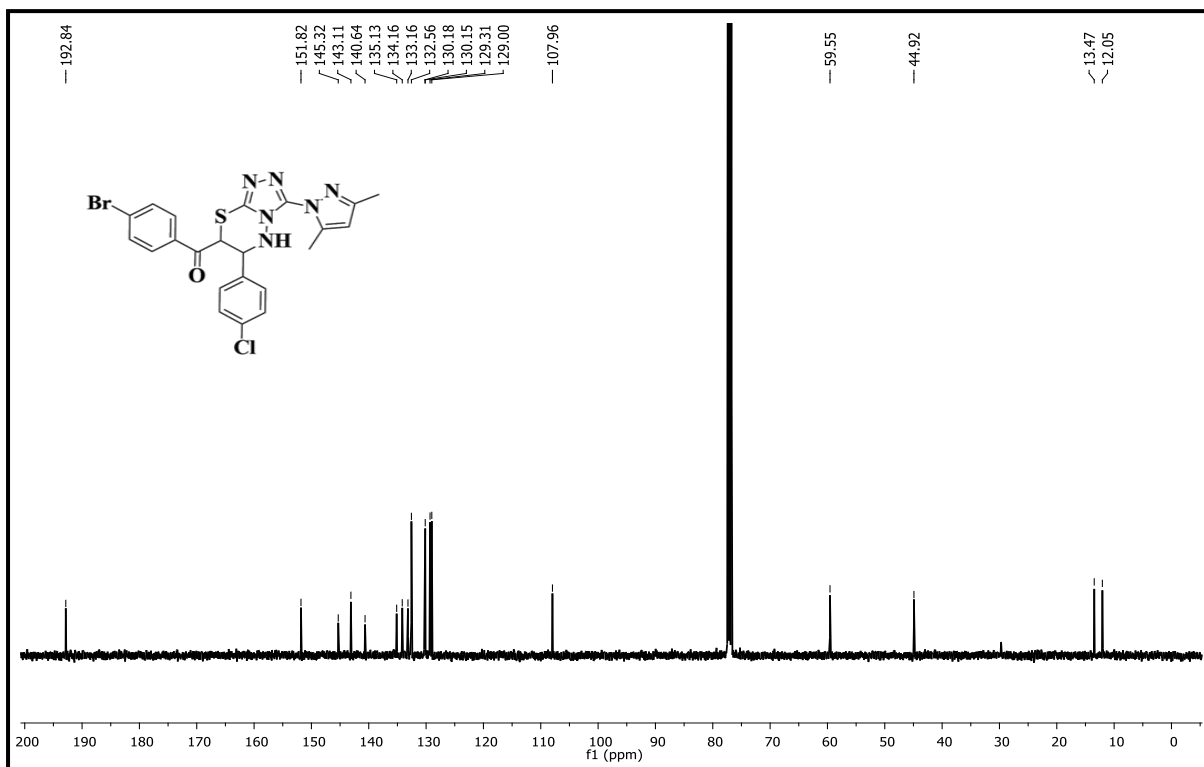


Fig: ¹³C-NMR Spectrum of Compound 34g (100 MHz, CDCl₃)

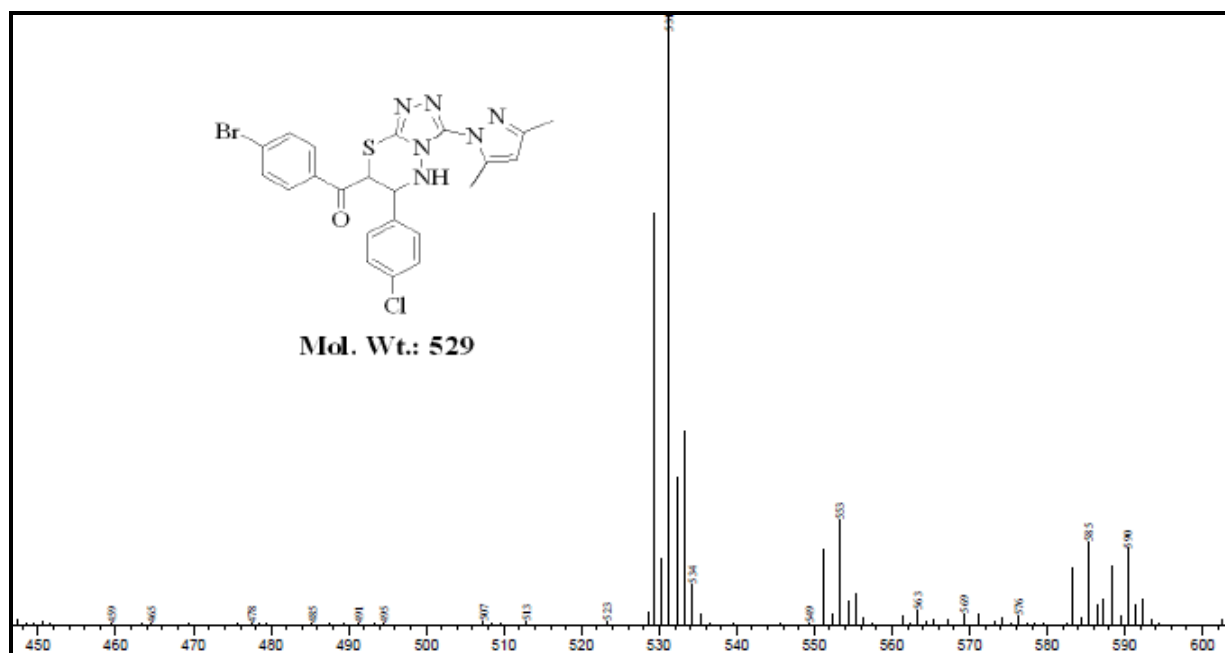


Fig: Mass Spectrum of Compound 34g

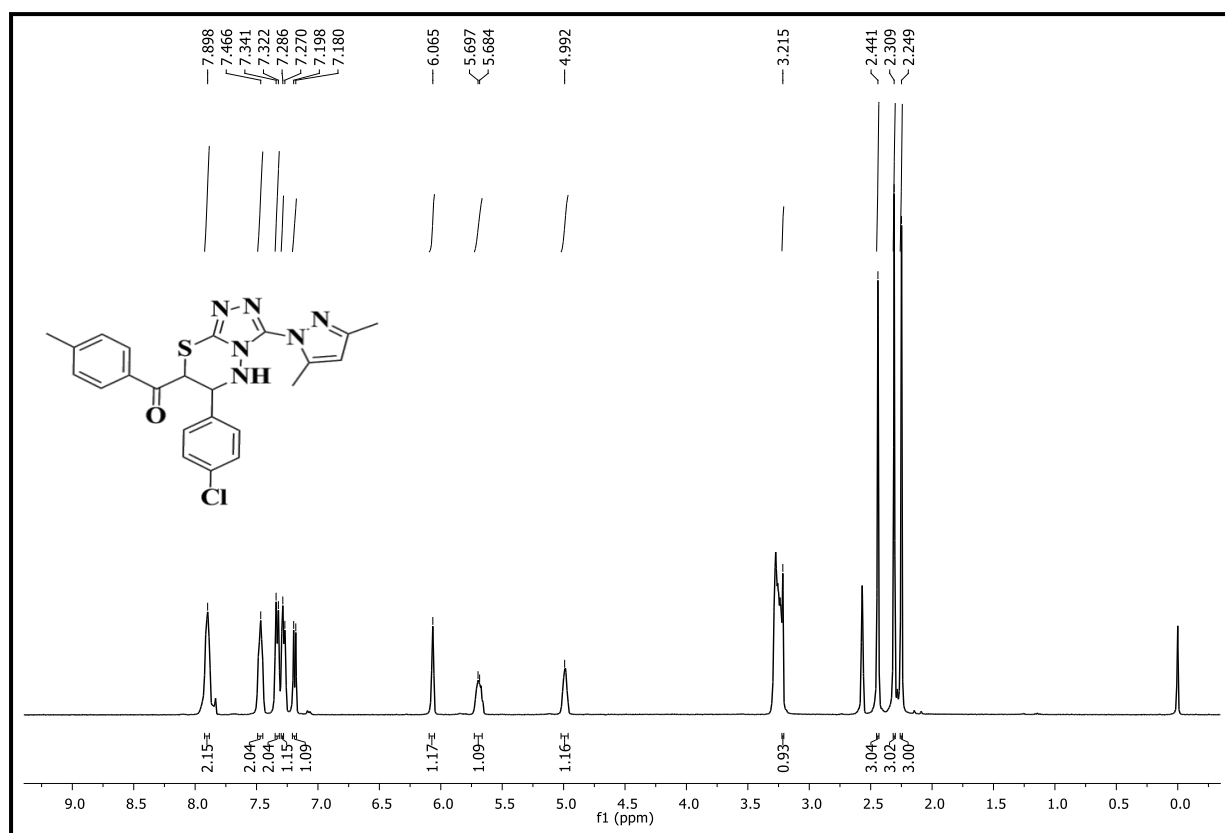


Fig: ^1H -NMR Spectrum of Compound 34h (400 MHz, $\text{CDCl}_3 + \text{DMSO}-d_6$)

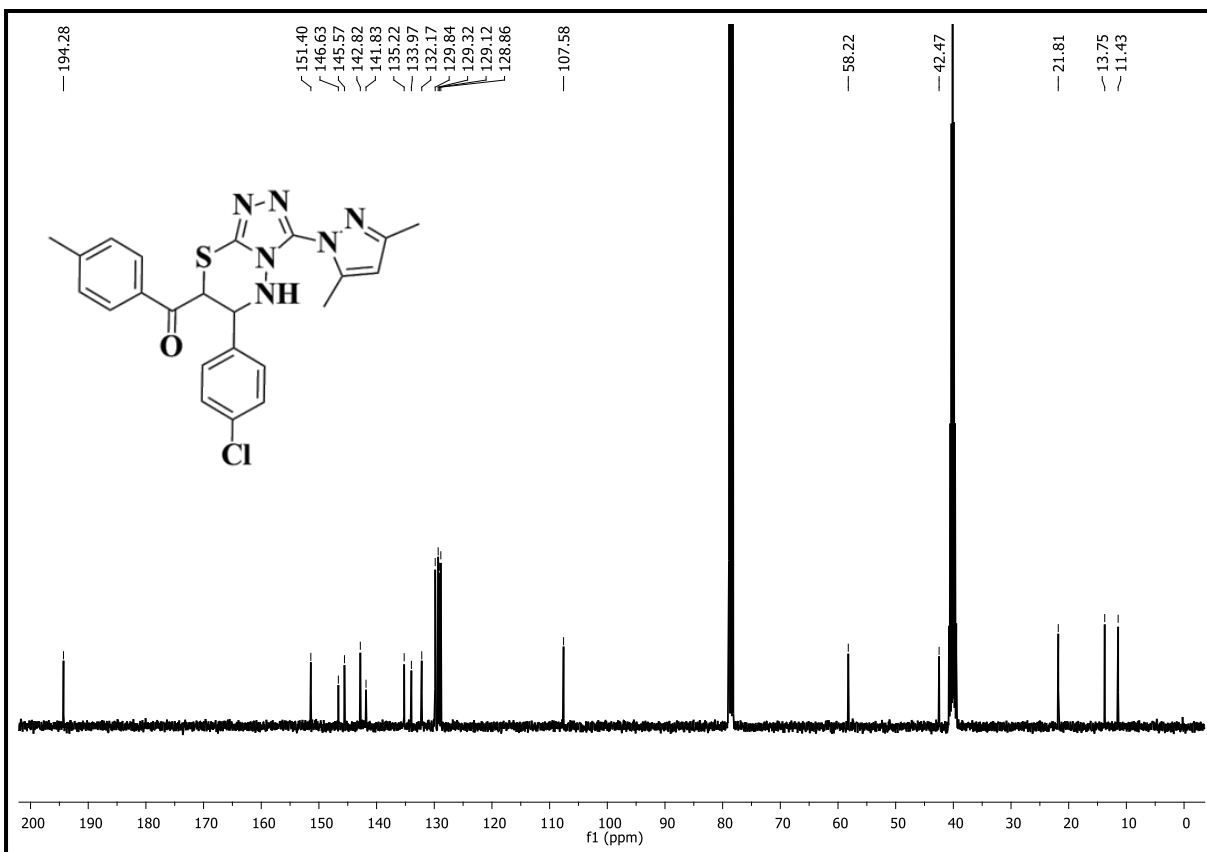


Fig: ¹³C-NMR Spectrum of Compound **34h** (100 MHz, CDCl₃+DMSO-*d*₆)

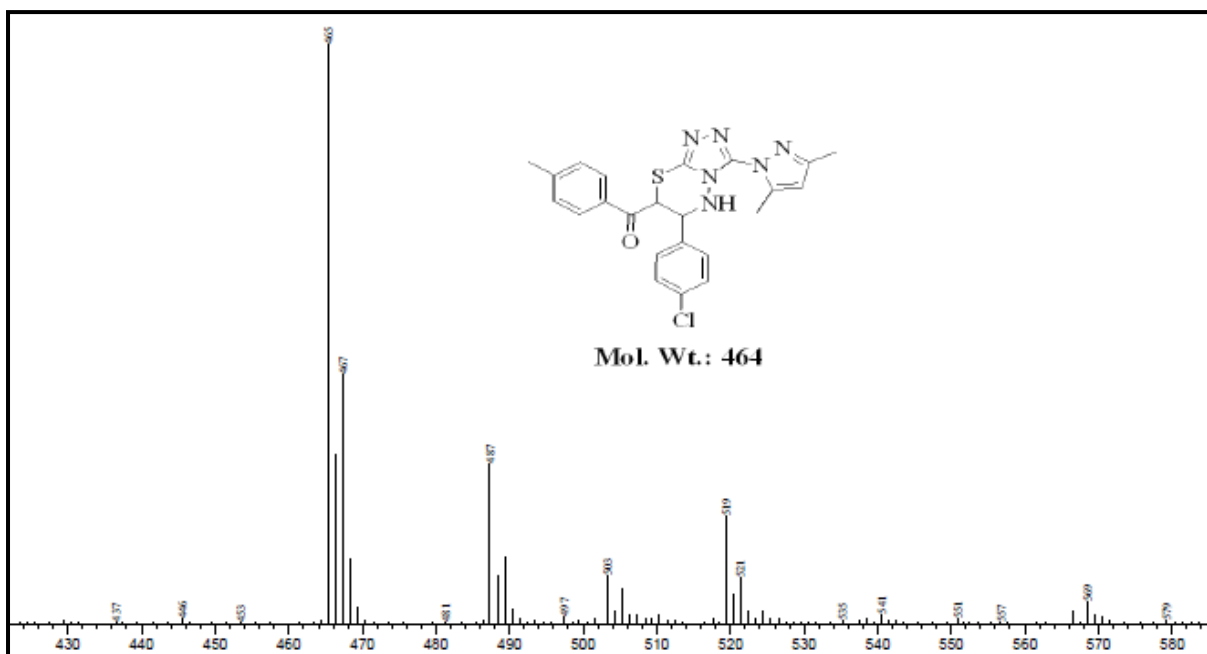


Fig: Mass Spectrum of Compound **34h**

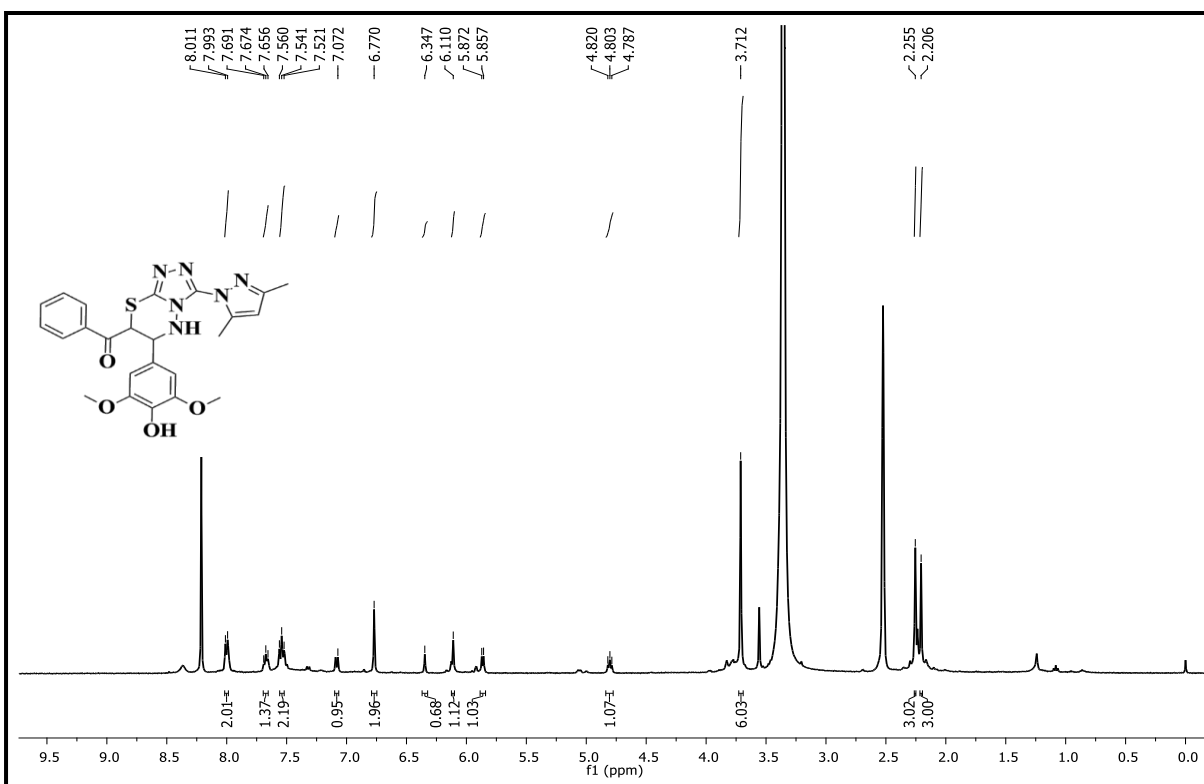


Fig: ¹H-NMR Spectrum of Compound **34i** (400 MHz, CDCl₃+DMSO-*d*₆)

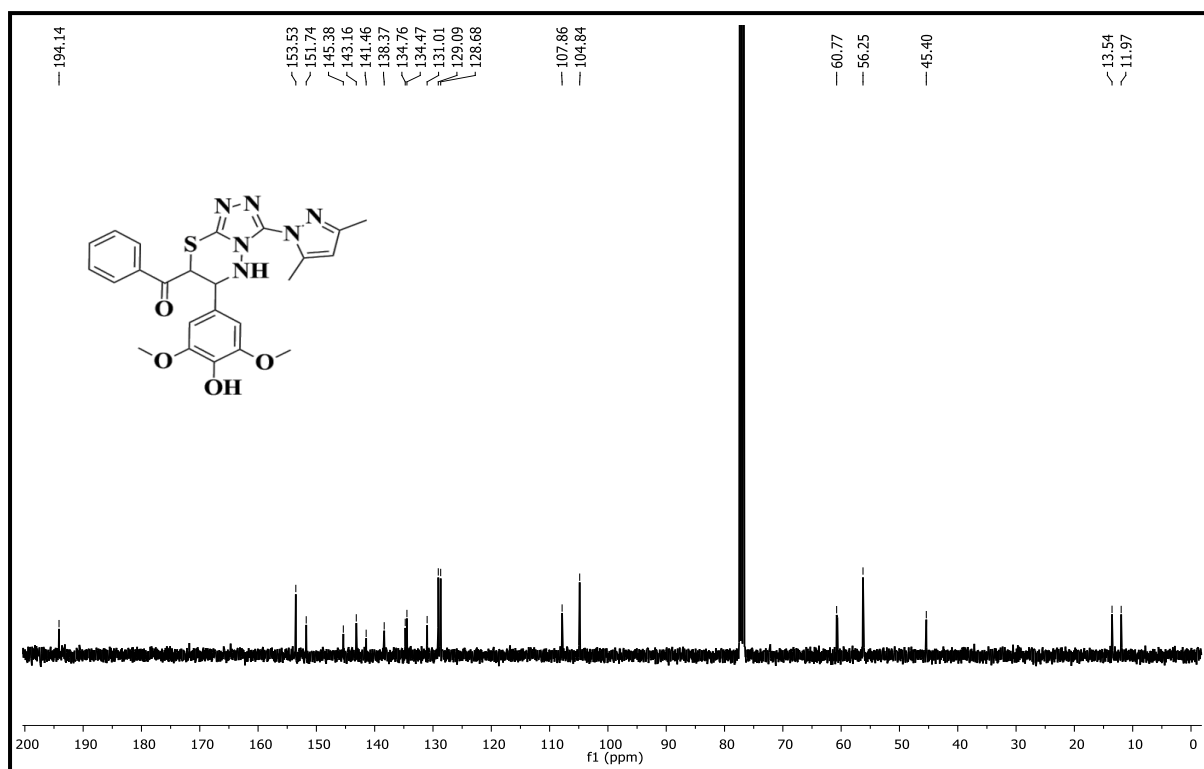


Fig: ¹³C-NMR Spectrum of Compound **34i** (100 MHz, CDCl₃)

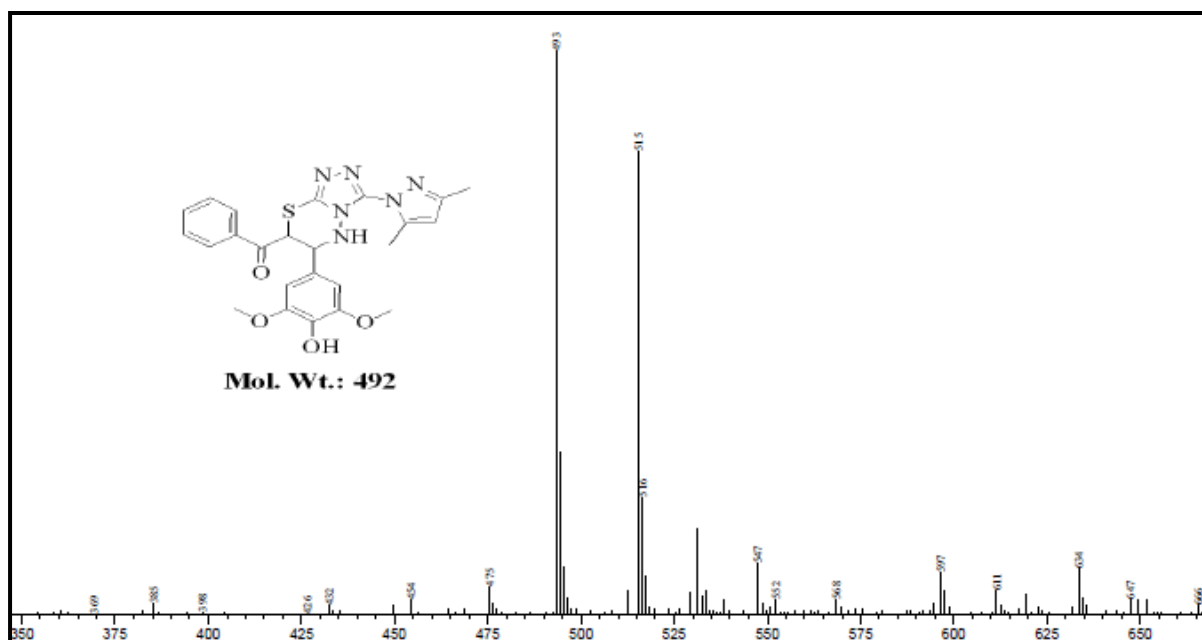


Fig: Mass Spectrum of Compound **34i**

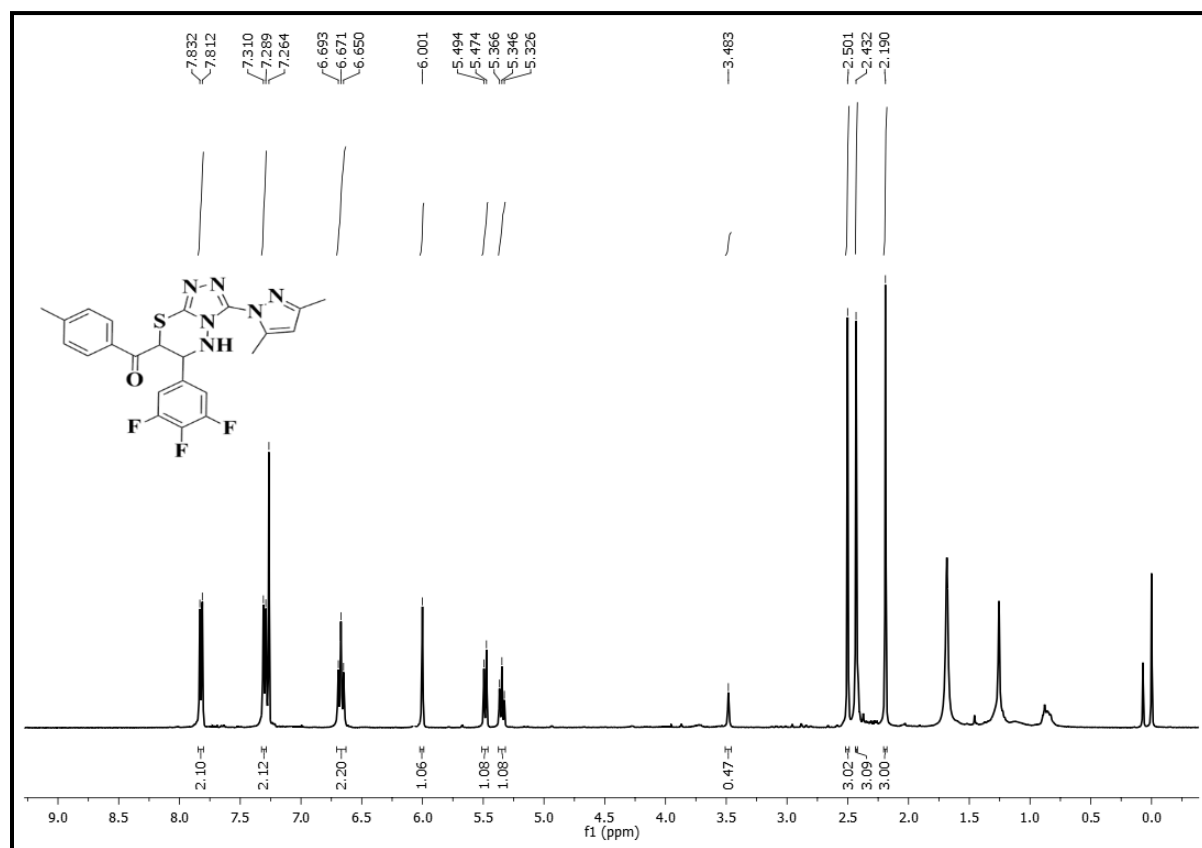


Fig: ^1H -NMR Spectrum of Compound **34j** (400 MHz, CDCl_3)

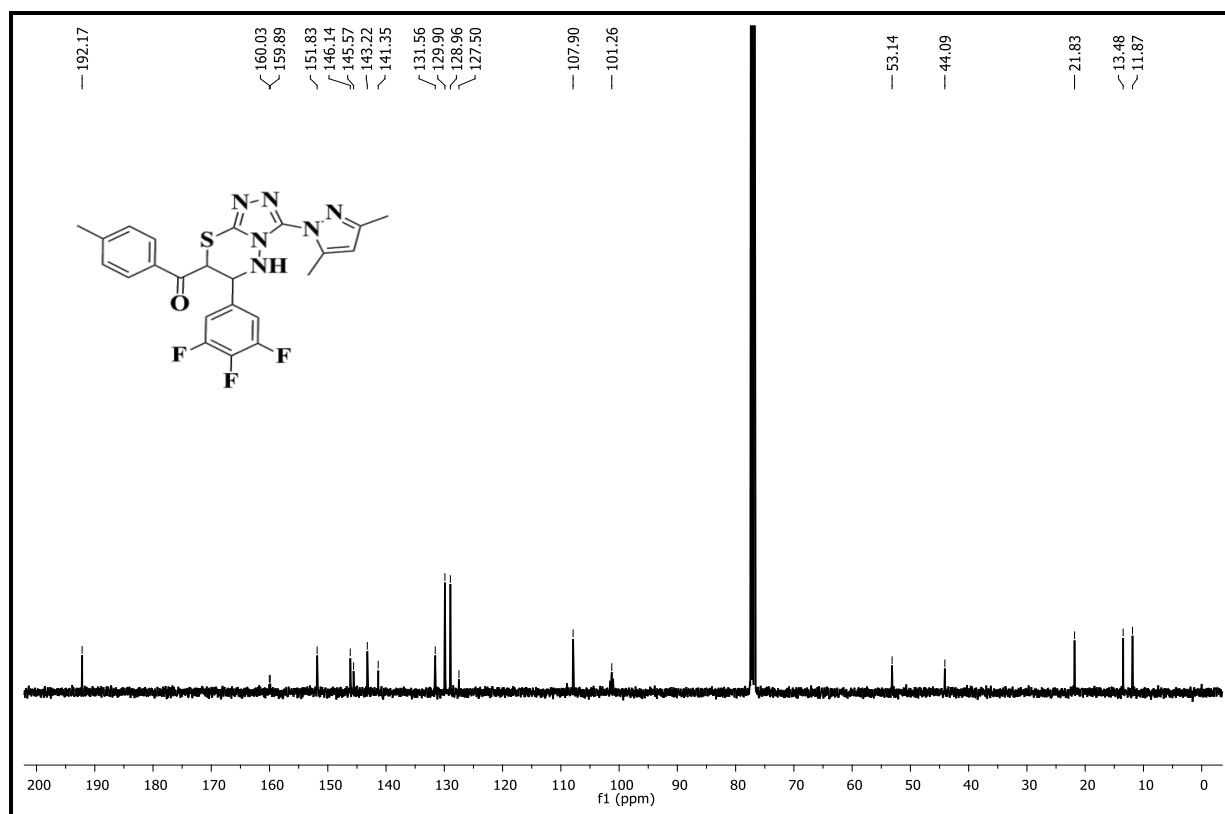


Fig: ¹³C-NMR Spectrum of Compound 34j (100 MHz, CDCl₃)

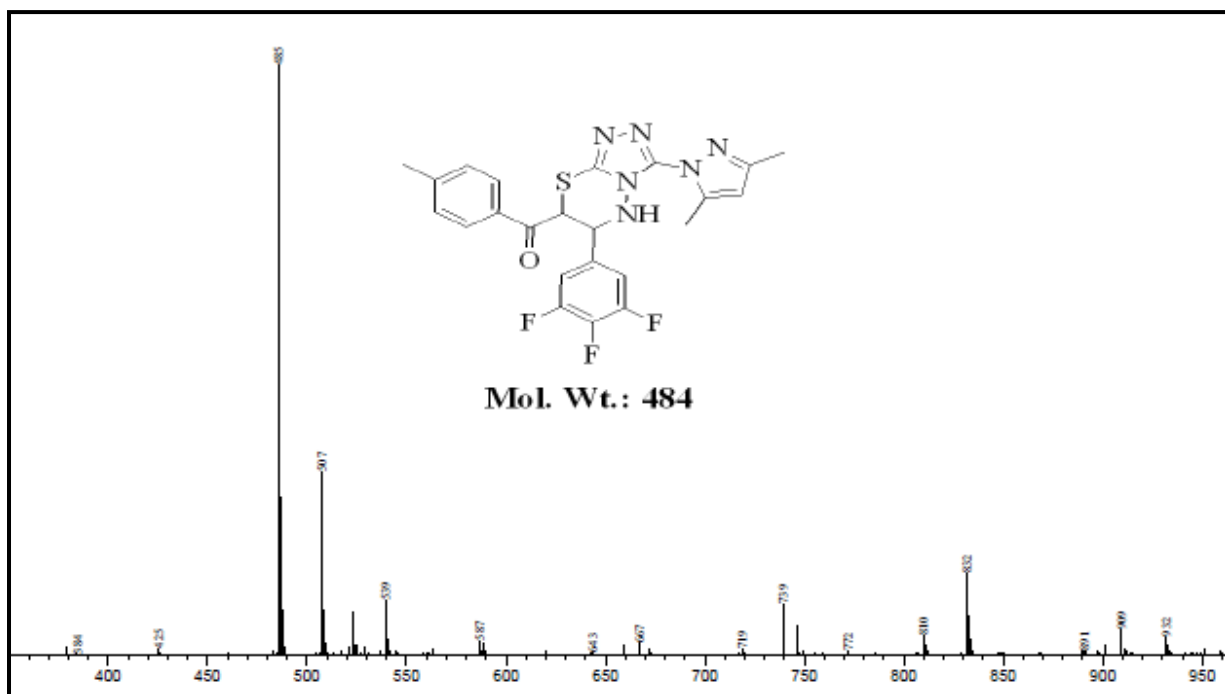


Fig: Mass Spectrum of Compound 34j

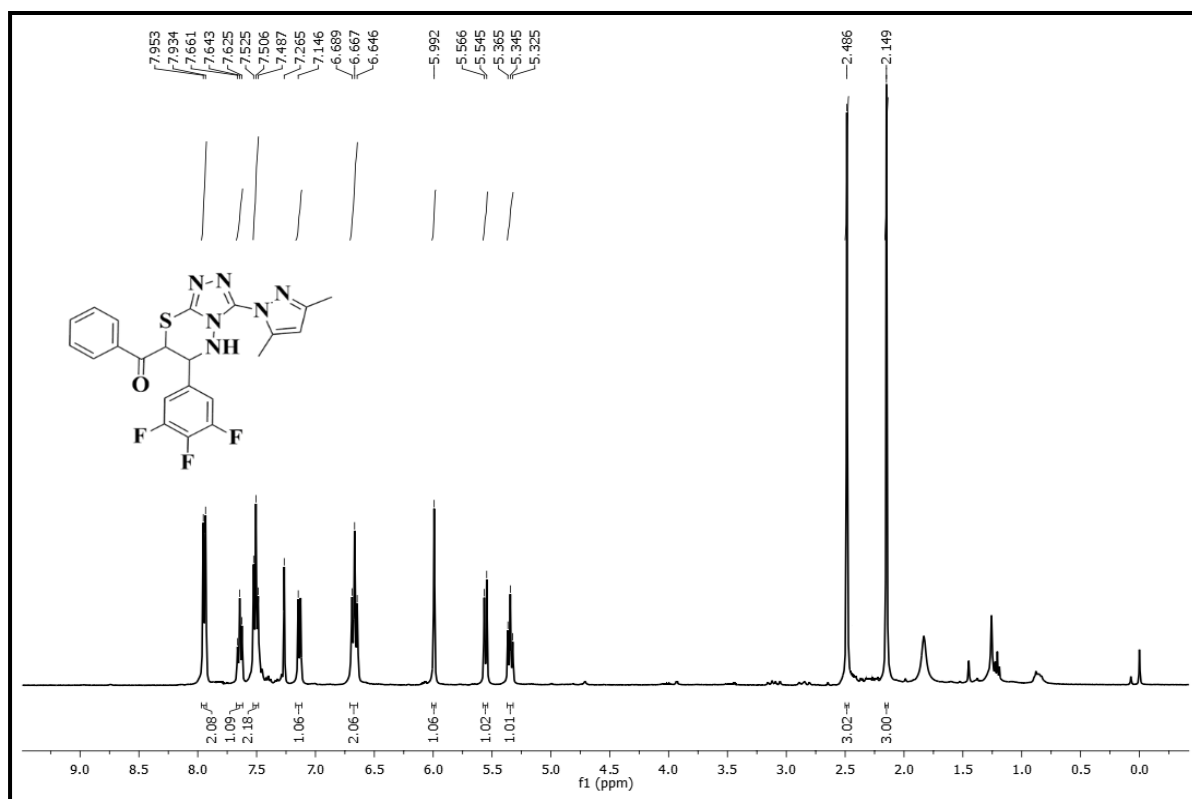


Fig: ¹H-NMR Spectrum of Compound **34k** (400 MHz, CDCl₃)

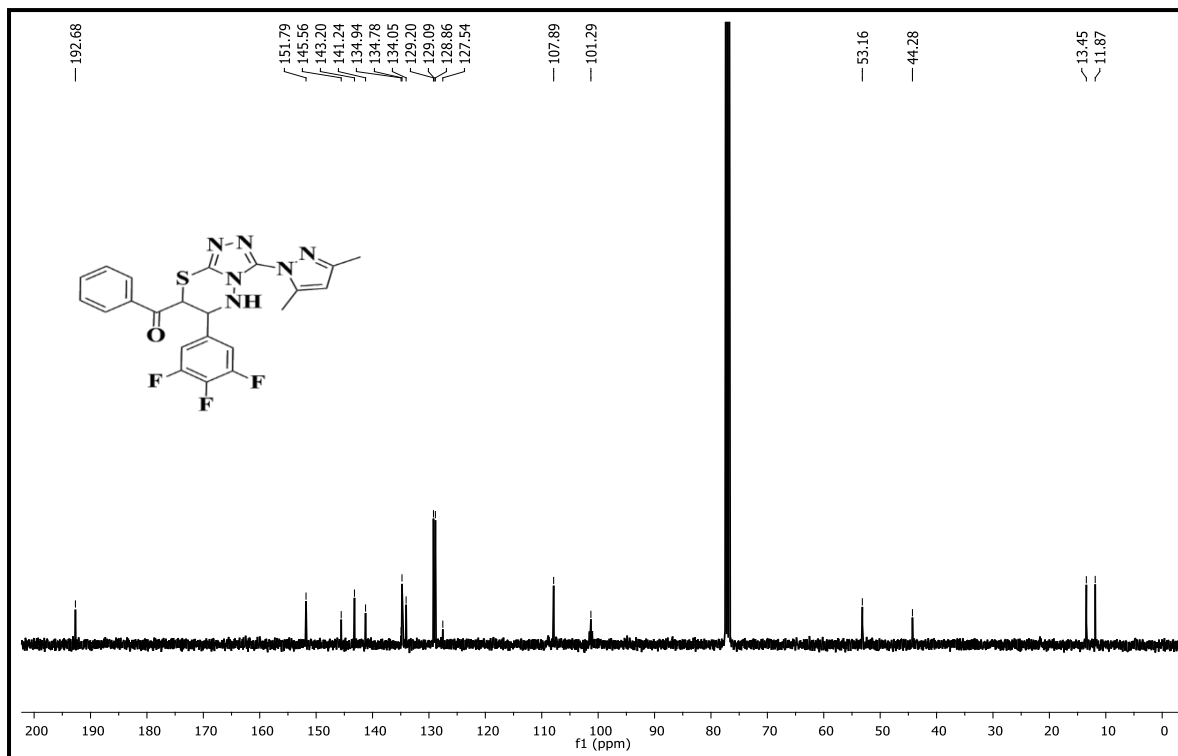


Fig: ¹³C-NMR Spectrum of Compound **34k** (100 MHz, CDCl₃)

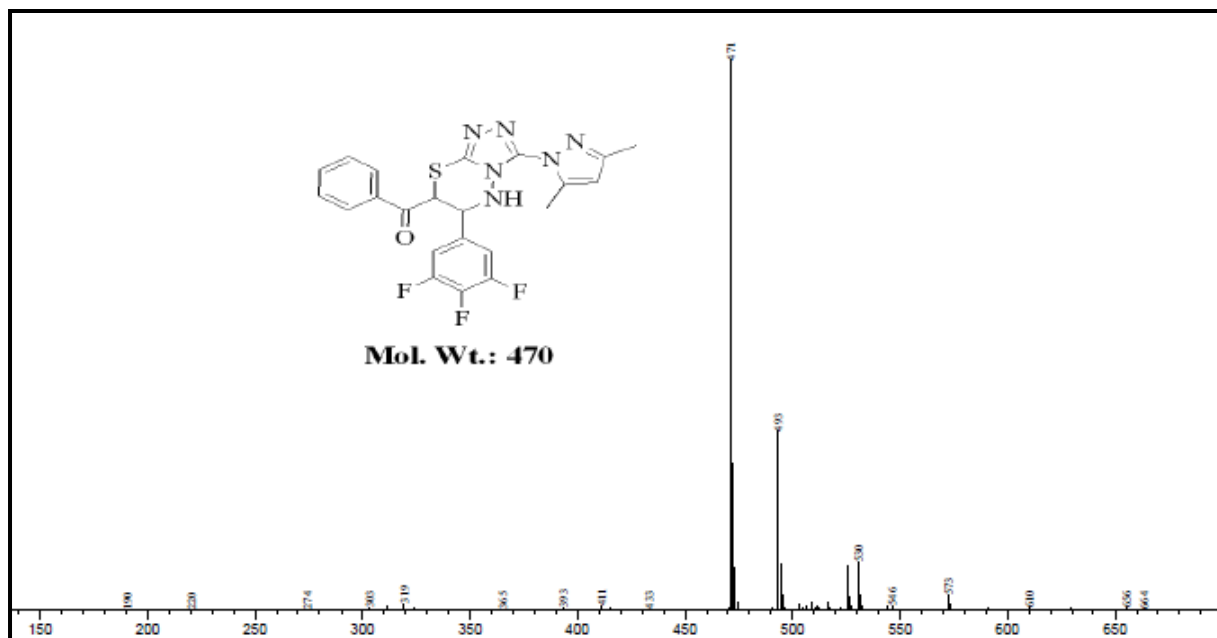


Fig: Mass Spectrum of Compound 34k

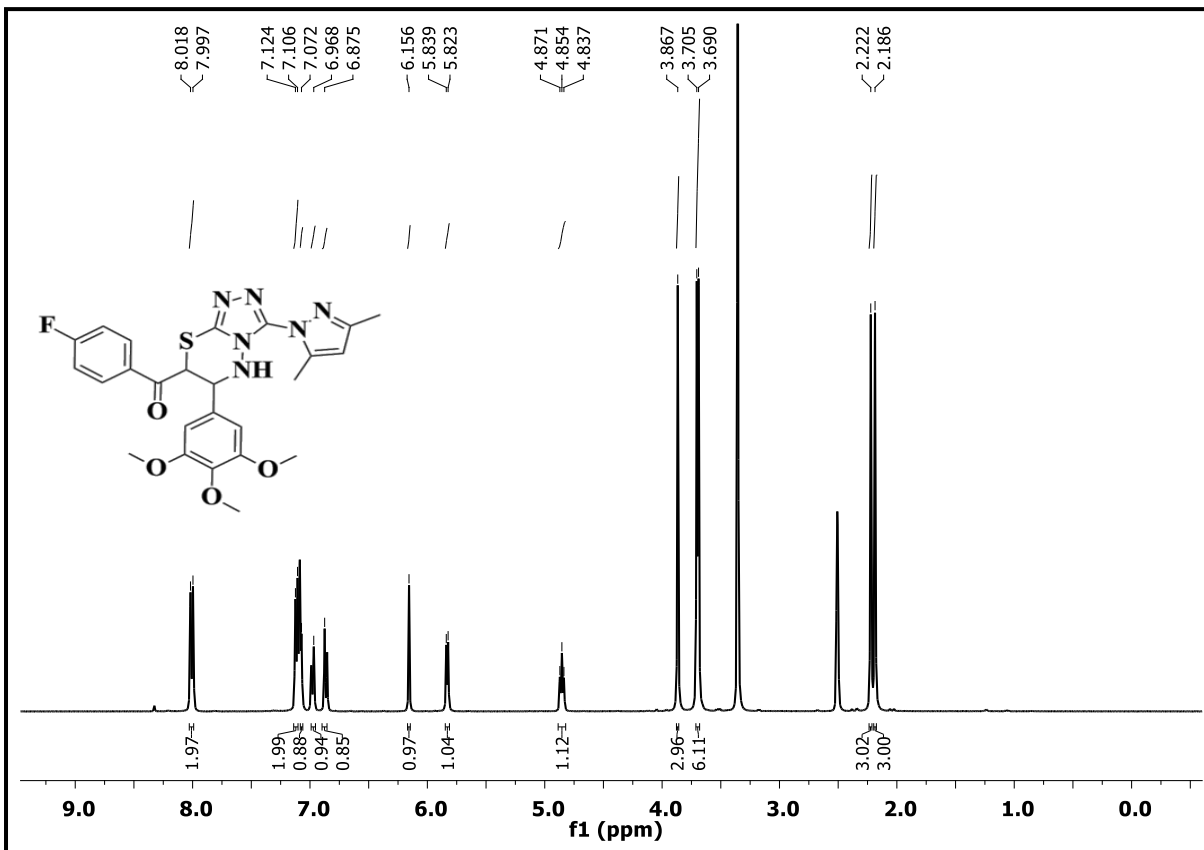


Fig: ^1H -NMR Spectrum of Compound 34l (400 MHz, $\text{CDCl}_3 + \text{DMSO}-d_6$)

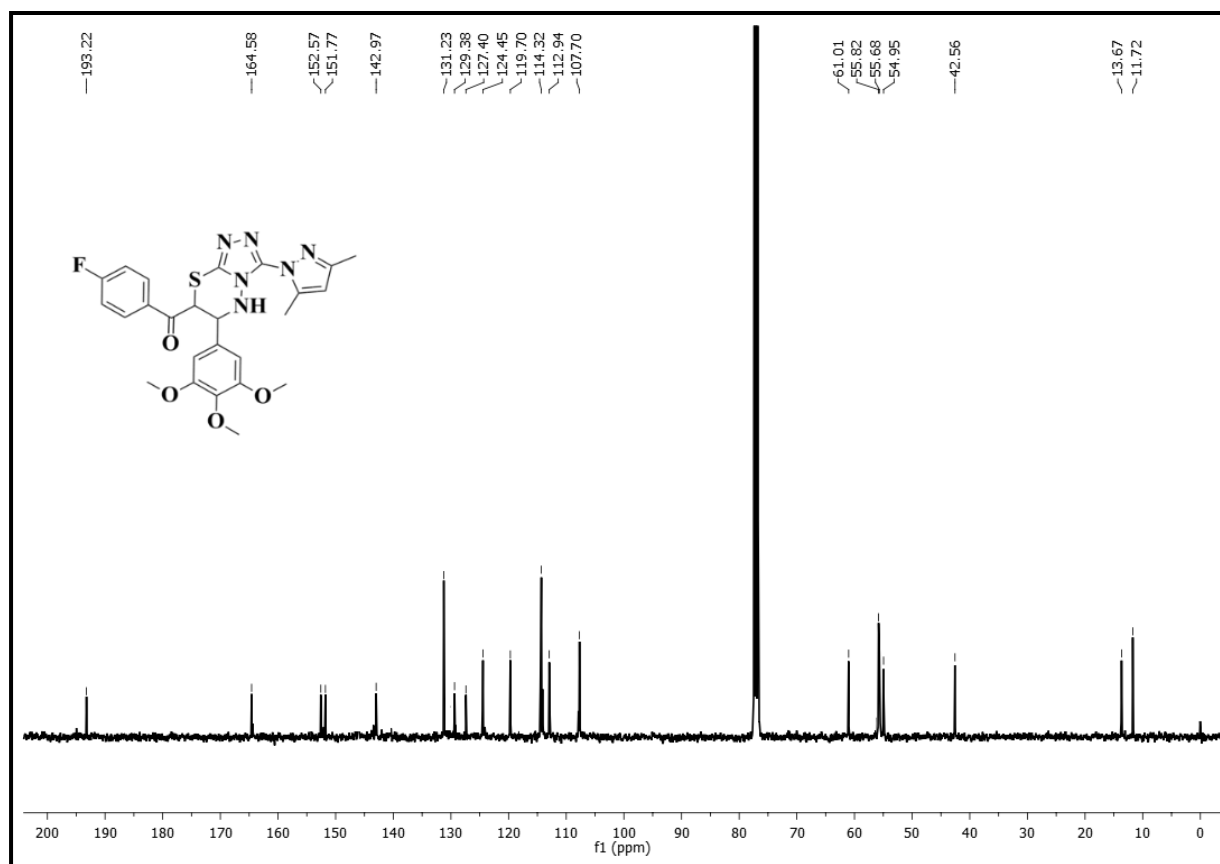


Fig: ¹³C-NMR Spectrum of Compound **34l** (100 MHz, CDCl₃)

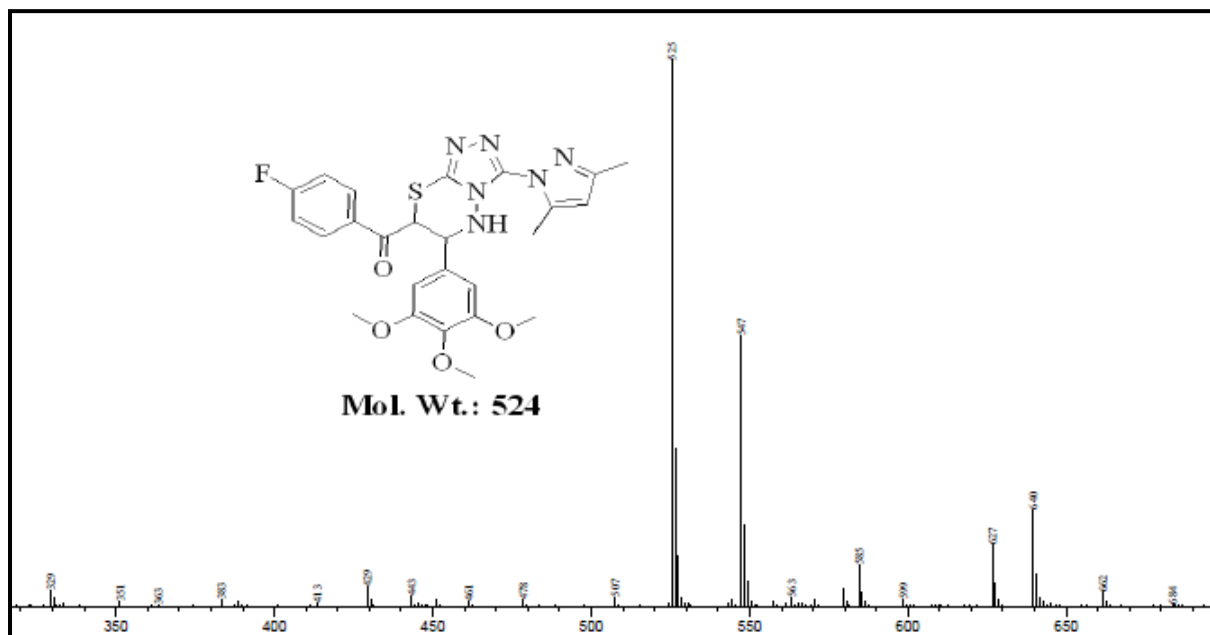


Fig: Mass Spectrum of Compound **34l**

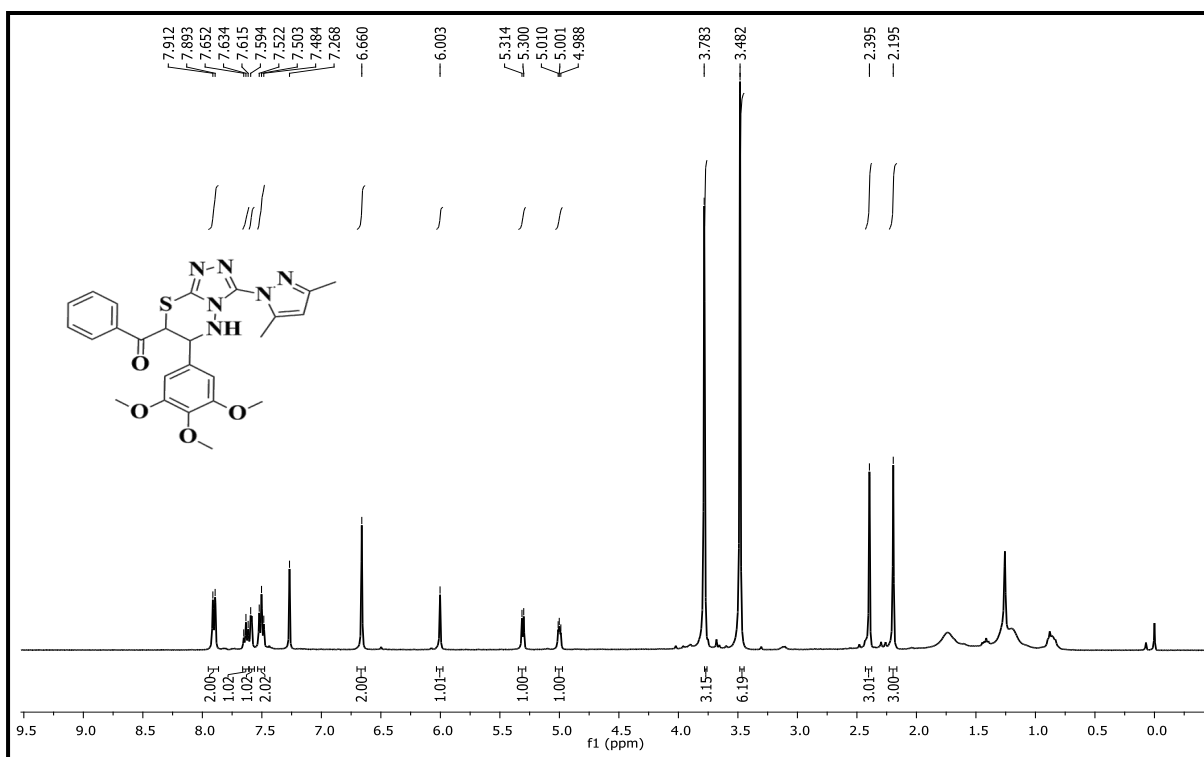


Fig: ^1H -NMR Spectrum of Compound **34m** (400 MHz, CDCl_3)

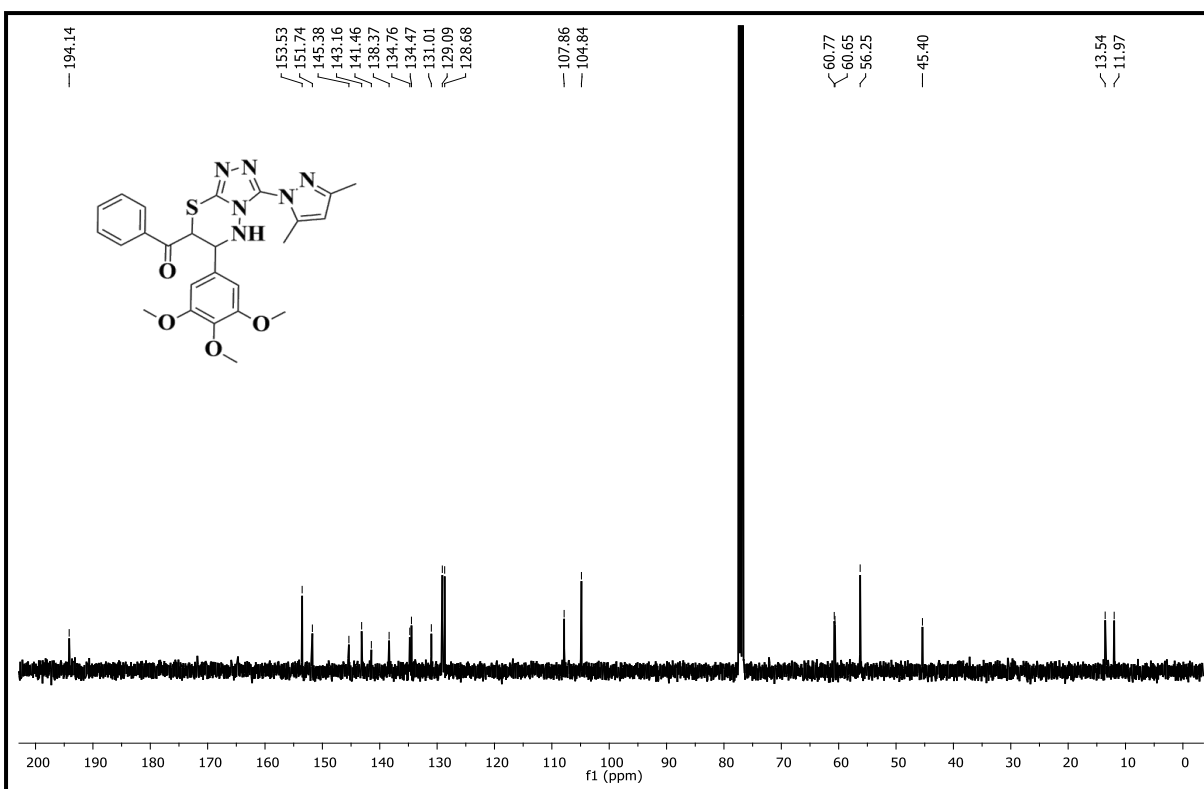


Fig: ^{13}C -NMR Spectrum of Compound **34m** (100 MHz, CDCl_3)

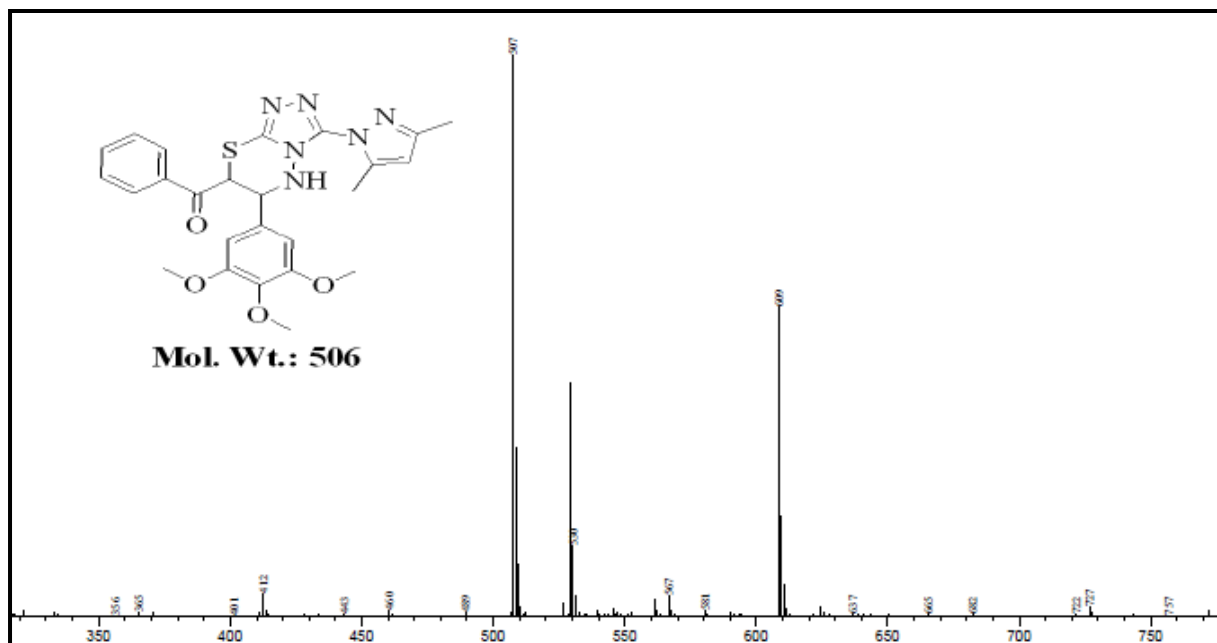


Fig: Mass Spectrum of Compound **34m**

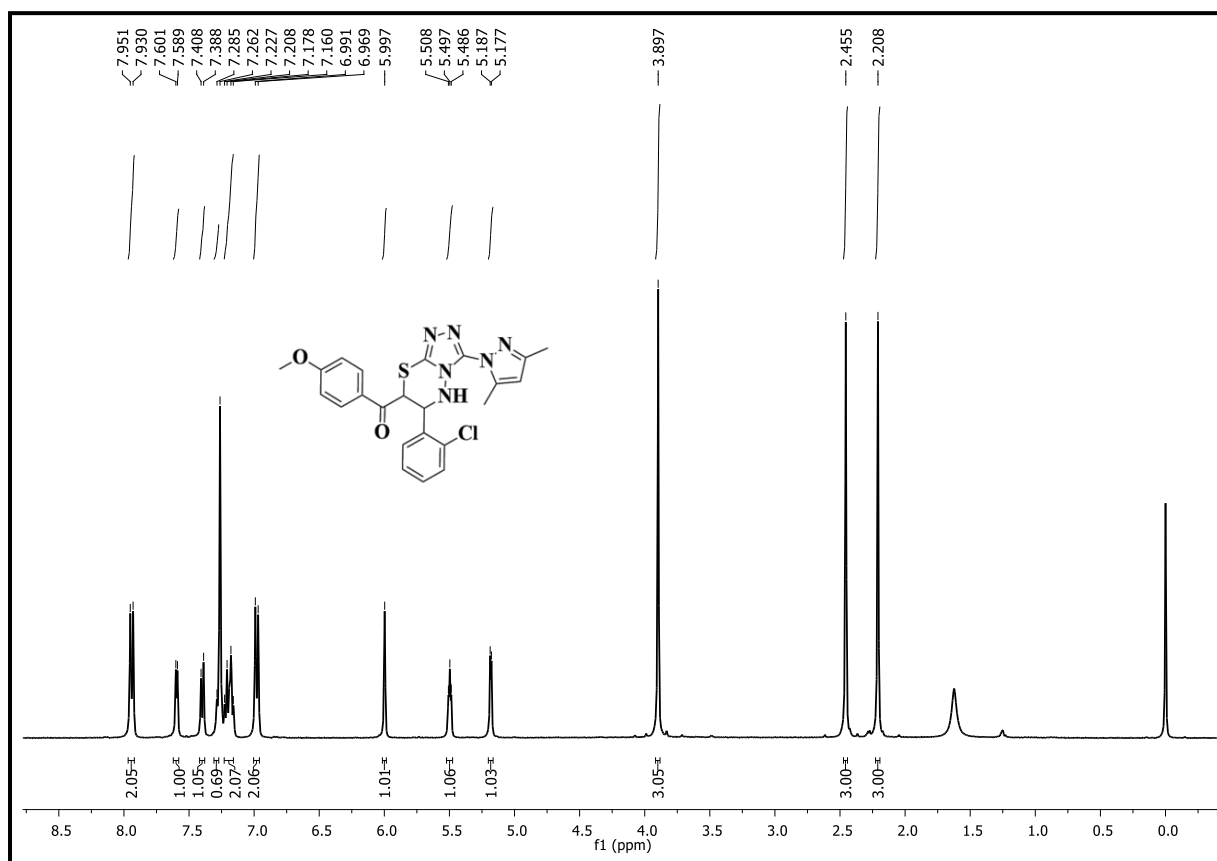


Fig: ^1H -NMR Spectrum of Compound **34n** (400 MHz, CDCl_3)

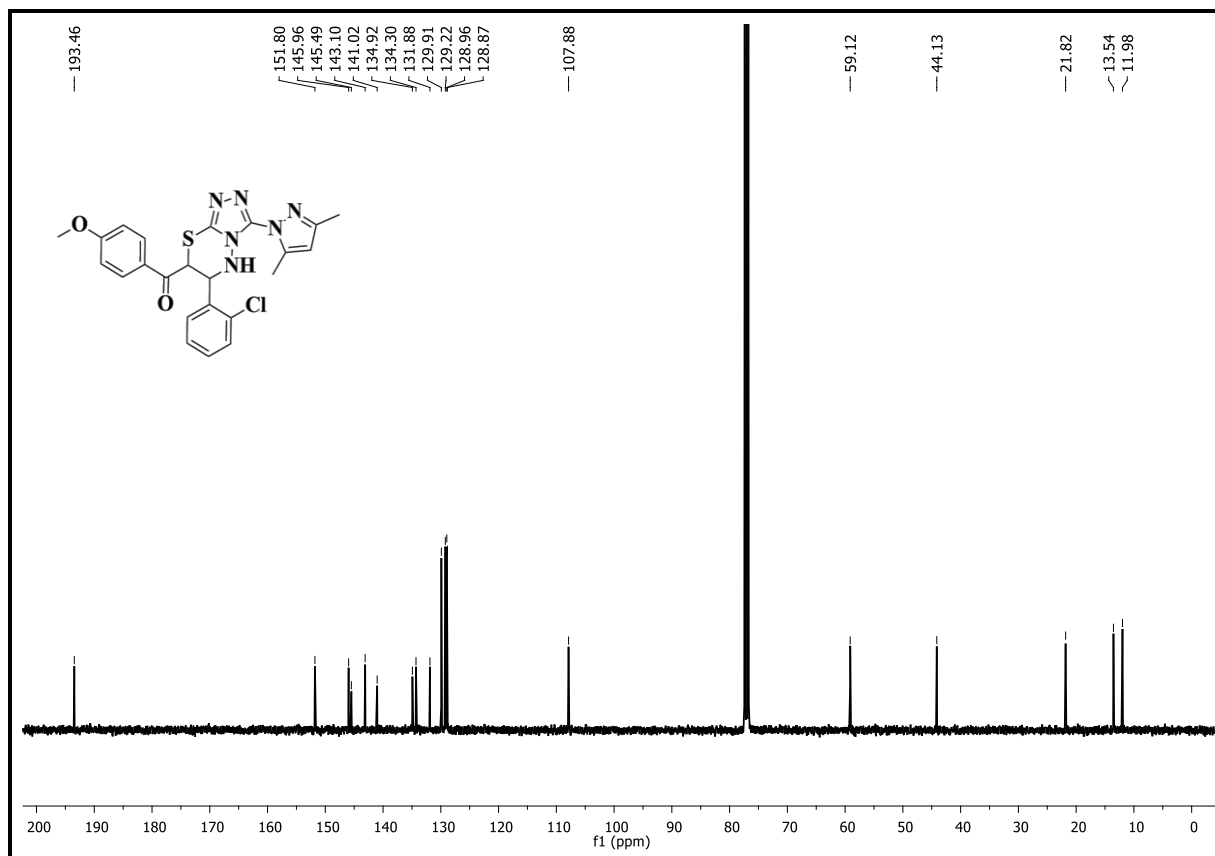


Fig: ¹³C-NMR Spectrum of Compound 34n (100 MHz, CDCl₃)

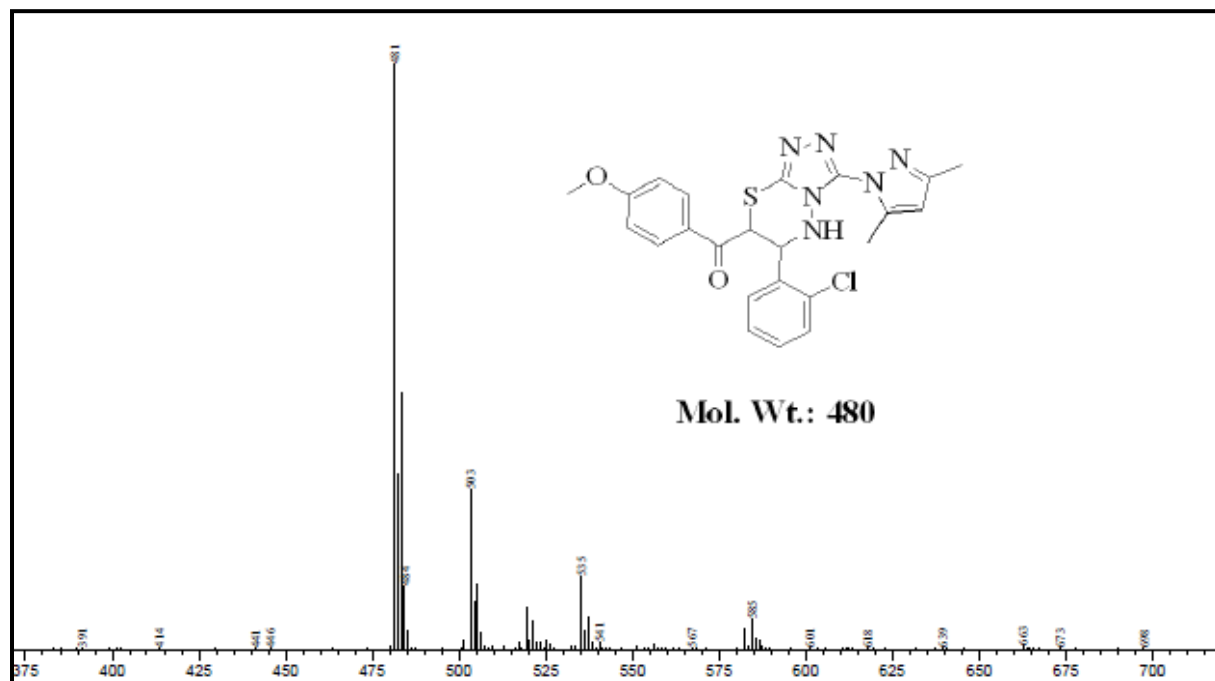


Fig: Mass Spectrum of Compound 34n

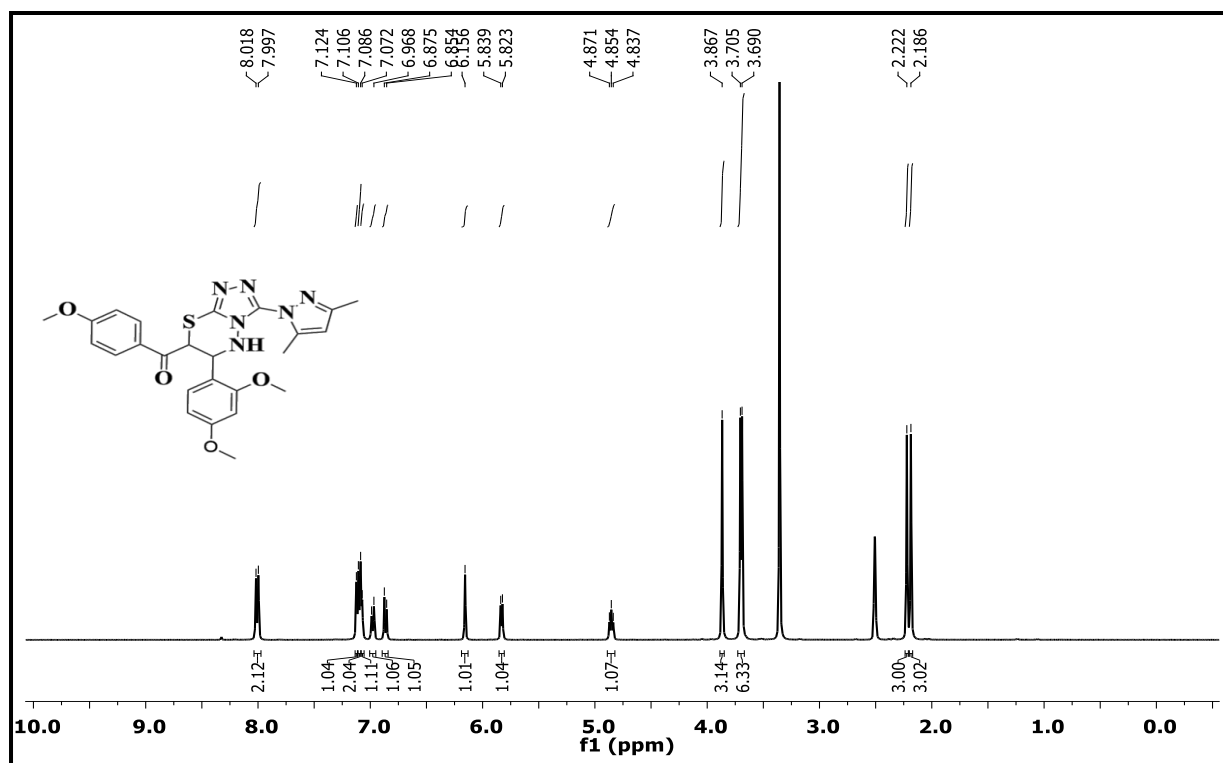


Fig: ¹H-NMR Spectrum of Compound **34o** (400 MHz, DMSO-*d*₆)

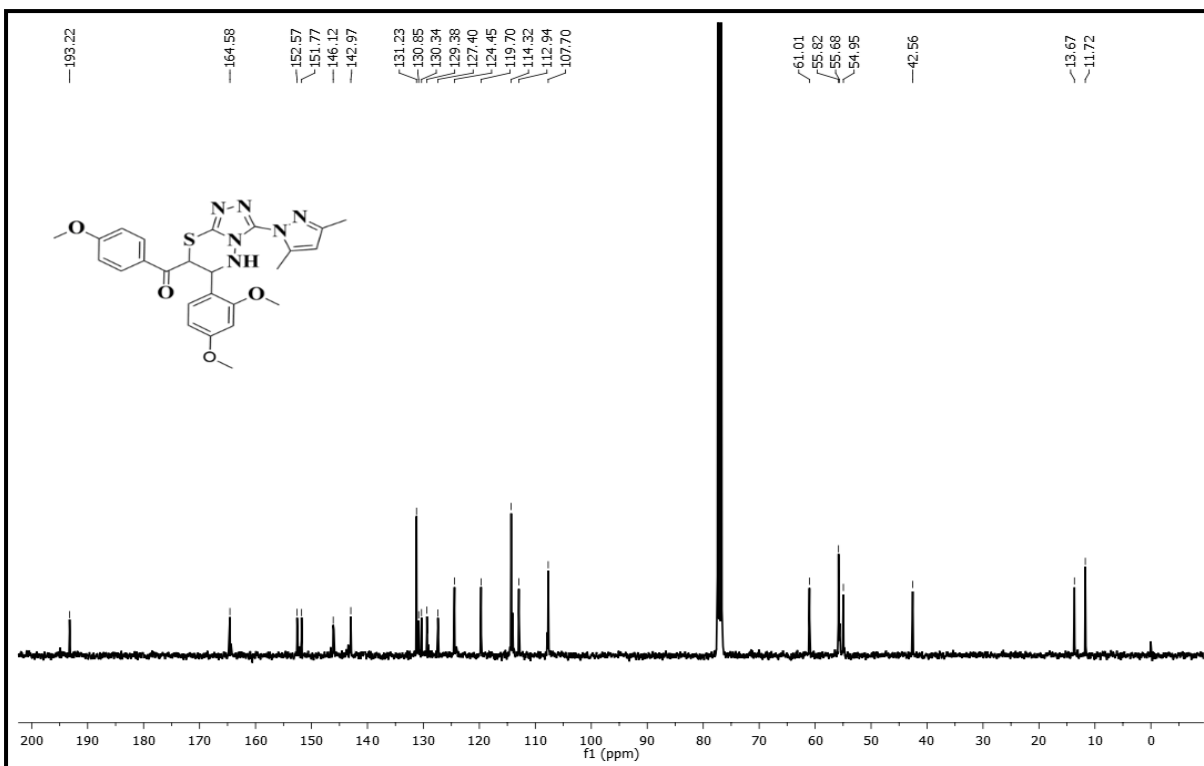


Fig: ¹³C-NMR Spectrum of Compound **34o** (100 MHz, CDCl₃)

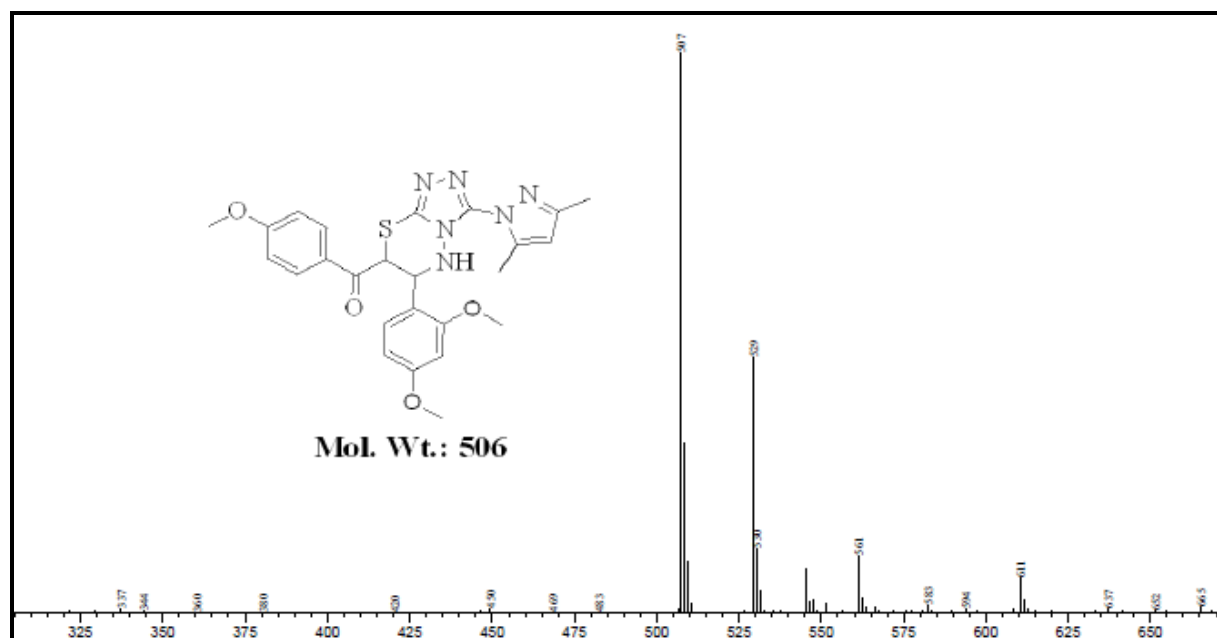


Fig: Mass Spectrum of Compound **34o**

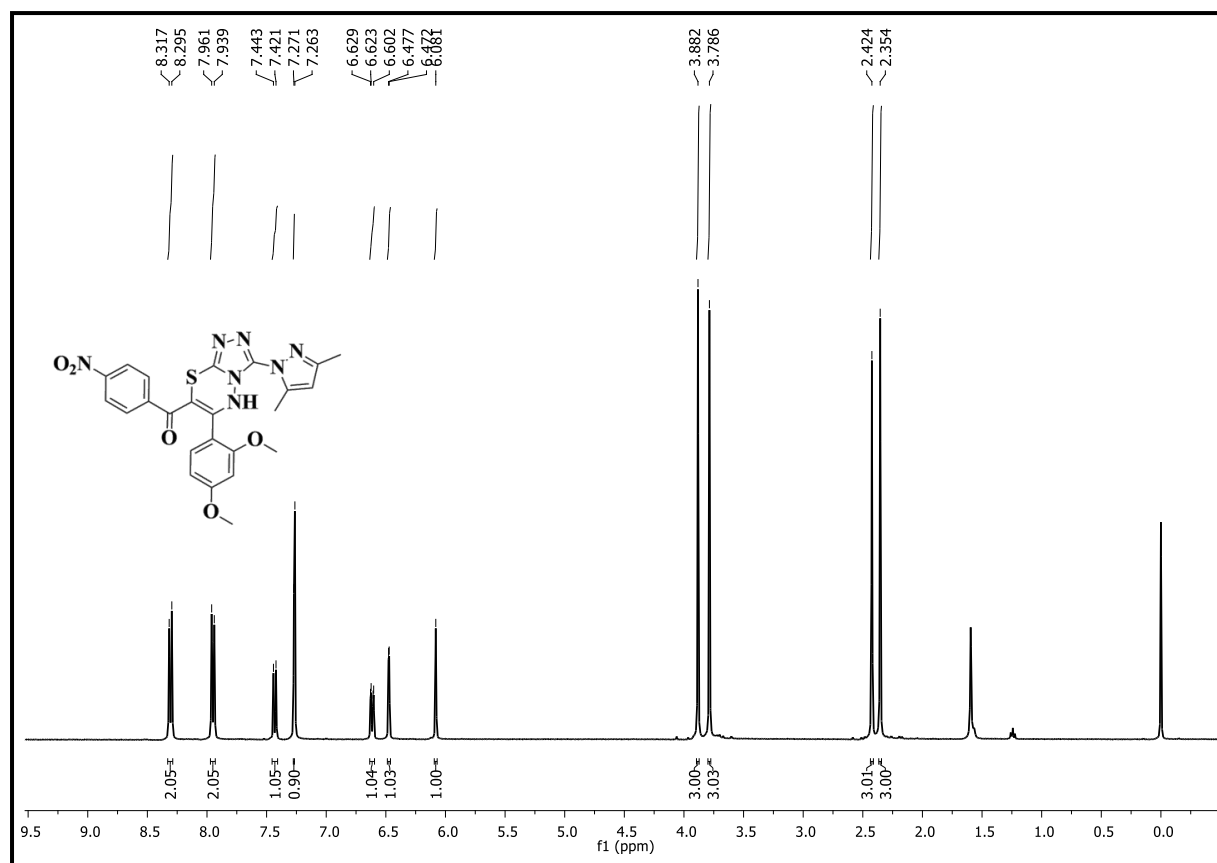


Fig: ^1H -NMR Spectrum of Compound **34p** (400 MHz, CDCl_3)

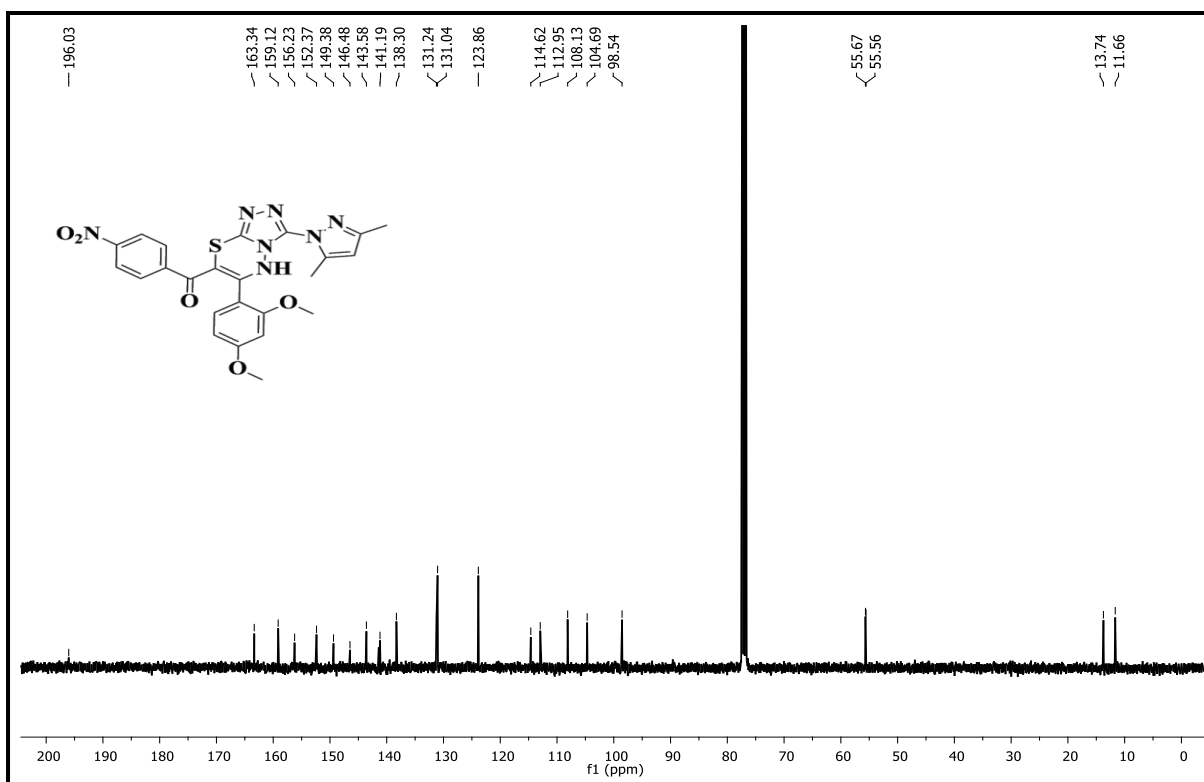


Fig: ¹³C-NMR Spectrum of Compound 34p (100 MHz, CDCl₃)

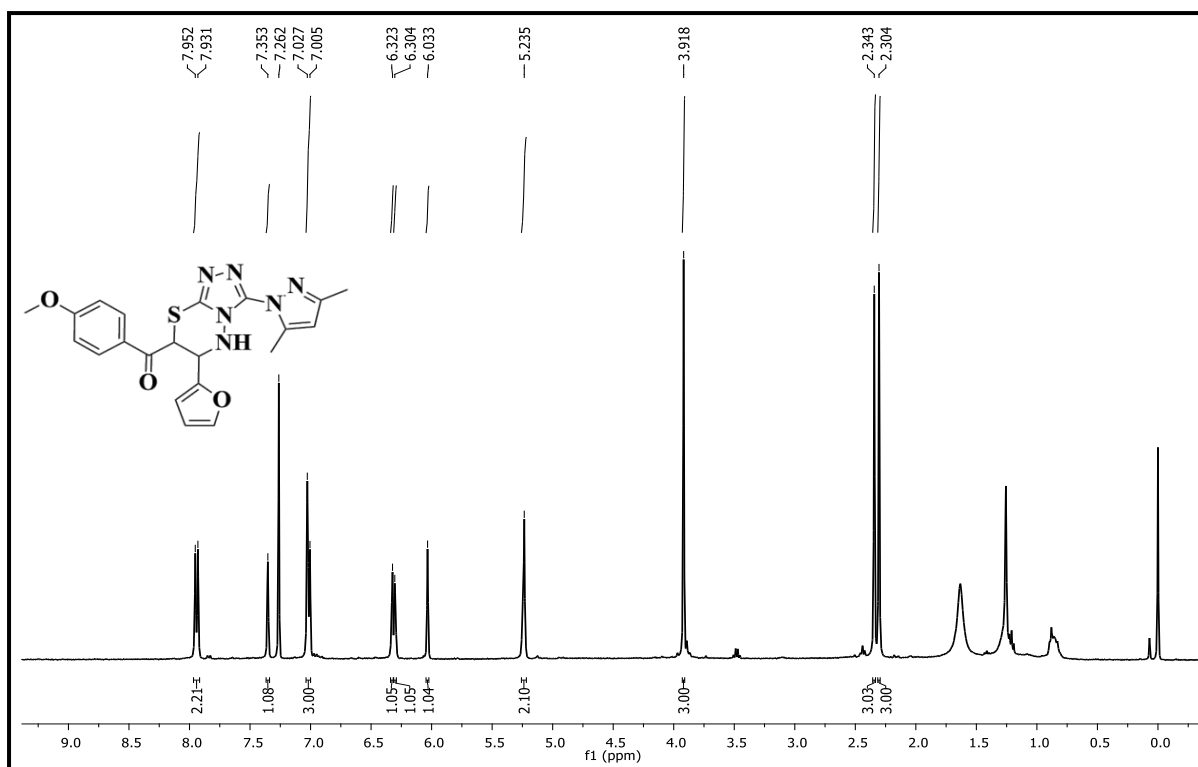


Fig: ¹H-NMR Spectrum of Compound 34q (400 MHz, CDCl₃)

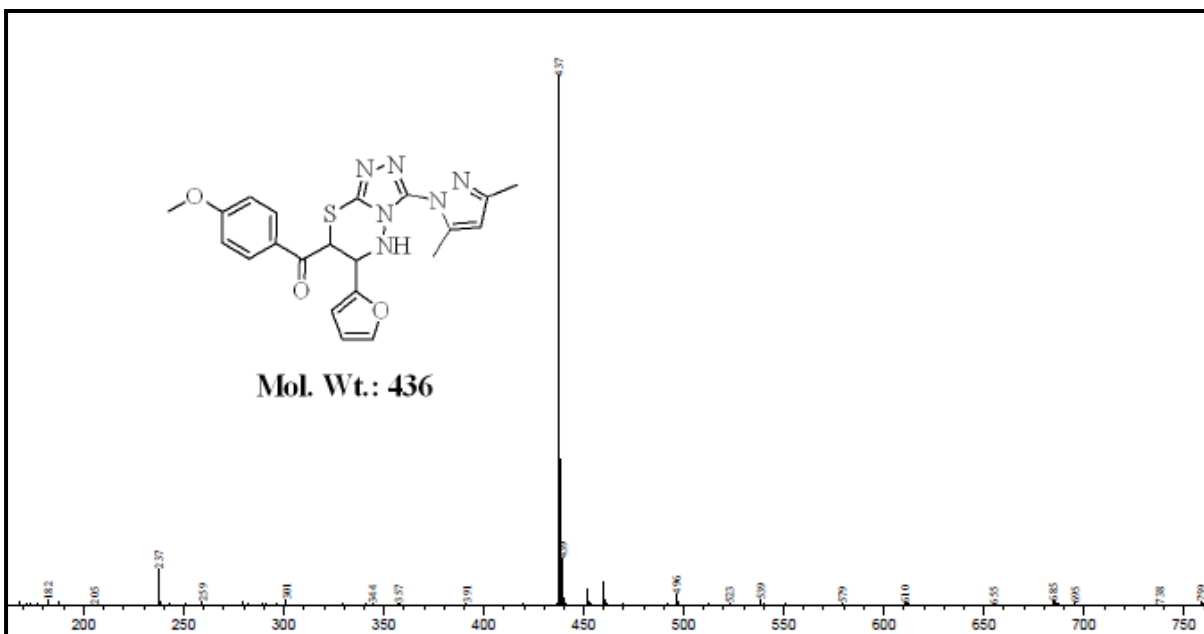


Fig: Mass Spectrum of Compound 34q

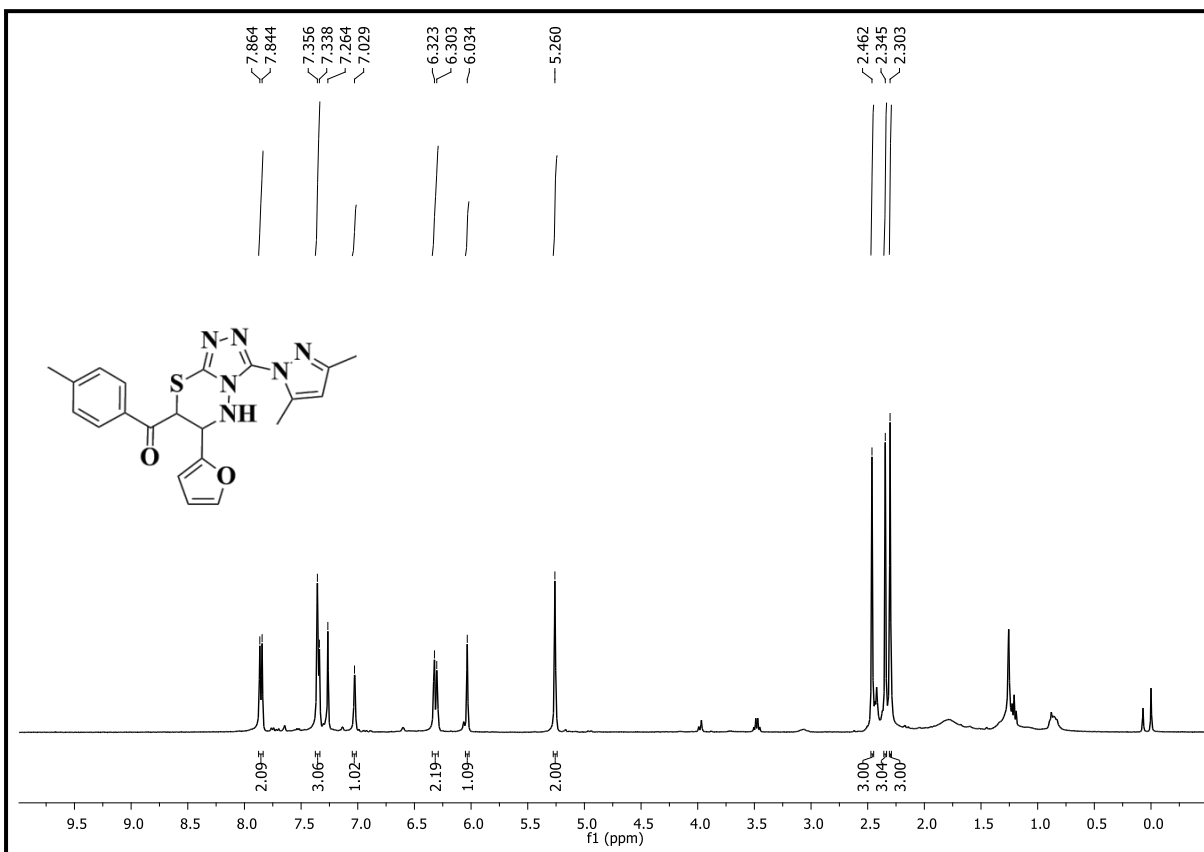


Fig: ^1H -NMR Spectrum of Compound 34r (400 MHz, CDCl_3)

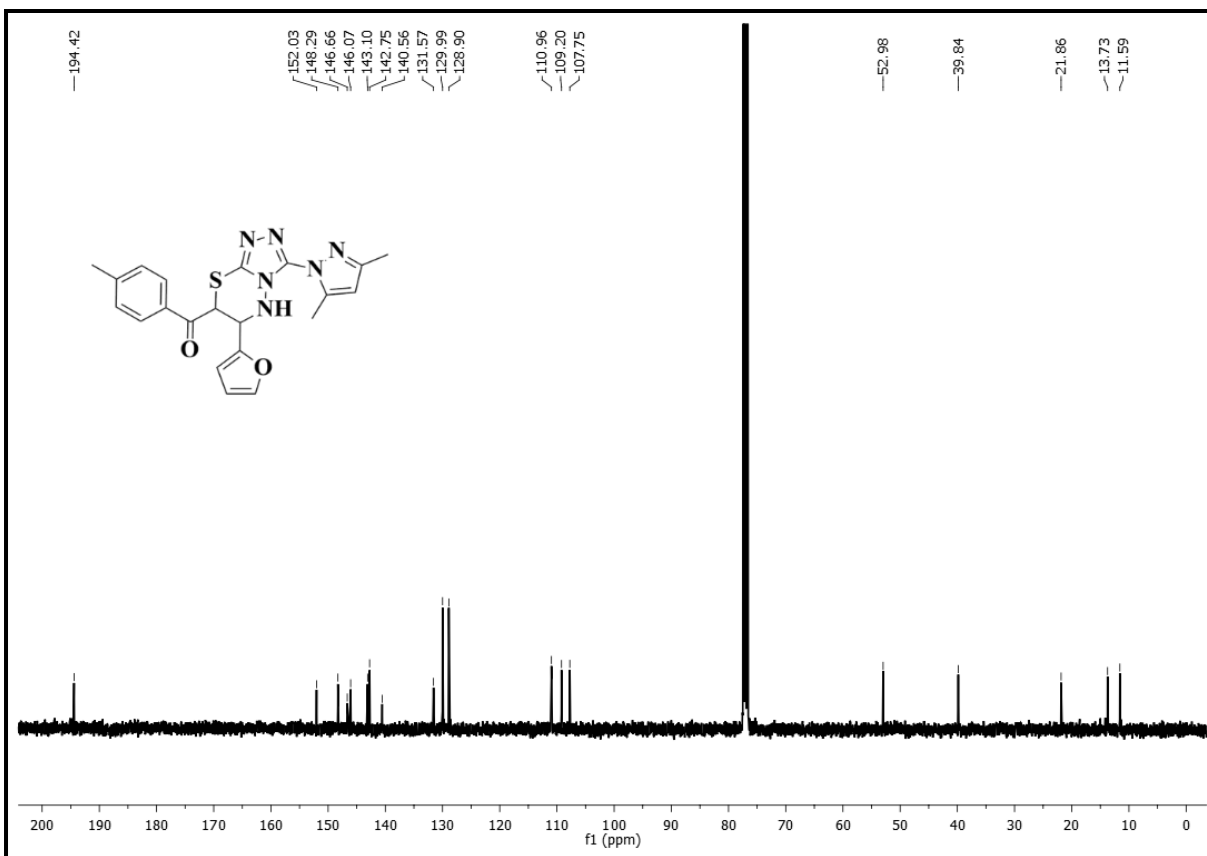


Fig: ¹³C-NMR Spectrum of Compound 34r (100 MHz, CDCl₃)

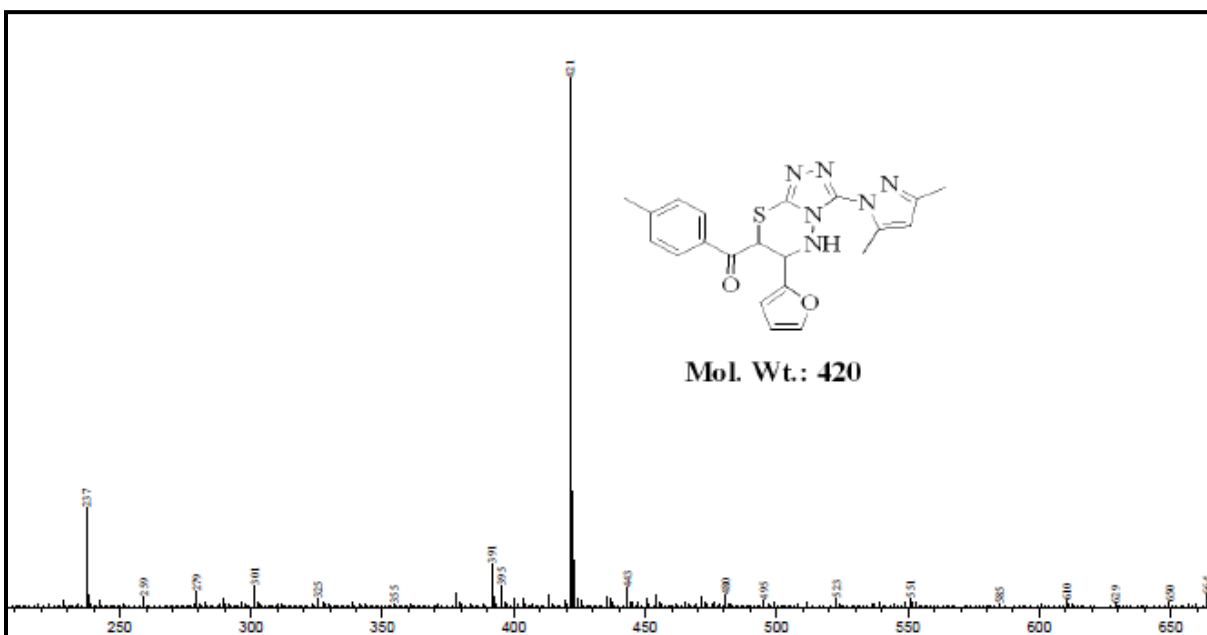


Fig: Mass Spectrum of Compound 34r

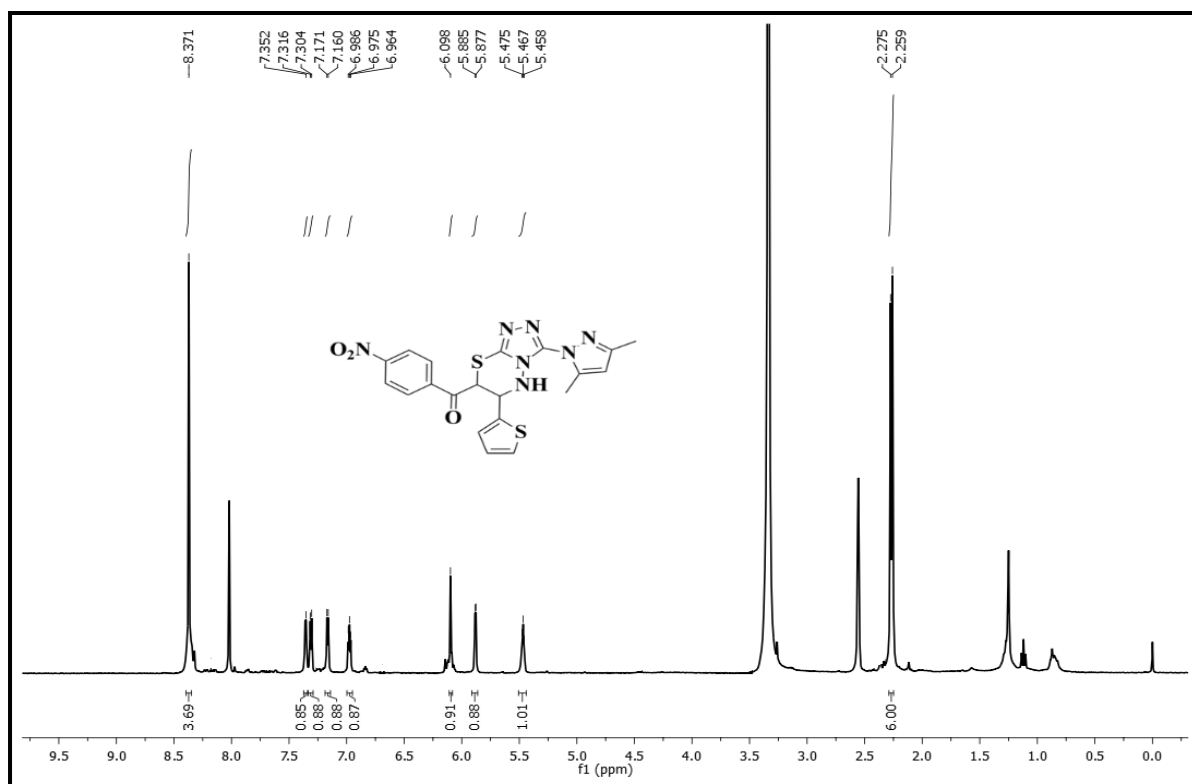


Fig: ¹H-NMR Spectrum of Compound 34s (400 MHz, CDCl₃+DMSO-*d*₆)

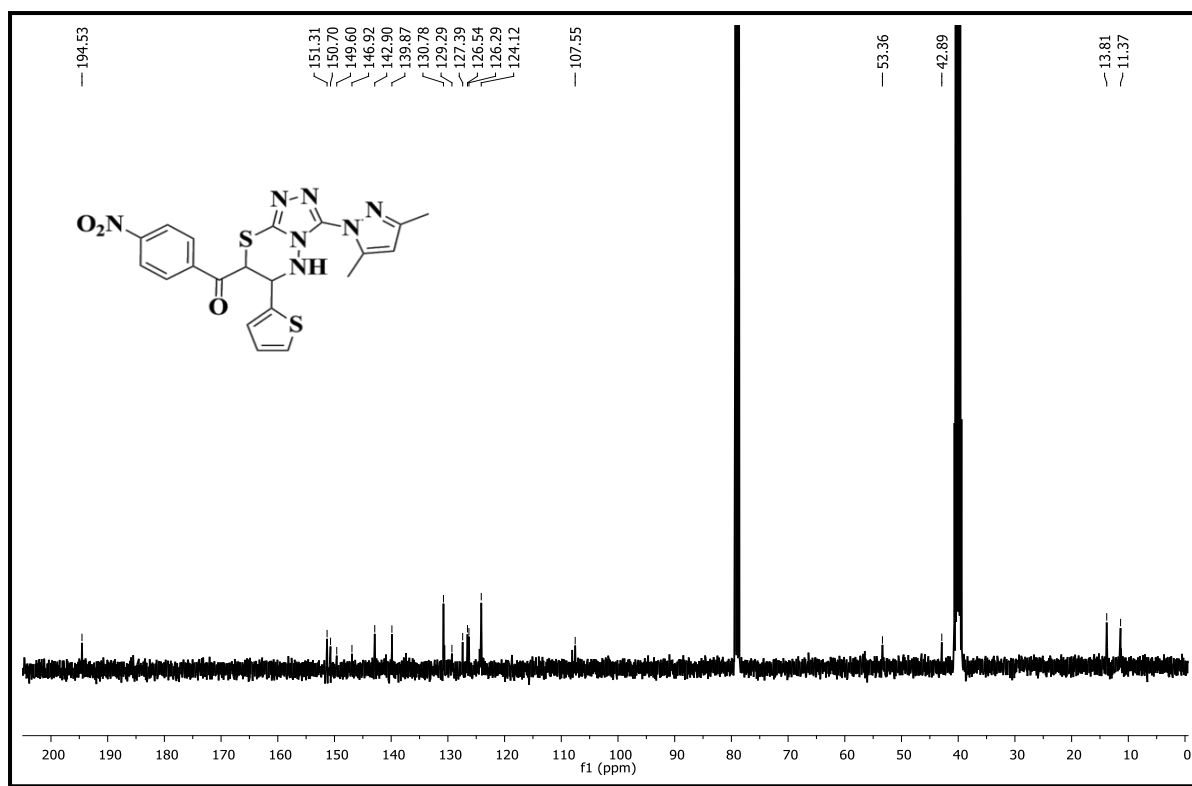


Fig: ¹³C-NMR Spectrum of Compound 34s (100 MHz, CDCl₃+DMSO-*d*₆)

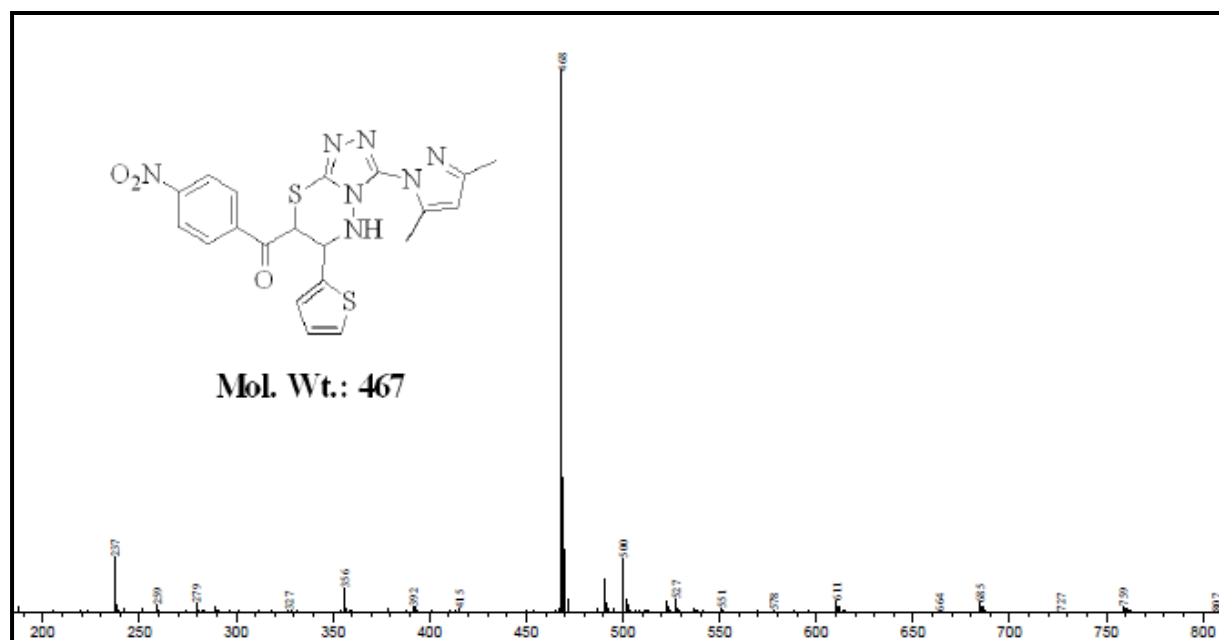


Fig: Mass Spectrum of Compound 34s

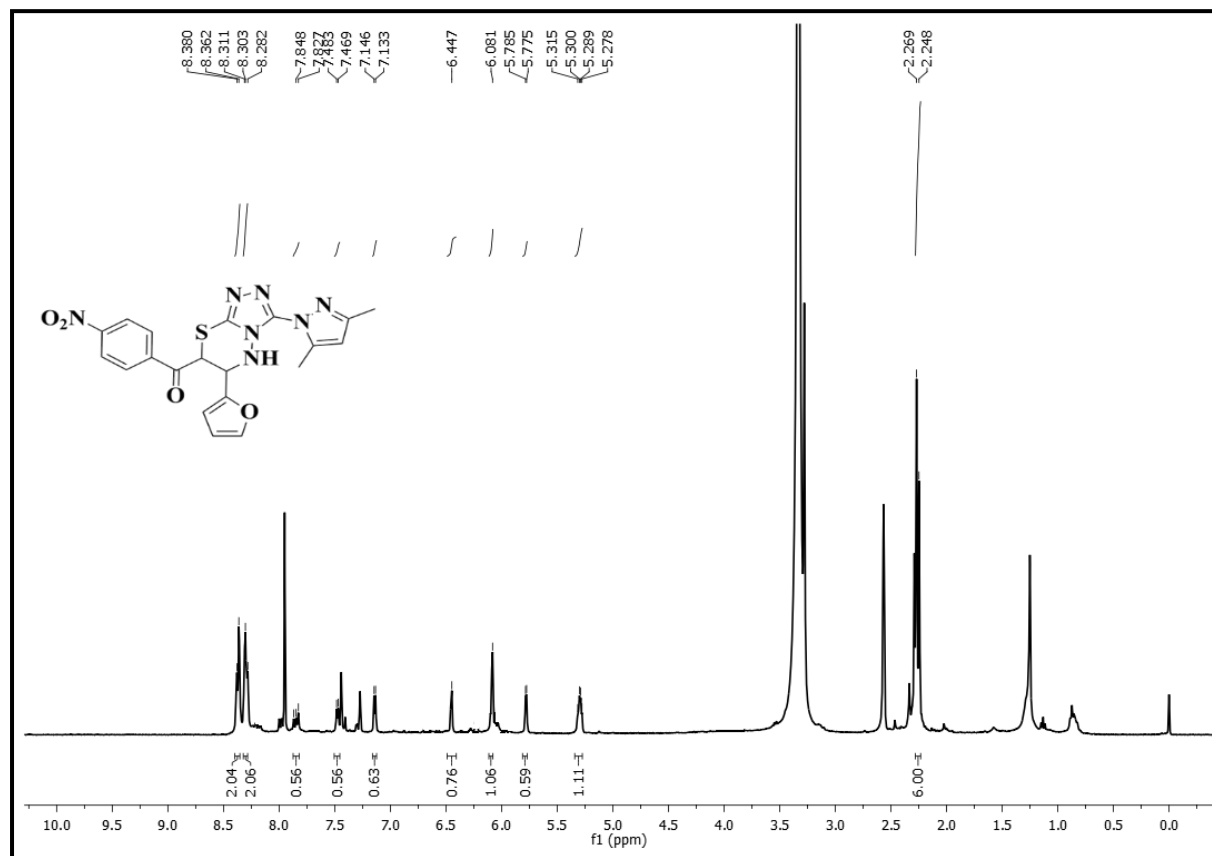


Fig: ^1H -NMR Spectrum of Compound 34t (400 MHz, $\text{CDCl}_3 + \text{DMSO}-d_6$)

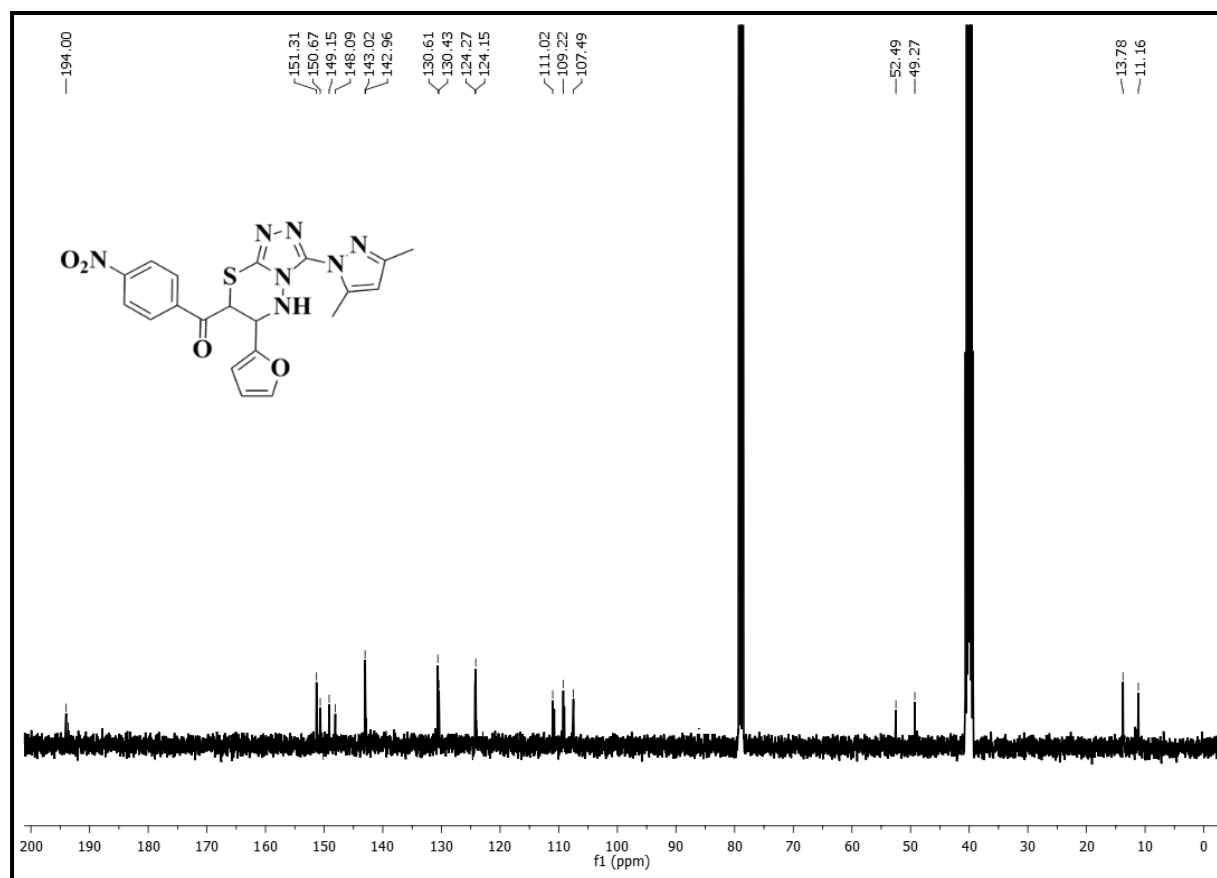


Fig: ¹³C-NMR Spectrum of Compound **34t** (100 MHz, CDCl₃+DMSO-*d*₆)

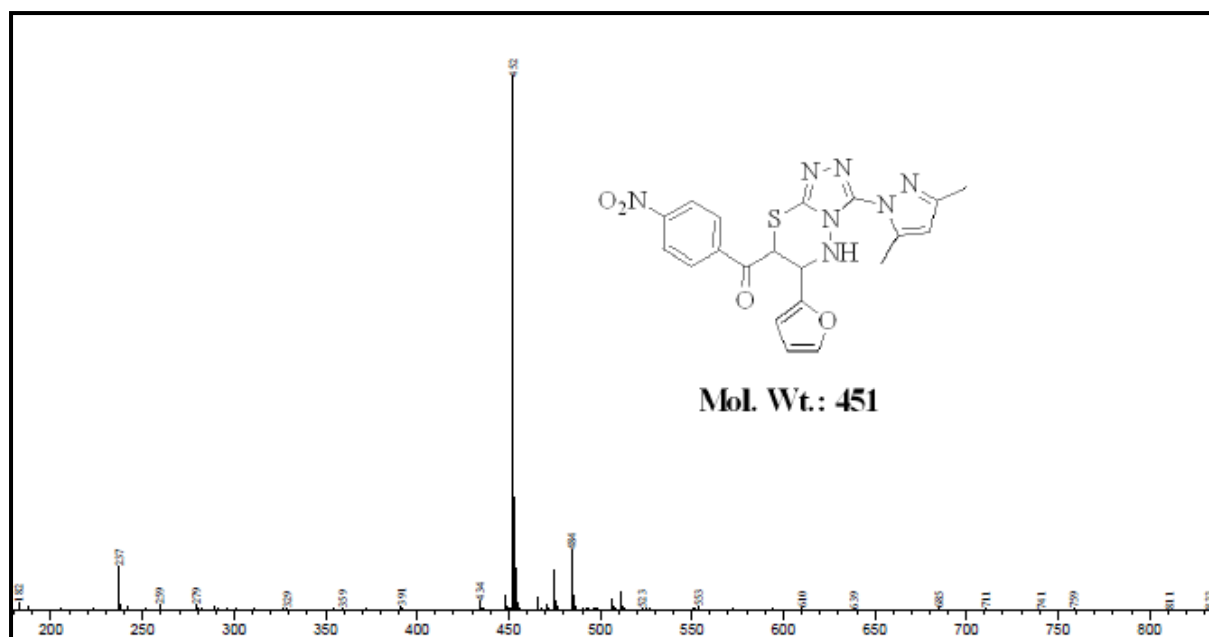


Fig: Mass Spectrum of Compound **34t**

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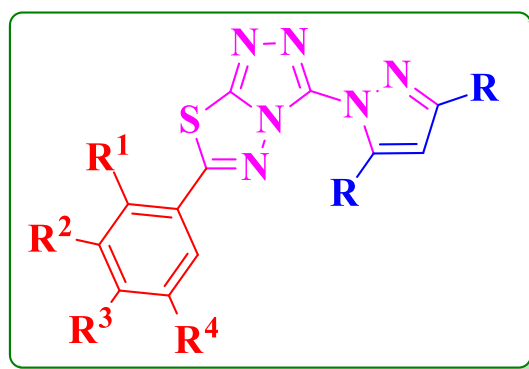
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CHAPTER-V

AN EFFICIENT ONE-POT SYNTHESIS OF 6-PHENYL-3-(1*H*-PYRAZOL-1-YL)-[1,2,4]TRIAZOLO[3,4-*b*][1,3,4]THIADIAZOLE DERIVATIVES AND THEIR ANTIMICROBIAL EVALUATION AND MOLECULAR DOCKING STUDIES



INTRODUCTION:

Pyrazole belongs to an azole class of five-membered heterocyclic compounds. Pyrazole structure is a composition of two adjacent nitrogen atoms and three carbon atoms. The synthetic protocol of pyrazole is very easy and simple^{1,2}. Literature review reveals that the pyrazole derivatives are showing extensive biological, pharmaceutical activities such as apoptotic inducers³, anti-inflammatory^{4,5}, anti-depressant⁶, anti-malarial⁷, anti-cancer⁸, anti-diabetic⁹, antimicrobial-antianalgesic¹⁰, and anti-HBV (hepatitis B virus)¹¹ and anti-tumor¹² activities.

From the past few decades, 1,3,4-thiadiazole derivatives have shown remarkable applications in the various areas of medicinal, agriculture, and material chemistry¹³. 1,3,4-Thiadiazole is a five-membered heterocyclic compound with one S and two N-atoms. 1,2,4-Triazolo[3,4-*b*][1,3,4]thiadiazole is a fused heterocyclic core motif in the numerous biologically active compounds and drugs (**Fig. 1**)¹⁴. Based on the literature survey, there are many synthetic procedures for the preparation of 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazole derivatives^{15,16}. 1,2,4-Triazolo[3,4-*b*][1,3,4]thiadiazoles have wide range of biological properties such as anti-cancer¹⁷⁻²⁰, anti-microbial^{21,22}, analgesic-antiinflammatory²³⁻²⁵, anti-tubercular²⁶, anti-viral²⁷, anti-tumor²⁸, anti-depressant²⁹, COX-2 inhibitor³⁰, cytotoxic^{31,32}, and pharmacological^{33,34} activities.

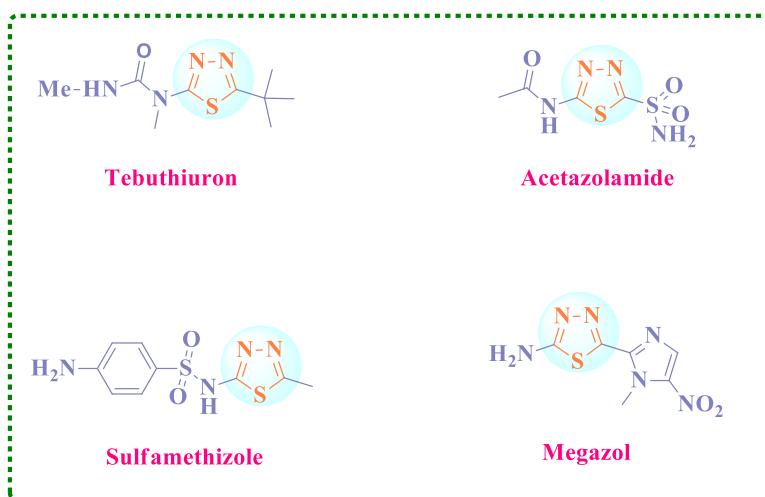
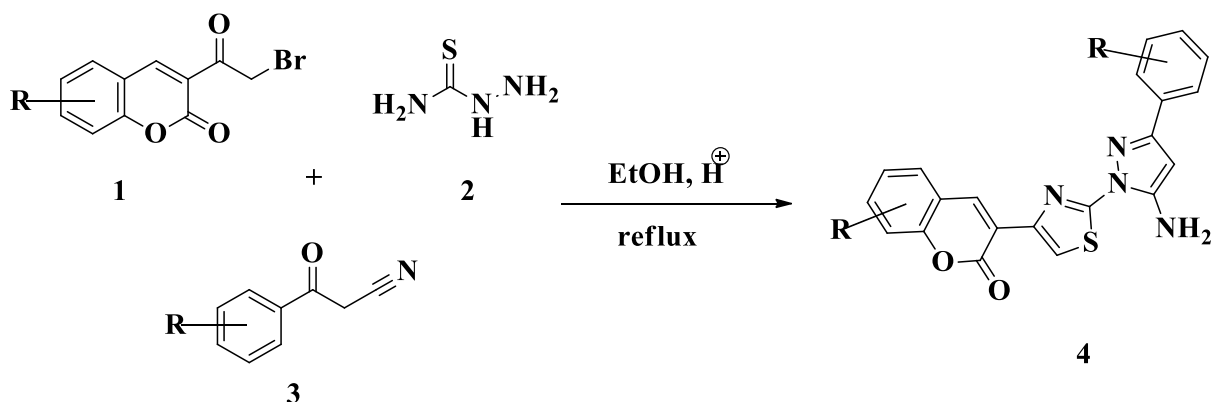


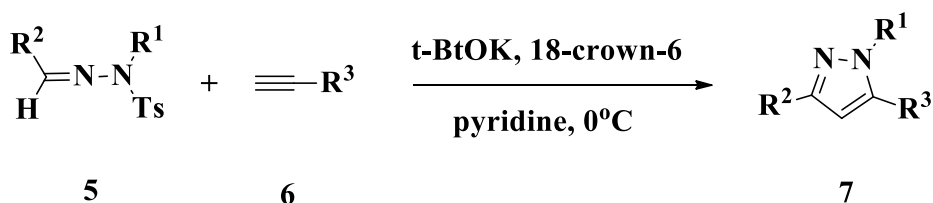
Fig. 1: Commercially available thiadiazole containing drugs.

Herein some of the synthetic procedures and biological applications of pyrazoles and thiadiazoles from the literature survey are presented below.

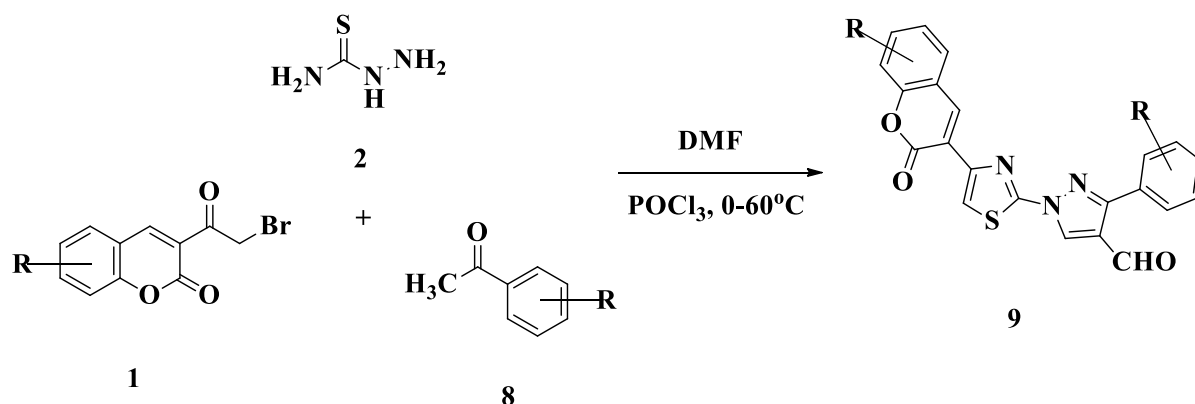
Vaarla³⁵ et al. carried out the synthesis of 3-(2-(5-amino-3-phenyl-1*H*-pyrazol-1-yl)thiazol-4-yl)-2*H*-chromen-2-one (**4**) derivatives using 3-(2-bromoacetyl)-2*H*-chromen-2-one (**1**), hydrazinecarbothioamide (**2**), and 3-oxo-3-phenylpropanenitrile (**3**) in ethanol and the presence of HCl under reflux temperature in good yields. The target compounds were evaluated for their anti-cancer activity with promising results.



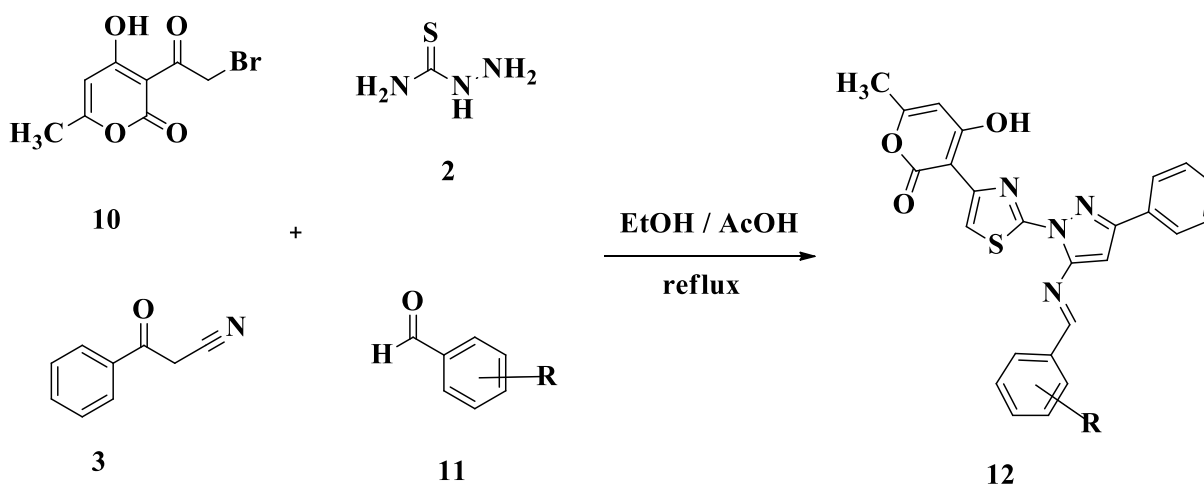
1,3,5-Trisubstituted pyrazoles (**7**) were synthesized by **Kong³⁶ et al.** by the reaction of various tosylhydrazones (**5**) with different terminal alkynes (**6**) in the t-BtOK, 18-crown-6, and pyridine at 0°C temperature in maximum yields.



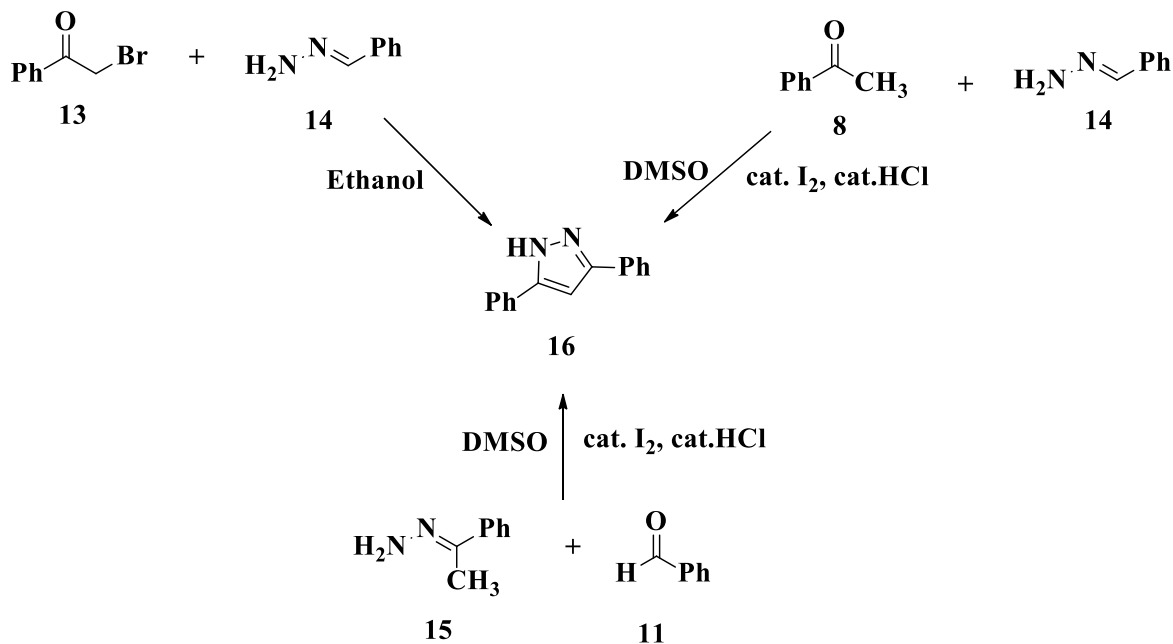
Krishnaiah³⁷ et al. prepared the substituted 1-(4-(2-oxo-2*H*-chromen-3-yl)thiazol-2-yl)-3-phenyl-1*H*-pyrazole-4-carbaldehydes (**9**) from the reaction of different 3-(2-bromoacetyl)coumarins (**1**), thiosemicarbazide (**2**) and substituted acetophenones (**8**) using Vilsmeier-Haack conditions up to 85% yields. The final analogs were screened for their biological evaluation with good results.



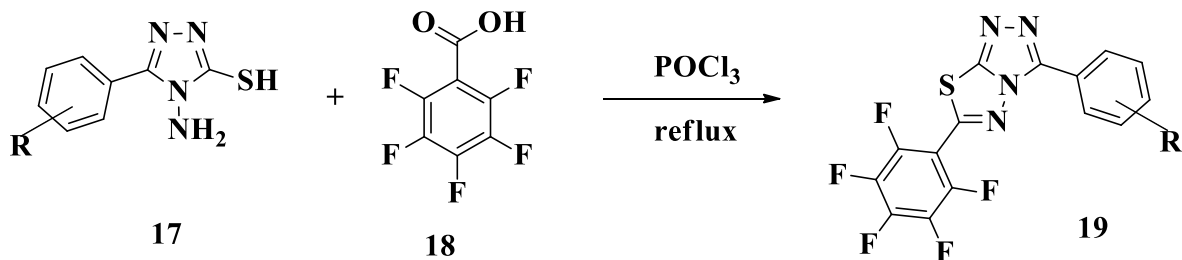
Bade and Vedula³⁸ synthesized the substituted (*E*)-3-(2-(5-(benzylideneamino)-3-phenyl-1*H*-pyrazol-1-yl)thiazol-4-yl)-4-hydroxy-6-methyl-2*H*-pyran-2-ones (**12**) via a one-pot, multi-component reaction starting from 3-(2-bromoacetyl)-4-hydroxy-6-methyl-2*H*-pyran-2-one (**10**), hydrazinecarbothioamide (**2**), phenacylcyanide (**3**) and various aromatic aldehydes (**11**) in ethanol and few drops of AcOH.



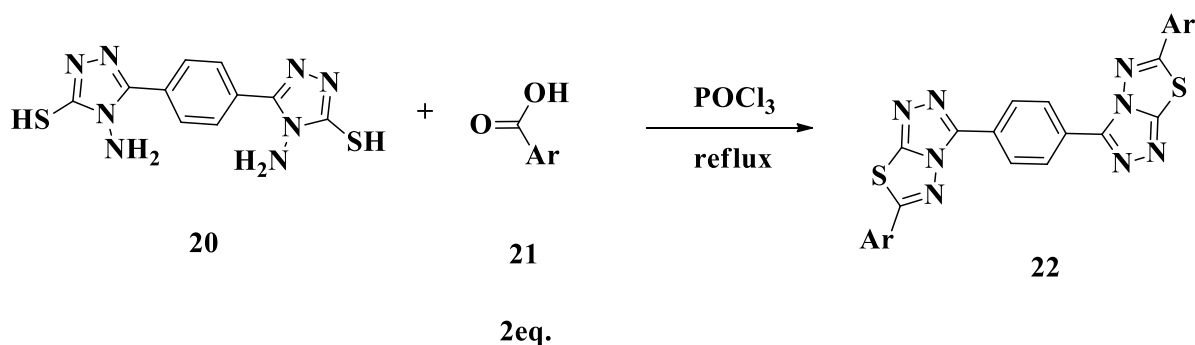
Mohamady³⁹ *et al.* described the synthesis of disubstituted pyrazoles (**16**) from the different methods using phenacyl bromides (**13**), phenylhydrazones (**14**), acetophenones (**8**), keto hydrazones (**15**) and aromatic aldehydes in their respective one-pot, green synthetic methods.



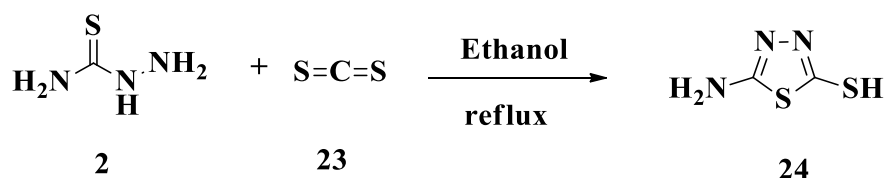
4-Amino-5-phenyl-4*H*-1,2,4-triazole-3-thiols (**17**) and 2,3,4,5,6-pentafluorobenzoic acid (**18**) were refluxed in POCl_3 to produce the 6-(perfluorophenyl)-3-phenyl-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles⁴⁰ (**19**).



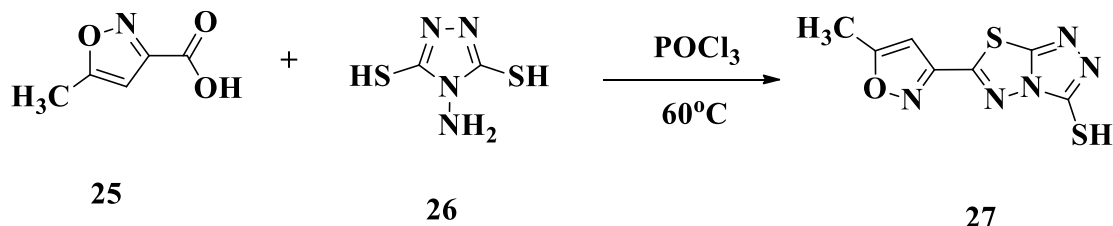
Palekar⁴¹ *et al.* synthesized the substituted bis 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazoles (**22**) from the reflux of 5,5'-(1,4-phenylene)bis(4-amino-4*H*-1,2,4-triazole-3-thiol) (**20**) and aromatic carboxylic acids (**21**) in presence of POCl_3 and these compounds exhibited good anti-bacterial activity.



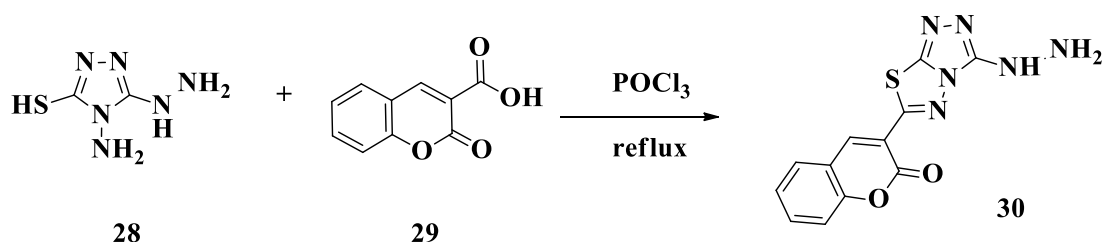
Simple 5-amino-1,3,4-thiadiazole-2-thiol (**24**) was synthesized from the reaction of thiosemicarbazide (**2**) and carbon disulfide (CS₂) at the reflux temperature of ethanol by **Vudhgiri⁴² et al.**



Vaarla and Vedula⁴³ conducted the cyclization reaction between the 5-methylisoxazole-3-carboxylic acid (**25**) and 4-amino-4*H*-1,2,4-triazole-3,5-dithiol (**26**) in POCl₃ at 60°C to generate the 6-(5-methylisoxazol-3-yl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole-3-thiol (**27**). Further, compound **27** was used as starting material for the synthesis of S-alkylated products.



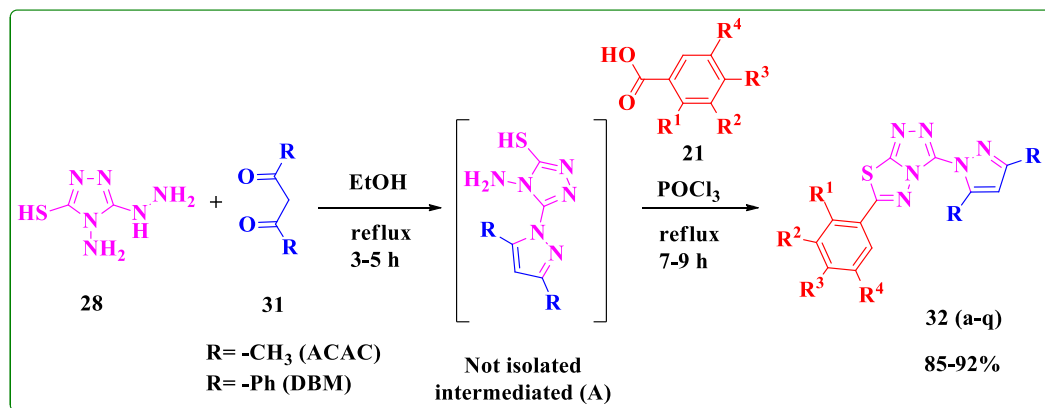
Sharma⁴⁴ et al. reported the synthesis of 3-(3-hydrazinyl-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-6-yl)-2*H*-chromen-2-one (**30**) from the cyclization reaction of 4-amino-5-hydrazinyl-4*H*-1,2,4-triazole-3-thiol (**28**) and 2-oxo-2*H*-chromene-3-carboxylic acid (**29**) in the presence of POCl₃ under the reflux condition. Compound **30** was acted as an intermediate for the synthesis of pyrazolyltriazolothiadiazole.



PRESENT WORK:

In view of the biological applications of the 1,2,4-triazole, pyrazole, and 1,3,4-thiadiazole moieties, we have developed a new synthetic route for the establishment of these motifs in a single compound *via* a one-pot, three-component condensation method. Novel hybrid molecules containing three biological active scaffolds were expected to possess good anti-bacterial activities. We have synthesized a new hybrid template of pyrazolyl[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole in search of promising anti-bacterial agents.

In the current method, the anticipated target pyrazolyl[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole derivatives (**32a-q**) were synthesized using readily available starting compounds purpald (**28**), acetylacetone/dibenzoyl methane (DBM) (**31**), and substituted benzoic acids (**21**) *via* a one-pot, sequential addition of three-component reaction (**Scheme-1**).



Scheme-1: One-pot, three-component synthesis of target title derivatives **32a-q**.

Initially, we have conducted a reaction between purpald (**28**) and acetylacetone/DBM (**31**) in presence of dry ethanol under reflux for 3-5 h to generate intermediate (**A**). The solvent was evaporated and dried to get the intermediate (**A**) followed by sequential addition of various benzoic acids (**21**) and phosphorous oxychloride (POCl_3)^{45,46} reflux for 7-9 h produced the target

title derivatives (**32a-q**) with high yields (85-92%, **Table-1**) through a one-pot, three-component reaction.

Table-1: Synthesis of pyrazolyl[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole derivatives (**32a-q**).

Compound	R	R ¹	R ²	R ³	R ⁴	Time (h)	Yield (%)
32a	CH ₃	H	H	H	H	10.00	88
32b	CH ₃	H	H	CH ₃	H	10.30	90
32c	CH ₃	H	H	F	H	11.10	86
32d	CH ₃	H	NO ₂	H	H	12.50	85
32e	CH ₃	Cl	H	H	H	11.40	87
32f	CH ₃	Br	H	H	H	11.00	90
32g	CH ₃	H	H	Cl	H	10.30	88
32h	CH ₃	H	H	I	H	10.30	86
32i	CH ₃	H	H	NO ₂	H	11.10	85
32j	CH ₃	H	NO ₂	H	NO ₂	11.40	89
32k	Ph	H	H	NH ₂	H	12.50	86
32l	Ph	H	H	Cl	H	11.50	90
32m	Ph	H	H	I	H	12.20	89
32n	Ph	H	H	CH ₃	H	11.30	92
32o	Ph	H	H	NO ₂	H	13.15	87
32p	Ph	H	NO ₂	H	NO ₂	14.00	86
32q	Ph	Cl	H	H	H	12.30	88

When the reaction is operated between **28**, **31**, and **21** in the above-mentioned reaction conditions there would be a possibility to form alternative products such as **33** and **34** (**Fig. 2**). In the present investigation, compound **32** was formed alone as evidenced by thin-layer chromatography as a single spot. Based on the spectral data and analytical data the possible products **33** and **34** were declined.

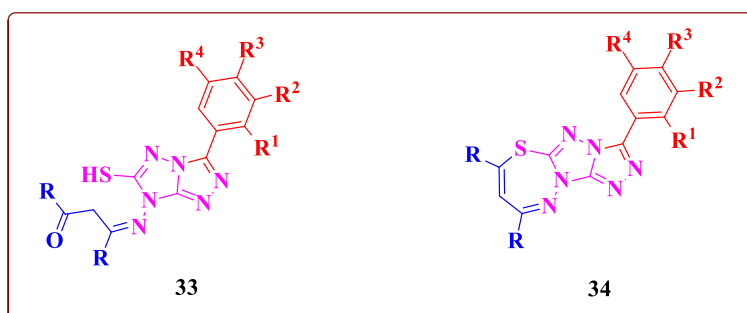
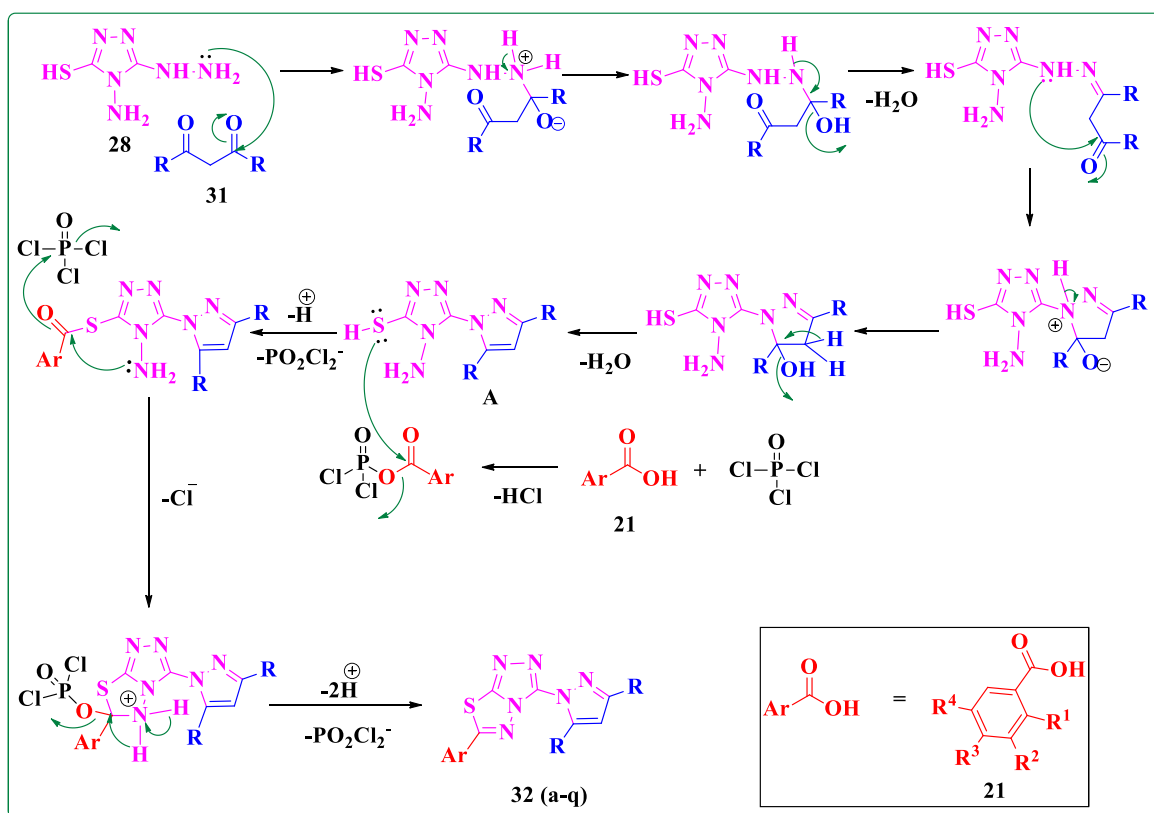


Fig. 2: Alternative other probable products for **32**.



Scheme-2: Plausible reaction pathway for the synthesis of pyrazolyl[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles (**32a-q**) via a one-pot three component reaction⁴⁷.

In the current investigation, initially, the pyrazole ring was formed by the cyclo condensation between the hydrazino group (-NH-NH₂) of purpald **28** and diketo compounds **31a-b**. Sequentially added third components such as substituted aromatic carboxylic acids **21a-k** also underwent cyclization reaction with intermediate **A** to form the fused [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole ring. The role of POCl₃ in the cyclization reaction is that it converts aromatic carboxylic acid into its benzoic or substituted benzoic phosphorodichloridic anhydride. This attacks on thiol group of **A** to yield the thioester with the loss of phosphorodichloridate (PO₂Cl₂⁻) anion. Then the second molecule of POCl₃ attacks on thioester followed by ring closure reaction with the amino group by the elimination of another PO₂Cl₂⁻ anion to yield final products **4a-q** (**Scheme-2**)⁴³.

All the structures of target molecules **32a-q** were established based on their analytical and spectral studies. Compound **32a** is taken as an example to explain the FT-IR spectrum. The compound showed a characteristic peak due to C=N at 1614 cm⁻¹. The ¹H-NMR (400 MHz, CDCl₃+DMSO-*d*₆) spectrum of the molecule **32a** exhibits two individual singlets at δ 2.33 and 2.46 for both -CH₃ groups on pyrazole ring, characteristic pyrazole ring proton appeared as a singlet at δ 6.18 ppm. The remaining five aromatic protons displayed peaks in the aromatic range from δ 7.58 to 7.64 ppm. The ¹³C-NMR (100 MHz, CDCl₃+DMSO-*d*₆) spectrum of compound **32a** recorded two peaks at δ 11.6 and 13.8 for the -CH₃ carbons and peak at δ 108.5 for the pyrazole carbon. The sp² hybridized carbon peaks appeared in the aromatic region from δ 126.7-152.0 ppm. The ESI-MS of compound **32a** displayed a molecular ion peak at *m/z*: 297 [M+H]⁺.

Anti-microbial evaluation:

Assay of *in vitro* anti-bacterial activity, bacterial strains were purchased from a national collection of industrial microorganisms, Pune, India. Antibacterial activity was tested against Gram-positive bacteria *Staphylococcus aureus*, *Bacillus cereus*, and Gram-negative bacteria *Escherichia coli*, *Salmonella paratyphi* using streptomycin as the reference drug.

The sterilized nutrient agar medium was distributed 100 mL each in two 250 mL conical flasks and allowed to cool to room temperature. To these media, 18-24 h grown bacterial sub-cultures were added and shaken thoroughly to ensure uniform distribution of organisms throughout the medium.

Then, agar medium was distributed in equal portions, in sterilized Petri dishes, ensuring that each petri dish contains about 900 µg/ml of the medium. The medium was then allowed for solidification. The cups were made with the help of a sterile cork borer (6 mm diameter) punching into the set of agar media. The solutions of required concentrations (100 µg/ml) of test compounds were prepared by dissolving the compounds in DMSO were filled into the cups with 1 mL of respective solution. Then, the Petri dishes were kept for incubation in an inverted position for 24-48 h at 37°C in an incubator. When growth inhibition zones were developed surrounding each cup, their diameter in mm was measured and compared with that of the standard drugs. Each experiment was made in triplicate using DMSO as a control⁴⁸.

Results:

All the newly synthesized compounds (**32a-q**) were investigated for their antibacterial activity. Compounds **32a**, **32g**, and **32p** showed good activity against *Staphylococcus aureus*, and compounds **32e**, **32f**, and **32j** showed good activity against *Bacillus cereus*. Gram-positive bacteria had a thick cell wall, containing a high amount of peptidoglycan and Gram-negative bacteria had two layers of cell membrane: the inner membrane contains peptidoglycan and the outer membrane contains lipopolysaccharides (**Table-2**).

Table-2: Anti-bacterial evaluation for the newly synthesized compounds **32a-q**.

Compd	Conc. µg/ml	Minimum Inhibitory Concentration			
		<i>Escherichia coli</i>	<i>Salmonella paratyphi</i>	<i>Bacillus cereus</i>	<i>Staphylococcus aureus</i>
32a	900	450	200	200	150
32b	900	400	300	200	450
32c	900	400	450	600	200
32d	900	700	600	370	300
32e	900	160	260	550	500
32f	900	400	500	140	400
32g	900	410	340	210	140
32h	900	700	650	600	640
32i	900	500	420	500	550

32j	900	600	400	130	400
32k	900	00	00	560	700
32l	900	700	500	400	440
32m	900	00	00	00	500
32n	900	00	400	00	00
32o	900	00	00	700	00
32p	900	00	700	00	120
32q	900	00	500	00	650
Standard (Streptomycin)	900	110	100	100	100

Molecular Docking Studies:

Molecular docking studies are very useful to examine and to gain a wise observation in the way of binding interactions of each ligand molecules (**32a-q**) with receptor structure. All the chemical structures (new ligands) were drawn by using ChemDraw Ultra 12.0 and 2D structures drawn and converted to mol2 format by using Open Babel GUI version 2.3.2 (OpenBableGUI; Chris Morley, USA), Molecular energy was minimized using the Energy Minimization module of Maestro Tool(Schrodinger software) under the Chemistry at Harvard Macromolecular Mechanics(CHARMM) force field. The three-dimensional structure of the receptor structure was retrieved from the RCSB database (PDB ID: 1M17). The structure preparation and correction of protein were performed using the protein preparation suite. The target protein file was prepared by removing the structural water molecule, heteroatoms, and co-factors by leaving only the residues associated with protein. The protein preparation suite (wizard) tool was used to prepare the target protein file by addition of polar hydrogen atoms to the macromolecule, an essential step to correct the calculation of partial charge by keeping all other values as default. Further, the grid was prepared and molecular docking was performed using the Glide docking module, and the results obtained were scrutinized based on the highest dock score and number of H-bonds by visualizing in Pymol.

In silico molecular docking studies are made interactions between the donors and acceptors. In general, Hydrogen bonds are formed between a hydrogen atom bound to a small,

highly electronegative atom and another small, highly electronegative atom with an unshared electron pair. The elements that frequently contribute to hydrogen bonds are nitrogen, oxygen, and fluorine. In our study also proven that the receptor and ligand have made interactions between the oxygen and nitrogen atoms and the distance measured in angstrom (Å) as shown in **Table-3**.

Epidermal Growth Factor Receptor (EGFR) is found on the surface of the normal cells and is involved in cell growth. It is found to be at high levels on some types of cancer cells, which causes these cells to grow and divide. Blocking EGFR may keep cancer cells from growing. Some EGFR tyrosine kinase inhibitors are used to treat cancer.

Table-3: Molecular interactions with ligands against Epidermal Growth Factor Receptor tyrosine kinase (1M17.pdb).

Ligand	Receptor Interaction Atoms	Ligand Atoms	Distance (Å)	Docking (Kcal/mol)
32a	ALA719-O	NH	2.66	-89.324
	LEU764-O	NH	2.88	
32b	ALA719-O	NH	2.66	-88.141
	LEU764-O	NH	2.88	
32c	ALA719-O	NH	2.70	-88.019
	LEU764-O	NH	2.89	
32d	ASP831-OD2	NH	2.70	-87.130
	THR830-OG1	NH	2.85	
32e	ALA719-O	NH	2.69	-89.001
	LEU764-O	NH	2.88	
32f	ALA719-O	NH	2.70	-89.0125
	LEU764-O	NH	2.89	
32g	ALA719-O	NH	2.69	-89.254
	LEU764-O	NH	2.87	
32h	ALA719-O	NH	2.69	-88.911
	LEU764-O	NH	2.90	

32i	ALA719-O	NH	2.91	-87.021
	THR766-OG1	NH	2.94	
32j	ASP831-NH	O	2.54	-89.351
	THR766-OG1	NH	2.91	
32k	MET769-O	NH	2.80	-87.958
32l	ASP831-OD2	NH	2.80	-88.214
	ASP831-OD2	NH	2.83	
32m	ASP831-OD2	NH	2.79	-86.274
	THR830-OG1	NH	2.95	
32n	MET769-O	NH	2.79	-88.124
32o	THR830-OG1	NH	2.91	-88.017
	ASP831-OD2	NH	2.60	
	ASP831-OD2	NH	2.74	
32p	ASP831-OD2	NH	2.69	-89.098
32q	ALA719-O	NH	2.95	88.316
	LEU764-O	NH	2.85	

Our molecular docking studies revealed that all the synthesized molecules exhibited excellent binding energies towards the receptor active sites. Molecular docking results were identified basis on the ideal interacted ligands were scrutinized based on the greatest ligand binding poses were identified using the low binding energy, high docking score, and the number of H-bonding, hydrophobic interactions at receptor site i.e., **32a**, **32e**, **32f**, **32g**, **32j**, and **32p** (**Fig. 3**). **Table-3** represents the docking score, Hydrogen bond distance, and interacting atoms. All the compounds were found to be buried.

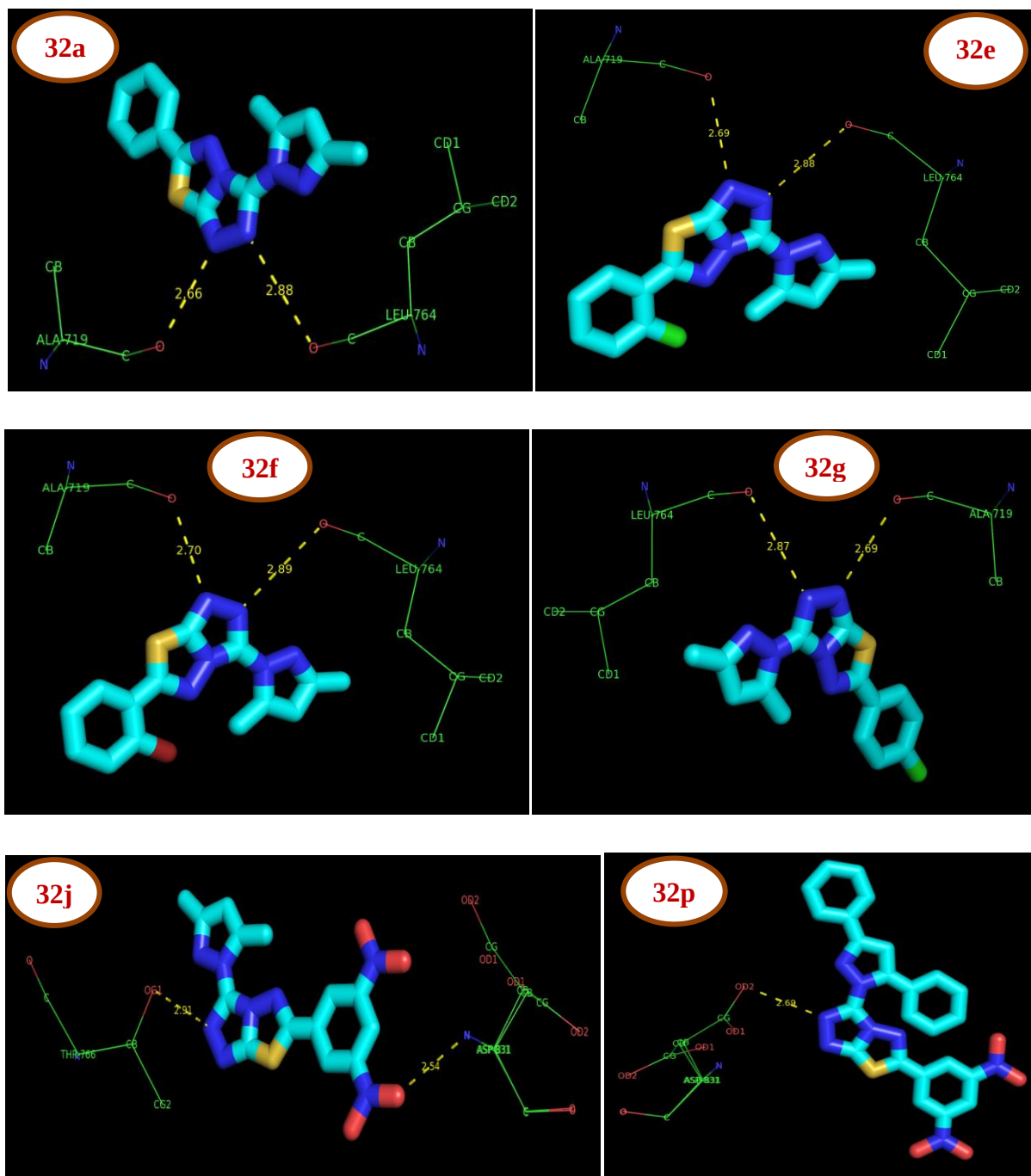


Fig. 3: Good docking interaction images of compounds 32a, 32e, 32f, 32g, 32j, and 32p with receptor 1M17.

In summary, we have synthesized pyrazolyl[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole derivatives (4a-q) through a one-pot sequential addition of three-component reaction with maximum yields (85-92%) using readily available starting compounds in the laboratory. We

have developed a tricyclic system *via* a one-pot multi-component approach. Further, all the compounds were evaluated for the antibacterial activity against Gram-positive and Gram-negative bacteria, among these **4a**, **4e**, **4f**, **4g**, **4j**, and **4p** gave good results against respective bacteria. Additionally, *In silico* molecular docking studies were carried out using EGFR-tyrosine kinase receptor (1M17) with the newly synthesized compounds. Among all the compounds **4a**, **4e**, **4f**, **4g**, **4j**, and **4p** were exhibited good docking interactions.

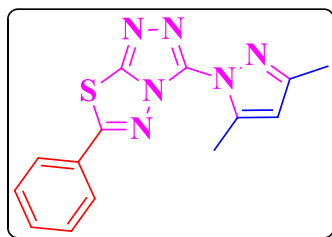
EXPERIMENTAL:

General procedure for the synthesis of 6-phenyl-3-(1*H*-pyrazol-1-yl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole derivatives (32a-q).

A mixture with purpald **1** (1mmol) and diketone **2** (1mmol) was refluxed for 3-5 h in presence of 4 mL of ethanol in a 25 mL RB-flask. The solvent was evaporated, to the same flask substituted benzoic acid **3** (1mmol) was added sequentially, and continued the reaction in presence of 2 mL of POCl₃ under the reflux condition for 7-9 h. After the completion of the reaction was cooled to room temperature, neutralized with NaHCO₃ solution, the separated solid was filtered and recrystallized from 5-8 mL ethanol.

SPECTRAL DATA:

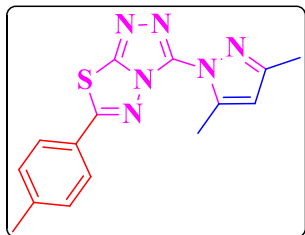
3-(3,5-Dimethyl-1*H*-pyrazol-1-yl)-6-phenyl-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (32a):



Grey color solid (0.260g, 88%); m.p.: 175-177°C; **IR (KBr, $\nu_{\text{max}}/\text{cm}^{-1}$):** 1614 (C=N); **¹H-NMR (400 MHz, CDCl₃+DMSO-*d*₆, δ ppm):** 2.33 (s, 3H, -CH₃), 2.46 (s, 3H, -CH₃), 6.18 (s, 1H, -CH- of pyrazole ring), 7.58 (t, 3H, *J*=7.6 Hz, Ar-H), 7.64 (d, 2H, *J*=8.8 Hz, Ar-H); **¹³C-NMR (100 MHz, CDCl₃+DMSO-*d*₆, δ ppm):**

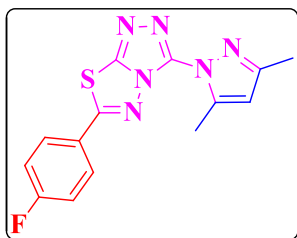
11.6, 13.8, 108.5, 126.7, 127.7, 129.1, 129.5, 129.6, 130.1, 133.5, 143.5, 152.0; **ESI-MS (*m/z*):** 297 [M+H]⁺; Analytical calculated formulae C₁₄H₁₂N₆S: C, 56.74; H, 4.08; N, 28.36; S, 10.82; Found: C, 56.70; H, 4.14; N, 28.40; S, 10.86.

3-(3,5-Dimethyl-1H-pyrazol-1-yl)-6-(p-tolyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (32b):



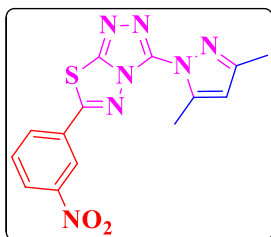
Pale green color solid (0.279g, 90%); m.p.: 170-172°C; **IR (KBr, $\nu_{\max}/\text{cm}^{-1}$):** 1610 (C=N); **$^1\text{H-NMR}$ (400 MHz, CDCl_3 , δ ppm):** 2.29 (s, 3H, -CH₃), 2.33 (s, 3H, -CH₃), 2.42 (s, 3H, -CH₃), 6.06 (s, 1H, -CH- of pyrazole ring), 7.25 (d, 2H, $J=7.6$ Hz, Ar-H), 7.66 (d, 2H, $J=7.6$ Hz, Ar-H); **$^{13}\text{C-NMR}$ (100 MHz, CDCl_3 , δ ppm):** 11.3, 13.6, 21.8, 107.9, 129.1, 129.4, 129.6, 129.7, 129.9, 143.5, 143.7, 152.7, 164.4; **ESI-MS (m/z):** 311 $[\text{M}+\text{H}]^+$; Analytical calculated formulae $\text{C}_{15}\text{H}_{14}\text{N}_6\text{S}$: C, 58.05; H, 4.55; N, 27.08; S, 10.33; Found: C, 58.16; H, 4.51; N, 27.14; S, 10.37.

3-(3,5-Dimethyl-1H-pyrazol-1-yl)-6-(4-fluorophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (32c):



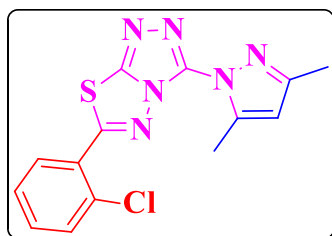
Light green color solid (0.270g, 86%); m.p.: 162-164°C; **IR (KBr, $\nu_{\max}/\text{cm}^{-1}$):** 1602 (C=N); **$^1\text{H-NMR}$ (400 MHz, $\text{CDCl}_3+\text{DMSO}-d_6$, δ ppm):** 2.35 (s, 3H, -CH₃), 2.49 (s, 3H, -CH₃), 6.16 (s, 1H, -CH- of pyrazole ring), 7.28 (t, 2H, $J=8.8$ Hz, Ar-H), 7.97 (dd, 2H, $J=8.8$ Hz, $J=5.2$ Hz, Ar-H); **$^{13}\text{C-NMR}$ (100 MHz, $\text{CDCl}_3+\text{DMSO}-d_6$, δ ppm):** 11.6, 13.8, 108.4, 116.8, 117.0, 117.2, 125.5, 130.2, 134.5, 143.2, 152.1, 166.6; **ESI-MS (m/z):** 315 $[\text{M}+\text{H}]^+$; Analytical calculated formulae $\text{C}_{14}\text{H}_{11}\text{FN}_6\text{S}$: C, 53.49; H, 3.53; N, 26.74; S, 10.20; Found: C, 53.53; H, 3.57; N, 26.70; S, 10.24.

3-(3,5-Dimethyl-1H-pyrazol-1-yl)-6-(3-nitrophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (32d):



Yellow color solid (0.289g, 85%); m.p.: 168-170°C; **IR (KBr, $\nu_{\max}/\text{cm}^{-1}$):** 1614 (C=N); **$^1\text{H-NMR}$ (400 MHz, $\text{DMSO}-d_6$, δ ppm):** 2.35 (s, 3H, -CH₃), 2.48 (s, 3H, -CH₃), 6.19 (s, 1H, -CH- of pyrazole ring), 7.89 (t, 1H, $J=8.4$ Hz, Ar-H), 8.35 (d, 1H, $J=8.8$ Hz, Ar-H), 8.47 (d, 1H, $J=9.2$ Hz, Ar-H), 8.79 (s, 1H, Ar-H); **$^{13}\text{C-NMR}$ (100 MHz, $\text{CDCl}_3+\text{DMSO}-d_6$, δ ppm):** 11.6, 13.7, 108.4, 121.9, 128.5, 129.7, 132.4, 133.9, 134.4, 141.2, 143.2, 144.5, 152.0, 166.7; **ESI-MS (m/z):** 342 $[\text{M}+\text{H}]^+$; Analytical calculated formulae $\text{C}_{14}\text{H}_{11}\text{N}_7\text{O}_2\text{S}$: C, 49.26; H, 3.25; N, 28.72; S, 9.39; Found: C, 49.30; H, 3.29; N, 28.76; S, 9.36.

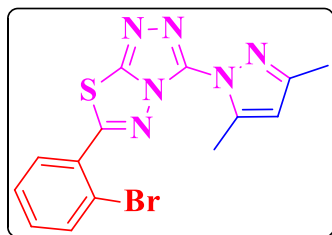
6-(2-Chlorophenyl)-3-(3,5-dimethyl-1*H*-pyrazol-1-yl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (32e):



Pale yellow color solid (0.287g, 87%); m.p.: 165-167°C; **IR (KBr, $\nu_{\max}/\text{cm}^{-1}$):** 1614 (C=N); **$^1\text{H-NMR}$ (400 MHz, $\text{CDCl}_3+\text{DMSO-}d_6$, δ ppm):** 2.31 (s, 3H, -CH₃), 2.49 (s, 3H, -CH₃), 6.15 (s, 1H, -CH- of pyrazole ring), 7.51-7.55 (m, 2H, Ar-H), 7.77-7.81 (m, 2H, Ar-H); **$^{13}\text{C-NMR}$ (100 MHz, $\text{CDCl}_3+\text{DMSO-}d_6$, δ ppm):** 11.3, 13.7,

108.5, 127.5, 128.6, 130.4, 131.4, 132.0, 132.7, 134.2, 143.4, 152.1, 164.5, 168.5; **ESI-MS (*m/z*):** 331 [M+H]⁺; Analytical calculated formulae C₁₄H₁₁ClN₆S: C, 50.83; H, 3.35; N, 25.41; S, 9.69; Found: C, 50.80; H, 3.30; N, 25.45; S, 9.65.

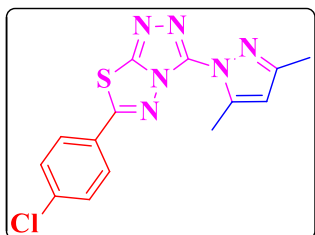
6-(2-Bromophenyl)-3-(3,5-dimethyl-1*H*-pyrazol-1-yl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (32f):



Pale yellow color solid (0.337g, 90%); m.p.: 159-161°C; **IR (KBr, $\nu_{\max}/\text{cm}^{-1}$):** 1608 (C=N); **$^1\text{H-NMR}$ (400 MHz, $\text{CDCl}_3+\text{DMSO-}d_6$, δ ppm):** 2.31 (s, 3H, -CH₃), 2.49 (s, 3H, -CH₃), 6.15 (s, 1H, -CH- of pyrazole ring), 7.51-7.55 (m, 2H, Ar-H), 7.80 (d, 1H, *J*=8.8 Hz, Ar-H), 7.91 (d, 1H, *J*=9.2 Hz, Ar-H); **$^{13}\text{C-NMR}$ (100 MHz, $\text{CDCl}_3+\text{DMSO-}d_6$, δ ppm):** 11.7, 13.8, 108.4, 121.9, 128.6, 129.7, 132.4, 133.9, 134.4, 141.2,

143.2, 144.5, 152.1, 166.7; **ESI-MS (*m/z*):** 377 [M+2]⁺; Analytical calculated formulae C₁₄H₁₁BrN₆S: C, 44.81; H, 2.95; N, 22.40; S, 8.55; Found: C, 44.85; H, 2.91; N, 22.44; S, 8.51.

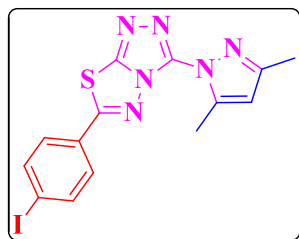
6-(4-Chlorophenyl)-3-(3,5-dimethyl-1*H*-pyrazol-1-yl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (32g):



Pale yellow color solid (0.290g, 88%); m.p.: 163-165°C; **IR (KBr, $\nu_{\max}/\text{cm}^{-1}$):** 1614 (C=N); **$^1\text{H-NMR}$ (400 MHz, $\text{CDCl}_3+\text{DMSO-}d_6$, δ ppm):** 2.34 (s, 3H, -CH₃), 2.47 (s, 3H, -CH₃), 6.17 (s, 1H, -CH- of pyrazole ring), 7.57 (d, 2H, *J*=8.0 Hz, Ar-H), 7.93 (d, 2H, *J*=8.0 Hz, Ar-H); **$^{13}\text{C-NMR}$ (100 MHz, $\text{DMSO-}d_6$, δ ppm):** 11.3, 13.7 108.7,

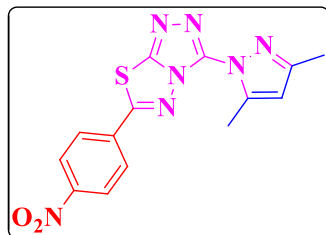
127.9, 129.5, 129.7, 129.9, 130.2, 138.3, 143.7, 152.4, 167.1; **ESI-MS (*m/z*):** 331 [M+H]⁺; Analytical calculated formulae C₁₄H₁₁ClN₆S: C, 50.83; H, 3.35; N, 25.41; S, 9.69; Found: C, 50.80; H, 3.31; N, 25.45; S, 9.72.

3-(3,5-Dimethyl-1H-pyrazol-1-yl)-6-(4-iodophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (32h):



Yellow color solid (0.362g, 86%); m.p.: 174-176°C; **IR (KBr, $\nu_{\max}/\text{cm}^{-1}$):** 1595 (C=N); **$^1\text{H-NMR}$ (400 MHz, DMSO- d_6 , δ ppm):** 2.32 (s, 3H, -CH₃), 2.45 (s, 3H, -CH₃), 6.18 (s, 1H, -CH- of pyrazole ring), 7.71 (d, 2H, $J=8.8$ Hz, Ar-H), 7.94 (d, 2H, $J=8.4$ Hz, Ar-H); **$^{13}\text{C-NMR}$ (100 MHz, CDCl₃+DMSO- d_6 , δ ppm):** 11.4, 13.8, 108.5, 129.2, 129.6, 132.1, 133.8, 138.9, 140.7, 143.6, 152.2, 167.1; **ESI-MS (m/z):** 424 [M+2]⁺; Analytical calculated formulae C₁₄H₁₁IN₆S: C, 39.82; H, 2.63; N, 19.90; S, 7.59; Found: C, 39.86; H, 2.67; N, 19.94; S, 7.55.

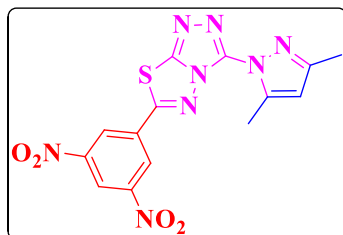
3-(3,5-Dimethyl-1H-pyrazol-1-yl)-6-(4-nitrophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (32i):



Brown color solid (0.289g, 85%; m.p.: 182-184°C; **IR (KBr, $\nu_{\max}/\text{cm}^{-1}$):** 1593 (C=N); **$^1\text{H-NMR}$ (400 MHz, CDCl₃+DMSO- d_6 , δ ppm):** 2.33 (s, 3H, -CH₃), 2.46 (s, 3H, -CH₃), 6.16 (s, 1H, -CH- of pyrazole ring), 7.55 (d, 2H, $J=5.6$ Hz, Ar-H), 7.91 (d, 2H, $J=5.6$ Hz, Ar-H); **$^{13}\text{C-NMR}$ (100 MHz, CDCl₃+DMSO- d_6 , δ ppm):**

11.5, 13.7, 108.7, 127.9, 129.5, 130.2, 138.3, 141.1, 143.7, 152.4, 167.1; **ESI-MS (m/z):** 342 [M+H]⁺; Analytical calculated formulae C₁₄H₁₁N₇O₂S: C, 49.26; H, 3.25; N, 28.72; S, 9.39; Found: C, 49.22; H, 3.29; N, 28.76; S, 9.36.

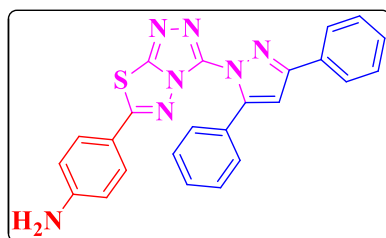
3-(3,5-Dimethyl-1H-pyrazol-1-yl)-6-(3,5-dinitrophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (32j):



Cream color solid (0.343g, 89%); m.p.: 188-190°C; **IR (KBr, $\nu_{\max}/\text{cm}^{-1}$):** 1604 (C=N); **$^1\text{H-NMR}$ (400 MHz, CDCl₃+DMSO- d_6 , δ ppm):** 2.33 (s, 3H, -CH₃), 2.47 (s, 3H, -CH₃), 6.23 (s, 1H, -CH- of pyrazole ring), 9.09 (s, 2H, Ar-H), 9.12 (s, 1H, Ar-H); **$^{13}\text{C-NMR}$ (100 MHz, DMSO- d_6 , δ ppm):** 16.4, 18.6, 113.4, 126.9,

132.7, 136.9, 146.2, 148.3, 153.9, 156.9, 159.2, 169.2; **ESI-MS (m/z):** 387 [M+H]⁺; Analytical calculated formulae C₁₄H₁₀N₈O₄S: C, 43.52; H, 2.61; N, 29.00; S, 8.30; Found: C, 43.55; H, 2.64; N, 28.97; S, 8.33.

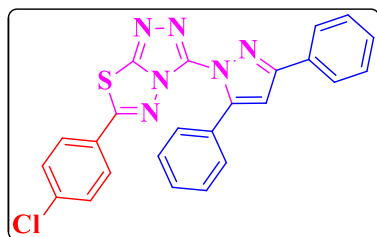
4-(3-(3,5-Diphenyl-1H-pyrazol-1-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-yl)aniline (32k):



Green color solid (0.374g, 86%); m.p.: 154-156°C; **IR (KBr, $\nu_{\max}/\text{cm}^{-1}$):** 1603 (C=N); **$^1\text{H-NMR}$ (400 MHz, $\text{CDCl}_3+\text{DMSO}-d_6$, δ ppm):** 5.70 (s, 2H, -NH₂), 6.67 (d, 2H, $J=8.0$ Hz, Ar-H), 7.12 (s, 1H, -CH- of pyrazole ring), 7.35-7.49 (m, 10H, Ar-H), 7.94 (d, 2H, $J=8.8$ Hz, Ar-H); **$^{13}\text{C-NMR}$ (100**

MHz, $\text{CDCl}_3+\text{DMSO}-d_6$, δ ppm): 110.5, 118.8, 119.7, 131.0, 132.8, 133.4, 134.1, 134.2, 134.5, 136.7, 152.9, 158.9, 159.2, 173.9; **ESI-MS (m/z):** 436 $[\text{M}+\text{H}]^+$; Analytical calculated formulae $\text{C}_{24}\text{H}_{17}\text{N}_7\text{S}$: C, 66.19; H, 3.93; N, 22.51; S, 7.36; Found: C, 66.23; H, 3.90; N, 22.55; S, 7.40.

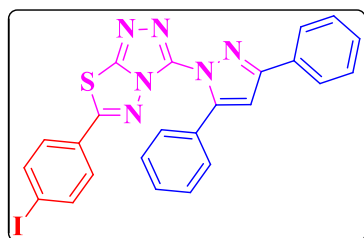
6-(4-Chlorophenyl)-3-(3,5-diphenyl-1H-pyrazol-1-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (32l):



White color solid (0.408g, 90%); m.p.: 11-153°C; **IR (KBr, $\nu_{\max}/\text{cm}^{-1}$):** 1591 (C=N); **$^1\text{H-NMR}$ (400 MHz, $\text{CDCl}_3+\text{DMSO}-d_6$, δ ppm):** 6.59 (s, 1H, -CH- of pyrazole ring), 7.15-7.30 (m, 6H, Ar-H), 7.60 (d, 2H, $J=9.2$ Hz, Ar-H), 7.82 (d, 2H, $J=6.0$ Hz, Ar-H), 7.94 (d, 4H, $J=8.8$ Hz, Ar-H); **$^{13}\text{C-NMR}$ (100 MHz,**

$\text{CDCl}_3+\text{DMSO}-d_6$, δ ppm): 106.0, 126.3, 127.7, 128.1, 128.3, 129.3, 129.5, 131.3, 131.9, 132.9, 134.3, 136.1, 147.6, 148.2, 154.7, 165.4, 166.3, 167.2; **ESI-MS (m/z):** 455 $[\text{M}+\text{H}]^+$; Analytical calculated formulae $\text{C}_{24}\text{H}_{15}\text{ClN}_6\text{S}$: C, 63.36; H, 3.32; N, 18.47; S, 7.05; Found: C, 63.31; H, 3.35; N, 18.51; S, 6.96.

3-(3,5-Diphenyl-1H-pyrazol-1-yl)-6-(4-iodophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (32m):

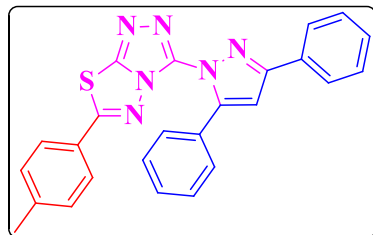


Brown color solid (0.485g, 89%); m.p.: 158-160°C; **IR (KBr, $\nu_{\max}/\text{cm}^{-1}$):** 1598 (C=N); **$^1\text{H-NMR}$ (400 MHz, $\text{CDCl}_3+\text{DMSO}-d_6$, δ ppm):** 6.59 (s, 1H, -CH- of pyrazole ring), 7.30 (d, 2H, $J=7.2$ Hz, Ar-H), 7.37-7.53 (m, 4H, Ar-H), 7.59 (d, 2H, $J=8.0$ Hz, Ar-H), 7.72 (d, 2H, $J=8.0$ Hz, Ar-H), 7.81 (d, 2H, $J=7.6$ Hz,

Ar-H), 7.94 (d, 2H, $J=8.8$ Hz, Ar-H); **$^{13}\text{C-NMR}$ (100 MHz, $\text{CDCl}_3+\text{DMSO}-d_6$, δ ppm):** 101.2, 106.0, 126.2, 127.7, 127.9, 128.1, 128.3, 129.2, 129.5, 130.7, 131.5, 132.9, 137.9, 138.9, 147.7,

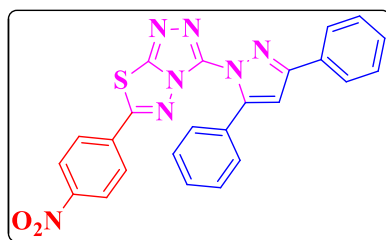
148.2, 154.5, 167.4; **ESI-MS (m/z):** 547 $[M+H]^+$; Analytical calculated formulae $C_{24}H_{15}IN_6S$: C, 52.76; H, 2.77; N, 15.38; S, 5.87; Found: C, 52.72; H, 2.73; N, 15.34; S, 5.82.

3-(3,5-Diphenyl-1H-pyrazol-1-yl)-6-(p-tolyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (32n):



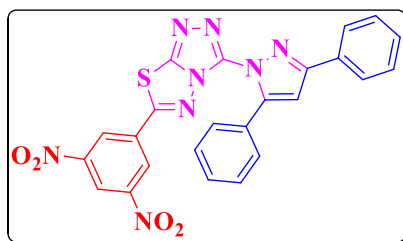
Light yellow color solid (0.399g, 92%); m.p.: 173-175°C; **IR (KBr, $\nu_{\max}/\text{cm}^{-1}$):** 1610 (C=N); **$^1\text{H-NMR}$ (400 MHz, $\text{CDCl}_3+\text{DMSO}-d_6$, δ ppm):** 2.43 (s, 3H, $-\text{CH}_3$), 7.26 (s, 1H, $-\text{CH}-$ of pyrazole ring), 7.43-7.49 (m, 6H, Ar-H), 7.74 (d, 2H, $J=9.2$ Hz, Ar-H), 7.85 (d, 2H, $J=7.2$ Hz, Ar-H), 7.96 (d, 2H, $J=8.0$ Hz, Ar-H), 8.11 (d, 2H, $J=9.2$ Hz, Ar-H); **$^{13}\text{C-NMR}$ (100 MHz, $\text{CDCl}_3+\text{DMSO}-d_6$, δ ppm):** 21.6, 105.9, 126.3, 127.6, 128.1, 128.3, 128.6, 129.1, 129.2, 129.3, 129.5, 129.7, 130.5, 131.9, 144.2, 148.9, 154.6, 168.7; **ESI-MS (m/z):** 435 $[M+H]^+$; Analytical calculated formulae $C_{15}H_{18}N_6S$: C, 69.10; H, 4.18; N, 19.34; S, 7.38; Found: C, 69.14; H, 4.14; N, 19.30; S, 7.33.

3-(3,5-Diphenyl-1H-pyrazol-1-yl)-6-(4-nitrophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (32o):



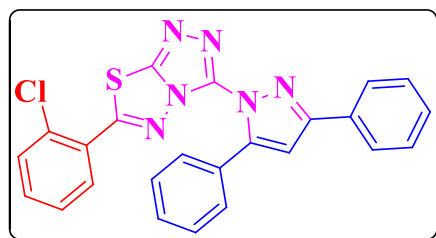
Yellow color solid (0.404g, 87%); m.p.: 159-161°C; **IR (KBr, $\nu_{\max}/\text{cm}^{-1}$):** 1614 (C=N); **$^1\text{H-NMR}$ (400 MHz, $\text{CDCl}_3+\text{DMSO}-d_6$, δ ppm):** 6.88 (s, 1H, $-\text{CH}-$ of pyrazole ring), 7.43 (unresolved singlet, 6H, Ar-H), 7.57 (m, 2H, Ar-H), 7.83 (d, 2H, $J=6.0$ Hz, Ar-H), 8.17 (d, 2H, $J=7.6$ Hz, Ar-H), 8.25 (d, 2H, $J=7.6$ Hz, Ar-H); **$^{13}\text{C-NMR}$ (100 MHz, $\text{CDCl}_3+\text{DMSO}-d_6$, δ ppm):** 106.5, 124.4, 126.3, 128.5, 128.6, 129.2, 129.3, 129.6, 129.8, 129.9, 131.5, 136.5, 144.5, 148.1, 150.4, 154.9, 164.4, 168.4; **ESI-MS (m/z):** 466 $[M+H]^+$; Analytical calculated formulae $C_{24}H_{15}N_7O_2S$: C, 61.93; H, 3.25; N, 21.06; S, 6.89; Found: C, 61.97; H, 3.21; N, 19.96; S, 6.85.

6-(3,5-Dinitrophenyl)-3-(3,5-diphenyl-1H-pyrazol-1-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (32p):



Light yellow color solid (0.438g, 86%); m.p.: 160-162°C; **IR** (KBr, $\nu_{\max}/\text{cm}^{-1}$): 1615 (C=N); **$^1\text{H-NMR}$** (400 MHz, $\text{CDCl}_3+\text{DMSO-}d_6$, δ ppm): 6.91 (s, 1H, -CH- of pyrazole ring), 7.40 (unresolved singlet, 4H, Ar-H), 7.57 (d, 2H, $J=2.4$ Hz, Ar-H), 7.84 (d, 2H, $J=5.6$ Hz, Ar-H), 8.93 (s, 1H, Hz, Ar-H), 9.11 (d, 2H, $J=10$ Hz, Ar-H), 9.44 (s, 2H, Ar-H); **$^{13}\text{C-NMR}$** (100 MHz, $\text{CDCl}_3+\text{DMSO-}d_6$, δ ppm): 107.5, 122.9, 126.3, 128.3, 128.5, 129.2, 129.3, 129.4, 129.9, 133.4, 134.8, 144.3, 148.1, 148.9, 155.0, 157.8, 162.4, 168.3; **ESI-MS** (m/z): 511 $[\text{M}+\text{H}]^+$; Analytical calculated formulae $\text{C}_{24}\text{H}_{14}\text{N}_8\text{O}_4\text{S}$: C, 56.47; H, 2.76; N, 21.95; S, 6.28; Found: C, 56.42; H, 3.00; N, 21.91; S, 6.24.

6-(2-Chlorophenyl)-3-(3,5-diphenyl-1H-pyrazol-1-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (32q):



Brown color solid (0.399g, 88%); m.p.: 154-156°C; **IR** (KBr, $\nu_{\max}/\text{cm}^{-1}$): 1612 (C=N); **$^1\text{H-NMR}$** (400 MHz, $\text{CDCl}_3+\text{DMSO-}d_6$, δ ppm): 6.84 (s, 1H, -CH- of pyrazole ring), 7.31 (unresolved singlet, 2H, Ar-H), 7.42-7.56 (m, 8H, Ar-H), 7.69 (unresolved single, 2H, Ar-H), 7.98(unresolved single, 2H, Ar-H); **$^{13}\text{C-NMR}$** (100 MHz, $\text{CDCl}_3+\text{DMSO-}d_6$, δ ppm): 106.3, 126.4, 127.5, 128.6, 129.2, 129.3, 129.5, 129.7, 129.8, 130.5, 131.0, 131.8, 132.8, 144.7, 148.1, 154.8, 165.1, 168.5; **ESI-MS** (m/z): 455 $[\text{M}+\text{H}]^+$; Analytical calculated formulae $\text{C}_{24}\text{H}_{15}\text{ClN}_8\text{S}$: C, 63.36; H, 3.32; N, 18.47; S, 7.05; Found: C, 63.32; H, 3.36; N, 18.44; S, 6.95.

SPECTRA

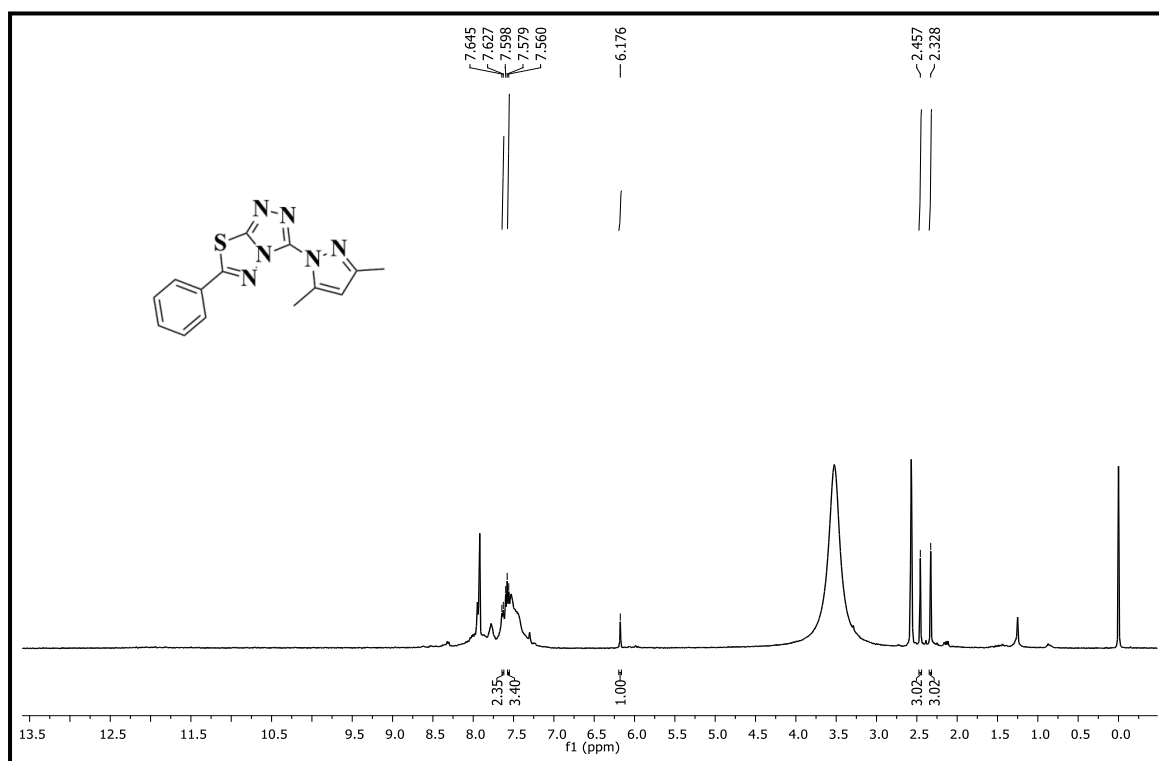


Fig: ¹H-NMR Spectrum of Compound 32a (400 MHz, CDCl₃+DMSO-*d*₆)

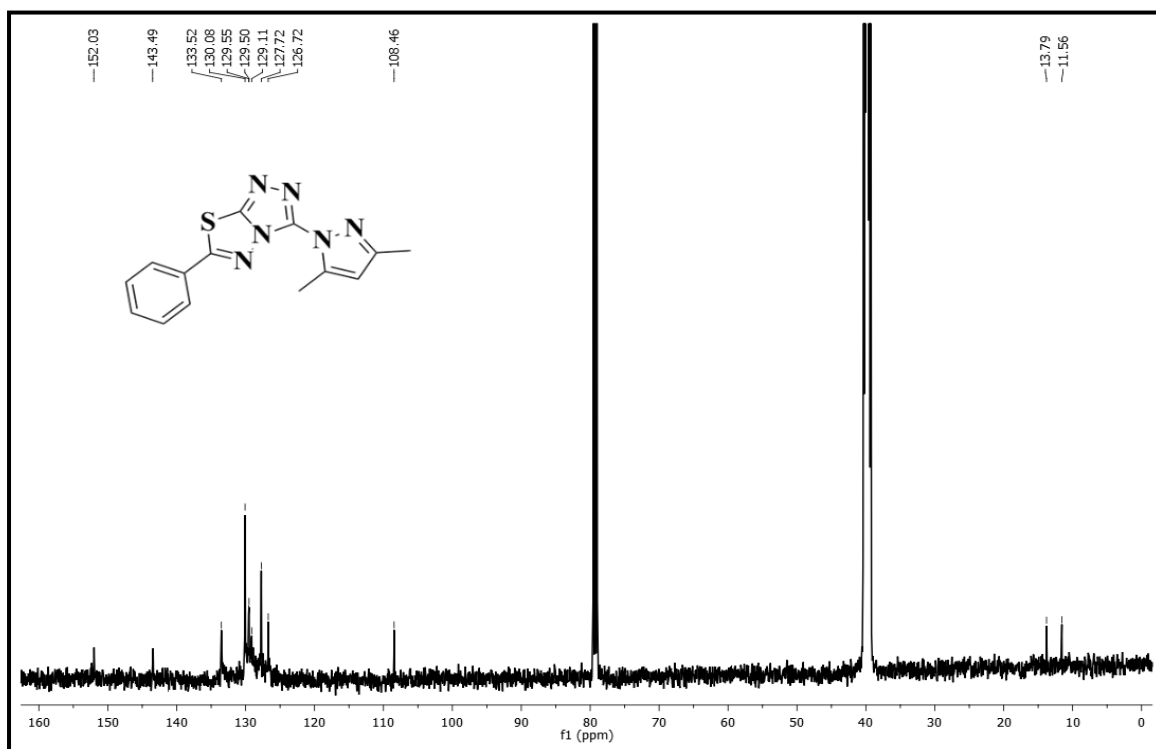


Fig: ¹³C-NMR Spectrum of Compound 32a (100 MHz, CDCl₃+DMSO-*d*₆)

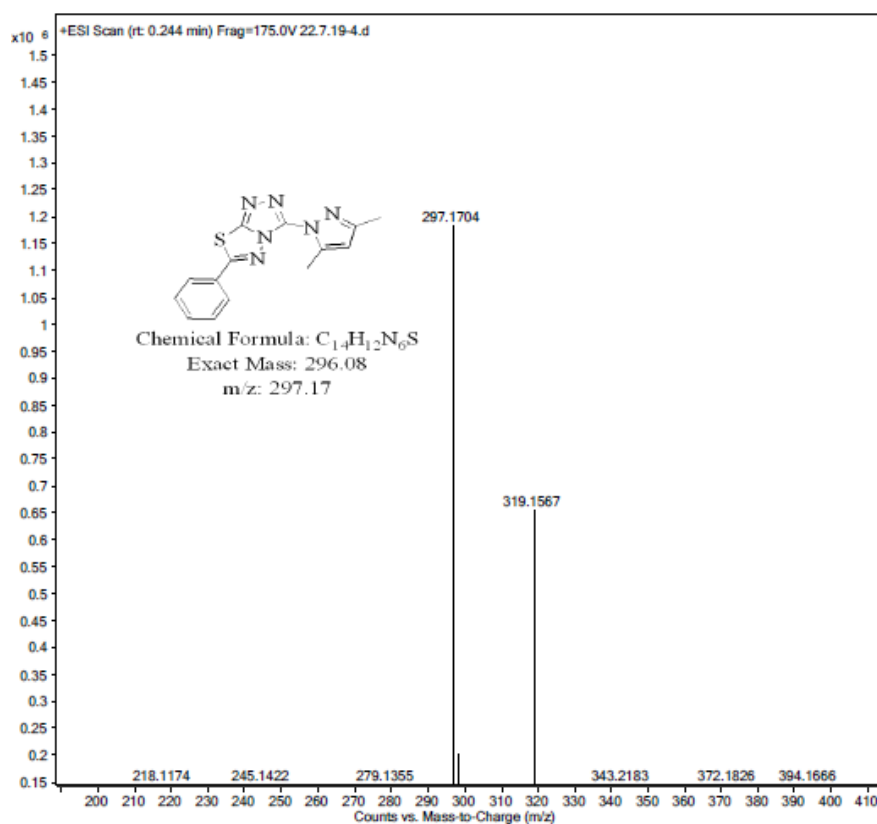


Fig: Mass Spectrum of Compound 32a

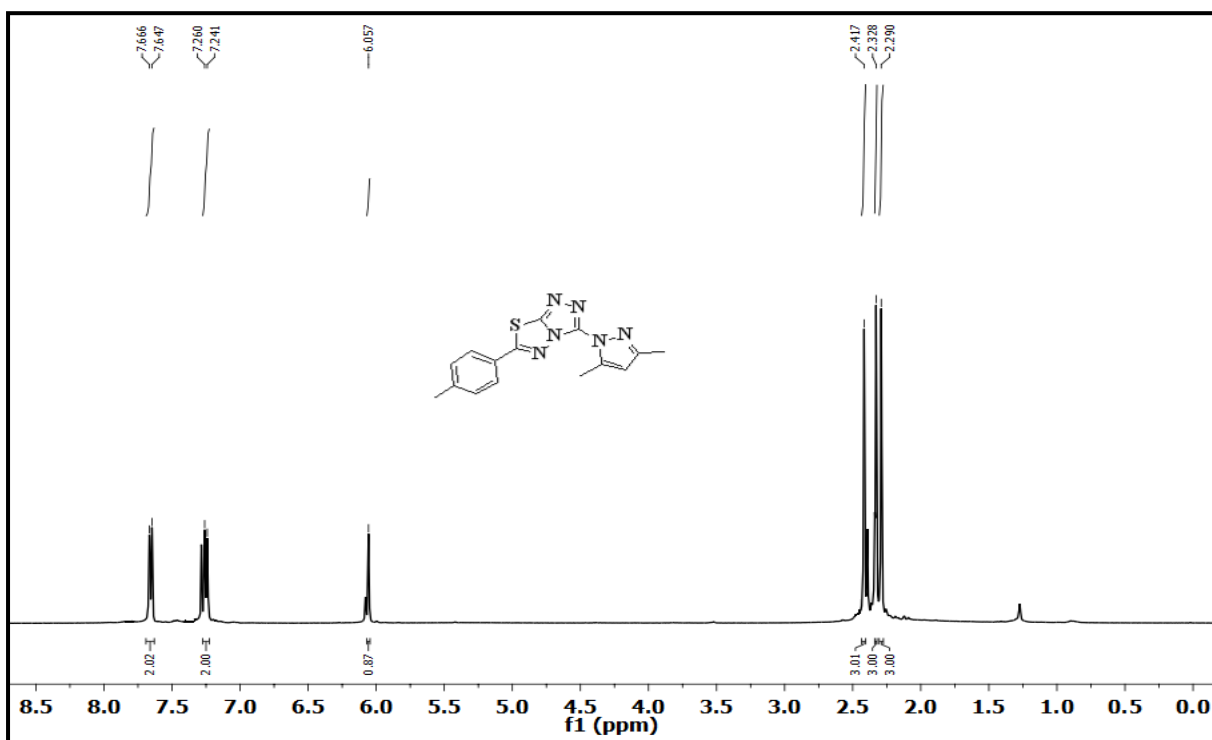


Fig: 1H -NMR Spectrum of Compound 32b (400 MHz, $CDCl_3$)

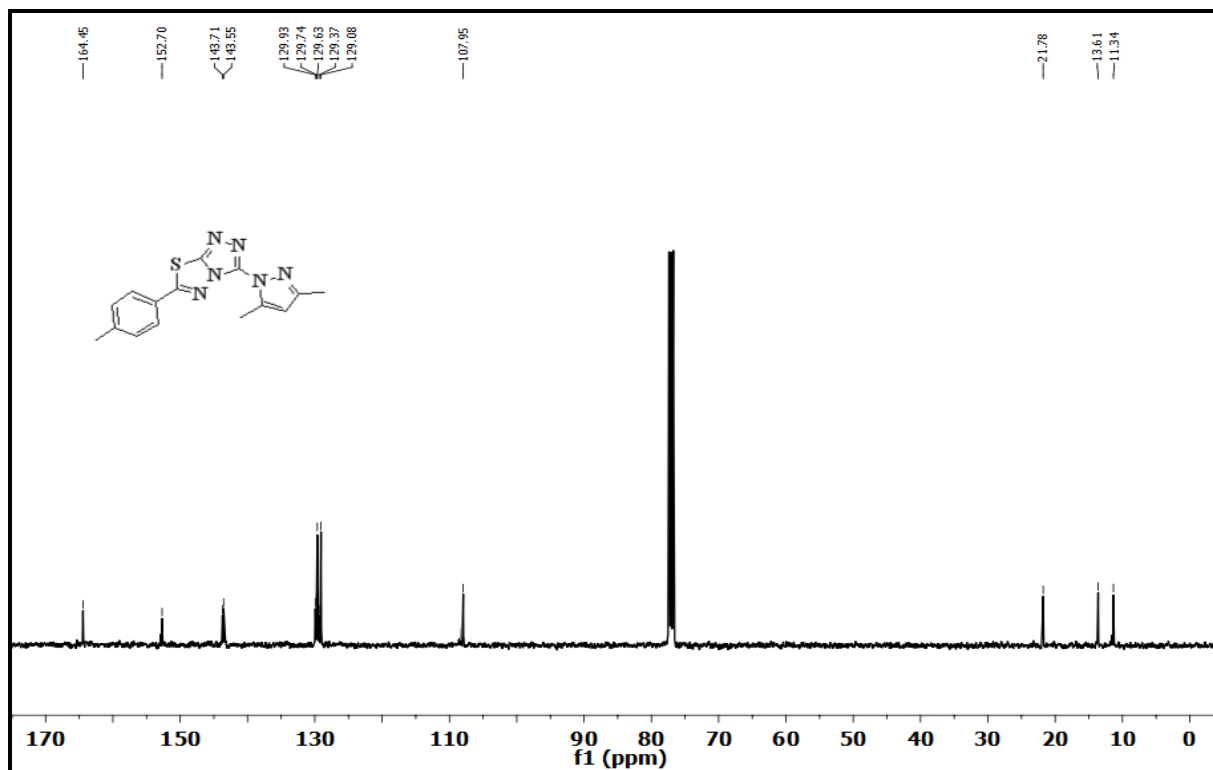


Fig: ¹³C-NMR Spectrum of Compound 32b (100 MHz, CDCl₃)

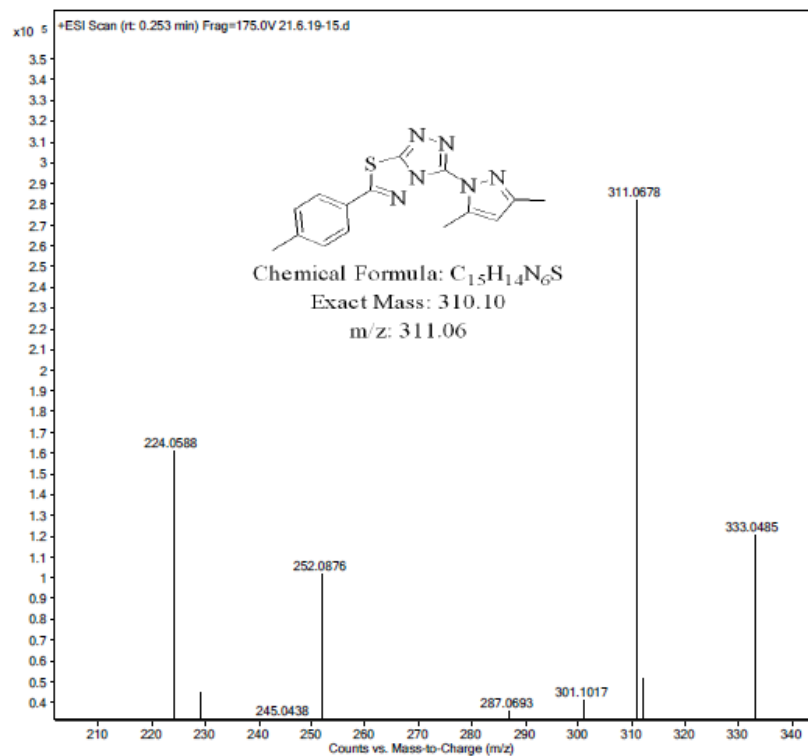


Fig: Mass Spectrum of Compound 32b

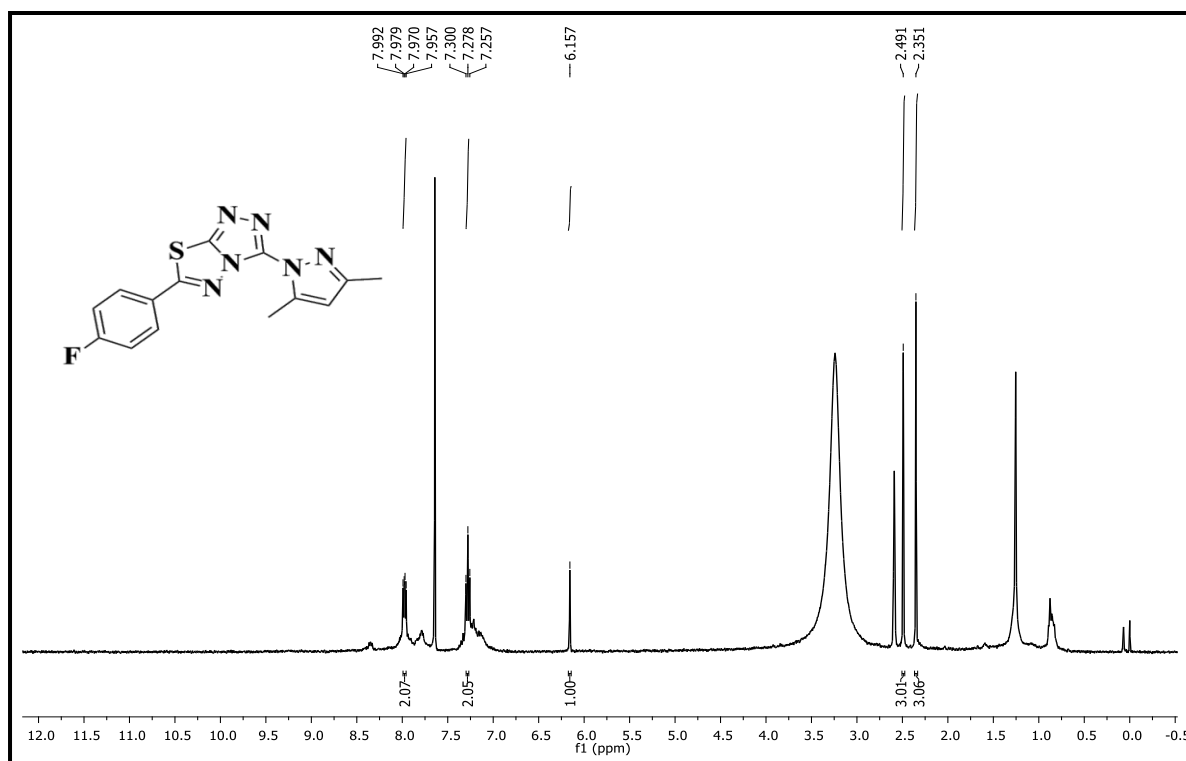


Fig: ¹H-NMR Spectrum of Compound **32c** (400 MHz, CDCl₃+DMSO-*d*₆)

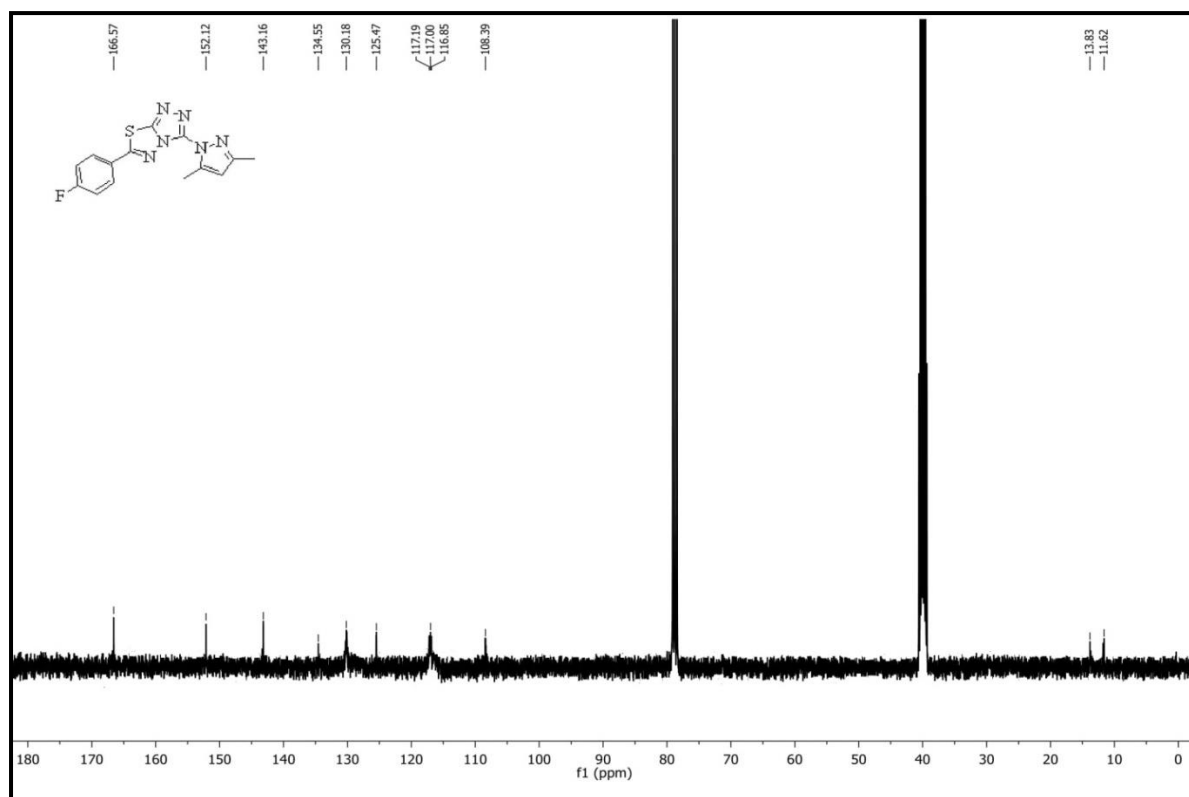


Fig: ¹³C-NMR Spectrum of Compound **32c** (100 MHz, CDCl₃+DMSO-*d*₆)

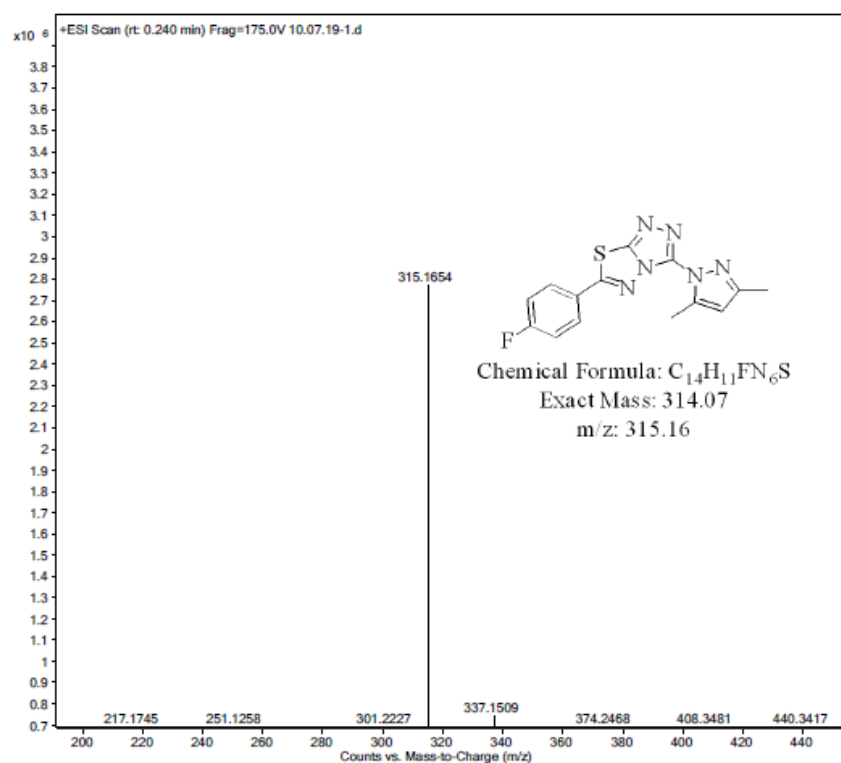


Fig: Mass Spectrum of Compound 32c

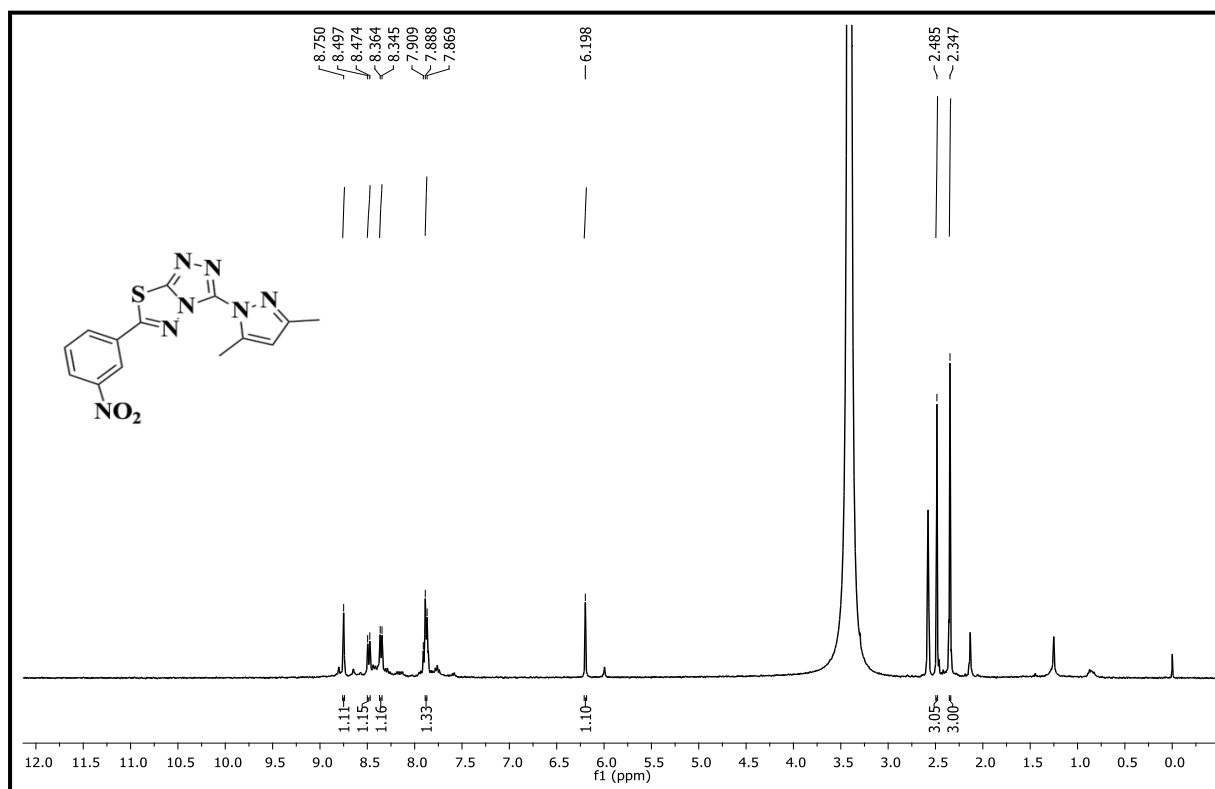


Fig: 1H -NMR Spectrum of Compound 32d (400 MHz, $DMSO-d_6$)

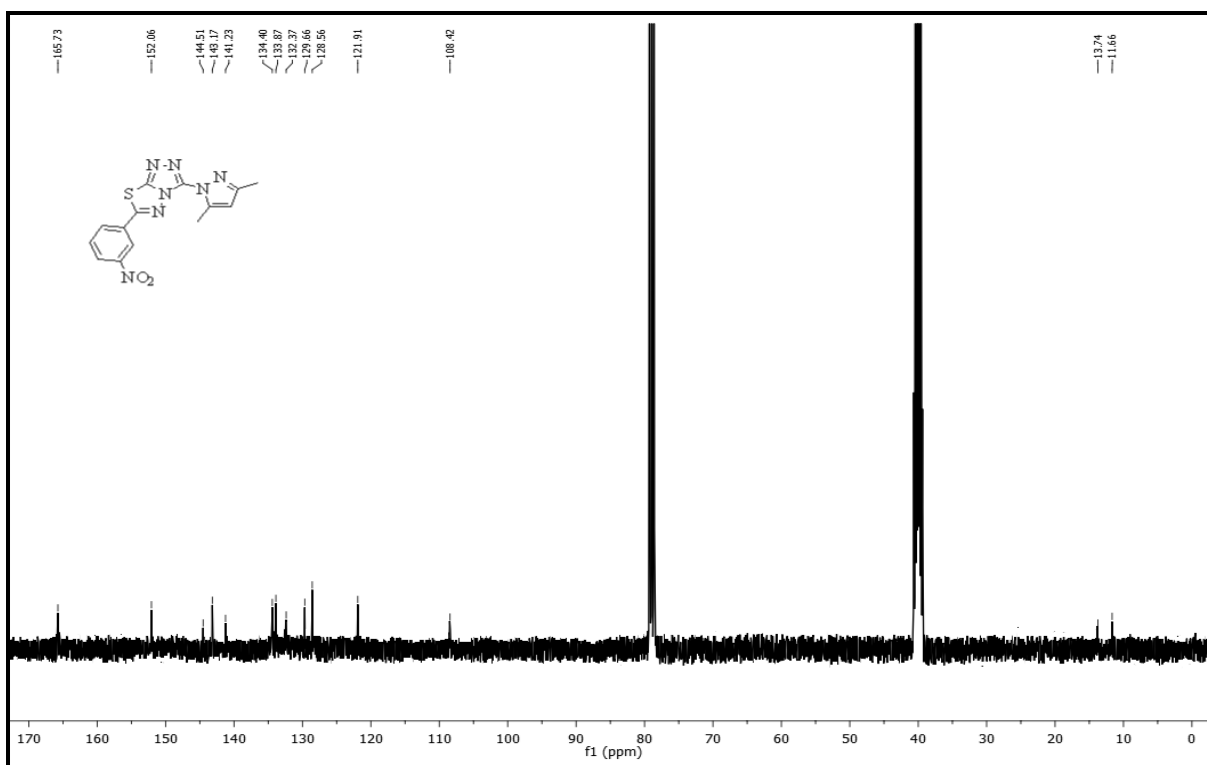


Fig: ¹³C-NMR Spectrum of Compound **32d** (100 MHz, CDCl₃+DMSO-*d*₆)

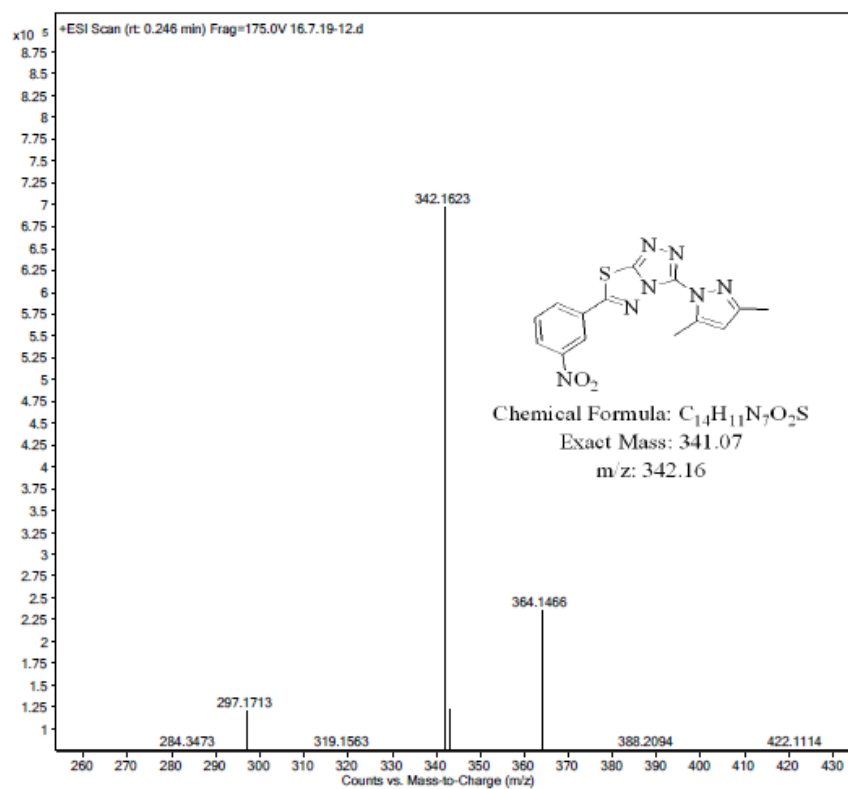


Fig: Mass Spectrum of Compound **32d**

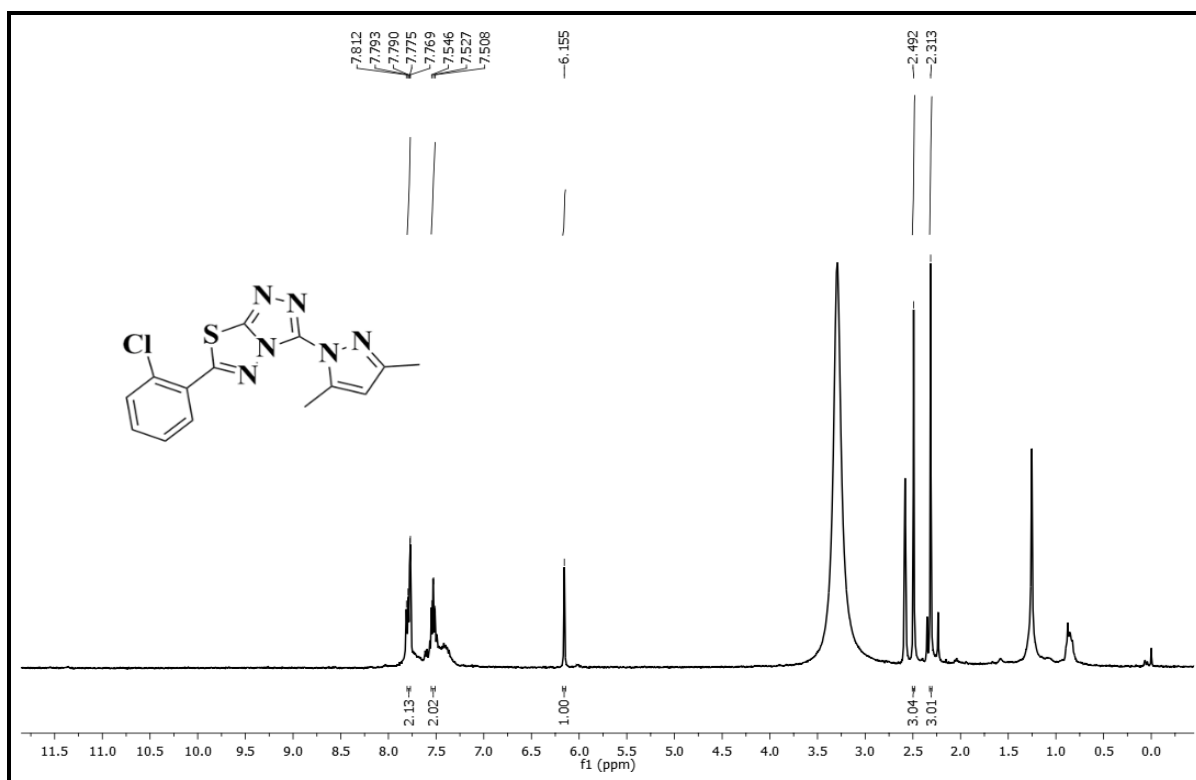


Fig: ¹H-NMR Spectrum of Compound **32e** (400 MHz, CDCl₃+DMSO-*d*₆)

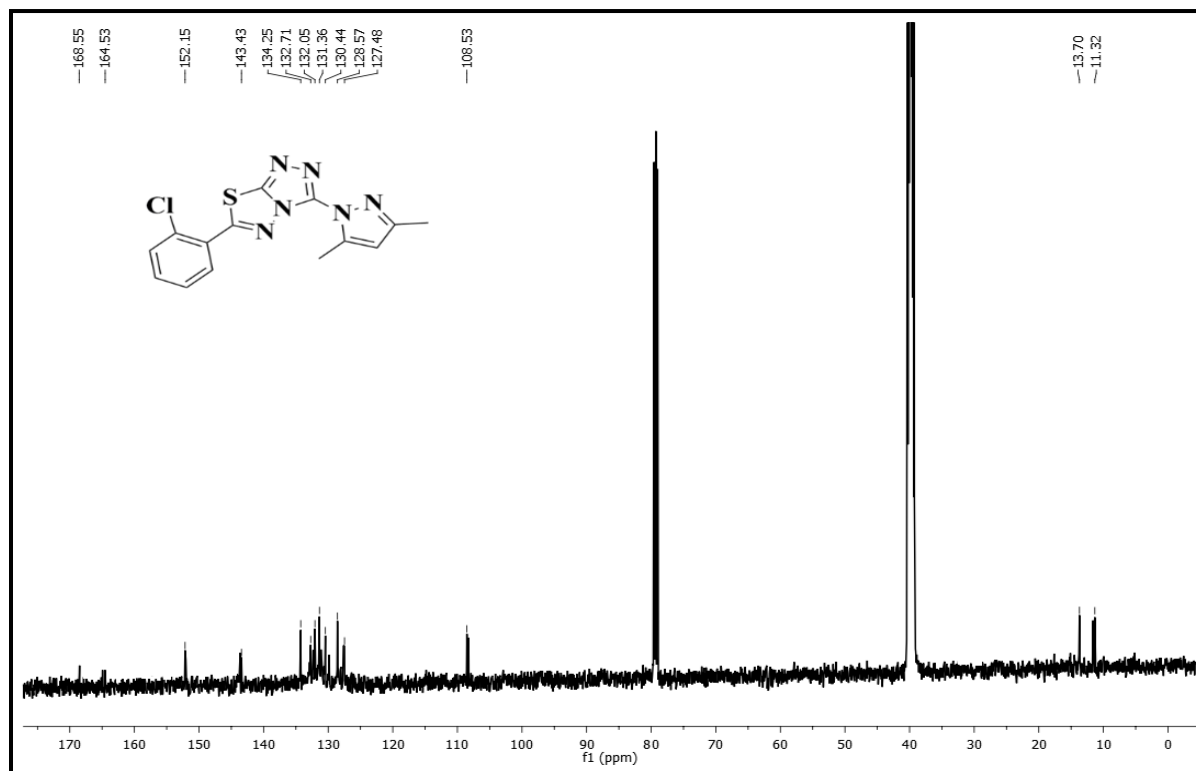


Fig: ¹³C-NMR Spectrum of Compound **32e** (100 MHz, CDCl₃+DMSO-*d*₆)

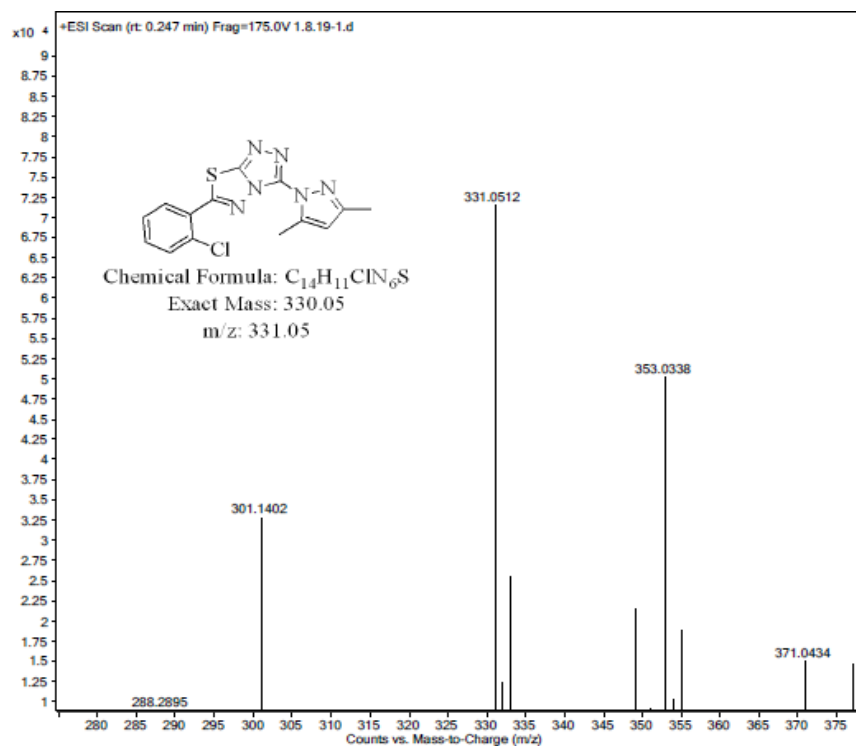


Fig: Mass Spectrum of Compound 32e

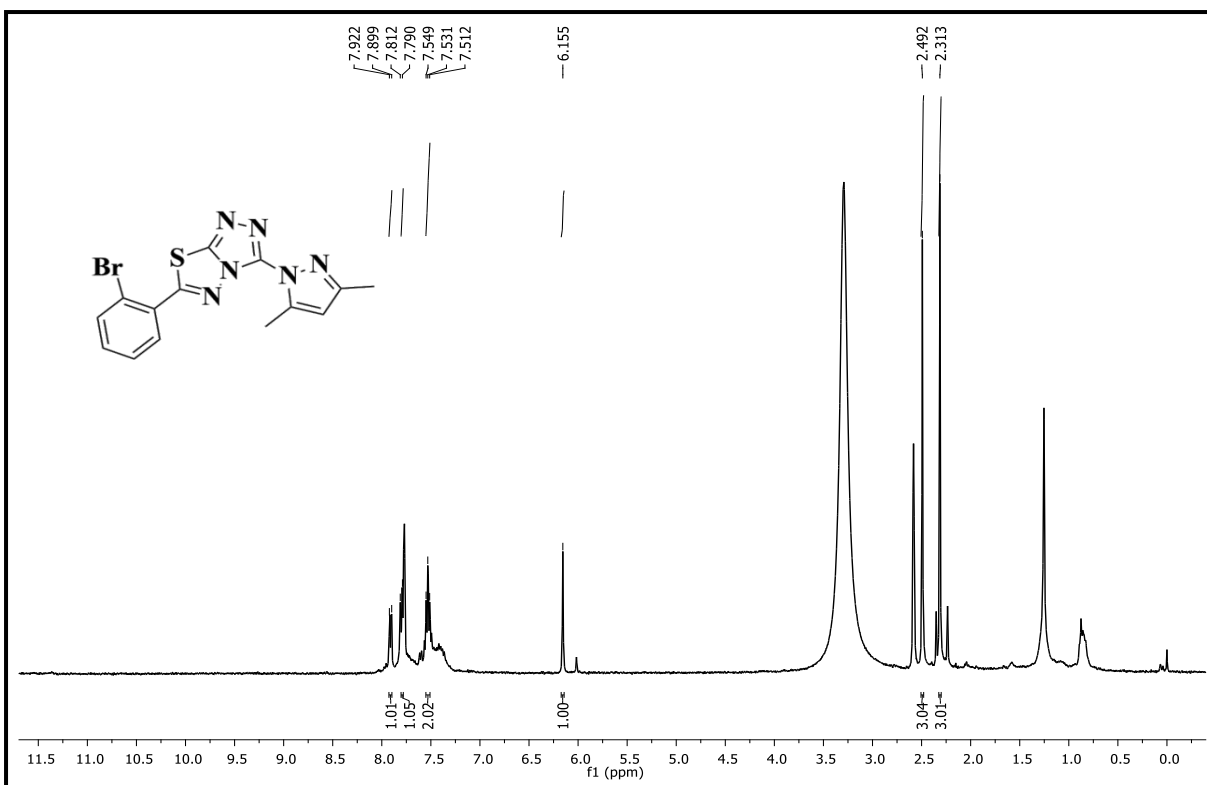


Fig: 1H -NMR Spectrum of Compound 32f (400 MHz, $CDCl_3 + DMSO-d_6$)

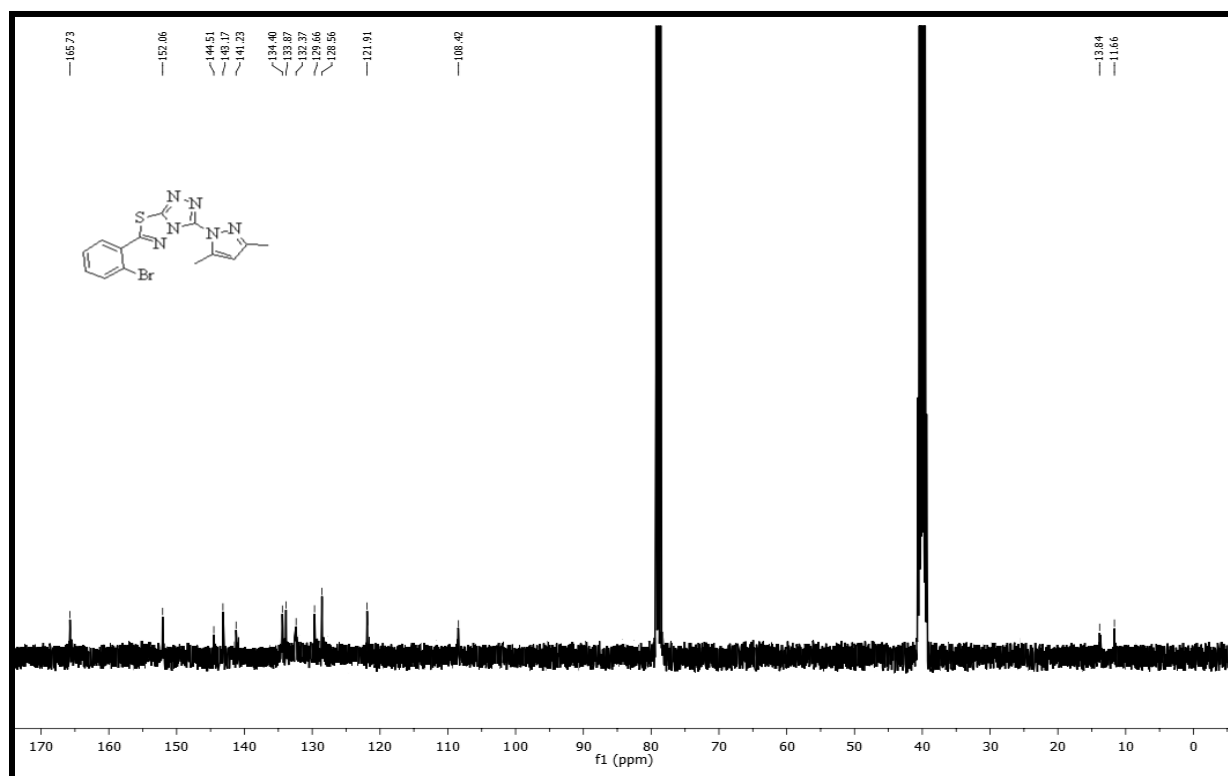


Fig: ^{13}C -NMR Spectrum of Compound **32f** (100 MHz, $\text{CDCl}_3+\text{DMSO}-d_6$)

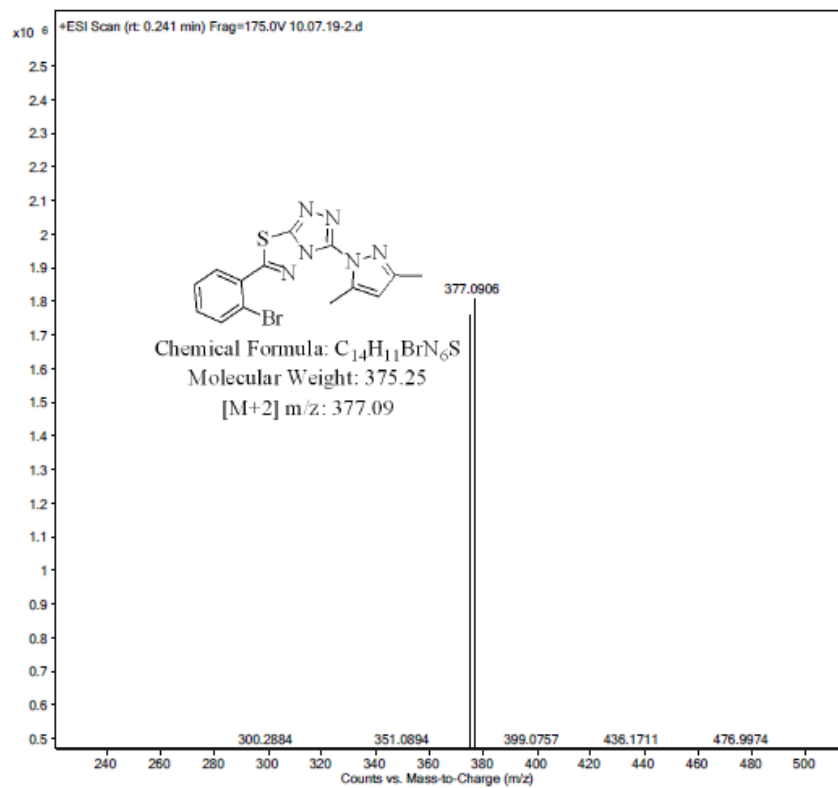


Fig: Mass Spectrum of Compound **32f**

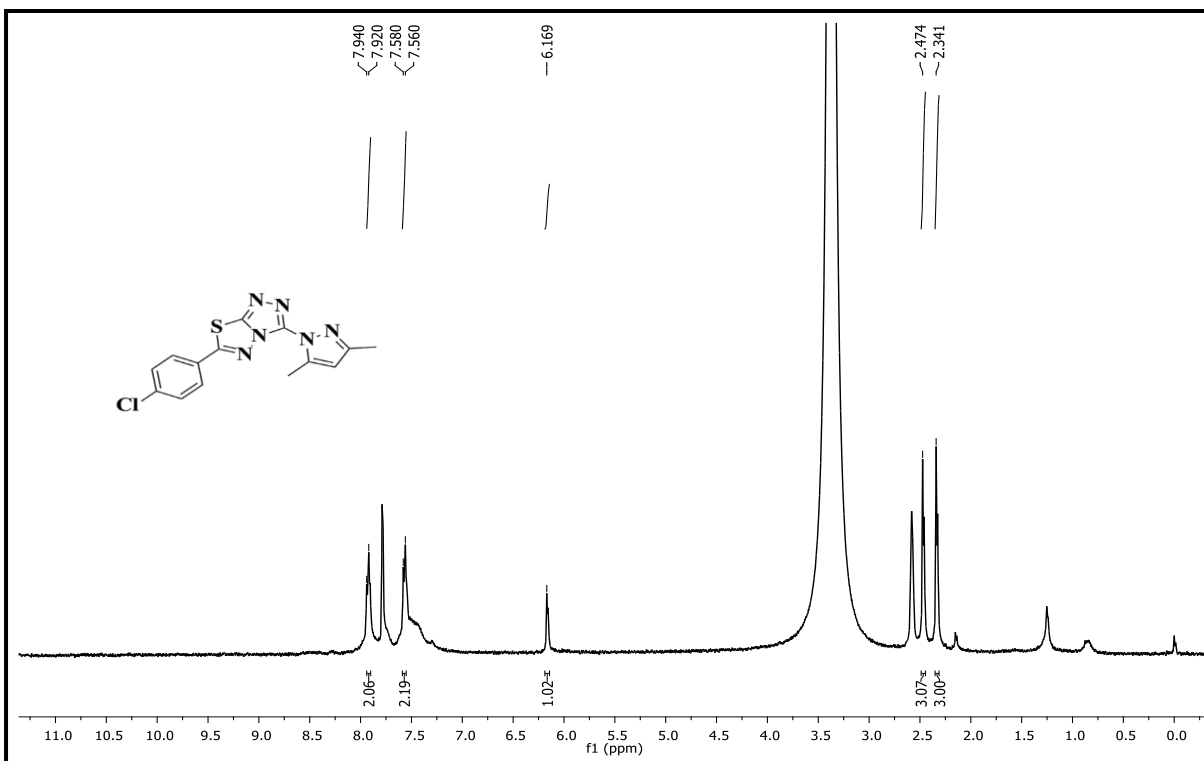


Fig: ¹H-NMR Spectrum of Compound **32g** (400 MHz, CDCl₃+DMSO-*d*₆)

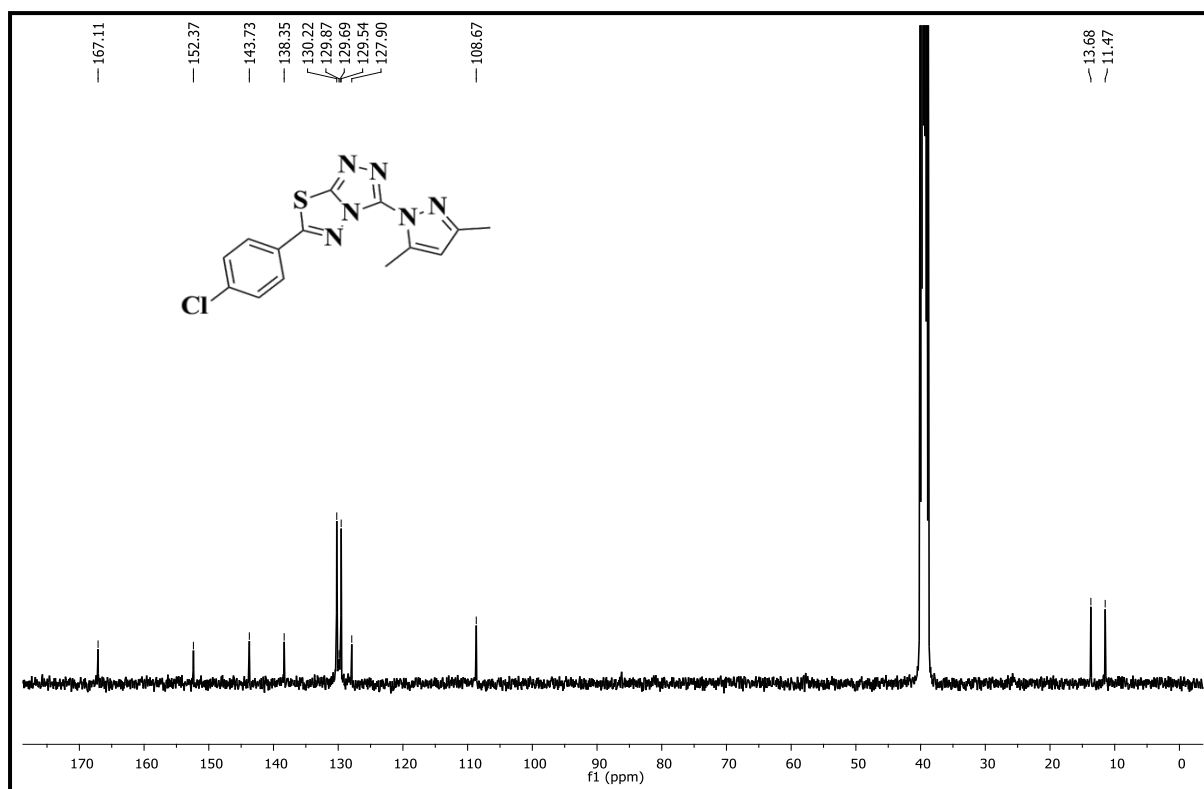


Fig: ¹³C-NMR Spectrum of Compound **32g** (100 MHz, DMSO-*d*₆)

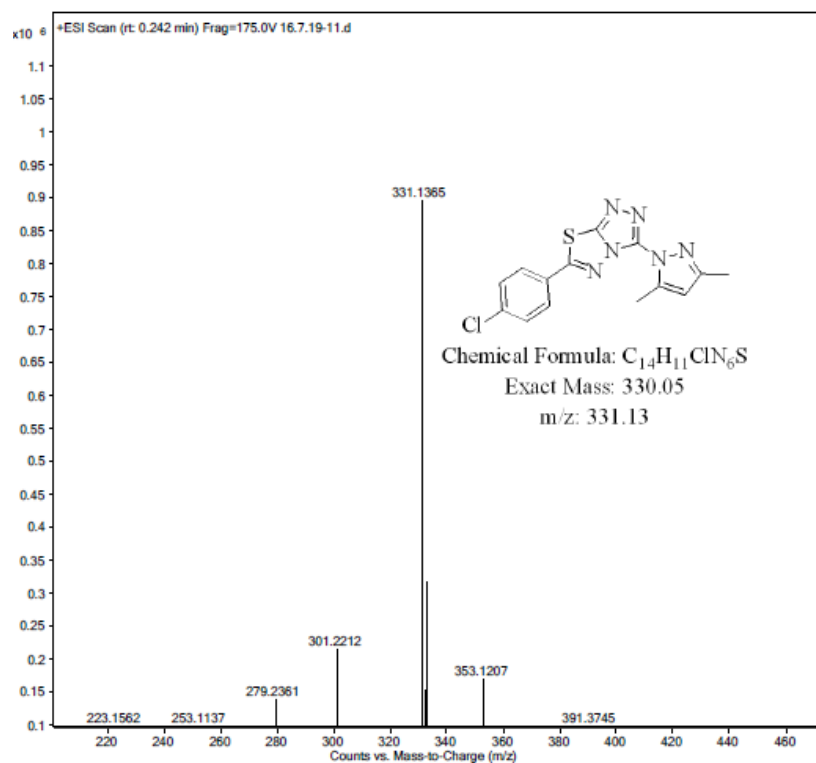


Fig: Mass Spectrum of Compound 32g

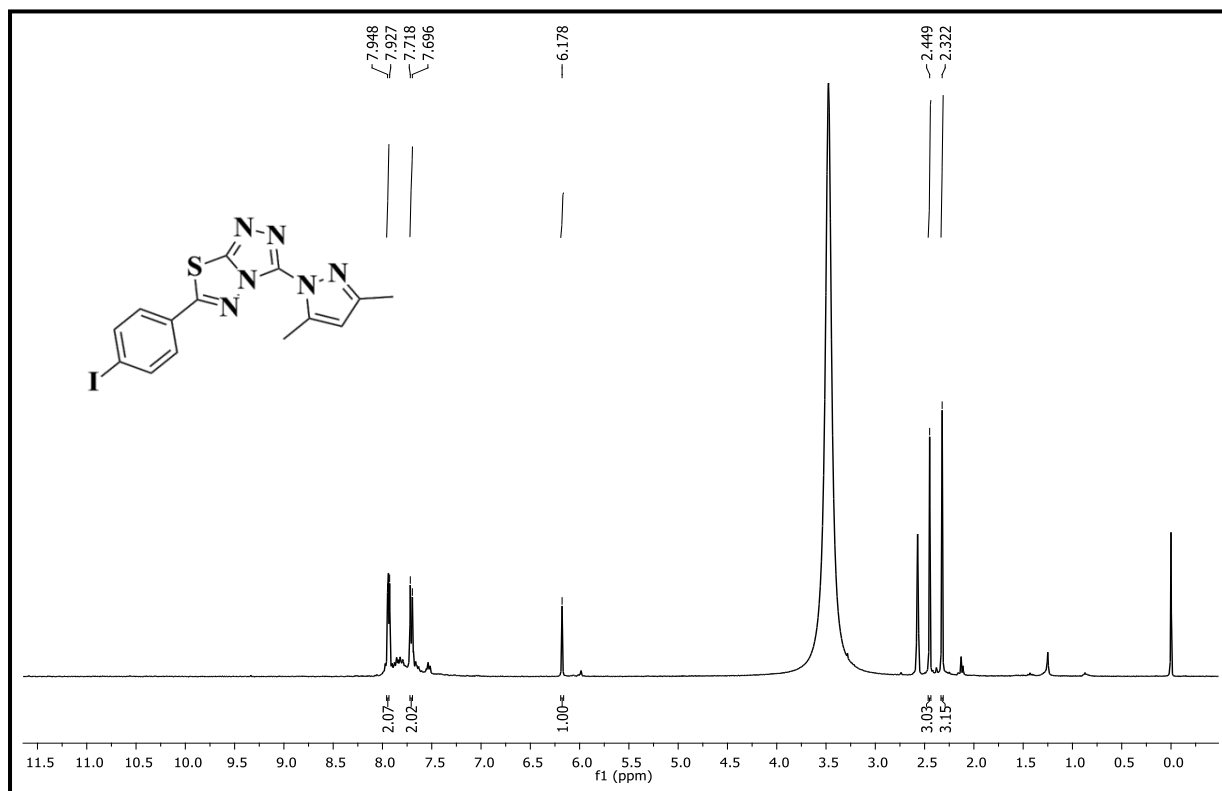


Fig: 1H -NMR Spectrum of Compound 32h (400 MHz, $DMSO-d_6$)

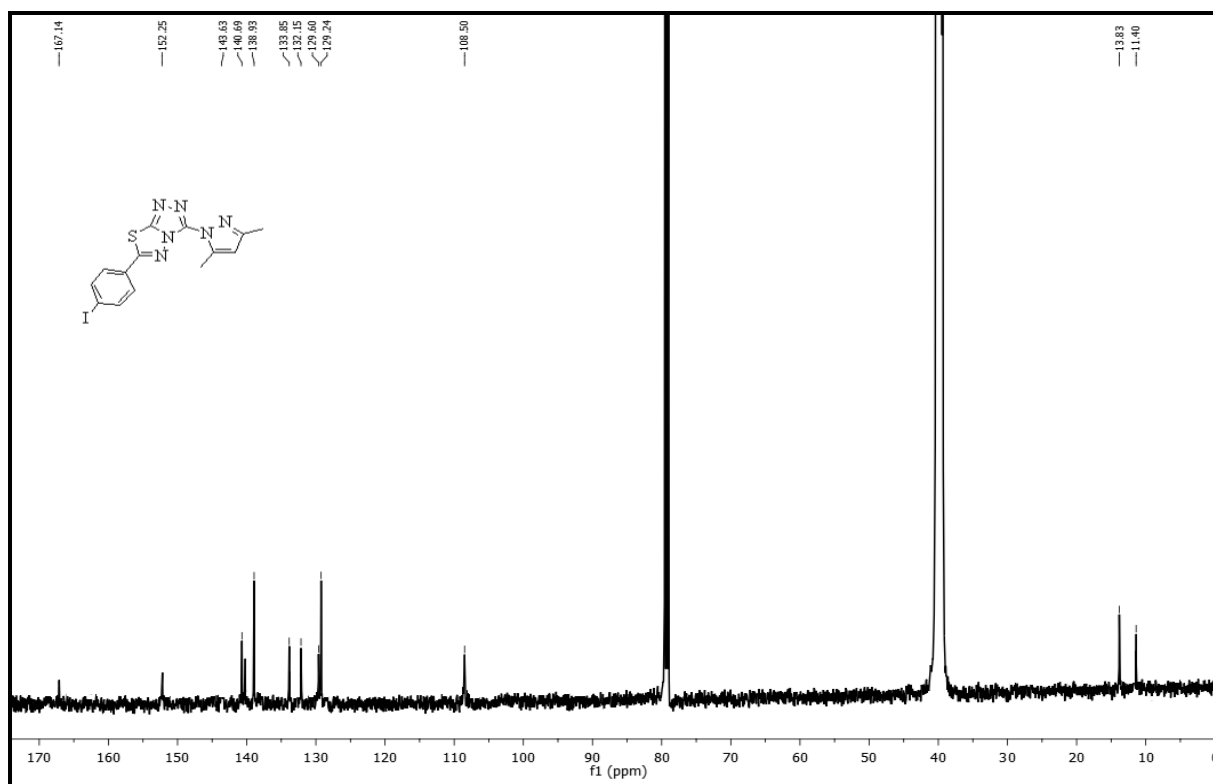


Fig: ¹³C-NMR Spectrum of Compound **32h** (100 MHz, CDCl₃+DMSO-*d*₆)

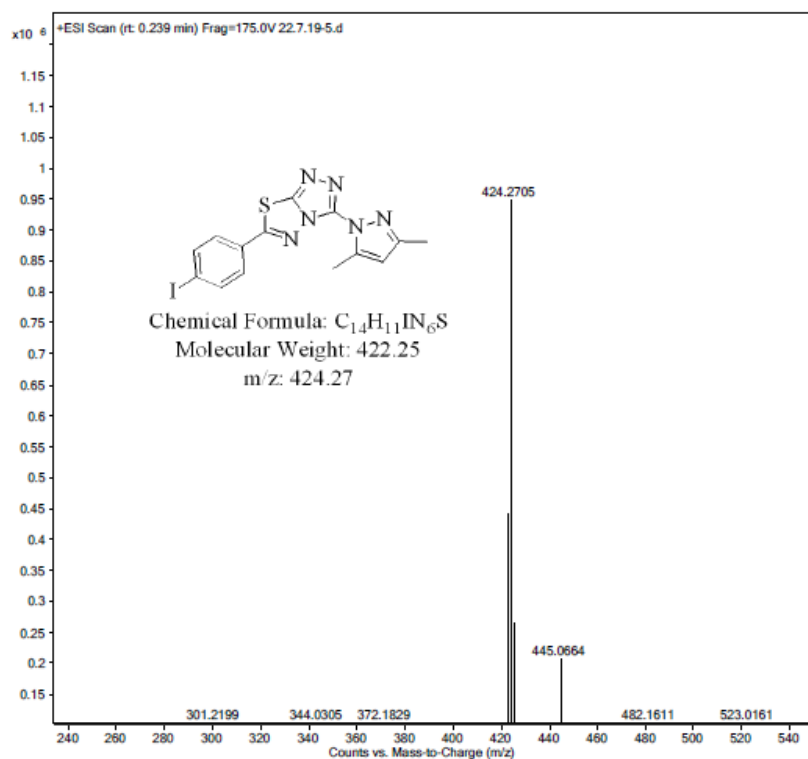


Fig: Mass Spectrum of Compound **32h**

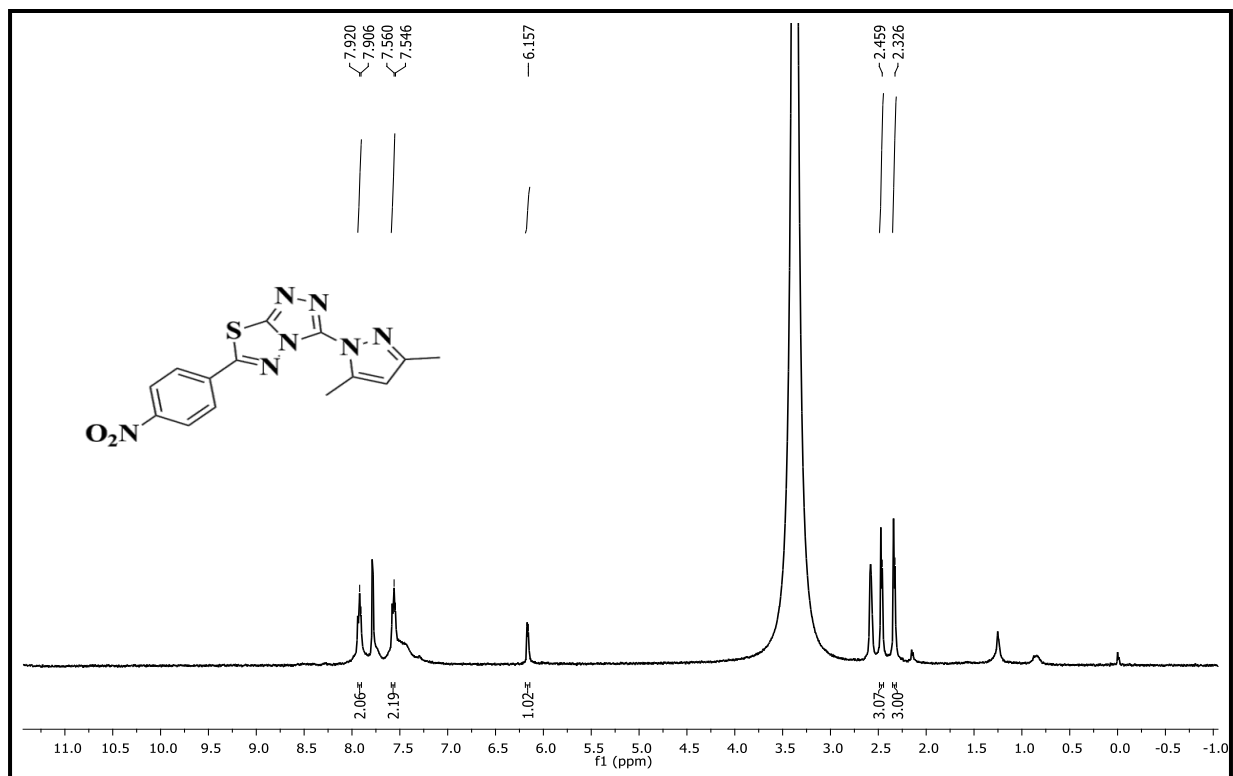


Fig: ¹H-NMR Spectrum of Compound 32i (400 MHz, CDCl₃+DMSO-*d*₆)

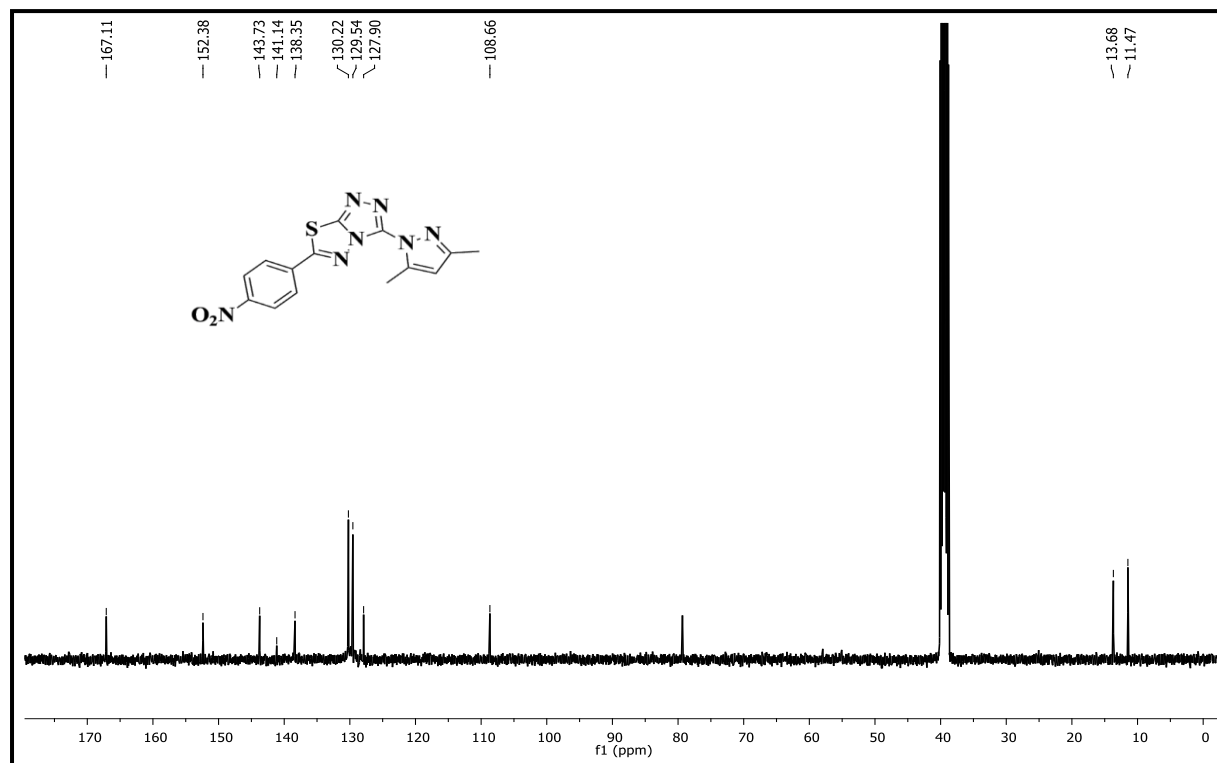


Fig: ¹³C-NMR Spectrum of Compound 32i (100 MHz, CDCl₃+DMSO-*d*₆)

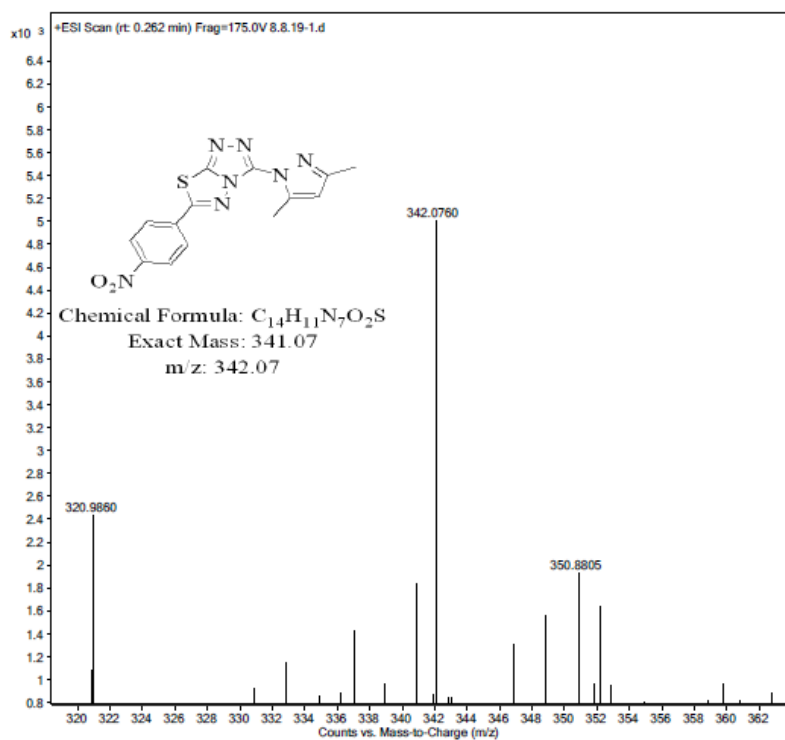


Fig: Mass Spectrum of Compound 32i

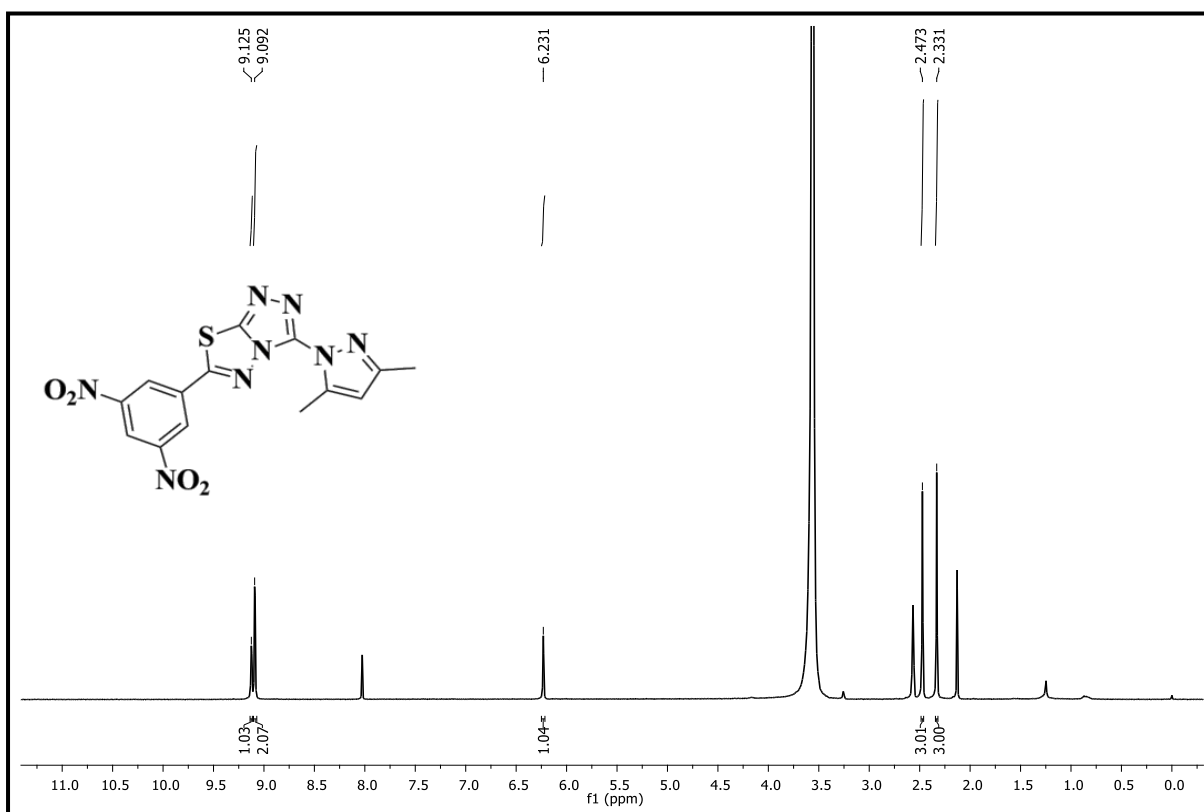


Fig: ^1H -NMR Spectrum of Compound 32j (400 MHz, $\text{CDCl}_3 + \text{DMSO}-d_6$)

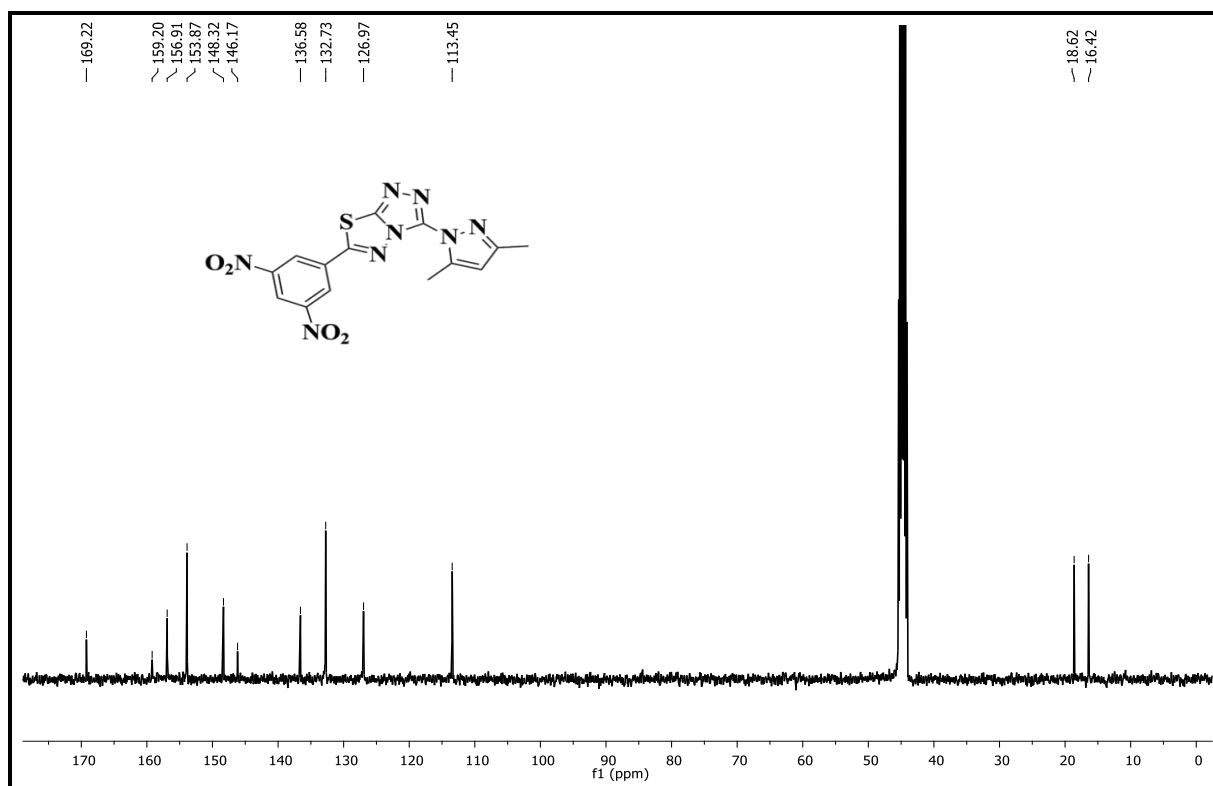


Fig: ¹³C-NMR Spectrum of Compound 32j (100 MHz, DMSO-*d*₆)

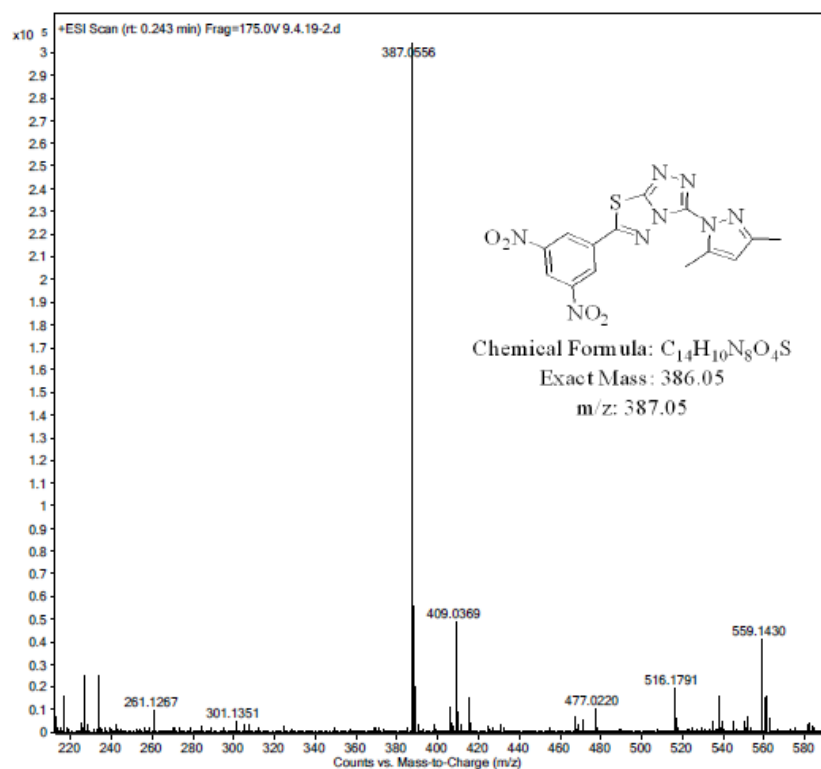


Fig: Mass Spectrum of Compound 32j

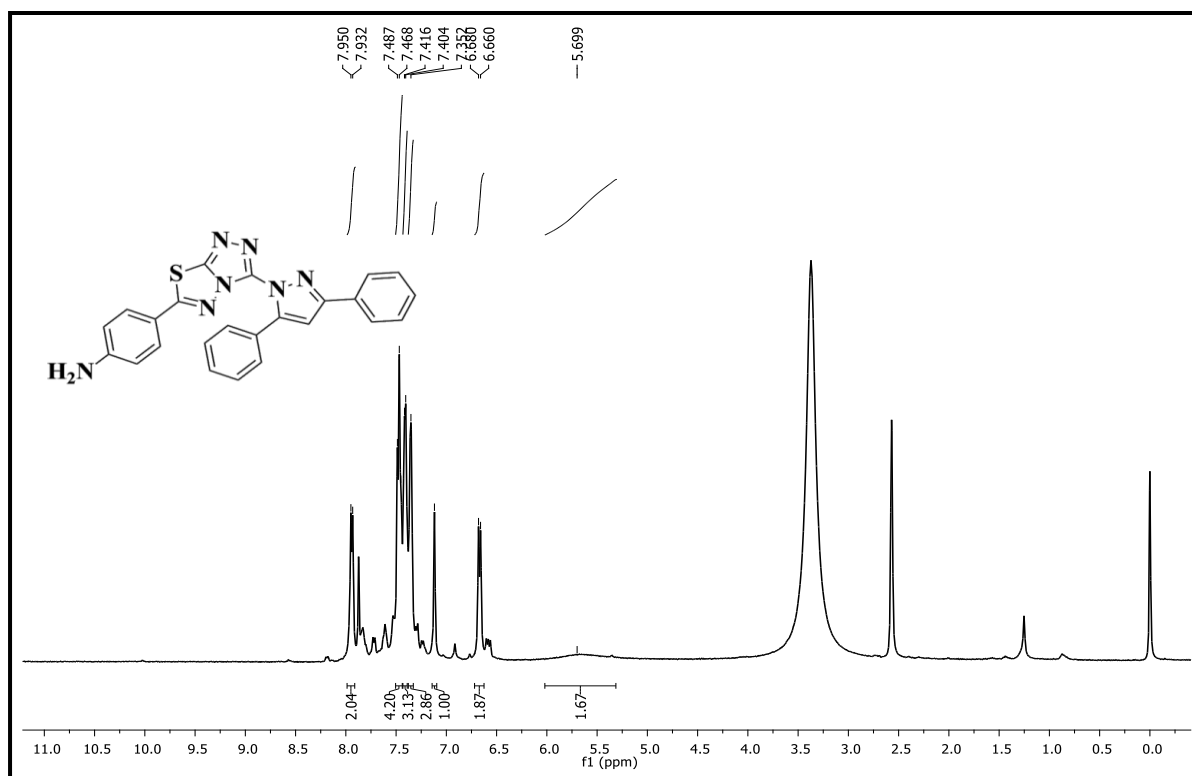


Fig: ¹H-NMR Spectrum of Compound **32k** (400 MHz, CDCl₃+DMSO-*d*₆)

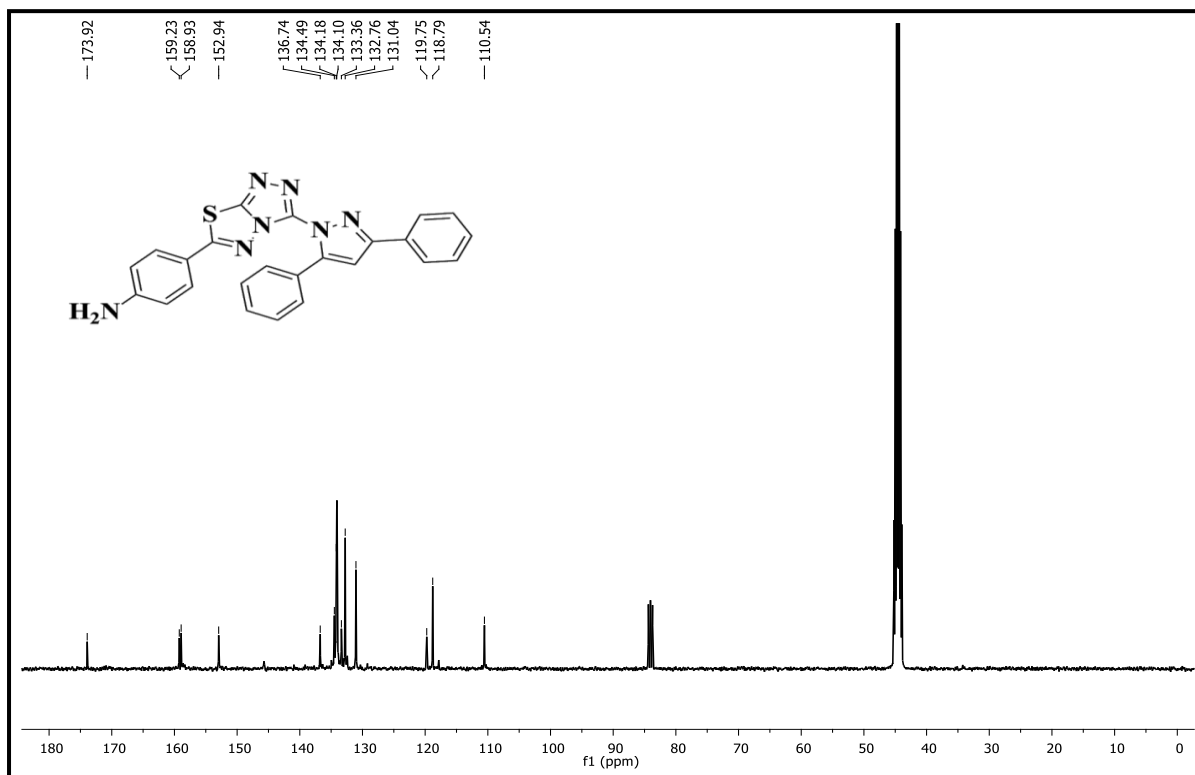


Fig: ¹³C-NMR Spectrum of Compound **32k** (100 MHz, CDCl₃+DMSO-*d*₆)

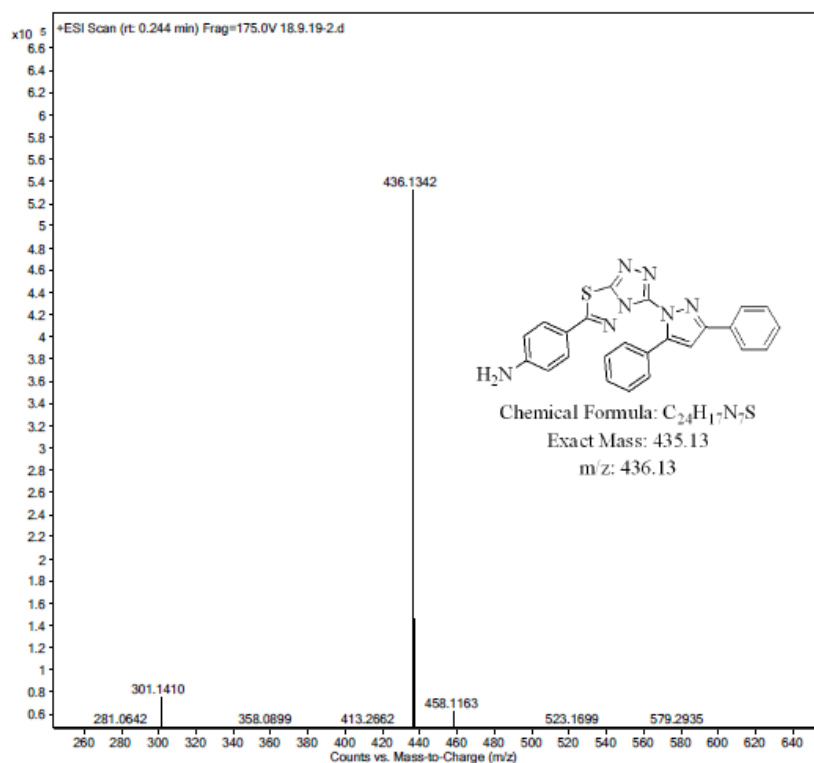


Fig: Mass Spectrum of Compound 32k

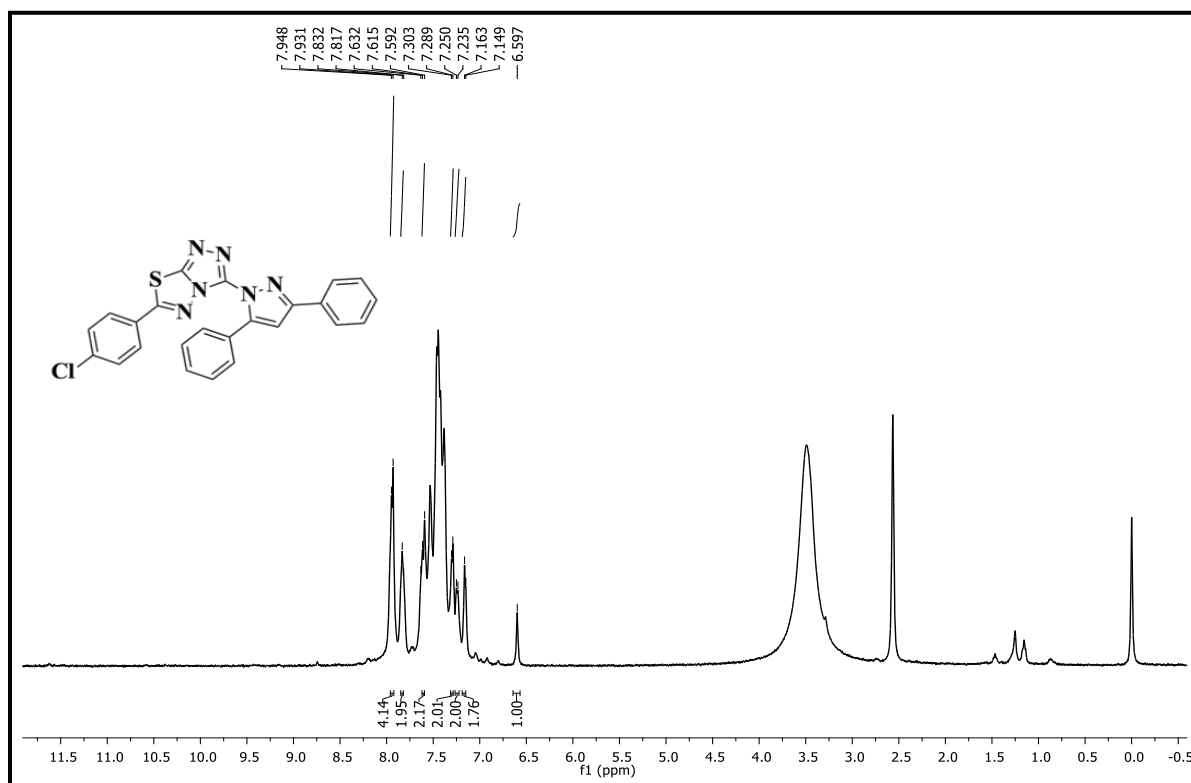


Fig: 1H -NMR Spectrum of Compound 32l (400 MHz, $CDCl_3 + DMSO-d_6$)

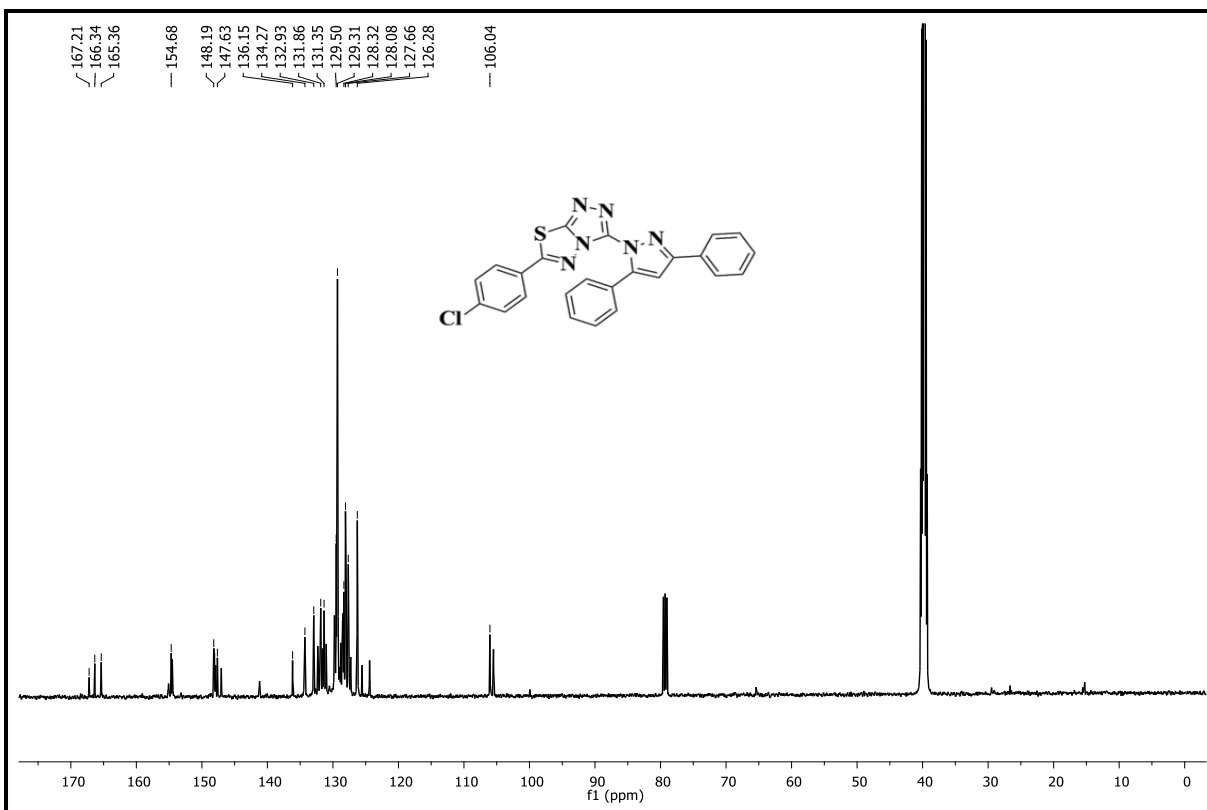


Fig: ^{13}C -NMR Spectrum of Compound **32I** (100 MHz, $\text{CDCl}_3 + \text{DMSO}-d_6$)

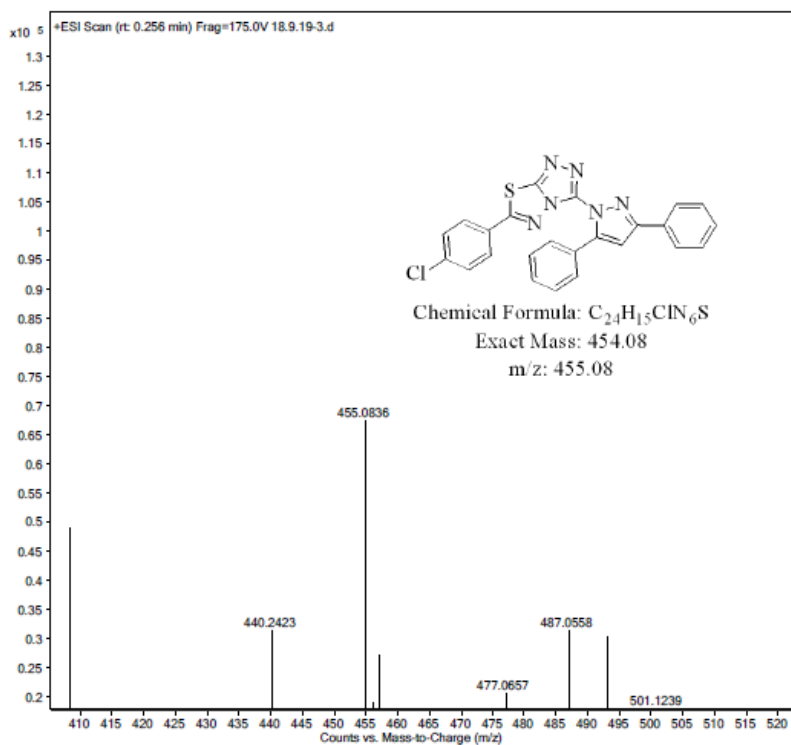


Fig: Mass Spectrum of Compound **32I**

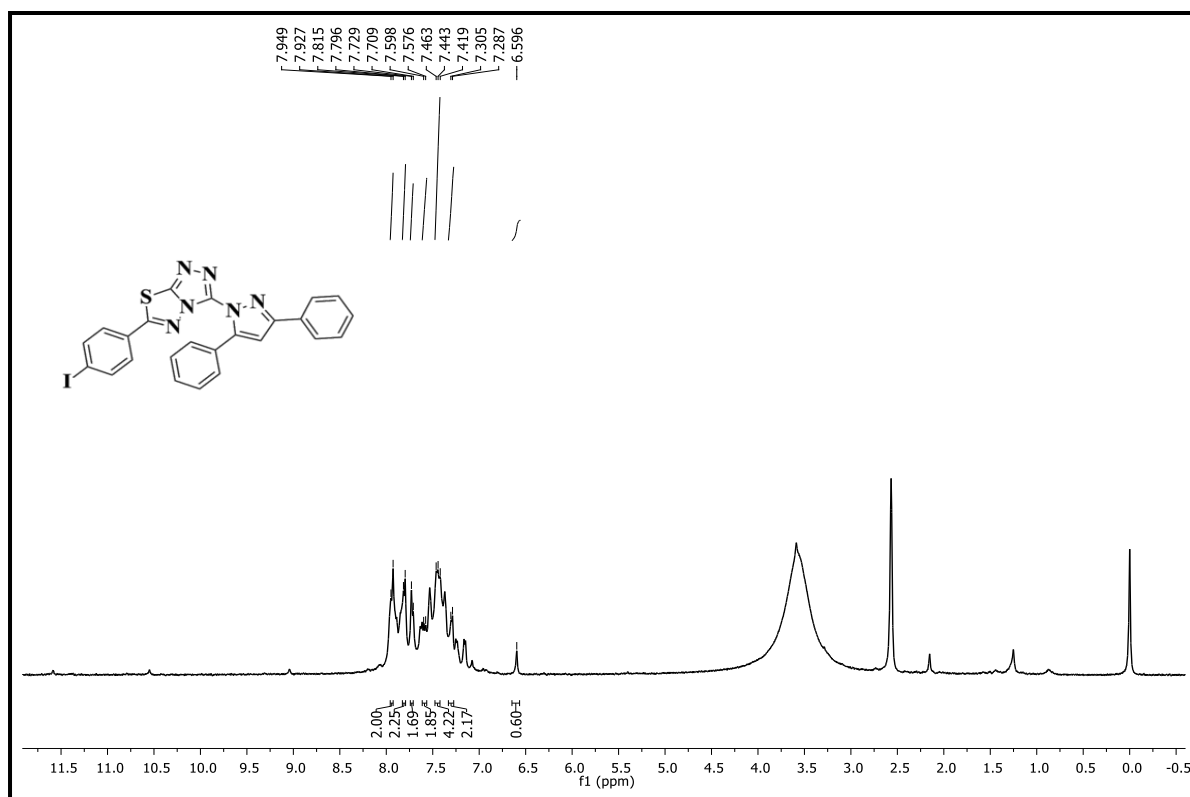


Fig: ¹H-NMR Spectrum of Compound **32m** (400 MHz, CDCl₃+DMSO-*d*₆)

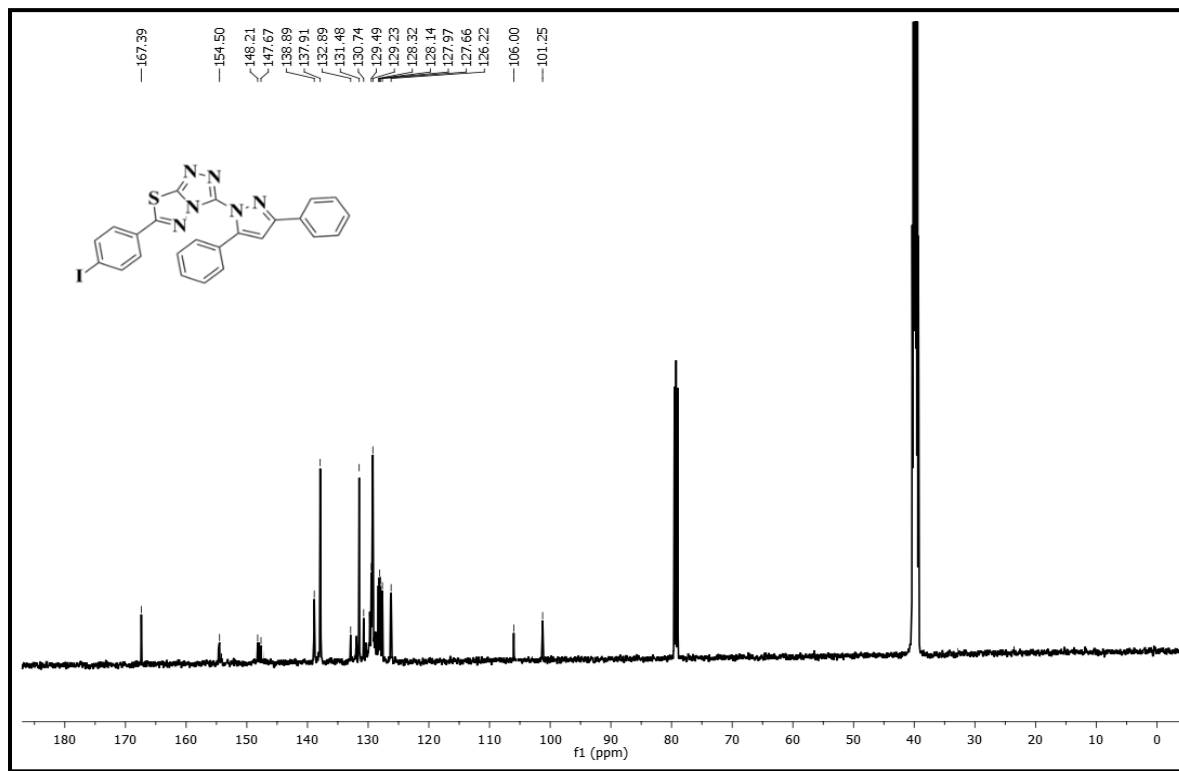


Fig: ¹³C-NMR Spectrum of Compound **32m** (100 MHz, CDCl₃+DMSO-*d*₆)

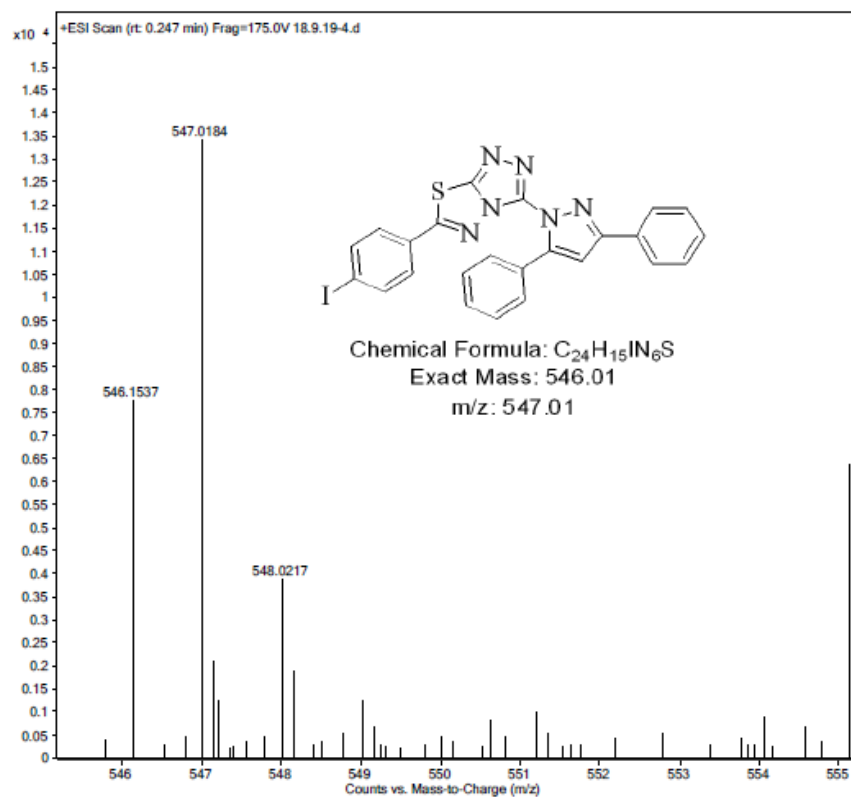


Fig: Mass Spectrum of Compound 32m

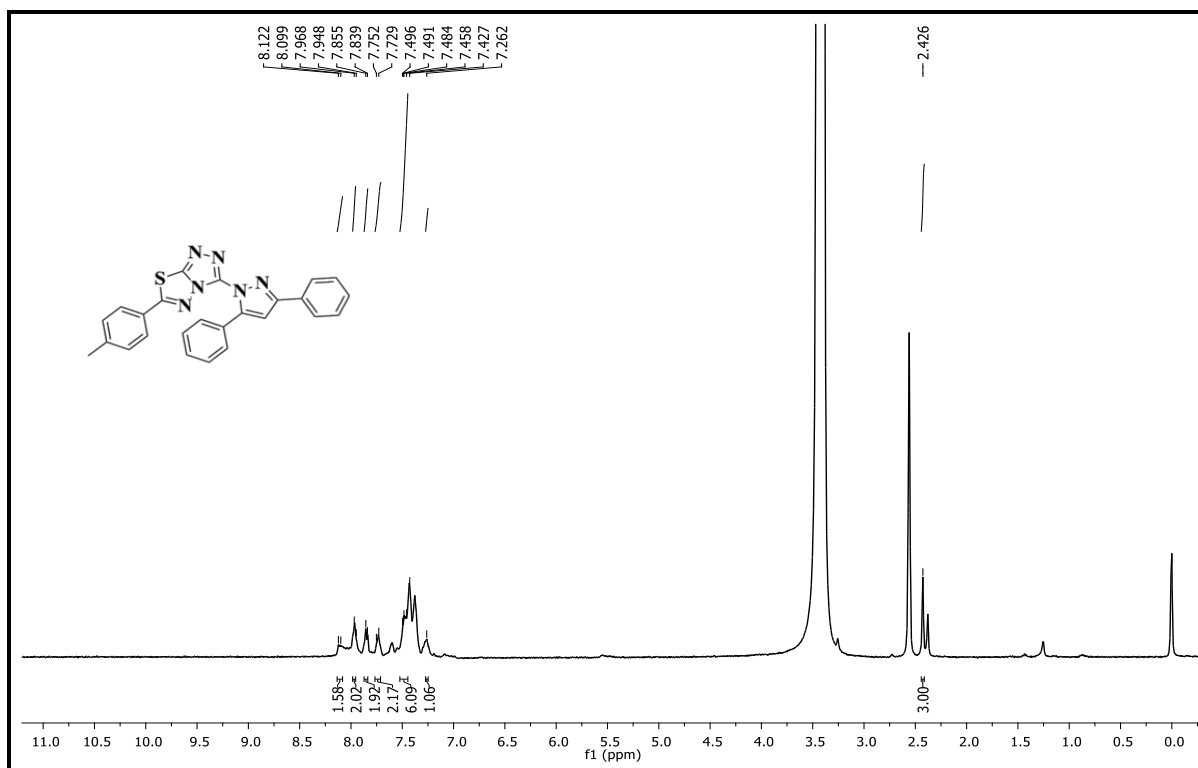


Fig: 1H -NMR Spectrum of Compound 32n (400 MHz, $CDCl_3$ +DMSO- d_6)

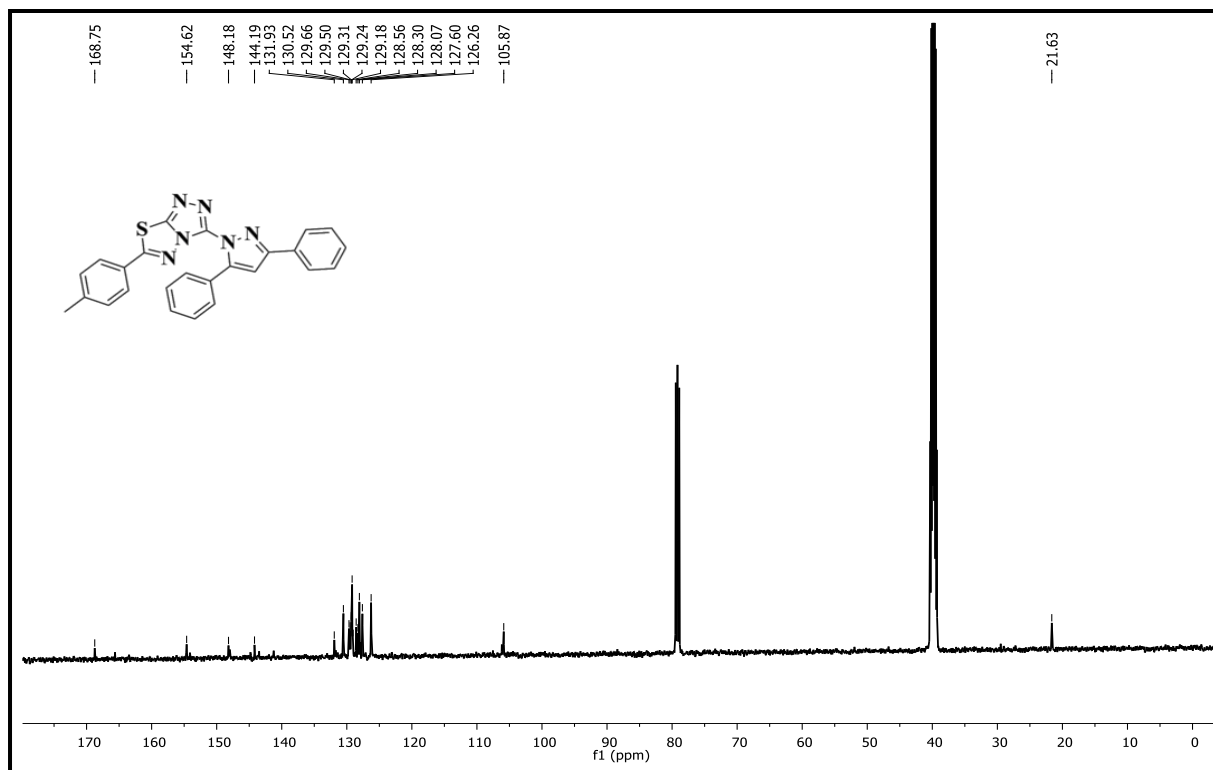


Fig: ^{13}C -NMR Spectrum of Compound **32n** (100 MHz, $\text{CDCl}_3+\text{DMSO}-d_6$)

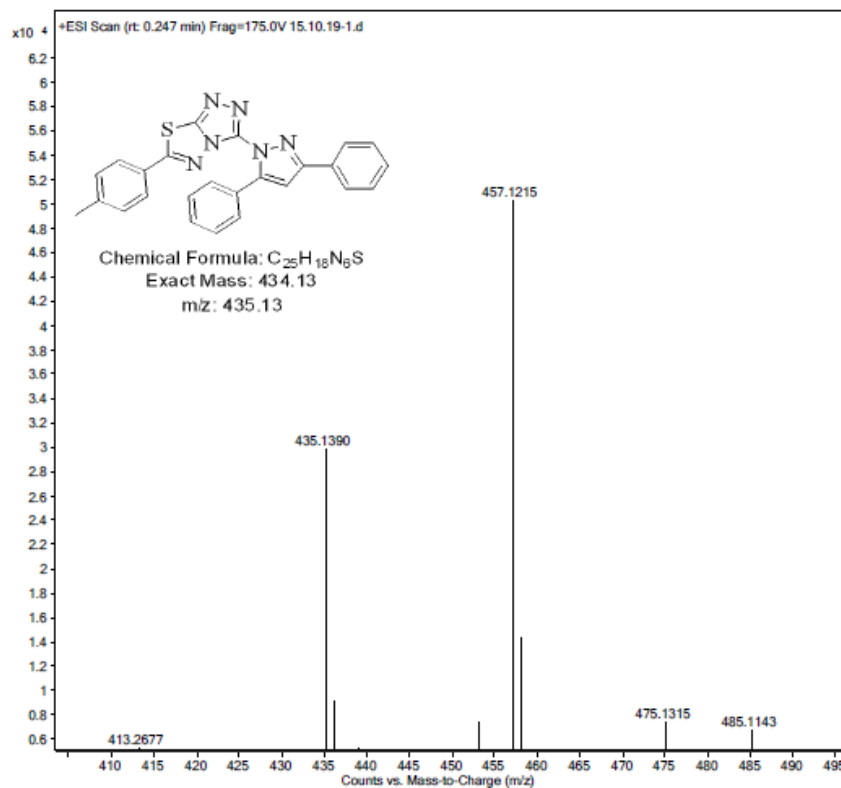


Fig: Mass Spectrum of Compound **32n**

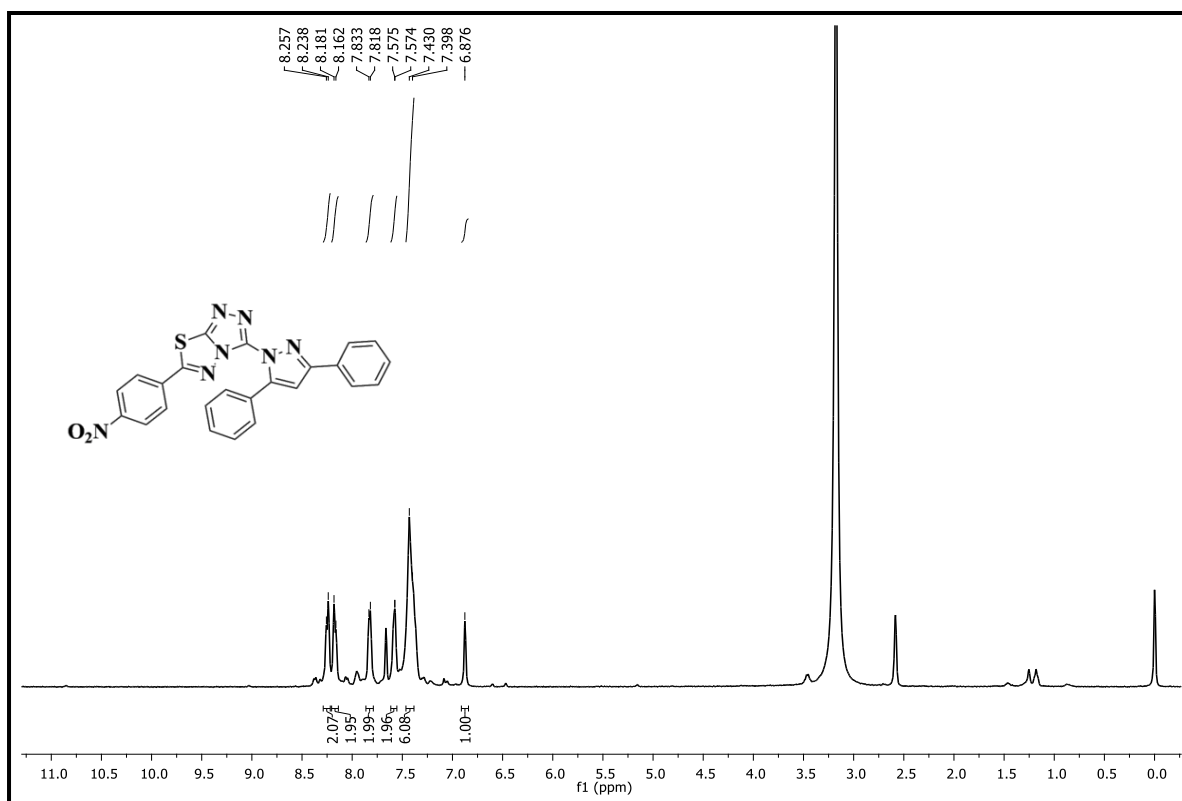


Fig: ¹H-NMR Spectrum of Compound **32o** (400 MHz, CDCl₃+DMSO-*d*₆)

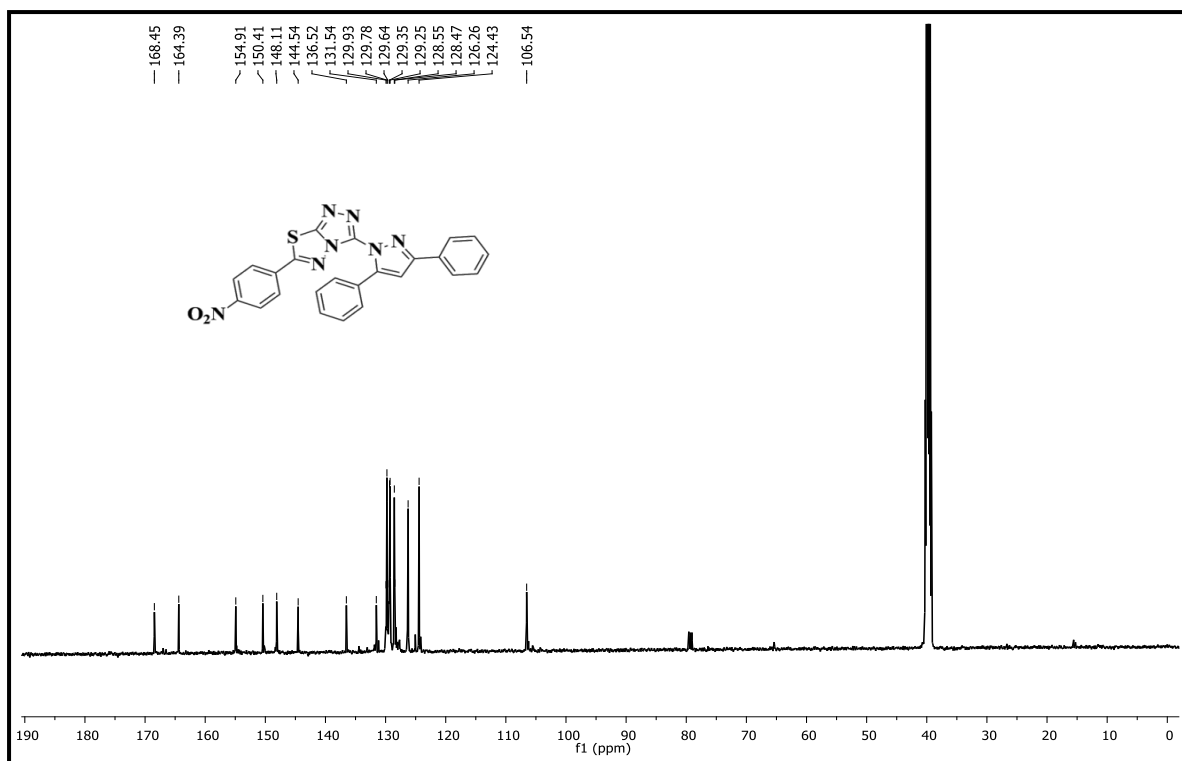


Fig: ¹³C-NMR Spectrum of Compound **32o** (100 MHz, CDCl₃+DMSO-*d*₆)

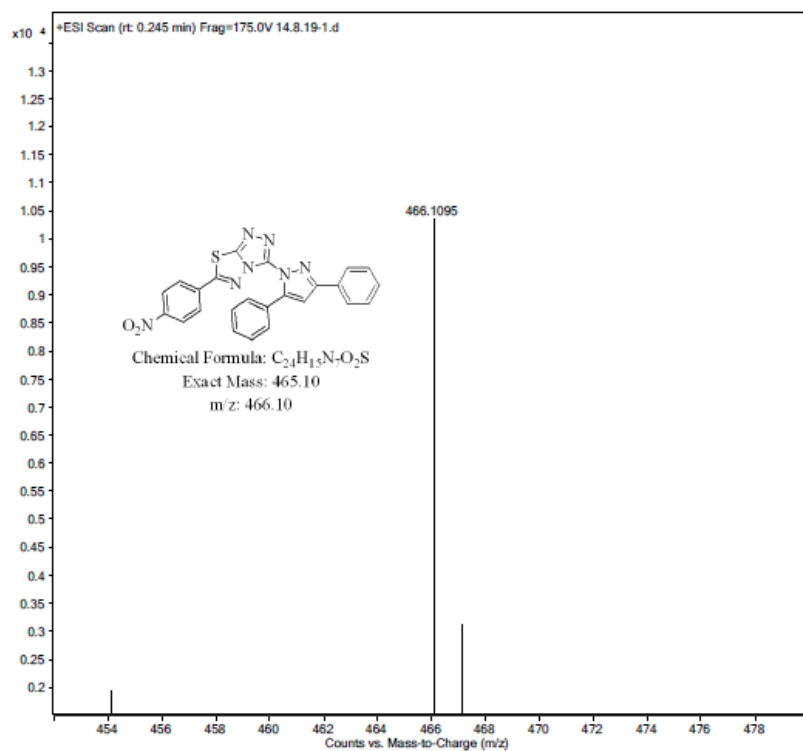


Fig: Mass Spectrum of Compound 32o

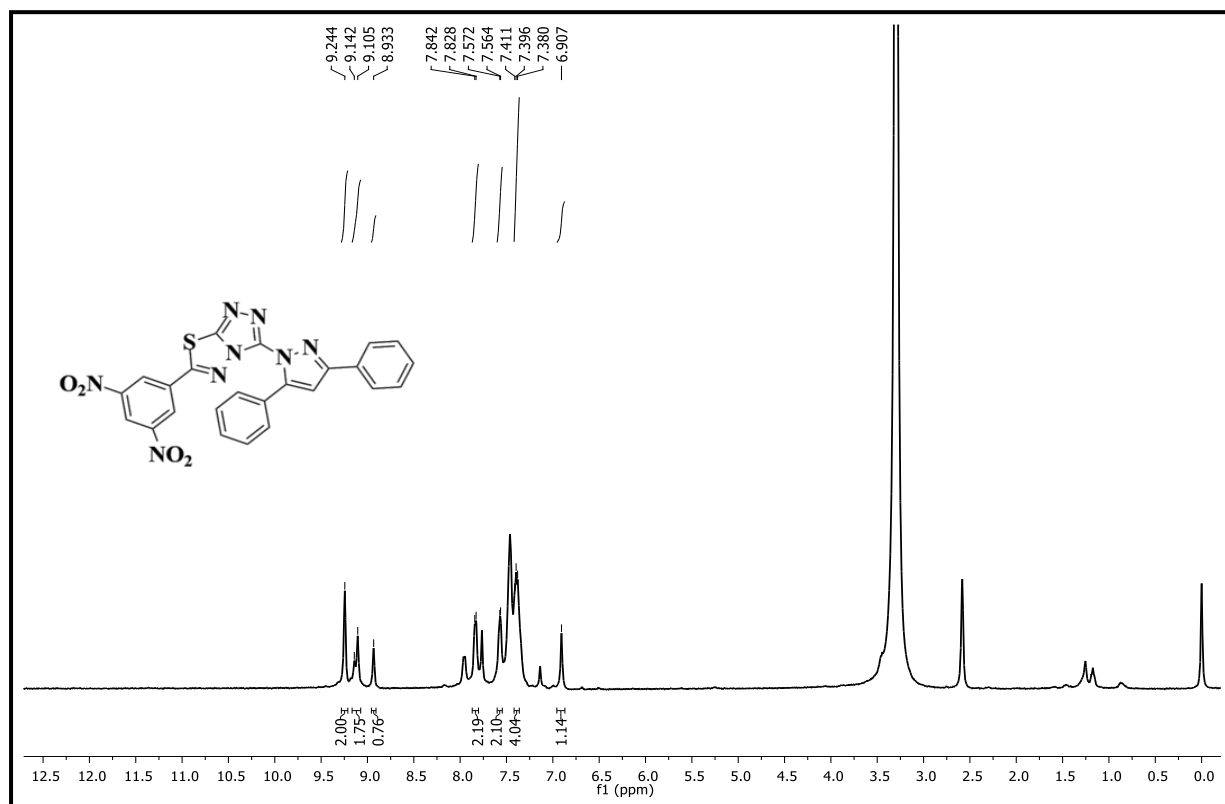


Fig: ¹H-NMR Spectrum of Compound 32p (400 MHz, CDCl₃+DMSO-*d*₆)

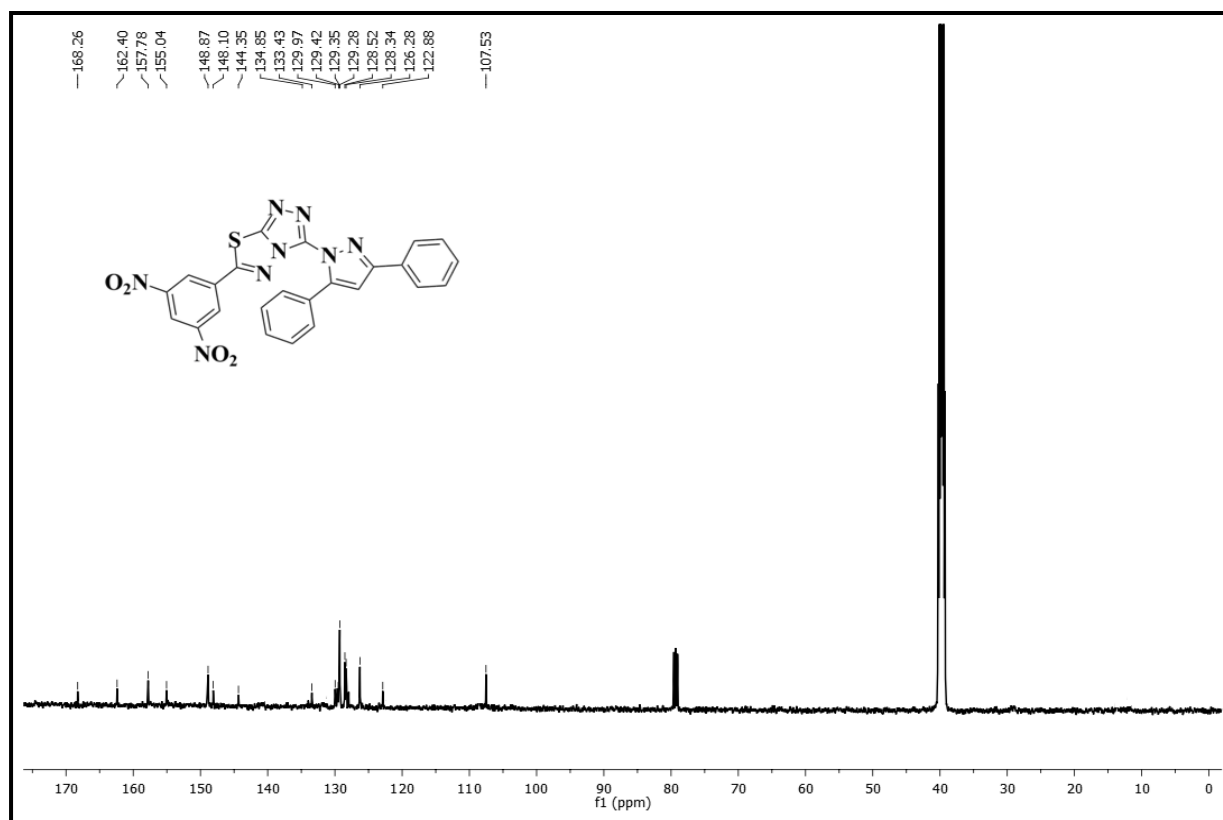


Fig: ^{13}C -NMR Spectrum of Compound **32p** (100 MHz, CDCl_3 +DMSO- d_6)

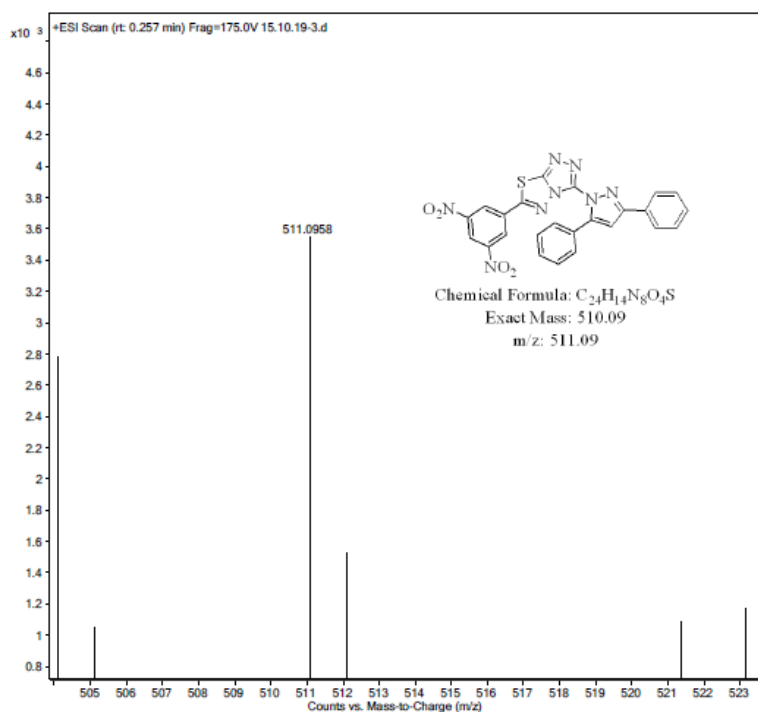


Fig: Mass Spectrum of Compound **32p**

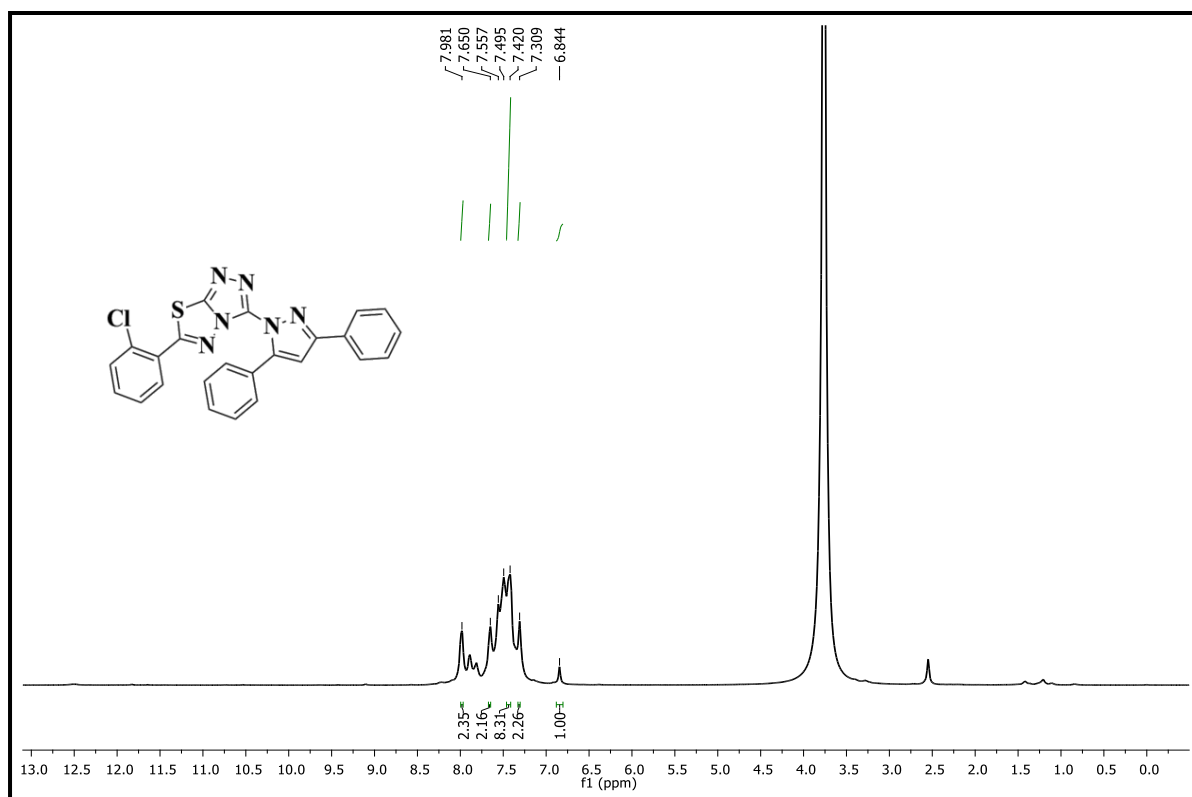


Fig: ¹H-NMR Spectrum of Compound **32q** (400 MHz, CDCl₃+DMSO-*d*₆)

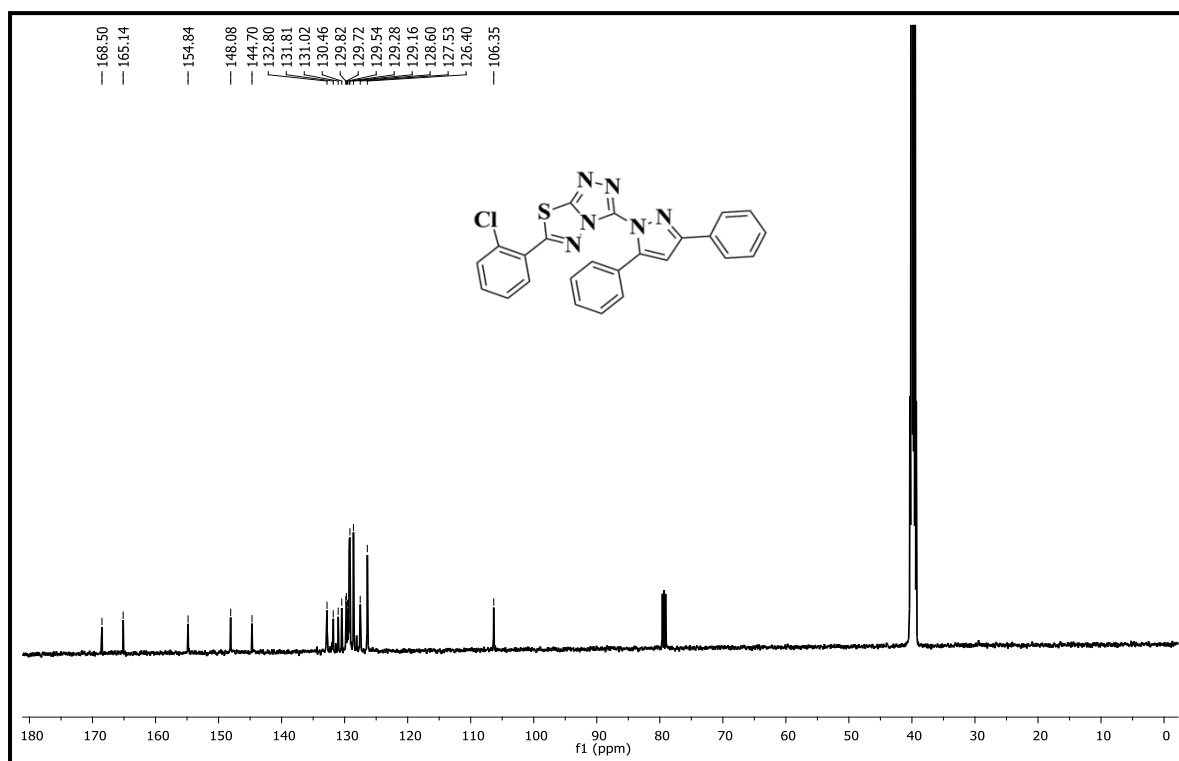


Fig: ¹³C-NMR Spectrum of Compound **32q** (400 MHz, CDCl₃+DMSO-*d*₆)

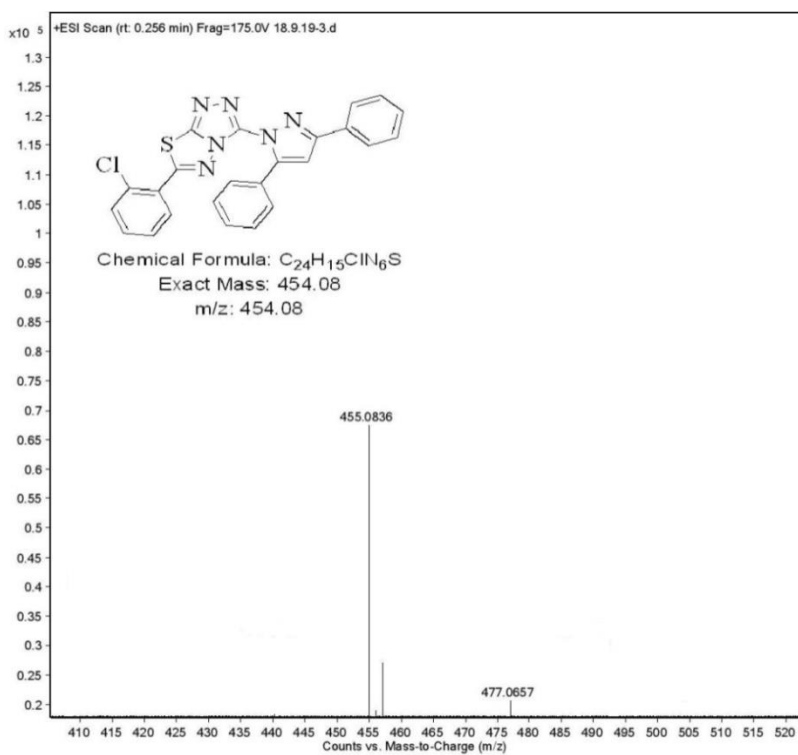


Fig: Mass Spectrum of Compound **32q**

References:

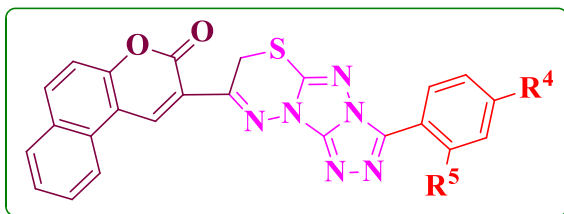
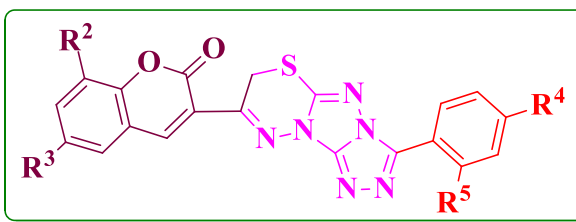
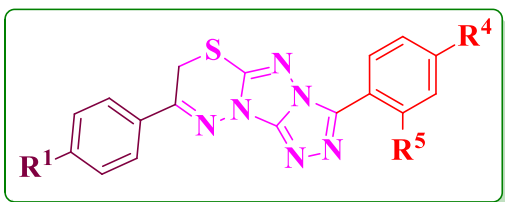
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CHAPTER-VI

NOVEL ONE-POT, THREE COMPONENT SYNTHESIS OF 7*H*- [1,2,4]TRIAZOLO[4',3':1,5][1,2,4]TRIAZOLO[3,4-*b*][1,3,4]THIADIAZINE DERIVATIVES



INTRODUCTION:

1,2,4-Triazole is a heterocyclic compound having three nitrogen atoms in the five-membered ring^{1,2}. Most of the 1,2,4-triazole derivatives exhibit various biological, pharmaceutical, and agrochemical applications. 1,2,4-Triazoles play an important role in the medicinal chemistry due their wide range of biological activities such as anti-bacterial^{3,4}, anti-fungal^{5,6}, anti-viral^{7,8}, anti-tumor⁹, anti-inflammatory¹⁰⁻¹³, anti-tubercular^{14,15}, anti-oxidant¹⁶⁻¹⁸, anti-proliferative¹⁹ and insecticidal²⁰ activities (**Fig. 1**). When two rings of triazoles are fused that results in the formation of 1,2,4-triazolo[4,3-*b*][1,2,4]triazole. These are fused heterocyclic rings are nitrogen ring compounds. They are nitrogen-rich with good biological activity.

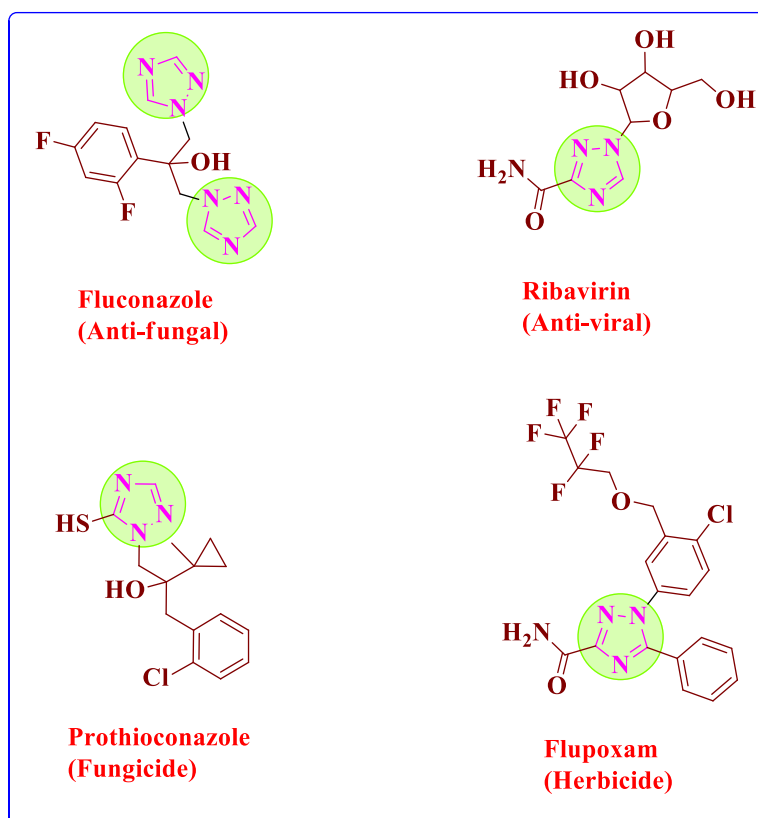


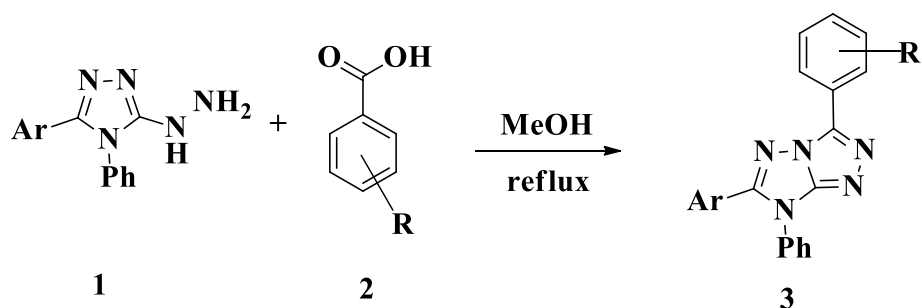
Fig. 1: Commercially available drugs bearing triazole motifs.

Triazolothiadiazine is a fused heterocyclic scaffold having five-membered triazole and six-membered thiadiazine core moieties. Important class of triazolothiadiazines are 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines. They find extensive therapeutic applications such as anti-

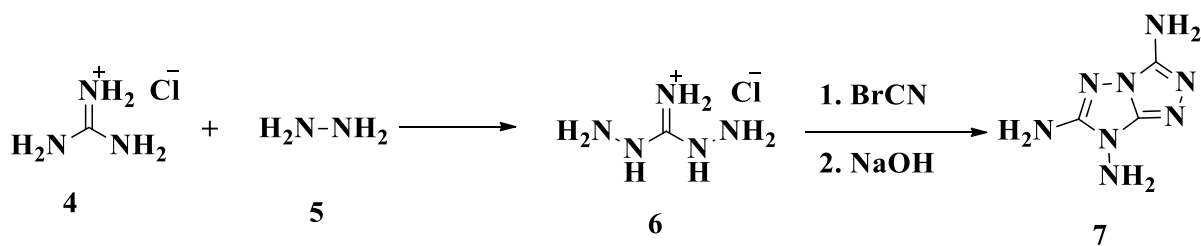
cancer^{21,22}, anti-HIV²³, anti-inflammatory^{24,25}, anti-tubercular²⁶, anti-microbial^{27,28}, anti-oxidant²⁹, analgesic³⁰, anti-viral³¹ and PDE4 inhibitors³².

The following is a brief literature survey on the synthesis of fused triazolotriazoles and triazolothiadiazines.

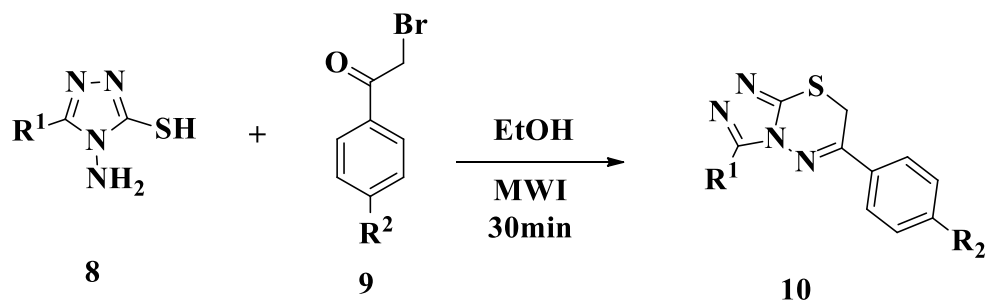
Ghattas³³ *et al.* described the synthesis of [1,2,4]triazolo[4,3-*b*][1,2,4]triazoles (**3**) from the reaction of different 3-hydrazinyl-1,2,4-triazoles (**1**) and substituted aromatic benzoic acids (**2**) in presence of methanol under reflux condition in good yields.



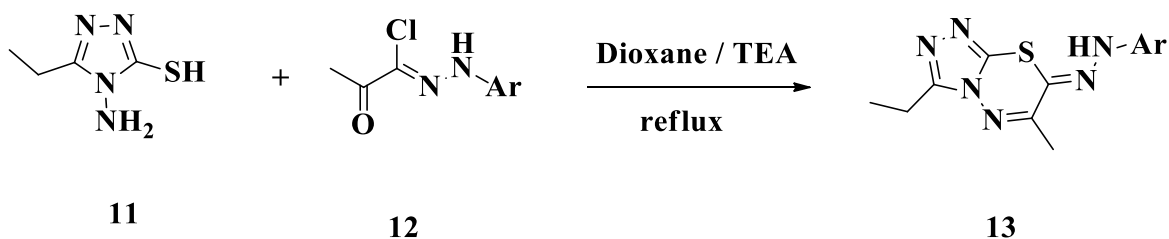
Chengming³⁴ *et al.* reported the preparation of 7*H*-[1,2,4]triazolo[4,3-*b*][1,2,4]triazole-3,6,7-triamine (**7**) from two-step reaction. In the first step diaminomethaniminium chloride (**4**) reacted with hydrazine (**5**) to form compound **6**, further, it was converted into compound **7**.



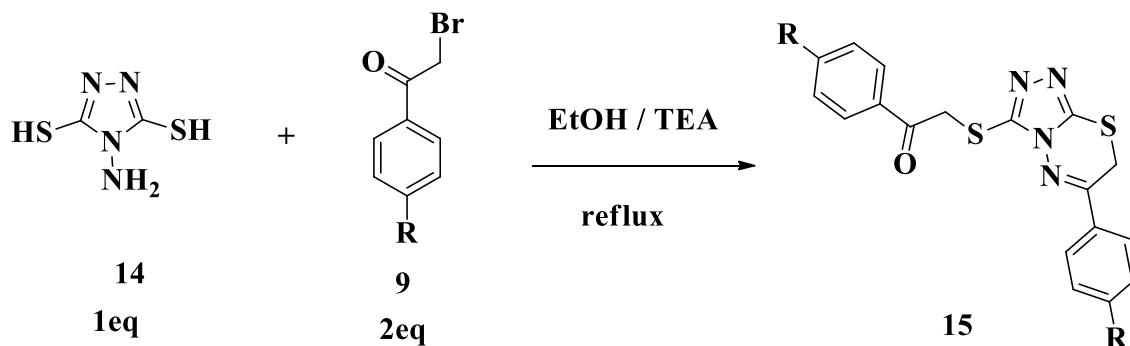
Ziqiang³⁵ *et al.* described the synthesis of various [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines (**10**) using substituted 4-amino-1,2,4-triazole-3-thiols (**8**) and different phenacyl bromides (**9**) in presence of ethanol under microwave irradiation (MWI) condition. The final compounds were evaluated for their anti-tubercular activity with good results.



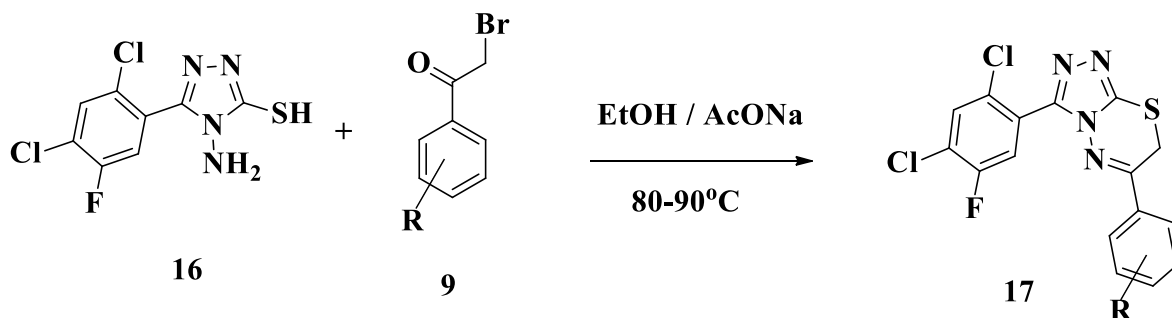
Fathy³⁶ et al. reported the synthesis of different (*Z*)-3-ethyl-7-hydrazono-6-methyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines (**13**) using a mixture of 4-amino-5-ethyl-4*H*-1,2,4-triazole-3-thiol (**11**) and various (*Z*)-2-oxopropanehydrazonoyl chlorides (**12**) in dioxane/triethylamine under reflux condition.



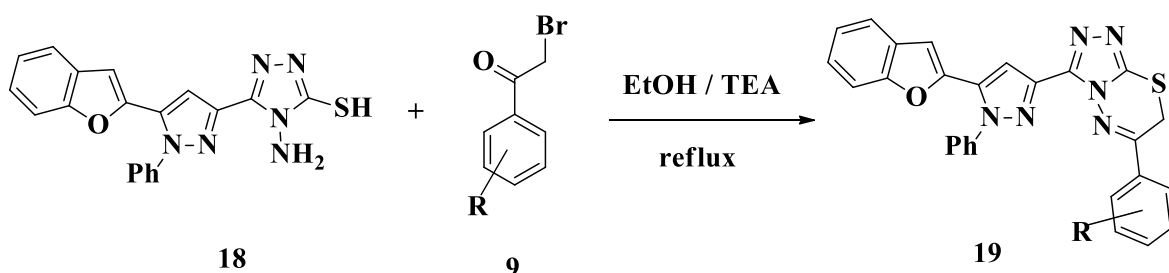
Varun and Rajeswar³⁷ described the synthesis of a series of 1-phenyl-2-((6-phenyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)thio)ethanones (**15**) from the reaction of 4-amino-4*H*-1,2,4-triazole-3,5-dithiol (**14**) with two equivalents substituted phenacyl bromides (**9**) in presence of ethanol having triethylamine under reflux condition via a one-pot, multi-component reaction. Further, the designed target compounds were screened for their anti-cancer activity and they exhibited moderate results.



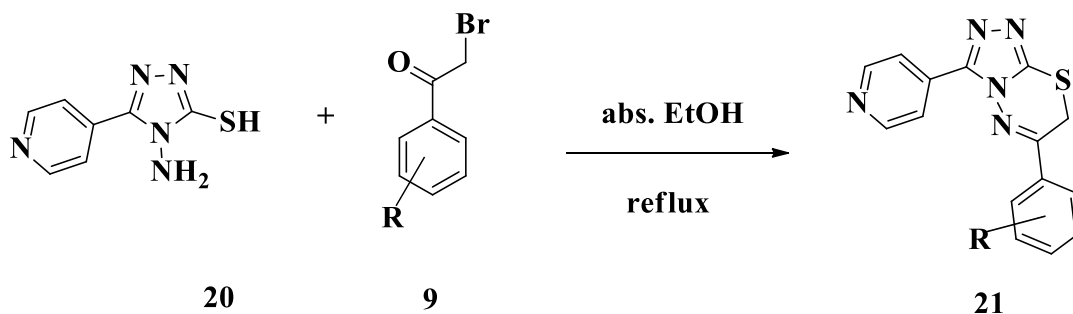
Subrahmanya³⁸ et al. reported the synthesis and anti-tumor activity of substituted 3-(2,4-dichloro-5-fluorophenyl)-6-phenyl-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazines (**17**) using a mixture of 4-amino-5-(2,4-dichloro-5-fluorophenyl)-4H-1,2,4-triazole-3-thiol (**16**) and various phenacyl bromides (**9**) in ethanol with sodium acetate at 80-90°C temperature.



Bakr³⁹ et al. published the synthesis of 1,2,4-triazolo[3,4-b][1,3,4]-thiadiazine derivatives (**19**) and these compounds were evaluated for their anti-microbial activity.



Mona⁴⁰ and co-worker described the synthesis of 6-phenyl-3-(pyridin-4-yl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (**21**) analogues from the reaction of 4-amino-5-(pyridin-4-yl)-4H-1,2,4-triazole-3-thiol (**20**) with appropriate phenacyl bromides (**9**) in the presence of absolute ethanol under reflux condition. The final products were tested for their anti-cancer activity.



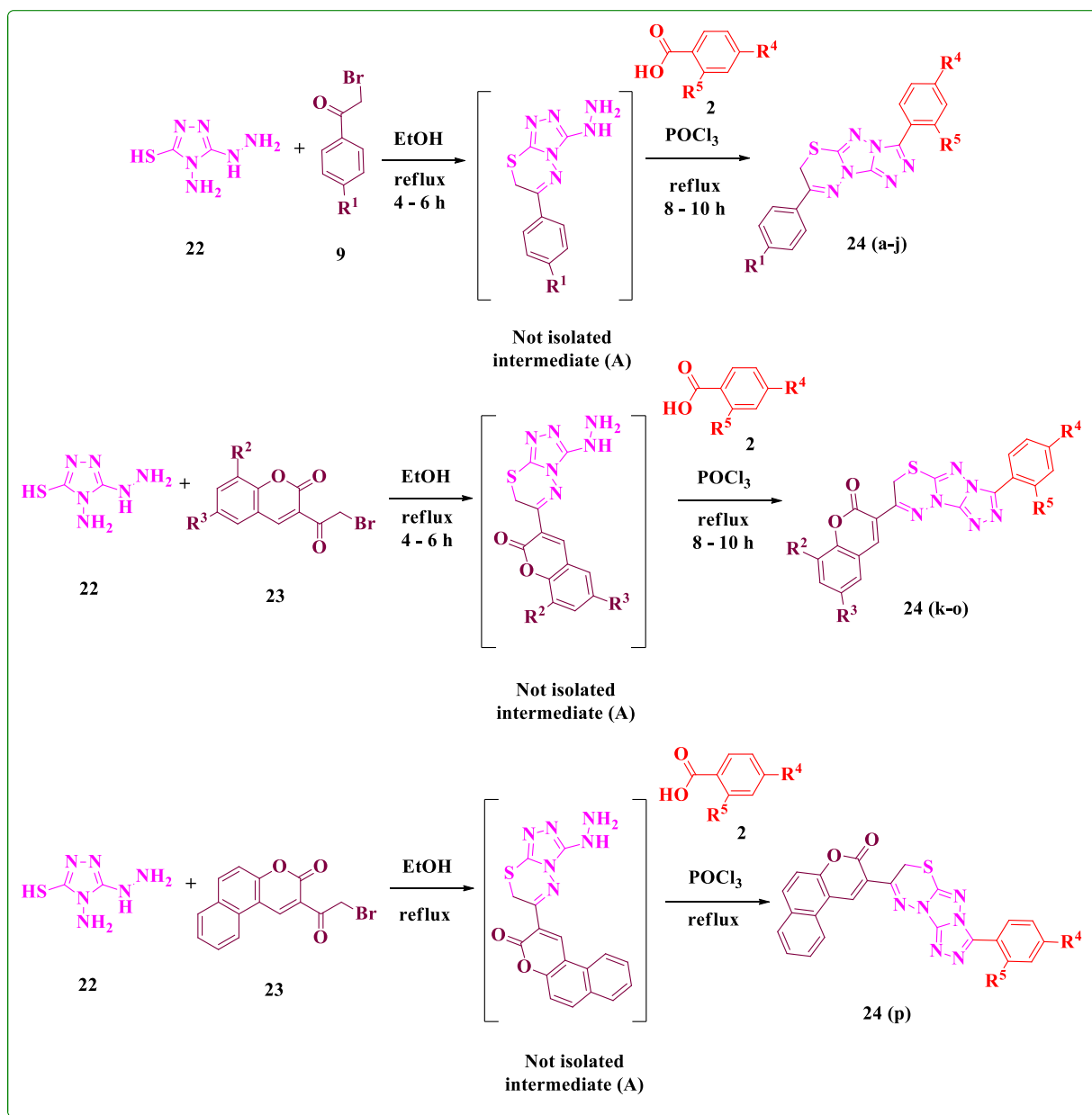
PRESENT WORK:

Taking into consideration of significant biological and pharmaceutical applications of fused triazole and triazolothiadiazine scaffolds, it was decided to incorporate all these core units in a single molecule *via* a one-pot, multi-component approach.

The present chapter describes a facile one-pot, three-component synthesis of 7*H*-[1,2,4]triazolo[4',3':1,5][1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines (**24a-p**). The final compounds were synthesized by the reaction of readily available starting compounds 4-amino-5-hydrazinyl-4*H*-1,2,4-triazole-3-thiol (**22**), phenacyl bromides (**9**) or 3-(2-bromoacetyl)-2*H*-chromen-2-ones (**23**) and appropriate benzoic acids (**2**) in absolute ethanol and POCl₃ under reflux temperature with good yields (**Scheme-1**).

In continuation of previous work⁴¹ we have developed one-pot multi-component synthesis of [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines by the reaction of 4-amino-5-hydrazinyl-4*H*-1,2,4-triazole-3-thiol (**22**) and various phenacyl bromides (**9a-h**) or 3-(2-bromoacetyl)-2*H*-chromen-2-ones (**23i-p**) in presence of absolute ethanol under reflux. When the reaction was carried out between **22** and **9** or **23** there is an initial formation of the intermediate **A**. This, later on, undergoes cyclization with benzoic acids (**2**) in presence of POCl₃ to give the desired products (**24a-p**) with good to excellent yields (80-91%, **Table-1**).

When the reaction is carried out between **22** and **9** or **23** there is a possibility of the formation of other alternative products like **25** or **26** or **27** or all of them (**Fig. 2**) by the way in which the reaction is taking place. But we got only one product **24** (By TLC). All the other possible products (**25**, **26**, and **27**) were rejected on the ground of their spectral information. The uniqueness of this reaction is that there is a formation of several bonds like two C=N, C-S, and C-N in one-pot. Due to the presence of many nitrogen atoms as high energy materials.



Scheme-1: Novel one-pot, three-component synthesis of 7H-[1,2,4]triazolo[4',3':1,5][1,2,4]triazolo[3,4-b][1,3,4]thiadiazine derivatives (**24a-p**).

The formation of the final target compounds can be described by a plausible reaction mechanism⁴² (**Scheme-2**). The highly nucleophilic thiol (-SH) functional group of 4-amino-5-hydrazinyl-4H-1,2,4-triazole-3-thiol (**22**) substitutes the bromine atom of phenacyl bromides (**9**) or 3-(2-bromoacetyl)-2H-chromen-2-ones (**23**) to yield the open chain thioketone. This suffers from the loss of water molecule to give the intermediate **A**. The role of POCl₃ in the cyclization

reaction is that it converts aromatic carboxylic acid into its benzoic or substituted benzoic phosphorodichloridic anhydride. This attacks on the hydrazino group of **A** to yield the hydrazide with the loss of phosphorodichloridate (PO_2Cl_2^-) anion. Then the second molecule of POCl_3 attacks on hydrazide followed by ring closure reaction with the nitrogen of triazole ring by the elimination of another PO_2Cl_2^- anion to yield final products **24a-p**.

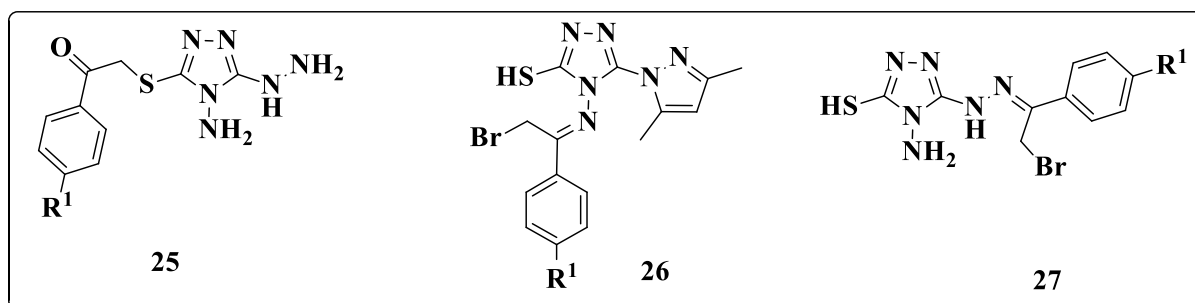
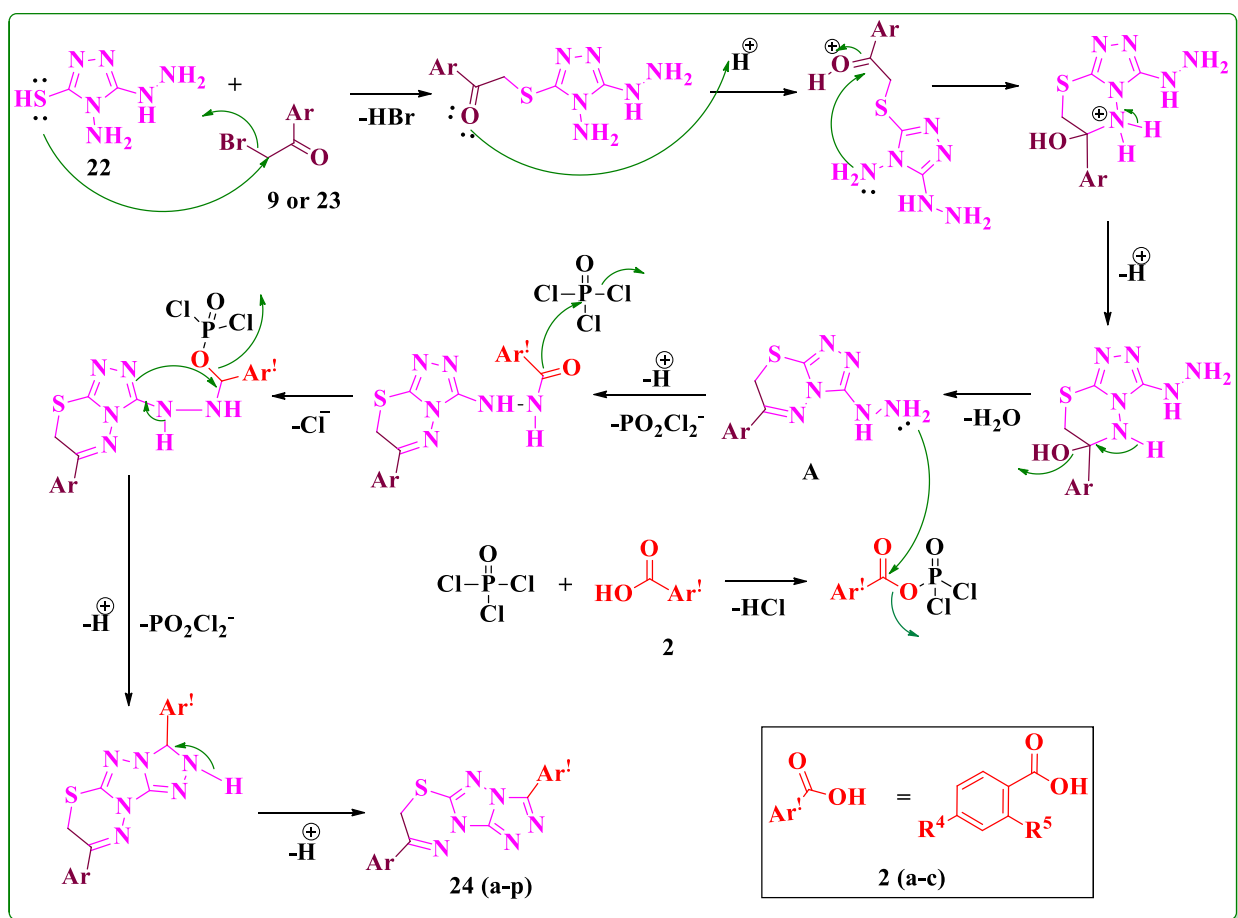


Fig. 2: Other possible alternative products.

Table-1: 7H-[1,2,4]triazolo[4',3':1,5][1,2,4]triazolo[3,4-b][1,3,4]thiadiazines (**24a-p**).

Product	R ¹	R ²	R ³	R ⁴	R ⁵	Time (h)	Yield (%)
24a	H	—	—	CH ₃	H	12.10	88
24b	CH ₃	—	—	CH ₃	H	12.30	91
24c	OCH ₃	—	—	CH ₃	H	12.40	86
24d	F	—	—	CH ₃	H	13.10	83
24e	Cl	—	—	CH ₃	H	13.20	87
24f	Br	—	—	CH ₃	H	13.00	84
24g	NO ₂	—	—	CH ₃	H	14.20	80
24h	Ph	—	—	CH ₃	H	13.30	89
24i	OCH ₃	—	—	F	H	14.00	81

24j	Br	—	—	H	Br	13.50	83
24k	—	H	H	CH ₃	H	15.00	90
24l	—	OCH ₃	H	CH ₃	H	15.20	85
24m	—	H	Cl	CH ₃	H	15.10	82
24n	—	H	Br	CH ₃	H	15.30	84
24o	—	Br	Br	CH ₃	H	15.50	81
24p	—	—	—	CH ₃	H	15.30	86



Scheme-2: Plausible reaction pathway for the synthesis of target derivatives (24a-p).

All the structures of the newly synthesized derivatives were confirmed by their analytical and spectral data. For example, the FT-IR spectrum of compound **24a** showed a stretching band at 1608 cm^{-1} corresponding to C=N. The ^1H -NMR (400 MHz, $\text{CDCl}_3+\text{DMSO}-d_6$) spectrum of the compound **24a** displayed a singlet at δ 2.43 ppm for the methyl group ($-\text{CH}_3$) on the benzene ring, characteristic thiadiazine methylene protons appeared as a singlet at δ 4.34 ppm. The remaining nine aromatic protons appeared in the downfield from δ 7.32 to 8.15 ppm. The ^{13}C -NMR (100 MHz, $\text{CDCl}_3+\text{DMSO}-d_6$) spectrum of the product **24a** showed a peak at δ 21.6 ppm for the methyl carbon on the aromatic ring and the methylene ($-\text{CH}_2-$) carbon of the thiadiazine ring appeared at δ 23.0 ppm. The remaining sp^2 hybridized carbons gave peaks in the downfield from δ 114.6 to 162.8 ppm. In the ESI-MS compound **24a** exhibited the base peak at m/z : 347 $[\text{M}+\text{H}]^+$.

In summary, we have synthesized 7*H*-[1,2,4]triazolo[4',3':1,5][1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines (**24a-p**) via a one-pot, multi-component reaction approach with readily available starting compounds under the simple reaction conditions and good yields. All the newly synthesized compounds were confirmed by their analytical and spectral data.

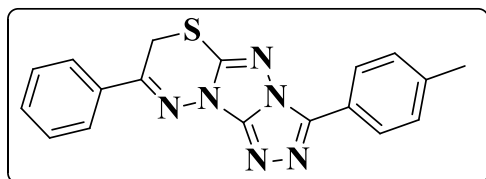
EXPERIMENTAL:

Typical reaction procedure for the synthesis of 7*H*-[1,2,4]triazolo[4',3':1,5][1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine derivatives (24a-p):

A mixture of 4-amino-5-hydrazinyl-4*H*-1,2,4-triazole-3-thiol **22** (1 mmol) and substituted phenacyl bromide (1 mmol) or 3-(2-bromoacetyl)-2*H*-chromen-2-one (1 mmol) was taken in a 50 mL round bottom flask having 5 mL of absolute ethanol and the reaction mixture was refluxed for 4-6 h. Then the solvent was evaporated, appropriate benzoic acid (1 mmol) was added to the same reaction mixture in RB flask having POCl_3 (2 mL). Then the reaction was continued under the reflux condition for 8-10 h. After completion, the reaction mixture was cooled to room temperature then neutralized with NaHCO_3 solution and the separated solid was filtered and dried. The final compound was recrystallized from 6-8 mL ethanol.

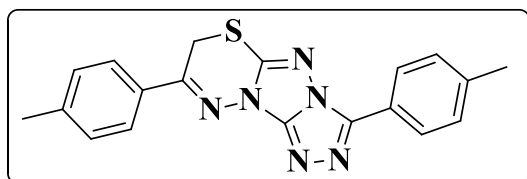
SPECTRAL DATA:

8-Phenyl-3-(*p*-tolyl)-7*H*-[1,2,4]triazolo[4',3':1,5][1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine (24a):



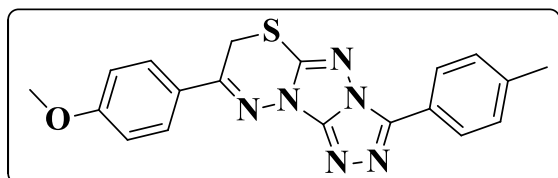
Coffee color solid (0.304g, 88%); m.p.: 157-159°C; **IR** (KBr, $\nu_{\max}/\text{cm}^{-1}$): 1608 (C=N); **¹H-NMR** (400 MHz, CDCl₃+DMSO-*d*₆, δ ppm): 2.43 (s, 3H, -CH₃), 4.34 (s, 2H, -CH₂-), 7.33 (d, 2H, *J*=7.6 Hz, Ar-H), 7.52-7.59 (m, 3H, Ar-H), 8.02 (d, 2H, *J*=7.2 Hz, Ar-H), 8.14 (d, 2H, *J*=8.0 Hz, Ar-H); **¹³C-NMR** (100 MHz, CDCl₃+DMSO-*d*₆, δ ppm): 21.6, 23.05, 114.6, 122.6, 125.7, 129.0, 129.4, 129.7, 129.8, 140.4, 143.2, 151.1, 153.1, 162.8; **ESI-MS** (*m/z*): 347 [M+H]⁺; Analytical calculated formulae C₁₈H₁₄N₆S: C, 62.41; H, 4.07; N, 24.26; S, 9.26; Found: C, 62.46; H, 4.12; N, 24.30; S, 9.21.

3,8-Di-*p*-tolyl-7*H*-[1,2,4]triazolo[4',3':1,5][1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine (24b):



Coffee color solid (0.327g, 91%); m.p.: 162-164°C; **IR** (KBr, $\nu_{\max}/\text{cm}^{-1}$): 1610 (C=N); **¹H-NMR** (400 MHz, CDCl₃+DMSO-*d*₆, δ ppm): 2.43 (s, 3H, -CH₃), 2.44 (s, 3H, -CH₃), 4.41 (s, 2H, -CH₂-), 7.33-7.36 (m, 4H, Ar-H), 7.93 (d, 2H, *J*=8.8 Hz, Ar-H), 8.12 (d, 2H, *J*=8.0 Hz, Ar-H); **¹³C-NMR** (100 MHz, CDCl₃+DMSO-*d*₆, δ ppm): 21.5, 23.2, 123.6, 125.4, 127.7, 128.3, 130.2, 130.6, 130.9, 140.1, 142.7, 151.2, 154.3; **ESI-MS** (*m/z*): 361 [M+H]⁺; Analytical calculated formulae C₁₉H₁₆N₆S: C, 63.31; H, 4.47; N, 23.32; S, 8.90; Found: C, 63.35; H, 4.50; N, 23.36; S, 8.94.

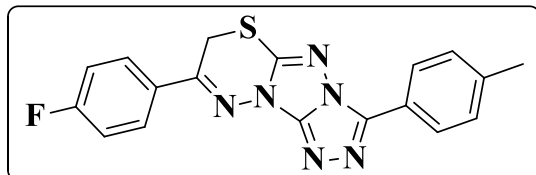
8-(4-Methoxyphenyl)-3-(*p*-tolyl)-7*H*-[1,2,4]triazolo[4',3':1,5][1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine (24c):



Pale yellow color solid (0.323g, 86%); m.p.: 165-167°C; **IR** (KBr, $\nu_{\max}/\text{cm}^{-1}$): 1607 (C=N); **¹H-NMR** (400 MHz, CDCl₃+DMSO-*d*₆, δ ppm): 2.43 (s, 3H, -CH₃), 3.89 (s, 3H, -OCH₃), 4.39 (s, 2H, -CH₂-), 7.04 (d, 2H, *J*=8.4 Hz, Ar-H), 7.34 (d, 2H, *J*=7.6 Hz, Ar-H), 8.01 (d, 2H, *J*=8.4 Hz, Ar-H), 8.12 (d, 2H, *J*=7.6 Hz, Ar-H); **¹³C-NMR** (100 MHz, CDCl₃+DMSO-*d*₆, δ ppm): 21.6,

23.0, 55.7, 114.6, 122.6, 125.7, 129.0, 129.4, 129.7, 129.8, 140.4, 143.2, 151.1, 153.1, 162.8; **ESI-MS (*m/z*):** 377 [M+H]⁺; Analytical calculated formulae C₁₉H₁₆N₆OS: C, 60.62; H, 4.28; N, 22.33; S, 8.52; Found: C, 60.57; H, 4.24; N, 22.37; S, 8.57.

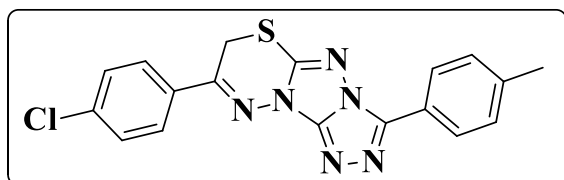
8-(4-Fluorophenyl)-3-(*p*-tolyl)-7*H*-[1,2,4]triazolo[4',3':1,5][1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine (24d):



Brown color solid (0.302g, 83%); m.p.: 158-160°C; **IR (KBr, $\nu_{\text{max}}/\text{cm}^{-1}$):** 1608 (C=N); **¹H-NMR (400 MHz, CDCl₃+DMSO-*d*₆, δ ppm):** 2.43 (s, 3H, -CH₃), 4.44 (s, 2H, -CH₂-), 7.29 (t, 2H,

$J=8.4$ Hz, Ar-H), 7.34 (d, 2H, $J=8.0$ Hz, Ar-H), 8.11 (d, 4H, $J=7.6$ Hz, Ar-H); **¹³C-NMR (100 MHz, CDCl₃+DMSO-*d*₆, δ ppm):** 26.3, 36.5, 121.3, 130.2, 134.7, 134.9, 135.2, 137.2, 144.5, 155.6, 160.9, 161.4, 166.6, 169.7; **ESI-MS (*m/z*):** 365 [M+H]⁺; Analytical calculated formulae C₁₈H₁₃FN₆S: C, 59.33; H, 3.60; N, 23.06; S, 8.80; Found: C, 59.28; H, 3.56; N, 23.11; S, 8.84.

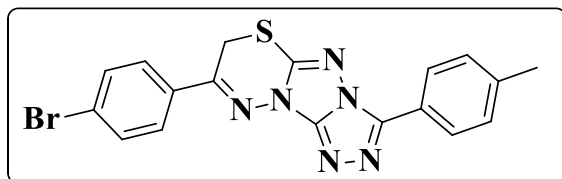
8-(4-Chlorophenyl)-3-(*p*-tolyl)-7*H*-[1,2,4]triazolo[4',3':1,5][1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine (24e):



Light yellow color solid (0.330g, 87%); m.p.: 163-165°C; **IR (KBr, $\nu_{\text{max}}/\text{cm}^{-1}$):** 1609 (C=N); **¹H-NMR (400 MHz, CDCl₃+DMSO-*d*₆, δ ppm):** 2.40 (s, 3H, -CH₃), 4.54 (s, 2H, -CH₂-),

7.38 (d, 2H, $J=7.6$ Hz, Ar-H), 7.64 (d, 2H, $J=8.0$ Hz, Ar-H), 8.06 (d, 4H, $J=8.8$ Hz, Ar-H); **¹³C-NMR (100 MHz, CDCl₃+DMSO-*d*₆, δ ppm):** 21.5, 23.3, 127.8, 128.2, 129.9, 130.9, 137.7, 138.6, 142.6, 142.7, 144.9, 153.1, 161.5, 163.9; **ESI-MS (*m/z*):** 381 [M+H]⁺; Analytical calculated formulae C₁₈H₁₃ClN₆S: C, 56.77; H, 3.44; N, 22.07; S, 8.42; Found: , 56.80; H, 3.40; N, 22.03; S, 8.47.

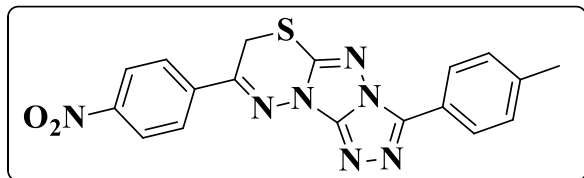
8-(4-Bromophenyl)-3-(p-tolyl)-7H-[1,2,4]triazolo[4',3':1,5][1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (24f):



Pale yellow color solid (0.357g, 84%); m.p.: 156-158°C; **IR (KBr, $\nu_{\max}/\text{cm}^{-1}$):** 1611 (C=N); **$^1\text{H-NMR}$ (400 MHz, $\text{CDCl}_3+\text{DMSO-}d_6$, δ ppm):** 2.41 (s, 3H, -CH₃), 4.52 (s, 2H, -CH₂-),

7.37 (d, 2H, $J=8.0$ Hz, Ar-H), 7.77 (d, 2H, $J=8.4$ Hz, Ar-H), 7.99 (d, 2H, $J=8.4$ Hz, Ar-H), 8.07 (d, 2H, $J=8.0$ Hz, Ar-H); **$^{13}\text{C-NMR}$ (100 MHz, $\text{CDCl}_3+\text{DMSO-}d_6$, δ ppm):** 21.6, 23.0, 114.6, 122.6, 125.6, 129.0, 129.4, 129.7, 129.8, 140.3, 143.2, 151.1, 151.8, 153.1 ; **ESI-MS (m/z):** 425 $[\text{M}+\text{H}]^+$; Analytical calculated formulae $\text{C}_{18}\text{H}_{13}\text{BrN}_6\text{S}$: C, 50.83; H, 3.08; N, 19.76; S, 7.54; Found: C, 50.87; H, 3.02; N, 19.73; S, 7.58.

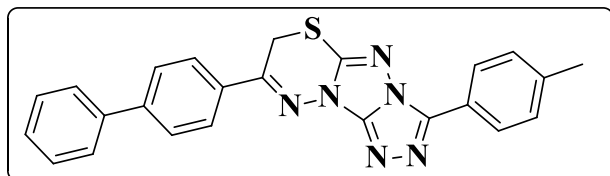
8-(4-Nitrophenyl)-3-(p-tolyl)-7H-[1,2,4]triazolo[4',3':1,5][1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (24g):



Yellow color solid (0.312g, 80%); m.p.: 155-157°C; **IR (KBr, $\nu_{\max}/\text{cm}^{-1}$):** 1610 (C=N); **$^{13}\text{C-NMR}$ (100 MHz, $\text{CDCl}_3+\text{DMSO-}d_6$, δ ppm):** 26.3, 34.1, 122.5, 128.9, 129.5, 130.3 133.0,

134.4, 134.5, 134.9, 135.9, 139.3, 148.4, 172.7; **ESI-MS (m/z):** 392 $[\text{M}+\text{H}]^+$; Analytical calculated formulae $\text{C}_{18}\text{H}_{13}\text{N}_7\text{O}_2\text{S}$: C, 55.23; H, 3.35; N, 25.05; S, 8.19; Found: C, 55.20; H, 3.31; N, 25.09; S, 8.15.

8-([1,1'-Biphenyl]-4-yl)-3-(p-tolyl)-7H-[1,2,4]triazolo[4',3':1,5][1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (24h):

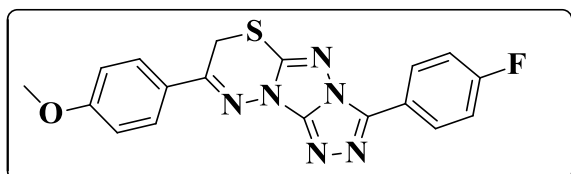


Coffee color solid (0.375g, 89%); m.p.: 172-174°C; **IR (KBr, $\nu_{\max}/\text{cm}^{-1}$):** 1598 (C=N); **$^1\text{H-NMR}$ (400 MHz, $\text{CDCl}_3+\text{DMSO-}d_6$, δ ppm):** 2.38 (s, 3H, -CH₃), 4.51 (s, 2H, -CH₂-)

, 7.32 (d, 2H, $J=8.4$ Hz, Ar-H), 7.38 (t, 1H, $J=7.2$ Hz, Ar-H), 7.47 (t, 4H, $J=7.6$ Hz, Ar-H), 7.69 (d, 2H, $J=7.6$ Hz, Ar-H), 7.80 (d, 2H, $J=8.4$ Hz, Ar-H), 8.06 (d, 2H, $J=8.0$ Hz, Ar-H); **$^{13}\text{C-NMR}$ (100 MHz, $\text{CDCl}_3+\text{DMSO-}d_6$, δ ppm):** 21.6, 23.0, 122.6, 125.7, 128.3, 129.0, 129.2, 129.3,

129.4, 129.7, 129.8, 130.4, 140.4, 143.2, 143.5, 151.1, 151.8, 153.1, 162.8; **ESI-MS (m/z):** 423 $[M+H]^+$; Analytical calculated formulae $C_{24}H_{18}N_6S$: C, 68.23; H, 4.29; N, 19.89; S, 7.59; Found: C, 68.26; H, 4.25; N, 19.85; S, 7.55.

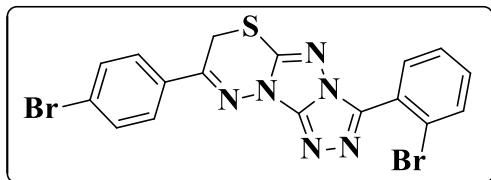
3-(4-Fluorophenyl)-8-(4-methoxyphenyl)-7H-[1,2,4]triazolo[4',3':1,5][1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (24i):



Yellow color solid (0.307g, 81%); m.p.: 179-181°C; **IR (KBr, ν_{max}/cm^{-1}):** 1604 (C=N); **^{13}C -NMR (100 MHz, $CDCl_3$ +DMSO- d_6 , δ ppm):** 23.3, 56.0, 114.9, 116.2, 116.3, 116.7, 125.8,

129.8, 130.3, 131.4, 132.6, 150.8, 155.0, 162.8; **ESI-MS (m/z):** 381 $[M+H]^+$; Analytical calculated formulae $C_{18}H_{13}FN_6OS$: C, 56.83; H, 3.44; N, 22.09; S, 8.43; Found: C, 56.80; H, 3.40; N, 22.04; S, 8.40.

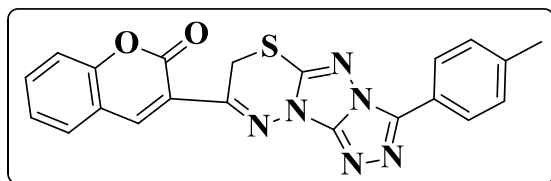
3-(2-Bromophenyl)-8-(4-bromophenyl)-7H-[1,2,4]triazolo[4',3':1,5][1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (24j):



Pale yellow color solid (0.406g, 83%); m.p.: 185-187°C; **IR (KBr, ν_{max}/cm^{-1}):** 1607 (C=N); **1H -NMR (400 MHz, $CDCl_3$ +DMSO- d_6 , δ ppm):** 4.35 (s, 2H, -CH₂-), 7.57 (t, 1H, J =8.8 Hz, Ar-H), 8.10 (t, 1H, J =8.8

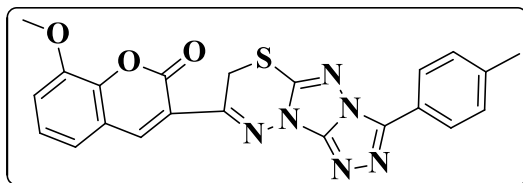
Hz, Ar-H), 8.91 (d, 2H, J =5.2 Hz, Ar-H), 9.00 (d, 2H, J =5.6 Hz, Ar-H), 9.03 (d, 1H, J =2.8 Hz, Ar-H), 9.10 (d, 1H, J =6.8 Hz, Ar-H); **^{13}C -NMR (100 MHz, $CDCl_3$ +DMSO- d_6 , δ ppm):** 23.0, 114.6, 122.6, 125.6, 129.0, 129.4, 129.7, 129.8, 140.4, 143.2, 143.5, 151.1, 151.8, 153.1, 162.8; **ESI-MS (m/z):** 493 $[M+3]^+$; Analytical calculated formulae $C_{18}H_{13}Br_2N_6S$: C, 41.65; H, 2.06; N, 17.14; S, 6.54; Found: C, 41.61; H, 2.02; N, 17.12; S, 6.50.

3-(3-(*p*-Tolyl)-7*H*-[1,2,4]triazolo[4',3':1,5][1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-8-yl)-2*H*-chromen-2-one (24k):



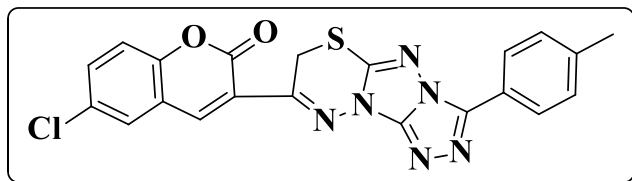
Light orange color solid (0.372g, 90%); m.p.: 192-194°C; **IR (KBr, $\nu_{\max}/\text{cm}^{-1}$):** 1608 (C=N), 1720 (C=O); **^{13}C -NMR (100 MHz, DMSO- d_6 , δ ppm):** 21.6, 24.9, 116.8, 125.4, 128.7, 129.6, 129.8, 130.2, 130.4, 134.3, 134.4, 134.7, 144.9, 153.7, 154.5, 159.1, 167.8, 169.9; **ESI-MS (m/z):** 415 [M+H]⁺; Analytical calculated formulae C₂₁H₁₄N₆O₂S: C, 60.86; H, 3.40; N, 20.28; S, 7.74; Found: C, 60.82; H, 3.35; N, 20.31; S, 7.78.

8-Methoxy-3-(3-(*p*-tolyl)-7*H*-[1,2,4]triazolo[4',3':1,5][1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-8-yl)-2*H*-chromen-2-one (24l):



Yellow color solid (0.377g, 85%); m.p.: 195-197°C; **IR (KBr, $\nu_{\max}/\text{cm}^{-1}$):** 1609 (C=N), 1719 (C=O); **^1H -NMR (400 MHz, CDCl₃+DMSO- d_6 , δ ppm):** 2.36 (s, 3H, -CH₃), 3.95 (s, 3H, -OCH₃), 4.42 (s, 2H, -CH₂-), 7.76 (d, 1H, J =7.6 Hz, Ar-H), 7.81 (d, 2H, J =8.0 Hz, Ar-H), 7.90 (t, 1H, J =8.4 Hz, Ar-H), 8.04 (d, 2H, J =8.0 Hz, Ar-H), 8.13 (d, 1H, J =6.4 Hz, Ar-H), 8.56 (s, 1H, Ar-H); **^{13}C -NMR (100 MHz, CDCl₃+DMSO- d_6 , δ ppm):** 26.3, 26.4, 61.4, 124.0, 125.9, 126.8, 130.2, 130.4, 130.6, 133.1, 133.4, 134.3, 134.5, 134.9, 135.3, 135.5, 144.5, 148.3, 151.5, 172.6; **ESI-MS (m/z):** 445 [M+H]⁺; Analytical calculated formulae C₂₂H₁₆N₆O₃S: C, 59.45; H, 3.63; N, 18.91; S, 7.21; Found: C, 59.42; H, 3.67; N, 18.95; S, 7.25.

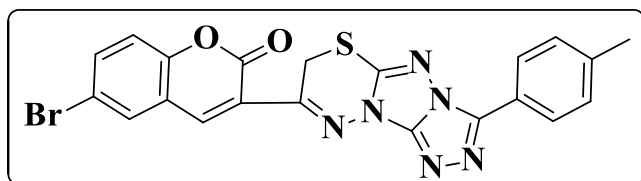
6-Chloro-3-(3-(*p*-tolyl)-7*H*-[1,2,4]triazolo[4',3':1,5][1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-8-yl)-2*H*-chromen-2-one (24m):



Yellow color solid (0.367g, 82%); m.p.: 189-191°C; **IR (KBr, $\nu_{\max}/\text{cm}^{-1}$):** 1610 (C=N), 1727 (C=O); **^1H -NMR (400 MHz, CDCl₃+DMSO- d_6 , δ ppm):** 2.42 (s, 3H, -CH₃), 4.40 (s, 2H, -CH₂-), 7.24 (d, 2H, J =8.0 Hz, Ar-H), 7.33 (d, 2H, J =7.2 Hz, Ar-H), 7.64 (d, 1H, J =8.0 Hz, Ar-H), 7.76 (s, 1H, Ar-H), 8.11 (d, 1H, J =8.0 Hz, Ar-H), 8.59 (s, 1H, Ar-H); **^{13}C -**

NMR (100 MHz, CDCl₃+DMSO-*d*₆, δ ppm): 21.6, 24.3, 118.2, 125.5, 128.3, 128.7, 129.0, 129.1, 129.2, 129.7, 130.0, 130.1, 133.7, 143.1, 143.9, 150.7, 152.9, 168.1; **ESI-MS (*m/z*):** 449 [M+H]⁺; Analytical calculated formulae C₂₁H₁₃ClN₆O₂S: C, 56.19; H, 2.92; N, 18.72; S, 7.14; Found: C, 56.15; H, 2.96; N, 18.68; S, 7.17.

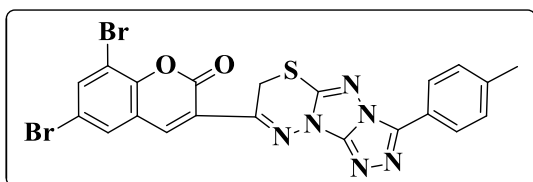
6-Bromo-3-(3-(*p*-tolyl)-7*H*-[1,2,4]triazolo[4',3':1,5][1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-8-yl)-2*H*-chromen-2-one (24n):



Yellow color solid (0.414g, 84%); m.p.: 203-205°C; **IR (KBr, $\nu_{\max}/\text{cm}^{-1}$):** 1610 (C=N), 1728 (C=O); **¹H-NMR (400 MHz, CDCl₃+DMSO-*d*₆, δ ppm):** 2.43 (s, 3H, -

CH₃), 4.39 (s, 2H, -CH₂-), 7.24 (d, 1H, *J*=8.0 Hz, Ar-H), 7.33 (d, 2H, *J*=8.0 Hz, Ar-H), 7.97 (d, 1H, *J*=8.0 Hz, Ar-H), 8.04 (s, 1H, Ar-H), 8.10 (d, 2H, *J*=8.0 Hz, Ar-H), 8.58 (s, 1H, Ar-H); **¹³C-NMR (100 MHz, CDCl₃+DMSO-*d*₆, δ ppm):** 21.6, 29.5, 117.2, 119.0, 125.4, 127.9, 128.1, 128.4, 128.6, 129.4, 129.5, 129.6, 129.8, 130.2, 132.2, 143.5, 153.5, 163.7, 167.8; **ESI-MS (*m/z*):** 495 [M+2]⁺; Analytical calculated formulae C₂₁H₁₃BrN₆O₂S: C, 51.13; H, 2.66; N, 17.04; S, 6.50; Found: C, 51.17; H, 2.62; N, 17.08; S, 6.54.

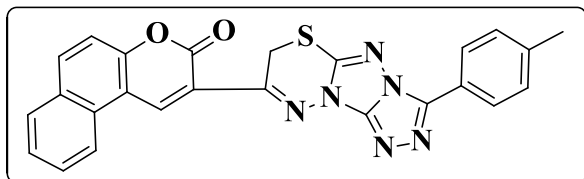
6,8-Dibromo-3-(3-(*p*-tolyl)-7*H*-[1,2,4]triazolo[4',3':1,5][1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-8-yl)-2*H*-chromen-2-one (24o):



Yellow color solid (0.463g, 81%); m.p.: 216-218°C; **IR (KBr, $\nu_{\max}/\text{cm}^{-1}$):** 1611 (C=N), 1730 (C=O); **¹H-NMR (400 MHz, CDCl₃+DMSO-*d*₆, δ ppm):** 2.43 (s, 3H, -CH₃), 4.37 (s, 2H, -CH₂-), 7.32

(d, 2H, *J*=8.0 Hz, Ar-H), 7.94 (s, 1H, Ar-H), 8.01 (s, 1H, Ar-H), 8.12 (d, 2H, *J*=8.0 Hz, Ar-H), 8.55 (s, 1H, Ar-H); **¹³C-NMR (100 MHz, CDCl₃+DMSO-*d*₆, δ ppm):** 21.6, 24.2, 110.7, 117.5, 121.3, 125.5, 128.1, 128.3, 129.0, 129.1, 129.4, 129.6, 129.7, 131.7, 143.1, 143.5, 150.2, 157.8, 168.0; **ESI-MS (*m/z*):** 573 [M+H]⁺; Analytical calculated formulae C₂₁H₁₂Br₂N₆O₂S: C, 44.08; H, 2.11; N, 14.69; S, 5.60; Found: C, 44.04; H, 2.15; N, 14.64; S, 5.64.

2-(3-(*p*-Tolyl)-7*H*-[1,2,4]triazolo[4',3':1,5][1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-8-yl)-3*H*-benzo[*f*]chromen-3-one (24p):



Yellow color solid (0.399g, 86%); m.p.: 208-210°C; **IR (KBr, $\nu_{\text{max}}/\text{cm}^{-1}$):** 1610 (C=N), 1719 (C=O); **$^1\text{H-NMR}$ (400 MHz, $\text{CDCl}_3+\text{DMSO-}d_6$, δ ppm):** 2.42 (s, 3H, -

CH_3), 4.45 (s, 2H, $-\text{CH}_2-$), 7.32 (d, 2H, $J=8.0$ Hz, Ar-H), 7.64 (t, 2H, $J=7.2$ Hz, Ar-H), 7.78 (t, 2H, $J=7.2$ Hz, Ar-H), 7.98 (d, 1H, $J=7.6$ Hz, Ar-H), 8.13 (d, 2H, $J=8.4$ Hz, Ar-H), 8.44 (d, 1H, $J=8.4$ Hz, Ar-H), 9.32 (s, 1H, Ar-H); **$^{13}\text{C-NMR}$ (100 MHz, $\text{CDCl}_3+\text{DMSO-}d_6$, δ ppm):** 21.6, 35.1, 113.1, 113.9, 116.9, 117.0, 117.1, 122.8, 124.3, 125.6, 127.1, 128.4, 128.8, 129.5, 129.6, 129.8, 130.4, 143.6, 154.5, 155.3, 163.6, 167.9, 176.4; **ESI-MS (m/z):** 465 $[\text{M}+\text{H}]^+$; Analytical calculated formulae $\text{C}_{25}\text{H}_{16}\text{N}_6\text{O}_2\text{S}$: C, 64.64; H, 3.47; N, 18.09; S, 6.90; Found: C, 64.72; H, 3.44; N, 18.04; S, 6.94.

SPECTRA

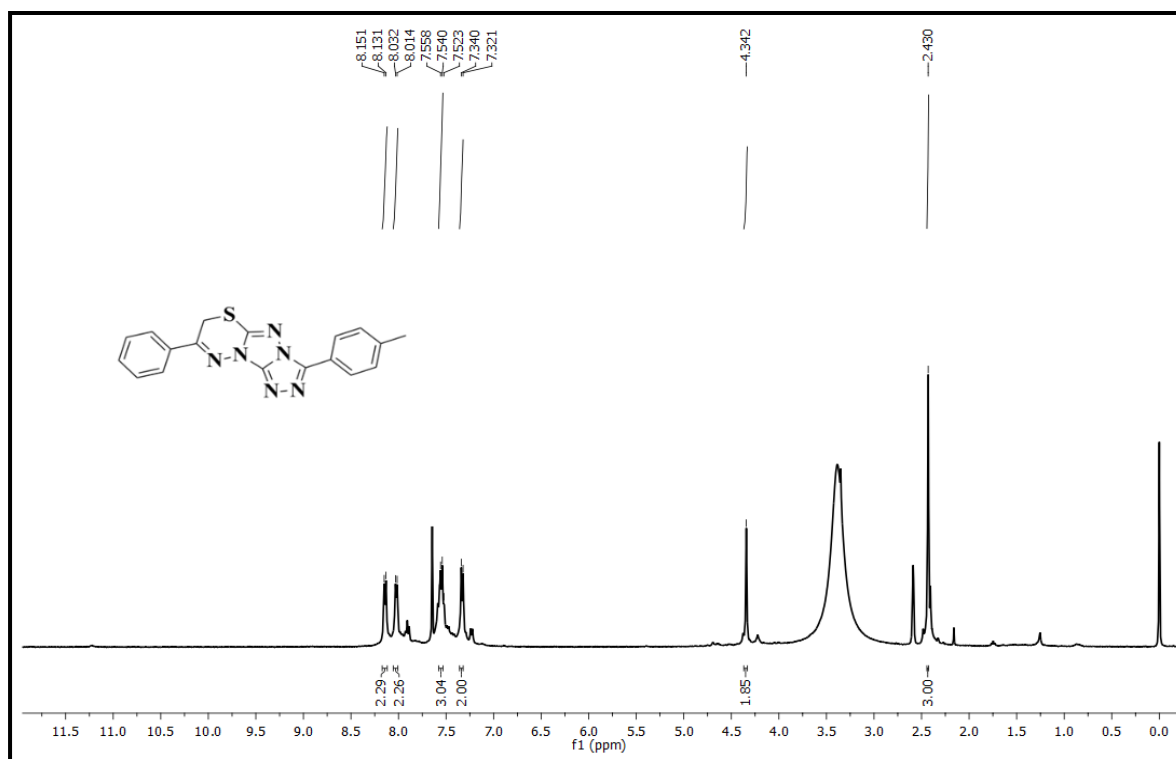


Fig: ¹H-NMR Spectrum of Compound **24a** (400 MHz, CDCl₃+DMSO-*d*₆)

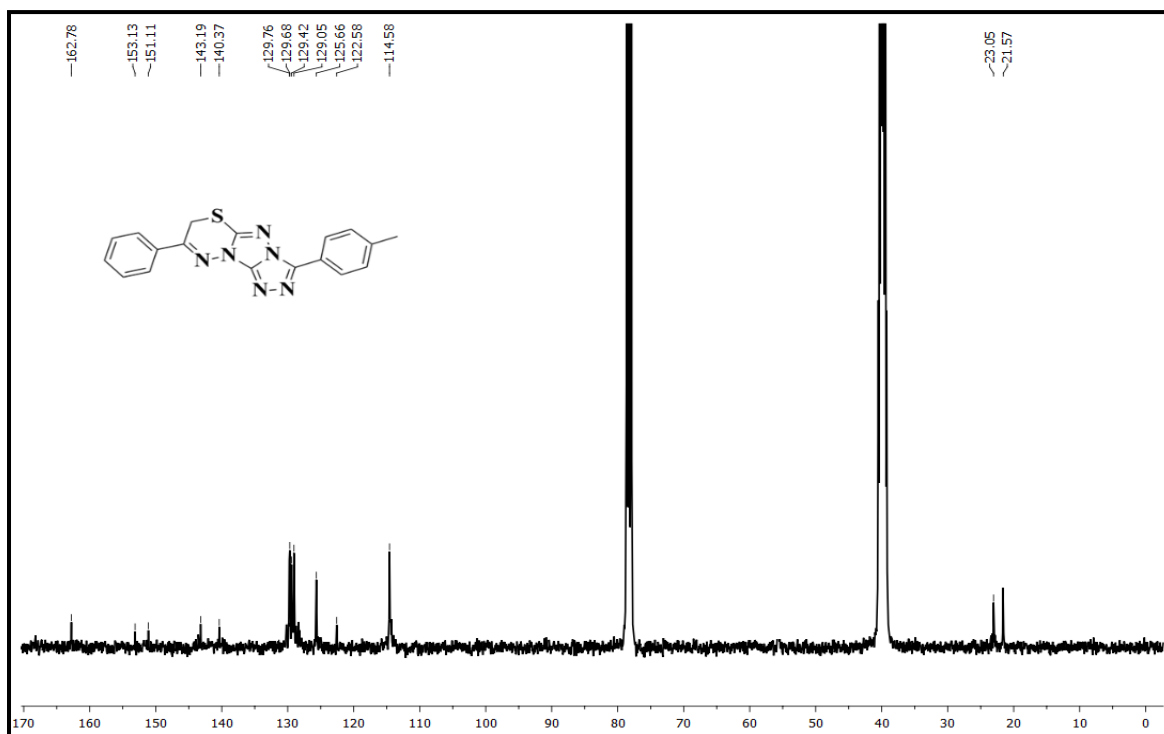


Fig: ¹³C-NMR Spectrum of Compound **24a** (100 MHz, CDCl₃+DMSO-*d*₆)

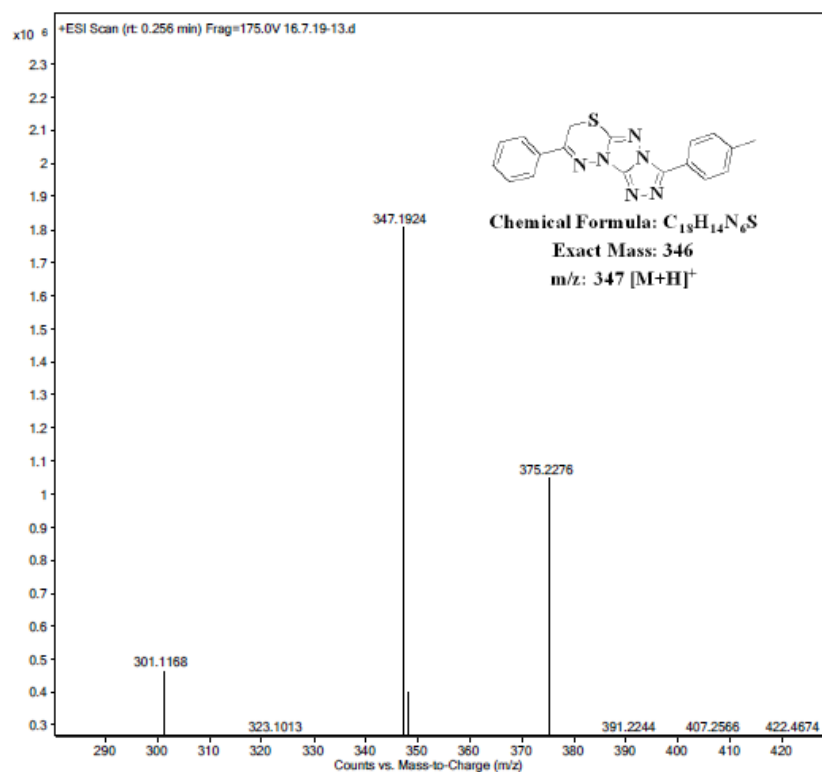


Fig: Mass Spectrum of Compound **24a**

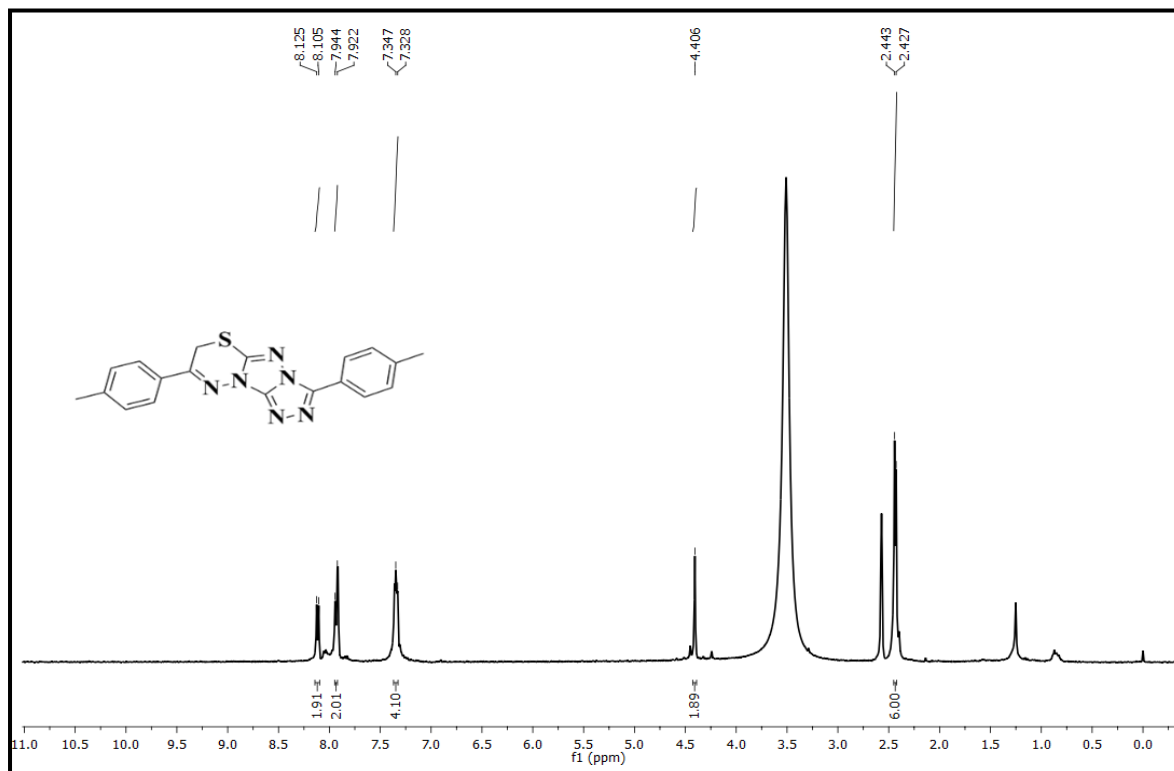


Fig: 1H -NMR Spectrum of Compound **24b** (400 MHz, $CDCl_3$ + $DMSO-d_6$)

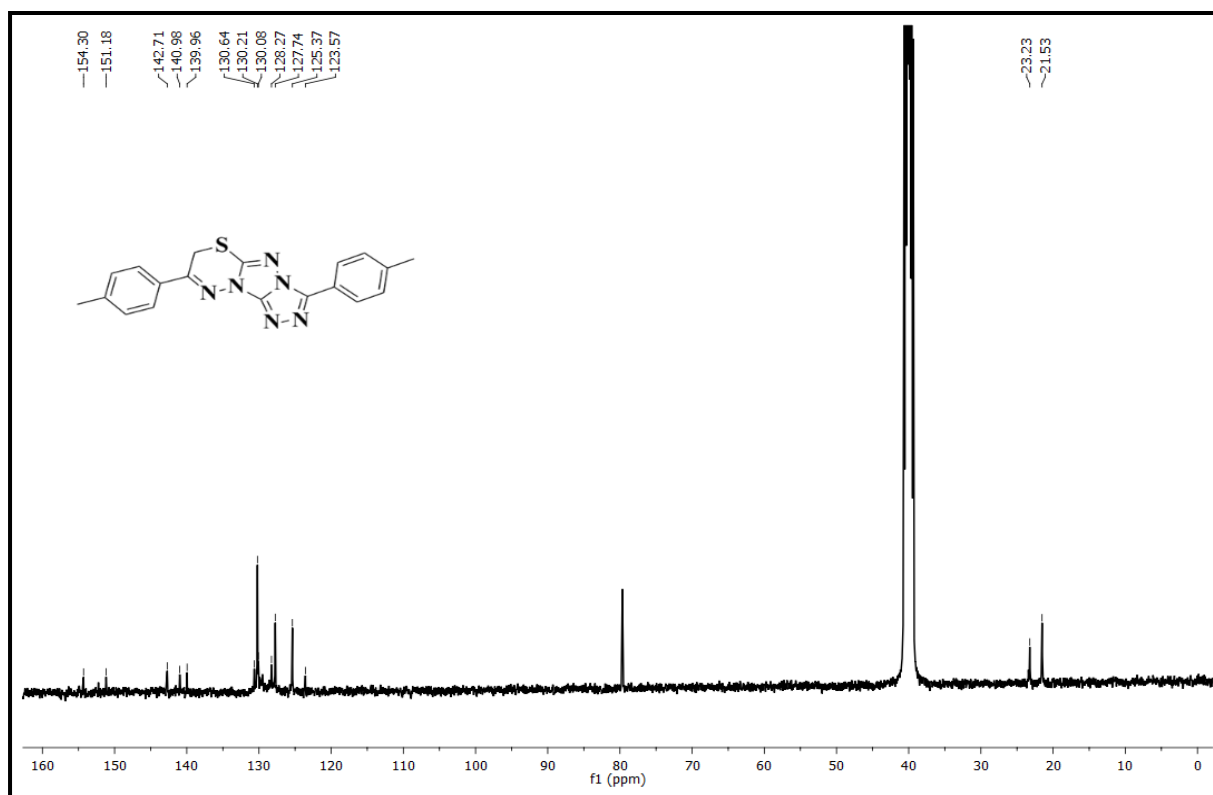


Fig: ¹³C-NMR Spectrum of Compound **24b** (100 MHz, CDCl₃+DMSO-*d*₆)

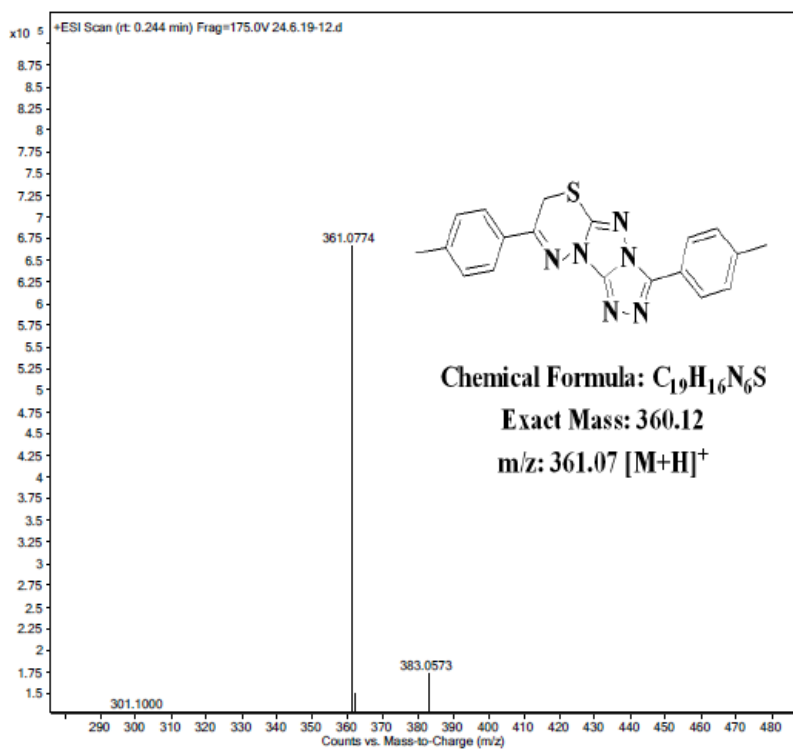


Fig: Mass Spectrum of Compound **24b**

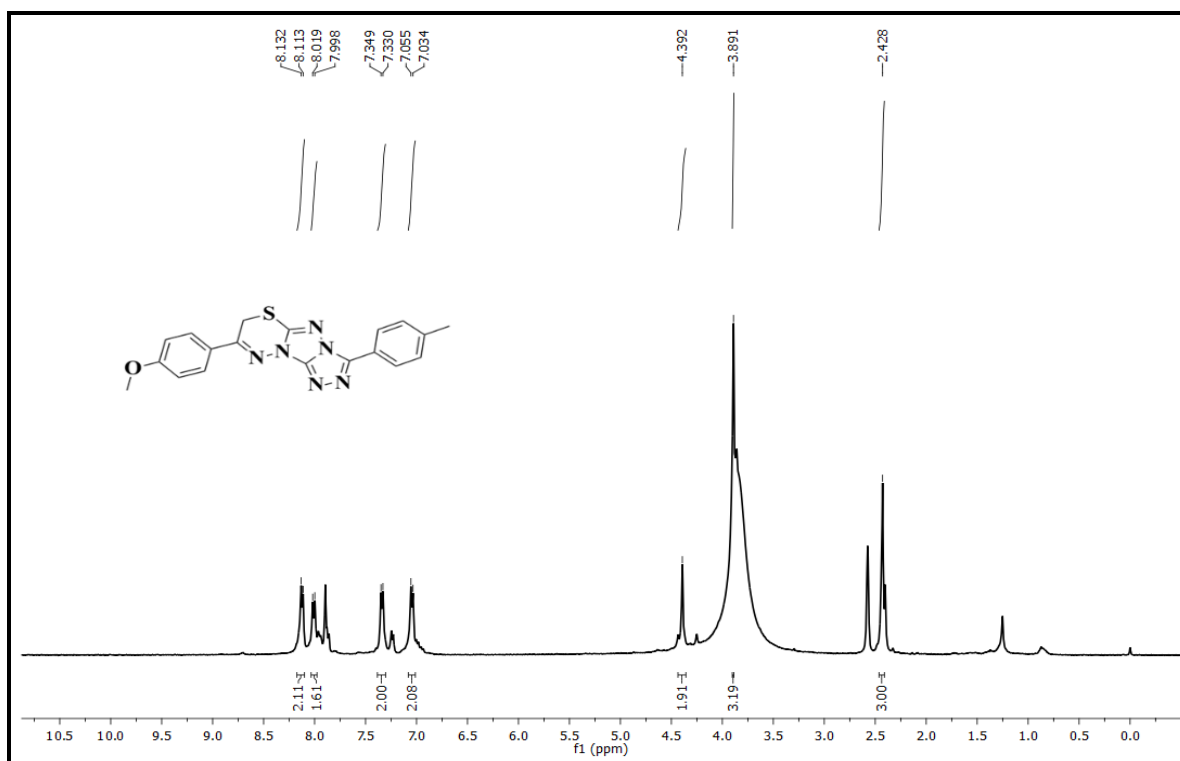


Fig: ¹H-NMR Spectrum of Compound **24c** (400 MHz, CDCl₃+DMSO-*d*₆)

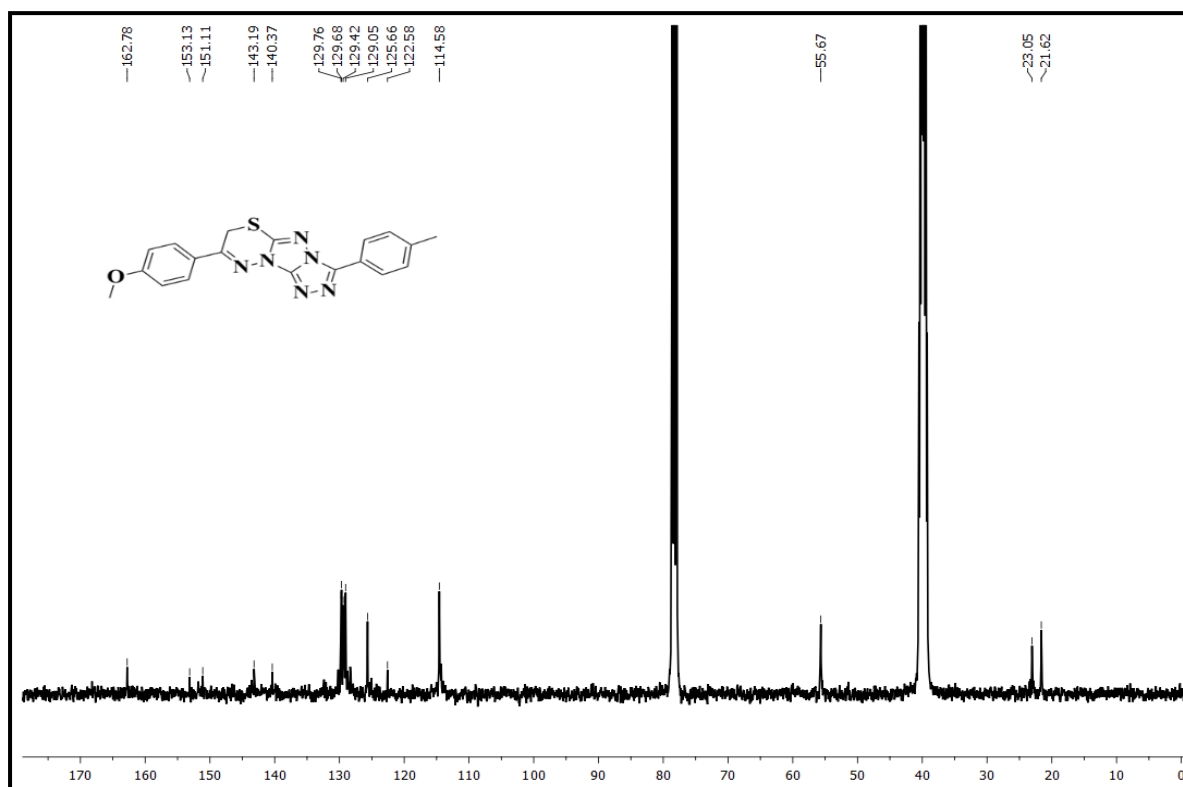


Fig: ¹³C-NMR Spectrum of Compound **24c** (100 MHz, CDCl₃+DMSO-*d*₆)

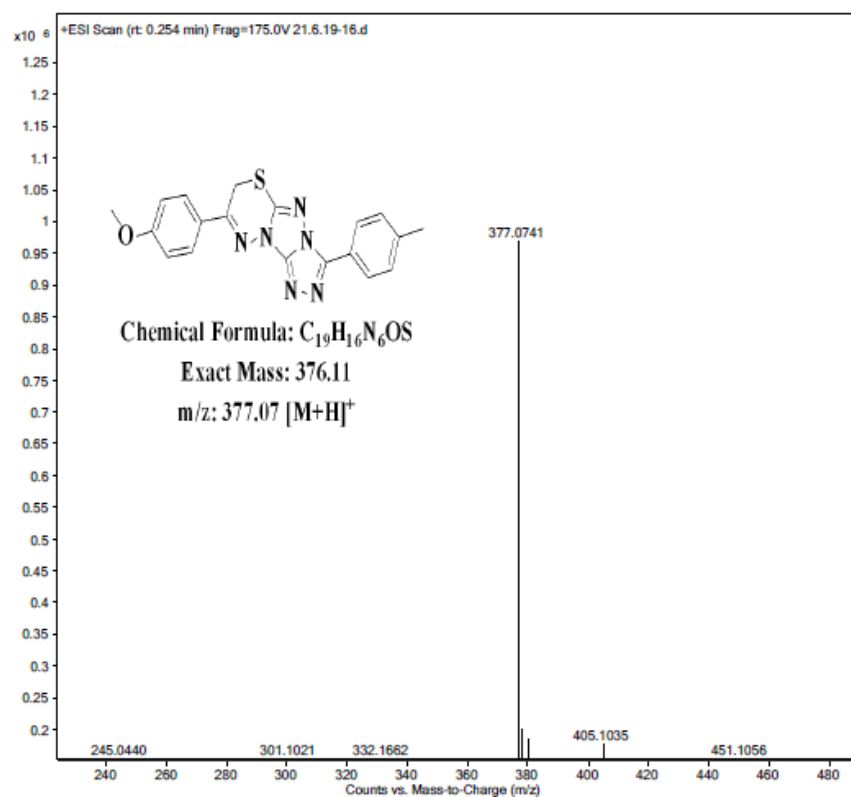


Fig: Mass Spectrum of Compound **24c**

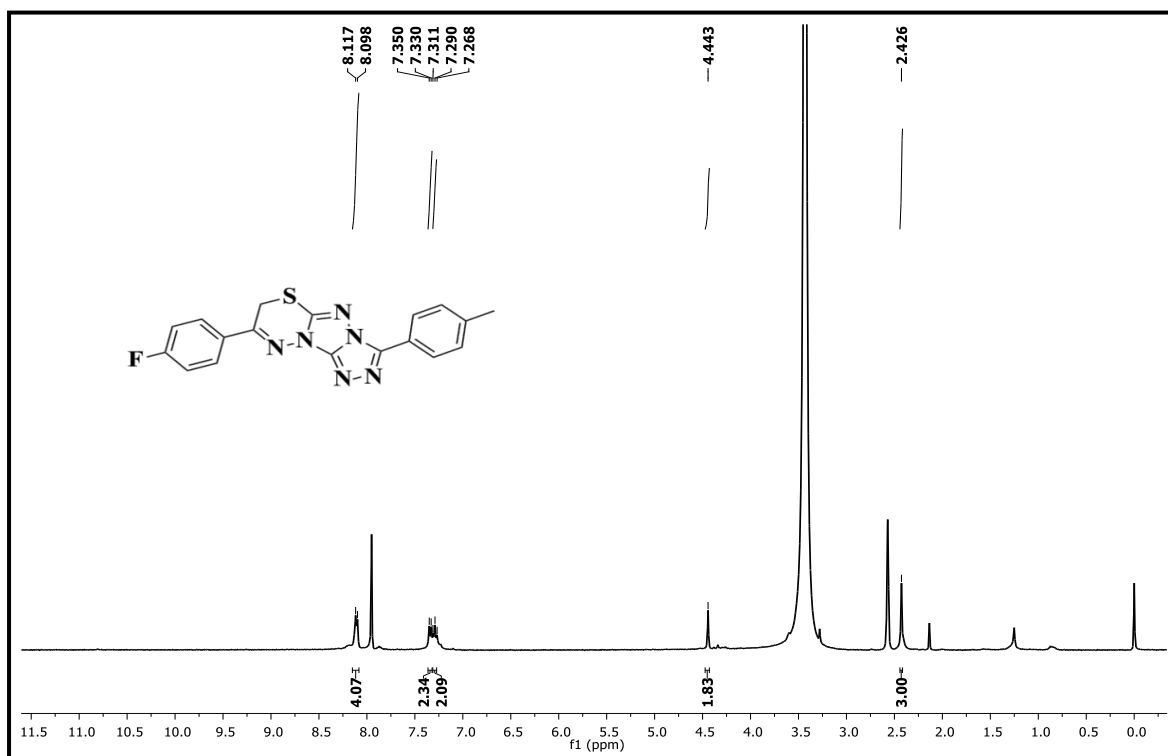


Fig: 1H -NMR Spectrum of Compound **24d** (400 MHz, $CDCl_3 + DMSO-d_6$)

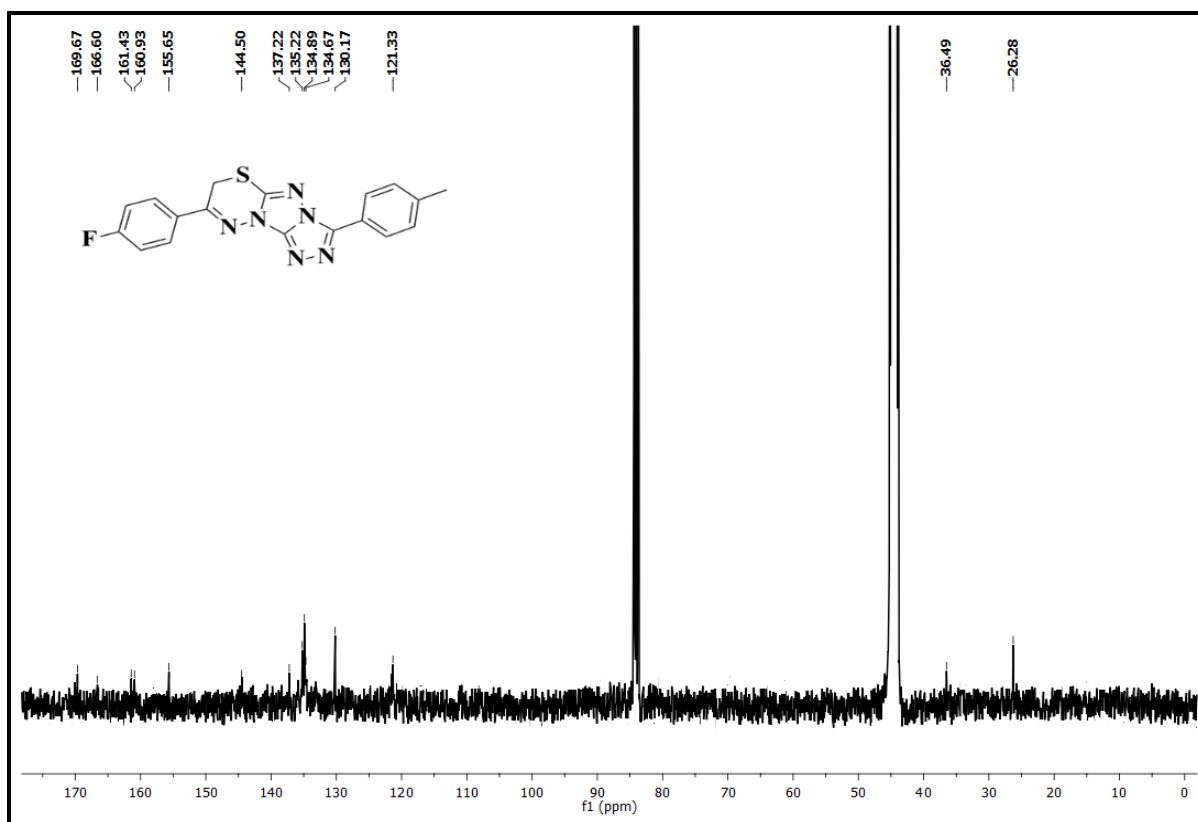


Fig: ¹³C-NMR Spectrum of Compound **24d** (100 MHz, CDCl₃+DMSO-*d*₆)

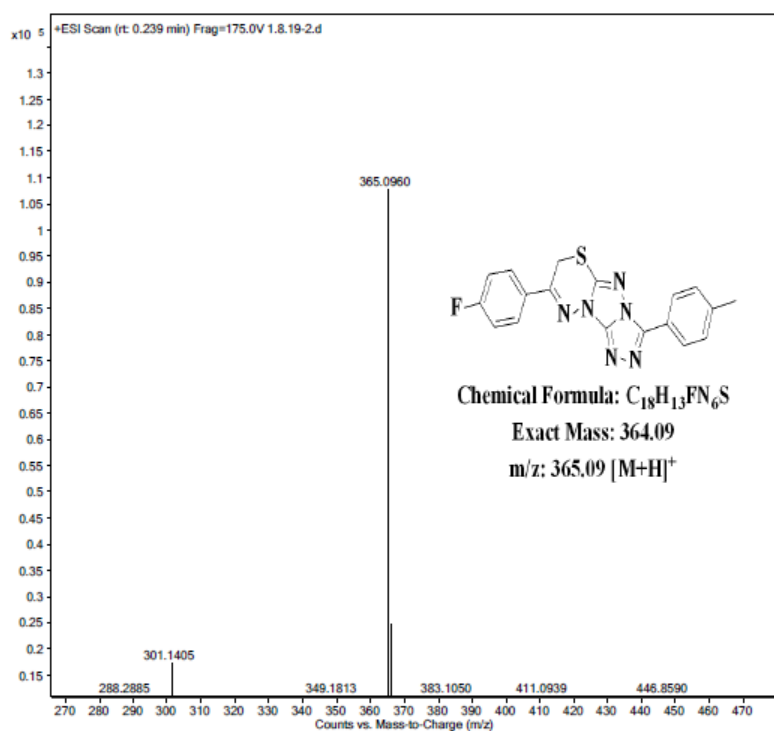


Fig: Mass Spectrum of Compound **24d**

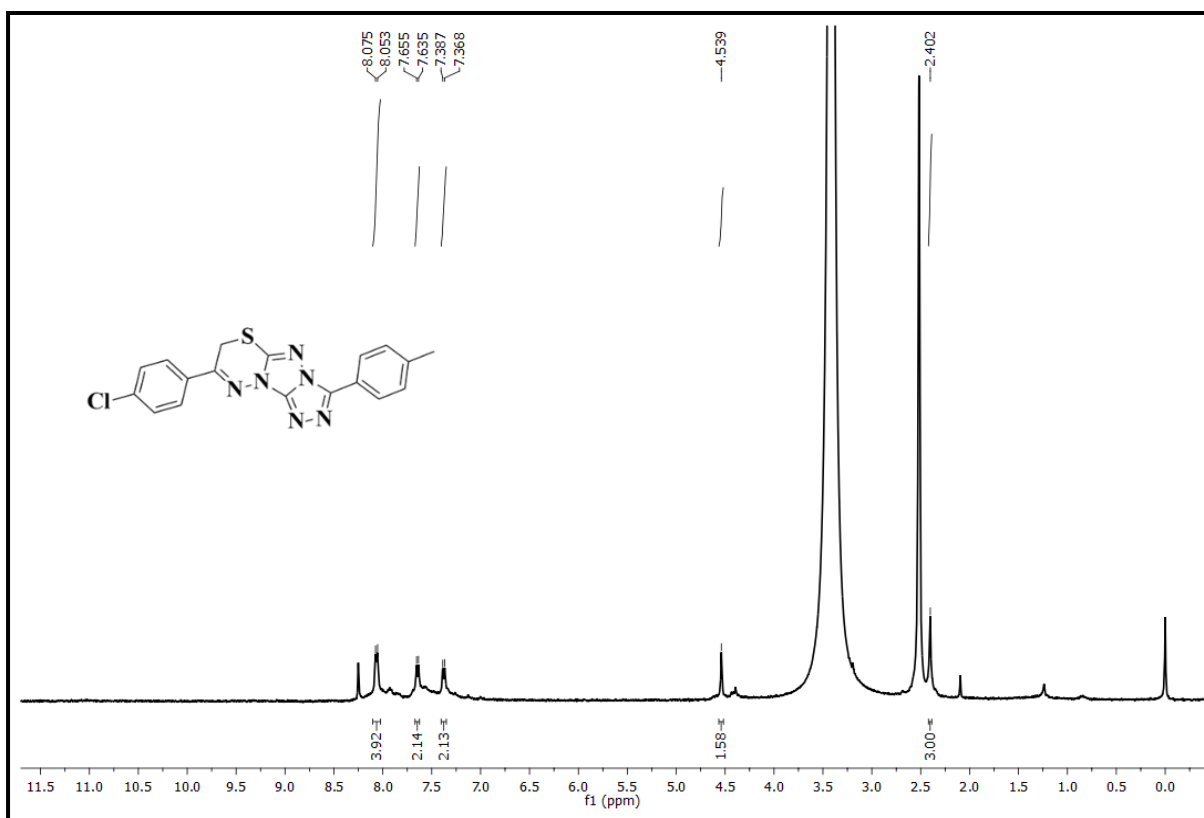


Fig: ¹H-NMR Spectrum of Compound **24e** (400 MHz, CDCl₃+DMSO-*d*₆)

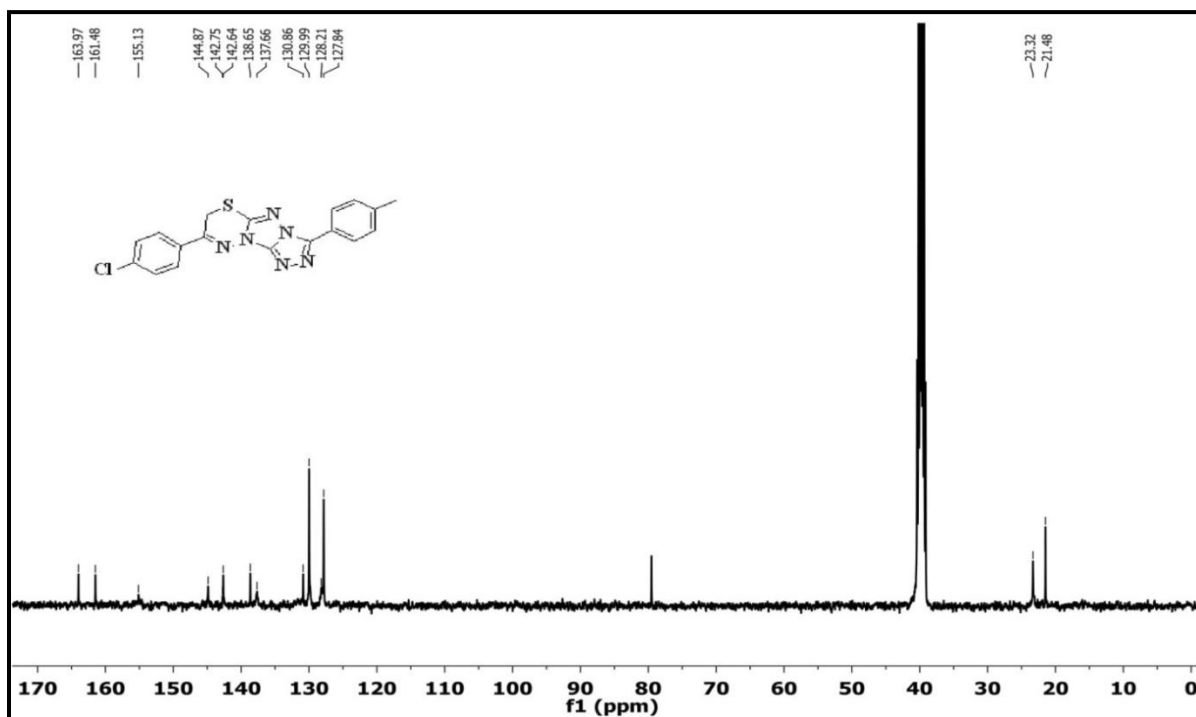


Fig: ¹³C-NMR Spectrum of Compound **24e** (100 MHz, CDCl₃+DMSO-*d*₆)

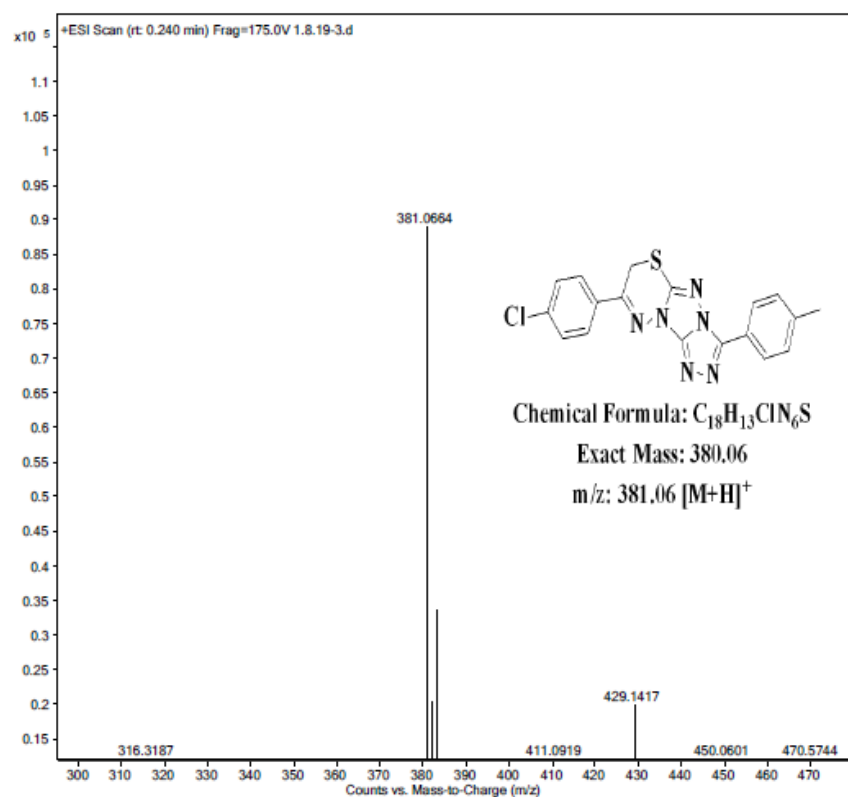


Fig: Mass Spectrum of Compound **24e**

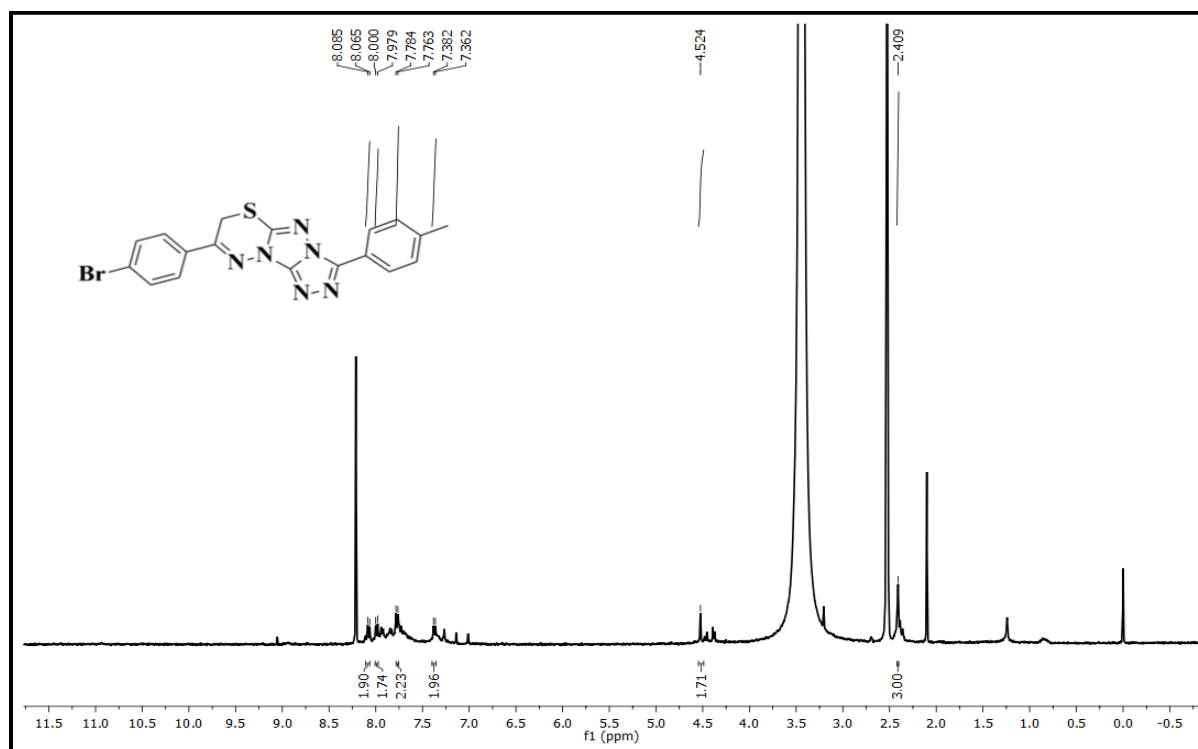


Fig: 1H -NMR Spectrum of Compound **24f** (400 MHz, $CDCl_3+DMSO-d_6$)

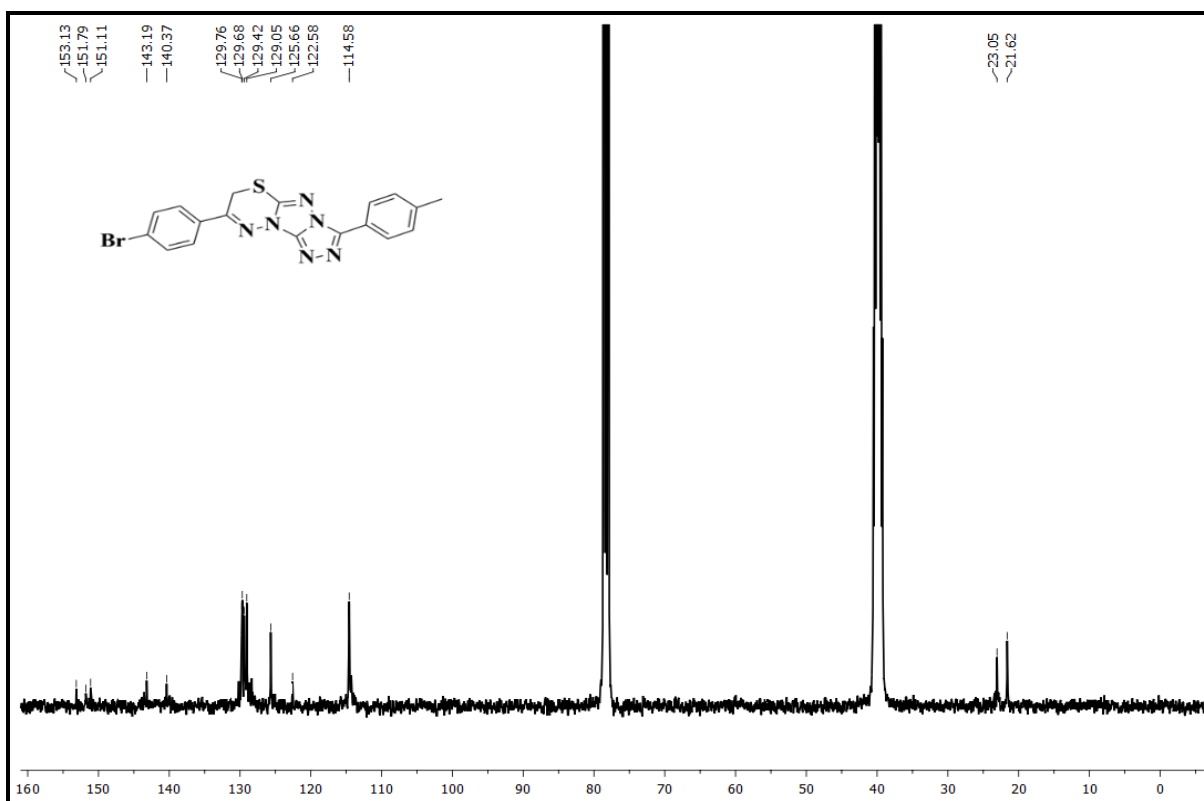


Fig: ¹³C-NMR Spectrum of Compound 24f (100 MHz, CDCl₃+DMSO-*d*₆)

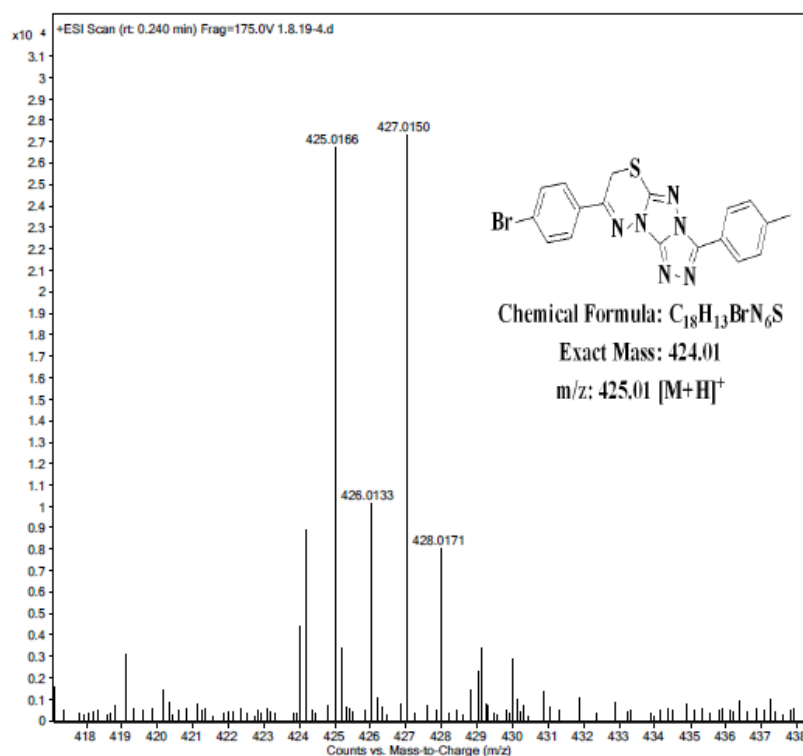


Fig: Mass Spectrum of Compound 24f

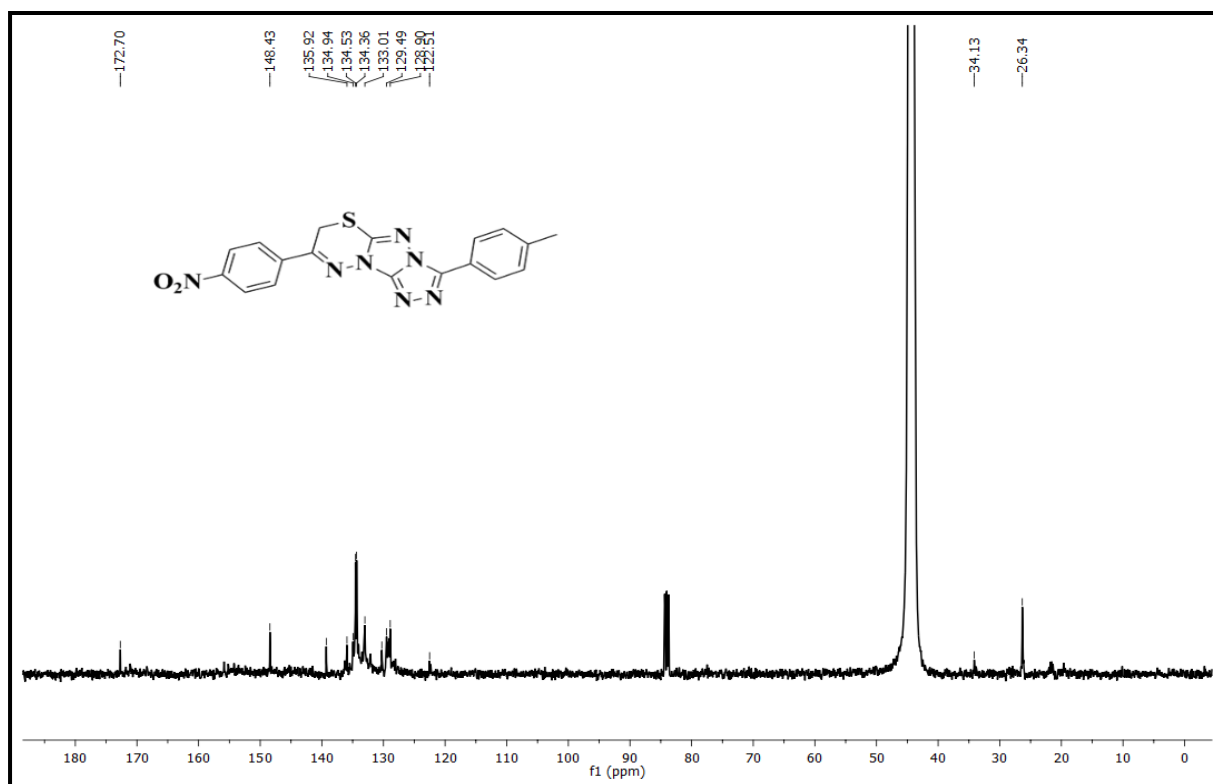


Fig: ¹³C-NMR Spectrum of Compound 24g (100 MHz, CDCl₃+DMSO-*d*₆)

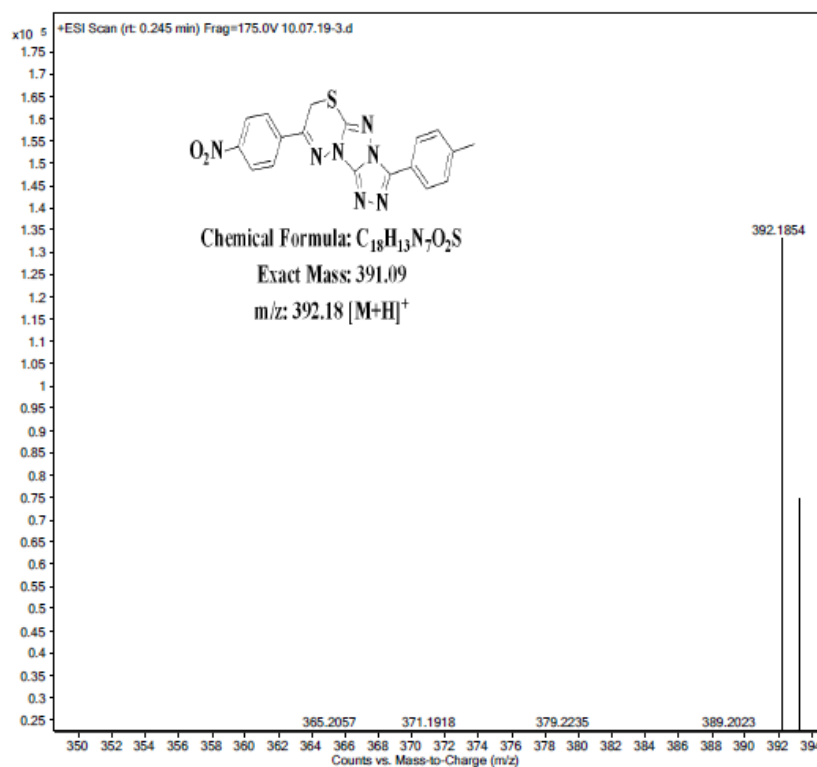


Fig: Mass Spectrum of Compound 24g

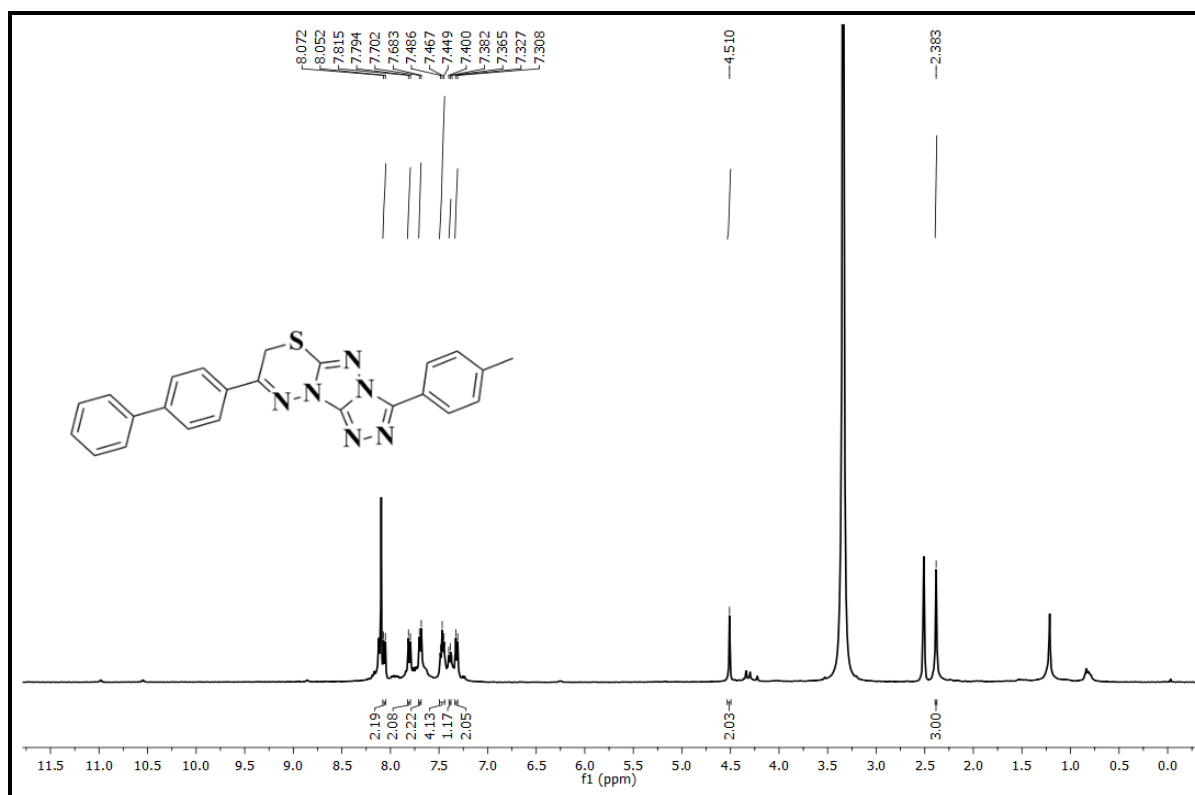


Fig: ¹H-NMR Spectrum of Compound **24h** (400 MHz, CDCl₃+DMSO-*d*₆)

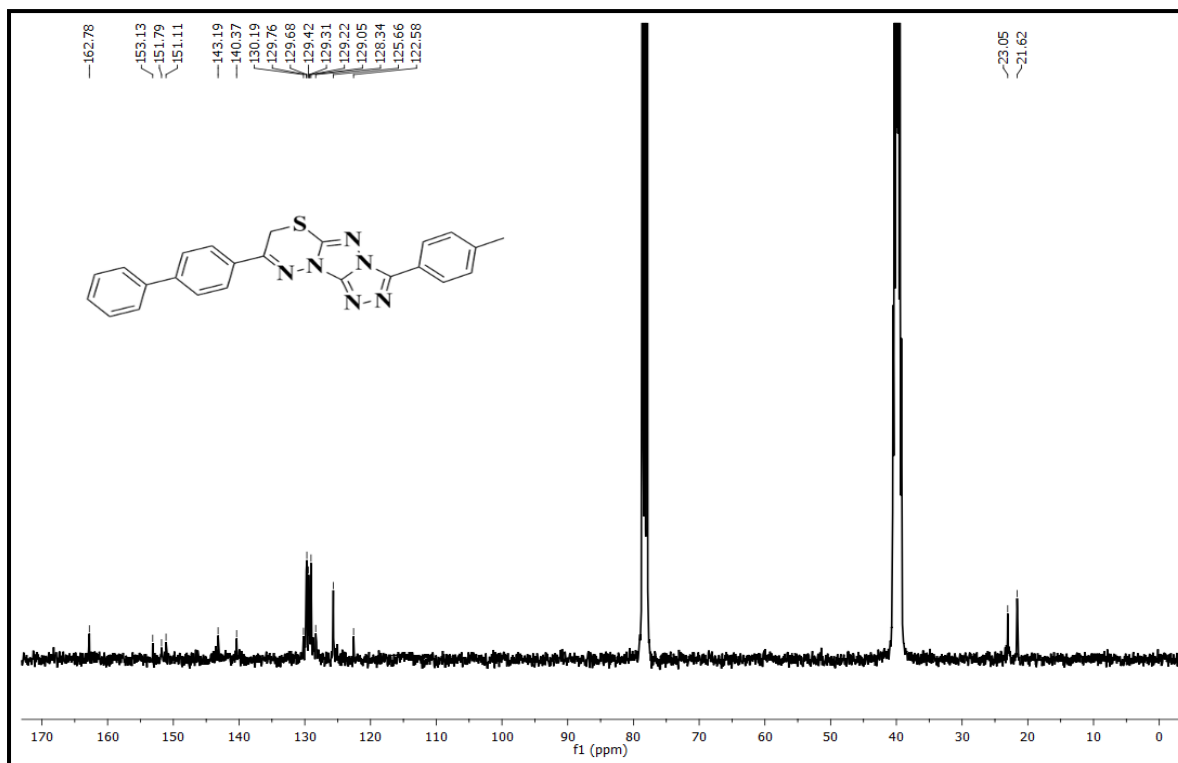


Fig: ¹³C-NMR Spectrum of Compound **24h** (100 MHz, CDCl₃+DMSO-*d*₆)

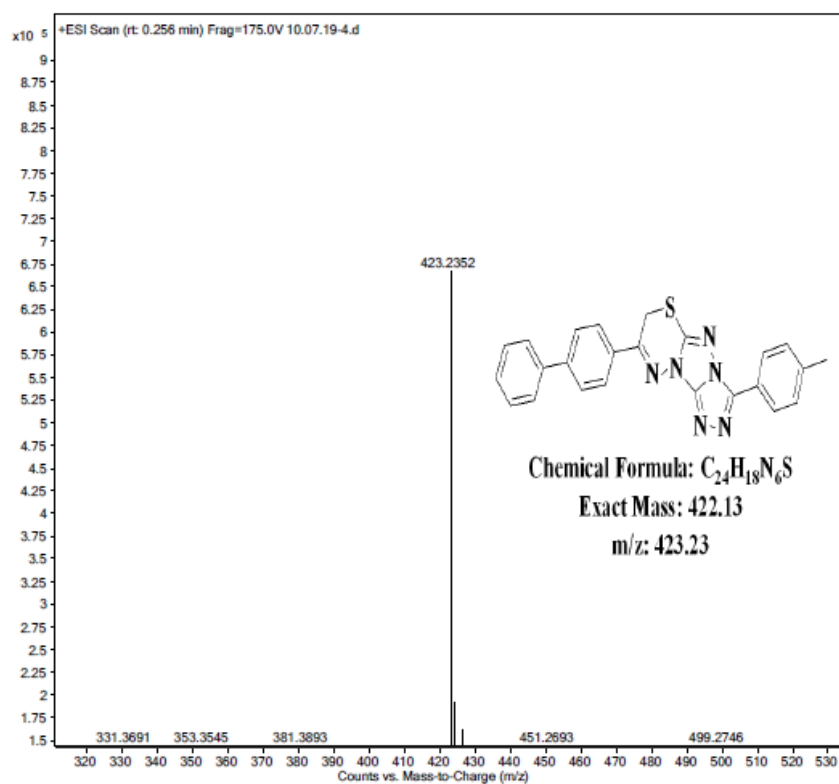


Fig: Mass Spectrum of Compound **24h**

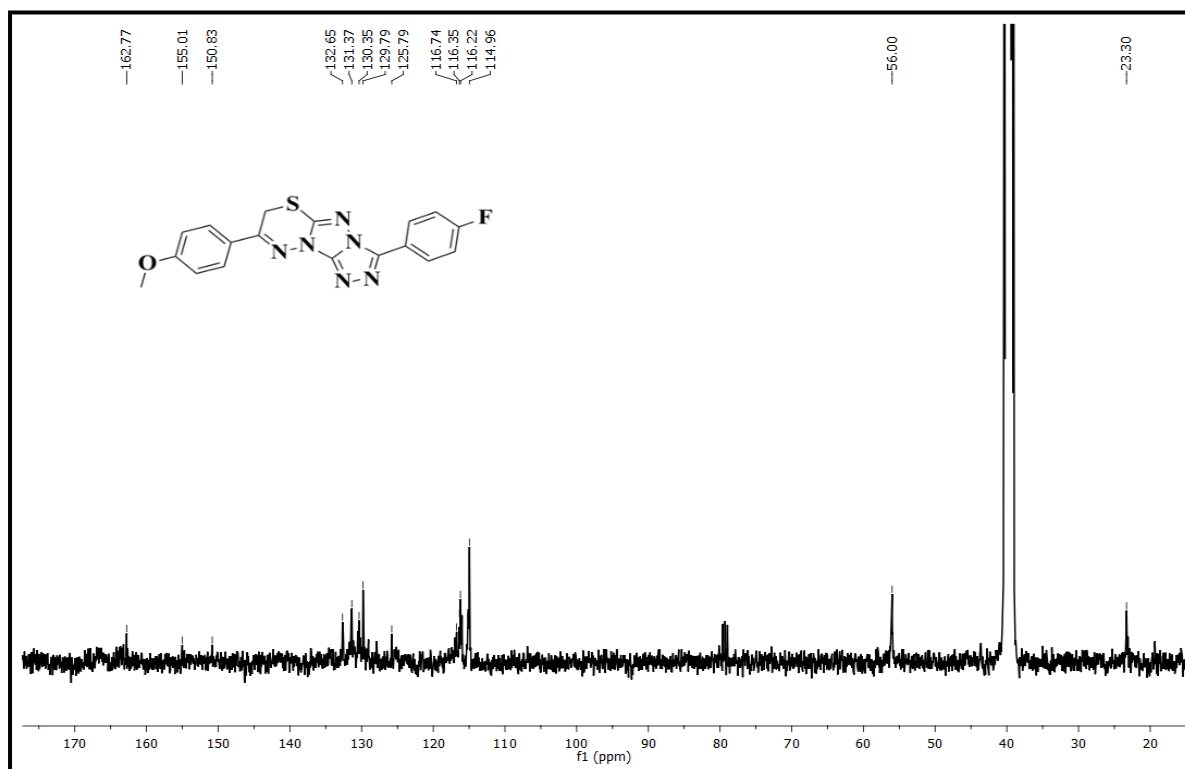


Fig: ^{13}C -NMR Spectrum of Compound **24i** (100 MHz, $CDCl_3 + DMSO-d_6$)

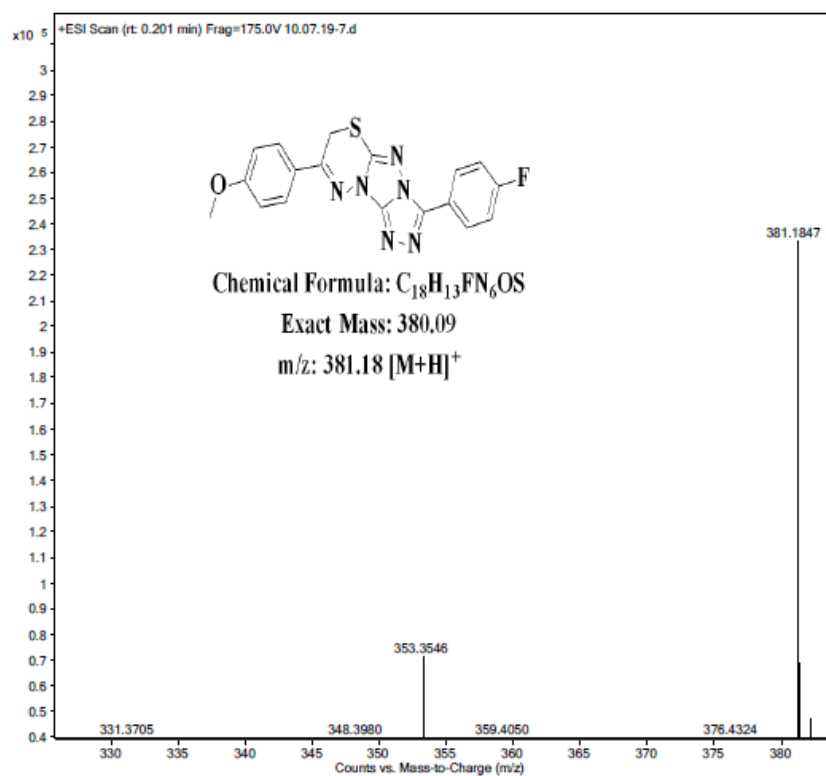


Fig: Mass Spectrum of Compound **24i**

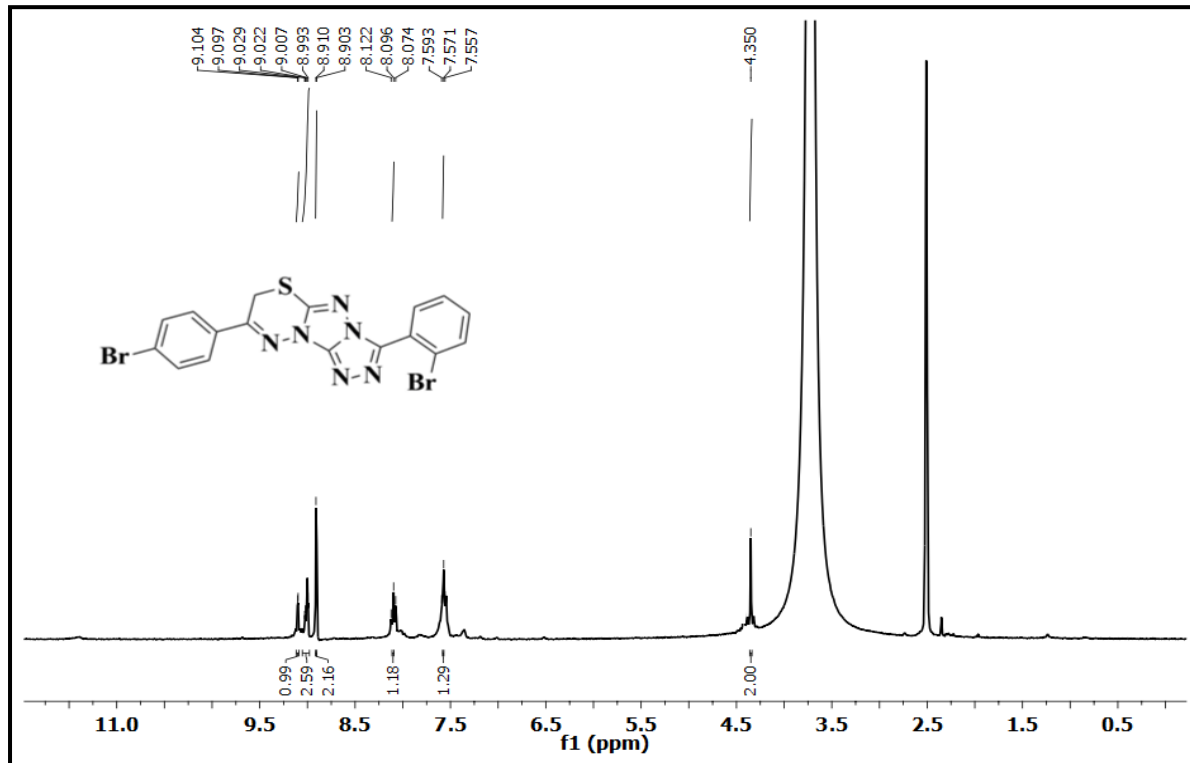


Fig: 1H -NMR Spectrum of Compound **24j** (400 MHz, $CDCl_3+DMSO-d_6$)

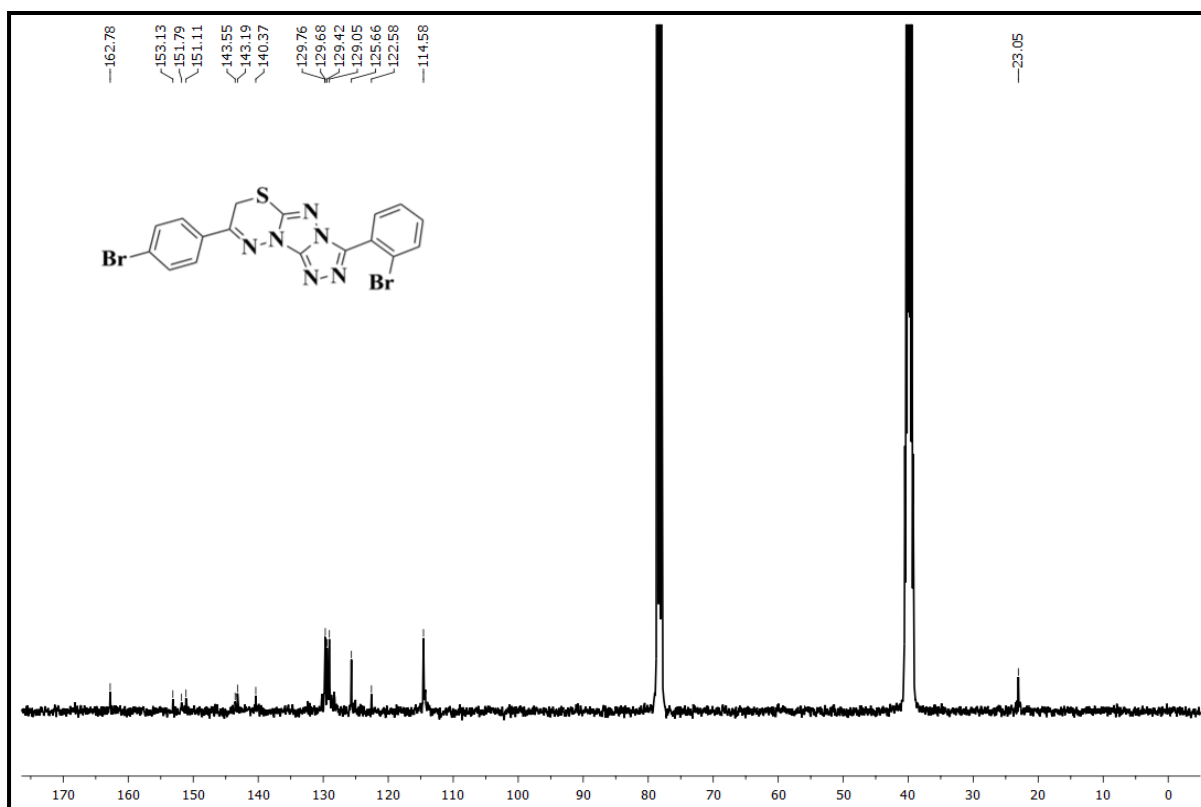


Fig: ¹³C-NMR Spectrum of Compound 24j (100 MHz, CDCl₃+DMSO-*d*₆)

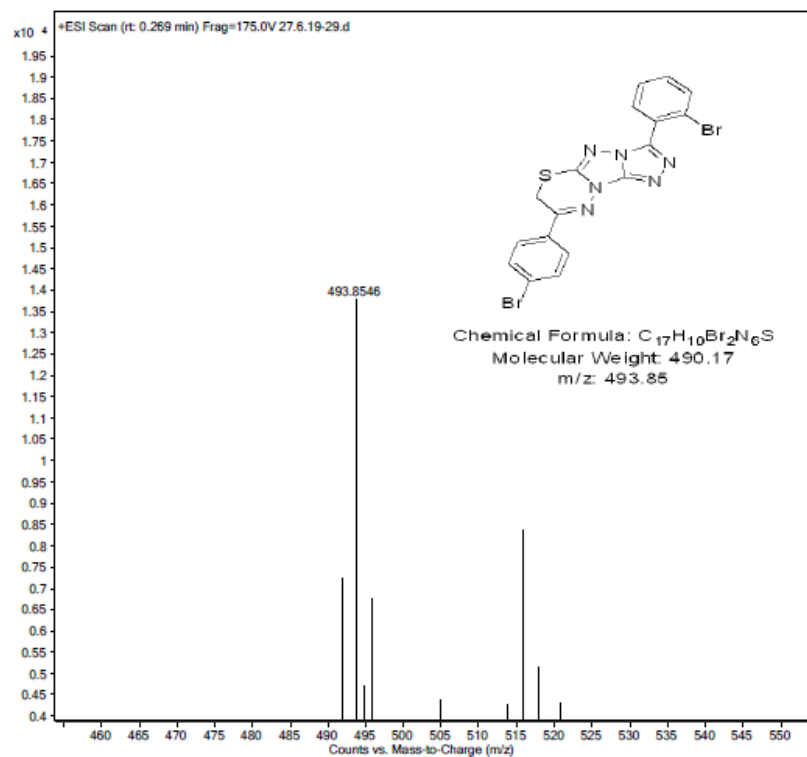


Fig: Mass Spectrum of Compound 24j

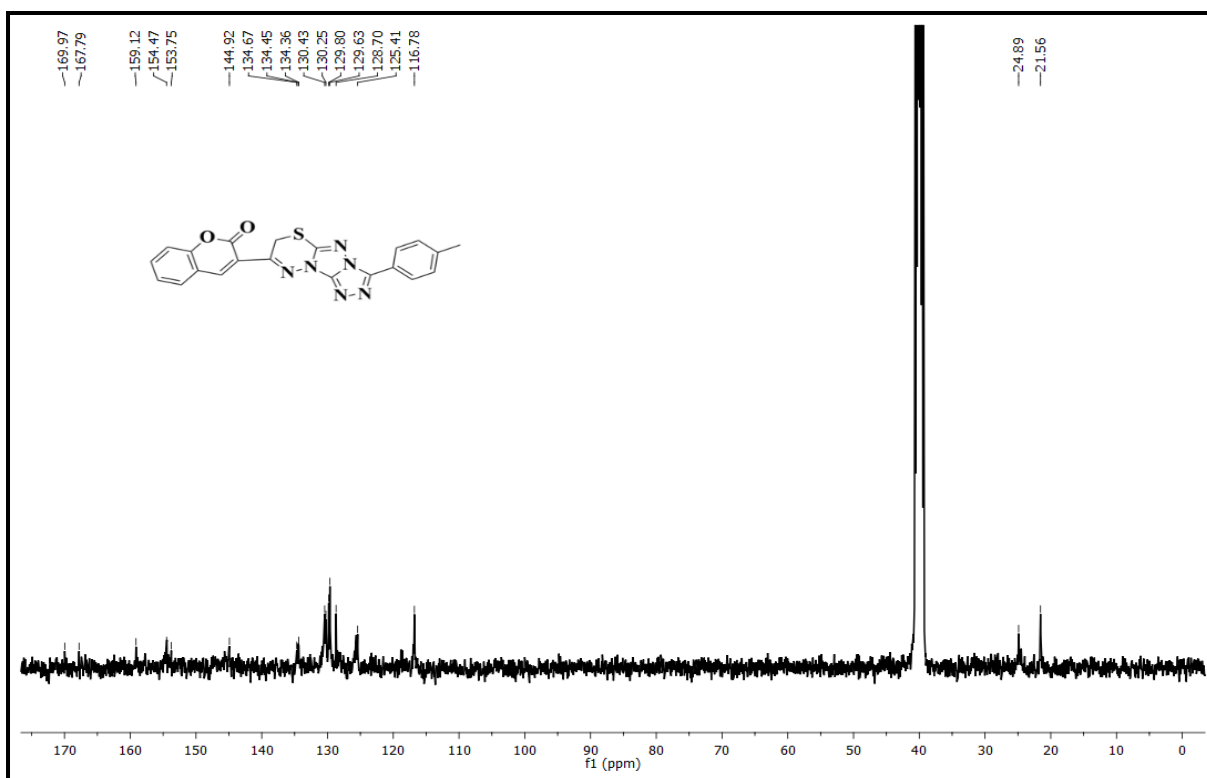


Fig: ¹³C-NMR Spectrum of Compound **24k** (100 MHz, DMSO-*d*₆)

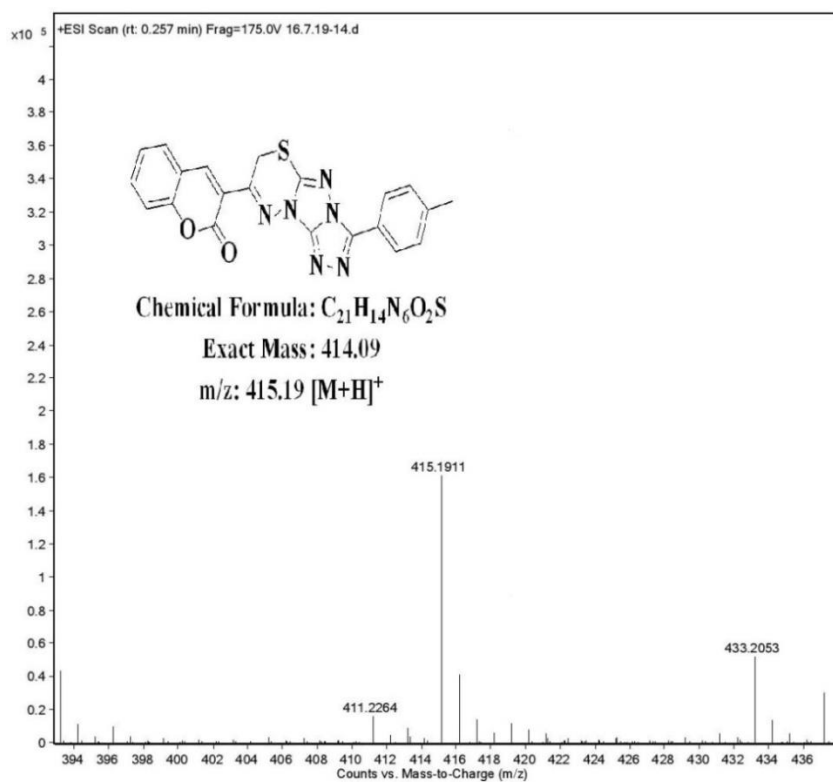


Fig: Mass Spectrum of Compound **24k**

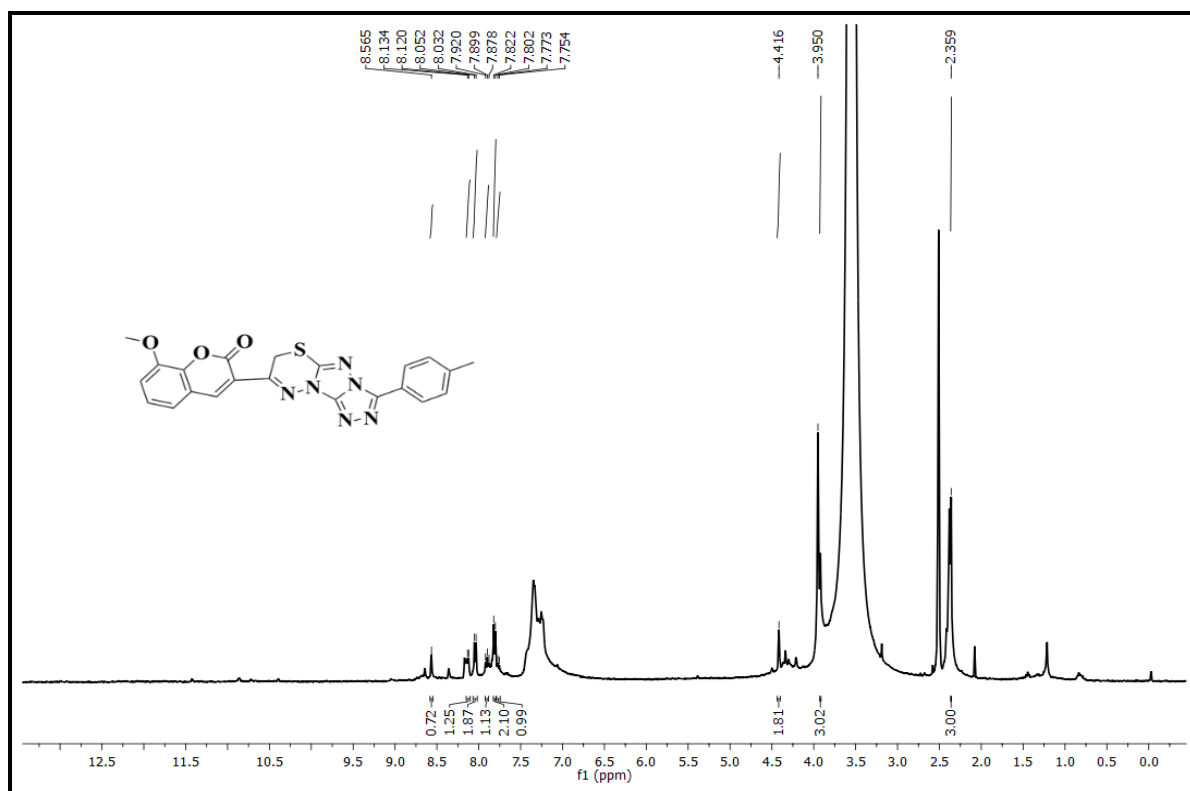


Fig: ¹H-NMR Spectrum of Compound **24I** (400 MHz, CDCl₃+DMSO-*d*₆)

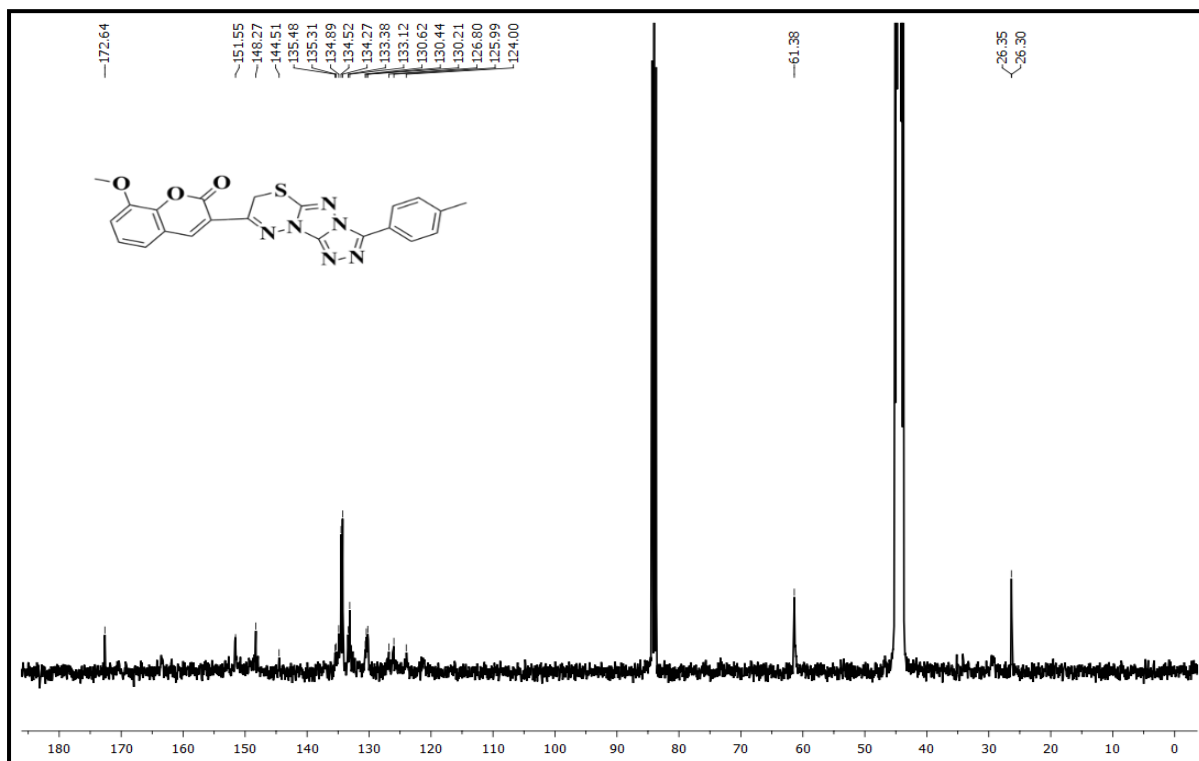


Fig: ¹³C-NMR Spectrum of Compound **24I** (100 MHz, CDCl₃+DMSO-*d*₆)

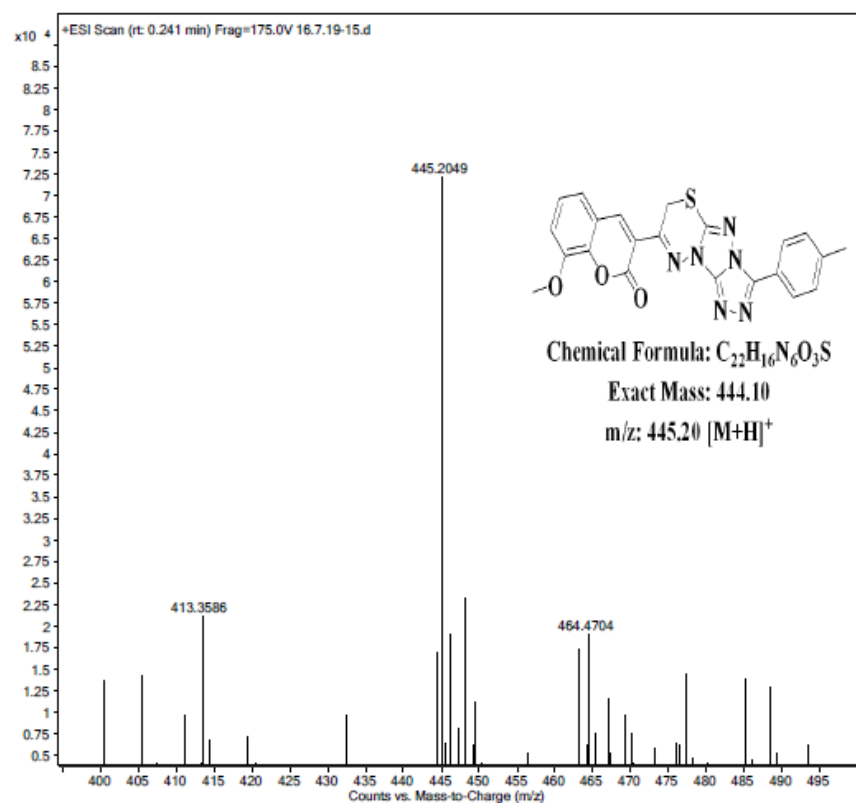


Fig: Mass Spectrum of Compound **24l**

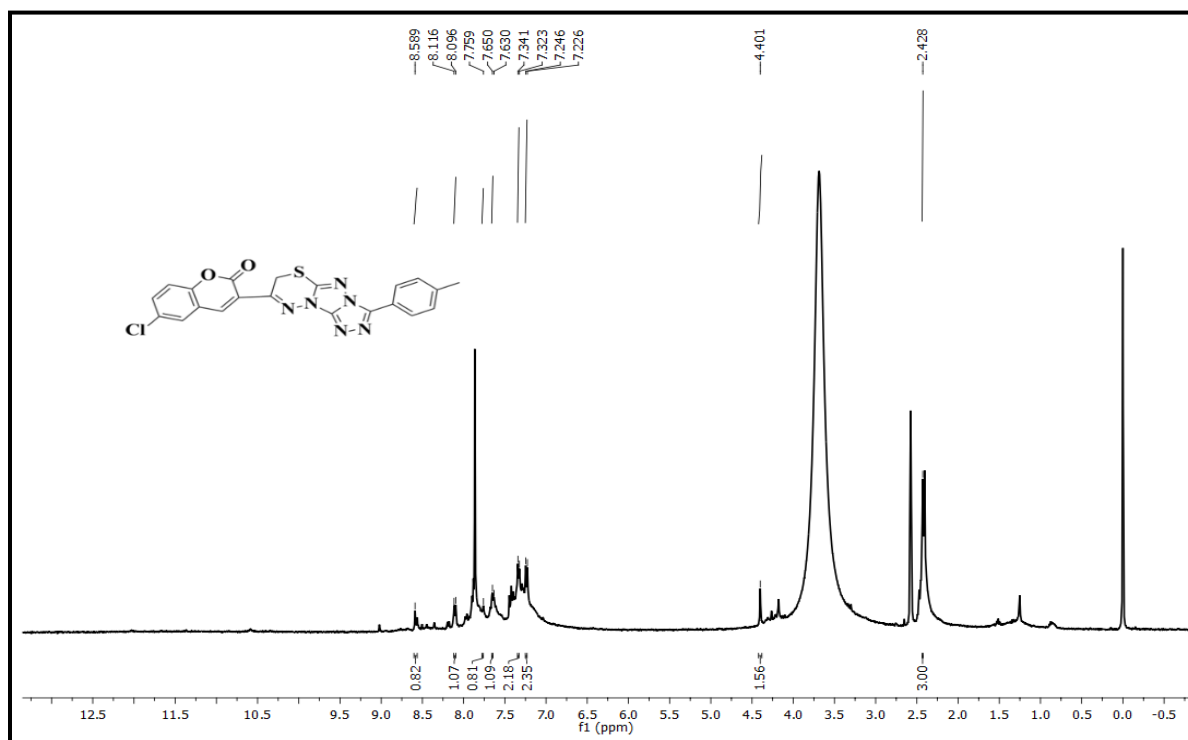


Fig: ¹H-NMR Spectrum of Compound **24m** (400 MHz, CDCl₃+DMSO-*d*₆)

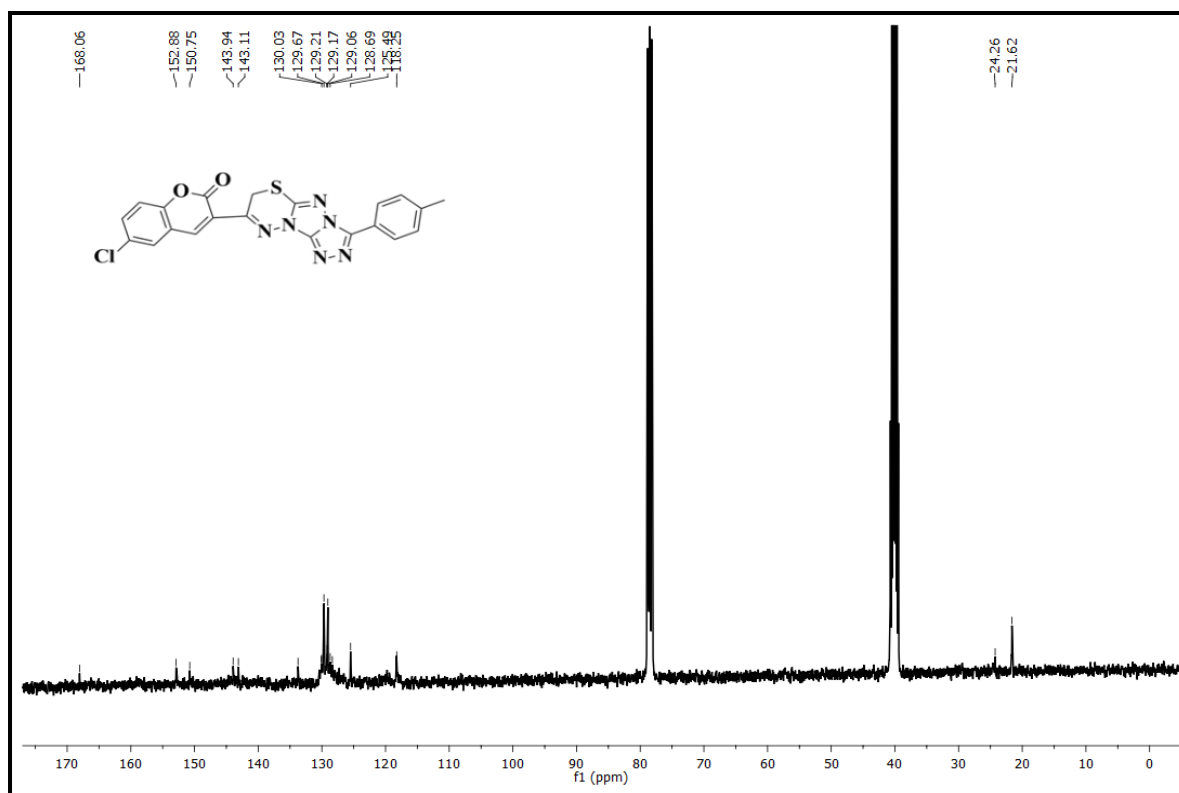


Fig: ¹³C-NMR Spectrum of Compound 24m (100 MHz, CDCl₃+DMSO-*d*₆)

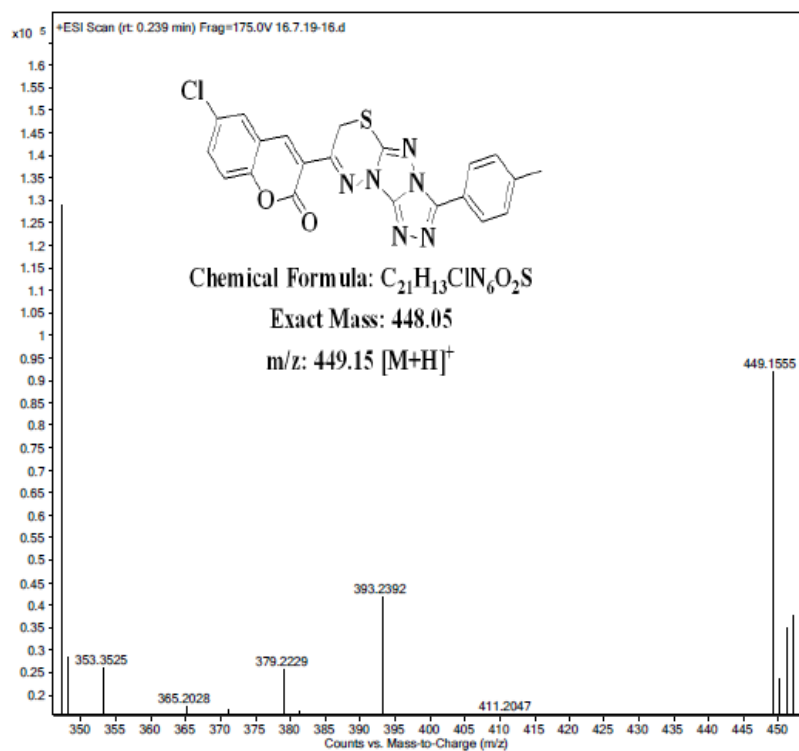


Fig: Mass Spectrum of Compound 24m

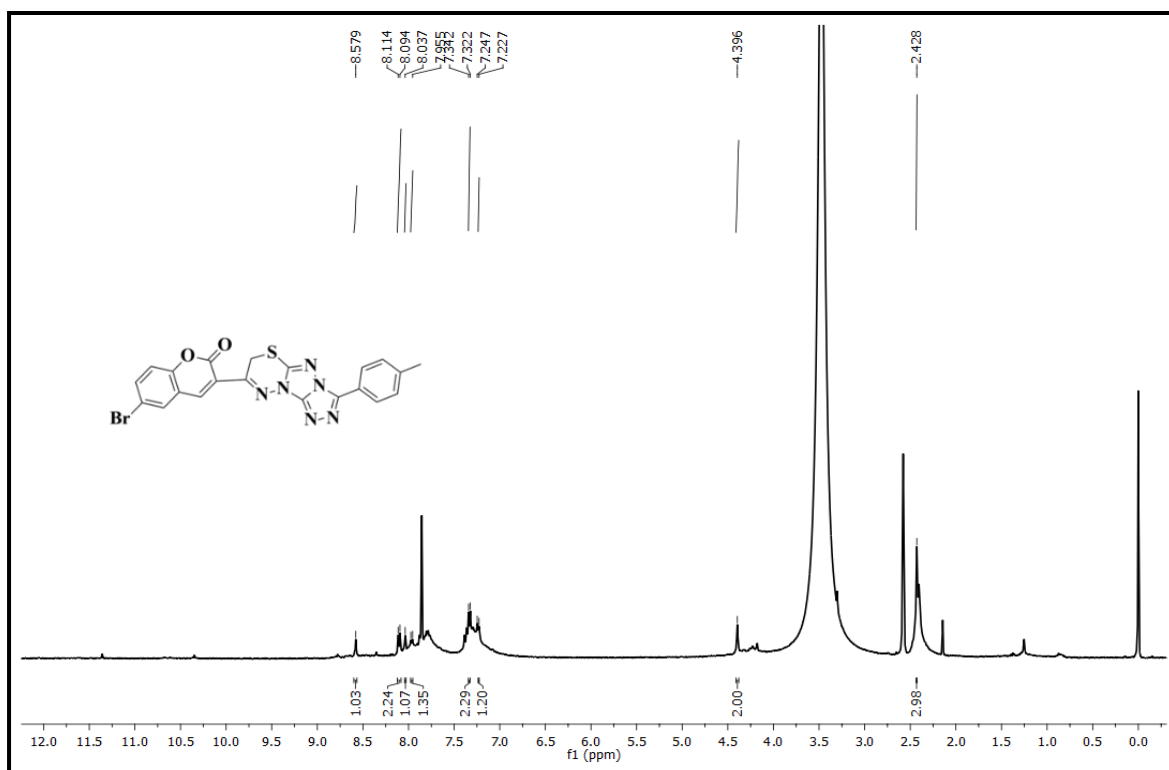


Fig: ¹H-NMR Spectrum of Compound **24n** (400 MHz, CDCl₃+DMSO-*d*₆)

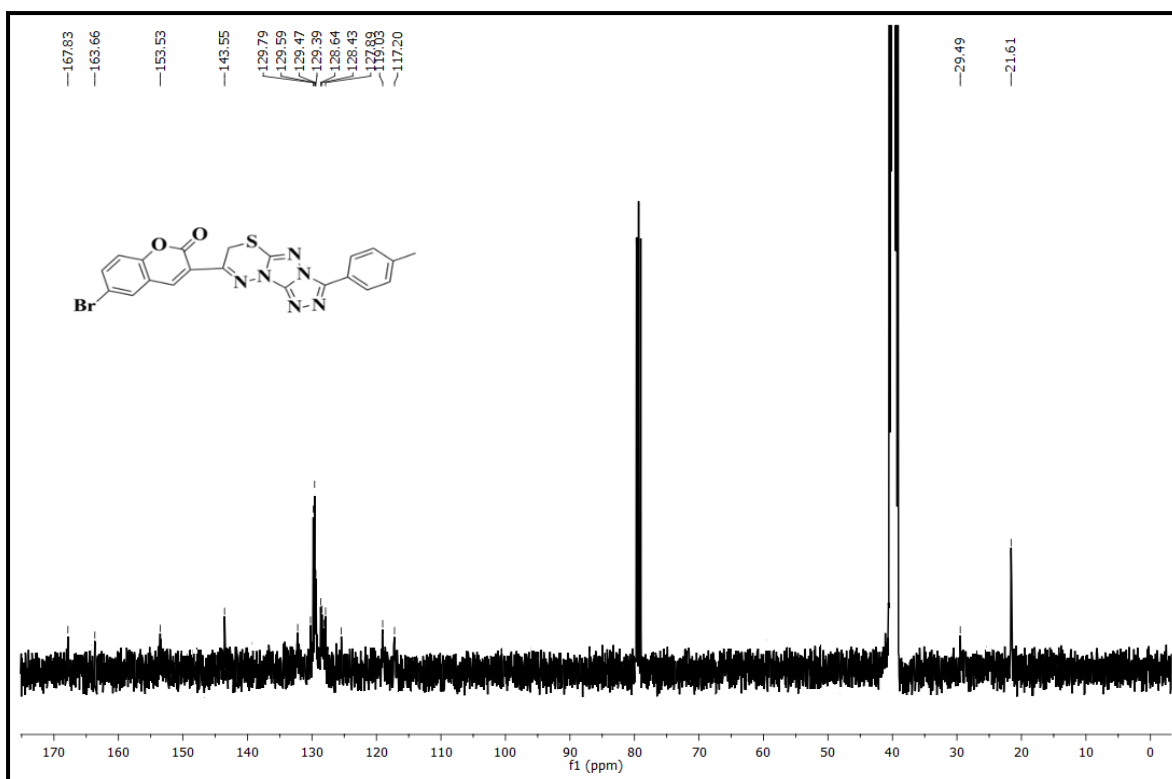


Fig: ¹³C-NMR Spectrum of Compound **24n** (100 MHz, CDCl₃+DMSO-*d*₆)

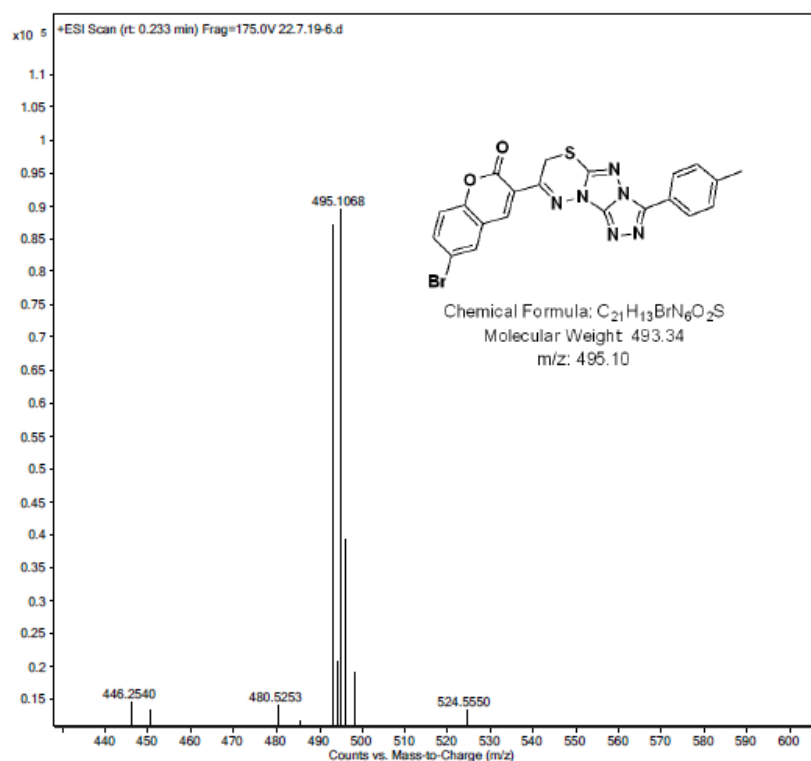


Fig: Mass Spectrum of Compound **24n**

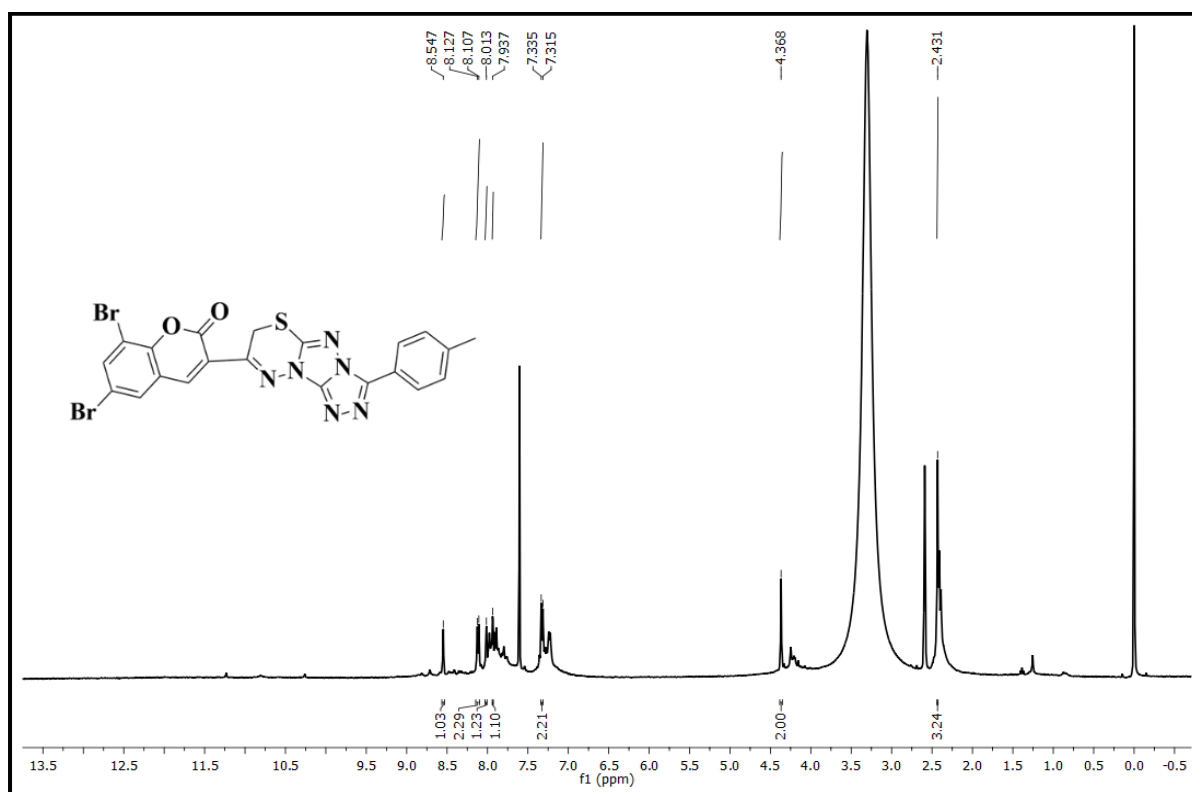


Fig: 1H -NMR Spectrum of Compound **24o** (400 MHz, $CDCl_3$ + $DMSO-d_6$)

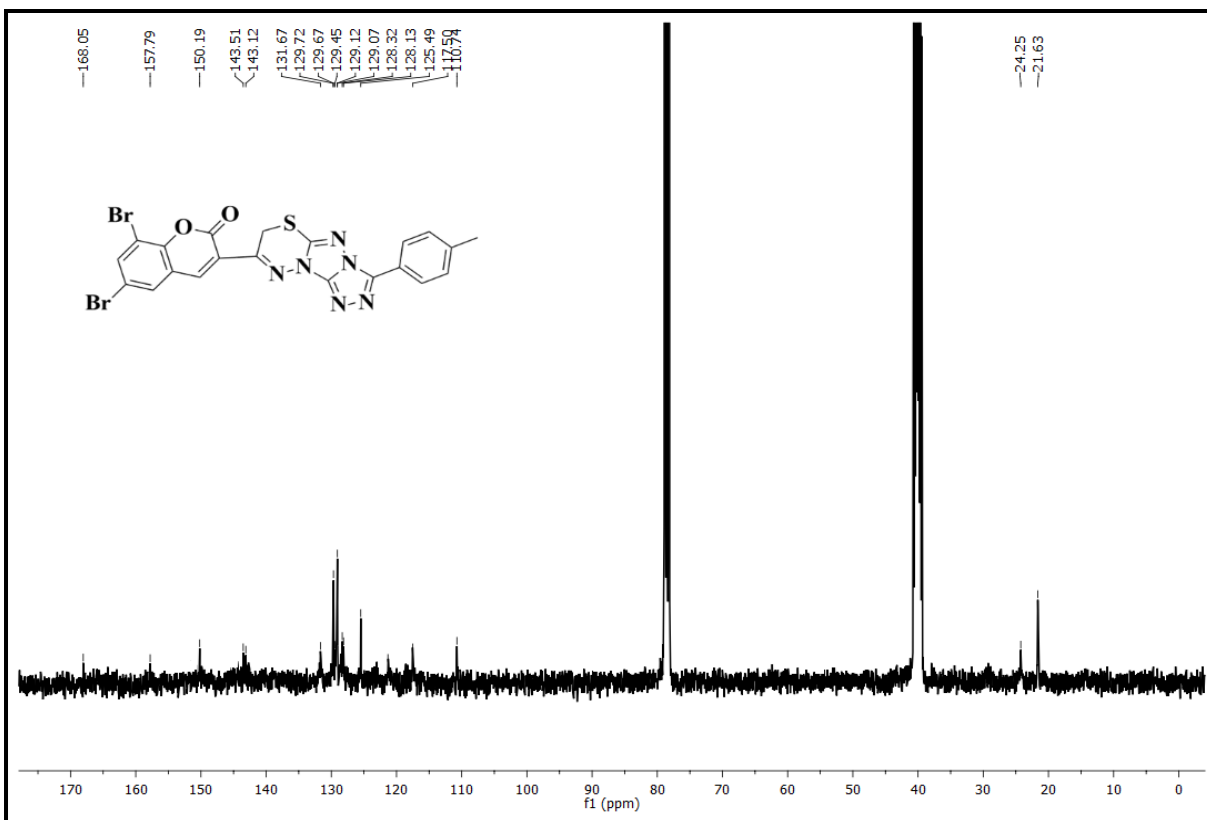


Fig: ^{13}C -NMR Spectrum of Compound **24o** (400 MHz, $\text{CDCl}_3+\text{DMSO}-d_6$)

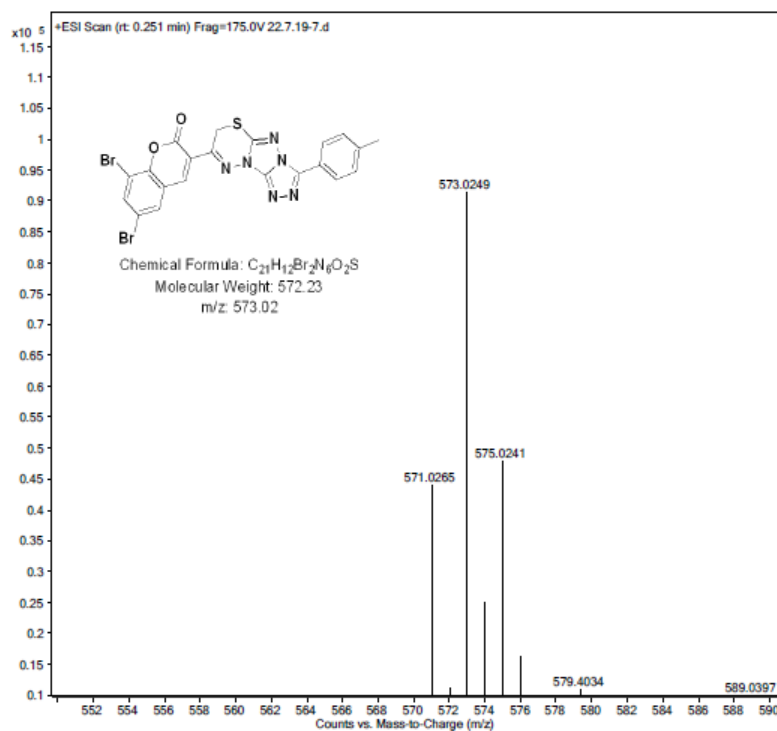


Fig: Mass Spectrum of Compound **24o**

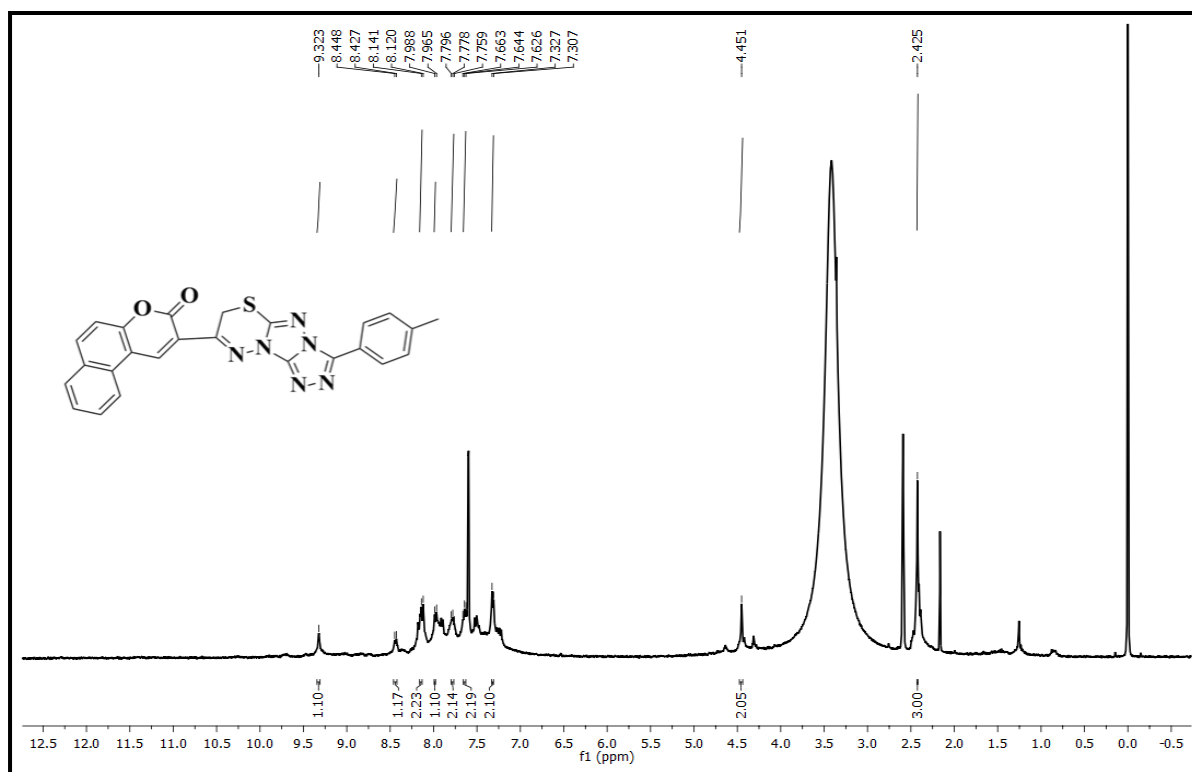


Fig: ¹H-NMR Spectrum of Compound **24p** (400 MHz, CDCl₃+DMSO-*d*₆)

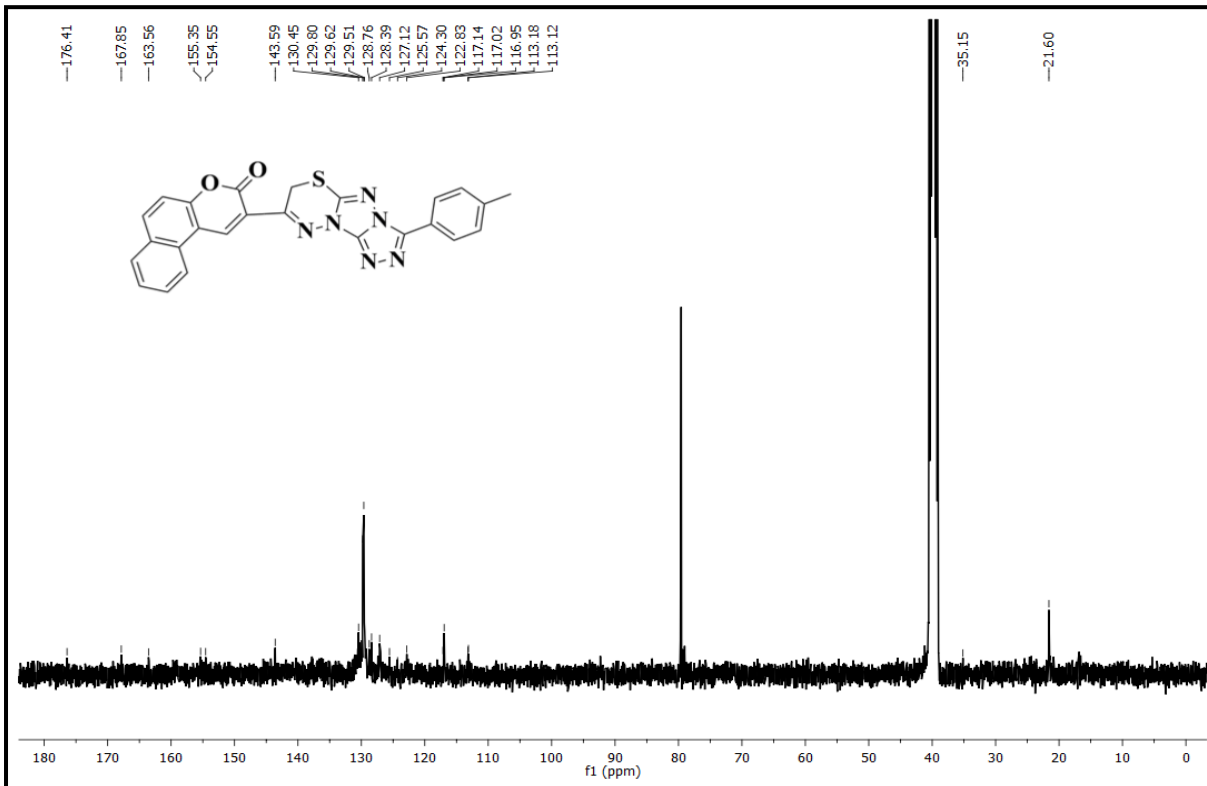


Fig: ¹³C-NMR Spectrum of Compound **24p** (100 MHz, CDCl₃+DMSO-*d*₆)

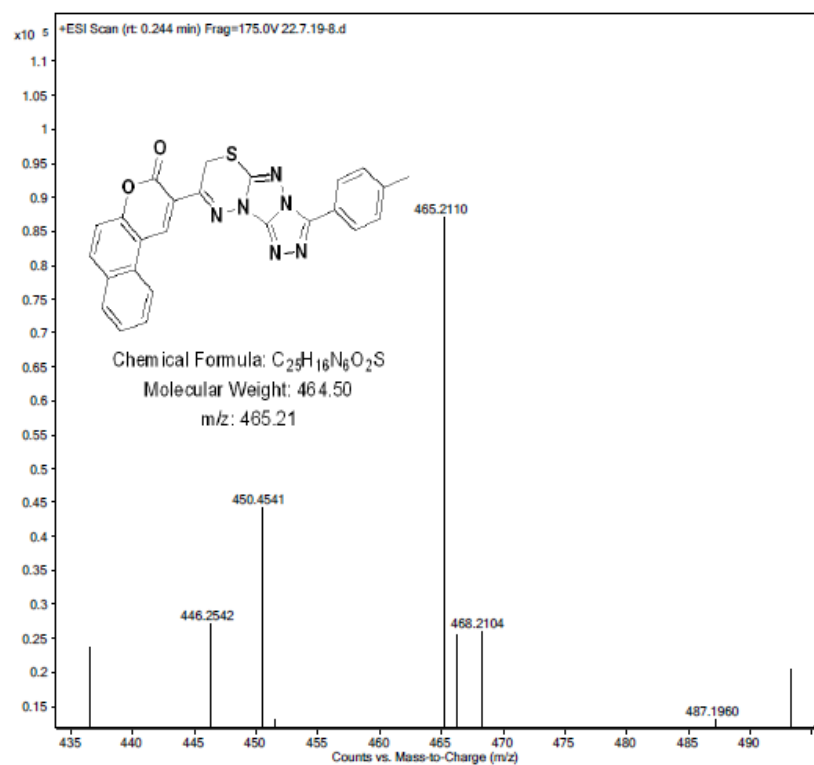


Fig: Mass Spectrum of Compound **24p**

References:

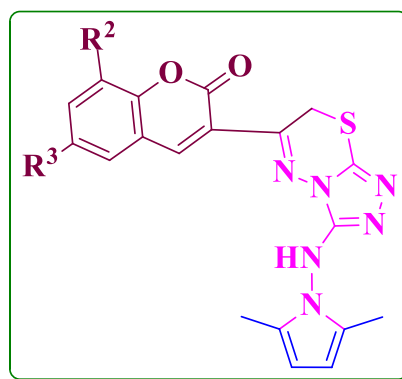
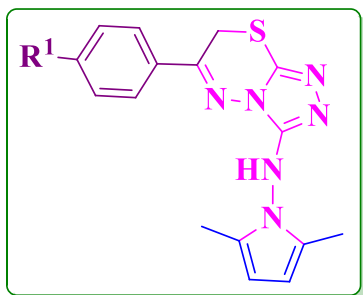
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CHAPTER-VII

AN EFFICIENT ONE-POT THREE COMPONENT SYNTHESIS OF N-(2,5-DIMETHYL-1H-PYRROL-1-YL)-7H-[1,2,4]TRIAZOLO[3,4-*b*][1,3,4]THIADIAZIN-3-AMINE DERIVATIVES



INTRODUCTION:

Heterocyclic chemistry is one of the most important branches of organic chemistry. Most of the heterocyclic compounds contain nitrogen and sulfur atoms in five and six-membered isolated and fused ring systems. These compounds have gained significant attention in the fields of organic synthesis, medicinal chemistry, pharmaceutical, and agrochemical industries. Numerous heterocyclic compounds exhibit a variety of biological applications.

Thiadiazine skeleton is made up of two nitrogen atoms and one sulfur atom in the six-membered heterocyclic ring systems. The amino ($-NH_2$) and thiol ($-SH$) groups of 1,2,4-triazole participate in the reaction with α -halo ketones to produce the fused triazolothiadiazine as core heterocyclic unit. Generally, the 1,3,4-thiadiazine ring is combined with 1,2,4-triazole to form the 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines ring system with five and six-membered fused heterocyclic moiety. Triazolothiadiazines possess therapeutic importance due to their wide range of biological, medicinal applications such as anti-inflammatory¹, anti-HIV², anti-cancer^{3,4}, anti-bacterial^{5,6}, anti-proliferative⁷, anti-tubercular⁸, anti-oxidant⁹, anti-viral, anti-analgesic¹¹, and plant growth regulators¹².

Pyrrole is a well known five-membered aromatic heterocyclic ring having a nitrogen atom. Many of the natural products and macrocyclic complexes such as haemoglobin, chlorophyll, vitamin-B12, and bile pigments were constructed with a pyrrole ring as a core unit. Pyrrole ring combines with different pharmacophores that lead to the formation of important heterocyclic compounds. Pyrrole derivatives exhibit remarkable pharmaceutical and biological activities such as anti-coccidial¹³, anti-convulsant¹⁴, anti-proliferative^{15,16}, anti-fungal¹⁷, anti-mycobacterial¹⁸, anti-cancer^{19,20}, anti-inflammatory²¹ and anti-tubercular²² activities (**Fig. 1**).

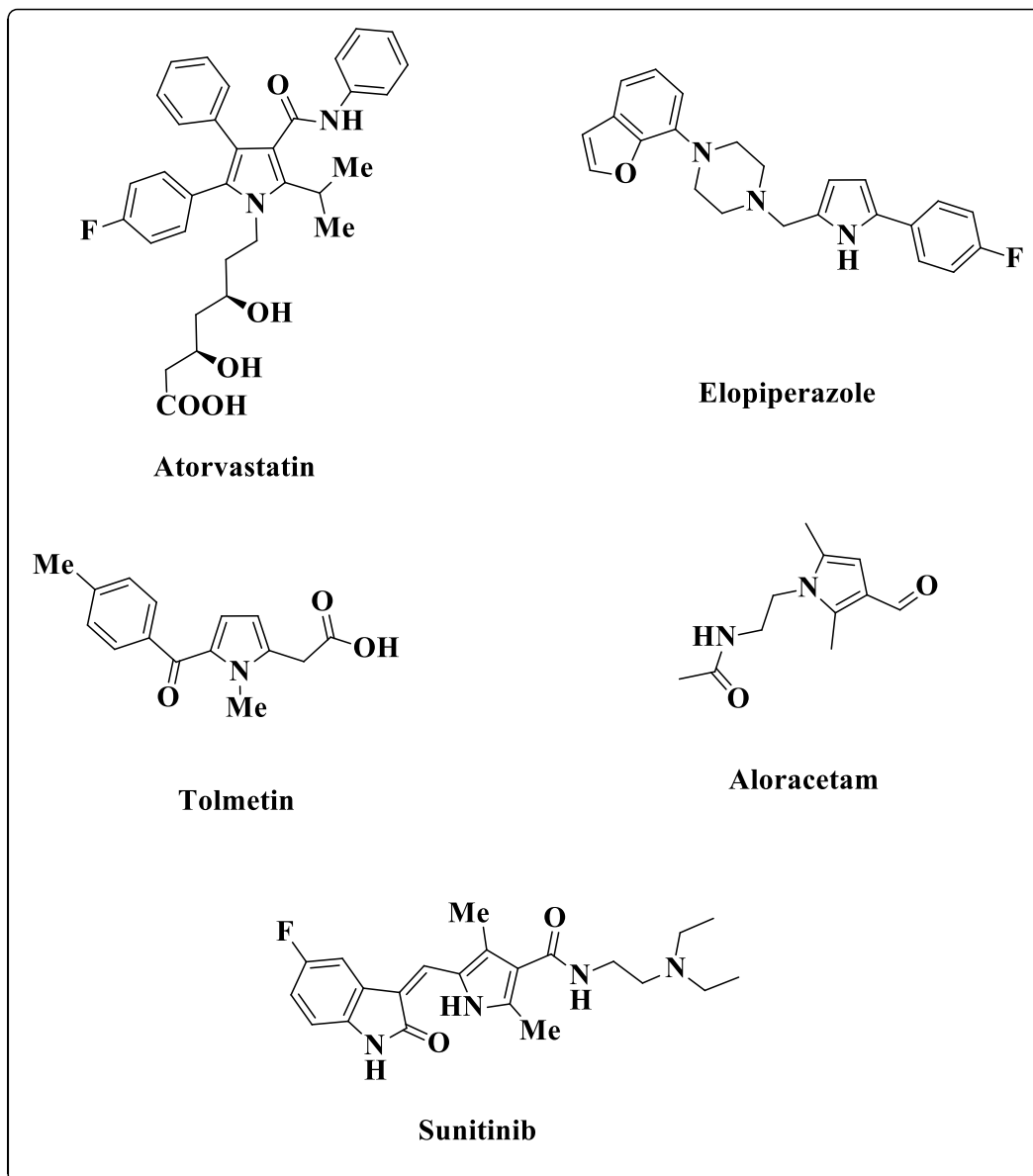
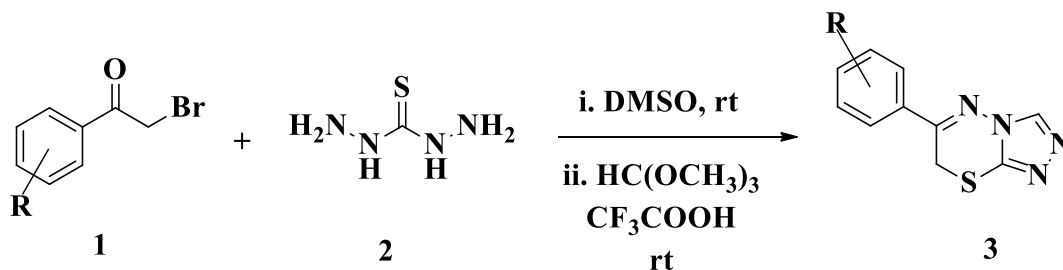


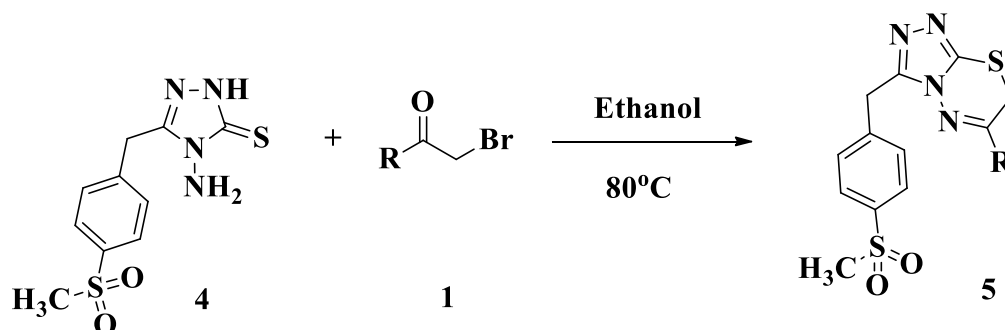
Fig. 1: Commercially available drugs having pyrrole ring.

The following literature survey reveals the synthesis of 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines and pyrrole derivatives.

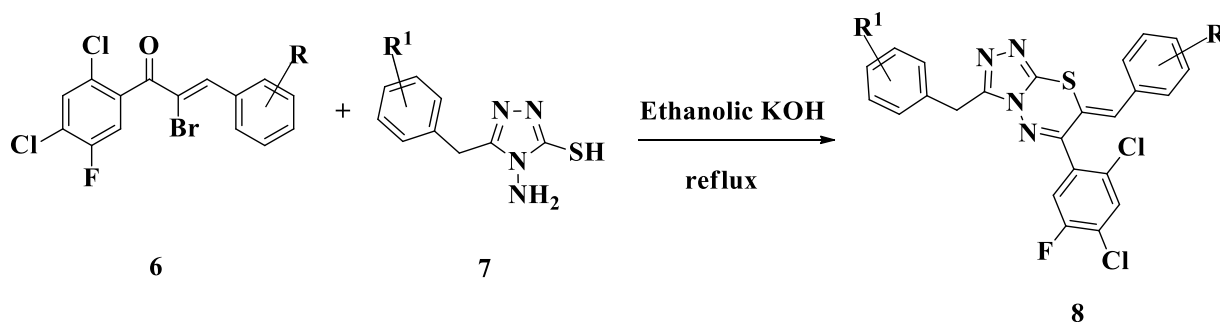
Alexander²³ et al. explained the synthesis substituted 6-phenyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines (**3**) from the reaction of different phenacyl bromides (**1**) with thiocarbohydrazide (**2**) in presence of DMSO at room temperature.



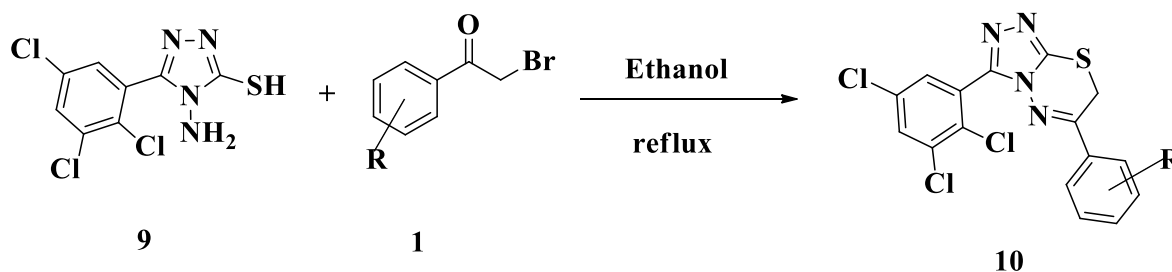
Sumangala²⁴ et al. carried out the reaction between 4-amino-3-(4-(methylsulfonyl)benzyl)-1*H*-pyrazole-5(4*H*)-thione (**4**) and various phenacyl bromides (**1**) in the presence of absolute ethanol at 80°C temperature to yield the 3-(4-(methylsulfonyl)benzyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine derivatives (**5**). The target compounds were screened for their cytotoxic and anti-microbial activity with good results.



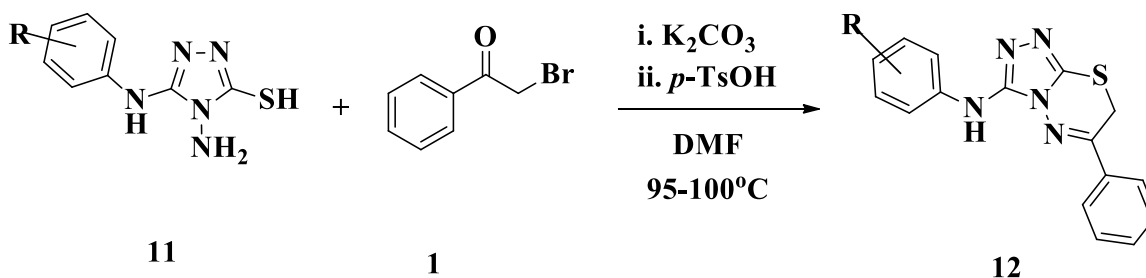
Holla²⁵ et al. reported the synthesis, anti-bacterial, anti-fungal and anti-cancer activities of the different (*Z*)-3-benzyl-7-benzylidene-6-(2,4-dichloro-5-fluorophenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine (**8**) derivatives from the reaction between substituted (*Z*)-2-bromo-1-(2,4-dichloro-5-fluorophenyl)-3-phenylprop-2-en-1-one (**6**) and different 4-amino-5-benzyl-4*H*-1,2,4-triazole-3-thiol (**7**) in presence of ethanolic KOH solution under reflux condition.



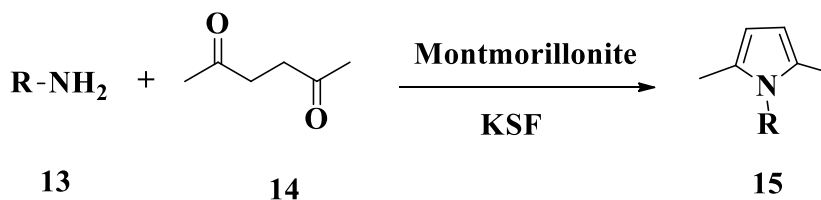
Prakash²⁶ et al. published the synthesis, anti-microbial and anti-inflammatory activity of substituted 6-phenyl-3-(2,3,5-trichlorophenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4] thiadiazines (**10**) from the reaction of 4-amino-5-(2,3,5-trichlorophenyl)-4*H*-1,2,4-triazole-3-thiol (**9**) with substituted phenacyl bromides (**1**) in the ethanol under reflux condition with good yields.



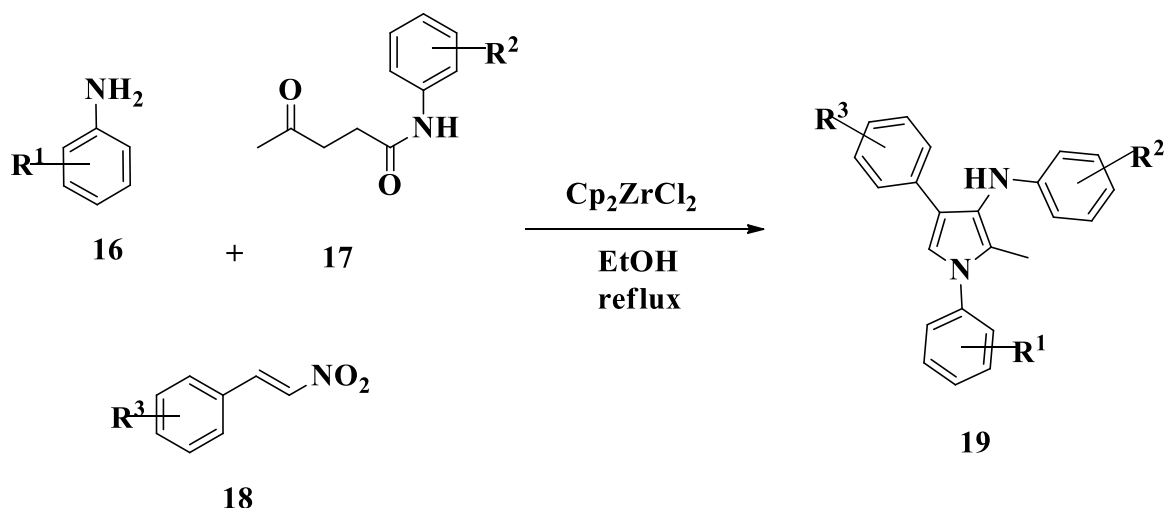
A series of *N*-6-diphenyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-amine (**12**) were synthesized by **Suyog²⁷ and co-worker** by the reaction of 4-amino-5-(phenylamino)-4*H*-1,2,4-triazole-3-thiol (**11**) with phenacyl bromide (**1**) using potassium carbonate, *p*-TsOH, and DMF at 95-100°C with moderate yields.



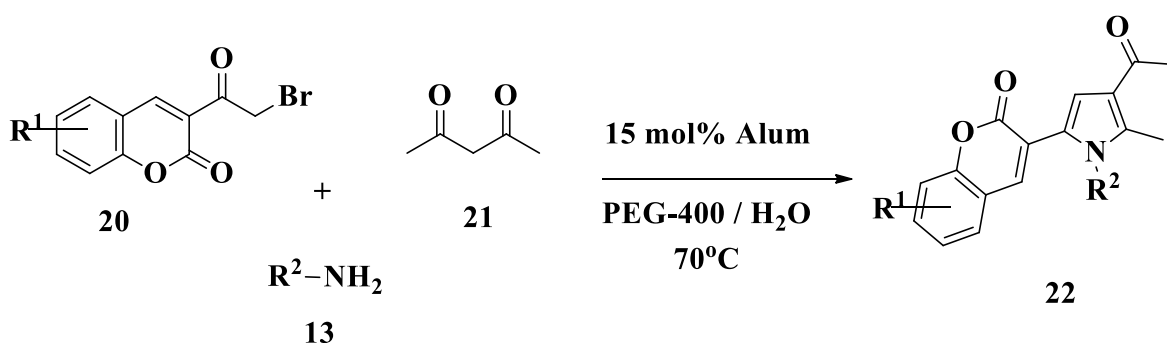
Bimal²⁸ et al. reported the synthesis of substituted 2,5-dimethyl-1*H*-pyrroles (**15**) from the reaction of different primary amines (**13**) with hexane-2,5-dione (**14**) using montmorillonite-KSF clay with high yields.



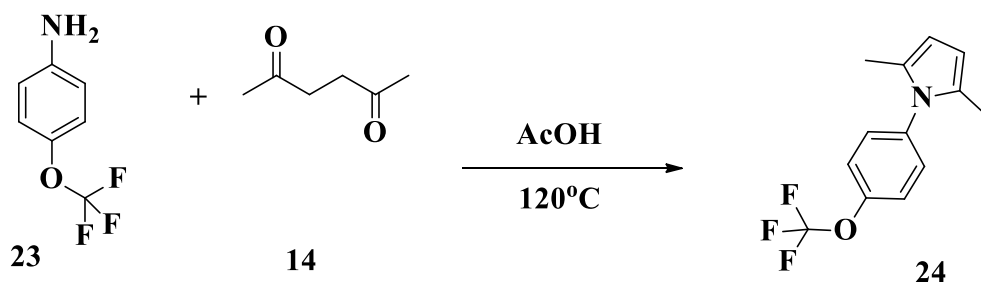
Derivatives of 2-methyl-*N*,1,4-triphenyl-1*H*-pyrrol-3-amines (**19**) were synthesized by **Sandeep**²⁹ *et al.* via a one-pot reaction of substituted anilines (**16**), different 4-oxo-*N*-phenylpentanamides (**17**) and (*E*)-(2-nitrovinyl)benzenes (**18**) using Zirconocene chloride catalyst in ethanol under reflux condition with good yields.



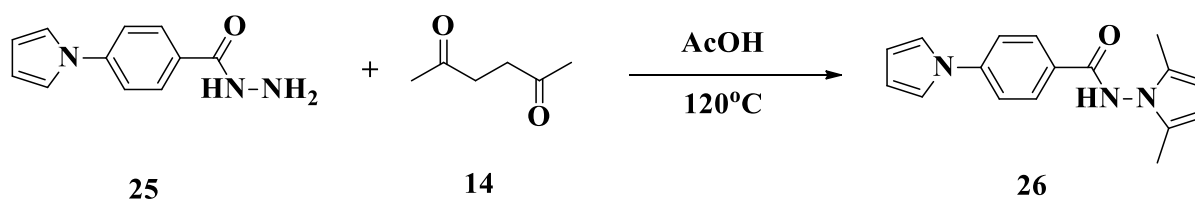
Veronica³⁰ *et al.* carried out the multi-component reaction between the substituted 3-(2-bromoacetyl)-2*H*-chromen-2-ones (**20**), acetylacetone (**21**) and different primary amines (**13**) using alum promoted reaction in presence of polyethyleneglycol-400 (PEG-400) and water at 70°C temperature to produce high yields of the 3-(4-acetyl-5-methyl-1*H*-pyrrol-2-yl)-2*H*-chromen-2-one (**22**) derivatives.



Mykhaylo³¹ *et al.* conducted the reaction between 4-(trifluoromethoxy)aniline (**23**) with hexane-2,5-dione (**14**) in presence of acetic acid at 120°C temperature to produce the 2,5-dimethyl-1-(4-(trifluoromethoxy)phenyl)-1*H*-pyrrole (**24**).



Joshi³² *et al.* carried the synthesis *N*-(2,5-dimethyl-1*H*-pyrrol-1-yl)-4-(1*H*-pyrrol-1-yl)benzamide (26) from the reaction 4-(1*H*-pyrrol-1-yl)benzohydrazide (25) with acetonylacetone (14) in presence of glacial acetic acid under heating condition.



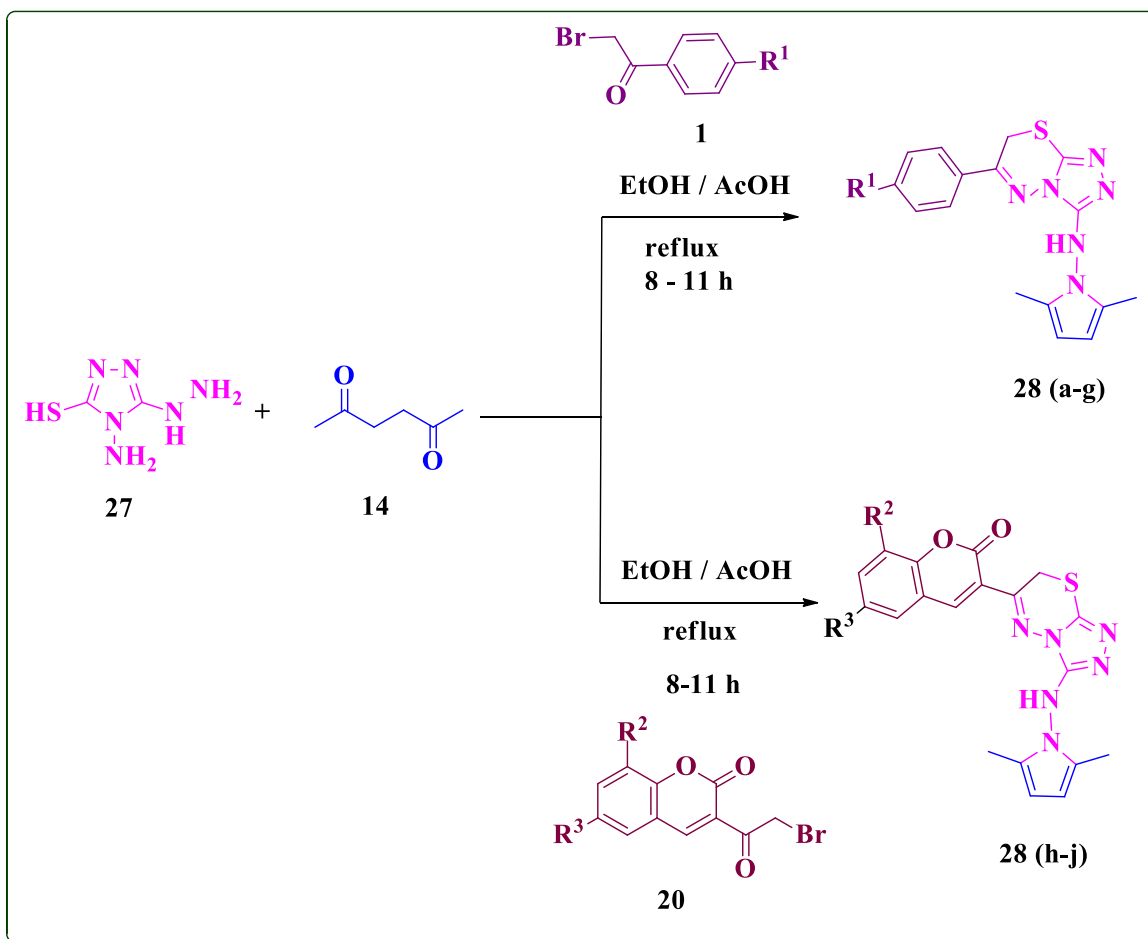
PRESENT WORK:

The present chapter delineates the synthesis of a series of *N*-(2,5-dimethyl-1*H*-pyrrol-1-yl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-amine derivatives (28a-j) via a one-pot multi-component reaction pathway.

In view of the importance of triazolothiadiazines and pyrroles in various fields, we are interested to develop the compounds with triazolothiadiazine and pyrrole rings in single heterocyclic scaffolds. There are many procedures reported for the synthesis of the pyrrole ring. In the current investigation, we have developed the pyrrole ring through Paal-Knorr synthesis. Initially, we have carried out the reaction between 4-amino-5-hydrazinyl-4*H*-1,2,4-triazole-3-thiol (27) and substituted phenacyl bromides (1) or 3-(2-bromoacetyl)-2*H*-chromen-2-ones (20) in presence of ethanol at reflux temperature, then hexane-2,5-dione (14) and acetic acid were added to this reaction mixture in the same reaction vessel under reflux condition to produce the substituted *N*-(2,5-dimethyl-1*H*-pyrrol-1-yl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-amines (28a-j) with excellent yields (Scheme-1).

Based on the previous work³³, we have conducted the reaction in absolute ethanol and acetic acid without optimization with other solvents. The designed target compounds were obtained by the reaction of purpald (27), phenacyl bromides (1), or 3-(2-bromoacetyl)-2*H*-chromen-2-ones (20), and acetonylacetone (14) in absolute ethanol containing acetic acid under reflux for 8-11 h. The yields of the products were 86-94% (Table-1). The solvent selection and reaction condition have played an important role in the formation of a heterocyclic ring.

The other alternative products that are possible between the reaction of 27, 1, or 2 and 14 would be 29 or 30 or both. In the present investigation, we got a single product (28) as evidenced by TLC. The expected alternative products 29 and 30 (Fig. 2) can be deleted based on the analytical and spectral (IR, ¹H-, ¹³C-NMR, and Mass) studies. The specialty of this reaction is the formation of thiadiazine ring and pyrrole rings. Many bands like C-S, C=N, and N-C bonds are formed in one-pot.



Scheme-1: One-pot synthesis of *N*-(2,5-dimethyl-1*H*-pyrrol-1-yl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-amine derivatives (28a-j).

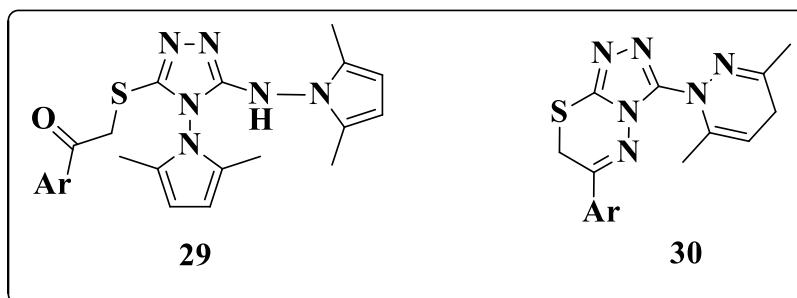


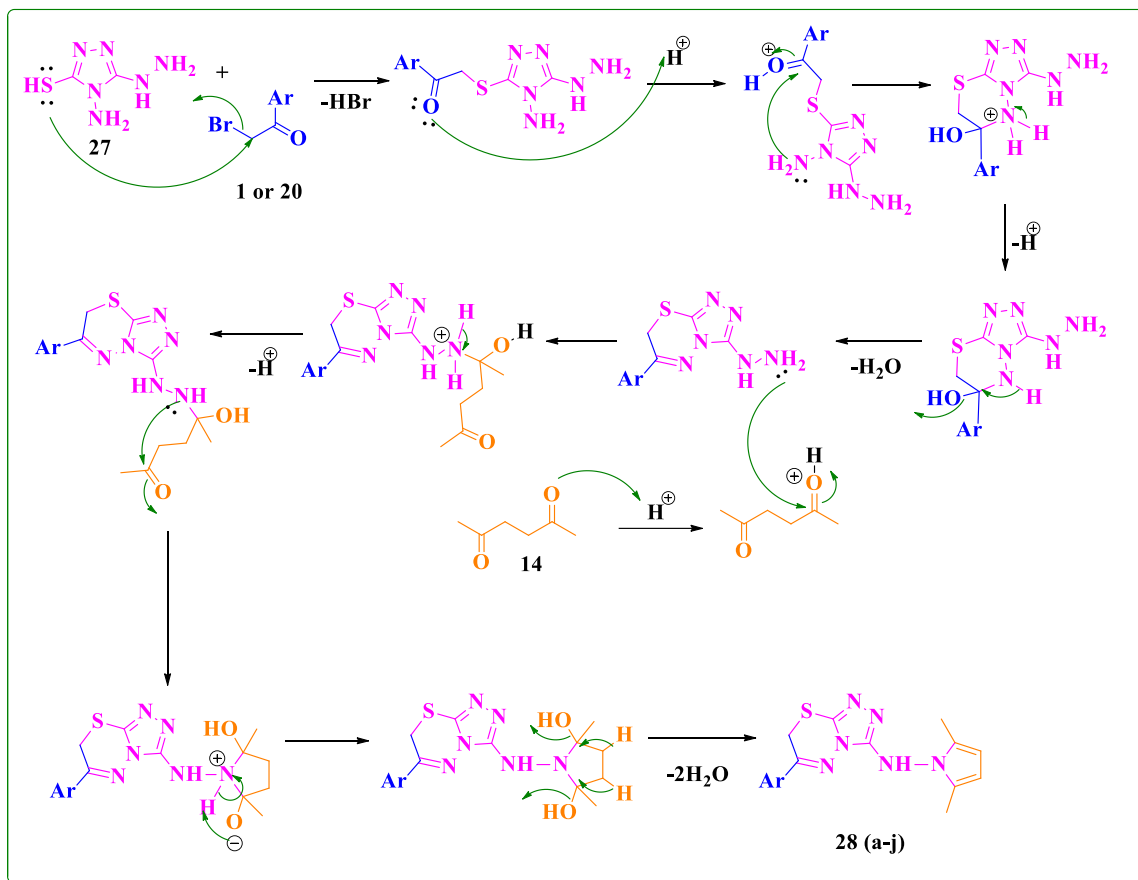
Fig. 2: Possible alternative products.

Table-1: Series of *N*-(2,5-dimethyl-1*H*-pyrrol-1-yl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-amines (**28a-j**).

Compound	R ¹	R ²	R ³	Time (h)	Yield (%)
28a	H	—	—	8.30	90
28b	CH ₃	—	—	8.40	94
28c	OCH ₃	—	—	8.30	92
28d	F	—	—	9.00	87
28e	Cl	—	—	9.50	91
28f	Br	—	—	9.30	90
28g	NO ₂	—	—	10.00	87
28h	—	H	H	10.20	89
28i	—	OCH ₃	H	10.40	86
28j	—	H	Cl	11.00	88

In the present investigation, the plausible reaction mechanism was proposed for the formation of *N*-(2,5-dimethyl-1*H*-pyrrol-1-yl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-amines (**28a-j**) via a one-pot multi-component reaction approach (**Scheme-2**). Due to the high nucleophilicity and reactivity of both thiol (–SH) and amino (–NH₂) functional groups in the starting material **28** they participated in the cyclo condensation reaction with α-bromo ketone

compounds **1** or **20** to give fused 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine ring. The hydrazino (–NH–NH₂) group of compound **28** underwent the Paal-Knorr pyrrole reaction with acetonylacetone (**14**) to generate the pyrrole ring.



Scheme-2: Reaction pathway for the synthesis of designed compounds **28a-j**.

The structures of newly synthesized derivatives were confirmed by their analytical and spectral studies. In the FT-IR spectrum, the compound **28b** gave stretching band at 3294 cm⁻¹ belongs to the –NH– group. The ¹H-NMR (400 MHz, CDCl₃) spectrum of the molecule **28b** exhibited the characteristic singlet for the symmetrical two methyls (six protons, –CH₃) groups on pyrrole at δ 2.19 ppm, another singlet recorded at δ 2.44 ppm for the methyl on the benzene ring. The characteristic thiadiazine methylene (–CH₂–) two protons appeared as a singlet at δ 3.95 ppm. The –NH– proton showed a peak at δ 4.13 ppm and the two symmetrical olefinic protons on the pyrrole ring displayed as a singlet at δ 5.79 ppm. The remaining four protons appeared as two doublet peaks in the downfield δ 7.29 to 7.73 (aromatic region). In the ¹³C-NMR (100 MHz, CDCl₃) spectrum of compound **28b**, the characteristic peaks appeared at δ 11.5, 21.6 and 23.8 ppm belongs to two symmetrical methyl carbons on pyrrole, –CH₃ carbon on benzene

ring and thiadiazine carbon respectively. The sp^2 -hybridized two symmetrical carbons on the pyrrole ring showed a single peak at δ 104.1 ppm. The remaining sp^2 carbons appeared in the aromatic region from 127.1 to 153.2 ppm. The molecular ion peak of the compound **28b** recorded at m/z : 339 $[M+H]^+$ in the ESI-MS.

In the conclusion, we have developed a fused 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine and pyrrole rings in a single compound through a one-pot multi-component reaction strategy. The reaction protocol is very easy to operate, with simple reaction conditions, without metals, and a high atom economy.

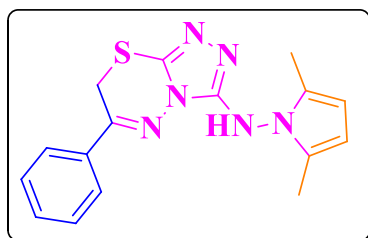
EXPERIMENTAL:

General reaction procedure for an efficient one-pot three component synthesis of *N*-(2,5-dimethyl-1*H*-pyrrol-1-yl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-amine derivatives (28a-j):

A mixture of equal amounts of 4-amino-5-hydrazinyl-4*H*-1,2,4-triazole-3-thiol **28** (0.001 mol) and substituted phenacyl bromide **1** (0.001 mol) or 3-(2-bromoacetyl)-2*H*-chromen-2-one **20** (0.001 mol) and hexane-2,5-dione **14** (0.001 mol) was refluxed in the presence of absolute 4 mL ethanol containing 2 mL glacial acetic acid in a 25 mL round bottom flask at 70-85°C for 9-11 h to produce the target molecules (**28**). After completion of the reaction, the mixture was cooled to the ambient temperature, the separated solid was filtered. The dry final product was recrystallized from the 6-8 mL ethanol.

SPECTRAL DATA:

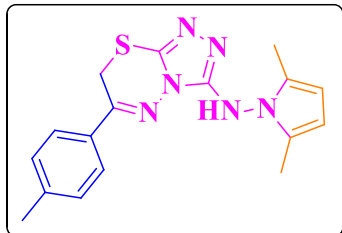
***N*-(2,5-Dimethyl-1*H*-pyrrol-1-yl)-6-phenyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-amine (28a):**



Yellow solid (0.291g, 90%); m.p.: 179-181°C; IR (KBr, $\nu_{\max}/\text{cm}^{-1}$): 3329 (-NH-), 1610 (C=N); $^1\text{H-NMR}$ (400 MHz, CDCl_3 , δ ppm): 2.21 (s, 6H, two -CH₃), 4.00 (s, 2H, -CH₂-), 4.04 (s, 1H, -NH-), 5.82 (s, 2H, two =CH- of pyrrole ring), 7.51-7.56 (m, 3H, Ar-H), 7.83 (d, 2H, $J=7.2$ Hz, Ar-H); $^{13}\text{C-NMR}$

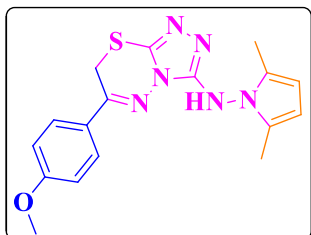
(100 MHz, CDCl₃, δ ppm): 11.5, 23.7, 104.2, 128.4, 128.5, 129.4, 131.7, 137.7, 138.5, 152.1, 152.2; **ESI-MS (m/z):** 325 [M+H]⁺; Analytical calculated formulae C₁₆H₁₆N₆S: C, 59.24; H, 4.97; N, 25.91; S, 9.88; Found: C, 59.28; H, 4.95; N, 25.95; S, 9.84.

***N*-(2,5-Dimethyl-1*H*-pyrrol-1-yl)-6-(*p*-tolyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-amine (28b):**



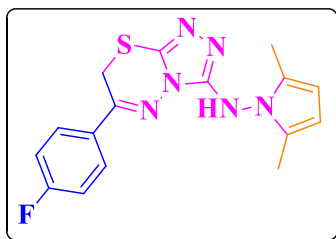
Light yellow solid (0.317g, 94%); m.p.: 192-194°C; **IR (KBr, $\nu_{\max}/\text{cm}^{-1}$):** 3294 (-NH-), 1581 (C=N); **¹H-NMR (400 MHz, CDCl₃, δ ppm):** 2.19 (s, 6H, two -CH₃), 2.44 (s, 3H, -CH₃), 3.95 (s, 2H, -CH₂-), 4.19 (s, 1H, -NH-), 5.79 (s, 2H, two =CH- of pyrrole ring), 7.31 (d, 2H, *J*=7.6 Hz, Ar-H), 7.72 (d, 2H, *J*=8.4 Hz, Ar-H); **¹³C-NMR (100 MHz, CDCl₃, δ ppm):** 11.5, 21.6, 23.8, 104.1, 127.2, 128.4, 129.9, 130.5, 137.9, 142.9, 152.1, 153.2; **ESI-MS (m/z):** 339 [M+H]⁺; Analytical calculated formulae C₁₇H₁₈N₆S: C, 60.33; H, 5.36; N, 24.83; S, 9.47; Found: C, 60.37; H, 5.40; N, 24.80; S, 9.51.

***N*-(2,5-Dimethyl-1*H*-pyrrol-1-yl)-6-(4-methoxyphenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-amine (28c):**



Yellow solid (0.325g, 92%); m.p.: 198-200°C; **IR (KBr, $\nu_{\max}/\text{cm}^{-1}$):** 3427 (-NH-), 1604 (C=N); **¹H-NMR (400 MHz, DMSO-*d*₆, δ ppm):** 2.11 (s, 6H, two -CH₃), 3.42 (s, 3H, -OCH₃), 4.47 (s, 2H, -CH₂-), 5.75 (s, 2H, two =CH- of pyrrole ring), 7.62 (s, 2H, Ar-H), 8.15 (s, 2H, Ar-H), 8.38 (s, 1H, -NH-); **¹³C-NMR (100 MHz, DMSO-*d*₆, δ ppm):** 11.7, 23.1, 56.0, 103.5, 114.7, 126.0, 127.9, 129.9, 137.6, 152.9, 154.0, 162.6; **ESI-MS (m/z):** 355 [M+H]⁺; Analytical calculated formulae C₁₇H₁₈N₆OS: C, 57.61; H, 5.12; N, 23.71; S, 9.05; Found: C, 57.58; H, 5.16; N, 23.75; S, 9.00.

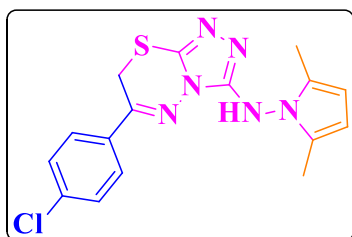
***N*-(2,5-Dimethyl-1*H*-pyrrol-1-yl)-6-(4-fluorophenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-amine (28d):**



Brown solid (0.297g, 87%); m.p.: 186-188°C; **IR (KBr, $\nu_{\text{max}}/\text{cm}^{-1}$):** 3416 (-NH-), 1599 (C=N); **$^1\text{H-NMR}$ (400 MHz, CDCl_3 , δ ppm):** 2.19 (s, 6H, two -CH₃), 3.96 (s, 2H, -CH₂-), 5.81 (s, 2H, two =CH- of pyrrole ring), 7.19 (d, 2H, $J=8.4$ Hz, Ar-H), 7.22 (s, 1H, -NH-), 7.83 (dd, 2H, $J=6.8$ Hz, $J=3.6$ Hz, Ar-H); **$^{13}\text{C-NMR}$**

(100 MHz, $\text{DMSO}-d_6$, δ ppm): 11.7, 23.3, 103.5, 116.3, 116.5, 127.9, 130.8, 153.4, 155.9, 163.4, 165.9; **ESI-MS (m/z):** 343 [$\text{M}+\text{H}$]⁺; Analytical calculated formulae $\text{C}_{16}\text{H}_{15}\text{FN}_6\text{S}$: C, 56.13; H, 4.42; N, 24.54; S, 9.36; Found: C, 56.10; H, 4.45; N, 24.59; S, 9.40.

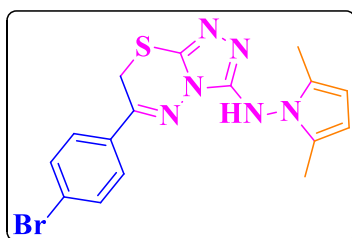
***N*-(2,5-dimethyl-1*H*-pyrrol-1-yl)-6-(4-chlorophenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-amine (28e):**



Light yellow solid (0.325g, 91%); m.p.: 194-196°C; **IR (KBr, $\nu_{\text{max}}/\text{cm}^{-1}$):** 3227 (-NH-), 1583 (C=N); **$^1\text{H-NMR}$ (400 MHz, CDCl_3 , δ ppm):** 2.19 (s, 6H, two -CH₃), 3.95 (s, 2H, -CH₂-), 5.80 (s, 2H, two =CH- of pyrrole ring), 7.40 (s, 1H, -NH-), 7.48 (d, 2H, $J=8.4$ Hz, Ar-H), 7.76 (d, 2H, $J=8.8$ Hz, Ar-H); **$^{13}\text{C-NMR}$**

(100 MHz, CDCl_3 , δ ppm): 11.5, 23.7, 104.2, 128.4, 128.5, 129.4, 131.7, 137.7, 138.5, 152.1, 152.2; **ESI-MS (m/z):** 359 [$\text{M}+\text{H}$]⁺; Analytical calculated formulae $\text{C}_{16}\text{H}_{15}\text{ClN}_6\text{S}$: C, 53.55; H, 4.21; N, 23.42; S, 8.94; Found: C, 53.58; H, 4.25; N, 23.46; S, 8.90.

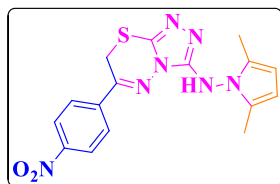
***N*-(2,5-dimethyl-1*H*-pyrrol-1-yl)-6-(4-bromophenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-amine (28f):**



Yellow solid (0.362, 90%); m.p.: 202-204°C; **IR (KBr, $\nu_{\text{max}}/\text{cm}^{-1}$):** 3427 (-NH-), 1586 (C=N); **$^1\text{H-NMR}$ (400 MHz, CDCl_3 , δ ppm):** 2.19 (s, 6H, two -CH₃), 3.95 (s, 2H, -CH₂-), 5.80 (s, 2H, two =CH- of pyrrole ring), 7.63 (s, 1H, -NH-), 7.65-7.69 (m, 4H, Ar-H); **$^{13}\text{C-NMR}$ (100 MHz, CDCl_3 , δ ppm):** 11.5, 29.7, 104.2,

126.5, 128.6, 129.9, 132.2, 132.4, 153.3, 158.8, 160.1; **ESI-MS (*m/z*):** 405 [M+2]⁺; Analytical calculated formulae C₁₆H₁₅BrN₆S: C, 47.65; H, 3.75; N, 20.84; S, 7.95; Found: C, 47.61; H, 3.78; N, 20.80; S, 7.98.

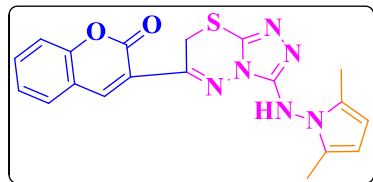
***N*-(2,5-Dimethyl-1*H*-pyrrol-1-yl)-6-(4-nitrophenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-amine (28g):**



Yellow solid (0.321g, 87%); m.p.: 208-210°C; **IR (KBr, ν_{max} /cm⁻¹):** 3249 (-NH-), 1578 (C=N); **¹H-NMR (400 MHz, CDCl₃+DMSO-*d*₆, δ ppm):** 2.16 (s, 6H, two -CH₃), 4.29 (s, 2H, -CH₂-), 5.72 (s, 2H, two =CH- of pyrrole ring), 8.33 (s, 4H, Hz, Ar-H), 9.93 (s, 1H, -NH-); **¹³C-**

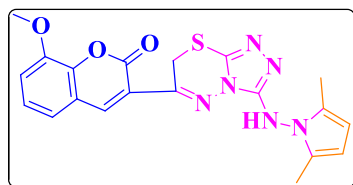
NMR (100 MHz, CDCl₃+DMSO-*d*₆, δ ppm): 11.6, 23.3, 103.9, 106.1, 124.3, 127.9, 129.4, 149.5, 153.1, 166.0, 172.8; **ESI-MS (*m/z*):** 370 [M+H]⁺; Analytical calculated formulae C₁₆H₁₅N₇O₂S: C, 52.02; H, 4.09; N, 26.54; S, 8.68; Found: C, 52.07; H, 4.04; N, 26.58; S, 8.72.

3-(3-((2,5-Dimethyl-1*H*-pyrrol-1-yl)amino)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-6-yl)-2*H*-chromen-2-one (28h):



Pale yellow solid (0.348g, 89%); m.p.: 201-203°C; **IR (KBr, ν_{max} /cm⁻¹):** 3426 (-NH-), 1719 (C=O), 1607 (C=N); **¹³C-NMR (100 MHz, CDCl₃+DMSO-*d*₆, δ ppm):** 21.5, 30.1, 116.5, 119.4, 121.2, 125.7, 125.8, 132.2, 137.1, 143.7, 146.7, 146.9, 152.0, 153.1, 154.8, 159.0; **ESI-MS (*m/z*):** 393 [M+H]⁺; Analytical calculated formulae C₁₉H₁₆N₆O₂S: C, 58.15; H, 4.11; N, 21.42; S, 8.17; Found: C, 58.19; H, 4.15; N, 21.46; S, 8.13.

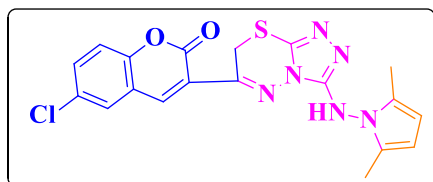
3-(3-((2,5-Dimethyl-1*H*-pyrrol-1-yl)amino)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-6-yl)-8-methoxy-2*H*-chromen-2-one (28i):



Brown solid (0.362g, 86%); m.p.: 218-220°C; **IR (KBr, ν_{max} /cm⁻¹):** 3415 (-NH-), 1719 (C=O), 1607 (C=N); **¹H-NMR (400 MHz, CDCl₃+DMSO-*d*₆, δ ppm):** 2.16 (s, 6H, two -CH₃), 4.00 (s, 3H, -OCH₃), 4.21 (s, 2H, -CH₂-), 5.74 (s, 2H, two =CH- of pyrrole ring), 7.27-7.35 (m, 3H, Ar-H), 8.61 (s, 1H, -NH-), 9.72 (s, 1H, Ar-H); **¹³C-NMR (100 MHz, CDCl₃+DMSO-*d*₆, δ ppm):** 21.5, 30.1, 56.7, 116.5, 119.4, 121.2, 125.7, 125.8, 132.2, 137.1,

143.7, 146.7, 146.9, 152.0, 153.1, 154.8, 159.0; **ESI-MS (m/z):** 423 $[M+H]^+$; Analytical calculated formulae $C_{20}H_{18}N_6O_3S$: C, 56.86; H, 4.29; N, 19.89; S, 7.59; Found: C, 56.85; H, 4.26; N, 19.85; S, 7.62.

7-Chloro-3-(3-((2,5-dimethyl-1*H*-pyrrol-1-yl)amino)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-6-yl)-2*H*-chromen-2-one (28j):



Yellow solid (0.374g, 88%); m.p.: 213-215°C; **IR (KBr, $\nu_{\max}/\text{cm}^{-1}$):** 3415 (-NH-), 1725 (C=O), 1606 (C=N); **^1H -NMR (400 MHz, $\text{CDCl}_3+\text{DMSO}-d_6$, δ ppm):** 2.08 (s, 6H, two -CH₃), 4.16 (s, 2H, -CH₂-), 5.65 (s, 2H, two =CH- of pyrrole ring), 7.35-7.39 (m, 1H, Ar-H), 7.59 (d, 1H, $J=7.2$ Hz, Ar-H), 7.71 (s, 1H, -NH-), 8.53 (s, 1H, Ar-H), 9.79 (s, 1H, Ar-H); **^{13}C -NMR (100 MHz, $\text{CDCl}_3+\text{DMSO}-d_6$, δ ppm):** 21.2, 24.3, 118.3, 119.6, 123.9, 128.7, 128.9, 130.1, 130.2, 130.3, 133.5, 133.7, 152.8, 152.9, 158.6, 158.8; **ESI-MS (m/z):** 427 $[M+H]^+$; Analytical calculated formulae $C_{19}H_{15}ClN_6O_2S$: C, 53.46; H, 8.31; N, 19.69; S, 7.51; Found: C, 53.49; H, 8.28; N, 19.65; S, 7.56.

SPECTRA:

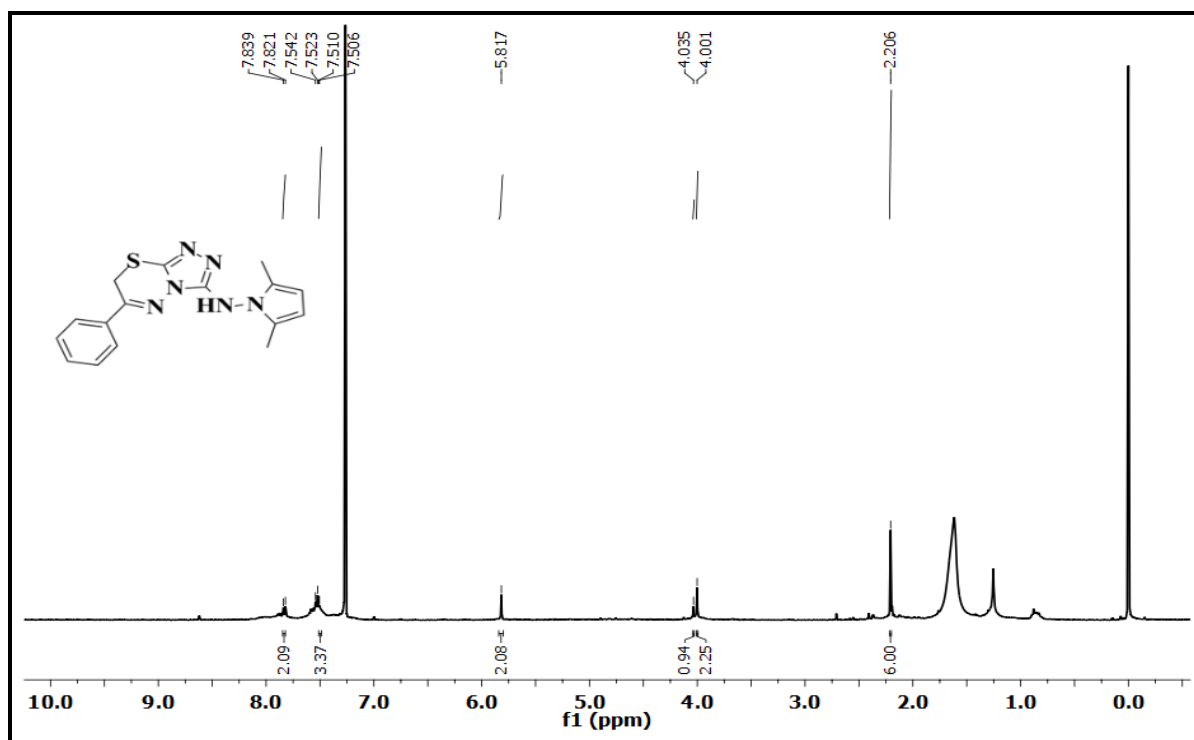


Fig: ^1H -NMR Spectrum of Compound **28a** (400 MHz, CDCl_3)

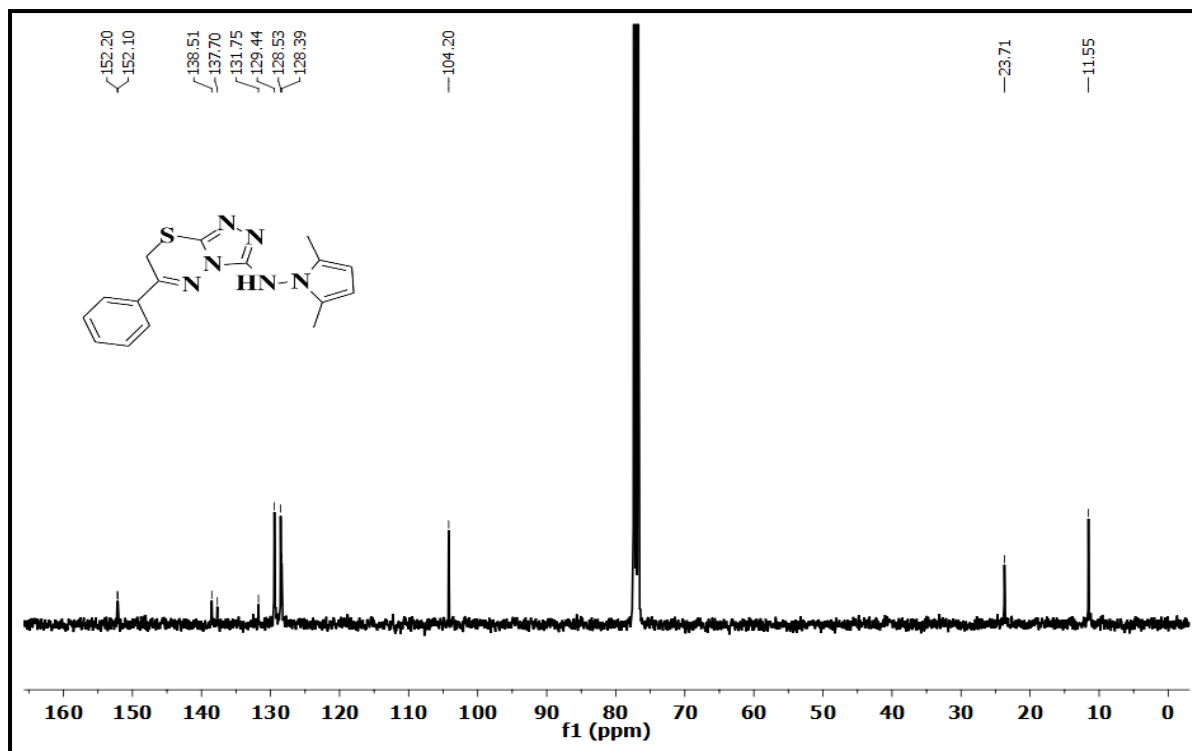


Fig: ^{13}C -NMR Spectrum of Compound **28a** (100 MHz, CDCl_3)

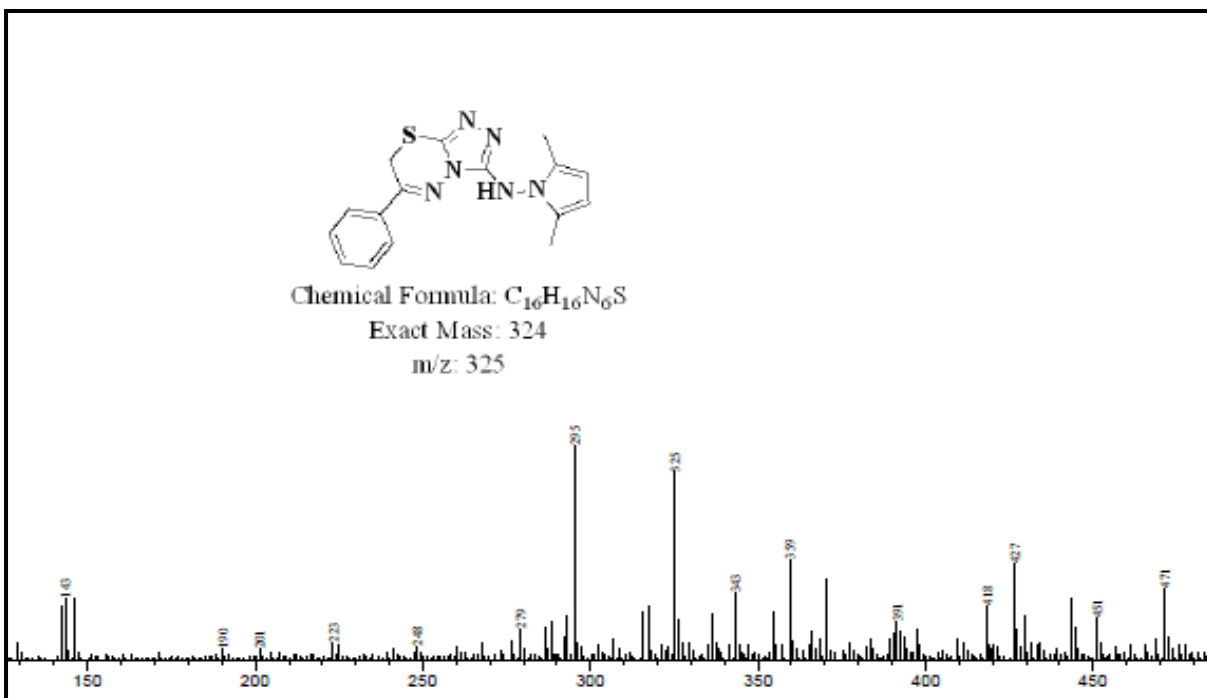


Fig: Mass Spectrum of Compound **28a**

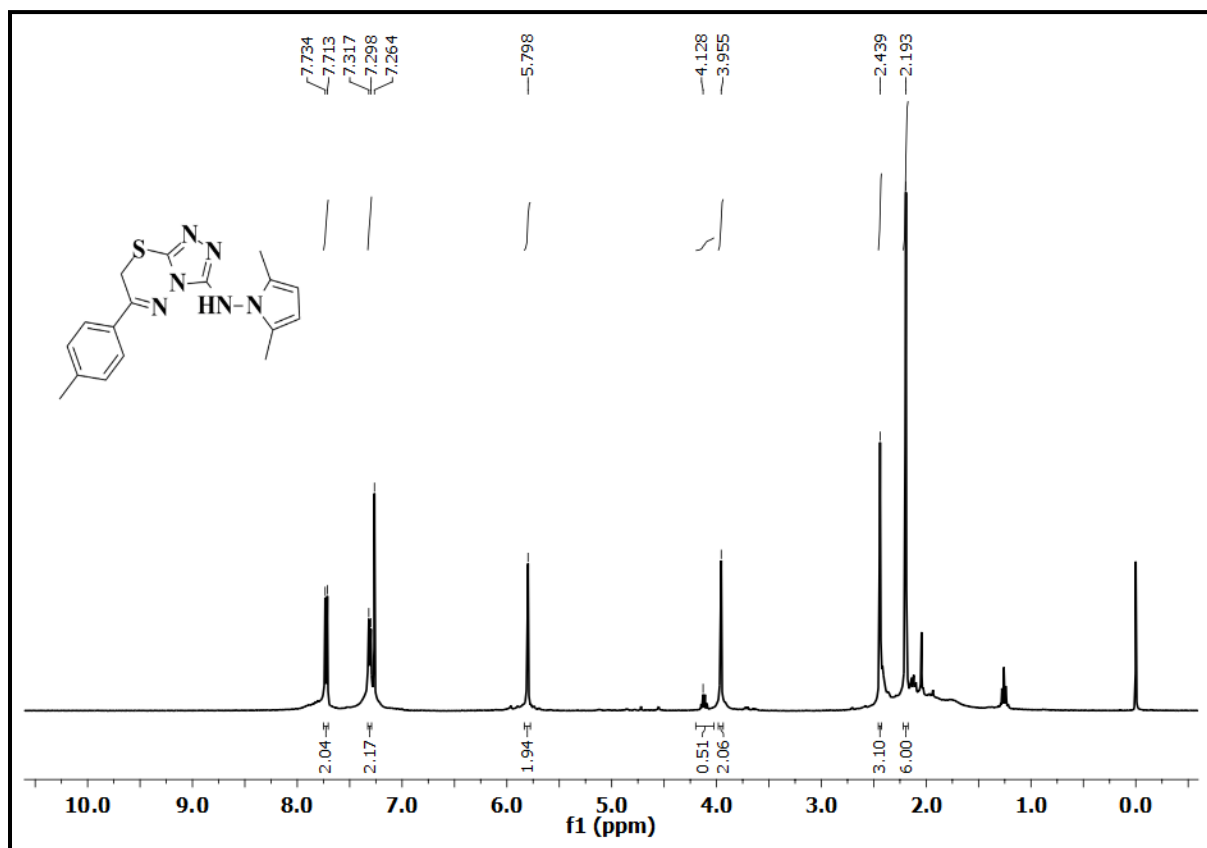


Fig: ^1H -NMR Spectrum of Compound **28b** (400 MHz, CDCl_3)

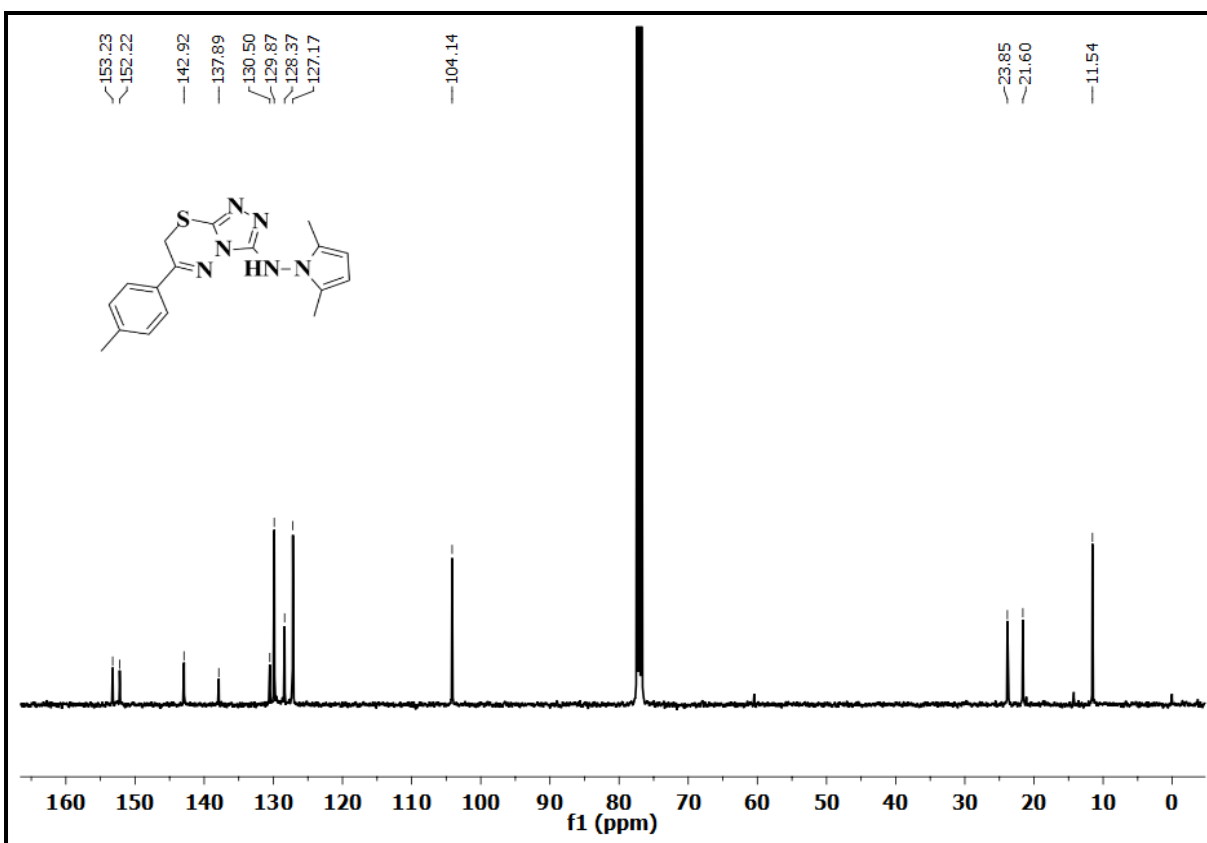


Fig: ^{13}C -NMR Spectrum of Compound **28b** (100 MHz, CDCl_3)

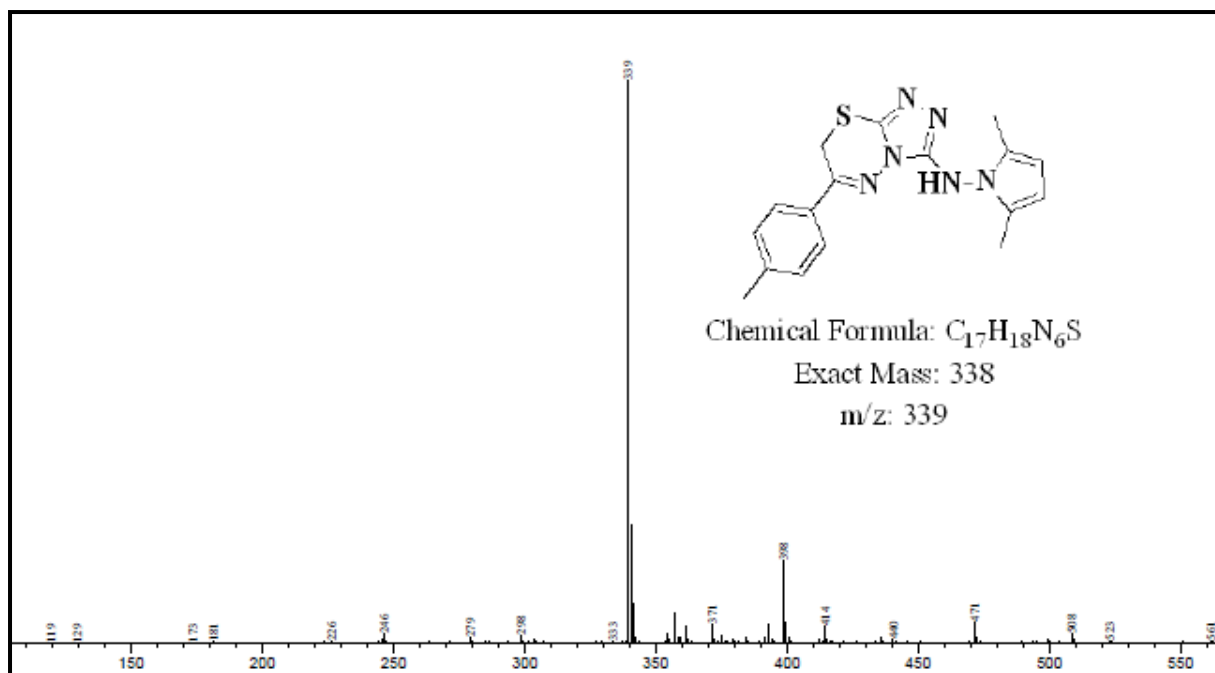


Fig: Mass Spectrum of Compound **28b**

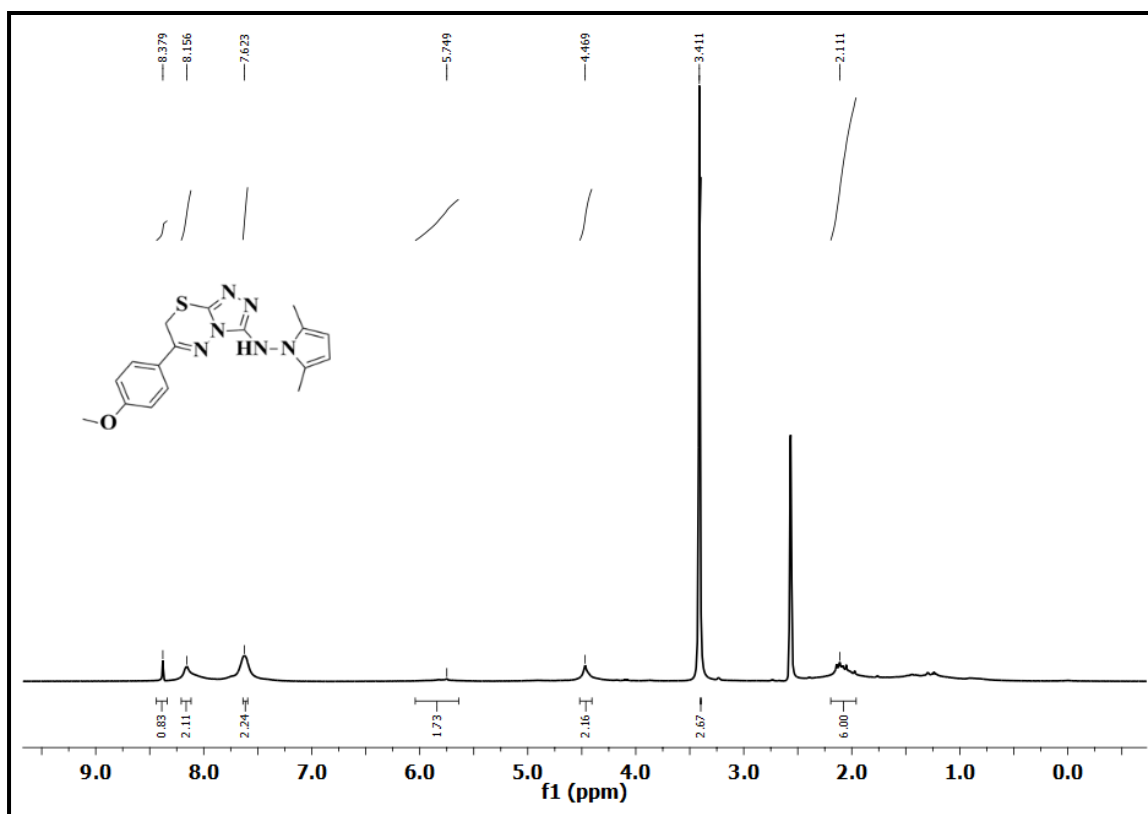


Fig: ¹H-NMR Spectrum of Compound **28c** (400 MHz, DMSO-*d*₆)

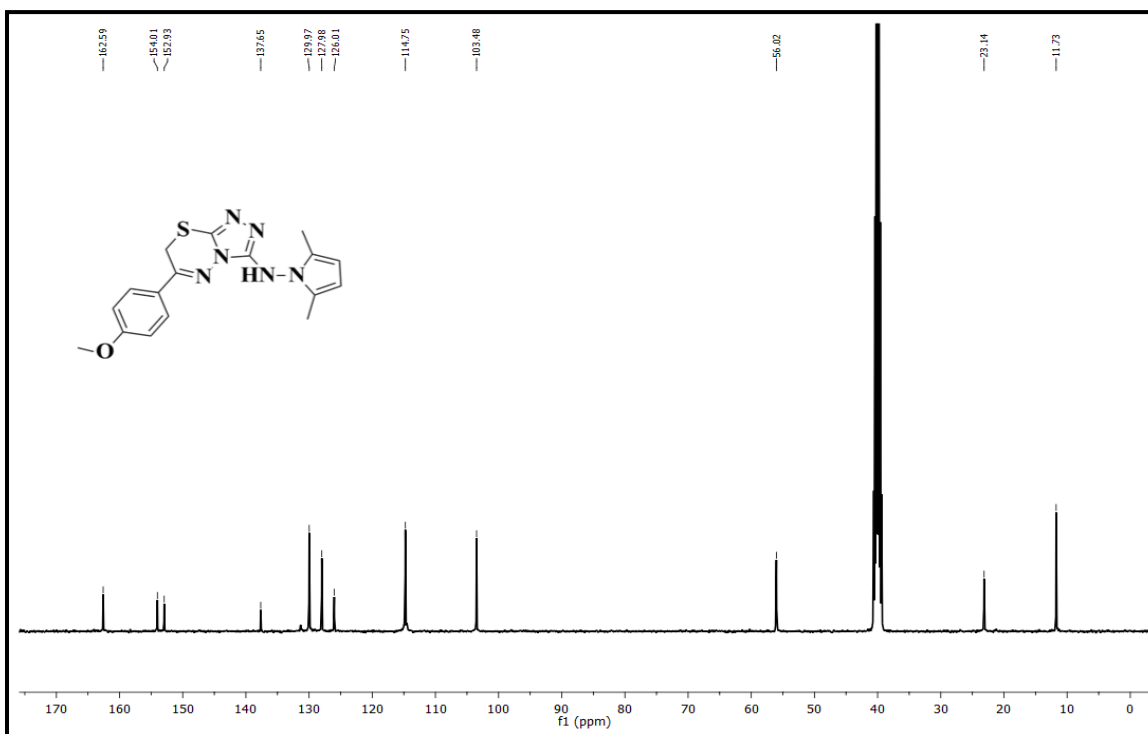


Fig: ¹³C-NMR Spectrum of Compound **28c** (100 MHz, DMSO-*d*₆)

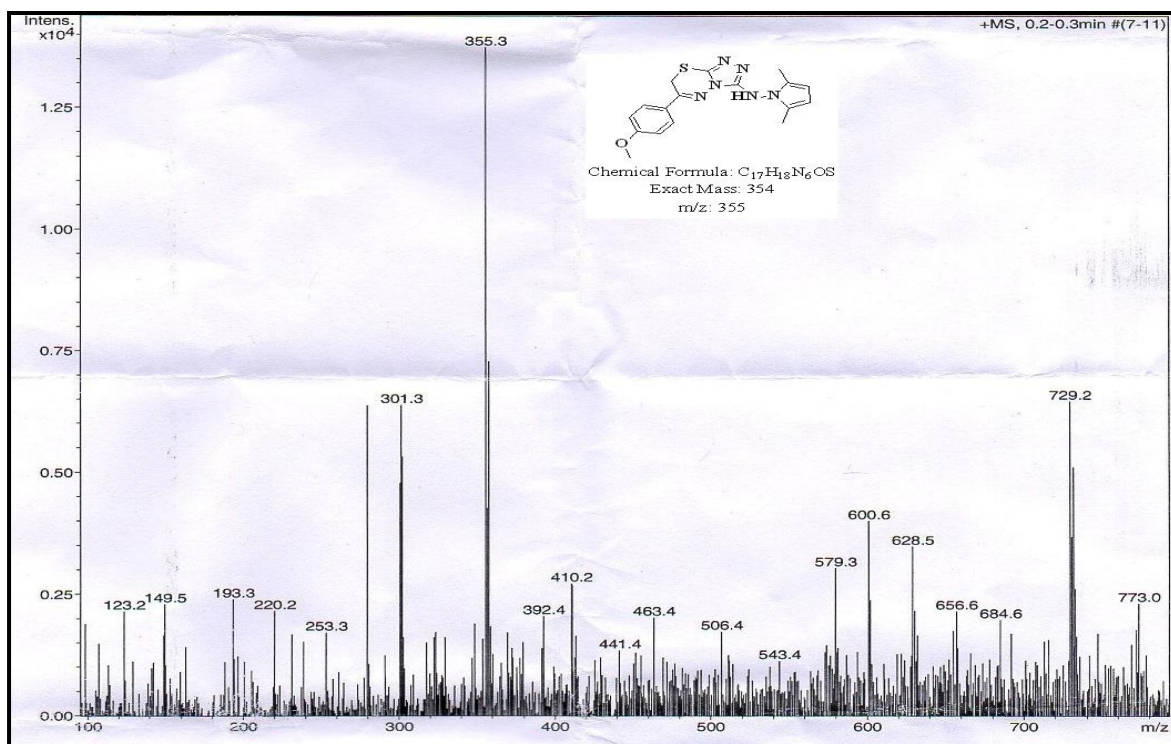


Fig: Mass Spectrum of Compound **28c**

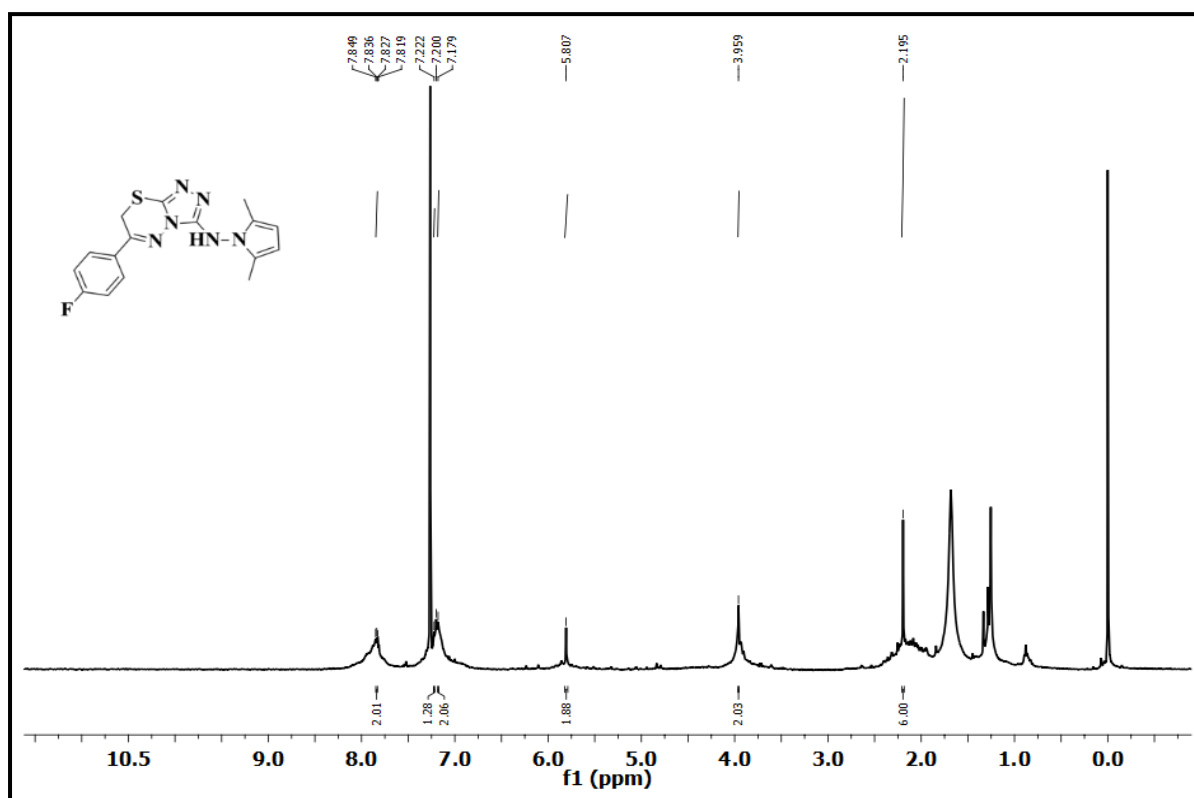


Fig: ^1H -NMR Spectrum of Compound **28d** (400 MHz, CDCl_3)

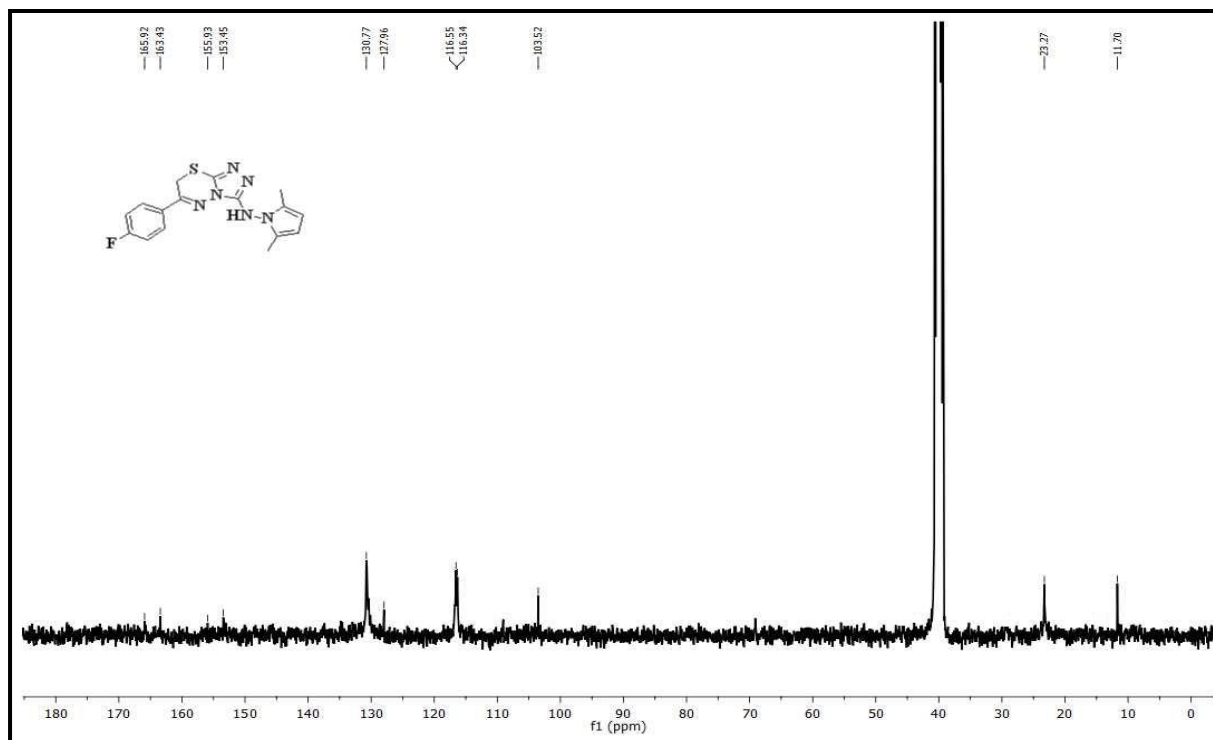


Fig: ¹³C-NMR Spectrum of Compound **28d** (100 MHz, DMSO-*d*₆)

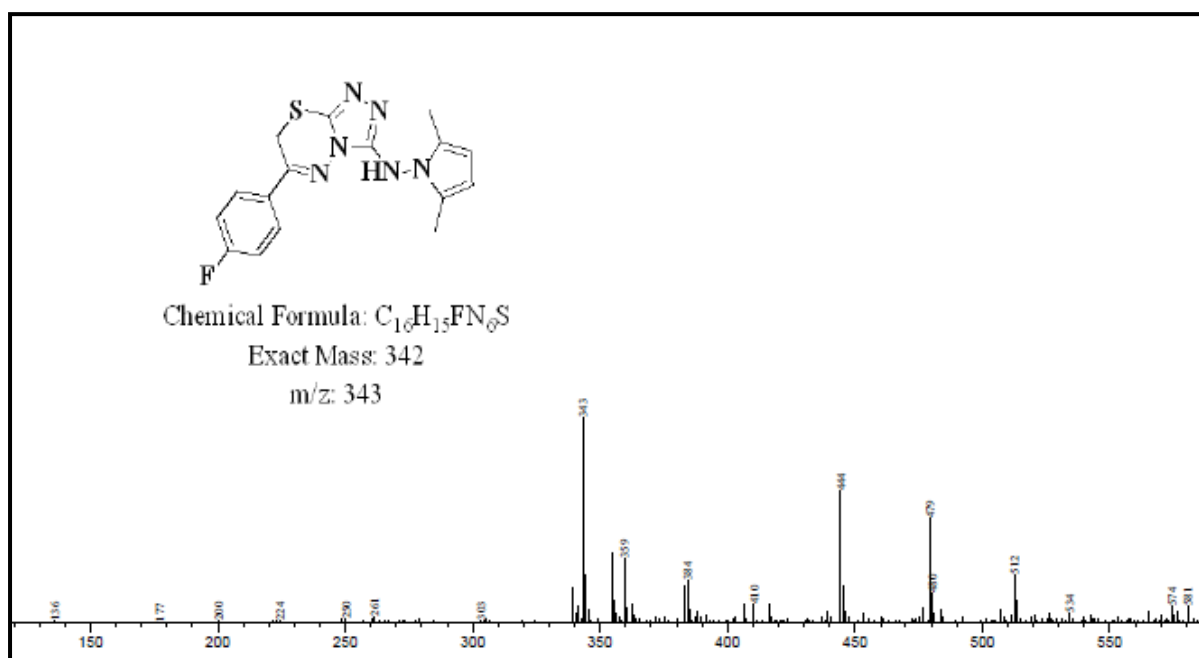


Fig: Mass Spectrum of Compound **28d**

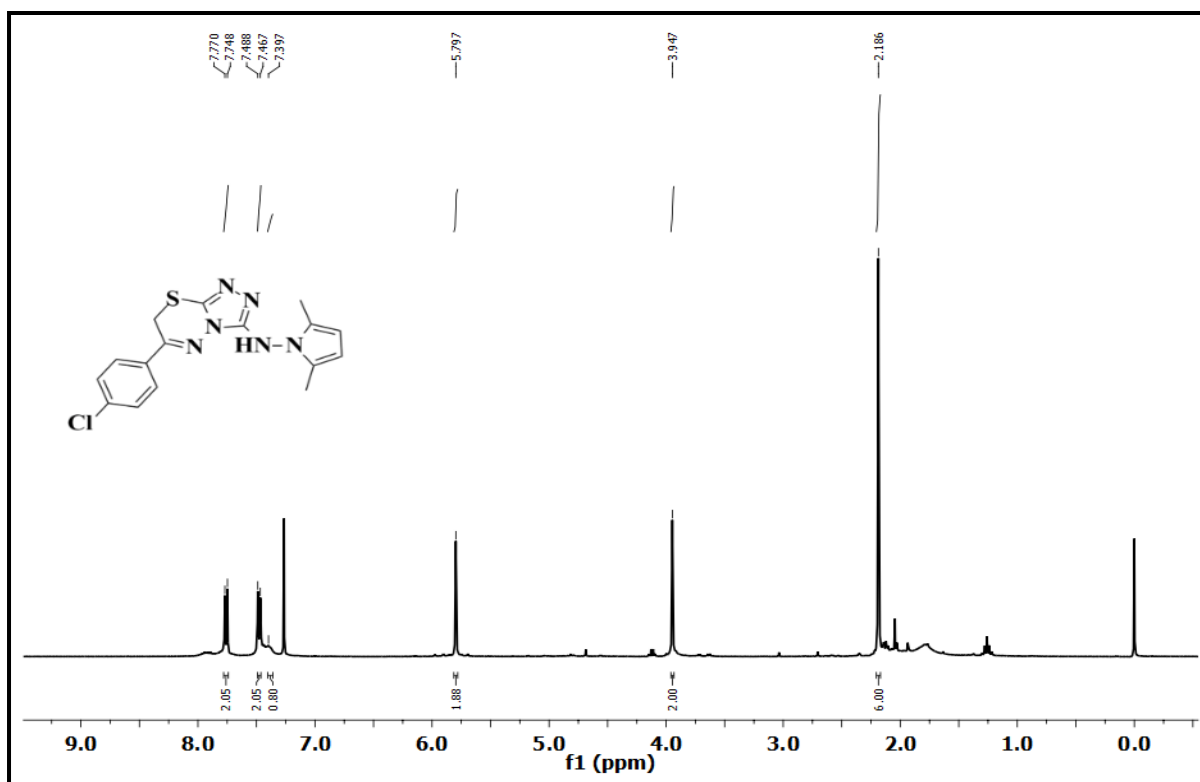


Fig: ¹H-NMR Spectrum of Compound **28e** (400 MHz, CDCl₃)

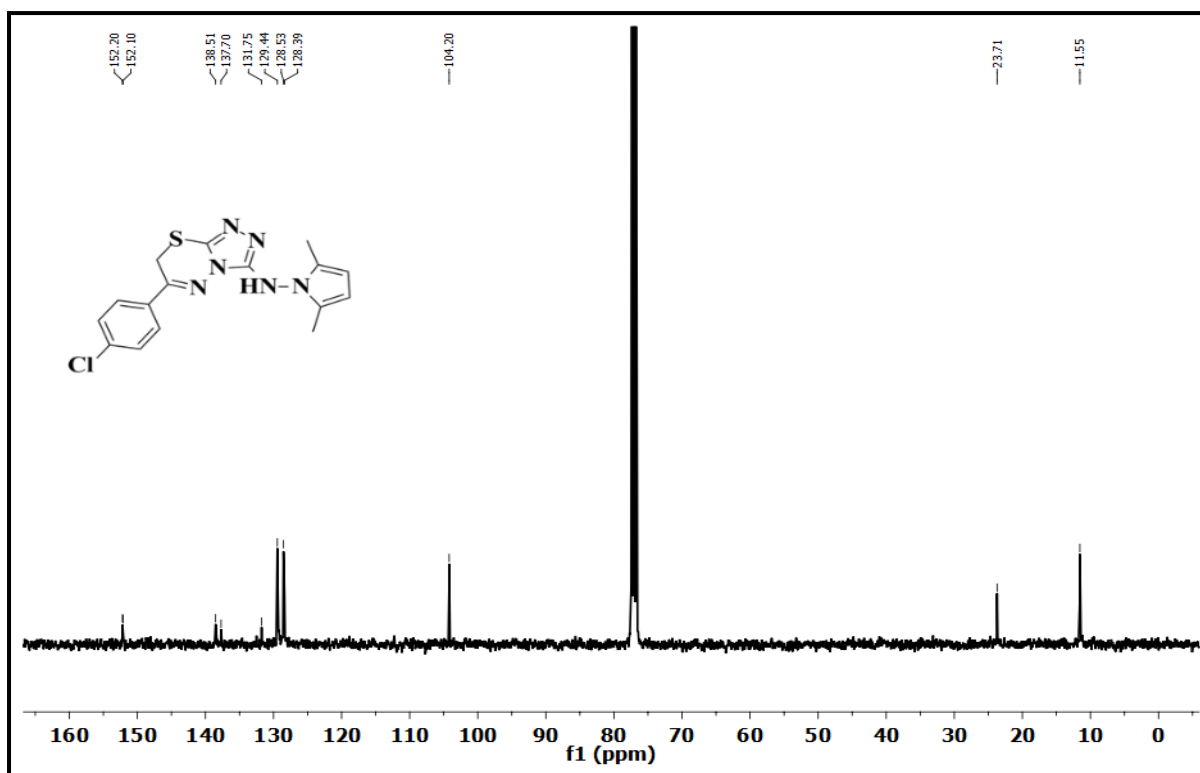


Fig: ¹³C-NMR Spectrum of Compound **28e** (100 MHz, CDCl₃)

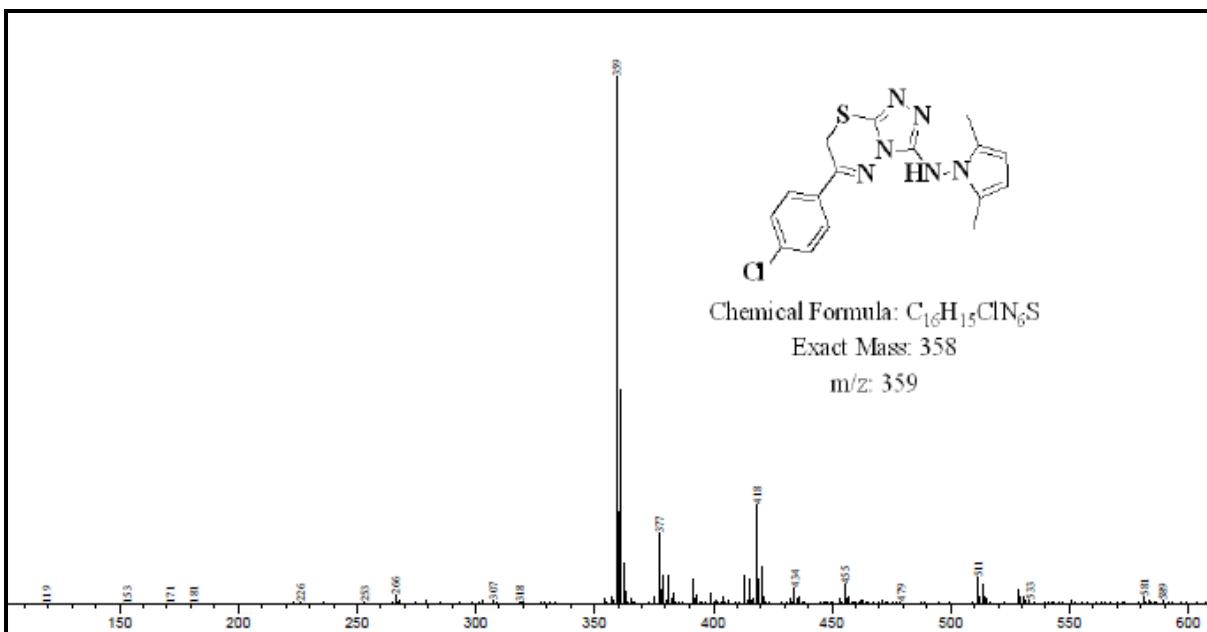


Fig: Mass Spectrum of Compound **28e**

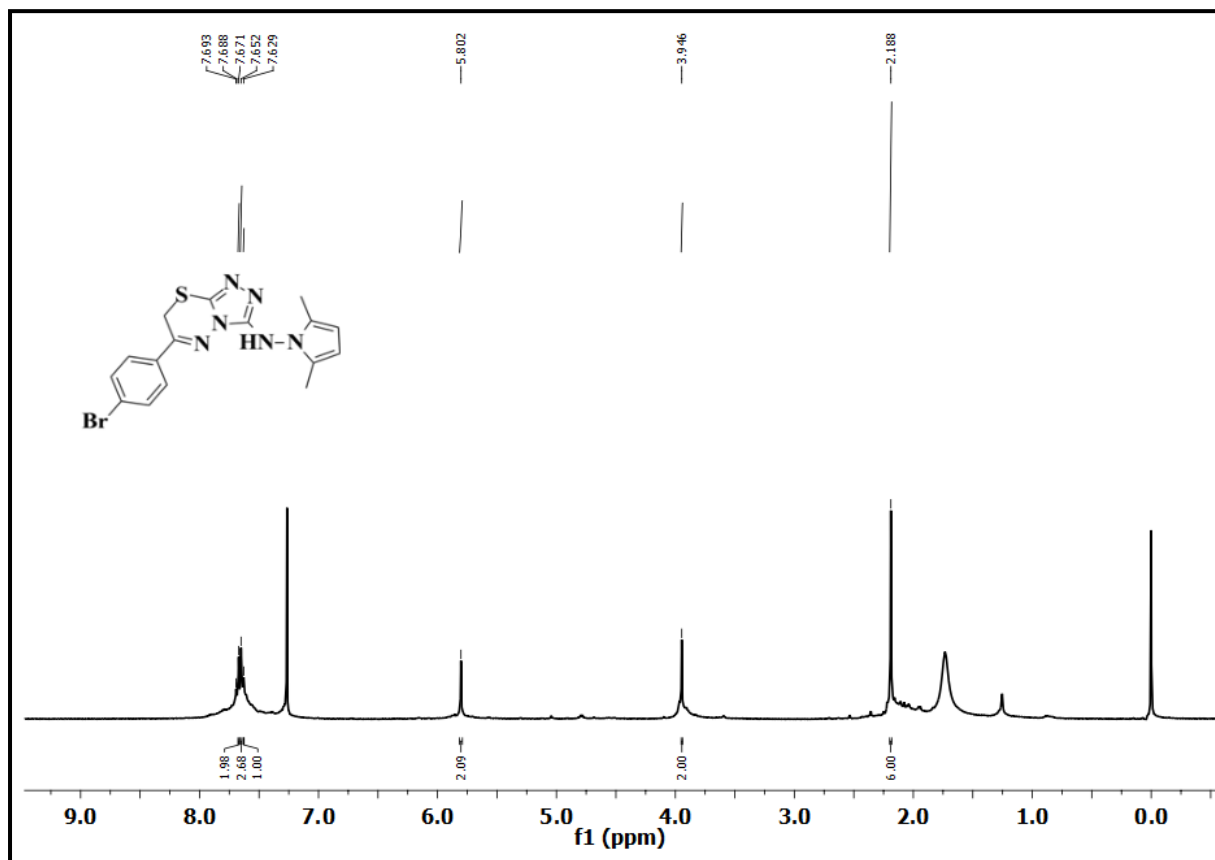


Fig: 1H -NMR Spectrum of Compound **28f** (400 MHz, $CDCl_3$)

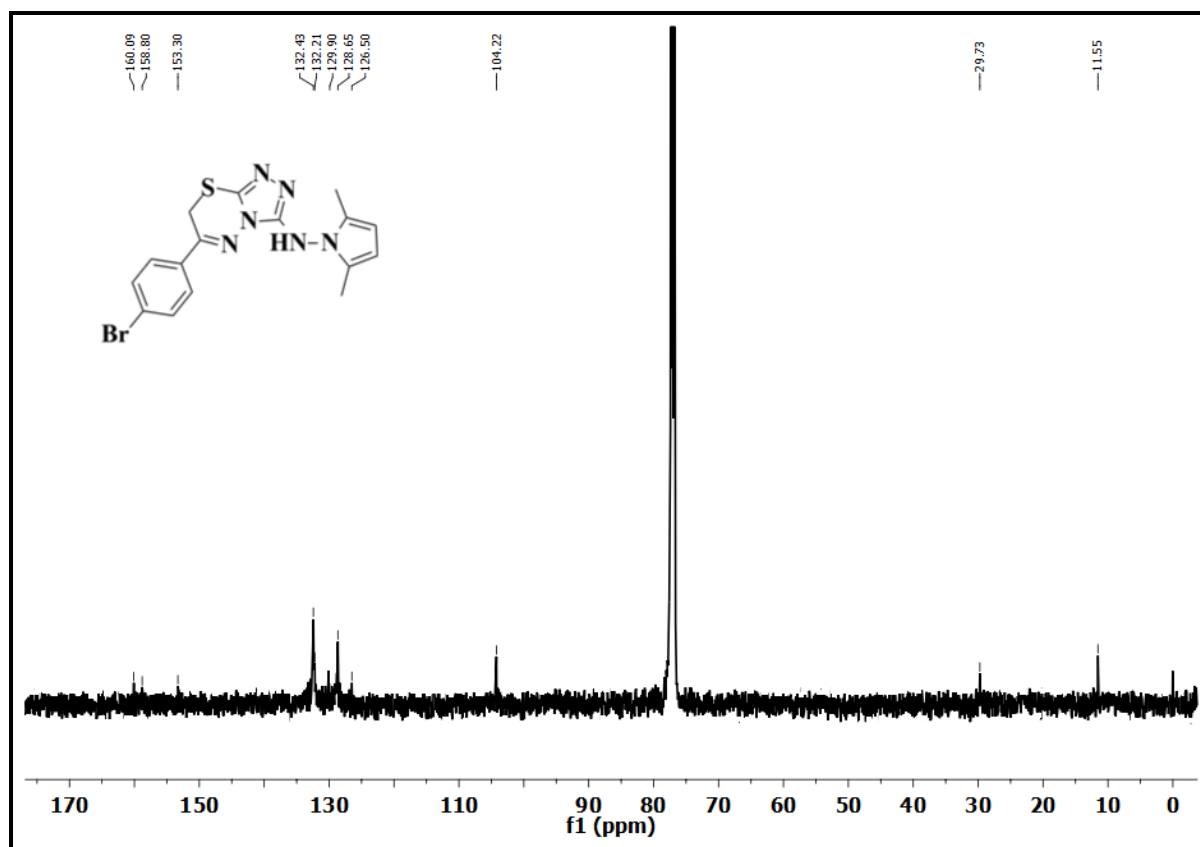


Fig: ¹³C-NMR Spectrum of Compound 28f (100 MHz, CDCl₃)

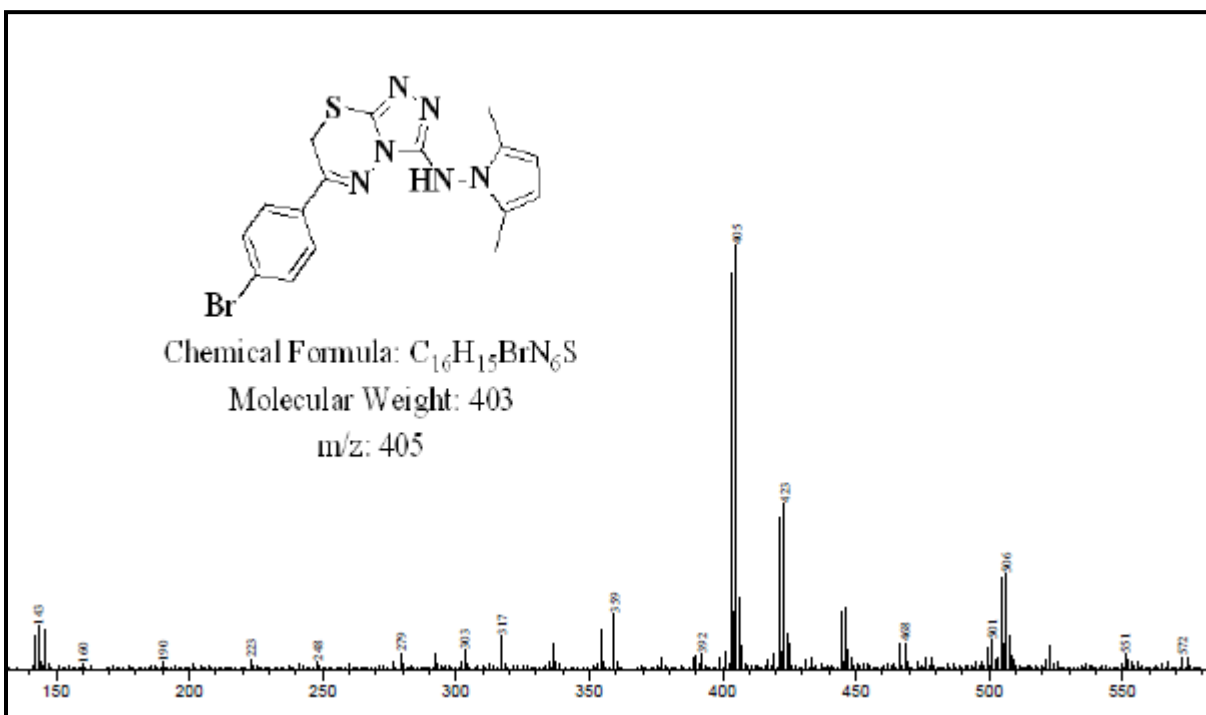


Fig: Mass Spectrum of Compound 28f

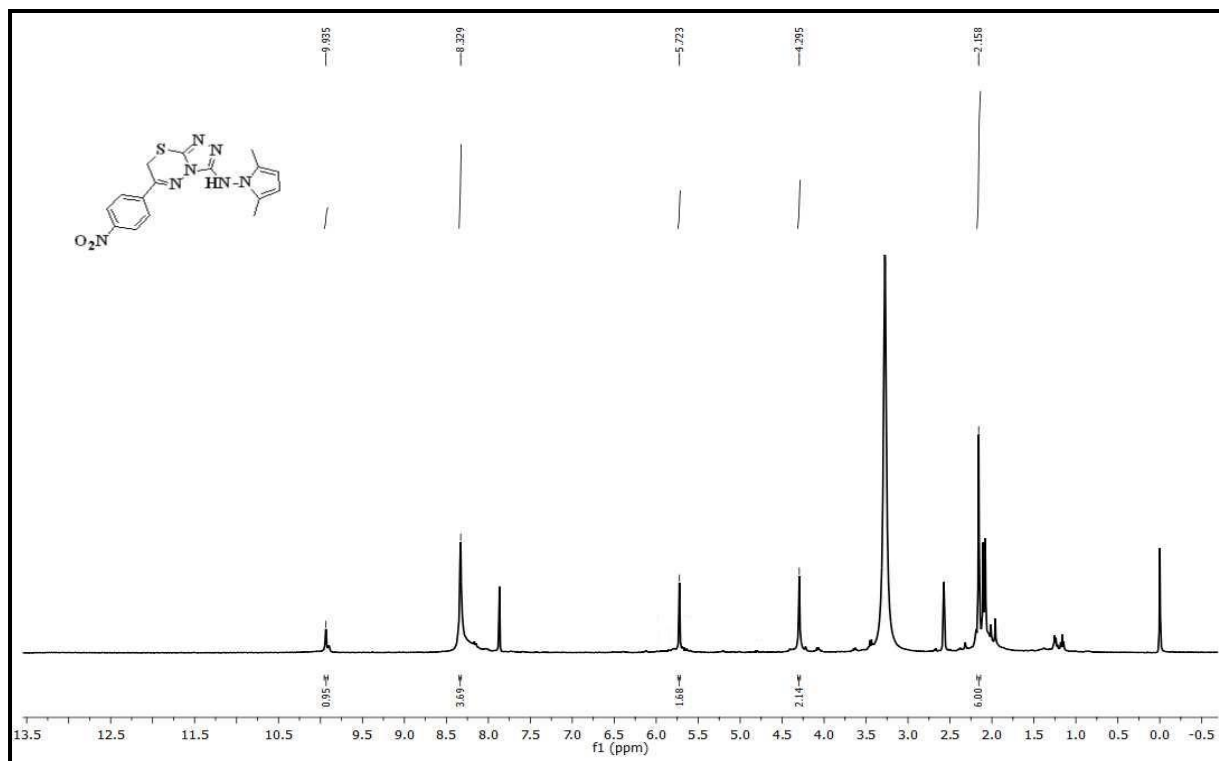


Fig: ¹H-NMR Spectrum of Compound **28g** (400 MHz, CDCl₃+DMSO-*d*₆)

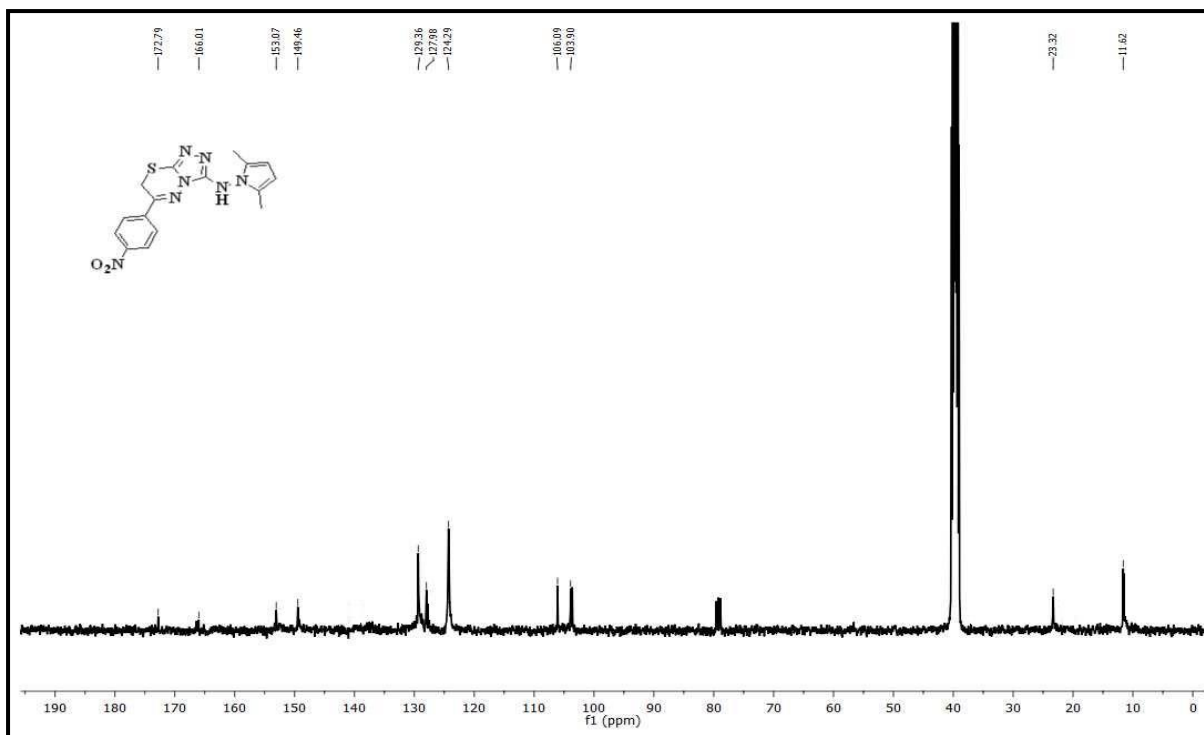


Fig: ¹³C-NMR Spectrum of Compound **28g** (100 MHz, CDCl₃+DMSO-*d*₆)

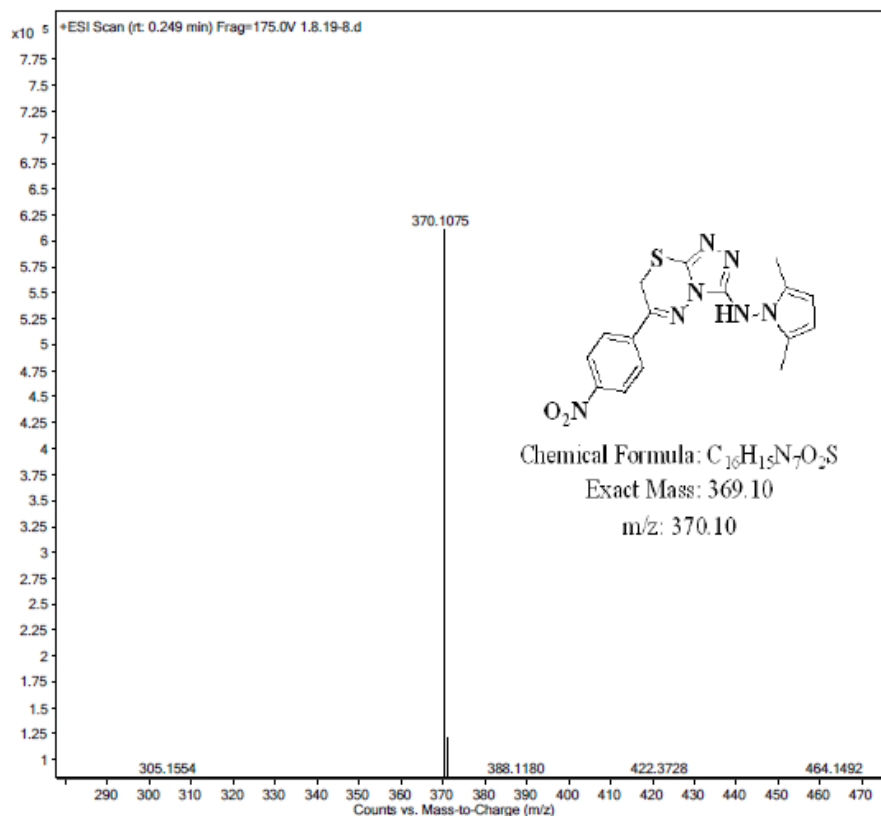


Fig: Mass Spectrum of Compound **28g**

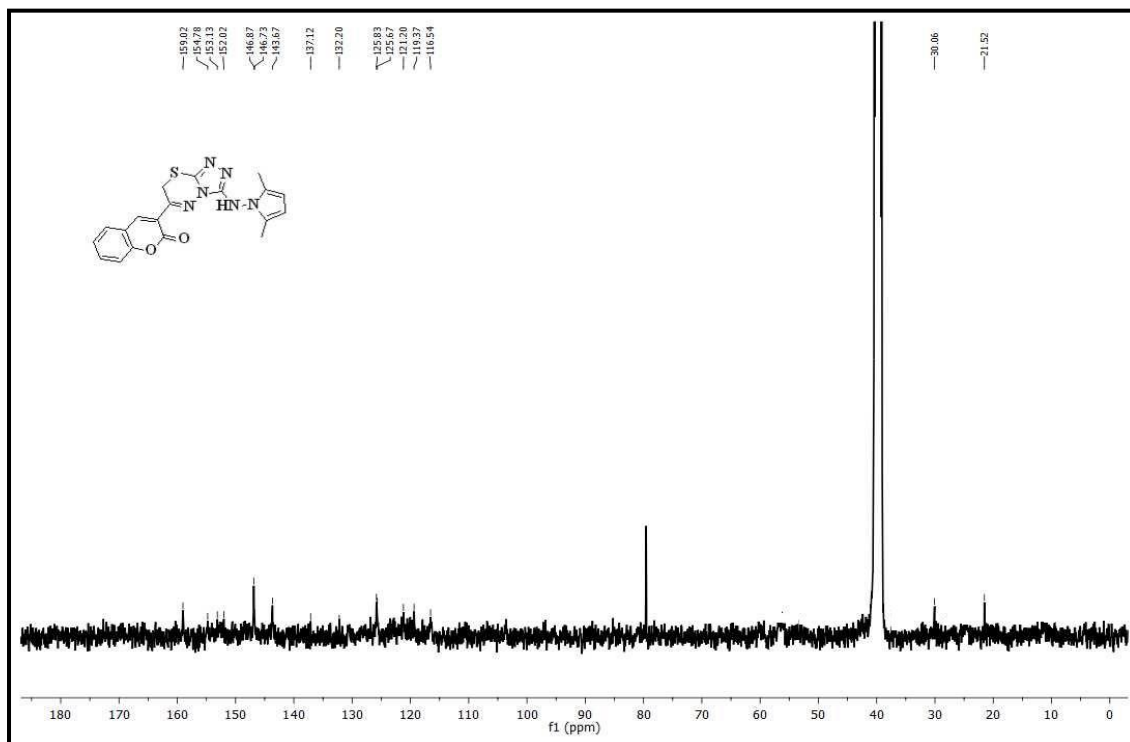


Fig: ^{13}C -NMR Spectrum of Compound **28h** (100 MHz, $CDCl_3$ +DMSO- d_6)

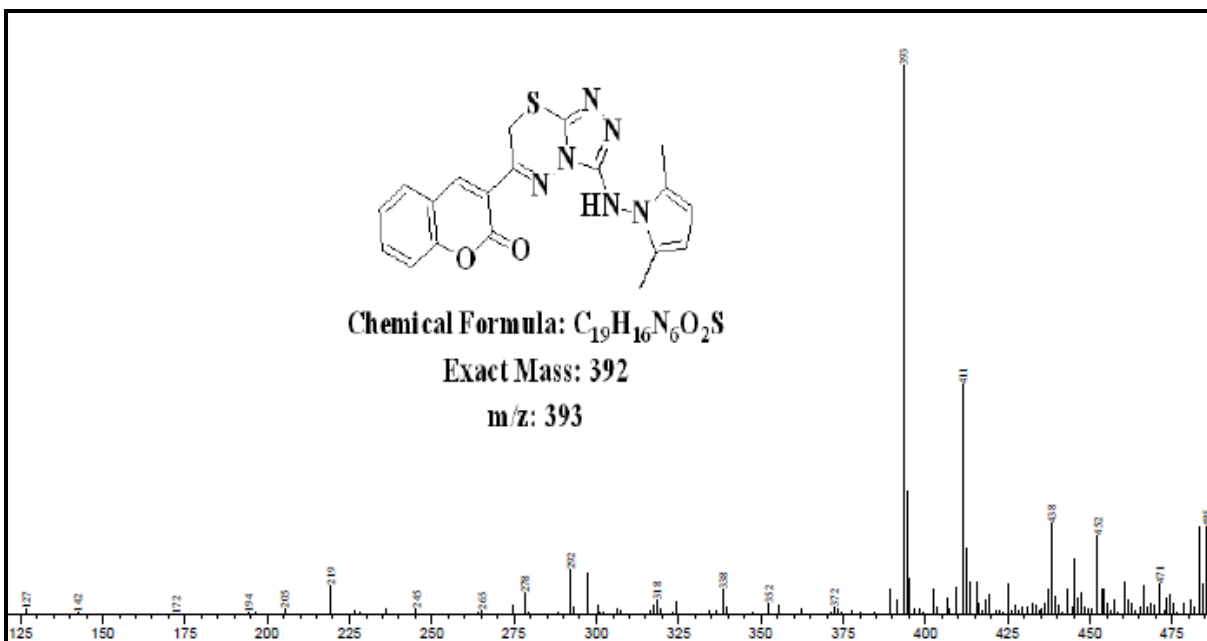


Fig: Mass Spectrum of Compound **28h**

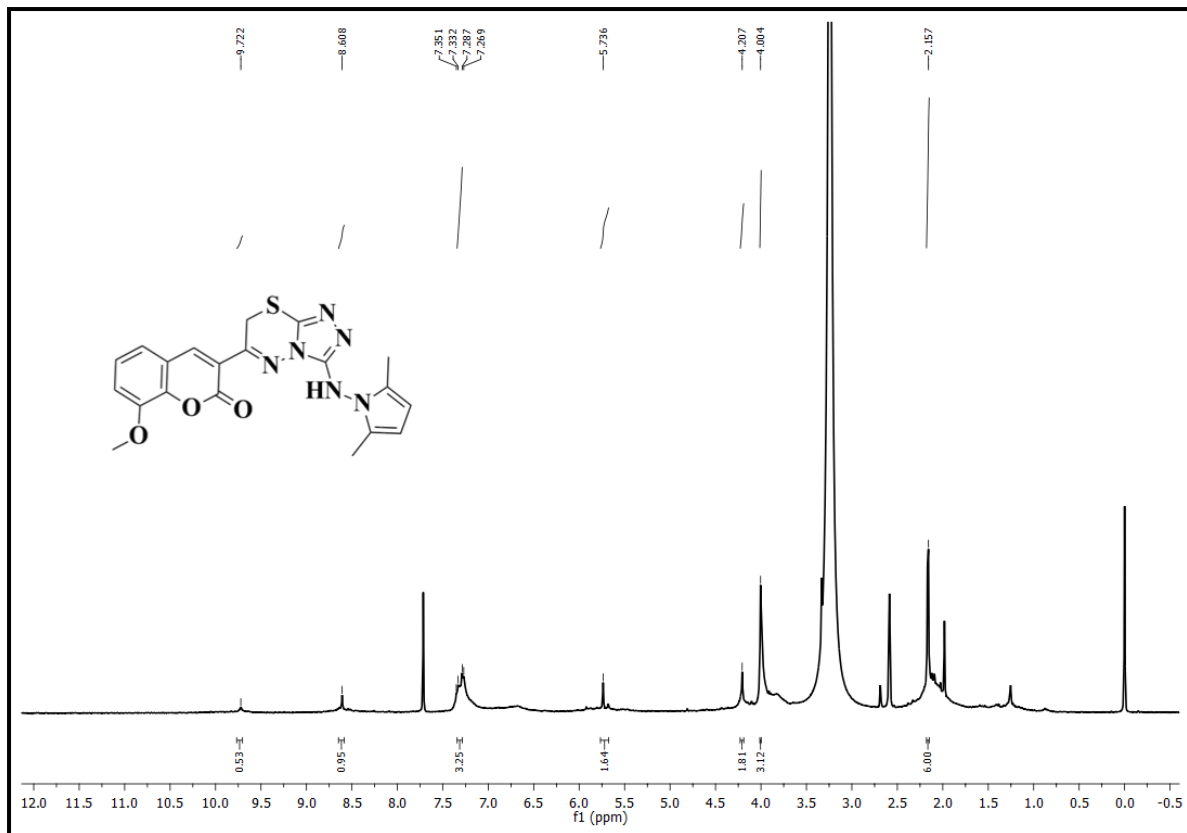


Fig: ^1H -NMR Spectrum of Compound **28i** (400 MHz, $\text{CDCl}_3+\text{DMSO}-d_6$)

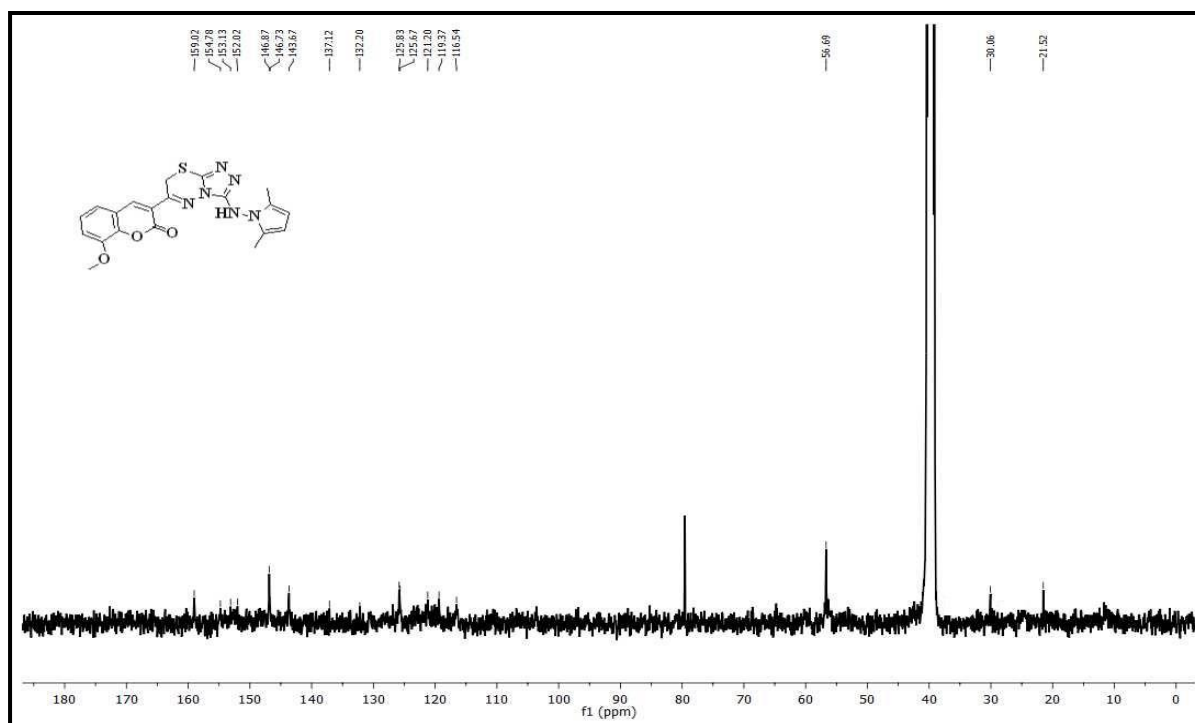


Fig: ¹³C-NMR Spectrum of Compound 28i (100 MHz, CDCl₃+DMSO-*d*₆)

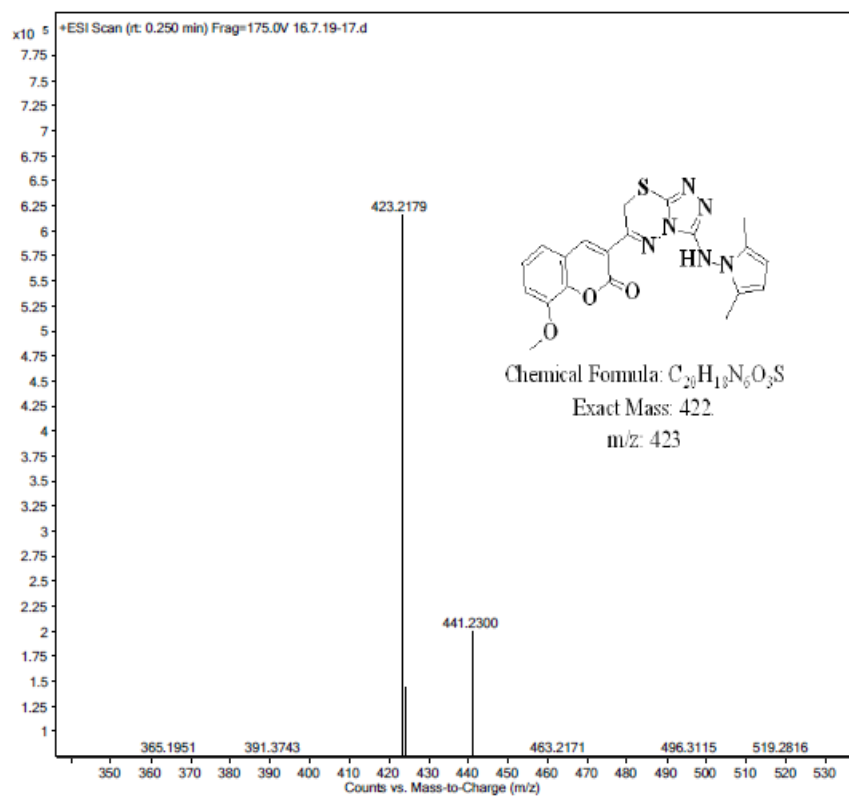


Fig: Mass Spectrum of Compound 28i

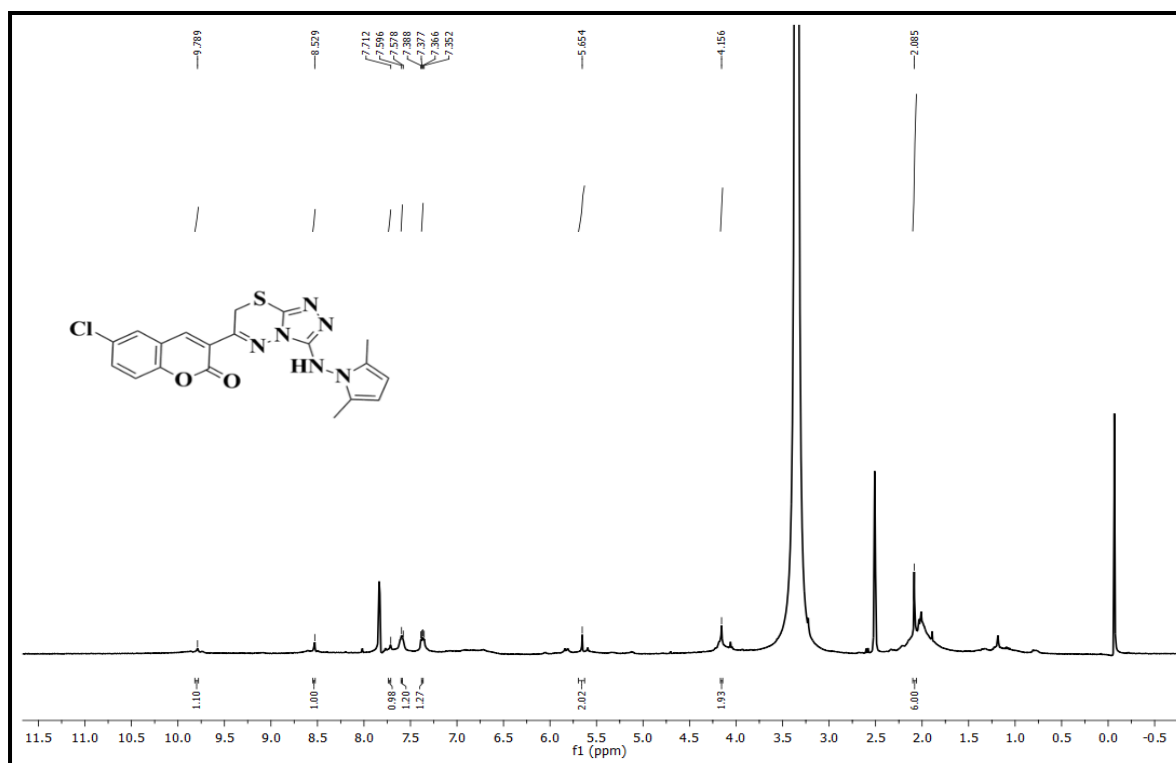


Fig: ¹H-NMR Spectrum of Compound **28j** (400 MHz, CDCl₃+DMSO-*d*₆)

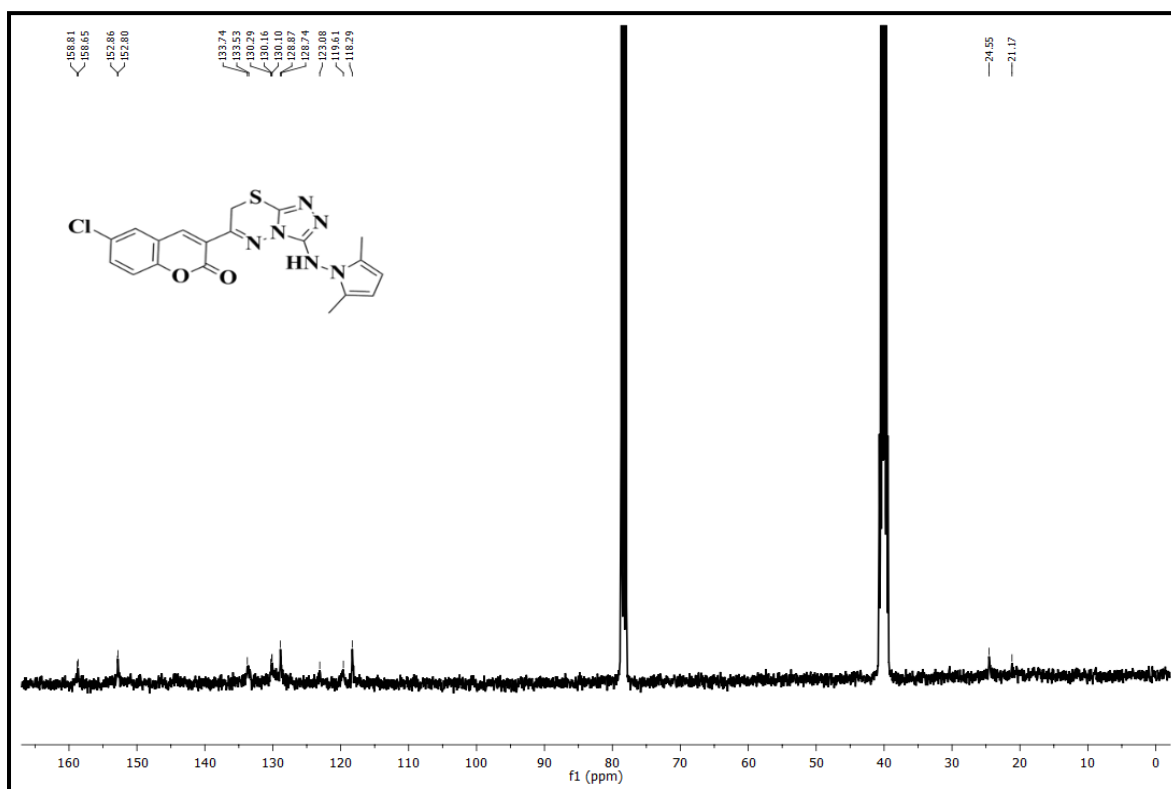


Fig: ¹³C-NMR Spectrum of Compound **28j** (100 MHz, CDCl₃+DMSO-*d*₆)

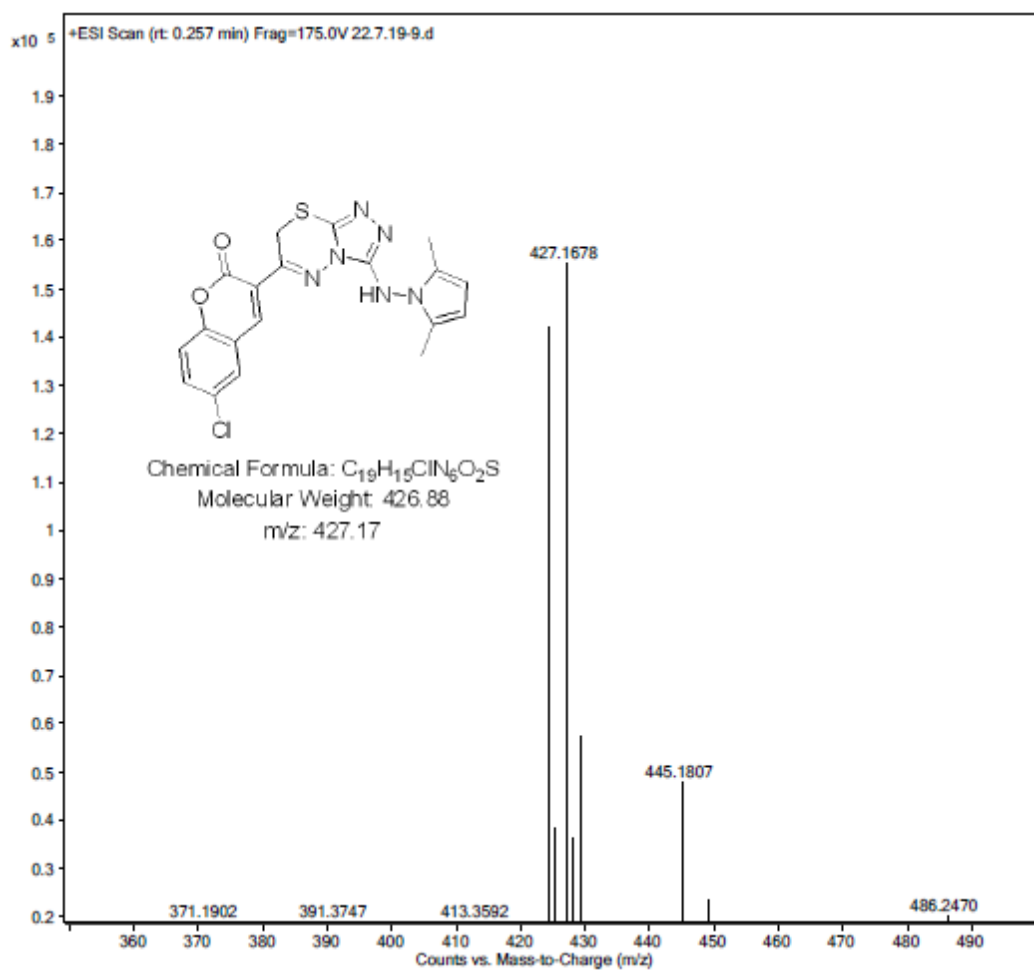


Fig: Mass Spectrum of Compound **28j**

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SUMMARY

SUMMARY

The thesis entitled “**MCR Approach for the synthesis of new heterocyclic compounds from 4-amino-5-hydrazinyl-4H-1,2,4-triazole-3-thiol**” consists of seven chapters, out of which chapter-I describes multi-component reactions and their utility in the synthesis of biologically active compounds. The remaining six chapters deal with the synthesis of different heterocyclic compounds relying on the use of a multi-component approach as a common theme.

CHAPTER-I: A micro review on multi-component reactions and their utility in the synthesis of biologically active compounds.

Chapter-I describes multi-component reactions (MCRs). MCRs are also known as Multi-Component Assembly Process (MCAP). Multi-component reactions find outstanding results in organic chemistry. These reactions involve the condensation of three or more functional groups of the molecules in a single reaction vessel. The protocol of the MCRs is very simple and easy to operate the reaction with high yields. Most of the MCRs are used for the formation of C-C, C-N, C-O, and, C-S bonds in the different types of heterocyclic and hetero acyclic compounds. Plenty of advantages are there for the MCR process to produce the single target compound without by-products, duration of the reaction is less, green solvents, eco-friendly, metal-free, economical effective, mild reaction conditions, effortless, etc. Multi-component reactions are used in several fields such as agrochemicals, combinatorial chemistry, medicinal chemistry, natural products, and polymers synthesis. Most of the biologically active compounds have been effectively synthesized through multi-component reactions.¹⁻¹⁵

However, the functionalization of heterocyclic compounds with diversified pharmacophores to develop effective therapeutic scaffolds with efficient, rapid, and clean synthetic strategies is of great importance. Without any doubt, the most efficient, and rapid synthetic techniques involve MCRs¹⁶⁻¹⁹, which have evolved as an efficient tool for the introduction of molecular diversity in therapeutically important heterocyclic structures.

In this research work, we have synthesized various new heterocyclic compounds *via* a one-pot, multi-component approach and developed novel synthetic methodologies. The entire research work is converted into the thesis.

Objectives of the present work are mentioned and outlines of the work carried out in the present investigations are also given.

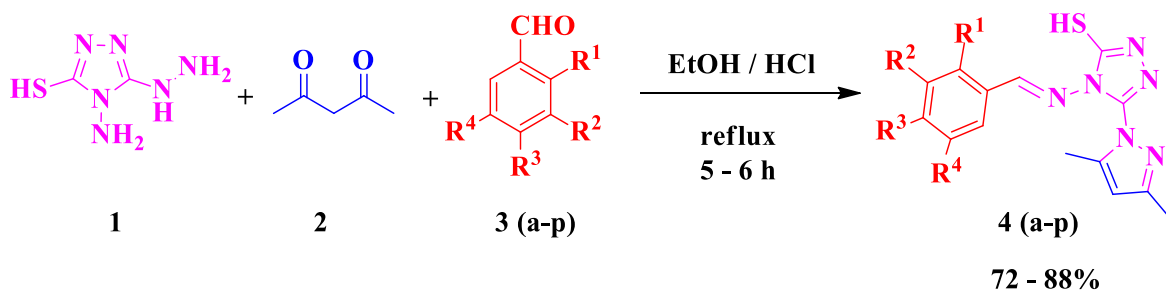
4-Amino-5-hydrazinyl-4*H*-1,2,4-triazole-3-thiol²⁰, 3-acetyl coumarins^{21,22} and 3-(2-bromoacetyl) coumarins²³⁻²⁴ were prepared and used as starting materials for the synthesis of heterocyclic compounds.

CHAPTER-II: Synthesis of benzylideneamino-3,5-dimethyl-1*H*-pyrazoles, synthesis of various aralkyl/alkyl thio-3,5-dimethyl-1*H*-pyrazolyl-4*H*-1,2,4-triazol-4-amines and their docking studies, and four-component, one-pot synthesis of (*E*)-*N*-benzylidene-3-(benzylthio)-5-(3,5-dimethyl-1*H*-pyrazol-1-yl)-4*H*-1,2,4-triazol-4-amines and their DNA binding and molecular docking studies.

Chapter-II is divided into three parts.

PART-A: A facile one-pot, three-component synthesis of benzylideneamino-3,5-dimethyl-1*H*-pyrazoles.

The title compounds were synthesized by the reaction of 4-amino-5-hydrazino-4*H*-[1,2,4]triazole-3-thiol (**1**), acetylacetone (**2**), and aromatic aldehydes (**3**) in absolute ethanol containing two drops of concentrated HCl. The products were obtained in good yields (**Scheme-1**). All the synthesized compounds were well characterized by analytical and spectral (IR, ¹H-NMR, ¹³C-NMR, and Mass) data.



<u>Entry</u>	<u>Product</u>	<u>R¹</u>	<u>R²</u>	<u>R³</u>	<u>R⁴</u>
1	4a	H	H	CH ₃	H
2	4b	H	NO ₂	H	H
3	4c	OH	H	H	H
4	4d	OH	OCH ₃	H	H

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5	4e	Cl	H	H	H
6	4f	OH	H	N(C ₂ H ₅) ₂	H
7	4g	H	OCH ₃	OCH ₃	H
8	4h	OCH ₃	OCH ₃	H	H
9	4i	OCH ₃	H	OCH ₃	H
10	4j	H	OCH ₃	OCH ₃	OCH ₃
11	4k	H	OCH ₃	H	H
12	4l	OH	OC ₂ H ₅	H	H
13	4m	H	H	OCH ₃	H
14	4n	H	H	Cl	H
15	4o	H	H	OH	H
16	4p	H	H	NO ₂	H

Scheme-1: One-pot synthesis of substituted benzylideneamino-3,5-dimethyl-1*H*-pyrazoles (**4a-p**).

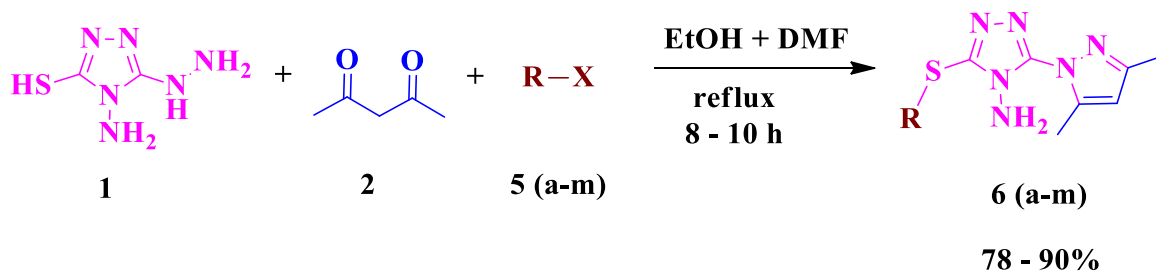
In conclusion, a facile, one-pot three-component reaction for the synthesis of benzylideneamino-3,5-dimethyl-1*H*-pyrazoles (**4a-p**) has been achieved *via* a multi-component approach using readily available starting materials. This method provides various advantages, such as good yields, neat reaction conditions, easy workup, and purification. The synthesized heterocyclic compounds may be beneficial for drug discovery.

PART-B: One-pot, multi-component cascade reaction for the synthesis of various aralkyl/alkyl thio-3,5-dimethyl-1*H*-pyrazolyl-4*H*-1,2,4-triazol-4-amines and their docking studies.

The title compounds were obtained by the reaction of 4-amino-5-hydrazino-4*H*-[1,2,4]triazole-3-thiol (**1**), acetylacetone (**2**), and aralkyl/alkyl halides (**5**) in a mixture of dry

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ethanol and dimethylformamide (DMF) (1:1) under reflux. By using this condition 13 derivatives (**6a-m**) were synthesized (**Scheme-2**). All the synthesized compounds were well characterized by analytical and spectral data such as IR, $^1\text{H-NMR}$, $^{13}\text{C-NMR}$, and Mass spectral data.



<u>Entry</u>	<u>Product</u>	<u>R-</u>
17	6a	
18	6b	
19	6c	$\text{HC}\equiv\text{C}-\text{CH}_2-$
20	6d	$\text{H}_2\text{C}=\overset{\text{H}}{\text{C}}-\overset{\text{H}_2}{\text{C}}-$
21	6e	
22	6f	
23	6g	$\text{H}_3\text{C}-(\text{H}_2\text{C})_{12}-\overset{\text{H}_2}{\text{C}}-$
24	6h	$\text{H}_3\text{C}-(\text{CH}_2)_{10}-\overset{\text{H}_2}{\text{C}}-$

SUMMARY

25	6i	$\text{H}_3\text{C}-(\text{H}_2\text{C})_8-\overset{\text{H}_2}{\text{C}}-$
26	6j	$\text{H}_3\text{C}-(\text{CH}_2)_6-\overset{\text{H}_2}{\text{C}}-$
27	6k	$\text{H}_3\text{C}-(\text{H}_2\text{C})_5-\overset{\text{H}_2}{\text{C}}-$
28	6l	$\text{H}_3\text{C}-(\text{H}_2\text{C})_4-\overset{\text{H}_2}{\text{C}}-$
29	6m	$\text{H}_3\text{C}-(\text{H}_2\text{C})_3-\overset{\text{H}_2}{\text{C}}-$

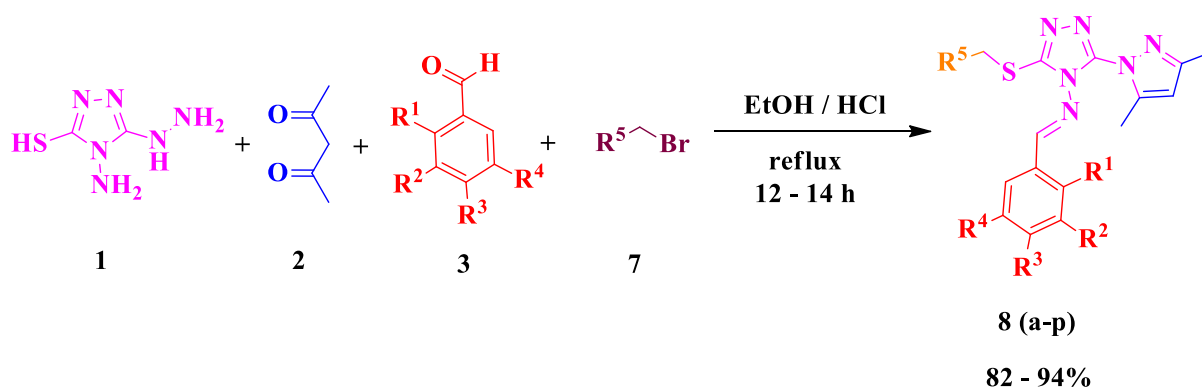
Scheme-2: Synthesis of various aralkyl/alkyl thio-3,5-dimethyl-1*H*-pyrazolyl-4*H*-1,2,4-triazol-4-amines (**6a-m**).

In summary, a facile and simple procedure for the synthesis of various aralkyl/alkyl thio-3,5-dimethyl-1*H*-pyrazolyl-4*H*-1,2,4-triazol-4-amines (**6a-m**) has been achieved via a multi-component approach using readily available chemicals. The attractive and notable features of this approach are that good yields, neat reaction conditions, easy product purification, metal-free, atom economy, and avoiding toxic catalyst.

PART-C: Four-component, one-pot synthesis of (*E*)-*N*-benzylidene-3-(benzylthio)-5-(3,5-dimethyl-1*H*-pyrazol-1-yl)-4*H*-1,2,4-triazol-4-amines and their DNA binding and molecular docking studies.

The target compounds **8a-p** were synthesized by the reaction of 4-amino-5-hydrazinyl-4*H*-1,2,4-triazole-3-thiol (**1**) with pentane-2,4-dione (**2**), substituted aromatic aldehydes (**3**), and aralkyl/allyl/propargyl bromides (**7**) via a one-pot, four-component condensation as shown in the **Scheme-3**. All the synthesized compounds were well characterized by analytical and spectral (IR, ¹H-NMR, ¹³C-NMR, and Mass) data.

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<u>Entry</u>	<u>Compound</u>	<u>R¹</u>	<u>R²</u>	<u>R³</u>	<u>R⁴</u>	<u>R⁵</u>
30	8a	H	H	OH	H	Benzyl
31	8b	OH	OC ₂ H ₅	H	H	Benzyl
32	8c	H	OCH ₃	OCH ₃	OCH ₃	4-Nitro benzyl
33	8d	H	NO ₂	H	H	4-Nitro benzyl
34	8e	H	H	Cl	H	Benzyl
35	8f	H	H	Cl	H	4-Nitro benzyl
36	8g	H	NO ₂	H	H	Benzyl
37	8h	H	H	OCH ₃	H	4-Nitro benzyl
38	8i	H	F	F	F	Propargyl
39	8j	OCH ₃	OCH ₃	H	H	4-Nitro benzyl
40	8k	OH	H	H	NO ₂	Propargyl
41	8l	OH	OCH ₃	H	NO ₂	Propargyl
42	8m	OH	H	H	NO ₂	Benzyl
43	8n	OH	OCH ₃	H	NO ₂	Benzyl

44	8o	H	H	OCH ₃	H	Allyl
45	8p	H	H	CH ₃	H	Allyl

Scheme-3: A one-pot, four-component synthesis of (*E*)-*N*-benzylidene-3-(benzylthio)-5-(3,5-dimethyl-1*H*-pyrazol-1-yl)-4*H*-1,2,4-triazol-4-amines (**8a-p**).

In conclusion, (*E*)-*N*-benzylidene-3-(benzylthio)-5-(3,5-dimethyl-1*H*-pyrazol-1-yl)-4*H*-1,2,4-triazol-4-amines (**8a-p**) were synthesized in good yields via a one-pot four-component reaction by readily available starting materials, and no column chromatography is needed for the purification of the products. This method is operationally simple. Further, DNA binding studies were also carried out by using UV-Visible and Fluorescence experiments. Furthermore, molecular docking studies were confirmed the binding interactions with the EGFR receptor.

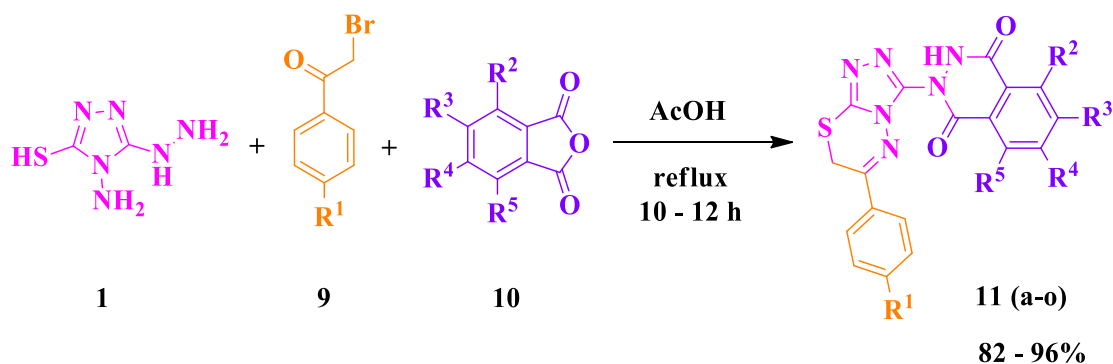
CHAPTER-III: One-pot, multi-component synthesis of substituted 2-(6-phenyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)-2,3 dihydrophthalazine-1,4-diones.

This chapter describes brief literature on the synthesis and biological applications of dihydro-1,4-phthalazinediones and 1,3,4-thiadiazines.

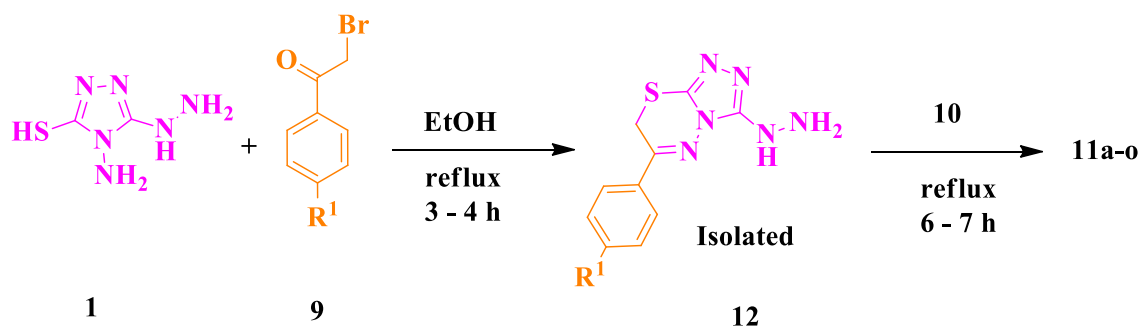
To synthesize the title compounds initially, we have screened the reaction conditions using compound **1**, 2-bromo-1-(4-methoxyphenyl)ethanone (**9**), and isobenzofuran-1,3-dione (phthalic anhydride) (**10**) as starting materials. The reaction was initially conducted in the water under reflux condition and no product was observed even after 10 hours. When the same reaction was operated in methanol only a 10% yield of the product was observed. On the other hand, when the same reaction was carried out in ethanol under reflux condition the yield of the product was 20%. Finally, when the reaction was operated in the acetic acid (AcOH) under reflux condition gave the desired compound **11a** in 86% yield. Hence, the 2-(6-phenyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)-2,3-dihydrophthalazine-1,4-diones (**11a-o**) were synthesized in acetic acid under reflux condition (**Method I, Scheme-4**). All the synthesized compounds were well characterized by analytical and spectral (IR, ¹H-NMR, ¹³C-NMR, and Mass) data.

SUMMARY

Method I:



Method II: Alternative route for the synthesis of **11a-o**



<u>Entry</u>	<u>Product</u>	<u>R¹</u>	<u>R²</u>	<u>R³</u>	<u>R⁴</u>	<u>R⁵</u>
46	11a	OCH ₃	H	H	H	H
47	11b	CH ₃	H	H	H	H
48	11c	NO ₂	H	H	H	H
49	11d	Br	H	H	H	H
50	11e	Cl	H	H	H	H
51	11f	F	H	H	H	H
52	11g	CH ₃	H	NO ₂	H	H
53	11h	F	H	NO ₂	H	H

SUMMARY

54	11i	H	Br	Br	Br	Br
55	11j	OCH ₃	Br	Br	Br	Br
56	11k	CH ₃	Br	Br	Br	Br
57	11l	F	Br	Br	Br	Br
58	11m	Cl	Br	Br	Br	Br
59	11n	Br	Br	Br	Br	Br
60	11o	H	H	H	H	H

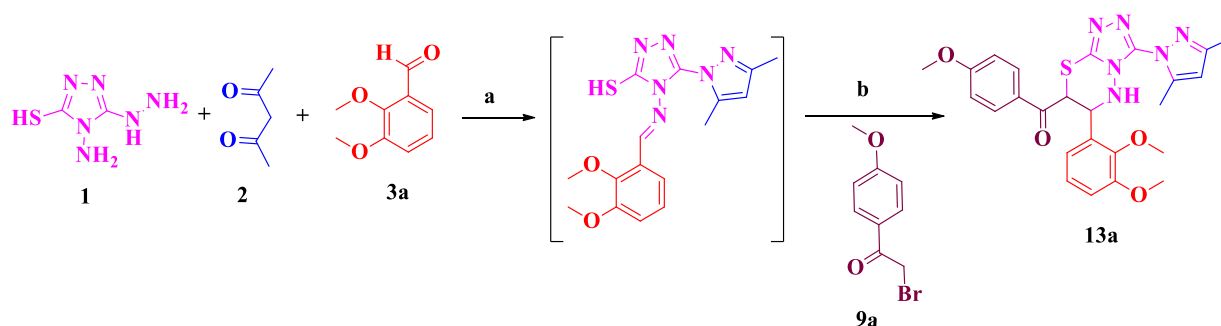
Scheme-4: Synthesis of 2-(6-phenyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)-2,3-dihydrophthalazine-1,4-dione (**11a-o**) derivatives.

The target compounds **11** can also be synthesized through an alternative method involving condensation of 4-amino-5-hydrazino-4*H*-[1,2,4]triazole-3-thiol (**1**) with substituted 2-bromo-1-phenylethanone (**9**) to produce the respective intermediates 3-hydrazinyl-6-phenyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines. These intermediates were subsequently converted into final products by condensation with phthalic anhydrides (**10**). The products obtained by **Method I** and **Method II** were the same (by mixed melting points and co-TLC). In the present investigation, **Method-I** was preferred over **Method II**, because of the high yields of the products in **Method-I**.

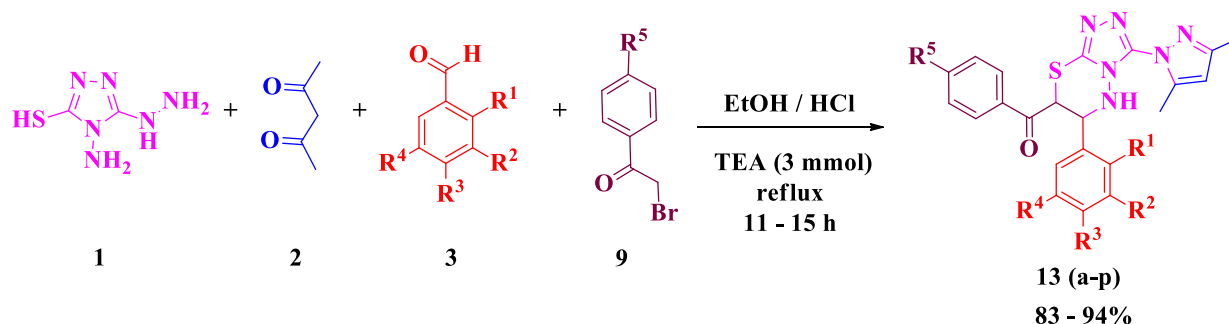
In conclusion, we have synthesized 2-(6-phenyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)-2,3-dihydrophthalazine-1,4-diones **11a-o** via a one-pot, multi-component reaction using readily available starting materials. This approach is very simple to operate, it involves clean and simple reaction conditions with high yields. The advantage of this method is that the reaction was carried out without using metals and toxic catalysts and the products were obtained in a single step with the high atom-economy.

CHAPTER-IV: Discovery of [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-7-yl(phenyl)methanone derivatives with promising anti-coronavirus and anti-tumoral activity.

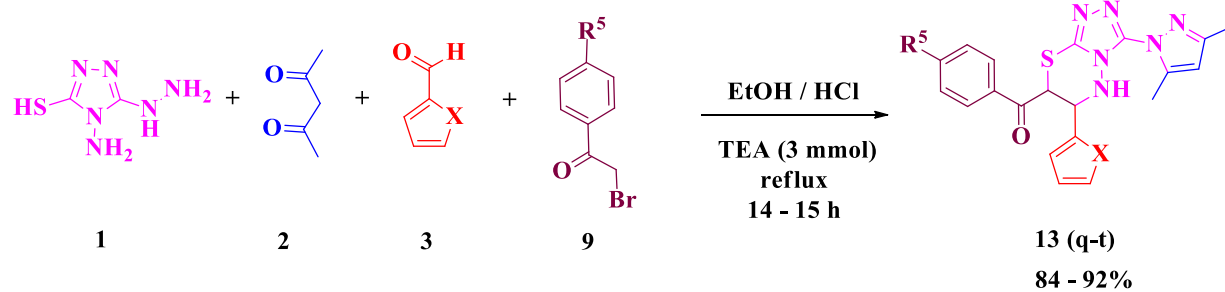
The synthesis of the [1,2,4]triazolo[3,4-*b*][1,3,4]dihydrothiadiazin-7-yl-(phenyl)methanone derivatives was carried out in a one-pot procedure. To optimize the chemistry, a model reaction using 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole (**1**), acetylacetone (**2**), 2,3-dimethoxybenzaldehyde (**3a**), and 4-methoxyphenacyl bromide (**9a**) as starting materials was performed. The first step of the reaction was carried out in ethanol at reflux temperature, in the presence of a catalytic amount of HCl yielding the intermediate 5-(3,5-dimethyl-1*H*-pyrazol-1-yl)-4-((4-methoxybenzylidene)amino)-4*H*-1,2,4-triazole-3-thiol. The intermediate was converted into the final product upon reaction with 4-methoxyphenacyl bromide.



Model reaction. **Reaction conditions:** a) **1** (1 mmol), **2** (1 mmol), **3a** (1 mmol), EtOH, HCl ; b) **9a** (1 mmol), TEA (3 mmol), EtOH, reflux.



SUMMARY



<u>Entry</u>	<u>Product</u>	<u>R¹</u>	<u>R²</u>	<u>R³</u>	<u>R⁴</u>	<u>R⁵</u>	<u>X</u>
61	13a	OCH ₃	OCH ₃	H	H	OCH ₃	—
62	13b	H	H	CH ₃	H	CH ₃	—
63	13c	H	H	NO ₂	H	NO ₂	—
64	13d	H	OCH ₃	H	H	Cl	—
65	13e	Br	H	H	H	F	—
66	13f	Br	H	H	H	CH ₃	—
67	13g	H	H	Cl	H	Br	—
68	13h	H	H	Cl	H	CH ₃	—
69	13i	H	OCH ₃	OH	OCH ₃	H	—
70	13j	H	F	F	F	CH ₃	—
71	13k	H	F	F	F	H	—
72	13l	H	OCH ₃	OCH ₃	OCH ₃	F	—
73	13m	H	OCH ₃	OCH ₃	OCH ₃	H	—
74	13n	Cl	H	H	H	OCH ₃	—

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75	13o	OCH ₃	H	OCH ₃	H	OCH ₃	—
76	13p	OCH ₃	H	OCH ₃	H	NO ₂	—
77	13q	—	—	—	—	OCH ₃	O
78	13r	—	—	—	—	CH ₃	O
79	13s	—	—	—	—	NO ₂	S
80	13t	—	—	—	—	NO ₂	O

Scheme-5: One-pot, four-component synthesis of pyrazolyl-dihydro[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine derivatives (**13a-t**).

Using this methodology, a series of compounds were prepared with various substituted benzaldehydes and phenacyl bromides. This approach is simple and affords the desired products in yields ranging from 83 to 94% (**Scheme-5**). All the synthesized compounds were well characterized by analytical and spectral (IR, ¹H-NMR, ¹³C-NMR, and Mass) data. The compound **13h** structure was further confirmed by single-crystal X-ray analysis (**Fig. 1**).

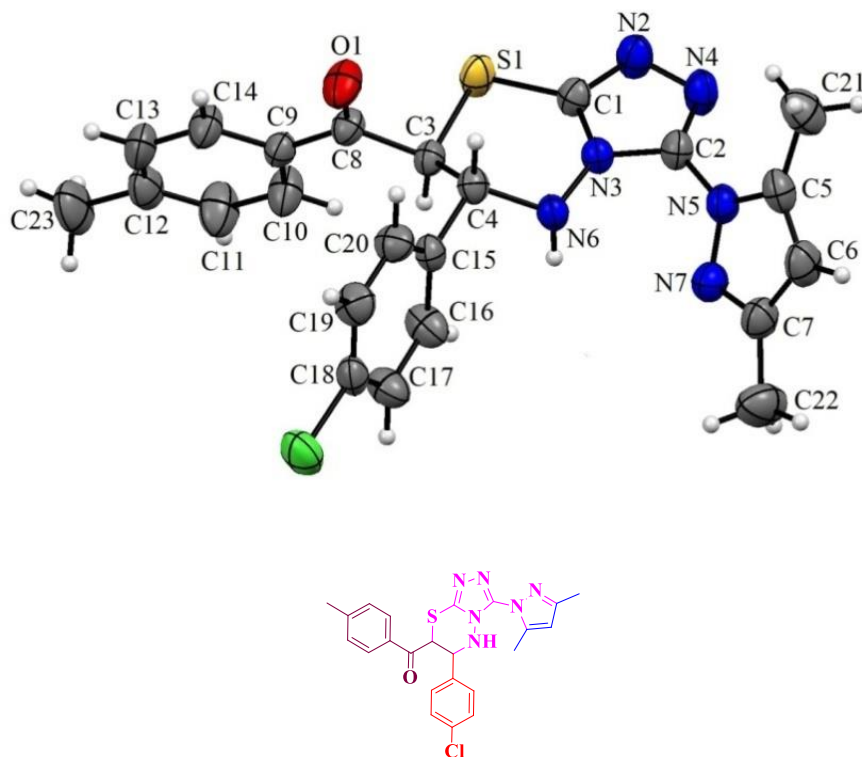


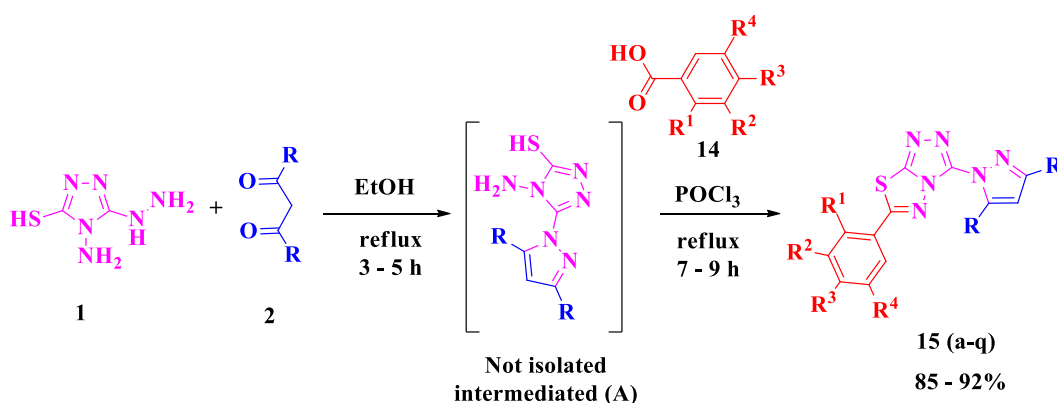
Fig. 1: ORTEP representation of compound **13h**.

SUMMARY

In summary, the synthesis of a series of [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-7-yl)(phenyl)methanone derivatives was carried out in excellent yields *via* a one-pot, four-component method using readily available starting materials. The reactions proceeded with high atom economy, leading to the formation of three C–N, one C–C, and one C–S bonds in a single operation, giving multi-annulated products. Some of the compounds showed the anti-viral activity against the human coronavirus 229E (hCoV229E) in HEL cells. The compounds **13b** and **13f** display promising activity with EC₅₀ values of 4.7 and 3.2 μ M, respectively. From anti-tumoral screening, it was observed that two derivatives (compounds **13j** and **13p**) showed low μ M activity against the different cell lines.

CHAPTER-V: An efficient one-pot synthesis of 6-phenyl-3-(1*H*-pyrazol-1-yl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole derivatives and their antimicrobial evaluation and molecular docking studies.

In this chapter pyrazolyl[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole derivatives (**15a-q**) were synthesized using readily available starting compounds. Compound **1**, acetylacetone/dibenzoyl methane (**2**), and substituted benzoic acids (**14**) *via* a one-pot, sequential addition of three-component reaction. The yields of the products were 85-92% (**Scheme-6**). All the synthesized compounds were well characterized by analytical and spectral (IR, ¹H-NMR, ¹³C-NMR, and Mass) data.



SUMMARY

<u>Entry</u>	<u>Compound</u>	<u>R</u>	<u>R¹</u>	<u>R²</u>	<u>R³</u>	<u>R⁴</u>
81	15a	CH ₃	H	H	H	H
82	15b	CH ₃	H	H	CH ₃	H
83	15c	CH ₃	H	H	F	H
84	15d	CH ₃	H	NO ₂	H	H
85	15e	CH ₃	Cl	H	H	H
86	15f	CH ₃	Br	H	H	H
87	15g	CH ₃	H	H	Cl	H
88	15h	CH ₃	H	H	I	H
89	15i	CH ₃	H	H	NO ₂	H
90	15j	CH ₃	H	NO ₂	H	NO ₂
91	15k	Ph	H	H	NH ₂	H
92	15l	Ph	H	H	Cl	H
93	15m	Ph	H	H	I	H
94	15n	Ph	H	H	CH ₃	H
95	15o	Ph	H	H	NO ₂	H
96	15p	Ph	H	NO ₂	H	NO ₂
97	15q	Ph	Cl	H	H	H

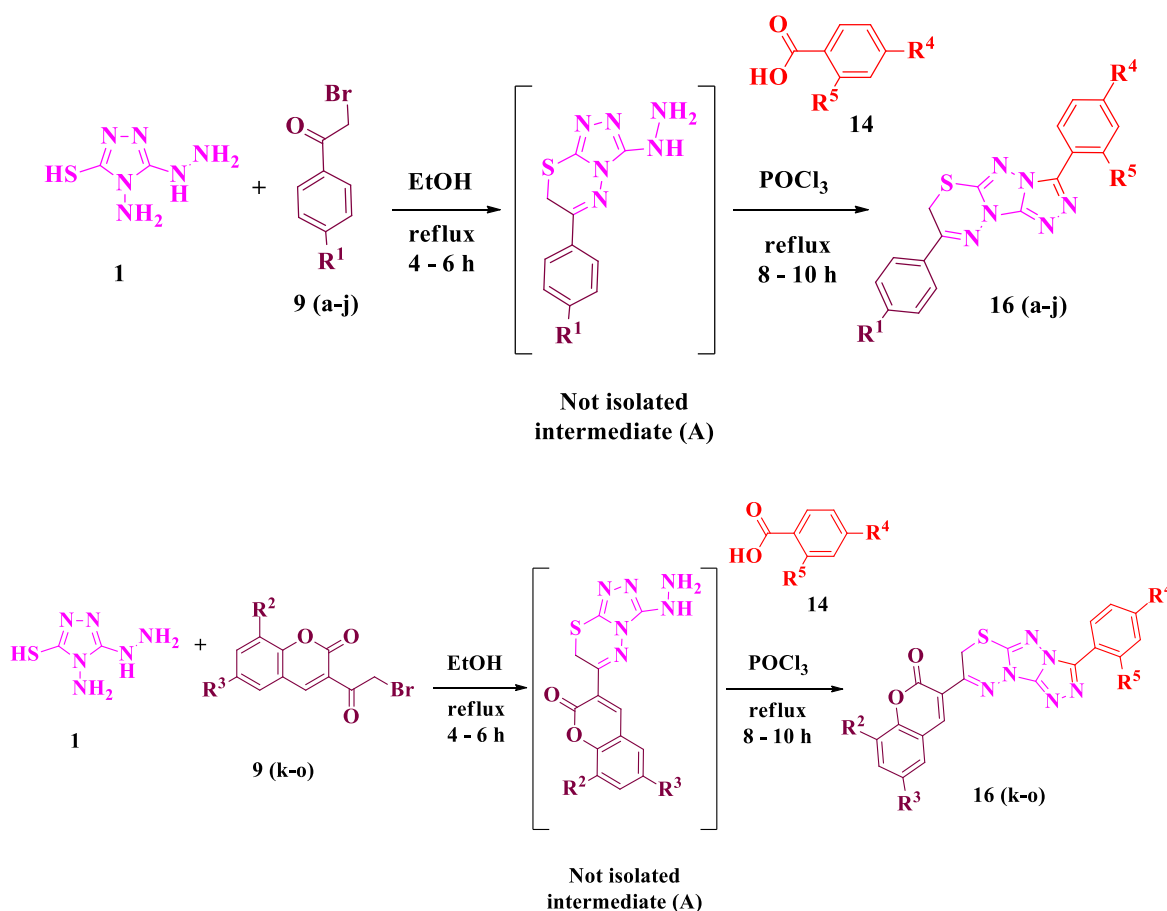
Scheme 6: One-pot, three-component synthesis of title derivatives **15a-q**.

In gist, we have synthesized new pyrazolyl[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole derivatives (**15a-q**) through a one-pot sequential addition of three-component reaction with good yields (85-92%). Further, all the synthesized compounds were evaluated for their antibacterial

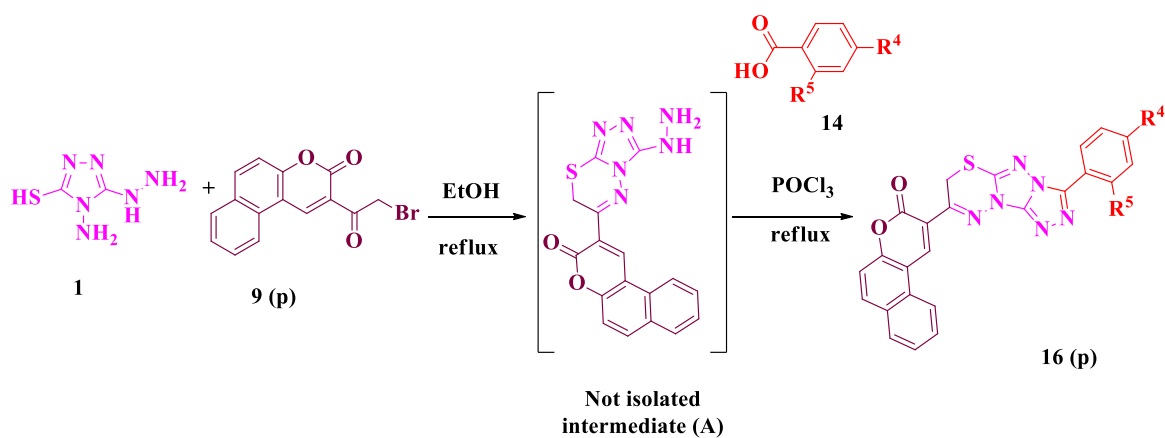
activity against Gram-positive and Gram-negative bacteria. Among these **15a**, **15e**, **15f**, **15g**, **15j**, and **15p** gave good results against Gram-positive and Gram-negative bacteria.

CHAPTER-VI: One-pot, three component synthesis of new 7H-[1,2,4]triazolo[4',3':1,5][1,2,4]triazolo[3,4-b][1,3,4]thiadiazines.

In the present chapter, we have synthesized the 7H-[1,2,4]triazolo[4',3':1,5][1,2,4]triazolo[3,4-b][1,3,4]thiadiazine derivatives (**16a-p**) via a one-pot, three-component reaction using compound **1**, various aromatic bromoethanones (**9**) and substituted benzoic acids (**14**) in ethanol and POCl₃ with good yields (**Scheme-7**). All the synthesized compounds were well characterized by analytical and spectral (IR, ¹H-NMR, ¹³C-NMR, and Mass) data.



SUMMARY



<u>Entry</u>	<u>Product</u>	<u>R¹</u>	<u>R²</u>	<u>R³</u>	<u>R⁴</u>	<u>R⁵</u>
98	16a	H	—	—	CH ₃	H
99	16b	CH ₃	—	—	CH ₃	H
100	16c	OCH ₃	—	—	CH ₃	H
101	16d	F	—	—	CH ₃	H
102	16e	Cl	—	—	CH ₃	H
103	16f	Br	—	—	CH ₃	H
104	16g	NO ₂	—	—	CH ₃	H
105	16h	Ph	—	—	CH ₃	H
106	16i	OCH ₃	—	—	F	H
107	16j	Br	—	—	H	Br
108	16k	—	H	H	CH ₃	H
109	16l	—	OCH ₃	H	CH ₃	H
110	16m	—	H	Cl	CH ₃	H
111	16n	—	H	Br	CH ₃	H

SUMMARY

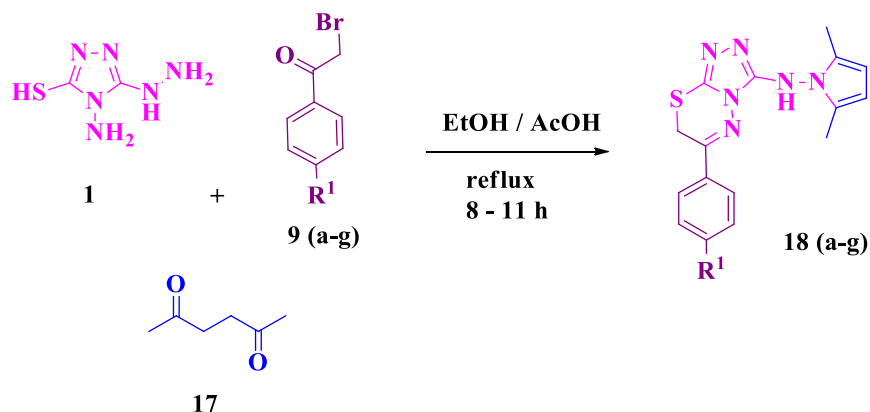
112	16o	—	Br	Br	CH ₃	H
113	16p	—	—	—	CH ₃	H

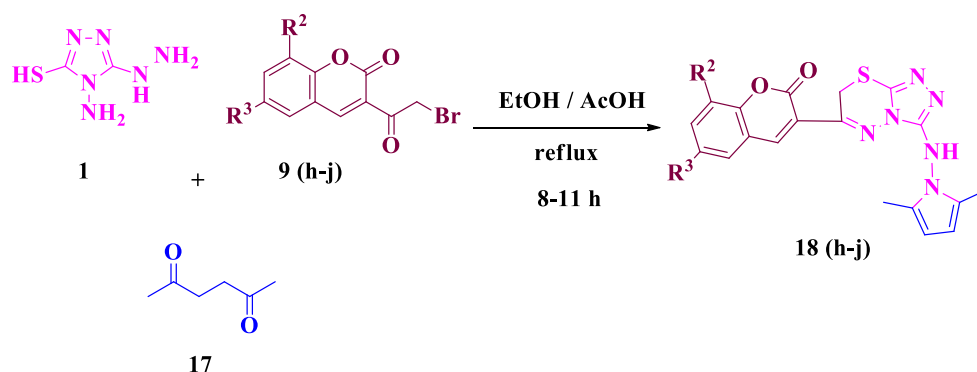
Scheme-7: One-pot, three-component synthesis of 7*H*-[1,2,4]triazolo[4',3':1,5] [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine derivatives (**16a-j**, **16k-o** and **16p**).

In summary, we have synthesized novel 7*H*-[1,2,4]triazolo[4',3':1,5][1,2,4] triazolo[3,4-*b*][1,3,4]thiadiazine derivatives (**16a-j**, **16k-o** and **16p**) through a one-pot multi-component reaction in good yields. In the present investigation, we have developed a methodology for the synthesis of fused heterocyclic compounds.

CHAPTER-VII: An efficient one-pot three component synthesis of *N*-(2,5-dimethyl-1*H*-pyrrol-1-yl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-amine derivatives.

In view of the importance of triazolothiadiazines and pyrroles in various fields such as therapeutic, medicinal, and biological applications, we became interested to synthesize the title compounds with triazolothiadiazine and pyrrole rings in a single heterocyclic scaffold by a one-pot synthesis. There are many procedures reported in the literature for the synthesis of pyrroles and triazolothiadiazine rings. But no report is available to synthesize the title compounds by the MCR process. The present investigation involves the reaction between 4-amino-5-hydrazinyl-4*H*-1,2,4-triazole-3-thiol (**1**), substituted phenacyl bromides or 3-(2-bromoacetyl)-2*H*-chromen-2-ones (**9**) and hexane-2,5-dione (**17**) in a mixture of ethanol and acetic acid to give final products **18a-j** in excellent yields (**Scheme-8**). All the synthesized compounds were well characterized by analytical and spectral (IR, ¹H-NMR, ¹³C-NMR, and Mass) data.





<u>Entry</u>	<u>Compound</u>	<u>R¹</u>	<u>R²</u>	<u>R³</u>
114	18a	H	—	—
115	18b	CH ₃	—	—
116	18c	OCH ₃	—	—
117	18d	F	—	—
118	18e	Cl	—	—
119	18f	Br	—	—
120	18g	NO ₂	—	—
121	18h	—	H	H
122	18i	—	OCH ₃	H
123	18j	—	H	Cl

Scheme-8: One-pot synthesis of *N*-(2,5-dimethyl-1*H*-pyrrol-1-yl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-amine derivatives (**18a-j**).

In conclusion, we have synthesized **18a-j** by a one-pot multi-component reaction strategy. The reaction protocol is very easy to operate, simple reaction conditions, without application of metals, with high atom economy.

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List of International and National Conferences and Workshops Participated

1. **P. C. Jilloju** and R. R. Vedula* presented a paper entitled *“One-pot, Multi-component Synthesis of Substituted 2-(6-phenyl-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)-2,3-dihydrophthalazine-1,4-diones and their Molecular Docking Studies”*, International Conference on “Advances in Chemical Sciences and Technologies” (**ACST-2019**), 23rd to 25th September-2019, Department of Chemistry, NIT Warangal, Telangana.
2. **P. C. Jilloju** and R. R. Vedula*, presented a paper entitled *“A Facile One-Pot Three-Component Synthesis of Benzylideneamino-3,5-dimethyl-1H-Pyrazoles”*, International Conference on “Chemistry For Sustainable Future” (**CFSF-2018**), 7th to 9th August-2018, Palamuru University, Mahabubnagar, Telangana.
3. **P. C. Jilloju** and R. R. Vedula* presented a paper entitled *“One-pot, Multi-Component Cascade Reaction for the Synthesis of Various Aralkyl/Alkyl Thio-3,5-dimethyl-1H-pyrazolyl-4H-1,2,4-triazol-4-amine and their Docking Studies”*, Two day National Conference on “Recent Trends in Pharmaceutical Sciences and Research” (**RTPSR-2019**), 23rd and 24th November-2019, Hanamkonda, Warangal, Telangana.
4. **P. C. Jilloju** and R. R. Vedula* presented a paper entitled *“One-pot, Multi-Component Cascade Reaction for the Synthesis of Various Aralkyl/Alkyl Thio-3,5-dimethyl-1H-pyrazolyl-4H-1,2,4-triazol-4-amine and their Docking Studies”*, Two day National Seminar on “Advances in Chemical Research” (**ACR-19**), 29th and 30th March-2019, Kakatiya University, Warangal, Telangana.
5. Participated in Medicinal Chemistry Conference cum Workshop (**MED CHEM-2015**) on “Anti-diabetic Drug Discovery and Development”, 29th to 30th October-2015, IIT Madras, Chennai.
6. Attended an International Conference on “Technology for Education”, T4E-2015 7th IEEE, NIT Warangal.
7. Participated in National Conference on “Frontiers in Chemical Science and Technology” (FCST-2016), NIT Warangal.
8. Participated in National Conference on “Recent Advances in Organic Synthesis” (RAOS-2016), NIT Warangal.
9. Participated in National Symposium "Recent Advances in Chemical and Material Science" (RACMS-2016), Rajiv Gandhi University of Knowledge Technologies-Basar.

CONFERENCES AND WORKSHOPS ATTENDED

10. Attended a National Conference on “Recent Development in Chemical Sciences and Allied Technologies” (RDCSAT-2017), NIT Warangal.
11. Six-day workshop on “Teaching and Learning of Modern Methods of Organic Chemistry through Hands-On Experience” 24th – 29th Feb 2020 at Teaching and Learning Centre (TLC), National Institute of Technology, Warangal.
12. Six-day Workshop on “Instructional Strategies in Physical Methods of Chemical Analysis with Hands-on Experiments”, 2nd -7th March 2020 at Teaching and Learning Centre (TLC), National Institute of Technology, Warangal.

LIST OF PUBLICATIONS

1. **Parameshwara Chary Jilloju**, **R. R. Vedula***, “A facile one-pot three-component synthesis of benzylideneamino-3,5-dimethyl-1*H*-pyrazoles”, *Synthetic Communications*, **2018**, *48*, 1739-1746.
2. **Parameshwara Chary Jilloju**, A. Vinaykumar, P. Shyam, **R. R. Vedula***, “One-pot, Multicomponent Cascade Reaction for the Synthesis of Various Aralkyl/alkylthio-3,5-dimethyl-1*H*-pyrazolyl-4*H*-1,2,4-triazol-4-amine and Their Docking Studies”, *Journal of Heterocyclic Chemistry*, **2019**, *56*, 1012-1019.
3. **Parameshwara Chary Jilloju**, M. Srikanth, S. V. Kumar, **R. R. Vedula***, “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”, *Polycyclic Aromatic Compounds*, **2019**, DOI: <https://doi.org/10.1080/10406638.2019.1709212>.
4. **Parameshwara Chary Jilloju**, P. Shyam, A. Sanjeev, **R. R. Vedula***, “Four-component, one-pot synthesis of (*E*)-*N*-benzylidene-3-(benzylthio)-5-(3,5-dimethyl-1*H*-pyrazol-1-yl)-4*H*-1,2,4-triazol-4-amines and their DNA binding and molecular docking studies”, *Journal of Molecular Structure*, **2021**, *1225*, 1291420.
5. **Parameshwara Chary Jilloju**, P. Shyam, C. Raju, **R. R. Vedula***, “One-pot synthesis, characterization, antimicrobial evaluation and molecular docking studies of 6-phenyl-3-(1*H*-pyrazol-1-yl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole derivatives”, *Polycyclic Aromatic Compounds*, **2021**, DOI: <https://doi.org/10.1080/10406638.2021.1886127>.
6. M. Srikanth, S. R. Peddi, R. K. Aravilli, **Parameshwara Chary Jilloju**, V. Manga, **R. R. Vedula***, “Microwave irradiated one-pot, three-component synthesis of a new series of hybrid coumarin based thiazoles: Antibacterial evaluation and molecular docking studies”, *Journal of Molecular Structure*, **2021**, *1225*, 129114.

A facile one-pot three-component synthesis of benzylideneamino-3,5-dimethyl-1H-pyrazoles

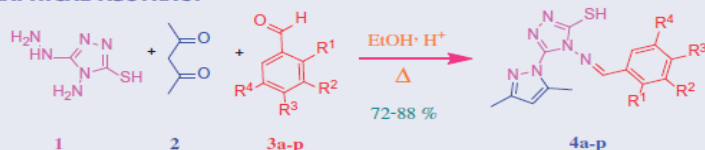
Parameshwara Chary Jilloju and Rajeswar Rao Vedula

Department of Chemistry, National Institute of Technology, Warangal, India

ABSTRACT

A facile, one-pot three-component protocol for the synthesis of substituted 4-(benzylideneamino)-5-(3,5-dimethyl-1H-pyrazol-1-yl)-4H-1,2,4-triazole-3-thiols have been reported by the reaction of 4-amino-5-hydrazinyl-4H-1,2,4-triazole-3-thiol with acetyl acetone and various substituted benzaldehydes via multi component reaction. The newly synthesized derivatives were characterized by their elemental analysis and spectral data. The current strategy provides heterocyclic compounds in good yields with broad substrate scope.

GRAPHICAL ABSTRACT



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4-Amino-5-hydrazinyl-4H-1,2,4-triazole-3-thiol; acetyl acetone; 3,5-dimethyl-1H-pyrazole; three-component

Introduction

Multi-component reactions (MCRs) are convergent reactions, in which three or more starting materials react to form a complex product, where basically all or most of the atoms contribute to the newly formed single product. In an MCR, a product is assembled according to a cascade of elementary chemical reactions. Multi-component reactions play an insignia part in organic chemistry because they produce maximum yield of product and selectivity, at the same time produce very less by-products compared to stepwise synthesis. Additionally, multi-component reactions are operationally simple, less expensive, rapid, economical and involve easy experimental procedures.^[1]

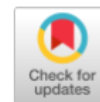
Triazole and its hetero cyclic compounds exhibits many biological activities,^[2] such as antimicrobial, anti-HIV, CNS-stimulatory, antiviral, analgesic and antifungal activities.^[3–6] Likewise, there are known drugs consisting the triazole ring, e.g., Triazolam, Alprazolam, Etizolam, and Furacylin.^[7–10] Pyrazoles are hetero cyclic compounds with two nitrogen

CONTACT Rajeswar Rao Vedula ✉ vrajesw@yahoo.com Department of chemistry, NIT Warangal, 506 004 Warangal, Telangana, India.

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Month 2019 One-pot, Multicomponent Cascade Reaction for the Synthesis of Various Aralkyl/alkylthio-3,5-dimethyl-1*H*-pyrazolyl-4*H*-1,2,4-triazol-4-amine and Their Docking Studies

Parameshwara Chary Jilloju,^a Allam Vinaykumar,^c Perugu Shyam,^b and Rajeswar Rao Vedula^{a*}

^aDepartment of Chemistry, National Institute of Technology, Warangal, Telangana 506004, India

^bDepartment of Biotechnology, National Institute of Technology, Warangal, Telangana 506004, India

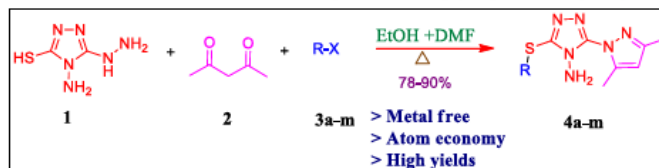
^cIndian Institute of Chemical Technology, Hyderabad, Telangana 500007, India

*E-mail: vragesw@yahoo.com

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A facile and simple one-pot procedure for the synthesis of various aralkyl/alkylthio-3,5-dimethyl-1*H*-pyrazolyl-4*H*-1,2,4-triazol-4-amine has been described *via* a multicomponent reaction of 4-amino-5-hydrazinyl-4*H*-1,2,4-triazole-3-thiol, acetylacetone, and various aryl/alkyl halides in good yields. All the newly synthesized compounds were characterized by using analytical and spectral studies. Our *in silico* studies confirmed that 4e, 4f, 4g, and 4j have the best inhibition activity among the synthesized compounds with a high selective index against the Tubulin protein and showed best interactions with receptor structure. The present study provides a novel series of compounds with a promising inhibitor to prevent on Tubulin protein.

J. Heterocyclic Chem., 00, 00 (2019).

INTRODUCTION

Chemical reactions that use three or more different starting materials as chemical structures and yields the final product in a one-pot procedure are usually called as multicomponent reactions. In multi component reaction (MCR), a product is accomplished according to a cascade of elementary chemical reactions. MCRs play an insignia part in organic chemistry because they produce the maximum yield of product and selectivity. They produce very less by-products compared with stepwise synthesis. Additionally, MCRs are operationally simple, cost-effective, rapid, and involve easy experimental procedures [1].

Triazole heterocyclic compounds is having a wide range of biological activities [2], like antifungal, CNS-stimulatory, antimicrobial, anticancer, antiviral, anti-HIV, and analgesic activities [3–6]. Likewise, there are known drugs consisting the triazole core ring, for example, etizolam, triazolam, flurazepam, and alprazolam [7–10]. Pyrazole, which is a five-membered heterocyclic compound consisting of two nitrogen atoms at adjacent positions, and synthesis of pyrazoles were reported using various procedures in the literature [11]. Pyrazole motifs have been exhibiting interesting biological activities such as anticancer, analgesic, antimicrobial, antidiabetic, anti-inflammatory, and immunosuppressive activity [12–17].

Organosulfur chemistry is one of the most useful and important branch in organic synthesis. Compounds

possessing C–S bonds are important in organic chemistry [18–21]. Thioethers are beneficial and important compounds in different branches such as materials, agriculture, industry, pharmaceutical, medicine, heterocyclic chemistry, and biological processes [22–30]. In the field of medicine, organosulfur compounds are broadly used for treatment of different types of diseases such as Alzheimer's, cancer, tuberculosis, and Parkinson [31–34].

RESULTS AND DISCUSSION

In view of the importance of triazoles, pyrazoles, and thioethers, we would like to incorporate all these units in single heterocyclic system so that the resulting molecule may exhibit good biological activity. In continuation of earlier work on MCR [35–37], in the present work, we are reporting the one-pot, multicomponent cascade reaction for the synthesis of various aralkyl/alkylthio-3,5-dimethyl-1*H*-pyrazolyl-4*H*-1,2,4-triazol-4-amine and their docking studies.

For the optimization of reaction conditions, the reaction was conducted with 4-amino-5-hydrazino-4*H*-[1,2,4] triazole-3-thiol (1), acetylacetone (2), and aralkyl/alkyl halides (3) in the water under reflux condition, and no product was formed. On the other hand, when the same reaction was carried out in a mixture of equal amount of



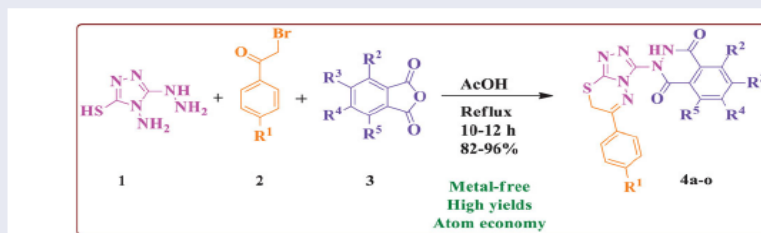
One-Pot, Multi-Component Synthesis of Substituted 2-(6-Phenyl-7H-[1,2,4]Triazolo[3,4-b][1,3,4]Thiadiazin-3-yl)-2,3-Dihydrophthalazine-1,4-Diones

Parameshwara Chary Jilloju^a, Mamidala Srikanth^a, Sthalam Vinay Kumar^b, and Rajeswar Rao Vedula^a

^aDepartment of Chemistry, National Institute of Technology, Warangal, India; ^bIndian Institute of Chemical Technology, Hyderabad, India

ABSTRACT

A series of 2-(6-phenyl-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)-2,3-dihydrophthalazine-1,4-diones (**4a–4o**) have been synthesized *via* a one-pot multi-component reaction. The reaction of 4-amino-5-hydrazineyl-4H-1,2,4-triazole-3-thiol (**1**), substituted 2-bromo-1-phenylethanone (**2**), and phthalic anhydride (**3**) in the presence of acetic acid under reflux conditions afforded the title compounds in excellent yields. All the synthesized compounds were fully characterized.



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Multi-component reaction; phthalazine; phthalic anhydride; thiadiazine; triazole

Introduction

Multi-component reactions (MCRs) are those reactions in which three or more reactants come together to form a new product with complex architecture in one vessel. MCRs are efficient and effective methods in the sustainable and diversity-oriented synthesis of heterocyclic compounds. MCRs are new methods for the one-pot synthesis of various new heterocyclic compounds and nowadays MCRs are widely used in the drug discovery also. There are many environmental benign protocols producing excellent yields in mild reaction conditions and require short reaction timings, operationally simple medium. Generally, these are easy to operate, very simple experimental procedure to carry out the reaction without by-products, rapid, and low cost reactions. These are also referred as multi-component assembly process (MCAP).¹

Nitrogen heterocycles are one of the important class in the organic compounds particularly, 1,2,4-triazoles and their derivatives are showing promising biological activities.^{2–4} Fused heterocyclic compounds related to 1,2,4-triazoles have many applications in various fields such as

CONTACT Rajeswar Rao Vedula ✉ rajeswarnitw@gmail.com Department of Chemistry, National Institute of Technology, Warangal, Telangana 506004, India.

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Four-component, one-pot synthesis of (E)-N-benzylidene-3-(benzylthio)-5-(3,5-dimethyl-1H-pyrazol-1-yl)-4H-1,2,4-triazol-4-amines and their DNA binding and molecular docking studies

Parameshwara Chary Jilloju^a, Perugu Shyam^b, Ananthula Sanjeev^c, Rajeswar Rao Vedula^{a,*}

^aDepartment of Chemistry, National Institute of Technology, Warangal, Telangana, 506004, India

^bDepartment of Biotechnology, National Institute of Technology, Warangal, Telangana, 506004, India

^cChemistry Department, Osmania University, Hyderabad, Telangana, 500007, India

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ABSTRACT

An efficient four-component strategy has been developed for the synthesis of a novel series of (E)-N-benzylidene-3-(benzylthio)-5-(3,5-dimethyl-1H-pyrazol-1-yl)-4H-1,2,4-triazol-4-amines in good yields. The method is effective for the synthesis of benzylidene aralkyl/alkyl/propargyl thio pyrazolyl-1,2,4-triazol-4-amines in a one-pot operation. This method is operationally simple, metal-free, and works under reflux conditions with diverse substrates. Among all the synthesized compounds **5a**, **b**, **c**, **h**, **j**, and **n** were shown good results in silico studies. **5n** is showing good results in both DNA binding and molecular docking studies.

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1. Introduction

In recent years multi-component reactions (MCRs) are growing interest and these are also referred as multi-component assembly process. In these three or more reactants assemble in a one-pot to generate complex molecules. In these process by-products formation is very less and produces maximum yields of products because all the reactants participate in the reaction. Most of the multi-component reactions produce pure products and there is no need of column chromatography purification. MCRs are eco-friendly, atom economy, involve short reaction time, selective and high yields of products are formed in a single vessel with minimum effort. Now a day's most of chemists prefer multi-component reactions for the synthesis of new drugs [1–2].

1,2,4-Triazoles, their fused heterocycle derivatives have been playing a remarkable role in the pharmaceutical industry. Most of the 1,2,4-triazole containing compounds are showing biological activities such as anti-tuberculosis [3], anti-inflammatory [4], anti-cancer [5], anti-microbial [6], anti-fungal [7], anti-oxidant [8], anti-HIV [9], anti-convulsant [10], anti-analgesic [11] and ant-malarial [12]. Some of the drugs having 1,2,4-triazole ring is core moiety

for example Fluconazole, Ribavirin, Flupoxam and Prothioconazole (Fig. 1).

Among heterocycles, pyrazoles are interesting skeletons. There are many literature procedures to synthesise the pyrazoles [13–16]. Compounds possessing pyrazole moiety play a vital role due to its pharmaceutical and biological activities such as anti-bacterial [17], anti-angiogenic [18], anti-proliferative [19], anti-cancer [20], anti-microbial [21], insecticidal [22], anti-viral [23], and anti-diabetic [24]. Few pyrazole-based potent biological active drugs were shown in the Fig. 2 [25].

Condensation of carbonyl compounds with primary amines produces Schiff bases. They have imine or an azomethine functional group [26–29]. In the formation of metal complexes Schiff bases act as ligands [30]. Compounds with Schiff base are exhibiting a wide range of applications such as biological, catalysts, dyes and stabilizers [31]. Many compounds having Schiff bases are reported to exhibit broad range of biological activities such as anti-viral [32], anti-bacterial [33], anti-oxidant [34], anti-proliferative [35], anti-microbial [36], anti-tuberculosis [37], herbicidal [38], insecticidal [39]. Compounds having thio-ether linkage are of important in organic chemistry, agrochemicals, pharmaceuticals and they possess good biological applications [40–44].

* Corresponding author.

E-mail address: rajeswarmitw@gmail.com (R.R. Vedula).

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Research Article

An Efficient One-Pot Synthesis of 6-Phenyl-3-(1*H*-Pyrazol-1-yl)-[1,2,4]Triazolo[3,4-*b*][1,3,4]Thiadiazole Derivatives and Their Antimicrobial Evaluation and Molecular Docking Studies

Parameshwara Chary Jilloju, Perugu Shyam, Chedupaka Raju &

Rajeswar Rao Vedula 

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Abstract

An efficient rapid synthesis of a new class of diversely functionalized 6-phenyl-3-(1*H*-pyrazol-1-yl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole derivatives (**4a-q**) is described via a facile one-pot, three-component cascade reaction with high yields. It is a multi-

functional cyclization reaction to form two new heterocycles. The structures of newly formed compounds were confirmed by using spectral and analytical studies. Simple

reaction conditions, the good isolated yield of the product, and no column chromatographic purification are attractive features of the present protocol. Further the newly synthesized compounds were screened for anti-microbial activity and molecular docking interactions.



BIO-DATA



Parameshwara Chary Jilloju,

Present: C/o. Prof. V. Rajeswar Rao, Department of Chemistry, National Institute of Technology Warangal, Telangana, India. PIN-506004, **Residence:** H.No: 2-40, Madgula Pally, Nalgonda, Telangana, India. PIN-508374.

+91-9959528376

charymmt@gmail.com

Languages knew: Telugu, English and Hindi; **Marital Status:** Married

PROFILE: DOB: 10.05.1989

Father's Name: Raghunadha Chary Jilloju

Mother's Name: Dhanalakshmi Jilloju

EDUCATION

+ Doctor of Philosophy, July 2015 – March 2021

Department of Chemistry, National Institute of Technology (NIT) Warangal, India

Specialization: Organic Synthesis

+ Master of Science, July 2010 – May 2012 (78%)

Kakatiya University Warangal, Telangana, India

Specialization: Organic Chemistry

+ Bachelor of Science, July 2007 – June 2010 (71%)

Osmania University, Hyderabad, Telangana, India

Subjects: Botany, Zoology, and Chemistry

+ Intermediate, June 2005 – April 2007 (88%)

Vaasavi Junior College, Miryalaguda, Telangana, India

Subjects: Biology, Physics, and Chemistry

+ SSC, June 2004 – April 2005 (82%)

Z.P.H.S. Madgula Pally, Telangana, India

Subjects: Mathematics, General Science, and Social Studies

ACADEMIC ACHIEVEMENTS

- ✓ Qualified in All India level CSIR-UGC-NET (LS) Examination–2012, 2014
- ✓ Qualified in All India level Graduate Aptitude Test in Engineering (GATE)–2013, 2015
- ✓ Qualified State Level Eligibility Test (SLET) for Assistant Professor/Lectureship Examination-2013
- ✓ Secured highest marks in 10th class at the School level.

RESEARCH EXPERIENCE

 **Doctoral Research Scholar: July 2015 – March 2021** Department of Chemistry, National Institute of Technology (NIT) Warangal, India

Thesis Title: “*MCR Approach for the synthesis of new heterocyclic compounds from 4-amino-5-hydrazinyl-4H-1,2,4-triazole-3-thiol*”.

TEACHING EXPERIENCE

- Assisted in the smooth conduction of Organic Chemistry Laboratory to the undergraduate and Post graduate students at NIT Warangal for five years.
- Assisted in guiding Post graduate student’s project during their final semester in the Department of Chemistry, NIT Warangal.