SYNTHESIS AND BIOLOGICAL ACTIVITY STUDIES OF NEW HETEROCYCLIC COMPOUNDS USING MULTICOMPONENT STRATEGIES

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BY

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CERTIFICATE

This is to certify that the research work presented in this thesis entitled "**Synthesis and Biological Activity Studies of New Heterocyclic Compounds using Multicomponent Strategies**" Submitted by Mr. Papisetti Venkatesham for the award of Degree of Doctor of Philosophy in Chemistry. National Institute of Technology, Warangal (Telangana) under my supervision and that the same has not been submitted elsewhere for any degree

VRRao 14-06-23

Date: 14-06-2023

Prof. V. Rajeswar Rao

Place: NIT Warangal

Thesis supervisor

DECLARATION

I hereby declare by that the work presented in this thesis entitled "**Synthesis and Biological Activity Studies of New Heterocyclic Compounds using Multicomponent Strategies**" is based entirely on the results of the investigations and research work carried out by me under the supervision of Prof. Vedula Rajeswar Rao, Department of Chemistry, National Institute of Technology, Warangal. I declare this work is original and has not been submitted in part or full, for any degree or diploma to this or any other University.

Date: 14-06-2023 (**Papisetti Venkatesham**)

Place: NIT Warangal

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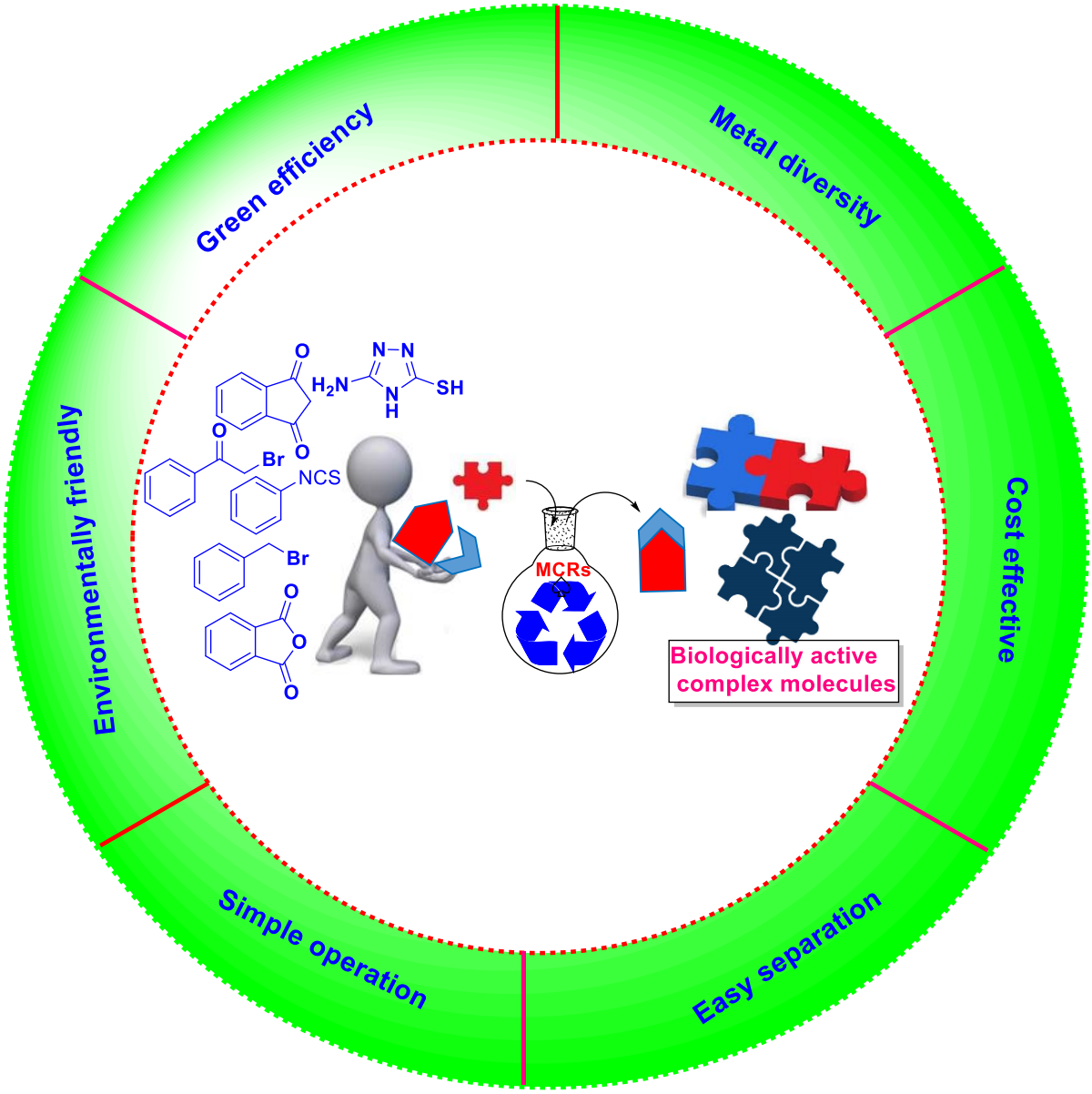
CHAPTER-I

A review on Multicomponent Reactions and its Applications in the Synthesis of Biologically Active Heterocyclic Compounds



1.1. Introduction

Multi-component reactions are known as a group of chemical transformations in which the rapid assembly of polysubstituted systems occurs without isolation of undesirable intermediates and most of the atoms participate in the newly formed bonds that lead to get target compound with high percentage of the yield in a single reaction step and shorter reaction time. The main aim of MCRs is for the development of heterocyclic compounds,[1] cycloaddition reactions,[2] natural products,[3] and generate a carbon-carbon bonds.[4] These reactions are classified as 3CC, 4CC, 5CC etc., Many of the MCRs products are used as a drugs in pharmaceutical industries and these compounds are biologically active. Due to this the MCRs have attained a crucial importance even in late 20th century. The factors such as solvent, temperature, catalyst, concentration, type of functional groups and starting material are particularly important for development and discovery of novel MCRs.

History of MCRs

The organo-chemical reactions and their products were documented over 4.6 million years ago. The Miller experiment has illustrated that the chemical compounds in nature are formed not only by the conventional chemical reaction of two components but also a variety of MCRs products are formed.[5] The Strecker three-component reaction is a first MCRs introduced in 1850. Examples for MCRs are 1. Laurent and Gerhardt reaction, 2. Hantzsch dihydropyridine synthesis, 3. Hantzsch pyrrole synthesis, 4. Radziszewski imidazole synthesis, 5. Biginelli dihydro pyrimidine synthesis,[6]. Gewald thiophene synthesis, 6. Assinger thiazole synthesis, 7. Bucherer-Bergs 4CC reaction, 7. Mannich reaction, 8. Passerini α-acyloxycarbamide synthesis, 9. Ugi 4CC reaction, 10. Groebke-Blackburn-Bienayme imidazole 3CC reaction.

Name reactions on MCRs.

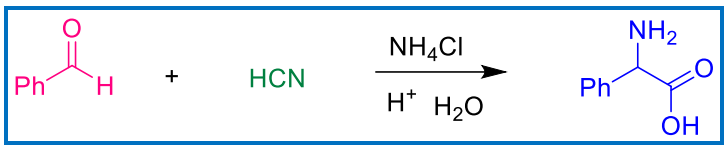
Laurent and Gerhardt reaction

In 1838 Laurent and Gerhardt[7] introduced the first MCR product of benzoyl azotide from the reaction of two equivalents of benzaldehyde and hydrogen cyanide, ammonia. In which 2-amino phenyl acetonitrile was first generated and then reaction with another equivalent of benzaldehyde to form a Schiff base products 1a.

Scheme-1.0

Strecker amino acid synthesis. [8]

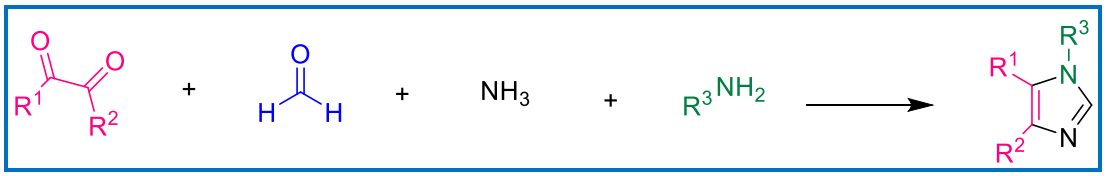
Formally in 1850 Strecker established a modern MCR for the synthesis of *α*–amino acids in which initially NH_4Cl dissociate to generate ammonia after aldehyde (aromatic or aliphatic) reaction with hydrogen cyanide, ammonia followed by hydrolysis to form a α-amino acid **Scheme-1.1**.



Radziszewski imidazole reaction. [9]

The four component condensation reaction of 1,2-diketones with ammonia, formalin and primary amine to afford substituted five membered Imidazoles heterocycles (Scheme-1.2)

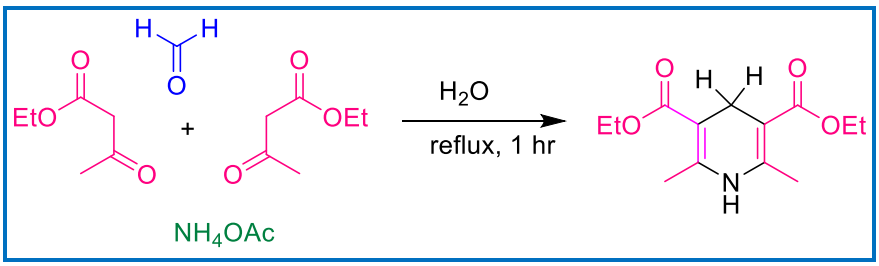
Scheme-1.2



Hantzsch dihydropyridine synthesis [10]

In 1881 the German chemist Arthur Rudolf Hantzsch established a cyclized product of dihydropyridine. In this reaction two equivalents of ethyl acetoacetate were condensed with aldehyde and ammonium acetate to form a dihydropyridine shown in Scheme-1.3

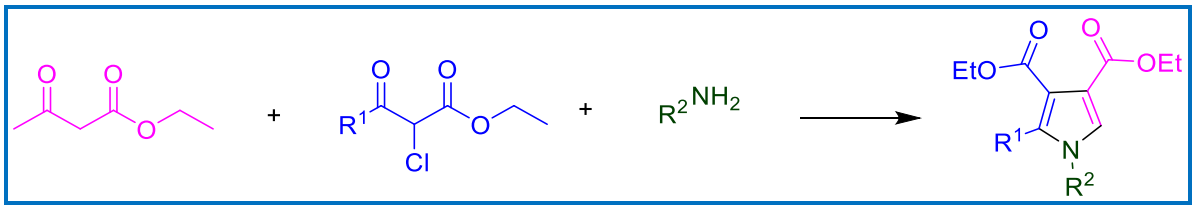
Scheme-1.3



Hantzsch pyrrole reaction. [11]

Arthur Rudolf Hantzsch developed synthesis of an aromatic nitrogen containing five-membered heterocyclic compound from the reaction of active methylene compound (*β*-ketoester) and *α*-halo ester with primary amine has shown in scheme-1.4

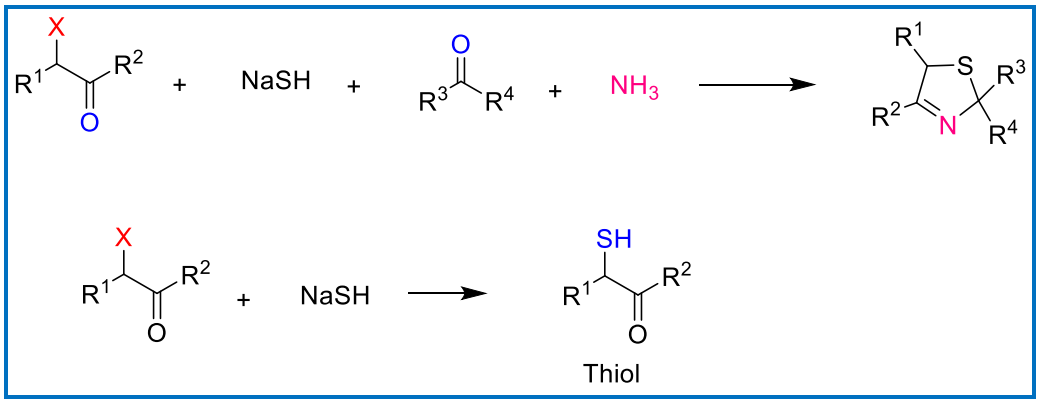
Scheme-1.4



Asinger reaction. [12]

In 1956 Friedrich Asinger reported a four component MCR of 3-thiazolines. In this reaction α-halogenated carbonyl compounds were reacted with NaSH (sodium hydrogensulphide) and insitu generated thiols, ammonia and another mole of carbonyl compound to form a thiazoline shown in scheme-1.5

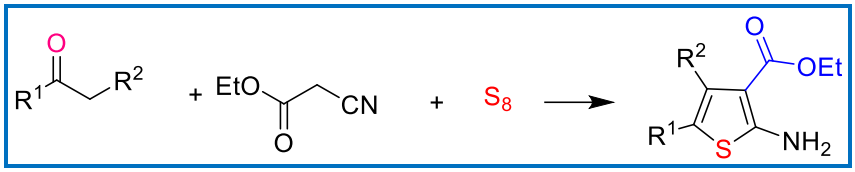
Scheme-1.5



Gewald thiophene synthesis. [13]

Karl Gewald *et al* reported a sulfur (S_8) involved three component condensation reaction. The reaction between α-methylene carbonyl compound and ethyl cyano acetate, elemental sulfur in presence of base to generate a five membered heterocyclic compound thiophene (scheme-1.6)

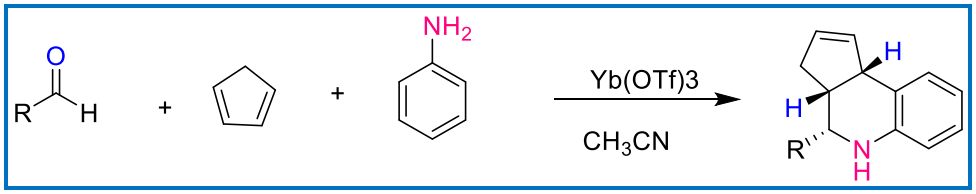
Scheme-1.6



Grieco 3CC reaction. [14]

Paul Grieco established a three component reaction in 1985. The reaction consists of condensation of aldehyde with aromatic amine and cyclopentadiene (electron rich alkene) to give a cyclised six membered product containing nitrogen atom in presence of Lewis acid or trifluoro acetic acid **scheme-1.7**

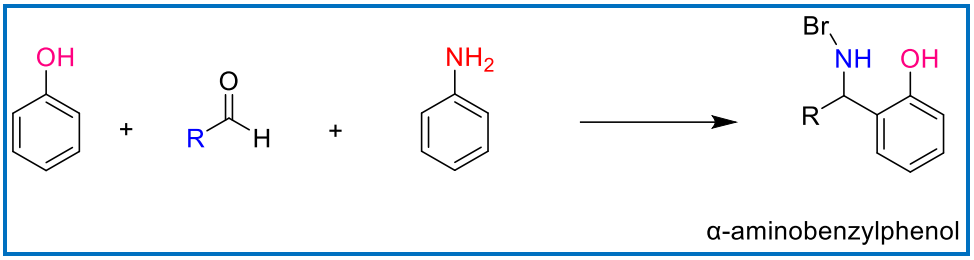
Scheme-1.7



Betti three component reaction. [15]

Mario Betti introduced a three component reaction in1900. In this reaction Phenol on reaction with aldehydes and aromatic primary amines (aniline) to afford a α-amino benzyl phenol. (Scheme-1.8)

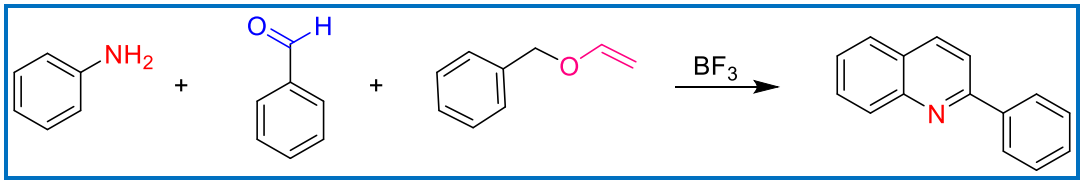
Scheme-1.8



Povarov quinoline synthesis; [16]

The synthesis consists of quinoline based molecules by using aniline, benzaldehyde to generate imine compound, then reaction with electron rich alkenes (enol ether or enamine) in presence of Lewis acid BF_3 (activate the imine) **scheme-1.9**

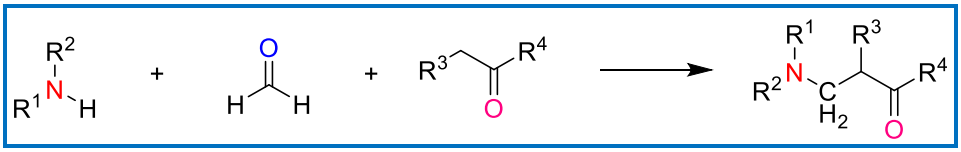
Scheme-1.9



Mannich reaction. [17]

Amino methylation of active methylene carbonyl compounds, formaldehyde and primary or secondary amines to form a C-C single bond in a one-pot three component condensation process have shown in **scheme-1.10**

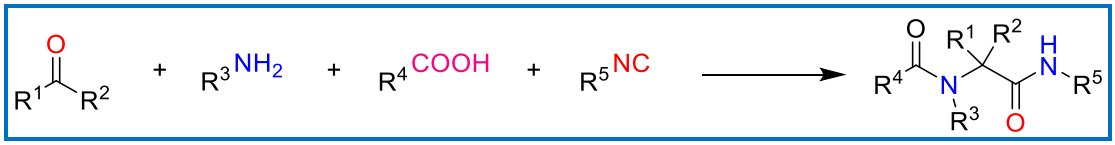
Scheme-1.10



Ugi reaction. [18]

Ivar Karl Ugi in 1959 has developed the isocyanide involved one-pot four component condensation reaction. In which the reaction between aldehyde, ketone, carboxylic acid and isocyanide to produce a bis-amide (peptide bond). **Schame-1.11**

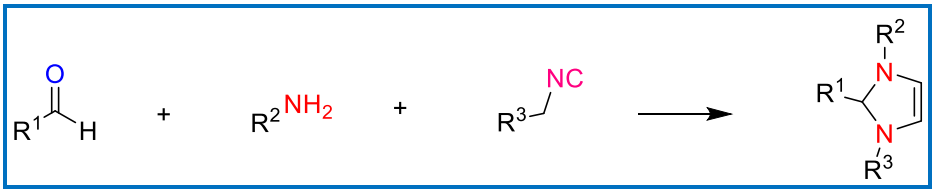
Scheme-1.11



Orru imidazole synthesis. [19]

Orru *et al* introduced an efficient modern one-pot MCRs for the synthesis of imidazoles. Condensation of aldehydes, primary amines and active methylene containing isocyanides lead to the formation of an imidazole represented in **scheme-1.12**

Scheme-1.12



1.2. Classification of MCRs.

Multi-component reactions are classified depending on the number of reactants participated during the reaction time into three component, four component, five component etc., MCRs are mainly devided in to type-1, type-II, type-III and their sub classes A and B.[6] These are i.) Imine based multicomponent reactions. ii) Isocyanide based multicomponent reactions

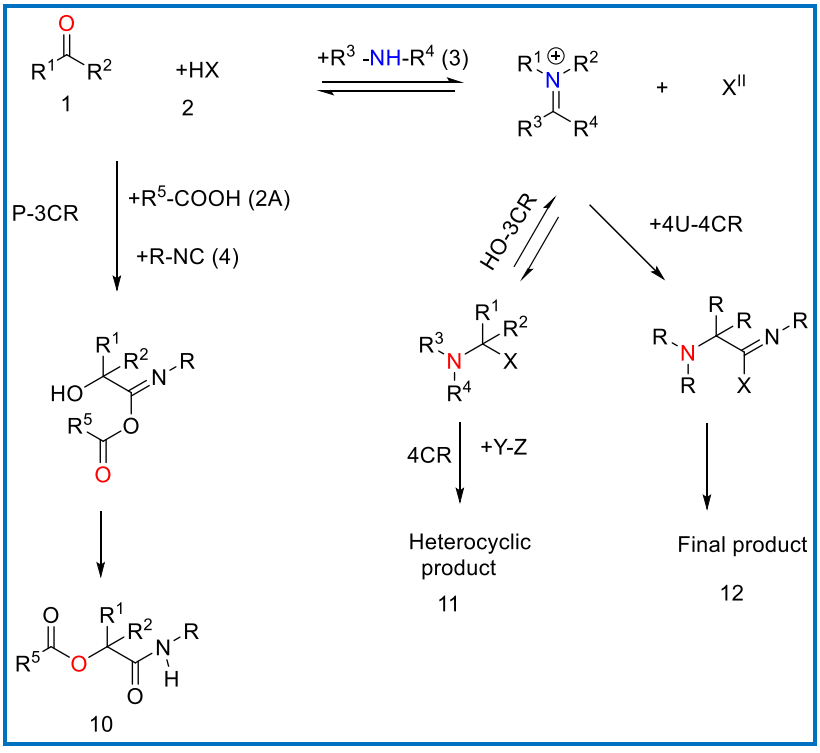
Type-IA: In this type of MCRs products were not to be isolated and all the reactants and products are involved in mobile equilibria.

Type-IB: In this separation of products was possible, with only intermediates and starting materials participating in the equilibrium.

Type-IIA: Type-IA & IB intermediates irreversibly react to form a multifunctional components and heterocyclic product formed with irreversibly. In which every adduct becomes a part of heterocyclic compound finally total no. of chemical bonds constant.

Type-IIB: The isocyanide based MCRs are examples for type-IIB. In this type of MCRs type-IIA intermediate reaction with IB the final product formed irreversibly with another adduct and in which the number of chemical bonds have been increased

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



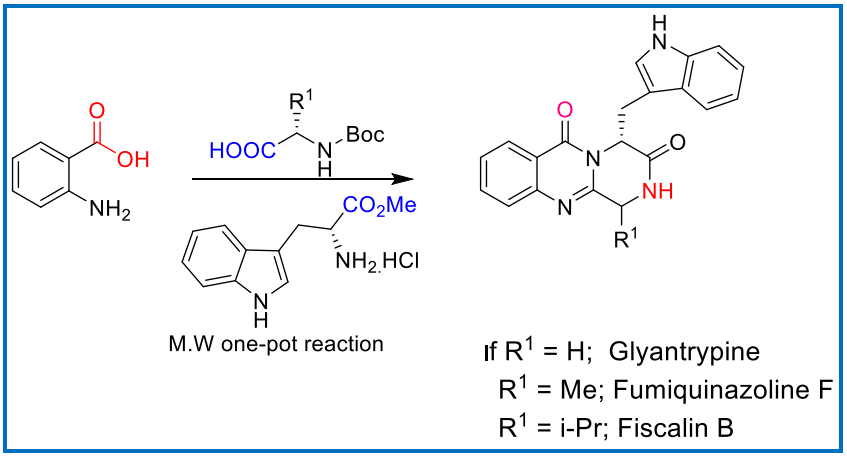
1.3. Different methods involved in MCRs

A variety of techniques have been used for the development of MCR reactions, over conventional method to ease of handling the reaction i.e minimization of time, purification of compound, improve percentage yield, reduce by-products and minimize cost-effective chemicals.

Microwave assisted MCRs

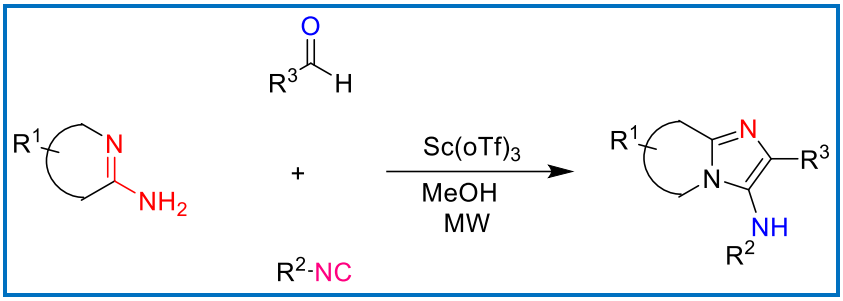
The MCR reaction in combined with microwave method dramatically helpful to reduce the chemical wastage, require time for completion of the reaction is decreases. The microwave method is well-known process for the synthesis of heterocyclic ring containing natural products. Pyrazine [2,1-*b*] quinazoline-3,6-dione basic moiety were synthesized by the reaction of 2-amino benzoic acid with amino acid esters and Boc-amino acids to give pyrazinoquinazoline heterocyclic molecule. When the change of R^1 functional group If R^1 = H; Glyantrypine, R^1 = Me; Fumiquinazoline F and R^1 = i-Pr; Fiscaline B are the biologically active natural products[20] shown in **scheme-1.13.**

Scheme-1.13



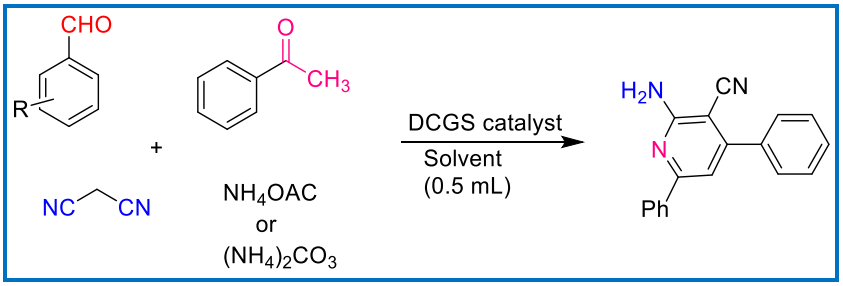
Jiang *et al* [21] developed the Groebke-Blackburn-Bienayme type multi-component reaction under microwave irradiation for the construction of five-member heterocyclics. The reaction of imidines with isocyanides and aldehydes catalyzed by scandium triflate in MeOH to afford a fused 3-amino imidazole as represented in **scheme-1.14**

Scheme-1.14



Tamaddon *et al* [22] synthesized the MCR microwave employing one-pot four component cyclocondensation reaction of aromatic aldehydes, acetophenones, malanonitrile and ammonium acetate or ammonium carbonate in the presence of DCGC (0.1 mol%) as a catalyst in water under reflux condition for 1 h to form a Hantzsch product of 2-amino-4,6-diphenylnicotinonitrile with 96% of the yield which has shown in **Scheme-1.15.**

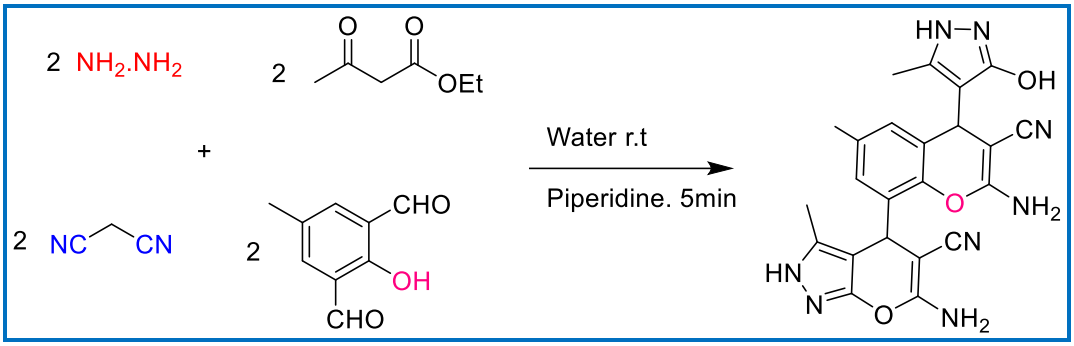
Scheme-1.15.



Aqueous medium MCRs.

The MCR reactions were carried out by using water as a solvent, offers many advantages. It is a green solvent and increase the rate and efficiency of the reaction compared with organic solvent. After completion of the reaction water is removed by simple filtration, final products were formed in crystalline solids. Ugi was the first to introduce higher order complex MCR architectures. In this regard water assisted as a solvent for pseudo eight component synthesis of pyranopyrazole-substituted 4*H*-chromene complex molecule. This was synthesized by condensation of two equivalents of hydrazine hydrate, 2 equivalents of ethyl acetoacetate, two equivalents of malanonitrile and 2 equivalents of 2-hydroxy-5-methylisopthalaldehyde in one-pot using water as a solvent and add 10 mol% of base piperidine, stir for 5 minutes to produce a complex structure molecule [23] shown in **scheme-1.16**.

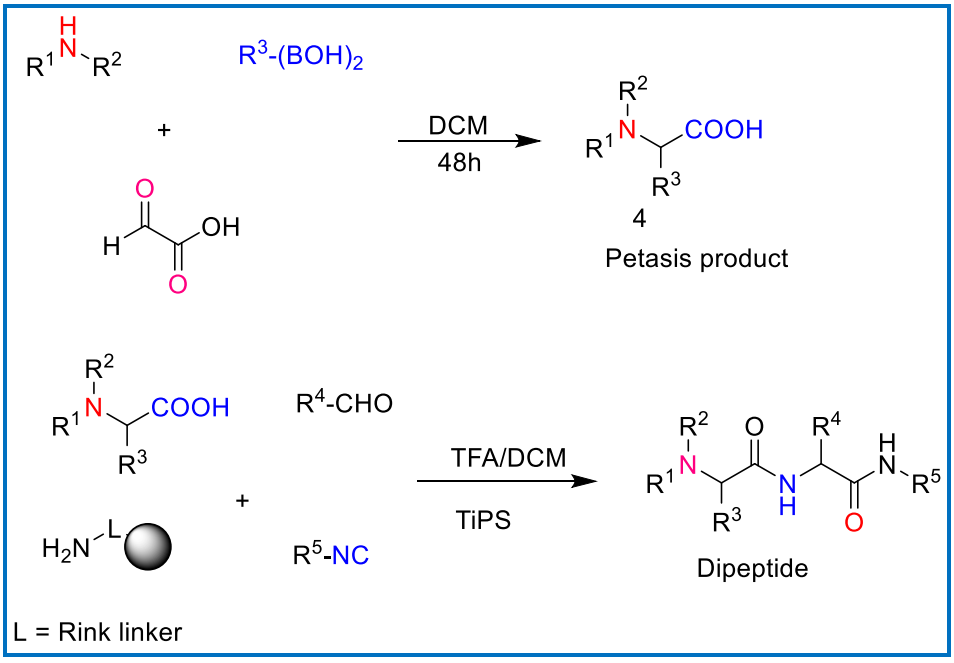
Scheme-1.16



Solid phase MCRs

The solid phase MCR reactions are important for the preparation of a library of chemical entities. In this MCR method different polymer resins are used for the preparation of peptides. In which the dipeptide has been synthesized through Petasis reaction followed by Ugi 4CC. Initially the preparation of α-amino acid by tandem Petasis reaction of secondary amine, boronic acid and glyoxylic aid in DCM 48 h to give a tertiary α-amino acid. This was carried out in solution phase. And the Petasis products were subjected to Ugi 4CC reaction in presence of free amine Rink linker to afford a dipeptide. This step was carried out in solid phase as shown in **Scheme-1.17** [24]

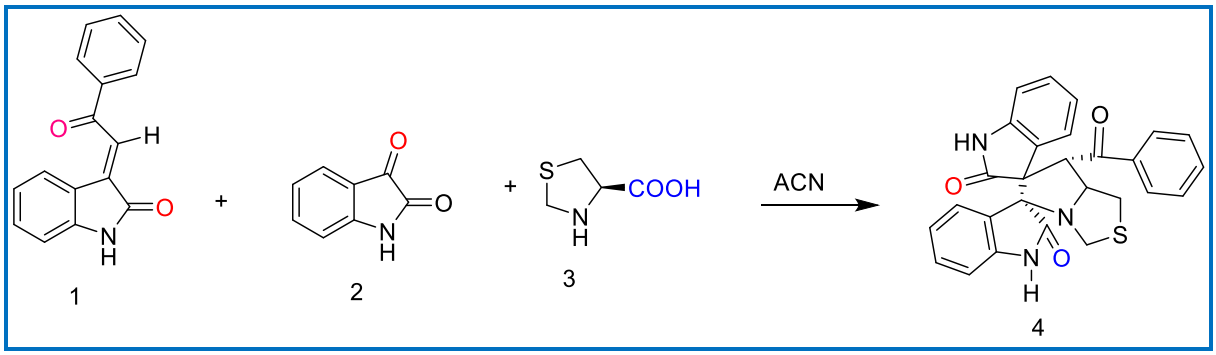
Scheme-1.17



Ultra sonic assisted MCRs.

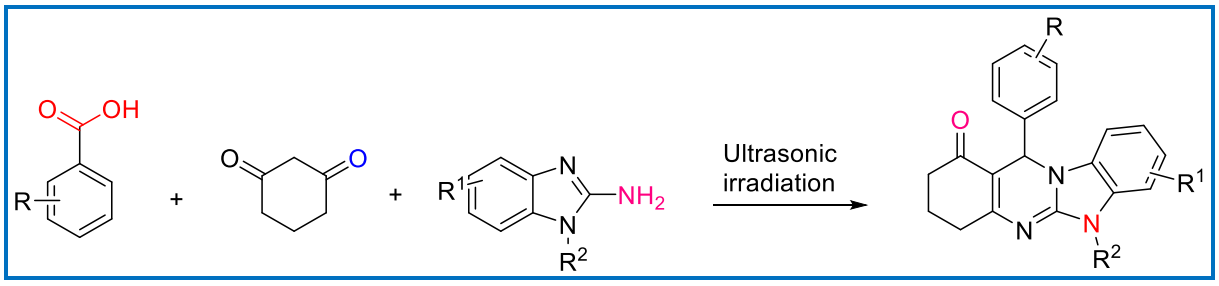
Babu *et al* [25a] reported the spiro heterocycles (**Scheme-1.18**) using Ultrasonic assisted MCR method. The mixture of L-proline and isatin was condensed in presence of acetonitrile to form a azomethine ylied. The generated ylied subsequently undergo cyclocondensation reaction with 2-oxo-2-phenylethylidene) indolin-2-one to achieved a spiro heterocyclic molecule with good percentage of the yield.

Scheme-1.18



Banarjee [25b] developed the ultrasonic assisted synthesis of substituted aromatic aldehydes. 1,3 cyclohexanone and 2-amino benzimidazole in presence of isopropanol. Which was represented in **scheme-1.19**.

Scheme-1.19



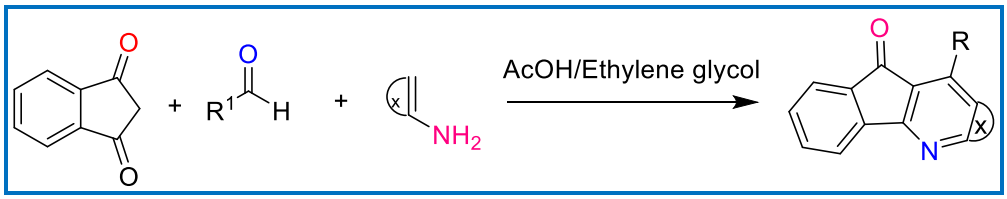
1.4. Applications of MCRs

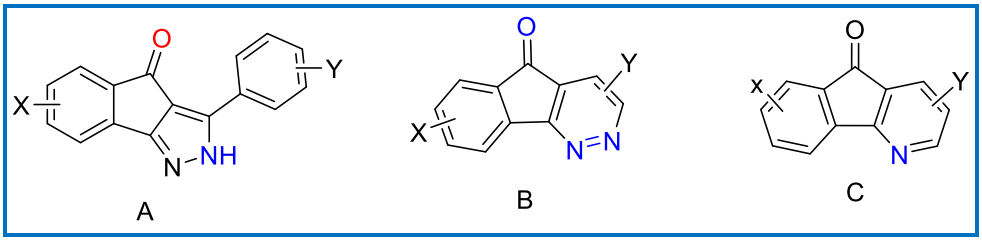
MCRs involved in natural product synthesis.

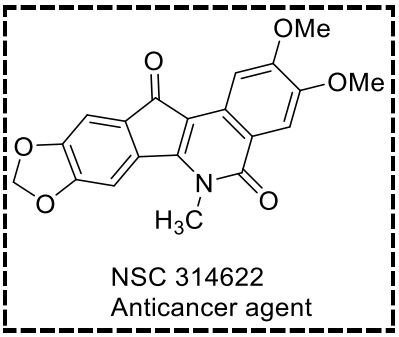
MCRs are used in different fields of chemistry. In this context the natural products have placed an important role in the medicinal chemistry field. MCRs are essential for the synthesis of various natural products. In this method quantitative yields were obtained and easy to isolate the final products in pure form. Many of the polycyclic indenopyridine alkaloid derivatives are biologically active natural products. The synthetic protocol of the indenopyridone

heterocyclics involve one-pot three component condensation of 1,3 indane dione with aldehydes and amino heterocycles or aniline to afford polycyclic heteroaromatic molecule (Scheme-1.20). The following indenopyridazine B and indenopyrazole A are identified as cyclin-dependent kinase inhibitors[26a]. And indenopyridazone B is monoamine oxidase inhibitor.[26b] Further the multiple activity of indenopyridine C to act as a calcium modulating activity, [26c] cytotoxic [27] and the lastly the NSC 314622 is an important compound for the development of anticancer agent Topoisomerase-I. [28]

Scheme-1.20

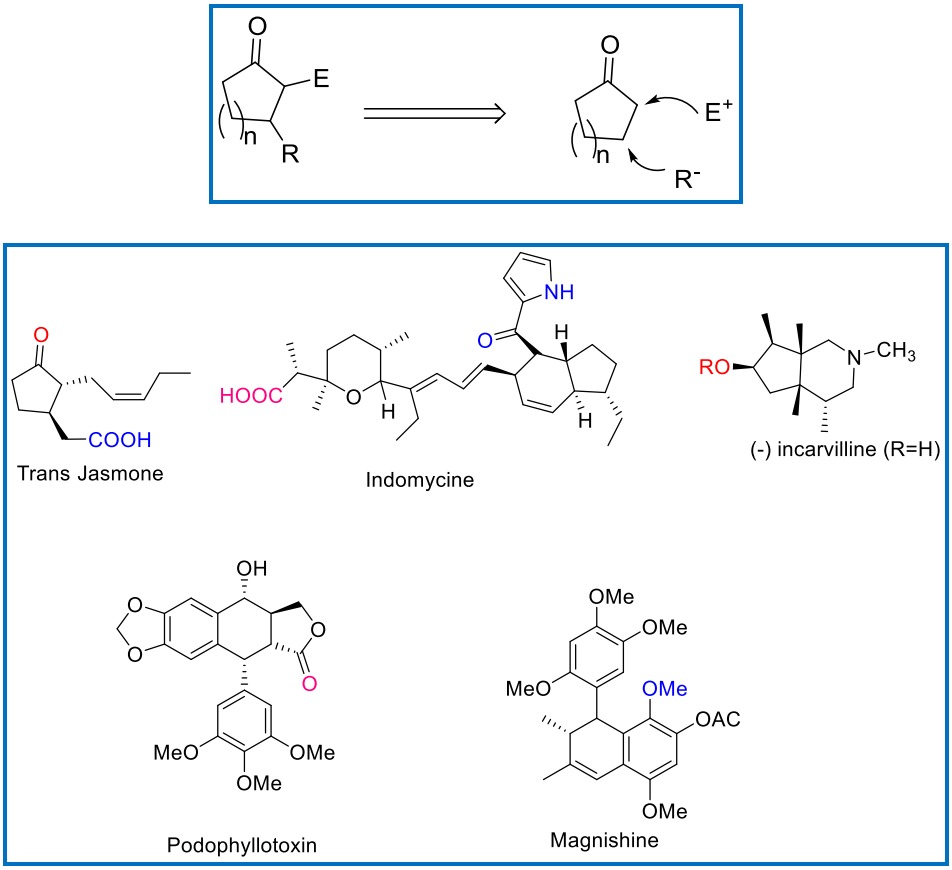






The conjugate cycloaddition of cyclic enones followed by electrophilic trapping to form a enolate is useful for the development of natural product alkaloid [29a, 29b, 29c] such as trans jasmine, indanomycin, podophyllotoxin, incarvilline, magnoshinine. This group of alkaloids show various biological activities [30a, 30b].

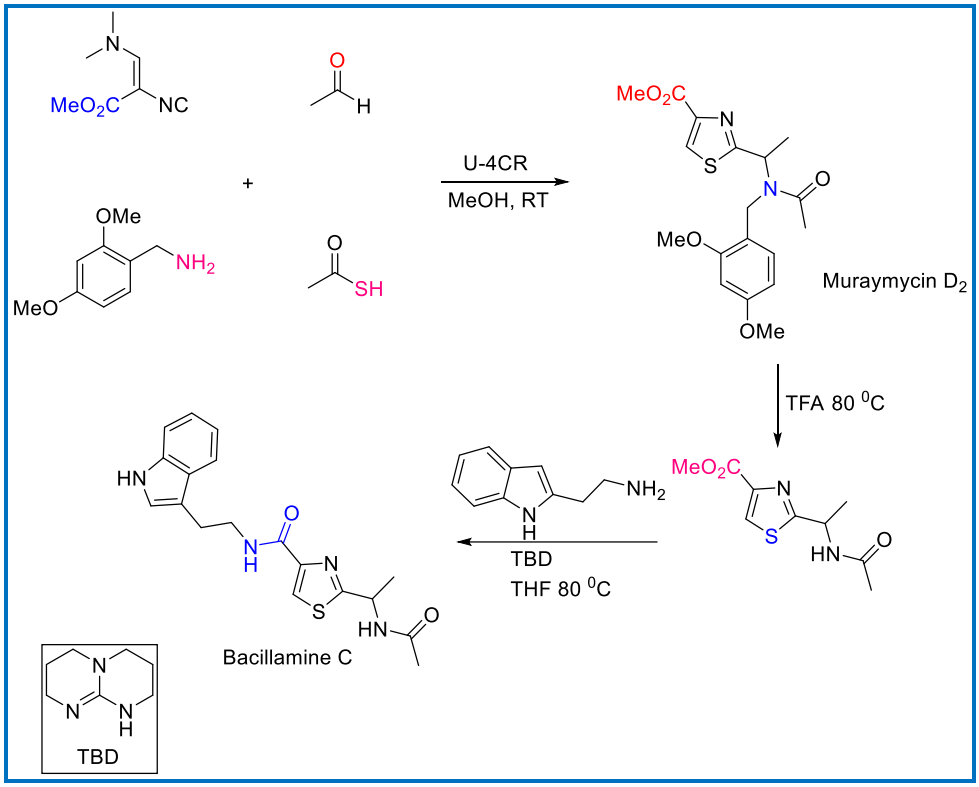
Scheme-1.21



Synthesis of Bacillamide C natural product.

Wang *et al* [31] reported the natural product Bacillamide C. The one-pot four component reaction between substituted isocyanides, aldehydes, 2,4-dimethoxy benzyl amine and thioacetic acid in presence of methanol to generate a thiazole compound. Muraymycin D2 in a single step. Subsequently the removal of di methoxy group and TBD mediated amide bond formed to afford a target product Bacillamide C explain in **scheme-1.22.**

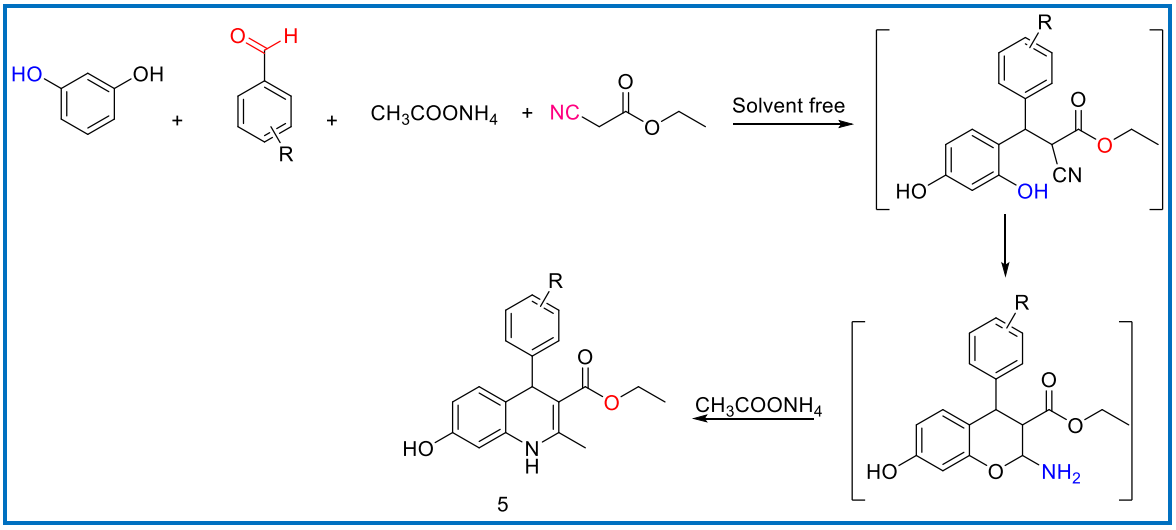
Scheme-1.22

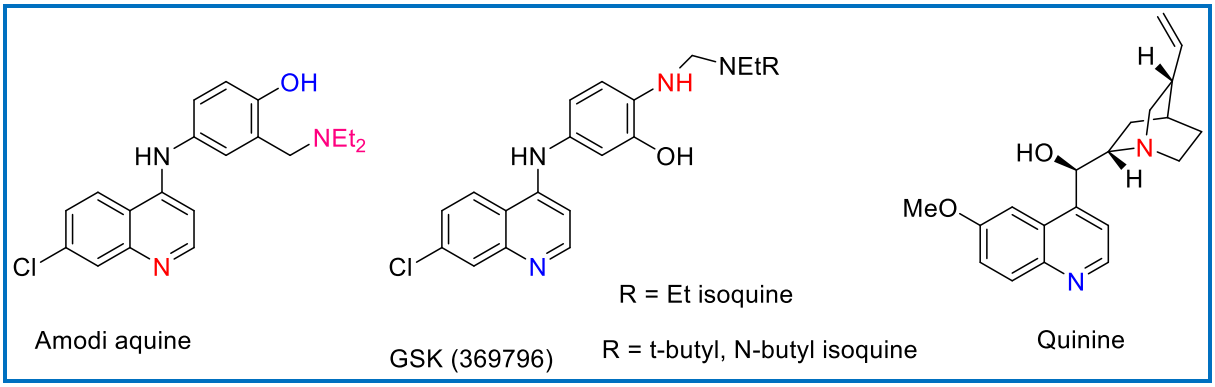


Synthesis of quinoline alkaloids

EI-Saghier *et al* [32] developed the quinine based alkaloids *via* MCRs approach. The quinoline compound 5 has been synthesized from Skrup synthesis by the reaction aromatic aldehydes, resorcinol, β-cyanoester and aromatic aliphatic amines in the presence of solvent free conditions. These derivatives of aminoquine and N-tertiary butyl isoquine (GSK 369796) and quinine are showing antimalarial activity having quinoline moiety [33,34, 35] **Scheme 1.23**

Scheme-1.23

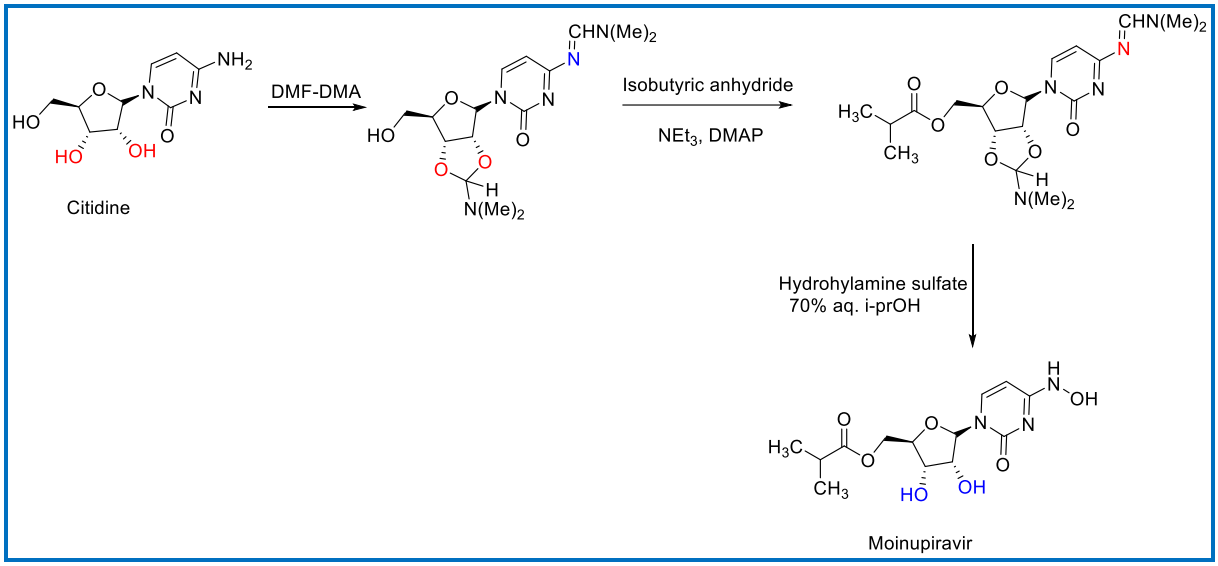




MCRs involved in drugs

Multi-component reactions are also efficiently used for the synthesis of biologically active drug molecules. In the MCRs method the yield of the final product is increased compared to conventional method. Concise synthesis of an important antiviral drug Molnupiravir using one-pot process [36a] from cytidine. The reaction of cytidine in presence of DMF-DMA in pyridine in which the selective protection of 2', 3'-dihydroxyls and primary amine group of cytidine, after protection of hydroxy group with iso-butyric anhydride further deprotect the diol group to achieve a Molnupiravir with good percentage of yield 61%. Molnupiravir is useful for examine anti-SARS-CoV2 effect by lethal mutation[36 b, 36 c] **Scheme-1.24**

Scheme-1.24

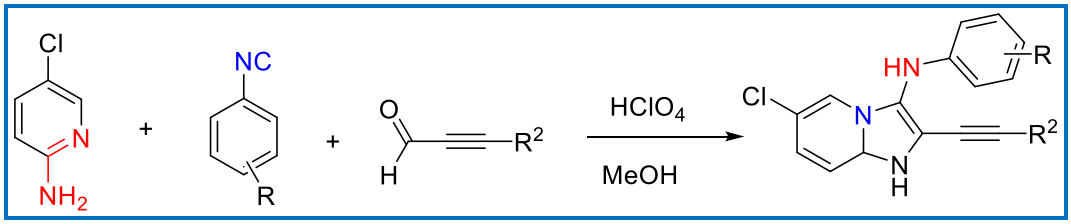


GBB (Groebke-Blackburn-Bienayme) Reaction used in drug discovery

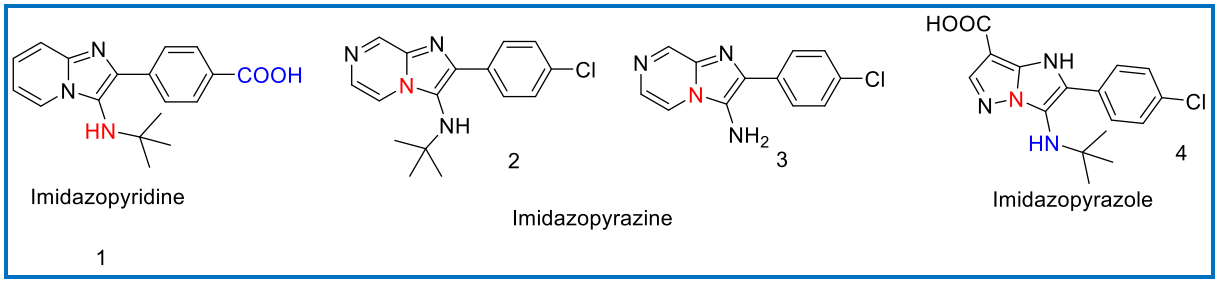
The above reaction is used to evaluate therapeutic bridgehead nitrogen containing fused imidazoles by the reaction of aldehydes, isocyanides, amidines to produce a building blocks of imidazopyridines and imidazopyrazines [37] as shown in **scheme-1.25a**

Baviskar *et al* [38] reported the fused imidazopyridines and imidazopyrazine 2, and imidazopyrazole-3,4 motifs using GBB reaction and evaluated their anticancer activity against brest cancer and kidney cancer activity. **Scheme-1.25b**

Scheme-1.25a

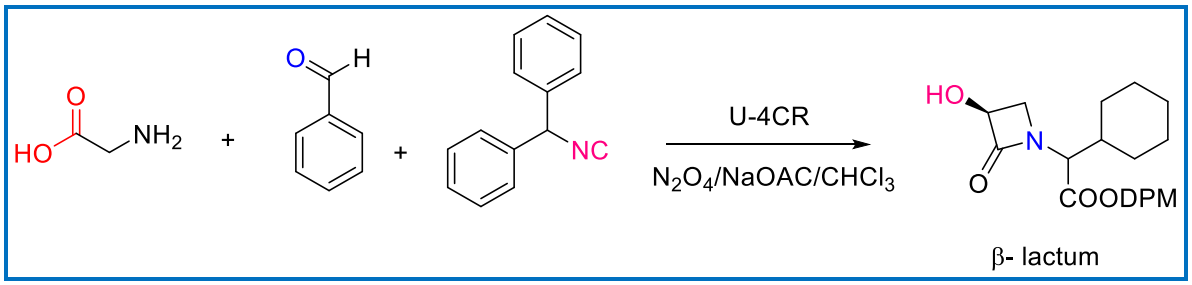


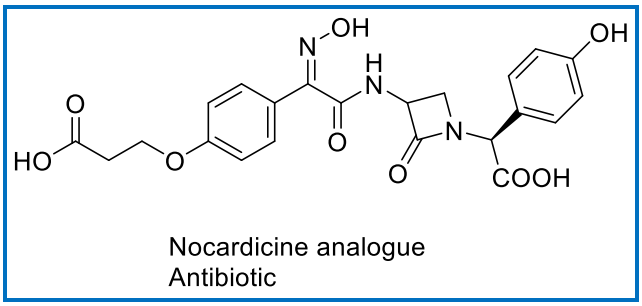
Scheme-1.25b



Weber [39] has developed the MCR synthesis of biologically active β-lactum derivatives by the reaction of amino acid, aldehyde and isocyanide, dinitrogen tetroxide to form a carboxylic acid derivative nocardicine, it is an antibiotic agent.[40]

Scheme-1.26

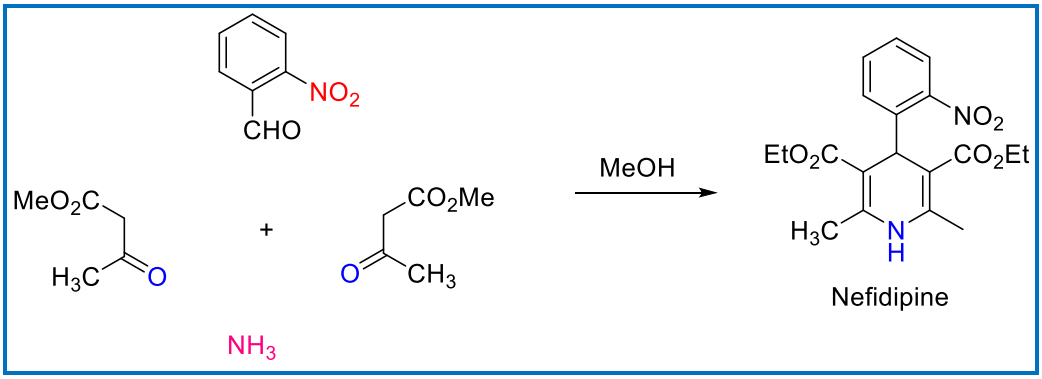




Synthesis of calcium channel blocking agent Nefidepine *via* Biginelli reaction.

Guisnet [41 a] has carried out the synthesis of 4-aryl dihydropyridine *via* one-pot three component process. In this condensation of two equivalents of β-keto ester with aldehydes to afford a 4-aryl dihydropyridine-3,5-dicarboxilic acid ester named as Nefidepine. It is a first medicinal MCRs product can advantageously use for calcium antagonist [41 b]

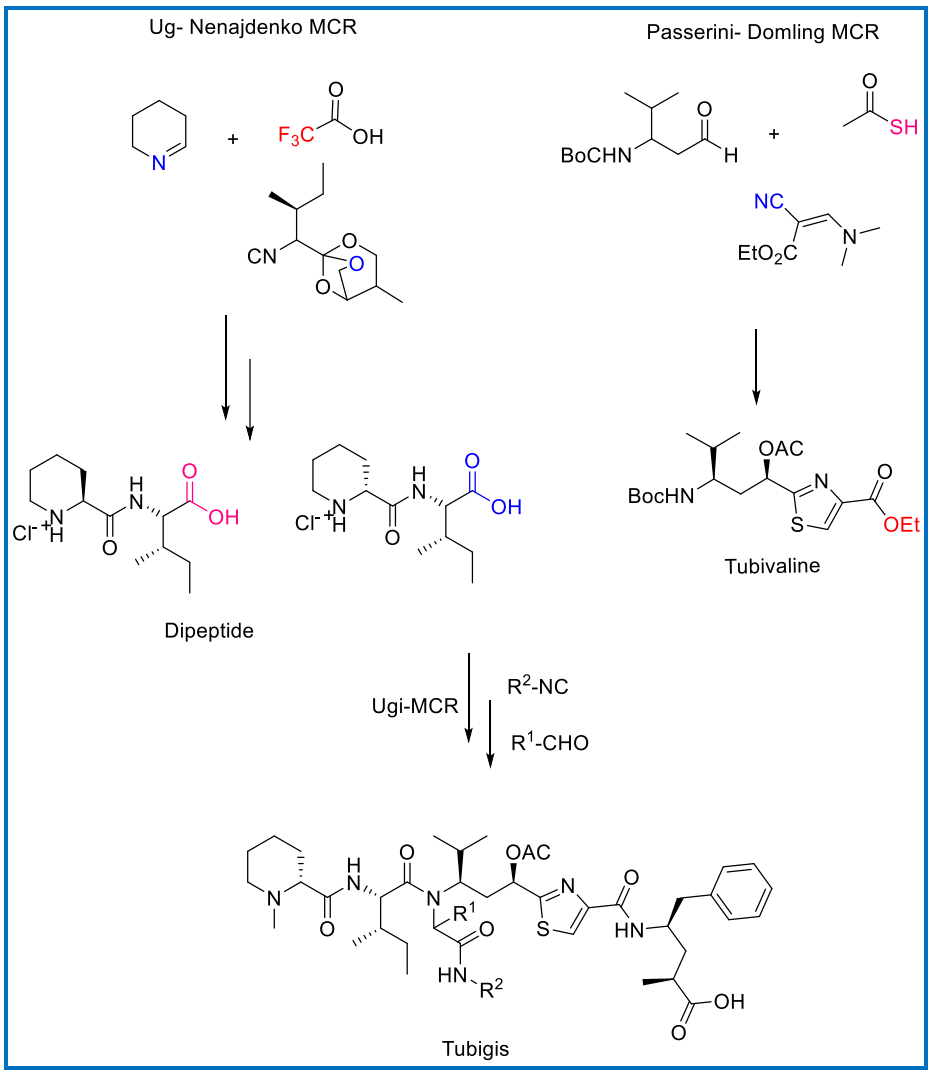
Scheme-1.27



Synthesis of anticancer drugs *via* MCR approach

Synthesis of anticancer agent Tubugis by a multicomponent method was reported by **pando *et al***.[42] It is commonly used in medicinal and combinatorial chemistry. The Tubugis synthetic protocol involves Ugi-Nenajdenko, Passerini-Domling MCR and Ugi-MCR reactions as shown in scheme-1.28**.**

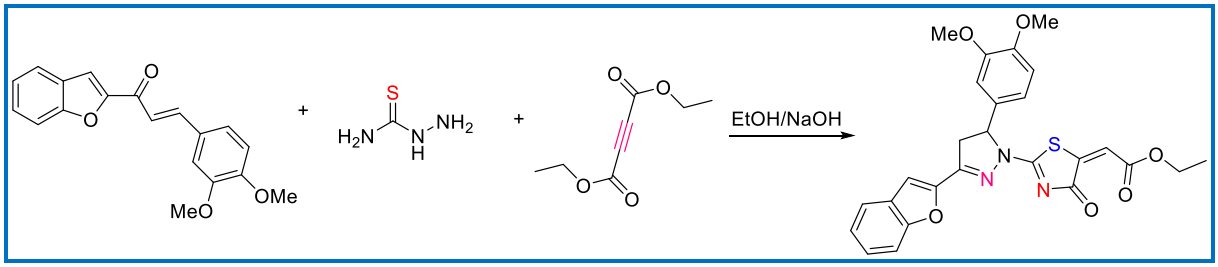
Scheme-1.28



Recent literature reports of biologically active MCR products.

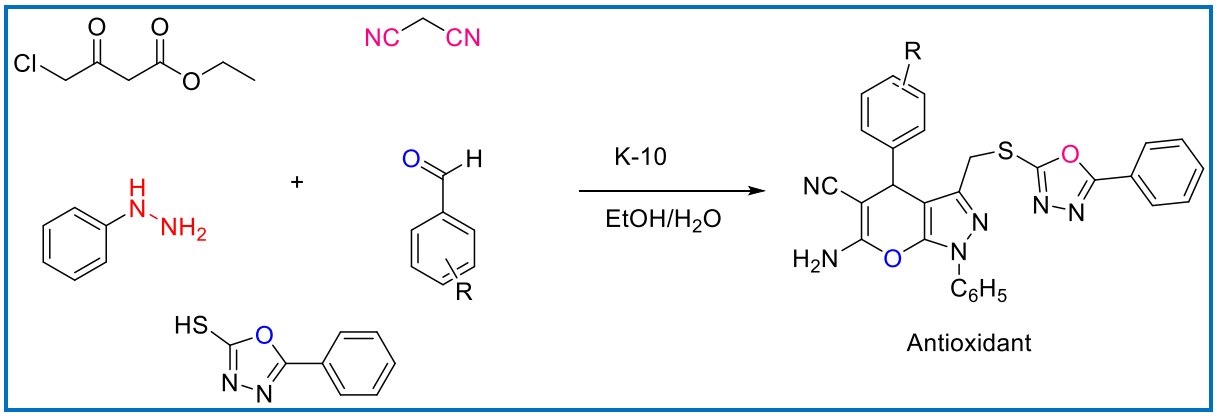
Yakaiah *et al* [43] published the pyrazolo-oxothiazolodine *via* a one-pot synthesis of substituted chalcones, thiosemicarbazide and diethyl acetylene dicarboxilate under EtOH/ NaOH. These thiazolo-pyrazole derivatives were acted as a antiproliferative agents against human lung cancer. (Scheme-1.29)

Scheme-1.29



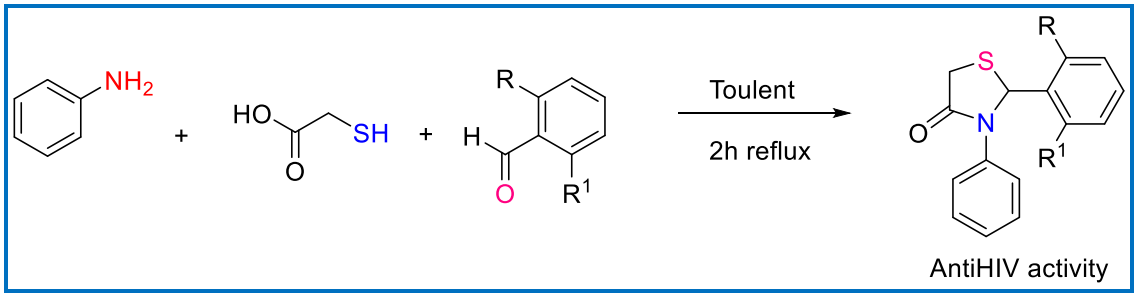
Reddy *et al* [44] have developed the one-pot five component protocol for the synthesis of 1,4-dihydropyrano pyrazoles by the reaction of chloromethyl acetoacetate, malanonitrile, phenyl hydrazine and aromatic aldehydes and 5-phenyl oxadiazoles 2-thiol in presence of EtOH. The final compounds were screened for their biological activity and they have shown antioxidant property. (Scheme-1.30)

Scheme-1.30



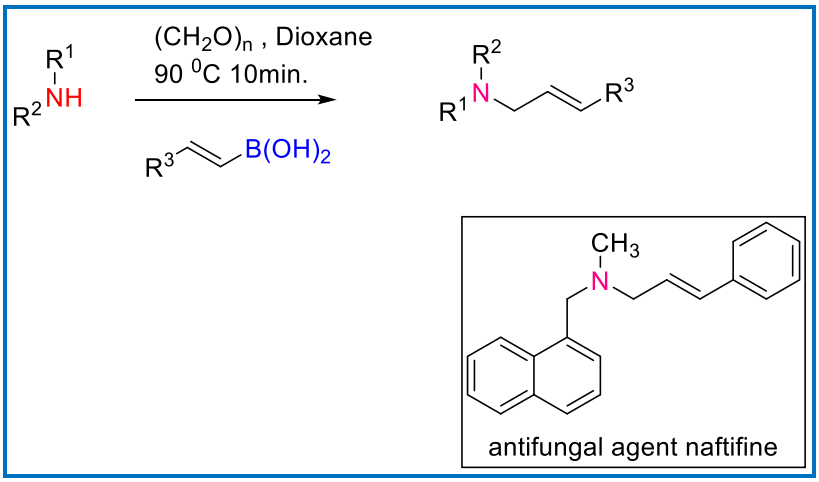
Barreca *et al*. [45] synthesized the thiazole heterocyclic containing scaffolds. The reaction between aniline and thioglycolic acid aromatic aldehydes in toluene under reflux for 2 hours to give a thiazole compound. Further these compounds were screened for their antiviral activity and these type of moieties exhibiting promising anti HIV activity. (Scheme-1.31)

Scheme-1.31



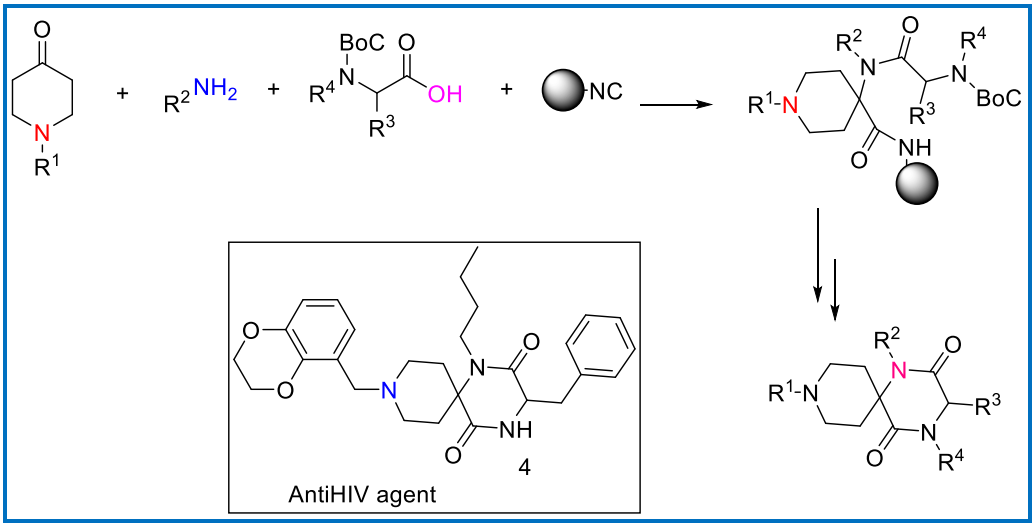
Batey [46] has synthesized the biologically active secondary amine product by using a reaction between secondary amine, paraformaldehyde and *E*- boronic acid in dioxane. In this reaction formation of C-N bond takes place. The reactions have first reported by Petasis. This is also known as Petasis borono-Mannich reaction. The analogues of this type of derivative Naftifine exhibiting potent antifungal activity shown in scheme-1.32.

Scheme-1.32



Habashita *et al* [47] synthesized the spirodiketopiperazine compounds through Ugi-4CC approach. The spirodiketopiperazine antagonists were used for the treatment of anti HIV.[48] (Scheme-1.33)

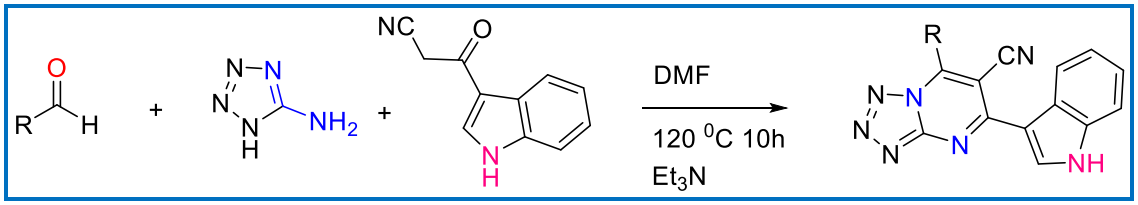
Scheme-1.33



Radwan *et al* [49] efficiently synthesized the tetrazolopyrimidine-6-carbonitrile *via* a one-pot multi-component condensation of various aldehyde, tetrazole-5- amine and 3-cyano indole in

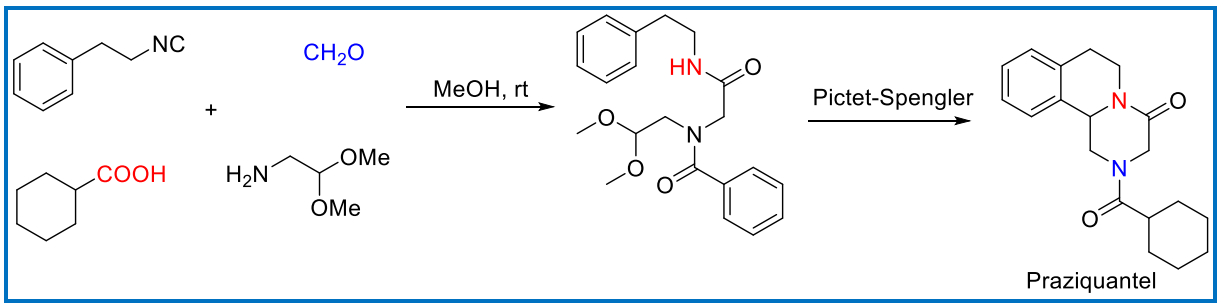
the presence of DMF/Et_3N at 120 °C for 10 h to produce a fused tetrazolopyrimidine-6-carbonitrile (Scheme-1.34). These moieties have shown predominant cytotoxic activity. [50]

Scheme-1.34



Cao *et al* [51] developed the schistosomiasis drug praziquantel which is having a isoquinoline structure. The one-pot four component condensation of paraformaldehyde, (2-isocyanoethyl) benzene, cyclohexyl carboxylic acid and 2,2dimethoxy ethyl amine followed by Pictet-Spengler reaction to give a praziquantel as shown in scheme-1.35**.**

Scheme-1.35



1.5. 1,2,4-triazoles

Five-membered ring bearing nitrogen atoms at 1,2,4 positions is calld as symmetrical triazole. These are occupied an important role in pharmaceutical industry, substituted 1,2,4 triazole compounds are exhibiting good biological activity. Many of the anticancer, antiviral, antifungal, antidepressants and antianalgesic drugs having a 1,2,4-triazole moiety the **Fig-2** showing regularly using triazole ring containing drugs. The vorozole, letrozole, anastrozole are the examples for triazole involved anticancer drugs[52] and viramidine, ribavirin, temsavir, doravirin [53] are the examples for antiviral triazole comprising drugs. [54a, b] Paclobutranol, fluconazole, voriconazole are the antifungal drugs [55] and triazolam, alprazolam, estrazolam are the diazepam ring skeleton triazole containing antidepressant drugs .[56]

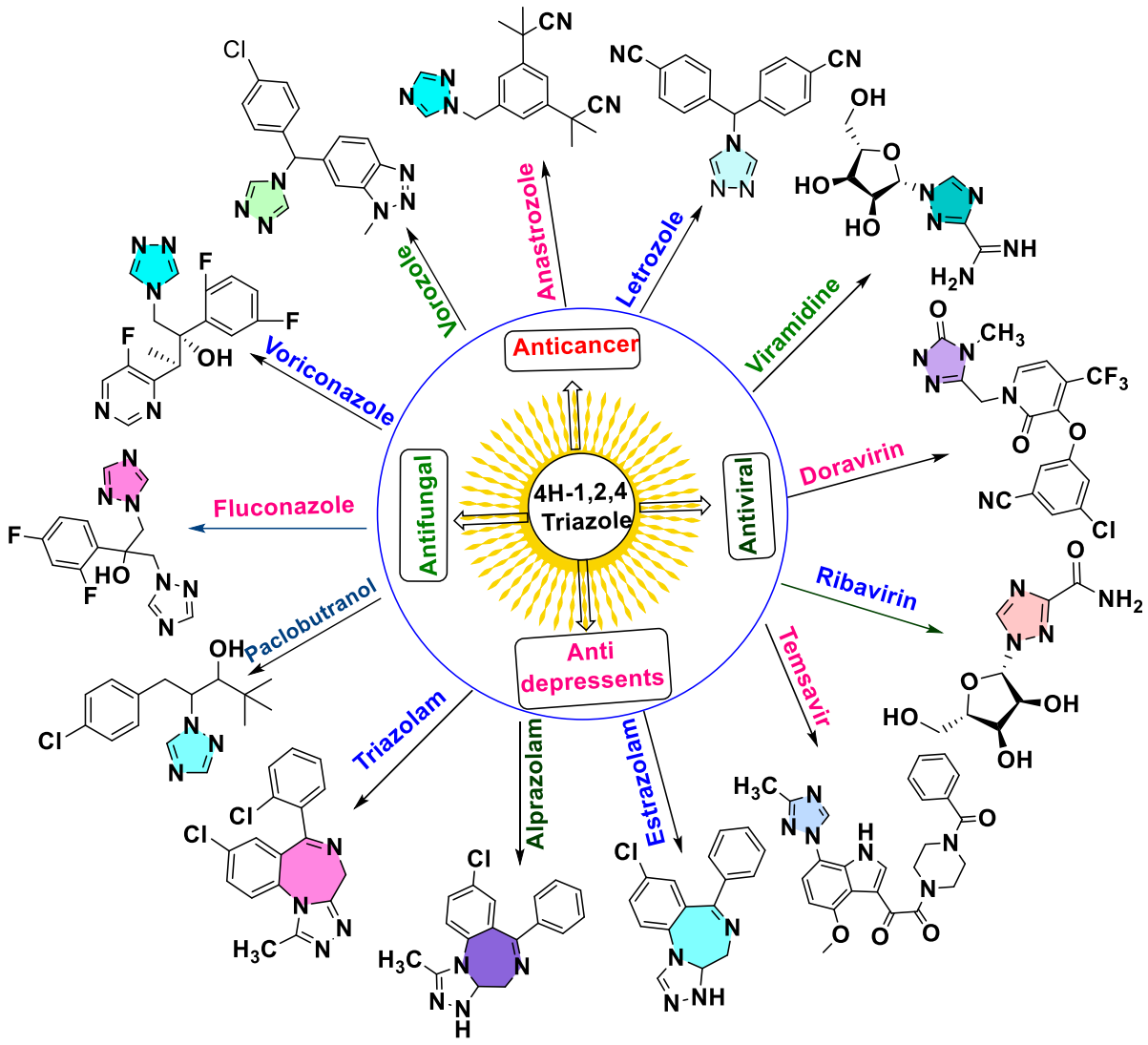
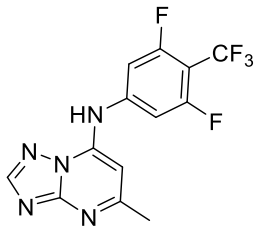


Fig-2. Pharmaceutically active triazole ring containing drug molecules**.**

Biological applications of 1,2,4-Triazole scaffold.

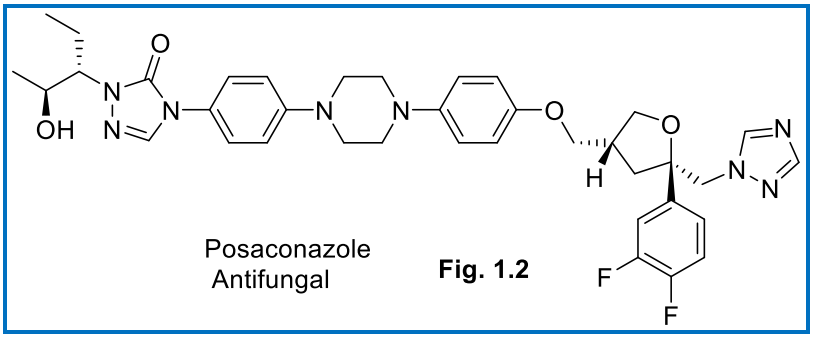
Gujjar *et al* [57] carried out the synthesis of the fused triazolopyrimidine moiety and evaluated for their antimalarial activity (**Fig. 1.1**). These compounds were screened against for human, plasmodium falciparum enzymes and also evaluated against for plasmodium falciparum 3D7 cells, and good results were obtained.



Antimalarial activity

Fig. 1.1

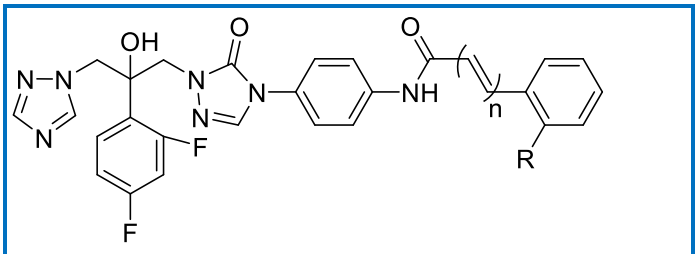
Franklim *et al* [58] reported the synthesis of antifungal agent Posaconazole [**Fig 1.2**], this has shown broad panel of antifungal activity and it is currently entering into phase-II clinical trials.



Posaconazole
Antifungal

Fig. 1.2

Zhou *et al* [59] developed the triazolone substituted compound (**Fig. 1.3**) in which the triazolone ring linked to piperazine. It exhibits promising *in-vitro* antifungal activity against candida albicanes and candida parapsilosis activity. The nitro substituted compound (R = NO_2) showed good activity with MIC_{80} values are 0.00024, 0.0039 µg/mL.

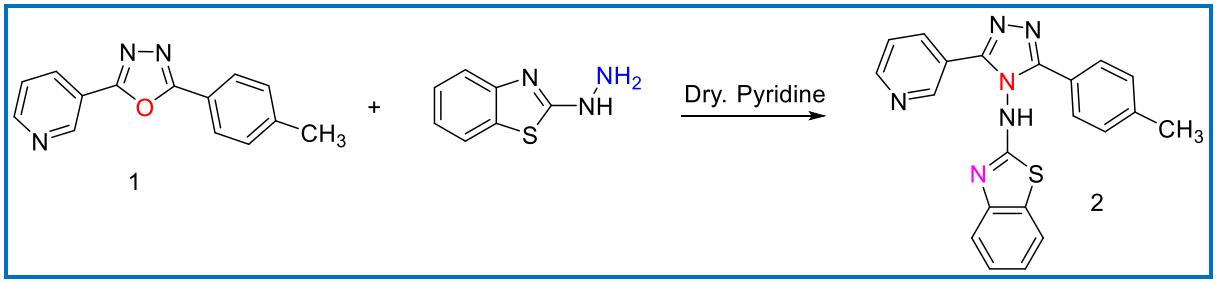


Candida albicanes/ Candida parapsilosis activity

Fig. 1.3

Patel *et al* [60] reported good antitubercular activity for the following compound-2. It was synthesized by the reaction of 2,5 di substituted oxadiazoles, and 2-hydrazino 1,3-benzothiazole. The 1,2,4-triazole having the pyridine ring substitution and substituted 4-amino benzothiazole moiety has shown remarkable antituberculosis activity against tested *Staphylococcus aureus* MTCC 96 Gram +Ve bacteria and *Escherichia coli* MTCC 443 Gram –Ve bacteria.

Scheme-1.36



Havaldar *et al*. [61a] reported the substituted triazole compound possessing antifungal activity and antimalarial activity (**Fig 1.4**). If R^1 = H, R^2 = NO_2 exhibit their good activity against *aspergillus niger* and if R^1, R^2 = F showing antimicrobial activity against plasmodium falciparum strain. And also 1,2,4-triazoles are valuable biologically active compounds [61b]

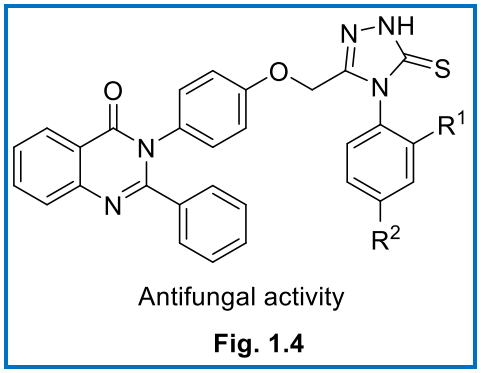
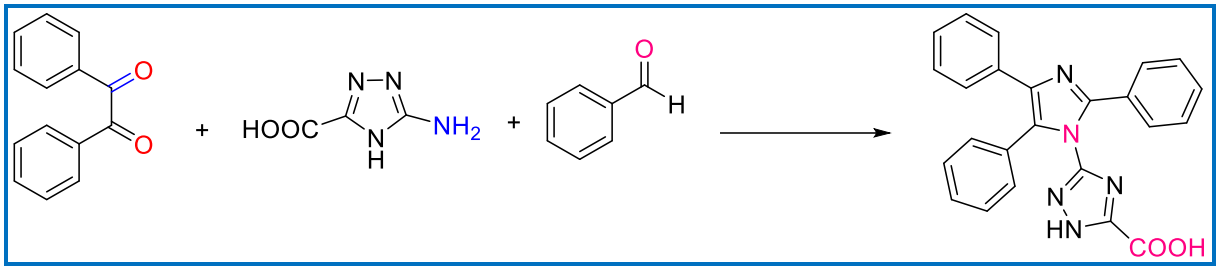


Fig. 1.4

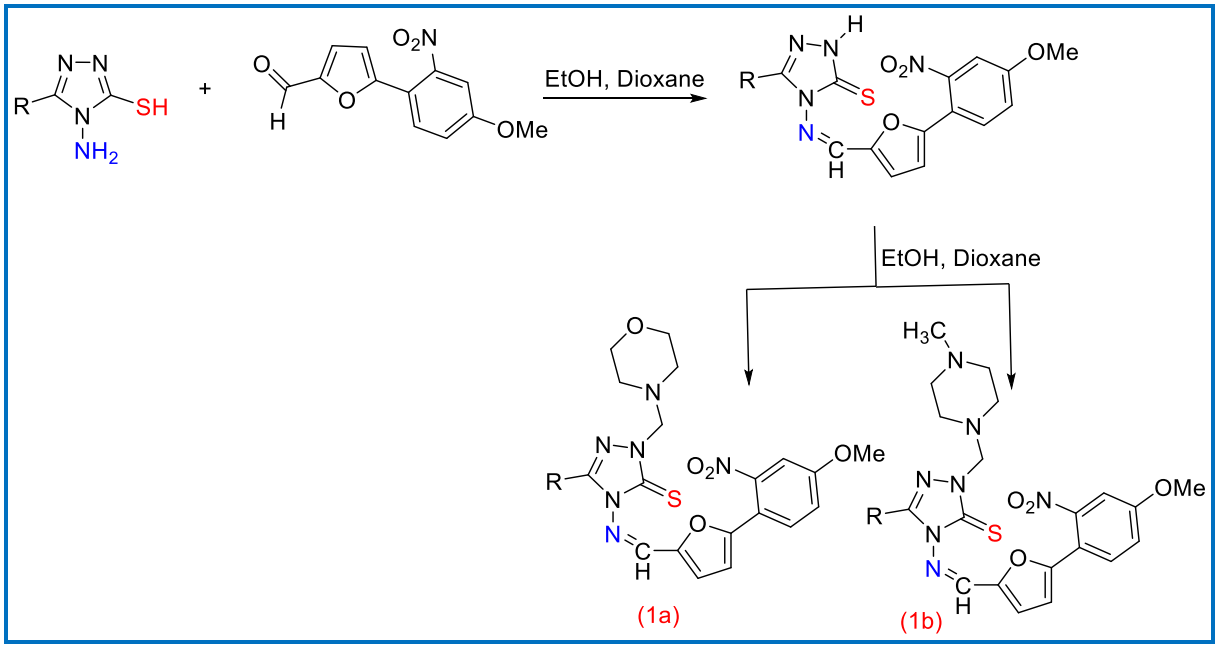
Nikalje *et al* [62] synthesized the triazolo imidazole compound and tested for its antibacterial activity against *Streptococcus pneumonia* (Gram +Ve), *Escherichia coli* (Gram –Ve) strains compared with Ampicillin which was taken as a standard reference drug.

Scheme-1.37



Holla *et al* [63] reported the target compounds (Scheme-1.38) by the condensation of amino mercapto 1,2,4 triazole and substituted aldehydes in EtOH, dioxane to form a Schiff bases then amino methylation takes place with different secondary amines by following Mannich reaction. The final compounds were screened for their anticancer activity against various cancer cell lines.

Scheme-1.38



Liao *et al* [64] developed the neuroprotective agent 1,3,5-tri substituted 1,2,4-triazole. Due to its higher bioavailability the compound exhibits promising neuroprotective activity to treat ischemic stock (**Fig. 1.5**).

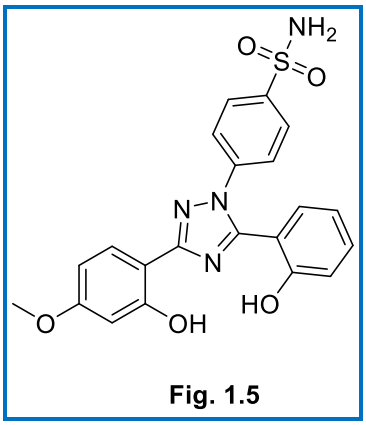


Fig. 1.5

Karabanovich *et al* [65] reported the synthesis of following 1,2,4-triazole derivative (**Fig. 1.6**). The antimycobacterial activity of the compound was screened against the standard *M. tuberculosis* $H_{37}Rv$ strain and nontuberculous *Mycobacterium avium*, *Mycobacterium kansasi* strains.

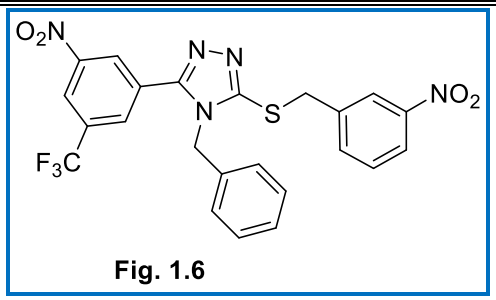
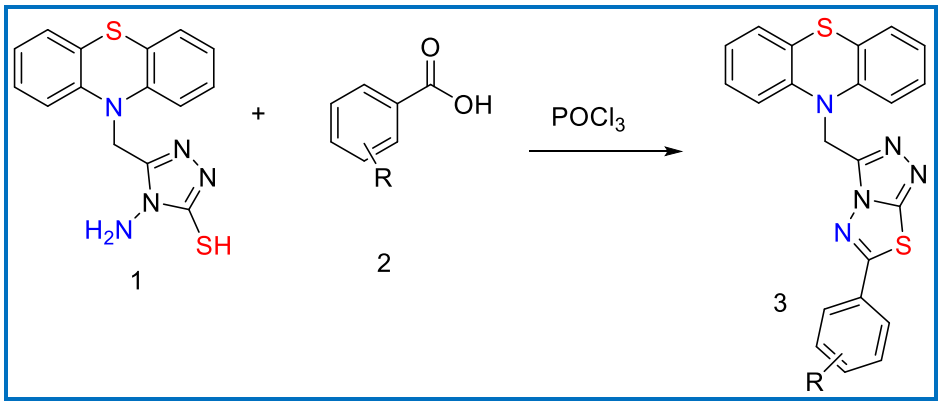


Fig. 1.6

Maddila *et al* [66] carried out the synthesis of triazolothiazoles through a one-pot process by reaction of aromatic acids with triazole having free amino and mercapto groups in presence of $POCl_3$ to generate a fused triazolothiazole heterocyclic moiety (Scheme-1.39). Further these compounds were screened for their anti-inflammatory activity against standard reference drug indomethacin.

Scheme-1.39



Wang *et al* [67] reported the analogues of ciprofloxacin-triazole derivative (**Fig. 1.7**) and screened for their antibacterial activity against bacterial strains such as *S. aureus, M. luteus, B. subtilis, B. proteus, E. coli*. The tested compounds exhibited high inhibitory against Gram +Ve and Gram –Ve bacteria with the range of MIC values 0.25 – 32 µg/mL.

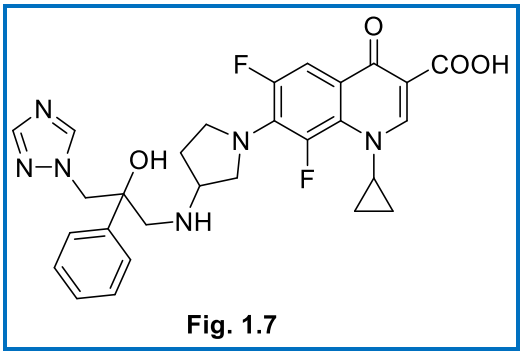


Fig. 1.7

Turan-zitouni *et al* [68] reported of the synthesis the antipyrene structured compound (**Fig. 1.8**) and tested for their antinociceptive activity. The obtained results are in higher as activity compared to dipyrone drug. The greater maximum possibility effective (MPE%) values were found.

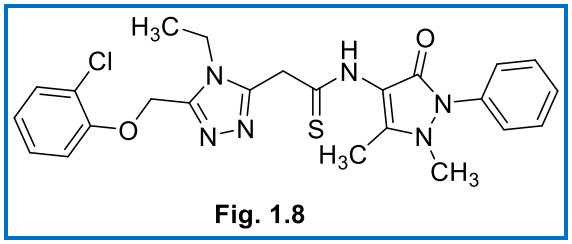


Fig. 1.8

Mustafa *et al* [69] published the antitubline and antiproliferative activity for the compound (**Fig.1.9**). These are analogues of natural product combretastatin. The cytotoxicity of the compound was tested using MTT assay with Hep2 and HL-60 human cancer cell lines. The obtained IC_{50} values were greater than that of carbonic anhydrase 4 (CA4) in HepG2 cells.

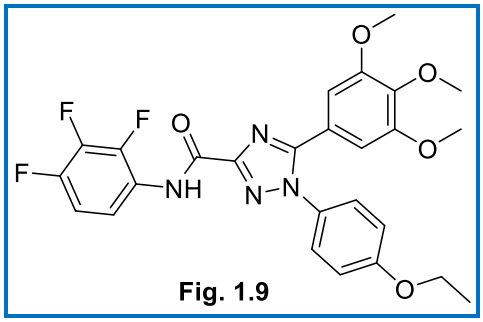


Fig. 1.9

Sreenu *et al* [70] reported the synthesis of the bis (Coumarinyl trizolothiadiazinyl) ethane (**Fig. 1.10**) in a multi-component process by reaction of thiocarbohydrazide, tartaric acid and 3(2-bromo acetyl) coumarin in presence of EtOH. These compounds were tested for their broad antiviral activity by using different cell cultures. The obtained EC_{50} values are high compared with standard drugs.

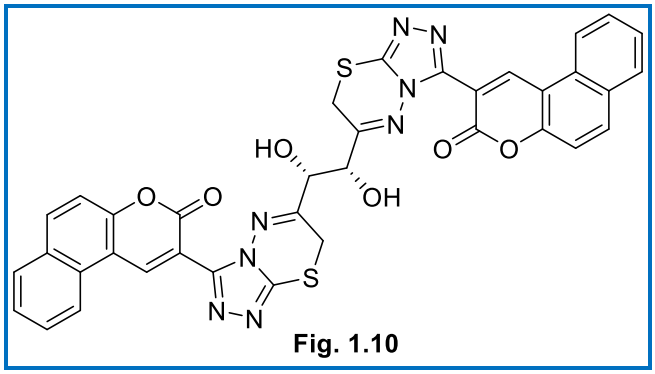


Fig. 1.10

Vaarla *et al* [71] developed the one-pot multicomponent synthesis of trizolothiadiazinyl coumarins (**Fig. 1.11**). In this reaction condensation of various carboxylic acids, thiocarbohydrazide and 3(2-bromo acetyl) coumarin under solvent free conditions. The compounds were screened for their antimicrobial activity against Gram +Ve, Gram –Ve bacterial strains. The obtained MIC μg/mL are good compared to the standard reference.

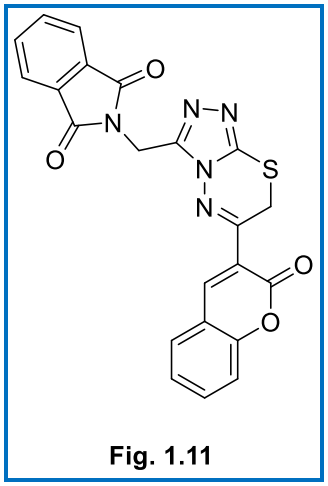


Fig. 1.11

Ashid *et al* [72] synthesized a series of triazolothiazole compound (**Fig. 1.12**) and screened for their antimicrobial and antifungal activity against *Bacillus subtilis*, *Klebsiella pneumoniae*, *Escherichia coli*, *Proteus mirabilis* bacterial strains and *Aspergillus Fumigatus* and *Candida albicanes* are antifungal strains. Ciprofloxacin and Flucanazole as a reference drugs.

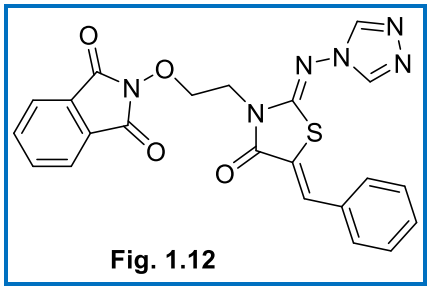


Fig. 1.12

1.6. PRESENT WORK.

The above overview shows that the multicomponent reactions have gained particular importance due to the development of high-throughput screening methods that allowed for the rapid identification of potential therapeutic medications among large collection of organic molecules. For this, it is essential to develop new methods to synthesize organic compounds. The techniques that would enable quick access to highly potent libraries of compounds were most popular for MC reactions, making them ideal for this concept. In general, conventional process involves loss of large amount of compounds and also required significant amount of solvents during purification, isolation of intermediates and it is time taking process.

The modern society also follows strict environmental requirements in scientific and industrial production of pharmaceutical compounds. In this context the multicomponent approaches are environmentally safe to design chemical compounds and they reduce isolation of intermediates. They are helpful to increase in the yield of final products and in these reactions more number of chemical bonds are formed in a single step.

In this research work we desire to develop fused, unfused new heterocyclic compounds which follow MCR (multicomponent reaction) method. And synthesized compounds were evaluated for their biological activity.

Need for the present study.

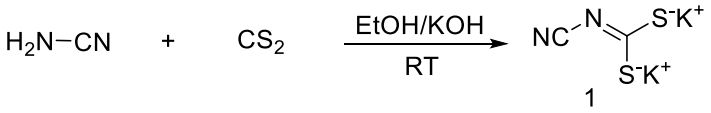
Owing to the biological significance of the chemical moieties in the organic compounds, the synthesis of compounds embedded with this scaffold has been gaining interest. In this regard, in the present research work, it was anticipated to synthesize a newer series of 1,2,4-triazoles, fused[3,2-*b*][1,2,4]triazoles, [1,2,4]triazolo[1,5-*a*]pyrimidine-7-(4*H*)-ones and [1,2,4] triazolo [3,4-*b*][1,3,4]-6-amino thiadiazines which would exhibit biological properties.

Scope of the work.

a) To synthesize an efficient methodologies using MCR approach.
b) To synthesize prominent and diversified organic compounds, embedded with biologically active scaffolds.
c) To organize biological activity studies such as anticancer, antiviral, antibacterial, DNA binding properties.

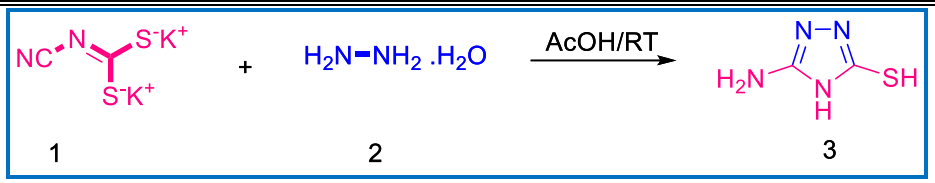
Preparation of Starting materials.

1). The dipotassium cyanodithioimidocarbonate salt (1) has prepared based on the literature procedure[73] in which the cyanamide on reaction with carbon disulphide in ethanolic potassium hydroxide under one-pot process to form a yellow color compound-1

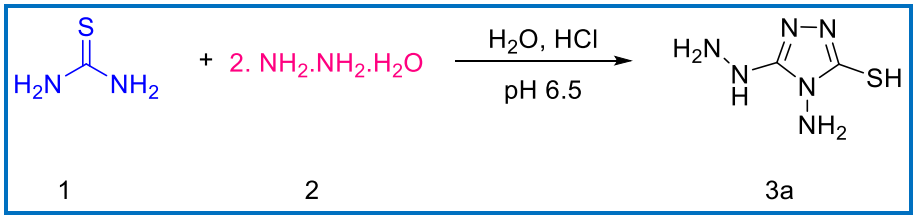


1

2). 1,2,4-triazole compound-3 has been prepared according to literature reports. In this the dipotassium cyanodithioimidocarbonate salt (1) was reacted with hydrazine hydrate (2) in presence of acetic acid at RT to produce a white color solid compound 5-amino-4*H*-1,2,4-triazole-3-thiol.



3). 4-Amino-5-hydrazienyl-4*H*-1,2,4-triazole-3-thiol was prepared by using literature procedure.[74]



Aims and objectives of the work.

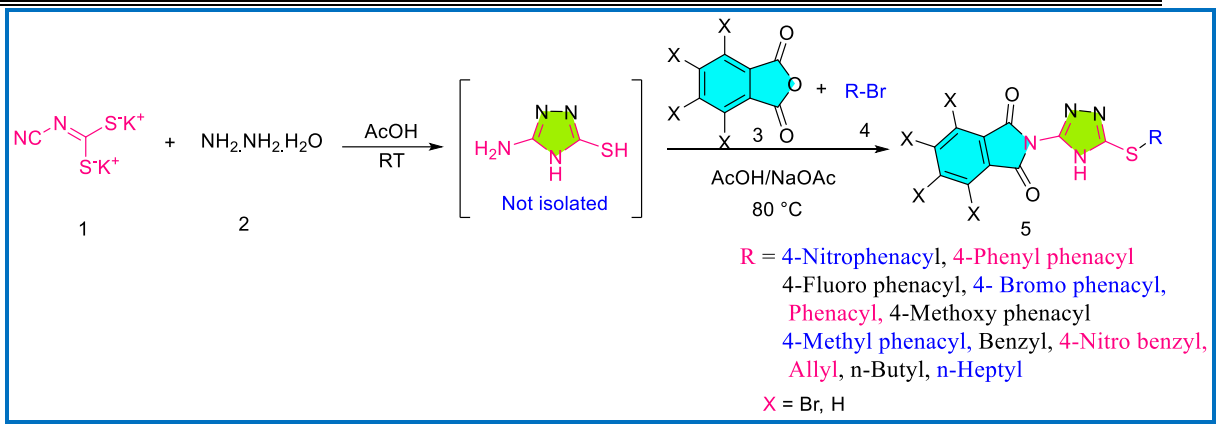
From the preceding review, MCRs are important for the synthesis of new heterocyclic compounds. MCRs provide rapid access to high quality compound libraries. MCRs ideally suited the new demand, and this in turn fueled more interest in the earlier developed reactions and in the invention of similar or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

1. To develop an efficient, environmentally benign facile methods for the synthesis of biologically potent molecules.
2. Evaluation of pharmacological activities of the newly synthesized heterocyclic compounds.
3. To develop facile, efficient eco-friendly synthetic methods for the preparation of different heterocyclic motifs.
4. To characterize the synthesized compounds using FT-IR, ^{1}H NMR, ^{13}C NMR, HRMS spectroscopic techniques.

The present research work briefly covers the synthesis of nitrogen and sulfur heterocyclic compounds and their biological activity studies. The present work is divided into seven chapters.

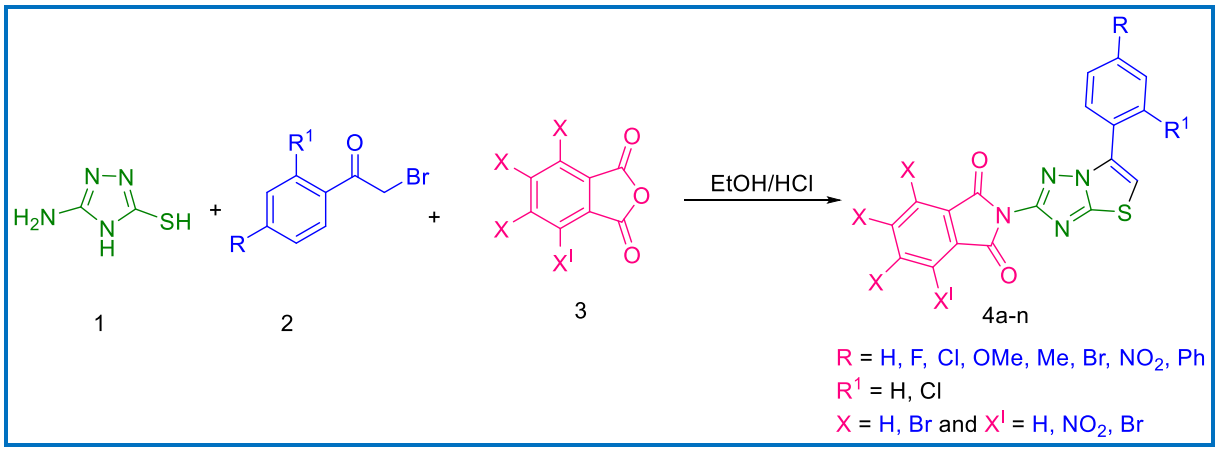
CHAPTER-I: A Review on Multicomponent Reactions and its Applications in the Synthesis of Biologically Active Heterocyclic Compounds.

CHAPTER-II: A facile one-pot four component synthesis of thio alkyl/aryl/benzyl 1,2,4-triazolo isoindoline-1,3-diones, and their biological evaluation, molecular docking studies and DFT calculations

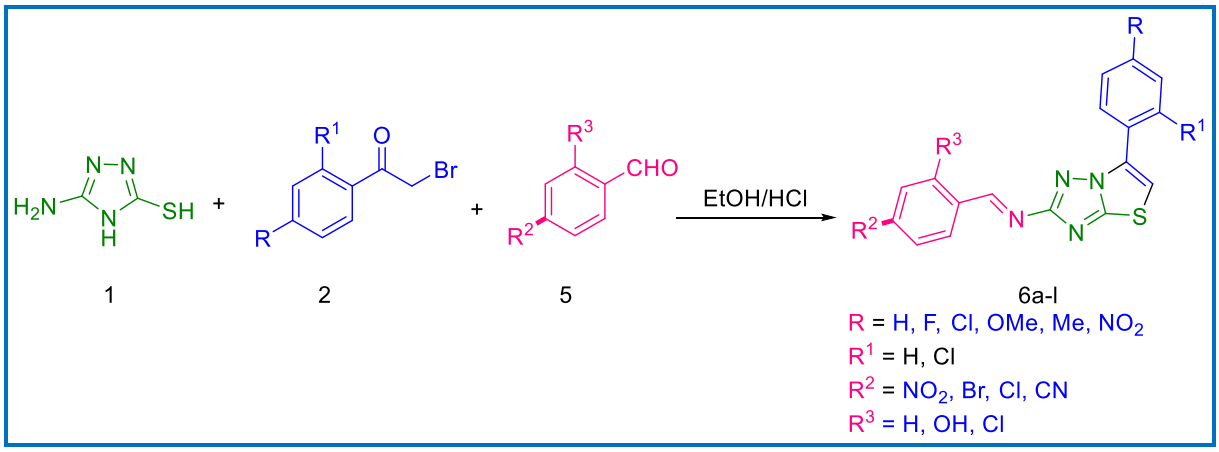


CHAPTER-III: One-pot three component synthesis of fused [3,2-*b*] [1,2,4] triazolothiazole isoindolines and Schiff bases, characterization and targeting glioma *in-vitro* anticancer activity, molecular docking study

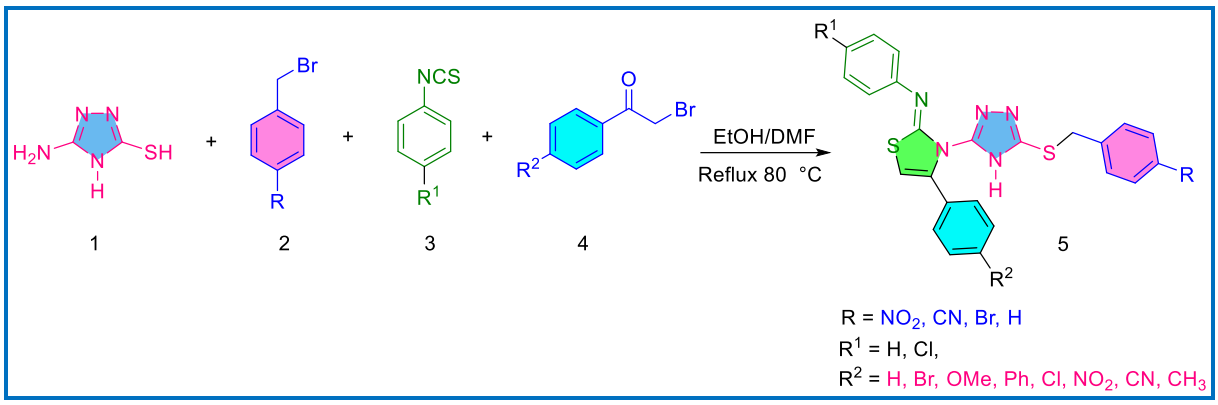
Scheme-2.1



Scheme-2.2



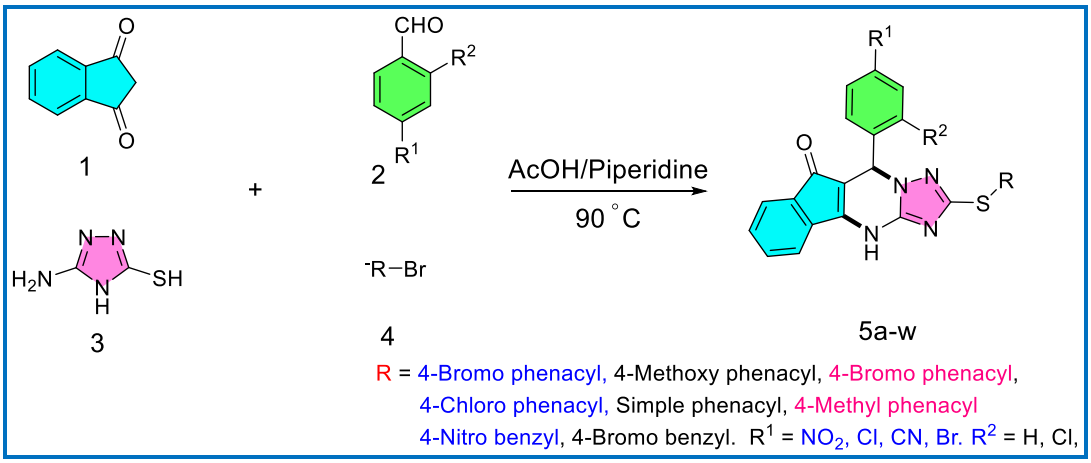
CHAPTER-IV: Synthesis of novel thioalkylated triazolothiazoles and their promising *in-vitro* antiviral activity



CHAPTER-V: This chapter consists of section-A and section- B

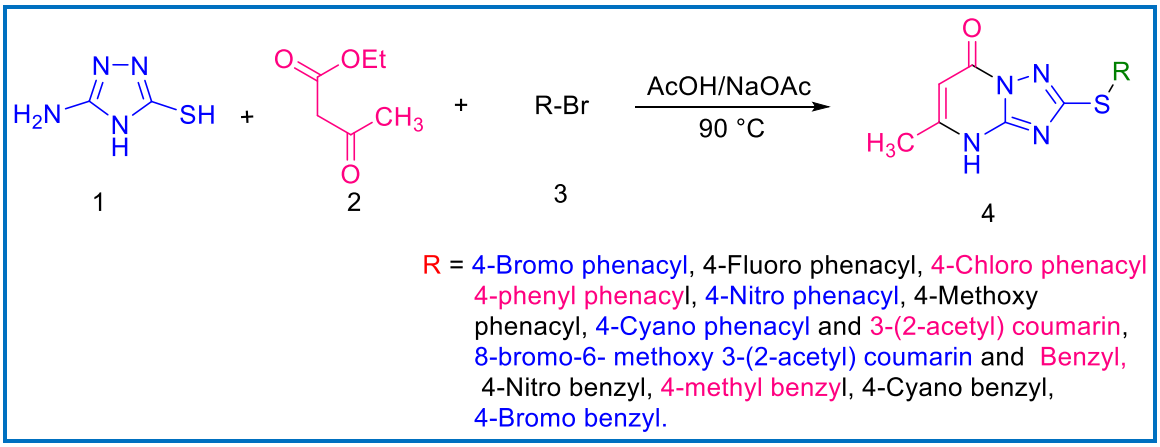
Section-A: Describes the novel one-pot four component synthesis of 1,2,4-triazolo[1,5-*a*] pyrimidines, and their *in-vitro* anticancer evaluation and molecular docking studies

Scheme-4.1

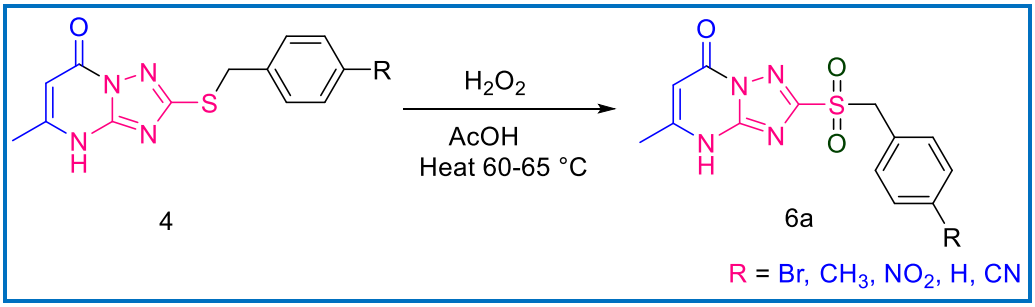


Section-B: Describes the synthesis of new thioalkylated triazolopyrimidinones, sulfones and their biological activity

Scheme-4.2

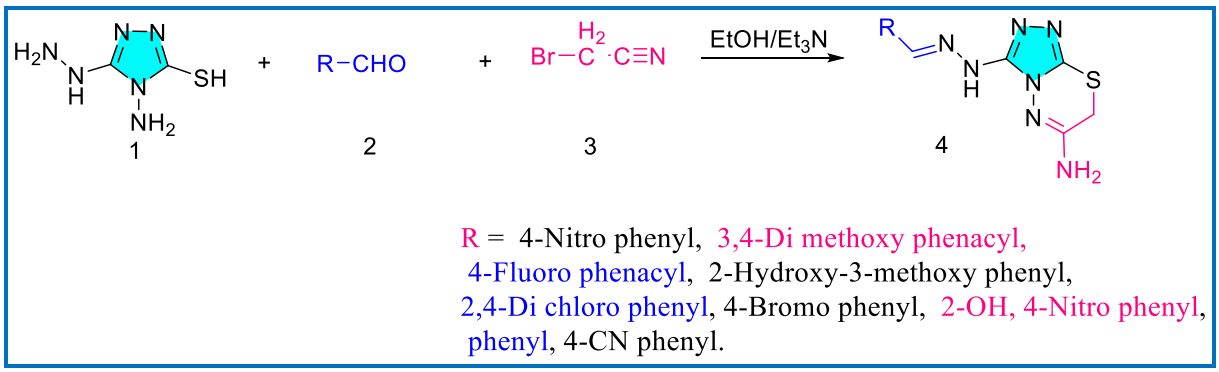


Scheme-4.2b:

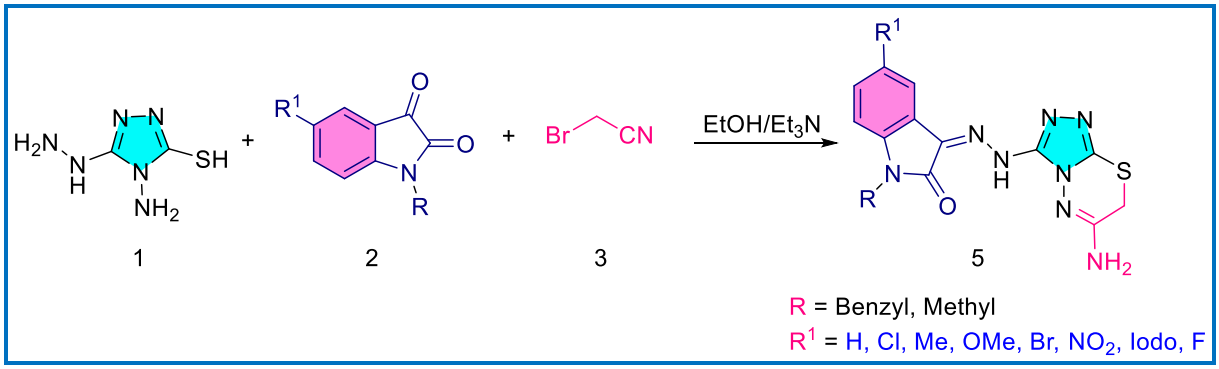


CHAPTER-VI: Novel one-pot synthesis, characterization, DNA binding studies of fused [1,2,4] triazolo [3,4-b][1,3,4] 6-aminothiadiazines and their hydrazineylidene indolin-2-ones, Schiff bases

Scheme-6.0

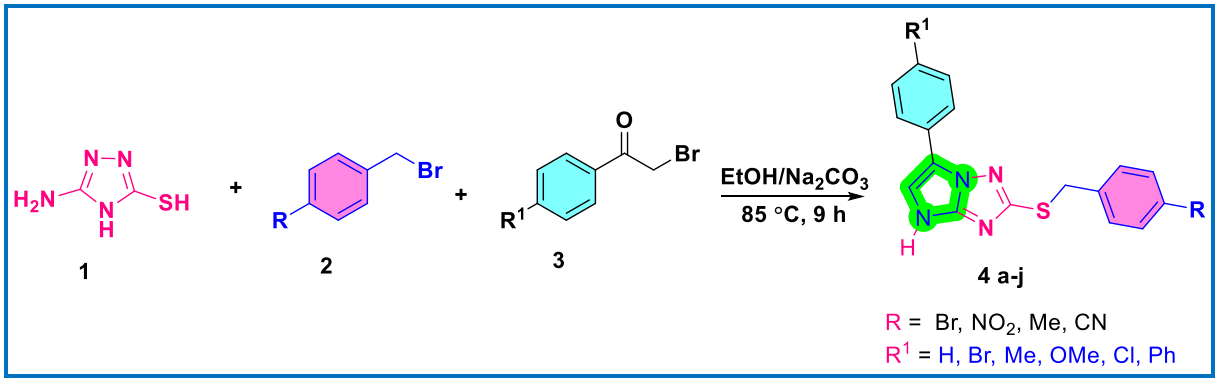


Scheme-6.1

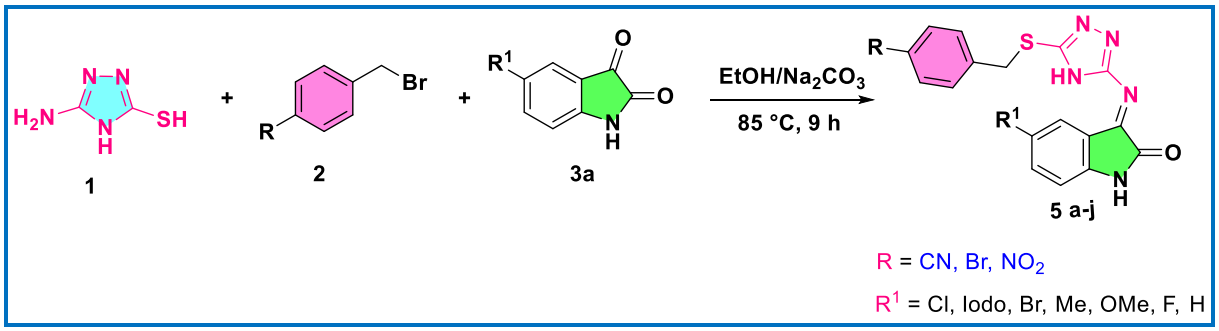


CHAPTER-VII: Novel one-pot synthesis of imidazo[2,1-b][1,2,4]triazoles, 1,2,4-triazolo iminoindoline-2-ones and their in-vitro antibacterial activity, B-DNA study

Scheme-7.1



Scheme-7.2



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CHAPTER-II

A facile one-pot four component synthesis of thio alkyl/benzyl/ phenacyl 1,2,4-triazolo isoindoline-1,3-diones, and their In-vitro cytotoxic activity, molecular docking studies, and DFT calculations

2.1. Introduction

Nitrogen containing heterocyclic systems have widespread applications in the field of both synthetic and medicinal chemistry. In addition to that phthalimide (isoindoline) also known to have significant biological activities. It is used as a precursor for the synthesis azo dyes, anthranilic acid, and saccharin. And also these phthalimide compounds were known to develop various *N*-alkylated substrates, for the construction of a novel heterocyclic compounds for example Click Chemistry. Maleimide, succinamide and phthalimides are having a five membered ring nitrogen atom flanked by two carbonyl groups these are also known as cyclic imides. The cyclic imides were able to produce intramolecular aldol condensation through the formation of C-C bond and C-N bond. In general, the phthalimide (isoindoline) ring is prepared by the reaction between phthalic anhydride and various aliphatic or aromatic primary amines in presence of suitable solvent [1]. The basic core structure of the cyclic imides is having the CO-N(R)-CO unit, and these are hydrophobic, neutral in nature. The phthalimide ring containing compounds contains various biological activities[2,3]. And many of them are used in drugs, one of the best example, the thalidomide is a drug containing isoindoline heterocyclic system and it exists in two enantiomeric forms. (*S*) enantiomer is teratogenic in nature while (*R*) enantiomer is a sedative drug. The R form prevents the birth defect in pregnant women's. Many of the marine natural products contain isoindoline structural component [4]. The **Fig.1** explains the biologically active phthalimide ring containing drug molecules [5-10]. Isoindoline-1,3-diones exhibit various biological activities such as antifungal [11], anti-inflammatory [12.13], anticancer [14,15], antimicrobial [16], antibacterial [17], activity, anticonvulsant [18], cyclooxygenase inhibitor [19], anticholinesterase activity [20], antialzheimer's activity [21] etc. For instance, isoindoline-1,3-dione moiety attached with 1,2,4-Triazole rings are find much attention in the medicinal, and pharmaceutical industry [22,23]. And also the isoindoline ring connected to the substituted sulfone functional group their activity significantly has been increased [24,25]. Owing to the importance of phthalimide and its derivatives in pharmacology, a new synthetic protocols to synthesize this moiety dragged considerable attention in the field of organic chemistry.

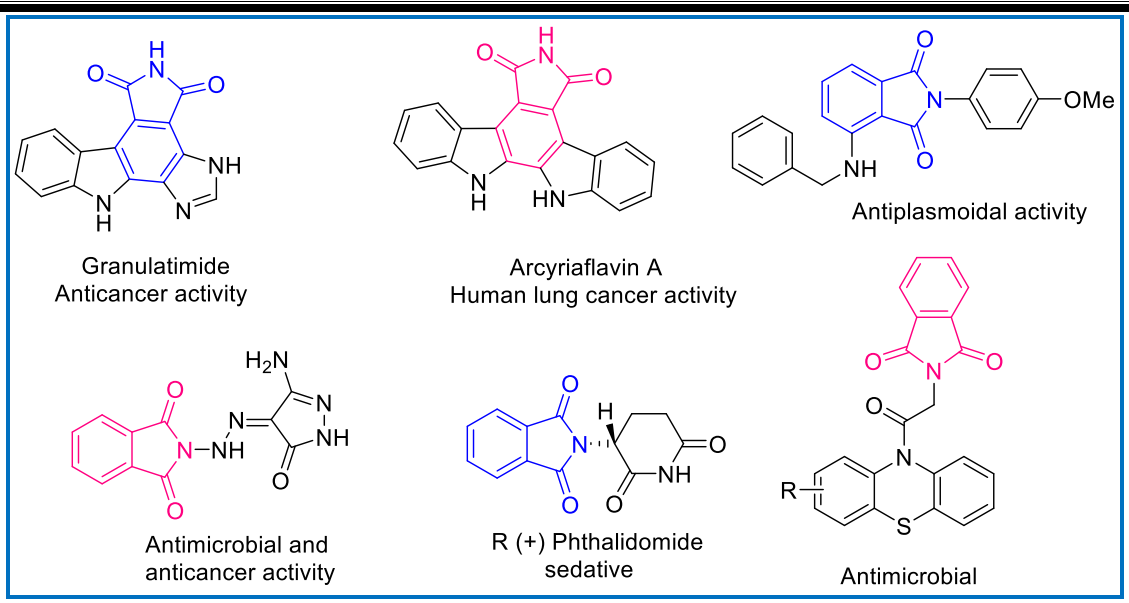
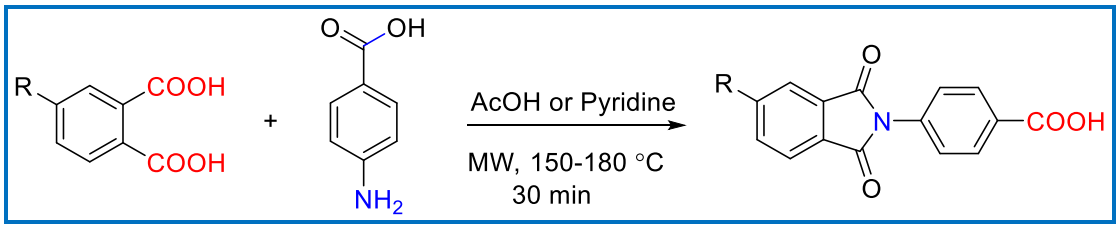


Fig.1. The biologically active drug molecules having substituted phthalimide ring.

The literature reports of phthalimides synthesis

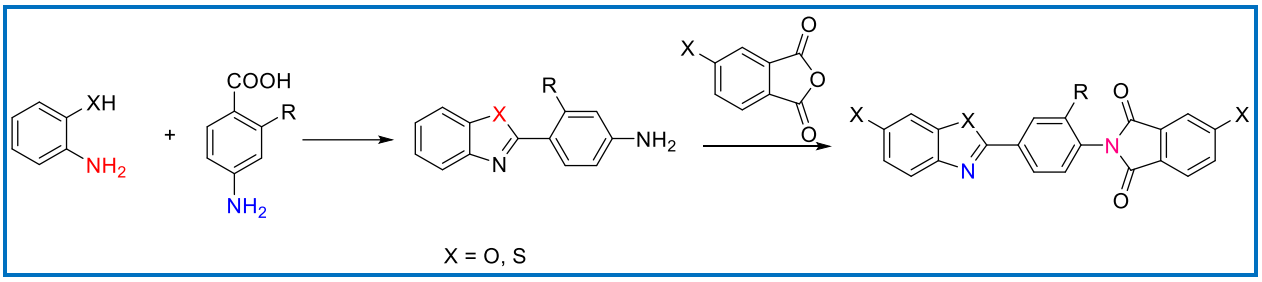
Chorell and co-worker[26] have reported the one-pot synthesis of a series of 2-substituted phthalimides from phthalic acid. In this reaction the condensation of phthalic acid with para-substituted benzoic acid using AcOH or pyridine under microwave irradiation at 150-180 °C leads to produce substituted phthalimides (Scheme-1.1).

Scheme-1.1



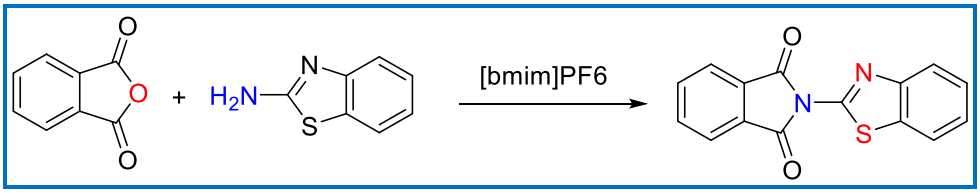
Philoppes and co-worker [27] described an efficient synthesis of the PPA cyclization reaction of ortho-amino phenol with para-amino benzoic acid at 222 °C to obtain a 2-phenyl benzoxazole or 2-phenyl thiazole followed by condensation with phthalic anhydride to achieve a phthalimide derivative. These compounds are exhibiting good antitumor activity in MCF-7cancer cell lines (Scheme-1.2).

Scheme-1.2



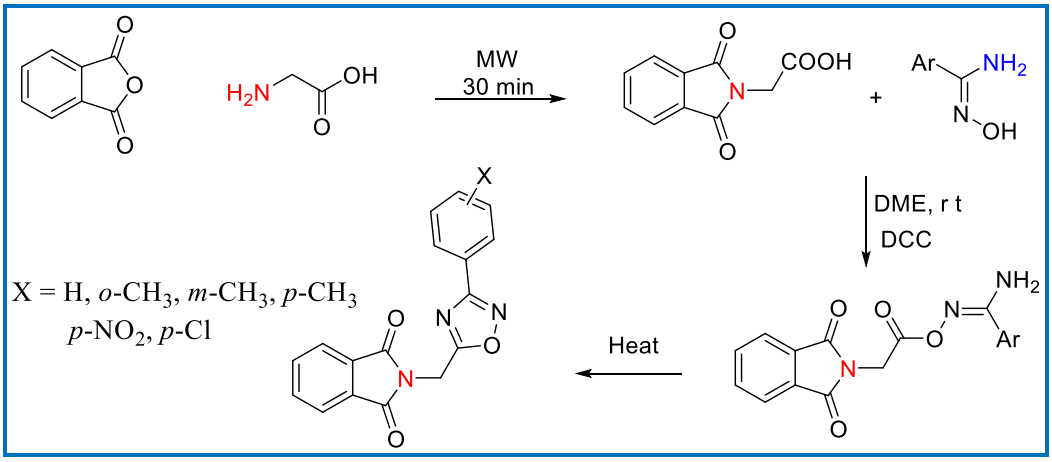
Nagarajan *et al* [28] efficiently synthesized the benzo thiazole substituted isoindolines by the reaction of phthalic anhydride with 2-aminobenzothiazole in presence of ionic liquid [bmim]PF6 at 110 °C for 4 hours to afford the good yield of the product. And these compounds have been found to have anti-angiogenic activity (Scheme-1.3)

Scheme-1.3



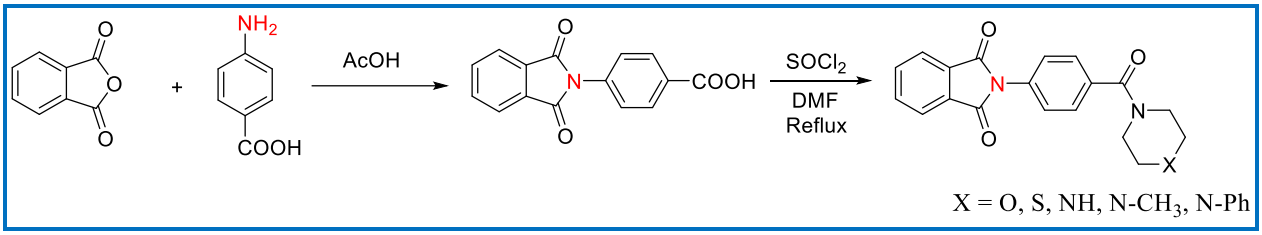
Antunes *et al*[29] developed the microwave assisted synthesis of phthalimide oxadiazole derivatives. The condensation reaction of phthalic anhydride with amino acid (glycine) in the absence of solvent to form *N*-phthaloyl glycine then followed by reaction with oxime under DCC/DMF to produce the oxime of ester. After cyclization to obtained the phthalimide oxadiazoles. These derivatives have shown antianalgesic activity (Scheme-1.4)

Scheme-1.4



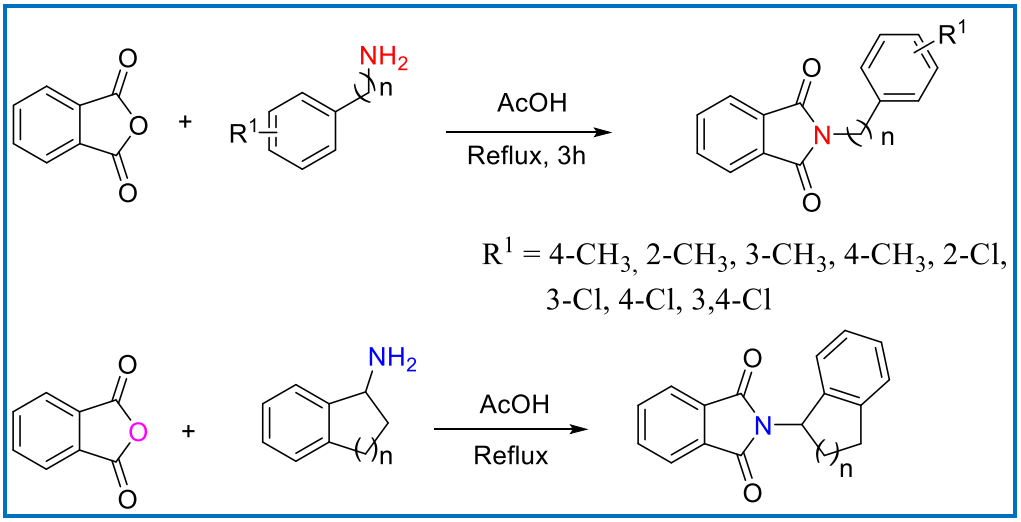
Lima *et al* [30] published a functionalized phthalimide derivative in which the reaction of phthalic anhydride with *p*-amino benzoic acid in acetic acid under reflux condition to generate a isoindoline benzoic acid then reaction with $SOCl_2$ in DMF under reflux condition to give a piperazine substituted phthalimide compounds. These derivatives have shown anti-inflammatory activity has shown in Scheme-1.5.

Scheme-1.5



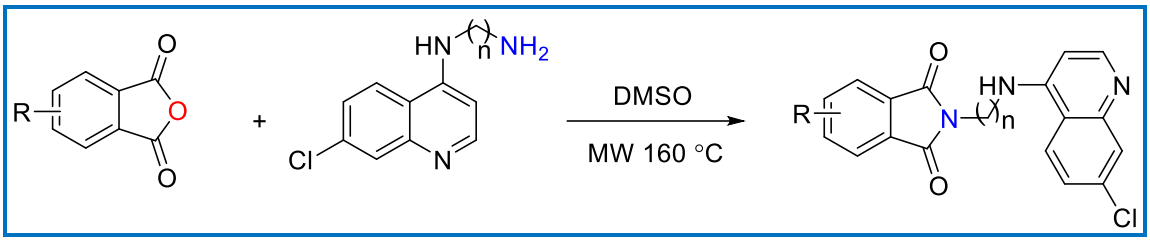
Capitosti *et al* established a series of phthalimides.[31] In this the reaction phthalic anhydride with primary amines in glacial acetic acid under reflux for 3 h to obtain a phthalimide derivatives (Scheme-1.6). These analogue compounds demonstrated angiogenesis and prostate cancer activity.

Scheme-1.6



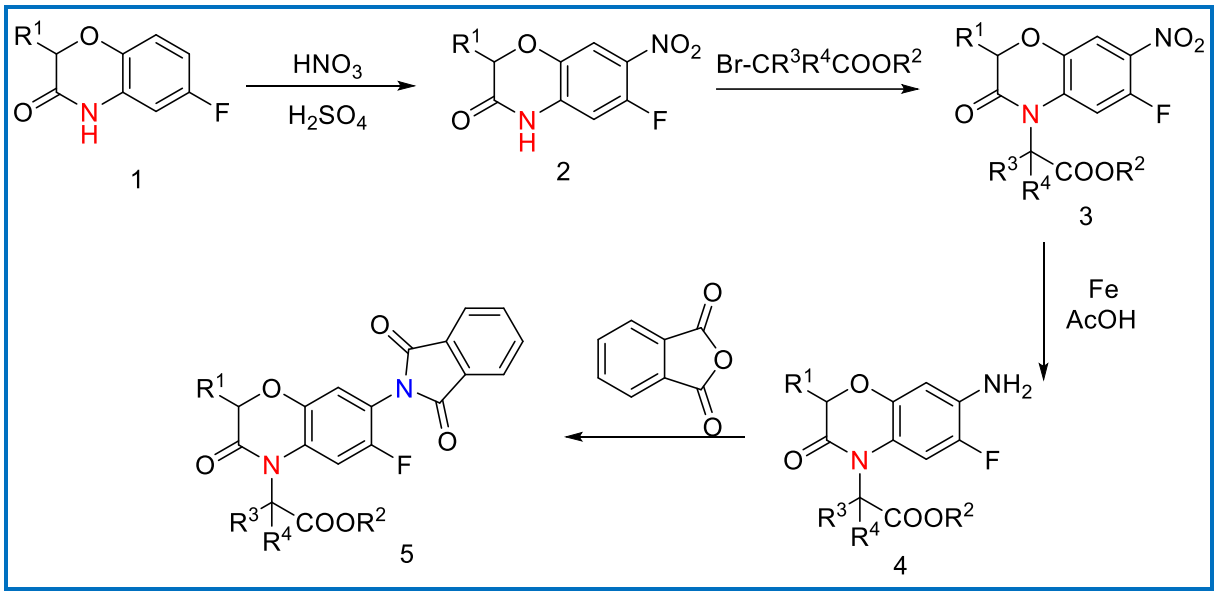
Rani *et al* [32] reported microwave synthesis of 4-amino quinoline phthalimides by the reaction of substituted phthalic anhydrides with 4-aminoquinolines in DMSO which lead to produce a quinoline substituted phthalimides. These molecules have displayed promising anti-plasmoidal activity (Scheme-1.6)

Scheme-1.7



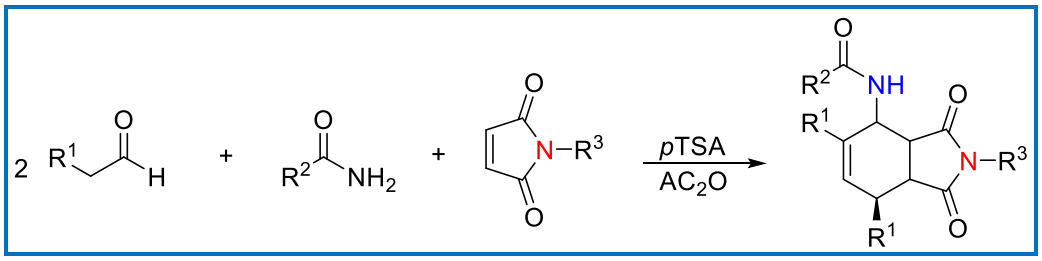
Huang *et al* [33] published the isoindoline ring containing final compounds. Benzoxazinone on reaction with HNO_3 followed by *N*-alkylation then NO_2 group has been reduced by Fe/AcOH to afford a primary amine compounds. This on, further reaction with phthalic anhydride to produce a phthalimide derivatives 5. These compounds were exhibited promising herbicidal activity.

Scheme-1.8



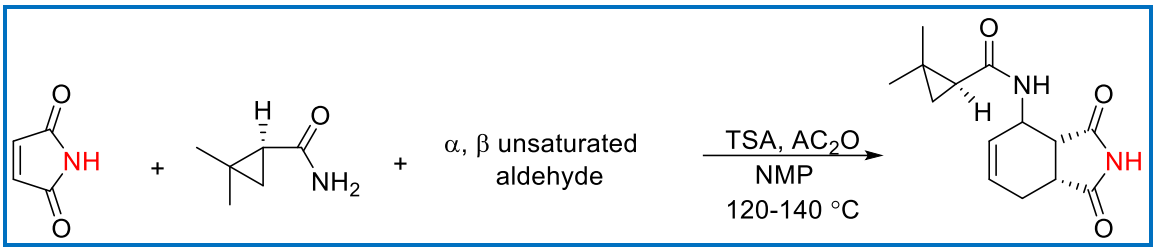
Neumann *et al* [34] synthesized one-pot protocol for the synthesis of cyclohexane substituted phthalimide derivatives *via* hetero Diels-Alder cycloaddition reaction of acetamide, two equivalents of active methylene containing aldehydes with maleimide in presence of *p*TSA/AC_2O to produce a phthalimide analogue compounds with good yield 90-92% shown in scheme-1.9.

Scheme-1.9



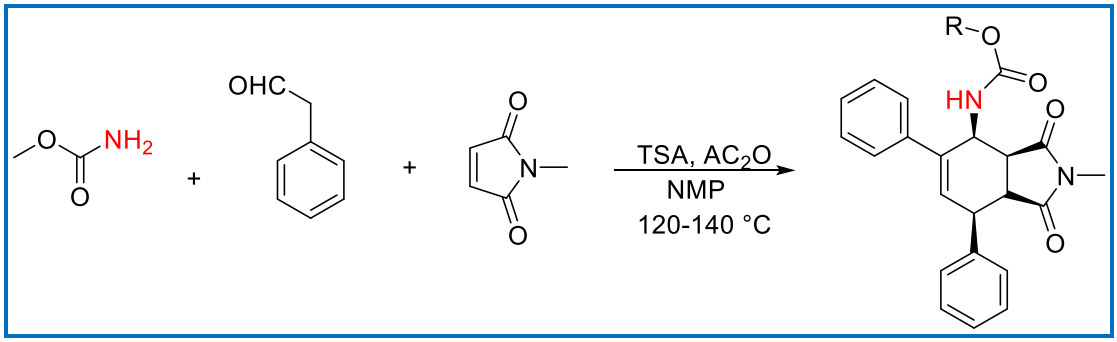
Jacobi Von Wangelin *et al* [35] reported an efficient one-pot protocol for phthalimide derivatives by reacting chiral amides and chiral α, β unsaturated aldehydes with maleimide in presence of TSA/AC_2O at 120-140°C. (Scheme-1.10).

Scheme-1.10



Strubing *et al* [36] developed the multicomponent synthesis of carbon-heterocyclic imides by the reaction of amido ester, α, β unsaturated aldehydes with maleimide in presence of (dipolar aprotic solvent) NMP at 140 °C. Which has shown in scheme-1.11

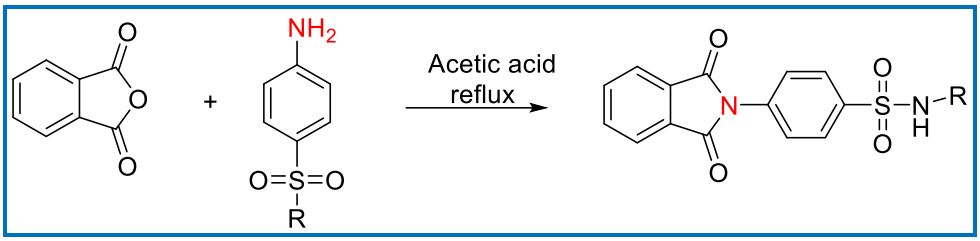
Scheme-1.11



Santos *et al* [37] synthesized substitute sulfonamides the condensation of phthalic anhydride with different substituted sulfonamide's in acetic acid at reflux to produce a phenyl sulfonamide

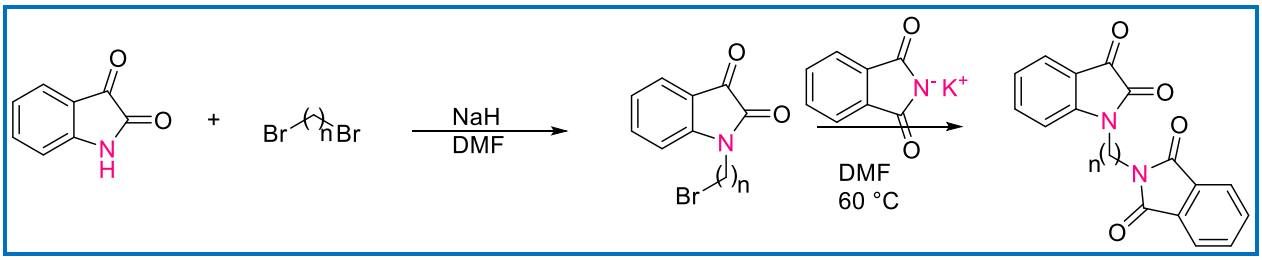
substituted phthalimide compounds (Scheme-1.12). And these derivatives have identified as antimycobacterium tuberculosis activity.

Scheme-1.12



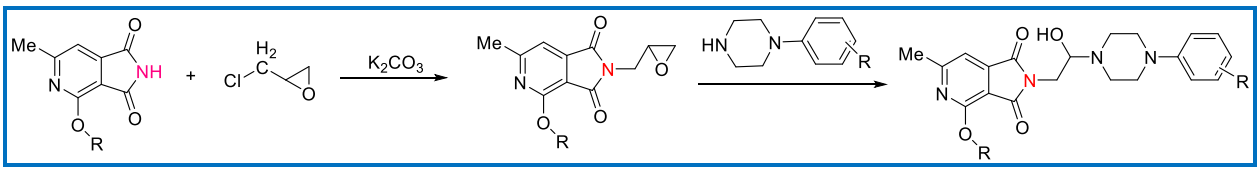
Singh *et al* [38] developed the synthesis of bromo alkyl isatins. These were synthesized through the reaction of isatin with di bromo alkane in NaH/DMF to form a *N*-alkyl isatin then subsequent reaction with potassium phthalimide in DMF at 60 °C (Scheme-1.13). These substrates have shown cytotoxic activity.

Scheme-1.13



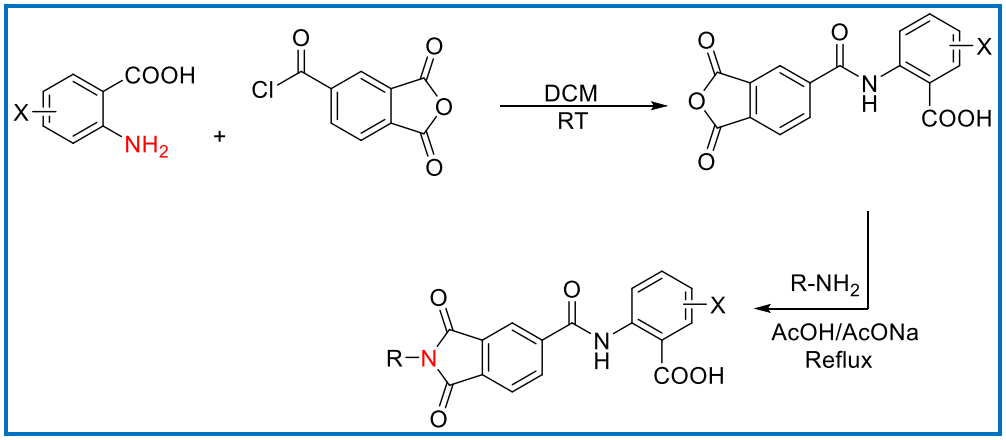
Sladowska *et al* [39] developed pyrrolo pyridine 1,3-diones by reaction with epichloro hydride in potassium carbonate to form a *N*-2,3-epoxy propyl derivative then treated with *N*-alkyl piperazine in presence of EtOH/NaOEt to produce a target product with good yields. And these compounds show pharmacophore properties (Scheme-1.14).

Scheme-1.14



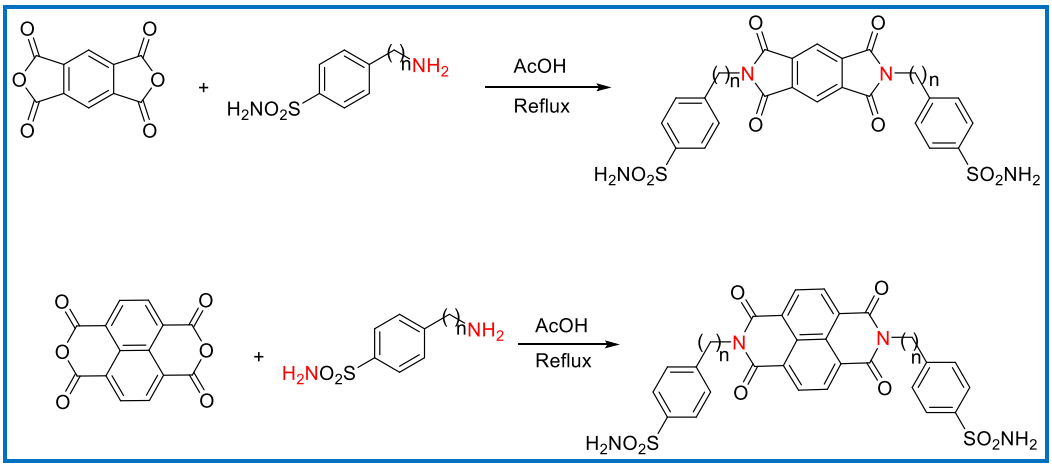
El-Azab *et al* [40] synthesized a series of phenyl isoindoline-5-carbaximido benzoic acid from trimellitic anhydride reaction with anthranilic acid in presence of DCM at rt initially to generate a 1,3-dihydro iso benzo furan-5-carboxamide benzoic acid. These compounds on further reaction with different primary amines in AcOH/NaOAc under reflux condition to give the title compounds (Scheme-1.15).

Scheme-1.15



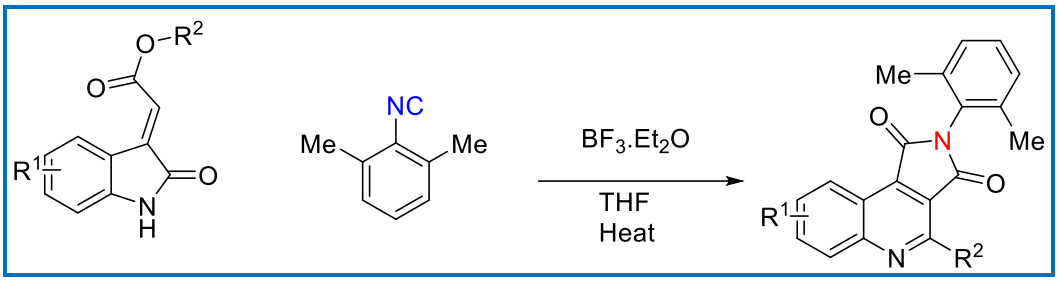
Angeli *et al* [41] published tetra hydro benzophenanthrolines and dihydro pyrrole isoindole dual sulfonamides by using the reaction of polycyclic 1,3-diones with benzene sulfonamide to generate cyclic imides. These moieties show carbonic anhydrase inhibitory activity. (Scheme-1.16).

Scheme-1.16



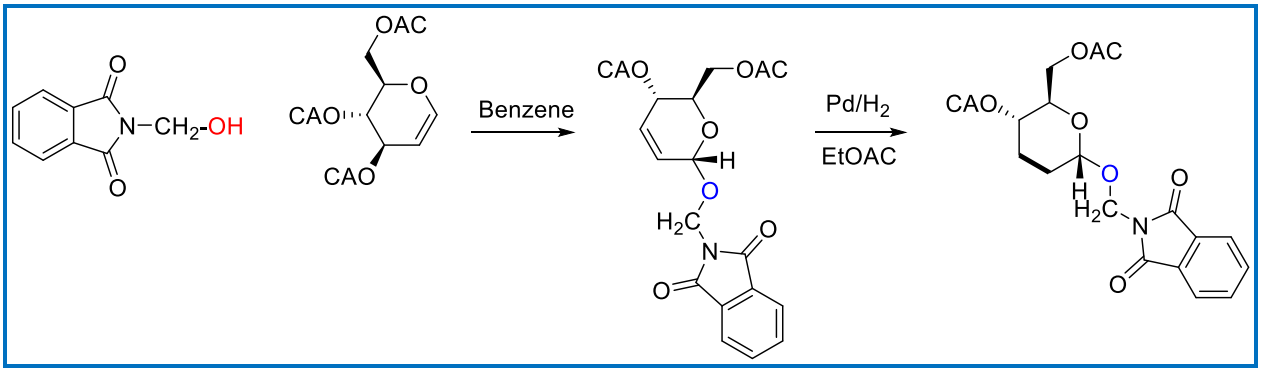
Li *et al* [42] synthesized quinazoline derivatives the cycloaddition reaction of methyleneindolinone with isocyanide in the presence of boron trifluoride etherate ($BF_3.OEt_3$) under heating condition.

Scheme-1.17



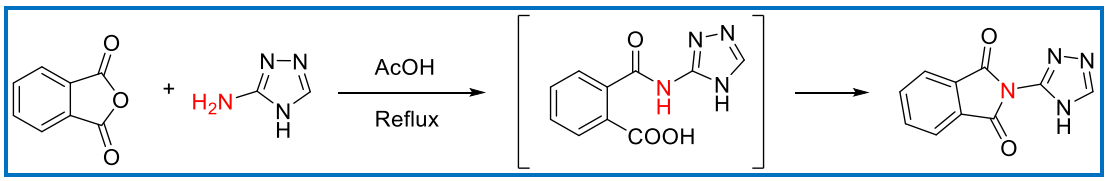
Srivastava *et al* [43] efficiently developed diacetyl-2,3-di deoxy-α-D erythro-hex-2-enopyranoside compound by condensation of N-hydroxy methyl phthalimide with pyranose substrate in presence of benzene/BF_3.OEt_2 at RT by Ferriers method. Then the double bond of sugar was reduced with Pd/H_2 to give saturated derivative with good yield. These compounds active against hypolipidemic activity shown in scheme1.18.

Scheme-1.18



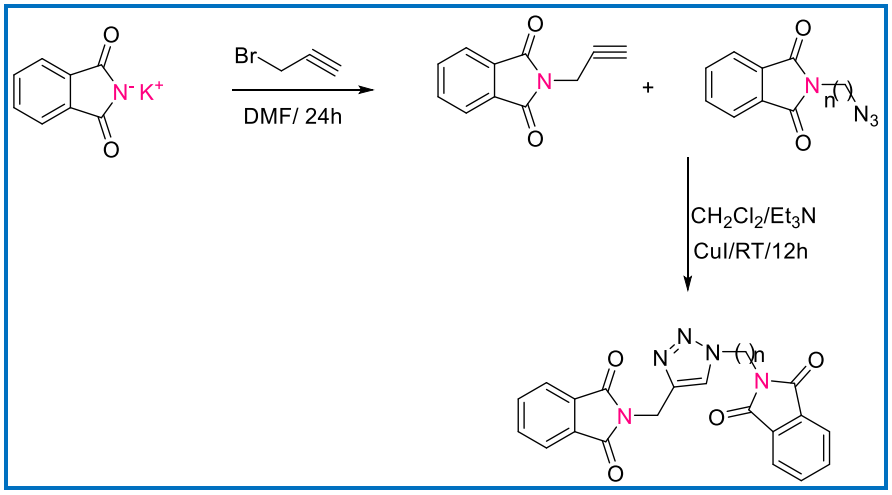
Sena *et al* [44] reported the reaction of phthalic anhydride with amino 1,2,4-triazole under reflux condition in presence of acetic acid. Overall in this initially ring opening reaction take place then cyclization leads to afford a 1,2,4-triazolo phthalimide derivative with high percentage of yield.

Scheme-1.19



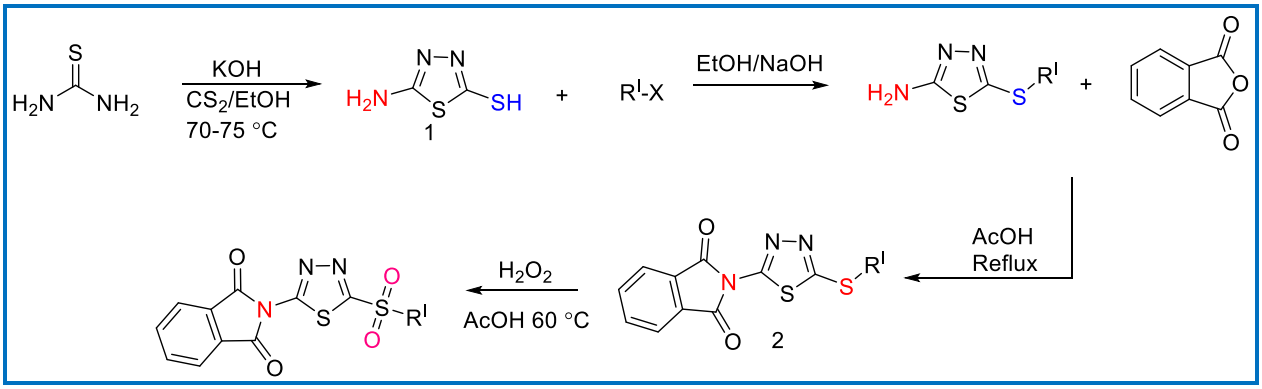
Assis *et al* [45] developed the reaction of potassium phthalimide with propargyl bromide in the presence of DMF/rt to generate N-propargyl phthalimide. This compound further involves in click reaction with *N*-azido alkyl phthalimides in presence of CuI to form a bis-phthalimide 1,2,3-triazole shown in scheme-1.20.

Scheme-1.20



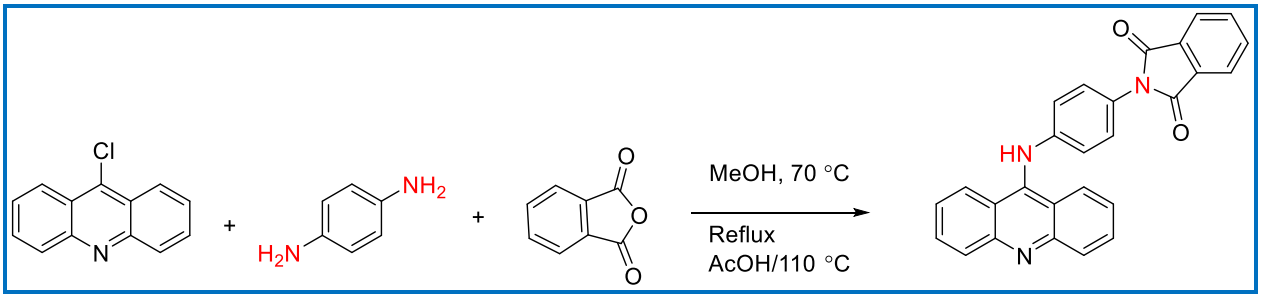
Wang *et al* [46] reported the synthesis of 2-*N*-phthalimido 5-sulfonyl 1,3,4-thiadiazoles. The condensation of thiourea and carbon disulfide under reflux with KOH/EtOH at 70 -75 °C to produce a five membered cyclized intermediate 1,3,4-thiadiazole, the free NH_2 and SH functional groups on compound further reaction with alkyl halides and phthalicanhydride to form the corresponding compound-2. Furthermore, the sulphide group converted into sulfone by using H_2O_2 in the presence of acetic acid at 60 °C. These derivatives have shown anticancer activity. (Scheme-1.21)

Scheme-1.21



Mane *et al* [47] reported a series of new anticancer compounds acridine derivatives through the one-pot reaction of 9-chloroacridine, benzene-1,4-diamine and phthalic anhydride in presence of methanol and acetic acid at 110 °C has shown in scheme-1.22.

Scheme-1.22



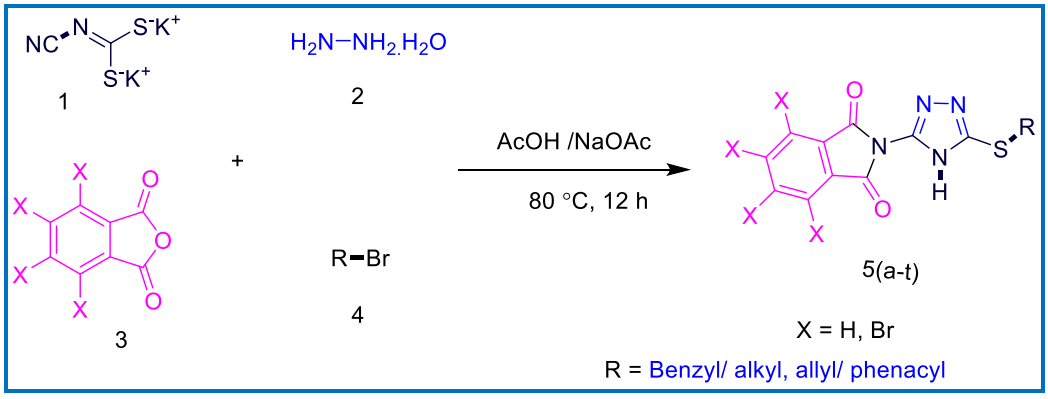
2.2. Present work

Dipotassium cyanodithioimidocarbonate salt (**1**) was prepared by the reaction of carbon disulphide, cyanamide in ethanol/KOH and stirred at rt for 24 h to form a yellow color solid product (1) [48, 49]. And phthalic anhydride, alkyl halides, phenacyl bromides were purchased form chemical suppliers.

Synthesis of thioalkyl 1,2,4-triazolo isoindoline-1,3-diones. (Scheme-1)

Alkyl/aralkyl/phenacyl thiotriazolyl isoindoline-1,3-diones were synthesized by the reaction of dipotassium cyanodithioimidocarbonate (1) salt with hydrazine hydrate (2), phthalic anhydride (3) and alkyl/aralkyl/phenacyl bromides (4) using acetic acid and sodium acetate *via* a one-pot four-component synthesis. The good yields of the products were obtained in a short period of time.

Scheme-1: Synthesis of alkyl 1,2,4-triazolo isoindolines.

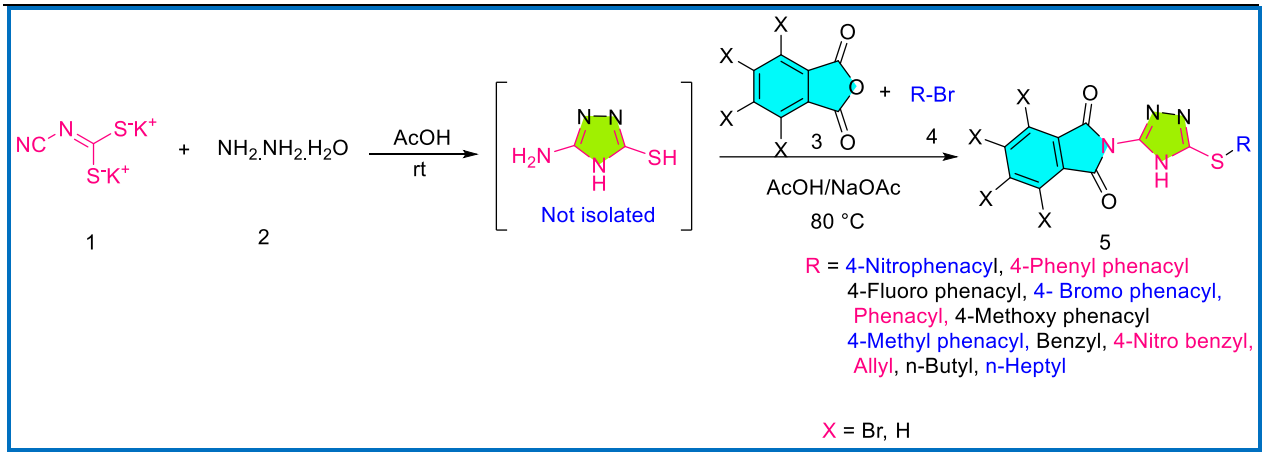


Reaction conditions*:* AcOH/NaOAc at 80 °C for 12 h under multicomponent process.

2.2.1 Results and discussion:

Keeping in view of the biological importance of phthalimides and triazoles we became interested in exploring these motifs for their biological activity by attaching simple thioether linkage at the C_2 position of 1,2,4-triazole. The target compounds (**Scheme-1**) were synthesized by a four-component one-pot synthesis involving condensation of dipotassium cyanodithioimidocarbonate salt (1), hydrazine hydrate (2), phthalic anhydride (3) followed by the addition of alkyl/aralkyl/phenacyl halides (4) in AcOH/NaOAc at 80 °C. The uniqueness of this reaction is that without isolation of potential Intermediate-I, we have carried out the reaction. In order to reduce the number of steps and the reaction time for the formation of **5 a-t**.

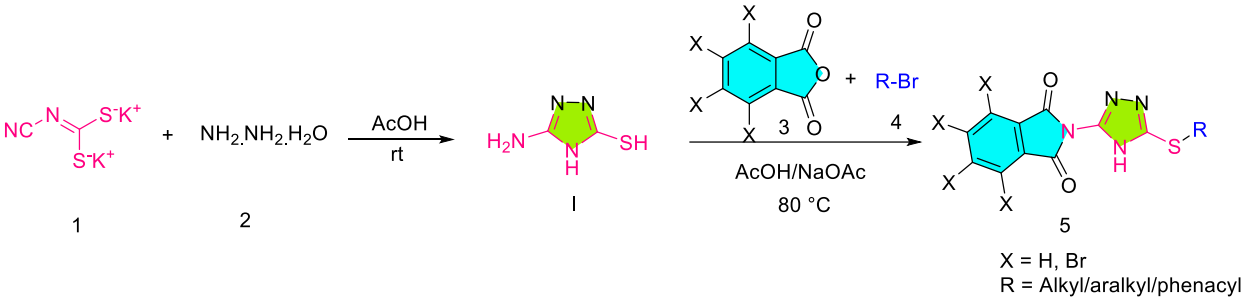
Scheme-1: Method-I Outline schematic representation of final compounds (**5 a-t**).



Reaction conditions: **1** (1.0 mmol), **2** (1.5 mmol), **3** (1.0 mmol), **4** (1.0 mmol) was taken in AcOH/NaOAc heat at 80 ˚C for 12 h.

Method-I: The final compounds **5a-t** can also be synthesized by an alternative procedure (Method-II) involving condensation of **1** and **2** in presence of acetic acid to give an intermediate **I** (Isolated). This is further reacted with alkyl/aralkyl/phenacyl bromides and phthalic anhydride using acetic acid and NaOAc to give a final compounds **5a-t.** In this one-pot three-component condensation reaction yield of the products was less (70%) compared to that by Method-I. Compounds obtained by both methods were found to be identical by their mixed m.p measurements, Co-TLC and superimposable IR spectra. In the present study out of two methods, we have followed Method-I for the synthesis of **5 a-t.** This is due to its formation of high yields, and short reaction time.

Scheme-2: Method-II represents the reaction was carried out without isolation of Intermediate-I



Scheme-2: Method-II explains one-pot four-component synthesis.

The optimization of the reaction was carried out for compound **5j** (**Table-2**) in methanol, ethanol and acetic acid in this conditions the yields are low. On the other hand, when toluene was used as solvent there is no reaction even at 60 °C. Whereas acetic acid in ethanol, acetic acid in HCl were used, there is no appreciable change in the yield of the product. Finally, we have carried out the reaction with 1.0 mmol of sodium acetate in acetic acid. Fortunately, high yield of the product was obtained with 85% yield at 80 °C.

Table 1 The optimization reaction conditions[a]. Solvent and catalyst screening for the one-pot four component synthesis using sodium acetate as a catalyst.

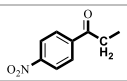
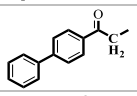
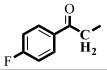
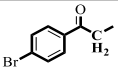
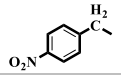
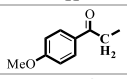
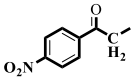
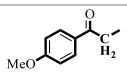
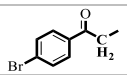
Entry	Solvent	Catalyst	Temp (°C)	Time (h)	Yield (%)[b]
1.	MeOH	-	60	12	12
2.	EtOH	-	60	12	18
3.	AcOH	-	60	12	20
4.	Toluene	-	60	12	n.r[c]
5.	AcOH	HCl	70	14	30
6.	AcOH	EtOH	70	14	39
7.	AcOH	NaOAc(0.5 mmol)	65	14	50
8.	AcOH	NaOAc (0.1 mmol)	70	14	80
9.	AcOH	NaOAc(1.5 mmol)	80	12	85[d]

[a]**Reaction conditions**: 1 (1.0 mmol), 2 (1.0 mmol), 3 (1.0 mmol), 4 (1.0 mmol). [b]Percentage yield of the product. [c]n. r = no reaction. [d]AcOH/NaOAc (1.5 mmol) at 80 °C for 12 h to get 85% yield.

Therefore, among the screened solvents, used catalysts, and conditions AcOH and NaOAc under reflux gave desired product with 85% yield in 12 hours. The optimized conditions of the title compound **5j** as shown in **Table-1**.

After getting the optimal conditions we confirmed sodium acetate as the best catalyst in acetic acid, by using optimization conditions we have generalized this method and the scope of this reaction has been extended to other phenacyl bromides, alkyl, allyl and benzyl bromides to acquire the final compounds (**5 a-t)** in good yields with the above mentioned enthusiastic results as an incitement and considering the significance of this methodology, it was delineated to synthesize the products with various substituents. The variation in the yields of the final products probably due to the electronic factors.

Table 2: Substrate scope of alkyl/aralkyl/phenacyl thiotriazolyl isoindoline-1,3-diones (**5a-t**).

Product	X	R	Time (h)	Yield (%)
5a	H		15	85
5b	H		16	89
5c	H		13	80
5d	H		14	88
5e	H		14	89
5f	H	$H_3C-(CH_2)_2\cdot CH_2-$	15	81
5g	H		13	84
5h	Br		10	90
5i	H	$-H_2C-\underset{H}{C}=CH_2$	15	86
5j	H		12	85
5k	Br		10	91
5l	Br		10	90
5m	Br	$-H_2C-\underset{H}{C}=CH_2$	11	88
5n	Br		10	87
5o	Br		10	92

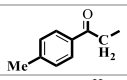
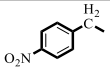
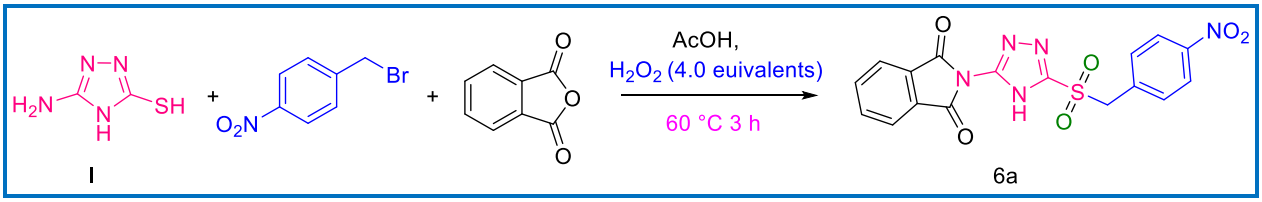
5p	Br	$H_3C-(CH_2)_2-CH_2-$	11	80
5q	Br		10	85
5r	H		14	82
5s	Br	$H_3C-(CH_2)_5-CH_2-$	10	81
5t	H	$H_3C-(CH_2)_5-CH_2-$	15	81

Table-2. Shows the synthesized derivatives with their yields and conditions.

Further, we have derivatized the sulfide compound into sulfones with the use of oxidizing reagent H_2O_2. In this context, we have converted the sulfide compound 5r (**scheme-1**) into sulfone **6a** with H_2O_2 in acetic acid/EtOH reaction medium. The structure 6a was confirmed by its spectral data. (IR, ^{1}H-NMR, ^{13}C-NMR, Mass spectrum). In the proton NMR spectrum of compound **6a,** the sulfone attached methylene (SO_2-CH_2) two protons appeared as a singlet in the downfield compared with the corresponding thioether attached methylene (S-CH_2) protons.

Scheme-3: Synthesis of sulfone by one-pot three-component condensation reaction.



Reaction conditions*:* **I** (1.0 mmol), 4-nitro benzyl bromide (1.0 mmol), Phthalic anhydride (1.0 mmol), H_2O_2 was taken (4.0 mmol) in EtOH/AcOH heat at 60 °C.

2.2.2 *In-vitro* cytotoxic activity:

The cytotoxicity study has been carried out using *HeLa* cell lines and are expressed MTT assay [50]. Among the tested compounds the three compounds **5m, 5p, 5r** were displayed a notable cytotoxicity in *HeLa* cell line. In the present study, we have observed that the treatment of the *HeLa* cancer cell lines with isoindoline series of compounds at different concentrations by taking 100µL has a control. The obtained results from cytotoxic tests against *HeLa* cell line indicated that all the three compounds **5m, 5p, 5r** exhibited significant cytotoxic effects in comparison with the control. Although significant changes between different compounds in the same concentrations

were not observed (**5m, 5p, 5r**), in compounds **5m, 5p** and **5r** at the highest tested concentrations demonstrated mild cytotoxic effects in *Hela* cell line has shown in the **Fig.2**.

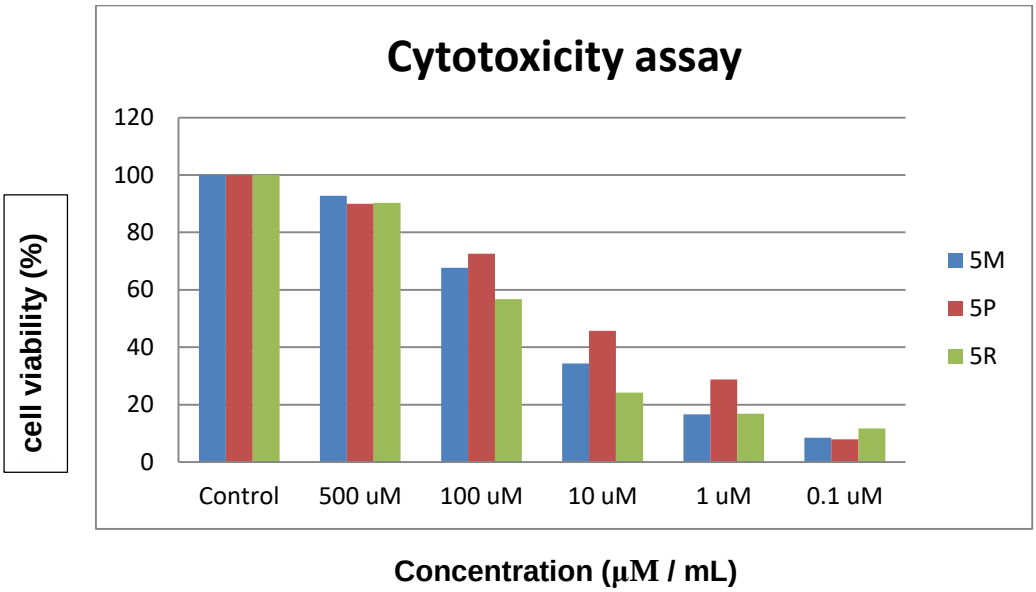


Fig.2: Cytotoxic assay of the compounds **5m, 5p, 5r** at different concentrations

Table-3: Effects of synthesized compounds **5m, 5p, 5r** proliferation was determined using MTT assay.

Compound	Control	100µM	10µM	1µM	0.1µM	IC_{50} µM
5m	100µM	67.65	34.33	16.64	8.44	102.7059
5p	100µM	72.56	45.67	28.77	7.89	68.0517
5r	100µM	56.77	24.23	16.84	11.76	206.875

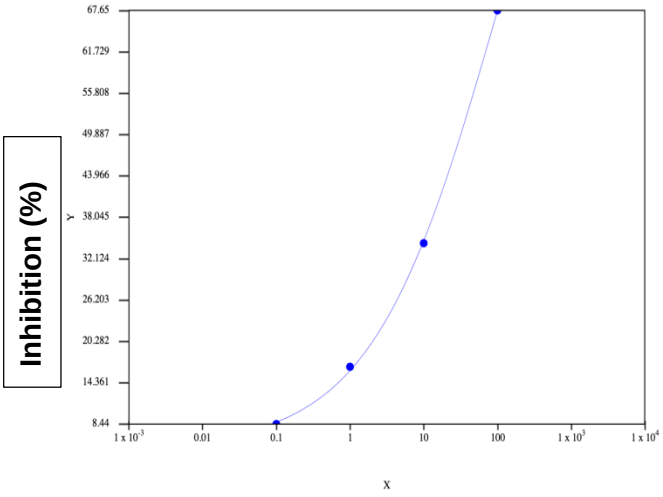
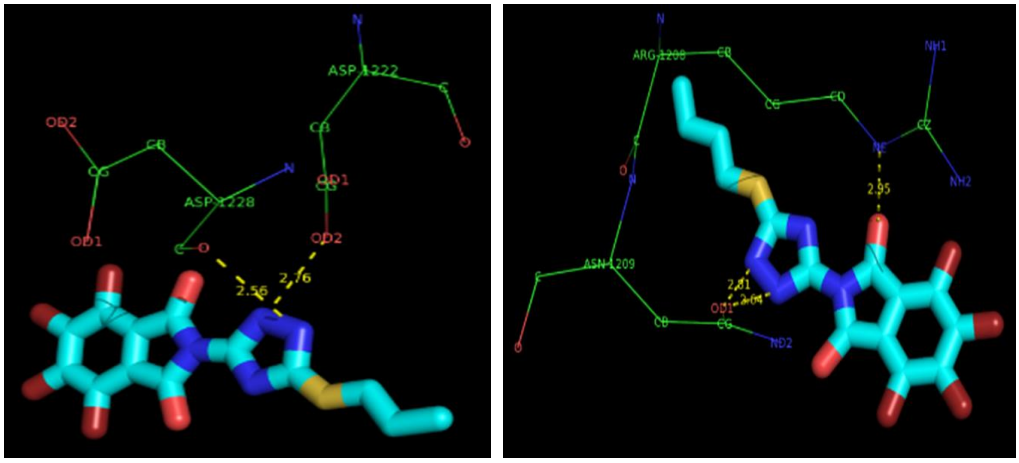


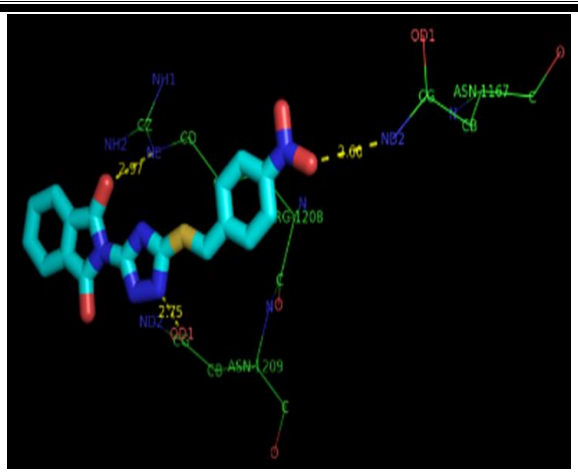
Fig.3: Represents the inhibition coefficient of the **5m, 5p, 5r** molecules in *HeLa* cell lines.

2.2.3 Molecular Docking Studies.

In silco studies revealed that, all the synthesized compounds displayed an excellent binding energies towards the receptor active sites. Molecular docking results were identified on the basis of ideal interacted ligands were examined based on the highest ligand binding poses were identified using the high docking score [51]. Among all the compounds **5m, 5p, 5r** displayed good docking scores. The number of H-bonds, hydrophobic interactions at receptor site of **5m, 5p, 5r** compounds shown in **Fig. 4.** The **Table-4** represents the docking energy, interacting atoms and Hydrogen bond distance.



Compound 5m **Compound 5p**



Compound 5r

Fig.4 Molecular interactions of the receptor with ligands **5m, 5p, 5r**

Table-4 Docking energy values with different interacting atoms and ligands.

Analog	Receptor(4GG5) (Interacting atoms)	Ligand (atoms)	H-bond Distance (Å)	Docking energy (Kcal/mol)
5a	Tyr 1230-NH	O	3.09	-74.652
	Arg 1208-NE	O	3.02	
	Asn 1209-NE	O	3.15	
5b	Asn 1167-ND2	O	2.64	-80.657
	Asp 1164-OD1	NH	3.10	
5c	Arg 1208-O	NH	2.89	-83.986
5d	Asp 1222-N	O	3.10	-76.154
	Asn 1209-OD1	NH	3.61	
5e	Asn 1209-O	NH	2.92	-79.785
	Asp 1222-N	O	3.12	
5f	Met 1160-NH	O	3.10	-80.863
	Pro 1158-O	NH	3.29	-79.851
5g	Asp 1204-OD1	NH	2.65	-83.192
	Asp 1228-O	NH	3.00	
5h	Met 1160-N	O	3.10	-75.804
	Pro 1158-O	NH	3.25	
5i	Asp 1222-N	O	2.79	-81.421
	Ala 1226-O	NH	3.56	
5j	Asp 1204-OD2	NH	2.53	-80.970
	Asp 1222-OD2	NH	2.86	

	Arg 1227-NE	O	3.18	
5k	Asp 1222-N	O	2.82	-75.214
	Arg 1208-O	NH	3.57	
5l	Asp 1222-N	O	2.80	-82.385
	Arg 1208-O	NH	3.11	
5m	Asp 1228-O	NH	2.56	-86.214
	Asp 1222-OD2	NH	2.76	
5n	Asn 1209-OD1	NH	3.00	-76.500
5o	Arg 1208-O	NH	3.07	-80.896
5p	Asn 1209-OD1	NH	2.64	-83.564
	Asn 1209-OD1	OD1	2.81	
	Arg 1208-NE	O	2.95	
5q	Asn 1209-OD1	NH	2.86	-82.645
5r	Asn 1167-ND2	O	2.60	-85.502
	Asn 1209-OD1	NH	2.75	
	Arg 1208-NE	O	2.97	
5s	Asn 1209-OD1	NH	2.86	-82.645

Table-4: Docking energy values of the ligand atoms with amino acid residues.

2.2.4 DFT Calculations:

The geometries of various reactants, intermediates (IMs), transition structures (TSs), and products were fully optimized without any geometrical/symmetrical constraints using density functional theory (DFT) based Becke's three parameter hybrid exchange functional and Lee–Yang–Parr correlation functional (B3LYP) employing the 6-31G* basis set [52, 53]. The stationary points on potential energy surface were characterized as local minima by the frequency calculations at the same level of theory. The TSs were confirmed by the existence of a characteristic single imaginary frequency. All the thermodynamic parameters were calculated in the gas phase at 298.15 K temperature and 1 atm pressure. All the calculations were performed using the Gaussian16 suite of programs. [54]

The proposed mechanism of the formation of **5(a-t)** from **I** and **3** is shown in below**.** The same mechanism is investigated using density functional theory based calculation to probe the energetics of formation of product.

Plausible reaction mechanism (**Fig. 5a**) for the synthesis of title compounds. (**5 a-t**)

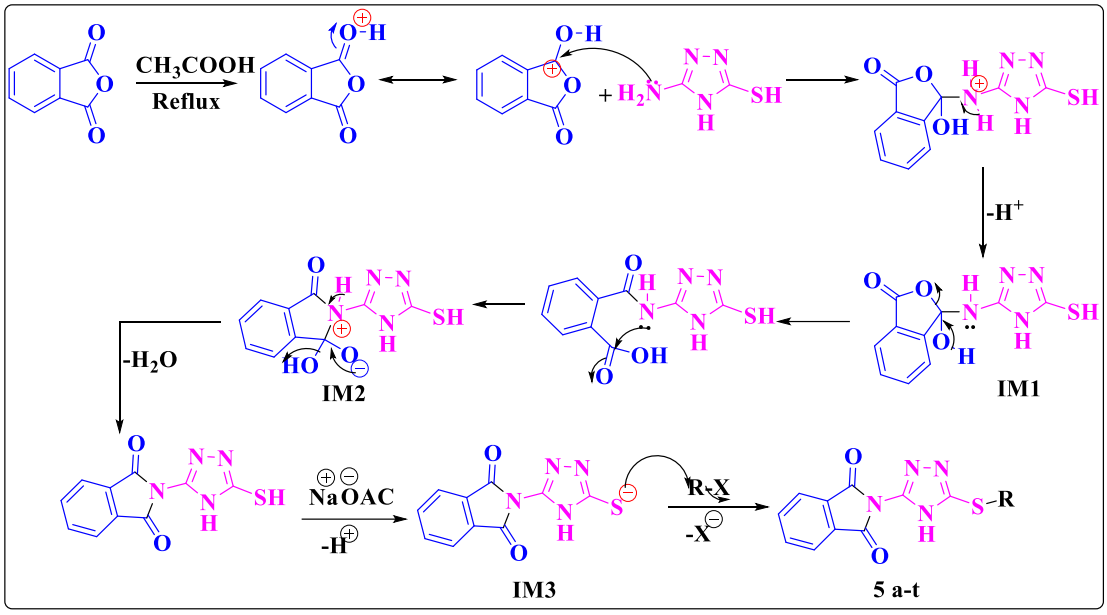


Fig. 5a: mechanism of the reaction

It can be noted from the proposed mechanism that the intermediate **IM3** is vital in the process of product formation. Therefore, the relative energy of formation of **IM3 was** analyzed exclusively. The relative energy profile for the formation of intermediate **IM3** from **I** and **3** *via* various intermediates are shown in **Fig.5**. The same figure also presents the optimized geometries of various TSs. Optimized geometries of reactants and various intermediates are provided in electronic supplementary information along with important geometrical parameters.

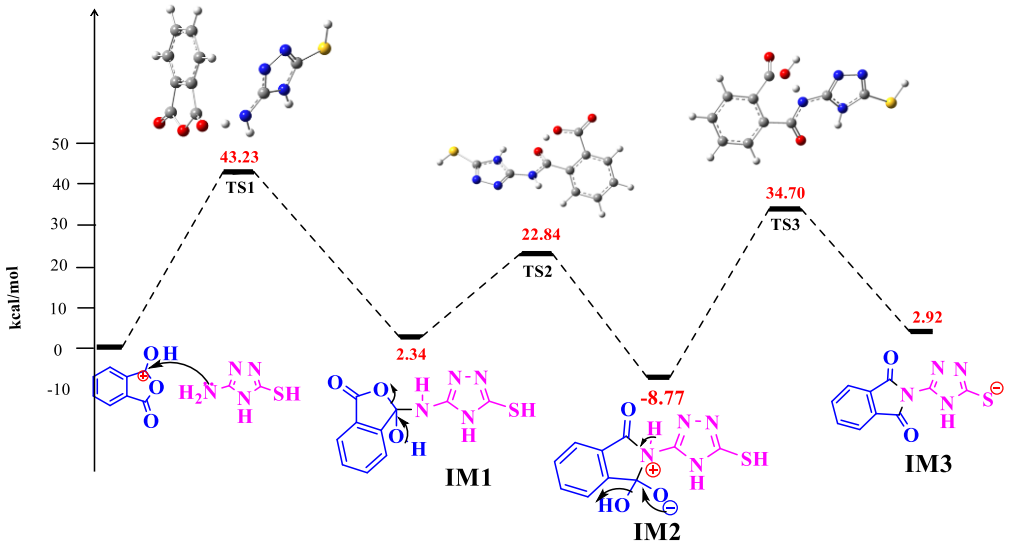


Fig.5: Relative energy profile of step-1 i.e. formation IM3 from reactants **I** and **3**.

This reaction involves a two-step process/mechanism for the completion of the reaction. In the first step of the reaction, the lone pair of N-atom of 5-amino-4*H*-1,2,4-triazole 3-thiol reacts with one of the carbonyl carbon of the phthalic anhydride in the presence of acetic acid give raise to the first intermediate (IM1) having an energy of 2.34 kcal/mol. The bond distance between N of 5-amino-4*H*-1,2,4-triazole-3-thiol and a carbonyl carbon atom of the phthalic anhydride is found to be 1.42 Å. The ring opening in IM1 leads to the form intermediate state (IM2). The calculated energy barrier for the proton rearranging is 25.18 kcal/mol. Comparative analysis of relative energies clearly indicates that the IM2 is energetically more stable than that of reactants. Typically, the calculated relative energy of IM2 is -8.77 kcal/mol. The active intermediate (IM3) formed *via* dehydration of IM2. Calculated relative energy of TS3 and IM3 are 34.70 and 2.92 kcal/mol, respectively. As mentioned, the IM3 involves in the second step of reaction to form a product.

The IM3 reacts with different alkyl bromides to yield the products. The potential energy profile of the formation of a product from IM3-Br_4 is given in **Fig.6**. The geometries of transition state and products are also shown in the same. It can be noted from the same figure that the relative energy of formation of TS needs 24.4 kcal/mol energy. Furthermore, the product **5k** is energetically more stable when compared with the IM3-Br_4.

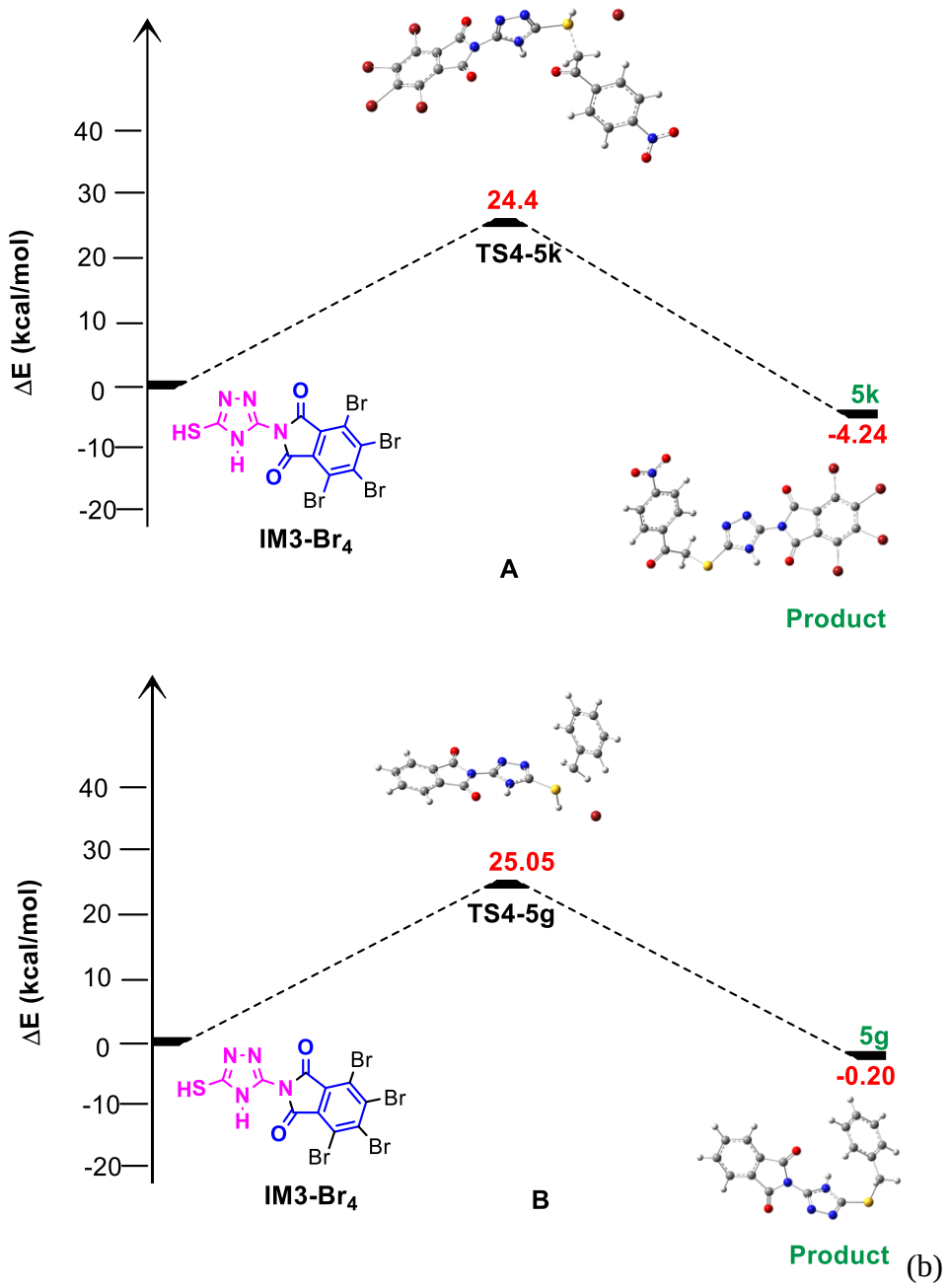


Fig.6*:* Relative energy profile of formation of (A) **5k** and (B) **5g**.

Comparative analysis of the energetics of formation of **5k** and **5g.** This clearly indicates the energetic stability of **5k**. Indeed, the energetics obtained using DFT calculations are clearly akin with that of experimental yield.

2.3. Conclusions

We have developed a novel substituted 1,2,4-triazolo isoindolines containing thioether linkages with a Gabriel phthalimide reaction by using multicomponent approach. The MCR reactions have several advantages, such as no need of drastic reaction conditions, easy work-up procedure, no application of column chromatography for purification of the compounds and products were obtained in a good yield. The newly synthesized compounds were evaluated for them *in-vitro* anticancer activity against *Hela* cell lines. The compounds **5m, 5p, 5r** have shown potent cytotoxic activity. Molecular docking studies and DFT calculations were carried out. The docking analysis for synthesized compounds were showing good binding interaction with amino acid residues.

2.4. Experimental

2.4 a. ***General procedure for the synthesis of 2-(5-((2-oxo/alkyl-2-phenylethyl) thio)-4H-1,2,4-triazol-3-yl) isoindoline-1,3-dione. (5a-t).*** Method-I

Dipotassium cyanodithioimidocarbonate salt [1] (1.0 mmol) was taken in 5ml of acetic acid and then dropwise addition of hydrazine hydrate [**2**] (1.5 mmol) was carried out with stirring at rt for 24 hrs to form a white color solid 5-amino-4*H*-1,2,4-triazole-3-thiol (**I**). without isolation of this intermediate phthalic anhydride [**3**] (1.0 mmol) was added, and refluxed for 8-10 h. Then alkyl/aralkyl/phenacyl bromides (1.0 mmol) and NaOAc (1.0 mmol) were added and refluxed for 4-5 h, by monitoring the TLC. After completion of the reaction, the reaction mixture was poured into ice cold water. Solid separated was filtered and recrystallized from ethanol.

2.4 b. ***Synthesis of 2-(5-((2-oxo/alkyl-2-phenylethyl) thio)-4H-1,2,4-triazol-3-yl) isoindoline-1,3-dione. (5a-t).*** Method-II

Dipotassium cyanodithioimidocarbonate salt [1] (1.0 mmol) was taken in 5ml of acetic acid and then dropwise addition of hydrazine hydrate [**2**] (1.5 mmol) was carried out stirring at rt for 24 hrs to form a white color solid 5-amino-4*H*-1,2,4-triazole-3-thiol (**I**). The compound **I** was isolated and recrystallized from ethanol. The isolated intermediate **I** (1.0 mmol) was reacted with phthalic anhydride [**3**] (1.0 mmol) in presence of acetic acid solvent under reflux for 8-10 h. Then alkyl/phenacyl bromides (1.0 mmol) and NaOAc (1.0 mmol) were added and refluxed for another 4-5 h, by monitoring the TLC. After completion of the reaction, the reaction mixture was poured into ice cold water. Solid separated was filtered, recrystallized with ethanol.

2.4 c. ***Synthesis of 2-(5-((4-nitrobenzyl)-4H-1,2,4-triazol-3-yl) isoindoline-1,3-dione. (6a)***

A mixture of 5-amino-4*H*-1,2,4-triazole-3-thiol (**I**) (1 mmol) and *p*-NO_2 benzyl bromide (1.0 mmol) was taken in EtOH (1mL) and refluxed for 2 hours by monitoring TLC. After completion of the reaction, ethanol was evaporated and then 3 mL of acetic acid, phthalic anhydride was added (1.0 mmol) and refluxed for another 5-6 hours followed by addition of H_2O_2 (4.0 mmol). The reaction was further refluxed for 2-3 hours. After completion of the reaction as checked by TLC the solid separated was filtered and washed with water and recrystallized from methanol.

2.4 d. Experimental procedure for cytotoxic activity.

The test samples were dissolved in distilled dimethyl sulphoxide (DMSO) and maintained a medium to get a stock solution of 1 mg/mL concentration. The cytotoxicity assays were carried out on 96 well microtitre plates containing 10,000 cells/well. Medium containing different concentrations of the test compounds were added after 24 h of partial monolayer cell formation. Control cells were incubated in a culture medium without test compounds. The microtitre plates were incubated at 370 ^{0}C in a humidified incubator with 5% CO_2 for 72 h. After incubation for 72 h, 20μL of MTT solution (2mg/mL in PBS) was added to the plates and was further incubated for 4 h at 37 ^{0}C. MTT-formazon crystals formed were dissolved in 100 μL of DMSO and the optical density was read with a microtitre plate reader (Biorad) at 570nm. To perceive growth inhibition by the series of compounds, cells were seeded onto 96-well plates at a density of 5×10^3/well before compound treatment. Cytotoxicity of the series of compounds in a variety of cell lines was determined using the MTT assay after incubation of cells with these compounds at various concentrations for 3 days. The concentration of 50% inhibition of cell growth (**IC_{50}**) was determined by interpolation of the dose-response curves.

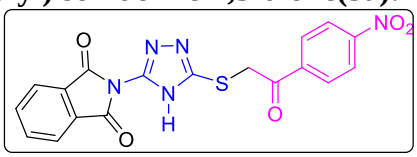
2.4 e. Experimental procedure for molecular docking

All the synthesized chemical (ligand) compounds of thio alkylated 1,2,4-triazole isoindoline-1,3-diones were drawn 2D models using Chemdraw software and converted into 3D structures using Open Babel GUI version 2.3.2 (Open Bable GUI; Chris Morley, USA). Molecular energy was minimized using the Energy minimization module of Maestro Tool (Schrodinger software) under the CHARMM force field. The Crystal structure of c-Met in the complex was retrieved from Protein Data Bank (PDB ID: 4GG5.pdb). 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 by using protein preparation suite (wizard) tool was used to prepare target protein file addition of polar hydrogens 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.

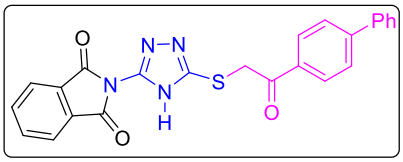
2.5. Characterization data of synthesized compounds (5a-t)

2-(5-((2-(4-Nitrophenyl)-2-oxoethyl)thio)-*4H*-1,2,4-triazol-3-yl)isoindoline-1,3-dione(5a):



Yellow solid; yield 85%; mp: 218–219 °C; IR (KBr) cm^{-1}: 3229 (NH), 1790 (C=O), 1727 (C=O), 1704 (C=O), 1600 (C=N), 1527 (NO_2 asymmetric), 1320 (NO_2 symmetric); ^{1}H-NMR (400MHz, DMSO-d_6, δ ppm): 5.06 (s, 2H, S–CH_2), 7.94-8.02 (m, 4H, Ar-H), 8.25 (d, *J* = 8.4 Hz, 2H, Ar-H), 8.37 (d, *J* = 8.4 Hz, 2H, Ar-H), 14.57 (brs, 1H, NH); ^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm):19.0, 124.3, 124.5, 130.3, 131.4, 135.8, 140.4, 150.6, 166.2, 193.0; (ESI-HRMS) (*m/z*): $[M+H]^+$ 410.0558 Anal. Calcd for $C_{18}H_{11}N_5O_5S$: C, 52.81; H, 2.71; N, 17.11; S, 7.83. Found: C, 52.85; H, 2.75; N, 17.14; S, 7.86.

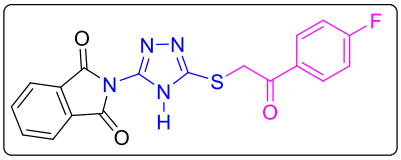
2-(5-((2-([1,1-Biphenyl]-4-yl)-2-oxoethyl)thio)-*4H*-1,2,4-triazol-3-yl)isoindoline-1,3-dione (5b):



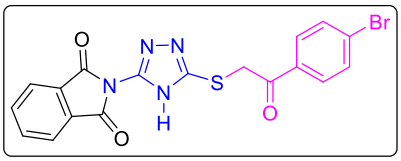
White solid; yield 89%; mp: 230-231 °C; IR (KBr) cm^{-1}: 3061 (NH), 2908 (C-H), 1797 (C=O), 1733 (C=O), 1693 (C=O), 1601 (C=N); ^{1}H NMR (400MHz, DMSO-d_6, δ ppm): 5.07 (s, 2H, S-CH_2), 7.42-7.53 (m, 4H), 7.76 (d, *J* = 7.6Hz, 2H), 7.86 (d, *J* = 8Hz, 2H), 7.96-8.00 (m, 3H,), 8.11(d, *J* = 8Hz, 2H),14.56 (brs, 1H, NH); ^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 23.0, 124.5, 127.4, 127.5, 129.0, 129.5, 129.6, 131.4, 134.4, 135.8, 139.2, 145.6, 150.6, 153.3, 166.4, 193.0; (ESI-HRMS) (*m/z*): $[M+H]^+$ 441.1015; Anal. Calcd for $C_{24}H_{16}N_4O_3S$: C, 65.44; H, 3.66; N, 12.72; S, 7.28. Found: C, 65.49; H, 3.62; N, 12.75; S, 7.24.

2-(5-((2-(4-Fluorophenyl)-2-oxoethyl)thio)-*4H*-1,2,4-triazol-3-yl)isoindoline-1,3-dione (5c): White solid; yield 80%; mp: 225-226 °C; IR (KBr) cm^{-1}: 3335 (NH), 2914 (C-H), 1794 (C=O), 1768 (C=O), 1680 (C=O), 1542 (C=N); ^{1}H-NMR (400MHz, DMSO-d_6, δ ppm): 5.01 (s, 2H, S-

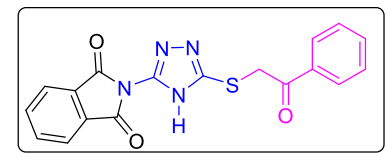
CH_2), 7.39 (t, *J* = 8.8 Hz, 2H), 7.95-8.00 (m, 2H), 8.10-8.13 (m, 2H), 8.13 - 8.10 (m, 2H), 14.53 (brs, 1H, NH); ^{13}C-NMR (100 MHz, DMSO-*d*$_6$, δ ppm): 21.4, 116.2, 116.4, 124.5, 131.3, 131.9, 132.0, 132.3, 135.9, 164.5, 166.3, 172.6, 167.0, 192.1; (ESI-HRMS) (*m/z*): $[M+H]^+$ 383.0612; Anal. Calcd for $C_{18}H_{11}FN_4O_3S$: C, 56.54; H, 2.90; N, 14.65; S, 8.38. Found: C, 56.57; H, 2.93; N, 14.69; S, 8.34.



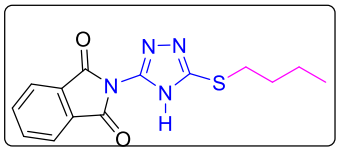
2-(5-((2-(4-Bromophenyl)-2-oxoethyl) thio)-*4H*-1,2,4-triazol-3-yl)isoindoline-1,3-dione (5d): Orange solid; yield 88%; mp: 209-210 ºC; IR (KBr) cm^{-1}: 3277 (NH), 3066 (C-H), 1796 (C=O), 1734 (C=O), 1701 (C=O), 1564 (C=N); ^{1}H-NMR (400MHz, DMSO-*d*$_6$, δ ppm) : 5.00 (s, 2H, S-CH_2), 7.78 (d, *J* = 8.4 Hz, 2H),7.94-7.96 (m, 4H), 8.00 (d, *J* = 2.4 Hz, 2H), 14.53 (brs, 1H, NH); ^{13}C-NMR (100 MHz, DMSO-*d*$_6$, δ ppm): 21.5, 121.7, 128.4, 130.8, 130.8, 131.1, 132.2, 132.3, 132.3, 134.6, 137.6, 152.6, 162.2, 192.7; (ESI-HRMS) (*m/z*): $[M+H]^+$ 442.9811; Anal. Calcd for $C_{18}H_{11}BrN_4O_3S$: C, 48.77; H, 2.50; N, 12.64; S, 7.23. Found: C, 48.74; H, 2.53; N, 12.60; S, 7.27.



2-(5-((2-Oxo-2-phenylethyl)thio)-*4H*-1,2,4-triazol-3-yl)isoindoline-1,3-dione (5e): Light yellow solid; yield 89%; mp: 205-206 ºC; IR (KBr) cm^{-1}: 3064 (NH), 2914 (C-H), 1794 (C=O),1735 (C=O), 1698 (C=O), 1567 (C=N); ^{1}H-NMR (400MHz, DMSO-*d*$_6$, δ ppm): 5.02 (s, 2H, S-CH_2), 7.28-7.41 (m, 5H), 7.98-8.03 (m, 4H); 14.53 (brs, 1H, NH); ^{13}C-NMR (100 MHz, DMSO-*d*$_6$, δ ppm): 27.7, 124.5, 128.8, 129.3, 131.3, 134.3, 135.5, 135.9, 154.3, 166.3, 193.5; (ESI-HRMS) (*m/z*): $[M+H]^+$ 365.0707; Anal. Calcd for $C_{18}H_{12}N_4O_3S$: C,59.33; H, 3.32; N, 15.38; S, 8.80. Found: C, 59.30; H, 3.35; N, 15.35; S, 8.84.

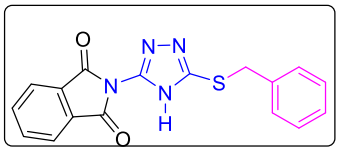


2-(5-(Butylthio)-*4H*-1,2,4-triazol-3-yl)isoindoline-1,3-dione (5f): White solid; yield 81%; mp: 230-231 ºC; IR (KBr) cm^{-1}: 3098 (NH), 2954 (C-H),1743 (C=O), 1714 (C=O): 1619 (C=N); ^{1}H-NMR (400MHz, DMSO-*d*$_6$, δ ppm): 0.93 (t, *J* = 7.4 Hz, 3H), 1.44 -1.48 (m, 2H), 1.69 (t, 2H), 3.18 (t, *J* = 7.0 Hz, 2H), 7.91–8.02 (m, 4H), 14.33 (brs, 1H, NH); ^{13}C-NMR (100 MHz, DMSO-*d*$_6$, δ ppm):



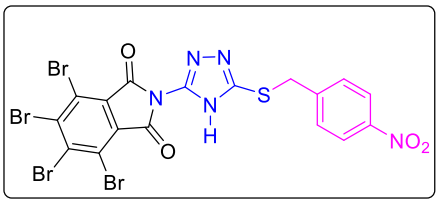
13.8, 21.4, 31.6, 124.5, 131.4, 135.8, 150.4, 165.9; (ESI-HRMS) (*m/z*): $[M+H]^+$ 303.0906; Anal. Calcd for $C_{14}H_{14}N_4O_2S$: C, 55.62; H, 4.67; N, 18.53; S, 10.60. Found: C, 55.65; H, 4.70; N, 18.49; S, 10.63.

2-(5-(Benzylthio)-*4H*-1,2,4-triazol-3-yl)isoindoline-1,3-dione (5g):



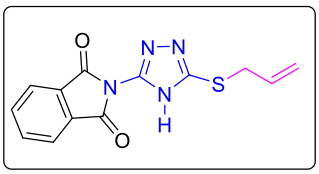
White solid; yield 84%; mp: 210-211 ºC; IR (KBr) cm^{-1}: 2930 (NH), 2886 (C-H), 1743 (C=O), 1714 (C=O), 1600 (C=N); ^{1}H-NMR (400MHz, DMSO-d_6, δ ppm): 4.46 (s, 2H, S-CH_2), 7.28-7.41 (m, 5H), 7.98-8.03 (m, 4H), 14.57 (brs, 1H, NH); ^{13}C-NMR (100 MHz, DMSO-d_6, ppm): 35.5 (C_3), 123.9 (C_{12} & C_{15}), 124.0 (C_7), 124.5 (C_5 & C_9), 130.3 (C_6 & C_8), 130.7 (C_{11} & C_{16}), 131.4 (C_{13} & C_{14}), 135.9 (C_4), 145.9 (C_2), 147.2 (C_1), 166.2 (C_{10} & C_{17}); (ESI-HRMS) (*m/z*): $[M+H]^+$ 337.1552; Anal. Calcd for $C_{17}H_{12}N_4O_2S$: C, 60.70; H, 3.60; N, 16.66; S, 9.53. Found: C, 60.73; H, 3.57; N, 16.70; S, 9.50.

4,5,6,7-Tetrabromo-2-(5-((4-nitrobenzyl)thio)-*4H*-1,2,4-triazol-3-yl)isoindoline-1,3-dione (5h):



Yellow solid; yield 90%; mp: 207-208 ºC; IR (KBr) cm^{-1}: 3115 (NH), 2850 (C-H), 1744 (C=O), 1698 (C=O), 1598 (C=N), 1518 (NO_2 asymmetric), 1343 (NO_2symmetric); ^{1}H-NMR (400MHz, $CDCl_3$+DMSO-d_6, δ ppm): 4.51 (s, 2H, S-CH_2), 7.63 (d, *J* = 6.8 Hz, 2H), 8.13 (d, *J* = 8.4 Hz, 2H), 14.49 (brs, 1H, NH); ^{13}C-NMR (100MHz, DMSO-d_6, δ ppm): 35.7, 121.8, 124.0, 130.6, 131.2, 137.6, 145.8, 147.2, 162.2; (ESI-HRMS) (*m/z*): $[M+H]^+$ 693.7025; Anal. Calcd for $C_{17}H_7Br_4N_5O_4S$: C, 29.30; H, 1.01; N, 10.05; S, 4.60. Found: C, 29.33; H, 1.05; N, 10.09; S, 4.64.

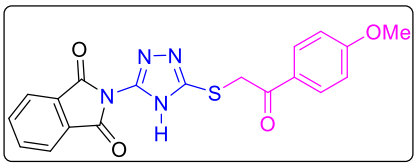
2-(5-(Allylthio)-*4H*-1,2,4-triazol-3-yl)isoindoline-1,3-dione (5i):



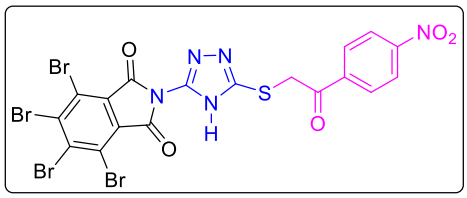
White solid; yield 86%; mp: 224-225 ºC; IR (KBr) cm^{-1}: 3084 (NH), 2925 (C-H stretch), 1743 (C=O), 1714 (C=O), 1535 (C=N), 1503 (C=C); ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 3.84 (d, *J* = 6.8 Hz, 2H), 5.12 (d, *J* = 10.0 Hz, 1H), 5.28 (d, *J* = 17.2 Hz, 1H), 5.88-5.98 (m, 1H), 7.95-8.02 (m, 4H), 14.56 (brs, 1H, NH); ^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 35.1, 119.2, 124.5, 131.4, 133.4,

135.8, 150.8, 152.6, 166.4; (ESI-HRMS) (*m/z*): $[M+H]^+$ 287.1379; Anal. Calcd for $C_{13}H_{10}N_4O_2S$: C, 54.54; H, 3.52; N, 19.57; S, 11.20. Found: C, 54.50; H, 3.55; N, 19.54; S, 11.23.

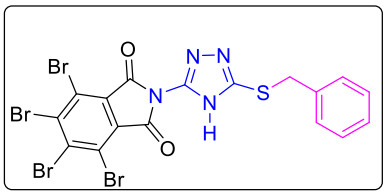
2-(5-((2-(4-Methoxyphenyl)-2-oxoethyl)thio)-*4H*-1,2,4-triazol-3-yl)isoindoline-1,3-dione (5j): White solid; yield 85%; mp: 222-223 °C; IR (KBr) cm^{-1}: 3442 (NH), 1795 (C=O), 1733 (C=O), 1698 (C=O), 1572 (C=N); ^{1}H-NMR (400MHz, DMSO-d_6, δ ppm): 3.85 (s, 3H, OCH_3), 4.96 (s, 2H, S-CH_2), 7.07 (d, *J* = 8.8 Hz, 2H, ArH), 7.95-8.02 (m, 6H, ArH); 14.53 (brs, 1H, NH); ^{13}C-NMR (100MHz, DMSO-d_6, δ ppm): 26.3, 56.0, 114.5, 124.5, 128.4, 131.3, 131.4, 135.8, 150.7, 164.1, 166.4, 191.7; (ESI-HRMS) (*m/z*): $[M+H]^+$ 395.0817; Anal. Calcd for $C_{19}H_{14}N_4O_4S$: C, 57.86; H, 3.58; N, 14.21; S, 8.13. Found: C, 57.90; H, 3.55; N, 14.24; S, 8.16.



4,5,6,7-Tetrabromo-2-(5-((2-(4-nitrophenyl)-2-oxoethyl)thio)-*4H*-1,2,4-triazol-3-yl)isoindoline-1,3-dione (5k): Yellow solid; yield 91%; mp: 255-256 °C; IR (KBr) cm^{-1}: 3342 (NH), 2925 (C-H stretch), 1743 (C=O), 1693 (C=O), 1583 (C=N), 1527 (NO_2 asymmetric), 1342 (NO_2 symmetric); ^{1}H-NMR (400MHz, DMSO-d_6, δ ppm): 5.07 (s, 2H, S-CH_2), 8.25(d, *J* = 8.8 Hz, 2H), 8.37 (d, *J* = 8.4 Hz, 2H), 14.62 (brs, 1H, NH); ^{13}C-NMR (100MHz, DMSO-d_6, δ ppm): 16.3, 121.7, 122.0, 124.3, 130.2, 130.7, 130.9, 131.1, 136.7, 137.7, 150.5, 162.1, 166.4, 192.9; (ESI-HRMS) (*m/z*): $[M+H]^+$ 721.6986; Anal. Calcd for $C_{18}H_7Br_4N_5O_5S$: C, 29.82; H, 0.97; N, 9.66; S, 4.42. Found: C, 29.86; H, 0.93; N, 9.43; S, 4.45.

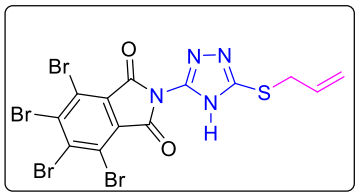


2-(5-(Benzylthio)-*4H*-1,2,4-triazol-3-yl)-4,5,6,7-tetrabromoisoindoline-1,3-dione(5l): Yellow solid; yield 90%; mp: 215-216 °C; IR (KBr) cm^{-1}: 3132 (NH), 2933 (C-H stretch), 1779 (C=O), 1741 (C=O), 1550 (C=N), 663 (C-Br stretch); ^{1}H-NMR (400MHz, DMSO-d_6, δ ppm): 4.45 (s, 2H, S-CH_2), 7.27 – 7.39 (m, 5H, Ar-H), 14.61 (brs, 1H, NH); ^{13}C-NMR (100MHz, DMSO-d_6, δ ppm): 21.5, 121.7, 128.0, 129.0, 129.3, 131.2,

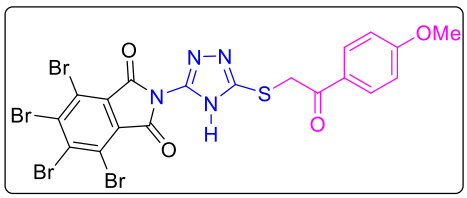


137.3, 137.6, 150.4, 152.9, 162.3, 172.4; (ESI-HRMS) (*m/z*): $[M+H]^+$ 650.7170; Anal. Calcd for $C_{17}H_8Br_4N_4O_2S$: C, 31.32; H, 1.24; N, 8.59; S, 4.92. Found: C, 31.35; H, 1.26; N, 8.62; S, 4.95.

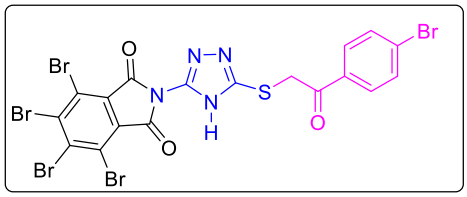
2-(5-(Allylthio)-*4H*-1,2,4-triazol-3-yl)-4,5,6,7-tetrabromoisoindoline-1,3-dione (5m): Yellow solid; yield 88%; mp: 296-297 °C; IR (KBr) cm^{-1}: 3107 (NH), 2926 (C-H stretch), 1782 (C=O), 1741 (C=O), 1693 (C=O), 1551 (C=N), 1500 (C=C); ^{1}H-NMR (400MHz, $CDCl_3$ +DMSO-d_6, δ ppm): 3.81 (d, *J* = 6.8 Hz, 2H, CH_2). 5.12 (d, *J* = 10.0 Hz, 1H), 5.27 (d, *J* = 17.2 Hz, 1H), 5.88-5.98 (m, 1H), 14.37 (brs, 1H, NH); ^{13}C-NMR (100MHz, DMSO-d_6, δ ppm): 35.1, 119.2, 121.7, 131.2, 133.4, 137.6, 150.7, 162.3; (ESI-HRMS) (*m/z*): $[M+H]^+$ 598.7012; Anal. Calcd for $C_{13}H_6Br_4N_4O_2S$: C, 25.94; H, 1.00; N, 9.31; S, 5.33. Found: C, 25.91; H,1.04; N, 9.34; S, 5.30



4,5,6,7-Tetrabromo-2-(5-((2-(4-methoxyphenyl)-2-oxoethyl)thio)-*4H*-1,2,4-triazol-3-yl)isoindoline-1,3-dione (5n): Yellow solid; yield 87%; mp: 254-255 °C; IR (KBr) cm^{-1}: 3098 (NH), 2906 (C-H), 1742 (C=O), 1658 (C=O), 1594 (C=N); ^{1}H-NMR (400MHz, DMSO-d_6, δ ppm): 3.85 (s, 3H, OCH_3), 4.97 (s, 2H, S-CH_2), 7.08 (d, *J* = 8.8 Hz, 2H), 8.00 (d, *J* = 8.8 Hz, 2H), 14.59 (brs, 1H, NH); ^{13}C-NMR (100MHz, DMSO-d_6, δ ppm): 26.5, 56.0, 114.5, 121.7, 128.3, 131.0, 131.3, 137.6, 162.2, 164.1, 191.6; (ESI-HRMS) (*m/z*): $[M+Na]^+$ 730.7029; Anal. Calcd for $C_{19}H_{10}Br_4N_4NaO_4S$: C, 32.14; H, 1.42; N, 7.89; S, 4.52. Found: C, 32.15; H, 1.45; N, 7.85; S, 4.55.

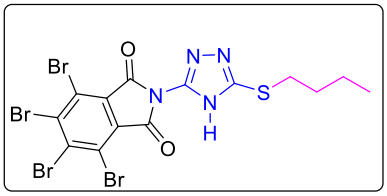


4,5,6,7-Tetrabromo-2-(5-((2-(4-bromophenyl)-2-oxoethyl)thio)-*4H*-1,2,4-triazol-3-yl) isoindoline-1,3-dione (5o): Yellow solid; yield 92%; mp: 334-335 °C; IR (KBr) cm^{-1}: 3324 (NH), 2964 (C-H), 1783 (C=O), 1743 (C=O), 1683 (C=O), 1584 (C=N); ^{1}H-NMR (400MHz, DMSO-d_6, δ ppm): 5.00 (s, 2H, S-CH_2), 7.78 (d, *J* = 8.4 Hz, 2H), 7.95 (d, *J* = 8.4 Hz, 2H), 14.60 (brs, 1H, NH); ^{13}C-NMR (100MHz, DMSO-d_6, δ ppm): 21.5, 121.7, 128.4, 130.8, 130.8, 131.1, 132.2, 132.3, 134.6, 137.6, 162.2,



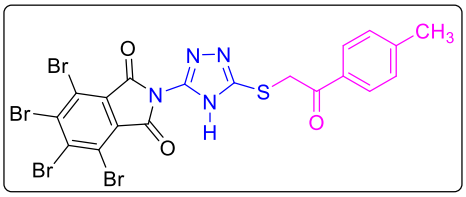
192.7; (ESI-HRMS) (*m/z*) : $[M+H]^+$ 756.6221; Anal. Calcd for $C_{18}H_7Br_5N_4O_3S$: C, 28.49; H, 0.93; N, 7.38; S, 4.22. Found: C, 28.45; H, 0.97; N, 7.34; S, 4.26.

4,5,6,7-Tetrabromo-2-(5-(butylthio)-*4H*-1,2,4-triazol-3-yl)isoindoline-1,3-dione(5p):



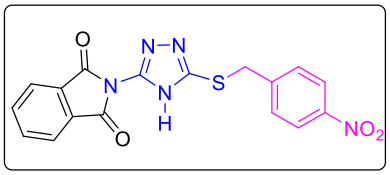
White solid; yield 80%; mp: 256-257 °C; IR (KBr) cm^{-1}: 3320 (NH), 3100 (C-H), 1789 (C=O), 1737 (C=O), 1612 (C=N); ^{1}H-NMR (400MH_Z, DMSO-d_6, δ ppm): 0.88 (t, *J* = 7.2 Hz, 3H), 1.37-1.42 (m, 2H),1.60-1.66 (m, 2H), 3.18 (t, *J* = 7.0 Hz, 2H), 14.55 (brs, 1H, NH); ^{13}C-NMR (100MH_Z, DMSO-d_6, δ ppm): 13.8, 21.4, 31.5, 32.0, 121.7, 122.1, 131.0, 131.2, 136.5, 137.6, 162.3, 166.3; (ESI-HRMS) (*m/z*): $[M+H]^+$ 614.7330; Anal. Calcd for $C_{14}H_{10}Br_4N_4O_2S$: C, 27.21; H, 1.63; N, 9.07; S, 5.19. Found: C, 27.25; H, 1.66; N, 9.04; S, 5.15.

4,5,6,7-Tetrabromo-2-(5-((2-oxo-2-(p-tolyl)thio)-*4H*-1,2,4-triazol-3-yl)isoindoline-1,3-dione (5q):



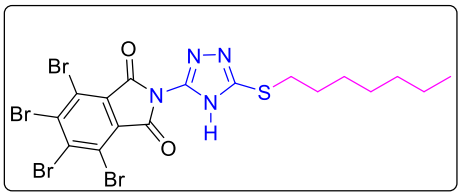
Yellow solid; yield 85%; mp: 264-265 °C; IR (KBr) cm^{-1}: 3330 (NH), 2972 (C-H), 1780 (C=O), 1742 (C=O), 1677 (C=O), 1602 (C=N); ^{1}H-NMR (400MH_Z, DMSO-d_6, δ ppm): 2.38 (s, 3H, CH_3), 4.99 (s, 2H, S-CH_2), 7.36 (d, *J* = 8.0 Hz, 2H), 7.92 (d, *J* = 8.0 Hz, 2H), 14.67 (brs, 1H, NH); ^{13}C-NMR (100MH_Z, DMSO-d_6, δ ppm): 21.6, 79.4, 121.7, 128.8, 128.9, 129.7, 129.8, 131.0, 132.9, 137.6, 145.0, 162.2, 192.8; (ESI-HRMS) (*m/z*): $[M+H]^+$ 690.8; Anal. Calcd for $C_{19}H_{10}Br_4N_4O_3S$: C, 32.88; H, 1.45; N, 8.07; S, 4.62. Found: C, 32.85; H, 1.48; N, 8.03; S, 4.58.

2-(5-((4-Nitrobenzyl)thio)-*4H*-1,2,4-triazol-3-yl)isoindoline-1,3-dione (5r):

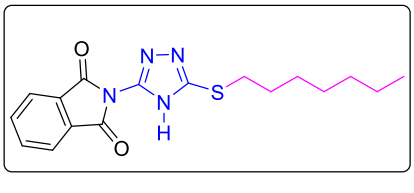


Yellow solid; yield 82%; mp: 257-258 °C; IR (KBr) cm^{-1}: 3200 (NH), 2945 (C-H), 1727 (C=O), 1673 (C=O), 1600 (C=N), 1526 (NO_2 asymmetric), 1320 (NO_2 symmetric); ^{1}H-NMR (400 MHz, $CDCl_3$+DMSO-d_6, δ ppm): 4.49 (s, 2H, S-CH_2), 7.63 (d, *J* = 8.4 Hz, 2H), 7.90 -7.98 (m, 4H), 8.13 (d, *J* = 8.4 Hz, 2H), 14.32 (brs, 1H, NH); ^{13}C-NMR (100MH_Z, DMSO-d_6, δ ppm): 35.5, 123.9, 124.0, 124.5, 130.6, 131.4, 135.9, 145.9, 147.2, 166.2; (ESI-HRMS) (*m/z*): $[M+H]^+$ 382.0612; Anal. Calcd for $C_{17}H_{11}N_5O_4S$: C, 53.54; H, 2.91; N, 18.36; S, 8.41. Found: C, 53.51; H, 2.95; N, 18.32; S, 8.45.

4,5,6,7-Tetrabromo-2-(5-(heptylthio)-*4H*-1,2,4-triazol-3-yl)isoindoline-1,3-dione(5s): Yellow solid; yield 81%; mp: 259-260 °C; IR (KBr) cm^{-1}: 3324 (NH), 2925 (C-H), 1742 (C=O), 1693 (C=O) 1583 (C=N); ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 0.85 (t, J = 6.2 Hz, 3H), 1.25-1.37 (m, 6H), 1.64-1.67 (m, 2H), 2.08 (m, 2H), 3.17 (t, J = 7.2 Hz, 2H), 14.58 (brs, 1H, NH); ^{13}C-NMR (100MHz, DMSO-d_6, δ ppm): 14.3, 22.4, 28.2, 28.5, 29.5, 31.5, 79.6, 121.7, 131.2, 137.6, 150.9, 162.4, 165.9; (ESI-HRMS) (m/z): $[M+H]^+$ 656.7829; Anal. Calcd for $C_{17}H_{16}Br_4N_4O_2S$: C, 30.94; H, 2.44; N, 8.49; S, 4.86. Found: C, 30.90; H, 2.41; N, 8.45; S, 4.82.

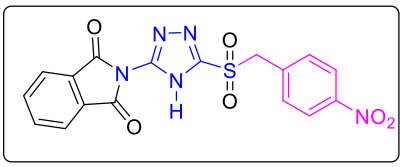


2-(5-(Heptylthio)-*4H*-1,2,4-triazol-3-yl)isoindoline-1,3-dione (5t): White solid; yield 81%; mp: 230-231 °C; IR (KBr) cm^{-1}: 2925 (NH), 2872 (C-H), 1743 (C=O), 1714 (C=O), 1534 (C=N); ^{1}H-NMR (400 MHz, $CDCl_3$+DMSO-d_6, δ ppm) :0.87 (s, 3H), 1.28-1.41 (m, 8H), 1.71 (t, J = 6.8 Hz, 2H), 3.17 (t, J = 5.2 Hz, 2H), 7.88-7.96 (m, 4H), 14.22 (brs, 1H, NH); ^{13}C-NMR (100MHz, DMSO-d_6 δ ppm): 14.3 (C_9), 22.4 (C_8), 28.2 (C_5), 28.5 (C_6), 29.5 (C_4), 31.5 (C_7), 32.3 (C_3), 124.5 (C_{12} & C_{14}), 131.4 C_{11} & C_{16}), 135.8 (C_{13} & C_{15}), 150.8 (C_2), 153.5 (C_1), 166.5 (C_{10} & C_{17}); (ESI-HRMS) (m/z): $[M+H]^+$ 345.2272; Anal. Calcd for $C_{17}H_{20}N_4O_2S$: C, 59.28; H, 5.85; N, 16.27; S, 9.31. Found: C, 59.25; H, 5.82; N, 16.23; S, 9.34.



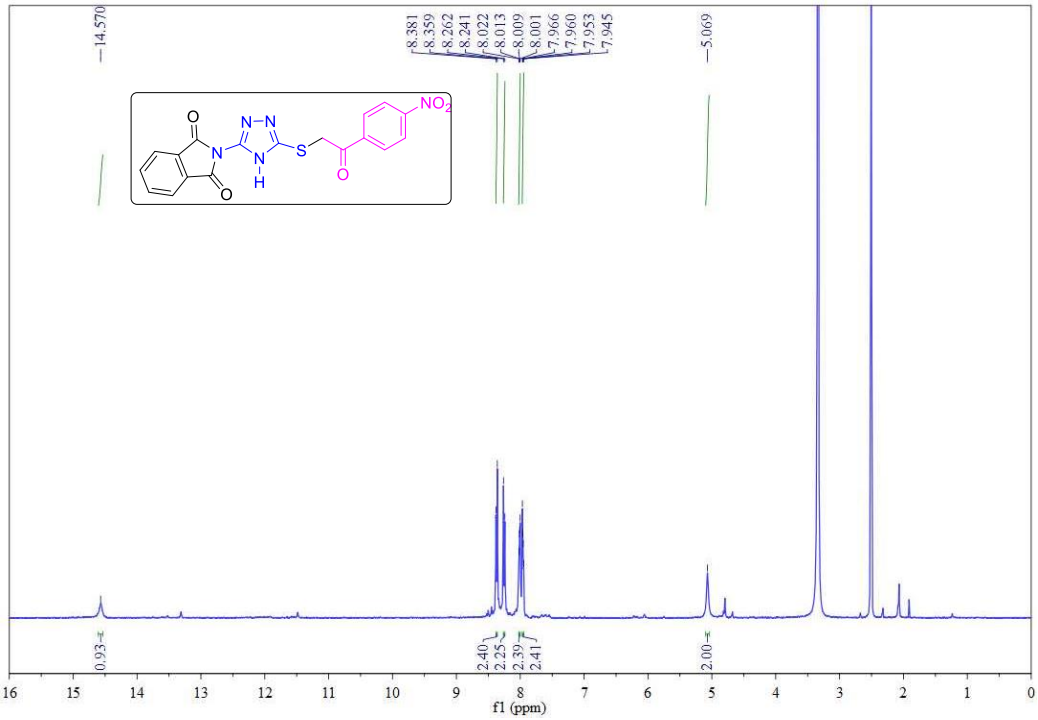
2-(5-(Benzylsulfonyl)-4*H*-1,2,4-triazol-3-yl)isoindoline-1,3-dione: (6a)

White solid; yield 84%; mp:242-243 °C; IR (KBr) cm^{-1}: 3075 (NH), 2928 (C-H), 1734 (C=O), 1692 (C=O),1595 (C=N), 1574 (NO_2 asymmetric), 1348 (NO_2 symmetric), 1316 (SO_2); ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 5.06 (s, 2H, S-CH_2), 7.63-7.65 (m, 2H), 7.67-7.75 (m, 3H), 7.98-8.00 (m, 1H), 8.29 (d, J = 8.8 Hz, 2H), 12.44 (brs, 1H, NH); ^{13}C-NMR (100MHz, DMSO-d_6, δ ppm): 60.1, 124.8, 128.3, 128.9, 129.2, 131.1, 131.6, 133.2, 133.8, 136.2, 165.2, 166.7; (ESI) +Ve, (m/z): 413.98; Anal. Calcd for $C_{17}H_{11}N_5O_6S$: C, 49.40; H, 2.68; N, 16.94; S, 7.76. Found: C, 49.44; H, 2.72; N, 16.90; S, 7.72.

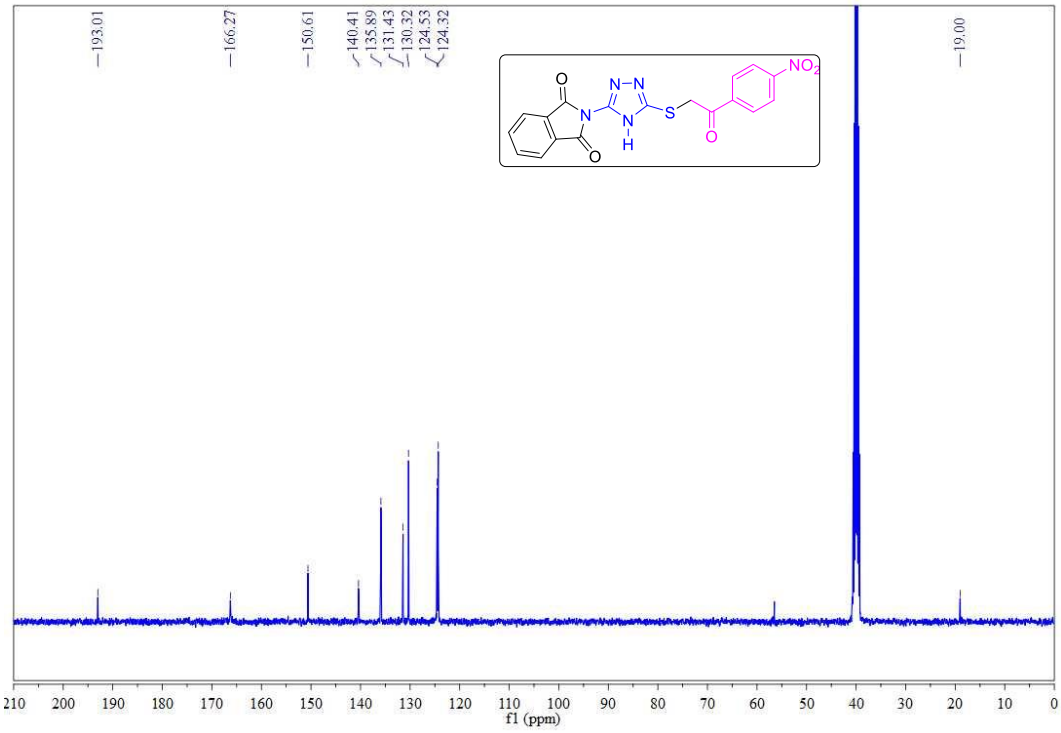


2.6. Copies of the spectral data.

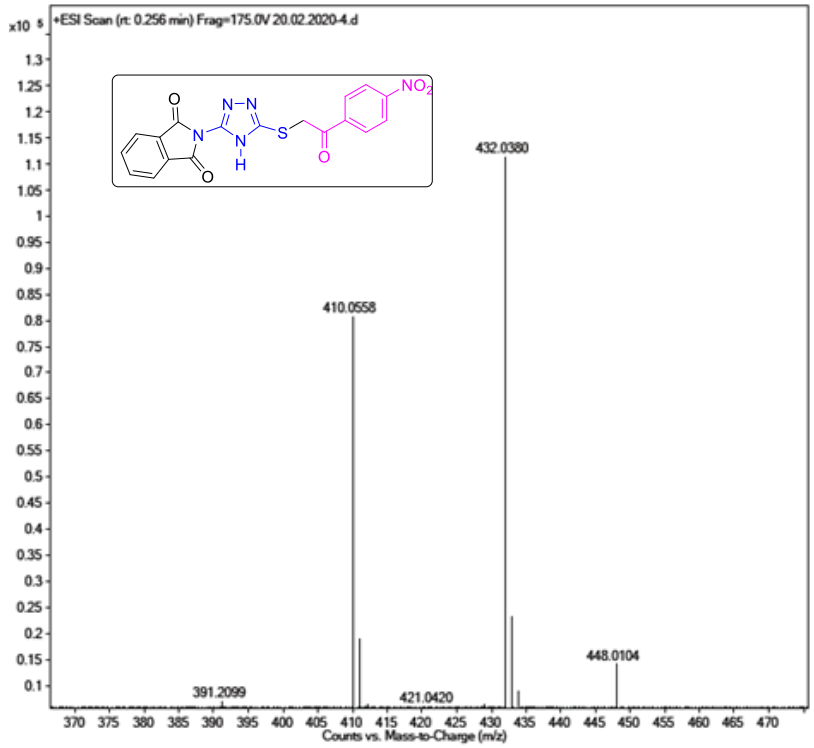
^{1}H NMR Spectrum of compound 5a (DMSO-d_6 400 MHz)



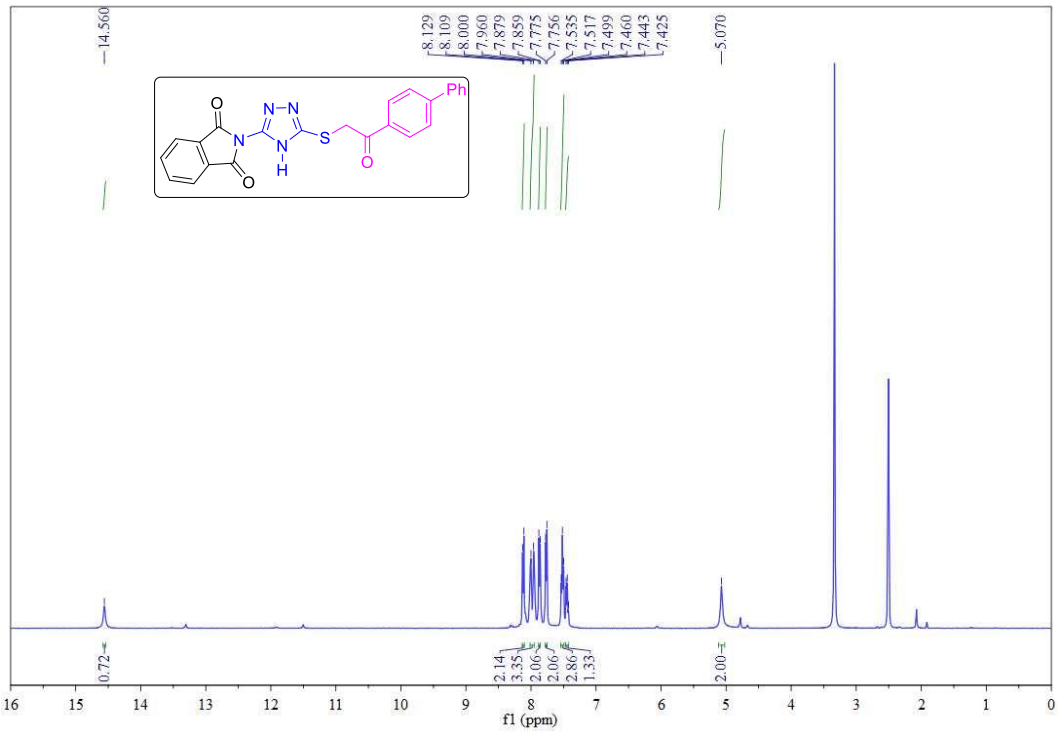
^{13}C NMR Spectrum of compound 5a (DMSO-d_6 100 MHz)



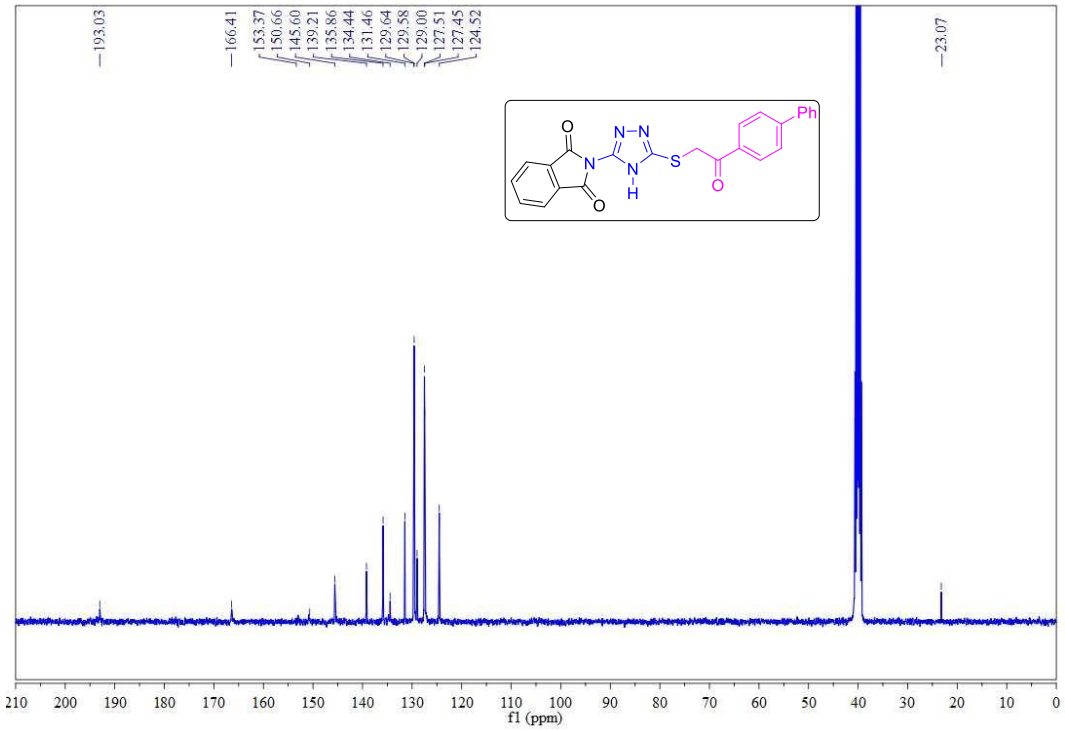
Mass Spectrum of compound 5a



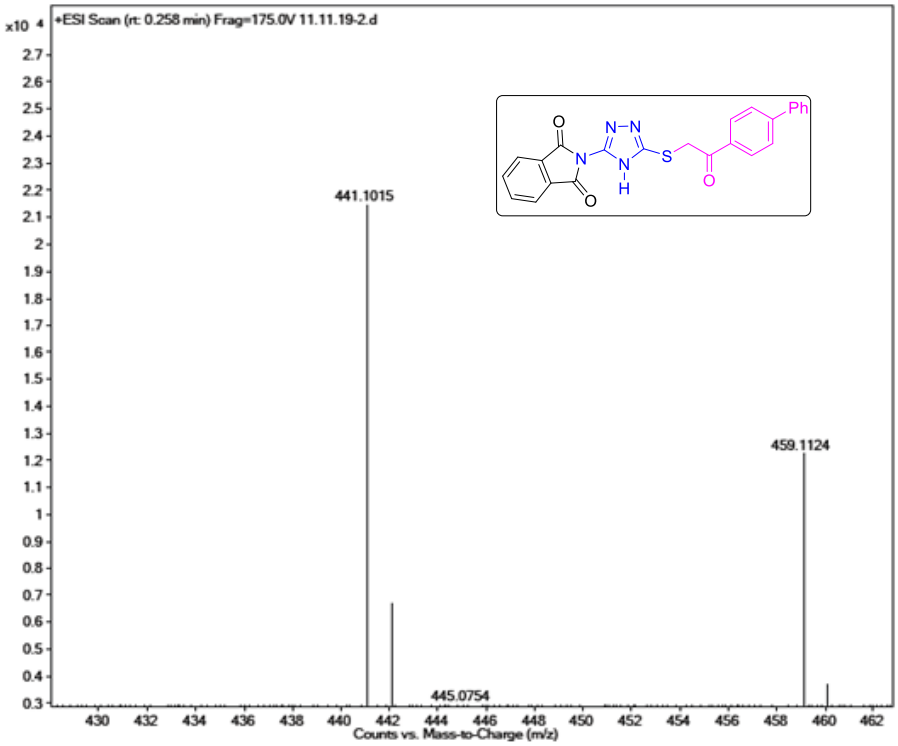
^{1}H NMR Spectrum of compound 5b (DMSO-d_6 400 MHz)



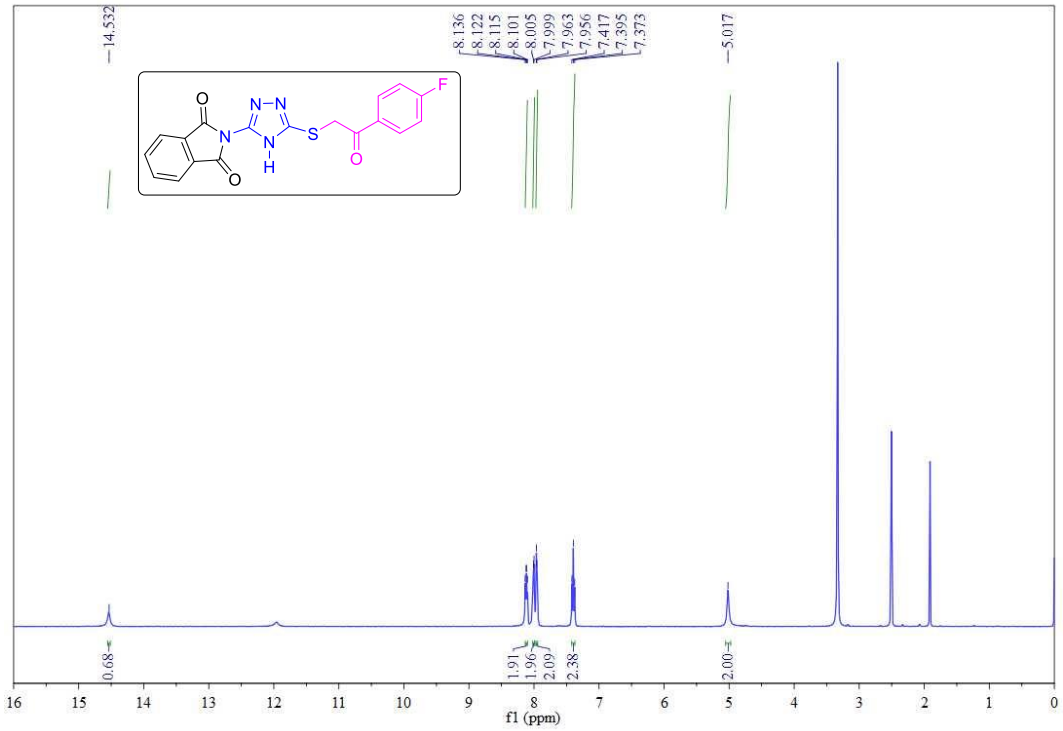
^{13}C NMR Spectrum of compound 5b (DMSO-d_6 100 MHz)



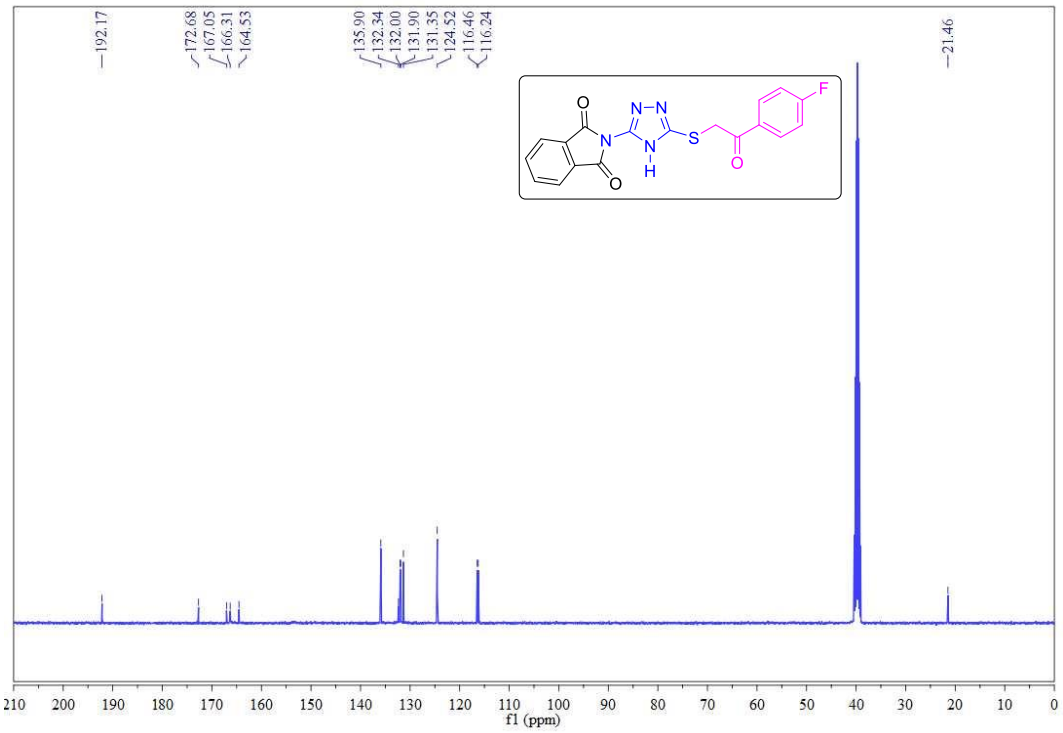
Mass Spectrum of compound 5b



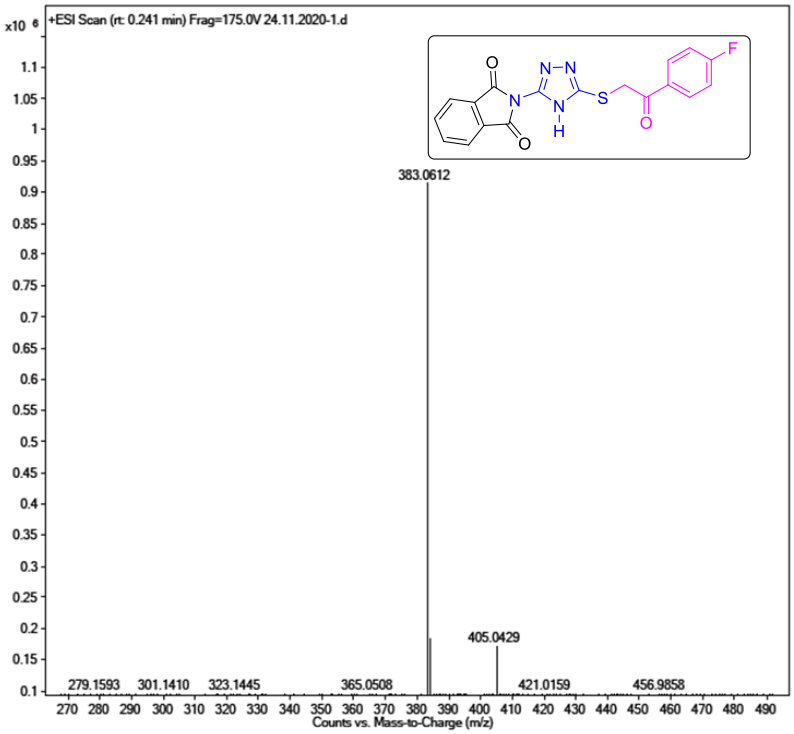
^{1}H NMR Spectrum of compound 5c (DMSO-d_6 400 MHz)



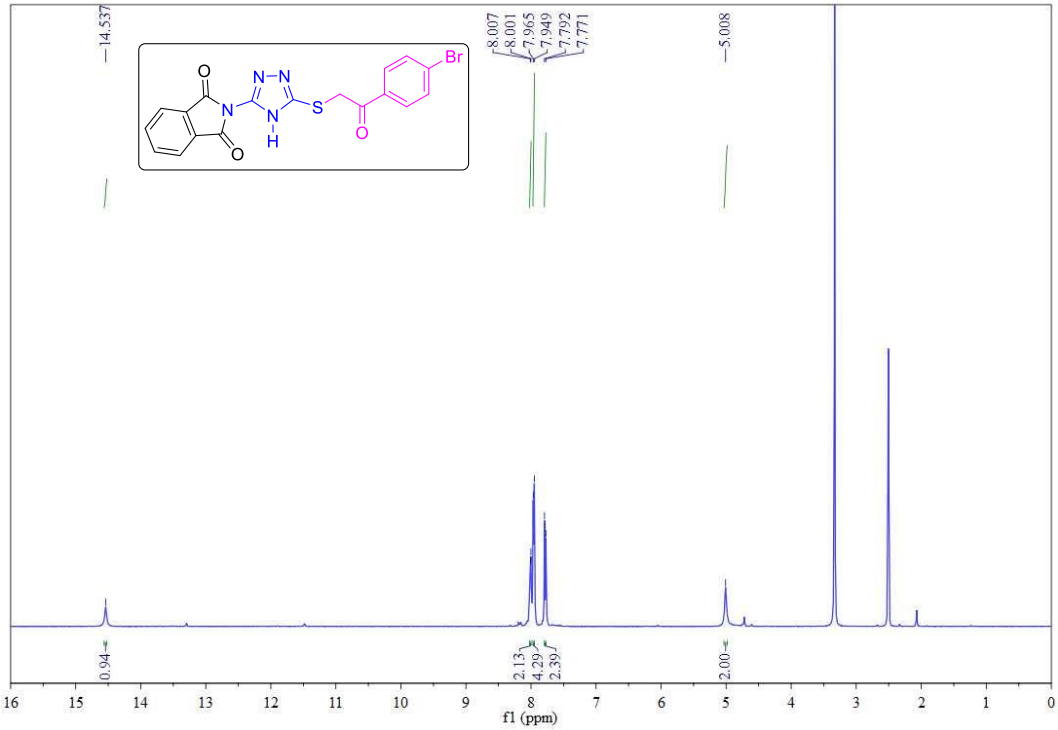
^{13}C NMR Spectrum of compound 5c (DMSO-d_6 100 MHz)



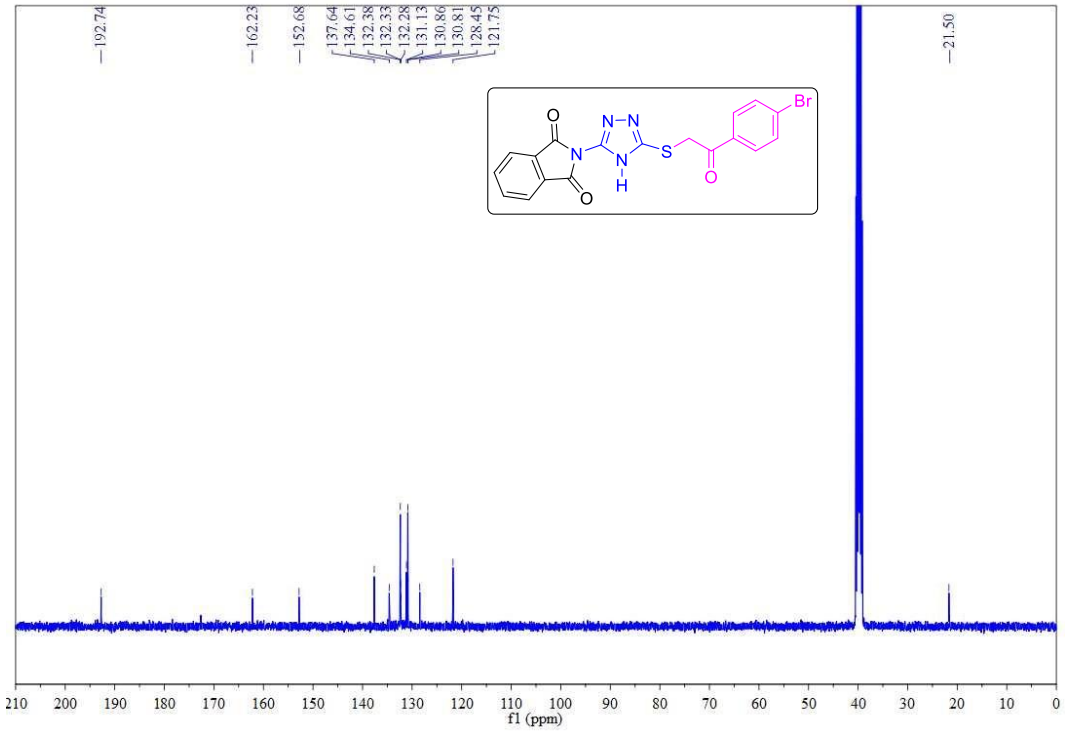
Mass Spectrum of compound 5c



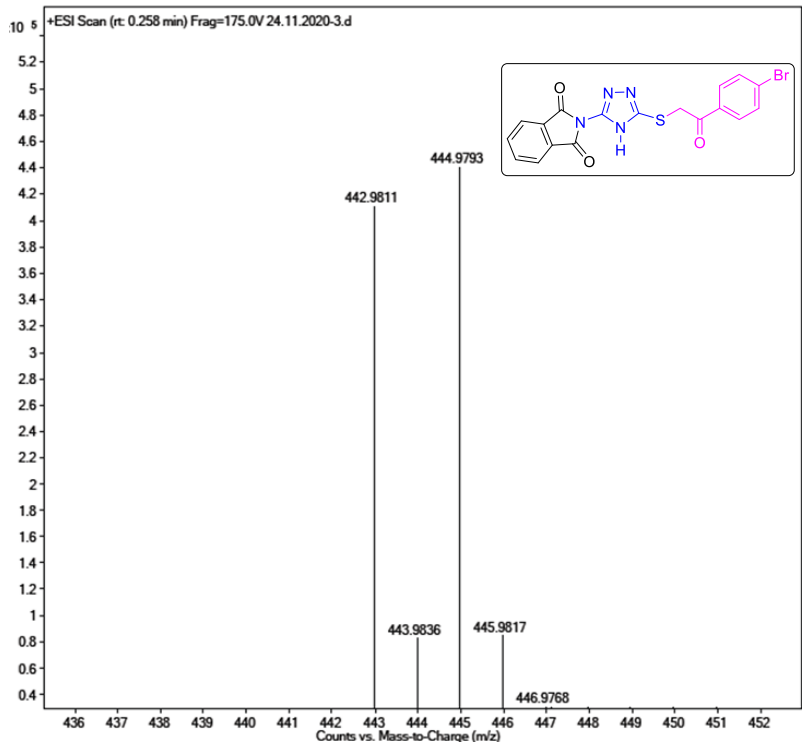
^{1}H NMR Spectrum of compound 5d (DMSO-d_6 400 MHz)



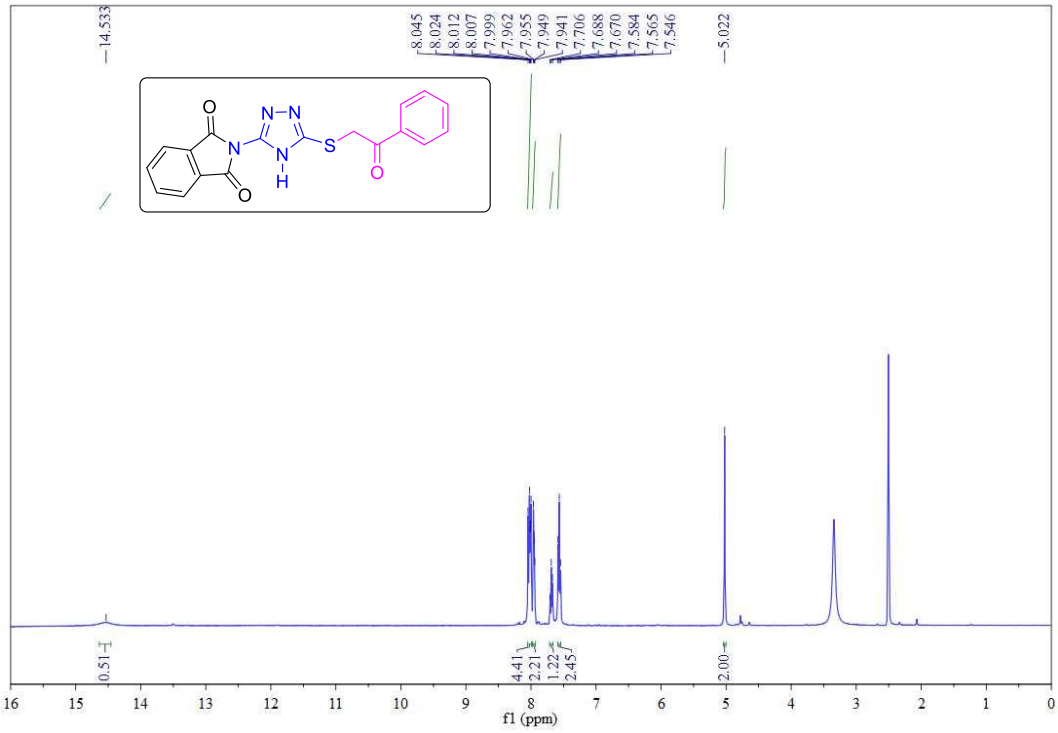
^{13}C NMR Spectrum of compound 5d (DMSO-d_6 100 MHz)



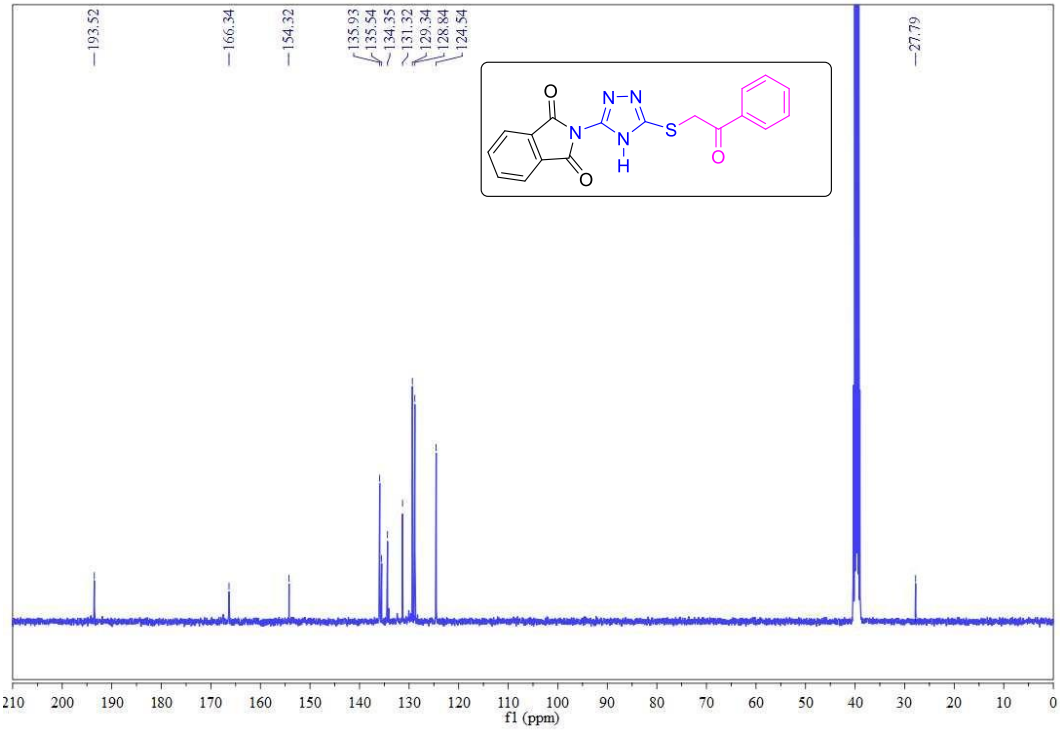
Mass Spectrum of compound 5d



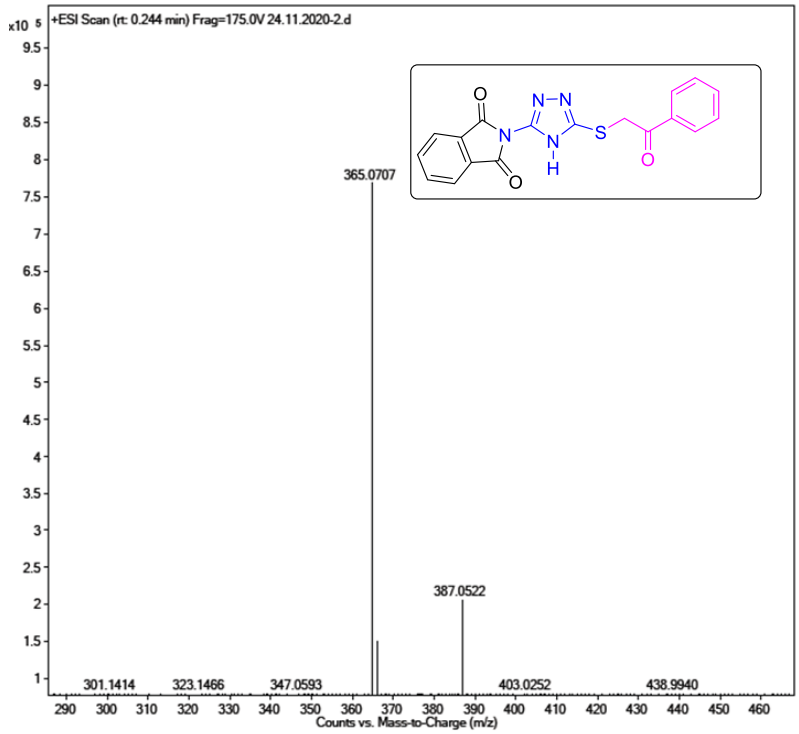
^{1}H NMR Spectrum of compound 5e (DMSO-d_6 400 MHz)



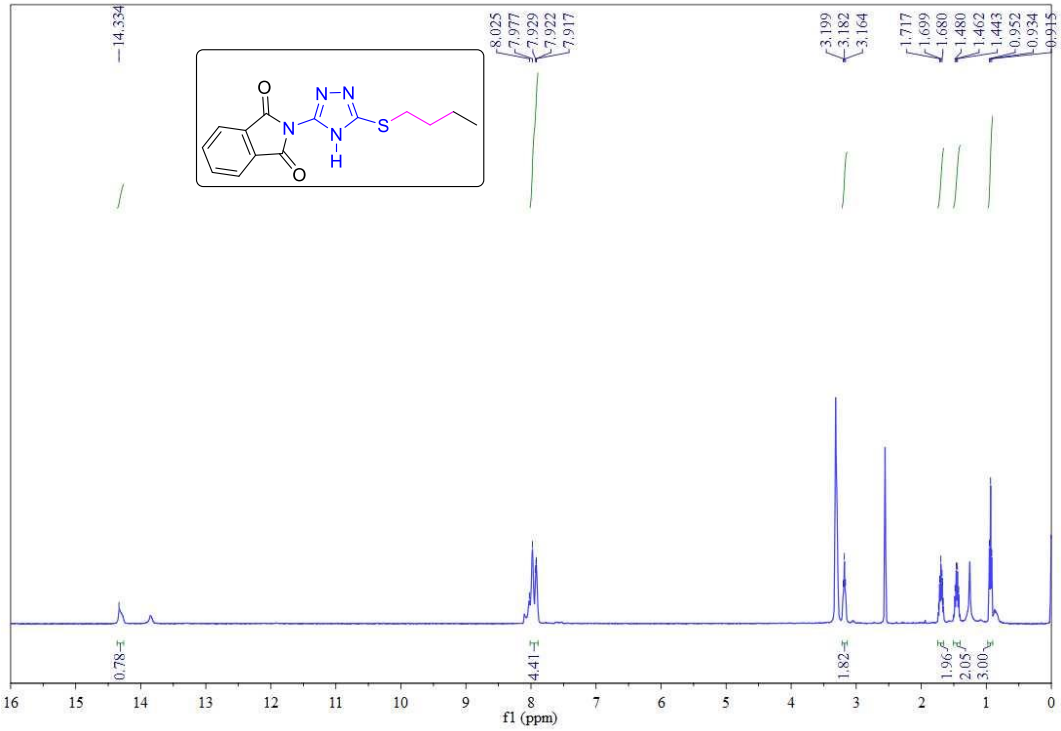
^{13}C NMR Spectrum of compound 5e (DMSO-d_6 100 MHz)



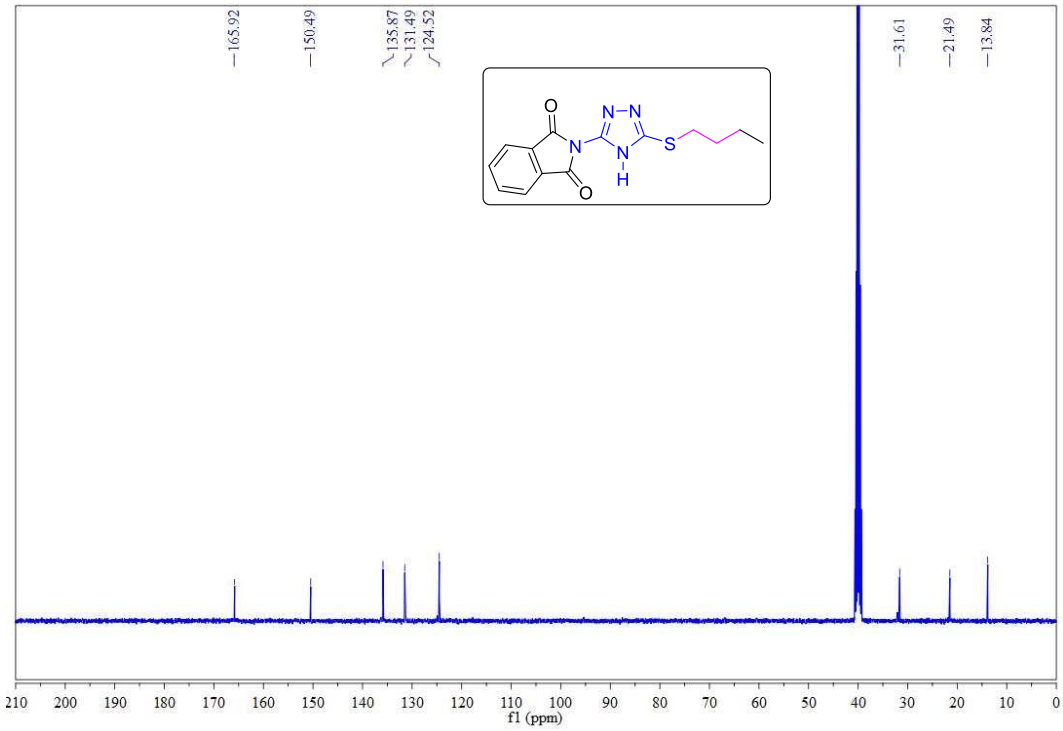
Mass Spectrum of compound 5e



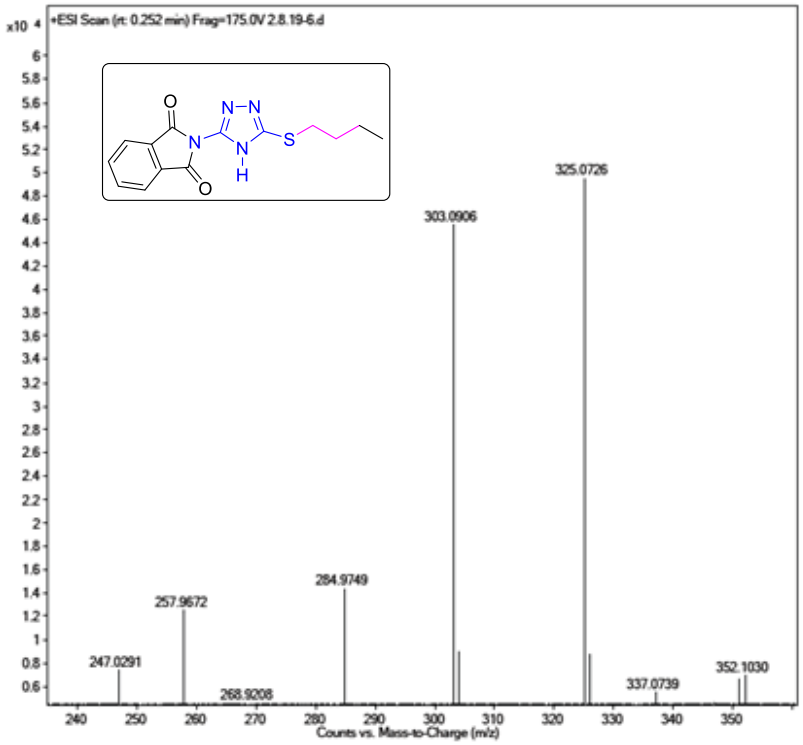
^{1}H NMR Spectrum of compound 5f (DMSO-d_6 400 MHz)



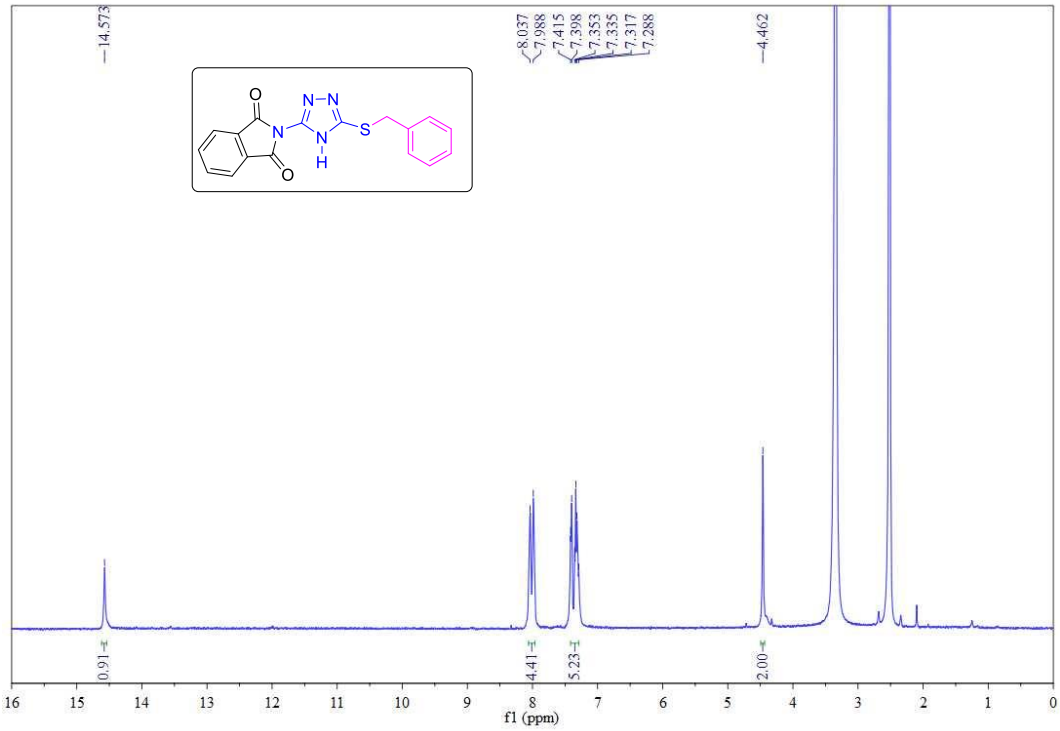
^{13}C NMR Spectrum of compound 5f (DMSO-d_6 100 MHz)



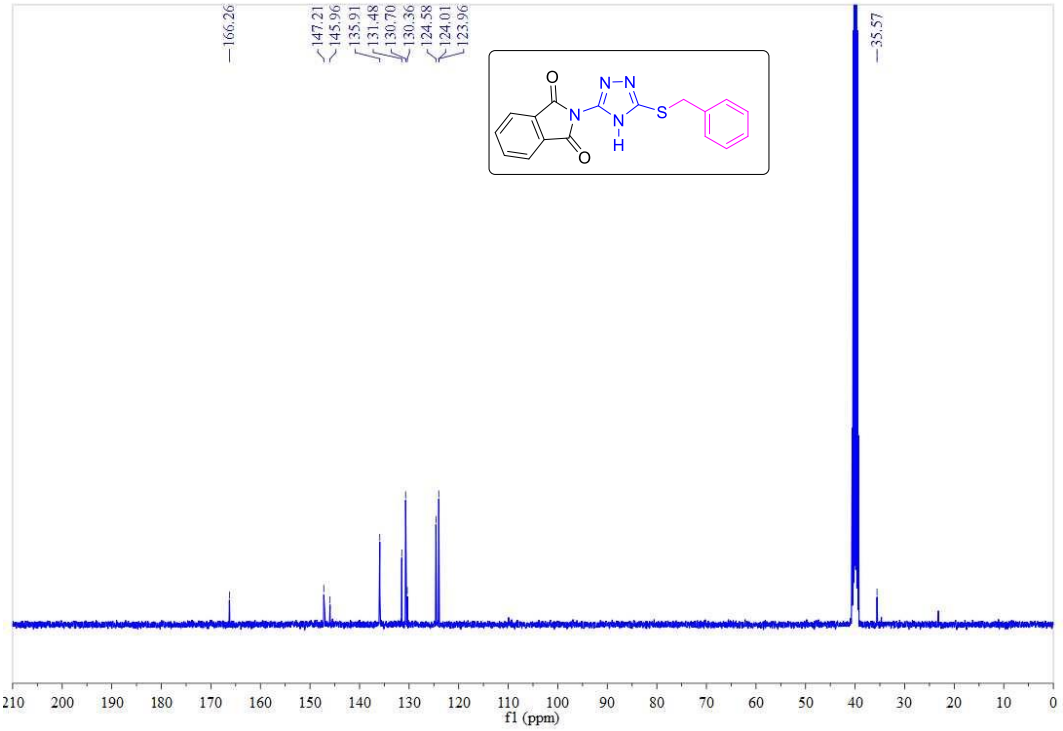
Mass Spectrum of compound 5f



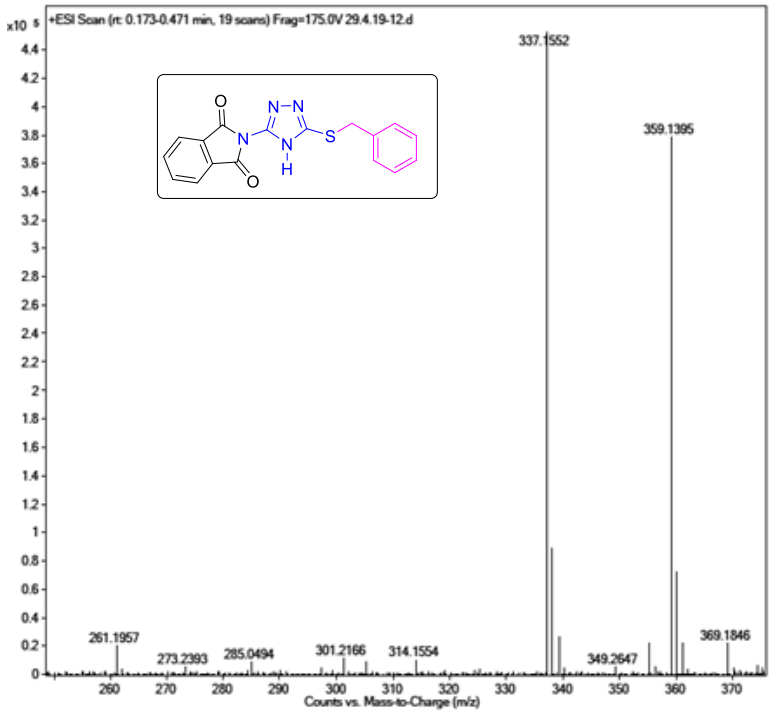
^{1}H NMR Spectrum of compound 5g (DMSO-d_6 400 MHz)



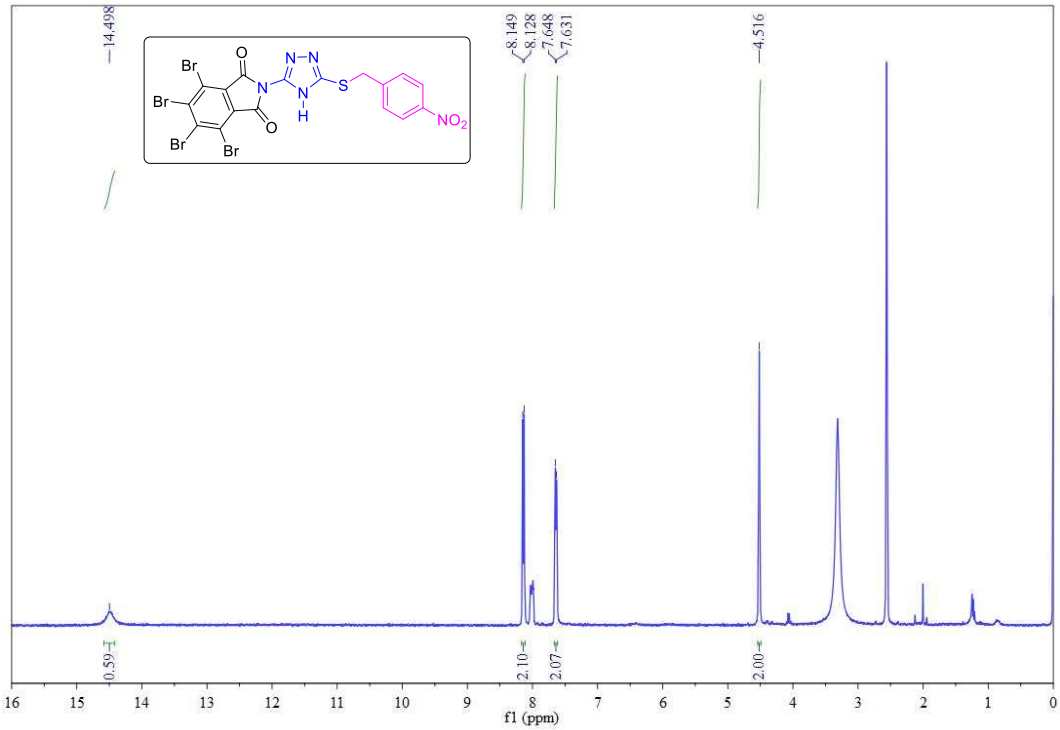
^{13}C NMR Spectrum of compound 5g (DMSO-d_6 100 MHz)



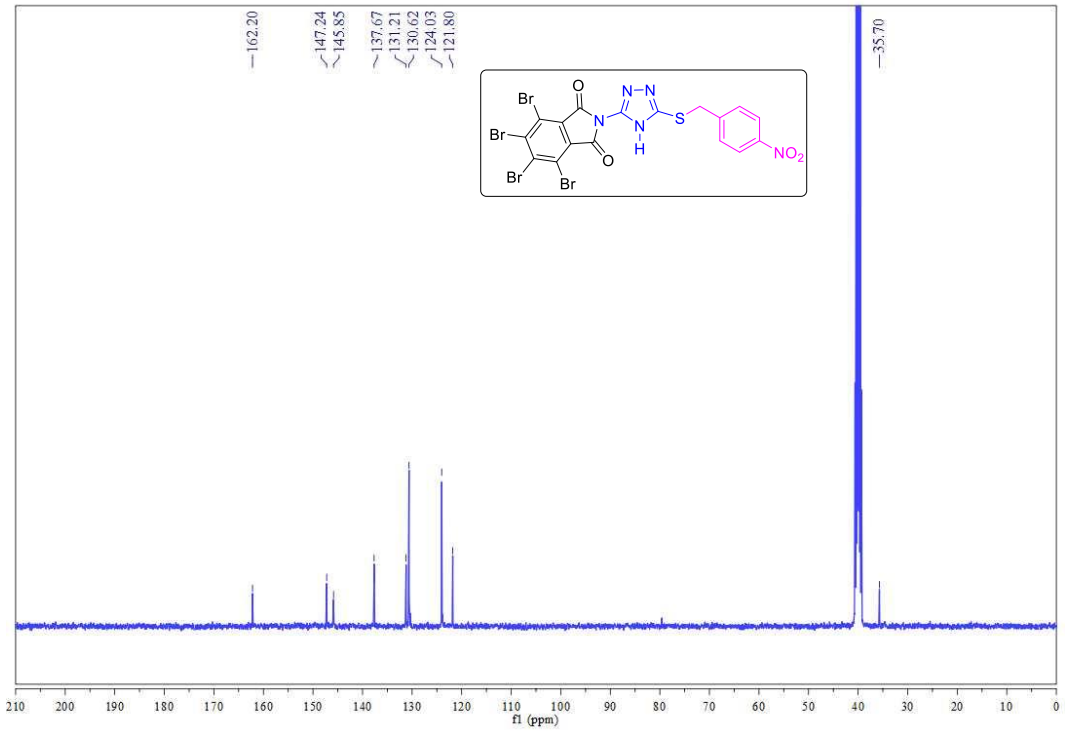
Mass Spectrum of compound 5g



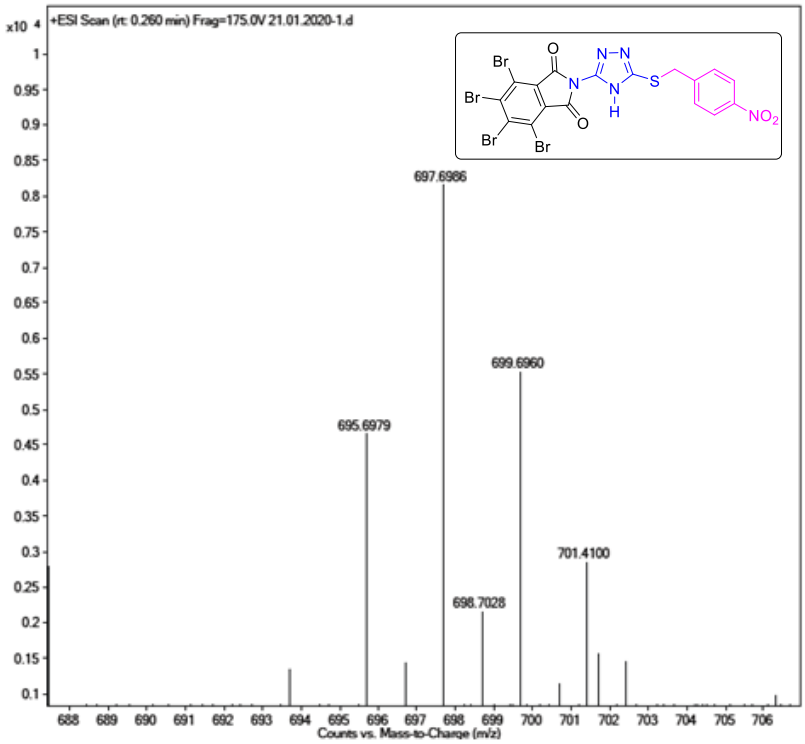
^{1}H NMR Spectrum of compound 5h (DMSO-d_6 400 MHz)



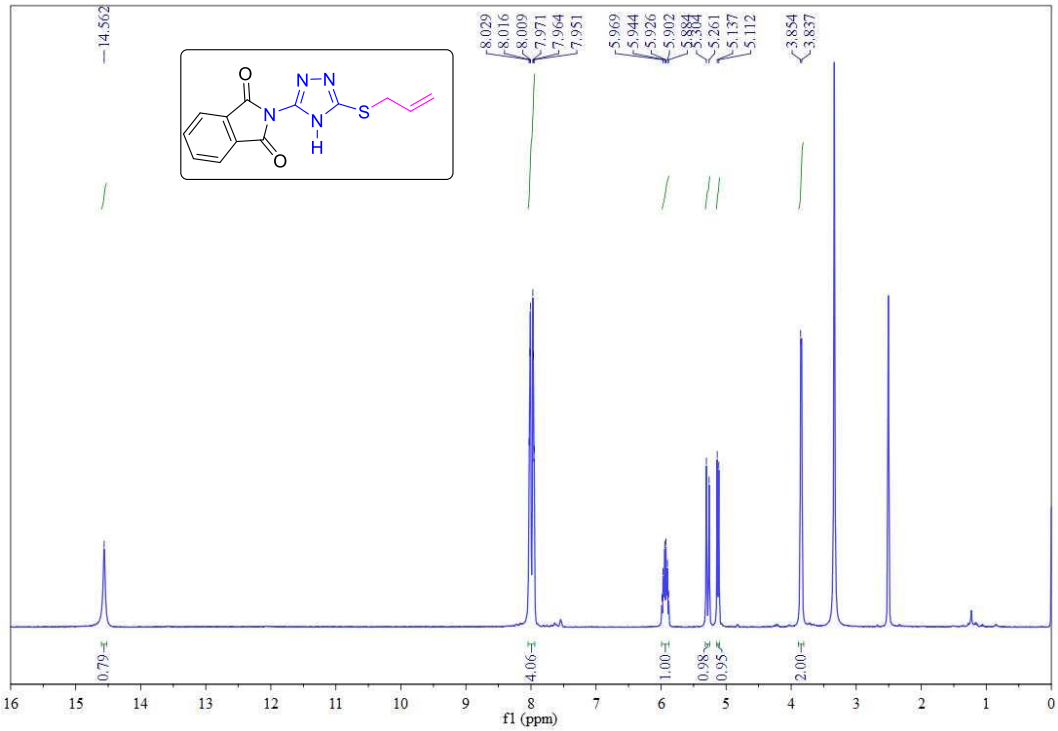
^{13}C NMR Spectrum of compound 5h (DMSO-d_6 100 MHz)



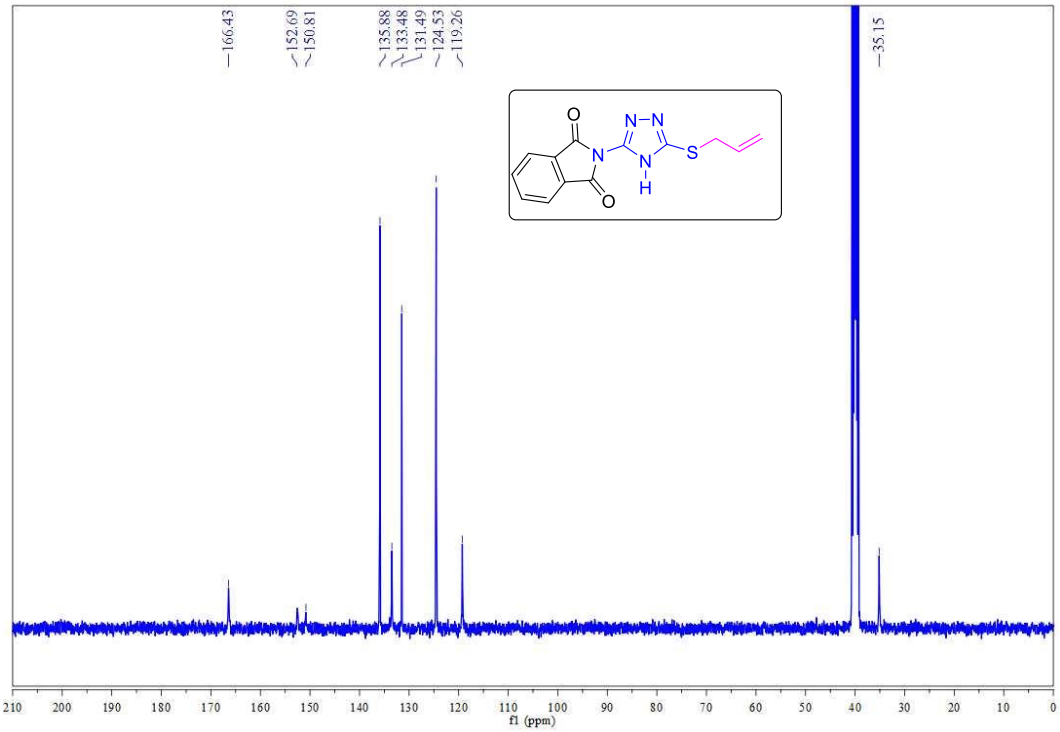
Mass Spectrum of compound 5h



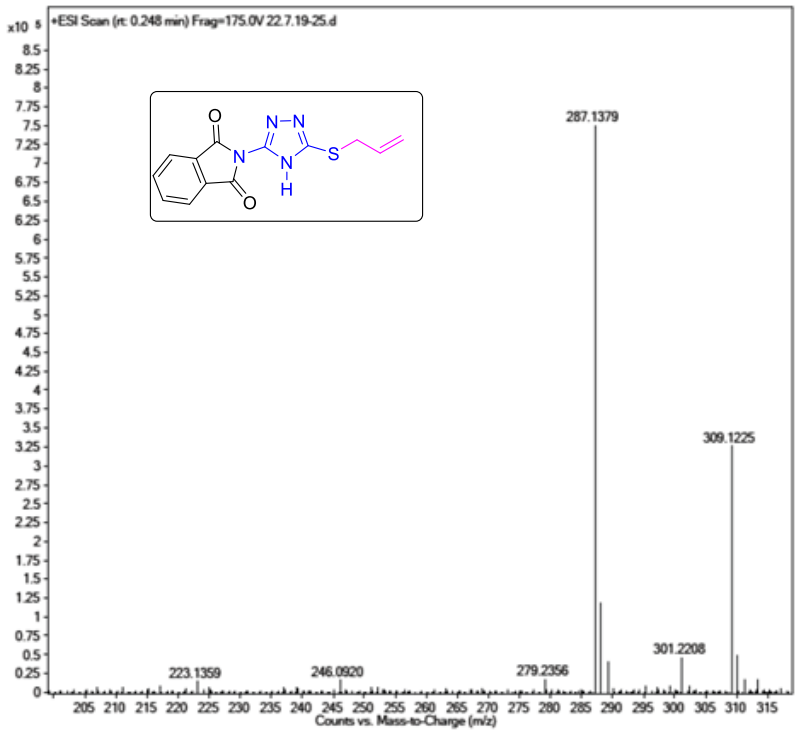
^{1}H NMR Spectrum of compound 5i (DMSO-d_6 400 MHz)



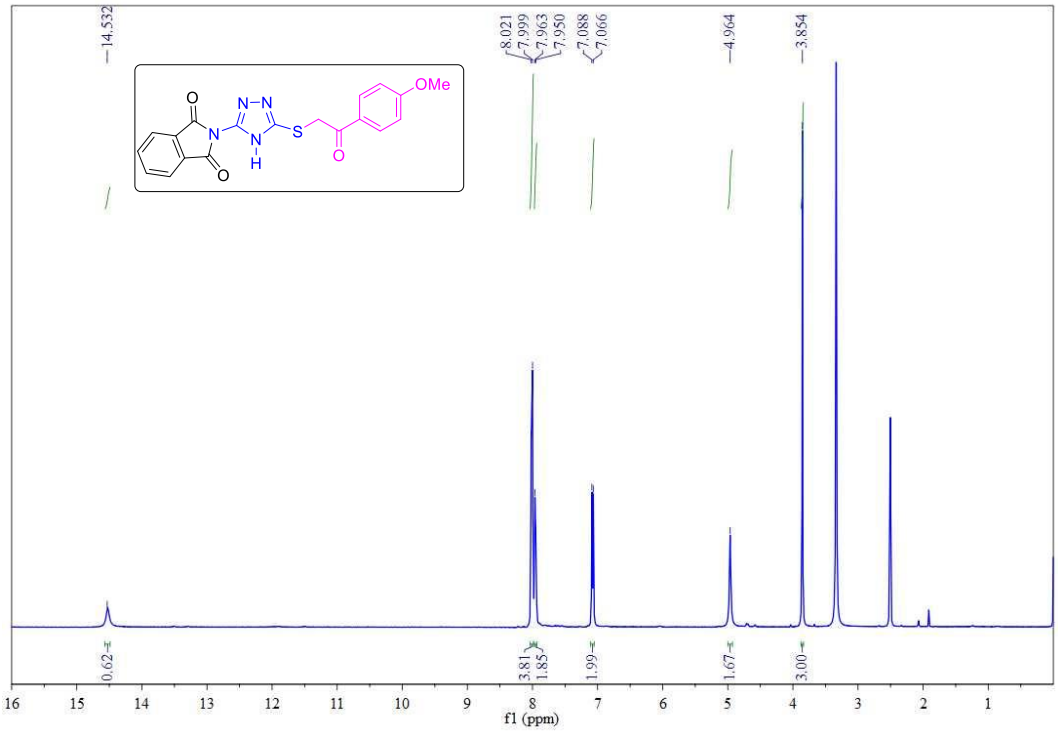
^{13}C NMR Spectrum of compound 5i (DMSO-d_6 100 MHz)



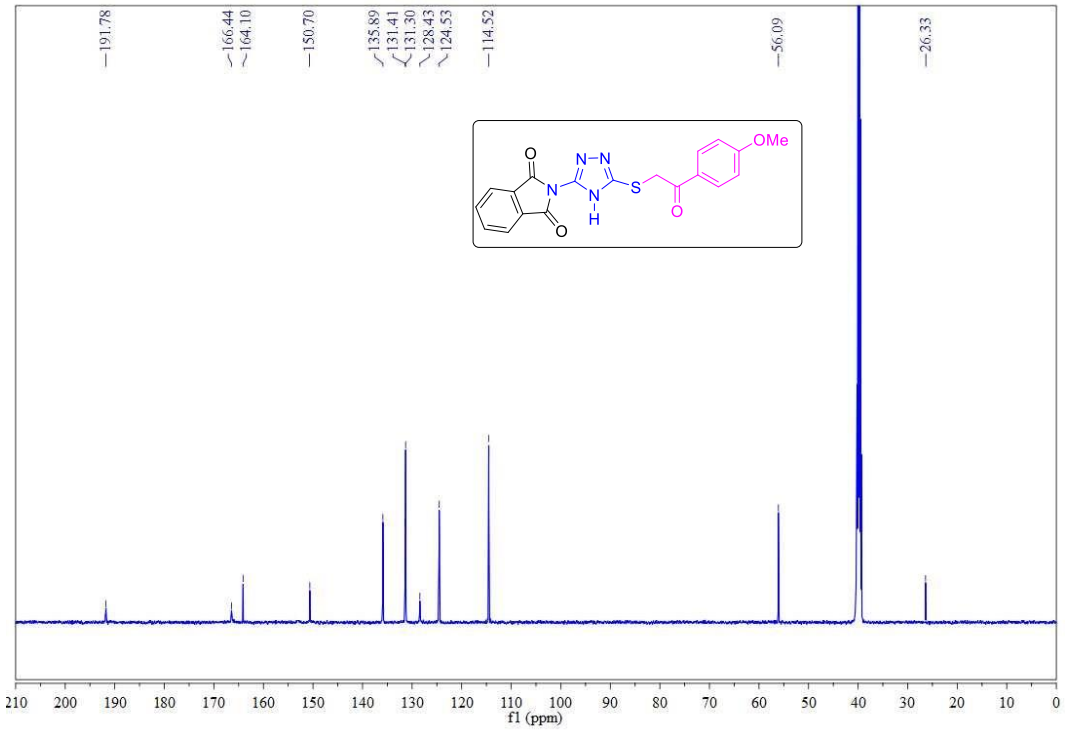
Mass Spectrum of compound 5i



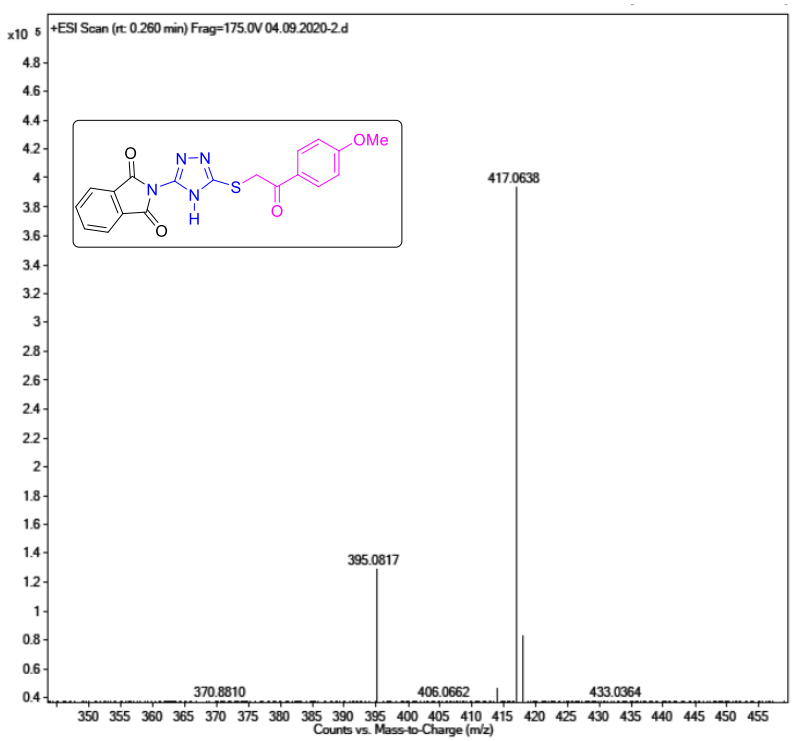
^{1}H NMR Spectrum of compound 5j (DMSO-d_6 400 MHz)



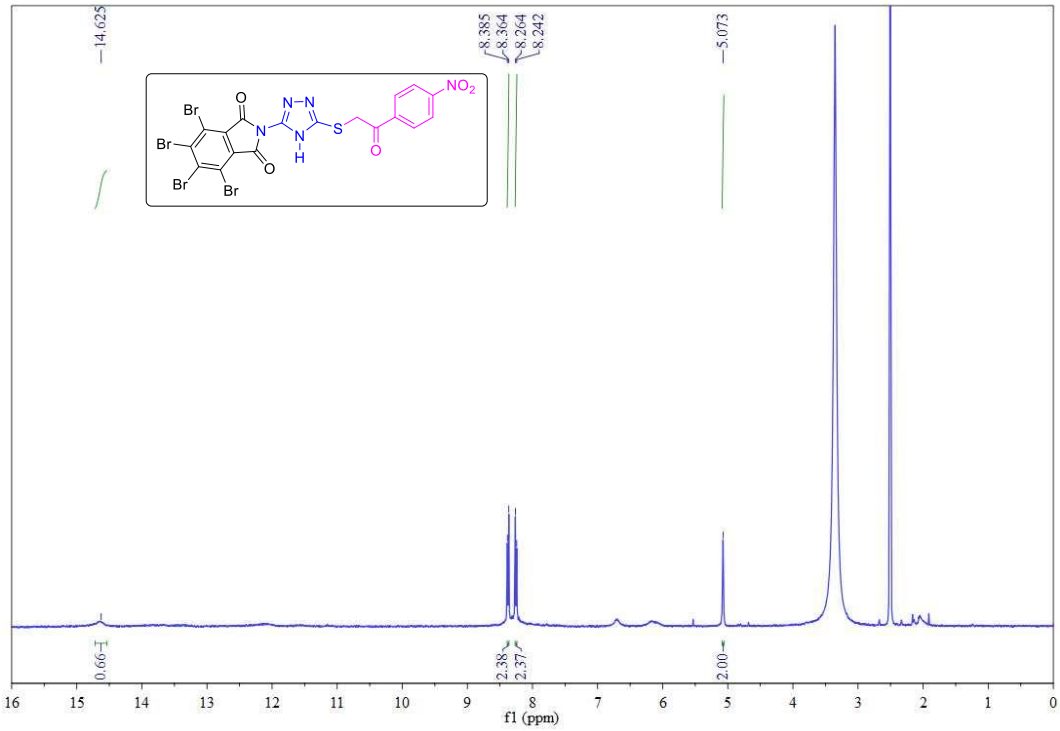
^{13}C NMR Spectrum of compound 5j (DMSO-d_6 100 MHz)



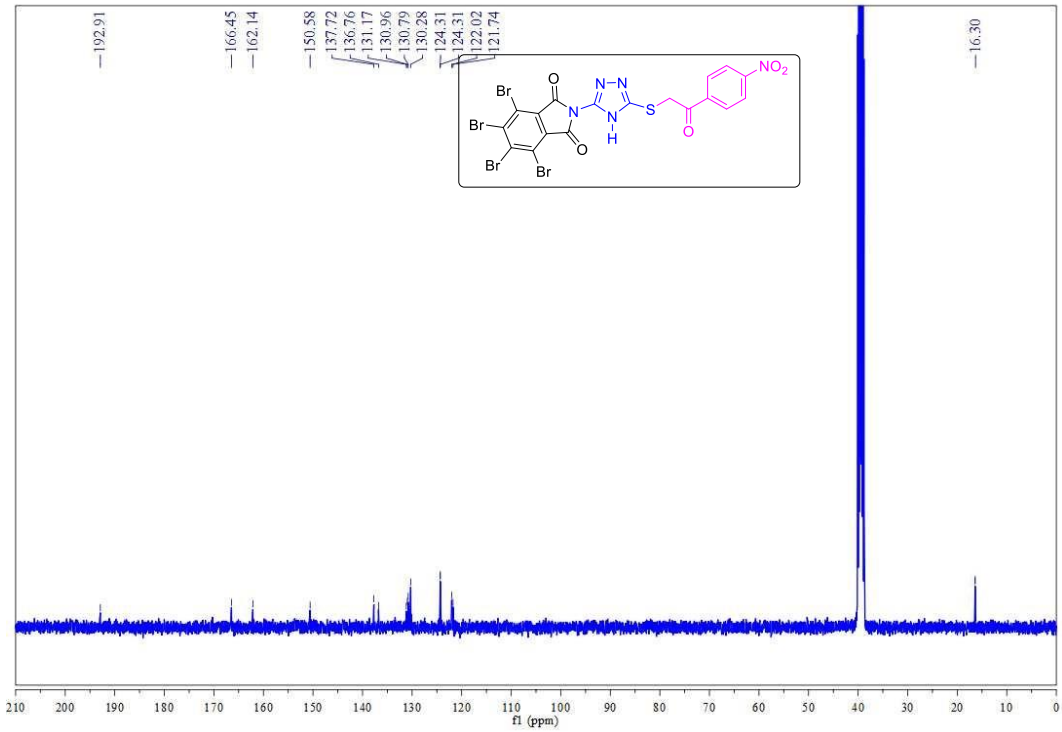
Mass Spectrum of compound 5j



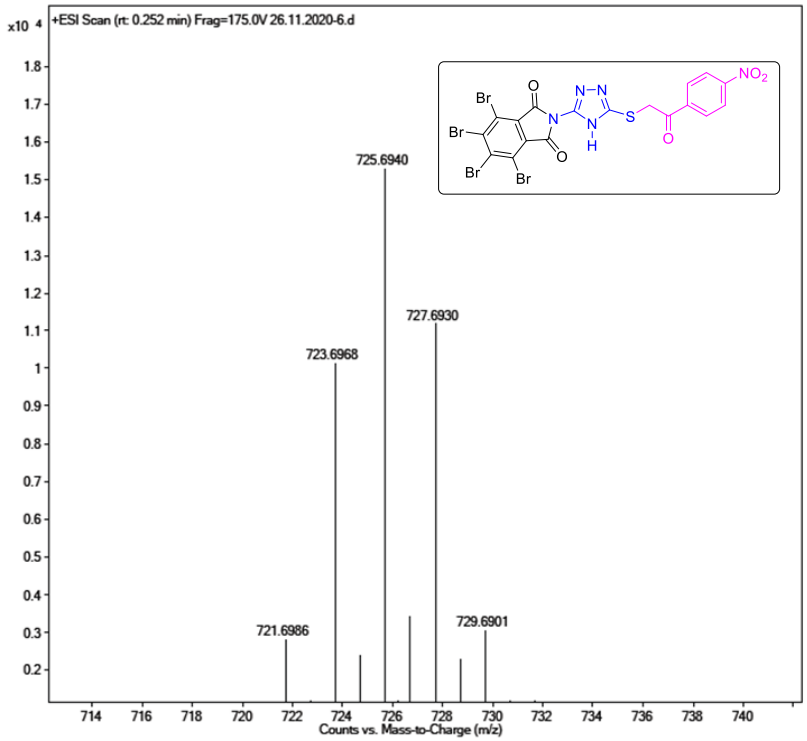
^{1}H NMR Spectrum of compound 5k (DMSO-d_6 400 MHz)



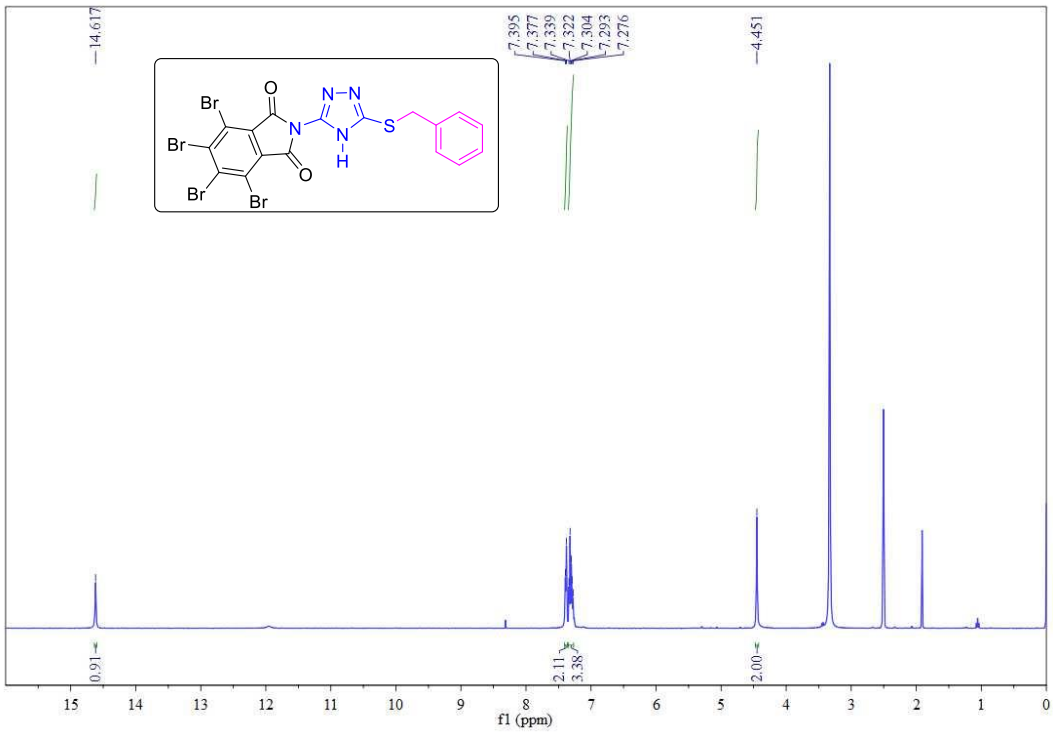
^{13}C NMR Spectrum of compound 5k (DMSO-d_6 100 MHz)



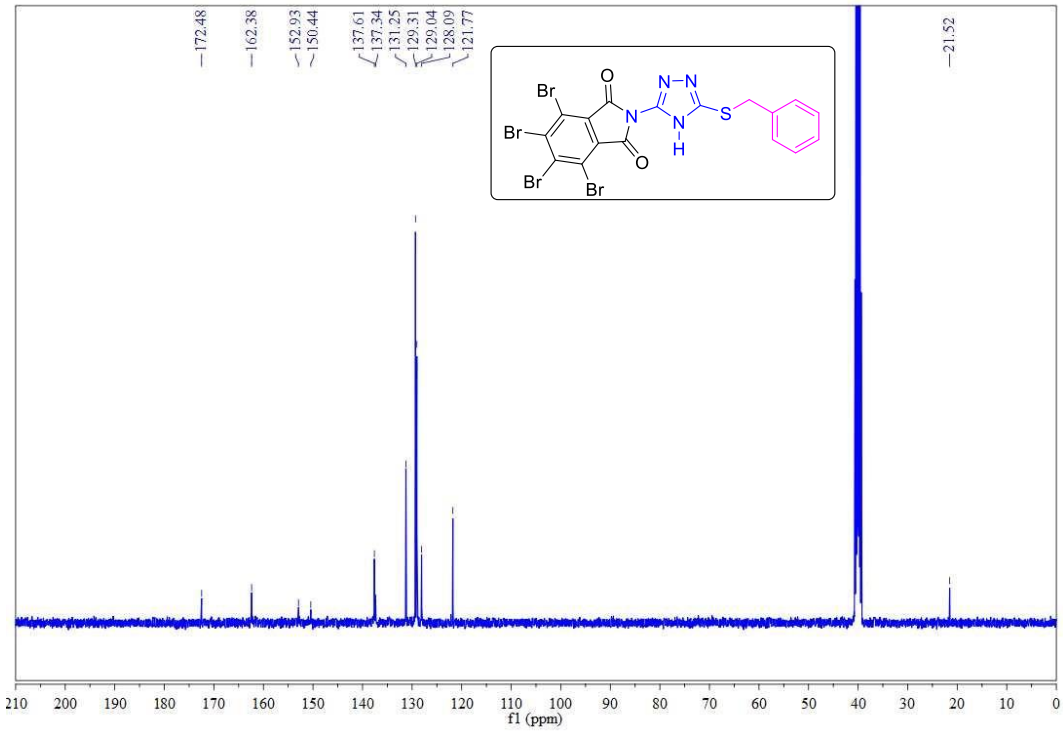
Mass Spectrum of compound 5k



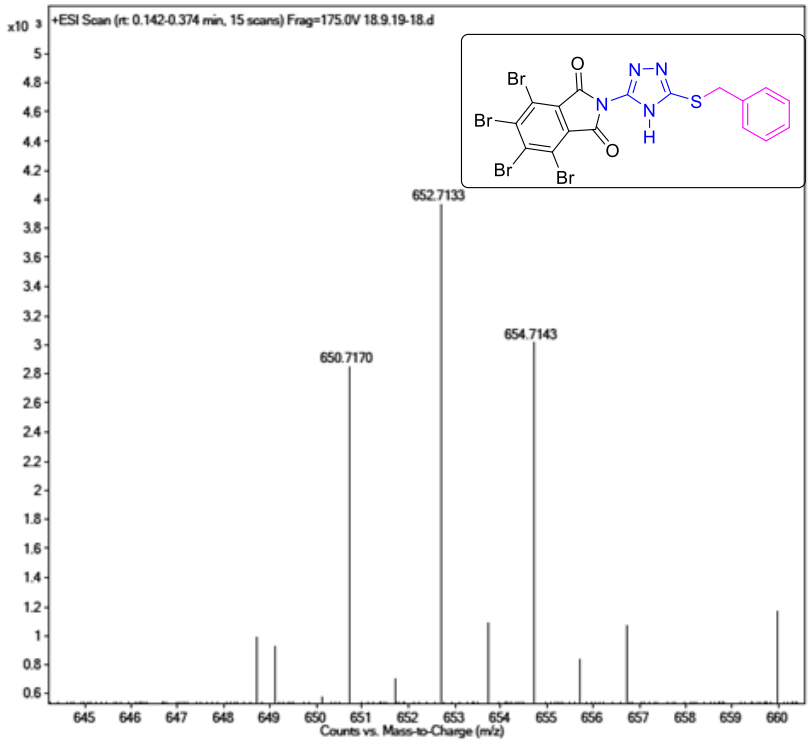
^{1}H NMR Spectrum of compound 5l (DMSO-d_6 400 MHz)



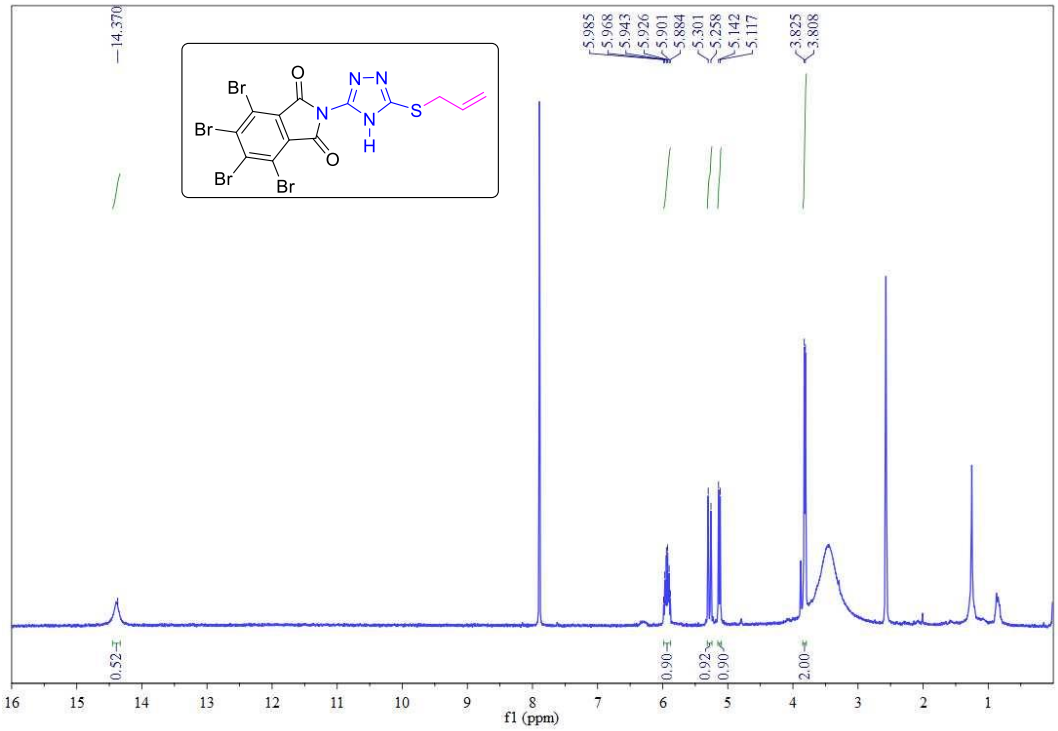
^{13}C NMR Spectrum of compound 5l (DMSO-d_6 100 MHz)



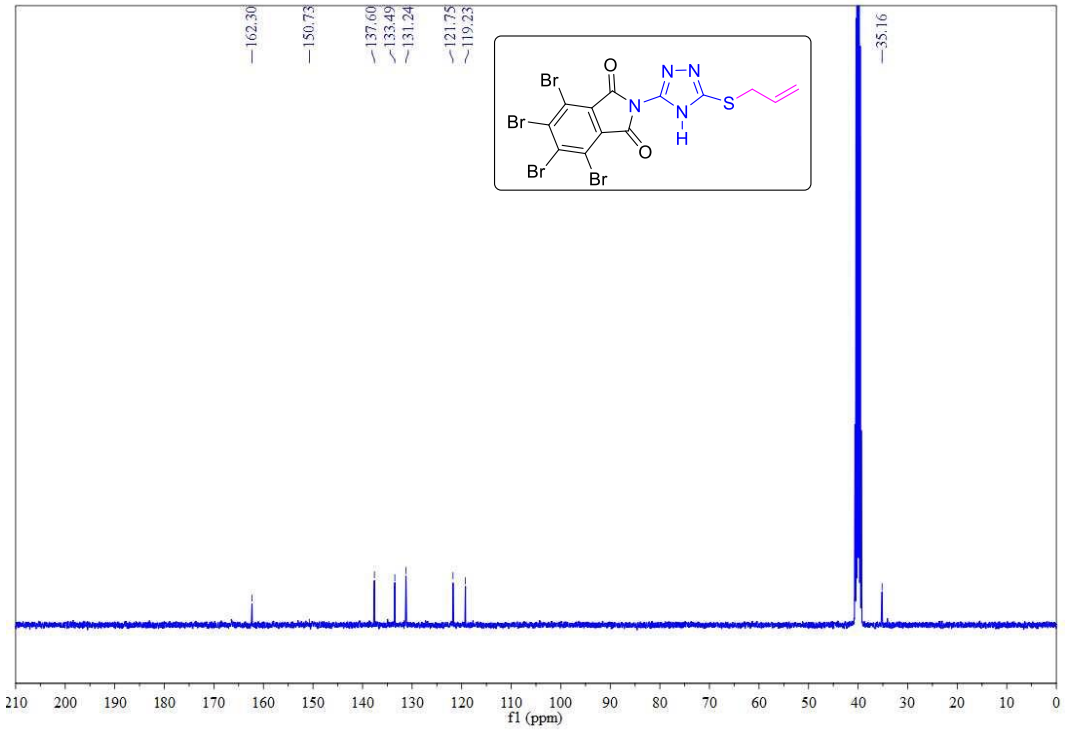
Mass Spectrum of compound 5l



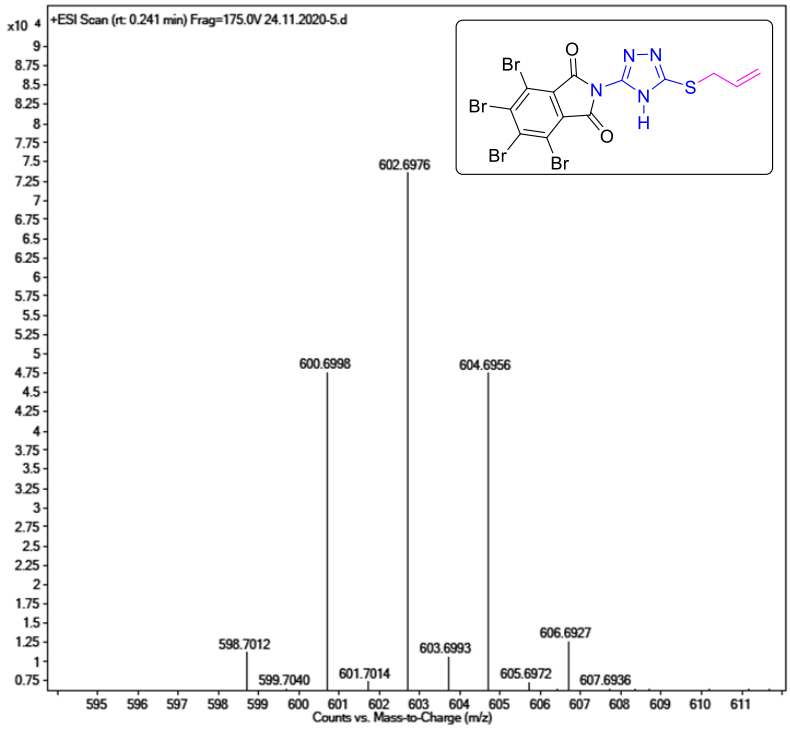
^{1}H NMR Spectrum of compound 5m (DMSO+$CDCl_3$ 400 MHz)



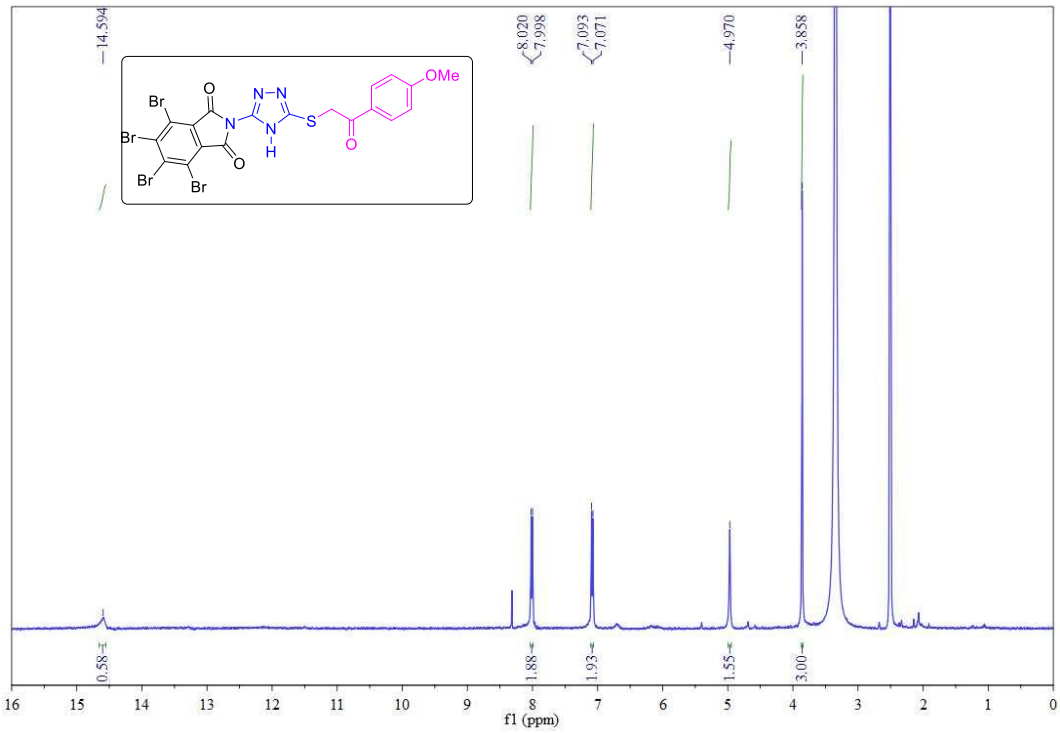
^{13}C NMR Spectrum of compound 5m (DMSO-d_6 100 MHz)



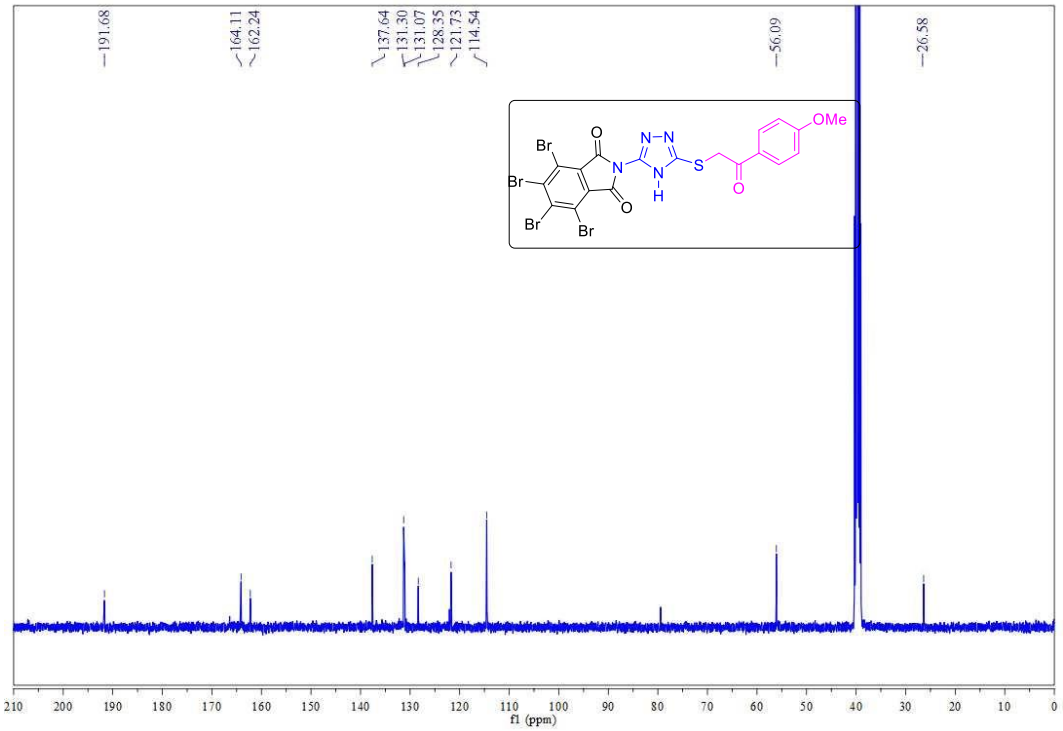
Mass Spectrum of compound 5m



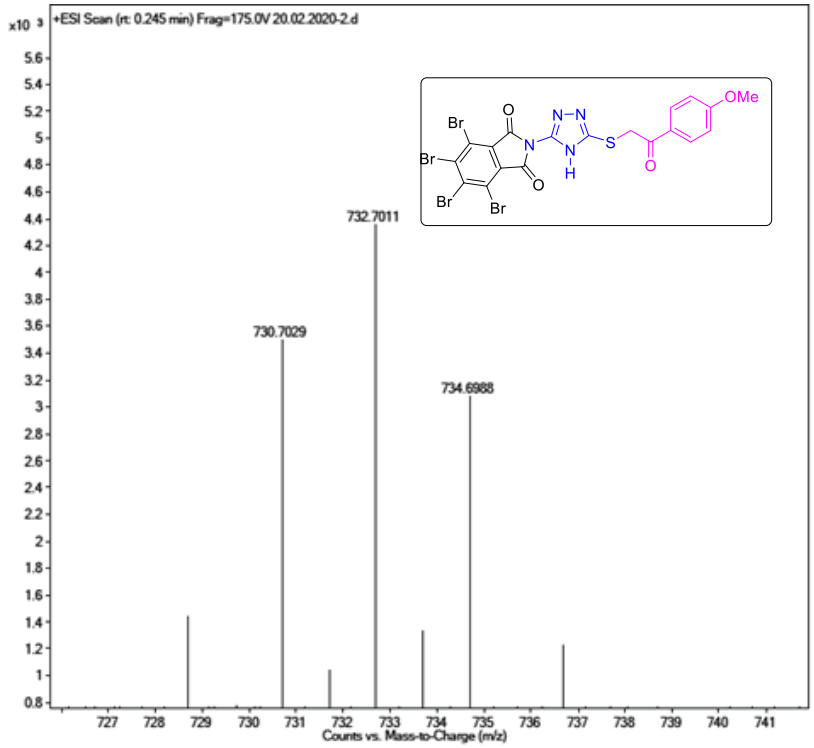
^{1}H NMR Spectrum of compound 5n (DMSO-d_6 400 MHz)



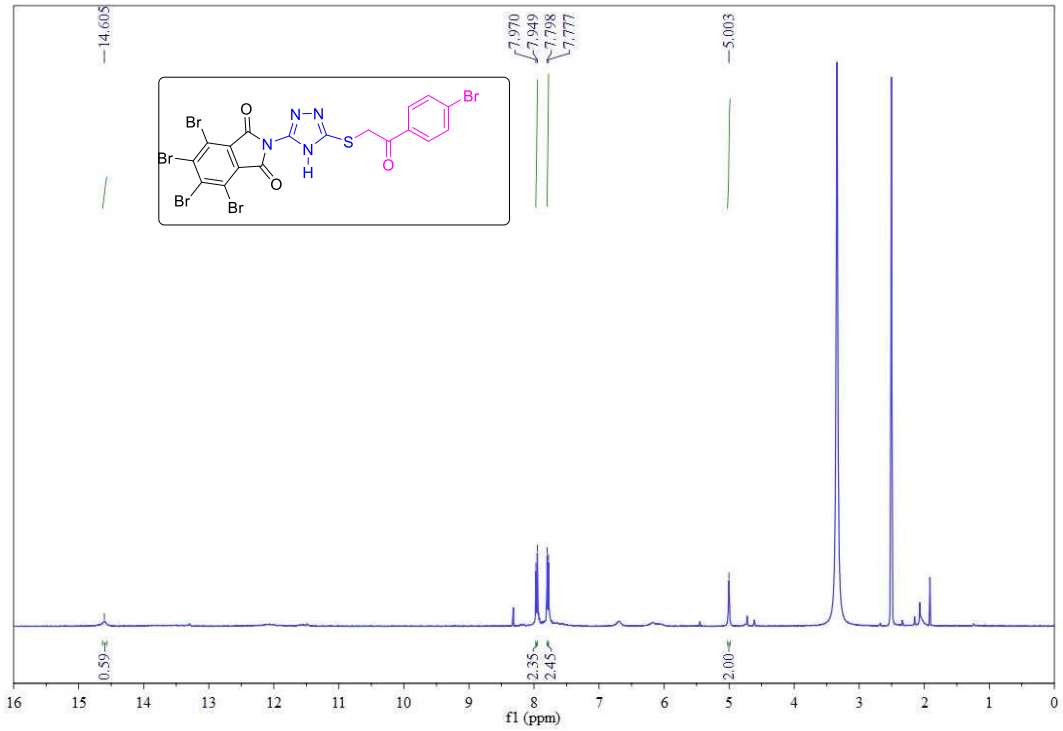
^{13}C NMR Spectrum of compound 5n (DMSO-d_6 100 MHz)



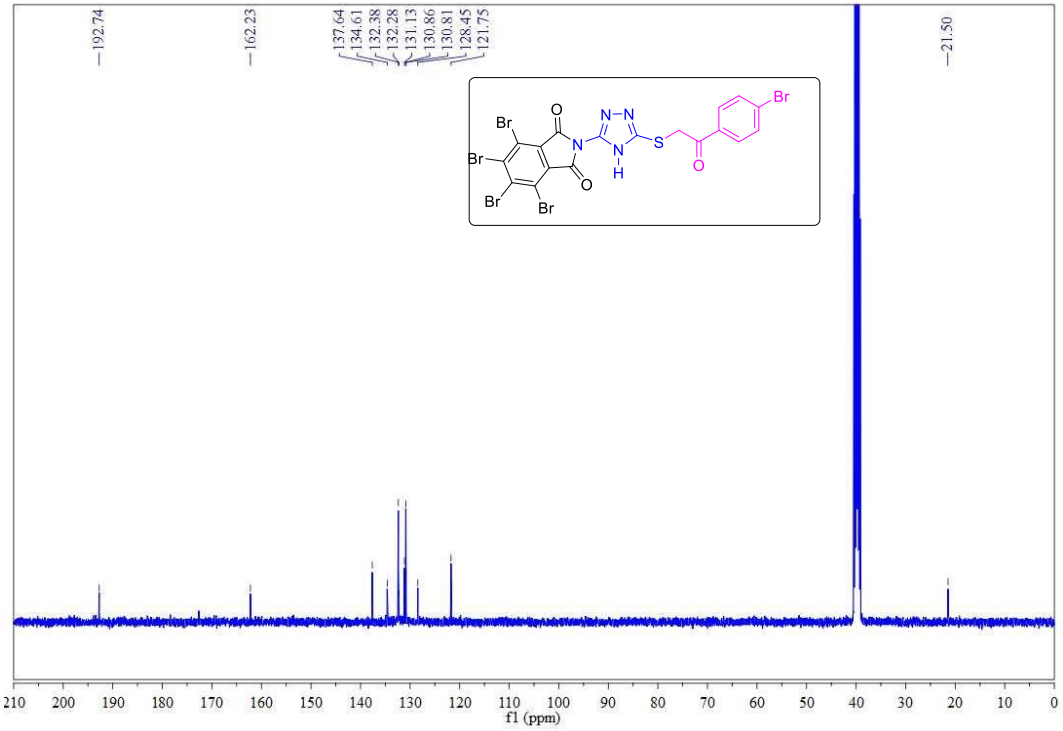
Mass Spectrum of compound 5n



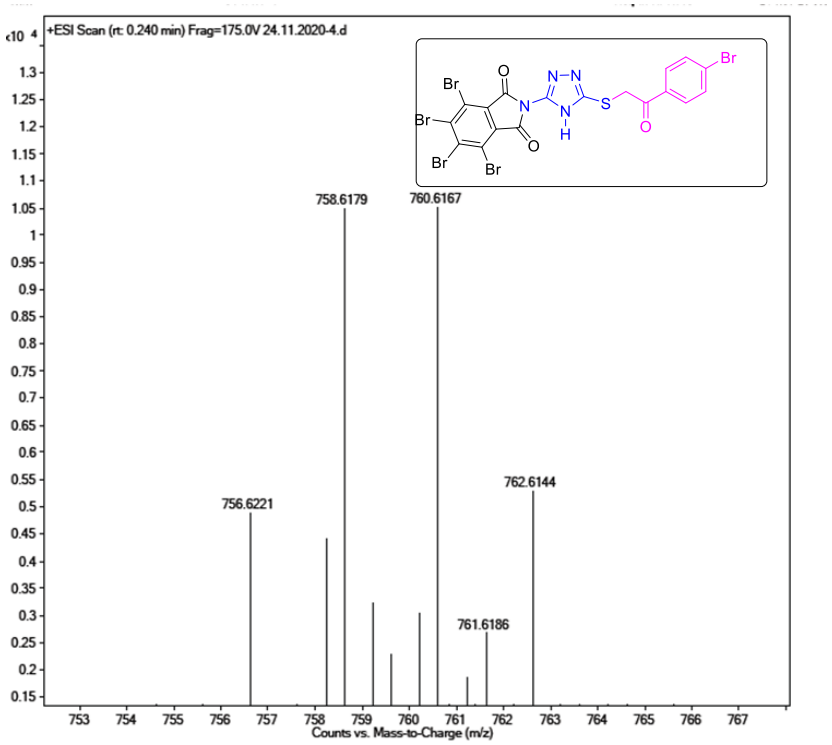
^{1}H NMR Spectrum of compound 5o (DMSO-d_6 400 MHz)



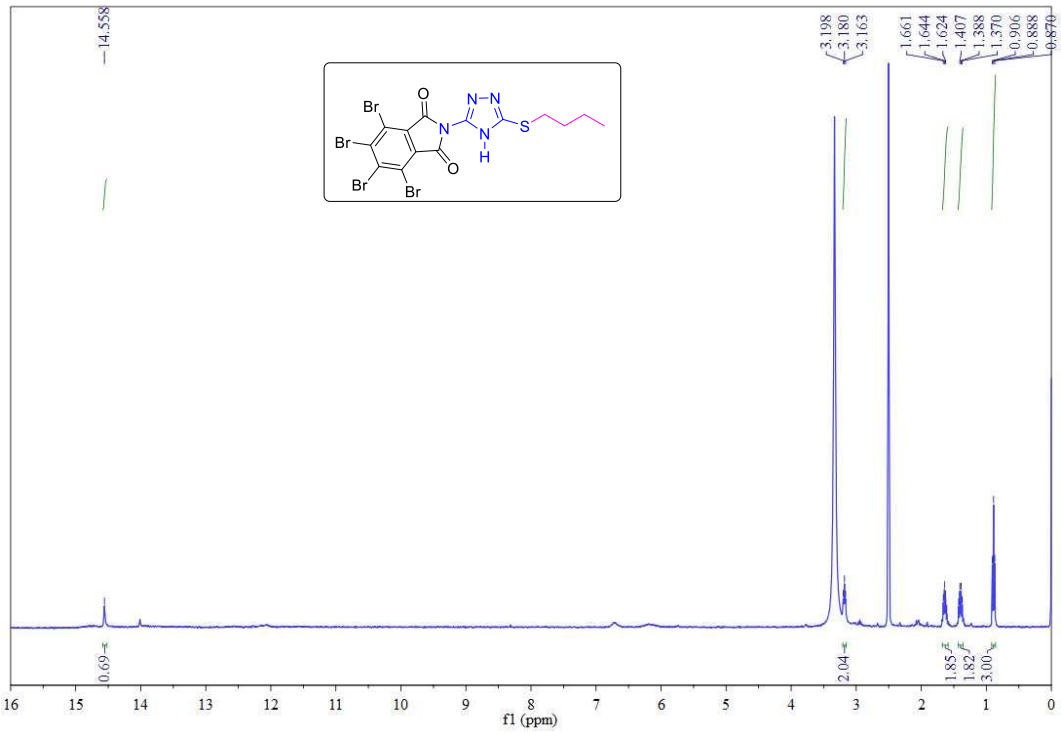
^{13}C NMR Spectrum of compound 5o (DMSO-d_6 100 MHz)



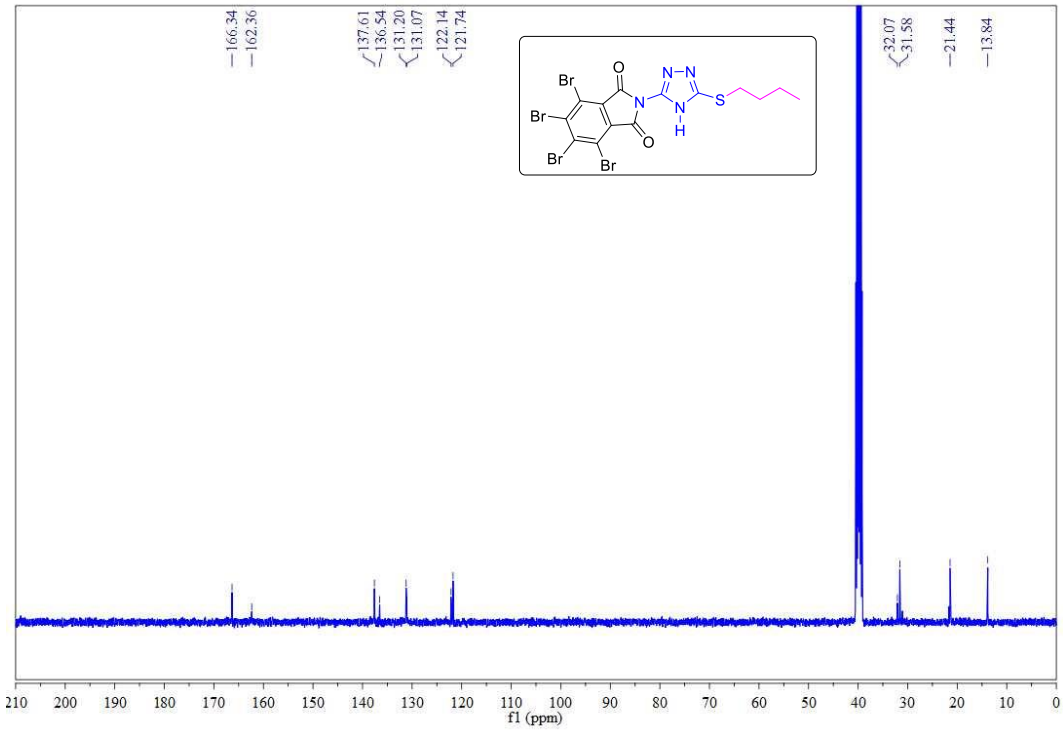
Mass Spectrum of compound 5o



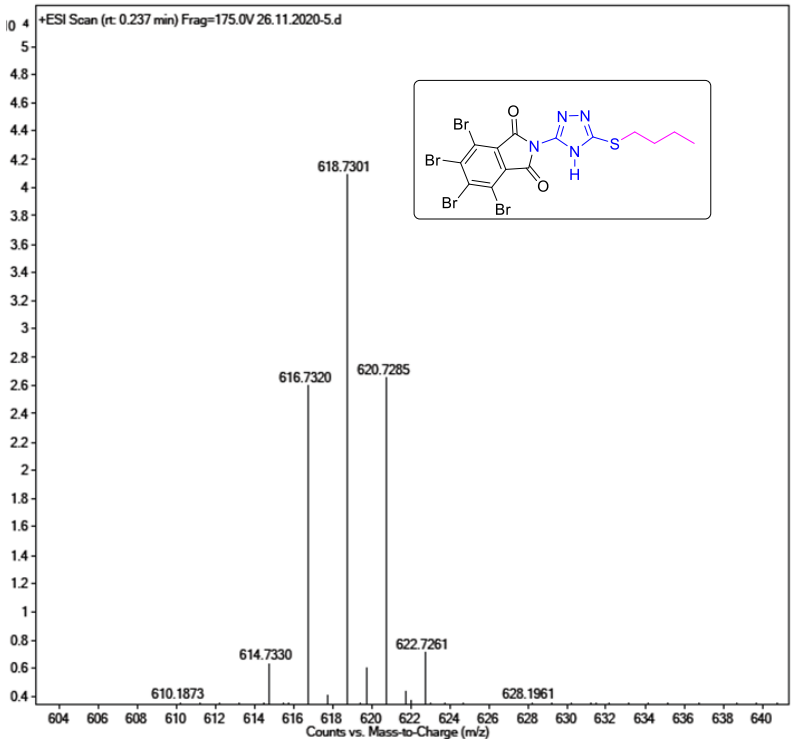
^{1}H NMR Spectrum of compound 5p (DMSO-d_6 400 MHz)



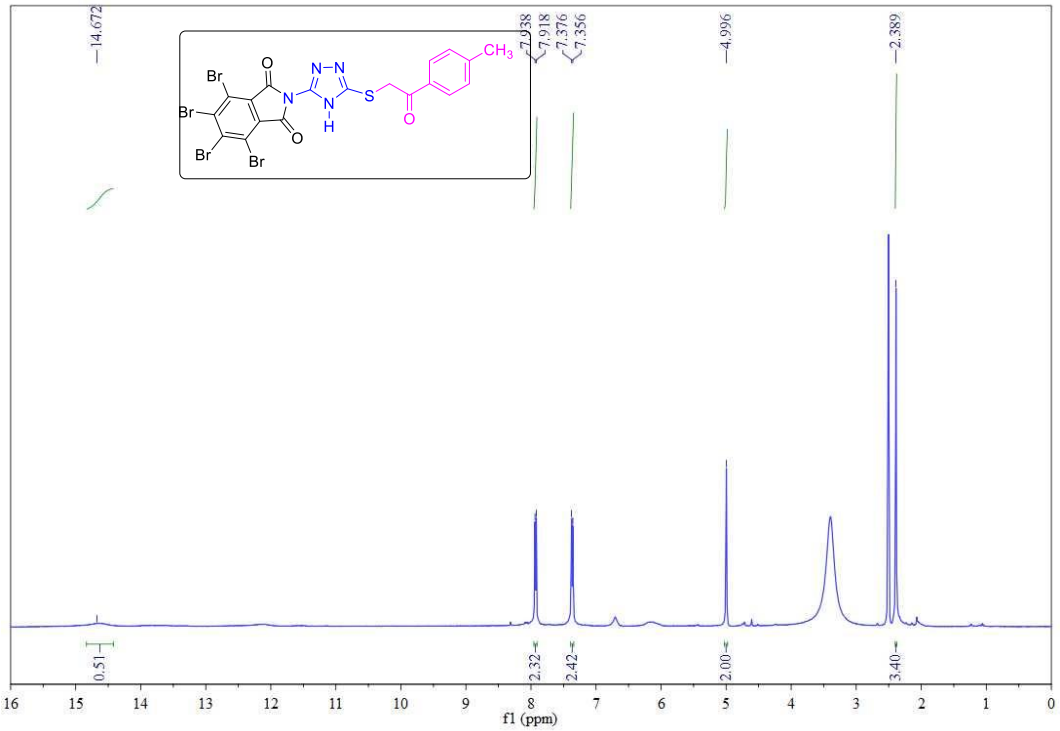
^{13}C NMR Spectrum of compound 5p (DMSO-d_6 100 MHz)



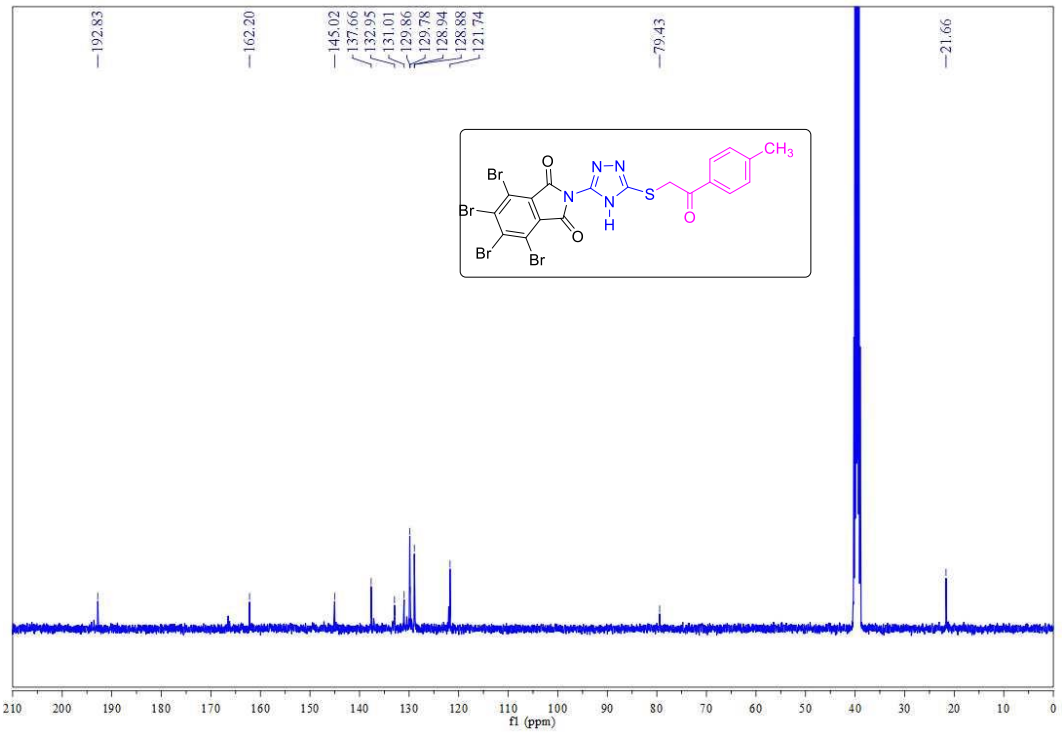
Mass Spectrum of compound 5p



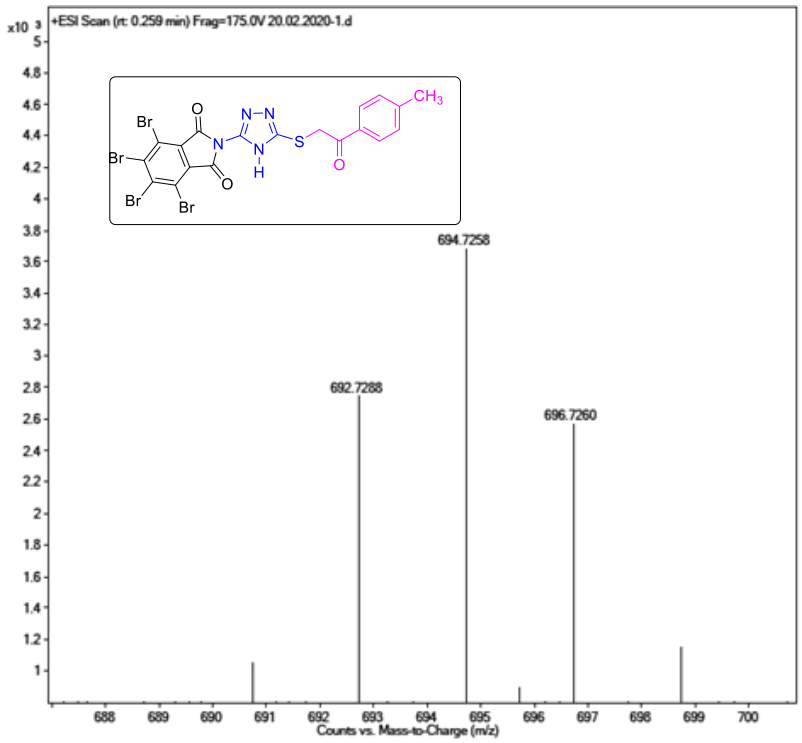
^{1}H NMR Spectrum of compound 5q (DMSO-d_6 400 MHz)



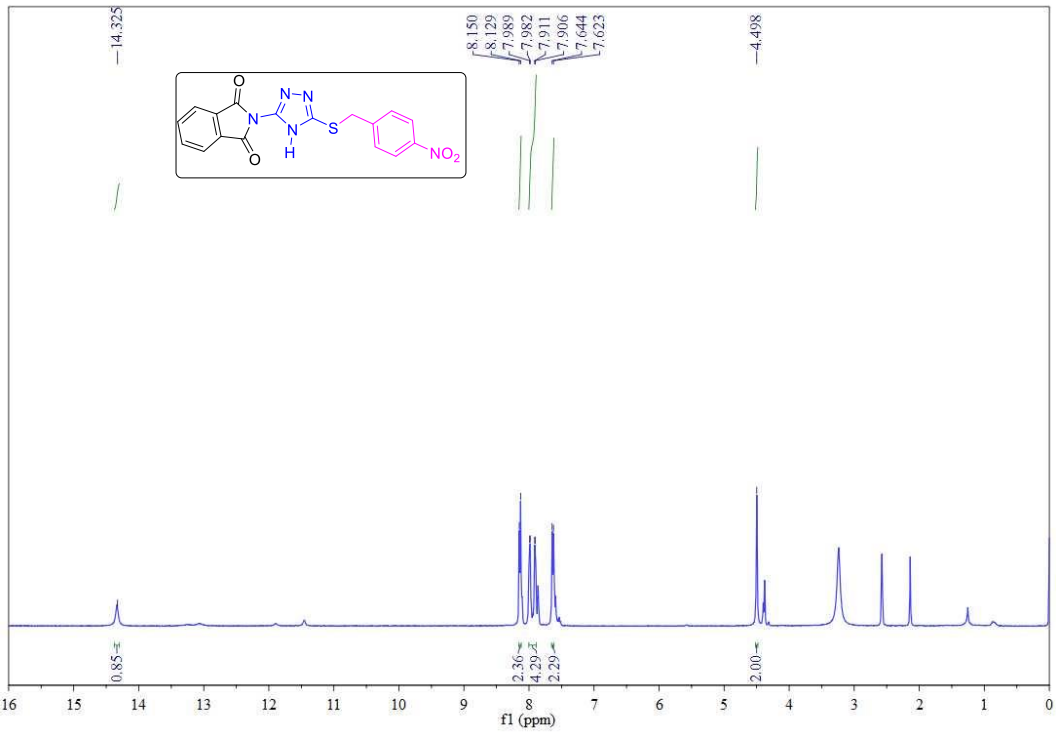
NMR Spectrum of compound 5q (DMSO-d_6 100 MHz)



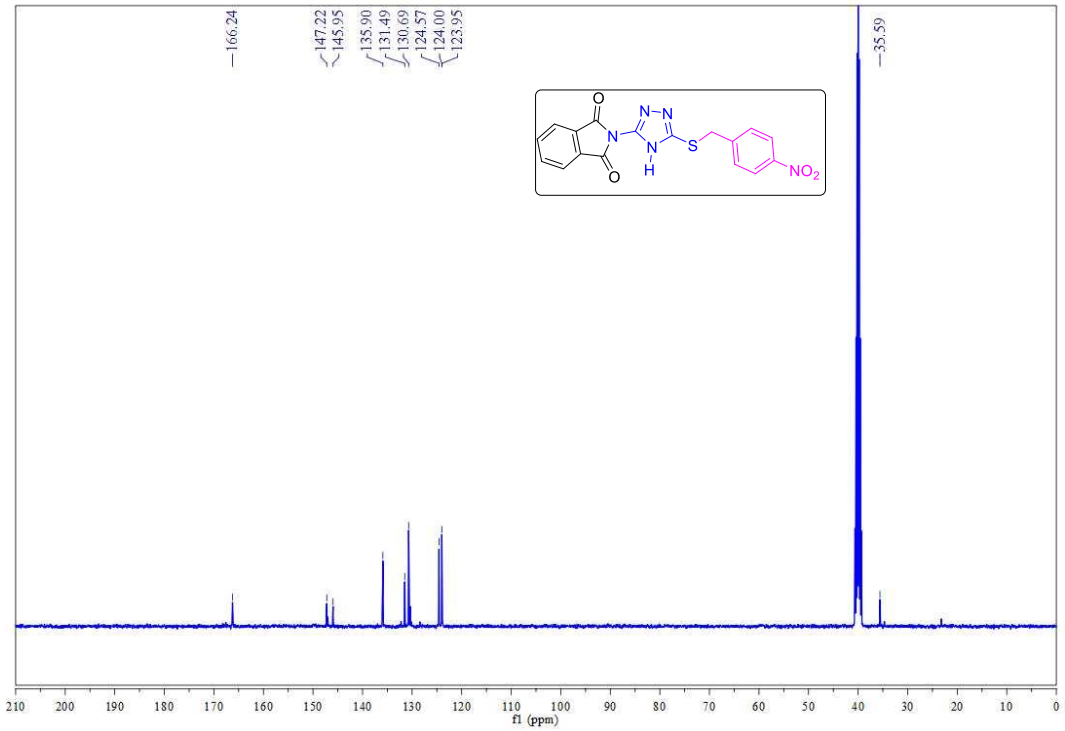
Mass Spectrum of compound 5q



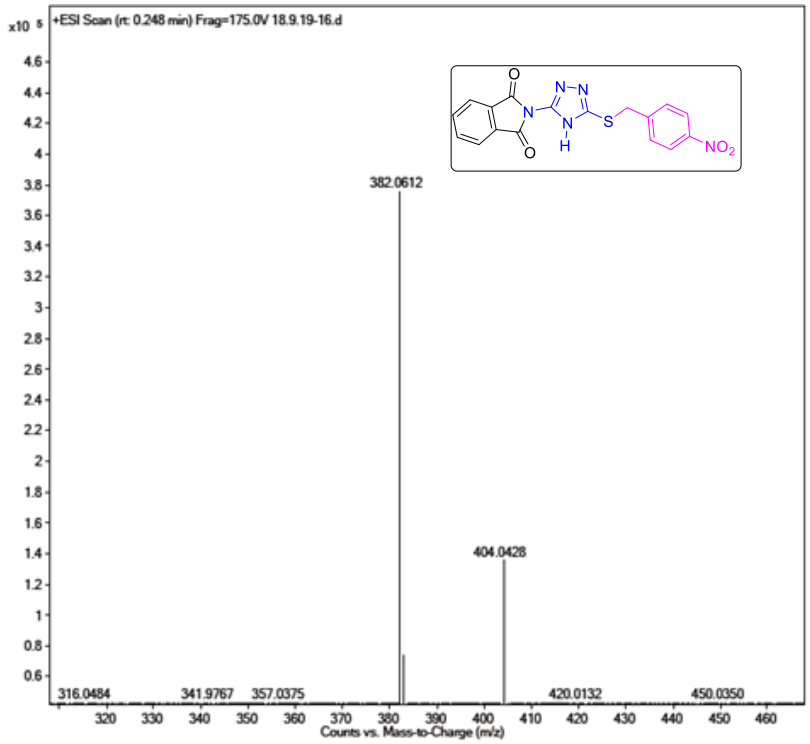
^{1}H NMR Spectrum of compound 5r (DMSO+ $CDCl_3$ 400 MHz)



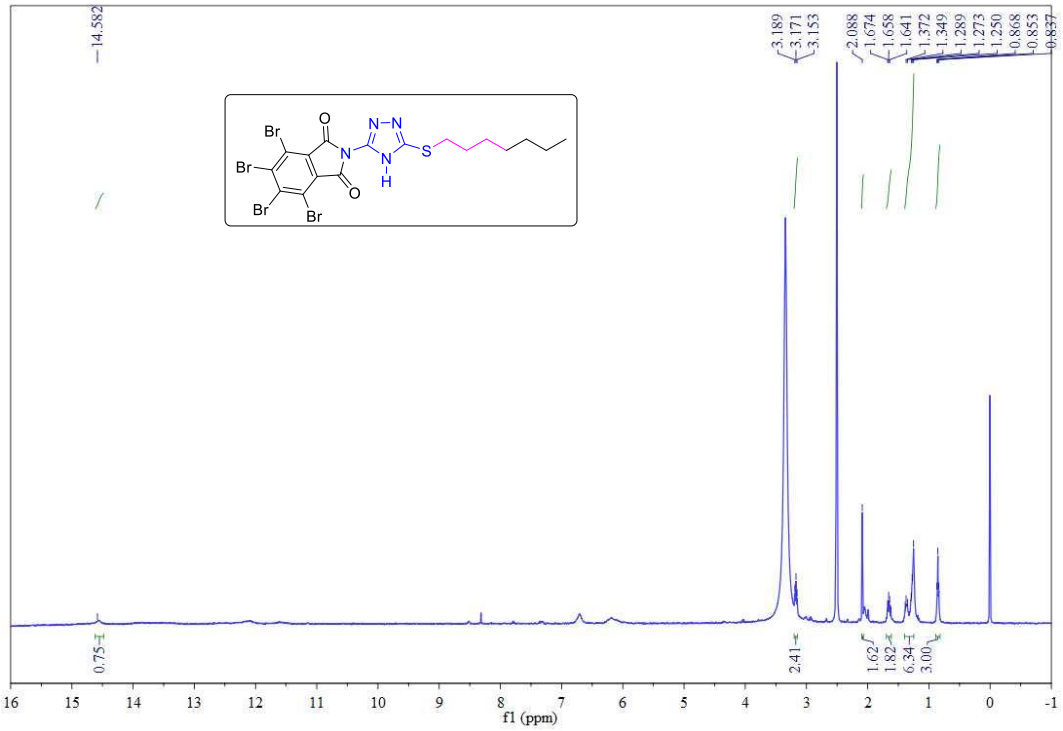
^{13}C NMR Spectrum of compound 5r (DMSO-d_6 100 MHz)



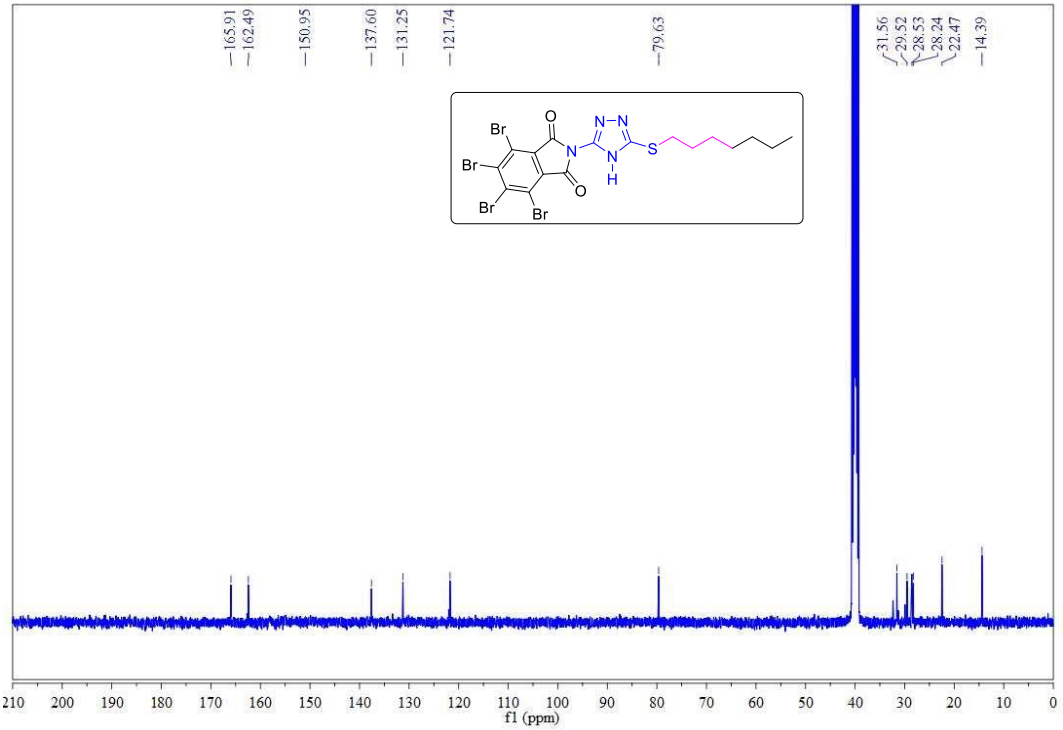
Mass Spectrum of compound 5r



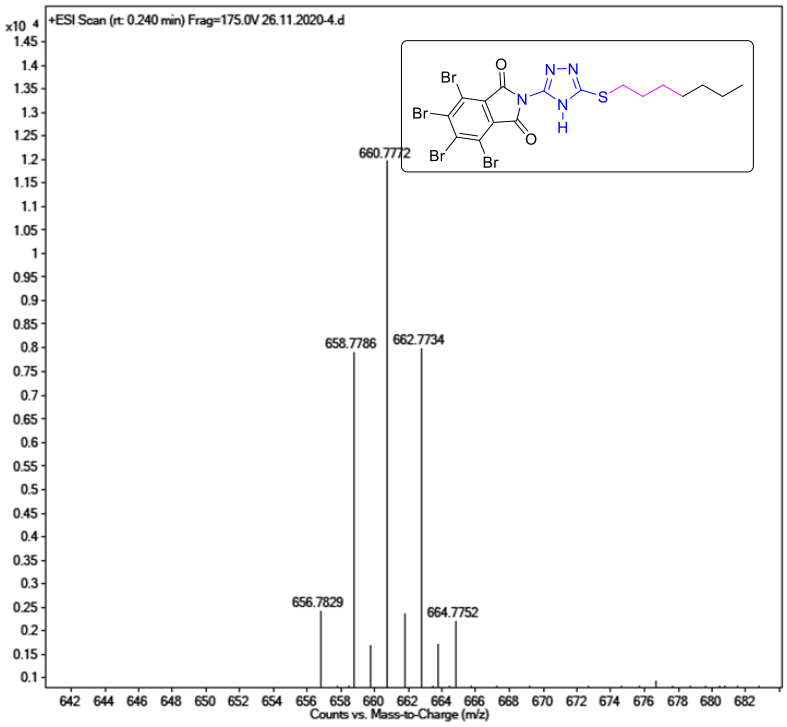
^{1}H NMR Spectrum of compound 5s (DMSO-d_6 400 MHz)



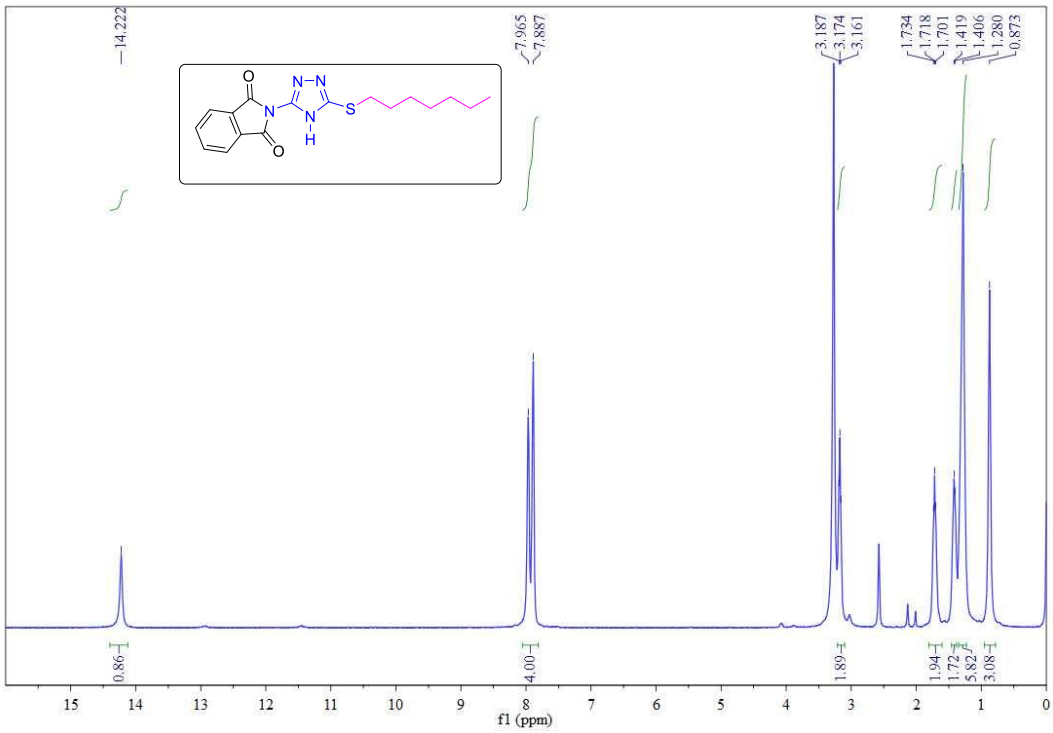
^{13}C NMR Spectrum of compound 5s (DMSO-d_6 100 MHz)



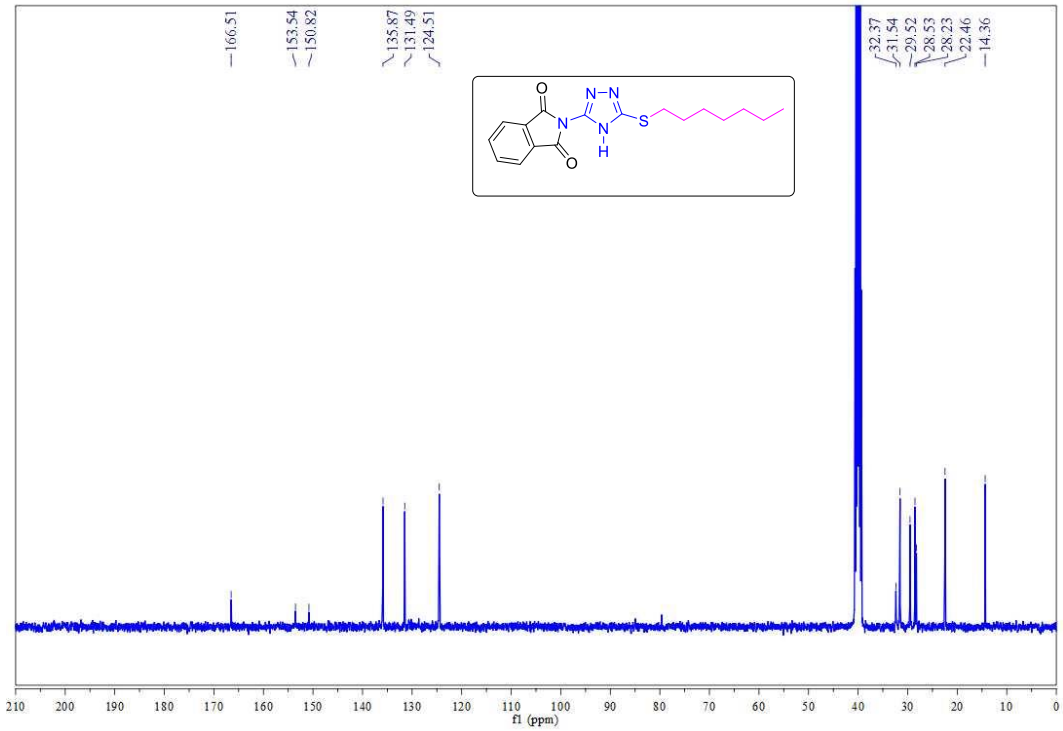
Mass Spectrum of compound 5s



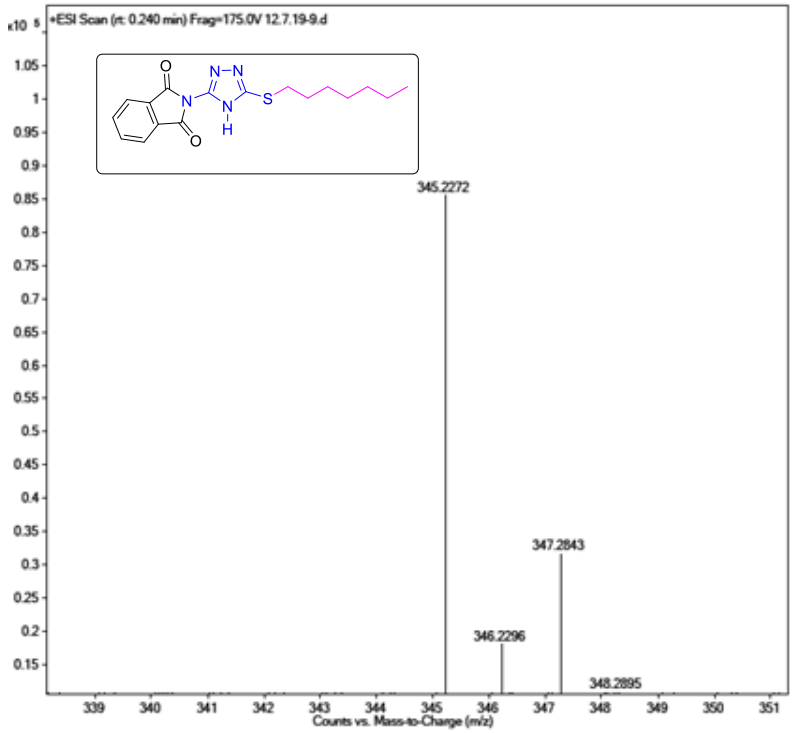
^{1}H NMR Spectrum of compound 5t (DMSO-d_6 400 MHz)



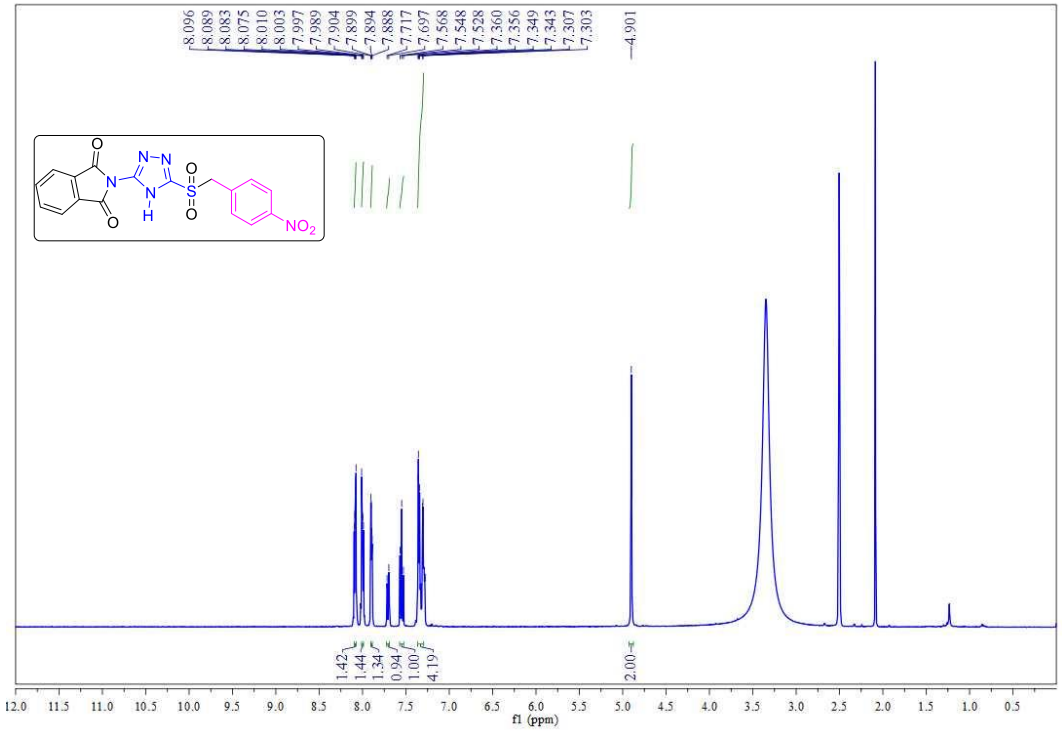
^{13}C NMR Spectrum of compound 5t (DMSO-d_6 100 MHz)



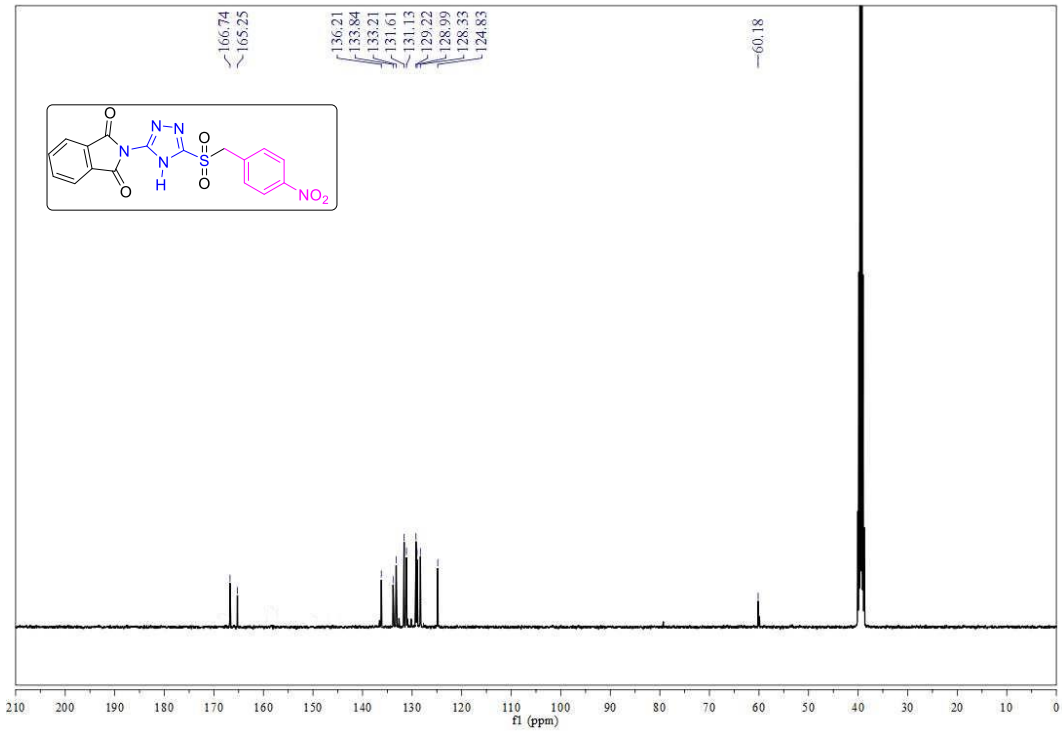
Mass Spectrum of compound 5t



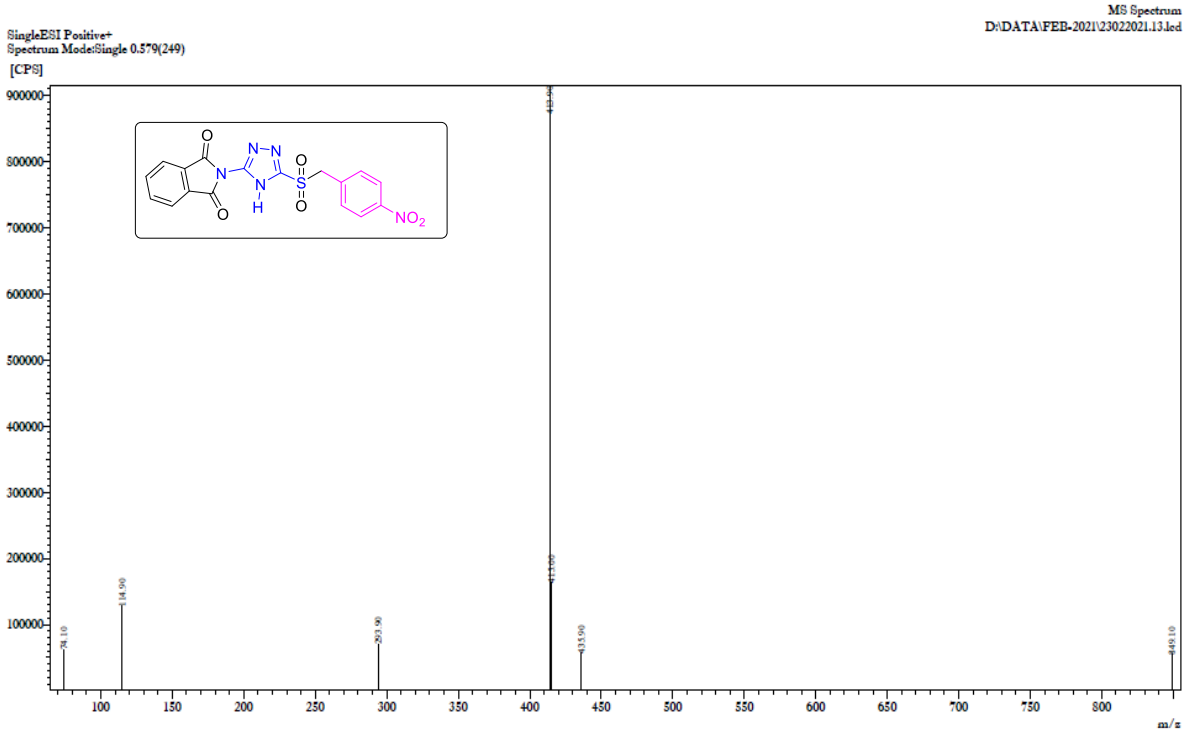
^{1}H NMR spectrum of compound 6a (DMSO-d_6 400 MHz)



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



Mass spectrum of compound 6a



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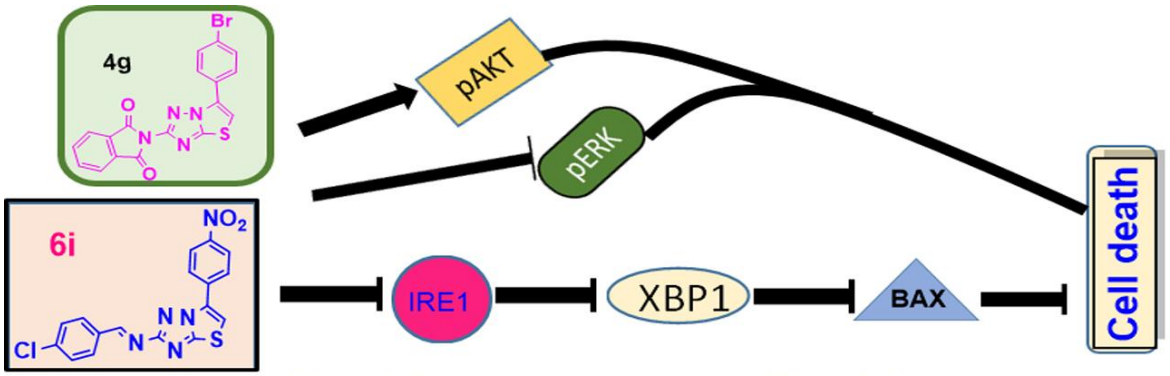
CHAPTER-III

One-pot three component synthesis of fused [3,2-b] [1,2,4]-triazolo-thiazole isoindolines and Schiff bases, characterization and targeting glioma in-vitro anticancer activity, molecular docking study.



4g. IC_{50} μM; C6 = 8.091±0.43 **6i.** IC_{50} μM; C6 = 8.74 ±0.68
IC_{50} μM; LN18 = 12.68±0.17 IC_{50} μM; LN18 = 12.56±0.21

3.1 Introduction.

The phthalimide (isoindoline) ring has been identified as most important scaffold in synthetic organic chemistry and in which nitrogen atom flanked by two carbonyl groups (-CO-N(R)-CO-).[1] The *N*-substituted cyclic imides has emerged as a promising moiety in the medicinal chemistry field due to its wide spectrum of therapeutic effects.[2-6] Furthermore, the *N*-substituted imines (Schiff bases), compounds having a general core structure is R-N=CH-R. and these were show the wide range of applications in organic synthesis. Moreover, the Schiff bases are an important intermediate for the development of various heterocyclic compounds such as imidazoles, thiazoles, oxazoles etc., For example GBB reaction is extensively used for the synthesis of bicyclic imidazoles from substituted Schiff bases. And also these were just as important in terms of pharmacological properties.[7-8]

The five membered ring *N*-bridged heterocyclic compounds are acquired as a biologically active scaffolds. Fused heterocyclic compounds with N and S heteroatoms have attracted a lot of interest in the field of medicinal chemistry.[9-16] The possible existing types of five membered ring fused heterocyclic systems are [3,2-*b*][1,2,4]-triazolothiazoles, [2,1-*b*][1,3,4]-thiadiazole, imidazo[1,2-*b*][1,2,4]-triazoles.[17] The antitumor properties of the 2-amino-1,3,4-thiadiazole skeletons are well recognized[18], and its fused systems with the imidazo [3,2-*b*][1,2,4]-triazole ring systems are likewise known to possess remarkable anticancer activities.[19] Further, triazolothiazoles exhibit well known applications in medicinal chemistry research some of them have antibacterial,[20] anti-inflammatory, [21] antifungal,[22] analgesic [23-24] and anticancer [25] and antiviral [26] activities. Hybrid molecules created by combining distinct pharmacophores could lead to compounds with interesting biological properties.

Compounds 1 and 2 **Fig.1** were found to exhibit potent anticancer activity after a nitrogen containing heterocyclic system has introduced into the isoindoline-1,3-diones.[27-28] Fluorine substituted compound 3 **Fig.1** demonstrates significant prostate cancer activity, [29] and compound 4 **Fig.1** is identified as potential antitumor agent inhibiting cyclin dependent kinase. [30] In the **Fig.1** the compounds from 5-10 was showing antimicrobial and antibacterial, anticancer activities. [31-35]

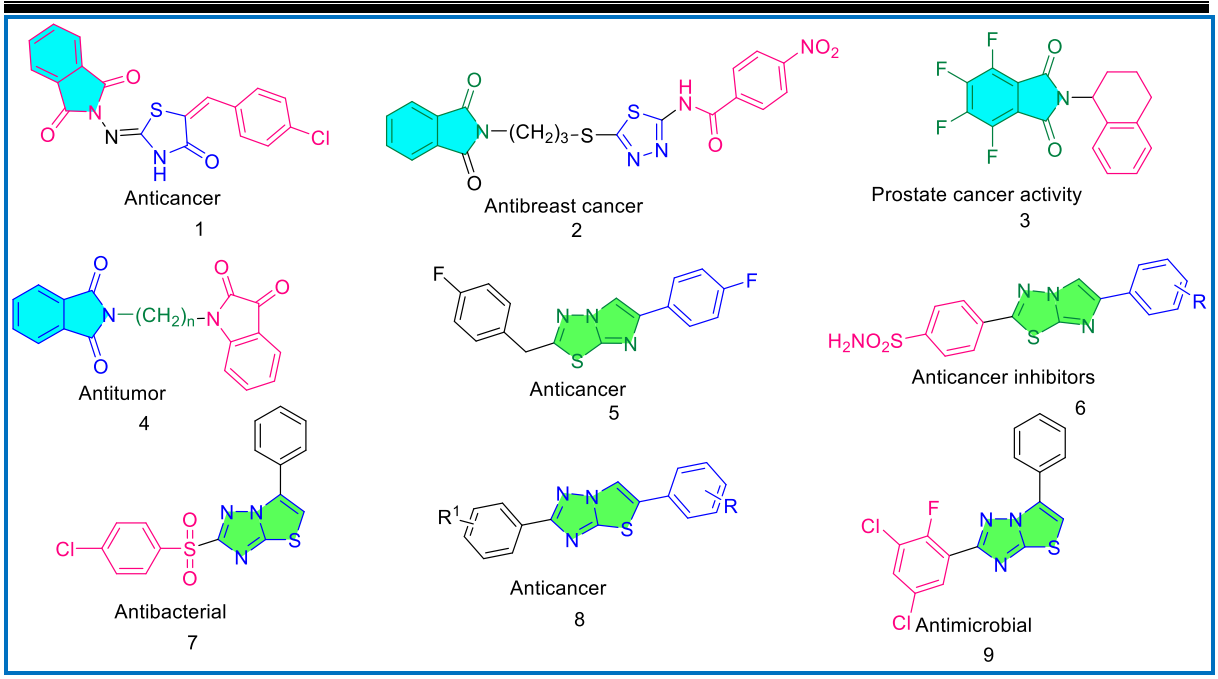
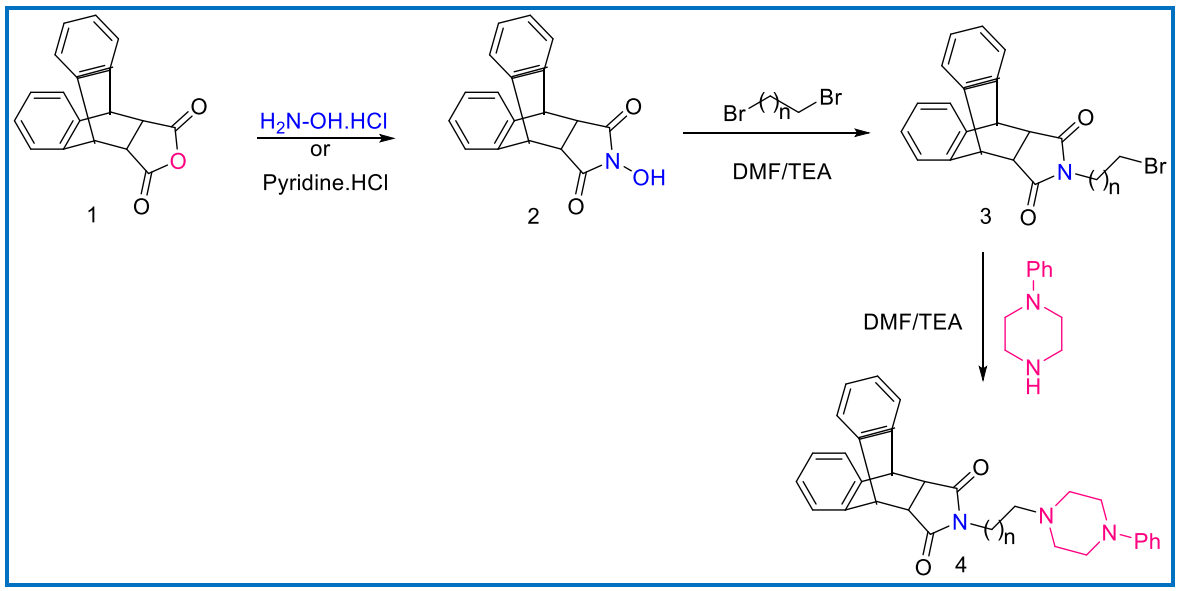


Fig.1. Explains similar reported anticancer compounds.

Literature reports of isoindolines, fused five membered heterocyclics, Schiff bases.

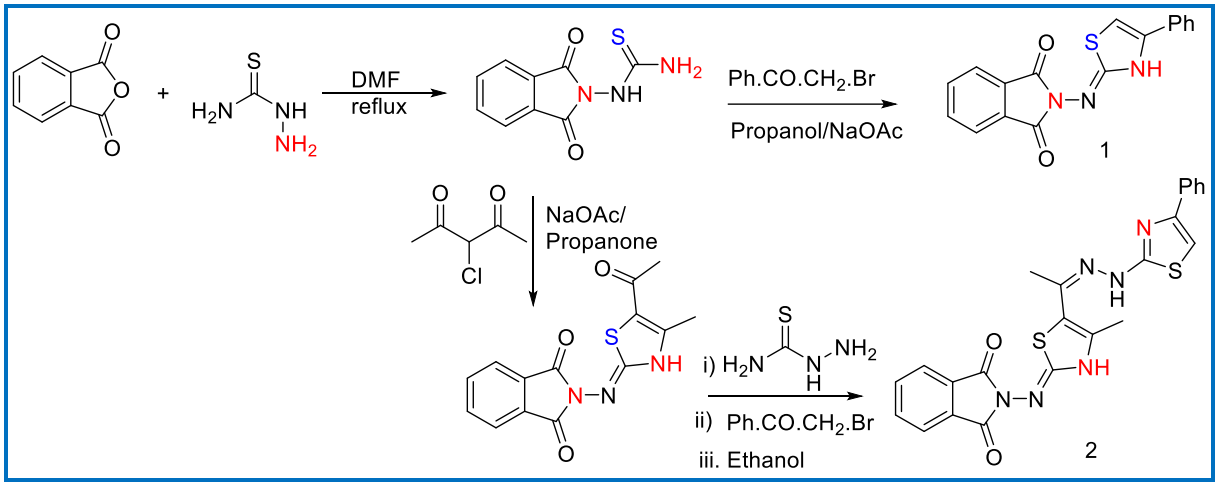
Abu-Hashem *et al* [36] reported the reaction of phthalic anhydride containing bicyclic compound with hydroxyl amine hydrochloride to produce a derivative of oxime substrate. This on reaction with 1,3 di bromo propane in DMF/TEA gave a bromo *N*-alkyl phthalimide. This free alkyl group again reaction with *N*-phenyl piparazine leads to form a corresponding phthalimide substituted phenyl piparazine-1-yl propyl compound. These compounds show good anti-inflammatory activity.

Scheme-1.1



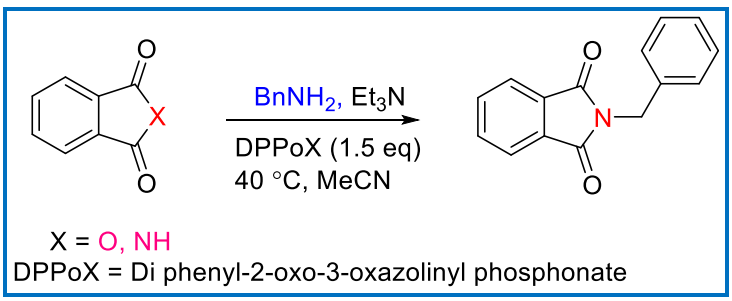
Oliveira *et al* [37] published the synthesis of compound 2. In this phthalimide ring substituted 1,3 thiazoles were prepared initially by the reaction of phthalic anhydride with thiosemicarbazide in DMF reflux to give a phthalimide attached thiourea intermediate. This on reaction with 2-bromo acetophenone to produce isoindoline thiazole compound (1). The intermediate on reaction with 3-chloro acetyl acetone and thiosemicarbazide, phenacyl bromide to obtained a compound 2. The final compounds thiazole substituted isoindoline derivatives have shown potent anticancer activity shown in scheme-1.2.

Scheme-1.2



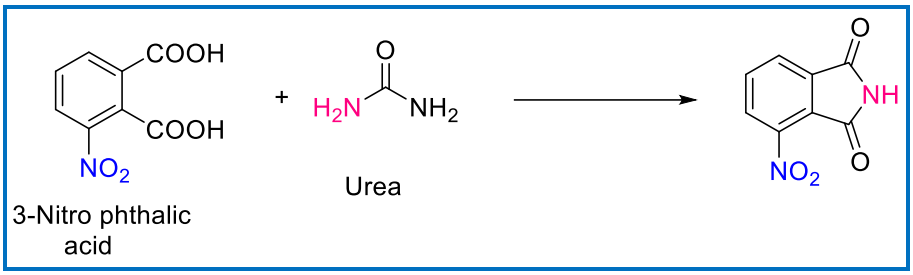
Alaa *et al* [38] developed the DPPoX promoted synthesis of *N*-benzyl phthalimide form the reaction of phthalimide or phthalic anhydride and benzyl amine in CH_3CN by using equal amount of DPPoX and Et_3N at 40 °C to obtain a corresponding substituted phthalimides. In this reaction when they used primary amines the yield of the product was increased when compared to secondary amines (scheme-1.13). And these compounds have exhibit promising anticancer activity.

Scheme-1.3



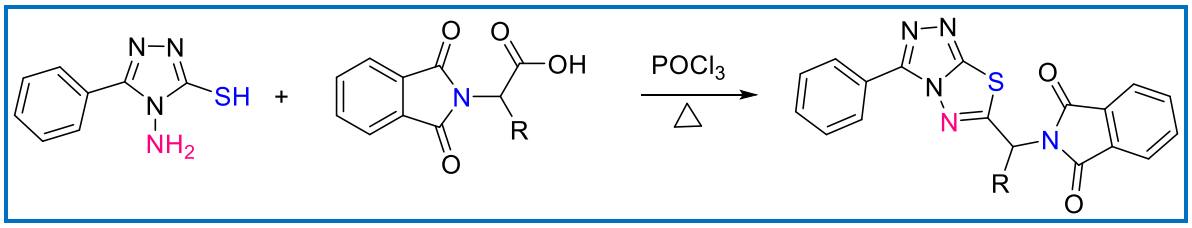
Hassanzadeh *et al* [39] reported a reaction of 3-nitro phthalic acid with urea in presence of ethylene glycol mono methyl ether under reflux to afford an yellow solid 3-nitro phthalimide with notable yield. Further this exhibit antianxiety activity (Scheme-1.4)

Scheme-1.4



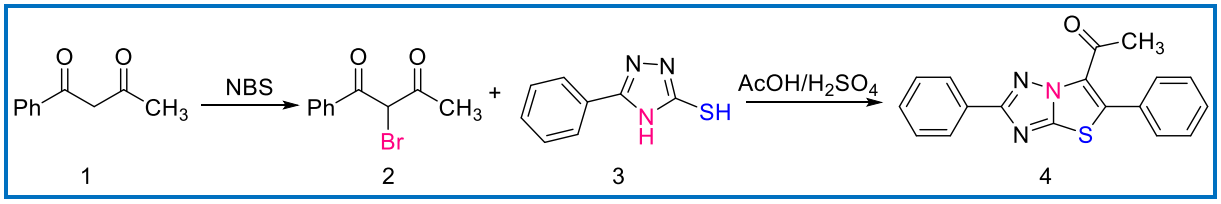
Barbary *et al* [40] published the reaction of 4-amino-5-phenyl 1,2,4-triazole-3-thiol with *N*-carboxylic acid isoindoline in the presence of $POCl_3$ at reflux to generate a fused triazolothiazole phthalimide heterocyclic compounds (Scheme-1.5). These compounds posess potent antiviral activity.

Scheme-1.5



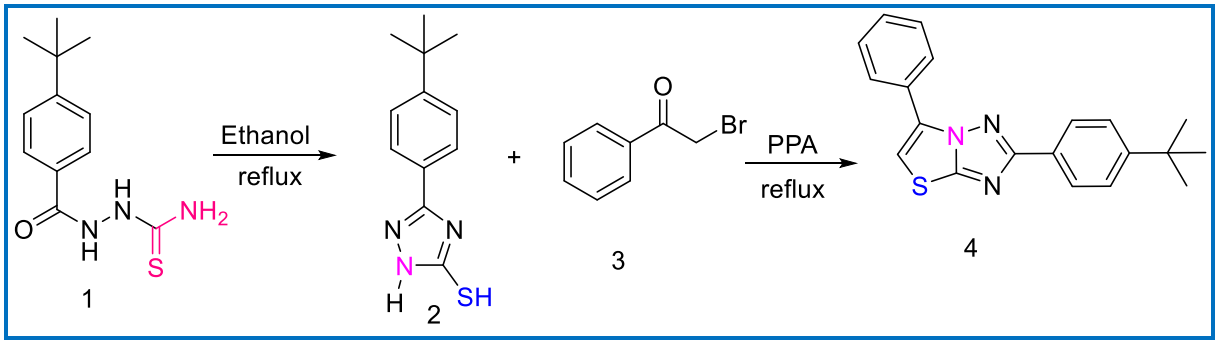
Agarwal *et al* [41] reported a one-pot synthesis of fused thiazolo [3,2-*b*][1,2,4]triazole via the reaction of 1-phenyl 1,3-butane dione with NBS and 5-phenyl triazole-3-thiol under $AcOH/H_2SO_4$ at reflux temperature to obtain a fused triazolothiazole moiety (4) with good yield.(Scheme-1.6)

Scheme-1.6



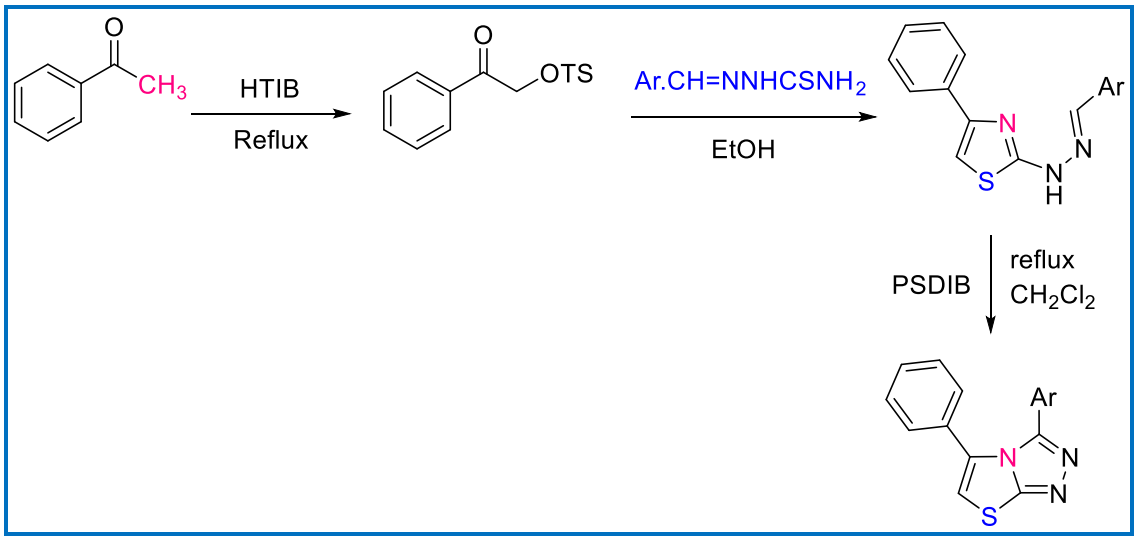
Jag Mohan [42] reported the condensation reaction of 5-mercapto-3-[p-(t-butyl phenyl)] s-triazole with 2-halo aceto phenone in presence of ethanol under reflux condition for 3 h initially to produce the un-cyclized thioalkylated compound. This was subsequently cyclization with strong acid (PPA) to give thiazolo [3,2-*b*]-s-triazole. The final compounds were tested for their antimicrobial activity against gram-negative bacteria *E. coli.* and gram-positive bacteria *S. aureus.* (Scheme-1.17)

Scheme-1.17



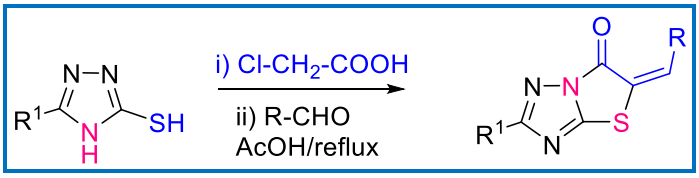
Liu *et al* [43] reported reaction of acetophenone with HTIB [(hydroxy(tosyloxy)iodo] benzene in CAN to yield α-tosyloxy acetophenone. This again on reaction with arene carbaldehyde thiosemicarbazone gave Schiff base. And this compound on cyclization with PSDIB (poly[(4-diacetoxyiodo)-styrene] to form fused triazolothiazole heterocyclic derivative. (Scheme-1.8)

Scheme-1.8



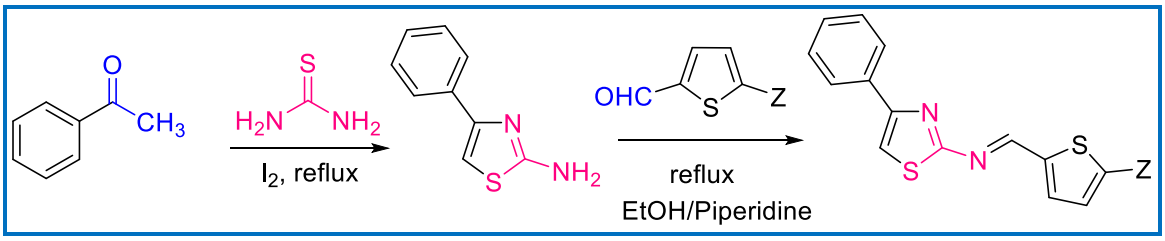
Slivka *et al* [44] published the one-pot three component reaction between 5-aryl 1,2,4-triazole-3-thiol with chloroacetic acid and different aldehydes in presence of AcOH at reflux to form a 5-arylidene substituted fused [3,2-*b*][1,2,4]triazoles. Many of these substrates were possess good biological activity.

Scheme-1.9



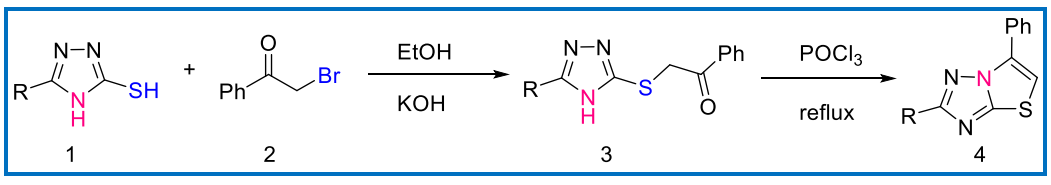
Amorim *et al* [45] developed the synthesis of Schiff bases from 4-phenyl 2-amino thiazoles. In which initially the acetophenone was reacted with thiourea in presence of I_2 under reflux to form a 4-aryl -2-amino thiazole followed by reaction with aromatic/heteroaromatic aldehydes in presence of EtOH/piperidine at reflux to produce a thiazole substituted Schiff bases. These have been explored as potential biological properties such as antimicrobial, anticancer etc.

Scheme-1.10



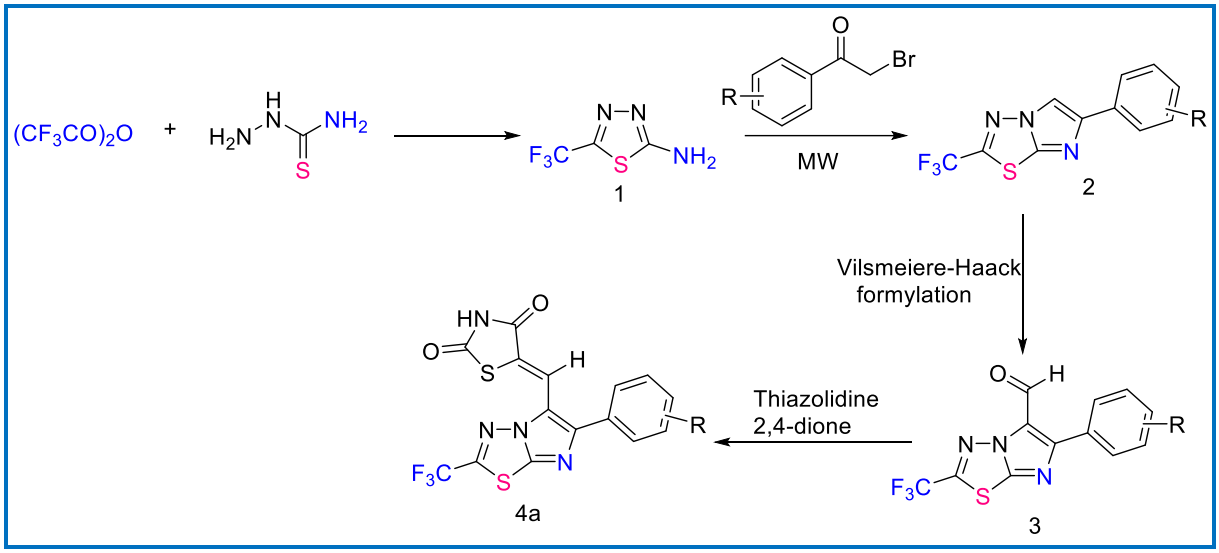
Pignatello *et al* [46] reported the synthesis of fused thiazolo[3,2-*b*][1,2,4]-triazole derivatives. These were synthesized by the reaction between 5-alkyl-4*H*-1,2,4-triazole-3-thiol and α-halogenated ketones such as 2-bromo acetophenone, 2-chloro aceto acetate, chloro acetone in methanol under reflux to develop a thioalkylated product which were subjected to cyclization reaction with $POCl_3$ to obtained a bicyclic thiazolotriazole moiety. These compounds were associated with good anti-inflammatory, antibacterial, analgesic activities. (Scheme-1.11)

Scheme-1.11



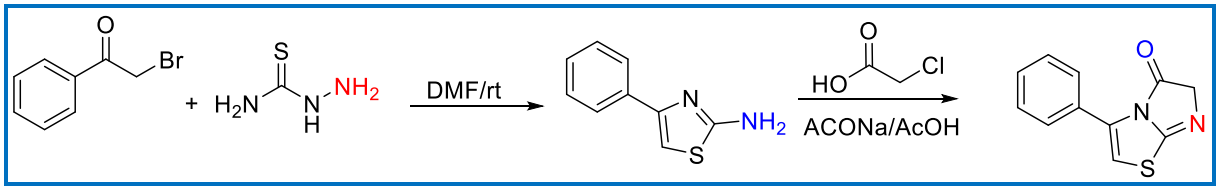
Alegaon *et al* [47] published the synthesis of imidazo fused 1,3,4 thiadiazoles. reaction between trifluoro acetic anhydride and thiosemicarbazide to give a 2-trifluoromethyl-5-amino thiadiazole. This was subjected to cyclization with phenacyl bromide under microwave irradiation followed by Vilsmeiere-Haack formylation to generate a formyl substituted fused heterocyclic compound and then subsequent reaction with thiadiazolidine-2,4-dione gave a thiazole derivative. These substrates were demonstrate promising antitubercular activity. (Scheme-1.12)

Scheme-1.12



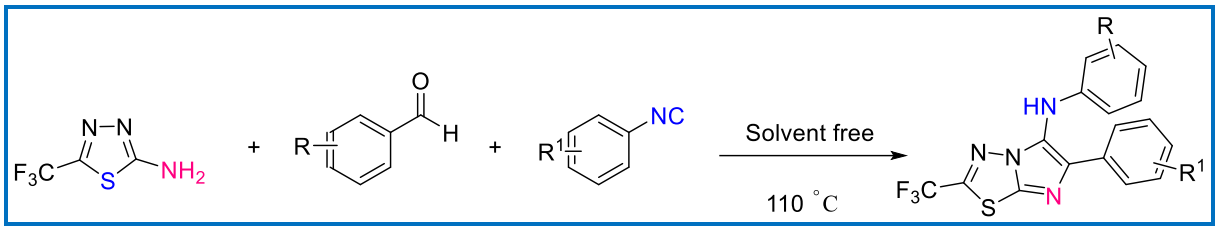
Fascio *et al* [48] describe the reaction between phenacyl bromides with thiourea in DMF to generate a 2-amino-4-arylthiazoles. These were subject to cyclization with chloro acetic acid to obtained a imidazothiazole derivative. These compounds exhibit broad spectrum of biological activities. (Scheme-1.13)

Scheme-1.13



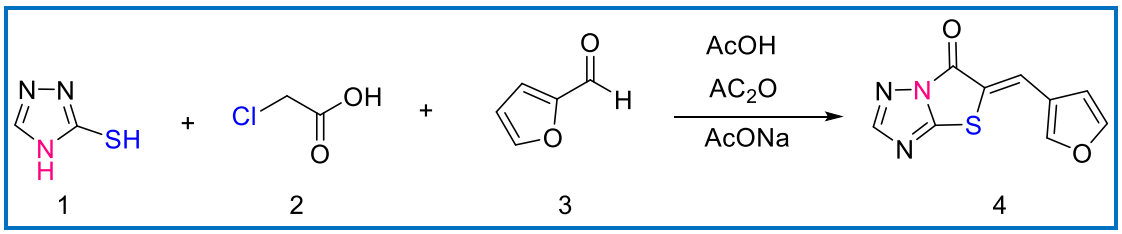
Sarchahi *et al* [49] developed an efficient isocyanide based one-pot three component synthesis of fused imidazo[2,1-*b*][1,3,4]-thiadiazoles by the reaction of 5-triflouromethyl 2-amino thiadiazole with aromatic aldehydes, phenyl isocyanide in the presence of solvent free conditions at 110°C. (Scheme-1.14)

Scheme-1.14



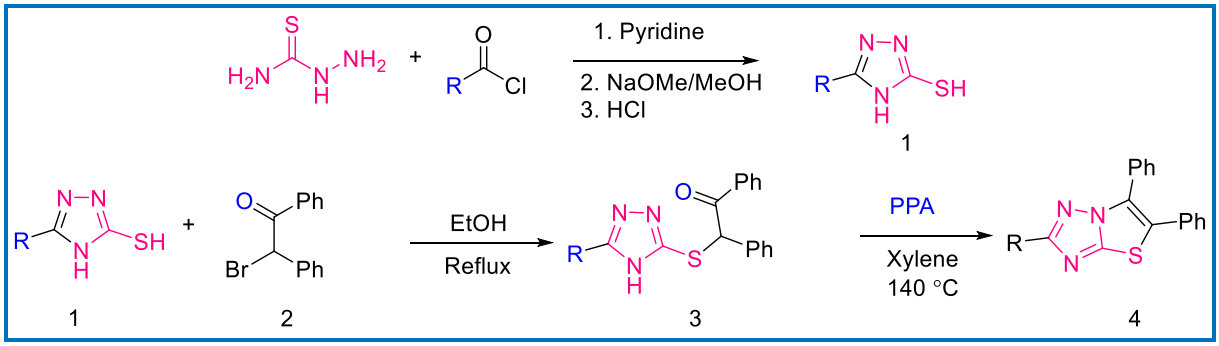
Holota *et al* [50] published thiazolo[3,2-*b*][1,2,4]-triazole-6-ones by a one-pot three component reaction of 4*H*-1,2,4-triazole-3-thiol, chloro acetic acid and various substituted aldehydes in AcOH/NaOAc/AC_2O. (Scheme-1.15)

Scheme-1.15



2-Mercapto 1,2,4-triazole (1) has been synthesized by condensation of thiosemicarbazide, various acid chlorides in Pyridine/NaOMe/MeOH/HCl. The compound 1 was reaction with diaryl bromo ketone in EtOH under reflux then subsequently cyclization with PPA to afford a fused [3,2-*b*][1,2,4]-triazolothiazoles (Scheme-1.16). These compounds posess potent COX-2 enzyme inhibitors [51].

Scheme-1.16



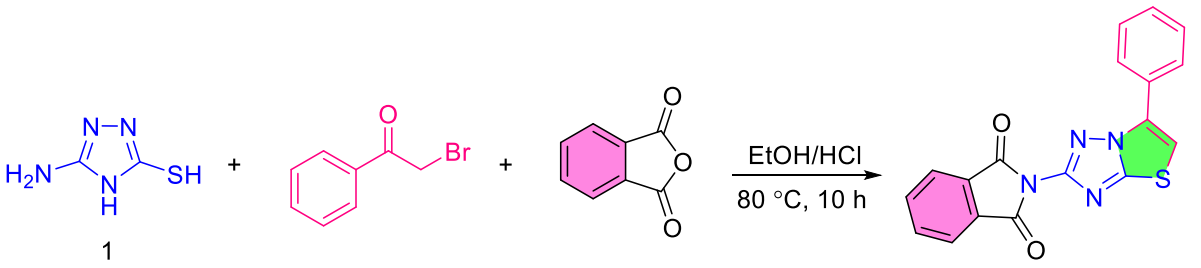
3.2. Present work

3.2.1 Chemistry

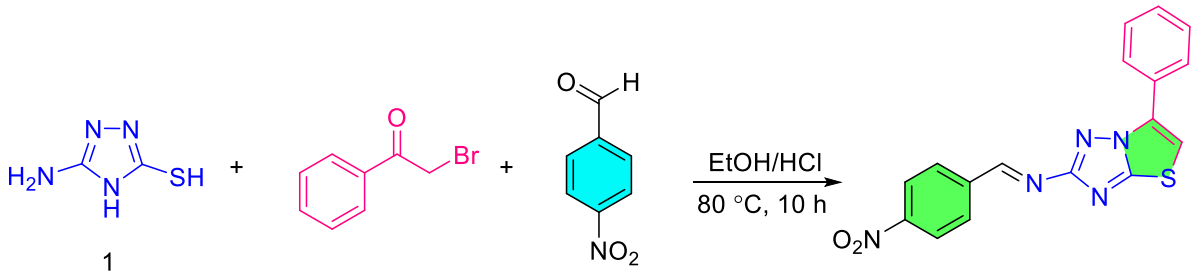
The molecular hybridization is a new research topic in drug design and development of new hybrid core by combination of two or more pharmacophore moieties to generate enhanced affinity, efficiency, and decrease side effects, when compared to parental drug. Moreover, this approach can outcome result in compounds presenting modified selectivity profile, changed dual modes of action and reduce side effects.

Inspired by biological activities of substituted thiazolo[3,2-*b*][1,2,4]-triazol-2-yl systems, we decided to synthesize target molecules and evaluated their anticancer properties. In a multi-component approach, 5-amino-4*H*-1,2,4-triazole-3-thiol (1) was reacted with phenacyl bromide (2) and phthalic anhydride (3), to produce novel bicyclic triazolothiazole isoindoline moieties 4 a-n (Scheme-1). On the other hand, when compound (1) reacted with various phenacyl bromides (2) and different aromatic aldehydes (5) to give triazolothiazole Schiff bases 6 a-l (**Scheme-2**)

Schematic representation of triazolothiazole isoindolines. **Scheme-1& Scheme-2**



Scheme-1



Scheme-2

Table-1. Optimization study of the Scheme-1&2 for **4a** and **6a** compounds

S.no	Solvent	Catalyst(mol%)	Temp (°C)	Time (h)	Yield(%)[a]
1	EtOH	-	rt	10	20
2	AcOH	-	60	8	n.r
3	DMF	-	60	8	n.r
4	DMF	K_2CO_3	90	8	10
5	MeOH	HCl	60	8	22
6	EtOH	-	60	8	40
7	EtOH	*p*TSA	80	9	35
8	EtOH	HCl(0.3)	70	10	60[b]
9	EtOH	HCl(0.5)	80	12	90[c]
10	EtOH	$POCl_3$(1.0)	80	10	65

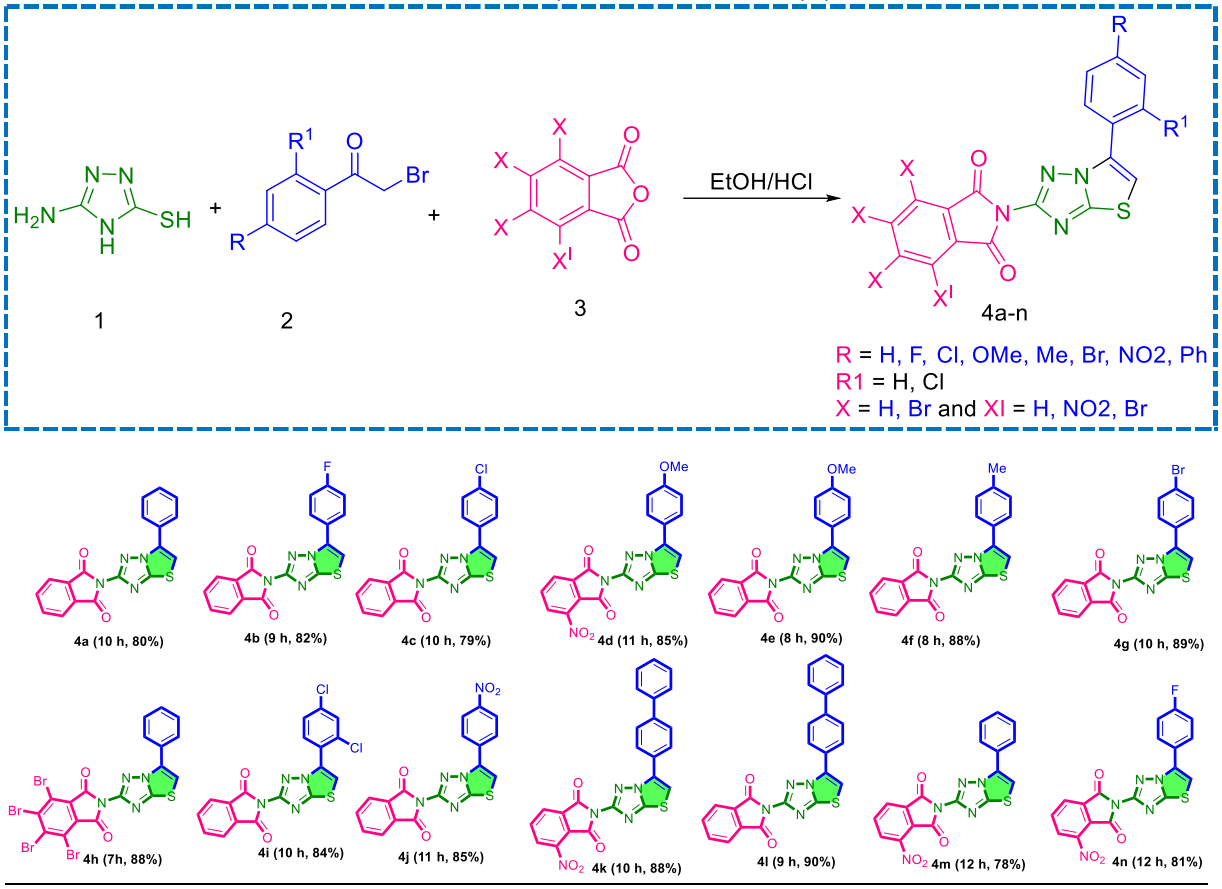
Reaction conditions: Amino mercapto-1,2,4-triazole 1 (1 mmol), phenacyl bromide (1 mmol), phthalic anhydride (1 mmol) and *p*-nitro benzaldehyde (1 mmol), catalyst (5 mol%), ethanol solvent (4 mL), 12 h.

[a]Isolated yield; [b]Hydrochloric acid (0.3 mol%); [c]Hydrochloric acid (0.5 mol%) used; n.r = No reaction.

The reaction was optimized for scheme-1 and 2 in different solvents with change of catalysts and temperature at different time intervals. The high percentage of yields was obtained in EtOH in HCl 5 mol% at 80 °C for 12 hrs.(**Table-1**)

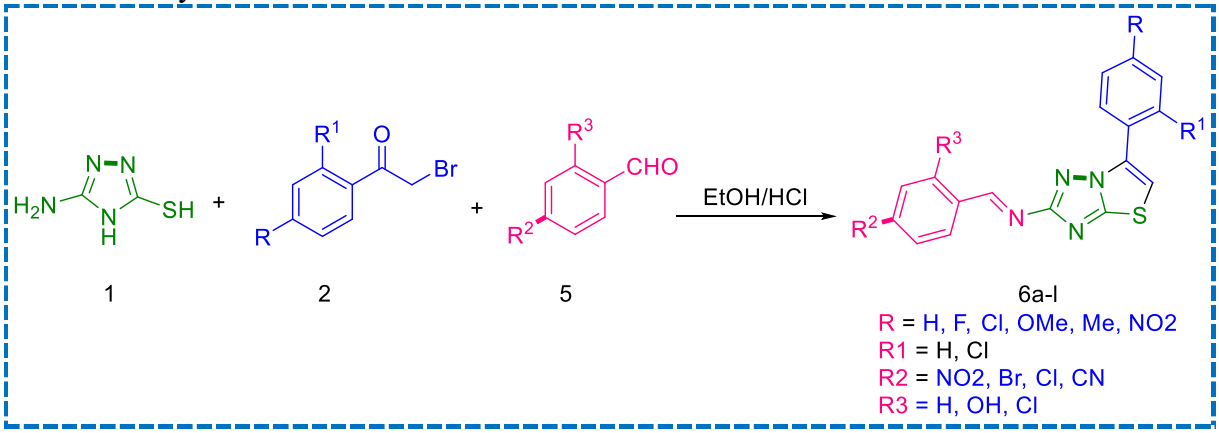
The mechanism of the formation of final compounds was explained for scheme-1 and 2. The bromine atom of phenacyl bromide were replaced by more nucleophilic thiol group of 1 to give corresponding phenacyl thio compounds. the reaction proceeds through SN^2 type mechanism. This undergo acid catalysed ring closure to give 6-phenyl thiazolo[3,2-*b*][1,2,4]-triazol-2-amine. Which on further condensation with phthalicanhydride 3, aromatic aldehyde 5 to give a fused triazolothiazole isoindoline and Schiff base products. The speciality of this reaction is two C-N, one C-S bonds are formed at a time (**4a-n**) and also C=N, C-S, N-C bonds are formed (**6a-l**)

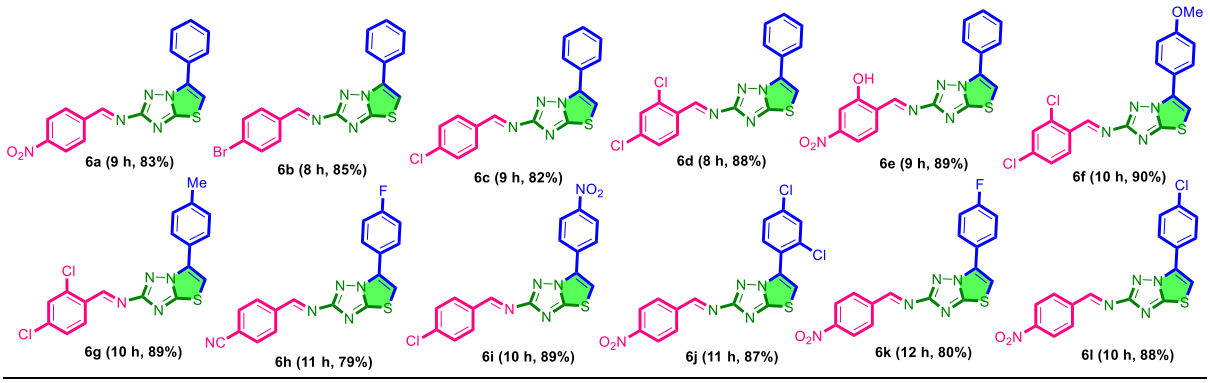
Scheme-1: Derivatized fused isoindoline-1,3-dione thiazolo-1,2,4-triazoles:



Reaction conditions: Amino mercapto-1,2,4-triazole 1 (1 mmol), phenacyl bromides 2 (1 mmol), phthalic anhydrides 3 (1 mmol) EtOH in HCl 5 (mol%) 80 °C.

Scheme-2: Synthesized derivatives of fused triazolothiazole Schiff bases:





Reaction conditions: Amino mercapto-1,2,4-triazole 1 (1 mmol), α-halo acetophenones 2 (1 mmol), aromatic aldehydes 5 (1 mmol) EtOH in HCl 5 (mol%) 80 °C.

All the new compound structures were characterized by physical and spectral data such as m.ps, FT-IR, ^{1}H NMR, ^{13}C NMR, HRMS and C, H, N elemental analysis. The IR spectra of the compounds 4a-n and 6a-l, the thiazole ring alkene C-H stretching vibrational frequency appears between 2900-3100 cm^{-1}, isoindoline ring C=O stretching frequency is at 1740-1750 cm^{-1}, Schiff base C-H stretching vibrational frequency appears at 1590-1600 cm^{-1}, NO_2 group unsymmetric and symmetric stretching frequencies appear at 1313-1540 cm^{-1}, OCH_3 group vibrational frequency appears at 1030-1040 cm^{-1}. In the ^{1}H NMR spectra of the compound the characteristic thiazole ring containing C-H proton appears a singlet at 7.90-8.00 δ ppm, Imine (-N=CH-) singlet proton appears at 9.33-10.31 δ ppm, aromatic protons appear at 7.30-8.45 δ ppm. In ^{13}C NMR spectra of compounds 4a-n the thiazole ring C-H carbons appears at 109-114 δ ppm, isoindoline ring imide carbonyl carbons at 166-168 δ ppm, triazole ring carbons appears at 150-160 δ ppm, aromatic carbons represent at 120-140 δ ppm and O-CH_3 carbons show between 50-60 δppm. The imine carbon resonances showed at 165-170 δ ppm. Mass spectra of all the compounds calculated mass matched with the found mass $[M+H]^{+}$. Further, the compound 4e structure was confirmed with a single crystal X-ray diffraction study. The crystal system is Triclinic (r.m.s deviation = 0.003 A^{0}) and twisted from C17---C16---O3---C19, C15---C16---O3---C19. (CCDC No. 2171369). The geometric parameters of the crystal data is given in supplementary information.

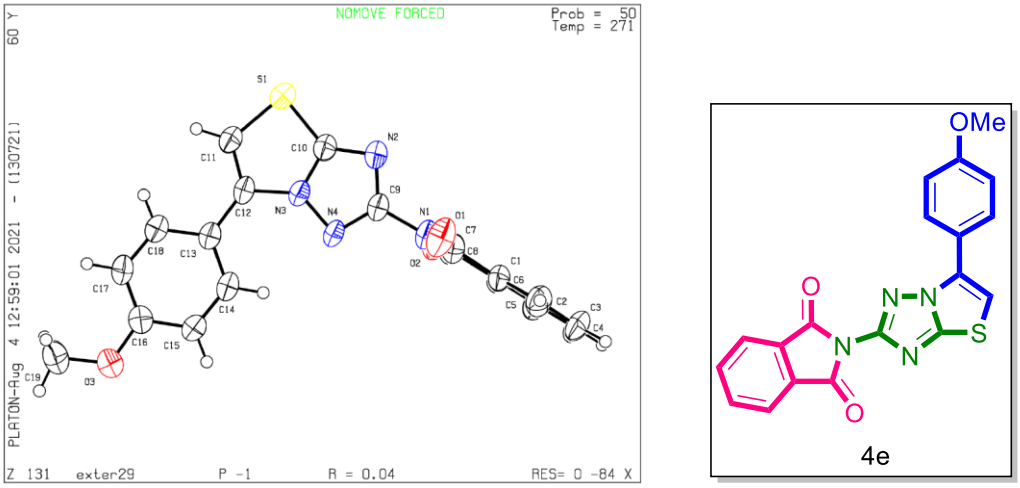


Fig. 2. The ORTEP diagram for single crystal data of the compound 4e.

Table-2: Single crystal data of the compound 4e

Identification Code	Compound 4e
Empirical formula	$C_{19}H_{12}N_4O_3S$
Formula weight	376.3900
Temperature/K	271 K
Crystal system	Triclinic
Space group	P-1
Unit cell dimensions a/Å	8.3955(3)
b/Å	11.2273(3)
c/Å	12.1153(3)
α/°	76.322(2)
β/°	78.263(2)
γ/°	79.632(3)
Volume	1075.90(6)
Z	2
D_{calc} Mg/m^3	1.162

μ/mm^{-1}	0.174
F(000)	388.0
2Θ range for data collection/°	25.027
Index ranges	$-9 \leq h \leq 9$ $-13 \leq k \leq 13$ $-14 \leq l \leq 14$
Data/restraints/parameters	245
Goodness-of-fit on F^2	1.099
Final R indexes [I>=2σ (I)]	3782
Final R indexes [all data]	3050
CCDC	2171369

Table-2: Single crystal X-ray crystal structure data of compound 4e.

3.2.2. Anticancer activity.

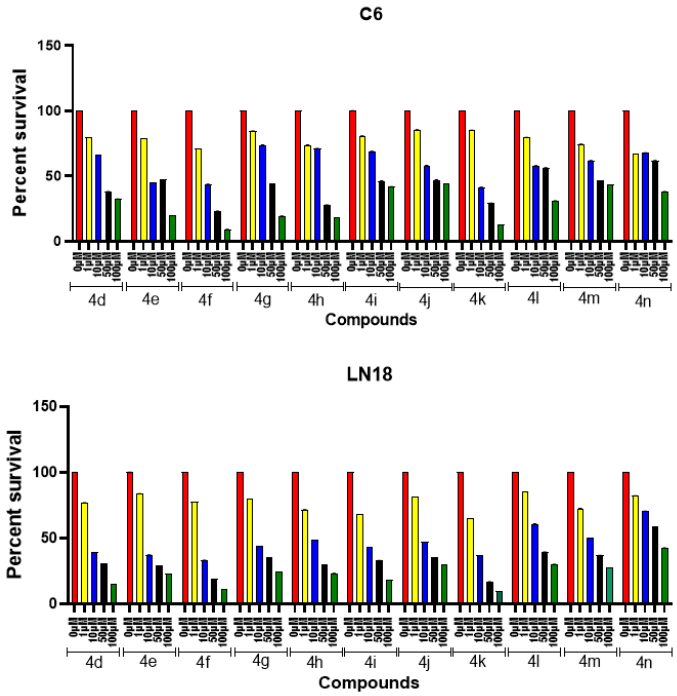
We initially evaluated the cytotoxic effects of the target hybrid compounds on C6 rat and LN18 human glioblastoma cell lines at different doses. The cell survival was analyzed using MTT [3-(4,5dimetylthiazol-2-yl)-2,5-diphenyl-tetrazolim bromide] assay. As represented in **Table.2** and **Fig.3** all the compounds have shown dose dependent inhibition. The best inhibitory effect was shown by compound **4l, 4g** in the first series and **6c** and **6i** amongst the second series in both cell lines. Compounds 4l and 6i were not completely soluble in DMSO hence we have selected **4g** and **6i** for further studies

Table-3. IC_{50} values of the scheme-1 and scheme-2 series compounds.

Scheme-1 Code	**IC_{50} (µM) C6**	**IC_{50} (µM) LN18**	**Scheme-2 Code**	**IC_{50} (µM) C6**	**IC_{50} (µM) LN18**
4a	>50	>50	**6a**	>50	>50
4b	>50	>50	**6b**	> 50	>50
4c	>50	>50	**6c**	22.40 ±0.35	23.69 ± 0.19
4d	>50	>50	**6d**	40.78 ±0.71	39.18 ± 0.40

4e	38.2±0.42	41.14±0.29	**6e**	36.58 ±0.52	41.35± 0.14
4f	28.15±0.78	33.17±0.54	**6f**	7.564 ±1.11	21.51±0.36
4g	8.091±0.43	12.68±0.17	**6g**	28.32 ±0.52	35.99±0.43
4h	38.32±0.82	44.49±0.28	**6h**	56.88 ±0.71	52.48±0.24
4i	29.84±0.83	31.89±0.26	**6i**	8.74 ±0.68	12.56±0.21
4j	20.73±0.52	25.67±0.16	**6j**	41.84 ±0.93	44.79±0.31
4k	22.06±0.66	25.19±0.35	**6k**	37.28 ±1.40	39.69±0.40
4l	5.791-0.64	8.97-0.24	**6l**	19.22 ±0.68	29.77±0.35
4m	29.12±0.54	35.78±0.43			
4n	39.91±0.83	44.41±0.83			

Table-3. IC_{50} values of the scheme-1&2 compounds in C6-rat and LN18 glioma cell lines



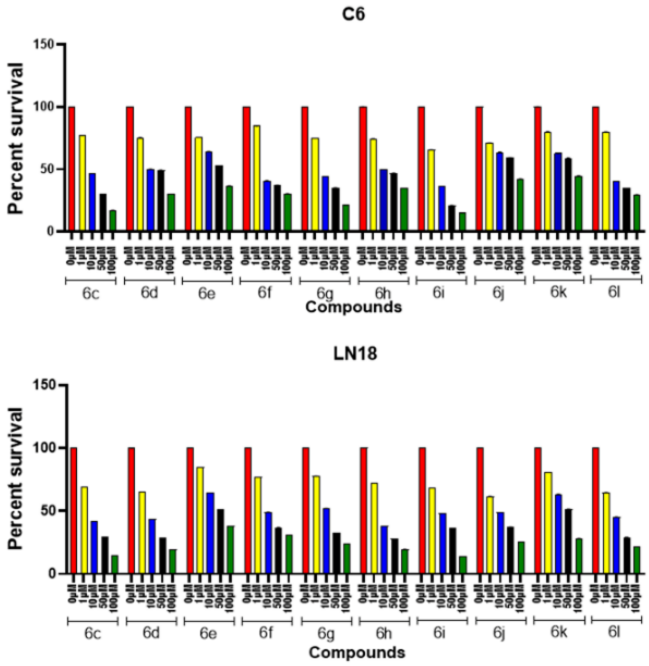


Fig.3. *In-vitro* anti-cytotoxic activity of the compounds at different mentioned concentrations in C6 rat and LN18 human GBM cell lines (n=3, one representative experiment is shown).

3.2.2A. Cytotoxic anti-migratory efficacy and colony forming ability of compounds in glioma cells.

To further understand the cytotoxic effect of the synthesised compounds on GBM cell lines, we treated the cells with various doses of the compounds and measured cell growth, proliferation and migration. TMZ-treated cells were used to compare the efficacy of the novel compounds against the GBM cells. The treatment with 4g and 6i significantly mitigated the proliferation of C6 and LN18 cells as compared to the vehicle treated or TMZ treated cells (**Fig. 4**).

The respective treatments also increased the cytotoxicity in the C6 and LN18 GBM cells in a concentration dependent manner and hence resulted in significant reduction in the colony forming ability of cells from a single cell The graphical representation demonstrates the colony forming ability of cells at respective treatments was shown in (**Fig. 5**).

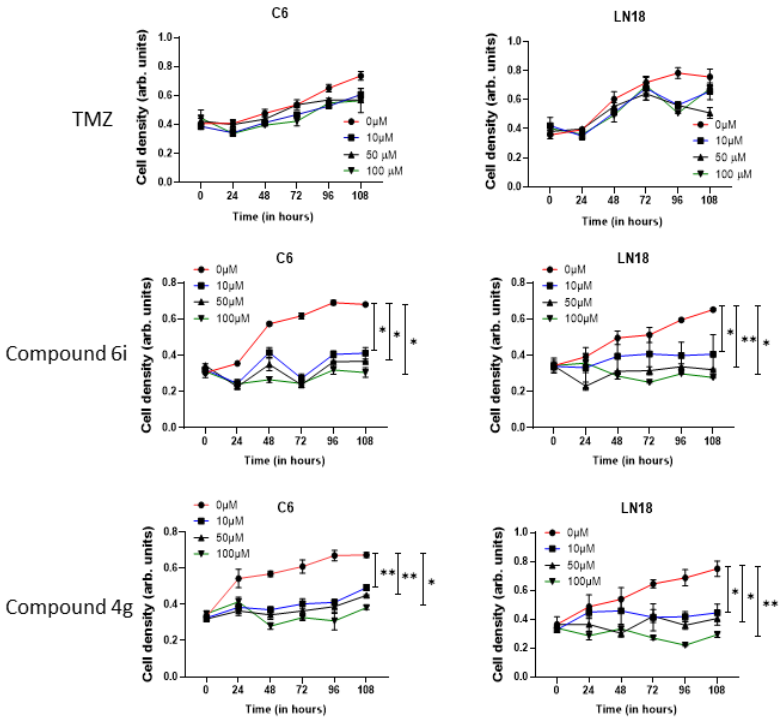


Fig.4. Cell viability was analysed using MTT at indicated time points with TMZ treatment and with mentioned compounds in order to analyse anti-proliferative effect (n=3, one representative experiment is shown). Statistical data of the experiments is shown as mean ± sem * $p < 0.05$, ** $p < 0.01$.

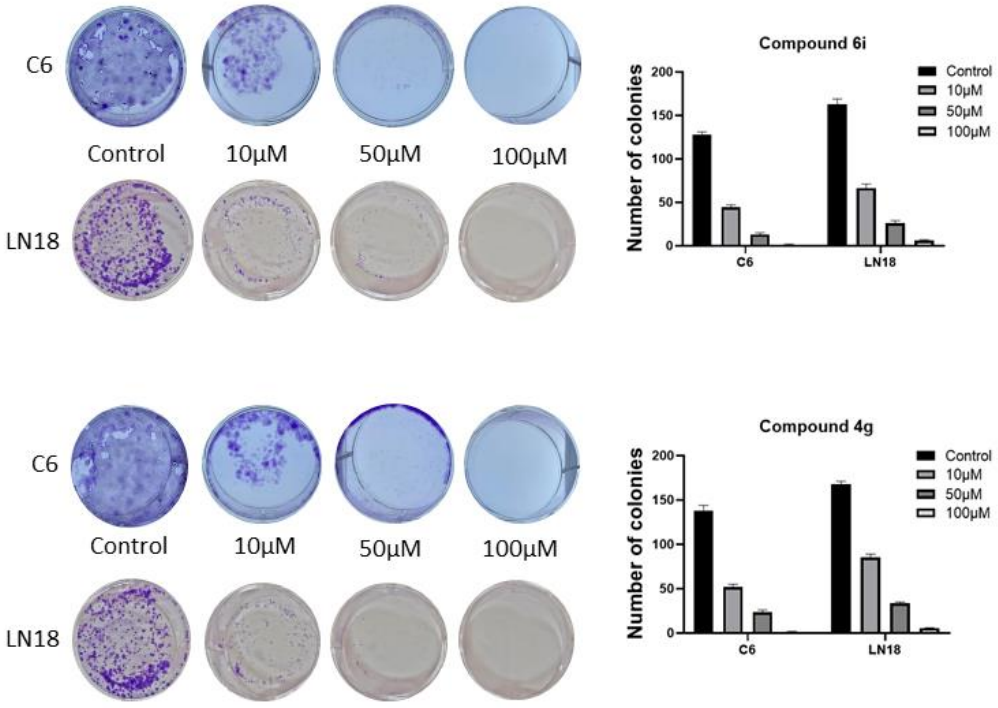
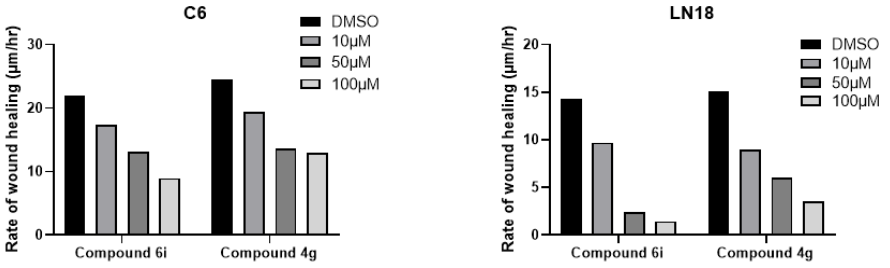
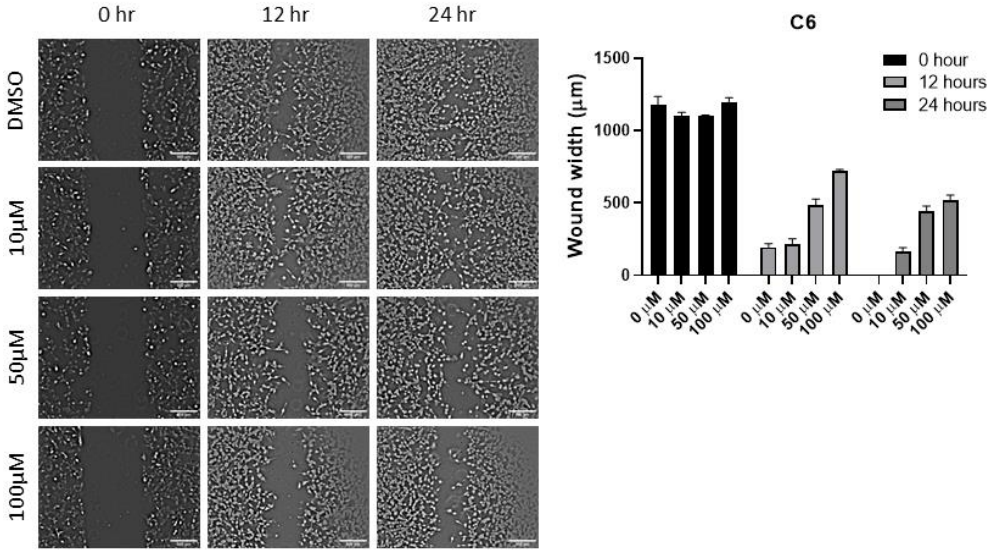


Fig.5. Clonogenic assay. Respective treatment of the compounds significantly reduces the colony forming ability of the glioma cells. The cells were treated for 48 hours and cultured under optimal conditions to analyse the colony forming abilities at different treatment concentrations (n=3, one representative experiment is shown.

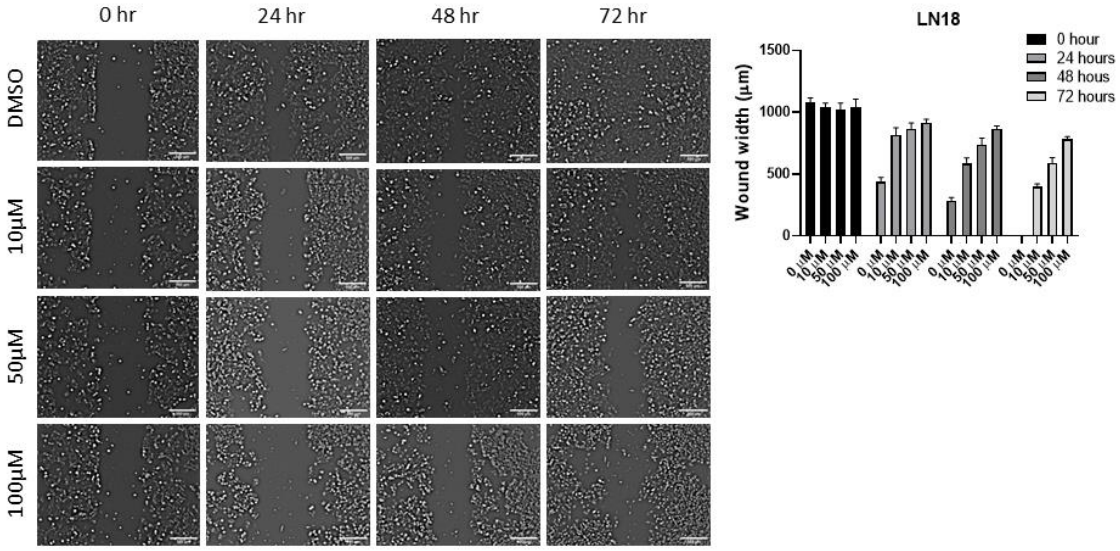
The wound healing assay demonstrates the ability of the cells to migrate after creating a scratch wound in the confluent bed of cells. The migration ability of cells treated with 4g and 6i was inhibited as shown in wound healing assay. Vehicle treated cells has the highest rate of migration in C6 cells (21.95µM/hr and 24.46µM/hr) compared to 100µM treatment (8.96µM/hr and 13.02µM/hr) and in LN18 cells (14.36µM/hr and 15.04µM/hr) compared to (1.44µM/hr and 3.51 µM/hr) with 4g and 6i treatment respectively (**Fig**.**6**). This shows that the rate of proliferation in the control cells is significantly more as compared to the 4g and 6i treated cells.



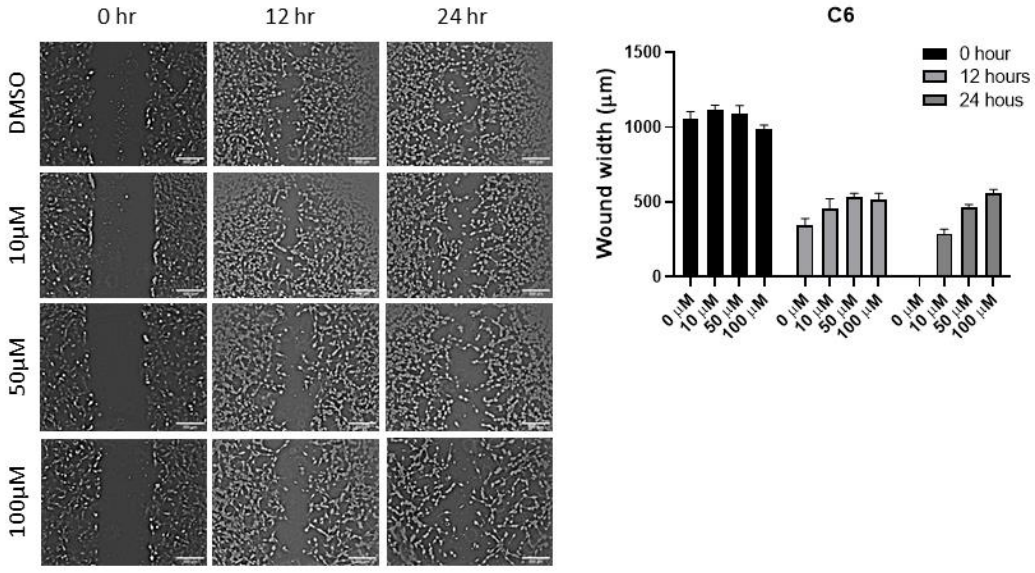
C6_Compound 4g



LN18_Compound 4g



C6_Compound 6i



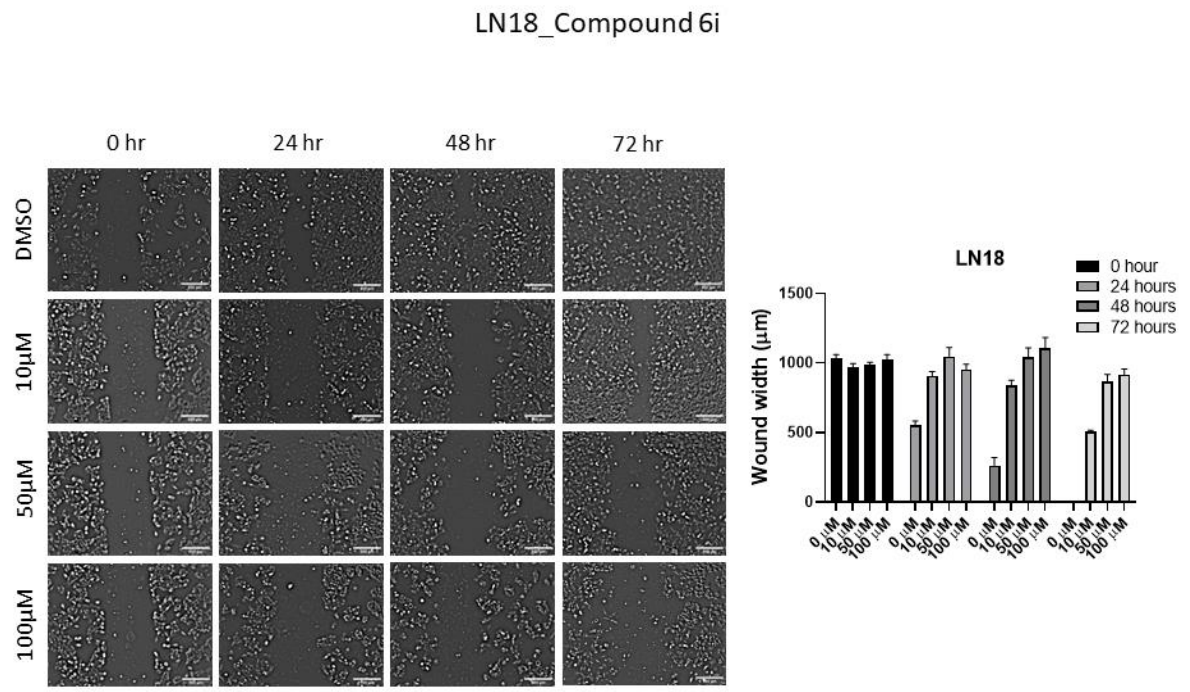


Fig. 6. Treatment leads to mitigation of proliferation in glioma cells. Wound healing scratch assay in vehicle treated (DMSO) and cells treated with 10µM, 50µM and 100µM of indicated compounds (n=3, one representative experiment is shown)

3.2.2B. Cell cycle arrest.

We analyzed the effect of most active compounds, 4g and 6i on cell cycle in the GBM cell lines (Fig.7). Treatment of the cells with 10µM concentration of compound 6i increased the cell population in sub-G1 phase (66.5% and 50%) compared to 60% and 44% in the control C6 and LN18 GBM cells respectively. Whereas the 4g treatment at 10µM resulted in the arrest of cells in G2-M phase of the cell cycle (31.9% and 54.9%) compared to 19.3% and 39.4% in the control C6 and LN18 GBM cells respectively. This indicates that cells were arrested at G1 phase by 6i and G2-M phase by 4g confirming that cell cycle arrest is one of the mechanisms by which the compounds are inhibiting the cell proliferation. Furthermore, treatment with higher concentrations (50µM and 100µM) of the respective compounds resulted in a significant increase in the population of cells in the sub-G1 phase indicating cells are undergoing apoptosis. Apoptotic cells were observed in approximately 35% and 47% of 6i and 4g-treated C6 and LN18 GBM cells, respectively, compared to 2-7 percent in control C6 and LN18 GBM cells. Apoptosis plays a crucial role in the elimination of mutated and hyper-proliferating neoplastic cells and hence considered as a protective mechanism against cancer progression. It has been also reported as the process of significant cell death after cytotoxic drug treatment in various

types of cancers.[53] Our results show that the compounds induce apoptosis in a dose dependent manner in C6 and LN18 GBM Cell lines.

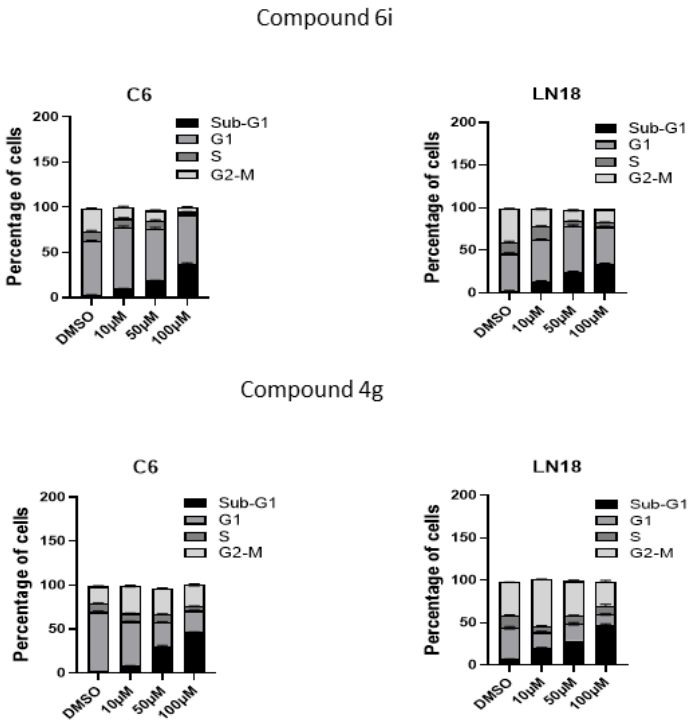


Fig-7. Treatment leads to the modulation of cell cycle distribution. Cell cycle analysis displaying different phases of cell cycle in C6 and LN18 cells in vehicle treated (DMSO) and cells treated with 10μM, 50μM and 100μM of indicated compounds (n=3, one representative experiment is shown).

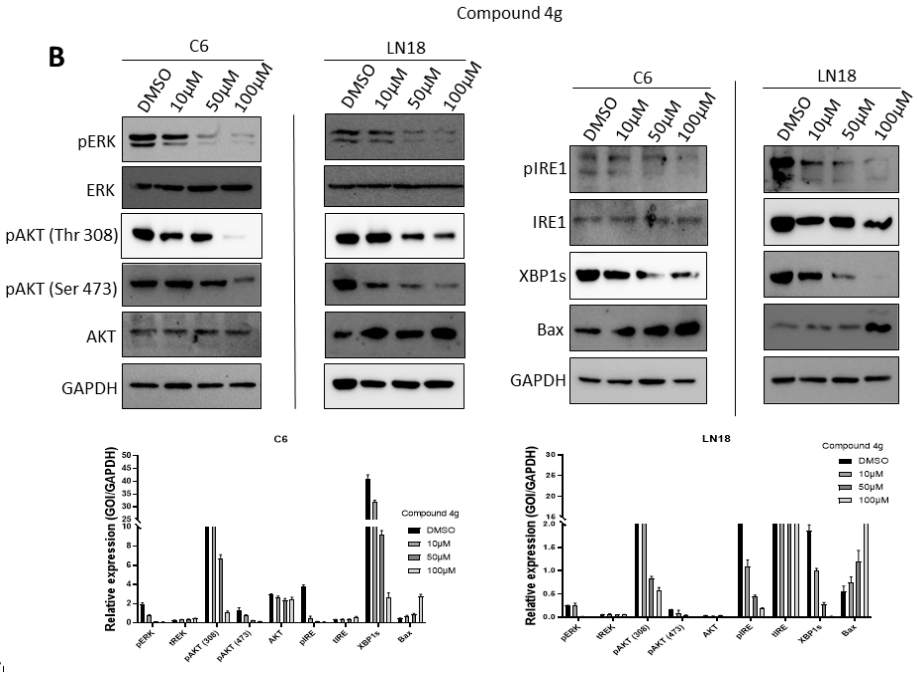
3.2.2C. Targets ERK, AKT and UPR pathway.

We subsequently investigated its involvement in the cancer associated ERK and AKT signalling pathways. Both compounds 6i (**Fig**. 8A) and 4g (**Fig**. 8B) reduced phosphorylation of ERK and AKT (Thr 308 and Ser 473) in a concentration dependent manner as compared to vehicle (DMSO) treated cells. Interestingly, we also found that the treatment resulted in the reduction in expression of the unfolded protein response sensory protein, IRE1 and its downstream protein spliced XBP1 which ultimately leads to increased apoptosis as seen by enhanced Bax expression in **Fig.** 8A and 8B.

4g and 6i are able to regulate the survival by modulating the expression of one of the endoplasmic reticulum (ER) resident sensors, Inositol requiring enzyme 1(IRE-1), which is involved in the unfolded protein response, attenuating protein translation and enhancing protein folding

and quality control which leads to the increased clearance capacity of ER.[54, 55] IRE1 overexpression leads to the survival of cancer cells, predominantly mediating by its RNase activity through non-conventional splicing of X-box binding protein (sXBP1) mRNA and ultimately regulating pro-apoptotic pathways.[56] Phosphorylation of ERK leads to its activation which is associated with cell survival and proliferation. ERK1/2 also regulates AKT activity. We observed significant downregulation in the pERK and pAKT levels that represents that ERK activation was reversed by 6g and 4i treatment leading to cell death. IRE1 is a conserved ER stress sensor. IRE1 phosphorylation leads to nonconventional splicing of the mRNA, encoding XBP-1, leading to the expression of XBP1 spliced (XBP1s), a potent transcription factor. Higher IRE1/XBP1activity results in tumor progression and aggressiveness in most cancers such as leukemia, GBM, myeloma, renal and breast cancers and has been associated with poor prognosis. XBP1 also promotes tumor invasion and drug resistance in cancer. We observed significant downregulation in IRE1/XBP1 expression with respective treatments of 4g and 6i in C6 and LN18 cell lines, which suggests that the treatments resulted in reduction of ER stress and hence can help in mitigating gliomagenesis.

The downstream regulator of the MEK/ERK or PI3K/AKT signalling pathways is GSK-3β. [57] Inhibition of ERK or AKT causes GSK-3 to remain in an inhibited state, resulting in the interaction of GSK-3 and SNAIL [58] and hence GSK-3β was chosen for docking studies.



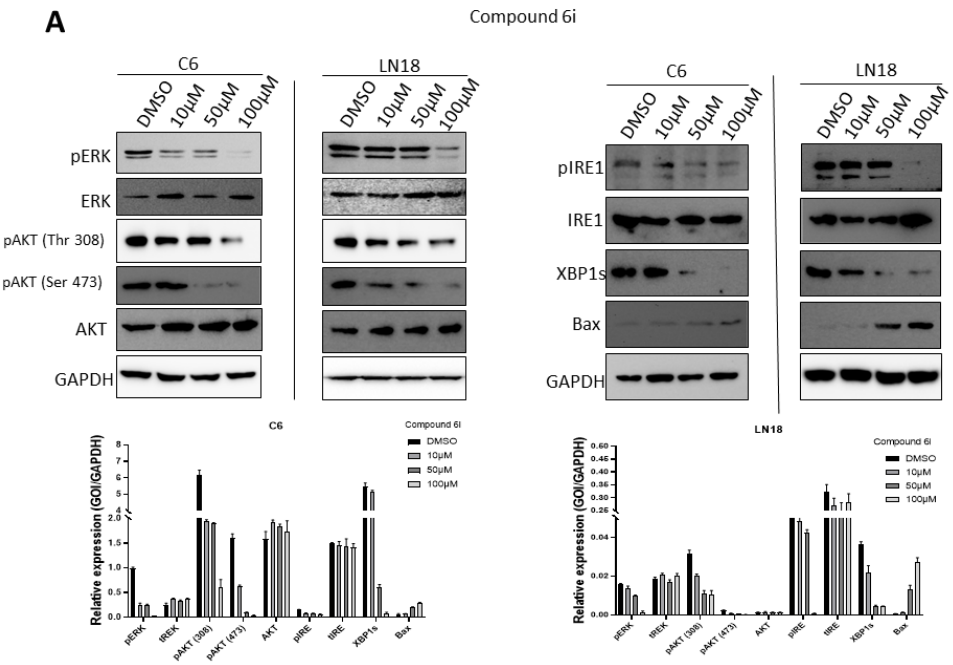


Fig.8 A, B. Treatment modulated the activity of glioma-associated signalling pathways. Western blot of pERK, ERK, pAKT (Thr 308 and Ser 473), AKT, pIRE1, IRE1, XBP1s in vehicle treated (DMSO) and cells treated with 10μM, 50μM and 100μM of indicated compounds. GAPDH serves as the loading control (n=3, one representative experiment is shown).

3.3. Molecular docking study.

Molecular docking studies were done by GSK-3β inhibitor complexed with the inhibitor 6QH4001 protein retrieved from the protein data bank with PDB ID: 5K5N.[59]

The binding site interactions of GSK-3β enzyme have been determined for both the standard drug temozolamide and two series of compounds. Temozolamide has shown 2 polar hydrogen bond interactions with Val 135 amino acid as shown in **Fig.9** with a dock score of -5.158Kcals/mole and binding free energy of -40.294 Kcals/mole respectively. This indicates that the synthesized compounds have higher affinity towards the receptor with good dock scores and binding energies which are higher than temozolamide. 6i exhibited one polar hydrogen bond interaction with Val135 and oxygen of nitro group attached to benzene ring, one hydrophobic interaction with Lys85 and nitrogen of triazole ring the dock score -6.432 Kcals/mole binding energy of -60.185 Kcals/mole and also exhibited additional π-π stacking interaction with Phe 67 of benzene ring (**Fig.11**). Whereas, 4g shows hydrogen bond interaction with carbonyl of isoindoline and Thr138, one hydrogen bond interaction with triazolothiazole with Arg141. The *p*-bromo phenyl group is entrenched in the hydrophobic cavity with a dock score of -4.711 Kcals/mole and binding energy of -74.621 Kcals/mole which are higher than

that of the standard molecule(Fig-10). Both 4g, 6i interacted with amino acid residue by one hydrophobic interaction at the active site of target protein i.e., with the carbonyl oxygen of isoindoline ring and amino acid residue of Thr138. There by we can conclude that these compounds may serve as good inhibitors towards glioma.

Structure Activity Relationship (SAR)

with a molar volume of 154.7 cm^3, while the bromophenyl group having a molar volume of 105.6 cm^3. In the series 1 (4a-4n) it is observed that the presence of the hydrophobic phenyl group attached to fused thiazole ring has a greater impact on the dock score. The molecule 4l having a biphenyl substitution showed a highest biological activity with IC_{50} value of 5.791 μM with dock score of -5.134 Kcal/mol and binding free energy (MM-GBSA) of -76.414 Kcal/mol. The bromo substituted phenyl group on the thiazolotriazole (4g) having a IC_{50} value of 8.091 μM, show a dock score of -4.711 Kcal/mol and MM-GBSA value of -74.621 Kcal/mol. This comparison shows that the hydrophobic moiety biphenyl is entrenching deep into the hydrophobic cavity.

The activity of molecules 4h and 4i having tetrabromophenyl substitution on isoindoline dione ring showed lower IC_{50} values of 40.88 and 38.32 μM respectively suggesting that the tetrabromo substitution reduces the activity of compounds although they had biphenyl and bromo phenyl substitution on the azole ring. When the electron donating groups is present at para position on the phenyl group like methyl or methoxy the biological activity reduced considerably and the IC_{50} values are 38.2 and 28.15 μM respectively, accordingly the docking score and MM/GBSA values reduced, binding energy values suggest that the compound 4g and 4i are in correlation with the biological activity results and also implies that the 2,4-dichloro substitution on the phenyl attached to the thiazole ring of the thiazolotriazole showed reduced biological activity and reduced dock score (-4.852 kcal/mol) due to steric clash.

In series-2 (6a-6l) 6-phenylmethylidine [1,3] thiazolo [1,2,4] triazol-2-imine it is observed that the chloro substitution at the 4^{th} position on the 6-phenylmethylidene (6e) and on the same molecule when a nitro group was substituted at the 4^{th} positon of the phenyl attached to the thiazole ring (6i) show good biological activity with the IC_{50} value of 22.40 and 8.74 μM respectively. These compounds have shown dock score of -6.739 and -6.432 kcal/mol respectively and MM/GBSA values of -63.049 and -60.185 kcal/mol respectively.

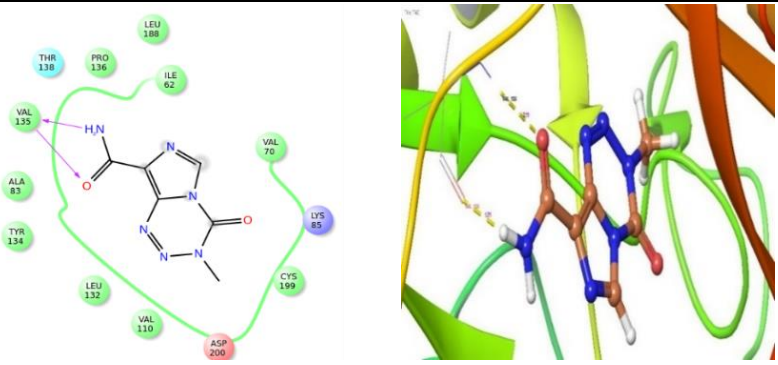


Fig.9. 2D & 3D ligand interaction diagrams of the standard drug temozolamide showing two polar hydrogen bond interactions with Val. 135.

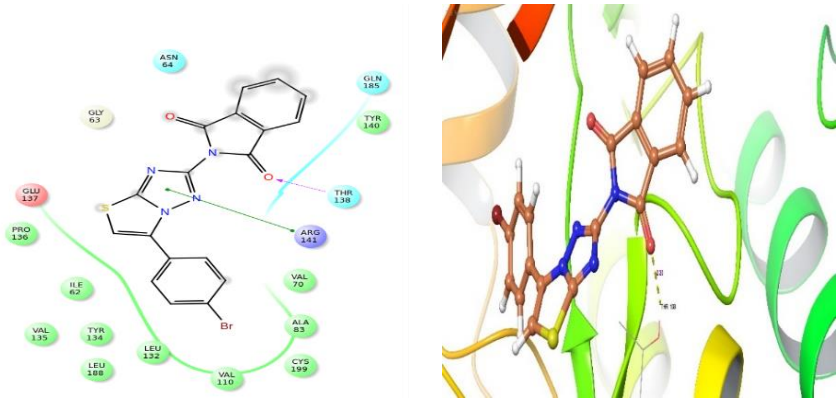


Fig. 10. 2D & 3D ligand interaction diagrams of molecule 4g from the scheme-1 showing a hydrogen bond interaction with carbonyl of isoindoline dione and Thr138, one hydrogen bond interaction with triazolothiazole with Arg 141. The *p*-bromophenyl group is entrenched in the hydrophobic cavity.

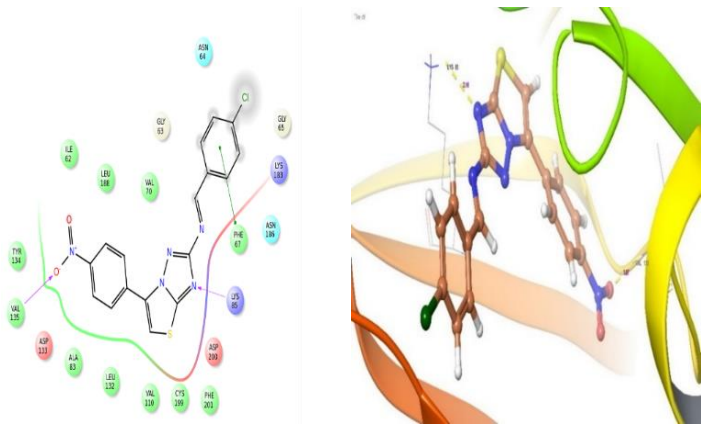


Fig. 11. 2D & 3D ligand interaction diagrams of molecule 6i from the scheme-2 showing one polar hydrogen bond interaction with Val 135, one hydrophobic interaction with Lys 85 and one π-π stacking interaction with Phe 67.

The compound 6f has shown an IC_{50} value of 7.564 with dock score of -6.575 and MM/GBSA values -68.125 kcal/mol. This implies that the substitution of methoxy group on 4th position respectively has improved the activity and also it is in compliance with the MM/GBSA values.

Table.3. Dock scores and binding free energies for the synthesized compounds of scheme 1 and 2

Scheme-1 Code	docking score	Prime MMGBSA DG bind	Scheme-2 Code	docking score	Prime MMGBSA DG bind
4a	-5.211	-58.152	**6a**	-5.236	-62.935
4b	-5.282	-59.542	**6b**	-5.304	-62.952
4c	-4.869	-74.907	**6c**	-6.739	-63.049
4d	-5.640	-60.604	**6d**	-7.185	-70.858
4e	-5.089	-63.727	**6e**	-6.105	-57.904
4f	-5.135	-70.974	**6f**	-6.575	-68.125
4g	**-4.711**	**-74.621**	**6g**	-7.010	-71.038
4h	-5.120	-59.867	**6h**	-5.429	-61.660
4i	-6.826	-74.908	**6i**	**-6.432**	**-60.185**
4j	-4.852	-82.402	**6j**	-6.501	-75.854
4k	-5.678	-71.704	**6k**	-5.026	-65.166
4l	-5.134	-76.414	**6l**	-5.243	-68.366
4m	-5.608	-79.199	STD TMZ	-5.158	-40.294
4n	-5.887	-64.819			
STD TMZ	-5.158	-40.294			

Table.4. Qikprop values of scheme 1 and scheme 2 synthesized compounds. For **scheme-1**

Code	MW	QPlog Po/w	QPlogS	QPP-Caco	QPlog BB	QPPMDCK	% Human Oral Ab-sorption
4a	346.363	3.16	-4.803	743.215	-0.45	734.657	96.836
4b	364.353	3.401	-5.179	744.125	-0.345	1331.795	100
4c	380.808	3.667	-5.572	744.078	-0.301	1816.977	100
4d	391.36	2.405	-4.84	88.962	-1.526	74.065	75.915

4e	376.389	3.223	-4.959	743.573	-0.543	735.041	100
4f	360.389	3.486	-5.415	743.043	-0.485	734.474	100
4g	425.259	3.746	-5.692	744.182	-0.292	1953.93	100
4h	391.36	2.53	-4.773	134.879	-1.322	116.135	79.881
4i	661.947	5.231	-7.535	1064.431	0.243	10000	85.838
4j	415.253	4.106	-6.186	748.415	-0.173	3735.515	100
4k	421.386	2.592	-4.933	134.007	-1.439	115.324	80.194
4l	422.46	4.833	-6.931	743.615	-0.626	735.086	100
4m	467.458	4.203	-6.901	134.943	-1.572	116.195	89.681
4n	409.351	2.797	-5.259	133.823	-1.255	208.472	81.382
TMZ	194.152	-1.21	-1.382	58.452	-1.405	22.983	51.486

For scheme-2

Code	**MW**	**QPlogPo/w[a]**	**QPlogS[b]**	**QPP-Caco[c]**	**QPlogBB[d]**	**QPPMDCK[e]**	**% Human Oral Ab-sorption[f]**
6a	349.366	3.721	-5.295	417.772	-1.03	394.151	95.643
6b	383.265	5.062	-6.148	3494.863	0.223	10000	100
6c	338.814	4.983	-6.028	3494.753	0.212	9667.967	100
6d	373.259	5.418	-6.594	3518.345	0.361	10000	100
6e	365.366	3.196	-5.34	155.185	-1.567	135.141	84.873
6f	403.281	6.661	-8.168	3493.778	0.106	9665.054	100
6g	387.286	5.744	-7.205	3518.031	0.348	10000	100
6h	347.369	3.875	-6.408	725.375	-0.676	1295.502	100
6i	383.811	4.228	-6.066	417.692	-0.886	973.1	100
6j	418.256	4.67	-6.681	428.736	-0.752	2047.939	100
6k	367.357	3.961	-5.671	417.841	-0.927	713.692	100
6l	383.811	4.227	-6.065	417.83	-0.886	973.732	100
TMZ	194.152	-1.21	-1.382	58.452	-1.405	22.983	51.486

[a]. QPlogPo/w Predicted octanol/water partition coefficient (Acceptable range-2.0 to 6.5).

[b]. QPlogS (aqueous solubility) (Acceptable range -6.5 to 0.5).

[c]. QPPCaco cell permeability (Acceptable range <25 is poor and >80% is high).

[d]. QPlogBB Predicted Blood Brain Barrier permeability (Acceptable range-3 to 1.2)

[e]. QPPMDCK (Acceptable range <25 is poor and >80% is high).

[f]. %HOA: Percentage of human oral absorption (Acceptable range: <25 is poor and >80% is **high).**

3.4. Conclusions:

In conclusion, we have developed a one-pot synthesis of [3,2-*b*][1,2,4]triazolothiazole isoindolines and Schiff bases by using a multi-component approach. These compounds show antiproliferative effect, anti-migratory effect and cell cycle arrest in a dose dependent manner in C6 and LN18 GBM cell lines by modulating MAP kinase pathway. The molecular docking studies demonstrate that 4g and 6i were more effective in binding with GSK-3β and were in agreement with *in-vitro* results.

3.5. Experimental.

All the chemicals were purchased from commercially available sources i.e. Sigma Aldrich, TCI, Spectrochem, Finar and used without further purification. The solvents were purchased from Sigma Aldrich, TCI and stored over a molecular sieve prior to use. Progress of the reaction was monitored with Thin Layer Chromatography (TLC) using aluminium-foil backed silica gel plates. FT-IR spectra were recorded on a Perkin Elmer spectrometer using KBr disc and values were represented in cm^{-1}. ^{1}H NMR spectra were recorded on Bruker's AVANCE 400 MHz spectrometer using DMSO-d_6 as a solvent and Tetra methyl silane (TMS) as an internal standard reference. The abbreviations were used to explain the splitting pattern; s = singlet, d = doublet, t = triplet, q = quartet and coupling constant (*J*) units expressed in Hz. ^{13}C NMR spectra recorded on Bruker's AVANCE 100 MHz spectrometer and it is fully decoupled with broad band proton decoupling the chemical shifts values (δ) were represented in ppm with reference to the centre line of a triplet at 77.16 ppm for chloroform-d_6 and septet at 39.52 ppm for DMSO-d_6. SMP30 (Stuart, Staffordshire, UK) apparatus was used for identification of Melting points and are uncorrected. Molecular mass of the compounds was confirmed with HRMS (ESI-TOF) Agilent technologies. The purity of the compounds >90 percent was checked by elemental analysis.

3.5.1. ***General procedure for the synthesis of 2-(6-phenylthiazolo[3,2-b][1,2,4]triazol-2-yl) isoindoline-1,3-diones (4a-n).***

A mixture of 5-amino-4*H*-1,2,4-triazol-3-thiol 1 (1 mmol), substituted phenacyl bromide 2 (1 mmol) and 2 drops of concentrated hydrochloric acid in ethanol (3 mL) was stirred at room temperature for 1 h and then reaction mixture was refluxed at 80 °C for 7 h. After consumption of the reactant's phthalic anhydride 3 (1 mmol) was added to the same reaction mixture and refluxed for additional 4 h. After completion of the reactants the reaction mixture was cooled and neutralized with 5 % aqueous $NaHCO_3$ solution, solid product was filtered, dried and recrystalized from ethanol.

3.5.2. ***General procedure for the synthesis of 1-phenyl-N-(6-phenylthiazolo[3,2-b][1,2,4]triazol-2-yl)methanimine (6 a-l).***

A mixture of 5-amino-4*H*-1,2,4-triazol-3-thiol 1 (1 mmol), substituted phenacyl bromide 2 (1 mmol) and 2 drops of concentrated hydrochloric acid in ethanol (3 mL) was stirred at room temperature for 1 h and then the reaction mixture was refluxed at 80 °C for 7 h. After consumption of the reactant's substituted aromatic aldehyde 5 (1 mmol) was added to the same reaction mixture and refluxed for additional 3 h. After completion of the reactants, the reaction mixture was cooled and neutralized with 5 % aqueous $NaHCO_3$ solution, solid product was filtered, dried and recrystalized from ethanol

3.5.3. Cell culture and reagents.

Rat glioma cell lines C6 and LN18 were cultured in Dulbecco's Modified Eagles Medium (DMEM) media, supplemented with 10% heat inactivated fetal bovine serum (Gibco, NY, USA). Antibiotics such as penicillin (100 IU/mL) and streptomycin (100 mg/mL) were supplemented. The cells were maintained at 37 °C and humidified atmosphere in 5% CO_2 incubator.

3.5.4. Cell viability assay.

Cell viability was determined by MTT assay wherein live cells reduce yellow MTT to formazon crystals. For C6 and LN18 cell lines, 5,000 and 7,500 cells respectively were plated in distinct 96 wells plates followed by the drug treatment. Cell viability was assessed by incubating the cells in methyl thiazole tetrazolium (MTT, 5 mg/mL; Sigma Aldrich, Taufkirchen, Germany) for 4 h at 37 °C, the formazan crystals thus formed were solubilized in DMSO and absorbance was measured at 570 nm.

3.5.5. Cell proliferation assay.

Cell proliferation was analysed using MTT assay. For C6 and LN18 cell lines, 2,500 and 5,000 cells respectively were plated at a density of 1 X 104 cells/ well in distinct 96 wells plates. The cells were incubated overnight followed by the drug treatment for 48 hours. 10 µL of MTT (5mg/mL) was added at different time points i.e., 24 hours, 48 hours, 72 hours, 96 hours and 108 hours. The cells were further incubated for 4 hours at 37 °C. DMSO was added to dissolve the formazon crystals and the absorbance was taken at 570 nm. We also used FDA approved chemotherapeutic agent for glioma, Temozolomide (TMZ) as a control for our experiments to compare the efficacy of the potential anti-cancerous compounds.

3.5.6. Wound healing assay.

For wound healing assay, 6 wells plates were used. 3×105 cells of both C6 and LN 18 cell lines were plated in each well. The cells were incubated overnight so that they adhere the surface and form a monolayer. When the cells were 80% confluent, the monolayer was scratched with the help of autoclaved 200µl microtip. This was followed by capturing images at distinct time points of 0 hour, 24 hours, 48 hours for C6 cell line and additionally 72 hours for LN18 cell line. The captured images were compared to quantify the rate of migration of the cells after the wound.

3.5.7. Clonogenic assay.

To determine clonogenic survival, were seeded in 6-well plate (C6: 500 cells/well and LN18: 1000 cells/well). The cells were treated with respective drugs at different concentrations for 48 hours and further cells were supplemented with fresh complete media. The cells were incubated at optimal conditions post treatment unless the colonies were seen in the cultures. The cells were washed with 1× PBS, further the colonies were stained with 0.5% crystal violet for 10 min. Further, the cells were washed with water to remove the excess stain and imaged for analysis.

3.5.8. Western blotting.

Protein concentrations were determined and quantified by Bradford assay reagent (Sigma, USA). 30µg of protein samples were subjected for 10% SDS-PAGE and electro-transferred onto the nitrocellulose membrane. Membrane was blocked for 1 h at room temperature using 5% skimmed milk and was incubated overnight at 4 °C with primary antibodies (1:1000) against respective antibodies: pERK, pAKT (Thr 308 and Ser 473), AKT (Cell Signaling Technology Europe, Frankfurt/Main, Germany), ERK (Santa Cruz Biotechnology, Dallas, TX,

USA), pIRE, IRE, XBP1 (Abcam, Cambridge, UK). After washing, the membranes were incubated with HRP-conjugated secondary antibodies (1:2000) and blots were developed using luminescence detection reagents (Thermo scientific, USA).

3.5.9. Cell cycle analysis.

The cells were plated in 60 mm plate and were allowed to adhere for 24 hours. Drug treatment was given to the cells for 48 hours. Trypsinization of the cells was done to obtain single cell suspension. The cells were centrifuged at 2000 rpm for 5 mins. The pelleted cells were fixed using 70% ethanol with gentle vortexing and were transferred to -20 °C for overnight. The cells were centrifuged, ethanol was discarded and the pelleted cells were washed with PBS thrice to remove the ethanol completely. Followed by PI staining (PI) mixture (50 µg/mL PI, 1% triton-X-100, 50 µg/mL RNase A) and incubated for 30 mins in the dark at room temperature. The cells were washed and resuspended with PBS. Then, PI-stained cells were subjected to flow cytometry using a FACS Calibur (BD Biosciences). Cell cycle parameters were analysed using Flowjo software.

3.5.10. Statistical analysis.

Statistical analysis was performed using GraphPad Prism 9.0 (GraphPad Software Inc., CA, USA). The representative experimental data shown is repeated at least three times unless mentioned otherwise. The statistical analysis was performed using multiple pairwise comparisons for in vitro data. The statistical significance between two groups was evaluated using Student t test. The results are represented as mean ± standard error mean (SEM). p-values of <0.05 are considered as statistically significant (ns: not significant; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

3.5.11. Docking Analysis.

3.5.11a. Ligand preparation: All the structures of the derivatives, the standard Temozolamide were sketched in chemsketch and imported into the Maestro build panel of Schrodinger suite. They were converted to three-dimensional structures and prepared using Ligprep module. This generated all possible states at a physiological pH range 7 ± 2 and produces lowest potential energy conformer of the ligand using an OPLS 2005 force field. All these ligands post preparation was used for molecular docking studies

3.5.11b. Protein preparation: In this current studies, GSK-3β inhibitor complexed with the inhibitor 6QH4001 protein has been retrieved from the protein data bank with PDB ID: 5K5N.

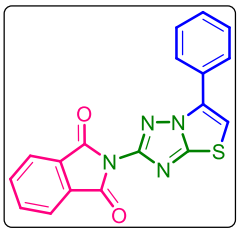
Protein preparation wizard module of Schrodinger Suite was used to prepare the protein. Hydrogens were added, the hetero atoms and water molecules were deleted from the crystal structure. The complex was minimized to relieve steric clashes using the OPLS 2005 force field.

3.5.11c. Molecular docking: A set of derivatives synthesized were taken and constructed using Maestro build panel and prepared by Ligprep application in the Schrodinger 2010 suite. GLIDE 5.6 (Glide, Version 5.6. New York, NY) was used for molecular docking studies. The Grid was generated at ligand binding region of the 5K5N and receptor van der Waals scaling for the nonpolar atoms was set to 0.9 and ligand molecule was picked so that it could be excluded from grid generation. The molecular docking of all the derivatives into the generated grid was performed by using the extra precision (XP) docking mode. The dimensions of the grid box were set to 10 Å × 10 Å × 10 Å. In initial phase of docking, 5000 poses per ligand were taken out of which top 800 poses per ligand were passed on for energy minimization. During energy minimization, the maximum number of minimization steps were set to 100, and distance-dependent dielectric constant was set to 2.0

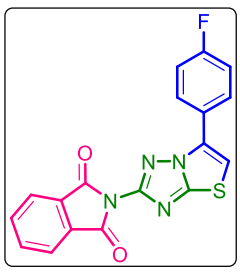
3.6. Characterization data of the synthesized compounds

SCHEME-1

2-(6-Phenylthiazolo[3,2-b][1,2,4]triazol-2-yl)isoindoline-1,3-dione (4a): White solid; yield 80%; mp: 220-221 °C; IR (KBr) cm^{-1}: 3119 (alkene C-H), 1747 (imide C=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.53-7.59 (m, 3H), 7.98-8.00 (m, 2H), 8.05-8.07 (m, 2H), 8.10 (s, 1H), 8.19 (d, J = 6.8 Hz, 2H); $^{13}C\{H\}$NMR (100MHz, DMSO-d_6) δ (ppm): 111.9, 124.6, 126.8, 127.6, 129.5, 130.4, 131.4, 132.4, 136.0, 153.4, 157.2, 166.3; HRMS (ESI-TOF) (*m/z*): Calculated for $C_{18}H_{10}N_4O_2S$ $[M+H]^+$ 347.0597; found 347.0603. C, H, N Analysis: Calculated C, 62.42; H, 2.91; N, 16.18; found: C, 62.46; H, 2.95; N, 16.22.

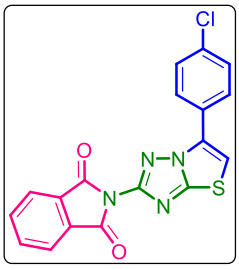


2-(6-(4-Fluorophenyl) thiazolo[3,2-b] [1,2,4] triazol-2-yl) isoindoline-1,3-dione (4b): White solid; Yield 82%; m.p: 238-239 °C; IR (KBr) cm^{-1}: 3110 (alkene C-H), 1747 (imide C=O), 1076 (C-F); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.45 (t, J = 8.8 Hz, 2H), 7.98 - 8.00 (m, 2H), 8.05-8.07 (m, 2H), 8.09 (s, 1H), 8.23-8.27 (m, 2H); $^{13}C\{H\}$NMR (100MHz, DMSO-d_6) δ (ppm): 111.8, 116.4, 116.7, 124.3, 124.6, 129.2, 129.3, 131.4, 135.9, 153.4, 157.2, 161.9, 166.3; HRMS (ESI-TOF) (*m/z*): Calculated for

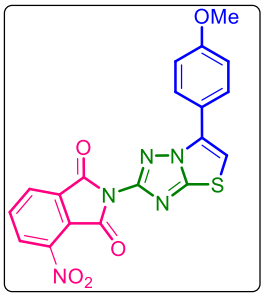


$C_{18}H_9FN_4O_2S$ $[M+H]^+$ 365.0503; found 365.0505. C, H, N Analysis: Calculated C, 59.34; H, 2.49; N, 15.38; found: C, 59.30; H, 2.52; H, 15.35.

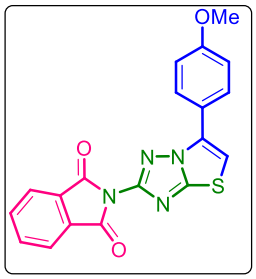
2-(6-(4-Cholrophenyl) thiazolo[3,2-b] [1,2,4] triazol-2-yl) isoindoline-1,3-dione (4c): White solid; Yield 79%; mp: 279-280 °C; IR (KBr) cm^{-1}: 3118 (alkene C-H), 1746 (imide C=O), 628 (C-Cl); ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.67 (d, J = 8.8Hz, 2H), 8.00-7.98 (m, 2H), 8.05-8.07 (m, 2H), 8.17 (s, 1H), 8.23 (d, J = 8.8 Hz, 2H); $^{13}C\{H\}$NMR (100MH_Z, DMSO-d_6) δ (ppm): 112.6, 124.5, 124.7, 128.2, 128.5, 129.2, 129.4, 129.5, 130.7, 131.3, 135.9, 136.0, 166.3; HRMS (ESI-TOF) (m/z): Calculated for $C_{18}H_9ClN_4O_2S$ $[M+H]^+$ 381.0208; found 381.0206. C, H, N Analysis: Calculated C, 56.77; H, 2.38; N, 14.71; found: C, 56.80. H; 2.35; N, 14.76.



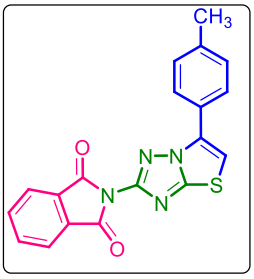
2-(6-(4-Methoxyphenyl) thiazolo[3,2-b] [1,2,4] triazol-2-yl)-4-nitroisoindoline-1,3-dione (4d): Yellow solid; Yield 85%; m.p: 220-221 °C; IR (KBr) cm^{-1}: 3124 (alkene C-H), 1723 (imide C=O), 1517, 1338 (NO_2), 1116 (C-O-C); ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 3.83 (s, OCH_3, 3H), 7.07-7.15 (m, 4H), 7.37 (s, 1H), 8.13 (d, J = 8.8 Hz, 3H); $^{13}C\{H\}$NMR (100MHz, DMSO-d_6) δ (ppm): 57.7, 103.2, 106.9, 114.6, 114.7, 120.6, 121.1, 128.1, 128.3, 132.0, 132.1, 155.7, 160.4, 160.6, 168.6; HRMS (ESI-TOF) (m/z): Calculated for $C_{19}H_{11}N_5O_5S$ $[M+H]^+$ 422.0561; found 422.0567. C, H, N Analysis: Calculated C, 54.16; H, 2.63; N, 16.62; O; found: C, 54.13; H, 2.67; N, 16.66.



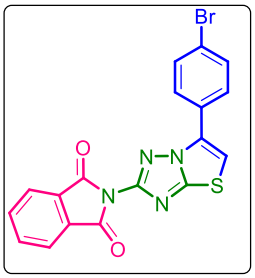
2-(6-(4-Methoxyphenyl) thiazolo[3,2-b] [1,2,4] triazol-2-yl) isoindoline-1,3-dione (4e): Light yellow solid; Yield 90 %; m.p: 237-238 °C; IR (KBr) cm^{-1}: 3119 (alkene C-H), 1747 (imide C=O), 1036 (C-O-C); ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 3.84 (s, OCH_3, 3H), 7.14 (d, J = 8.8 Hz, 2H), 7.94 (s, 1H), 7.98 -8.00 (m, 2H), 8.05-8.07 (m, 2H), 8.13 (d, J = 8.8 Hz, 2H); $^{13}C\{H\}$NMR (100MH_Z, DMSO-d_6) δ (ppm): 55.8, 109.5, 114.9, 120.1, 124.6, 128.3, 128.4, 131.4, 132.3, 135.9, 153.3, 157.1, 160.8, 166.3; HRMS (ESI-TOF) (m/z): Calculated for $C_{19}H_{12}N_4O_3S$ $[M+H]^+$ 377.0703; found 377.0711. C, H, N Analysis: Calculated C, 60.63; H, 3.21; N, 14.89; found: C, 60.67; H, 3.26; N, 14.85.



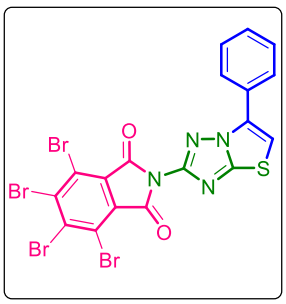
2-(6-(p-Tolyl) thiazolo[3,2-b] [1,2,4] triazol-2-yl) isoindoline-1,3-dione (4f): Light yellow solid; Yield 88%; m.p: 242-243 °C; IR (KBr) cm^{-1}: 3107 (alkene C-H), 1742 (imide C=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 3.83 (s, 3H), 7.14 (d, J = 9.2 Hz, 2H), 7.94 (s, 1H), 7.97-7.99 (m, 2H), 8.05-8.07 (m, 2H), 8.11-8.14 (m, 2H); $^{13}C\{H\}NMR$ (100MH_Z, DMSO-d_6) δ (ppm): 23.8, 109.5, 114.8, 120.1, 124.6, 128.3, 128.4, 131.4, 132.3, 135.9, 153.3, 157.1, 160.8, 166.3; HRMS (ESI-TOF) (*m/z*): Calculated for $C_{19}H_{12}N_4O_2S$ $[M+H]^+$ 361.0754; found 361.0971. C, H, N Analysis: Calculated C, 63.32; H, 3.36; N, 15.55; found: C, 63.36; H, 3.32; N, 15.59.



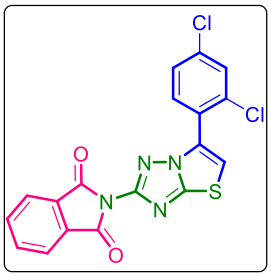
2-(6-(4-Bromophenyl) thiazolo[3,2-b] [1,2,4] triazol-2-yl) isoindoline-1,3-dione (4g): Light yellow solid; Yield 89%; m.p: 278-279 °C; IR (KBr) cm^{-1}: 3099 (alkene C-H), 1745 (imide C=O), 531 (C-Br); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.81 (d, J = 8.8 Hz, 2H), 7.98-8.00 (m, 2H), 8.05-8.07 (m, 2H), 8.16 (d, J = 8.4 Hz, 2H), 8.18 (s, 1H); $^{13}C\{H\}NMR$ (100MH_Z, DMSO-d_6) δ (ppm): 112.3, 123.7, 124.5, 127.0, 128.6, 128.8, 131.6, 132.3, 132.4, 135.9, 153.8, 157.3, 166.1; HRMS (ESI-TOF) (*m/z*): Calculated for $C_{18}H_9BrN_4O_2S$ $[M+H]^+$ 424.9702; found 424.9704. C, H, N Analysis: Calculated C, 50.84; H, 2.13; N, 13.17; found: C, 50.88; H, 2.16; N, 13.13.



4,5,6,7-Tetrabromo-2-(6-phenylthiazolo[3,2-b][1,2,4]triazol-2-yl)isoindoline-1,3-dione (4h): Yellow solid; Yield 88%; m.p: 313-314 °C; IR (KBr) cm^{-1}: 3120 (alkene C-H), 1738 (imide C=O), 664 (C-Br); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.55-7.62 (m, 3H), 8.11 (s, 1H), 8.22 (d, J = 7.2 Hz, 2H); $^{13}C\{H\}NMR$ (100MH_Z, DMSO-d_6) δ (ppm): 112.1, 121.8, 126.8, 127.6, 128.8, 129.5, 130.4, 131.2, 132.4, 137.7, 152.9, 157.2, 162.2; HRMS (ESI-TOF) (*m/z*): Calculated for $C_{18}H_6Br_4N_4O_2S$ $[M+H]^+$ 658.7018; found 660.7020(M^{+2}). C, H, N Analysis: Calculated C, 32.66; H, 0.91; N, 8.46; found: C, 32.62; H, 0.95; N, 8.42.

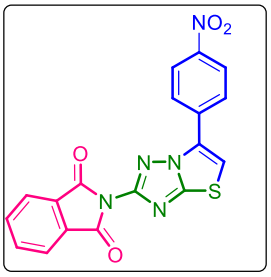


2-(6-(2,4-Dichlorophenyl)thiazolo[3,2-b][1,2,4]triazol-2-yl)isoindoline-1,3-dione (4i): Light brown solid; Yield 84%; m.p: 224-225 °C; IR (KBr) cm^{-1}: 3117 (alkene C-H), 1748 (imide C=O), 707 (C-Cl); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.88 (d, J = 8.4 Hz, 1H),7.98-8.00 (m, 2H), 8.05-8.07 (m, 2H), 8.23 (d, J = 8.4 Hz, 1H), 8.31 (s, 1H), 8.50 (s, 1H); $^{13}C\{H\}$NMR (100MHz, DMSO-d_6) δ (ppm): 114.1, 124.6, 126.8, 128.1, 128.3, 130.3, 131.4, 131.7, 132.3, 132.8, 136.0, 153.5, 157.2, 166.2; HRMS (ESI-TOF) (*m/z*): Calculated for $C_{18}H_8Cl_2N_4O_2S$ $[M+H]^+$ 414.9818; found 414.9823. C, H, N Analysis: Calculated C, 52.06; H, 1.94; N, 13.49; found: C, 52.10; H, 1.92; N, 13.52.

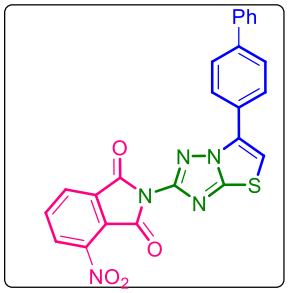


2-(6-(4-Nitrophenyl) thiazolo[3,2-b] [1,2,4] triazol-2-yl) isoindoline-1,3-dione (4j):

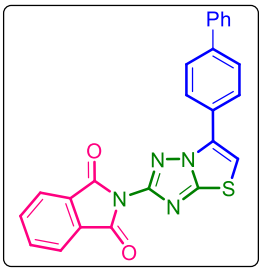
Yellow solid; Yield 85%; m.p: 270-271 °C; IR (KBr) cm^{-1}: 3107 (alkene C-H), 1748 (imide C=O), 1525, 1347 (NO_2); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.99-8.01 (m, 2H), 8.06-8.08 (m, 2H), 8.31 (s, 1H), 8.45 (d, J = 1.6 Hz, 2H), 8.50-8.52 (m, 2H); $^{13}C\{H\}$NMR (100MHz, DMSO-d_6) δ (ppm): 109.8, 116.2, 124.5, 124.7, 127.4, 127.8, 130.5, 131.4, 133.5, 134.4, 136.0, 156.0, 157.4, 166.2; HRMS (ESI-TOF) (*m/z*): Calculated for $C_{18}H_9N_5O_4S$ $[M+H]^+$ 392.0448; found 392.0455. C, H, N Analysis: Calculated C, 55.24; H, 2.32; N, 17.90; found: C,55.20; H, 2.36; N, 17.94.



2-(6-([1,1'-Bipheny]-4-yl)thiazolo[3,2-b]1,2,4]triazol-2-yl)-4-nitroisoindoline-1,3-dione (4k): Yellow solid; yield 88%; m.p: 198-199 °C; IR (KBr) cm^{-1}: 3030 (alkene C-H), 1741 (imide C=O), 1527, 1346 (NO_2); ^{1}H NMR (400 MHz, DMSO-d_6,) δ (ppm): 7.49-7.51 (m, 3H), 7.61 (s, 1H), 7.77 (d, J = 7.2 Hz, 3H), 7.85 (d, J = 8.4 Hz, 2H), 7.91 (d, J = 9.6 Hz, 2H), 8.29-8.31 (m, 2H); $^{13}C\{H\}$NMR (100MHz, DMSO-d_6) δ (ppm): 105.4, 109.2, 127.0, 127.1, 127.2, 127.3, 127.4, 127.5, 128.3, 128.4, 129.5, 131.8, 139.6, 141.2, 155.9, 168.8; HRMS (ESI-TOF) (*m/z*): Calculated for $C_{24}H_{13}N_5O_4S$ $[M+H]^+$ 468.076; found 468.0763. C, H, N Analysis: Calculated C, 61.67; H, 2.80; N, 14.98; found: C, 61.70; H, 2.84; N, 14.95.

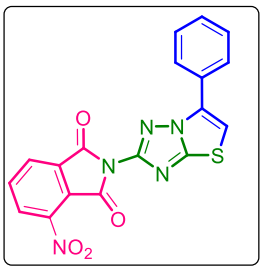


2-(6-([1,1'-Biphenyl]-4-yl)thiazolo[3,2-b] [1,2,4] triazol-2-yl) isoindoline-1,3-dione (4l):



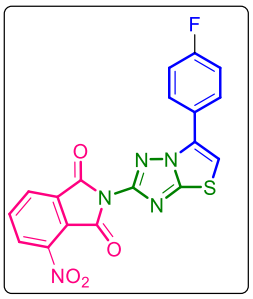
Light yellow solid; Yield 90%; m.p: 198-199 °C; IR (KBr) cm^{-1}: 3108 (alkene C-H), 1745 (imide C=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.49-7.53 (m, 3H), 7.77 (d, J = 7.2 Hz, 2H), 7.90 (d, J = 8.8 Hz, 2H),7.98-8.00 (m, 2H), 8.06-8.08 (m, 2H), 8.17 (s, 1H), 8.30 (d, J = 8.8 Hz, 2H); $^{13}C\{H\}$NMR (100MH_Z, DMSO-d_6) δ (ppm): 111.8, 124.6, 126.6, 127.0, 127.1, 127.3, 127.6, 128.5, 129.5, 131.4, 132.1, 136.0, 139.4, 141.8, 153.4, 157.2, 166.3; HRMS (ESI-TOF) (*m/z*): Calculated for $C_{24}H_{14}N_4O_2S$ $[M+H]^+$ 423.0910; found 423.0913. C, H, N Analysis: Calculated C, 68.23; H, 3.34; N, 13.26; found: C, 68.20; H, 3.36; N, 13.29.

4-Nitro-2-(6-phenylthiazolo[3,2-b] [1,2,4] triazol-2-yl) isoindoline-1,3-dione(4m):



Light Yellow solid; Yield 78%; m.p: 215-216 °C; IR (KBr) cm^{-1}: 3050 (alkene C-H), 1741 (imide C=O), 1541, 1358 (NO_2); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.53-7.59 (m, 3H), 8.11 (s, 1H), 8.18 (d, J = 8.0 Hz, 3H), 8.33 (d, J = 7.2 Hz, 1H), 8. 42 (d, J = 8.0 Hz, 1H); $^{13}C\{H\}$ NMR (100MH_Z, DMSO-d_6) δ (ppm): 106.4, 126.6, 127.8, 128.2, 129.3, 130.1, 130.9, 132.5, 135.8, 146.1, 156.3, 166.8, 167.6; HRMS (ESI-TOF) (*m/z*): Calculated for $C_{18}H_9N_5O_4S$ $[M+H]^+$ 392.0448; found 392.0448. C, H, N Analysis: Calculated C, 55.24; H, 2.32; N, 17.90; found: C, 55.28; H; 2.35; N, 17.93.

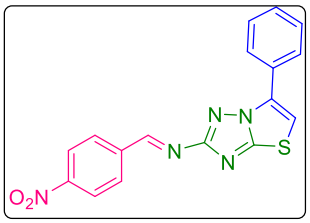
2-(6-(4-fluorophenyl)thiazolo[3,2-b][1,2,4]triazol-2-yl)-4-nitroisoindoline-1,3-dione(4n):



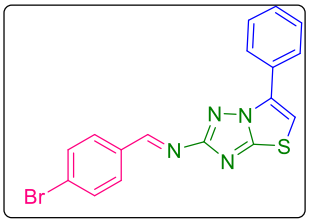
Yellow solid; Yield 81%; m.p: 220-221 °C; IR (KBr) cm^{-1}: 3107 (alkene C-H), 1742 (imide C=O), 1541, 1357 (NO_2) 1100 (C-F); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.42-7.45 (m, 2H), 8.10 (s, 1H), 8.16 (t, J = 8.0 Hz, 1H), 8.24-8.26 (m, 2H), 8.32 (d, J = 7.2 Hz, 1H), 8.41 (d, J = 8 Hz, 1H); $^{13}C\{H\}$NMR (100MH_Z, DMSO-d_6) δ (ppm): 112.0, 116.4, 116.7, 123.3, 128.3, 129.3, 129.7, 131.4, 133.5, 137.4, 145.2, 152.9, 157.2, 161.7, 164.3; HRMS (ESI-TOF) (*m/z*): Calculated for $C_{18}H_8FN_5O_4S$ $[M+H]^+$ 410.0354; found 410.0354. C, H, N Analysis: Calculated C, 52.81; H, 1.97; N, 17.11; found: C, 52.85; H, 1.94; N, 17.14.

6.1. SCHEME-2

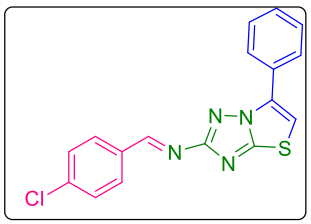
(E)-1-(4-Nitrophenyl)-N-(6-phenylthiazolo[3,2-b][1,2,4]triazol-2-yl)methanimine(6a): Yellow solid; Yield 83%; m.p: 195-196 °C; IR (KBr) cm^{-1}: 2929 (alkene C-H), 1610 (imine), 1534, 1313 (NO_2); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.58-7.62 (m, 3H), 7.95 (s, 1H), 8.23-8.25 (m, 2H), 8.30-8.34 (m, 2H), 8.38-8.40 (m, 2H), 9.49 (s, 1H); ^{13}C{H}NMR (100MHz, DMSO-d_6) δ (ppm): 106.0, 124.5, 126.6, 128.1, 129.3, 130.1, 131.0, 132.3, 140.2, 151.0, 156.2, 168.0; HRMS (ESI-TOF) (*m/z*): Calculated for $C_{17}H_{11}N_5O_2S$ $[M+H]^+$ 350.0706; found 350.0725. C, H, N Analysis: Calculated C, 58.44; H, 3.17; N, 20.05; found: C, 58.40; H, 3.20; N, 20.09.



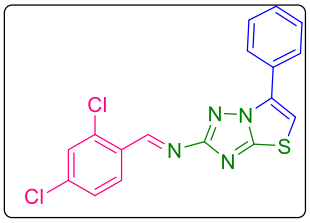
(E)-1-(4-Bromophenyl)-N-(6-phenylthiazolo[3,2-b][1,2,4]triazol-2-yl)methanimine(6b): White solid; Yield 85%; m.p: 189-190 °C; IR (KBr) cm^{-1}: 3044 (alkene C-H), 1618 (imine), 597 (C-Br); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.53-7.60 (m, 4H), 7.78-7.85 (m, 2H), 7.94 (s, 1H), 8.03 (d, *J* = 8.4 Hz, 1H), 8.20 (d, *J* = 7.6 Hz, 1H), 8.25 (d, *J* = 7.2 Hz, 1H), 9.35 (s, 1H); ^{13}C{H}NMR (100MHz, DMSO-d_6) δ (ppm): 109.2, 126.5, 126.7, 128.1, 129.2, 129.3, 130.1, 131.7, 132.2, 132.7, 155.9, 160.2, 168.7; HRMS (ESI-TOF) (*m/z*): Calculated for $C_{17}H_{11}BrN_4S$ $[M+H]^+$ 382.9961; found 382.9967. C, H, N Analysis: Calculated C, 53.28; H, 2.89; N, 14.62; found: C, 53.25; H, 2.85; N, 14.65.



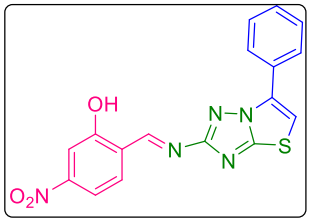
(E)-1-(4-Chlorophenyl)-N-(6-phenylthiazolo[3,2-b][1,2,4]triazol-2-yl)methanimine(6c): White solid; Yield 91%; m.p: 212-213 °C; IR (KBr) cm^{-1}: 3102 (alkene C-H), 1681 (imine), 727 (C-Cl); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.50-7.66 (m, 5H), 7.84 (s, 1H), 8.09-8.25 (m, 4H), 10.69 (s, 1H); ^{13}C{H}NMR (100 MHz, DMSO-d_6) δ (ppm): 109.2, 126.5, 126.7, 128.1, 129.2, 129.3, 129.7, 129.8, 130.1, 131.6, 132.2, 155.9, 160.2, 168.8; HRMS (ESI-TOF) (*m/z*): Calculated for $C_{17}H_{11}ClN_4S$ $[M+H]^+$ 339.0466; found 339.0470. C, H, N Analysis: Calculated C, 60.27; H, 3.27; N, 16.54; found: C, 60.30; H, 3.24; N, 16.50.



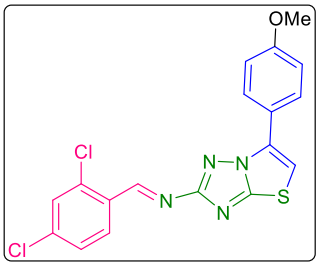
(E)-1-(2,4-Dichlorophenyl)-N-(6-phenylthiazolo[3,2-b][1,2,4]triazol-2-yl)methanimine (6d): White solid; Yield 93%; m.p: 192-193 °C; IR (KBr) cm^{-1}: 3100 alkene C-H), 1677 (imine), 727 (C-Cl); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.55-7.58 (m, 2H), 7.84 (s, 1H), 7.95 (s, 1H), 8.18-8.24 (m, 4H), 8.29 (d, J = 8.4 Hz, 1H), 9.64 (s, 1H); $^{13}C\{H\}$NMR (100MH_Z, DMSO-d_6) δ (ppm): 109.2, 126.5, 128.0, 128.7, 129.2, 129.3, 129.7, 130.1, 130.7, 131.5, 132.2, 160.4, 168.7; HRMS (ESI-TOF) (m/z): Calculated for $C_{17}H_{10}Cl_2N_4S$ $[M+H]^+$ 373.0076; found 373.0087. C, H, N Analysis: Calculated C, 54.70; H, 2.70; N, 15.01; found: C, 54.73; H, 2.74; N, 15.05.



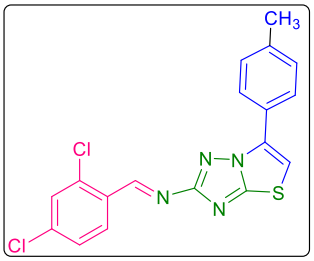
(E)-5-Nitro-2-((6-phenylthiazolo[3,2-b][1,2,4]triazol-2-yl)imino)methyl)phenol (6e): Yellow solid; Yield 81%; m.p: 203-204 °C; IR (KBr) cm^{-1}: 3074 (alkene C-H), 1610 (imine), 1527, 1339 (NO_2); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.20 (d, J = 9.2 Hz, 1H), 7.54-7.62 (m, 5H), 7.95 (s, 1H), 8.24 (d, J = 7.6 Hz, 2H), 8.87 (s, 1H), 9.67 (s, 1H); $^{13}C\{H\}$NMR (100MH_Z, DMSO-d_6) δ (ppm): 105.4, 118.8, 122.5, 125.0, 126.5, 126.7, 128.5, 129.2, 129.3, 129.8, 131.1, 140.2, 166.0; HRMS (ESI-TOF) (m/z): Calculated for $C_{17}H_{11}N_5O_3S$ $[M+H]^+$ 366.0655; found 366.0660. C, H, N Analysis: Calculated C, 55.89; H, 3.03; N, 19.17; found: C, 55.85; H, 3.07; N, 19.20.



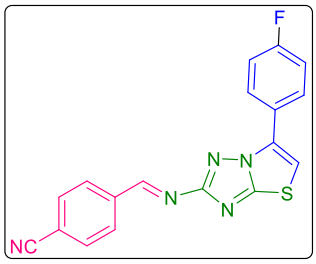
(E)-1-(2,4-Dichlorophenyl)-N-(6-(4-methoxyphenyl)thiazolo[3,2-b][1,2,4]triazol-2-yl) methanimine (6f): White solid; Yield 90%; m.p: 220-221°C; IR (KBr) cm^{-1}: 3119 (alkene C-H), 1620 (imine), 729 (C-Cl), 1257 (O-CH_3); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 3.85 (s, 3H), 7.13-7.15 (m, 2H), 7.67 (s, 1H), 7.78 (s, 1H), 8.13-8.18 (m, 4H), 9.62 (s, 1H); $^{13}C\{H\}$ NMR (100MH_Z, DMSO-d_6) δ (ppm): 79.6, 109.5, 125.2, 126.6, 128.7, 129.9, 130.1, 131.2, 132.3, 137.2, 138.3, 139.8, 160.2, 169.2; HRMS (ESI-TOF) m/z: Calculated for $C_{18}H_{12}Cl_2N_4OS$ $[M+H]^+$ 403.0182; found 403.0191. C, H, N, Analysis: Calculated C, 53.61; H, 3.00; N, 13.89; found: C, 53.64; H, 2.97; N, 13.86.



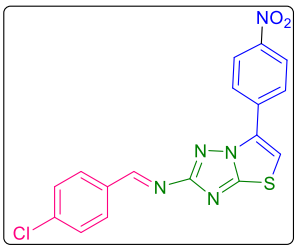
(E)-1-(2,4-Dichlorophenyl)-N-(6-(p-tolyl)thiazolo[3,2-b][1,2,4]triazol-2-yl)methanimine (6g)*:* Whit solid; Yield 89%; m.p: 218-219 °C; IR (KBr) cm^{-1}: 2920 (alkene C-H), 1615 (imine), 727 (C-Cl); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.39 (s, 3H), 7.37 (d, J = 8.4 Hz, 2H), 7.67 (s, 1H), 7.73 (d, J = 2.4 Hz, 1H), 7.77 (s, 1H), 8.09 (d, J = 8.4 Hz, 2H), 8.26 (d, J = 8.4 Hz, 1H), 9.60 (s, 1H); $^{13}C\{H\}$NMR (100MHz, DMSO-d_6) δ (ppm): 23.8, 106.9, 108.2, 114.7, 114.8, 120.6, 128.3, 128.4, 128.8, 130.2, 132.1, 138.4, 160.3, 160.7, 169.2; HRMS (ESI-TOF) m/z: Calculated for $C_{18}H_{12}Cl_2N_4S$ $[M+H]^+$ 387.0232; found 387.0241. C, H, N analysis: Calculated C, 55.82; H, 3.12; N, 14.47; found: C, 55.86; H, 3.15; N, 14.44.



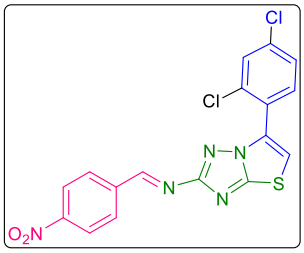
(E)-4-(((6-(4-Fluorophenyl)thiazolo[3,2-b][1,2,4]triazol-2-yl)imino)methyl)benzonitrile (6h)*:* White solid; Yield 79%; m.p: 240-241 °C; IR (KBr) cm^{-1}: 3106 (alkene C-H), 1611 (imine), 1018 (C-F); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) : 7.40-7.45 (m, 4H), 7.83 (s, 1H), 8.24-8.31 (m, 4H), 10.70 (s, 1H); $^{13}C\{H\}$NMR (100MHz, DMSO-d_6) δ (ppm): 109.1, 116.3, 116.5, 124.66, 124.69, 129.10, 129.18, 131.3, 155.9, 160.2, 161.8, 164.2; HRMS (ESI-TOF) m/z: Calculated for $C_{18}H_{10}FN_5S$ $[M+H]^+$ 348.0714; found 348.0929. C, H, N Analysis: Calculated C, 62.24; H, 2.90; N, 20.16; found: C, 62.20; H, 2.87; N, 20.20.



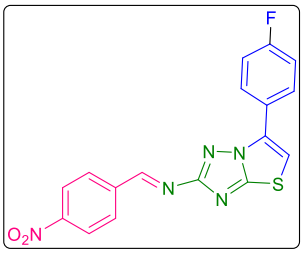
(E)-1-(4-Chlorophenyl)-N-(6-(4-nitrophenyl)thiazolo[3,2-b][1,2,4] triazol-2-yl)methanimine (6i)*:* White solid; Yield 89%; m.p: 233-234 °C; IR (KBr) cm^{-1}: 3074 (alkene C-H), 1616 (imine), 1545, 1344 (NO_2), 729 (C-Cl); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.64 (d, J = 8.4 Hz, 2H), 8.09 (d, J = 8.4 Hz, 2H), 8.28 (s, 1H), 8.40-8.42 (m, 2H), 8.49-8.52 (m, 1H), 8.55-8.58 (m, 1H), 9.36 (s, 1H); $^{13}C\{H\}$NMR (100MHz, DMSO-d_6) δ (ppm): 109.8, 124.5, 124.6, 127.3, 127.5, 129.8, 130.11, 131.6, 134.4, 147.6, 147.8, 156.0, 168.9; HRMS (ESI-TOF) (*m/z*): Calculated for $C_{17}H_{10}ClN_5O_2S$ $[M+H]^+$ 384.0316; found 384.0328. C, H, N Analysis: Calculated C, 53.20; H, 2.63; N, 18.25; found: C, 53.24; H, 2.67; N, 18.29.



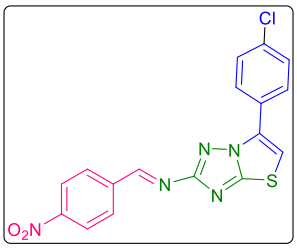
(E)–N-(6-(2,4-dichlorophenyl)thiazolo[3,2-b][1,2,4]triazol-2-yl)-1-(4-nitrophenyl) methanimine (6j): Yellow solid; Yield 87%; m.p: 230-231°C; IR (KBr) cm^{-1}: 3098 (alkene C-H), 1621 (imine), 1520, 1344 (NO_2), 730 (C-Cl); ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.77-7.85 (m, 2H), 8.06 (s, 1H), 8.17-8.22 (m, 2H), 8.34-8.43 (m, 2H), 8.54-8.55 (m, 1H), 10.75 (s, 1H); $^{13}C\{H\}$NMR (100MH_Z, DMSO-d_6) δ (ppm): 107.3, 111.1, 124.4, 126.3, 127.6, 127.8, 128.6, 130.8, 131.3, 131.8, 155.7, 168.5; HRMS (ESI-TOF) (*m/z*): Calculated for $C_{17}H_9Cl_2N_5O_2S$ $[M+H]^+$ 417.9927; found 417.3179. C, H, N Analysis: Calculated C, 48.82; H, 2.17; N, 16.74; found: C, 48.85; H, 2.21; N, 16.73.



(E)-N-(6-(4-Fluorophenyl)thiazolo[3,2-b][1,2,4]triazol-2-yl)-1-(4-nitrphenyl)methanimine (6k): Yellow solid; Yield 80%; m.p: 198-199 °C; IR (KBr) cm^{-1}: 3072 (alkene C-H), 1675 (imine), 1505, 1325 (NO_2), 1163 (C-F); ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.42-7.45 (m, 2H), 7.83 (s, 1H), 8.31-8.34 (m, 4H), 8.38-8.40 (m, 2H), 9.49 (s, 1H); $^{13}C\{H\}$NMR (100MH_Z, DMSO-d_6) δ (ppm): 109.1, 116.3, 116.5, 124.5, 124.7, 128.9, 129.0, 129.1, 130.8, 131.1, 131.2, 140.5, 155.8, 164.2, 168.8; HRMS (ESI-TOF) (*m/z*): Calculated for $C_{17}H_{10}FN_5O_2S$ $[M+H]^+$ 368.0612; found 368.0614. C, H, N Analysis: Calculated C, 55.58; H, 2.74; N, 19.06; found: C, 55.54; H, 2.77; N, 19.03.

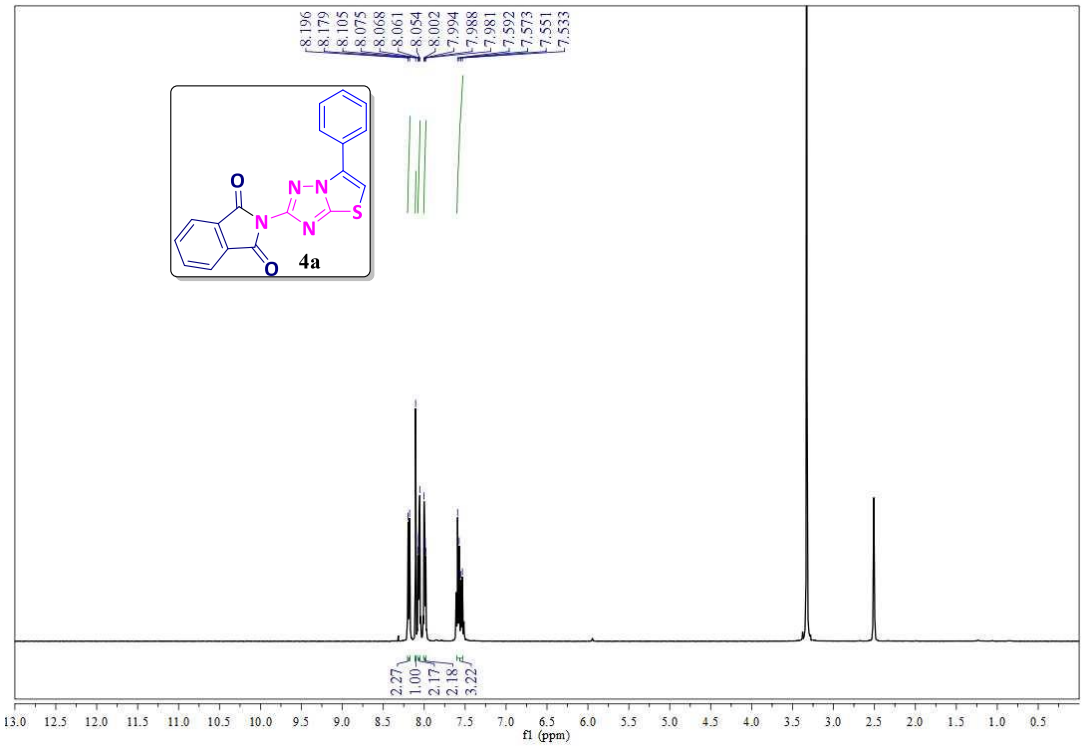


(E)-N-(6-(4-Chlorophenyl)thiazolo[3,2-b][1,2,4]triazol-2-yl)-1-(4-nitrophenyl)methanimine (6l): Yellow solid: Yield 88%; m.p: 211-212 °C; IR (KBr) cm^{-1}: 3074 (alkene C-H), 1614 (imine), 1520, 1344 (NO_2), 730 (C-Cl); ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.64-7.67 (m, 2H), 7.92 (s, 1H), 8.22-8.25 (m, 2H), 8.29-8.38 (m, 4H), 9.49 (s, 1H); $^{13}C\{H\}$NMR (100MH_Z, DMSO-d_6) δ (ppm): 106.1, 110.0, 124.7, 126.9, 128.2, 128.3, 129.2, 129.4, 131.0, 140.4, 151.0, 155.9, 168.8; HRMS (ESI-TOF) (*m/z*): Calculated for $C_{17}H_{10}ClN_5O_2S$ $[M+H]^+$ 384.0316; found 384.0322. C, H, N Analysis: Calculated C, 53.20; H, 2.63; N, 18.25; found: C, 53.23; H, 2.60; N, 18.22.

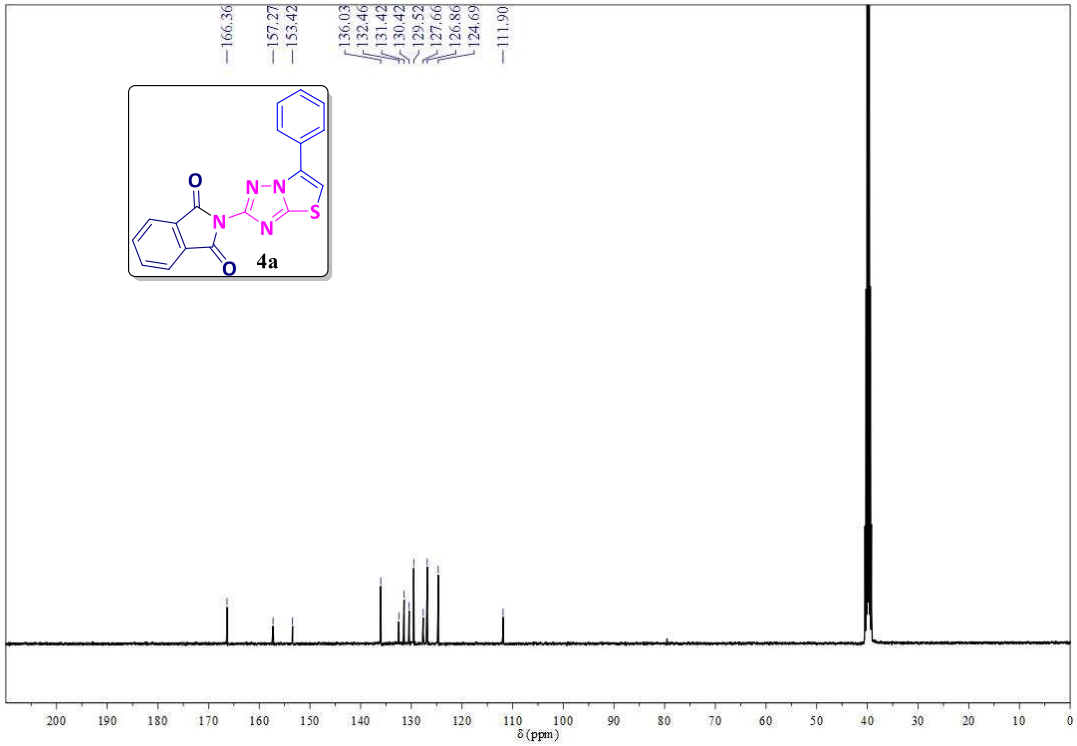


3.7. Copies of spectral data. (SCHEME-1)

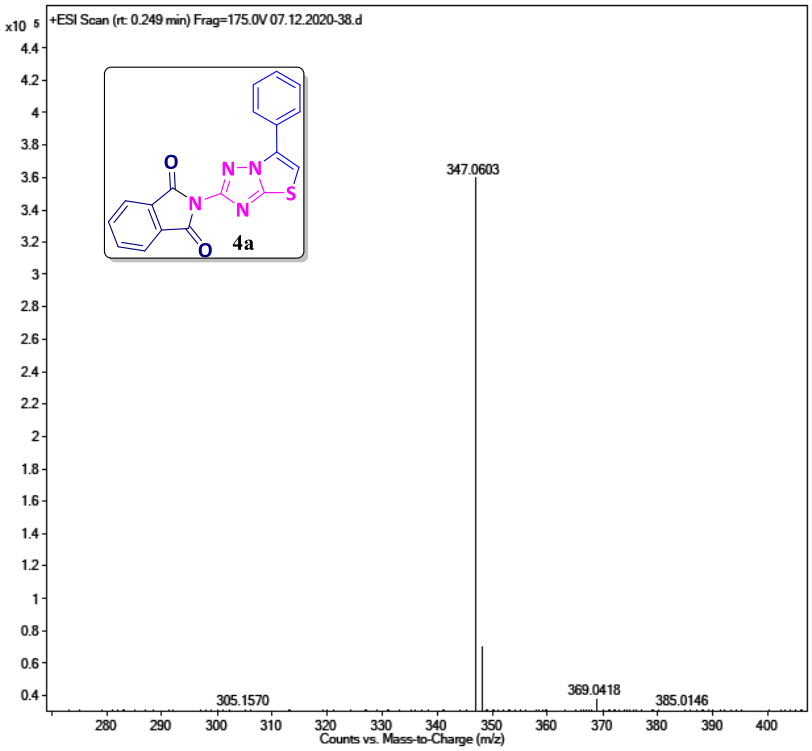
^{1}H NMR spectrum of compound **4a. (DMSO-d_6) 400 MHz**



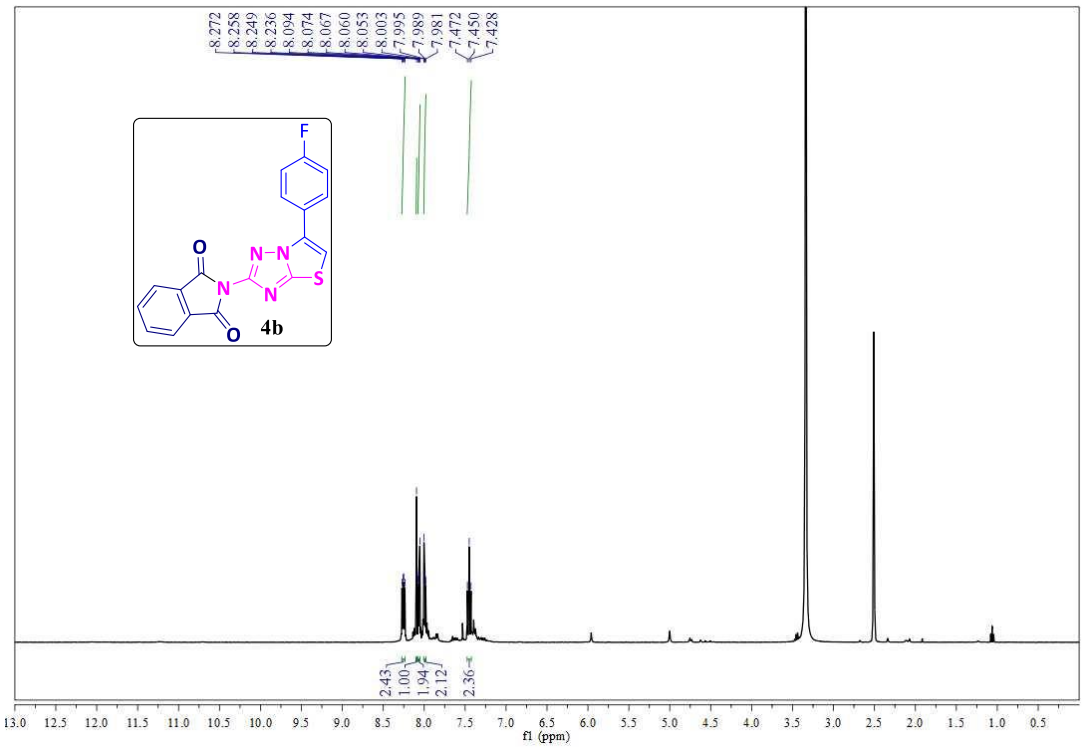
^{13}C NMR spectrum of compound **4a. (DMSO-d_6) 100 MHz**



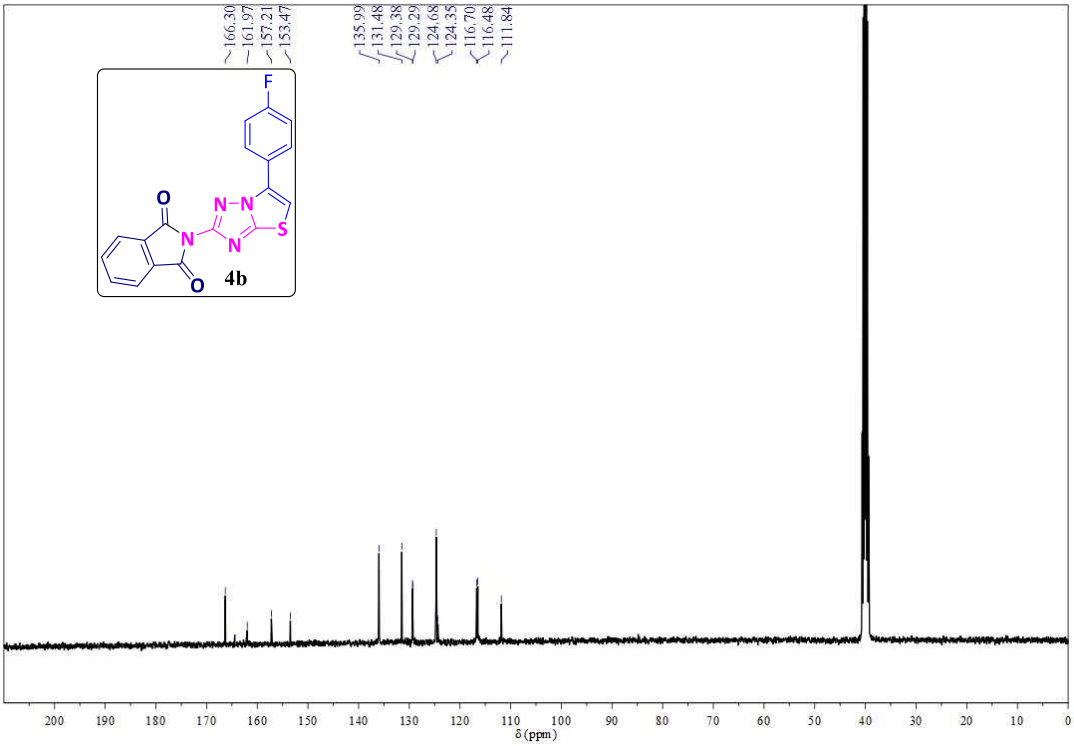
Mass spectrum of compound **4a**



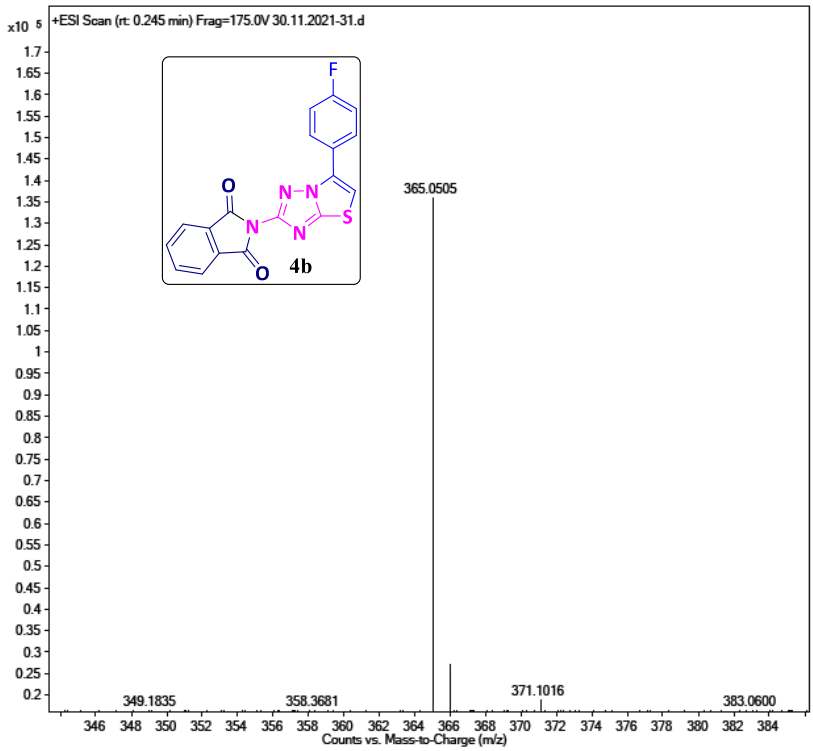
^{1}H NMR spectrum of compound **4b. (DMSO-d_6) 400 MHz**



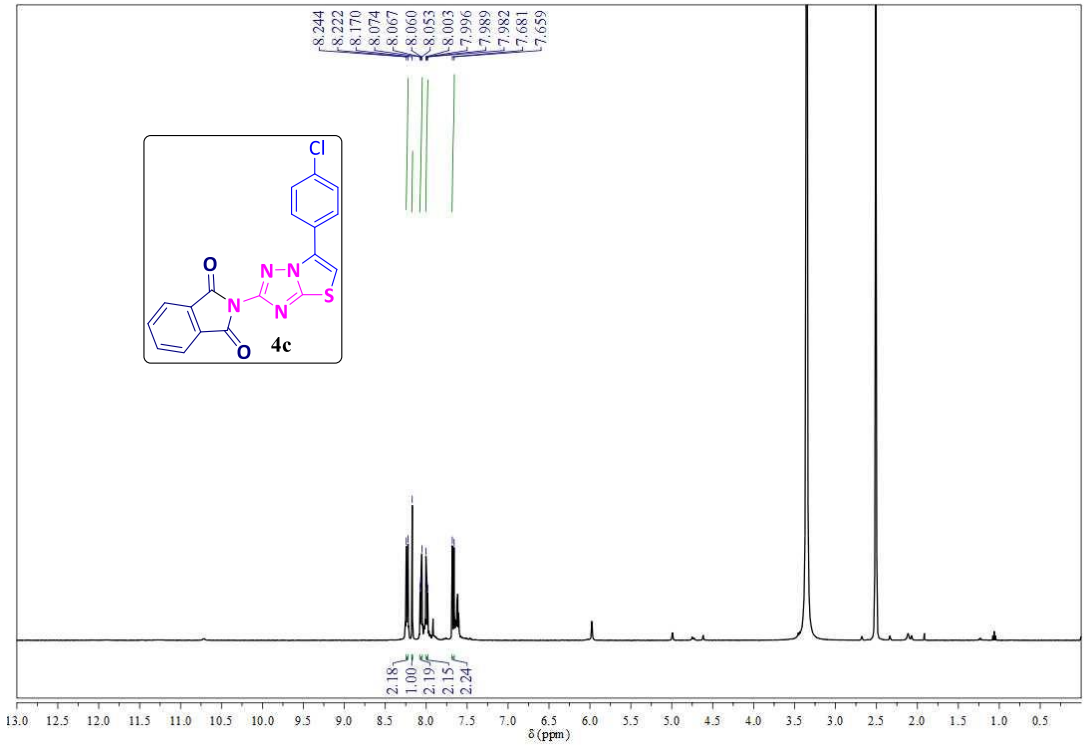
^{13}C NMR spectrum of compound **4b. (DMSO-d_6) 100MHz**



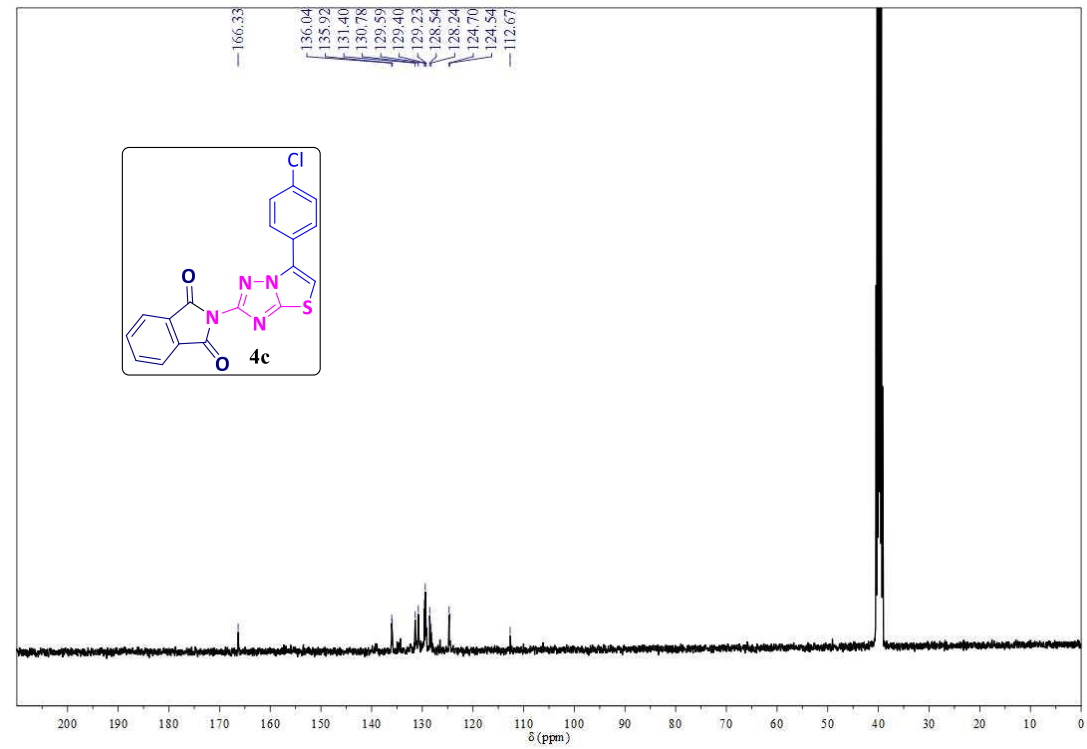
Mass spectrum of compound **4b**



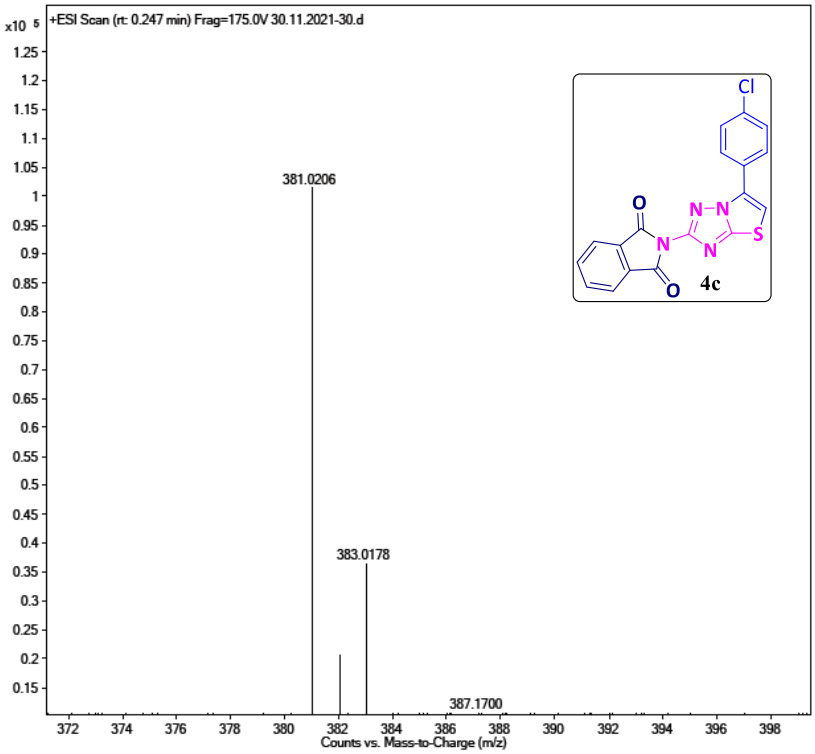
^{1}H NMR spectrum of compound **4c. (DMSO-d_6) 400MHz**



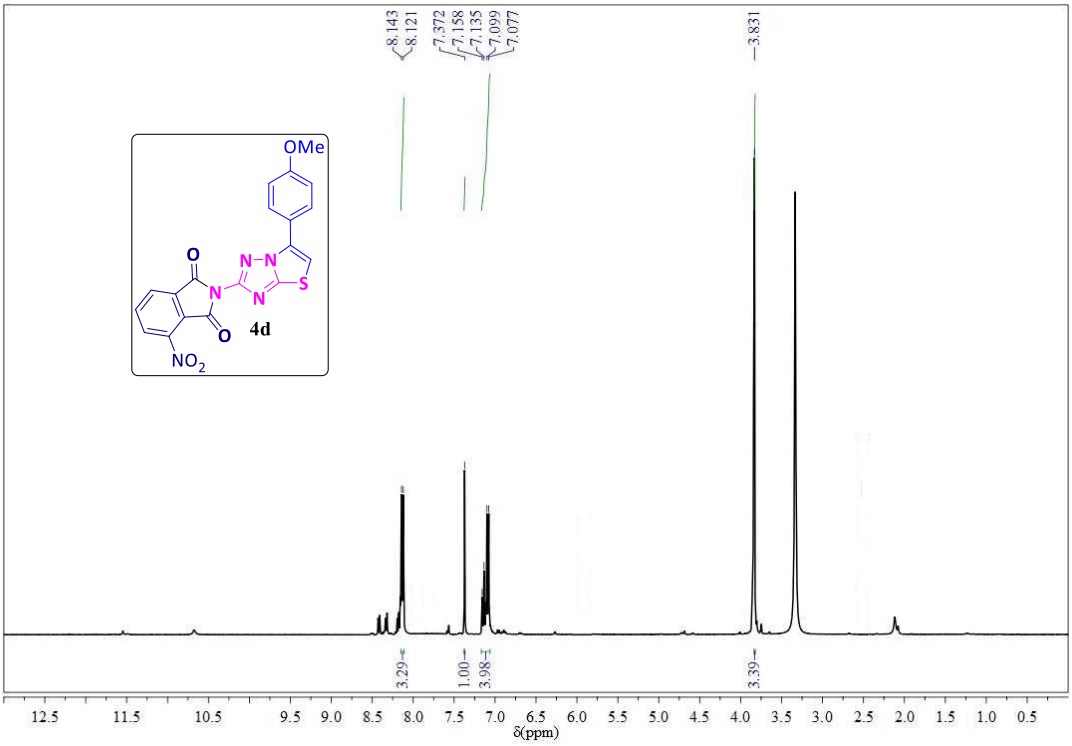
^{13}C NMR spectrum of compound **4c. (DMSO-d_6) 100 MHz**



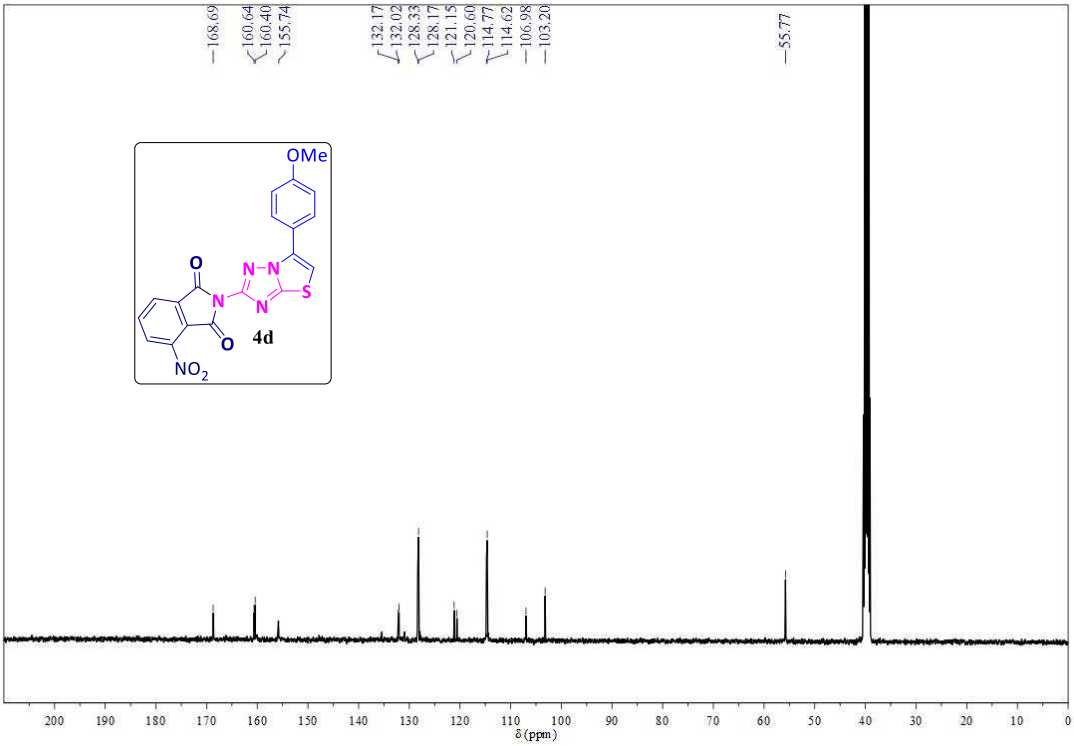
Mass spectrum of compound **4c**



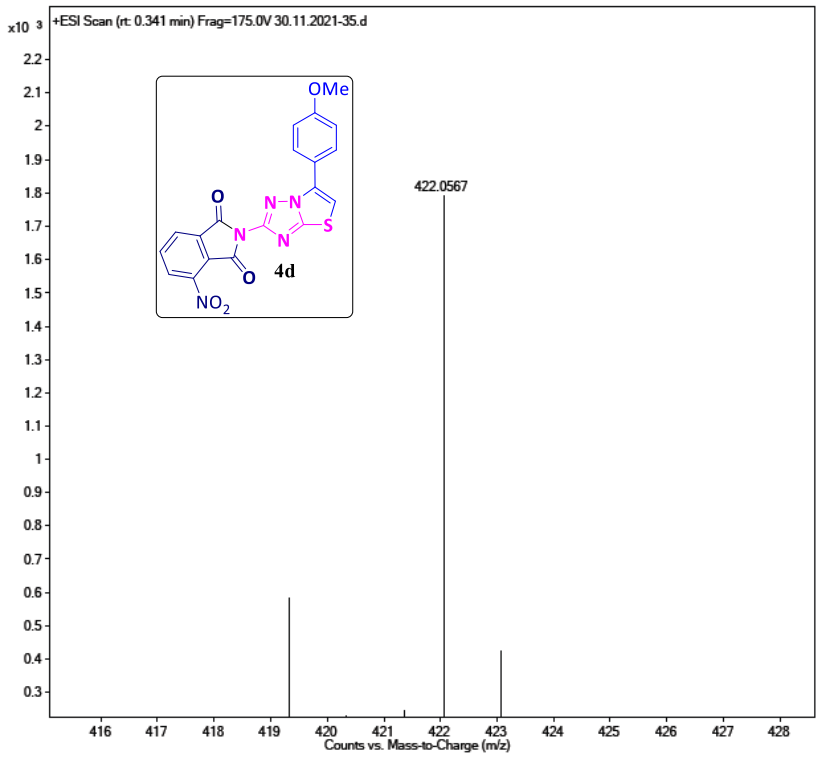
^{1}H NMR spectrum of compound **4d. (DMSO-d_6) 400 MHz**



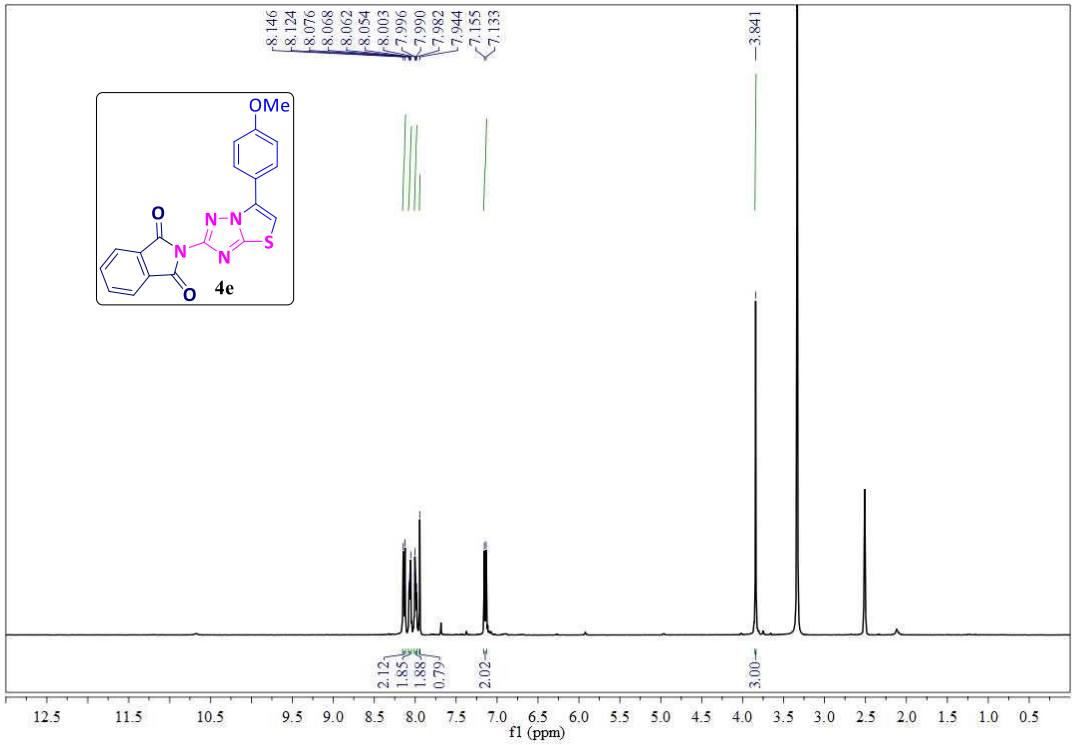
^{13}C NMR spectrum of compound **4d. (DMSO-d_6) 100 MHz**



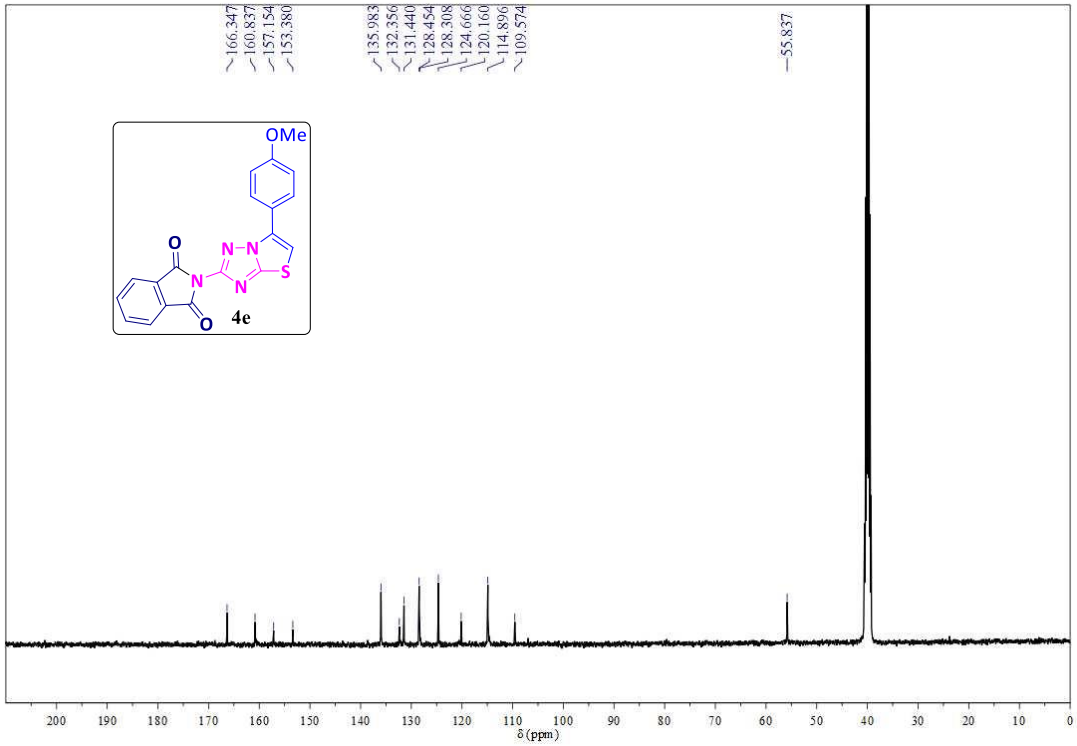
Mass spectrum of compound **4d**



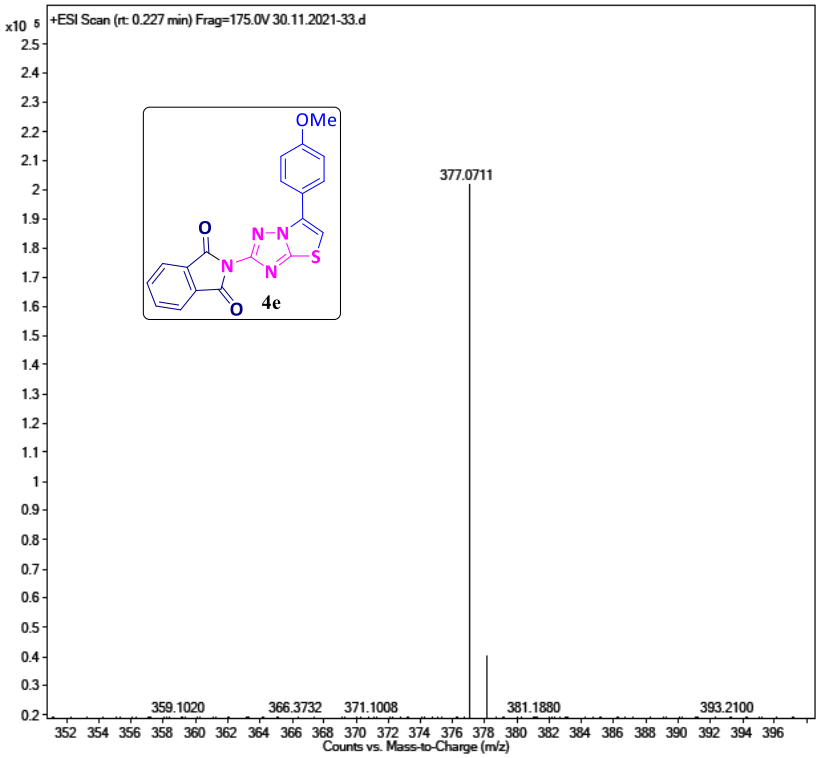
^{1}H NMR spectrum of compound **4e. (DMSO-d_6) 400 MHz**



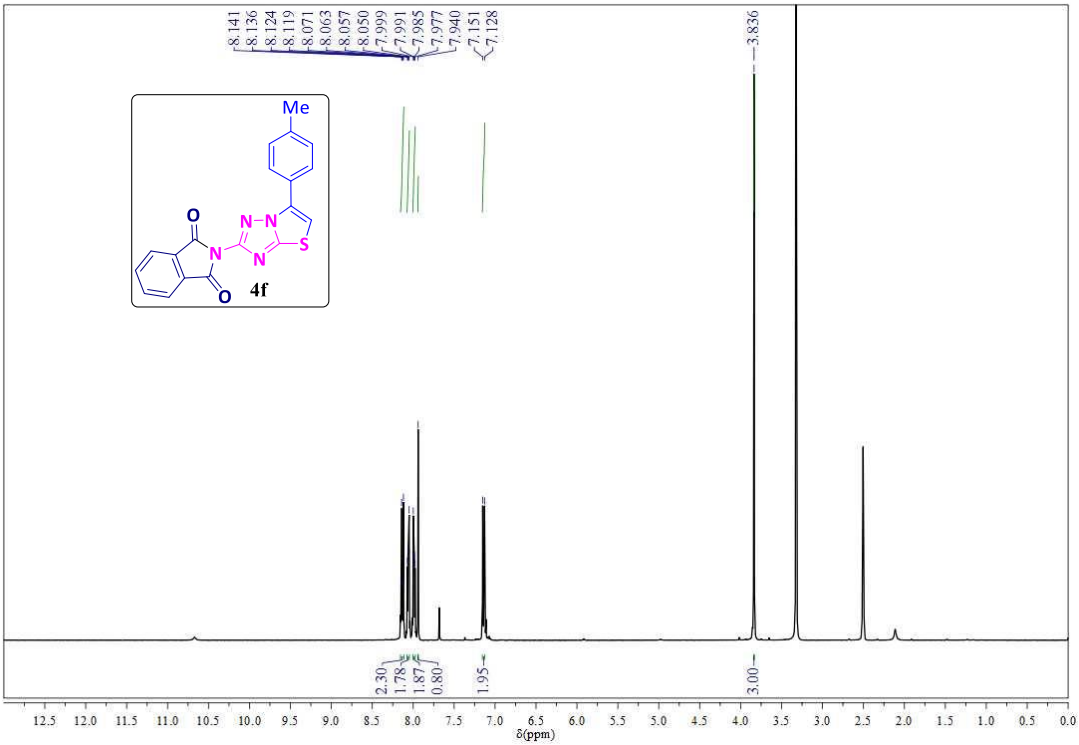
^{13}C NMR spectrum of compound **4e. (DMSO-d_6) 100 MHz**



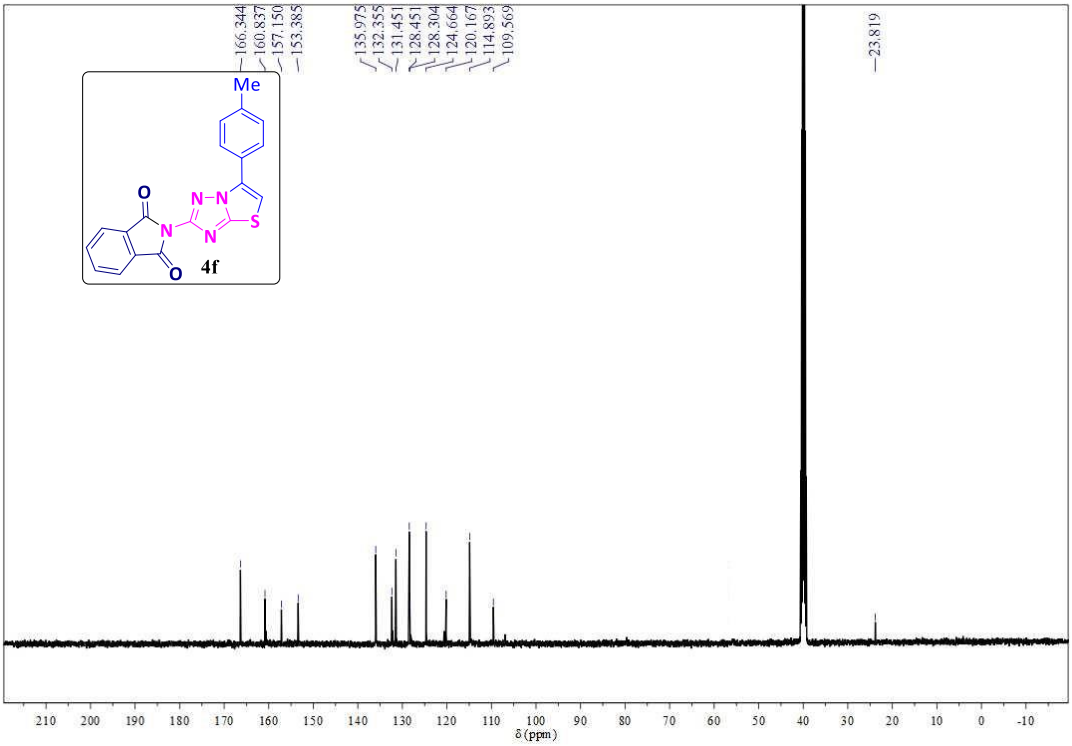
Mass spectrum of compound **4e.**



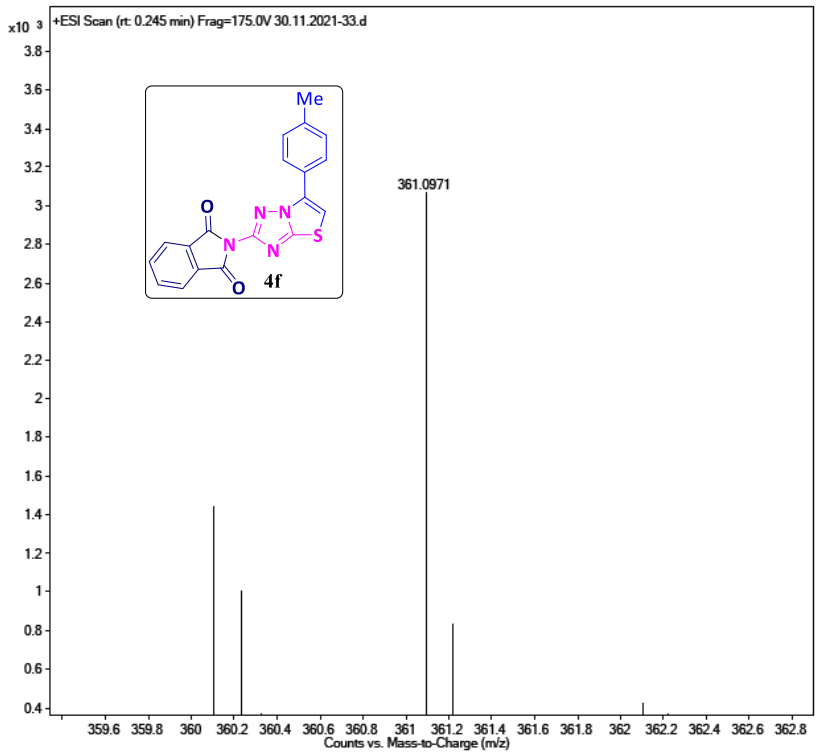
^{1}H NMR spectrum of compound **4f. (DMSO-*d*$_6$) 400 MHz**



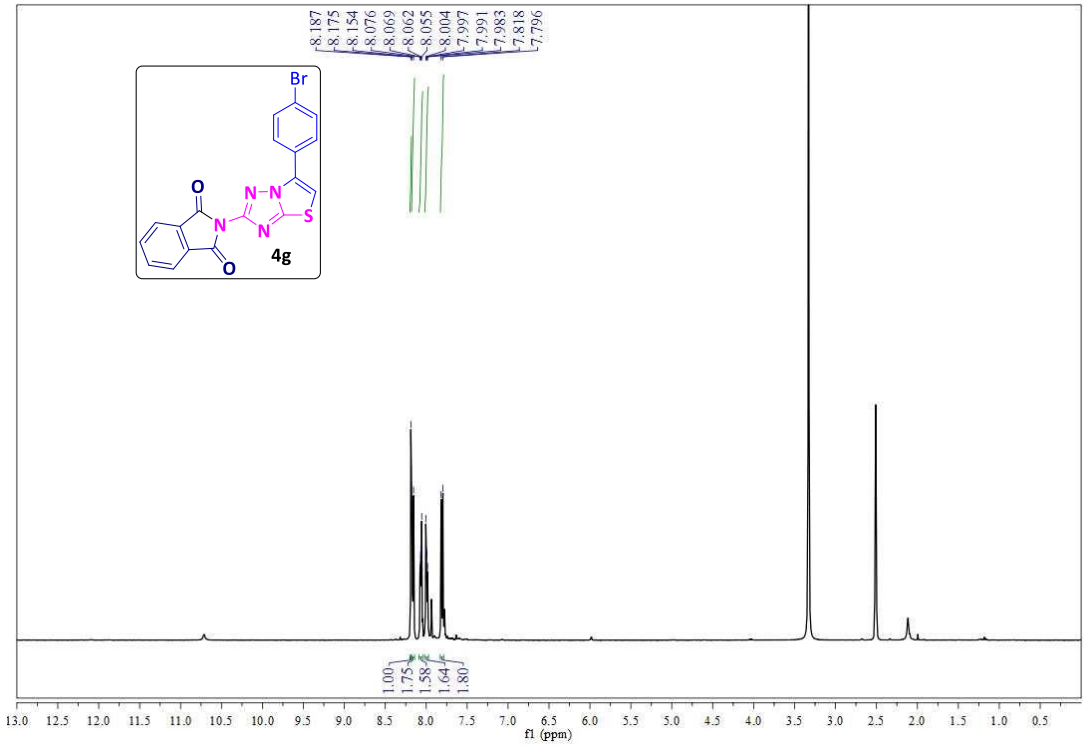
^{13}C NMR spectrum of compound **4f. (DMSO-d_6) 100 MHz**



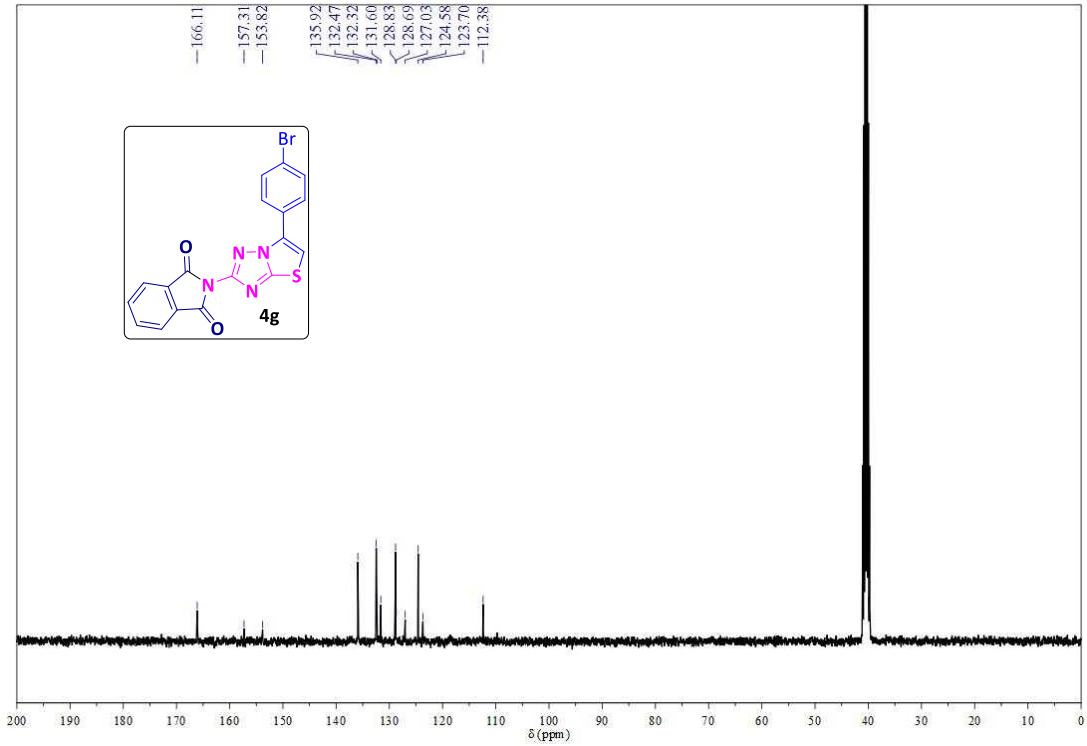
Mass spectrum of compound **4f.**



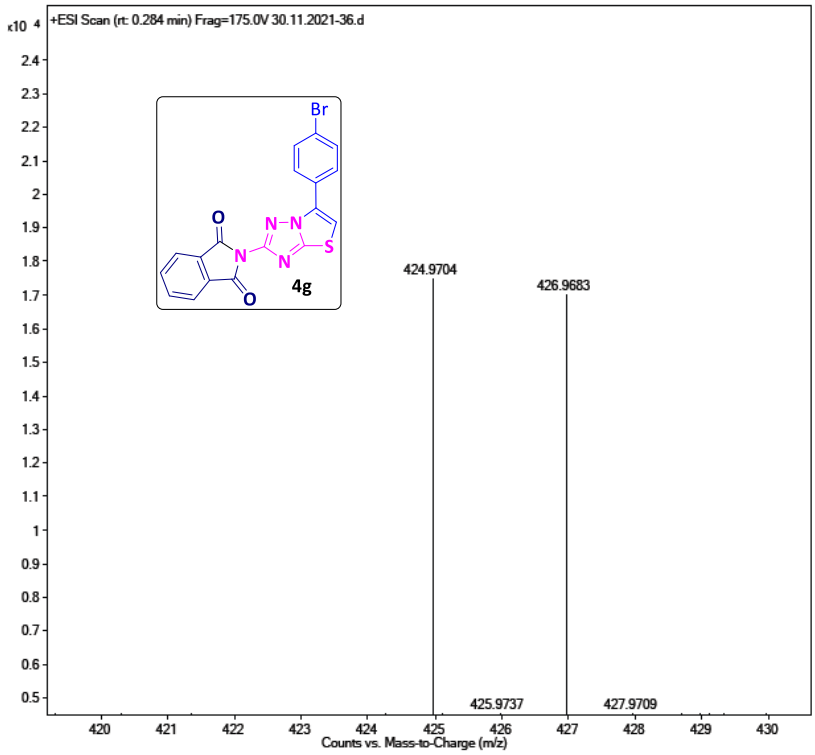
^{1}H NMR spectrum of compound **4g (DMSO-d_6) 400 MHz**



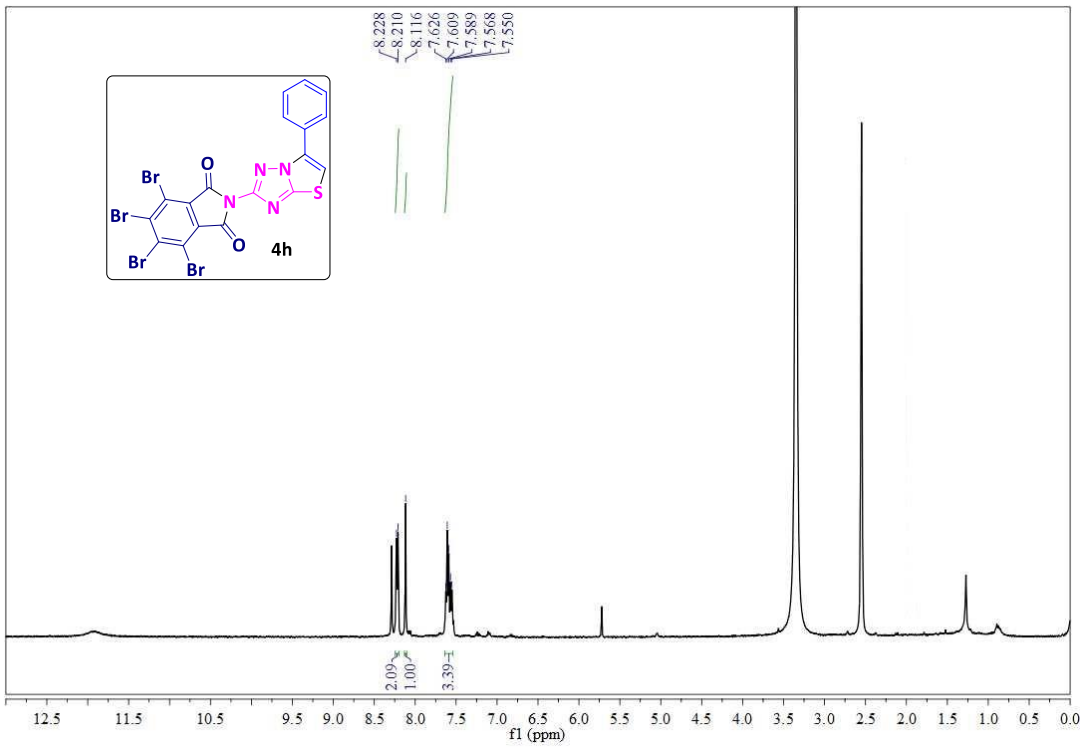
^{13}C NMR spectrum of compound **4g. (DMSO-d_6) 100 MHz**



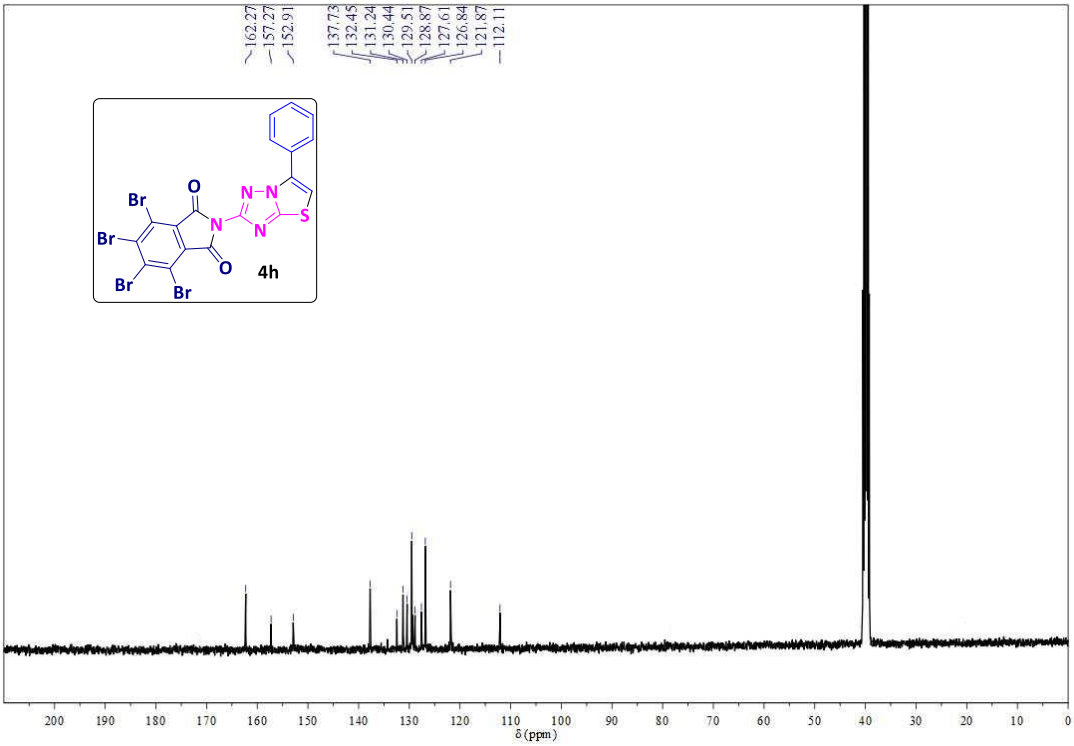
Mass spectrum of compound **4g**



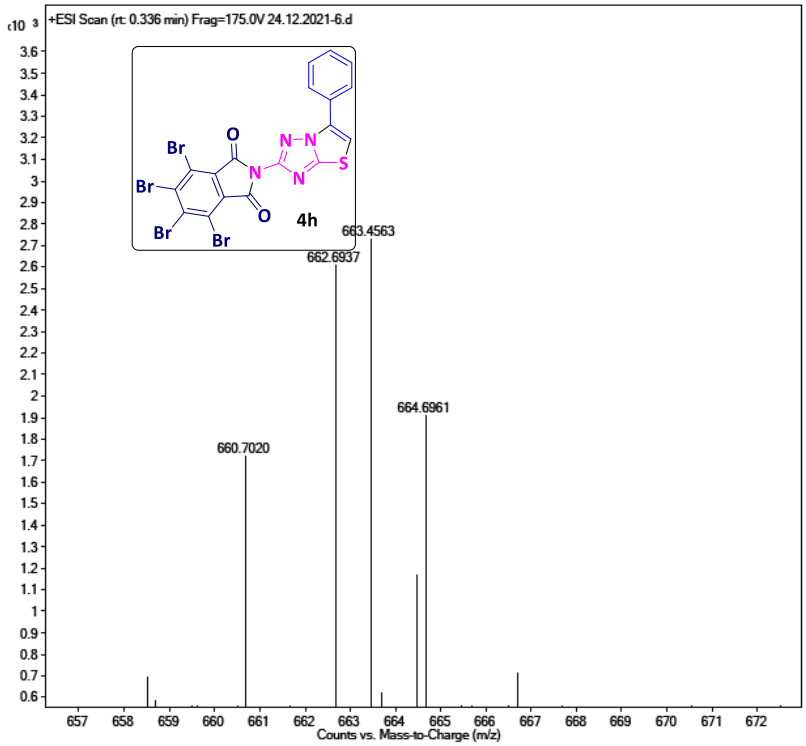
^{1}H NMR spectrum of compound **4h. (DMSO-d_6) 400 MHz**



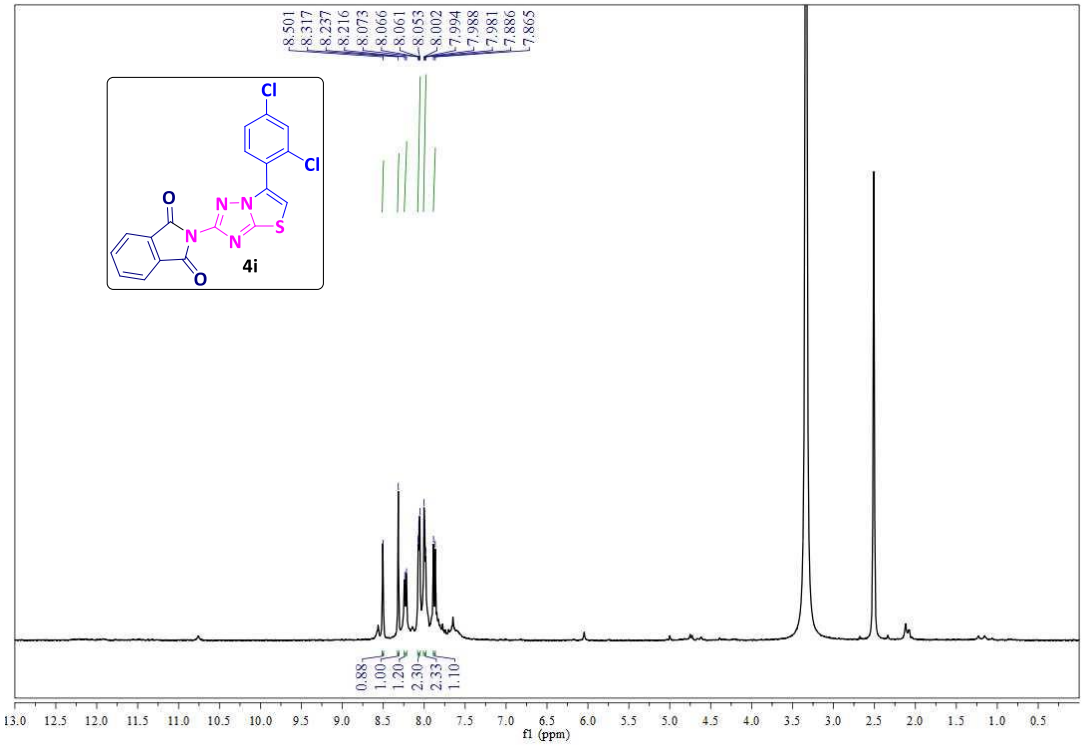
^{13}C NMR spectrum of compound **4h. (DMSO-d_6) 100 MHz**



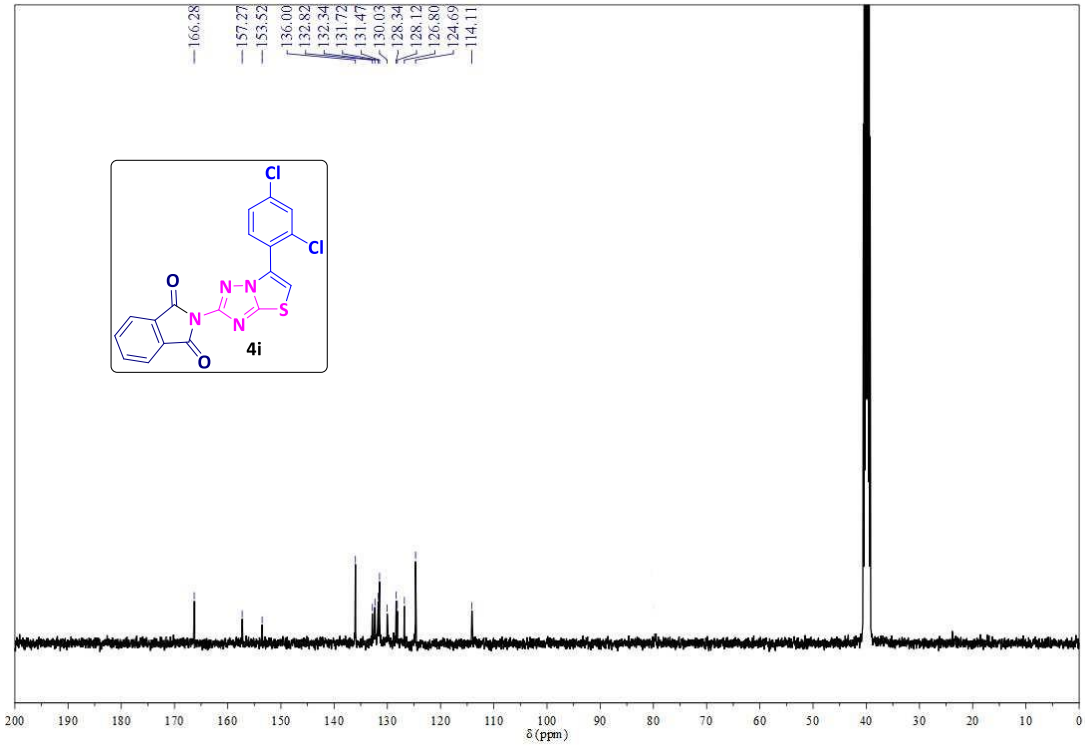
Mass spectrum of compound **4h**



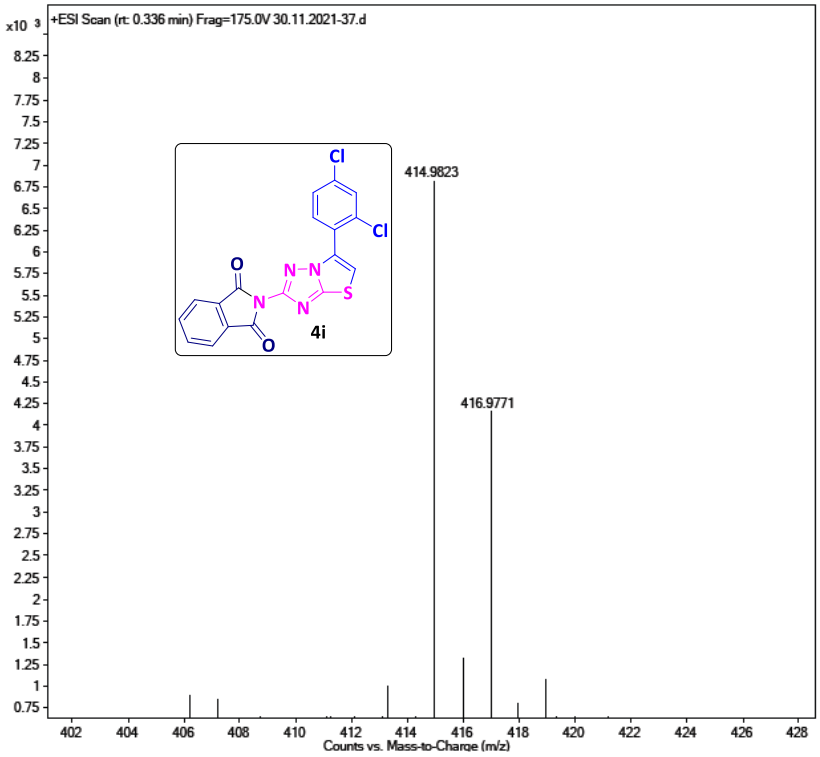
^{1}H NMR spectrum of compound **4i. (DMSO-d_6) 400 MHz**



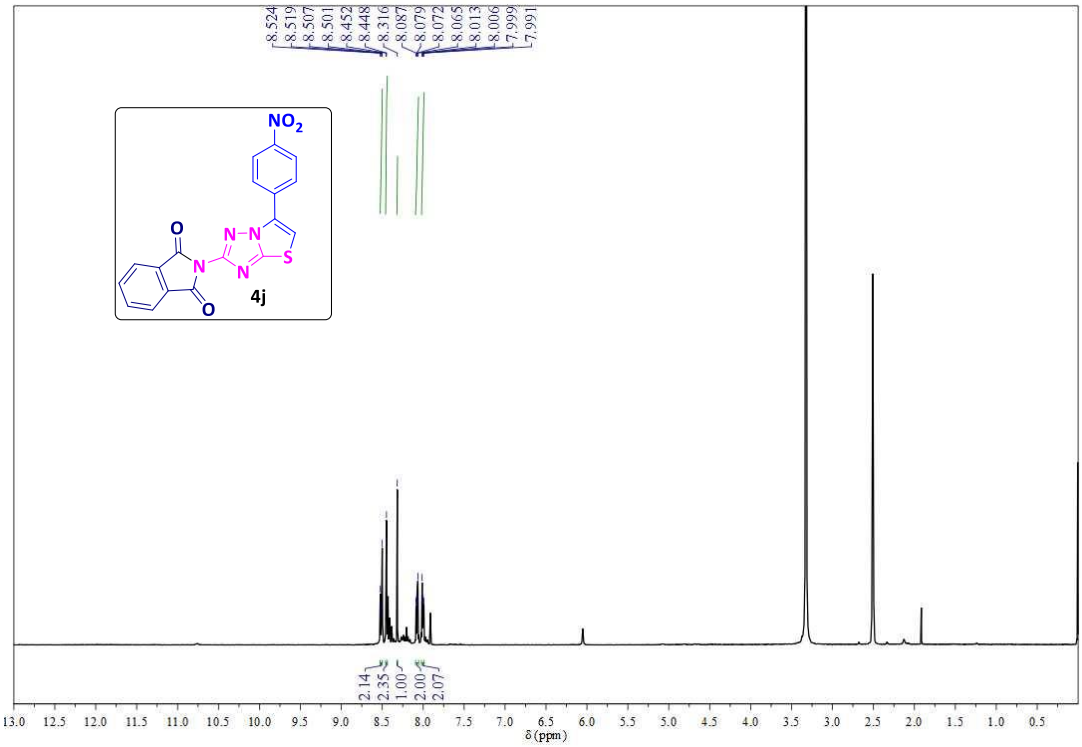
^{13}C NMR spectrum of compound **4i. (DMSO-d_6) 100 MHz**



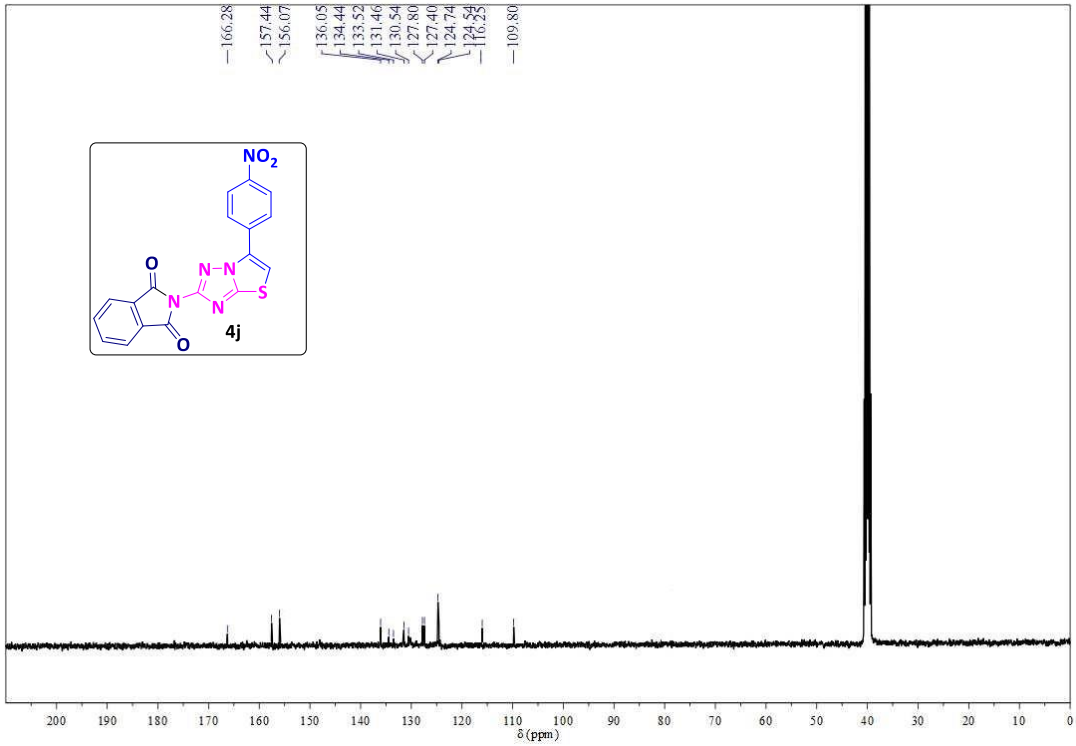
Mass spectrum of compound **4i**



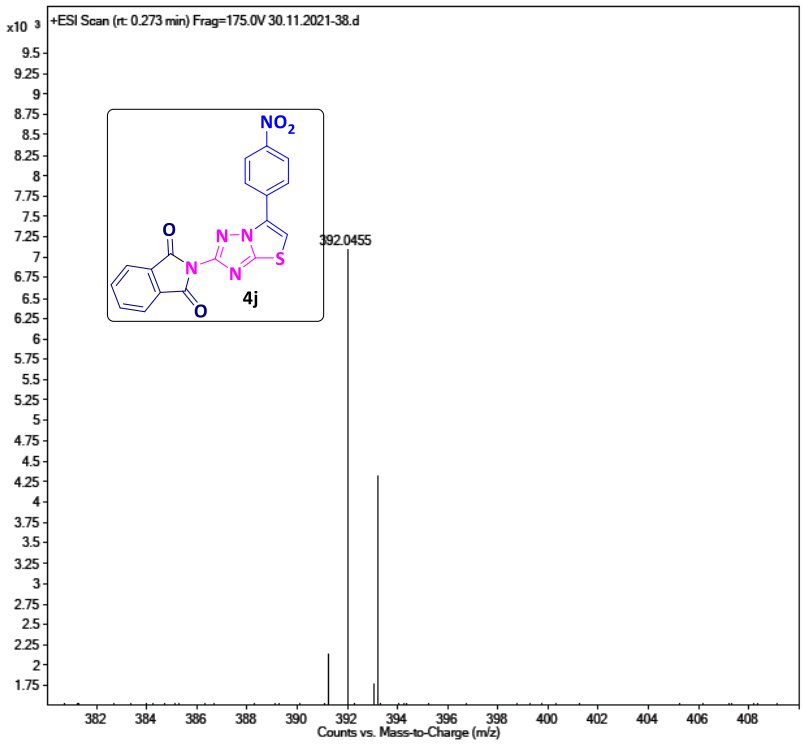
^{1}H NMR spectrum of compound **4j. (DMSO-*d*$_6$) 400 MHz**



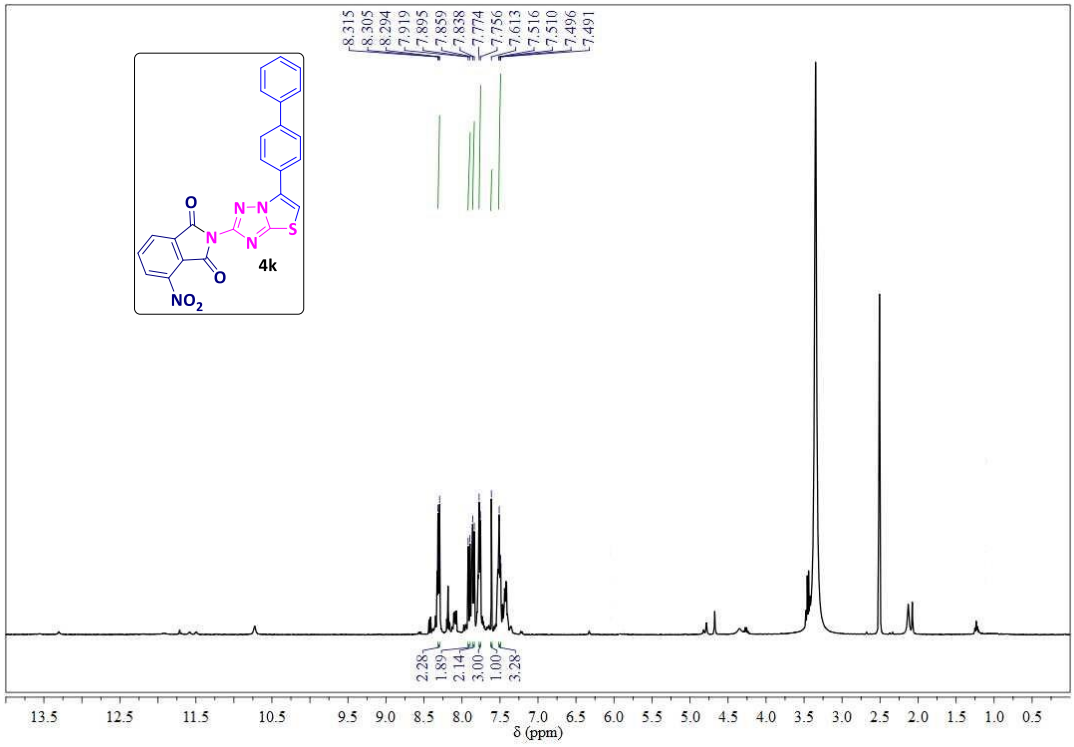
^{13}C NMR spectrum of compound **4j. (DMSO-d_6) 100 MHz**



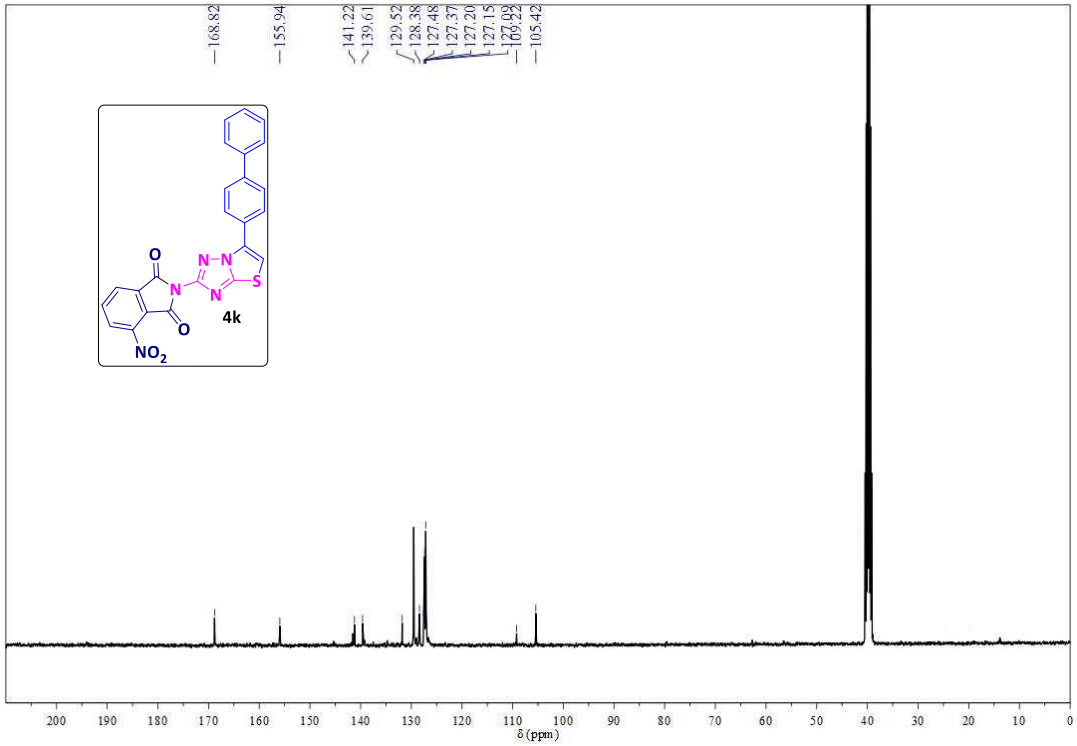
Mass spectrum of compound **4j**



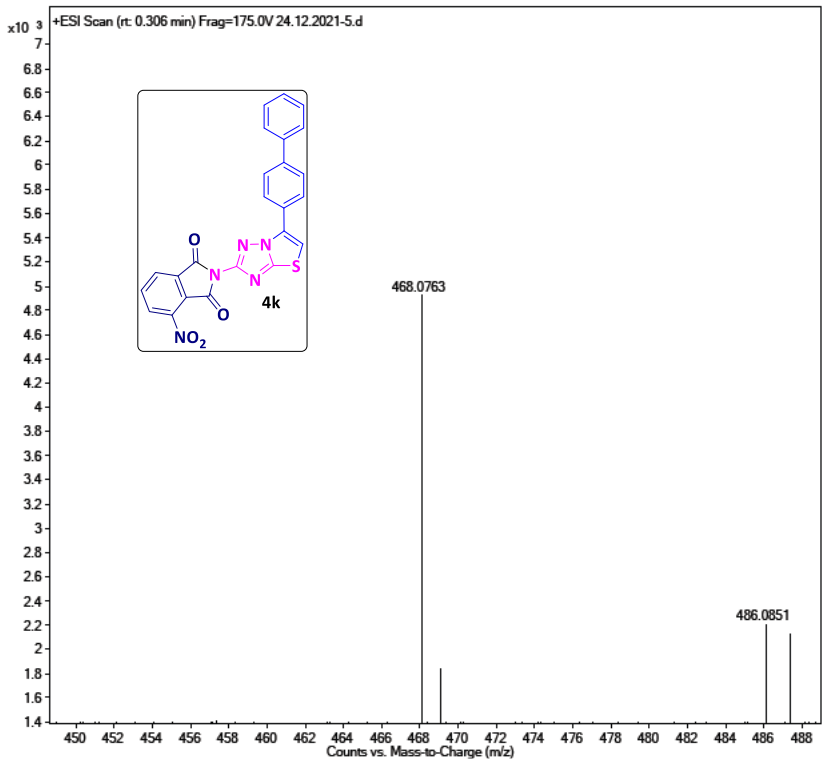
^{1}H NMR spectrum of compound **4k. (DMSO-d_6) 400 MHz**



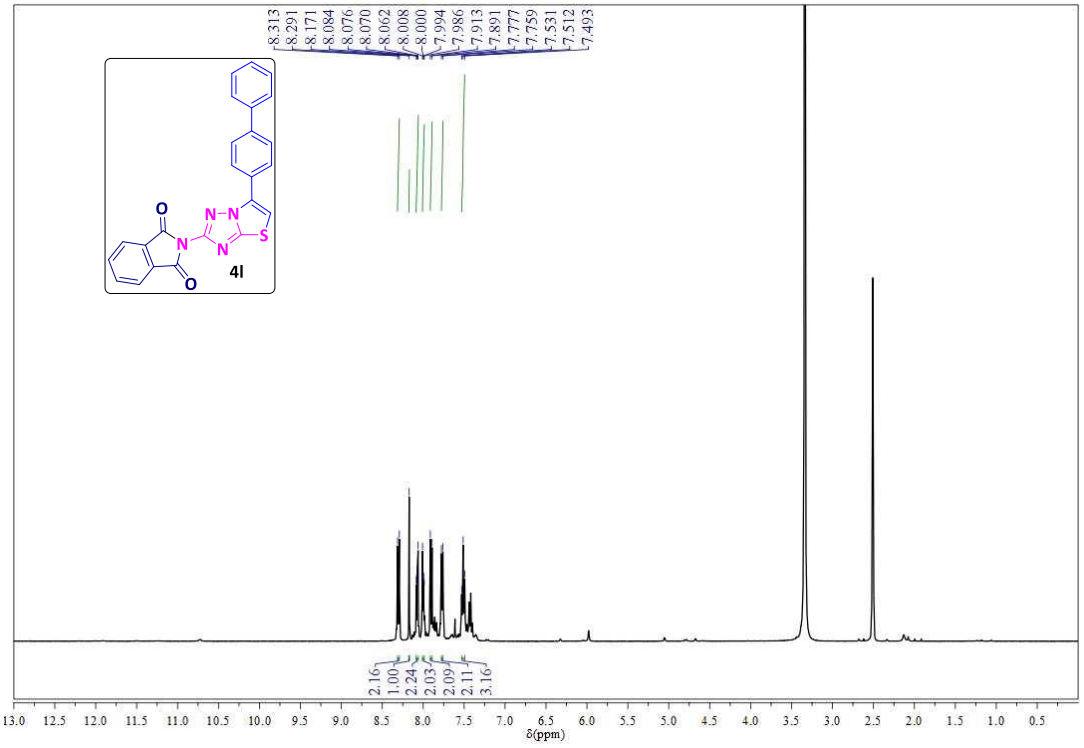
^{13}C NMR spectrum of compound **4k. (DMSO-d_6) 100 MHz**



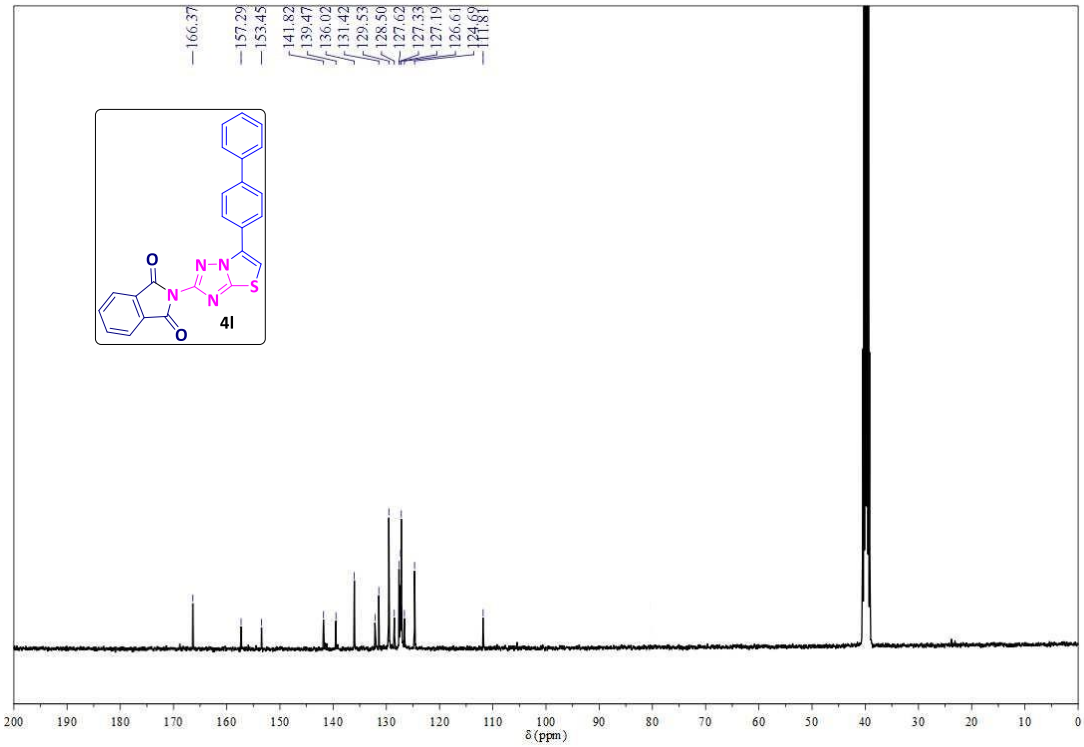
Mass spectrum of compound **4k**



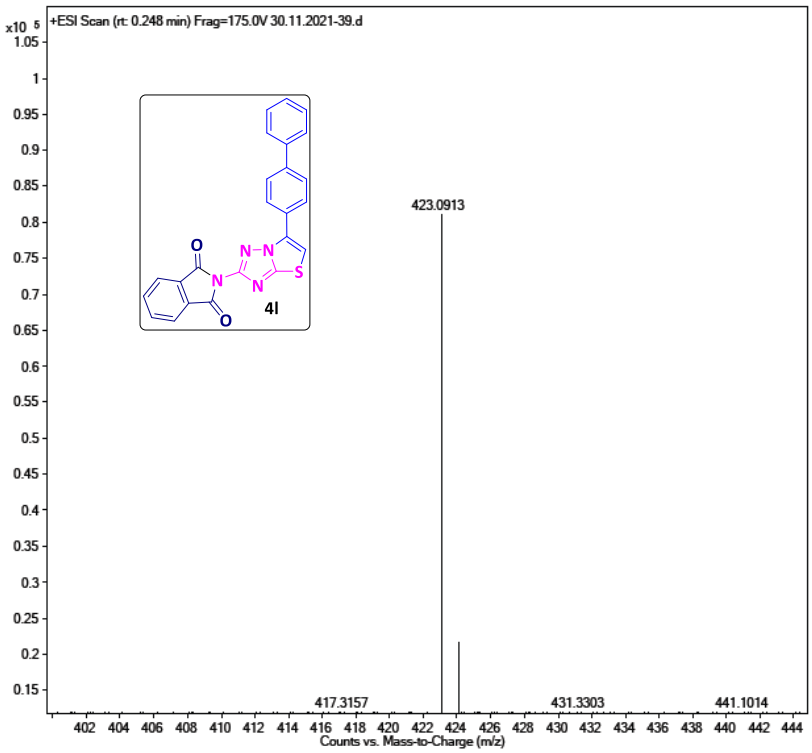
^{1}H NMR spectrum of compound **4l. (DMSO-*d*$_6$) 400 MHz**



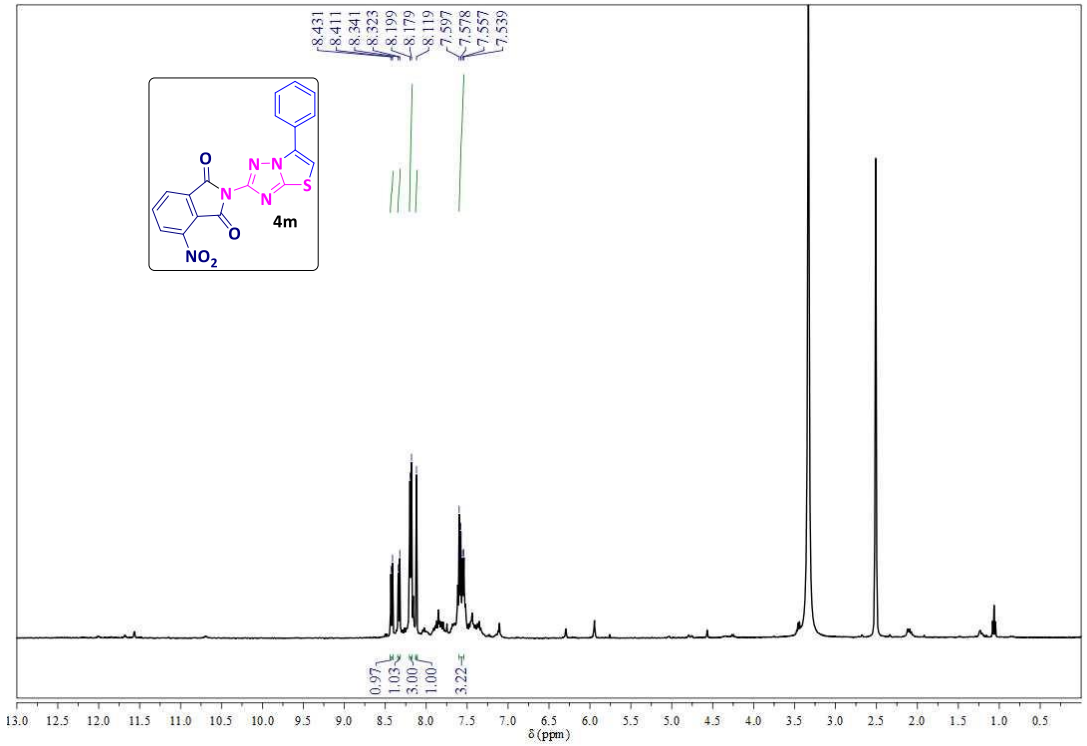
^{13}C NMR spectrum of compound **4l. (DMSO-d_6) 100 MHz**



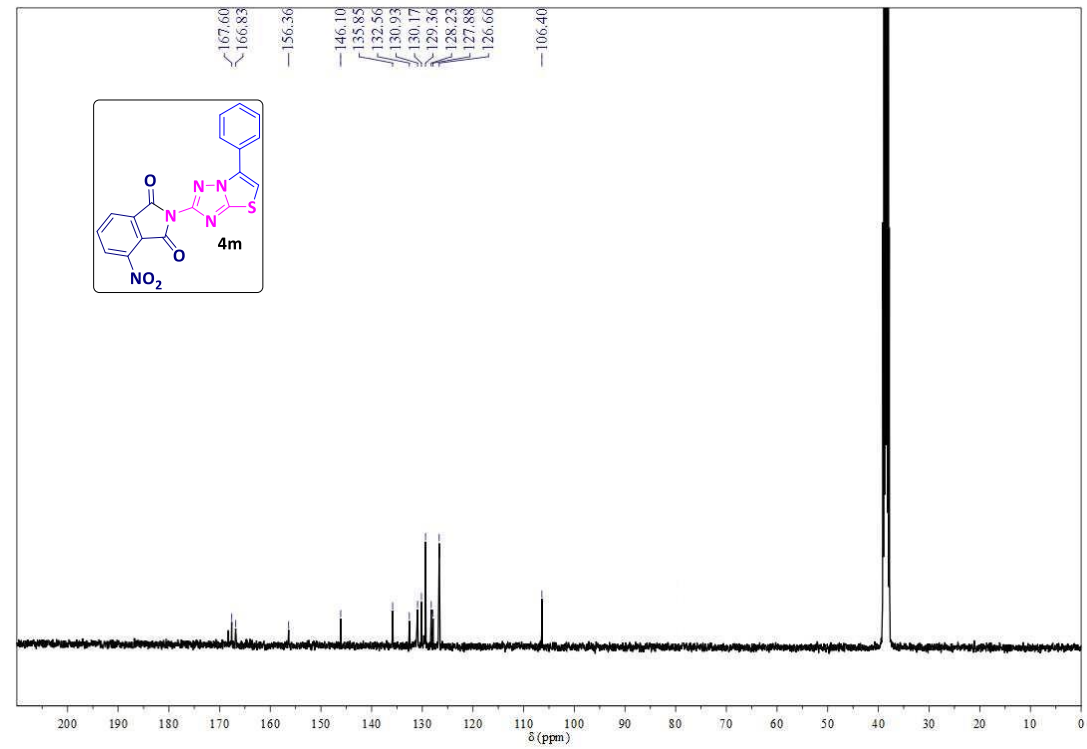
Mass spectrum of compound **4l**



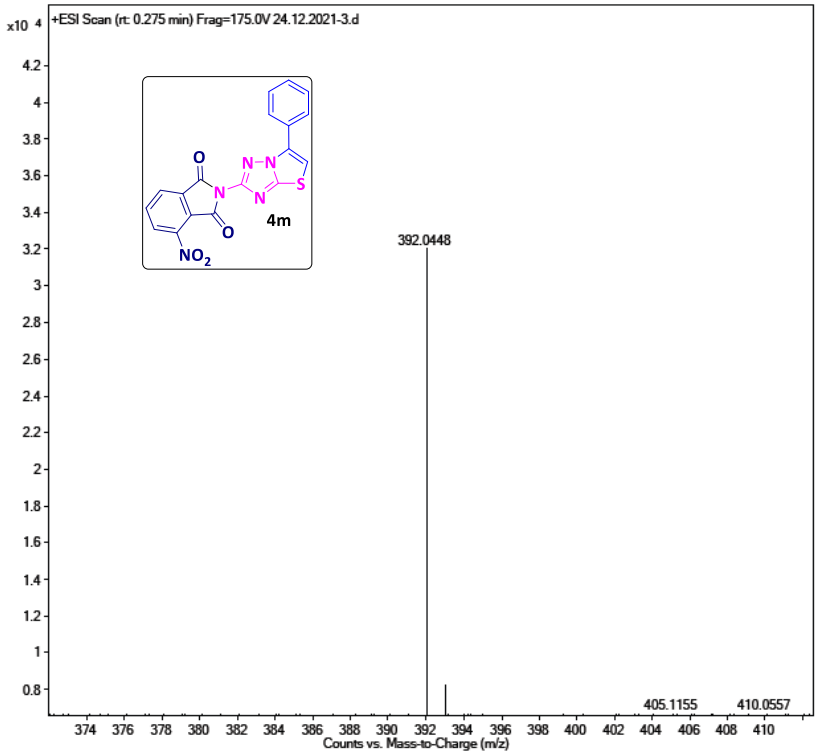
^{1}H NMR spectrum of compound **4m. (DMSO-d_6) 400 MHz**



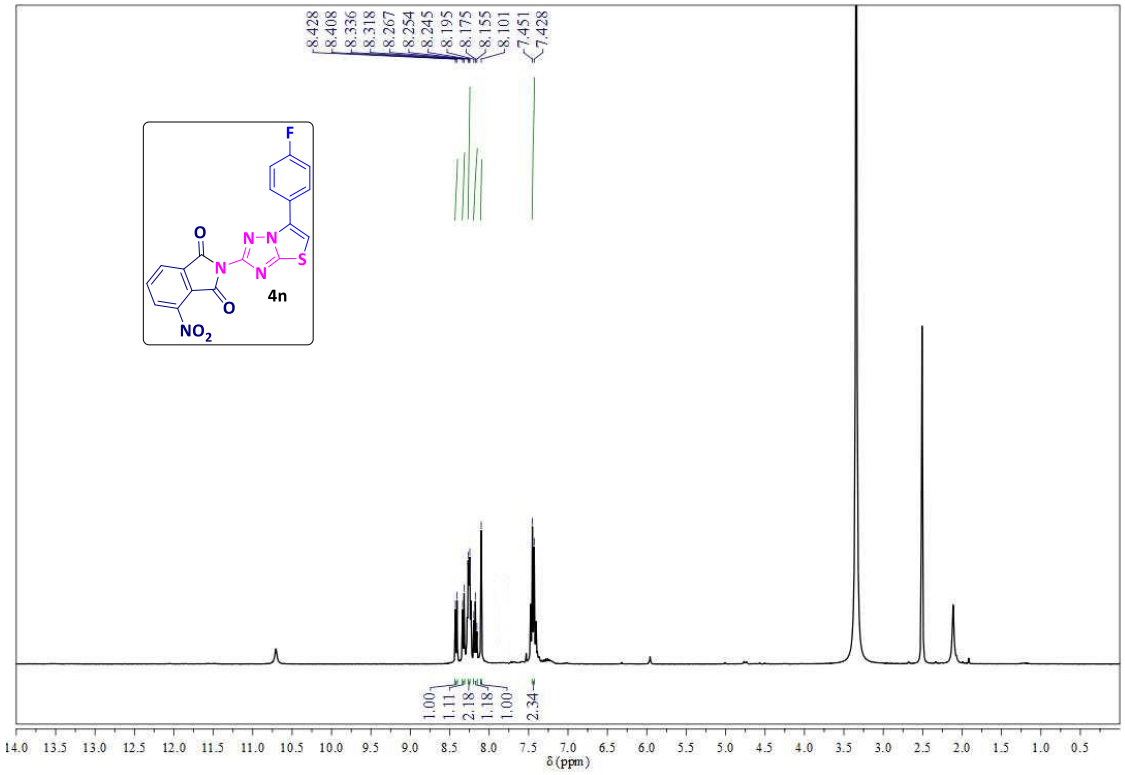
^{13}C NMR spectrum of compound **4m. (DMSO-d_6) 100 MHz**



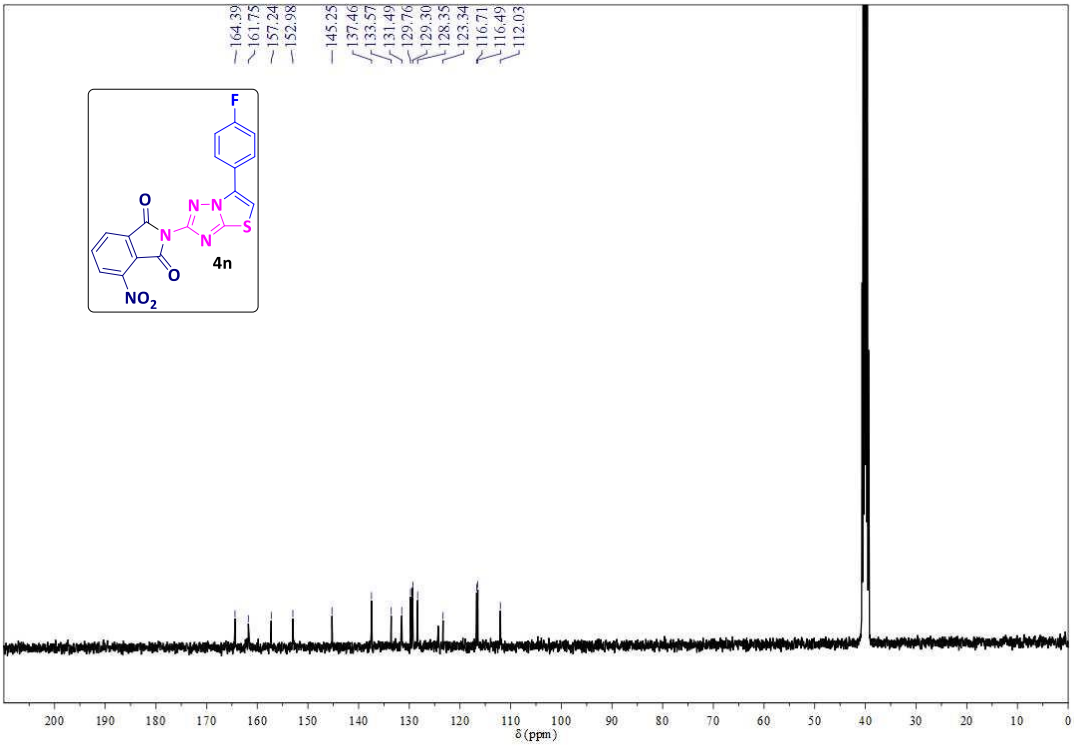
Mass spectrum of compound **4m**



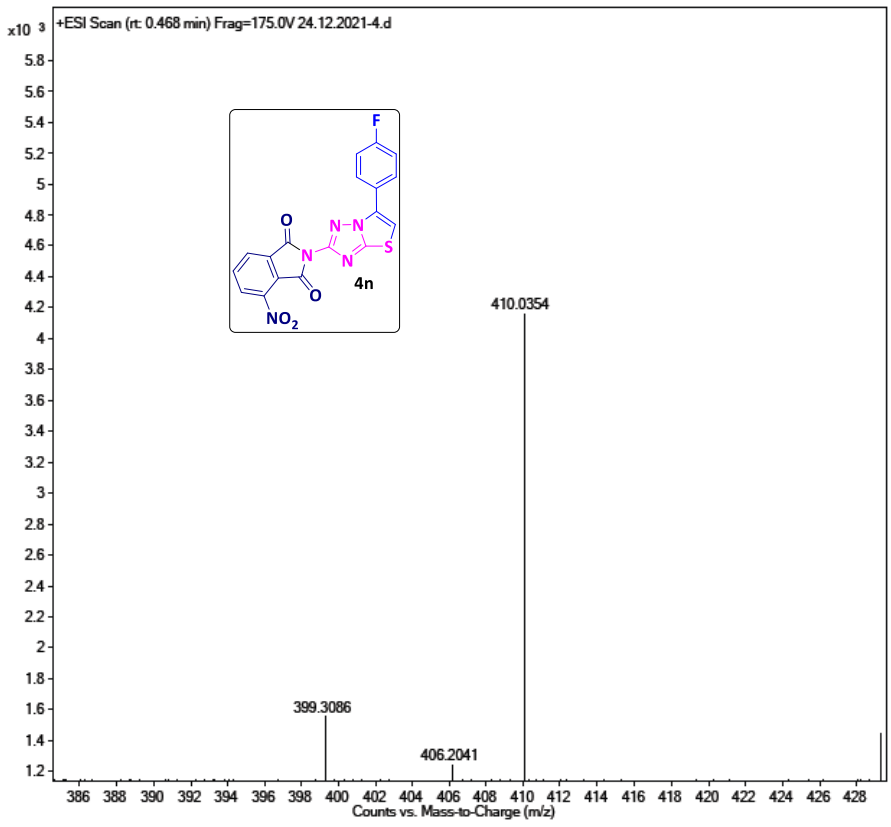
^{1}H NMR spectrum of compound **4n. (DMSO-d_6) 400 MHz**



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

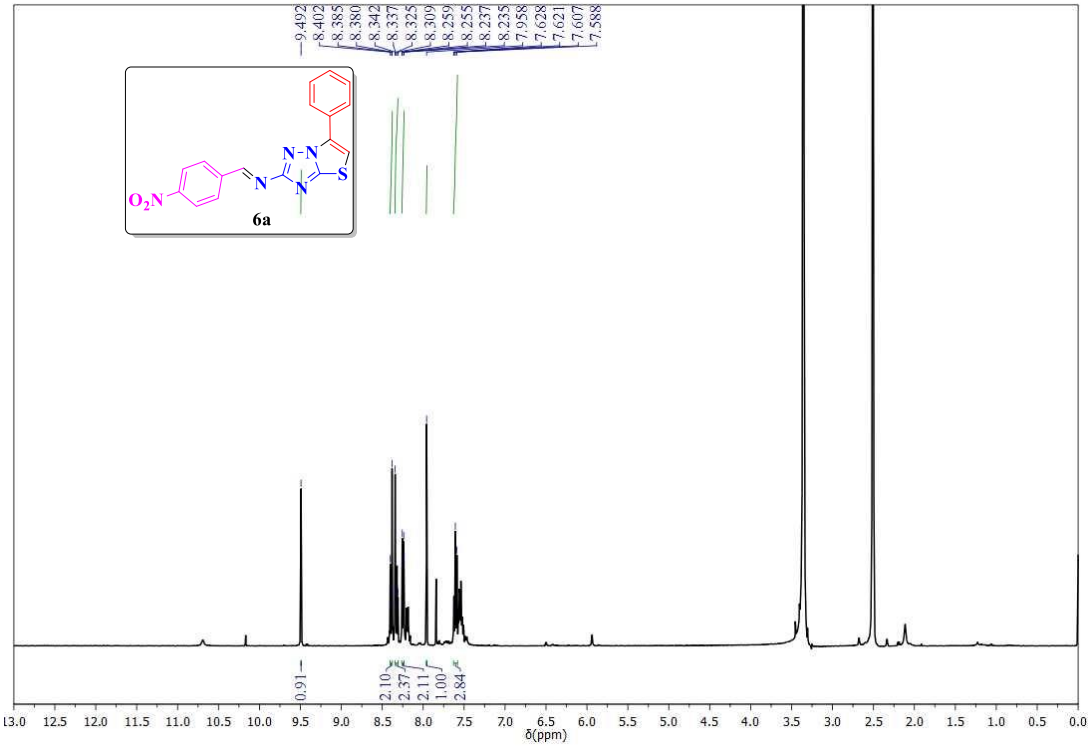


Mass spectrum of compound **4n**

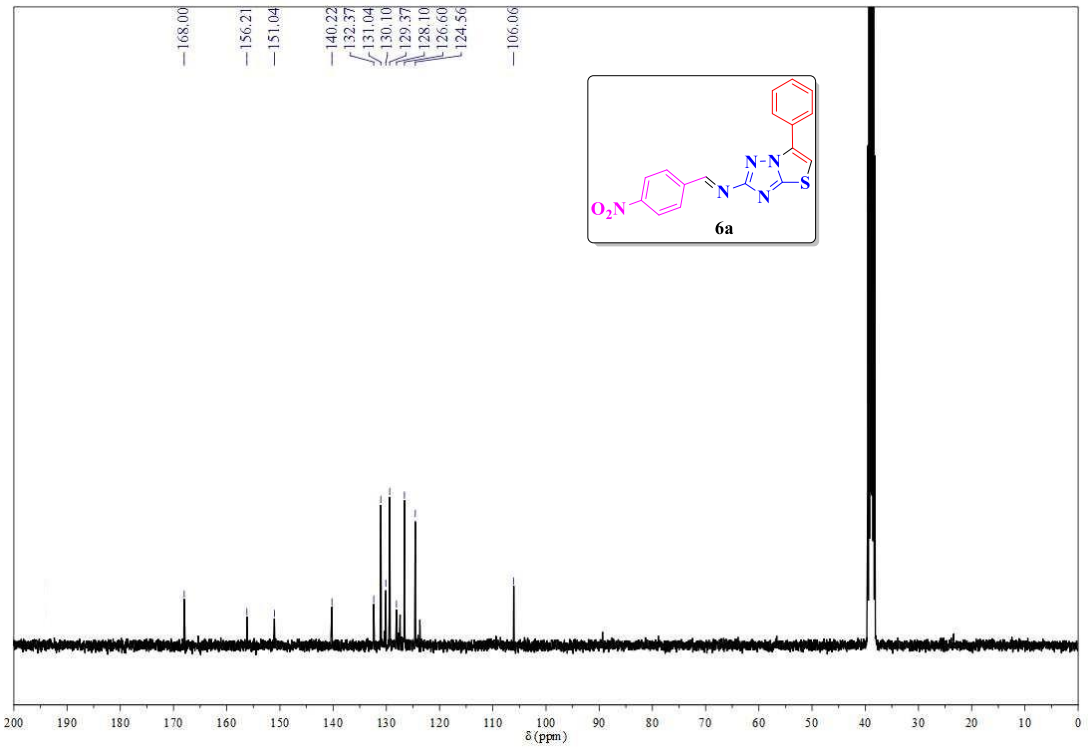


Scheme-2

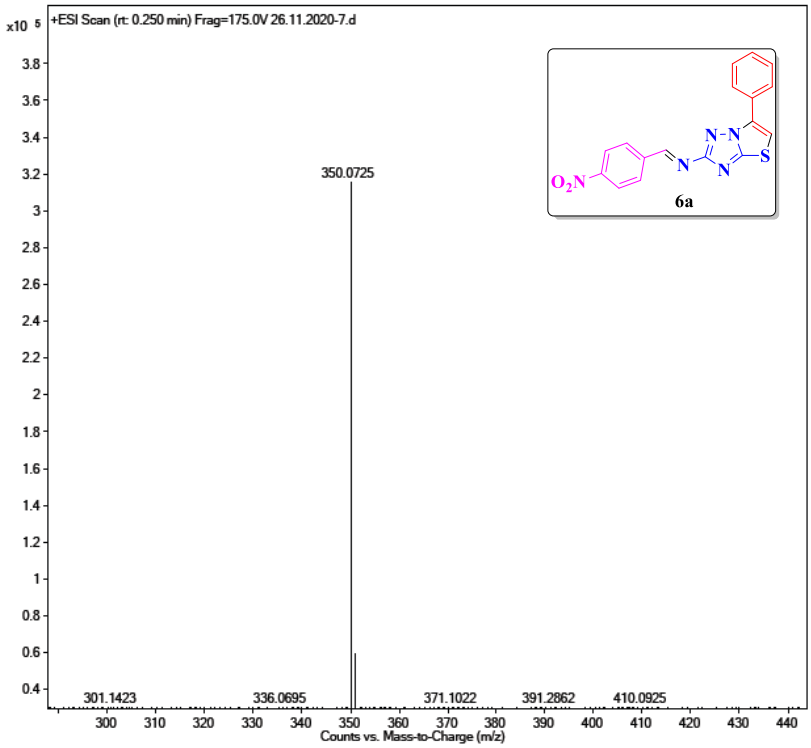
^{1}H NMR spectrum of compound 6a. (DMSO-d_6) 400 MHz



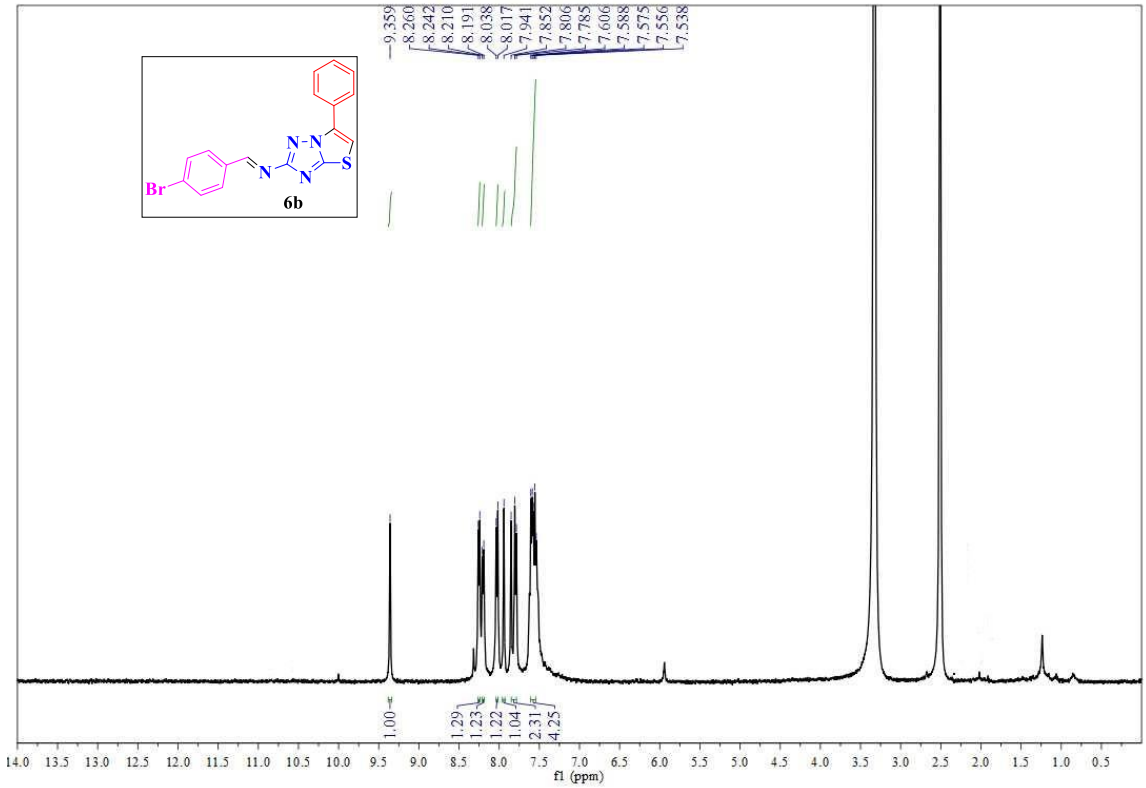
^{13}C NMR spectrum off compound **6a. (DMSO-d_6) 100 MHz**



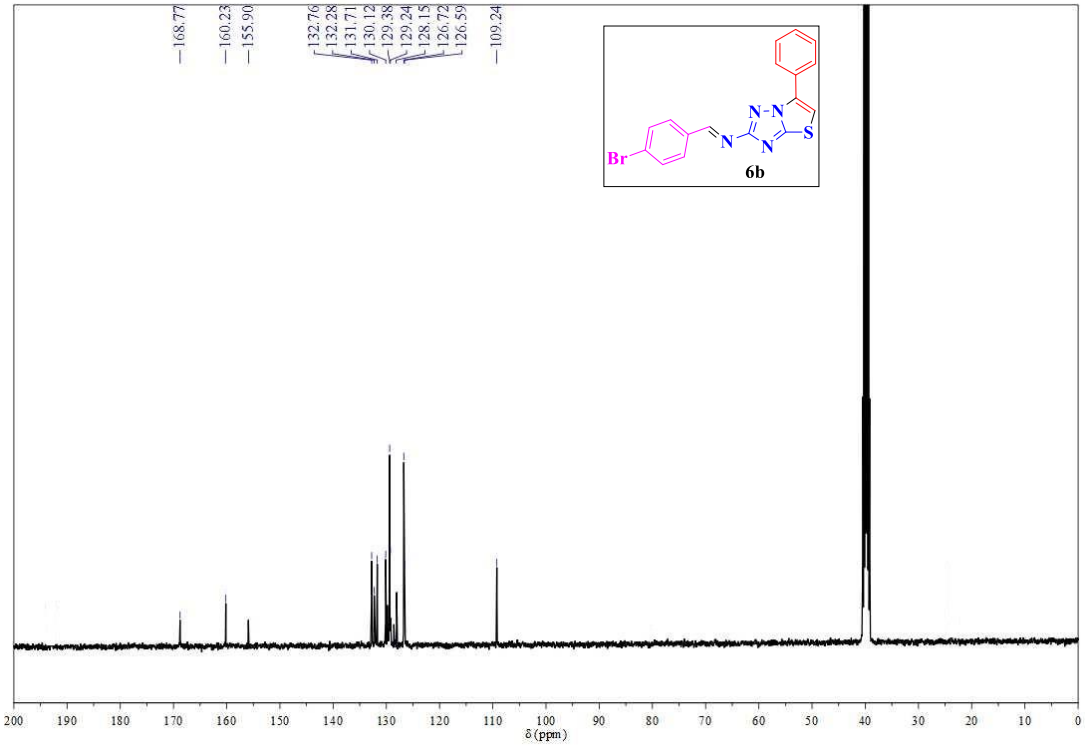
Mass spectrum of compound **6a**



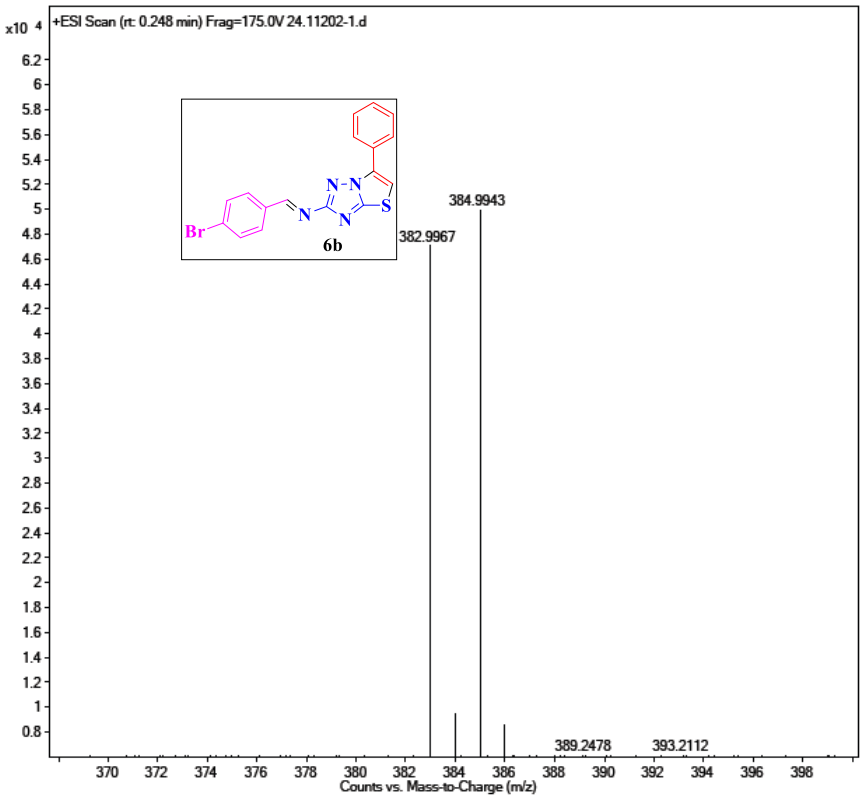
^{1}H NMR spectrum of compound **6b. (DMSO-d_6) 400 MHz**



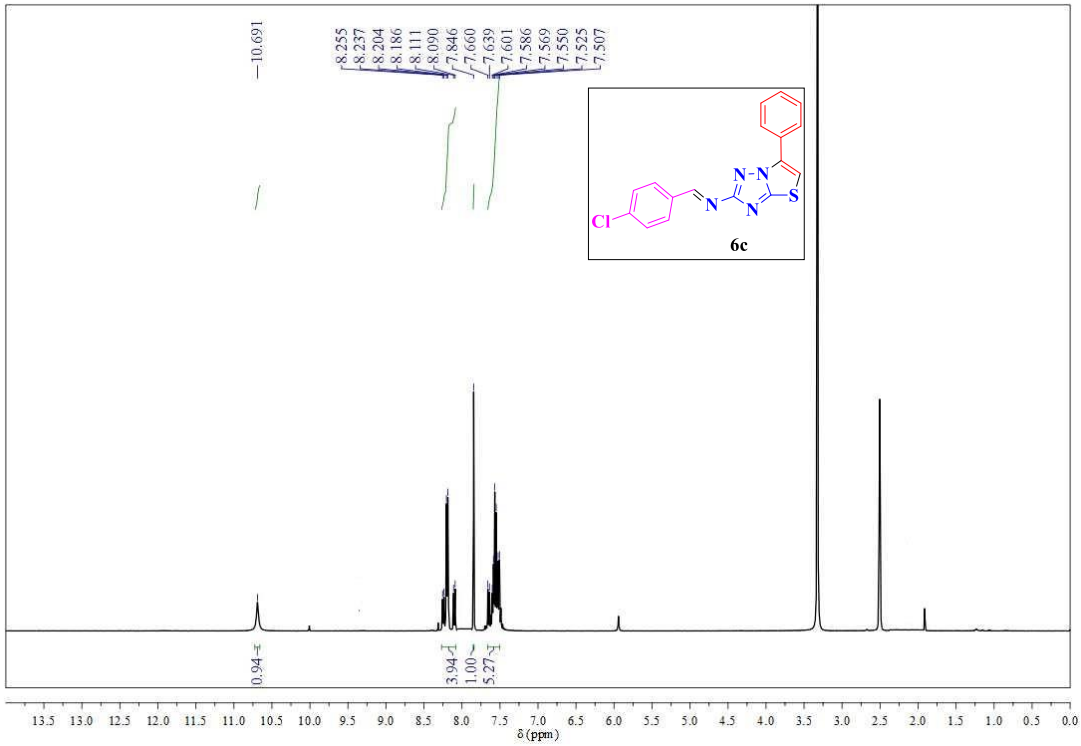
^{13}C NMR spectrum of compound **6b. (DMSO-d_6) 100 MHz**



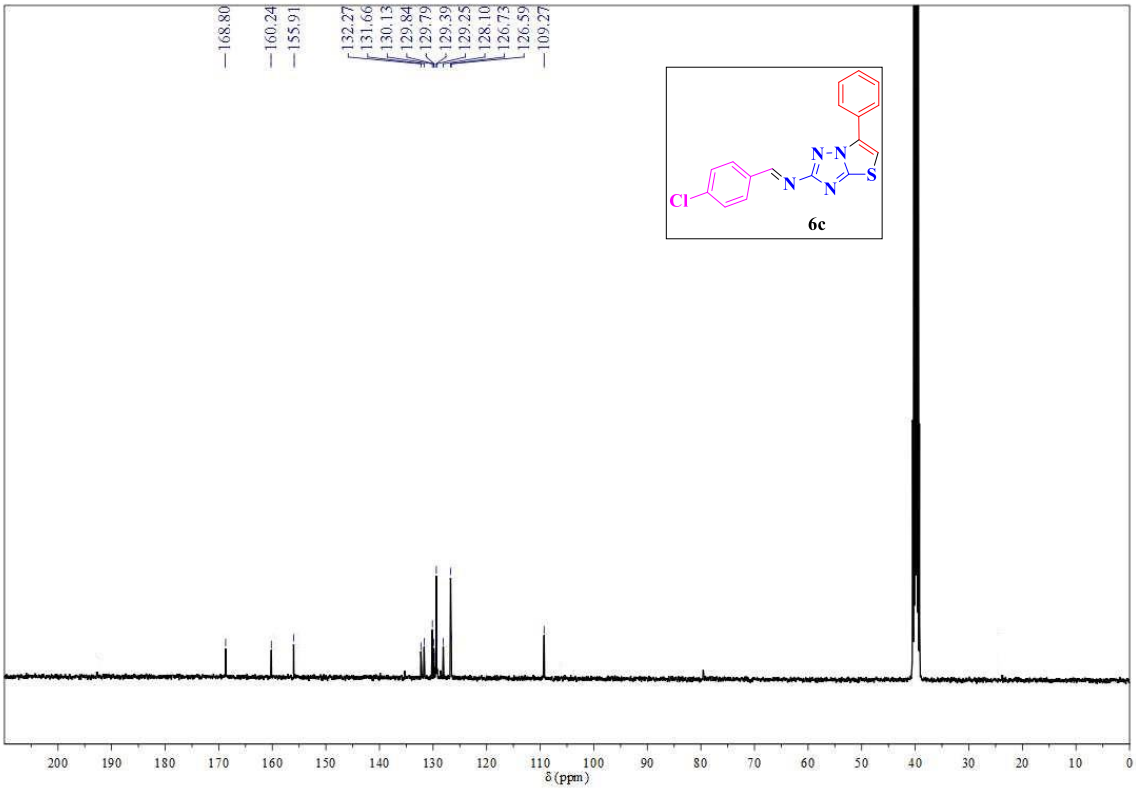
Mass spectrum of compound **6b**



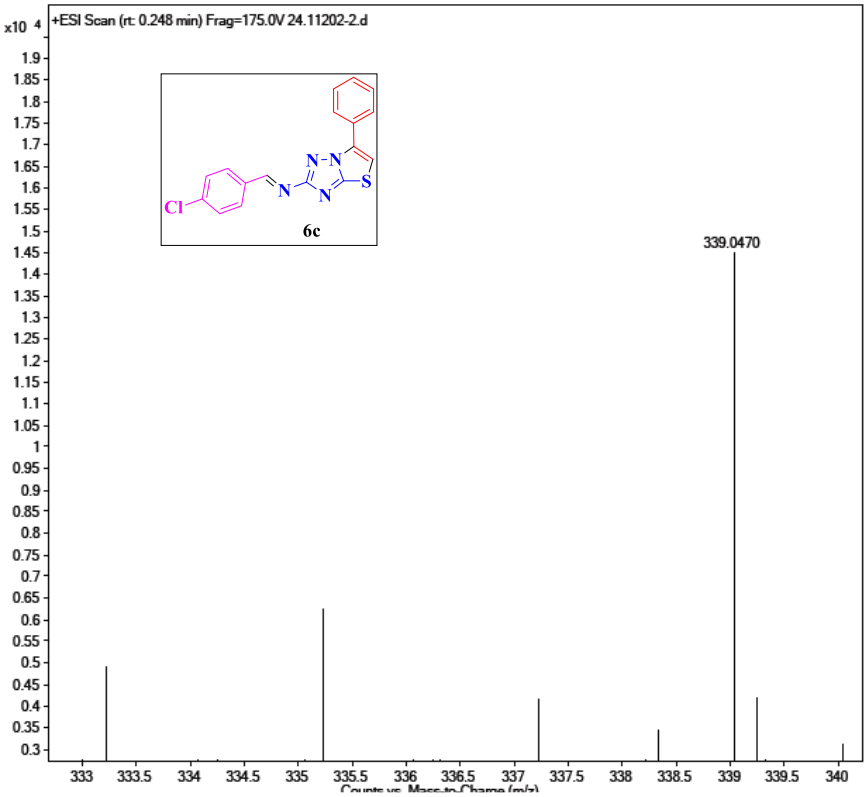
^{1}H NMR spectrum of compound **6c. (DMSO-d_6) 400 MHz**



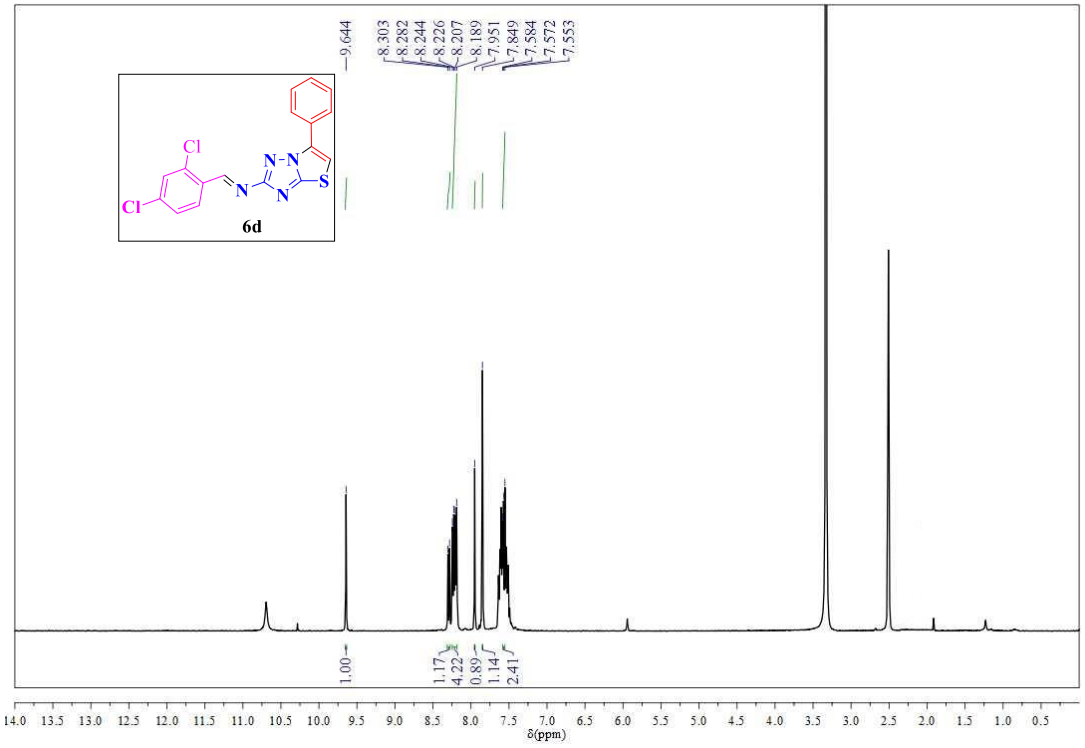
^{13}C NMR spectrum of compound **6c. (DMSO-d_6) 100 MHz**



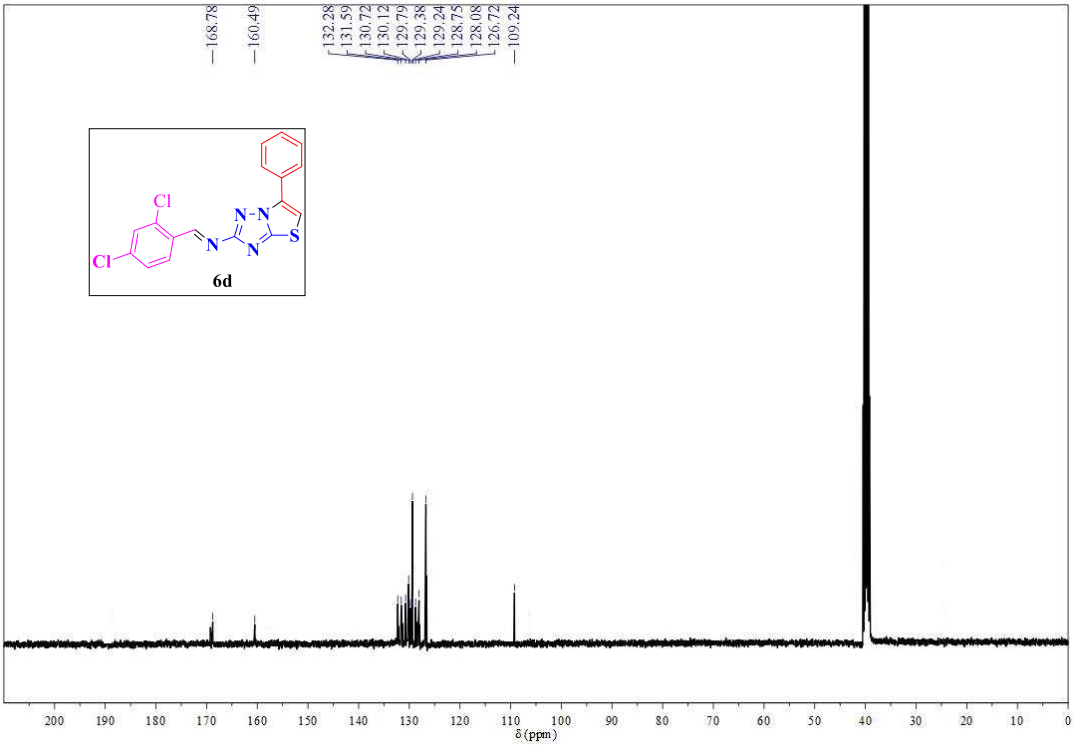
Mass spectrum of compound **6c**



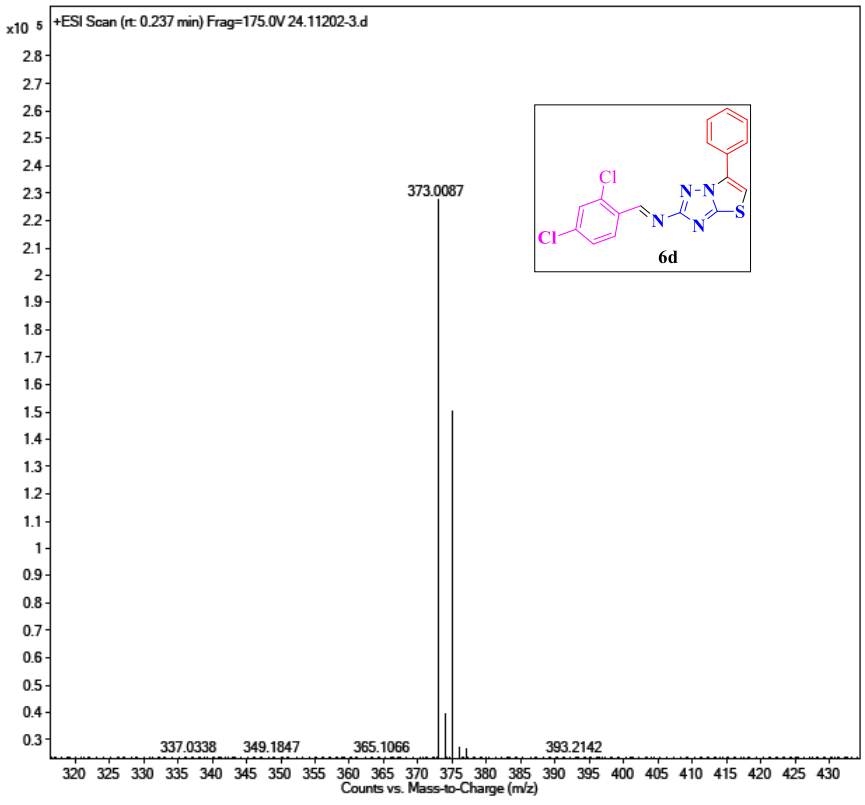
^{1}H NMR spectrum of compound **6d. (DMSO-d_6) 400 MHz**



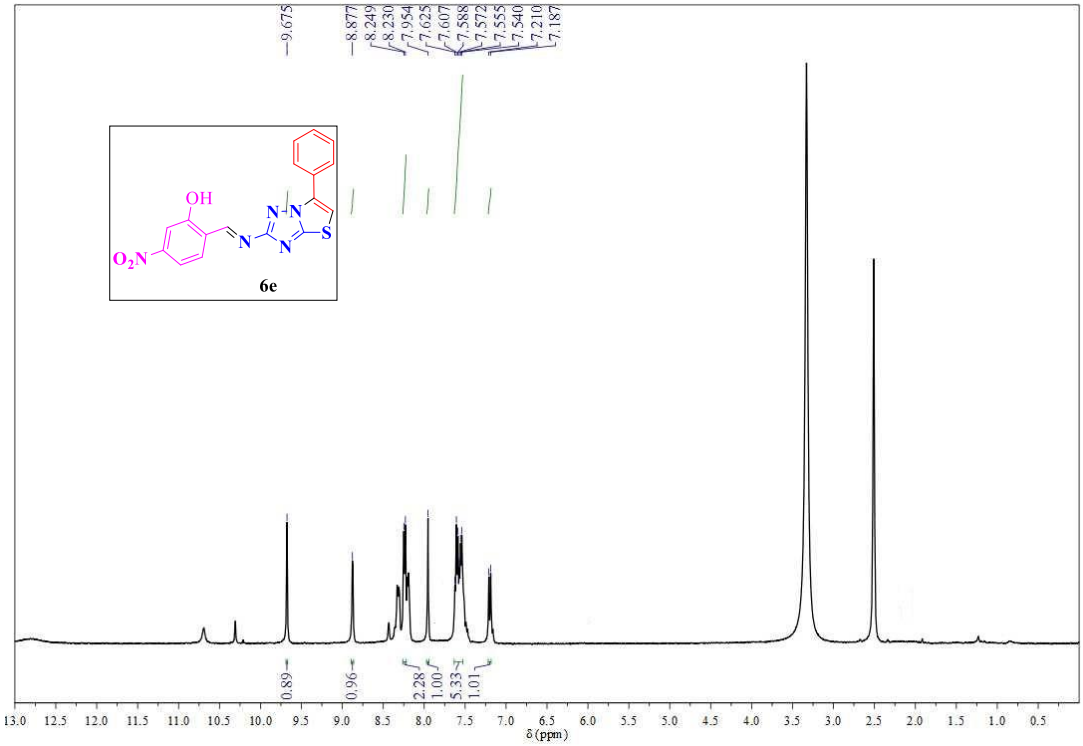
^{13}C NMR spectrum of compound **6d. (DMSO-d_6) 100 MHz**



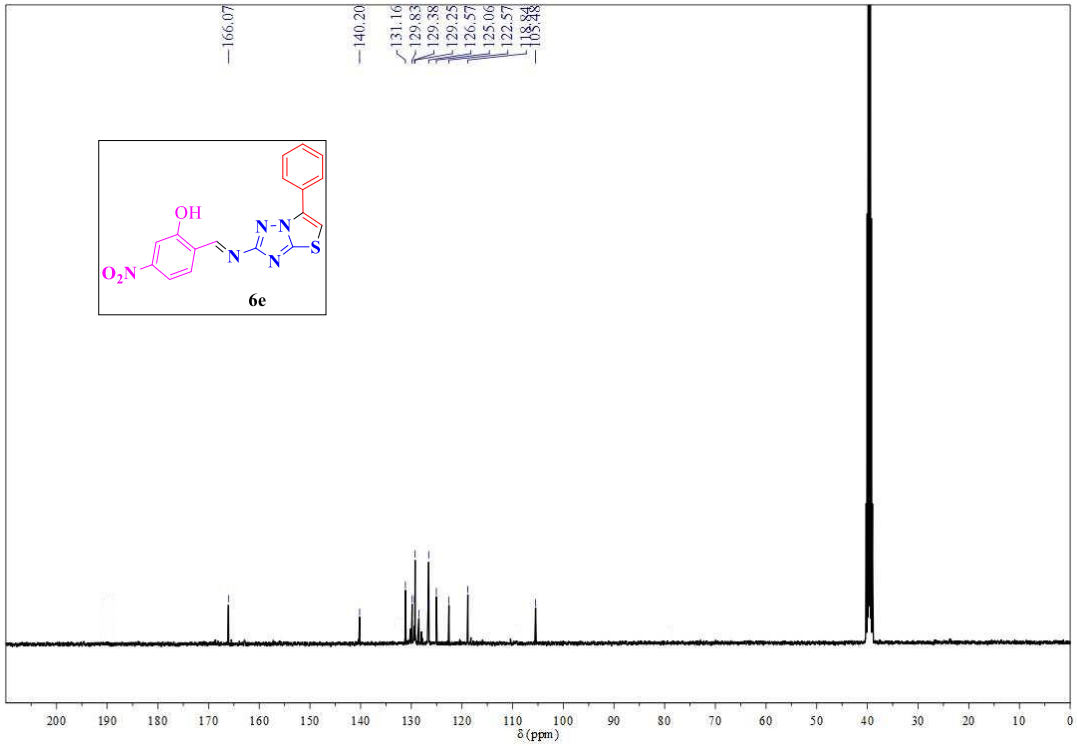
Mass spectrum of compound **6d**



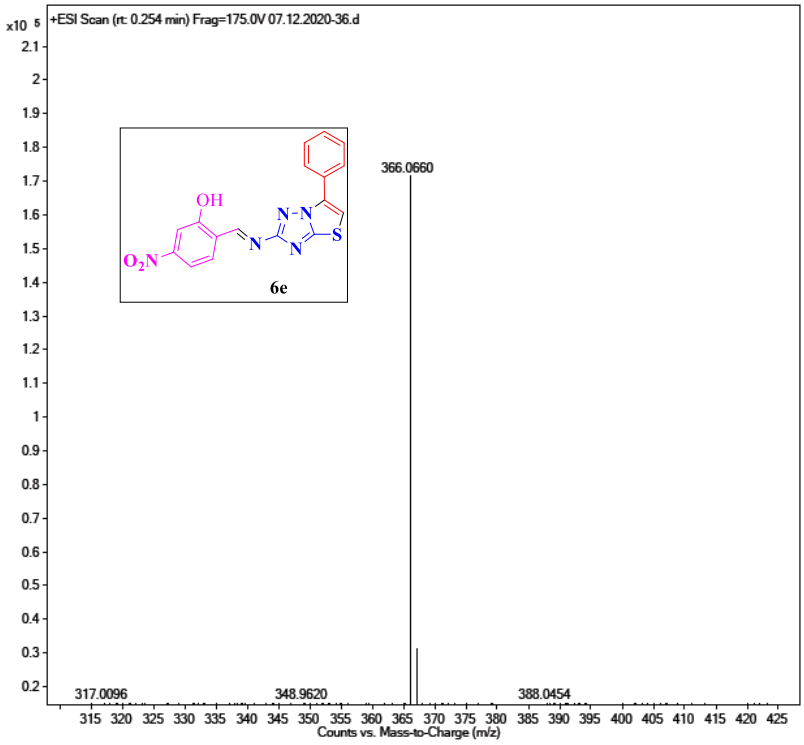
^{1}H NMR spectrum of compound **6e. (DMSO-d_6) 400 MHz**



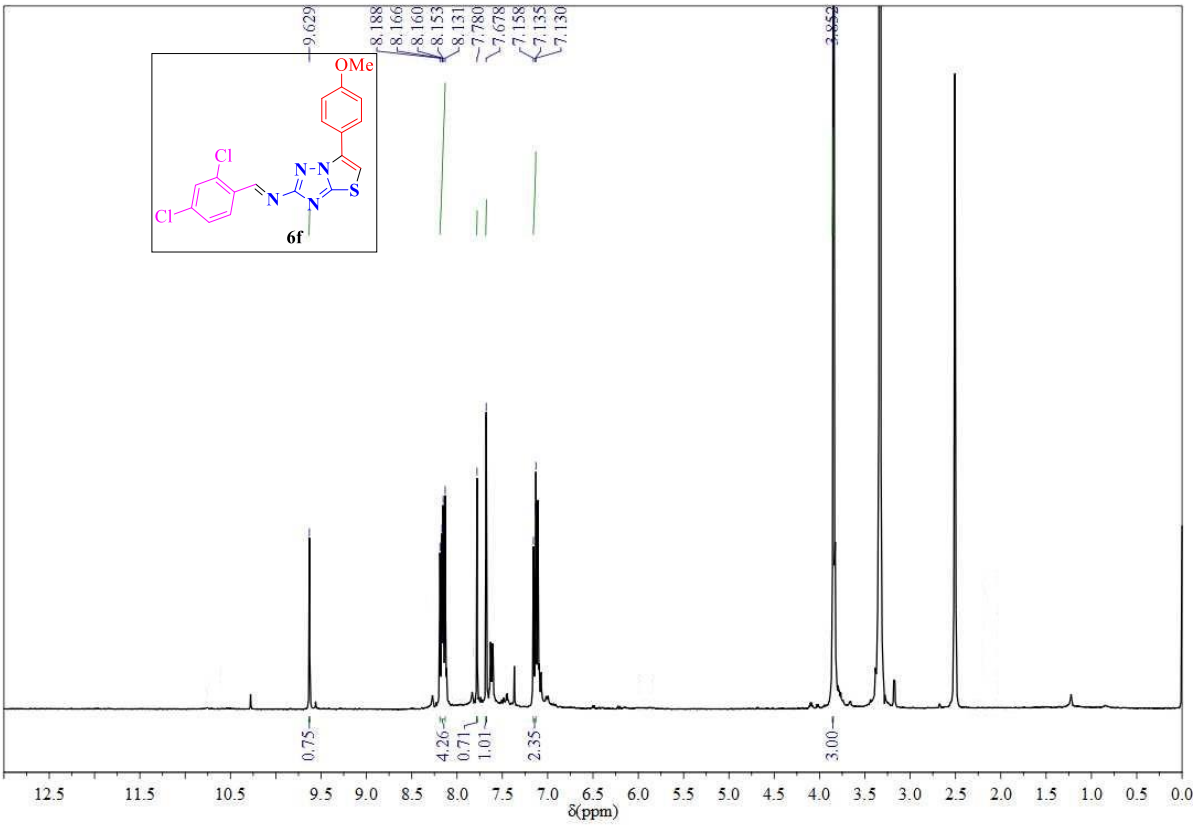
^{13}C NMR spectrum of compound **6e. (DMSO-d_6) 100 MHz**



Mass spectrum of compound **6e**



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



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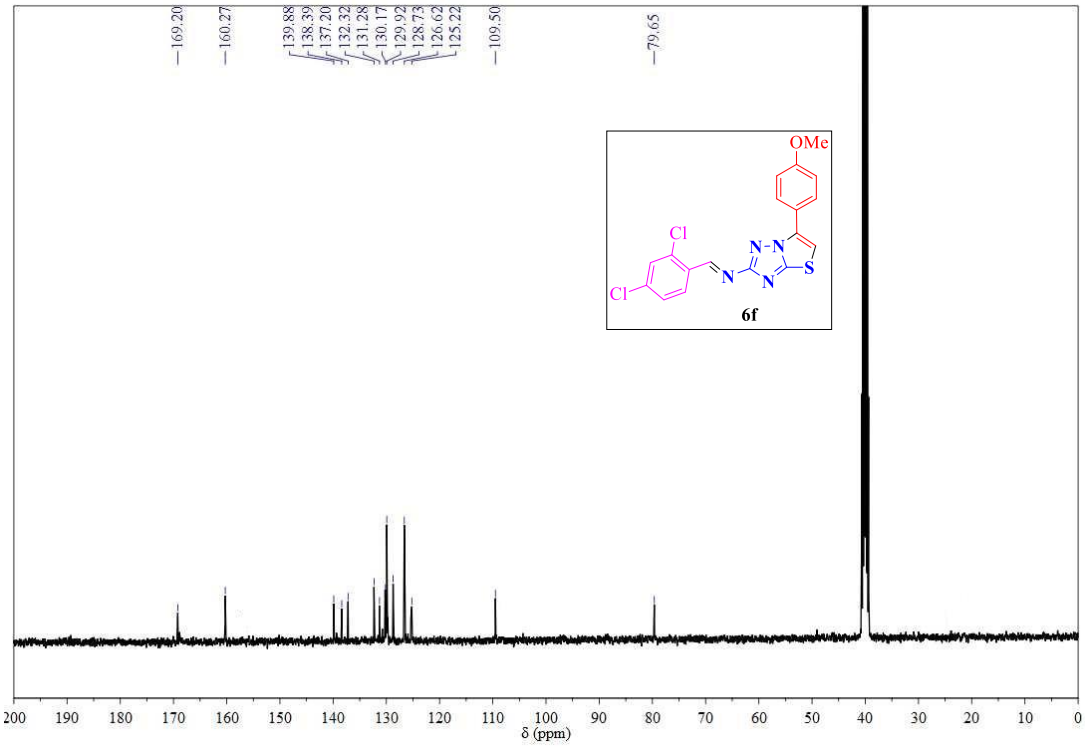
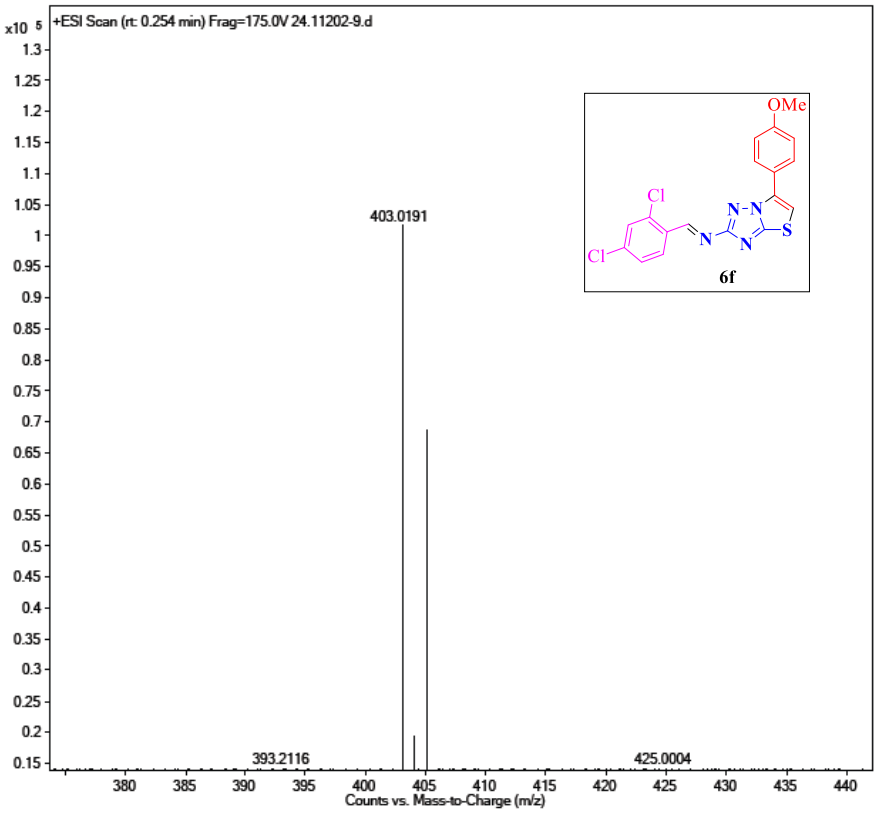


Mass spectrum of compound **6f.**



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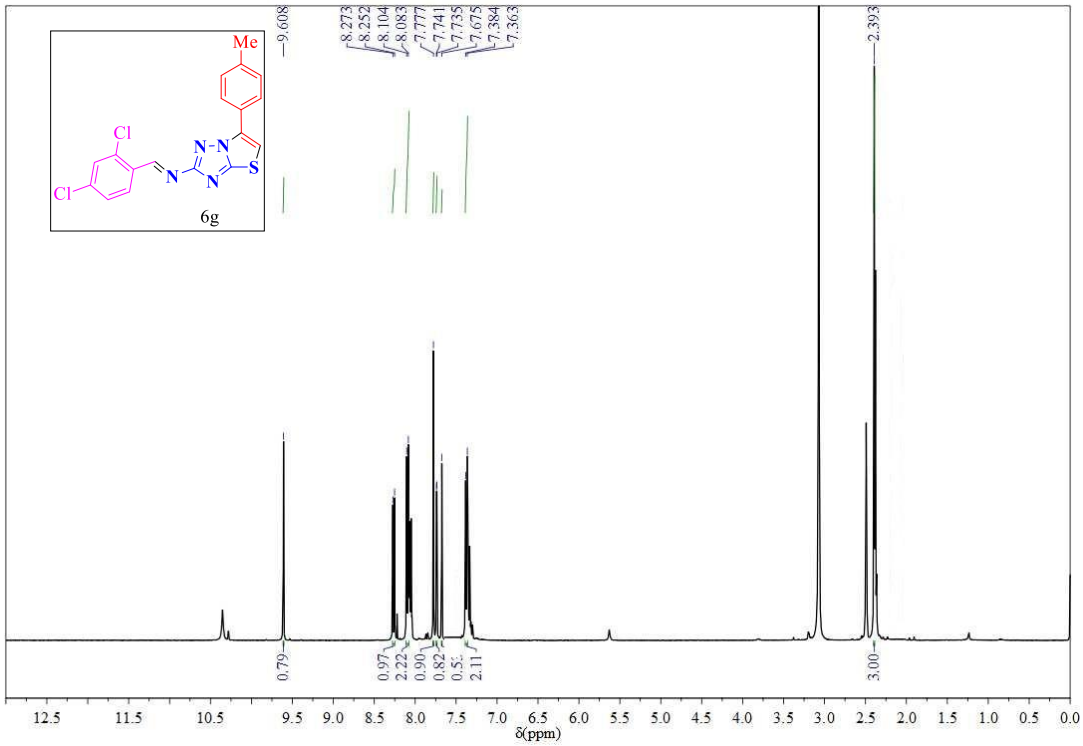
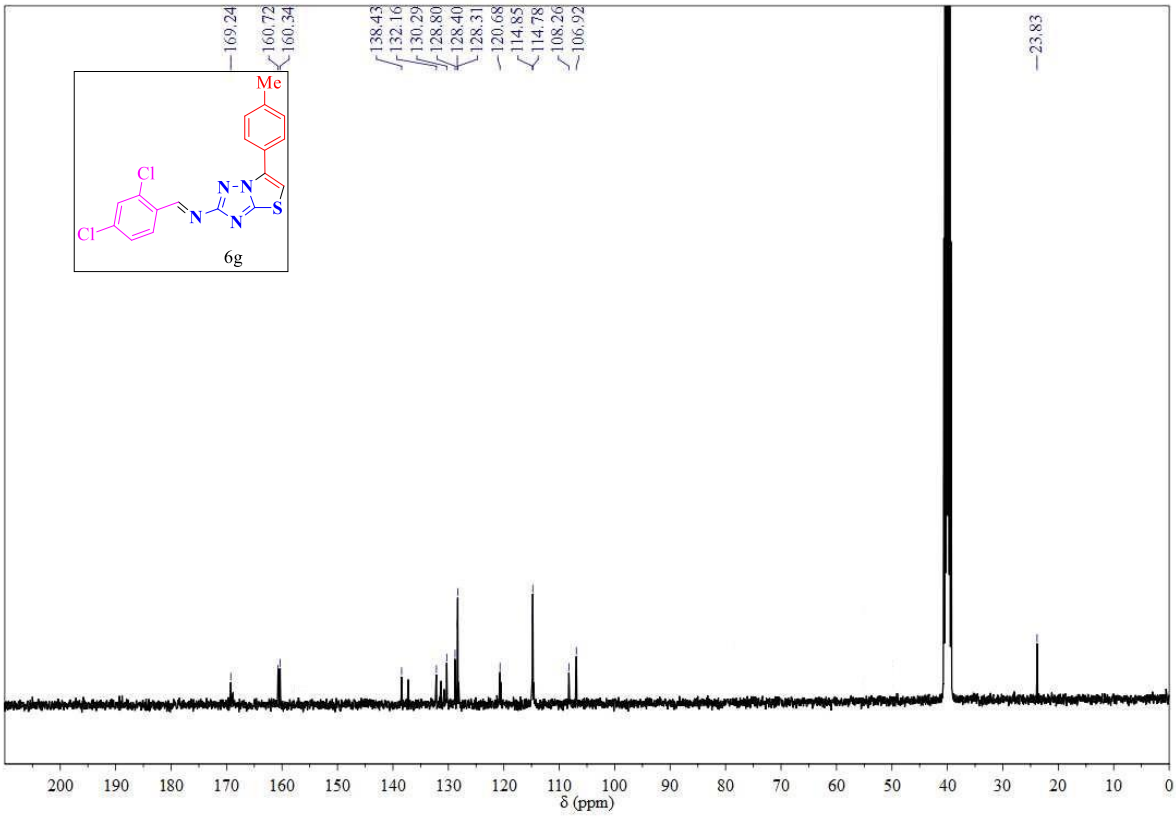
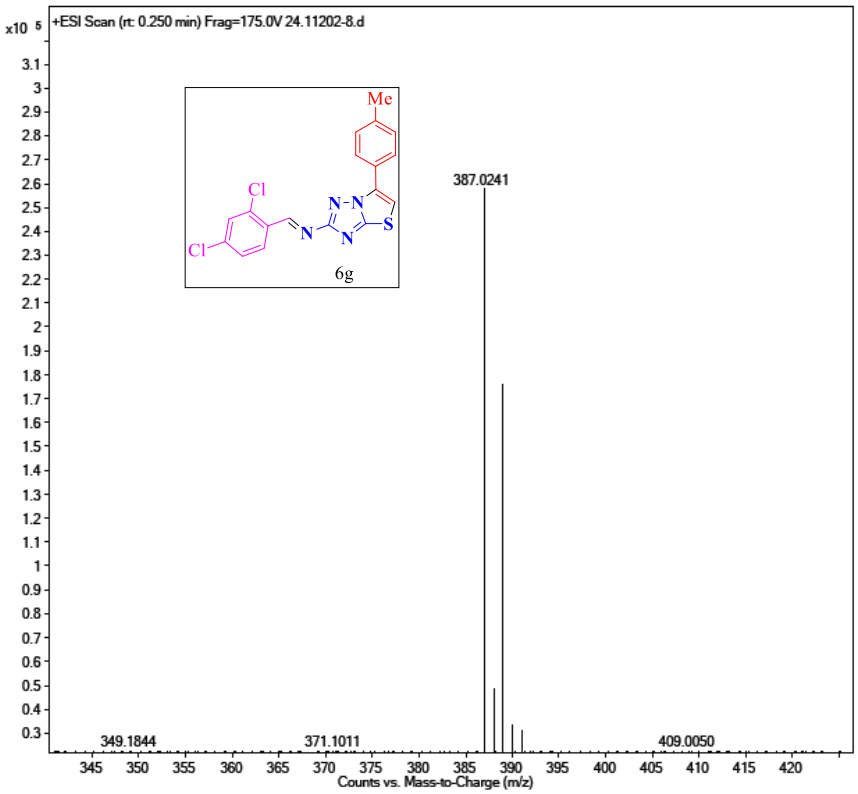


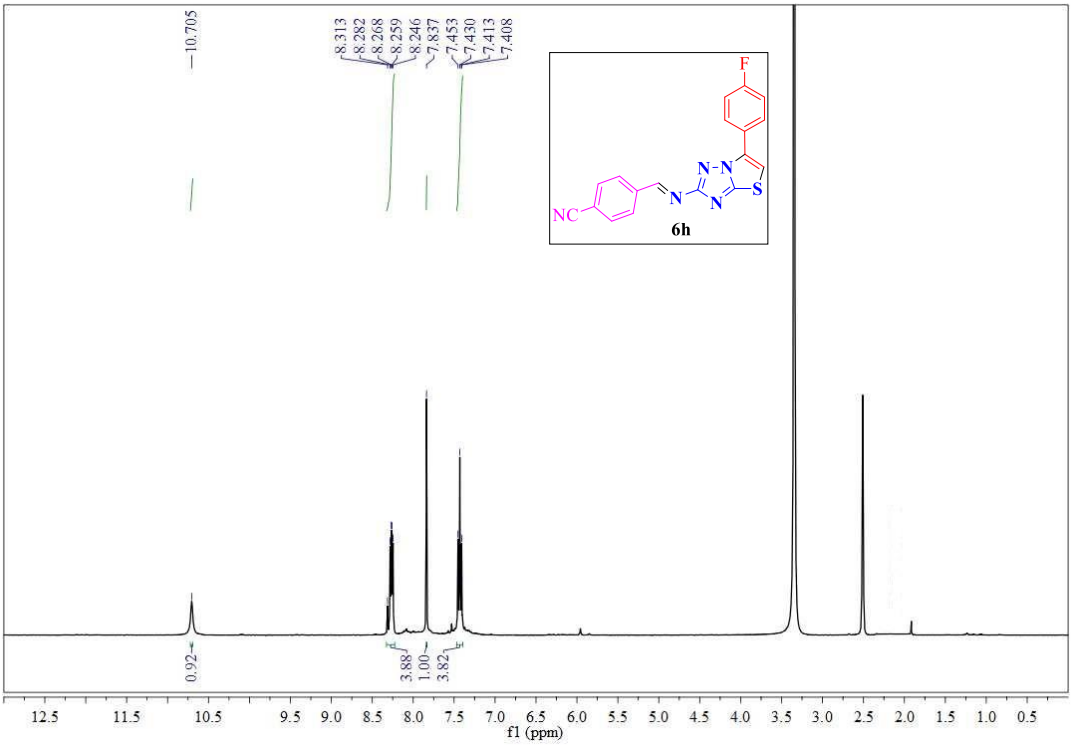
^{13}C NMR spectrum of compound **6g. (DMSO-d_6) 100 MHz**



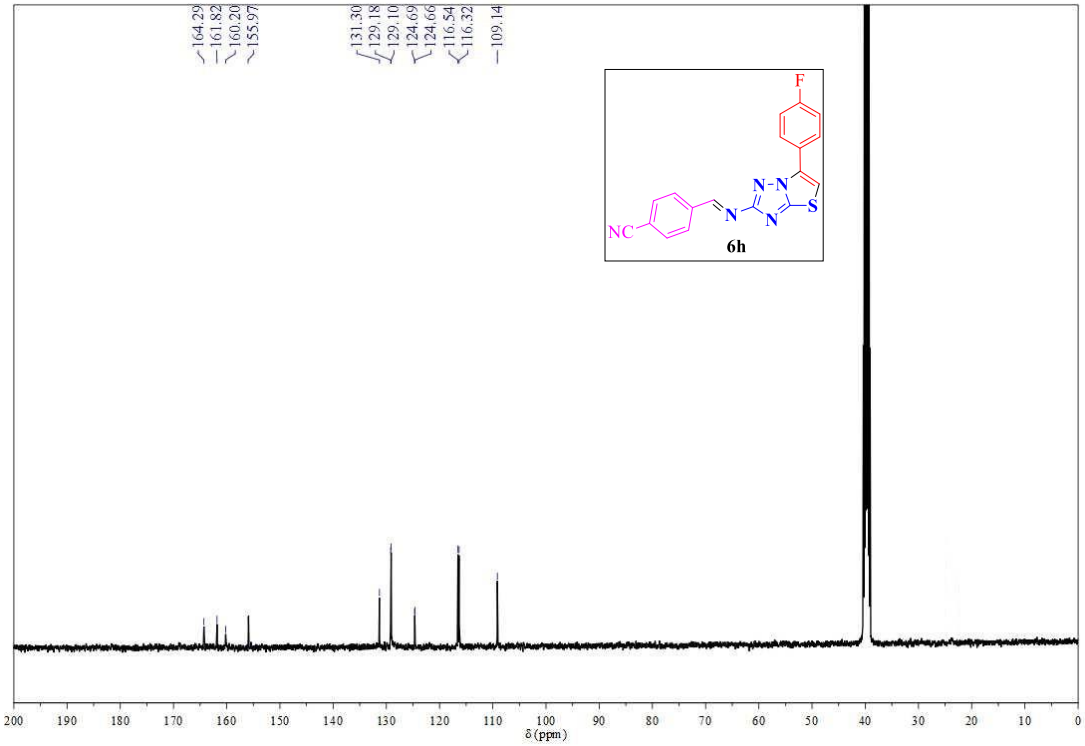
Mass spectrum of compound **6g**



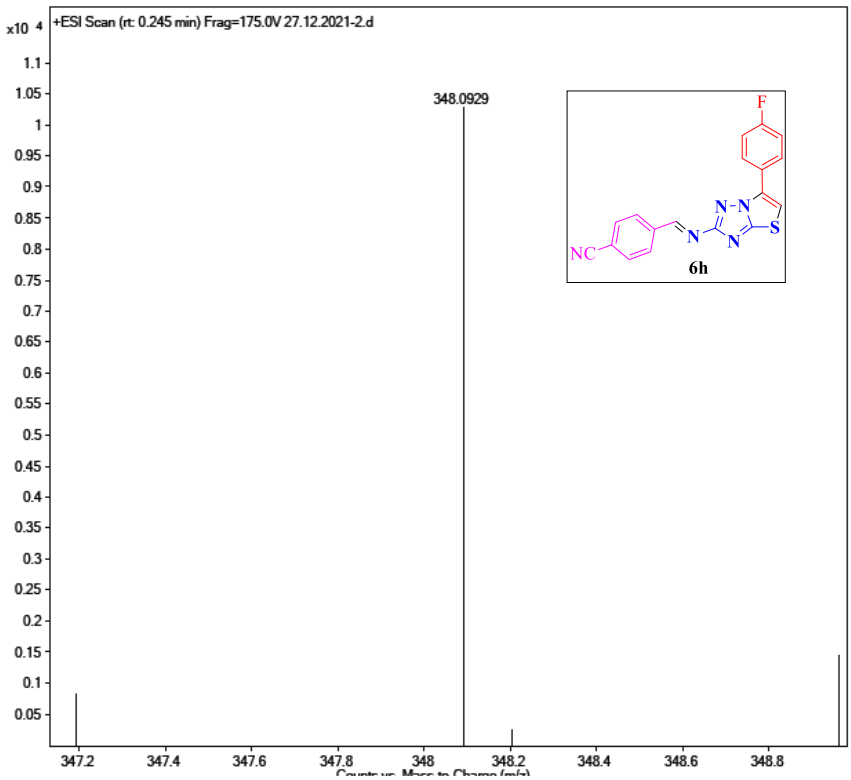
^{1}H NMR spectrum of compound **6h. (DMSO-d_6) 400 MHz**



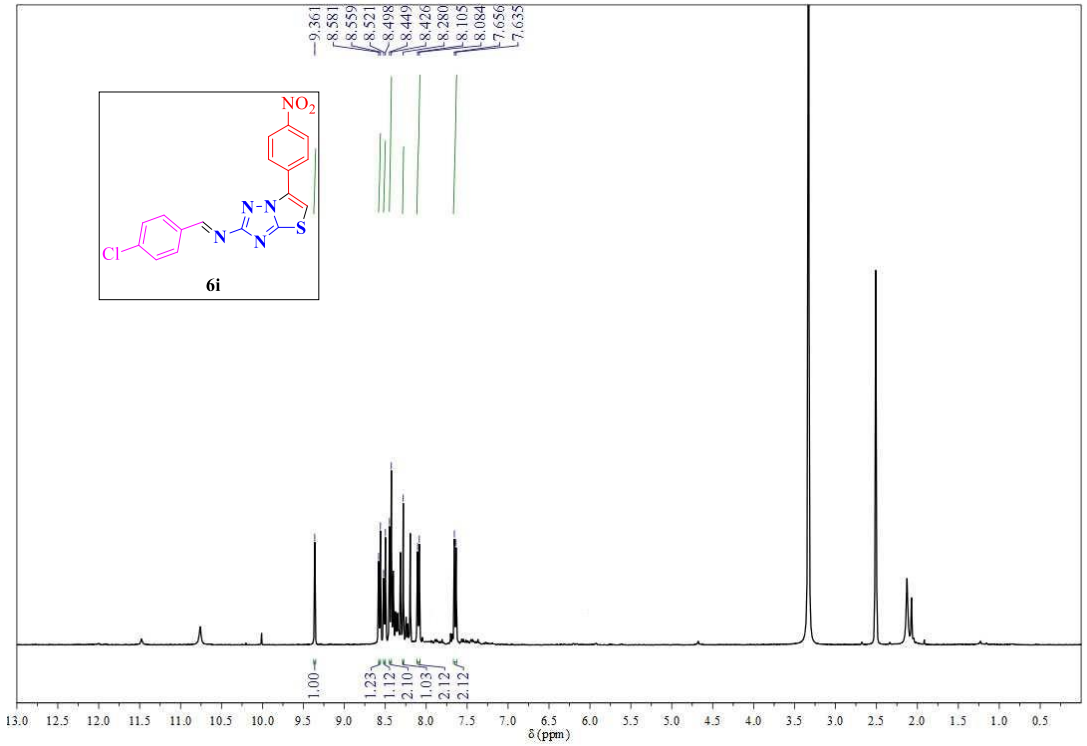
^{13}C NMR spectrum of compound **6h. (DMSO-d_6) 100 MHz**



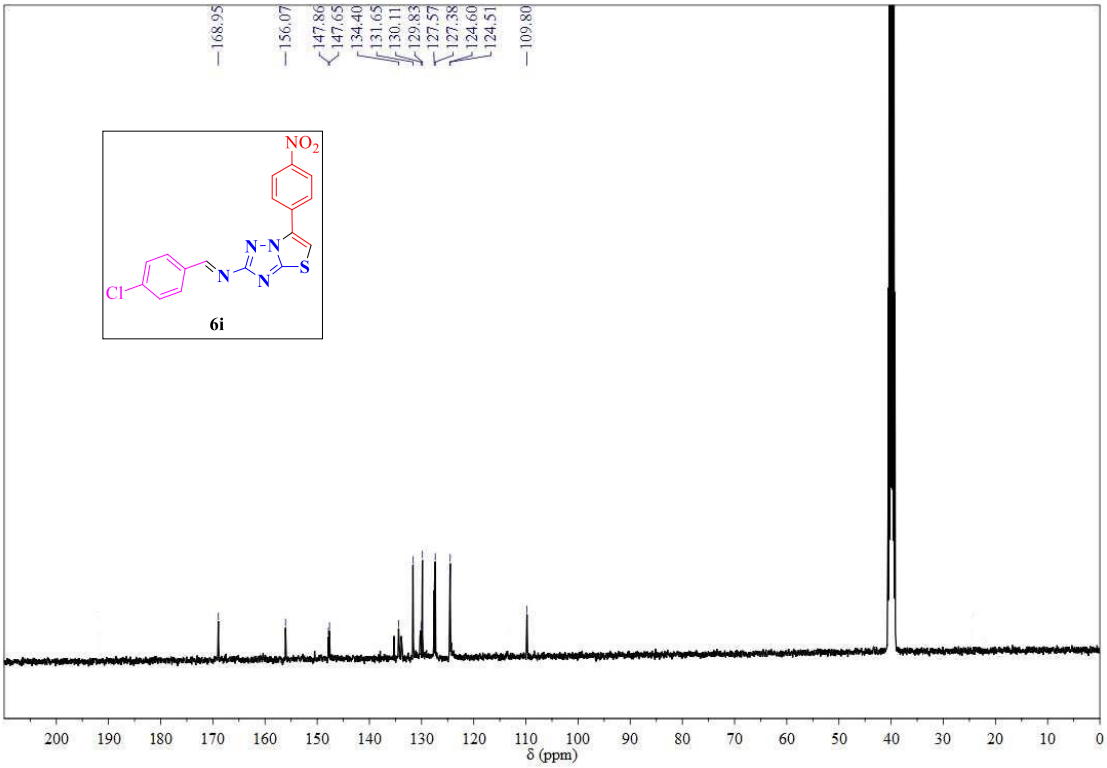
Mass spectrum of compound **6h**



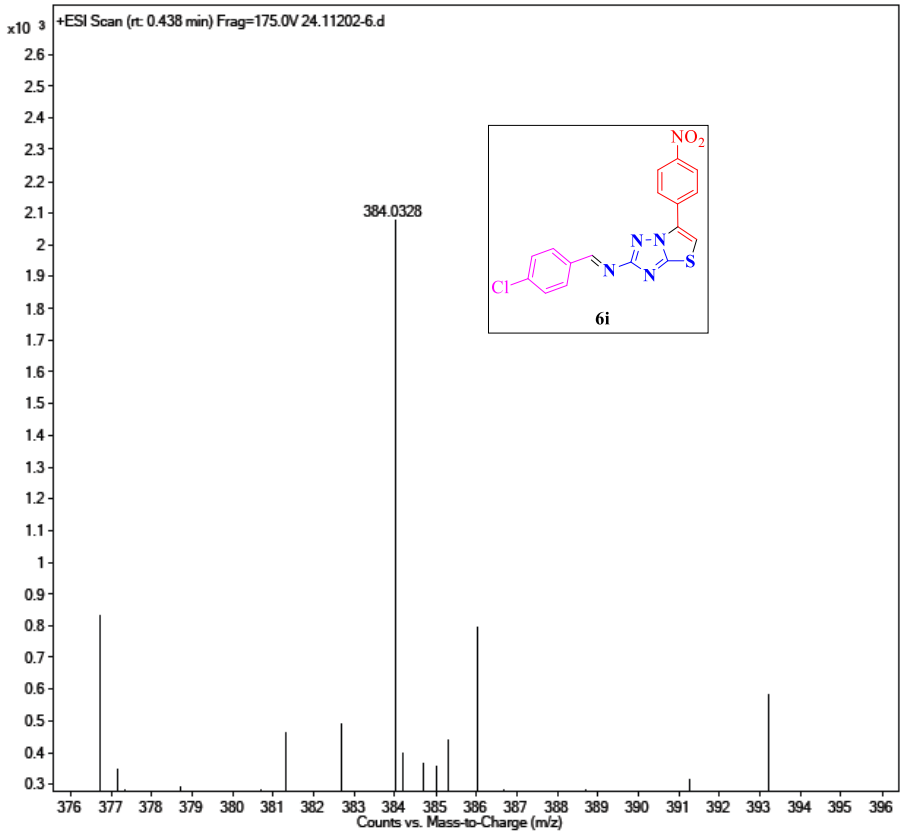
^{1}H NMR spectrum of compound **6i. (DMSO-d_6) 400 MHz**



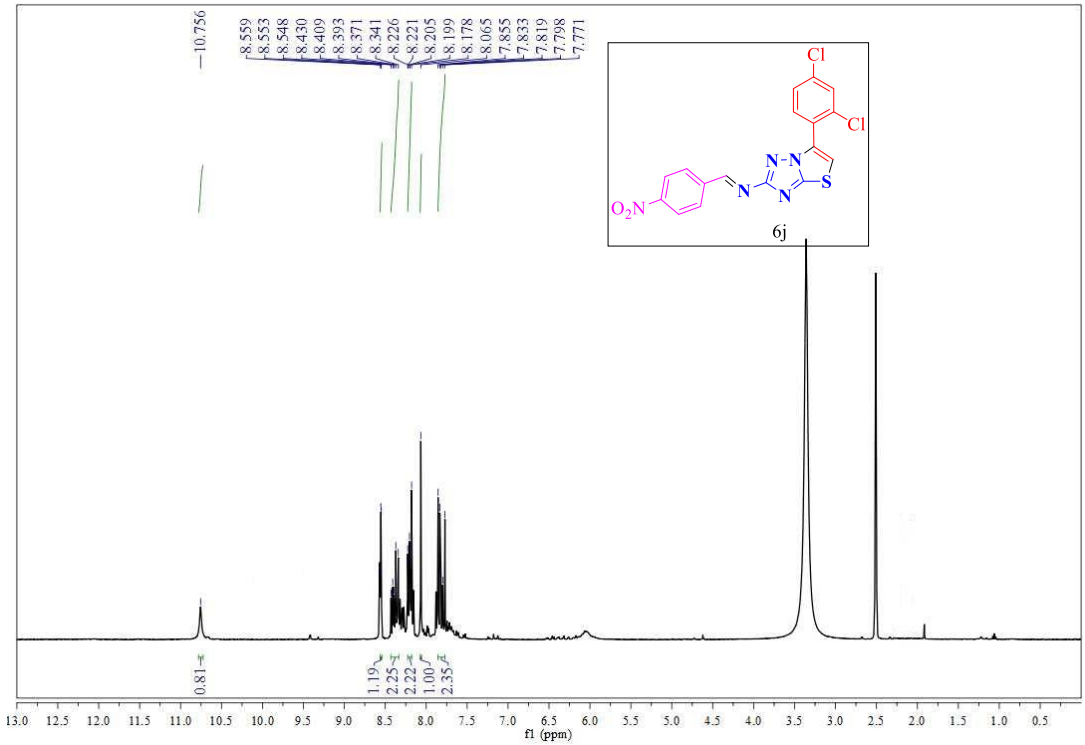
^{13}C NMR spectrum of compound **6i. (DMSO-d_6) 100 MHz**



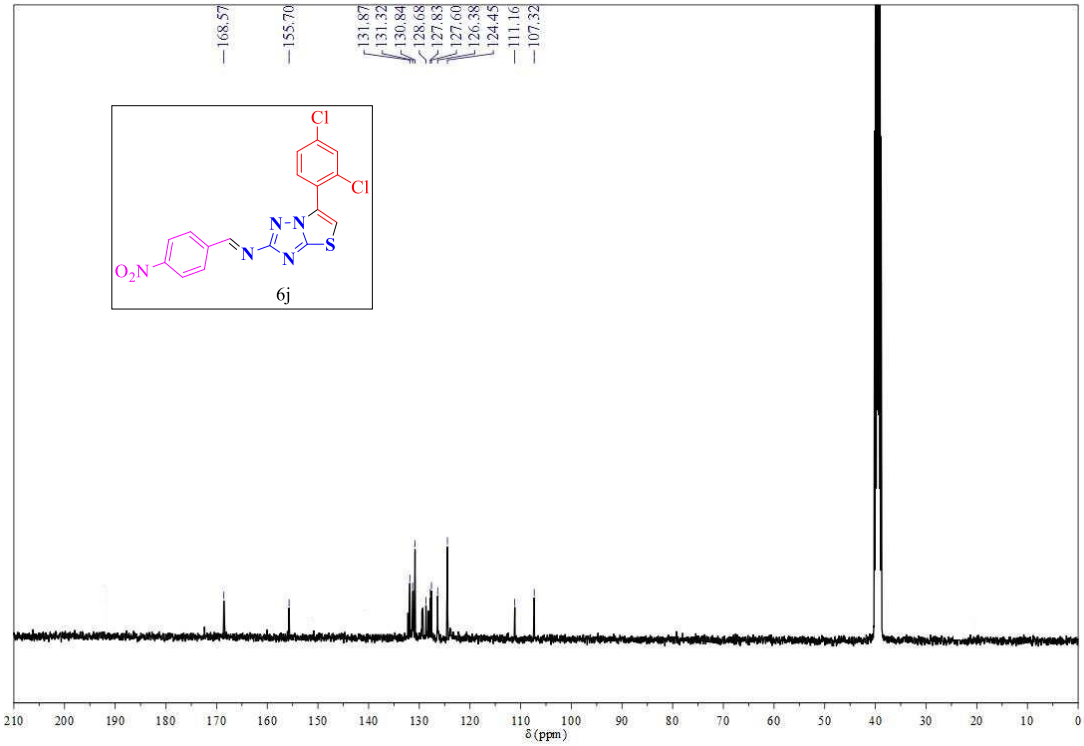
Mass spectrum of compound **6i**



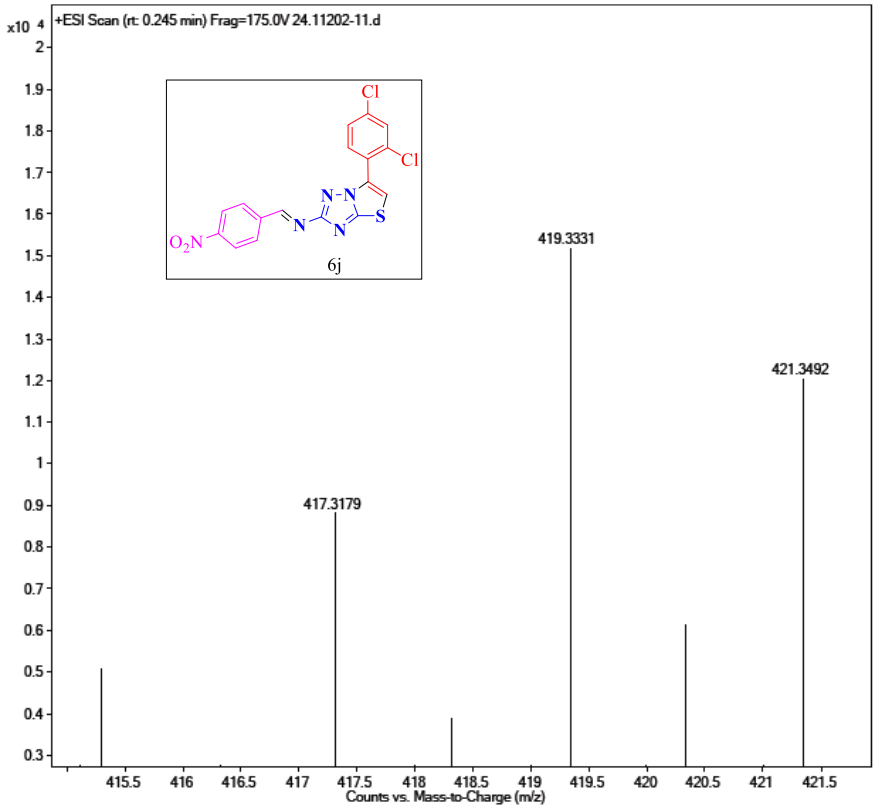
^{1}H NMR spectrum of compound **6j. (DMSO-*d*$_6$) 400 MHz**



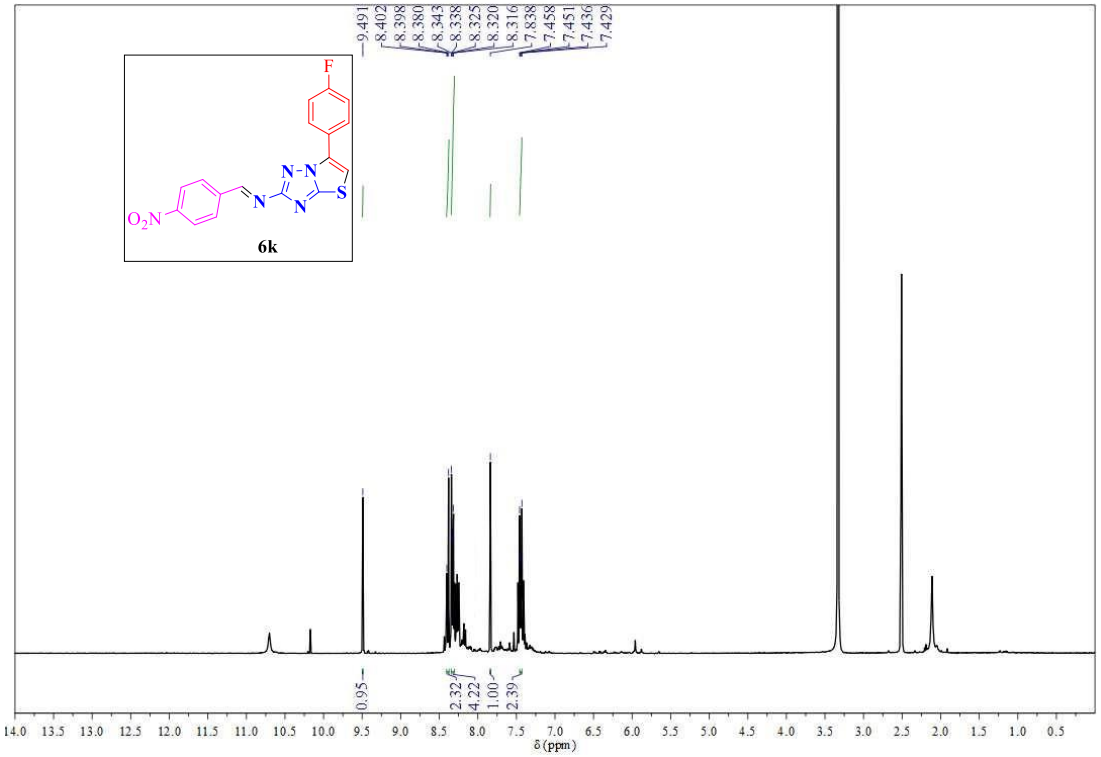
^{13}C NMR spectrum of compound **6j. (DMSO-d_6) 100 MHz**



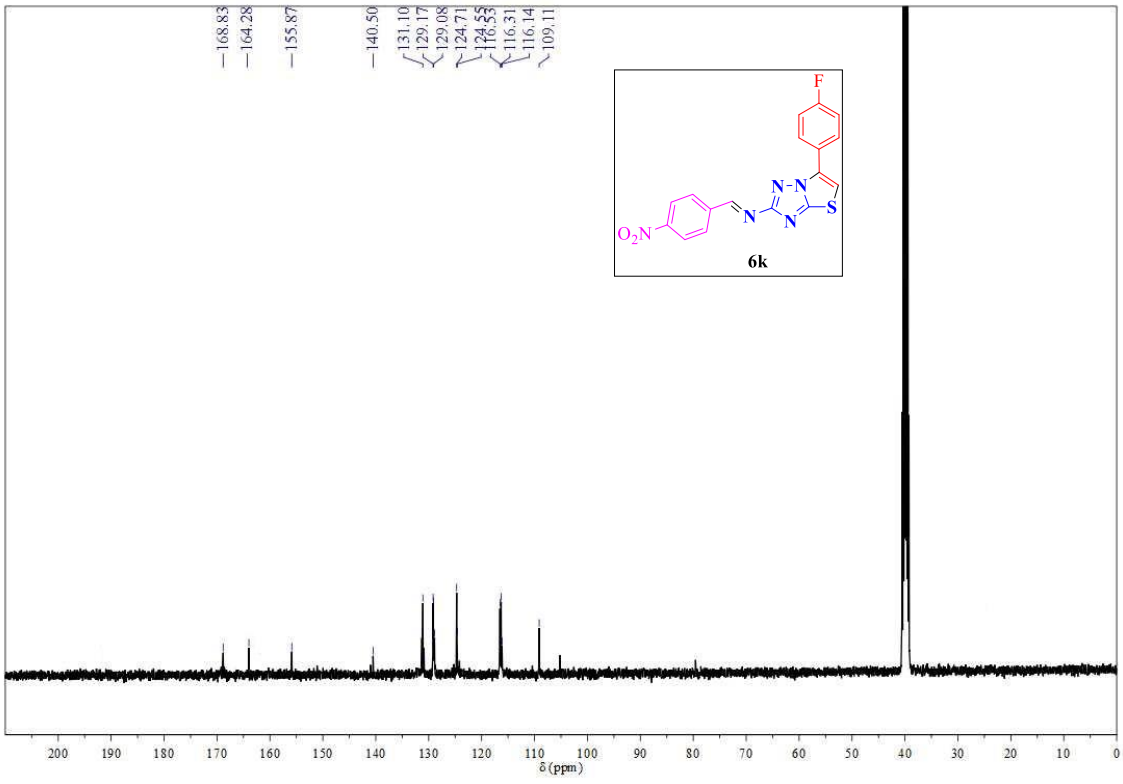
Mass spectrum of compound **6j**



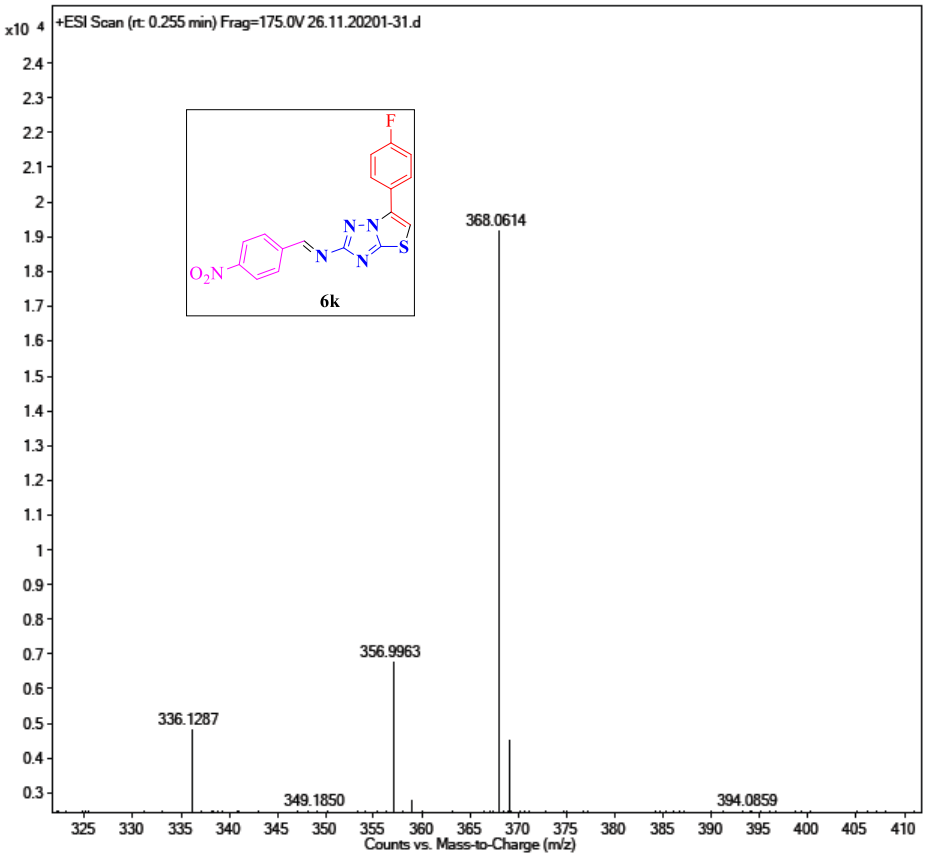
^{1}H NMR spectrum of compound **6k. (DMSO-d_6) 400 MHz**



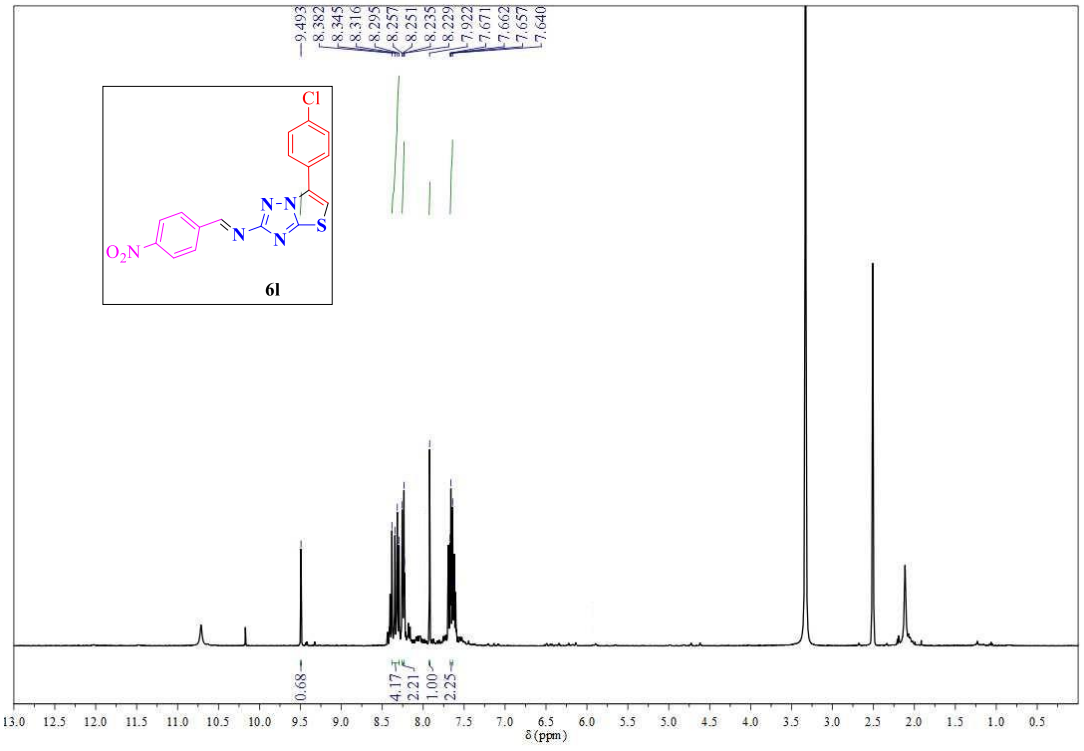
^{13}C NMR spectrum of compound **6k. (DMSO-d_6) 100 MHz**



Mass spectrum of compound **6k**



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



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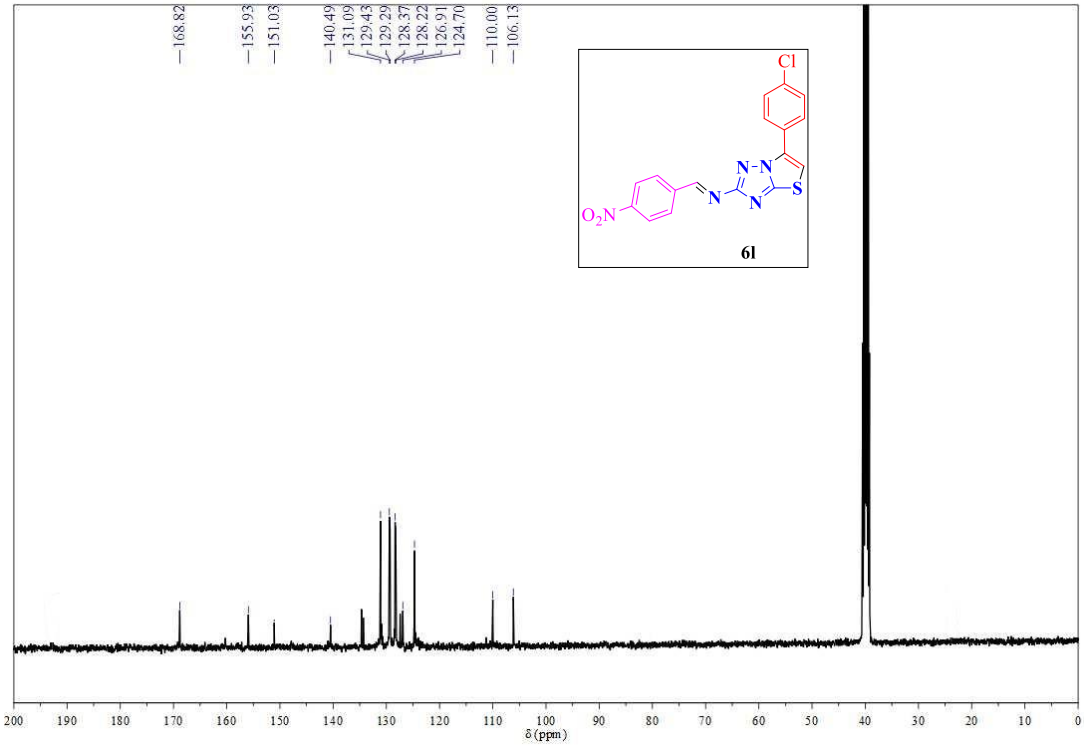
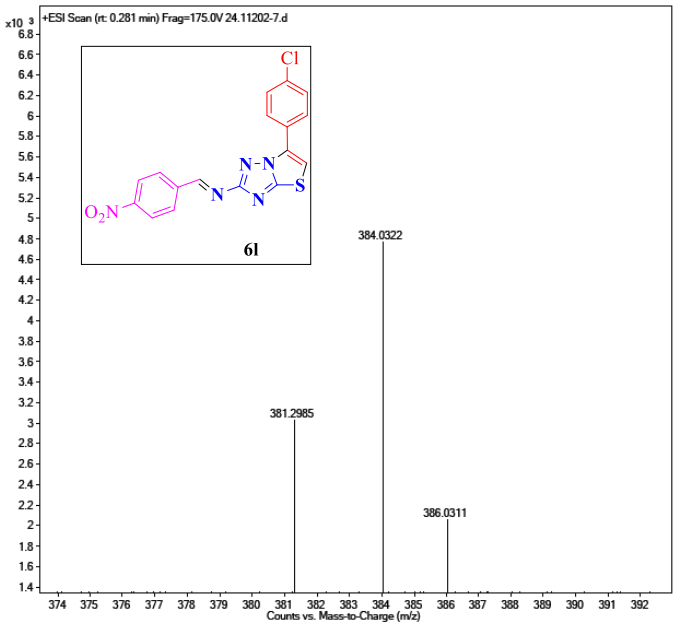


Mass spectrum of compound **6l**



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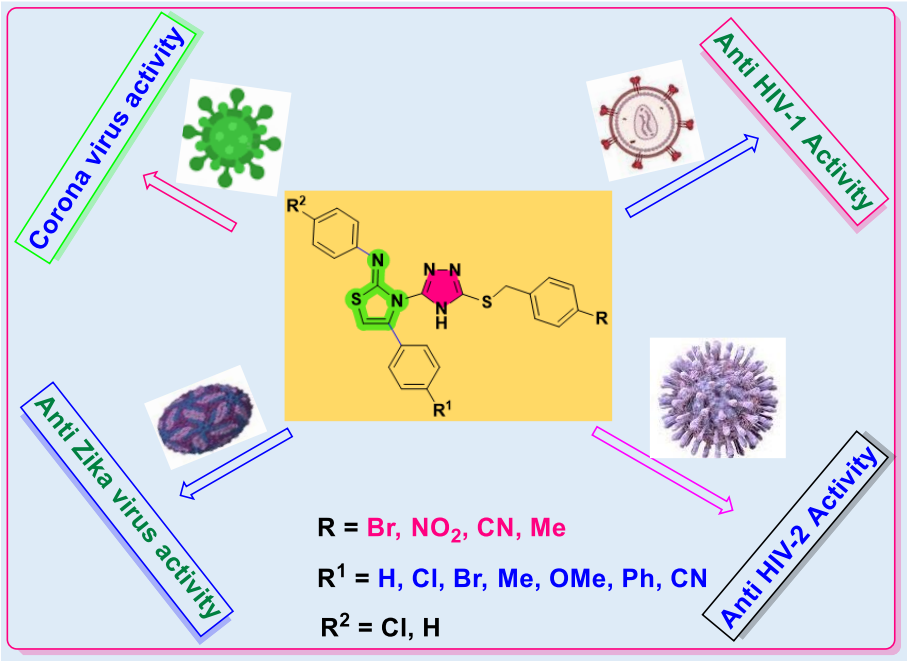
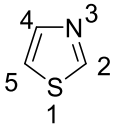
CHAPTER-IV

Synthesis of novel thioalkylated triazolothiazoles and their promising in-vitro antiviral activity



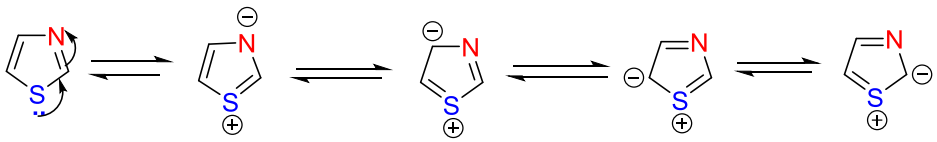
4.1. Introduction

Hantzsch thiazole method is used for the construction of simple thiazoles[1]. In the thiazole ring S atom is having the delocalized lone pair of electrons and it satisfies Huckel (4n+2) rule. Therefore, the thiazole moiety is an aromatic compound in which five membered ring contains two hetero atoms (S, N) it is possible to exist two isomeric structures i.e. 1,3-thiazole and 1,2 thiazole (isothiazole) The presence of acidic proton at C_2 position of 1,3-thiazole ring feasible to develop the new chemical entities such as formation of C-C, bond and ring cyclization reactions [2, 3]. And the 1,3-thiazoles have tremendous biological applications. The thiazolium salts are well known important catalysts in organic reactions to make the variety of the compounds[4].



Isomeric structures of thiazole molecule

Possible resonance structures of thiazole



The broad biological activity of thiazoles are used in many of the pharmaceutically active drug molecules to cure various diseases. Some of the biologically active thiazole containing drugs are the Penicillin is an antibiotic,[5] ritonavir is antiviral drug,[6] sulfathiazole is antibacterial drug,[7] abafungin is antifungal drug,[8] triazofurin, belomycin are the anticancer drugs.[9] [10] Thiazole moiety is an essential heterocyclic core unit for the development of biologically active molecules.[11] Many of the thiazole containing molecules possesses various biological activities

such as antimalarial,[12] antiviral,[13-15] anticancer,[16 16a] FabH inhibitors,[17] antimicrobial,[18 18a] anti-inflammatory,[19] anticytostatic agents,[20] antimiscellaneous properties,[21] CNS active agents,[22] and antihypertensive. [23]

In particular, the thiazole 2-imine derivatives have received a lot of attention in medicinal chemistry because these substances display drug-like properties and most of them are extensively used in the pharmaceutical industry and natural products.[24-31]

1,2,4-triazole moiety associated with a thiazole heterocyclic ring has been identified as potential biological activity molecule.[32] Thio alkylated triazolothiazole 2-imines are mainly used in the medicinal and pharmaceutical field and possess antiviral,[33] anticandidal,[34] and antituberculosis activity[35] among others. **Fig.1** describes the different bioactive thiazoles and triazolothiazole motifs.[36-44]

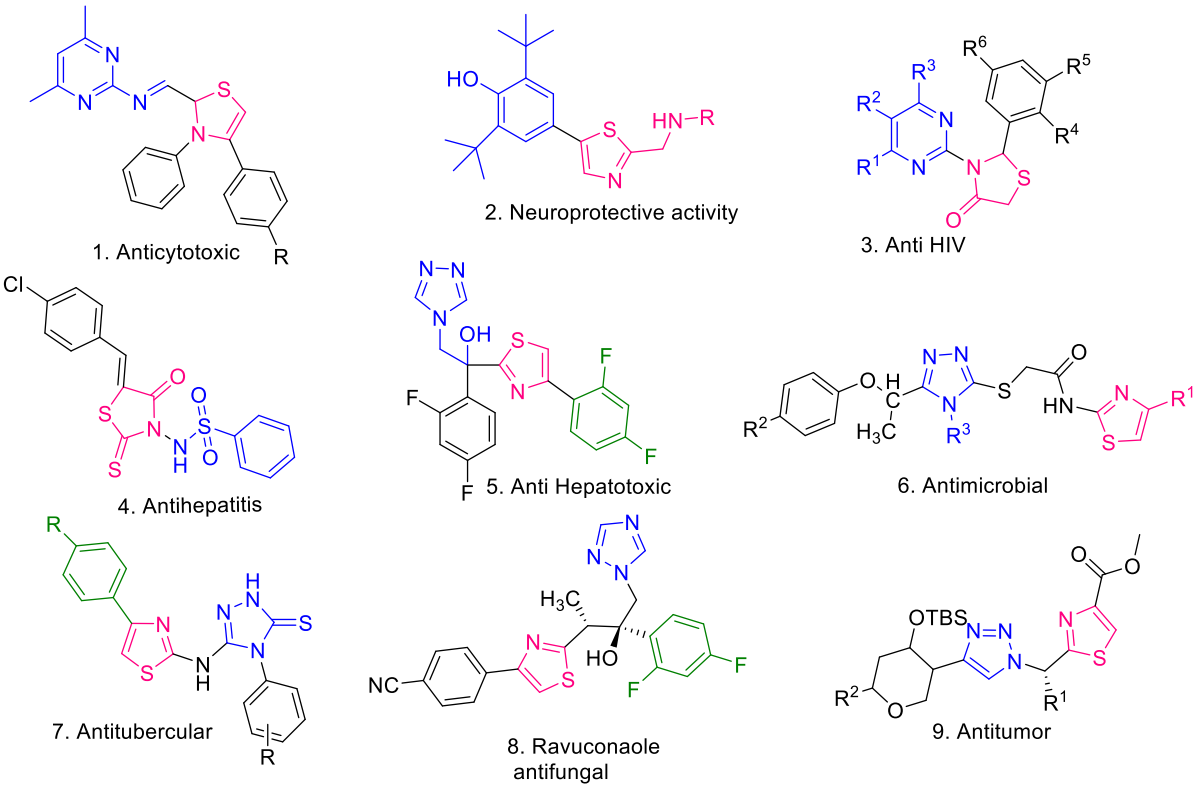
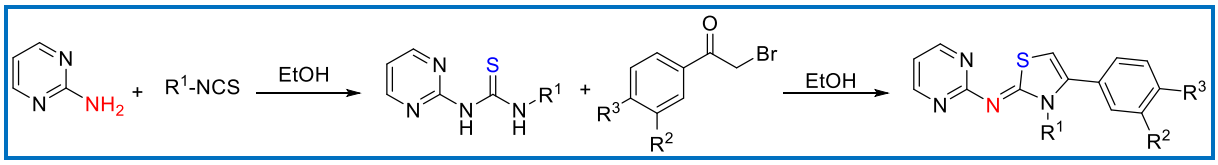


Fig.1. Biologically active thiazoles and triazolothiazole heterocyclic molecules.

Following are the literature reports of thiazoles.

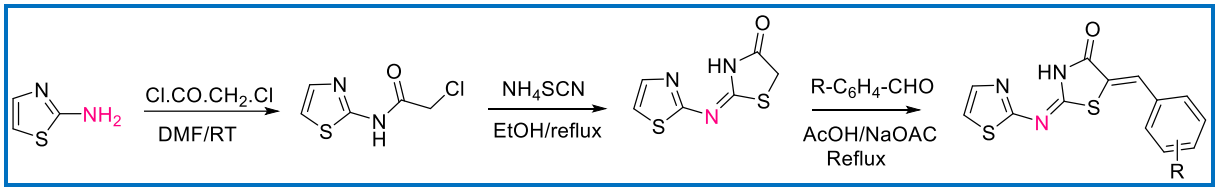
2-amino pyrimidines on reaction with phenyl isothiocyanates in EtOH initially to afford a thiourea derivative then it was treated various 2-halo acetophenones in same reaction to form a five membered heterocyclic compounds thiazoles. The final products were screened for their antiviral activity and most of the compounds were exhibited good viral activity.[45] (Scheme-1.1)

Scheme-1.1



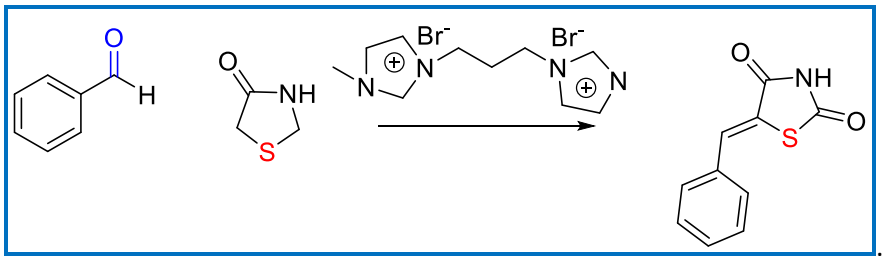
Vicini *et al* [46] reported the synthesis of 2(thiazol-2-yl imino (thiazolidine-4-ones by the reaction of 2-amino thiazole with chloroacetyl chloride in presence of DMF and reflux to produce the 2-halo *N*-acetyl compound. This again on reaction with ammonium thiocyanate in EtOH under reflux resulted in the formation of triazolyl thiazolidinone. These on further reaction with various aldehydes in presence of a mixture of AcOH/NaOAc to obtain final compounds. (Scheme-1.2)

Scheme-1.2



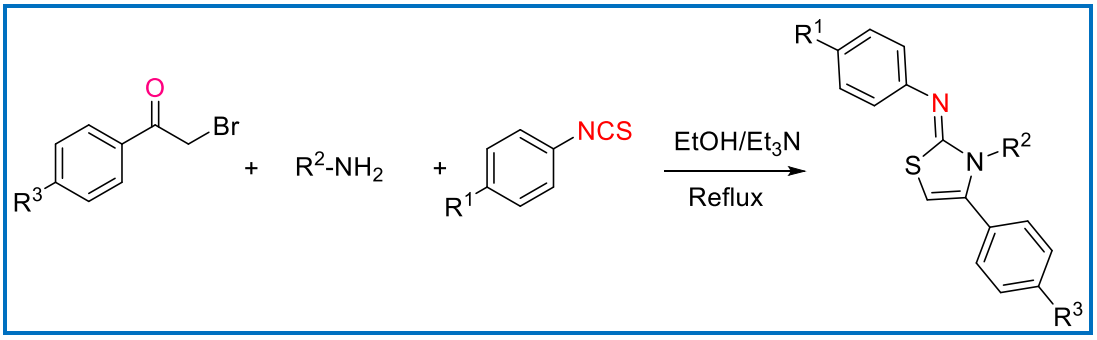
Jawale *et al* [47] published an efficient route for the applications of thiazole substrates in organic synthesis by using cationic liquids. The Knovenagal condensation reaction between aldehydes and 2,4 thiazolidinones in presence of ionic liquid to develop the 5-arylidine-2,4-thiazolidines leading to the formation of C-C double bond. (Scheme-1.3)

Scheme-1.3



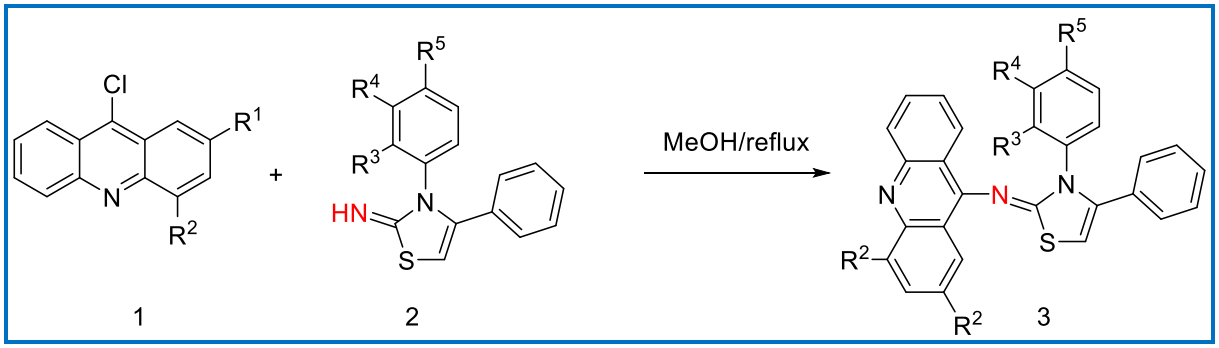
Heravi *et al* [48] synthesized a series of thiazole-2-imines with good percentage of yield in a short period of time by a one-pot three component reaction of α-bromo acetophenone, aromatic/aliphatic primary amines and phenyl isothiocyanates in EtOH/Et_3N at reflux condition. (Scheme-1.4)

Scheme-1.4



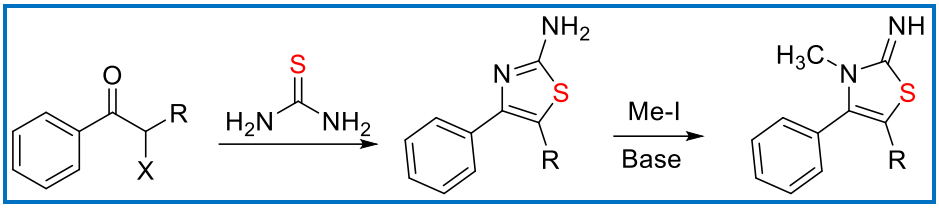
Sondhi *et al* [49] reported the synthesis of acridine 9-substituted thiazole 2-imine derivatives by the reaction of 9-chloro acridine with thiazole imine compound (2) in presence of methanol at reflux to afford a nucleophilic addition product (3). The final compounds were tested for their anti-inflammatory, kinase inhibitory and analgesic activity. (Scheme-1.5)

Scheme-1.5



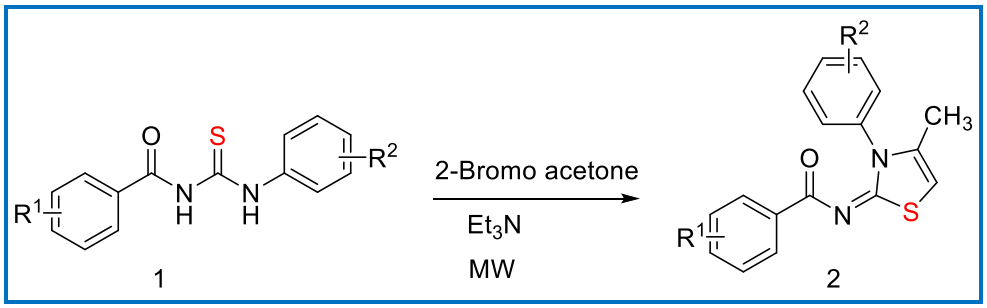
Kimpe *et al* [50] developed an unambiguous synthesis of 2-amino 4-thiazolines form the reaction of 2-bromo 1-phenyl propanone with thiourea in EtOH at reflux temperature to give the 2-amino thiazole. This have been reaction with alkyl halide (Me-I) in the same solvent to give new *N*-alkyl 2-imino thiazole. (Scheme-1.6)

Scheme-1.6



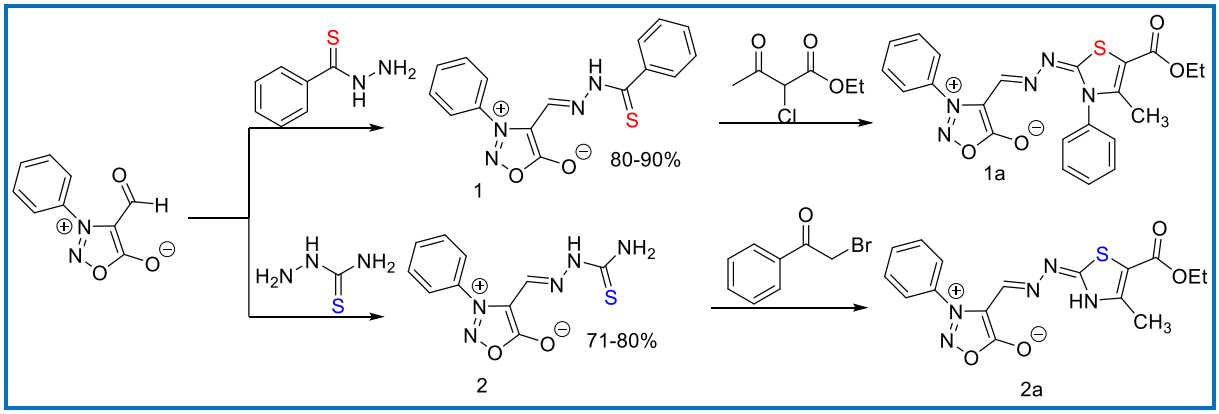
Saeed *et al* [51] reported the synthesis of thiazole ring containing benzimidazoles by condensation of the carbonyl compounds such as 2-bromo acetone cyclocondensation reaction with thiourea derivative of compound 1 in presence of Et_3N under microwave conditions to give a thiazole ring containing benzamide compound 2 shown in scheme-1.7.

Scheme-1.7



3-Aryl-4-formyl sydnones on reaction with *N*-substituted thiosemicarbazone in presence of ethanol/NaOAc/AcOH to make a thiourea substituted sydnone derivatives 1 and 2. Which were subsequently reaction with chloro ethyl acetate or *α*-halo acetophenone in ethanol to afford a corresponding thiazoles. These derivatives were shown antioxidant activity.[52] (Scheme-1.8)

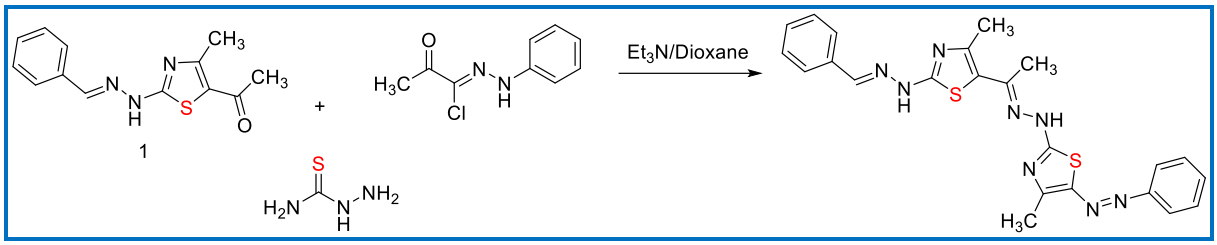
Scheme-1.8



The multicomponent reaction of acetyl thiazole compound 1 with thiosemicarbazide and appropriate hydrazonyl chloride in the presence of Et_3N/dioxane at reflux temperature to

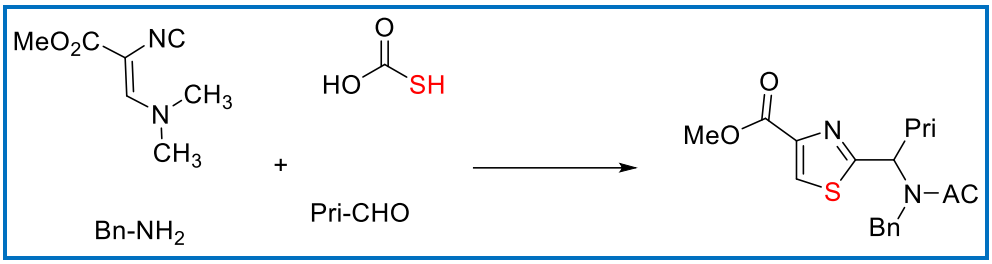
produce the corresponding aryl hydrazothiazolones. These final substrates were demonstrated as promising anticancer activity.[53] (Scheme-1.9)

Scheme-1.9



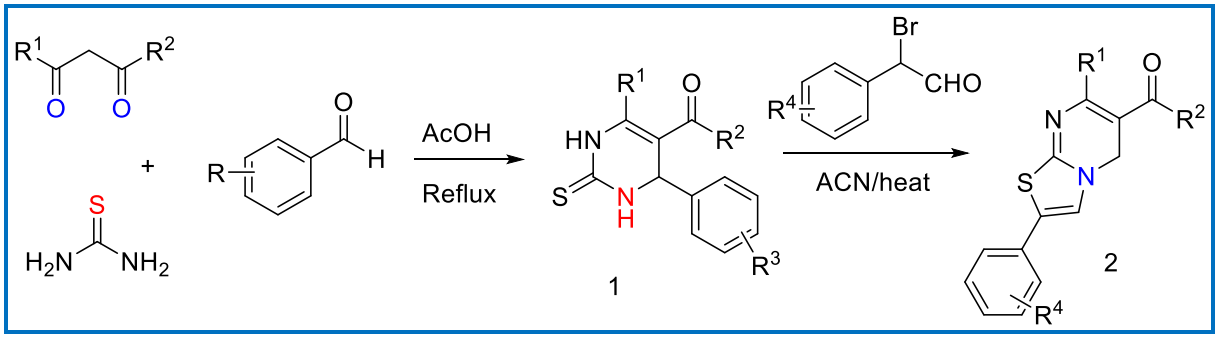
The combinatorial four component synthesis of 2,4-isubstituted thiazole derivatives by the reaction of β-dimethyl amino α-iso acrylate, thioglycolic acid, various aldehydes and benzyl amines. The final products were formed with good yield.[54] (Scheme-1.10)

Scheme-1.10



Wichmann *et al* [55] reported one-pot Biginelli type reaction of 1,3-di carbonyl compound, thiourea and aldehydes in AcOH at reflux conditions. Then followed by reaction with bromo-1- phenacylated aldehyde in CH_3CN to produce thiazole product 2. The pyrimidine attached thiazole entities shows metabotropic glutamate receptor antagonist properties. (Scheme-1.11)

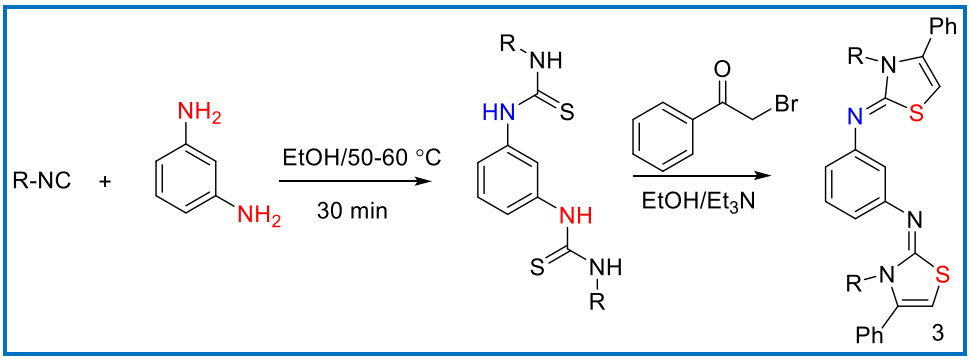
Scheme-1.11



Shiran *et al* [56] published the synthesis of bis-thiazole compounds form the reaction of different isocyanides with 1,3-diamino benzene in EtOH under heating for 30 min to form a respective

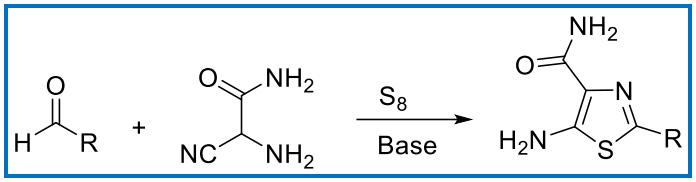
1,3-di substituted di thiourea derivatives. This on further reaction with 2-bromo acetophenone to produce the corresponding bis thiazole. These exhibit antibacterial activity. (Scheme-1.12)

Scheme-1.12



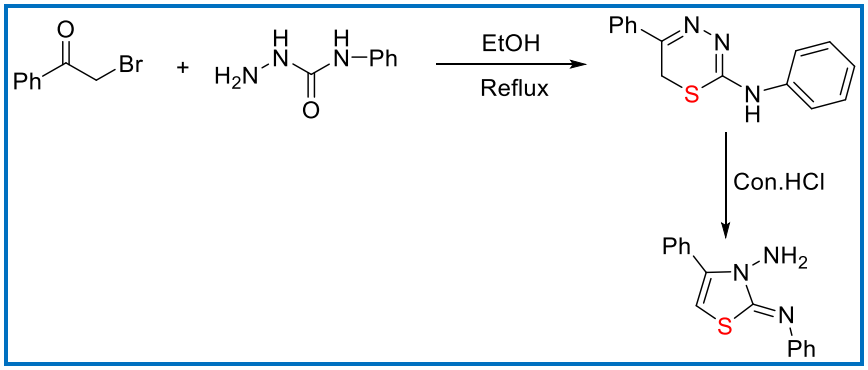
Childers *et al* [57] developed a one-pot synthesis of various 5-amino-4-carboxamide thiazoles via a one-pot multi-component approach by the combination of various aldehydes and 2-amino-2-cyano acetamide and S_8 elemental sulfur in presence of basic conditions shown in scheme-1.13.

Scheme-1.13



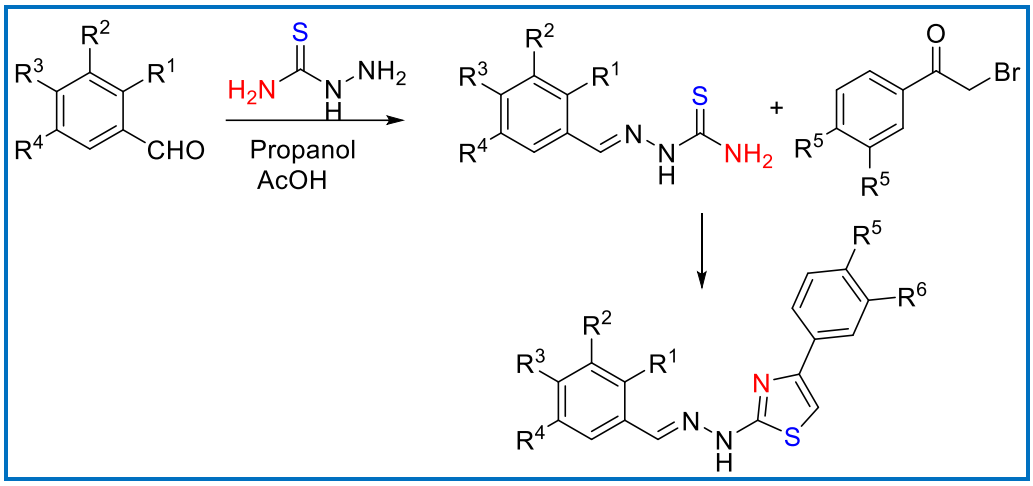
Pfeiffer *et al* [58] reported the synthesis of *N*-5-diphenyl-6*H*-1,3,4-thiadiazin-2-amine from α-bromo acetophenone and 4-phenyl thiosemicarbazide in EtOH. Later on this compound was treated with con. HCl to give a 2-hydrazono-3,4-diphenyl-2,3-dihydro thiazole represented in schemr-1.14.

Scheme-1.14



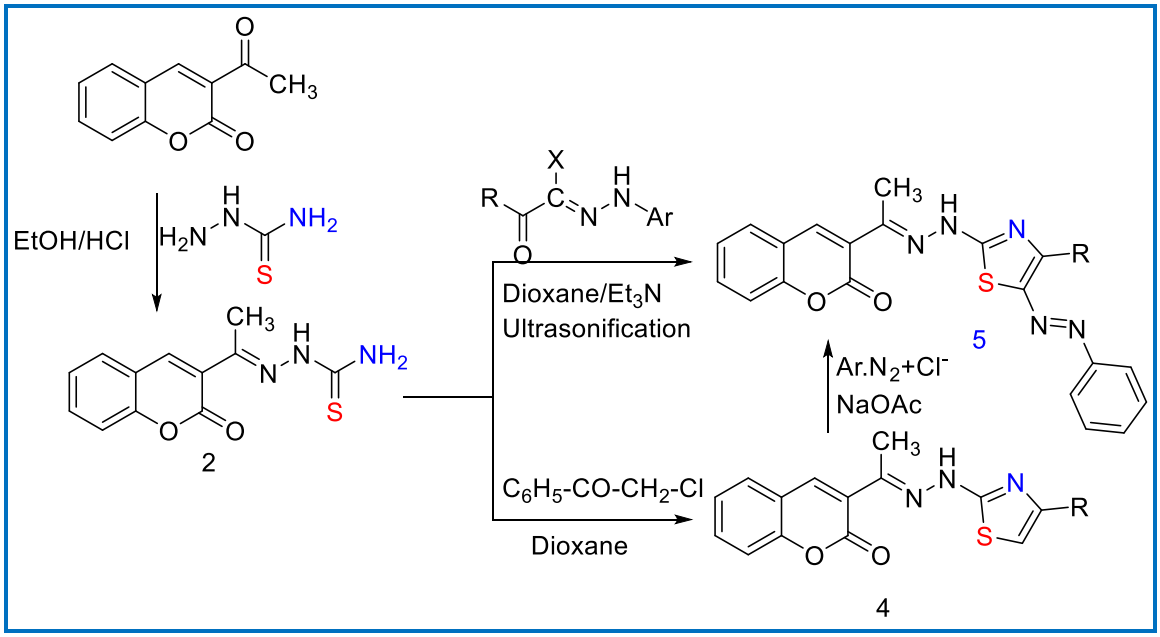
LV *et al* [59] reported the reaction of substituted aromatic aldehydes with thiosemicarbazide, 2-bromo acetophenones in presence of acetic acid/propanol to afford a substituted thiazole compound with good yields. The final compounds have shown potential antibacterial activity.

Scheme-1.15



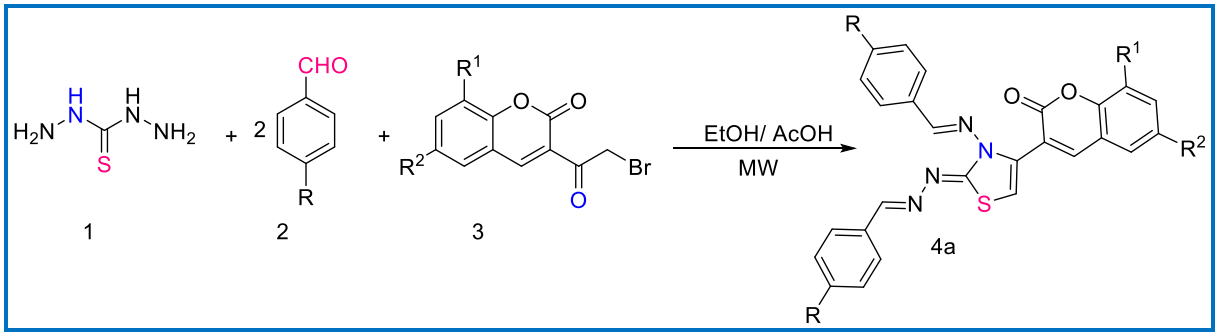
Gomha *et al* [60] synthesized the thiazole ring substituted compounds *via* a one-pot synthesis of 3-acetyl coumarin, thiosemicarbazide and hydrazonyl halide in dioxane/Et_3N under Ultra sonification condition. On the other hand, the final compound can be prepared by the reaction of thiosemicarbazone of 3-acetyl coumarin with 2-bromo acetophenones followed by condensation with aryl diazonium halides in dioxane. These compounds have shown cytotoxic activity. (Scheme-1.16)

Scheme-1.16



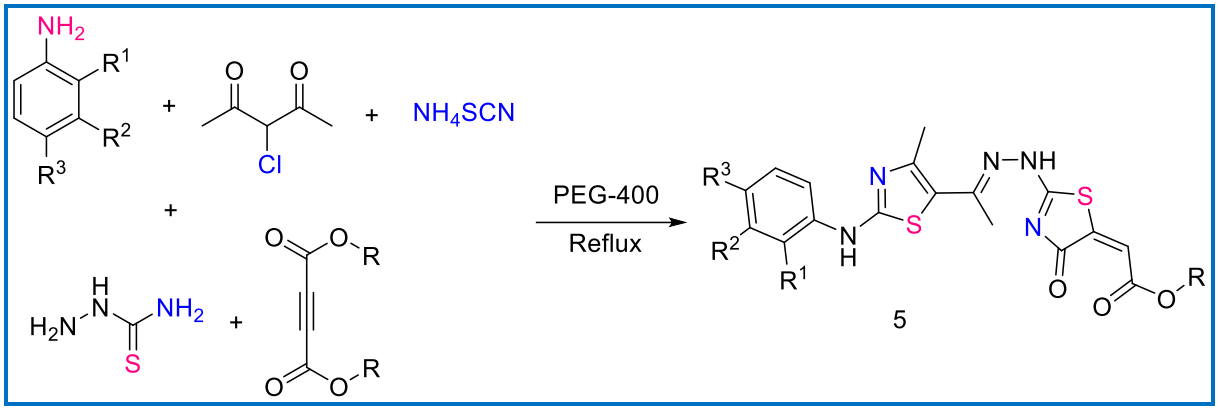
Mamidala *et al* [61] reported the pseudo three component synthesis of thiocarbohydrazide, aromatic aldehydes and 3-(2-bromo acetyl) coumarins in EtOH/AcOH under, microwave irradiation to give a thiazole derivatives with good yield. These compounds have shown good antibacterial activity. (Scheme-1.17)

Scheme-1.17



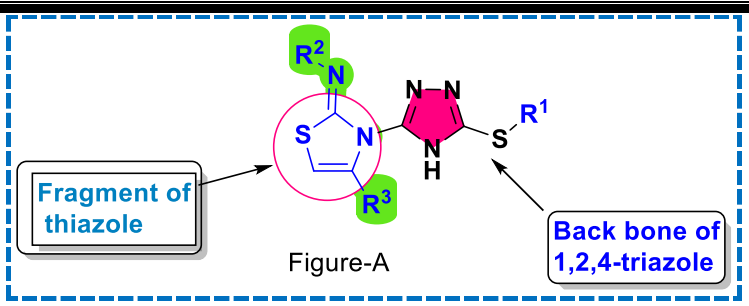
Scheme-1.18

Sujatha *et al* [62] carried out the multi-component synthesis of thiazoles by the condensation of aromatic amines, 3-chloro acetyl acetone, thiosemicarbazide, ammonium thiocyanate and di alkyl (Me, Et) acetylene di carboxylate in polyethylene glycol-400. (Scheme-1.18)



4.2. Present work.

In view of these observations and in continuation of our current work interest in the synthesis of thiazole heterocycles for biological evaluations[62a]. We have made an attempt to design and synthesis of new molecules with better antiviral properties as depicted in the following **fig. A.**

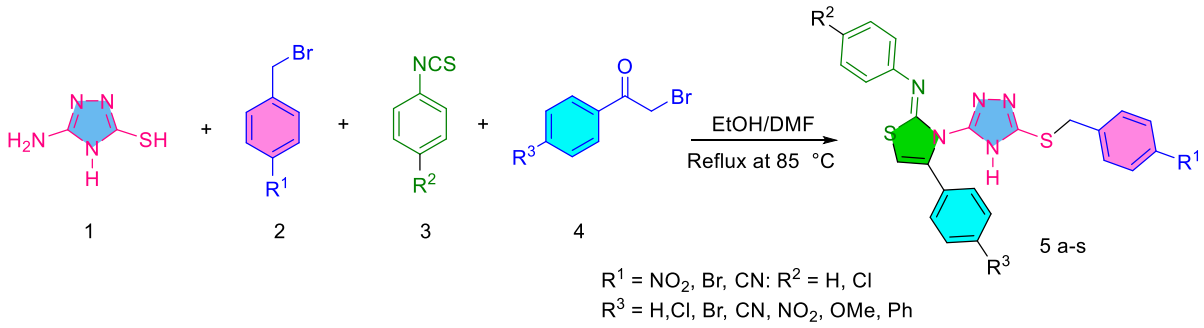


The present work was planned to synthesize some new thiazole derivatives bearing 1,2,4-triazole moiety. The newly synthesized compounds were investigated as antiviral agents.

4.2.1. Synthesis of 1,2,4-triazolothiazoles.

An efficient novel four component synthesis of 3-(5-(benzylthio)-4*H*-1,2,4-triazol-3-yl)-N,4-diphenylthiazol-2(3H)-imines was carried out by a one-pot multicomponent approach. Schematic representation of target compounds is depicted in scheme-1. The reaction of 5-amino-4H-1,2,4-triazole-3-thiol (1) with benzyl bromides (2) phenyl isothiocyanates (3) and 2-bromo acetophenones (4) using ethanol and DMF (8:2) gave novel target triazolothiazoles (5).

Scheme-1: Outline schematic representation of triazolothiazole synthesis.



4.2.2. Results and discussion.

The one-pot four component synthesis of triazolothiazole (5) can be achieved by the reaction of 1 with *p*-substituted benzyl bromides to form a thiobenzylated compound, then further reaction with phenylisothiocyanate and various 2-bromo acetophenones in EtOH/DMF at reflux temperature produced the corresponding (Z)-3-(5-(benzylthio)-4*H*-1,2,4-triazol-3-yl)-N,4-diphenylthiazol-2(3*H*)-imines with good yields.

In the optimization study the reaction was initially carried out between 5-amino-4*H*-[1,2,4]-triazole-3-thiol (1), benzyl bromide (2), phenylisothiocyanate (3), and simple phenacylbromide using various solvents such as methanol, AcOH, EtOH, DMF (Table-1 entries 1-4). In this

preliminary study it was found that in EtOH (Table-1 entry 3) the product has been formed to some extent. Further we have tried to improve yield of the product by introducing a mixture of solvents at different time intervals by varying the temperature (Table-1 entries 5-12). Out of the screened solvents (**entries 5-12**) EtOH + DMF (entry12) at 60 °C for 10 h produced good yield of the product. When gradually the temperature has been increased to 85 °C and time was maintained for 9h the maximum yield of the product was obtained (entry 14). Therefore, the optimized conditions for the formation of the product was 85 °C for 9 h with 90% yield. The optimization conditions were summarized in **Table-1**

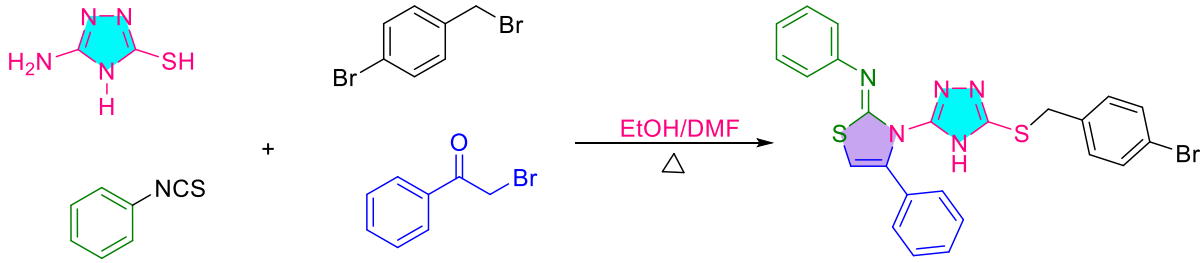


Table-1: The optimization conditions[a].

S.No	Solvent	Temp (°C)	Time (h)	Yield (%)[b]
1	MeOH	60	10	12
2	AcOH	60	12	n.r[c]
3	EtOH	60	8	20
4	DMF	60	9	trace
5	EtOH + AcOH(8:2)	70	10	n.r
6.	EtOH + HCl(8:2)	70	13	10
7	EtOH + Et_3N(8:2)	70	11	25
8	AcOH + Piperidine(8:2)	70	11	15
9	DMSO + EtOH(8:2)	70	15	n.r
10	AcOH + DMF(8:2)	70	10	22
11	EtOH + aq. KOH (0.1N)	70	8	14
12	EtOH + DMF(8:2)	60	9	65
13	EtOH + DMF(8:2)	70	9	73
14	EtOH + DMF(8:2)	85	9	90[d]

[a]***Reaction conditions***: Amino mercapto 1,2,4 triazole (1) (1 mmol), *p*-bromo benzyl bromide (2) (1 mmol), phenyl isothiocyanate (3) (1 mmol), phenacyl bromide (4) (1 mmol) using different mixture of solvents and temperatures, time. [b]Yield of the product; [c] n.r = no reaction; [d]85 °C for 9 hours EtOH + DMF, 90%.

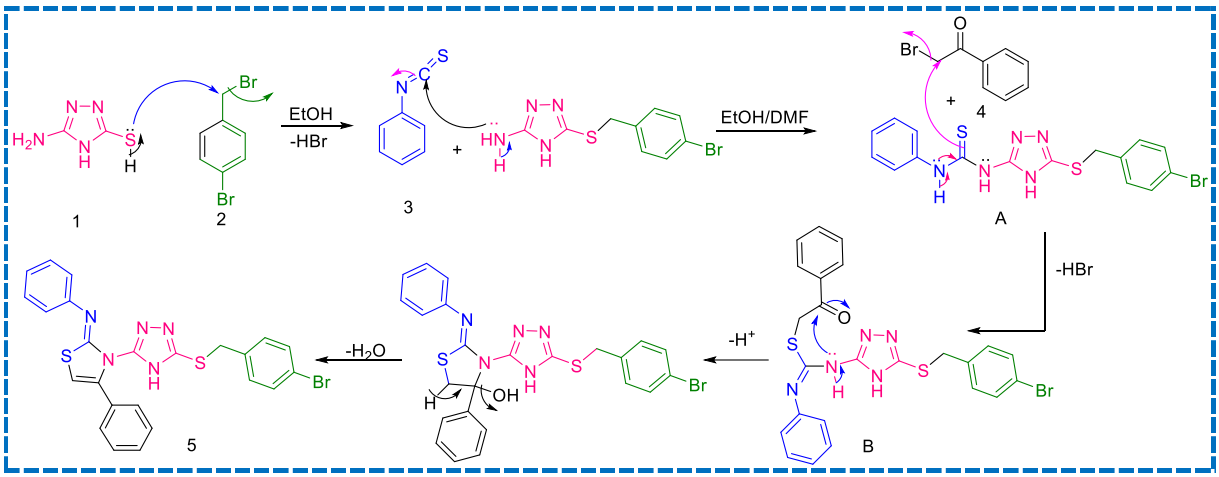
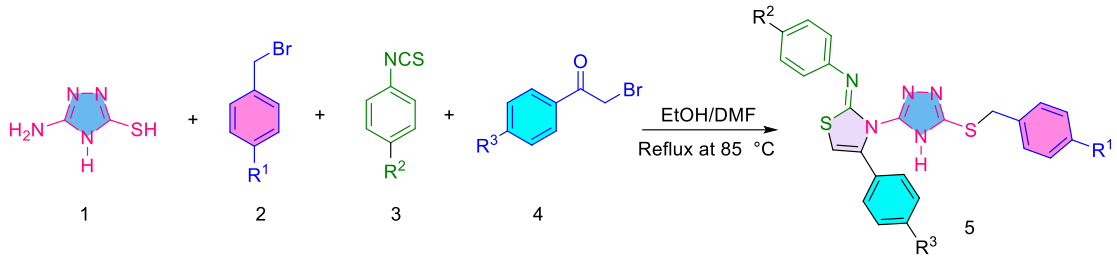
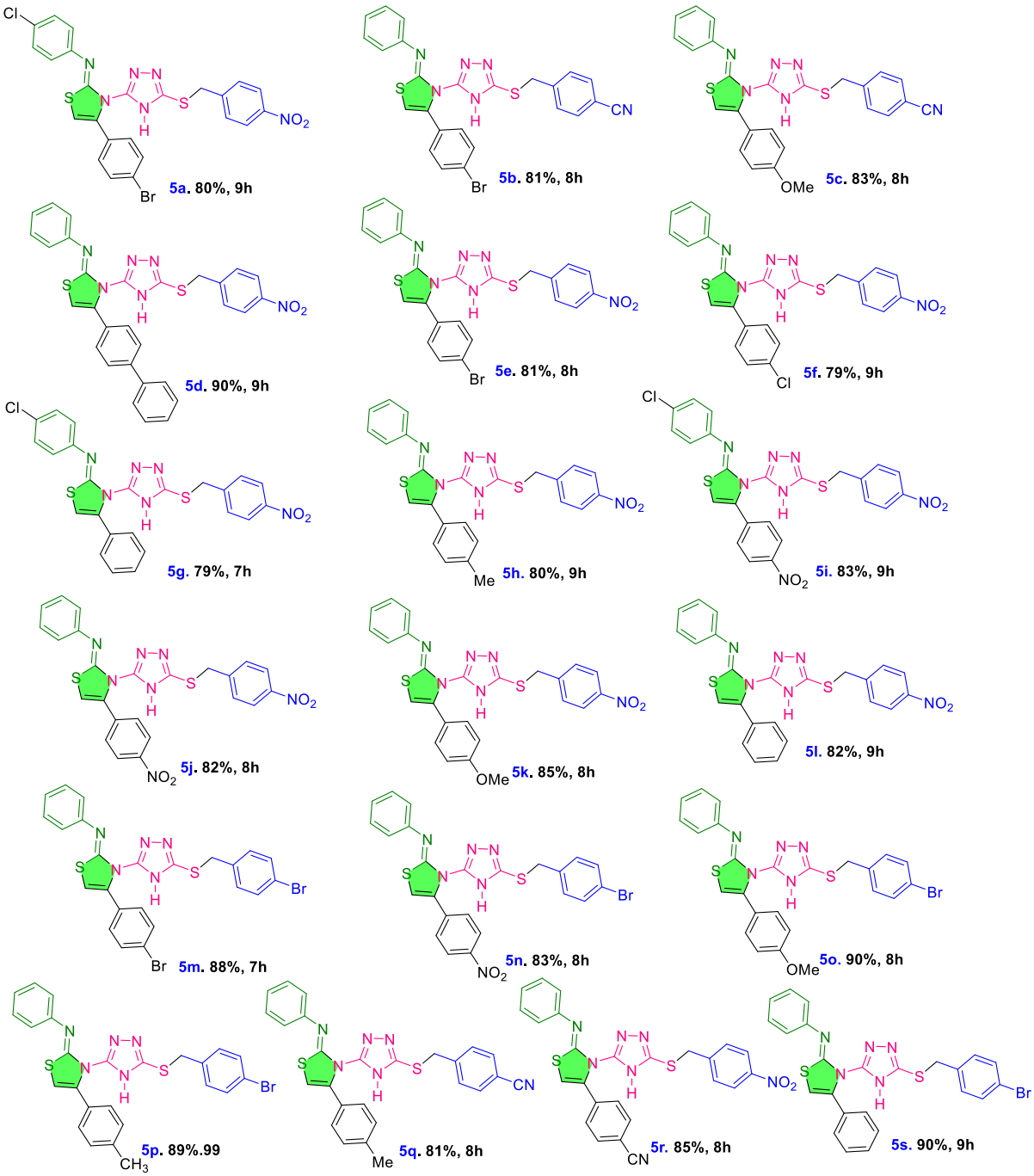


Fig.2. The plausible mechanism for the formation of compounds 5a-s.

Plausible mechanism for the formation of compound 5 is depicted in **Fig.2**. The molecule 1 has both amino and thiol reacting functional groups in which the more nucleophilic thiol group (Soft nucleophile) attacks on bromine atom of benzyl bromide (soft electrophilic centre) to form a thioalkylated product which follows SN^1 reaction mechanism with the elimination of HBr. Then the free amino (NH_2) group lone pair electrons attacks on the carbon atom of phenyl isothiocyanate to build a phenyl thiourea intermediate A. Subsequently the thiol group of A displaces the bromine of 2-bromoacetophenone to give intermediate B followed by intramolecular cyclocondensation reaction through the elimination of water molecule to produce a thiazole five membered heterocyclic ring. The advantage of this reaction is that there is a simultaneous formation of two C-N and one C-S bonds.

Table-2. One-pot four component synthesis of triazolo thiazole substrate scope.





Reaction conditions: 5-amino-4*H*-1,2,4-triazolo-3-thiol 1 (1 mmol), benzyl bromides 2 (1 mmol), Phenylisothiocyanates 3 (1 mmol), Phenacylbromides 4 (1 mmol), in EtOH + DMF refluxed at 85 ˚C.

Under the optimized reaction conditions, a study on the substrate scope was carried out and the results were presented in the **Table-2**. It can be seen from the reaction that a wide range of phenacyl bromides and aryl isothiocyanates are most suitable for this 4 component reaction. Phenacyl bromides and benzyl bromides tethered with both electron withdrawing and electron donating substrates gave the desired products. In general, when we employed electron withdrawing groups on phenacylbromide and benzyl bromide the yields were higher compared to electron withdrawing groups. The *p*-OCH_3 and biphenyl substituted derivatives 5o, 5d, 5s has shown high yield of the product.

The structures of the newly synthesised compounds (**5a-s**) were established by their physical and analytical data. The IR spectra of the compounds **5a-s** showed the presence of amine (N-H), alkene C-H, imine (C=N), nitro (NO_2), ether (C-O-C), and halo (C-Cl, C-Br) functional groups in the range of 3300 – 3421 cm^{-1}, 3021-3105 cm^{-2}, 1600-1640 cm^{-1}, NO_2 (asymmetric 1530-1544 cm^{-1}, symmetric 1320-1350 cm^{-1}), 1100-1250 cm^{-1}, and 680-721 cm^{-1} respectively. The proton NMR spectra of the thio alkylated methylene two protons appear as a singlet in the range of 4.42–4.49 δ ppm, the newly formed characteristic aromatic thiazole ring one singlet proton appears in the range of 5.44 –7.77 δ ppm and also the methoxy protons appear in the range of 3.60–3.69 δ ppm, whereas the triazole ring N-H singlet proton appears in the range of 12.95–13.06 δ ppm. All aromatic protons appear in the range of 7.10–8.18 δ ppm. Further, the carbon NMR spectra of **5a-s** the characteristic thiazole ring alkene carbon appears at 102.9-104.9 δ ppm, the thiazole ring imine carbon appears in the range of 160.0-165.4 δ ppm, -S-CH_2- carbon appears in the range of 34.44-35.89 δ ppm, while the aromatic carbons appear at 120.5-158.9 δ ppm. Molecular mass of all the compounds were matched with their $[M+H]^+$ ion peaks

4.3. Antiviral activity.

3-(5-(benzylthio)-4H-1,2,4-triazol-3-yl)-N,4-diphenylthiazol-2(3H)-imines were tested for their potential antiviral activity in various host cell cultures. Their activity has been compared to that of standard antiviral drugs. (AMD3100, Remdesivir, Ribavirin, Zanamivir, Rimantadine, Acyclovir, DS-10,000). The MT-4 $CD4^+$ T cell culture was used to evaluate the compounds against human immune deficient virus (HIV) The virus-induced cytopathogenic

effect (CPE) was measured colorimetrically AMD3100 was used as a reference drug. The compounds **5a** and **5i** exhibited potent activity against both HIV-1(NL4.3 strain) and HIV-2 (ROD strain). Seven other derivatives **5c**, **5e**, **5f**, **5k**, **5o**, **5q**, **5r** showed promising selective anti HIV-2 activity with EC_{50} values below 10 μM. The summary of 50% cytotoxic concentration (CC_{50}) and 50% effective concentration (EC_{50}) values are represented in **Table-3.**

Table-3. EC_{50}[a] and CC_{50}[b] values of the compounds tested against HIV replication in MT-4 $CD4^+$ T cell line

Compound	**Cellular toxicity**	**HIV-1**	**HIV-2**
		NL4.3	**ROD**
	CC_{50} (μM)	EC_{50} (μM)	EC_{50} (μM)
5a	>100	**6.8**	24.3
5b	>100	>100	11
5c	>100	>100	2.7
5d	58.9	>58.9	>58.9
5e	>100	>100	9.4
5f	>100	>100	7.8
5g	>100	>100	15.5
5h	>100	>100	61.7
5i	>100	**3.7**	21.3
5j	>100	>100	>100
5k	>100	>100	**1.8**
5l	44.7	>44.7	>44.7
5m	46.3	>46.3	>46.3
5n	44.9	>44.9	>44.9
5o	>100	>100	8.5
5p	>100	>100	>100
5q	>100	>100	2.2
5r	>100	>100	5.1
5s	>100	>100	72.4
Reference compound	CC_{50} (ng/mL)	EC_{50} (ng/mL)	EC_{50} (ng/mL)

AMD3100	>10000	9.9	3.9

[a] EC_{50}: 50% Inhibitory concentration or compound concentration required to inhibit HIV-induced cytopathogenic effects by 50% in MT-4 cell line.

[b] CC_{50}: 50% Cytotoxic concentration of the compounds also evaluated in the MT-4 cell line.

In addition to HIV, we also evaluate the whole set of newly synthesized derivatives (**5a-s**) against a broad set of viruses. Human coronaviruses 229E and OC43, as well as herpes simplex virus type 1 (HSV-1 strain KOS) were tested using HEL 299 cell cultures. Here remdesivir was used as a reference drug for human coronavirus and acyclovir and dextran sulphate (MW 10,000) were included as reference compounds for HSV-1. **5h** exhibited weak but selective activity against HCoV-OC43. Unfortunately, the tested compounds proved inactive against HCoV-229E and HSV-1 virus. All experimental EC_{50} and CC_{50} values are summarised in **Table-4**.

Table-4: Cytotoxicity and antiviral activity of the compounds in HEL 299 cell culture

Compound	**Cytotoxicity[a]** **CC_{50} (µM)**	**EC_{50}[b] (µM)**		
		Human coronavirus (229E)	**Human coronavirus (OC43)**	**Herpes simplex virus-1 (KOS)**
5a	50	>50	>50	>50
5b	50	>50	>50	>50
5c	47.3	>50	>50	>50
5d	50	>50	>50	>50
5e	>50	>50	>50	>50
5f	>50	>50	>50	>50
5g	>50	>50	>50	>50
5h	>50	>50	**43.3**	>50
5i	>50	>50	>50	>50
5j	>50	>50	>50	>50
5k	>50	>50	>50	>50
5l	>50	>50	>50	>50
5m	>50	>50	>50	>50

5n	37.4	>50	>50	>50
5o	25.6	>50	>50	>50
5p	>50	>50	>50	>50
5q	>50	>50	>50	>50
5r	>50	>50	>50	>50
5s	>50	>50	>50	>50
Remdesivir	>10	0.16	0.12	-
Acyclovir	>100	-	-	0.7
DS-10,000	>100	-	-	0.4

[a] CC_{50}: 50% Cytotoxic concentration of the compound evaluated in HEL 299 cell culture

[b] EC_{50}: 50% Effective concentration or compound concentration required to inhibit human coronavirus and herpes simplex virus-1 induced cytopathogenic effect by 50% in HEL 299 cell culture.

The human hepatoma cell line Hep3B was used to test against human coronavirus NL63, Zika virus (strain mr766) and yellow fever virus (vaccine strain 17D). Remdesivir and ribavirin are included as reference compounds. Among the tested compounds derivative **5k** showed mild activity against human coronavirus NL63 (EC_{50} 49.1 µM). Compound **5f** displayed promising activity against the replication of Zika virus, with an average EC_{50} value of 9.3 µM. All compounds (**5a-s**) were inactive against yellow fever virus. The summarized EC_{50} and CC_{50} values are shown in **Table-5.**

Table-5: Cytotoxicity and antiviral activity of the compounds in Hep3B cell culture.

Compound	**Cytotoxicity[a] CC_{50} (µM)**	**EC_{50}[b] (µM)**		
		Human coronavirus NL63	**Zika virus mr766**	**Yellow fever virus 17D**
5a	>50	>50	>50	>50
5b	8.1	>50	>50	>50
5c	34.3	>50	>50	>50
5d	20.7	>50	>50	>50
5e	36.9	>50	>50	>50
5f	>50	>50	**9.3**	>50

5g	34.1	>50	>50	>50
5h	24.6	>50	>50	>50
5i	>50	>50	>50	>50
5j	39.1	>50	>50	>50
5k	>50	**49.1**	>50	>50
5l	>50	>50	>50	>50
5m	>50	>50	>50	>50
5n	20.8	>50	>50	>50
5o	13.7	>50	>50	>50
5p	9	>50	>50	>50
5q	>50	>50	>50	>50
5r	26.5	>50	>50	>50
5s	>50	?50	>50	>50
AMD3100	>50	>50	>50	>50
Remdesivir	>10	0.28	2.85	1.1
Ribavirin	143.1	-	49.1	89.4

[a] Cytotoxic concentration (50%) determined by measuring the cell viability with colorimetric formazan-based MTS assay.

[b]50% Effective concentration or compound concentration required to inhibit virus induced cytopathogenic effect by 50% in Hep3B cell culture.

Lastly, we also tested the compounds against three subtypes of influenza virus (H1N1, H3N2 and B) and respiratory syncytial virus (RSV A strain Long) using MDCK and Hep2 cell cultures, respectively. Standard reference drugs remdesivir, ribavirin, zanamivir, rimantadine, and DS-10,000 were included as positive controls. Unfortunately, the derivatives **5a-s** did not show any activity against influenza viruses or respiratory syncytial virus in these host cell lines. EC_{50} and CC_{50} values are represented in **Table-6**.

Table-6: Cytotoxicity (CC_{50}) and antiviral activity (EC_{50}) of the compounds in MDCK and Hep2 cell cultures.

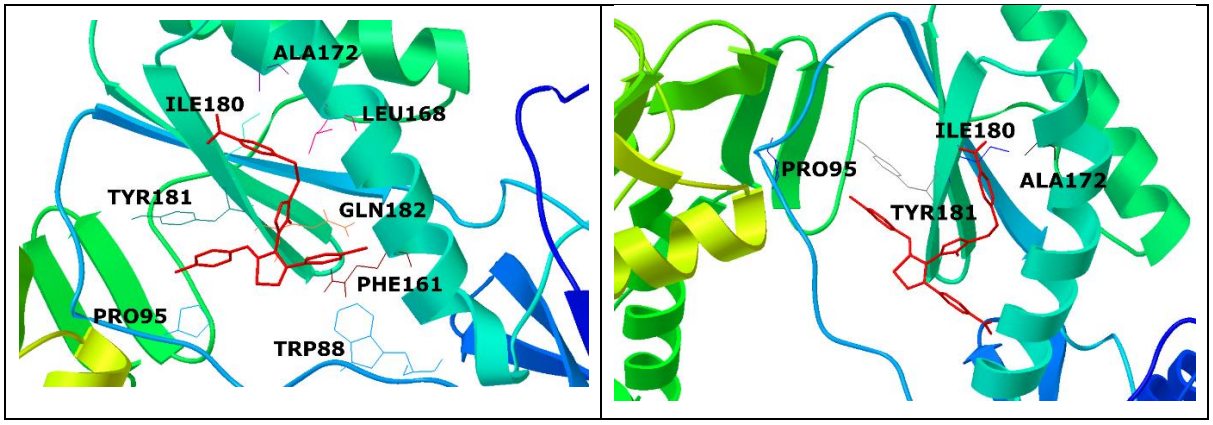
Compound	Cytotoxicity[a] CC_{50} (µM)		EC_{50}[b] (µM)			
	MDCK	Hep2	Influenza H_1N_1 MDCK	Influenza H_3N_2 MDCK	Influenza B MDCK	RSV A Long Hep2
5a	>50	>50	>50	>50	>50	>50
5b	>50	>50	>50	>50	>50	>50
5c	>50	44.1	>50	>50	>50	>50
5d	29	>50	>50	>50	>50	>50
5e	>50	>50	>50	>50	>50	>50
5f	>50	>50	>50	>50	>50	>50
5g	>50	>50	>50	>50	>50	>50
5h	17	20.1	>50	>50	>50	>50
5i	>50	>50	>50	>50	>50	>50
5j	>50	>50	>50	>50	>50	>50
5k	1.12	>50	>50	>50	>50	>50
5l	>50	>50	>50	>50	>50	>50
5m	>50	>50	>50	>50	>50	>50
5n	20.8	19.2	>50	>50	>50	>50
5o	23.3	28	>50	>50	>50	>50
5p	>50	>50	>50	>50	>50	>50
5q	>50	>50	>50	>50	>50	>50
5r	>50	>50	>50	>50	>50	>50
5s	>50	>50	>50	>50	>50	>50
Remdesivir	-	>10	-	-	-	0.08
Ribavirin	98.2	98.3	8.2	11.1	8.4	6.8
Zanamivir	>100	-	0.15	0.15	0.10	-
Rimantadine	>100	-	0.02	0.09	>100	-
DS-10,000	-	>100	-	-	-	0.07

[a]50% cytotoxic concentration of the compound evaluated in MDCK and Hep2 cell cultures

[b] Required to reduce virus induced cytopathicity by 50%.

4.4. Structure-based molecular docking

The X-ray crystallographic structure of HIV-1 reverse transcriptase (PDB: 4h4m) was considered as the receptor protein *en route* to analyse the bio-activity of 5a and 5i. The docked pose of these compounds along with the interacting amino acids at the active site of the receptor protein are depicted in **Fig.3.** The calculated binding affinity value for both the compounds is -8.3 kcal/mol which may be attributed due to analogy in the structures of the two compounds. Both the compounds show hydrogen bonding interactions with ILE180 at the active site of the protein. The compound 5a shows hydrophobic interactions with TRP88, PRO95, GLN161, LEU168, ALA172, TYR181, and GLN182 whereas 5i interacts with PRO95, ALA172, and TYR181.



5a **5i**

Fig. 3. The docked pose and the interacting amino acids, compound 5a and compound 5i at the active site of HIV-1 reverse transcriptase (4h4m).

The compound 5k was analysed for the anti-HIV-2 activity using the crystal structure of HIV-2 reverse transcriptase (PDB: 1mu2) as a receptor protein. The docked pose of the compound at the active site along with the interacting amino acids is displayed in **Fig. 4**. The 5k shows hydrogen bonding interactions with the TRP266 and hydrophobic interactions with GLN269, THR328, LYS340, LEU421, and VAL422. Further, the calculated binding affinity value of 5k is found to be -8.0 kcal/mol.

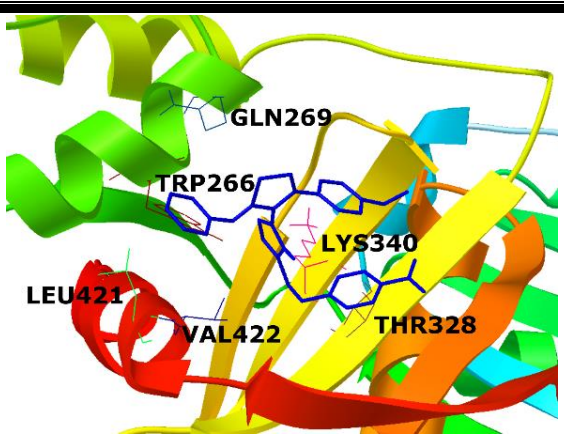


Fig. 4. The docked pose and the interacting amino acids, compound **5k** at the active site of HIV-2 reverse transcriptase (1mu2),

The X-ray crystal structure of Zika NS2B-NS3 protease (6l50) was used to study the anti Zikavirus activity of the compound 5f. **Fig.5** depicts the docked pose of the compound 5f at the active site of the protein. It has been observed that the compound shows hydrogen bonding with ASN152 and hydrophobic interactions with GLN74, ALA88, TRP89, ILE123, THR166, LYS169, and ARG170. The calculated binding affinity value of the compound 5f at the active site of the Zika NS2B-NS3 protease is -7.9 kcal/mol.

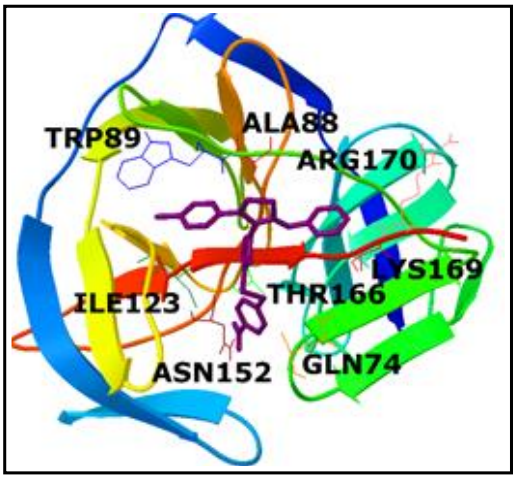
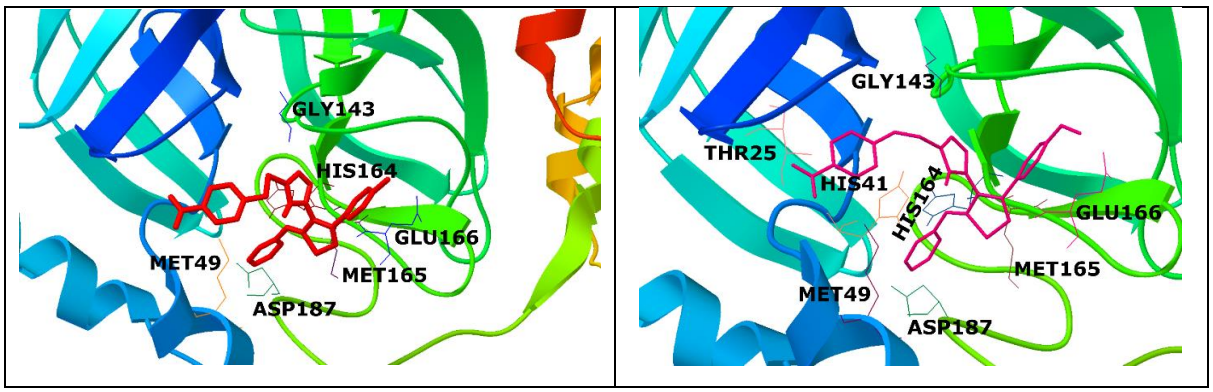


Fig.5. Compound **5f** at the active site of Zika NS2B-NS3 protease (6l50).

The anti-SARS-CoV-2 properties were studied for the compounds 5h and 5k using the crystallographic structure of COVID-19 main protease (PDB: 6lu7) as the receptor protein. Both these compounds have the same binding affinity of -8.5 kcal/mol at the active site which may be due to the similarity in their structures. The best docking pose of these compounds along with the interacting amino acids at the active site of the protein are presented in **Fig.6** The compound 5h show hydrophobic interactions with MET49, GLY143, HIS164, MET165,

GLU166, which ASP187 while 5k interacts with THR25, HIS41, MET49, GLY143, HIS164, MET165, GLU166, and ASP187 residues of the protein. These results demonstrate that the compounds 5h and 5k could serves as a potential candidate against SARS-CoV-2 main protease.



5h **5k**

Fig 6. The docked pose and the interacting amino acids, compound 5h and compound 5k at the active site of COVID-19 main protease (PDB: 6lu7).

To further shed light on the binding affinity of other compound for its anti-viral activities, the molecular docking simulations were also performed for compound 5p. The calculated binding affinity of compound 5p is -6.6, -6.8, -7.6, and -7.9 kcal/mol with the COVID-19 main protease, Zika NS2B-NS3 protease, HIV-1 reverse transcriptase, and HIV-2 reverse transcriptase, respectively. It has been noticed that the compound shows lower affinity and thus less effective as anti-SARS-CoV-2 and anti-Zika virus agent. However, the calculated binding score for HIV proteins are higher and comparatively closer to the best compounds. This may be mainly attributed due to the marginal structural variation and these derivatives may be well-suited as an anti-HIV agent.

The *N*-atom of triazole ring in compound 5p shows the hydrogen bonding interaction with GLN110 (2.16 Å), Br atom shows halogen bonding with ARG105 (3.97 Å) of the COVID-19 main protease. The C atoms in phenyl ring shows hydrophobic interactions with ILE106 (3.69 Å), GLN107 (3.60 Å), ILE249 (3.72 Å), PRO293 (3.87Å), and PHE294 (3.69 Å). The C atoms of the phenyl ring shows hydrophobic interactions with GLN74 (3.54 Å), LEU78 (3.71 Å), ALA88 (3.90 Å), TRP89 (3.26 Å), ILE123 (3.02 Å), ILE165 (3.55 Å), and GLN167 (3.52 Å) of the Zika NS2B-NS3 protease. The bromo-phenyl ring shows π-stacking interactions with the TYR319 (5.27 Å) and C atoms of the phenyl rings shows hydrophobic interactions with ALA101 (3.84 Å), HIS317 (3.85 Å), LEU348 (3.81) Å), and TRP382 (3.45 Å) residues of the

HIV-2 reverse transcriptase. The N atom of *N*-ring shows hydrogen bonding with the GLN91 (2.28 Å), benzyl group shows π-stacking interactions with the TYR181 (3.76 Å), and Br atoms show halogen bonding interaction with HIS96 (3.38 Å). Mostly, the C atoms in the phenyl ring shows hydrophobic interactions with the PRO95 (3.62 Å), LEU100 (3.67 Å), GLN161 (3.73 Å), and GLN182 (3.73 Å) residues of the HIV-1 reverse transcriptase.

4.5. Conclusions:

The synthesis of novel thioalkylated triazolothiazoles **5a-s** was carriedout by a one-pot four component method using easily available starting materials. Compounds **5a-s** were screened against a broad panel of viruses, such as HIV-1, HIV-2, coronavirus, Zika virus, RSV, influenza virus and yellow fever virus by using different host cell lines. Two newly synthesized derivatives possess potent activity against both HIV-1 and HIV-2, and seven others were selectively active against HIV-2. Of these, **5f** also displayed promising activity against Zika virus. The two compounds **5k** (**Table-5**) **5h (Table-4)** show moderate active against corona virus. All presented data show that these novel compounds are promising candidates for further optimization in order to developed promising antiviral agents.

4.6. Experimental:

4.6.1. Chemistry

All the starting materials and solvents were used i.e., Merk, Spectrochem, TCI, Finar, and used without purification. The solvents were stored in $4A^0$ molecular sieves. The progress of the reaction was monitored by thin layer chromatography (TLC) with silica gel coated aluminium foil plates (E. Merck, Mumbai, India) using ethyl acetate and n-hexane (3:7) ratio. The compounds' melting points were checked with the Stuart Staffordshire, UK (SMP30) instrument and are uncorrected. A Perkin Elmer spectrometer was used to record FT-IR spectra, KBr was used as a standard reference compound, and values were given in cm^{-1}. ^1H NMR spectra were recorded on a BRUCKER 400 MHz spectrophotometer, TMS was used as an internal standard reference compound and DMSO-d_6 was used as a solvent. The splitting pattern of the protons was represented on s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, and chemical shift values (δ) were expressed in ppm reference to quintet for DMSO-d_6 at 2.5 ppm, triplet for $CDCl_3$ at 7.26 ppm, and coupling constant units (*J*) were represented with Hz. Broad band proton decoupled carbon NMR spectra was recorded on the BRUCKER 100 MHz spectrophotometer and chemical shift values were shown on δ ppm. The peak appears as a septet for DMSO-d_6 at 39.7 ppm and a triplet for $CDCl_3$ solvent at 77.16. The Carlo Erba

EA 1108 instrument was used for C, H, N elemental analysis. Mass spectrum (HRMS) were recorded on the Agilent Technologies Instrument ESI (+Ve mode).

4.6.2. Antiviral assays

Antiviral assays were performed towards Herpes simplex virus-1 (HSV-1 KOS), and human coronavirus (HCoV-229E and -OC43) in HEL 299 cell cultures, respiratory syncytial virus A in Hep-2 cells, yellow fever virus, Zika virus and human coronavirus (HCoV-NL63) in Hep3B cell cultures and influenza A/H1N1 (A/Ned/378/05), influenza A/H3N2 (A/HK/7/87), influenza B (B/Ned/537/05) in MDCK cell cultures. On the day of the infection, growth medium was aspirated and replaced by serial dilutions of the test compounds. The virus was then added to each well, diluted to obtain a viral input of 100 $CCID_{50}$ ($CCID_{50}$ being the virus dose that is able to infect 50% of the cell cultures). Mock-treated cell cultures receiving solely the test compounds were included on each cell line, to determine the cytotoxicity of the test compounds. After 3 to 7 days of incubation, the virus-induced cytopathogenic effect (CPE) was measured colorimetrically by the formazan-based 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) cell viability assay (CellTiter 96 AQueous One Solution Cell Proliferation Assay from Promega, Madison, WI), and the antiviral activity was expressed as the 50% effective concentration (EC_{50}). In parallel, the 50% cytotoxic concentration (CC_{50}) of the compounds was derived from the mock-infected cells. The activities were compared with the activities of reference antiviral drugs: AMD3100, remdesivir, ribavirin, zanamivir, rimantadine, acyclovir and Dextran sulfate (DS-10,000).

The anti-HIV-1 and anti-HIV-2 activity of each compound were evaluated in MT-4 $CD4^+$ T cell cultures was determined by a tetrazolium-based colorimetric assay. Briefly, 3-fold dilutions of various test compounds were added in a 96-well plate and preincubated for 20 min at 37°C with MT-4 cells (1×10^6 cells/ml). Next, various concentrations of virus, HIV-1 NL4.3 and HIV-2 ROD were given depending on the $TCID_{50}$ of the virus stock. Five days' post-infection, cytopathic effects (CPE) were scored microscopically and antiviral activity was measured by MTS/PES using a Spectramax 96-well plate reader (Molecular Devices) as described previously [63].

4.6.3. Docking study.

In silico molecular docking simulations were performed for the synthesized compounds that have exhibited the best antiviral properties in the biological activity analysis. The molecular docking simulations were carried out using Auto Dock Vina with an effectiveness value of 16

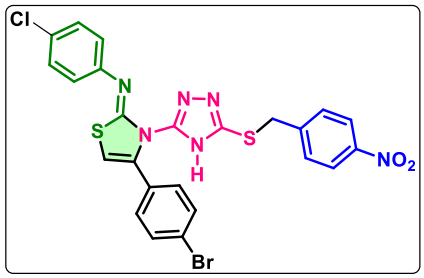
[64]. The receptor viral proteins were obtained from the protein data bank; the bound ligand and water molecules were removed, and the polar hydrogens were added to facilitate docking

4.6a. General procedure for the synthesis of thiobenzyl 1,2,4-triazole phenyiimino thiazoles

5-Amino-4-1,2,4-triazole-3-thiol (1 mmol), *p*-bromo benzyl bromide (1 mmol), Phenylisothiocyanate (1 mmol) and phenacyl bromide (1 mmol) were taken in ethanol 4mL and added 1 mL of DMF and refluxed for 9 hours at 85 °C. After completion of the reaction (check by the TLC) the reaction mixture was poured in to ice water. The white color solid was isolated and recrystalized from 5-8 mL ethanol.

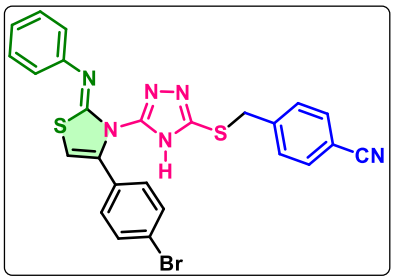
4.7. Characterization data of synthesized compounds.

(Z)-4-(4-Bromophenyl)-N-(4-chlorophenyl)-3-(5-((4-nitrobenzyl)thio)-4H-1,2,4-triazol-3yl)thiazol-2(3H)-imine 5a



White solid; Yield 80%; mp: 237-238 °C; IR (KBr) cm^{-1}: 3403 (N-H), 3034 (Alkene C-H), 1598 (Imine C=N), 1540, 1344 (Unsymmetric, Symmetric NO_2); ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 4.48 (s, 2H), 6.96 (s, 1H), 7.16 (d, J = 8.5 Hz, 2H), 7.26 – 7.27 (m, 1H), 7.43 (d, J = 5.7 Hz, 2H), 7.50 (d, J = 8.5 Hz, 2H), 7.61 (s, 1H), 7.70 (d, J = 8.7 Hz, 2H), 8.17 (d, J = 8.7 Hz, 2H), 13.09 (s, 1H); $^{13}C\{^1H\}$NMR (100MH_Z, DMSO-d_6) δ (ppm): 34.33, 104.94, 122.35, 123.52, 128.12, 128.89, 129.53, 129.63, 130.02, 130.76, 130.93, 131.35, 133.27, 137.15, 138.55, 146.51, 147.24, 155.12, 157.75, 164.50. HRMS (ESI-TOF) (*m/z*): Calculated for $C_{24}H_{16}BrClN_6O_2S_2$ $[M+H]^+$ 598.9726; found 598.9727; C, H, N Analysis calculated C, 48.05, H, 2.69, N, 14.01; found; C, 48.09, H, 2.71, N, 14.05.

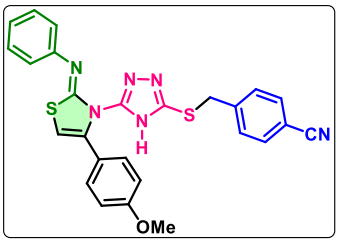
(Z)-4-(((5-(4-Bromophenyl)-2-(phenylimino)thiazol-3(2H)-yl)-4H-1,2,4-triazol-3-yl)methyl)benzonitrile 5b



White solid; Yield 81%; mp: 240-241 °C; IR (KBr) cm^{-1}: 3387 (N-H), 3066 (Alkene C-H), 2225 (CN), 1601 (Imine C=N), 691 (C-Br);^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 4.42 (s, 2H), 6.95 (s, 1H), 7.11 (d, J = 8.5 Hz, 2H), 7.30 – 7.33 (m, 2H), 7.37-7.45 (m, 5H), 7.61 (d, J = 8.3 Hz, 2H), 7.77 (d, J = 8.3 Hz, 2H), 13.04 (s, 1H); $^{13}C\{^1H\}$ NMR (100MH_Z, DMSO-d_6) δ (ppm): 35.48, 104.91, 110.37, 119.09, 122.53, 128.95, 129.58, 129.65, 130.17, 130.48, 131.61, 132.62, 137.94, 137.99, 145.34, 164.34. HRMS (ESI-TOF) (*m/z*):

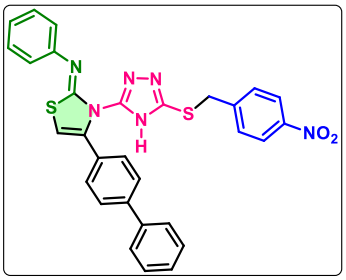
Calculated for $C_{25}H_{17}BrN_6S_2$ $[M+H]^+$ 545.0217; found 545.0219; C, H, N Analysis calculated C, 55.05, H, 3.14, N, 15.41; found; C, 55.09, H,3.17, N,15.45.

(Z)-4-(((5-(4-Methoxyphenyl)-2-(phenylimino)thiazol-3(2H)-yl)-4H-1,2,4-triazol-3-yl)methyl)benzonitrile 5c



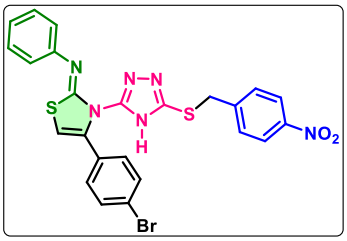
White solid; Yield 83%; mp: 233-234 °C; IR (KBr) cm^{-1}: 3210 (N-H), 3021 (Alkene C-H), 2219 (CN), 1601 (Imine C=N), 1210 ($O-CH_3$); ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 3.68 (s, 3H), 4.42 (s, 2H), 6.77 (s, 1H), 6.79 (s, 2H), 7.08 (d, J = 8.9 Hz, 2H), 7.28 – 7.40 (m, 5H), 7.61 (d, J = 8.3 Hz, 2H), 7.76 (d, J = 8.4 Hz, 2H), 13.00 (s, 1H); $^{13}C\{^1H\}$ NMR (100MH_Z, DMSO-d_6) δ (ppm): 35.03, 55.58, 103.39, 110.17, 114,07, 119.40, 123.34, 128.94, 129.60, 129.74, 130.21, 130.65, 132.74, 138.07, 139.01, 145.54, 155.50, 158.41, 159.75, 165.07. HRMS (ESI-TOF) (m/z): Calculated for $C_{26}H_{20}N_6OS_2$ $[M+H]^+$ 497.1218; found 497.1221; C, H, N Analysis calculated C, 62.88, H, 4.06, N, 16.92; found; C, 62.87, H,4.08, N,16.88.

(Z)-4-([1,1'-Biphenyl]-4-yl)-3-(5-((4-nitrobenzyl)thio)-4H-1,2,4-triazol-3-yl)-N-phenylthiazol-2(3H)-imine 5d



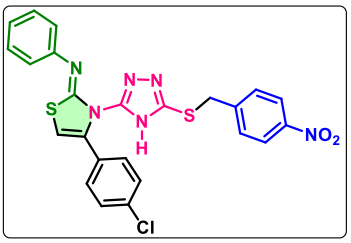
White solid; Yield 90%; mp: 251-252 °C; IR (KBr) cm^{-1}: 3040 (N-H), 3040 (Alkene C-H), 1621 (Imine C=N), 1560, 1343 (NO_2 Unsymmetric, Symmetric); ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 4.49 (s, 2H), 6.96 (s, 1H), 7.25 (d, J = 8.5 Hz, 2H), 7.35-7.36 (m, 4H), 7.41 – 7.45 m, 4H), 7.55 (d, J = 8.5 Hz, 2H), 7.62 (d, J = 7.1 Hz, 2H), 7.70 (d, J = 8.8 Hz, 2H), 8.17 (d, J = 8.8 Hz, 2H), 13.05 (s, 1H); $^{13}C\{^1H\}$NMR (100MH_Z, DMSO-d_6) δ (ppm): 34.79, 104.68, 123.94, 126.74, 127.00, 128.30, 129.06, 129.45, 129.68, 130.07, 130.44, 137.99, 138.78, 139.31, 140.44, 146.92, 147.68, 165.07. HRMS (ESI-TOF) (m/z): Calculated for $C_{30}H_{22}N_6O_2S_2$ $[M+H]^+$ 563.1324; found 563.1327; C, H, N Analysis calculated C, 64.04, H, 3.94, N, 14.94; found; C, 64.08, H,3.98, N,14.90.

(Z)-4-(4-Bromophenyl)-3-(5-((4-nitrobenzyl)thio)-4H-1,2,4-triazol-3-yl)-N-phenylthiazol-2(3H)-imine 5e



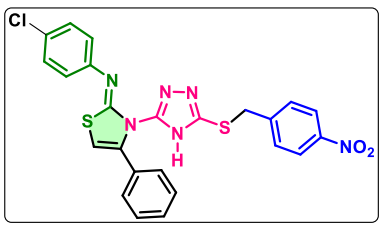
White solid; Yield 81%; mp: 253-254 °C; IR (KBr) cm^{-1}: 3201 (N-H), 3006 (Alkene C-H), 1605 (Imine C=N), 1540, 1345 (Unsymmetric, Symmetric NO_2), 684 (C-Br); ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 4.48 (s, 2H), 6.96 (s, 1H), 7.12 (d, J = 8.8 Hz, 2H), 7.32 (d, J = 6.8 Hz, 2H), 7.40-7.46 (m, 3H), 7.64 (d, J = 8.4 Hz, 1H), 7.70 (d, J = 8.8 Hz, 1H), 7.81 (d, J = 8.8 Hz, 1H), 7.94 (d, J = 8.4 Hz, 1H), 8.17 (d, J = 8.4 Hz, 2H), 13.05 (s, 1H); $^{13}C\{^1H\}$NMR (100MH_Z, DMSO-d_6) δ (ppm): 34.78, 105.09, 122.57, 123.94, 129.12, 129.65, 129.71, 130.44, 131.23, 131.65, 132.42, 137.72, 137.94, 146.93, 146.66, 157.77, 164.95. HRMS (ESI-TOF) (*m/z*): Calculated for $C_{24}H_{17}BrN_6O_2S_2$ $[M+H]^+$ 565.0116; found 565.0124; C, H, N Analysis calculated C, 50.98, H, 3.03, N, 14.86; found; C, 50.95, 3.07, N, 14.88.

(Z)-4-(4-Chlorophenyl)-3-(5-((4-nitrobenzyl)thio)-4H-1,2,4-triazol-3-yl)-N-phenylthiazol-2(3H)-imine 5f



White solid; Yield 79%; mp: 238-239 °C; IR (KBr) cm^{-1}: 3420 (N-H), 3105 (Alkene C-H), 1584 (Imine C=N), 1542, 1347 (Unsymmetric, Symmetric NO_2), 697 (C-Cl); ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 4.48 (s, 2H), 6.95 (s, 1H), 7.18 (d, J = 8.4 Hz, 2H), 7.30-7.35 (m, 5H), 7.41 (d, J = 7.6 Hz, 2H), 7.70 (d, J = 8.4 Hz, 2H), 8.16 (d, J = 8.4 Hz, 2H), 13.05 (s, 1H); $^{13}C\{^1H\}$ NMR (100MH_Z, DMSO-d_6) δ (ppm): 34.75, 105.12, 123.94, 128.73, 129.11, 129.66, 129.70, 129.94, 130.44, 131.03, 133.89, 137.74, 137.89, 146.93, 147.70, 155.46, 158.33, 164.96. HRMS (ESI-TOF) (*m/z*): Calculated for $C_{24}H_{17}ClN_6O_2S_2$ $[M+H]^+$ 521.0621; found 521.0626; C, H, N Analysis calculated C, 55.33, H, 3.29, N, 16.13; found; C,55.30, H, 3.26, N,16.17.

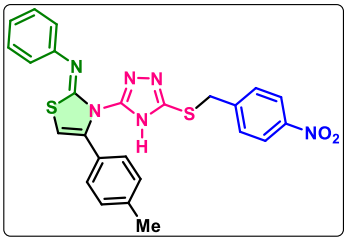
(Z)-N-(4-Chlorophenyl)-3-(5-((4-nitrobenzyl)thio)-4H-1,2,4-triazol-3-yl)-4-phenylthiazol-2(3H)-imine 5g



White solid; Yield 79%; mp: 231-232 °C; IR (KBr) cm^{-1}: 3370 (N-H), 3033 (Alkene C-H), 1559 (Imine C=N), 1522, 1347 (Unsymmetric, Symmetric NO_2), 780 (C-Cl); ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 4.48 (s, 2H), 6.90 (s, 1H), 7.19-7.21 (m, 2H), 7.27 – 7.29 (m, 4H), 7.40 – 7.41 (m, 2H),

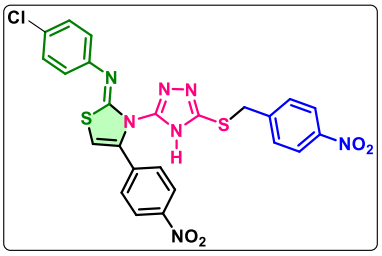
7.55-7.56 (m, 1H), 7.70 (d, *J* = 8.8 Hz, 2H), 8.17 (d, *J* = 8.8 Hz, 2H), 13.07 (s, 1H); $^{13}C\{^{1}H\}$ NMR (100MHz, DMSO-d_6) δ (ppm): 34.77, 107.78, 123.92, 128.42, 128.58, 128.76, 129.31, 129.46, 129.86, 130.37, 130.43, 131.06, 131.29, 133.61, 133.85, 136.72, 137.03, 138.76, 139.17, 146.93, 147.58, 155.63, 158.06, 164.85. HRMS (ESI-TOF) (*m/z*): Calculated for $C_{24}H_{17}ClN_6O_2S_2$ $[M+H]^+$ 521.0621; found 521.0623; C, H, N Analysis calculated C, 55.33, H, 3.29, N, 16.13; found; C, 55.36, H, 3.31, N, 16.16.

(Z)-3-(5-((4-Nitrobenzyl)thio)-4H-1,2,4-triazol-3-yl)-N-phenyl-4-(p-tolyl)thiazol-2(3H)-imine 5h



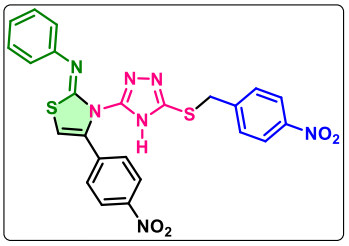
White solid; Yield 80%; mp: 234-235 °C; IR (KBr) cm^{-1}: 3422 (N-H), 3045 (Alkene C-H), 1601 (Imine C=N), 1542, 1347 (Unsymmetric, Symmetric NO_2); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.21 (s, 3H), 4.47 (s, 2H), 6.83 (s, 1H), 7.04 (s, 4H), 7.28-7.39 (m, 5H), 7.69 (d, *J* = 8.7 Hz, 2H), 8.16 (d, *J* = 8.8 Hz, 2H), 13.01 (s, 1H); $^{13}C\{^{1}H\}$ NMR (100MHz, DMSO-d_6) δ (ppm): 21.15, 34.74, 103.94, 123.96, 128.23, 128.96, 129.08, 129.23, 129.60, 129.70, 130.45, 138.03, 138.58, 139.19, 146.94, 147.74, 155.41, 158.40, 165.10. HRMS (ESI-TOF) (*m/z*): Calculated for $C_{25}H_{20}N_6O_2S_2$ $[M+H]^+$ 501.1167; found 501.1168; C, H, N Analysis calculated C, 59.98, H, 4.03, N, 16.79; found; C, 59.65, H, 4.07, N, 16.76.

(Z)-N-(4-Chlorophenyl)-3-(5-((4-nitrobenzyl)thio)-4H-1,2,4-triazol-3-yl)-4-(4-nitrophenyl)thiazol-2(3H)-imine 5i



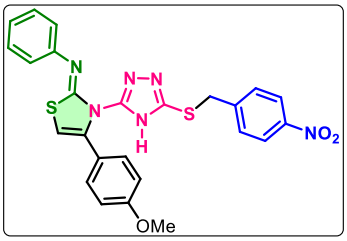
Yellow solid; Yield 83%; mp: 225-226 °C; IR (KBr) cm^{-1}: 3401 (N-H), 3058 (Alkene C-H), 1598 (Imine C=N), 1536, 1348 (Unsymmetric, Symmetric NO_2) 702 (C-Cl); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 4.49 (s, 2H), 7.17 (s, 1H), 7.43 – 7.49 (m, 4H), 7.65-7.71 (m, 4H), 8.13-8.18 (m, 4H), 13.14 (s, 1H); $^{13}C\{^{1}H\}$ NMR (100MHz, DMSO-d_6) δ (ppm): 34.76, 107.76, 123.91, 123.95, 128.45, 129.45, 129.91, 130.39, 130.44, 131.28, 133.82, `136.73, 137.06, 138.81, 146.95, 147.60, 155.61, 158.06, 164.83. HRMS (ESI-TOF) (*m/z*): Calculated for $C_{24}H_{16}ClN_7O_4S_2$ $[M+H]^+$ 566.0472; found 566.0478; C, H, N Analysis calculated C, 50.93, H, 2.85, N, 17.32; found; C, 5.93, H, 2.88, N,17.39.

(Z)-3-(5-((4-Nitrobenzyl)thio)-4H-1,2,4-triazol-3-yl)-4-(4-nitrophenyl)-N-phenylthiazol-2(3H)-imine 5j



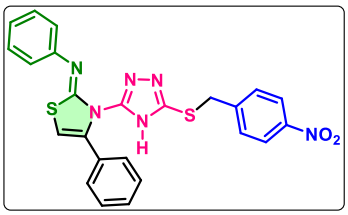
Yellow solid; Yield 82%; mp: 221-222 °C; IR (KBr) cm^{-1}: 3401 (N-H), 3033 (Alkene C-H), 1604 (Imine C=N), 1541, 1347 (Unsymmetric, Symmetric NO_2); ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 4.53 (s, 2H), 7.22 (s, 1H), 7.40-7.42 (m, 2H), 7.47-7.50 (m, 5H), 7.75 (d, J = 8.8 Hz, 2H), 8.14 (d, J = 8.9 Hz, 2H), 8.22 (d, J = 8.8 Hz, 2H), 13.10 (s, 1H); $^{13}C\{^1H\}$ NMR (100MH_Z, DMSO-d_6) δ (ppm): 34.79, 107.54, 123.77, 123.93, 129.27, 129.55, 129.83, 130.25, 130.44, 137.08, 137.28, 137.53, 146.93, 147.44, 147.59, 158.45, 164.82. HRMS (ESI-TOF) (m/z): Calculated for $C_{24}H_{17}N_7O_4S_2$ $[M+H]^+$ 532.0861; found 532.0867; C, H, N Analysis calculated C, 54.23, H, 3.22, N, 18.45; found; C,54.26, H, 3.27, N, 18.48.

(Z)-4-(4-Methoxyphenyl)-3-(5-((4-nitrobenzyl)thio)-4H-1,2,4-triazol-3-yl)-N-phenylthiazol-2(3H)-imine 5k



White solid; Yield 85%; mp: 247-248 °C; IR (KBr) cm^{-1}: 3204 (N-H), 3131 (Alkene C-H), 1589 (Imine C=N), 1540, 1348 (Unsymmetric, Symmetric NO_2), 1180 (O-CH_3); ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 3.69 (s, 3H), 4.48 (s, 2H), 6.77 (s, 1H), 6.79 (s, 2H), 7.08 (d, J = 8.8 Hz, 2H), 7.28 – 7.30 (m, 2H), 7.34 – 7.41 (m, 3H), 7.70 (d, J = 8.8 Hz, 2H), 8.17 (d, J = 8.8 Hz, 2H), 13.01 (s, 1H); $^{13}C\{^1H\}$ NMR (100MH_Z, DMSO-d_6) δ (ppm): 34.75, 55.58, 103.35, 114.07, 123.33, 123.95, 128.94, 129.60, 129.73, 130.45, 130.64, 138.07, 139.00, 146.93, 147.73, 155.40, 158.48, 159.75, 165.07. HRMS (ESI-TOF) (m/z): Calculated for $C_{25}H_{20}N_6O_3S_2$ $[M+H]^+$ 517.1116; found 517.1116; C, H, N Analysis calculated C, 58.13, H, 3.90, N, 16.27; found; C, 58.17, H, 3.94, N, 1631.

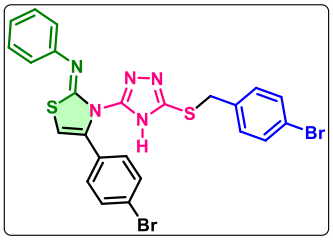
(Z)-3-(5-((4-Nitrobenzyl)thio)-4H-1,2,4-triazol-3-yl)-N,4-diphenylthiazol-2(3H)-imine 5l



Light yellow solid; Yield 82%; mp: 246-247 °C; IR (KBr) cm^{-1}: 3403 (N-H), 3025 (Alkene C-H), 1615 (Imine C=N); ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 4.48 (s, 2H), 6.44 (s, 1H), 6.89-6.92 (m, 5H), 7.00-7.03(m, 2H), 7.35-7.39 (m, 5H), 8.17 (d, J = 8.8 Hz, 2H), 13.04 (s, 1H); $^{13}C\{^1H\}$ NMR (100MH_Z, DMSO-d_6) δ (ppm): 34.75, 104.47, 121.44, 123.41, 123.95, 128.08, 128.61, 128.70, 129.28, 129.51,

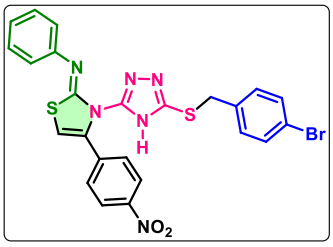
130.00, 130.45, 131.61, 138.35, 139.63, 146.93, 147.73, 151.86, 155.44, 158.40, 159.88, 165.08. HRMS (ESI-TOF) (*m/z*): Calculated for $C_{24}H_{18}N_6O_2S_2$ $[M+H]^+$ 487.1011; found 487.1017; C, H, N Analysis calculated C, 59.24, H, 3.73, N, 17.27; found; C,59.27, H, 3.76, N,17.30.

(Z)-3-(5-((4-Bromobenzyl)thio)-4H-1,2,4-triazol-3-yl)-4-(4-bromophenyl)-N-phenylthiazol-2(3H)-imine 5m



White solid; Yield 88%; mp: 229-230 °C; IR (KBr) cm^{-1}: 3421 (N-H), 3037 (alkene C-H), 1600 (C=N), 696 (C-Br); ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 4.32 (s, 2H), 6.95 (s, 1H), 7.11 (d, J = 8.5 Hz, 2H), 7.32 (d, J = 7.0 Hz, 2H), 7.35 – 7.38 (m, 3H), 7.41 (d, J = 7.6 Hz, 2H), 7.45 (d, J = 8.5 Hz, 2H), 7.49 (d, J = 8.4 Hz, 2H), 13.02 (s, 1H); $^{13}C\{^1H\}$NMR (100MHz, DMSO-d_6) δ (ppm): 34.75, 105.14, 120.54, 122.56, 129.10, 129.69, 130.33, 131.25, 131.44, 131.65, 137.77, 137.93, 138.88, 155.83, 158.26, 164.91. HRMS (ESI-TOF) (*m/z*): Calculated for $C_{24}H_{17}Br_2N_5S_2$ $[M+H]^+$ 597.9371; found 597.9364; found C, H, N Analysis calculated C, 48.10, H, 2.86, N, 11.68; found; C, 48.14, H, 289, N,11.65.

(Z)-3-(5-((4-Bromobenzyl)thio)-4H-1,2,4-triazol-3-yl)-4-(4-nitrophenyl)-N-phenylthiazol-2(3H)-imine 5n

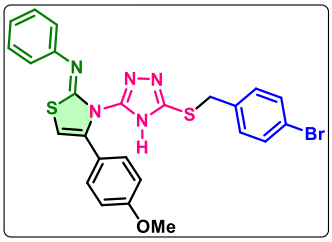


White solid; Yield 83%; mp: 222-223 °C; IR (KBr) cm^{-1}: 3308 (N-H), 3054 (Alkene C-H), 1605 (Imine C=N), 1544, 1340 (Unsymmetric, Symmetric NO_2) 985 (C-Br); ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 4.33 (s, 2H), 7.16 (s, 1H), 7.35 – 7.38 (m, 5H), 7.41 – 7.45 (m, 4H), 7.48 – 7.50 (m, 2H), 8.07 – 8.10 (m, 2H), 13.06 (s, 1H); $^{13}C\{^1H\}$ NMR (100MHz, DMSO-d_6) δ (ppm): 34.80, 107.58, 120.57, 123.78, 124.52, 129.23, 129.61, 129.81, 130.26, 131.44, 131.68, 131.85, 131.91, 137.07, 137.34, 137.61, 138.82, 147.50, 164.75. HRMS (ESI-TOF) (*m/z*): Calculated for $C_{24}H_{17}BrN_6O_2S_2$ $[M+H]^+$ 565.0116; found 565.0121; C, H, N Analysis calculated C, 50.98, H, 3.03, N, 14.86; found; C, 50.95, H, 3.07, N,14.85.

(Z)-3-(5-((4-Bromobenzyl)thio)-4H-1,2,4-triazol-3-yl)-4-(4-methoxyphenyl)-N-phenylthiazol-2(3H)-imine 5o

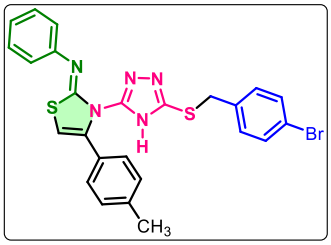
White solid; Yield 90%; mp: 252-253 °C; IR (KBr) cm^{-1}: 3402 (N-H), 3012 (Alkene C-H), 1599 (Imine C=N), 1145 (O-CH_3), 698 (C-Br); ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 3.69

(s, 3H), 4.33 (s, 2H), 5.44 (s, 1H), 6.77-6.80 (m, 3H), 7.07 – 7.11 (m, 3H), 7.29 – 7.31 (m, 2H), 7.37-7.41 (m, 3H), 7.47 – 7.50 (m, 2H), 12.98 (s, 1H); $^{13}C\{^{1}H\}$ NMR (100MHz, DMSO-d_6) δ (ppm): 34.75, 55.58, 103.40, 114.07, 114.57, 129.60, 129.74, 130.64, 130.88, 131.45, 131.64, 131.68, 138.09, 138.53, 138.92, 155.29, 155.78, 157.77, 158.36, 159.74, 164.09, 165.03. HRMS (ESI-TOF) (*m/z*): Calculated for $C_{25}H_{20}BrN_5OS_2$ $[M+H]^+$ 550.0371; found 550.0374; C, H, N Analysis calculated C, 54.55, H, 3.66, N, 12.72; found; C, 54.59, H,3.69, N,12.76.

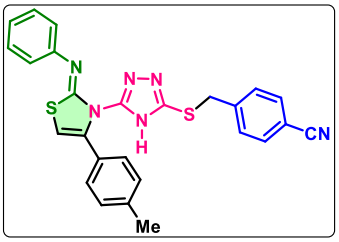


(Z)-3-(5-((4-Bromobenzyl)thio)-4H-1,2,4-triazol-3-yl)-N-phenyl-4-(p-tolyl)thiazol-2(3H)-imine. 5p

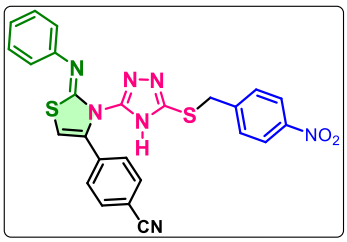
White solid; Yield 89%; mp: 239-240 °C; IR (KBr) cm^{-1}: 3401 (N-H), 3034 (Alkene C-H), 1593 (Imine C=N);^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.22 (s, 3H), 4.33 (s, H), 6.83 (s, 1H), 7.29 – 7.31 (m, 3H), 7.37 – 7.41 (m, 6H), 7.46 – 7.50 (m, 4H), 12.99 (s, 1H); $^{13}C\{^{1}H\}$ NMR (100MHz, DMSO-d_6) δ (ppm): 21.15, 34.76, 103.94, 120.54, 128.25, 128.62, 128.94, 129.07, 129.23, 129.59, 129.71, 129.87, 134.45, 131.68, 138.05, 138.57, 138.90, 139.16, 155.78, 158.34. HRMS (ESI-TOF) (*m/z*): Calculated for $C_{25}H_{20}BrN_5S_2$ $[M+H]^+$ 534.0421; found 534.0433. C, H, N Analysis calculated C, 56.18, H, 3.77, N, 13.10; found; C, 56.15, H, 3.75, N, 13.14.



(Z)-4-(((5-(2-(Phenylimino)-4-(p-tolyl)thiazol-3(2H)-yl)-4H-1,2,4-triazol-3-yl) thio) methyl) benzonitrile ***5q*****.** White solid; Yield 81%; mp: 244-245 °C; IR (KBr) cm^{-1}: 3161 (N-H), 3021 (Alkene C-H), 2240 (CN), 1589 (Imine C=N); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.21 (s, 3H), 4.42 (s, 2H), 6.83 (s, 1H), 7.03 (s, 4H), 7.28 – 7.30 (m, 2H), 7.33 -7.40 (m, 3H), 7.61 (d, J = 8.4 Hz, 2H), 7.76 (d, J = 8.4 Hz, 2H), 13.02 (s, 1H); $^{13}C\{^{1}H\}$ NMR (100MHz, DMSO-d_6) δ (ppm): 21.15, 35.06, 103.91, 110.18, 119.29, 128.24, 128.95, 129.08, 129.23, 129.60, 129.71, 130.21, 132.72, 138.04, 138.58, 139.19, 145.51, 165.03. HRMS (ESI-TOF) (*m/z*): Calculated for $C_{26}H_{20}N_6S_2$ $[M+H]^+$ 481.1269; found 481.1262; C, H, N Analysis calculated C, 64.98, H, 4.19, N, 17.49; found; C, 64.94, H, 4.15,N, 17.53.

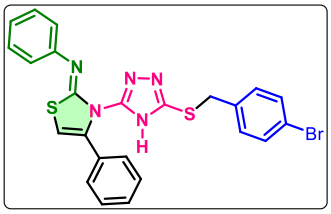


Z)-3-(5-((4-Nitrobenzyl)thio)-4H-1,2,4-triazol-3-yl)-2-(phenylimino)-2,3-dihydrothiazol-4-yl)benzonitrile 5r



White solid; Yield 85%; mp: 237-238 °C; IR (KBr) cm^{-1}: 3402 (N-H), 3035 (Alkene C-H), 2221 (CN), 1594 (Imine C=N), 1544, 1341 (Unsymmetric, Symmetric NO_2); ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 4.48 (s, 2H), 7.11 (s, 1H), 7.34-7.37 (m, 4H), 7.42 (d, J = 7.6 Hz, 2H), 7.69-7.7.74 (m, 4H), 8.05 (d, J = 8.3 Hz, 1H), 8.17 (d, J = 8.7 Hz, 2H), 13.14 (s, 1H); $^{13}C\{^1H\}$ NMR (100MHz, DMSO-d_6) δ (ppm): 34.76, 106.97, 111.54, 118.74, 123.96, 128.78, 129.21, 129.61, 129.77, 129.89, 130.45, 132.58, 133.26, 135.52, 137.45, 137.61, 146.94, 147.64, 155.27, 158.36, 164.81. HRMS (ESI-TOF) (*m/z*): Calculated for $C_{25}H_{17}N_7O_2S_2$ $[M+H]^+$ 512.5858; found 512.0976; C, H, N Analysis calculated C, 58.70, H, 3.35, N, 19.17; found; C, 58.71, H,3.38, N, 19.16

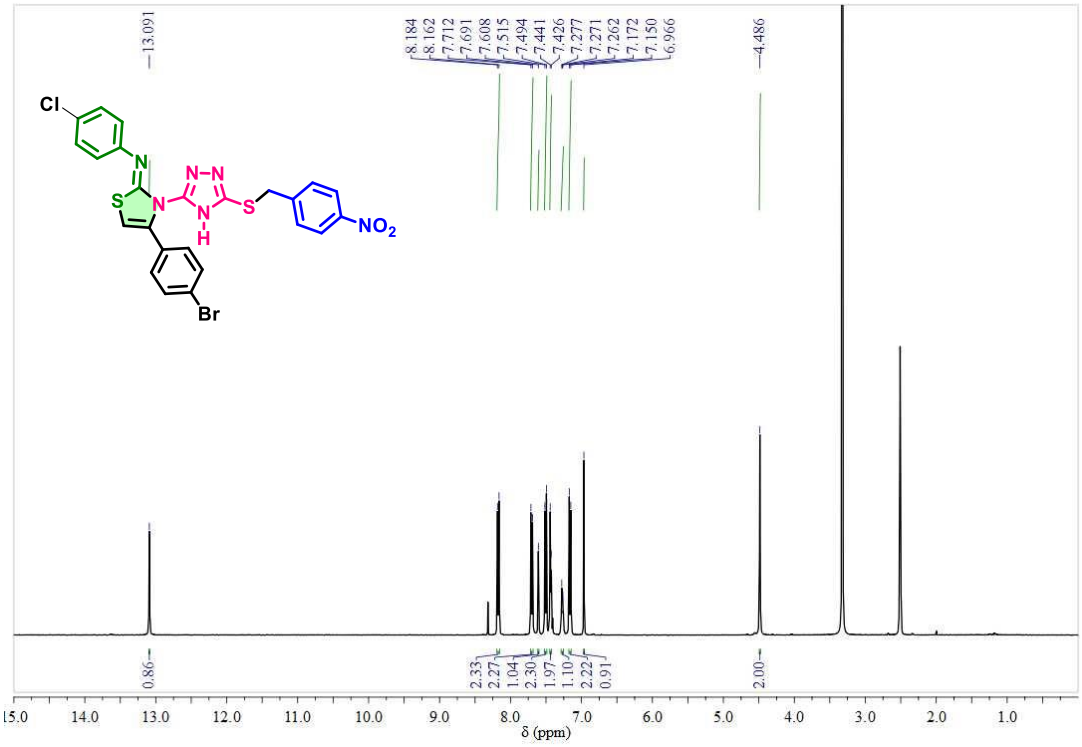
Z)-3-(5-((4-Bromobenzyl)thio)-4H-1,2,4-triazol-3-yl)-N,4-diphenylthiazol-2(3H)-imine. 5s



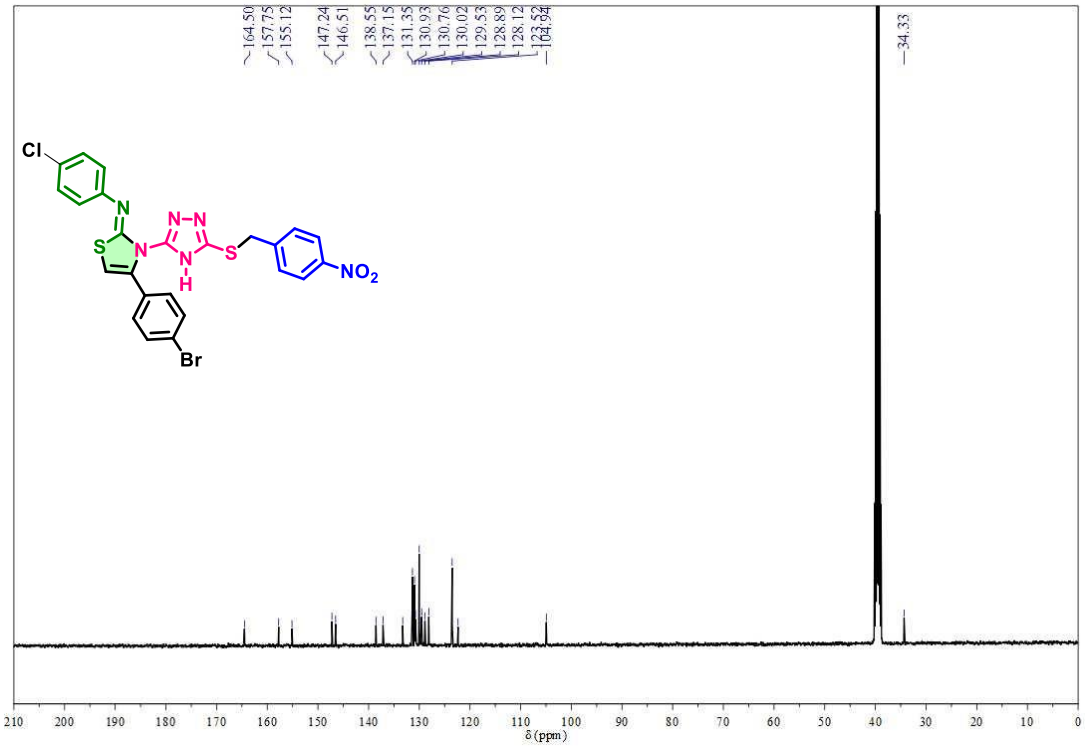
White solid; Yield 90%; mp: 232-233 °C; IR (KBr) cm^{-1}: 3385 (N-H), 3032 (Alkene C-H), 1589 (Imine C=N), 749 (C-Br): ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 4.33 (s, 2H), 6.89 (s, 1H), 7.15-7.17 (m, 2H), 7.24 (d, J = 6.6 Hz, 3H), 7.30-7.33 (m,3H), 7.37-7.40 (m, 4H), 7.49 (d, J = 8.4 Hz, 2H), 13.00 (s, 1H); $^{13}C\{^1H\}$ NMR (100MHz, DMSO-d_6) δ (ppm): 34.80, 107.58, 120.57, 123.78, 124.52, 129.23, 129.61, 129.81, 130.26, 131.44, 131.68, 137.07, 137.34, 137.61, 138.82, 147.50, 164.34. HRMS (ESI-TOF) (*m/z*): Calculated for $C_{24}H_{18}BrN_5S_2$ $[M+H]^+$ 520.0265; found 520.0272; C, H, N Analysis calculated C, 55.39, H, 3.49, N, 13.46; found;C,55.42, H, 3.52, N,13.43.

4.8. Copies of spectral data

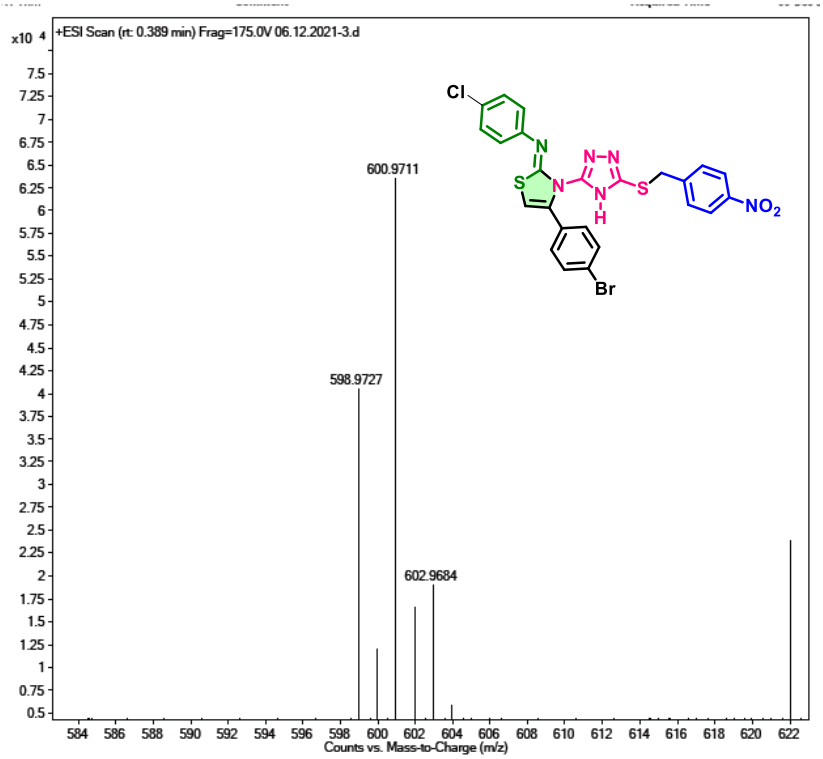
^{1}H NMR spectrum of compound (DMSO-d_6) *5a* (400 MHz)



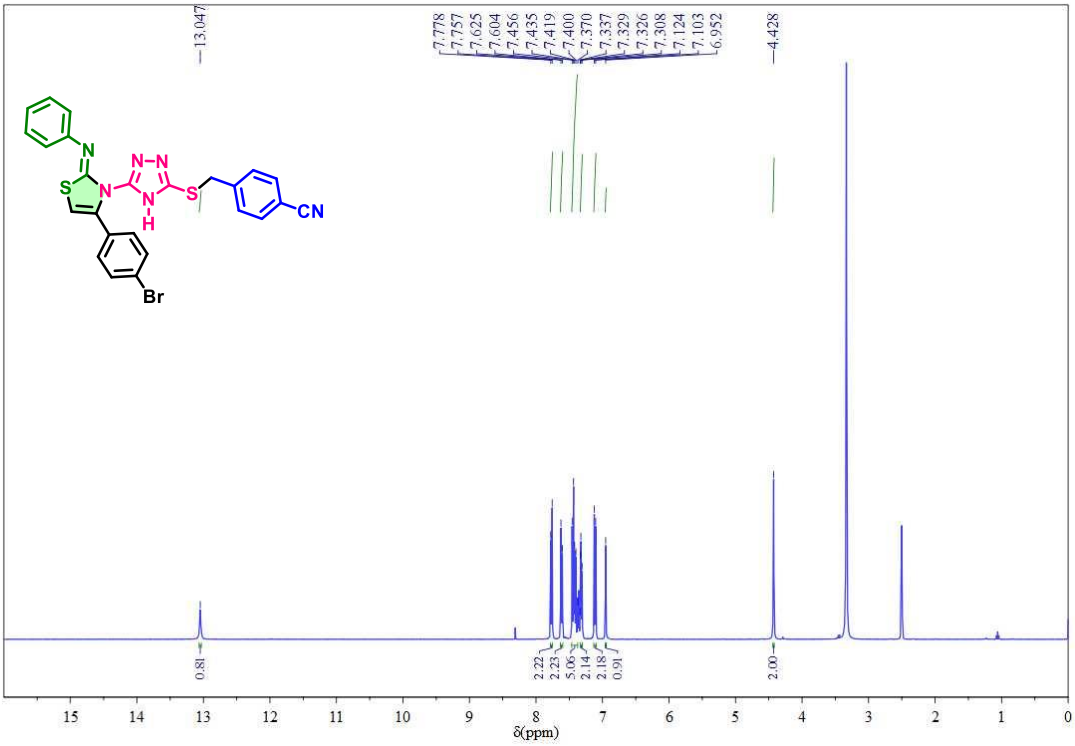
^{13}C NMR spectrum of compound (DMSO d_6) *5a* (100 MHz)



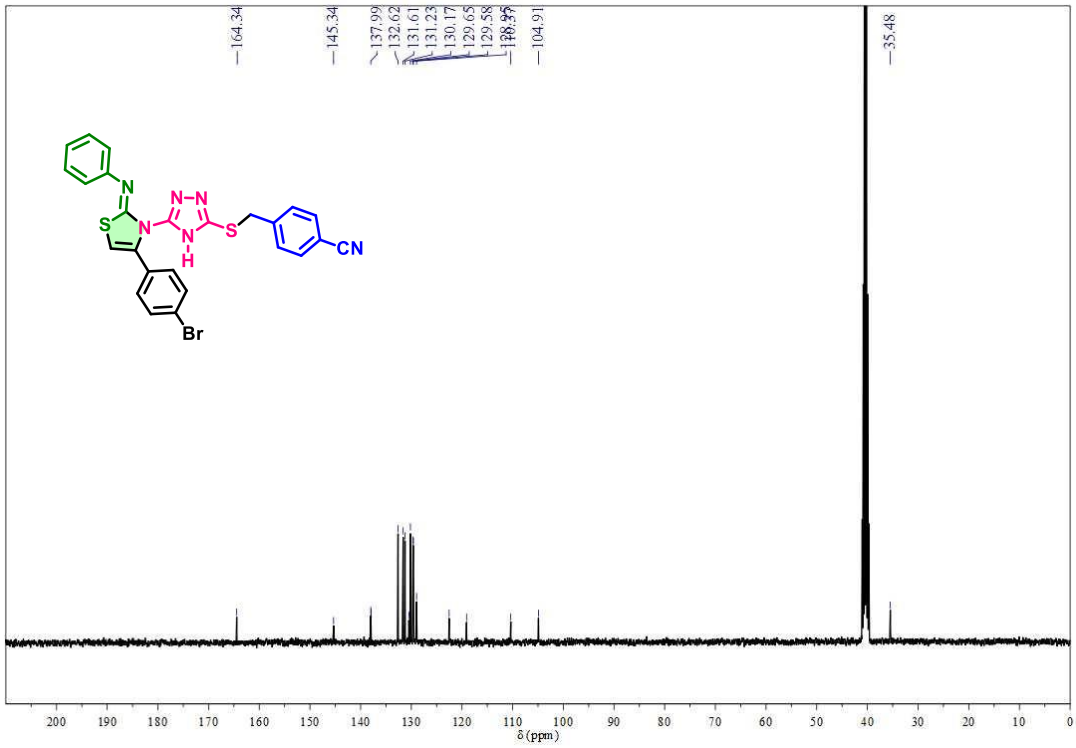
Mass spectrum of compound 5a



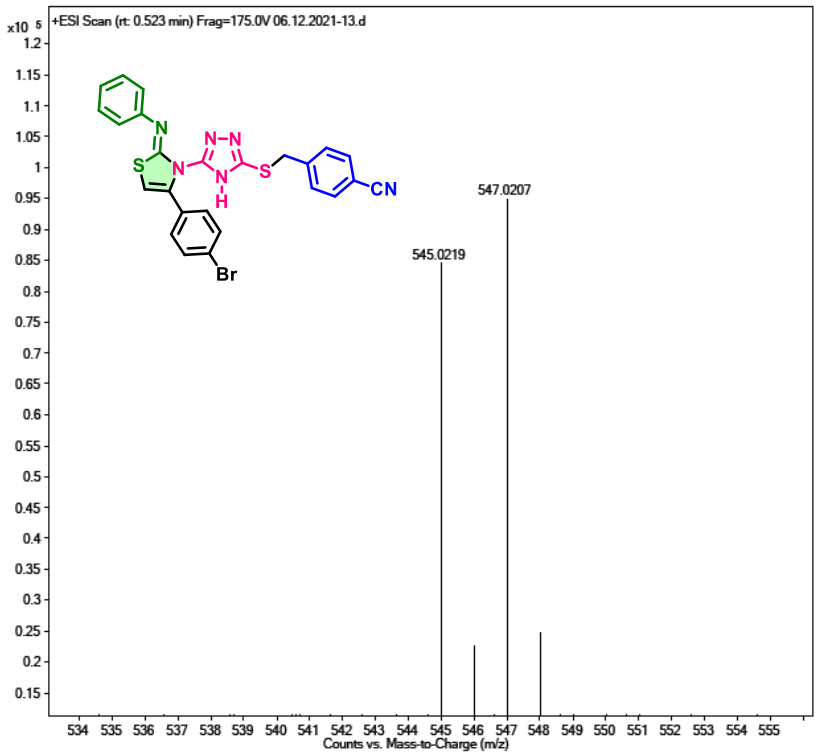
^{1}H NMR spectrum of compound (DMSO d_6) 5b (400 MHz)



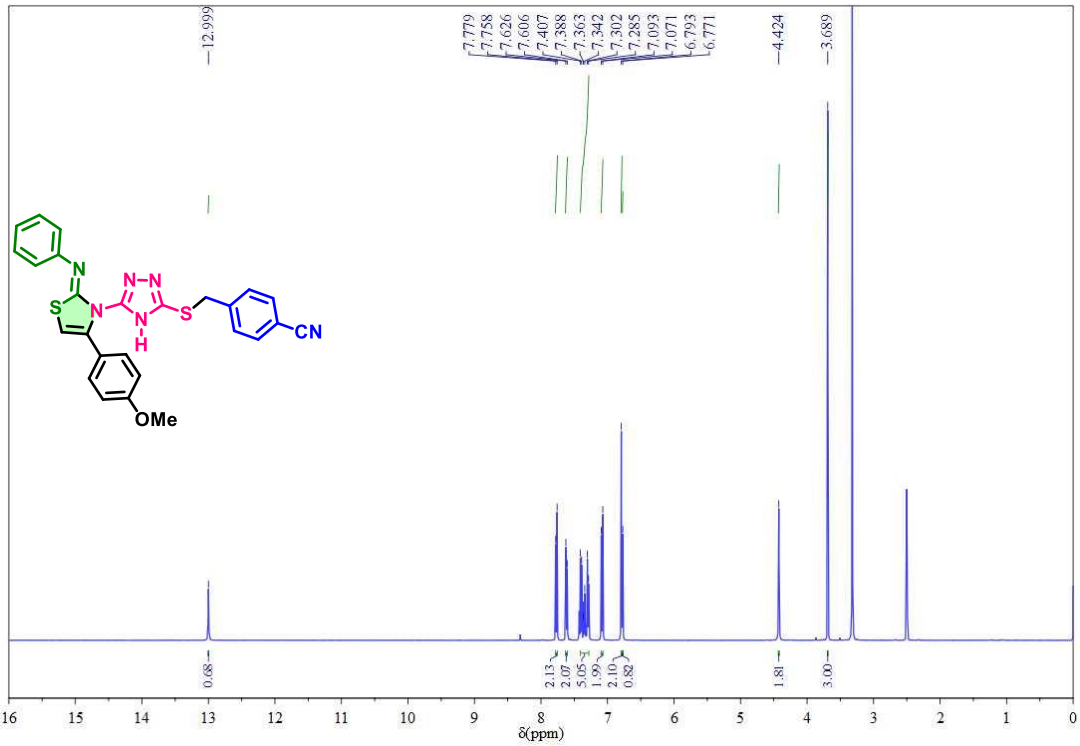
^{13}C NMR spectrum of compound (DMSO d_6) 5b (100 MHz)



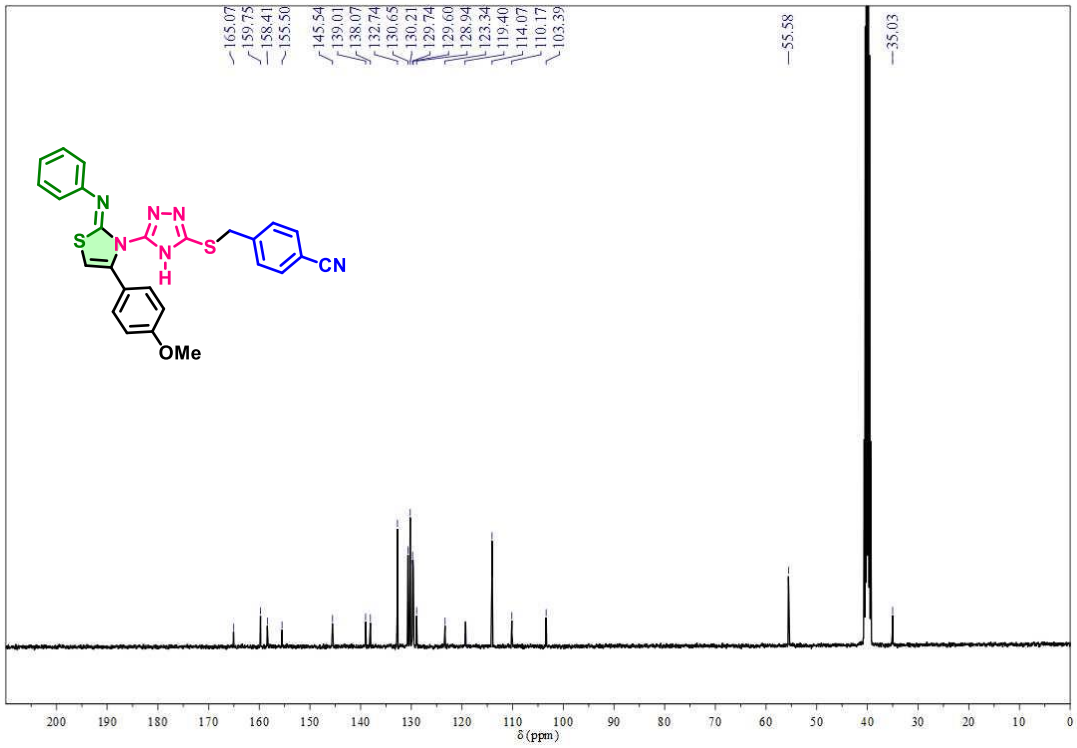
Mass spectrum of compound 5b



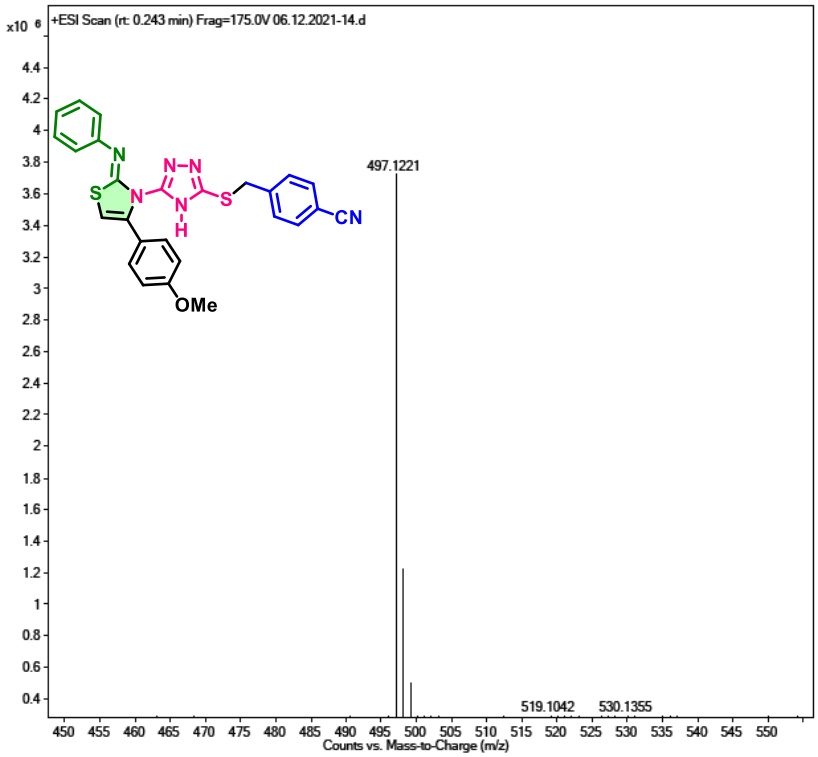
^{1}H NMR spectrum of compound (DMSO-d_6) *5c* (400 MHz)



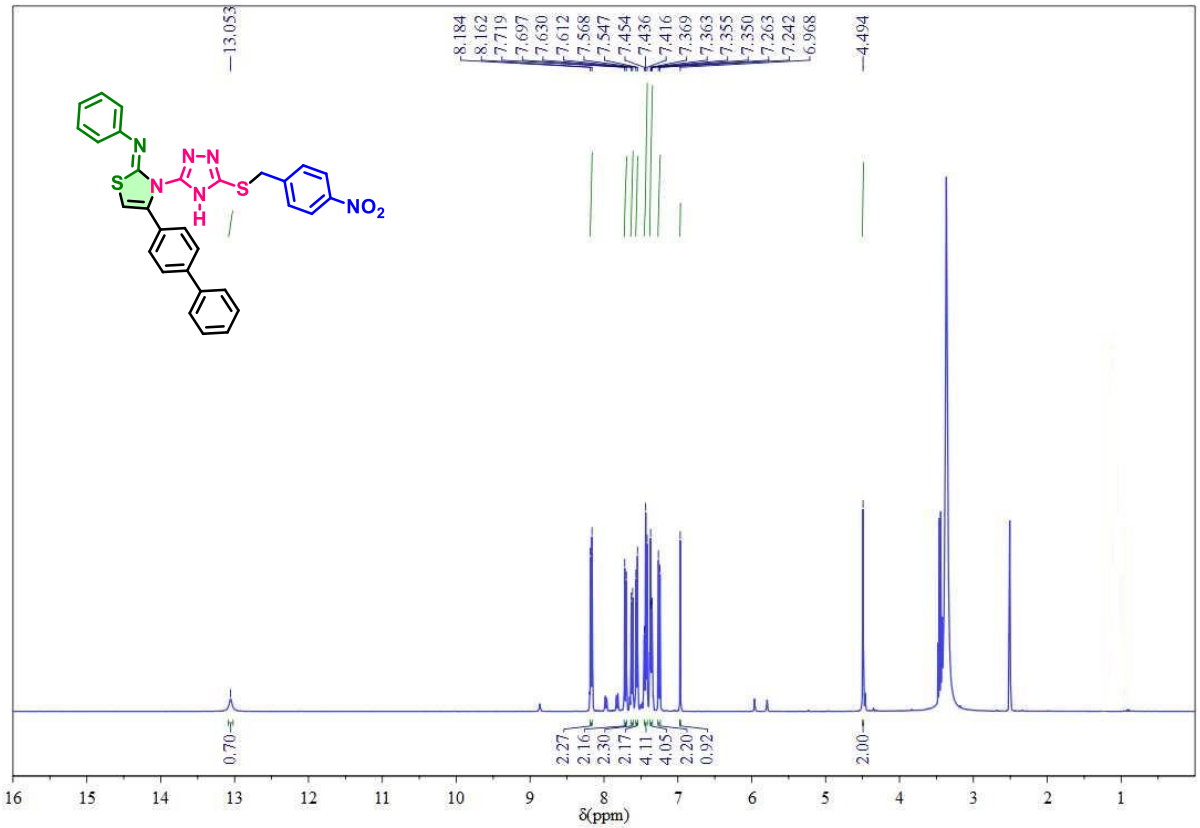
^{13}C NMR spectrum of compound (DMSO-d_6) 5c (100 MHz)



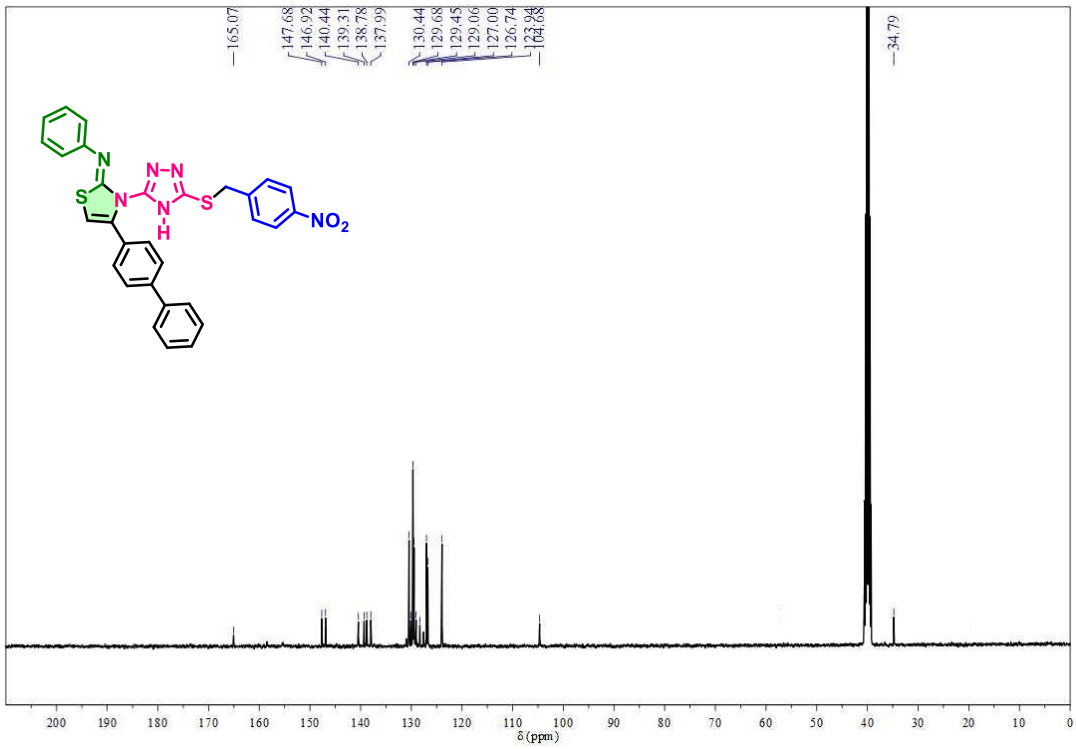
Mass spectrum of compound 5c



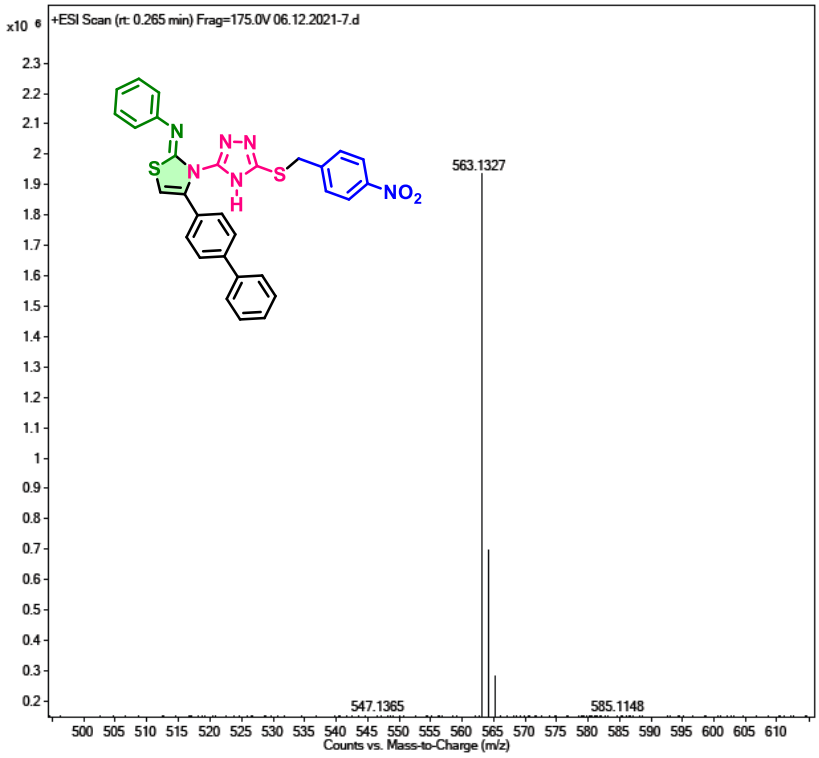
^{1}H NMR spectrum of compound (DMSO-d_6) 5d (400 MHz)



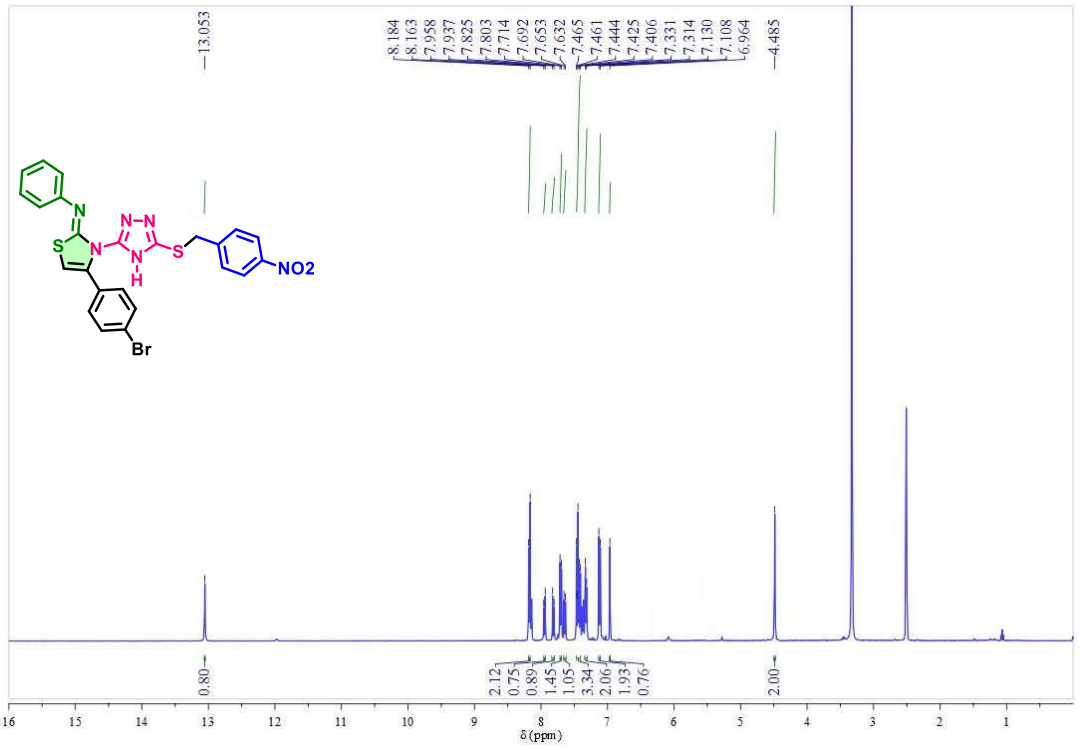
^{13}C NMR spectrum of compound (DMSO-d_6) *5d* (100 MHz)



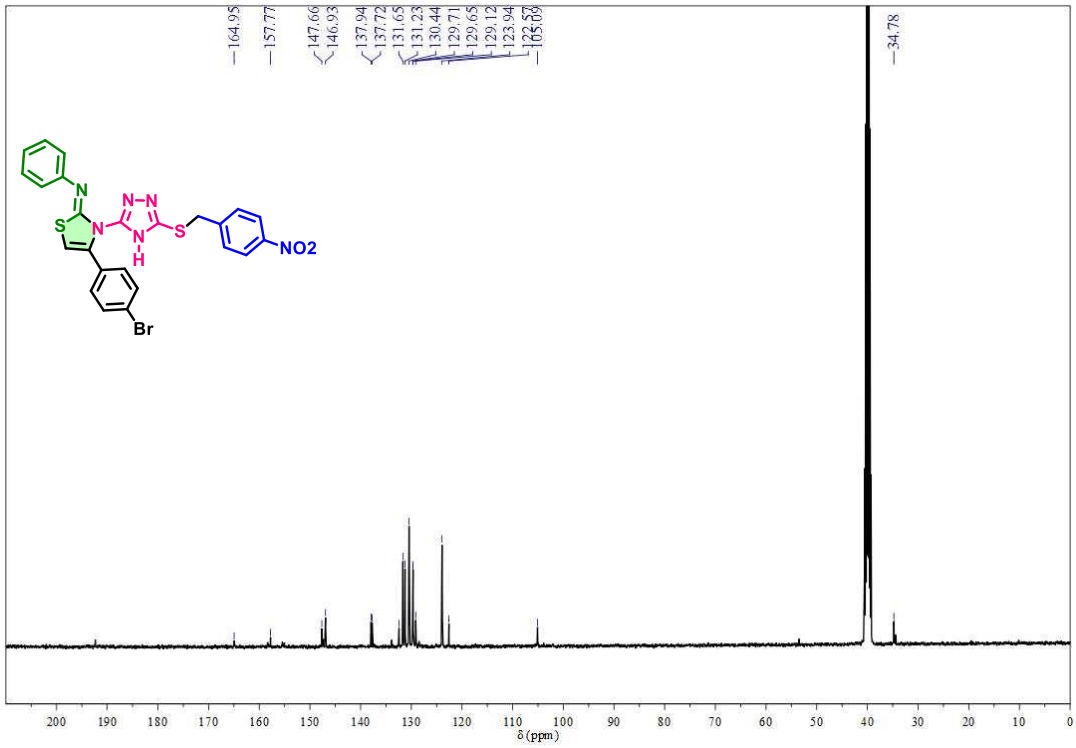
Mass spectrum of compound *5d*



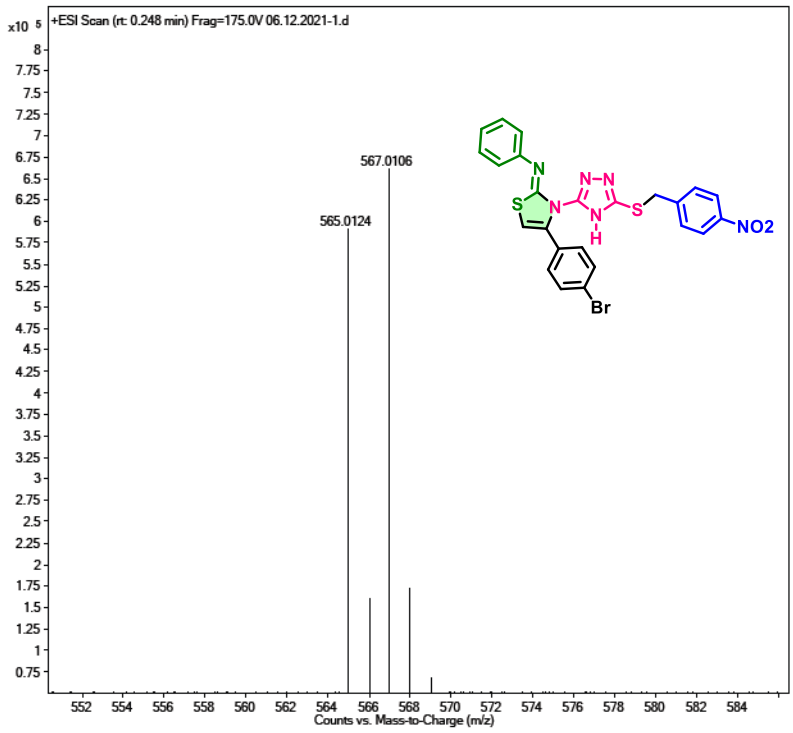
^{1}H NMR spectrum of compound (DMSO-d_6) *5e* (400 MHz)



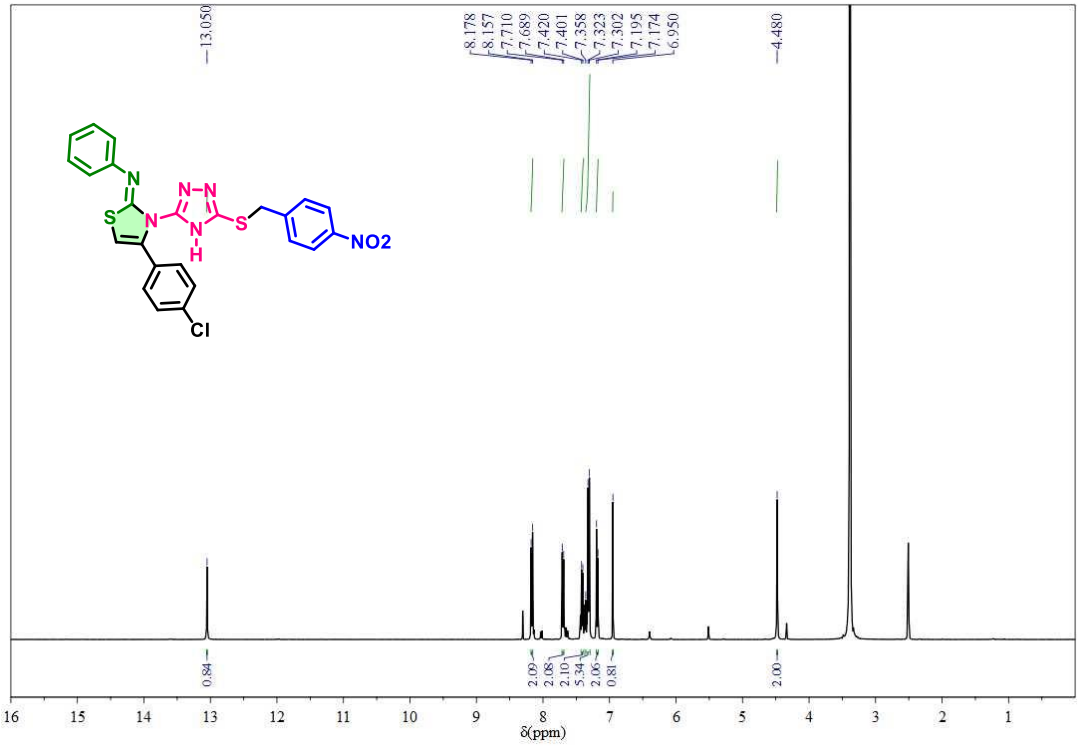
^{13}C NMR spectrum of compound (DMSO-d_6) *5e* (100 MHz)



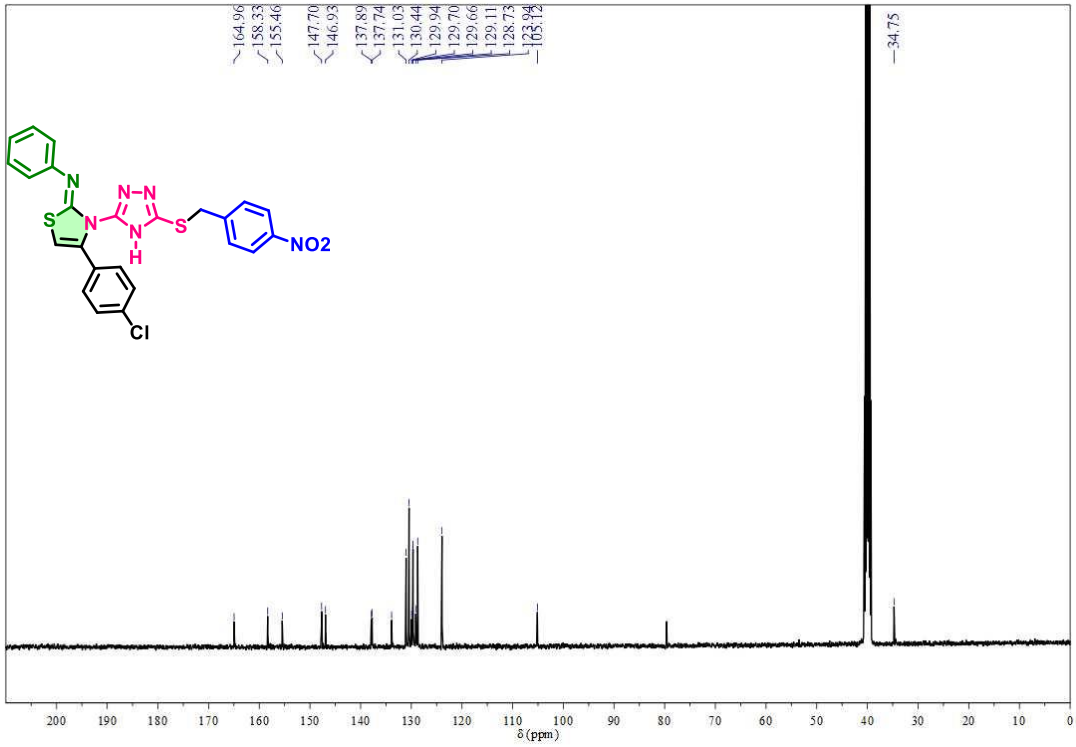
Mass spectrum of compound *5e*



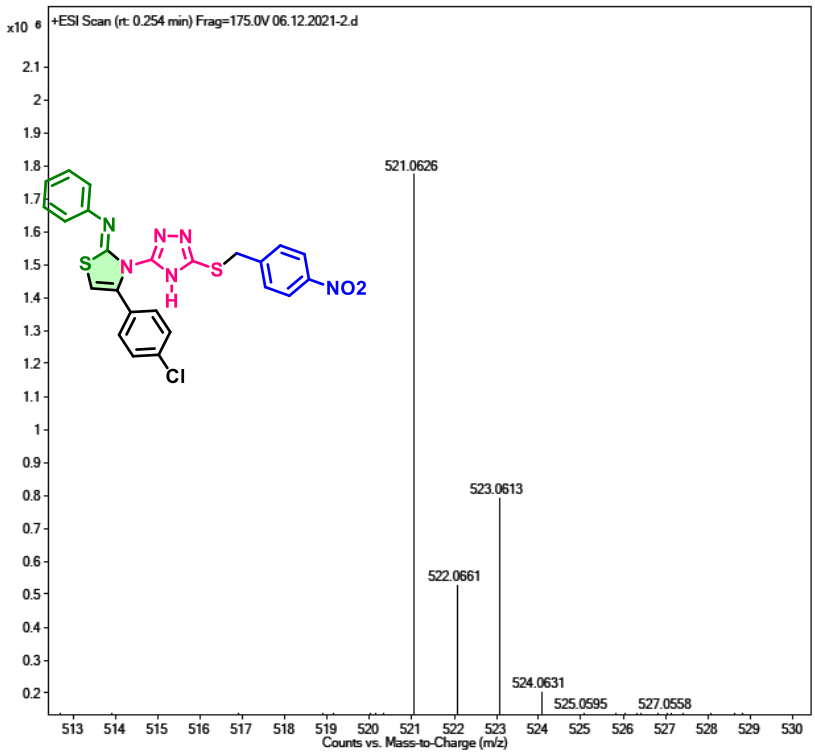
^{1}H NMR spectrum of compound (DMSO-d_6) *5f* (400 MHz)



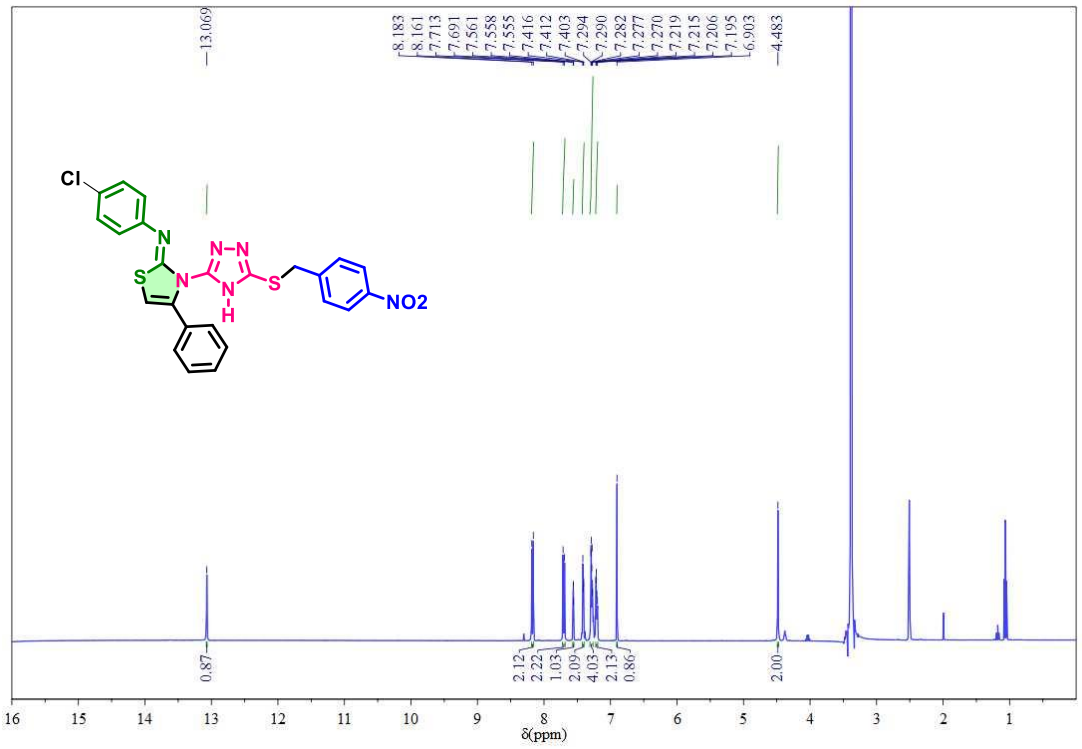
^{13}C NMR spectrum of compound (DMSO-d_6) *5f* (100 MHz)



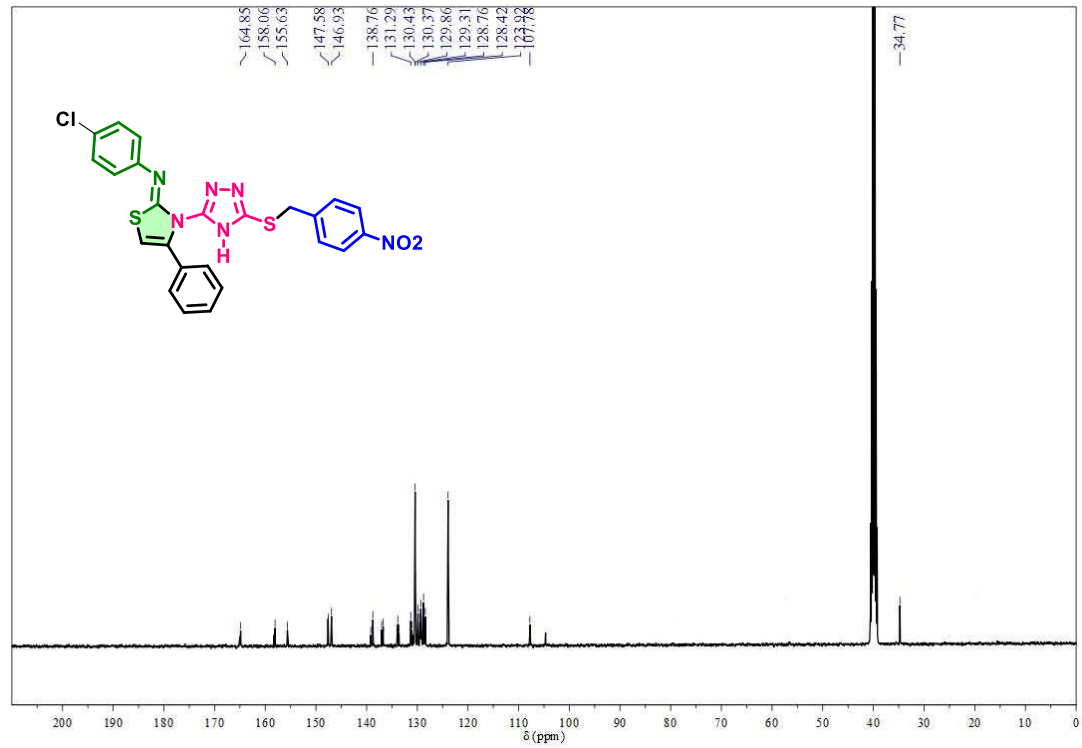
Mass spectrum of compound *5f*



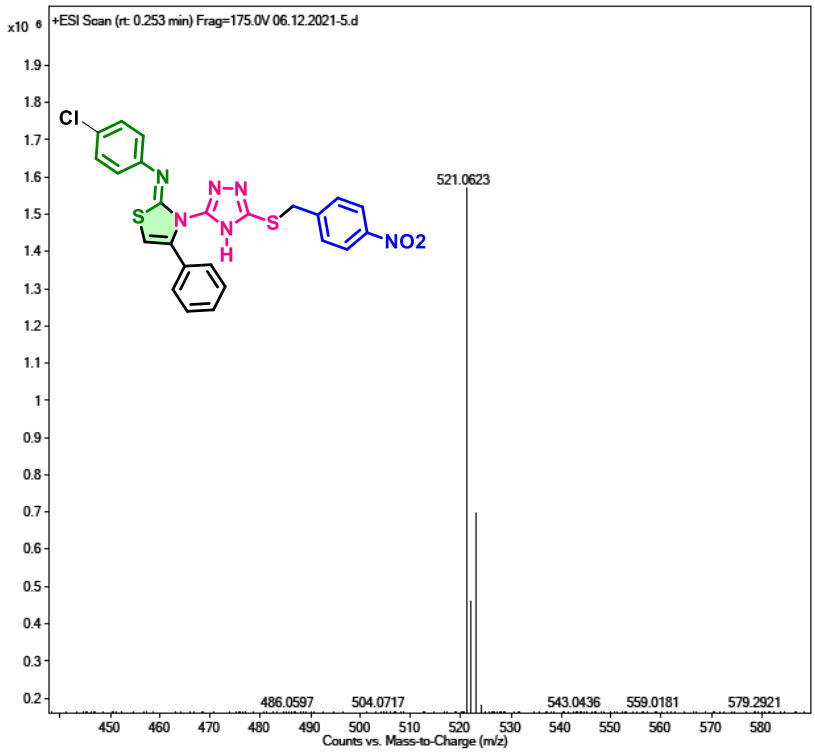
^{1}H NMR spectrum of compound (DMSO-d_6) *5g* (100 MHz)



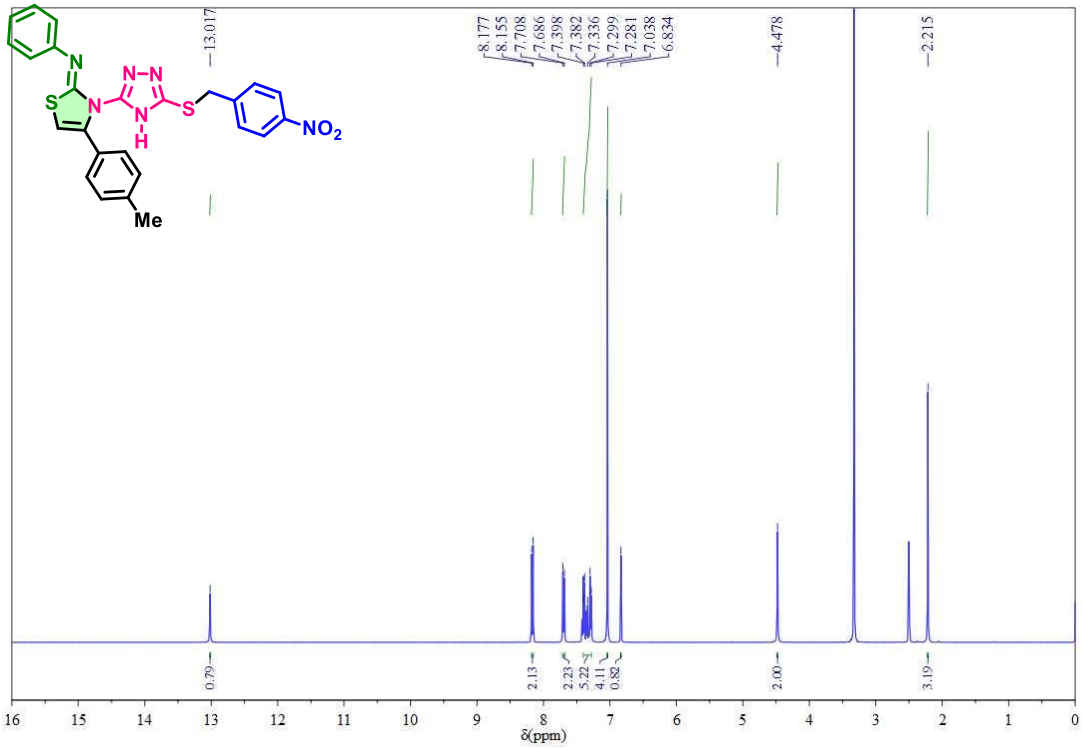
^{13}C NMR spectrum of compound (DMSO-d_6) *5g* (100 MHz)



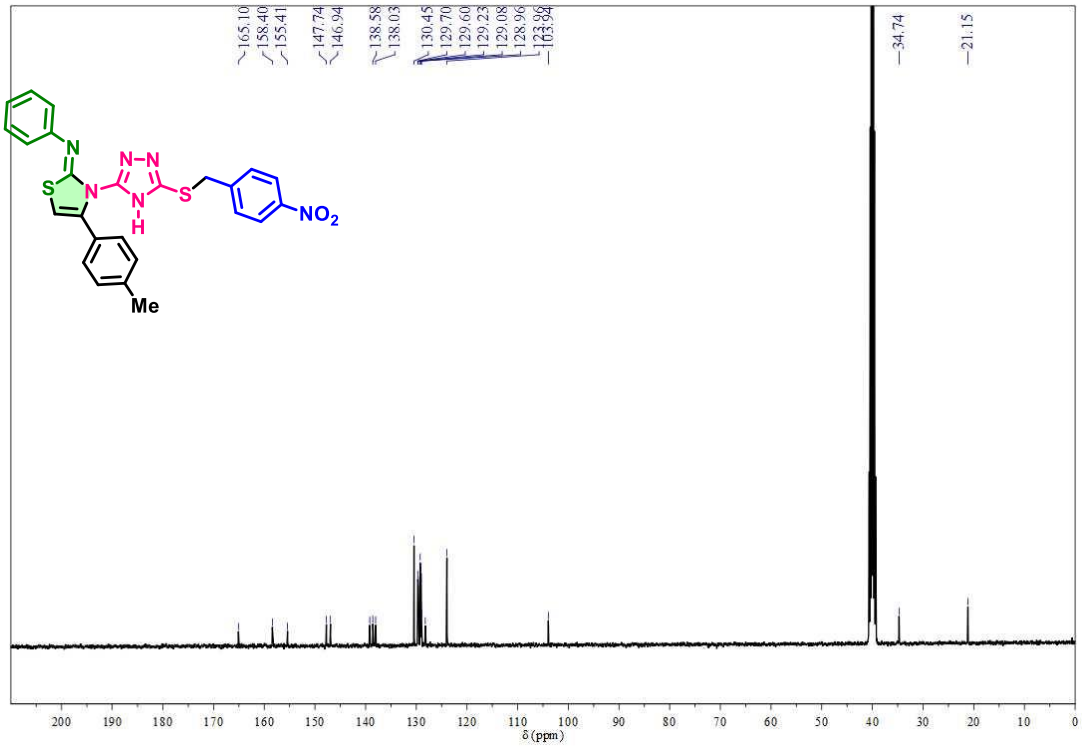
Mass spectrum of compound *5g*



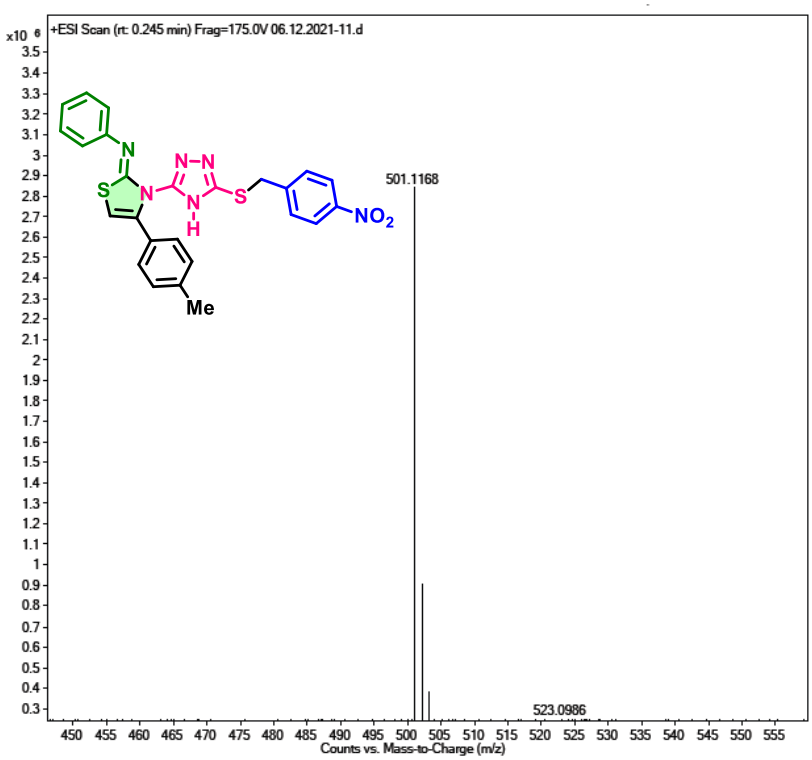
^{1}H NMR spectrum of compound (DMSO-d_6) *5h* (400 MHz)



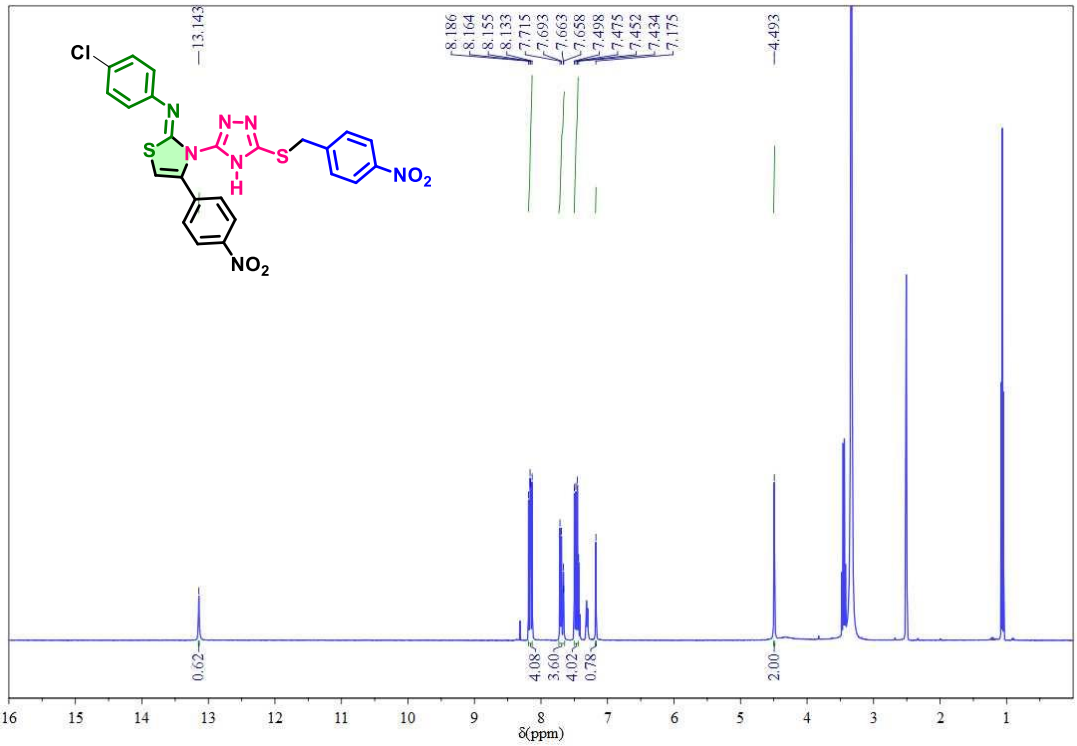
^{13}C NMR spectrum of compound (DMSO-d_6) *5h* (100 MHz)



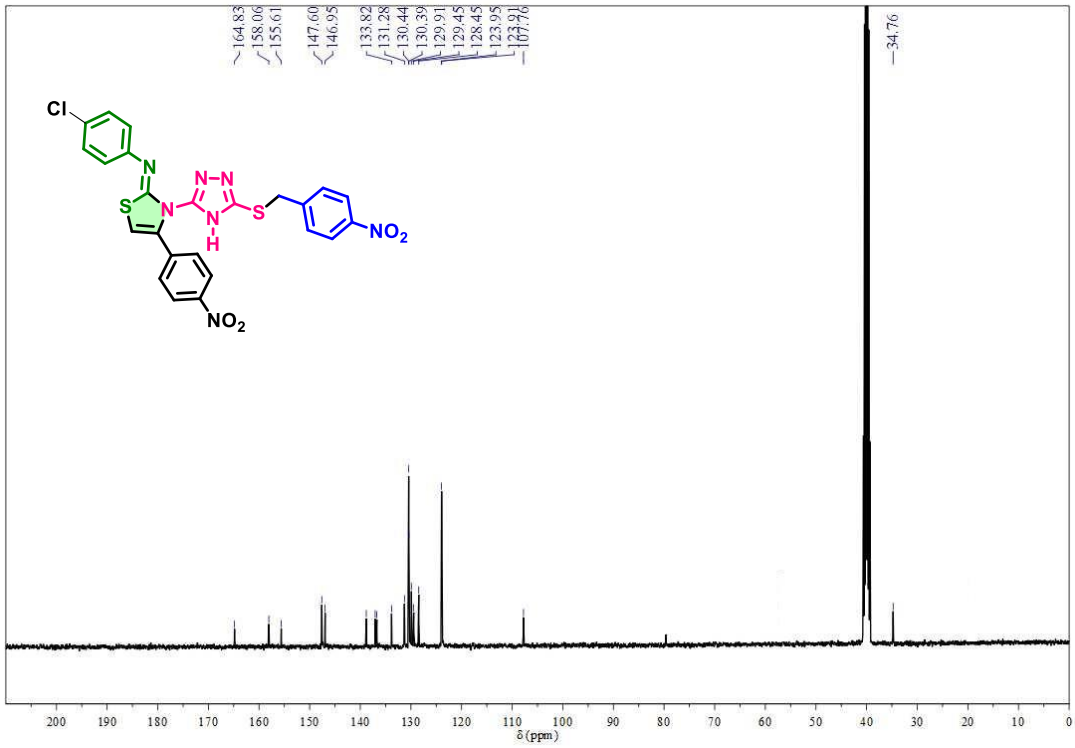
Mass spectrum of compound *5h*



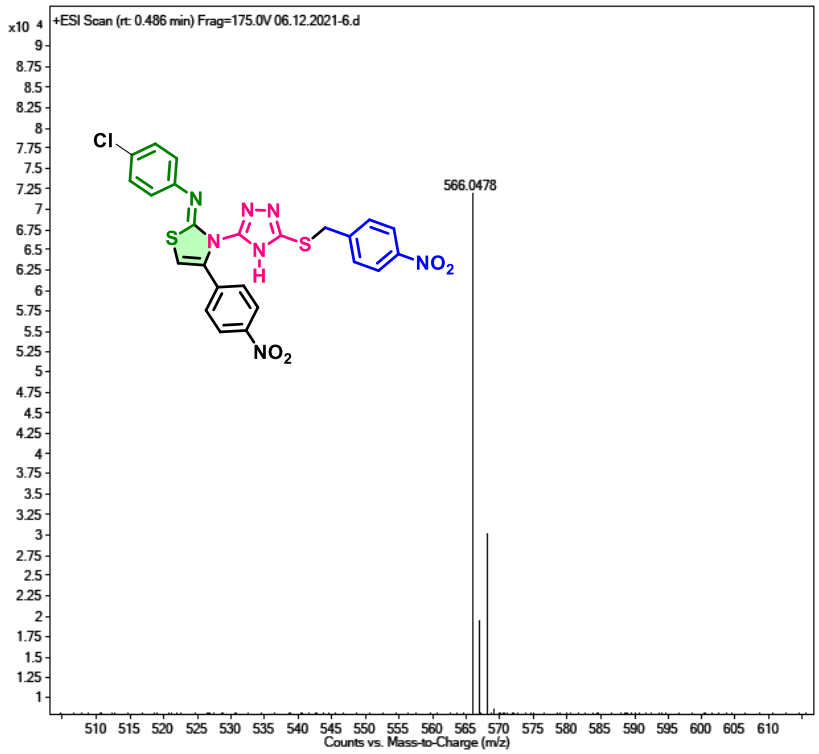
^{1}H NMR spectrum of compound (DMSO-d_6) *5i* (400 MHz)



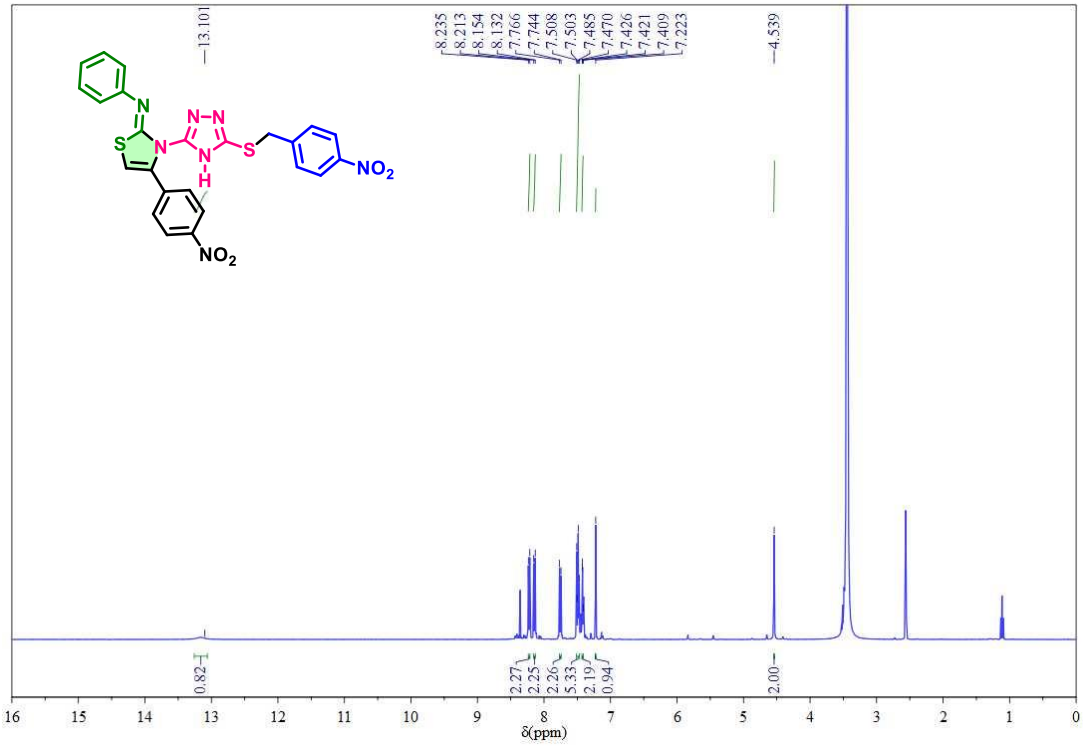
^{13}C NMR spectrum of compound (DMSO-d_6) *5i* (100 MHz)



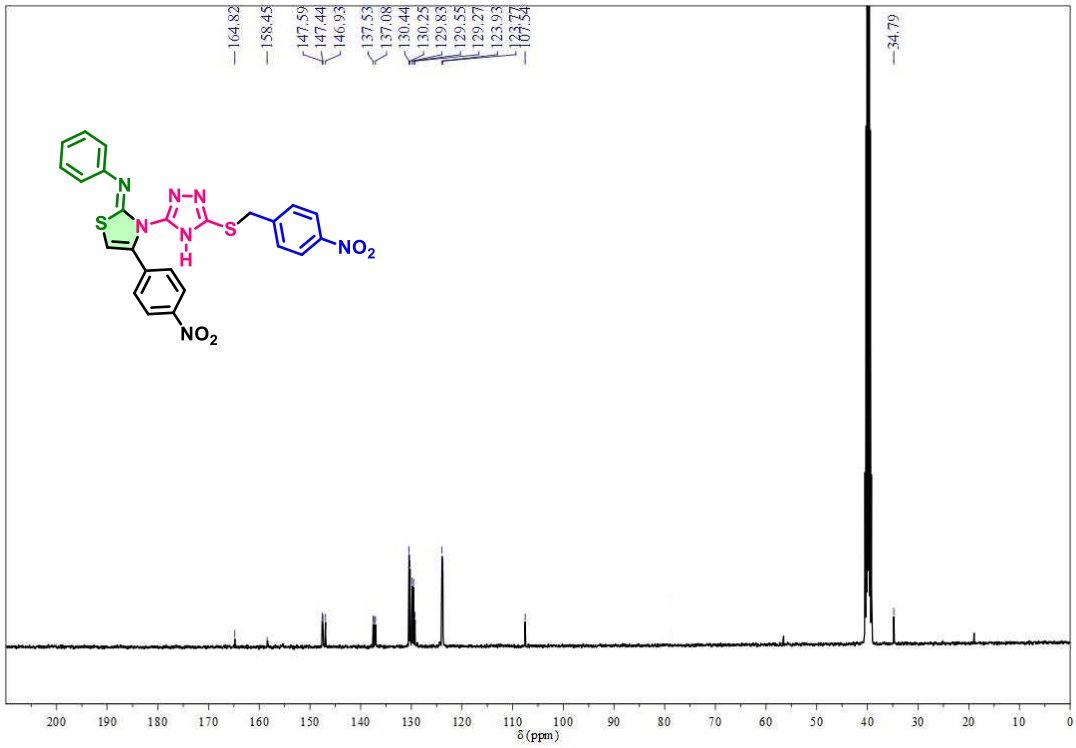
Mass spectrum of compound *5i*



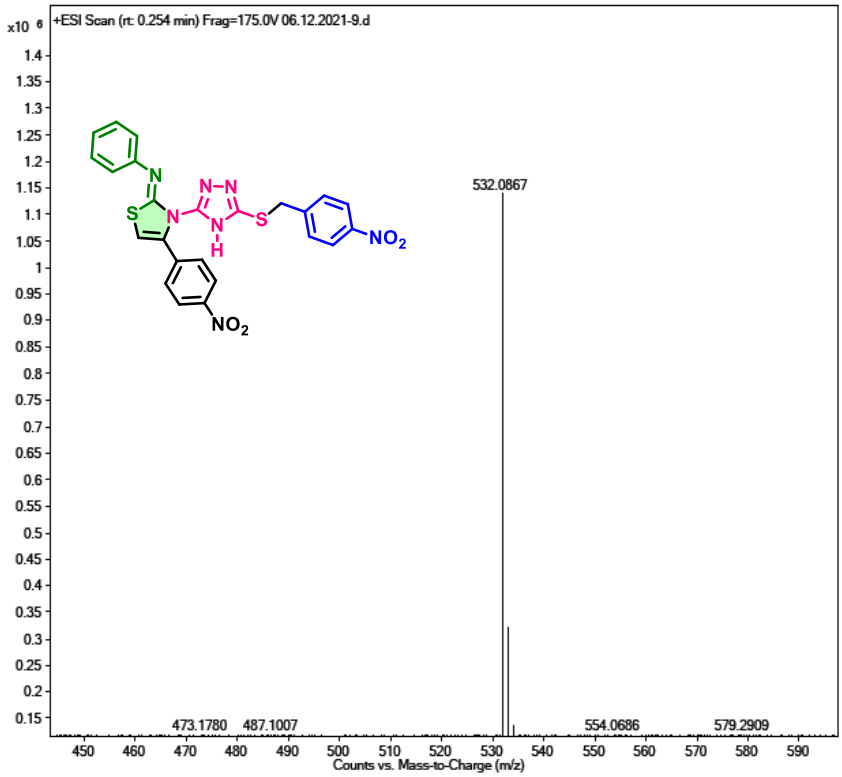
^{1}H NMR spectrum of compound (DMSO-d_6) *5j* (400 MHz)



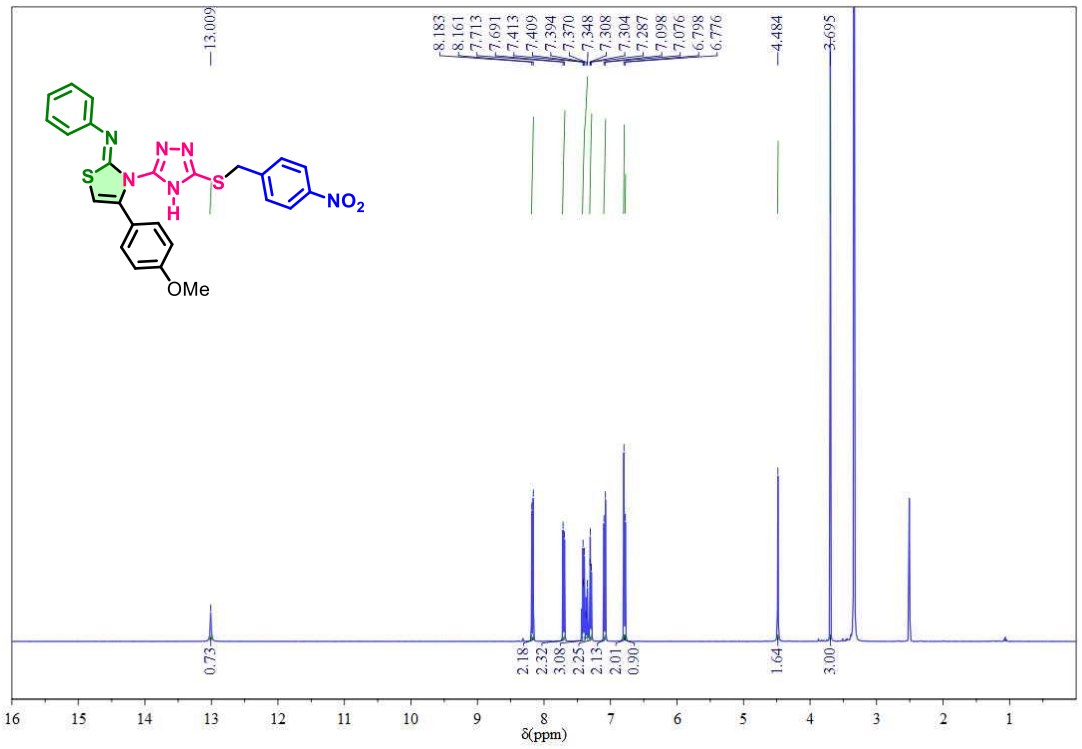
^{13}C NMR spectrum of compound (DMSO-d_6) *5j* (100 MHz)



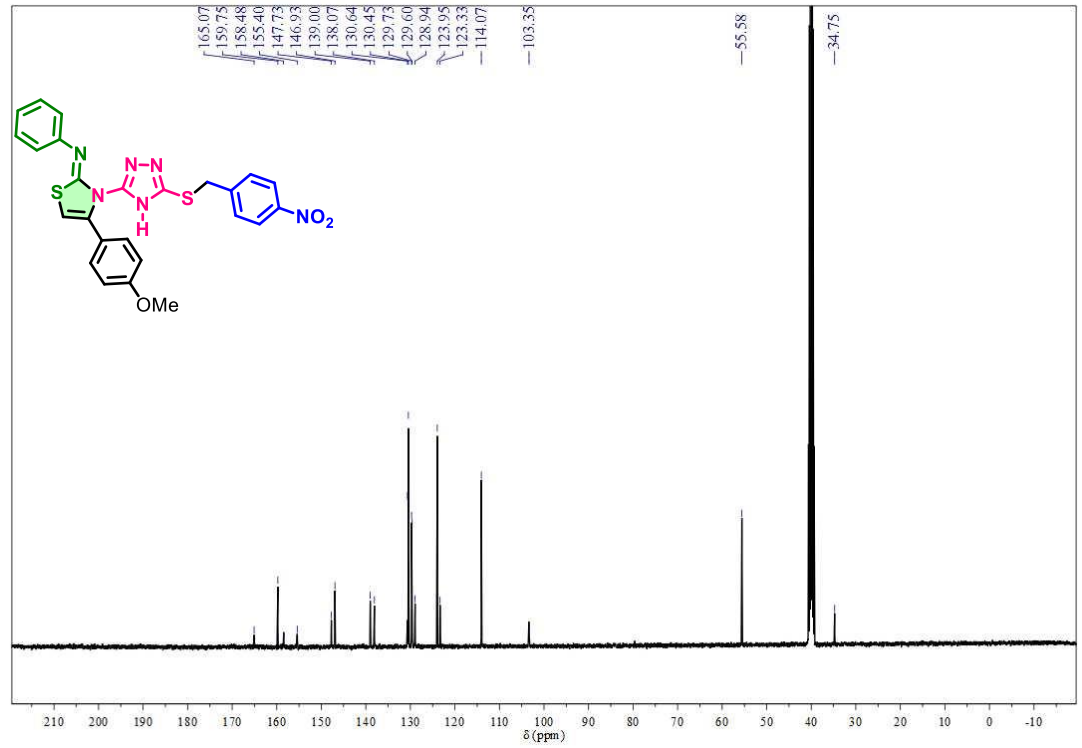
Mass spectrum of compound *5j*



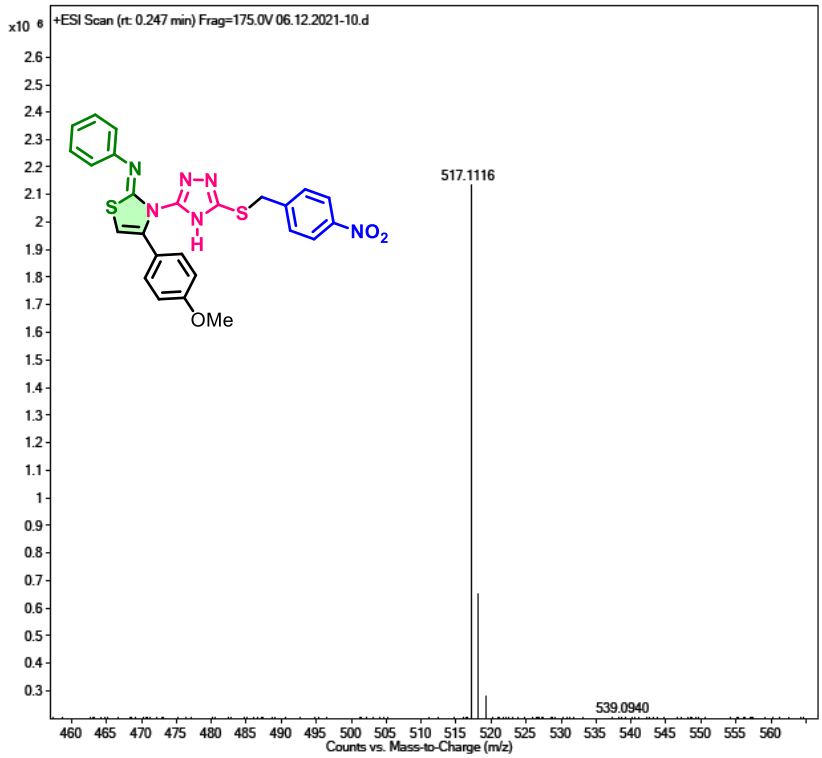
^{1}H NMR spectrum of compound *5k* (DMSO-d_6) (400 MHz)



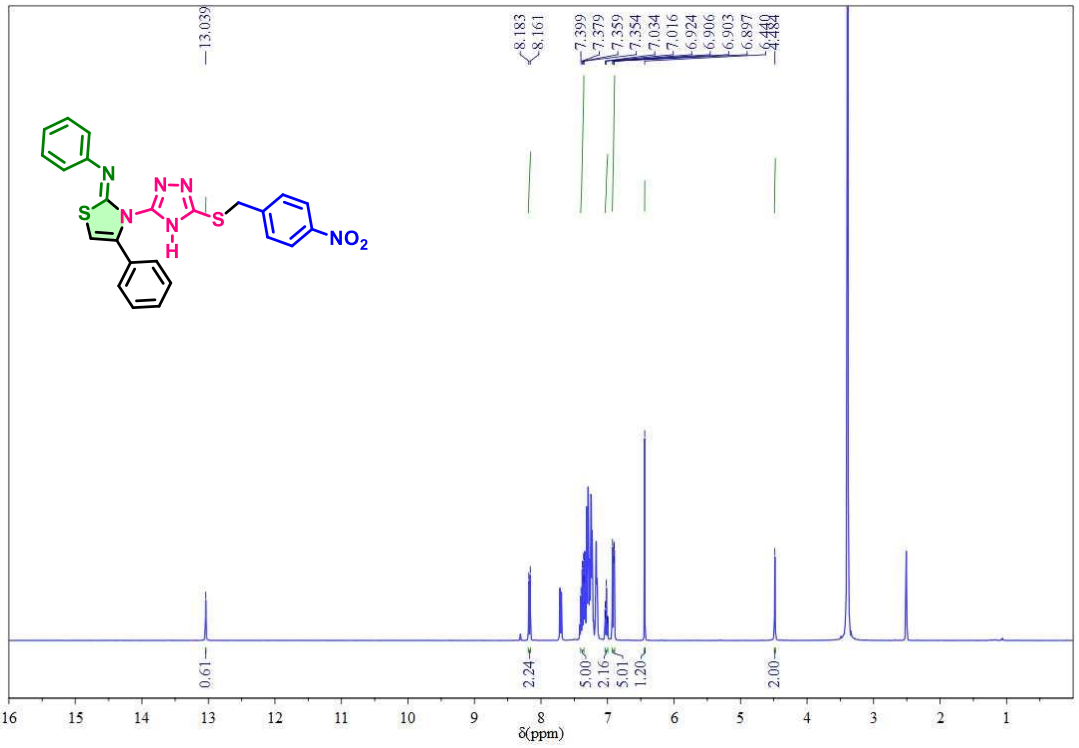
^{13}C NMR spectrum of compound *5k* (DMSO-d_6) (100 MHz)



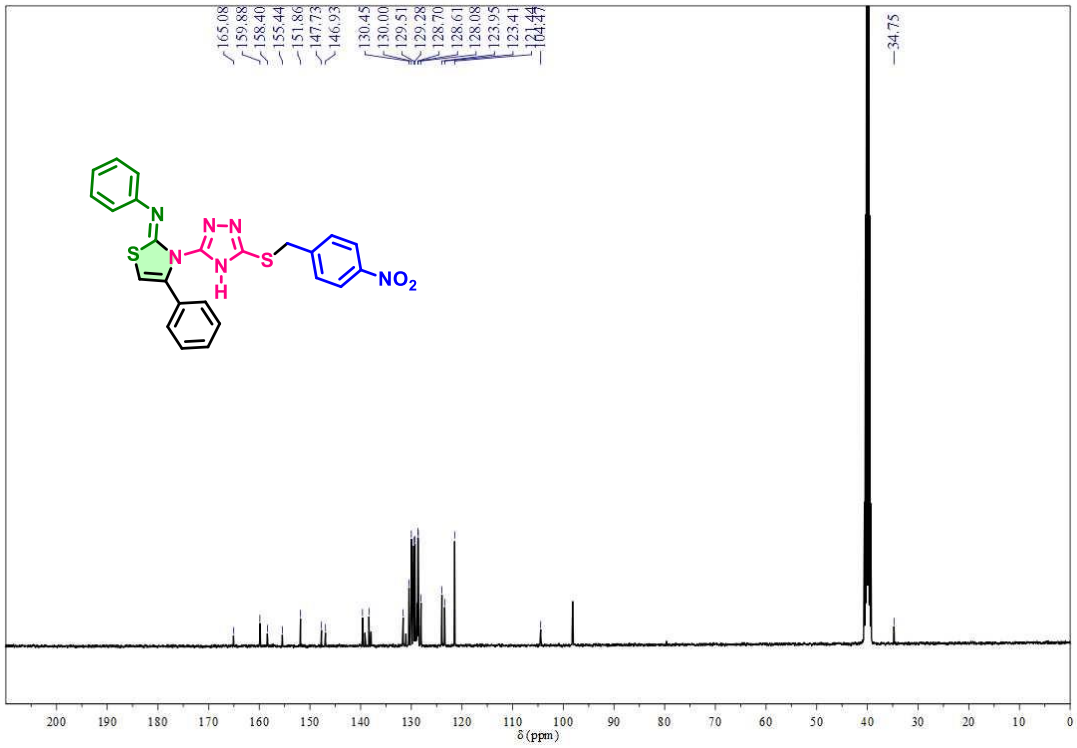
Mass spectrum of compound *5k*



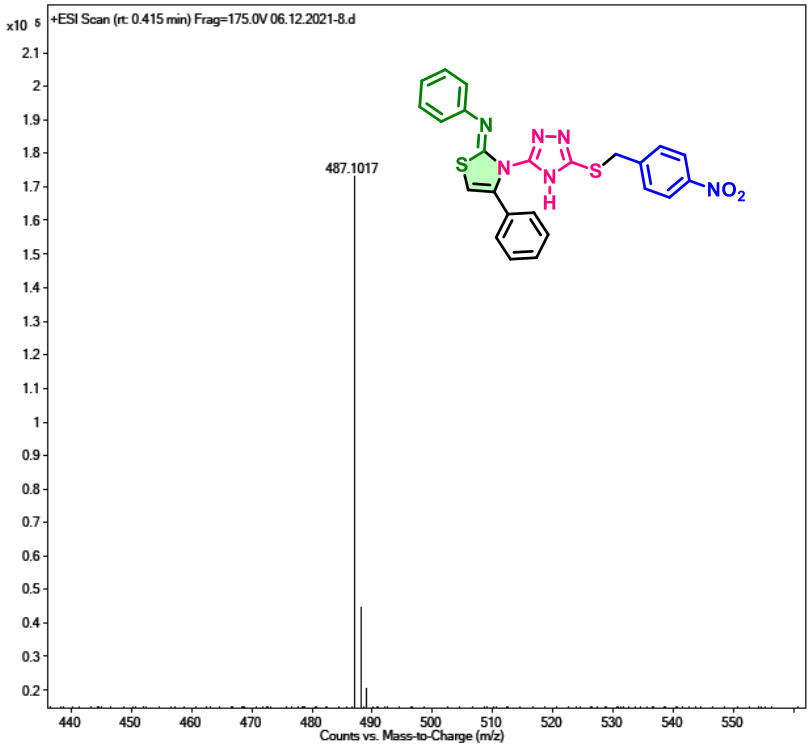
^{1}H NMR spectrum of compound *5l* (DMSO-d_6 400 MHz)



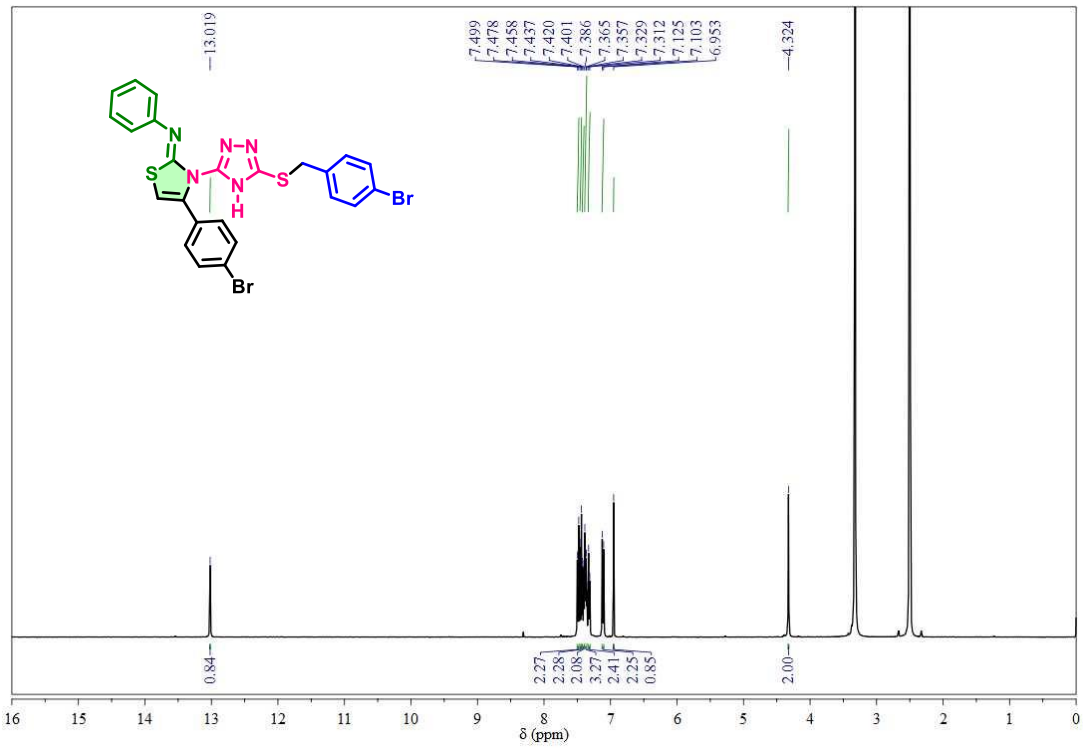
^{13}C NMR spectrum of compound *5l* (DMSO-d_6) (100 MHz)



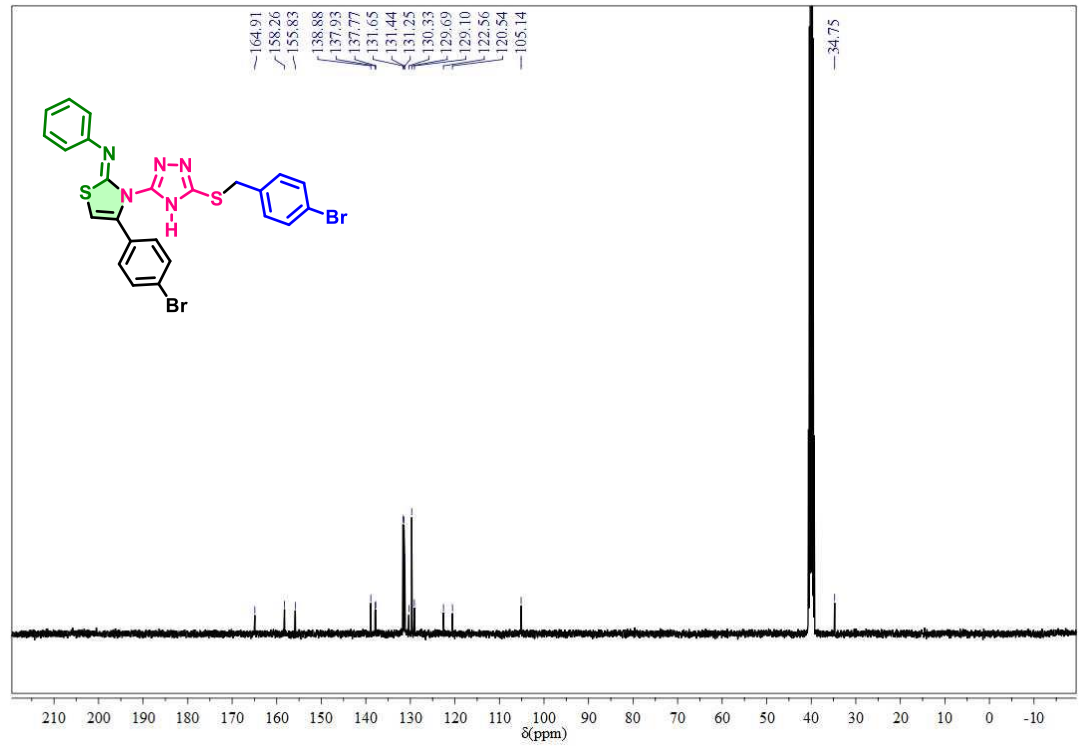
Mass spectrum of compound *5l*



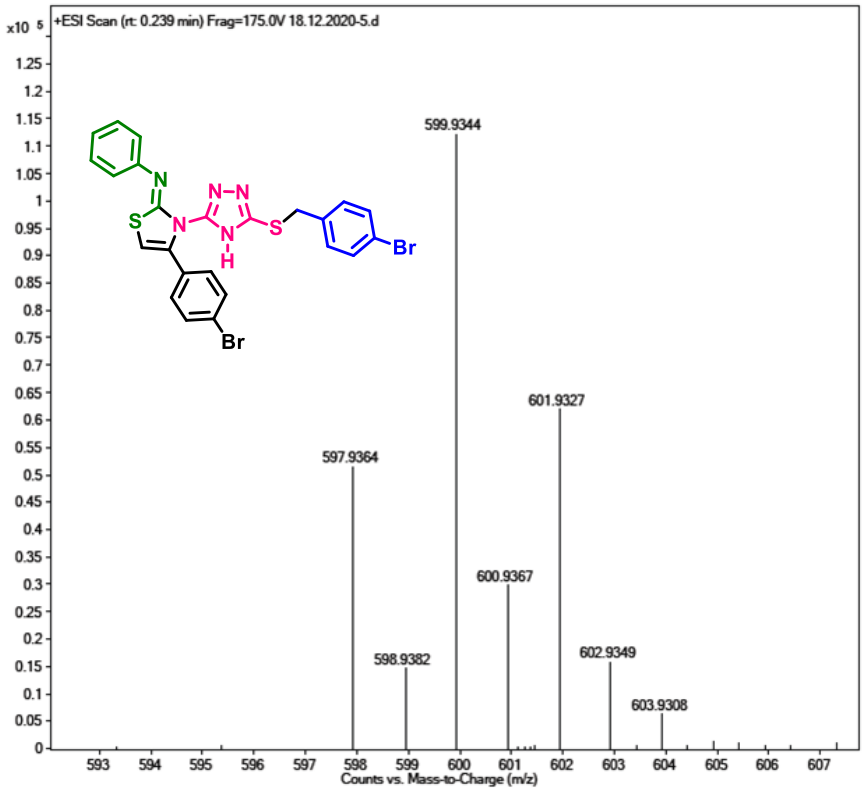
^{1}H NMR spectrum of compound *5m* (DMSO-*d*$_6$) (400 MHz)



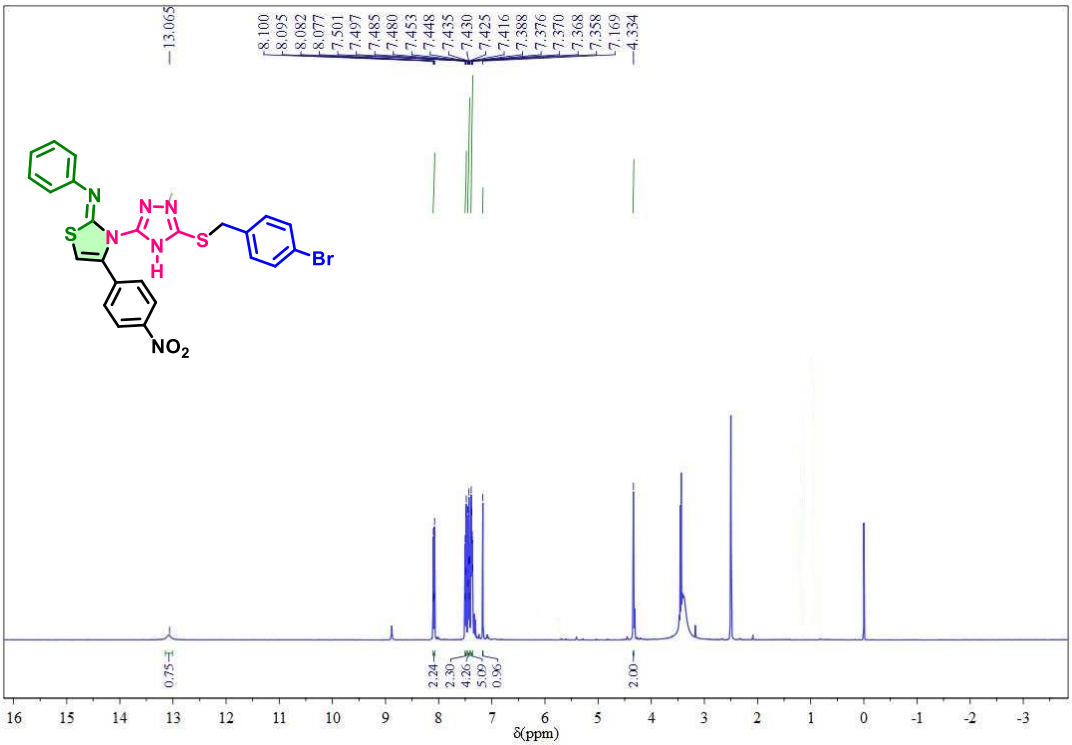
^{13}C NMR spectrum of compound *5m* (DMSO-*d*$_6$) (100 MHz)



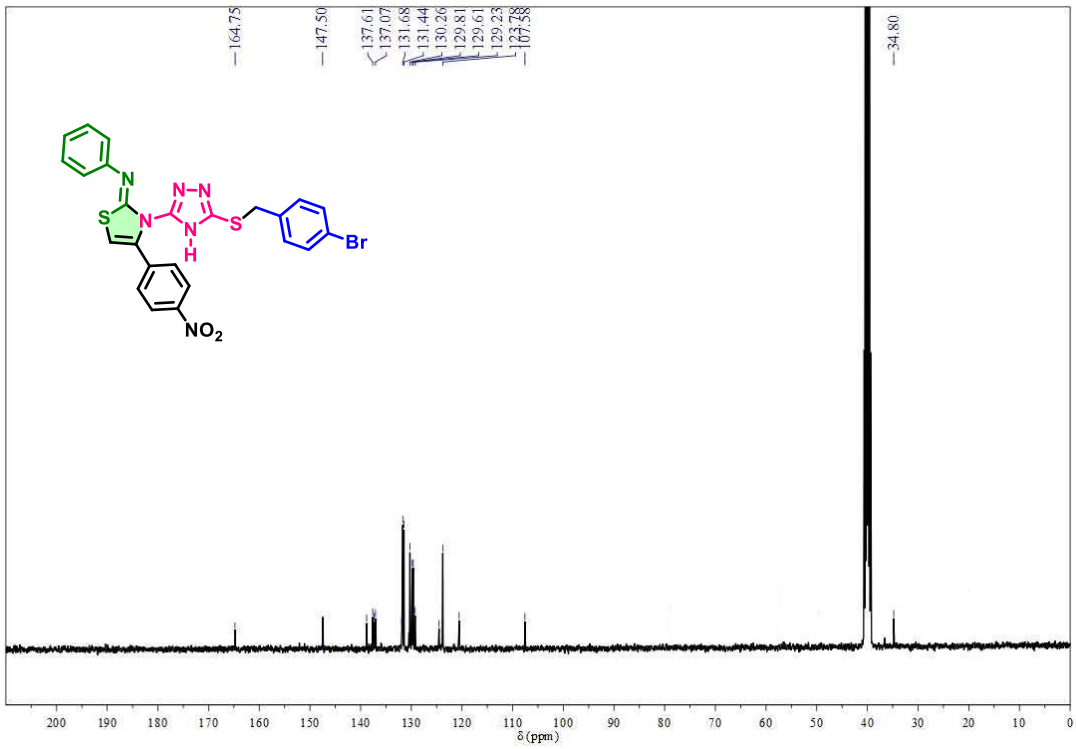
Mass spectrum of compound *5m*



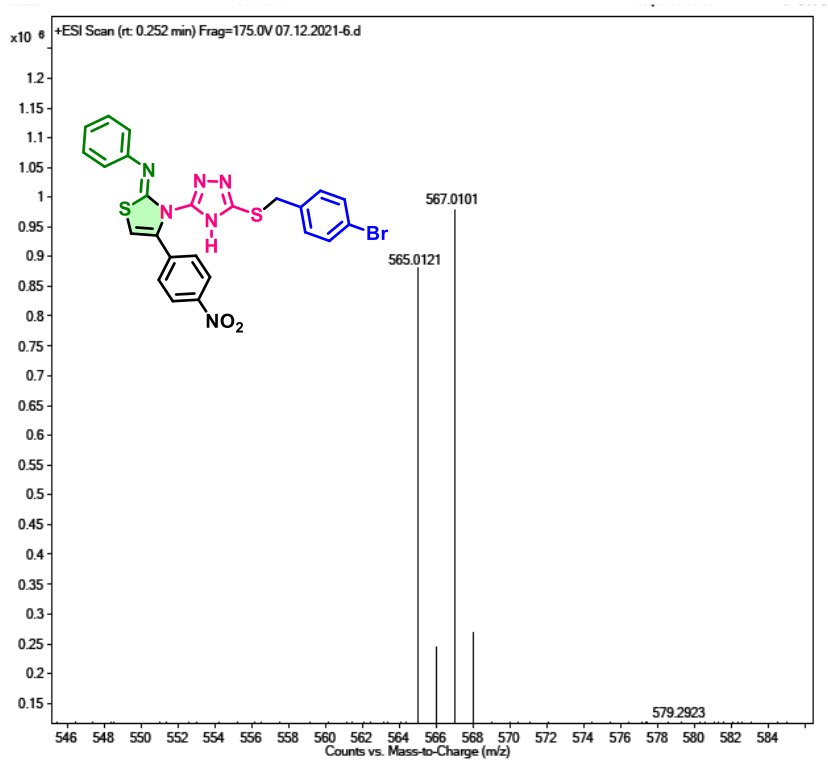
^{1}H NMR spectrum of compound *5n* (DMSO-d_6) (400 MHz)



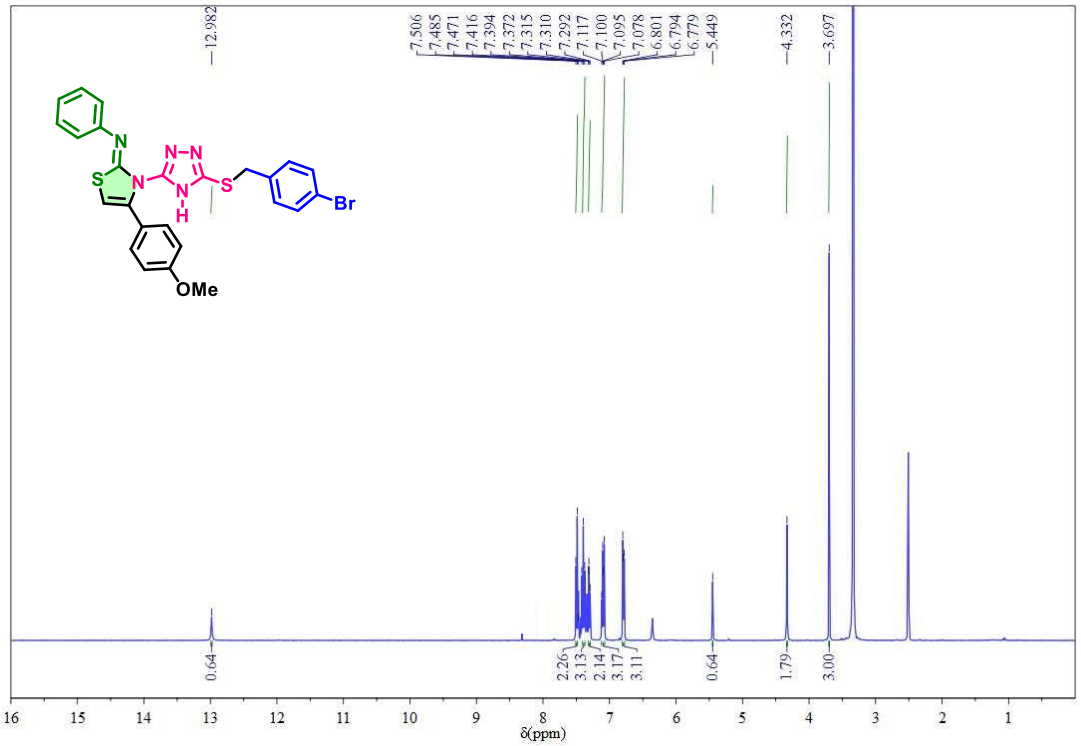
^{13}C NMR spectrum of compound *5n* (DMSO-*d*$_6$) (100 MHz)



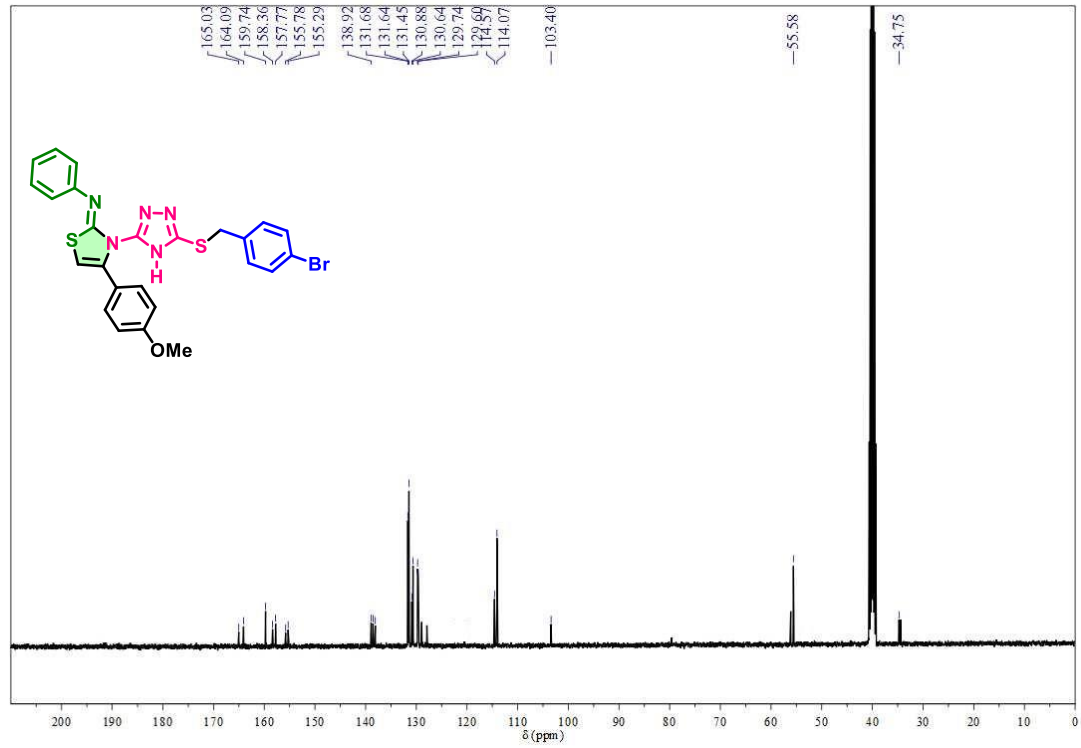
Mass spectrum of compound *5n*



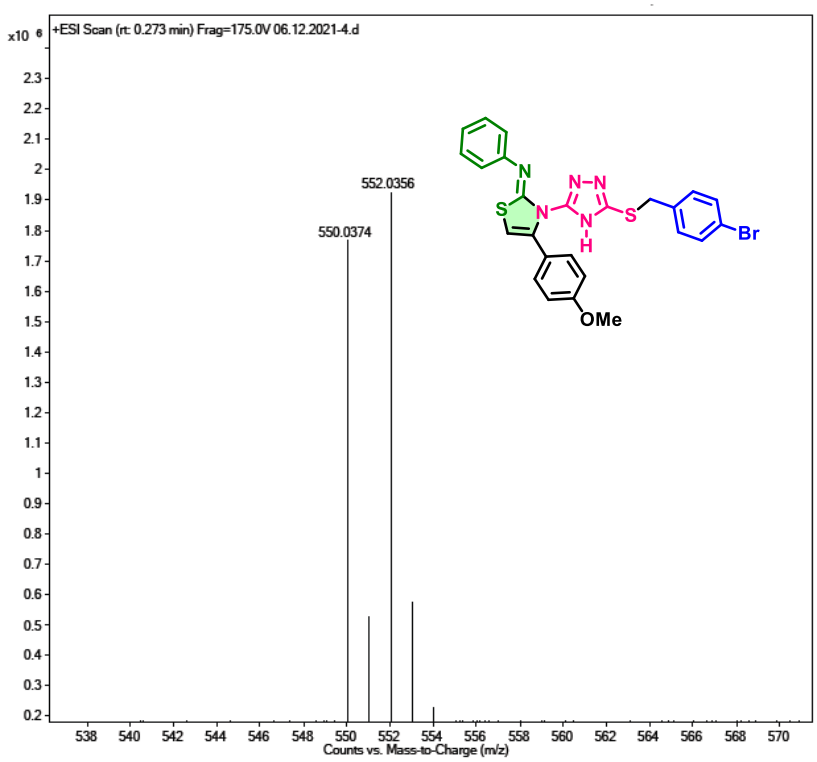
^{1}H NMR spectrum of compound *5o* (DMSO-d_6) (400 MHz)



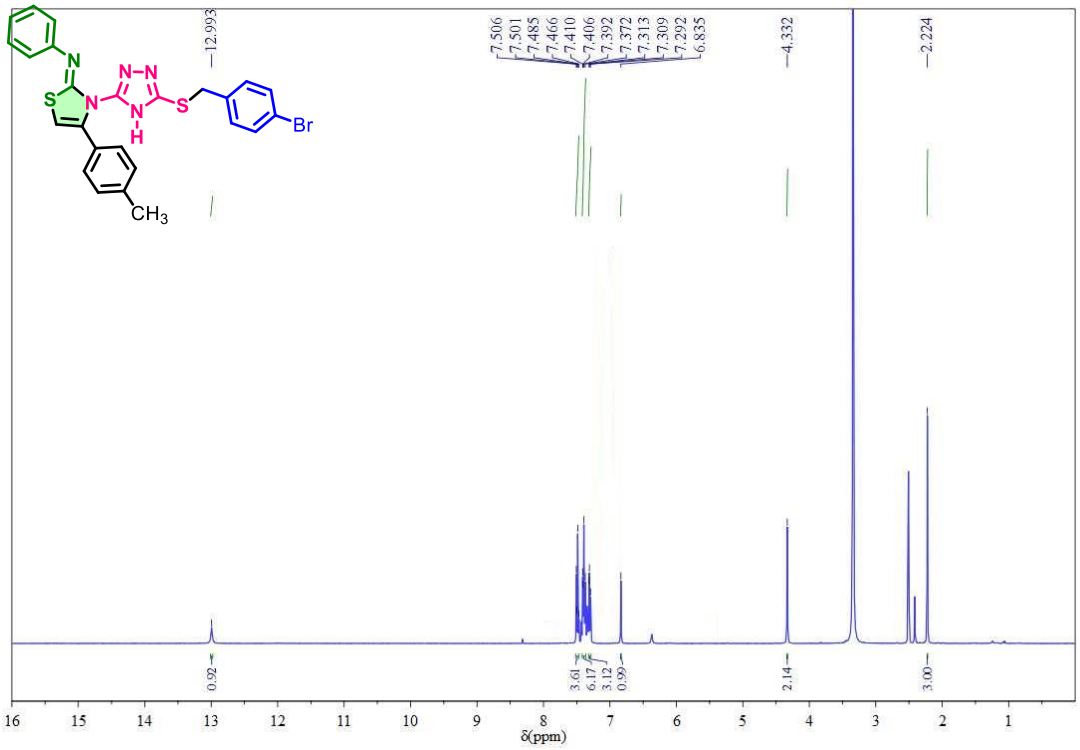
^{13}C NMR spectrum of compound *5o* (DMSO-d_6) (100 MHz)



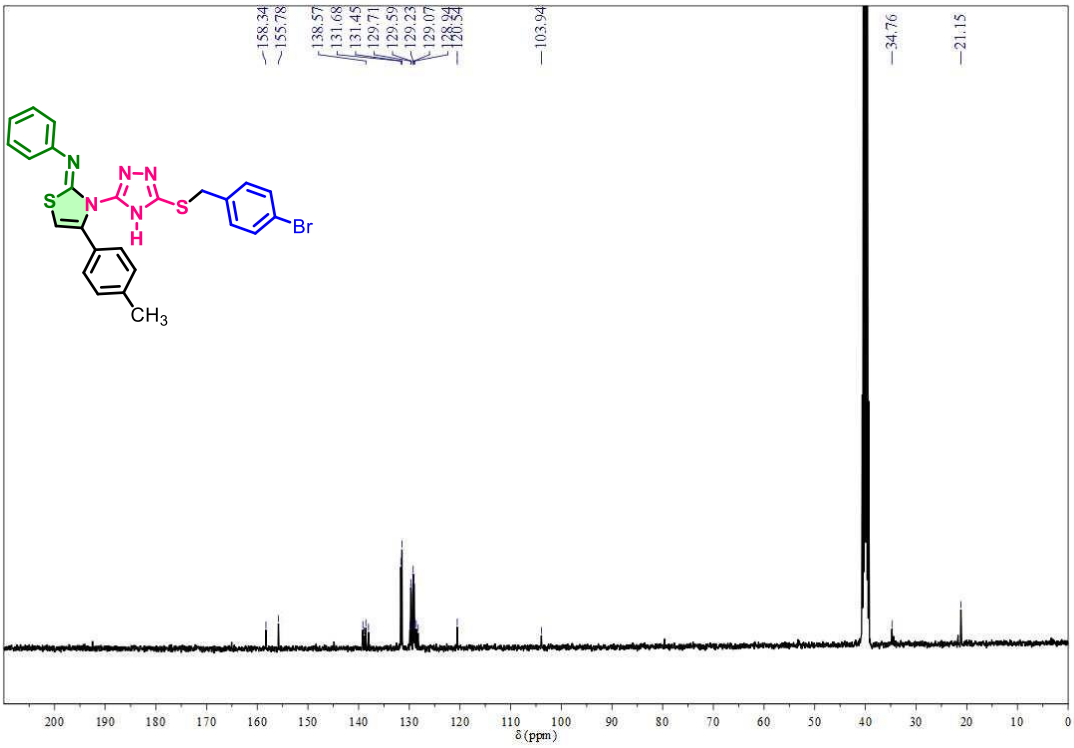
Mass spectrum of compound *5o*



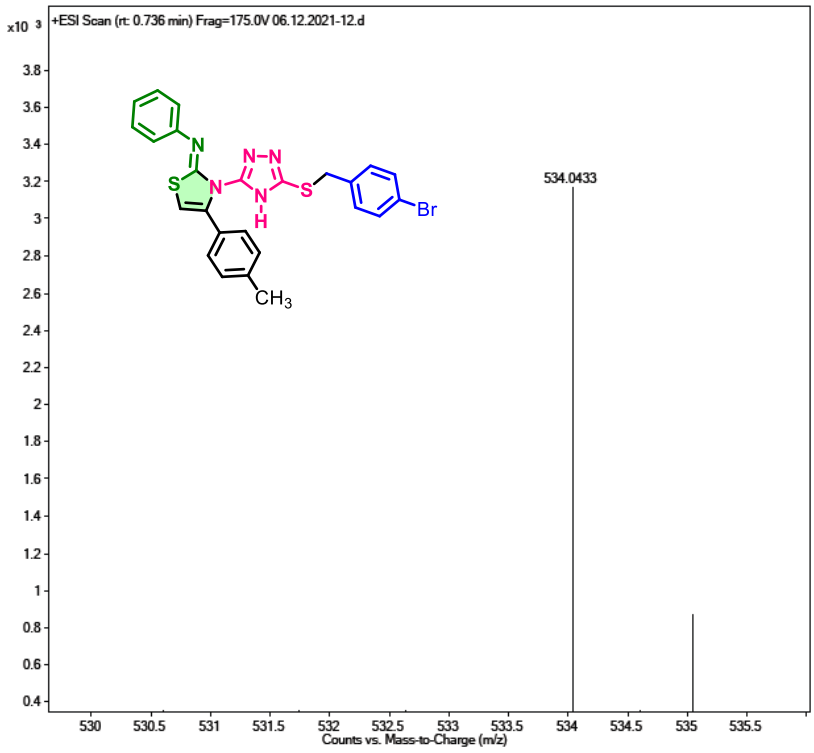
^{1}H NMR spectrum of compound *5p* (DMSO-d_6) (400 MHz)



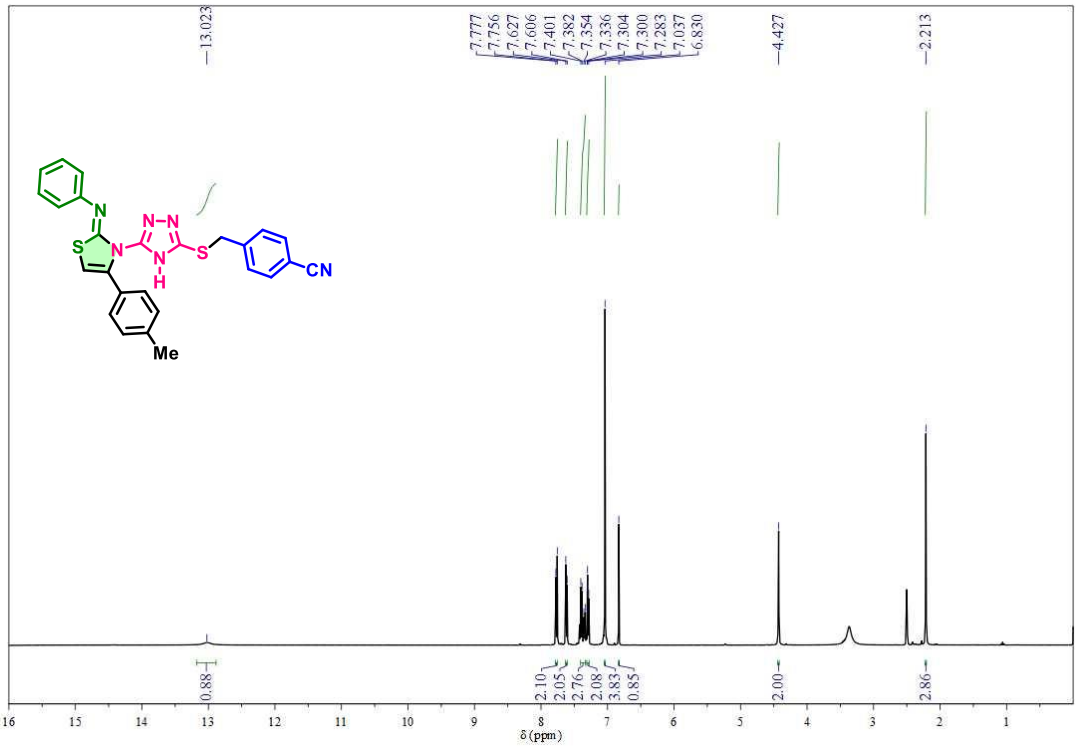
^{13}C NMR spectrum of compound *5p* (DMSO-*d*$_6$) (100 MHz)



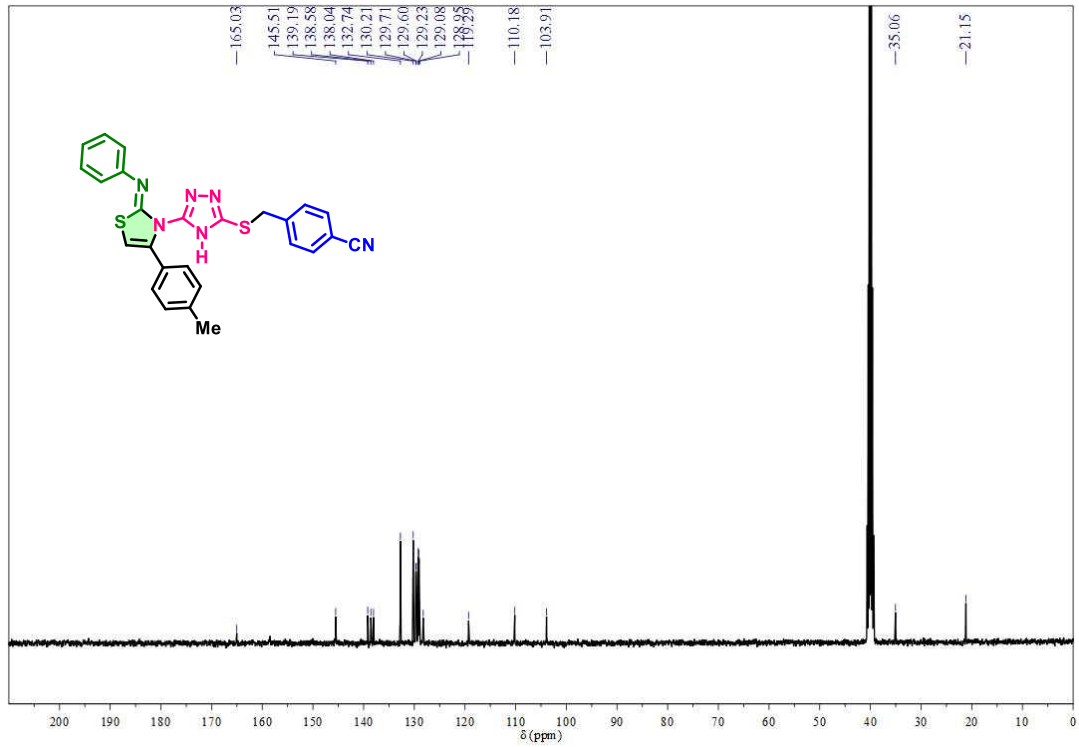
Mass spectrum of compound *5p*



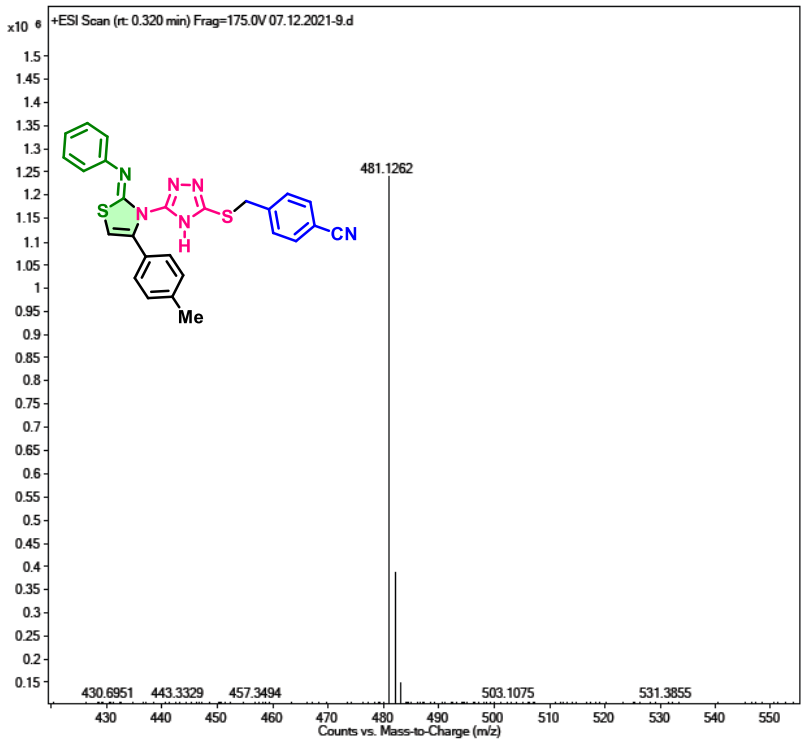
^{1}H NMR spectrum of compound *5q* (DMSO-*d*$_6$) (400 MHz)



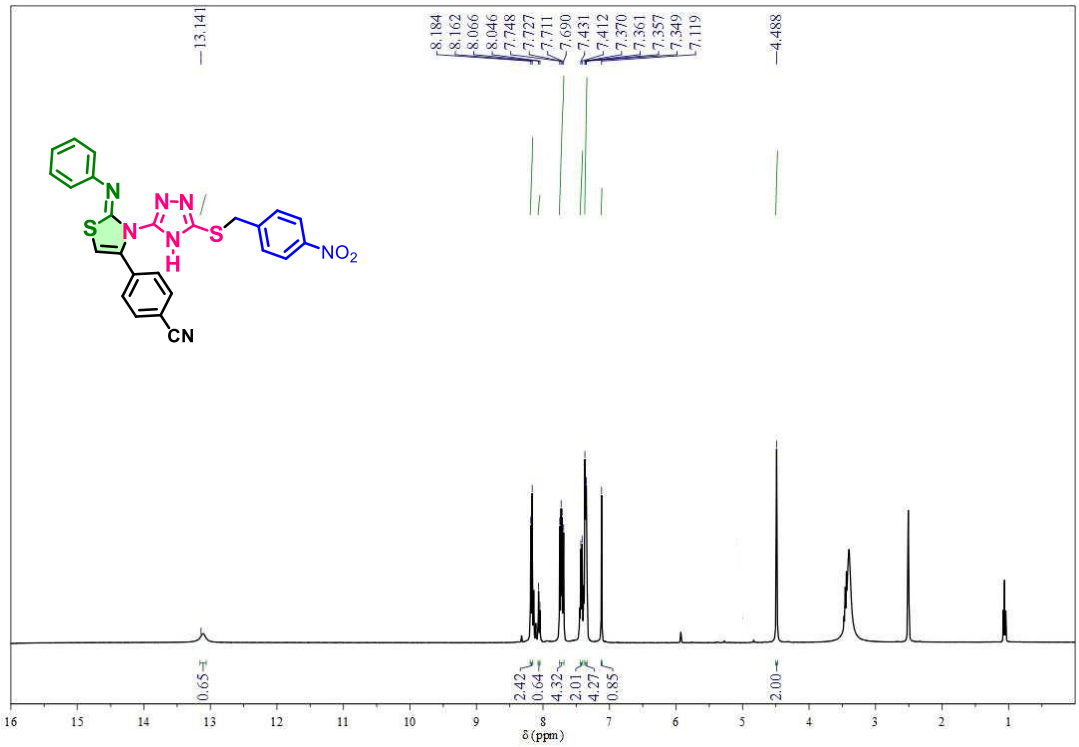
^{13}H NMR spectrum of compound *5q* (DMSO-*d*$_6$) (100 MHz)



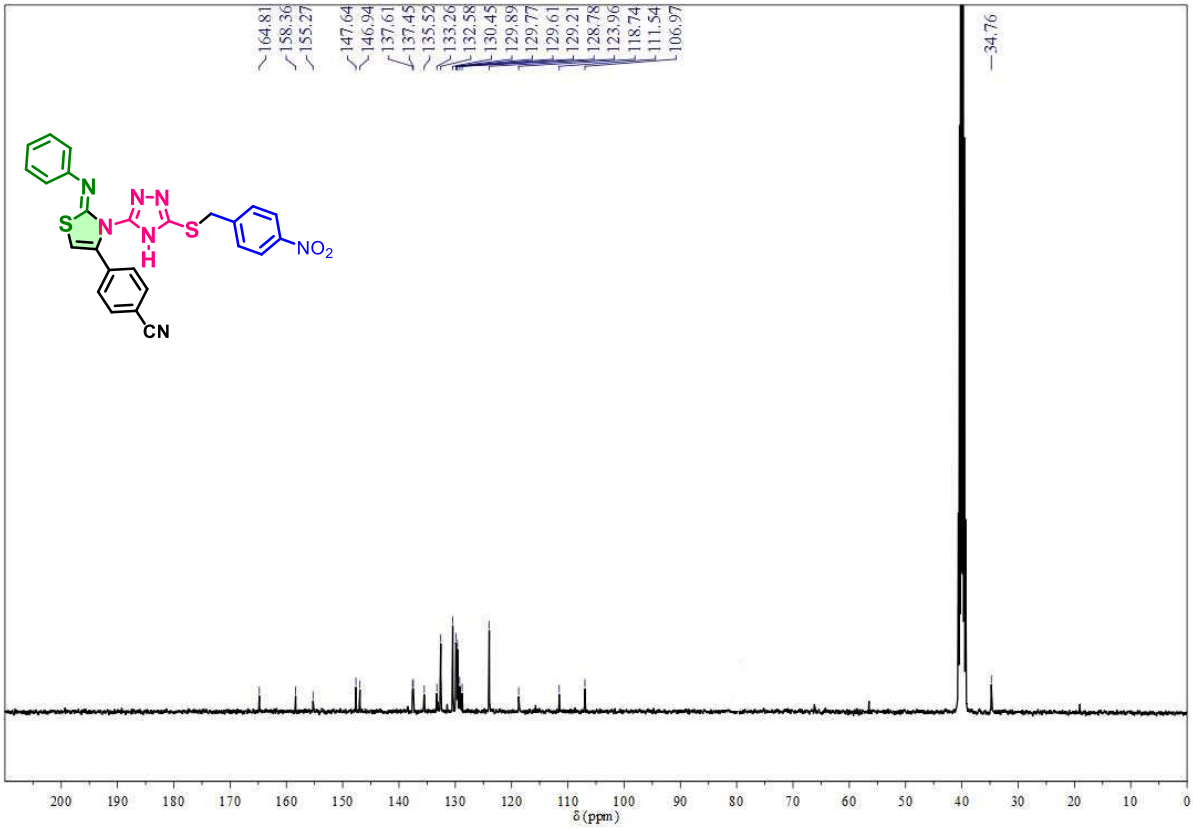
Mass spectrum of compound *5q*



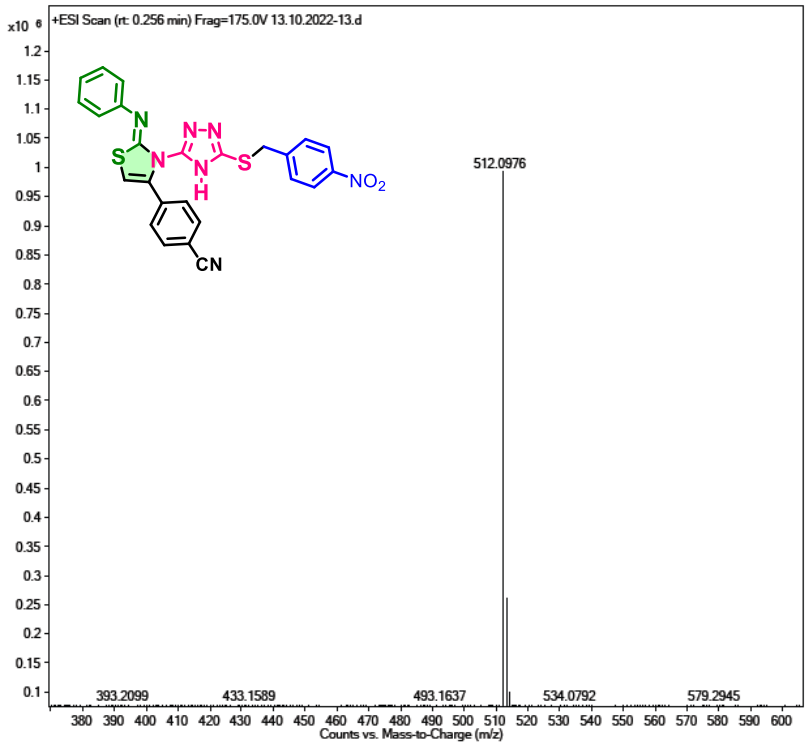
^{1}H NMR spectrum of compound *5r* (DMSO-*d*$_6$) (400 MHz)



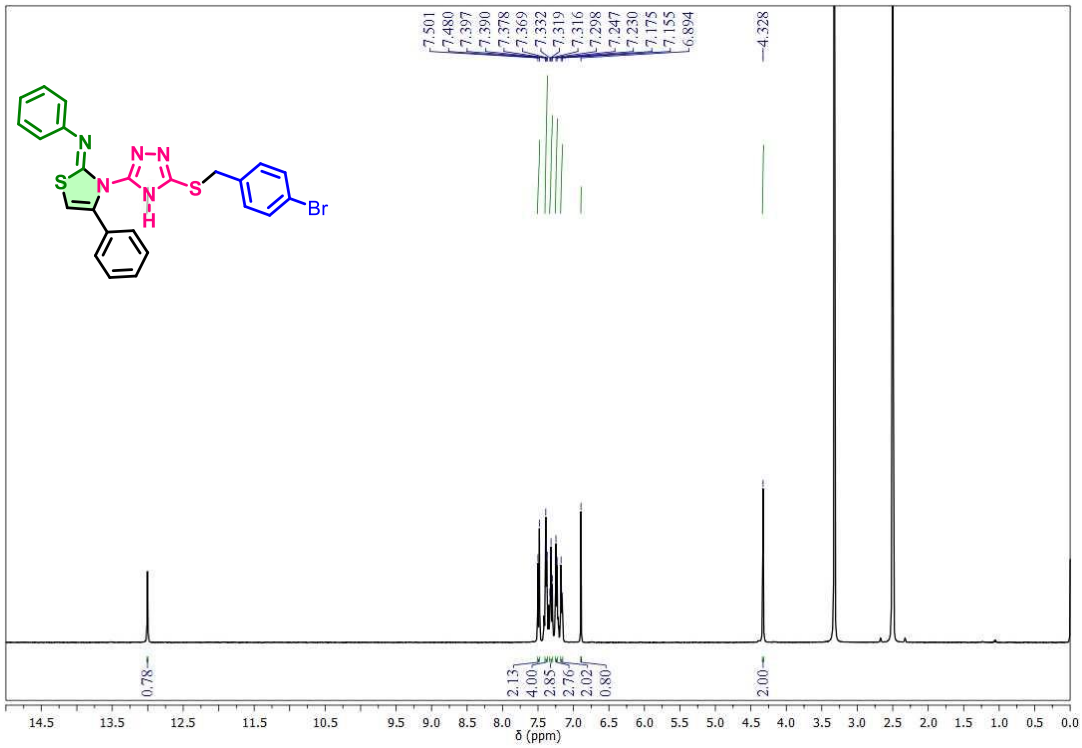
^{13}C NMR spectrum of compound *5r* (DMSO-d_6) (100 MHz)



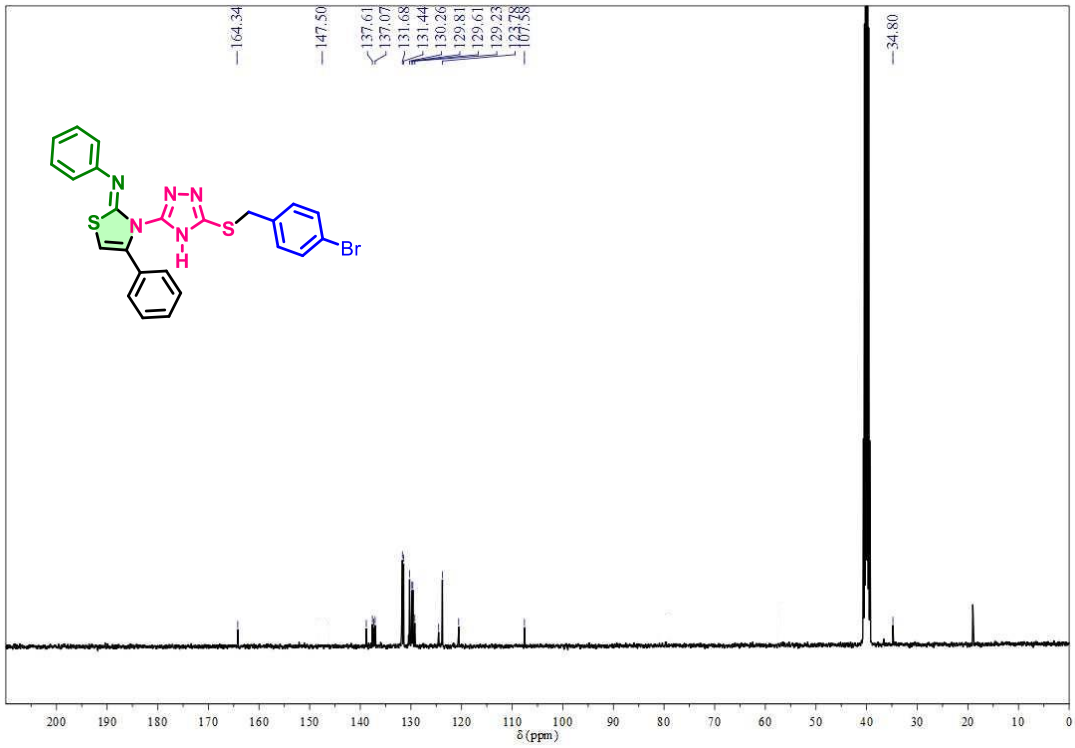
Mass spectrum of compound *5r*



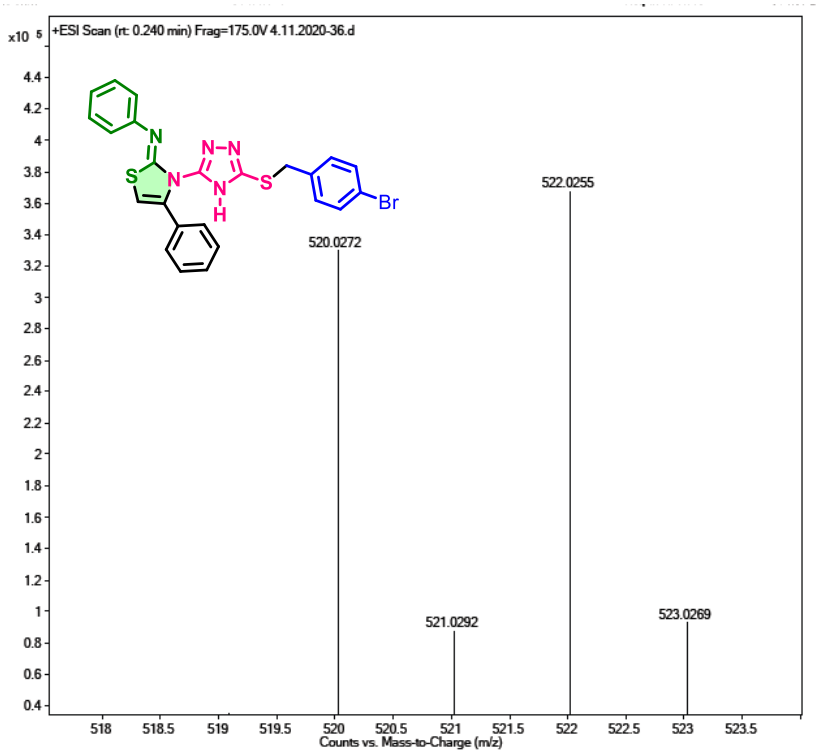
^{1}H NMR spectrum of compound *5s* (DMSO-*d*$_6$) (400 MHz)



^{13}C NMR spectrum of compound *5s* (DMSO-*d*$_6$) (100 MHz)



Mass spectrum of compound 5s



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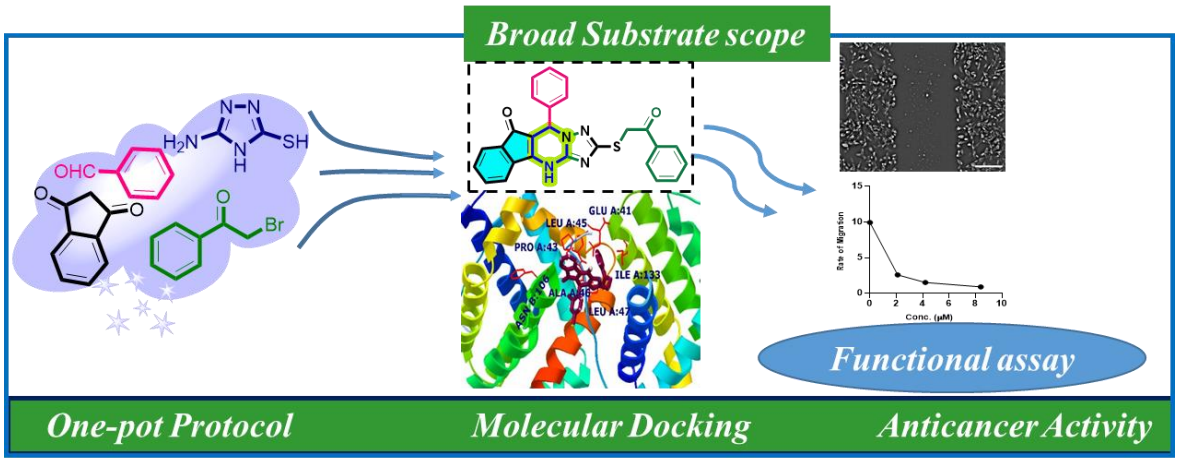
CHAPTER-V (SECTION-A)

Novel one-pot four component synthesis of 1,2,4-triazolo[1,5-a] pyrimidines, and their in-vitro anticancer evaluation, molecular docking studies



5A.1.Introduction

1,2,4-triazolo[1,5-*a*] pyrimidine (TP) bicyclic *N*-heteroarenes belong to an interesting class of fused heterocyclic systems with 10-π electrons aromatic ring. Which are composed of electron excess five membered triazole ring (6-π electron) fused to an electron deficient six membered pyrimidine ring (4- π electron) [1-2]. Depending on the structural alignment [1,5-*c*], [4,3-*c*], [4,3-*a*], [1,5-*a*] isomers are possible for TP heterocyclic rings. Among these the 1,2,4-triazol[1,5-*a*] pyrimidines have well established applications in agriculture, and medicinal chemistry [3]. When many heterocyclic rings combine into a single structure, the molecules exhibit potential therapeutic prospects (2) For instance Trapidil (**Fig-1**) is used for the treatment of kidney, coronary heart and liver diseases as well as an antiplatelet agent and vasodilator. Recently, it has been utilised in the treatment of cancer and neurological disorders [4]. the biologically active triazolopyrimidine containing moieties are shown in **Fig-1** [5]. the trifluoro methyl substituted compound 7 shows promising antiparasitic activity [6]. And the compound 8 exhibits antitubercular activity as it is having phenyl group ortho to pyrimidine ring [7].

In the field of pharmaceutical and medical chemistry, 1,2,4-triazolo[1,5-*a*] pyrimidines with fused N and S hetero atoms have a diverse range of pharmacological applications [8-15]. The antitumor properties of 1,2,4-triazolo[1,5-*a*] pyrimidines are well recognized [16-18] and are associated with [4,3-*a*], [4,3-*c*], [1,5-*c*] systems [19- 21]. A large number of TPs also exhibit antiviral [22], anti-Alzheimer's [23], antiCNS activity [24], antibacterial [25], antioxidant [26], antimalarial [27], antifungal [28], antidiabetic [29], activity and as agrochemical agents [30].

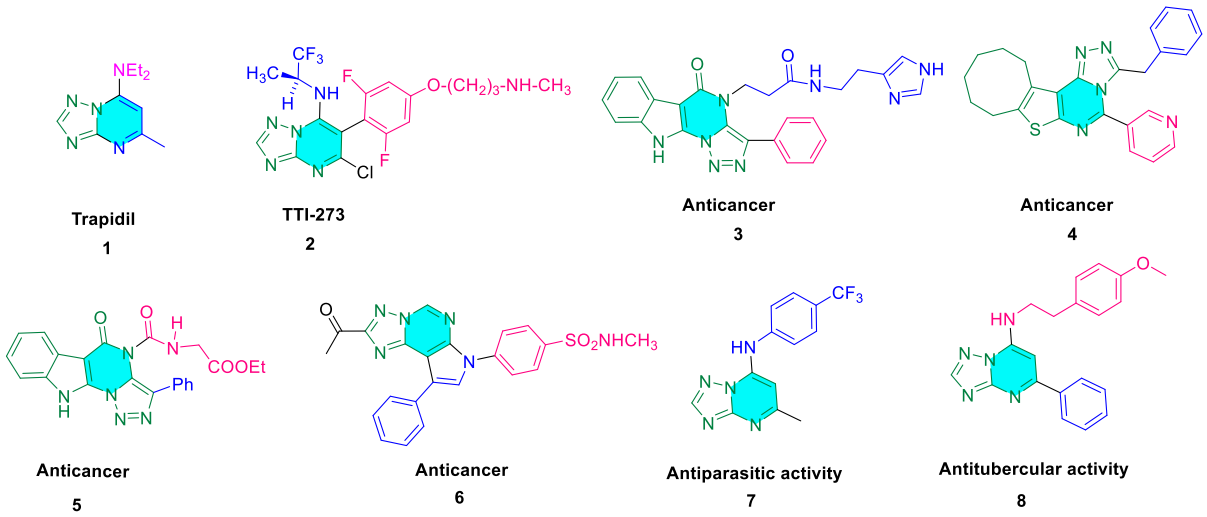
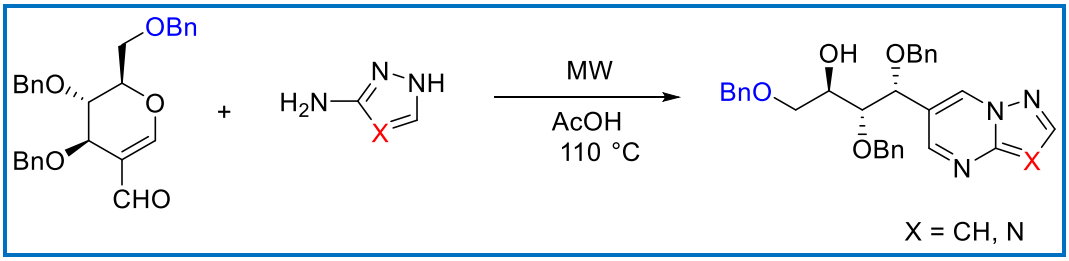


Fig-1 Similar type of fused triazolo pyrimidine moieties**.**

Literature reports:

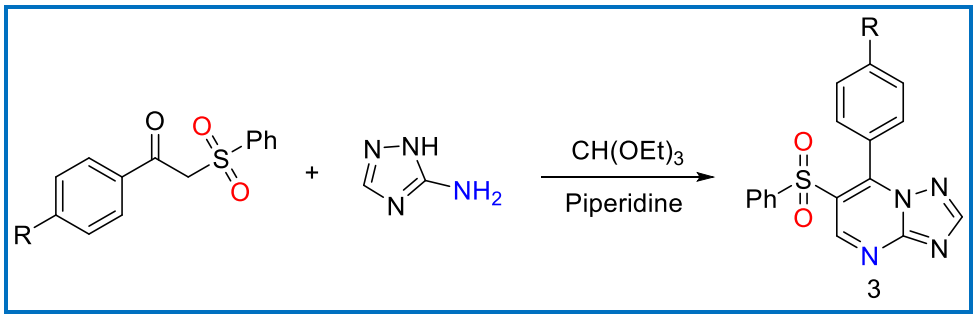
Sagar *et al* [31] published an efficient one-pot stereo divergent drug like carbohydrate 1,2,4-triazolo[1,5-*a*] pyrimidines, pyrazolo[1,5-*a*] pyrimidine from the reaction of 2C-formyl glucal and 3-amino-1,2,4-triazole, 3-aminopyrazoles under microwave irradiation by using AcOH at 110 ˚C. (Scheme-1.1)

Scheme-1.1



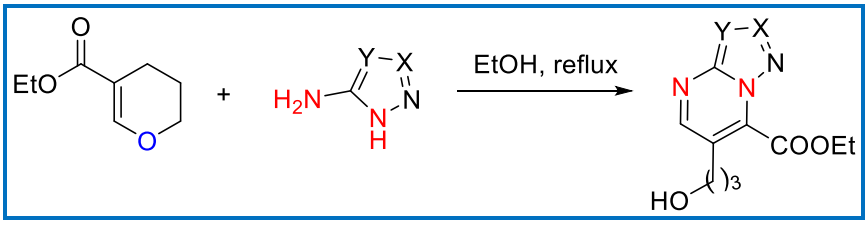
Shaaban *et al* [32] developed the sulfonated compound 3. The compound 3 was obtained form (1-aryl-2-(phenyl) sulfonyl) ethanone and 3-amino 1,2,4-triazole in the presence of triethyl ortho formate and piperidine. Further, these derivatives were screened against for colon tumor anticancer activity. (Scheme-1.2)

Scheme-1.2



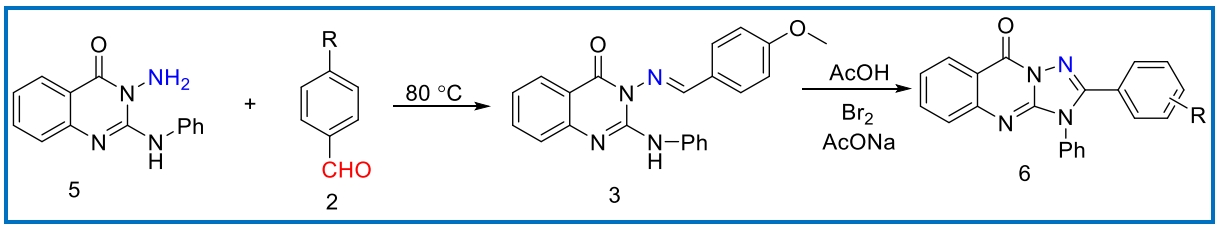
Stepaniuk *et al* [33] established the one-pot synthesis of fused triazolopyrimidine heterocyclic compound. In this 2-pyrene 3-carboxylate on reaction with pyrazole amine or triazole-2-amine compound-2 in ethanol under reflux for 36 h to give the corresponding bicyclic triazolo pyrimidines-4 or pyrazoles. (Scheme-1.3)

Scheme-1.3



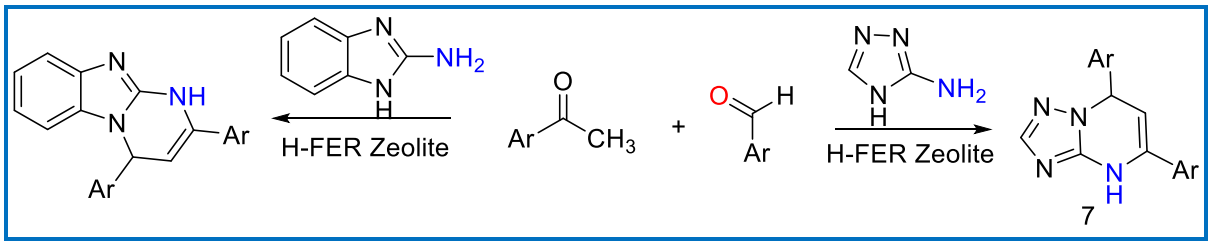
Fischer [34] reported the linear triazolo quinazoline heterocyclic compound-6. In this reaction the combination of di amino quinazolone 4-one (5) on reaction with substituted aromatic aldehydes to generate imine derivative-3, after cyclization reaction with AcOH/NaOAc a triazolo pyrimidinone compound-5 with high yield. (Scheme-1.4)

Scheme-1.4



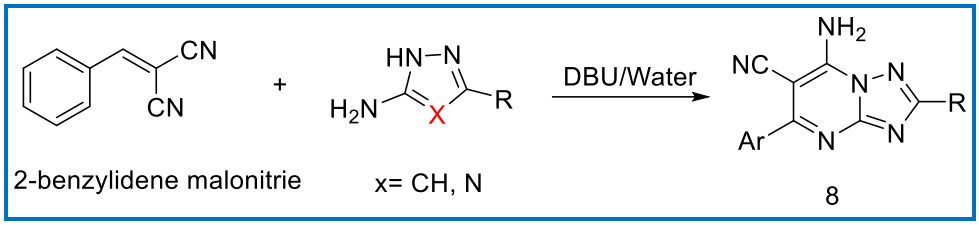
Hassane *et al* [35] carried out the one-pot three component synthesis of ferrierite zeolite catalytic mediated pyrimido [1,2-*a*] benzimidazole 1,2,4-triazolo[1,5-*a*] pyrimidines (7). Condensation of acetophenone, aromatic aldehydes and 2-amino benzimidazole or 2-amino-1,2,4-triazole by using catalytic amount of H-FER zeolite catalyst in presence of water under reflux to obtain the title compounds. (Scheme-1.5)

Scheme-1.5



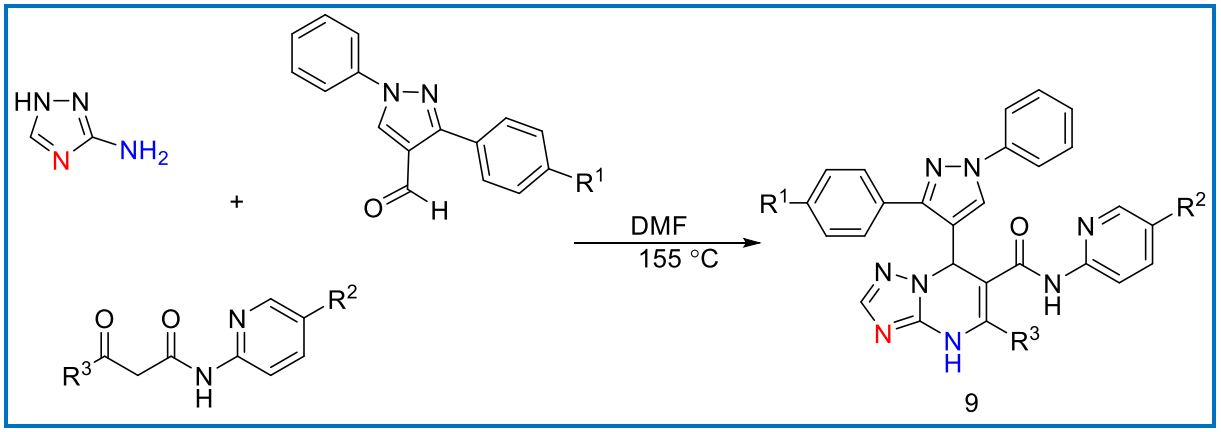
Gol *et al* [36] synthesized the 1,2,4-triazolo[1,5-*a*] pyrimidine systems by the reaction of 2-benzylidine malanonitrile and 1*H*-pyrazolo 5-amine or triazole 2-amine by using DBU as a base and water as a solvent under heating to generate a corresponding pyrimidine compound 8 with good yield. (Scheme-1.6)

Scheme-1.6



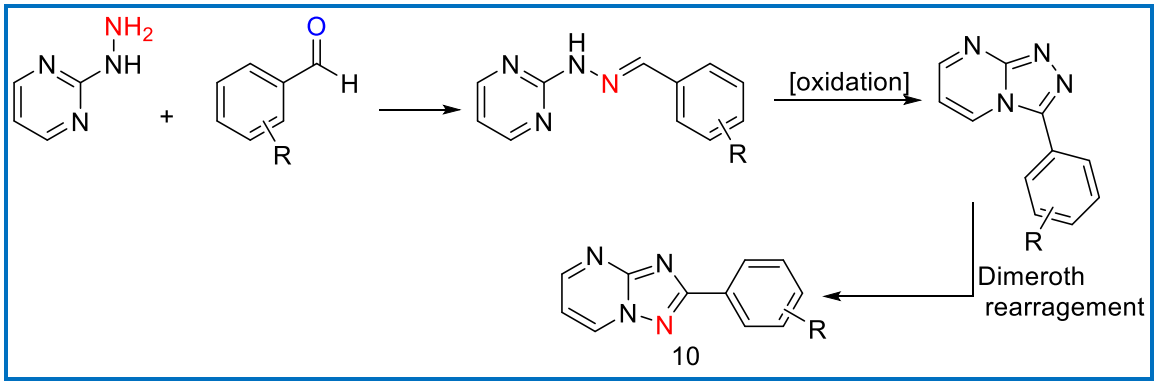
Bhatt *et al* [37] developed pyrazole substituted triazolo pyrimidine type derivatives (9) via one-pot multi-component process. In this they have condensed pyrazole carbaldehyde, triazole amine and pyridine-2-yl-3-oxobutanamide in DMF at reflux to yield the final compounds-9. Furthermore, these final substrates were evaluated for their antitubercular activity. (Scheme-1.7)

Scheme-1.7



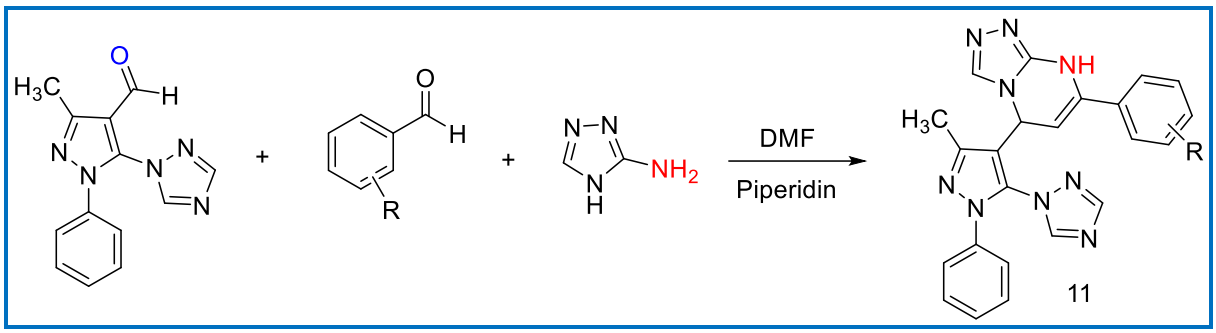
Salgado *et al* [38] reported the microwave irradiation reaction of 2-hydrazineylpyrimidine with various substituted aromatic aldehydes to produce the corresponding *N*-benzylidene-*N*-pyrimidin-2-yl hydrazine on Dimroth rearrangement give compounds 10. (Scheme-1.8)

Scheme-1.8



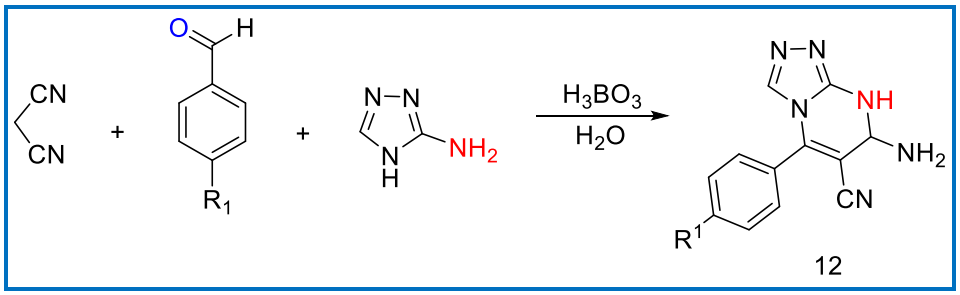
Pogaku *et al* [39] reported the one-pot three component condensation of triazole based substituted pyrazole carbaldehyde with aromatic aldehydes and 1,2,4-triazole amine in presence of DMF/Piperidine at reflux temperature to generate a triazolopyrimidines-11 with notable yields. And these compounds have shown antidiabetic activity. (Scheme-1.9)

Scheme-1.9



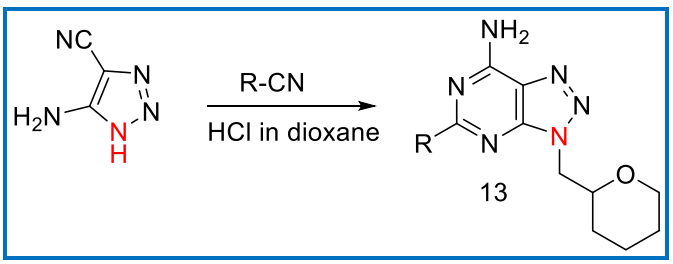
Singh *et al* [40] developed the one-pot three component condensation of malanonitrile, aromatic aldehydes and 1,2,4-triazole 3-amine in boronic acid as a catalyst in aqueous miscellaneous medium. The products are triazolo pyrimidines (12) with good yields. (Scheme-1.10)

Scheme-1.10



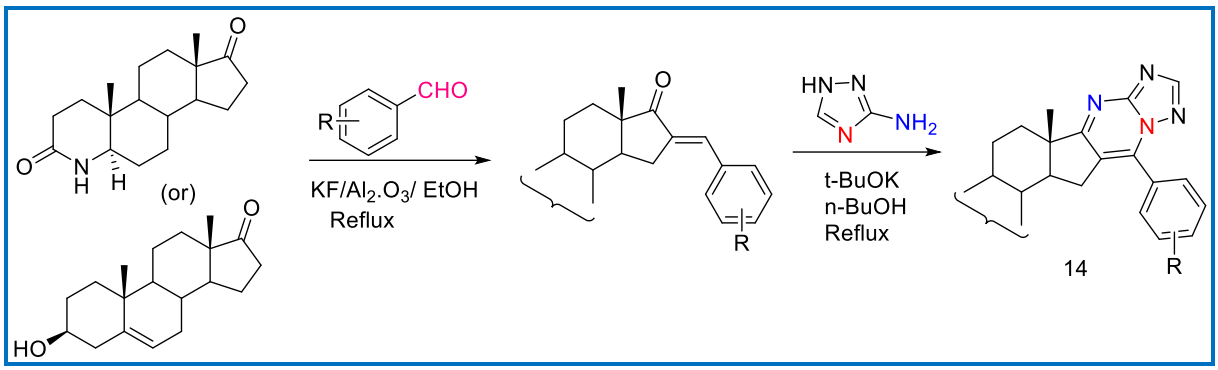
Deninno *et al* [41] established a one-pot protocol for the construction of triazolopyrimidine heterocyclic compounds-13 by the reaction of 4-cyano triazolo-5-amine with different substituted cyanides in presence of HCl/Dioxane. These compounds have exhibited antidiabetic activity. (Scheme-1.11)

Scheme-1.11



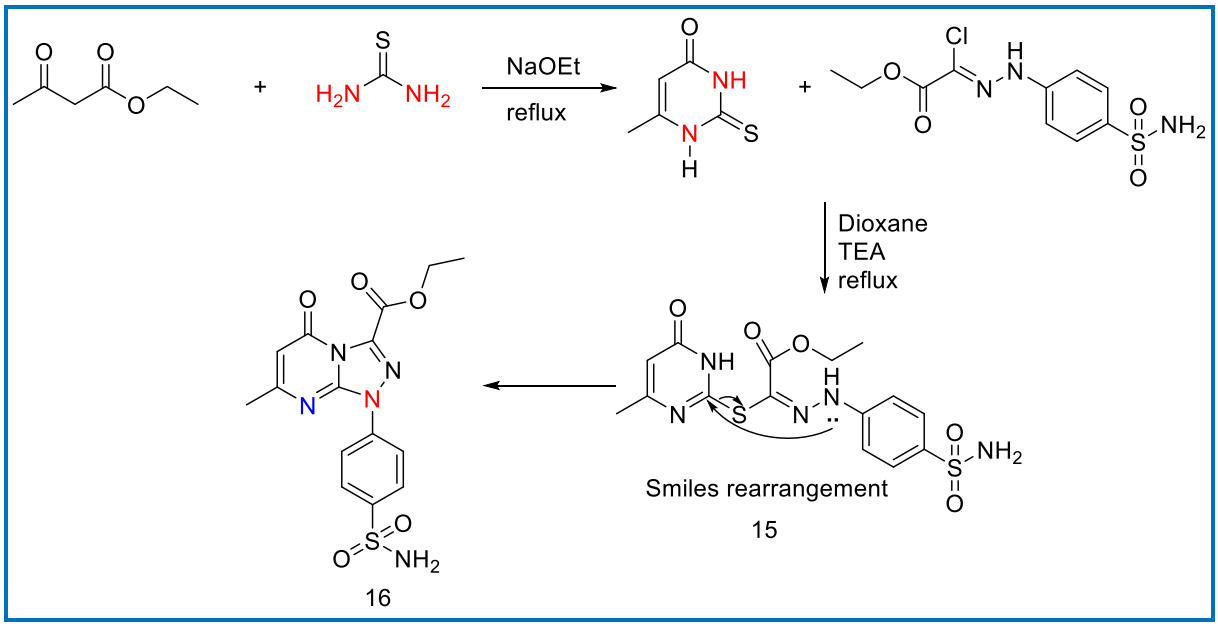
Huang *et al* [42] synthesized the triazolo pyrimidines (14) by the reaction of 4-aza androstane-3, 17-dione (or) DHEA dehydro epiandrosterone with different aromatic aldehydes in KF/Al_2O_3 to produce the corresponding aldol products. Further, these intermediate on reaction with traizole amine in presence of *t*-BuOK/*n*-BuOH at reflux gave a triazolopyrimidine compounds-14. The final compounds have screened for their anti-inflammatory activity. (Scheme-1.12)

Scheme-1.12



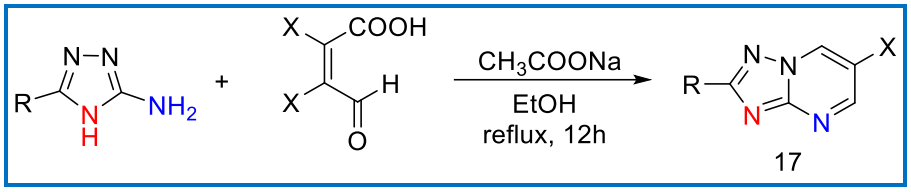
Said *et al* [43] published the synthesis of triazolo pyrimidones-15. Cyclo condensation of EAA, thiourea in the presence of NaOEt to result the 6-methyl thio uracil. Subsequently this compound on reaction with hydrazonyl chloride in dioxane and Et_3N yields thioalkylated compound 15. This undergoes Smiles rearrangement to produce the final compound-16. These molecules have shown good anticancer activity. (Scheme-1.13)

Scheme-1.13.



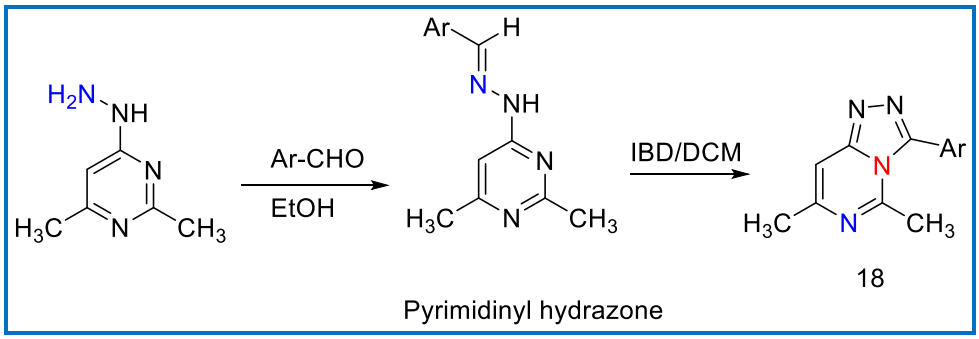
Krishnaraj *et al* [44] synthesized the triazolo[4,3-*a*] pyrimidines-17 by the reaction of 1,2,4-triazole amine with 2,3 dihalo-4-enoic acid (unsaturated acid) under EtOH/NaOAc at reflux temperature. The final compounds were subjected for their antibacterial activity against *E. coli,* and *S. aureus.* (Scheme-1.14)

Scheme-1.14



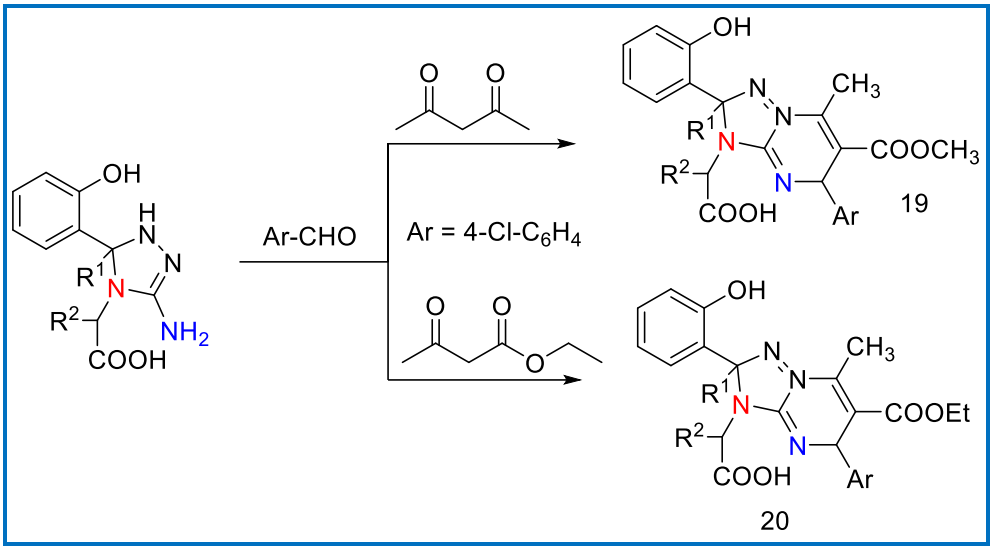
Kumar *et al* [45] reported the condensation of 2,6-di methyl 4-hydrazino pyrimidine with aromatic aldehydes in presence of EtOH to form Schiff bases. compound 2. These on oxidative cyclocondensation reaction in presence of IBD/DCM to give a 1,2,4-triazole[4,3-c] pyrimidines (18). The final derivatives possess antibacterial activity against *B. subtilis* is Gram +Ve bacteria and *E. coli* is Gram –Ve bacteria.

Scheme-15.



Mohamed *et al* [46] established a one-pot three component reaction of 3-amino-5-phenyl triazolo-4-propanoic acid with aldehydes, acetyl acetone (or) ethyl acetoacetate to produce the corresponding bicyclic triazolopyrimidine molecules 19 and 20. These were evaluated for their antibacterial activity by using Cephalothin, Chloramphenicol used as reference drugs.

Scheme-16



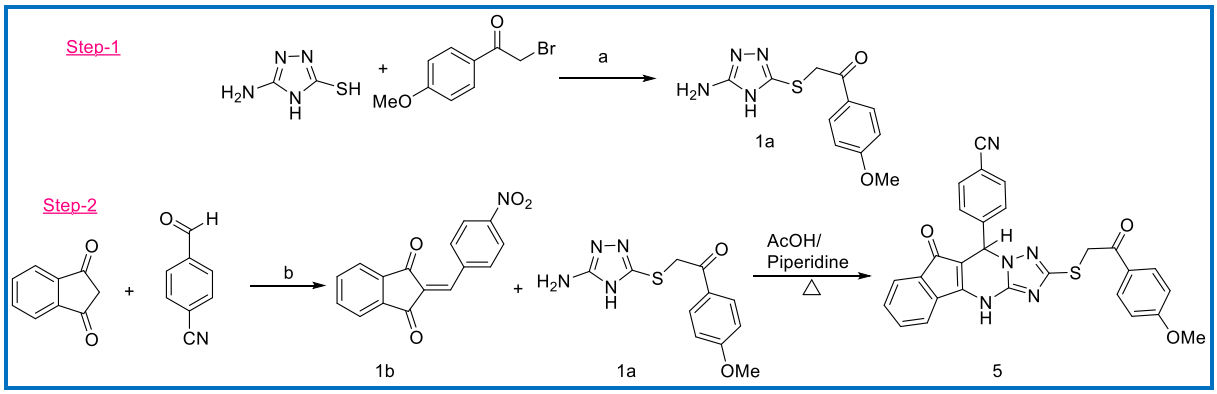
5A.2. Present work.

Earlier mentioned literature reports reveal that the triazolo pyrimidine derivatives are significant entities in pharmaceutical industry and used as a drugs for curing diseases. In view of biological importance of TPs in the present work we have developed a new class of thioalkylated (benzyl/phenacyl) 1,2,4-triazolo[1,5-*a*] pyrimidine analogues under multicomponent approach.

5A.2.1. Synthesis: Method-1

In the method-1 the reaction of 5-amino-4*H*-1,2,4-triazole-3-thiol with 4-methoxy phenacyl bromide in ethanol at 80 °C for 6 h to give intermediate compound **1a**.

Scheme-1: **method-1.** Synthesis of fused triazolopyrimidines.



Reaction conditions: (a). Ethanol 60 °C, (b). AcOH/ Piperidine at 90 °C

In this reaction the more nucleophilic thiol group displaces the bromine atom of 4-methoxy phenacyl bromide to give thioalkylated compound instead of *N*-alkylated product. This is due to more nucleophilicity of thiol group. In step-2 the reaction of 1,3-indane dione with 4-cyano benzaldehyde in presence of AcOH/piperidine under reflux conditions gave an intermediate **1b.** To the **1b** the first product **1a** was added and again refluxed for 7-8 h, where the yellow solid product was formed with 65 percentage yield. The synthetic protocol is depicted in **Scheme-1**.

Method-2: Schematic representation of thioalkylated triazolopyrimidines.

Alternative synthesis to method-1 is method-2. In this process we have carried out 4CC reaction between 1,3-indane dione, *p*-cyano benzaldehyde, 5-amino-4*H*-1,2,4-triazolo-3-thiol and *p*-methoxy phenacyl bromide in acetic acid/piperidine (4:1) at 90 ˚C. In this reaction there is simultaneous formation of two nitrogen-carbon bonds, one C-C and one C-S bonds. In this method-2 the high % of yield of the products were formed in a short reaction time compare to method-1. We followed the method-2 for the synthesis of final compounds. The outline schematic representation of one-pot four component process is shown in **scheme-2**.

Scheme-2

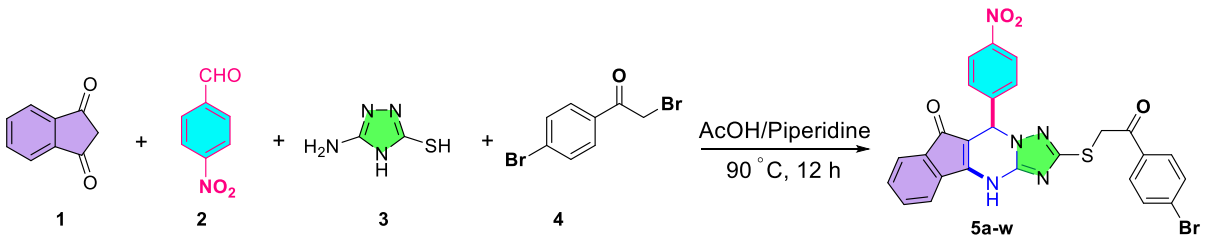


Table -1: Optimization conditions for the synthesized compounds[a].

S.No	Solvent	Temp (°C)	Time (h)	Yield (%)[b]
1	CH_3CN	65	8	n.r
2	EtOH	65	8	8
3	MeOH	65	8	n.r
4	AcOH	65	8	15
5	DMF	70	10	10
6	EtOH+ Et_3N (4:1)	75	10	20
7	EtOH+ HCl (4:1)	80	10	15

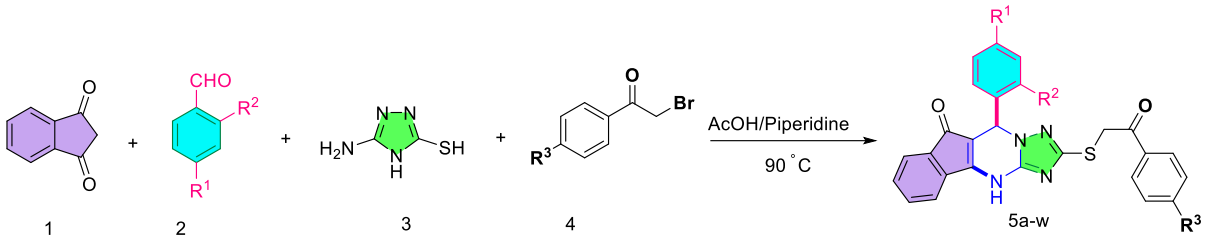
8	AcOH+ H_2SO_4 (4:1)	80	10	20
9	DMF+ EtOH (4:1)	90	12	25
10	AcOH+ Piperidine (4:1)	80	11	40[c]
11	AcOH +Piperidine (4:1)	80	14	60[d]
12	AcOH +Piperidine(4:1)	80	16	95[e]

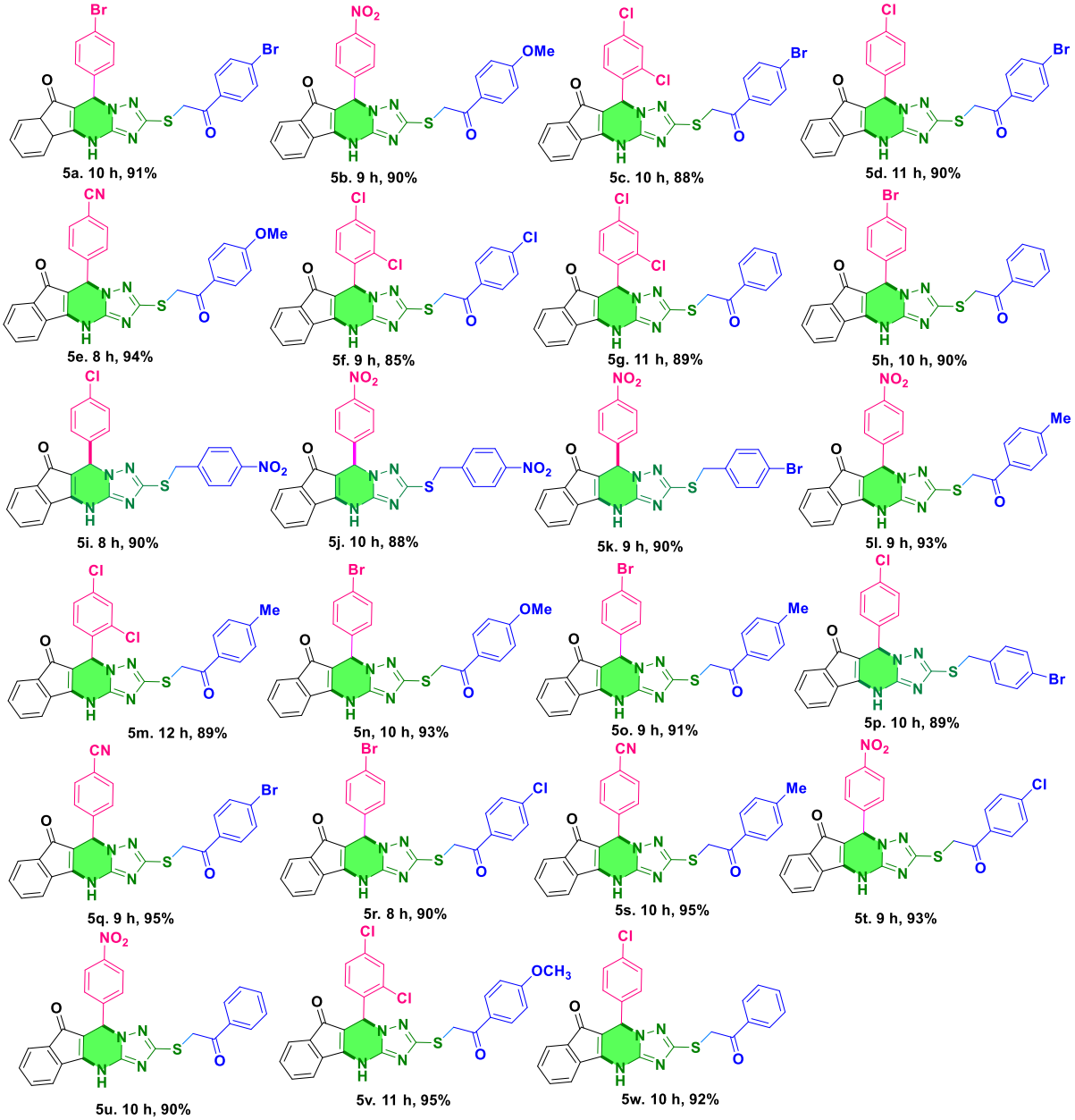
[a]**Reaction conditions:** 1,3 indane dione (1 mmol), 4-cyano benzaldehyde aldehyde (1 mmol), 5-amino-4*H*-1,2,4-triazole-3-thiol (1 mmol), 4-Ome phenacyl bromide (1 mmol) taken in solvent AcOH, Piperidine (4:1). [b]Isolated yield, [c]AcOH + piperidine, [d]AcOH + piperidine, [e]AcOH + piperidine at 90 °C for 16 h. n.r = no reaction.

We have carried out the optimization study of the reaction by changing of mixture of solvents, and temperature at different time intervals. Firstly, we have examined the reaction using CH_3CN, EtOH, MeOH, AcOH, DMF at constant temperature and time as shown in**Table-1**, **entry 1-5**. Based on this preliminary observation it was found that the reaction proceeds in AcOH smoothly. Further, we tried to improve the yield of the product by addition of mixture of solvents (**Table-1 entry 6-10**). Among all the tested solvents AcOH with piperidine (4:1) at 65 °C gave 40 percentage yield of the product. The optimum yield 90% of the product was formed at 90 °C for 16 h (**entry 12**). Therefore, the optimization conditions of the reaction is AcOH/piperidine (4:1) at 90°C for 16 h, to get the 95% yield of the product. The screened conditions were summarised in **Table-1**

The newly synthesized compounds structures (**5a-w**) were confirmed by their IR, ^{1}H NMR, ^{13}C NMR, HRMS. In the IR spectra pyrimidine ring N-H appears as a broad peak at 3103 - 3181 cm^{-1}. Alkane C-H stretching frequency is at 3057-3075 cm^{-1}, nitrile frequency is at 2210 cm^{-1}, carbonyl stretching frequency appear at 1670-1730 cm^{-1}, NO_2 group asymmetric, symmetric stretching vibrational frequencies appears at 1518, 1347 cm^{-1}, C-O-C (ether group) stretching frequency at 1050-1270 cm^{-1}. In the ^{1}H NMR spectrum the compound **5e** OCH_3 group three hydrogens appears as a singlet at 3.86 δ ppm, thioalkylated CH_2 protons appears as a quartet at 4.61-4.73 δ ppm. The characteristic newly formed pyrimidine ring alkane C-H one singlet proton appear at 6.39 δ ppm. The protons from 7.03-7.93 δ ppm correspond to aromatic and the N-H proton appears at 12.62 δ ppm. In the ^{13}C NMR spectrum of compound **5e** sulphur attached CH_2 carbon appear at 38.8 δ ppm, O-CH_3 carbon appears at 56.0 δ ppm, pyrimidine ring characteristic tertiary carbon appear at 58.3 δ ppm, the carbonyl carbons appear at 188.9 and 192.1 δ ppm respectively.

Table-2 synthesized derivatives





Reaction conditions: 1,3 indane dione (1 mmol), aromatic aldehydes (1 mmol), 5-amino-4*H*-1,2,4-triazole-3-thiol (1 mmol), phenacyl bromides (1 mmol) was taken in AcOH/Piperidine under reflux at 90 °C.

The carbons from 102.7–163.8 δ ppm indicate aromatic carbons. Furthermore, the compound **5e** has also by confirmed by heteronuclear single quantum coherence (**HSQC**) spectrum. The ^{1}H NMR of thioalkylated quartet CH_2 protons correlated with 38.8 δ ppm peak of ^{13}C NMR and the ^{1}H NMR spectrum of pyrimidine ring tertiary carbon attached singlet proton (6.39 δ ppm) has correlates with ^{13}C NMR spectrum of 58.5 δ ppm peak and also in the ^{1}H NMR spectrum the methoxy protons correlates with ^{13}C NMR of 56.0 peak. Based on these findings, we have confirmed 58.5 δ ppm (^{13}C NMR) peak corresponds to pyrimidine ring tertiary carbon. (spectra are available in supplementary data). All the final compounds structures were confirmed with their HRMS (+Ve mode) spectra.

The plausible mechanism for the formation of final compounds was established. The Knovenagal condensation reaction between 1,3-indane dione and aromatic aldehydes takes place to form α, β unsaturated ketone (**1b**) with the elimination of water molecule to produce a C=C. Further the intermediate **1b** involves cyclocondensation reaction with 5-amino-4*H*-1,2,4-triazole-3-thiol to generate a triazolopyrimidine six membered ring product *via* Biginelli reaction with the elimination of water molecule. In this step initially the triazole amine was condensed with carbonyl group of **1b.** This if followed by ring closure. Later the free SH group of triazole participate in nucleophilic substitution reaction with phenacyl bromides with the elimination of HBr. Presence of electron-donating substituents in *p*-position of the phenacyl moiety to affords a good percentage yield of the product (94-95%).

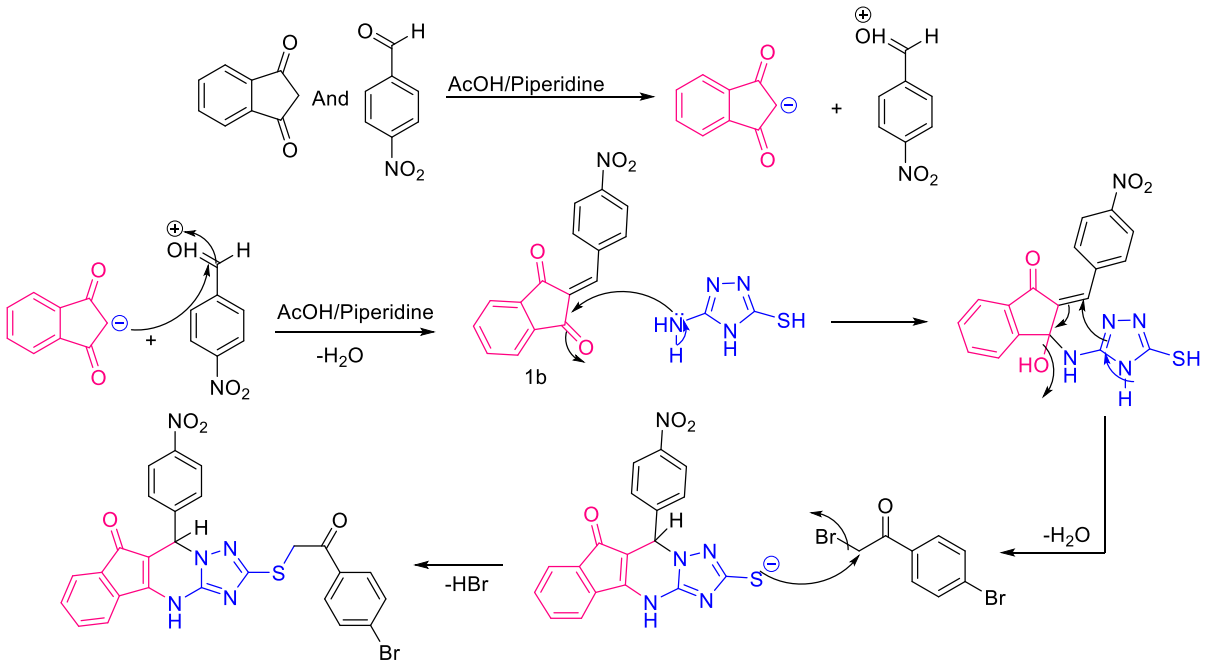


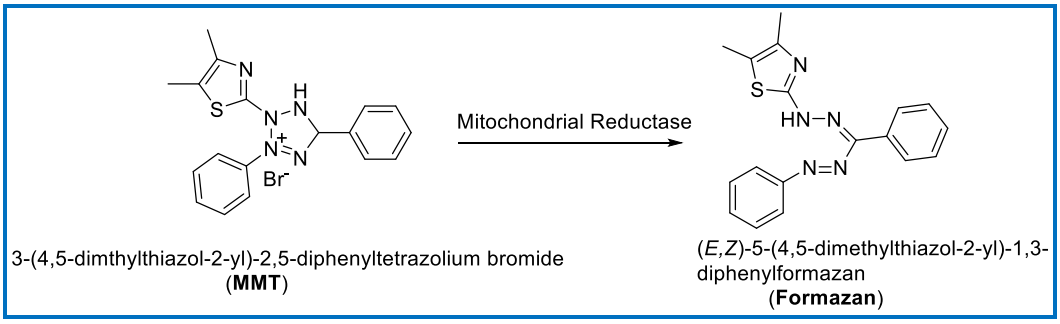
Fig-2. Plausible mechanism for the formation of compounds (5a-w).

5A.2.2 MTT Assay

A colorimetric technique called the MTT Cell Viability assay is used to measure the metabolic activity of cells. Living cells transform the yellow tetrazole, MTT into the purple formazan. This transformation of tetrazolium salts into coloured formazan happens only by living (metabolically active) cells but not by dead cells. As a result, tetrazolium salt-based colorimetric tests can detect only live cells. These metabolic activity assays are widely employed to detect drug-induced cytotoxicity or cell necrosis since a cytotoxic substance would decrease the rate of tetrazolium salt cleavage by a population of cells. Cells that divide quickly, such as cancer cells, have high rates of MTT decrease [47].

Principle

Numerous *in vitro* tests are based on measurements of cell viability and proliferation. The MTT assay is one of them and was first described by Mosmann in 1983. The MTT, which is 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, is based on the ability of a mitochondrial dehydrogenase enzyme from viable cells to cleave the tetrazolium rings of the pale yellow MTT and form a dark blue formazan crystal that is largely impermeable to cell membranes, leading to its accumulation within healthy cells. By using spectrophotometric techniques, the resulting intracellular purple formazan can be solubilized and measured. The insoluble purple formazan result is converted into a coloured solution by adding a solubilization solution, which is typically either dimethyl sulfoxide, an acidified ethanol solution, or a solution of the detergent sodium dodecyl sulfate in diluted hydrochloric acid that is added to dissolve the insoluble purple formazan product into a colored solution. Now the colour can be measured using a simple colorimetric assay at a specific wavelength (often between 500 and 600 nm). A multiwell scanning spectrophotometer (ELISA reader) can be used to read the data. The MTT assay tracks how quickly cells divide and, in the contrary, when metabolic processes cause reduction in cell viablity.



In-Vitro Anticancer activity

Preliminary investigations were done by screening the compounds for anti-cancer activity on breast cancer cell line (human MCF-7) and immortal glioma cell line LN18. All the compounds were evaluated for their anti-proliferative activity by performing tetrazolium reduction. This assay provides a quantitative measure of the number of cells with metabolically active mitochondria and is based on the reduction of a tetrazolium bromide salt, MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]. The cells were treated with different concentrations of compound and cell viability was measured. Since the compounds were dissolved in DMSO, cells treated with DMSO alone served as vehicle control. Drug dose-response curves were plotted after calculating survival fraction. The results were analyzed for the survival of the glioma cells survival against the final concentration. All treatments were performed in triplicate and results expressed as mean ± S.D. For each compound, six concentrations were used for treatment (DMSO, 5 µM, 10 µM, 50 µM, 100 µM and 200 µM). Analysis of data showed that the compounds showed IC_{50} values less than 10 µM in MCF cell lines (**Table-3**) These cells show more sensitivity towards these series as compared to LN18 cells (**Table-4**). 13 compounds significantly diminished the cell viability over time in a dose dependent manner. Percent survival (**Fig-3**) of MCF 7 and LN18 (**Fig-4**) cells were plotted to indicate the contrast survival of untreated cells with other drug concentrations for each compound.

Table-3. IC_{50} values of the compounds 5a-w

Compound code	MCF-7 Cells IC_{50} Value (µM)	Compound code	MCF-7 Cells IC_{50} Value (µM)
5a	>100	**5m**	4.18
5b	3.3	**5n**	>100
5c	>100	**5o**	2.7
5d	>100	**5p**	3.38
5e	9.03	**5q**	>100
5f	3.46	**5r**	>100
5g	4.2	**5s**	4.33
5h	>100	**5t**	4.62
5i	3.84	**5u**	>100

5j	2.7	**5v**	>100
5k	6.16	**5w**	>100
5l	3.92		

Table 3. IC_{50} values (in µM) of different compounds in MCF-7 breast cancer cell line

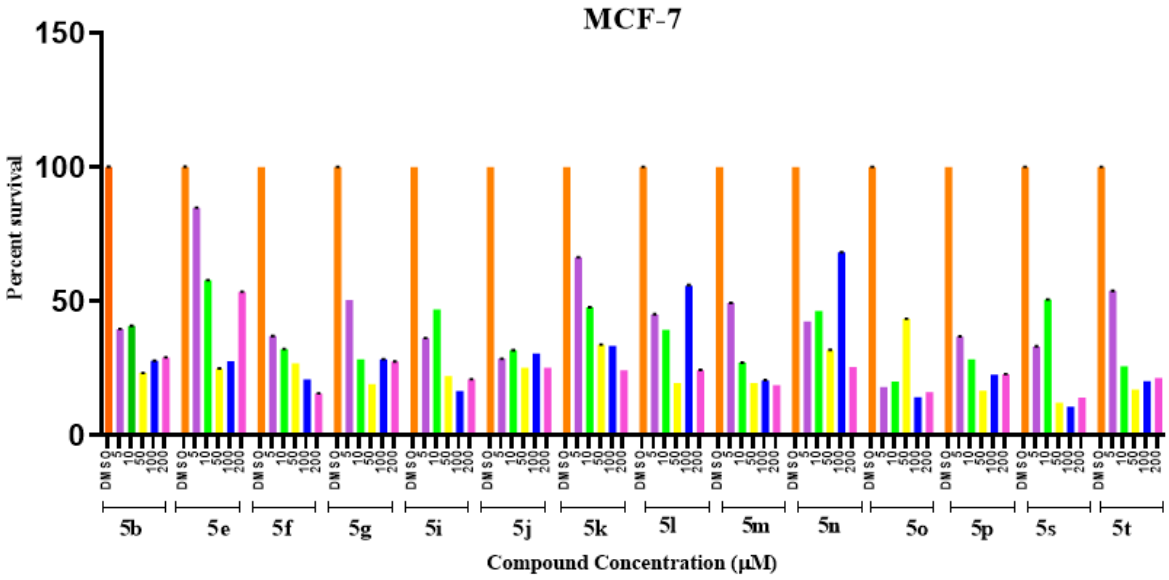


Fig-3. Bar graph showing the percent survival of MCF-7 cells upon treatment of different compounds at different concentrations (Vehicle control, 5µM, 10µM, 50µM, 100µM, 200µM). DMSO treated cells show 100% viability. Other concentrations show comparative percentage of cell survival.

Table-4. IC_{50} values of the compounds 5a-w in LN18 cell lines

Compound code	LN18 Cells IC_{50} Value (µM)	Compound code	LN18 Cells IC_{50} Value (µM)
5a	>100	**5m**	36.34
5b	13.77	**5n**	50.55
5c	>100	**5o**	25.33
5d	>100	**5p**	14.45
5e	9.3	**5q**	>100
5f	20.98	**5r**	>100

5g	7.51	**5s**	16.69
5h	>100	**5t**	14.72
5i	49.48	**5u**	>100
5j	6.5	**5v**	>100
5k	20.53	**5w**	>100
5l	10.39		

Table 4. IC_{50} values (in µM) of different compounds in LN18 human patient derived glioma cell line.

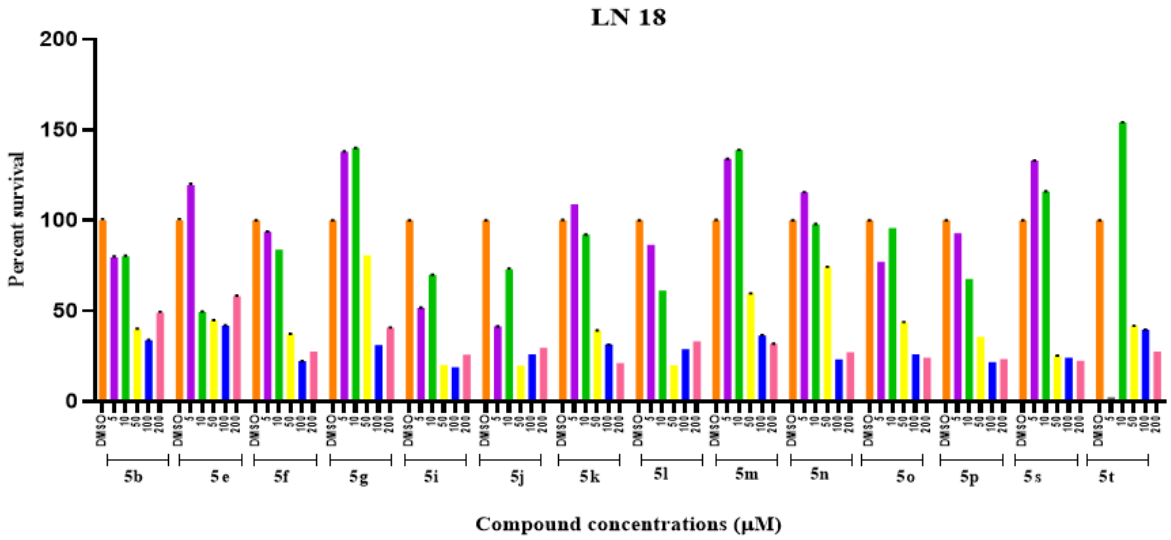


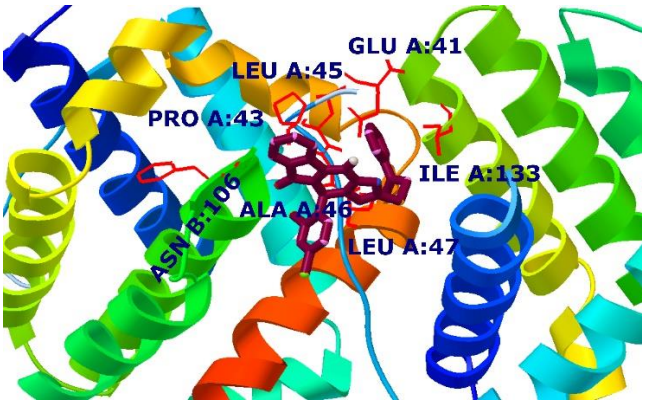
Fig.4. Bar graph showing the percent survival of LN18 cells upon treatment of different compounds at different concentrations (Vehicle control, 5µM, 10µM, 50µM, 100µM, 200µM). DMSO treated cells show 100% viability. Other concentrations show comparative percentage of cell survival.

5A.2.3 Molecular docking results

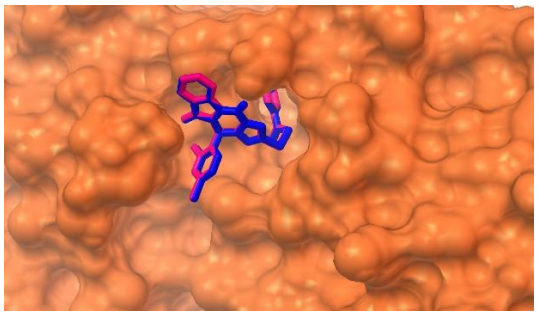
En route to forecast the anticancer activity of the synthesized compounds, *in silico* molecular docking simulations of compound **5g** have also been performed in the present report. The X-ray crystallographic structure of Bax (PDB ID: 4S0O), BCL-2 (PDB ID:2W3L), Caspase 3 (PDB ID: 5I9B), Caspase 8 (PDB ID: 1QDU), Cyt *c* (PDB ID: 1HRC), p53 (PDB ID: 2OCJ), and PARP (PDB ID: 6I8M) proteins obtained from the protein data bank were considered for the study. The bound ligands and water molecules were removed and polar hydrogen were

added in the proteins prior to the molecular docking simulations. The simulations were performed employing the Auto Dock Vina software[1] and the anticancer activity is predicted based on the magnitude of binding affinity values and interacting amino acid residues at the active site of the receptor protein. The reproducibility of the docking pose at the active site of the receptor proteins were also assured with the root mean square deviation (RMSD) estimated between two docked poses.[48]

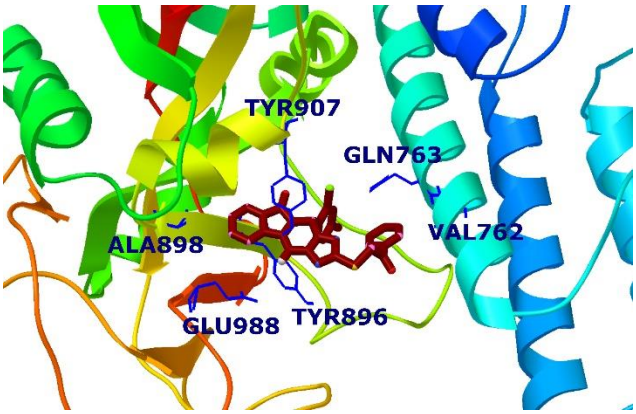
Fig.5 displays docked pose of the compound **5g** along with the interacting amino acids at the active site of Bax and PARP proteins and surface view of the protein with two overlapping conformers of the compound **5g**. The calculated binding affinity values, RMSD, interacting amino acid residues for **5g** with all the considered proteins are systematically tabulated in **Table 5.**



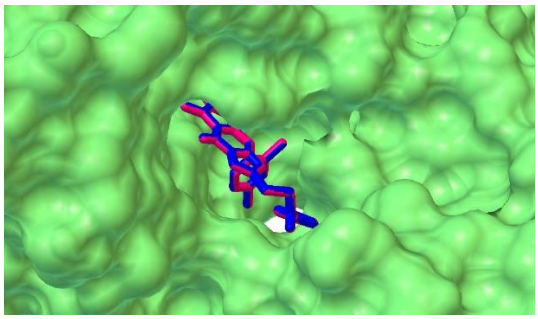
(a)



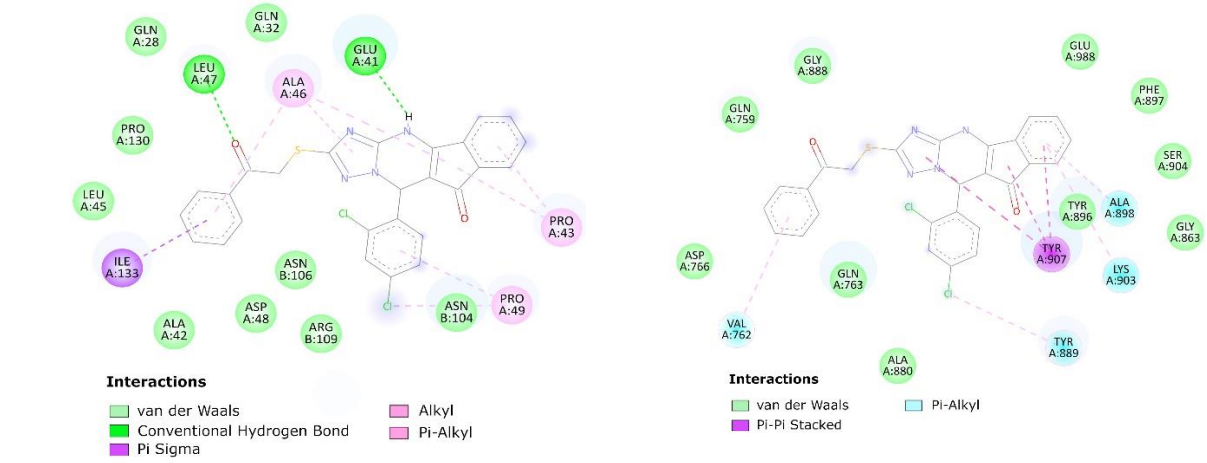
(b)



(c)



(d)



(e) **(f)**

Fig.5. (a) The complex of protein (Bax) with compound **5g** illustrating the interacting amino acid residues, (b) Overlapping docked poses of compound **5g** at the binding site of Bax, (c) The complex of protein (PARP) with compound **5g** illustrating the interacting amino acid residues, (b) Overlapping docked poses of compound **5g** at the binding site of PARP, (e) The 2D display of the interacting amino acids of compound **5g** at the binding site of Bax protein, and (f) The 2D display of the interacting amino acids of compound **5g** at the binding site of PARP protein.

The calculated magnitude of binding affinity (-10.8 kcal/mol) for compound **5g** is found to be the highest with PARP protein. Close analysis of the PARP-**5g** complex reveals the hydrophobic interactions with VAL762, GLN763, TYR896, ALA898, and GLU988 amino acid residues at the binding site. However, no hydrogen bonding interactions have been between **5g** and PARP has been noticed. Thus, outcomes of the docking simulations exclusively emphasize that the compound **5g** shows high affinity towards PARP protein based on the binding affinity values and the nature of interaction between **5g** at the binding site is mostly hydrophobic.

Furthermore, the compound **5g** also shows good binding affinities with the Caspase 3 (-9.4 kcal/mol) and displays hydrogen bonding interaction with HIS121, CYS163, and ARG207 and hydrophobic interactions with THR62, GLU123, PHE128, TYR204, TRP206, PHE256. The compound **5g** have the binding affinity of -8.8 kcal/mol with BCL-2 and shows hydrogen bonding interaction with SER75 and hydrophobic interactions with ASP62, PHE71, ALA72, VAL115, TYR161. Moreover, **5g** binds with Bax protein with a binding affinity of -8.6 kcal/mol showing hydrogen bonding interactions with GLU41, LEU47 and hydrophobic interactions with PRO43, LEU45, ALA46, ILE133, and ASN106.

To ensure the reproducibility of the docking poses at the binding site of all the studied receptor proteins, the docking simulation was reperformed. The RMSD between two docked conformers of **5g** is calculated and the results are provided in **Table 5**. It is evident from the table that the RMSD between two docked conformers is very small and ranges within 0.408 to 1.626. These RMSD value advocates the reproducibility of the docking poses and can be clearly visualized from the overlapping conformers of the compound **5g** at the binding site of Bax and PARP proteins depicted in **Fig.4**. Therefore, the outcomes of the molecular docking simulations exclusively emphasize that the synthesized compound **5g** could be considered as a potential anticancer agent.

Table 5. Binding Affinity, RMSD Between Two Docked Conformers of the Compounds 5g and Interacting Amino Acid Residues at the binding Site of the Various Proteins Considered in the Present Study.

Protein	Binding affinity (kcal/mol)	RMSD	Interacting Amino Acids (Distance in Å)	
			Hydrogen bonding	Hydrophobic Interaction
Bax	-8.6	0.933	GLU41 (1.91), LEU47 (2.34)	PRO43 (3.60), LEU45 (3.78), ALA46 (4.00), ILE133 (Pi-sigma, 3.58), ASN106 (Halogen bond, 3.47)
BCL-2	-8.8	0.488	SER75 (2.26)	ASP62 (3.57), PHE71 (3.49), ALA72 (3.68) VAL115 (3.07), TYR161 (4.75)
Caspase3	-9.4	1.115	HIS121 (1.93), CYS163 (Pi-Donor, 5.66), ARG207 (1.80)	THR62 (3.80), GLU123 (Pi-Anion, 3.61), PHE128 (3.75), TYR204 (Pi-Pi Stacked, 3.41), TRP206 (Pi-Pi Stacked, 3.70), PHE256 (3.42),
Caspase8	-8.5	0.986	HIS237 (3.67), ARG341 (2.21)	ARG177 (3.55), TYR244 (3.75), TYR290 (3.61), HIS237 (Pi-Pi Stacked, 5.30), TYR290 (Halogen Bond, 3.40), TYR340 (Pi-Pi Stacked, 4.82)
Cyt *c*	-6.3	0.408	ALA15 (3.28), LYS7 (Carbon Hydrogen Bond, 4.46), LYS27 (2.42)	PHE10 (3.68), VAL11 (Pi-Sigma, 3.72), GLU21 (Pi-Anion, 6.01), TYR97 (Halogen Bond, 3.59)
p53	-6.9	1.626	GLN100 (2.32), THR102 (3.21), SER269 (3.00)	LEU111 (3.41), LEU252 (3.62), ASN268 (3.95), GLU271 (3.61)
PARP	-10.8	0.581	--	VAL762 (Pi-Alkyl, 3.66), GLN763 (3.92), TYR896 (3.65), ALA898 (3.74), TYR907 (Pi-Pi Stacked, 3.86), GLU988 (3.98)

5A.3 Conclusions:

In summary, we have synthesized the final compounds in a multicomponent process. In which one pot-four component reaction between 1,3-indane dione, aromatic aldehydes, 5-amino-4*H*-1,2,4-triazole-3-thiol, and various phenacyl bromides were reacted to produce high yield of product fused1,2,4-triazolopyrimidinones (5a-w) in a short period of time. The advantages of MCRs method is an economical, broad substrate and ecofriendly synthesis. The final compounds structures were confirmed by their spectral data. All the synthesized compounds (5a-w) were screened against *in-vitro* antibreast cancer activity with the help of LN18 and MCF-7cell lines. Molecular docking simulation was also carried out for all the synthesized compounds.

5A.4 Experimental:

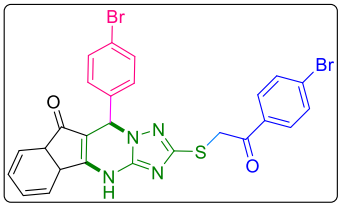
The starting materials were commercially purchased form Sigma-Aldrich, Alfa Aesar, TCI, Spectrochem and used without additional purification. The solvents were purchased from Finar, Merck and stored over a 4 Å molecular sieves. The progress of the reaction was checked with Thin-layer chromatography (TLC) on silica gel coated aluminium plates using ethyl acetate and n-hexane (2:8) ratio. The FI-IR spectra were recorded on Perkin Elmer spectrometer using solid KBr disk and values were expressed in cm^{-1}. Proton NMR spectra was recorded on Bruker AVANCE 400 MHz spectrometer with the use of DMSO-d_6 solvent and TMS (Tetra methyl silane) as an internal reference standard compound. The abbreviations were used to explain the splitting pattern; s = singlet, d = doublet, t = triplet, m = multiplet, q = quartet, and the chemical shift (δ) were expressed in ppm reference to centre line of quintet at 2.5 ppm for DMSO-d_6 and triplet at 7.26 ppm for $CDCl_3$ solvent, coupling constant (J) units represented in Hz. Carbon NMR spectra was recorded on Bruker AVANCE 100 MHz spectrometer and chemical shift (δ) values were represented in ppm and it is fully broad bond decoupled proton NMR spectra. Melting points of the compounds were checked with Stuart Staffordshire, UK (SMP30) Instrument and were uncorrected. The molecular mass of the compounds was checked with HRMS (ESI +Ve mode) Agilent Technologies Instrument.

5A.4.1 *General procedure for the synthesis of thioalkylated fused triazolopyrimidines (5a-w)*

A mixture of 1,3-Indane dione (1 mmol) 4-nitro aromatic aldehyde (1 mmol) in the presence of AcOH (4 mL) was an added catalytic amount of piperidine (10 mol%), refluxed for 30 minutes. After consumption of the reaction 5-amino-4*H*-1,2,4-triazole-3-thiol and the reaction mixture was continued to reflux for another 5-6 hours (vide TLC) and then added *p*-bromo phenacyl bromides under same solvent conditions and refluxed for another 4 hours. After completion of the reaction (vide TLC) the crystalline solid product was filtered and washed with water and recrystalized from methanol.

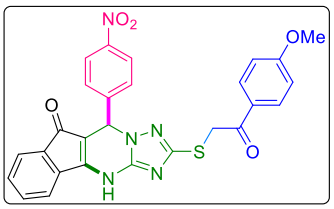
5A.5 Characterization data of synthesized compounds (5a-w)

10-(4-Bromophenyl)-2-((2-(4-bromophenyl)-2-oxoethyl) thio)-4,10-dihydro-9H-indeno[1,2-d][1,2,4]triazolo[1,5-a]pyrimidin-9-one. (**5a**)



Yellow solid; Yield 91%; mp: 263-264 °C; IR (KBr) cm^{-1}: 3173 (N-H), 3069 (alkane C-H), 1701, 1685 (C=O), 766 (C-Br); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) : 4.69 (q, J = 16.8 Hz, 2H), 6.26 (s, 1H), 7.24 (d, J = 8.4 Hz, 2H), 7.32 (d, J = 6.8 Hz, 1H), 7.48 – 7.51 (m, 3H), 7.67 (d, J = 7.2 Hz, 1H), 7.74 (d, J = 8.8 Hz, 2H), 7.83-7.87 (m, 2H), 8.44 (d, J = 8.8 Hz, 1H), 12.54 (s, 1H); ^{13}C NMR (100MHz, DMSO-d_6) δ (ppm): 38.9, 58.2, 103.1, 120.6, 121.4, 122.0, 126.6, 128.1, 130.2, 130.7, 131.8, 132.2, 132.7, 133.7, 135.0, 135.7, 139.2, 148.4, 154.7, 158.4, 189.0, 193.3. HRMS (ESI-TOF) (*m/z*): Calculated for $C_{26}H_{16}Br_2N_4O_2S$ $[M+H]^+$ 606.9439; found 606.9430.

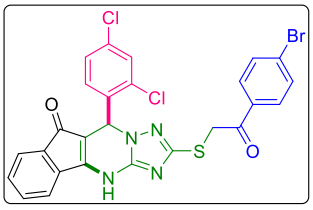
2-((2-(4-Methoxyphenyl)-2-oxoethyl) thio)-10-(4-nitrophenyl)-4,10-dihydro-9H-indeno[1,2-d][1,2,4]triazolo[1,5-a]pyrimidin-9-one (**5b**).



Yellow solid; yield 90%; mp: 234-235 °C; IR (KBr) cm^{-1}: 3177 (N-H), 3069 (alkane C-H), 1689, 1676 (C=O), 1518, 1310 (NO_2), 1277 (C-O-C); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 3.85 (s, 3H), 4.66 (q, J = 16.8 Hz, 2H), 6.46 (s, 1H), 7.02 (d, J = 8.8 Hz, 2H), 7.58 (d, J = 8.8 Hz, 2H), 7.91 (d, J = 8.8 Hz, 2H), 8.15 (d, J = 8.8 Hz, 2H), 8.35 (d, J = 8.8 Hz, 2H), 8.60 (d, J = 8.8 Hz, 2H), 12.66 (s, 1H); ^{13}C NMR (100MHz, DMSO-d_6) δ (ppm): 38.8, 56.0, 58.2, 102.6, 114.3, 123.8, 129.4, 131.1,

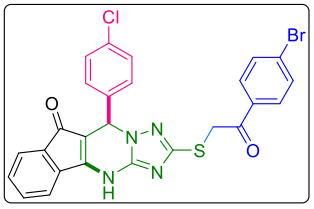
132.8, 134.6, 136.7, 139.0, 140.2, 142.0, 142.6, 147.7, 149.3, 154.9, 159.0, 163.8, 188.6, 192.1. HRMS (ESI-TOF) (*m/z*): Calculated for $C_{27}H_{19}N_5O_5S$ $[M+H]^+$ 526.1180; found 526.1155.

2-((2-(4-Bromophenyl)-2-oxoethyl) thio)-10-(2,4-dichlorophenyl)-4,10-dihydro-9H-indeno [1,2-d][1,2,4]triazolo[1,5-a]pyrimidin-9-one. (**5c**)



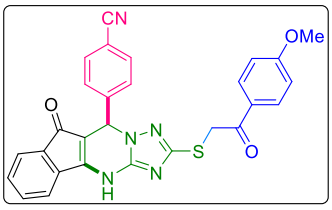
Yellow solid; Yield 88%; mp: 215-216 °C; IR (KBr) cm^{-1}: 3173 (N-H), 3070 (alkane C-H), 1729, 1691 (C=O), 733 (C-Cl), 702 (C-Br); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 4.68 (q, J = 17.2, Hz, 2H), 6.56 (s, 1H), 7.30 (d, J = 6.8 Hz, 1H), 7.61 – 7.64 (m, 2H), 7.74 (d, J = 8.8 Hz, 2H), 7.83 – 7.85 (m, 4H), 8.56 (d, J = 8.4 Hz, 2H), 12.62 (s, 1H); ^{13}C NMR (100MHz, DMSO-d_6) δ (ppm): 38.9, 61.3, 123.8, 127.7, 129.6, 129.8, 130.6, 132.1, 132.2, 134.9, 136.6, 136.8, 137.1, 137.9, 138.1, 140.2, 142.5, 188.3, 189.1. HRMS (ESI-TOF) (*m/z*): Calculated for $C_{26}H_{15}BrCl_2N_4O_2S$ $[M+H]^+$ 596.9554; found 596.9549.

2-((2-(4-Bromophenyl)-2-oxoethyl) thio)-10-(4-chlorophenyl)-4,10-dihydro-9H-indeno[1,2-d][1,2,4]triazolo[1,5-a]pyrimidin-9-one (**5d**).



Yellow solid; Yield 90%; mp: 260-261°C; IR (KBr) cm^{-1}: 3171 (N-H), 3068 (alkane C-H), 1701, 1685 (C=O), 765 (C-Cl), 710 (C-Br); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 4.70 (q, J = 17.0, Hz, 2H), 6.28 (s, 1H), 7.29 – 7.38 (m, 6H), 7.42 (d, J = 7.2 Hz, 1H), 7.67 (d, J = 7.2 Hz, 1H), 7.74 (d, J = 8.8 Hz, 2H), 7.86 (d, J = 8.4 Hz, 2H). 12.55 (s, 1H); ^{13}C NMR (100MHz, DMSO-d_6) δ (ppm): 39.0, 58.3, 103.2, 120.6, 121.4, 128.1, 128.8, 129.9, 130.7, 131.4, 132.2, 132.7, 133.4, 133.7, 135.0, 135.7, 138.8, 148.4, 154.7, 158.4, 189.0, 193.3. HRMS (ESI-TOF) (*m/z*): Calculated for $C_{26}H_{16}BrClN_4O_2S$ $[M+H]^+$ 562.9944; found 562.9936.

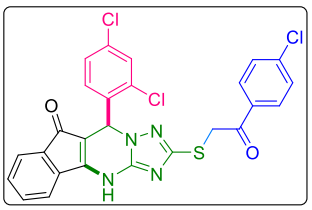
4-(2-((2-(4-Methoxyphenyl)-2-oxoethyl) thio)-9-oxo-9,10-dihydro-4H-indeno[1,2-d][1,2,4] triazolo[1,5-a]pyrimidin-10-yl)Benzonitrile (**5e**).



Yellow solid; Yield 94%; mp: 230-231 °C; IR (KBr) cm^{-1}: 3110 (N-H), 3098 (alkane C-H), 1735, 1690 (C=O), 1251 (C-O-C); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 3.86 (s, 3H), 4.67 (q, J = 16.8 Hz, 2H), 6.39 (s, 1H), 7.04 (d, J = 8.8 Hz, 2H), 7.32 (d, J = 6.8 Hz, 1H), 7.42 (t, J = 7.6 Hz, 1H), 7.49 – 7.51 (m, 3H), 7.68 (d, J = 7.2 Hz, 1H), 7.78 (d, J = 8.4 Hz, 2H), 7.92 (d, J = 8.8 Hz, 2H), 12.62 (s, 1H); ^{13}C

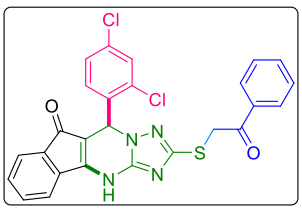
NMR (100MHz, DMSO-d_6) δ (ppm): 38.8, 56.0, 58.5, 102.7, 111.5, 114.4, 119.0, 120.7, 121.5, 128.8, 129.0, 131.1, 131.5 ,132.8, 133.0, 133.7, 135.6, 144.9, 148.5, 154.9, 158.9, 163.8, 188.9, 192.1. HRMS (ESI-TOF) (*m/z*): Calculated for $C_{28}H_{19}N_5O_3S$ $[M+H]^+$ 506.1287; found 506.1275.

2-((2-(4-Chlorophenyl)-2-oxoethyl) thio)-10-(2,4-dichlorophenyl)-4,10-dihydro-9H-indeno [1,2-d][1,2,4]triazolo[1,5-a]pyrimidin-9-one (**5f**)



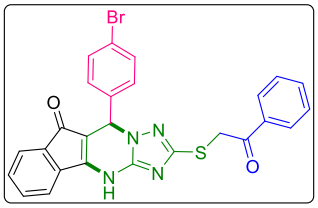
Yellow solid; Yield 85%; mp: 218-219 °C; IR (KBr) cm^{-1}: 3177 (N-H), 3074 (alkane C-H), 1729, 1692 (C=O), 733 (C-Cl); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 4.67 (q, J = 16.8, Hz, 2H), 6.55 (s, 1H), 7.37-7.42 (m, 2H), 7.57-7.60 (m, 2H), 7.91 (d, J = 8.4 Hz, 2H), 7.97 – 7.99 (m, 4H), 8.55 (d, J = 8.4 Hz, 1H), 12.64 (s, 1H); ^{13}C NMR (100MHz, DMSO-d_6) δ (ppm): 38.7, 63.7, 109.6, 123.7, 127.7, 129.3, 129.6, 129.7, 130.5, 132.0, 134.9, 136.7, 136.8, 137.0, 138.1, 140.1, 140.4, 142.5, 157.8, 188.3, 189.2. HRMS (ESI-TOF) (*m/z*): Calculated for $C_{26}H_{15}BrCl_3N_4O_2S$ $[M+H]^+$ 553.0059; found 553.0069.

10-(2,4-Dichlorophenyl)-2-((2-oxo-2-phenylethyl) thio)-4,10-dihydro-9H-indeno[1,2-d] [1,2,4] triazolo[1,5-a] pyrimidin-9-one (**5g**)



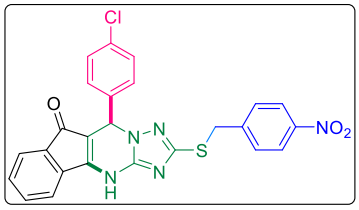
Yellow solid; Yield 89%; mp: 218-219 °C; IR (KBr) cm^{-1}: 3181 (N-H), 3070 (alkane C-H), 1730, 1691 (C=O), 733 (C-Cl); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 4.72 (q, J = 16.8, Hz, 2H), 6.57 (s, 1H), 7.53 – 7.54 (m, 3H), 7.61 (d, J = 2.0 Hz, 1H), 7.66 (d, J = 7.2 Hz, 2H), 7.84 (d, J = 2.4 Hz, 2H), 8.57 (d, J = 8.4 Hz, 4H), 12.62 (s, 1H); ^{13}C NMR (100MHz, DMSO-d_6) δ (ppm): 39.1, 62.8, 101.5, 120.6, 121.4, 123.8, 127.7, 128.6, 129.1, 131.5, 132.1, 132.7, 133.9, 134.9, 136.0, 136.6, 136.8, 137.0, 138.1, 155.3, 158.7, 189.1, 193.8. HRMS (ESI-TOF) (*m/z*): Calculated for $C_{26}H_{16}BrCl_2N_4NaO_2S^+$ $[M+H]^+$ 541.0271; found 541.0271.

10-(4-Bromophenyl)-2-((2-oxo-2-phenylethyl) thio)-4,10-dihydro-9H-indeno[1,2-d][1,2,4] triazolo[1,5-a]pyrimidin-9-one (**5h**)



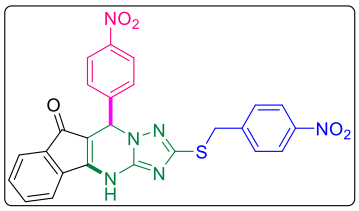
Yellow solid; Yield 90%; mp: 290-291 °C; IR (KBr) cm^{-1}: 3169 (N-H), 3062 (alkane C-H), 1700, 1686 (C=O), 707 (C-Br); ^{1}H NMR (400 MHz, DM SO-d_6) δ (ppm): 4.74 (q, J = 17.0 Hz, 2H), 6.28 (s, 1H), 7.25 (d, J = 8.4 Hz, 2H), 7.33 (d, J = 6.8 Hz, 1H), 7.41 (t, J = 7.4 Hz, 1H), 7.49-7.55 (m 5H), 7.67 (d, J = 6.4 Hz, 2H), 7.95 (d, J = 7.2 Hz, 2H), 12.55 (s, 1H); ^{13}C NMR (100MHz, DMSO-d_6) δ (ppm): 39.2, 58.3, 103.1, 120.6, 121.4, 122.0, 128.7, 129.2, 130.2, 131.4, 131.8, 132.7, 133.7, 134.0, 135.7, 136.0, 139.2, 148.4, 154.7, 158.5, 189.0, 193.9. HRMS (ESI-TOF) (*m/z*): Calculated for $C_{26}H_{17}BrN_4O_2S$ $[M+H]^+$529.0334; found 529.0331.

10-(4-Chlorophenyl)-2-((4-nitrobenzyl) thio)-4,10-dihydro-9H-indeno[1,2-d][1,2,4]triazolo [1,5-a]pyrimidin-9-one (**5i**)



Orange solid; Yield 90%; mp: 250-251 °C; IR (KBr) cm^{-1}: 3135 (N-H), 3071 (alkane C-H), 1690 (C=O), 1562, 1343 (NO_2), 763 (C-Cl); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 4.36 (q, J = 14.0 Hz, 2H), 6.34 (s, 1H), 7.34 – 7.42 (m, 6H), 7.49-7.52 (m, 3H), 7.69 (d, J = 7.2 Hz, 1H), 8.03 (d, J = 8.4 Hz, 2H), 12.58 (s, 1H); ^{13}C NMR (100MHz, DMSO-d_6) δ (ppm): 33.5, 57.8, 102.7, 120.1, 121.0, 123.2, 128.4, 129.5, 129.9, 131.0, 132.3, 133.6, 133.2, 135.3, 138.4, 146.3, 148.1, 154.3, 157.4, 188.5. HRMS (ESI-TOF) (*m/z*): Calculated for $C_{25}H_{16}ClN_5O_3S$ $[M+H]^+$ 502.0740; found 502.0730.

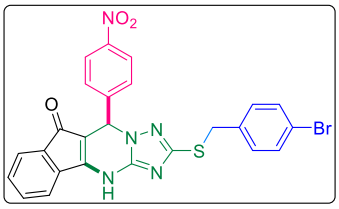
2-((4-Nitrobenzyl) thio)-10-(4-nitrophenyl)-4,10-dihydro-9H-indeno[1,2-d][1,2,4]triazolo [1,5-a]pyrimidin-9-one (**5j**)



Orange solid; Yield 88%; mp: 221-222 °C; IR (KBr) cm^{-1}: 3160 (N-H), 3069 (alkane C-H), 1689 (C=O), 1574, 1347 (NO_2); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 4.36 (q, J = 14.2 Hz, 2H), 6.51 (s, 1H), 7.34 (d, J = 6.8 Hz, 1H), 7.43 (t, J = 7.4 Hz, 1H), 7.50-7.53 (m, 3H), 7.62 (d, J = 8.8 Hz, 2H), 7.71 (d, J = 7.2 Hz, 1H), 8.01 (d, J = 8.8 Hz, 2H), 8.20 (d, J = 8.8 Hz, 2H), 12.69 (s, 1H); ^{13}C NMR (100MHz, DMSO-d_6) δ (ppm): 34.4, 58.2, 102.7, 120.7, 121.5, 123.6, 123.9, 124.1,

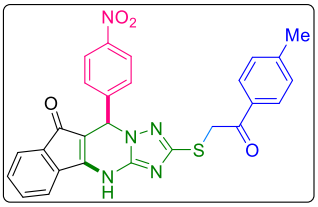
129.5, 130.4, 131.6, 132.8, 133.7, 134.6, 135.6, 146.6, 146.8, 147.8, 148.8, 155.0, 158.2, 188.9: HRMS (ESI-TOF) (*m/z*): Calculated $C_{25}H_{16}N_6O_5S$ $[M+H]^+$ 513.0981; found 513.0978.

2-((4-Bromobenzyl) thio)-10-(4-nitrophenyl)-4,10-dihydro-9H-indeno[1,2-d][1,2,4]triazolo [1,5-a]pyrimidin-9-one (**5k**)



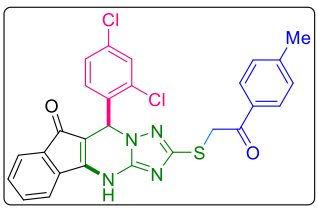
Orange solid; Yield 90%; mp: 201-202 °C; IR (KBr) cm^{-1}: 3114 (N-H), 3069 (alkane C-H), 1689 (C=O), 1574, 1347 (NO_2), 713 (C-Br); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 4.21 (q, J = 12.4 Hz, 2H), 6.51 (s, 1H), 7.21 (d, J = 8.4 Hz, 2H), 7.32 – 7.36 (m, 3H), 7.63 (d, J = 8.4 Hz, 2H), 7.71 (d, J = 7.2 Hz, 1H), 8.21 (d, J = 8.8 Hz, 2H), 8.36 (d, J = 8.8 Hz, 1H), 8.61 (d, J = 8.8 Hz, 1H), 12.69 (s, 1H); ^{13}C NMR (100MHz, DMSO-d_6) δ (ppm): 34.4, 58.2, 102.7, 120.7, 121.5, 123.8, 124.1, 129.5, 131.4, 132.8, 133.7, 134.6, 135.6, 137.9, 140.2, 142.0, 146.8, 147.8, 148.7, 155.0, 158.6, 188.9: HRMS (ESI-TOF) (*m/z*): Calculated $C_{25}H_{16}BrN_5O_3S$ $[M+H]^+$ 546.0235; found 546.0245.

10-(4-Nitrophenyl)-2-((2-oxo-2-(p-tolyl) ethyl) thio)-4,10-dihydro-9H-indeno[1,2-d][1,2,4] triazolo[1,5-a]pyrimidin-9-one (**5l**)



Yellow solid; Yield 93%; mp: 248-249 °C; IR (KBr) cm^{-1}: 3169 (N-H), 3074 (alkane C-H), 1731, 1688 (C=O), 1518, 1347 (NO_2); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.38 (s, 3H), 4.69 (q, J = 17.0 Hz, 2H), 6.46 (s, 1H), 7.32 (t, J = 7.2 Hz, 3H), 7.58 (d, J = 8.8 Hz, 2H), 7.69 (d, J = 7.2 Hz, 1H), 7.83 (d, J = 8.0 Hz, 2H), 8.15 (d, J = 8.8 Hz, 2H), 8.36 (d, J = 8.8 Hz, 1H), 8.61 (d, J = 8.8 Hz, 1H), 12.65 (s,1H); ^{13}C NMR (100MHz, DMSO-d_6) δ (ppm): 21.6, 39.0, 58.2, 102.6, 120.7, 121.5, 123.9, 124.1, 128.8, 129.4, 129.7, 131.5, 133.4, 133.7, 134.6, 135.6, 136.8, 142.0, 144.5, 146.7, 147.7, 148.5, 154.9, 158.9, 188.9, 193.3: HRMS (ESI-TOF) (*m/z*): Calculated $C_{27}H_{19}N_5O_4S$ $[M+H]^+$ 510.1236; found 510.1235.

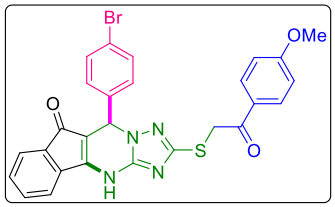
10-(2,4-Dichlorophenyl)-2-((2-oxo-2-(p-tolyl) ethyl) thio)-4,10-dihydro-9H-indeno[1,2-d] [1,2,4] triazolo[1,5-a] pyrimidin-9-one (**5m**)



Yellow solid; Yield 89%; mp: 223-224 °C; IR (KBr) cm^{-1}: 3173 (N-H), 3074 (alkane C-H), 1729, 1691 (C=O), 733 (C-Cl); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.39 (s, 3H), 4.68 (q, J = 16.8 Hz, 2H), 6.58 (s, 1H), 7.32 (d, J = 7.6 Hz, 2H), 7.4 1 (t, J = 7.8 Hz, 2H), 7.62 (d, J = 6.8 Hz, 2H), 7.81 – 7.85 (m, 3H), 8.56

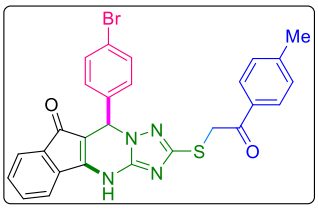
(d, *J* = 8.4 Hz, 2H), 12.62 (s, 1H); ^{13}C NMR (100MHz, DMSO-d_6) δ (ppm): 21.2, 38.5, 60.5, 120.1, 121.0, 123.3, 127.2, 128.3, 131.6, 132.3, 134.4, 135.1, 136.2, 136.3, 136.6, 137.4, 137.6, 139.7, 142.1, 143.9, 154.8, 188.7, 192.9: HRMS (ESI-TOF) (*m/z*): Calculated $C_{27}H_{18}Cl_2N_4O_2S$ $[M+H]^+$ 533.0606; found 533.0593.

10-(4-Bromophenyl)-2-((2-(4-methoxyphenyl)-2-oxoethyl) thio)-4,10-dihydro-9H-indeno [1,2-d][1,2,4]triazolo[1,5-a]pyrimidin-9-one (**5n**)



Yellow solid; Yield 93%; mp: 198-199 °C; IR (KBr) cm^{-1}: 3177 (N-H), 3086 (alkane C-H), 1725, 1692 (C=O), 1185 (C-O-C), 735 (C-Br);^{1}H NMR (4 00 MHz, DMSO-d_6) δ (ppm): 3.86 (s, 3H), 4.67 (q, *J* = 16.8 Hz, 2H), 6.28 (s, 1H), 7.05 (d, *J* = 9.2 Hz, 2H), 7.26 (d, *J* = 8.4 Hz, 2H), 7.50 (d, *J* = 8.4 Hz, 2H), 7.78 (d, *J* = 8.4 Hz, 4H), 8.43 (d, *J* = 8.8 Hz, 2H), 12.56 (s, 1H); ^{13}C NMR (100MHz, DMSO-d_6) δ (ppm): 38.9, 56.1, 58.3, 103.1, 114.4, 123.6, 123.7, 127.6, 130.2, 130.4, 131.1, 131.8, 132.3, 132.4, 135.9, 136.4, 136.5, 140.0, 142.4, 144.3, 189.0, 189.6: HRMS (ESI-TOF) (*m/z*): Calculated $C_{27}H_{19}BrN_4O_3S$ $[M+H]^+$ 559.0439; found 559.0452.

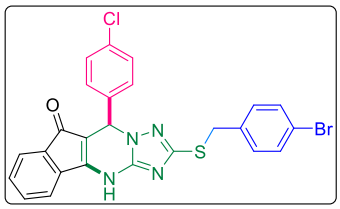
10-(4-Bromophenyl)-2-((2-oxo-2-(p-tolyl) ethyl) thio)-4,10-dihydro-9H-indeno[1,2-d][1,2,4] triazolo[1,5-a]pyrimidin-9-one (**5o**)



Yellow solid; Yield 91%; mp: 278-289 °C; IR (KBr) cm^{-1}: 3165 (N-H), 3057 (alkane C-H), 1698, 1684 (C=O), 736 (C-Br); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.39 (s, 3H), 4.69 (q, *J* = 17.0 Hz, 2H), 6.27 (s, 1H), 7.25 (d, *J* = 8.4 Hz, 2H), 7.32 – 7.34 (m, 3H), 7.49 – 7.51 (m, 3H), 7.67 (d, *J* = 7.2 Hz, 1H), 7.84 (d, *J* = 8.0 Hz, 2H), 12.55 (s, 1H); ^{13}C NMR (100MHz, DMSO-d_6) δ (ppm): 21.6, 39.0, 58.3, 103.1, 120.6, 121.4, 122.0, 128.8, 129.7, 130.2, 131.4, 131.8, 132.7, 133.4, 135.7, 139.2, 144.4, 148.3, 154.7, 158.6, 189.0, 193.4: HRMS (ESI-TOF) (*m/z*): Calculated $C_{27}H_{19}BrN_4O_2S$ $[M+H]^+$ 543.0490; found 543.0495.

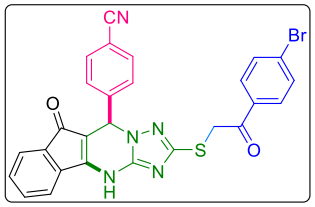
2-((4-Bromobenzyl)thio)-10-(4-chlorophenyl)-4,10-dihydro-9H-indeno[1,2-d][1,2,4]triazolo [1,5-a]pyrimidin-9-one (**5p**)

Orange solid; Yield 89%; mp: 221-222 °C; IR (KBr) cm^{-1}: 3179 (N-H), 3065 (alkane C-H), 1695 (C=O), 744 (C-Cl), 708 (C-Br);, DMSO-^{1}H N MR (400 MHz d_6) δ (ppm): 4.20 (q, *J* = 13.6 Hz, 2H), 6.34 (s, 1H), 7.20 (d, *J* = 8.4 Hz, 2H), 7.33-7.35 (m, 6H), 7.40-7.43 (m, 3H),



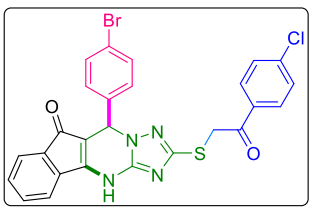
7.70 (d, *J* = 7.2 Hz, 1H), 12.58 (s, 1H); ^{13}C NMR (100MHz, DMSO-d_6) δ (ppm): 34.4, 58.3, 103.2, 120.6, 121.5, 128.9, 130.0, 131.5, 132.7, 133.4, 133.7, 135.7, 138.0, 139.0, 148.5, 154.7, 158.3, 172.4, 189.0: HRMS (ESI-TOF) (*m/z*): Calculated $C_{25}H_{16}BrClN_4OS$ $[M+H]^+$ 534.9995; found 534.9965.

4-(2-((2-(4-Bromophenyl)-2-oxoethyl)thio)-9-oxo-9,10-dihydro-4H-indeno[1,2-d][1,2,4] triazolo[1,5-a]pyrimidin-10-yl)Benzonitrile (**5q**)



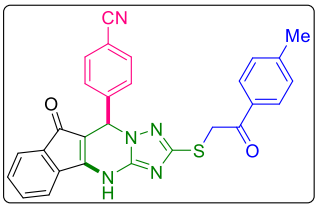
Yellow solid; Yield 95%; mp: 240-241°C; IR (KBr) cm^{-1}: 3113 (N-H), 3089 (alkane C-H), 2209 (CN), 1723, 1672 (C=O), 744 (C-Br); ^{1}H NMR (400 MH z d_6) δ (ppm): 4.69 (q, *J* = 17.0 Hz, 2H), 6.38 (s, 1H), 7.32 (d, *J* = 7.2 Hz, 1H), 7.41 (t, *J* = 7.4 Hz, 1H), 7.47-7.52 (m, 3H), 7.68 (d, *J* = 7.2 Hz, 1H), 7.72-7.78 (m, 4H), 7.86 (d, *J* = 8.8 Hz, 2H), 12.60 (s, 1H); ^{13}C NMR (100MHz, DMSO-d_6) δ (ppm): 39.0, 58.5, 102.7, 111.5, 118.9, 120.7, 121.5, 128.1, 129.0, 130.7, 131.5, 132.2, 132.8, 132.9, 133.7, 135.0, 135.6, 144.9, 148.6, 154.9, 158.6, 188.9, 193.3. HRMS (ESI-TOF) (*m/z*): Calculated $C_{27}H_{16}BrN_5O_2S$ $[M+H]^+$ 554.0286; found 554.0290.

10-(4-Bromophenyl)-2-((2-(4-chlorophenyl)-2-oxoethyl)thio)-4,10-dihydro-9H-indeno[1,2-d] [1,2,4]triazolo[1,5-a]pyrimidin-9-one (**5r**)



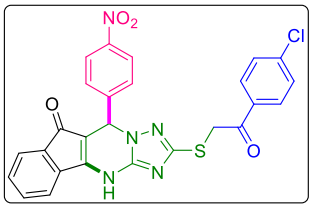
Yellow solid; Yield 90%; mp: 228-229°C; IR (KBr) cm^{-1}: 3127 (N-H), 3012 (alkane C-H), 1733, 1662 (C=O), 789 (C-Cl), 744 (C-Br); ^{1}H NMR (400 MHz d_6) δ (ppm): 4.69 (q, *J* = 16.8 Hz, 2H), 6.26 (s, 1H), 7.23 (d, *J* = 8.4 Hz, 2H), 7.32 (d, *J* = 6.8 Hz, 1H), 7.40 (t, *J* = 7.8 Hz, 1H), 7.47-7.50 (m, 3H), 7.59 (d, *J* = 8.4 Hz, 2H), 7.67 (d, *J* = 7.2 Hz, 1H), 7.94 (d, *J* = 8.8 Hz, 2H), 12.54 (s, 1H); ^{13}C NMR (100MHz, DMSO-d_6) δ (ppm): 39.0, 58.4, 103.1, 120.6, 121.4, 122.0, 129.3, 130.2, 130.6, 131.4, 131.8, 132.7, 133.7, 134.7, 135.7, 138.9, 139.2, 148.4, 154.7, 158.4, 189.0, 193.1. HRMS (ESI-TOF) (*m/z*): Calculated $C_{26}H_{16}BrClN_4O_2S$ $[M+H]^+$ 562.9944; found 562.9935.

4-(9-Oxo-2-((2-oxo-2-(p-tolyl) ethyl)thio)-9,10-dihydro-4H-indeno[1,2-d][1,2,4]triazolo[1,5-a]pyrimidin-10-yl)Benzonitrile (**5s**)



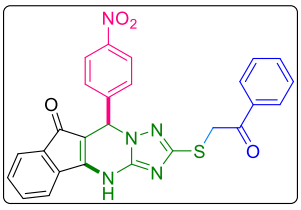
Yellow solid; Yield 95%; mp: 251-252 °C; IR (KBr) cm^{-1}: 3145 (N-H), 3098 (alkane C-H), 2198 (CN), 1714, 1684 (C=O); ^{1}H NMR (400 MHz d_6) δ (ppm): 2.39 (s, 3H), 4.69 (q, J = 16.8 Hz, 2H), 6.38 (s, 1H), 7.31-7.33 (m, 3H), 7.40 (t, J = 7.4 Hz, 1H), 7.47-7.50 (m, 3H), 7.67 (d, J = 7.2 Hz, 1H), 7.77 (d, J = 8.4 Hz, 2H), 7.83 (d, J = 8.0 Hz, 2H), 12.61 (s, 1H); ^{13}C NMR (100MHz, DMSO-d_6) δ (ppm): 21.6, 39.0, 58.5, 102.7, 111.5, 119.0, 120.7, 121.5, 128.8, 129.0, 129.7, 131.5, 132.7, 132.9, 133.4, 133.7, 135.6, 144.4, 144.9, 148.5, 154.9, 158.8, 188.9, 193.3. HRMS (ESI-TOF) (*m/z*): Calculated $C_{28}H_{19}N_5O_2S$ $[M+H]^+$ 490.1337; found 490.1332.

2-((2-(4-Chlorophenyl)-2-oxoethyl) thio)-10-(4-nitrophenyl)-4,10-dihydro-9H-indeno[1,2-d][1,2,4]triazolo[1,5-a]pyrimidin-9-one (**5t**)



Yellow solid; Yield 93%; mp: 236-237 °C; IR (KBr) cm^{-1}: 3172 (N-H), 3012 (alkane C-H), 1728, 1653 (C=O), 1340, 1532 (NO2), 785 (C-Cl); ^{1}H NMR (400 MHz d_6) δ (ppm): 4.70 (q, J = 16.8 Hz, 2H), 6.45 (s, 1H), 7.32 (d, J = 6.8 Hz, 1 H), 7.41 (t, J = 7.8 Hz, 1H), 7.51 (t, J = 7.6 Hz, 1H), 7.56-7.58 (m, 4H), 7.69 (d, J = 7.2 Hz, 1H), 7.93 (d, J = 8.8 Hz, 2H), 8.15 (d, J = 8.8 Hz, 2H), 12.65 (s, 1H); ^{13}C NMR (100MHz, DMSO-d_6) δ (ppm): 38.9, 58.2, 102.6, 120.7, 121.5, 124.0, 129.2, 129.4, 130.6, 131.5, 132.8, 133.7, 134.6, 135.6, 138.9, 146.6, 147.8, 148.6, 154.9, 158.7, 188.9, 193.0. HRMS (ESI-TOF) (*m/z*): Calculated for $C_{26}H_{16}ClN_5O_4S$ $[M+H]^+$ 530.069; found 530.0669.

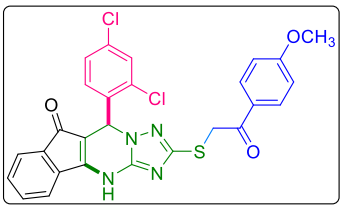
10-(4-nitrophenyl)-2-((2-oxo-2-phenylethyl)thio)-4,10-dihydro-9H-indeno[1,2-d][1,2,4] triazolo[1,5-a]pyrimidin-9-one (**5u**)



Yellow solid; Yield 90%; mp: 265-266 °C; IR (KBr) cm^{-1}: 3194 (N-H), 3010 (alkane C-H), 1745, 1679 (C=O), 1318, 1504 (NO2); ^{1}H NMR (400 MHz d_6) δ (ppm): 4.73(q, J = 17.0 Hz,2H), 6.45 (s, 1H), 7.32 (d, J = 6.8 Hz, 1H), 7.41 (t, J = 7.4 Hz, 1H), 7.50-7.53 (m, 3H), 7.58 (d, J = 8.8 Hz, 2H), 7.64-7.69 (m, 2H), 7.93 (d, J = 7.2 Hz, 2H), 8.15 (d, J = 8.8 Hz, 2H), 12.65 (s, 1H); ^{13}C NMR (100MHz, DMSO-d_6) δ (ppm): 39.1, 58.2, 102.6, 120.7, 121.5, 124.1, 128.7, 129.1, 129.4, 131.5, 132.8, 133.7,

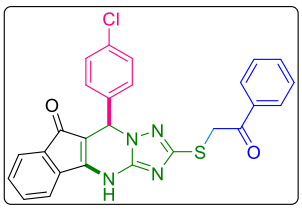
134.0, 135.6, 135.9, 146.7, 147.8, 148.6, 155.0, 158.9, 188.9, 193.8; HRMS (ESI-TOF) (*m/z*): Calculated for $C_{26}H_{17}N_5O_4S$ $[M+H]^+$ 496.1074; found 496.4142.

10-(2,4-Dichlorophenyl)-2-((2-(4-methoxyphenyl)-2-oxoethyl)thio)-4,10-dihydro-9H-indeno[1,2-d][1,2,4]triazolo[1,5-a]pyrimidin-9-one (**5v**)



Yellow solid; Yield 95%; mp: 224-225 °C; IR (KBr) cm^{-1}: 3103 (N-H), 3070 (alkane C-H), 1729, 1692 (C=O), 1289 (C-O-C), 733 (C-Cl); ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 3.86 (s, 3H), 4.66 (q, J = 16.8 Hz, 2H), 6.59 (s, 1H), 7.03 (d, J = 8.8 Hz, 2H), 7.30 (d, J = 6.8 Hz, 1H), 7.41 (t, J = 7.4 Hz, 2H), 7.48-7.52 (m, 2H), 7.55 (d, J = 2.4 Hz, 1H), 7.67 (d, J = 7.2 Hz, 1H), 7.91 (d, J = 8.8 Hz, 2H), 12.63 (s, 1H); ^{13}C NMR (100MHz, DMSO-d_6) δ (ppm): 38.8, 56.0, 114.4, 123.8, 127.7, 129.6, 129.8, 131.0, 132.1, 134.9, 136.6, 136.8, 137.0, 137.9, 138.1, 140.2, 142.5, 188.3, 189.1. HRMS (ESI-TOF) (*m/z*): Calculated for $C_{27}H_{18}BrCl_2N_4O_3S$ $[M+H]^+$ 549.0555; found 549.0556.

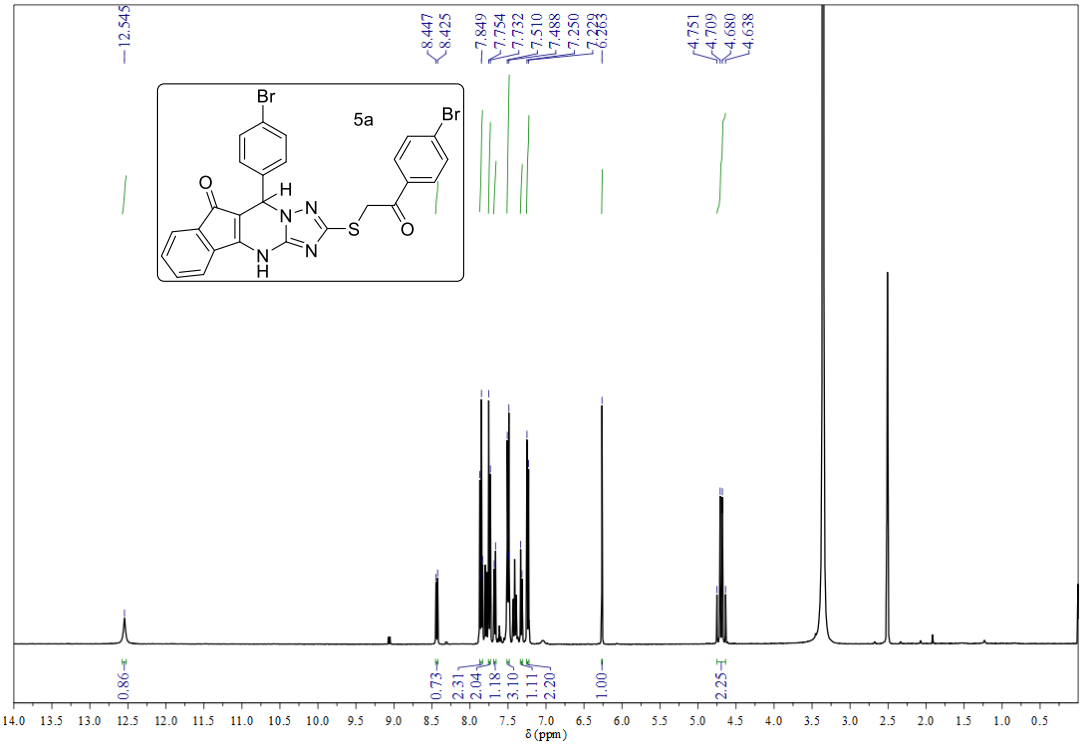
10-(4-Chlorophenyl)-2-((2-oxo-2-phenylethyl)thio)-4, 10-dihydro-9H-indeno[1,2-d][1,2,4] triazolo[1,5-a]pyrimidin-9-one (**5w**)



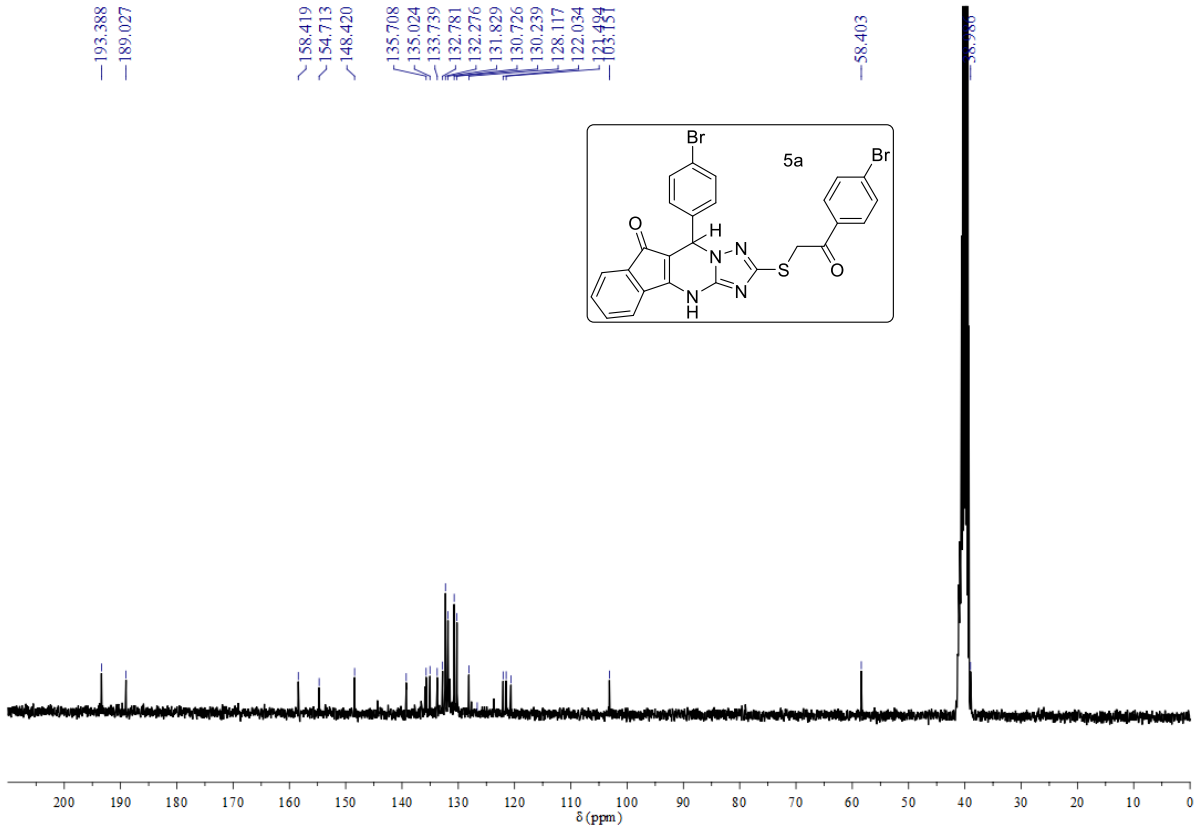
Yellow solid; Yield 92%; mp: 245-246 °C; IR (KBr) cm^{-1}: 3173 (N-H), 3068 (alkane C-H), 1703, 1686 (C=O), 746 (C-Cl); ^1H NMR (400 MHz d_6) δ (p pm): 4.74 (q, J = 17.0 Hz, 2H), 6.29 (s, 1H), 7.31-7.35 (m, 5H), 7.47 – 7.56 (m, 4H), 7.67 (d, J = 6.8 Hz, 2H), 7.95 (d, J = 7.2 Hz, 2H), 12.55 (s, 1H); ^{13}C NMR (100MHz, DMSO-d_6) δ (ppm): 39.2, 58.3, 103.2, 120.6, 121.4, 128.7, 128.9, 129.2, 129.9, 131.4, 132.7, 133.4, 133.7, 134.0, 135.7, 136.0, 138.8, 148.3, 154.8, 158.6, 189.0, 193.9: HRMS (ESI-TOF) (*m/z*): Calculated $C_{26}H_{17}ClN_4O_2S$ $[M+H]^+$ 485.0839; found 485.0844.

5A.6 Copies of spectral data

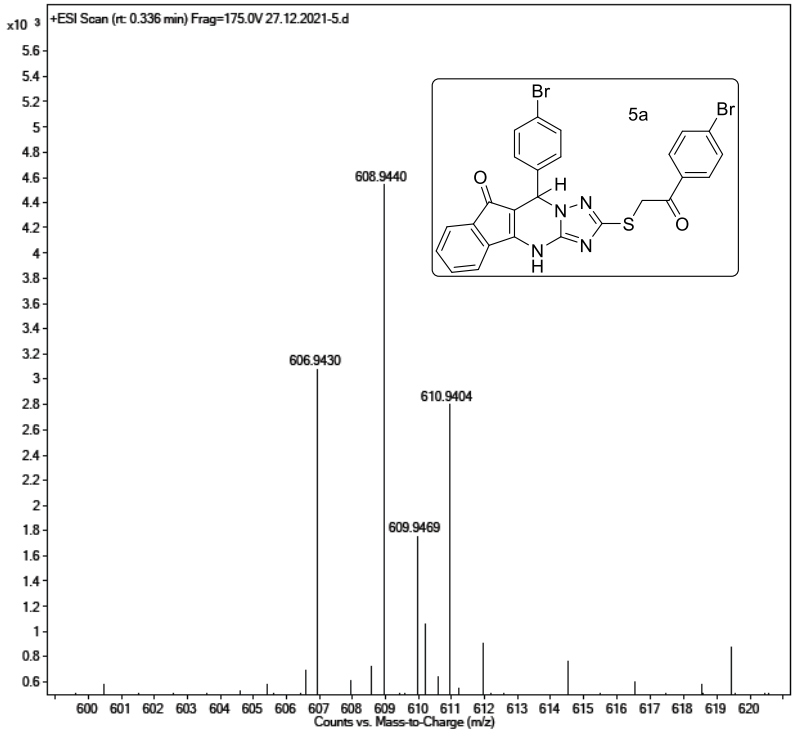
^{1}H NMR spectrum of compound 5a (DMSO-d_6) 400 MHz



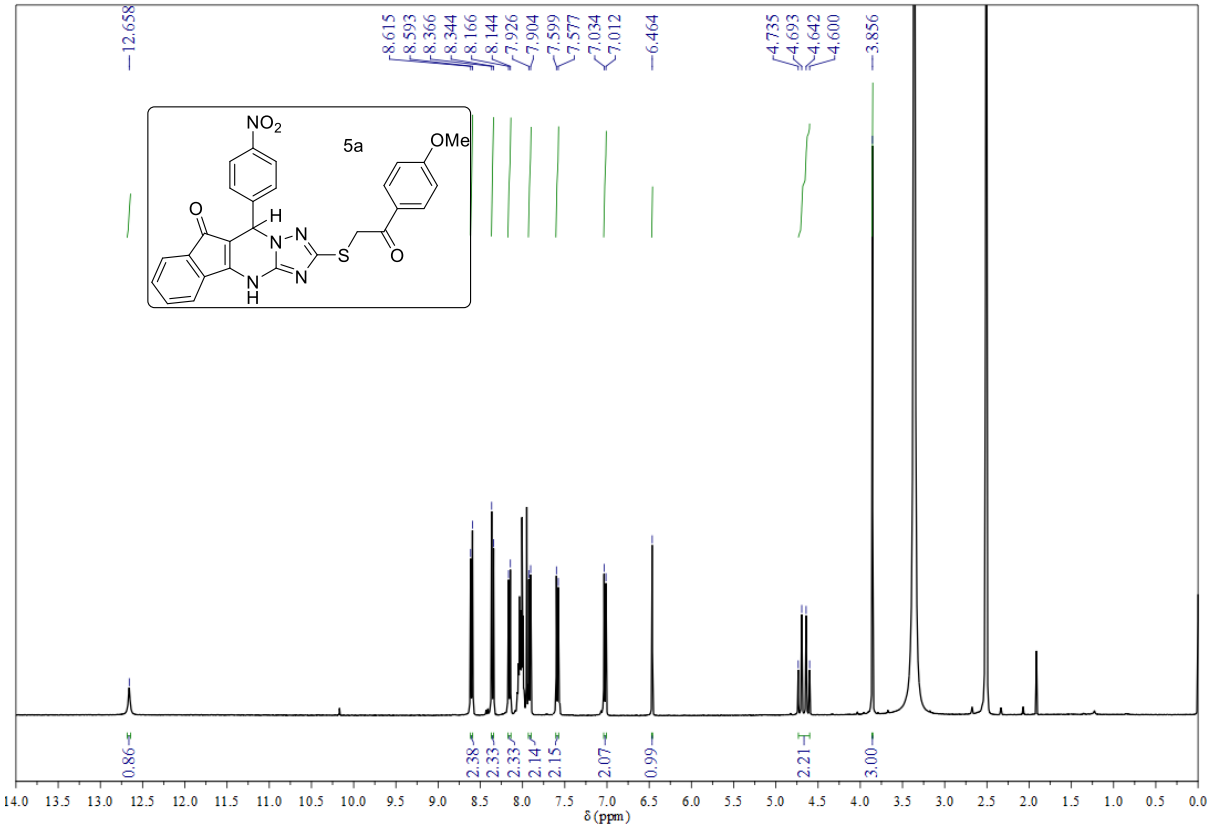
^{13}C NMR spectrum of compound 5a (DMSO-d_6) 100 MHz



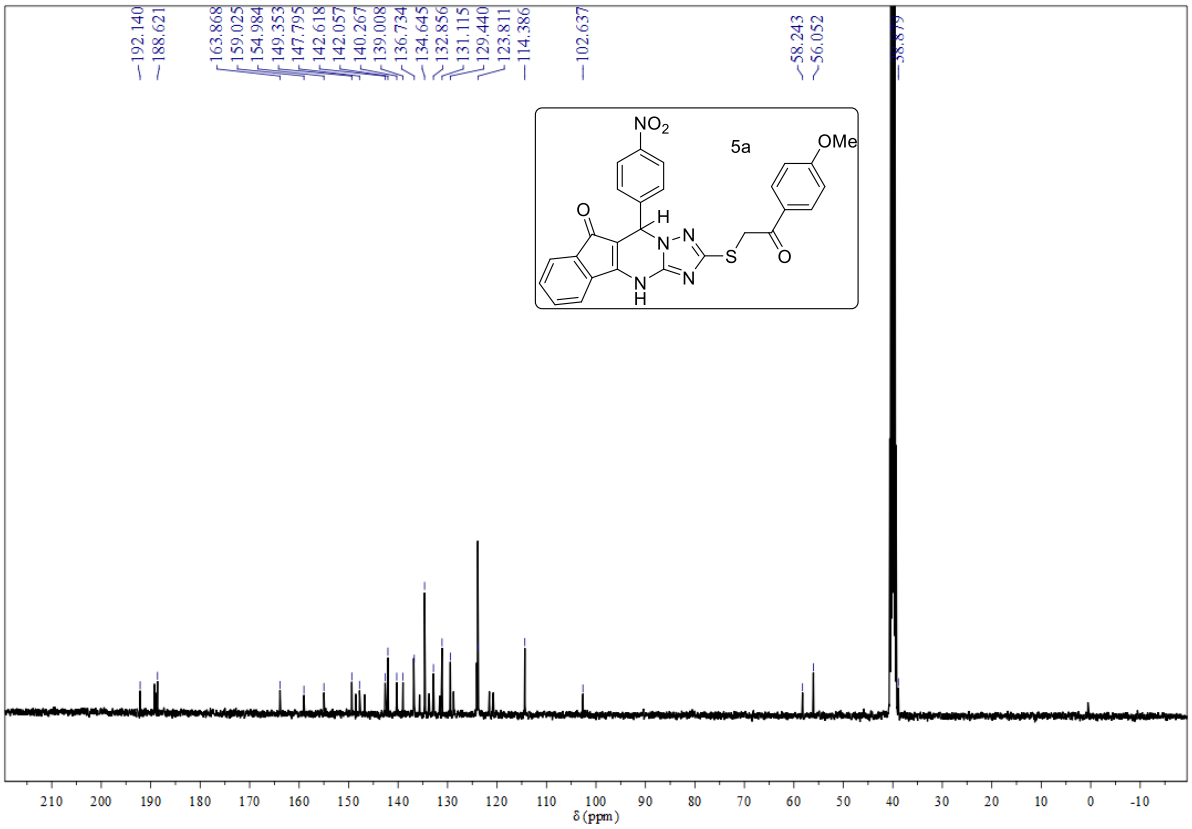
Mass spectrum of compound 5a



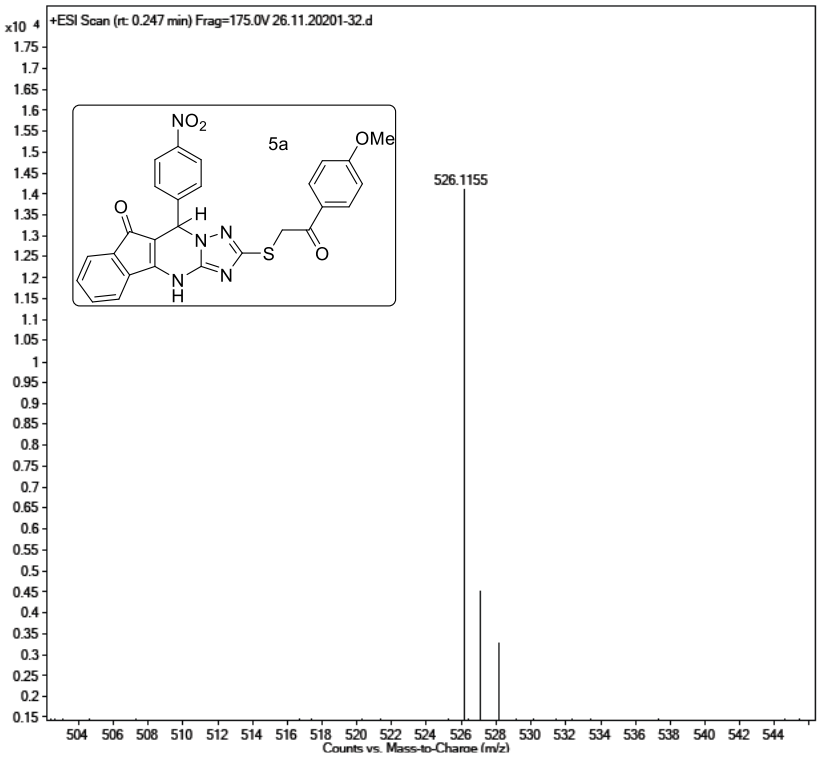
^{1}H NMR spectrum of compound 5b (DMSO-d_6) 400 MHz



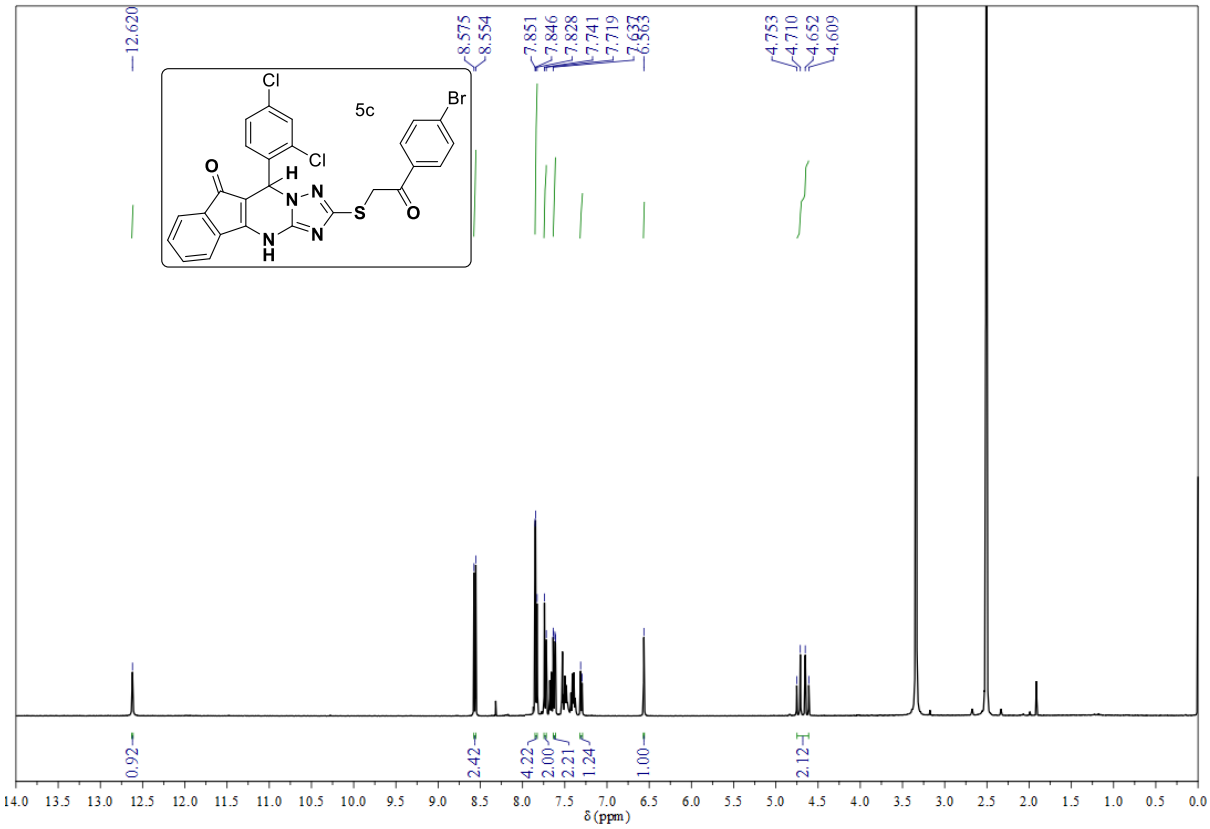
^{13}C NMR spectrum of compound 5b (DMSO-d_6) 100 MHz



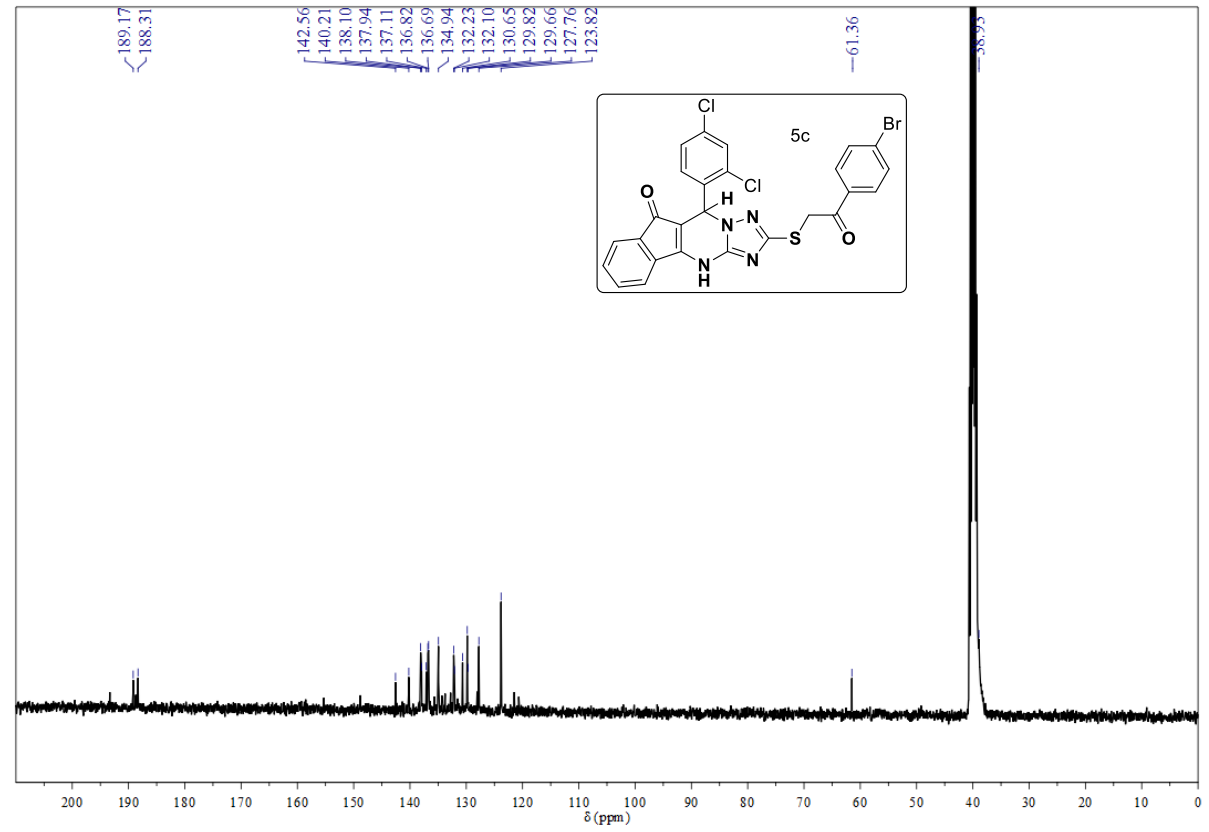
Mass spectrum of compound 5b



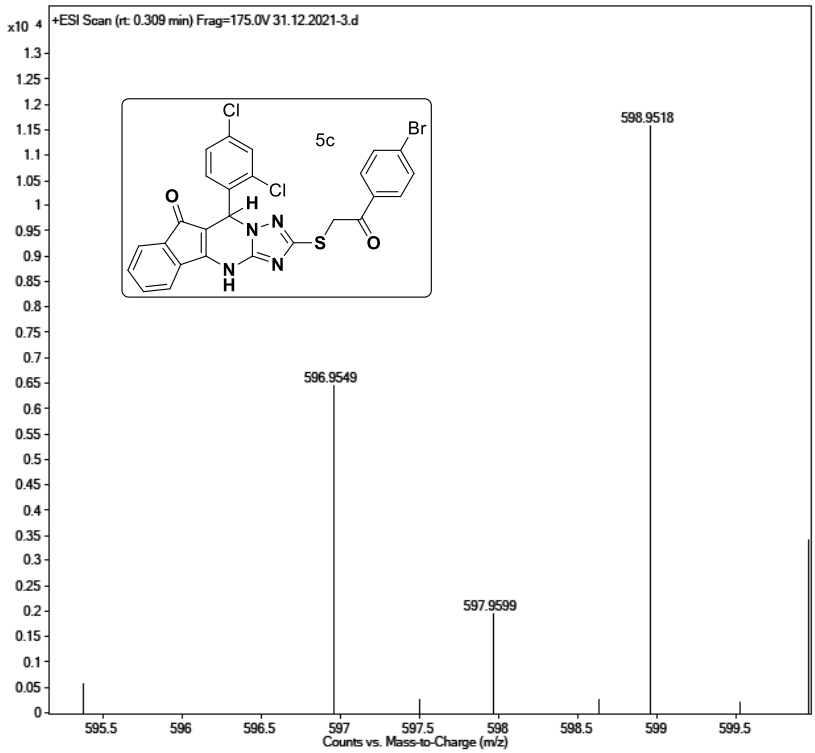
^{1}H NMR spectrum of compound 5c (DMSO-d_6) 400 MHz



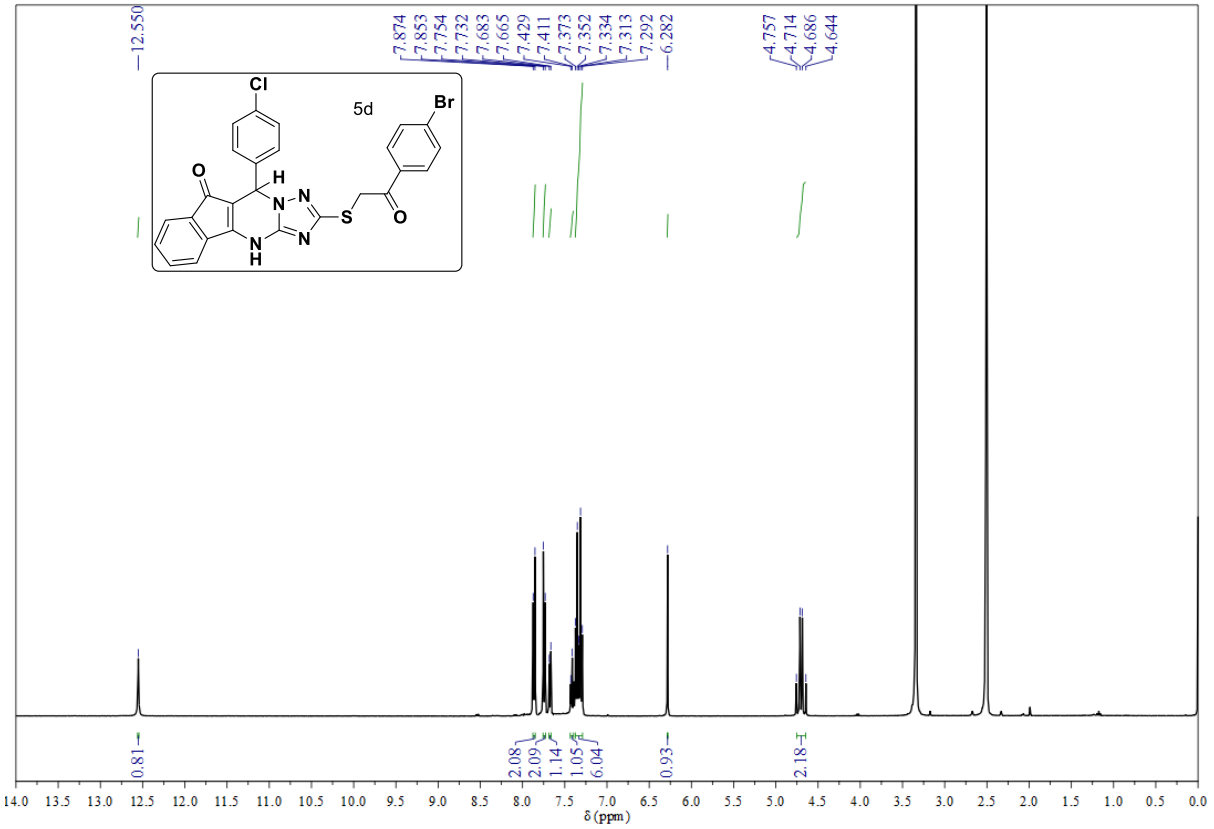
^{13}C NMR spectrum of compound 5c (DMSO-d_6) 100 MHz



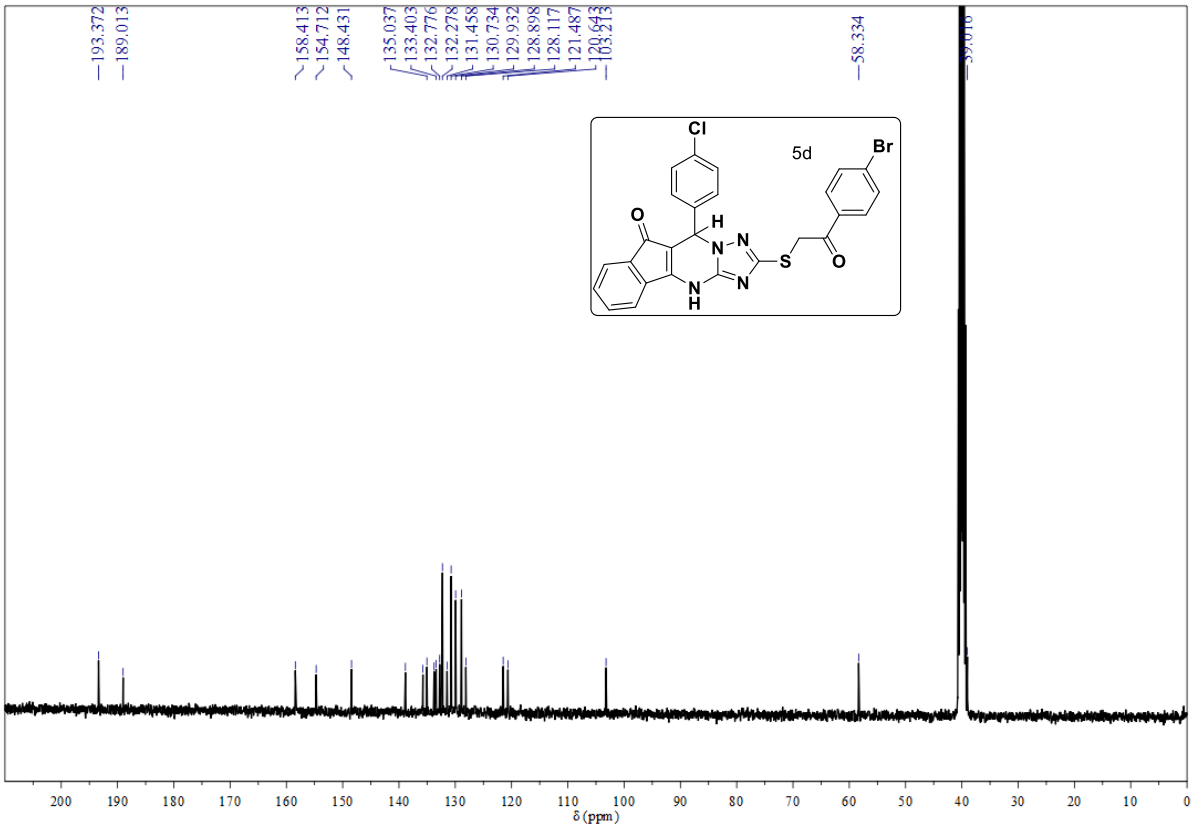
Mass spectrum of compound 5c



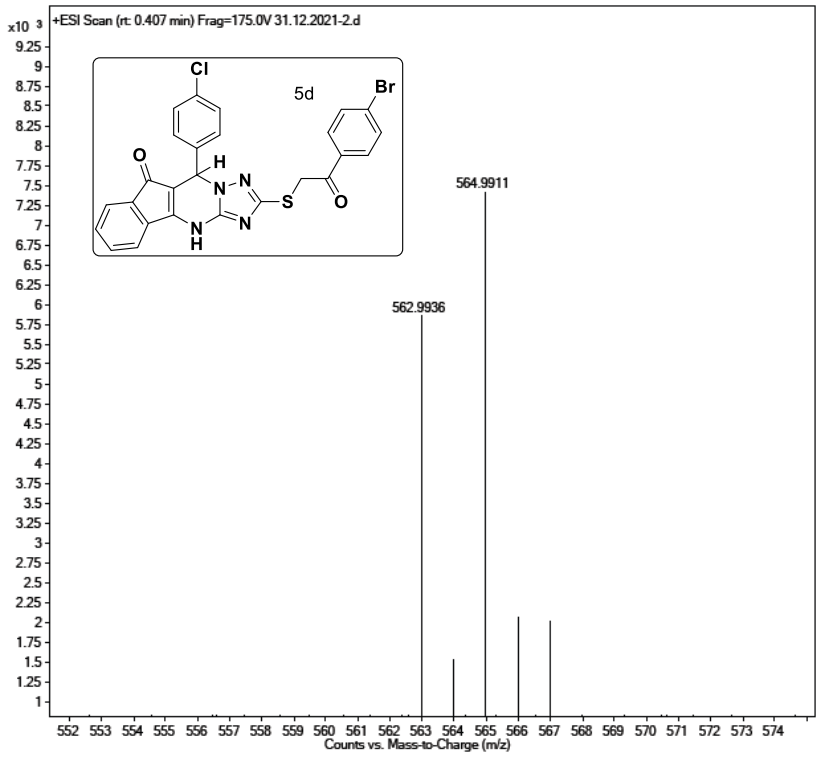
^{1}H NMR spectrum of compound 5d (DMSO-d_6) 400 MHz



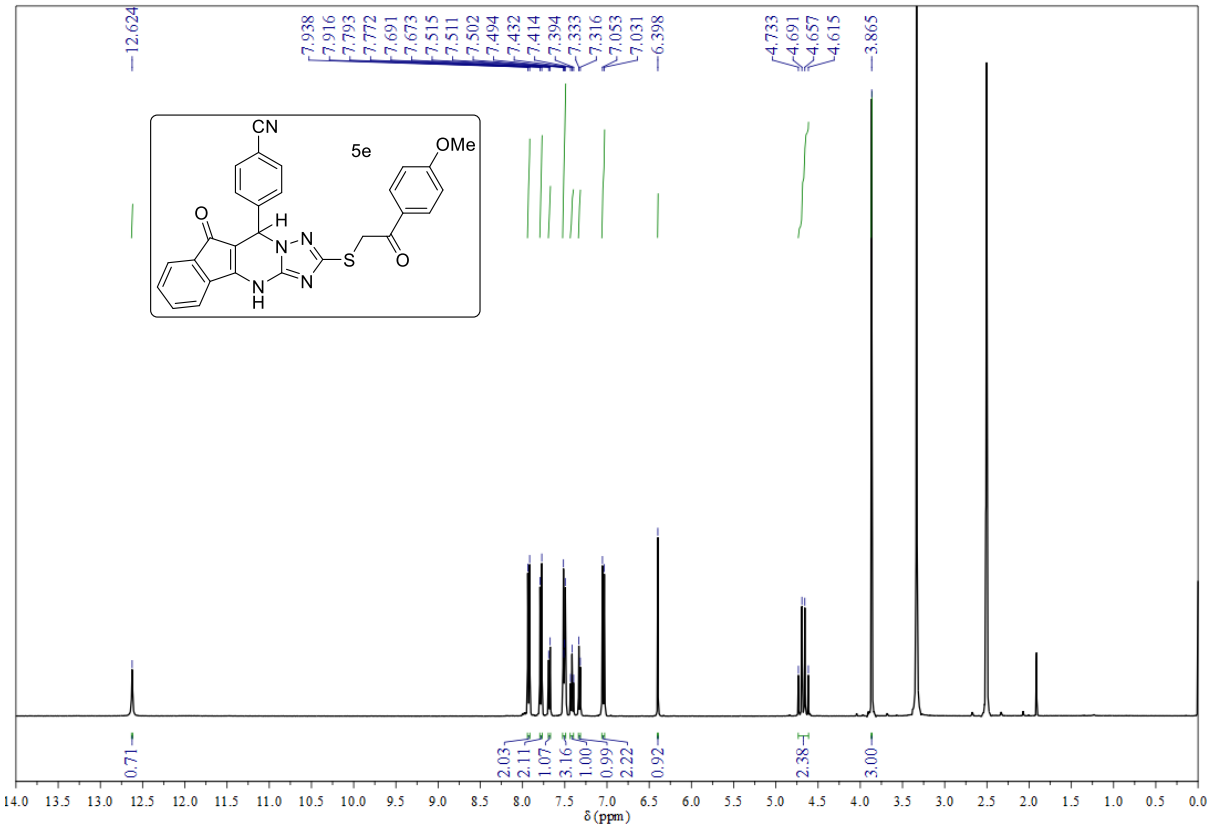
^{13}C NMR spectrum of compound 5d (DMSO-d_6) 100 MHz



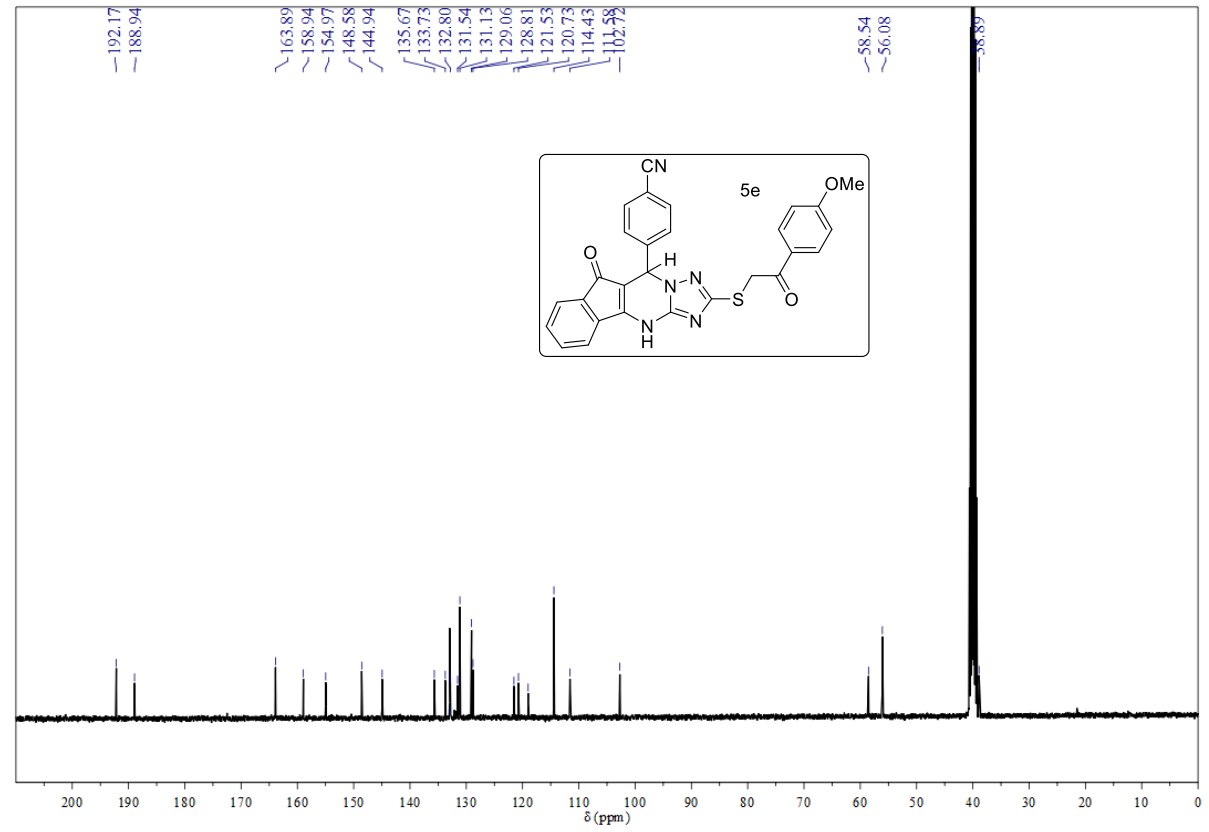
Mass spectrum of compound 5d



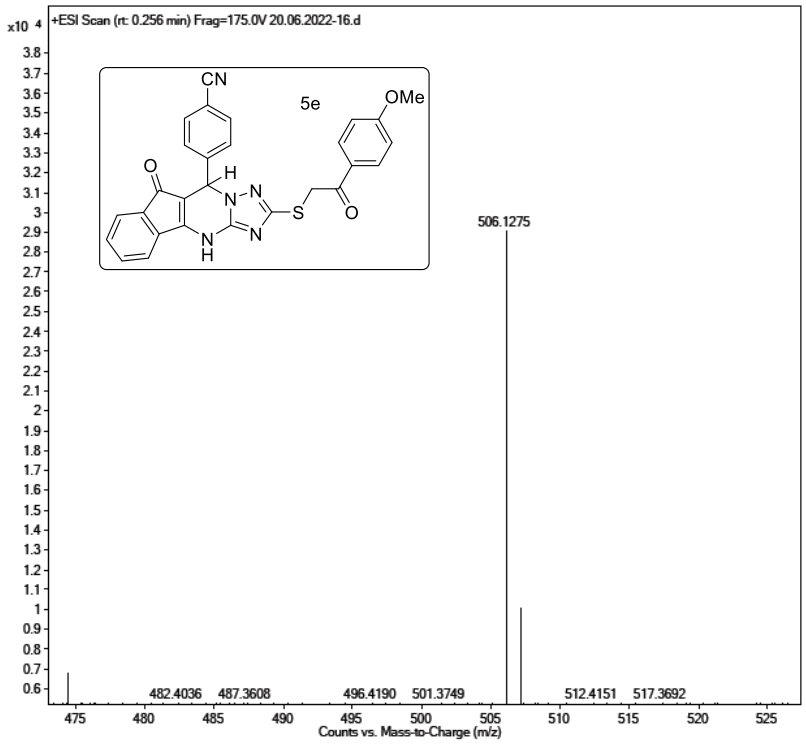
^{1}H NMR spectrum of compound 5e (DMSO-d_6) 400 MHz



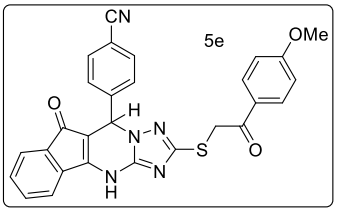
^{13}C NMR spectrum of compound 5e (DMSO-d_6) 100 MHz

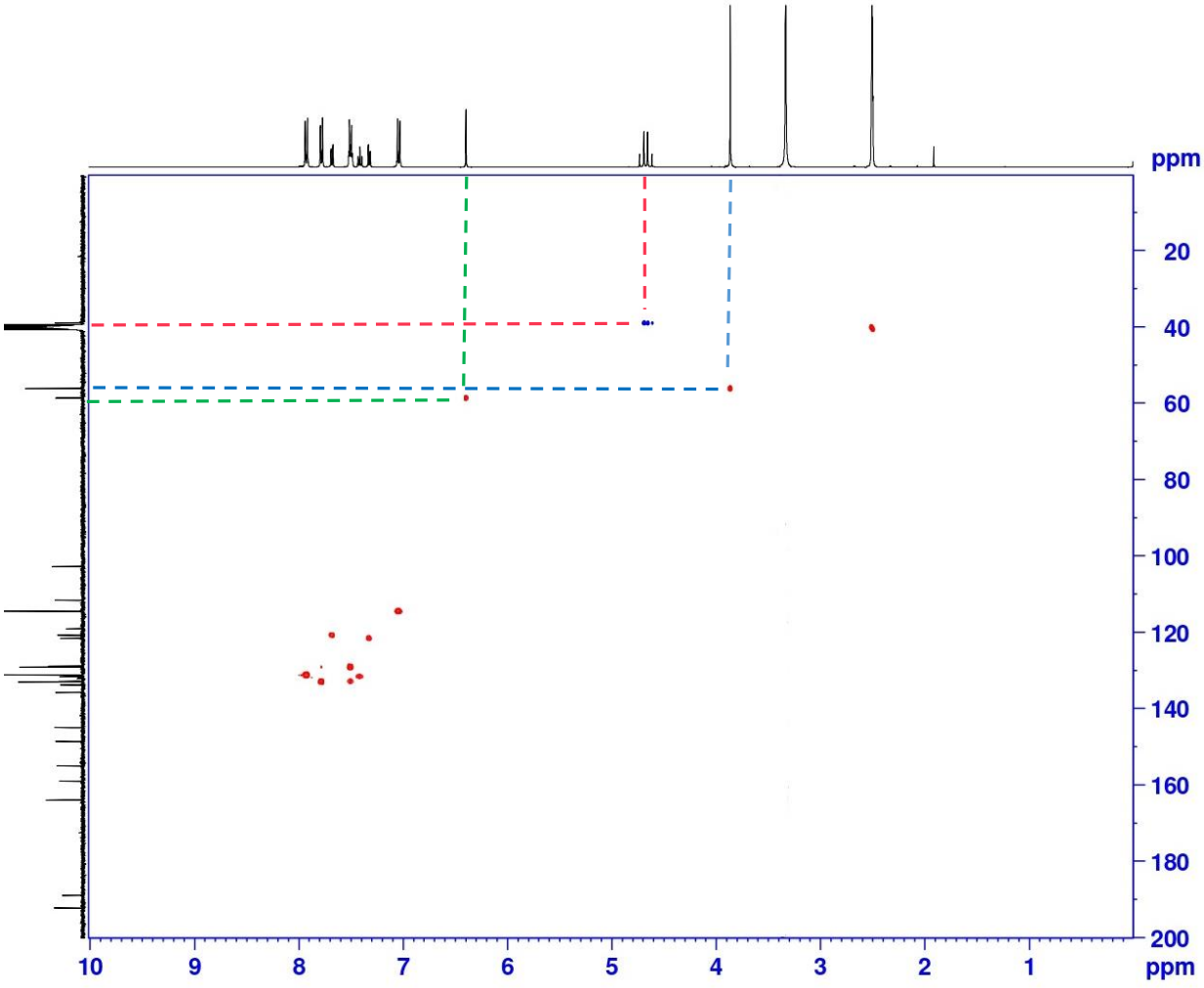


Mass spectrum of compound 5e

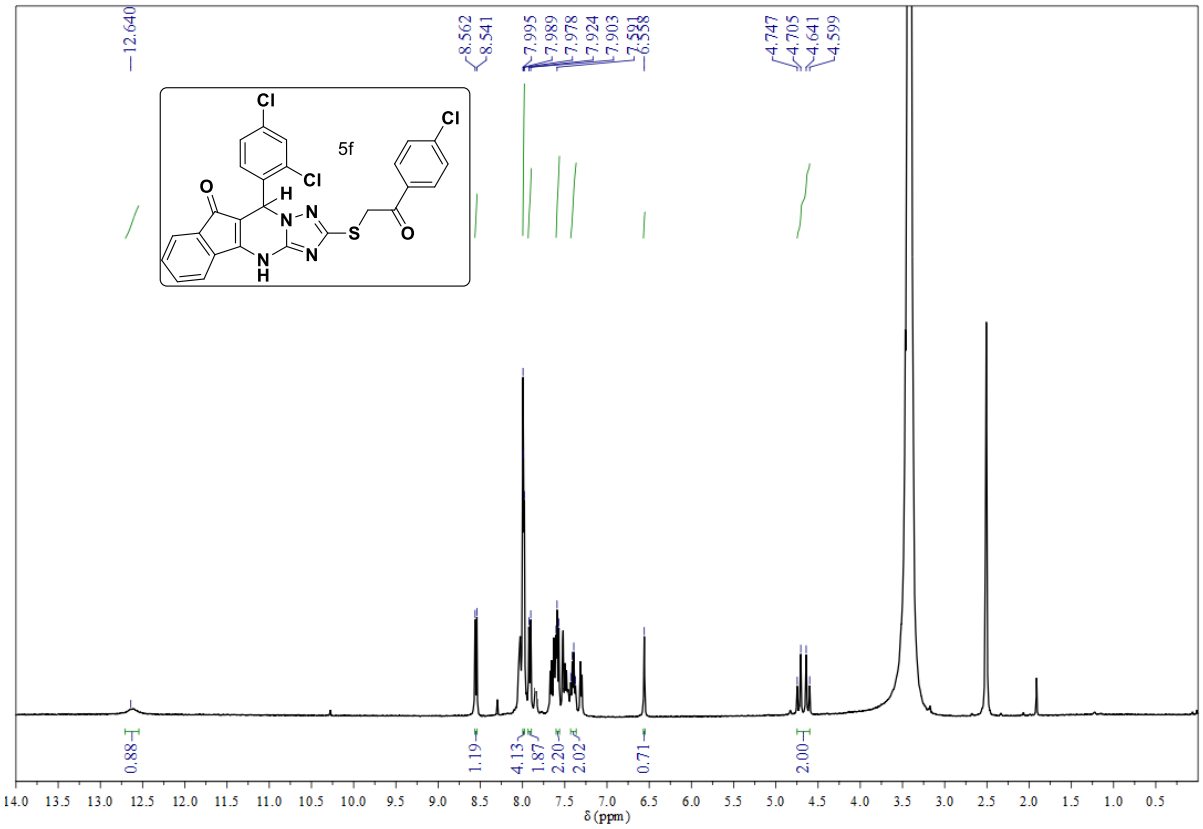


HSQC spectrum of compound 5e (DMSO- d_6)

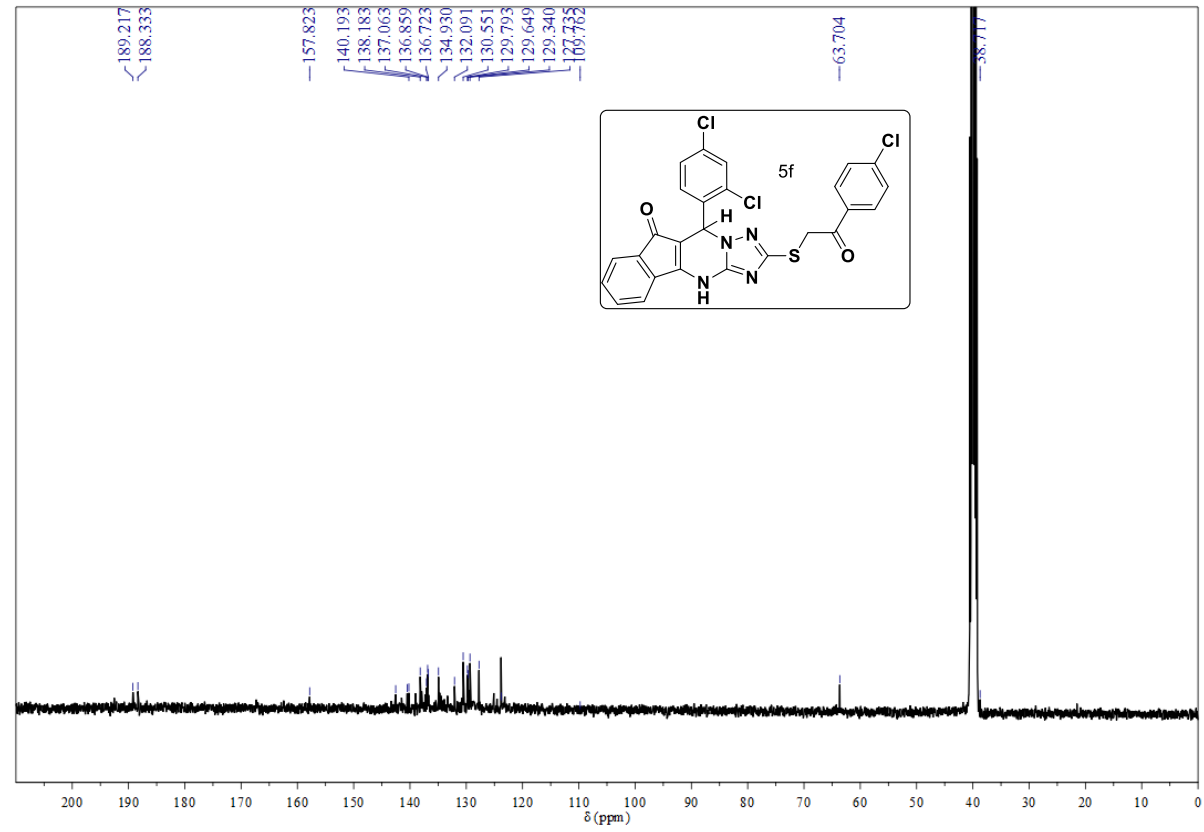




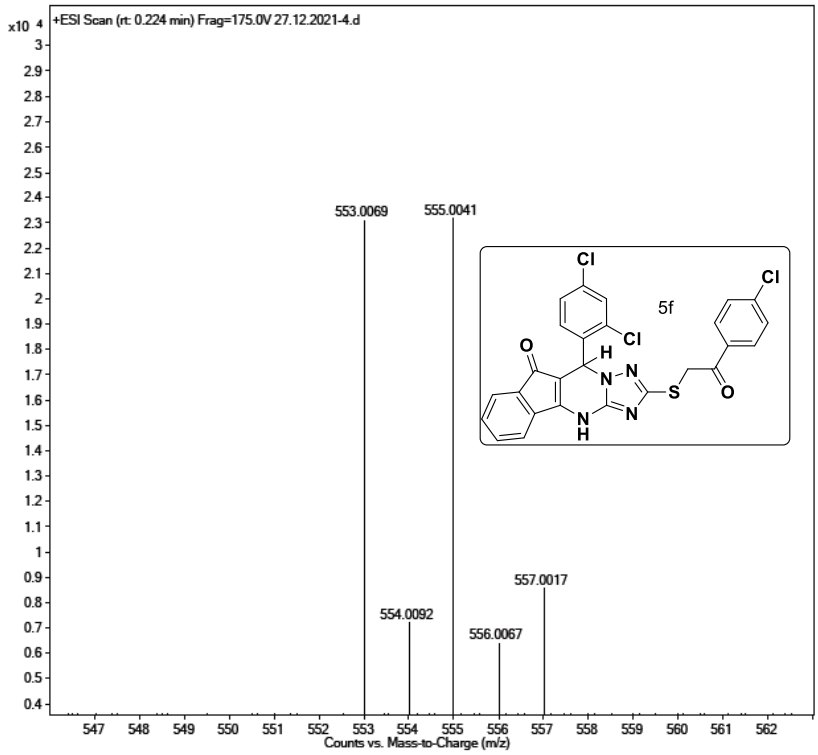
^{1}H NMR spectrum of compound 5f (DMSO- d_6) 400 MHz



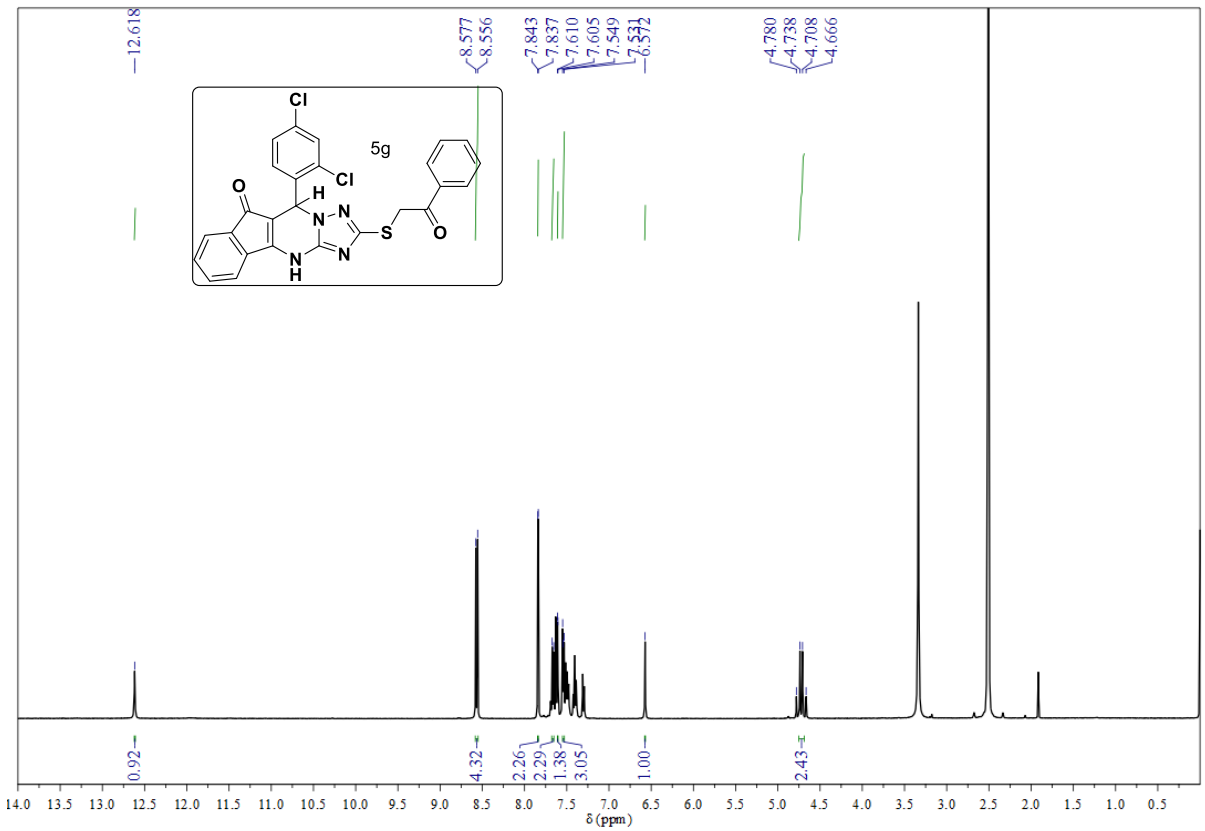
^{13}C NMR spectrum of compound 5f (DMSO- d_6) 100 MHz



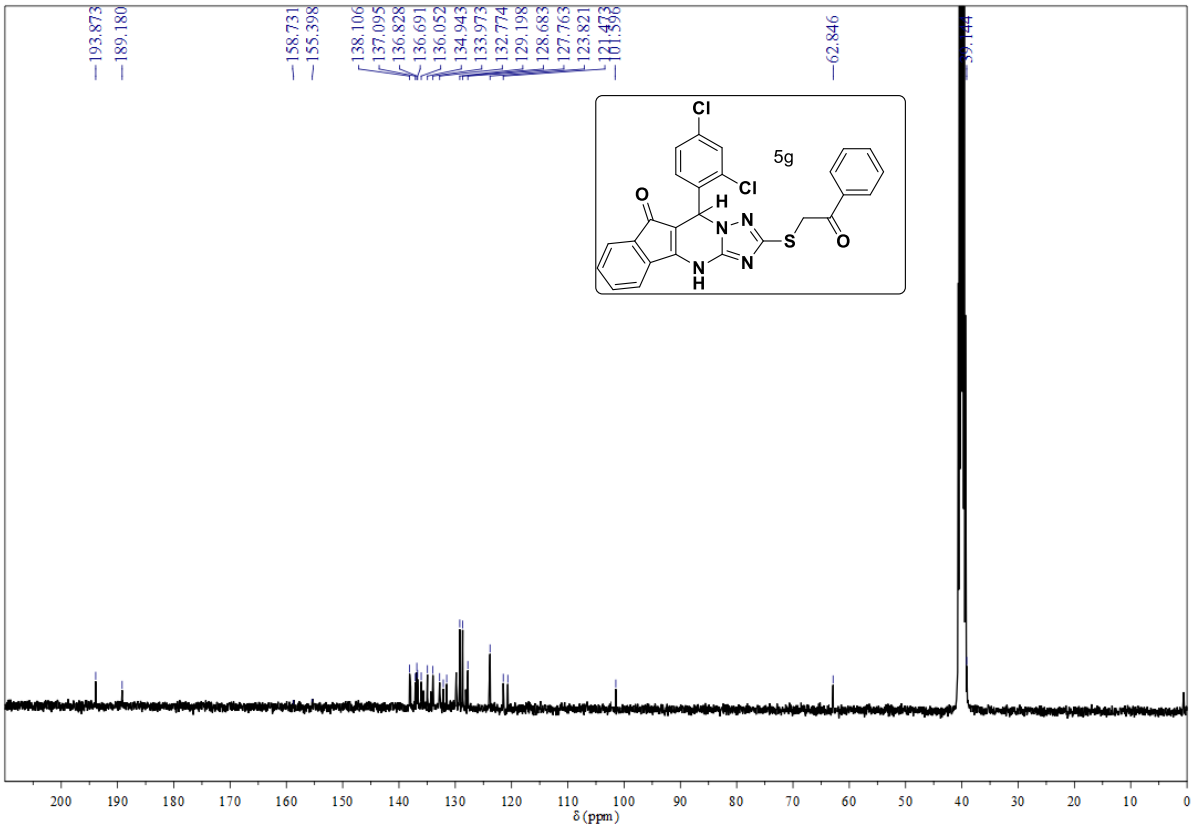
Mass spectrum of compound 5f



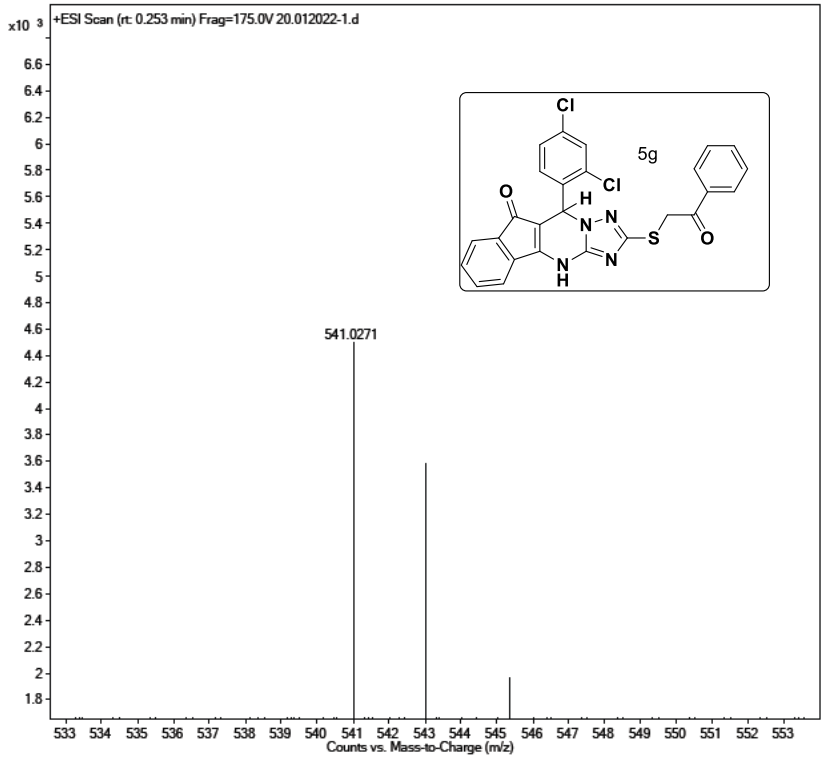
^{1}H NMR spectrum of compound 5g (DMSO- d_6) 400 MHz



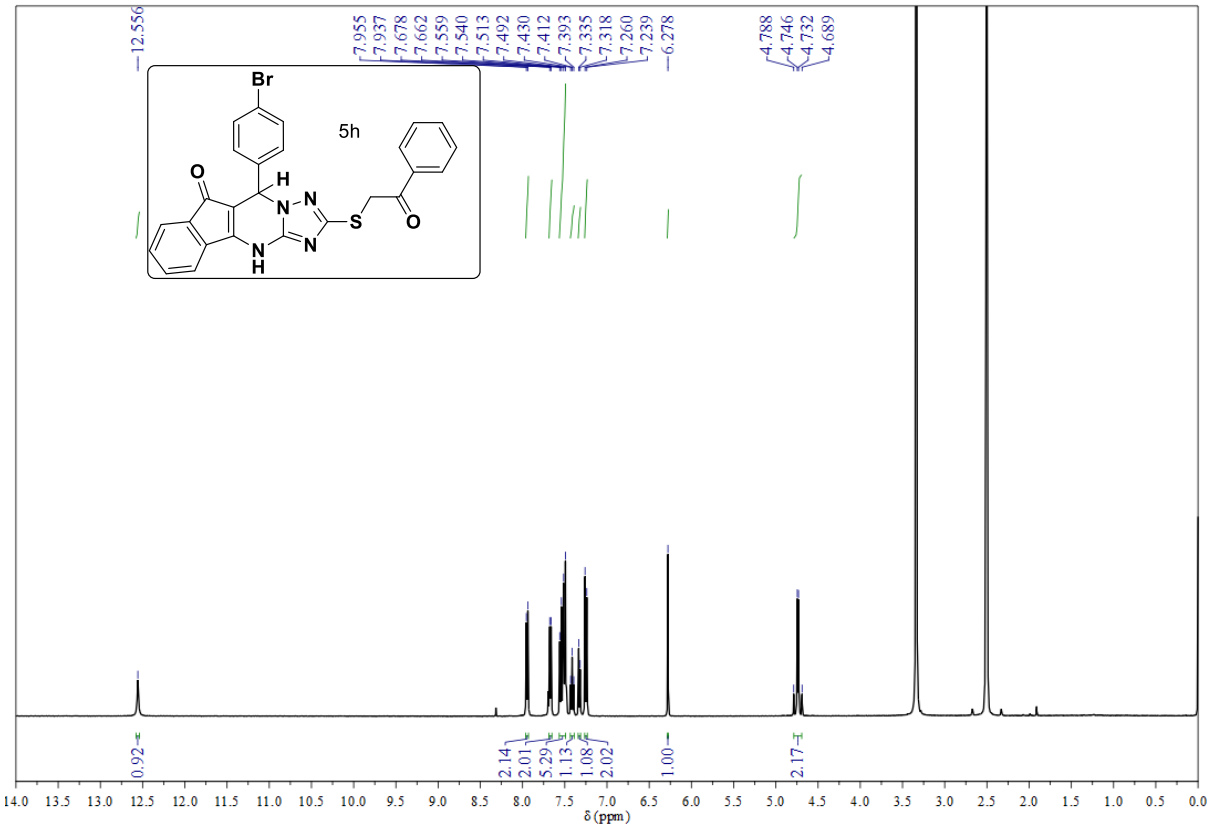
^{13}C NMR spectrum of compound 5g (DMSO- d_6) 100 MHz



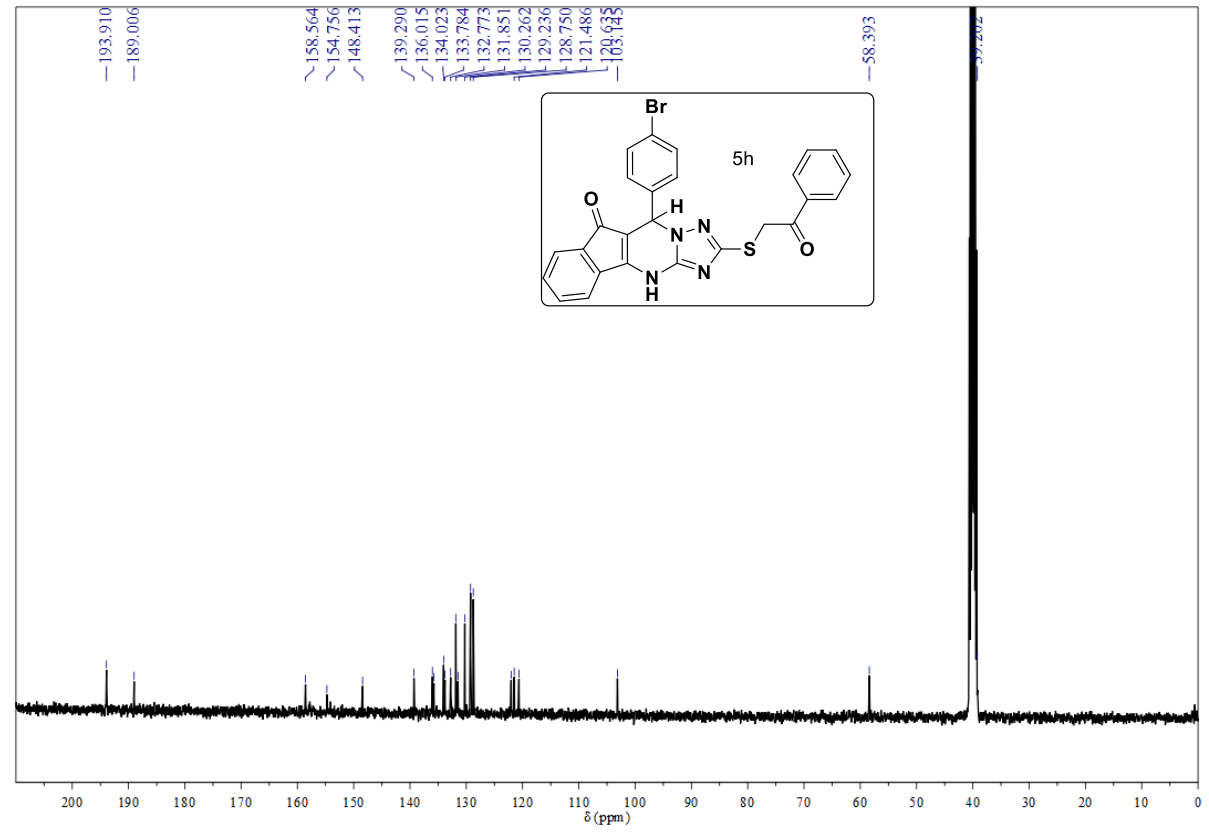
Mass spectrum of compound 5g



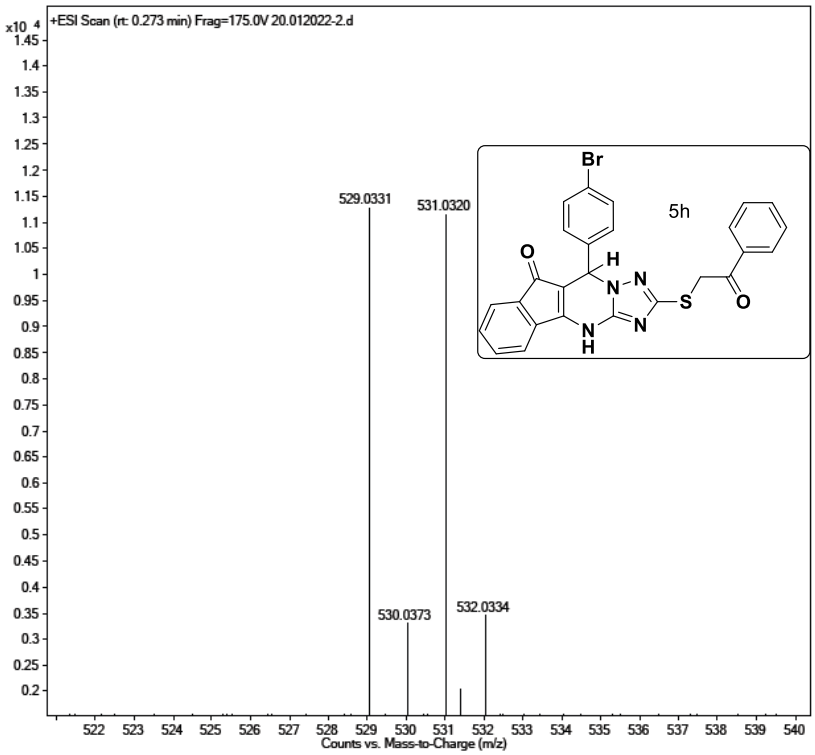
^{1}H NMR spectrum of compound 5h (DMSO- d_6) 400 MHz



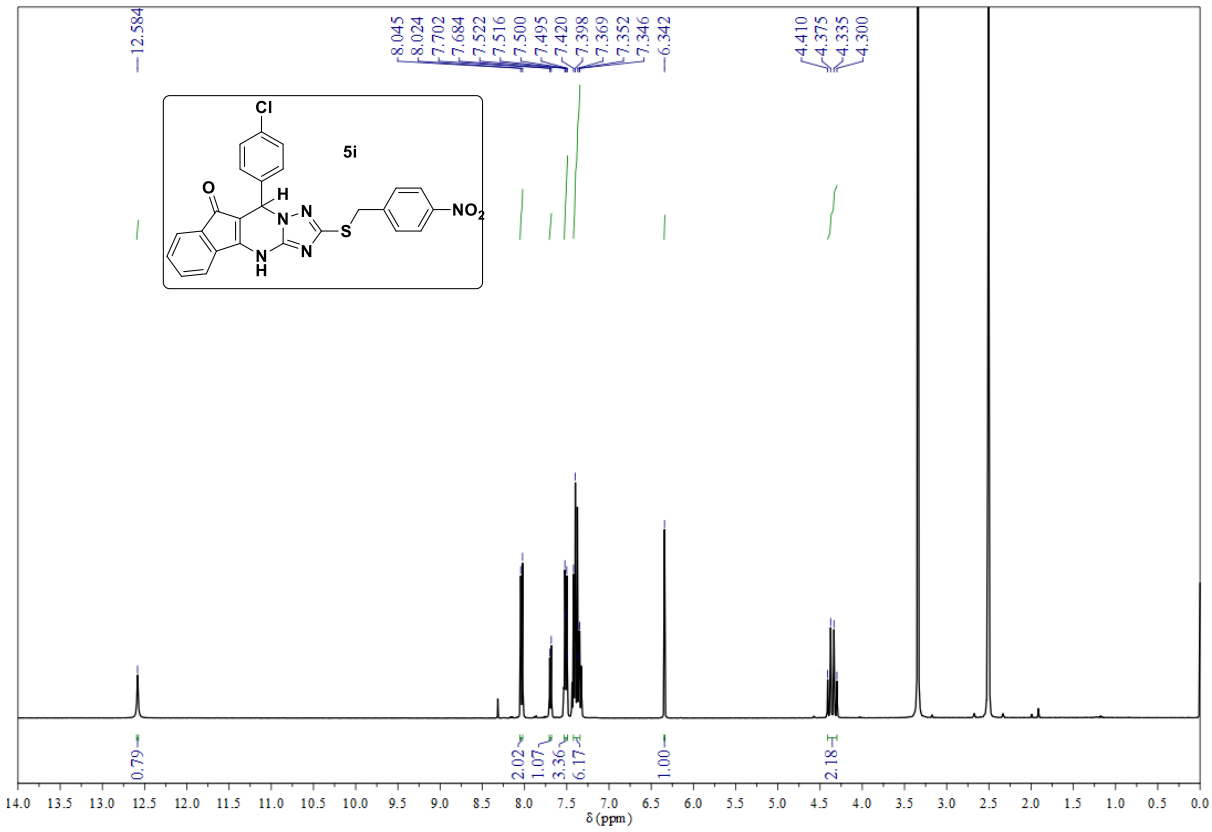
^{13}C NMR spectrum of compound 5h (DMSO- d_6) 100 MHz



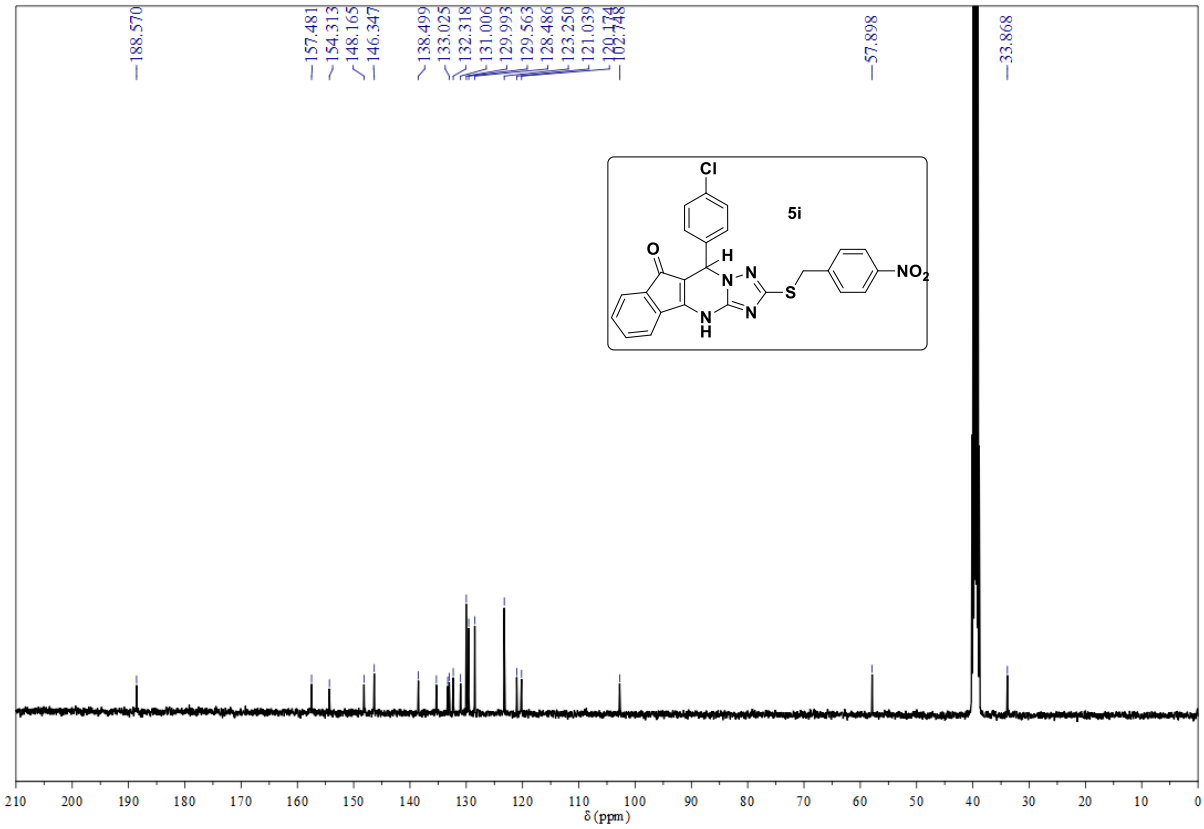
Mass spectrum of compound 5h



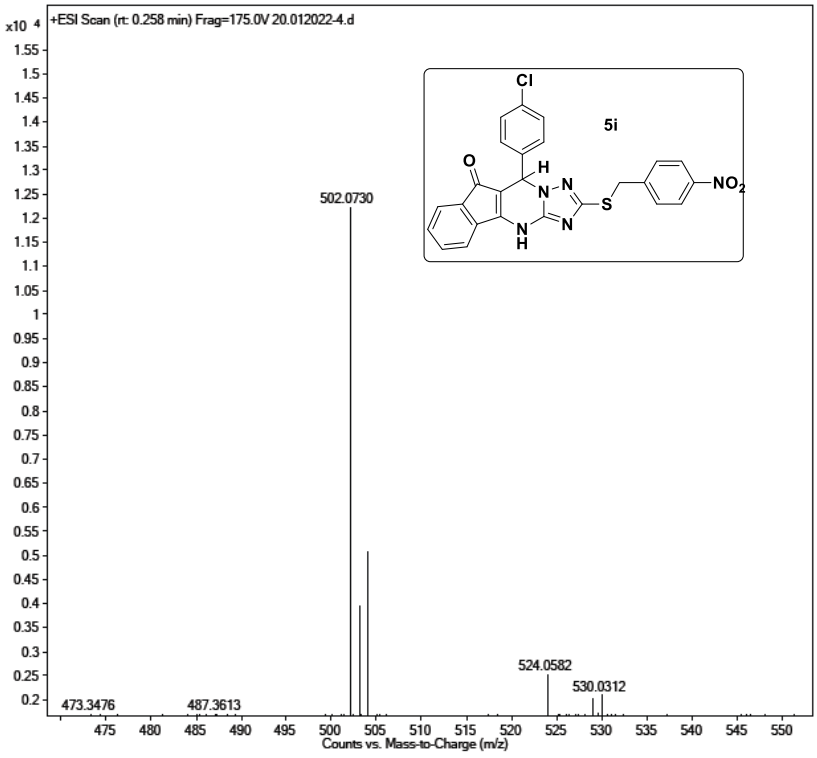
^{1}H NMR spectrum of compound 5i (DMSO- d_6) 400 MHz



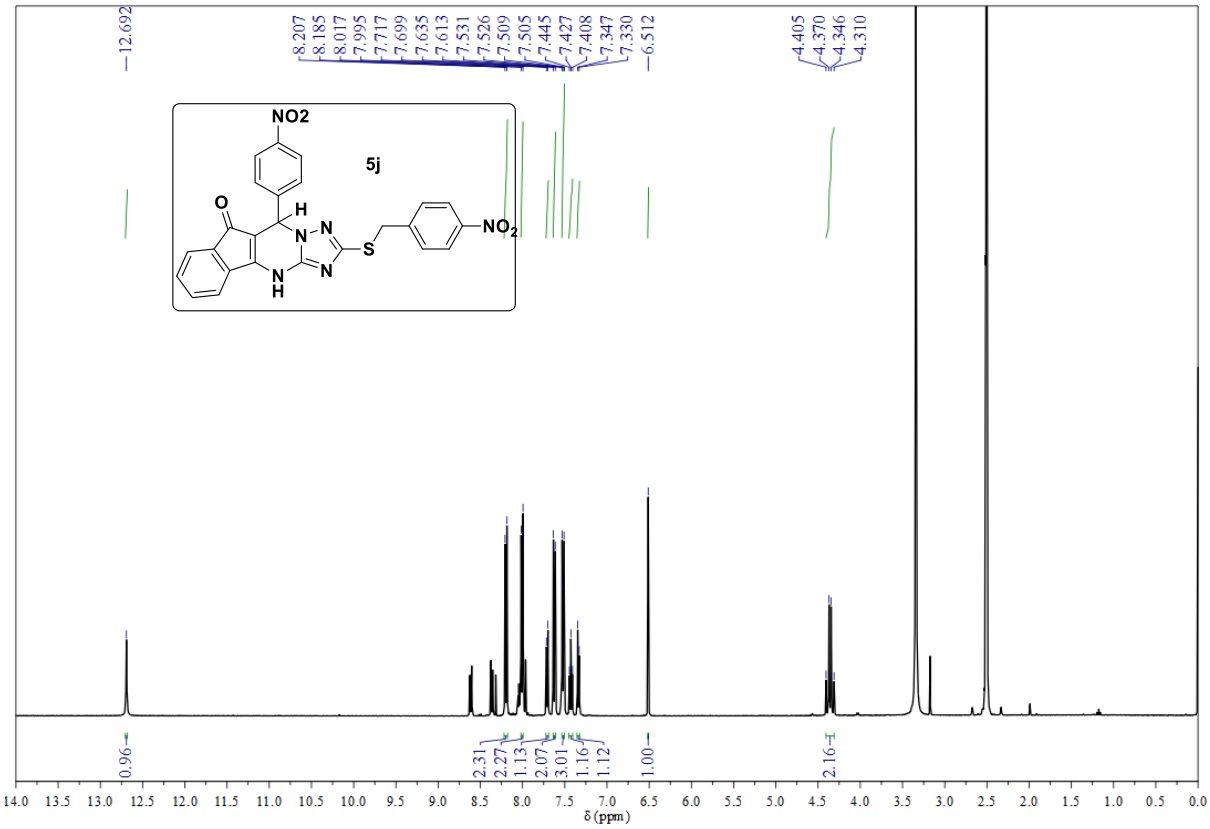
^{13}C NMR spectrum of compound 5i (DMSO- d_6) 100 MHz



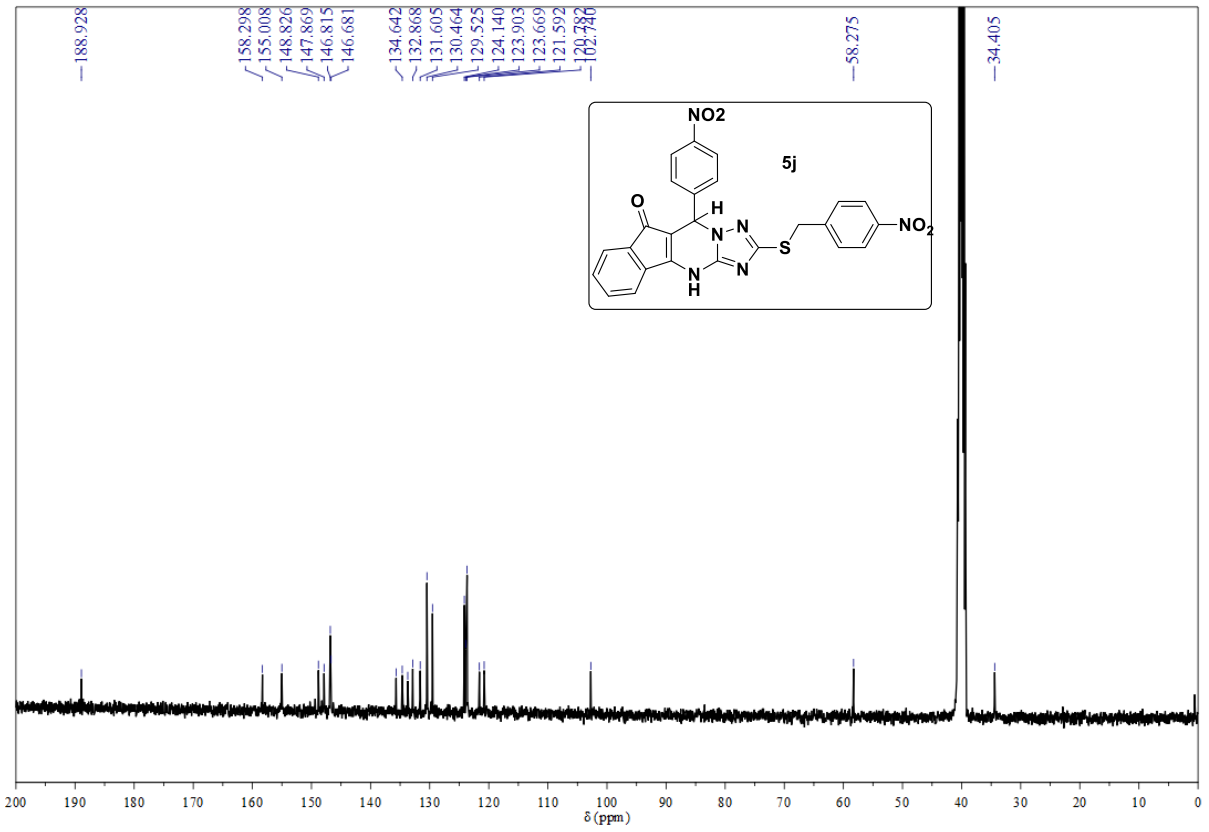
Mass spectrum of compound 5i



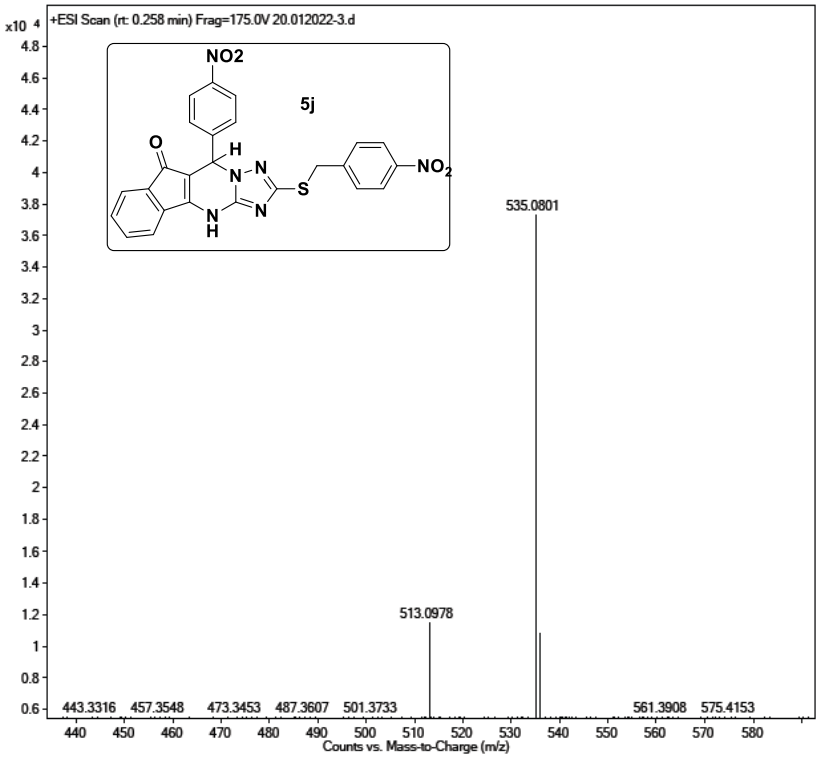
^{1}H NMR spectrum of compound 5j (DMSO- d_6) 400 MHz



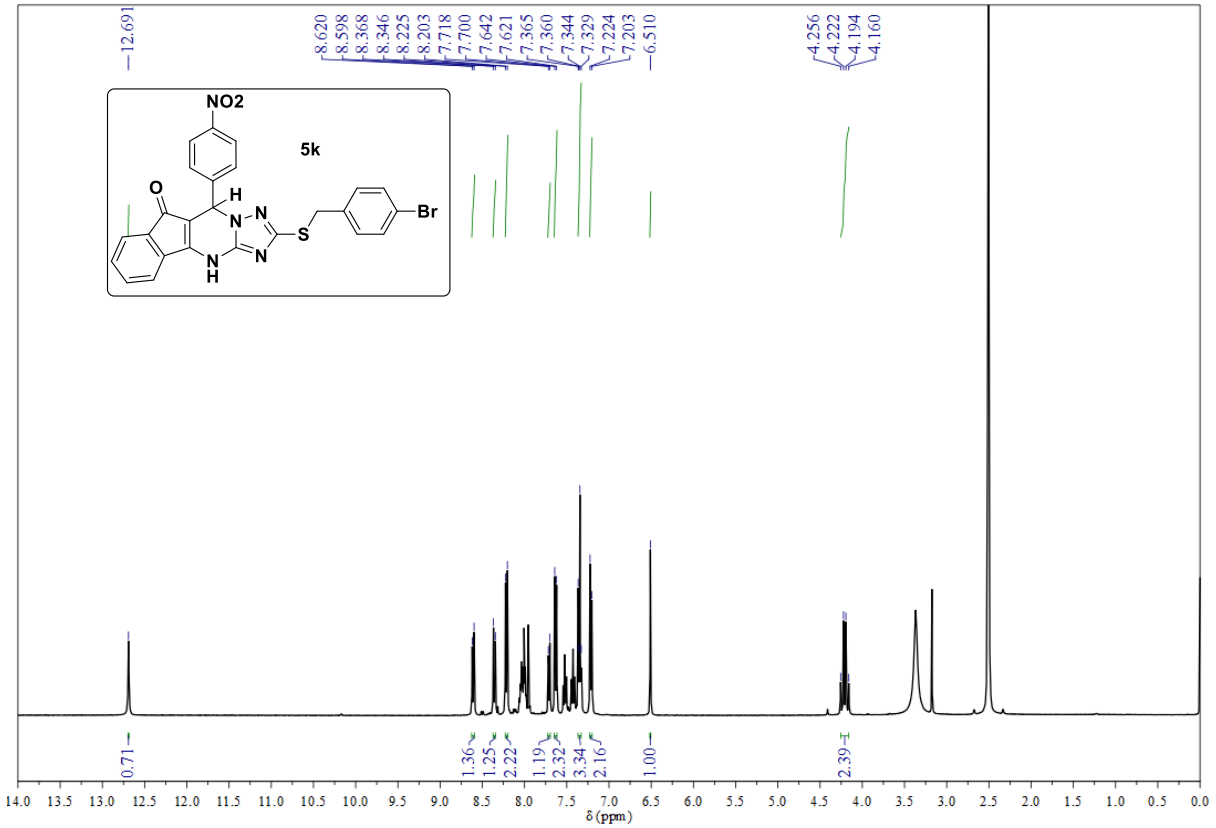
^{13}C NMR spectrum of compound 5j (DMSO- d_6) 100 MHz



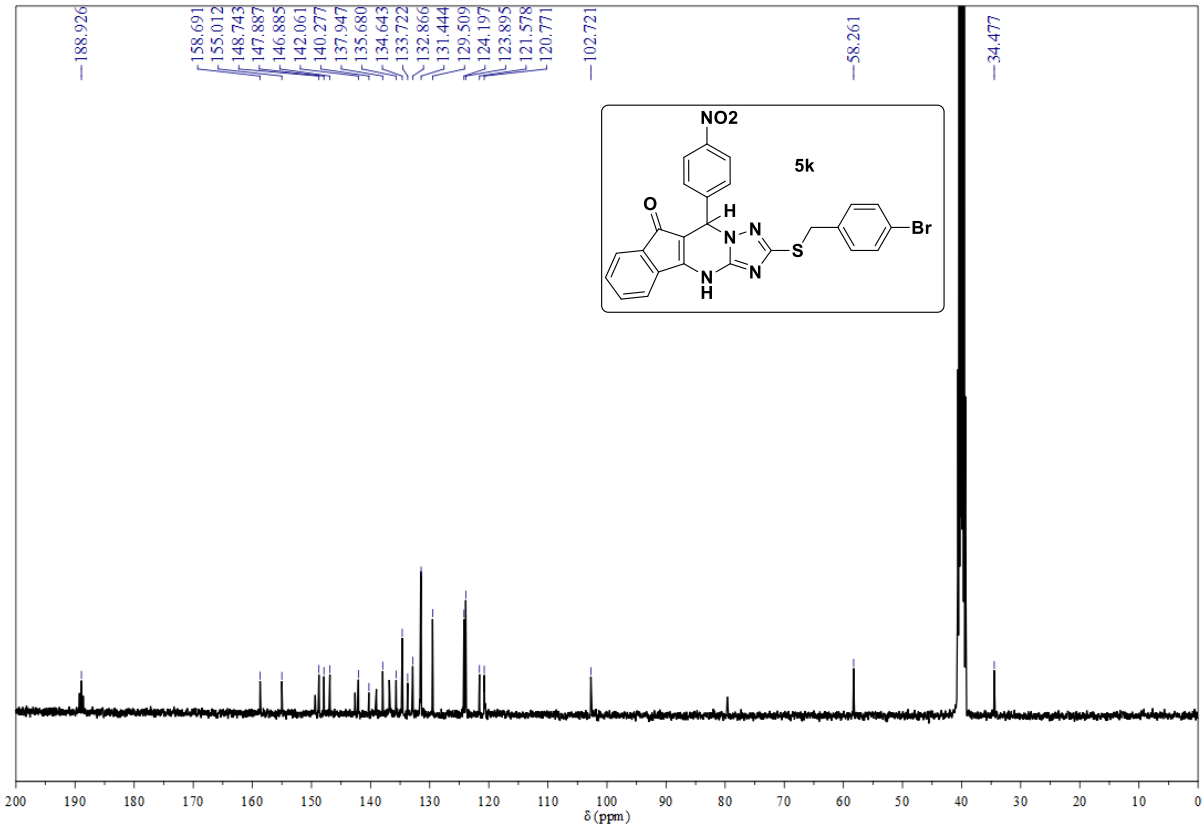
Mass spectrum of compound 5j



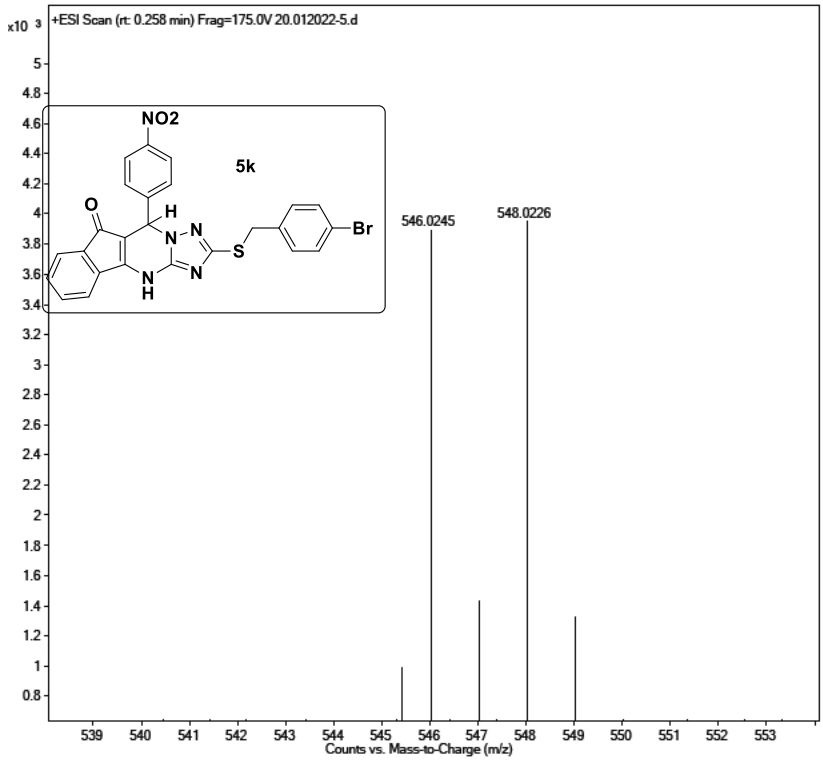
^{1}H NMR spectrum of compound 5k (DMSO- d_6) 400 MHz



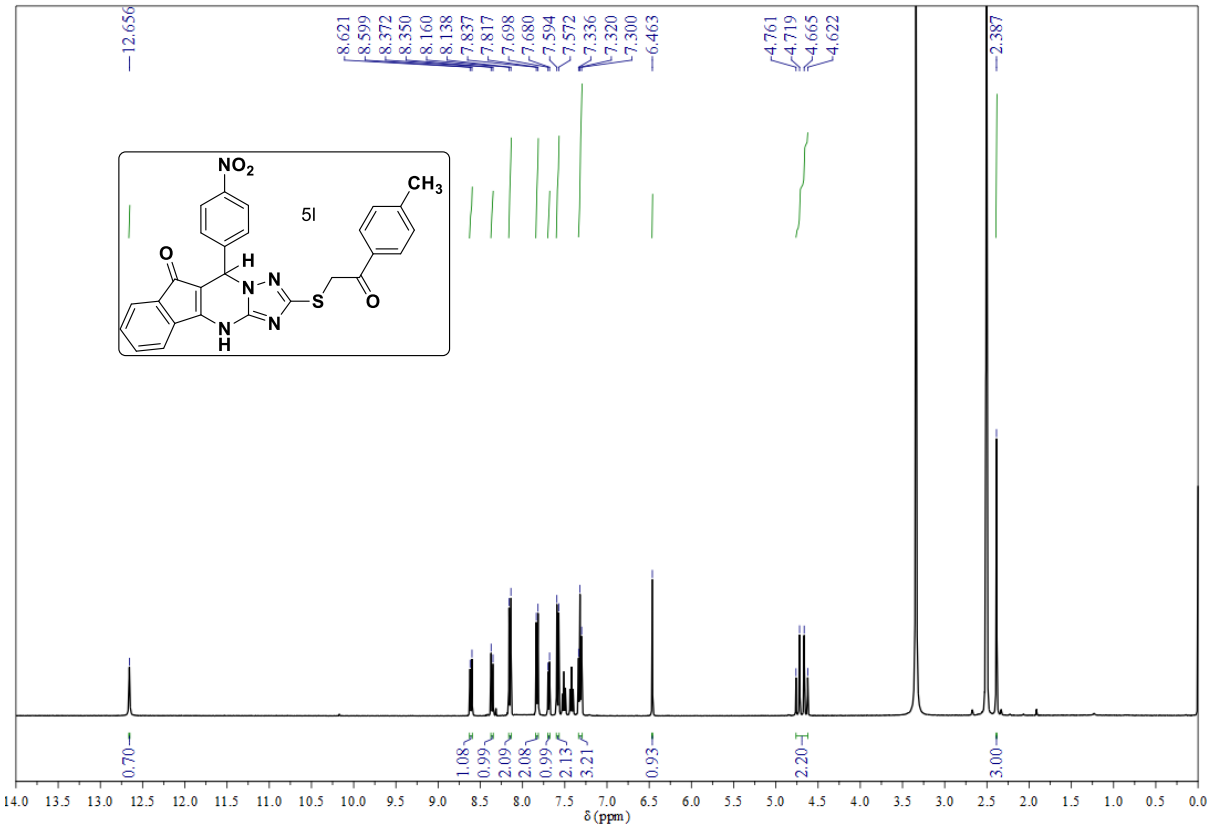
^{13}C NMR spectrum of compound 5k (DMSO- d_6) 100 MHz



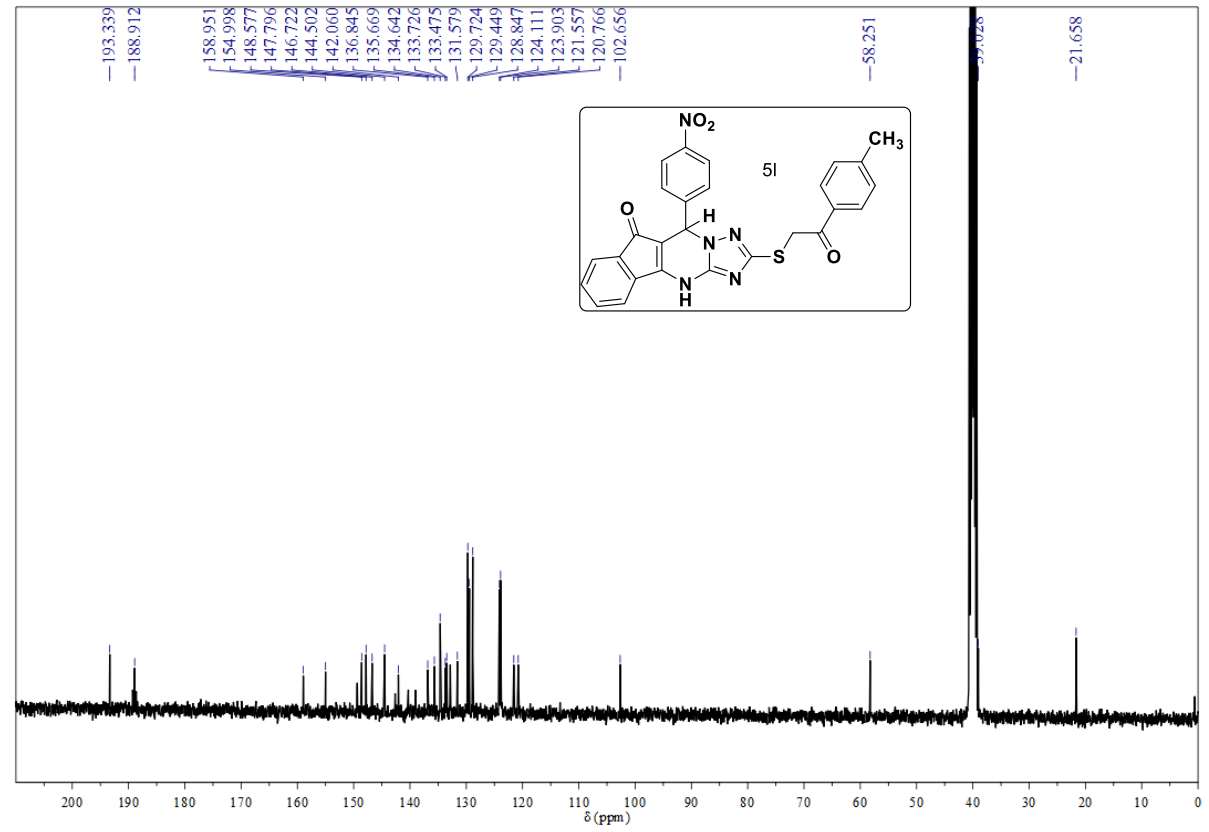
Mass spectrum of compound 5k



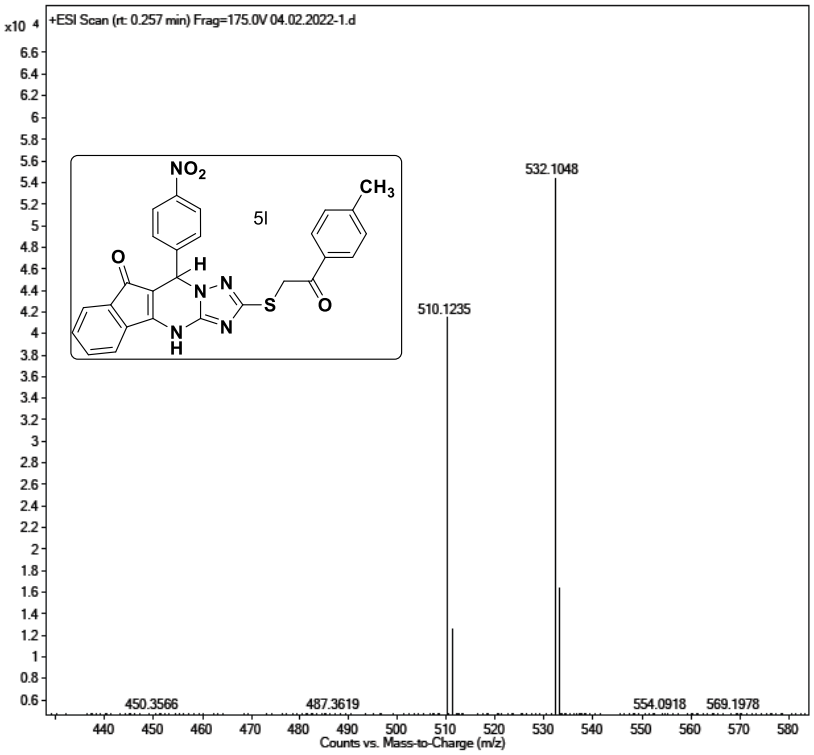
^{1}H NMR spectrum of compound 5l (DMSO- d_6) 400 MHz



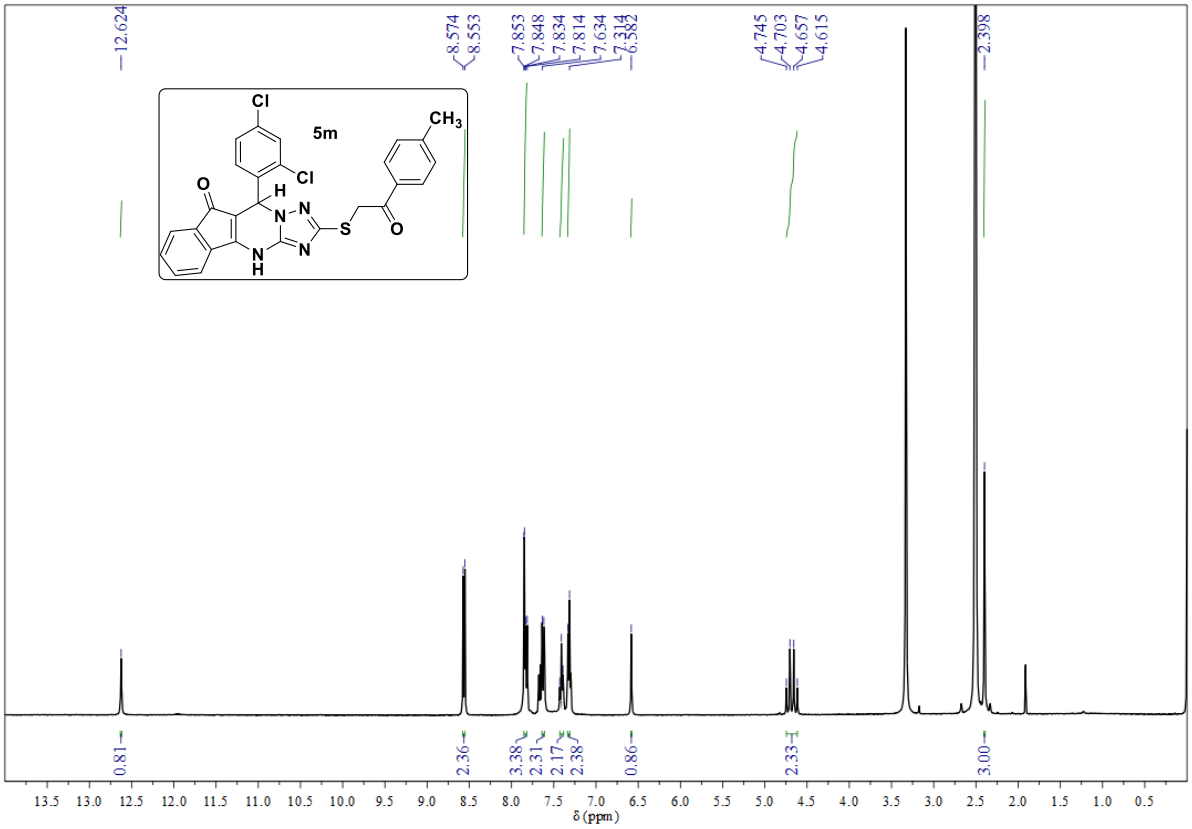
^{13}C NMR spectrum of compound 5l (DMSO- d_6) 100 MHz



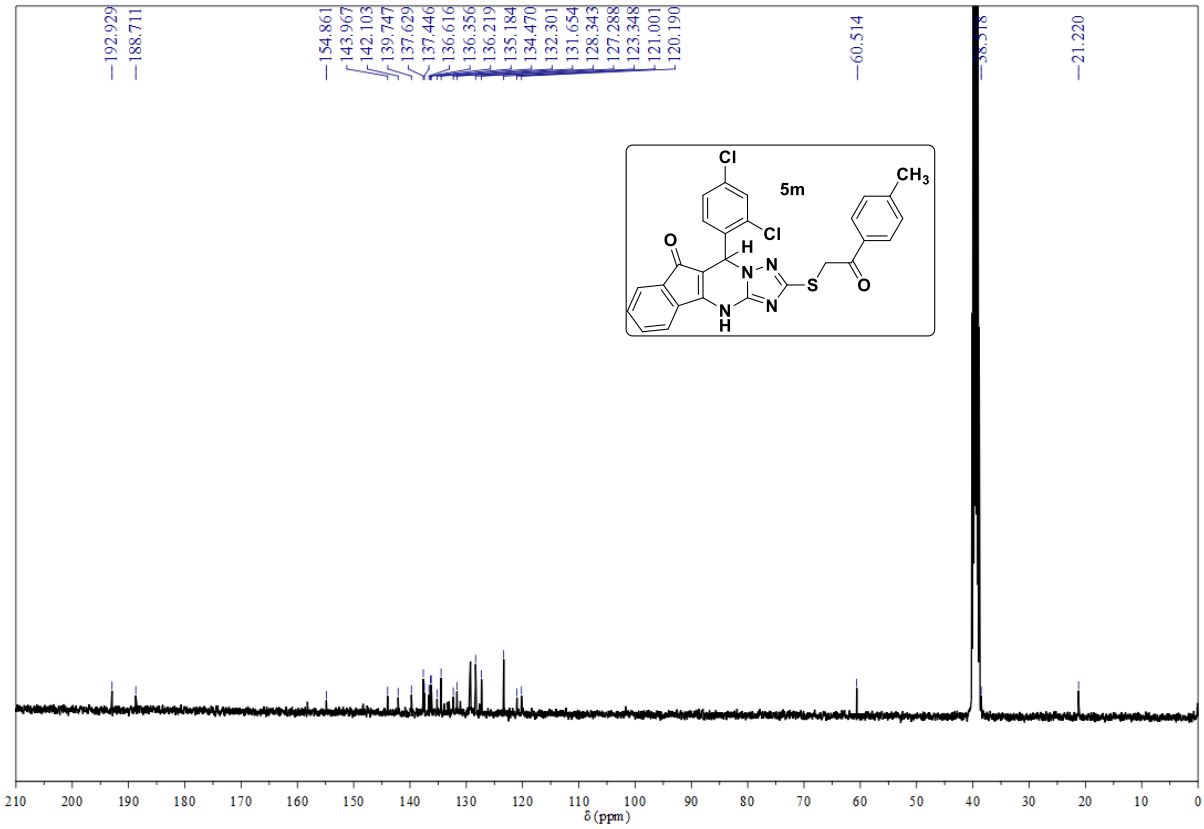
Mass spectrum of compound 5l



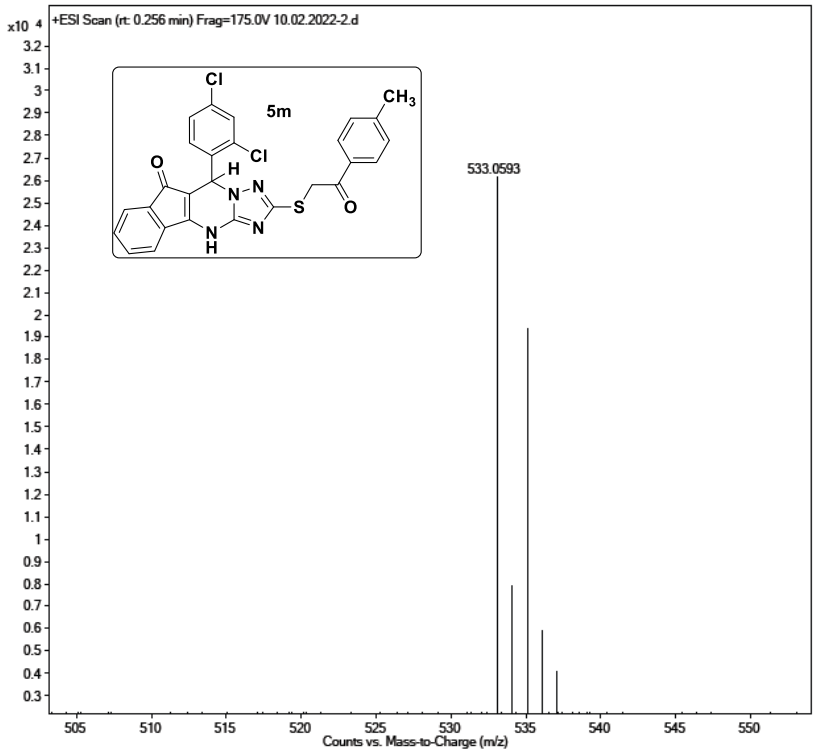
^{1}H NMR spectrum of compound 5m (DMSO- d_6) 400 MHz



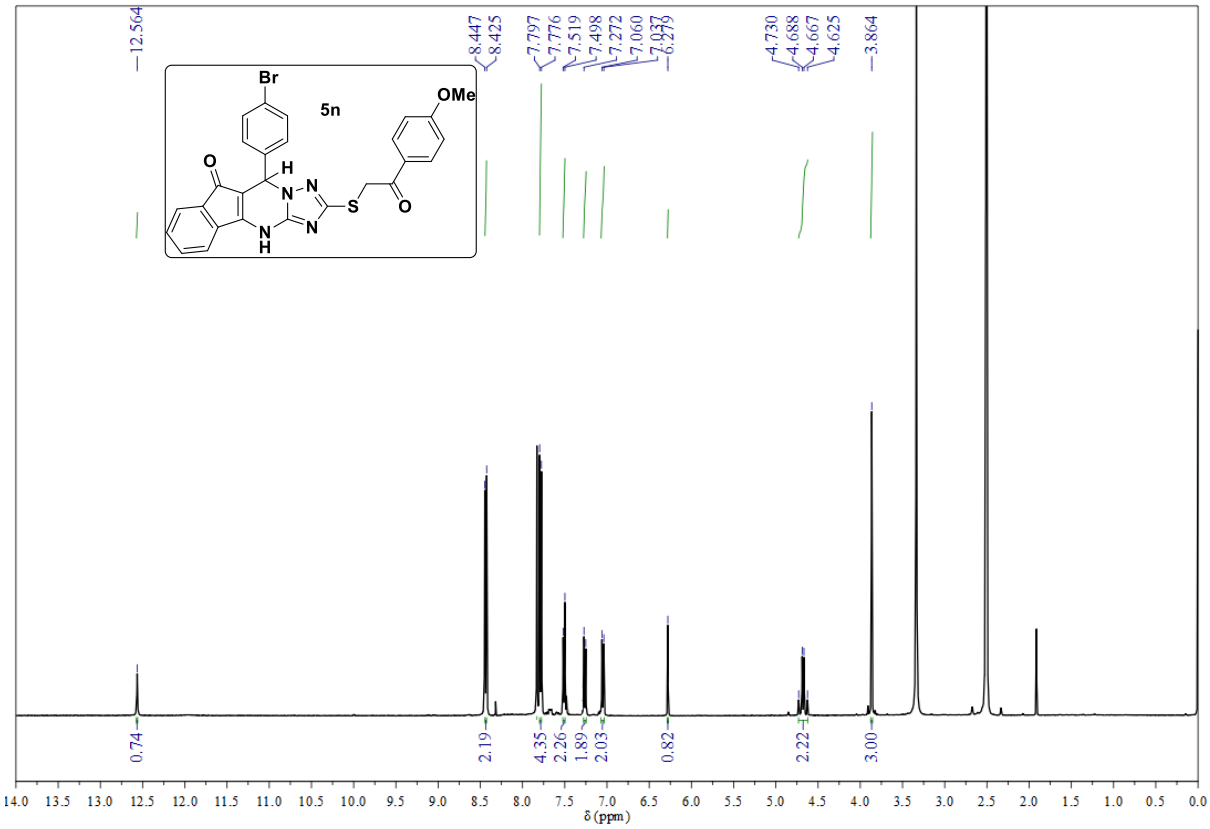
^{13}C NMR spectrum of compound 5m (DMSO- d_6) 100 MHz



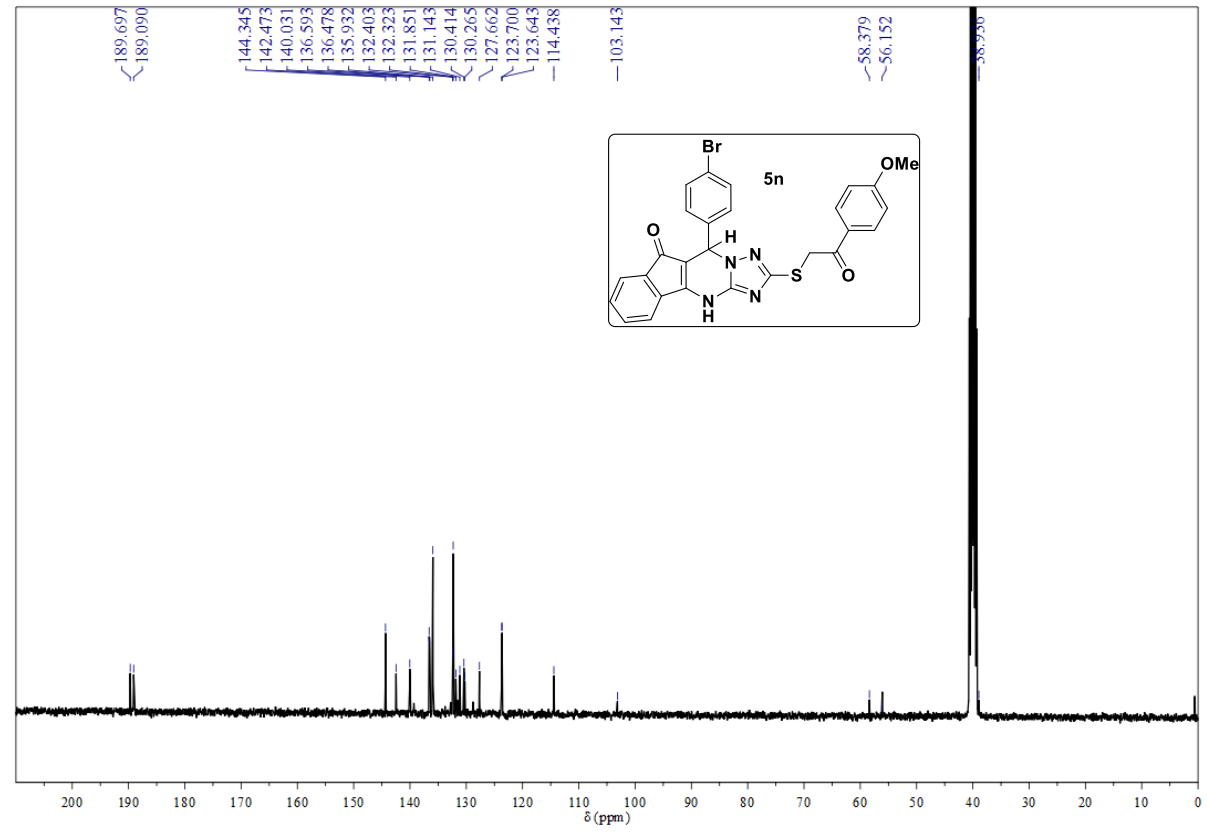
Mass spectrum of compound 5m



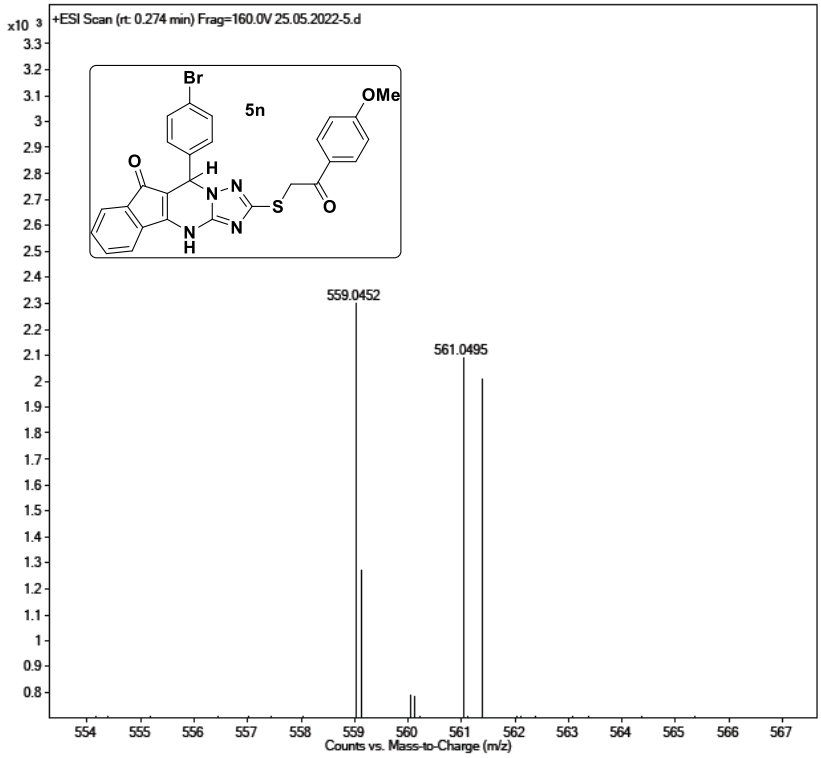
^{1}H NMR spectrum of compound 5n (DMSO- d_6) 400 MHz



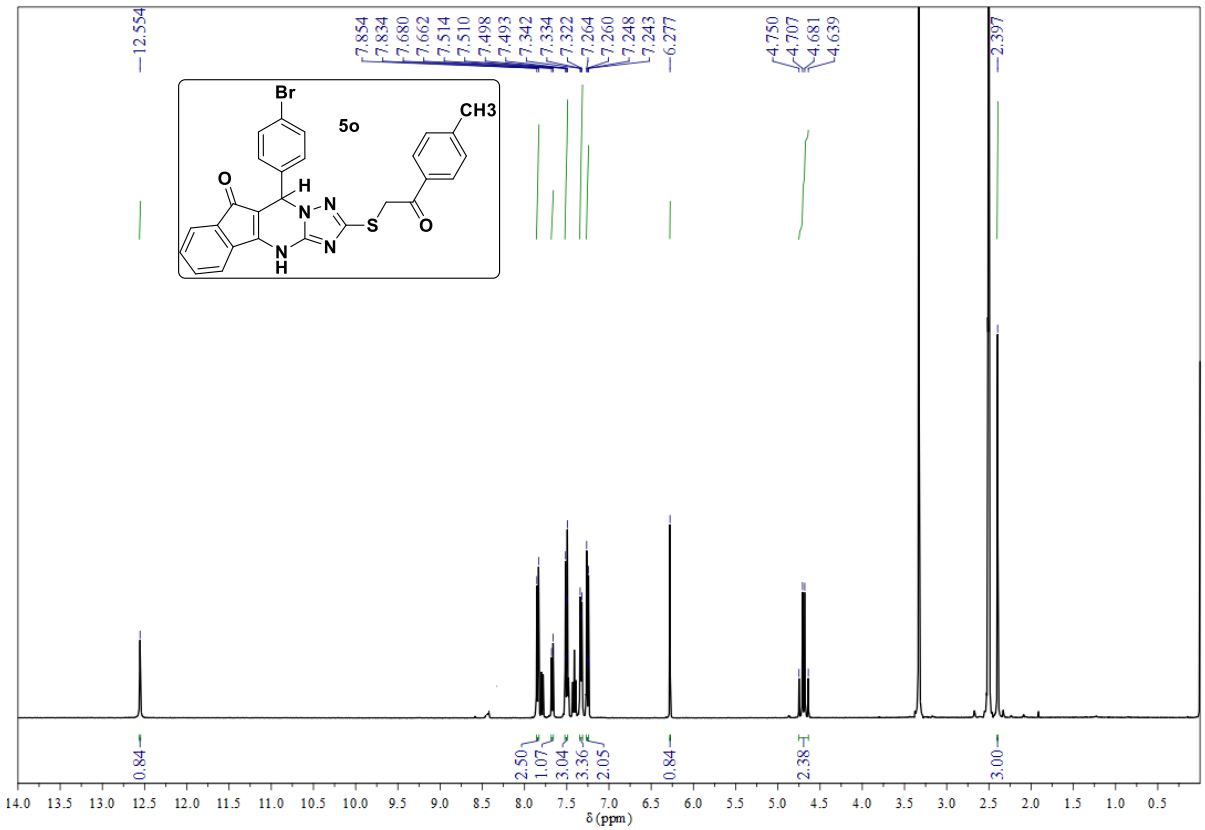
^{13}C NMR spectrum of compound 5n (DMSO- d_6) 100 MHz



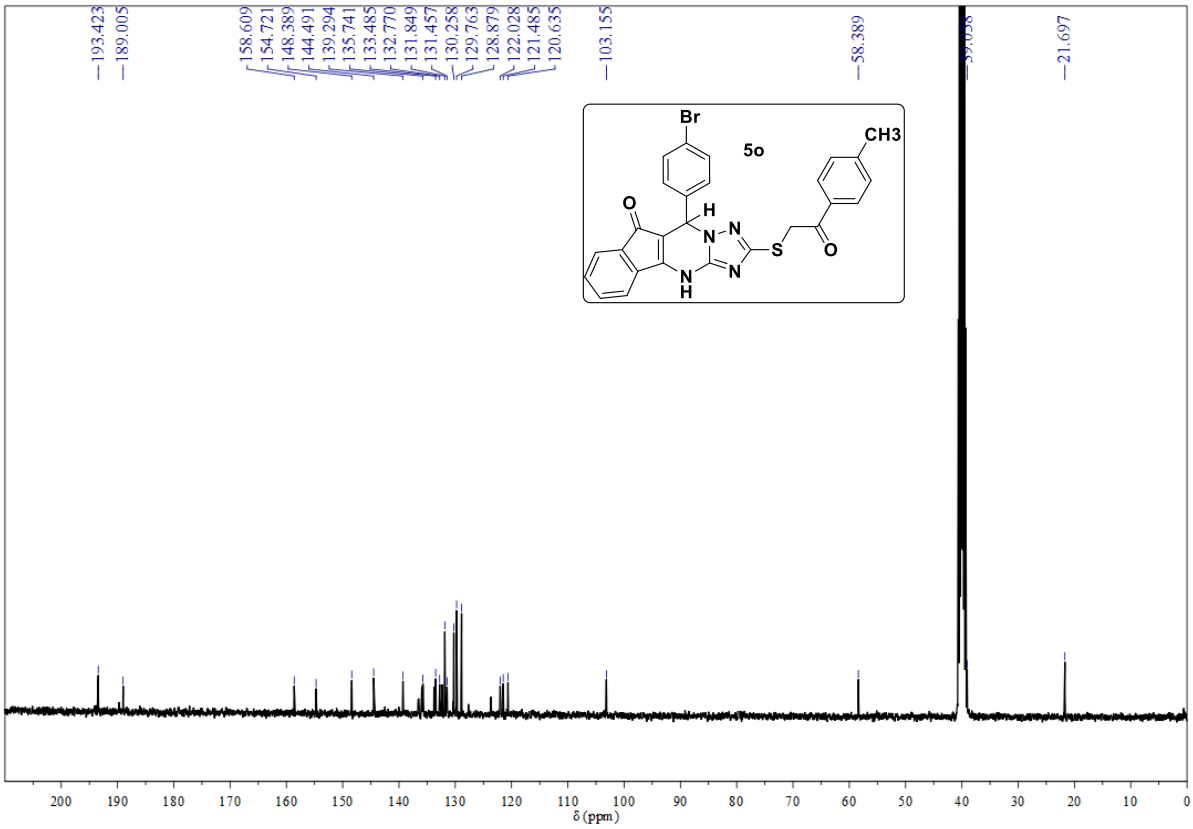
Mass spectrum of compound 5n



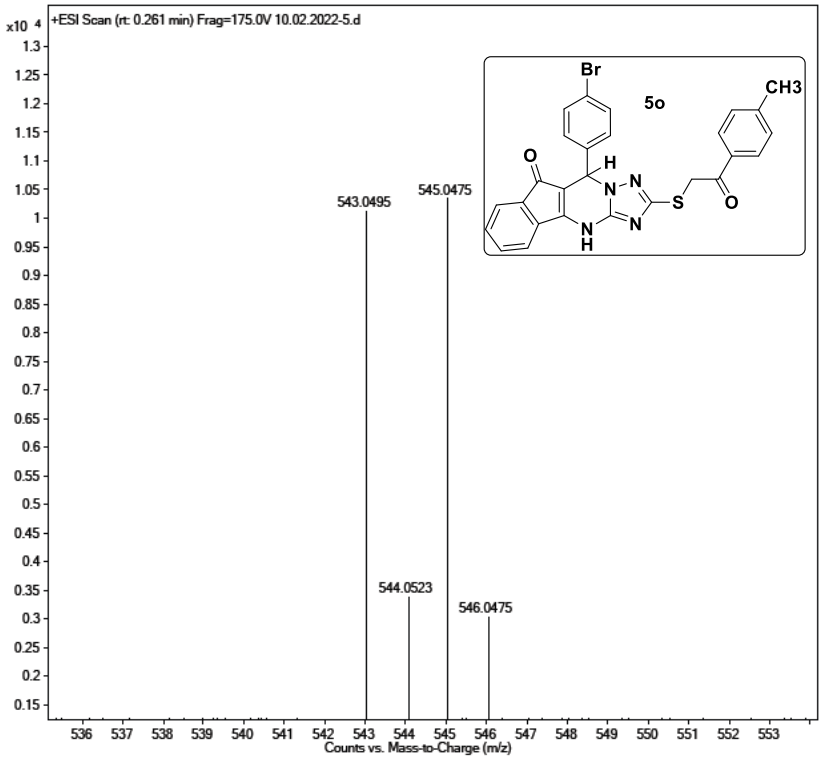
^{1}H NMR spectrum of compound 5o (DMSO- d_6) 400 MHz



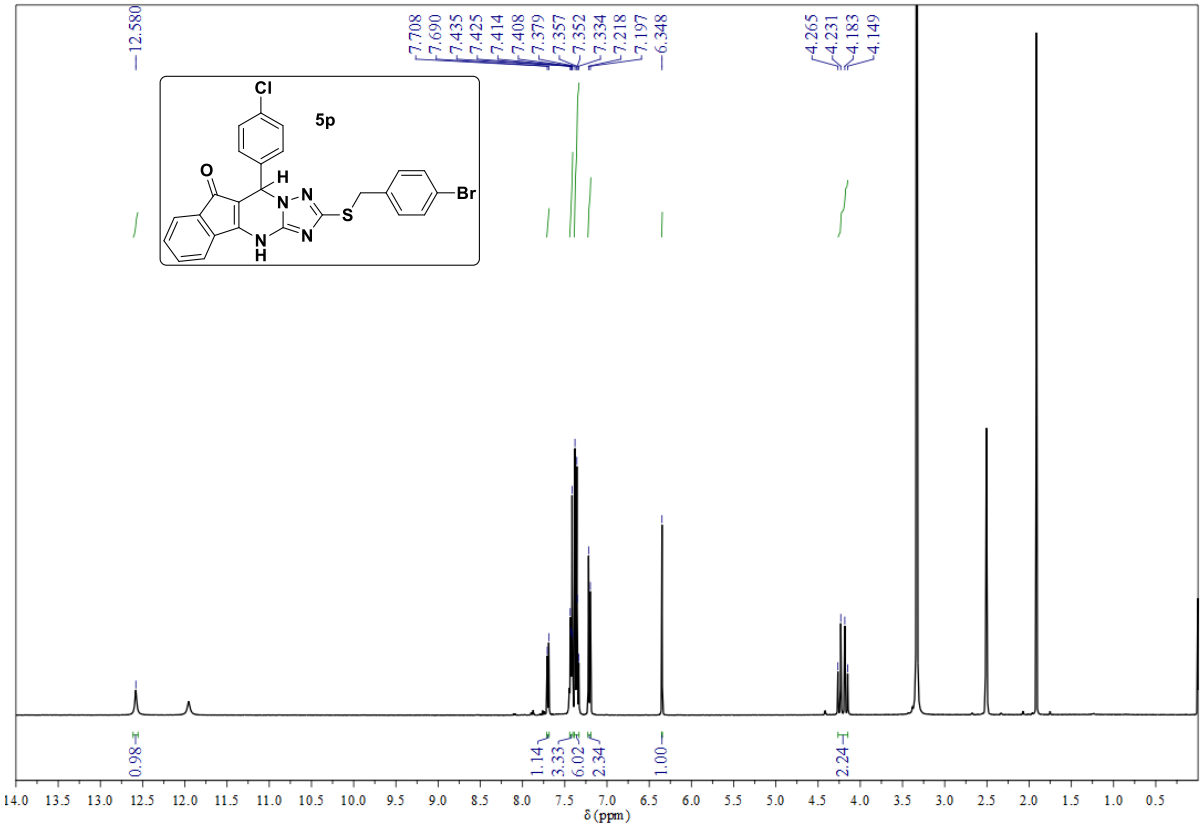
^{13}C NMR spectrum of compound 5o (DMSO- d_6) 100 MHz



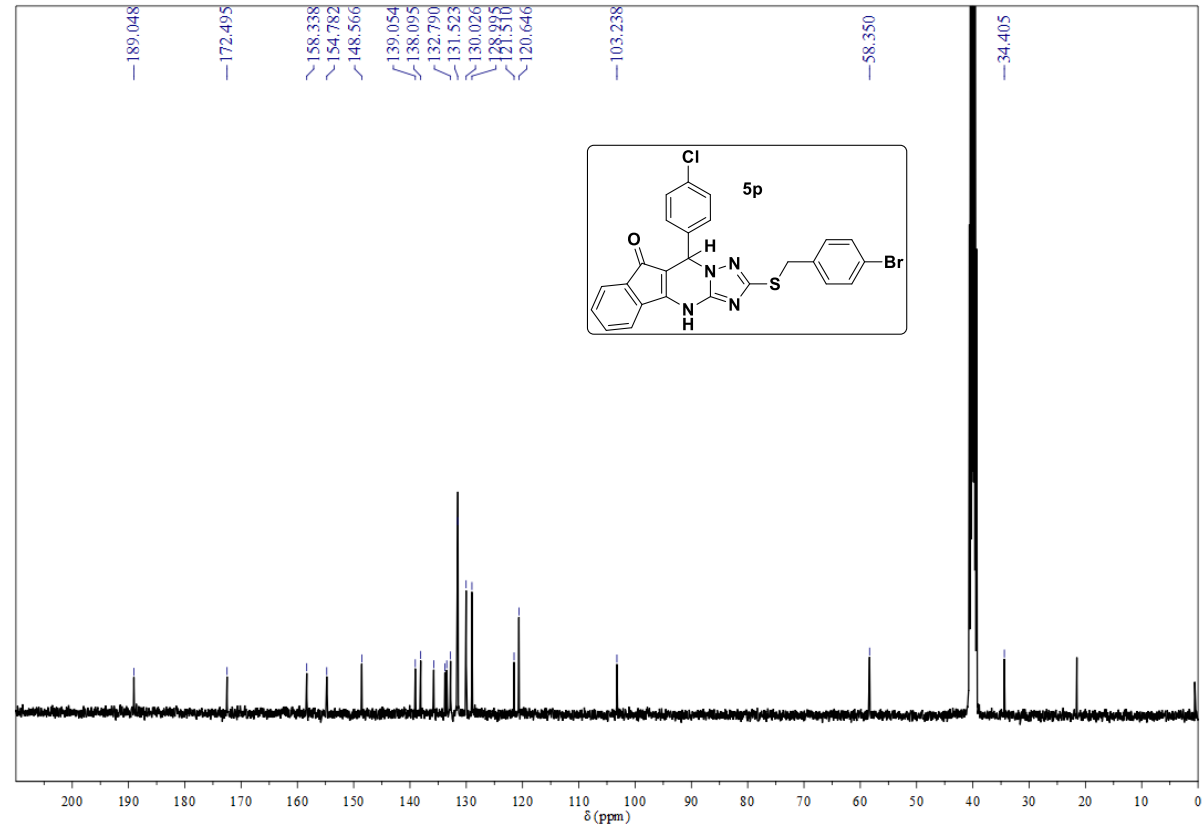
Mass spectrum of compound 5o



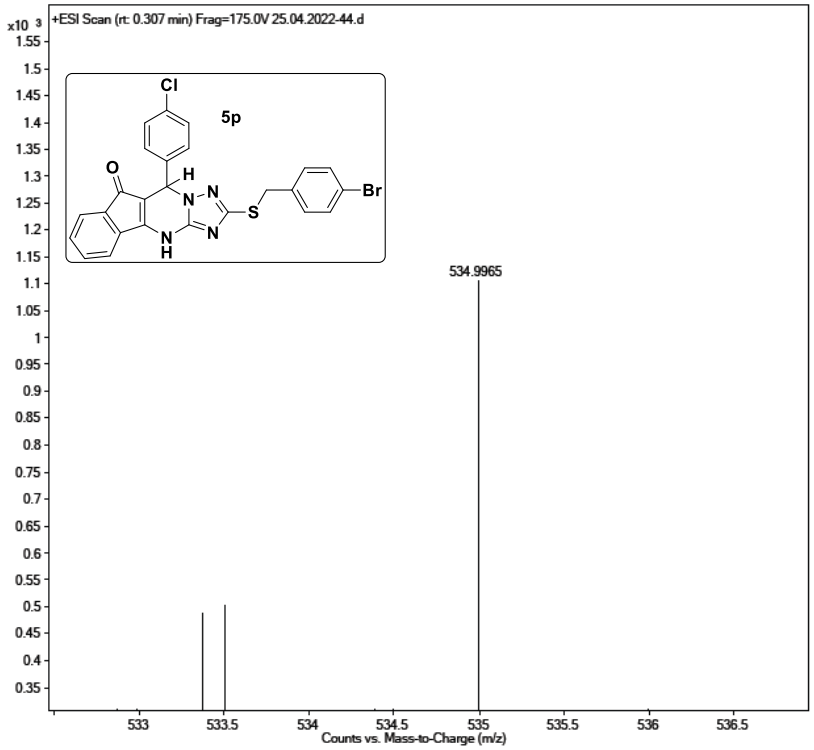
^{1}H NMR spectrum of compound 5p (DMSO- d_6) 400 MHz



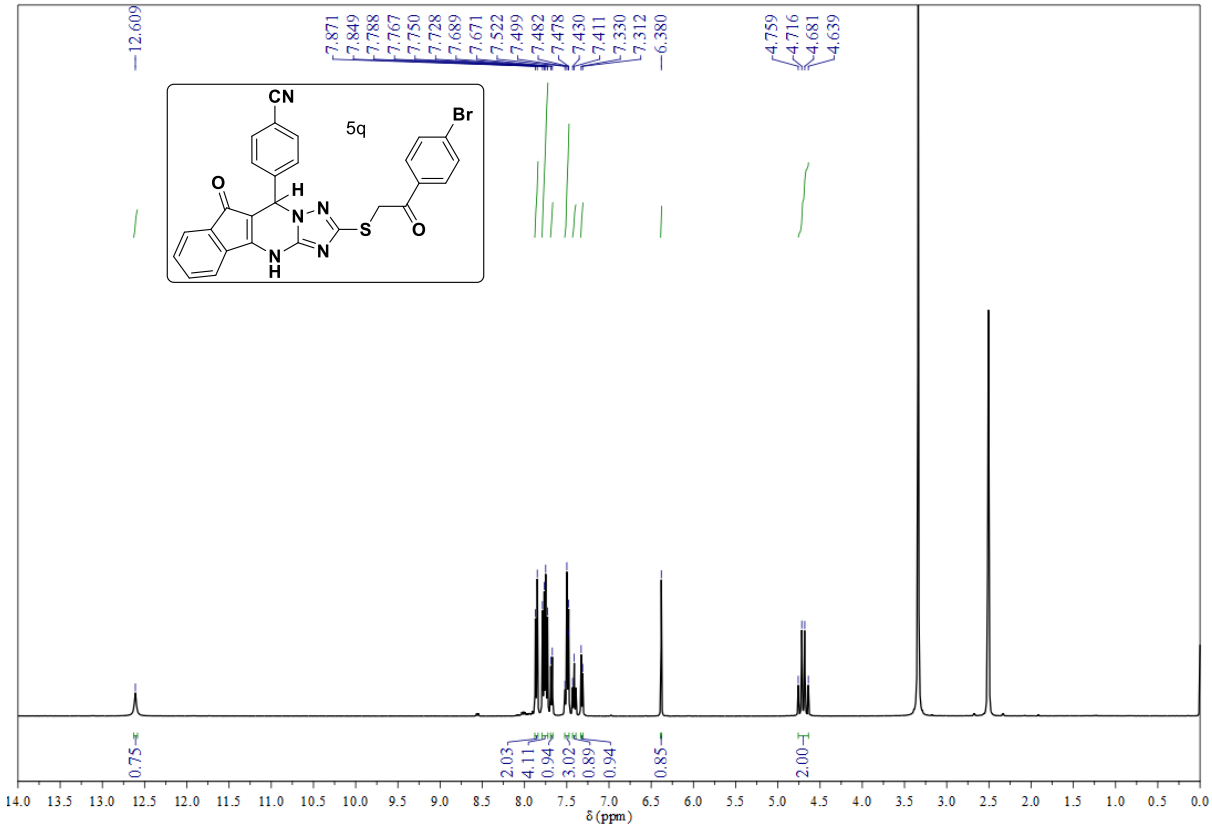
^{13}C NMR spectrum of compound 5p (DMSO- d_6) 100 MHz



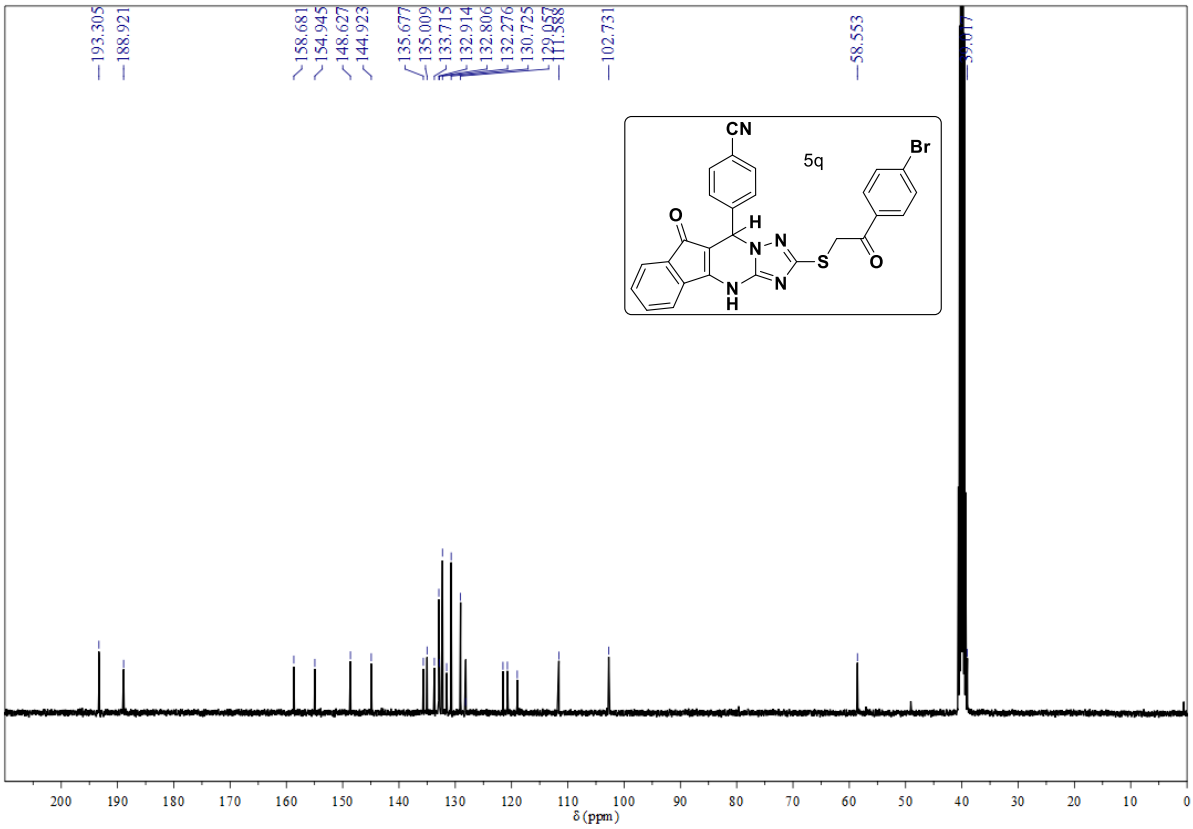
Mass spectrum of compound 5p



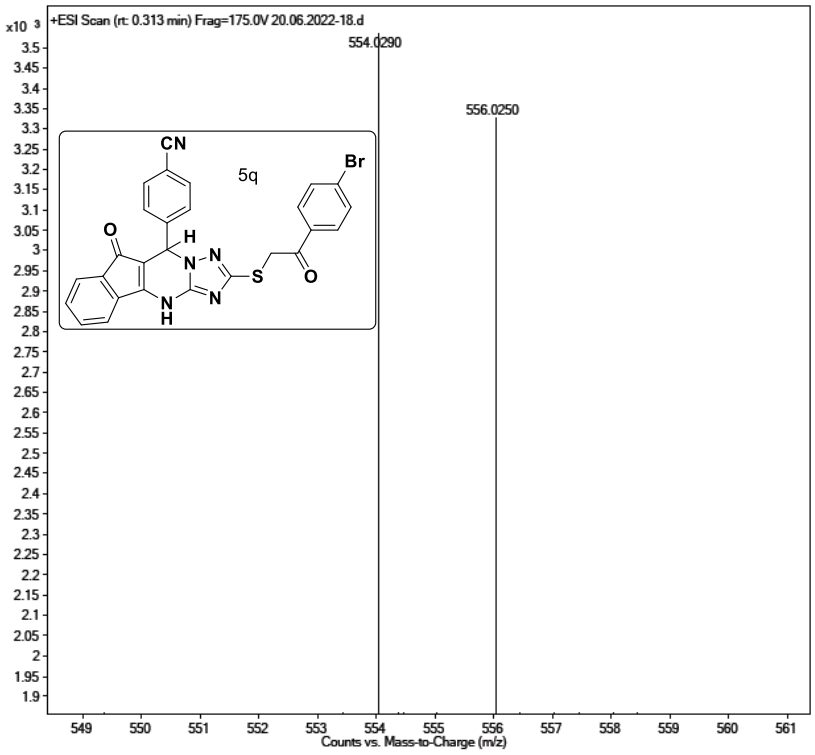
^{1}H NMR spectrum of compound 5q (DMSO- d_6) 400 MHz



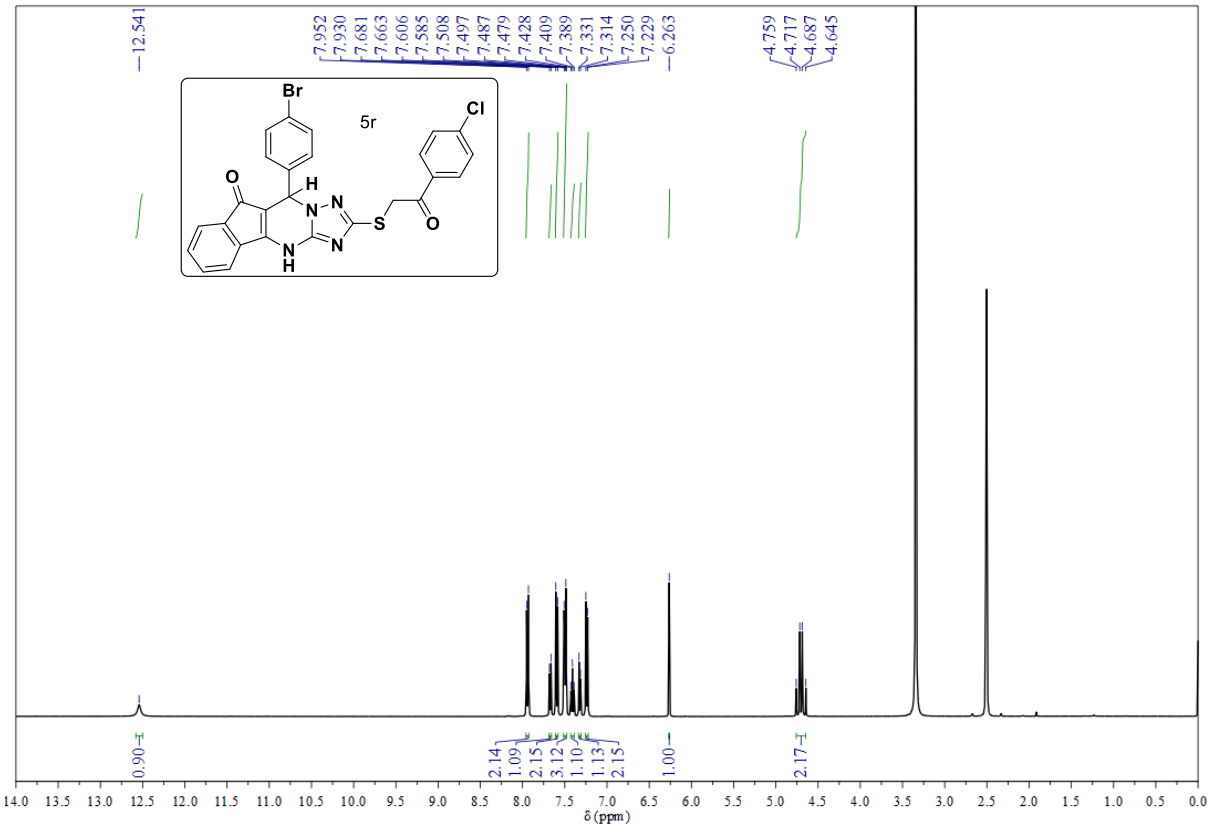
^{13}C NMR spectrum of compound 5q (DMSO- d_6) 100 MHz



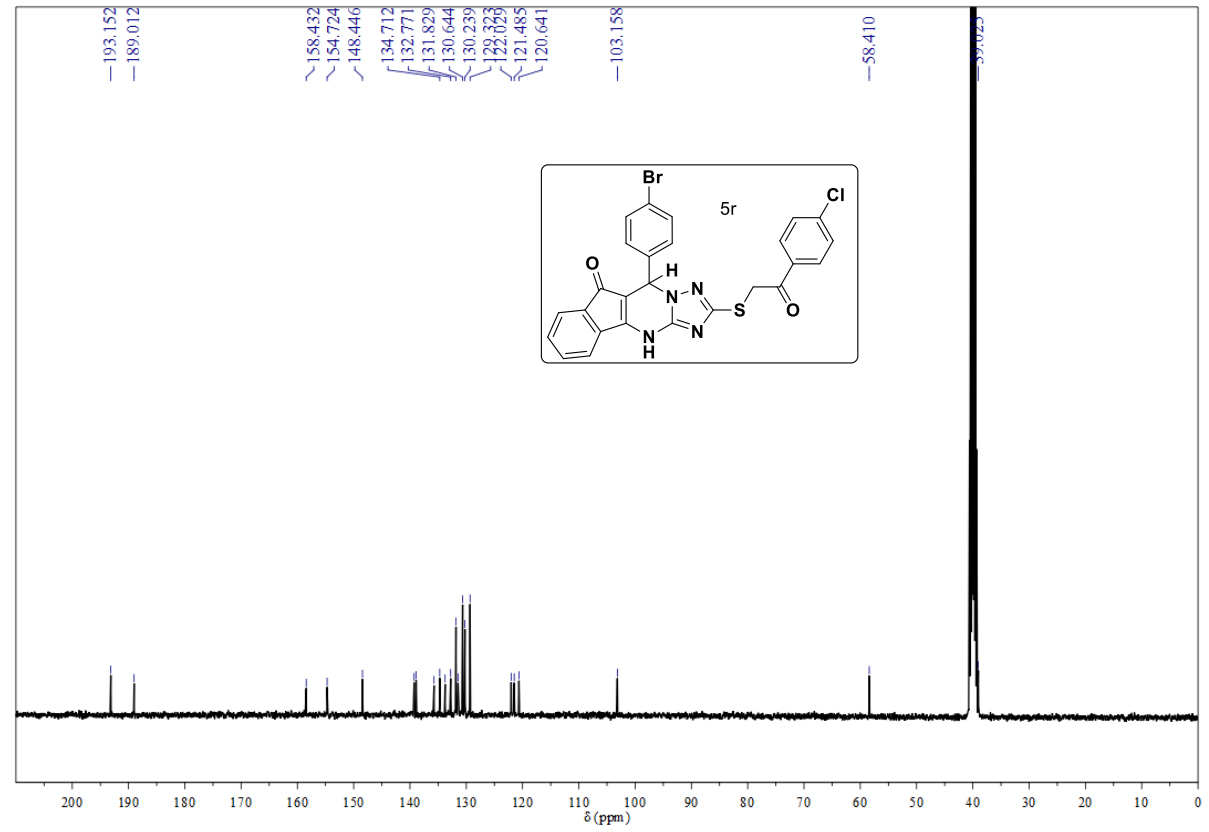
Mass spectrum of compound 5q



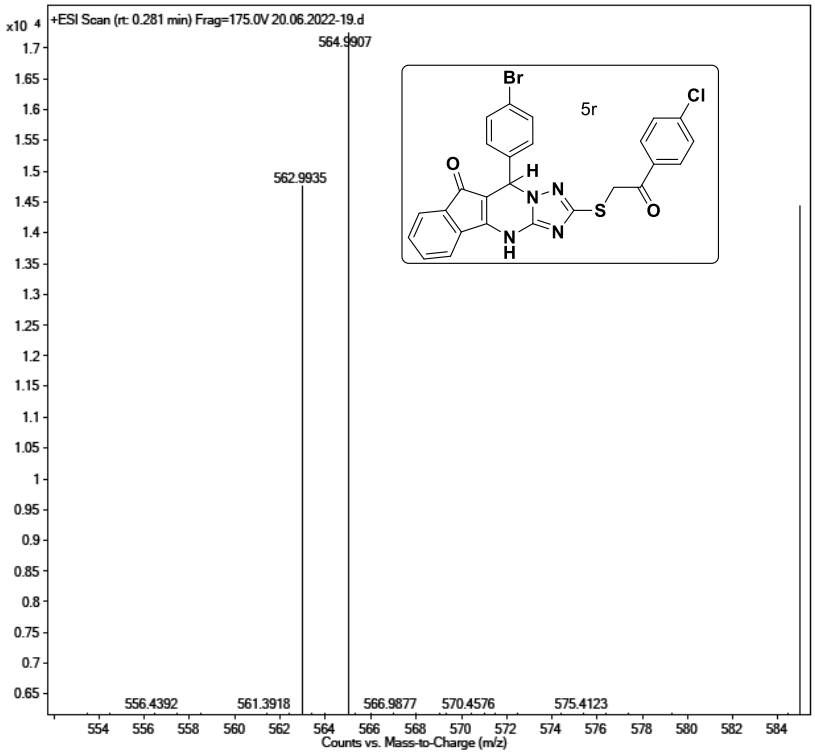
^{1}H NMR spectrum of compound 5r (DMSO- d_6) 400 MHz



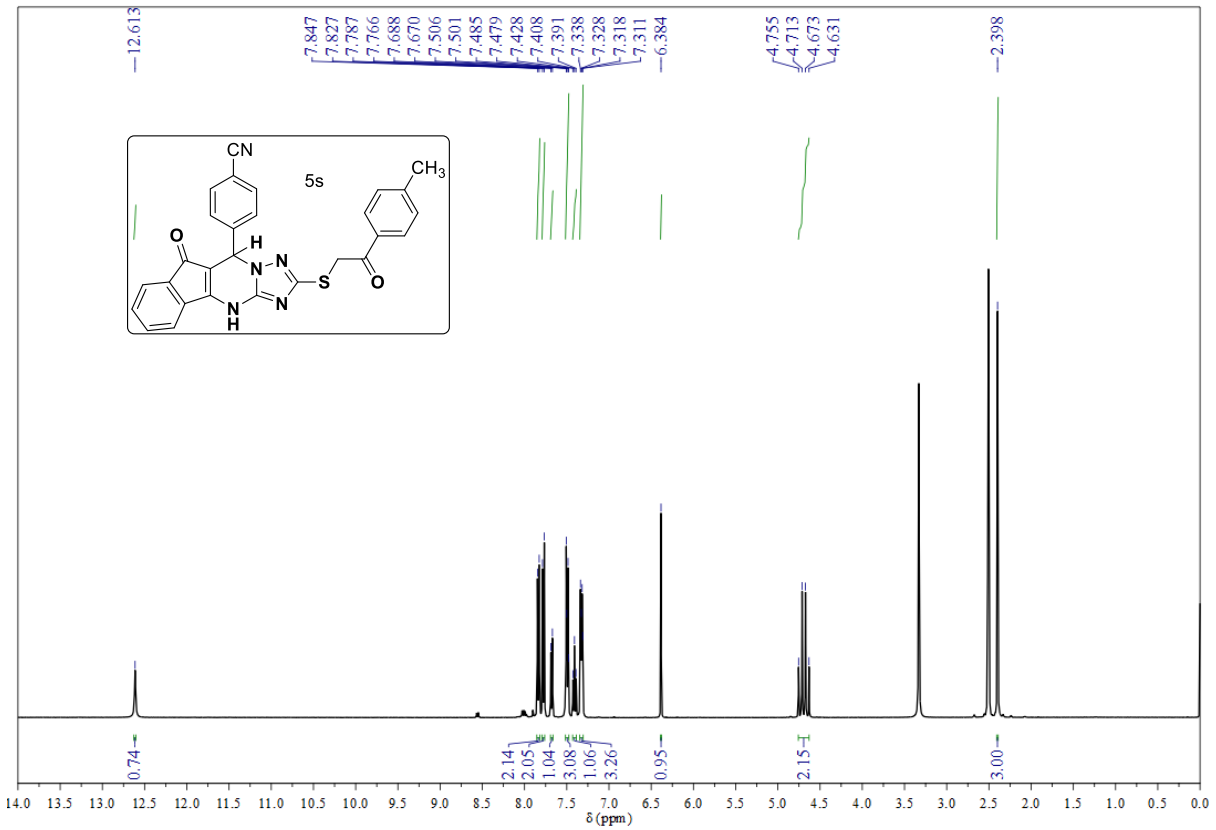
^{13}C NMR spectrum of compound 5r (DMSO- d_6) 100 MHz



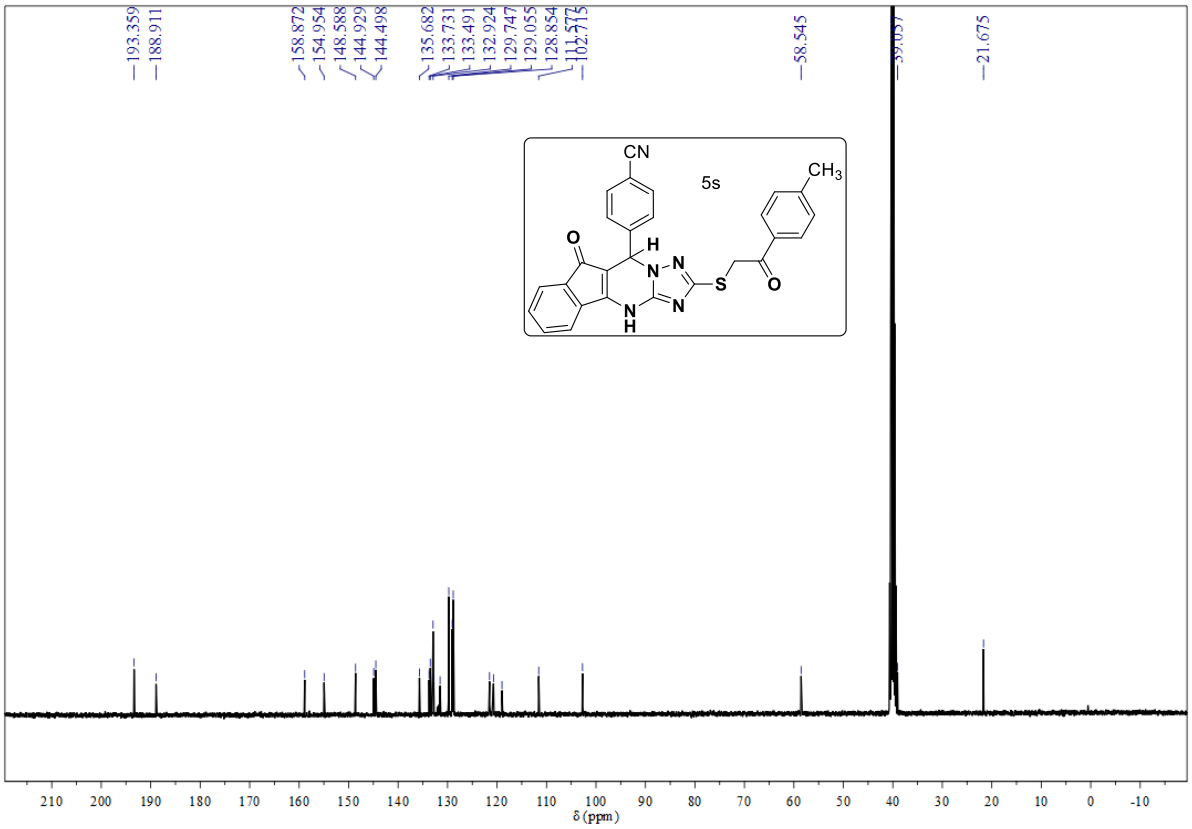
Mass spectrum of compound 5r



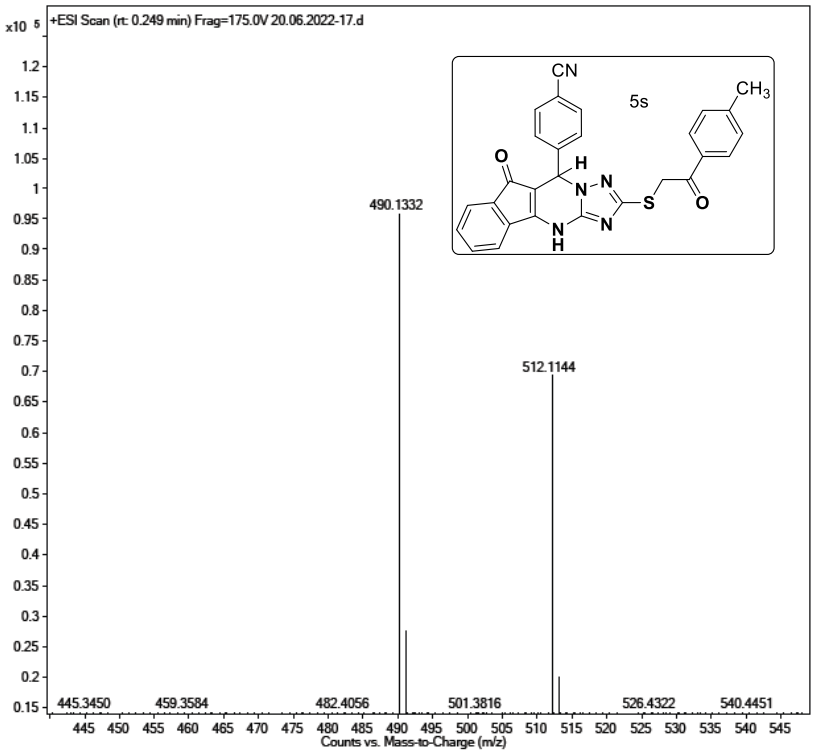
^{1}H NMR spectrum of compound 5s (DMSO- d_6) 400 MHz



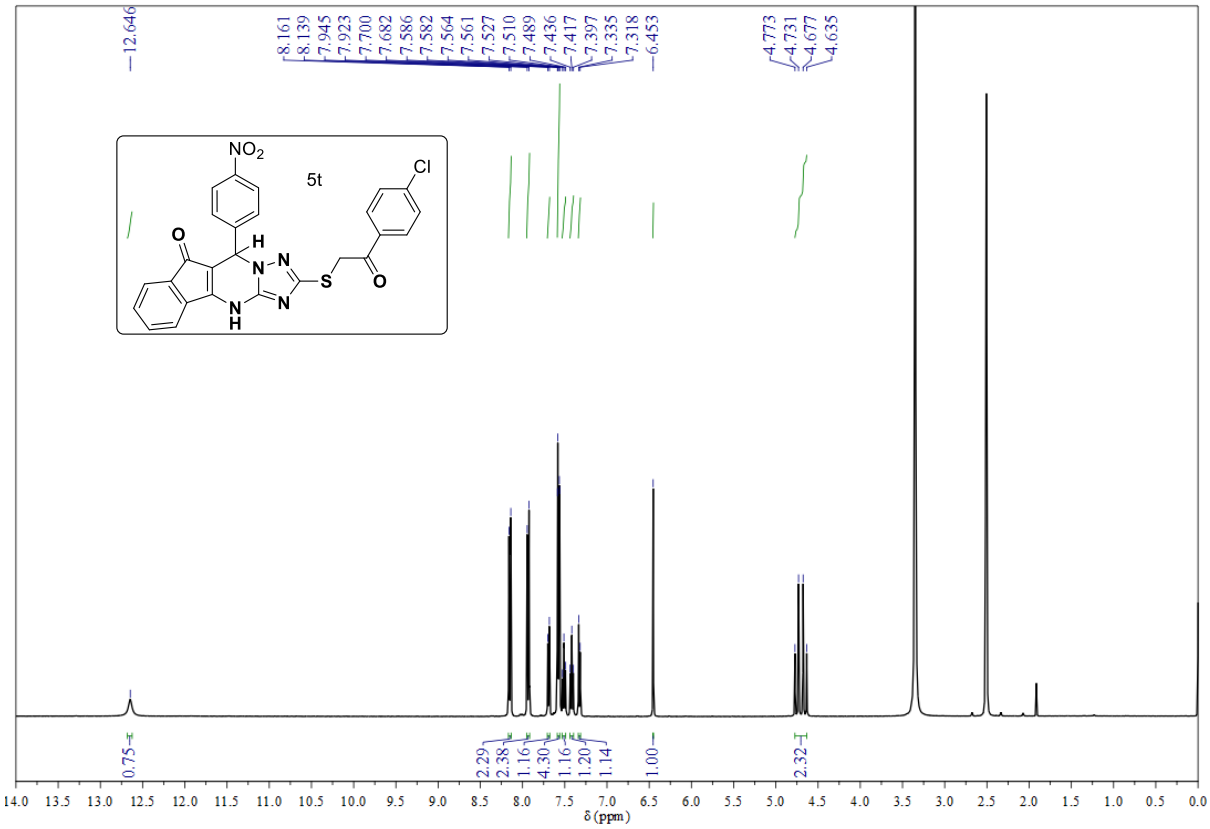
^{13}C NMR spectrum of compound 5s (DMSO- d_6) 100 MHz



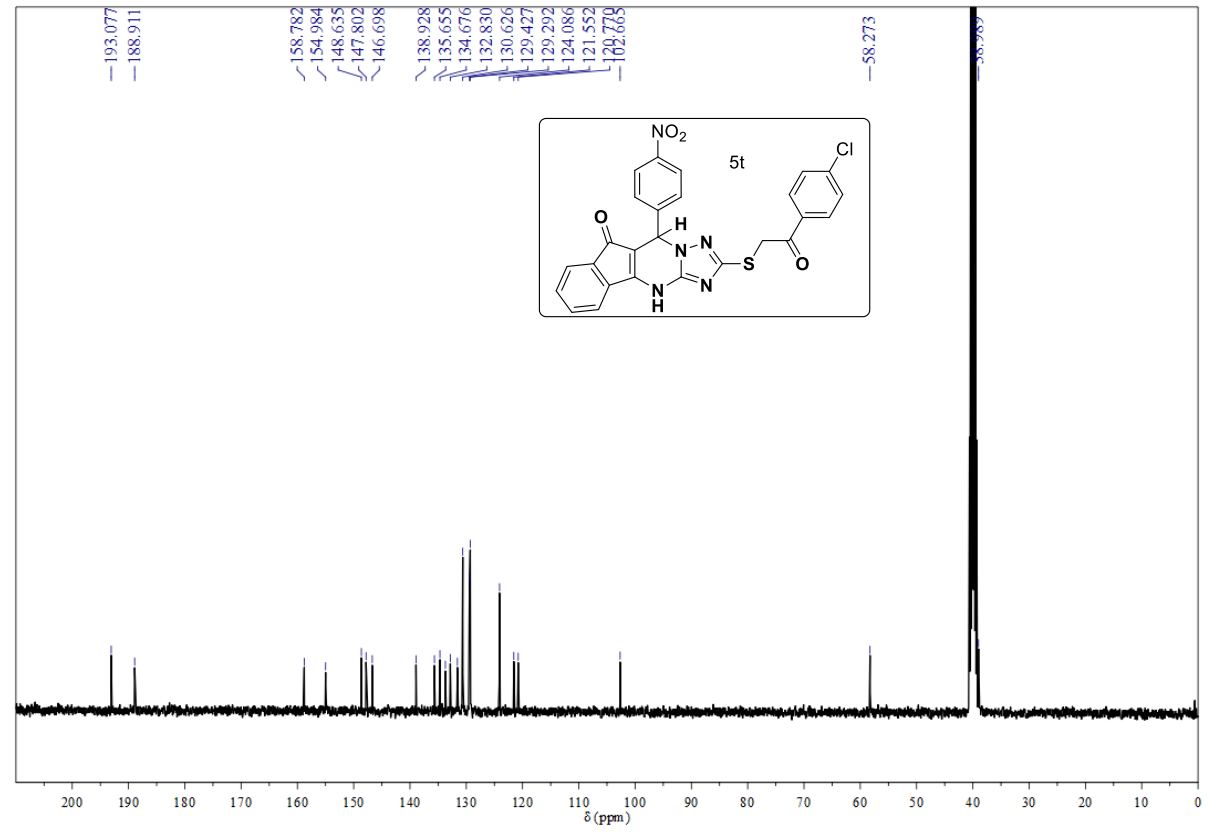
Mass spectrum of compound 5s



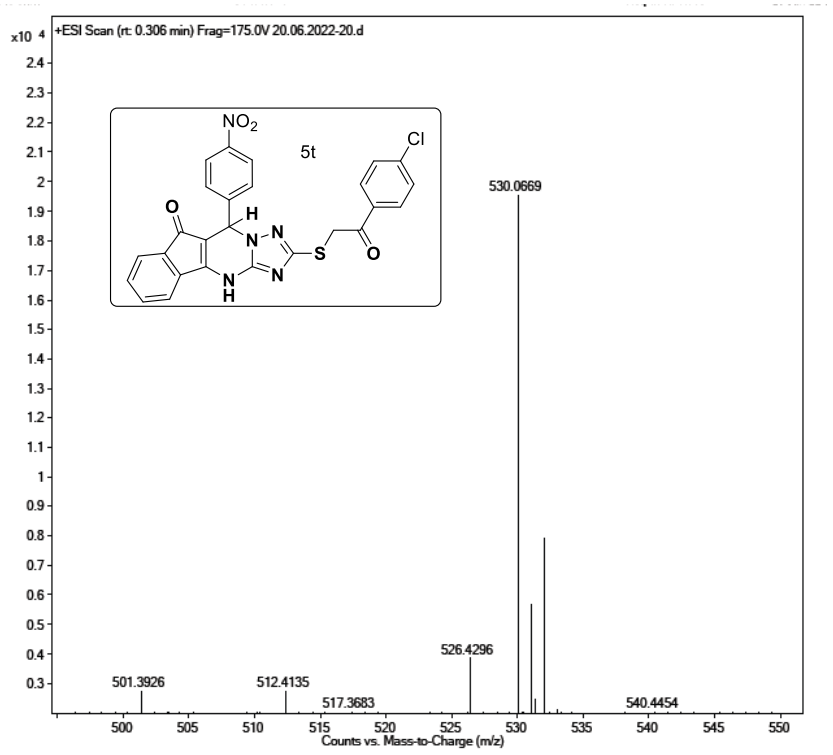
^{1}H NMR spectrum of compound 5t (DMSO- d_6) 400 MHz



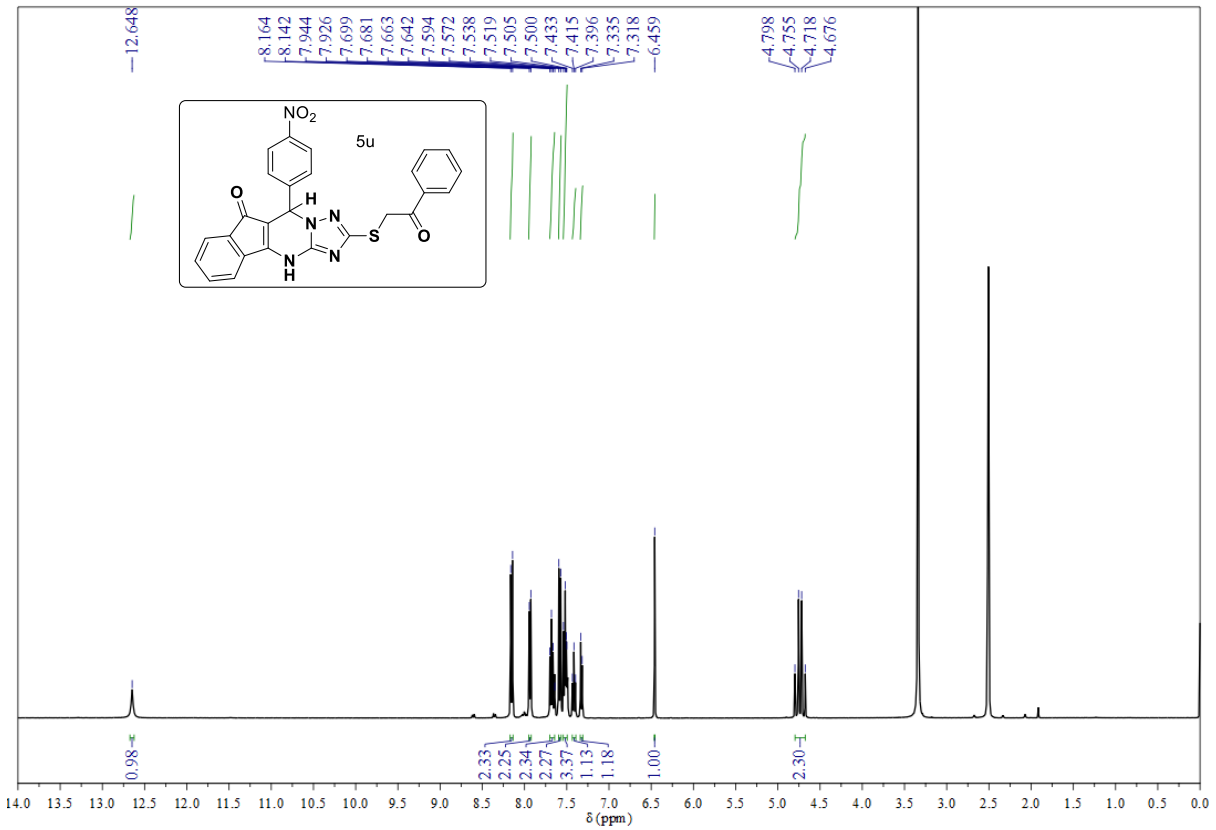
^{13}C NMR spectrum of compound 5t (DMSO- d_6) 100 MHz



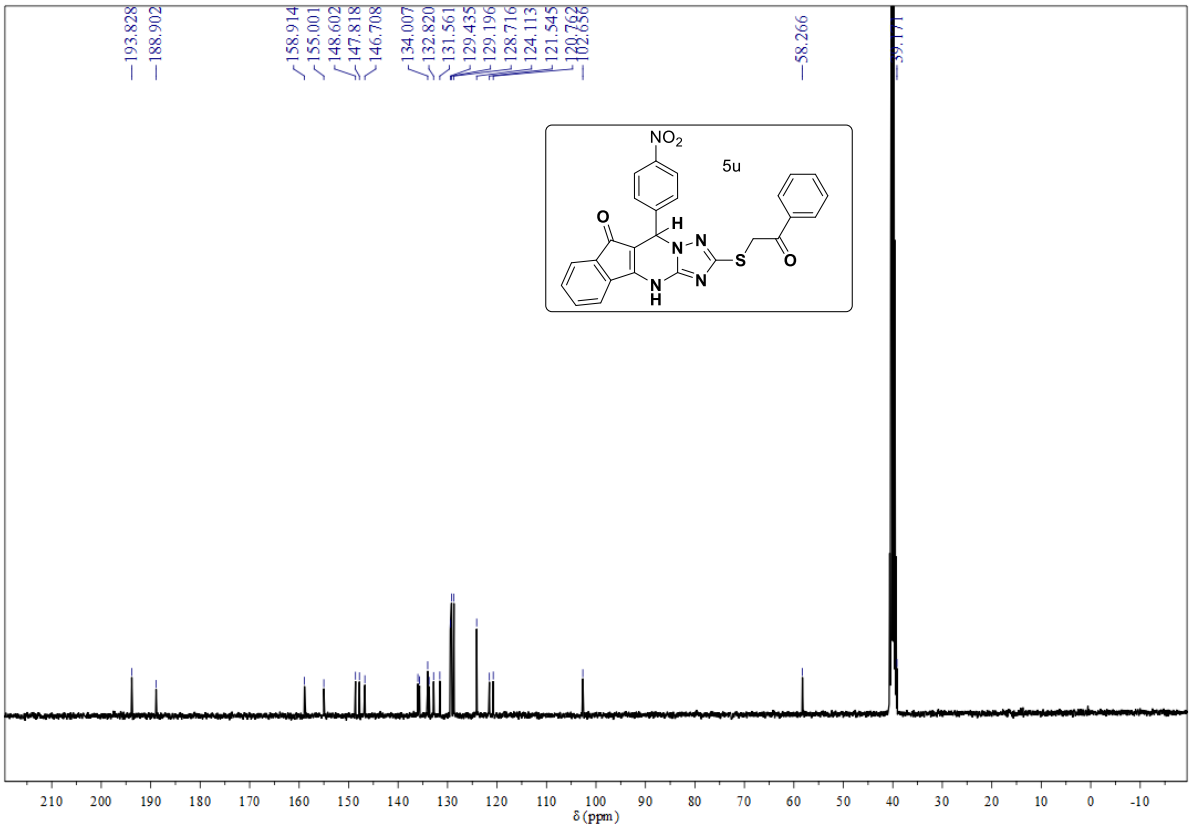
Mass spectrum of compound 5t



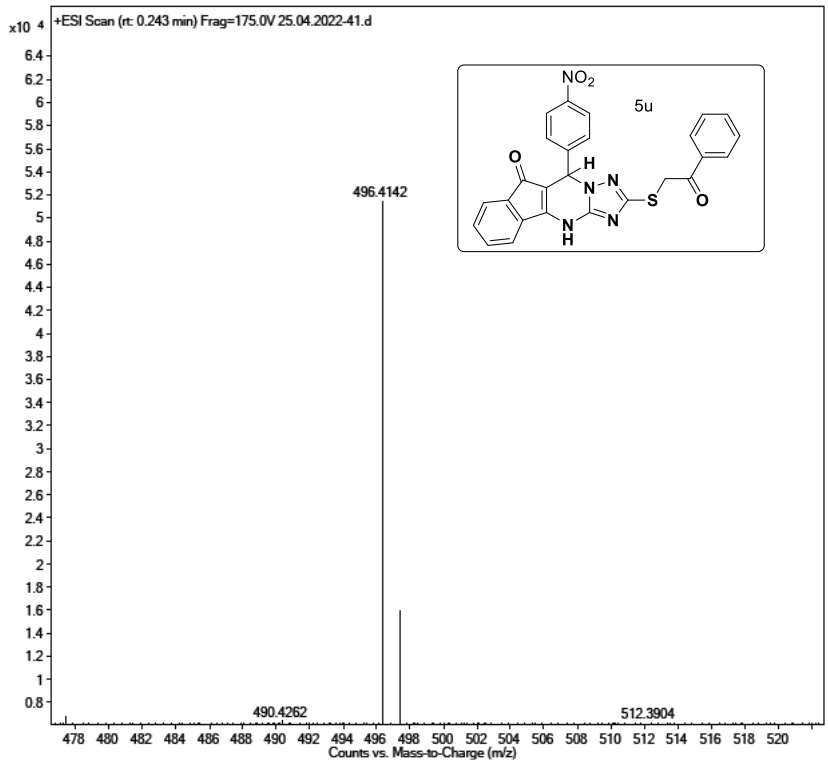
^{1}H NMR spectrum of compound 5u (DMSO- d_6) 400 MHz



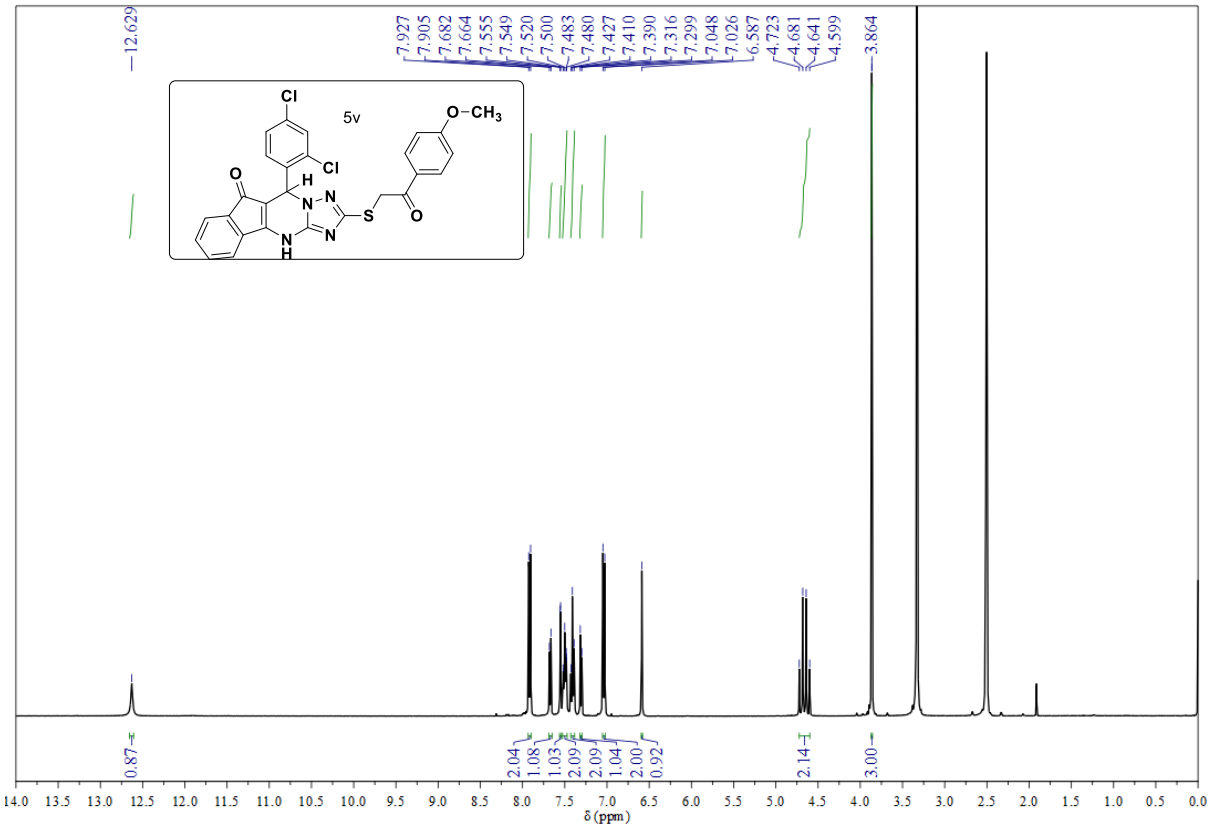
^{13}C NMR spectrum of compound 5u (DMSO- d_6) 100 MHz



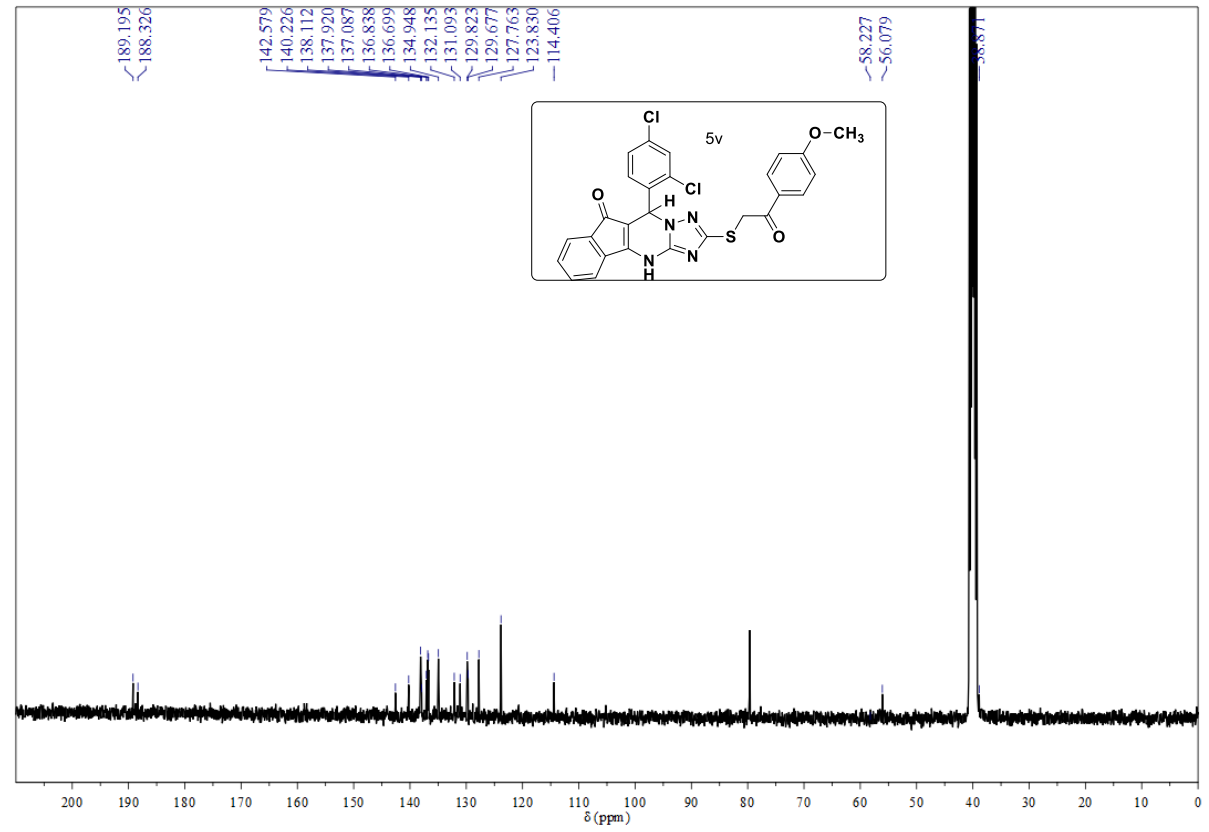
Mass spectrum of compound 5u



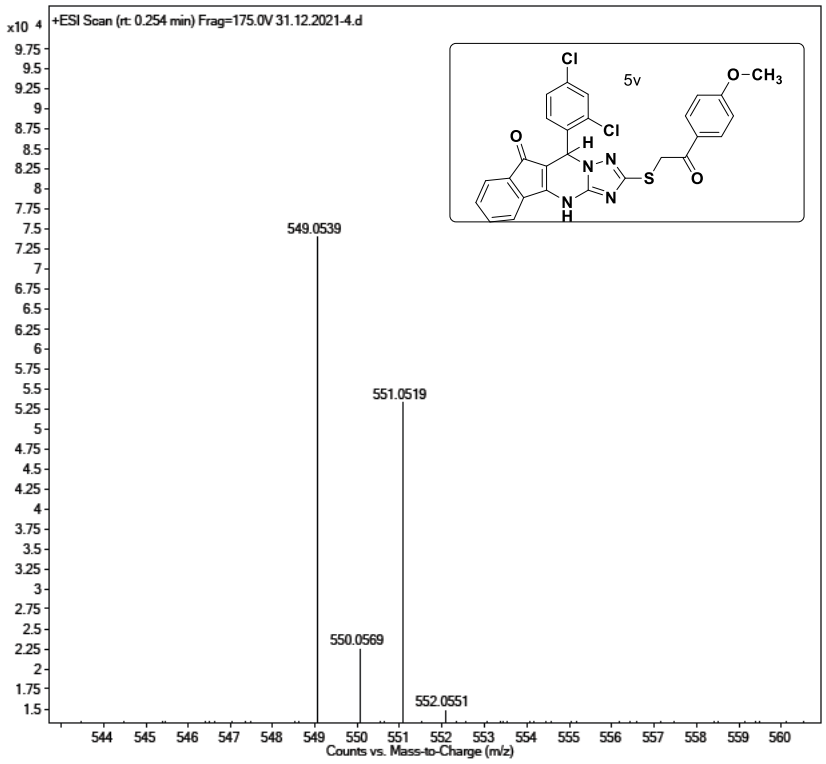
^{1}H NMR spectrum of compound 5v (DMSO- d_6) 400 MHz



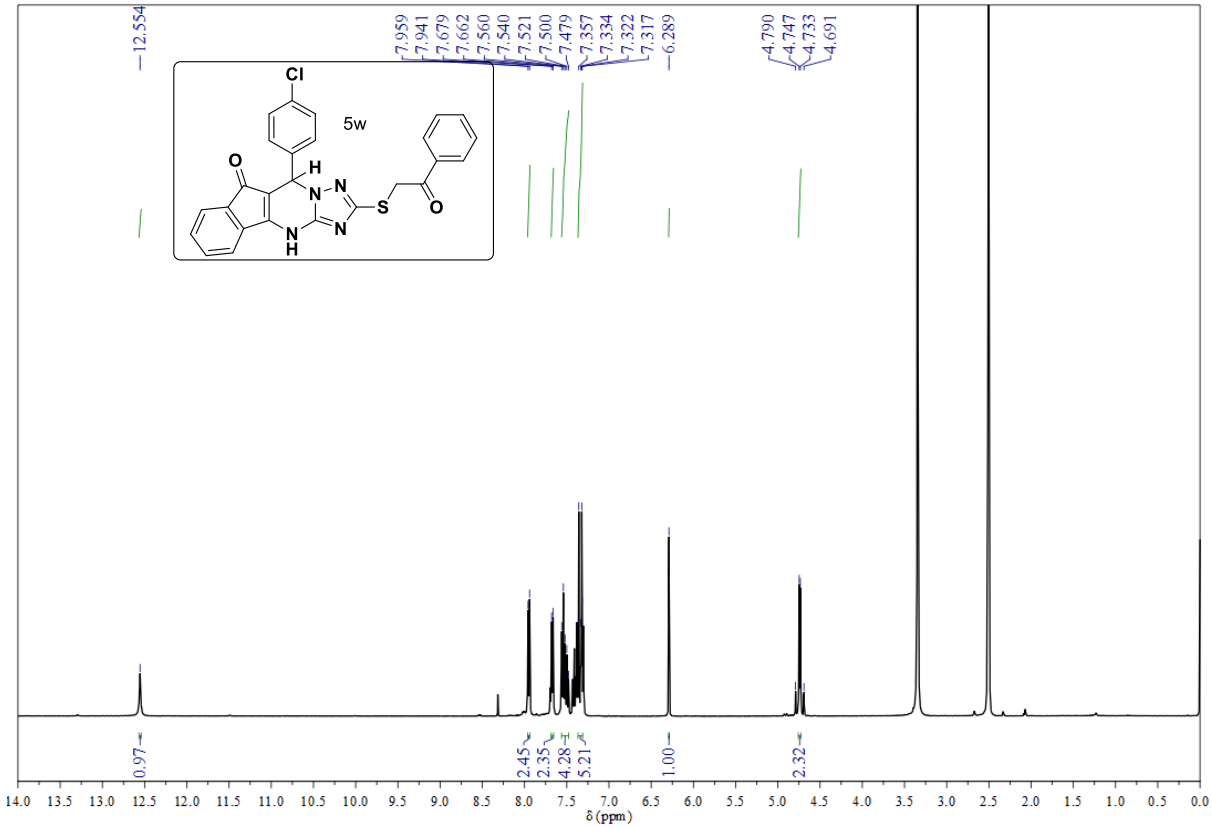
^{13}C NMR spectrum of compound 5v (DMSO- d_6) 100 MHz



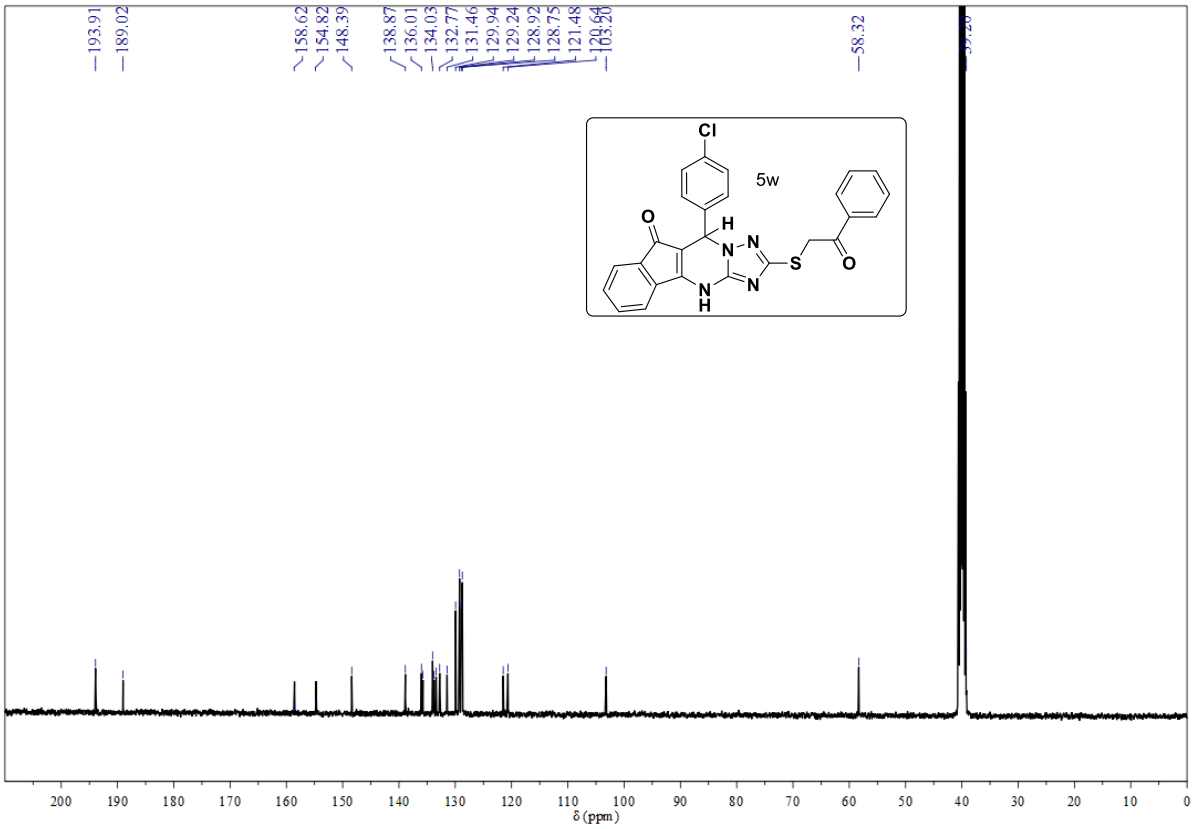
Mass spectra of compound 5v



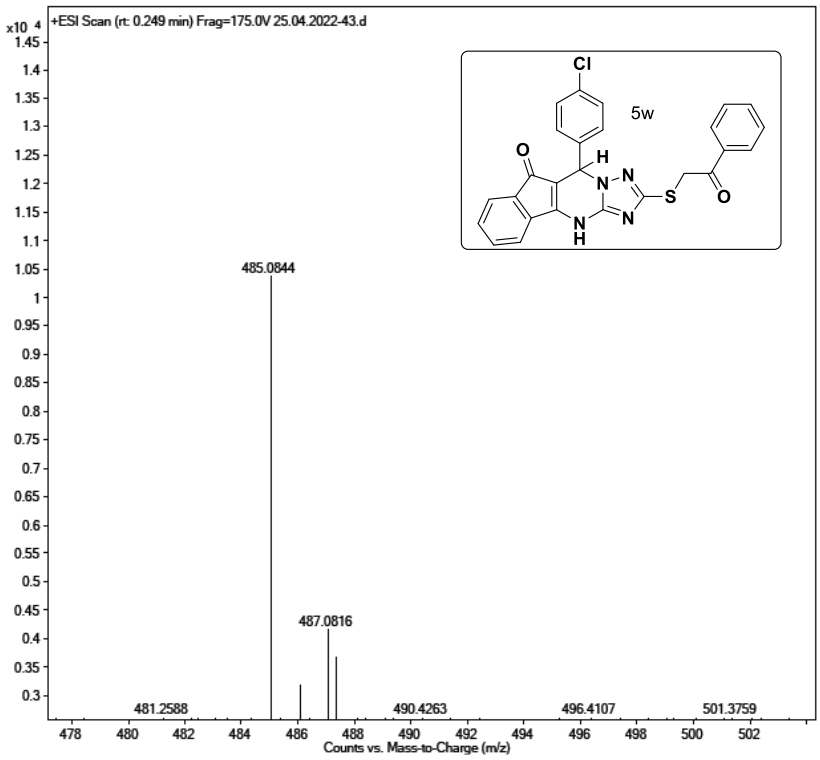
^{1}H NMR spectrum of compound 5w (DMSO- d_6) 400 MHz



^{13}C NMR spectrum of compound 5w (DMSO- d_6) 100 MHz



Mass spectrum of compound 5w



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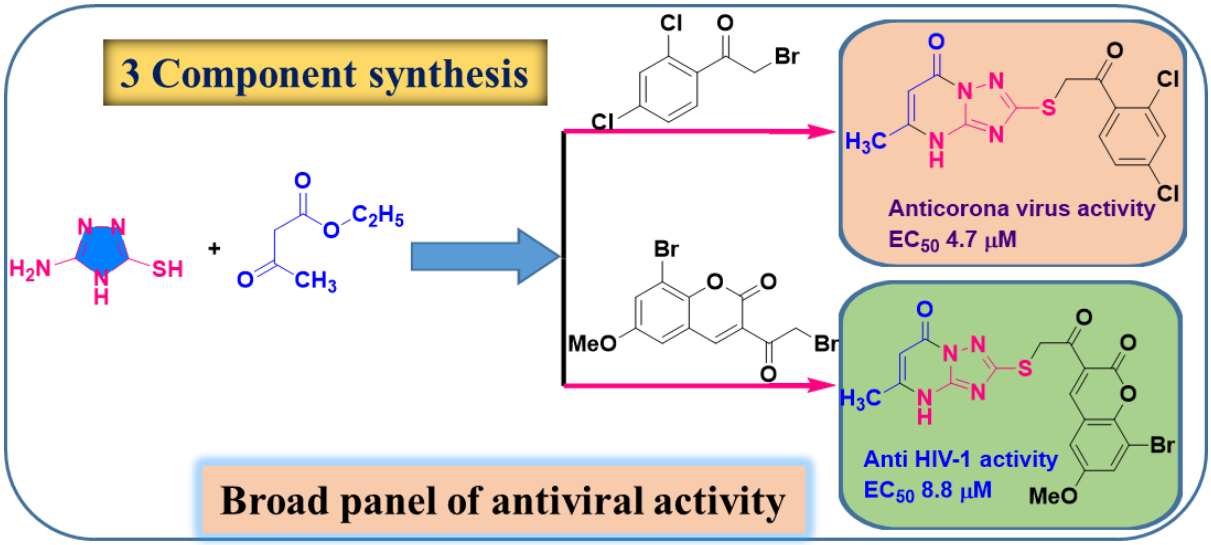
CHAPTER-V

SECTION-B

Synthesis of novel thioalkylated triazolopyrimidinones, sulfones and their biological activity



5B.1 Introduction

Over the past few years we have seen variety of viral diseases in people throughout the world including Ebola virus in Africa [1], Zikavirus spread in America [2] and recently COVID-2019 pandemic[3]. The prevention of antiviral infections is a major challenge in research because the ability of the viruses to mutate within the genome of existing one [4]. Namely the viruses are two types DNA and RNA viruses. Herpus simplex virus, Small pox virus, Adenovirus falls under DNA and coronavirus, HIV, Zika virus, Ebola virus, RSV-1 has RNA containing viruses [5-6]. Evaluation of antiviral resistance has led to continued interest in design of innovative antiviral medications.

Two heterocyclic rings within the molecule such as five membered triazole comprised with six membered pyrimidine ring containing compounds are attracted great attention in synthetic point of view and also these were shows significance biological activities then unfused heterocyclic moieties [7-10]. Triazolo pyrimidinone (TP) scaffold can be synthesized by using Biginelli reaction to make a wide variety of structural isomers [11] among the possible isomers of fused triazolopyrimidines the 1,2,4-triazolo [1,5-a] pyrimidine system exhibits broad range of medicinal applications such as anticancer [12], antiviral [13], antioxidant [14], antimalarial [15], antiasthma [16], antimicrobial [17-18], cardiovascular agents [19], Herbicidal [20]. Furthermore, sulfones also play a key role in synthetic organic chemistry because of these functional groups have various biological applications. [21-22] Apart from these the sulfone group bound to triazole or pyrimidine ring their biological efficiency are increased. [23-26] Some of the similar bioactive compounds [27-32] have shown in **Fig.1.**

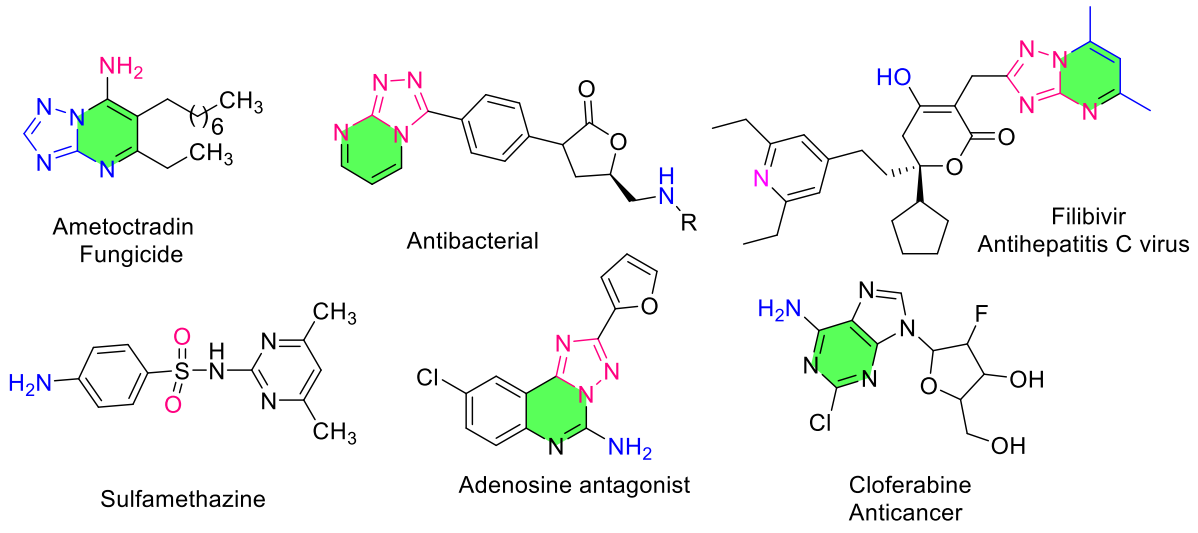


Fig-1. Relative biologically active drug molecules.

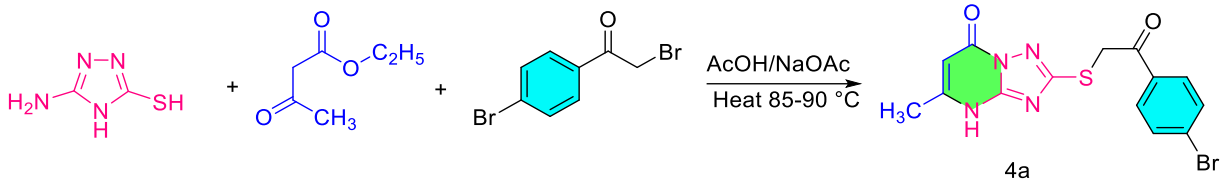
In view of the importance of triazolopyrimidines and sulfones and in continuation of our earlier work we have developed thiophenacyl 1,2,4-triazolopyrimidinones and 2-(benzyl sulfonyl)-5-methyl [1,2,4]-triazolo[1,5-*a*] pyrimidin-7(4*H*)-ones and screened against for antiviral and antibacterial activity. These two series of compounds were synthesized by using multi-component approach. The advantages of MCR method is more reliable than conventional methodology. In which the products are formed with high percentage of yield and lessening of the reaction time. [33-36]

5B.2 Present work

5B.2.1. Chemistry

Keeping in view of the importance of triazolo pyrimidinones and continuous of efforts we have development a new series of triazolo pyrimidinones. These compounds were synthesized by a one-pot three component condensation of 5-amino-4*H*-1,2,4-triazol-3-thiol, *β*-ketoester and various phenacyl bromides in the presence of a mixture of AcOH/NaOAc leading to the formation of a novel thioalkyl (phenacyl/3-2-bromoacetyl coumarin) triazolopyrimidinones with notable yields. (**Sheme-1**). The final compound structures were confirmed by their spectral analysis such as IR, ^{1}H NMR, ^{13}C NMR, HRMS.

Scheme-1



Optimization of the reaction.

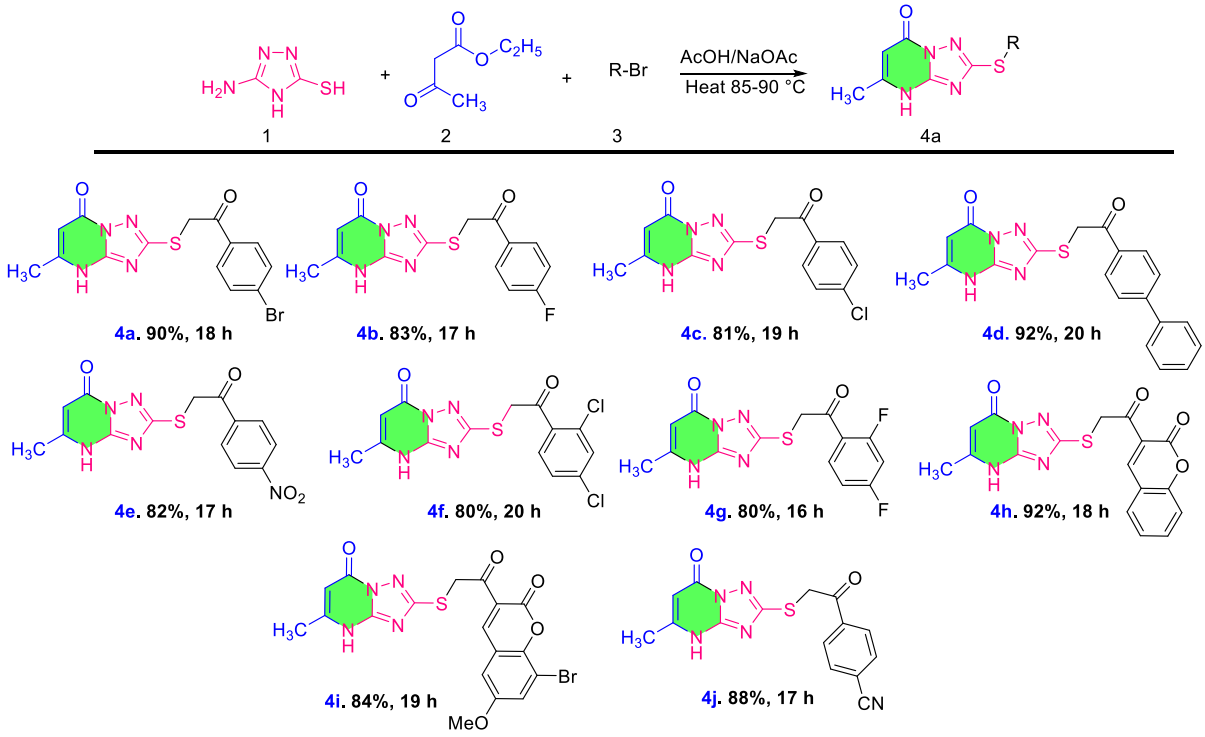
S.No	Solvent	Catalyst (mol %)	Time (h)	Temp (°C)	Yield (%)[a]
1	AcOH	-	10	60	15
2	MeOH	-	10	60	10
3	CH_3CN	-	10	70	n.r
4	DMF	-	10	70	n.r[b]
5	EtOH	HCl (0.1 N)	10	60	n.r
6	DMF	K_2CO_3 (5)	12	80	n.r
7	AcOH	Piperidine (3)	10	90	18
8	AcOH	NaOAc (5)	15	90	40

9	AcOH	NaOAc (10)	15	90	70
10	AcOH	NaOAc (15)	15	90	92[c]

Reaction conditions: 5-amino-4*H*-1,2,4-traizole-3-thiol (1 mmol), ethyl acetoacetate (1 mmol), phenacylbromide (1 mmol) in AcOH/NaOAc; [a] Yield of the product, [b] n.r = no reaction, [c] 92% yield at 90 °C for 15 h.

In the optimization study of the reaction firstly the 5-amino-4*H*-1,2,4-triazol-3-thiol was allowed to reaction with ethyl acetoacetate and 4-bromo phenacyl bromide in acetic acid under heating at 60 °C for 10 h to get the expected product **(4a)** with 15% yield. Further, we have tried by changing of the solvents and conditions. Such as MeOH, CH_3CN, DMF solvents without addition of any bases at variable temperatures (**Table-1 entry 2-4**) in this conditions we did not get the product. In EtOH + HCl (0.1 N), DMF +K_2CO_3 (5 mol%) in this conditions also product not formed (**entry 5-6**). Based on the preliminary observation (**entry 1-6)** the reaction is moving in AcOH. Further, our delight to improve the yield we have screened the reaction in AcOH with the addition of catalysts i.e., pyridine (3 mol%) and NaOAc (5 mol%, 10 mol%, 15 mol%) (**Table-1 entry 7-10**). Among these conditions AcOH + NaOAc (15 mol %) for 15 h at 90 °C to get the 92% yield of the product. (**Table-1**)

Synthesized derivatives.



Reaction conditions: 1 (1 mmol), 2 (1 mmol), 3 (1mmol) mixture taken in AcOH/NaOAc, reflux at 90 °C.

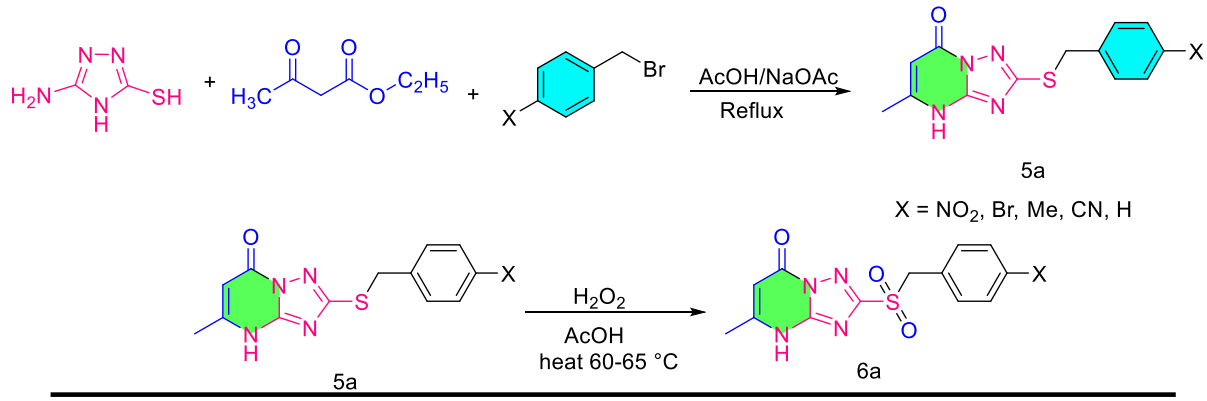
By using the optimized conditions of thio alkyl triazolopyrimidinones we checked the substrate scope of the reaction by changing the various para substituted phenacyl bromides and 3(2-bromo acetyl) coumarins. Phenacylbromides having both electron donating and electron withdrawing substituents at *p*-position have good effect on the yield of the product. Electron donating groups at para position gave good yields of the products when compared with electron withdrawing groups on para position of phenacyl bromides. And also 3-(2-bromo acetyl) coumarin derivatives produced good yields (>90%) in a shorter period of reaction time

Scheme-II

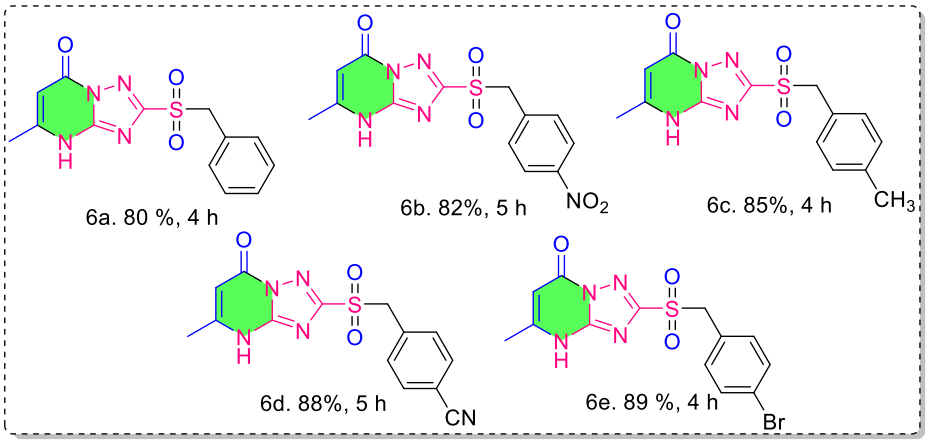
In this scheme-II the 5-amino-4*H*-1,2,4-triazole-3-thiol was cyclocondensation with ethyl acetoacetate in presence of acetic acid under reflux conditions (90 ^{o}C) for 14-15 h lead to formation of a bicyclic triazolothiazole heterocyclic compound. Then we have added fused NaOAc and substituted benzyl bromides and refluxed for 4-5h to form a thiobenzylated triazolo pyrimidinones (5a) with good yield. The isolated sulphide compound 5a was further converted in to sulfones by oxidation with hydrogen peroxide in acetic acid at 60-65 ^{o}C for 4-5 h. The formation of sulfonated compound 6a was depicted in **scheme-II**. And 6a structure was confirmed with FT-IR, ^{1}H NMR, ^{13}C NMR and HRMS, spectral analysis. The proton NMR spectrum of 6a sulfonated attached methylene two protons appear as singlet in the range of 4.5 -5.2 δppm. And triazolo pyrimidinone ring C-H singlet proton appears at down filed compared with sulphide compound 5a due to the electron withdrawing nature of the SO_2 group. (see supplementary file for copies of spectral data). Based on these findings the compound 6a was confirmed

All the synthesized compounds structures (Scheme-I and Scheme-II) were confirmed by their spectral data. The IR spectra of Scheme-I and scheme-II indicate the presence of amine, alkene, nitrile, lactone, lactam, carbonyl, nitro, and sulfone, halogen functional groups. The stretching frequency of (N-H) is observed at 3240-3425 cm^{-1}, (alkene C-H) 2825-2965 cm^{-1}, (CN) 2190-2285 cm^{-1}, (lactone C=O) 1710 – 1733 cm^{-1}, (C=O)1620-1634 cm^{-1}, (N-CO) 1605 -1614 cm^{-1}, NO_2 (Unsymmetric 1520-1545 cm^{-1}, Symmetric 1315- 1340 cm^{-1}), and SO_2 1295 – 1325 cm^{-1}, and C-X 690-895 cm^{-1} respectively.

Scheme-II: Schematic representation of compound 6a synthesis.



Synthesized derivatives



Reaction conditions: 5-amino-4*H*-1,2,4-triazole-3-thiol (1 mmol), EAA (1 mmol), *p*-substituted benzyl bromides (1 mmol) were taken in AcOH/NaOAc, and refluxed at 90 °C for 15 h. After 5a (1 mmol), H_2O_2 (2 mmol) were taken in AcOH and heat at 60-65 °C to form 6a.

In the ^{1}H NMR spectra the methyl singlet protons appear at 2.27-2.37 δppm, thioalkylated CH_2 singlet protons appears at 4.80-4.98 δppm, the characteristic pyrimidinone ring alkene C-H one singlet proton appear at 5.79-6.05 δppm, aromatic protons are appeared in the range of 7.25-8.28 δppm, N-H singlet proton is shown at 13.15- 13.36 δppm. Proton decoupled ^{13}C NMR spectra of methyl carbon appear at 18.8-19.1 δppm, S-CH_2 carbon appear at 37-46 δppm, the pyrimidinone ring alkene C-H carbon is shown at 98.1-99.2 δppm, while the remaining all aromatic carbons are appears in between 115- 159.0 δppm and imide carbonyl is shown at 160.0-162.2, carbonyl carbon has represented at 190-192.0δppm. Molecular mass of all the compounds were matched with their $[M+H]^+$ ion peak**.**

5B.2.2. Antiviral activity

The Scheme-I compounds were screened for their broad panel of antiviral activity by using various cell cultures. The HIV-1 and HIV-2 viral activity was carried out using MT-4 $CD4^+$ T cell lines and AMD3100 was takes as a standard reference drug. The Human corona virus activity was screened with the use of HELL299, Hep-3β cell culture and their activity have been compared with standard drugs Remdesivir, Ribavirin. The anti Zika virus activity and anti-influenza virus activity, anti Herpus Simplex Virus activity-1 and Yellow fever virus, Respiratory syncytial virus (RSV) activity was carried out using Hep-3β, MDCK, HEL299. The activity has been compared to that of reference antiviral drugs i.e., Ribavirin, Zanamivir, Rimantadine, Acyclovir, DS-10,000. All the screened compounds their activity were represented table-2 and 3. Among the tested compounds the compound active against Human corona virus and the compound 4i exhibited promising anti HIV-1 activity.

Table-2 Broad panel of antiviral activity results in various cell culture

S.No	Cytotoxicity(CC_{50})[a] µM				Antiviral activity (EC_{50})[b] µM									
					Human corona virus (HCoV)			Influenza virus			RSV	HSV-1	Yellow fever virus	Zika virus
	Hel299	He3β	MDCK	Hep2	229E	OC43	NL63	H1N1	H3N2	B	Along	KOS	17D	Mr766
					HEL229	HEL229	Hep3β	MDCK			Hep2	HEL229	Hep3β	Hep3β
4a	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
4b	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
4c	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
4d	>50	>50	**28**	**35.2**	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
4e	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
4f	>50	>50	**27.7**	>50	>50	**4.7**	>50	>50	>50	>50	>50	>50	>50	>50
4g	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
4h	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
4i	85.2	**43.4**	**34**	**13.5**	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
4j	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50

Standard reference drugs used														
A	>10	>10	-	>10	0.16	0.12	0.28	-	-	-	0.08	-	1.1	2.8
B	-	143.1	98.2	98.3	-	-	-	8.2	11.1	8.4	6.8	-	89.4	49.1
C	-	-	>100	-	-	-	-	0.15	0.15	0.10	-	-	-	-
D	-	-	>100	-	-	-	-	0.02	0.09	>100	-	-	-	-
E	>100	-	-	-	-	-	-	-	-		-	0.7	-	-
F	>100	-	-	>100							0.07	0.4		

A = Remdesivir, **B** = Ribavirin, **C** = Zanamivir, **D** = Rimantadine, **E** = Acyclovir, **F** = DS-10,000;

[a] Cytotoxic concentration (50%) of the compound evaluated in Hel299, Hep3β, MDCK, Hep2 cell culture

[b]50% Effective concentration or compound concentration required to inhibit virus induced cytopathogenic effect by 50% in various cell culture.

Table-3. EC_{50}[a] and CC_{50}[b] values of the compounds tested against HIV replication in MT-4 $CD4^+$ T cell line

Compound	**Cellular toxicity**	**HIV-1**	**HIV-2**
		NL4.3	**ROD**
	CC_{50} (µM)	EC_{50} (µM)	EC_{50} (µM)
4a	>100	>100	>100
4b	>100	>100	>100
4c	>100	>100	>100
4d	>100	>100	>100
4e	>100	>100	>100
4f	>100	>100	>100
4g	>54.0	>54.0	54.0
4h	>100	>100	>100
4i	**43.7**	**8.8**	**>43.7**
4j	>100	>100	>100
Reference compound AMD3100	CC_{50} (ng/mL)	EC_{50} (ng/mL)	EC_{50} (ng/mL)

[a] EC_{50}: 50% Inhibitory concentration or compound concentration required to inhibit HIV-induced cytopathogenic effects by 50% in MT-4 cell line.
[b] CC_{50}: 50% Cytotoxic concentration of the compounds also evaluated in the MT-4 cell line.

The MT-4 $CD4^+$ T cell culture was used to evaluate the compounds against human immune deficient virus (HIV) The virus-induced cytopathogenic effect (CPE) was measured colorimetrically, AMD3100 was used as a reference drug. Among the tested derivatives the compound 4k is promising activity against HIV-1(NL 4.3 strain). And also the compounds were tested for against Zikavirus, Herpus simplex virus-1, Human corona virus, yellow fever virus, RSV virus, Influenza virus with respective cell culture medium. Among the all screened compounds with different strains the compound **4g** is potent activity against HCoV (Human corona virus) the average experimental-1, experimental-2 EC_{50} value is 4.7 µM by using HEL 299 cell line. And the compound 4k has showed promising activity against HIV-1 the EC_{50} value is 8.8 µM. The used cell cultures, strains and compounds EC_{50} and cytotoxic (CC_{50}) values were summarized in **table-2 and 3.**

5B.3. Conclusions:

In the conclusion section the Scheme-I thiophenacylated triazolopyrimidinones were successfully synthesized *via* a one-pot multicomponent process. The choosen multicomponent method has some advantages in which product was formed with high percentage of the yield, reaction completed in single step, and the reaction take less time to complete. Further, the **4a-j** compounds were screened for their broad panel of *in-vitro* antiviral activity in various cell culture medium. The compounds 4f has shown human corona virus activity (**EC_{50} 4.7 µM**) and 4h exhibited anti HIV-1 activity (**EC_{50} 8.8 µM**) in respective cell culture. Furthermore, the scheme-II sulphonated compounds also successfully synthesized by using MCRs method.

5B.4.Experimental.

5B.4.1. Chemistry

All the starting materials were commercially purchased from chemical sources and used without further purification. The completion of the reaction was checked with TLC coated with silica gel aluminium foil plates by using ethyl acetate and n-hexane (3:7). The melting points of the compounds were checked with Stuart Staffordshire, UK (SMP30) instrument. The Perkin-Elmer spectrophotometer is used for record IR and KBR as a reference, the units were represented in cm^{-1}. Proton NMR spectra have been recording on BRUKER-400MHz spectrophotometer and TMS as a standard reference compound, the chemical shift values were represented in δppm. And the coupling constant (*J*) values represented in HZ. The proton decoupled ^{13}C NMR spectra was recorded by using BRUCKER-100MHz spectrophotometer and the chemical shift values expressed in δppm. The mass spectrum (HRMS) were recorded on the Agilent Technologies Instrument ESI (+Ve mode).

5B.4.2. Antiviral assay.

Antiviral assays were performed towards herpes simplex virus-1 (HSV-1 KOS), and human coronavirus (HCoV-229E and -OC43) in HEL 299 cell cultures, yellow fever virus, respiratory syncytial virus A in Hep-2 cells, Zika virus and human coronavirus (HCoV-NL63) in Hep3B cell cultures and influenza A/H1N1 (A/Ned/378/05), influenza B (B/Ned/537/05) in MDCK cell cultures, influenza A/H3N2 (A/HK/7/87). On the day of the infection, growth medium was aspirated and replaced by serial dilutions of the test compounds. The virus was then added to each well, diluted to obtain a viral input of 100 $CCID_{50}$ ($CCID_{50}$ being the virus dose that is

able to infect 50% of the cell cultures). Mock-treated cell cultures receiving solely the test compounds were included on each cell line, to determine the cytotoxicity of the test compounds. After 3 to 7 days of incubation, the (CPE) 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 (CellTiter 96 AQueous One Solution Cell Proliferation Assay from Promega, Madison, WI), and the antiviral activity was expressed as the (EC_{50}) 50% effective concentration. In parallel, the (CC_{50}) 50% cytotoxic concentration of the compounds was derived from the mock-infected cells. The activities were compared with the activities of reference antiviral drugs. AMD3100, ribavirin, remdesivir, acyclovir, zanamivir, rimantadine and dextran sulfate (DS-10,000).

The anti-HIV-2 and anti-HIV-1 activity of each compound were evaluated in MT-4 $CD4^+$ T cell cultures was determined by a tetrazolium-based colorimetric assay. Briefly, 3-fold dilutions of various test compounds were added in a 96-well plate and preincubated for 20 min at 37°C with MT-4 cells (1×10^6 cells/ml). Next, various concentrations of virus, HIV-1 NL4.3 and HIV-2 ROD were given depending on the $TCID_{50}$ of the virus stock. Five days' post-infection, (CPE) cytopathic effects were scored microscopically and antiviral activity was measured by MTS/PES using a Spectramax 96-well plate reader (Molecular Devices) as described previously.[37]

4. General procedure for the synthesis of thiophenacyl/3-acetyl-2H-chromene-2-one 1,2,4-triazolopyrimidinones.5a

A mixture of 5-Amino-4*H*-1,2,4-triazolo-3-thiol (1 mmol), ethylacetoacetate (1 mmol), phenacyl bromide/3-(2-bromo acetyl)-2*H*-chromen-2-one (1 mmol) was taken in acetic acid and added 20 mole percentage of NaOAc refluxed for 20 h then we checked the TLC After completion of the reaction the reaction mixture was poured into ice cold water the white colour solid product was isolated and recrystalized from methanol. 90% of the pure product was formed.

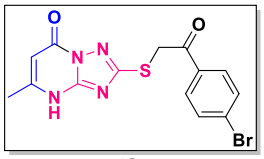
General procedure for the synthesis 2-(benzylsulfonyl)-5-methyl-[1,2,4]triazolo[1,5-a] pyrimidin-7(4H)-one. 6a

A mixture of 5-Amino-4*H*-1,2,4-triazolo-3-thiol (1 mmol), benzyl bromide (1 mmol) ethylacetoacetate (1 mmol) was taken in acetic acid and 20 mole percentage of NaOAc was added then refluxed for 20 h after checking the TLC and after completion of the reaction mixture poured into ice water the white crystalline product was isolated and dried. The isolated

product (1 mmol) was taken in acetic acid and H_2O_2 (2.0 mmol) was added and heated at 60 °C for 4-5 h, the white solid was filtered and wash with water.

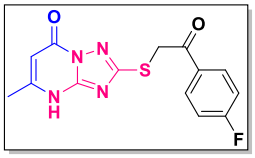
5B.5. Characterization data of synthesized compounds

2-((2-(4-Bromophenyl)-2-oxoethyl)thio)-5-methyl-[1,2,4]-triazolo[1,5-a]pyrimidin-7(4H)-one 4a



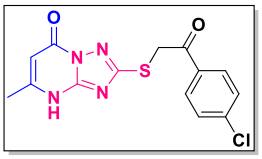
White solid; Yield 90%; mp: 250-251 °C; IR (KBr) cm^{-1}: 3288 (N-H), 2904 (alkene C-H), 1706 (C=O), 1682 (Imide C=O), 665 (C-Br); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.33 (s, 3H), 4.95 (s, 2H), 5.85 (s, 1H), 7.84 (d, J = 8.7 Hz, 2H), 8.04 (d, J = 8.6 Hz, 2H), 13.27 (s, 1H); ^{13}C{H} NMR (100 MHz, DMSO-d_6) δ (ppm): 18.9, 41.9, 99.0, 128.3, 130.8, 132.33, 132.39, 134.91, 151.32, 151.54, 155.33, 162.11, 193.09: HRMS (ESI-TOF) (*m/z*): Calculated for $C_{14}H_{11}BrN_4O_2S$ $[M+H]^+$ 378.98364; found 378.9818

2-((2-(4-Fluorophenyl)-2-oxoethyl)thio)-5-methyl-[1,2,4]-triazolo[1,5-a]pyrimidin-7(4H)-one 4b.



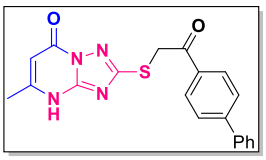
White solid; Yield 83%; mp: 255-256 °C; IR (KBr) cm^{-1}: 3509 (N-H), 2910 (alkene C-H), 1721 (C=O), 1590 (Imide C=O), 833 (C-F); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.27 (s, 3H), 4.93 (s, 2H), 5.79 (s, 1H), 7.40 (t, J = 8.8 Hz, 2H), 8.14 (m, 2H), 13.15 (s, 1H); ^{13}C{H} NMR (100 MHz, DMSO-d_6) δ (ppm): 18.9, 42.1, 99.0, 116.2, 116.4, 131.8, 151.2, 151.5, 155.2, 162.1, 164.4, 166.9, 192.3: HRMS (ESI-TOF) (*m/z*): Calculated for $C_{14}H_{11}FN_4O_2S$ $[M+H]^+$ 319.0665; found 319.0673

2-((2-(4-Chlorophenyl)-2-oxoethyl)thio)-5-methyl-[1,2,4]-triazolo[1,5-a]pyrimidin-7(4H)-one 4c.



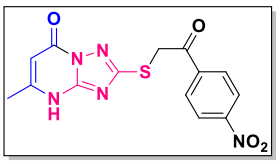
White solid; Yield 81%; mp: 249-250 °C; IR (KBr) cm^{-1}: 3288 (N-H), 2906 (alkene C-H), 1701 (C=O), 1680 (Imide C=O) 732 (C-Cl); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.27 (s, 3H), 4.92 (s, 2H), 5.79 (s, 1H), 7.64 (d, J = 8.4 Hz, 2H), 8.06 (d, J = 8.4 Hz, 2H), 13.15 (s, 1H); ^{13}C{H} NMR (100 MHz, DMSO-d_6) δ (ppm): 18.9, 42.7, 99.0, 129.4, 130.7, 134.6, 139.0, 151.2, 151.5, 155.2, 162.0, 192.8: HRMS (ESI-TOF) (*m/z*): Calculated for $C_{14}H_{11}ClN_4O_2S$ $[M+H]^+$ 335.0369; found 335.0376.

2-((2-([1,1'-Biphenyl]-4-yl)-2-oxoethyl)thio)-5-methyl-[1,2,4]-triazolo[1,5-a]pyrimidin-7(4H)-one 4d



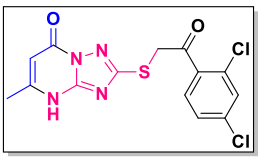
White solid; Yield 92%; mp: 260-261 °C; IR (KBr) cm^{-1}: 3403 (N-H), 2905 (alkene C-H), 1712 (C=O), 1602 (Imide C=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.28 (s, 3H), 4.98 (s, 2H), 5.80 (s, 1H), 7.44 - 7.46 (m, 1H), 7.50 - 7.52 (m, 2H), 7.76 - 7.79 (m, 2H), 7.88 (d, J = 8.5 Hz, 2H), 8.14 (d, J = 8.4 Hz, 2H), 13.16 (s, 1H); $^{13}C\{H\}$ NMR (100 MHz, DMSO-d_6) δ (ppm): 18.9, 43.2, 99.0, 127.5, 128.9, 129.5, 134.7, 39.2, 145.4, 151.1, 151.5, 155.2, 162.2, 193.1: HRMS (ESI-TOF) (m/z): Calculated for $C_{20}H_{16}N_4O_2S$ $[M+H]^+$ 377.1072; found 377.1065

5-Methyl-2-((2-(4-nitrophenyl)-2-oxoethyl)thio)-[1,2,4]-triazolo[1,5-a]pyrimidin-7(4H)-one 4e.



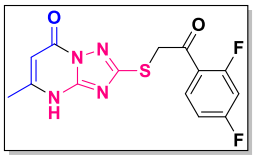
Light yellow solid; Yield 82%; mp: 258-259 °C; IR (KBr) cm^{-1}: 3514 (N-H), 2914 (alkene C-H), 1709 (C=O), 1682 (Imide C=O), NO_2 (1524 Unsymmetric, 1345 symmetric); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.27 (s, 3H), 4.99 (s, 2H), 5.79 (s, 1H), 8.28 (d, J = 8.9 Hz, 2H), 8.38 (d, J = 8.9 Hz, 2H), 13.16 (s, 1H); $^{13}C\{H\}$ NMR (100 MHz, DMSO-d_6) δ (ppm): 18.9, 42.4, 99.0, 124.3, 130.2, 140.6, 150.5, 151.2, 151.5, 155.2, 161.9, 193.2: HRMS (ESI-TOF) (m/z): Calculated for $C_{14}H_{11}N_5O_4S$ $[M+H]^+$ 346.061; found 346.0620.

2-((2-(2,4-Dichlorophenyl)-2-oxoethyl)thio)-5-methyl-[1,2,4]-triazolo[1,5-a]pyrimidin-7(4H)-one 4f



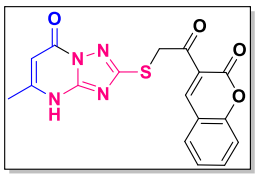
White solid; Yield 80%; mp: 247-248 °C; IR (KBr) cm^{-1}: 3214 (N-H), 2910 (alkene C-H), 1688 (C=O), 1684 (Imide C=O), 707 (C-Cl); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.28 (s, 3H), 4.93 (s, 2H), 5.80 (s, 1H), 7.84 – 7.86 (m, 2H), 8.27 (d, J = 2.0 Hz, 1H), 13.16 (s, 1H); $^{13}C\{H\}$ NMR (100 MHz, DMSO-d_6) δ (ppm): 18.9, 46.5, 99.0, 128.8, 130.7, 131.6, 136.1, 151.5, 155.2, 161.9, 192.2: HRMS (ESI-TOF) (m/z): Calculated for $C_{14}H_{10}Cl_2N_4O_2S$ $[M+H]^+$ 368.998 found 368.9971

2-((2-(2,4-Difluorophenyl)-2-oxoethyl)thio)-5-methyl-[1,2,4]-triazolo[1,5-a]pyrimidin-7(4H)-one 4g



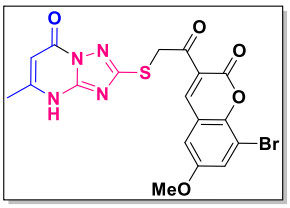
White solid; Yield 80%; mp: 240-241 °C; IR (KBr) cm^{-1}: 3402 (N-H), 2910 (alkene C-H), 1721 (C=O), 1689 (Imide C=O), 833 (C-F); ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.27 (s, 3H), 4.81 (s, 2H), 5.79 (s, 1H), 7.25 – 7.30 (m, 1H), 7.45 -7.51 (m, 1H), 7.98 – 8.0 (m, 1H), 13.31 (s, 1H); $^{13}C\{H\}$ NMR (100 MHz, DMSO-d_6) δ (ppm): 19.0, 42.7, 98.9,105.7, 113.1, 133.3, 151.4, 151.7, 155.3, 162.1, 190.4: HRMS (ESI-TOF) (*m/z*): Calculated for $C_{14}H_{10}F_2N_4O_2S$ $[M+H]^+$ 337.0571; found 337.0570

5-Methyl-2-((2-oxo-2-(2-oxo-2H-chromen-3-yl)ethyl)thio)-[1,2,4]-triazolo[1,5-a]pyrimidin-7(4H)-one 4h



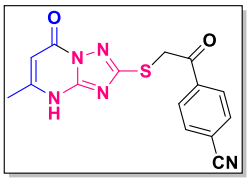
Light brown solid; Yield 92%; mp: 265-266 °C; IR (KBr) cm^{-1}: 3623 (N-H), 2909 (alkene CH), 1702 (C=O), 1603 (lactone C=O), 1555 (Imide C=O), 1172 (C-O-C) ; ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.28 (s, 3H), 4.86 (s, 2H), 5.80 (s, 1H), 7.45 (t, *J* = 7.5 Hz, 1H), 7.52 (d, *J* = 8.3 Hz, 1H), 7.79 (t, *J* = 7.8 Hz, 1H), 8.00 (d, *J* = 7.7 Hz, 1H), 8.81 (s, 1H), 13.19 (s, 1H); $^{13}C\{H\}$ NMR (100 MHz, DMSO-d_6) δ (ppm): 18.9, 42.5, 99.0, 116.7, 118.5, 123.6, 125.6, 131.4, 135.4, 148.7, 151.2, 151.5, 155.1, 155.2, 158.9, 162.0, 191.4: HRMS (ESI-TOF) (*m/z*): Calculated for $C_{17}H_{12}N_4O_4S$ $[M+H]^+$ 369.0657; found 369.0660

2-((2-(8-Bromo-6-methoxy-2-oxo-2H-chromen-3-yl)-2-oxoethyl)thio)-5-methyl-[1,2,4]-triazolo[1,5-a]pyrimidin-7(4H)-one 4i



Light brown solid; Yield 84%; mp: 260-261 °C; IR (KBr) cm^{-1}: 3341 (N-H), 2937 (alkene C-H), 1731 (C=O), 1695 (lactone C=O), 1634 (Imide C=O), 1239 (C-O-C), 731 (C-Br); ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.96 (s, 3H), 3.99 (s, 3H), 4.60 (s, 2H), 5.85 (s, 1H), 7.50-7.51 (m, 1H), 7.54-7.56 (m, 1H), 8.73 (s, 1H), 11.98 (s, 1H); HRMS (ESI-TOF) (*m/z*): Calculated for $C_{18}H_{13}BrN_4O_5S$ $[M+H]^+$ 476.9768; found 476.9741.

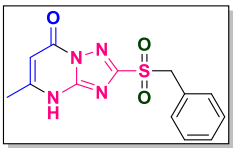
4-(2-((5-Methyl-7-oxo-4,7-dihydro-[1,2,4]-triazolo[1,5-a]pyrimidin-2-yl)thio)acetyl) benzonitrile *4j*



White solid; Yield 88%; mp: 231-232 °C; IR (KBr) cm^{-1}: 3531 (N-H), 2914 (alkene C-H), 2228 (CN), 1705 (C=O), 1682 (Imide C=O); ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.27 (s, 3H), 4.95 (s, 2H), 5.79 (s, 1H), 8.05 (d, *J* = 8.4 Hz, 2H), 8.19 (d, *J* = 8.4 Hz, 2H), 13.15 (s,

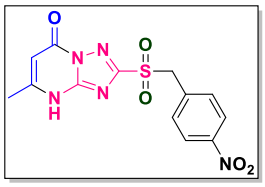
1H); $^{13}C\{H\}$ NMR (100 MHz, DMSO-d_6) δ (ppm): 18.9, 37.1, 99.0, 115.9, 118.5, 129.4, 133.3, 139.1, 151.2, 151.5, 155.2, 161.9, 193.3: HRMS (ESI-TOF) (*m/z*): Calculated for $C_{15}H_{11}N_5O_2S$ $[M+H]^+$ 326.0711; found 326.0722.

2-(Benzylsulfonyl)-5-methyl-[1,2,4]-triazolo[1,5-a]pyrimidin-7(4H)-one. 6a



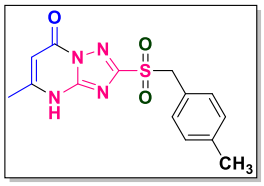
White solid; Yield 82%; mp: 238-239 °C; IR (KBr) cm^{-1}: 3070 (N-H), 2824 (Alkene C-H), 1698 (pyrimidine C=O), 1333 (SO_2); ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.37 (s, 3H), 4.89 (s, 2H), 6.01 (s, 1H), 7.37 – 7.32 (m, 5H), 13.66 (s, 1H); $^{13}C\{H\}$ NMR (100 MHz, DMSO-d_6) δ (ppm): 19.3, 59.8, 99.8, 127.4, 128.9, 129.2, 130.9, 131.8, 151.8, 153.5, 155.7, 160.9: HRMS (ESI-TOF) (*m/z*): Calculated for $C_{13}H_{12}N_4O_3S$ $[M+H]^+$ 305.0708; found 305.0707

5-Methyl-2-((4-nitrobenzyl)sulfonyl)-[1,2,4]-triazolo[1,5-a]pyrimidin-7(4H)-one. 6b



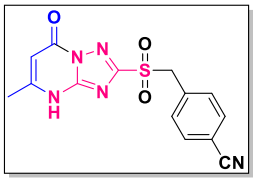
White solid; Yield 82%; mp: 235-236 °C; IR (KBr) cm^{-1}: 3377 (N-H), 2968 (Alkene C-H), 1626 (pyrimidine C=O), NO_2 (1566 Unsymmetric, 1348 symmetric), 1317 (SO_2); ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.36 (s, 3H), 5.14 (s, 2H), 6.01 (s, 1H), 7.61 (d, J = 8.8 Hz, 2H), 8.21 (d, J = 8.8 Hz, 2H), 13.63 (s, 1H); $^{13}C\{H\}$ NMR (100 MHz, DMSO-d_6) δ (ppm): 19.4, 59.2, 99.7, 123.9, 133.0, 134.3, 148.2, 151.9, 155.2, 156.7, 160.6: HRMS (ESI-TOF) (*m/z*): Calculated for $C_{13}H_{11}N_5O_5S$ $[M+H]^+$ 349.0481; found 350.0559

5-Methyl-2-((4-methylbenzyl)sulfonyl)-[1,2,4]-triazolo[1,5-a]pyrimidin-7(4*H*)-one. 6c



White solid; Yield 85%; mp: 231-232 °C; IR (KBr) cm^{-1}: 3254 (N-H), 2980 (alkene C-H), 1582 (pyrimidine C=O), 1332 (SO_2); ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.13 (s, 3H), 2.27 (s, 3H), 4.67 (s, 2H), 7.10-7.15 (m, 4H), 11.87 (s, 1H), 14.25 (s, 1H); $^{13}C\{H\}$ NMR (100 MHz, DMSO-d_6) δ (ppm): 21.1, 23.2, 59.6, 102.1, 124.7, 129.5, 131.4, 138.5, 150.1, 157.9, 170.0: HRMS (ESI-TOF) (*m/z*): Calculated for $C_{14}H_{14}N_4O_3S$ $[M+H]^+$ 319.0865; found 319.0672

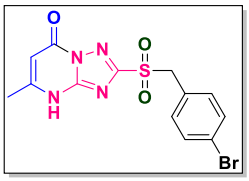
4-(((5-Methyl-7-oxo-4,7-dihydro-[1,2,4]-triazolo[1,5-a]pyrimidin-2-yl)sulfonyl) methyl) Benzonitrile. 6d



White solid; Yield 88%; mp: 230-231 °C; IR (KBr) cm^{-1}: 3531 (N-H), 2914 (alkene C-H), 2228 (CN), 1682 (pyrimidine C=O), 1294 (SO_2); ^1H NMR (400 MHz, DMSO-d_6) δ (ppm):2.14 (s, 3H), 4.59 (S, 3H), 7.44 (d, J = 8.3 Hz, 2H), 7.80 (d, J = 8.3 Hz, 2H), 11.83 (s, 1H), 14.04 (s, 1H); $^{13}C\{H\}$ NMR (100 MHz, DMSO-d_6) δ (ppm): 23.2, 58.0, 99.8, 111.2, 119.0, 131.8, 132.5,

132.7, 136.5, 169.7; HRMS (ESI-TOF) (*m/z*): Calculated for $C_{14}H_{11}N_5O_3S$ $[M+H]^+$ 330.1661; found 330.0672

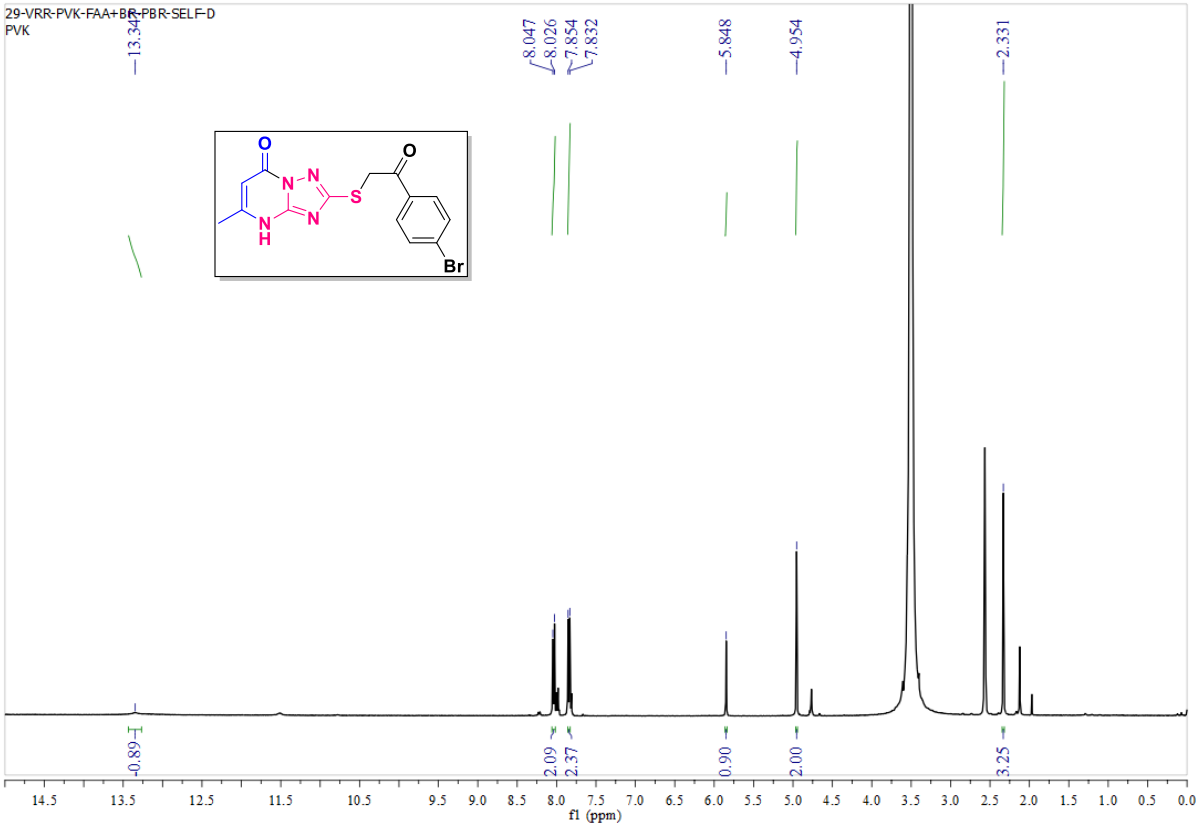
2-((4-Bromobenzyl)sulfonyl)-5-methyl-[1,2,4]-triazolo[1,5-a]pyrimidin-7(4H)-one. 6e



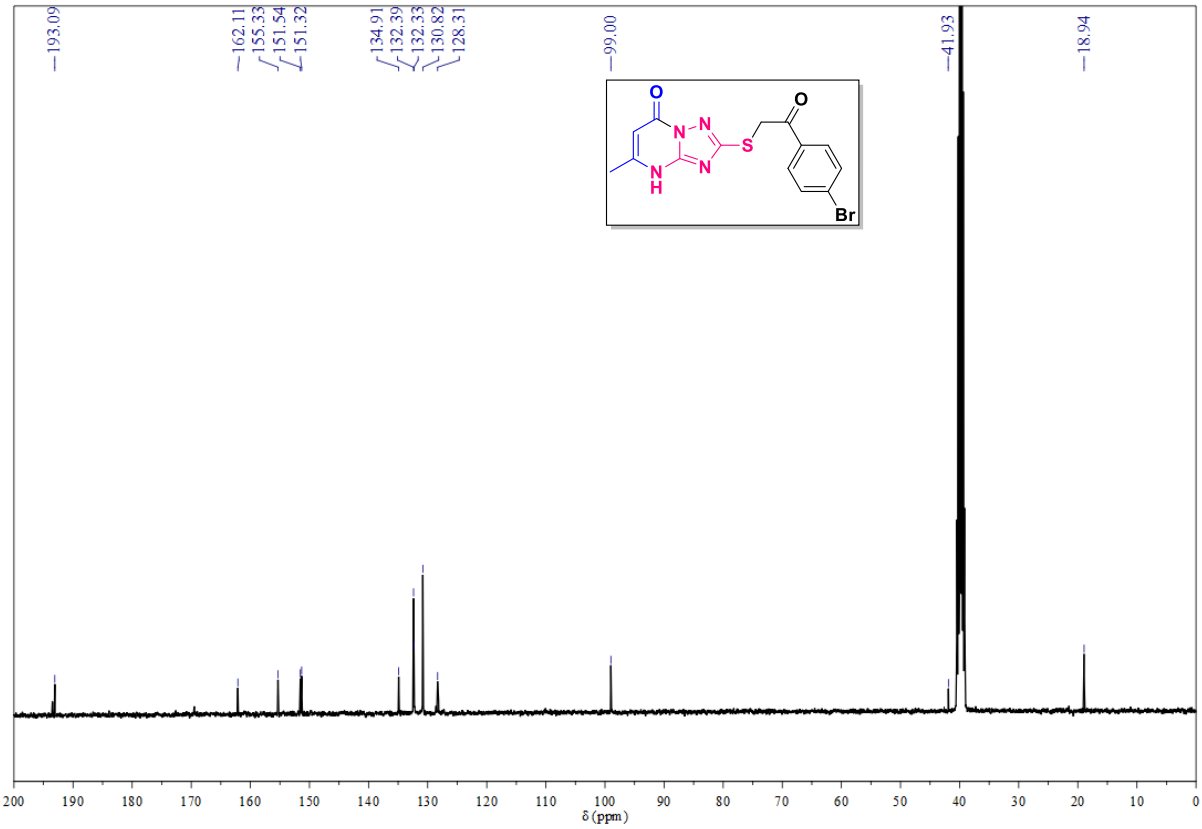
White solid; Yield 89%; mp: 238-239 °C; IR (KBr) cm^{-1}: 3070 (N-H), 2894 (alkene C-H), 1625 (pyrimidine C=O), 1337 (SO_2), 675 (C-Br); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.36 (s, 3H), 4.91 (s, 2H), 6.01 (s, 1H), 7.56 (d, J = 8.4 Hz, 2H), 7.27 (d, J = 8.5 Hz, 2H),13.61 (s, 1H); HRMS (ESI-TOF) (*m/z*): Calculated for $C_{13}H_{11}BrN_4O_3S$ $[M+H]^+$ 382.9813; found 382.9801

5B.6. Spectral data

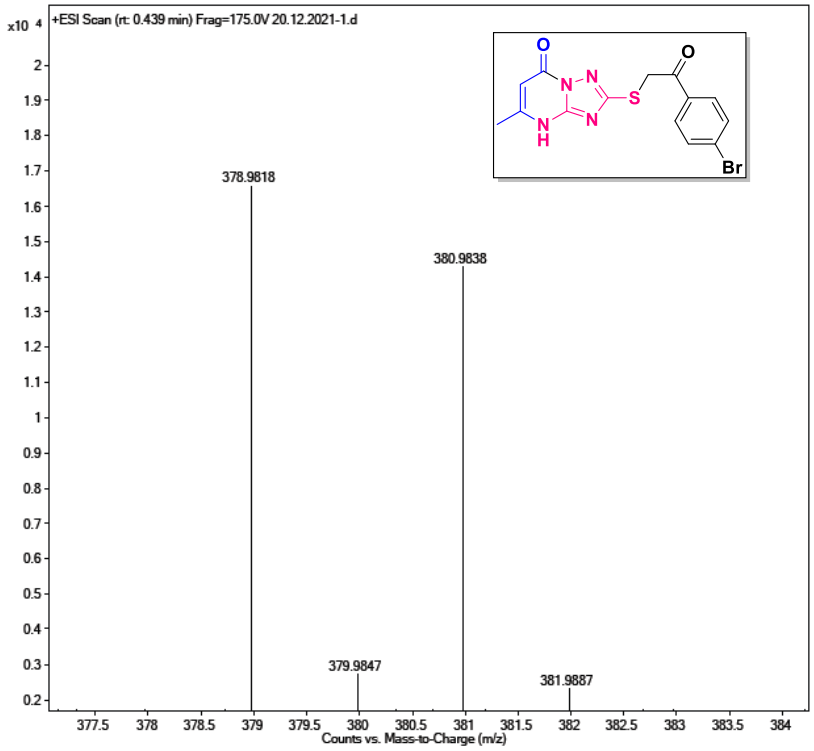
^{1}H NMR spectrum of compound 4a (DMSO-d_6 400 MHz) (**SCHEME-I**)



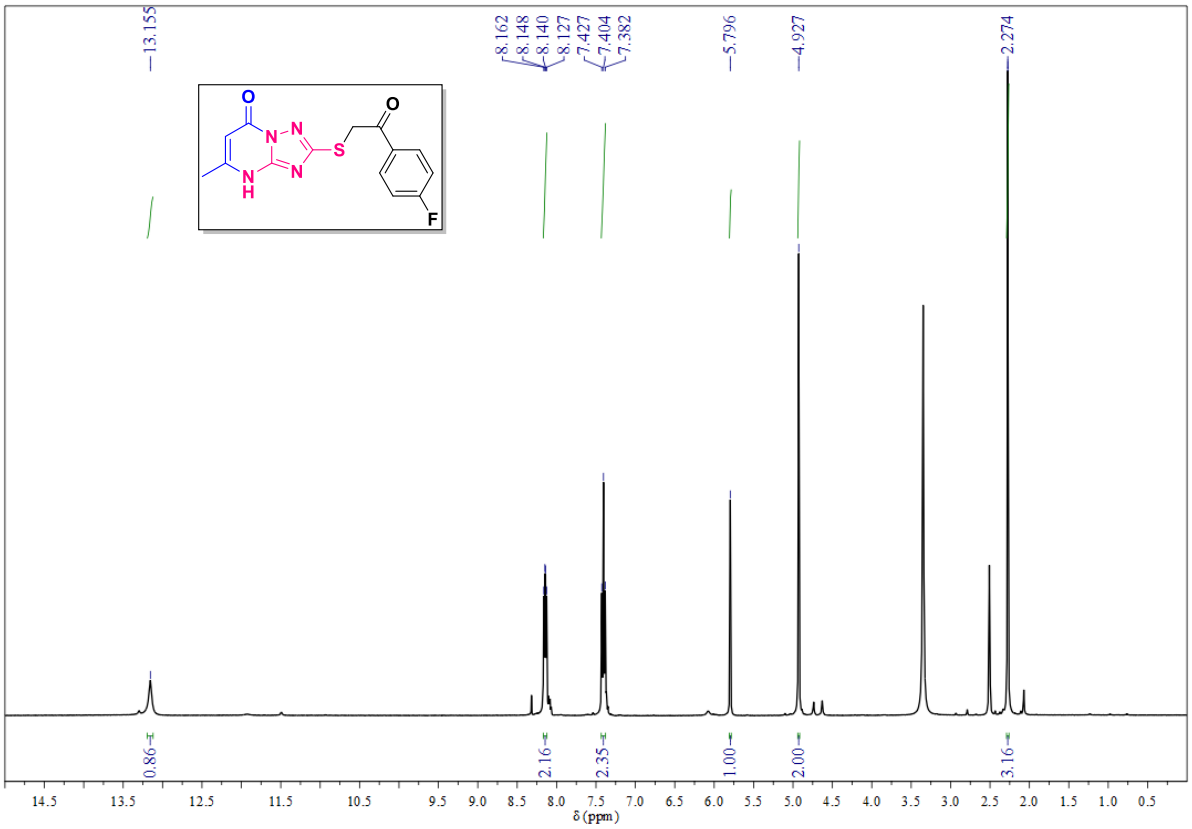
^{13}C NMR spectrum of compound 4a (DMSO-d_6 100 MHz)



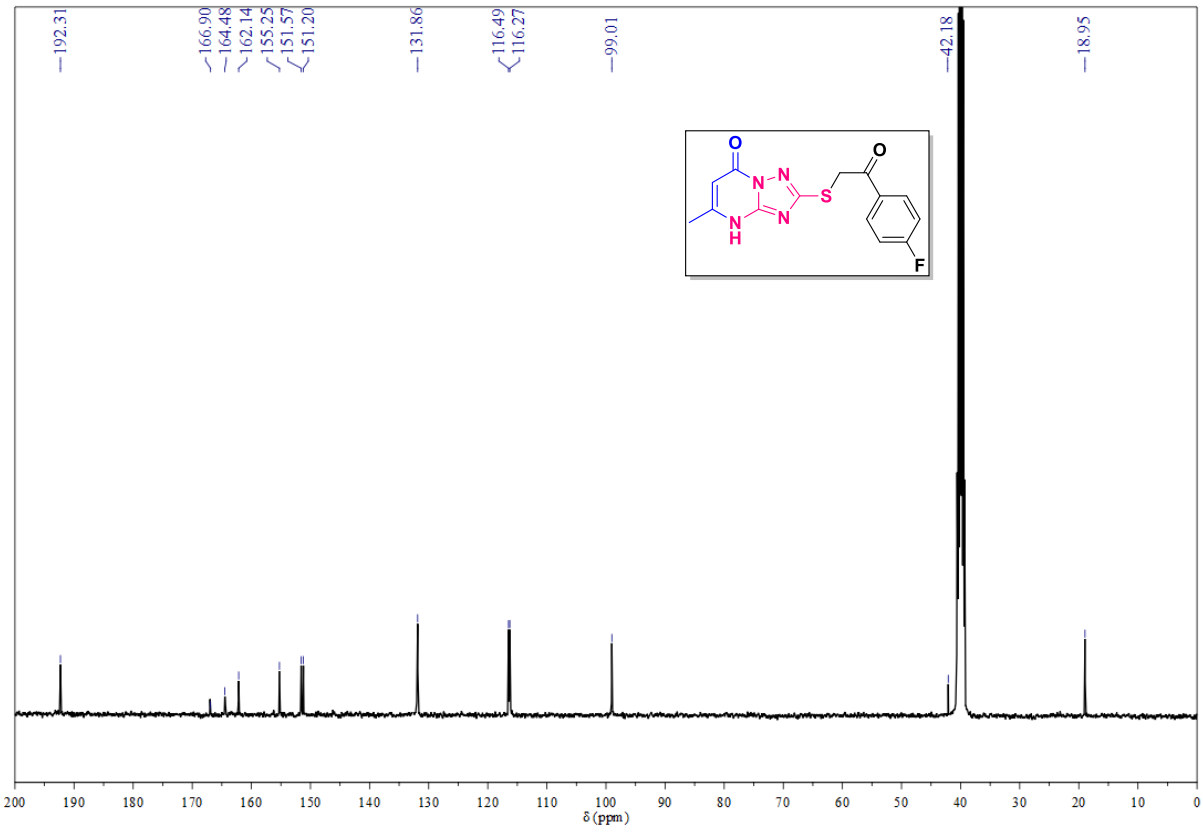
Mass spectrum of compound 4a



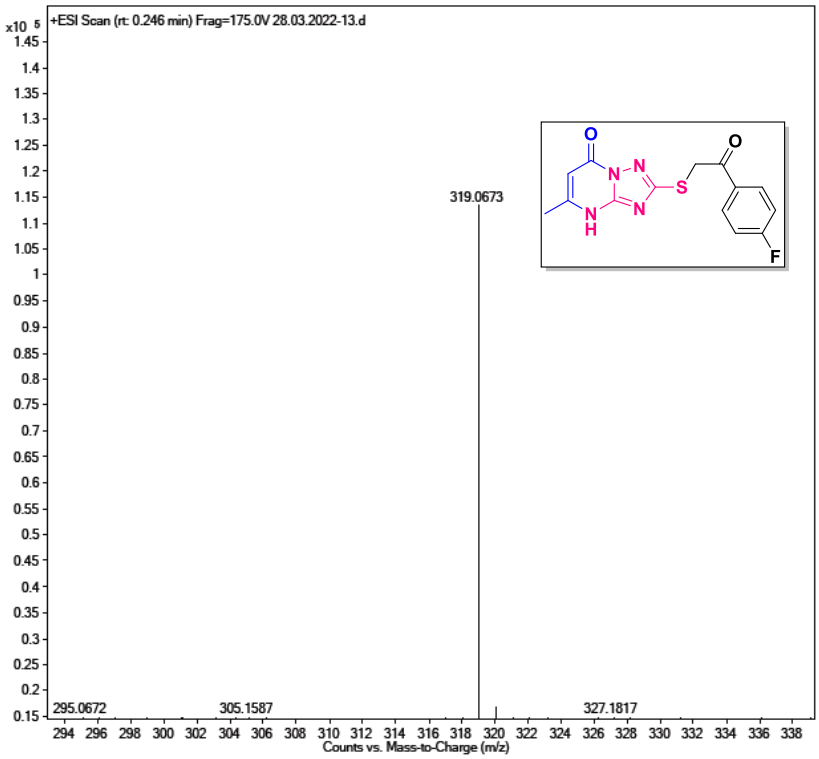
^{1}H NMR Spectrum of compound 4b (DMSO-d_6 400 MHz)



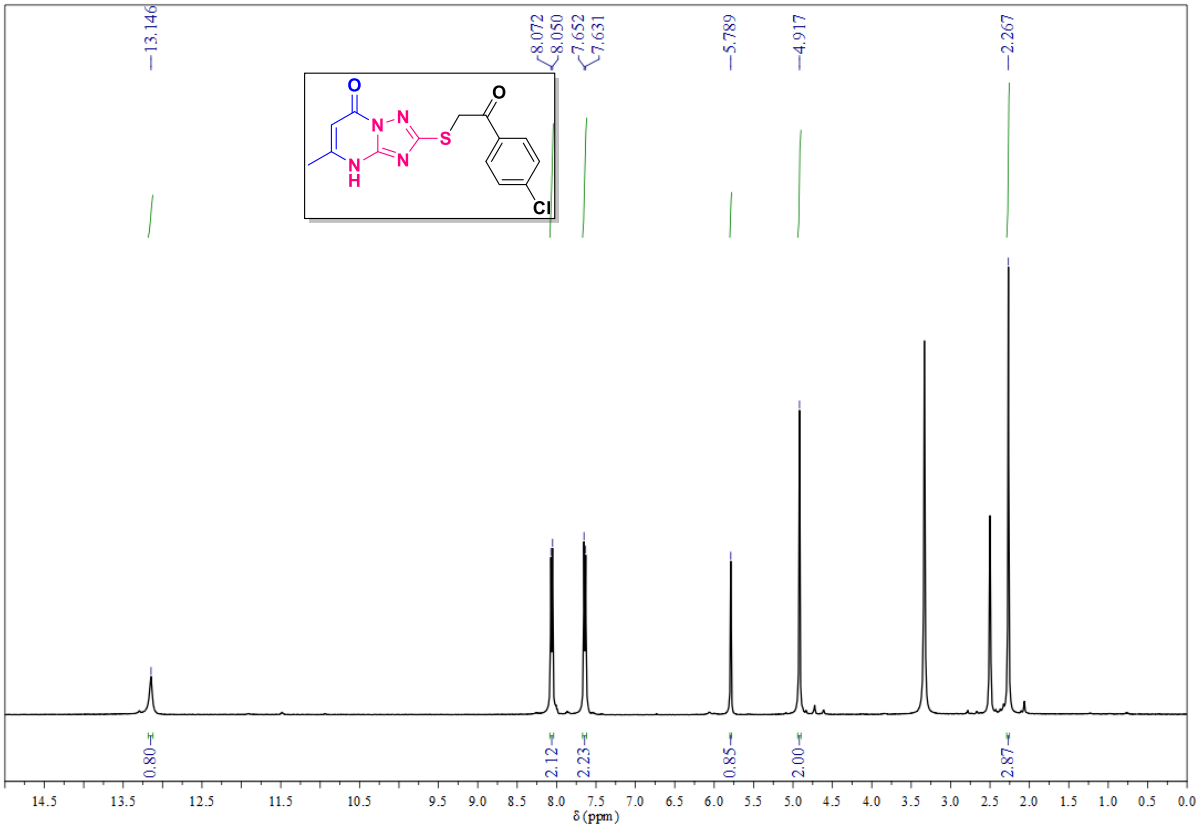
^{13}C NMR Spectrum of compound 4b (DMSO-d_6 100 MHz)



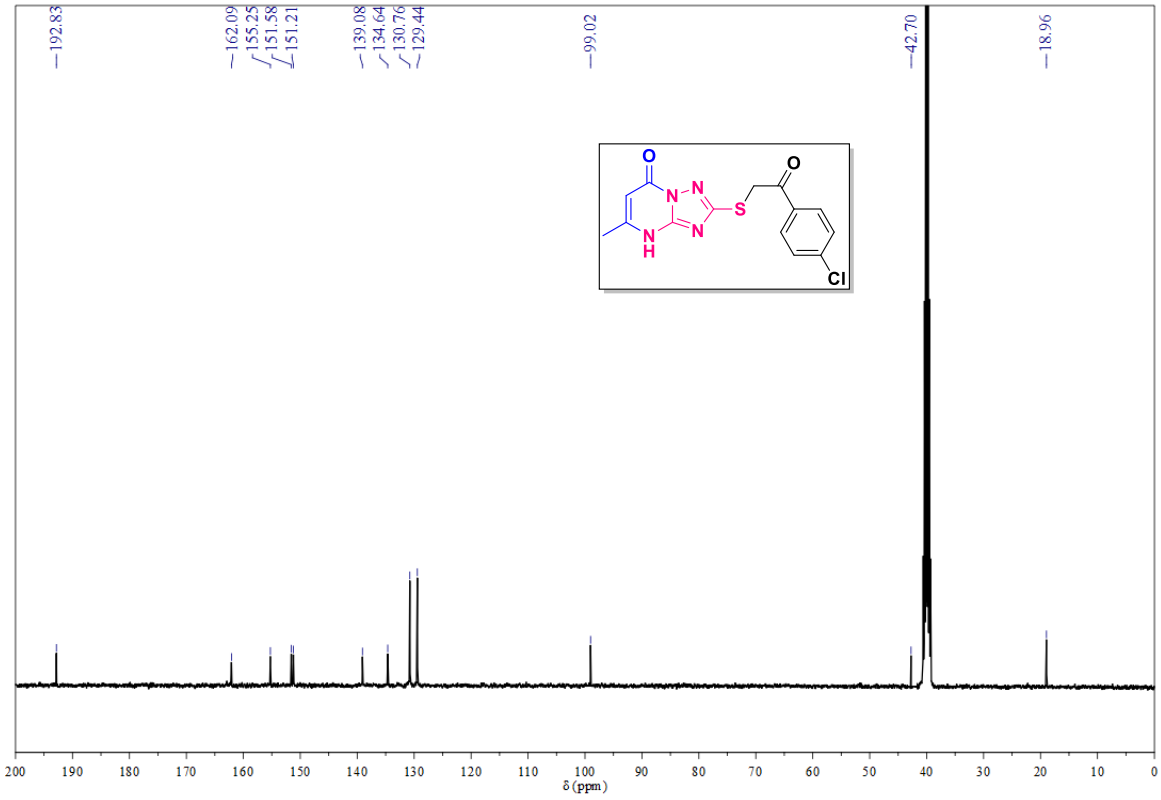
HRMS spectrum of compound 4b



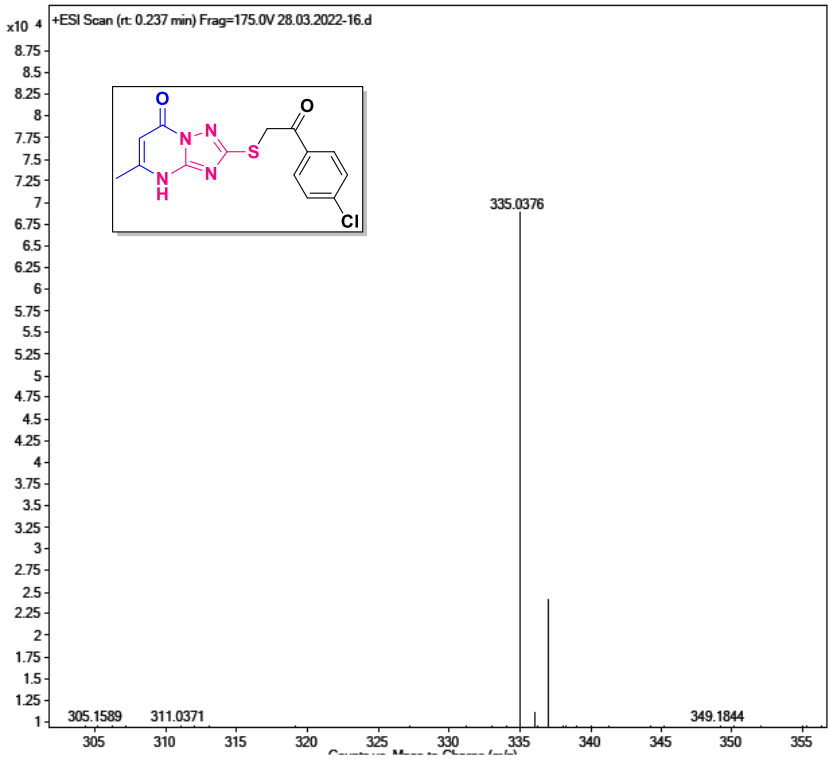
^{1}H NMR Spectrum of compound 4c (DMSO-d_6 400 MHz)



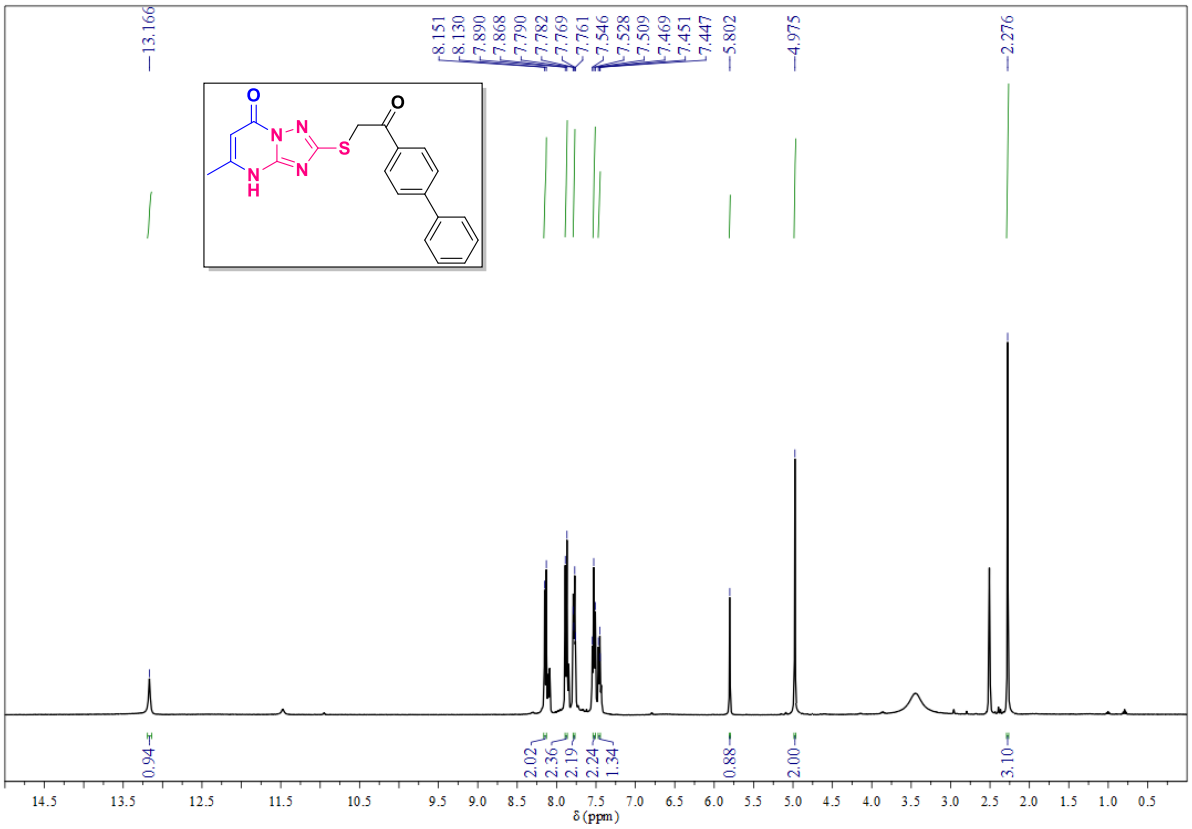
^{13}C NMR Spectrum of compound 4c (DMSO-d_6 100 MHz)



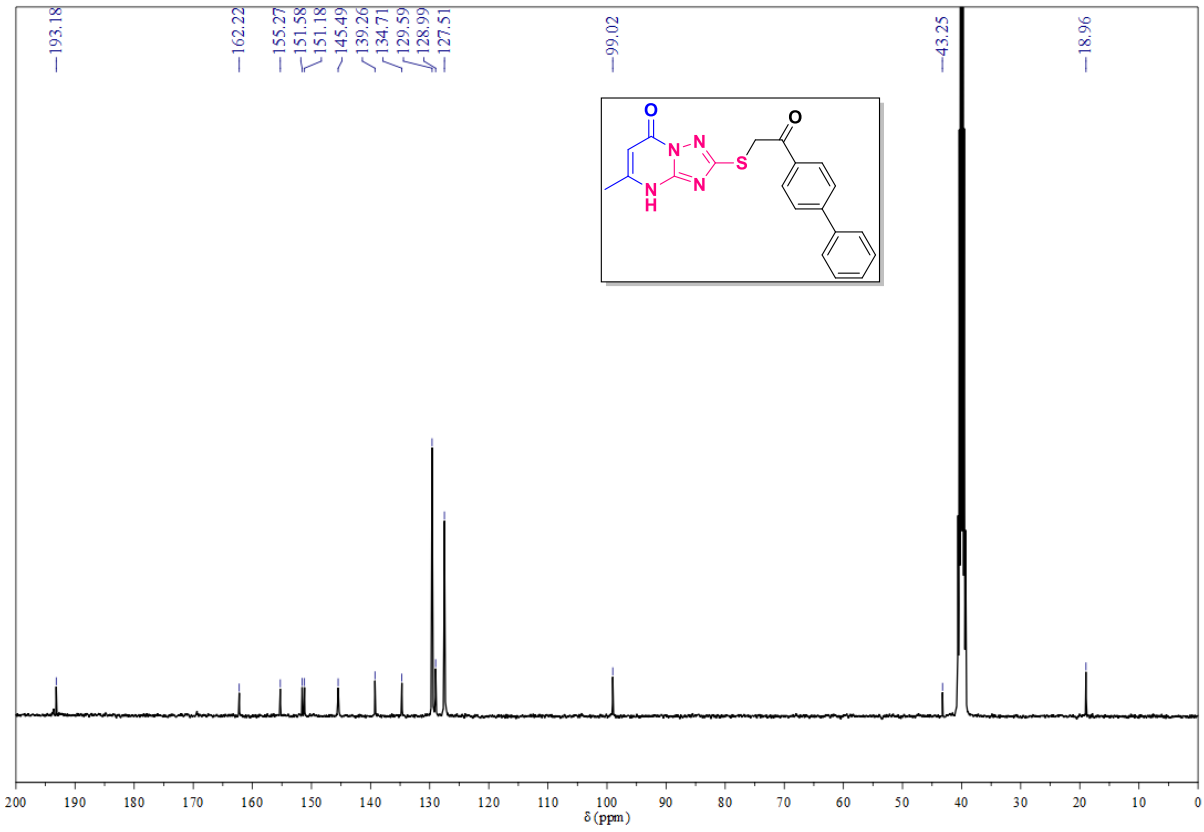
HRMS spectrum of compound 4c



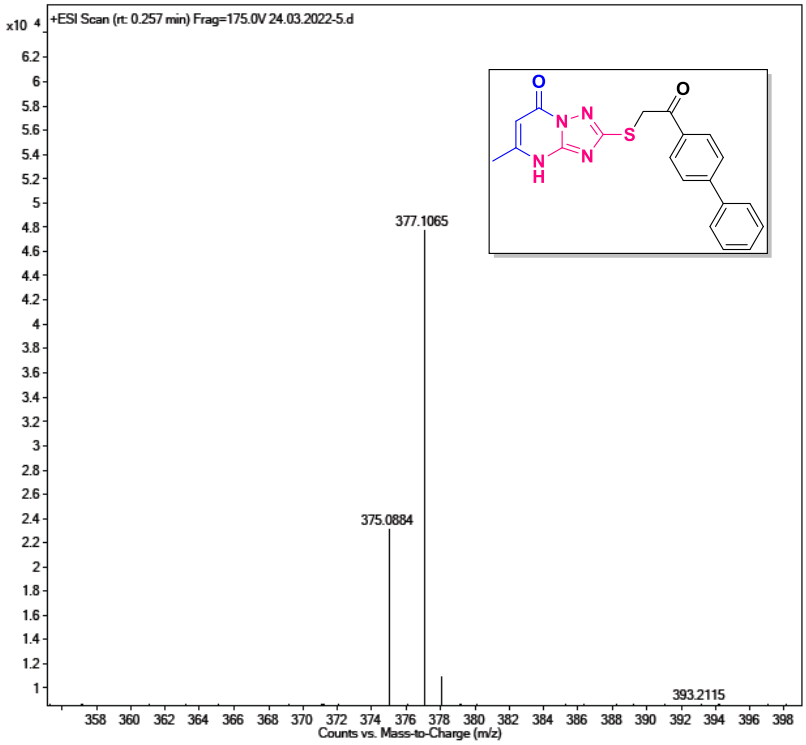
^{1}H NMR Spectrum of compound 4d (DMSO-d_6 400 MHz)



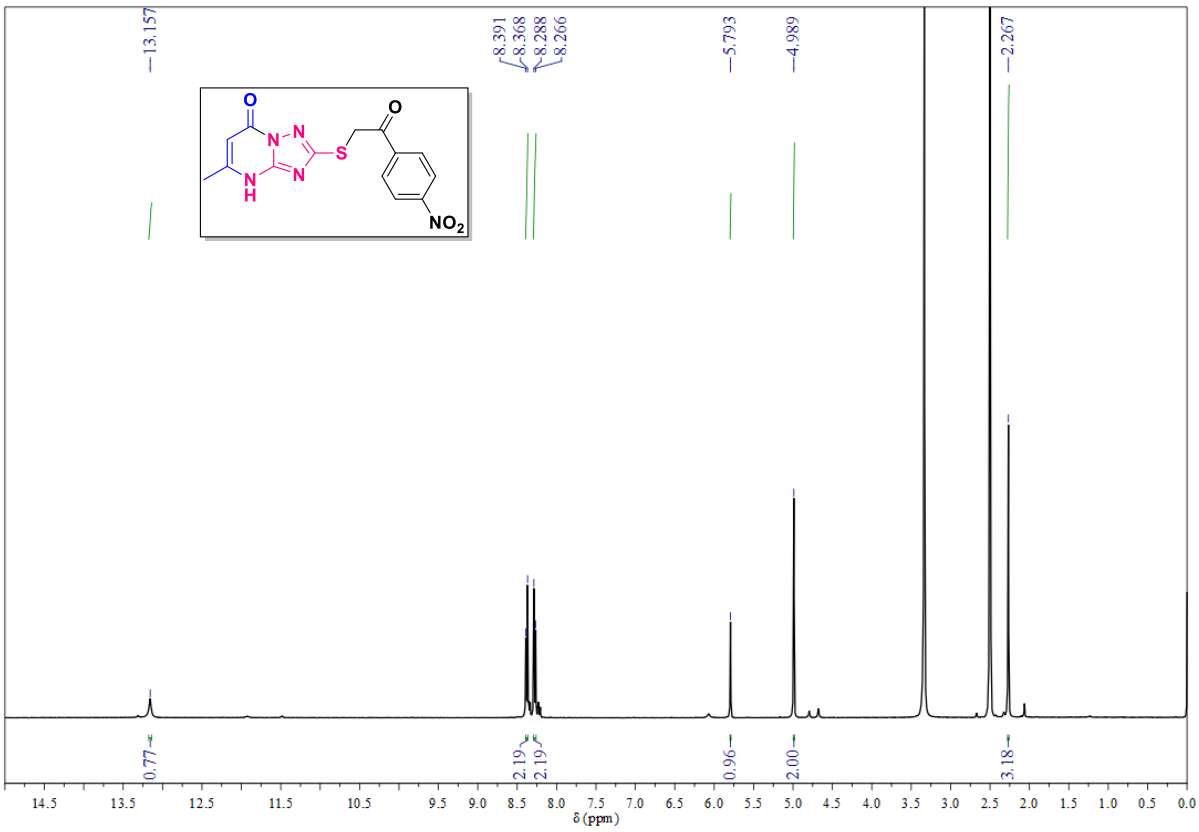
^{13}C NMR spectrum of compound 4d (DMSO-d_6 100 MHz)



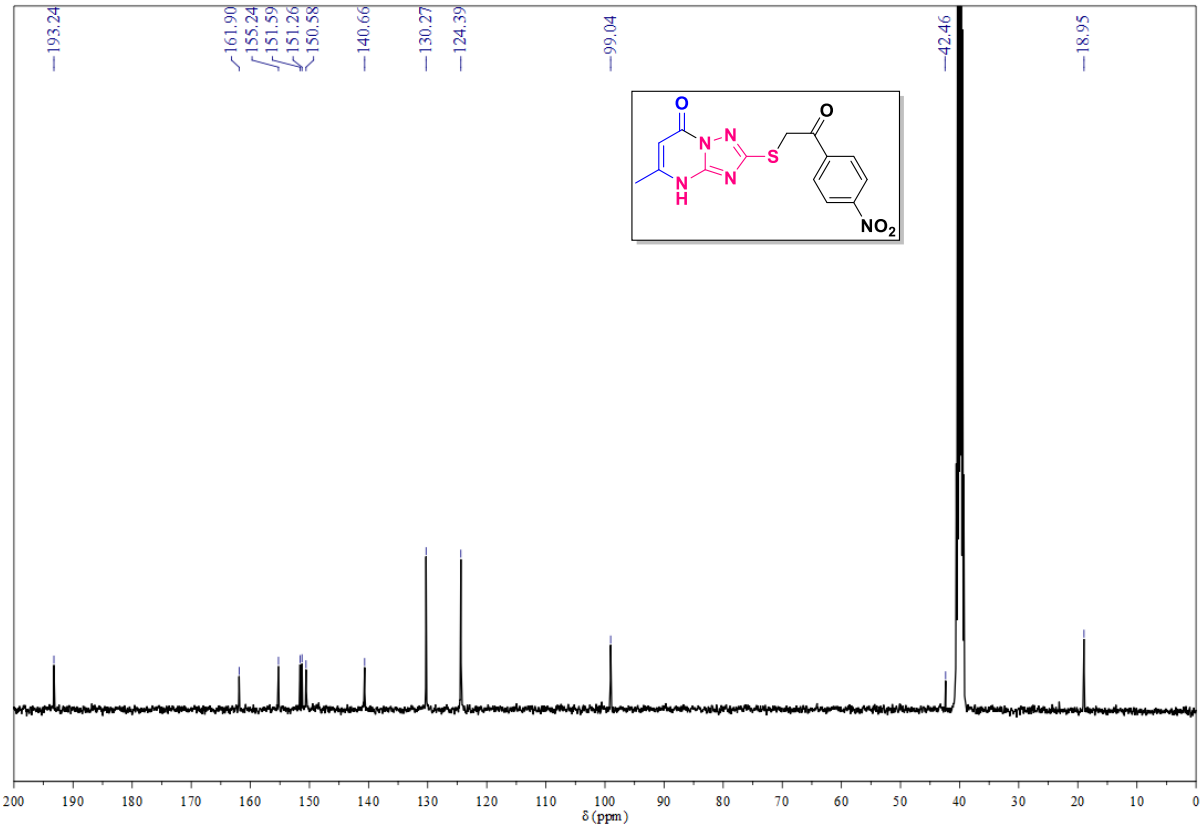
HRMS spectrum of compound 4d



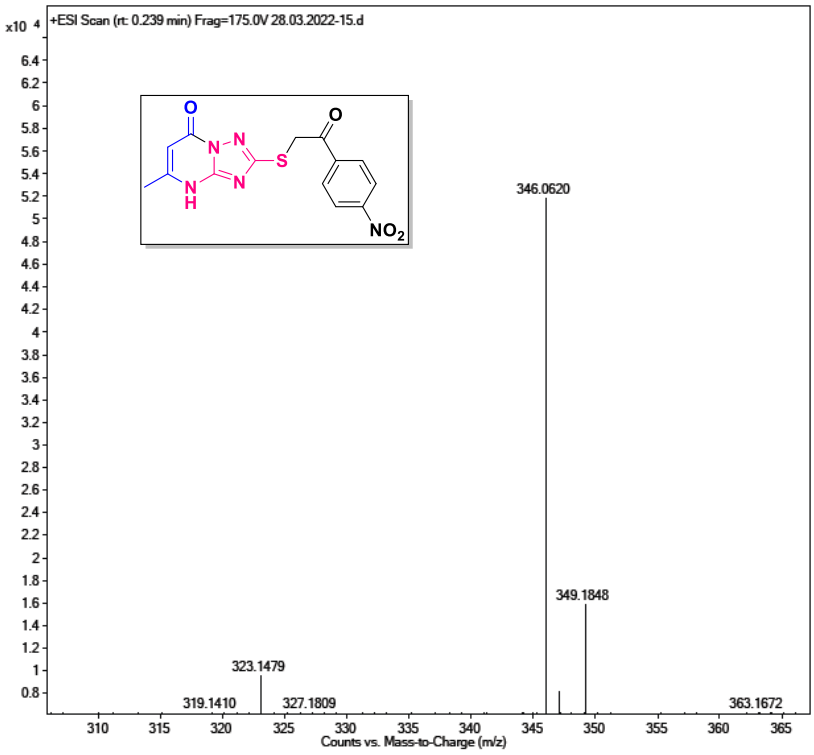
^{1}H NMR spectrum of compound 4e (DMSO-d_6 400 MHz)



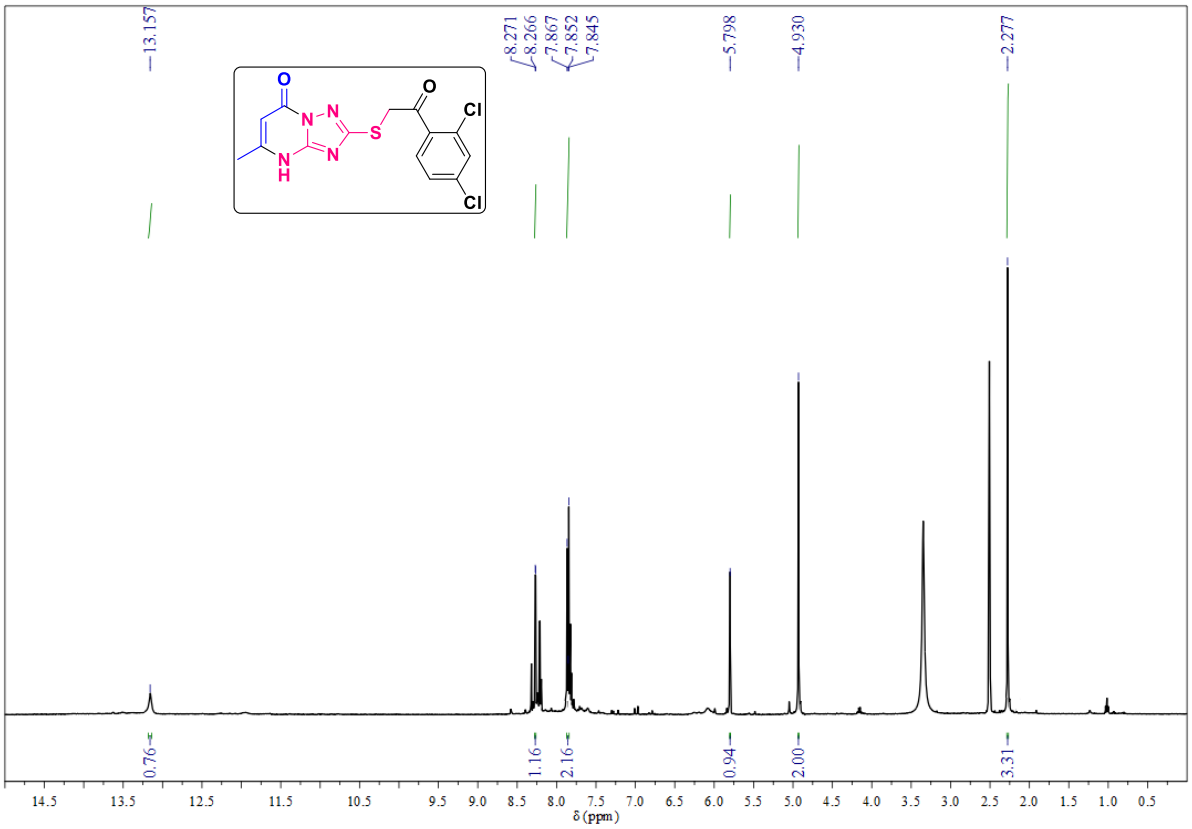
^{13}C NMR Spectrum of compound 4e (DMSO-d_6 100 MHz)



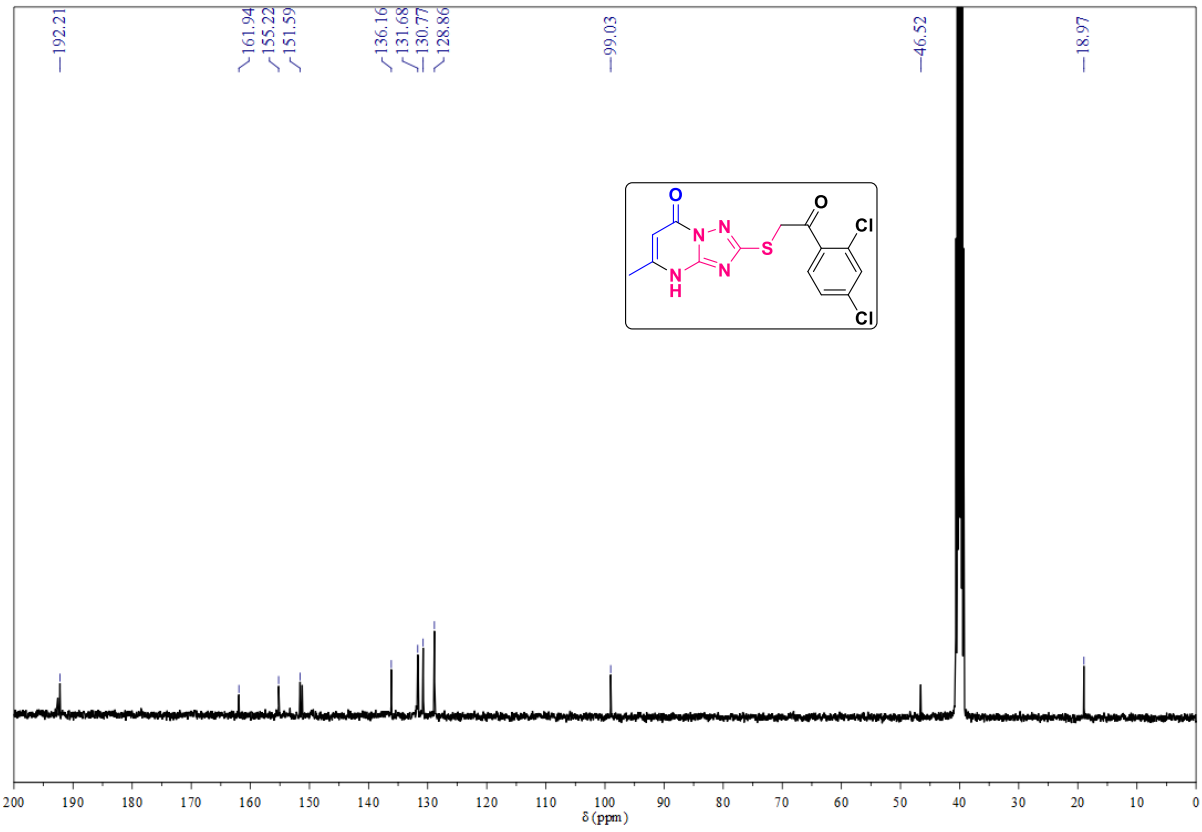
HRMS spectrum of compound 4e



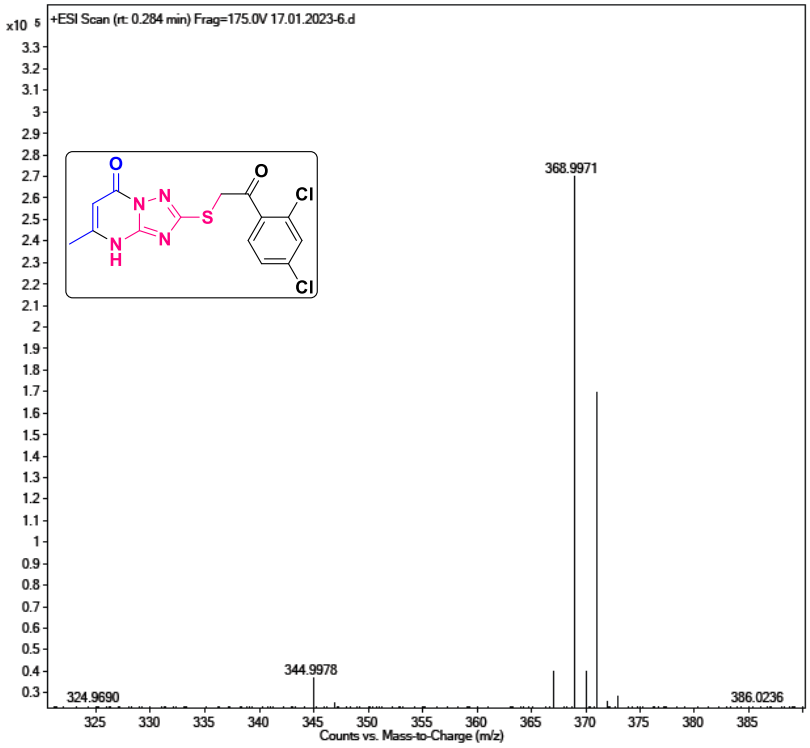
^{1}H NMR Spectrum of compound 4f (DMSO-d_6 400 MHz)



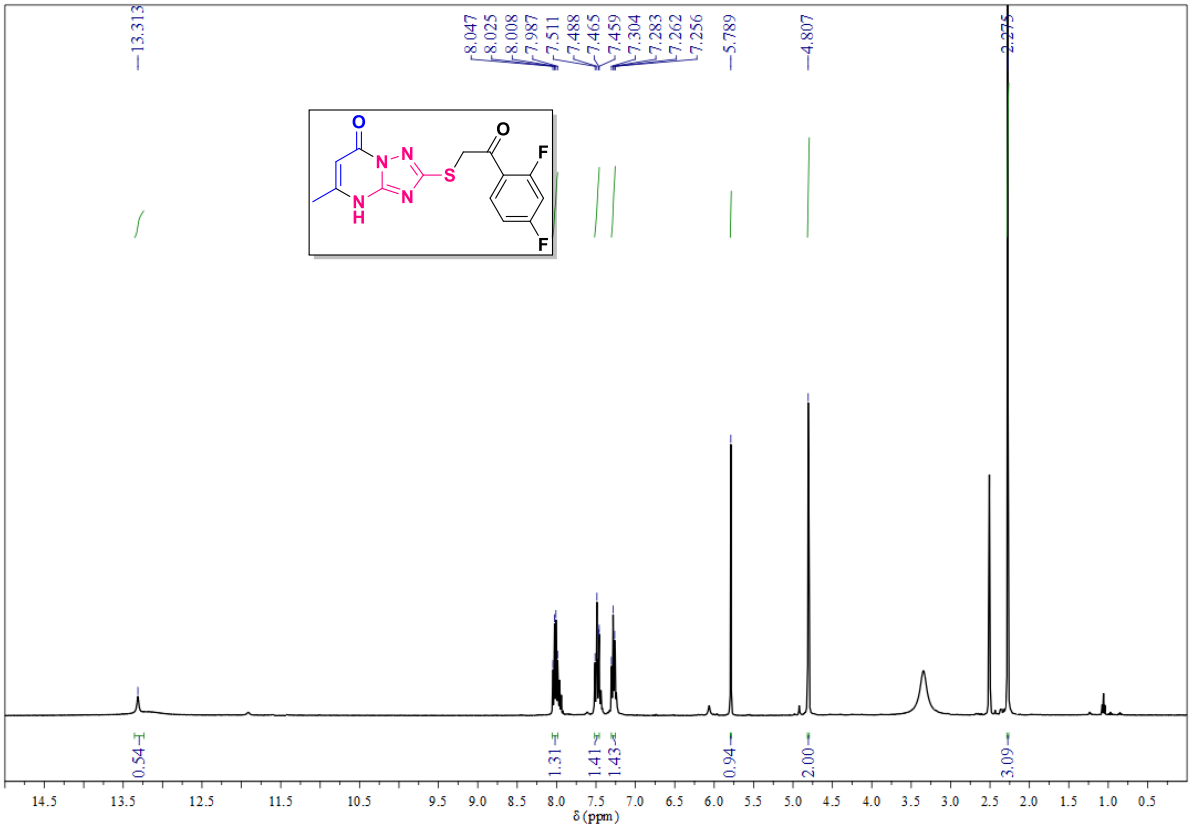
^{13}C NMR Spectrum of compound 4f (DMSO-d_6 100 MHz)



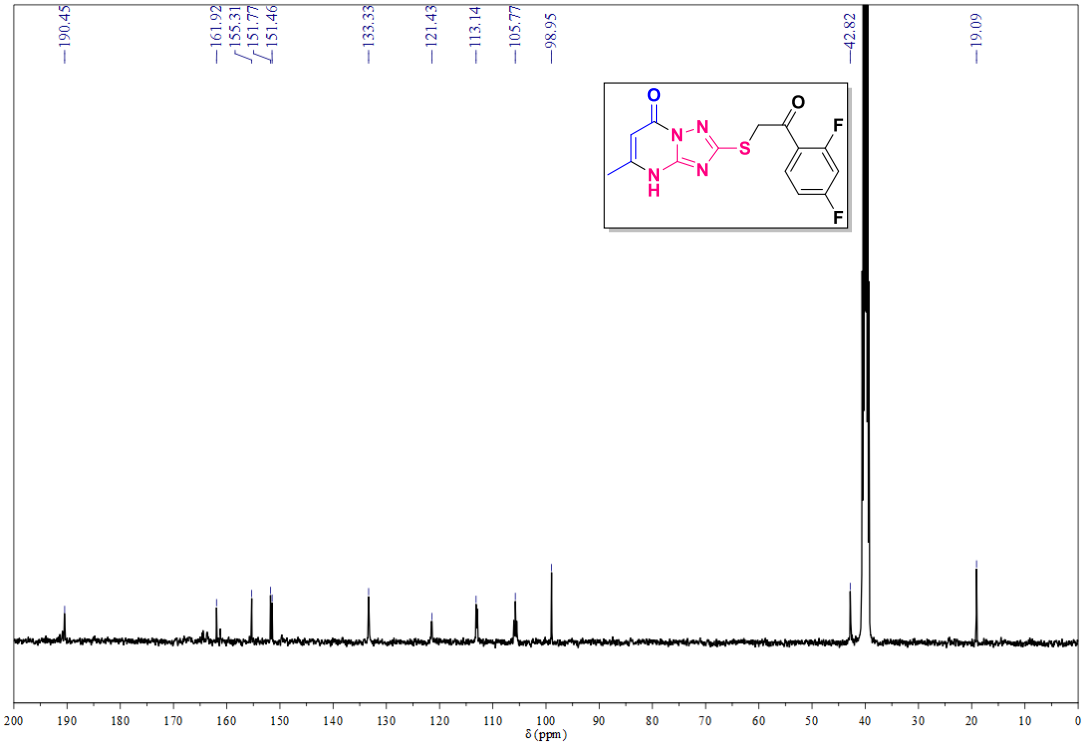
HRMS spectrum of compound 4f



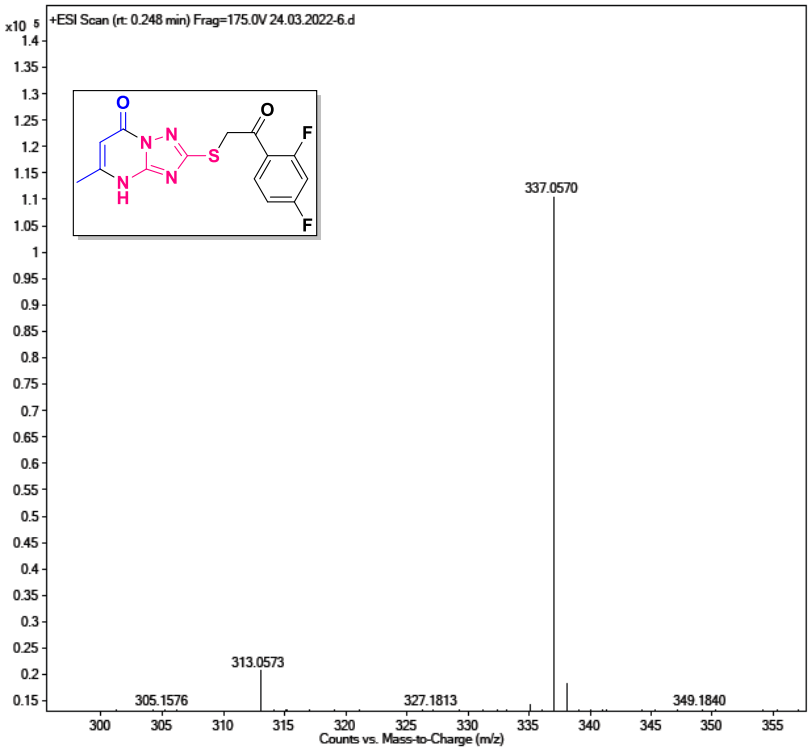
^{1}H NMR Spectrum of compound 4g (DMSO-d_6 400 MHz)



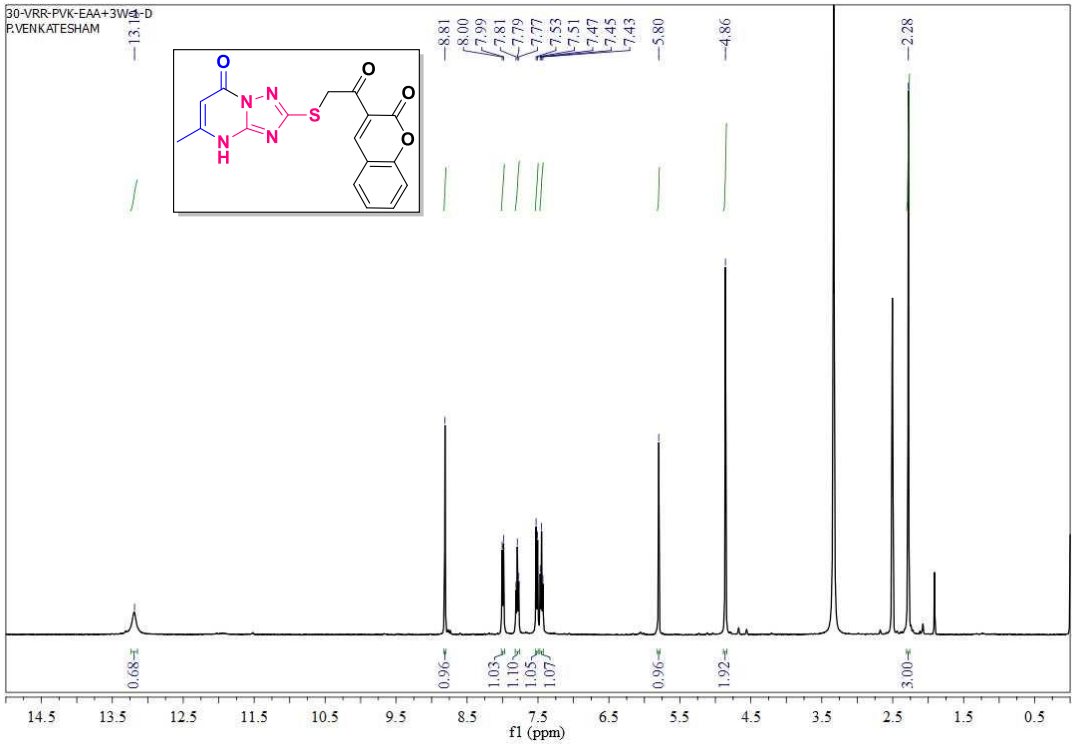
^{13}C NMR spectrum of compound 4g (DMSO-d_6 100 MHz)



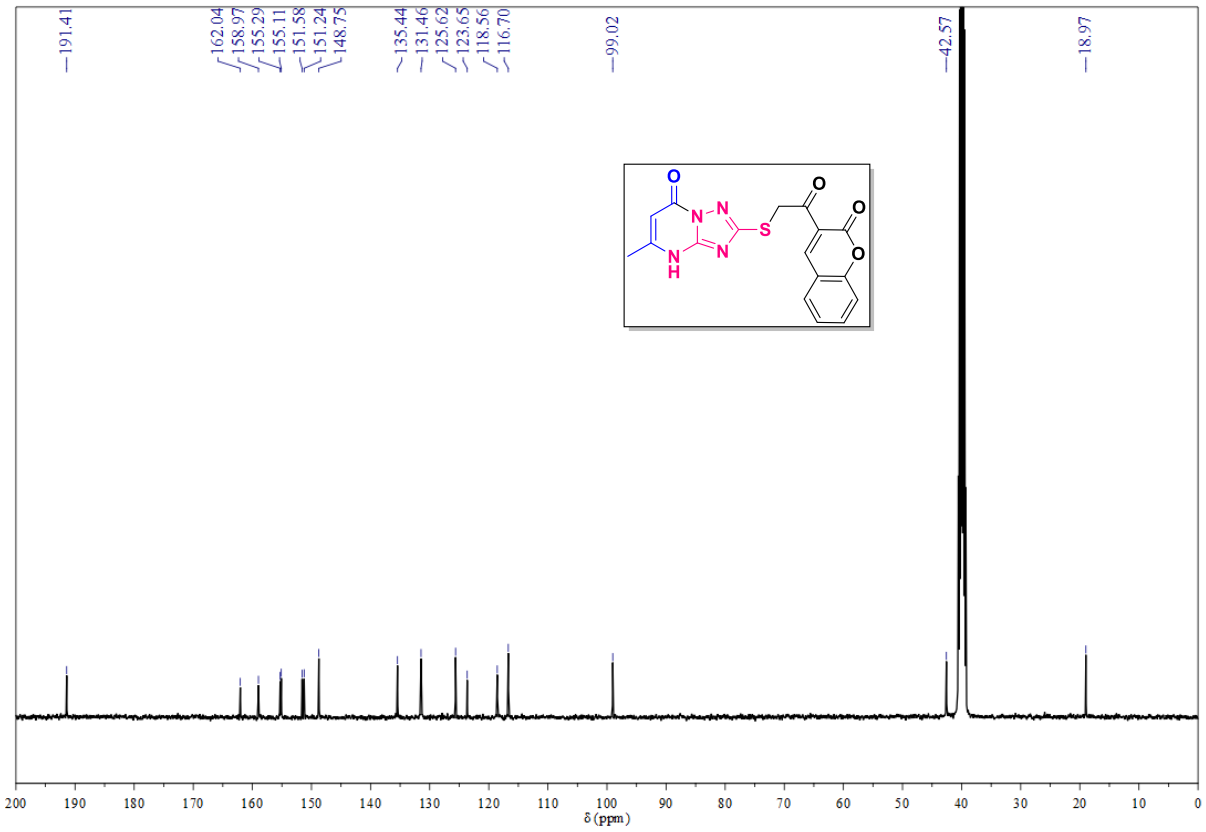
HRMS Spectrum of compound 4g



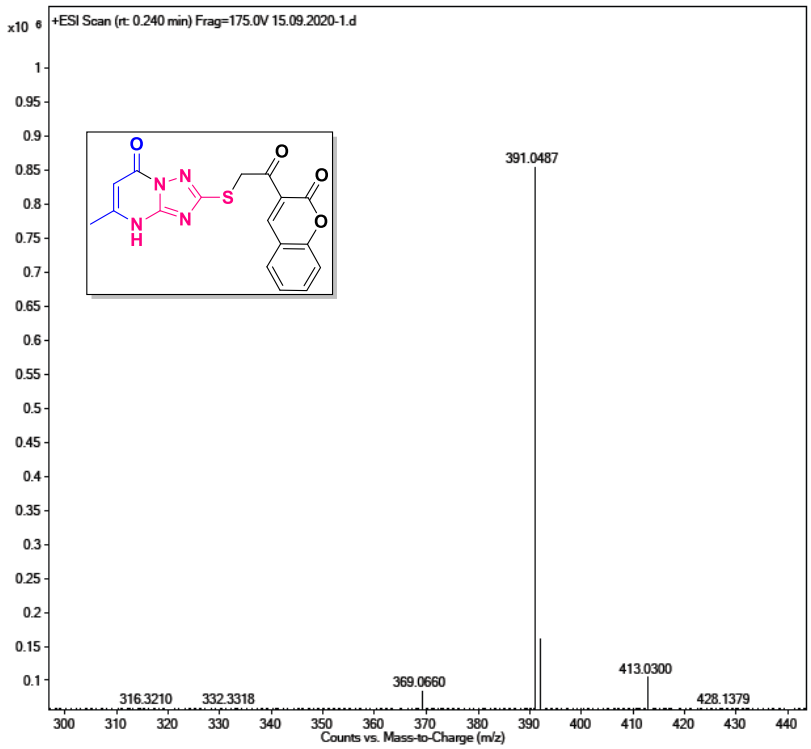
^{1}H NMR Spectrum of compound 4h (DMSO-d_6 400 MHz)



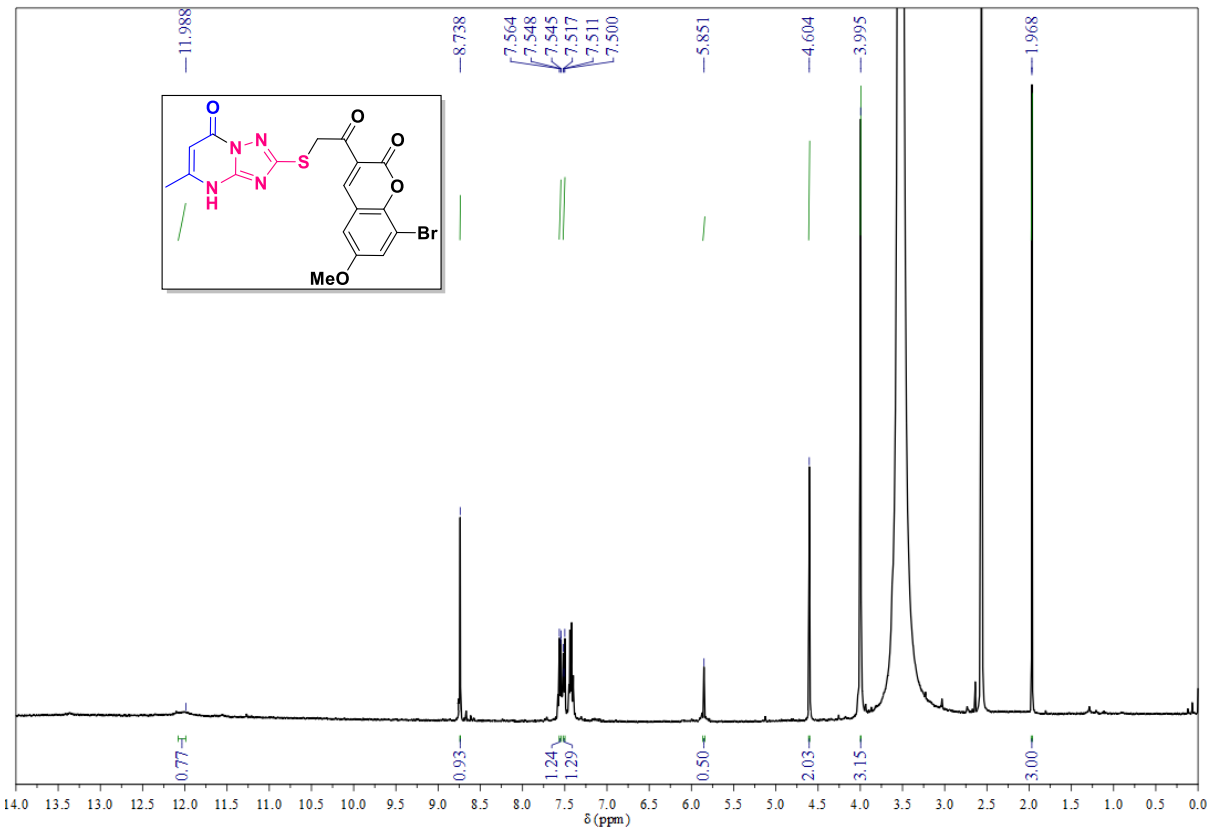
^{13}C NMR spectrum of compound 4h (DMSO-d_6 100 MHz)



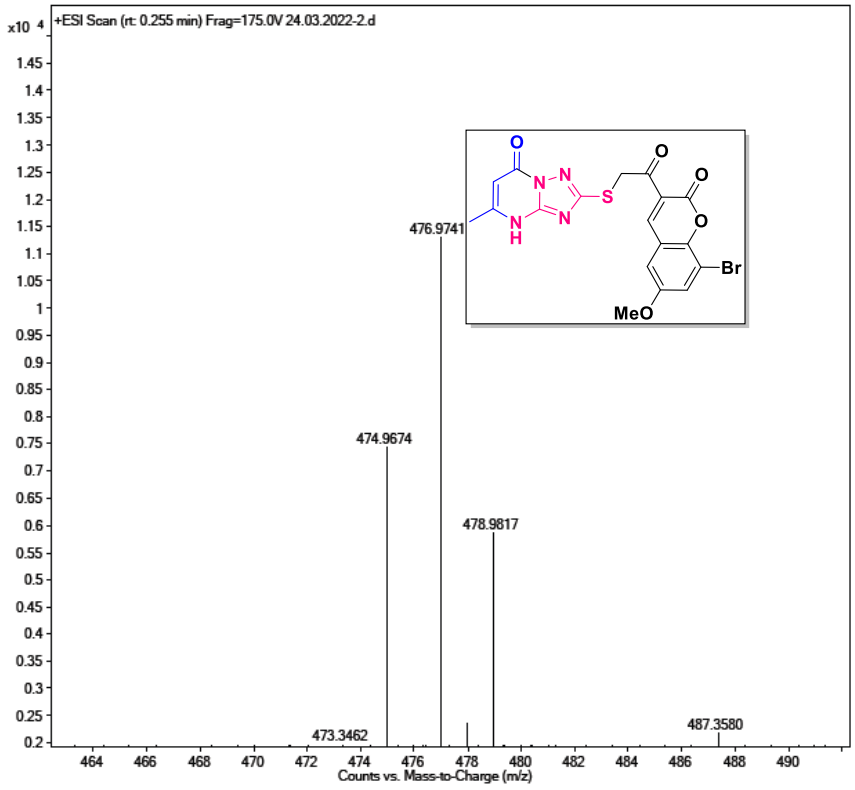
HRMS spectrum of compound 4h



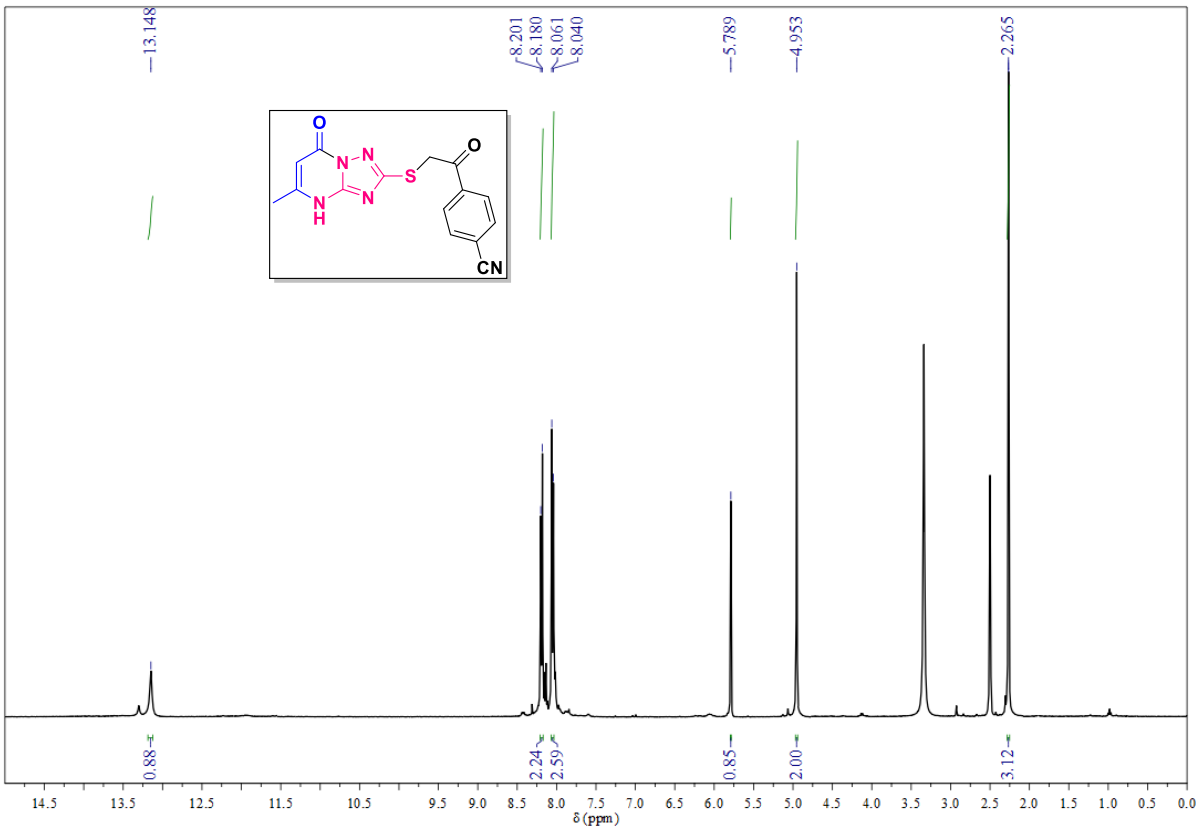
^{1}H NMR Spectrum of compound 4i (DMSO-d_6 400 MHz)



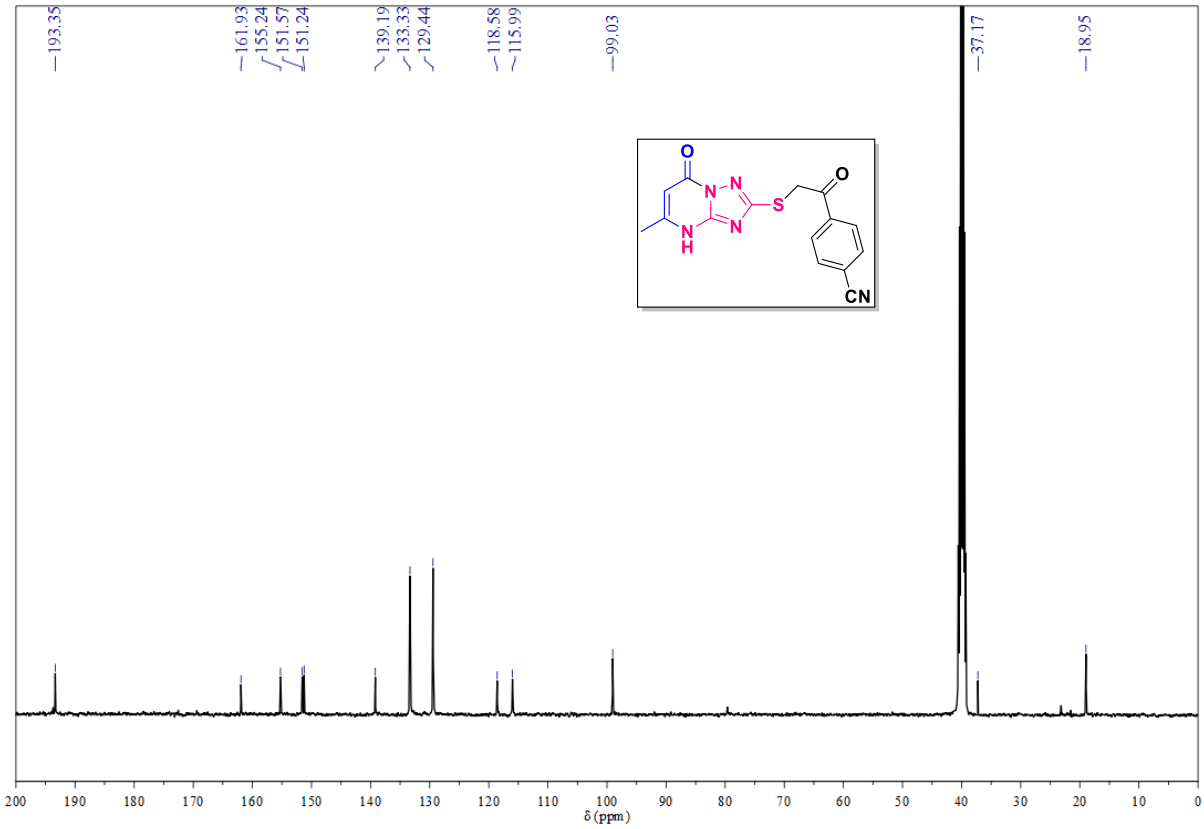
HRMS spectrum of compound 4i



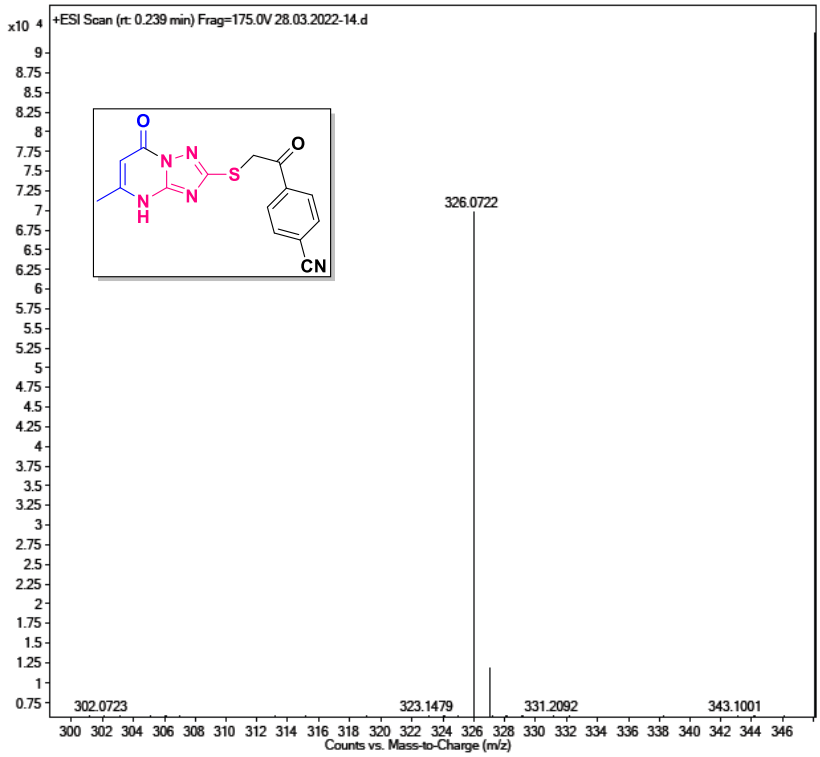
^{1}H NMR spectrum of compound 4j (DMSO-d_6 400 MHz)



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

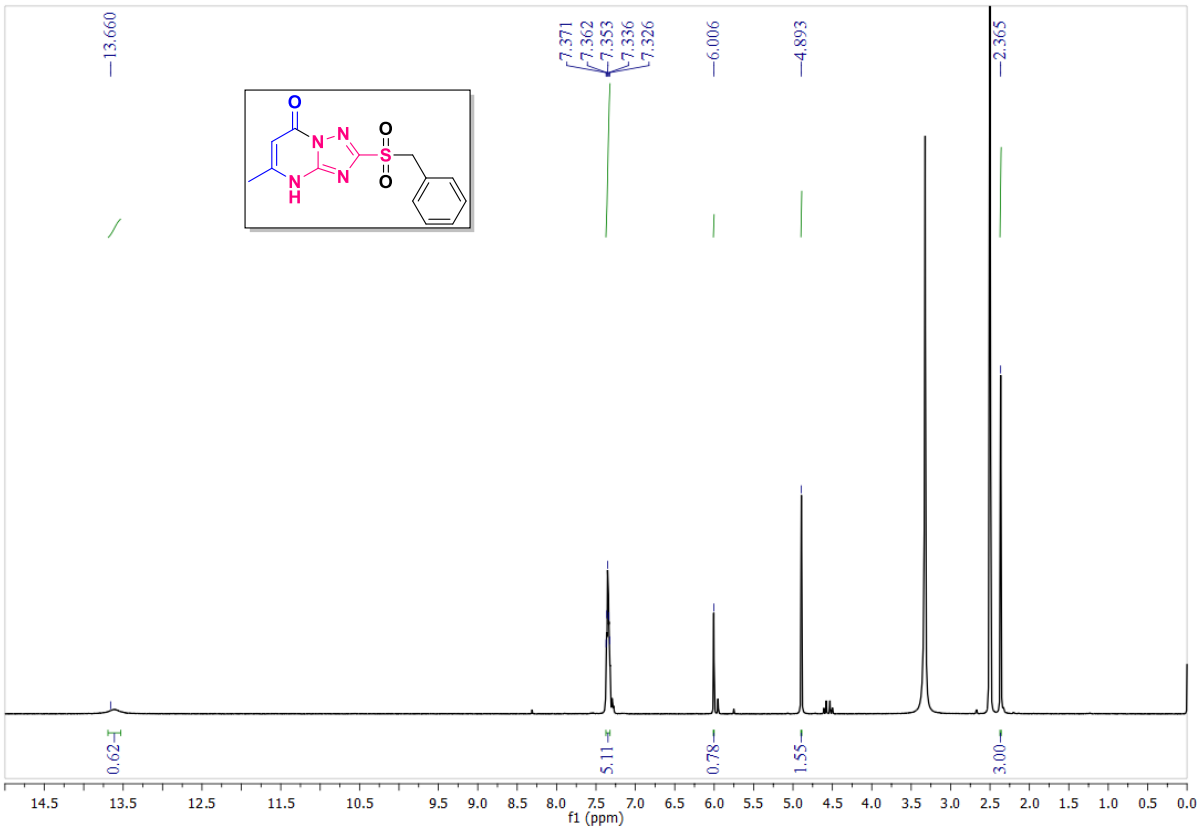


HRMS Spectrum of compound 4j

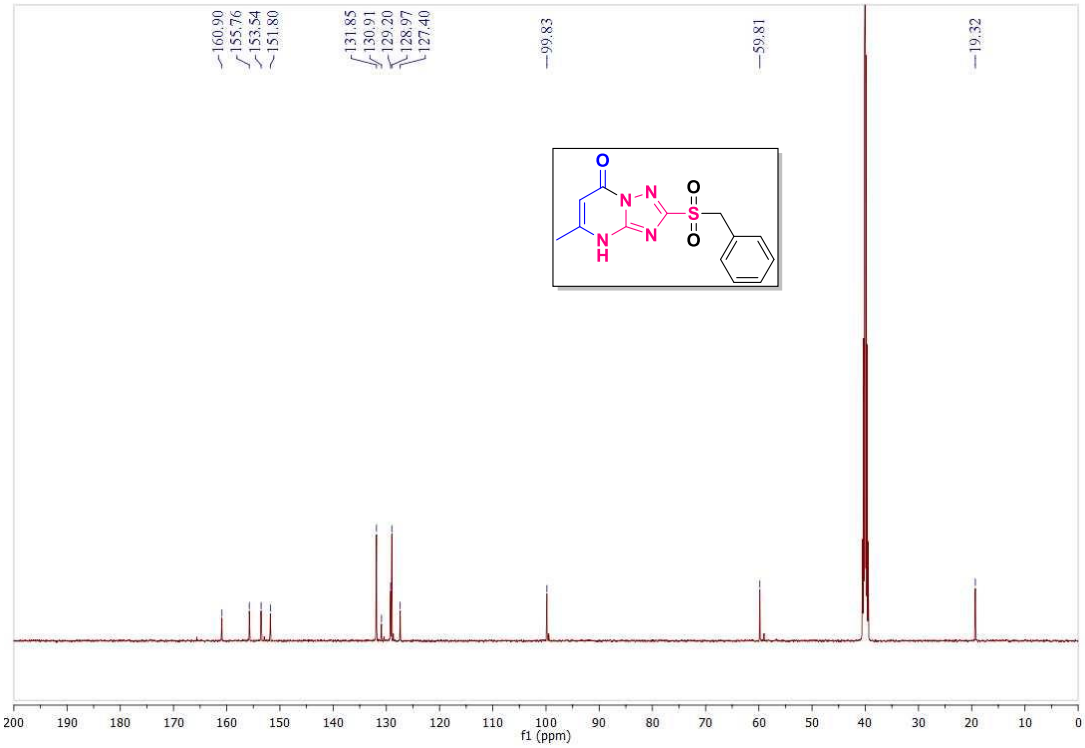


SCHEME-II (SULFONES)

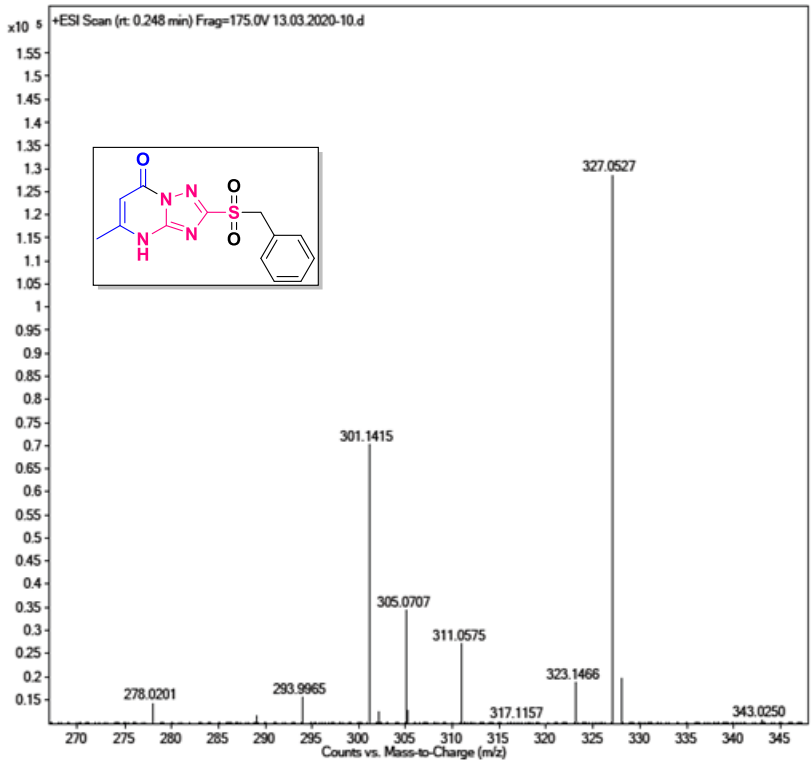
^{1}H NMR Spectrum of compound 6a (DMSO-d_6 400 MHz)



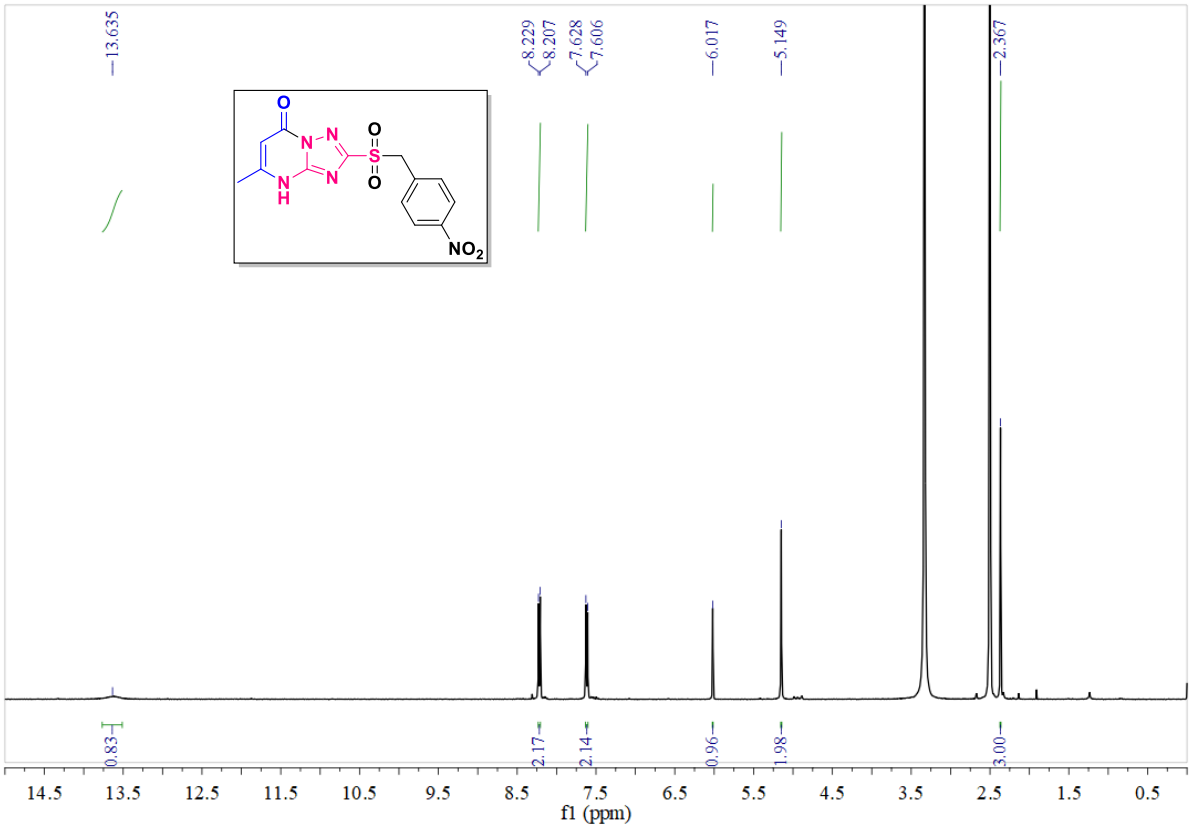
^{13}C NMR Spectrum of compound 6a (DMSO-d_6 100 MHz)



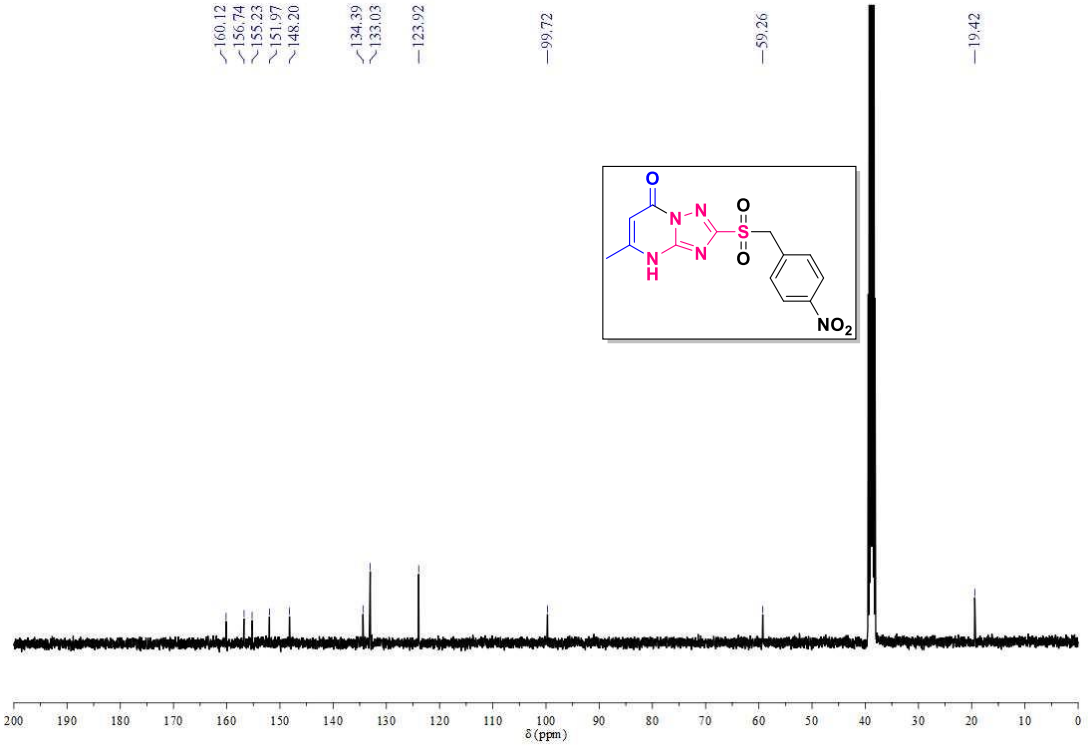
HRMS spectrum of compound 6a



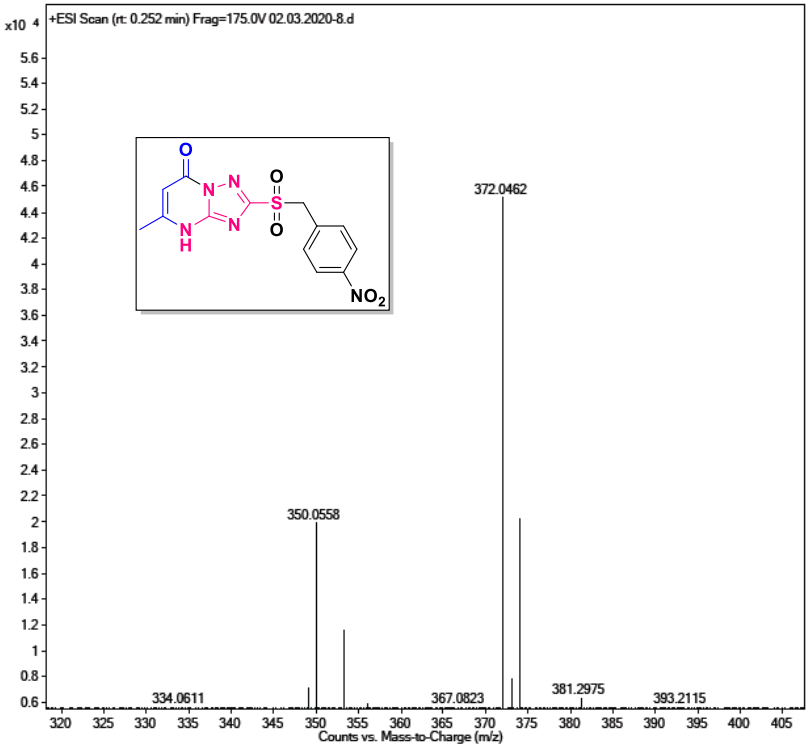
^{1}H NMR Spectrum of compound 6b (DMSO-d_6 400 MHz)



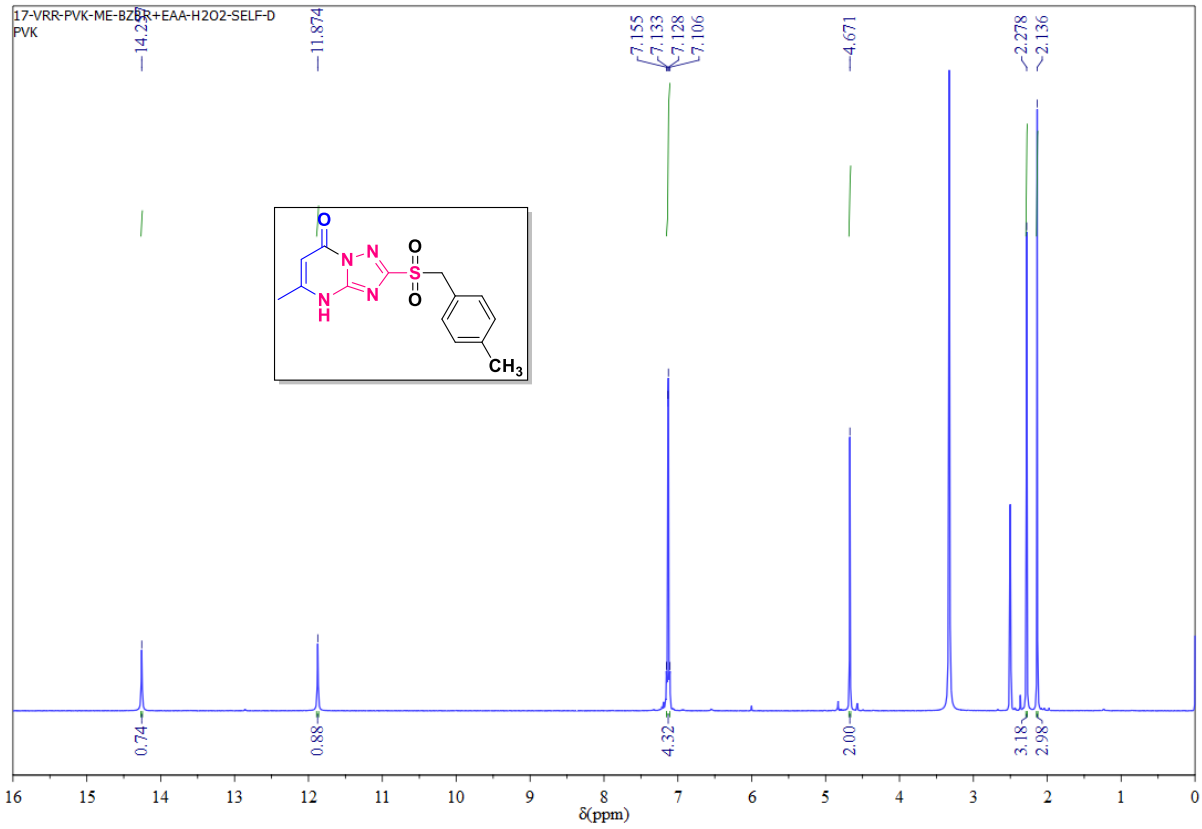
^{13}C NMR Spectrum of compound 6b(DMSO-d_6 100 MHz)



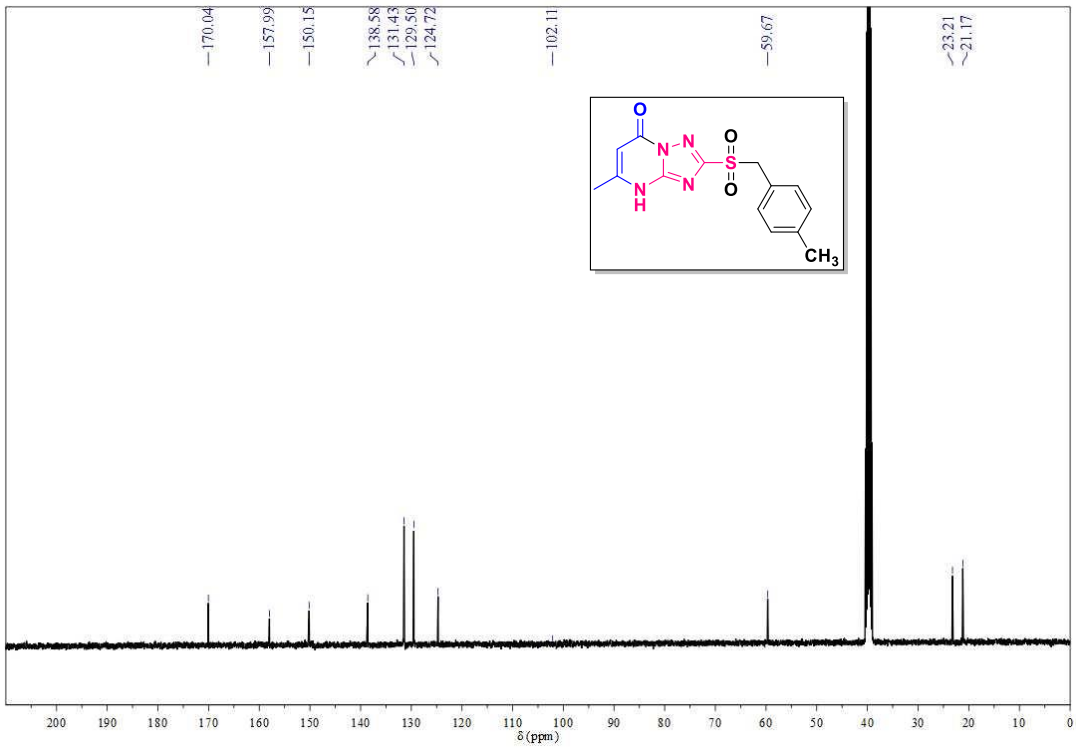
HRMS Spectrum of compound 6b



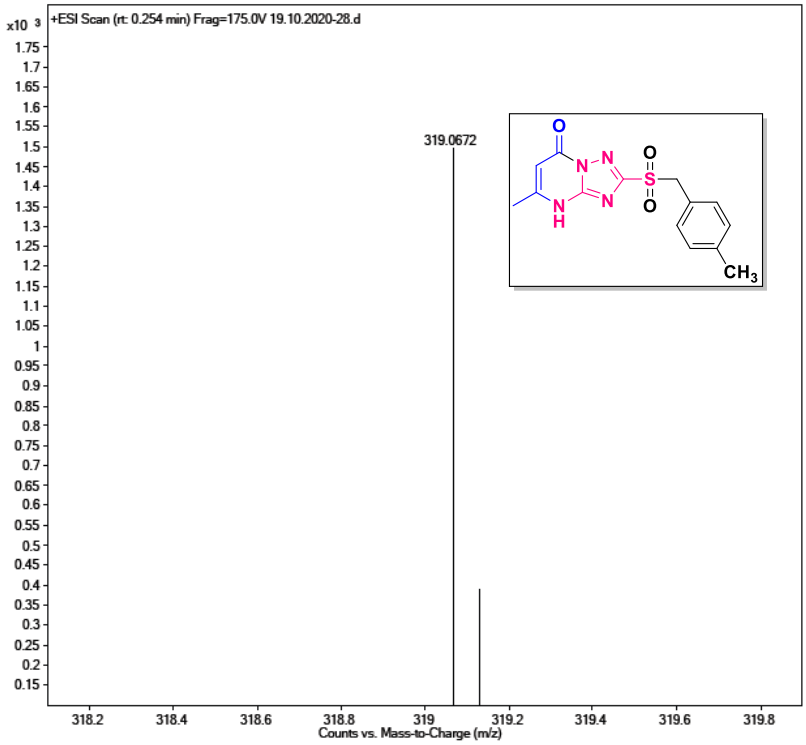
^{1}H NMR Spectrum of compound 6c (DMSO-d_6 400 MHz)



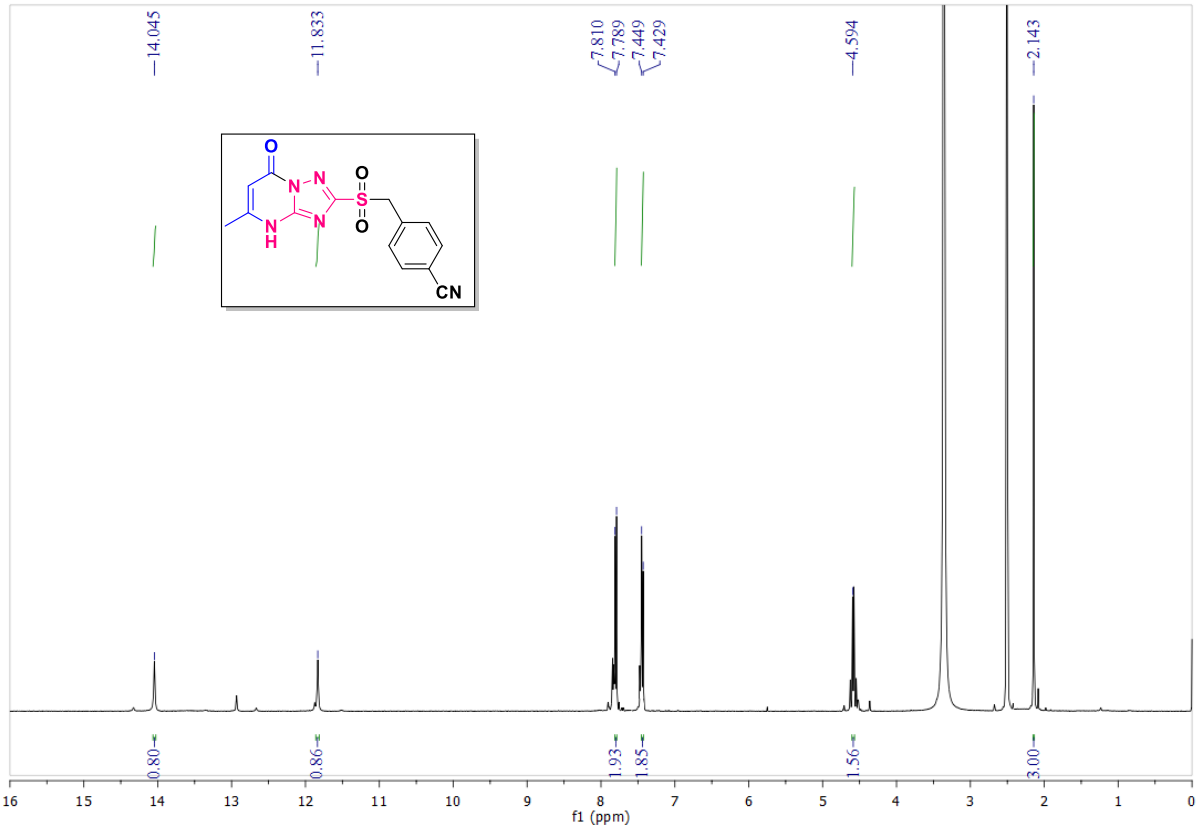
^{13C} NMR Spectra of compound 6c (DMSO-d_6 100 MHz)



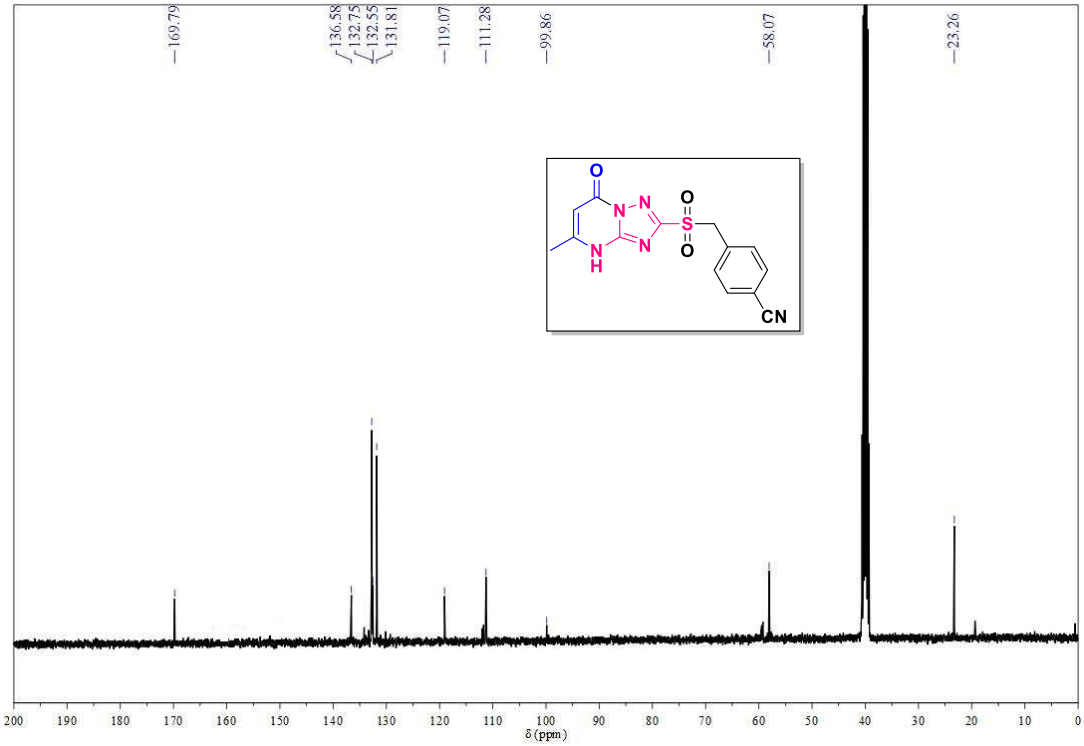
HRMS Spectrum of compound 6c



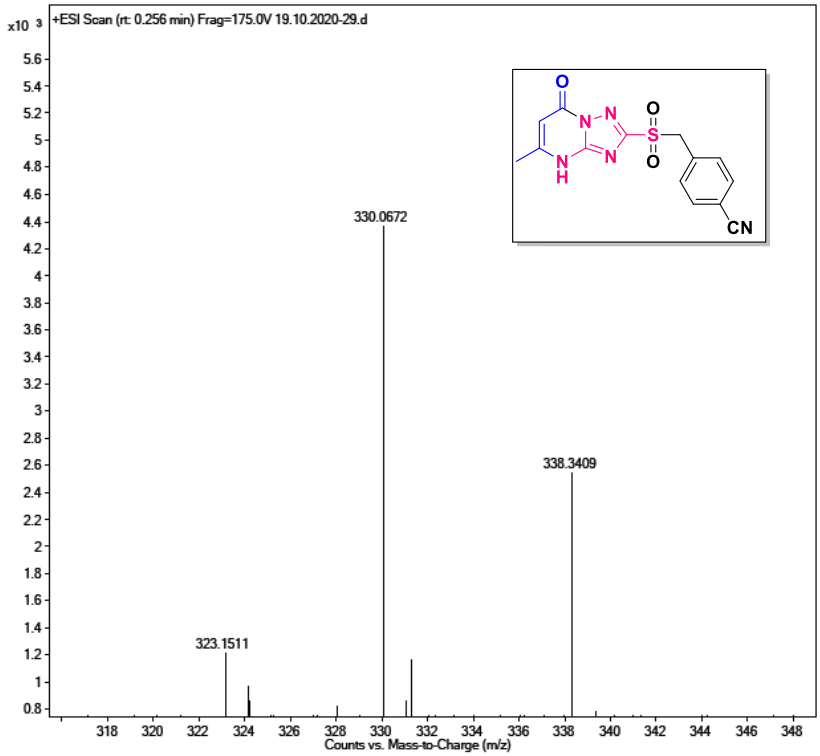
^{1}H NMR Spectrum of compound 6d (DMSO-d_6 400 MHz)



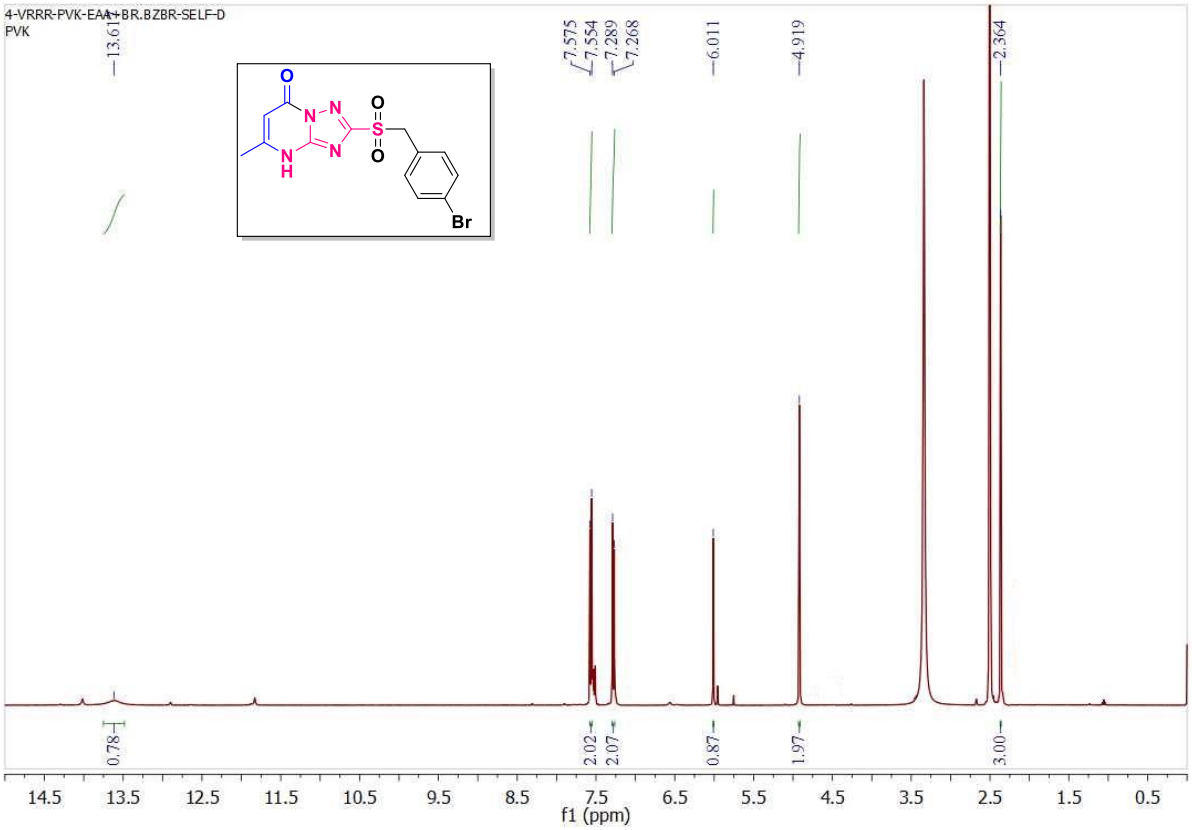
^{13}C NMR Spectrum of compound 6d (DMSO-d_6 100 MHz)



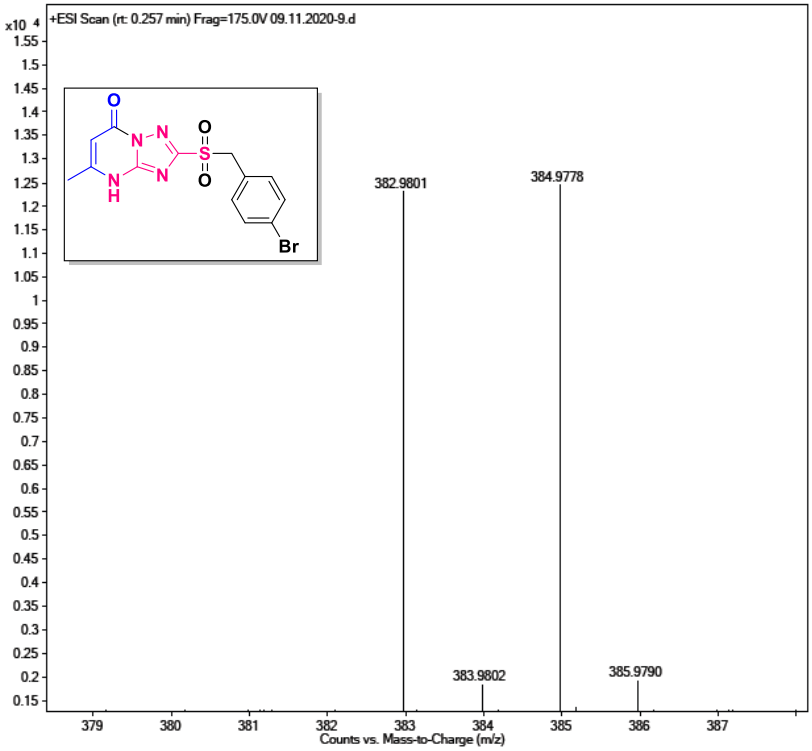
HRMS spectrum of compound 6d



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



HRMS Spectrum of compound 6e



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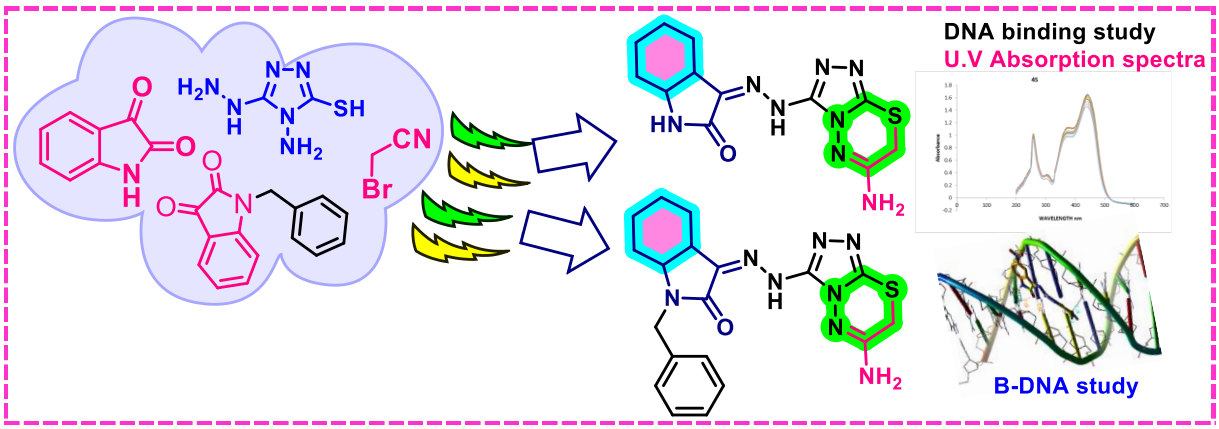
CHAPTER-VI

Novel one-pot synthesis, characterization, DNA binding studies of fused [1,2,4]-triazolo [3,4-b][1,3,4] 6-aminothiadiazines and their hydrazineylidene indolin-2-ones, Schiff bases



6.1 Introduction

Hetero aromatic moieties comprising nitrogen and sulphur atoms have significant role in the realm of heterocyclic chemistry over oxygen hetero atom[1]. Literature reports also reveals that the sulphur and nitrogen containing heteroatoms exhibit broad spectrum of biological and medicinal applications [2,3]. Due to various structural and biological activities of these thiadiazine unit, researchers have interested to elaborate a novel triazolothiadiazines by using various methods[4]. Thiol and amino functional groups are interestingly involved in the synthesis of triazolothiadiazoles[5], triazolothiadiazines[6], thiadiazipines[7]. Specially the triazolothiadiazines have potential applications in medicinal and pharmaceutical industry [8-10]. The possible isomeric structures for triazolothiadiazines are 1,2,4-triazolo [5,1-*b*][1,3,5]thiadiazine, 1,2,4-triazolo[3,4-*b*][1,3,4] thiadiazine, 1,2,4-triazolo [5,1-*b*][1,3,4] thiadiazine, 1,2,4-triazolo[1,5-*c*][1,3,5]thiadiazines[11]. Among these 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines were found to have a wide range of therapeutic and biological uses[12-14]. These moieties have ability to show hydrogen bond donating and accepting characteristics and this core makes as précised pharmacophore for the interaction of target proteins [15-17].

Triazolothiadiazines have shown promising biological activities such as antiviral [18], antioxidant [19], anti-inflamatory [20], antibacterial [21], anticancer [22], antihelminthic [23], anticandidal [24], antitubercular [25], antimicrobial[26] etc. **Fig.1** shows the biologically active triazolothiadiazine molecules. The phenyl substituted thiadiazine compound-1 demonstrate anticancer activity [27], the ester functional group containing fused thiadiazine compound 2 is having a potent antibacterial agent [28], The pyridine ring attached triazole moiety compound 7 shows antileishmanial activity [29], and the compounds 3,4,5,6,8 exhibit various biologically activities [30-34]. In the present research work by considering the importance of triazolothiadiazines we were specially choosen for the synthesis of new pharmacologically active triazolothiadiazine heterocyclic compounds through multicomponent approach. The advantages of this method in which most of the atoms participated in newly formed bonds and these reactions are atom economic, good substrate scope, required time for the completion of the reaction is less [35, 36].

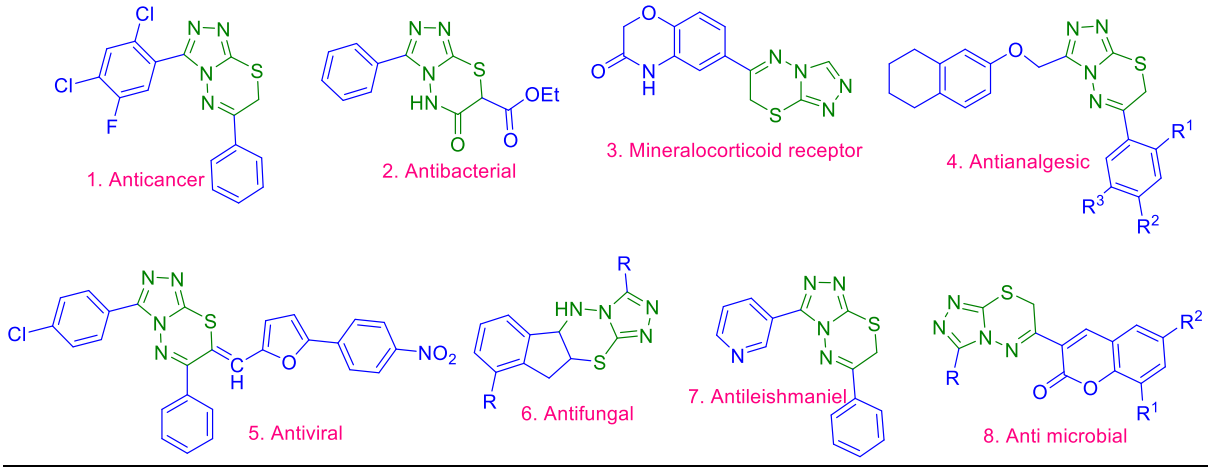


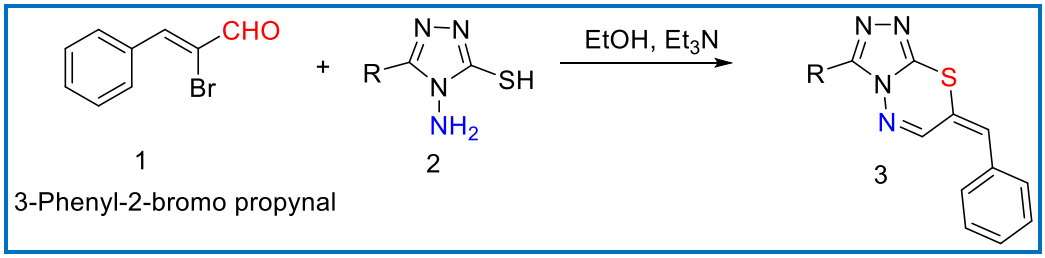
Fig.1. Reported biologically active thiadiazine compounds.

Furthermore, the isatin derivatives have outstanding building block in organic synthesis. six-membered triazolothiadiazine is linked to isatin or Schiff base the biological activity of such molecules increases [37].

Literature reports of 1,2,4-triazolothiadiazines

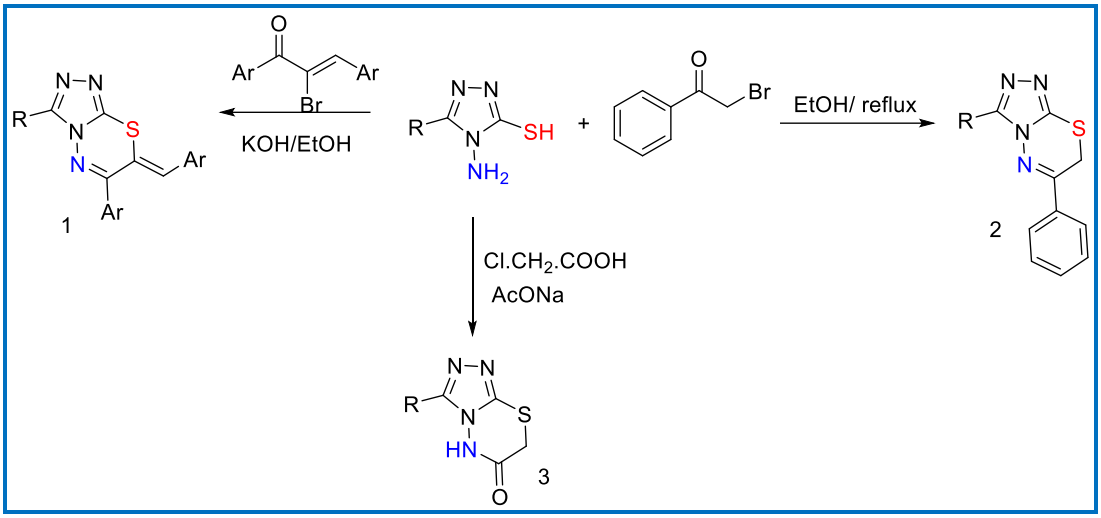
Kharamchikhin *et al* [38] reported a one-pot protocol for the development of 3-R-7(phenyl methylene)-s-triazolo[3,4-b][1,3,4]thiadiazine-3 which has depicted in **scheme-1.1**. 2-Bromo-3-phenyl acryl aldehyde was condensed with mercapto amino 1,2,4-triazole compound 2 to give an expected product 3 with high yield. These substrates have shown antiviral activity.

Scheme-1.1



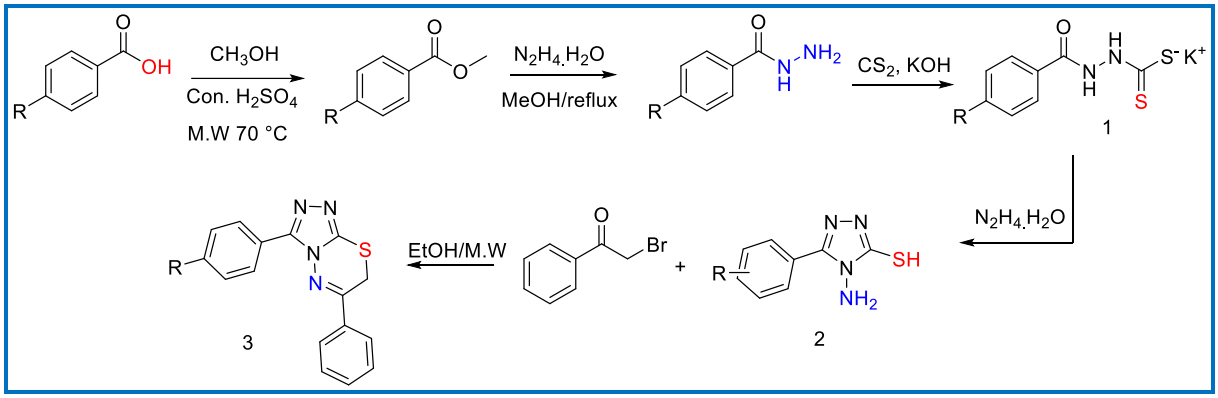
Shehry *et al* [39] reported the synthesis of 1,2,4-triazolothiadiazole 1,2,3 final compounds from the reaction of mercapto amino triazole-1with phenacyl bromides, chloroacetic acid and 2-bromo-1,3-diphenyl prop-2-en-1-ones under suitable reaction conditions was represented in **scheme-1.2.** Further these derivatives were tested for their anti-inflammatory activity**.**

Scheme-1.2



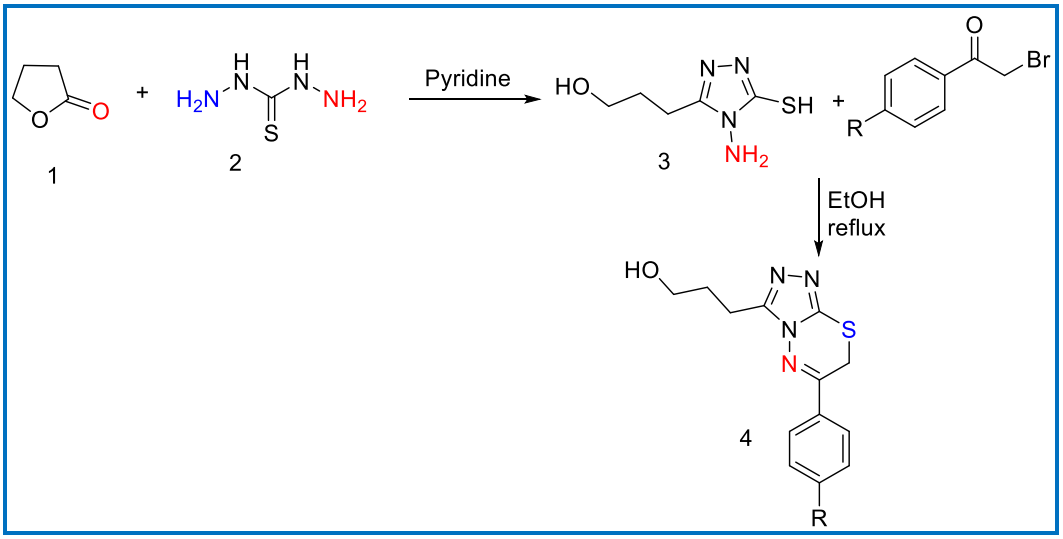
XU *et al* [40] reported an efficient step wise protocol for the synthesis of 3,6 diphenyl fused-7*H*-[1,2,4]-triazolo [3,4-*b*] [1,3,4] thiadiazines-3 was shown in scheme-3. In this initially benzoic acid converts into ester then converts into benzo hydrazide. This is on reaction with CS_2 to form a carbodithioate-1 again cyclocondensation reaction takes place and subsequently reaction with 2-halo aceto phenone to obtain bridged heterocyclic system-3. (Scheme-1.3).

Scheme-1.3



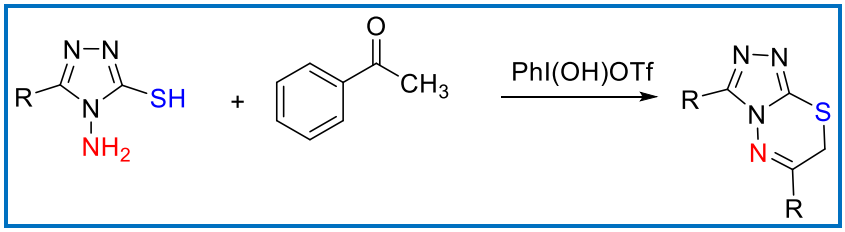
Jin *et al* [41] reported the synthesis of the dihydro furan 2(3*H*)-one, by the condensation of thiocarbohydrazide and γ-butyro lactone under in pyridine and reflux for 6 h. The white color compound 4-amino-3-(3-hydroxy propyl)-5-mercapto-1,2,4-triazole was separate and then treated with appropriate 2-bromo acetophenones in ethanol to produce a 3,6 di substituted 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazole-4. These were promising inhibitory activity on the growth of radish, wheat seeds. (Scheme-1.4)

Sheme-1.4



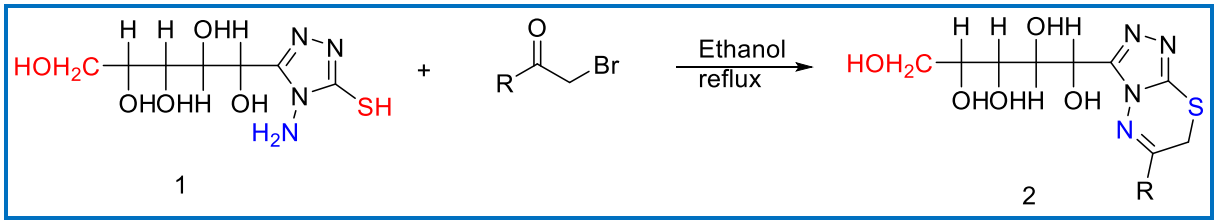
Singh *et al* [42] reported that ketones on oxidation reaction with [hydroxy(tosyloxy)iodo] benzene to form an intermediate α-tosyloxy ketones these on subsequently reaction with 4-amino-5-mercapto-1,2,4-triazole in presence of CH_3CN at reflux temperature to form triazolothiadiazine final compound. (Scheme-1.5)

Scheme-1.5



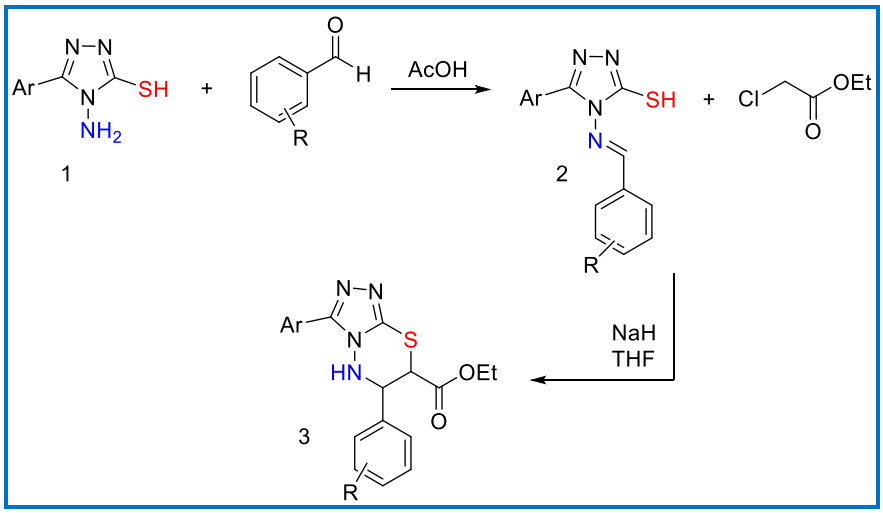
Xiong *et al* [43] developed the reaction of 4-amino-5-mercapto-3-(D-gluco-pentitol-1-yl)-1,2,4-triazole-1 and 2-bromo acetophenone in the presence of ethanol and reflux for 3 h to generate a 6-aryl-3-(D-glucopentitol-1-yl)-7*H*-1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines-2. (Scheme-1.6)

Scheme-1.6



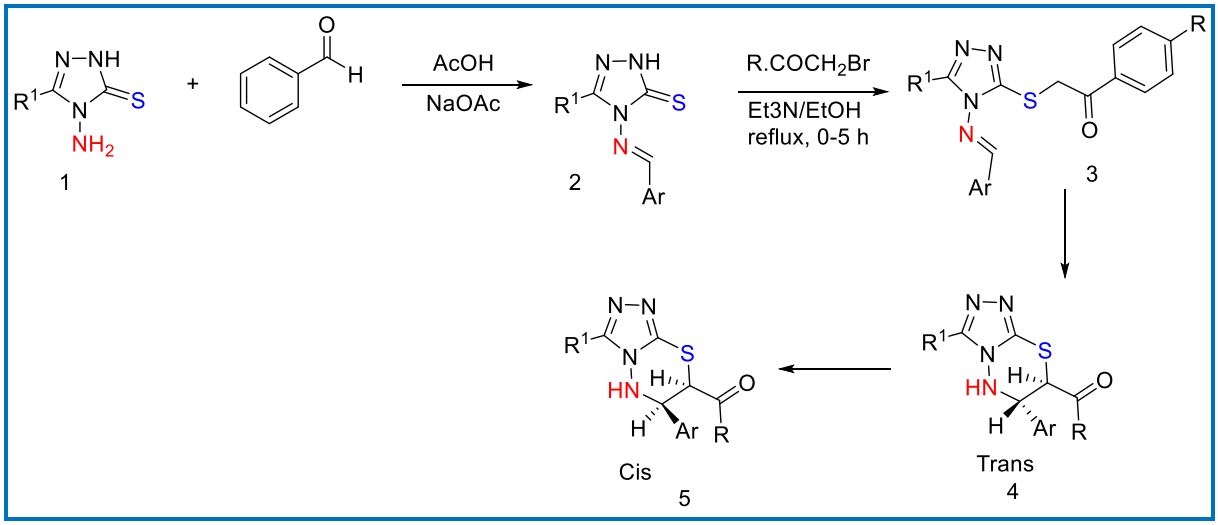
Foroughifar *et al* [44] published the two-step process for the synthesis of 1,2,4-triazolothiadiazole carboxylate compound-3. In this first step the aromatic aldehydes on reaction with mercapto amino 1,2,4-triazole-1 in the presence of acetic acid to form a Schiff base derivative-2. This on further cyclization with chloro ethyl acetate in dry THF/NaH to obtain the desired products. (Scheme-1.7)

Scheme-1.7



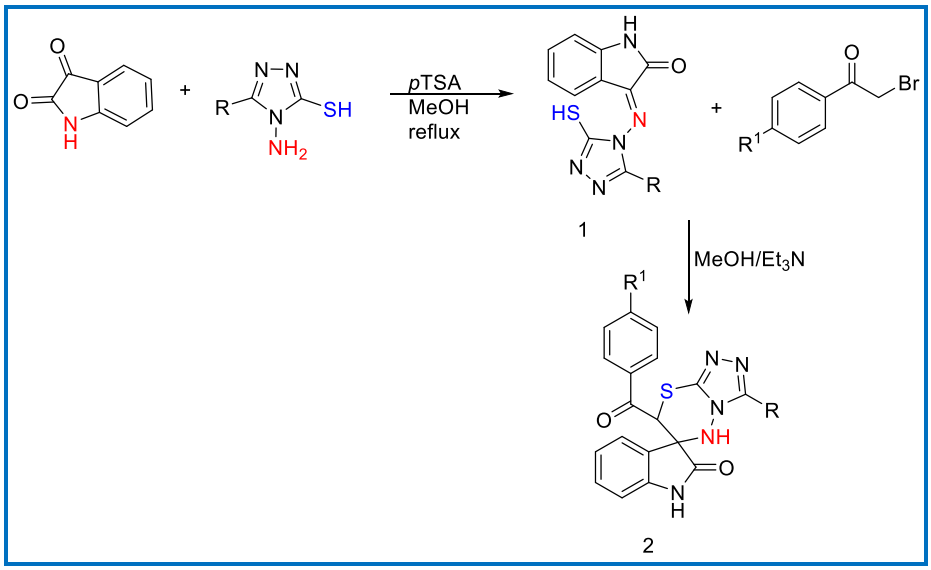
Al-Etaibi *et al* [45] developed base catalysed intramolecular C-C bond formation reaction of 3-alkyl-4-amino-5-mercapto triazole compound-1 on reaction with aromatic aldehydes in acetic acid/NaOAc to give a Schiff base-2. Then it is on condensation with 2-halo acetophenone (or) ethyl bromo acetate under ethanol/Et_3N exclusively to produce a stereo selective product trans-6,7-dihydro-5*H*-[1,2,4]-triazolo[3,4-*b*][1,3,4]thiadiazole-4-ones. (Scheme-1.8)

Scheme-1.8.



Ji *et al* [46] announciated the synthesis spiro oxindoles based [1,2,4]-triazolo[3,4-*b*][1,3,4]thiadiazoles form the reaction between isatin and mercapto triazole amine in the presence of *p*TSA/CH_3OH at reflux to form an intermediate-1. This was subsequently reaction with α-halo ketones in MeOH/Et_3N under reflux to generate spiro oxindole substituted triazolothiadiazine-2. And this substrate was exhibited anticancer activity against DU145, EC109, MGC803, MCF-7 cell lines. (Scheme-1.9)

Scheme-1.9



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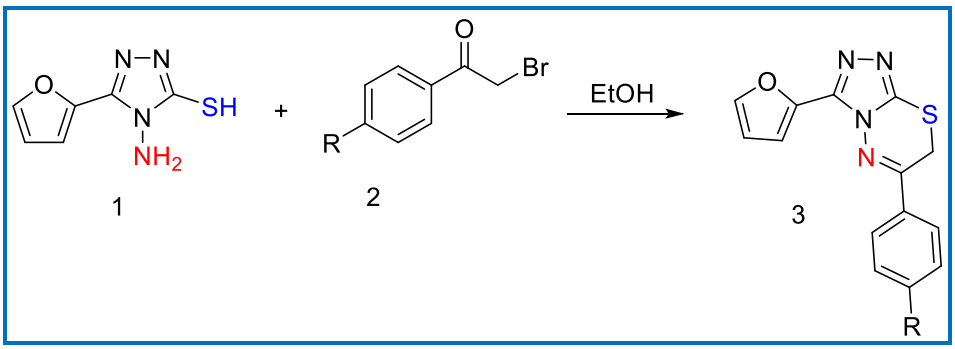
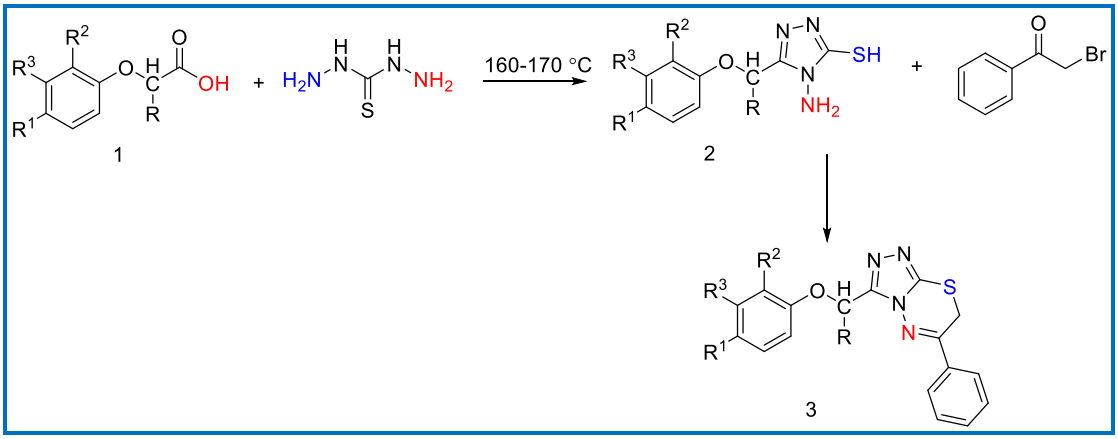
EI Shery *et al* [49] published the 4-amino-5-(furan-2-yl)-4*H*-1,2,4-triazole-3-thiol with 2-bromo acetophenones in presence of dry. EtOH to give a 3-(furan-2-yl)-6-phenyl-7*H*-[1,2,4]-triazolo[3,4-*b*][1,3,4]thiadiazines. And these compounds were evaluated for their antimicrobial activity against *staphylococcus epidermidis* and *Staphylococcus aureus*. (Scheme-1.10)

Scheme-1.10



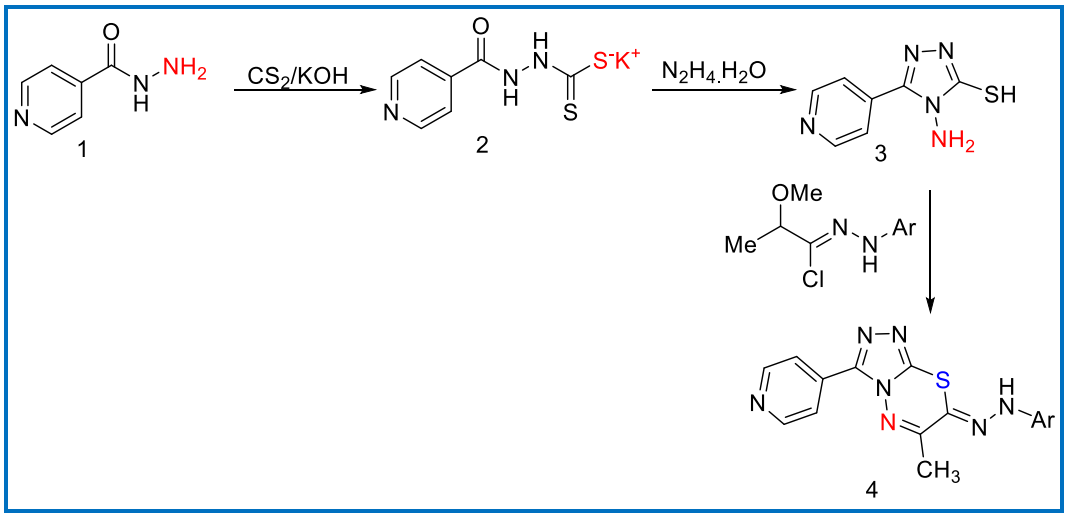
Prasad *et al* [47] reported the synthesis of 3-aryloxy alkyl-6-aryl-7*H*-s-triazolo[3,4-*b*][1,3,4]thiadiazine-5 in a single step by the reaction of aryloxy alkyl carboxylic acid-1, thiocarbohydrazide mixture heated on mantle at 160-170 °C for 1h to form a triazole compound-2. This was again on reaction with α-bromo acetophenone in the presence of ethanol under heating to produce a triazolothiadiazole-3. Additionally, these has been screened for their anti-inflammatory and analgesic activity. (Scheme-1.11)

Scheme-1.11



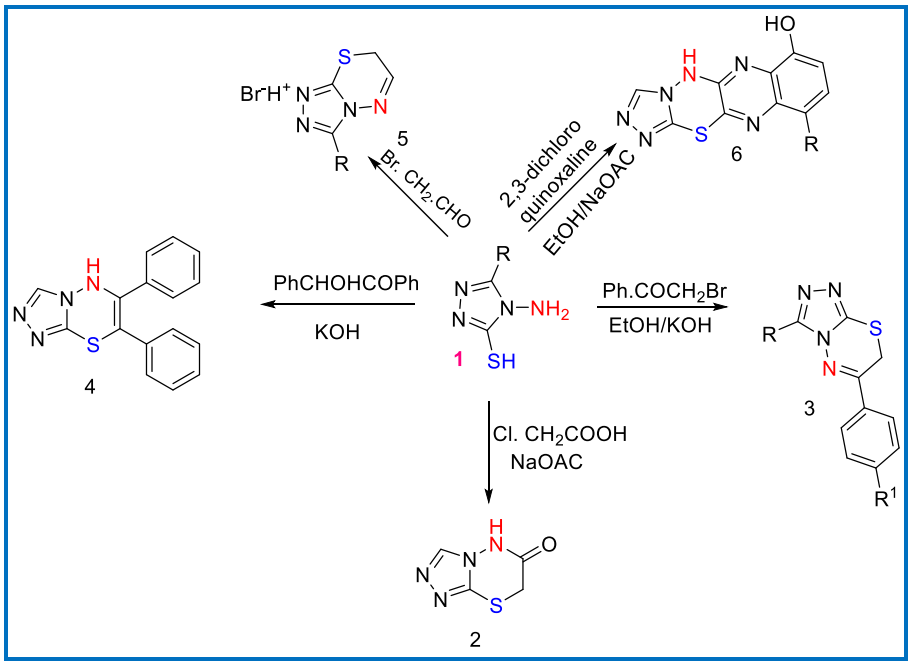
Bhat *et al* [48] reported the synthesis of triazolo thiadiazine derivative-4 starting from the reaction of isonicotinohydrazide with CS_2/KOH and subsequent condensation with hydrazine hydrate to obtained the corresponding triazole derivative-3. This was on further reaction with aryl hydrazonyl chloride in EtOH/Et_3N to generate final compound-4. The *in-vitro* anticandidal activity of these compounds was screened with reference to ketoconazole standard drug.

Scheme-1.12



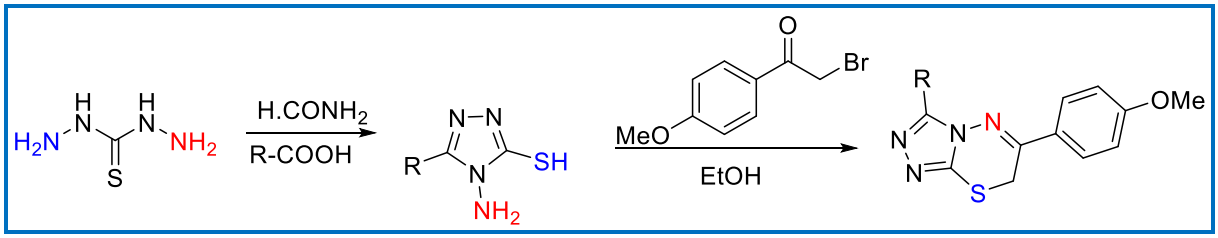
Mohan *et al* [50] developed numerous substituted bridgehead nitrogen heterocyclic systems with aid of the using of 3-alkyl-4-amino-5-mercapto 1,2,4-triazole. The designed substrates and conditions has shown in **scheme-1.13.**

Scheme-13



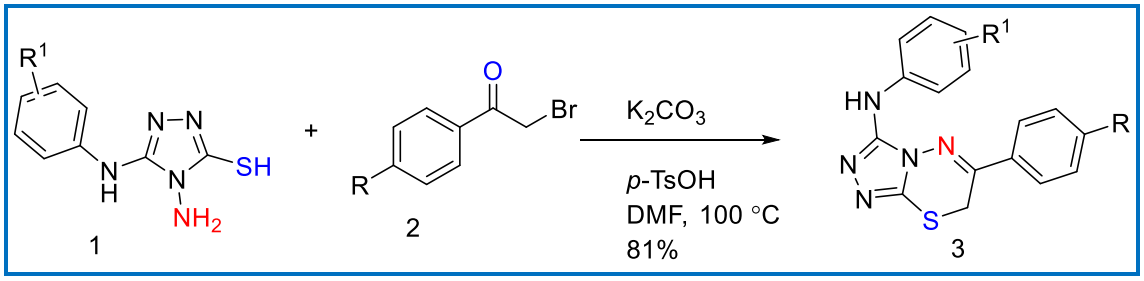
Yanchenko *et al* [51] reported the bridged nitrogen heterocyclic triazolothiadiazoles. By the reaction of 3-aklyl-4-amino-5-mercapto compound with various 2-halo acetophenones in presence of EtOH at reflux temperature. These derivatives were tested for their anticancer activity. (Scheme-1.14)

Scheme-1.14.



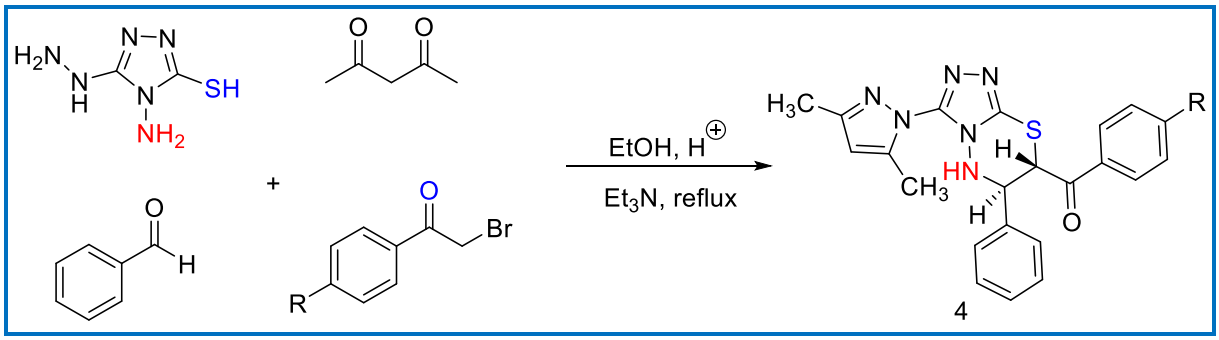
Marathe *et al* [52] published the condensation of 4-amino-5-(arylamino)-4*H*-1,2,4-triazole-3-thiol-1 with α-bromo acetophenones in presence of DMF/K_2CO_3 by using catalytic amount of *p*-TsOH under reflux to give the corresponding substituted triazolothiadiazole with good yields. (Scheme-1.15)

Scheme-15



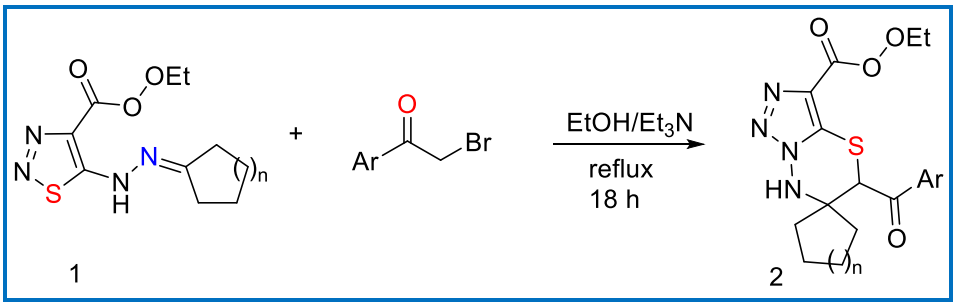
Jilloju *et al* [53] published a one-pot multi-component synthesis of triazolothiadiazines-4 by the reaction of 5-hydrazino-3-mercapto-4-amino-1,2,4-triazole, acetyl acetone, aromatic aldehydes and different phenacyl bromides under EtOH/Et_3N reflux condition to produce the 90% yield shown in scheme-16. Later these compounds were screened for their promising anti-coronavirus activity. (Scheme-1.16)

Scheme-1.16



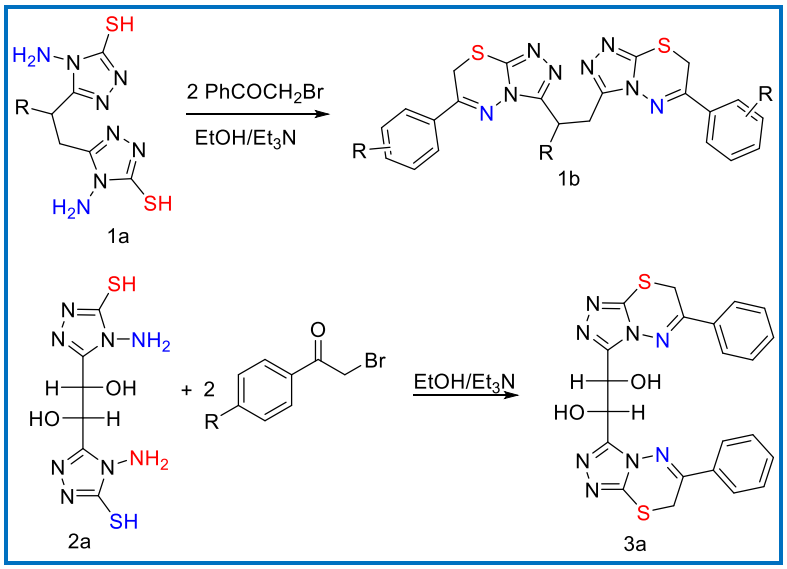
Kalinina *et al* [55] reported the synthesis of spirocyclic 6,7-dihydro-5*H*-[1,2,4] triazolo[5,1-*b*][1,3,4]thiadiazines *via* Dimroth rearrangement of hydrazone substituted 1,2,3-thiadiazolyl compound-1 with different bromo acetophenones in EtOH/Et_3N. These derivatives possess antiproliferative activity. (Scheme-1.17)

Scheme-1.17.



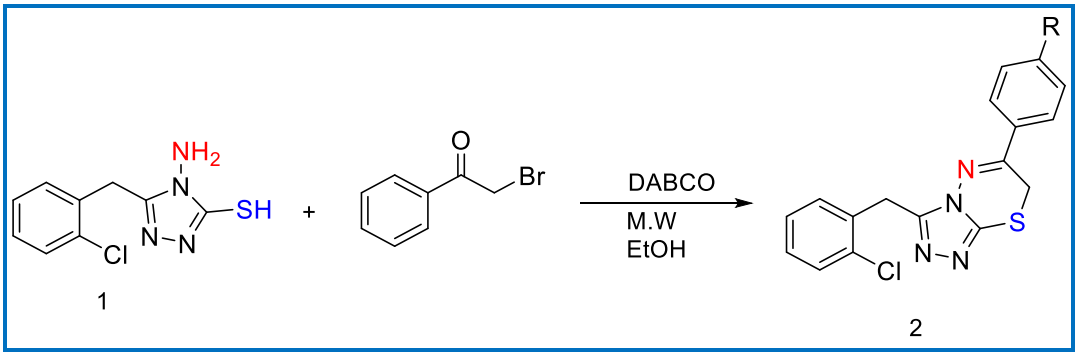
Abdel-Wahab *et al* [54] published the pseudo multicomponent synthesis of 1,2-bis(6-phenyl-7*H*-[1,2,4]-triazolo [3,4-*b*][1,3,4]thiadiazin-3-yl)ethane-**1b** by the reaction of 1a with 2 equivalents of α-bromo acetophenone under reflux in EtOH/Et_3N. Whereas the 3a was synthesized from the cyclocondensation of 1R, 2S 1,2-bis (4-amino-5-mercapto-4*H*-1,2,4-triazole-3yl) with 2 equivalents of phenacyl bromide in EtOH/Et_3N. (Scheme-1.18)

Scheme-1.18



Bhatt *et al* [56] published the one-pot expenditious green protocol for the preparation of triazolothiadiazines by microwave irradiation of substituted 4-amino-3-mercapto triazole and 2-bromo acetophenones in presence of reusable catalyst i.e., DABCO, EtOH to form desired product 3-benzyl substituted triazolothiadiazoles These compounds have shown moderate antimicrobial activity. (Scheme-1.19)

Scheme-1.19



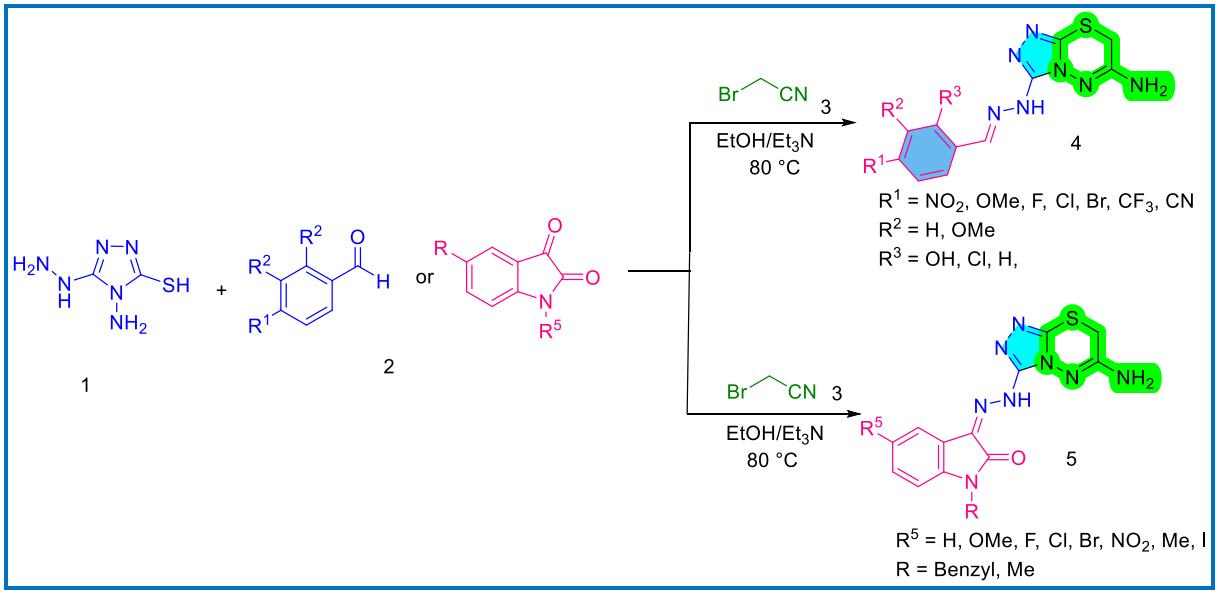
6.2. Present work

Various methods have been applied previously for the synthesis of [1,2,4]-triazolo[3,4-*b*] [1,3,4] thiadiazines which paved us the way to synthesize the title compounds. In view of the literature and inspired by the environmentally benign synthesis of triazolothiadiazines we have taken up the synthesis of title compounds. In the present research work we have synthesized 1-((6-amino-7*H*- [1,2,4]-triazolo [3,4-*b*][1,3,4]thiadiazin-3-yl)amino-2*H*-benzo[*d*]imidazol-2-one (**scheme-2**) and 3-(2-benzylidenehydrazineyl)-7*H*-[1,2,4]-triazolo[3,4-*b*][1,3,4]thiadiazin-6-amine (**scheme-1**) by multicomponent process.

6.2.1 Synthesis

Condensation of 4-amino-5-hydrazinyl-4*H*-1,2,4-triazole-3-thiol (1), substituted isatins and aromatic aldehydes (2), bromo acetonitrile (3) in ethanol and catalytic amount of triethyl amine at 90 ˚C to give the desired product with high yield. The different *N*-substituted isatins have been prepared by the reaction of isatin and various benzyl, alkyl halides (R-X) in the presence of DMF/K_2CO_3 at room temperature.

Schematic representation of triazolothiadiazine imines and triazolothiadiazine hydrazineylidene indoline-2-ones.



The optimization of the reaction was conducted by the variation of solvents with the change of catalysts and temperature at different time intervals. In the initial screening we have carried out the reaction by using MeOH, DMF, AcOH, EtOH and PEG-400 (**Table-1 entry 1-5**). In EtOH some amount of the product was obtained (**entry-3**). Moreover, the reaction has carried out in

ethanol by changing different bases and different temperatures, at different time intervals. Among these screened conditions (**Table-1 entry 6-11**) good percentage of the yield 91% was produced in EtOH/Et_3N. Hence, the optimized condition of the reaction is EtOH, Et_3N (10 mol%) at 90 ˚C for 8 h.

Table-1: Optimization of the reaction conditions for compound **4a**.

S.No	Solvent	Base (mol%)	Temp (°C)	Time (h)	Yield (%)[a]
1	MeOH	-	65	8	n. d
2	AcOH	-	65	8	10
3	EtOH	-	65	7	20
4	PEG 400		65	9	n. d
5	DMF	K_2CO_3(3)	65	10	n. d
6	AcOH	NaOAC (3)	65	8	15
7	EtOH	DMF(3)	90	10	15
8	EtOH	KOH(6)	80	9	12
9	EtOH	Et_3N (3)	70	8	55[b]
10	EtOH	Et_3N (5)	75	8	77[c]
11	EtOH	Et_3N(10)	80	8	82[d]
12	EtOH	Et_3N(10)	90	8	91[e]

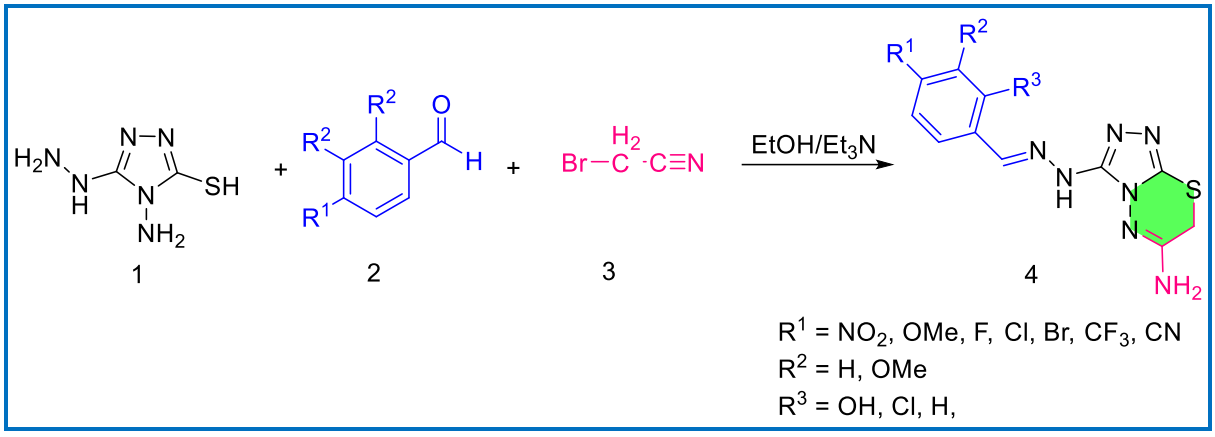
***Reaction conditions**:*1 (1 mmol), 2 (1 mmol),3 (1 mmol), Base (10 mol%), Ethanol (3mL), [a]Isolated yield, [b]Et_3N (3 mol%), [c]Et_3N (5 mol%), [d]Et_3N (10 mol%), [e]Et_3N (10 mol% for 9h). n. d = not detected.

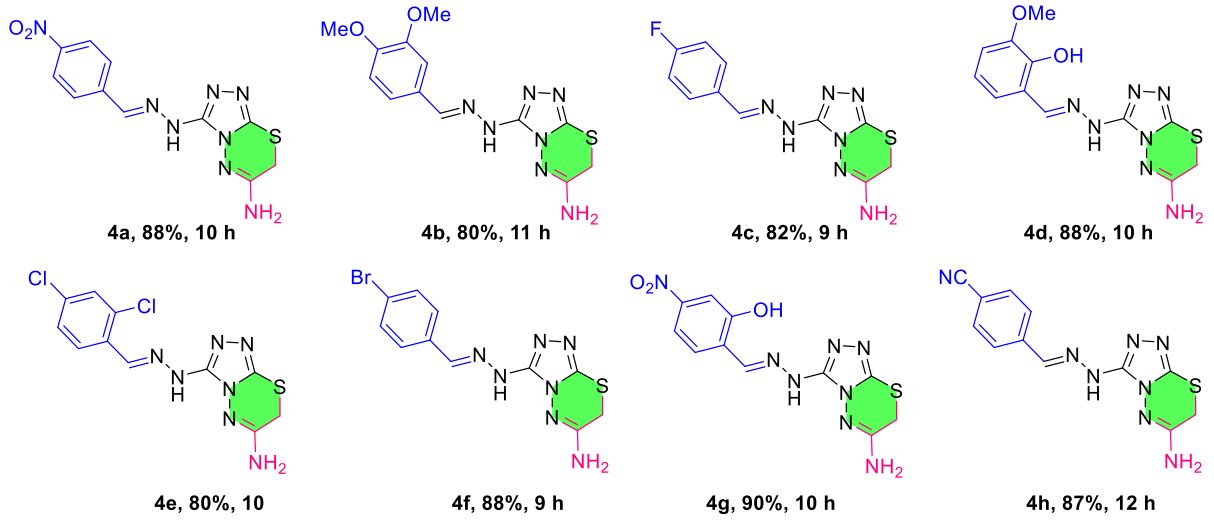
The plausible mechanism for the formation of product (4), was explained. Initially 4-amino-5-hydrazinyl-4*H*-1,2,4-triazole-3-thiol (1) reacts with isatins or aromatic aldehydes resulting in the formation of therefore, the regeoselective product 4-amino 5-benzylidenehydrazineyl 3-thiol or hydrazono derivative of isatin. In which the more nucleophilic hydrazino group of compound-1 readily reacts with electrophilic carbonyl carbon to produce Schiff bases. Further, the attack of more nucleophilic thiol group on bromo acetonitrile to produce a thioalkylated derivative over *N*-alkylated product, subsequent cyclization reaction with free N-amino group of 1 leads to six membered ring product [1,2,4]-traizole [1,3,4] thiadiazine imines with good yields. In this reaction simultaneously one C-S, two C-N bonds were formed.

The synthesized final compounds structures were fully characterized by their analytical and spectral data such as FT-IR, ^{1}H NMR, ^{13}C NMR, and HRMS spectra. In the IR spectra of thiadiazine ring NH_2 peak appears at 3230-3351 cm^{-1}, hydrazono group attached N-H peak at

3098-3115 cm^{-1}, isatin ring N-H stretching frequency appears at 3015-3127 cm^{-1}, isatin carbonyl stretching frequency appears at 1621-1681 cm^{-1}, NO_2 symmetric, asymmetric stretching frequency is at 1344, 1514 cm^{-1}. The ^1H NMR spectra of thiadiazine ring CH_2 two protons appeared as singlet at 4.30 δ ppm. NH_2 two protons appeared as broad singlet peak at 6.12 δ ppm, aromatic ring protons appeared at 6.98 – 9.25 δ ppm, hydrazono group N-H appeared as broad singlet at 10.13-11.05 δ ppm, oxindole ring N-H singlet proton is appeared as singlet at 13.89 δ ppm. In the proton decoupled ^{13}C NMR of thiadiazine ring CH_2 carbon appeared at 16.10 δ ppm, the thiadiazine ring NH_2 attached carbon showed at 148.50 δ ppm oxindoles hydrazone carbon appeared at 166.83 δ ppm, molecular mass of all the compounds were matched with their $[M+H]^+$ ion peaks.

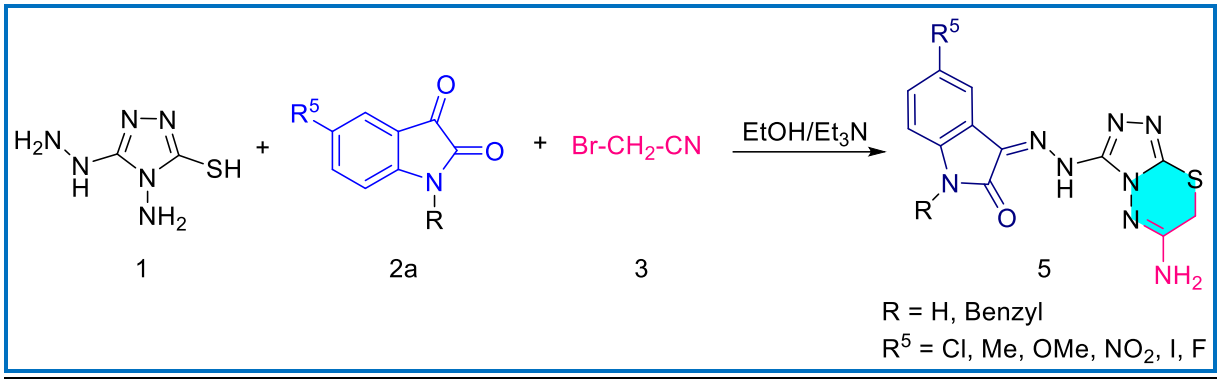
Scheme-1:

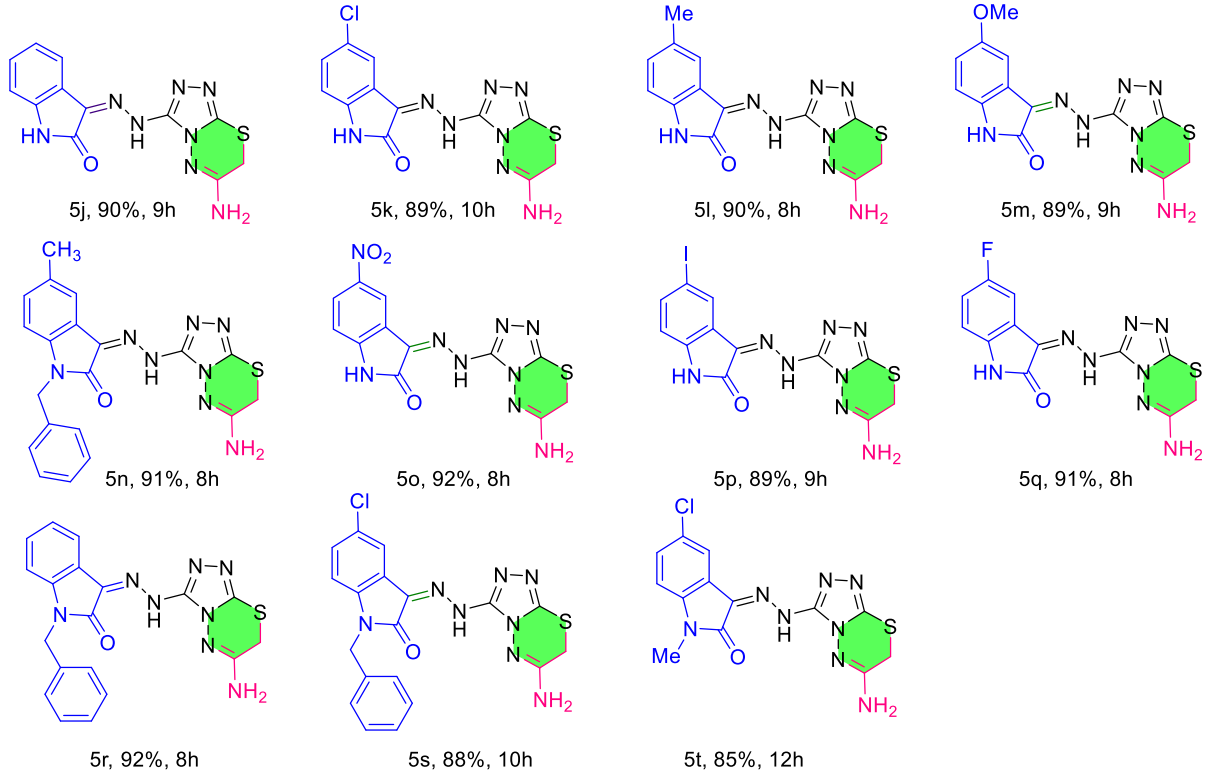




Reaction conditions: 1,2,4-triazole 1 (1 mmol), aldehyde 2 (1 mmol), Bromo acetonitrile 3 (1 mmol), EtOH/Et_3N.

Scheme-2





***Reaction conditions**:* 1 (1 mmol), 2a (1 mmol), 3 (1 mmol). EtOH/Et_3N.

6.2.2. DNA binding study.

Electronic absorption spectroscopic studies:

Electronic absorption study is one of the most reliable techniques to examine the binding affinity and binding mode of metal complexes with CT-DNA.[57,58] In this study, absorption titration concentration of CT-DNA is kept constant while the concentration of compound is varied. Absorption spectra of **5l, 5m, 5o, 5r** and **5s** are shown in **Fig-2**. Upon increasing concentration of CT-DNA to the synthesized compounds, absorbance decreased (hypochromism) and wavelength shifted towards long wavelength (red shift) due to a strong stacking interaction between the aromatic chromophore of the compounds and the adjacent

base pairs of DNA.[59] The extent of the hypochromism commonly parallels with the intercalative binding strength. The electronic absorption spectra of 5l (437 nm), 5m (363 nm), 5s (444 nm), 5r (441 nm) and 5o (427 nm) show intense absorption bands. While measuring the absorption spectra, a proper amount of CT-DNA was added to both compound solution and the reference solution to eliminate the absorbance of CTDNA itself. From the absorption titration data, the binding constant (Kb) was determined using following equation.

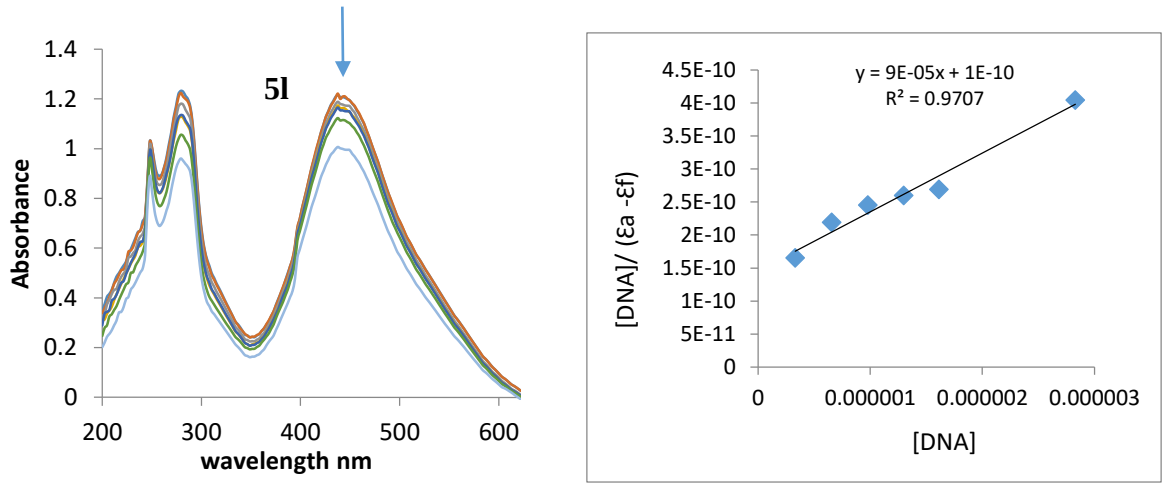
$$[DNA]/(\varepsilon a - \varepsilon f) = [DNA]/(\varepsilon b - \varepsilon f) + 1/Kb(\varepsilon b - \varepsilon f)$$

Here, Kb is the binding constant, [DNA] is the concentration of DNA in the base pairs, εa is apparent coefficient equal to Aobsd/[compound], εf and εb correspond to the extinction coefficients of the free and fully bound forms of the compounds, respectively. In plots of [DNA]/ (εa – εf) versus [DNA], Kb is given by the ratio of slope to the intercept and shown in **Table-2**. The adsorption results reveal that out of 20 synthesized analogues 5 compounds showed greater binding affinity with CT-DNA.

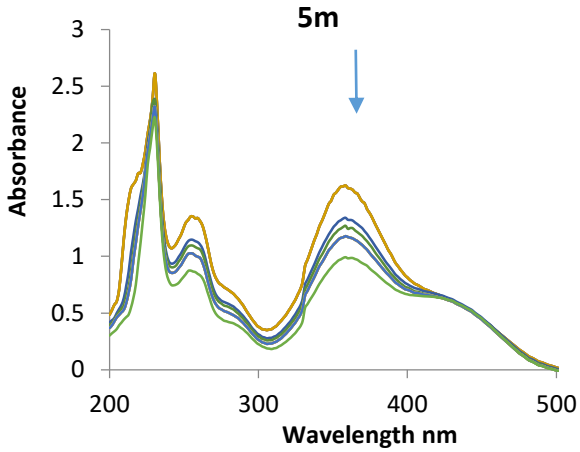
Table-2: Intrinsic binding constant (K_b) of the compounds 5l, 5m, 5o, 5r, 5s

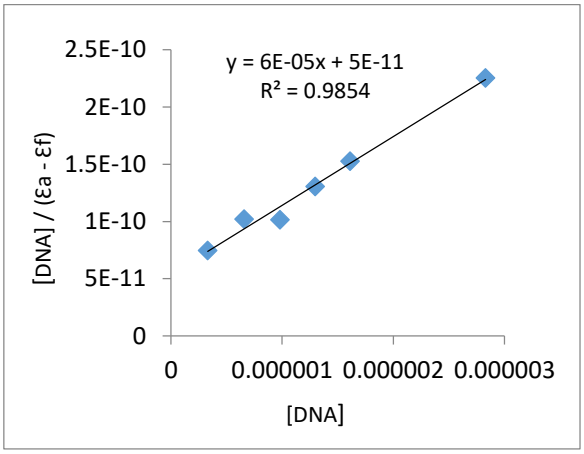
Compound code	Kb
5l	2.1548 x 10^4 M^{-1}
5m	2.36.3 x 10^4 M^{-1}
5o	6.3406 x 10^4 M^{-1}
5r	8.9742 x 10^4 M^{-1}
5s	7.878 x 10^4 M^{-1}

Compound-5l

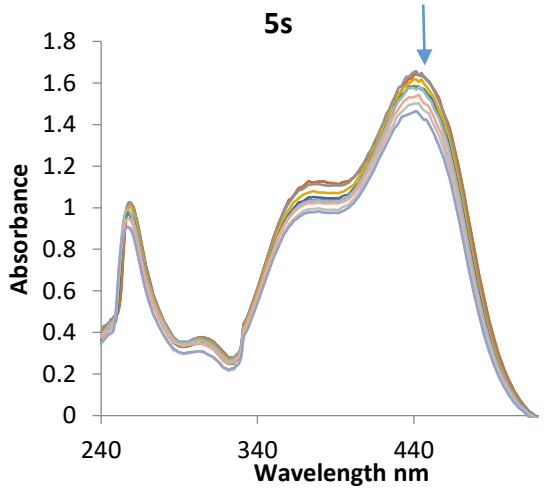


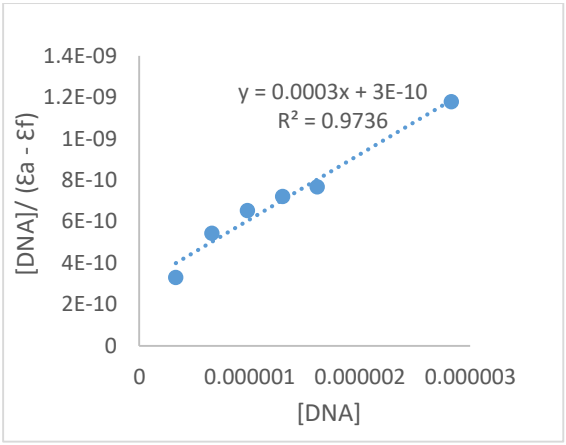
Compound-5m



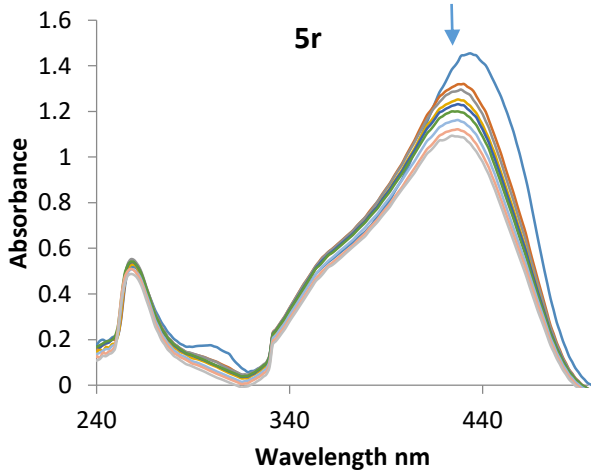


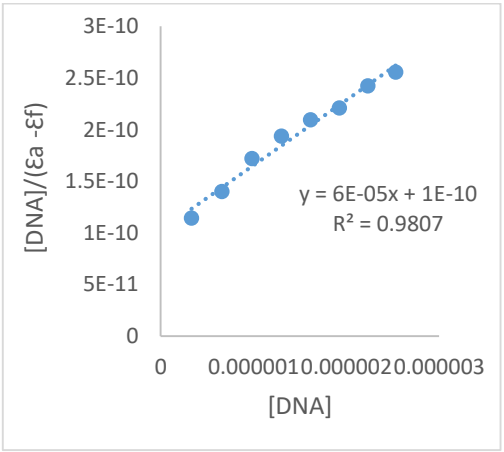
Compound-5s





Compound-5r





Compound-5o

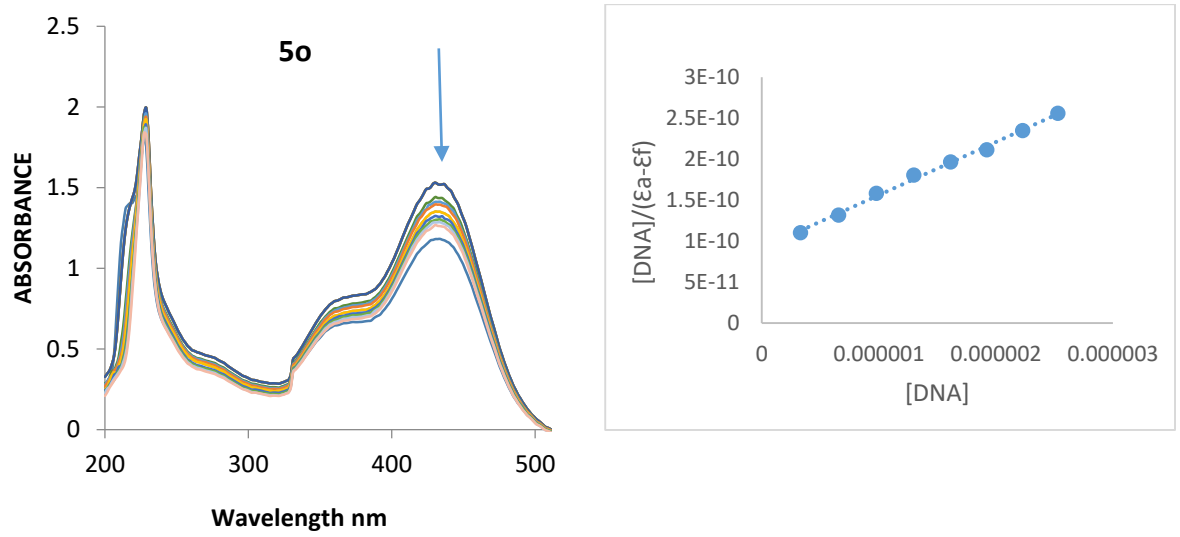


Fig-2. Absorption spectra of synthesized compounds 5l, 5m, 5s, 5r, 5o in tris HCl / NaCl buffer 5 mM Tris–HCl; 50 mM NaCl, pH 7.1 on addition of increasing concentration of CT-DNA. Arrow shows decrease in the absorbance upon increasing amounts of CT-DNA.

Conditions: [DNA] = 10-100 μM, [Compounds] = 0-100 μM

6.2.3. Molecular Docking Studies:

Using molecular docking studies, the best DNA intercalated compounds were assessed for their DNA binding affinity, which is a potent computational analysis tool in bio-informatics. The B-form DNA sequence d(CGCGAATTCGCG)2 from the protein databank with PDB ID 1bna was retrieved.[60] The DNA and ligand molecules' files were provided using the Flare molecular docking program with default settings. The macromolecule was prepared using the protein preparation wizard and the ligands were minimized by using XED force field. The docked poses were generated by the docking program, and the binding mode with the lowest atomic energy conformation was chosen. The 3D and 2D interactions was generated by using the Flare Cresset visualizer.[61]

The five best DNA intercalated compounds, **5l, 5m, 5o, 5r,** and **5s**, performed molecular docking studies on the X-ray crystal structure of a B-DNA dodecamer with a resolution of 1.9 Å (PDB ID: 1BNA). Through the use of molecular docking analysis, we gained a better understanding of how the reported fleximers compounds binds to the two DNA strands. The docked compounds preferably bound to the minor groove of the DNA due to their narrow structure, electrostatic properties, and reduced steric repulsions. The binding affinity (LFdG) for the docked compounds was found to be in the range of -6.89 to -7.90 kcal/mol, and the LF

Rank score ranged from -6.75 to -9.48. The docking scores and interactions are given in Table-1. Among all the docked compounds, compound **5o**, bearing 5-nitroindole at the hydrophilic end, displayed the LF Rank score of -9.49 and LF dG of -6.90 kcal/mol. As shown in Figure-1, **5o** has intercalated with DNA and exhibits interactions with DT7, DT19, DC21, and DA5 residues. Similarly, compound **5l**, bearing 5-methyl indole, displayed the LF Rank score of -9.98 and LF dG of -7.07 kcal/mol. Compound **5l** exhibits interactions with DT7, DT19, DA5, DC21, and DT20 (**Fig-3**).

Table-2: Molecular Docking studies of Best compounds with B-DNA dodecamer (PDB ID: 1bna)

Title	LF Rank Score	LF dG	LF VSscore	LF LE	Interactions
5o	-9.49	-6.90	-7.32	-0.28	DT7, DT19, DC21, DA5
5m	-7.96	-7.91	-8.11	-0.33	DC21, DT7, DT7, DA18, DT19, DA5
5l	-9.98	-7.07	-7.97	-0.31	DT7, DT19, DA5, DC21, DT20
5r	-7.05	-5.62	-6.48	-0.19	DC3, DA5, DC21, DT20, DG4,
5s	-6.75	-6.11	-6.94	-0.20	DC3, DA5, DC21, DG4, DT20, DC3, DG4, DT20

Compound-5l

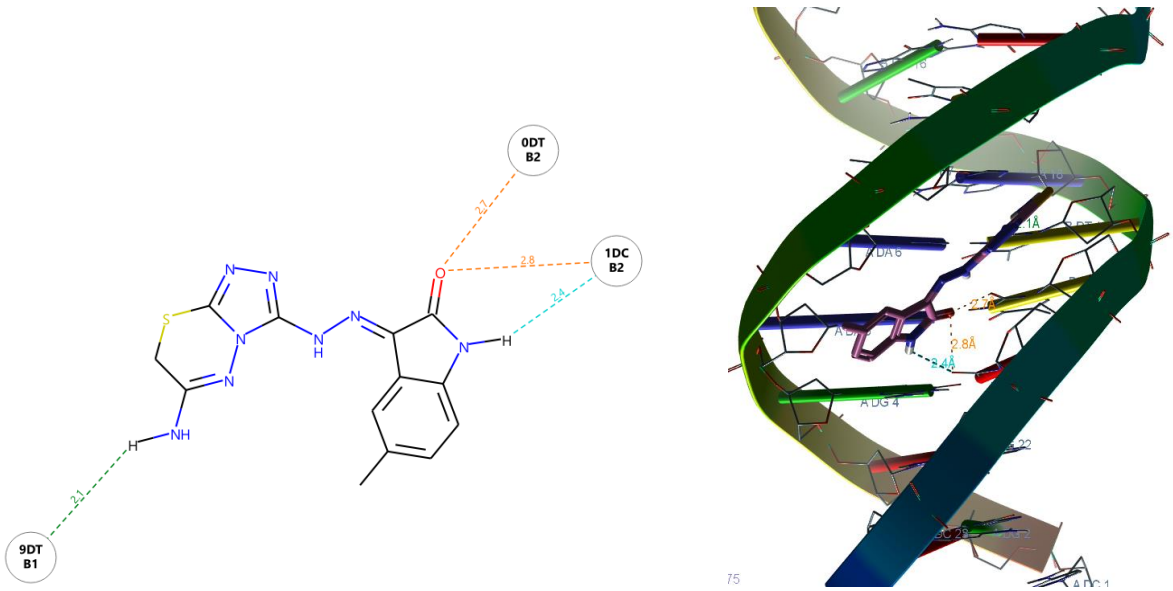


Fig-3: Docking interaction of compound 5l with B-DNA dodecamer (PDB ID: 1bna)

Compound-5m

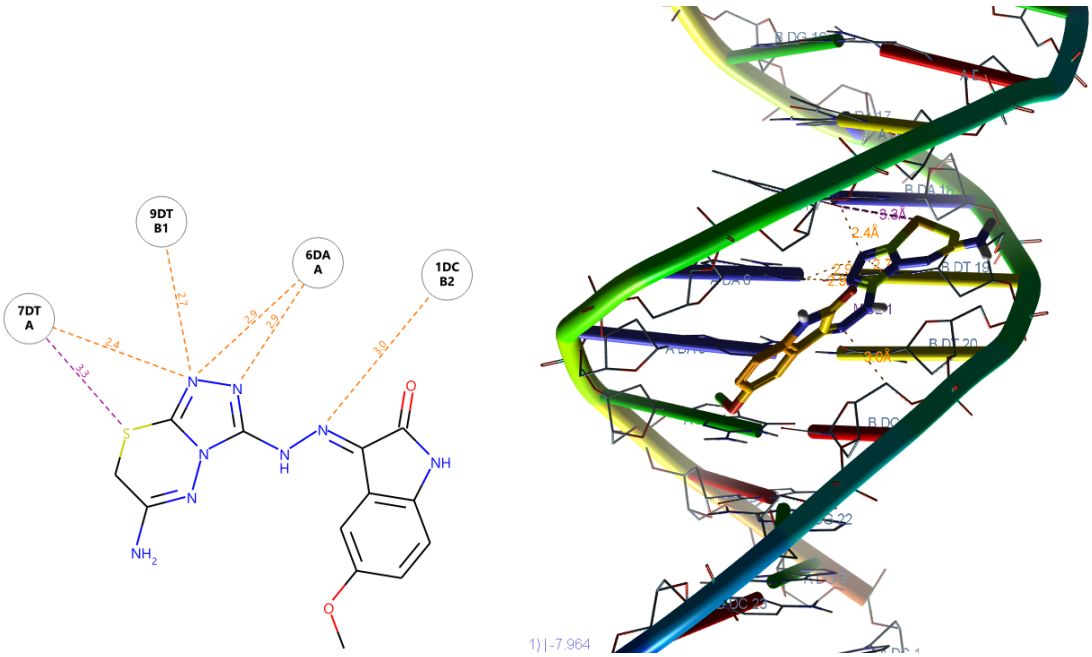


Fig-4: Docking interaction of compound 5m with B-DNA dodecamer (PDB ID: 1bna)

Compound-5o

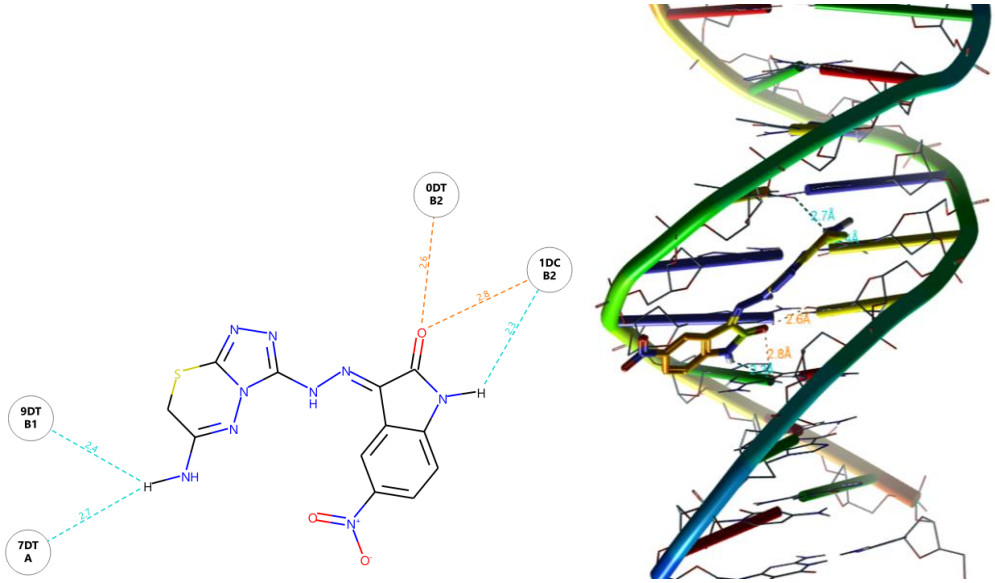


Fig-5: Docking interaction of compound 5o with B-DNA dodecamer (PDB ID: 1bna)

Compound-5r

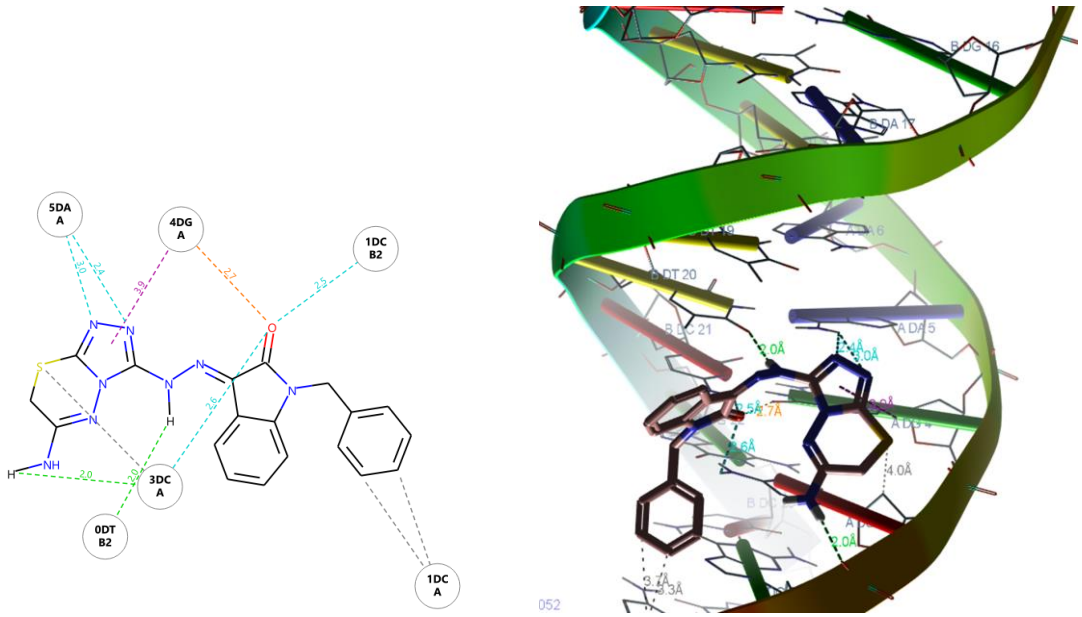


Fig-6: Docking interaction of compound 5r with B-DNA dodecamer (PDB ID: 1bna)

Compound-5s

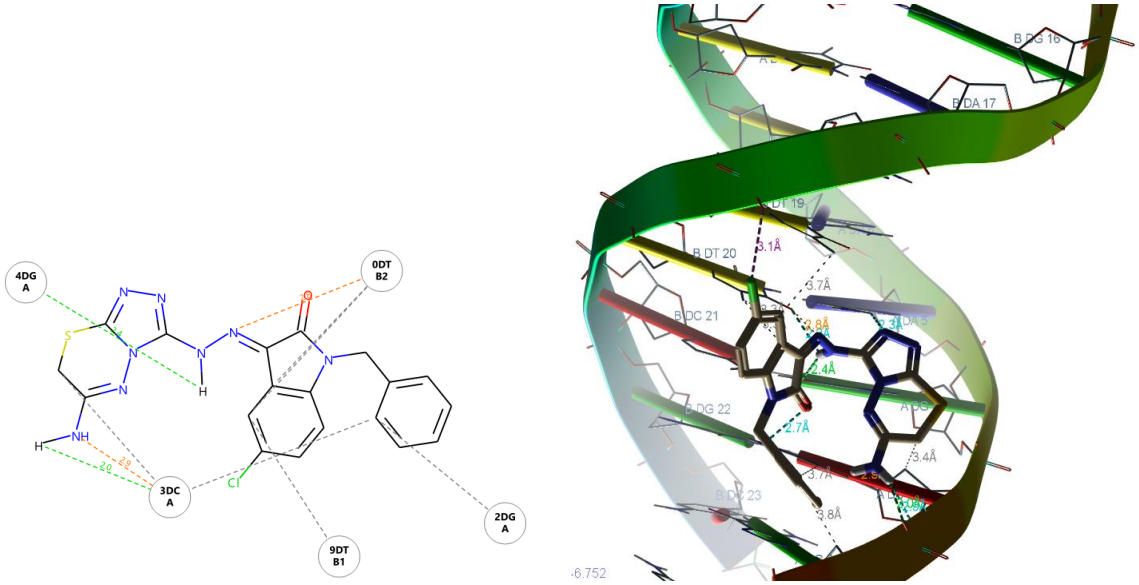


Fig-7: Docking interaction of compound 5s with B-DNA dodecamer (PDB ID: 1bna)

6.3. Conclusions.

The synthesis of 1,2,4-triazolo [3,4-*b*][1,3,4] 6-aminothiadiazines and their hydrazineylidene indolin-2-ones, Schiff bases were synthesized by using multicomponent process. The advantages of the present method are high yields of the products, good substrate scope of the reaction and environmentally benign. All the synthesized compounds were studied for electronic absorption spectra. Among these the compounds **5l, 5m, 5o, 5r, 5s** have shown greater binding affinity with CT-DNA. Molecular docking results were also revealed that the compounds **5l, 5m, 5o, 5r, 5s** having good binding interactions with respect to amino acids.

6.4. Experimental:

The required chemicals were procured from chemical suppliers i.e Merk, Alfa acer, TCI, Spectrochem and used without purification. The solvents were procured from Finar, Aldrich chemical suppliers and stored over a 4 Å molecular sieves. The progress of the reaction was monitored by using Silica gel-coated aluminium TLC plates using ethyl acetate, n-hexane (2:8 ratio). Melting points of the compounds were checked with Stuart Staffordshire, UK (SMP30) Instrument and are uncorrected. The FT-IR Spectra of the compounds were recorded on Perkin Elmer spectrometer using KBr disk and values were represented by cm^{-1}. The proton NMR spectra of the compounds were recorded on Brucker AVANCE 400 MHz spectrophotometer using DMSO-d_6 solvent (centered at 2.5 δ ppm quintet) and Chemical shift values were represented by δ ppm. TMS was taken as the internal standard reference compound (0 δ ppm). The notations used for splitting pattern of the protons was shown with s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. The coupling constant values were expressed in Hz. Proton decoupled ^{13}C NMR spectra were recorded on Brucker 100 MHz spectrophotometer using DMSO-d_6 solvent. And the peak appeared septet centered at 39.52 δ ppm. ESI-HRMS spectra were recorded for all the final compounds by using Agilent Technology Instrument made in Japan.

6.4.1 Preparation of DNA solution

The UV-Vis titration experiments were performed by maintaining a constant concentration of the synthesized compounds at 10 μM throughout experiment (5 mM Tris-HCl/50 mM NaCl buffer at pH 7.4). The ratio of 1.8–1.9 of UV absorbance at 260 and 280 nm was given by CT-DNA in tris HCl-NaCl buffer solution, indicating that the DNA was sufficiently free of protein. [62] The concentration of the source DNA is 1 mg/mL. We determined the concentration of CT-DNA stock solution by employing a molar absorptivity (6600 $M^{-1}cm^{-1}$) at A260 nm, after 1:30

dilution of source DNA with 5 mM Tris-HCl/50 mM NaCl buffer at pH = 7.2. [63] Thus, the concentration of the source DNA (1 mg/mL) is estimated to be 5700 μM. In experiments, the concentration of CT-DNA was varied between 0–10 μM keeping the total volume of the reaction mixture constant (3 mL). After each addition of CT-DNA to the synthesized compounds, the resulting solution was allowed to equilibrate at 25 ˚C for 5 min followed by recording of absorption spectrum. The binding constants *(*Kb*)* were calculated from the spectroscopic titration data by the plot between [DNA] / (εa − εf*)* and [DNA]. [64]

6.4.2 General procedure for the synthesis of 3-(2-(6-amino-7*H*-[1,2,4]-triazolo[3,4-*b*][1,3,4]thiadiazin-3 yl)hydrazineylidene)indolin-2-ones and imines.

A mixture of 4-amino-5-hydrazinyl-4*H*-1,2,4-triazole-3-thiol (1 mmol), isatin or aromatic aldehyde (1 mmol), bromo acetonitrile (1 mmol) was taken in ethanol and 10 mol% of triethyl amine, the reaction mixture was refluxed for 8 hours. After completion of the reaction was checked by TLC, the yellow solid product was filtered and recrystalized with ethanol.

6.4.2a *General Procedure for the preparation of N-benzylindoline-2,3-dione*.

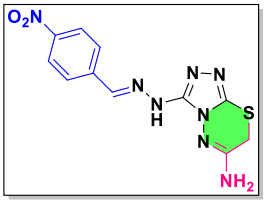
A mixture of benzyl bromide (1 mmol) and isatin (1 mmol) was taken in DMF 2mL solvent, potassium carbonate (1 mmol) was added and stirred for 12 h at room temperature After completion of the reaction the reaction mixture was poured in to ice cold water and solid separated was filtered.

6.4.2b *General procedure for the preparation of 1-methylindoline-2,3-dione*

The isatin (1 mmol) was taken in 50 mL of round bottom flask, added 3 mL acetone solvent and kept in ice bath then methyl iodide (1 mmol) was added and stirred at room temperature for overnight. After completion of the reaction (as checked by TLC) the reaction mixture was poured in to ice cold water and the solid product obtained was filtered.

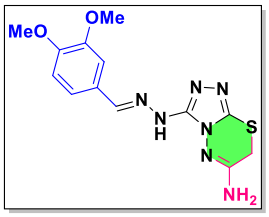
6.5. characterization data of synthesized compounds

(E)-3-(2-(4-nitrobenzylidene)hydrazineyl)-7H-[1,2,4]-triazolo[3,4-b][1,3,4]thiadiazin-6-amine: 4a. Yellow solid; Yield 88%; mp: 252-253 °C; IR (KBr) cm^{-1}: 3263 (NH_2), 3117, (NH), 1611 (schiff base)), 1513,1339 (NO_2); ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 4.23 (s, 2H), 5.92 (s, 2H broad), 7.93 (d, J = 8.4 Hz, 2H), 8.25 (d, J = 8.4 Hz, 2H),

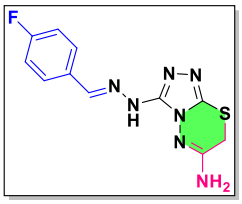


8.35 (s, 1H), 11.20 (s, 1H); ^{13}C NMR (100MHz, DMSO-d_6) δ (ppm): 17.31, 118.00, 124.43, 127.50, 140.45, 141.92, 146,13, 147.40, 153.94. HRMS (ESI-TOF) (*m/z*): Calculated for $C_{11}H_{10}N_8O_2S$ $[M+H]^+$ 319.0725; found 319.0726.

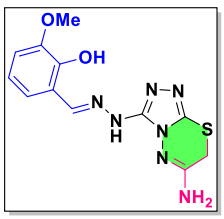
(E)-3-(2-(3,4-dimethoxybenzylidene)hydrazineyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-6-amine: 4b. Yellow solid; Yield 80%; mp: 240-241 °C; IR (KBr) cm^{-1}: 3301 (NH_2), 3100 (NH), 1623 (schiff base), 1106 (C-O-C); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 3.78 (s, 3H), 3.80 (s, 3H), 4.22 (s, 2H), 5.84 (s, 2H broad), 6.98 (d, *J* = 8.4 Hz, 1H), 7.13 (dd, *J* = 8.3, 1.8 Hz, 1H), 7.31 (s, 1H), 8.15 (s, 1H), δ 10.51 (s, 1H); ^{13}C NMR (100MHz, DMSO-d_6) δ (ppm): 17.3, 55.93, 55.99, 108.5, 111.9, 118.0, 121.1, 128.1, 145.3, 149.4, 150.3, 154.7. HRMS (ESI-TOF) (*m/z*): Calculated for $C_{13}H_{15}N_7O_2S$ $[M+H]^+$ 334.1086; found 334.1086.



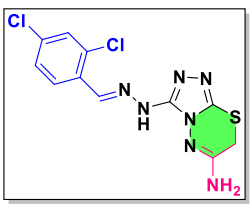
(E)-3-(2-(4-fluorobenzylidene)hydrazineyl)-7H-[1,2,4]-triazolo[3,4-b][1,3,4]thiadiazin-6-amine: 4c.Yellow solid; Yield 82%; mp: 235-236 °C; IR (KBr) cm^{-1}: 3342 (NH_2), 3119(NH), 1615 (schiff base), 813 (C-F); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 4.31 (s, 2H), 7.25 (t, *J* = 8.8 Hz, 2H), 7.37 (t, *J* = 8.8 Hz, 2H), 8.32 (s, 2H broad), 10.03 (s, 1H), 12.70 (s, 1H): ^{13}C NMR (100MHz, DMSO-d_6) δ (ppm): 17.26, 118.04, 126.03, 127.28, 127.68, 139.76, 145.89, 148.46, 154.17. HRMS (ESI-TOF) (*m/z*): Calculated for $C_{11}H_{10}FN_7S$ $[M+H]^+$ 292.0798; found 292.0780.



(E)-2-((2-(6-amino-7H-[1,2,4]-triazolo[3,4-b][1,3,4]thiadiazin-3-yl)hydrazineylidene)methyl)-6-methoxyphenol: 4d. Yellow solid; Yield 88%; mp: 237-238 °C; IR (KBr) cm^{-1}: 3512 (OH), 3313 (NH_2), 3010 (NH), 1624 (schiff base), 1104 (C-O-C); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 3.80 (s, 3H), 4.20 (s, 2H), 5.84 (s, 2H), 6.82 (t, *J* = 7.9 Hz, 2H), 7.10 (d, *J* = 7.3 Hz, 1H), 8.47 (s, 1H), 10.45 (s, 1H), 12.99 (s, 1H); ^{13}C NMR (100MHz, DMSO-d_6) δ (ppm): 17.66, 56.25, 113.31, 113.65, 117.95, 119.57, 119.97, 120.88, 146.52, 148.26, 154.47, 165.12. HRMS (ESI-TOF) (*m/z*): Calculated for $C_{12}H_{13}N_7O_2S$ $[M+H]^+$ 320.0929; found 320.0928.

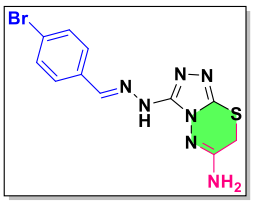


(E)-3-(2-(2,4-dichlorobenzylidene)hydrazineyl)-7H-[1,2,4]-triazolo[3,4-b][1,3,4]thiadiazin-6-amine: 4e.Yellow solid; Yield 80%; mp: 241-242 °C; IR (KBr) cm^{-1}: 3310 (NH_2), 3095 (NH), 1602 (schiff base), 716 (C-Cl); ^{1}H NMR (400 MHz, DMSO-d_6) δ

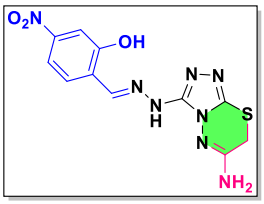


(ppm): 4.24 (s, 2H), 5.88 (s, 2H), 7.47 (m, 1H), 7.66 (d, *J* = 2.1 Hz, 1H), 8.04 (d, *J* = 8.6 Hz, 1H), 8.60 (s, 1H), 11.22 (s, 1H); ^{13}C NMR (100MHz, DMSO-*d*$_6$) δ (ppm): 17.32, 117.96, 128.21, 129.56, 131.67, 133.22, 134.35, 138.47, 146.05, 154.30. HRMS (ESI-TOF) (*m/z*): Calculated for $C_{12}H_{13}N_7O_2S$ $[M+H]^+$ 342.0095; found 342.0089.

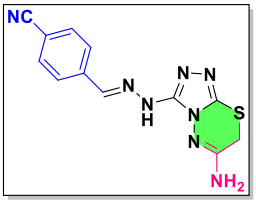
(E)-3-(2-(4-bromobenzylidene)hydrazineyl)-7H-[1,2,4]-triazolo[3,4-b][1,3,4]thiadiazin-6-amine: 4f.Yellow solid; Yield 88%; mp: 234-235 °C; IR (KBr) cm^{-1}: 3317 (NH_2), 3100 (NH), 1612 (schiff base), 697 (C-Br); ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ (ppm): 4.28 (s, 2H), 6.00 (s, 2H), 7.59 (d, *J* = 9.6 Hz, 1H), 7.62-7.65 (m, 2H), 7.71 (d, *J* = 8.8Hz, 1H), 8.27 (s, 1H), 10.79 (s, 1H); ^{13}C NMR (100MHz, DMSO-*d*$_6$) δ (ppm): 17.43, 117.78, 123.32, 128.71, 129.16, 132.20, 134.02, 134.45, 149.97, 153.22, 164.96. HRMS (ESI-TOF) (*m/z*): Calculated for $C_{11}H_{10}BrN_7S$ $[M+H]^+$ 351.9980; found 351.9977. C, H, N Analysis:



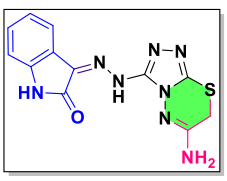
(E)-2-((2-(6-amino-7H-[1,2,4]-triazolo[3,4-b][1,3,4]thiadiazin-3-yl)hydrazineylidene)methyl)-5-nitrophenol: 4g.Yellow solid; Yield 90%; mp: 244-245 °C; IR (KBr) cm^{-1}. 3354 (OH), 3273 (NH_2), 3079 (NH), 1623 (schiff base), 1574, 1399 (NO_2); ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ (ppm): 4.22 (s, 2H), 5.85 (s, 2H), 7.08 (d, *J* = 9.1 Hz, 2H), 8.08-8.11 (m, 1H), 8.52 (d, *J* = 2.8 Hz, 1H), 8.55 (s, 1H), 11.94 (s, 1H); ^{13}C NMR (100MHz, DMSO-*d*$_6$) δ (ppm): 17.50, 117.25, 117.94, 121.21, 123.57, 124.41, 125.95, 127.49, 140.45, 147.38, 162.26. HRMS (ESI-TOF) (*m/z*): Calculated for $C_{12}H_{13}N_7O_2S$ $[M+H]^+$ 335.0675; found 335.0671.



(E)-4-((2-(6-amino-7H-[1,2,4]-triazolo[3,4-b][1,3,4]thiadiazin-3-yl)hydrazineylidene)methyl)Benzonitrile: 4h. Yellow solid; Yield 87%; mp: 243-244 °C; IR (KBr) cm^{-1}. 3362 (NH_2), 3288 (NH), 2223 (CN), 1624 (schiff base); ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ (ppm): 4.15 (s, 2H), 5.82 (s, 2H), 7.86 – 7.79 (m, 4H), 8.21 (s, 1H), 12.98 (s, 1H); ^{13}C NMR (100MHz, DMSO-*d*$_6$) δ (ppm): 17.35, 111.07, 117.94, 119.39, 127.39, 133.02, 146.02, 154.43, 157.45. HRMS (ESI-TOF) (*m/z*): Calculated for $C_{12}H_{10}N_8S$ $[M+H]^+$ 299.0827; found 299.0821.

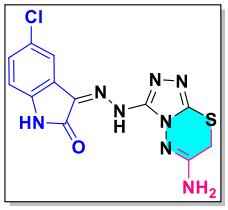


(E)-3-(2-(6-amino-7H-[1,2,4]-triazolo[3,4-b][1,3,4]thiadiazin-3-yl)hydrazineylidene)indolin-2-one: 5j. Yellow solid; Yield 90%; mp: 232-233 °C; IR (KBr) cm^{-1} 3351(NH_2), 3115 (NH), 3057 (lactam ring NH), 2973, 1642 (lactam ring C=O), 1616 (N=C); ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ (ppm): 4.26 (s, 2H), 6.11 (s, 2H), 6.96 (d, *J* = 7.6 Hz, 1H),

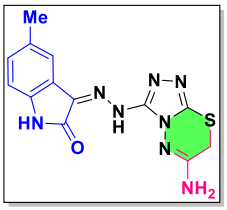


7.10 (t, *J* = 7.6 Hz, 1H), 7.34 (t, *J* = 8.2 Hz, 1H), 7.56 (d, *J* = 7.6 Hz, 1H), 11.23 (s, 1H), 12.87 (s, 1H); ^{13}C NMR (100MHz, DMSO-*d*$_6$) *δ* (ppm): 17.90, 109.55, 120.36, 121.58, 122.88, 127.18, 129.43, 130.69, 147.63, 158.85, 163.54, 166.69. HRMS (ESI-TOF) (*m/z*): Calculated for $C_{12}H_{10}N_8OS$ $[M+H]^+$ 315.0776; found 315.0778.

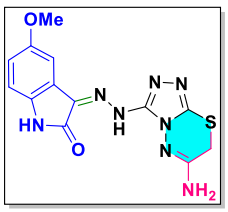
(E)-3-(2-(6-amino-7H-[1,2,4]-triazolo[3,4-b][1,3,4]thiadiazin-3-yl)hydrazineylidene)-5-chloroindolin-2-one: 5k. Yellow solid; Yield 89%; mp: 239-240 °C; IR (KBr) cm^{-1} : 3310 (NH_2), 3151 (NH), 3010 (lactam ring NH), 1620 (lactam ring C=O), 1611 (N=C), 789 (C-Cl); ^{1}H NMR (400 MHz, DMSO-*d*$_6$) *δ* (ppm): 4.29 (s, 2H), 6.07 (s, 2H), 6.82 (d, *J* = 8.2 Hz, 1H), 7.21 (dd, *J* = 8.2, 2.3 Hz, 1H), 8.52 (s, 1H), 10.44 (s, 1H), 13.63 (s, 1H); ^{13}C NMR (100MHz, DMSO-*d*$_6$) *δ* (ppm): 16.00, 110.93, 117.73, 119.92, 125.59, 125.96, 128.63, 137.15, 140.10, 148.10, 159.13, 166.53. HRMS (ESI-TOF) (*m/z*): Calculated for $C_{12}H_9ClN_8OS$ $[M+H]^+$ 349.0387; found 349.0380.



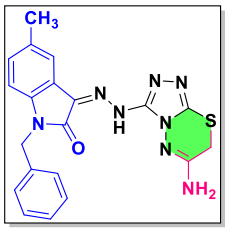
(E)-3-(2-(6-amino-7H-[1,2,4]-triazolo[3,4-b][1,3,4]thiadiazin-3-yl)hydrazineylidene)-5-methylindolin-2-one: 5l. Yellow solid; Yield 90%; mp: 244-245 °C; IR (KBr) cm^{-1} . 3347 (NH_2), 3116 (NH), 3002 (lactam ring NH), 1620 (lactam ring (C=O), 1615 (N=C), ^{1}H NMR (400 MHz, DMSO-*d*$_6$) *δ* (ppm): 2.31 (s, 3H), 4.25 (s, 2H), 6.09 (s, 2H), 6.84 (d, *J* = 7.9 Hz, 1H), 7.14 (d, *J* = 7.9 Hz, 1H), 7.38 (s, 1H), 11.09 (s, 1H), 12.84 (s, 1H); ^{13}C NMR (100MHz, DMSO-*d*$_6$) *δ* (ppm): `17.91, 21.36, 111.15, 120.74, 127.75, 131.88, 133.33, 139.30, 146.54, 147.54, 153.14, 163.63. HRMS (ESI-TOF) (*m/z*): Calculated for $C_{13}H_{12}N_8OS$ $[M+H]^+$ 329.0933; found 329.0931.



(E)-3-(2-(6-amino-7H-[1,2,4]-triazolo[3,4-b][1,3,4]thiadiazin-3-yl)hydrazineylidene)-5-metho xyindolin-2-one: 5m. Yellow solid; Yield 89%; mp: 234-235 °C; IR (KBr) cm^{-1} . 3397 (NH_2), 3213 (NH), 3015 (lactam ring NH), 1626 (lactam ring (C=O), 1597 (N=C), 1128 (C-O-C); ^{1}H NMR (400 MHz, DMSO-*d*$_6$) *δ* (ppm): 3.77 (s, 3H), 4.26 (s, 2H), 6.11 (s, 2H), 6.87 (d, *J* = 8.4 Hz, 1H), 6.91 (d, *J* = 2.5 Hz, 1H), 7.12 (d, *J* = 2.5 Hz, 1H), 11.03 (s, 1H), 12.91 (s, 1H); ^{13}C NMR (100MHz, DMSO-*d*$_6$) *δ* (ppm): 19.00, 55.87, 105.35, 109.90, 113.16, 119.41, 135.39, 139.22, 147.73, 154.82, 155.77, 166.87. HRMS (ESI-TOF) (*m/z*): Calculated for $C_{13}H_{12}N_8O_2S$ $[M+H]^+$ 345.0882; found 345.0885.

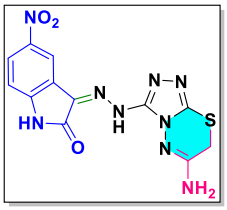


***(E)-3-(2-(6-amino-7H-[1,2,4]-triazolo[3,4-b][1,3,4]thiadiazin-3-yl)hydrazineylidene)-1-benzyl-5-methylindolin-2-one* :5n**

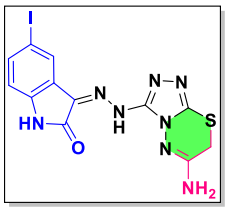


Yellow solid; Yield 84%; mp: 223-224 °C; IR (KBr) cm^{-1}. 3359 (NH_2), 3184 (NH), 1619 (lactam C=O), 1572 (N=C); ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.31 (s, 3H), 4.28 (s, 2H), 5.01 (s, 2H), 6.15 (s, 2H), 6.98 (d, J = 8.0 Hz, 1H), 7.15 (d, J = 8.0 Hz, 1H), 7.32 – 7.37 (m, 5H), 7.45 (s, 1H), 12.82 (s, 1H); ^{13}C NMR (100MH_Z, DMSO-d_6) δ (ppm): 21.03, 21.31, 42.95, 110.51, 117.92, 120.68, 127.61, 127.84, 128.06, 129.05, 129.20, 136.47, 137.64, 139.64, 146.74, 147.78, 153.04, 161.67. HRMS (ESI-TOF) (*m/z*): Calculated for $C_{20}H_{18}N_8OS$ $[M+H]^+$ 419.1402; found 419.1411.

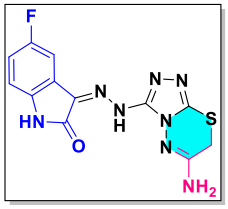
(E)-3-(2-(6-amino-7H-[1,2,4]-triazolo[3,4-b][1,3,4]thiadiazin-3-yl)hydrazineylidene)-5-nitro indolin-2-one: 5o. Yellow solid; Yield 92%; mp: 232-233 °C; IR (KBr) cm^{-1}. 3377 (NH_2),3045 (NH), 2994 (lactam ring NH),1623 (lactam ring C=O), 1590 (N=C), 1516, 1336 (NO_2); ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 4.30 (s, 2H), 6.12 (s, 2H), 7.00 (d, J = 8.6 Hz, 1H), 8.14 (dd, J = 8.6, 2.5 Hz, 1H), 9.25 (d, J = 2.4 Hz, 1H), 11.02 (s, 1H), 13.89(s, 1H); ^{13}C NMR (100MH_Z, DMSO-d_6) δ (ppm): 16.10, 109.56, 117.71, 118.55, 121.19, 125.59, 135.66, 142.24, 146.70, 148.50, 159.12, 166.83. HRMS (ESI-TOF) (*m/z*): Calculated for $C_{12}H_9BrN_8OS$ $[M+H]^+$ 360.0627; found 360.0626.



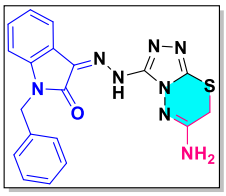
(E)-3-(2-(6-amino-7H-[1,2,4]-triazolo[3,4-b][1,3,4]thiadiazin-3-yl)hydrazineylidene)-5-iodoindolin-2-one: 5p.Yellow solid; Yield 89%; mp: 240-241 °C; IR (KBr) cm^{-1} . 3325 (NH_2), 3113 (NH), 3015 (lactam ring NH), 1630 (lactam ring C=O), 1593 (N=C), 590 (C-I); ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 4.28 (s, 2H), 6.06 (s, 2H), 6.67 (d, J = 8.1 Hz, 1H), 7.50 (dd, J = 8.1, 1.8 Hz, 1H), 8.80 (s, 1H), 10.42 (s, 1H), 13.56 (s, 1H); ^{13}C NMR (100MH_Z, DMSO-d_6) δ (ppm): 16.01, 112.02, 117.74, 120.94, 134.25, 137.36, 140.94, 148.05, 159.06, 166.04. HRMS (ESI-TOF) (*m/z*): Calculated for $C_{12}H_9IN_8OS$ $[M+H]^+$ 440.9743; found 440.9760.



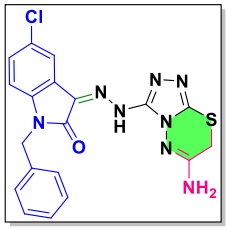
(E)-3-(2-(6-amino-7H-[1,2,4]-triazolo[3,4-b][1,3,4]thiadiazin-3-yl)hydrazineylidene)-5-fluoro indolin-2-one: 5q. Yellow solid; Yield 91%; mp: 243-244 °C; IR (KBr) cm^{-1} . 3361 (NH_2), 3058 (NH), 2937 (lactam ring NH), 1630 (lactam ring C=O), 1600 (N=C), 787 (C-F); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 4.26 (s, 2H), 6.12 (s, 2H), 7.17 (t, J = 9.0 Hz, 1H), 7.38 (dd, J = 8.1, 2.4 Hz, 1H), 8.33 (d, J = 8.9 Hz, 1H), 10.33 (s, 1H), 11.24 (s, 1H); ^{13}C NMR (100MHz, DMSO-d_6) δ (ppm): 15.97, 110.00, 113.58, 115.11, 117.74, 119.32, 137.73, 147.97, 156.84, 158.98, 166.80. HRMS (ESI-TOF) (*m/z*): Calculated for $C_{12}H_9FN_8OS$ $[M+H]^+$ 333.0718; found 333.0715.



(E)-3-(2-(6-amino-7H-[1,2,4]-triazolo[3,4-b][1,3,4]thiadiazin-3-yl)hydrazineylidene)-1-benz ylindolin-2-one: 5r. Yellow solid; Yield 92%; mp: 243-244 °C; IR (KBr) cm^{-1} . 3313 (NH_2), 3193 (NH), 1615 (lactam ring C=O), 1574 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 4.276(s, 2H), 5.03 (s, 2H), 6.14 (s, 2H), 7.08 – 7.16(m, 2H), 7.29– 7.37 (m, 6H), 7.62 (d, J = 7.3 Hz, 1H), 12.82 (s, 1H); ^{13}C NMR (100MHz, DMSO-d_6) δ (ppm): 17.84, 42.80, 108.75, 110.70, 122.27, 127.64, 127.89, 129.09, 129.23, 137.59, 147.83, 153.01, 165.32. HRMS (ESI-TOF) (*m/z*): Calculated for $C_{19}H_{16}N_8OS$ $[M+H]^+$ 405.1246; found 405.1248.

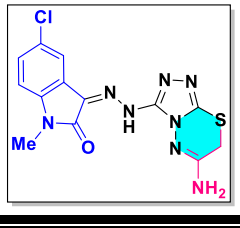


(E)-3-(2-(6-amino-7H-[1,2,4]-triazolo[3,4-b][1,3,4]thiadiazin-3-yl)hydrazineylidene)-1-benz yl-5-chloroindolin-2-one 5s. Yellow solid; Yield 88%; mp: 250-251 °C; IR (KBr) cm^{-}1. 3317 (NH_2), 3190 (NH), 1610 (lactam ring C=O), 1594 (N=C), 752 (Cl); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 4.29 (s, 2H), 4.99 (s, 2H), 6.17 (s, 2H), 7.12 (d, J = 8.5 Hz, 1H), 7.31– 7.33 (m, 4H), 7.35-7.37 (m, 3H), 12.81 (s, 1H); ^{13}C NMR (100MHz, DMSO-d_6) δ (ppm): 19.02, 42.86, 110.13, 117.74, 119.32, 125.83, 126.41, 127.60, 127.79, 128.34, 129.13, 135.93, 137.30, 140.09, 148.36, 159.12, 165.07. HRMS (ESI-TOF) (*m/z*): Calculated for $C_{19}H_{15}ClN_8OS$ $[M+H]^+$ 439.0856; found 439.0867.



(E)-3-(2-(6-amino-7H-[1,2,4]-triazolo[3,4-b][1,3,4]thiadiazin-3-yl)hydrazineylidene)-5-chloro-1-methylindolin-2-one 5t.

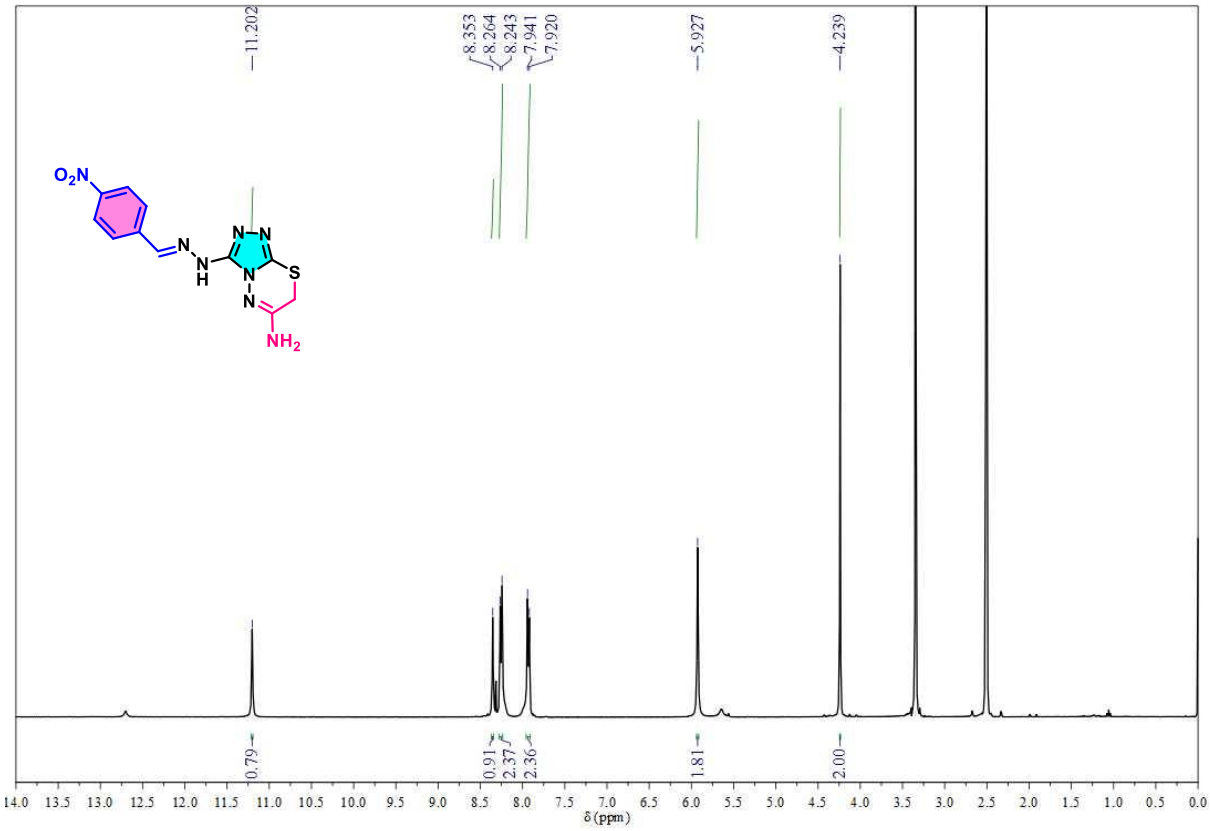
Yellow solid; Yield 85%; mp: 233-234 °C; IR (KBr) cm^{-1}. 3372 (NH_2), 3012 (NH), 1623 (lactam ring C=O), 1611 (N=C), 696 (Cl), ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 3.26 (s, 3H), 4.28 (s, 2H), 6.15 (s, 2H), 7.19 (d, J = 8.4 Hz, 1H), 7.45 – 7.47 (m, 1H), 7.59 (d, J = 1.9 Hz, 1H),



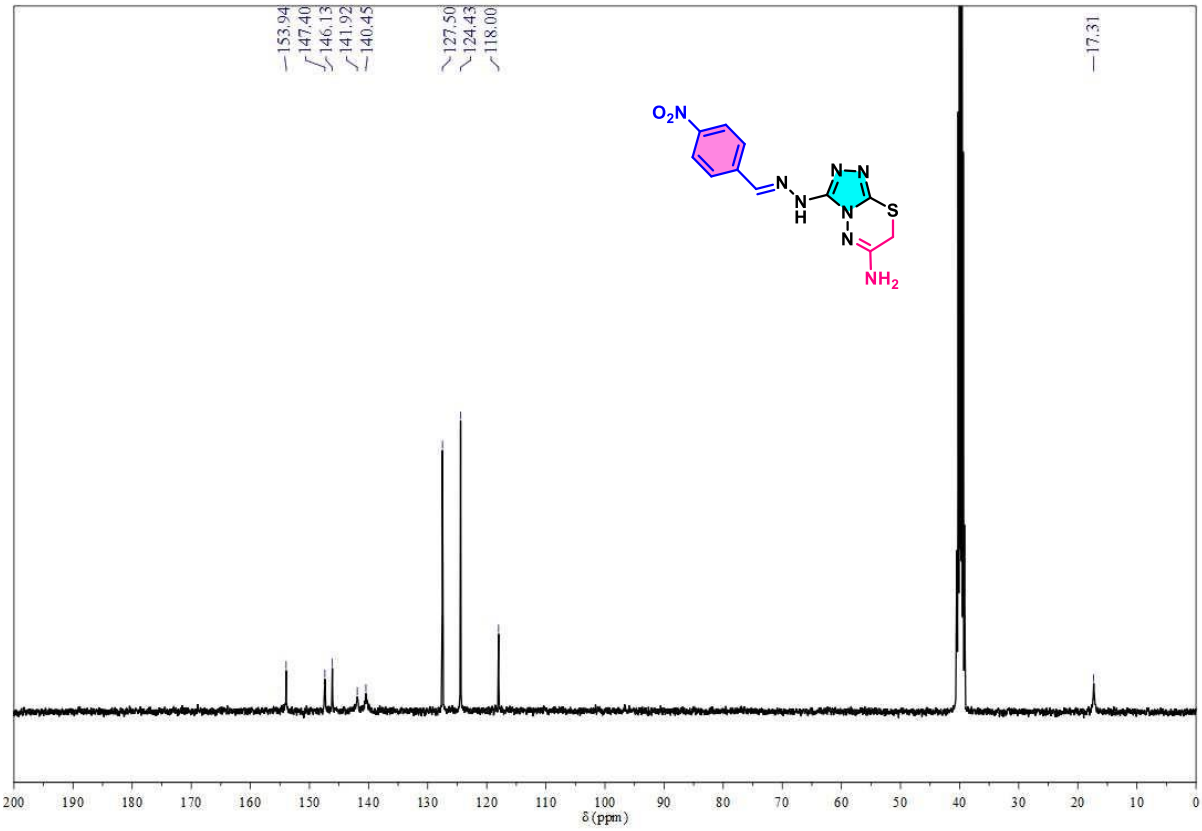
12.80 (s, 1H); ^{13}C NMR (100MHz, DMSO-d_6) δ (ppm): 15.99, 26.26, 109.56, 117.74, 119.06, 125.77, 126.20, 128.47, 136.38, 141.25, 148.19, 159.09, 164.96. HRMS (ESI-TOF) (*m/z*): Calculated for $C_{13}H_{11}ClN_8OS$ $[M+H]^+$ 363.0543; found 363.0553.

6.6. Copies of spectral data ^{1}H NMR, ^{13}C NMR, HRMS of synthesized compounds.

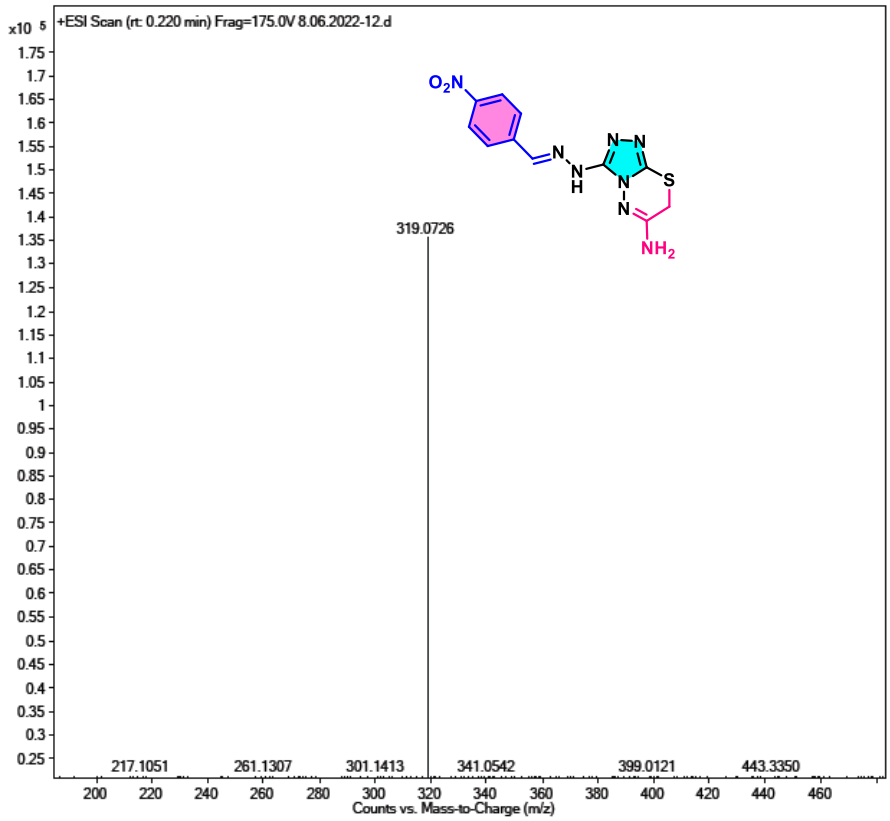
^{1}H NMR spectrum of compound 4a (DMSO-d_6) 400 MHz



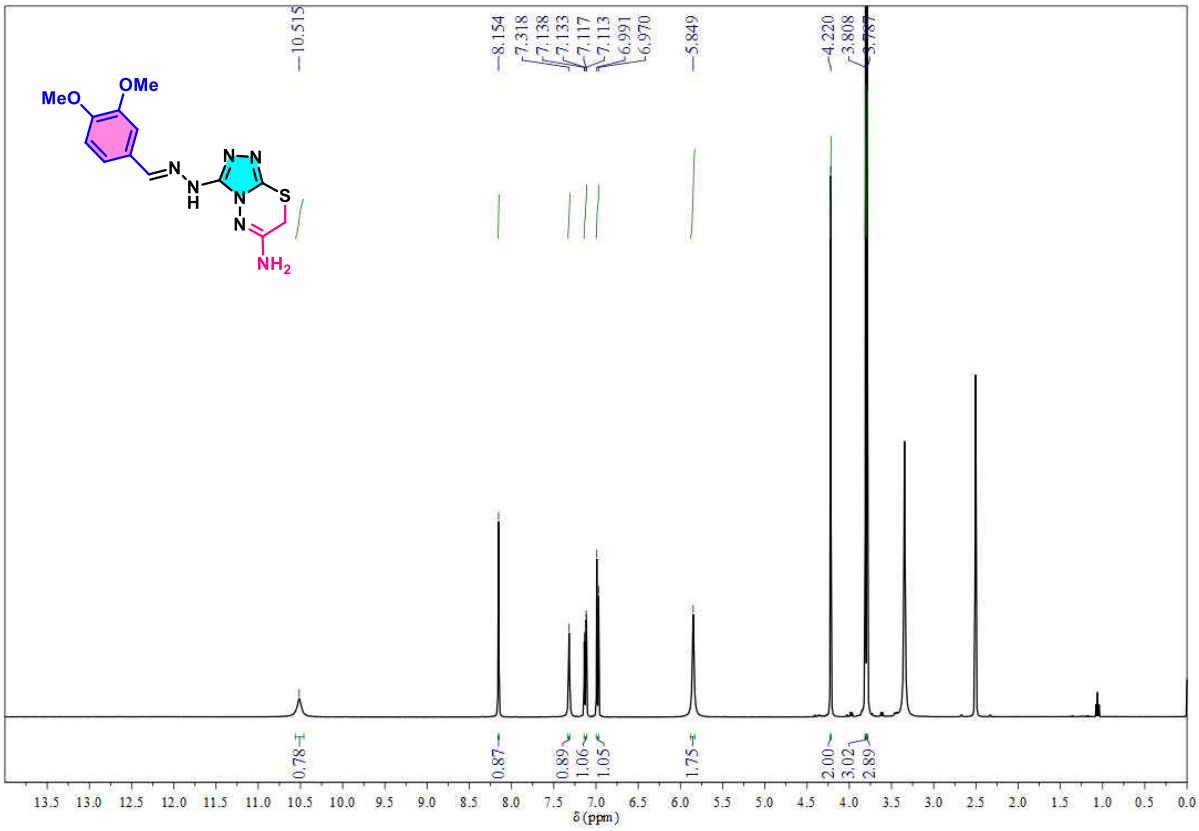
^{13}C NMR spectrum of compound 4a (DMSO-d_6) 100 MHz



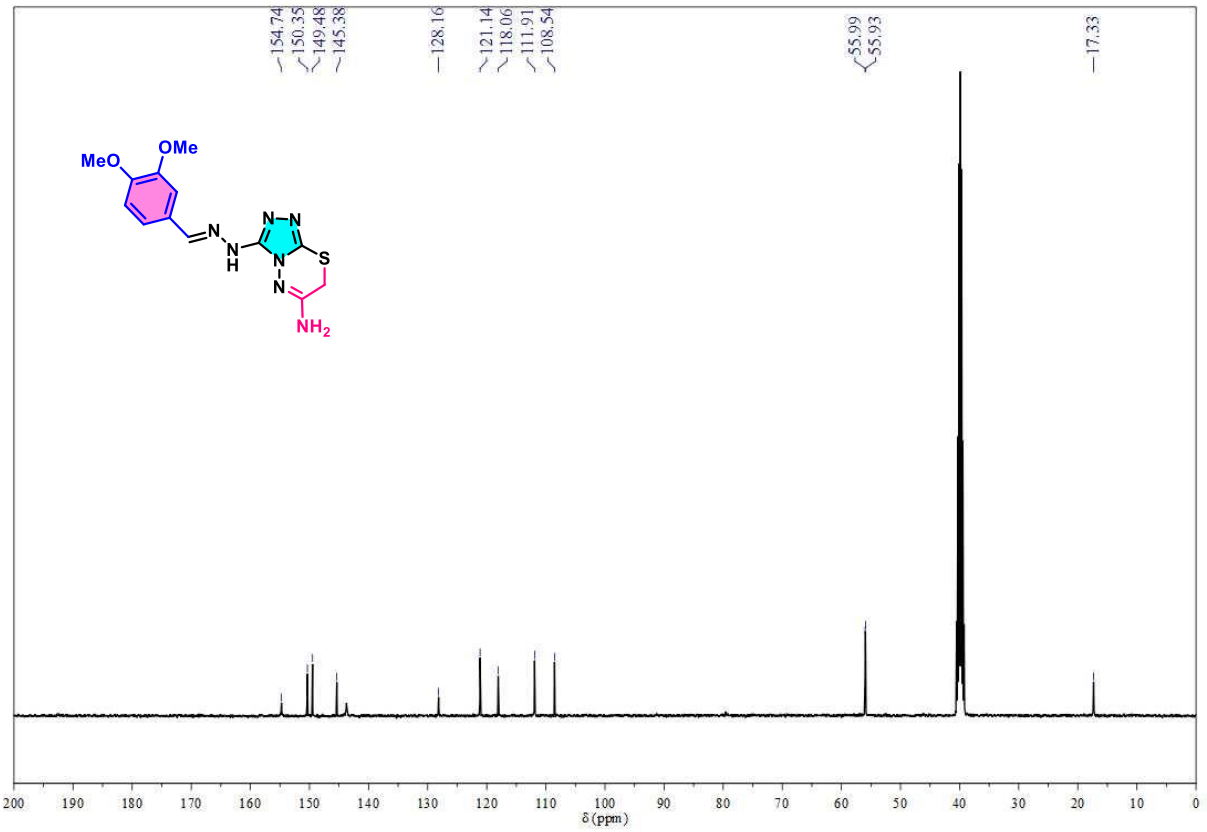
Mass Spectrum of compound 4a



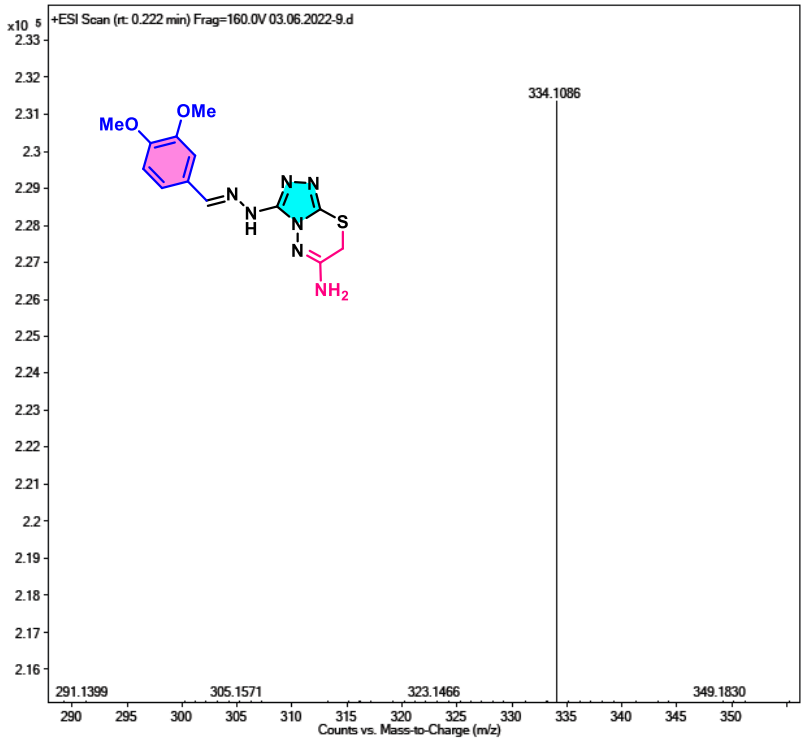
^{1}H NMR spectrum of compound 4b (DMSO-d_6) 400 MHz



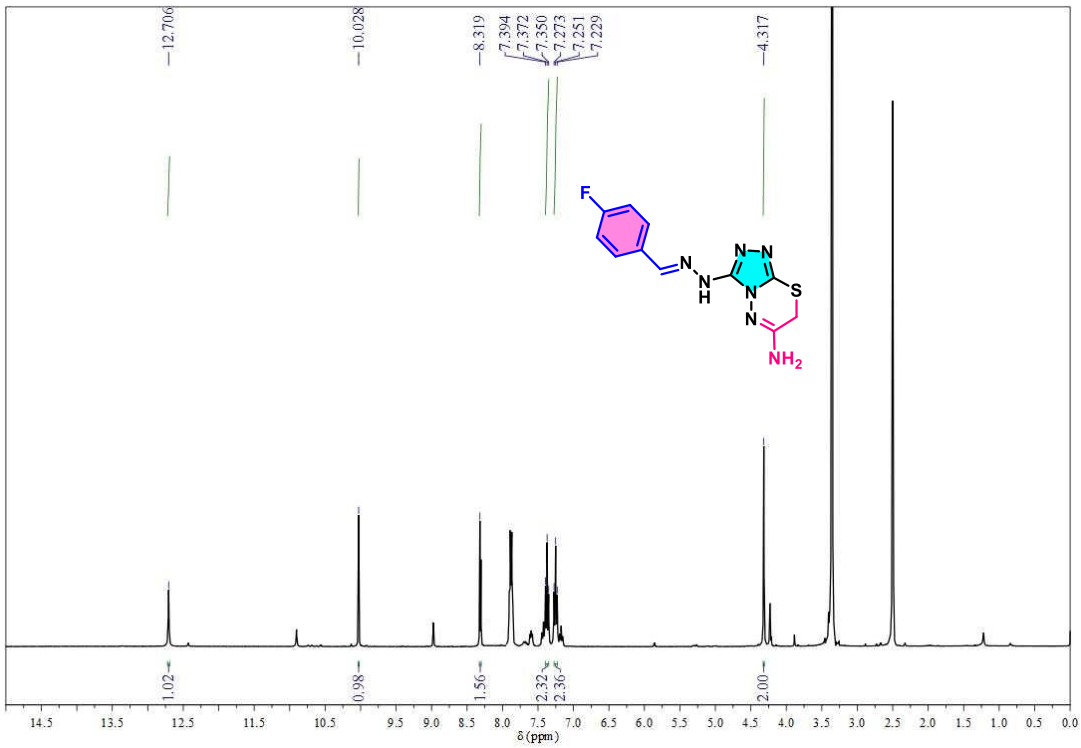
^{13}C NMR Spectrum of compound 4b (DMSO-d_6) 100 MHz



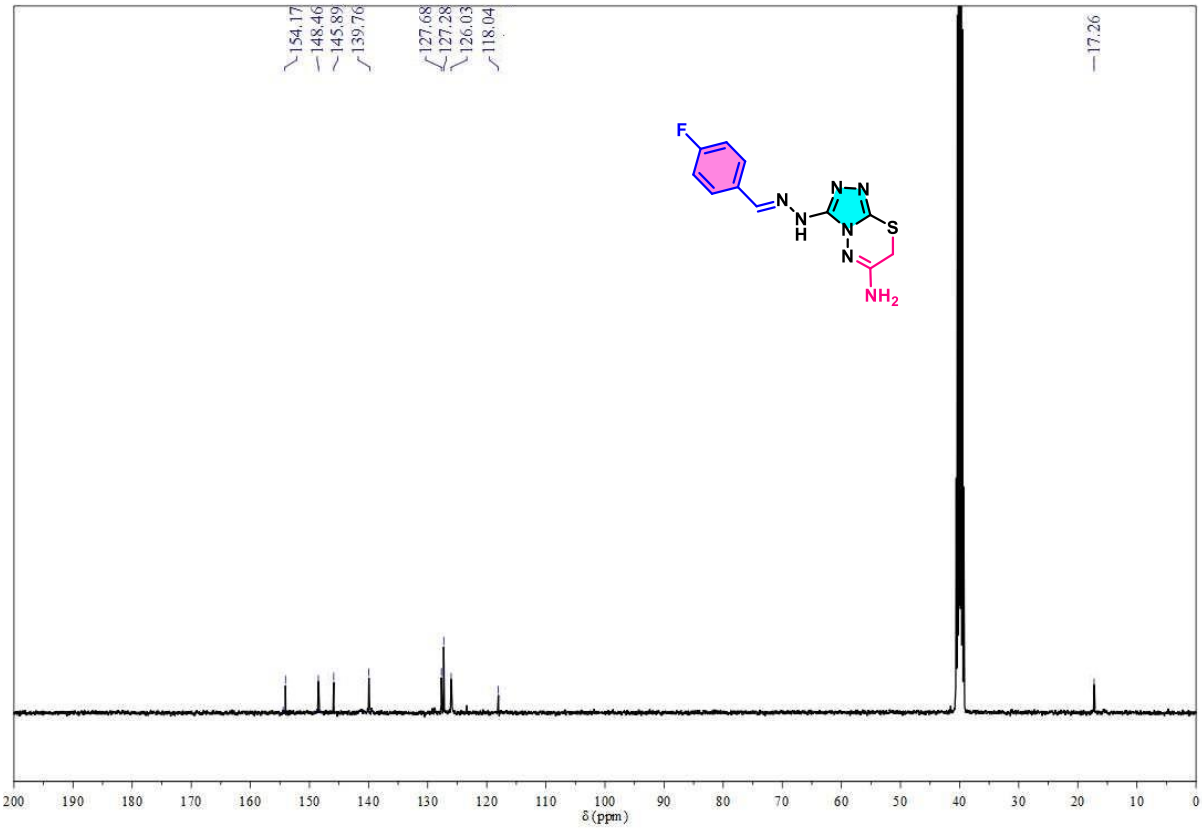
Mass spectrum of compound 4b



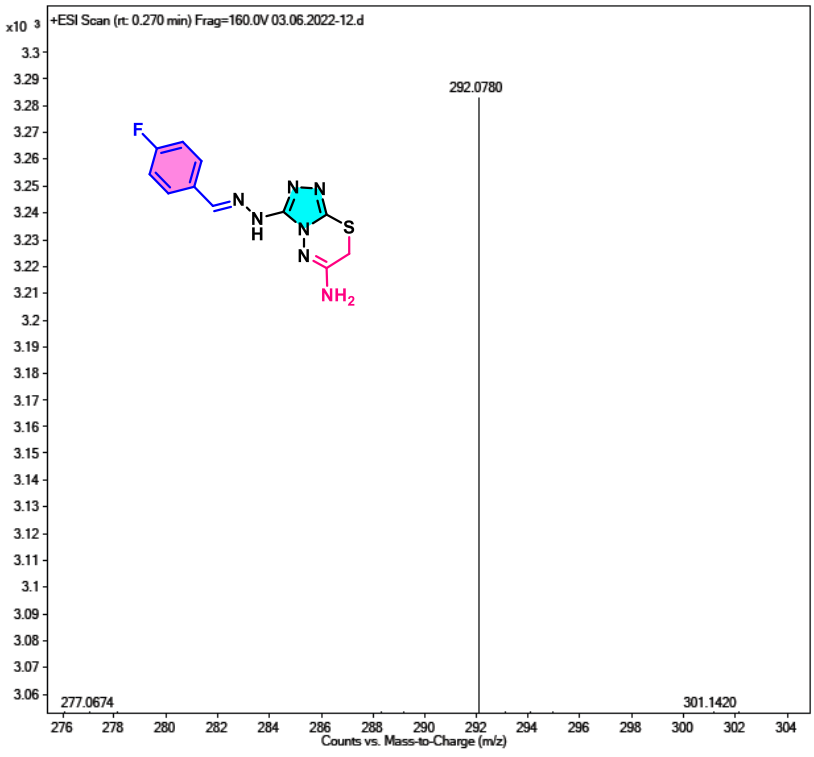
^{1}H NMR spectrum of compound 4c (DMSO-d_6) 400 MHz



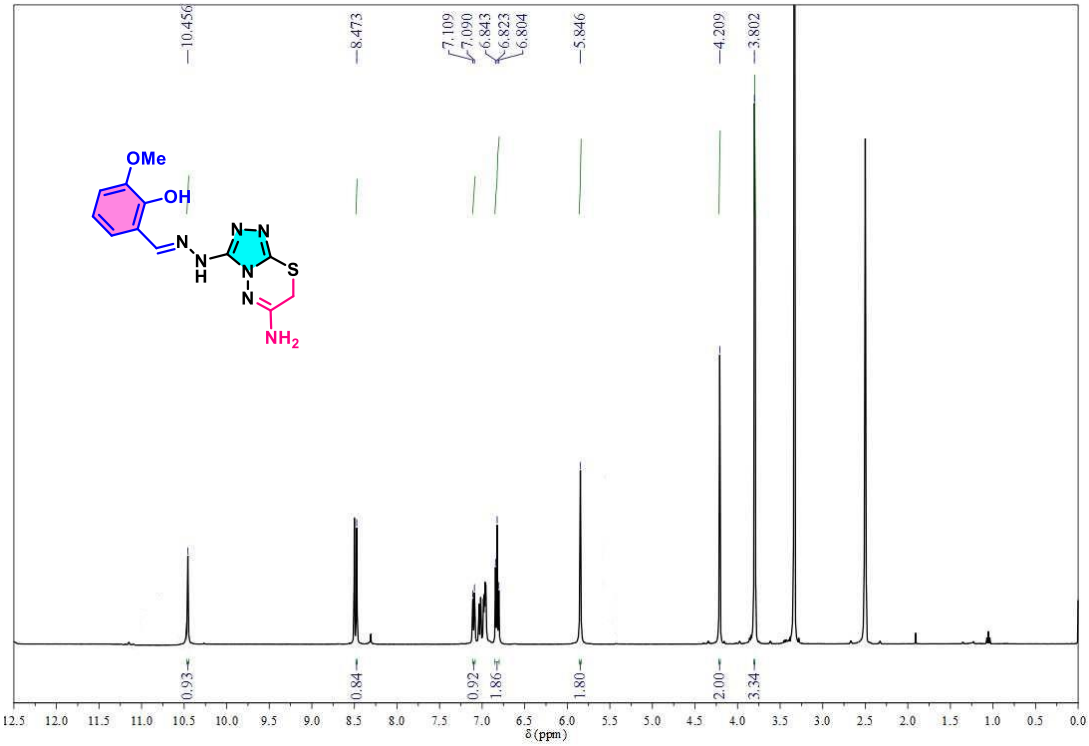
^{13}C NMR spectrum of compound 4c (DMSO-d_6) 100 MHz



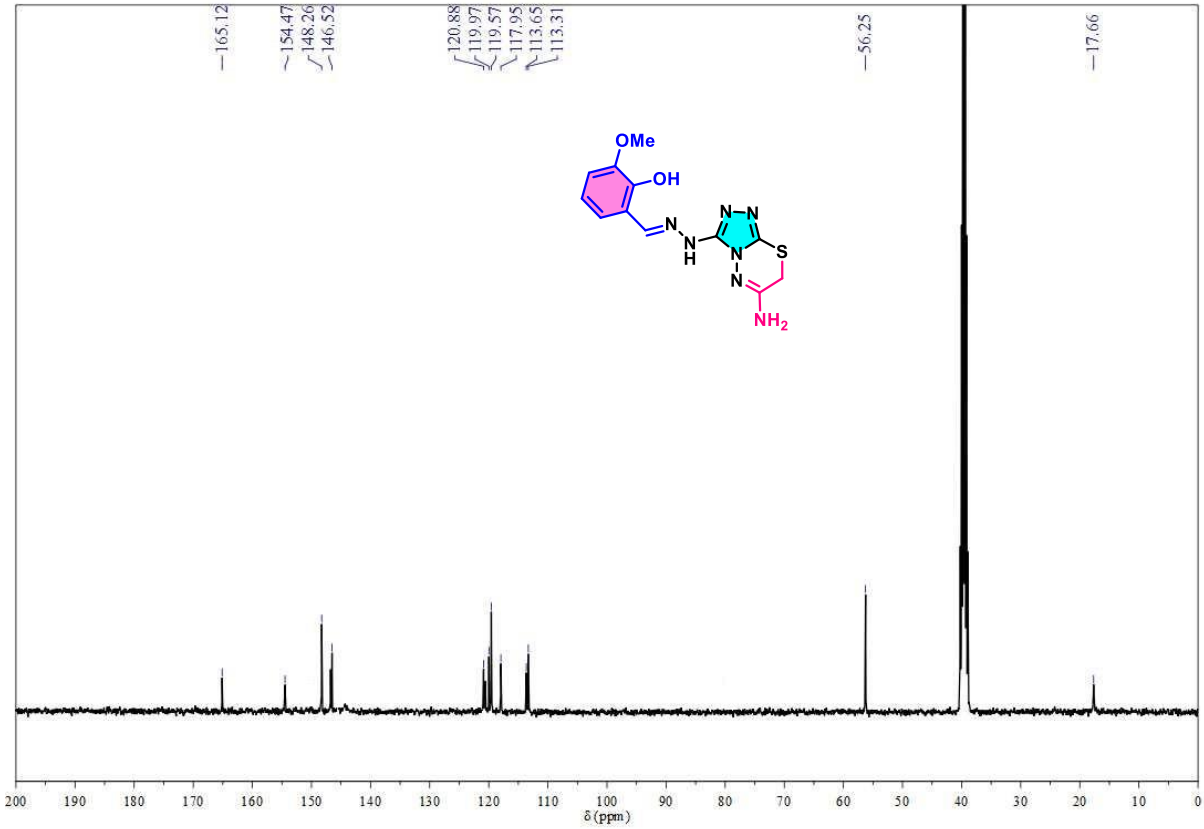
Mass spectrum of compound 4c



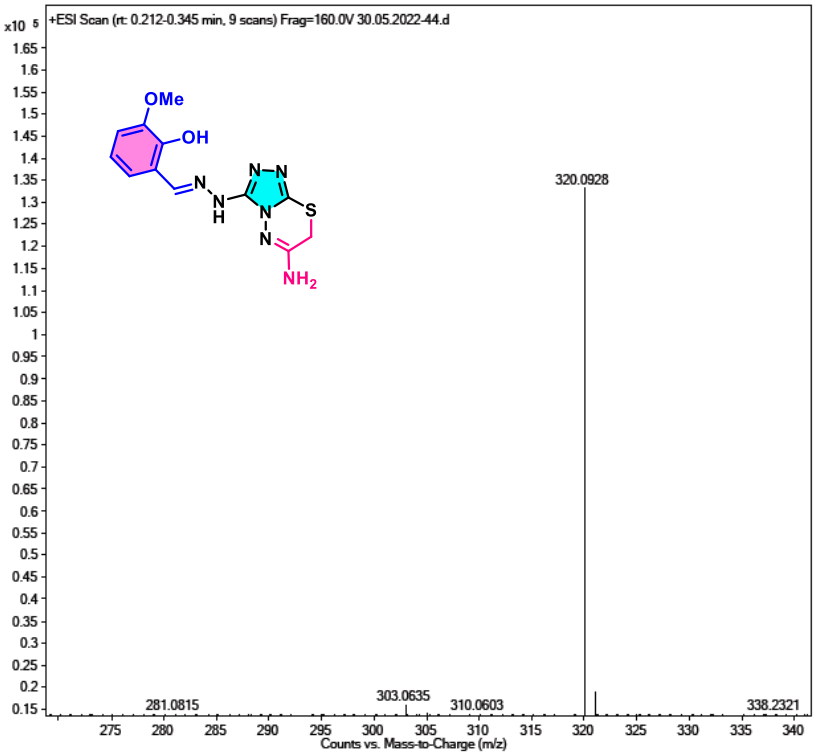
^{1}H NMR Spectrum of compound 4d (DMSO-d_6) 400 MHz



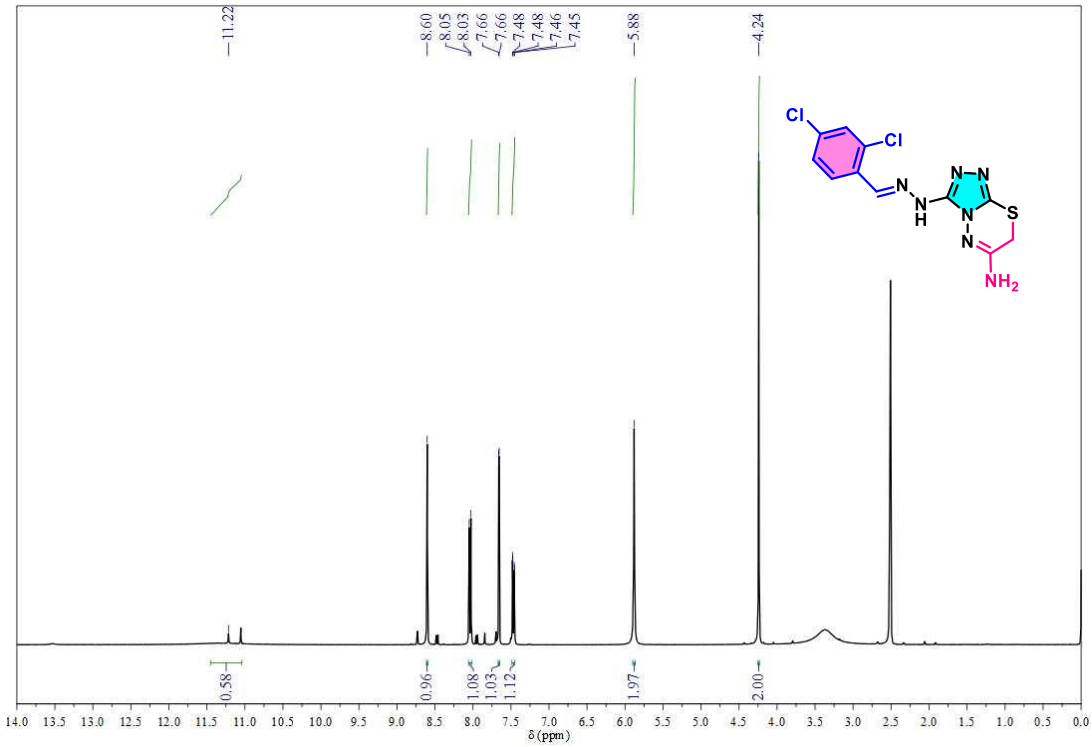
^{13}C NMR spectrum of compound 4d (DMSO-d_6) 100 MHz



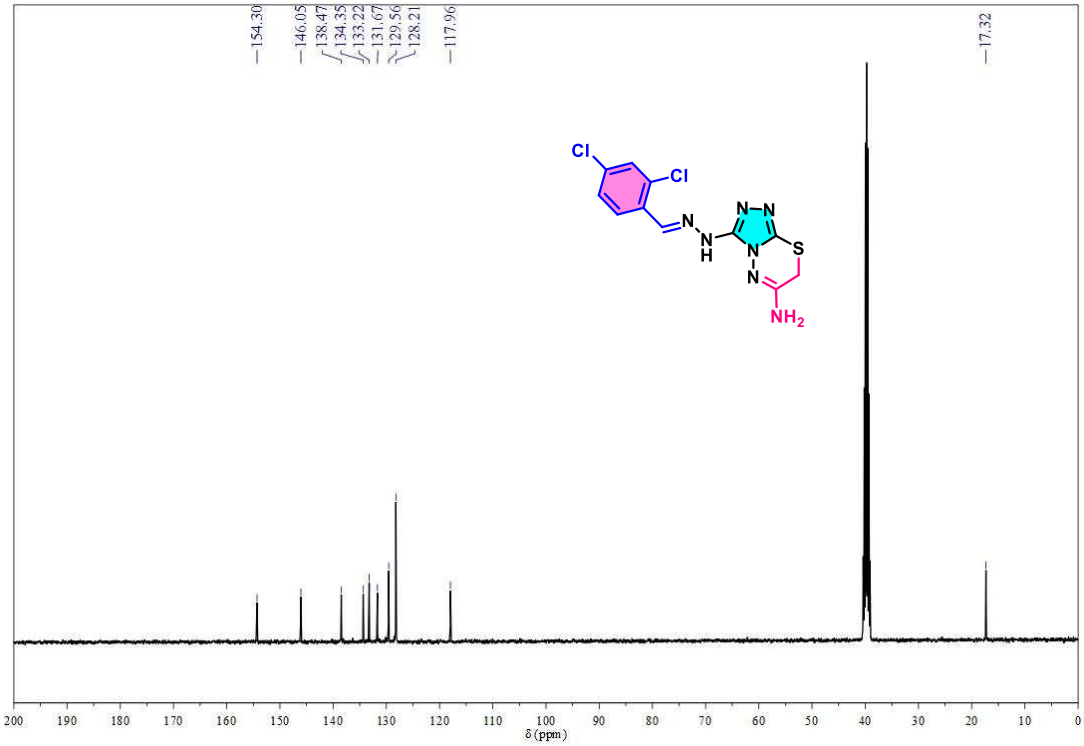
Mass spectrum of compound 4d



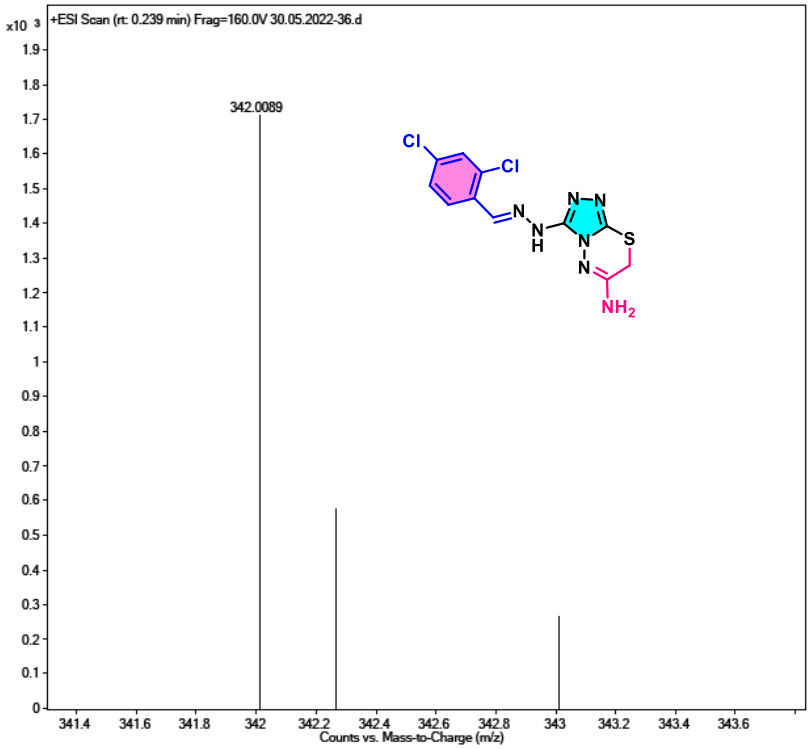
^{1}H NMR spectrum of compound 4e (DMSO-d_6) 400 MHz



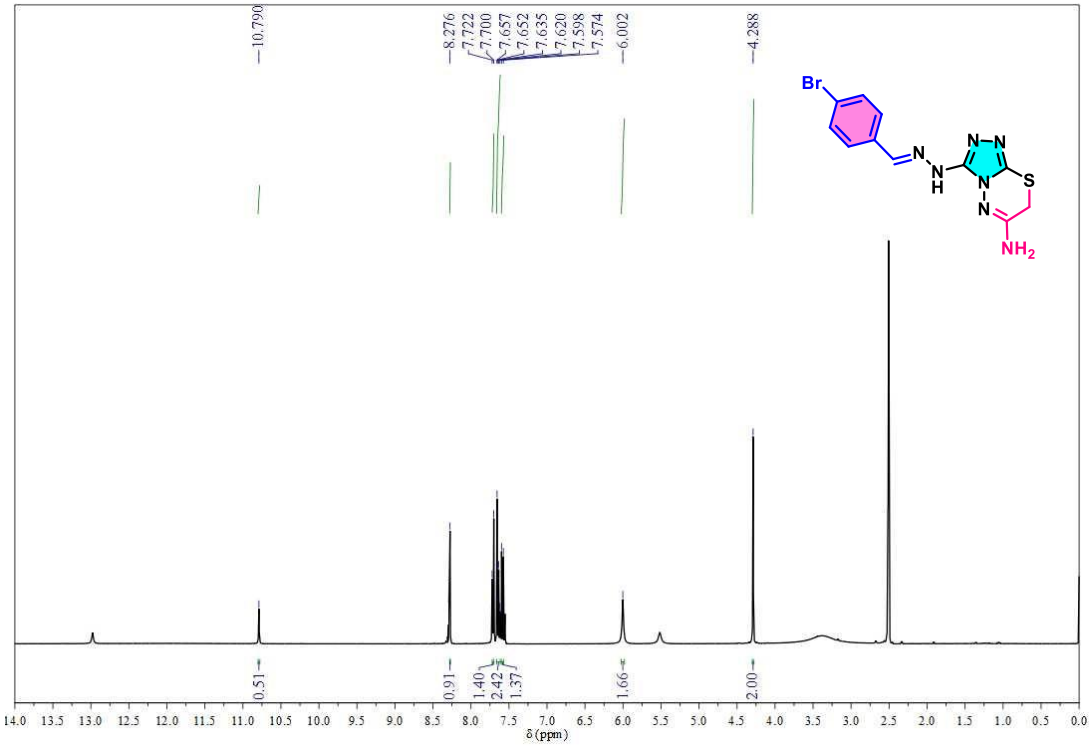
^{13}C NMR spectrum of compound 4e (DMSO-d_6) 100 MHz



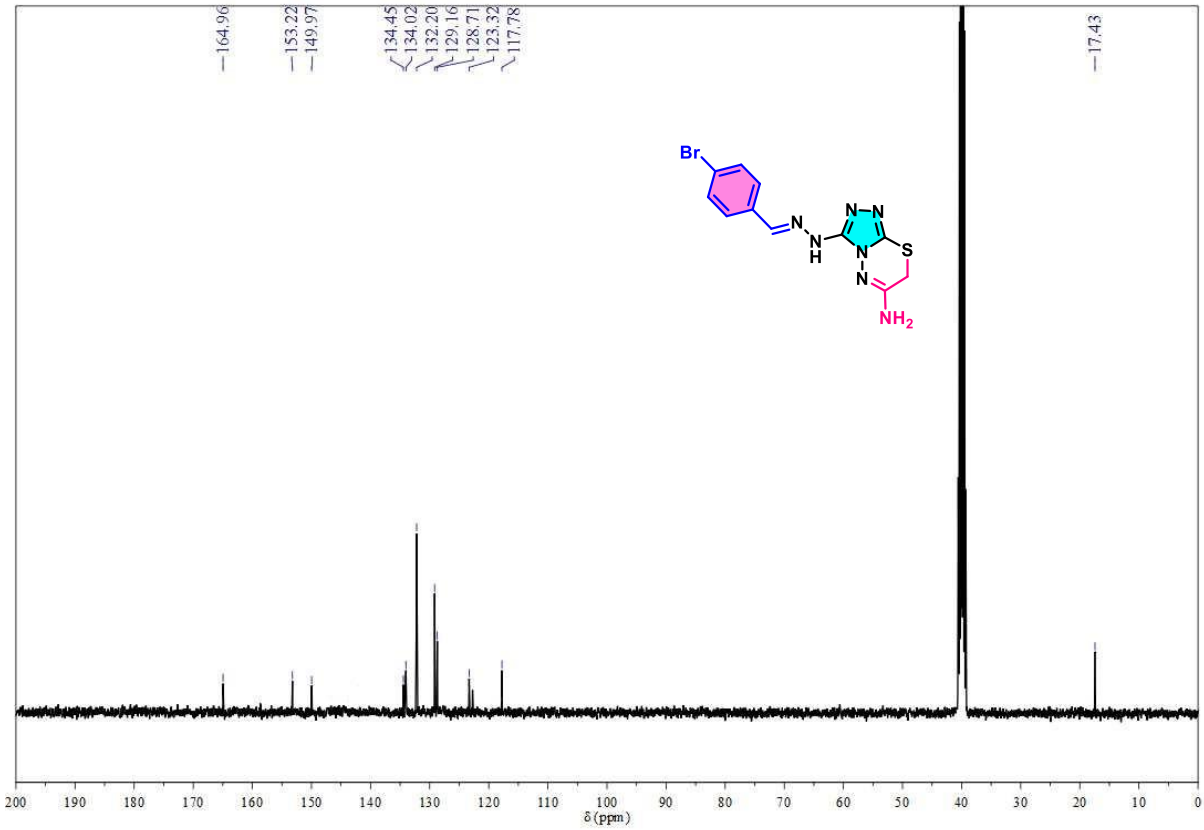
Mass spectrum of compound 4e



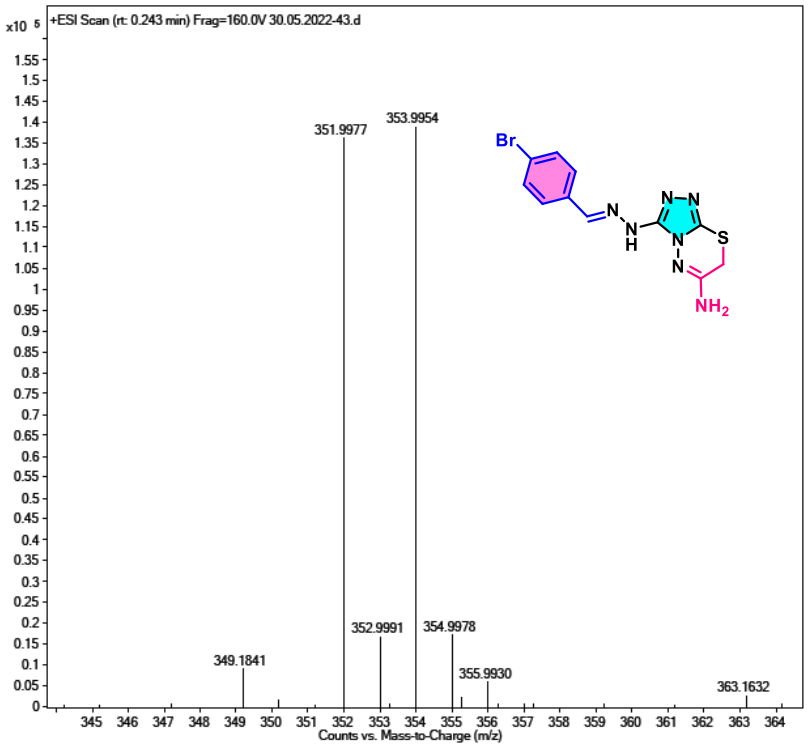
^{1}H NMR Spectrum of compound 4f (DMSO-d_6) 400 MHz



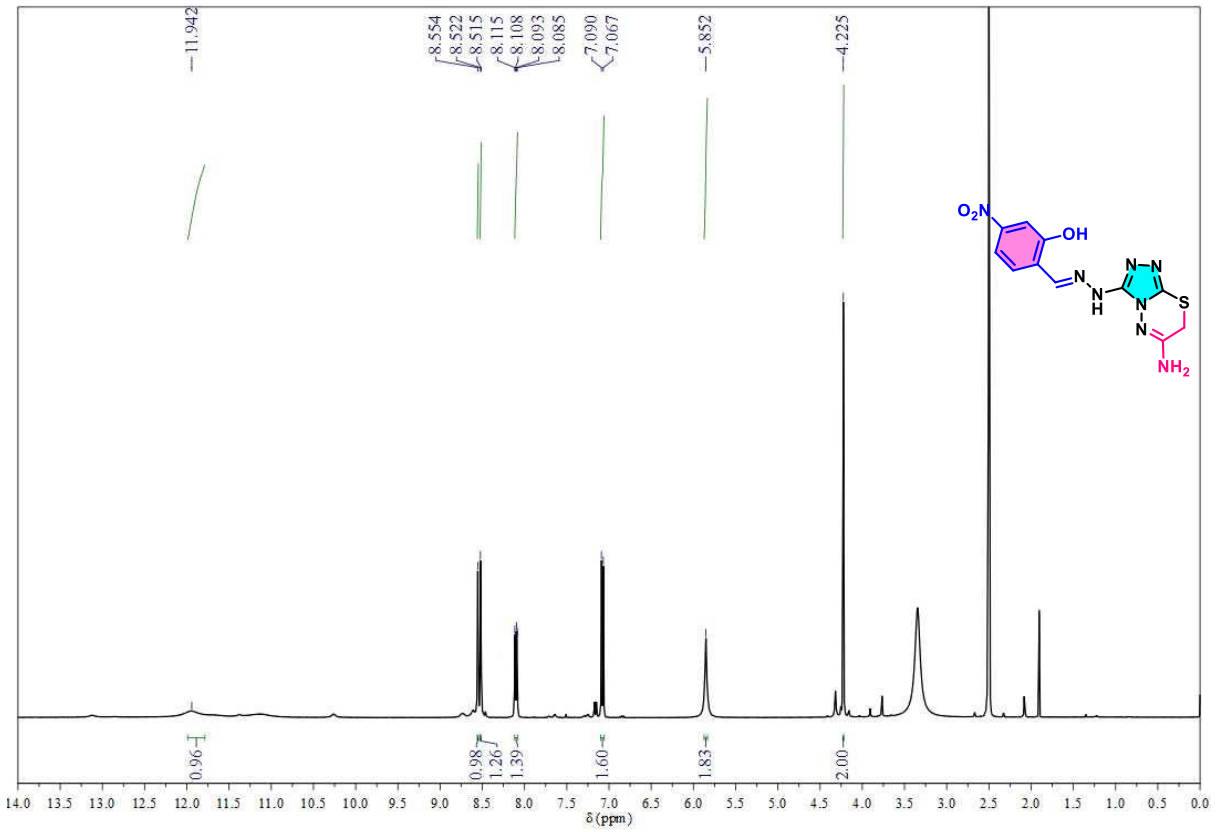
^{13}C NMR spectrum of compound 4f (DMSO-d_6) 100 MHz



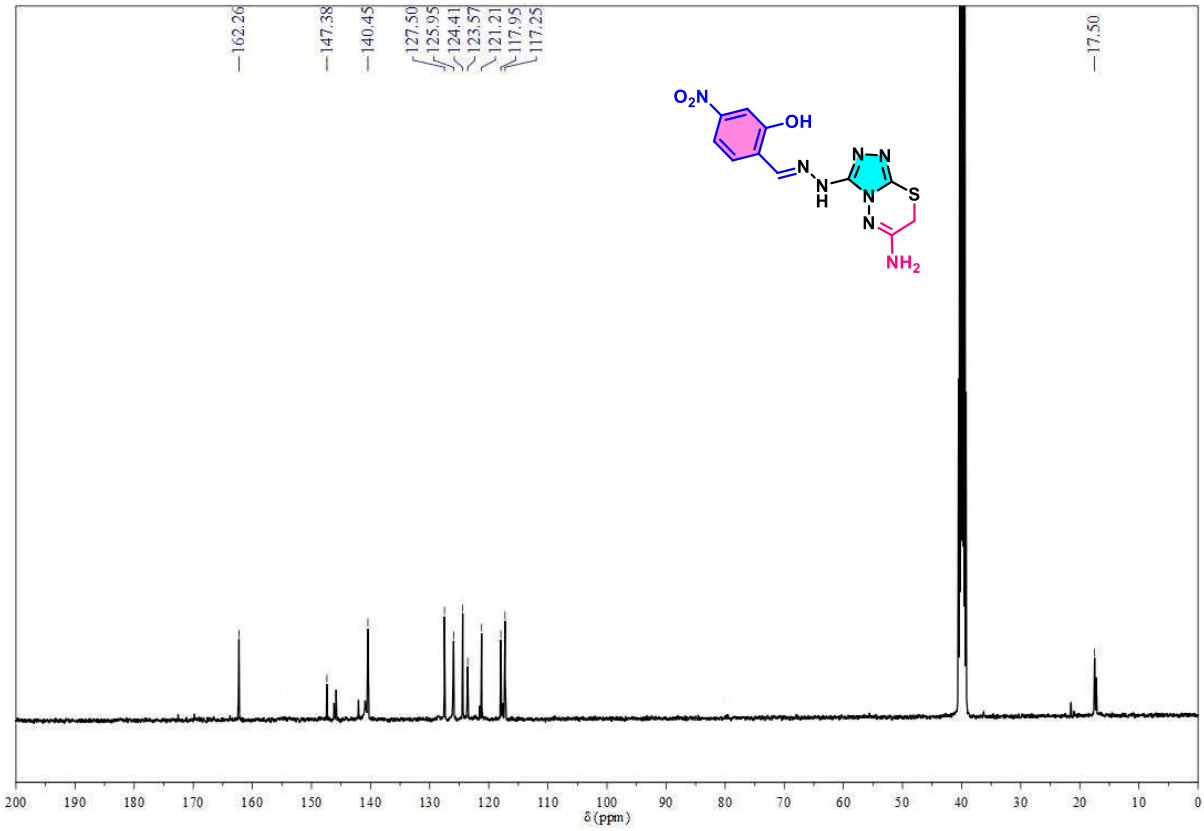
MASS Spectrum of compound 4f



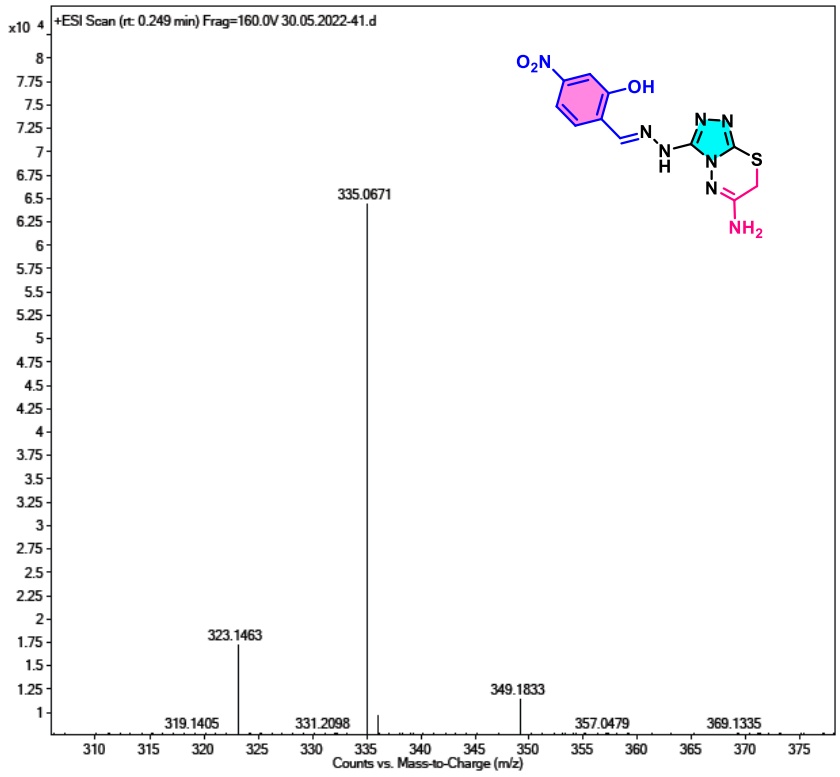
^{1}H NMR Spectrum of compound 4g (DMSO-d_6) 400 MHz



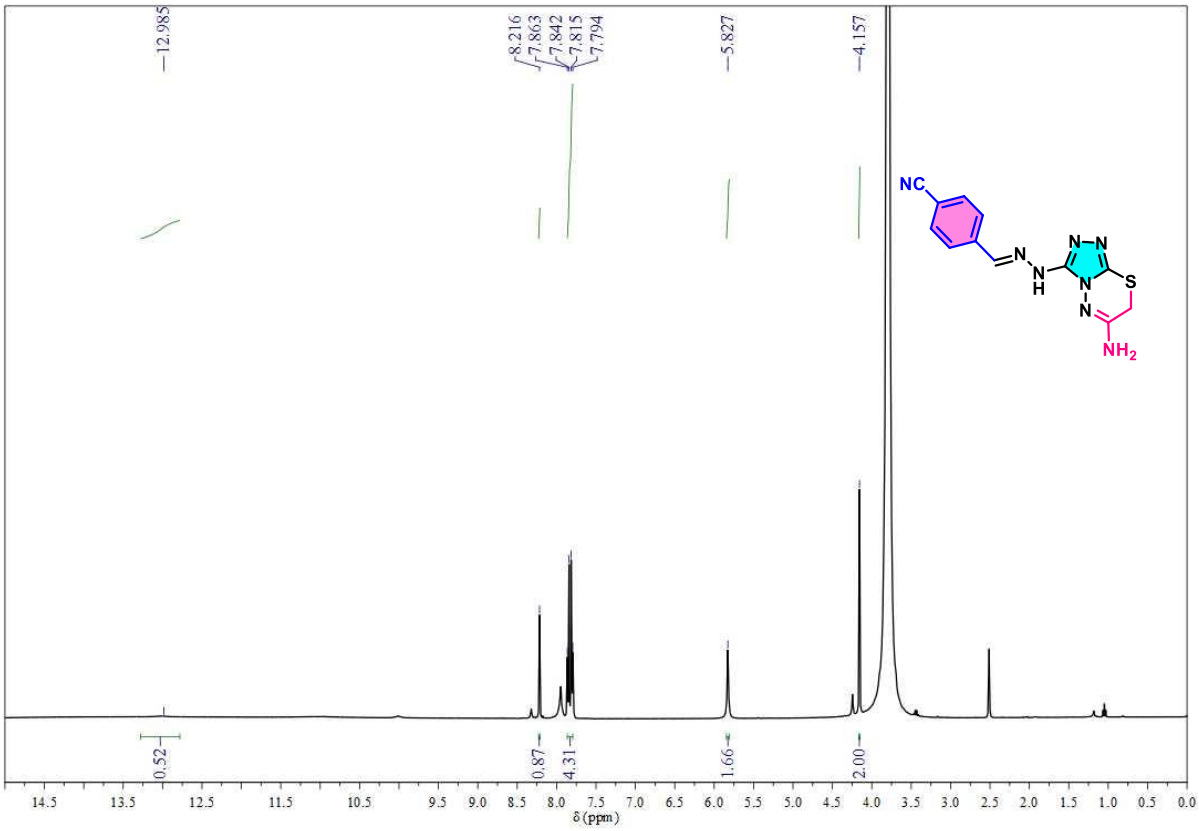
^{13}C NMR spectrum of compound 4g (DMSO-d_6) 100 MHz



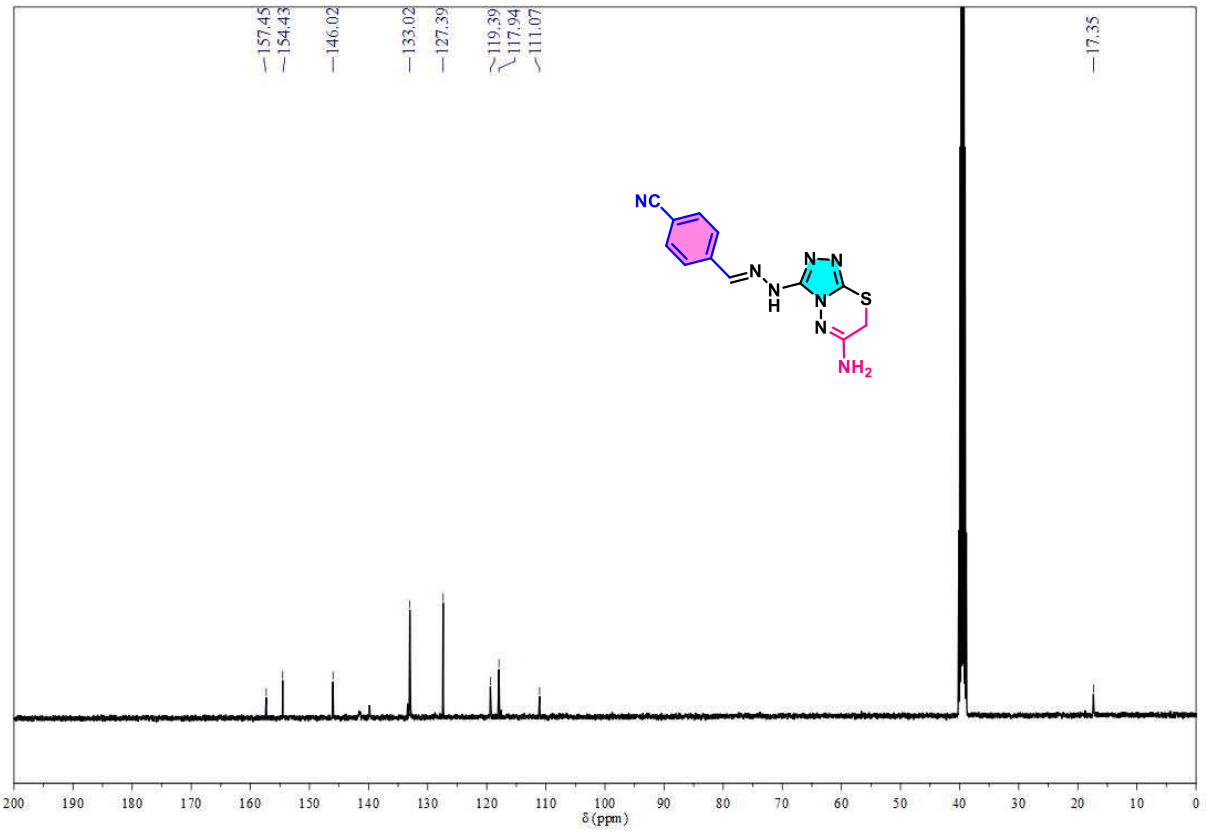
Mass spectrum of compound 4g



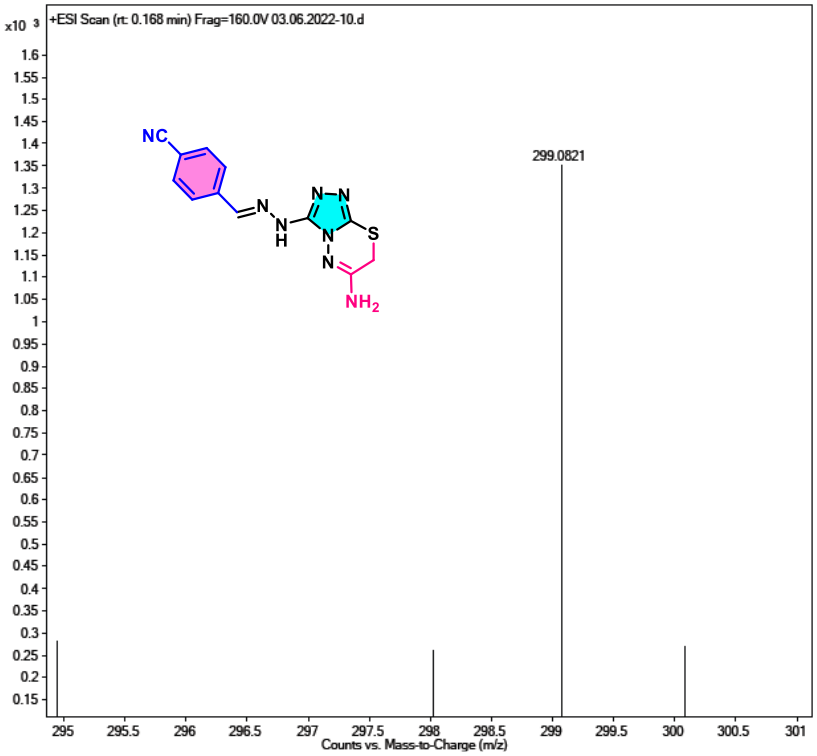
^{1}H NMR Spectrum of compound 4h (DMSO-d_6) 400 MHz



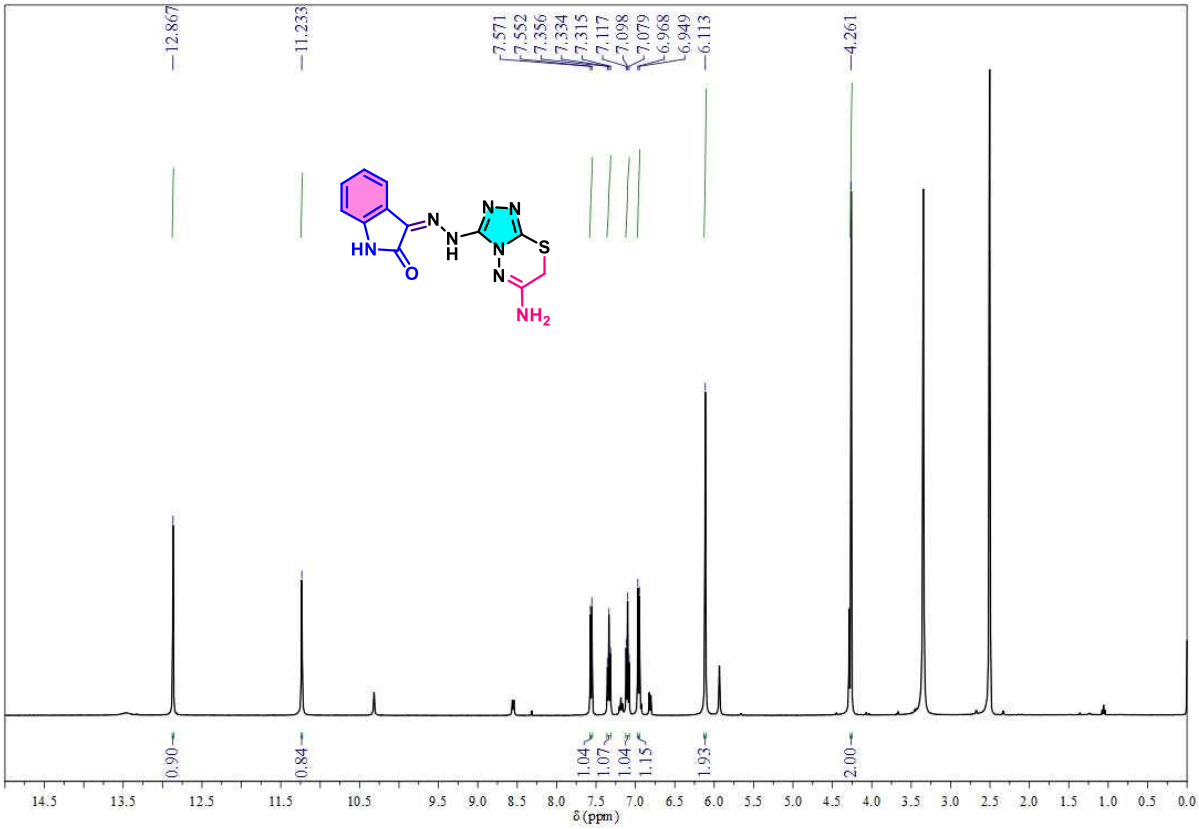
^{13}C NMR spectrum of compound 4h (DMSO-d_6) 100 MHz



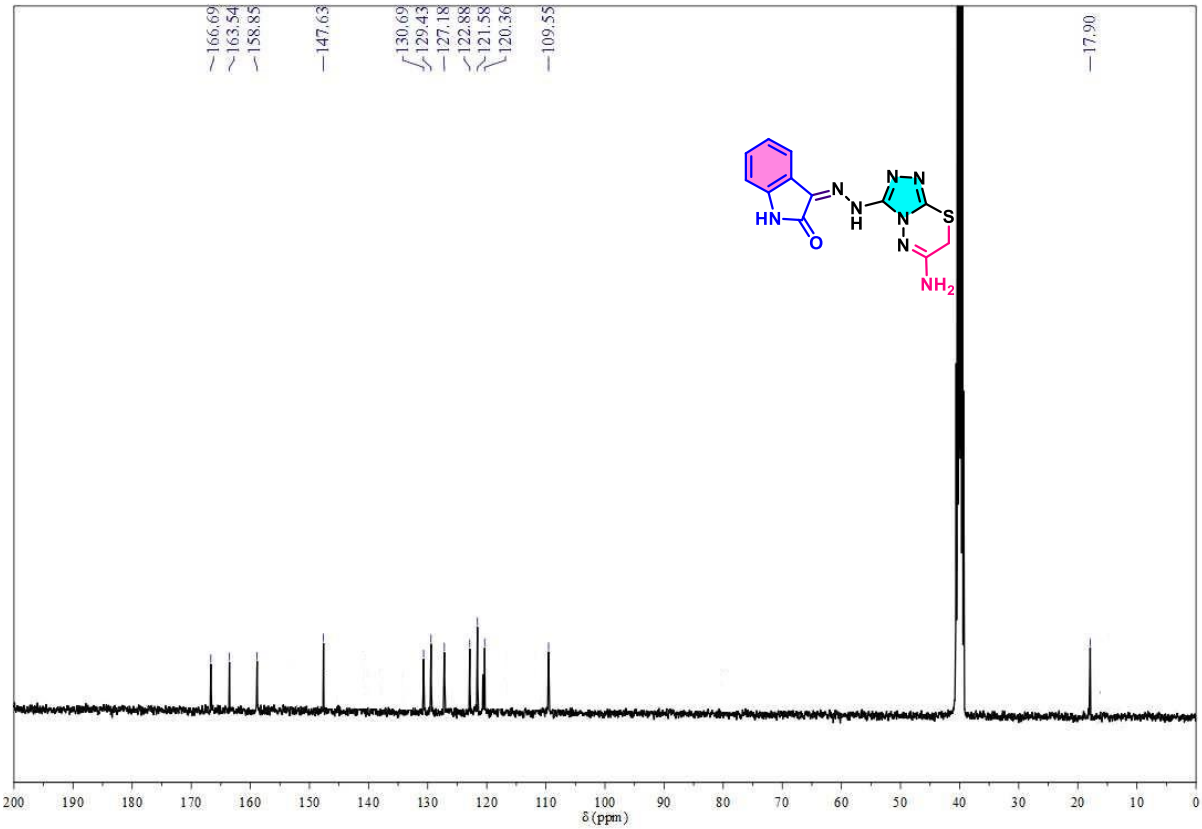
Mass spectrum of compound 4h



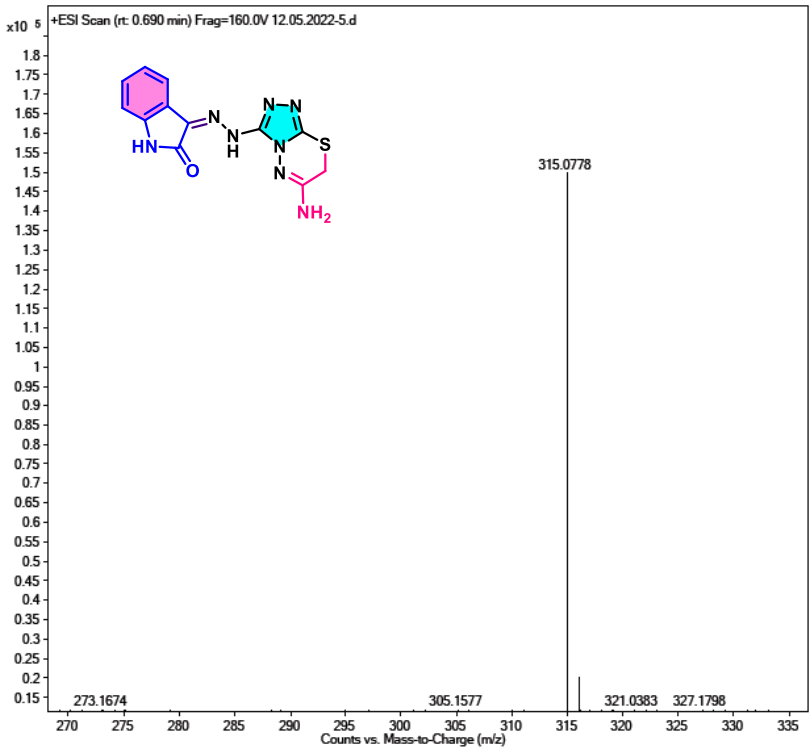
^{1}H NMR Spectrum of compound 5j (DMSO-d_6) 400 MHz



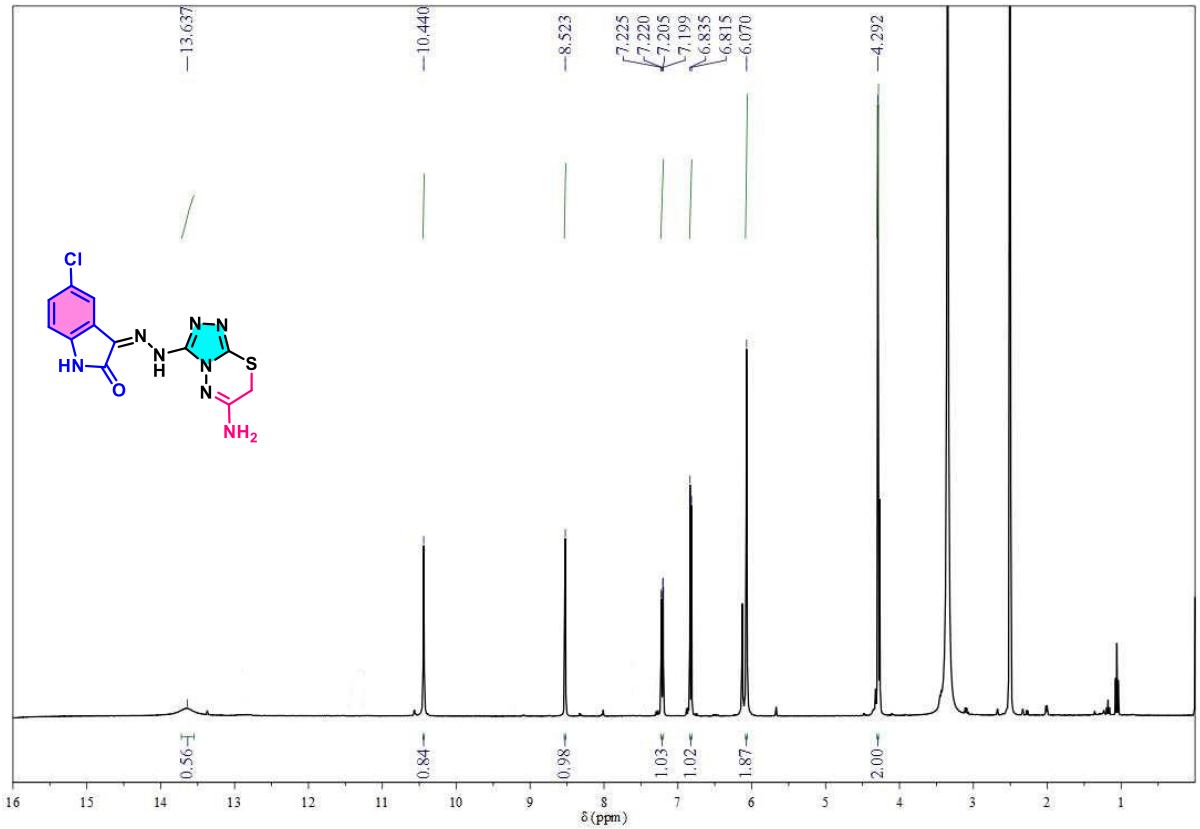
^{13}C NMR Spectrum of compound 5j (DMSO-d_6) 100 MHz



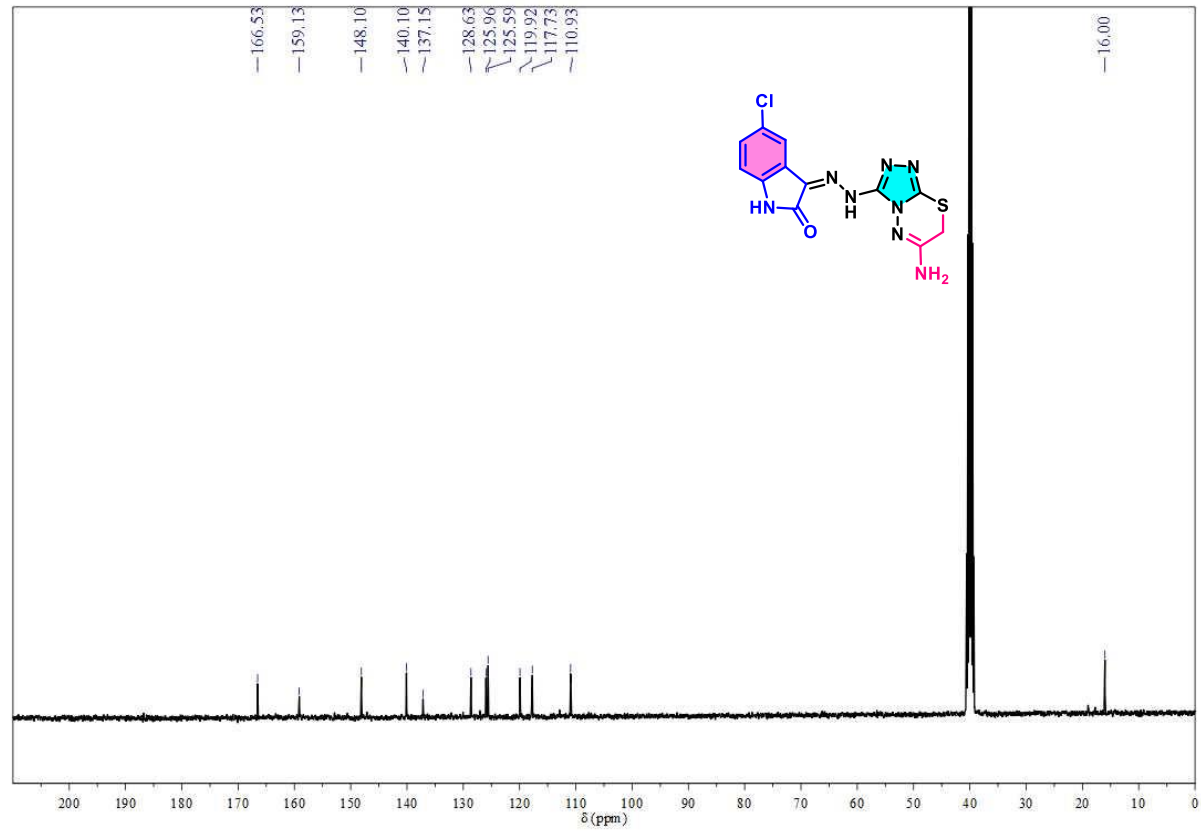
Mass spectrum of compound 5j



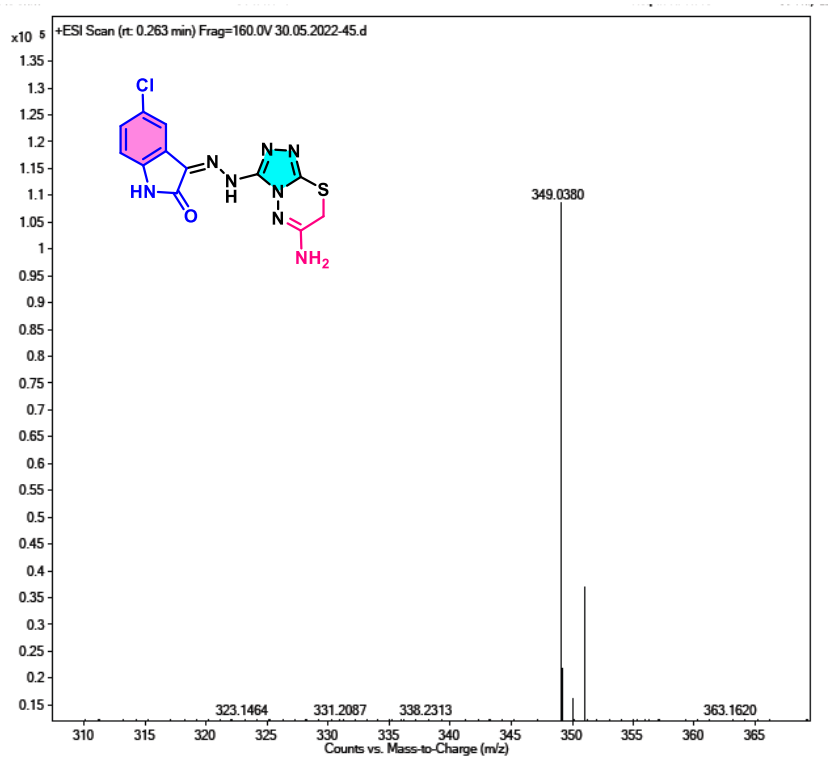
^{1}H NMR Spectrum of compound 5k (DMSO-d_6) 400 MHz



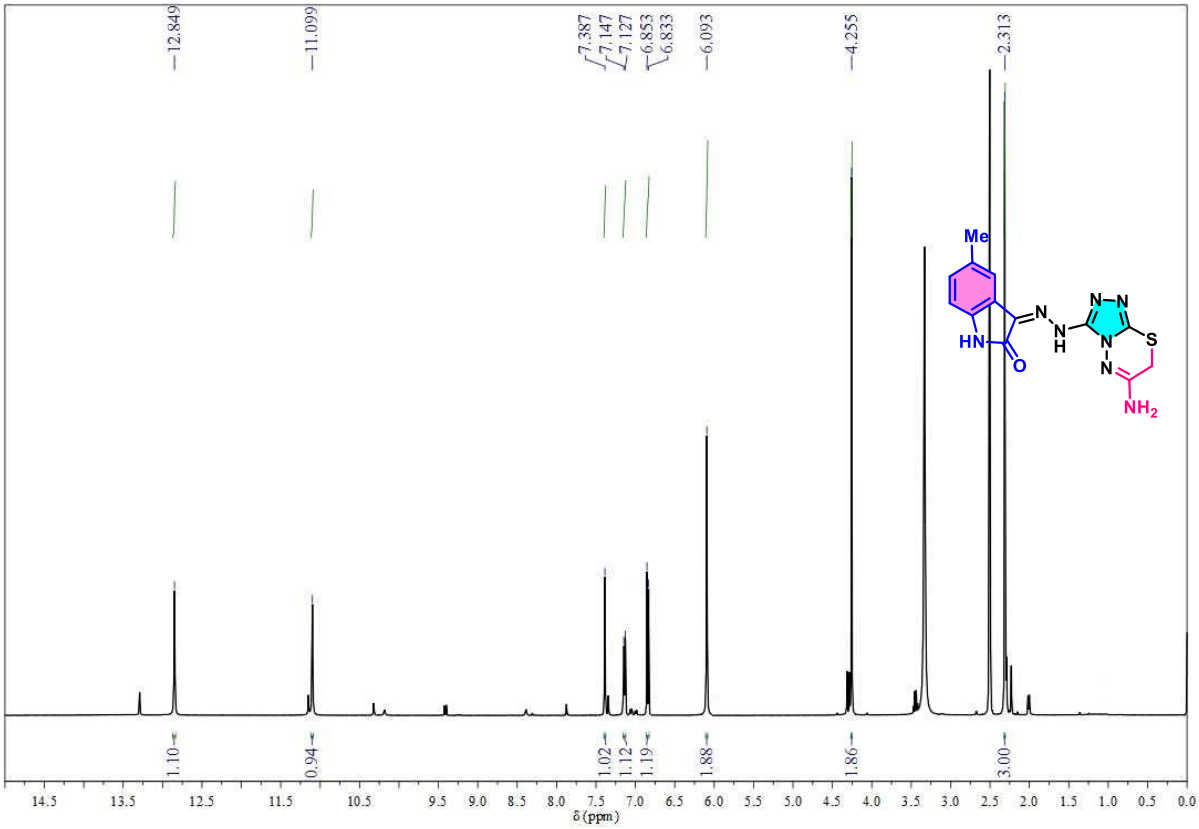
^{13}C NMR spectrum of compound 5k (DMSO-d_6) 100 MHz



Mass Spectrum of compound 5k



^{1}H NMR Spectrum of compound 5l (DMSO-d_6) 400 MHz



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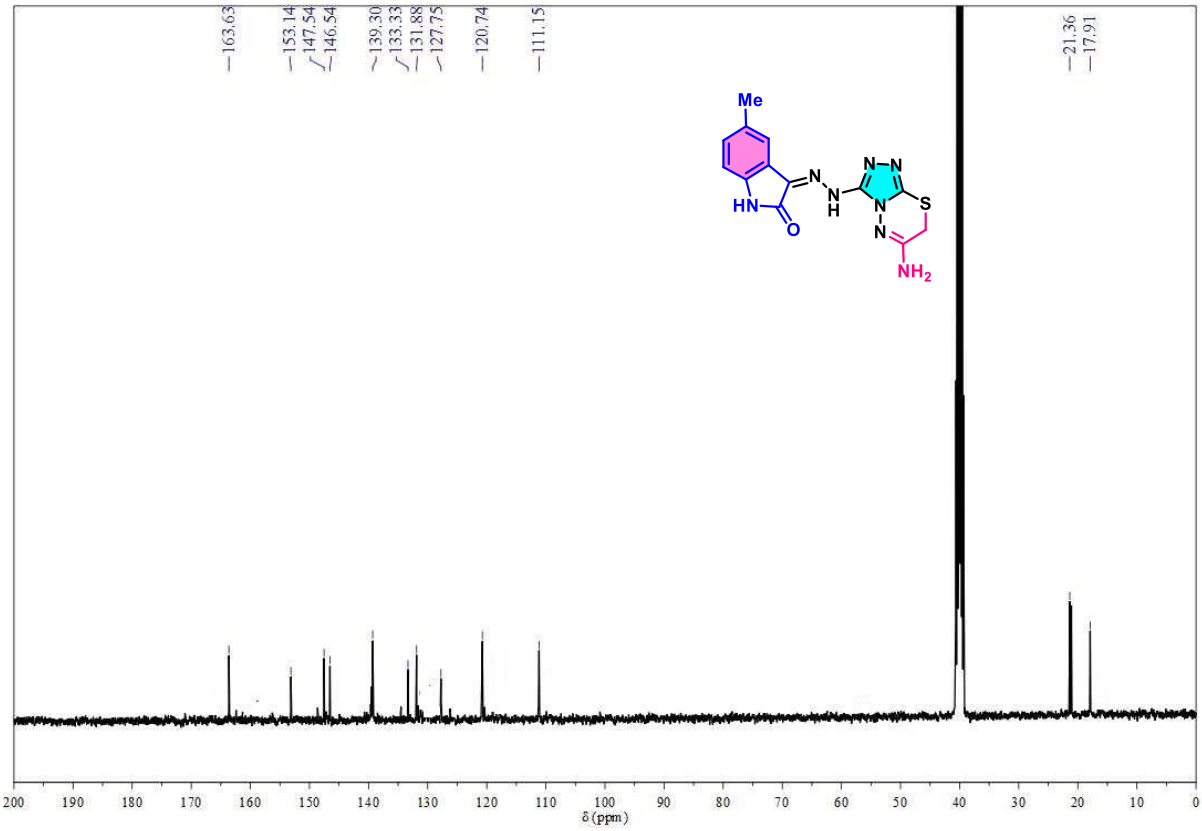
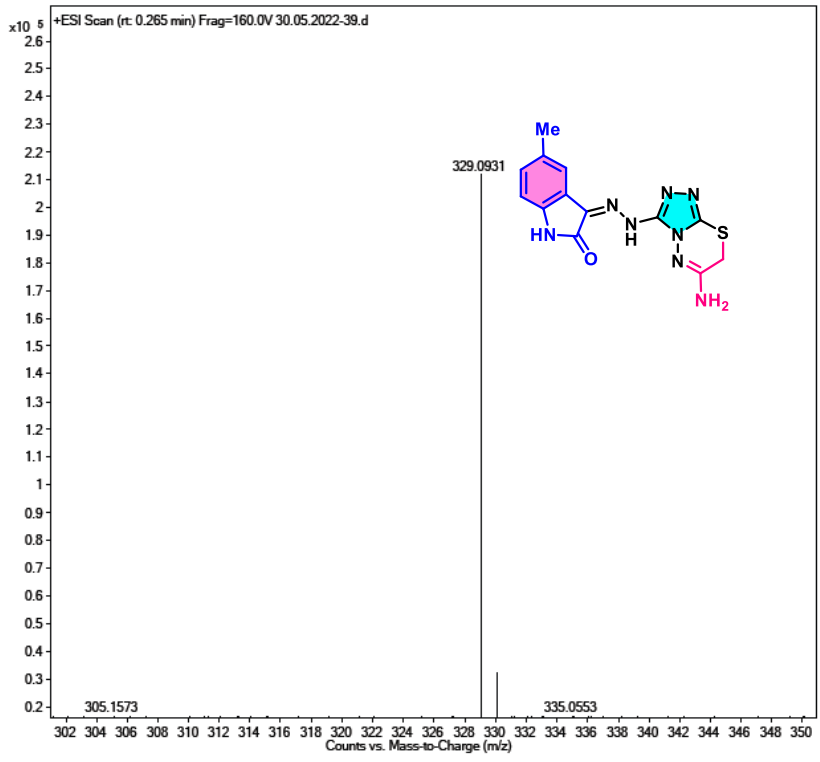
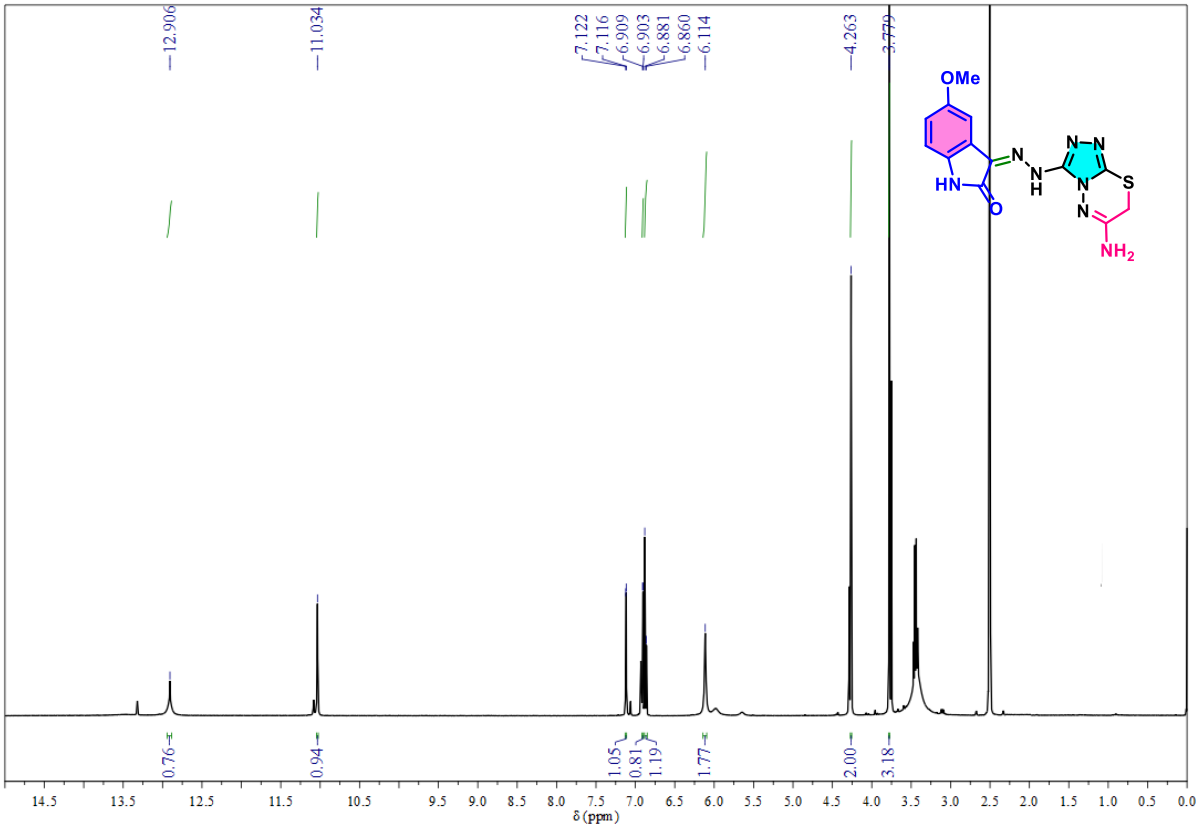


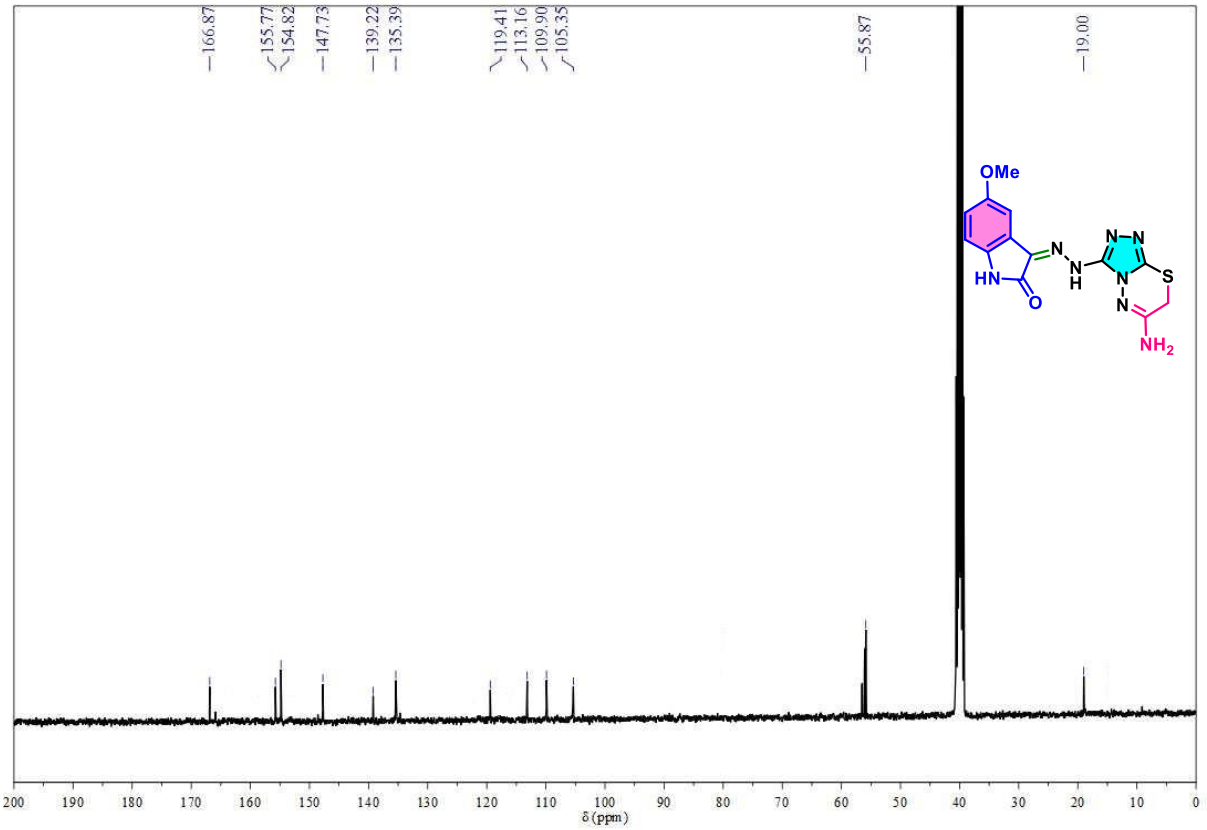
Mass Spectrum of compound 5l



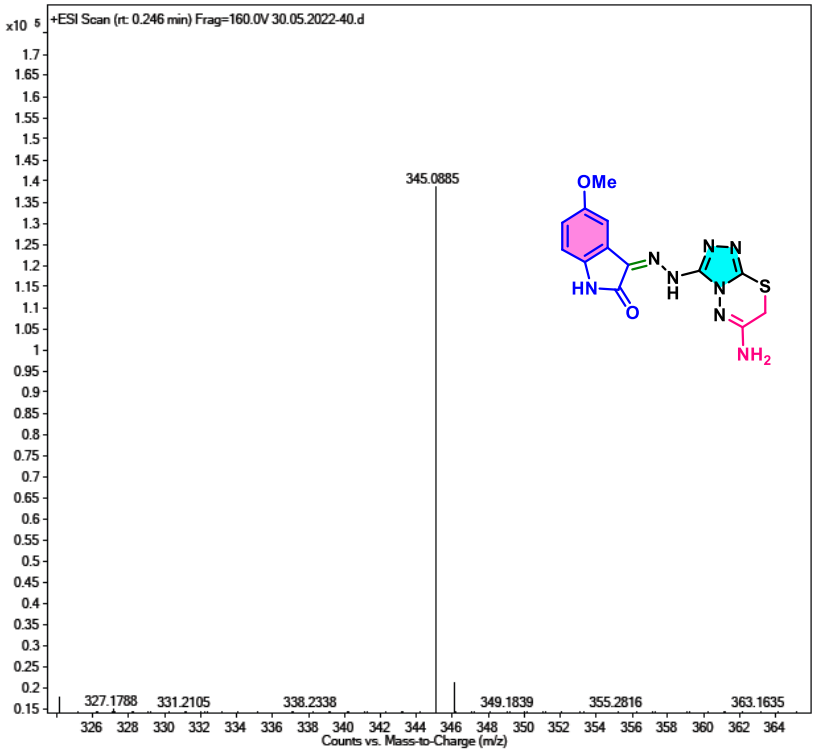
^{1}H NMR Spectrum of compound 5m (DMSO-d_6) 400 MHz



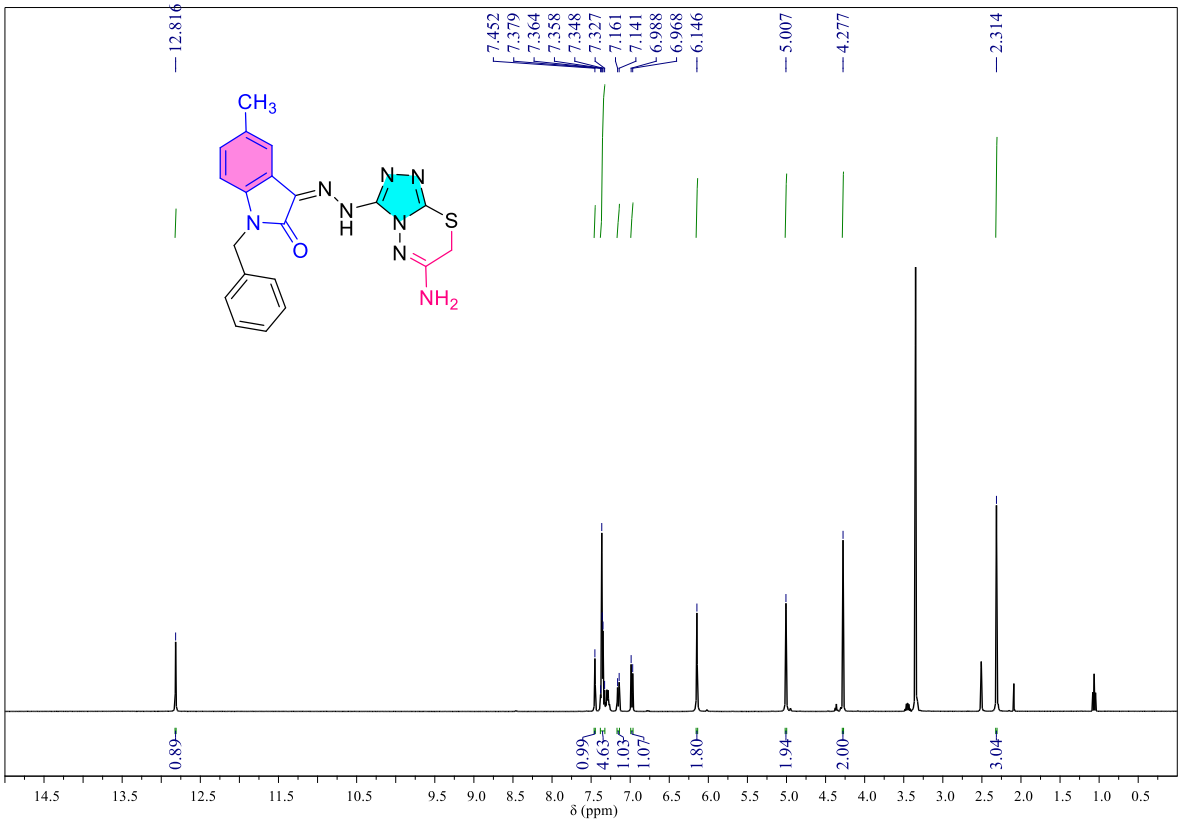
^{13}C NMR spectrum of compound 5m (DMSO-d_6) 100 MHz



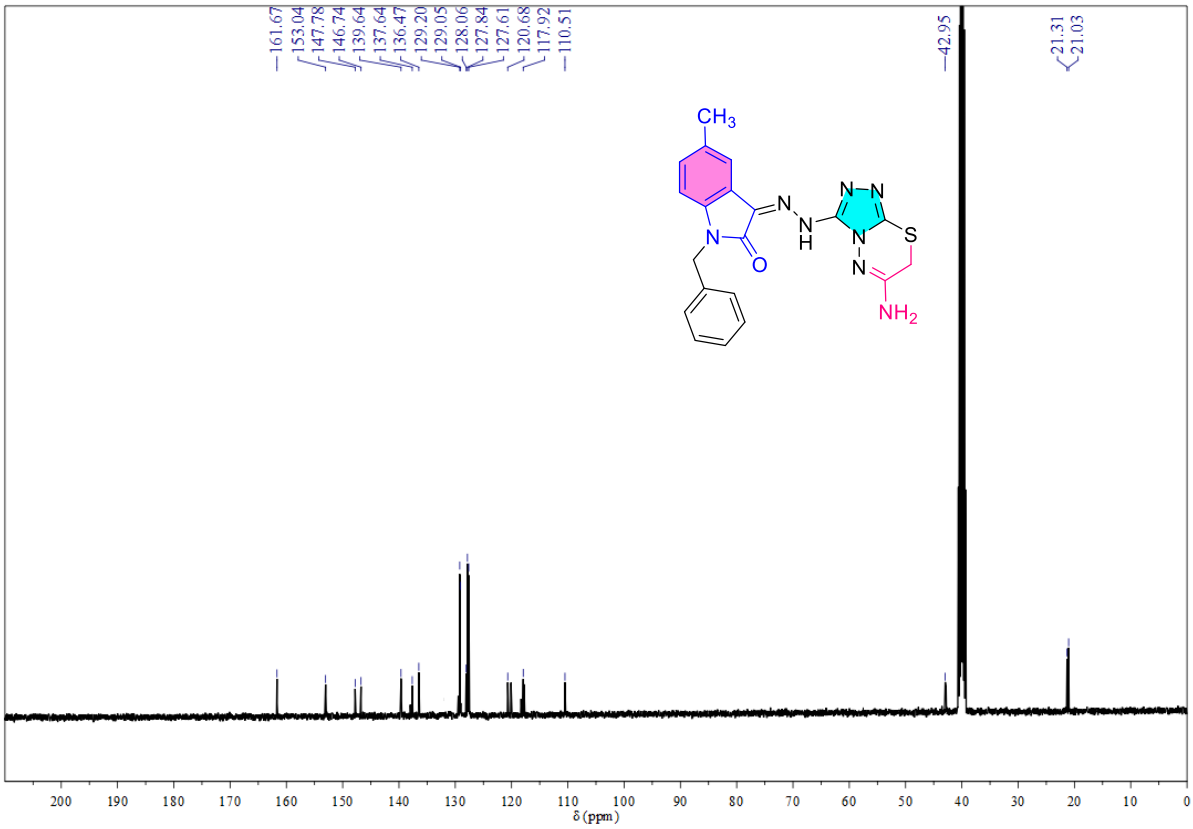
Mass spectrum of compound 5m



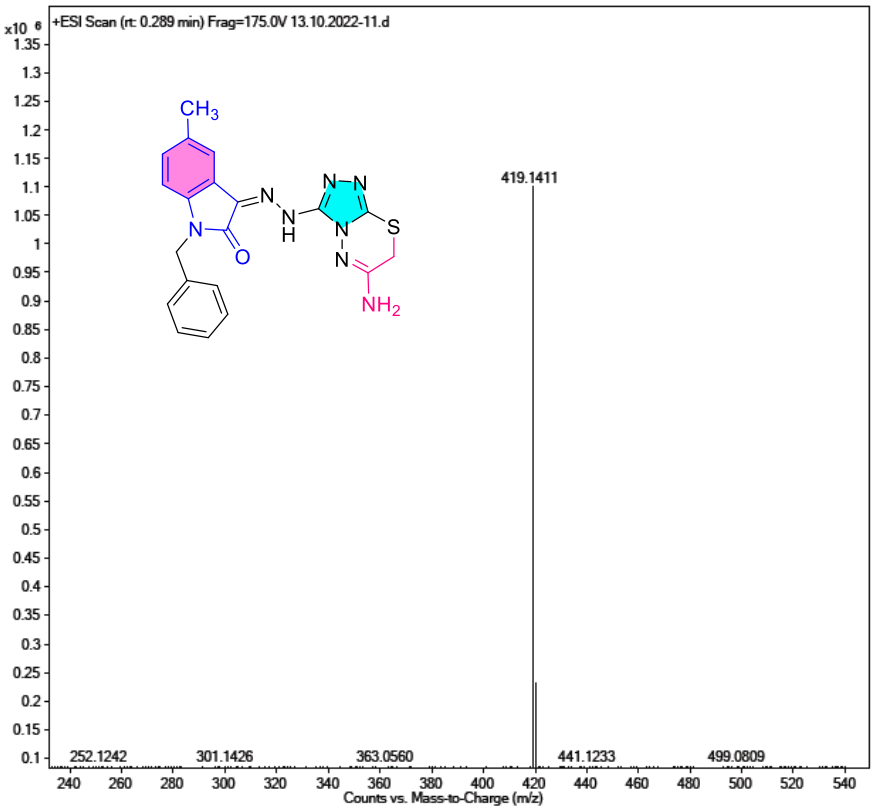
^{1}H NMR spectrum of compound 5n (DMSO-d_6) 400 MHz



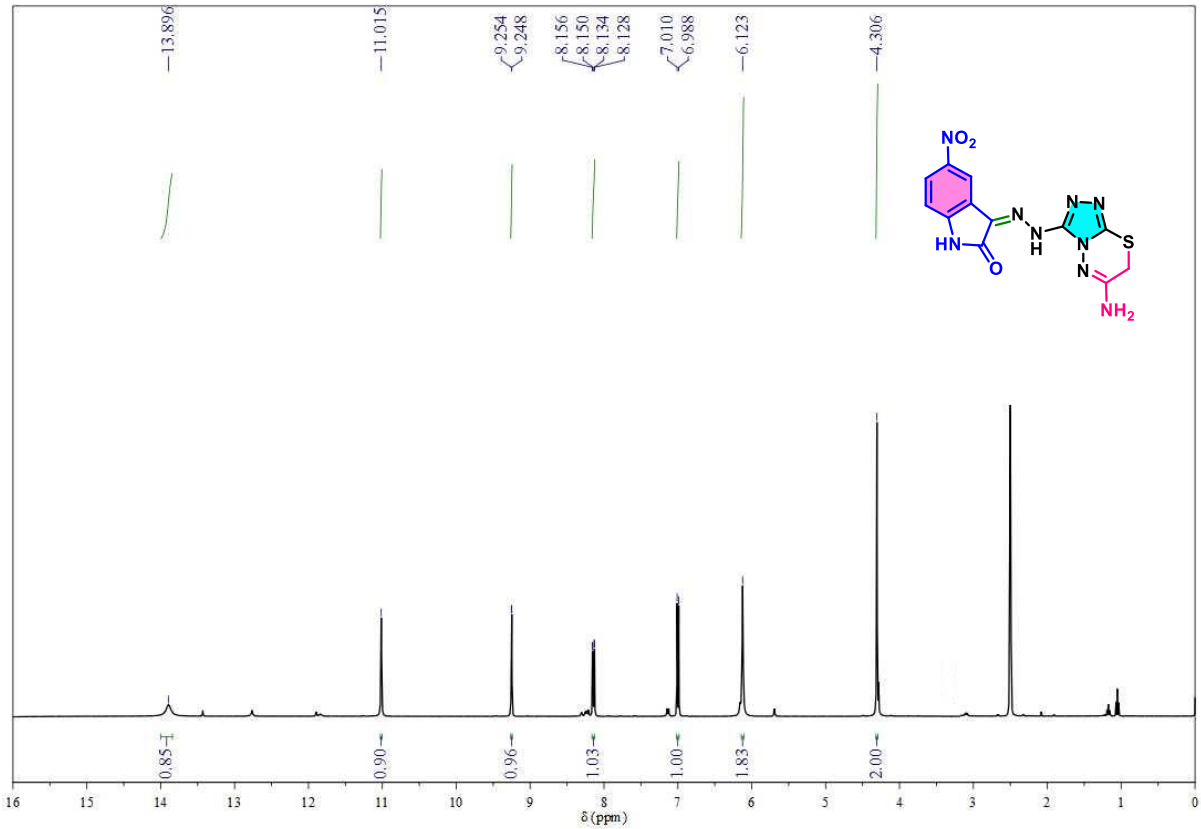
^{13}C NMR spectrum of compound 5n (DMSO-d_6) 100 MHz



Mas spectrum of compound 5n



^{1}H NMR Spectrum of compound 5o (DMSO-d_6) 400 MHz



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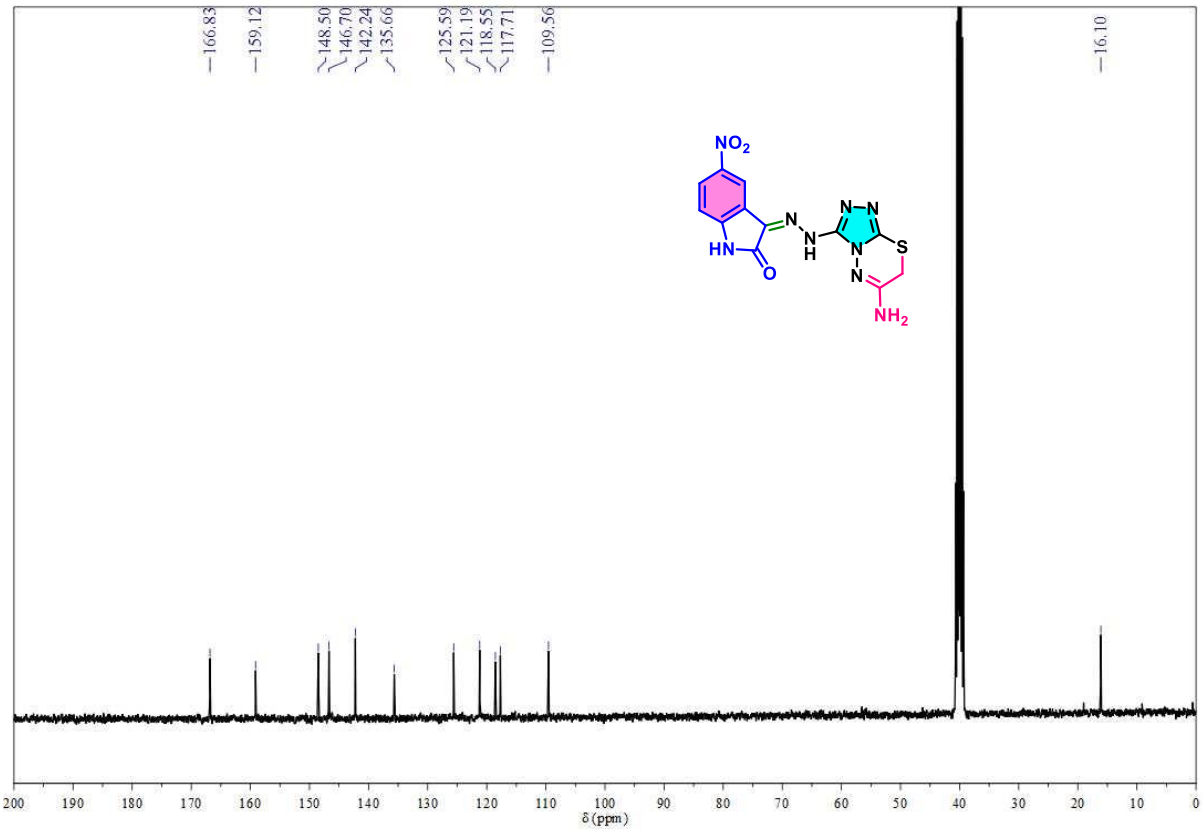
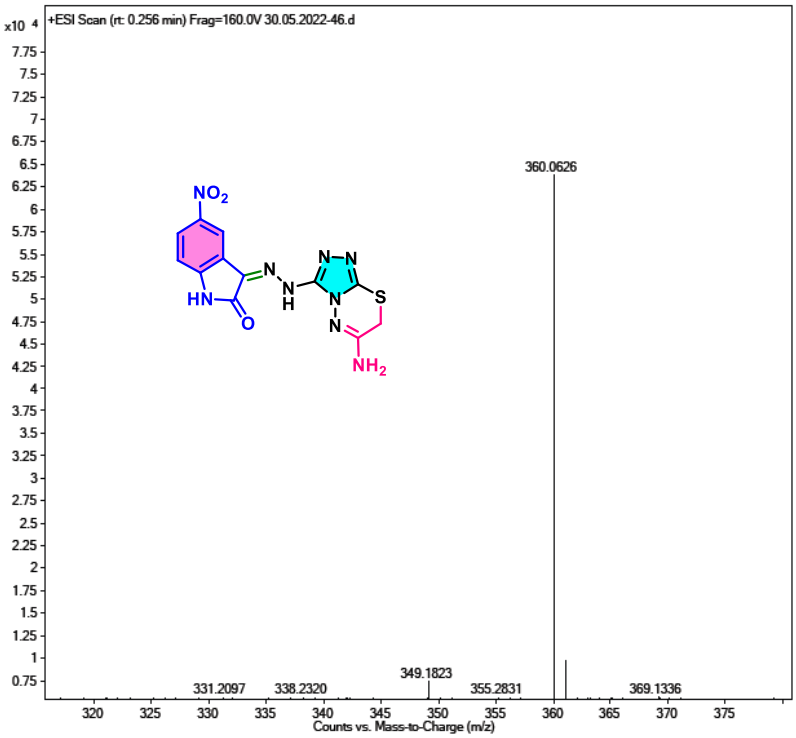
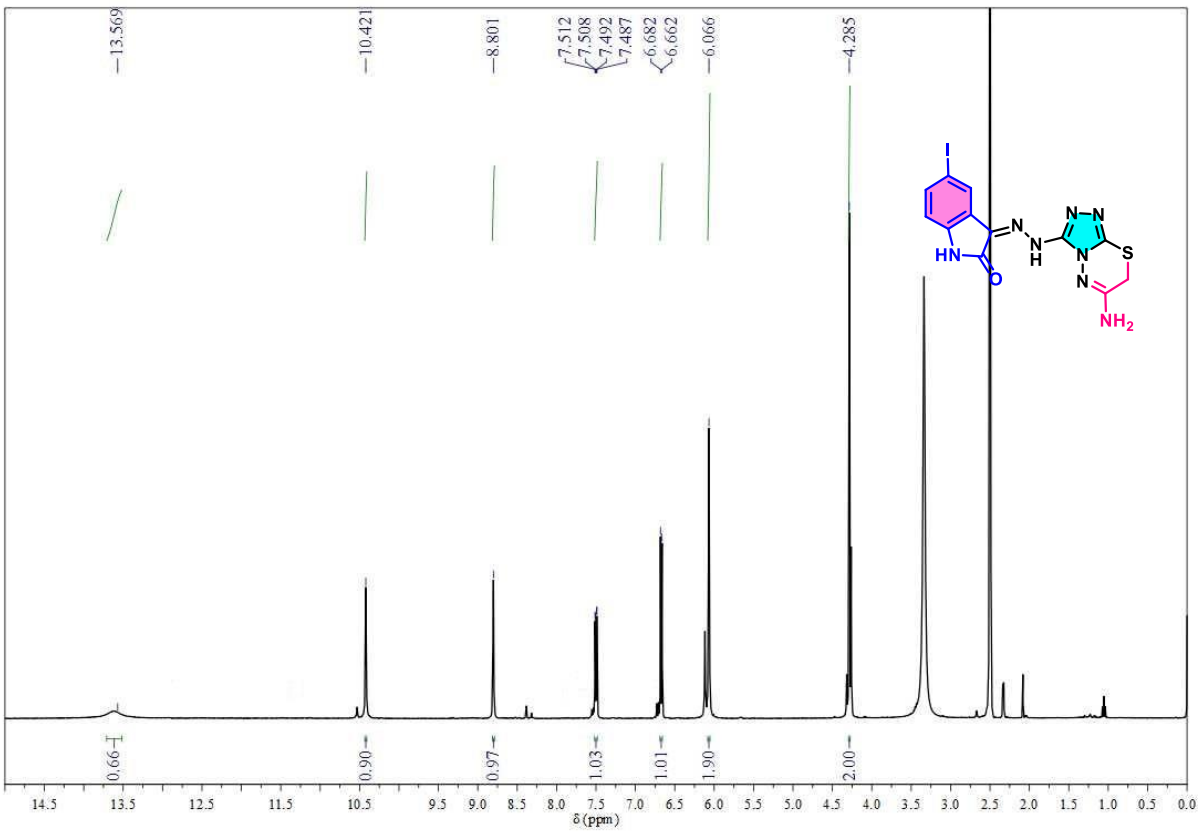


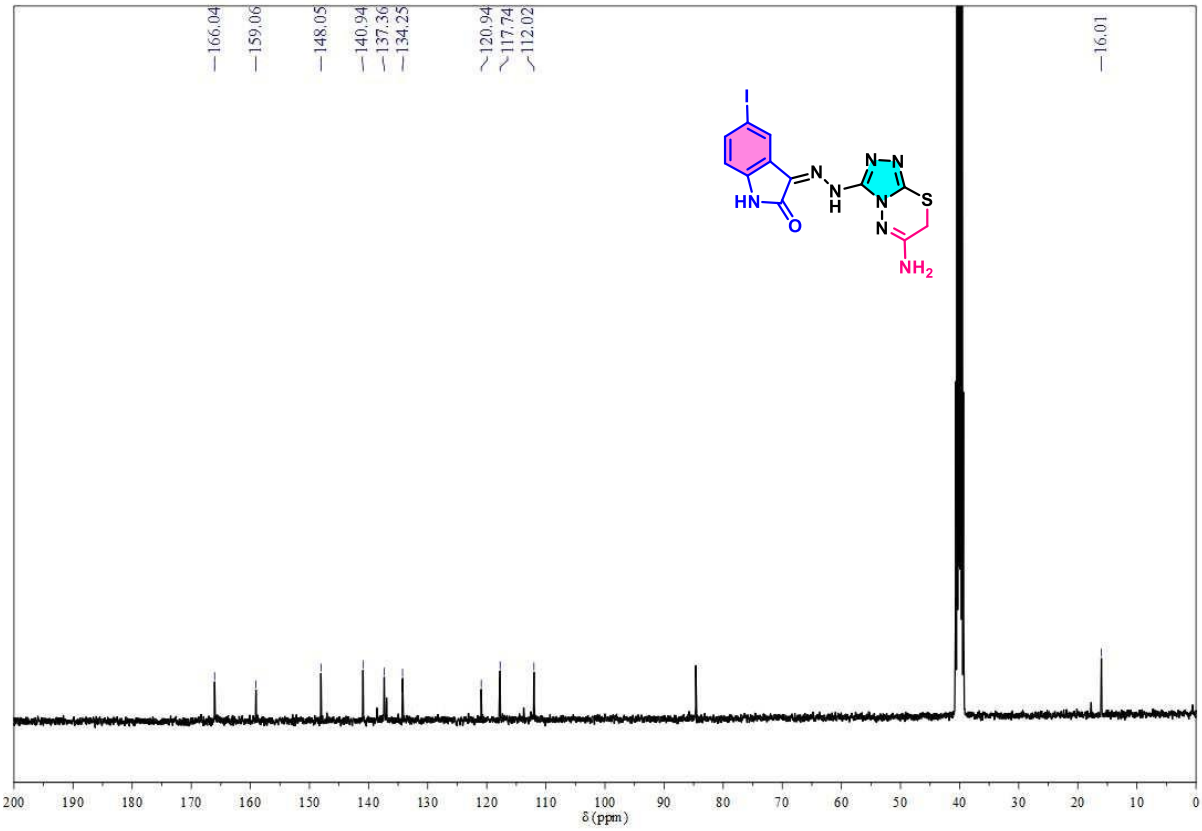
Mass spectrum of compound 5o



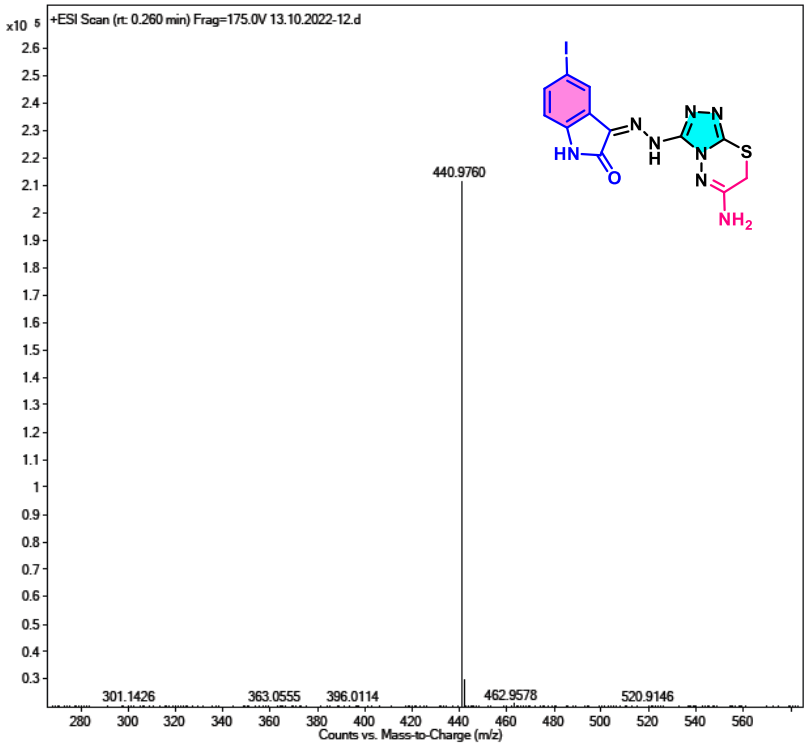
^{1}H NMR spectrum of compound 5p (DMSO-d_6) 400 MHz



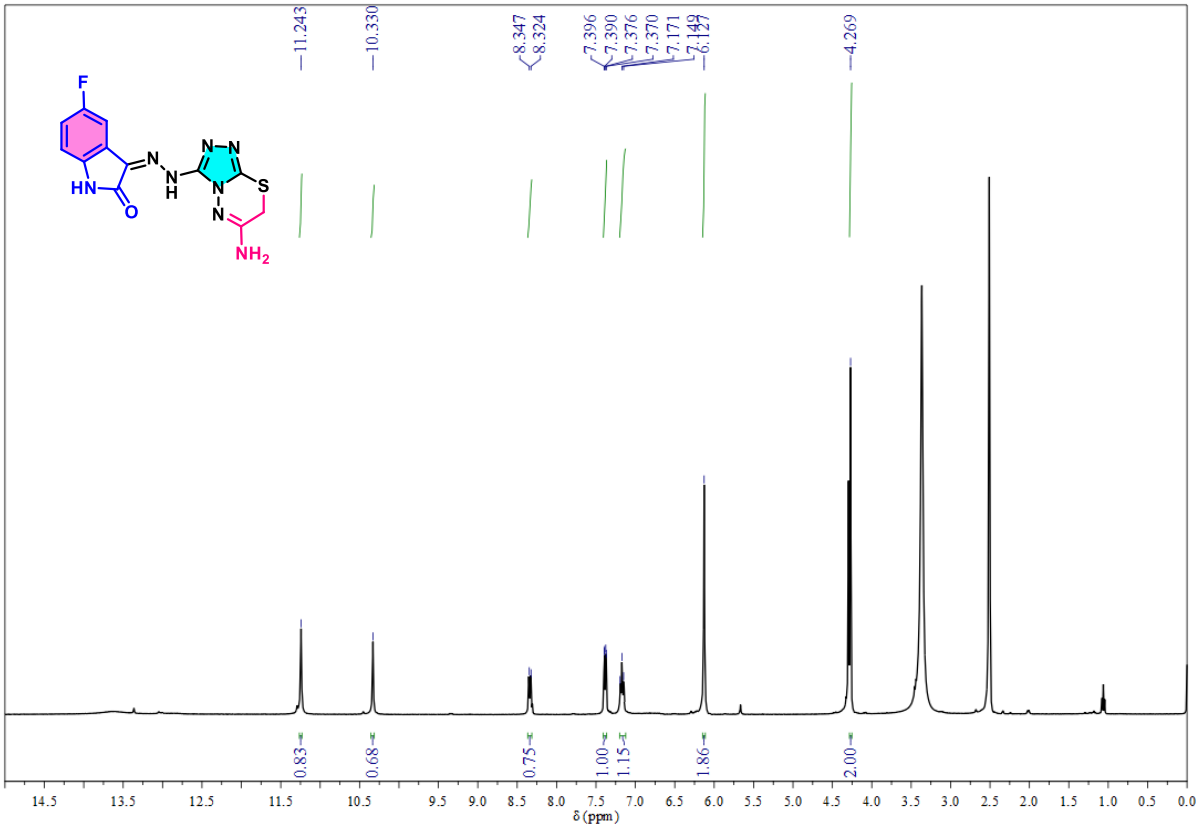
^{13}C NMR spectrum of compound 5p (DMSO-d_6) 100 MHz



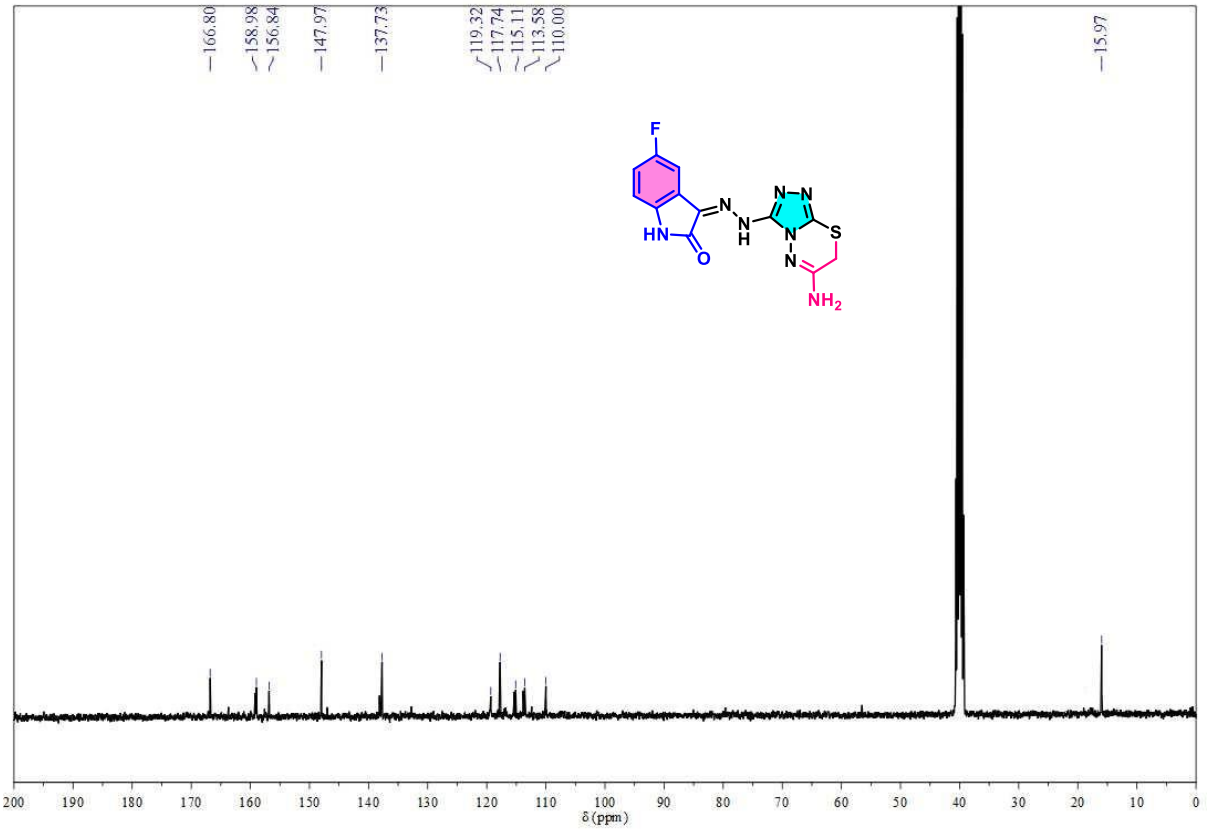
Mass Spectrum of compound 5p



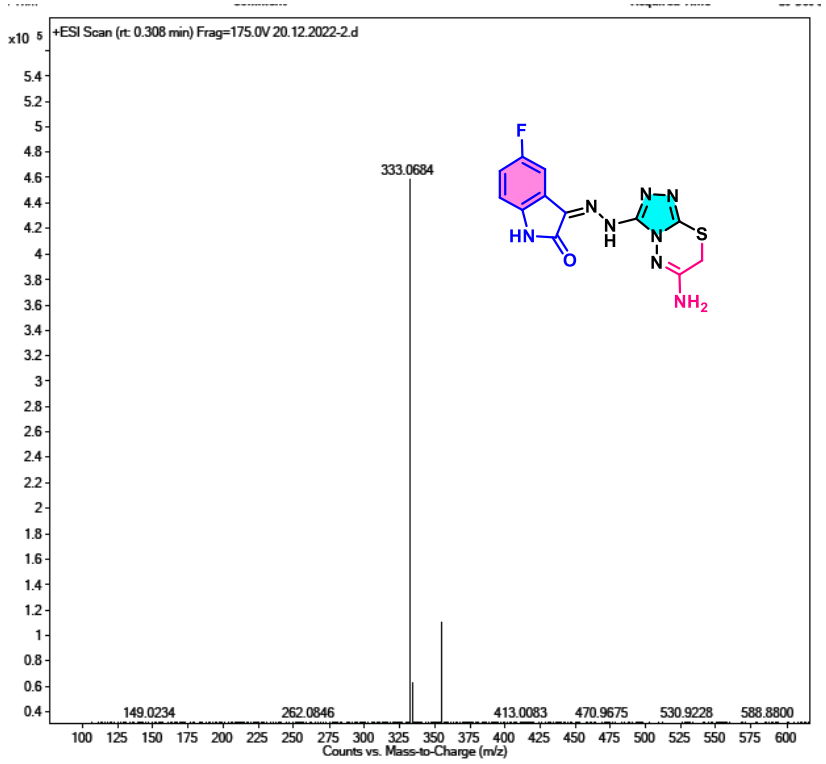
^{1}H NMR spectrum of compound 5q (DMSO-d_6) 400 MHz



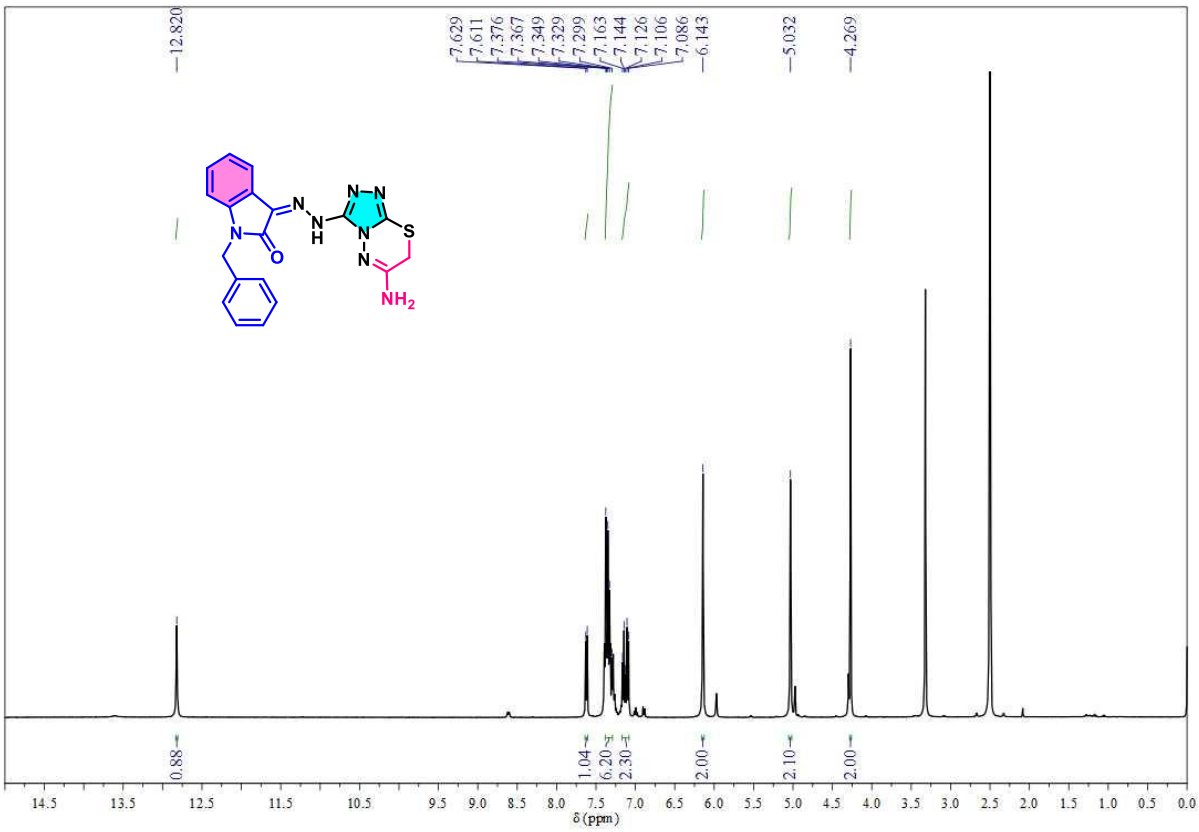
^{13}C NMR spectrum of compound 5q (DMSO-d_6) 100 MHz



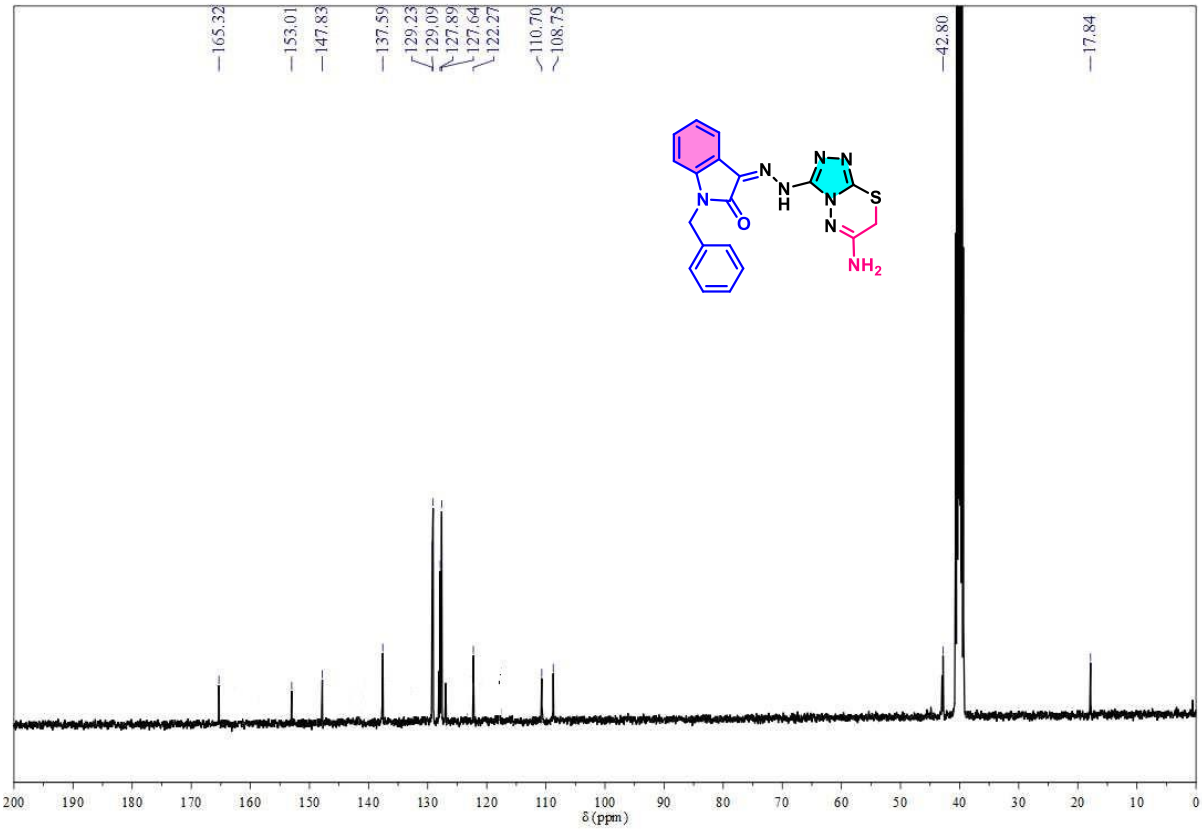
Mass spectrum of 5q



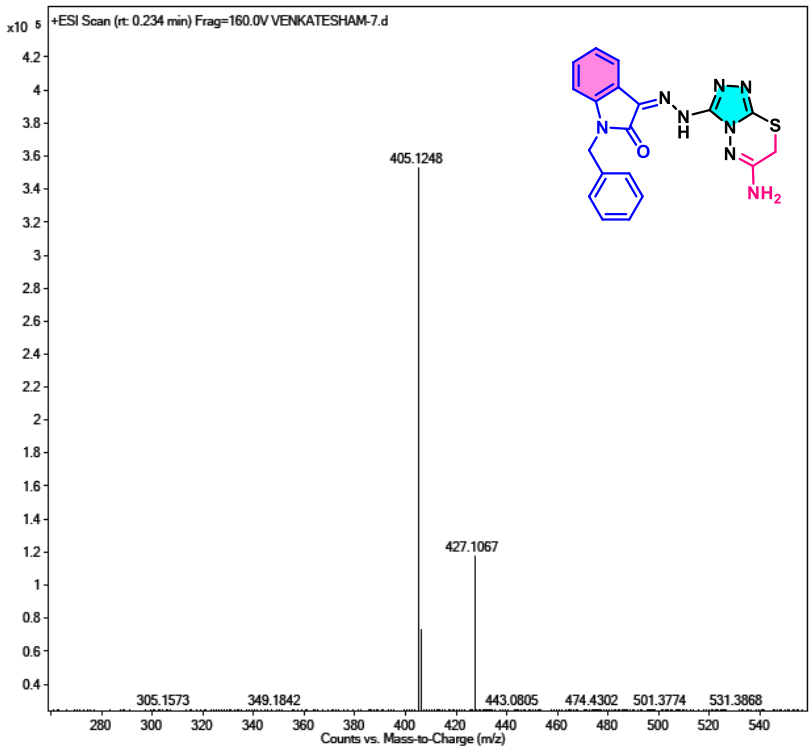
^{1}H NMR spectrum of compound 5r (DMSO-d_6) 400 MHz



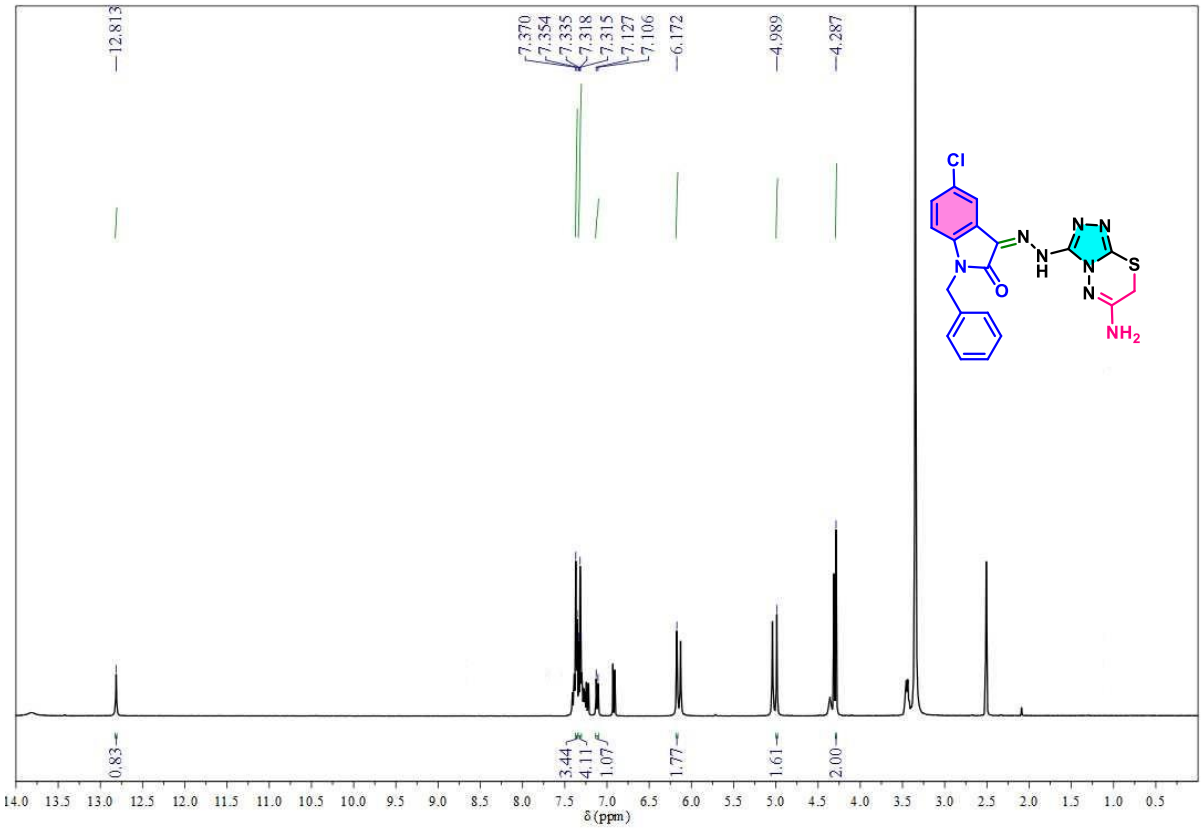
^{13}C NMR spectrum of compound 5r (DMSO-d_6) 100 MHz



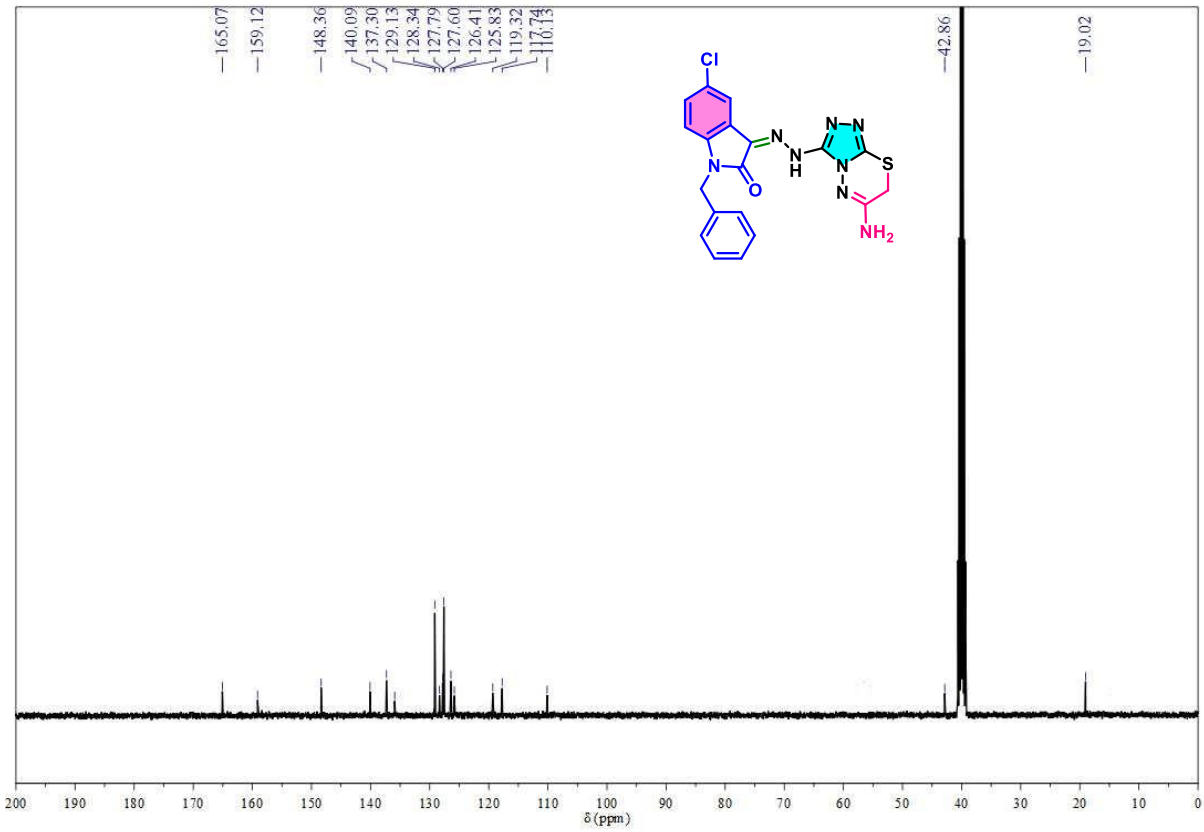
Mass spectrum of compound 5r



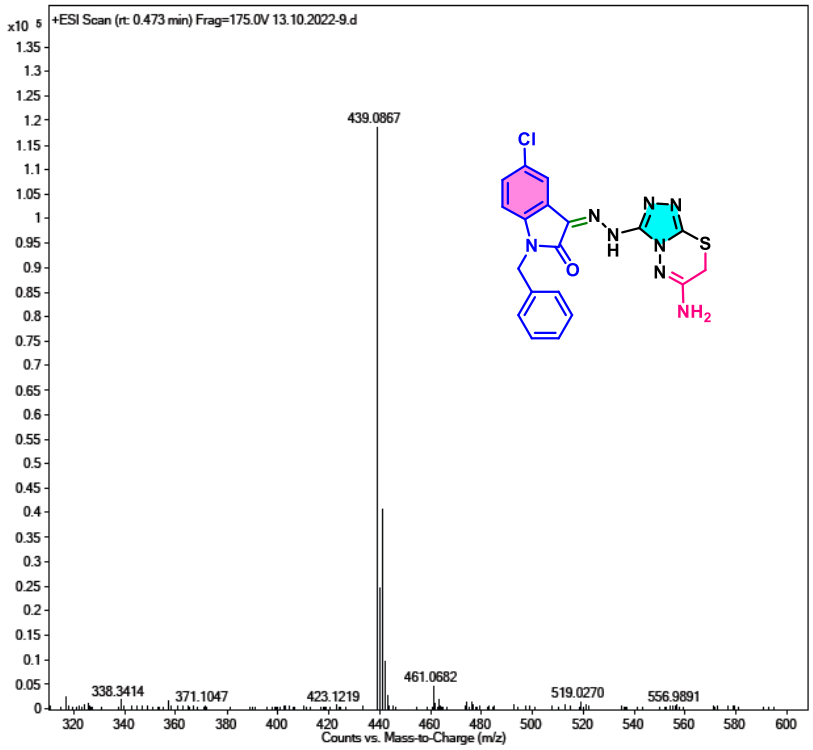
^{1}H NMR spectrum of compound 5s (DMSO-d_6) 400 MHz



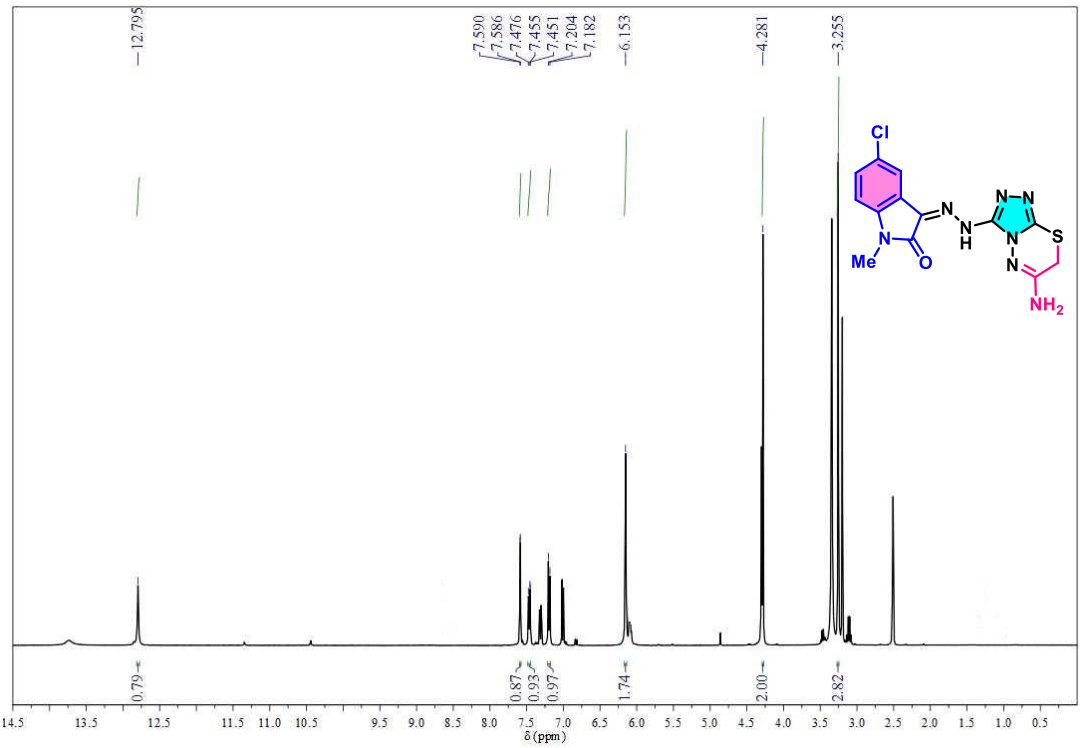
^{13}C NMR spectrum of compound 5s (DMSO-d_6) 100 MHz



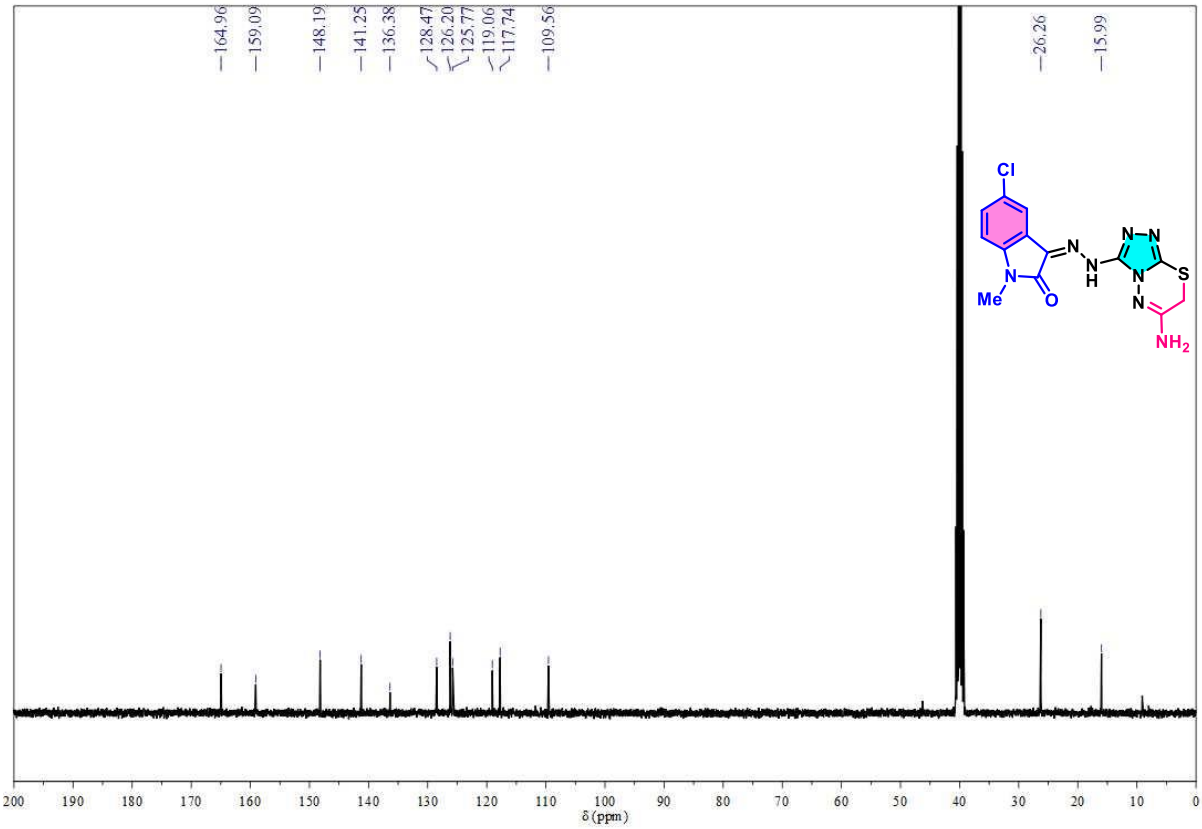
Mass spectrum of compound 5s



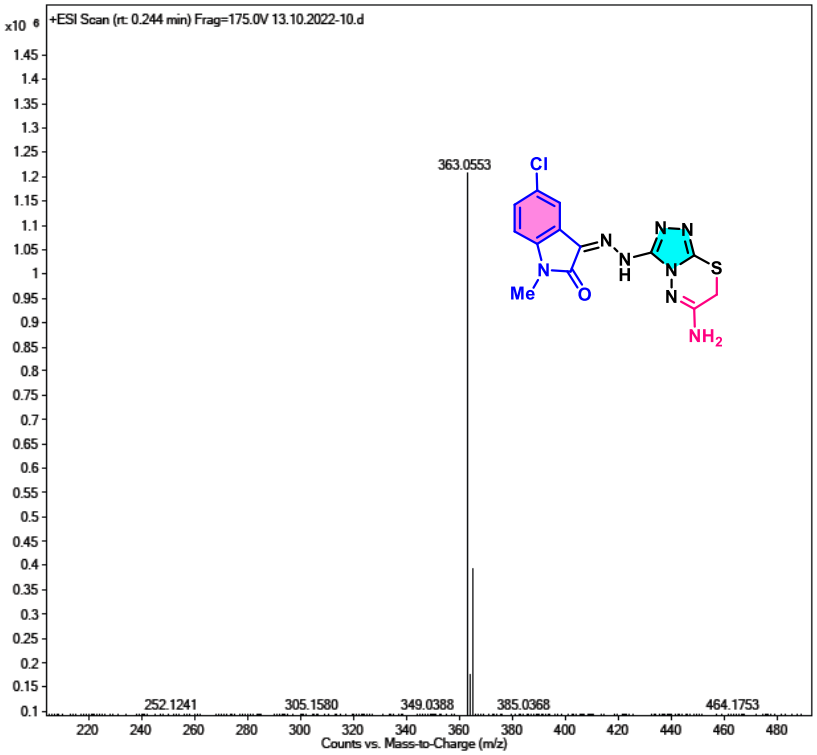
^{1}H NMR spectrum of compound 5s (DMSO-d_6) 400 MHz



^{13}C NMR spectrum of compound 5t. (DMSO-d_6) 100 MHz



Mass spectrum of compound 5t



6.7. References

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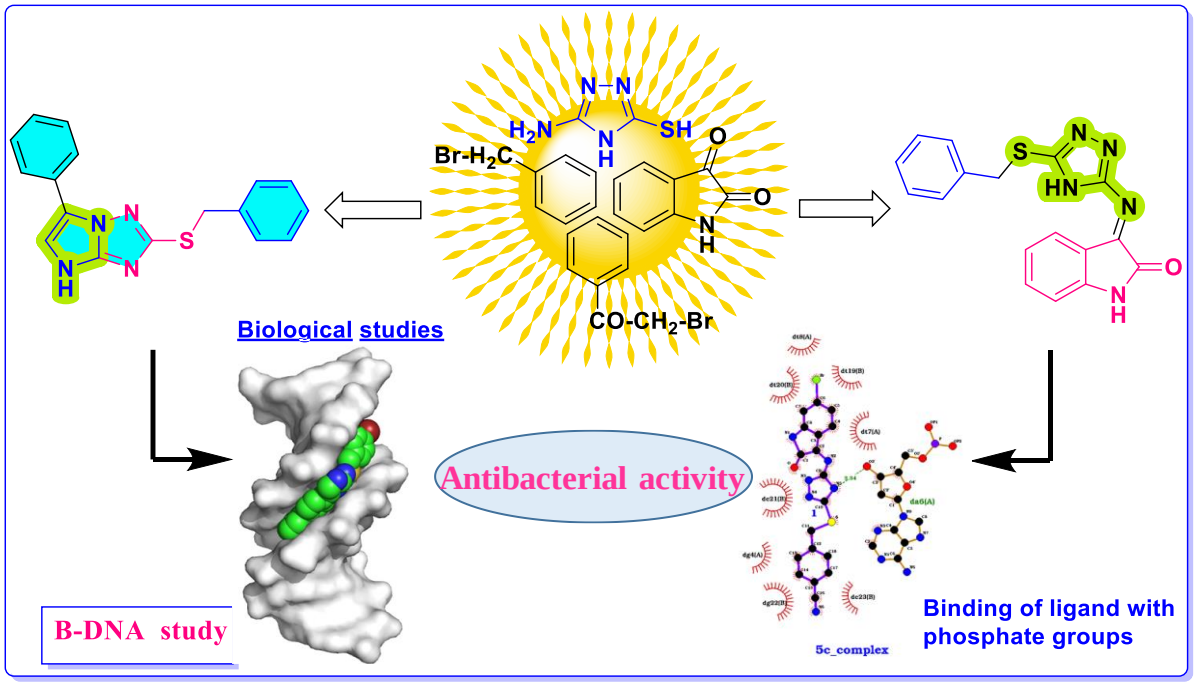
CHAPTER-VII

Novel one-pot synthesis of imidazo[2,1-b][1,2,4]-triazoles, 1,2,4-triazolo iminoindoline-2-ones and their in-vitro antibacterial activity, B-DNA study.



7.1 Introduction

The 1,2,4-triazole moiety has received special attention for the development of various heterocyclic systems,[1] while the 4*H*-1,2,4-triazole moiety is having free amino functional group at third position of triazole ring which enhance the possibility to develop variety of fused, unfused five and six membered heterocyclic compounds such as imidazole's, pyrimidines, thiazoles, pyridines.[2-9] Furthermore, the primary amines are helpful for the development of Schiff bases and Mannich bases, which are significant intermediates in organic synthesis.[10] The literature study reveals that the imine core moiety containing compounds are particular importance in medicinal chemistry, and exhibits potential biological activities. And also oxindoline substituted imines are showing potent pharmacological activities.[11-14]

Further, the imidazole heterocyclic compounds having two nitrogen atoms located at the 1,3 positions of five membered ring. The presence of active C_2 hydrogen of imidazole nucleus allows it to C_2 functionalization reactions to build a C-C, C-N bonds, which show promising biological applications.[15-19] Moreover, the 1,2,4-triazole moiety fused with imidazole nucleus such type of bicyclic five membered heterocyclic compounds demonstrates its phenomenal biological applications.[20] The possible isomeric structures of bicyclic 1,2,4-triazoloimidazoles are [2,1-*c*][1,2,4], [2,1-*b*][1,2,4], [5,1-*c*][1,2,4].[21-24] Among these [2,1-*b*][1,2,4] triazoloimidazoles have much importance in medicinal chemistry, pharmaceutical industry.[25] **Fig.1** represents biologically active imidazoles, imino oxindolines and bicyclic triazoloimidazoles.[16-33] The [2,1-*b*] imidazole compound 9 displayed potential anticancer properties[34] and also the imino oxindoline substituted molecule 2 and 5 demonstrates their good antibacterial activity.[35]

Keeping all the above findings and the importance of fused imidazoles, iminoindoline-2-ones in the present work the choosen starting material was choosen in such a way that it has both the co-ordinating groups of amine and thiol, functional groups which may facilitate to synthesize a different kinds of substrates. The 2-(benzylthio)-6-phenyl-4*H*-imidazo[1,2-*b*][1,2,4]-triazoles and 3-((5-(benzylthio)-4*H*-1,2,4-triazol-3-yl)imino)indolin-2-ones have been synthesized by using multi-component method. The present method has several advantages over conventional methodology. In this MCRs method high yield of the products were produced and possible to reduce the number of reaction steps, the reaction has completed in a short period of time.[36-37]

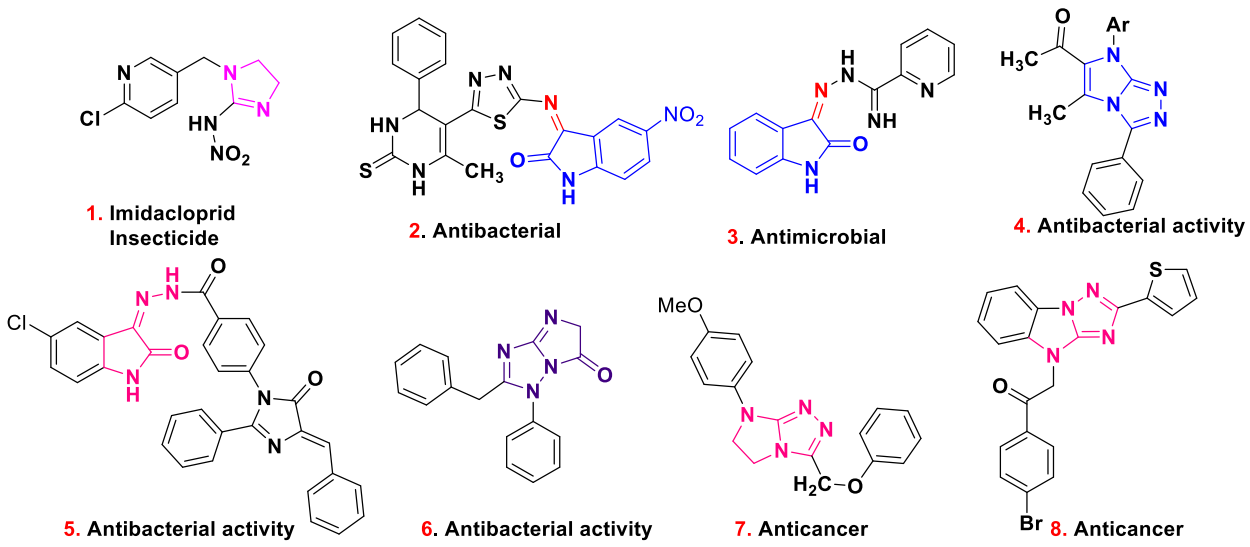
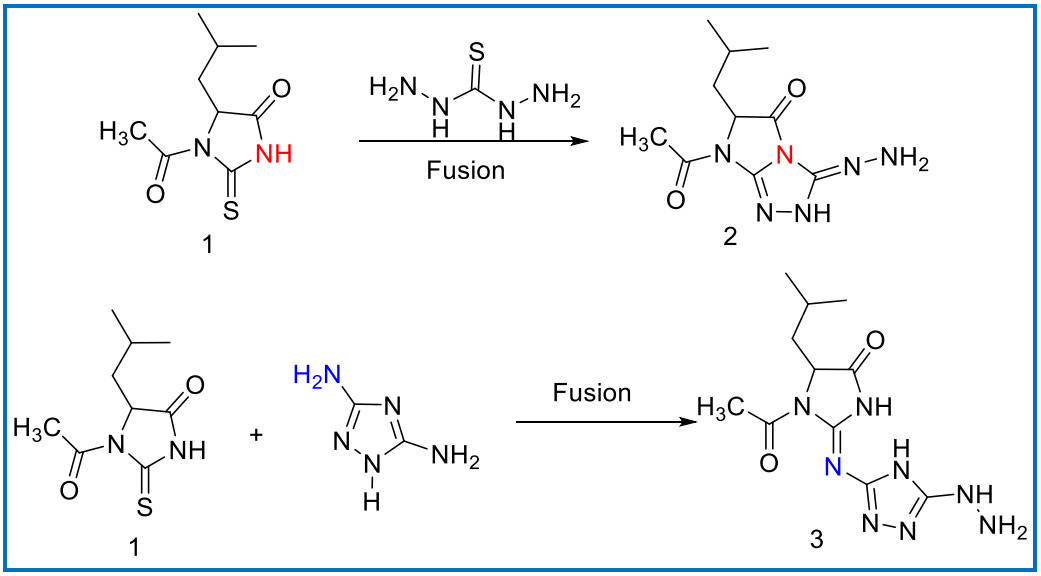


Fig.1. Reported biologically active imidazoles and mannich base compounds.

Literature reports

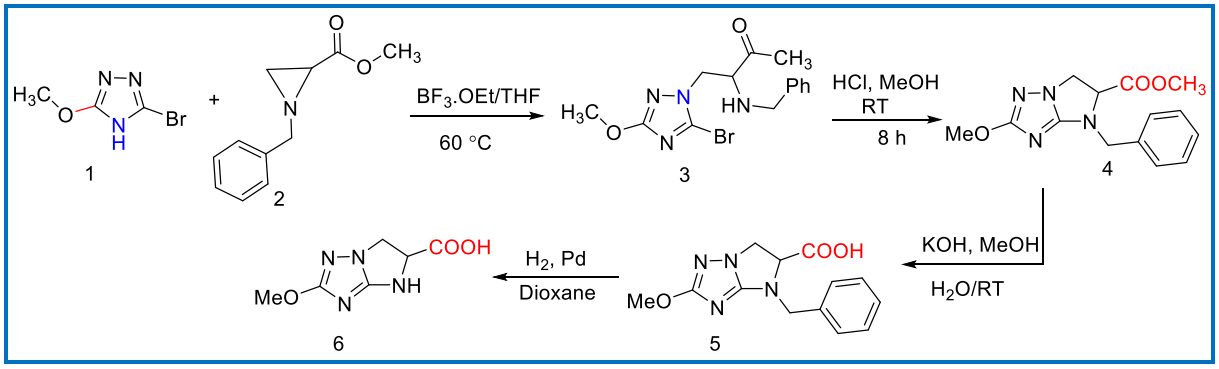
Hassan *et al* [38] reported the solvent free protocol for the synthesis of either fused or unfused imidazole molecules 2 and 4. In which the 1-acetyl-5-isobutyl-2-thioxoimidazolidin-4-(3*H*)-one (1) was cyclocondensed with thiocarbohydrazide to afford a bicyclic 2-iminoimidazoline compound-2. Whereas the 1-acetyl-5-isobutyl-2-thioxoimidazolidin-4-(3*H*)-one (1) on fusion with 1*H*-1,2,4-triazole-3,5-diamine to produce the corresponding compound-3. And also these derivatives have shown antiviral activity. (Scheme-1)

Scheme-1



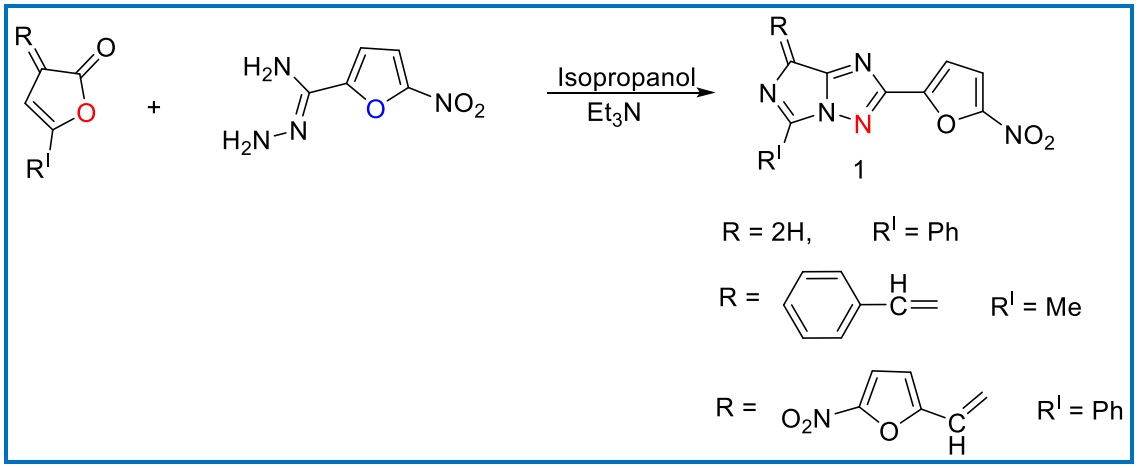
Farooa *et al* [39] published the fused 1,2,4-triazoloimidazole 5-carboxylic acid compound by the reaction of 3-bromo-5-methyl-1*H*-1,2,4-triazole and aziridines-1-carboxylate *via* various functional group interconversions and cycloaddition reaction which leads to produce target compound. (Scheme-2)

Scheme-2



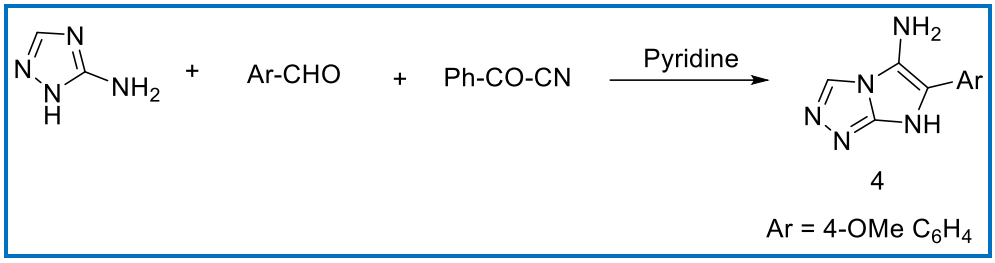
Pavlov *et al* [40] synthesized the biologically active fused triazolo imidazole compound-1 by the reaction of 5-substituted nitro furfural and 2-phenyl-5-oxazolone in presence of isopropanol/Et_3N to give the 2-phenyl-4-(5-nitro-2-furfurylidene)-5-oxazolone. The final compounds exhibit antimicrobial activity. (Scheme-3)

Scheme-3



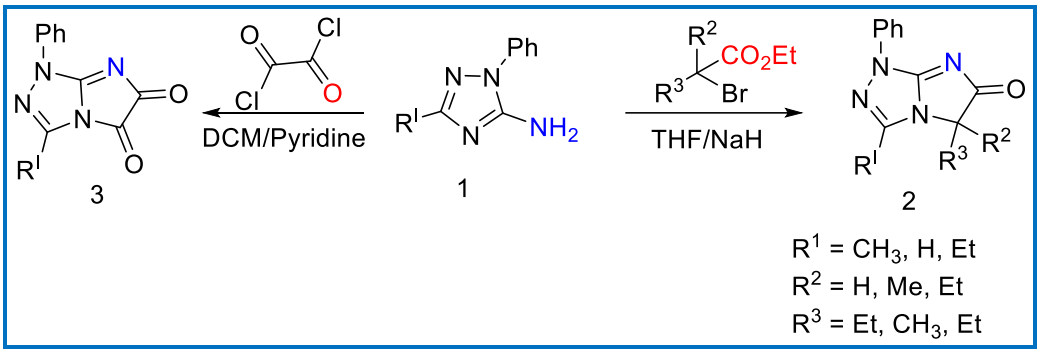
Sadek *et al* [41] reported the microwave irradiation of 3-amino-4*H*-1,2,4-triazole, aromatic aldehyde and phenacyl cyanide in pyridine to generate the corresponding bicyclic 5-amino 1,2,4-triazolo imidazole compound-4 with good yield. (Scheme-4)

Scheme-4



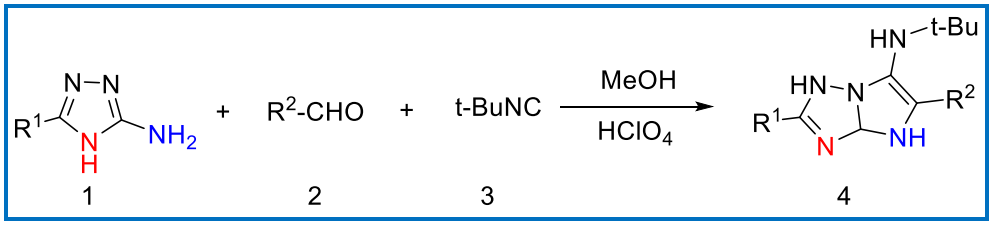
Allouche *et al* [42] developed the one-pot reaction of triazole amine compound-1 with substitute α-bromo ester in THF/NaH to form bridgehead nitrogen containing imidazole molecule-2. On the other hand the triazole compound-1 cyclocondensation with oxalyl chloride in DCM/pyridine resulted to produce the imidazo[2,1-*c*][1,2,4]-triazol-5,6-dione compound-3 with high yield. (Scheme-5)

Scheme-5



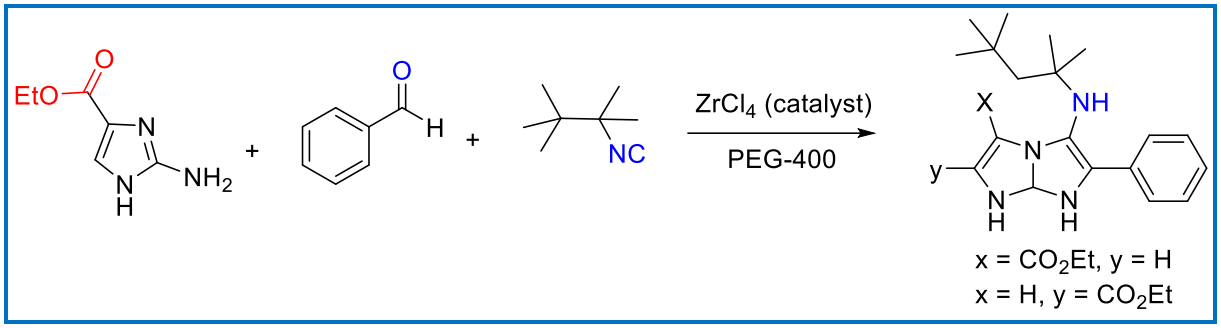
Huang *et al* [43] reported the isocyanide based one-pot three component cyclo condensation reaction of 1,2,4-triazole amine with different types of aromatic aldehydes and tertiary butyl isocyanide in MeOH/$HClO_4$ to form bicyclic 1,2,4-triazolo imidazole final compound-4 have shown in scheme-6.

Scheme-6



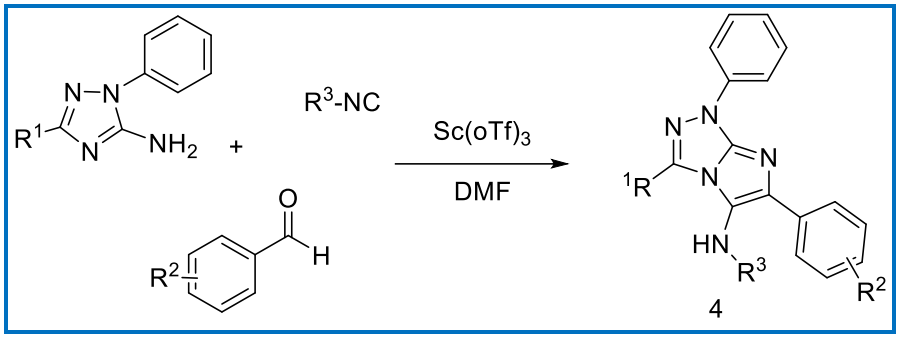
Driowya *et al* [44] developed an efficient one-pot method for the synthesis of 5-imino imidazo[1,2-a] imidazoles. In which the 2-amino imidazole 4- carboxylate with substituted isocyanides and aldehydes in PEG-400 and $ZrCl_4$ was used as a catalyst. (Scheme-7)

Scheme-7



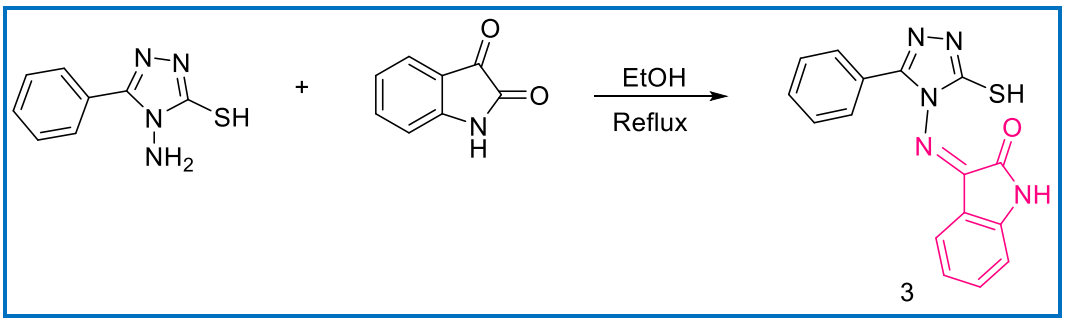
Aouali *et al* [45] synthesized the fused 1,2,4-triazolo imidazole heterocyclic molecule-4 from the reaction of 5-amino-1,2,4-triazole, substituted isocyanide and aldehyde in presence of $Sc(oTf)_3$ in DMF. And also these substrates have been evaluated for antioxidant antimicrobial activity this were shown in scheme-8.

Scheme-8



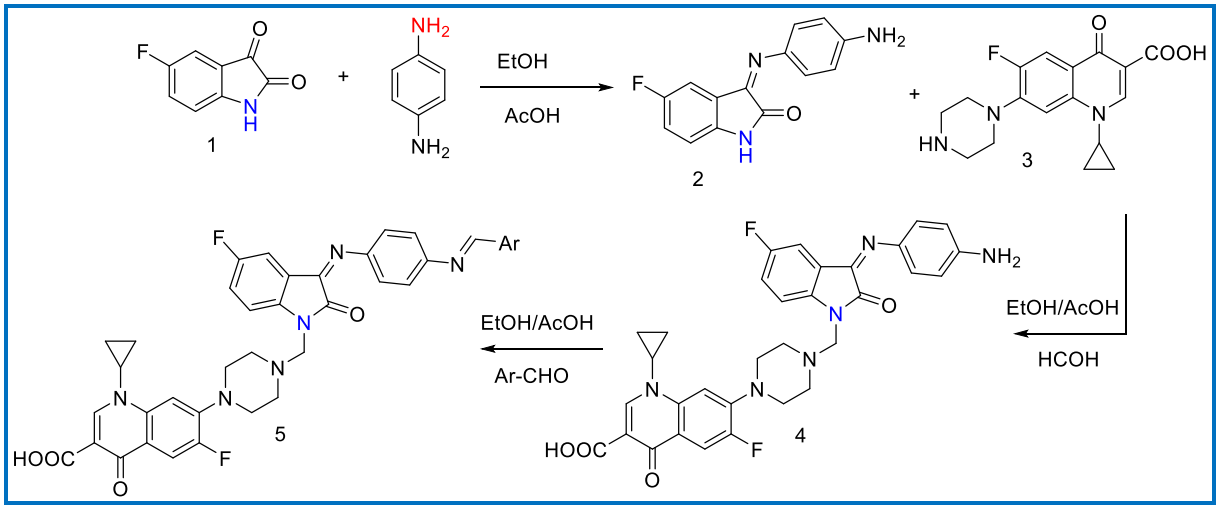
Ain *et al* [46] reported the reaction of 4-amino 3-mercapto triazole with isatin under ethanol reflux conditions to give the 3-((3-mercapto-5-phenyl-4*H*-1,2,4-triazol-4-yl) imino) indolin-2-one molecule-3 with high yields. These compounds were shown good antifungal and antibacterial activities. (Scheme-9)

Scheme-9



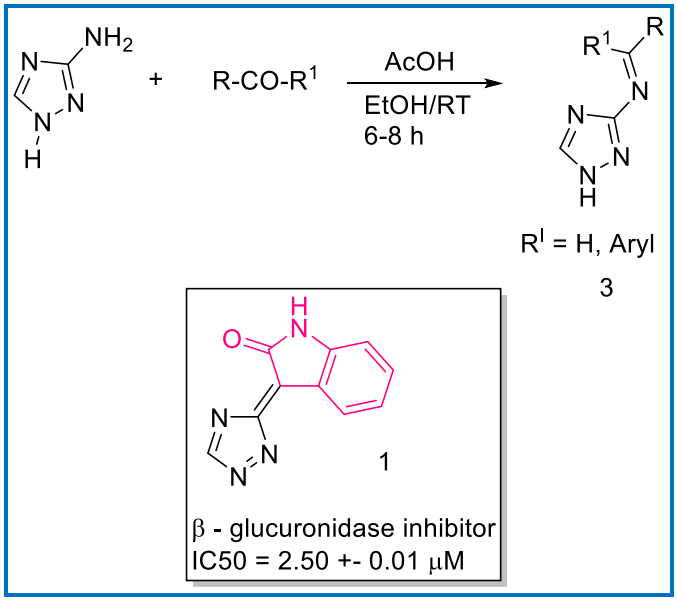
Prakash and co-worker [47] reported the Ciprofloxacin methylene isatin compound-4 incorporating with various aromatic aldehydes in EtOH/AcOH to afford the Mannich base of isatin containing Schiff base compounds-5. These compounds have shown good antibacterial activity against Gram +ve and Gram –ve bacteria. (Scheme-10)

Scheme-10



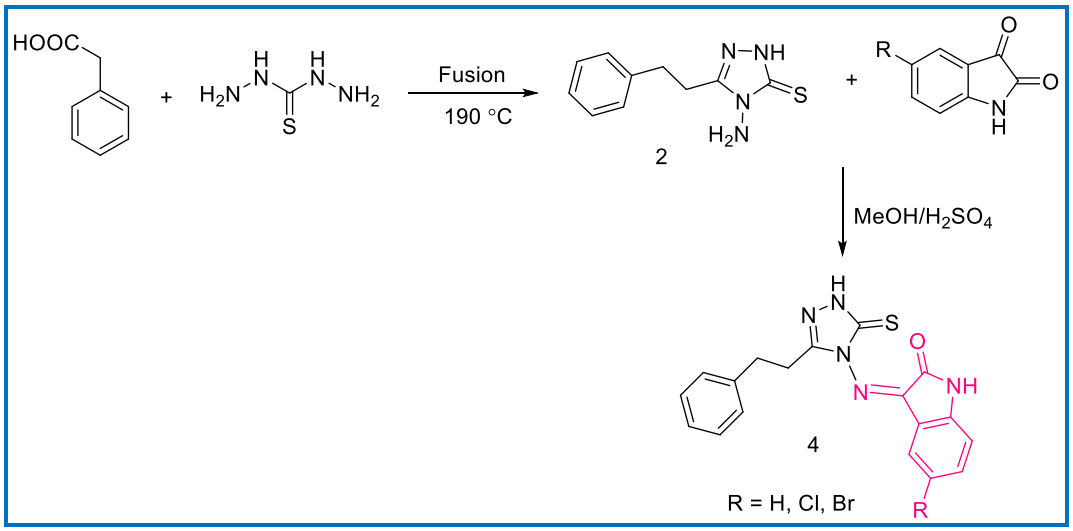
Jamil *et al* [48] synthesized a series of 1,2,4-triazole hydrazones by the reaction of 2-amino 1,2,4-triazole with aryl carbonyl derivatives in presence of EtOH/AcOH. Further these substrates tested for their β-glucuronidase inhibitor activity. (Scheme-11)

Scheme-11



Murthy *et al* [49] synthesized the reaction of phenyl acetic acid with thiocarbohydrazide to form a benzyl-1,2,4-triazole-3-thiol-2. This was subsequently reacted with isatin in MeOH/H_2SO_4 leads to generate 1,2,4-triazole based Mannich product of isatin derivative-4. (Scheme-12)

Scheme-12

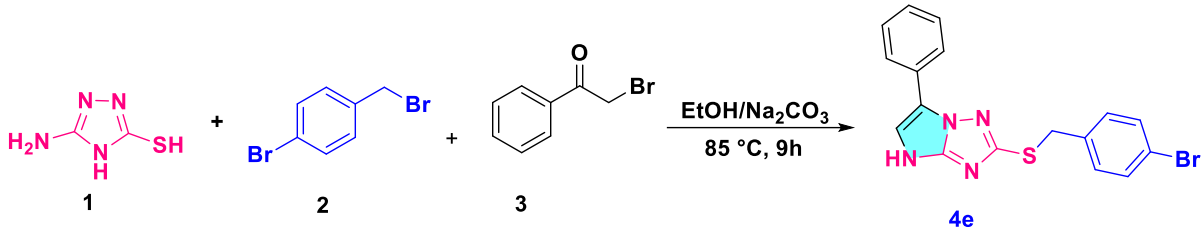


7.2. Present work

Keeping in view of the pharmacological importance of imidazo[2,1-b][1,2,4]triazoles, 1,2,4-triazoloimino indoline-2-ones and in continuation of our research programme on the addition of new series of bioactive heterocycles here in we are reporting the synthesis of thiobenzylated fused 1,2,4-triazolo imidazoles, 1,2,4-triazolo imino indoline 2-ones and their molecular docking studies and *in-vitro* antibacterial activity.

Synthesis of thiobenzylated bicyclic imidazo[2,1-*b*][1,2,4]-triazoles (**4a-j**) compounds were synthesized by one-pot three component condensation reaction of 5-amino-4*H*-1,2,4-triazole-3-thiol **1** (1.0 mmol), *p*-bromo benzyl bromide **2** (1.0 mmol), phenacyl bromide **3** (1.0 mmol), in the presence of ethanol, fused Na_2CO_3 as a base affording the corresponding desired products (**4a-j**) in a single step which have been shown in **Scheme-1.** Further, the scheme-2 thiobenzylated 1,2,4-triazolo iminoindoline 2-ones were synthesized by the reaction of 5-amino-4*H*-1,2,4-triazolo-3-thiol **1** (1.0 mmol), *p*-bromo benzyl bromide **2** (1.0 mmol), isatin **3a** (1.0 mmol) taken in EtOH/Na_2CO_3 and refluxed at 85 °C for 9 h to produce the corresponding target compounds **5a-i.** The optimization conditions for both the schemes are similar and represented in **Table-1**

Scheme-I. Schematic representation scheme-1



S**cheme-2.** Schematic representation scheme-2

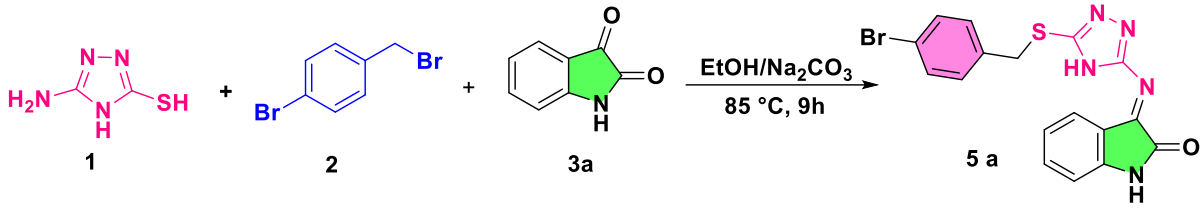


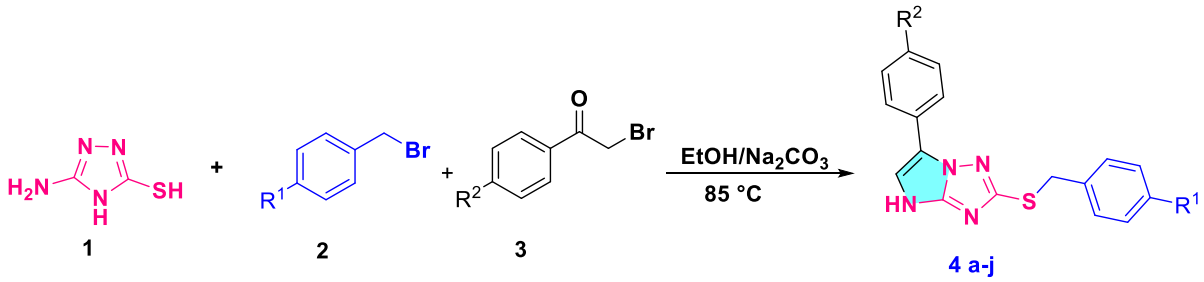
Table-1. Optimization study of the reaction conditions of scheme-1 &2[a].

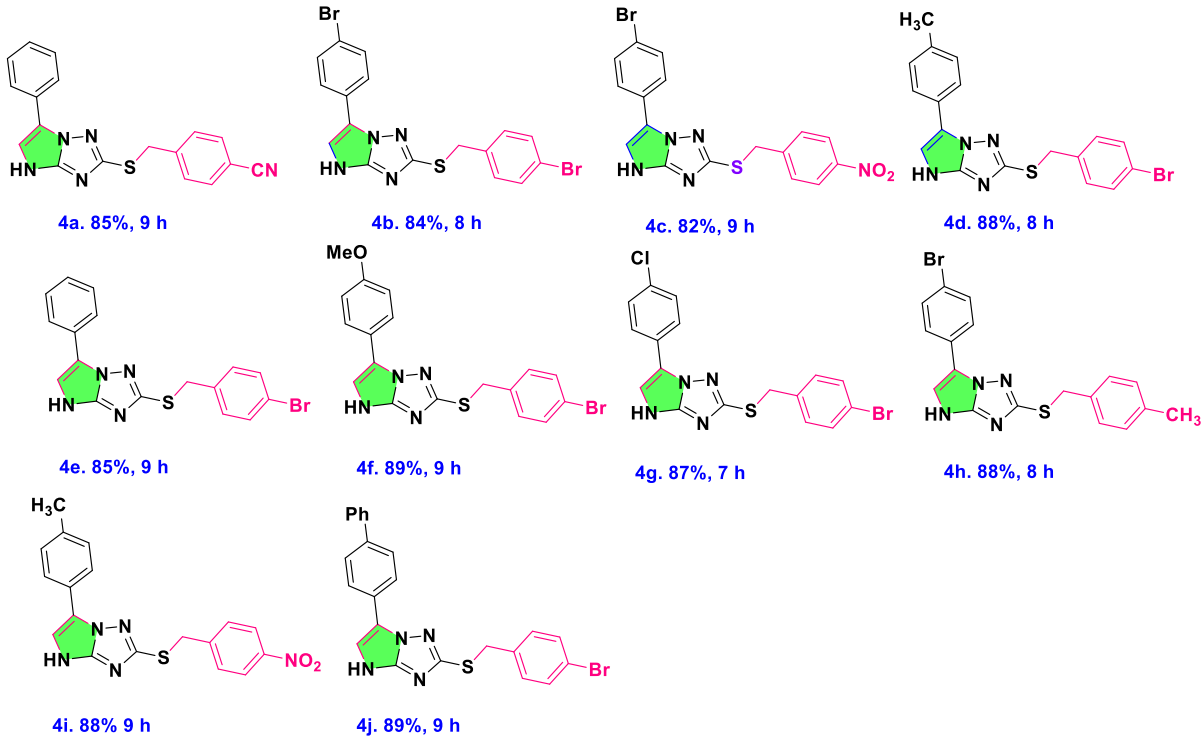
S.no.	Solvent	Acid/base (Equiv)	Temp (°C)	Time (h)	Yield (%)[b]
1	CH_3CN	-	60	10	n.r
2	MeOH	-	60	10	n.r
3	EtOH	-	60	10	n.r
4	DMF	K_2CO_3 (2)	80	10	10
5	AcOH	NaOAc (2)	80	10	12
6	AcOH	H_2SO_4 (0.1N)	80	12	n.r
7	EtOH	K_2CO_3 (2.5)	80	10	15
8	EtOH	HCl (0.1N)	80	10	n.r
9	EtOH	Na_2CO_3 (1)	80	9	35
10	EtOH	Na_2CO_3 (1.5)	80	9	80
11	**EtOH**	**Na_2CO_3 (2)**	**85**	**9**	**89 (Scheme-1 92 (Scheme-2)**

[a]***Reaction conditions***: **Scheme-1.** 5-amino-4*H*-1,2,4-triazolo-3-thiol (1.0 mmol), *p*-bromo benzyl bromide (1.0 mmol), phenacyl bromide (1.0 mmol) taken in EtOH/Na_2CO_3 reflux at 85 ˚C for 9 h to produce 89% yield. [b] Yield of the isolated product. n.r = no reaction. **Scheme-2.** 5-amino-4*H*-1,2,4-triazolo-3-thiol (1.0 mmol), *p*-bromo benzyl bromide (1.0 mmol), isatin (1.0 mmol) taken in EtOH/Na_2CO_3 reflux at 85 °C reflux at 85 °C for 9 h give 92 % yield.

The optimization study has been carried out for compound **4e** in scheme-1 and compound **5i** for scheme-2. In firstly we have carried out the reaction in ACN, MeOH, EtOH (**entry 1-3**) by using this conditions the formation of product was not observed. On the other hand, we have tested in DMF, AcOH, EtOH by changing the acids and bases (**entry 4-9**). Among these tested solvents and bases/acids EtOH by using of fused Na_2CO_3 (1 equiv) some amount of the product was extended at 80 °C. Further, our delight to improve the yield of the product by applying 2 equivalents of fused Na_2CO_3 and increase temperature good yield was formed at 85 °C for 9 h (**entry 11**).

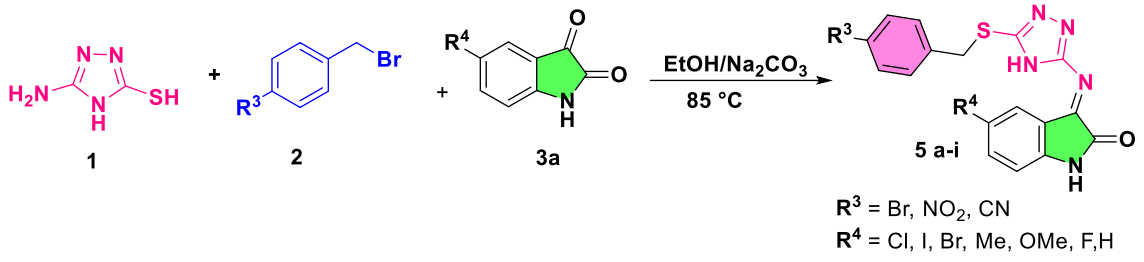
Table-2a. Derivatization of fused triazolo imidazoles (scheme-1 **4a-j**)

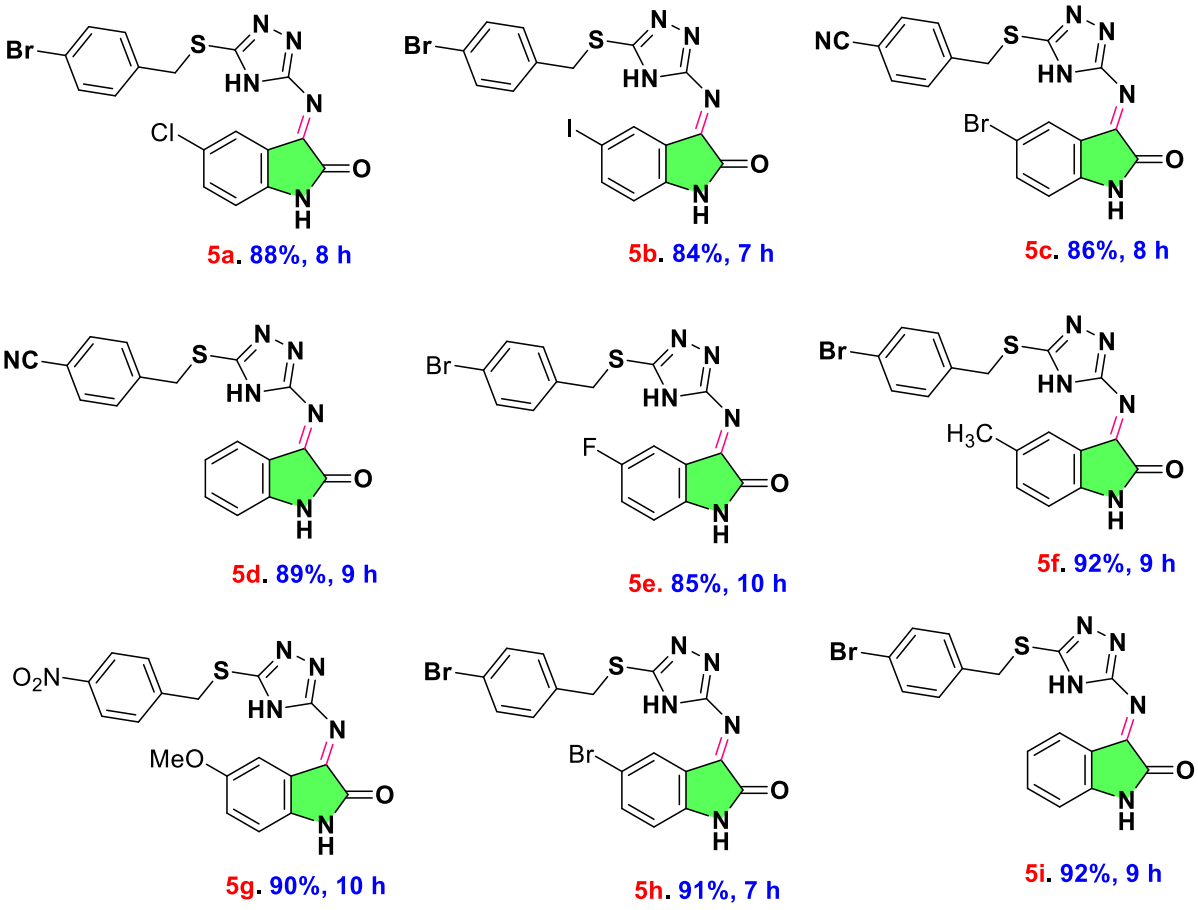




Reaction conditions: 5-amino-4*H*-1,2,4-triazole-3-thiol 1 (1.0 mmol), *p*-substituted benzyl bromides 2 (1.0 mmol), *p*-substituted phenacyl bromides 3 (1.0 mmol) were taken in 2 mL ethanol/Na_2CO_3 refluxed at 85 °C.

Table-2b: Substrate scope of the **Scheme-2** reaction (**5 a-i**)





Reaction conditions: 5-amino-4*H*-1,2,4-triazole-3-thiol 1 (1.0 mmol), *p*-substituted benzyl bromides 2 (1.0 mmol), 5-substituted isatins **3a** (1.0 mmol) mixture was taken in 2 mL EtOH/Na_2CO_3 and reflux at 85 °C.

Thus, the optimization conditions of the reaction for scheme-1 is triazole amine compound **1 (**1.0 mmol**),** *p*- bromo benzyl bromide **2 (**1.0 mmol**),** phenacyl bromide **3 (**1.0 mmol) and for scheme-2, 1,2,4-triazole amine compound **1 (**1.0 mmol**),** *p*-benzyl bromide **2 (**1.0 mmol**),** isatin **4 (**1.0 mmol) in 2 mL of EtOH /Na_2CO_3 (2 equiv.) at 85 °C for 9 h. 89% product was formed in scheme-1 and 92% product was produced in scheme-2 as shown in **Table-1**.

By using the optimization conditions, we have explored the substrate scope and generality of the reaction with various substituted phenacyl bromides, benzyl bromides and isatins (**Table-2a, b**). Electron donating substituents on benzene ring such as -OCH_3, Me, Br, groups increases the yield of the product (**Table-2a. Scheme-1**). Whereas electron withdrawing *p*-nitro group in phenacyl bromide did not offer the cyclization product even by the addition of excess amount of base. In the scheme-1 one C-S bond and two C-N bonds were formed simultaneously. In the case of scheme-2 -OCH_3, Me, F substituted isatin compounds have been produced with high yield (**Table-2b. Scheme-2**). Unfortunately, in scheme-2 when we put the reaction with *p*-nitro benzyl bromide and methyl isatin the reaction was not moved. The main advantage of this reaction is that the products were formed in a single step with high yields, scheme-1 and 2 follows similar reactions conditions.

In scheme-1, scheme-2 the final compound structures were confirmed by analytical and spectral studies i.e IR, ^{1}H NMR, ^{13}C{H}NMR, and HRMS. In the IR spectra for the compounds in **scheme-1** the imidazole ring N-H stretching was appeared at 3100-3200 cm^{-1}, and the characteristic imidazole alkene C-H stretching shows at 2890-2980 cm^{-1}, CN stretching frequency appear at 2200-2230 cm^{-1}, NO_2 unsymmetric stretching at 1530-1540 cm^{-1} and symmetric NO_2 stretching appear at 1330-1340 cm^{-1}, C-O-C stretching frequency observed at 1150-1250 cm^{-1}, C-Br stretching appear at 712-790 cm^{-1}. For scheme-2 compounds the triazole ring N-H stretching appear at 3200-3300 cm^{-1}, oxindoline ring N-H stretching frequency was observed at 3100-3190 cm^{-1}, and C=N stretching was shown at 1520-1560 cm^{-1}. In the proton NMR spectra S-CH_2 protons appeared as singlet at 4.20-4.95 δ ppm. The characteristic imidazole ring C-H proton was observed as a singlet at 7.90 – 8.25 δ ppm and the imidazole ring N-H proton appear at 12.10-12.90 δ ppm. In **scheme-2** the triazole N-H proton was observed at 11.90 -12.30 δ ppm and isatin N-H proton appeared at 10.20-11.15 δ ppm. In the ^{13}C NMR spectra the characteristic imidazole alkene C-H carbon shows at 105-110 δ ppm. S-CH_2 carbon appeared at 33.0-35.2 δ ppm. Isatin C=O carbon shows at 160-165.0 δ ppm. O-CH_3 carbon appear at 58.0-59.2 δ ppm. The HRMS spectra of all the compounds molecular masses matched with $[M+H]^+$ ion peak.

7.2.1. Antibacterial assay.

The *In-vitro* antibacterial activity of both the synthesized compounds bicyclic thiobenzyl 1,2,4-triazolo imidazoles (**scheme-1**) and thiobenzyl 1,2,4-triazolo iminoindolines (**scheme-2**) were screened against Gram negative bacteria *Escherichia coli* (ATCC-25922) and Gram positive bacteria *Bacillus subtilis* (ATCC-9372). And results are indicated that the tested compounds in both the series haven't shown any significant activity against *E. coli* at any applied concentration. However, the **scheme-2** compounds exhibited potent antibacterial activity against *B. subtilis*, (Gram +ve) with the potency being proportional to the size of the zone of inhibition (ZOI). Streptomycin displayed the maximum ZOI (14 mm), while dimethyl sulfoxide (DMSO) didn't produce any detectable ZOI. Among the screened compounds, the compound 5f have shown the highest ZOI (11 mm), while 5i displayed the lowest ZOI (7 mm). These findings are presented in **Table 3** and **Fig.2.**

Table-3. The *in-vitro* antibacterial activity of the compounds against Gram-positive *B. subtilis*

Plate	compound	concentration (ug)	ZOI (mm)
A	5a	200	10
	5a	20	8
	5a	2	8
	5b	200	9
B	5b	20	6
	5b	2	9
	5c	200	8
	5c	20	5
	5c	2	5
C	5d	200	10
	5d	20	8
	5d	2	0
	5e	200	9
	5e	20	8
D	5e	2	6
	5f	200	11
	5f	20	8
	5f	2	5
	5g	200	9
E	5g	20	8
	5g	2	6
	5h	200	8
	5h	20	8
	5h	2	8
F	5i	200	7
	5i	20	6

5i	2	0
DMSO		0
Streptomycin		14

Table-3: Antibacterial assay zone of inhibition results of scheme-2 (**5a-i**) compounds with three different concentrations (μg).

The minimum concentration of antibacterial agent required for the inhibition of bacterial growth is calld MIC (minimum inhibition concentration). The MIC values of the scheme-2 were represented in **Table-4** compared with standard (Streptomycin) drug.

Table-4 Minimum Inhibitory concentration (mg/ mL) of compounds.

Compound	Concentration (ug)	ZOI (mm)	MIC (mg/mL)
5a	2	8	0.1
5b	2	9	0.1
5c	2	5	0.1
5d	20	8	1.0
5e	2	6	0.1
5f	2	5	0.1
5g	2	6	0.1
5h	2	8	0.1
5i	20	6	1.0

MIC (mg/mL) values of the scheme-2 (5a-i series) of compounds.

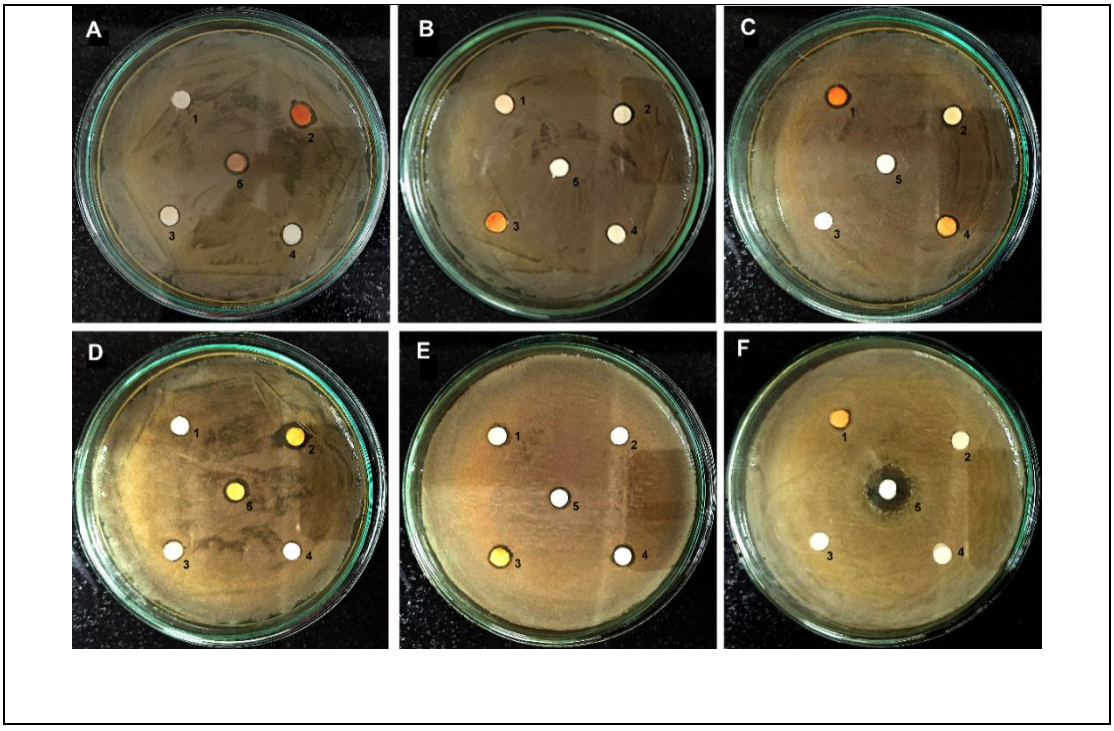
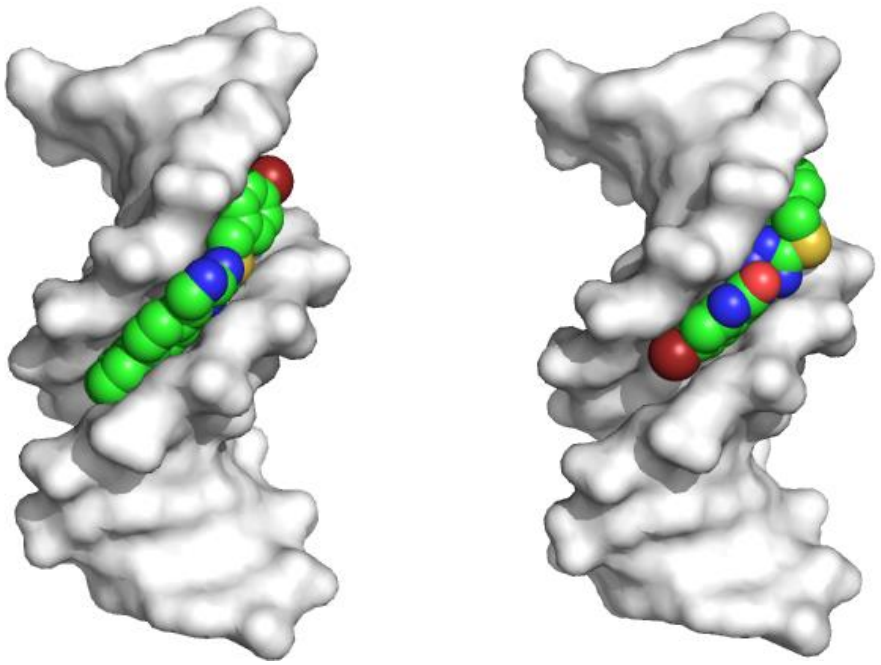


Fig.2. Disc diffusion method antibacterial assay of the compounds against Gram-positive *B. subtilis.*

7.2.2. Docking compounds to B-DNA

Molecular Docking studies was carried out for the scheme-1&2 series of compounds with the DNA. Compounds that bind to either DNA or RNA can be used for repression of replication or transcription and hence explored widely in antibacterial or anticancer studies. DNA structure is comprised of stacks of nucleotide bases and sugar phosphate backbone. The DNA binding compounds could either bind at the minor or major groove of the double stranded molecule, they could intercalate between two nucleotide stacks or the binding could also be non-specific and electrostatic in nature[50]. We performed blind docking of the ten compounds of scheme-1 and nine from scheme-2 to the DNA. The crystal structure of B-DNA (1BNA) was obtained from the protein data bank (PDB). The compounds were then docked one by one using Auto dock 4.0 (details under materials and methods). The binding energies are given in **Table 5** and **Table 5a.** The highest binding energy of scheme-1 was found to be **4j** (-12.95 kcal/mol) and that of scheme-2 was found to be **5c** (-10.59 kcal/mol) respectively. Interestingly, all the docked complexes of the scheme-1&2 series preferred being bound at the minor groove of DNA (**Fig.3 (a) and (b)**).



4j 5c

Fig. 3. Binding of B-DNA with (a) 4j and (b) 5c which show the highest binding energies of 12.95kcal/mol and -10.59 kcal/mol respectively**.**

It was found that both the highest binding interactions occurred at the central part of the DNA molecule. Compound 4 makes large number of non-bonded interactions. The DNA residues 4-9 and 18-22 interact with the compound 4j. The compound 5c shows one H-bonded interaction with the DNA residue number 6 where the N_4 from the compound interacts with the oxygen molecule (O_2') of Adenine 6 nitrogenous base. The binding energies of scheme-2 with the DNA was found to be consistently lower than scheme-1. In both cases, the binding of the compounds was with the nitrogenous base alone and no intercation with the sugar phosphate backbone could be observed (**Fig. 4 (a) and (b)**).

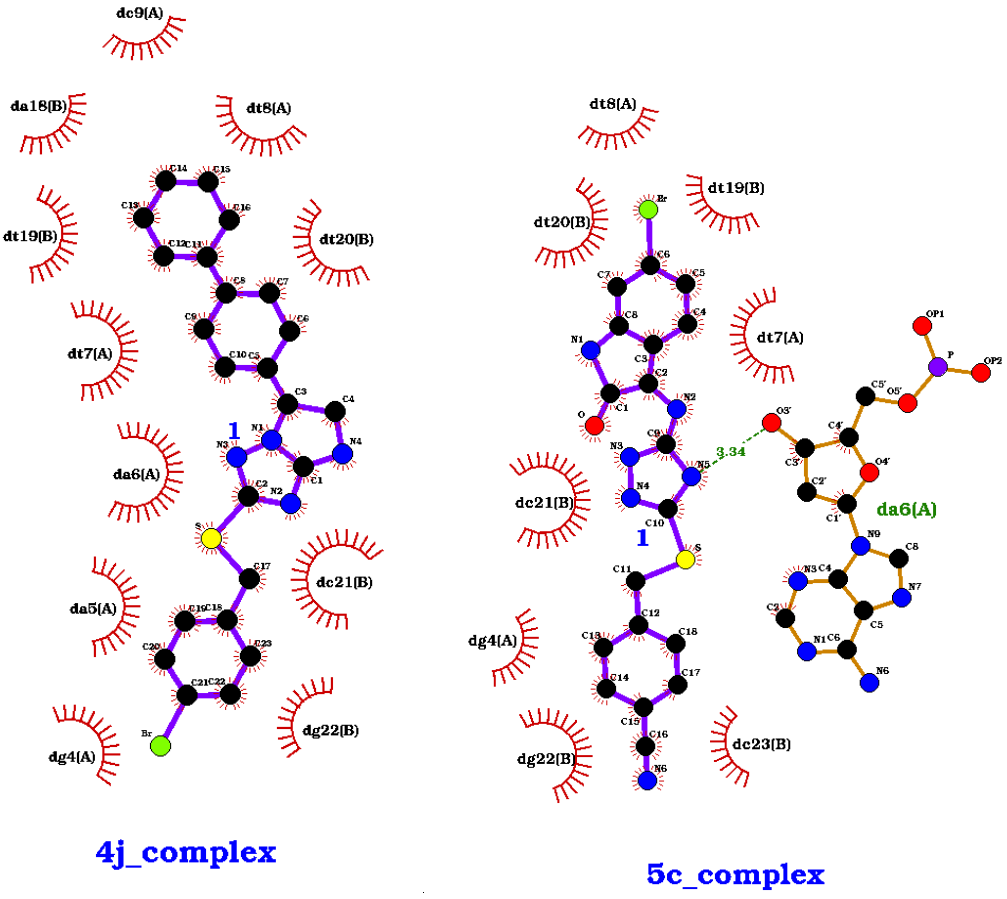


Figure-4. Interaction of residues of B-DNA with (a) 4j and (b) 5c. H-bonds are shown as green dotted lines and non-polar interactions are shows as rays of red lines arising from the residues. All atoms are colored as per CPK.

Table 5. Docking energies of scheme-1 with B-DNA

Serial No.	Compound	Docking Energy
1	4a	-11.2
2	4b	-11.82

3	4c	-11.75
4	4d	-11.54
5	4e	-10.74
6	4f	-12.01
7	4g	-11.78
8	4h	-12.22
9	4i	-11.14
10	4j	**-12.95**

Table 5a. Docking energies of scheme-2 with B-DNA

Serial No.	Compound	Docking Energy
1	5a	-10.25
2	5b	-10.12
3	5c	**-10.59**
4	5d	-9.92
5	5e	-9.4
6	5f	-9.81
7	5g	-9.85
8	5h	-9.73
9	5i	-10.55

Apart from the ability of the compounds to bind to the DNA molecule, we also checked the propensity of the prepared molecules to bind to other potential proteins and enzymes so that these molecules could be further explored for their drug-like properties. We found that 4j compounds were capable of binding to GPCRs, membrane receptors and nuclear receptors and also to enzymes like kinases, transferases and phosphodiesterase's. However, we found that compound 5c has a very good potential of binding to enzymes including kinases (53.3% and oxidoreductases (26.7%) apart from binding to phosphodiesterase's and other enzymes. Its ability to bind to GPCRs is very less (~6.7%). Since, most of the compounds of the series have similar chemical backbone, comparable binding affinities are expected from other compounds

of the series with the protein receptors and enzymes. Hence, 2-(benzylthio)-6-phenyl-4*H*-imidazo[1,2-*b*][1,2,4]-triazoles and benzylthio-4*H*-1,2,4-triazolo iminoimdoline-2-ones compounds could be further explored in the drug discovery studies.

7.3. Conclusions.

The synthesis of scheme-1 and scheme-2 final compounds were carried out in a one-pot three component process. Furthermore, the similar optimization reaction conditions we were used for both the schemes. The Scheme-1 and scheme-2 series of compounds were screened for their antibacterial activity against *E. coli* and *B. subtilis* by using Streptomycin as reference drug. The **5 a-i** series of compounds have shown their good **MIC** values against ***B. subtilis*** (Gram +Ve). The B-DNA binding studies are also carried out for all the **scheme-1, scheme-2** compounds. Among these the 4j, 5c exhibit good binding interactions with amino acid residues.

7.4. Experimental.

7.4.1. Chemistry.

The required chemicals, and solvents for the synthesis of final compounds in Scheme-1 and scheme-2 were sought from chemical suppliers i.e., Merk, Spectrochem, TCI, Finar. The completion of the reaction was checked by using silica gel coated aluminium foil plates (TCL) in ethyl acetate, n-hexane (8:2). Stuart Staffordshire, UK (SMP30) instrument was used to check m.p and are uncorrected. FT-IR spectra were recorded by using Perkin Elmer spectrometer with reference to KBr and values were represented in cm^{-1}. BRUKER 400 MHz spectrophotometer was used to record ^{1}H NMR spectra, with respect to standard internal reference compound TMS. The chemical shift values were represented in δ ppm, and coupling constant (*J*) values were indicated by Hz. Proton decoupled $^{13}C\{H\}$NMR spectra were recorded by using BRUKER 100 MHz spectrophotometer with respect to TMS as internal standard reference compound and chemical shift values were shown in δ ppm. Mass spectra (HRMS) were recorded on the Agilent Technologies Instrument ESI (+Ve mode).

7.4.2 Antibacterial activity.

The *in-vitro* antibacterial activity of the compounds was evaluated against Gram negative *Escherichia coli* (*E. coli*) and Gram Positive *Bacillus subtilis* (*B. subtilis*) bacteria using the standard disc diffusion method.[51] The compounds were dissolved in dimethyl sulfoxide (DMSO) to prepare three different final concentrations of 200 mg/mL, 20 mg/mL, and 2 mg/mL. 10 µL of each dilution was loaded onto sterile Whatman-1 filter paper discs with a

diameter of 5 mm and allowed to dry [52]. DMSO and Streptomycin (200mg/mL) were taken as negative and positive controls respectively. 100 µL of freshly grown overnight bacterial cultures were spread onto sterile nutrient agar plates, and the compound-impregnated paper discs were placed on top of the plates. The plates were incubated at 37 °C for 24 hours. The size (in mm) of the zone of inhibition (ZOI) surrounding the disc was measured and recorded.

General procedure for the synthesis of scheme-1 compounds (4 a-j).

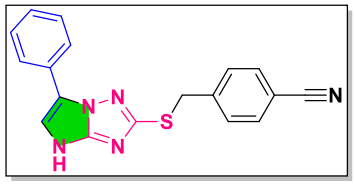
A mixture of 5-amino-4*H*-1,2,4-triazole-3-thiol **1** (1.0 mmol), *p*-bromo benzyl bromide **2** (1.0 mmol), phenacyl bromide **3** (1.0 mmol) was taken in a 50 mL round bottom flask and added 2 mL of ethanol and 2 equivalents of fused Na_2CO_3, refluxed at 85 °C for 9 h. Completion of the reaction was checked by TLC and the reaction mixture was poured in to ice cold water, the white color solid separated was filtered, dried and the compound was recrystalized from ethanol.

General procedure for synthesis of scheme-2 compounds (5 a-i).

A mixture of 5-amino-4*H*-1,2,4-triazole-3-thiol **1** (1.0 mmol), *p*-bromo benzyl bromide **2** (1.0 mmol), isatin **3a** (1.0 mmol) was taken in a 50 mL round bottom flask and added 2 mL of ethanol and 2 equivalents of fused Na_2CO_3, refluxed at 85 °C for 9 h. Completion of the reaction was checked by TLC and the reaction mixture was cooled and poured in to ice cold water, the light yellow color solid separated was filtered and recrystalized from ethanol.

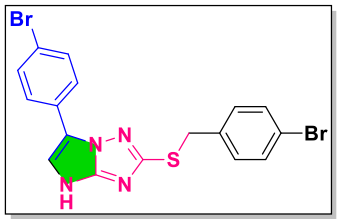
7.5. SCHEME-1. Characterization data of synthesized compounds

***4-(((6-Phenyl-4H-imidazo[1,2-b][1,2,4]-triazol-2-yl)thio)methyl)Benzonitrile.* 4a**



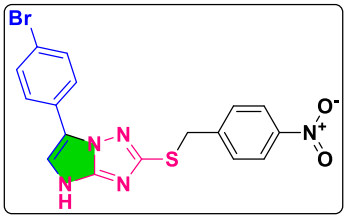
White solid; yield 85%; mp. 213°C; IR (KBr) cm^{-1}: 3391 (N-H), 3128 (alkene C-H), 2220 (CN), 1605 (C=N), 764 (C-Br): ^{1}H NMR (40 0 MHz, DMSO-d_6) δ ppm; 4.42 (s, 2H), 7.44-7.50 (m, 5H), 7.73 (d, *J* = 7.4 Hz, 2H), 7.81 (d, *J* = 8.2 Hz, 2H), 8.20 (s, 1H), 12.57 (s, 1H), ^{13}C{H}NMR (100MHz, DMSO-d_6) δ ppm; 35.3, 104.2, 124.8, 128.0, 128.5, 129.1, 129.5, 129.8, 130.6, 133.6, 142.1, 151.6, 161.6, 168.1: HRMS (ESI) (*m/z*) Calculated for $C_{18}H_{13}N_5S$ $[M+H]^+$ 332.0970; found 332.0969.

***2-((4-Bromobenzyl)thio)-6-(4-bromophenyl)-4H-imidazo[1,2-b][1,2,4]-triazole*. 4b**



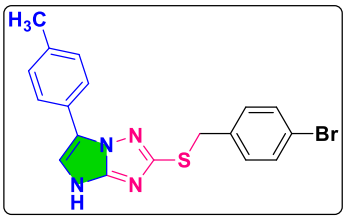
White solid; yield 84%; mp. 230 °C; IR (K Br) cm^{-1}: 3370 (N-H), 3156 (Alkene C-H), 1603 (C-Br), 743 (C-Br): ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm; 4.19 (s, 2H), 7.33 (d, *J* = 8.4 Hz, 2H), 7.48 (d, *J* = 8.1 Hz, 2H), 7.81 (d, *J* = 8.6 Hz, 2H), 7.95 (d, *J* = 8.6 Hz, 2H), 8.25 (s, 1H), 12.60 (s, 1H), $^{13}C\{H\}$ NMR (100MHz, DMSO-d_6) δ ppm; 34.5, 104.8, 120.6, 121.4, 126.7, 128.5, 130.5, 131.4, 131.6, 132.4, 134.0, 138.5, 151.6, 155.4, 157.7: HRMS (ESI) (*m/z*) Calculated for $C_{17}H_{12}Br_2N_4S$ $[M+H]^+$ 462.9227; found 462.9224.

***6-(4-Bromophenyl)-2-((4-nitrobenzyl)thio)-4H-imidazo[1,2-b][1,2,4]-triazole* .4c**



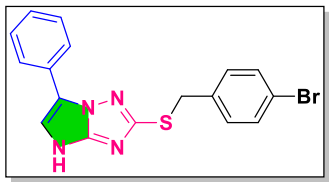
White solid; yield 82%; mp. 212 °C; IR (KBr) cm^{-1}: 3371 (N-H), 3160 (Alkene C-H), 1602 (C=N), 1591, 1346 (NO_2 Unsymmetric, Symmetric), 746 (C- Br): ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm; 4.34 (s, 2H), 7.64 (d, *J* = 8.8 Hz, 2H), 7.70 (d, *J* = 8.8 Hz, 2H), 7.81 (d, *J* = 8.6 Hz, 2H), 7.95 (d, *J* = 6.7 Hz, 2H), 8.25 (s, 1H), 12.64 (s, 1H), $^{13}C\{H\}$ NMR (100MHz, DMSO-d_6) δ ppm; 34.4, 104.4, 123.9, 126.7, 130.4, 130.5, 132.4, 133.9, 147.3, 151.6, 155.0, 157.8: HRMS (ESI) (*m/z*) Calculated for $C_{17}H_{12}BrN_5O_2S$ $[M+H]^+$ 429.9973; found 429.9962.

***2-((4-Bromobenzyl)thio)-6-(p-tolyl)-4H-imidazo[1,2-b][1,2,4]-triazole*. 4d**



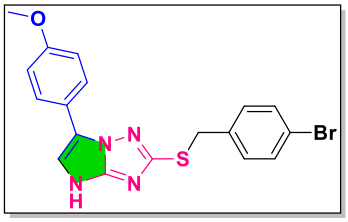
White solid; yield 88%; mp. 217 °C; IR (KBr) cm^{-1}: 3362 (N-H), 3140 (Alkene C-H), 1601 (C=N), 732 (C-Br): ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm; 2.33 (s, 3H), 4.35 (s, 2H), 7.27 (d, *J* = 8.0 Hz, 2H), 7.37 – 7.39 (m, 2H), 7.48 – 7.50 (m, 2H), 7.62 (d, *J* = 8.2 Hz, 2H), 8.11 (s, 1H),12.46 (s, 1H), $^{13}C\{H\}$NMR (100MHz, DMSO-d_6) δ ppm; 21.7, 34.9, 103.6, 120.6, 124.7, 126.9, 128.6, 130.0, 131.4, 131.6, 138.0, `151.3, 157.7, 161.3: HRMS (ESI) (*m/z*) Calculated for $C_{18}H_{15}BrN_4S$ $[M+H]^+$ 399.0279; found 399.0274.

***2-((4-Bromobenzyl)thio)-6-phenyl-4H-imidazo[1,2-b][1,2,4]-triazole*. 4e**



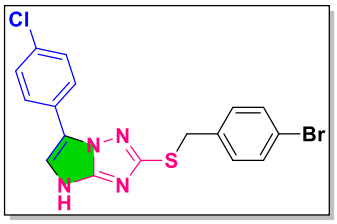
White solid; yield 89%; mp. 231°C; IR (KBr) cm^{-1}: 3120 (N-H), 2957 (C-H), 1584 (C=N), 783 (C-Br); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm; 4.39 (s, 2H), 7.43 (d, *J* = 8.5 Hz, 2H), 7.49 – 7.55 (m, 5H), 7.77 (d, *J* = 7.4 Hz, 2H), 8.20 (s, 1H), 12.60 (s, 1H), $^{13}C\{H\}$ NMR (100MHz, DMSO-d_6) δ ppm; 34.9, 104.2, 120.6, 124.7, 128.5, 129.4, 129.7, 130.6, 131.4, 131.6, 138.4, 151.5, 161.5: HRMS (ESI) (*m/z*) Calculated for $C_{17}H_{13}BrN_4S$ $[M+H]^+$ 385.0122; found 385.0122.

***2-((4-Bromobenzyl)thio)-6-(4-methoxyphenyl)-4H-imidazo[1,2-b][1,2,4]-triazole*. 4f**



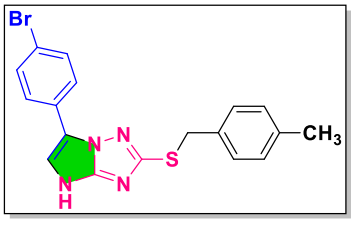
White solid; yield 89%; mp. 221°C; IR (KBr) cm^{-1}: 3367 (N-H), 3134 (Alkene C-H), 1601 (C=N), 1192 (C-O-C), 734 (C-Br): ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm; 3.79 (s, 3H), 4.34 (s, 2H), 7.03 (d, *J* = 8.9 Hz, 2H), 7.38 (d, *J* = 8.4 Hz, 2H), 7.50 (d, *J* = 8.4 Hz, 2H), 7.65 (d, *J* = 8.8 Hz, 2H), 8.03 (s, 1H), 12.41 (s, 1H), $^{13}C\{H\}$NMR (100MHz, DMSO-d_6) δ ppm; 35.0, 55.7, 102.9, 114.9, 120.5, 122.0, 126.4, 130.8, 130.9, 131.4, 131.6, 138.3, 151.2, 159.6, 160.9: HRMS (ESI) (*m/z*) Calculated for $C_{18}H_{15}BrN_4OS$ $[M+H]^+$ 415.0228; found 415.0253.

***2-((4-Bromobenzyl)thio)-6-(4-chlorophenyl)-4H-imidazo[1,2-b][1,2,4]-triazole*. 4g**



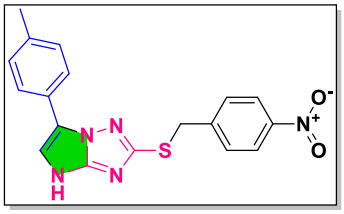
White solid; yield 87%; mp. 241°C; IR (KBr) cm^{-1}: 3247 (N-H),3103 (Alkene C-H), 1604 (C=N), 795 (C-Cl), 701 (C-Br): ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm; 4.19 (s, 2H), 7.33 (d, *J* = 8.4 Hz, 1H), 7.39 (d, *J* = 8.4 Hz, 1H), 7.48 (d, *J* = 8.1 Hz, 2H), 7.67 (d, *J* = 8.6 Hz 2H), 7.75 (d, *J* = 8.6 Hz, 1H), 8.24 (s, 1H), 12.61 (s, 1H); $^{13}C\{H\}$NMR (100MHz, DMSO-d_6) δ ppm; 34.5, 104.5, 120.6, 126.5, 129.4, 130.4, 131.4, 131.6, 133.4, 138.2, 139.3, 155.1, 157.5, 161.7: HRMS (ESI) (*m/z*) Calculated for $C_{17}H_{12}BrClN_4S$ [M+H]+ 418.9733; found 418.9746.

***6-(4-Bromophenyl)-2-((4-methylbenzyl)thio)-4H-imidazo[1,2-b][1,2,4]-triazole*.** **4h**White solid; yield 88%; mp. 220 °C; IR (KBr) cm^{-1}: 3104 (Alkene C-H), 1604 (C=N), 723 (C-Br): ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm; 2.27 (s, 3H), 4.18 (s, 2H), 7.10 (d, *J* = 8Hz, 2H), 7.24 (d, *J* = 7.9 Hz, 2H), 7.68 (s, H), 7.81 (d, *J* = 8.5 Hz, 2H), 7.95 (d, *J*



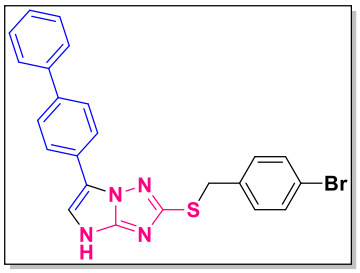
= 8.5 Hz, 2H), 12.59 (s, 1H); ^{13}C{H}NMR (100MHz, DMSO-d_6) δ ppm; 21.7, 34.4, 124.7, 126.9, 128.6, 129.8, 130.0, 130.8, 131.4, 131.6, 138.4, 144.8, 155.3, 157.7, 161.3: HRMS (ESI) (*m/z*) Calculated for $C_{18}H_{15}BrN_4S$ [M+H]+ 399.0279; found 399.0286.

2-((4-Nitrobenzyl)thio)-6-(p-tolyl)-4H-imidazo[1,2-b][1,2,4]-triazole. **4i**



White solid; yield 88%; mp. 215 °C; IR (KBr) cm^{-1}: 3361 (N-H), 3039 (Alkene C-H), 1605 (C=N), 1540, 1345 (NO_2 Unsymmetric, Symmetric): ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm; 2.27 (s, 3H), 4.18 (s, 2H), 7.10 (d, *J* = 8Hz, 2H), 7.24 (d, *J* = 7.9 Hz, 2H), 7.68 (s, H), 7.81 (d, *J* = 8.5 Hz, 2H), 7.95 (d, *J* = 8.5 Hz, 2H), 12.59 (s, 1H); ^{13}C{H}NMR (100MHz, DMSO-d_6) δ ppm; 21.2, 34.9, 103.6, 123.9, 124.7, 128.6, 129.8, 130.0, 130.4, 131.3, 131.6, 146.9, 147.2, 151.3, 160.8 : HRMS (ESI) (*m/z*) Calculated for $C_{18}H_{15}BrN_4S$ [M+H]+ 366.1019; found 366.1029.

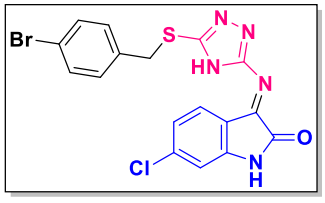
6-([1,1'-Biphenyl]-4-yl)-2-((4-bromobenzyl)thio)-4H-imidazo[1,2-b][1,2,4]-triazole. **4j**



White solid; yield 89%; mp. 222 °C; IR (KBr) cm^{-1}: 3324 (N-H), 3105 (Alkene C-H), 1607 (C=N), 724 (C-Br): ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm; 4.20 (s, 2H), 7.33 (d, *J* = 8.4 Hz, 1H), 7.40 (d, *J* = 8.5 Hz, 2H), 7.47 (s, 1H), 7.49 (d, *J* = 0.9 Hz, 2H), 7.51 (d, *J* = 2.1 Hz, 1H), 7.74 (s, 1H), 7.78-7.79 (m, 2H), 7.83 (d, *J* = 8.6 Hz, 1H), 7.90 (d, *J* = 8.5 Hz, 2H), 8.25 (s, 1H), 12.61 (s, 1H); ^{13}C{H}NMR (100MHz, DMSO-d_6) δ ppm; 34.4, 104.3, 125.2, 127.0, 127.4, 127.5, 127.6, 129.2, 129.4, 129.6, 131.4, 131.6, 131.7, 133.8, 145.6, 155.4, 157.7 : HRMS (ESI) (*m/z*) Calculated for $C_{23}H_{17}BrN_4S$ [M+H]+ 461.0440; found 461.0454.

7. 5.1. SCHEME-2.

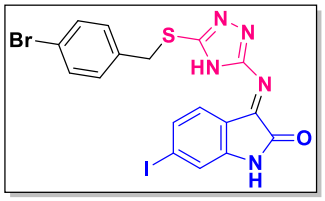
(E)-3-((5-((4-Bromobenzyl)thio)-4H-1,2,4-triazol-3-yl)imino)-6-chloroindolin-2-one. **5a**



Yellow solid; yield 88%; mp. 210 °C; IR (KBr) cm^{-1}: 3379 (Triazole N-H), 3092 (Oxindole ring N-H), 1619 (Imide C=O), 1584 (C=N): ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm; 4.39 (s, 2H), 6.88 (d, *J* = 8.2 Hz, 1H), 7.41 – 7.34 (m, 4H), 7.46 (d, *J* = 7.6 Hz, 2H), 9.83 (s, 1H), 10.82 (s, 1H); ^{13}C{H}NMR (100MHz, DMSO-d_6) δ ppm; 35.4, 111.8, 120.2, 120.3, 125.7, 129.5, 131.3, 131.6, 131.8, 139.3, 142.0, 144.4,

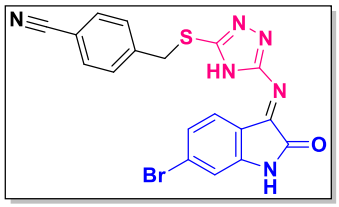
158.3, 165.1, 166.7: HRMS (ESI) (*m/z*) Calculated for $C_{17}H_{11}BrClN_5OS$ $[M+H]^+$447.9634; found 447.9632.

(E)-3-((5-((4-Bromobenzyl)thio)-4H-1,2,4-triazol-3-yl)imino)-6-iodoindolin-2-one. **5b**



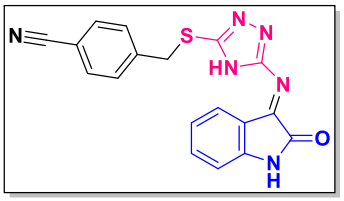
Light yellow solid; yield 84%; mp. 220 °C; IR (KBr) cm^{-1}: 3335 (Triazole N-H), 3089 (Oxindole ring N-H), 1721 (Imide C=O), 1601 (C=N), 709 (C-Br), 631 (C-I): ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm; 4.43 (s, 2H), 6.74 (d, *J* = 8.1 Hz, 1H), 7.39 (d, *J* = 8.3 Hz, 2H), 7.47 (d, *J* = 8.3 Hz, 2H), 7.66 (d, *J* = 8.1 Hz, 1H), 10.01 (s, 1H), 10.85 (s, 1H); ^{13}C{H}NMR (100MHz, DMSO-d_6) δ ppm; 35.6, 84.8, 113.0, 120.4, 120.9, 131.4, 131.6, 138.1, 138.8, 140.9, 145.7, 158.1, 165.8: HRMS (ESI) (*m/z*) Calculated for $C_{17}H_{11}BrIN_5OS$ $[M+H]^+$ 539.8990; found; 539.8981.

(E)-4-(((5-((6-Bromo-2-oxoindolin-3-ylidene)amino)-4H-1,2,4-triazol-3-yl)thio)methyl) Benzonitrile. **5c**



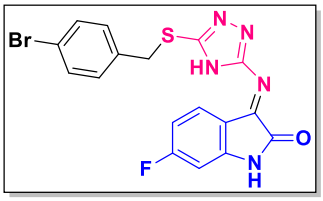
Light brown solid; yield 86%; mp. 219 °C; IR (KBr) cm^{-1}: 3170 (Triazole N-H), 3092 (Oxindole ring N-H), 2228 (CN), 1701 (Imide C=O), 1576 (C=N), 709 (C-Br): ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm; 4.49 (s, 2H), 6.83 (s, 1H), 7.80 – 7.48 (m, 6H), 9.83 (s, 1H), 10.83 (s, 1H); ^{13}C{H}NMR (100MHz, DMSO-d_6) δ ppm; 35.6, 110.0, 112.5, 113.5, 119.3, 120.4, 130.1, 132.3, 132.7, 135.1, 145.2, 145.7, 157.9, 166.2: HRMS (ESI) (*m/z*) Calculated for $C_{18}H_{11}BrN_6OS$ $[M+H]^+$ 438.9976; found 438.9971.

(E)-4-(((5-((2-Oxoindolin-3-ylidene)amino)-4H-1,2,4-triazol-3-yl)thio)methyl)Benzonitrile. 5d



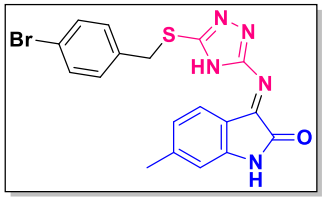
Light yellow solid; yield 89%; mp. 216 °C; IR (KBr) cm^{-1}: 3204 (Triazole N-H), 3092 (Oxindole ring N-H), 2209 (CN), 1698 (Imide C=O), 1601 (C=N): ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm; 4.42 (s, 2H), 6.83 (d, *J* = 7.7 Hz, 1 H), 6.88 – 6.92 (m, 1H), 7.30 (t, *J* = 8.2 Hz, 1H), 7.61 (d, *J* = 8.3 Hz, 2H), 7.73 (d, *J* = 8.3 Hz, 3H), 9.38 (s, 1H), 10.61 (s, 1H); ^{13}C{H}NMR (100MHz, DMSO-d_6) δ ppm; 35.7, 109.8, 110.3, 118.9, 119.3, 121.6, 129.7, 130.1, 132.5, 132.7, 146.3, 156.9, 166.7: HRMS (ESI) (*m/z*) Calculated for $C_{18}H_{12}N_6OS$ $[M+H]^+$ 361.0871; found 361.0872.

(E)-3-((5-((4-Bromobenzyl)thio)-4H-1,2,4-triazol-3-yl)imino)-6-fluoroindolin-2-one. 5e



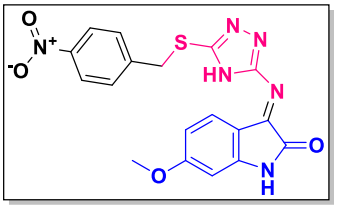
Light yellow solid; yield 85%; mp. 226 °C; IR (KBr) cm^{-1}: 3345 (Triazole N-H), 3059 (Oxindole ring N-H), 1701 (Imide C=O), 1603 (C=N), 809 (C-F), 723 (C-Br): ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm; 4.36 (s, 2H), 6.85 (s, 1H), 7.18 (td, *J* = 8.8, 2.8 Hz, 1H), 7.37 (d, *J* = 8.4 Hz, 2H), 7.46 (d, *J* = 8.4 Hz, 2H), 9.48 (s, 1H), 10.71 (s, 1H); ^{13}C{H}NMR (100MHz, DMSO-d_6) δ ppm; 35.3, 111.0, 111.1, 116.8, 118.8, 119.5, 120.3, 131.3, 131.5, 139.3, 142.2, 156.5, 157.9, 158.8, 164.7, 166.9: HRMS (ESI) (*m/z*) Calculated for $C_{17}H_{11}BrFN_5OS$ $[M+H]^+$ 431.9930; found 431.9924.

***(E)-3-((5-((4-Bromobenzyl)thio)-4H-1,2,4-triazol-3-yl)imino)-6-methylindolin-2-one*. 5f**



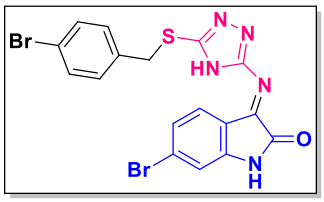
Yellow solid; yield 92%; mp. 215 °C; IR (KBr) cm^{-1}: 3295 (Triazole N-H), 3098 (Oxindole ring N-H), 1695 (Imide C=O), 1603 (C=N), 721 (C-Br): ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm; .16 (s, 3H), 4.39 (s, 2H), 7.31 (d, *J* = 8.4 Hz, 2H), 7.37 (d, *J* = 8.5 Hz, 2H), 7.45-7.48 (m, 3H), 9.18 (s, 1H),10.62 (s, 1H); ^{13}C{H}NMR (100MHz, DMSO-d_6) δ ppm; 21.1, 35.4, 110.3, 112.5, 118.6, 120.4, 125.2, 130.2, 130.3, 131.3, 131.62, 131.65, 139.1, 144.2, 166.4: HRMS (ESI) (*m/z*) Calculated for $C_{18}H_{14}BrN_5OS$ $[M+H]^+$428.0180; found 428.0185.

(*E*)-6-Methoxy-3-((5-((4-nitrobenzyl)thio)-4H-1,2,4-triazol-3-yl)imino)indolin-2-one*. 5g



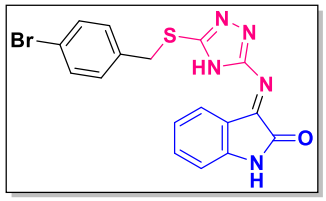
Yellow solid; yield 90%; mp. 231 °C; IR (KBr) cm^{-1}: 3345 (Triazole N-H), 3024 (Oxindole ring N-H), 1701 (Imide C=O), 1604 (C=N), 1540, 1345 (NO_2 Unsymmetric, Symmetric), 1194 (C-O-C); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 3.70 (s, 3H), 4.48 (s, 2H), 6.76 (d, *J* = 8.4 Hz, 1H), 6.90-6.93 (m, 1H), 7.67 (d, *J* = 8.8 Hz, 2H), 8.13 (d, *J* = 8.8 Hz, 3H), 9.32 (s, 1H), 10.46 (s, 1H), ^{13}C{H}NMR (100MHz, DMSO-d_6) δ ppm; 35.4, 55.9, 110.6, 116.1, 118.0, 119.5, 123.8, 130.3, 139.6, 146.7, 148.4, 154.7, 156.7, 166.9: HRMS (ESI) (*m/z*) Calculated for $C_{18}H_{14}N_6O_4S$ [M+H]+ 411.0875; found 411.0870.

(*E*)-6-Bromo-3-((5-((4-bromobenzyl)thio)-4H-1,2,4-triazol-3-yl)imino)indolin-2-one. 5h



Yellow solid; yield 91%; mp. 229 °C; IR (KBr) cm^{-1}: 3302 (Triazole N-H), 3045 (Oxindole ring N-H), 1701 (Imide C=O), 1609 (C=N), 721 (C-Br): ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm δ 10.70 (s, 1H), 9.25 (s, 1H), 7.47 (d, *J* = 8.4 Hz, 2H), 7.38 (d, *J* = 8.4 Hz, 2H), 7.32 (d, *J* = 6.6 Hz, 1H), 6.92 (t, *J* = 7. 5 Hz, 1H), 6.85 (d, *J* = 7.7 Hz, 1H), 4.35 (s, 2H). ^{13}C{H}NMR (100MHz, DMSO-d_6) δ ppm; 35.3, 113.6, 113.7, 119.1, 120.9, 131.4, 131.9, 132.7, 137.5, 138.1, 147.5, 164.5: HRMS (ESI) (*m/z*) Calculated for $C_{17}H_{11}Br_2N_5OS$ $[M+H]^+$ 491.9129; found; 491.9122.

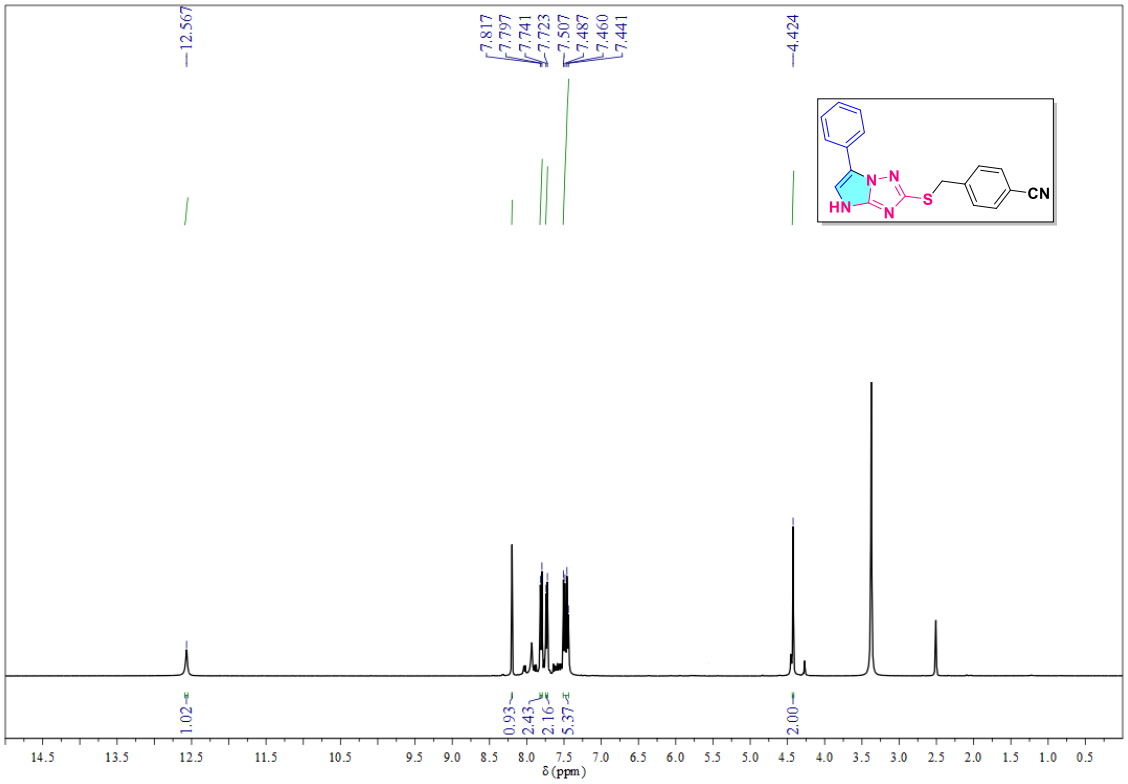
***(E)-3-((5-((4-Bromobenzyl)thio)-4H-1,2,4-triazol-3-yl)imino)indolin-2-one*. 5i**



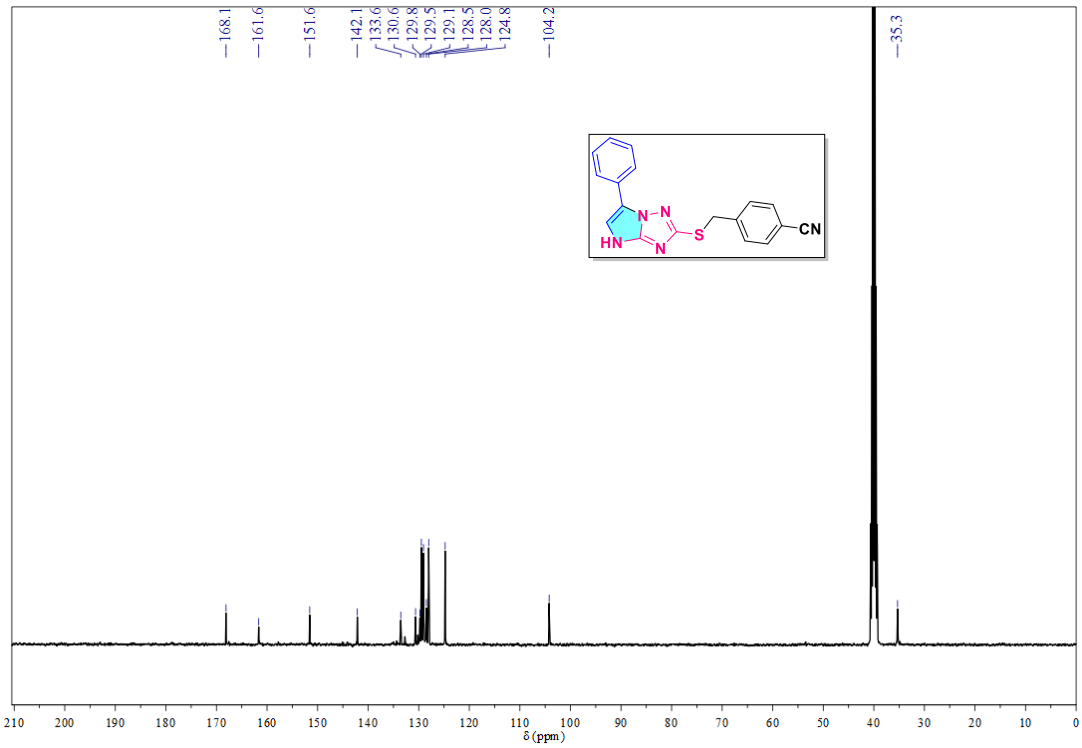
Brown solid; yield 92%; mp. 214 °C; IR (KBr) cm^{-1}: 3302 (Triazole N-H), 3024 (Oxindole ring N-H), 1698 (Imide C=O), 1609 (C=N), 721 (C-Br): ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 4.35 (s, 2H), 6.85 (d, *J* = 7.7 Hz, 1H), 6.90 - 6.94 (m, 1H), 7.30 – 7.35 (m, 2H), 7.37 (d, *J* = 11.6 Hz, 2H), 7.47 (d, *J* = 8.4 Hz, 2H), 9.20 (d, *J* = 7.7 Hz, 1H), 10.70 (s, 1H), ^{13}C{H}NMR (100MHz, DMSO-d_6) δ ppm;35.4, 110.6, 118.5, 120.3, 121.8, 129.7, 131.3, 131.5, 133.4, 139.2, 146.4, 157.3, 166.3 : HRMS (ESI) (*m/z*) Calculated for $C_{17}H_{12}BrN_5OS$ $[M+H]^+$ 414.0024; found; 414.0021.

7.6. Copies of spectral data.

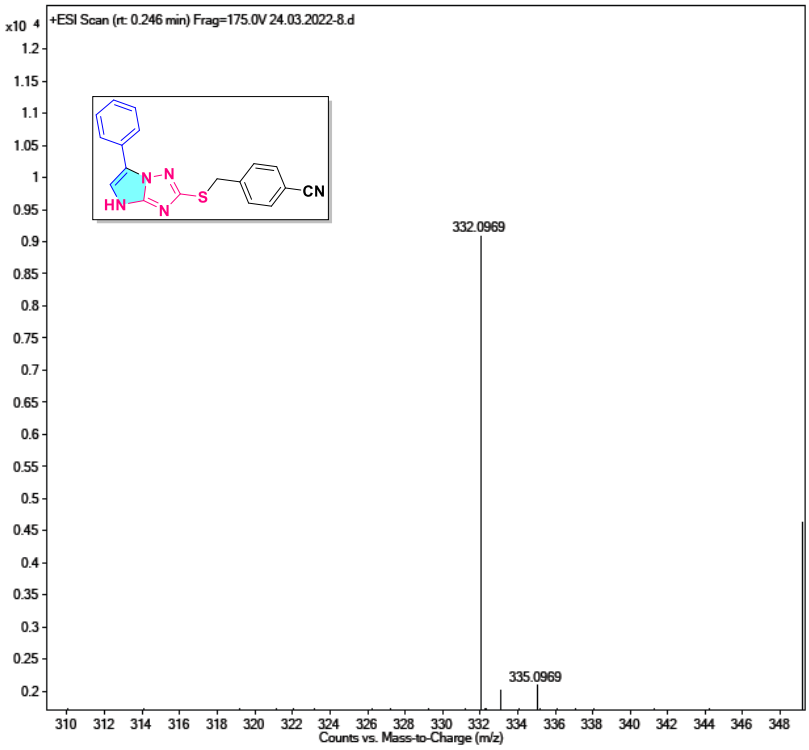
^{1}H NMR spectrum of compound 4a (DMSO-d_6, 400 MHz) [**SCHEME-1**]



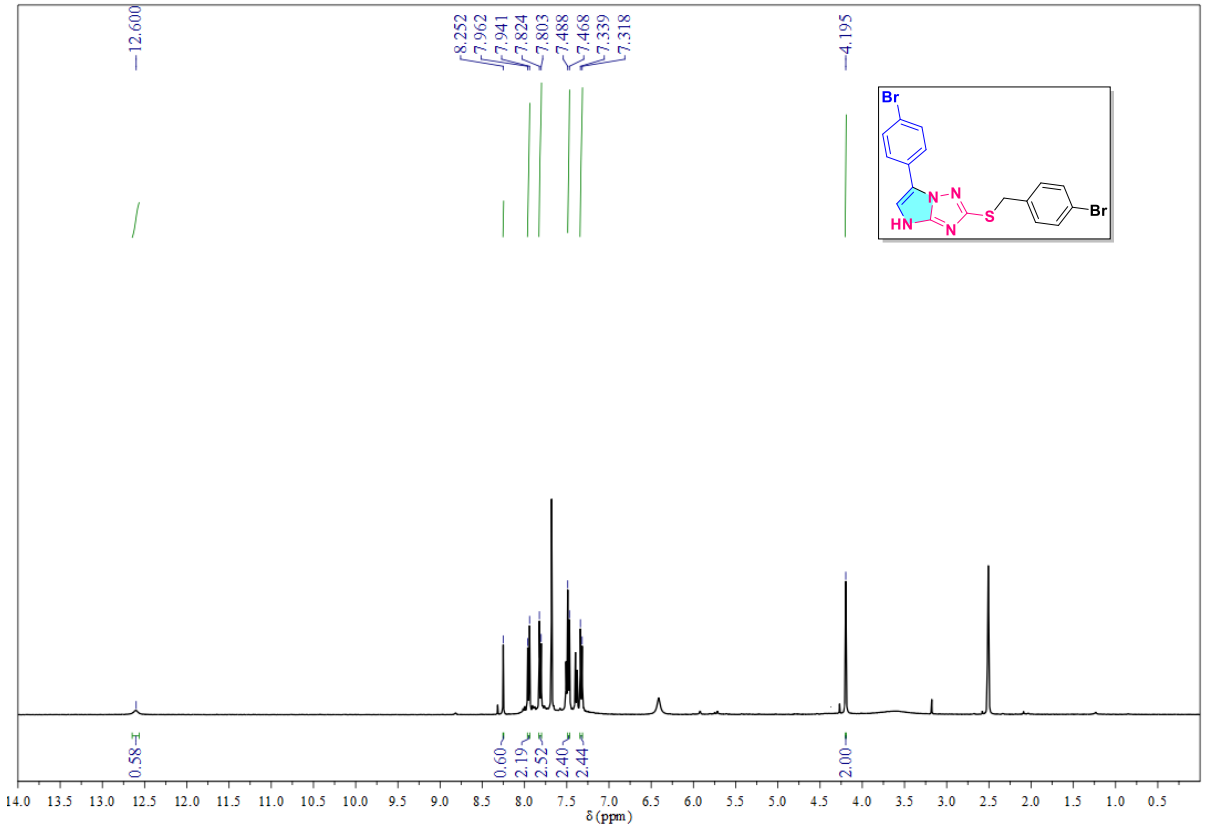
^{13}C NMR spectrum of compound 4a (DMSO-d_6, 100 MHz)



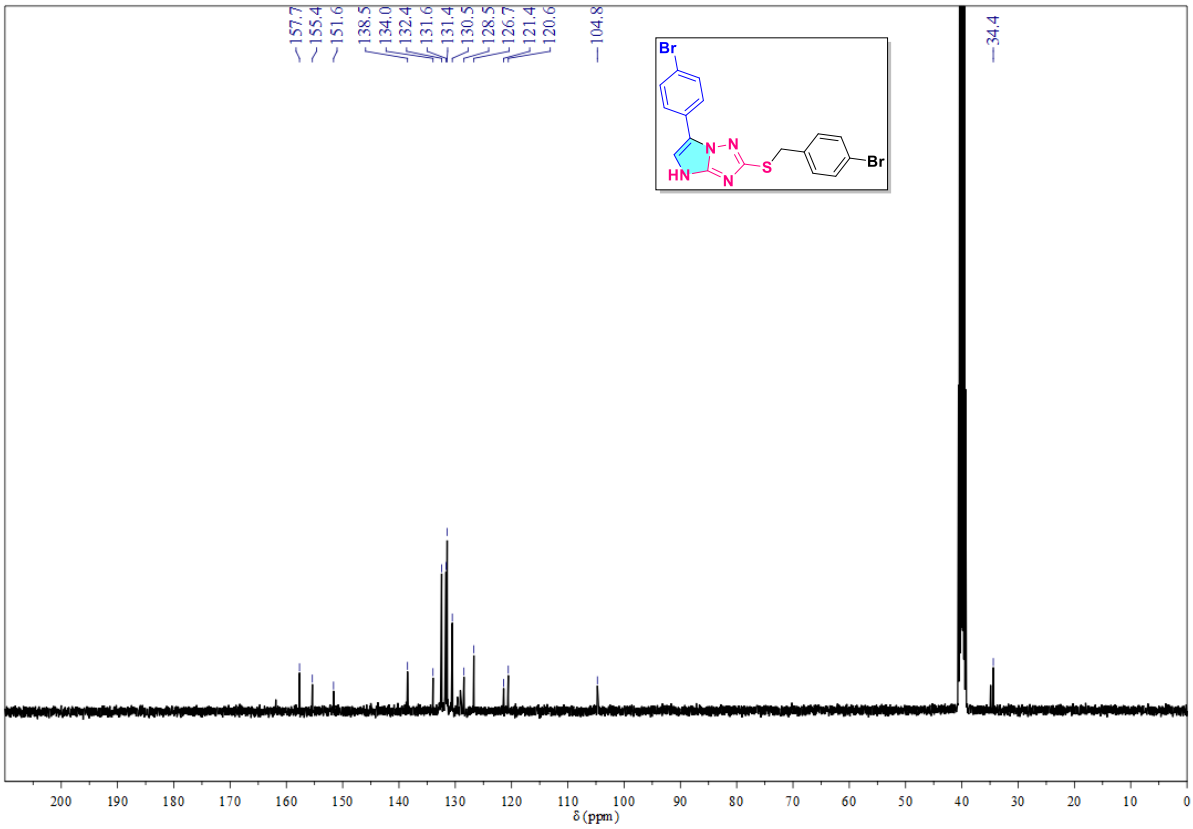
Mass spectrum of compound 4a



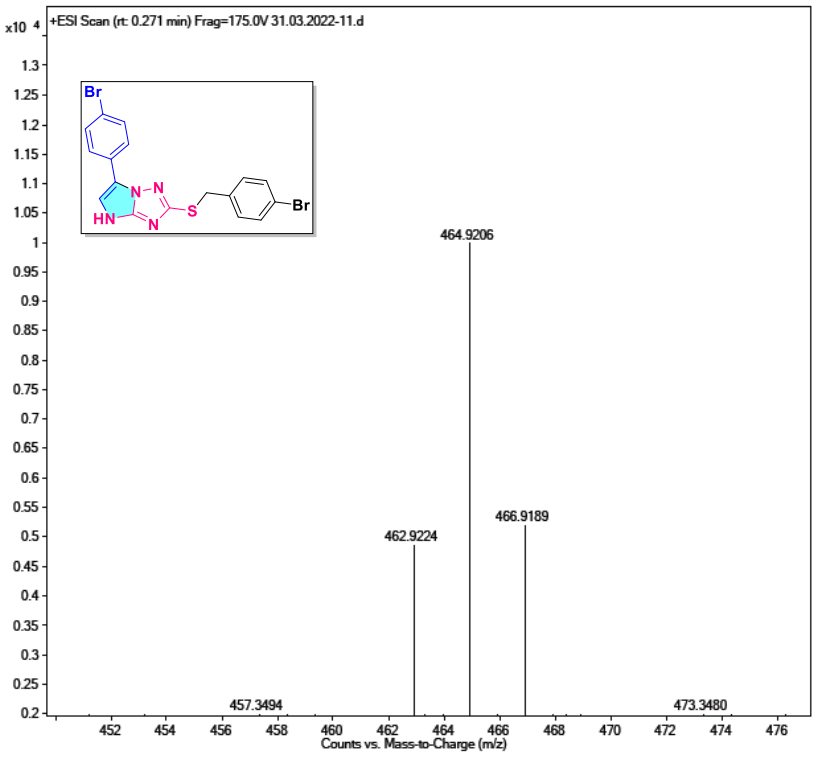
^{1}H NMR spectrum of compound 4b (DMSO-d_6, 400 MHz)



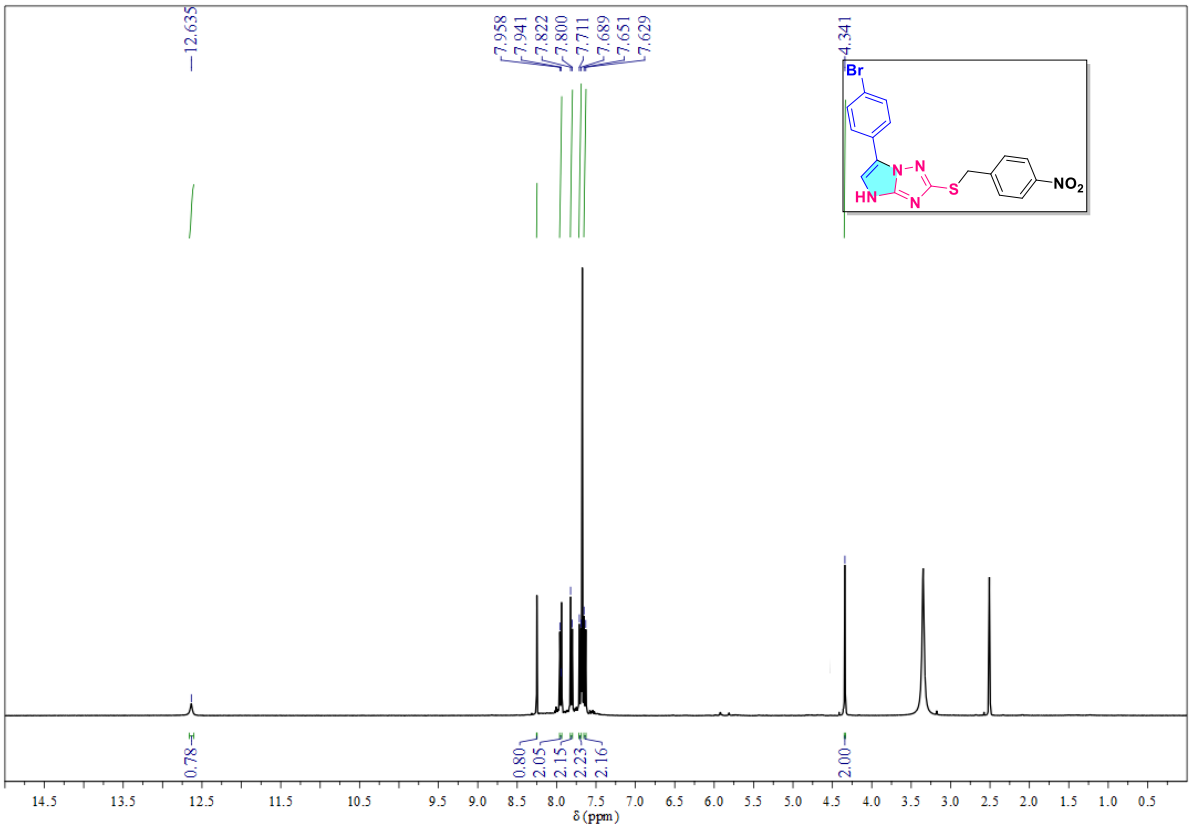
^{13}C NMR spectrum of compound 4b (DMSO-d_6, 100 MHz)



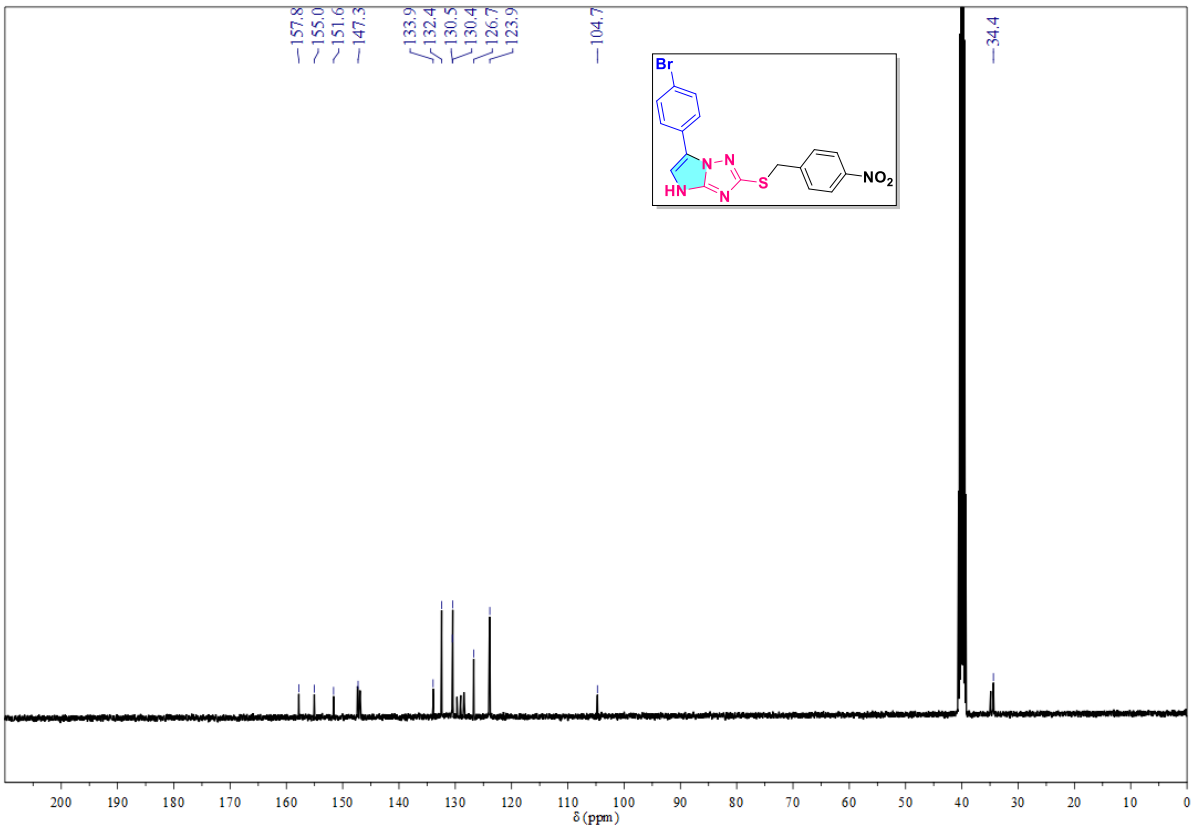
Mass spectrum of compound 4b



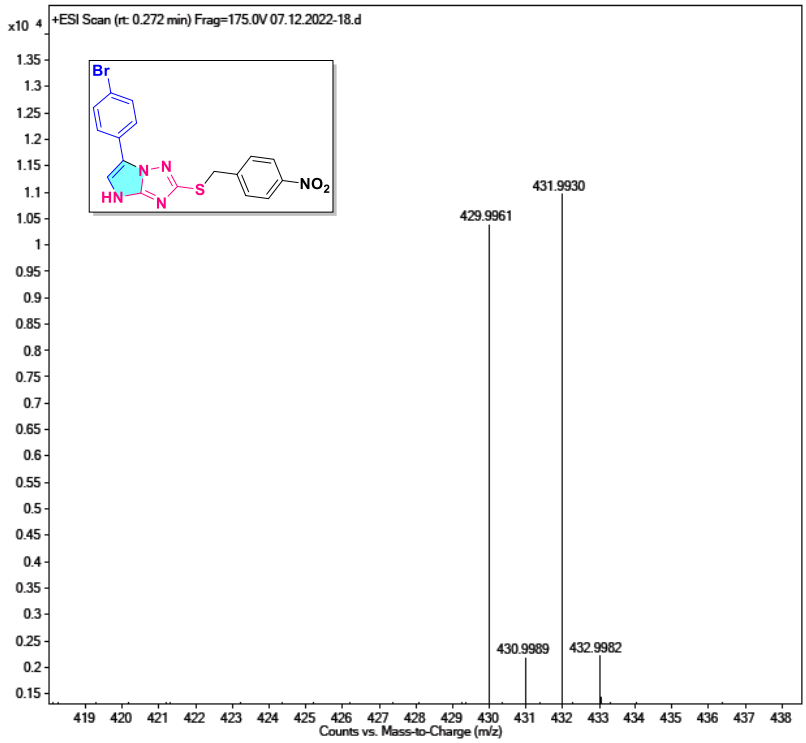
^{1}H NMR spectrum of compound 4c (DMSO-d_6, 400 MHz)



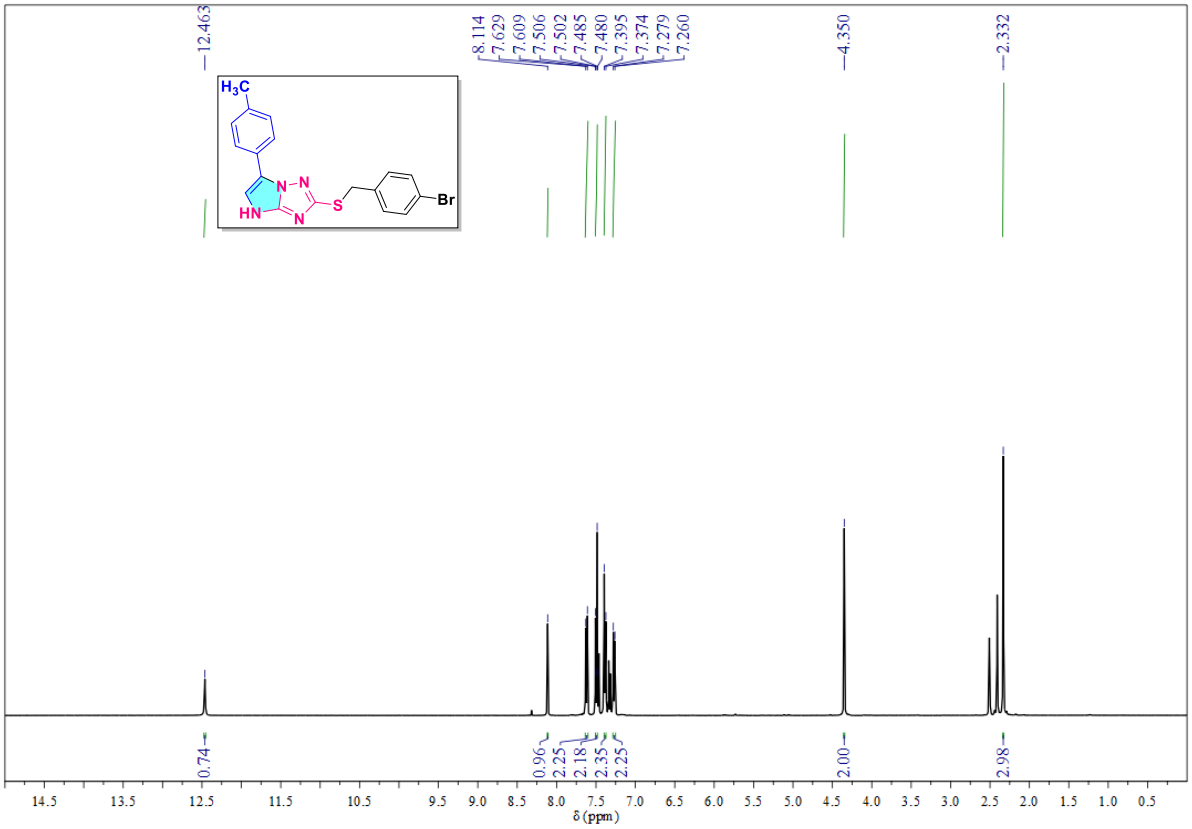
^{13}C NMR spectrum of compound 4c (DMSO-d_6, 100 MHz)



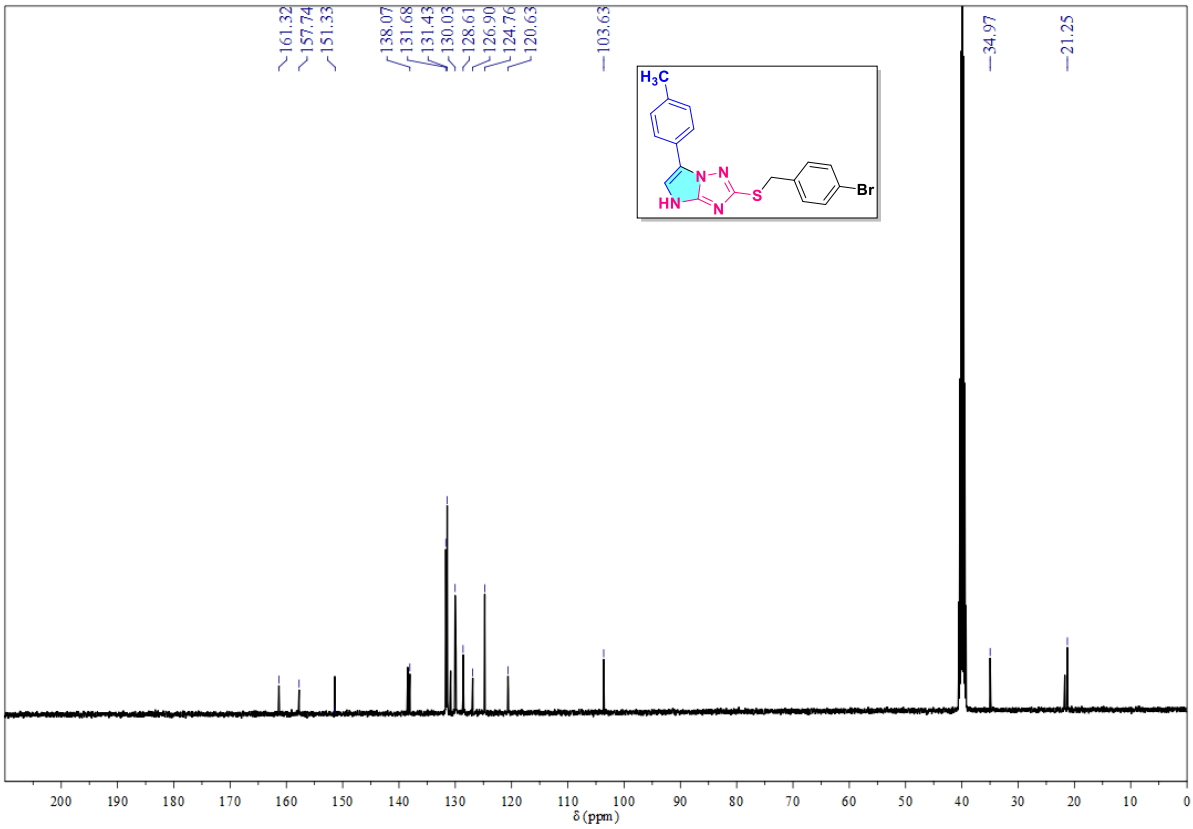
Mass spectrum of compound 4c



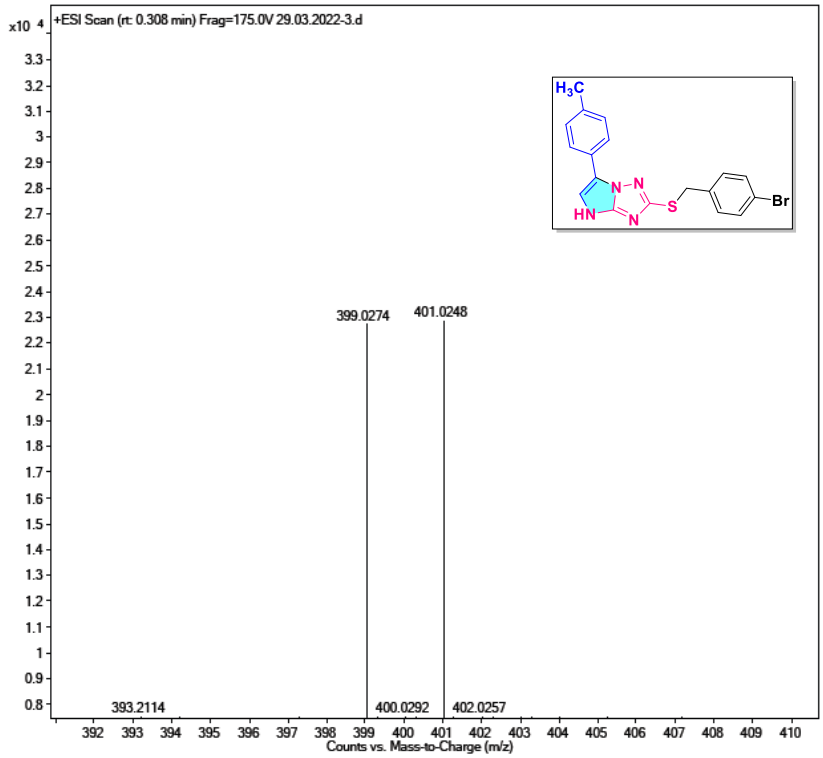
^{1}H NMR spectrum of compound 4d (DMSO-d_6, 400 MHz)



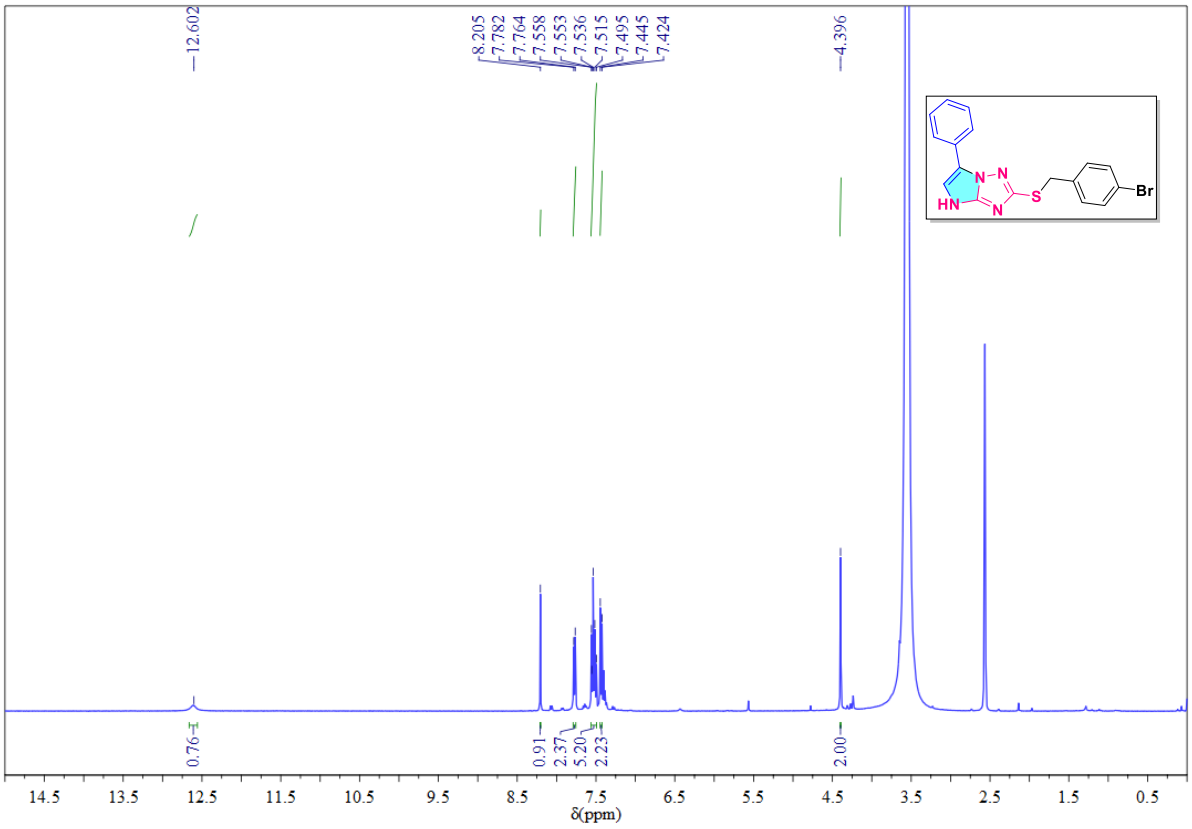
^{13}C NMR spectrum of compound 4d (DMSO-d_6, 100 MHz)



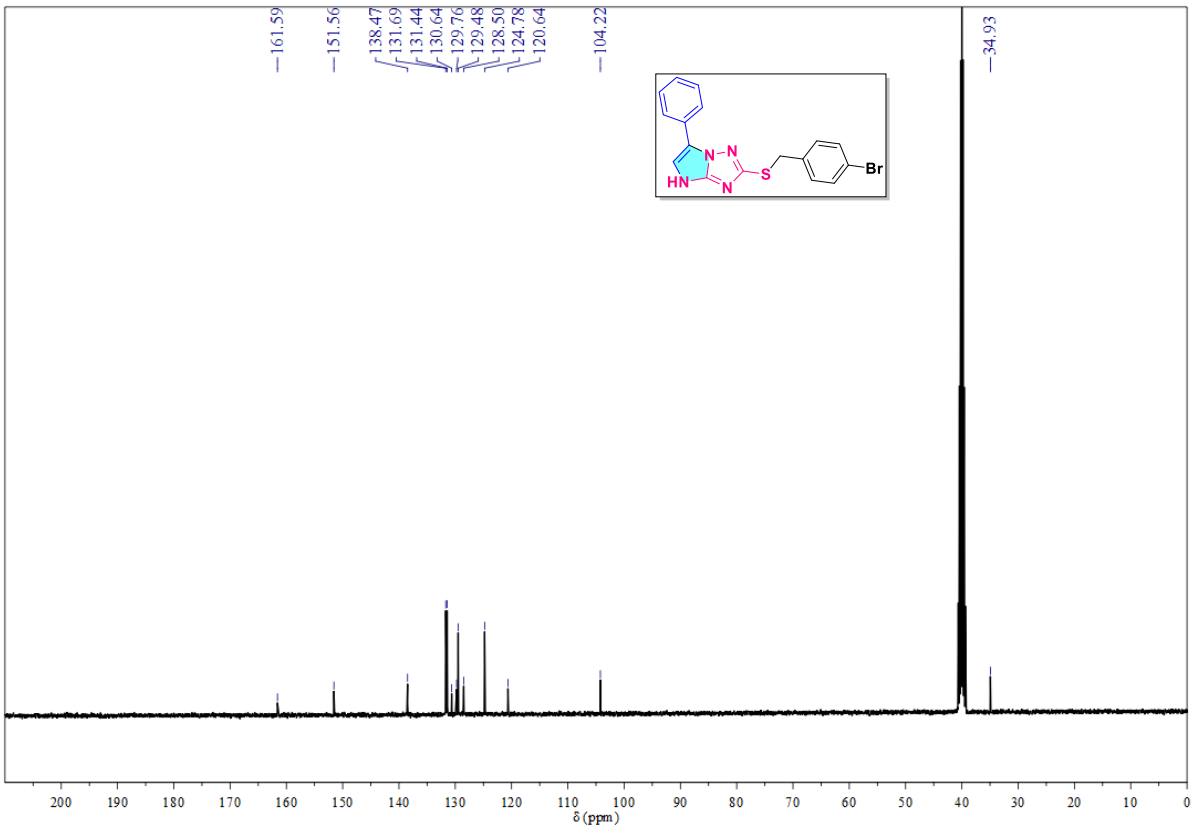
Mass spectrum of compound 4d



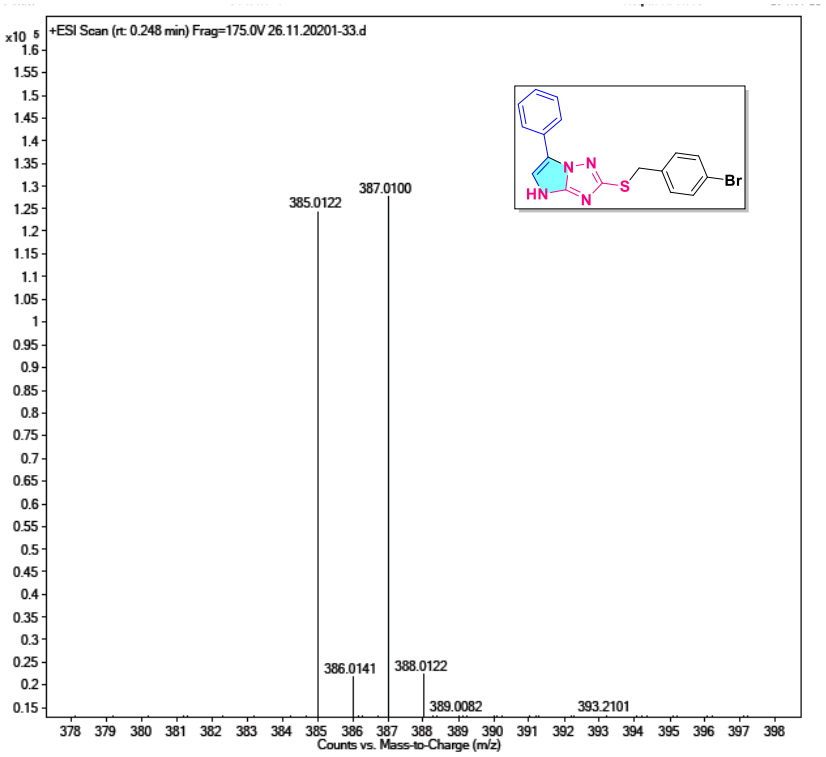
^{1}H NMR spectrum of compound 4e (DMSO-d_6, 400 MHz)



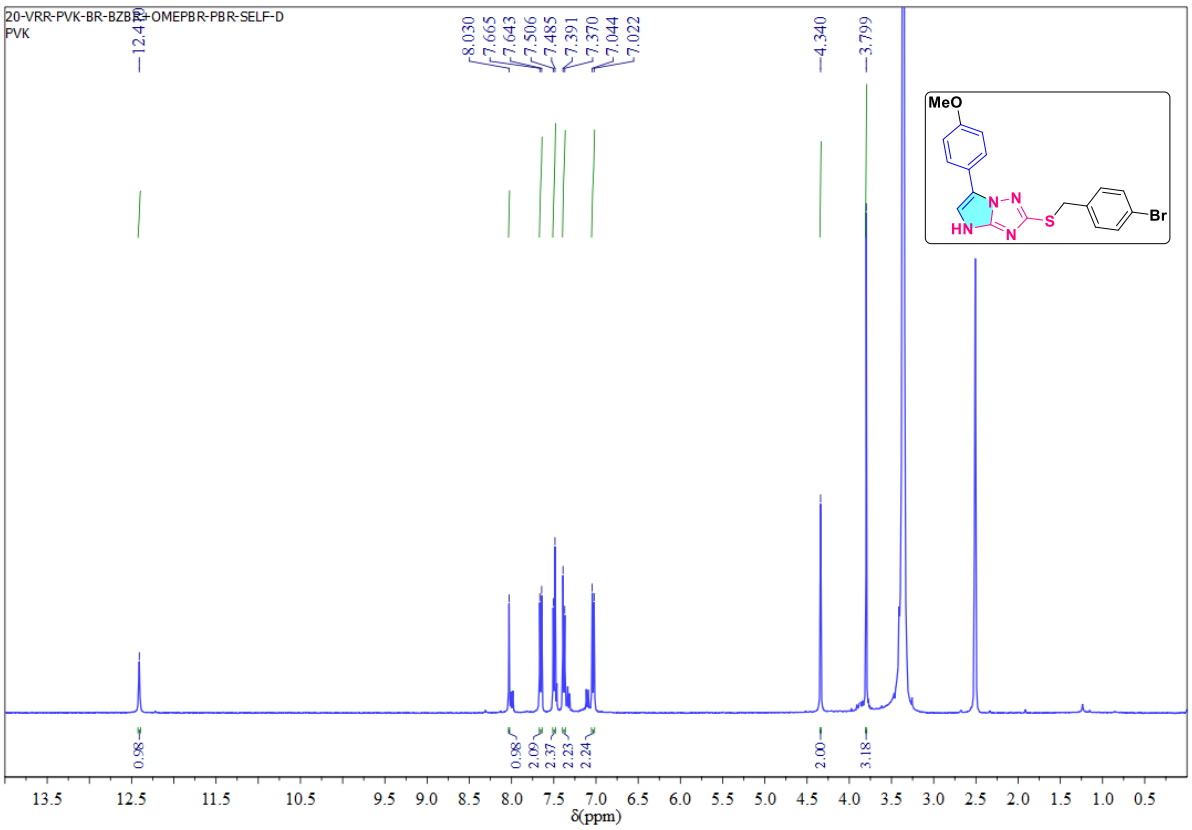
^{13}C NMR spectrum of compound 4e (DMSO-d_6, 100 MHz)



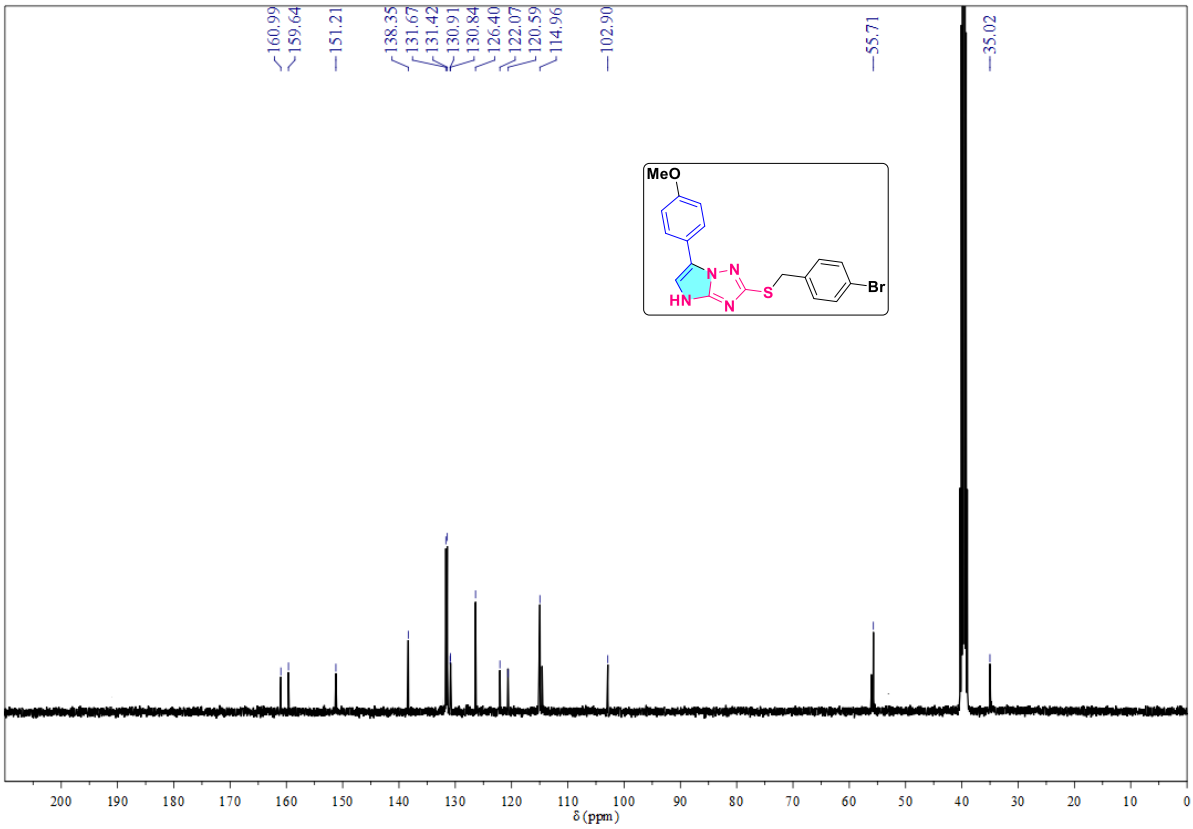
Mass spectrum of compound 4e



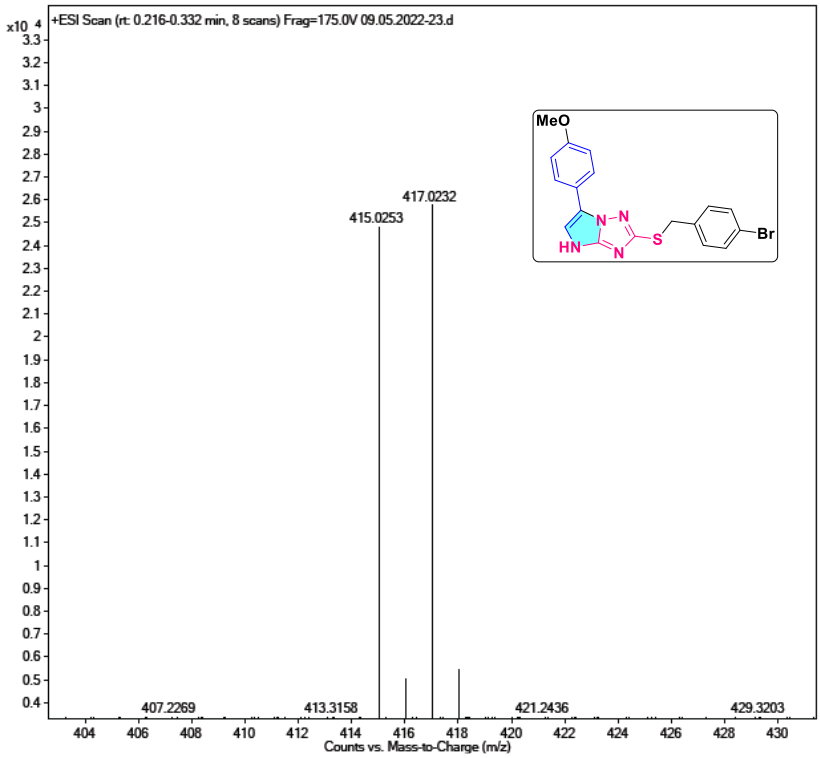
^{1}H NMR spectrum of compound 4f (DMSO-d_6, 400 MHz)



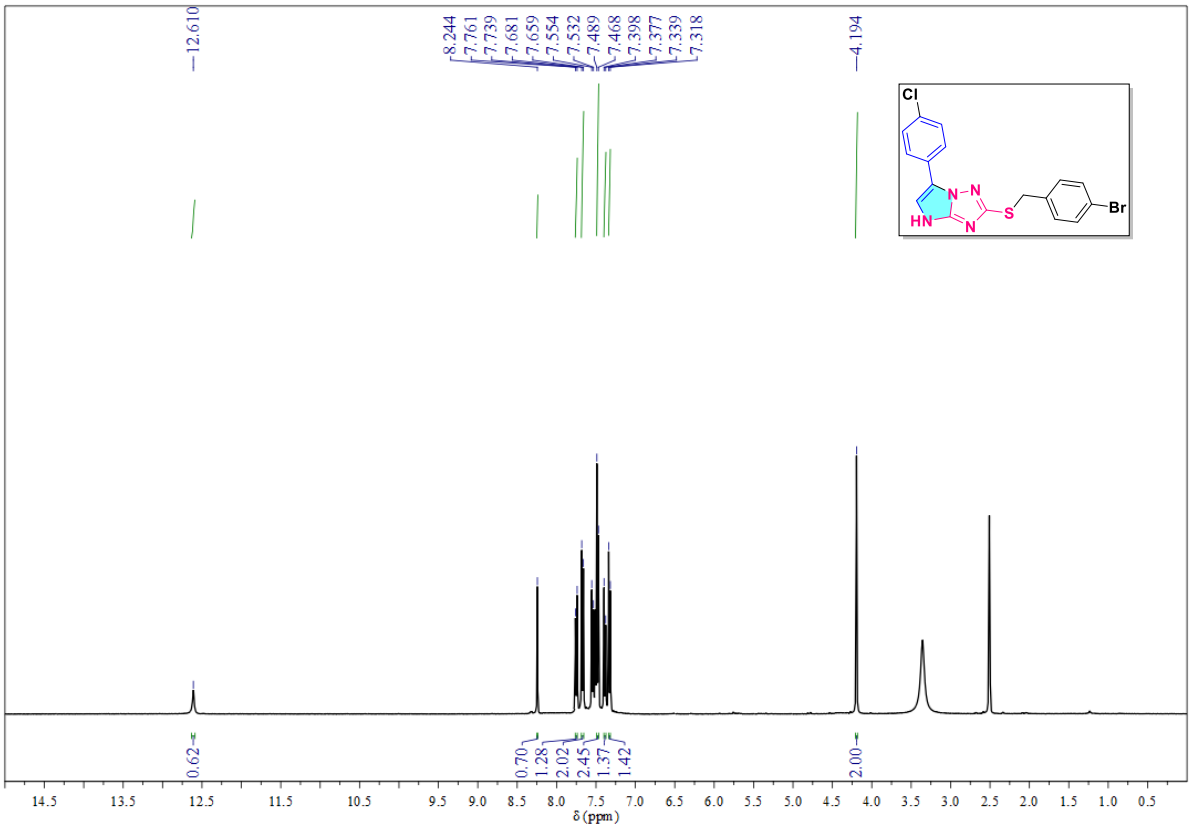
^{13}C NMR spectrum of compound 4f (DMSO-d_6, 100 MHz)



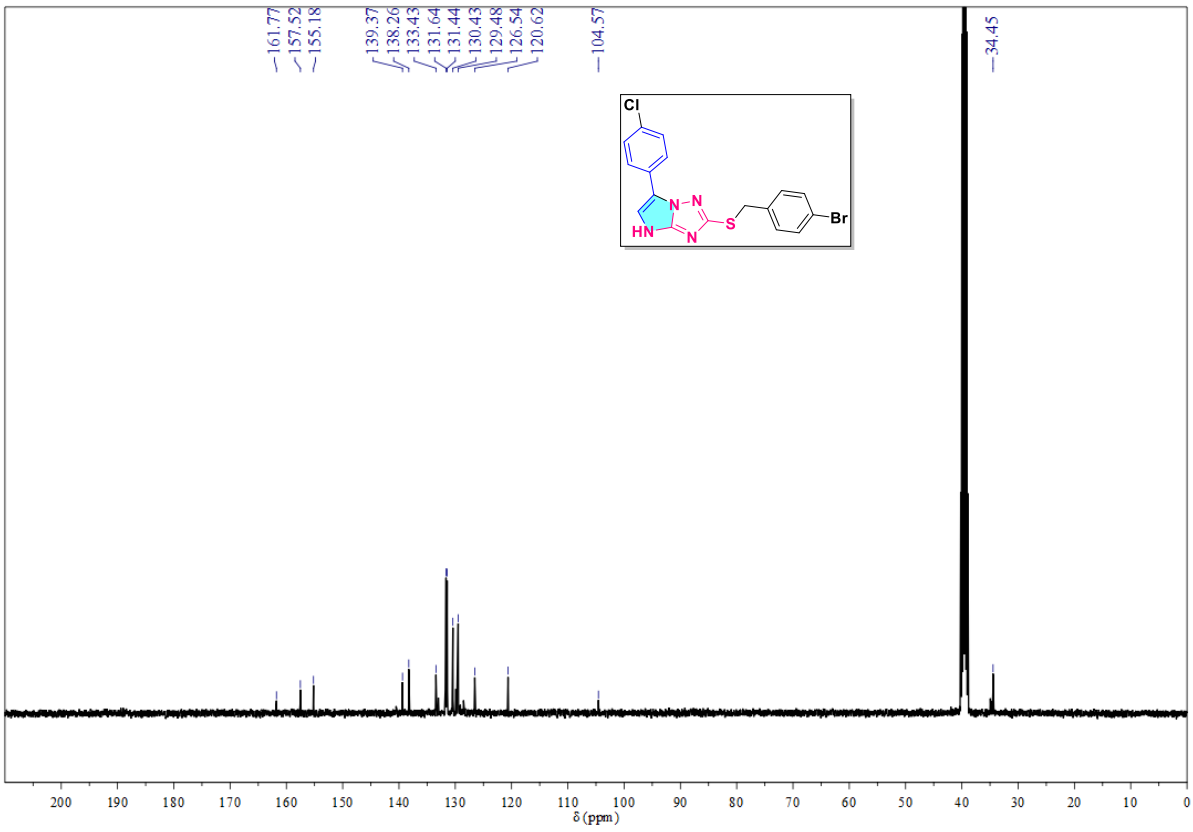
Mass spectrum of compound 4f



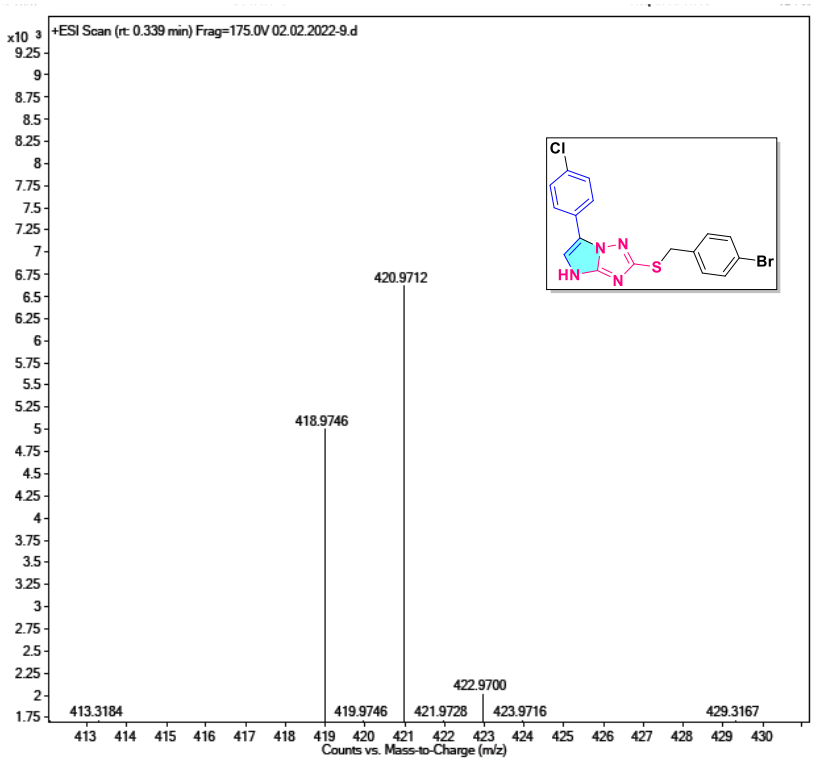
^{1}H NMR spectrum of compound 4g (DMSO-d_6, 400 MHz)



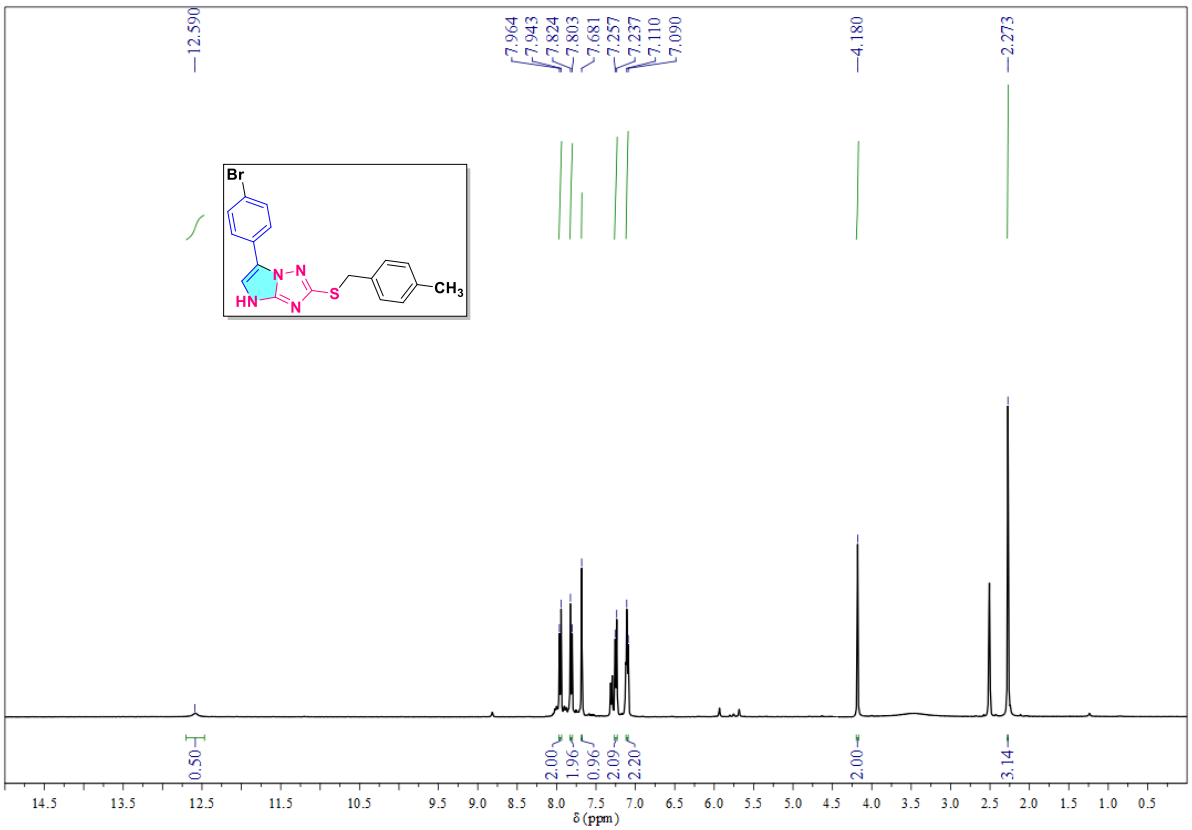
^{13}C NMR spectrum of compound 4g (DMSO-d_6, 100 MHz)



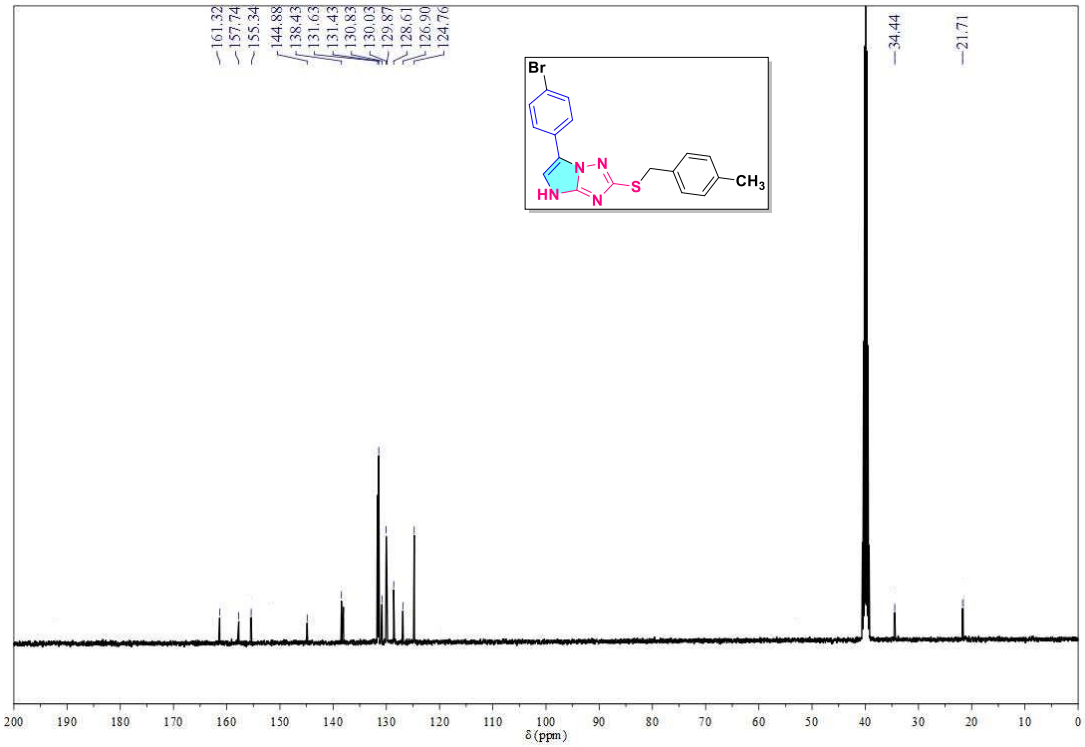
Mass spectrum of compound 4g



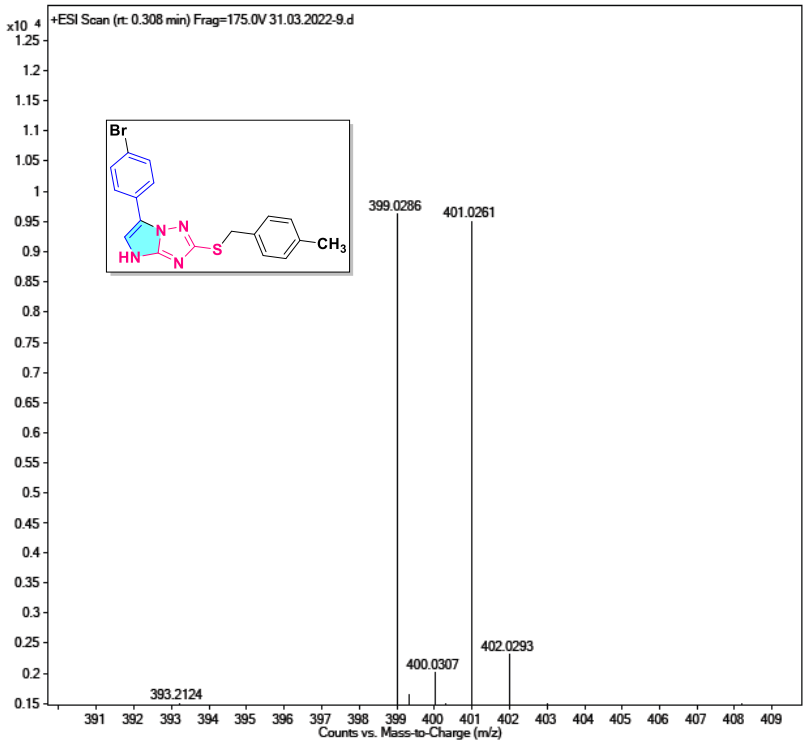
^{1}H NMR spectrum of compound 4h (DMSO-d_6, 400 MHz)



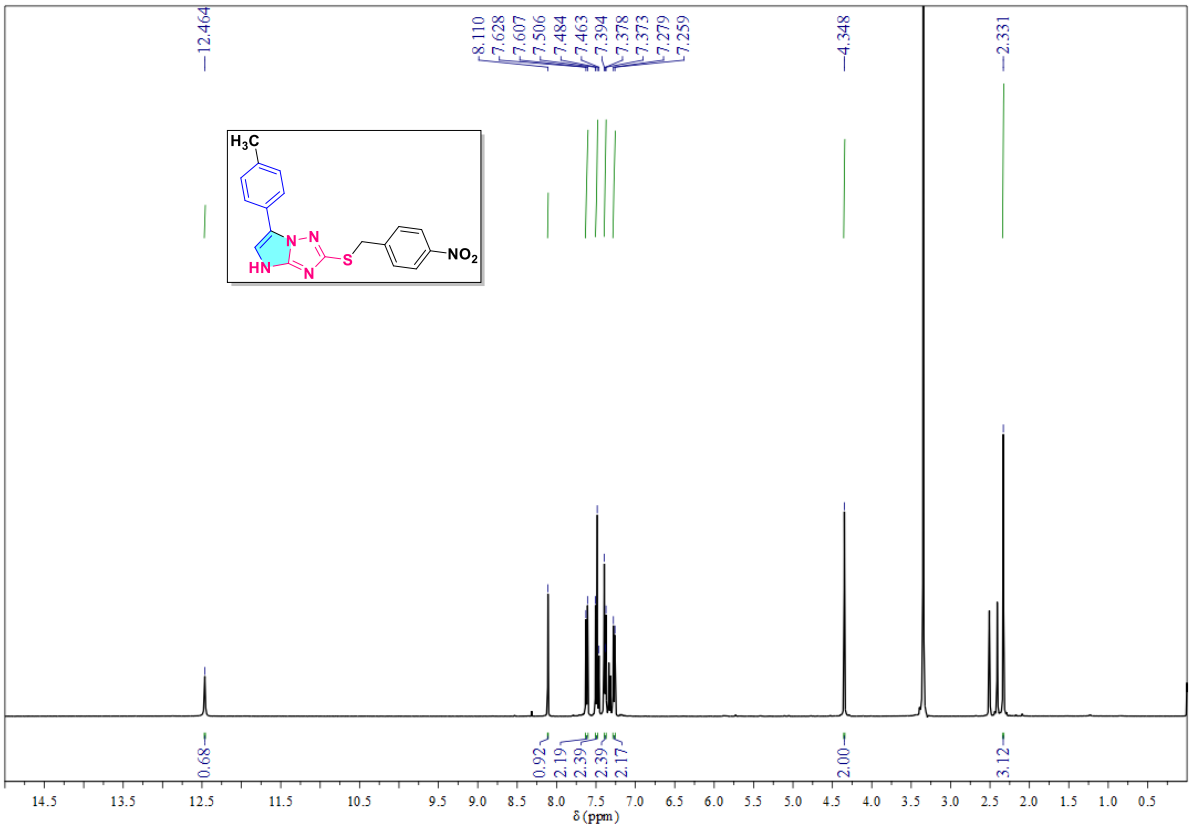
^{13}C NMR spectrum of compound 4h (DMSO-d_6, 100 MHz)



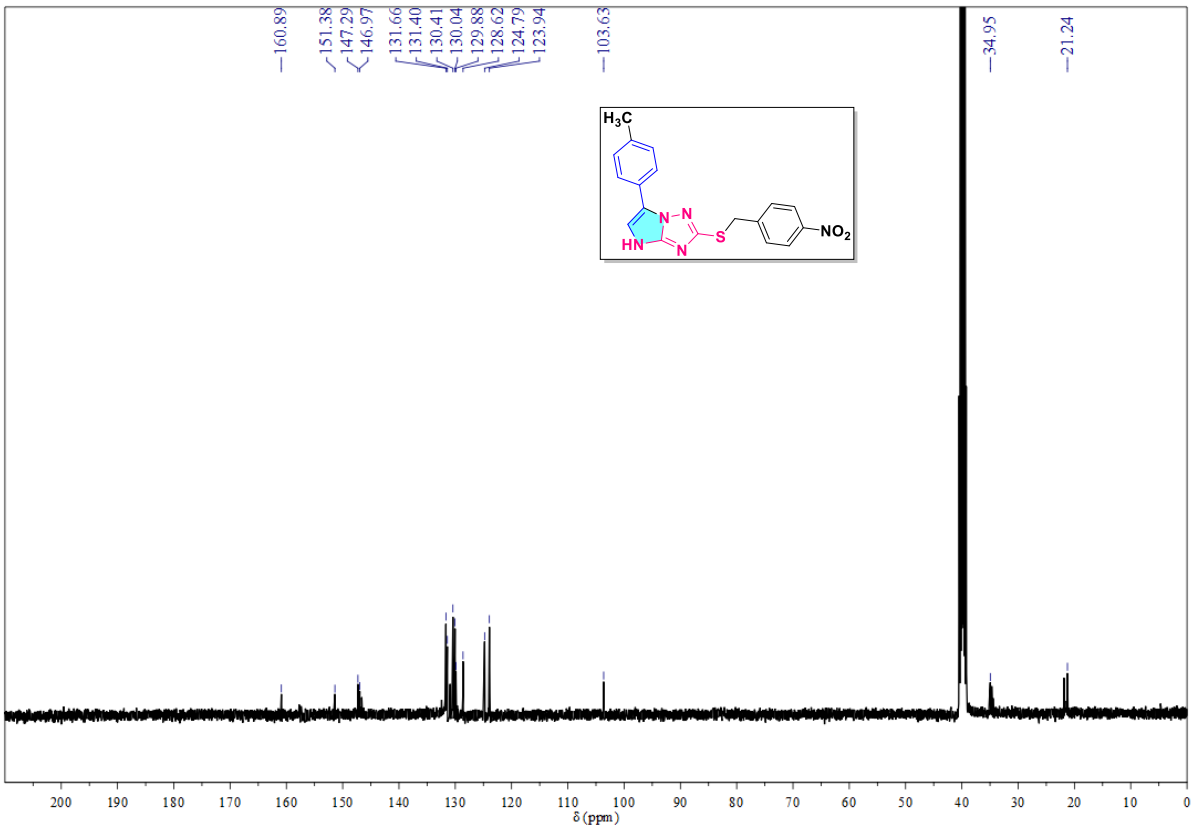
Mass spectrum of compound 4h



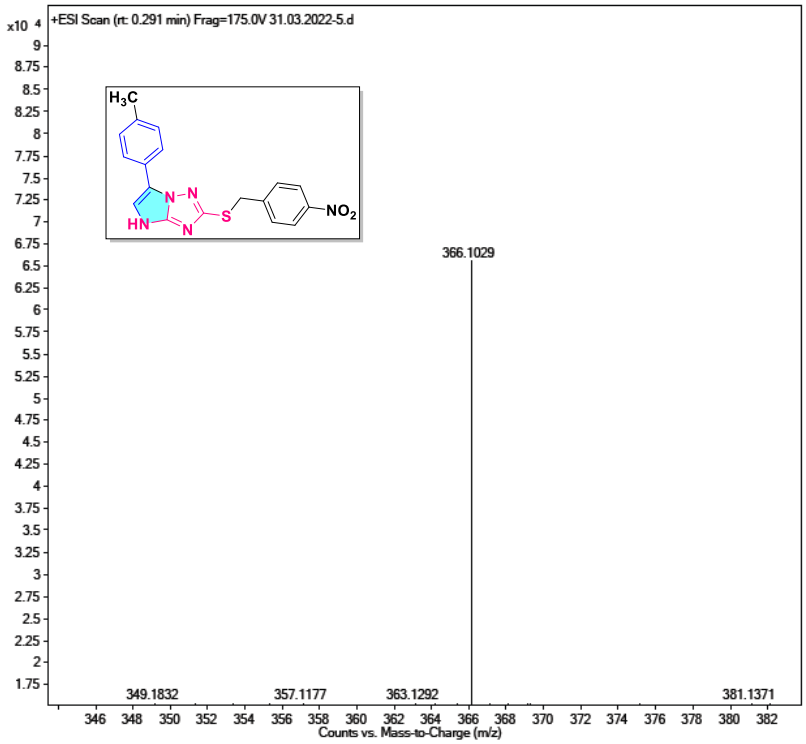
^{1}H NMR spectrum of compound 4i (DMSO-d_6, 400 MHz)



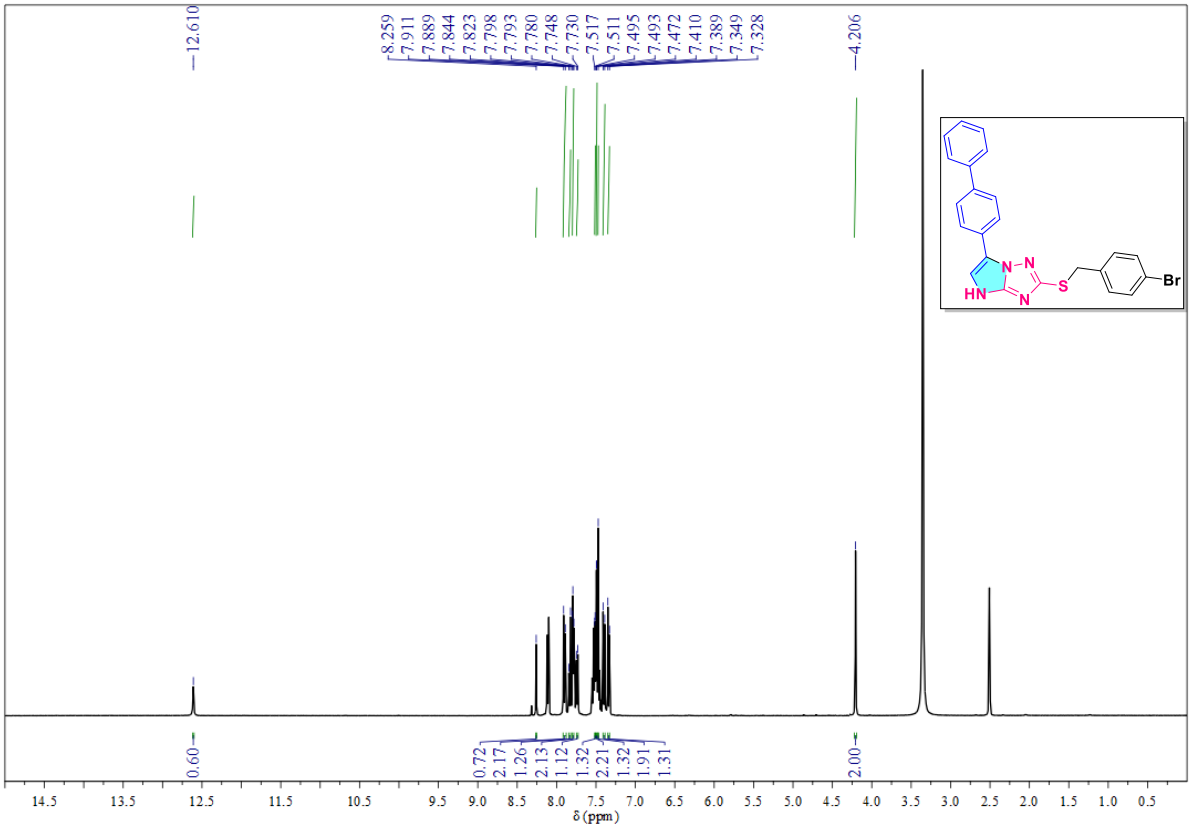
^{13}C NMR spectrum of compound 4i (DMSO-d_6, 100 MHz)



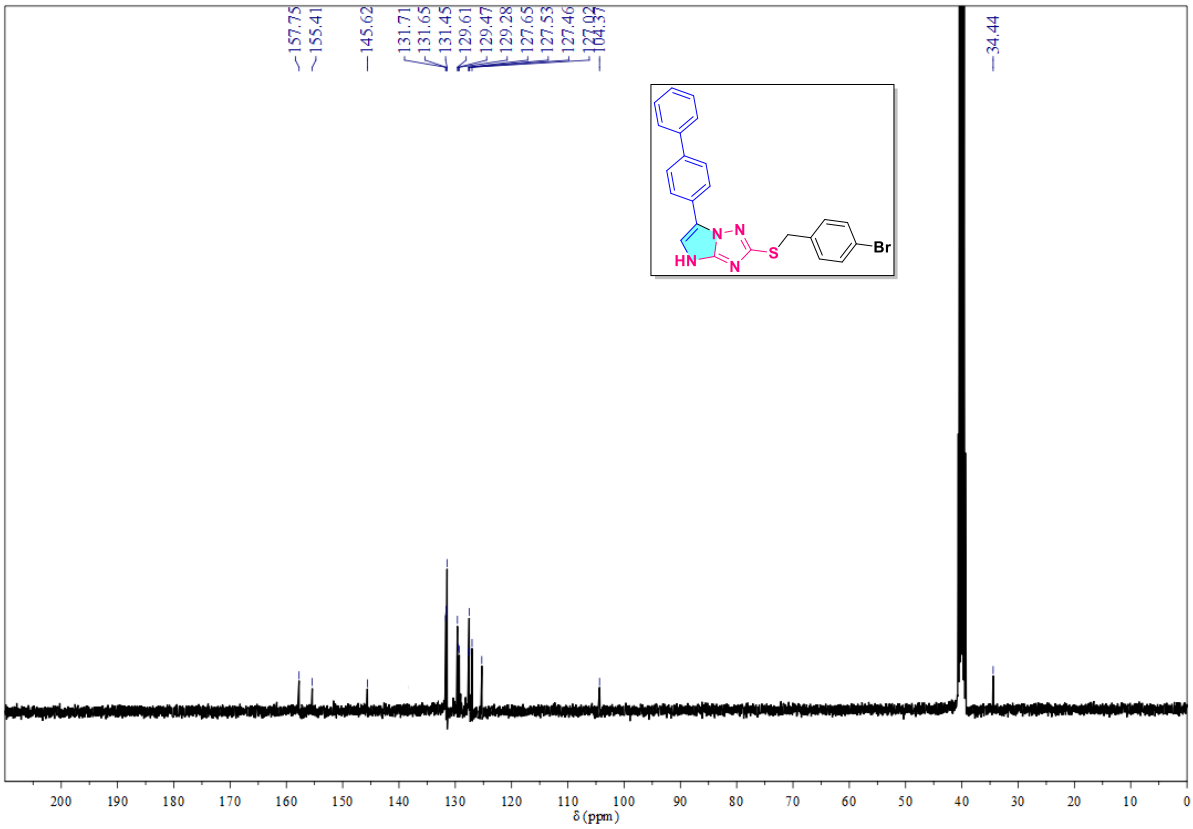
Mass spectrum of compound 4i



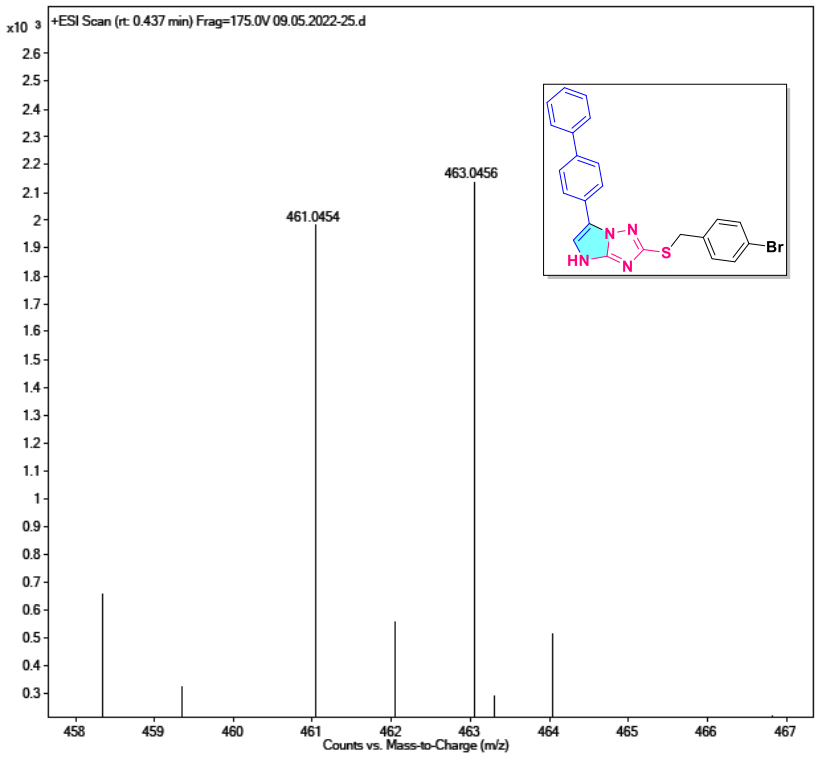
^{1}H NMR spectrum of compound 4j (DMSO-d_6, 400 MHz)



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

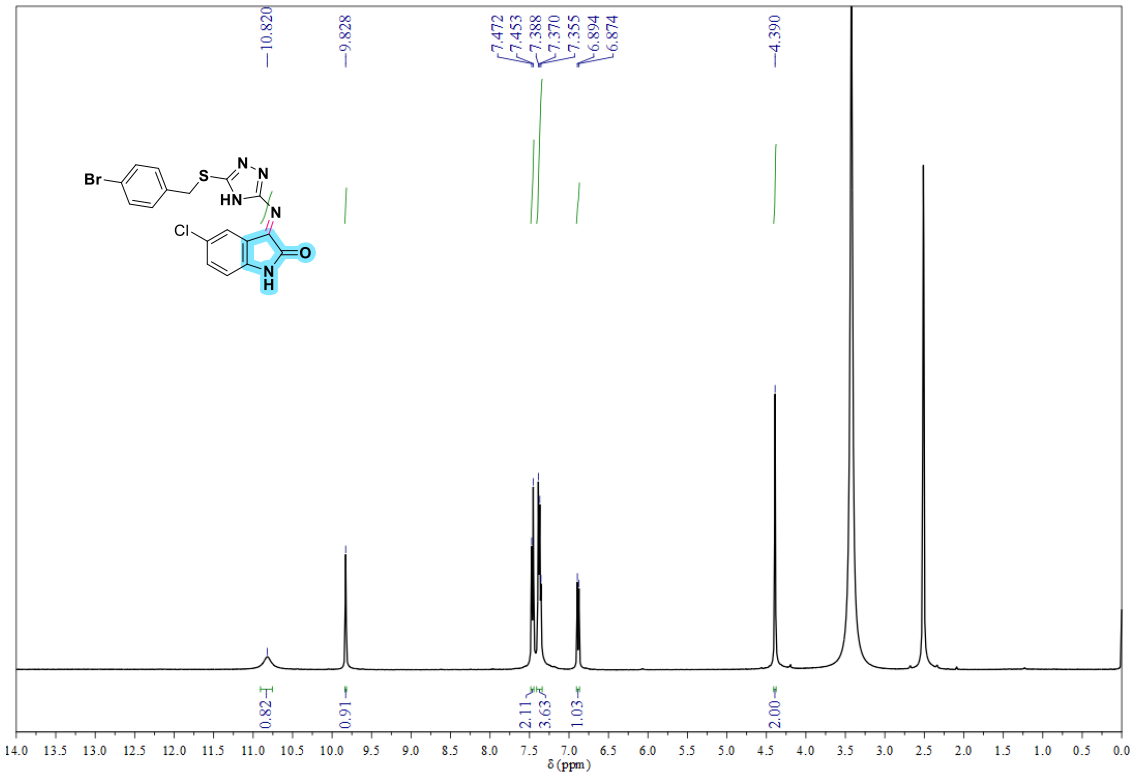


Mass spectrum of compound 4j

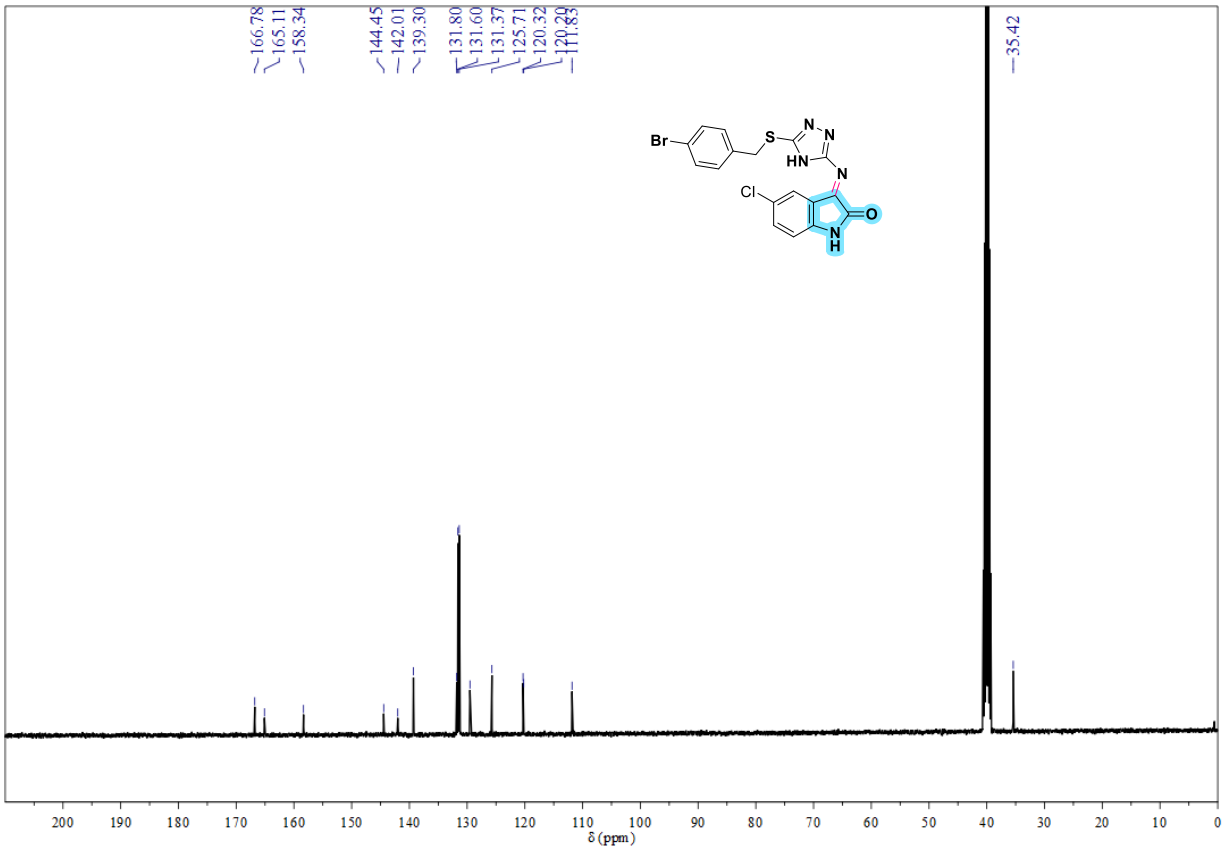


SCHEME-2

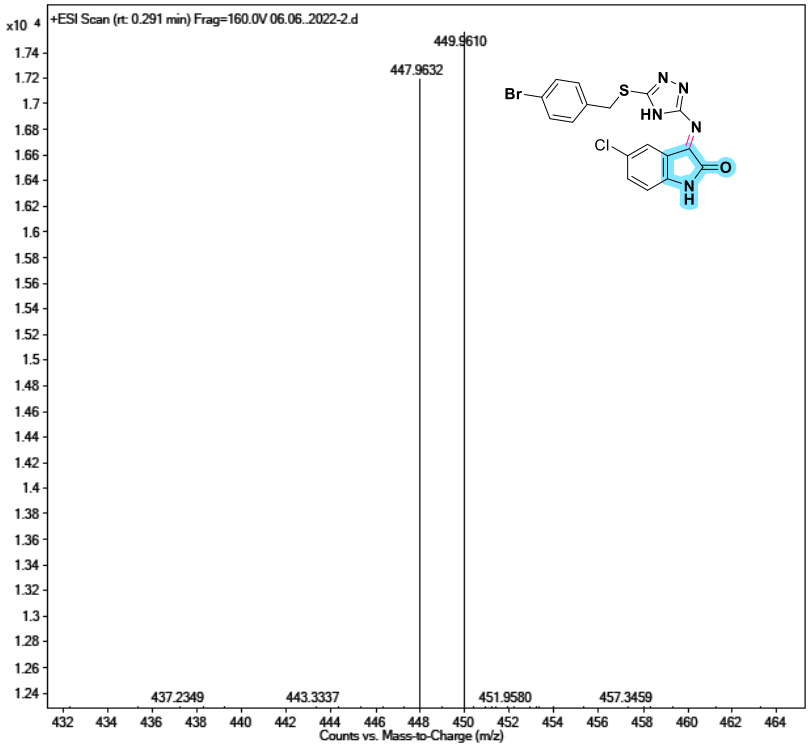
^{1}H NMR spectrum of compound 5a (DMSO-d_6, 400 MHz)



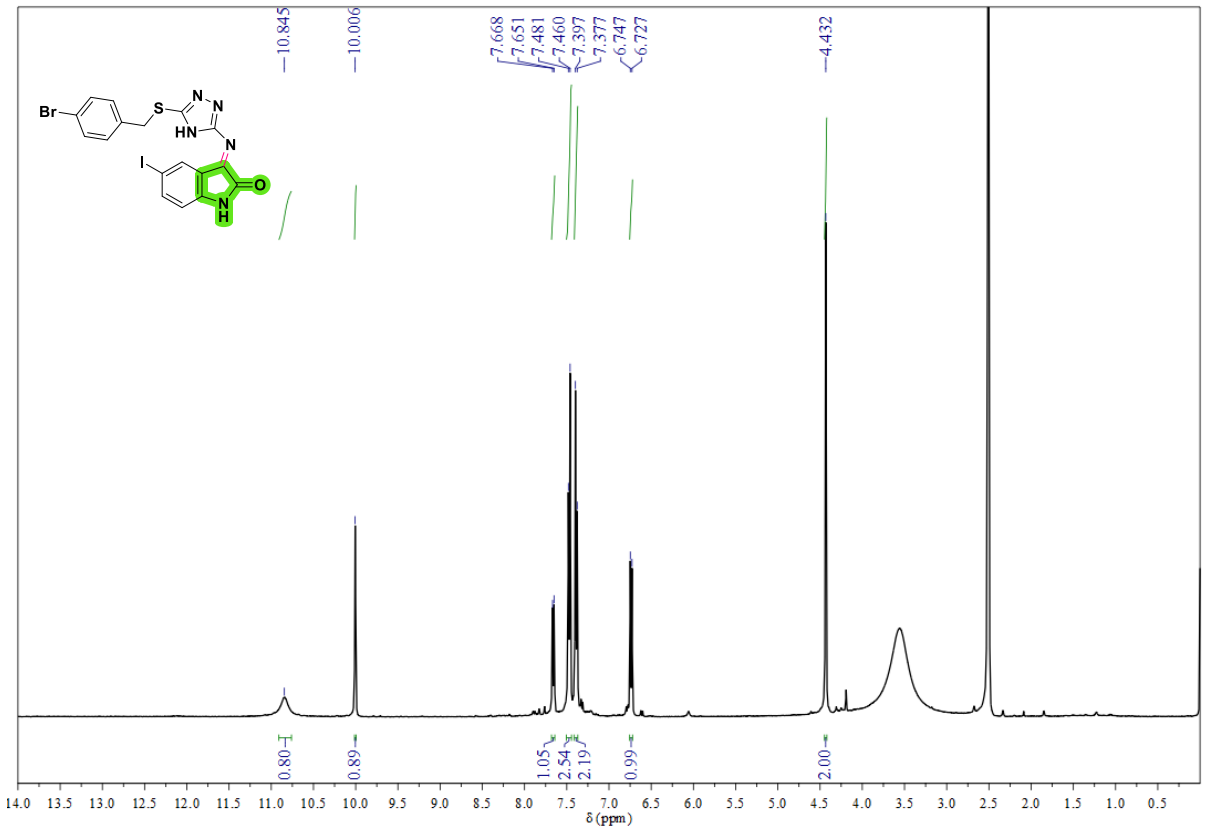
^{13}C NMR spectrum of compound 5a (DMSO-d_6, 100 MHz)



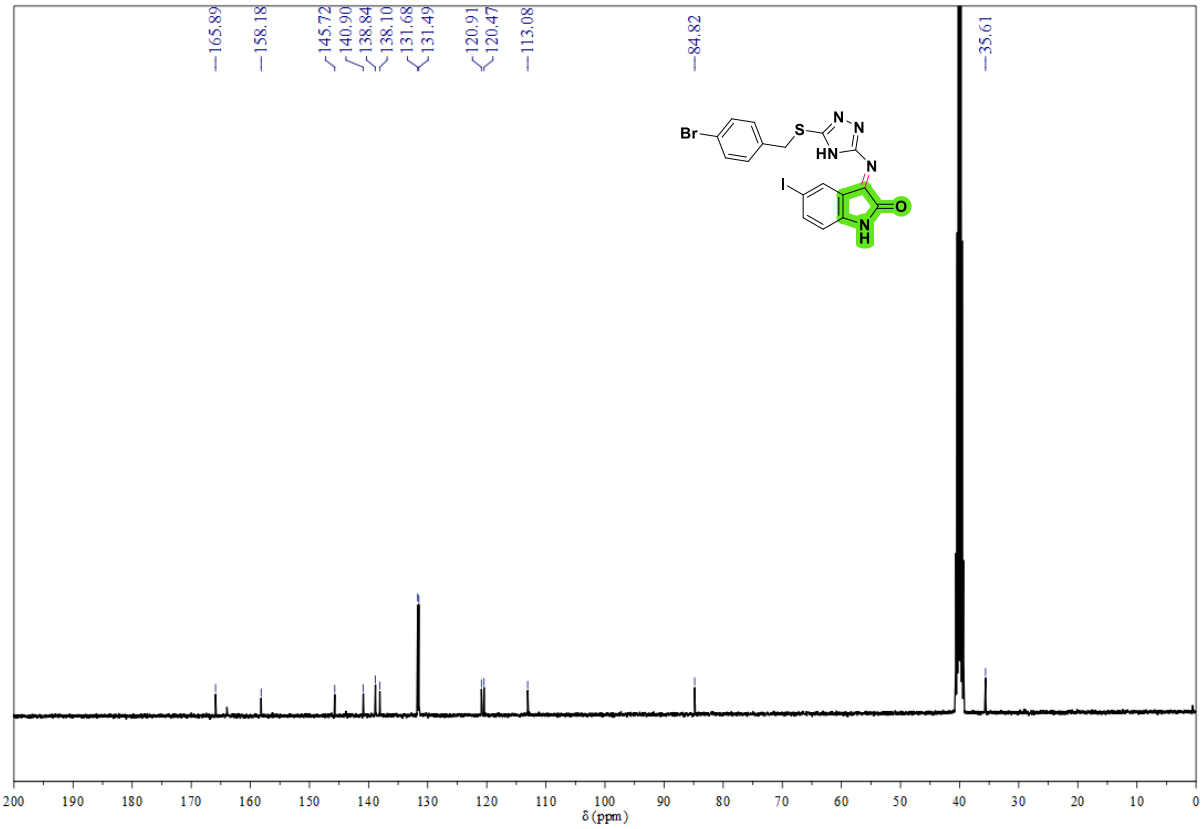
Mass spectrum of compound 5a



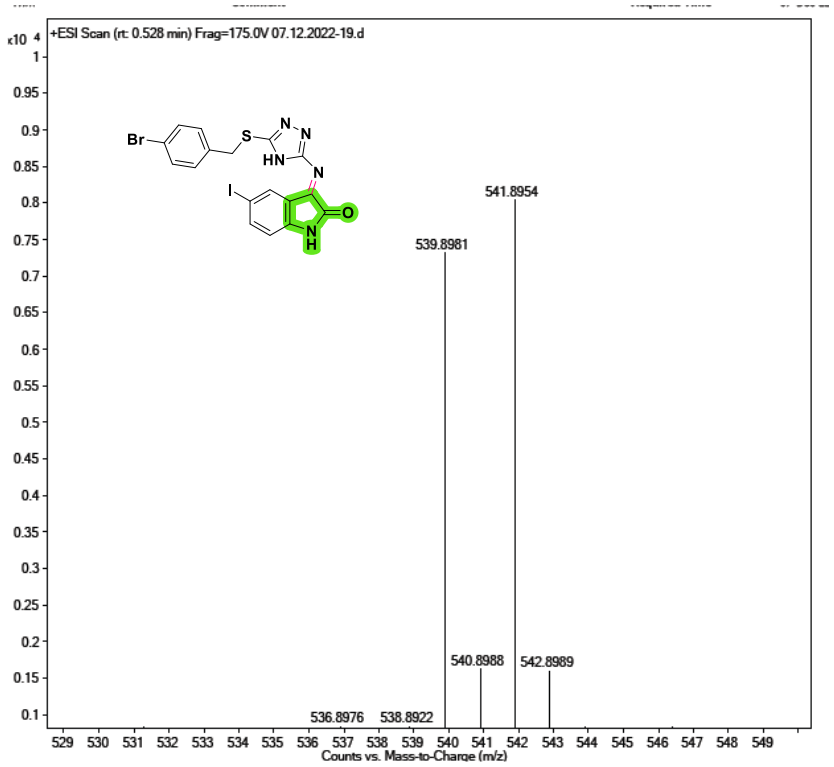
^{1}H NMR spectrum of compound 5b (DMSO-d_6, 400 MHz)



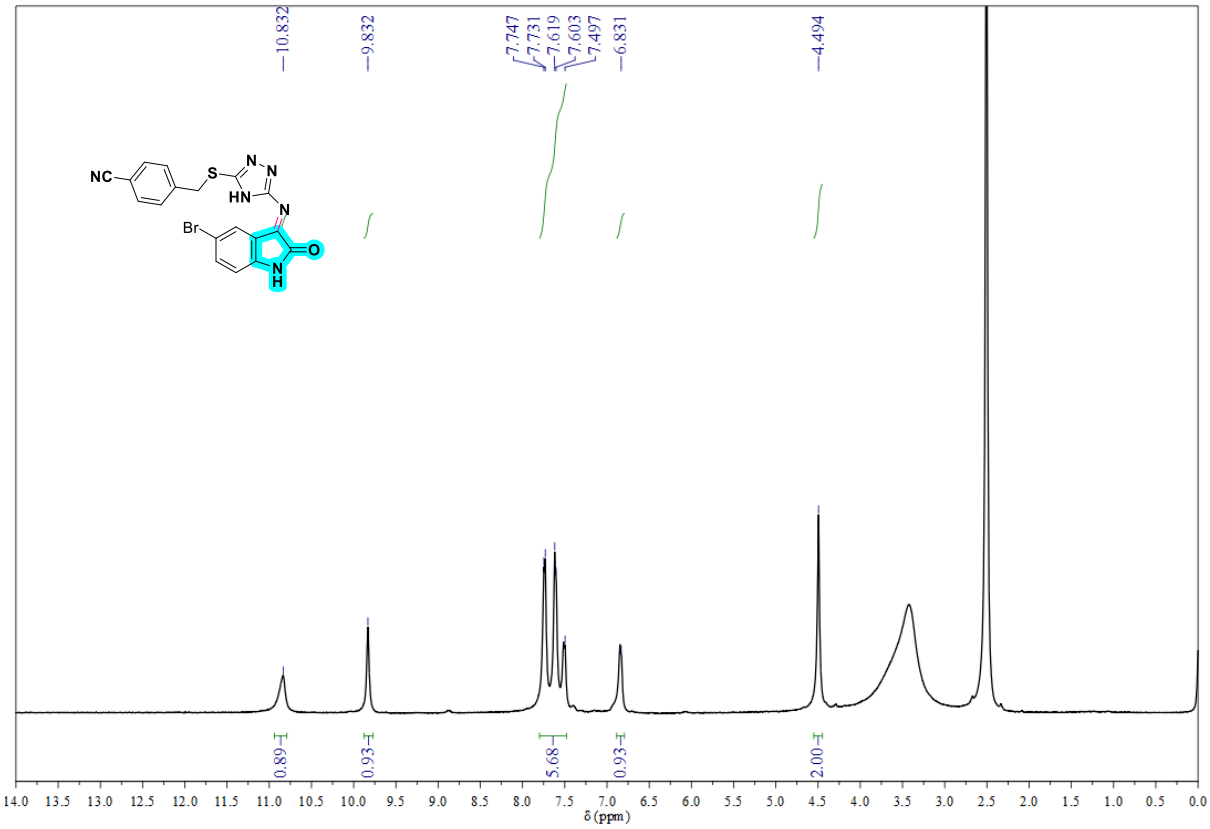
^{13}C NMR spectrum of compound 5b (DMSO-d_6, 100 MHz)



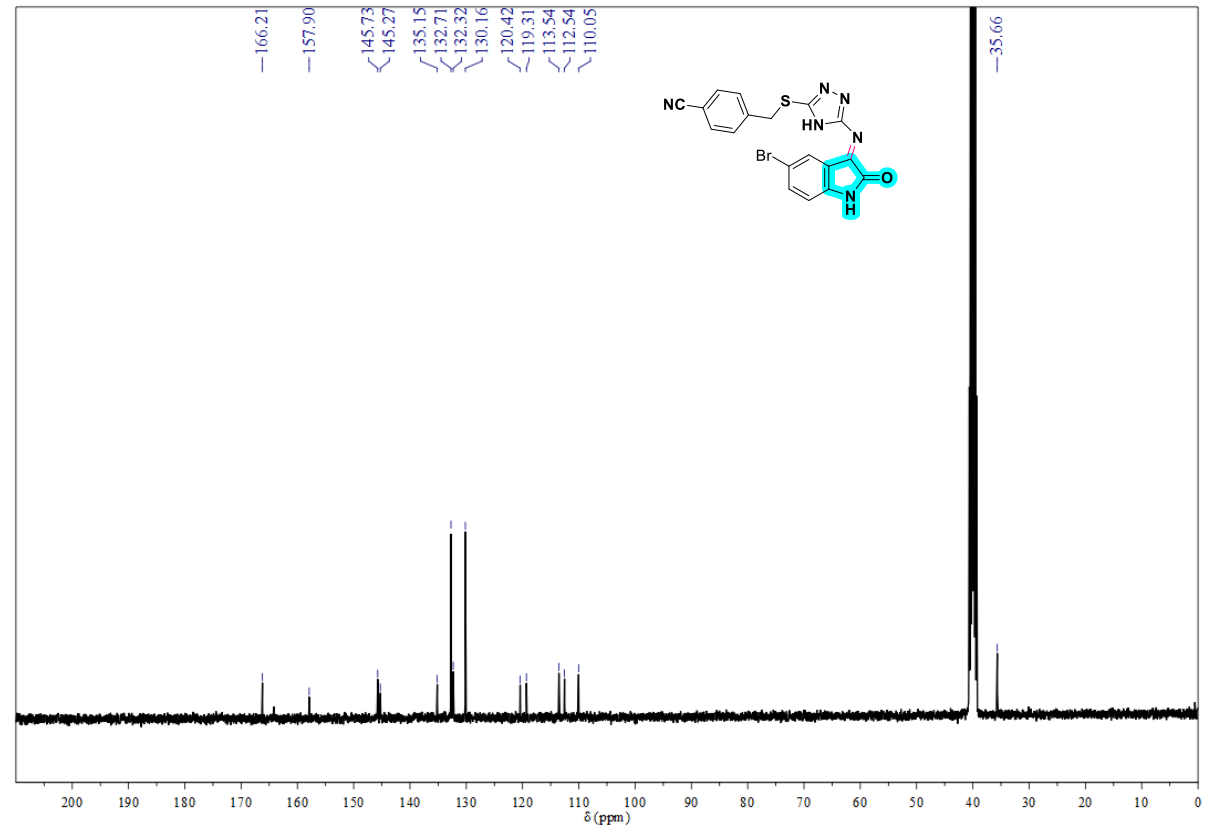
Mass spectrum of compound 5b



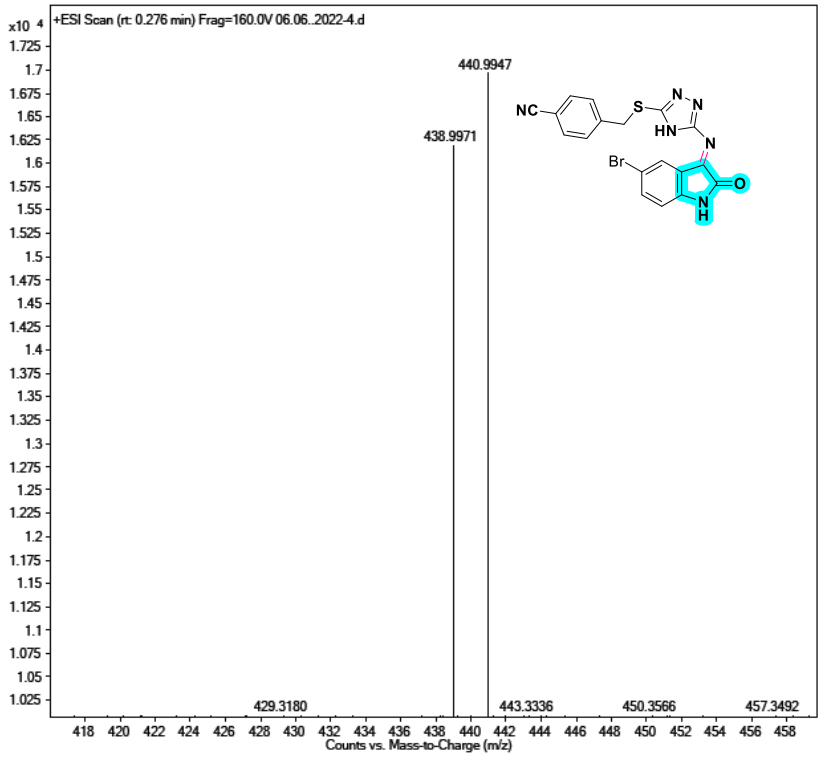
^{1}H NMR spectrum of compound 5c (DMSO-d_6, 400 MHz)



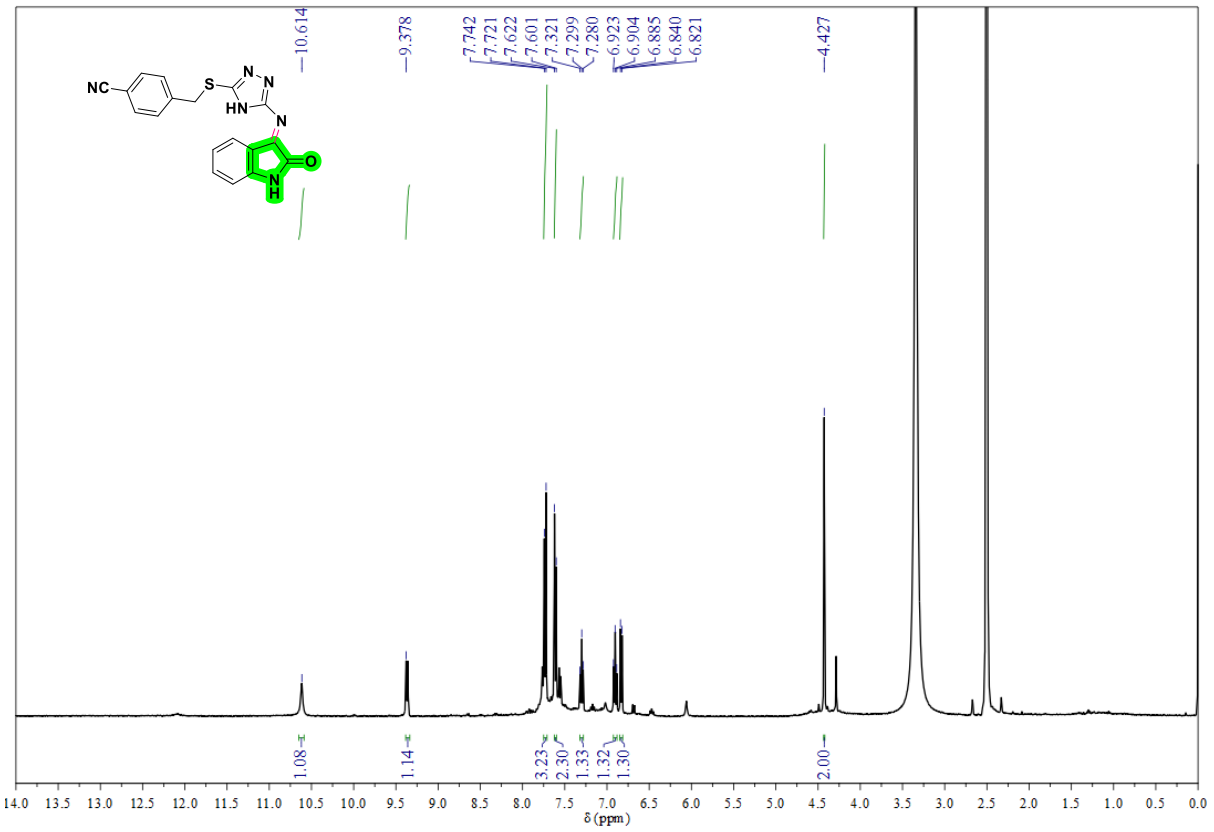
^{13}C NMR spectrum of compound 5c (DMSO-d_6, 100 MHz)



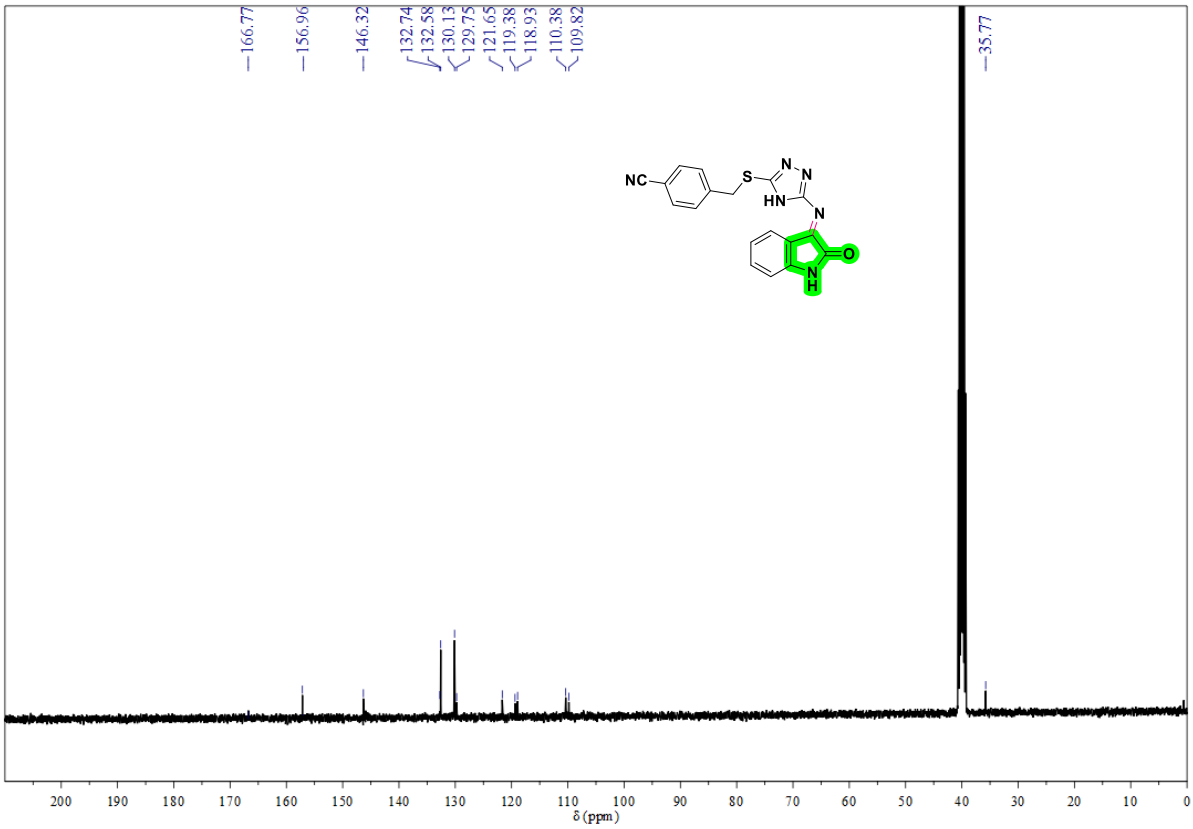
Mass spectrum of compound 5c



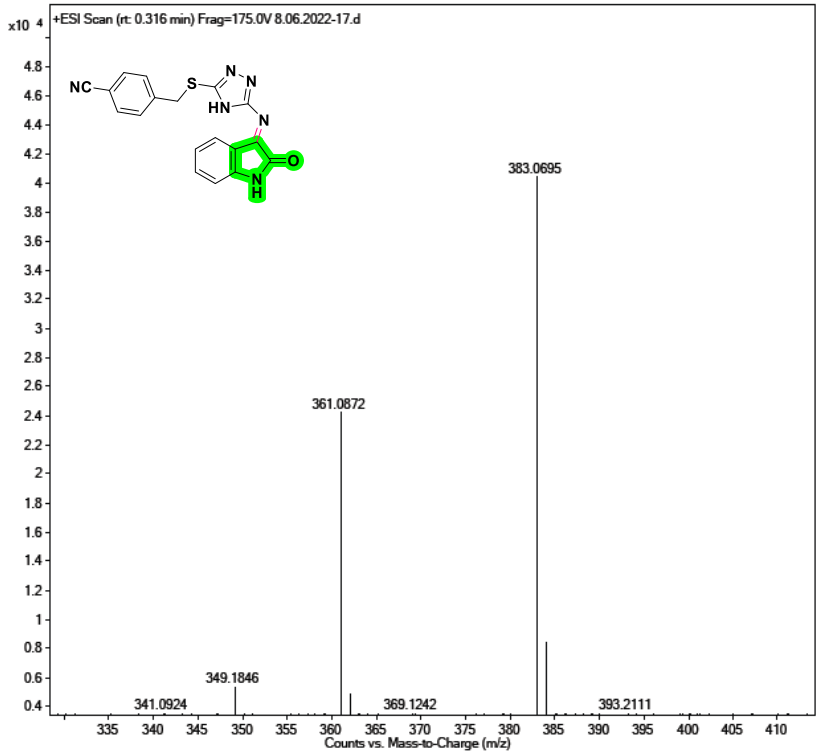
^{1}H NMR spectrum of compound 5d (DMSO-d_6, 400 MHz)



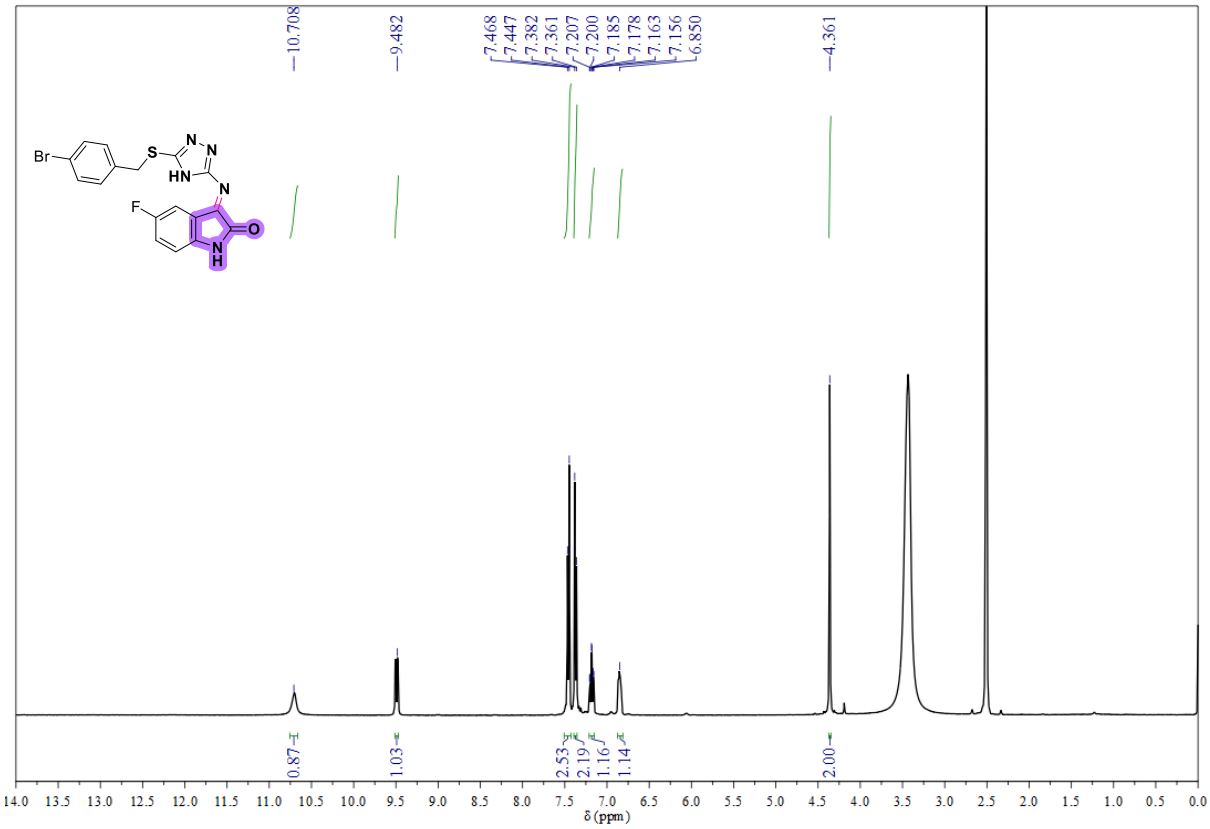
^{13}C NMR spectrum of compound 5d (DMSO-d_6, 100 MHz)



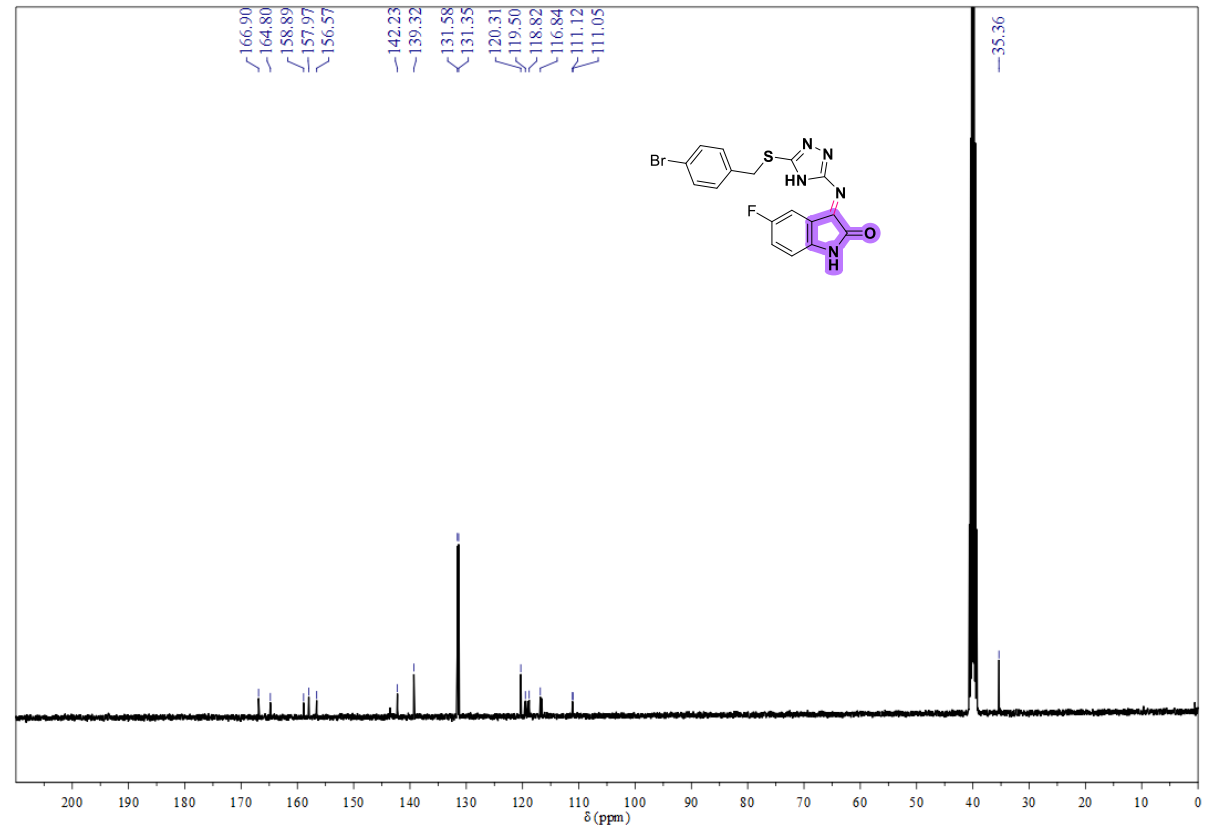
Mass spectrum of compound 5d



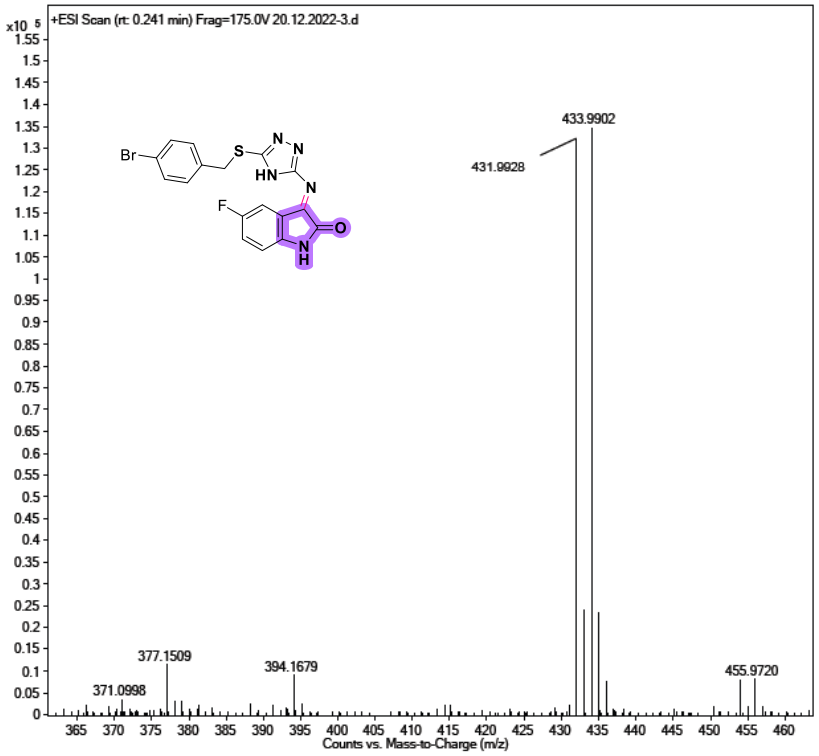
^{1}H NMR spectrum of compound 5e (DMSO-d_6, 400 MHz)



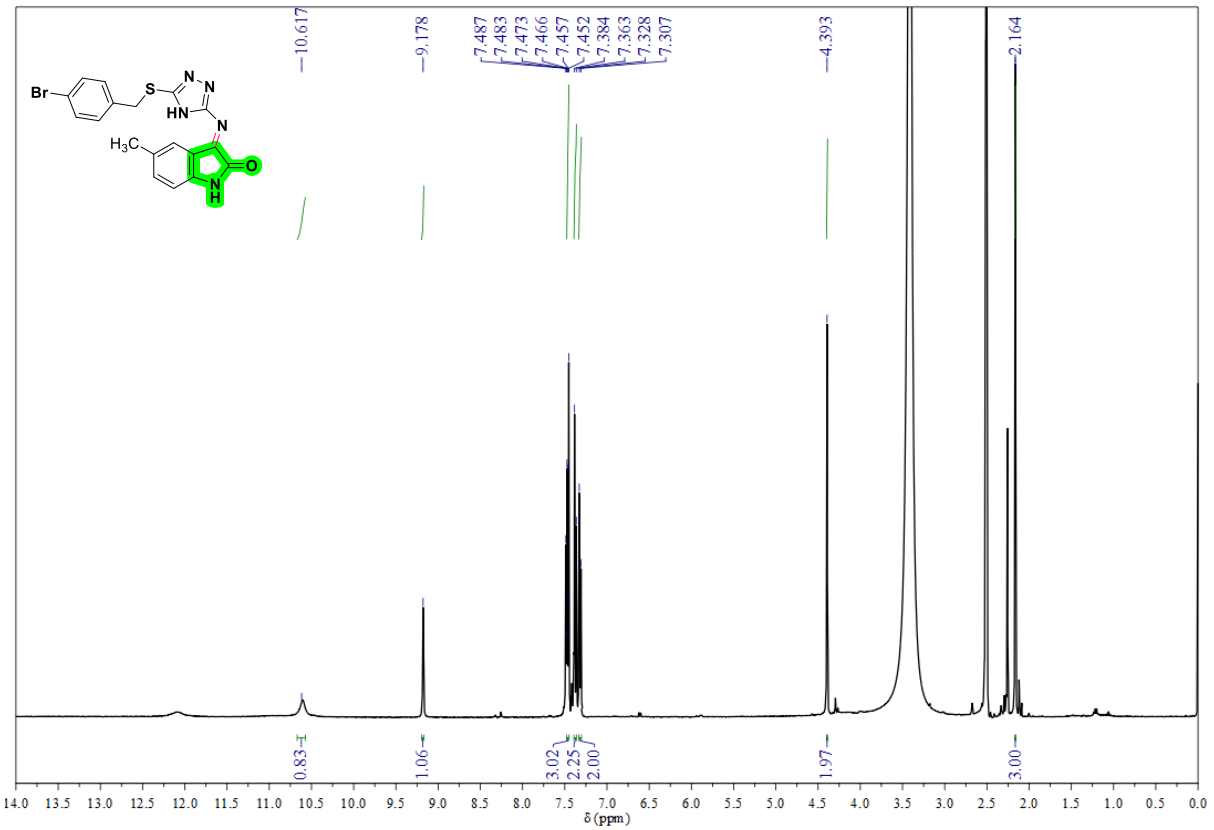
^{13}C NMR spectrum of compound 5e (DMSO-d_6, 100 MHz)



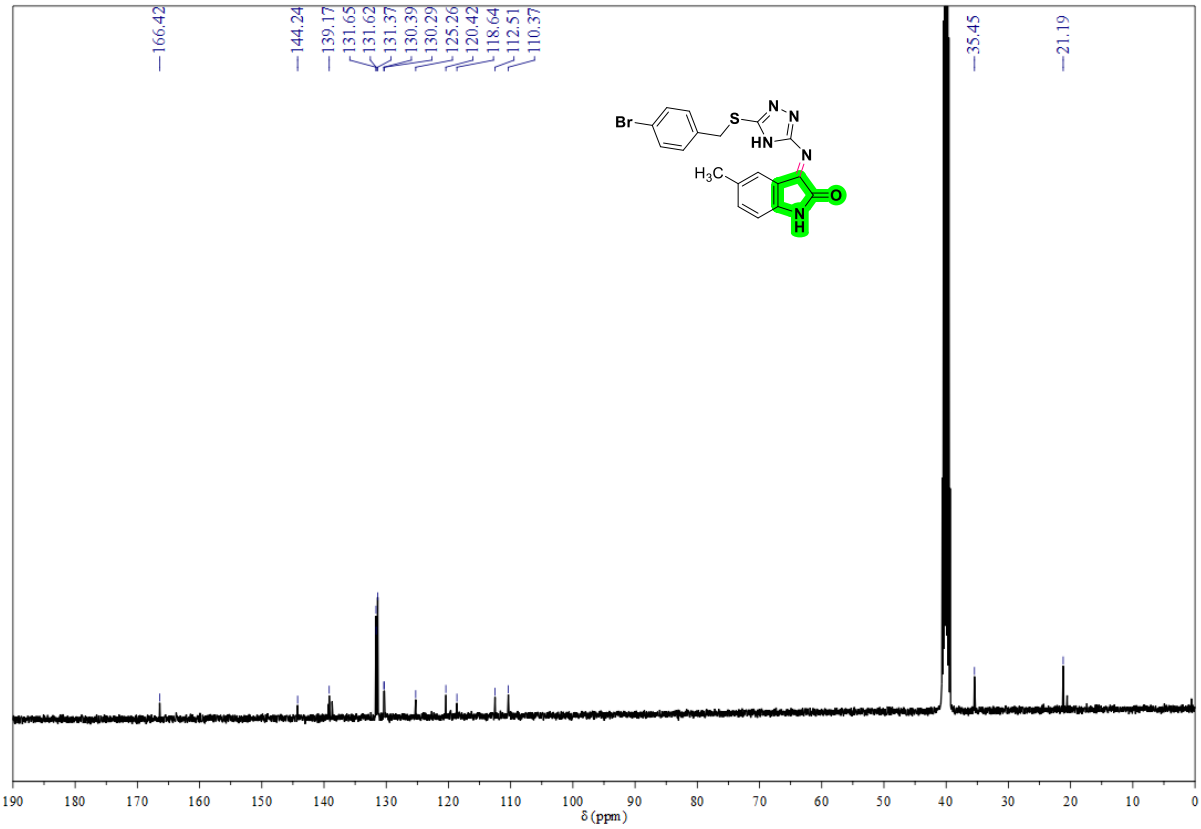
Mass spectrum of compound 5e



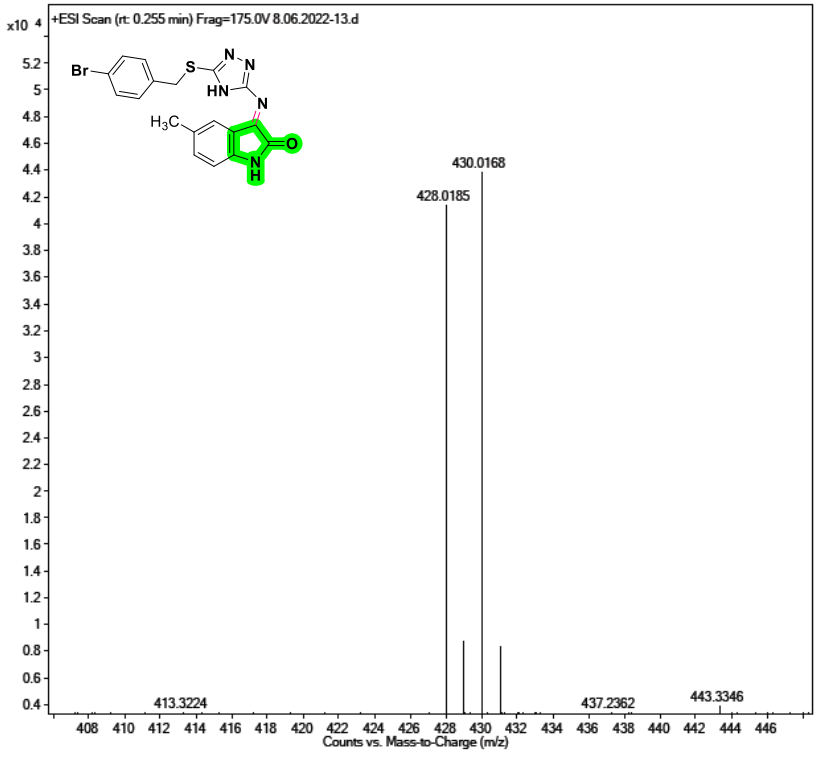
^{1}H NMR spectrum of compound 5f (DMSO-d_6, 400 MHz)



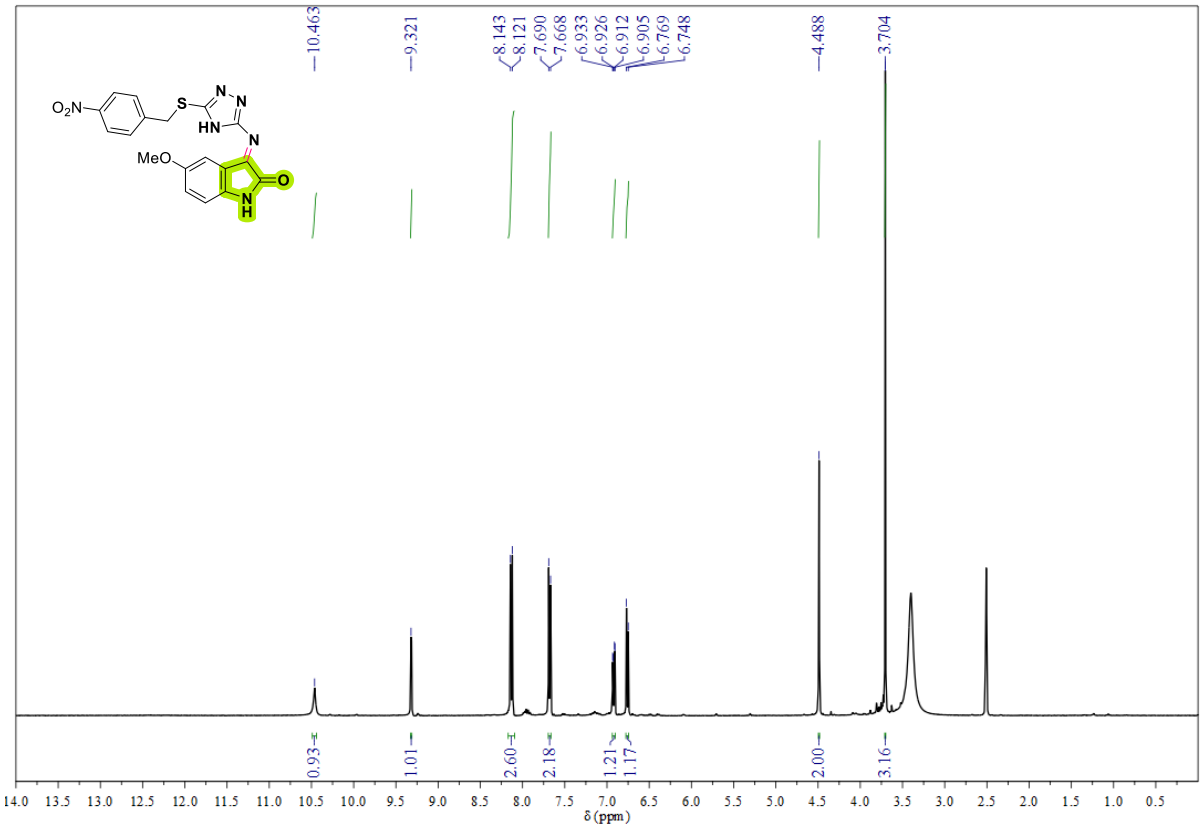
^{13}C NMR spectrum of compound 5f (DMSO-d_6, 100 MHz)



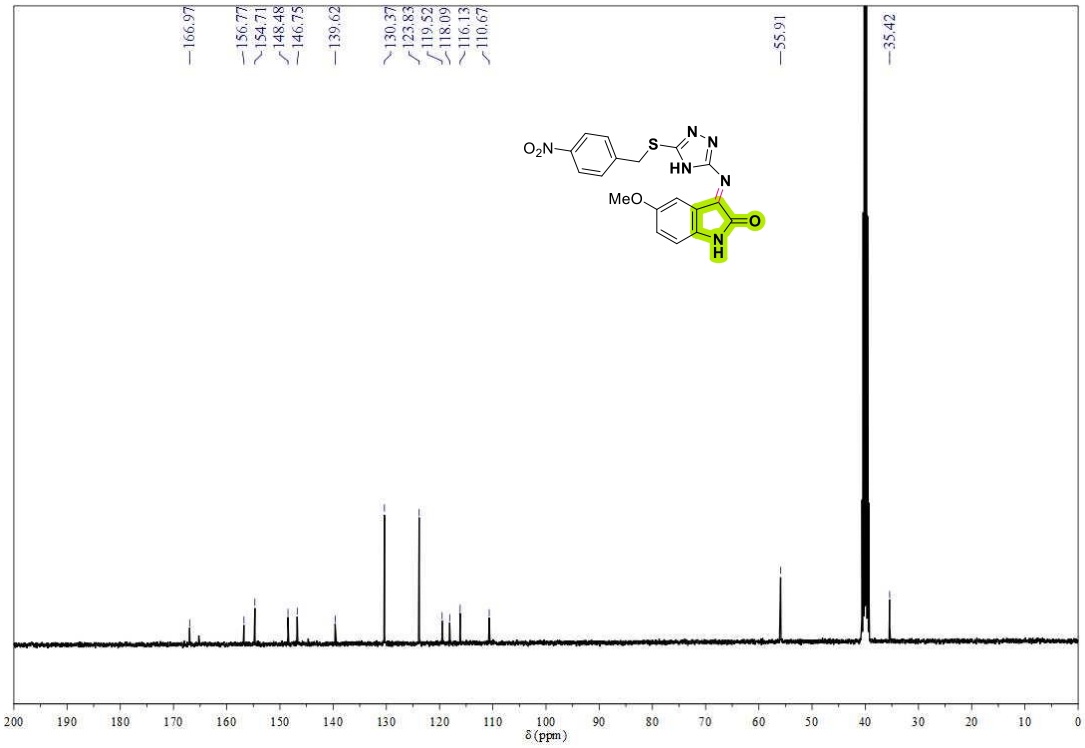
Mass spectrum of compound 5f



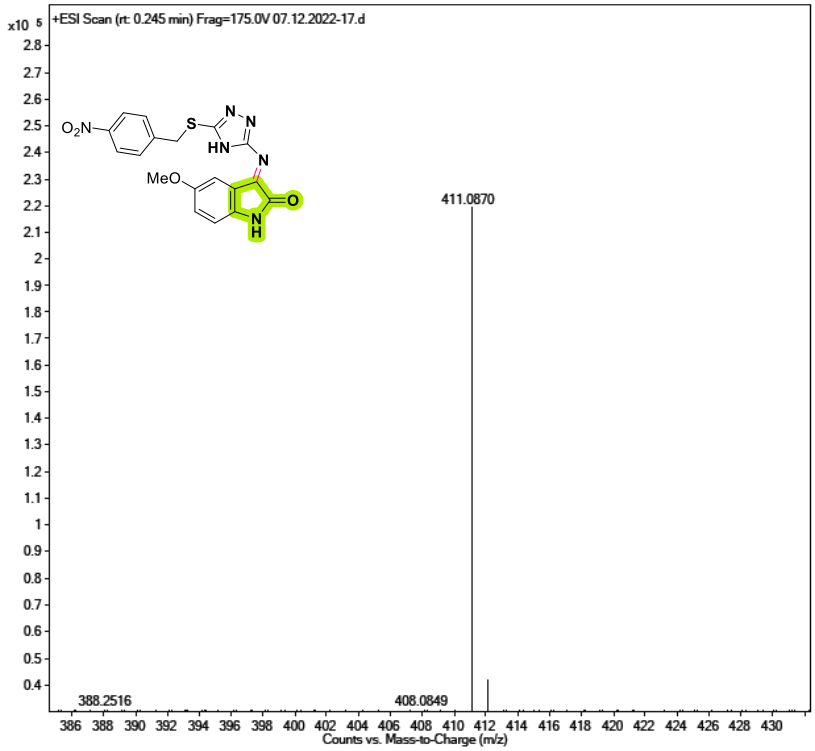
^{1}H NMR spectrum of compound 5g (DMSO-d_6, 400 MHz)



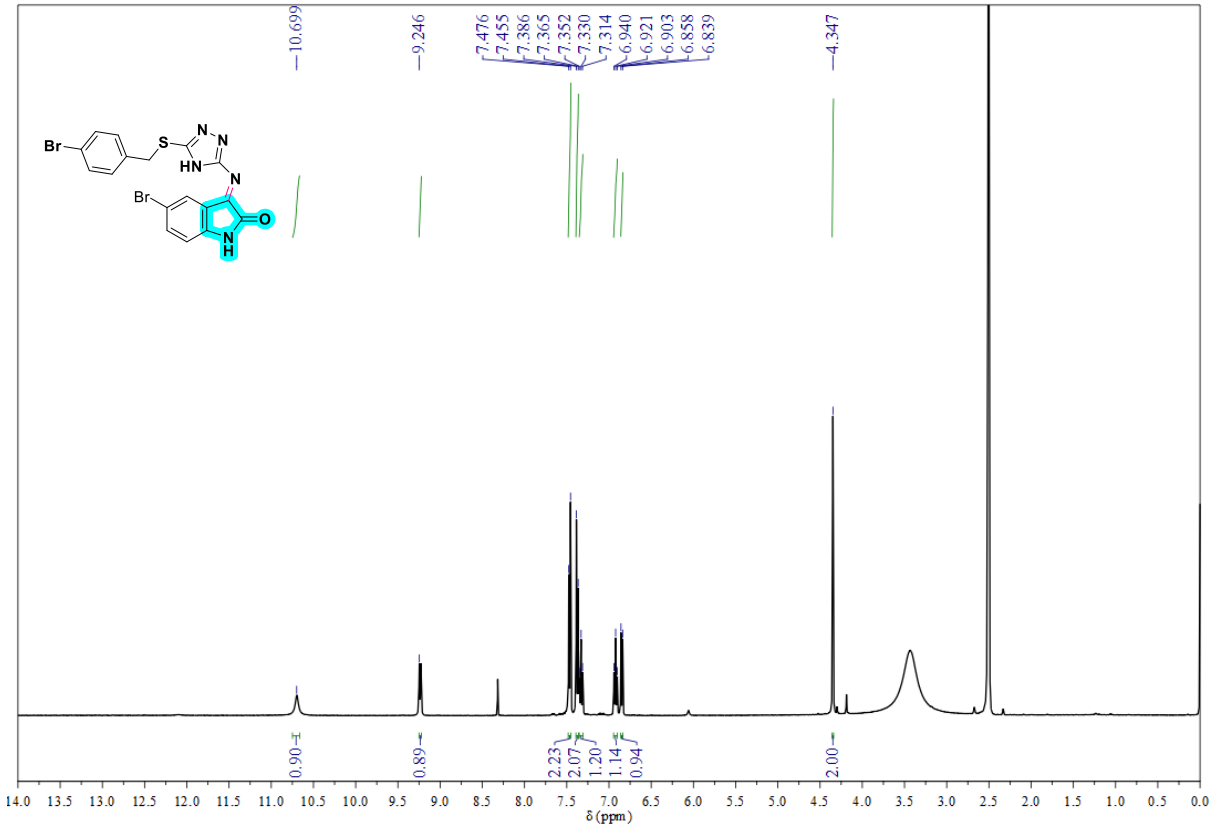
^{13}C NMR spectrum of compound 5g (DMSO-d_6, 100 MHz)



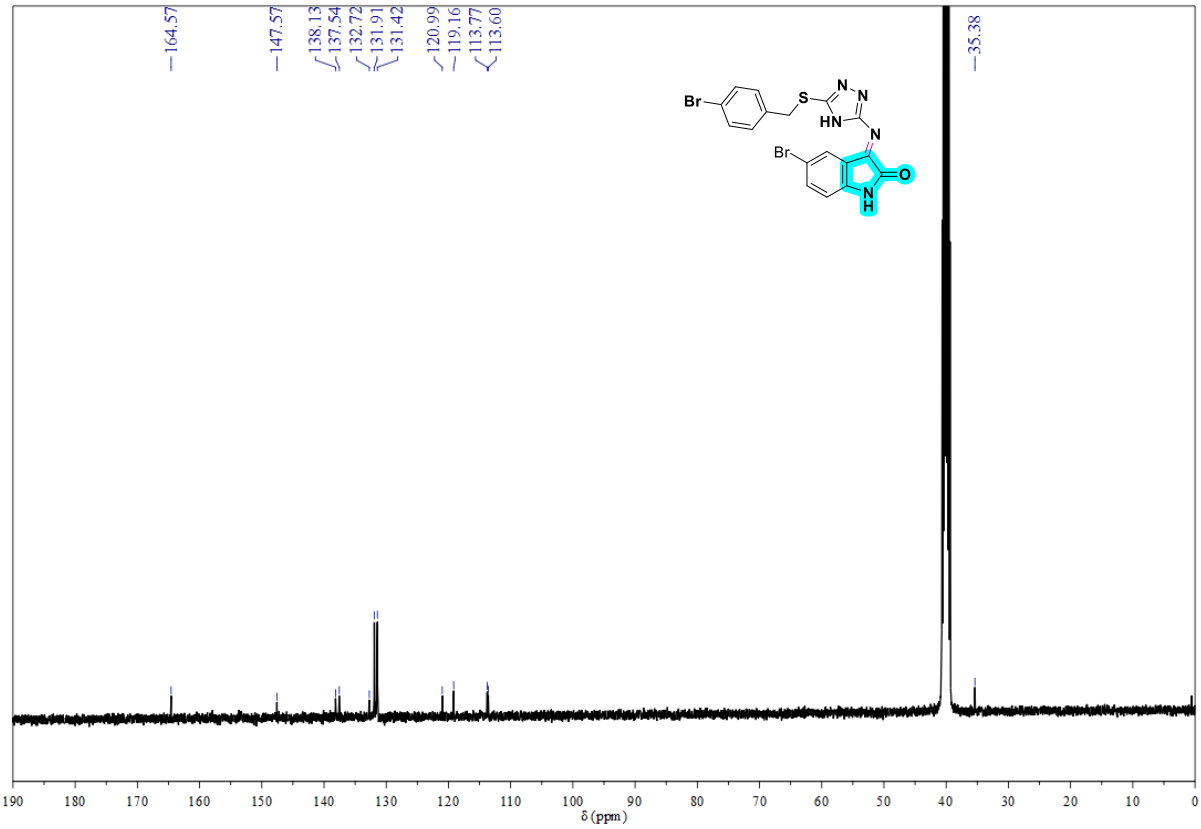
Mass spectrum of compound 5g



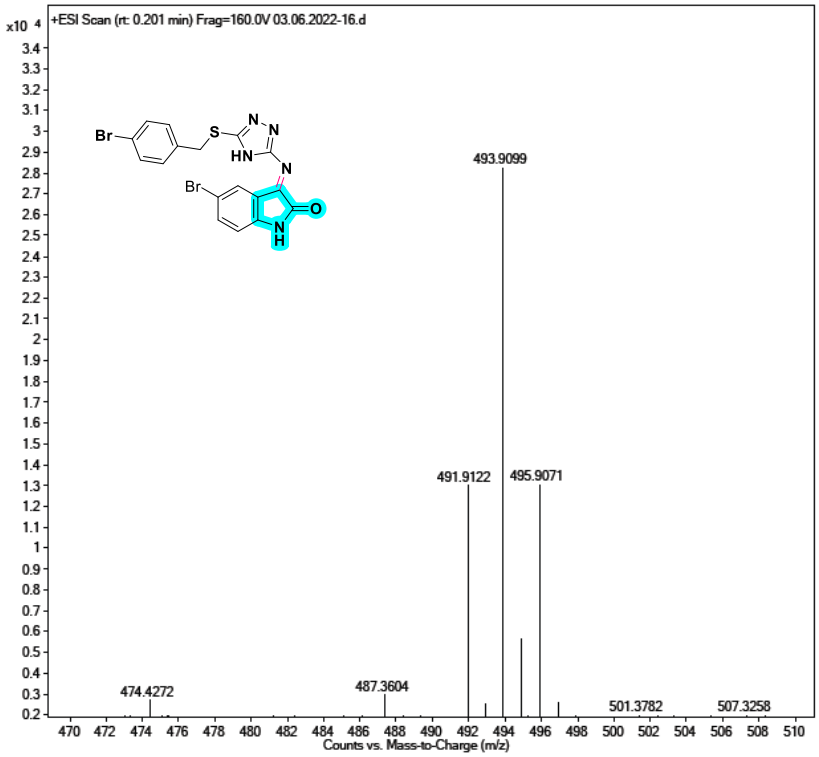
^{1}H NMR spectrum of compound 5h (DMSO-d_6, 400 MHz)



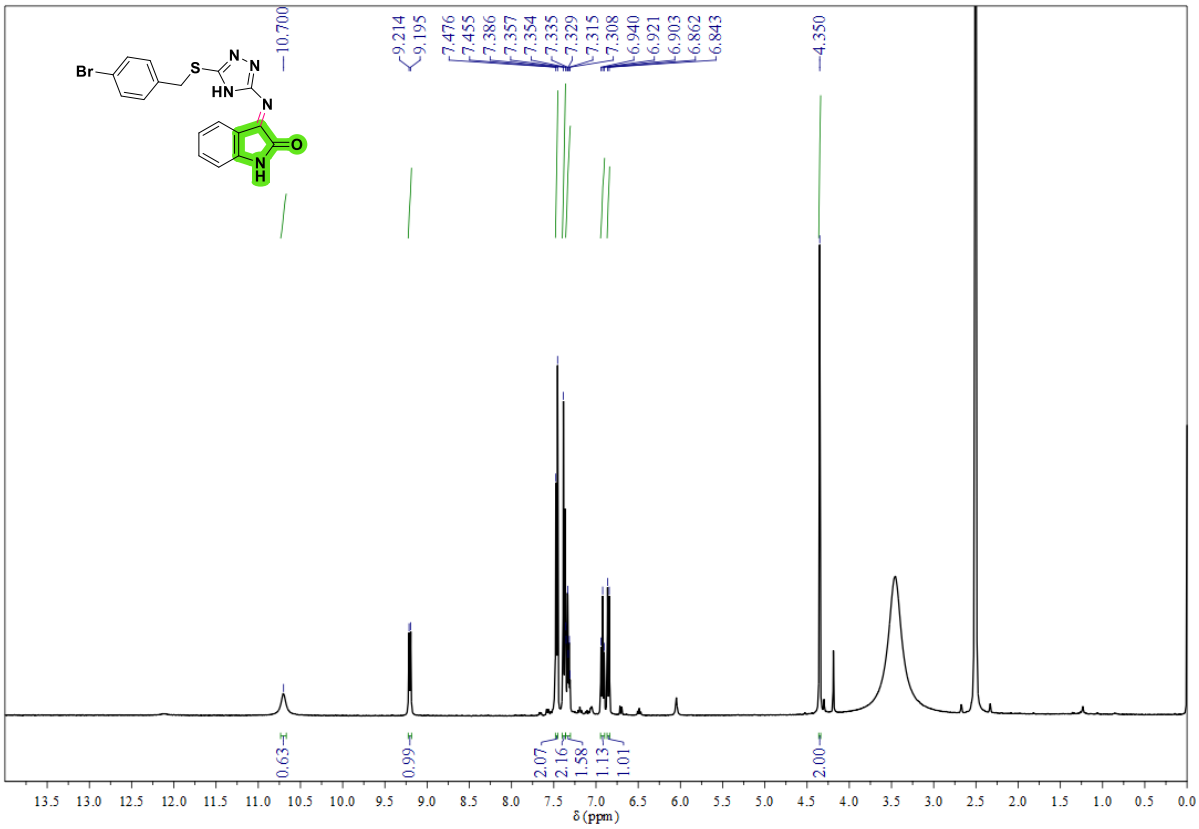
^{13}C NMR spectrum of compound 5h (DMSO-d_6, 100 MHz)



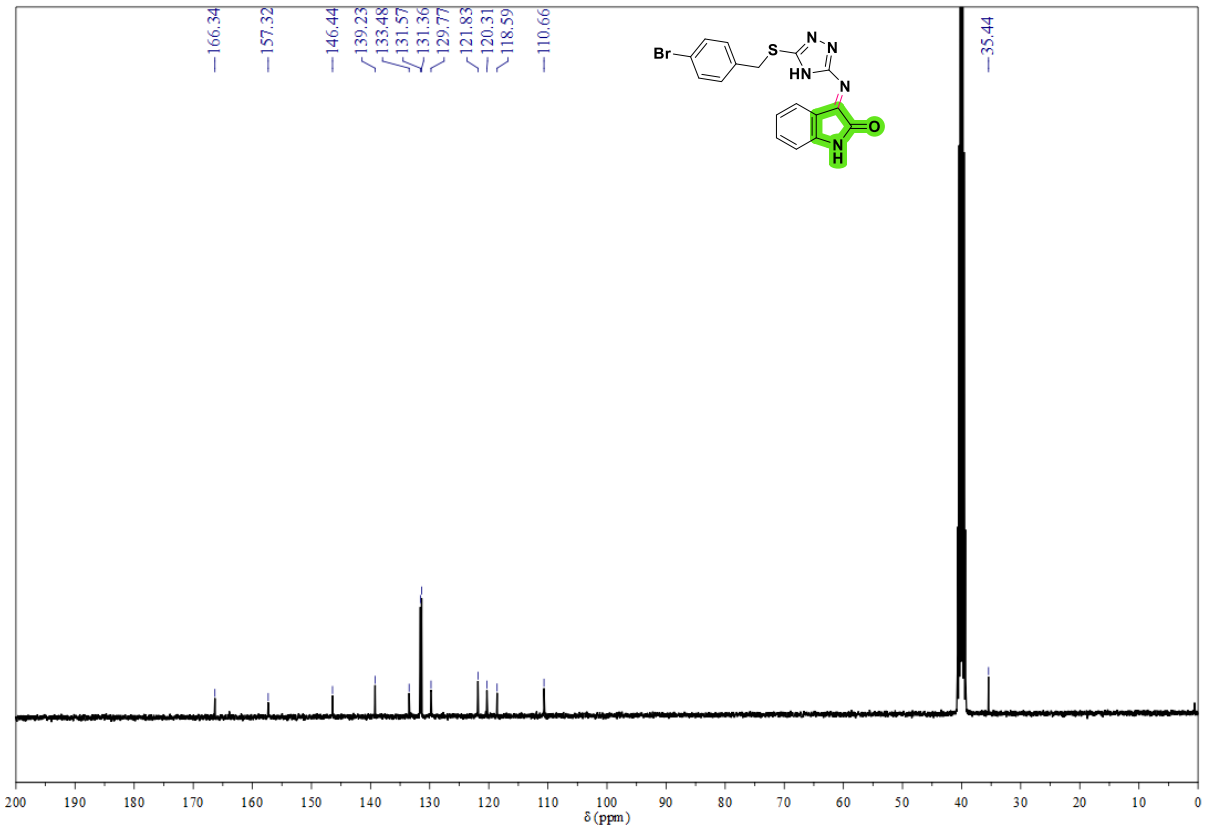
Mass spectrum of compound 5h



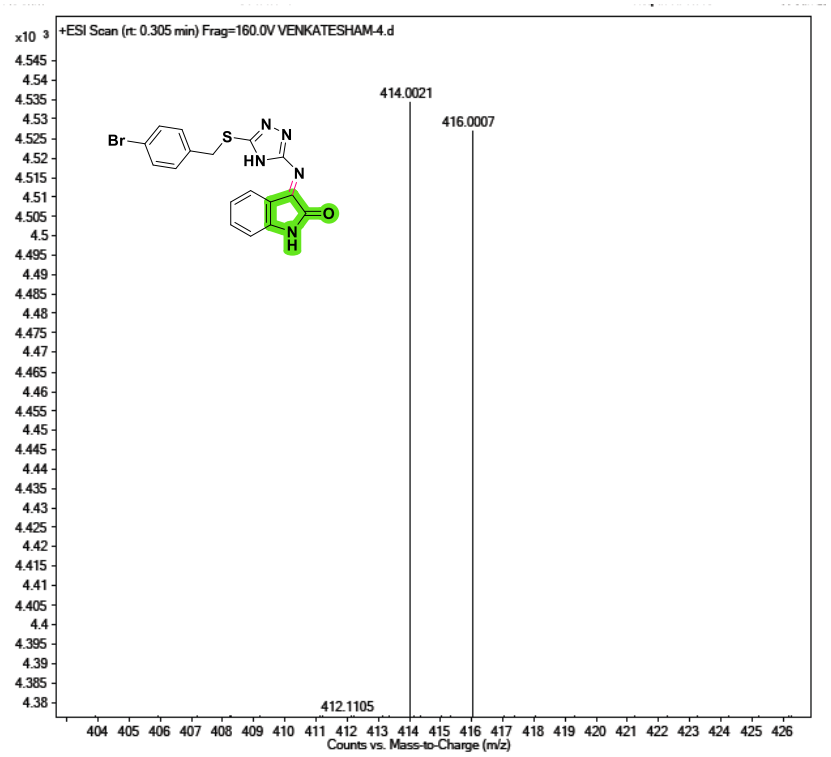
^{1}H NMR spectrum of compound 5i (DMSO-d_6, 400 MHz)



^{13}C NMR spectrum of compound 5i (DMSO-d_6, 100 MHz)



Mass spectrum of compound 5i



7.7. References.

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SUMMARY

CHAPTER-I

A review on Multicomponent Reactions and its Applications in the Synthesis of Biologically Active Heterocyclic Compounds

This chapter deals with the introduction to multicomponent reactions. These type of reactions are known as a group of chemical transformations by the rapid assembly of polysubstituted systems which occur without isolation of undesirable intermediates and most of the atoms participate in the newly formed bonds that lead to generate target compound with high percentage of the yield in a single reaction step and shorter reaction time [1-5]. These reactions are useful for the development of C-C, C-N, C-S bonds through single step chemical transformation [6], synthesis of various six membered and five membered heterocyclic compounds such as thiazoles, triazoles, pyrimidines, pyridines, pyrazoles *via* 3CC, 4CC, 5CC reactions and their medicinal applications in pharmaceutical industries [7-9]. And also chapter-1 discuss about different types of methods that are involving in multicomponent reactions such as solid phase MCRs, water medium MCRs[10], ultrasonic assisted MCRs, microwave assisted MCRs, and other types of multicomponent reactions, in the synthesis of natural products Moreover, this chapter also describes the brief introduction of 4*H*-1,2,4-triazole heterocyclic molecules for the synthesis of fused/unfused five and six membered N, S heterocyclic moieties which are relevant for the synthesis of pharmacologically active drugs [11-13].

The main aims and objectives of the research work

- To develop operationally easy and efficient and straight forward protocol for the production of bicyclic heterocyclics, isoindolines and schiff bases.

The aforementioned importance of triazoles here in we synthesized various fused/unfused 4*H*-1,2,4-triazole based heterocyclic compounds, in the part of this study thioalkylated triazolo isoindolines (phthalimides), fused [3,2-*b*] [1,2,4]-triazolothiazole isoindolines and schiff bases, triazolothiazoles, [1,2,4]-triazolo[1,5-*a*]pyrimidines, [1,2,4]-triazolo[1,5-*a*]pyrimidinones and their sulfones, [1,2,4]-triazolo[3,4-*b*[1,3,4]thiadiazoles, bicyclic 1,2,4-triazoloimidazoles, imines, schiff bases by using multicomponent reactions.

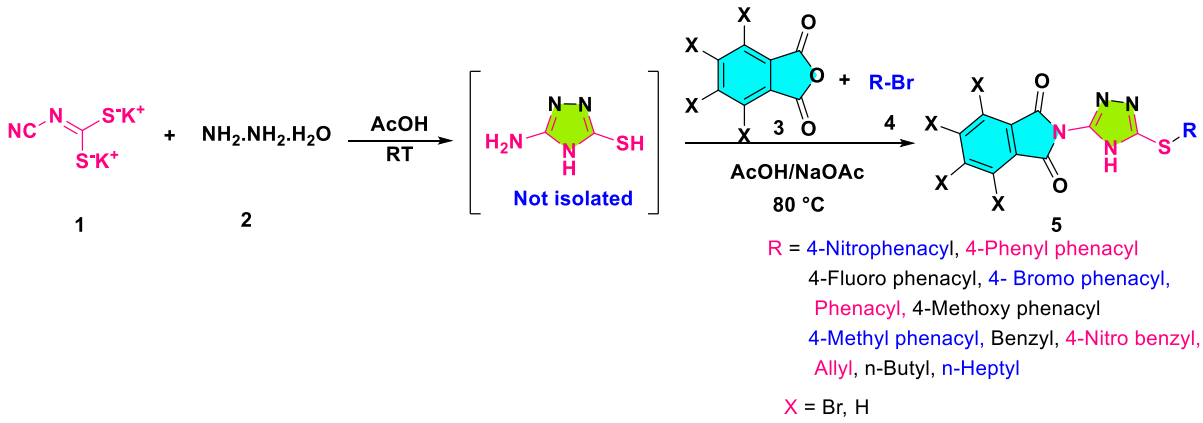
The required starting materials like 5-amino-4*H*-1,2,4-triazole-3-thiol[14], 3-acetyl coumarins[15], 3(2-bromo acetyl) coumarins[16] and 4-amino-5-hydrazineyl-4*H*-1,2,4-triazole-3-thiol[17] were synthesized by using literature methods. The objectives of the present work are mentioned and outline of the work carried out in the present investigation are presented.

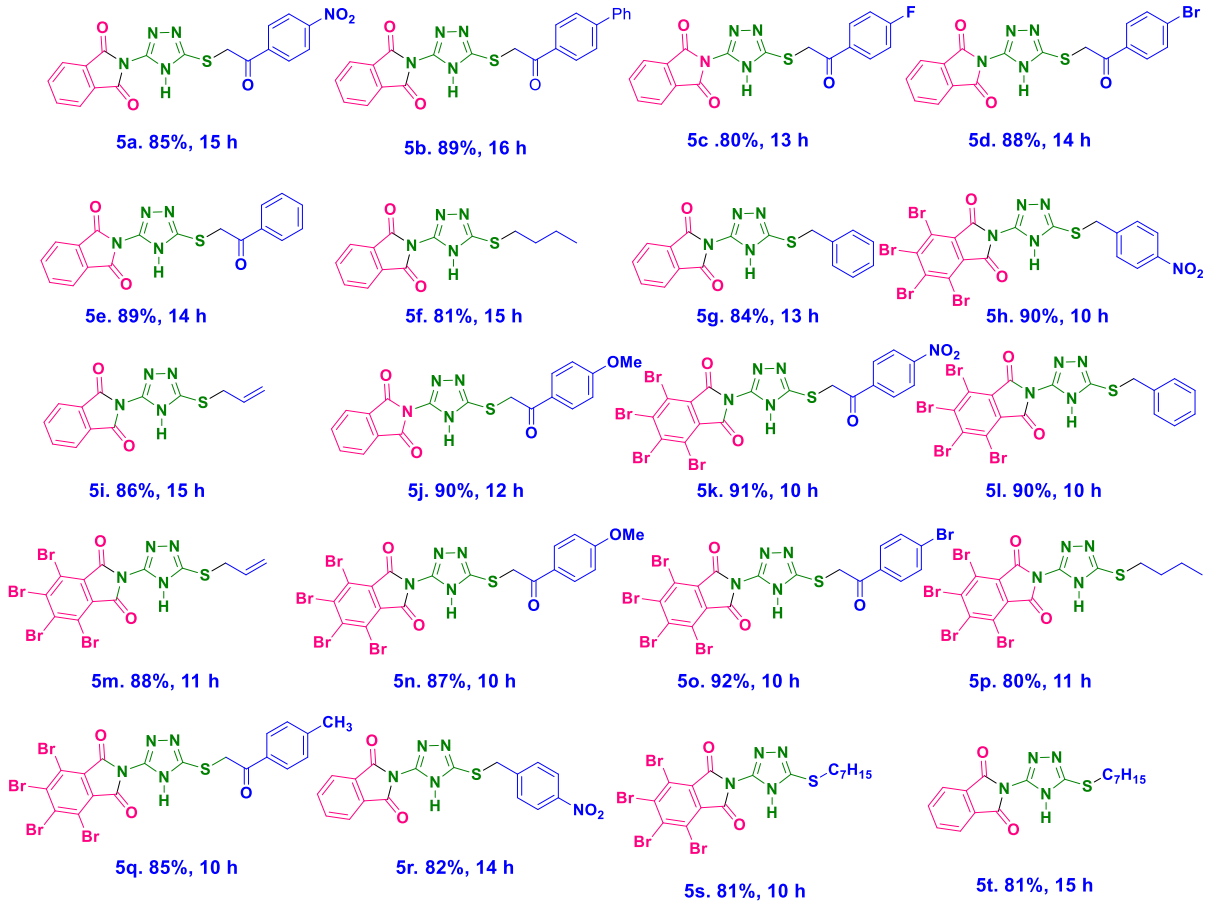
CHAPTER-II

A facile one-pot four component synthesis of thio alkyl/aryl/benzyl 1,2,4-triazolo isoindoline-1,3-diones, and their *In-vitro* cytotoxic activity, molecular docking studies, and DFT calculations

This chapter deals with the synthesis of title compounds (5) by the reaction of dipotassium cyanodithioimidocarbonate salt with hydrazine hydrate, phthalic anhydride and alkyl/aralkyl/phenacyl bromides using acetic acid and sodium acetate *via* a one-pot four-component synthesis (**method-1**). Alternatively, the same final products were also synthesized by the reaction of dipotassium cyanodithioimidocarbonate salt with hydrazine hydrate in presence of acetic acid to give intermediate 5-amino-4*H*-1,2,4-triazole-3-thiol [**I**]. (**method-2**) This compound was further reacted with phthalic anhydride, followed by a reaction with alkyl/aralkyl/phenacyl bromides to give the title compounds. **Scheme-1.**

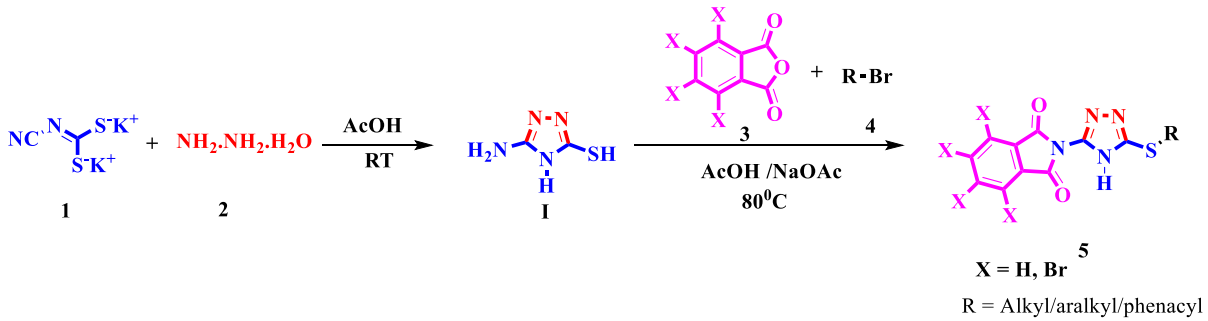
Scheme-1 (method-1)





Reaction conditions*:* **1** (1.0 mmol), **2** (1.5 mmol), **3** (1.0 mmol), **4** (1.0 mmol) was taken in AcOH/NaOAc

Method-2.



In the method-1 high yields of the products were obtained over method-2. In this chapter total 20 different final products were synthesized and all the new synthesized compound structures were confirmed by their spectral studies (FT-IR, ^{1}H-NMR, ^{13}C-NMR, HRMS).

***In-vitro* cytotoxic assay.** The *in-vitro* cytotoxic study was carriedout for all the synthesized compounds using *HeLa* cell lines. Among the screened compounds the **5m, 5p, 5r** has shown good cytotoxic activity.

Molecular docking study. *In silco* studies revealed that, all the synthesized compounds displayed excellent binding energies towards the receptor active sites. Molecular docking results were identified based on the ideal interacted ligands with protiene residues. The final compounds were drawn 2D models using Chemdraw software and converted into 3D structures using Open Babel GUI version 2.3.2 (Open Bable GUI; Chris Morley, USA). Molecular energy was minimized using the Energy minimization module of Maestro Tool (Schrodinger software) under the CHARMM force field. The Crystal structure of c-Met in the complex was retrieved from Protein Data Bank (PDB ID: 4GG5.pdb).

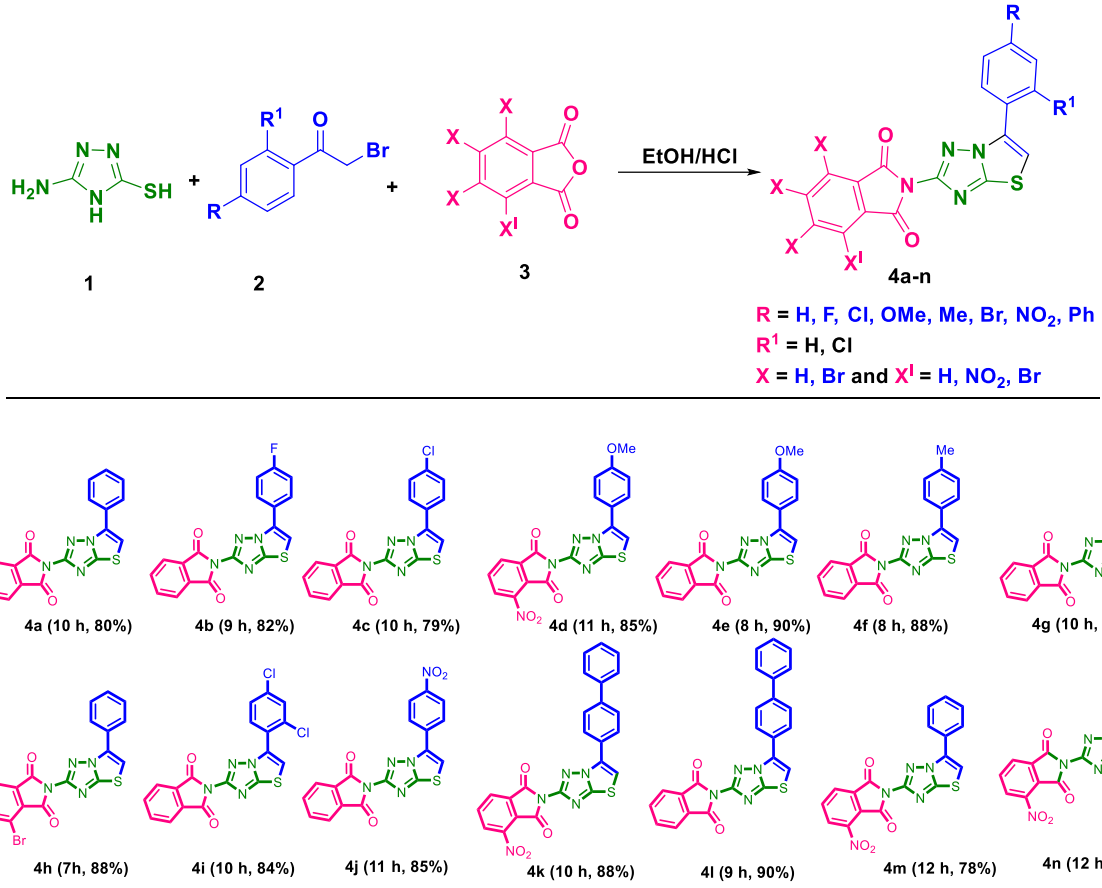
DFT calculations. The mechanism of the reaction was proposed using the density functional theory based to probe the energetics for formation of product. This study was useful for calculation of energy required for formation of various intermediates IM1(2.34), IM2(-8.77), IM3(2.92) and transition states TS1(43.23), TS2(22.84), TS3(34.70) throughout the reaction mechanism.

CHAPTER-III

One-pot three component synthesis of fused [3,2-*b*] [1,2,4]-triazolothiazole isoindolines and Schiff bases, characterization and targeting glioma *in-vitro* anticancer activity, molecular docking study.

This chapter describes the synthesis of a new series of fused triazolothiazole scaffolds bearing isoindoline and Schiff base moieties through multi-component approach, in which the 5-amino-4*H*-1,2,4-triazole-3-thiol (1) was reacted with phenacyl bromides (2) and phthalic anhydride (3), in the presence of EtOH/HCl to produce novel bicyclic triazolothiazole isoindoline moieties 4 a-n (**Scheme-2**). On the other hand, when compound (1) was reacted with various phenacyl bromides (2) and different aromatic aldehydes (5) in EtOH/HCl to give triazolothiazole Schiff bases 6 a-l (**Scheme-2.1).** And all the compounds were evaluated their for antitumor activity.

Scheme-2



Reaction conditions: Amino mercapto-1,2,4-triazole 1 (1 mmol), Phenacyl bromide 2 (1 mmol), Phthalic anhydride 3 (1 mmol) EtOH in HCl 5 (mol%) 80 °C.

In this **scheme-2** we have synthesized 14 new derivatives **4 a-n** and the structures of final compounds were confirmed by their IR, ^{1}H NMR, ^{13}C NMR, HRMS. And also the compound 4e was confirmed with single crystal data. It is a triclinic system, space group p-1, CCDC. 2171369

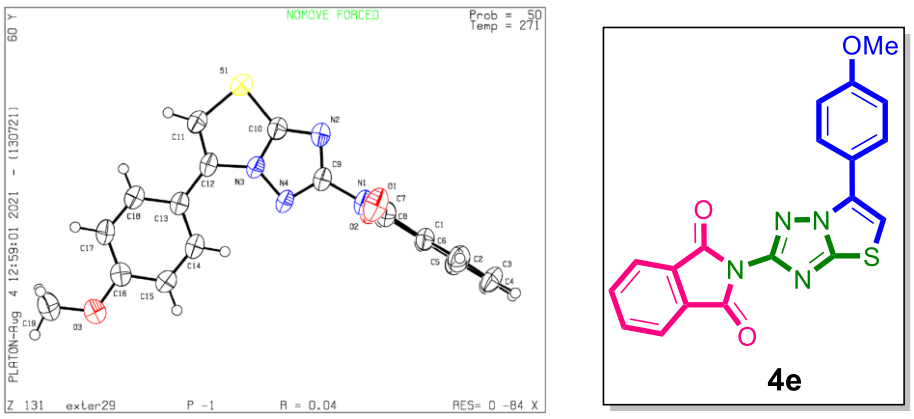
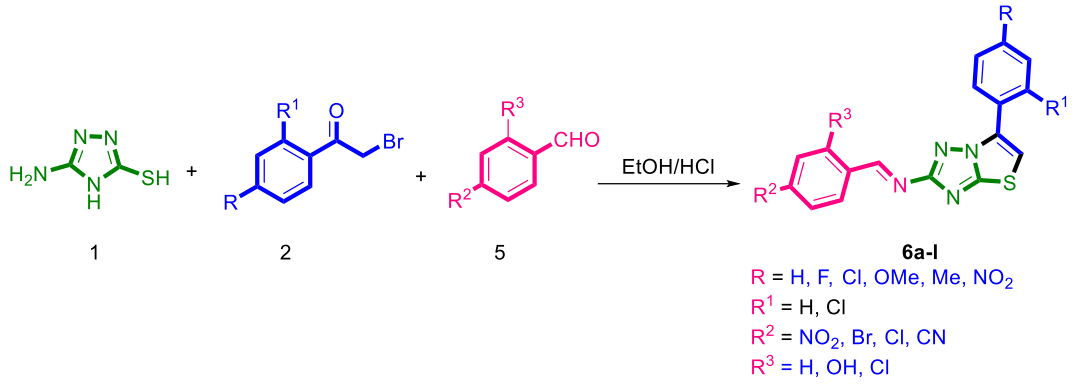
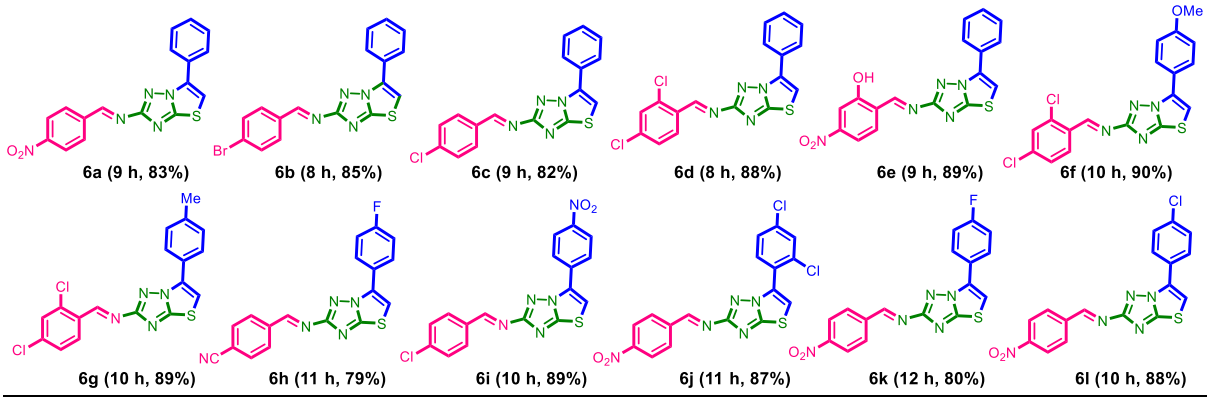


Fig. 1. The ORTEP diagram for single crystal data of the compound 4e

Scheme-2.1





Reaction conditions: Amino mercapto-1,2,4-triazole 1 (1 mmol), α-halo acetophenone 2 (1 mmol), aromatic aldehyde 5 (1 mmol) EtOH in HCl 5 (mol%) 80 °C.

***In vitro* anticancer activity assay:** All the scheme-2 & scheme-2.1 derivatives were screened for *in-vitro* brain cancer activity using C6 rat and LN18 human glioblastoma cell lines at different doses and temozolamide was takes as a standard drug. Both the series of compounds have shown good IC_{50} values and the MAP kinase pathway studies were also carriedout for compounds **4g**, **6i**.

Table.1 IC_{50} values of the **scheme-2** and **scheme-2.1** series of compounds.

Scheme-1 Code	**IC_{50} (µM) C6**	**IC_{50} (µM) LN18**	**Scheme-2 Code**	**IC_{50} (µM) C6**	**IC_{50} (µM) LN18**
4a	>50	>50	6a	>50	>50
4b	>50	>50	6b	> 50	>50
4c	>50	>50	6c	22.40 ±0.35	23.69 ± 0.19
4d	>50	>50	6d	40.78 ±0.71	39.18 ± 0.40
4e	38.2±0.42	41.14±0.29	6e	36.58 ±0.52	41.35± 0.14

4f	28.15±0.78	33.17±0.54	6f	7.564 ±1.11	21.51±0.36
4g	8.091±0.43	12.68±0.17	6g	28.32 ±0.52	35.99±0.43
4h	38.32±0.82	44.49±0.28	6h	56.88 ±0.71	52.48±0.24
4i	29.84±0.83	31.89±0.26	6i	8.74 ±0.68	12.56±0.21
4j	20.73±0.52	25.67±0.16	6j	41.84 ±0.93	44.79±0.31
4k	22.06±0.66	25.19±0.35	6k	37.28 ±1.40	39.69±0.40
4l	5.791-0.64	8.97-0.24	6l	19.22 ±0.68	29.77±0.35
4m	29.12±0.54	35.78±0.43			
4n	39.91±0.83	44.41±0.83			

Table-1. IC_{50} values of the synthesized compounds in C6 and LN18 human glioma cell lines

Molecular docking study: Scheme-2 and 2.1 compounds molecular docking studies were done by GSK-3β inhibitor complexed with the inhibitor 6QH4001 protein retrieved from the protein data bank with PDB ID: 5K5N.

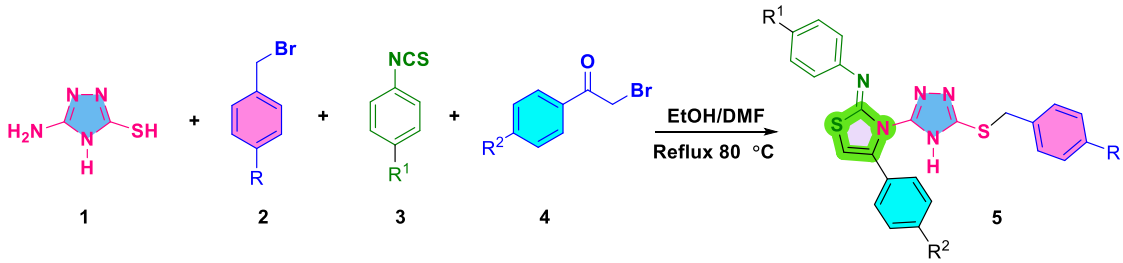
CHAPTER-IV

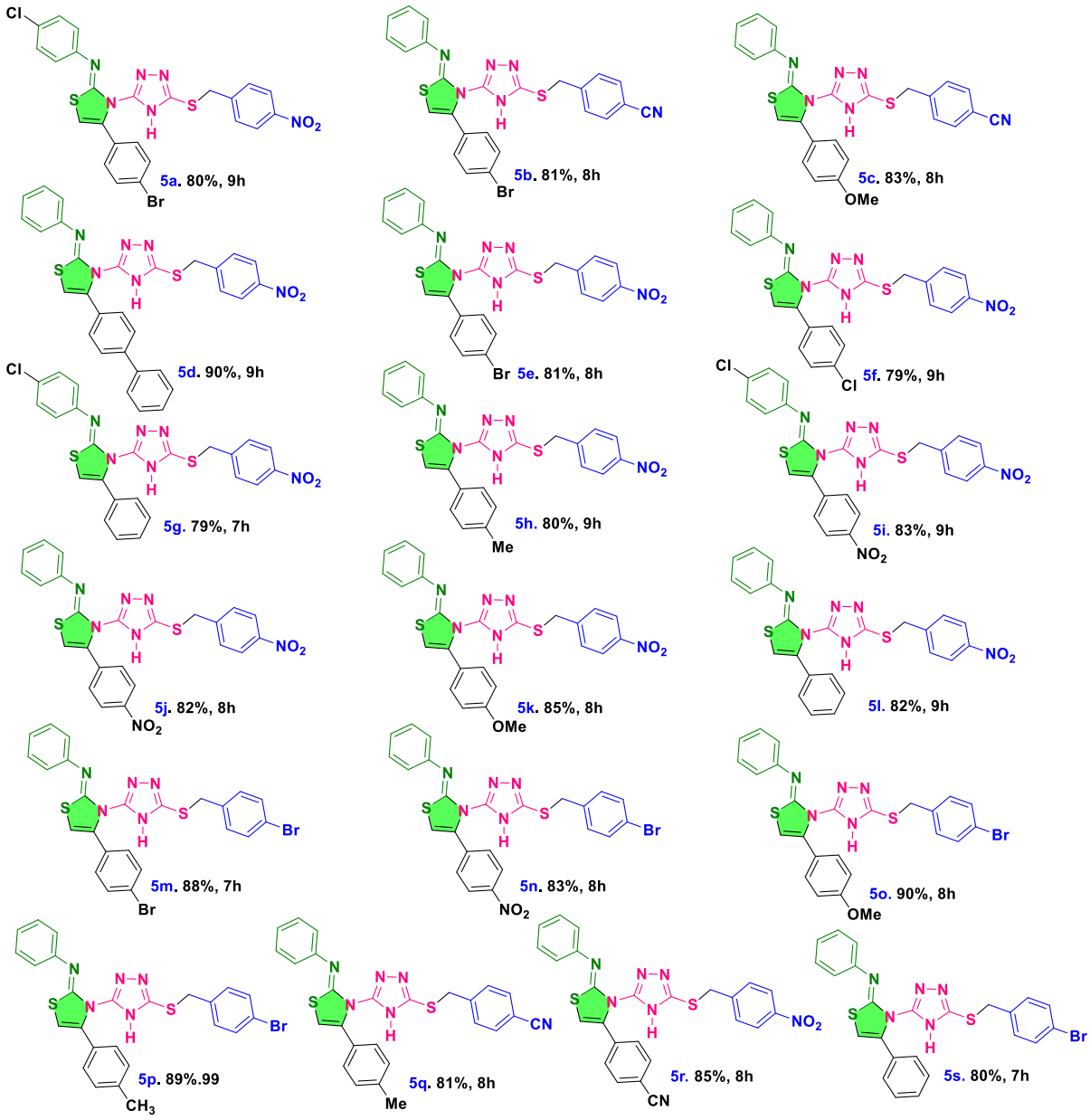
Synthesis of novel thioalkylated triazolothiazoles and their promising *in-vitro* antiviral activity

An efficient novel four component protocol for the synthesis of 3-(5-(benzylthio)-4*H*-1,2,4-triazol-3-yl)-N,4-diphenylthiazol-2(3*H*)-imines was carried out by one-pot multicomponent approach. The reaction of 5-amino-4*H*-1,2,4-triazole-3-thiol (1) with benzyl bromides (2) phenyl isothiocyanates (3) and 2-bromo acetophenones (4) in presence of ethanol and DMF (8:2) gave the novel target triazolothiazoles (5). The schematic representation is depicted in

scheme-3

Scheme-3.





Reaction conditions: 1 (1 mmol), 2 (1 mmol), 3 (1 mmol), 4 (1 mmol), in presence of EtOH + DMF under reflux condition.

In this chapter we have synthesized novel compounds 19 **5 a-s** by using various phenacyl bromides, benzyl bromides, phenylisothiocyanates and further these compounds structures were confirmed by their IR, ^{1}H NMR, ^{13}C NMR, HRMS.

***In-vitro* antiviral assay:** The final compounds were screened for their broad panel of *in-vitro* antiviral activity. Their activity has been compared with that of standard antiviral drugs. Such as (AMD3100, Remdesivir, Ribavirin, Zanamivir, Rimantadine, Acyclovir, DS-10,000). The MT-4 $CD4^{+}$ T cell culture was used to evaluate the compounds against human immune deficient virus (HIV) The virus-induced cytopathogenic effect (CPE) was measured colorimetrically AMD3100 was used as a reference drug. The compounds **5a** exhibited potent activity against both HIV-1 **6.8** μM (NL4.3 strain) and HIV-2 24.3 μM (ROD strain). and **5i 3.7** μM (NL4.3 strain) and 21.3 μM (ROD strain).

we have also evaluated the whole set of newly synthesized derivatives (**5a-s**) against a broad set of viruses. Human coronaviruses 229E and OC43, as well as *H*erpes simplex virus type 1 (HSV-1 strain KOS) were tested using HEL 299 cell cultures. Here, Remdesivir was used as a reference drug for human coronavirus and Acyclovir and Dextran sulphate (MW 10,000) were included as reference compounds for HSV-1. **5h** exhibited weak but selective activity against HCoV-OC43. The EC_{50} value of compound 5h against human corona virus is **43.3** μM**.**

Compound **5f** displayed promising activity against the replication of *Z*ika virus, with an average EC_{50} value of 9.3 μM against mr766 cell lines.

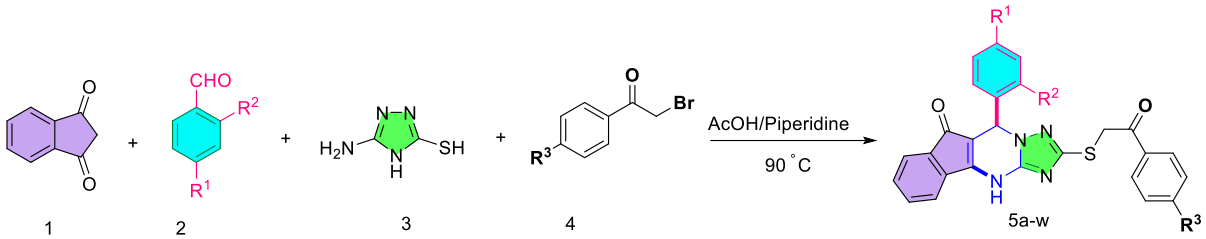
Lastly, we have also tested the synthesized compounds against three subtypes of influenza virus (H_1N_1, H_3N_2 and B) and respiratory syncytial virus (RSV A strain Long) using MDCK and Hep2 cell cultures respectively unfortunately, they didn't show activity against these viruses.

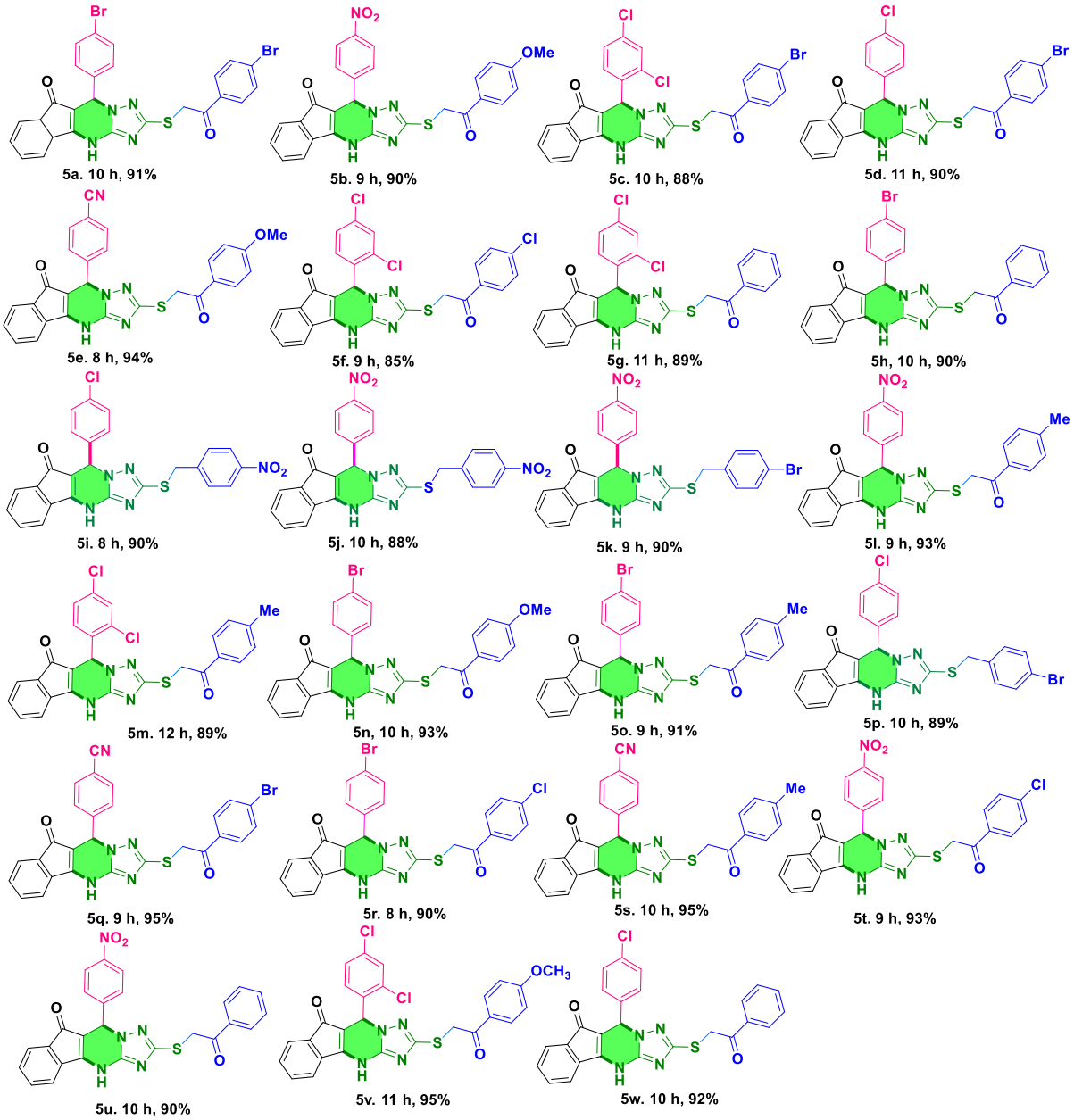
CHAPTER-V SECTION-A

Novel one-pot four component synthesis of 1,2,4-triazolo[1,5-*a*] pyrimidines, and their *in-vitro* anticancer evaluation, molecular docking studies

In this explains the one-pot four component synthesis of 2-((2-oxo-2-phenylethyl/benzyl) thio)-10-phenyl-4,10-dihydro-9*H*-indeno[1,2-*d*] [1,2,4]-triazolo[1,5-*a*] pyrimidin-9-ones by the reaction of 1,3-indane dione, aromatic aldehyde, 5-amino-4*H*-1,2,4-triazol-3-thiol and phenacyl bromides using acetic acid and a catalytic amount of piperidine at 90 °C for 13 h to obtained with high yields of the products. (scheme-4)

Scheme-4





Reaction conditions*:* 1,3 indane dione (1 mmol), aromatic aldehydes (1 mmol), 5-amino-4*H*-1,2,4-triazole-3-thiol (1 mmol), phenacyl bromides (1 mmol) was taken in AcOH/Piperidine under reflux at 90 °C.

In this scheme-1 we have synthesized 23 different substrates and all the synthesized compounds structures were confirmed with their spectral analysis. i.e. IR, ^{1}H NMR, ^{13}C NMR and Mass.

***In-vitro* anticancer activity:** The compounds were screened for their *in-vitro* anticancer activity by using LN18, MCF-7 brest cancer cell lines The compounds 5g and 5h showed best activity against brest cancer with the IC_{50} values are 3.8 and 2.7 µM respectively. For compound 5g using LN18 cell lines the IC_{50} values was found to be 7.51 µM while for 5h the value was found to be 6.5 µM using MCF-7 cell lines.

Table-3: IC_{50} values of the tested **scheme-4** compounds against brest cancer

Code	LN18 (IC_{50} µM)	MCF-7 (IC_{50} µM)
5b	3.3	13.77
5d	9.03	9.3
5e	3.46	20.98
5f	4.2	49.48
5g	**3.8**	**7.51**
5h	**2.7**	**6.5**
5i	6.16	20.53
5j	3.92	10.39
5m	4.18	36.34
5n	Interrupted	50.55
5o	2.73	25.33
5p	3.3	14.45
5s	4.33	16.69
5t	4.6	14.72

SECTION-VB

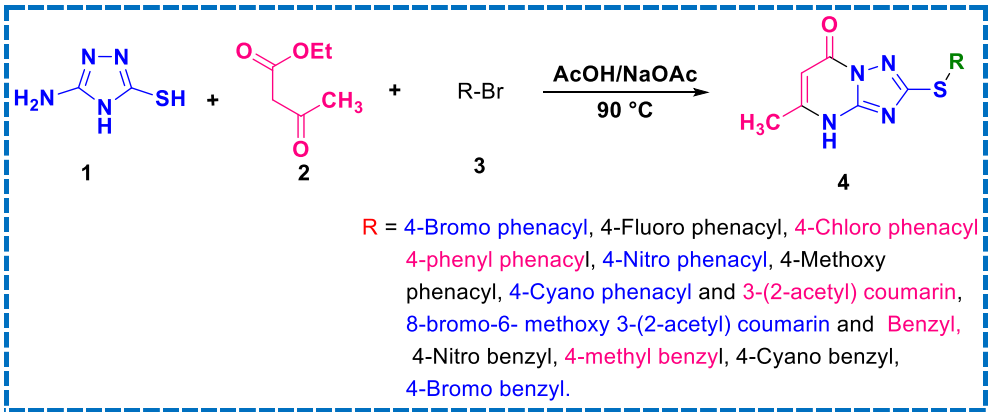
Synthesis of novel thioalkylated triazolopyrimidinones, sulfones and their biological activity

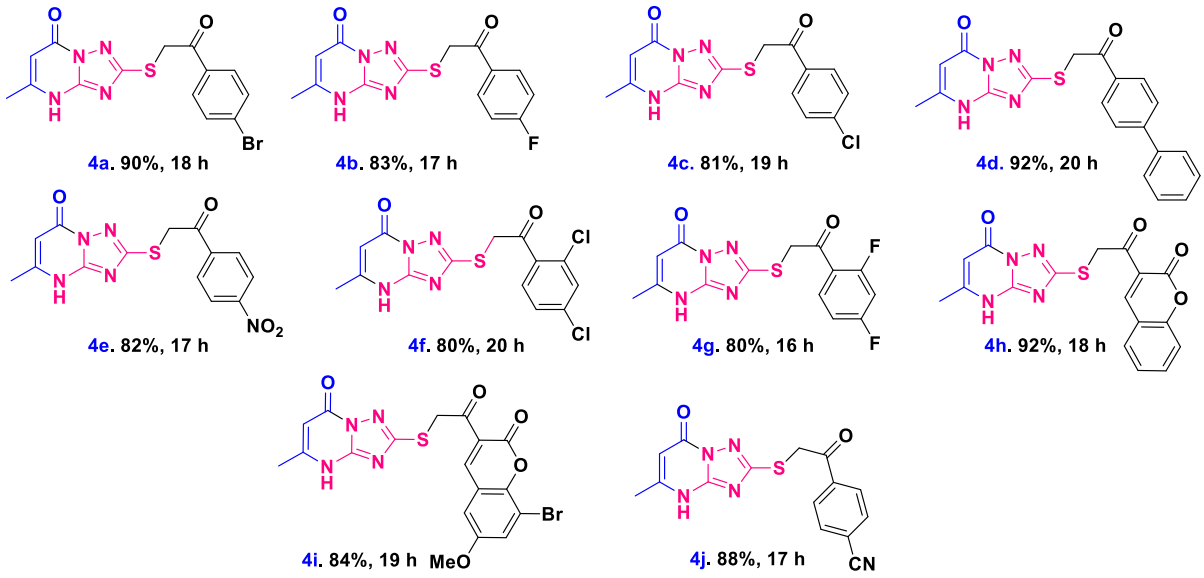
In this discussed one-pot three component synthesis of thioalkylated 1,2,4-triazlopyrimidines, sulfones and its biological activity.

Fused triazolopyrimidinones were synthesized *via* a one-pot three component condensation of 5-amino-4*H*-1,2,4-triazol-3-thiol, β-ketoester and various 2-bromo acetophenones, 3(2-bromo acetyl) coumarins, different benzyl bromides in the presence of a mixture of AcOH and NaOAc

lead to produce a novel thioalkyl (phenacyl/3-(2-bromoacetyl) coumarin/benzyl) triazolopyrimidinones with notable yields. (**Sheme-5**).

Scheme-5





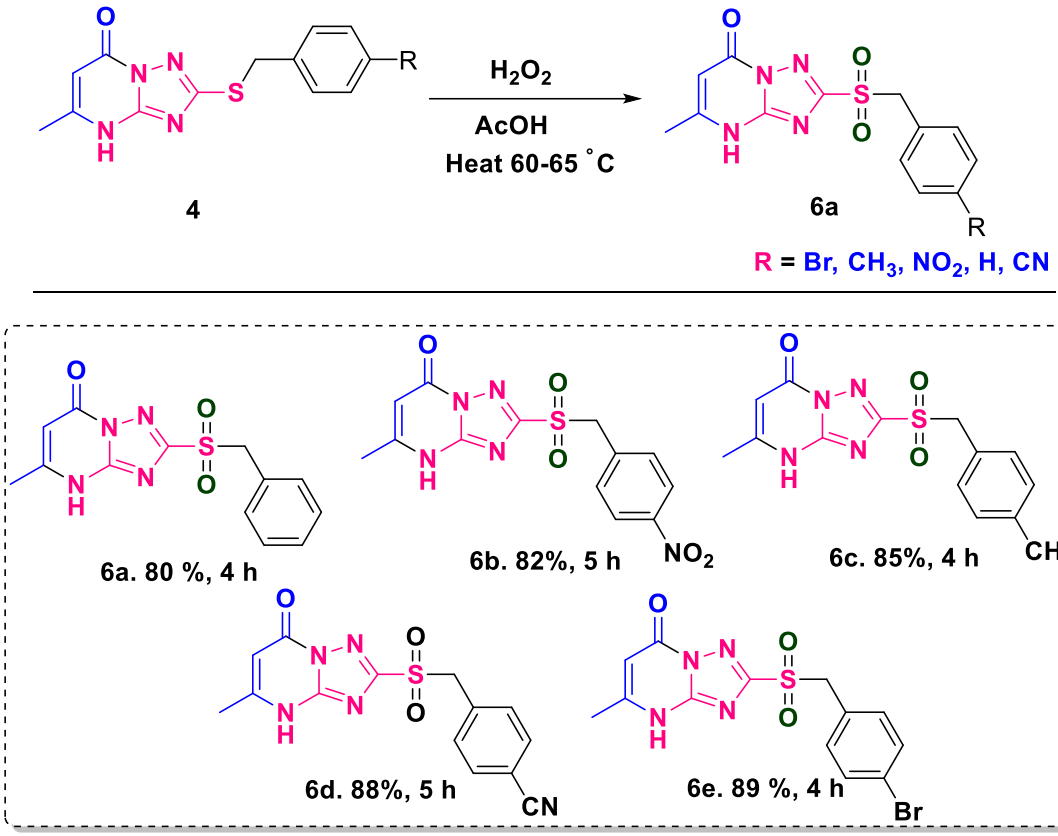
Reaction conditions: 1 (1 mmol), 2 (1 mmol), 3 (1mmol) mixture in AcOH/NaOAc reflux at 90 °C.

The synthesized final compounds (4a-l) were characterized by IR, ^{1}H NMR, ^{13}C NMR, HRMS

Scheme-5.1

In this scheme the above scheme-5 sulphide compounds such as 2-(substituted benzylthio)-5-methyl-[1,2,4]-triazolo[1,5-*a*]pyrimidin-7(4*H*)-ones were converted in to their sulfones using hydrogen peroxide in acetic acid at 60-65 °C. lead to produce the corresponding sulfone derivatives **6a-e**. The synthesis of sulfone compounds has depicted in **scheme-5.1**

Scheme-5.1



Reaction conditions: *Para*-substituted thiobenzyl 1,2,4-triazolo pyrimidinone (1 mmol), H_2O_2 (3 mmol), taken in AcOH heat at 65 ˚C.

The above synthesized final compounds in scheme-5, scheme-5.1 compounds have been confirmed by their spectral analysis such as FT-IR, ^{1}H NMR, ^{13}C NMR, HRMS.

Biological activity:

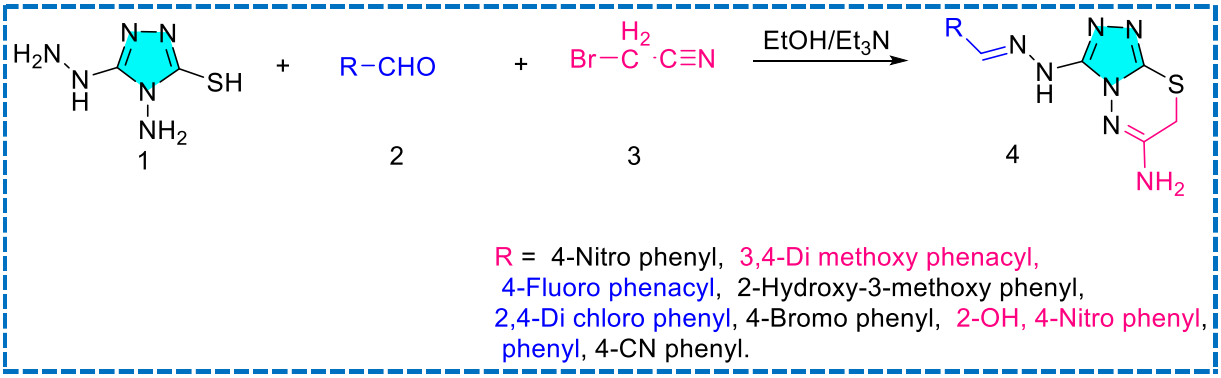
Further the scheme-5 compounds (**4a-j**) have screened for their broad panel of antiviral activity using different cell lines. The antiviral potency of the compounds was compared with standard reference antiviral drugs (AMD3100, Remdesivir, Ribavirin, Zanamivir, Rimantadine, Acyclovir, DS-10,000). The MT-4 CD+4 cell culture was used for anti HIV-1 and the compound **4k** has shown significant activity against HIV-1 the EC_{50} value is 8.8 µM. And also among all the screened compounds the compound **4g** exhibits promising HCoV (Human Corona virus) activity by using HEL 299 cell line the EC_{50} value is 4.7 µM. And also further both the scheme-5 & scheme-5.1 compounds were screened for their antimicrobial activity with respective to gram –Ve and gram +Ve bacterial strains.

CHAPTER –VI

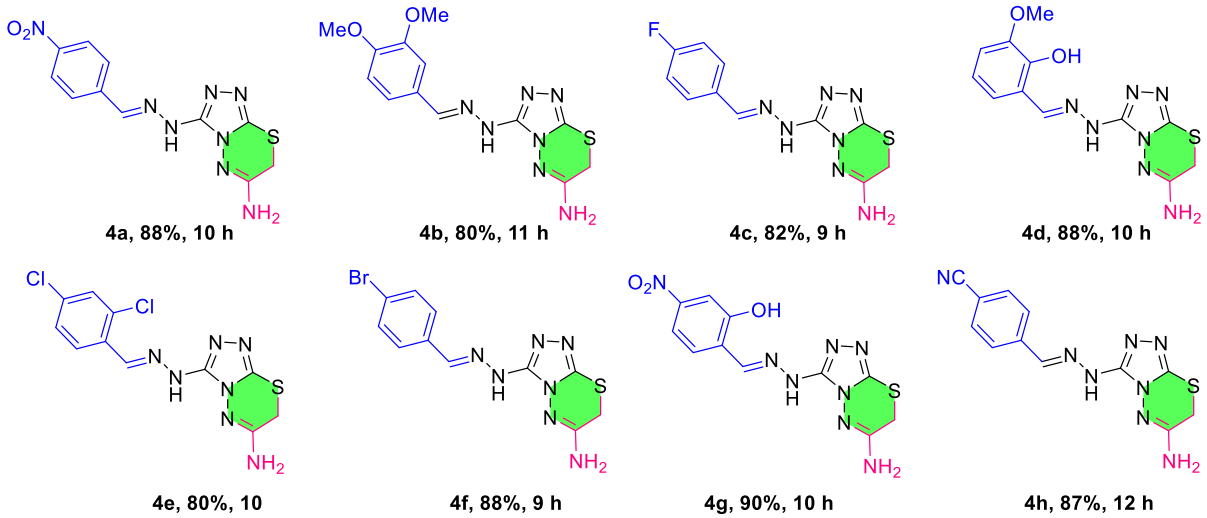
Novel one-pot synthesis, characterization, DNA binding studies of fused [1,2,4]-triazolo [3,4-b][1,3,4] 6-aminothiadiazines and their hydrazineylidene indolin-2-ones, Schiff bases

This chapter describes an efficient one-pot synthesis of fused [1,2,4]-triazolo[3,4-*b*][1,3,4] 6-aminothiadiazoles and their hydrazineylidene indolin-2-ones, Schiff bases. These compounds were synthesized by the reaction of 4-amino-5-hydrazinyl-4*H*-1,2,4-triazolo-3-thiol with different substituted isatins /aromatic aldehydes in presence of ethanol to gives Schiff bases and imines. These on cyclocondensation reaction with bromo acetonitrile and catalytic amount of Et_3N to afford fused heterocyclic compounds 6-aminothiadiazoles with high yields.

Scheme-6

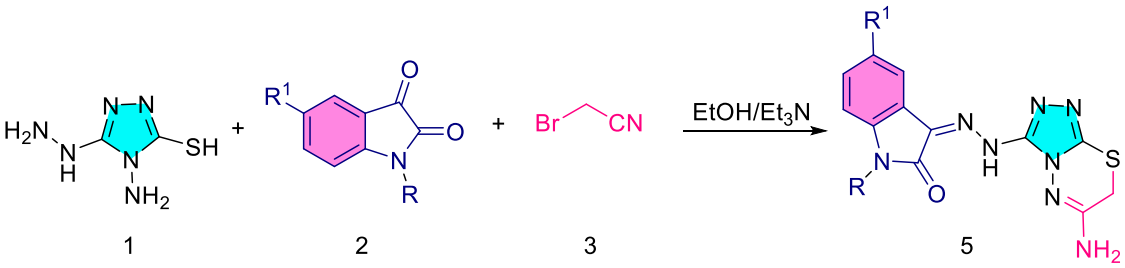


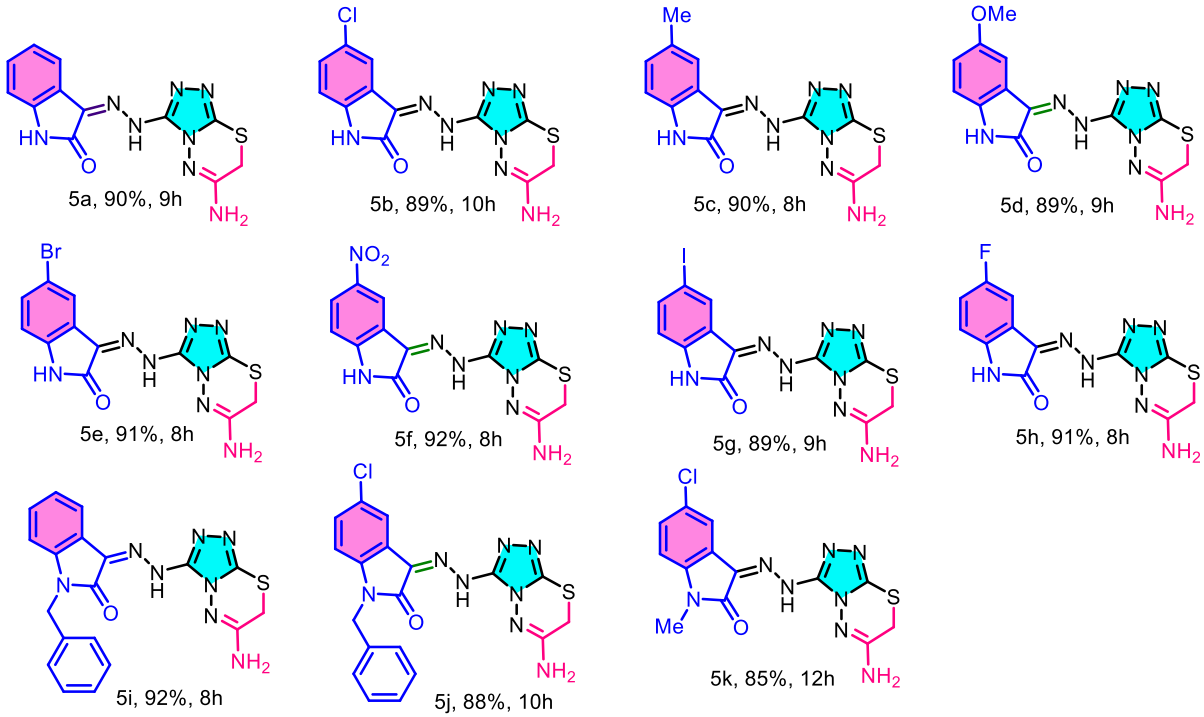
Substrate scope of the reaction.



Reaction conditions: 1,2,4-triazole 1 (1 mmol), aldehyde 2 (1 mmol), Bromo acetonitrile 3 (1 mmol), EtOH/Et_3N.

Scheme-6.1





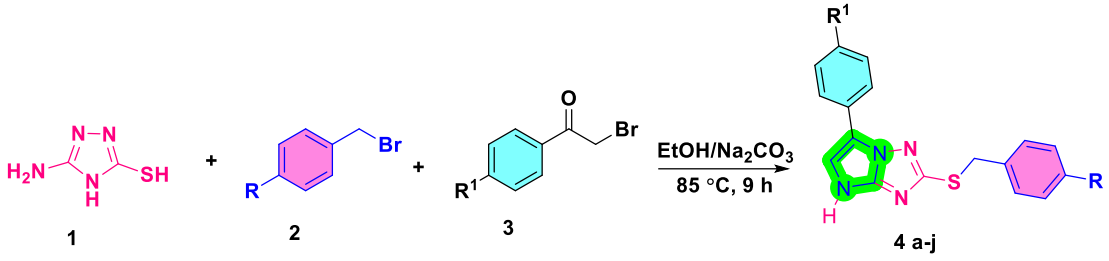
Reaction conditions: 1,2,4-triazole 1 (1 mmol), Isatin 2 (1 mmol), Bromo acetonitrile 3 (1 mmol). $EtOH/Et_3N$.

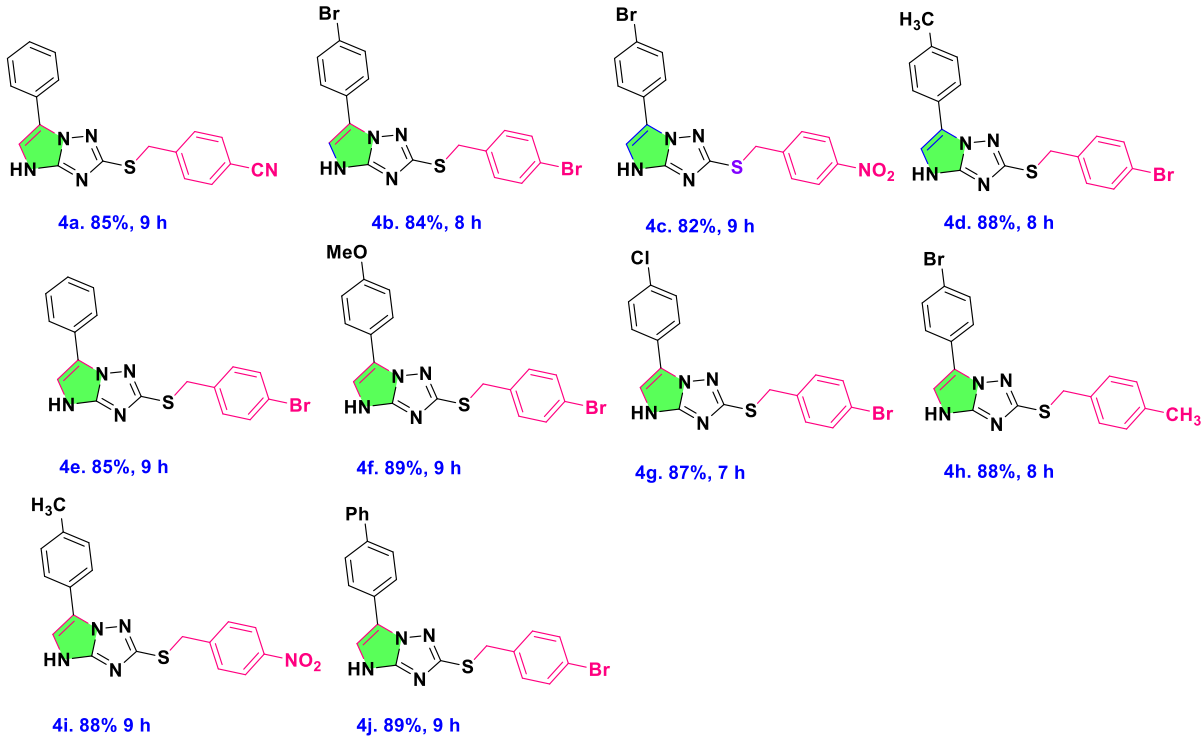
All the synthesized compounds were characterized by IR, ^1H NMR, ^{13}C NMR, HRMS. And also DNA binding studies, molecular docking simulations were carriedout for all the compounds.

CHAPTER-VII

Novel one-pot synthesis of imidazo[2,1-*b*][1,2,4]-triazoles, 1,2,4-triazolo iminoindoline-2-ones and their in-vitro antibacterial activity, B-DNA study

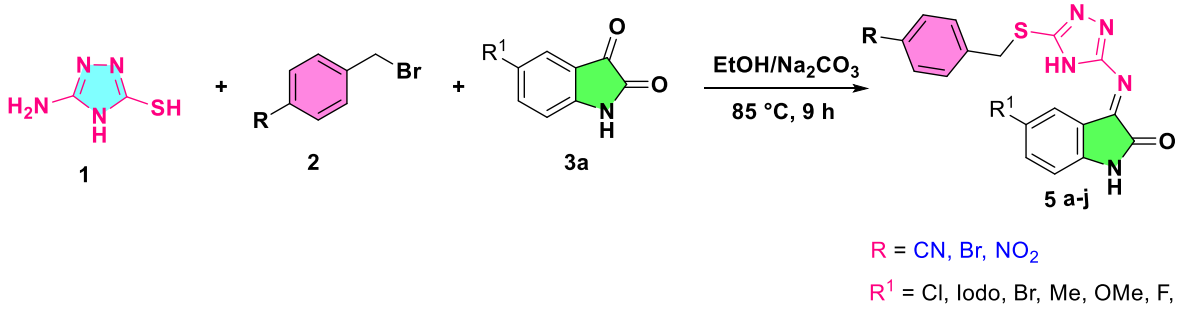
This chapter deals with the one-pot synthesis of fused[2,1-*b*][1,2,4]-triazoles and thioalkylated 1,2,4-triazolo oxindolines.

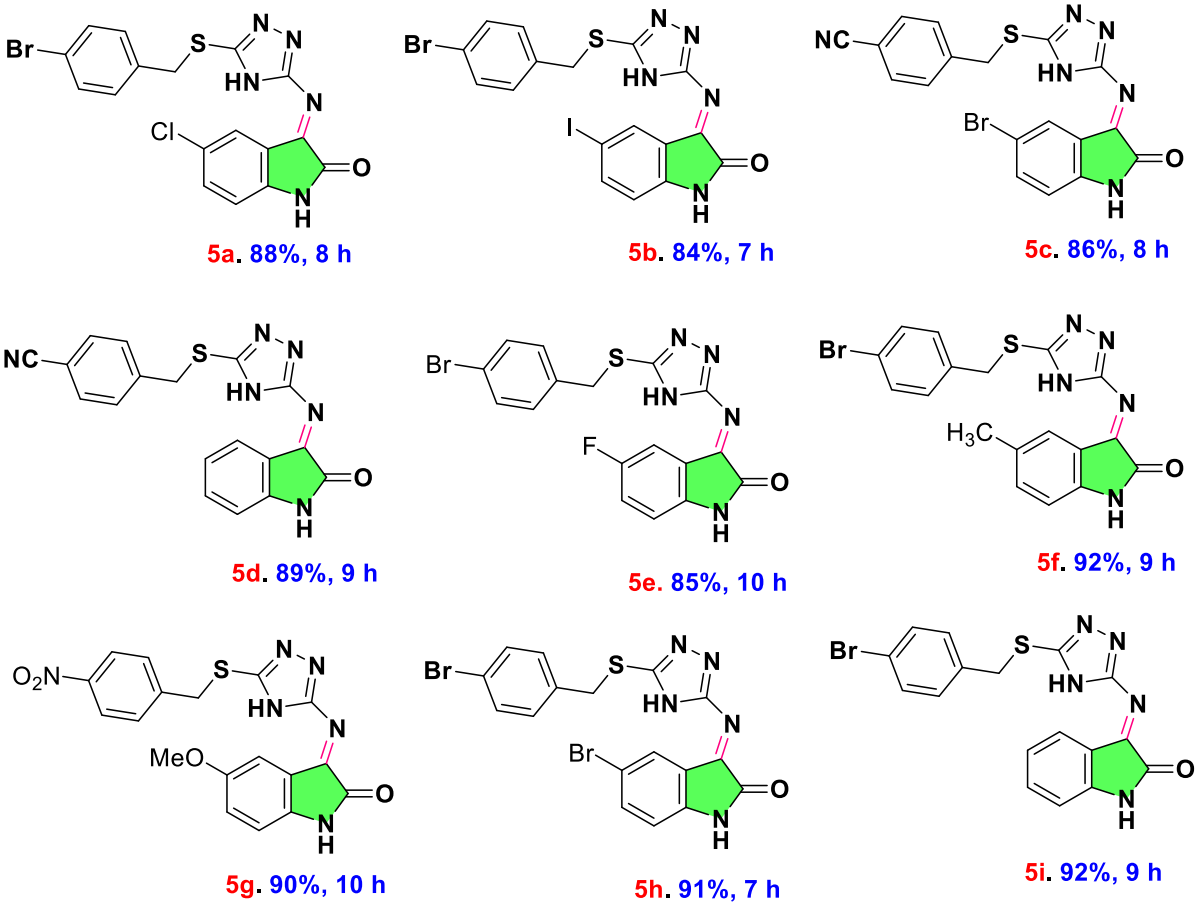




Reaction conditions: **1** (1 mmol), **2** (1 mmol), **3** (1 mmol) were taken in 2 mL Ethanol/Na_2CO_3 refluxed at 85 °C for 9 h to produce 89% of the yield of the products.

Scheme7.1





Reaction conditions: **1** (1 mmol), **2** (1 mmol), **3a** (1 mmol) mixture was taken in 2 mL EtOH/Na_2CO_3 and reflux at 85 °C for 9 h. 92% yield was obtained.

In this the reaction of 5-amino-4*H*-1,2,4-triazole-3-thiol with substituted benzyl bromides, and various phenacyl bromides using EtOH/Na_2CO_3 under reflux temperature to afford the corresponding bicyclic thiobenzylated triazolo imidazole heterocyclic compounds with good yields (**Scheme-7**). On the other hand, the condensation of 5-amino-4*H*-1,2,4-triazole-3-thiol with different substituted isatins, benzyl bromides in presence of similar reaction conditions such as EtOH/Na_2CO_3 lead to the thiobenzylated 1,2,4-triazolo oxindolines with high yields. (**Scheme-7.1**)

All the new synthesized derivatives of scheme-7 and scheme-7.1 structures have confirmed by their spectral analysis FT-IR, ^{1}H NMR, ^{13}C NMR and HRMS. Moreover, all the derivatives of the scheme-7 & 7.1 were screened for antibacterial activity and B-DNA binding studies. The scheme-7.1 compounds has shown good antibacterial activity against streptomycin Gram +Ve bacteria.

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APPENDICES

LIST OF PUBLICATIONS

1). **Facile one-pot Multi-Component synthesis, characterization, molecular docking studies, biological evaluation of 1,2,4-triazolo isoindoline-1,3-diones and their DFT calculations**

Papisetti Venkatesham, Perugu Shyam, Pooja, Raju Chedupaka, Rajeswar Rao Vedula *Polycyl. Aromat.Compd*, **2023**, *43*, 2283-2301. Doi.org/10.1080/10406638.2022.2042333

2). New class of fused [3,2-*b*][1,2,4]triazolothiazoles for targeting glioma *in vitro*

Papisetti Venkatesham, Nikhil Ranjan, Anwita Mudiraj, Vinutha Kuchana, Raju

Chedupaka, Vijjulatha Manga, Phanithi Prakash Babu*, Rajeswar Rao Vedula*

Bioorg. Med. Chem. Lett. **2023**, *80*, 129103. Doi.org/10.1016/j.bmcl.2022.129103

3). Synthesis of novel thioalkylated triazolothiazoles and their promising *in-vitro* antiviral activity

Papisetti Venkatesham, Dominique Schols, Leentje Persoons, Sandra Claes, Akanksha Ashok Sangolkar, Raju Chedupaka, Rajeswar Rao Vedula*

J. Mol. Struct. **2023**, *1286*, 135573. Doi.org/10.1016/j.molstruc.2023.135573

4). Synthesis, Characterization and Design Functional theory of novel one-pot thioalkylated benzimidazole linked 4-substituted mercapto imidazole molecular hybrids via multi-component approach

Raju Chedupaka, Ravinder pawar, **Papisetti Venkatesham**, Rajeswar Rao Vedula

Synth. Commun. **2022**, *52*, 1111-1121. Doi.org/10.1080/00397911.2022.2072745

5). A Facile one-pot synthesis of Benzimidazole Linked Pyrrole structured motifs via Multicomponent Approach. Design, Synthesis and Molecular docking studies

Raju Chedupaka, **Venkatesham Papisetti**, Akanksha Ashok Sangolkar, Rajeswar Rao Vedula

Polycycl. Aromat. Compd. **2022**, *42*, 7034-7048. Doi.org/10.1080/10406638.2021.1995010

Novel one-pot synthesis of thiobenzylated 1,2,4-triazolo imidazoles, iminoindoline-2-ones and their *in-vitro* antibacterial activity, B-DNA study

Papisetti Venkatesham, Sreenivasa Rao Parcha, Soumya Lipsa Rath, Raju Chedupaka, Rajeswar Rao Vedula*

Accepted in *J. Heterocycl. Chem.* (2023)

An Efficient Synthesis of 1,2,4-triazole Based [3,4-*b*][1,3,4] 6-Amino Thiadiazines and Their DNA Binding, Molecular Docking Studies.

Manuscript under review in Molecular diversity

Novel one-pot four component synthesis of 1,2,4-triazolo[1,5-*a*] pyrimidines, and their *in-vitro* anticancer evaluation, molecular docking studies

Minor comments received and submitted in ChemistrySelect.

Synthesis of novel thioalkylated triazolopyrimidinones, sulfones and their biological activity

Manuscript under preparation

LIST OF CONFERENCES AND WORKSHOPS ATTENDED

1. **A One Week Training Program on R & D Equipment under Synergistic Training Program Utilizing the scientific and Technological Infrastructure (STUTI-21)** Sponsored by Department of Science & Technology, Govt. of India. NIT Warangal, 4th – 10th April 2022.
2. **Lectures-Workshop on Recent Advances in Inter Disciplinary Chemical Sciences (RAICS-2022)** An international workshop May 24-25, 2022. Organized by Chemistry section, Mahila Mahavidyalaya BHU.
3. **Indian Council of Chemists 40th Annual National Conference** (29-30th December 2021) Department of Chemistry, Satavahana University, Karimnagar (TS). Oral presentation.
4. **1st International Virtual Conference on Recent Advances in Material Science and Organic Synthesis** (20-21th December 2021, RAMSOS-2021), NIT Raipur, Oral presentation**.**
5. **International Conference on Chemistry and Allied Sciences (ICCAS-2022)** 25-27, August, 2022, Organized by Department of Chemistry, Pingle Government College for women, Warangal. Participated in the Oral presentation.
6. **Participated in National Workshop on Spectral analysis of Organic Molecules. National Institute of Pharmaceutical Education and Research** (NIPER) Hyderabad. 16th – 17th July 2020**.**

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Facile One-Pot Multi-Component Synthesis, Characterization, Molecular Docking Studies, Biological Evaluation of 1,2,4-Triazolo Isoindoline-1,3-Diones and Their DFT Calculations

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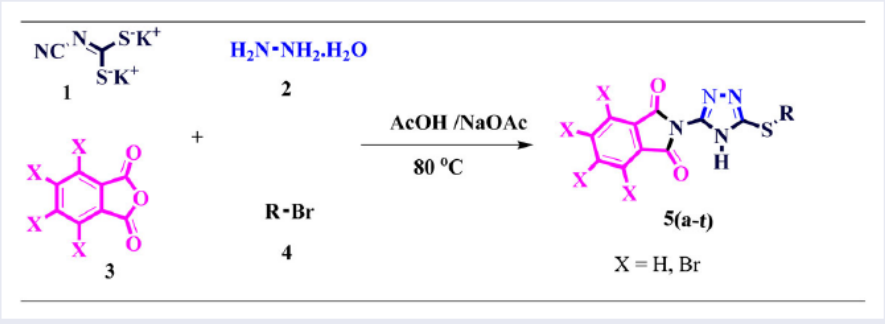
ABSTRACT

Alkyl/aralkyl/phenacyl thiotriazolyl isoindoline-1,3-diones were synthesized by the reaction of dipotassium cyanodithioimidocarbonate salt with hydrazine hydrate, phthalic anhydride and alkyl/aralkyl/phenacyl bromides using acetic acid and sodium acetate *via* a one-pot four-component synthesis. Alternatively, the same final products were also synthesized by the reaction of dipotassium cyanodithioimidocarbonate salt with hydrazine hydrate in presence of acetic acid to give intermediate 5-amino-4*H*-1,2,4-triazole-3-thiol [**I**]. This compound was further reacted with phthalic anhydride, followed by a reaction with alkyl/aralkyl/phenacyl bromides to give the title compounds in a two-step process. In this method, the yields are less compared to one-pot four-component synthesis. All the newly synthesized compounds were characterized by their spectral studies (FTIR, ^{1}H-NMR, ^{13}C-NMR, Mass). Further, the synthesized compounds were screened for their *in-vitro* anticancer activity. Compounds **5 m**, **5p**, **5r** showed good cytotoxic assay against *Hela* cancer cell lines. Furthermore, compounds **5(a-t)** were subjected to their docking analysis and DFT calculations.



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KEYWORDS
Isoindoline; Sulfone; Anti-cancer activity; Docking; DFT calculations

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New class of fused [3,2-b][1,2,4]triazolothiazoles for targeting glioma *in vitro*

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ARTICLE INFO

Keywords:
Fused heterocycles
Apoptosis
MAP kinase pathway
X-ray crystal data

ABSTRACT

Glioma is aggressive malignant tumor with limited therapeutic interventions. Herein we report the synthesis of fused bicyclic 1,2,4-triazolothiazoles by a one-pot multi-component approach and their activity against C6 rat and LN18 human glioma cell lines. The target compounds 2-(6-phenylthiazolo[3,2–b][1,2,4]triazol-2-yl) isoindoline-1,3-diones and (E)-1-phenyl-N-(6-phenylthiazolo[3,2–b][1,2,4]triazol-2-yl) methanimines were obtained by the reaction of 5-amino-4H-1,2,4-triazole-3-thiol with substituted phenacyl bromide, phthalic anhydride, and different aromatic aldehydes in EtOH/HCl under reflux conditions. In C6 rat glioma cell lines, compounds 4g and 6i showed good cytotoxic activity with IC_{50} values of 8.09 and 8.74 μM, respectively, resulting in G1 and G2-M phase arrest of the cell cycle and activation of apoptosis by modulating phosphorylation of ERK and AKT pathway.

Glioma is the most aggressive primary brain tumor with few treatment options and dismal prognosis. While standard treatment includes complete surgical resection followed by chemo-radiotherapy, recent scientific advances have led to the consideration of novel approaches like immunotherapy, gene therapy, altered signal transduction, and angiogenesis.[1] Despite all the available treatments, recurrence of GBM and drug resistance are its limitations and the reason for small median survival rate.[2] Hence, comprehensive analysis is required for a better understanding of this fatal disease. Genomic profiling of various tumors has revealed aberrant mutations in Mitogen-activated protein-kinase (MAPK) and associated pathways. such as AKT/mTOR pathway.[3,4] Overactivation of MAPK/ERK pathway promotes cell proliferation and subsequent phosphorylation of downstream substrates which can be related to tumor formation.[5] Elevated ERK expression has been detected in some of the common human cancers like ovarian, breast, brain and lung. But inhibition of ERK/MAPK path can significantly decrease the survival of tumor-forming cells and promote apoptosis.[6] MAPK and associated signaling pathways can lead to a response through ER stress signaling pathway.[7] Therefore, in this study we evaluated the *in vitro* activity of the synthesized triazolothiazoles against glioma cell lines as well as their mode of action.

The *N*-substituted imines and isoindolines have been identified as one of the most important scaffolds with R-CH = *N*-R, –CO-N(R)–CO– structures. The isoindoline unit makes them hydrophobic, neutral and can easily cross biological membranes.[8–10]

Because of their good biological activity, fused heterocyclic compounds with N and S have attracted a lot of interest in the field of medicinal chemistry.[11–16] The antitumor properties of the 2-amino-1,3,4-thiadiazole skeleton are well recognized, and its fused systems with the imidazo [3,2–b][1,2,4] triazole ring system are likewise known to possess remarkable anticancer activities.[17,18] Hybrid molecules created by combining distinct pharmacophores could lead to compounds with interesting biological characteristics. Fig. 1 shows similar reported anticancer moieties.[19–22]

Motivated by these findings and in continuation of our research in the synthesis of various bioactive heterocyclic units[23,24] we have synthesized a series of fused triazolothiazole scaffolds bearing isoindoline and schiff base moieties and evaluated their activity against C6 rat and LN18 human glioma cell lines.

The target compounds were synthesized by a multi-component

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Synthesis of novel thioalkylated triazolothiazoles and their promising in-vitro antiviral activity

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ARTICLE INFO

Keywords:
Triazolothiazole synthesis
Antiviral activity
Docking study

ABSTRACT

A novel series of 1,2,4-triazolothiazoles were efficiently synthesized by the reaction of 5-amino-4*H*-1,2,4-triazol-3-thiol, benzyl bromides, phenyl isothiocyanates and phenacyl bromides under one-pot process in the presence of EtOH/DMF. The products were obtained in pure form with high yields. All the newly synthesized compound structures were confirmed by spectral analysis i.e. IR, ^{1}H NMR, ^{13}C NMR and HRMS. All the synthesized derivatives were screened against a broad panel of viruses. Among them, two derivatives (5a and 5i) were capable of inhibiting both HIV-1 and HIV-2 replication in MT-4 cells, while seven other compounds showed selectivity towards HIV-2. Moreover, compound 5f has showed promising activity against the replication of Zika virus. Further, molecular docking studies were performed for the most active compounds.

1. Introduction

The past few years we have seen the emergence of several viral infections, including outbreaks of the Ebola virus in Africa, the Zika virus spreading across the Americas, and coronavirus disease 2019 (*COVID-19*), a pandemic caused by the novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), in people around the world [1]. Viruses can be classified by the type of viral genome they possess, namely DNA or RNA viruses. The DNA containing viruses include Herpes simplex virus -1 (HSV-1), Herpes simplex virus-2 (HSV-2), Adenoviruses, Smallpox viruses, Papillomaviruses [2]. The vast majority of viruses however have RNA genomes, and respiratory syncytial virus (RSV), parainfluenza virus-3, Zika virus, Ebola virus, Coronavirus, and HIV are among the most significant RNA viruses [3]. Respiratory syncytial virus is a major cause of respiratory illness, mainly in young children [4]. Zika virus is transmitted primarily by Aedes mosquitoes. Symptoms are generally mild, but there is an increased risk of neurologic complications such as Guillain-Barre syndrome or brain damage [5]. HIV is the virus that causes the chronic, potentially life-threatening acquired immunodeficiency syndrome (AIDS). This virus contains an RNA genome but passes through a DNA intermediate during its infection cycle [6]. HIV can be treated with antiretroviral medicines, but a combination of drugs is mostly used because the virus can quickly adapt and become resistant [7]. Evaluation of antiviral resistance has led to continued interest in design of innovative antiviral drugs, that are efficacious against various circulating resistant strains, have fewer side effects and high therapeutic efficiency.

Hantzsch thiazole method is used for the construction of simple thiazoles. The thiazole moiety is an important heterocyclic core unit for the development of biologically active molecules [8a, 8b]. In particular, the thiazole 2-imine derivatives have received a lot of attention in medicinal chemistry because these substances display drug-like properties and most of them are extensively used in the pharmaceutical industry and natural products [9–17]. Recent literature reveals that 1,2,4-triazoles are also important heterocyclic compounds in drug discovery and biological applications [18,19]. In the present study the chosen 1,2,4-triazole has both NH_2 and SH functional groups which are extremely straightforward to build novel triazolothiazole moieties. The 1,2,4-triazol ring structure possesses anticancer [20], antiviral [21], antifungal [22], antimicrobial [23], antioxidant [24], antibacterial [25] and antiproliferative activities [26], and is also used in agricultural industry [27]. Recently potent biological activity was reported for 1,2,4-triazole associated with a thiazole heterocyclic ring [28]. Thio alkylated triazolothiazole 2-imines were mainly used in the medicinal and pharmaceutical field and possess antiviral [29], anticandidal [30]- and antituberculosis activity [31], Anti-inflammatory [32] among others.

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