DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF NEW HETEROCYCLIC HYBRIDS

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By

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Dedicated to

…..My beloved parents

and my son Rudran

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CERTIFICATE

This is to certify that the research work presented in this thesis entitled **"DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF NEW HETEROCYCLIC HYBRIDS"** submitted by **Mr. Gondru Ramesh** for the award of the degree of Doctor of Philosophy in Chemistry, National Institute of Technology, Warangal (Telangana), under my guidance and supervision. This work has not been submitted earlier either in part or in full for any degree or diploma to this or any other university.

Date:

Place :

(**Prof. B. Rajitha)**

DECLARATION

I hereby declare that the research work presented in this thesis entitled **"DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF NEW HETEROCYCLIC HYBRIDS"** has been carried out by me under the supervision of **Dr. B. Rajitha**, Retired Professor, Ex-Emeritus Professor, Department of Chemistry, National Institute of Technology, and Warangal. I declare that this work is original and has not been submitted in part or full, for any degree or diploma to this or any other university.

Date:

Place: **(Gondru Ramesh)**

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(Gondru Ramesh)

ABBREVIATIONS

Ac	:	Acetyl
AcOH	:	Acetic acid
Ac_2O	:	Acetic anhydride
MeCN	:	Acetonitrile
AChE	:	Acetylcholine esterase
NH_4OAc	:	Ammonium acetate
Anhyd.	:	Anhydrous
Cat.	:	Catalytic
$CHCl_3$	:	Chloroform
Conc.	:	Concentration
CuI	:	Copper iodide
COX	:	Cyclooxygenase
DPPH	:	2,2-diphenyl-1-picrylhydrazyl
DHPM	:	3,4-Dihydropyrimidin-2(1*H*)-one
$CDCl_3$	:	Deuterated chloroform
DMSO-d_6	:	Deuterated Dimethyl sulfoxide
DMF	:	Dimethylformamide
DMSO	:	Dimethyl sulfoxide
MTT	:	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
DPPH	:	2,2-Diphenyl-1-picrylhydrazyl
CH_2Cl_2	:	Dichloromethane
DOX	:	Doxorubicin
EAC	:	Ehrlich ascites carcinoma
ESI	:	Electrospray ionization
EtOH	:	Ethanol
ESCMID	:	European Society of Clinical Microbiology and Infectious Diseases
FTIR	:	Fourier transform infrared
GI_{50}	:	50% Growth inhibition
IC_{50}	:	Half maximal inhibitory concentration
Hz	:	Hertz
HCl	:	Hydrochloric acid
H_2O_2	:	Hydrogen peroxide
IAEC	:	Institutional Animal Ethical Committee
ILs	:	Ionic liquids
LC_{50}	:	50% Lethal concentration

$[NMP]H_2PO_4$	:	*N*-Methyl-2-pyrrolidonium dihydrogen phosphate
MP	:	Melting point
MeOH	:	Methanol
MTCC	:	Microbial Type Culture Collection and Gene Bank
MIC	:	Minimum inhibitory concentration
MBC	:	Minimum bactericidal concentration
MFC	:	Minimum fungicidal concentration
MWI	:	Microwave irradiation
MLR	:	Multiple Linear Regressions
NCCLS	:	National Committee for Clinical Laboratory Standard
NMR	:	Nuclear magnetic resonance
NaOH	:	Sodium hydroxide
NaF	:	Sodium fluoride
NaOAc	:	Sodium acetate
NaOEt	:	Sodium ethoxide
$POCl_3$	:	Phosphoryl chloride
KOH	:	Potassium hydroxide
PPA	:	Polyphosphoric acid
PDB	:	Protein Data Bank
QSAR	:	Quantitative Structure Activity Relationship
ROS	:	Reactive oxygen species
rt	:	Room temperature
RCSB	:	Research Collaboratory for Structural Bioinformatics
SD	:	Standard deviation
SAR	:	Structure–activity relationship
H_2SO_4	:	Sulfuric acid
THF	:	Tetrahydrofuran
TCH	:	Thiocarbohydrazide
TSC	:	Thiosemicarbazide
TU	:	Thiourea
TLC	:	Thin Layer Chromatography
Et_3N	:	Triethylamine
TGI	:	Total growth inhibition
TMS	:	Tetramethylsilane
US	:	Ultra-sonication
WIPO	:	World Intellectual Property Organization
ZOI	:	Zone of inhibition

GENERAL REMARKS

1. Nuclear Magnetic Resonance (NMR) spectra were recorded on Bruker-400 MHz spectrometer (Bruker Corporation Ltd., Germany) using Tetramethylsilane (TMS) as the internal standard. Chemical shifts have been expressed in (δ) ppm units downfield from TMS. Selected data are reported as follows. Chemical shifts, multiplicity, (s = singlet, d = doublet, t = triplet, q = quartet and m = multiplet), coupling constants (*J* in Hz) as assignments.

2. Mass spectra were recorded on Jeol JMSD-300 spectrometer (Jeol Ltd., Tokyo, Japan).

3. Infrared (IR) spectra were recorded on Perkin-Elmer 100S (Perkin-Elmer Ltd. United Kingdom)/Thermo Nicolet Nexus 670 spectrometer (Thermo Electron Corporation Ltd., Waltham Massachusetts, US) using KBr pellets. Values have been expressed in cm^{-1}.

4. Elemental analyses (C, H, N) were performed on a Carlo-Erba model EA1108 analytical unit (Triad Scientific Ltd., New Jersey, USA).

5. All the melting points were recorded in open capillaries using Stuart SMP30 apparatus (Bibby Scientific Ltd. United Kingdom) and are uncorrected.

6. All evaporations were carried out under reduced pressure on Buchi/Heidolph rotary evaporator below 50 ^{o}C.

7. All the reactions were monitored by thin layer chromatography (TLC) with F_{254} silica-gel precoated sheets (Merck, Darmstadt, Germany) using hexane/ethyl acetate (8/2) as eluent. Visualization was accomplished with UV light and iodine vapours.

8. All the reagents and solvents were purchased from Aldrich/Merck/Spectrochem and used after purification (dry) following the procedure given in Vogel text book of Practical Organic Chemistry.

CONTENTS

CHAPTER-I

INTRODUCTION

INTRODUCTION

Heterocyclic chemistry is a branch of organic chemistry that deals with synthesis and chemical properties of heterocyclic compounds. Heterocyclic compounds are the cyclic organic compound comprises one or more heteroatom's (which is not Carbon or Hydrogen) and contain at least one carbon in their ring system. However, Borazine a six membered inorganic compound is an exception which has no carbon atoms and being termed as a heterocyclic. The most common hetero-atoms found in the heterocyclic ring systems are Nitrogen (N), oxygen (O), sulphur (S), phosphorous (P), and boron (B) etc. Among them, nitrogen, oxygen and sulphur containing heterocyclic systems are key building-blocks used to develop new materials possessing interesting electronic, mechanical or biological properties. Heterocyclic compounds may be aromatic, or partially or fully saturated. Nearly half of the known naturally occurring organic compounds contain at least one heterocyclic ring. Heterocyclic compounds are present in many pharmaceuticals, agrochemicals and in a wide variety of drugs, nucleic acids (DNA and RNA), vitamins (thiamine B_1, riboflavin B_2, nicotinamide B_3, pyridoxol B_6 and ascorbic acid C), heme and chlorophyll, penicillins, cephalosporins, macrolides *etc*., biologically active compounds including antimicrobial, anti-inflammatory, analgesic, antiepileptic, antiviral, antineoplastic, antihypertensive, antimalarial, antianxiety, antidepressant, antihistaminic, antioxidant, antitubercular, anti-Parkinson's, antidiabetic, antiobesity etc.[1] Many natural drugs such as quinine, papaverin, emetine, theophylline, atropine, procaine, codeine, morphine and reserpine are heterocyclic in nature. Almost all the compounds we now used as synthetic drugs such as diazepam, chlorpromazine, isoniazid, metronidazole, azidothymidine, barbiturates, antipyrine, captopril and methotrexate are also heterocyclic in nature. Some dyes, luminophores, pesticides and herbicides are also heterocyclic in nature. Because of their applications in medicine, agriculture, industry and academic research has made them attractive synthetic targets.

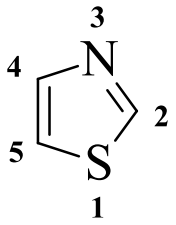
A brief introduction and applications of many pharmacologically active drugs embedding heterocycles like 3,4-dihydropyrimidinones, 3,4-dihydropyrimidine-thiones, coumarins, thiazolo[3,2-*a*]pyrimidines, thiazolo[3,2-*a*]pyrimidin-3(5*H*)-ones, coumarins, pyridines, pyrazoles, thiazoles, selenazoles, triazoles and pyridinium salts are discussed in this chapter. Many of them functions as calcium channel modulators, antimicrobial, antiviral, anticancer, antioxidant, antitumor, anticonvulsant, antiinflammatory, antiallergic,

antihypertensive, antineoplastic, antidepressant, antiproliferative, anticoagulant, antithyroid and anti-HIV agents, fungicidal, pesticidal, and herbicidal and plant growth regulating properties. Such compounds were also reported as inhibitors of enzymes like mitotic kinesin, CDC25 phosphatase, acetylcholinesterase (AChE), amino acid decarboxylase and histidine decarboxylase, and acts as antagonists of neuropeptide Y(NPY) and 5-HT_{2A} receptors.[2]

In view of aforementioned biological properties of heterocyclic compounds, prompted us to undertake the synthesis of, thiazoles, selenazoles, pyrazoles, triazoles, coumarins, thiazolo[3,2-*a*]pyrimidines, thiazolo[3,2-*a*]pyrimidin-3(5*H*)-ones and pyridinium salts, by adopting simple and cost-effective procedures. Most of the synthesized compounds were assessed for their *in vitro* antiproliferative, antibacterial, anti-biofilm, antifungal, antioxidant activities. A brief review on biologically importance of above mentioned heterocyclic scaffolds were also discussed in this chapter.

Thiazoles

Thiazole or 1,3-thiazole **(1)**, is a 5-membered heterocyclic compound that featuring both nitrogen (N) and sulphur (S) as part of the fully unsaturated aromatic ring. The thiazole scaffold has attained a considerable attention due to their numerous biologically activities including antiallergic, anti-hypertension, anti-inflammation, anti-schizophrenia, anti-bacterial, anti-cancer and anti-HIV activities.[3]



(1)

Fig. 1

Thiazole ring plays an important role in many of the biologically potent compounds. Some of the drugs and agrochemicals includes,[4] Penicillin (**2**), Ritonavir (**3**), Pramipexole (**4**), Tiazofurin (**5**), Cinalukast (**6**), Nizatidine (**7**), Ravuconazole (**8**), Fanetiazole (**9**), Meloxicam (**10**) and Thiamethoxam (**11**).

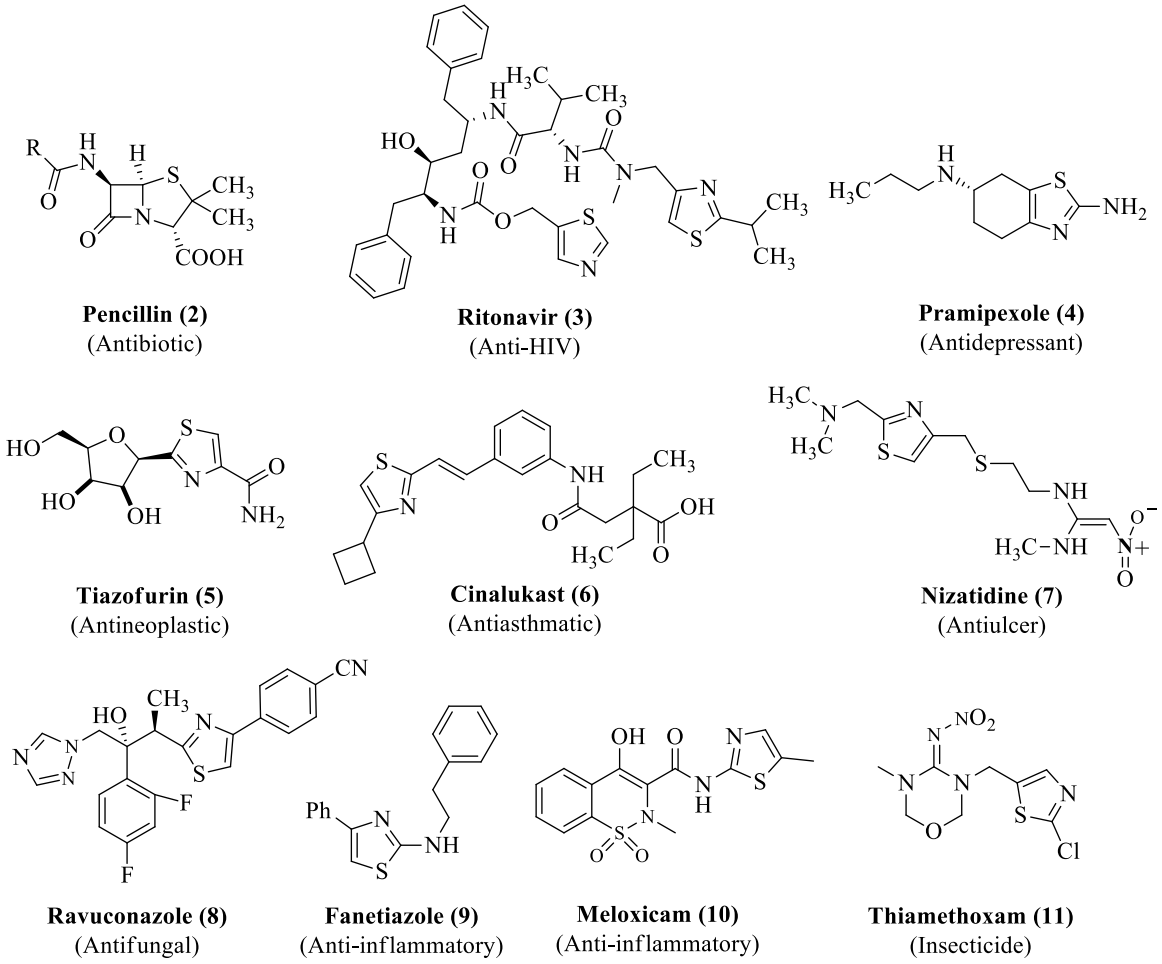
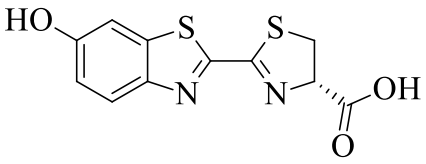


Fig. 2

Firefly luciferins are a class of light-emitting heterocyclic compounds found in bio-luminescencing organisms (*Lampyridae* species)[5] contains thiazole nucleus. Excited state of **Luciferin (12)** due to the enzyme-catalyzed oxidation emits characteristic yellow light by radio-active decay.

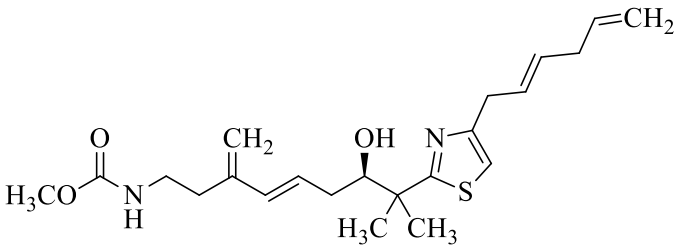


Firefly Luciferin (12)

Fig. 3

J. B. Morgan *et al.*[6] reported the marine sponge metabolite mycothiazole: a novel prototype mitochondrial complex I inhibitor. In this they have described the extraction of mycothiazole **(13)** from *petrosaspongia mycofijiensis* marine sponge and its biological

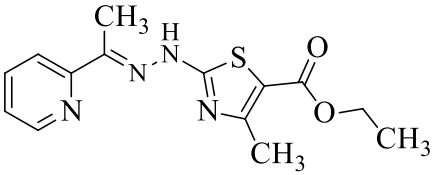
application as a inhibitor of hypoxic HIF-1 signalling in tumour cells with IC_{50} values 1 nM, which correlated with the suppression of hypoxia-stimulated tumour angiogenesis *in vitro*, exhibited pronounced *in vitro* neurotoxicity.



Mycothiazole (13)

Fig. 4

P. Makam *et al.*[7] reported the 2-(2-Hydrazinyl)thiazole derivatives: design, synthesis and *in vitro* antimycobacterial studies. Among the tested series of compounds, derivatives **(14)** and **(15)** were found to have promising activity with MIC values of 1.46 µM and 0.177 µM respectively.



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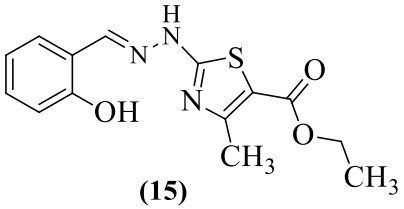
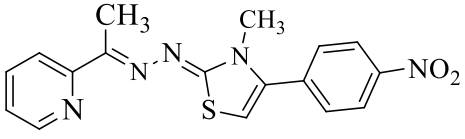


(15)

Fig. 5

E. B. da Silva and co-workers[8] reported the design and synthesis of potent anti-*Trypanosoma cruzi* agents new thiazoles derivatives which induce apoptotic parasite death. The compounds **16** and **17**, without affecting macrophages viability exhibited higher cytotoxic activity against the trypomastigote forms than the reference medicament benznidazole.



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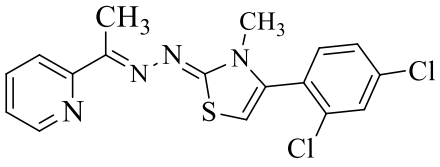
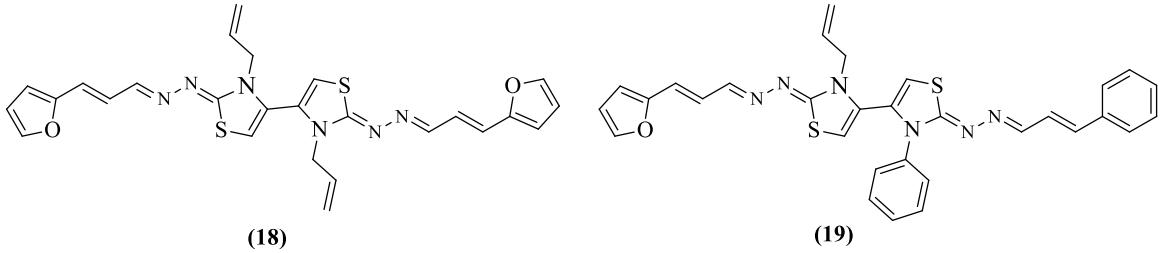


(17)

Fig. 6

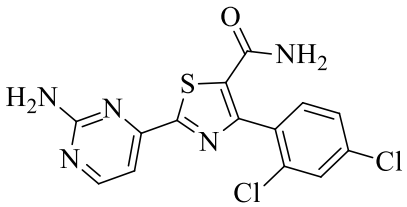
G. Alvarez *et al.*[9] described development of bis-thiazoles as inhibitors of triosephosphate isomerase from *Trypanosoma cruzi*. Identification of new non-mutagenic agents that are active *in vivo*. The bis-thiazoles **18** and **19** exhibited the best *in vitro* anti *T. cruzi* profile against amastigote form of the parasite than the reference drugs Nifurtimox and Benznidazole profile with a higher selectivity index.



(18) **(19)**

Fig. 7

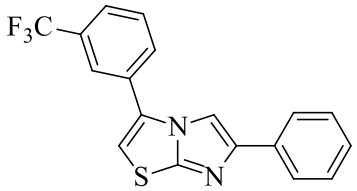
A. Reichelt and co-workers[10] reported Synthesis and structure-activity relationship of trisubstituted thiazoles as Cdc7 kinase inhibitors. From the SAR studies it was identified that, the compound **20** as a most potent selective Cdc7 inhibitor.



(20)

Fig. 8

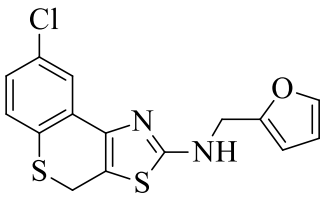
S. Koppireddi and co-workers[11] described the synthesis and anticancer evaluation of 3-aryl-6-phenylimidazo [2,1-*b*]thiazoles. Among the synthesized compounds the derivative **21** exhibited significant activity against HeLa cell-line, with IC_{50} as low as 6.5 µM.



(21)

Fig. 9

S. Bhat *et al.*[12] reported tricyclic thiazoles are a new class of angiogenesis inhibitors. The compounds, **22** and **23** were identified as potential lead antiangiogenic agents by the endothelial tube formation assay.



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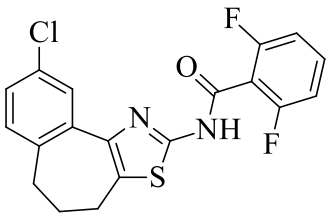
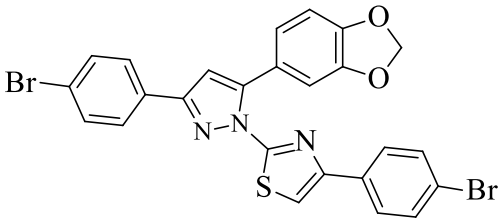


(23)

Fig. 10

H. H. Wang *et al.*[13] reported Synthesis, molecular docking and evaluation of thiazolyl-pyrazoline derivatives containing benzodioxole as potential anticancer agents. Among the synthesized compounds, derivative **24** have shown high antiproliferative activity against MCF-7 and B16-F10 cell lines, with IC_{50} value of 0.09 and 0.12 µM, respectively, being comparable with the positive control Erlotinib.

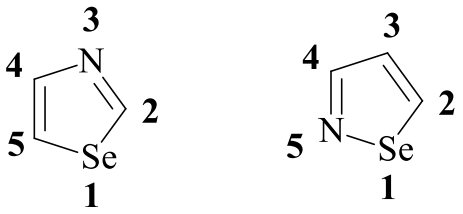


(24)

Fig. 11

Selenazoles

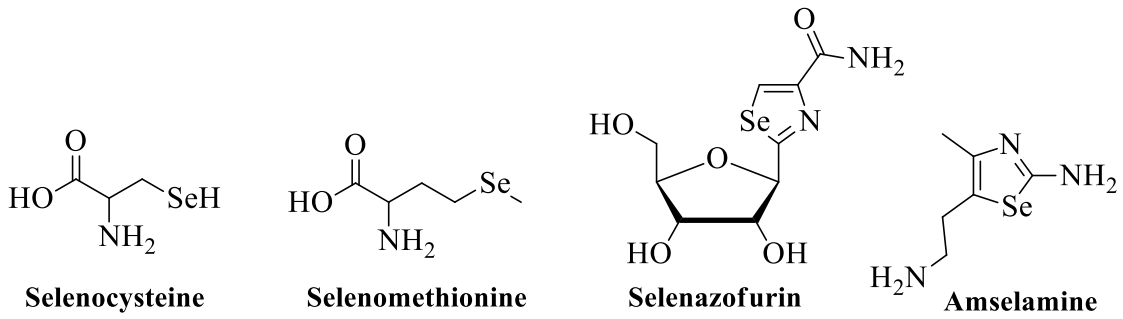
Selenium (Se) is an essential micronutrient for animals and is an integral component of several unusual amino acids (selenocysteine and selenomethionine), enzymes (glutathione peroxidises and thioredoxin reductase). Selenium is an essential trace element, it is toxic if taken in excess, its use as a nutritional supplement has been popularized recently due to its potential role as an antioxidant in low concentrations and in higher concentrations as an anticancer agent.[14]



(25) **(26)**

Fig. 12

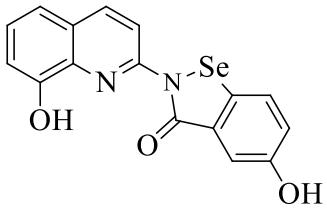
Among the selenium containing heterocyclic compounds (selenazoles **25** and isoselenazole **26**), selenazoles (1,3-selenazole) are the principal core structures present in a wide variety of natural products and have attracted much attention due to a wide variety of medicinal and biological properties. Prominent examples are selenazofurin (2-*β*-ribofuranosyl-1,3-selenazole-4-carboxamide) as antiviral agent[15] and amselamine [2-amino-5-(2-aminoethyl)-4-methyl-1,3-selenazole] as histamine H_2-agonist.[16] Recently, it was reported that 1,3-selenazole possess strong inhibitory activity against inducible nitric oxide synthase.[17] 2-amino-1,3-selenazoles are also good superoxide anion-scavengers.[18] Moreover, 2-dialkylamino-1,3-selenazole is an important starting material for preparing dyes.[19]



Selenocysteine **Selenomethionine** **Selenazofurin** **Amselamine**

Fig. 13

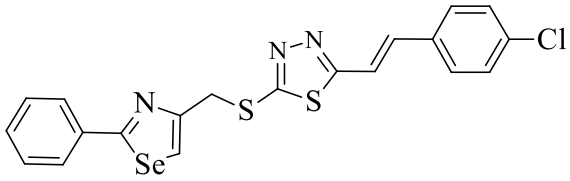
B. Wang and co-workers[20] described the synthesis and evaluation of 8-hydroxyquinolin derivatives substituted with (benzo[*d*][1,2]selenazol-3(2*H*)-one) as effective inhibitor of metal-induced Aβ aggregation and antioxidant. Among the tested series, compound **27** is the found to be most potent peroxide scavenger.



(27)

Fig. 14

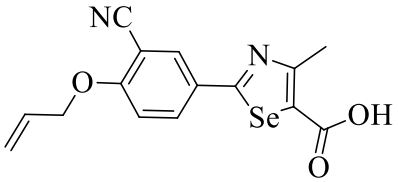
H. C. Zhao *et al.*[21] reported the synthesis and antitumor-evaluation of 1,3-selenazole-containing 1,3,4-thiadiazole derivatives. From the anticancer results it was found that the compound **28** was the most potent compound with IC_{50} value of 4.02 μM.



(28)

Fig. 15

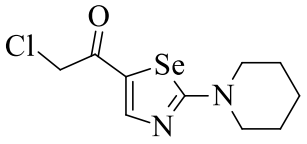
Q. Guan *et al.*[22] described the Synthesis and bioevaluation of 2-phenyl-4-methyl-1,3-selenazole-5-carboxylic acids as potent xanthine oxidase inhibitors. From the results, it was observed that the compound **29** emerged as the most potent xanthine oxidase inhibitor IC_{50}=5.5 nM in comparison to the standard febuxostat (IC_{50}=18.6 nM).



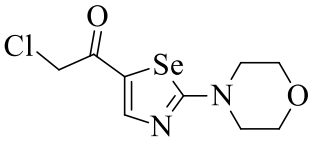
(29)

Fig. 16

K. N. Nam *et al.*[23] reported 5-Chloroacetyl-2-amino-1,3-selenazoles attenuate microglial inflammatory responses through NF-kappaB inhibition. The compounds **30** and **31** were found to be most potent antioxidant agents and strongly inhibit lipopolysaccharide-induced nitric oxide release from microglial cells.



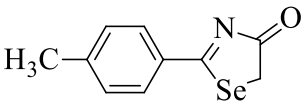
(30)



(31)

Fig. 17

M. Koketsu and co-workers[24] reported Inhibitory effects of 1,3-selenazol-4-one derivatives on mushroom tyrosinase. Among all the compounds, 2-(4-methylphenyl)-1,3-selenazol-4-one **(32)** exhibited the strongest inhibitory effect.

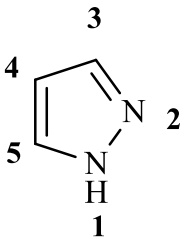


(32)

Fig. 18

Pyrazole

Pyrazoles[25] (**33**) are one of the important classes of 5-membered simple aromatic heterocyclic compounds with adjacent two nitrogen atoms in which one is basic and the other is neutral in nature. Pyrazole and its derivatives attained considerable popularity in drug discovery due to their appearance as the core structure in a large variety of compounds which are having versatile biological applications[26] that includes antimicrobial, anticonvulsant, anticancer, analgesic, antiinflammatory, antitubercular, cardiovascular agents etc.



(33)

Fig. 19

Some of the notable biologically potent[27] compounds and drugs embedding a pyrazole ring are given in the **Fig. 20**.

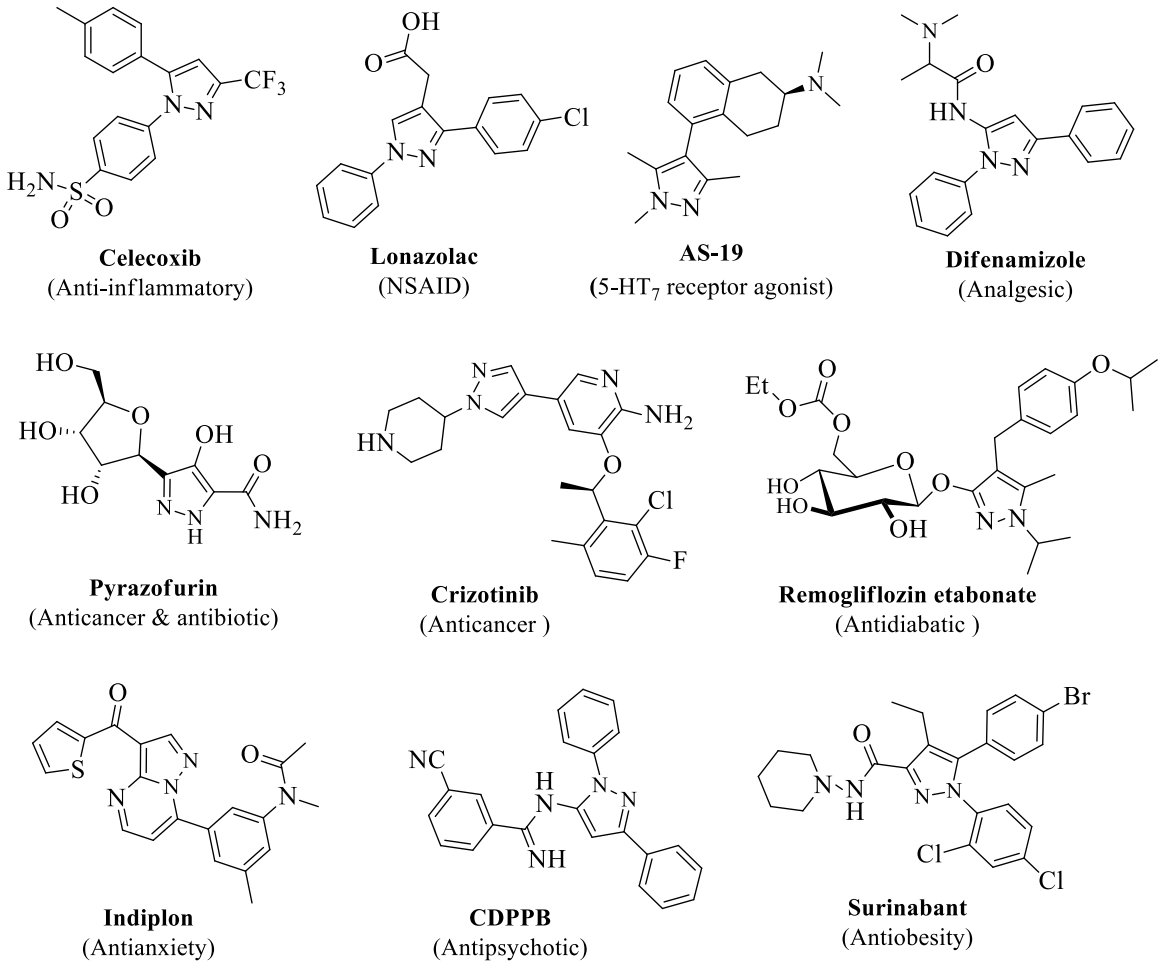


Fig. 20

Deiana V *et al.*[28] reported Tricyclic pyrazoles. Part 8. Synthesis, biological evaluation and modelling of tricyclic pyrazole carboxamides as potential CB_2 receptor ligands with antagonist/inverse agonist properties. Among the synthesized compounds, derivatives **34**, **35** and **36** exhibited the highest CB_2 receptor affinity with high selectivity.

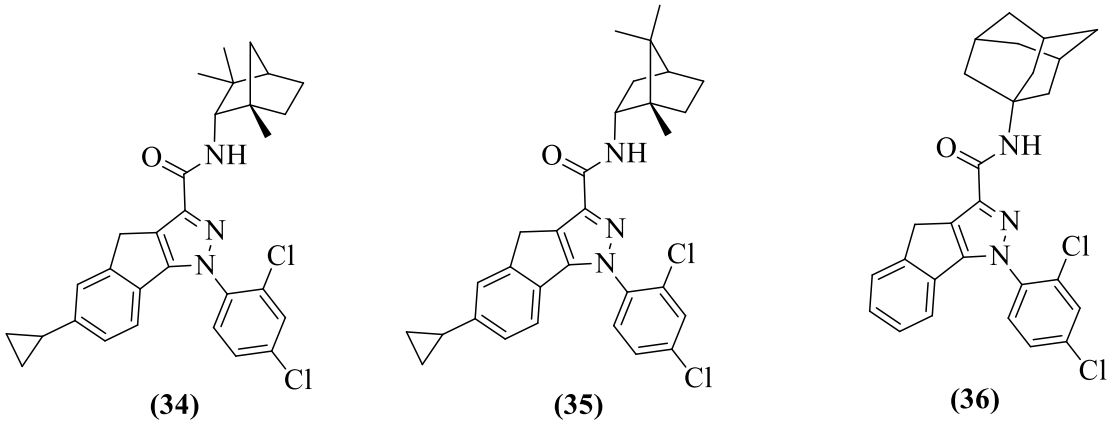


Fig. 21

B. Manjunatha *et al.*[29] described the design, synthesis and characterization of new 1,2,3-triazolyl pyrazole derivatives as potential antimicrobial agents *via* a Vilsmeier–Haack reaction approach. From the results, the compounds **37**, **38**, **39** and **40** were found to be having potent and broad spectrum antibacterial agents comparable to that of the positive control drug Ciprofloxacin.

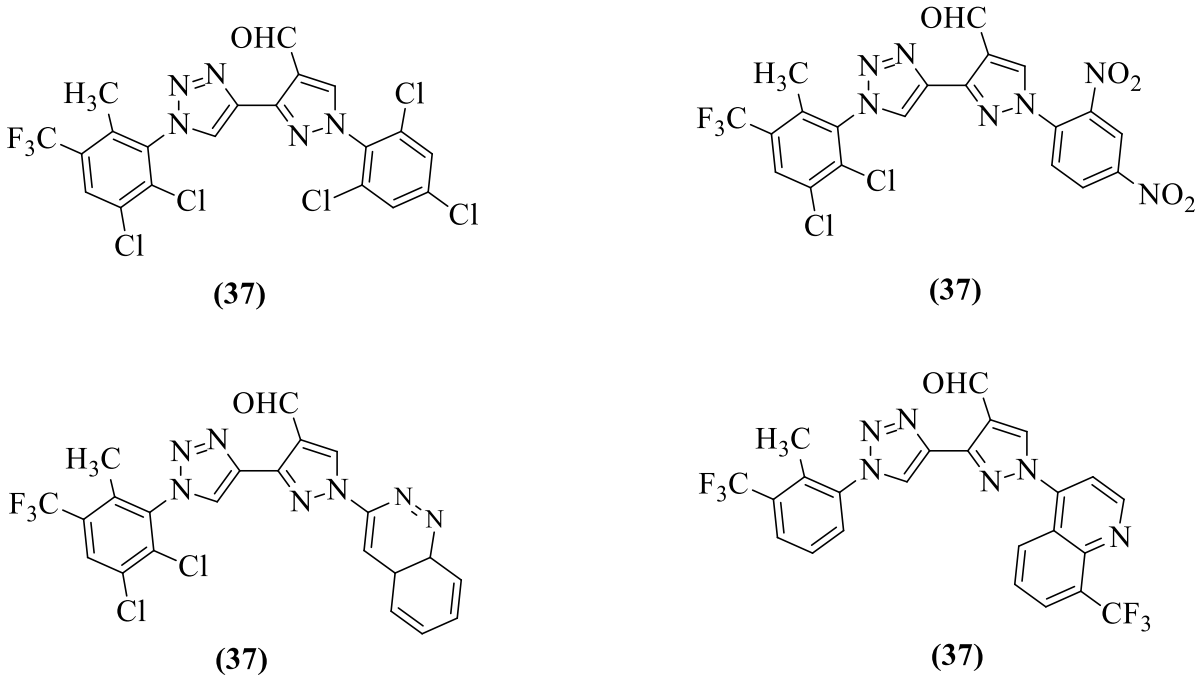
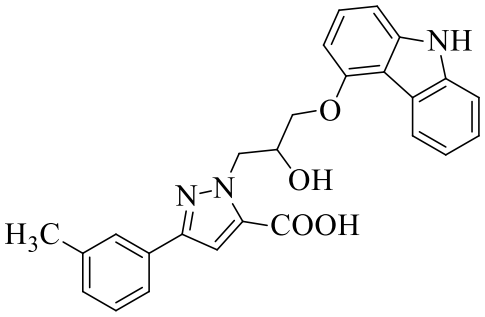


Fig. 22

N. Lingaiah *et al.*[30] reported the synthesis and cytotoxicity evaluation of 1-[3-(9*H*-carbazol-4-yloxy)-2-hydroxypropyl]-3-aryl-1*H*-pyrazole-5-carboxylic acid derivatives.

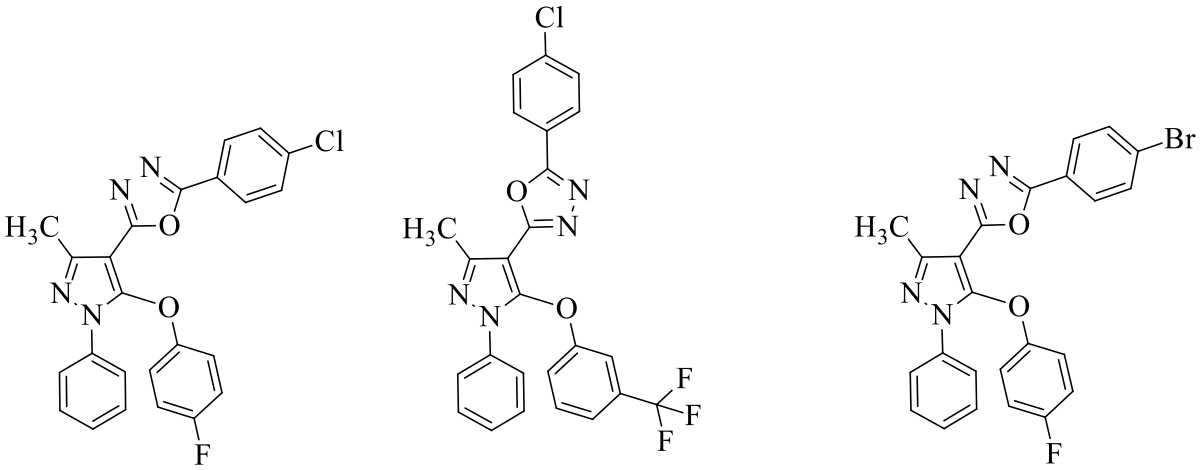
From the cytotoxicity results, the compound **38** was identified as a potential lead compound effective against the cell-line SK-N-SH human neuroblastoma (NB).



(38)

Fig. 23

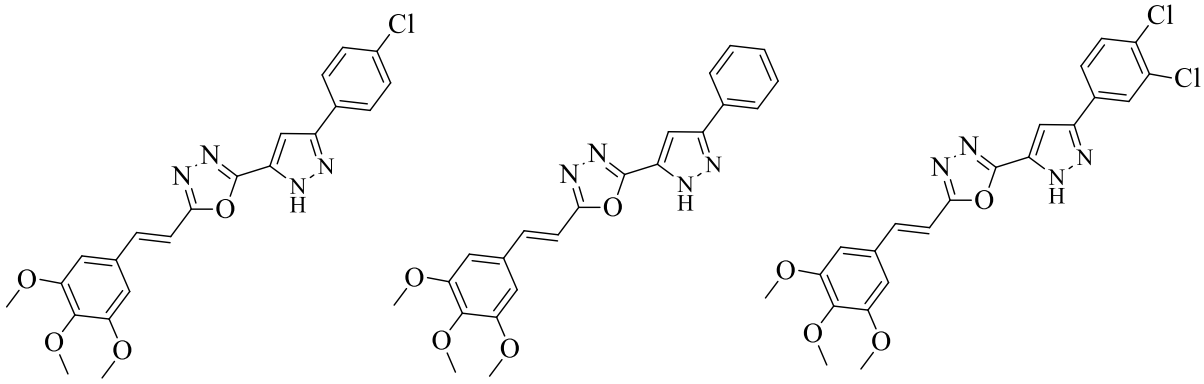
C. K. Sharad and co-workers[31] have reported the Design, Synthesis and characterization of fluoro substituted novel pyrazole nucleus clubbed with 1,3,4-oxadiazole scaffolds and their biological applications. From the antibacterial results, the compounds **39**, **40** and **41** were found to possess promising activity.



(39) **(40)** **(41)**

Fig. 24

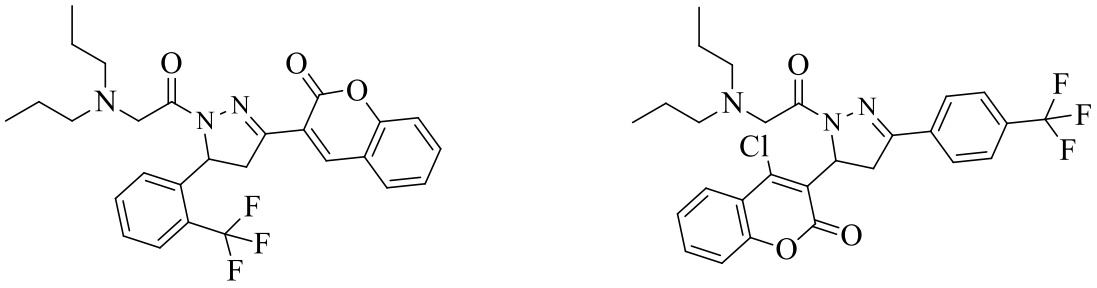
Ahmed Kamal and co-workers[32] reported the Pyrazole–oxadiazole conjugates: synthesis, antiproliferative activity and inhibition of tubulin polymerization. Among synthesized hybrids, **42**, **43** and **44** exhibited potent cytotoxicity with IC_{50} values ranging from 1.5 μM to 11.2 μM and tubulin polymerization inhibition with IC_{50} values of 1.3 μM, 3.9 μM and 2.4 μM respectively.



(42) **(43)** **(44)**

Fig. 25

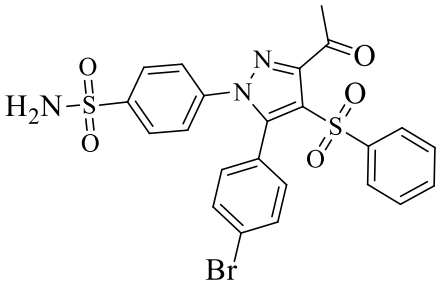
Dong zhi Qiang *et al.*[33] described novel 2*H*-chromen derivatives: design, synthesis and anticancer activity. The anti-proliferative results revealed that compounds **45** and **46** displayed strong inhibitory activity against HepG2 cell.



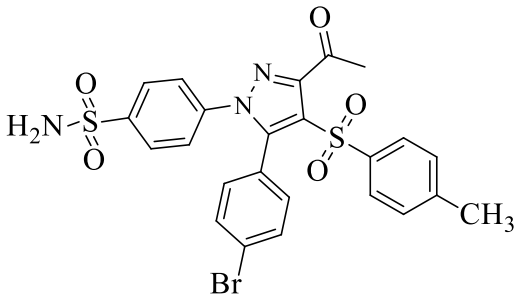
(45) **(46)**

Fig. 26

H. A. Abdel-Aziz *et al.*[34] reported the synthesis of *N*-benzenesulfonamide-1*H*-pyrazoles bearing arylsulfonyl moiety: novel celecoxib analogs as potent anti-inflammatory agents. The anti-inflammatory results showed that the compounds **47** and **48** showed excellent activity higher than that of celecoxib (ED_{50}=86±1.1 µM/kg) with $ED5_0$=68 ±2.2 and 51 ±0.7 µM/kg, respectively.



(47)



(48)

Fig. 27

Coumarins

Coumarins[35] (2*H*-chromen-2-one) are plant-derived polyphenolic compounds belongs to the benzopyrone chemical class, mainly found in plants of the family of *Rutaceae, Umbelliferae, Clusiaceae, Guttiferae, Caprifoliaceae, Oleaceae, Nyctaginaceae,* and *Apiaceae.* Coumarin (known as 1,2-benzopyrone or o-hydroxycinnamic acid-8-lactone) consisting of fused benzene and α-pyrone rings (**Fig. 28**) and gained momentous attention due to their presence as a core moiety in many of the natural[36] and synthetic[37] biologically active products and also due to their variety of biological applications that include anticancer,[38] antimicrobial,[39] anti-oxidant,[40] antiallergic, antithrombotic,[41] anti-inflammatory,[42] anti-TB,[43] anticoagulant,[44] vasorelaxant, anti-HIV,[45] cyclooxygenase and lipoxygenase inhibitors, antifertillity, anti-psychotic, MAO inhibitory activity and also as antiviral agents.[46]

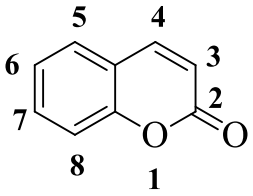


Fig. 28

Coumarin is a core structural motif present in numerous naturally occurring and synthetic pharmacologically potent compounds (**Fig. 29**).[47]

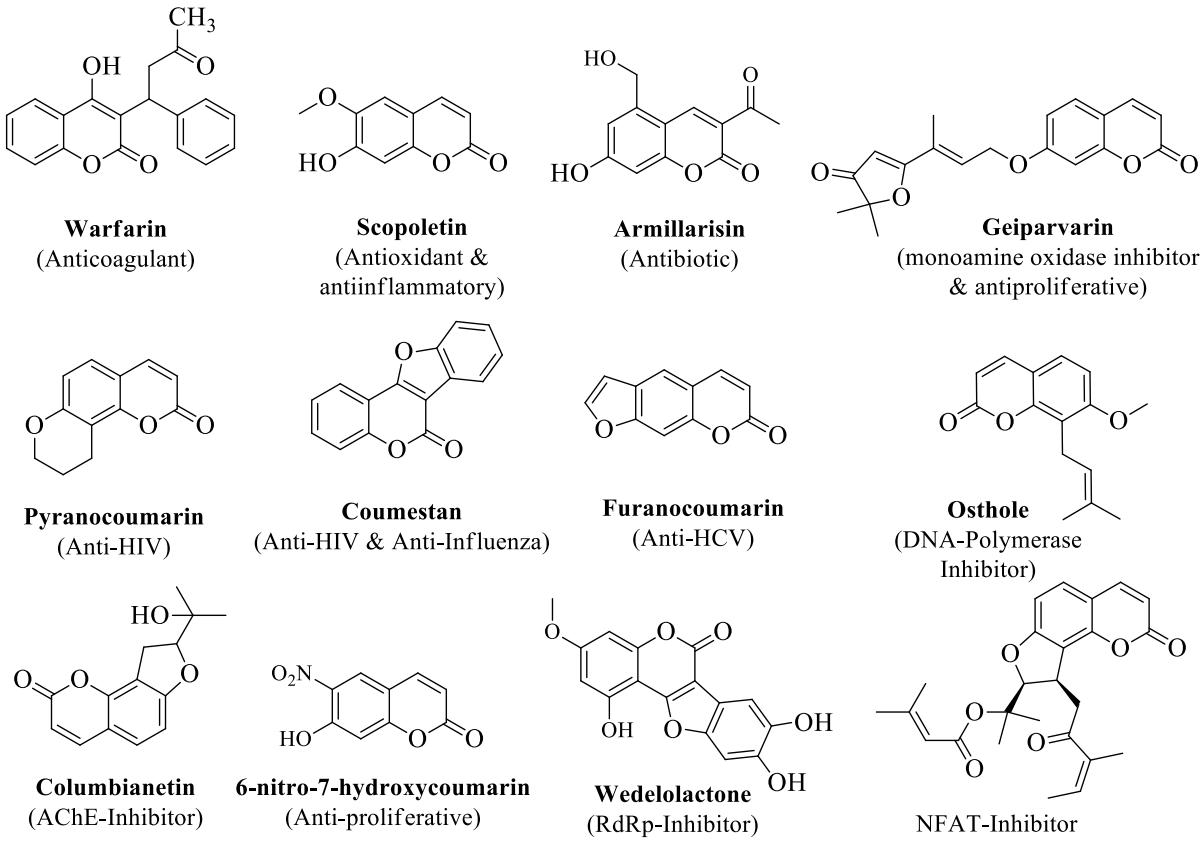
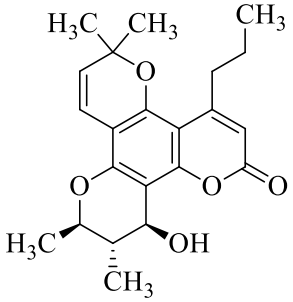


Fig. 29

Calanolide A is a non-nucleoside reverse transcriptase inhibitor (NNRTI) first isolated from *Calophyllum lanigerum* trees in Malaysia. Before it was identified as a potent anti-HIV inhibitor,[48] the U.S. National Cancer Institute tested calanolide A for anticancer activity, but the results were negative.

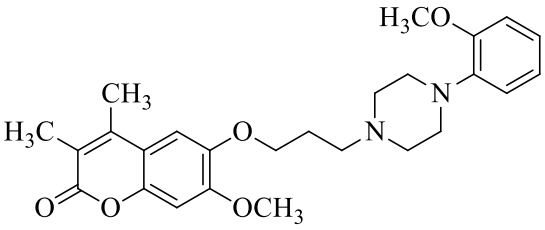


Calanolide A

Fig. 30

Ensaculin (KA-672) is a drug belongs to the coumarin family; initially it has been researched for the treatment for dementia which acts on a number of receptor systems,

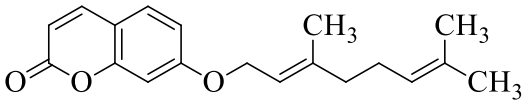
being both a weak NMDA antagonist and a $5HT_{1A}$ agonist.[49] *In vivo* study proved to be possessing potential nootropic effects.[50]



Ensaculin

Fig. 31

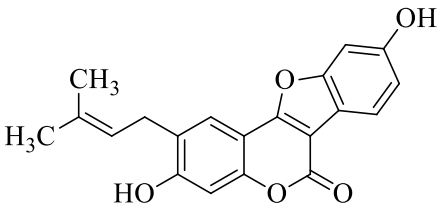
Auraptene (34) was first isolated from *Citrus* family plants and is known for curing degenerative diseases. It is naturally occurring bioactive monoterpene coumarin ether. Most of the literature studies have reported it as a chemo-preventative agent for variety of cancers like liver, skin, tongue, oesophagus, and colon in rodent models[51] and it was also reported as an inhibitor of cholesterol etherification and a modulator of oestrogen.[52]



Auraptene

Fig. 32

Psoralidin (37) is a natural phenolic compound and is the main constituent in the seeds of *Psoralea corylifolia*. It has the ability to inhibit *in vitro* against gastric, colon, prostate, and breast cancer cell-lines[53] and also regulates the insulin signalling by acting as a protein tyrosine phosphatase 1B inhibitor, which is a key metabolite involved in insulin signalling.[54]

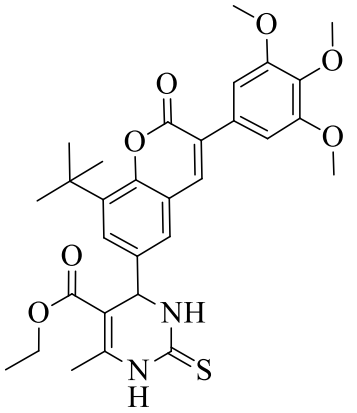


Psoralidin

Fig. 33

K. V. Sashidhara *et al.*[55] reported the discovery of coumarin-monastrol hybrid as potential antibreast tumor-specific agent. Among the synthesized compounds, the

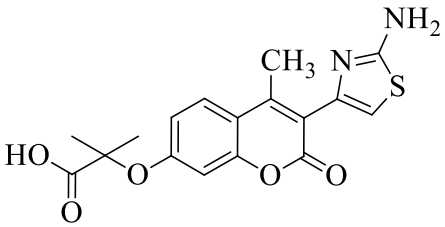
derivative **49** exhibited potent anti-proliferation against the breast cancer cell lines MCF-7 (IC_{50}~2.4 mM), T47D (IC_{50}~3.1 mM) and MDA-MB-231 (IC_{50}~3.9 mM) at all concentrations in a time dependant manner.



(49)

Fig. 34

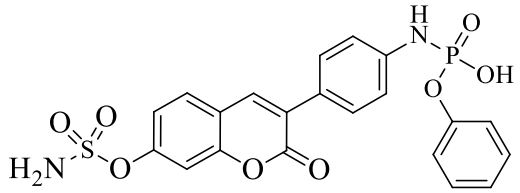
K. Venkata Sairam *et al.*[56] have reported the cytotoxicity studies of coumarin analogs: design, synthesis and biological activity. Compound **50** was identified as a potential lead compound with IC_{50} value of 2.4 and 4.8 µM for MCF-7 and MDA-231 respectively by MTT assay.



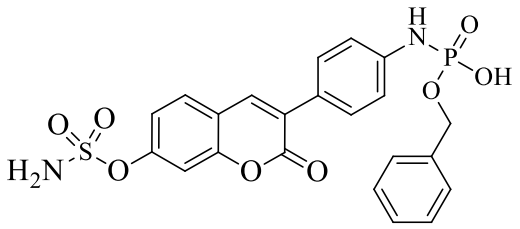
(50)

Fig. 35

D. Mateusz *et al.*[57] described the synthesis and steroid sulfatase inhibitory activities of *N*-phosphorylated 3-(4-aminophenyl)-coumarin-7-*O*-sulfamates. From the steroid sulfatase (STS) inhibiting results, the compounds **51** and **52** were found to be possess promising activity with IC_{50} values of 0.19 and 0.24 µM, respectively.



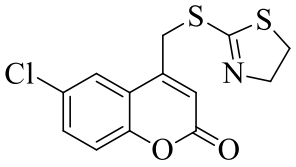
(51)



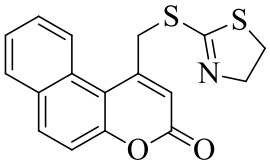
(52)

Fig. 36

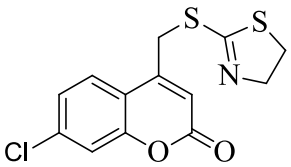
M. H. Kallappa and co-workers[58] reported a facile synthesis and evaluation of new biomolecule-based coumarinthiazoline hybrids as potent anti-tubercular agents, cytotoxicity, DNA cleavage and X-ray studies. Among the tested series, compound **53** exhibited promising anti-tubercular activity with MIC 0.09 µg/mL with very less cytotoxicity and the compounds **54** and **55** exhibited nuclease activity by cleaving the DNA.



(53)



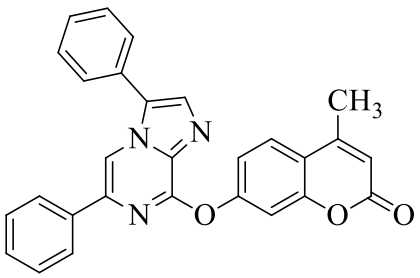
(54)



(55)

Fig. 37

G. Richa *et al.*[59] described the synthesis, *in vitro* anticancer activity and SAR studies of arylated imidazo[1,2-*a*]pyrazine-coumarin hybrids. Anticancer activity results revealed that the symmetrical diarylated hybrids **56** and **57** exhibited broad spectrum of activity against most of the tested cell-lines.



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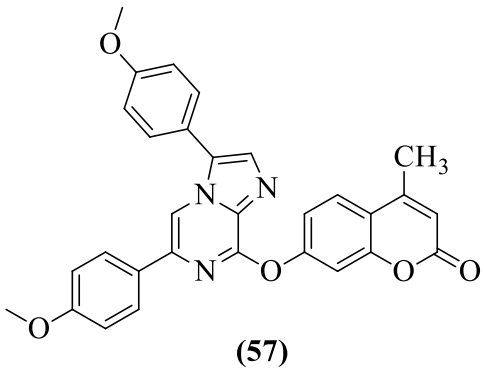


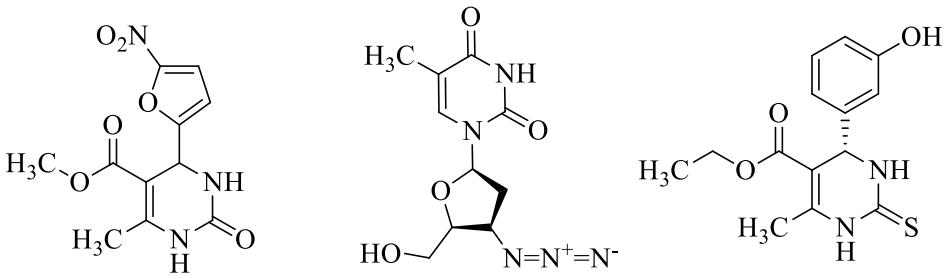
(57)

Fig. 38

3,4-Dihydropyrimidin-2(1*H*)-ones and Thiones

In 1891, Pietro Biginelli developed a multiple-component condensation reaction in order to synthesize 3,4-dihydropyrimidin-2(1*H*)-ones. This condensation reaction involves ethyl acetoacetate, an aryl aldehyde such as benzaldehyde and urea in alcohol and can be catalyzed by the Brønsted acids or by Lewis acids.[60]

In recent times, dihydropyrimidinone and their derivatives have gained considerable interest by the chemists due to their versatile pharmacological properties[61] including calcium channel blockers, antitumor, antiviral, antihypertensive and anti-inflammatory activities. **Nitractin (58)** was first reported in the 1960's as an antiviral agent specific against the trachoma group of viruses.[62] **Zidovudine** (ZDV) **(59)** and, also known as **azidothymidine** (AZT), is a first nucleoside antiretroviral drug used to prevent mother-to-child HIV spread during birth.[63] **Monastrol (60)** is a small, cell-permeable molecule that specifically inhibits the Kinesin-5 one of the family members, i.e., Eg5 there by restrict the cell-development during mitosis and it becomes emerged as a lead molecule to develop new anticancer drugs.[64]



Nitractin (58) **Zidovudine (59)** **Monastrol (60)**

Fig. 39

Thiazolo[3,2-*a*]pyrimidines and Thiazolo[3,2-*a*]pyrimidinones

In the recent years, the synthesis of thiazolo[3,2-*a*]pyrimidines and thiazolo[3,2-*a*]pyrimidinones have gained much attention due to the broad spectrum of biological activities such as antihypertensive,[65] anticancer,[66] anti-hyperglycaemic,[67] antimicrobial,[68] anti-inflammatory,[69] anti-arrhythmic, analgesic,[70] antiulcer,[71] antibacterial,[72] anti-HIV[73] and antitubercular activity.[74] Some of the natural occurring and synthetic biologically potent compounds embedding pyrimidine as core nucleus are illustrated in **Fig. 40**.

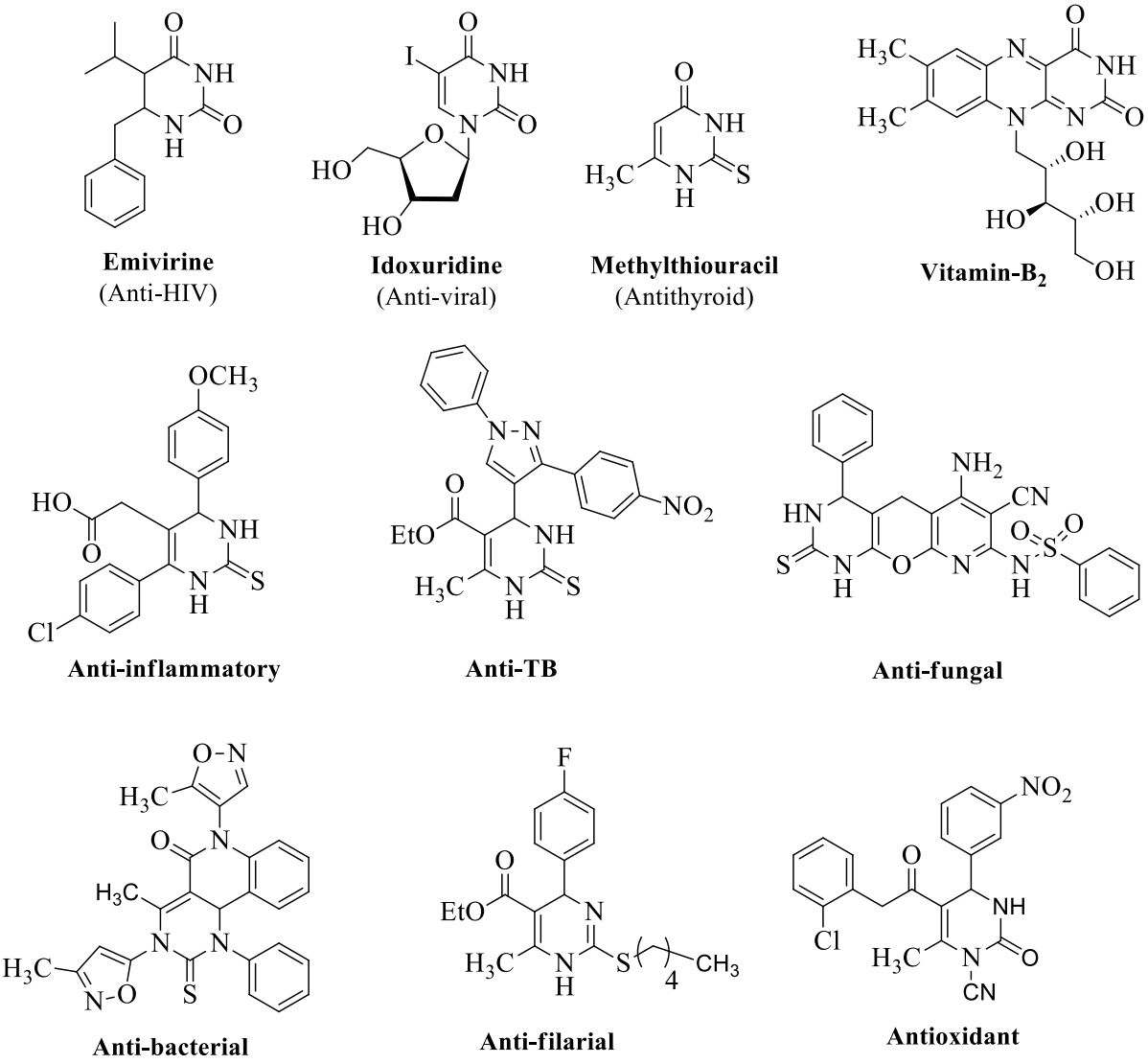
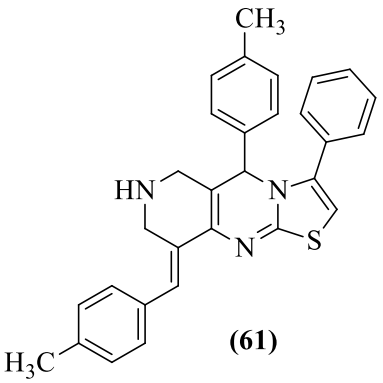
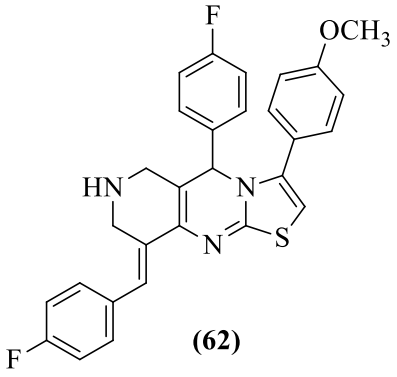


Fig. 40

A. Basiri and co-workers[75] reported the cholinesterase inhibitory activity versus aromatic core multiplicity: a facile green synthesis and molecular docking study of novel piperidone embedded thiazolopyrimidines. From the *in vitro* acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activity results, the compounds **61** against AChE and **62** against BChE were identified as most potent compounds than the standard drug galanthamine among the tested series with IC_{50} values 0.53 µM and 1.09 µM respectively.



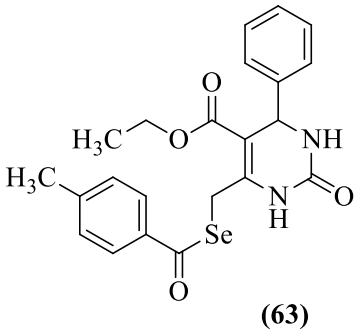
(61)



(62)

Fig. 41

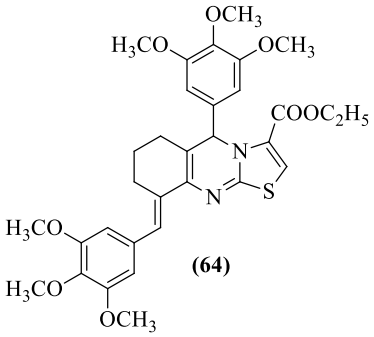
F. A. Barbosa *et al.*[76] reported the synthesis and evaluation of dihydropyrimidinone-derived selenoesters as multi-targeted directed compounds against Alzheimer's disease. The compound **63** was identified as a most promising compound among the synthesized series.



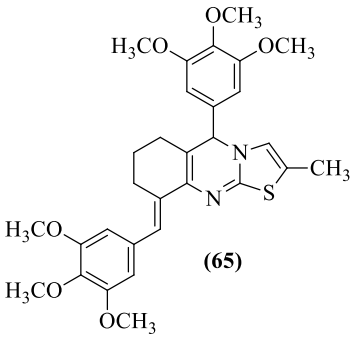
(63)

Fig. 42

G. S. Hassan[77] described the Synthesis and antitumor activity of certain new thiazolo [2,3-*b*]quinazoline and thiazolo[3,2-*a*]pyrimidine analogs. Antitumor activity results revealed that the compounds **64** and **65** were proved to 15 and 10-folds highly potent than the standard drug 5-flurouracil with GI_{50} MG-MID values of 1.5 and 2.4 µM respectively.



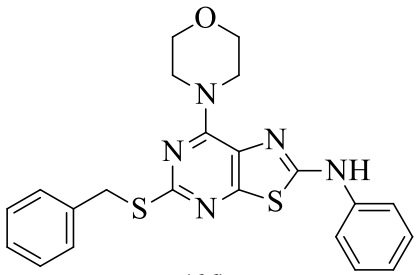
(64)



(65)

Fig. 43

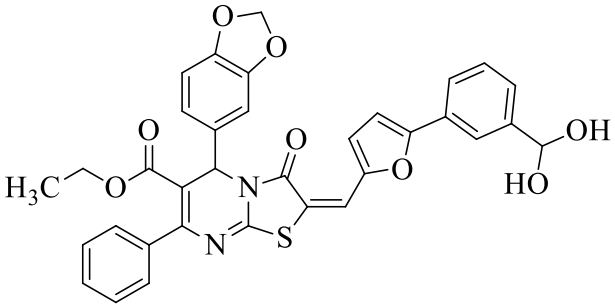
Z. H. Li *et al.*[78] described the Identification of thiazolo[5,4-*d*]pyrimidine derivatives as potent antiproliferative agents through the drug repurposing strategy. From the tested series of compounds, the derivative 66 exhibited potent anti-proliferative activity with high selectivity between cancer (MGC803) and normal cell. It also induced apoptosis in MGC803 cell-line.



(66)

Fig. 44

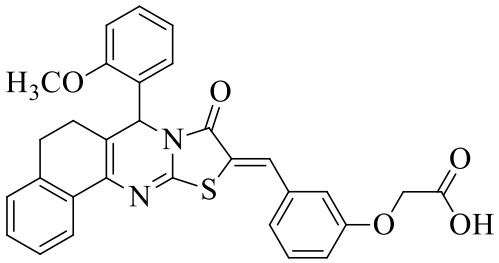
Z. Qin *et al.*[79] reported the structure-based discovery of inhibitors of the YycG histidine kinase: new chemical leads to combat Staphylococcus epidermidis infections. Among all the compounds, the thiazolo[3,2-*a*]pyrimidinone **(67)** has shown bactericidal effects on both planktonic and bio-film cells of *S.epidermidis* through inhibiting the autophosphorylation by binding to YycG protein.



(67)

Fig. 45

Y. Feng *et al.*[80] reported design, synthesis, and interaction study of quinazoline-2(1H)-thione derivatives as novel potential Bcl-xL inhibitors. In this work, the compound **(68)** was identified as a potent Bcl-x_L inhibitor with IC_{50} of 3.4 μM.

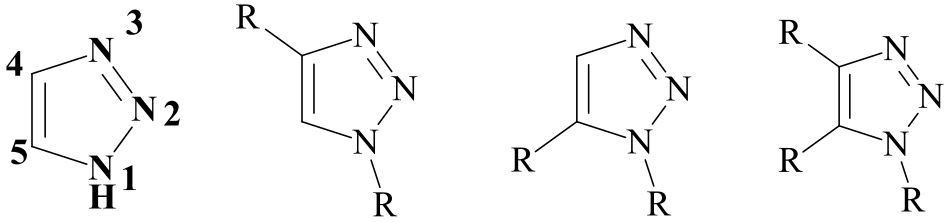


(68)

Fig. 46

1,2,3-triazoles

1,2,3-triazole[81] (**69**) is a five-membered basic aromatic heterocyclic[82] with three nitrogen atoms at 1, 2 and 3 positions. Depending up on the posiiton of substitution, substituted 1,2,3-triazoles are of three types, they are 1,4-disubstituted 1,2,3-triazoles (**70**), 1,5-disubstituted 1,2,3-triazoles (**71**) and 1,4,5-trisubstituted 1,2,3-triazoles **(72)**. Among the 1,2,3-triazole heterocyclic compounds, 1,4-disubstituted derivatives are the most common and can be acheived by the 1,3-dipolar cycloaddition reaction between azide and terminal or internal alkyne, which is also called as azide-alkyne Huisgen cycloaddition.



(69) **(70)** **(71)** **(72)**

Fig. 47

In the recent years, the synthesis of 1,2,3-triazole based heterocycles has gained much attention from the medicinal chemists and it becomes a cornerstone of drug discovery and medicinal chemistry due to their wide variety of potential biological activities[83] such as anticancer, antimicrobial, antioxidant, anti-HIV, anti-inflammatory, antifungal activities. Moreover, design and synthesis of many heterocyclic's linked with 1,2,3-triazole and their derivatives have received much attention due to their potential structural features includes moderate dipolar character, selectivity, rigidity, planarity, dipole moment, and

stability to metabolic degradation under *in-vivo* conditions and their binding ability through hydrogen bonding with various bio-molecular targets.[84] Some of the examples of triazole bearing commercial drugs and pharmacologically active molecules are given in **Fig. 48**.

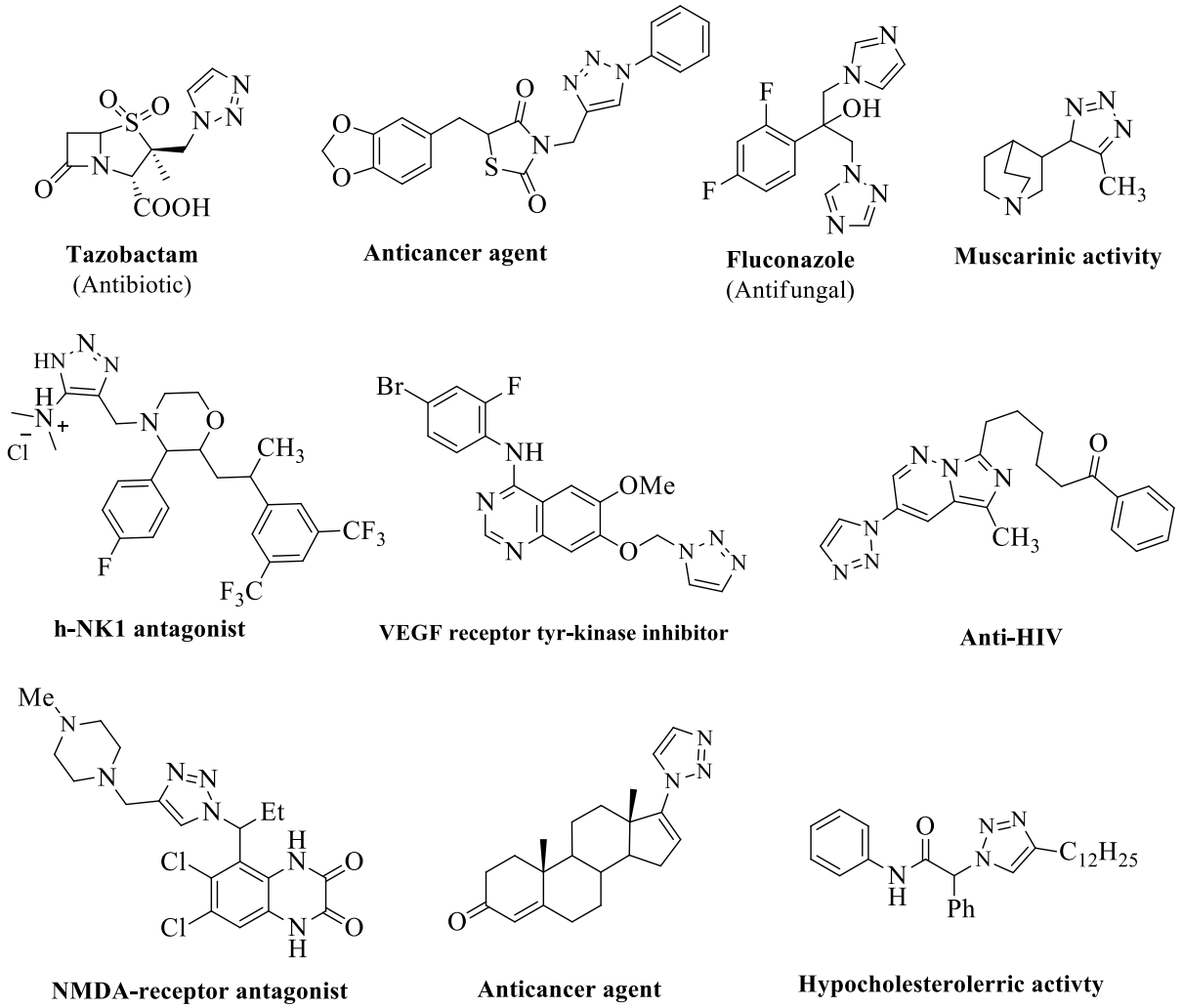


Fig. 48

F. Reck *et al.*[85] reported the identification of 4-substituted 1,2,3-triazoles as novel oxazolidinone antibacterial agents with reduced activity against monoamine oxidase A. In this work, they have found that the compounds **73**, **74**, **75** and **76** were good antibacterial agents with reduced activity against MAO-A, within the detection limit of the assay.

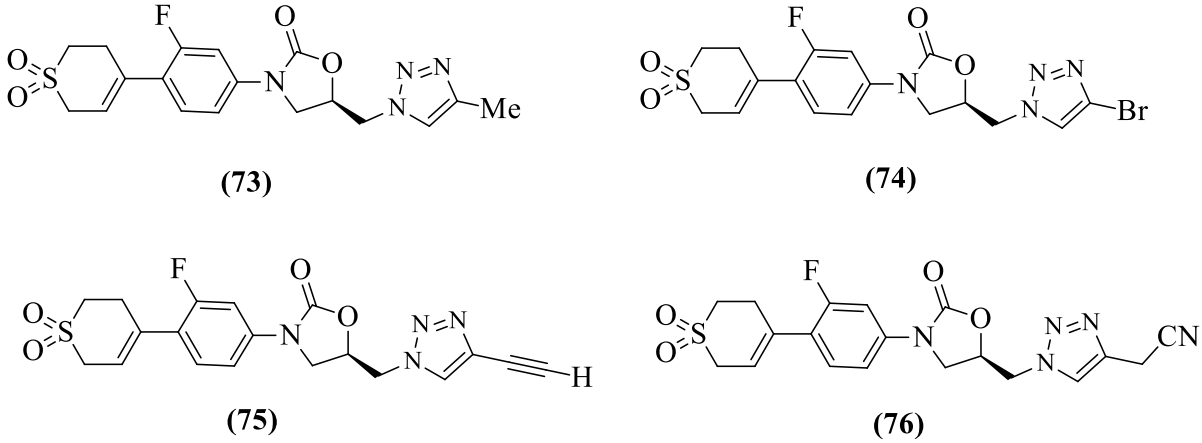


Fig. 49

C. P. Kaushik *et al.*[86] have reported the synthesis and antimicrobial evaluation of 1,4-disubstituted 1,2,3-triazoles with aromatic ester functionality. Compound **77** against bacterial and **78** and **79** against tested fungal strains showed significant activity.

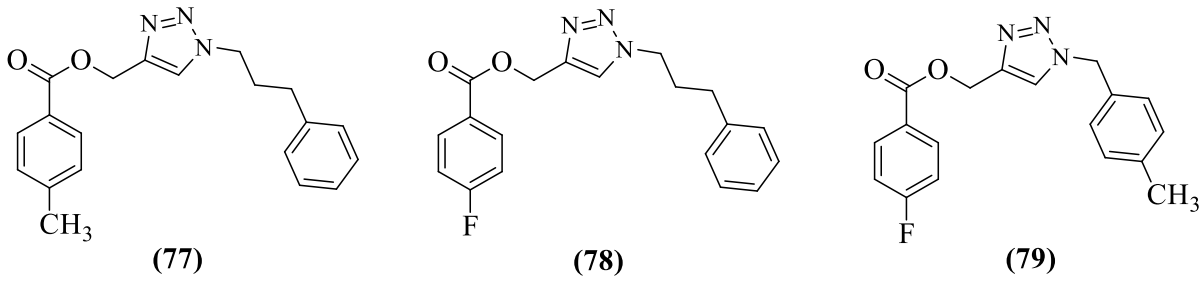


Fig. 50

S. A. Bakunov *et al.*[87] reported the synthesis and antiprotozoal activity of cationic 1,4-diphenyl-1*H*-1,2,3-triazoles. Compound **80** displayed antiplasmodial activity with IC_{50} value of 0.6 ηM and **81** showed better antitrypanosomal efficacies than melarsoprol.

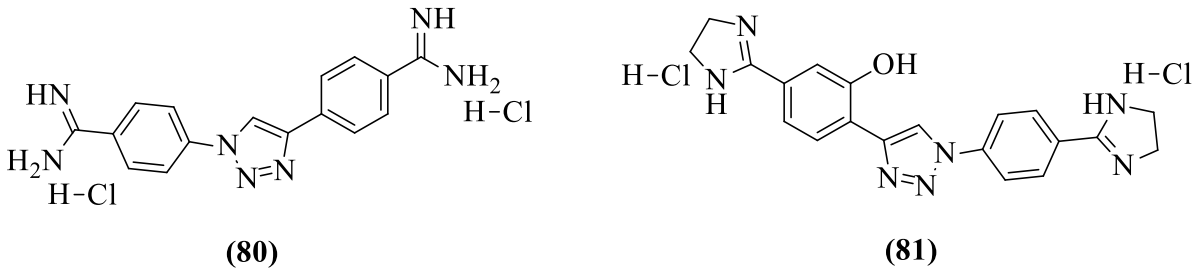
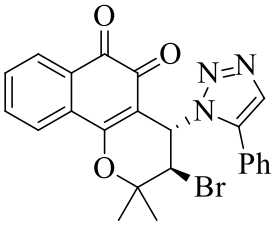


Fig. 51

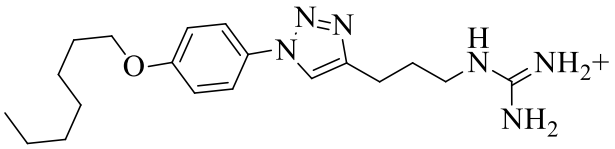
S. B. B. B. Bahia and co-workers[88] described molecular hybridization as a powerful tool towards multitarget quinoidal systems: synthesis, trypanocidal and antitumor activities of naphthoquinone-based 5-iodo-1,4-disubstituted-, 1,4- and 1,5-disubstituted-1,2,3-triazoles. Among the tested series, compound **82** was identified as a most potent against the tested cell-lines when compared to that of the β-lapachone precursor.



(81)

Fig. 52

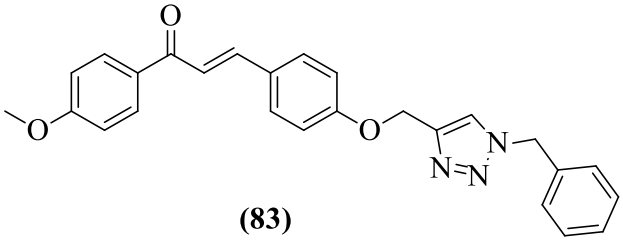
T. Bakka *et al.*[89] reported the Synthesis and antimicrobial evaluation of cationic low molecular weight amphipathic 1,2,3-triazoles. Compound **82** displayed potent inhibiting activity against *S. agalacticae, S. aureus* with MIC values 4 µg/mL.



(82)

Fig. 53

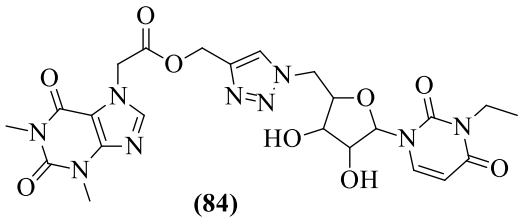
P. Yadav and co-workers[90] reported the green synthesis and anticancer potential of chalcone linked-1,2,3-triazoles. Compound **83** was identified as a most potent compound with better or comparable activity with that of positive control drug, against all the tested cancer cell lines with IC_{50} values in the range of 4-11 µM.



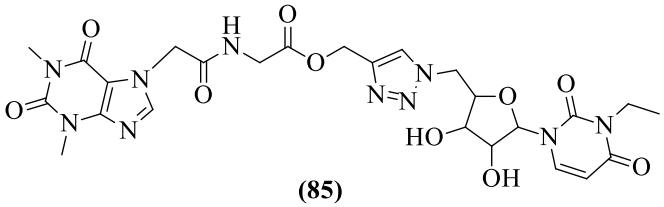
(83)

Fig. 54

R. R. Ruddarraju *et al.*[91] reported the design, synthesis, anticancer, antimicrobial activities and molecular docking studies of theophylline containing acetylenes and theophylline containing 1,2,3-triazoles with variant nucleoside derivatives. From the results it was identified that the compounds **84** and **85** displayed significant cytotoxic effect on all the tested cancer cell-lines (A549, HT-29, MCF-7 and A375) with IC_{50} values of 2.56, 2.19, 1.89, 4.89 μM and 3.57, 2.90, 2.10, 5.81 μM respectively.



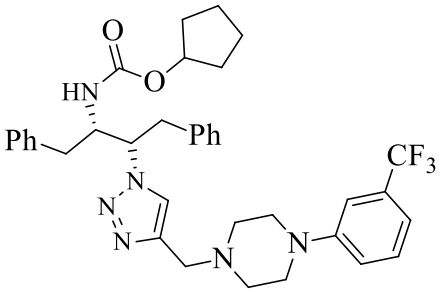
(84)



(85)

Fig. 55

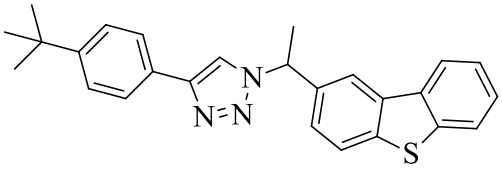
M. Whiting *et al.*[92] reported rapid discovery and structure-activity profiling of novel inhibitors of human immunodeficiency virus type 1 protease enabled by the copper(I)-catalyzed synthesis of 1,2,3-triazoles and their further functionalization.



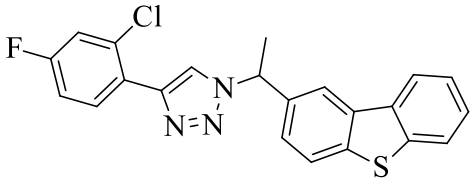
(84)

Fig. 56

S. R. Patpi and co-workers[93] described the design, synthesis, and structure-activity correlations of novel dibenzo[*b*,*d*]furan, dibenzo[*b*,*d*]thiophene, and *N*-methylcarbazole clubbed 1,2,3-triazoles as potent inhibitors of *Mycobacterium tuberculosis*. Out of all the tested compounds, derivatives **85** and **86** exhibited high profile of inhibitory activity against *M. tuberculosis* with MIC = 1.9 μM (0.78 μg/mL) which is 26-fold and 4-fold more active than pyrazinamide and ethambutol respectively with very low toxicity profile.



(85)

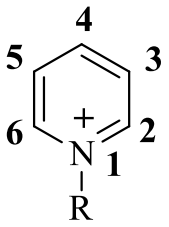


(86)

Fig. 57

Pyridinium salts

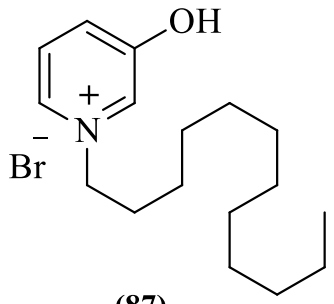
Quaternary pyridinium salts[94] are the unsaturated aromatic heterocyclic compounds which obeys Huckel's rule and are cationic conjugate acids of pyridine. Quaternization of pyridine ring can be achieved by the protonation or by simple alkylation. Pyridinium salts have various biological applications,[95] which include acetylcholinesterase (AChE), butyrylcholinesterase (BuChE) and AChE Carbamate inhibiting activity, which is used to cure neurodegenerative disorders like Alzheimer's disease (AD) and autoimmune disorder Myasthenia gravis (MG) respectively, anticancer, germicidal activity and antibacterial activities. Also they are known to have various industrial applications they are, applications in cosmetic, polymer industries and they are effectively utilizing as phase transfer catalysts (PTC's) and acylating agents.



R = H or Alkyl

Fig. 58

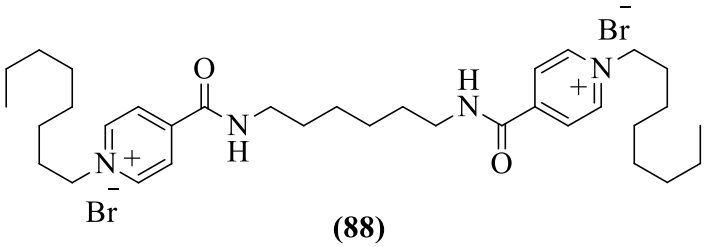
R. Dolezal *et al.*[96] reported their work towards understanding the mechanism of action of antibacterial *N*-alkyl-3-hydroxypyridinium salts: Biological activities, molecular modeling and QSAR studies. Among the tested compounds, the derivative **87** bearing *N*-dodecyl substituent showed MIC around 0.5 µM against methicillin resistant *S. aureus* (MRSA).



(87)

Fig. 59

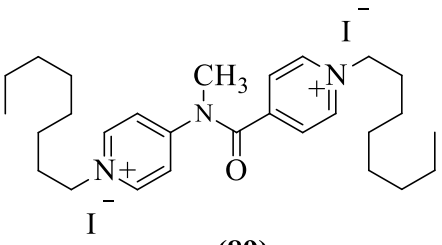
K. Fujimoto and co-workers[97] described antimalarial effect of bis-pyridinium salts, *N*,*N*'-hexamethylenebis(4-carbamoyl-1-alkylpyridinium bromide). All the synthesized compounds were assessed for their *in vitro P. falciparum* FCR-3 strain (ATCC 30932, chloroquine-sensitive). From the EC_{50} values, the compound **88** was identified as a most potent and ~10 fold more active than quinine (EC_{50} = 110 nM) and half as active as chloroquine (EC_{50} = 18 nM).



(88)

Fig. 60

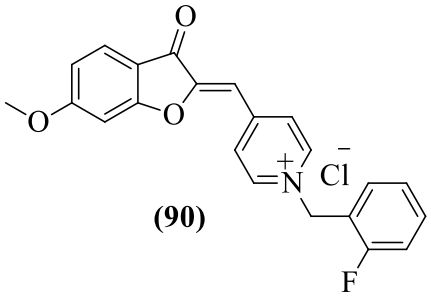
S. Fahs *et al.*[98] reported the development of a novel, multifunctional, membrane-interactive pyridinium salt with potent anticancer activity. From the anti-proliferative results, it was observed that the compound **89** exhibited potent anticancer activity through membrane lysis with EC_{50}s ranging from 9.8–312.5 µM on all the tested cell-lines.



(89)

Fig. 61

H. Nadri and co-workers[99] reported the design, synthesis and anticholinesterase activity of a novel series of 1-benzyl-4-((6-alkoxy-3-oxobenzofuran-2(3*H*)-ylidene) methyl) pyridinium derivatives. From the anticholinesterase activity results by using Ellman's method revealed that, among the tested compounds, the derivative **90** exhibited high levels of activity with IC_{50} = 10 ± 6.87 nM.



(90)

Fig. 62

Catalysis

Catalysis is a process in which the rate of chemical process increases due to the participation of additional substance i.e. catalyst.[100] The "**catalysis** (derived from Greek)" concept was first introduced by chemist **Elizabeth Fulhame** and later the term was coined by **Jons Jakob Berzelius**. According to him, the catalyst is a chemical substance (without being consumed in the process) which is needed in very small amounts to increase the rate of a chemical change by choosing a less activation energy pathway in order to reach transition state through less free-energy. Depending up on the miscibility of the catalyst with reaction medium, catalysts can be divided in to two categories, Homogeneous[101] (catalyst gets soluble in reaction medium to form single phase) and Heterogeneous catalysts[102] (whose molecules are not in single phase). Finally, all enzyme and bio-catalysts (protein based) comes under third category.

Ionic liquids

The design and development of new technologies and methodologies which are cleaner is a major concept in green chemistry. Here are the lists of principles developed by developed by **Paul Anastas** and **John Warner** to define greener conditions.[103]

1. Prevention of waste production
2. Atom Economy
4. Less Hazardous Chemical Syntheses
5. Designing Safer Chemicals
6. Safer Solvents and Auxiliaries
7. Design for Energy Efficiency
8. Use of Renewable Feedstocks
9. Reduce Derivatives
10. Catalysis
10. Design for Degradation
11. Real-time analysis for Pollution Prevention
12. Inherently Safer Chemistry for Accident Prevention

Among the several aspects of green chemistry, replacing the hazardous volatile organic solvents and polluting traditional homogeneous, heterogeneous catalysts with eco-friendly non-flammable recyclable task- specific dual solvent *i.e*. Ionic liquids[104] (ILs) have received considerable interest because of their unique properties[105] such as low volatilability, high thermal stability, recyclability, negligible vapour pressure, and ability to dissolve a wide range of materials.

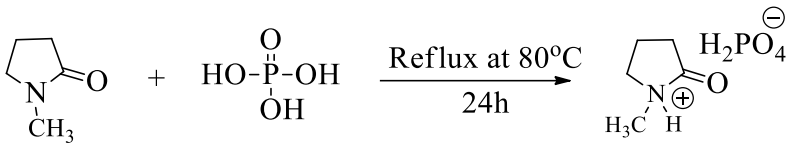
NEED AND OBJECTIVES OF THE PRESENT STUDY

Literature survey revealed that the heterocyclic compounds with 3,4-dihydropyrimidinones, 3,4-dihydropyrimidinethiones, thiazolo[3,2-*a*]pyrimidines, thiazolo[3,2-*a*]pyrimidin-3(5*H*)-ones, coumarins, pyridines, pyrazoles, thiazoles, selenazoles, triazoles and pyridinium salts known to as pharmacophores in many drugs which are in market. Aforementioned various biological applications of the scaffolds prompted us towards the design and synthesis of the molecules embedding one or more above pharmacophores utilizing either solvent-free conditions or in aqueous medium or under conventional method and evaluated for their possible biological activities.

The majority of the heterocyclic molecules described in this thesis were prepared by employing conventional methods. However some of the compounds were achieved by utilizing acidic ionic liquid [NMP]H_2PO_4.

_N_-Methyl-2-pyrrolidonium dihydrogen phosphate [NMP]H_2PO_4 acidic ionic liquid

NMP]H_2PO_4 was prepared according to the literature procedure[106]. *N*-methyl-2-pyrrolidinone was slowly added drop-wise to the cooled equimolar concentration of phosphoric acid, and the resulting mixture was heated at 80°C for 24h. The mixture was cooled to ambient temperature and washed with ether to remove any non-ionic residues. Then the residue is dried under high vacuum at 80°C on a rotary evaporator until the weight of the residue remains constant afforded the orange coloured, viscous ionic liquid.



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CHAPTER-II (SECTION-A)

3-(1-PHENYL-4-((2-(4-ARYLTHIAZOL-2-YL)HYDRAZONO)-METHYL)-1*H*-PYRAZOL-3-YL)-2*H*-CHROMEN-2-ONES: ONE-POT THREE COMPONENT CONDENSATION, *IN VITRO* ANTIMICROBIAL, ANTIOXIDANT AND MOLECULAR DOCKING STUDIES

INTRODUCTION

Most of the literature studies revealed that thiazoles and pyrazoles are the key moieties in heterocyclic chemistry and are the important structural backbone of various natural and synthetic biologically active molecules. They are known to possess a wide range of pharmacological activities that includes, antimicrobial,[1] anticancer,[2] anti-inflammatory,[3] antitubercular,[4] antihypertensive,[5] antidepressant,[6] anti-HIV,[7] anti-parkinsonian,[8] antiviral,[9] antiallergenic,[10] anticonvulsant,[11] antipyretic[12] and fibrinogen receptor antagonists with antithrombic activity.[13] Among the pyrazoles, especially 4-functionalized pyrazoles have been known to exhibit better antimicrobial and anti-inflammatory activities.[14] Similarly, coumarin is a core structural motif present in numerous naturally occurring compounds,[15] and have been reported to possess anticancer, anticoagulant, anti-inflammatory, antimicrobial, antioxidant, antiviral and cardiovascular activities[16,17] (**Fig. 1**).

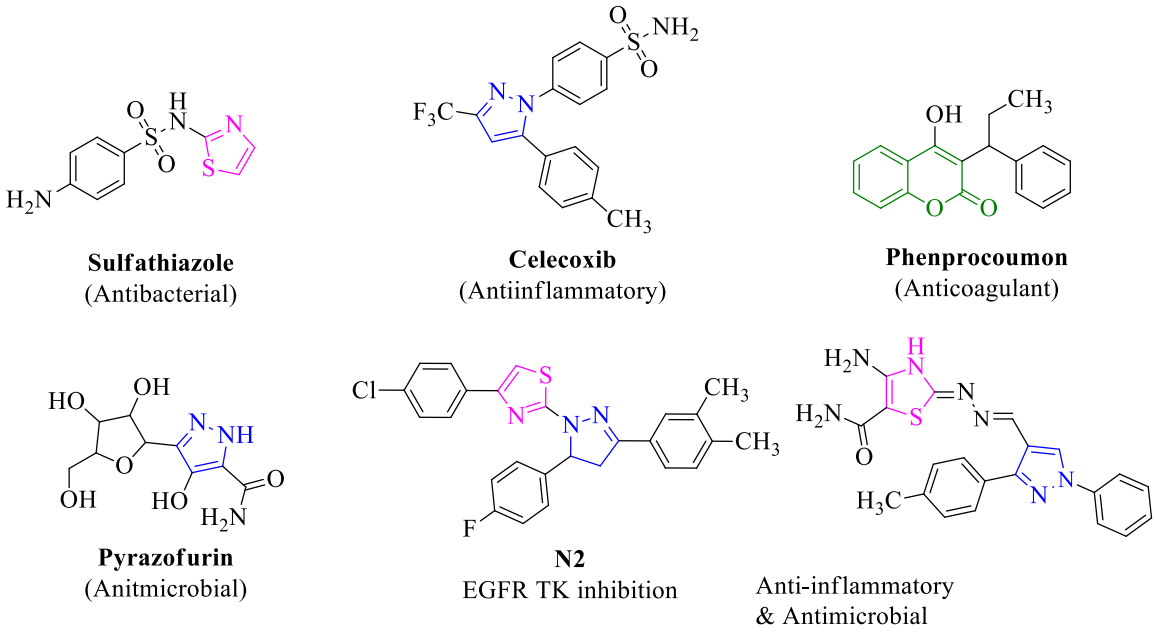
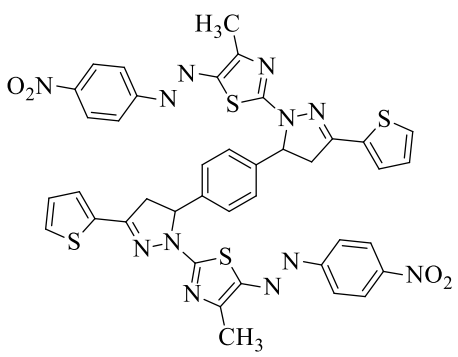


Fig. 1. Biologically active thiazole, pyrazole and coumarin derivatives.

Sobhi M. Gomha *et al.*[18] reported the synthesis and characterization of some new bis-pyrazolyl-thiazoles incorporating the thiophene moiety as potent anti-tumor agents. Among the synthesised compounds the derivatives **1**, **2** and **3** exhibited promising antitumor activity against Hepatocellular carcinoma (HepG2) cell line by MTT assay with IC_{50} values 1.37±0.15, 1.41±0.17, 1.62±0.20 μM, respectively.



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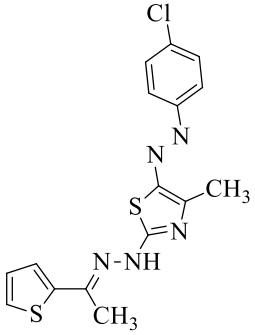

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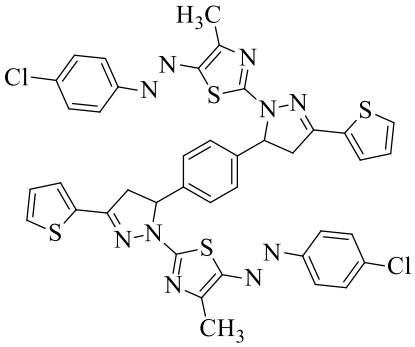
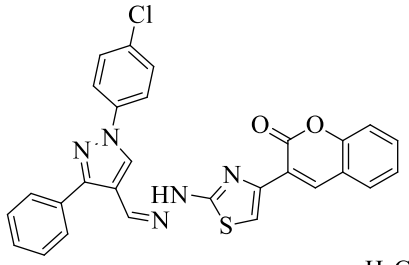


3

N. Harikrishna *et al.*[19] reported 1,3,4-Trisubstituted pyrazole bearing a 4-(chromen-2-one) thiazole: synthesis, characterization and it's biological studies. The synthesised compounds were assessed for their *in vitro* antibacterial and antifungal studies by the well diffusion method. Among the synthesised compounds it was found that the derivatives **4**, **5** and **6** were having Minimum Inhibitory Concentration (MIC) as low as 15.6 μgmL^{-1} against both bacterial and fungal strains.



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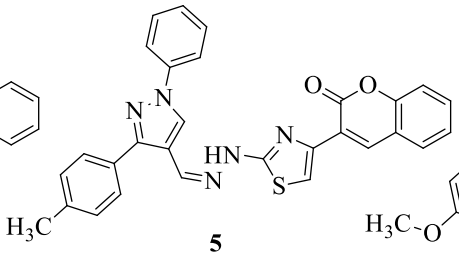

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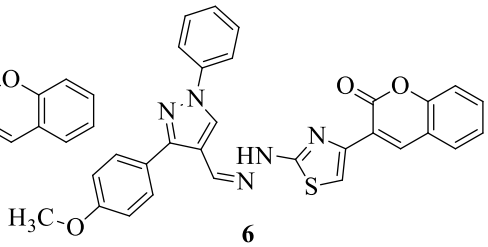
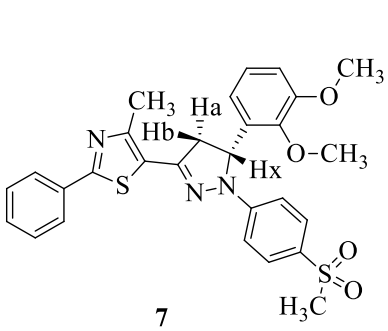


6

Eman K. A. Abdelall *et al.*[20] reported cyclooxygenase-2 and 15-lipoxygenase inhibition, synthesis, antiinflammatory activity and ulcer liability of new celecoxib analogues: Determination of region-specific pyrazole ring formation by NOESY. Among the synthesised series, the compounds **7**, **8** and **9** were found to be having higher anti-inflammatory and anti-COX-2/15-LOX inhibitory activities.



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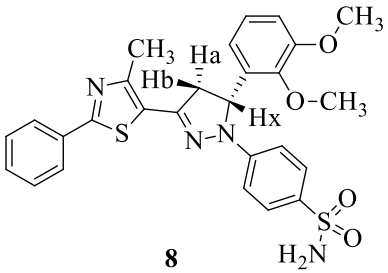

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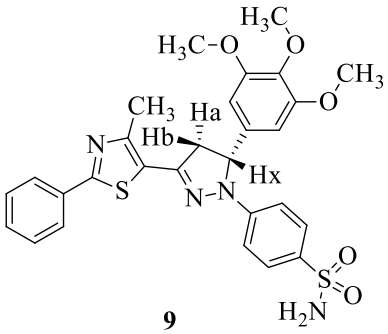
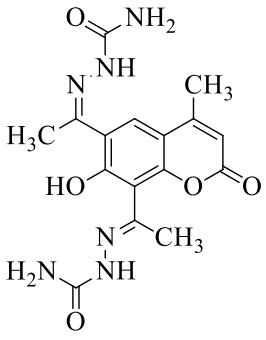


9

Renuka Nagamallu and co-workers.[21] described the synthesis of novel coumarin appended bis(formylpyrazole) derivatives: Studies on their antimicrobial and antioxidant activities. Among the tested series of compounds, **10** and **11** exhibited potent antimicrobial activity and the compounds **12** and **13** were found to be potent free radical scavenging activity (antioxidant) in DPPH and hydroxyl radical scavenging assay.



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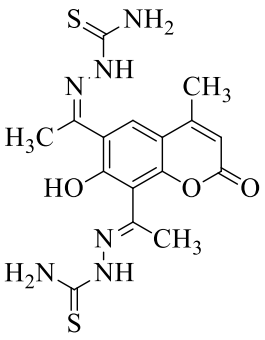

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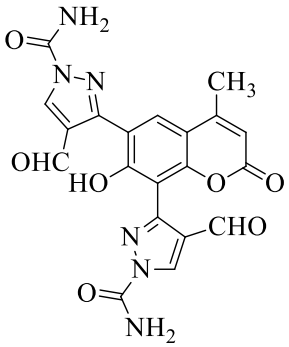

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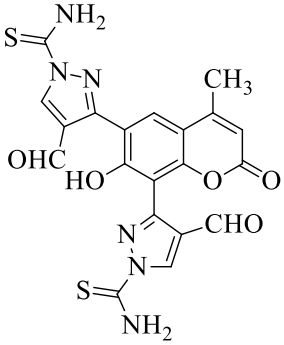
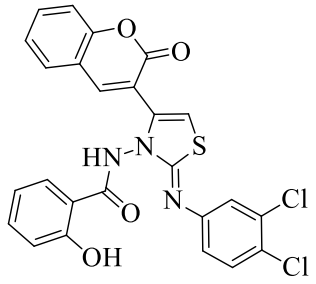


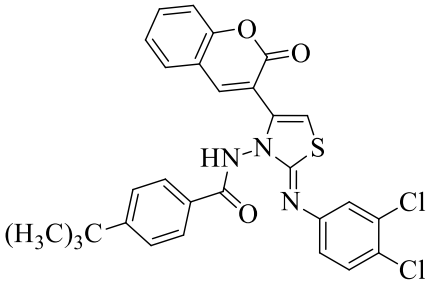
13

Uzma Salar *et al.*[22] reported the syntheses of new 3-thiazolyl coumarin derivatives, *in vitro* α-glucosidase inhibitory activity, and molecular modelling studies. From *in vitro* α-glucosidase inhibitory activity studies it was found that all the synthesised compounds exhibited superior activity in the range of IC_{50} = 0.12 ± 0.01-16.20 ± 0.23 μM as compared to that of the standard acarbose (IC_{50} = 38.25 ± 0.12 μM).



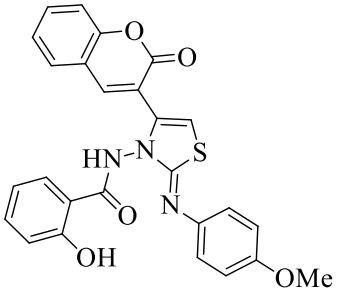
1

IC_{50} = 0.12 ± 0.01 μM



2

IC_{50} = 0.78 ± 0.01 μM

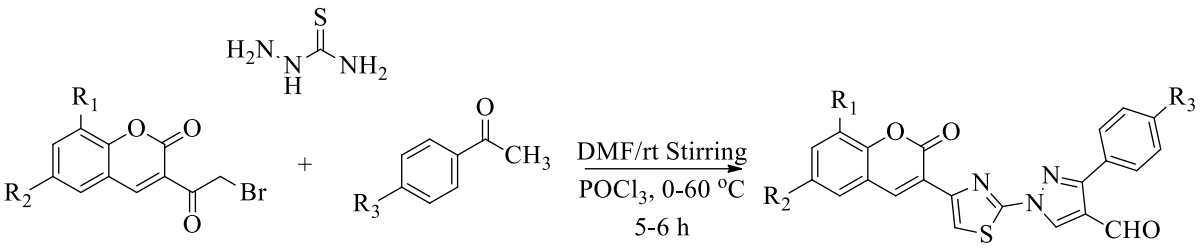


3

IC_{50} = 1.10 ± 0.01 μM

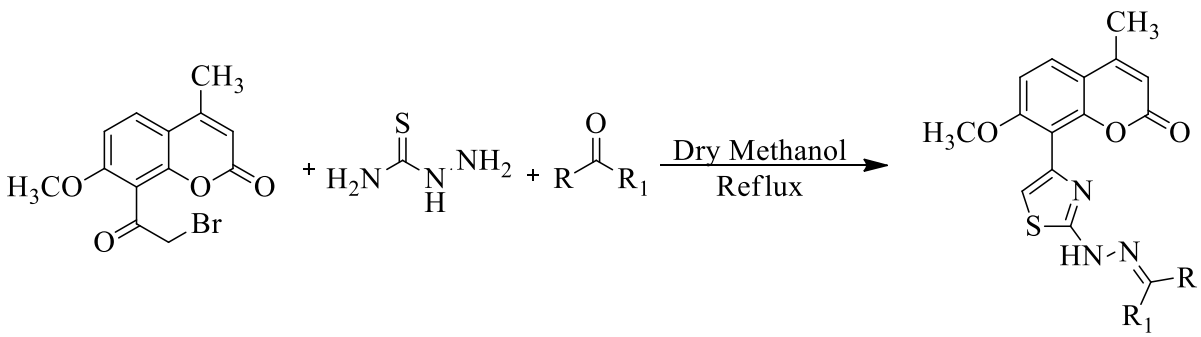
Various important approaches for the synthesis of thiazolyl coumarins.

From our laboratories, **Krishnaiah Vaarla and co-workers.**[23] described the synthesis, biological activity evaluation and molecular docking studies of novel coumarin substituted thiazolyl-3-aryl-pyrazole-4-carbaldehydes. The target compounds were achieved by involving 3-(2-bromoacetyl)coumarins, aminothiourea and substituted acetophenones utilising Vilsmeier–Haack condition *via* one-pot multicomponent approach.



Scheme-1

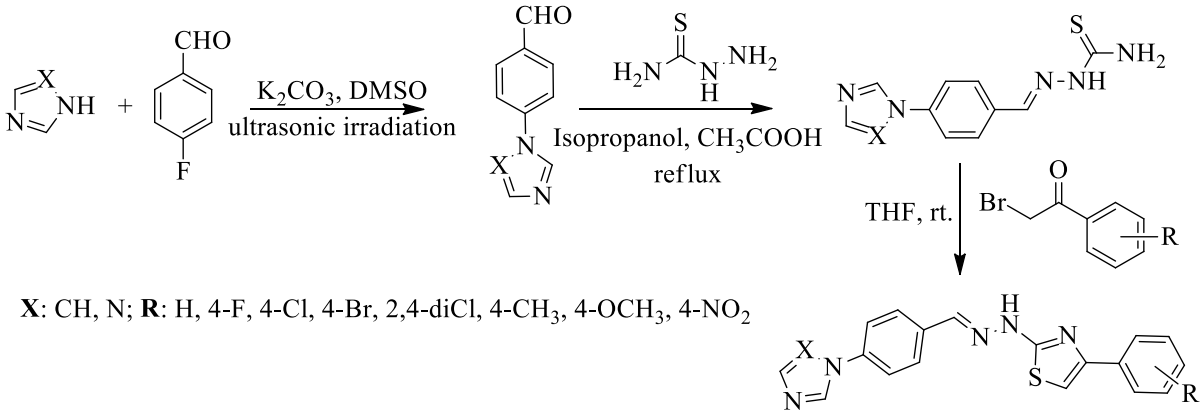
V. R. Rao and K. M. Reddy[24] reported a facile one-step synthesis of new types of 8-thiazolyl coumarins by the condensation of equimolar concentrations of 4-methyl-7-methoxy-8-(2-bromo-acetyl-chromen-2-one, thiosemicarbazide, and acetophenone or acetone under refluxing conditions in dry methanol.



R = H, CH_3; $\mathbf{R_1}$ = CH_3, C_6H_5, C_6H_4Cl, $OMeC_6H_4$, $(OMe)_3C_6H_2$, $(NMe)_2C_6H_3$

Scheme-2

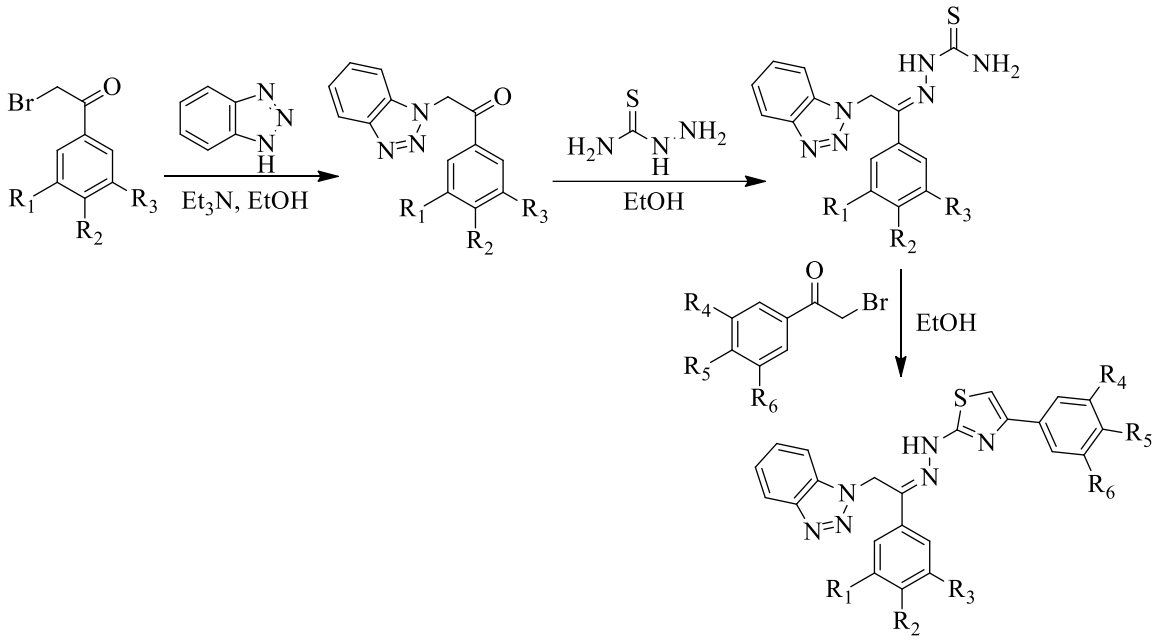
Keriman Ozadali *et al.*[25] described the synthesis and *in vitro* antimycobacterial activities of some new thiazolylhydrazone derivatives against *Mycobacterium tuberculosis* H37Rv.



X: CH, N; **R**: H, 4-F, 4-Cl, 4-Br, 2,4-diCl, 4-CH_3, 4-OCH_3, 4-NO_2

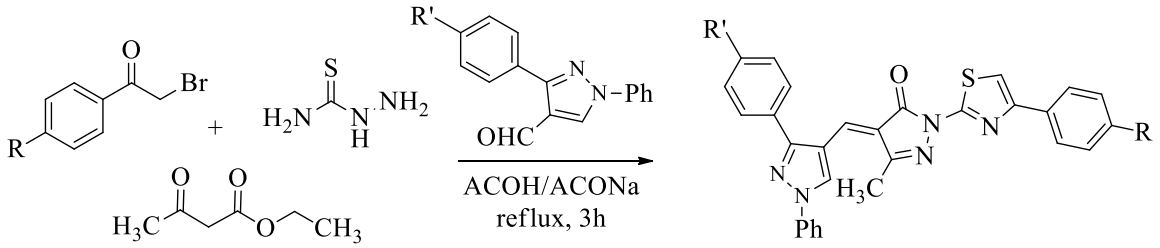
Scheme-3

Nitin D. Gaikwad *et al.*[26] reported the synthesis and biological evaluation of some novel thiazole substituted benzotriazole derivatives with the aim of investigating their antimicrobial activity.



Scheme-4

Rahul D. Kamble *et al*.[27] reported the synthesis and *in silico* investigation of thiazoles bearing pyrazole derivatives as anti-inflammatory agents. The target compounds were achieved by the four-component condensation of an equimolar quantity of phenacyl bromides, thiosemicarbazide, ethyl acetoacetate and hetero aldehyde in refluxing acetic acid in the presence of sodium acetate as a base.



Scheme-5

PRESENT WORK

During the past two decades, the world population suffering severely with the infectious diseases due to multi-drug resistance often results from the over-expression of a multidrug efflux system and their widespread usage.[28] Among them, microbial infections are the second most leading death causing diseases after a heart attack in the world, due to

their rapid spread, toxicity and resistance towards the existing antibiotic drugs. Hence there is an urgent need for the development of more potent, broad-spectrum antimicrobial novel drugs with no side effects and improved efficacy to cure microbial infections. In this context, the microbial target based synthesis of novel antimicrobial agents has attracted considerable interest in the drug discovery. In this regard, a well known key enzyme MurB, an NADPH-dependant enolpyruvyl reductase,[29] which is essential for the growth and biosynthesis of peptidoglycan polymeric layer of bacterial cell wall,[30] emerged as an important and attractive target for the development of new antibiotic drugs.[31] The MurB enzyme is unique to the prokaryotic cells and has no counterparts in eukaryotes. In addition, molecular docking technique also emerged as an important tool in drug designing and discovery of novel potential ligands. This computer aided drug designing suite is very much useful in studying the mechanism involved in the non-covalent interactions between the small molecule drug candidates and the binding site of a macromolecule and also to predict the accurate binding conformations of the ligands with the active pockets of macromolecules of pathogens.[32]

Antioxidants play a vital role in the body defence mechanism by regulating the generation and elimination of reactive oxygen species (ROS) such as hydroxyl radicals, superoxide radicals, singlet oxygen and hydrogen peroxide radicals those generated from excessive oxidative stress and normal metabolic activities. The regulating mechanism includes detoxification of excess ROS, if not the high concentrations of free radicals damages the normal cell structures, embedded proteins, lipids, carbohydrates and also damages the nitrogen bases of nucleic acids leading to mutations[33] and also causes cancer, ageing and neurodegenerative disorders such as Alzheimer's and Parkinson's diseases.[34] In addition to the body's defence mechanism includes superoxide dismutase (SOD), catalase and glutathione peroxidase, antioxidants also regulate the concentration of ROS by interacting with them and prevent their influence on other molecules. Thus, the discovery and development of novel synthetic radical scavengers attained great importance in organic chemistry.

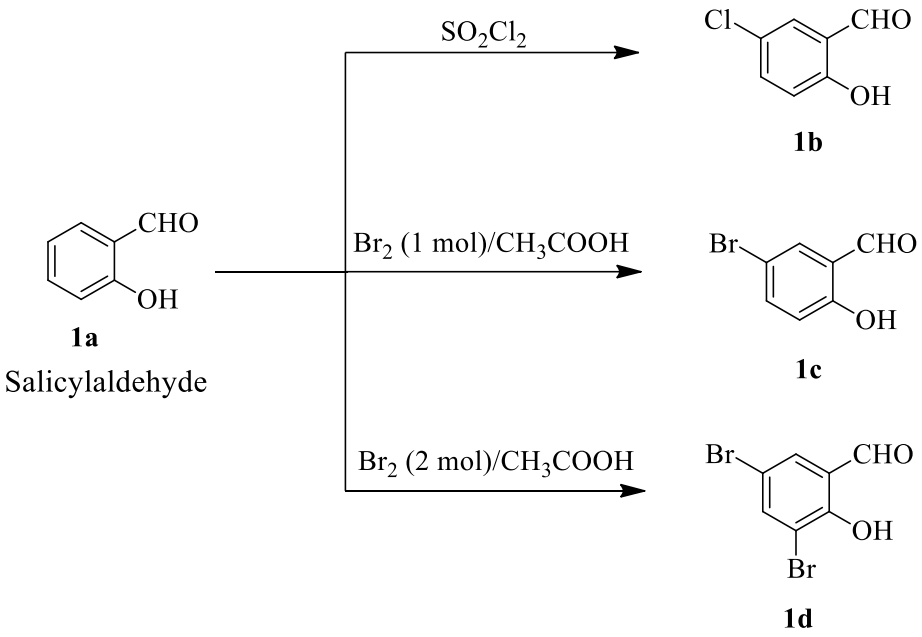
A further extension to our earlier works[35] and as a part of our endeavour towards the synthesis of biologically potent new heterocyclic scaffolds. Here in we report, the synthesis of novel heterocyclic scaffold bearing a coumarin nucleus with a pyrazole and 4-functionalized thiazole rings. This work is an expectation to find a new and more potent

antioxidant and antimicrobial agents which competitively inhibits the bacterial peptidoglycan biosynthesis by restricting the vital MurB enzyme.

Preparation of starting materials

Salicylaldehyde derivatives

The salicylaldehyde derivatives were prepared by according to the literature procedures. 5-Chloro salicylaldehyde **(1b)** was prepared by slow addition of sulfuryl chloride to the salicylaldehyde **(1a)** and acetic acid mixture in a molar proportion.[36] 5-bromo salicylaldehyde **(1c)**[37] and 3,5-dibromo salicylaldehyde **(1d)**[38] were exclusively prepared by the bromination of salicylaldehyde in acetic acid, with equimolar and two molar proportions of molecular bromine respectively.

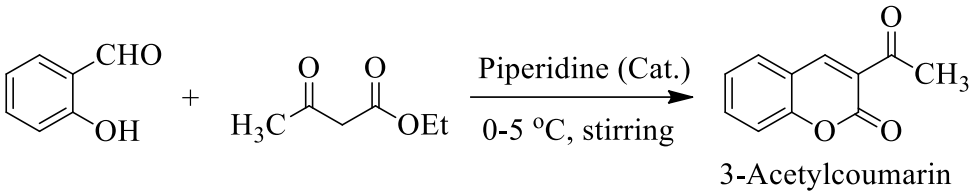


Scheme-6

3-Acetyl-2*H*-chromen-2-one and 3-(2-bromoacetyl)-2*H*-chromen-2-one

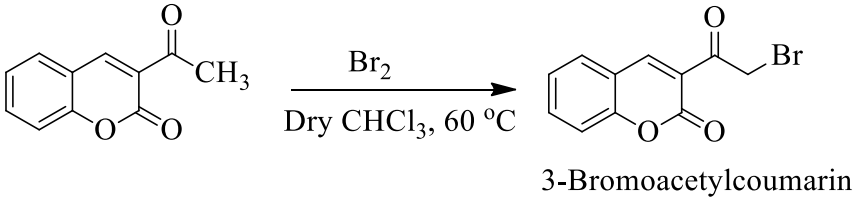
3-Acetylcoumarin and 3-Bromoacetylcoumarin were prepared by according to the literature report.[39] Piperidine (1 mL) was added dropwise to the ice cold ethylacetoacetate (0.1 mol) at 0-5 ^{o}C in a beaker, after Salicylaldehyde (0.1 mole) was added in portions while rapid stirring. The above reaction mixture was allowed to stand overnight at room temperature afforded the 3-acetylcouamrin. The separated yellow coloured solid was filtered under vacuum and washed with cold methanol and recrystallization with

methanol afforded the desired compound in the analytically pure form of 3-Acetyl-2*H*-chromen-2-one (85 %) (as pale yellow crystals, m.p.121-125 ^{o}C (Lit. m.p. 125-128 ^{o}C).



Scheme-7

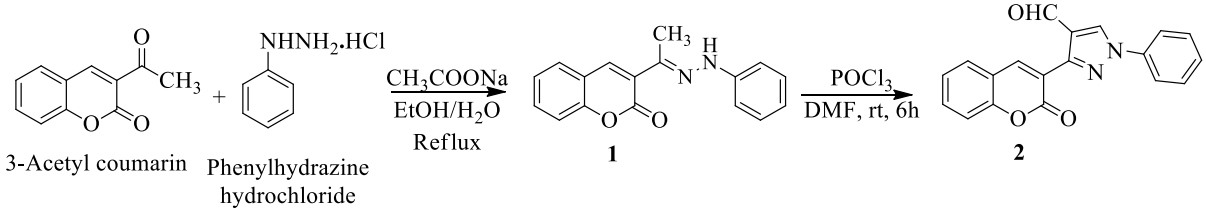
To a solution of 3-Acetylcoumarin (0.1 mole) in 100 mL alcohol-free chloroform (dry) was added molecular bromine (0.1 mole) in 20 mL dry chloroform dropwise by dropping funnel with eventual shaking and warming it on the heating mantle at 60 ^{o}C. After complete addition of bromine heated it for 20-30 min on a water bath to remove excess of HBr and allowed to cool and filtered the separated solid and washed with diethyl ether. Purification by recrystallization from glacial acetic acid, furnished the colourless needles of 3-(2-bromoacetyl)-2*H*-chromen-2-one **()** (80 %), m.p. 162-165 ^{o}C (Lit. M.p. 162 ^{o}C).



Scheme-8

Synthesis of 3-(2-oxo-2*H*-chromen-3-yl)-1-phenyl-1*H*-pyrazole-4-carbaldehyde[40] (2)

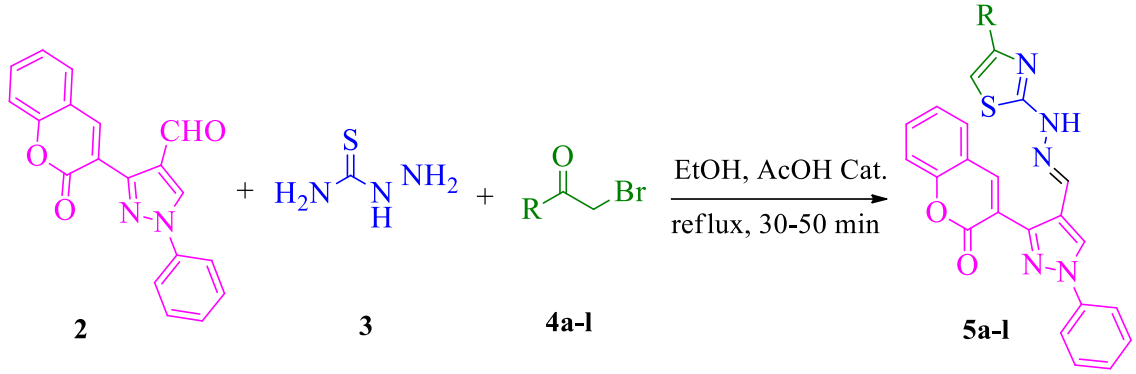
Refluxing an equimolar concentration of 3-Acetylcoumarin, Phenyl hydrazine hydrochloride and anhydrous sodium acetate in ethanol-water system yielded the hydrazone (**1**), which upon Vilsmeier-Haack formylation afforded the coumarin pyrazole aldehyde (**2**) in desirable yields.



Scheme-9

Synthesis of 3-(1-phenyl-4-((2-(4-arylthiazol-2-yl)hydrazono)methyl)-1*H*-pyrazol-3-yl)-2*H*-chromen-2-ones (5a-l)

An equimolar concentration of coumarin pyrazole aldehyde (**2**), thiosemicarbazide (**3**) and *α*-bromo ketones (**4a-l**) were condensed in refluxing ethanol in catalytic amount of acetic acid afforded the analytically pure products (**5a-l**) in good yields.



2 **3** **4a-l** **5a-l**

R:

a = C_6H_5 **b** = 4-MeC_6H_4 **c** = 4-$OMeC_6H_4$
d = 4-ClC_6H_4 **e** = 4-BrC_6H_4 **f** = 4-$NO_2C_6H_4$
g = 4-PhC_6H_4 **h** = Benzo[*f*]coumarin-3-yl **i** = Coumarin-3-yl
j = 6-Cl-Coumarin-3-yl **k** = 6-Br-Coumarin-3-yl **l** = 6,8-di-Br-Coumarin-3-yl

Scheme-10

Results and discussions

The synthetic protocol for the title compounds, 3-(1-phenyl-4-((2-(4-aryl/heteryl-thiazol-2-yl)hydrazono)methyl)-1*H*-pyrazol-3-yl)-2*H*-chromen-2-ones (**5a-l**) has outlined in Scheme 1, and were synthesized by the one-pot three-component condensation reaction of 3-(2-oxo-2*H*-chromen-3-yl)-1-phenyl-1*H*-pyrazole-4-carbaldehyde (**2**), thiosemicarbazide (**3**) and phenacyl bromides (**4a-g**)/2-(2-bromoacetyl)-3*H*-benzo[*f*]chromen-3-one (**4h**)/3-(2-bromoacetyl)-2*H*-chromen-2-ones (**4i-l**) in ethanol in the presence of catalytic amount of acetic acid under reflux conditions with good yields (85-92%) in shorter reaction times (30-50 min). The starting materials, 3-(2-oxo-2*H*-chromen-3-yl)-1-phenyl-1*H*-pyrazole-4-carbaldehyde (**2**), 2-(2-bromoacetyl)-3*H*-benzo[*f*]chromen-3-one (**4h**) and 3-(2-bromoacetyl)-2*H*-chromen-2-ones (**4i-l**) were synthesized by following the literature procedures.[35b,41] The physical data of the title compounds (**5a-l**) were presented in **Table 1**.

Structures of all the synthesised compounds (**5a-l**) were established with the aid of their spectral (IR, NMR and Mass) and (C, H and N) elemental analyses. Analytical and

spectral data of all the synthesized compounds were in full agreement with the proposed structures and also discussed for a representative compound **5d**: From the IR spectrum, the appearance of a broad absorption band at 3414 cm^{-1} and sharp bands at 1720 and 1628 cm^{-1} are ascribed to –N-H, -C=O and -C=N stretching frequencies respectively, confirming the formation of the proposed compound. From the ^{1}H NMR spectrum, the appearance of singlets at 12.02 ppm (NH proton), 8.92 ppm (pyrazole 5^{th} proton), 8.35 ppm (-CH=N proton), 8.06 ppm (coumarin 4^{th} proton) and 7.20 ppm (thiazole 5^{th} proton), and from the ^{13}C NMR the presence of signals at 168.1 ppm (thiazole -C=N carbon) & 158.8 ppm (lactone carbonyl carbon), and the molecular ion peak from the mass spectrum as well as elemental analyses data confirmed the formation of the product.

Table 1. Physical data of the title compounds (**5a-l**).[a]

Product	Time (min)	Yield[b] (%)	M.p. (ºC)
5a	35	89	175-177
5b	40	86	211-213
5c	30	88	195-197
5d	45	92	166-168
5e	45	90	189-191
5f	50	90	199-201
5g	40	88	210-212
5h	45	86	253-255
5i	35	89	257-259
5j	40	91	223-225
5k	45	86	233-235
5l	45	85	246-248

[a]Reaction conditions: Coumarin pyrazole aldehyde (**2**, 1 mmol), thiosemicarbazide (**3**, 1 mmol) and phenacyl bromides/3-(2-bromoacetyl)coumarins/2-(2-bromoacetyl)-3*H*-benzo[*f*] chromen-3-one (**4a-l**, 1 mmol), ethanol (5 mL), acetic acid (3 drops), reflux.

[b]Isolated yields.

Biological studies

***In vitro* antimicrobial activity**

All the synthesised compounds (**5a-l**) were screened for their *in vitro* antibacterial activity against four pathogenic microorganisms, including two Gram-positive bacteria, *Staphylococcus aureus* (MTCC 121) and *Bacillus subtilis* (MTCC 96), and two Gram-negative bacteria *Escherichia coli* (MTCC 40) and *Pseudomonas aeruginosa* (MTCC 2453). The standard pathogenic microbial cultures were procured from the Microbial Type Culture Collection (MTCC), Chandigarh, India and were incubated on sterile nutrient agar at room temperature and inoculated into the fresh nutrient broth of 10 mL, in order to yield a bacterial suspension of about 10-100 colony forming units (CFU) per mL. The inoculums size of approximately 10^6 CFU/plate was spread plated over the surface of the nutrient agar by diluting the initial microbial suspension 10 times with distilled water. 30 μL of an antibacterial suspension of 100 μgmL^{-1} concentration was transferred into the 6 mm diameter well made by the sterile cork borer and incubated for about 24 h at 37±1 ^{o}C. Antibacterial screenings were conducted in triplicates by a well-plate method in Mueller-Hinton Agar[42] at 100 μgmL^{-1} concentration for the synthesised compounds (**5a-l**) with respect to positive control Streptomycin at 30 μgmL^{-1}. Zone of inhibition (ZOI) values were measured in mm and Minimum inhibitory concentration (MIC) for the tested compounds, as well as standards, was measured in μgmL^{-1} by microdilution method.[43] DMSO used as a solvent control and the results are depicted in **Table 2**.

All the compounds (**5a-l**) were also screened for their *in vitro* antifungal activity against *Candida albicans*, *Aspergillus niger*, *Candida glabrata* and *Aspergillus parasiticus* fungal strains using positive control Clotrimazole.

Table 2. *In vitro* antimicrobial activity of **5a-l**.

Product	Antibacterial activity								Antifungal activity			
	S. a		***B. s***		***E. c***		***P. a***		***C. a***	***A. n***	***C. g***	***A. p***
	ZOI	**MIC**	**ZOI**	**MIC**	**ZOI**	**MIC**	**ZOI**	**MIC**	**ZOI**			
5a	17	50	16	50	19	50	17	50	10	14	8	8
5b	13	200	8	200	8	200	7	200	20	20	18	17
5c	16	100	7	200	8	200	8	200	10	8	10	10
5d	15	100	8	200	8	200	8	200	8	12	17	17
5e	12	200	8	200	8	200	8	200	8	8	12	10
5f	13	50	15	50	16	50	15	50	18	19	16	15
5g	9	200	8	200	8	200	13	100	8	10	10	8
5h	19	50	16	50	21	25	20	25	15	8	8	8
5i	8	200	8	200	8	200	8	200	10	8	10	12
5j	13	200	7	200	12	100	7	200	8	8	12	12
5k	22	50	18	50	22	12.5	17	50	12	12	10	12
5l	8	200	8	200	12	200	8	200	12	8	8	15
Streptomycin	22	25	21	12.5	20	12.5	20	12.5	–	–	–	–
Clotrimazole	–	–	–	–	–	–	–	–	24	20	22	20

Zone of inhibition (ZOI) values (in mm) for analogues (**5a-l**) at 100 μgmL^{-1} and positive control drugs Streptomycin and Clotrimazole at 30 μgmL^{-1}. MIC values were given in μgmL^{-1}.

Bacterial strains: ***S. a***– *Staphylococcus aureus*, ***B. s*** –*Bacillus subtilis*, ***E. c***–*Escherichia coli* and ***P. a***–*Pseudomonas aeruginosa*; Fungal strains: ***C. a***– *Candida albicans*, ***A. n***–*Aspergillus niger*, ***C. g***–*Candida glabrata* and ***A. p***–*Aspergillus parasiticus*.

'–' - Not performed.

Evaluation of antibacterial data (**Table 2**) revealed that most of the tested compounds exhibited moderate to excellent antibacterial and good to moderate antifungal activity against all the tested microbial strains. Among them, the compound **5k** has exhibited excellent activity against *E. coli* (ZOI = 22 mm and MIC = 12.5 μgmL^{-1}), good activity against *S. aureus* (ZOI = 22 mm and MIC = 50 μgmL^{-1}) and moderate activity against *B. subtilis* (ZOI = 18 mm and MIC = 50 μgmL^{-1}), and *P. aeruginosa* (ZOI = 17 mm and MIC = 50 μgmL^{-1}). Similarly, the compound **5h** has shown good activity against *E. coli* (ZOI = 21 mm and MIC = 25 μgmL^{-1}) and *P. aeruginosa* (ZOI = 20 mm and MIC = 25 μgmL^{-1}), and moderate inhibiting activity against *S. aureus* (ZOI = 19 mm and MIC = 50 μgmL^{-1}). The compound **5a** has also exhibited good activity against *E. coli* (ZOI = 19 mm and MIC = 50 μgmL^{-1}) and moderate activity against *S. aureus* (ZOI = 17 mm and MIC =

50 μgmL^{-1}) and *P. aeruginosa* (ZOI = 17 mm and MIC = 50 μgmL^{-1}) with respect to the standard antibacterial drug Streptomycin. From the antifungal results (**Table-2**) we have observed that the compounds **5b** (ZOI = 20 mm) and **5f** (ZOI = 19 mm) have shown good inhibiting activity against *A. niger* on comparing with the positive control drug Clotrimazole. Remaining compounds have shown moderate activity against all the tested microbial strains with ZOI ranging from 7-16 mm and MIC 50-200 μgmL^{-1} for bacteria, and ZOI 8-18 mm for fungi.

Structure-activity relationship of the compounds (**5a-l**) revealed that, the 4th position of thiazole ring bearing 6-bromo coumarinyl (**5k**), benzo[*f*]coumarinyl (**5h**) and simple phenyl (**5a**) were found to be potent antibacterial agents and the compounds bearing 4-methyl phenyl and 4-chloro phenyl were found to be good antifungal agents than the remaining compounds.

***In vitro* antioxidant activity**

In order to investigate the possible biological studies for the synthesised compounds (**5a-l**), also screened *in vitro* antioxidant activity in terms of hydrogen donating or radical scavenging ability by rapid and convenient technique *i.e.* 1,1-diphenyl-2-pic-ryl-hydrazyl (DPPH) assay[44] using Trolax and Ascorbic acid as standard drugs. Methanol (95%), DPPH solution and standard drugs were used as blank, control and reference respectively. Absorbance was calculated at 517 nm (at absorption maximum of DPPH) after keeping the mixture of 100 μL of synthesised compounds of concentration 10 μgmL^{-1} (dissolved in DMSO) and 900 μL of DPPH radical solution (0.004% w/v of DPPH in methanol) in a dark place for 30 min incubation period. Antioxidant activity was evaluated in IC_{50} in μM (the effective concentration at which 50% of the radicals were scavenged) and depicted in **Table 3**.

Table 3. Antioxidant activity of **5a-l** by DPPH Method.

Product	IC_{50} in µM
5a	12.79
5b	13.01
5c	16.80
5d	89.92
5e	67.85
5f	81.29
5g	15.51
5h	44.75
5i	13.89
5j	76.01
5k	74.56
5l	63.33
Trolox	14.22
Ascorbic acid	3.8

Evaluation of antioxidant activity revealed that most of the tested compounds exhibited moderate to strong DPPH radical scavenging ability compared with the positive controls Trolox and Ascorbic acid. Among them, the compounds **5a** bearing phenyl, **5b** bearing 4-methyl phenyl and **5i** having 2*H*-chromen-2-one were found to be more effective and potent DPPH radical scavenging ability with ~1.11, ~1.09, ~1.02 folds than positive control drug Trolax. Remaining all the compounds have shown good to moderate radical scavenging activity with IC_{50} values in the range of 15.51-89.92 μM. It was noticed that the compounds with electron donating groups on the phenyl ring were found to possess potent radical scavenging ability.

Molecular modelling studies

To explore and support the antibacterial mechanism, docking studies for the synthesised compounds (**5a-l**) was performed. This drug designing tool helpful to investigate and to gain a deep insight into the mode of binding interactions of each of these ligands (**5a-l**) with the receptor sites of UDP-*N*-acetylenolpyruvoylglucosamine reductase, MurB and

also to determine the best in silico conformation. Docking of the synthesised ligands was employed by using Lamarckian Genetic Algorithm (LGA),[45] inculcated in the docking program AutoDock 4.2. MurB is an essential enzyme that catalyses the reduction of enolpyruvyl uridine diphosphate *N*-acetyl glucosamine (EP-UNAG), an intermediate in the assembly of the UNAM-pentapeptide (m-A2pm) portion to uridine diphosphate *N*-acetyl muramic acid (UNAM), of cell wall precursor. Mur proteins (Mur A-F, Y and G) catalyse more than 10 biosynthetic transformations involved in the formation of the peptidoglycan layer of the cell walls of bacteria and they also conserved among several bacterial strains. Because of this, we selected the MurB enzyme as a target receptor.

The co-crystallized structure of target enzyme MurB (PDB id: 1MBT) was obtained from Protein Data Bank (RCSB) (http://www.rcsb.org/pdb). To carry out in silico studies, the 2D structures of the synthesised ligands (**5a-l**) were drawn in ChemBioOffice 2010 and converted to energy minimised 3D structures in pdb file format using MarvinSketch (ChemAxon). The target protein file was prepared by removing the structural water molecule, hetero atoms and co-factors by leaving only the residues associated with protein by using Discovery Studio 4.0 Visualizer (DSV). AutoDock 1.5.6 (MGL tools-1.5.6) tool was used to prepare target protein file that involves, assigning AD4 type atoms, calculating Gasteiger charges for every atom of the macromolecule, addition of polar hydrogen's to the macromolecule, an essential step to correct the calculation of partial charge by keeping all other values as default. The binding site of protein identification was carried out using CastP (serversts-fw.bioengr.uic.edu/castp/calculation.php). Docking simulations for the compounds **5a-l** were performed against the active site of the MurB enzyme. Then, finally, docking results were visualised using Maestro elements tutorial 1.8.

All inhibitors were compared out of 100 docking runs. The docking studies revealed that all the synthesised molecules exhibited excellent binding energies towards the receptor active pocket ranging from -9.02 to -11.15 kcalmol^{-1} and summarised in Table 4. Among them, the conformations with lowest binding energies and those ligands exhibiting well-established H-bonds with the closest range of 1.8-3.4 Å with one or more amino acids in the receptor active pocket were chosen as best docked ligand orientations (supporting file). Hence, the compounds **5a**, **5h** and **5k** were energetically favoured for MurB active site and are exhibiting bonds with amino acids of the active pocket of the receptor and

considered as the best docking poses. The ligand **5a** exhibited H-bonding with SER229, ARG214, ARG159 amino acids, whereas 5h with ARG214, ARG159, SER50 amino acids and 5k with SER116, CYS113, SER50, ARG159, ARG214, SER229 amino acids. Best docked orientations of synthesised ligands were shown in Fig. 2. The binding energies, inhibition constants and hydrogen bond interactions of all the compounds were tabulated in Table 4. These results revealed a variety of binding modes that may provide a sufficient explanation and good compromise between docking scores and *in vitro* results of the antibacterial activity.

Table 4. Autodock binding energies, no. of hydrogen bonds and residues involved in hydrogen bonding interaction of ligands for *E. Coli* (PDB id: 1MBT).

Product	Binding Energy ($kcalmol^{-1}$)	Inhibition Constant Ki (nM)	Residues involved in hydrogen bonding interactions (No. of hydrogen bonds)
5a	-10.93	9.77	SER229 (2), ARG214 (2), ARG159 (1)
5b	-10.44	22.39	SER229 (1), ARG214 (3), ARG159 (1), SER50 (1)
5c	-10.07	41.70	SER50 (2), SER229 (3), ARG214 (2)
5d	-10.34	26.44	ARG214 (3), SER229 (1), ARG159 (2), GLY123 (1)
5e	-10.08	40.66	ARG214 (3), SER229 (1), ARG159 (2), GLY123 (2)
5f	-10.85	11.21	SER229 (1), ARG214 (1), ARG159 (1), SER50 (1), GLU48 (1), GLY49 (1), CYS113 (1)
5g	-10.44	22.40	SER229 (4), ARG159(1)
5h	-11.14	6.84	ARG214 (3), ARG159 (2), SER50 (1)
5i	-9.83	62.09	SER116 (2), GLU48 (1), SER50 (3), ARG159 (1), ARG214 (2), SER229 (1)
5j	-10.23	31.68	SER116 (2), SER50 (1), CYS113 (1), ARG214 (1), SER229 (2)
5k	-11.15	6.71	SER116 (2), CYS113 (1), SER50 (1), ARG159 (2), ARG214 (1), SER229 (1)
5l	-9.02	244.22	SER229 (2), SER50 (2), SER116 (1)

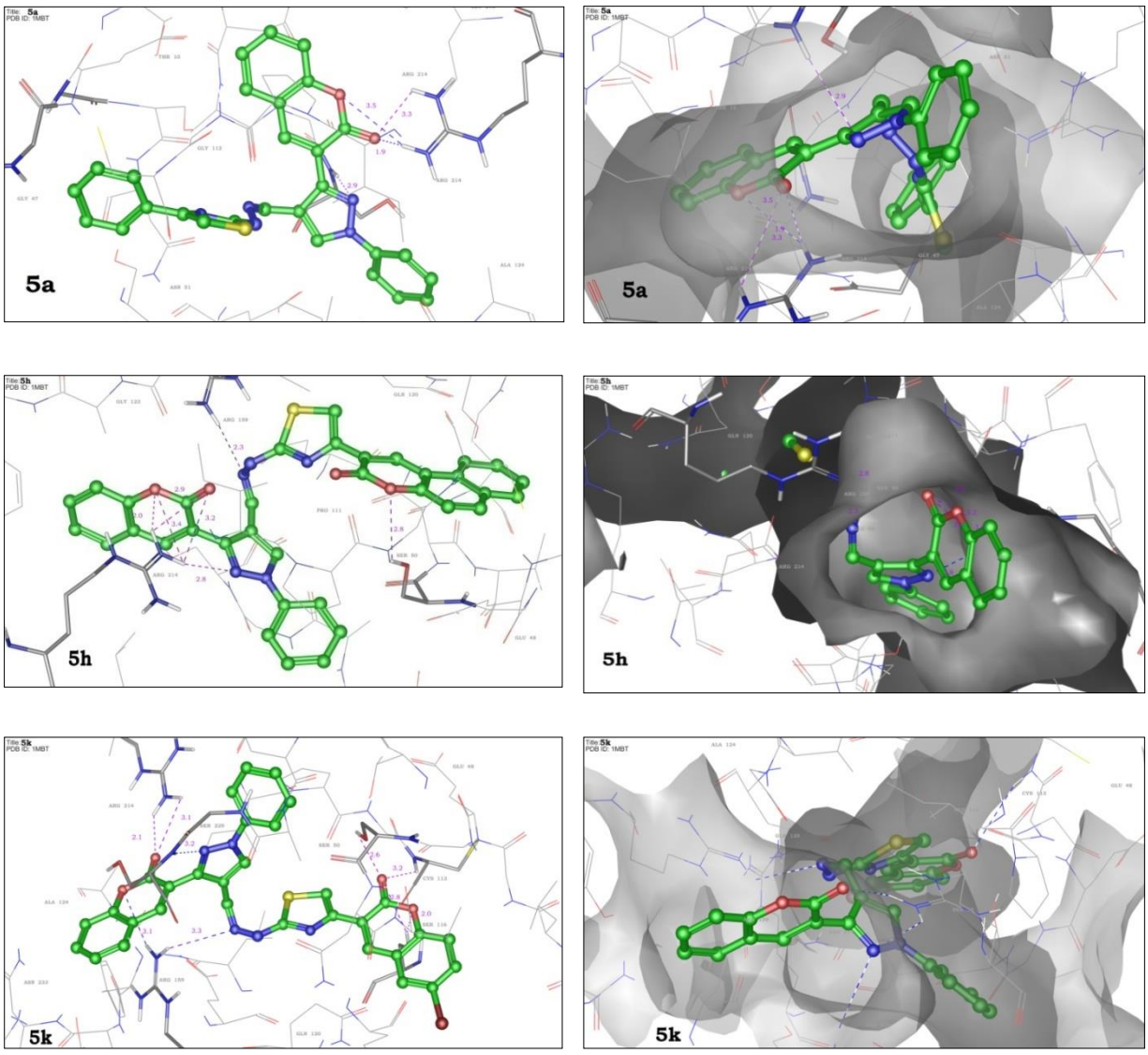


Fig. 2. Docking pose of **5a**, **5h** and **5k** (ball and stick) with UDP-*N*-acetylenolpyruvoylglucosamine reductase (MurB) (thin wire) with intermolecular H-bonding (pink and blue dotted lines) and 3D surface interaction (green) with the enzyme (represented in the molecular cloud).

Conclusion

In conclusion, a series of novel pyrazolyl coumarin bearing 2,4-disubstituted thiazole derivatives (**5a-l**) were reported in quantitative yields *via* MCR approach and evaluated for their *in vitro* antimicrobial and antioxidant studies. Among the series, compounds possessing 6-bromo coumarinyl (**5k**), benzo[*f*]coumarinyl (**5h**) and simple phenyl (**5a**) on thiazole ring were found to be potent and broad spectrum antibacterial agents with respect to standard drug Streptomycin. The compounds possessing 4-methyl phenyl (**5b**) and 4-

nitro phenyl (**5f**) on thiazole ring were found to be good antifungal agents. Antioxidant studies revealed that the compounds **5a**, **5b** and **5i** have excellent radical scavenging ability than the positive control Trolax. In order to support the *in vitro* antibacterial results, the synthesised compounds were docked into the plausible target UDP-*N*-acetylenolpyruvoylglucosamine reductase, a MurB enzyme. The binding energies and H-bond interactions with amino acids in the active site of target enzyme well supported the antibacterial inhibiting activity of **5k**, **5h** and **5a** and further helped to investigate the binding orientations of ligands with active pockets of an enzyme. All these results could be useful to evaluate novel antibacterial inhibitors and can be considered as a lead compounds for the development of antibacterial agents for the treatment of bacterial infection.

Experimental

General procedure for the synthesis of 3-(1-phenyl-4-((2-(4-arylthiazol-2-yl)hydrazono)methyl)-1*H*-pyrazol-3-yl)-2*H*-chromen-2-ones (5a-l)

A mixture of 3-(2-oxo-2*H*-chromen-3-yl)-1-phenyl-1*H*-pyrazole-4-carbaldehyde (**2**, 1 mmol), thiosemicarbazide (**3**, 1 mmol) and phenacyl bromides/3-(2-bromoacetyl)-2*H*-chromen-2-ones/2-(2-bromoacetyl)-3*H*-benzo[*f*] chromen-3-one (**4a-l**, 1 mmol) were dissolved in 5 mL of ethanol in the presence of catalytic amount of acetic acid (3 drops) and refluxed for about 30-50 min. The progress of the reaction was monitored by TLC. After completion of the reaction, the solid separated out was filtered, dried and washed with hot ethanol furnished the analytically pure products (**5a-l**) in good yields.

Spectral data

3-(1-Phenyl-4-((2-(4-phenylthiazol-2-yl)hydrazono)methyl)-1*H*-pyrazol-3-yl)-2*H*-chromen-2-one (5a)

Yellow solid; **IR** (KBr, cm^{-1}) υ_{max}: 3442 (NH), 1722 (C=O), 1630 (C=N); **^{1}H NMR** (400 MHz, DMSO-d_6): δ = 11.98 (s, 1H), 8.92 (s, 1H), 8.34 (s, 1H), 8.06 (s, 1H), 7.93 (d, *J* = 8.0 Hz, 2H), 7.88 (d, *J* = 7.2 Hz, 1H), 7.84 (d, *J* = 8.0 Hz, 2H), 7.68-7.78 (m, 2H), 7.50-7.58 (m, 3H), 7.35-7.45 (m, 4H), 7.27 (t, *J* = 7.2 Hz, 1H); **MS** (ESI) *m/z*: 490 $[M + H]^+$; Anal. calcd. for $C_{28}H_{19}N_5O_2S$: C, 68.70; H, 3.91; N, 14.31. Found: C, 68.49; H, 3.82; N, 14.58.

3-(1-Phenyl-4-((2-(4-(p-tolyl)thiazol-2yl)hydrazono)methyl)-1*H*-pyrazol-3-yl)-2*H*-chromen-2-one (5b)

Yellow solid; **IR** (KBr, cm^{-1}) υ_{max}: 3447 (NH), 1721 (C=O), 1629 (C=N); **^{1}H NMR** (400 MHz, DMSO-d_6): δ = 11.83 (s, 1H), 8.91 (s, 1H), 8.34 (s, 1H), 8.05 (s, 1H), 7.93 (d, *J* = 7.6 Hz, 2H), 7.86 (d, *J* = 6.4 Hz, 1H), 7.67-7.70 (m, 3H), 7.50-7.58 (m, 3H), 7.36-7.45 (m, 2H), 7.17 (d, *J* = 8.0 Hz, 2H), 7.03 (s, 1H), 2.30 (s, 3H); **MS** (ESI) *m/z*: 504 $[M + H]^+$; Anal. calcd. for $C_{29}H_{21}N_5O_2S$: C, 69.17; H, 4.20; N, 13.91. Found: C, 69.36; H, 4.03; N, 13.74.

3-(4-((2-(4-(4-Methoxyphenyl)thiazol-2-yl)hydrazono)methyl)-1-phenyl-1*H*-pyrazol-3-yl)-2*H*-chromen-2-one (5c)

Pale yellow solid; **IR** (KBr, cm^{-1}) υ_{max}: 3448 (NH), 1724 (C=O), 1627 (C=N), 1217 (C-O-C); **^{1}H NMR** (400 MHz, DMSO-d_6): δ = 12.03 (s, 1H), 8.91 (s, 1H), 8.34 (s, 1H), 8.05 (s, 1H), 7.85-7.94 (m, 3H), 7.71 (t, *J* = 8.8 Hz, 3H), 7.50-7.58 (m, 3H), 7.38-7.45 (m, 2H), 6.93 (d, *J* =9.6 Hz, 3H), 3.76 (s, 3H); **MS** (ESI) *m/z*: 520 $[M + H]^+$; Anal. calcd. for $C_{29}H_{21}N_5O_3S$: C, 67.04; H, 4.07; N, 13.48. Found: C, 67.31; H, 4.26; N, 13.25.

3-(4-((2-(4-(4-Chlorophenyl)thiazol-2-yl)hydrazono)methyl)-1-phenyl-1*H*-pyrazol-3-yl)-2*H*-chromen-2-one (5d)

Yellow solid; **IR** (KBr, cm^{-1}) υ_{max}: 3414 (NH), 1720 (C=O), 1628 (C=N), 750 (C-Cl); **^{1}H NMR** (400 MHz, DMSO-d_6): δ = 12.02 (s, 1H), 8.92 (s, 1H), 8.34 (s, 1H), 8.06 (s, 1H), 7.93 (d, *J* = 8.0 Hz, 2H), 7.80-7.87 (m, 3H), 7.68-7.72 (m, 1H), 7.38-7.58 (m, 7H), 7.20 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ = 168.0, 158.8, 153.5, 148.9, 145.3, 142.5, 138.9, 134.3, 133.3, 132.2, 131.8, 129.6, 128.8, 128.5, 128.1, 127.1, 126.9, 124.7, 121.7, 118.9, 118.7, 118.5, 116.0, 103.9; **MS** (ESI) *m/z*: 525 $[M]^+$; Anal. calcd. for $C_{28}H_{18}ClN_5O_2S$: C, 64.18; H, 3.46; N, 13.37. Found: C, 64.02; H, 3.63; N, 13.52.

3-(4-((2-(4-(4-Bromophenyl)thiazol-2-yl)hydrazono)methyl)-1-phenyl-1*H*-pyrazol-3-yl)-2*H*-chromen-2-one (5e)

Yellow solid; **IR** (KBr, cm^{-1}) υ_{max}: 3436 (NH), 1720 (C=O), 1630 (C=N), 684 (C-Br); **^{1}H NMR** (400 MHz, DMSO-d_6): δ = 12.00 (s, 1H), 8.91 (s, 1H), 8.34 (s, 1H), 8.06 (s, 1H), 7.85-7.94 (m, 2H), 7.74 (d, *J* = 8.8 Hz, 1H), 7.69 (d, *J* = 7.6 Hz, 3H), 7.37-7.58 (m, 7H), 7.21 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ = 168.0, 158.8, 153.5, 149.0, 145.3, 142.5, 138.9, 134.3, 133.7, 132.2, 131.4, 129.6, 128.7, 128.1, 127.4, 126.9, 124.7, 121.7,

120.4, 118.9, 118.7, 118.5, 116.0, 103.9; **MS** (ESI) *m/z*: 568 $[M]^+$; Anal. calcd. for $C_{28}H_{18}BrN_5O_2S$: C, 59.16; H, 3.19; N, 12.32. Found: C, 59.33; H, 3.01; N, 12.57.

3-(4-((2-(4-(4-Nitrophenyl)thiazol-2-yl)hydrazono)methyl)-1-phenyl-1*H*-pyrazol-3-yl)-2*H*-chromen-2-one (5f)

Brown solid; **IR** (KBr, cm^{-1}) υ_{max}: 3436 (NH), 1704 (C=O), 1632 (C=N), 1504, 1344 (NO_2); **^{1}H NMR** (400 MHz, DMSO-d_6): δ = 12.12 (s, 1H), 8.93 (s, 1H), 8.35 (s, 1H), 7.86-8.25 (m, 8H), 7.69-7.73 (m, 1H), 7.37-7.58 (m, 6H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ = 168.3, 158.8, 153.5, 148.3, 146.1, 145.3, 142.5, 140.5, 138.9, 134.5, 132.2, 129.6, 128.8, 128.2, 126.9, 126.2, 124.7, 124.0, 121.6, 118.9, 118.5, 116.0, 107.9; **MS** (ESI) *m/z*: 535 $[M + H]^+$; Anal. calcd. for $C_{28}H_{18}N_6O_4S$: C, 62.91; H, 3.39; N, 15.72. Found: C, 63.12; H, 3.16; N, 15.54.

3-(4-((2-(4-([1,1'-Biphenyl]-4-yl)thiazol-2-yl)hydrazono)methyl)-1-phenyl-1*H*-pyrazol-3-yl)-2*H*-chromen-2-one (5g)

Pale brown solid; **IR** (KBr, cm^{-1}) υ_{max}: 3444 (NH), 1737 (C=O), 1627 (C=N); **^{1}H NMR** (400 MHz, DMSO-d_6): δ = 12.00 (s, 1H), 8.92 (s, 1H), 8.35 (s, 1H), 8.08 (s, 1H), 7.85-7.95 (m, 5H), 7.68-7.71 (m, 5H), 7.34-7.58 (m, 9H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ = 167.9, 158.8, 153.5, 149.7, 145.3, 142.5, 139.5, 138.9, 134.2, 133.5, 132.2, 129.6, 128.8, 128.7, 128.1, 127.3, 126.9, 126.7, 126.4, 126.0, 124.6, 121.7, 118.9, 118.7, 118.5, 116.0, 103.3; **MS** (ESI) *m/z*: 566 $[M + H]^+$; Anal. calcd. for $C_{34}H_{23}N_5O_2S$: C, 72.19; H, 4.10; N, 12.38. Found: C, 72.53; H, 4.37; N, 12.19.

2-(2-(2-((3-(2-Oxo-2*H*-chromen-3-yl)-1-phenyl-1*H*-pyrazol-4-yl)methylene)hydrazinyl) thiazol-4-yl)-3*H*-benzo[*f*]chromen-3-one (5h)

Yellow solid; **IR** (KBr, cm^{-1}) υ_{max}: 3438 (NH), 1717 (C=O), 1638 (C=N); **^{1}H NMR** (400 MHz, DMSO-d_6): δ = 12.17 (s, 1H), 9.25 (s, 1H), 8.94 (s, 1H), 8.20-8.36 (m, 3H), 8.10 (d, *J* =7.6 Hz, 2H), 7.95 (d, *J* =7.6 Hz, 2H), 7.87 (d, *J* =6.4 Hz, 2H), 7.81 (s, 3H), 7.39-7.72 (m, 6H); **MS** (ESI) *m/z*: 608 $[M + H]^+$; Anal. calcd. for $C_{35}H_{21}N_5O_4S$: C, 69.18; H, 3.48; N, 11.53. Found: C, 69.39; H, 3.74; N, 11.36.

3-(2-(2-((3-(2-Oxo-2*H*-chromen-3-yl)-1-phenyl-1*H*-pyrazol-4-yl)methylene)hydrazinyl) thiazol-4-yl)-2*H*-chromen-2-one (5i)

Pale yellow solid; **IR** (KBr, cm^{-1}) υ_{max}: 3439 (NH), 1721 (C=O), 1633 (C=N); **^{1}H NMR** (400 MHz, DMSO-d_6): δ = 12.04 (s, 1H), 8.92 (s, 1H), 8.50 (s, 1H), 8.35 (s, 1H), 8.09 (s, 1H), 7.69-7.95 (m, 4H), 7.64 (t, *J* = 7.2 Hz, 1H), 7.36-7.60 (m, 9H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ = 167.4, 158.8, 158.6, 153.5, 152.2, 145.3, 143.8, 142.5, 138.9, 138.0, 134.6, 132.2, 131.6, 129.6, 128.7, 128.1, 126.9, 124.6, 121.5, 120.3, 119.1, 118.9, 118.5, 116.1, 115.8, 109.7; **MS** (ESI) *m/z*: 558 $[M + H]^+$; Anal. calcd. for $C_{31}H_{19}N_5O_4S$: C, 66.78; H, 3.43; N, 12.56. Found: C, 66.98; H, 3.22; N, 12.84.

6-Chloro-3-(2-(2-((3-(2-oxo-2*H*-chromen-3-yl)-1-phenyl-1*H*-pyrazol-4-yl)methylene) hydrazinyl)thiazol-4-yl)-2*H*-chromen-2-one (5j)

Pale brown solid; **IR** (KBr, cm^{-1}) υ_{max}: 3434 (NH), 1723 (C=O), 1607 (C=N), 751 (C-Cl); **^{1}H NMR** (400 MHz, DMSO-d_6): δ = 12.06 (s, 1H), 8.92 (s, 1H), 8.44 (s, 1H), 8.35 (s, 1H), 8.10 (s, 1H), 7.91-8.00 (m, 3H), 7.86 (d, *J* = 7.6 Hz, 1H), 7.69 (t, *J* = 7.2 Hz, 1H), 7.62 (t, *J* = 6.4 Hz, 1H), 7.39-7.58 (m, 7H); **MS** (ESI) *m/z*: 593 $[M]^+$; Anal. calcd. for $C_{31}H_{18}ClN_5O_4S$: C, 62.89; H, 3.06; N, 11.83. Found: C, 62.72; H, 3.26; N, 11.69.

6-Bromo-3-(2-(2-((3-(2-oxo-2*H*-chromen-3-yl)-1-phenyl-1*H*-pyrazol-4-yl)methylene) hydrazinyl)thiazol-4-yl)-2*H*-chromen-2-one (5k)

Brown solid; **IR** (KBr, cm^{-1}) υ_{max}: 3414 (NH), 1723 (C=O), 1600 (C=N), 685 (C-Br); **^{1}H NMR** (400 MHz, DMSO-d_6): δ = 12.06 (s, 1H), 8.92 (s, 1H), 8.42 (s, 1H), 8.35 (s, 1H), 8.11 (t, *J* = 6.8 Hz, 2H), 7.93 (t, *J* = 7.6 Hz, 1H), 7.85 (s, 2H), 7.68-7.73 (m, 1H), 7.51-7.56 (m, 4H), 7.40 (d, *J* = 8.8 Hz, 4H); **MS** (ESI) *m/z*: 636 $[M]^+$; Anal. calcd. for $C_{31}H_{18}BrN_5O_4S$: C, 58.50; H, 2.85; N, 11.00. Found: C, 58.63; H, 2.98; N, 11.25.

6,8-Dibromo-3-(2-(2-((3-(2-oxo-2*H*-chromen-3-yl)-1-phenyl-1*H*-pyrazol-4-yl)methylene) hydrazinyl)thiazol-4-yl)-2*H*-chromen-2-one (5l)

Yellow solid; **IR** (KBr, cm^{-1}) υ_{max}: 3437 (NH), 1702 (C=O), 1597 (C=N), 684 (C-Br); **^{1}H NMR** (400 MHz, DMSO-d_6): δ = 12.07 (s, 1H), 8.92 (s, 1H), 8.39 (s, 2H), 8.35 (s, 1H), 8.14 (t, *J* = 8 Hz, 2H), 7.94 (d, *J* = 8.4 Hz, 2H), 7.86 (d, *J* = 7.6 Hz, 1H), 7.71 (t, *J* = 8.4 Hz, 1H), 7.46-7.58 (m, 4H), 7.37-7.44 (m, 2H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ = 167.4, 158.8, 153.5, 145.3, 143.3, 142.6, 138.8, 136.3, 134.8, 132.3, 130.3, 129.6, 128.7, 128.1, 126.9, 124.7, 121.9, 121.5, 118.9, 118.5, 116.2, 116.1, 111.2, 109.7; **MS** (ESI) *m/z*: 715 $[M]^+$; Anal. calcd. for $C_{31}H_{17}Br_2N_5O_4S$: C, 52.05; H, 2.40; N, 9.79. Found: C, 52.22; H, 2.18; N, 9.96.

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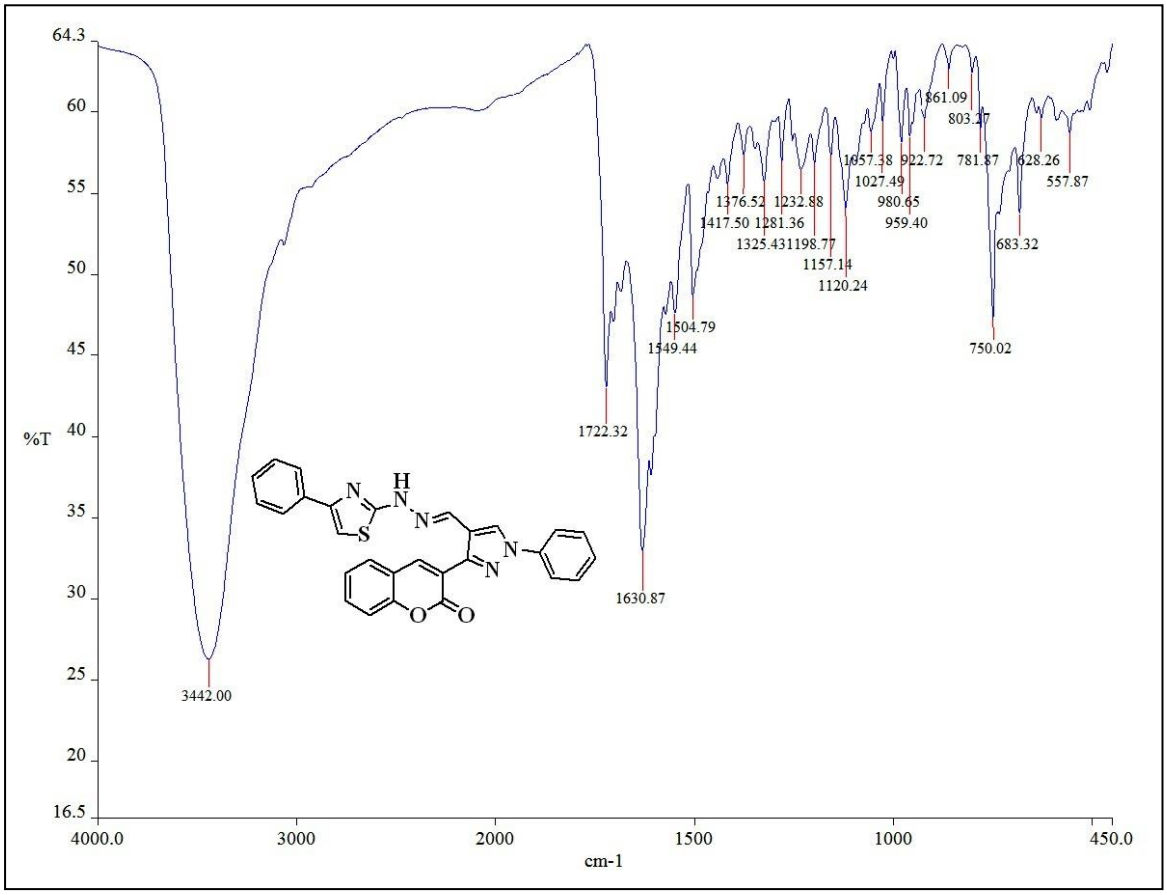
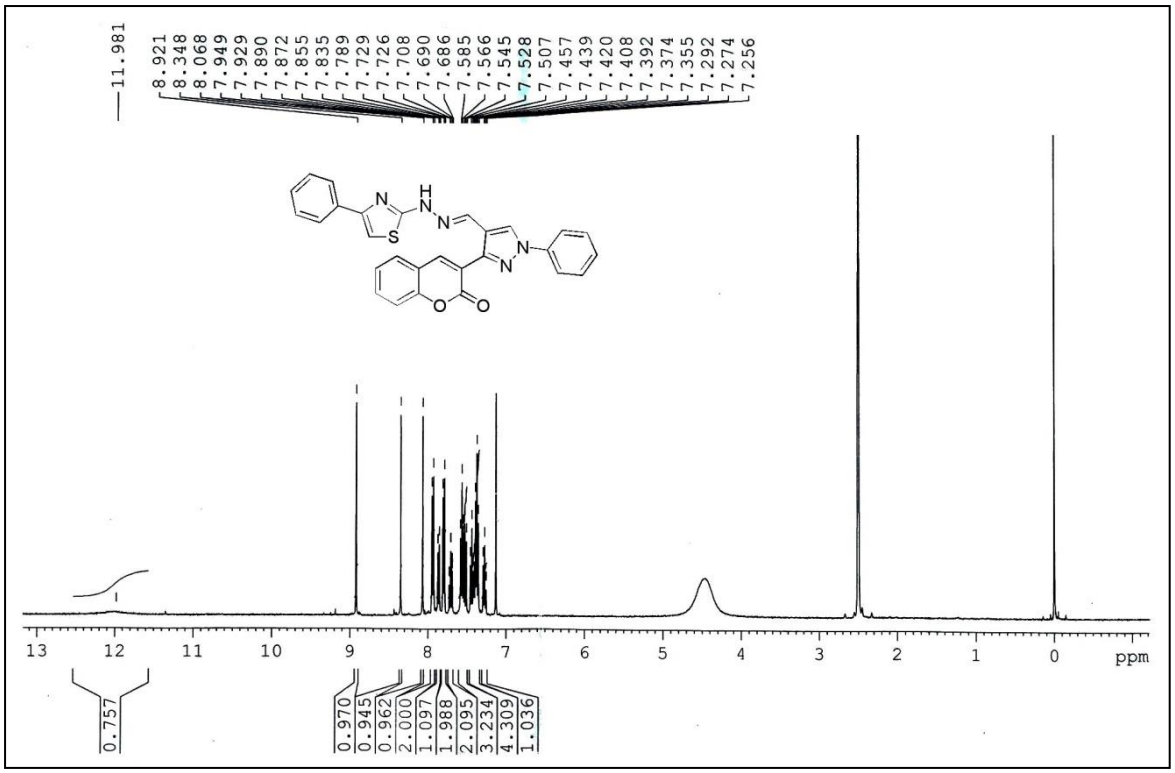
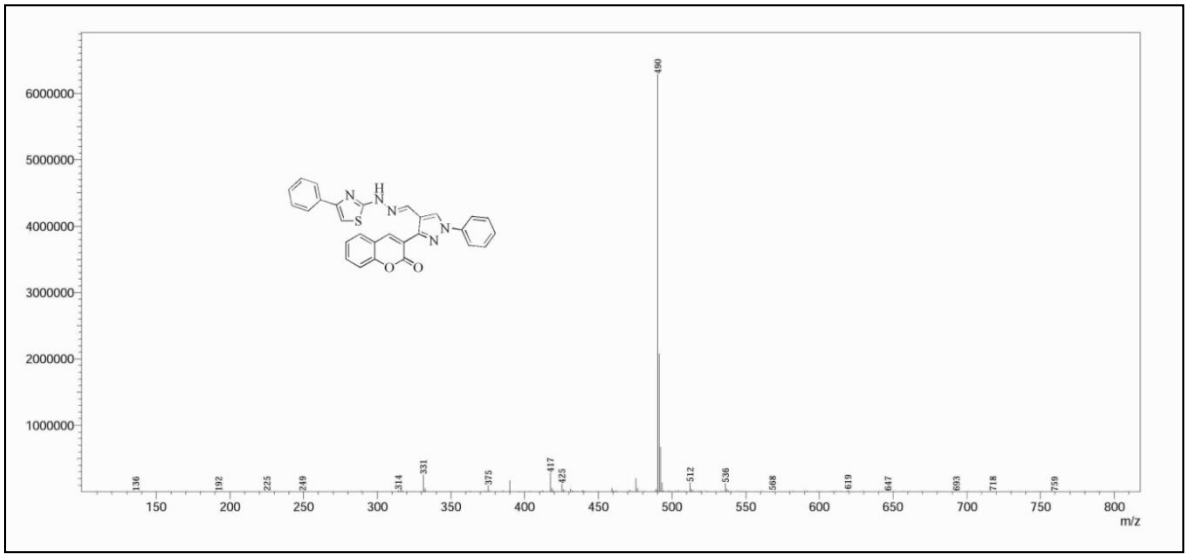


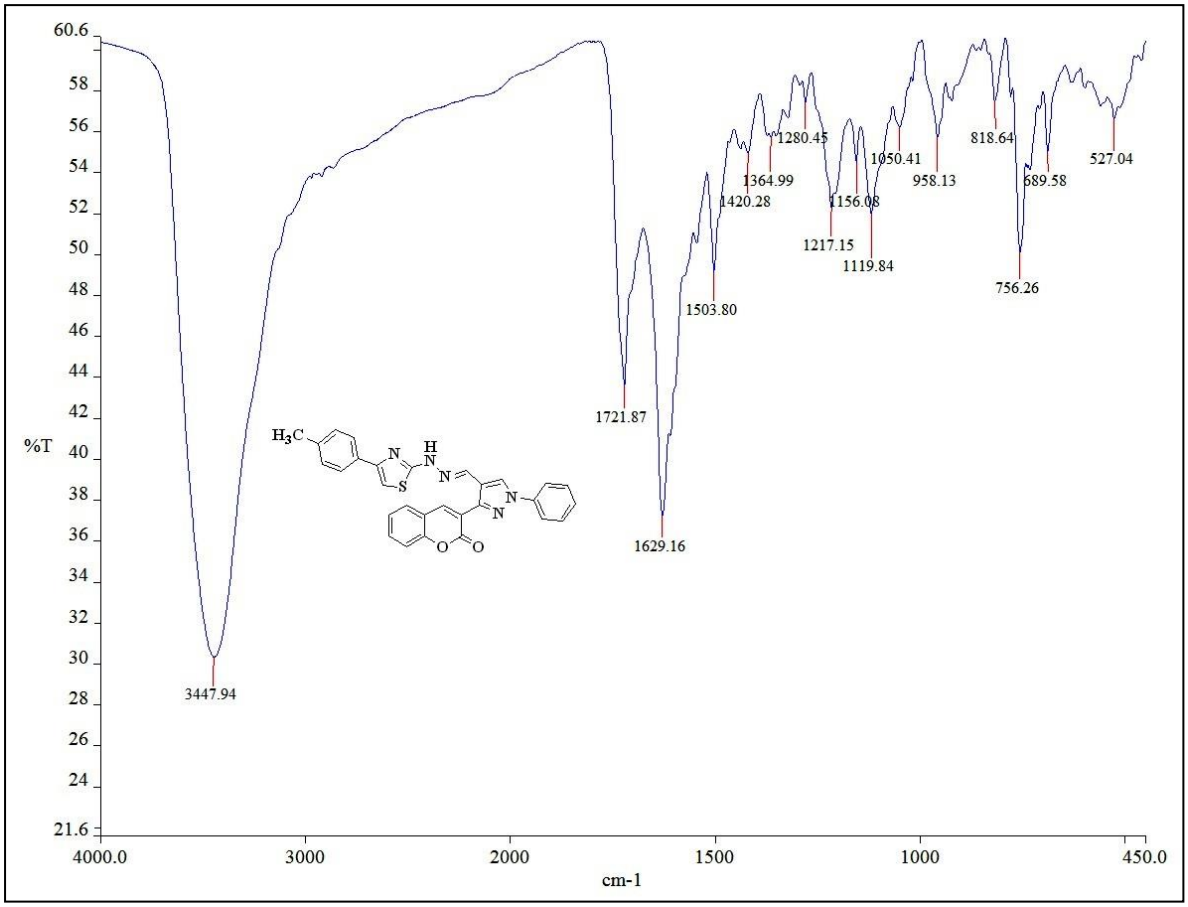
IR (KBr) spectrum of compound 5a



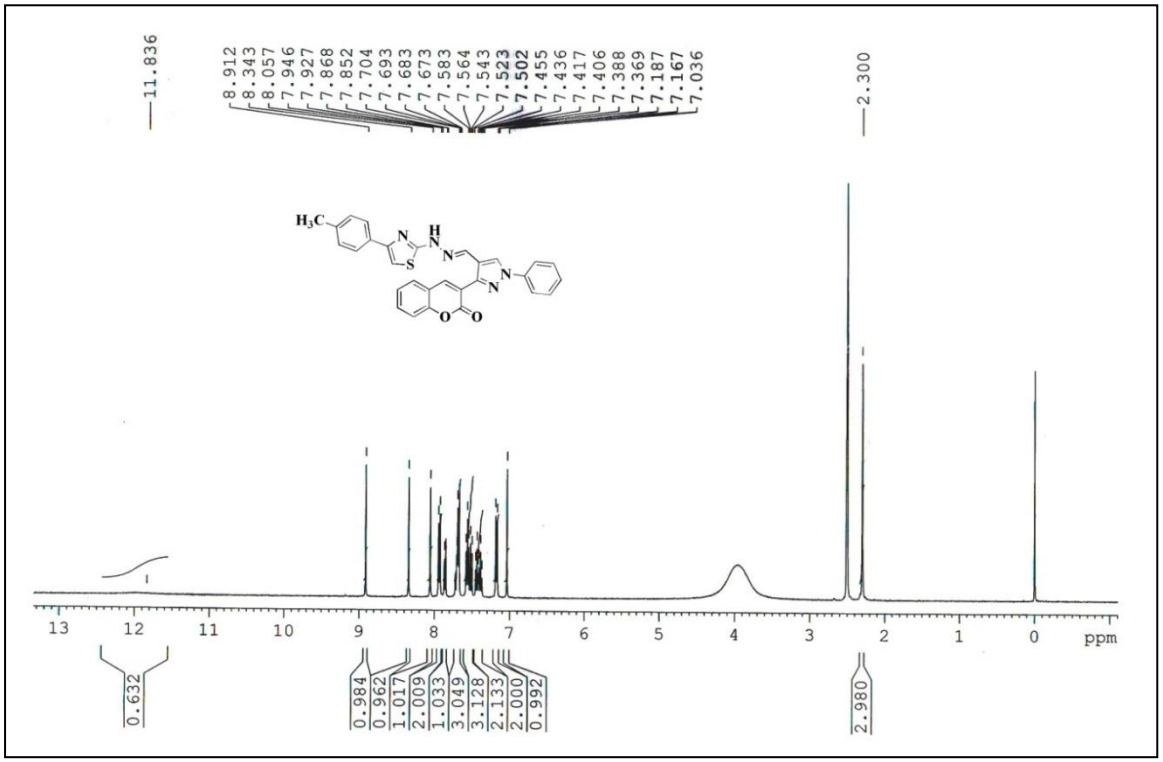
^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound 5a



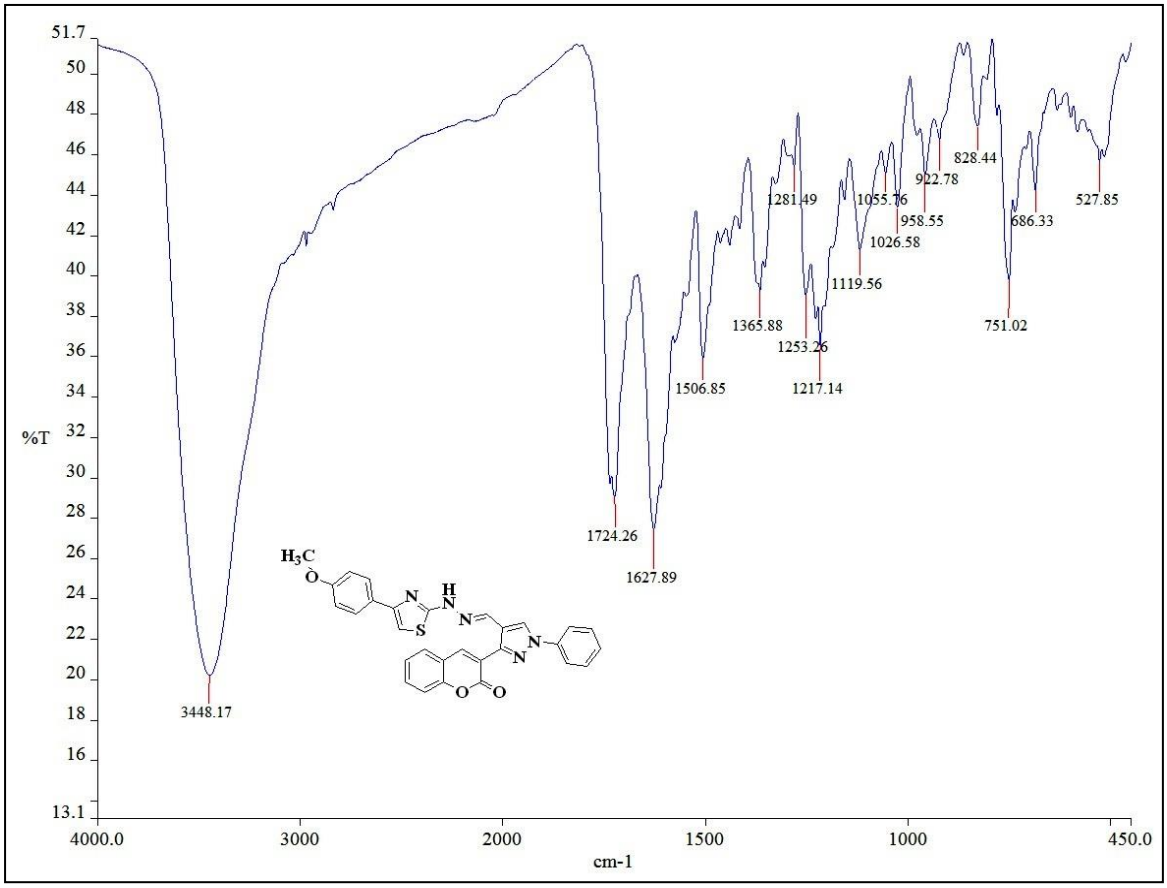
Mass spectrum of compound 5a (M.Wt: 489)



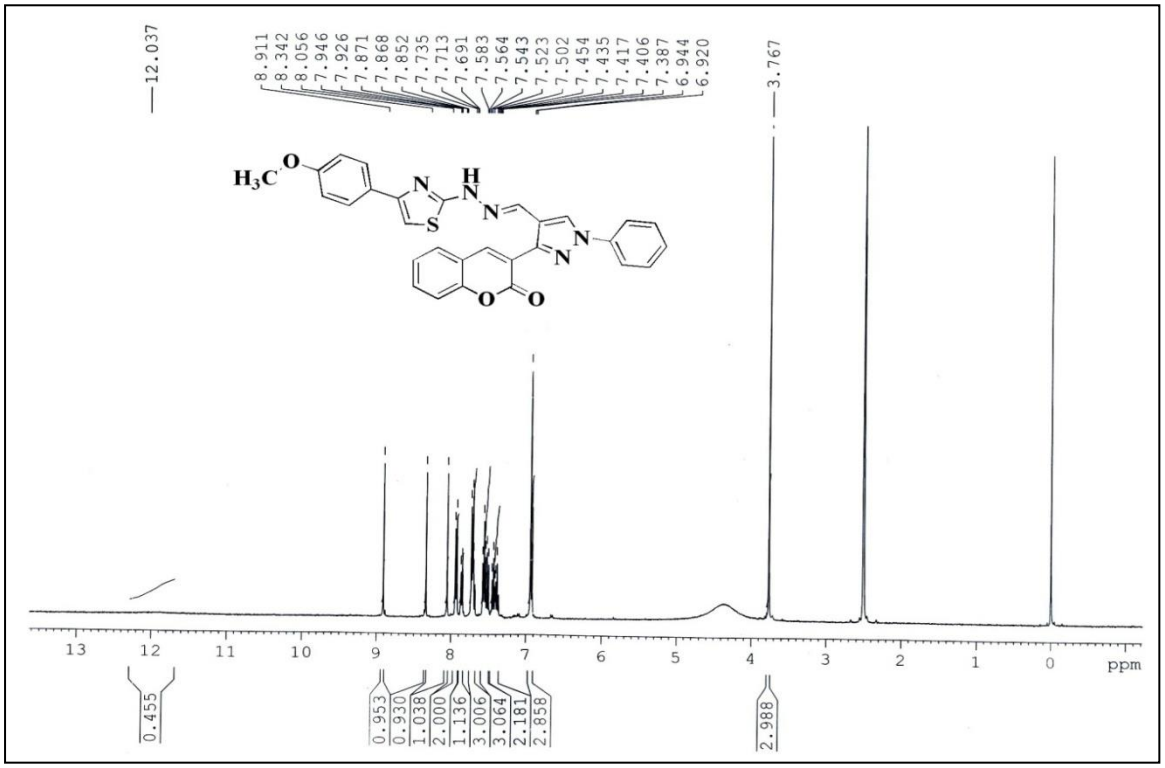
IR (KBr) spectrum of compound 5b



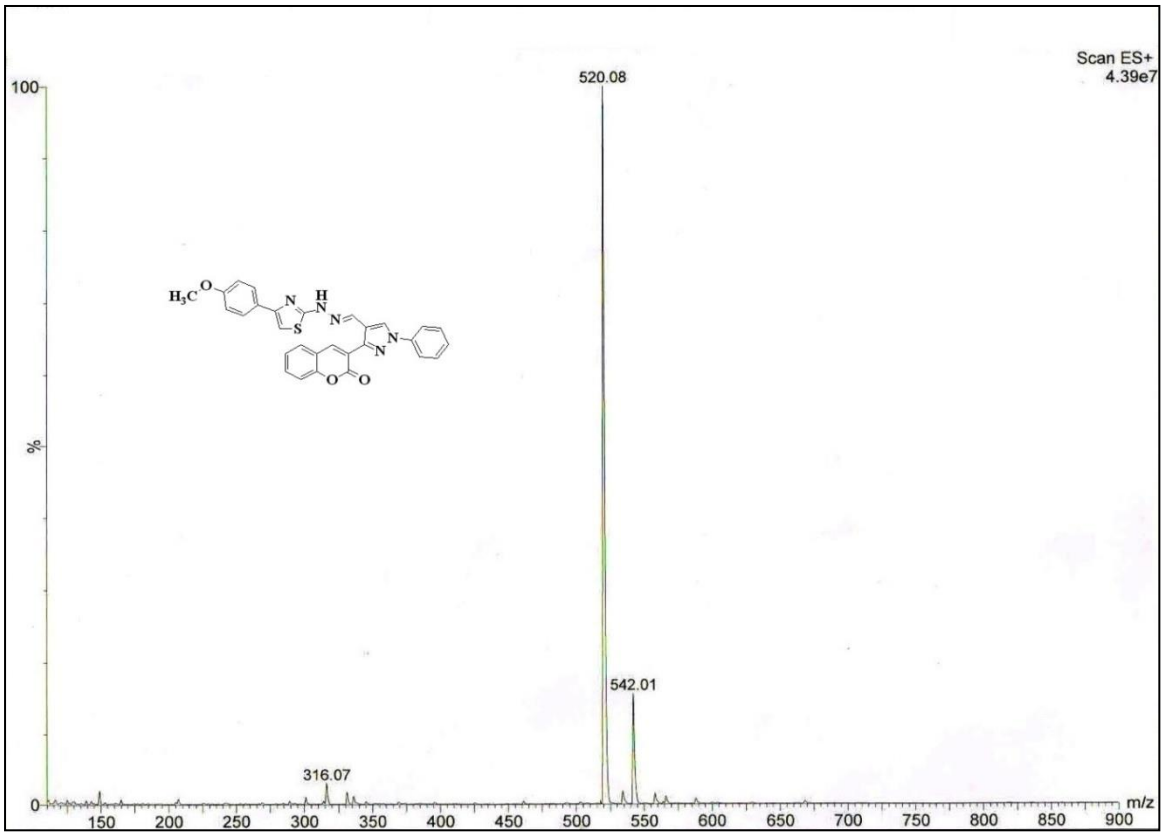
^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound 5b



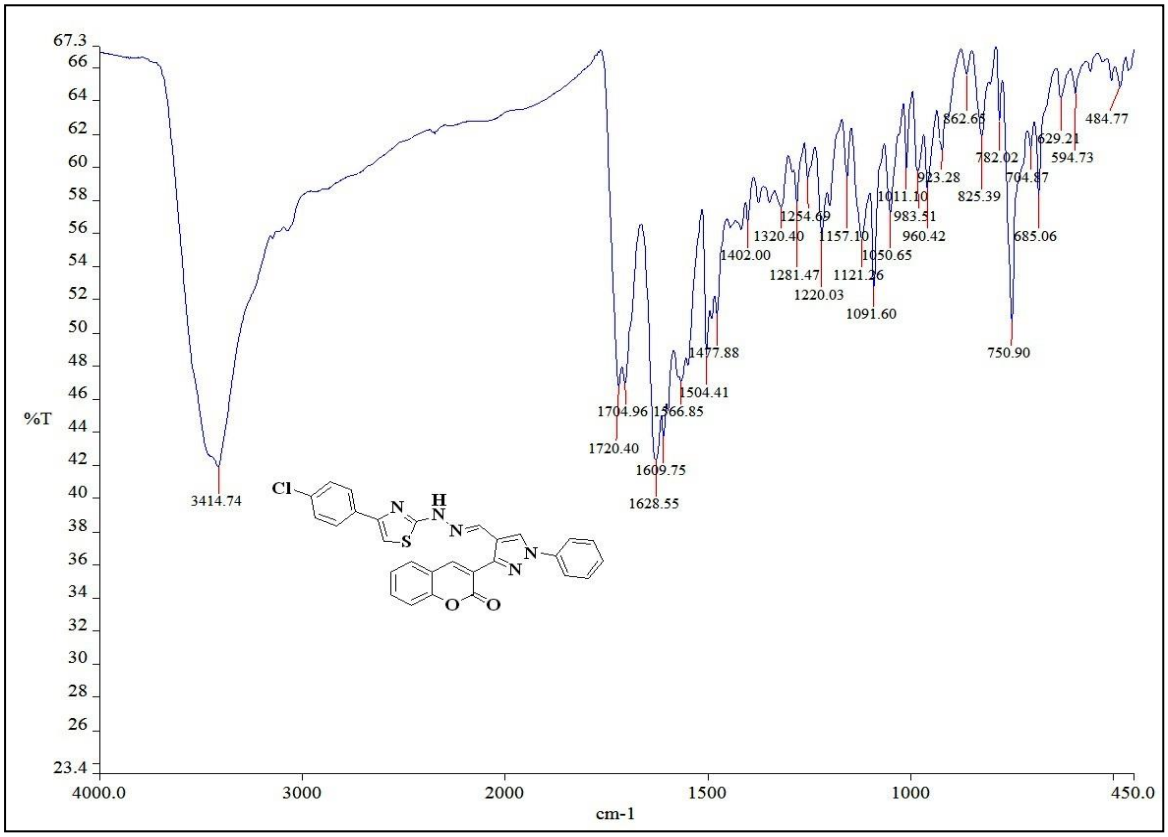
IR (KBr) spectrum of compound 5c



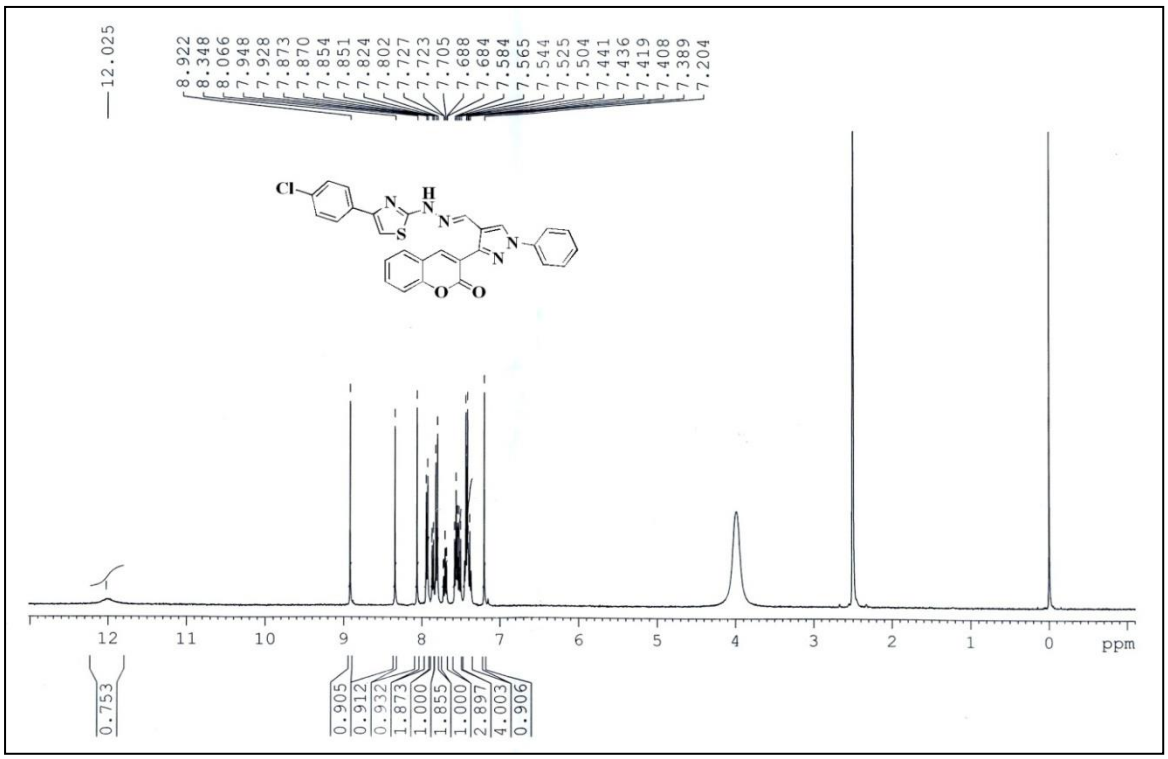
^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound 5c



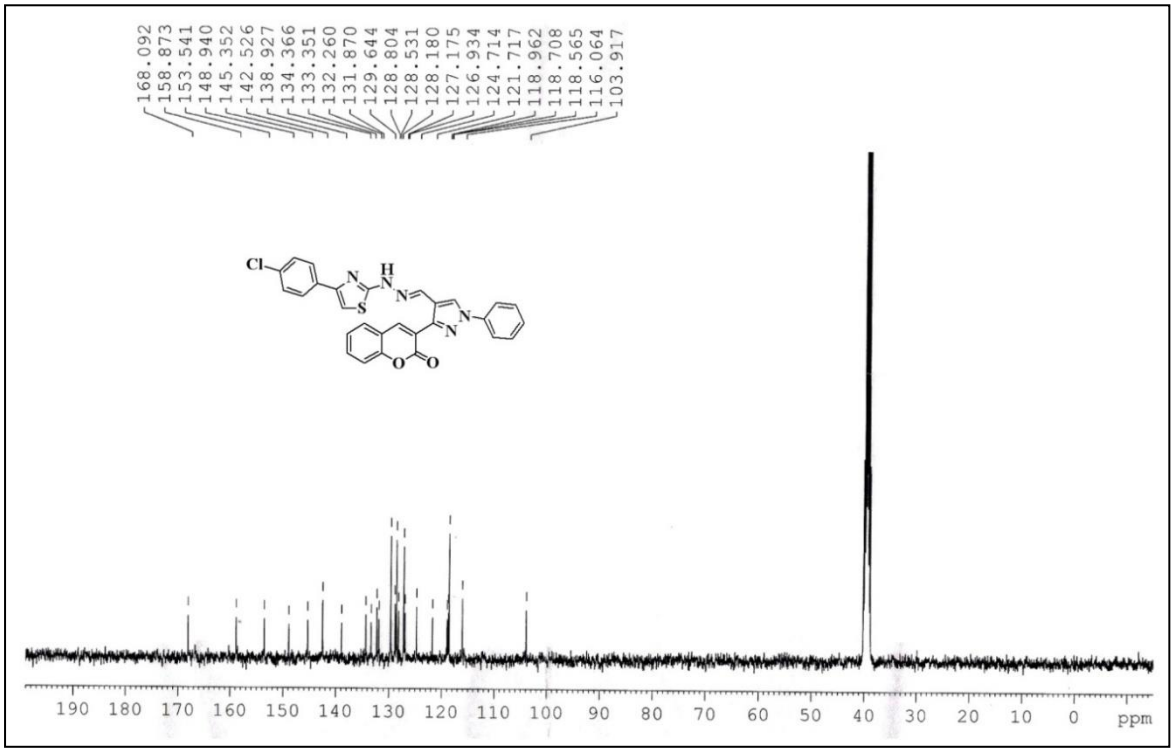
Mass spectrum of compound 5c (M.Wt: 519)



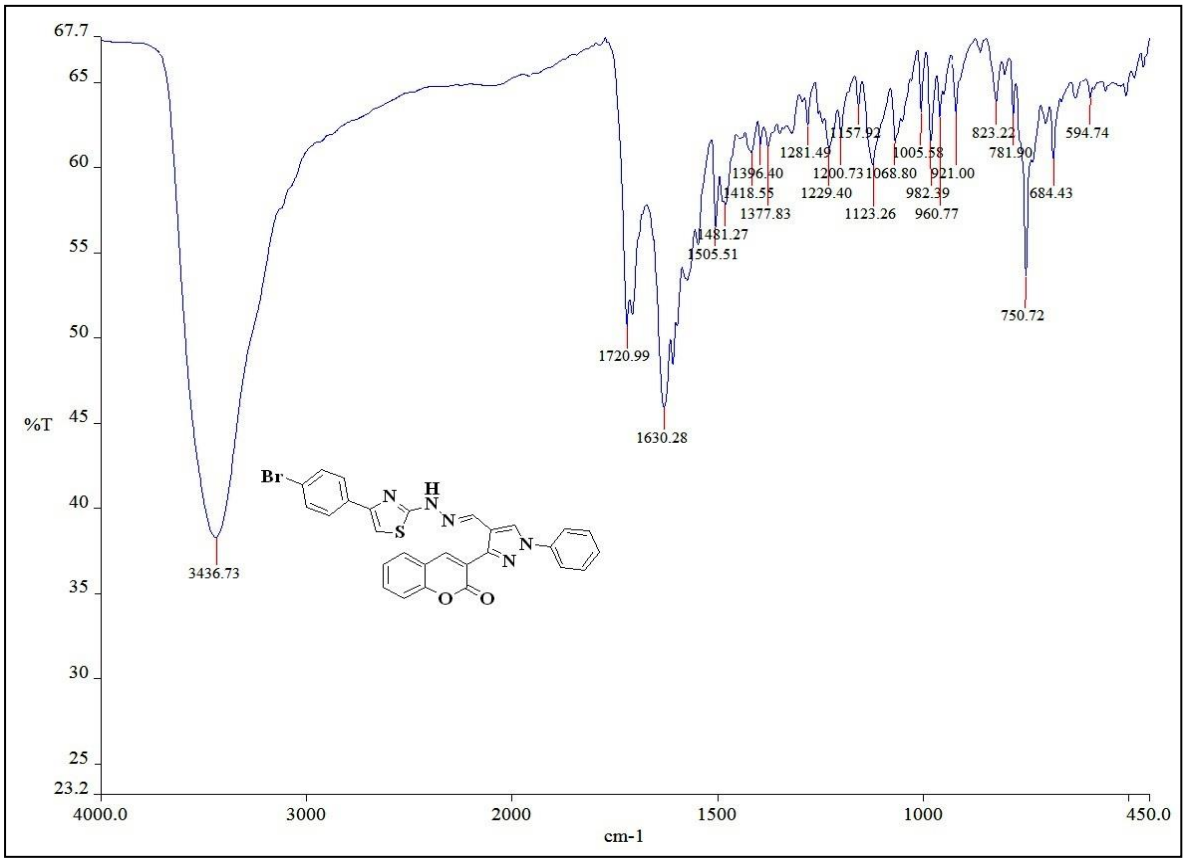
IR (KBr) spectrum of compound 5d



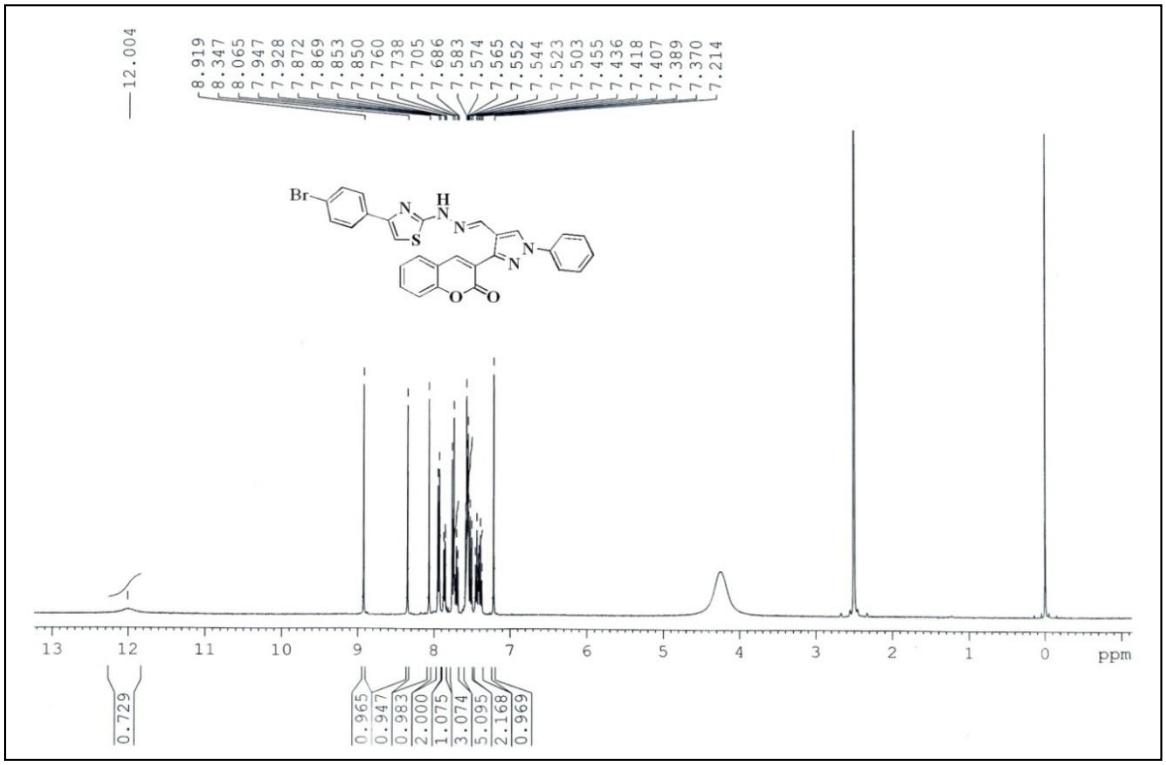
^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound 5d



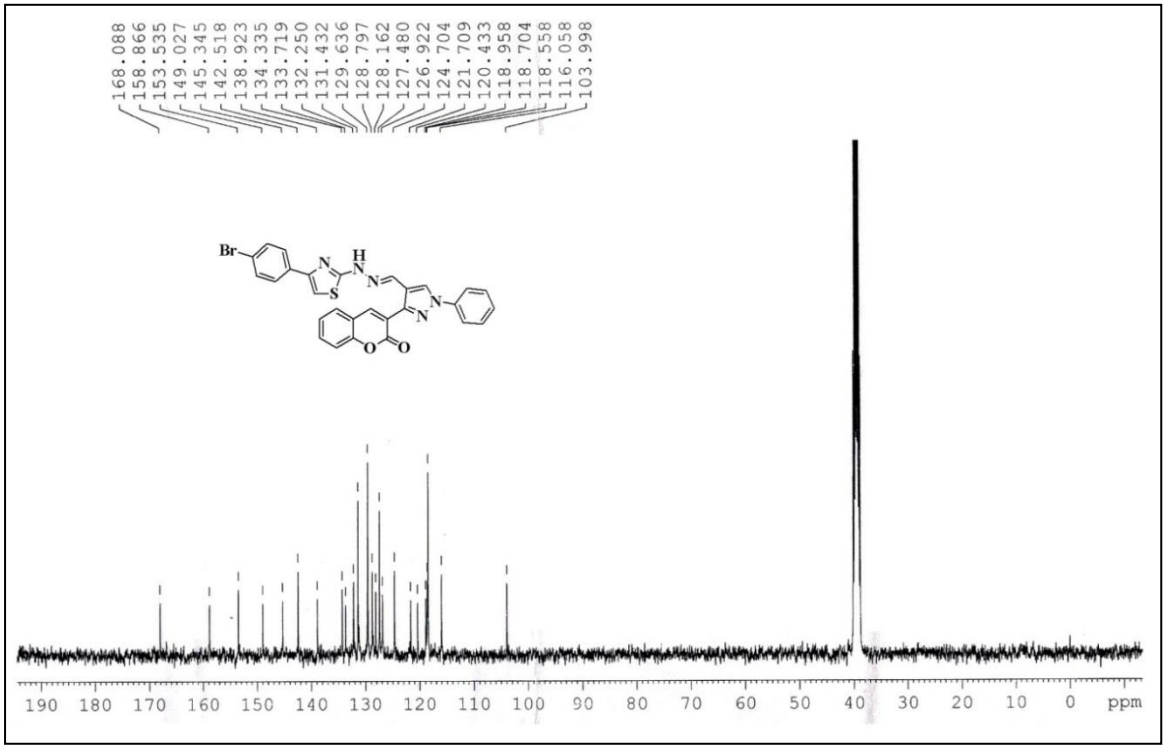
^{13}C NMR (100 MHz, DMSO-d_6) spectrum of compound 5d



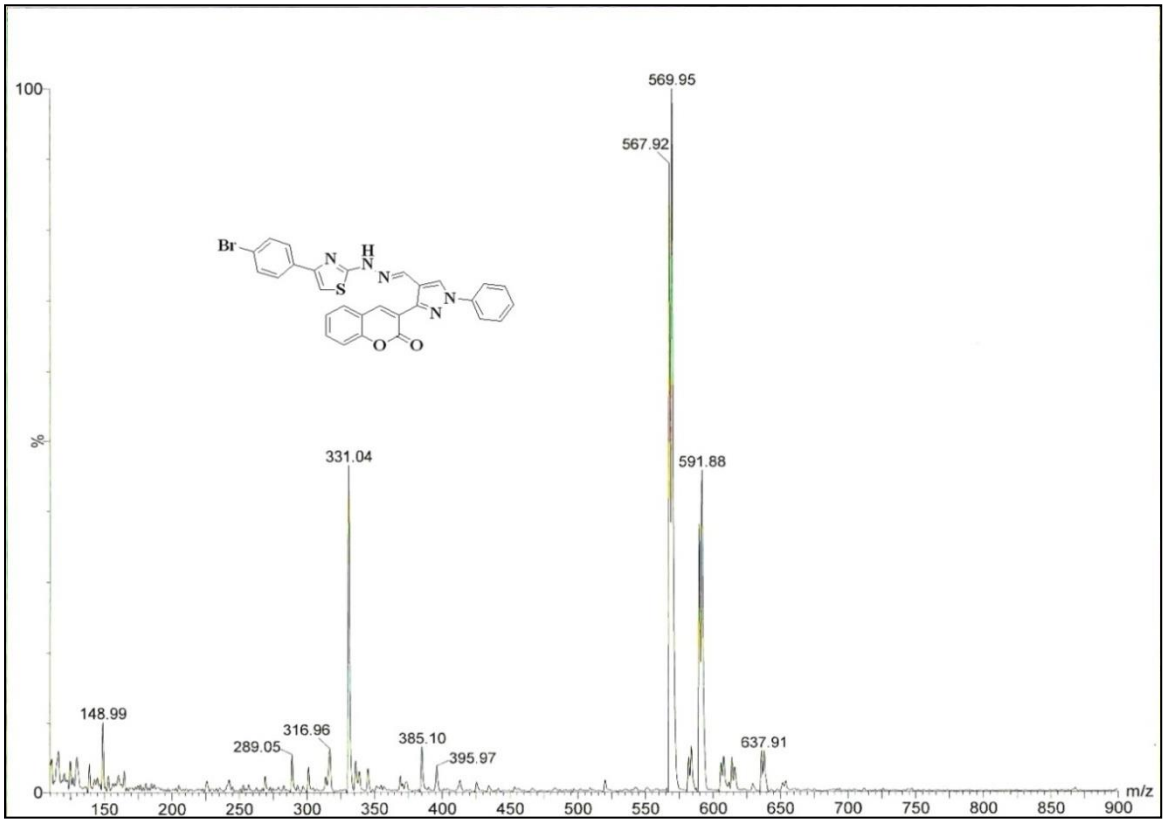
IR (KBr) spectrum of compound 5e



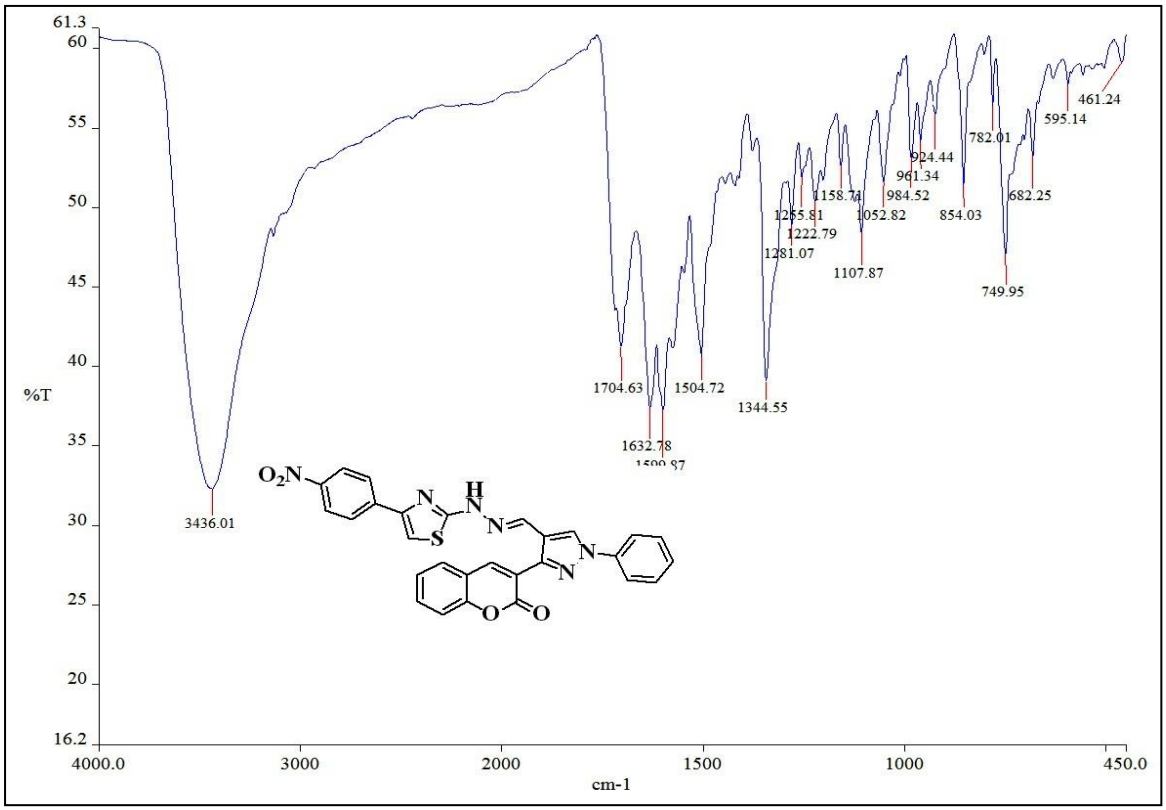
^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound 5e



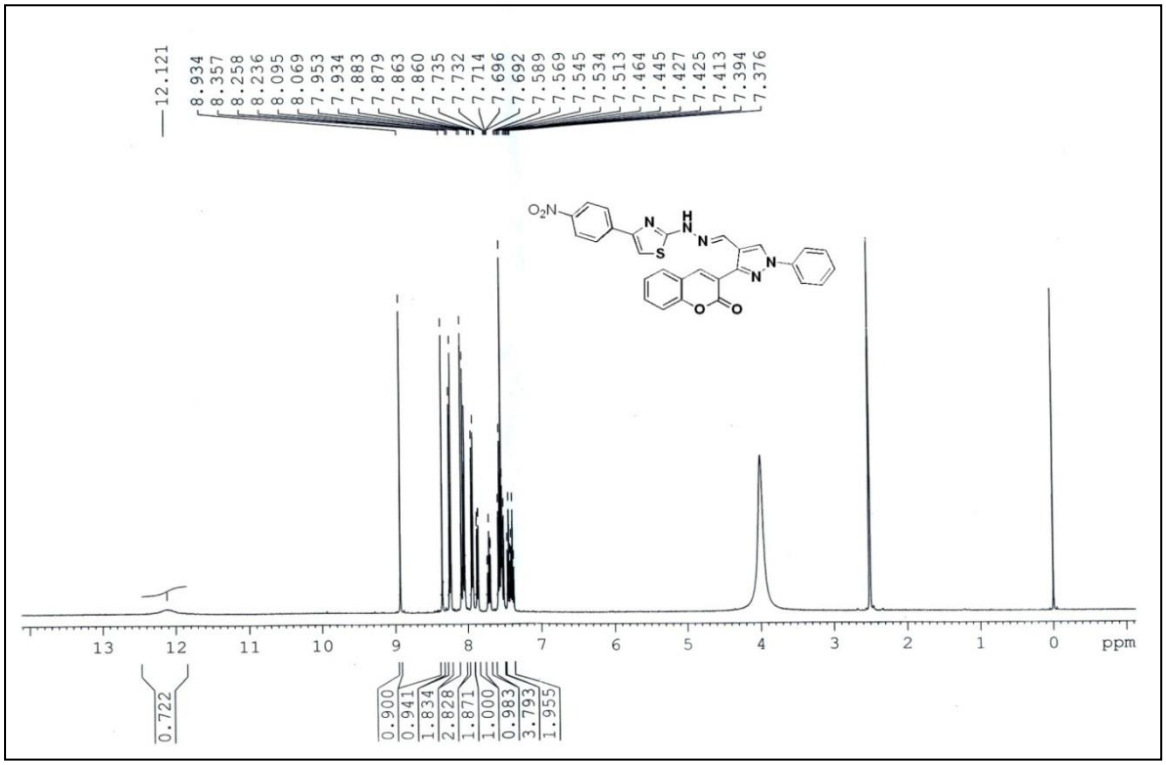
^{13}C NMR (100 MHz, DMSO-d_6) spectrum of compound 5e



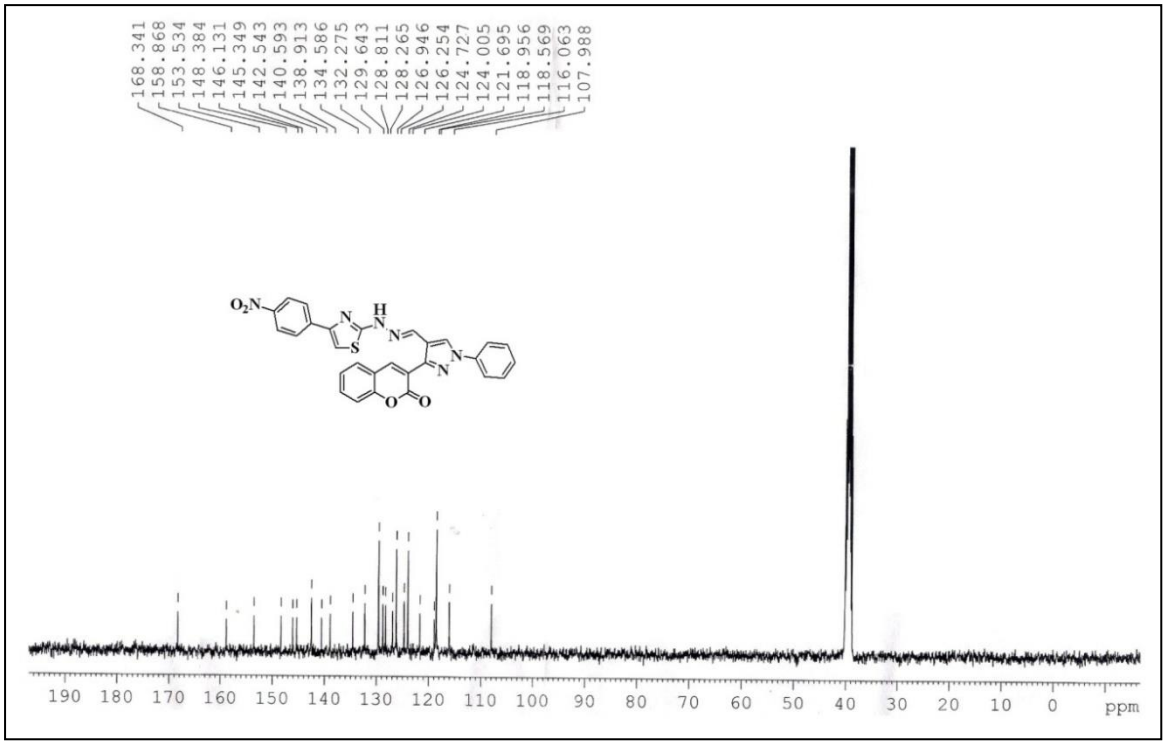
Mass spectrum of compound 5e (M.Wt: 568)



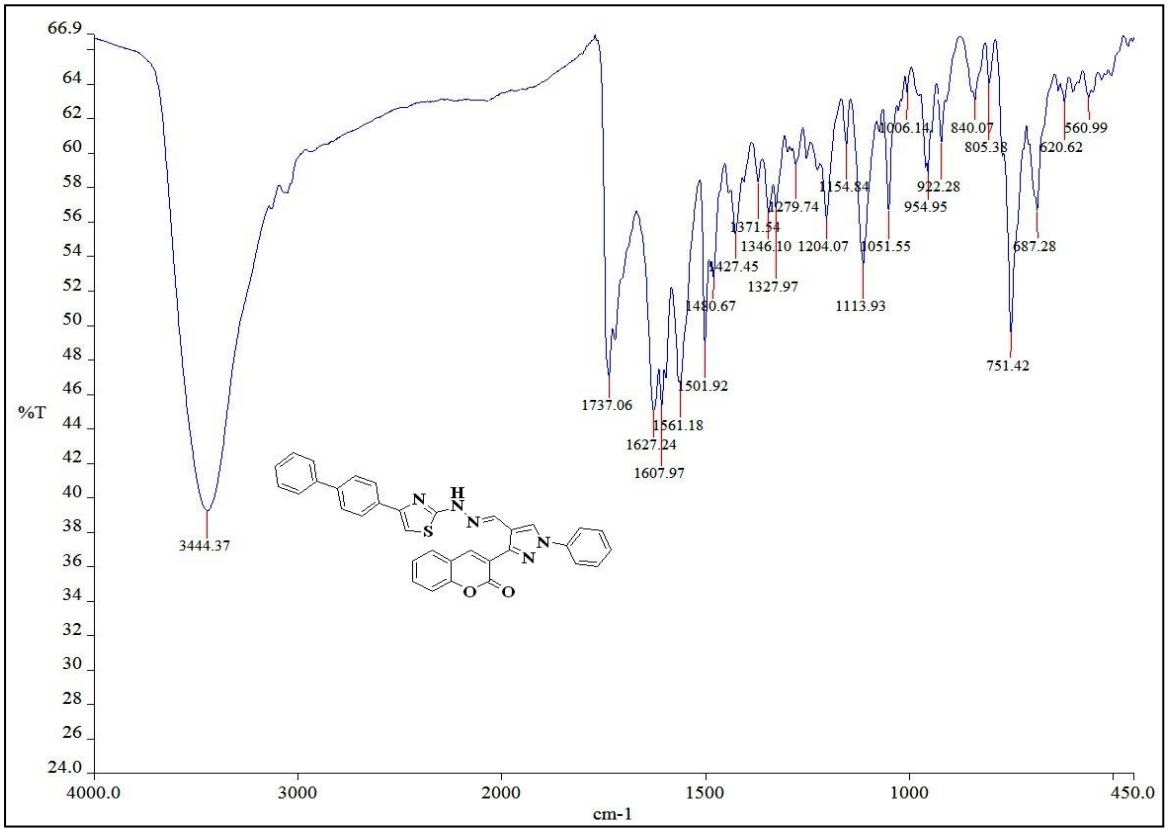
IR (KBr) spectrum of compound 5f



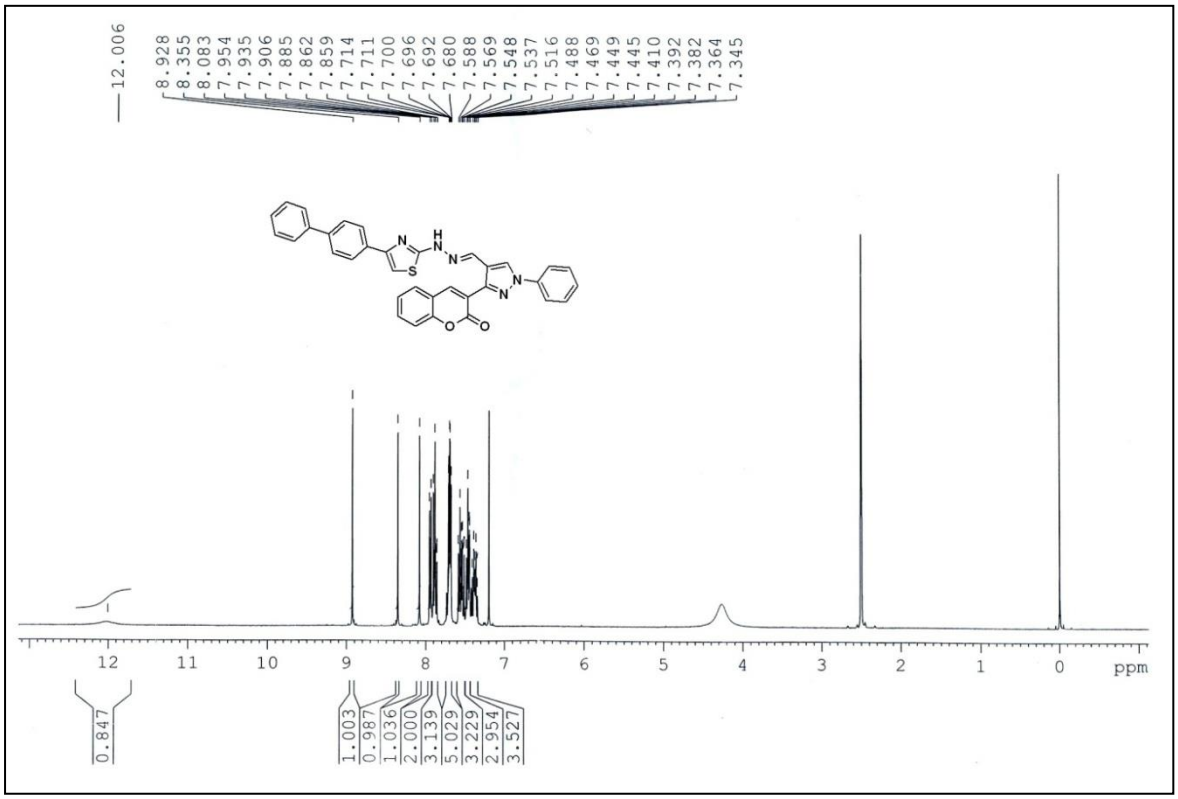
^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound 5f



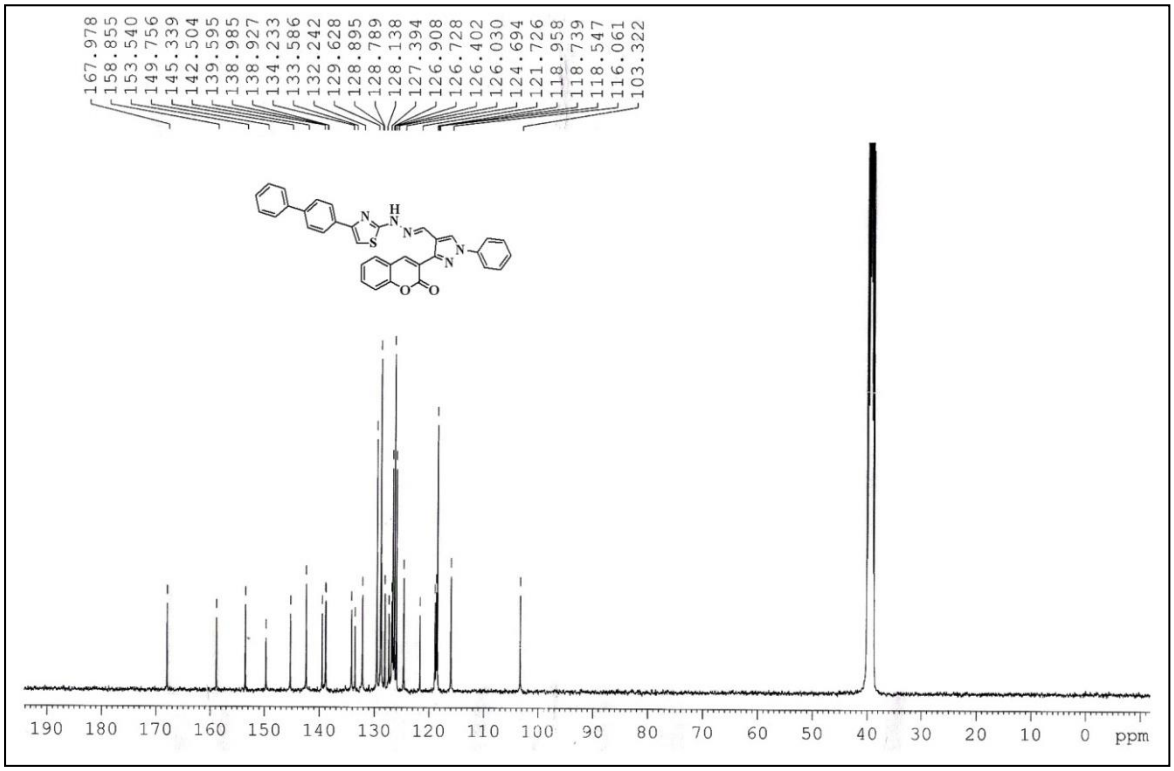
^{13}C NMR (100 MHz, DMSO-d_6) spectrum of compound 5f



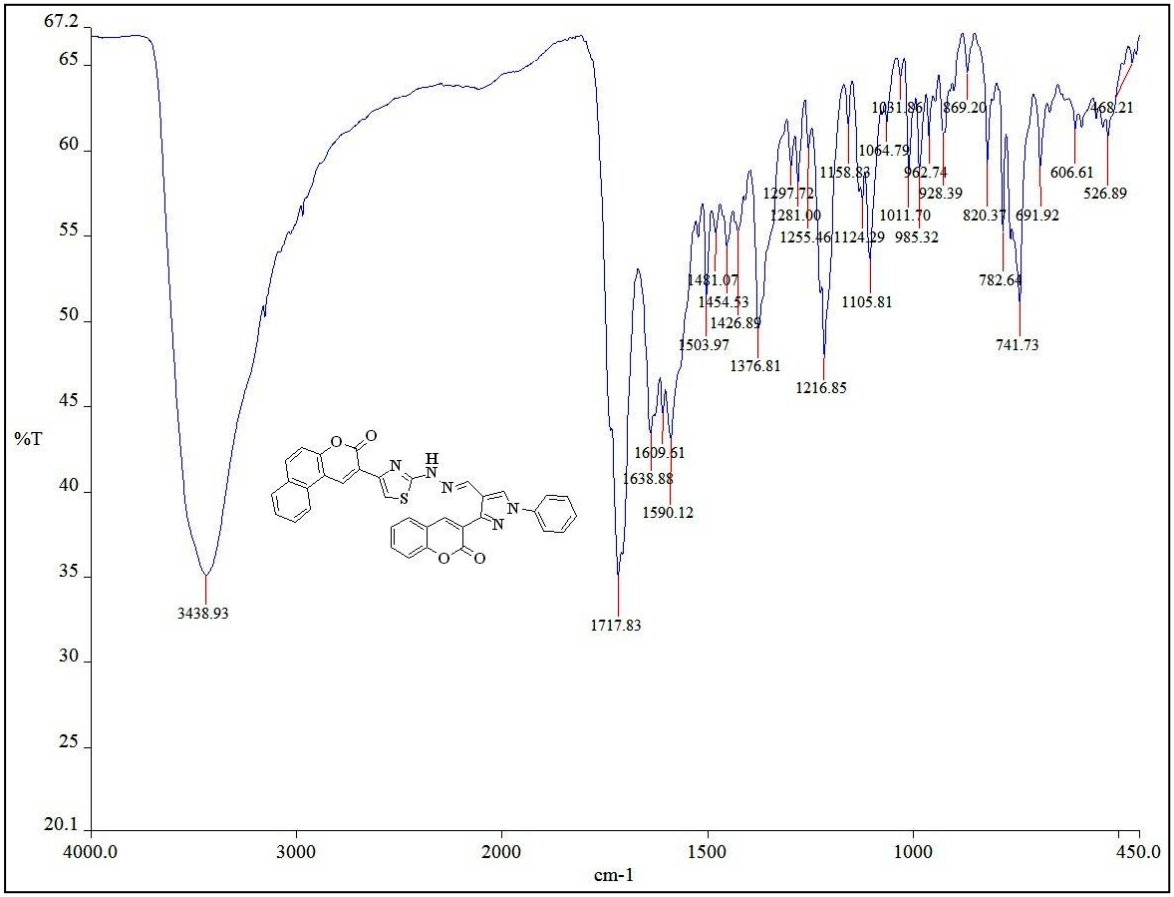
IR (KBr) spectrum of compound 5g



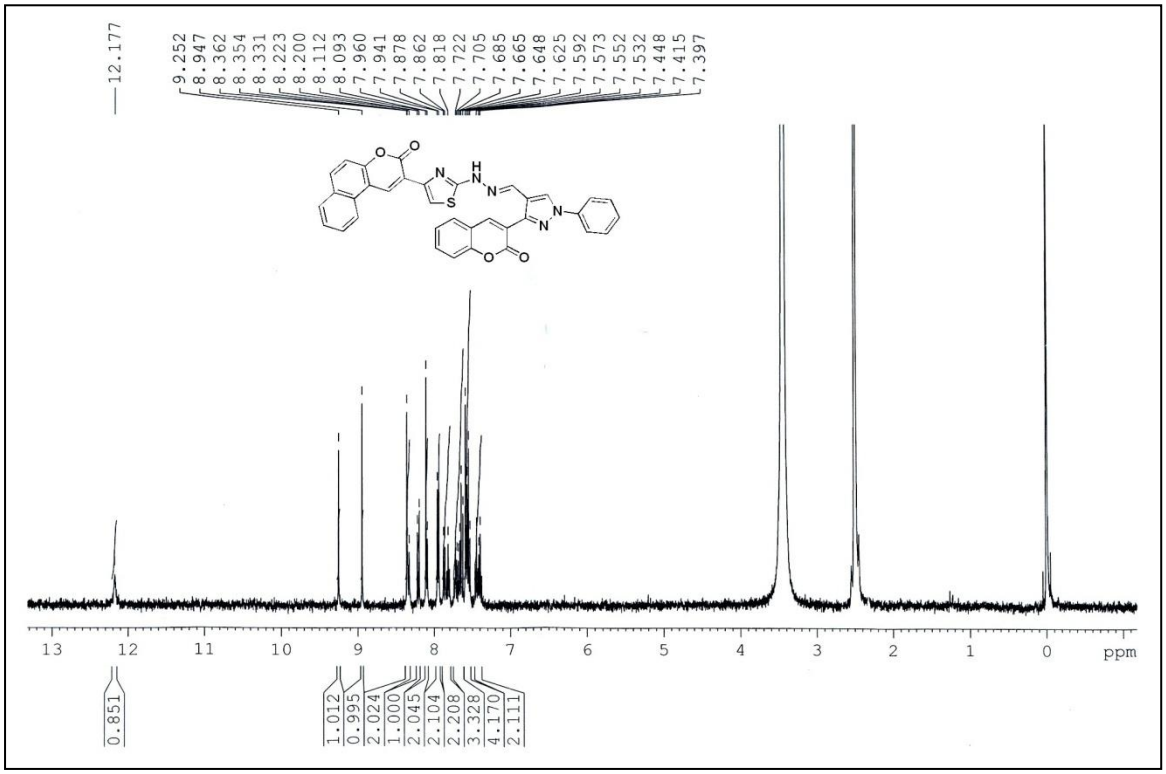
^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound 5g



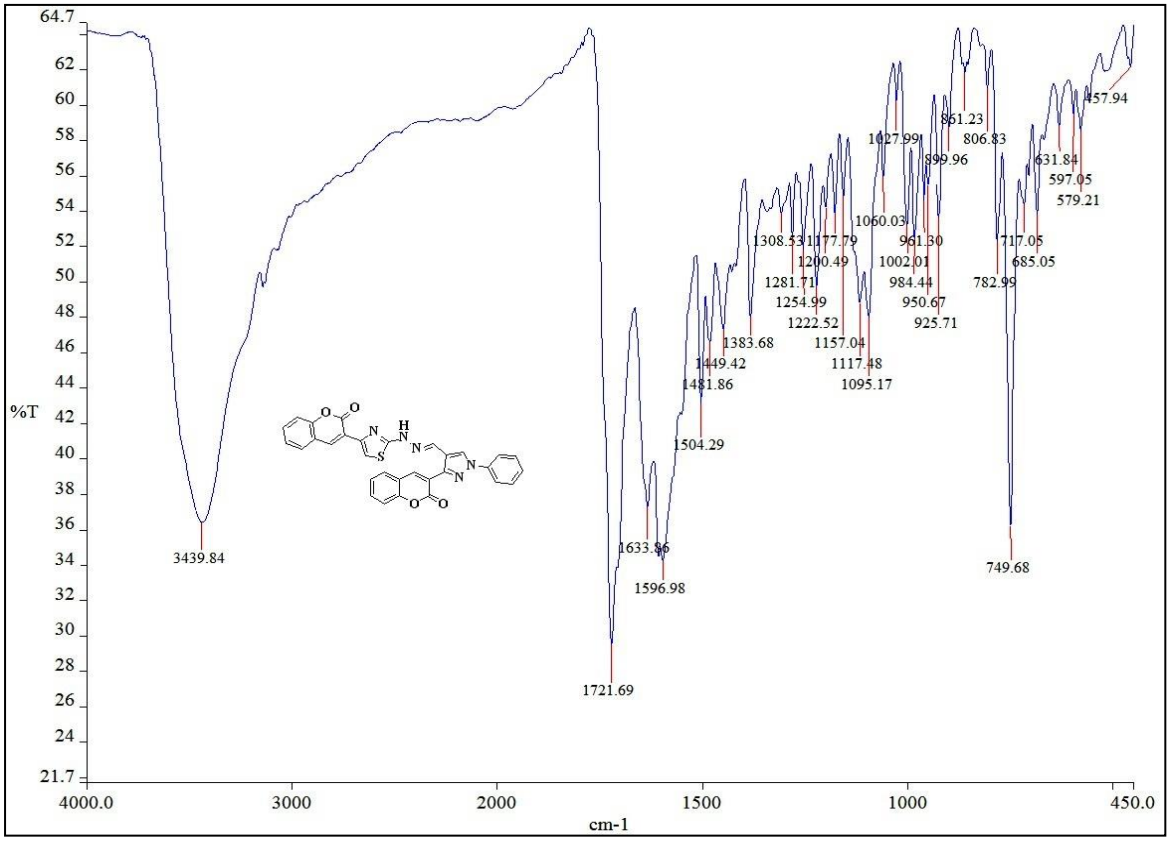
^{13}C NMR (100 MHz, DMSO-d_6) spectrum of compound 5g



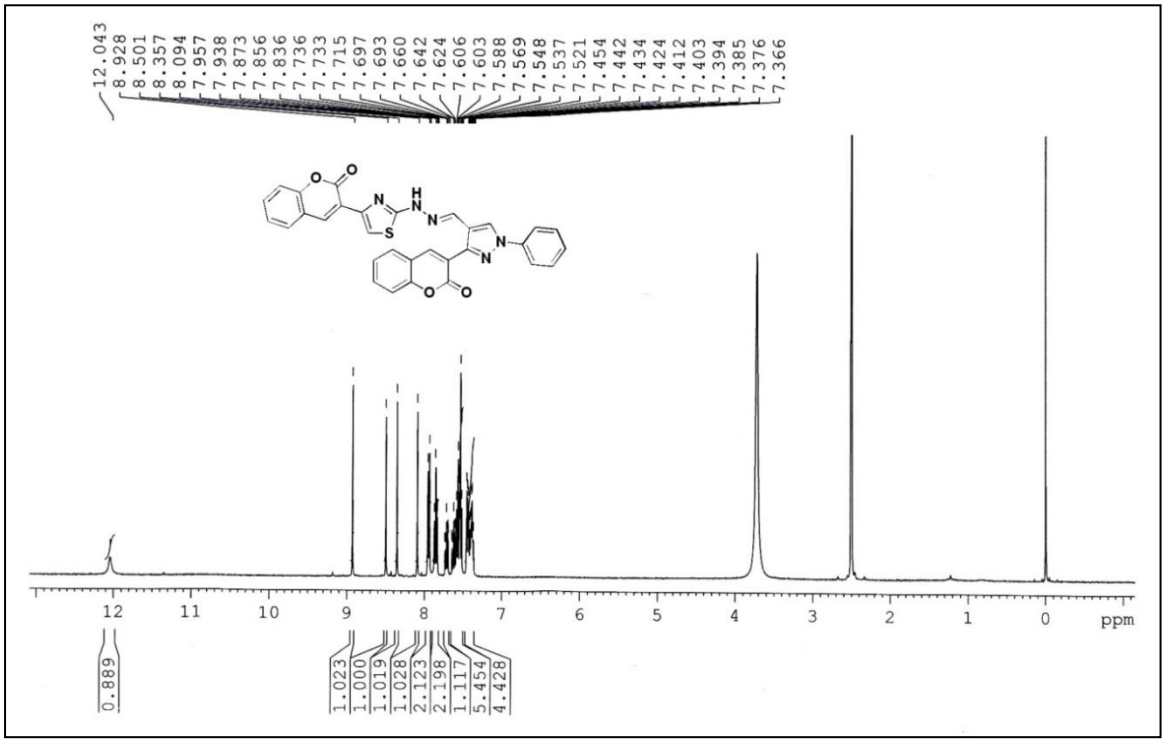
IR (KBr) spectrum of compound 5h



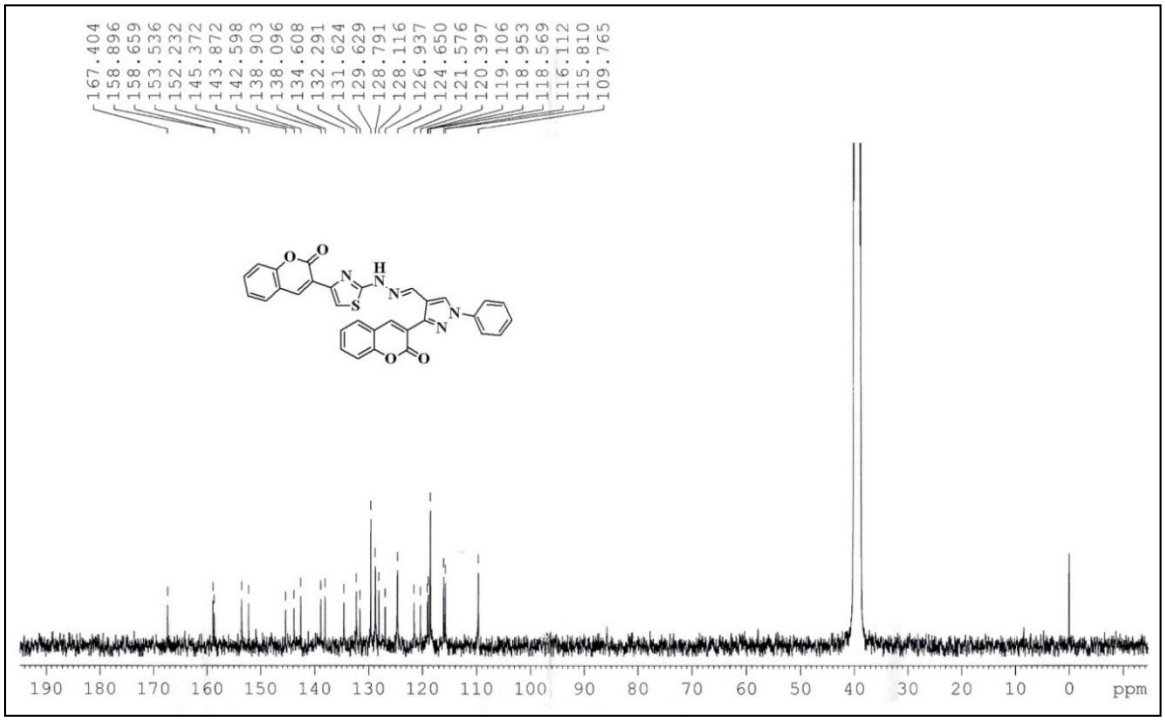
^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound 5h



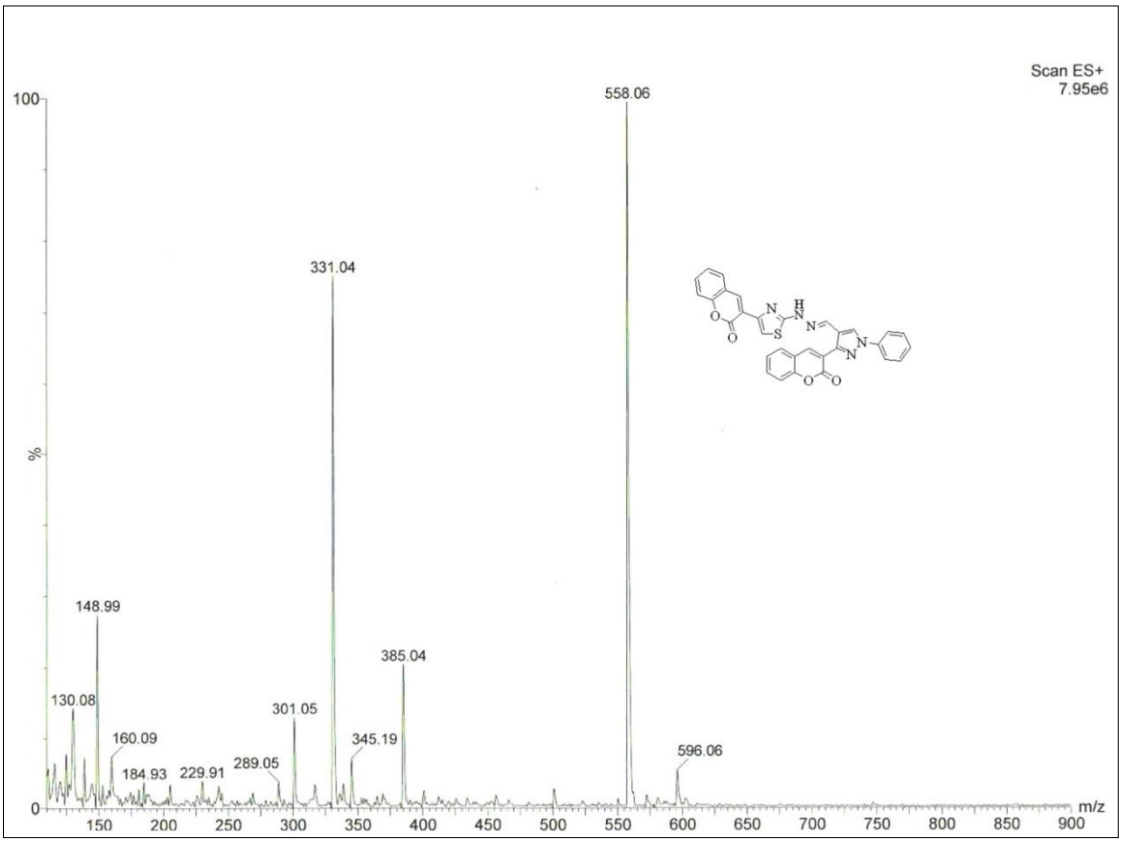
IR (KBr) spectrum of compound 5i



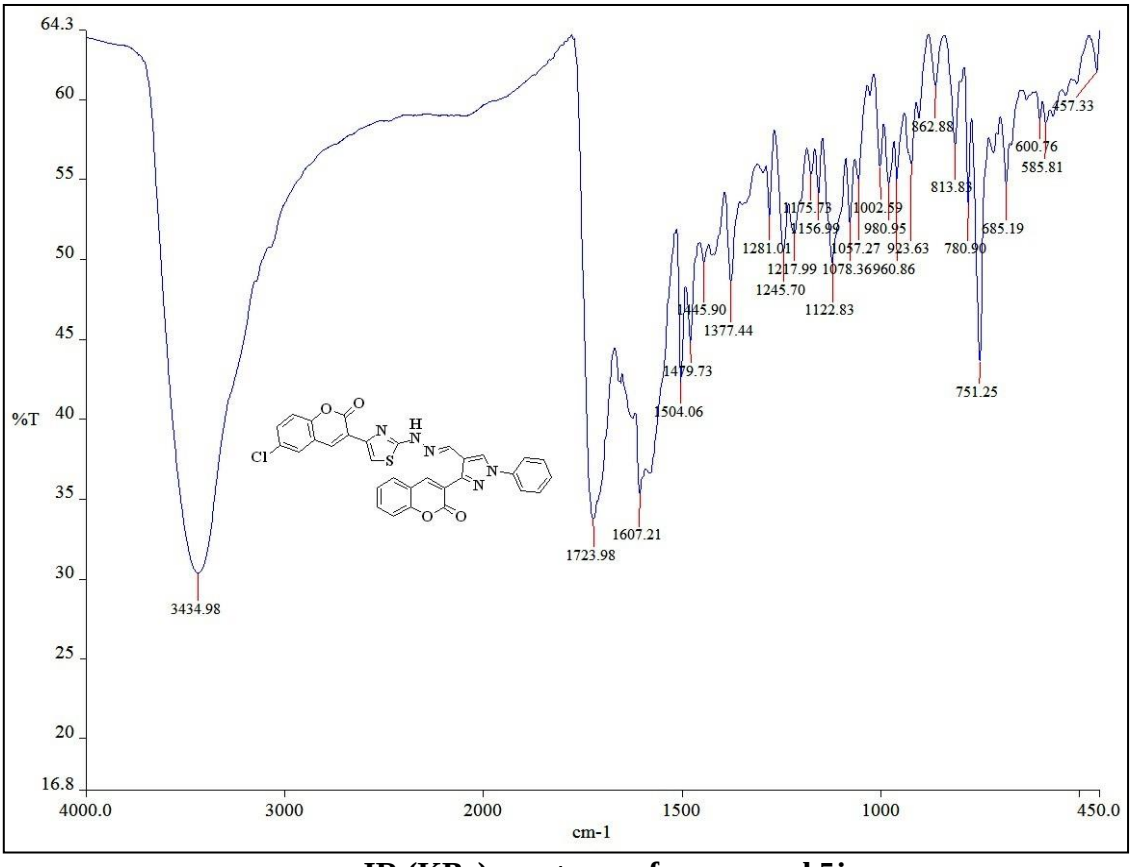
^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound 5i



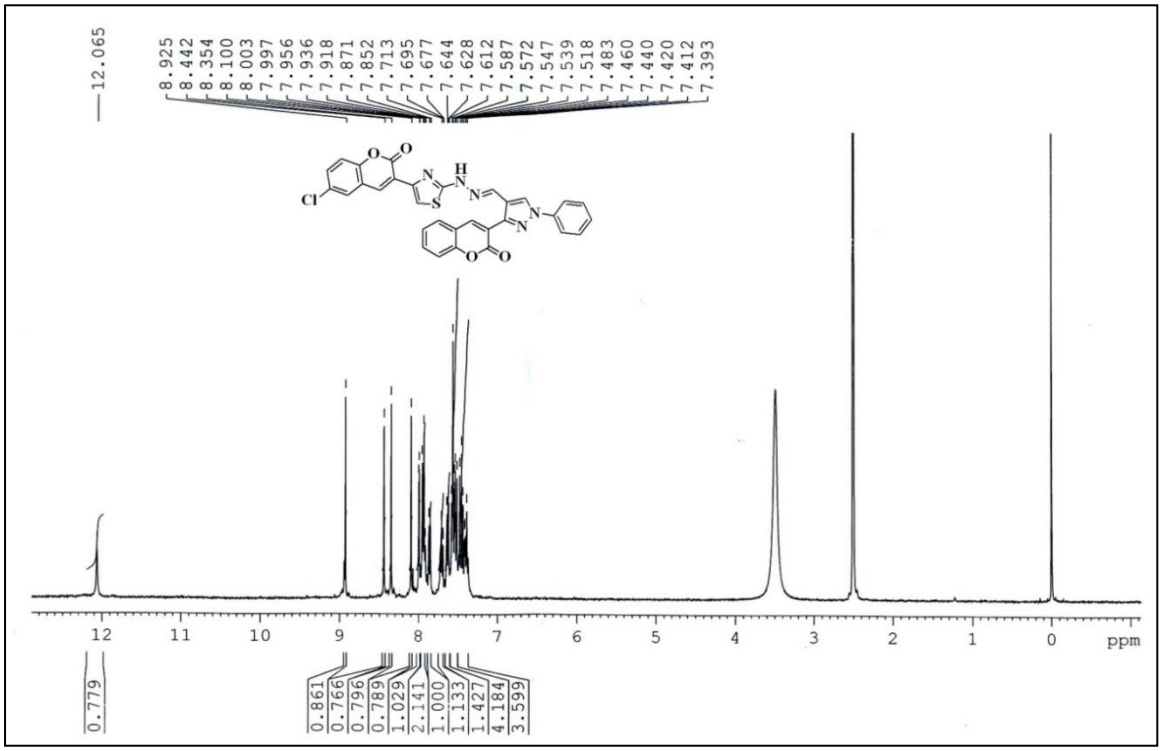
^{13}C NMR (100 MHz, DMSO-d_6) spectrum of compound 5i



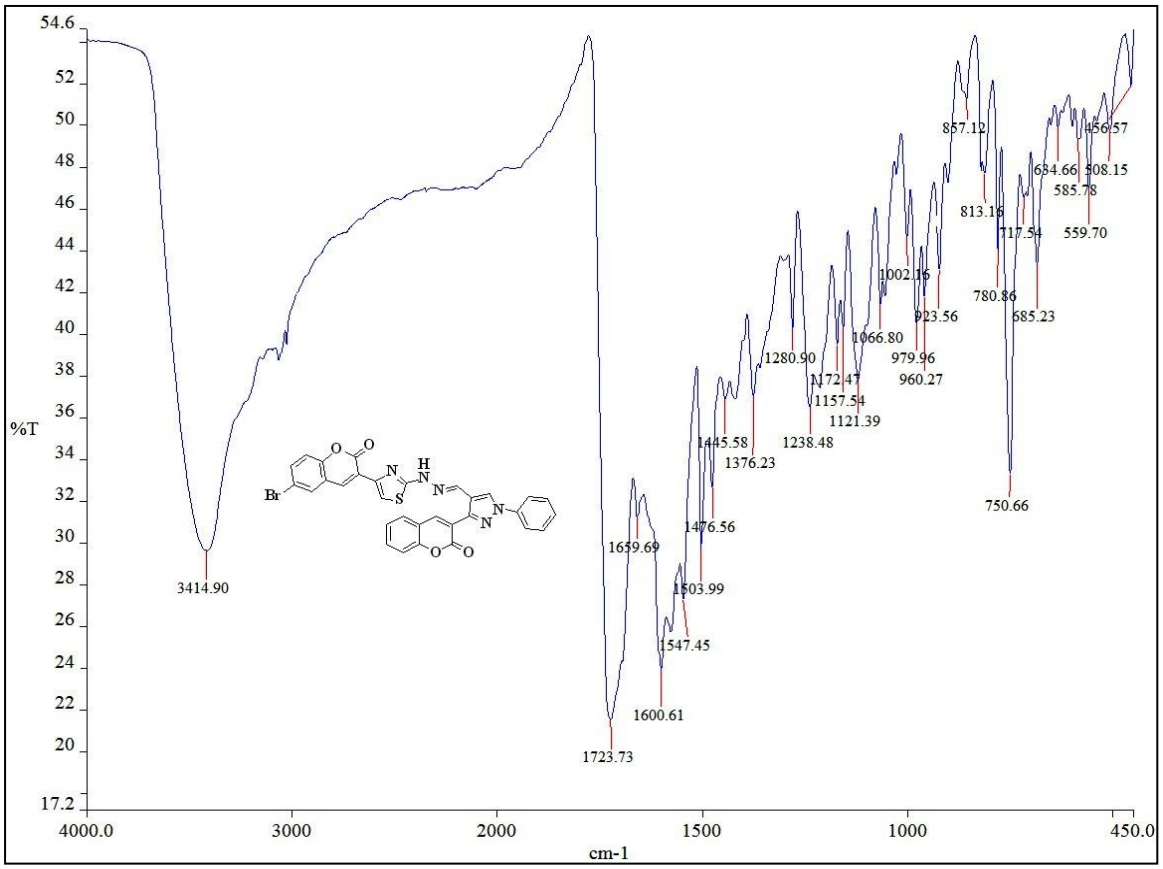
Mass spectrum of compound 5i (M.Wt: 558)



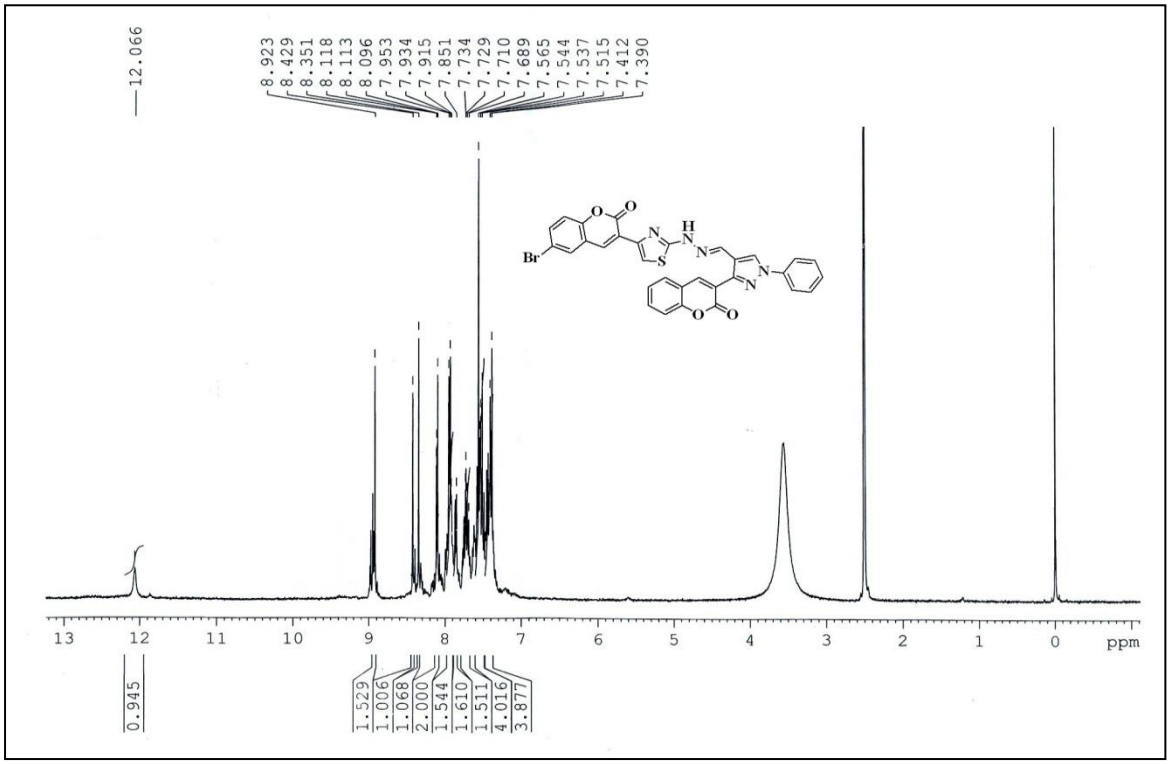
IR (KBr) spectrum of compound 5j



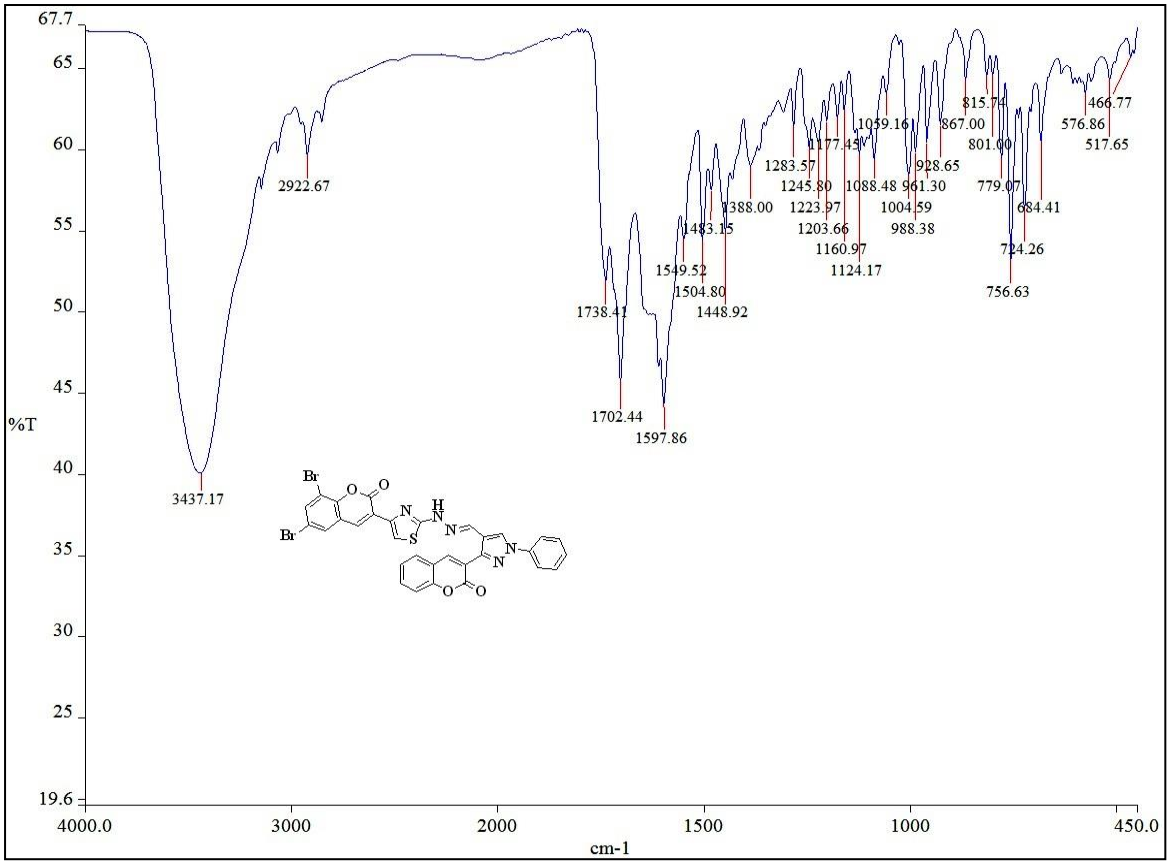
^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound 5j



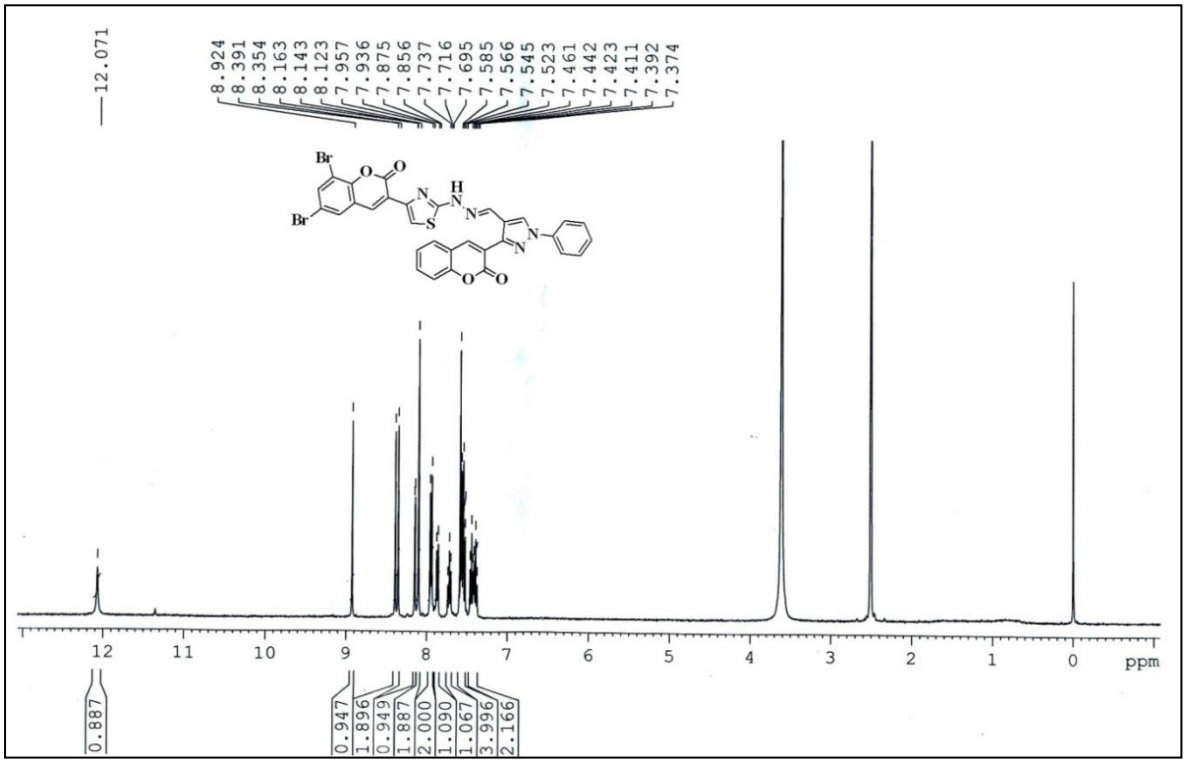
IR (KBr) spectrum of compound 5k



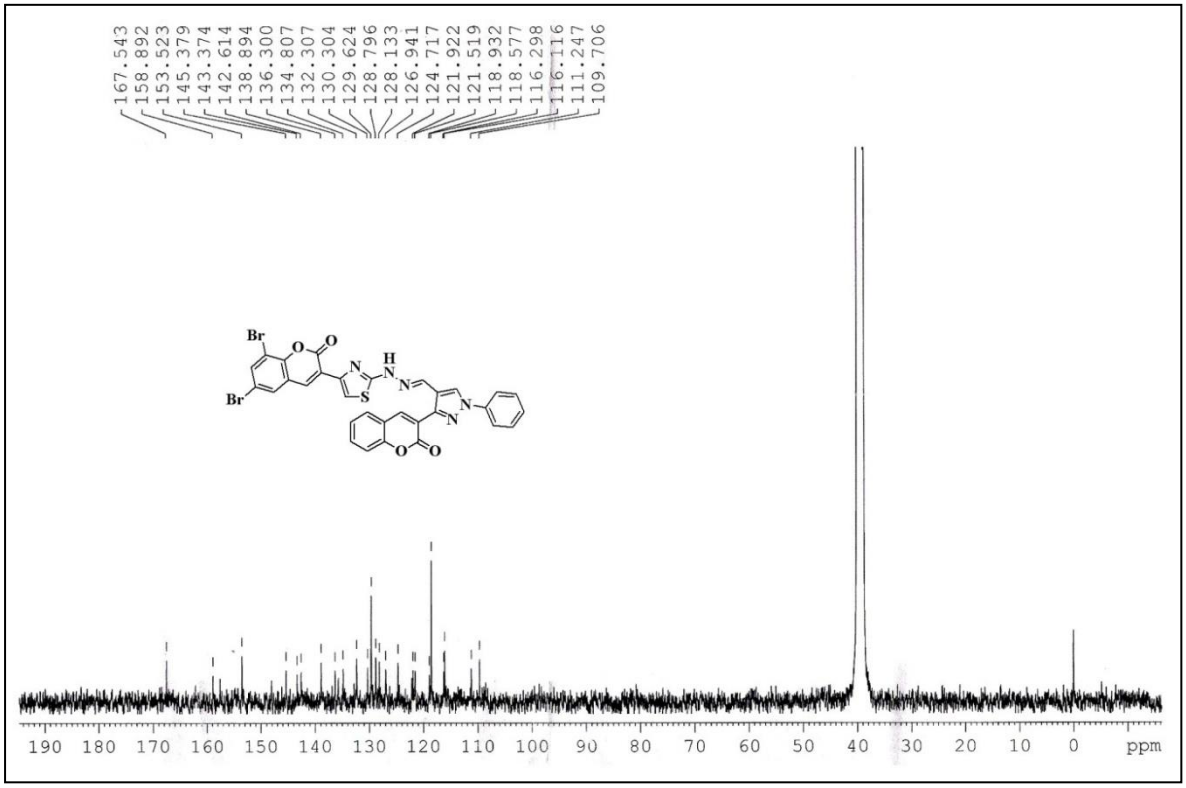
^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound 5k



IR (KBr) spectrum of compound 5l



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



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

CHAPTER-II (SECTION-B)

DESIGN, THREE COMPONENT ONE-POT SYNTHESIS AND *IN VITRO* BIOLOGICAL EVALUATION OF NOVEL 1,3-DISUBSTITUTED PYRAZOLE-2,4-DISUBSTITUTED THIAZOLE HYBRIDS EMBEDDING BENZOTHIAZOLE AND COUMARIN MOIETIES AS ANTIMICROBIAL, ANTI-BIOFILM AGENTS

INTRODUCTION

Despite several antibiotics and chemotherapeutics available, the treatment of pathogenic bacterial infections remains a pandemic and challenging therapeutic problem to the health care of the modern world.[1,2] The emerging of multi-drug resistant (MDR) superbugs or nightmare bacterial strains[3], which in turn resulted from the excessive prophylaxis of antibiotics in conjunction with mutations adopted by the pathogenic strains making paucity of chemotherapeutics and escalating an urgency of the development of alternative drug candidates.[4] In the past few decades, the interest towards the discovery of broad-spectrum novel antibiotics and antimicrobials with less toxicity, specificity, efficacy and a new multi-target mechanism of action[5], has gained much attention by the scientific and medical communities.

Furthermore, literature survey reveals that benzothiazole[6,7], coumarin[8,9], 1,2-pyrazole[10,11] and 1,3-thiazole[12,13] scaffolds are the most versatile class of compounds attained a great attention in the field of drug design and discovery owing to their vast variety of biological activities. In addition, they also make the core structure of various bioactive natural and synthetic drugs **Fig. 1.**

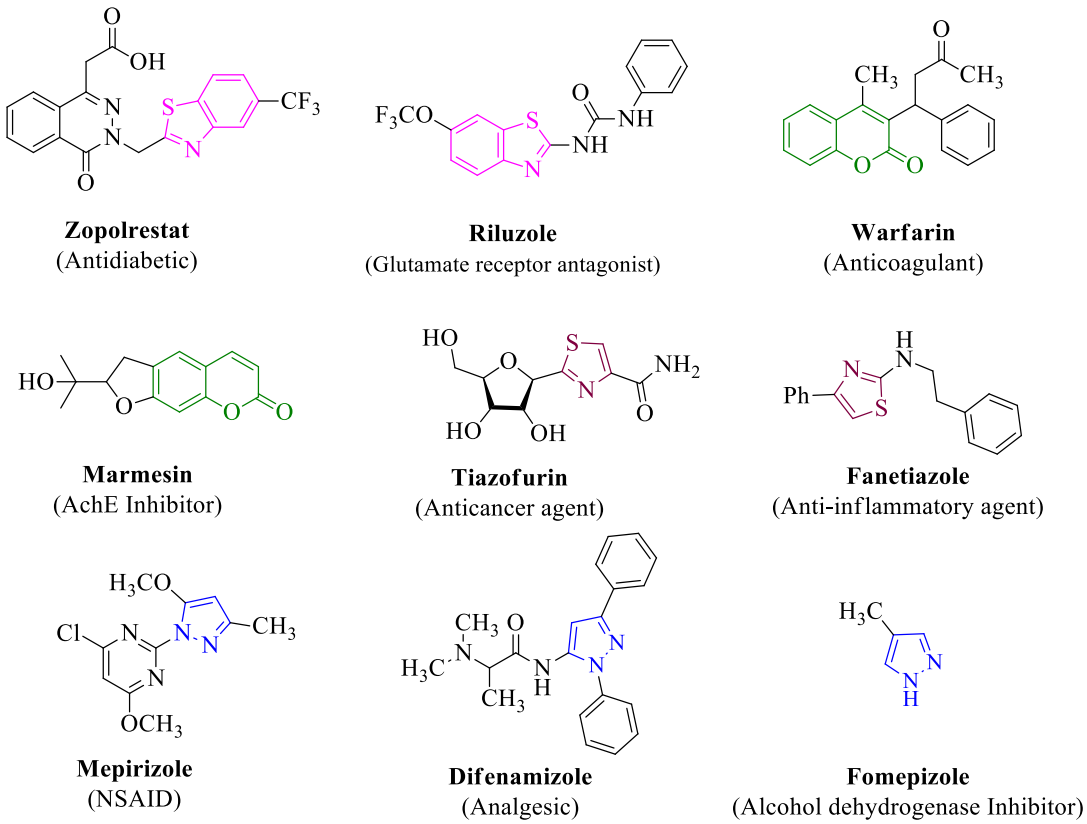
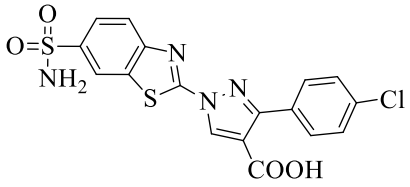


Fig. 1

Traditional combination therapy (Cocktail of drugs)[14,15] also has lead to the foundation for the formulation of such new hybrid chemical entities (ligands) in which two or more pharmacophore sub-units embedding their original characteristics in single multi-component biological entity[16], that can modulate several biological targets simultaneously with enhanced efficacy and lowers the risk of drug-drug interactions and are less prone to drug resistance.

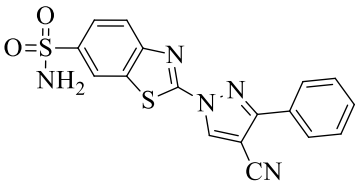
SitaRam and co-workers[17] have reported 4-functionalized 1,3-diarylpyrazoles bearing 6-aminosulfonylbenzothiazole moiety as potent inhibitors of carbonic anhydrase isoforms hCA I, II, IX and XII. Among the tested series of compound **3** exhibited 9–folds better inhibiting activity than the standard acetazolamide (AZA) against transmembrane, tumor associated isozyme hCA IX. And the compounds **3**, **4**, and **5** have comparable activity with reference drug AZA against another tumor associated isoform hCA XII.



3

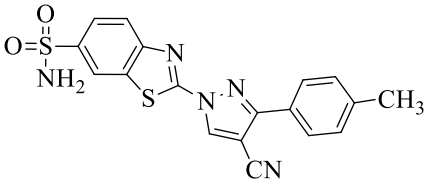
K_i = 2.8 nM against hCA IX

K_i = 5.5 nM against hCA IX



4

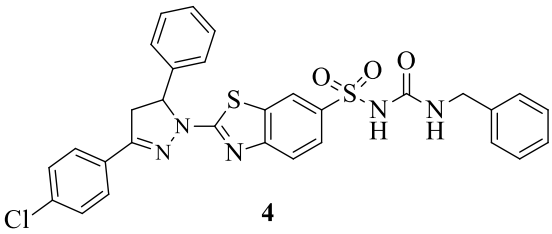
K_i = 6.6 nM



5

K_i = 7.2 nM

Chetna Kharbanda and their group[18] have described, novel benzothiazole based sulfonylureas/sulfonylthioureas: design, synthesis and evaluation of their antidiabetic potential. The target compounds were assayed for their *in vivo* oral glucose tolerance test (OGTT) on normoglycemic rat model. Bases on the OGTT results, also assessed *in vitro* PPAR-γ transactivation assay was performed on active compounds and they showed potent antidiabetic activity in the OGTT (better than standard drugs) and also showed a good dock score with the PPAR- γ receptor site. The above potent compounds were further evaluated for their antidiabetic potential on the STZ induced diabetic model. It was observed from the results that the four compounds **4**, **5**, **6** and **7** exhibited significant antidiabetic activity as compared to standard drugs without showing a much increase in body weight.



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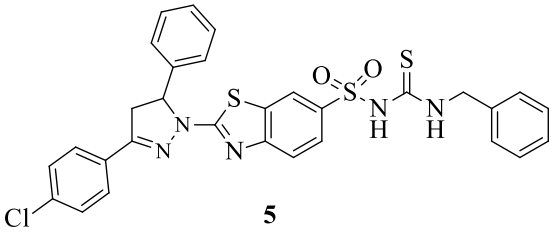

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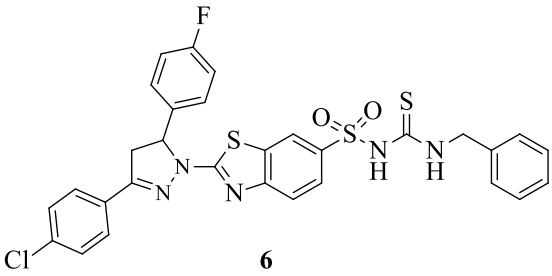

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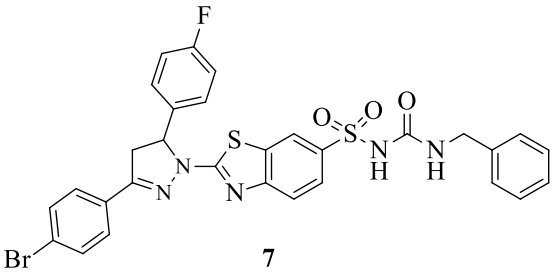
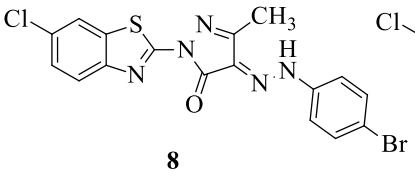


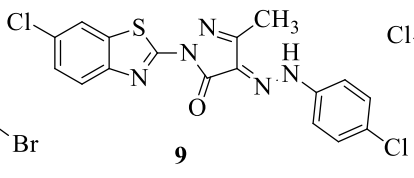
7

Mohd. Amir *et al*.[19] have reported the synthesis and antimicrobial activity of pyrazolinones and pyrazoles having benzothiazole moiety. The *in vitro* screening (by serial plate dilution method) results in MIC were represented in micro-molar concentration (μM) and revealed that the compounds **8**, **9** and **10** showed promising inhibiting activity particularly against the gram-positive bacteria strain *S. aureus* ATCC 25923.



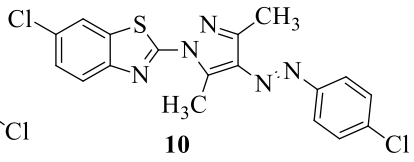
8

MIC = 13.95 μM



9

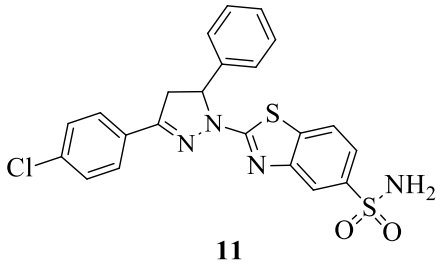
MIC = 31.01 μM



10

MIC = 15.58 μM

Chetna Kharbanda *et al.*[20] have reported synthesis and evaluation of pyrazolines bearing benzothiazole as anti-inflammatory agents. All the synthesized compounds were evaluated for their anti-inflammatory potential using carrageenan induced paw edema model. COX-2 enzyme assay, TNF-α assay and ulcerogenic risk evaluation were carried out for the potent compounds **11** and **12** to understand their mode of action and cytotoxicity.



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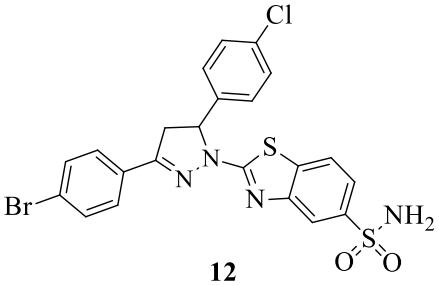
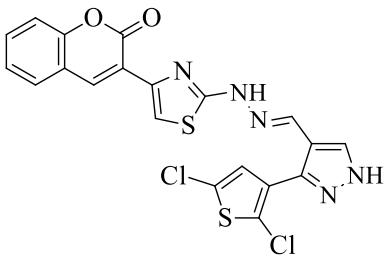
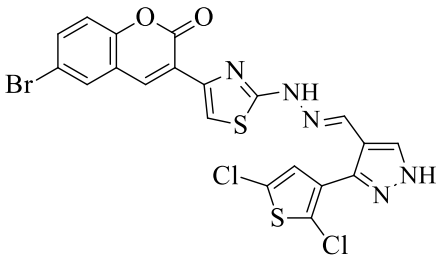


12

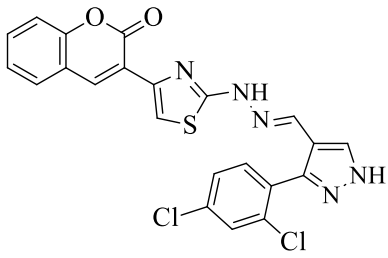
A. M. Vijesh *et al.*[21] described Synthesis, characterization and anti-microbial studies of some novel 2,4-disubstituted thiazoles. All the synthesized compounds were evaluated for their *in vitro* antimicrobial activity. From the results it was evident that, the compounds **13**, **14**, **15** and **16** have shown excellent antibacterial activity with MIC values 1.6125 µg/mL concentrations against *S. aureus* and *B. subtilis* bacteria when compared with the standard drug Ceftriaxone which is active at 3.125 µg/mL concentration.



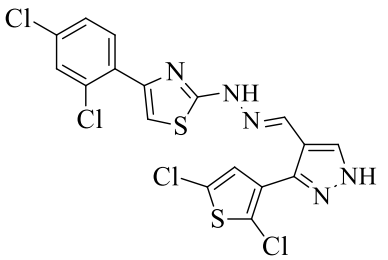
13



14



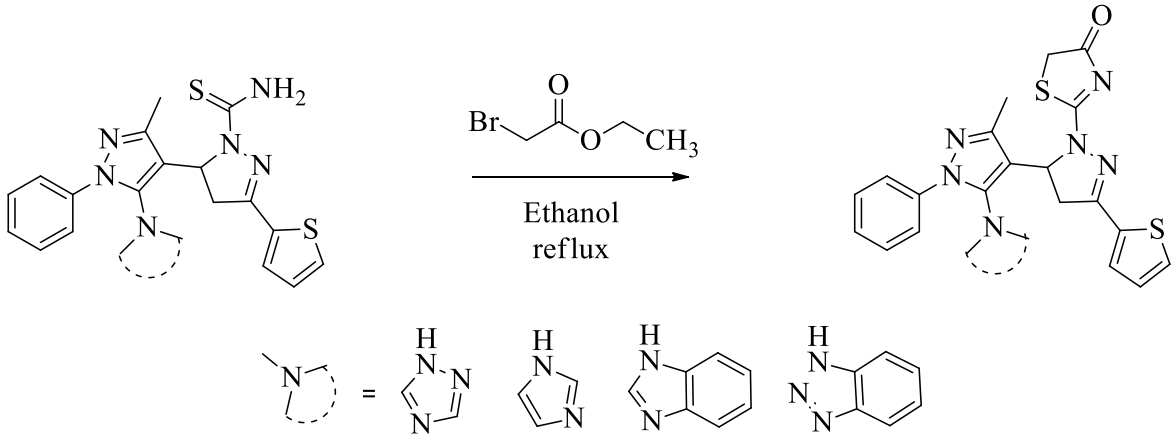
15



16

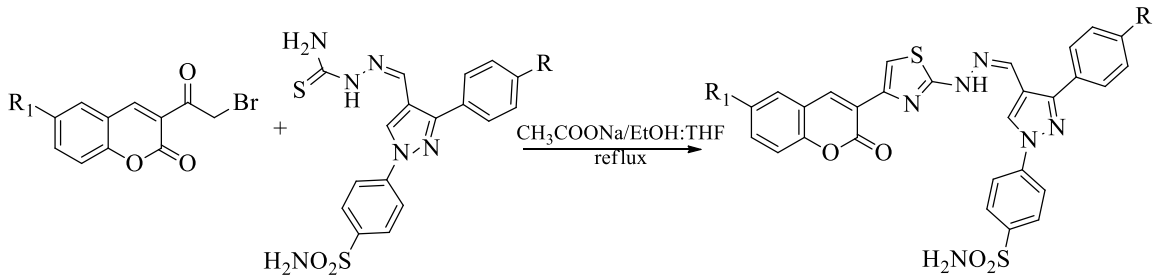
Various important approaches for the synthesis of disubstituted pyrazoles, thiazoles embedding benzothiazole and coumarin.

Piyush N. Kalaria and co-workers[22] reported design, synthesis and molecular docking of novel bipyrazolyl thiazolone scaffold as a new class of antibacterial agents. The target compounds were achieved by the cyclization of *N*-substituted thiourea with ethyl bromoacetate in refluxing ethanol.



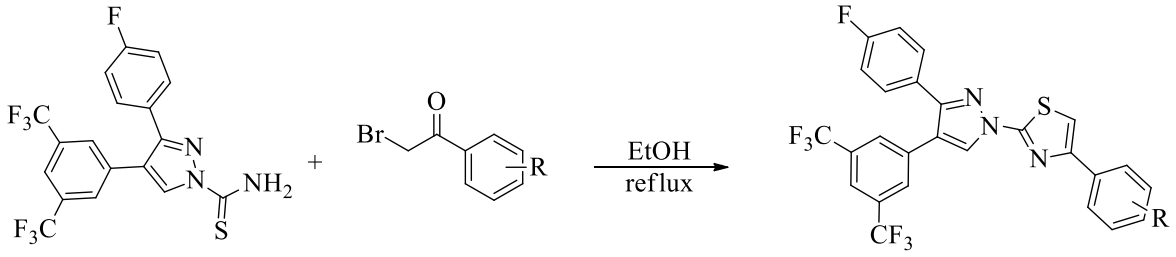
Scheme-1

Navneet Chandak *et al*.[23] described novel sulfonamide bearing coumarin scaffolds as selective inhibitors of tumor associated carbonic anhydrase isoforms IX and XII. The title compounds were afforded by the Hantzsch thiazole synthesis strategy.



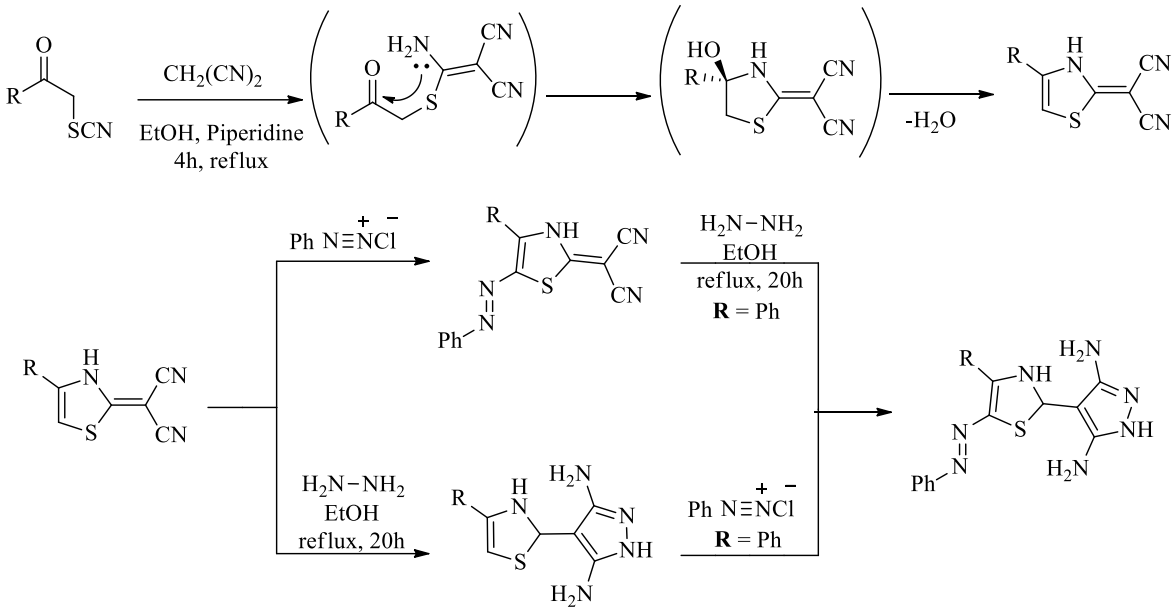
Scheme-2

Amar Patil and group[24] reported Synthesis and antifungal activity of 1, 5-diaryl pyrazole substituted thiazole derivatives.



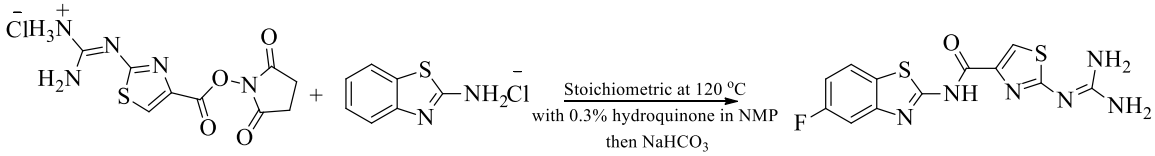
Scheme-3

Saleh Mohammed Al-Mousawi and co-workers[25] described the polyfunctional heteroaromatics: a route to dicyanomethylene thiazoles based on the reaction of α-thiocyanatoketones with malononitrile.



Scheme-4

Rodney C. Schnur *et. al*.[26] reported *N*-(5-fluorobenzothiazol- 2-yl)-2-guanidinothiazole-4-carboxamide. A novel, systemically active antitumor agent effective against 3LL Lewis lung carcinoma.



Scheme-5

PRESENT WORK

In light of above, and also as a part of our endeavour in the search of novel heterocyclic hybrids[27–29] which can overcome the limitations associated with original pharmacophore sub-units, herein we, by adopting the hybridization approach[30,31], engendered a new molecular hybrid by amalgamated the four pharmacophores (Pyrazole, thiazole, coumarin and benzothiazole motifs) in one molecular framework[32] with an expectation of enhanced antimicrobial activity by the synergistic effect of embedded moieties.

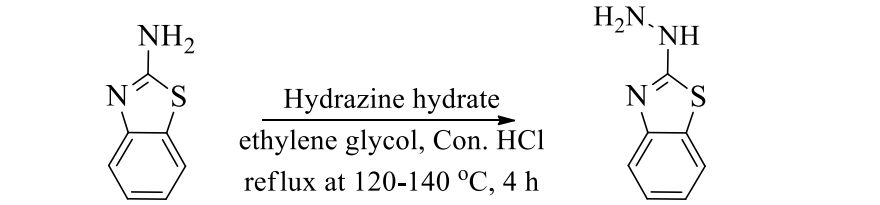
Preparation of starting materials

3-Acetyl-2*H*-chromen-2-one,2-Acetyl-3*H*-benzo[*f*]chromen-3-one,3-(2-Bromoacetyl)-2*H*-chromen-2-one and 2-(2-Bromoacetyl)-3*H*-benzo[*f*]chromen-3-one

The above important starting compounds were prepared according to the literature procedure as described in **Chapter-II**, **Section-A**.

2-hydrazinylbenzo[*d*]thiazole[33]

2-Hydrazinobenzothiazole was prepared by the reaction of 2-amino benzothiazole (1 eq) with hydrazine hydrate (1 eq) in refluxing ethylene glycol in the presence of conc. hydrochloric acid (2 mL). After the completion of the reaction (monitored by TLC) was cooled to room temperature and filtered the separated solid, washed with water and recrystallized with ethanol afforded the Starting compound.

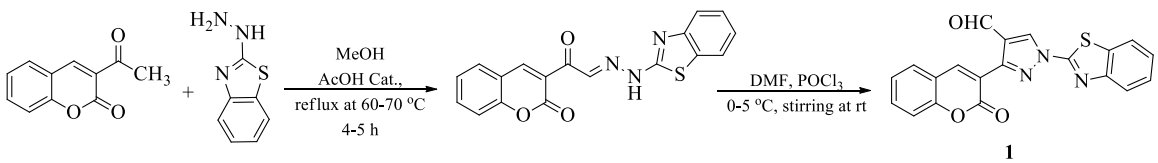


benzo[*d*]thiazol-2-amine 2-hydrazinylbenzo[*d*]thiazole

Scheme-6

Synthesis of 1-(benzo[*d*]thiazol-2-yl)-3-(2-oxo-2*H*-chromen-3-yl)-2*H*-pyrazole-4-carbaldehyde (1)[34]

Vilsmeier-Haack formylation of hydrazone afforded from the reaction of 3-Acetyl-2*H*-chromen-2-one and 2-Hydrazinobenzothiazole yielded the corresponding aldehyde **1**.

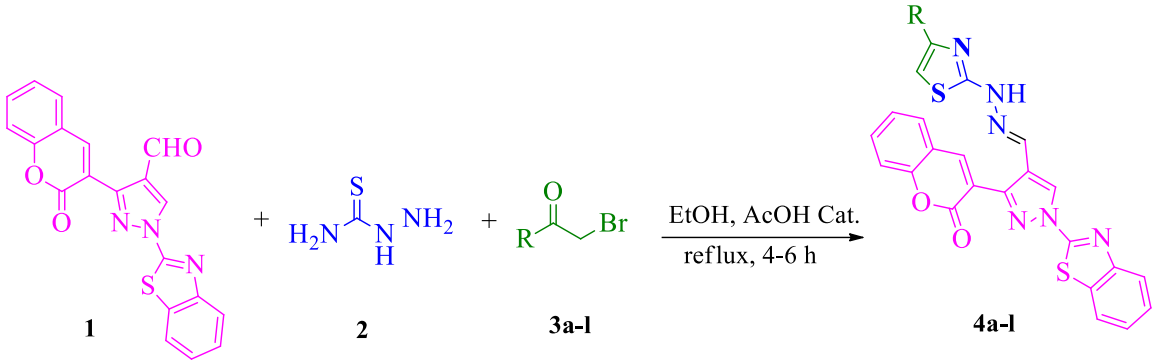


1

Scheme-7

Synthesis of 1,3-disubstituted pyrazole-2,4-disubstituted thiazole hybrids embedding benzothiazole and coumarin moieties (4a-l)

One pot three component condensation of aldehyde (**1**), aminothiourea (**2**) and α-bromo ketones (**3a-l**) in refluxing ethanol in the presence of catalytic amount of glacial acetic acid, furnished the corresponding target compounds in good to excellent yields **(Scheme-8)**.



R:

a = C_6H_5 **b** = 4-MeC_6H_4 **c** = 4-$OMeC_6H_4$
d = 4-PhC_6H_4 **e** = 4-FC_6H_4 **f** = 4-ClC_6H_4
g = 4-BrC_6H_4 **h** = 4-$NO_2C_6H_4$ **i** = coumarin-3-yl
j = benzo[*f*]coumarin-3-yl **k** = 8-Br-coumarin-3-yl **l** = 6,8-di-Br-coumarin-3-yl

Scheme-8

Results and discussions

The complete reaction conditions involved in the synthesis of titled compounds **4a-l** was illustrated in the **scheme-8**. The Vilsmeier-Haack formylation of hydrazone[35] achieved the key intermediate 1,3-disubstituted 4-functionalized pyrazole[36,37], which in turn afforded by the condensation of 3-acetyl coumarin[38] and benzothiazole hydrazine.[33,39] Further, the reaction of an aldehyde with thiosemicarbazide and various *α*-bromo ketones in refluxing ethanol in the presence of catalytic amount of glacial acetic acid afforded the target hybrid compounds **4a-l** in excellent yields (85-95%).

Structural assignments have been made on the basis of FTIR, NMR (^{1}H and ^{13}C), mass spectrometry and CHN analysis data, which is summarized in supporting information. Compound **4a**: In the IR spectrum, the bands corresponding to –C=N– was observed at 1604-1608 cm^{-1}. ^{1}H NMR spectra showed a characteristic singlet at s 12.20 ppm integrated for one proton of hydrazinyl –C=N–NH– group and s 9.10 ppm for –CH=N–

proton. In addition, ^{13}C NMR spectra displayed a significant signal at 168.3 for thiazole-C_2 and 143.4 for imine-C, thus structure of **4a** could be ruled out.

Biological evaluation

To explore the antimicrobial potential, all the derivatives of the library were assessed for their *in vitro* antimicrobial activities such as minimum inhibitory concentration (MIC), minimum bactericidal concentration, and minimum fungicidal concentration (MFC), and anti-biofilm properties.

Antibacterial activity

In vitro antimicrobial activity of the novel series of synthesized molecular hybrids (**4a-l**) was screened against seven bacterial and one fungal strain by employing agar well diffusion method.[40] Gram positive pathogenic bacterial strains used in the present work were [*Staphylococcus aureus* (MTCC 96), *Bacillus subtilis* (MTCC 121), *Staphylococcus aureus* MLS-16 (MTCC 2940) *and Micrococcus luteus* (MTCC 2470)]. Gram-negative [*Klebsiella planticola* (MTCC 530), *Escherichia coli* (MTCC 739), *Pseudomonas aeruginosa* (MTCC 2453)] pathogenic bacterial strains. The results of antibacterial activity are tabulated in **Table 1**.

Table 1

Antimicrobial activity (MIC and MBC) of the synthesized compounds (**4a-1)**.

Compds.	**Minimum Inhibitory Concentration**						
	MIC (MBC) µg/mL						
	Gram-positive bacteria				**Gram-negative bacteria**		
	A	**B**	**C**	**D**	**E**	**F**	**G**
4a	–	–	–	–	–	–	–
4b	7.8(15.6)	1.9 (7.8)	7.8(15.6)	3.9 (7.8)	3.9 (7.8)	7.8(3.9)	3.9 (7.8)
4c	7.8(15.6)	7.8(15.6)	7.8(15.6)	7.8(15.6)	7.8(15.6)	7.8(15.6)	7.8(15.6)
4d	7.8(15.6)	7.8(15.6)	7.8(15.6)	7.8(15.6)	–	7.8(15.6)	7.8(15.6)
4e	–	15.6(31.2)	–	–	–	–	–
4f	–	–	–	15.6(31.2)	–	–	–
4g	7.8(15.6)	3.9 (7.8)	7.8(15.6)	7.8(15.6)	7.8(15.6)	7.8(15.6)	–
4h	–	31.2(31.2)	–	–	–	–	–

4i	–	–	–	15.6(31.2)	–	–	–
4j	7.8(15.6)	–	7.8(15.6)	–	7.8(15.6)	7.8(15.6)	–
4k	7.8(15.6)	3.9 (7.8)	7.8(15.6)	7.8(15.6)	7.8(15.6)	7.8(15.6)	7.8 (15.6)
4l	–	3.9 (7.8)	7.8(15.6)	–	7.8(15.6)	7.8(15.6)	–
H	0.9 (0.9)	0.9 (0.9)	0.9(0.9)	0.9 (0.9)	0.9 (0.9)	0.9(0.9)	0.9 (0.9)

A: *S. aureus* MTCC 96; **B:** *B. subtilis* MTCC 121; **C:** *S. aureus* MLS16 MTCC 2940; **D:** *M. luteus* MTCC 2470; **E:** *K. Planticola* MTCC 530; **F:** *E. coli* MTCC 739; **G:** *P. aeruginosa* MTCC 2453.

H: Ciprofloxacin

(–) No activity

In general, all of the tested compounds exhibited a certain degree of inhibiting activity. From the results, it was revealed that, out of the tested series of compounds, notably, **4b**-**4d**, **4g**, and **4j**-**4l** exhibited a promising antimicrobial activity. The hybrid, **4b** with a tolyl substitution showed a very good to moderate activity with a MIC value of 1.9 µg/mL to 7.8 µg/mL against the tested pathogenic bacterial strains. The compound **4c** with a *p*-OCH_3 phenyl substitution showed a MIC of 7.8 µg/mL against all the test pathogens. The compound **4d** which is a biphenyl substituted thiazole derivative exhibited bacterial inhibiting activity with a MIC of 7.8 µg/mL against the test pathogens, except for *K. planticola* MTCC 530 and fungal strain *C. albicans* MTCC 3017. The compound **4g** a *p*-bromophenyl substitution exhibited a good antimicrobial activity against the test pathogens MICs ranging from 3.9 µg/mL to 7.8 µg/mL except for *P. aeruginosa* MTCC 2453 and *C. albicans* MTCC 3017. The compound **4k** with 8-bromocoumarinyl substitution was observed as a lead compound with promising antimicrobial activity against all the test pathogens with MIC values ranging from 3.9 µg/mL to 7.8 µg/mL. The compound **4l** with 6,8-dibromocoumarinyl substitution showed a good antimicrobial activity with MIC of 3.9 µg/mL and 7.8 µg/mL against two Gram-positive strains *B. subtilis* MTCC 121 and *S. aureus* MLS 16 MTCC 2940 and MIC of 7.8 µg/mL against *K. planticola* MTCC 530 and *E. coli* MTCC 739. Whereas the compounds **4a**, **4e**, **4f**, **4h** and **4i** exhibited bacterial inhibition against some of the test strains at higher concentrations ranging from 15.6 µg/mL to 62.5 µg/mL.

Antifungal activity

Candidiasis is the most common fungal infection in humans caused by any member of *Candida* spp. The chances of Candidiasis are propitious in infants and people with compromised immune system such as pregnant women, patients under medications for diabetes, chemo, HIV-AIDS. Statistics show around three out of every four women suffers from *Candida* infection for once at least in their life time. Also, *Candida* spp. has got the ability to form bio-films over medical devices such as cardiovascular catheters, contact lens, and voice prostheses etc.[41] Keeping the aforementioned facts in mind, our compounds **4a-4l** were also screened for antifungal activity against 12 panel of fungal strains using Miconazole as a standard (NCCLS, Wayne, 2000). The screening showed that the compounds **4b** and **4c** were moderately effective against *C. albicans* MTCC 3017 with MICs of 7.8 µg/mL each. Whereas the compound **4j** was effective against *C. albicans* MTCC 3017, *C. albicans* MTCC 227, *C. albicans* MTCC 1637 with MICs ranging between 3.9 µg/mL to 7.8 µg/mL. Further, the compound **4k** showed MIC of 7.8 µg/mL equivalent to the standard against *C. albicans* MTCC 3017 and with MIC of 3.9 µg/mL against *C. albicans* MTCC 227 that was more potent than the standard. The results are summarized in **Table 1** and **2**.

Table 2

Antifungal activity (MIC, MFC) of the synthesized compounds (**4a-l)**.

Compds.	**Minimum Inhibitory Concentration**											
	MIC (MFC) µg/mL											
	Fungal Strains											
	I	**J**	**K**	**L**	**M**	**N**	**O**	**P**	**Q**	**R**	**S**	**T**
4b	7.8/15.6	–	–	–	–	–	–	–	–	–	–	–
4c	7.8/15.6	–	–	–	–	–	–	–	–	–	–	–
4j	7.8/15.6	3.9/7.8	–	3.9/7.8	–	–	–	–	–	–	–	–
4k	7.8/15.6	3.9/7.8	–	–	–	–	–	–	–	–	–	–
U	7.8/7.8	7.8/7.8	7.8/7.8	7.8/7.8	7.8/7.8	7.8/7.8	7.8/7.8	7.8/7.8	7.8/7.8	7.8/7.8	7.8/7.8	7.8/7.8

I: *C. albicans* MTCC 3017; **J:** *C. albicans* MTCC-227; **K:** *C. albicans* MTCC-854; **L:** *C. albicans* MTCC-1637; **M:** *C. albicans* MTCC-3018; **N:** *C. albicans* MTCC-3958; **O:** *C. albicans* MTCC-4748; **P:** *C. parapsilosis* MTCC-1745; **Q:** *C. aaseri* MTCC-1962; **R:** *C. glabrata* MTCC-3019; **S:** *C. krusei* MTCC-3020; **T:** *Issatchenkia hanoiensis* MTCC-4755.

U: Miconazole

(–) No activity

MIC (usually reported as mg/L or µg/mL) is the lowest concentration of an antimicrobial agent that will inhibit the visible growth of a microbe (at which it has bacteriostatic activity) after overnight incubation. Regardless, it didn't know from the MIC data that, the microorganisms are completely killed or not. That's why once the MIC is determined; we have performed an extra set of steps in order to determine the lowest concentration at which an antimicrobial agent that reduces the viability of particular bacterium/fungi (MBC/MFC) by ≥99.9%.

Minimum bactericidal/fungicidal concentration (MBC/MFC)

The antimicrobials are normally regarded as bactericidal/fungicidal if the MBC/MFC is not more prominent than four circumstances the MIC.[42,43] Hence, considering the above MIC results of antibacterial and antifungal activity, using ciprofloxacin (MBC 0.9 µg/mL) and miconazole (MFC 7.8 µg/mL) as a reference drugs (positive controls) assayed for the MBC/MFC[2] for the compounds (**4b, 4c, 4d, 4g, 4j, 4k** and **4l)** which did not show any viability of the tested cells. It was observed from the results (**Table 1 and**) that, all the tested compounds exhibited good pattern of MBC (2xMIC = MBC) against the tested pathogenic strains. Especially, among them, the compound **4b**, **4c**, **4k** and **4l** has 2xMIC = MBC in case of all the tested bacterial strains except in case of *B. subtilis* for **4b**. However, the remaining other compounds **4d**, **4g**, **4j** and **4l** has also their bactericidal concentration below 2xMIC = MBC against some of the strains.

From the antifungal results, it was observed that the compounds **4b, 4c, 4j, 4k** showed fungicidal activity against *C. albicans* MTCC 3017 at MFC of 15.6 µg/mL. The compound **4j** showed MFC of 7.8 µg/mL against *C. albicans* MTCC 227 and *C. albicans* MTCC 1637 which equivalent to the standard. Further the compound **4k** showed a MFC of 7.8 µg/mL against *C. albicans* MTCC 227 which is equivalent to that of the positive control drug Miconazole.

Biofilm inhibition assay

Bio-films are self EPS (extra polymeric substance) based dynamic microbial communities with emerging characteristics that are different from single microbes. This collective matrix plays a role in increased resistance of microbial pathogens to antibiotic.[44] Majority of the in-dwelling device associated and nosocomial microbial infections are associated with bio-film formation and enhanced resistance to available drugs.[45] Bio-film formations

are reported in contact lenses, intra uterine devices, prosthetic heart valves, breast implants, dialysis catheters.[46] Apart from bacteria, Candidiasis is the most common human infection involving bio-film formation thus posing the risk of resistance to current anti fungal compounds.[47]

All these facts emphasize a great need for new compounds or hybrids of existing compounds that can destruct the bio-film matrix. Keeping in view of above facts, we explored the bio-film inhibiting property[48] of the synthesized molecular hybrids. In the current study, we tested compounds **4b, 4c, 4e, 4g, 4j** and **4k** against *M. luteus* MTCC 2470, *S. aureus* MTCC 96, *S. aureus* MLS16 MTCC2940, *B. subtilis* MTCC 121, *E. coli* and *K. planticola* MTCC 530, *Candida albicans* MTCC 3017, *P. aeruginosa* MTCC 2453. The results are tabulated in **Table 3**. The results indicated that, the compound **4b** inhibits the biofilm formation of *S. aureus* MLS 16 MTCC 2940 and *M. luteus* MTCC 2470 with IC_{50} values of 28.5 µM and 19 µM respectively. While the compound **4c** showed the biofilm inhibition of *S. aureus* MTCC 96, *B. subtilis* MTCC 121, *S. aureus* MLS 16 MTCC 2940, *M. luteus* MTCC 2470, *E.* coli MTCC 739 with IC_{50} values of 28 µM, 19 µM, 24 µM, 22 µM, 27.8 µM respectively. The compound **4d** inhibited the biofilm formation of *S. aureus* MTCC 96, *M. luteus* MTCC 2470 and *K. planticola* MTCC 530 at IC_{50} values of 75 µM, 56 µM and 51 µM respectively. The compound **4j** inhibited the biofilm formation of *S. aureus* MTCC 96 with IC_{50} value of 11.8 µM. While the compound **4k** showed the antibiofilm property against *S. aureus* MTCC 96, *S. aureus* MLS 16 MTCC 2940, *K. planticola* MTCC 530, *E.* coli MTCC 739 and *C. albicans* MTCC 3017.

Table 4

Biofilm inhibition assay of the synthesized compounds **4b-d**, **4g** and **4j-k**

Compd.	**IC_{50} values in (µM)**							
	S. aureus **MTCC 96**	***B. subtilis*** **MTCC 121**	***S. aureus MLS16*** **MTCC 2940**	***M. luteus*** **MTCC 2470**	***K. planticola*** **MTCC 530**	***E. coli*** **MTCC 739**	***P. aeruginosa*** **MTCC 2453**	***C. albicans*** **MTCC 3017**
4b	–	–	28.5±0.18	19 ± 0.21	–	–	–	–
4c	28 ±0.1	19± 0.22	24.2±0.19	22.8 ± 0.2	–	27.8 ±0.17	–	–
4d	75 ±0.32	–	–	56.9 ± 0.34	51 ± 0.26	–	–	–
4g	–	–	–	–	25.8 ± 0.21	27.9 ±0.22	–	–
4j	**11.8 ± 0.24**	–	–	–	–	–	–	–
4k	23 ±0.28	–	**12 ± 0.14**	–	**14.07 ± 0.19**	47.1±0.21	–	**16± 0.11**
Erythromycin	0.3±0.14	0.26±0.29	0.31±0.18	0.22±0.31	0.23 ± 0.17	0.31±0.15	0.35 ± 0.11	0.34 ± 0.20

Bold values refer to the promising results.
(–) No activity

Conclusion

We have designed and synthesized a novel series of molecular hybrids (**4a-4l**) by amalgamating the pyrazole, thiazole, coumarin and benzothiazole motifs for the first time. The scheme of the synthesis employed the Vilsmeier-Haack formylation for the one pot three component condensation reactions. The entire series of synthesized hybrids were screened for antibacterial, antifungal and anti biofilm properties. From the biological evaluation results, the compounds **4b** and **4k** showed promising antibacterial against the test pathogenic strains. The **4b** and **4k** also exhibited a very good antibiofilm property. Further the compound **4k** showed a good antifungal property against *Candida albicans* MTCC 3017 and *Candida albicans* MTCC 227.

Biological assays

Materials and methods for antimicrobial activity

Ciprofloxacin (Sigma) and Miconazole (Sigma) were used as positive controls against bacteria and fungi respectively. DMSO used as a negative or solvent control.

Tested microbial strains

Gram-positive [*Staphylococcus aureus* (MTCC 96), *Bacillus subtilis* (MTCC 121), *Staphylococcus aureus* MLS-16 (MTCC 2940) *and Micrococcus luteus* (MTCC 2470)]. Gram-negative [*Klebsiella planticola* (MTCC 530), *Escherichia coli* (MTCC 739),

Pseudomonas aeruginosa (MTCC 2453)] pathogenic bacterial strains and fungicidal strain *Candida albicans* (MTCC 227) were used in this experiment. The standard cultures of above pathogenic strains were procured from Microbial Type Culture Collection (MTCC), CSIR-Institute of Microbial Technology (IMTECH), Chandigarh, India-160 036.

Antimicrobial activity (MIC)

The *in vitro* antimicrobial studies were carried out by agar well diffusion method[1]. Mueller-Hinton agar plates were uniformly seeded with the reference pathogenic strains as 0.1mL culture suspension each equivalent to 0.5 McFarland standards ($1.5x10^8$ cfu/mL). Under sterile conditions in the laminar air flow chamber, 6 mm diameter wells were made on test strain seeded agar plates with a sterile cork borer. Under sterile conditions, wells were loaded with test samples dissolved in 10% DMSO at a 125-0.97 µg/mL dose range. Standard solutions of Ciprofloxacin and Miconazole at a 125-0.97 µg/well dose range were employed as a positive controls and DMSO was used as a negative control. These compound loaded plates were incubated at 37°C for 24h for bacteria and at 28°C for 48 h for fungi. After appropriate incubation, Minimum inhibitory concentration for the tested microbes was determined from minimum concentration of the tested series inhibiting the visual growth in the wells of agar plates. The whole experiment was performed in duplicate and their mean values are presented.

Minimum bactericidal concentration (MBC)/Minimum fungicidal concentration (MFC) studies

To determine the minimum bactericidal concentration (MBC)/minimum fungicidal concentration (MFC) studies for the tested pathogenic strains. 2mL micro-centrifuge tubes were used for performing the minimum bactericidal and fungicidal concentration assay. Range of concentrations of the test compounds from 0 to 125 µg/mL for bactericidal and 0 to 150 µg/mL dose range for fungicidal was prepared in Mueller Hinton broth and Sabouraud dextrose broth. 100 µL of overnight grown microbial cultures were added to each concentration of test compound in order to get 0.5 McFarland standards ($1.5x10^8$ cfu/mL) and incubated for 24hr at 37°C for bacteria and 28°C for 48 h for fungi. Following the incubation, 10µL of sample from each tube was seeded onto the Mueller Hinton agar plates (for bacteria) and Sabouraud dextrose agar plates (for fungi) to examine the growth at each concentration. Least concentration of test compound required to kill the reference bacteria and fungi is considered as MBC and MFC

respectively. MBC/MFC screenings were conducted in duplicate and presented their mean values.

Biofilm inhibition assay

The promising compounds identified from the antimicrobial activity, MBC and MFC assay were evaluated for biofilm inhibition by microtiter plate assay. The bacterial test strains were cultured in Muller Hinton Broth overnight at 37°C. The Candida strains were grown in the Sabouraud dextrose broth. The synthesized hybrids, Ciprofloxacin and Miconazole were dissolved in DMSO to get different concentrations ranging from 0-250 µg/mL. These hybrids and antibiotics were mixed with test pathogenic suspensions equivalent to 0.5 McFarland Standard. 100 µL of the compound treated microbial suspension was loaded into each well of the 96 well-plate and incubated without shaking for 24 h at 37°C. Following incubation, the medium was discarded to remove unattached microbial cells. The wells were loaded with 100 µL of 0.1% Crystal violet for 20 min at RT. This was followed by three washings with distilled water to remove excess stain and drying of plates at RT overnight in upright position over a blotting paper. After drying, 95% ethanol was added to each well and the absorbance was measured at 540nm using Infinite M200Pro microtitre plate reader (Tecan Group Ltd., Mannedorf, Switzerland) to determine the IC_{50} (µM). The experiments were carried out in triplicates and the mean values are considered for calculating the standard deviations.

Experimental

General procedures for the preparation of compounds (1)

An equimolar mixture of 3-acetyl-2*H*-chromen-2-one (6 mmol) and 2-hydrazinylbenzo[*d*]thiazole (6 mmol) in refluxing methanol in the presence of catalytic amount of glacial acetic acid was stirred for 4-5 h at 60-70 ^{o}C afforded the orange coloured solid hydrazone. The precipitate was filtered under vacuo, washed with chilled water several times, dried and used in the next step without further purification (Yield 96%).

Vilsmeier-Haack reagent was prepared by the drop-wise addition of $POCl_3$ (0.024 mmol) to the DMF (60 mmol) in round bottom flask which is cooled to 0 to 5 ^{o}C using ice-salt mixture over a period of 30 min and the mixture was continued to stir at same temperature for 30 min. The hydrazone was added (6 mmol) in small portions over a

period of 30 min to the Vilsmeier-Haack reagent and allowed to stir at the 0 to 5 ^{o}C for 30 min. Then removed the flask from the ice-bath and the reaction was brought to room-temperature and continued stirring for about 6h. After, ensuring the completion of reaction by the TLC. Poured the reaction mixture in to ice cold water, neutralized with $NaHCO_3$ and the yellow coloured solid obtained was stirred for one hour at ambient temperature and was filtered, washed with chilled-water and recrystallized from ethanol to afford pure 1,3-disubstituted pyrazole-4-carbaldehyde (**1**) (Yield 85%).

Spectral data

3-(1-(benzo[*d*]thiazol-2-yl)-4-((2-(4-phenylthiazol-2-yl)hydrazono)methyl)-1*H*-pyrazol-3-yl)-2*H*-chromen-2-one (4a)

A mixture of aldehyde (**1**, 1 mmol), *N*-aminothiourea (**2**, 1 mmol) and ω-bromoacetophenone (**3a**, 1 mmol) or 3-ω-bromoacetylcoumarins in absolute ethanol (5 mL) and catalytic amount of glacial acetic acid was refluxed for 4-6 h at 60-70 ^{o}C. The progress of the reaction was monitored by TLC. After the completion of reaction, the hot-reaction mixture was filtered off under vacuo and washed with water followed by the ethanol to afford the tile compound **4a**. Yellow solid; mp: 235-237 °C; **IR** (KBr, cm^{-1}) υ_{max}: 1712 (C=O), 1608 (C=N); **^{1}H NMR** (400 MHz, DMSO-*d6*): δ 12.20 (brs, 1H, =N-NH), 9.10 (s, 1H, –CH=N), 8.41 (s, 1H, pyrazole C5-H), 8.15 (d, *J* = 7.6 Hz, 1H, Ar-H), 8.09 (s, 1H, coumarin C4-H), 7.99 (d, *J* = 8.0 Hz, 1H, Ar-H), 7.90 (d, *J* = 6.8 Hz, 1H, Ar-H), 7.79 (d, *J* = 7.2 Hz, 2H, Ar-H), 7.74 (t, *J* = 7.2 Hz, 1H, Ar-H), 7.58 (t, *J* = 8.0 Hz, 1H, Ar-H), 7.53 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.47 (q, *J* = 7.6 Hz, 2H, Ar-H), 7.37 (t, *J* = 7.6 Hz, 2H, Ar-H); 7.27 (t, *J* = 7.2 Hz, 1H, Ar-H), 7.11 (s, 1H, Ar-H); **^{13}C NMR** (100 MHz, DMSO-*d6*): δ 168.31, 159.70, 159.12, 154.19, 150.83, 148.38, 143.48, 134.93, 133.44, 133.22, 133.13, 129.50, 129.03, 128.86, 128.01, 127.56, 125.98, 125.84, 125.31, 123.15, 122.71, 121.47, 121.19, 119.30, 116.66, 103.79; **MS** (ESI): m/z 548 $[M + H]^{+}$; Anal. calcd. for $C_{29}H_{18}N_6O_2S_2$: C, 63.72; H, 3.32; N, 15.37. Found: C, 64.01; H, 3.58; N, 15.12.

3-(1-(benzo[*d*]thiazol-2-yl)-4-((2-(4-(*p*-tolyl)thiazol-2-yl)hydrazono)methyl)-1*H*-pyrazol-3-yl)-2*H*-chromen-2-one (4b)

Yellow solid; mp: 248-250 °C; **IR** (KBr, cm^{-1}) υ_{max}: 1716 (C=O), 1604 (C=N); **^{1}H NMR** (400 MHz, DMSO-*d6*): δ 12.19 (brs, 1H, =N-NH), 9.08 (s, 1H, –CH=N), 8.40 (s, 1H, pyrazole C5-H), 8.14 (d, *J* = 8.0 Hz, 1H, Ar-H), 8.10 (s, 1H, coumarin C4-H), 7.98 (d, *J* =

8.0 Hz, 1H, Ar-H), 7.90 (d, *J* = 7.2 Hz, 1H, Ar-H), 7.73 (t, *J* = 7.6 Hz, 1H, Ar-H), 7.68 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.58 (t, *J* = 7.6 Hz, 1H, Ar-H), 7.53 (d, *J* = 8.0 Hz, 1H, Ar-H), 7.46 (q, *J* = 7.2 Hz, 2H, Ar-H), 7.17 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.02 (s, 1H, Ar-H); 2.29 (s, 3H, –$\underline{CH_3}$); **^{13}C NMR** (100 MHz, $CDCl_3$+DMSO-*d6*): δ 168.39, 159.31, 159.20, 154.13, 150.76, 148.21, 143.49, 134.15, 133.20, 132.68, 129.37, 129.08, 127.53, 127.02, 125.97, 125.45, 125.00, 122.58, 122.13, 120.82, 116.43, 102.37, 29.55; **MS** (ESI): m/z 562 $[M + H]^+$; Anal. calcd. for $C_{30}H_{20}N_6O_2S_2$: C, 64.27; H, 3.60; N, 14.99. Found: C, 64.03; H, 3.85; N, 15.22.

3-(1-(benzo[*d*]thiazol-2-yl)-4-((2-(4-(4-methoxyphenyl)thiazol-2-yl)hydrazono)methyl)-1*H*-pyrazol-3-yl)-2*H*-chromen-2-one (4c)

Orange solid; 240-242 °C; **^{1}H NMR** (400 MHz, DMSO-*d6*): δ 12.12 (brs, 1H, =N-$\underline{NH}$), 9.02 (s, 1H, –$\underline{CH}$=N), 8.35 (s, 1H, pyrazole C5-H), 8.09 (d, *J* = 9.2 Hz, 2H, Ar-H), 7.96 (d, *J* = 9.6 Hz, 1H, Ar-H), 7.88 (d, *J* = 7.6 Hz, 1H, Ar-H), 7.71 (d, *J* = 9.6 Hz, 3H, Ar-H), 7.55 (t, *J* = 7.6 Hz, 1H, Ar-H), 7.49 (d, *J* = 7.2 Hz, 1H, Ar-H), 7.44 (q, *J* = 7.2 Hz, 2H, Ar-H), 6.91 (d, *J* = 9.6 Hz, 2H, Ar-H), 6.85 (s, 1H, Ar-H); 3.77 (s, 3H, –$\underline{OCH_3}$); **MS** (ESI): m/z 578 $[M + H]^+$; Anal. calcd. for $C_{30}H_{20}N_6O_3S_2$: C, 62.49; H, 3.50; N, 14.57. Found: C, 62.80; H, 3.82; N, 14.27.

3-(4-((2-(4-([1,1'-biphenyl]-4-yl)thiazol-2-yl)hydrazono)methyl)-1-(benzo[*d*]thiazol-2-yl)-1*H*-pyrazol-3-yl)-2*H*-chromen-2-one (4d)

Yellow solid; 252-254 °C; **^{1}H NMR** (400 MHz, DMSO-*d6*): δ 12.19 (s, 1H, =N-$\underline{NH}$), 9.10 (s, 1H, –$\underline{CH}$=N), 8.42 (s, 1H, pyrazole C5-H), 8.15 (d, *J* = 8.0 Hz, 1H, Ar-H), 8.10 (s, 1H, coumarin C4-H), 7.99 (d, *J* = 7.6 Hz, 1H, Ar-H), 7.90 (t, *J* = 8.4 Hz, 3H, Ar-H), 7.74 (t, *J* = 7.2 Hz, 1H, Ar-H), 7.69 (t, *J* = 7.6 Hz, 4H, Ar-H), 7.58 (t, *J* = 7.6 Hz, 1H, Ar-H), 7.54 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.47 (q, *J* = 7.2 Hz, 4H, Ar-H), 7.36 (t, *J* = 7.2 Hz, 1H, Ar-H), 7.18 (s, 1H, Ar-H); **MS** (ESI): m/z 624 $[M + H]^+$; Anal. calcd. for $C_{35}H_{22}N_6O_2S_2$: C, 67.51; H, 3.56; N, 13.50. Found: C, 67.22; H, 3.83; N, 14.78.

3-(1-(benzo[*d*]thiazol-2-yl)-4-((2-(4-(4-fluorophenyl)thiazol-2-yl)hydrazono)methyl)-1*H*-pyrazol-3-yl)-2*H*-chromen-2-one (4e)

Yellow solid; 260-263 °C; **^{1}H NMR** (400 MHz, DMSO-*d6*): δ 12.20 (brs, 1H, =N-$\underline{NH}$), 9.09 (s, 1H, –$\underline{CH}$=N), 8.40 (s, 1H, pyrazole C5-H), 8.14 (d, *J* = 8.0 Hz, 1H, Ar-H), 8.10 (s, 1H, coumarin C4-H), 7.98 (d, *J* = 8.0 Hz, 1H, Ar-H), 7.90 (d, *J* = 7.6 Hz, 1H, Ar-H),

7.85-7.81 (m, 2H, Ar-H), 7.73 (t, *J* = 8.0 Hz, 1H, Ar-H), 7.58 (t, *J* = 7.6 Hz, 1H, Ar-H), 7.53 (d, *J* = 8.0 Hz, 1H, Ar-H), 7.46 (q, *J* = 7.2 Hz, 2H, Ar-H), 7.20 (t, *J* = 8.4 Hz, 2H, Ar-H); 7.10 (s, 1H, Ar-H); **^{13}C NMR** (100 MHz, DMSO-*d6*): δ 168.39, 159.71, 159.12, 154.19, 150.84, 148.38, 143.48, 133.41, 133.23, 133.13, 131.63, 129.51, 128.91, 127.99, 127.91, 127.58, 125.85, 125.32, 123.17, 122.72, 121.50, 121.19, 119.32, 116.67, 115.97, 115.76, 103.55; **MS** (ESI): m/z 566 $[M + H]^+$; Anal. calcd. for $C_{29}H_{17}FN_6O_2S_2$: C, 61.69; H, 3.03; N, 14.88. Found: C, 61.96; H, 2.82; N, 15.10.

3-(1-(benzo[*d*]thiazol-2-yl)-4-((2-(4-(4-chlorophenyl)thiazol-2-yl)hydrazono)methyl)-1*H*-pyrazol-3-yl)-2*H*-chromen-2-one (4f)

Yellow solid; 272-274 °C; **^{1}H NMR** (400 MHz, DMSO-*d6*): δ 12.17 (brs, 1H, =N-NH), 9.09 (s, 1H, –CH=N), 8.41 (s, 1H, pyrazole C5-H), 8.14 (d, *J* = 7.6 Hz, 1H, Ar-H), 8.09 (s, 1H, coumarin C4-H), 7.98 (d, *J* = 8.0 Hz, 1H, Ar-H), 7.90 (d, *J* = 7.2 Hz, 1H, Ar-H), 7.81 (d, *J* = 8.4 Hz, 2H, Ar-H), 7.73 (t, *J* = 8.4 Hz, 1H, Ar-H), 7.58 (t, *J* = 7.6 Hz, 1H, Ar-H), 7.52 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.47 (t, *J* = 8.0 Hz, 2H, Ar-H); 7.42 (d, *J* = 8.4 Hz, 2H, Ar-H); 7.18 (s, 1H, Ar-H); **MS** (ESI): m/z 582 $[M + H]^+$; Anal. calcd. for $C_{29}H_{17}ClN_6O_2S_2$: C, 59.94; H, 2.95; N, 14.46. Found: C, 60.25; H, 2.73; N, 14.67.

3-(1-(benzo[*d*]thiazol-2-yl)-4-((2-(4-(4-bromophenyl)thiazol-2-yl)hydrazono)methyl)-1*H*-pyrazol-3-yl)-2*H*-chromen-2-one (4g)

Yellow solid; 268-270 °C; **^{1}H NMR** (400 MHz, DMSO-*d6*): δ 12.18 (s, 1H, =N-NH), 9.09 (s, 1H, –CH=N), 8.41 (s, 1H, pyrazole C5-H), 8.15 (d, *J* = 7.6 Hz, 1H, Ar-H), 8.09 (s, 1H, coumarin C4-H), 7.99 (d, *J* = 8.0 Hz, 1H, Ar-H), 7.90 (d, *J* = 7.6 Hz, 1H, Ar-H), 7.73 (t, *J* = 8.4 Hz, 3H, Ar-H), 7.60-7.51 (m, 4H, Ar-H), 7.46 (q, *J* = 7.6 Hz, 2H, Ar-H), 7.20 (s, 1H, Ar-H); **MS** (ESI): m/z 625 $[M]^+$; Anal. calcd. for $C_{29}H_{17}BrN_6O_2S_2$: C, 55.68; H, 2.74; N, 13.44. Found: C, 55.88; H, 2.48; N, 13.67.

3-(1-(benzo[*d*]thiazol-2-yl)-4-((2-(4-(4-nitrophenyl)thiazol-2-yl)hydrazono)methyl)-1*H*-pyrazol-3-yl)-2*H*-chromen-2-one (4h)

Orange solid; mp: 286-288 °C; **^{1}H NMR** (400 MHz, DMSO-*d6*): δ 12.27 (s, 1H, =N-NH), 9.11 (s, 1H, –CH=N), 8.42 (s, 1H, pyrazole C5-H), 8.31 (s, 1H), 8.24 (d, *J* = 8.4 Hz, 2H, Ar-H), 8.15 (d, *J* = 8.0 Hz, 1H, Ar-H), 8.12 (s, 1H), 8.05 (d, *J* = 8.8 Hz, 1H, Ar-H), 7.99 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.90 (d, *J* = 7.6 Hz, 1H, Ar-H), 7.74 (t, *J* = 7.6 Hz, 1H, Ar-H), 7.58 (t, *J* = 8.4 Hz, 1H, Ar-H), 7.53 (d, *J* = 6.8 Hz, 2H, Ar-H); 7.47 (q, *J* = 7.6 Hz, 2H,

Ar-H); **MS** (ESI): m/z 593 $[M + H]^+$; Anal. calcd. for $C_{29}H_{17}N_7O_4S_2$: C, 58.87; H, 2.90; N, 16.57. Found: C, 59.17; H, 2.66; N, 16.26.

3-(2-(2-((1-(benzo[*d*]thiazol-2-yl)-3-(2-oxo-2*H*-chromen-3-yl)-1*H*-pyrazol-4-yl)methylene)hydrazinyl)thiazol-4-yl)-2*H*-chromen-2-one (4i)

Yellow solid; mp: 291-293 °C; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 12.21 (s, 1H, =N-NH), 9.10 (s, 1H, –CH=N), 8.50 (s, 1H,), 8.41 (s, 1H, pyrazole C5-H), 8.14 (t, *J* = 8.4 Hz, 2H, Ar-H), 7.99 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.90 (d, *J* = 7.6 Hz, 1H, Ar-H), 7.85 (d, *J* = 8.0 Hz, 1H, Ar-H), 7.74 (t, *J* = 8.0 Hz, 1H, Ar-H), 7.61 (q, *J* = 7.6 Hz, 2H, Ar-H); 7.54 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.50-7.43 (m, 4H), 7.38 (t, *J* = 7.6, 1H); **MS** (ESI): m/z 616 $[M + H]^+$; Anal. calcd. for $C_{32}H_{18}N_6O_4S_2$: C, 62.53; H, 2.95; N, 13.67. Found: C, 62.75; H, 2.67; N, 13.95.

2-(2-(2-((1-(benzo[*d*]thiazol-2-yl)-3-(2-oxo-2*H*-chromen-3-yl)-1*H*-pyrazol-4-yl)methylene)hydrazinyl)thiazol-4-yl)-3*H*-benzo[*f*]chromen-3-one (4j)

Yellow solid; mp: 288-290 °C; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 11.35 (s, 1H, =N-NH), 9.47 (s, 1H, –CH=N), 8.50 (s, 1H,), 8.47 (s, 1H, pyrazole C5-H), 8.18-8.12 (m, 3H, Ar-H), 8.04 (s, 1H, Ar-H), 7.97 (d, *J* = 7.6 Hz, 2H, Ar-H), 7.93 (d, *J* = 10.0 Hz, 2H, Ar-H), 7.75-7.67 (m, 2H, Ar-H), 7.59 (t, *J* = 8.0 Hz, 1H, Ar-H), 7.52 (t, *J* = 8.0 Hz, 2H, Ar-H); 7.46 (t, *J* = 7.6 Hz, 3H, Ar-H); **MS** (ESI): m/z 666 $[M + H]^+$; Anal. calcd. for $C_{36}H_{20}N_6O_4S_2$: C, 65.05; H, 3.03; N, 12.64. Found: C, 64.79; H, 3.31; N, 13.01.

3-(2-(2-((1-(benzo[*d*]thiazol-2-yl)-3-(2-oxo-2*H*-chromen-3-yl)-1*H*-pyrazol-4-yl)methylene)hydrazinyl)thiazol-4-yl)-8-bromo-2*H*-chromen-2-one (4k)

Orange solid; mp: 296-298 °C; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 11.36 (s, 1H, =N-NH), 9.48 (s, 1H, –CH=N), 8.47 (s, 1H, pyrazole C5-H), 8.16 (t, *J* = 8.0 Hz, 3H, Ar-H), 8.04 (s, 1H, Ar-H), 7.98 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.93 (d, *J* = 10.4 Hz, 1H, Ar-H), 7.73 (t, *J* = 7.6 Hz, 2H, Ar-H), 7.58 (t, *J* = 7.6 Hz, 1H, Ar-H); 7.53 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.50-7.44 (m, 3H, Ar-H); **MS** (ESI): m/z 693 $[M + H]^+$; Anal. calcd. for $C_{32}H_{17}BrN_6O_4S_2$: C, 55.42; H, 2.47; N, 12.12. Found: C, 55.72; H, 2.15; N, 12.33.

3-(2-(2-((1-(benzo[*d*]thiazol-2-yl)-3-(2-oxo-2*H*-chromen-3-yl)-1*H*-pyrazol-4-yl)methylene)hydrazinyl)thiazol-4-yl)-6,8-dibromo-2*H*-chromen-2-one (4l)

Orange solid; mp: 312-315 °C; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 11.36 (s, 1H, =N-NH), 9.48 (s, 1H, –CH=N), 8.48 (s, 1H, pyrazole C5-H), 8.17 (t, *J* = 7.6 Hz, 3H, Ar-H), 8.06 (s,

1H, Ar-H), 7.97 (d, *J* = 6.4 Hz, 2H, Ar-H), 7.93 (d, *J* = 9.6 Hz, 1H, Ar-H), 7.73 (t, *J* = 8.0 Hz, 2H, Ar-H), 7.60-7.51 (m, 4H, Ar-H); **MS** (ESI): m/z 693 $[M + H]^+$; Anal. calcd. for $C_{32}H_{16}Br_2N_6O_4S_2$: C, 49.76; H, 2.09; N, 10.88. Found: C, 50.09; H, 2.35; N, 10.58.

Biological assays

Materials and methods for antimicrobial activity

Ciprofloxacin (Sigma) and Miconazole (Sigma) were used as positive controls against bacteria and fungi respectively. DMSO used as a negative or solvent control.

Tested microbial strains

Gram-positive [*Staphylococcus aureus* (MTCC 96), *Bacillus subtilis* (MTCC 121), *Staphylococcus aureus* MLS-16 (MTCC 2940) *and Micrococcus luteus* (MTCC 2470)]. Gram-negative [*Klebsiella planticola* (MTCC 530), *Escherichia coli* (MTCC 739), *Pseudomonas aeruginosa* (MTCC 2453)] pathogenic bacterial strains and fungicidal strain *Candida albicans* (MTCC 227) were used in this experiment. The standard cultures of above pathogenic strains were procured from Microbial Type Culture Collection (MTCC), CSIR-Institute of Microbial Technology (IMTECH), Chandigarh, India-160 036.

Antimicrobial activity (MIC)

The *in vitro* antimicrobial studies were carried out by agar well diffusion method[1]. Mueller-Hinton agar plates were uniformly seeded with the reference pathogenic strains as 0.1mL culture suspension each equivalent to 0.5 McFarland standards ($1.5x10^8$ cfu/ml). Under sterile conditions in the laminar air flow chamber, 6 mm diameter wells were made on test strain seeded agar plates with a sterile cork borer. Under sterile conditions, wells were loaded with test samples dissolved in 10% DMSO at a 125-0.97 µg/mL dose range. Standard solutions of Ciprofloxacin and Miconazole at a 125-0.97 µg/well dose range were employed as a positive controls and DMSO was used as a negative control. These compound loaded plates were incubated at 37°C for 24h for bacteria and at 28°C for 48 h for fungi. After appropriate incubation, Minimum inhibitory concentration for the tested microbes was determined from minimum concentration of the tested series inhibiting the visual growth in the wells of agar plates. The whole experiment was performed in duplicate and their mean values are presented.

Minimum bactericidal concentration (MBC)/Minimum fungicidal concentration (MFC) studies

To determine the minimum bactericidal concentration (MBC)/minimum fungicidal concentration (MFC) studies for the tested pathogenic strains. 2mL micro-centrifuge tubes were used for performing the minimum bactericidal and fungicidal concentration assay. Range of concentrations of the test compounds from 0 to 125 µg/mL for bactericidal and 0 to 150 µg/mL dose range for fungicidal was prepared in Mueller Hinton broth and Sabouraud dextrose broth. 100 µL of overnight grown microbial cultures were added to each concentration of test compound in order to get 0.5 McFarland standards ($1.5x10^8$ cfu/mL) and incubated for 24hr at 37°C for bacteria and 28°C for 48 h for fungi. Following the incubation, 10µL of sample from each tube was seeded onto the Mueller Hinton agar plates (for bacteria) and Sabouraud dextrose agar plates (for fungi) to examine the growth at each concentration. Least concentration of test compound required to kill the reference bacteria and fungi is considered as MBC and MFC respectively. MBC/MFC screenings were conducted in duplicate and presented their mean values.

Biofilm inhibition assay

The promising compounds identified from the antimicrobial activity, MBC and MFC assay were evaluated for biofilm inhibition by microtiter plate assay. The bacterial test strains were cultured in Muller Hinton Broth overnight at 37°C. The Candida strains were grown in the Sabouraud dextrose broth. The synthesized hybrids, Ciprofloxacin and Miconazole were dissolved in DMSO to get different concentrations ranging from 0-250 µg/mL. These hybrids and antibiotics were mixed with test pathogenic suspensions equivalent to 0.5 McFarland Standard. 100 µL of the compound treated microbial suspension was loaded into each well of the 96 well-plate and incubated without shaking for 24 h at 37°C. Following incubation, the medium was discarded to remove unattached microbial cells. The wells were loaded with 100 µL of 0.1% Crystal violet for 20 min at RT. This was followed by three washings with distilled water to remove excess stain and drying of plates at RT overnight in upright position over a blotting paper. After drying, 95% ethanol was added to each well and the absorbance was measured at 540nm using Infinite M200Pro microtitre plate reader (Tecan Group Ltd., Mannedorf, Switzerland) to determine the IC_{50} (µM). The experiments were carried out in triplicates and the mean values are considered for calculating the standard deviations.

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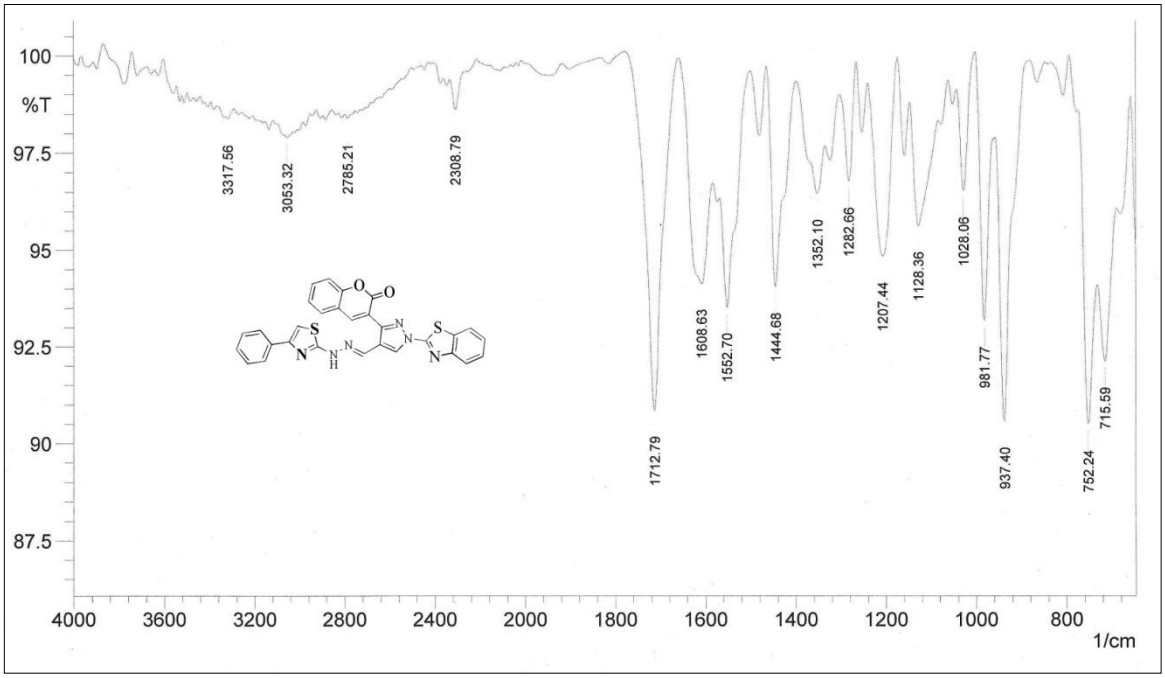
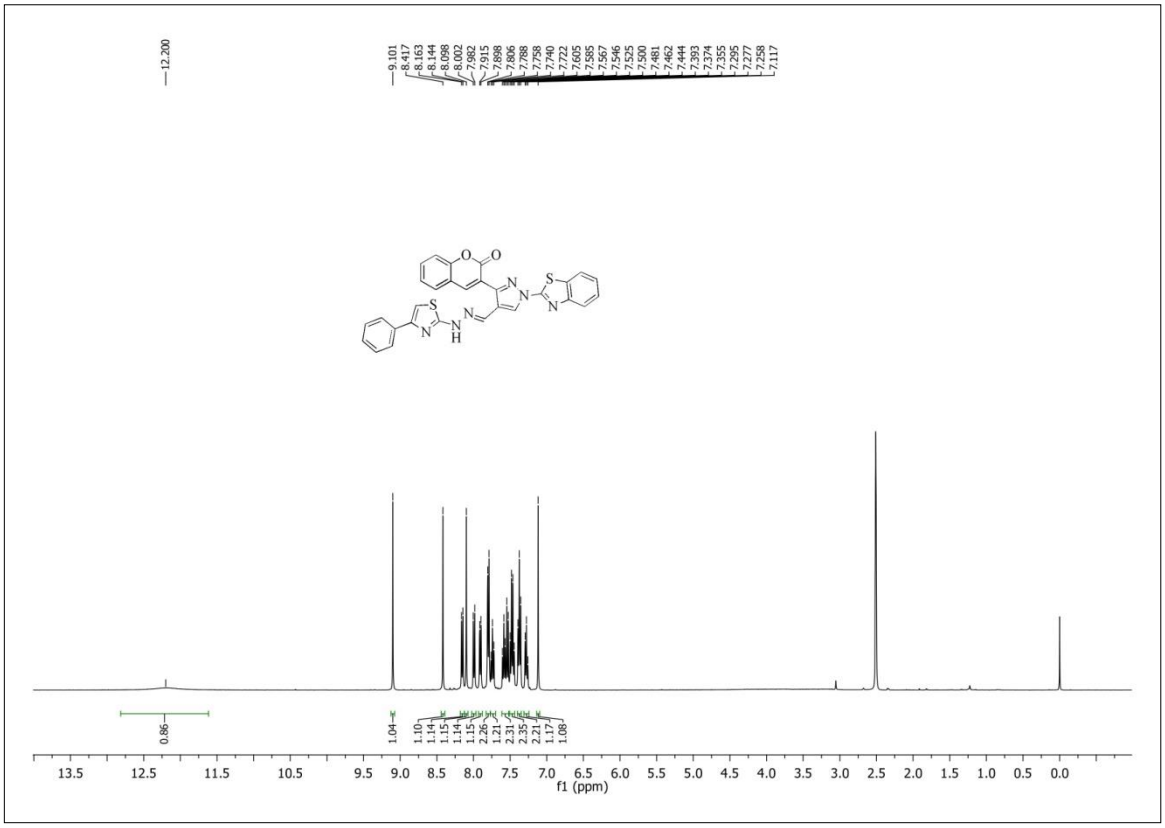
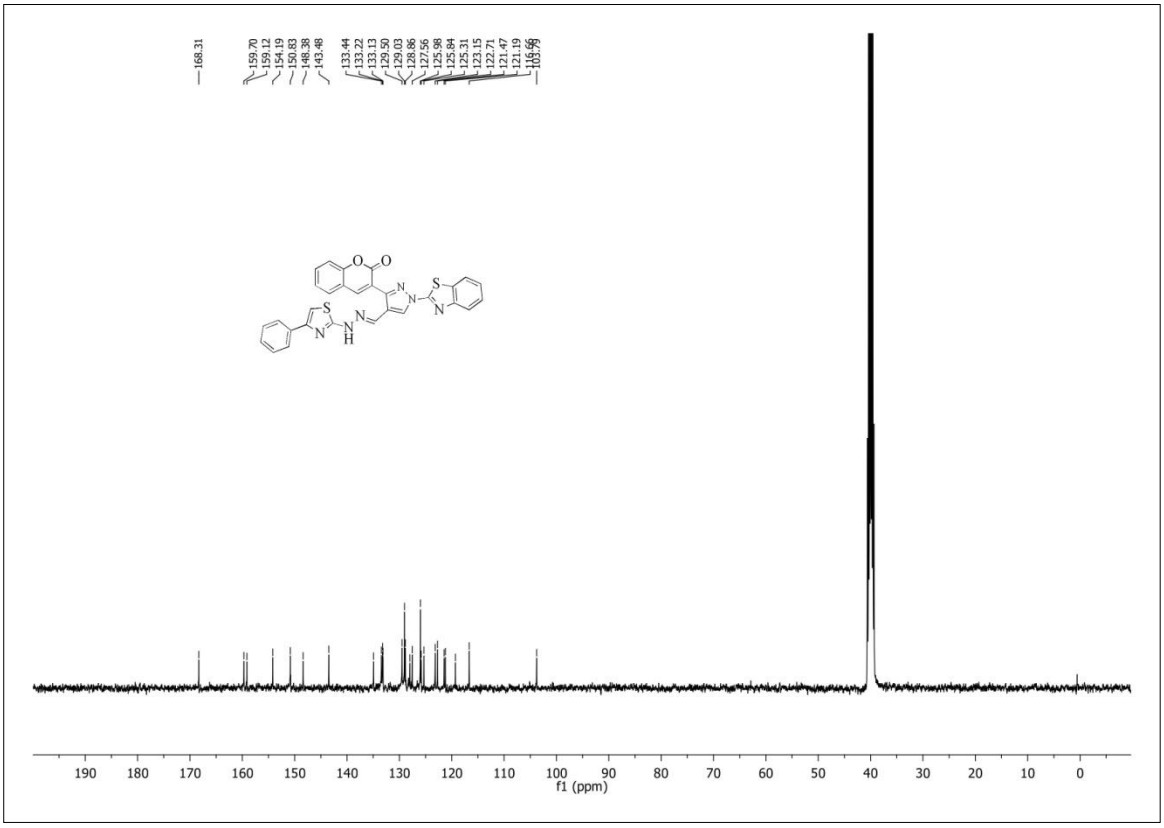


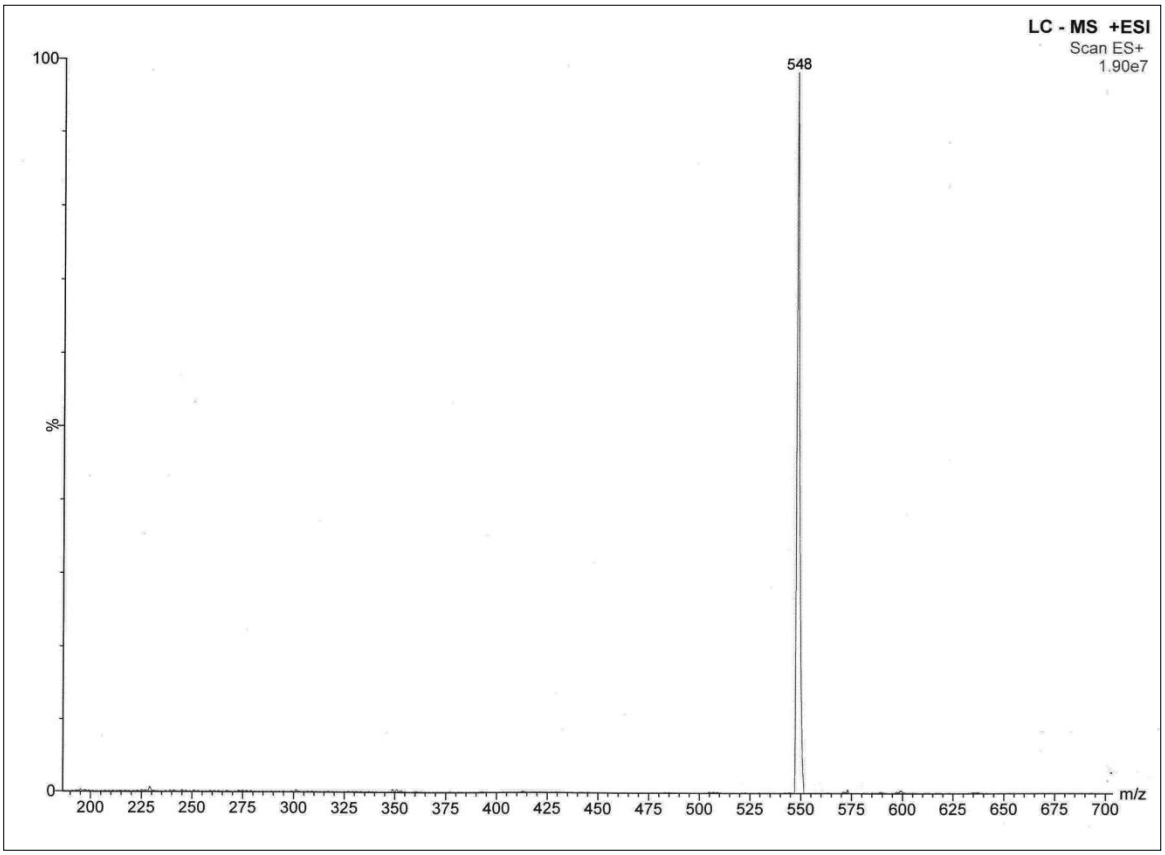
IR (KBr) spectrum of compound 4a



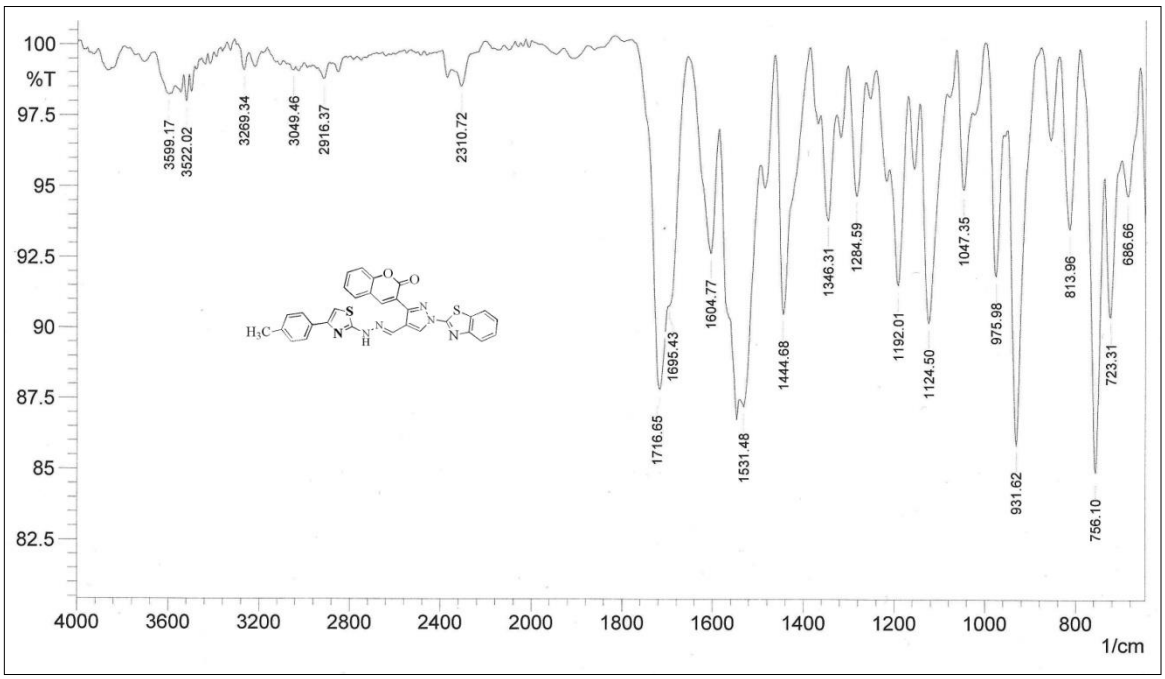
^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound 4a



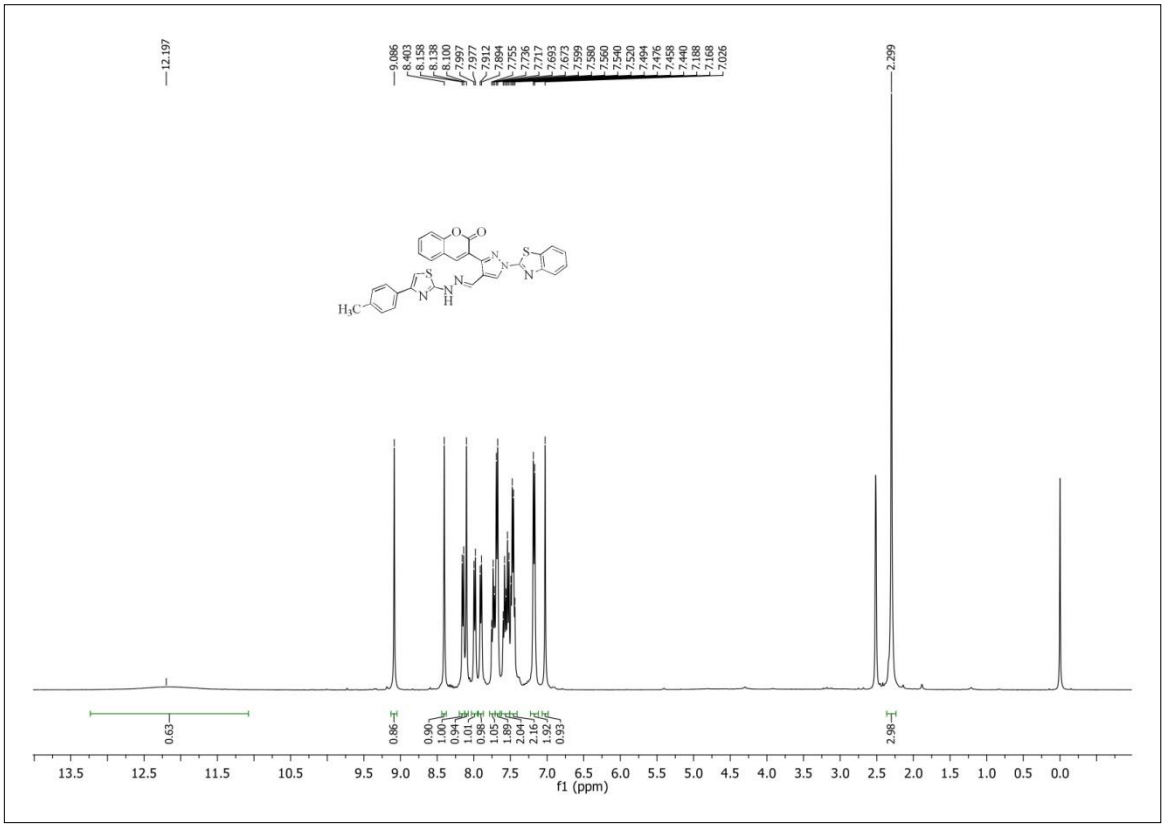
^{13}C NMR (100 MHz, DMSO-d_6) spectrum of compound 4a



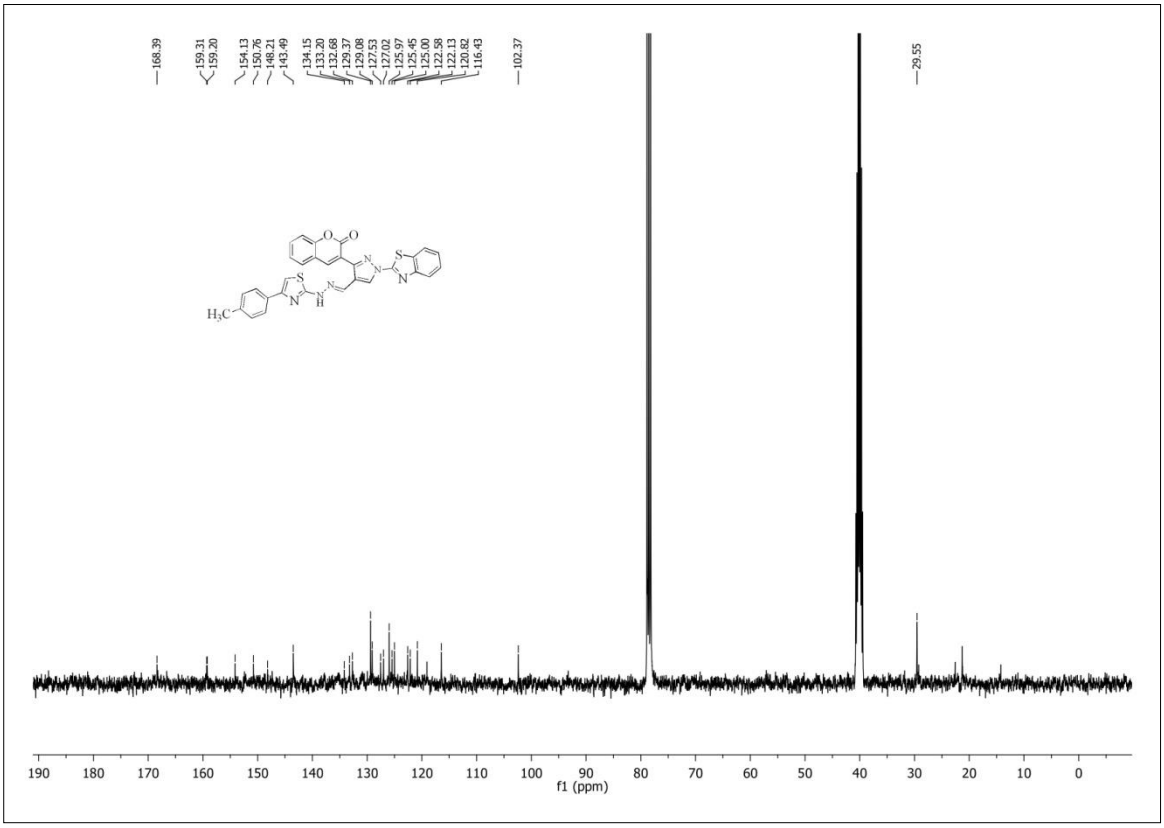
Mass spectrum of compound 4a (M.Wt: 547)



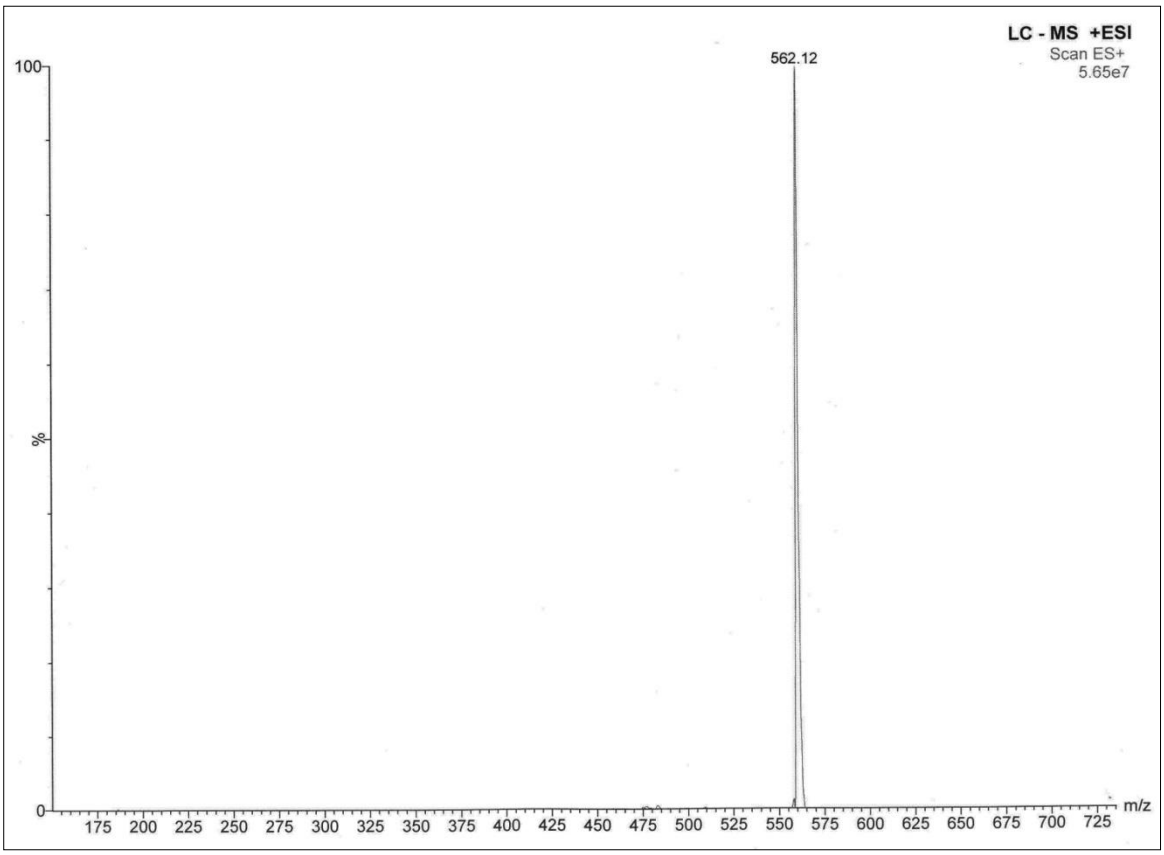
IR (KBr) spectrum of compound 4b



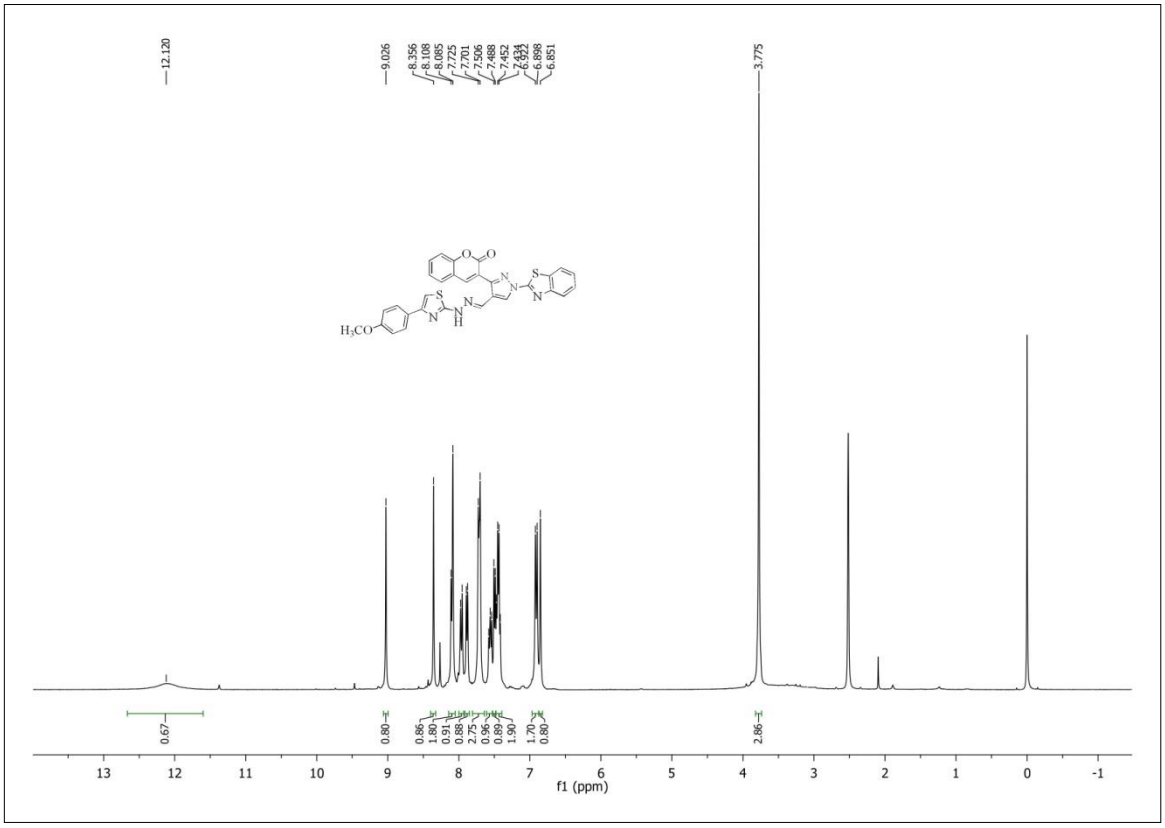
^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound 4b



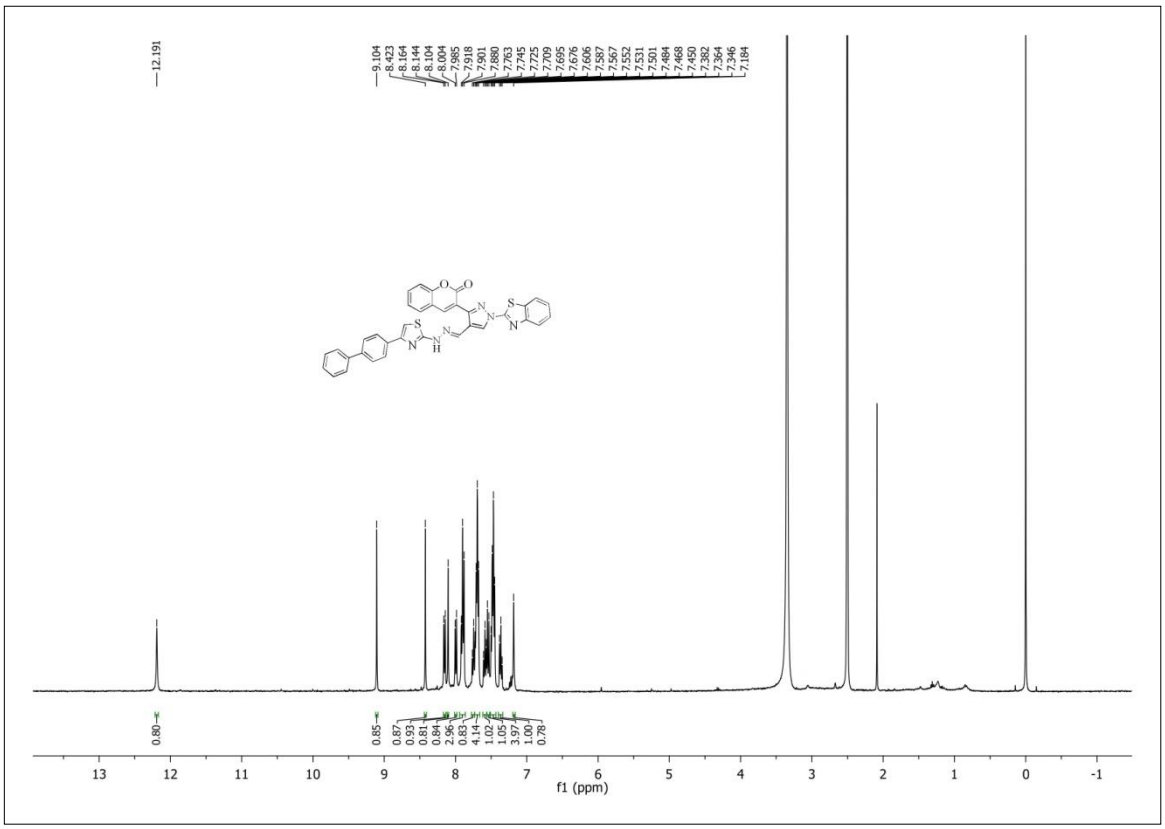
^{13}C NMR (100 MHz, DMSO-d_6) spectrum of compound 4b



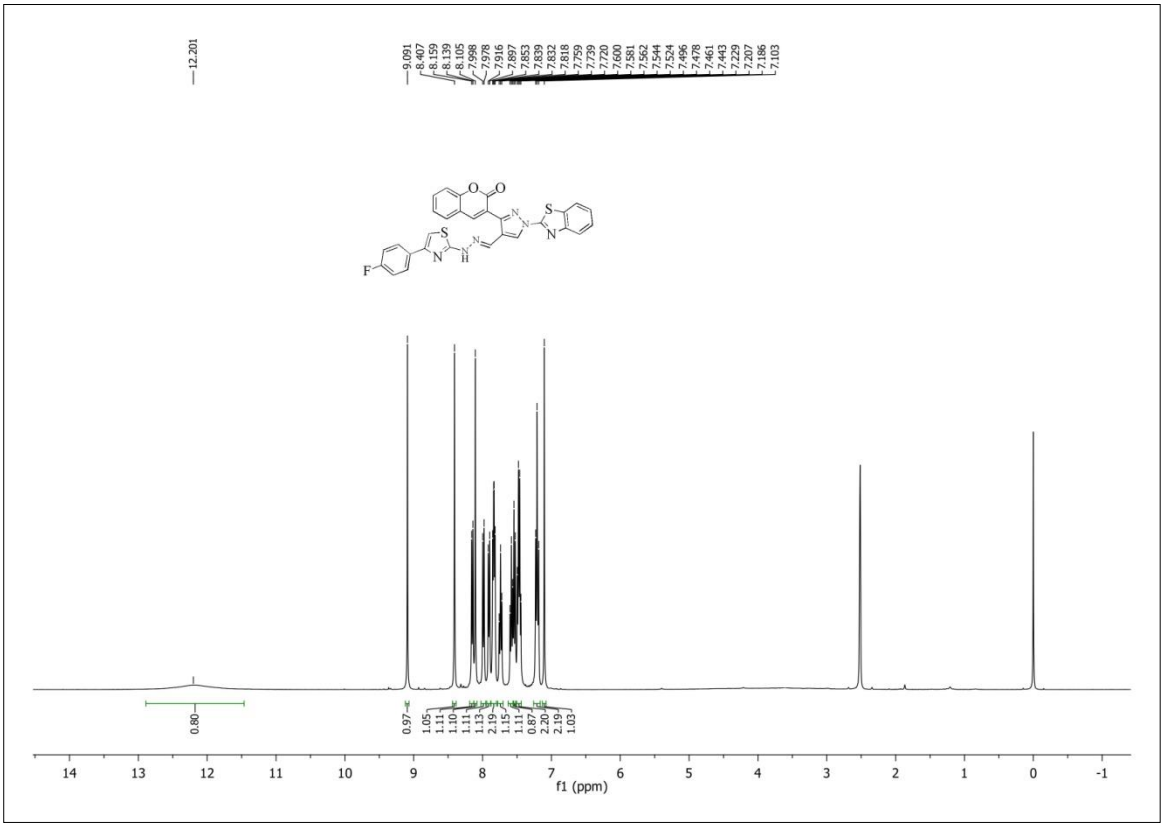
Mass spectrum of compound 4b (M.Wt: 561)



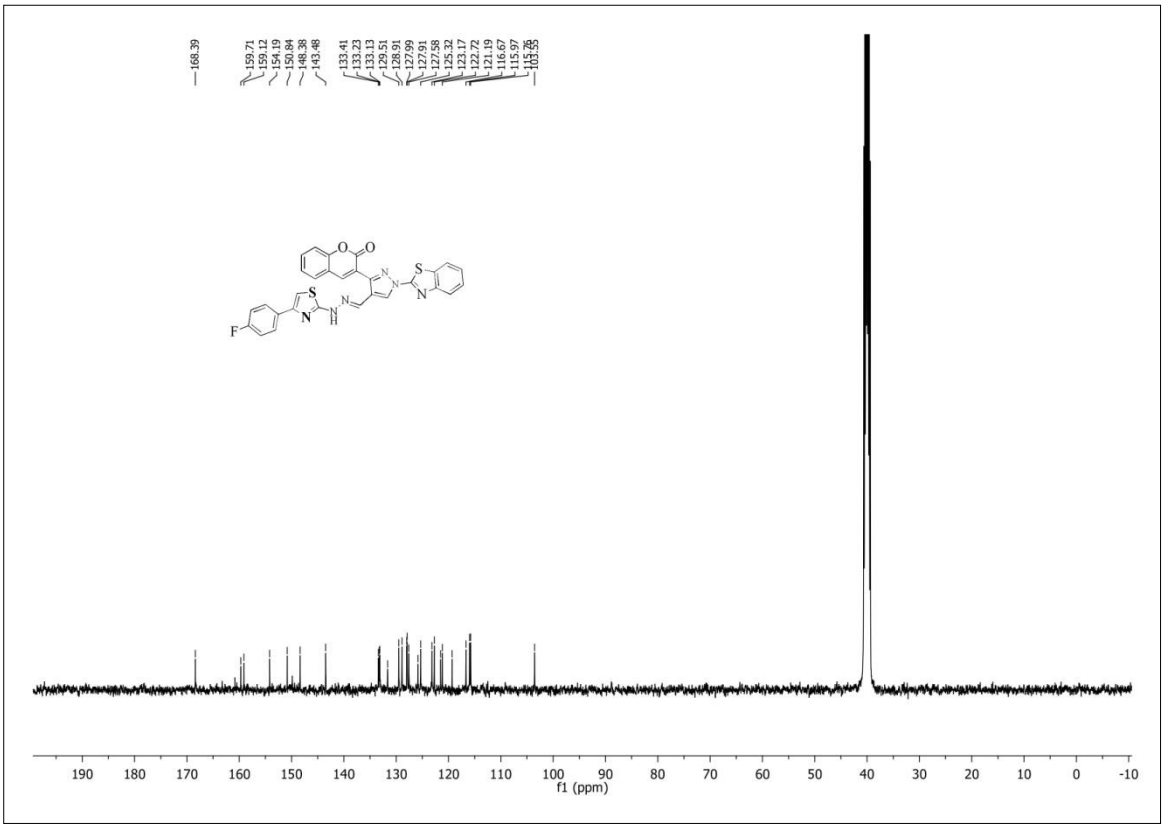
^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound 4c



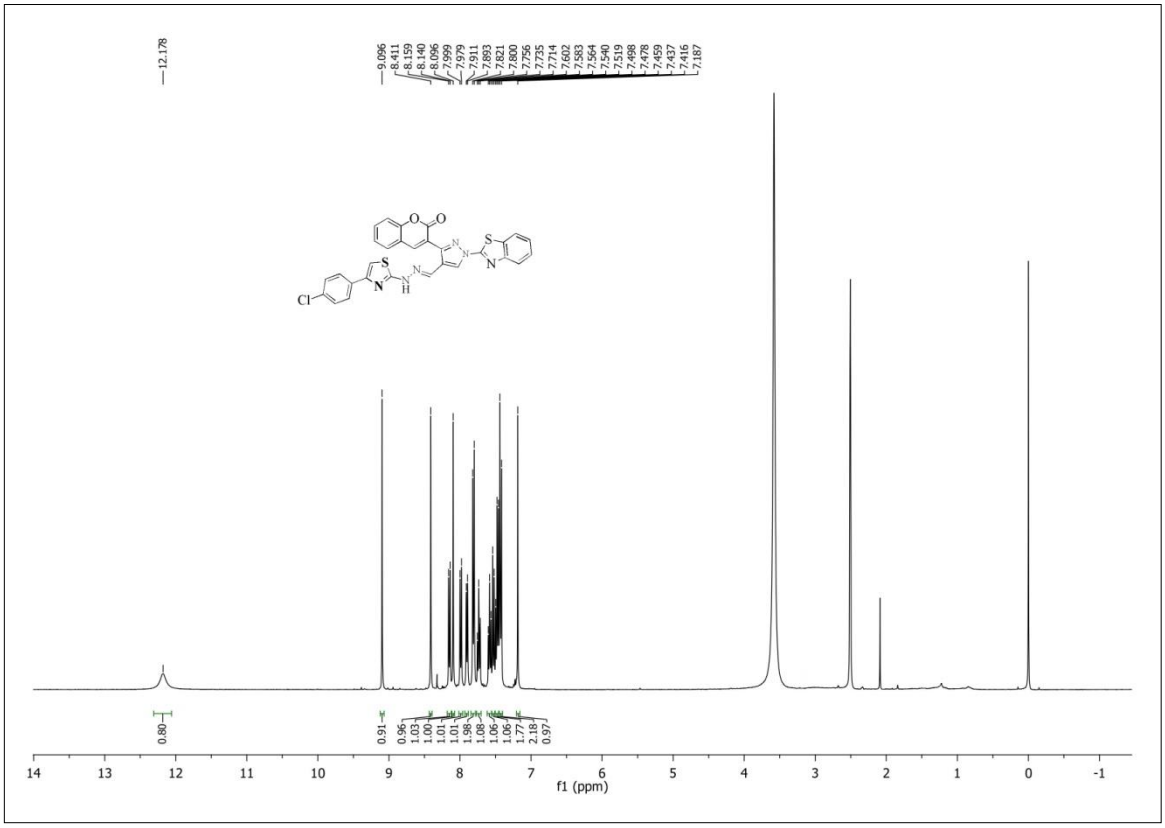
^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound 4d



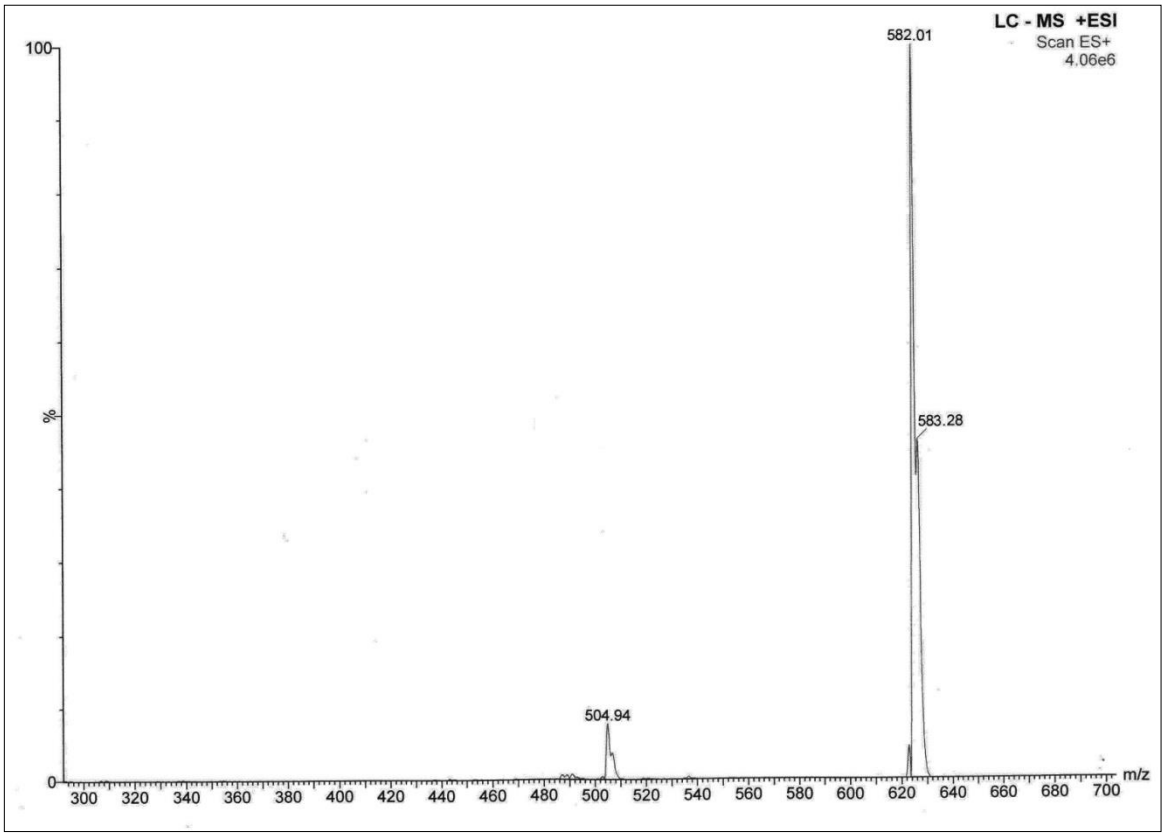
^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound 4e



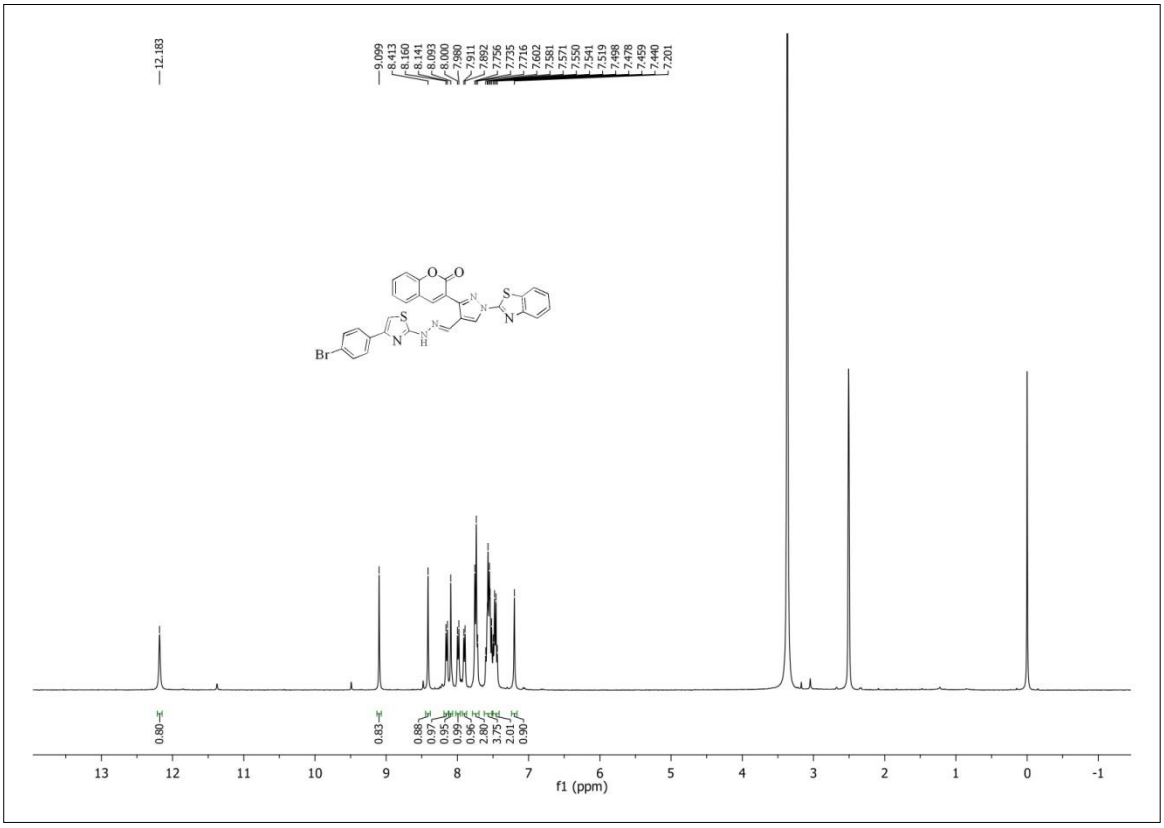
^{13}C NMR (100 MHz, DMSO-d_6) spectrum of compound 4e



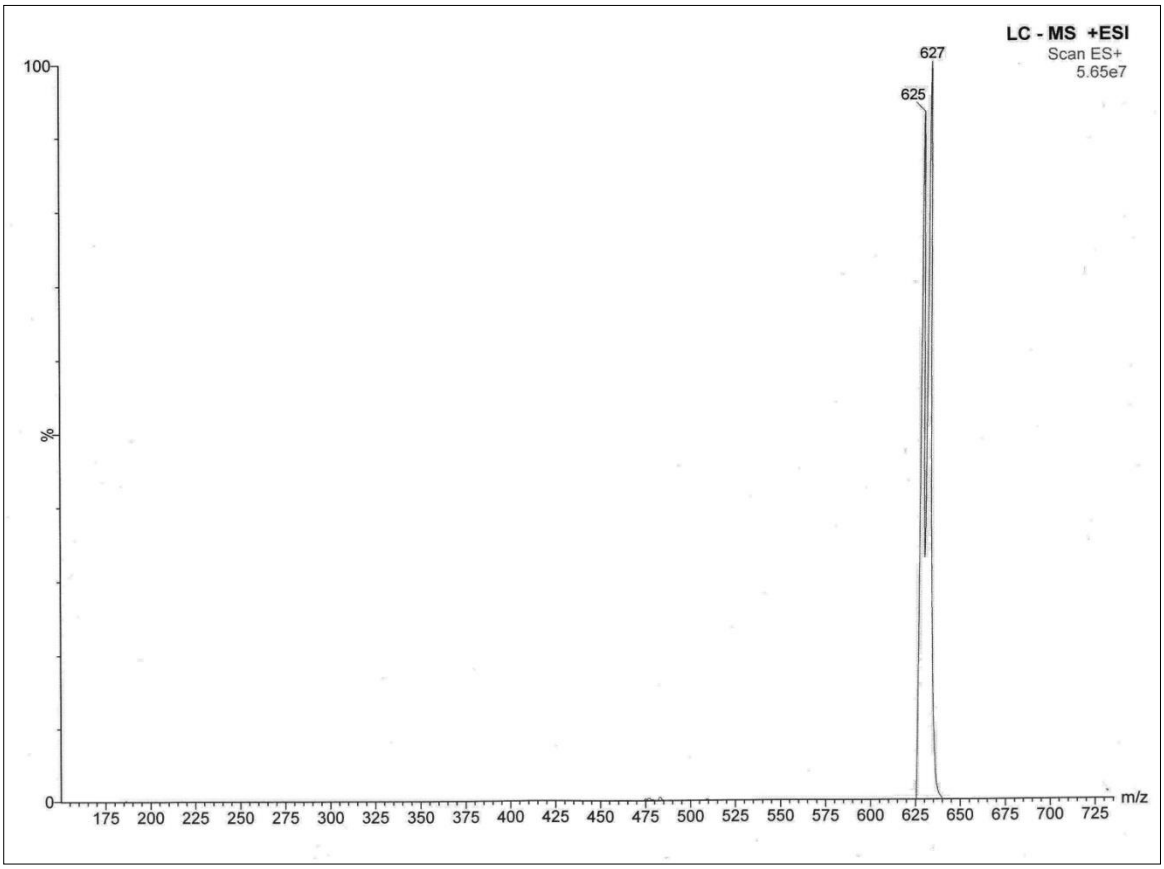
^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound 4f



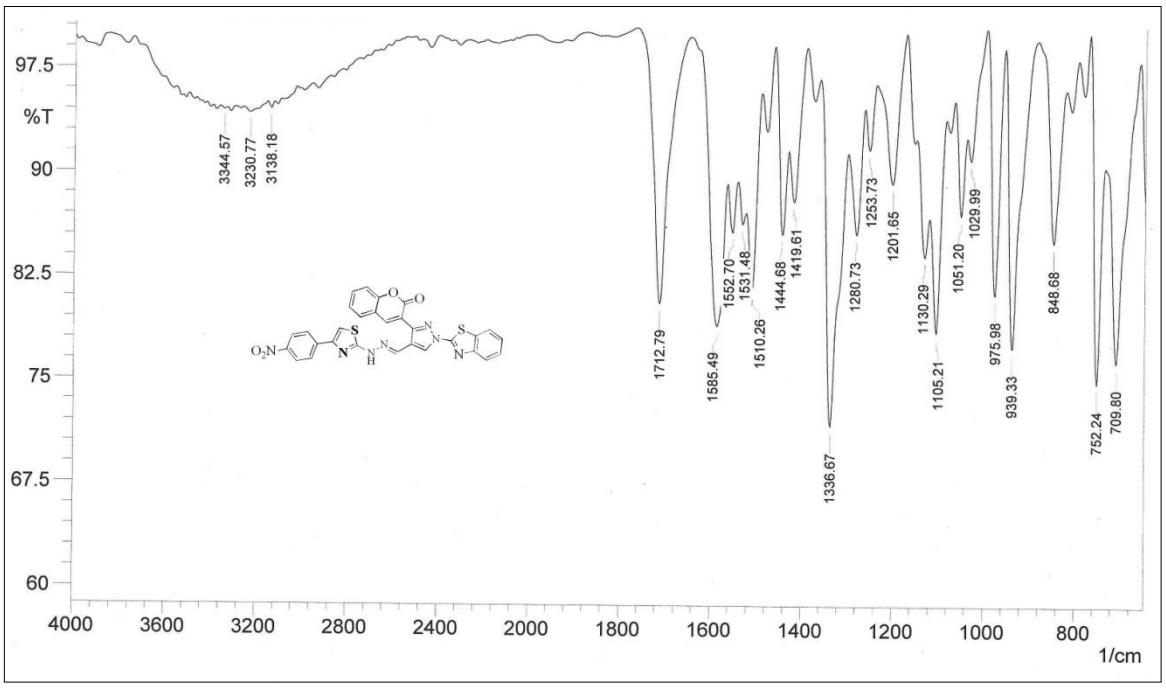
Mass spectrum of compound 4f (M.Wt: 581)



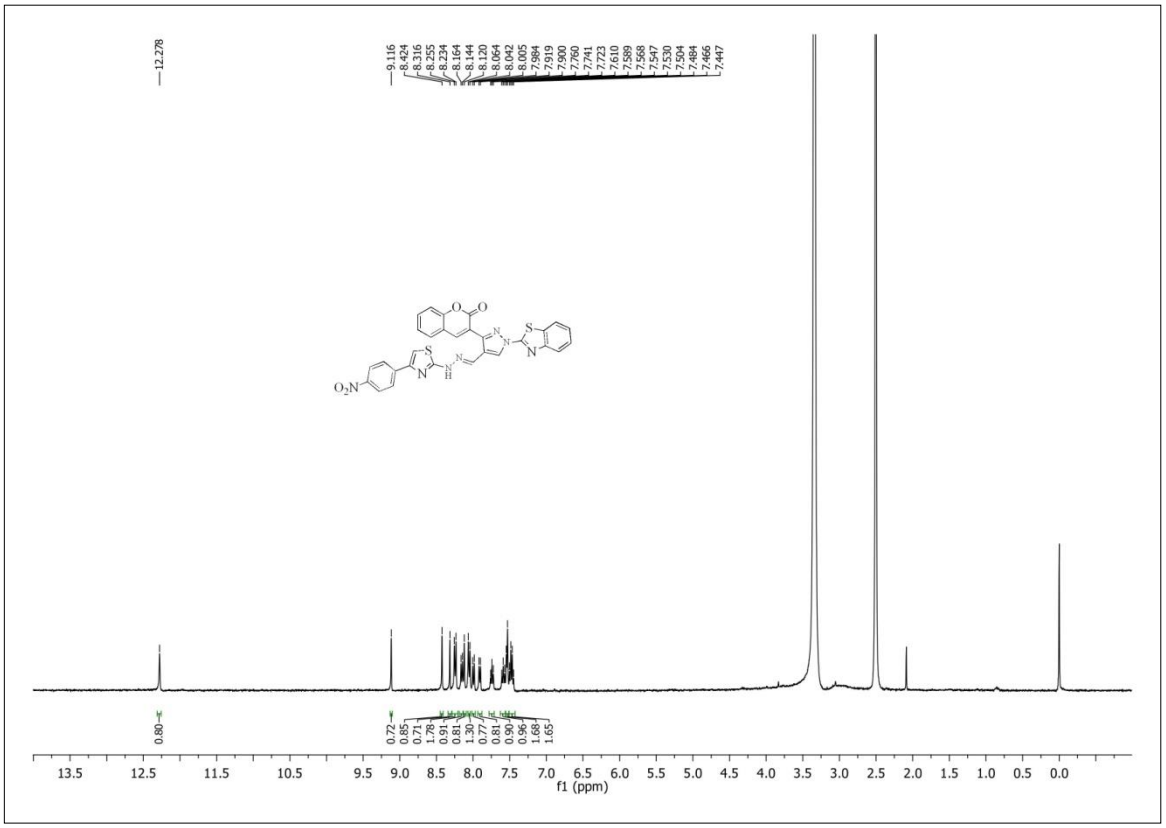
^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound 4g



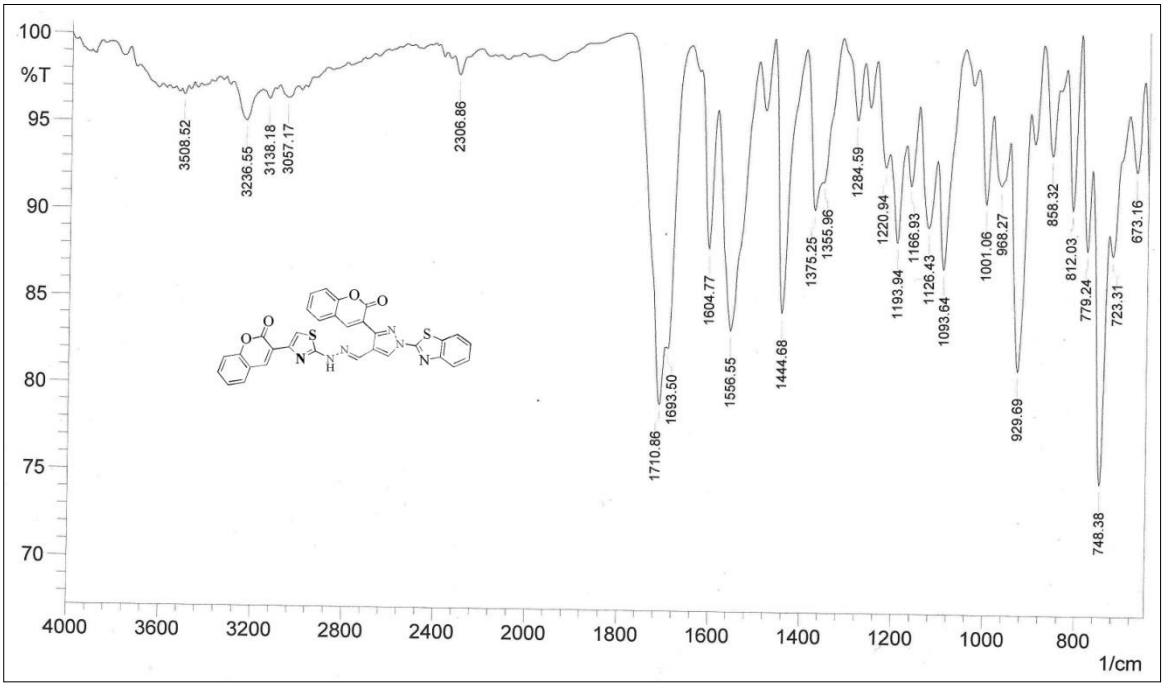
Mass spectrum of compound 4g (M.Wt: 625)



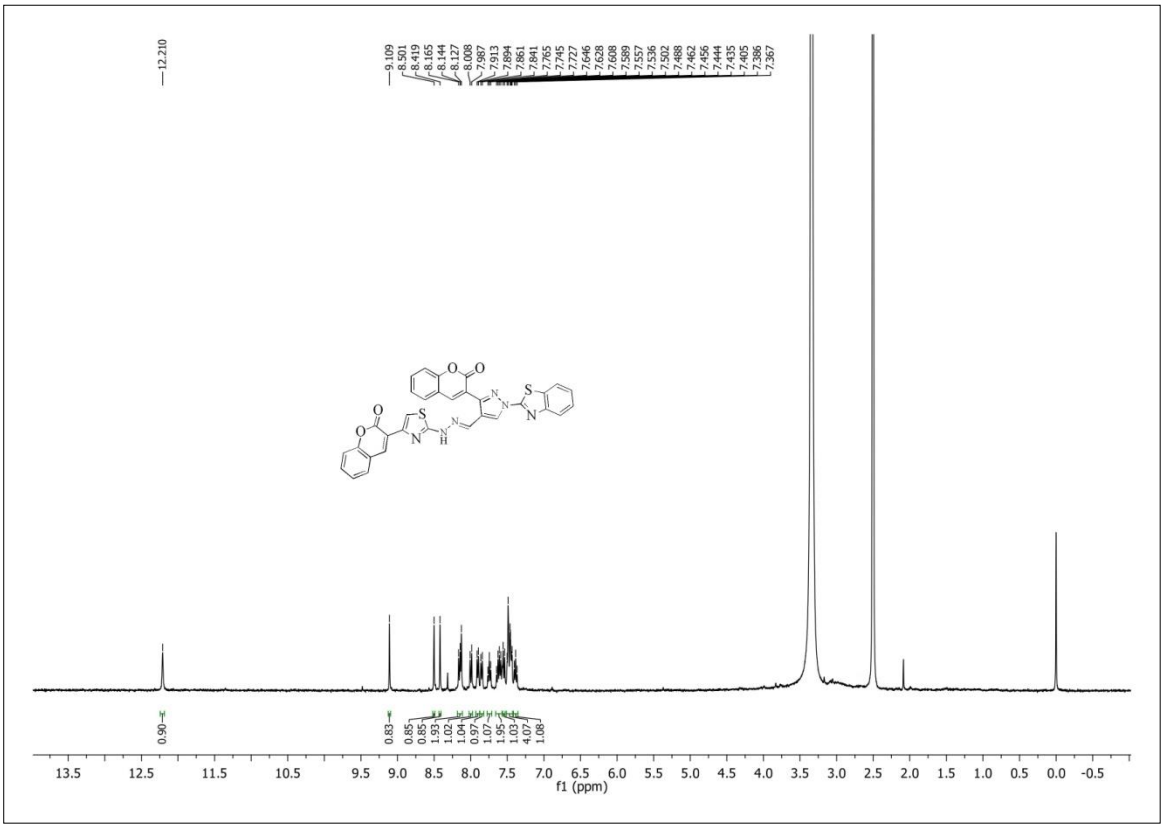
IR (KBr) spectrum of compound 4h



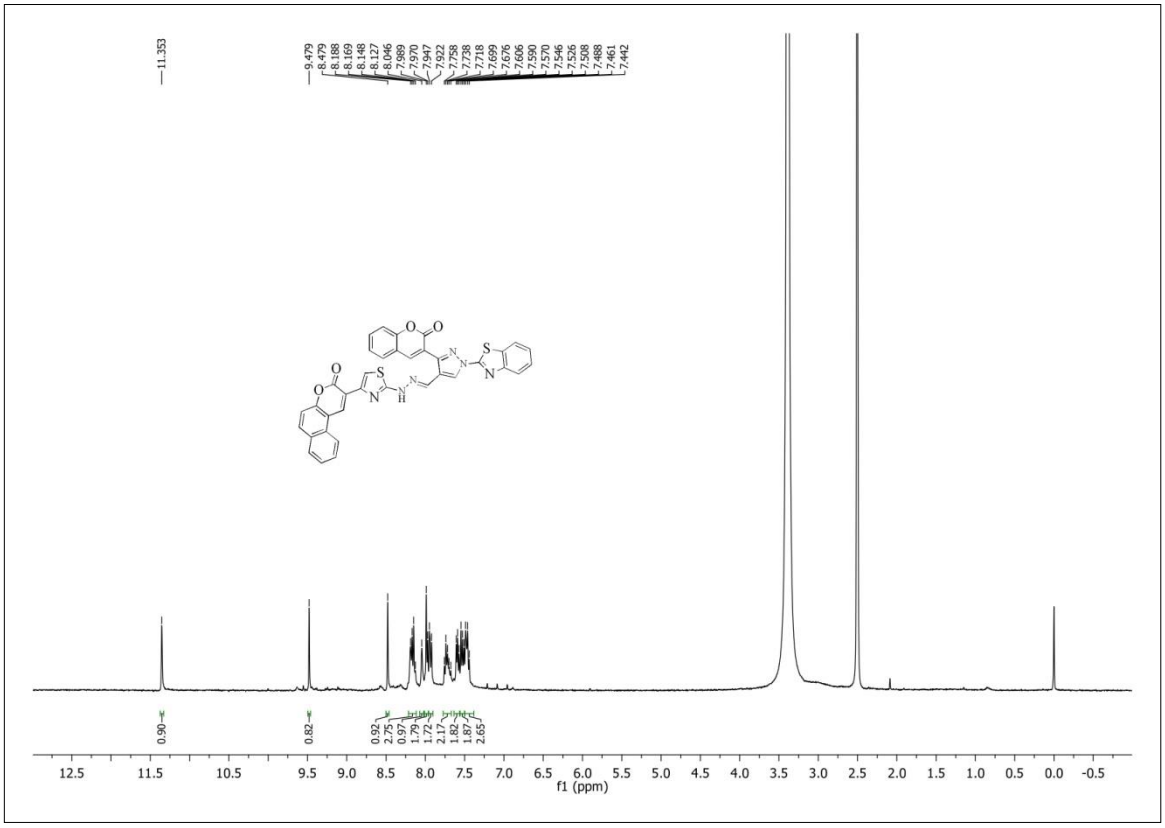
^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound 4h



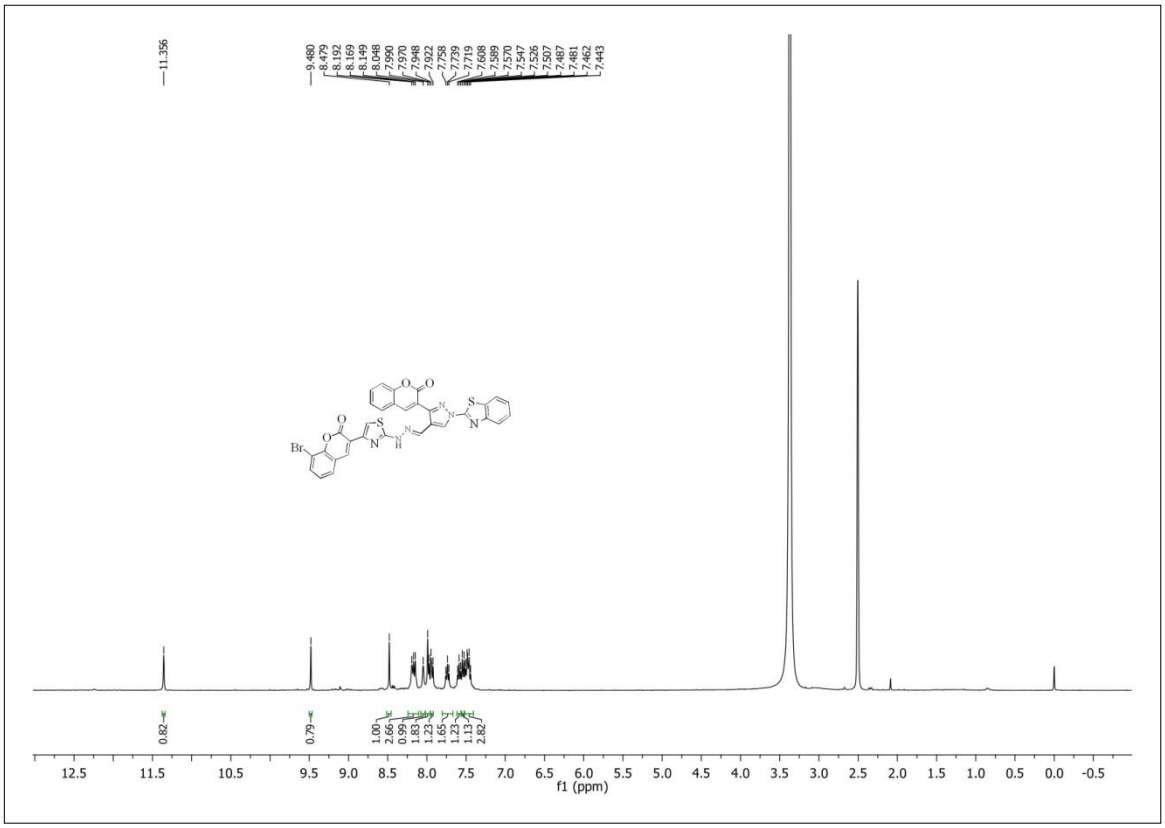
IR (KBr) spectrum of compound 4i



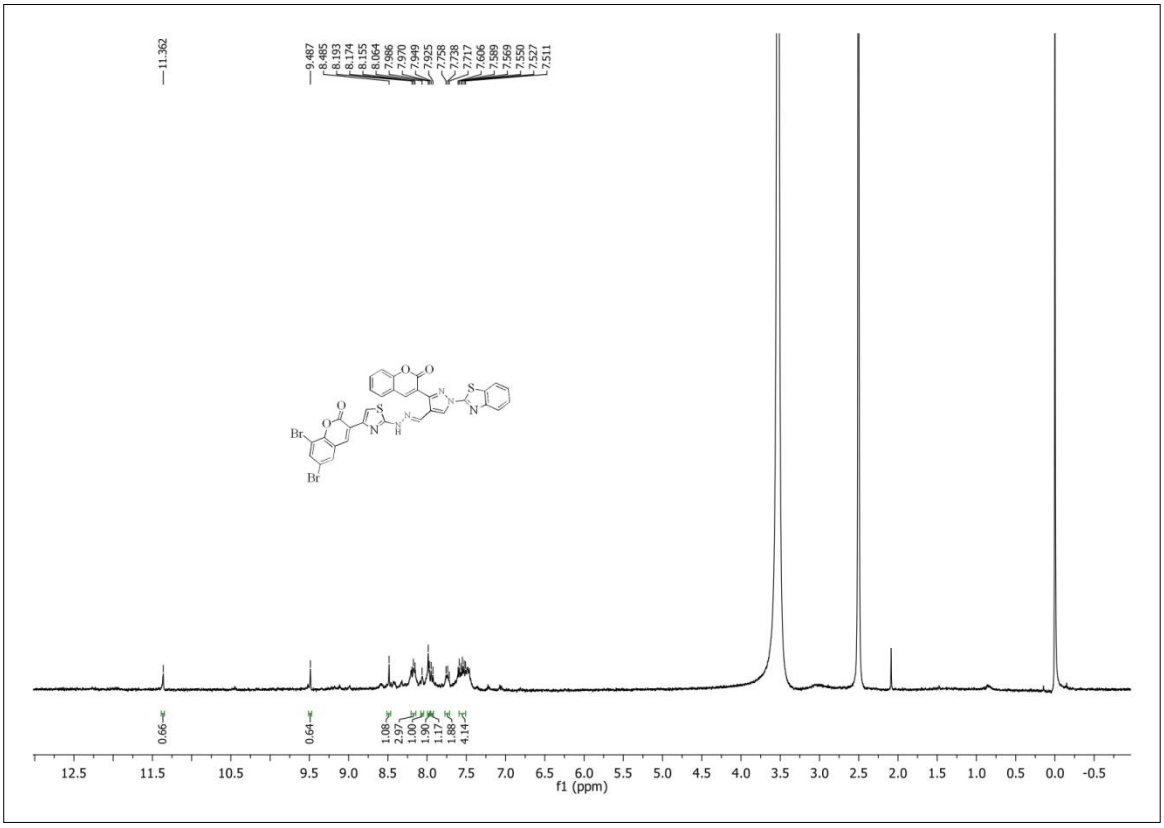
^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound 4i



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



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



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

CHAPTER-III (SECTION-A)

SYNTHESIS OF NEW 4-SUBSTITUTED 1,2,3-TRIAZOLE-HYDRAZINYL 1,3-THIAZOLE HYBRIDS BY EMPLOYING 'CLICK' CHEMISTRY: *IN VITRO* ANTIMICROBIAL AND ANTI-BIOFILM STUDIES

INTRODUCTION

Over the past few years, 1,2,3-Triazoles have received a great attention in medicinal chemistry and drug discovery field due to their versatile biological properties[1,2] such as antibacterial,[3,4] antimalarial,[5] anticancer,[6,7] antitubercular,[8] anntinociceptive,[9] anticonvulsant,[10] anti-inflammatory,[11] anti-allergic,[12] antineoplastic,[13] antimicrobial,[14] anti-HIV antiviral[15,16] and GSK-3β inhibitor activity.[17] Recently published reports on the biological potential of 1,2,3-Triazoles forced the research community towards their selection as a linker for the two functionalities.[18,19]

In addition, in the recent years, 1,3-thiazole[20,21] and coumarin-containing[22,23] derivatives which are elite class of naturally occurring compounds with promising therapeutic perspectives have been reported to exhibit a variety of biological activities. Some of the drug molecules embedding the 1,2,3-triazole,[24,25] 1,3-thiazole and coumarin ring are now available in the market or in the final stage of clinical trials which display a broad spectrum of biological properties (**Fig. 1**).

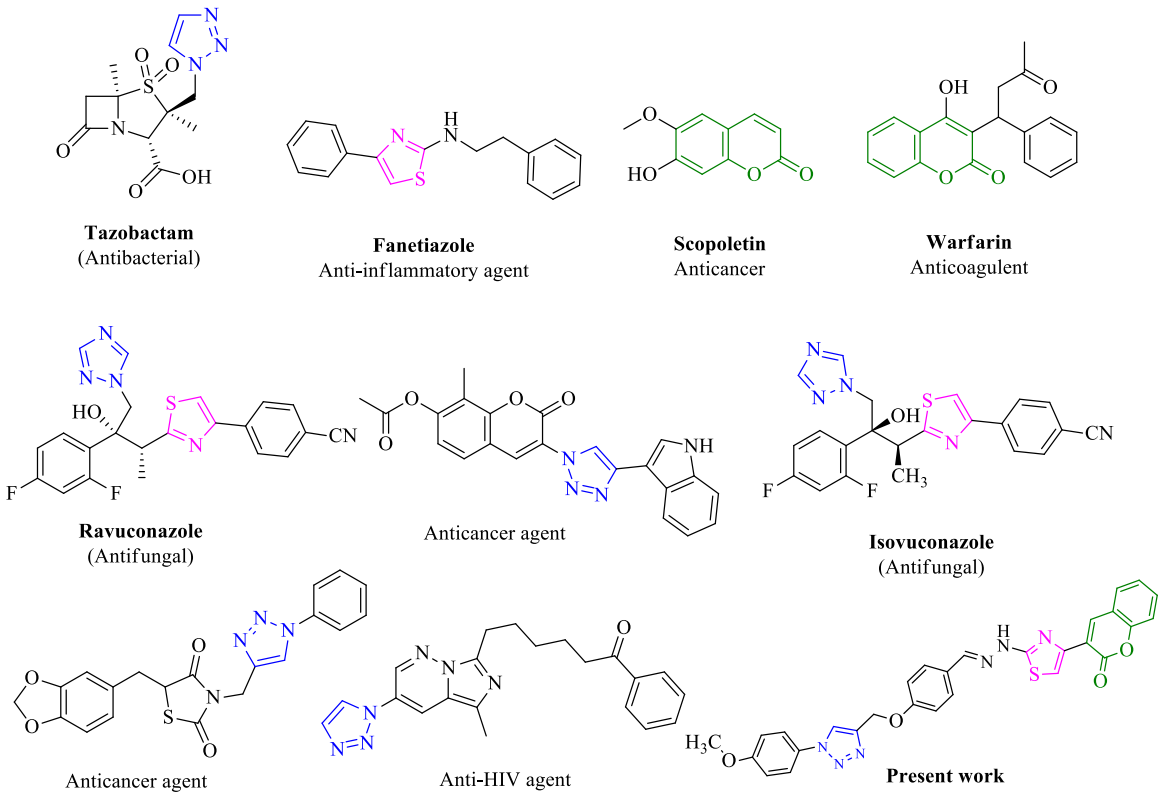
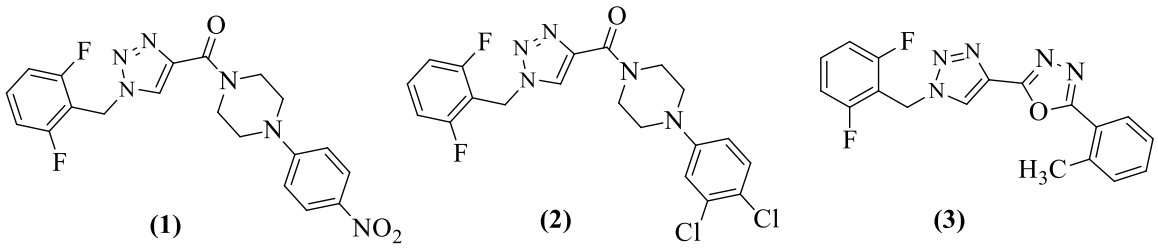


Fig. 1 Some of the examples of triazole, thiazole and coumarin bearing commercial drugs and bio-active molecules.

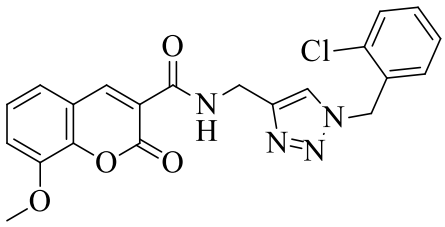
Rahul P. Jadhav *et al.*[26] Synthesis and biological evaluation of a series of 1,4-disubstituted 1,2,3-triazole derivatives as possible antimicrobial agents. Among the synthesized derivatives, compounds **1**, **2** and **3** were found to be having broad spectrum inhibiting activity against the tested strains.



(1) **(2)** **(3)**

Fig. 2

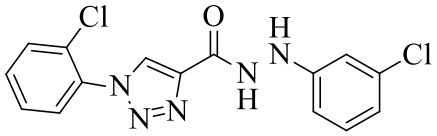
M. Saeedi co-workers[27] described the Synthesis of novel chromenones linked to 1,2,3-triazole ring system: Investigation of biological activities against Alzheimer's disease. From the results it was observed that, the Compound **4** is having good AchE activity when rivastigmine is used as a positive control drug and also the same compound has shown neuroprotective effect against H_2O_2-induced cell death in PC12 neurons.



(4)

Fig. 3

X. Wang[28] reported Synthesis of 1,2,3-triazole hydrazide derivatives exhibiting anti-phytopathogenic activity. From the *in vitro* anti-phytopathogenic activity results, compound **5** identified as a most potent against *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, *Fusarium graminearum*, and *Magnaporthe oryzae* with EC_{50} values of 0.18, 0.35, 0.37 and 2.25 µg/mL respectively.



(5)

Fig. 4

R. Kant *et al.*[29] reported the design, synthesis and biological evaluation of ciprofloxacin tethered bis-1,2,3-triazole conjugates as potent antibacterial agents. *In vitro* antibacterial results revealed that the compounds **6**, **7**, **8** and **9** exhibited 2-17 fold more potential inhibiting activity against most of the tested Gram +ve and Gram -ve species compared to the parent drug ciprofloxacin.

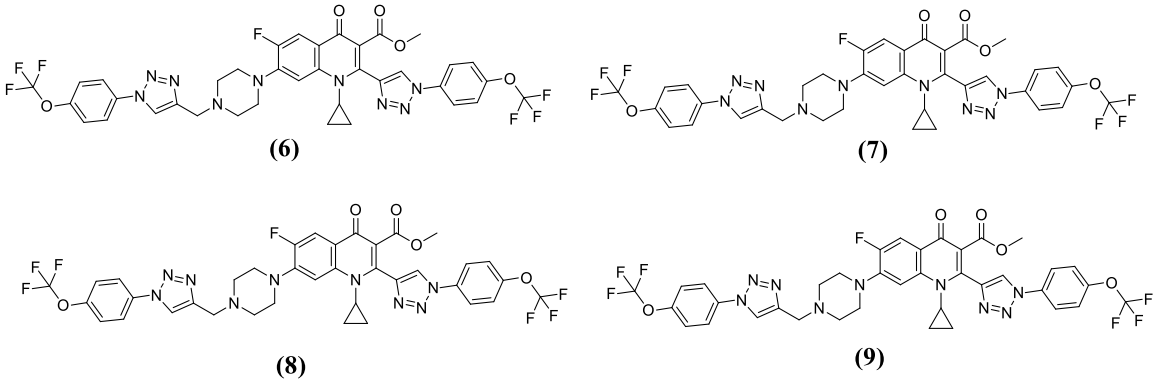
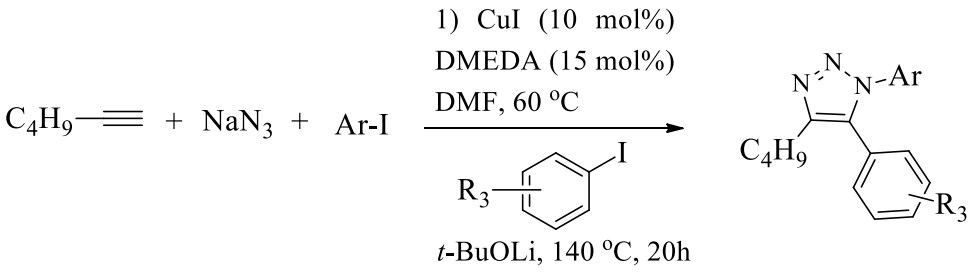


Fig. 5

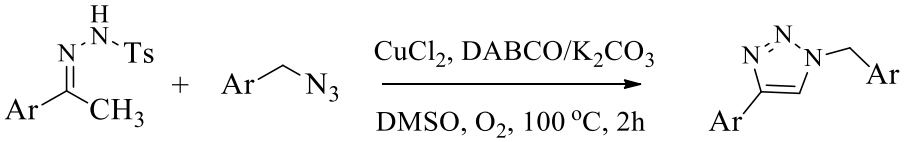
Various important approaches for the synthesis of 4-substituted 1,2,3-triazole derivatives

Fang We and c-workers[30] reported regioselective synthesis of multisubstituted 1,2,3-triazoles: moving beyond the copper-catalyzed azide–alkyne cycloaddition.



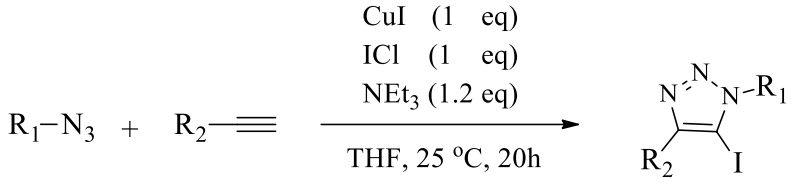
Scheme-1

Zhishuo Zheng and Lei Shi.[31] described An efficient regioselective copper-catalyzed approach to the synthesis of 1,2,3-triazoles from *N*-tosylhydrazones and azides.



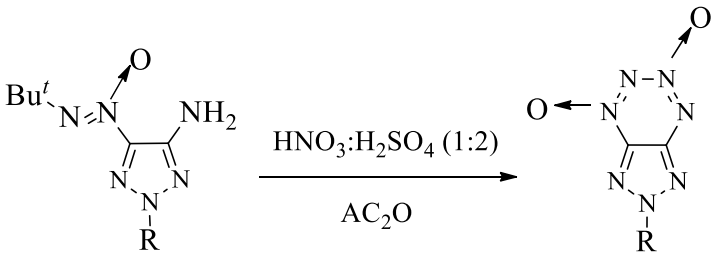
Scheme-2

L. Ackermann, H. K. Potukuchi[32] reported regioselective syntheses of fully-substituted 1,2,3-triazoles: the CuAAC/C-H bond functionalization nexus.



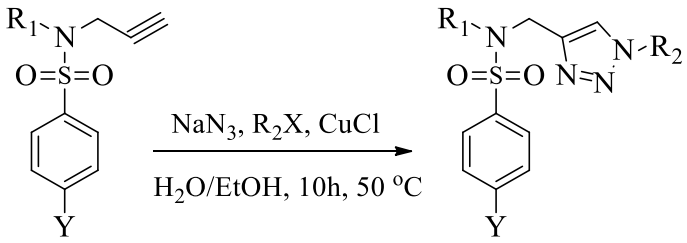
Scheme-3

A. V. Alexey *et al.*[33] described the Synthesis of 1,2,3,4-tetrazine 1,3-dioxides annulated with 1,2,3-triazoles and 1,2,3-triazole 1-oxides.



Scheme-4

Hamid Saeidian *et al.*[34] reported Versatile and green synthesis, spectroscopic characterizations, crystal structure and DFT calculations of 1,2,3-triazole based sulphonamides.



Scheme-5

PRESENT WORK

After the discovery of "click chemistry" concept individually by the Meldal and Sharpless in the early Twenties,[35-37] it has been utilizing in the various fields of chemistry to design a wide variety of triazole frameworks[38] including fused triazoles, triazolo heterocyclic frameworks, and macrocyclic triazoles ranging from enzyme inhibitors to molecular materials. Moreover in the recent years, the copper(I) catalyzed [3+2] azide-alkyne

cycloaddition (CuAAC) has gained considerable attention in the formation of 1,4-disubstituted 1*H*-1,2,3-triazoles due to their potential structural features includes moderate dipolar character, selectivity, rigidity, planarity, dipole moment, and stability to metabolic degradation under *in-vivo* conditions and their binding ability through hydrogen bonding with various bio-molecular targets.[39,40]

On the other hand, the emerging of life-threatening infections caused by multi-drug resistant[41] (MDR) becomes pandemic and making paucity of chemotherapeutics. Hence, there is an urgency to design and develop such alternative chemical entities with new multi-target mechanism of action or which can modulate more than one biological target with high specificity. An emerging strategy within medicinal chemistry and drug discovery is the molecular hybridization (MH) which is an analogous to traditional combination therapy. It is a structural modification strategy and is the only tool to design such molecular frame works with improved bioactivity by the adequate fusion of two or more bio-active pharmacophores.[42]

In view of aforementioned facts and in continuation of our interest towards the design and synthesis of biologically active new heterocyclic frameworks, herein we envisaged (**Fig. 2**) the incorporation of triazole, thiazole and coumarin scaffolds in to a single molecular framework. And synthesized a new series of hybrids by utilizing copper(I)-catalyzed azide-alkyne click chemistry with an expectation to find out the potential lead molecules and evaluated for their *in vitro* antimicrobial and anti-biofilm studies.

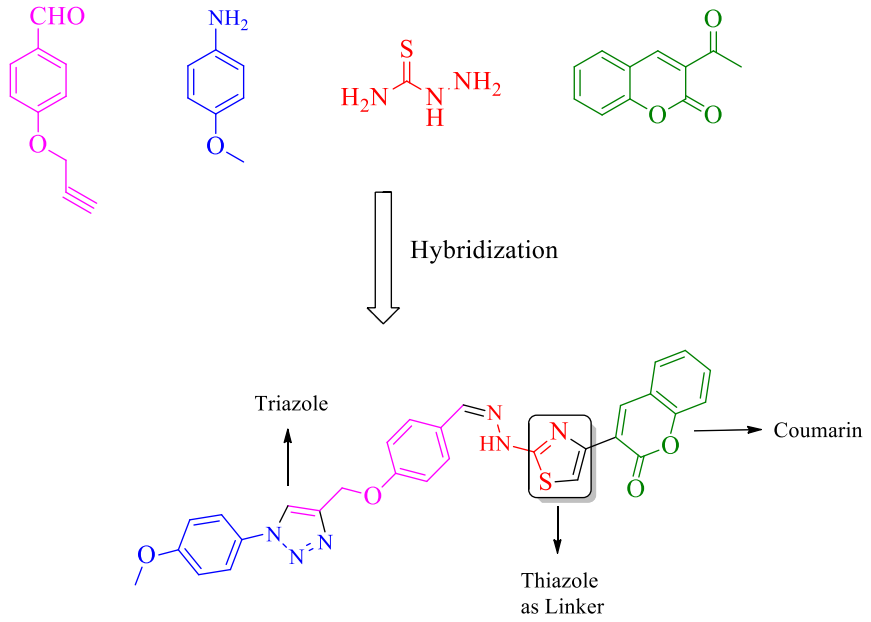


Fig. 2. Design strategy for 1, 2,3-triazole-1,2-thiazole-coumarin hybrids

Preparation of starting materials

3-Acetyl-2*H*-chromen-2-one,2-Acetyl-3*H*-benzo[*f*]chromen-3-one, 3-(2-Bromoacetyl)-2*H*-chromen-2-one and 2-(2-Bromoacetyl)-3*H*-benzo[*f*]chromen-3-one

The above important starting compounds were prepared according to the literature procedure as described in **Chapter-II**, **Section-A**.

4-(prop-2-yn-1-yloxy)benzaldehyde (2)

4-hydroxy benzaldehyde **1** (1.0 mmol) was dissolved in 0.5 mL of DMF in the presence of anhydrous potassium carbonate (1.2 mmol) was allowed to stir at room temperature for 30 min. Propargyl bromide (1.1 mmol) was added to the above reaction mixture and was stirred overnight at room temperature. After the completion of reaction (monitored by TLC using ethyl acetate: hexane as a solvent system). The reaction mixture was quenched by crushed ice and extracted with ethyl acetate (3x30 mL). The combined organic layers were washed with water (3x50 mL) and dried over anhydrous Na_2SO_4. The solvent was evaporated to dryness under reduced pressure to afford the crude product, which was then purified by re-crystallization by using chloroform to get colorless crystals of compound **2**.

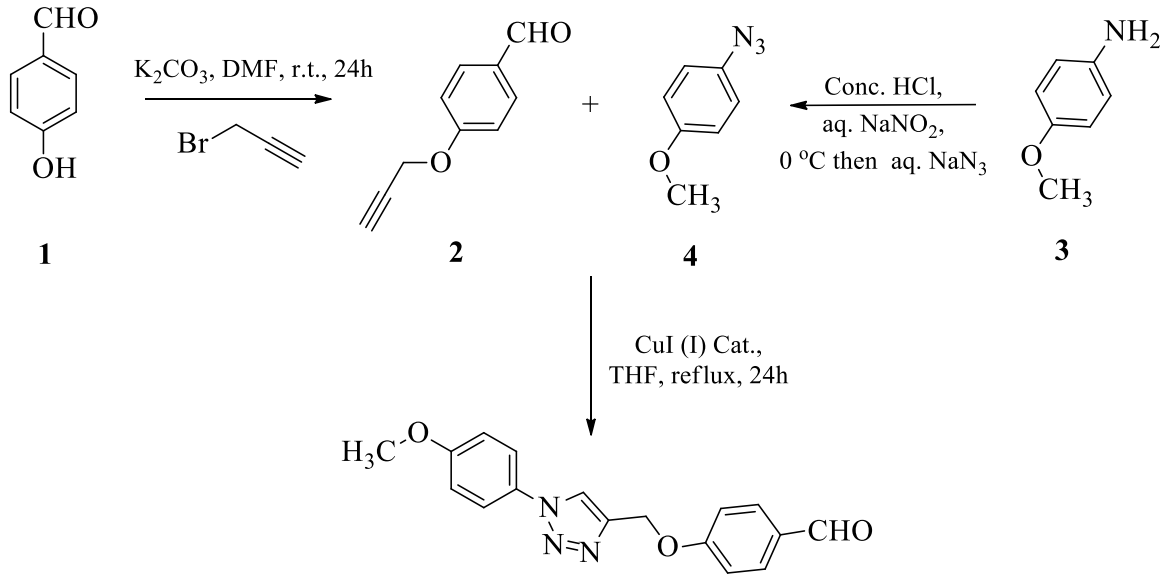
Preparation of 1-azido-4-methoxybenzene (4)

4-methoxy aniline **3** (1.0 mmol) was suspended in HCl/H_2O (1:1 v/v) in a 100 mL flask and cooled to 0-5 °C while stirring for 15 min. Aqueous $NaNO_2$ (1.5 mmol) was added drop-wise to the above-cooled solution and allowed to stir for about 15 min by maintaining the same temperature. After that was added aqueous solution of NaN_3 (1.5 mmol) gradually to the diazonium solution (Caution!) over a period of 15-20 min while stirring at 0-5 °C. The reaction mixture was stirred at room temperature for 1 hr and separated precipitate was extracted with *n*-hexane (3x30 mL) and the organic layer was washed with water, brine and dried over anhydrous Na_2SO_4. The solvent was removed under rotary evaporator to afford methoxyazidobenzene **4** as a dark red colored liquid, which was almost pure and used as such without further purification.

General procedure for the region-selective synthesis of 1,4-aldehyde 1,2,3-triazolyl aldehyde 5: Huisgen cycloaddition reaction

An equimolar concentration of alkyne **2** (1.0 mmol) and azide **4** (1.0 mmol) in a 100 mL round-bottomed flask, having THF as a solvent was stirred under reflux in the presence of

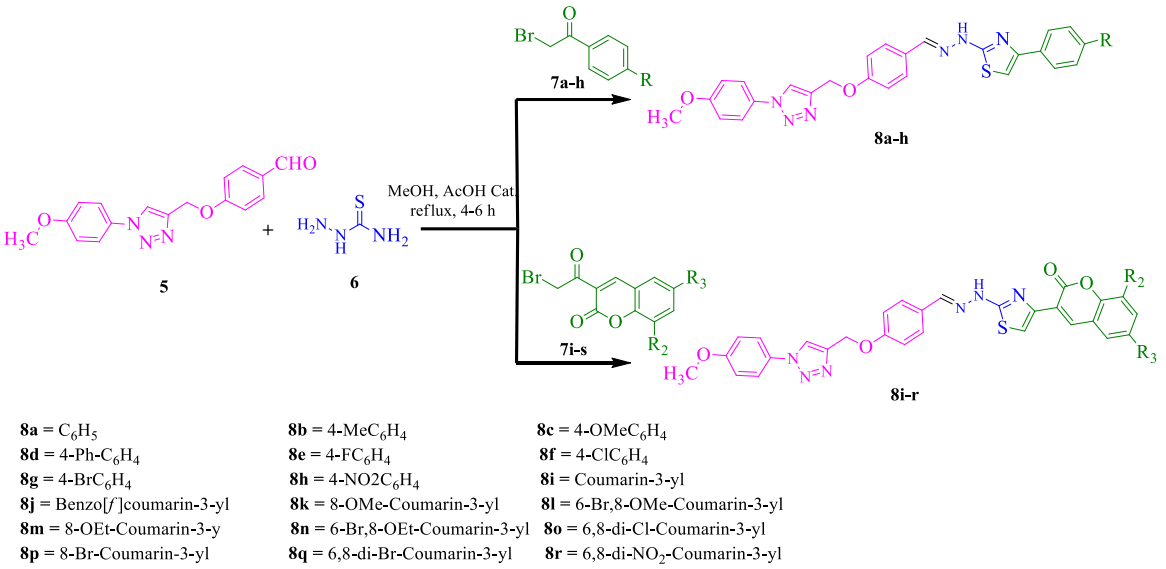
CuI (0.1 mmol) as a catalyst. After completion of the reaction (confirmed by TLC) and was cooled to room temperature and ice-cold water was added. Extracted with ethyl-acetate (3x30 mL). The combined organic layer was washed with water, brine and dried over anhydrous Na_2SO_4. The solvent was concentrated under reduced pressure afforded the crude product **5** as a brown colored solid. Washing, several times with *n*-hexane yielded the pure compound **5** as a light brown solid in 92% yield.



Scheme-6

General procedure for the synthesis of (8a-r)

A mixture of aldehyde **5** (1.0 mmol), thiosemicarbazide **6** (1.0 mmol) and phenacyl bromides/3-(2-bromoacetyl)-2*H*-chromen-2-ones/2-(2-bromoacetyl)-3*H*-benzo[*f*] chromen-3-one **7a-r** (1.0 mmol) in refluxing ethanol in the presence of catalytic amount of acetic acid was stirred for 4-6 hr. The progress of reaction was monitored by TLC. The separated solid was filtered under vacuo and washed with ethanol afforded the tilted compounds **8a-r** in almost analytically pure form.



8a = C_6H_5 **8b** = 4-MeC_6H_4 **8c** = 4-$OMeC_6H_4$
8d = 4-Ph-C_6H_4 **8e** = 4-FC_6H_4 **8f** = 4-ClC_6H_4
8g = 4-BrC_6H_4 **8h** = 4-$NO2C_6H_4$ **8i** = Coumarin-3-yl
8j = Benzo[*f*]coumarin-3-yl **8k** = 8-OMe-Coumarin-3-yl **8l** = 6-Br,8-OMe-Coumarin-3-yl
8m = 8-OEt-Coumarin-3-y **8n** = 6-Br,8-OEt-Coumarin-3-yl **8o** = 6,8-di-Cl-Coumarin-3-yl
8p = 8-Br-Coumarin-3-yl **8q** = 6,8-di-Br-Coumarin-3-yl **8r** = 6,8-di-NO_2-Coumarin-3-yl

Scheme-7

Results and discussion

Embracing the hybridization approach,[43] a series of novel hydrazinyl 1,3-thiazole-1,4-di-substituted 1,2,3-triazole hybrids (**8a-r**) were synthesized in quantitatively isolated yield. The synthetic pathway to achieve the title compounds **8a**–**r** is shown in **Scheme 6** and **7**. The three component condensation of aldehyde **5**, *N*-aminothiourea **6** and α-bromo ketones **7a-r** (4-substituted phenacyl bromides and substituted 3-(Bromoacetyl)coumarins under refluxing ethanol in the presence of catalytic amount of acetic acid. A key starting material in this work, 4-((1-(4-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)benzaldehyde **5** was achieved by the copper(I)-catalyzed region-selective Huisgen 1,3-dipolar cyclo-addition reaction[44] of a terminal alkyne **2** with an azide **4** *via* click chemistry approach (**Scheme 6**). The preparation of starting material **2** was afforded in excellent yield by the propargylation[45] of 4-hydroxy benzaldehyde with propargyl bromide in presence of potassium carbonate as a base in *N*,*N*-dimethylformamide (DMF) at room temperature and the azide **4** by employing the standard diazotization of corresponding aniline followed by *in situ* aromatic nucleophilic substitution by the sodium azide.[46]

With the key intermediate 1,4-disubstituted triazolyl aldehyde **5** in hand, all the title compounds **8a-r** were afforded in 82-95 % quantitative isolated yields. Structures of all the synthesized compounds were well established and they are in good agreement with their spectral (FTIR, ^{1}H NMR, ^{13}C NMR and ESI) and elemental analyses (C, H, and N).

In the FTIR spectrum of starting compound **5**, the disappearance of the absorption band at 1696 cm^{-1}, which is corresponding to the carbonyl group (-C=O) and appearance of bands ranging from 1604 to 1630 cm^{-1} for the imine (-C=N-) functional group in the compounds **8a-r** is an indication of the formation of the tiled compounds. From the ^{1}H NMR and ^{13}C NMR spectra of starting compound **5**, the absence of aldehydic proton as singlet at 9.89 ppm and the disappearance of peak corresponding to aldehydic carbonyl carbon at 190 ppm, and the appearance of singlet peak, corresponding to imine proton (-CH=N-) at 8.01 ppm and imine carbon signal at 142 ppm, confirming the formation of final products **8a-r**. In addition, molecular ion peak at *m/z* from the Electrospray ionization mass spectrometry (ESI-MS) and CHN-analyses data further confirmed the formation of products **8a-r**. Also found a characteristic singlet peak in ^{1}H NMR of all the final compounds **8a-r**, which is resonating around 8.87-8.89 ppm corresponded to the proton (C_5-H) of the triazole ring (Supporting file). Also the total number of protons in ^{13}C NMR and the number of signals in ^{13}C NMR equals the number of different carbons in the molecule matched perfectly with the structures.

Biological evaluation

Antimicrobial evaluation (MIC/MBC)

The in vitro antimicrobial activity of the novel series of synthesized molecular hybrids (**8a-r**) was screened against seven bacterial strains including four gram positive and three gram negative strains one fungal strain by employing agar well diffusion method.[47] The results of antimicrobial activity are illustrated in **Table 1**.

Table 1

Antimicrobial activity (MIC and MBC) of the synthesized compounds (**8i-r)**.

Compds.	**Minimum Inhibitory Concentration**						
	MIC (MBC) µg/mL						
	Gram-positive bacteria				**Gram-negative bacteria**		
	A	**B**	**C**	**D**	**E**	**F**	**G**
8a	–	–	–	–	–	–	–
8b	7.8(15.6)	–	–	–	–	–	–
8c	**3.9**(15.6)	7.8(15.6)	–	–	–	–	–
8d	7.8(15.6)	7.8(15.6)	–	–	–	–	–
8e	–	–	–	–	–	–	–
8f	7.8(15.6)	–	–	–	–	–	–
8g	–	7.8(31.2)	–	7.8(15.6)	–	–	15.6(62.5)
8h	7.8(15.6)	7.8(15.6)	–	7.8(15.6)	–	–	7.8 (31.2)
8i	7.8(15.6)	**3.9 (7.8)**	–	**3.9 (7.8)**	7.8(15.6)	–	7.8 (31.2)
8j	**3.9 (7.8)**	**3.9 (7.8)**	–	7.8 (15.6)	**1.9** (7.8)	**3.9**(15.6)	7.8 (31.2)
8k	**3.9 (7.8)**	**3.9 (7.8)**	**3.9(7.8)**	**3.9 (7.8)**	**1.9 (3.9)**	**3.9(3.9)**	7.8 (15.6)
8l	**3.9 (7.8)**	**3.9 (7.8)**	**3.9(7.8)**	**3.9 (7.8)**	**1.9 (3.9)**	**3.9(7.8)**	**3.9 (7.8)**
8m	–	–	–	–	–	–	–
8n	–	–	–	–	–	–	–
8o	–	–	–	–	–	–	–
8p	**3.9 (7.8)**	**3.9 (7.8)**	–	–	**3.9 (7.8)**	–	–
8q	–	**3.9 (7.8)**	–	**3.9 (7.8)**	–	**3.9(7.8)**	–
8r	–	–	–	–	–	–	–
H	0.9 (0.9)	0.9 (0.9)	0.9(0.9)	0.9 (0.9)	0.9 (0.9)	0.9(0.9)	0.9 (0.9)

A: *S. aureus* MTCC 96; **B:** *B. subtilis* MTCC 121; **C:** *S. aureus* MLS16 MTCC 2940; **D:** *M. luteus* MTCC 2470; **E:** *K. Planticola* MTCC 530; **F:** *E. coli* MTCC 739; **G:** *P. aeruginosa* MTCC 2453.

H: Ciprofloxacin

(–) No activity

In general, most of the synthesized compounds exhibited a certain degree of inhibiting activity (**Table 1**). It was observed from the activity results that, out of the tested series of

compounds, notably, **8c**, **8i-8l**, **8p** and **8q** displayed an appreciable antimicrobial activity. The hybrid, **8l** with a 6-bromo8-methoxycoumarinyl substitution and **8k** with 8-methoxycoumarinyl substitution exhibited excellent and broad spectrum of activity (8k, except against *P. aeruginosa*) against almost all the tested strains with MIC values ranging from 1.9 µg/mL to 3.9 µg/mL and MBC values ranging from 3.9 to 7.8 µg/mL. The remaining compounds (**8c**, **8i**, **8j**, **8p** and **8q**) exhibited good bacterial inhibiting activity with MICs of 3.9 µg/mL and MBC values ranging from 7.8 to 15.6 µg/mL against the test strains. Whereas the remaining compounds, exhibited moderate bacterial inhibition against the test strains with MIC values ranges from 7.8 to 15.6 µg/mL and MBC values ranging from 15.6 to 31.2 µg/mL.

Antifungal activity (MIC/MFC)

Keeping in mind about the aforementioned facts, our compounds **8a-4r** were also screened for *in vitro* antifungal activity against 12 panels of fungal strains using Miconazole as a standard. The screening results showed that the compounds **8i**, **8j**, **8l** and **8r** were effective against *C. albicans* strains with MICs of 3.9 µg/mL and MFCs 7.8 µg/mL. The results are summarized in **Table 2**.

Table 2

Antifungal activity (MIC, MFC) of the synthesized compounds (**8i-l** and **8r)**.

Compds.	**Minimum Inhibitory Concentration**											
	MIC (MFC) µg/mL											
	Fungal Strains											
	I	**J**	**K**	**L**	**M**	**N**	**O**	**P**	**Q**	**R**	**S**	**T**
8i	7.8/31.2	–	3.9/7.8	–	–	–	–	–	–	–	–	3.9/7.8
8j	7.8/31.2	–	3.9/7.8	–	–	–	–	–	–	–	–	3.9/7.8
8k	7.8/15.6	–	–	–	–	–	–	–	–	–	–	3.9/7.8
8l	7.8/15.6	–	–	3.9/7.8	3.9/7.8	–	–	–	3.9/7.8	3.9/7.8	–	–
8r	3.9/7.8	–	–	–	–	–	–	–	–	–	–	–
U	7.8/7.8	7.8/7.8	7.8/7.8	7.8/7.8	7.8/7.8	7.8/7.8	7.8/7.8	7.8/7.8	7.8/7.8	7.8/7.8	7.8/7.8	7.8/7.8

I: *C. albicans* MTCC 3017; **J:** *C. albicans* MTCC-227; **K:** *C. albicans* MTCC-854; **L:** *C. albicans* MTCC-1637; **M:** *C. albicans* MTCC-3018; **N:** *C. albicans* MTCC-3958; **O:** *C. albicans* MTCC-4748; **P:** *C. parapsilosis* MTCC-1745; **Q:** *C. aaseri* MTCC-1962; **R:** *C. glabrata* MTCC-3019; **S:** *C. krusei* MTCC-3020; **T:** *Issatchenkia hanoiensis* MTCC-4755.

U: Miconazole; (–) No activity.

Biofilm inhibition assay

Bio-films are self extra polymeric substance based dynamic microbial communities with emerging characteristics that are different from single microbes. This collective matrix plays a prominent role in protecting the microbial pathogens from antibiotics.[48] Majority of the in-dwelling device associated and nosocomial microbial infections are associated with bio-film formation pathogens.[49] Bio-film formations are reported in contact lenses, intra uterine devices, prosthetic heart valves, breast implants, dialysis catheters.[50] Apart from bacteria, Candidiasis can also form bio-film, thus posing the risk of resistance to current anti fungal compounds.[51]

Keeping in view of above facts, we explored the bio-film inhibiting property[52] of the synthesized compounds (**8a**-**8r**). In the present work, the compounds **8i-8r** were tested against *M. luteus* MTCC 2470, *S. aureus* MTCC 96, *S. aureus* MLS16 MTCC2940, *B. subtilis* MTCC 121, *E. coli* MTCC 739 and *K. planticola* MTCC 530, *Candida albicans* MTCC 3017, *P. aeruginosa* MTCC 2453. The results are tabulated in **Table 3**. The results indicated that, the compound **8i** against *B. subtilis* MTCC 121 with IC_{50} value 6.6 µM and **8l** and **8k** against *S. aureus* MTCC 96 with IC_{50} values 12.0 and 13.5 µM respectively, inhibited the biofilm formation.

Table 3

Biofilm inhibition assay of the synthesized compounds (**8i-r)**.

Compd.	**IC_{50} values in (µM)**							
	S. aureus **MTCC 96**	***B. subtilis*** **MTCC 121**	***S. aureus MLS16*** **MTCC 2940**	***M. luteus*** **MTCC 2470**	***K. planticola*** **MTCC 530**	***E. coli*** **MTCC 739**	***P. aeruginosa*** **MTCC 2453**	***C. albicans*** **MTCC 3017**
8i	–	6.6±0.11	–	–	35.7±0.09	31.3±0.20	19.5±0.11	63.5±0.14
8j	30.1±0.12	37.5±0.13	51.1±0.08	40.2±0.08	59.7±0.15	127.0±0.11	30.4±0.06	45.6±0.26
8k	13.5±0.14	16.6±0.16	–	155.3±0.24	40.2±0.28	68.0±0.24	47.3±0.22	35.6±0.2
8l	12.0±0.07	15.9±0.08	–	47.8±0.18	22.2±0.13	21.9±0.2	24.7±0.06	40.6±0.11
8m	–	–	–	–	–	–	–	–
8n	–	–	–	–	–	–	–	–
8o	–	–	–	–	–	–	–	–
8p	32.6±0.11	35.4±0.08	–	–	42.3±0.08	–	–	–
8q	–	42.6±0.23	–	45.1±0.09	–	–	110±0.14	–

8r	–	–	–	–	–	–	–	–
Ciprofloxacin	0.6±0.08	0.7±0.11	0.7±0.09	0.8±0.11	0.8±0.09	0.6±0.12	0.5±0.12	–
Miconazole	–	–	–	–	–	–	–	2.5 ± 0.09

(–) No activity

Conclusion

In summary, inspired by the hybridization approach, herein we have designed a simple and efficient protocol for the synthesis of 1,2,3-triazole-hydrazinyl 1,3-thiazole hybrids *via* multicomponent strategy. All the compounds were confirmed by spectral data and screened for *in vitro* antimicrobial and bio-film activities. Compounds **8c**, **8i-8l**, **8p** and **8q** were identified as a potential lead compounds to develop antibacterial, antifungal and anti-biofilm agents.

Experimental

General procedure for the synthesis of 8a-r

A mixture of aldehyde **5** (1.0 mmol), thiosemicarbazide **6** (1.0 mmol) and phenacyl bromides/3-(2-bromoacetyl)-2*H*-chromen-2-ones/2-(2-bromoacetyl)-3*H*-benzo[*f*] chromen-3-one **7a-r** (1.0 mmol) in refluxing ethanol in the presence of catalytic amount of acetic acid was stirred for 4-6 hr. The progress of reaction was monitored by TLC. The separated solid was filtered under vacuo and washed with ethanol afforded the tilted compounds **8a-r** in almost analytically pure form.

Spectral data

4-((1-(4-methoxyphenyl)-1H-1,2,3-triazol-4-yl)methoxy)benzaldehyde (**5**)

Light brown solid; 85% yield; mp: 175-177 °C; **IR**: 1696 cm^{-1} (C=O); **^{1}H NMR** (400 MHz, $CDCl_3$): δ 9.89 (s, 1H, Aldehydic-H), 8.01 (s, 1H, triazole C_5-H), 7.85 (d, *J* = 8.4 Hz, 2H), 7.63 (d, *J* = 8.8 Hz, 2H), 7.14 (d, *J* = 8.4 Hz, 2H), 7.02 (d, *J* = 8.4 Hz, 2H), 5.37 (s, 2H, –CH_2-O-), 3.87 (s, 3H, –OCH_3); **^{13}C NMR** (100 MHz, DMSO-*d6*) δ 190.80, 163.12, 160.03, 143.72, 132.06, 130.42, 130.28, 126.28, 122.29, 121.38, 115.12, 114.84, 62.17, 55.66; **MS** (ESI): m/z 310 $[M+H]^+$; Anal. calcd. for $C_{17}H_{15}N_3O_3$: C, 66.01; H, 4.89; N, 13.58. Found: C, 66.28; H, 4.68; N, 13.88.

2-(2-(4-((1-(4-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)benzylidene)hydrazinyl)-4-phenylthiazole (8a)

White solid; 92% yield; mp: 191-193 °C; **IR**: 3304 cm^{-1} (Hydrazinyl-NH), 1623 cm^{-1} (Imine C=N); **^{1}H NMR** (400 MHz, DMSO-*d6*): δ 12.16 (brs, 1H, =N-N-H), 8.89 (s, 1H, –CH=N-), 8.01 (s, 1H, triazole C_5-H), 7.84 (t, *J* = 9.6 Hz, 4H), 7.64 (d, *J* = 8.8 Hz, 2H), 7.41 (t, *J* = 7.6 Hz, 2H), 7.31 (t, *J* = 7.2 Hz, 2H), 7.16-7.14 (m, 4H), 5.28 (s, 2H, –CH_2-O-), 3.83 (s, 3H, –OCH_3); **^{13}C NMR** (100 MHz, DMSO-*d6*) δ 168.82, 159.80, 159.52, 150.23, 143.84, 142.24, 130.43, 129.09, 128.40, 128.13, 127.79, 126.06, 123.43, 122.31, 115.63, 115.37, 103.99, 61.63, 56.04; **MS** (ESI): m/z 483 $[M+H]^+$; Anal. calcd. for $C_{26}H_{22}N_6O_2S$: C, 64.71; H, 4.60; N, 17.42. Found: C, 64.48; H, 4.88; N, 17.69.

2-(2-(4-((1-(4-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)benzylidene)hydrazinyl)-4-(*p*-tolyl)thiazole (8b)

White solid; 88% yield; mp: 190-192 °C; **IR**: 3378 cm^{-1} (Hydrazinyl-NH), 1622 cm^{-1} (Imine C=N); **^{1}H NMR** (400 MHz, DMSO-*d6*): δ 12.11 (brs, 1H, =N-N-H), 8.89 (s, 1H, –CH=N-), 8.04 (s, 1H, triazole C_5-H), 7.82 (d, *J* = 8.8 Hz, 2H), 7.73 (d, *J* = 8.4 Hz, 2H), 7.64 (d, *J* = 8.8 Hz, 2H), 7.21-7.24 (m, 3H), 7.13-7.17 (m, 4H), 5.28 (s, 2H, –CH_2-O-), 3.83 (s, 3H, –OCH_3), 2.32 (s, 3H, –CH_3); **^{13}C NMR** (100 MHz, DMSO-*d6*) δ 168.74, 159.82, 159.52, 150.19, 143.86, 142.24, 137.45, 132.07, 130.45, 129.67, 128.41, 127.82, 126.02, 123.44, 122.33, 115.65, 115.39, 103.09, 61.65, 56.07, 21.31; **MS** (ESI): m/z 497 $[M+H]^+$; Anal. calcd. for $C_{27}H_{24}N_6O_2S$: C, 65.30; H, 4.87; N, 16.92. Found: C, 65.07; H, 5.09; N, 17.13.

4-(4-methoxyphenyl)-2-(2-(4-((1-(4-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)benzylidene)hydrazinyl)thiazole (8c)

Light brown solid; 90% yield; mp: 180-182 °C; **IR**: 3312 cm^{-1} (Hydrazinyl-NH), 1630 cm^{-1} (Imine C=N); **^{1}H NMR** (400 MHz, DMSO-*d6*): δ 12.10 (brs, 1H, =N-N-H), 8.88 (s, 1H, –CH=N-), 8.00 (s, 1H, triazole C_5-H), 7.77-7.83 (m, 4H), 7.63 (d, *J* = 8.8 Hz, 2H), 7.14 (d, *J* = 9.2 Hz, 5H), 6.97 (d, *J* = 8.8 Hz, 2H), 5.27 (s, 2H, –CH_2-O-), 3.83 (s, 3H, –OCH_3), 3.78 (s, 3H, –OCH_3); ^{13}C NMR (100 MHz, DMSO-*d6*) δ 169.23, 159.82, 159.53, 148.99, 146.65, 143.84, 142.21, 141.16, 130.41, 128.40, 126.79, 124.60, 123.43, 122.34, 115.64, 115.37, 108.81, 61.59, 56.04; **MS** (ESI): m/z 513 $[M+H]^+$; Anal. calcd. for $C_{27}H_{24}N_6O_3S$: C, 63.27; H, 4.72; N, 16.40. Found: C, 63.55; H, 4.91; N, 16.16.

4-([1,1'-biphenyl]-4-yl)-2-(2-(4-((1-(4-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)benzylidene)hydrazinyl)thiazole (8d)

Light brown solid; 88% yield; mp: 221-223 °C; **IR**: 3308 cm^{-1} (Hydrazinyl-NH), 1606 cm^{-1} (Imine C=N); **MS** (ESI): m/z 559 $[M+H]^+$; Anal. calcd. for $C_{32}H_{26}N_6O_2S$: C, 68.80; H, 4.69; N, 15.04. Found: C, 69.11; H, 4.48; N, 14.88.

4-(4-fluorophenyl)-2-(2-(4-((1-(4-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)benzylidene)hydrazinyl)thiazole (8e)

White solid; 86% yield; mp: 215-217 °C; **IR**: 3306 cm^{-1} (Hydrazinyl-NH), 1621 cm^{-1} (Imine C=N); **^{13}C NMR** (100 MHz, DMSO-*d6*) δ 168.90, 163.28, 160.86, 159.82, 159.47, 149.77, 143.87, 141.85, 131.74, 130.45, 128.33, 128.02, 127.94, 123.44, 122.34, 116.03, 115.81, 115.64, 115.39, 103.66, 61.65, 56.07; **MS** (ESI): m/z 501 $[M+H]^+$; Anal. calcd. for $C_{26}H_{21}FN_6O_2S$: C, 62.39; H, 4.23; N, 16.79. Found: C, 62.72; H, 4.48; N, 16.50.

4-(4-chlorophenyl)-2-(2-(4-((1-(4-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)benzylidene)hydrazinyl)thiazole (8f)

Light brown solid; 85% yield; yield; mp: 229-231 °C; **IR**: 3307 cm^{-1} (Hydrazinyl-NH), 1621 cm^{-1} (Imine C=N); mp: 222-224 °C; **MS** (ESI): m/z 517 $[M+H]^+$; Anal. calcd. for $C_{26}H_{21}ClN_6O_2S$: C, 60.40; H, 4.09; N, 16.26. Found: C, 60.68; H, 3.81; N, 16.51.

4-(4-bromophenyl)-2-(2-(4-((1-(4-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)benzylidene)hydrazinyl)thiazole (8g)

Light brown solid; 82% yield; yield; mp: 232-235 °C; **IR**: 3306 cm^{-1} (Hydrazinyl-NH), 1621 cm^{-1} (Imine C=N); mp: 245-247 °C; **MS** (ESI): m/z 561 $[M+H]^+$; Anal. calcd. for $C_{26}H_{21}BrN_6O_2S$: C, 55.62; H, 3.77; N, 14.97. Found: C, 55.92; H, 4.03; N, 14.75.

2-(2-(4-((1-(4-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)benzylidene)hydrazinyl)-4-(4-nitrophenyl)thiazole (8h)

Yellow solid; 82% yield; yield; mp: 258-260 °C; **IR**: 3308 cm^{-1} (Hydrazinyl-NH), 1606 cm^{-1} (Imine C=N); mp: 268-270 °C; **^{1}H NMR** (400 MHz, DMSO-*d6*): δ 12.18 (s, 1H, =N-N-H), 8.88 (s, 1H, triazole C_5-H), 8.28 (d, *J* = 9.2 Hz, 2H), 8.11 (d, *J* = 9.2 Hz, 2H), 8.02 (s, 1H, –CH=N-), 7.82 (d, *J* = 8.8 Hz, 2H), 7.71 (s, 1H), 7.64 (d, *J* = 8.8 Hz, 2H), 7.17-7.12 (m, 4H), 5.28 (s, 2H, –CH_2-O-), 3.83 (s, 3H, –OCH_3), **^{13}C NMR** (100 MHz, DMSO-*d6*) δ 169.23, 159.82, 159.53, 148.99, 146.65, 143.84, 142.21, 141.16, 130.41, 128.40, 126.79, 124.60, 123.43, 122.34, 115.64, 115.37, 108.81, 61.59, 56.04; **MS** (ESI): m/z 528

$[M+H]^+$; Anal. calcd. for $C_{26}H_{21}N_7O_4S$: C, 59.19; H, 4.01; N, 18.59. Found: C, 59.48; H, 4.28; N, 18.29.

3-(2-(2-(4-((1-(4-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)benzylidene)hydrazinyl)thiazol-4-yl)-2*H*-chromen-2-one (8i)

Light yellow solid; 83% yield; **IR**: 3297 cm^{-1} (Hydrazinyl-NH), 1716 (lactone C=O), 1607 cm^{-1} (Imine C=N); mp: 281-283 °C; **^{1}H NMR** (400 MHz, DMSO-*d6*): δ 12.09 (s, 1H, =N-N-H), 8.87 (s, 1H, –CH=N-), 8.54 (s, 1H, coumarin-C_4 proton), 8.03 (s, 1H, triazole C_5-H), 7.86-7.80 (m, 3H), 7.76 (s, 1H), 7.64 (d, *J* = 8.8 Hz, 3H), 7.45 (d, *J* = 8.4 Hz, 1H), 7.39 (t, *J* = 7.4 Hz, 1H), 7.17-7.12 (m, 4H), 5.28 (s, 2H, –CH_2-O-), 3.83 (s, 3H, OMe); **^{13}C NMR** (100 MHz, DMSO-*d6*) δ 168.29, 159.82, 159.53, 159.24, 152.78, 144.45, 143.87, 142.13, 138.61, 132.17, 130.46, 129.30, 128.38, 127.81, 125.20, 123.43, 122.34, 121.05, 119.68, 116.38, 115.66, 115.39, 110.90, 61.66, 56.07. **MS** (ESI): m/z 551 $[M+H]^+$; Anal. calcd. for $C_{29}H_{22}N_6O_4S$: C, 63.26; H, 4.03; N, 15.26. Found: C, 63.01; H, 3.82; N, 15.55.

2-(2-(2-(4-((1-(4-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)benzylidene)hydrazinyl)thiazol-4-yl)-3*H*-benzo[*f*]chromen-3-one (8j)

Light brown solid; 82% yield; mp: 285-287 °C; **IR**: 3387 cm^{-1} (Hydrazinyl-NH), 1718 (lactone C=O), 1626 cm^{-1} (Imine C=N); **MS** (ESI): m/z 601 $[M+H]^+$; Anal. calcd. for $C_{33}H_{24}N_6O_4S$: C, 65.99; H, 4.03; N, 13.99. Found: C, 66.28; H, 3.80; N, 13.77.

8-methoxy-3-(2-(2-(4-((1-(4-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)benzylidene)hydrazinyl)thiazol-4-yl)-2*H*-chromen-2-one (8k)

Yellow solid; 84% yield; yield; mp: 233-235 °C; **IR**: 3415 cm^{-1} (Hydrazinyl-NH), 1720 (lactone C=O), 1605 cm^{-1} (Imine C=N); **^{13}C NMR** (100 MHz, DMSO-*d6*) δ 168.30, 159.82, 159.53, 158.95, 146.75, 144.37, 143.87, 142.12, 142.06, 138.77, 130.46, 128.38, 127.81, 125.12, 123.43, 122.33, 121.17, 120.46, 120.23, 115.65, 115.39, 114.36, 110.99, 61.66, 56.58, 56.07; **MS** (ESI): m/z 581 $[M+H]^+$; Anal. calcd. for $C_{30}H_{24}N_6O_5S$: C, 62.06; H, 4.17; N, 14.47. Found: C, 62.32; H, 4.28; N, 14.71.

6-bromo-8-methoxy-3-(2-(2-(4-((1-(4-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)benzylidene)hydrazinyl)thiazol-4-yl)-2*H*-chromen-2-one (8l)

Orange solid; 82% yield; mp: 271-272 °C; **IR**: 3416 cm^{-1} (Hydrazinyl-NH), 1728 (lactone C=O), 1604 cm^{-1} (Imine C=N); **MS** (ESI): m/z 659 $[M+H]^+$; Anal. calcd. for $C_{30}H_{23}BrN_6O_5S$: C, 54.63; H, 3.52; N, 12.74. Found: C, 54.89; H, 3.22; N, 12.50.

8-ethoxy-3-(2-(2-(4-((1-(4-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)benzylidene)hydrazinyl)thiazol-4-yl)-2*H*-chromen-2-one (8m)

Yellow solid; 85% yield; mp: 265-267 °C; **IR**: 3425 cm^{-1} (Hydrazinyl-NH), 1729 (lactone C=O), 1627 cm^{-1} (Imine C=N); **MS** (ESI): m/z 596 $[M+H]^+$; Anal. calcd. for $C_{31}H_{26}N_6O_5S$: C, 62.61; H, 4.41; N, 14.13. Found: C, 62.38; H, 4.69; N, 13.87.

6-bromo-8-ethoxy-3-(2-(2-(4-((1-(4-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)benzylidene)hydrazinyl)thiazol-4-yl)-2*H*-chromen-2-one (8n)

Orange solid; 82% yield; mp: 289-291 °C; **IR**: 3522 cm^{-1} (Hydrazinyl-NH), 1728 (lactone C=O), 1605 cm^{-1} (Imine C=N); **MS** (ESI): m/z 674 $[M+H]^+$; Anal. calcd. for $C_{31}H_{25}BrN_6O_5S$: C, 55.28; H, 3.74; N, 12.48. Found: C, 55.59; H, 4.02; N, 12.78.

6,8-dichloro-3-(2-(2-(4-((1-(4-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)benzylidene)hydrazinyl)thiazol-4-yl)-2*H*-chromen-2-one (8o)

Yellow solid; 82% yield; mp: 284-286 °C; **IR**: 3405 cm^{-1} (Hydrazinyl-NH), 1732 (lactone C=O), 1622 cm^{-1} (Imine C=N); **MS** (ESI): m/z 620 $[M+H]^+$; Anal. calcd. for $C_{29}H_{20}Cl_2N_6O_4S$: C, 56.23; H, 3.25; N, 13.57. Found: C, 56.51; H, 3.02; N, 13.86.

8-bromo-3-(2-(2-(4-((1-(4-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)benzylidene)hydrazinyl)thiazol-4-yl)-2*H*-chromen-2-one (8p)

Yellow solid; 82% yield; mp: 303-305 °C; **IR**: 3409 cm^{-1} (Hydrazinyl-NH), 1730 (lactone C=O), 1604 cm^{-1} (Imine C=N); **MS** (ESI): m/z 630 $[M+H]^+$; Anal. calcd. for $C_{29}H_{21}BrN_6O_4S$: C, 55.33; H, 3.36; N, 13.35. Found: C, 55.57; H, 3.05; N, 13.63.

6,8-dibromo-3-(2-(2-(4-((1-(4-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)benzylidene)hydrazinyl)thiazol-4-yl)-2*H*-chromen-2-one (8q)

Orange solid; 83% yield; mp: 323-325 °C; **IR**: 3410 cm^{-1} (Hydrazinyl-NH), 1727 (lactone C=O), 1627 cm^{-1} (Imine C=N); **MS** (ESI): m/z 709 $[M+H]^+$; Anal. calcd. for $C_{29}H_{20}Br_2N_6O_4S$: C, 49.17; H, 2.85; N, 11.86;. Found: C, 49.47; H, 3.06; N, 11.64.

3-(2-(2-(4-((1-(4-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)benzylidene)hydrazinyl)thiazol-4-yl)-6,8-dinitro-2*H*-chromen-2-one (8r)

Orange solid; 82% yield; mp: 333-335 °C; **IR**: 3411 cm^{-1} (Hydrazinyl-NH), 1740 (lactone C=O), 1604 cm^{-1} (Imine C=N); **MS** (ESI): m/z 802 $[M+H]^+$; Anal. calcd. for $C_{39}H_{28}N_8O_{10}S$: C, 58.50; H, 3.52; N, 13.99;. Found: C, 58.77; H, 3.26; N, 13.70.

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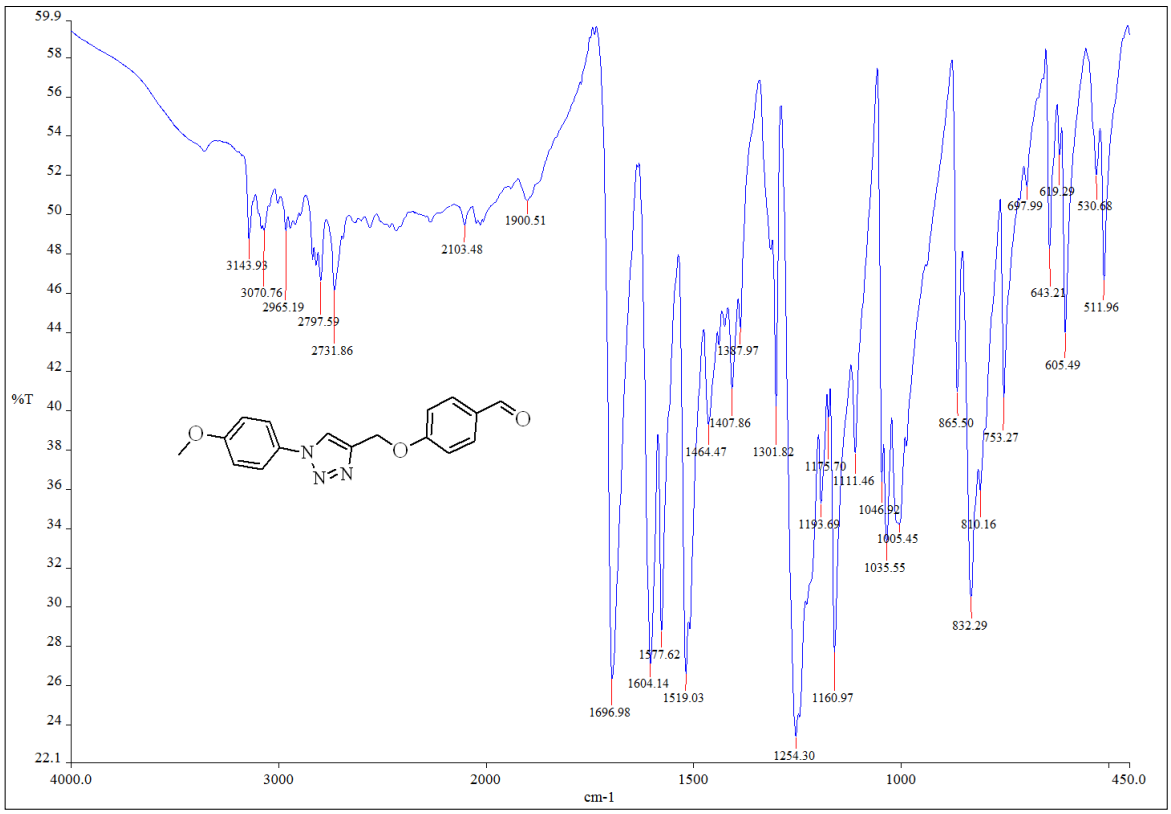
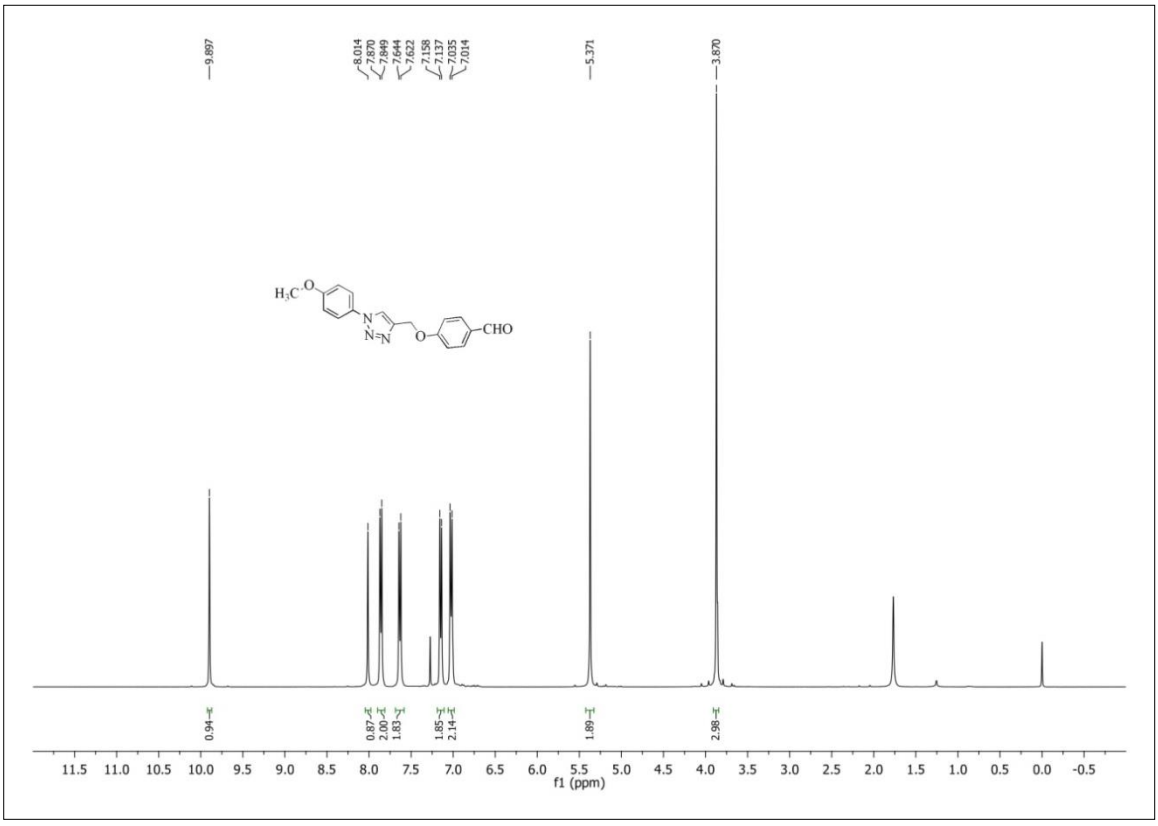
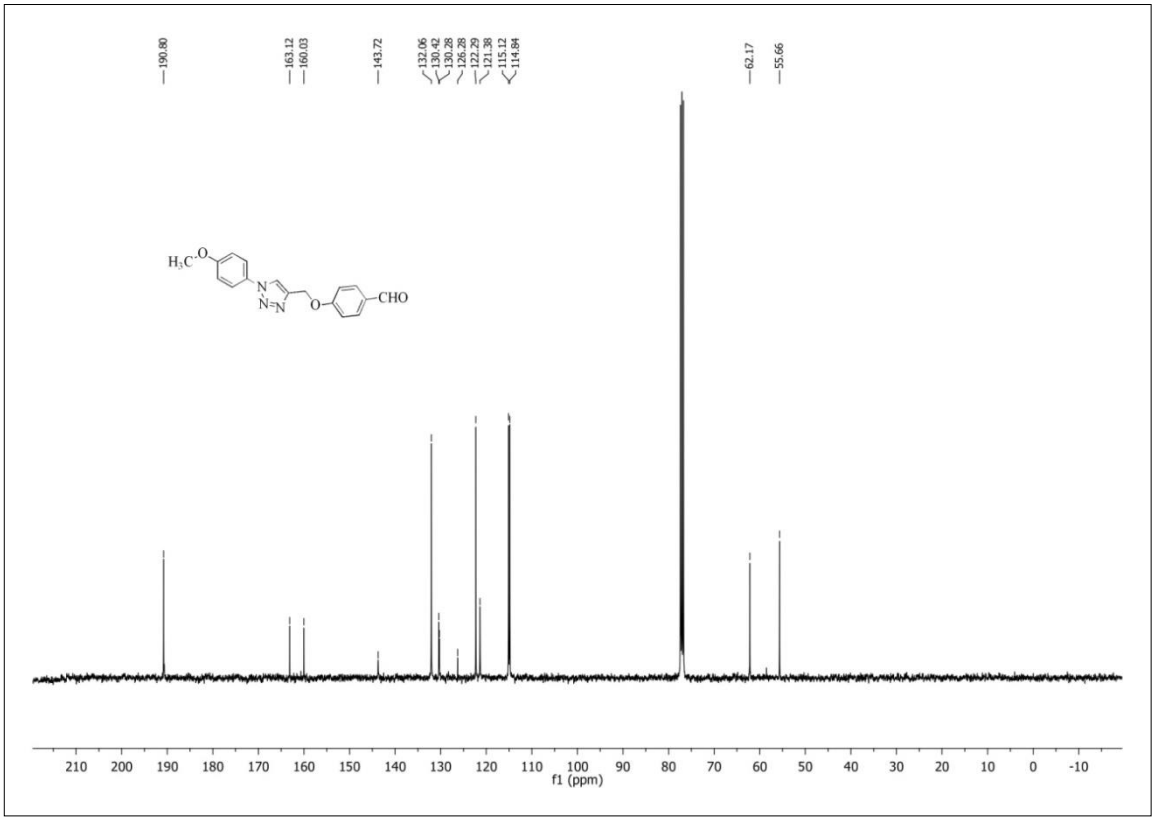


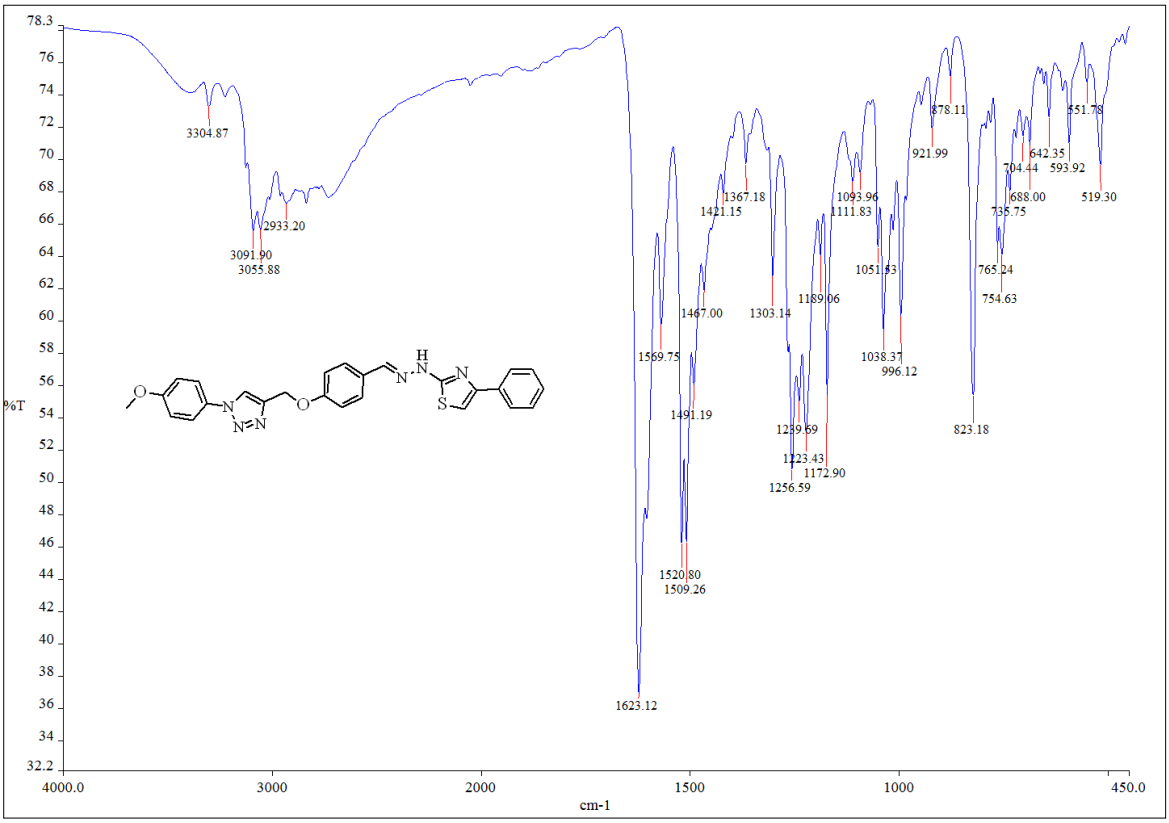
IR (KBr) spectrum of compound 5



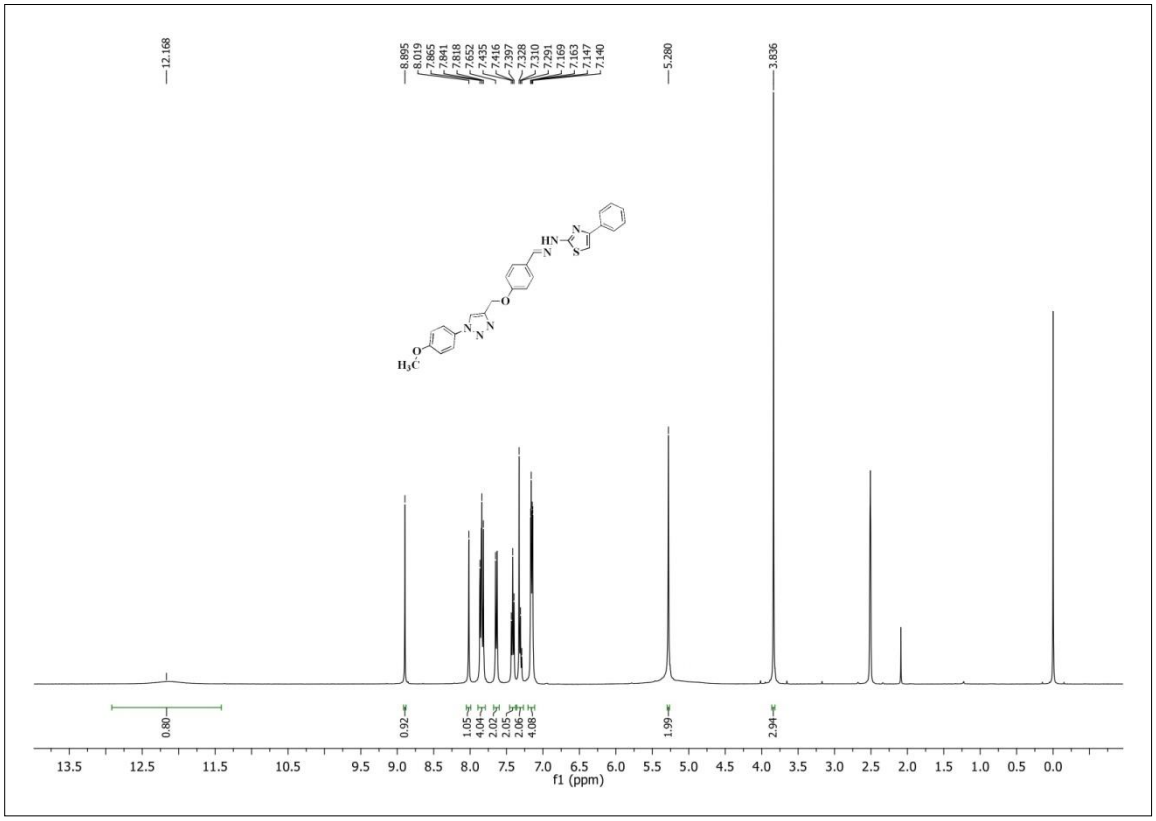
^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound 5



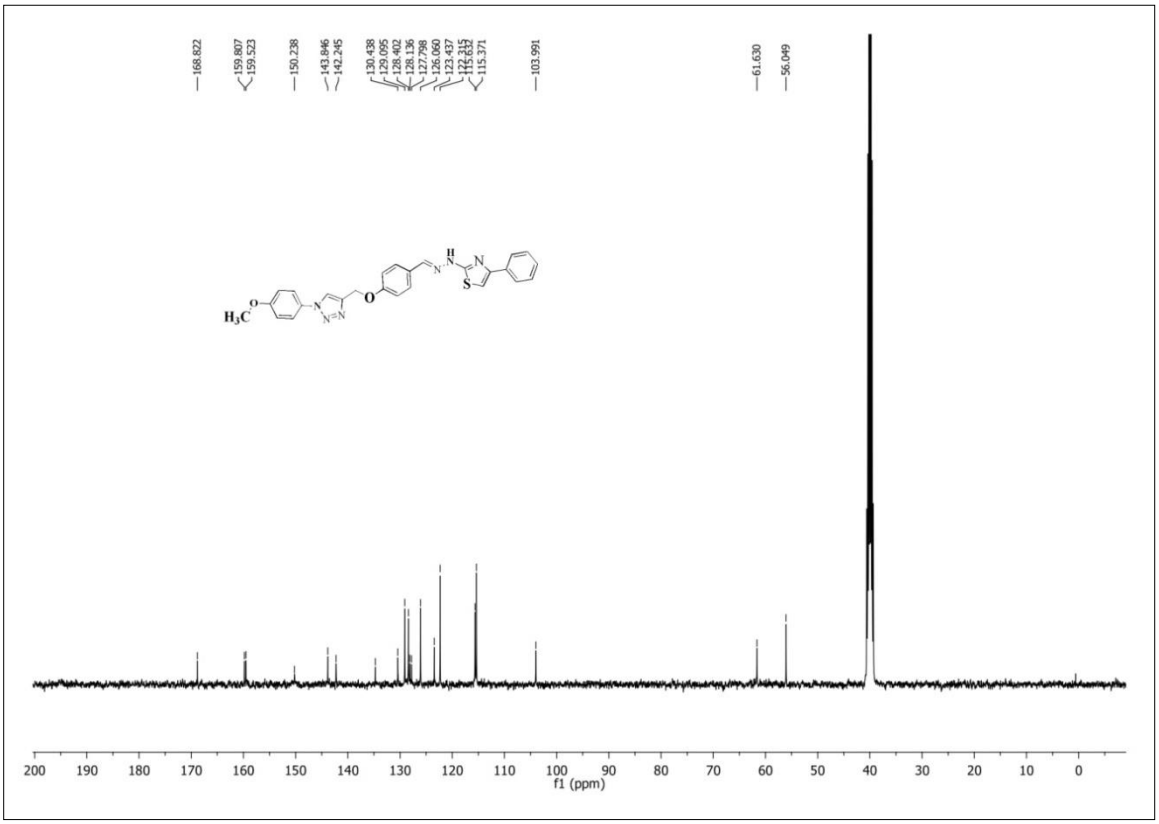
^{13}C NMR (100 MHz, $CDCl_3$) spectrum of compound 5



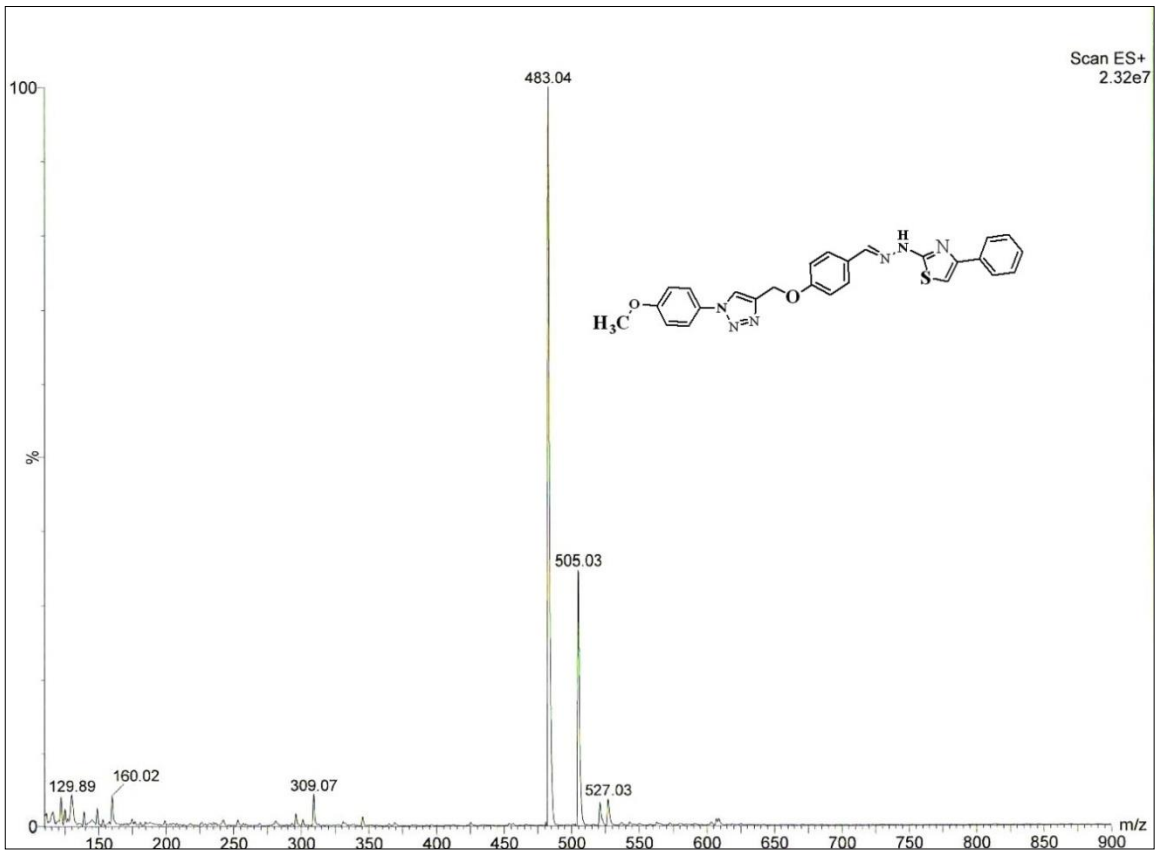
IR (KBr) spectrum of compound 8a



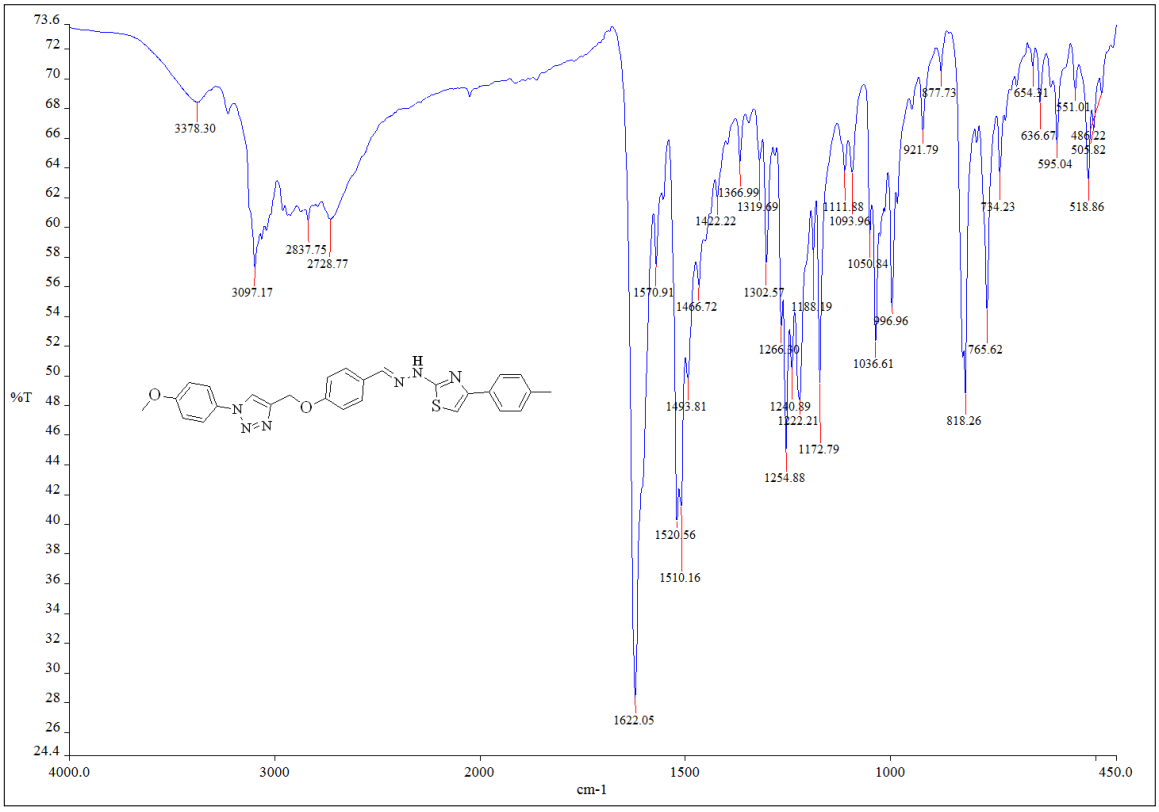
^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound 8a



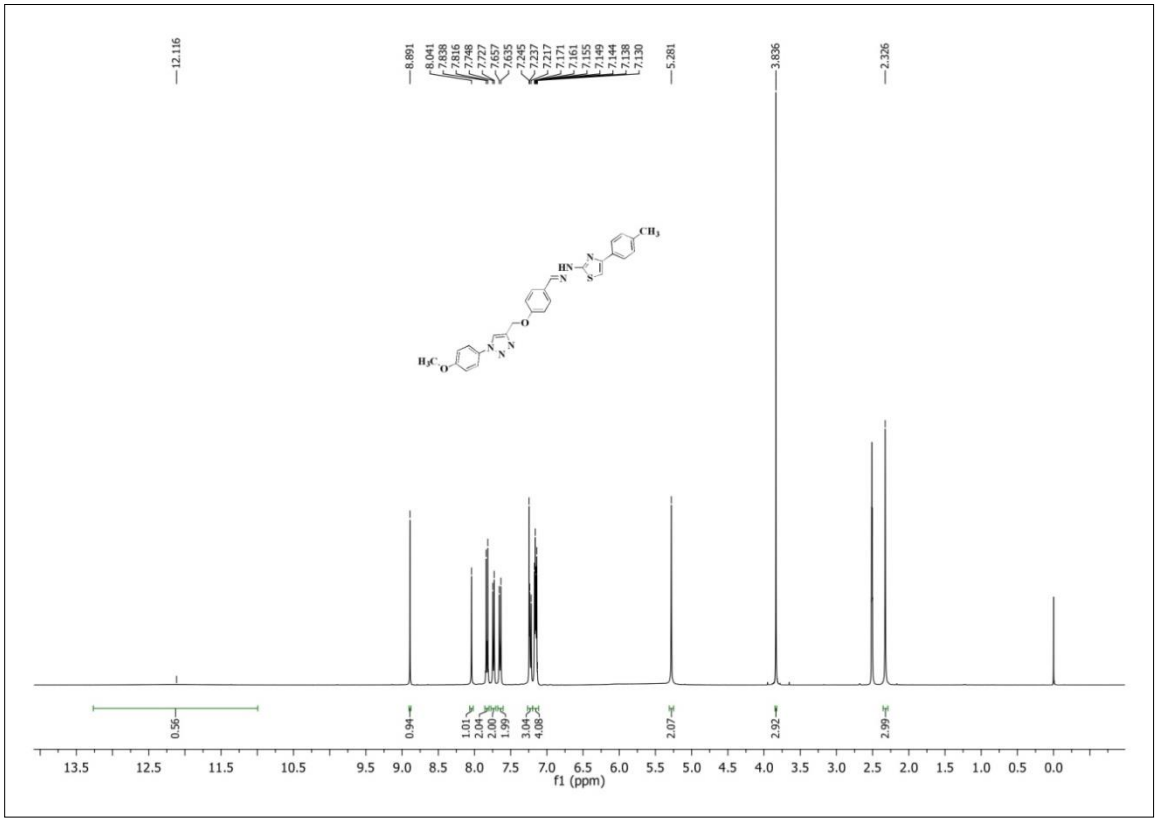
^{13}C NMR (100 MHz, DMSO-d_6) spectrum of compound 8a



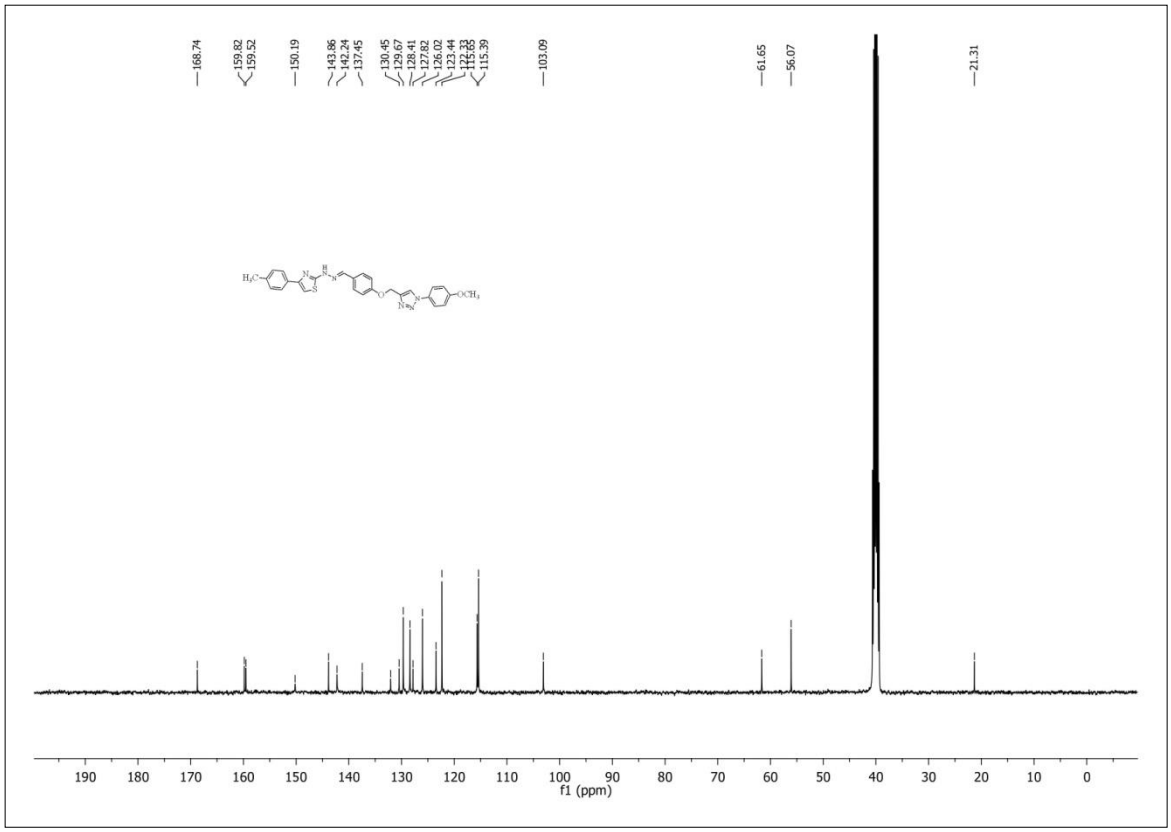
Mass spectrum of compound 8a (M.Wt: 582)



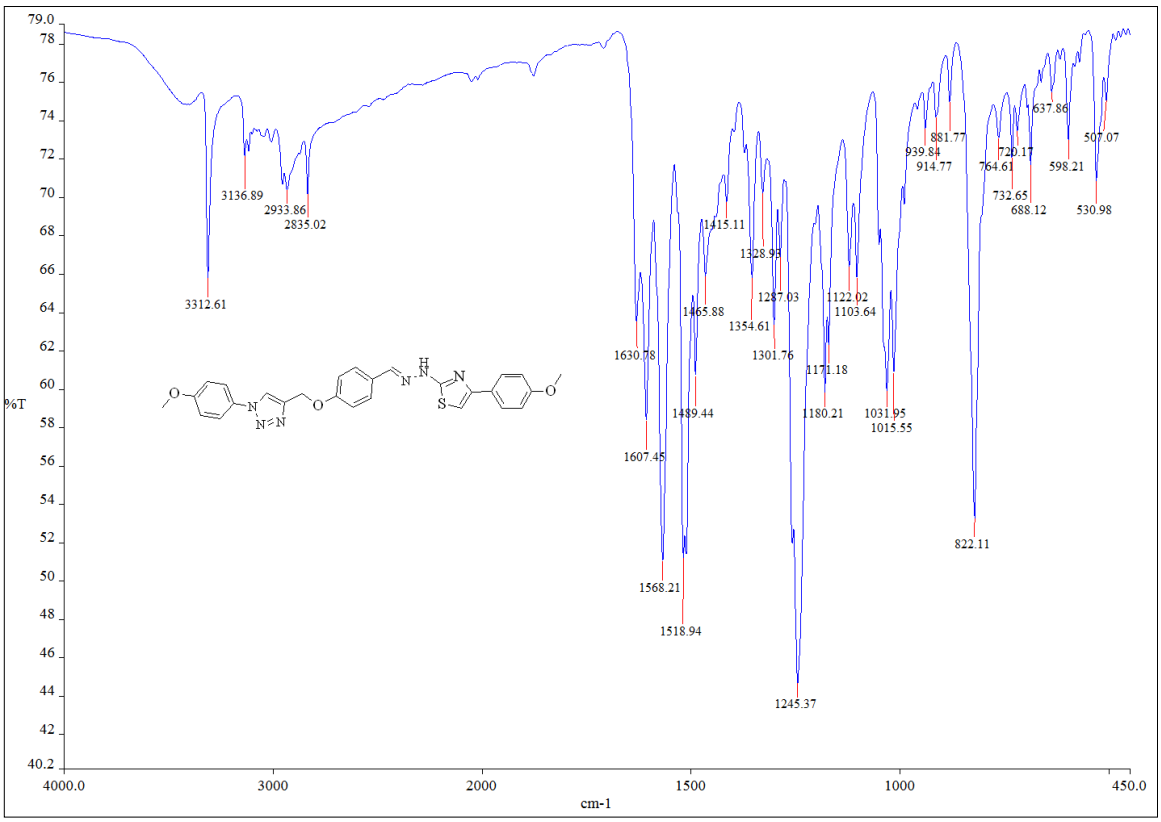
IR (KBr) spectrum of compound 8b



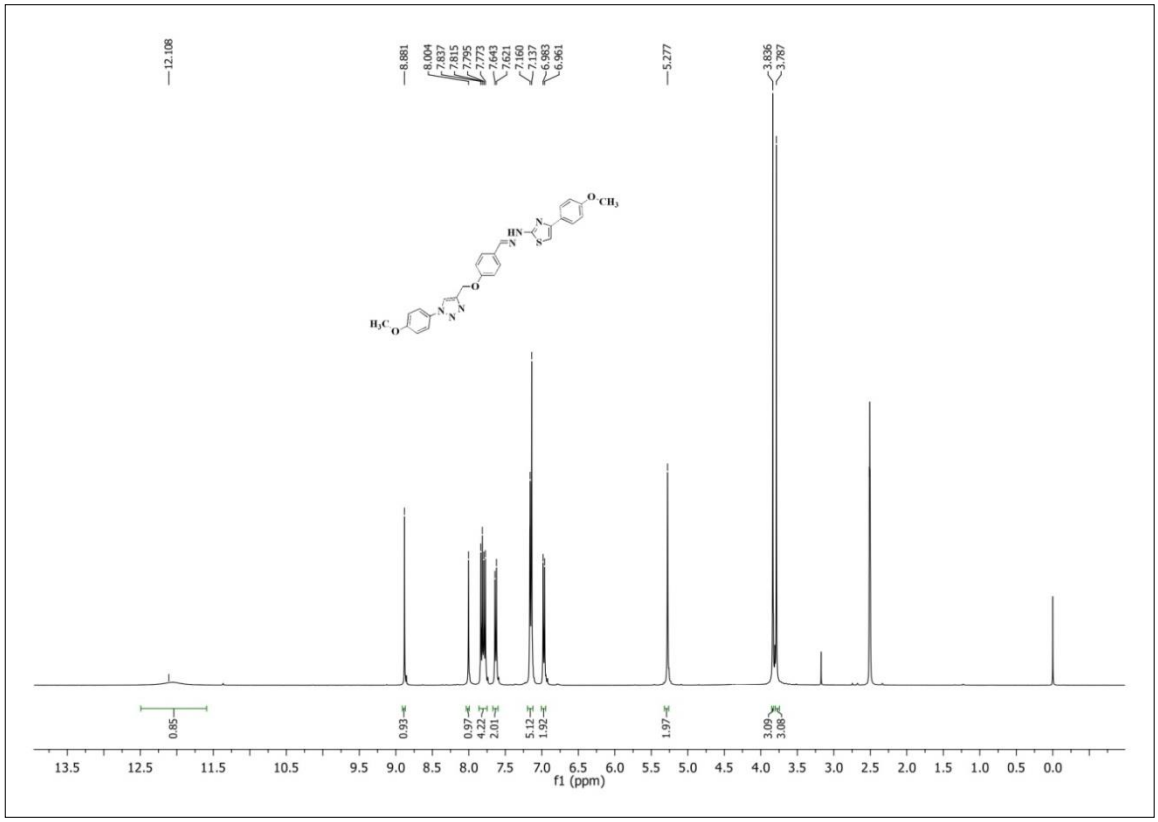
^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound 8b



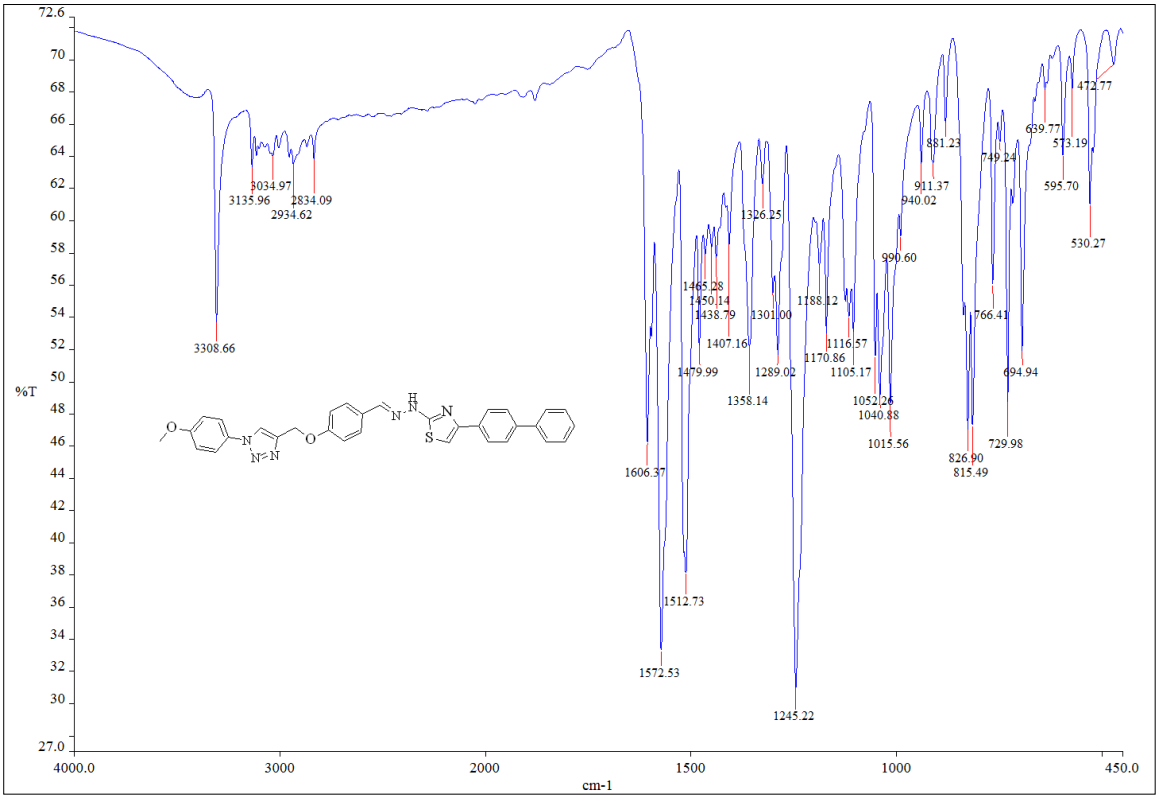
^{13}C NMR (100 MHz, DMSO-d_6) spectrum of compound 8b



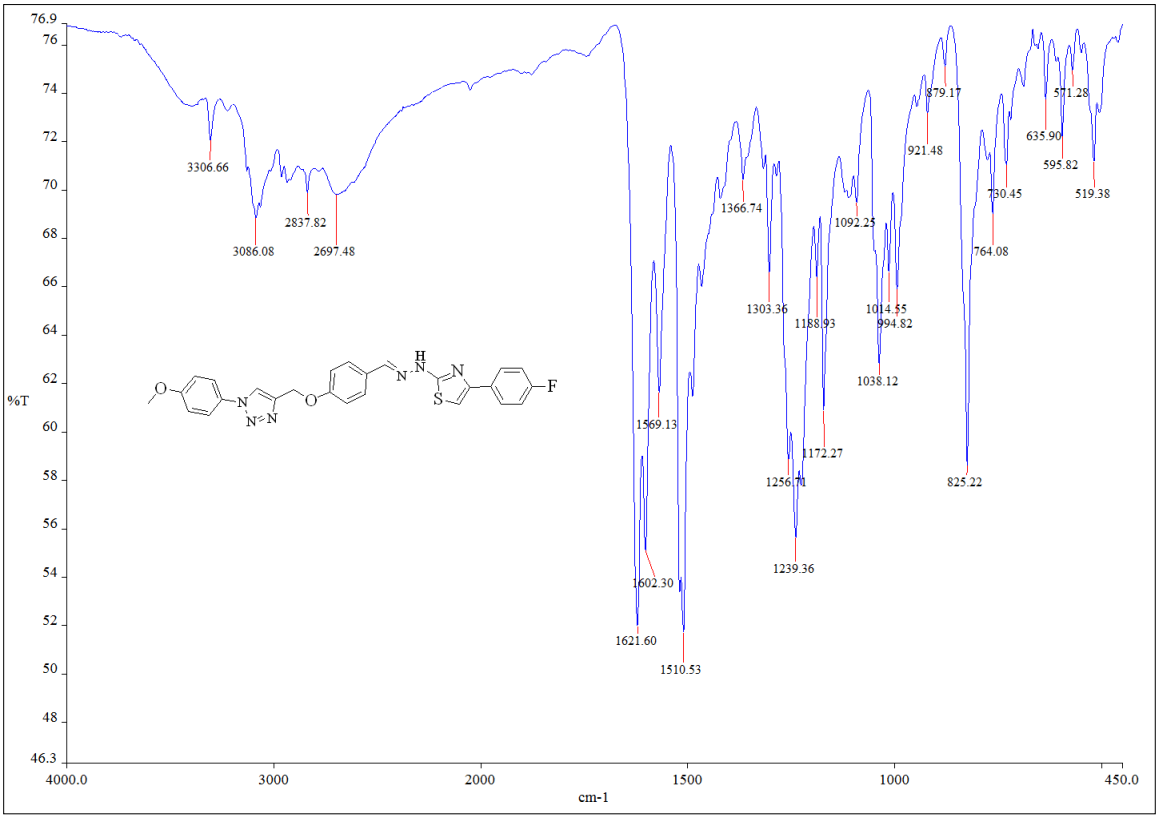
IR (KBr) spectrum of compound 8c



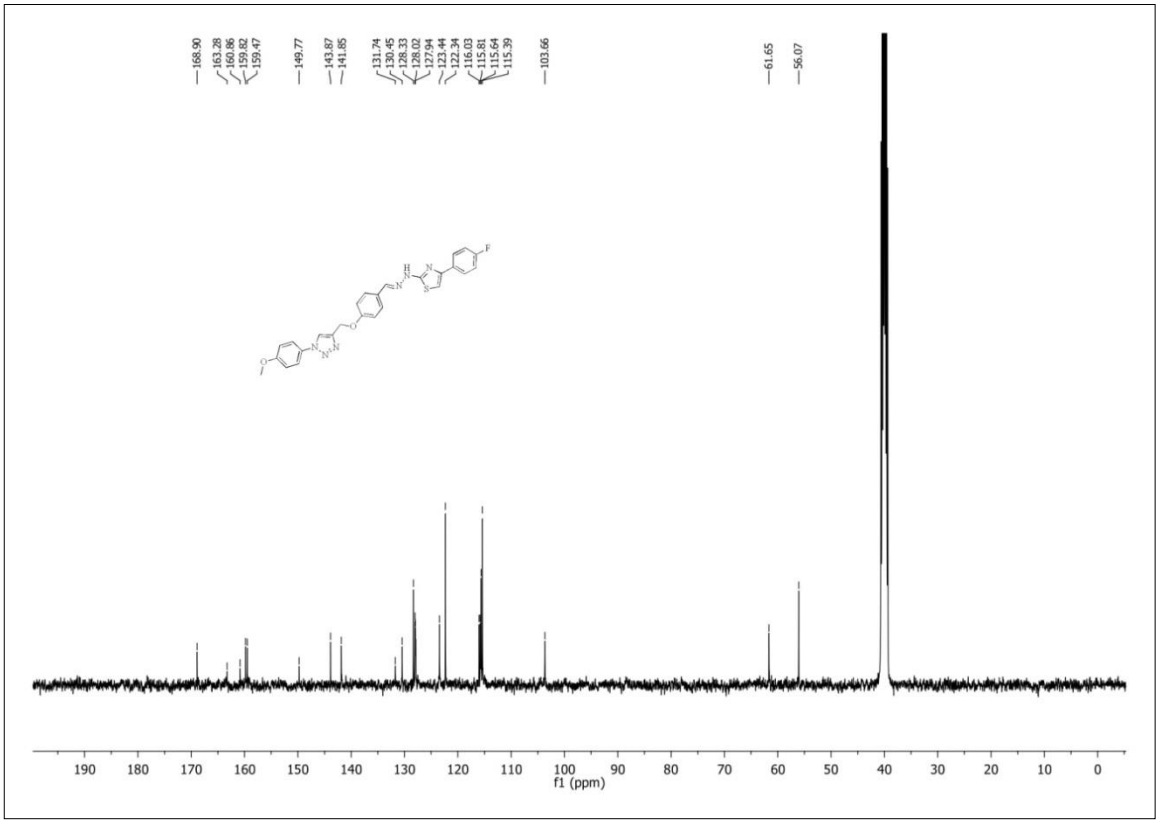
^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound 8c



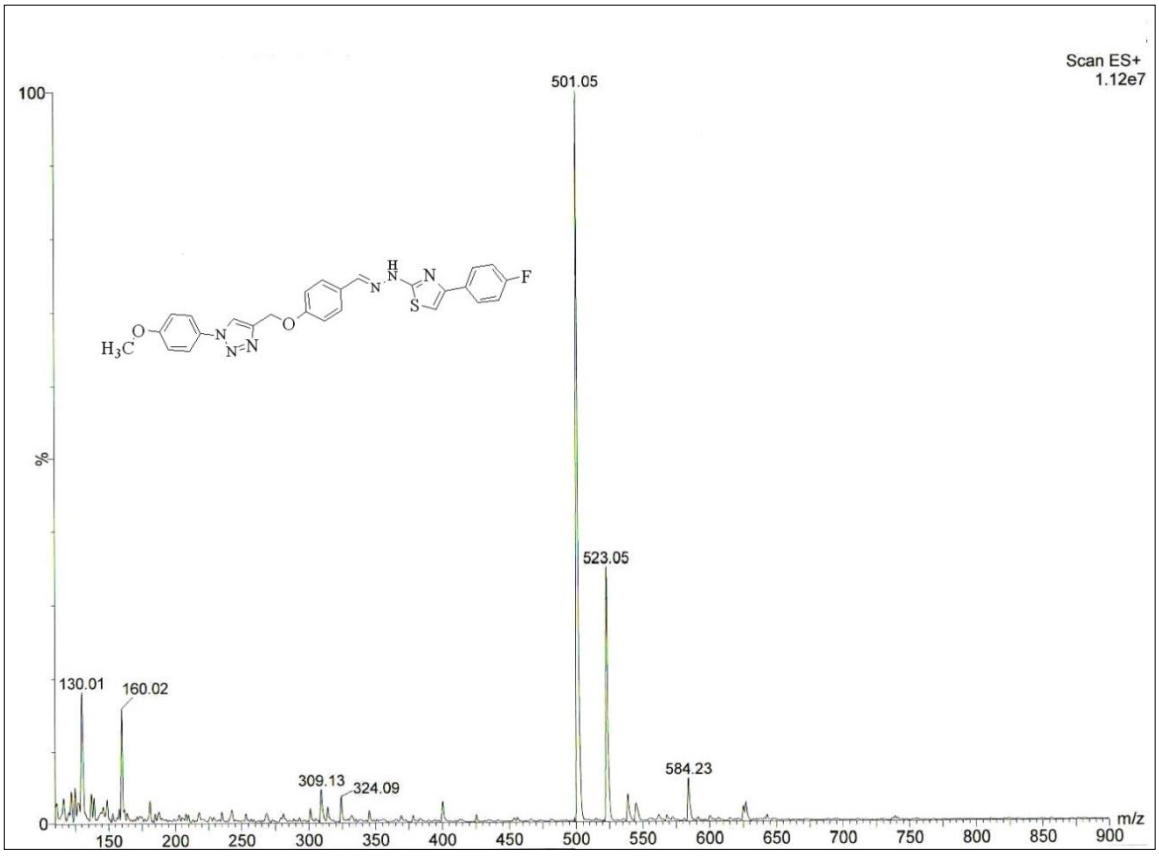
IR (KBr) spectrum of compound 8d



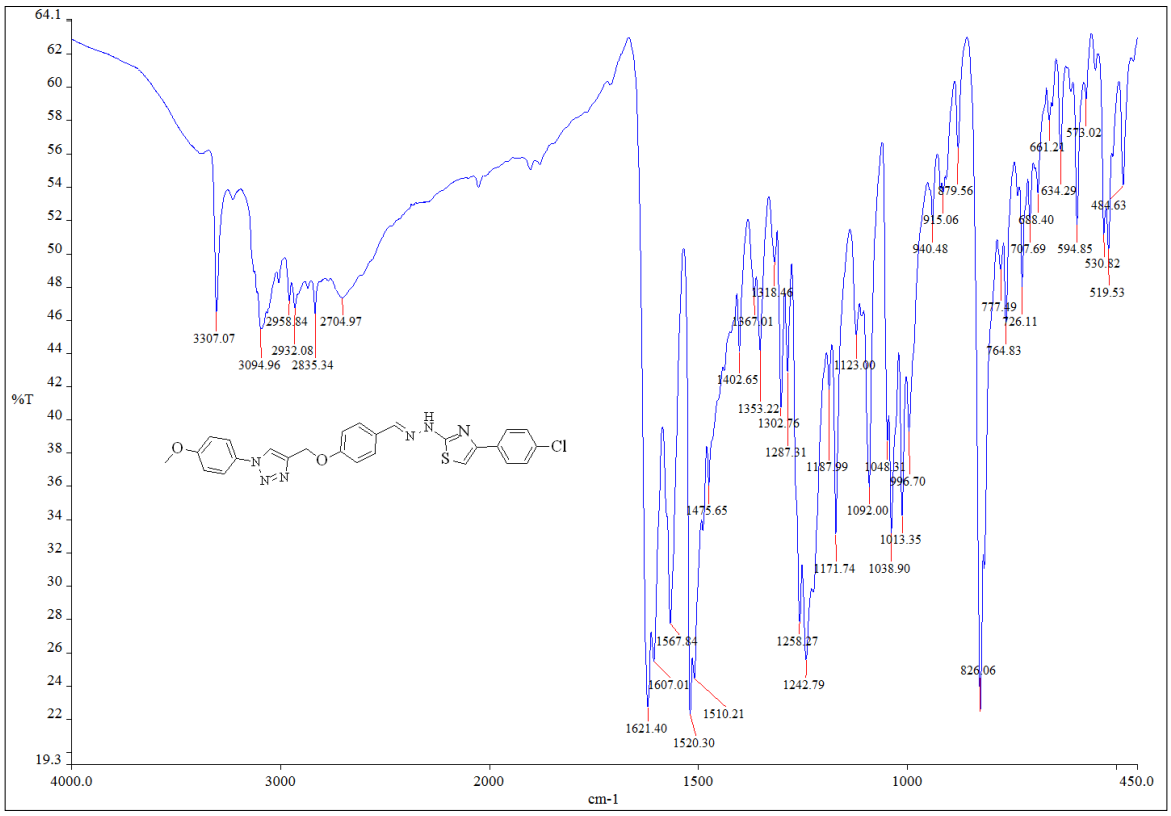
IR (KBr) spectrum of compound 8e



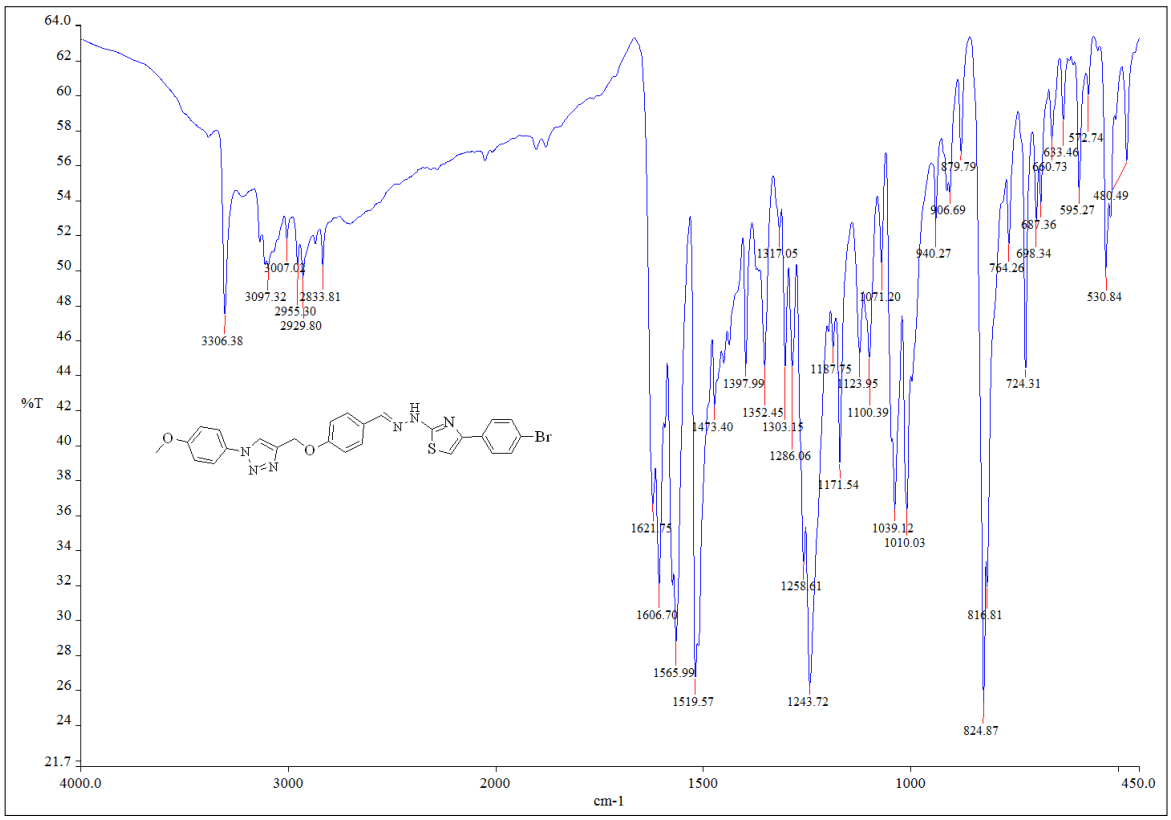
^{13}C NMR (100 MHz, DMSO-d_6) spectrum of compound 8e



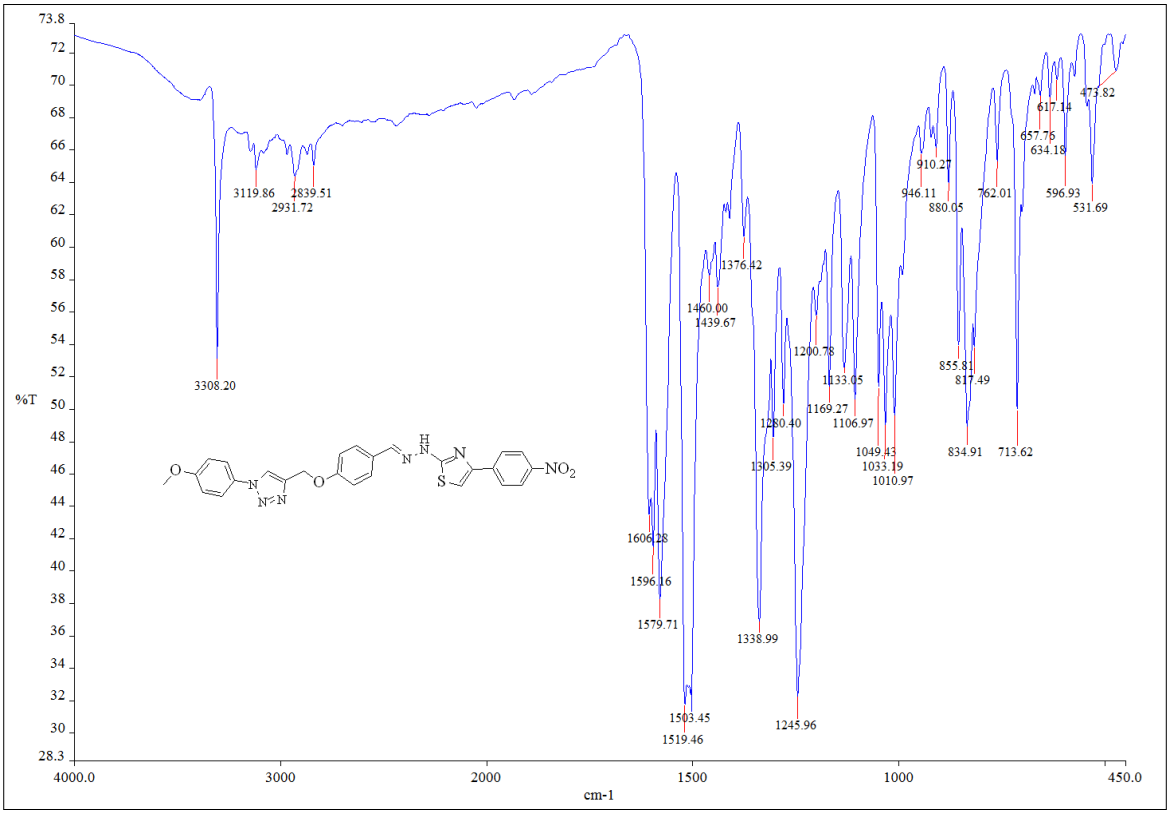
Mass spectrum of compound 8e (M.Wt: 500)



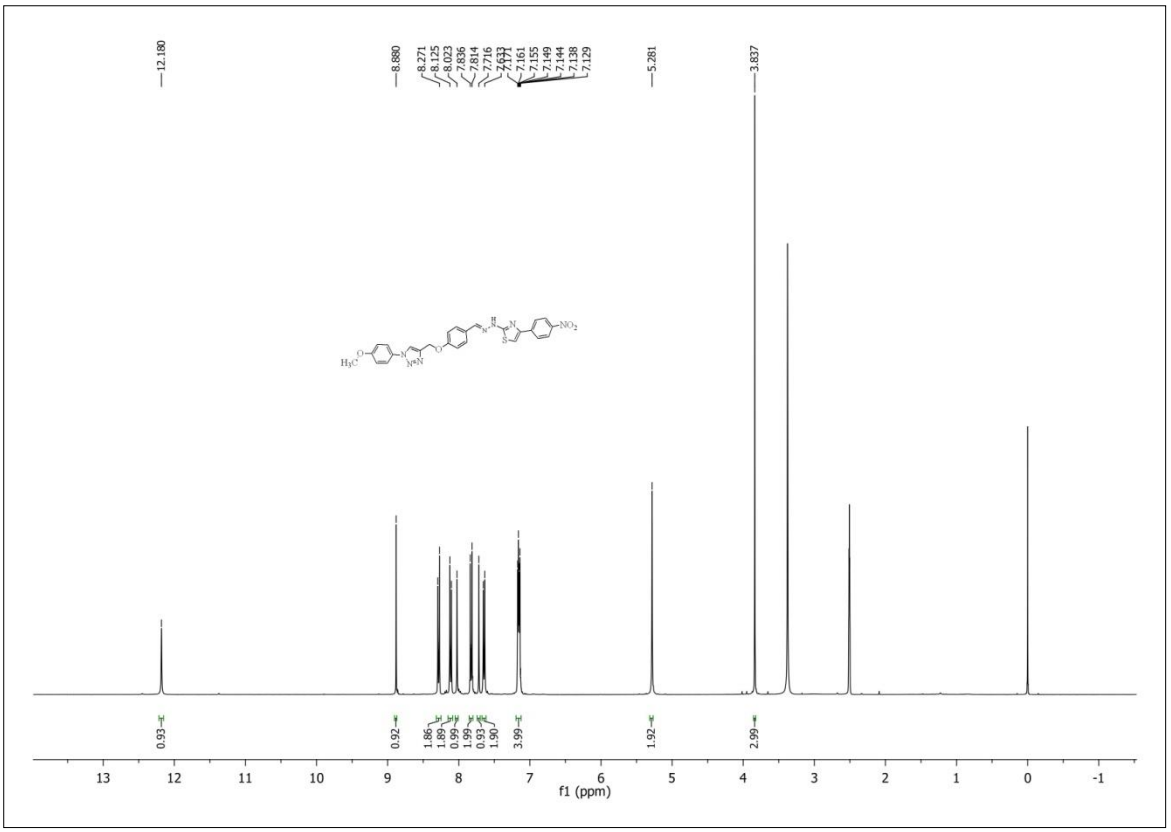
IR (KBr) spectrum of compound 8f



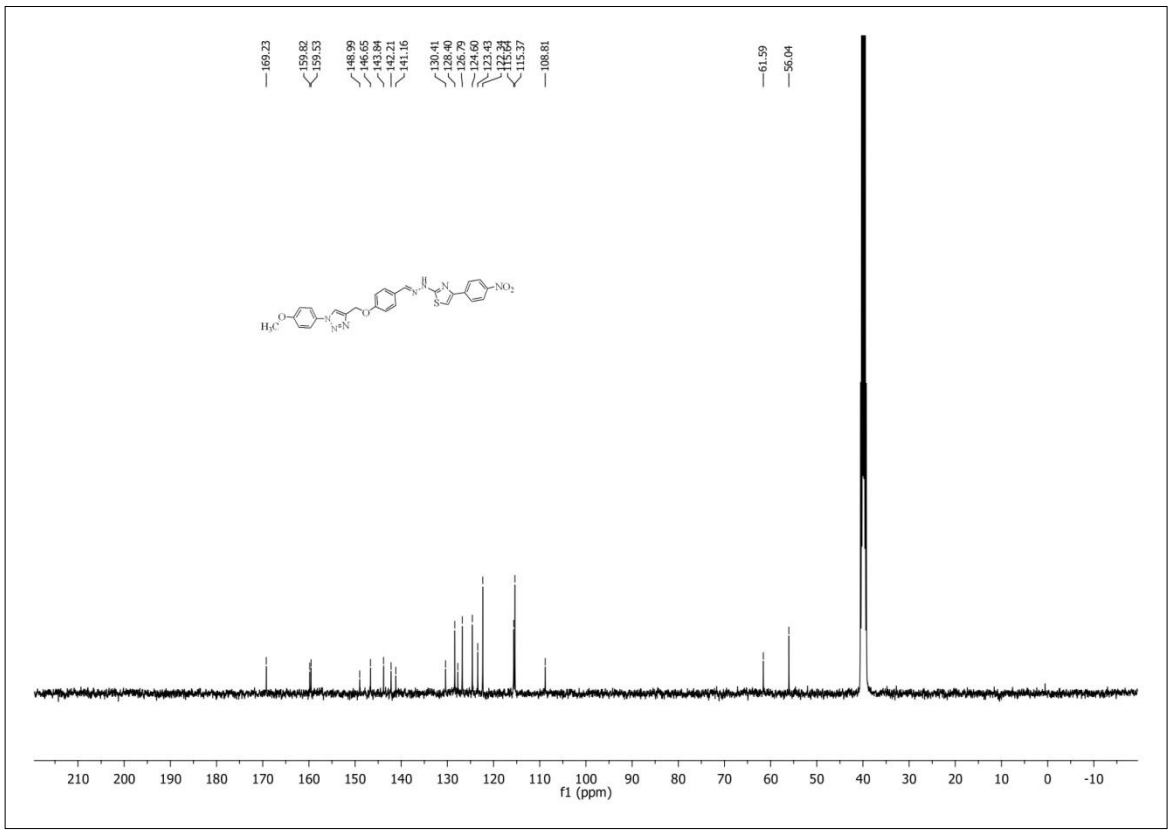
IR (KBr) spectrum of compound 8g



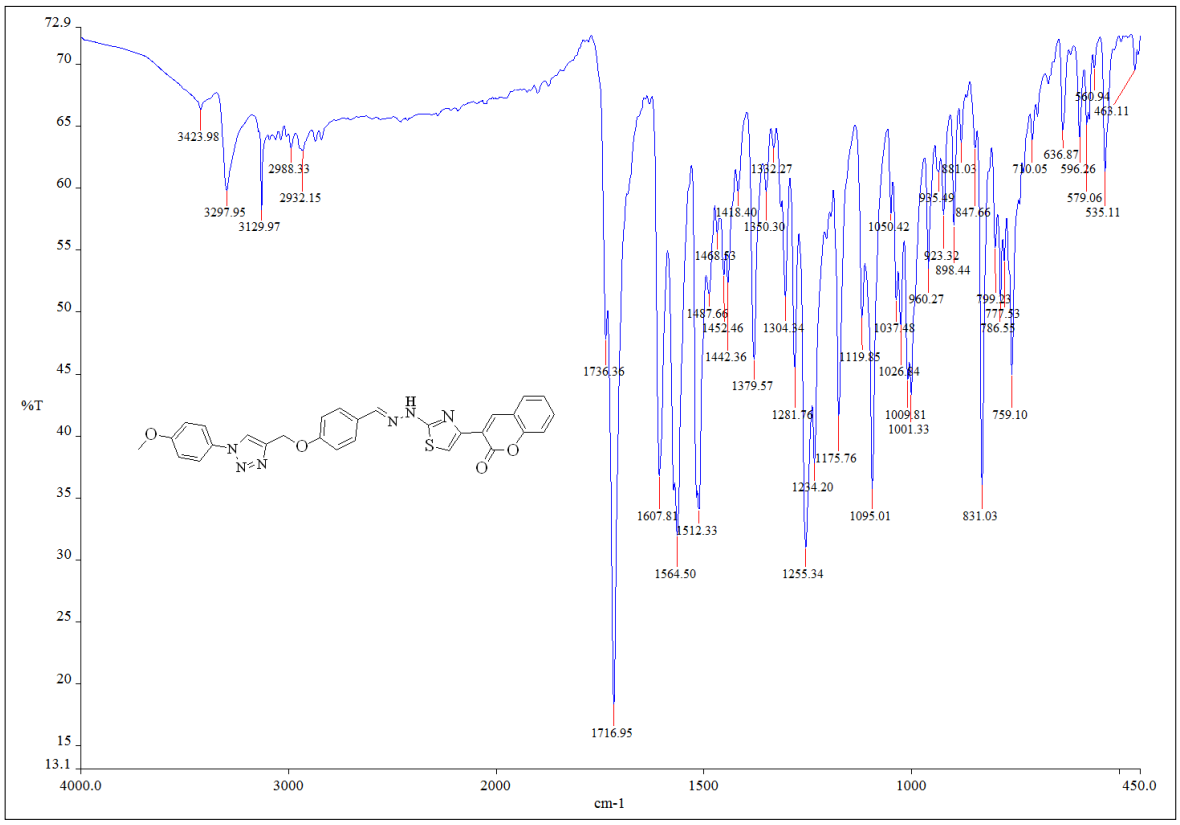
IR (KBr) spectrum of compound 8h



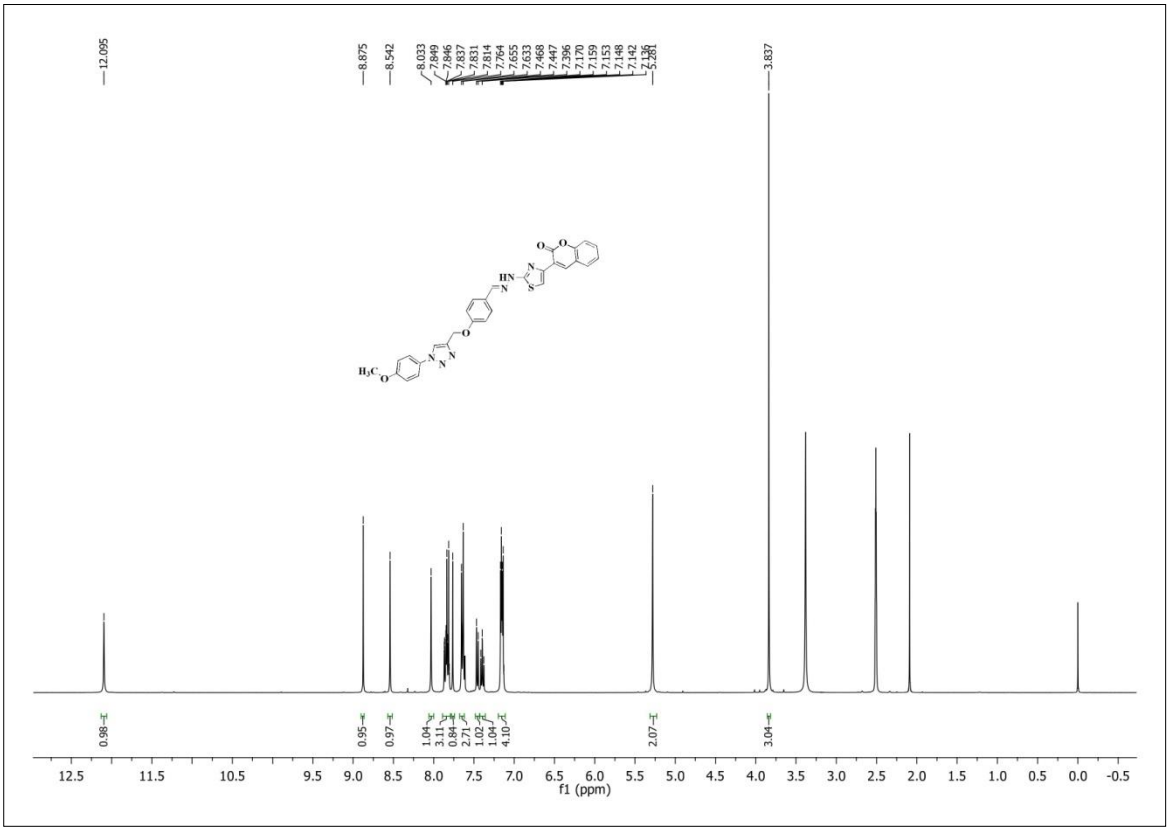
^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound 8h



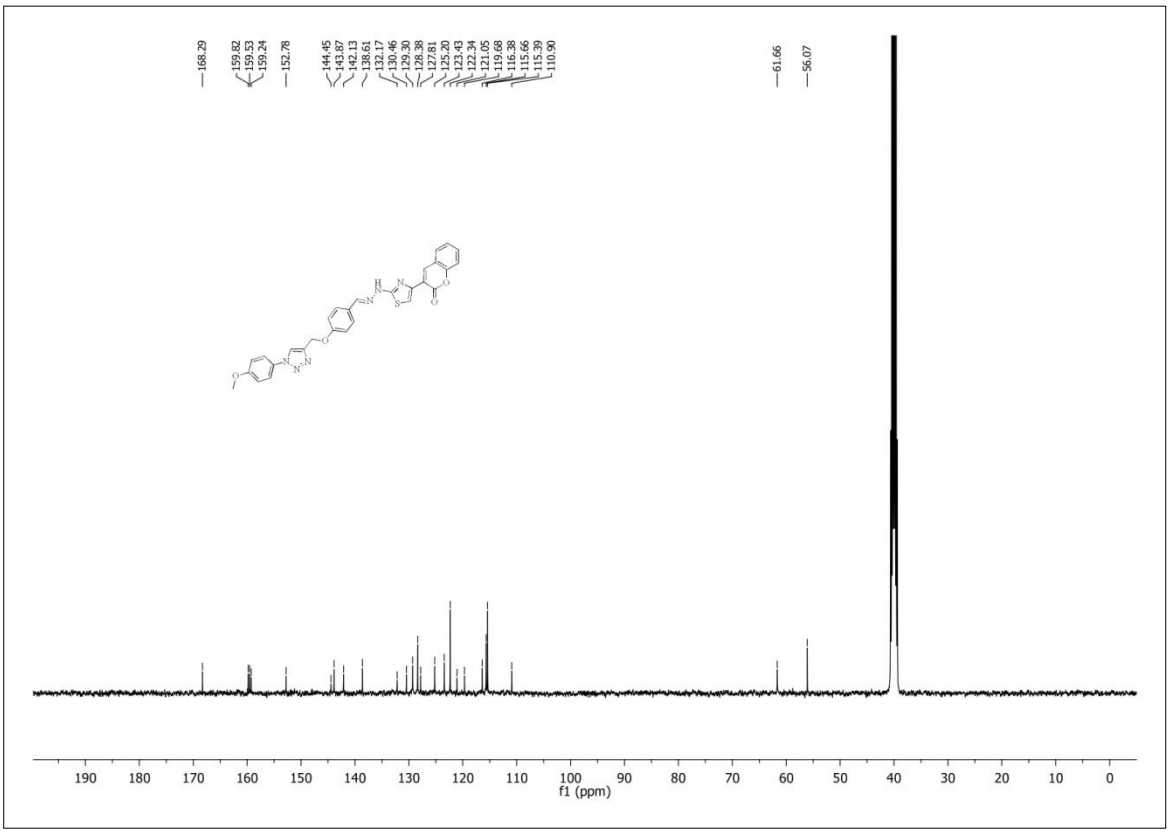
^{13}C NMR (100 MHz, DMSO-d_6) spectrum of compound 8h



IR (KBr) spectrum of compound 8i



^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound 8i



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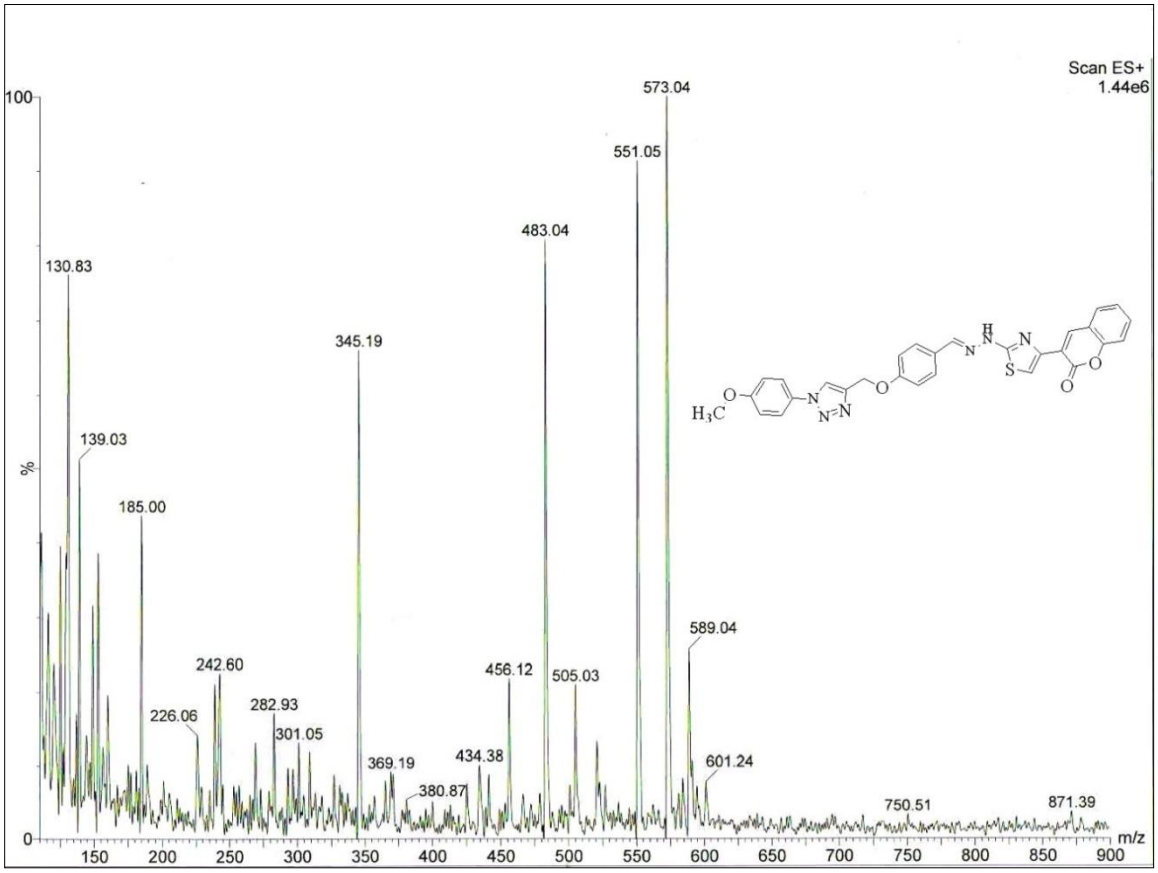
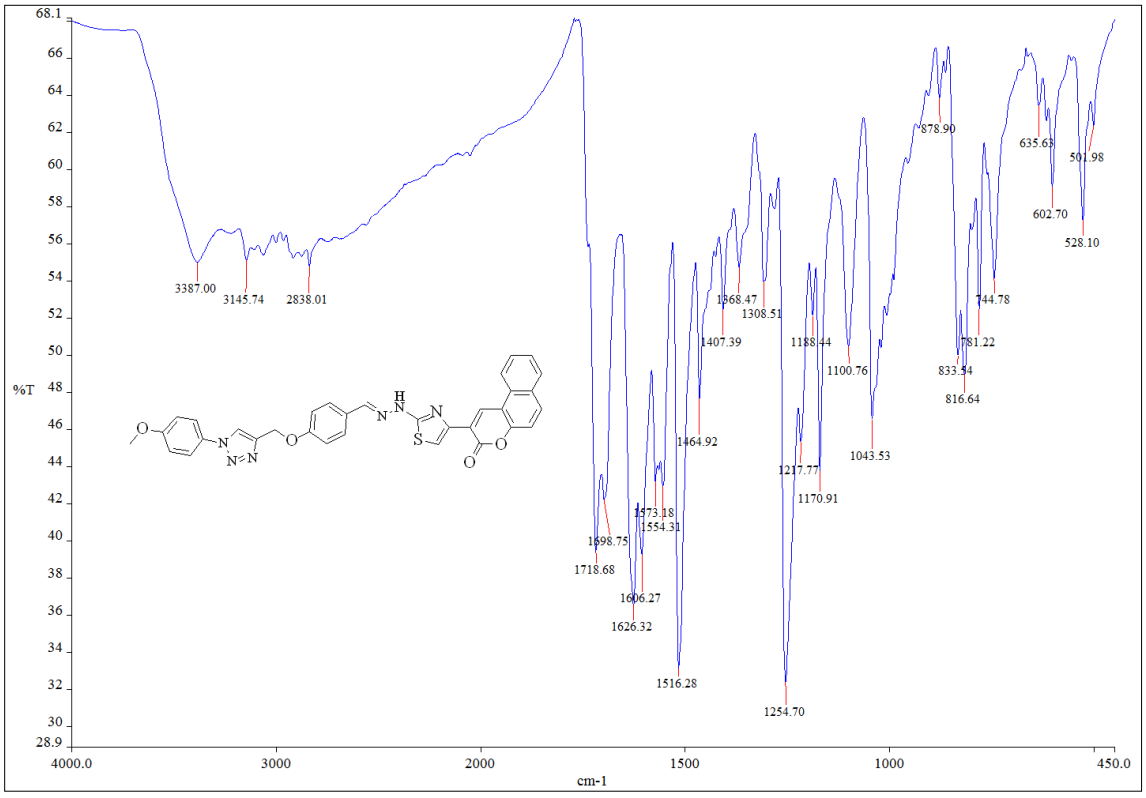
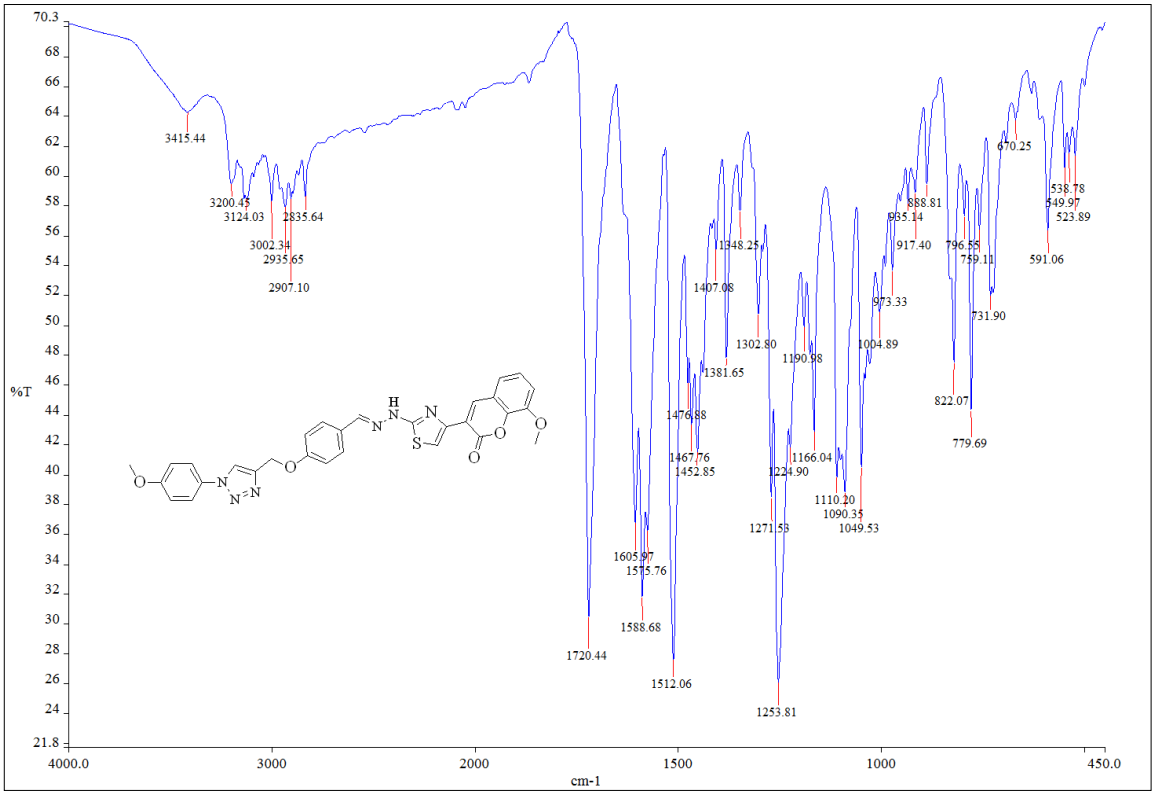


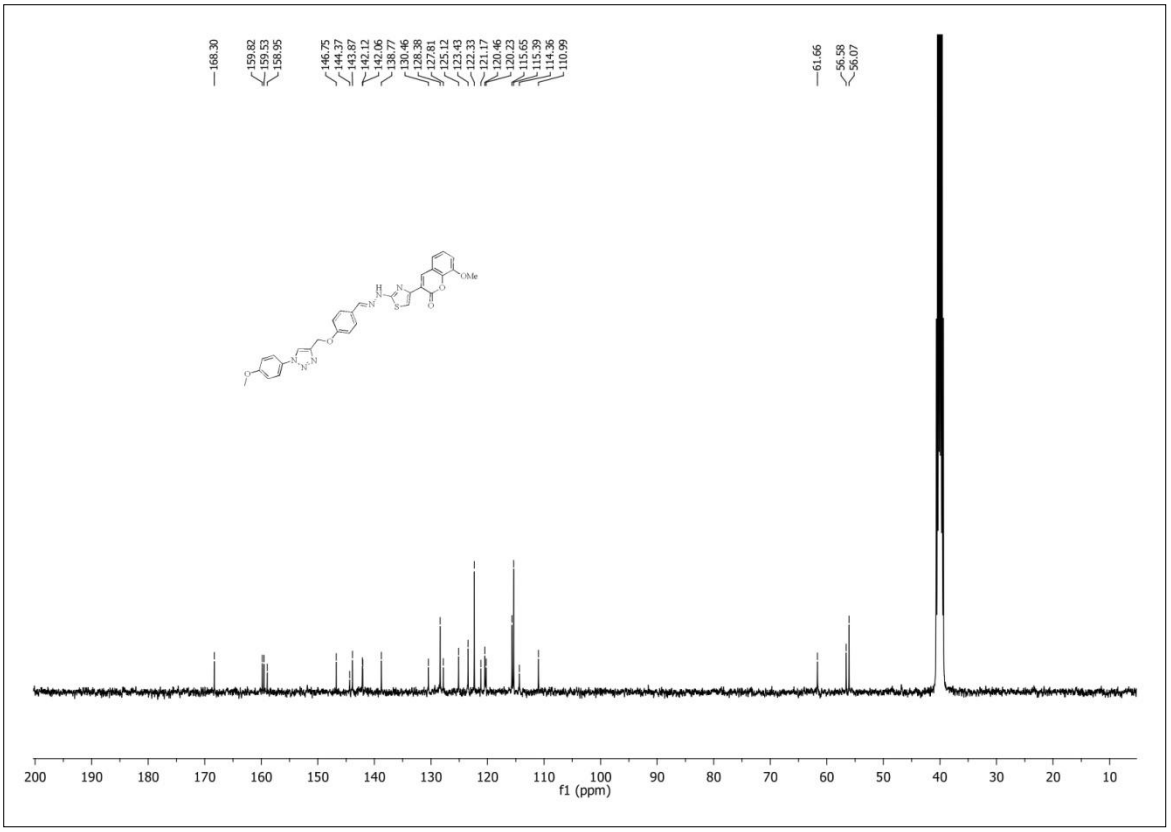
Mass spectrum of compound 8i (M.Wt: 550)



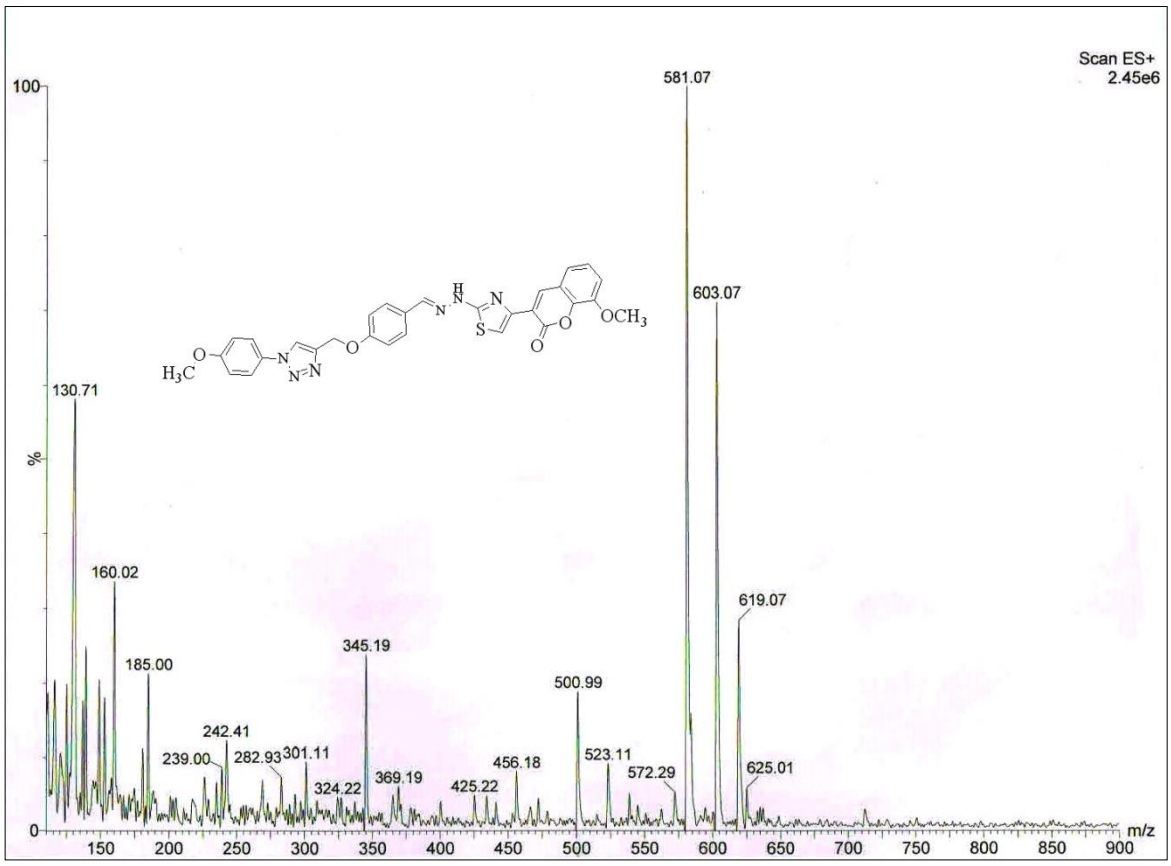
IR (KBr) spectrum of compound 8j



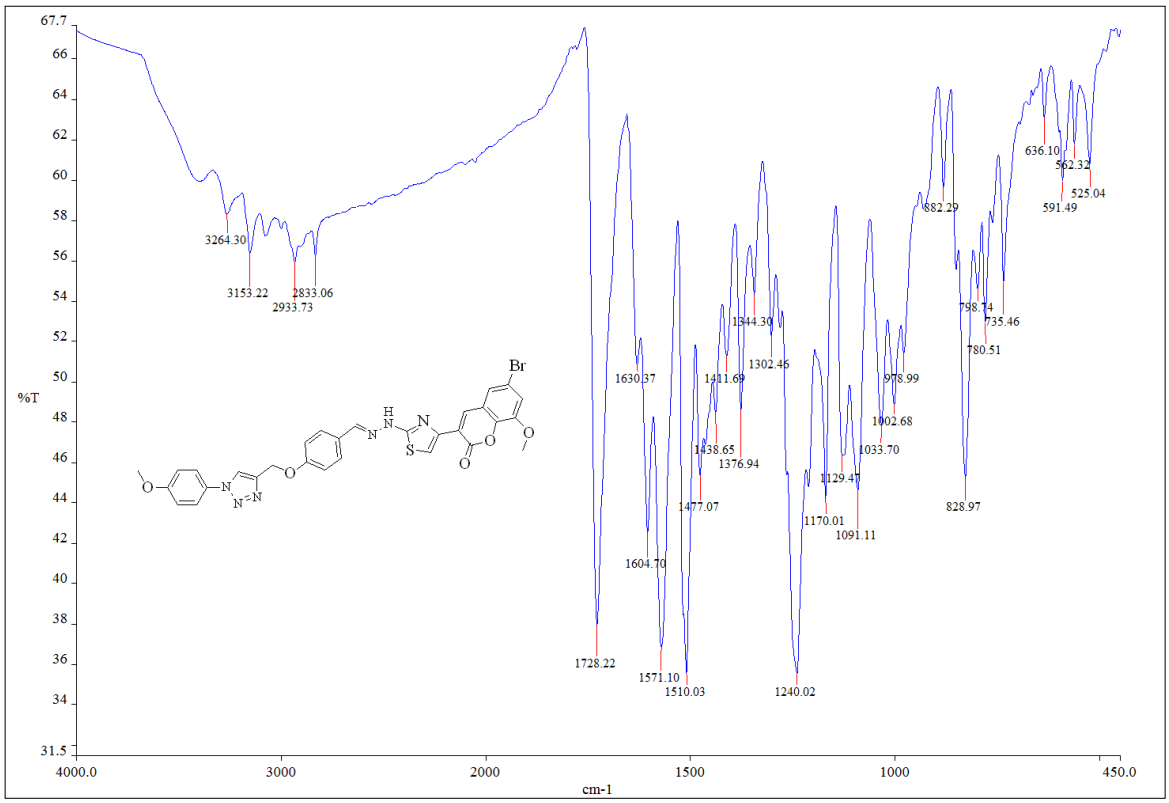
IR (KBr) spectrum of compound 8k



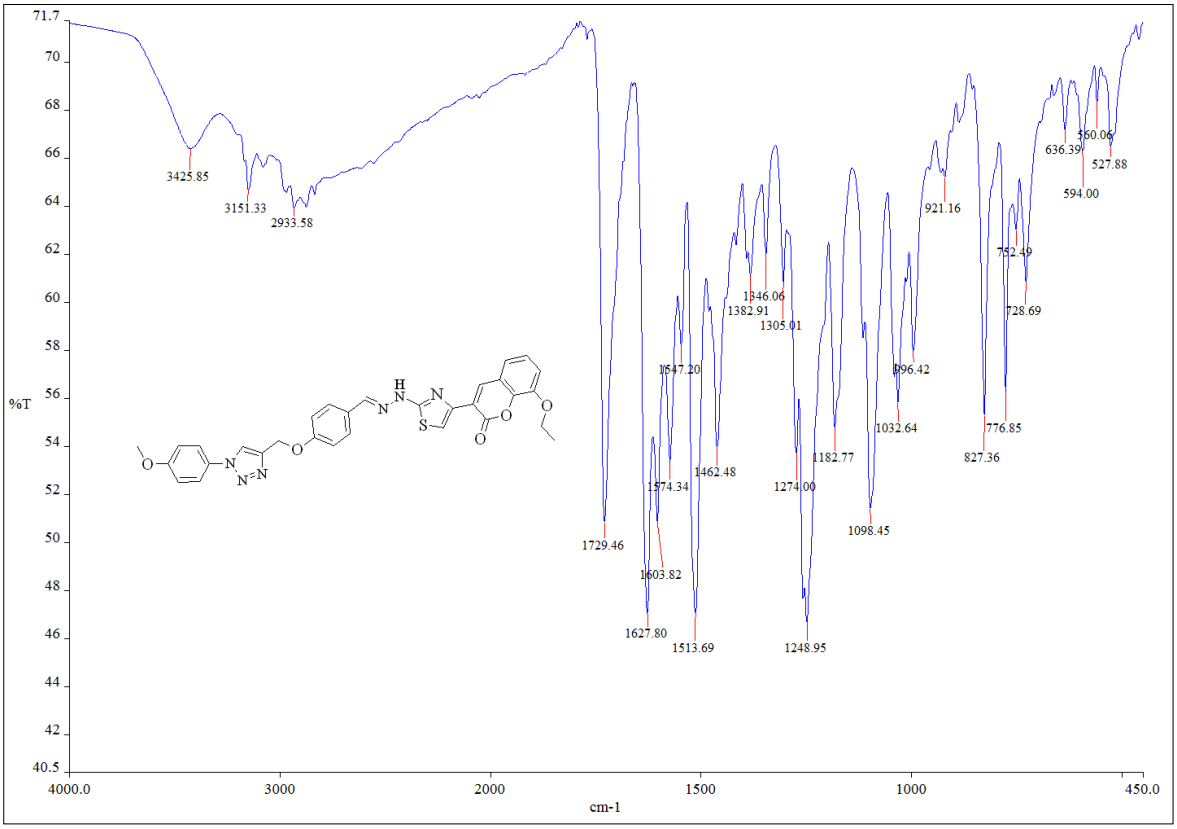
^{13}C NMR (100 MHz, DMSO-d_6) spectrum of compound 8k



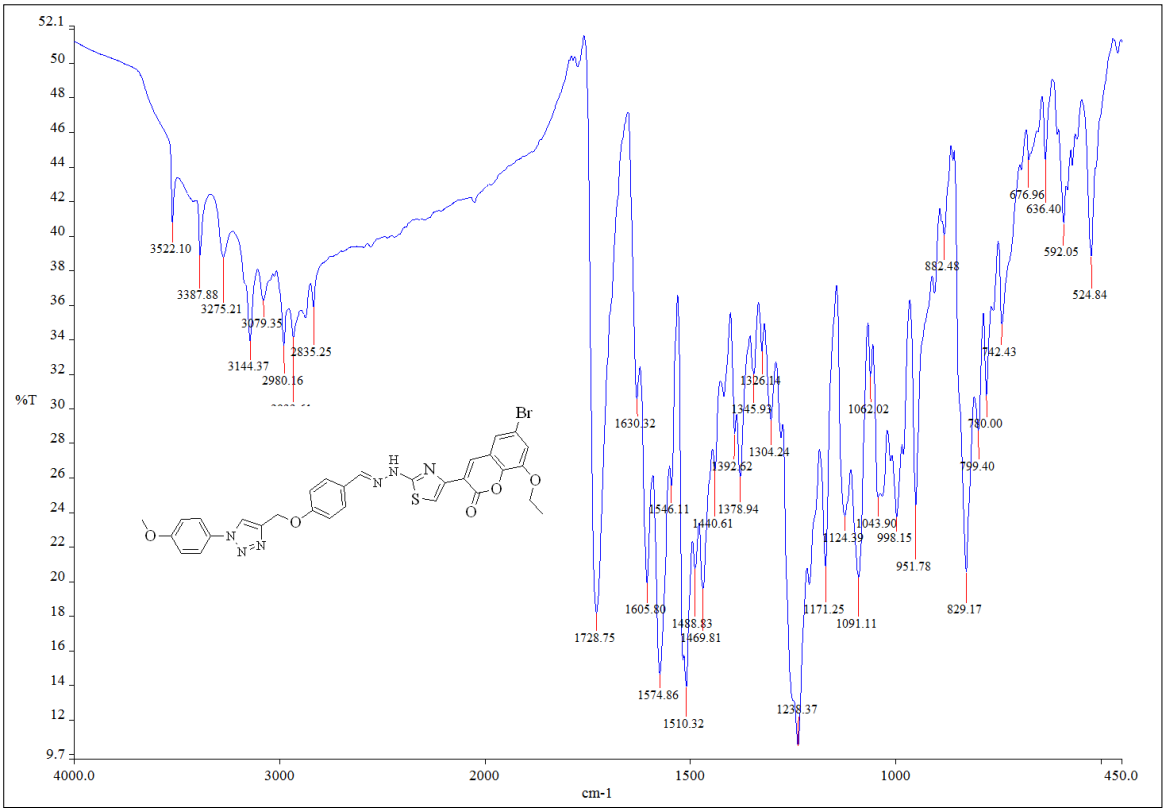
Mass spectrum of compound 8k (M.Wt: 580)



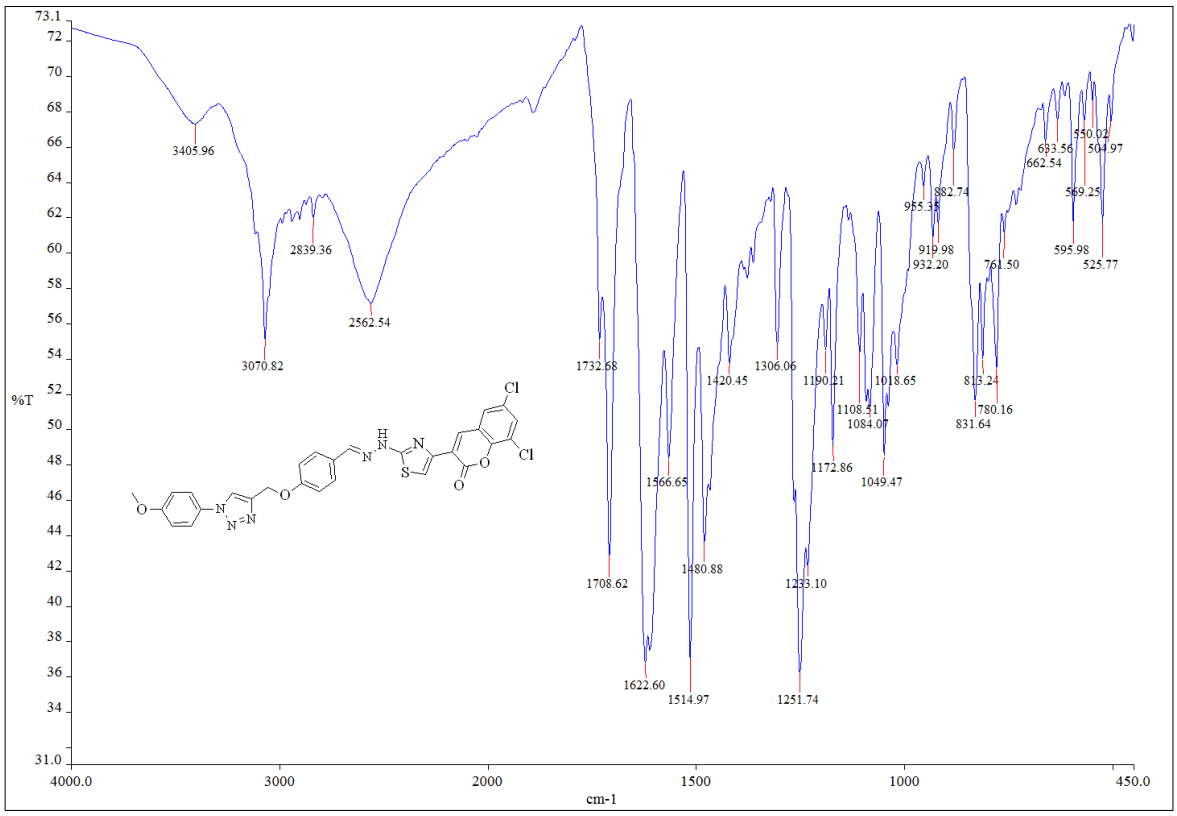
IR (KBr) spectrum of compound 8l



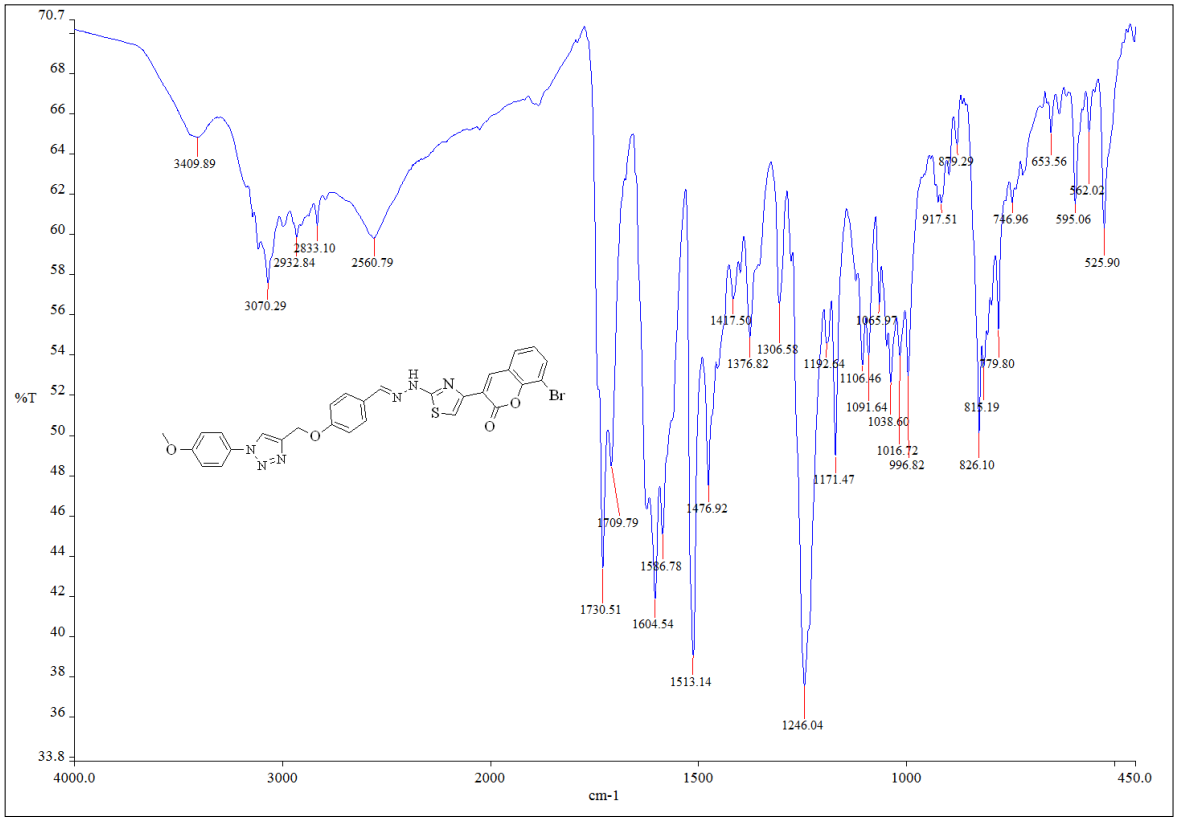
IR (KBr) spectrum of compound 8m



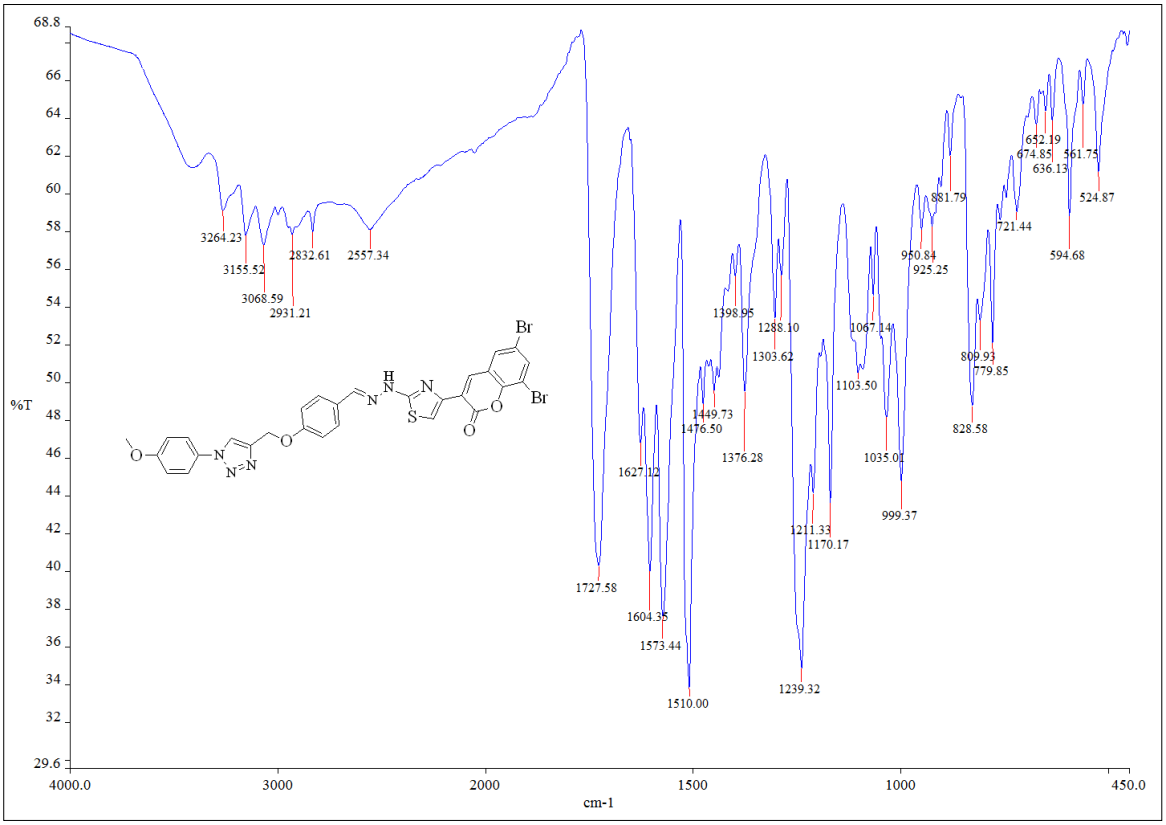
IR (KBr) spectrum of compound 8n



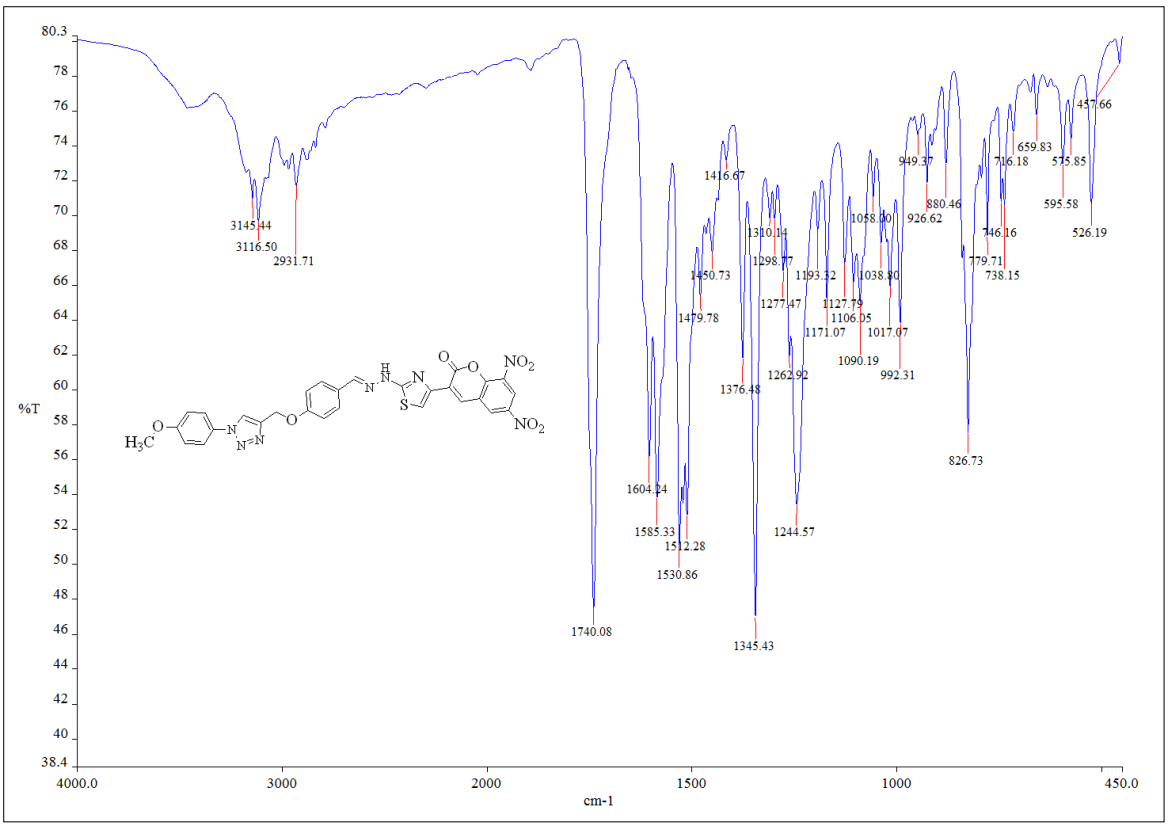
IR (KBr) spectrum of compound 8o



IR (KBr) spectrum of compound 8p



IR (KBr) spectrum of compound 8q



IR (KBr) spectrum of compound 8r

CHAPTER-III (SECTION-B)

SYNTHESIS OF NEW MORPHOLINE BASED HYDRAZINYL 1,3,4-THIADIAZIN-1,2,3-TRIAZOLE HYBRIDS

INTRODUCTION

In the recent years 1,2,3-triazole attained much attention from the researchers due to their valuable medicinal and biological properties, such as anti-bacterial,[1] anti-cancer,[2] anti-HIV,[3] anti-tubercular,[4] anti-neoplastic, anti-AchE,[5] antifungal,[6] antiallergic,[7] antimalarial,[8] α-glycosidase inhibition,[9] antimycobacterial,[10] anticoccidiostats,[11] anticonvulsant[12] activities. On the other hand, 1,3,4-thiadiazine and its derivatives also occupied a prominent place as a lead pharmacophores in the designing of new drug candidates because of their variety of biological properties[13] such as antimicrobial,[14] antifungal, anti-HIV,[15] cardiotonic and hypertensive activities.[16] Some of the drugs bearing triazole and 1,3,4-thiadiazine frame work are given below (**Fig. 1**).

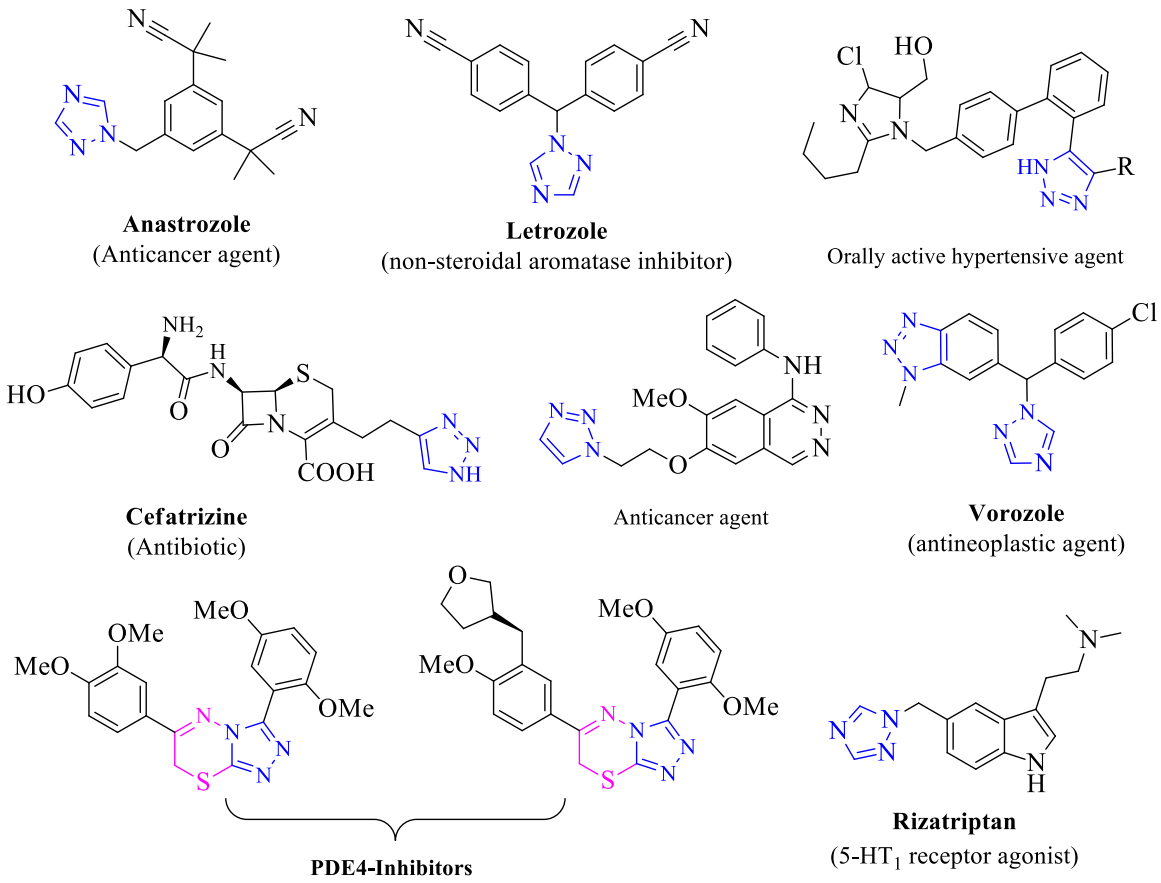
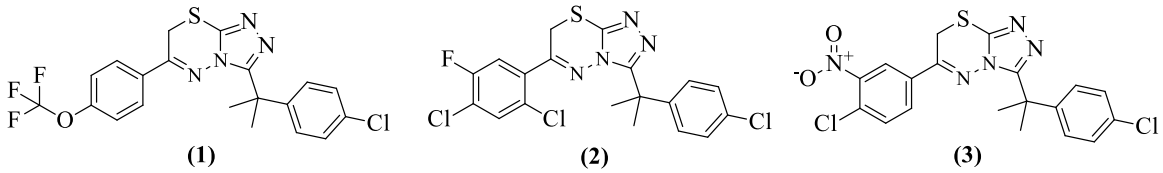


Fig. 1

Pushpan Puthiyapurayil and co-workers[17] reported the synthesis, spectral characterization and biological evaluation of a novel series of 6-arylsubstituted-3-[2-(4-substitutedphenyl)propan-2-yl]-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines. Among the tested series of compounds the derivative 1 displayed excellent anticancer activity against

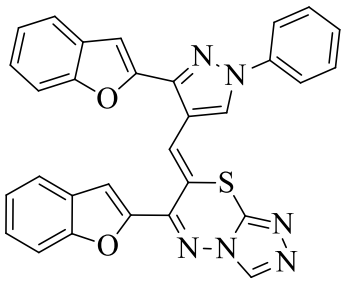
breast cancer cell-line with IC_{50} value 10.54μM. And the derivatives, 2 and 3 were found to be potent anthelmintic activity.



(1) **(2)** **(3)**

Fig. 2

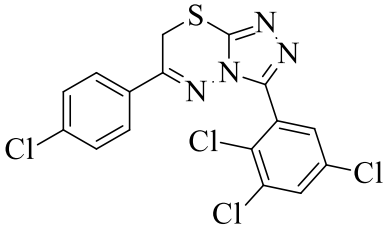
M. F. El Shehry and co-workers[18] reported the Synthesis and molluscicidal evaluation of some new pyrazole, isoxazole, pyridine, pyrimidine, 1,4-thiazine and 1,3,4-thiadiazine derivatives incorporating benzofuran moiety. From the *in vitro* results, it was identified that the compound **4** has promising molluscicidal activity.



(4)

Fig. 3

Prakash Karegoudar *et al.*[19] described the synthesis, antimicrobial and anti-inflammatory activities of some 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazoles and 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines bearing trichlorophenyl moiety.

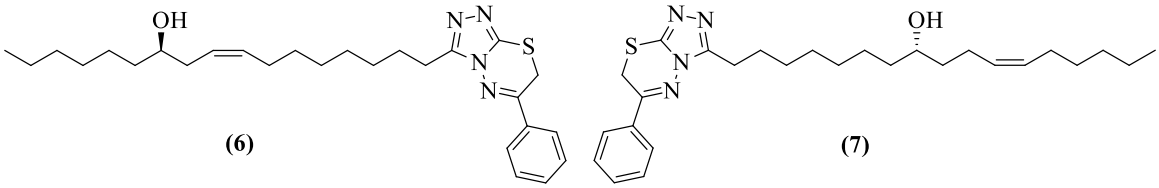


(5)

Fig. 4

Aiman Ahmad *et al.*[20] reported the Synthesis and anticancer activity of long chain substituted 1,3,4-oxadiazol-2-thione, 1,2,4-triazol-3-thione and 1,2,4-triazolo [3,4-b]-1,3,4-thiadiazine derivatives. Among the tested series of compounds, **6** and **7** were

identified as potential cytotoxic agents with IC_{50} values below 08.83 ± 1.4 µM against all the tested cell-lines.

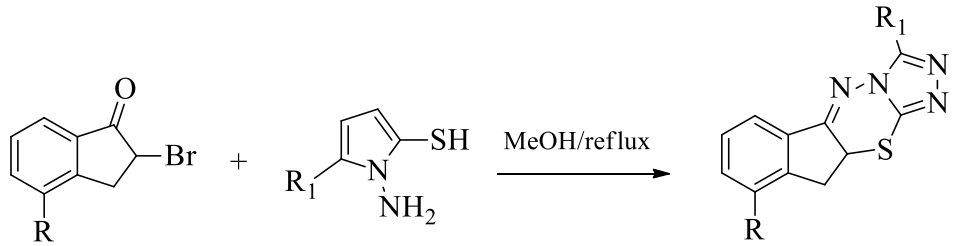


(6) **(7)**

Fig. 5

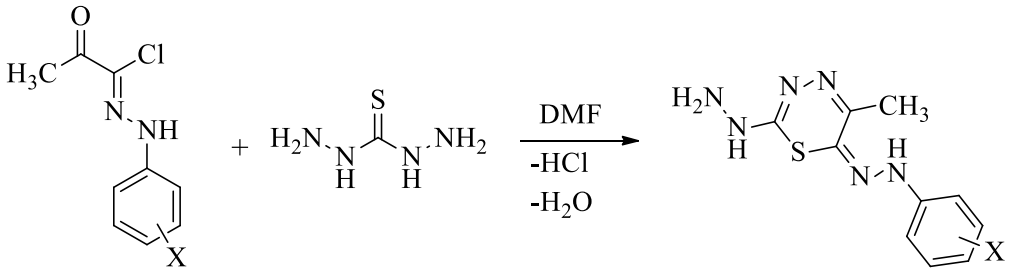
Various important approaches for the synthesis of 1,2,3-triazole-1,3,4-thiadiazine hybdrids.

O. Prakash *et al.*[21] described the synthesis and biological evaluation of dihydroindeno and indeno [1,2-*e*] [1,2,4]triazolo [3,4-*b*] [1,3,4]thiadiazines as antimicrobial agents.



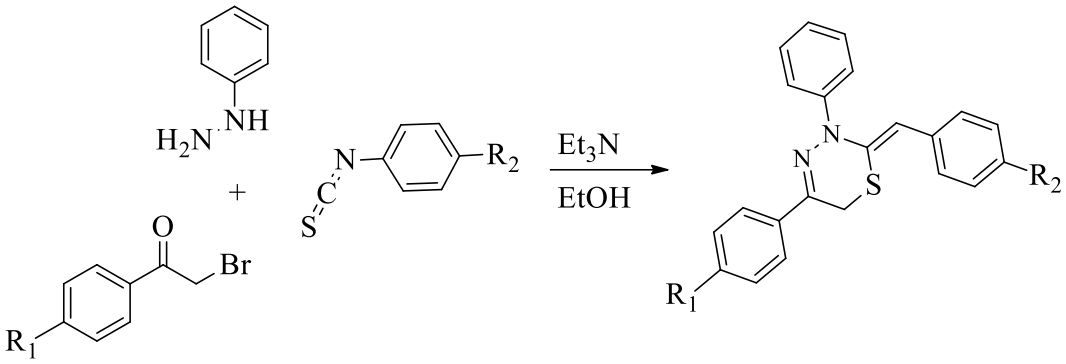
Scheme-1

Abdelwahed R. Sayed[22] reported the Synthesis of 1,3,4-thiadiazines, bis-1,3,4-thiadiazoles, [1,2,4]triazino[3,4-*b*][1,3,4] thiadiazine, thiazolines from carbonothioic dihydrazide.



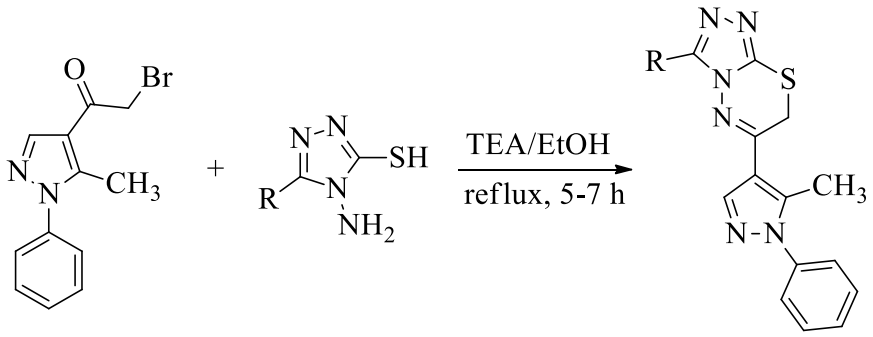
Scheme-2

Setareh Moghimi and co-workers[23] described a novel and easy route to 1,3,4-thiadiazine derivatives *via* the three-component reaction of phenylhydrazine, α-bromo aryl ketones and aryl isothiocyanates.



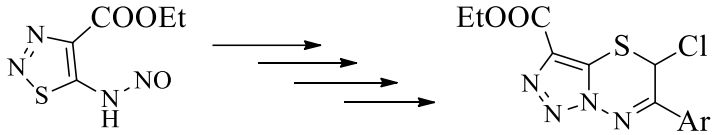
Scheme-3

Mostafa E. Salem *al.*[24] reported 2-Bromo-1-(1*H*-pyrazol-4-yl)ethanone: versatile precursors for novel mono-, bis- and poly{6-(1*H*-pyrazol-4-yl)-[1,2,4]triazolo [3,4-*b*][1,3,4]thiadiazines}.



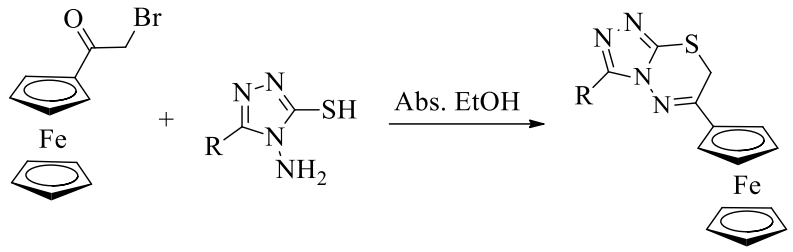
Scheme-4

Yury Yu. Morzherin and co-workers[25] developed a new ring transformation in the series of 1,2,3-thiadiazoles. Synthesis of 5*H*-[1,2,3]triazolo[5,1-*b*][1,3,4]thiazdiazines.



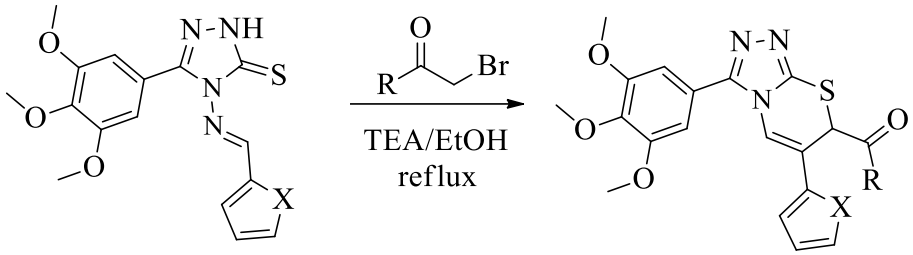
Scheme-5

Ruidong Miao *et al.*[26] reported Conjugation of substituted ferrocenyl to thiadiazine as apoptosis-inducing agents targeting the Bax/Bcl-2 pathway.



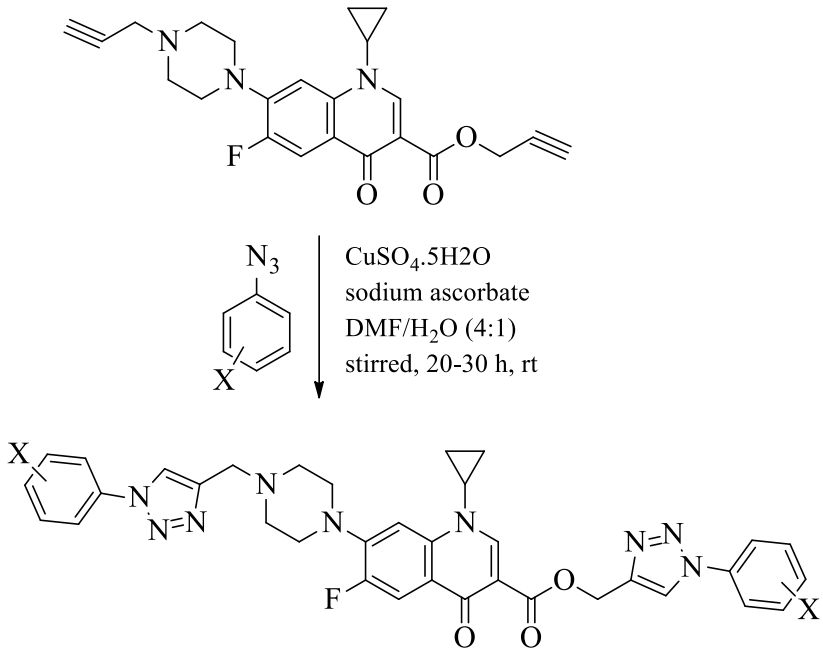
Scheme-5

Bei Zhang and co-workers[27] reported design, synthesis and biological evaluation of novel 1,2,4-triazolo[3,4-*b*][1,3,4] thiadiazines bearing furan and thiophene nucleus.



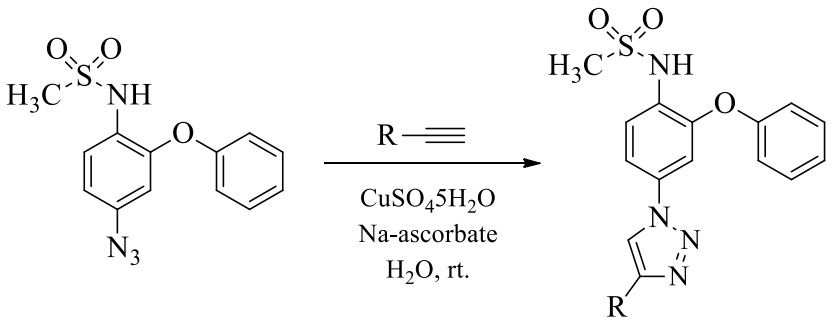
Scheme-6

Rama Kant *et al.*[28] reported design, synthesis and biological evaluation of ciprofloxacin tethered bis-1,2,3-triazole conjugates as potent antibacterial agents.



Scheme-7

Jyoti Mareddy *et al.*[29] reported 1,2,3-Triazole-nimesulide hybrid: Their design, synthesis and evaluation as potential anticancer agents.



Scheme-8

PRESENT WORK

Multi-component approach[30] (MCRs) have emerged as an efficient and powerful tool in modern synthetic organic chemistry which allows synthesis of small molecule libraries from two or more different starting materials in one step.[31] Such reactions offer a wide range of possibilities for the efficient construction of highly complex molecules in a single step; these reactions are best tools because of their productivity, simple procedures, and facile execution.[32] The interest over designing of new MCRs strategies has boosted during the past two decades because of its advantages over traditional step-wise synthesis.

Inspired from the molecular hybridization strategy (MH) and aforementioned diverse pharmacological applications of 1,2,3-triazoles and 1,3,4-thiadiazines forced us towards the design and synthesis of such heterocyclic hybrid with these two pharmacophores embodied in it. The target molecular frame-work was achieved by MCR approach.

Preparation of starting materials

3-Acetyl-2*H*-chromen-2-one,2-Acetyl-3*H*-benzo[*f*]chromen-3-one, 3-(2-Bromoacetyl)-2*H*-chromen-2-one and 2-(2-Bromoacetyl)-3*H*-benzo[*f*]chromen-3-one

The above important starting compounds were prepared according to the literature procedure as described in **Chapter-II**, **Section-A**.

4-(prop-2-yn-1-yloxy)benzaldehyde (2)

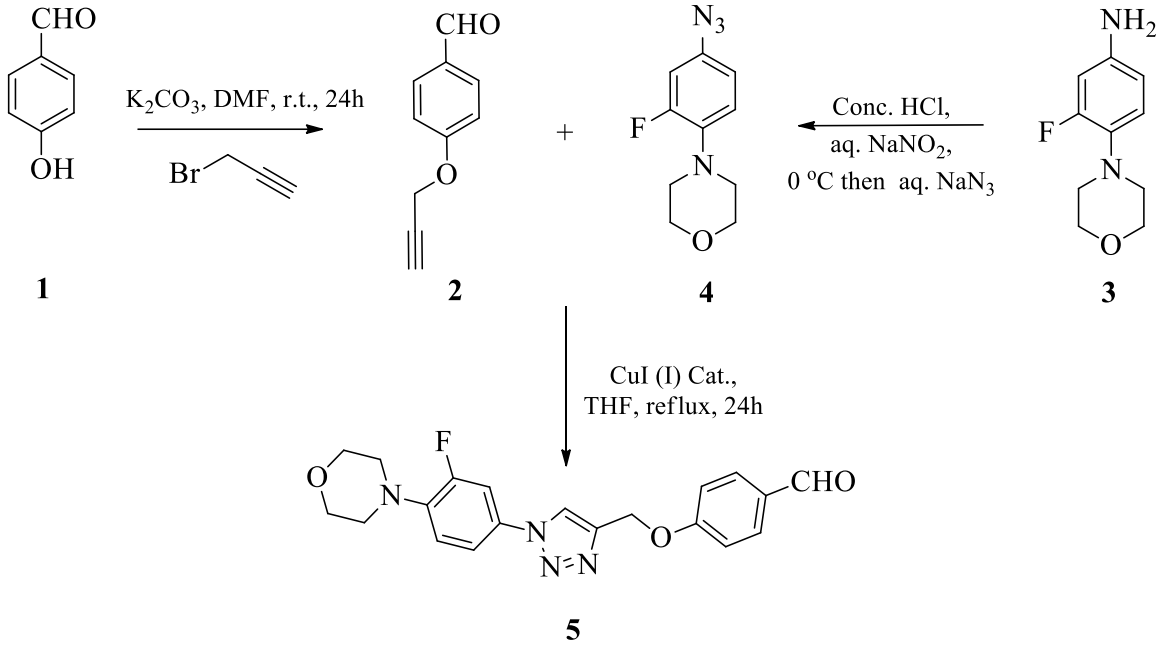
It was prepared by following the literature procedure described in **Chapter-III**, **Section-A**.

Preparation of 4-(4-azido-2-fluorophenyl)morpholine (4)

3-fluoro-4-morpholinoaniline **3** (1.0 mmol) was suspended in HCl/H_2O (1:1 v/v) in a 100 mL flask and cooled to 0-5 °C while stirring for 15 min. Aqueous $NaNO_2$ (1.5 mmol) was added drop-wise to the above-cooled solution and allowed to stir for about 15 min by maintaining the same temperature. After, was added aqueous solution of NaN_3 (1.5 mmol) gradually to the diazonium solution (Caution!) over a period of 15-20 min while stirring at 0-5 °C. The reaction mixture was stirred at room temperature for 1 hr and separated precipitate was extracted with hexane (3x30 mL) and the organic layer was washed with water, brine and dried over anhydrous Na_2SO_4. The solvent was removed under rotary evaporator to afford the target compound **4** as a yellow colored precipitate, which was almost pure and used as such without further purification.

General procedure for the region-selective synthesis of 4-((1-(3-fluoro-4-morpholinophenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)benzaldehyde 5: Huisgen cycloaddition reaction

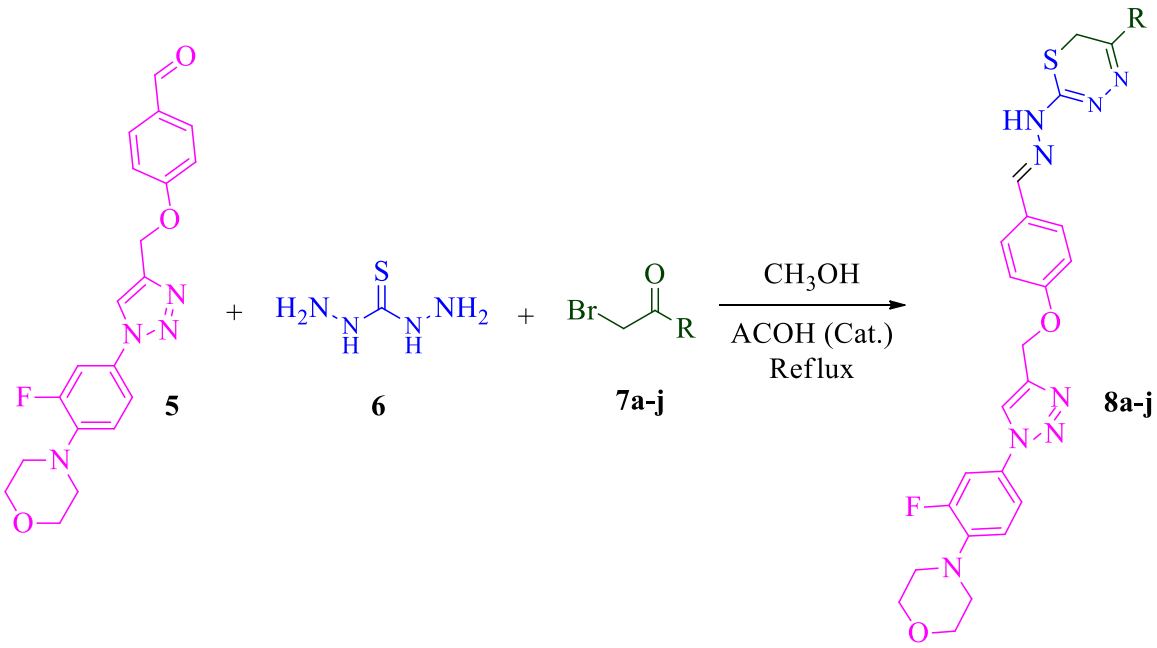
An equimolar concentration of 4-(prop-2-yn-1-yloxy)benzaldehyde **2** (1.0 mmol) and 4-(4-azido-2-fluorophenyl)morpholine **4** (1.0 mmol) in a 100 mL round-bottomed flask, having THF as a solvent was stirred under reflux in the presence of CuI (0.1 mmol) as a catalyst. After the completion of reaction ensured by TLC and was cooled to room temperature and ice-cold water was added. Extracted with ethyl acetate (3x30 mL). The combined organic layers were washed with water, brine and dried over anhydrous Na_2SO_4. The solvent was concentrated under reduced pressure afforded the crude product **5** as a brown colored solid. Washing, several times with ethyl acetate yielded the pure compound **5** as a white colored solid in 95 % yield.



Scheme-9

Thiocarbohyrazide (TCH)[33]

1.5 mL of carbon disulfide was added drop-wise to the solution of 5 mL of 85% hydrazine hydrate in 15 mL of water and stirred for 1h at room-temperature. Reflux the reaction mixture at 90 ^{o}C for 10 h, filter the separated precipitate on cooling, washed with excess of water and allowed to dry. Recrystallization with hot water yielded the pure white needles in yield 72-75 %.; M.P. 172-173 °C.



R:

8a = C_6H_5 **8b** = 4-MeC_6H_4 **8c** = 4-$OMeC_6H_4$

8d = 4-Ph-C_6H_4 **8e** = 4-FC_6H_4 **8f** = 4-ClC_6H_4

8g = 4-BrC_6H_4 **8h** = 4-$NO_2C_6H_4$ **8i** = Coumarin-3-yl

8j = Benzo[*f*]coumarin-3-yl

Scheme-10

Results and discussion

A general route for the synthesis of novel hydrazinyl 1,3,4-thiadiazinyl-1,4-di-substituted 1,2,3-triazole hybrids (**8a-j**) were potrayed in **Scheme 9** and **10.** The titled compounds were afforded in quantitative yields by the condensation of aldehyde **5**, TCH **6** and *α*-bromo ketones **7a-i** in refluxing methanol in the presence of catalytic amount of glacial acetic acid. 4-((1-(3-fluoro-4-morpholinophenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)benzaldehyde **5** the key intermediate in the present work was achieved by the Cu(I) catalyzed azide (**4**)-alkyne (**2**) Huisgen 1,3-dipolar cyclo-addition reaction[34] (**Scheme 9**). Propargylation[35] of *p*-hydroxy benzaldehyde with propargyl bromide in presence of K_2CO_3 in DMF at room temperature and diazotization[36] of corresponding aniline followed by *in situ* aromatic nucleophilic substitution by the sodium azide yielded the starting compounds **2** and **4**.

With the key intermediate 4-((1-(3-fluoro-4-morpholinophenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)benzaldehyde **5** in hand, all the target compounds **8a-j** were afforded in 80-95 % isolated yields. Structures of all the synthesized compounds were in accordance

with their spectral (FTIR, ^{1}H NMR, ^{13}C NMR and ESI) and elemental analyses (C, H, and N).

Conclusion

In conclusion, embracing the molecular hybridization approach, a series of hydrazinyl 1,3,4-thiadiazinyl-1,4-di-substituted 1,2,3-triazole hybrids (**8a-j**) were designed and synthesized successfully good to excellent yields by the one-pot three component condensation of an aldehyde **5**, TCH **6** and *α*-bromo ketones **7a-i** in refluxing methanol in catalytic amount of glacial acetic acid.

Experimental

General procedure for the synthesis of (8a-r)

Equimolar mixture of an aldehyde **5** (1.0 mmol), thiocarbohydrazide **6** (1.0 mmol) and *α*-bromoketones **7a-j** (1.0 mmol) in refluxing ethanol in the presence of catalytic amount of acetic acid was stirred for 4-6 hr. After, the completion of reaction (ensured by TLC). Filtered the separated solid under vacuo and washed with ethanol afforded the tilted compounds **8a-j** in almost analytically pure form (**Scheme-10**).

Spectral data

4-((1-(3-fluoro-4-morpholinophenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)benzaldehyde (5)
Grey colored solid; 88 % yield; mp: 192-194 °C; **^{1}H NMR** (400 MHz, DMSO-d_6): δ 8.95 (s, 1H), 8.42 (s, 1H), 8.00 (d, *J* = 8.4 Hz, 2H), 7.80 (d, *J* = 13.2 Hz, 1H), 7.69 (d, *J* = 8.4 Hz, 1H), 7.33 (d, *J* = 8.4 Hz, 2H), 7.23 (t, *J* = 8.8 Hz, 1H), 5.39 (s, 2H), 3.76 (s, 4H), 3.08 (s, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 190.75, 163.04, 156.54, 154.06, 144.04, 140.60, 132.06, 130.50, 121.00, 119.09, 116.46, 115.11, 109.77, 109.52, 66.82, 62.12, 50.61; **Mass** (ESI) *m/z*: 383 (M + H); Anal. calcd. for $C_{20}H_{19}FN_4O_3$: C, 62.82; H, 5.01; N, 14.65; Found: C, 62.61; H, 5.28; N, 14.86.

4-(2-fluoro-4-(4-((4-((2-(5-phenyl-6*H*-1,3,4-thiadiazin-2-yl)hydrazono)methyl)phenoxy)methyl)-1*H*-1,2,3-triazol-1-yl)phenyl)morpholine (8a)
Yellow solid; 88 % yield; mp: 215-217 °C; **IR** (KBr) υ_{max} (cm^{-1}): 1600 (C=N); **^{1}H NMR** (400 MHz, DMSO-d_6): δ 8.31 (s, 1H), 8.01 (d, *J* = 8.4 Hz, 1H), 7.71 (d, *J* = 8.4 Hz, 2H), 7.59-736 (m, 7H), 7.04-6.98 (m, 3H), 5.98 (s, 1H), 5.31 (s, 2H), 4.55 (s, 1H), 3.89 (s, 4H), 3.14 (s, 4H), 2.24 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.88, 156.14, 153.69,

147.09, 144.02, 140.27, 135.46, 131.22, 129.96, 129.31, 129.16, 128.55, 126.28, 123.35, 120.17, 116.94, 115.51, 109.44, 109.18, 66.53, 61.06, 50.73, 22.22; **Mass** (ESI) *m/z*: 572 (M + H); Anal. calcd. for $C_{29}H_{27}FN_8O_2S$: C, 61.04; H, 4.77; N, 19.64; Found: C, 61.27; H, 4.99; N, 19.40.

4-(2-fluoro-4-(4-((4-((2-(5-(*p*-tolyl)-6*H*-1,3,4-thiadiazin-2-yl)hydrazono)methyl)phenoxy)methyl)-1*H*-1,2,3-triazol-1-yl)phenyl)morpholine (8b)

Yellow solid; 92 % yield; mp: 220-222 °C; 1602 (C=N); **Mass** (ESI) *m/z*: 586 (M + H); Anal. calcd. for $C_{30}H_{29}FN_8O_2S$: C, 61.63; H, 5.00; N, 19.17; Found: C, 61.90; H, 4.79; N, 19.43.

4-(2-fluoro-4-(4-((4-((2-(5-(4-methoxyphenyl)-6*H*-1,3,4-thiadiazin-2-yl)hydrazono)methyl)phenoxy)methyl)-1*H*-1,2,3-triazol-1-yl)phenyl)morpholine (8c)

Yellow solid; 95 % yield; mp: 219-221 °C; **IR** (KBr) υ_{max} (cm^{-1}): 1600 (C=N); **^{1}H NMR** (400 MHz, DMSO-d_6): δ 8.32 (s, 1H), 7.99 (s, 1H), 7.70 (d, *J* = 8.4 Hz, 2H), 7.51 (s, 1H), 7.47 (d, *J* = 8.0 Hz, 2H), 7.43 (d, *J* = 8.8 Hz, 1H), 7.02 (t, *J* = 7.6 Hz, 3H), 6.95 (d, *J* = 8.0 Hz, 2H), 5.92 (s, 1H), 5.32 (s, 2H), 4.55 (s, 1H), 3.89 (s, 4H), 3.94 (s, 3H), 3.14 (s, 4H), 1.25 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.05, 160.19, 159.37, 156.56, 154.09, 151.51, 144.78, 140.93, 140.58, 130.15, 128.98, 128.88, 122.66, 120.84, 119.05, 116.49, 114.93, 113.78, 109.78, 109.52, 95.88, 66.86, 62.06, 55.39, 50.65, 30.96; **Mass** (ESI) *m/z*: 602 (M + H); Anal. calcd. for $C_{30}H_{29}FN_8O_3S$: C, 59.99; H, 4.87; N, 18.65; Found: C, 60.27; H, 4.67; N, 18.38.

4-(4-(4-((4-((2-(5-([1,1'-biphenyl]-4-yl)-6*H*-1,3,4-thiadiazin-2-yl)hydrazono)methyl)phenoxy)methyl)-1*H*-1,2,3-triazol-1-yl)-2-fluorophenyl)morpholine (8d)

Yellow solid; 92 % yield; mp: 230-233 °C; **IR** (KBr) υ_{max} (cm^{-1}): 1604 (C=N); **^{1}H NMR** (400 MHz, DMSO-d_6): δ 8.60 (s, 1H), 8.00 (d, *J* = 7.6 Hz, 1H), 7.81 (d, *J* = 8.0 Hz, 1H), 7.69-7.58 (m, 7H), 7.51-7.36 (m, 5H), 7.04-7.01 (m, 3H), 6.31 (s, 1H), 5.31 (s, 2H), 5.20 (s, 1H), 3.89 (s, 4H), 3.14 (s, 4H), 1.25 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.60, 160.13, 156.57, 154.09, 152.21, 145.07, 144.56, 142.58, 142.31, 140.61, 140.07, 131.16, 129.48, 128.96, 127.92, 127.50, 127.17, 120.93, 119.10, 119.06, 116.50, 115.04, 109.78, 109.53, 99.67, 66.86, 62.06, 50.65, 30.96; **Mass** (ESI) *m/z*: 648 (M + H); Anal. calcd. for $C_{35}H_{31}FN_8O_2S$: C, 65.00; H, 4.83; N, 17.33; Found: C, 65.28; H, 5.09; N, 17.12.

4-(2-fluoro-4-(4-((4-((2-(5-(4-fluorophenyl)-6*H*-1,3,4-thiadiazin-2-yl)hydrazono)methyl)phenoxy)methyl)-1*H*-1,2,3-triazol-1-yl)phenyl)morpholine (8e)

Yellow solid; 85 % yield; mp: 215-217 °C; **IR** (KBr) υ_{max} (cm^{-1}): 1600 (C=N); **^{1}H NMR** (400 MHz, DMSO-d_6): δ 8.32 (s, 1H), 7.99 (s, 1H), 7.71 (d, *J* = 8.0 Hz, 2H), 7.60-7.48 (m, 3H), 7.43 (d, *J* = 8.8 Hz, 1H), 7.12 (t, *J* = 8.8 Hz, 2H), 7.07-7.01 (m, 3H), 5.98 (s, 1H), 5.32 (s, 2H), 4.55 (s, 1H), 3.89 (s, 4H), 3.15 (s, 4H), 1.25 (s, 1H); **Mass** (ESI) *m/z*: 590 (M + H); Anal. calcd. for $C_{29}H_{26}F_2N_8O_2S$: C, 59.17; H, 4.45; N, 19.04; Found: C, 58.91; H, 4.67; N, 19.32.

4-(4-(4-((4-((2-(5-(4-chlorophenyl)-6*H*-1,3,4-thiadiazin-2-yl)hydrazono)methyl)phenoxy)methyl)-1*H*-1,2,3-triazol-1-yl)-2-fluorophenyl)morpholine (8f)

Yellow solid; 82 % yield; mp: 223-225 °C; **IR** (KBr) υ_{max} (cm^{-1}): 1602 (C=N); **Mass** (ESI) *m/z*: 605 (M^+); Anal. calcd. for $C_{29}H_{26}ClFN_8O_2S$: C, 57.56; H, 4.33; N, 18.52; Found: C, 57.26; H, 4.64; N, 18.29.

4-(4-(4-((4-((2-(5-(4-bromophenyl)-6*H*-1,3,4-thiadiazin-2-yl)hydrazono)methyl)phenoxy)methyl)-1*H*-1,2,3-triazol-1-yl)-2-fluorophenyl)morpholine (8g)

Yellow solid; 80 % yield; mp: 238-240 °C; **IR** (KBr) υ_{max} (cm^{-1}): 1600 (C=N); **^{1}H NMR** (400 MHz, DMSO-d_6): δ 8.41 (s, 1H), 7.99 (s, 1H), 7.70 (d, *J* = 7.2 Hz, 2H), 7.61-7.48 (m, 4H), 7.43 (d, *J* = 7.6 Hz, 2H), 7.03 (t, *J* = 7.6 Hz, 3H), 6.11 (s, 1H), 5.32 (s, 2H), 4.73 (s, 1H), 3.89 (s, 4H), 3.15 (s, 4H), 1.25 (s, 1H); **Mass** (ESI) *m/z*: 650 (M + H); Anal. calcd. for $C_{29}H_{26}BrFN_8O_2S$: C, 53.62; H, 4.03; N, 17.25; Found: C, 53.95; H, 3.80; N, 17.01.

4-(2-fluoro-4-(4-((4-((2-(5-(4-nitrophenyl)-6*H*-1,3,4-thiadiazin-2-yl)hydrazono)methyl)phenoxy)methyl)-1*H*-1,2,3-triazol-1-yl)phenyl)morpholine (8h)

Orange solid; 82 % yield; mp: 229-231 °C; **IR** (KBr) υ_{max} (cm^{-1}): 1602 (C=N**Mass** (ESI) *m/z*: 617 (M + H); Anal. calcd. for $C_{29}H_{26}FN_9O_4S$: C, 56.58; H, 4.26; N, 20.48; Found: C, 56.89; H, 4.04; N, 20.21.

3-(2-(2-(4-((1-(3-fluoro-4-morpholinophenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)benzylidene)hydrazinyl)-6*H*-1,3,4-thiadiazin-5-yl)-2*H*-chromen-2-one (8i)

Yellow solid; 83 % yield; mp: 244-246 °C; **IR** (KBr) υ_{max} (cm^{-1}): 1714 (C=O), 1602 (C=N); **Mass** (ESI) *m/z*: 650 (M + H); Anal. calcd. for $C_{32}H_{27}FN_8O_4S$: C, 60.18; H, 4.26; N, 17.54; Found: C, 60.47; H, 4.52; N, 17.22.

2-(2-(2-(4-((1-(3-fluoro-4-morpholinophenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)benzylidene)hydrazinyl)-6*H*-1,3,4-thiadiazin-5-yl)-3*H*-benzo[*f*]chromen-3-one (8j)

Yellow solid; 80 % yield; mp: 239-241 °C; **IR** (KBr) υ_{max} (cm^{-1}): 1716 (C=O), 1604 (C=N); **Mass** (ESI) *m/z*: 690 (M + H); Anal. calcd. for $C_{36}H_{29}FN_8O_4S$: C, 62.78; H, 4.24; N, 16.27; Found: C, 62.53; H, 4.52; N, 15.98.

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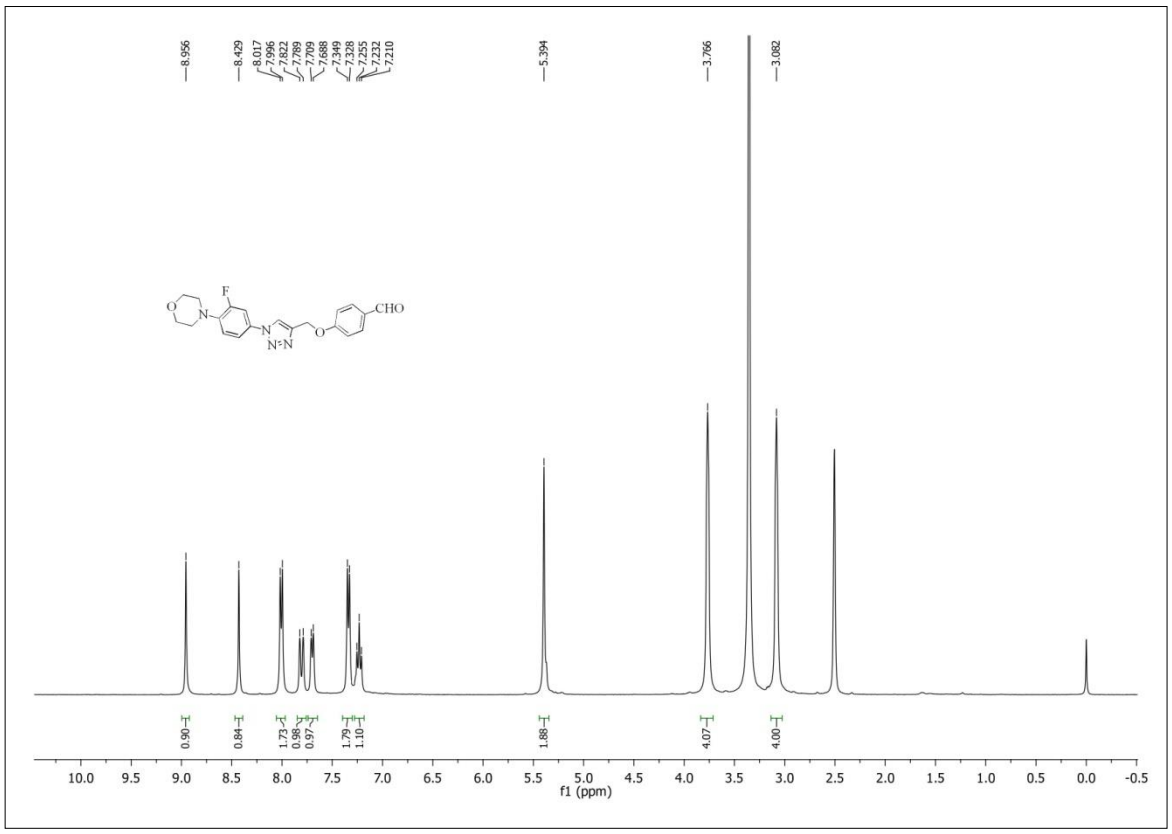
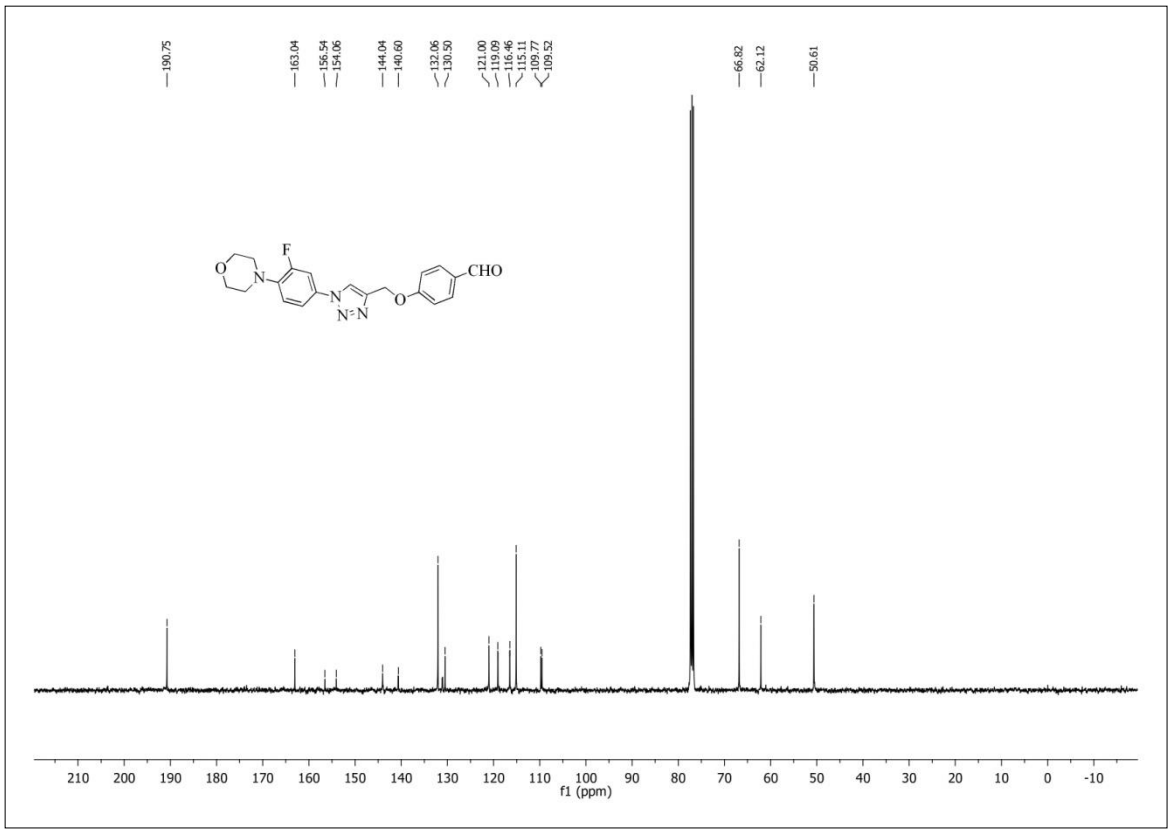
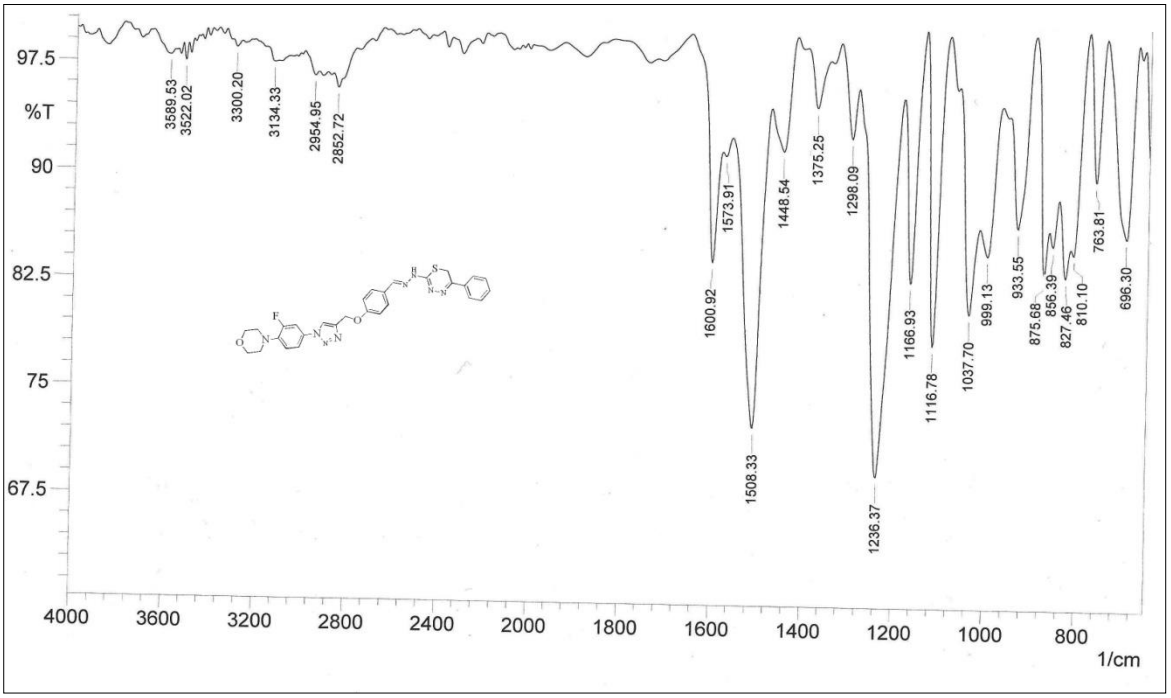


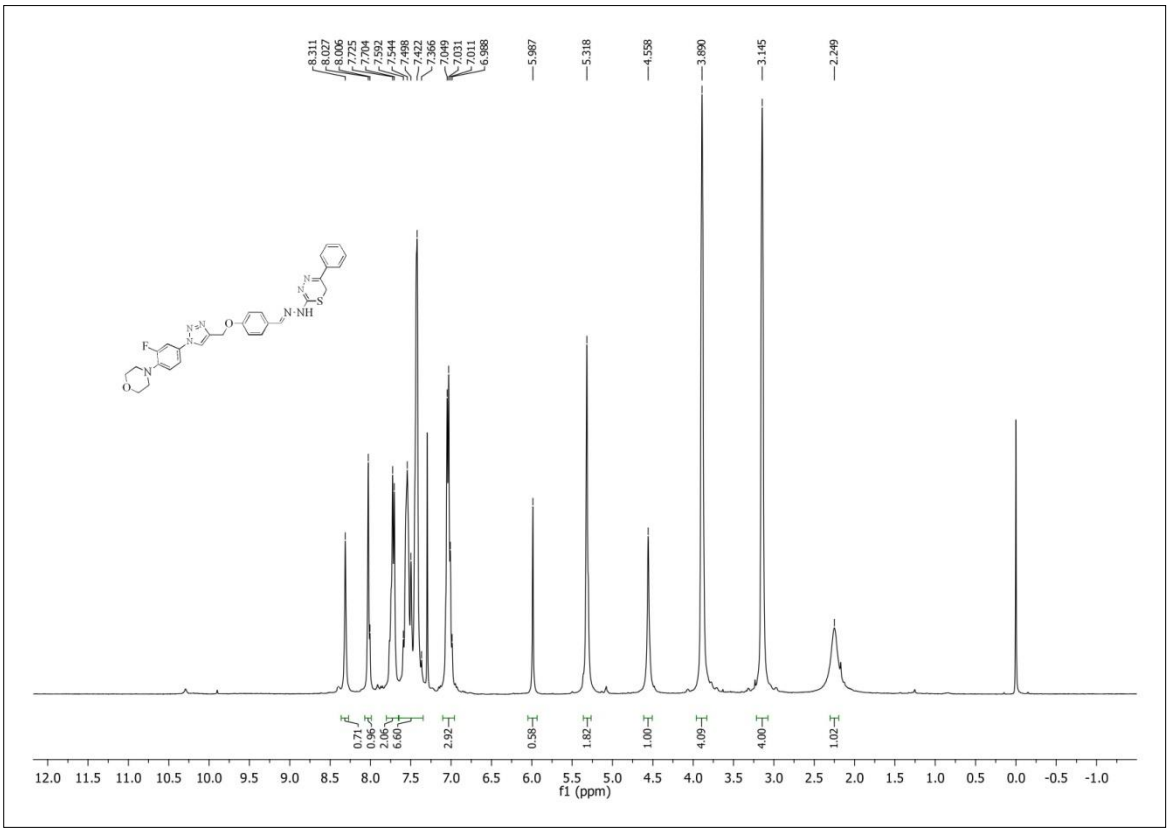
^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound 5



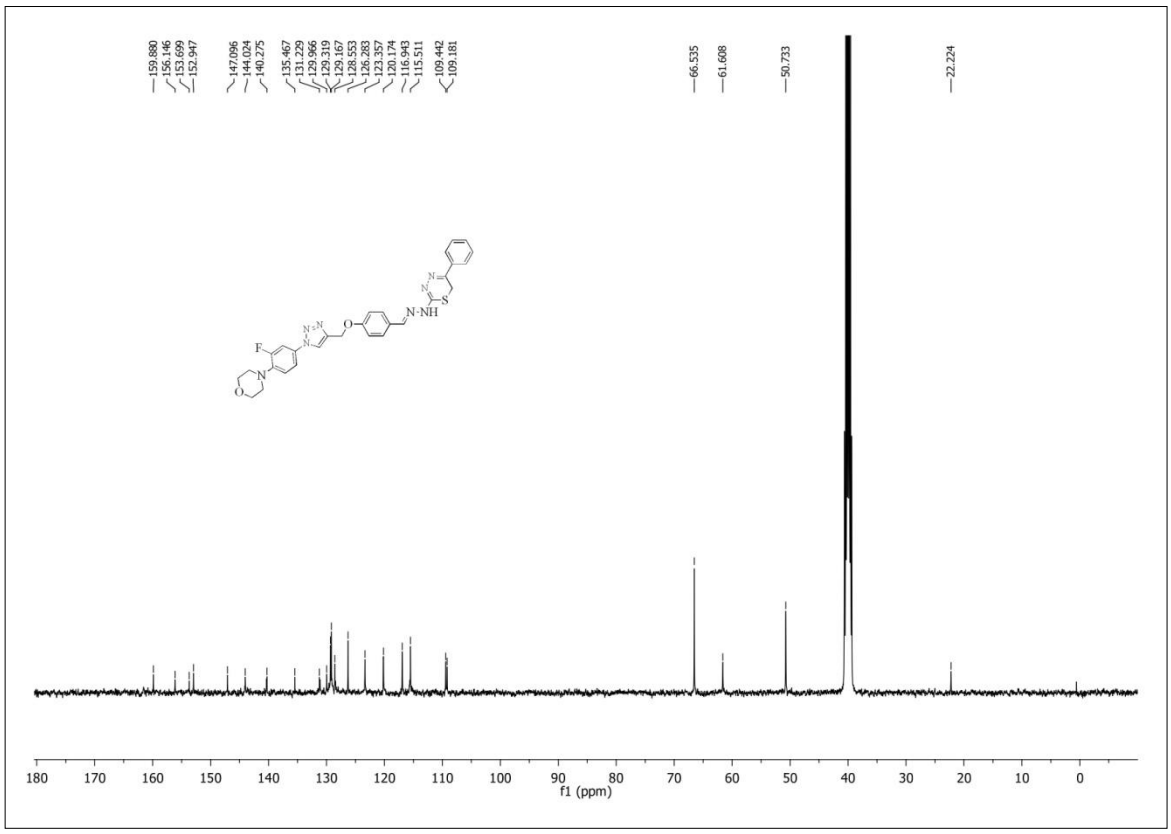
^{13}C NMR (100 MHz, DMSO-d_6) spectrum of compound 5



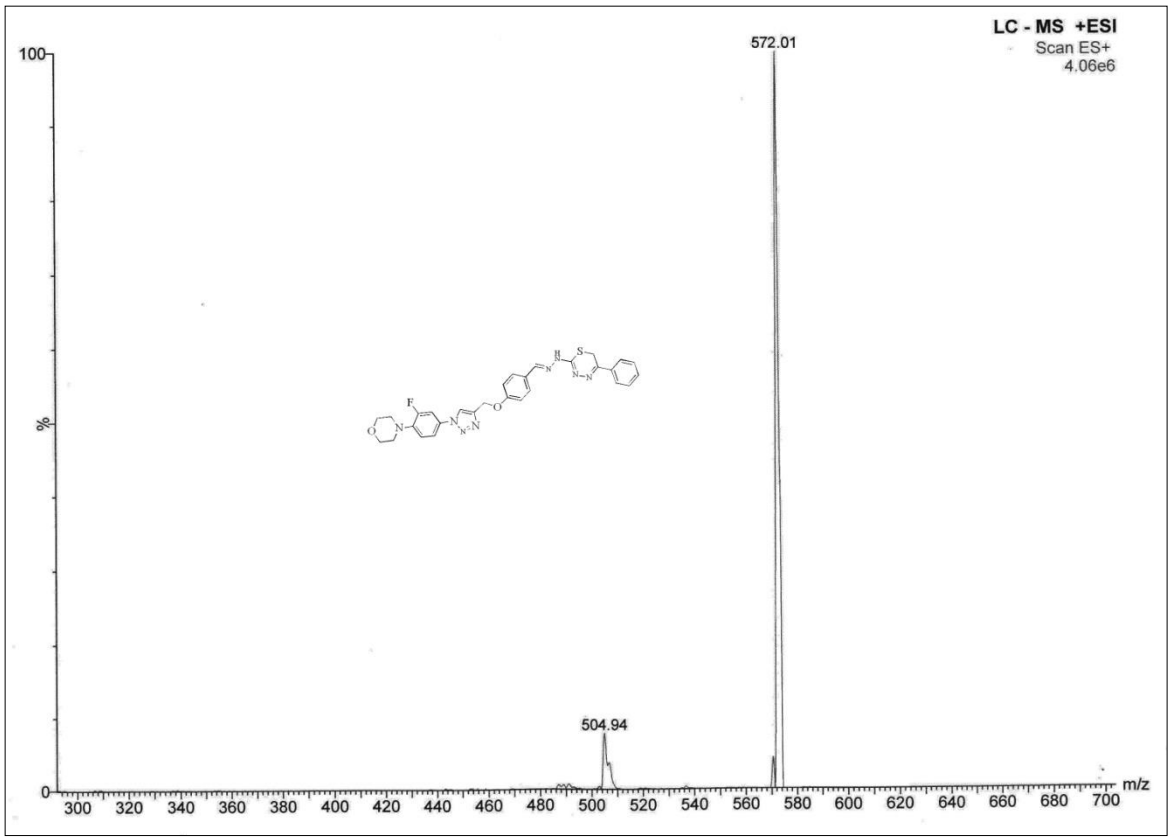
IR (KBr) spectrum of compound 8a



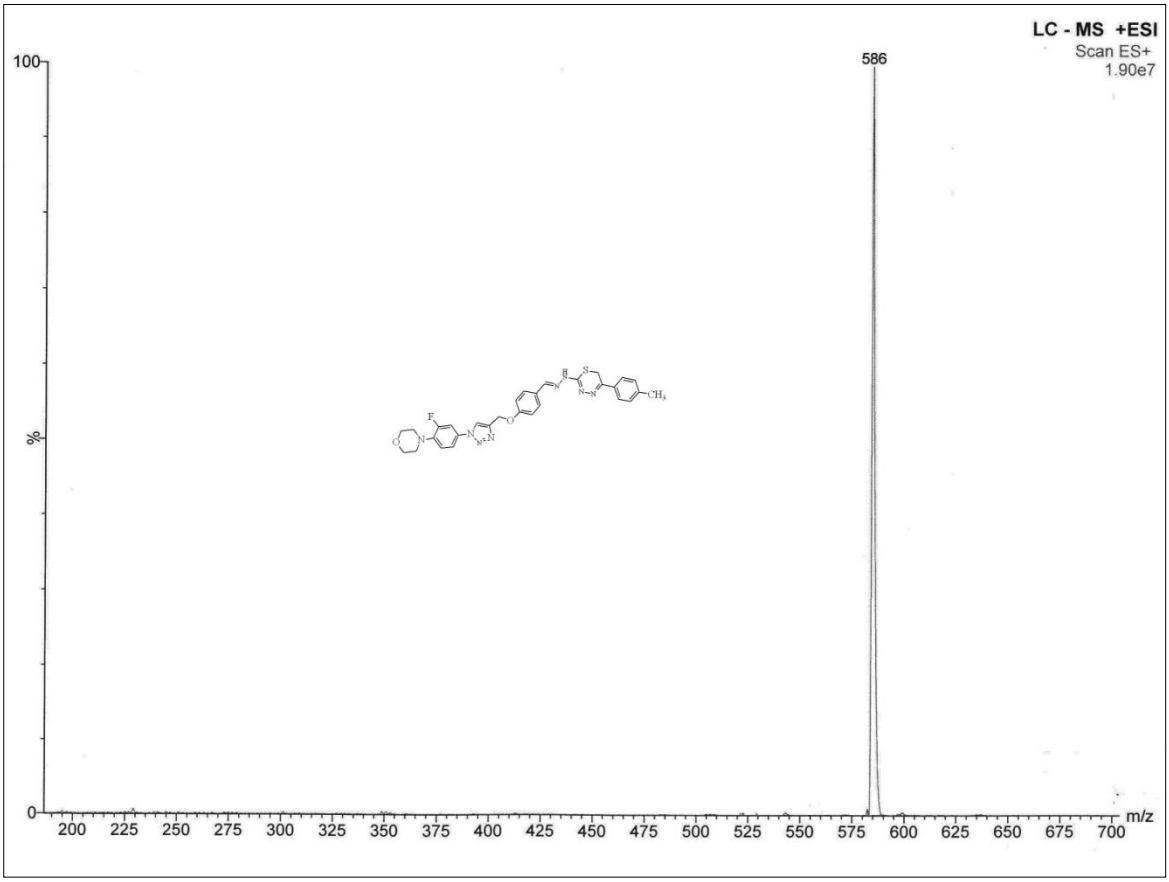
^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound 8a



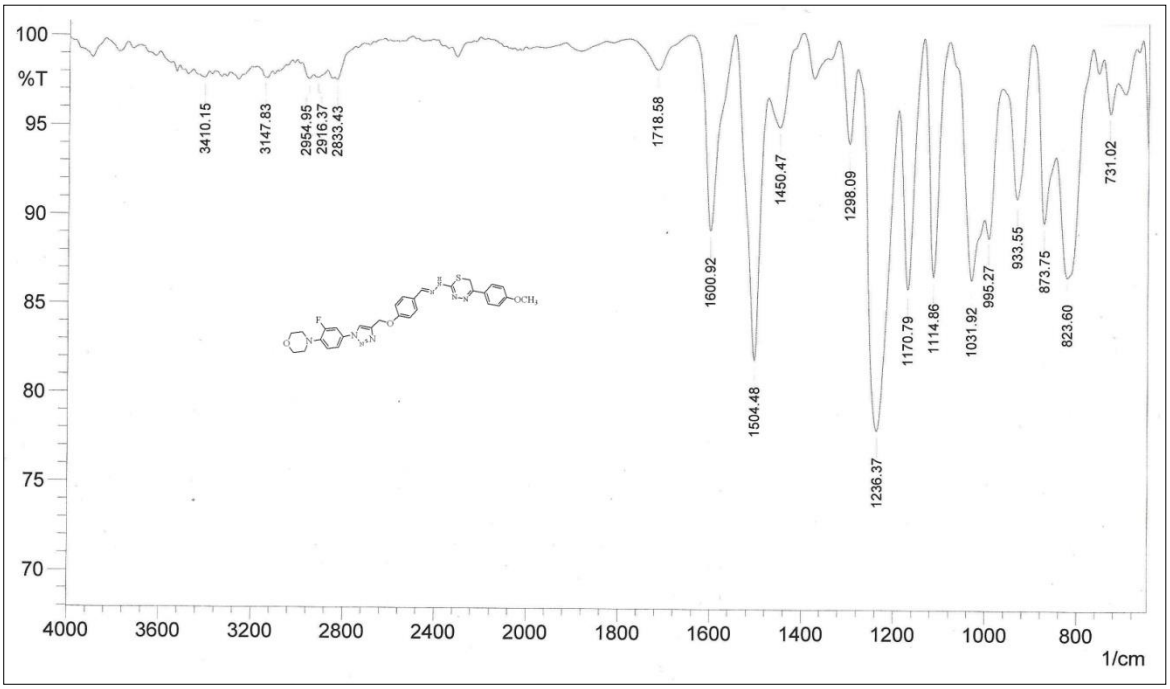
^{13}C NMR (100 MHz, $CDCl_3$) spectrum of compound 8a



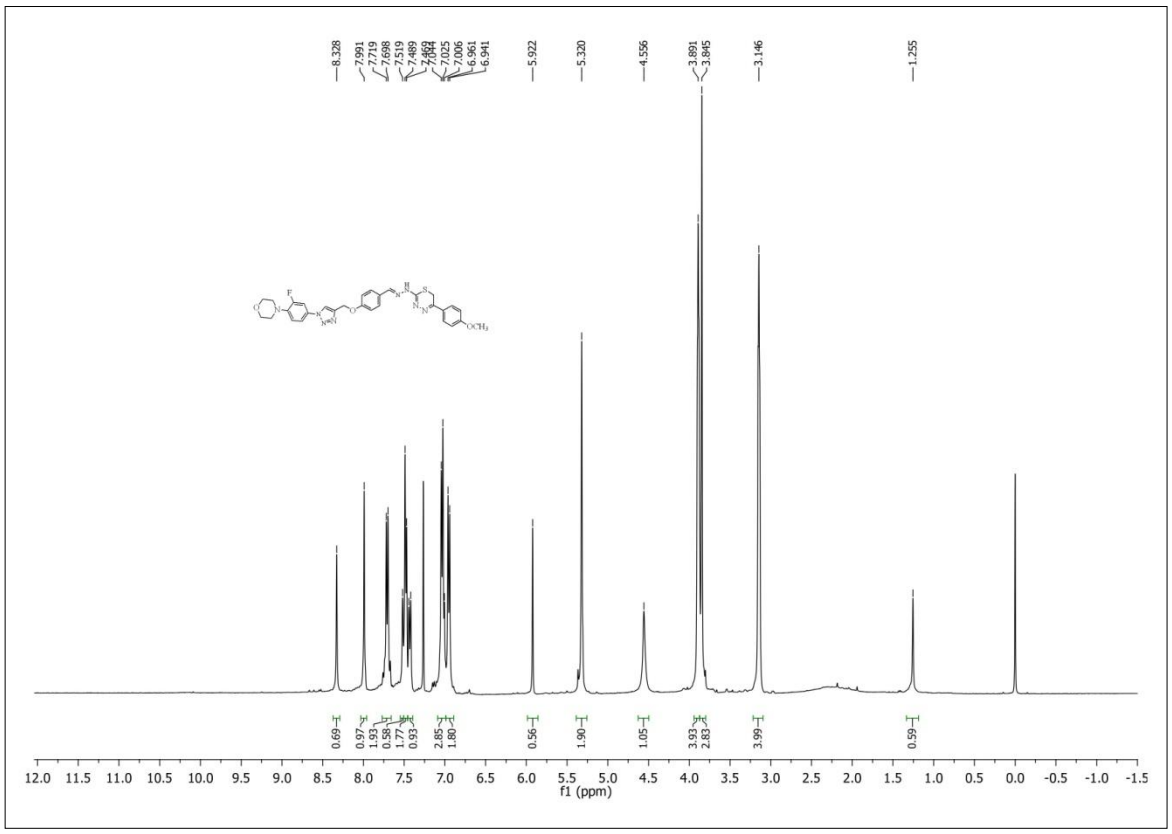
Mass spectrum of compound 8a (M.Wt: 571)



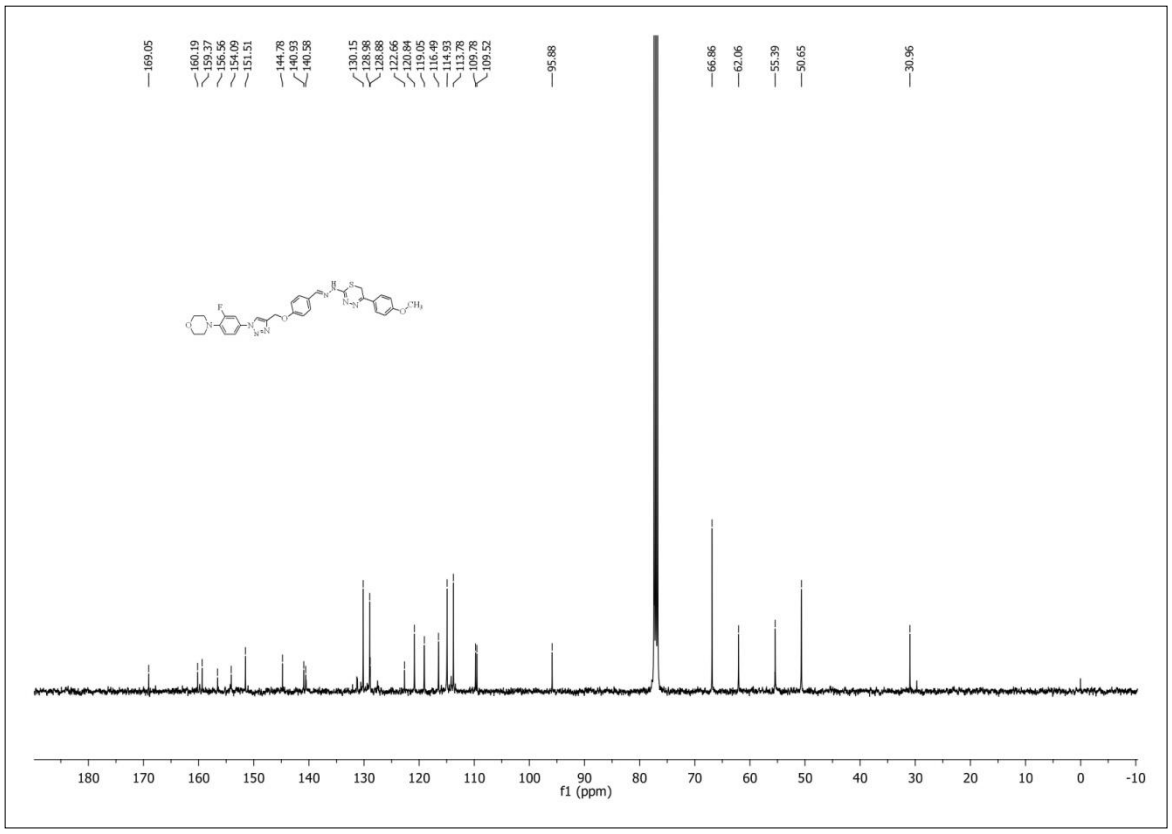
Mass spectrum of compound 8b (M.Wt: 585)



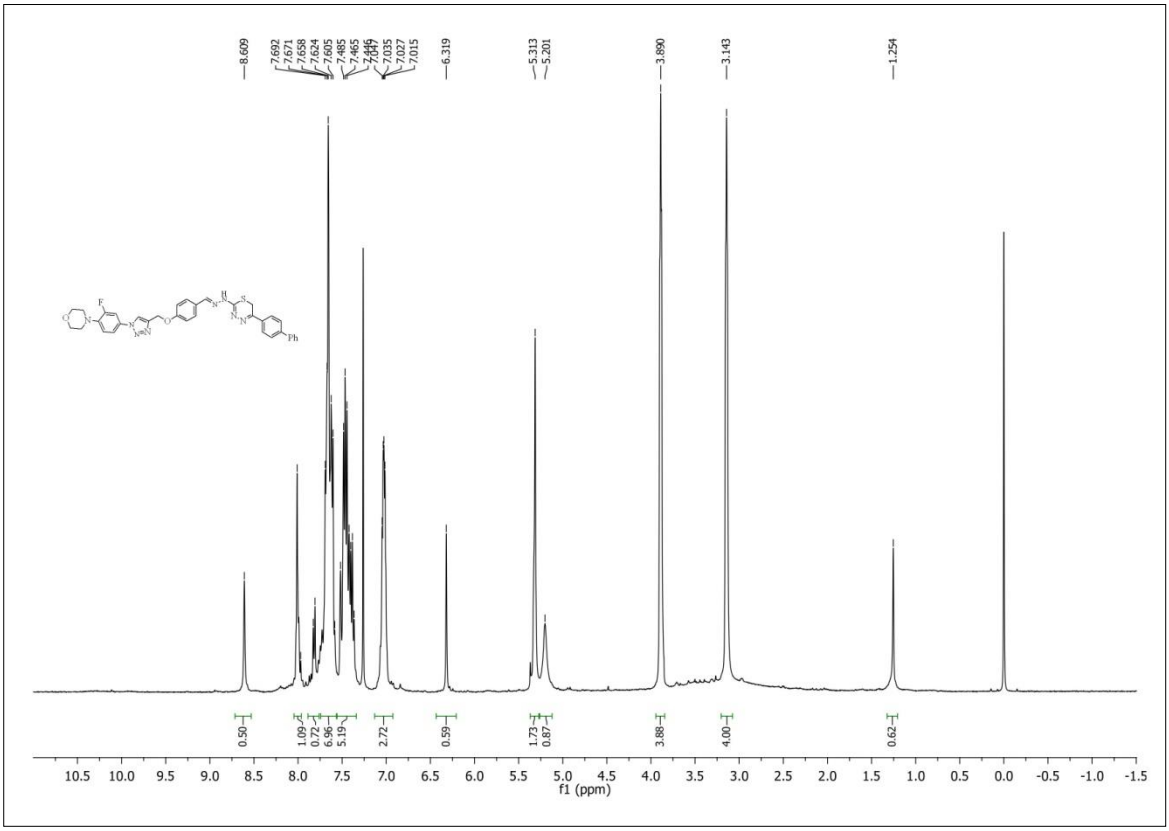
IR (KBr) spectrum of compound 8c



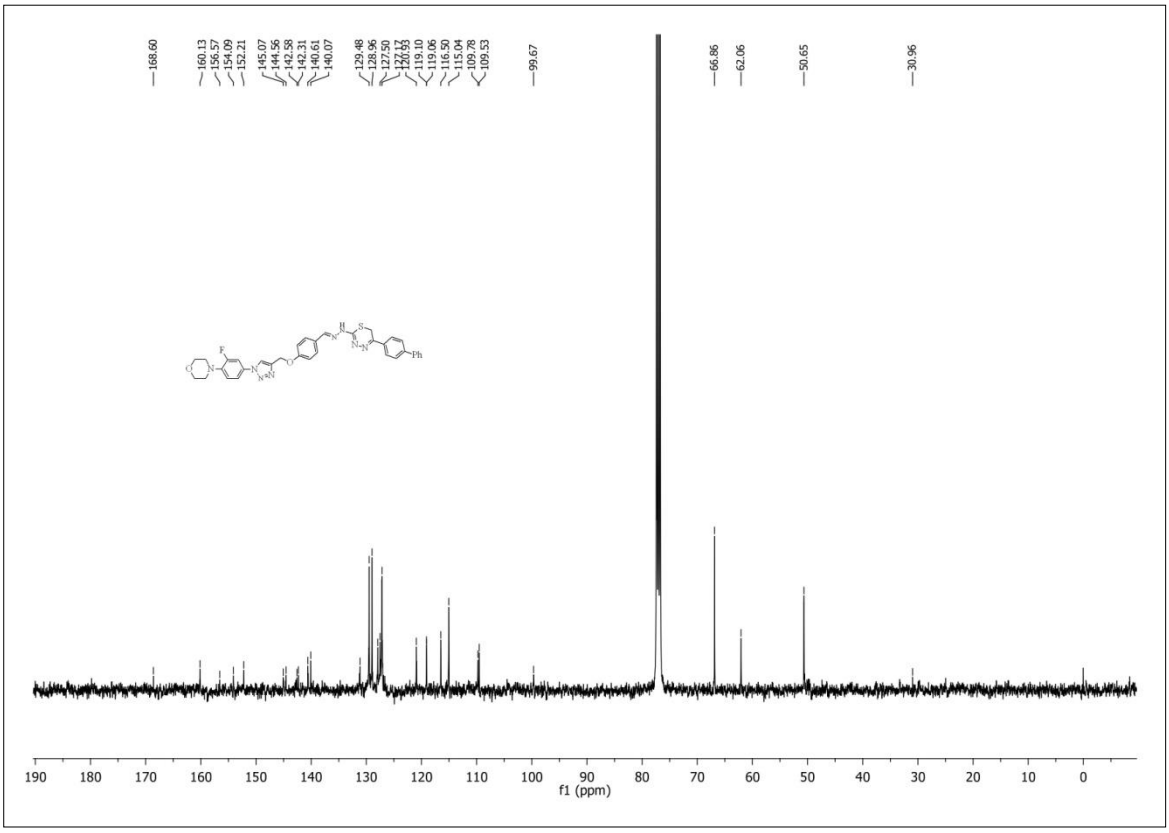
^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound 8c



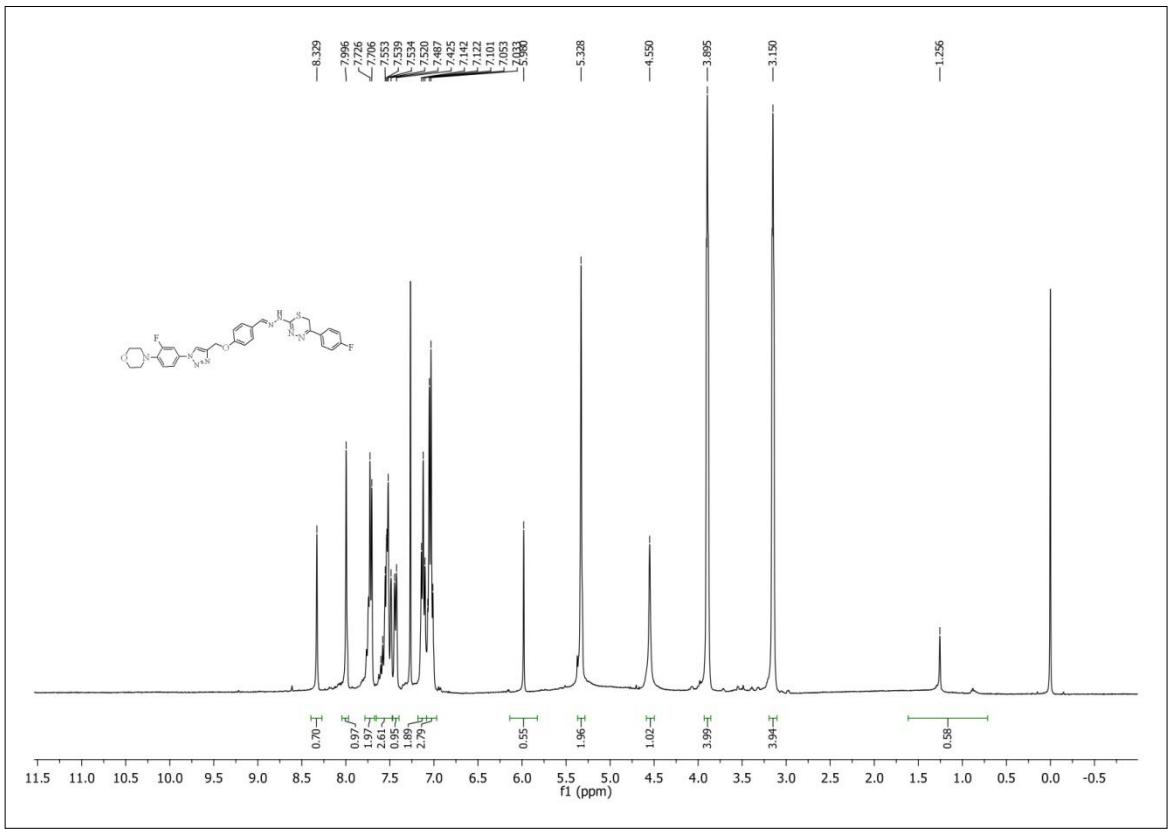
^{13}C NMR (100 MHz, $CDCl_3$) spectrum of compound 8c



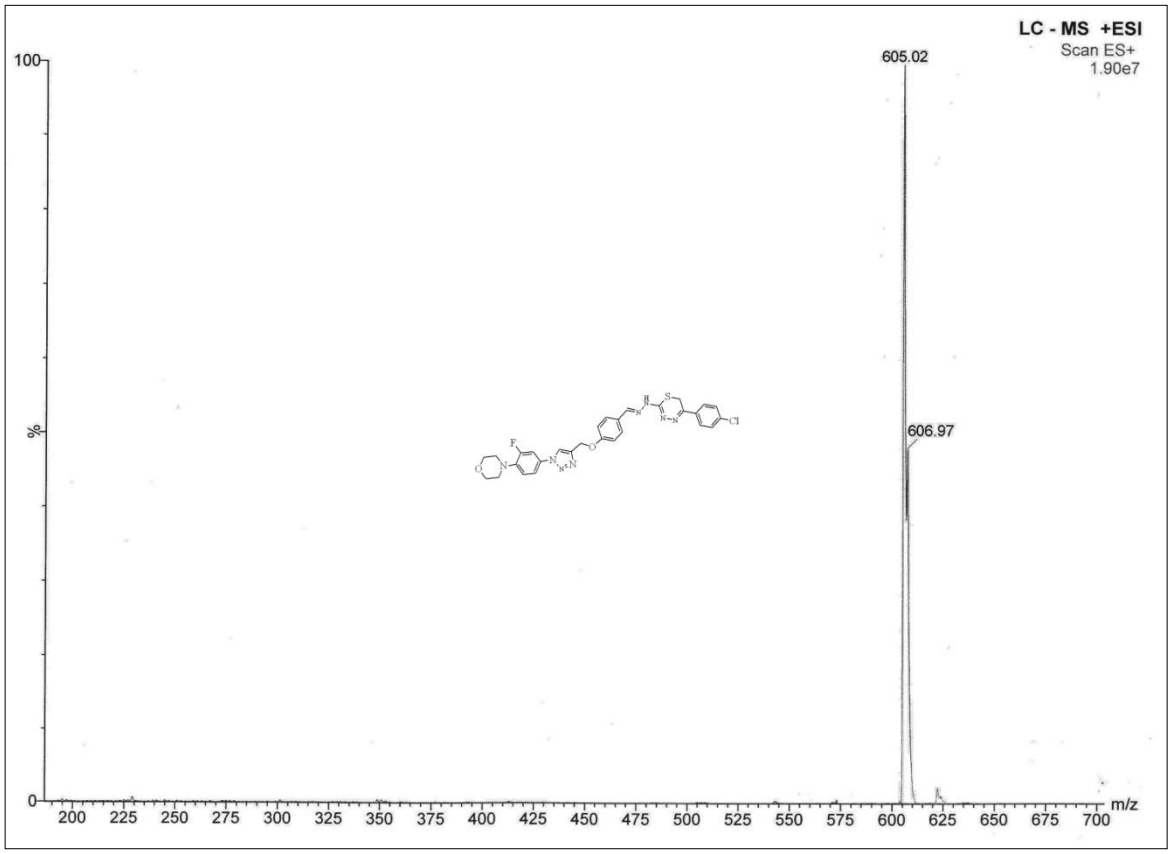
^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound 8d



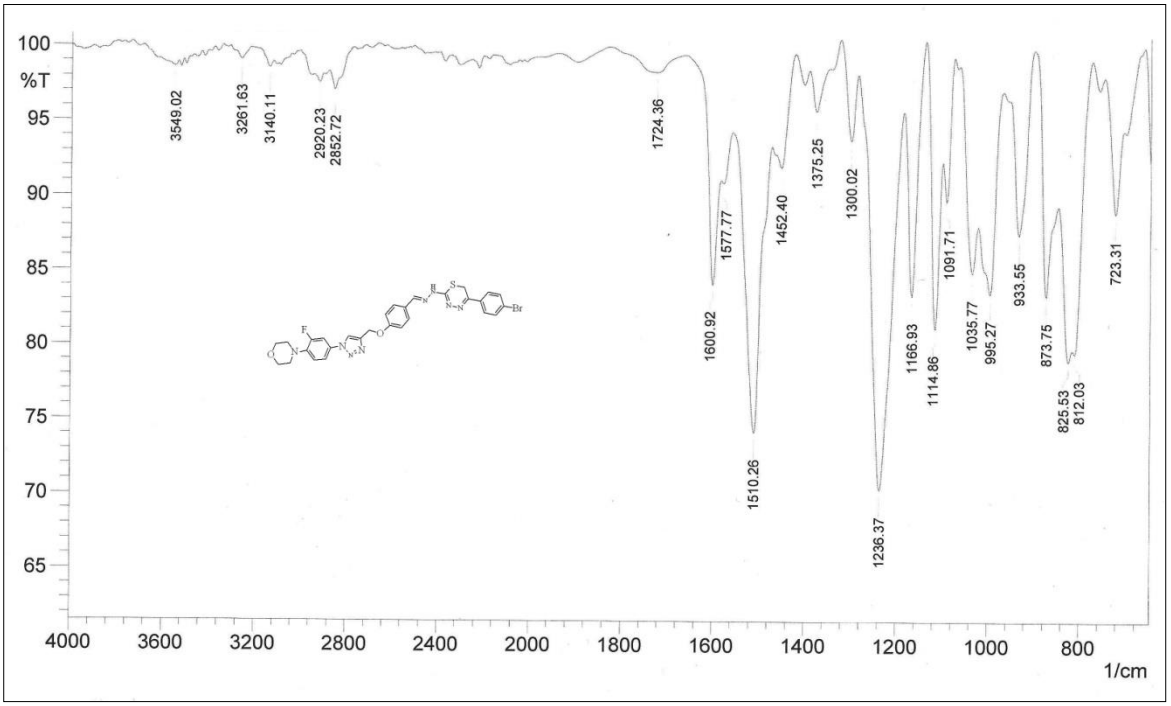
^{13}C NMR (100 MHz, $CDCl_3$) spectrum of compound 8d



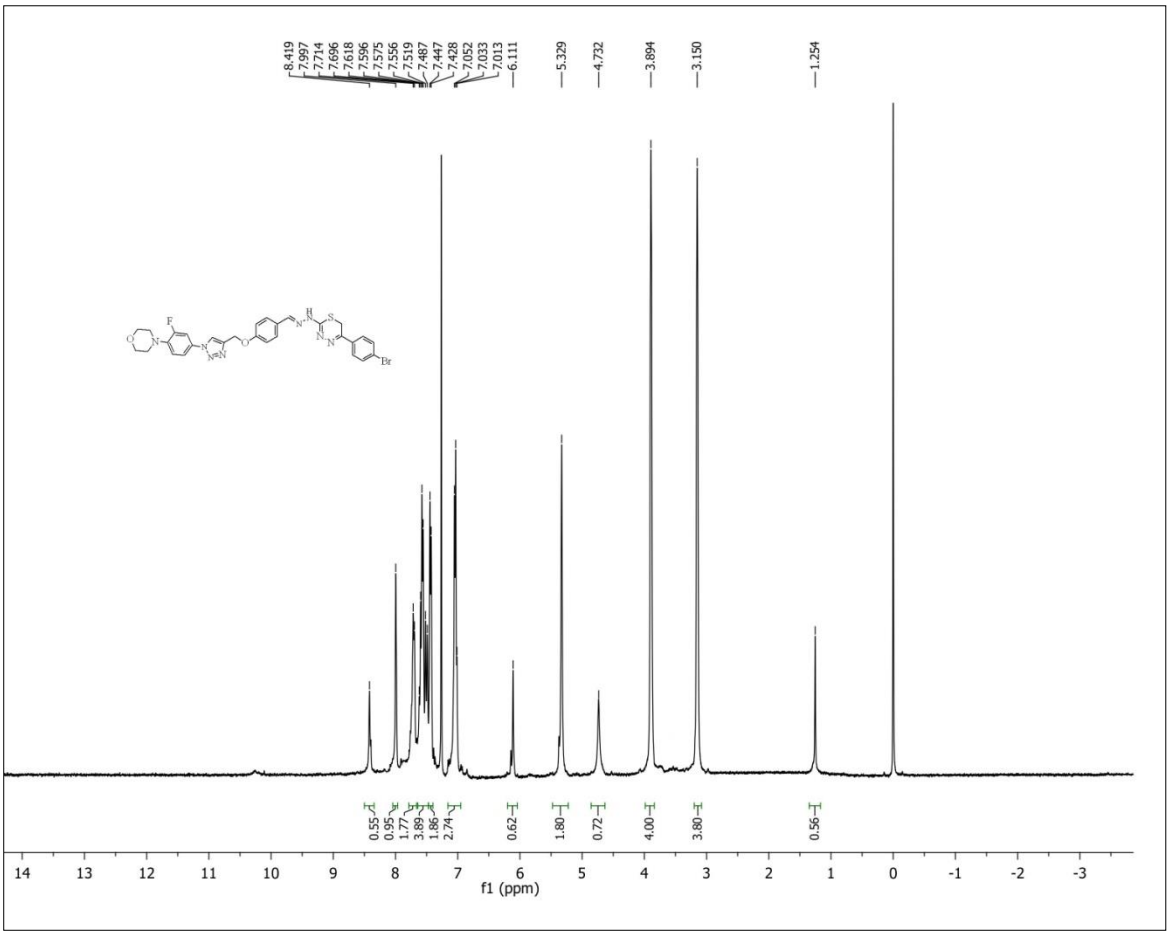
^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound 8e



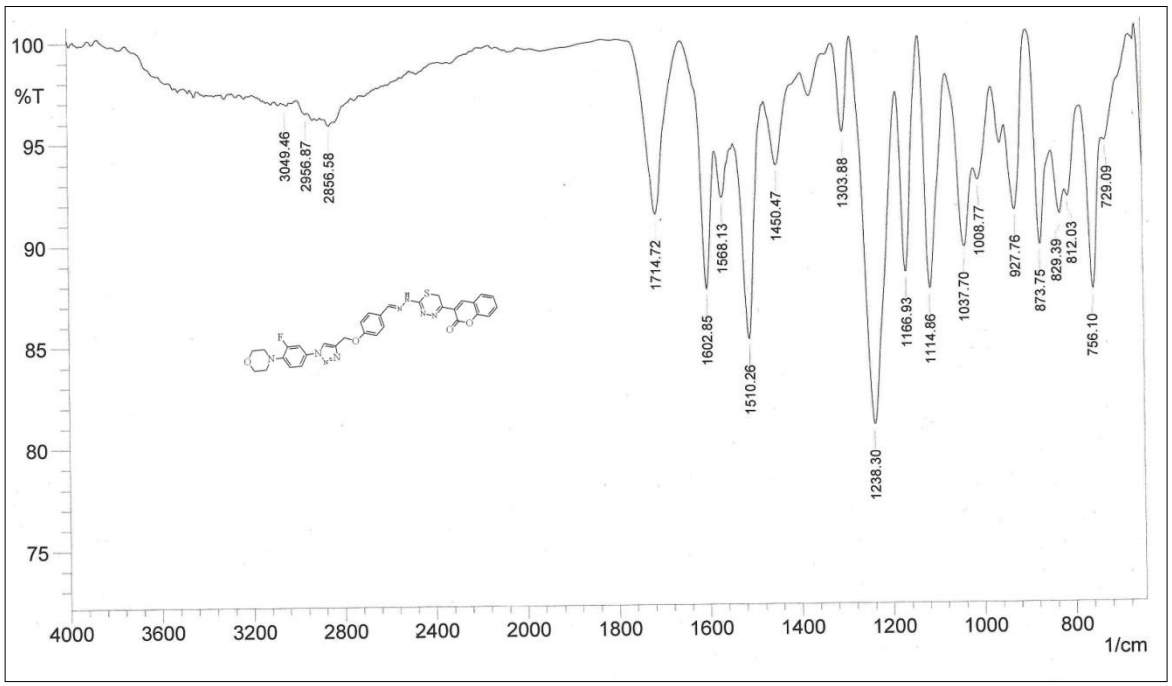
Mass spectrum of compound 8f (M.Wt: 605)



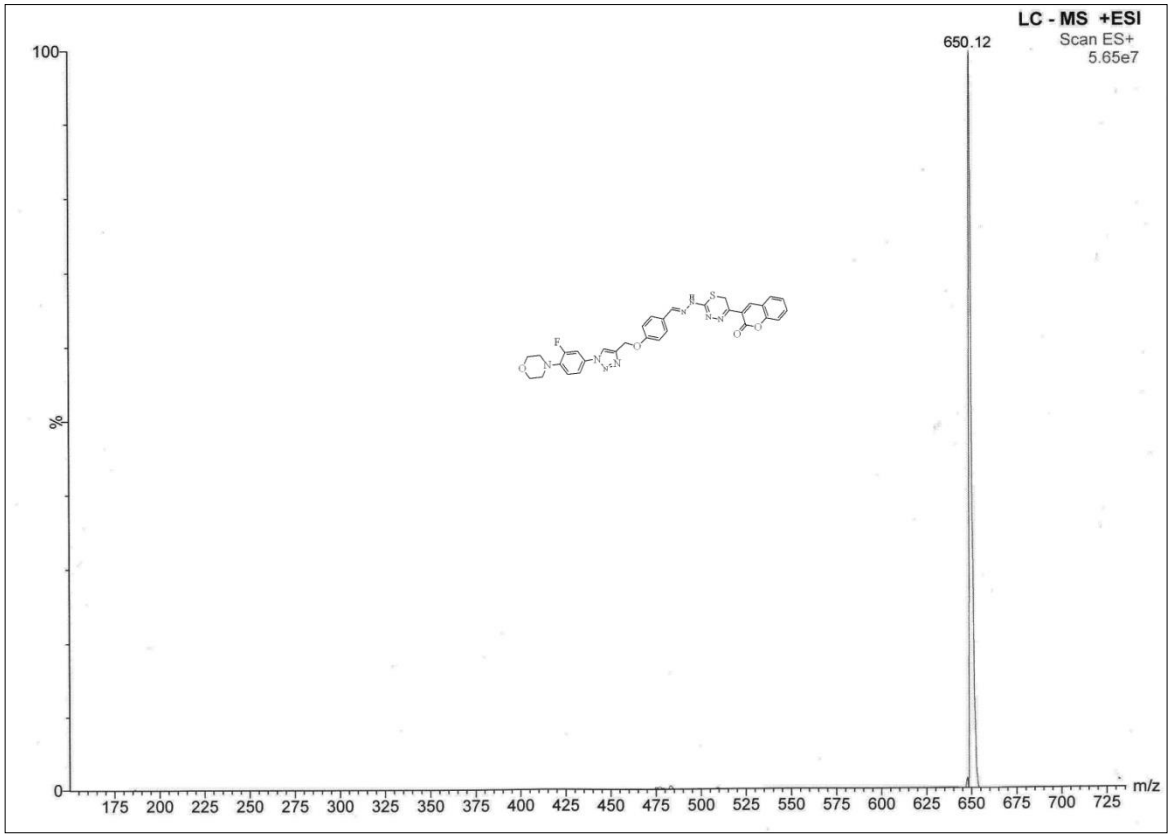
IR (KBr) spectrum of compound 8g



^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound 8g



IR (KBr) spectrum of compound 8i



Mass spectrum of compound 8i (M.Wt: 649)

CHAPTER-IV (SECTION-A)

ONE POT SYNTHESIS OF FUSED THIAZOLO[2,3-*b*]PYRIMIDINONE-PYRAZOLYLCOUMARIN HYBRIDS AND THEIR ANTICANCER, ANTIBACTERIAL ACTIVITY AND MOLECULAR DOCKING STUDIES

INTRODUCTION

Most of the literature surveys revealed that thiazolo[2,3-*b*]pyrimidinones have emerged as an important lead molecules in a variety of therapeutic areas, attracted the synthetic and medicinal chemists due to their broad spectrum of pharmacological properties[1] *viz*. Antibacterial[2-4], antiviral[5], antitumor[6,7], antioxidant[8], antinociceptive[9], antiparkinsonian[10], antimitotic[11,12], anti-inflammatory[13,14], antihypertensive[15], anticonvulsant[16], analgesic[17], and antibiofilm modulators[18]. Some of the related compounds have also been reported as calcium channel modulators[19,20], 5-HT_2 receptor antagonists[21,22] and also serve as inhibitors of xanthineoxidase[23], CDC25B phosphatase enzymes and the Bcl-2 family proteins[24].

Besides pyrazoles[25,26] and coumarin[27-31] derivatives are also gained much attention as bioactive pharmacophores associated with various biological activities[32]. On the other hand chalcones and their derivatives are also important structural motifs with a wide spectrum of biological activities[33]. **Fig. 1** represents a few of the literature reported and commercially important heterocyclic based drugs and bioactive molecules.

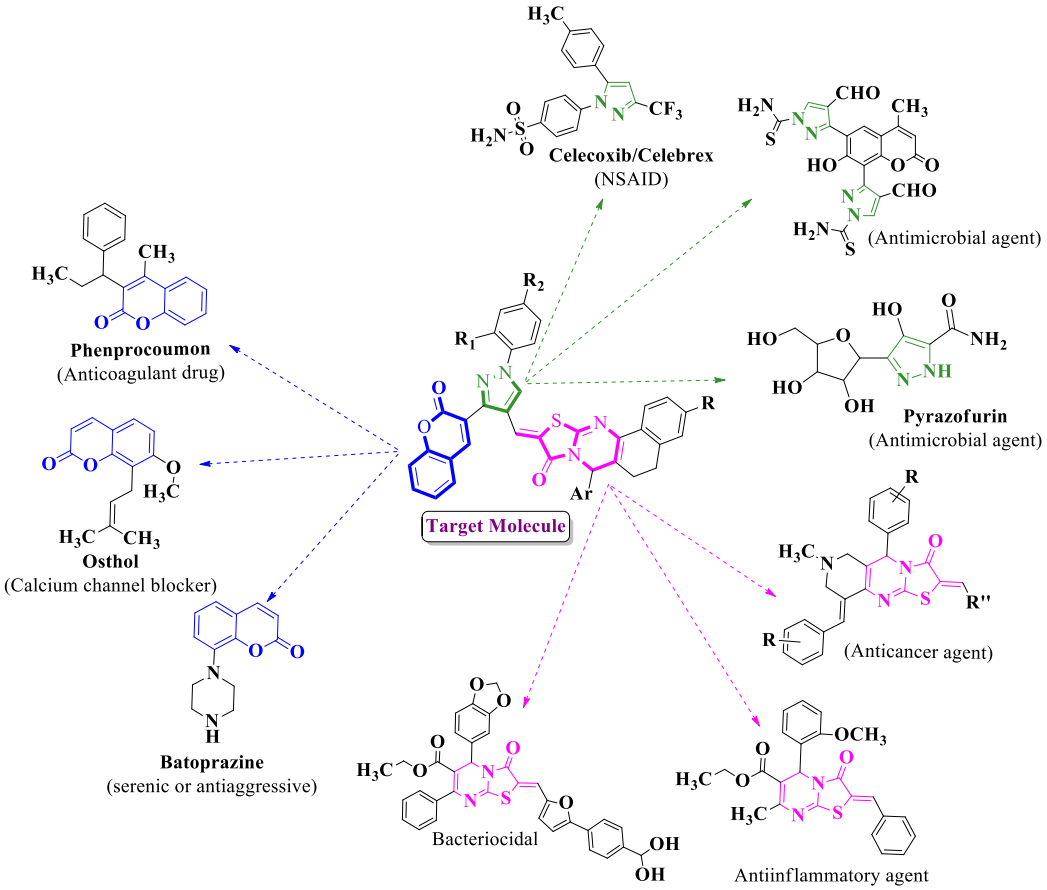


Fig. 1

Dan Zhao *et al.*[34] reported the biological evaluation of halogenated thiazolo[3,2-*a*]pyrimidin-3-one carboxylic acid derivatives targeting the YycG histidine kinase. The synthesized compounds were assessed for their *in vitro* antibacterial, antibiofilm and hemolytic activities. Most of the tested compounds exhibited good antibacterial and antibiofilm activity. In particular, compounds **1**, **2**, **3** and **4** exhibited promising inhibiting activity against *Staphylococcus epidermidis* and *Staphylococcus aureus* than the standard drug cefazolin (MIC= 17.6 µM) with MIC values of 1.56 µM.

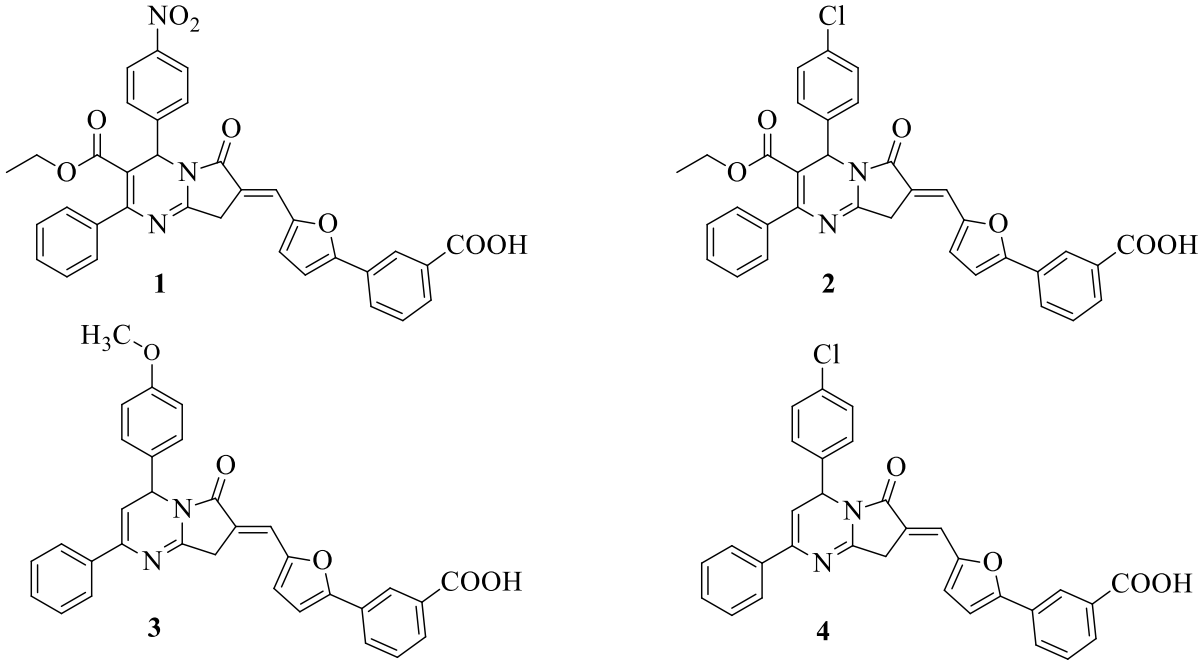


Fig. 2

Yaochun Xu and co-workers[35] reported the characterization of the stereo-chemical Structures of 2*H*-Thiazolo[3,2-*a*]pyrimidine compounds and their binding affinities for anti-apoptotic Bcl-2 family proteins. Interestingly, similar binding affinities for all three proteins were observed for both R and S enantiomers of most of the tested compounds.

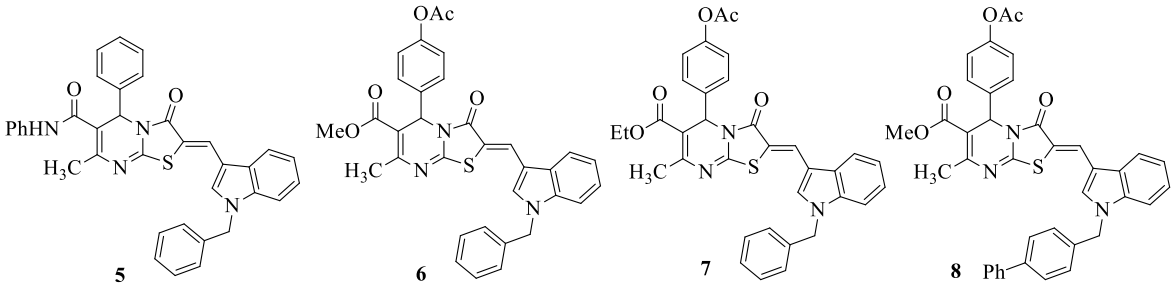


Fig. 3

Theivendran Panneer Selvam and his group[36] reported the synthesis and structure-activity relationship study of 2-(substitutedbenzylidene)-7-(4-fluorophenyl)-5-(furan-2-yl)-2*H*-thiazolo[3,2-*a*]pyrimidin-3(7*H*)-one derivatives as anticancer agents. The synthesized compounds were investigated for their *in vitro* anticancer activity on the U937-human histocytic lymphoma cell line by MTT assay by using gefitinib as standard. And from the results it was observed that the compounds **9-14** showed significant anticancer activity with IC_{50} values (μM) 3.04, 3.51, 4.04, 5.37, 5.81 and 6.42 respectively.

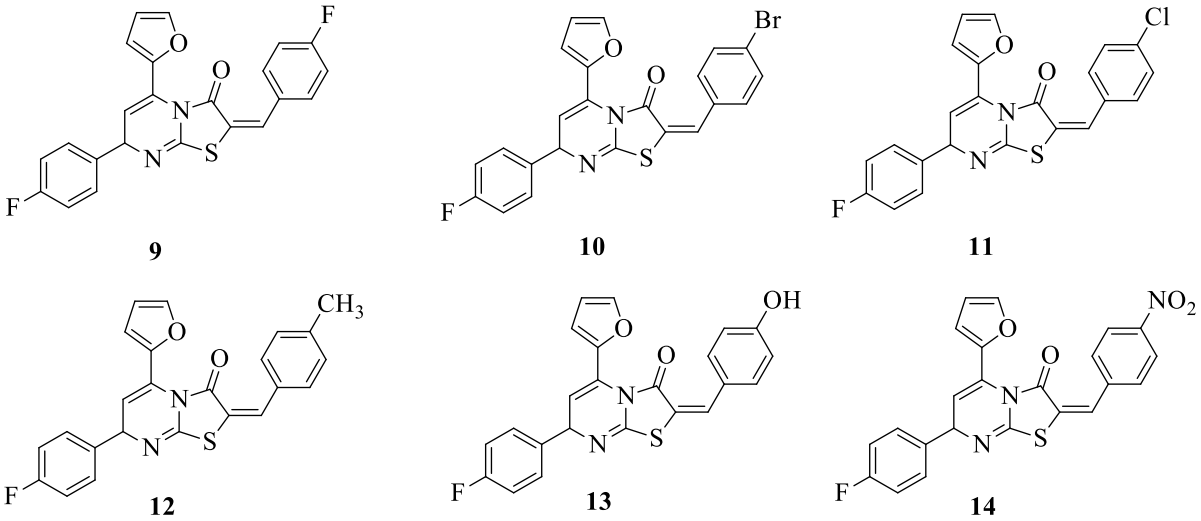
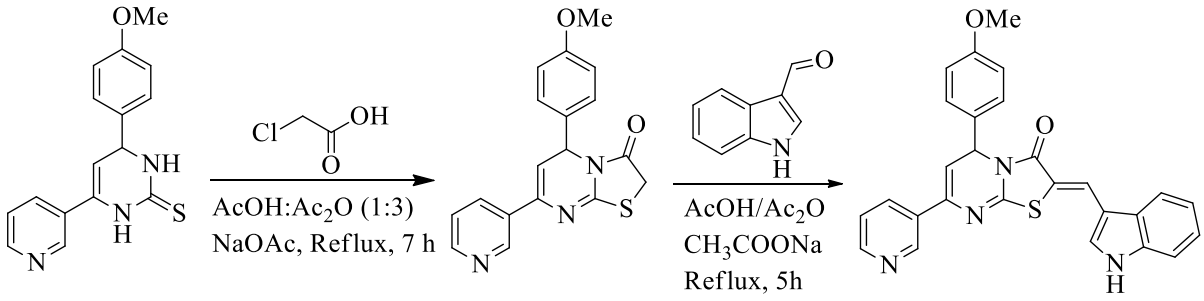


Fig. 4

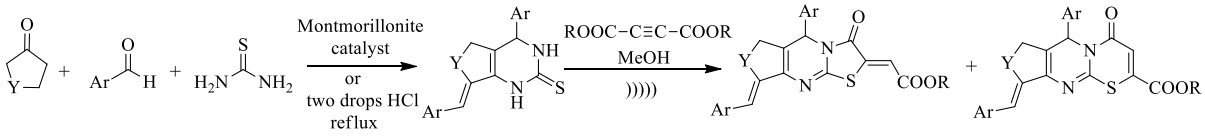
Various important approaches for the synthesis of thiazolo[2,3-*b*]pyrimidinones

N. A. Abdel-Latif and co-workers[37] reported the synthesis, analgesic, and antiparkinsonian profiles of some pyridine, pyrazoline, and thiopyrimidine derivatives.



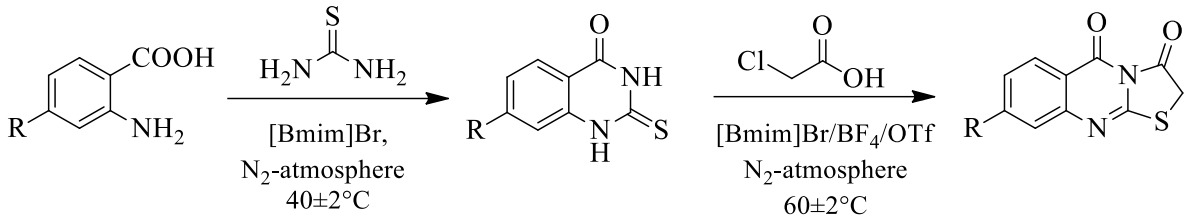
Scheme-1

A. Darehkordi *et al.*[38] described an efficient ultrasonic-assisted synthesis of the thiazolo[2,3-*b*] quinazoline and thiazolo[3,2-*a*] pyrimidine derivatives.



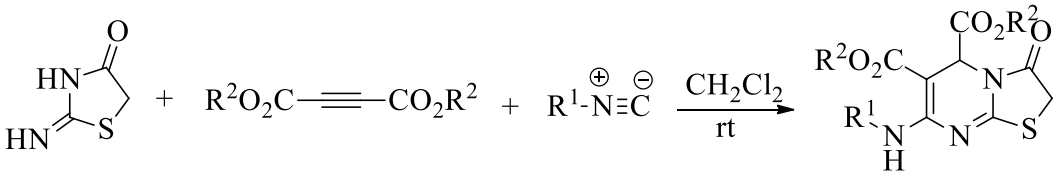
Scheme-2

A. K. Yadav *et al.*[39] developed an efficient ionic liquid mediated synthesis of substituted 5*H*[1,3]-thiazolo[2,3-*b*]quinazoline-3,5-(2*H*)-dione and 5*H*-thiazolo[2,3-*b*]quinazolin-5-one.



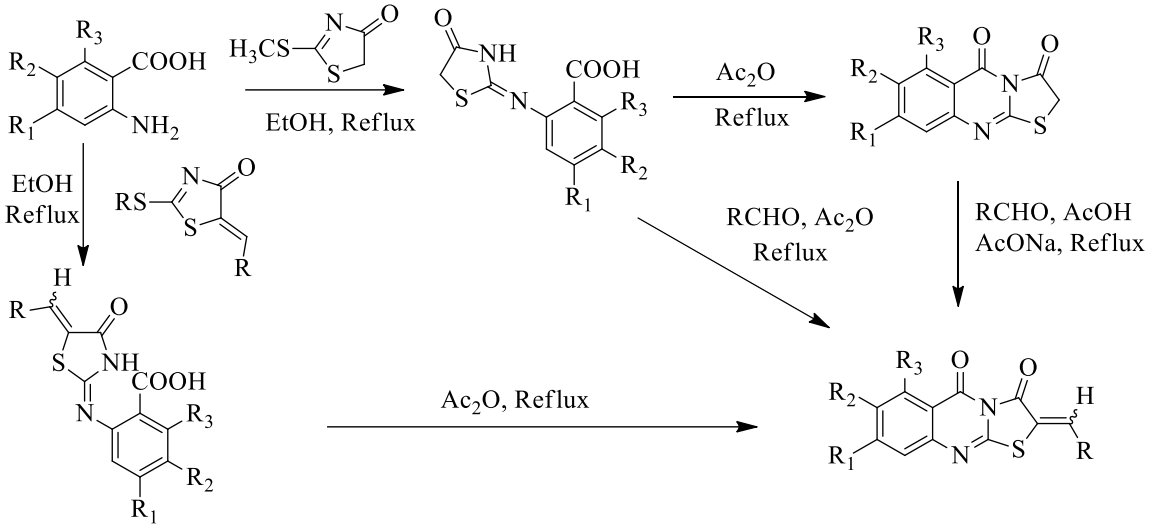
Scheme-3

A. A. Esmaeili and co-workers[40] reported an efficient regioselective synthesis of highly functionalized 3-oxo-2,3-dihydro-5*H*-thiazolo[3,2-*a*]pyrimidines *via* an isocyanide-based three-component reaction.



Scheme-4

A. I. Khodair[41] described a convenient synthesis of 2-Arylidene-5*H*-thiazolo[2,3-*b*]quinazo-line-3,5[2*H*]-diones and their benzoquinazoline derivatives.



Scheme-5

PRESENT WORK

After the cardiovascular diseases, cancer is the second most leading death causing multifactorial disease[42-44] resulted from a combined influence of genetic (inherited genes, genetic mutations and DNA damages) and environmental factors (exposure to UV-radiations, chemical agents and tobacco consumption). Malignancy is characterised by uncontrolled cell proliferation and differentiation[45]. High risk of toxicity, due to lack of specificity, causing adverse effects on rapidly dividing non-cancerous cells limiting the efficacy of most of the currently using chemotherapeutic in cancer therapy. The resistance of tumour cells towards the existing drugs also one of the major factors that escalating in search of new drugs. Hence, there is an emergency to find out and develop new anticancer lead molecules with target specificity, high rate of efficacy and with very low adverse side effects.

In addition, antimicrobial resistance (AMR) towards the existing antibiotics[46] becomes pandemic and also one of the major causes of mortality across the world. Excessive prophylaxis of antibiotics, new resistance mechanisms adopted by the microbes limiting the efficacy of existing chemotherapeutics. Hence it becomes a formidable challenge for the development of new and effective antimicrobial agents with a novel mode of action[47] in medicinal chemistry research.

Multicomponent reactions (MCRs)[48,49] are one of the interesting strategies that involve more than two easily accessible reactants join through covalent bonds to give multi-

functionalized complex structures in a single synthetic operation[50,51]. MCRs constitute one of the best tools for modern organic synthesis[52] because the product contains all of the essential parts of the starting materials (with the exception of condensation products)[53-55]. MCRs are one of the important sources for high degrees of molecular diversity[56]; hence this approach has become one of the powerful tools in modern drug discovery programme[57-62]. MCRs can offer significant advantages[63] over classical multi-step (step-wise synthetic route) synthesis[64] like fewer by-product production by reducing the number of synthetic steps, operational simplicity (workup, extraction and purification) under mild reaction conditions, cost, energy and time effective and rendering the transformations green[65-71]. Therefore the discovery of new MCRs with the green procedure is an interesting topic, especially in the areas of drug discovery, organic synthesis and combinatorial chemistry[72-74].

In the course of our longstanding research endeavour towards the development of new bioactive heterocycles[75-77], from the molecular design point of view and also by relying on the aforementioned biological data, here in we synthesized a series of novel heterocyclic hybrids (**6a-j**) embedding thiazolo[2,3-*b*]pyrimidinones and pyrazolyl chromenone moieties in their structural framework, hoping the synergistic influence of this combination on antibacterial and anticancer activity and to obtain new promising antibacterial and anticancer hits for further studies. All the synthesised compounds were assessed for their *in vitro* antibacterial studies against gram positive and gram negative bacteria and anticancer activity against diverse human cancer cell lines.

Molecular docking studies were also performed for the synthesised molecules in order to explore the possible binding modes and understand the mode of action of these compounds through their interactions with *E. coli* MurB enzyme, a key enzyme in peptidoglycan biosynthesis. The peptidoglycan layer of bacterial cell wall has been an important target in antibacterial chemotherapy since a long time. MurB, catalyzes the reduction of enolpyruvate moiety to a lactyl ether yielding UDP-*N*-acetylmuramate (UDP-MurNAc), a precursor of the cell wall[78,79]. Subsequently, this UDP-MurNAc is added with three amino acids and a dipeptide resulting in a pentapeptide. This pentapeptide when activated permits the cross-linking that gives cell wall its rigidity[80]. Further MurB along with other Mur proteins (Mur A-F, Y and G) catalyses various

biosynthetic transformations which are essential in the formation of the peptidoglycan layer of the bacterial cell wall[77,81]. Therefore MurB was selected as a target receptor.

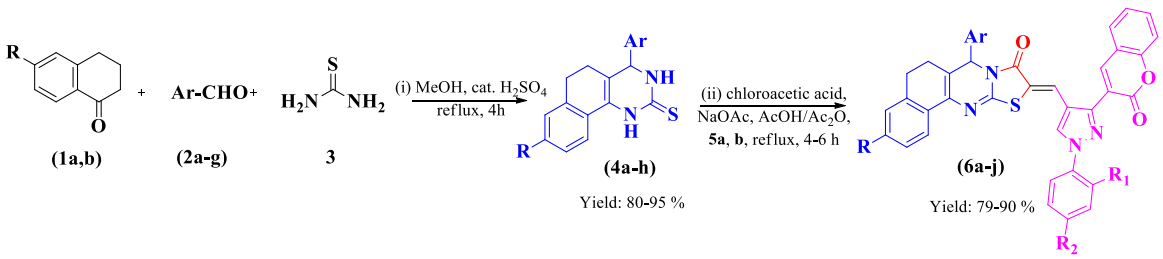
Preparation of starting materials

3-(2-oxo-2*H*-chromen-3-yl)-1-phenyl-1*H*-pyrazole-4-carbaldehyde 5a and 1-(2,4-dinitrophenyl)-3-(2-oxo-2*H*-chromen-3-yl)-1*H*-pyrazole-4-carbaldehyde 5b

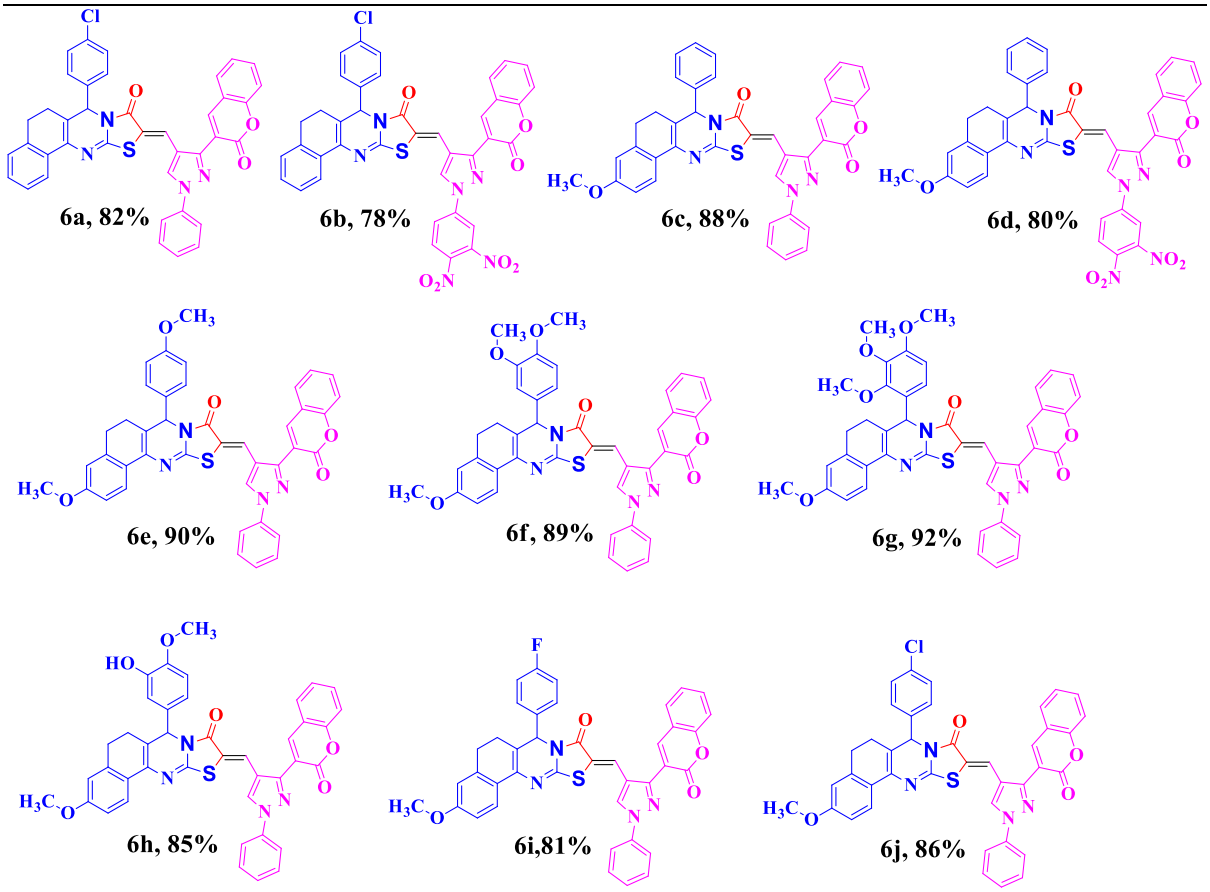
The key starting compounds were synthesized according to the literature procedure as described in **Chapter-II**, **Section-A**.

General procedure for the synthesis of 10-((3-(2-oxo-2*H*-chromen-3-yl)-1-aryl-1*H*-pyrazol-4-yl)methylene)-7-aryl-7,10-dihydro-5*H*-benzo[*h*]thiazolo[2,3-*b*]quinazolin-9(6*H*)-ones (6a-j)

A mixture of modified Biginelli products (**4a-h**), monochloroacetic acid, anhydrous sodium acetate, glacial acetic acid, acetic anhydride and aldehydes (**5a** or 5**b**) were heated for 4-6 h. The progress of the reaction was monitored by TLC; upon completion of the reaction, the reaction mixture was cooled to room temperature and poured in to the crushed ice with vigorous stirring. The obtained solid was filtered under suction, washed with cold water and recrystallized form glacial acetic acid afforded the analytically pure products (**6a-j)** in excellent yields.



Scheme-6



Isolated yield.

Results and discussion

The synthetic strategy adopted to obtain the titled compounds (**6a-j**) was outlined in **Scheme 6**. The target compounds were achieved by the one-pot three-component condensation of modified Biginelli product, fused 3,4-dihydropyrimidin-2(1*H*)-thiones (**4a-h**), mono chloroacetic acid and 3-(2-oxo-2*H*-chromen-3-yl)-1-aryl-1*H*-pyrazole-4-carbaldehyde (**5a**,**b**) in refluxing AcOH in the presence of Ac_2O and NaOAc furnished the desired products in good yields. The structure elucidation of newly synthesised compounds was well established by the FTIR, NMR and mass spectral studies as well as elemental analyses (C, H and N). In the IR spectrum of compound **6a**, the appearance of sharp bands at 1720 cm^{-1} (coumarin C=O stretching), 1709 cm^{-1} (thiazole C=O stretching), medium band at 1631 cm^{-1} (pyrimidine C=N stretching), sharp band at 1596 cm^{-1} (pyrazole C=N stretching)and the absorption band at 1532 cm^{-1} ascribed to C=C stretching frequency of α,β-unsaturated carbonyl. From the ^1H NMR spectrum, the presence of a singlet at 8.41 ppm (pyrazolyl methylene, =CH– proton) instead of a

multiplet for fused thiazole –CH_2– protons of unseparated intermediate and appearance of a signal at 144.00 ppm (pyrazolyl methylene, =CH– carbon) from ^{13}C NMR, and the molecular ion peak of the mass spectrum as well as elemental analyses data confirmed the formation of the product (**6a**).

Biological Activity

Anti-proliferative activity

In vitro cytotoxi*c* activity was carried out against human colorectal adenocarcinoma (Colo 205), chronic myelogenous leukaemia (K562), breast adenocarcinoma (MCF-7), breast adenocarcinoma (MDA-MB-231), hepatocellular Carcinoma Chronic Myelogenous (Hep G2), Human Embryonic Kidney 293 cells (HEK293). One of the most effective anticancer agents, Doxorubicin (DOX) was used as a positive control (reference) and the results were summarised in **Table 1**. The cell lines were obtained from the National Centre for Cell Sciences, Pune, India, and were cultured at a seeding density of 0.2×106 in DMEM/RPMI medium supplemented with 10% FBS, 100 UmL^{-1} penicillin, and 100 μgmL^{-1} streptomycin, respectively, and maintained in a humidified atmosphere with 5% CO_2 at 37±1 °C. The samples were dissolved in dimethylsulfoxide (DMSO; not exceeding the final concentration of 0.01%) and further diluted in cell culture medium. The antiproliferative response of the extract was assessed by the quantitative colorimetric 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay[82] Cells (∼10,000) were plated in 200 μL growth medium in the presence or absence of the test sample (10 μM concentration) in 96-well culture plates for 24 h. Then the culture plates were centrifuged at 2000 rpm for 10 min at room temperature. 100 μL of supernatant was discarded and 20 μL of MTT (5 $mgmL^{-1}$ in PBS) was added to each well and incubated for 4h at 37±1 °C. The viability of the cells was determined using a spectrophotometer at 570 nm. HEK 293 cells were screened to evaluate toxicity of the sample. The response parameter was expressed in the average percentage of inhibition of samples at 10 μM concentrations and the experiment was performed in triplicate and the results were taken as a mean ± standard deviation (SD) and are given in **Table 1**.

In vitro, cytotoxic activity results revealed that all of the tested samples exhibited moderate to weak inhibiting activity and most of the synthesised compounds were inactive. However, the derivatives **6a**, **6g**, **6h** and **6j** have shown moderate

antiproliferative activity when compared with reference drug doxorubicin (DOX) against the Hep G2 and Colo 205 tumour cell lines with an average percentage of inhibition (Avg % inh.) ranging from 35.54 to 40.34. Among the moderately potent test compounds, **6a**, **6h** and **6j** exhibited good antiproliferative activity against the Hep G2 with an average percentage of inhibition values, 40.34, 39.69 and 36.31 respectively, and the compound **6g** have shown good antiproliferative activity against both Colo 205 and Hep G2 tumor cell lines with an average percentage of inhibition values, 35.54 and 36.17 respectively (**Table 1**). On overall comparison, compounds derived from 4-chloro phenyl (**6a** and **6j**), 2,3,4-trimethoxy phenyl (**6g**) and 3-hydroxy4-methoxy phenyl (**6h**) have shown modest anticancer activity against the tested cell lines. Hence, further optimisations of the compounds may enhance their antiproliferative activity and they can be considered as a lead molecule for the development of new anticancer drugs.

Table-1. Antiproliferative activity (Avg. % of inhibition ± SD[a]**)** of compounds **6a-j** and reference Doxorubicin (DOX) against a panel of tumor cell lines.

	Cancer cell-lines					
Analogs	**Colo 205**	**K562**	**MCF-7**	**MDA-MB-231**	**Hep G2**	**HEK 293**
6a	NA	11.76±2.65	18.38±1.50	15.26±1.70	40.35±1.55	-2.60±1.50[b]
6b	7.29±1.75	17.73±1.32	14.90±0.90	20.86±1.27	21.67±3.33	-11.17±0.64
6c	7.94±1.65	15.78±1.50	15.39±1.14	15.46±1.36	15.23±2.96	-0.25±6.14
6d	9.31±1.87	22.22±1.65	10.31±1.84	15.22±2.43	14.29±2.07	-1.60±1.31
6e	13.25±1.51	11.17±0.97	11.00±1.43	17.98±6.62	13.40±1.98	-1.99±1.63
6f	14.88±3.78	12.63±2.47	19.78±2.65	21.75±1.56	22.46±2.21	-1.06±2.68
6g	35.54±1.21	7.12±2.13	15.32±3.16	20.37±1.39	36.18±1.88	-5.14±3.39
6h	3.41±3.53	15.19±2.66	18.11±1.75	NA	39.70±3.04	-4.05±1.82
6i	11.70±1.47	NA	10.65±2.27	NA	19.95±2.14	-10.78±3.07
6j	25.50±1.85	NA	28.48±0.91	NA	36.32±2.63	-12.49±3.80
DOX[c]	91.55±1.87	95.57±2.22	97.61±2.19	98.66±3.26	92.35±1.57	5.68±1.56

[a]Values are mean ± SD of three replicates; [b]Negative values indicates, samples were not toxic against normal cell line HEK 293; [c]DOX-Doxorubicin (positive control) and DMSO used as a negative control; NA-Not active.

Antibacterial Activity

All the synthesized compounds (**6a-j)** were assessed for their *in vitro* antibacterial activity against both Gram-positive (G^+) bacterial strains including *Staphylococcus aureus* (MTCC 121), *Bacillus subtilis* (MTCC 96) and *Staphylococcus epidermidis* (MTCC 2639) and Gram-negative (G^-) bacteria including *Escherichia coli* (MTCC 40), *Klebsiella pneumonia* (MTCC 109) and *Pseudomonas aeruginosa* (MTCC 2453). Standard pathogenic microbial cultures were procured from the Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, India, which was recognised by the World Intellectual Property Organization (WIPO). The experiments were carried out in triplicate and the results were taken as a mean ± standard deviation (SD). The minimum inhibitory concentrations (MICs) for all the synthesised compounds after 24 h incubation in the darkness at 37±1 ^oC in a humid atmosphere were reported in $\mu g\ ml^{-1}$ and the results were illustrated in **Table-2**. Antibacterial activity was assessed by the standard broth microdilution technique[83,84]. The antibiotics, penicillin and streptomycin were used as positive controls (standards) and DMSO was used as a negative control (solvent control) and they were also screened under identical conditions for the comparison of activity results.

Table 2. *In vitro* antibacterial activity data for the test compounds (**6a**–**j)**

Analogs	**MIC ($\mu g\ mL^{-1}$)**					
	S. aureus	***B. subtilis***	***S. epidermidis***	***E. coli***	***K. pneumonia***	***P. aeruginosa***
6a	25±0.11	–	25±0.15	–	50±0.56	50±0.29
6b	–	–	50±0.22	–	50±0.63	6.25±0.21
6c	–	50±0.45	25±0.36	12.5±0.25	12.5±0.61	_
6d	50±0.35	–	–	–	12.5±0.23	25±0.17
6e	25±0.62	25±0.46	12.5±0.9	50±0.32	50±0.79	6.25±0.79
6f	1.56±0.22	12.5±0.71	12.5±0.3	12.5±0.44	12.5±0.58	6.25±0.15
6g	1.56±0.35	1.56±0.45	3.12±0.66	6.25±0.70	6.25±0.4	12.5±0.23
6h	3.12±0.28	6.25±0.19	12.5±0.37	50±0.68	25±0.3	6.25±0.16
6i	–	50±0.82	–	25±0.15	12.5±0.33	–
6j	12.5±0.19	25±0.30	25±0.36	–	25±0.39	12.5±0.66
Streptomycin	6.25±0.25	6.25±0.70	3.125±0.45	6.25±0.82	3.125±0.96	1.562±0.69
Penicillin	1.562±0.21	1.562±0.65	3.125±0.22	12.5±0.35	6.25±0.88	12.5±0.74

MIC ($\mu g\ ml^{-1}$), minimum inhibitory concentration, i.e., the lowest concentration of the test compound to inhibit the growth of bacteria completely; "_" Indicates the concentration >100 $\mu g\ mL^{-1}$.

It is evident from **Table 2** that, the majority of the tested compounds (**6b**, **6c**, **6e**, **6f**, **6g**, **6h** and **6j)** exerted significant *in vitro* antibacterial inhibiting activity against all the tested bacterial strains with MICs ranging from 1.56 to 12.5 μg mL^{-1}. Among them, the compound **6g** with 2,3,4-trimethoxy substituents on phenyl ring was found to be stronger and have shown equipotent inhibitory efficacies and broader antibacterial spectrum than that of the reference drugs. Compound **6g** exhibited excellent inhibiting activity than the standard streptomycin (MIC = 6.25 μg mL^{-1}) and equipotent to that of penicillin (MIC = 1.562 μg mL^{-1}) against *S. aureus* and *B. subtilis* with MIC values 1.56 μg mL^{-1}, exerted nearly as active as positive control drugs (MIC = 3.125 μg mL^{-1}) against gram positive *S. epidermidis*(MIC = 3.12 μg mL^{-1}) and effectively inhibited the gram-negative *E. coli* and *K. pneumonia* (MIC = 6.25 μg mL^{-1}) and also demonstrated inhibitory potency against *P. aeruginosa* (MIC = 12.5 μg mL^{-1}) equal to that of the standard penicillin. Compounds **6f** and **6h** could effectively inhibit the growth of *S. aureus* with MIC values, (MIC = 1.56 and 3.12 μg mL^{-1} respectively) and *P. aeruginosa* (MIC = 6.25 μg mL^{-1}). Compound **6c** and **6f** exhibited inhibition of *E. coli* (MIC = 12.5 μg mL^{-1}) nearly to that of penicillin. Compounds **6b**, **6e**, **6f** and **6h** have shown bioactivity against *P. aeruginosa* (MIC = 6.25 μg mL^{-1}) which was better than penicillin. Finally, the compounds **6h** and **6j** have shown equipotent activity than that of standards streptomycin and penicillin respectively against *B. subtilis* and *P. aeruginosa* ((MIC = 6.25 and 12.5 μg mL^{-1}).While, the rest of the compounds (**6a**, **6d** and **6i**) have shown modest activity against all the tested strains with MIC values ranging from 12.5 to 50 μg mL^{-1}.

Structure activity relationship (SAR)

Interestingly, it was observed from experimental data (**Table 2**) that, the most of the analogues displayed potent bioactivity against gram-positive pathogenic strains, *i.e*., *B. subtilis*, *S. aureus* and *S. epidermidis* with MIC values ranging from 1.56 to 3.12 μg mL^{-1}and displayed moderate to good activity against gram-negative microorganisms, *i.e*., *E. coli*, *P. aeruginosa* and *K. pneumonia* with MICs ranging from 6.25 to 12.5 μg mL^{-1}. From the above *in vitro* results, overall we can conclude that the derivatives **6e**, **6f**, **6g** and **6h** derived from 4-,3,4-di, 2,3,4-trimethoxyphenyl and 3-hydroxy-4-methoxyphenyl moieties on the thiazoloquinazoline ring were found to be potent antibacterial agents.

Molecular docking study

Molecular docking studies were performed using Schrodinger suite 2010. Initially, the crystal structure of target enzyme MurB (PDB id: 1MBB) was obtained from protein data bank (http://www.rcsb.org/pdb). It was prepared, refined and minimised using protein preparation wizard available in the Schrodinger suite 2010. Later receptor grid was generated around the active site of the enzyme using GLIDE 5.6 (Schrödinger LLC, 2010), Glide, version 5.6. New York). During grid generation, the receptor Vander Waals scaling was set to 0.9[85]. Meanwhile, the ligands were drawn in Maestro build panel and prepared by LigPrep module available in the same suite. Finally, the low energy conformers of the prepared ligands were docked into the active site of the MurB enzyme. Docking results are tabulated in **Table 3**, energetically most favoured dock pose of each ligand was analyzed for interactions with the target receptor.

The docking studies clearly showed that the best active compound in the series (**6g**) can act as good anti-bacterial agent which is evident from its high dock score (-6.098 kcal mol^{-1}). It showed three hydrogen bond interactions with Tyr158, Lys 217, Lys 275 (**Figure 2**) and a π-π stacking interaction with Arg 159 (**Figure 3**). On the other hand compounds, **6c** and **6f** which possessed good activity values next to compound **6g** in the series showed dock scores of -5.005 and -5.248 kcal mol^{-1} respectively (**Table 3**). Compound **6c** showed two hydrogen bonds with Lys 217 and Gln 288 whereas compound **6f** showed three hydrogen bonds with Ser 229, Lys 262, Tyr 254 (**Figure 2**) and a π-cationic interaction with Lys 217 (**Figure 3**). Compounds **6h** and **6e** which exhibited poor biological activities showed poor dock scores of -3.656 and -2.968 kcal mol^{-1} respectively. Further compound **6h** showed only one hydrogen interaction with Gln 287 and hydrogen bond interactions were completely absent in compound **6e**. This might be one of the reasons for their low biological activity. Compound **6i** which exhibited relatively better activity compared to **6h** and **6e** showed a dock score of -4.294 kcal mol^{-1} and formed a hydrogen bond with Gln 288. It also showed a π-cationic interaction with Lys 262. The docking results well corroborated with *in vitro* antibacterial studies indicating that these compounds can be further optimised and developed as lead compounds.

Table 3. Dock scores and hydrogen bond interactions of ligands with *E. coli* (PDB id: 1MBB) obtained from docking studies.

Compound	MIC (μg mL^{-1})	Dock Score (kcal/mol)	Residues involved in hydrogen bonding
6c	12.5±0.25	-5.005	Lys 217, Gln 288
6e	50±0.32	-2.968	--
6f	12.5±0.44	-5.248	Ser 229, Tyr 254, Lys 262,
6g	6.25±0.70	-6.098	Tyr 158, Lys 217, Lys 275
6h	50±0.68	-3.656	Gln 287
6i	25±0.15	-4.294	Gln 288

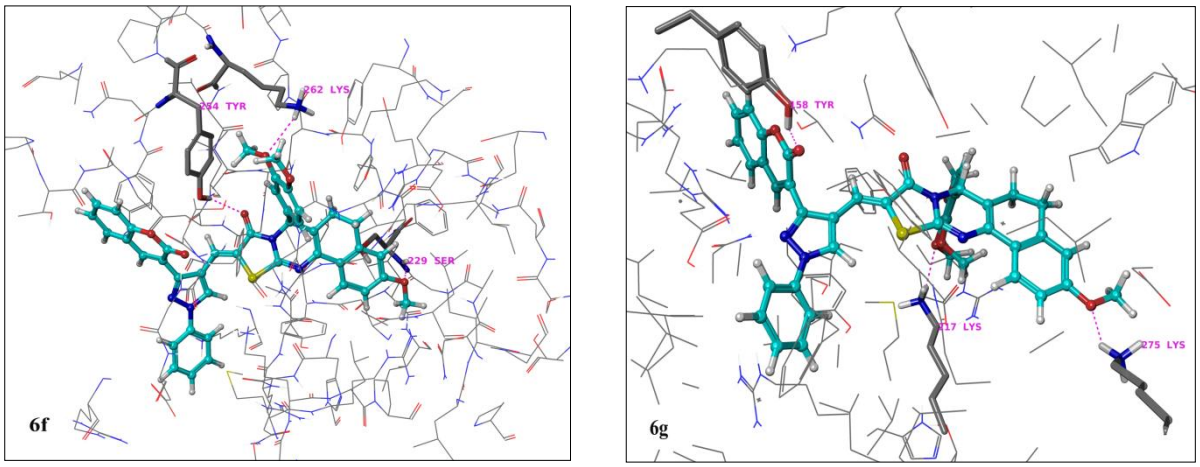


Figure 2. Docked conformations of **6f** and **6g** (ball and stick) in the active site of MurB (thin wire) showing hydrogen bond interactions (pink dotted lines).

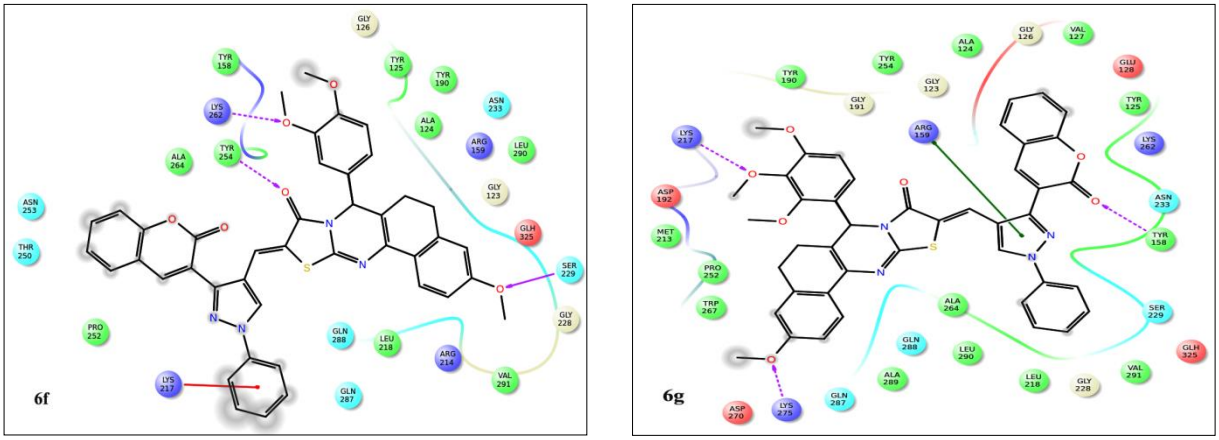


Figure 3. Ligand interaction diagram of compounds **6f** and **6g** showing hydrogen bond interactions (pink dotted and thick lines), π-π stacking interactions (green) and π-cationic interactions (red).

Conclusion

In summary, a series of novel 10-((3-(2-oxo-2*H*-chromen-3-yl)-1-aryl-1*H*-pyrazol-4-yl)methylene)-7-aryl-7,10-dihydro-5*H*-benzo[*h*]thiazolo[2,3-*b*]quinazolin-9(6*H*)-one derivatives (**6a-j**) were designed and synthesised by the one pot three-component approach with the hope of discovering new bioactive molecular hybrids with enhanced broad spectrum of pharmacological activities. All the newly synthesised compounds were well characterised by spectral and elemental analyses. And were investigated for their *in vitro* antiproliferative and antibacterial activities by MTT and broth microdilution technique respectively. From the experimental studies, it was revealed that, among the synthesized compounds (**6a-j**), the derivatives **6a**, **6b**, **6c**, **6e**, **6f**, **6g**, **6h** and **6j** were found to possess good biological activity. Among them, compounds **6a**, **6g**, **6h** and **6j** were found to have moderate antiproliferative activity against Hep G2 and Colo 205 cell lines. From the antibacterial data, compounds **6f**, **6g** and **6h** showed broad and excellent antibacterial efficacy against both G^+ and G^- strains comparable to that of the standards. The compounds **6b**, **6c**, **6e** and **6j** exhibited excellent inhibiting activity against G^- strains. The rest of the compounds **6a**, **6d** and **6i** displayed no antibacterial activity and were found to be inactive. These *in vitro* antibacterial studies were further supported by molecular docking. Overall, from the *in vitro* anticancer and antibacterial studies, we can conclude that the presence of 4-chlorophenyl, mono, di and tri-methoxy phenyl and 3-hydroxy-4-methoxyphenyl moieties on the thiazolo-quinazoline scaffold has been suggested to be responsible for the *in vitro* antiproliferative and antibacterial activities of the title compounds. Based on the above results, the synthesised series of compounds could be potential candidates for further development of novel anticancer and antimicrobial agents.

Experimental

General procedure for the synthesis of 10-((3-(2-oxo-2*H*-chromen-3-yl)-1-aryl-1*H*-pyrazol-4-yl)methylene)-7-aryl-7,10-dihydro-5*H*-benzo[*h*]thiazolo[2,3-*b*]quinazolin-9(6*H*)-ones (6a-j)

A mixture of compound **5** (1 mmol), mono chloroacetic acid (1.5 mmol), anhydrous sodium acetate (2 mmol), glacial acetic acid (2 ml), acetic anhydride (1.5 ml) and 1-(phenyl/2,4-dinitrophenyl)-3-(2-oxo-2*H*-chromen-3-yl)-1*H*-pyrazole-4-carbaldehyde (**5a**,**b**) (1 mmol) was refluxed at 60 ^oC for 4-6 h. The progress of the reaction was

monitored by TLC; upon completion of the reaction, the reaction mixture was cooled to room temperature and poured in to the crushed ice with vigorous stirring. The obtained precipitated solid was filtered under suction, washed with cold water and recrystallized form glacial acetic acid afforded the analytically pure products (**6a-j)** in excellent yields.

Spectral data:

7-(4-chlorophenyl)-10-((3-(2-oxo-2*H*-chromen-3-yl)-1-phenyl-1*H*-pyrazol-4-yl)methylene)-7,10-dihydro-5*H*-benzo[*h*]thiazolo[2,3-*b*]quinazolin-9(6*H*)-one (6a)

Yellow solid; Yield: 82 %; M.P.: 273-275; **IR** (KBr, cm^{-1}) υ_{max}: 1720 cm^{-1} (coumarin C=O), 1709 cm^{-1} (thiazole C=O), 1631 cm^{-1} (pyrimidine C=N), 1596 cm^{-1} (pyrazole C=N), 1532 cm^{-1} (C=C of *α*, *β*-unsaturated carbonyl); **^{1}H NMR** (400 MHz, DMSO-d_6): δ 1.90-2.33 (m, 2H), 2.63-2.78 (m, 2H), 5.86 (s, 1H), 7.14 (d, 1H, *J* = 6.8 Hz), 7.19-7.27 (m, 2H), 7.42-7.50 (m, 7H), 7.54-7.61 (m, 3H), 7.69 (d, 1H, *J* = 6.8 Hz), 7.80 (d, 2H, *J* = 7.2 Hz), 7.85 (d, 2H, *J* = 8.0 Hz), 8.05 (d, 1H, *J* = 8.0 Hz), 8.78 (s, 1H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 165.02, 159.81, 154.21, 150.22, 148.79, 144.02, 139.15, 137.51, 135.28, 134.78, 134.64, 132.52, 132.43, 129.75, 129.04, 128.48, 128.01, 127.89, 127.37, 127.22, 126.75, 124.77, 123.32, 121.64, 120.25, 119.72, 119.63, 118.91, 118.41, 116.78, 115.64, 59.35, 27.43, 24.99; **MS** (ESI) *m/z*: 665 $[M]^+$;Anal. calcd. for $C_{39}H_{25}ClN_4O_3S$: C, 70.42; H, 3.79; N, 8.42. Found: C, 70.71; H, 3.93; N, 8.72.

7-(4-chlorophenyl)-10-((1-(3,4-dinitrophenyl)-3-(2-oxo-2*H*-chromen-3-yl)-1*H*-pyrazol-4-yl)methylene)-7,10-dihydro-5*H*-benzo[*h*]thiazolo[2,3-*b*]quinazolin-9(6*H*)-one (6b)

Orange solid; Yield:79 %; M.P.: 236-238; **IR** (KBr, cm^{-1}) υ_{max}: 1719 cm^{-1} (coumarin C=O), 1706 cm^{-1} (thiazole C=O), 1633 cm^{-1} (pyrimidine C=N), 1597 cm^{-1} (pyrazole C=N), 1538 cm^{-1} (C=C of *α*, *β*-unsaturated carbonyl); **^{1}H NMR** (400 MHz, DMSO-d_6): δ 1.88-2.34 (m, 2H), 2.61-2.79 (m, 2H), 5.88 (s, 1H), 7.13-7.50 (m, 10H), 7.68-7.72 (m, 1H), 7.79-7.85 (m, 2H), 8.21 (s, 1H), 8.39 (d, 1H, *J* = 9.2 Hz), 8.72-8.74 (m, 1H); 8.93-8.95 (m, 2H); **MS** (ESI) *m/z*: 755 $[M]^+$;Anal. calcd. for $C_{39}H_{23}ClN_6O_7S$: C, 62.03; H, 3.07; N, 11.13. Found: C, 61.86; H, 3.22; N, 10.82.

3-methoxy-10-((3-(2-oxo-2*H*-chromen-3-yl)-1-phenyl-1*H*-pyrazol-4-yl)methylene)-7-phenyl-7,10-dihydro-5*H*-benzo[*h*]thiazolo[2,3-*b*]quinazolin-9(6*H*)-one (6c)

Yellow solid; Yield:88 %; M.P.: 268-270; **IR** (KBr, cm^{-1}) υ_{max}: 1717 cm^{-1} (coumarin C=O), 1711 cm^{-1} (thiazole C=O), 1635 cm^{-1} (pyrimidine C=N), 1606 cm^{-1} (pyrazole C=N), 1537 cm^{-1} (C=C of *α*, *β*-unsaturated carbonyl); **^{1}H NMR** (400 MHz, DMSO-d_6): δ 1.86-1.93 (m, 1H), 2.27-2.32 (m, 1H), 2.56-2.67 (m, 1H), 2.71-2.77 (m, 1H), 3.75 (s, 3H), 5.80 (s, 1H), 6.74-6.84 (m, 2H), 7.29-7.36 (m, 5H), 7.41-7.72 (m, 8H), 7.84 (d, 1H, *J* = 6.8 Hz), 8.05 (d, 2H, *J* = 8.4 Hz), 8.41 (s, 1H), 8.78 (s, 1H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 165.05, 159.42, 154.20, 148.73, 144.00, 139.24, 137.17, 134.09, 132.48, 129.74, 128.76, 128.46, 128.27, 127.84, 127.16, 125.70, 124.74, 124.60, 121.10, 120.29, 120.00, 119.72, 118.91, 118.51, 116.77, 113.79, 113.58, 111.23, 60.03, 55.32, 27.90, 24.99; **MS** (ESI) *m/z*: 662 $[M + H]^+$; Anal. calcd. for $C_{40}H_{28}N_4O_4S$: C, 72.71; H, 4.27; N, 8.48. Found: C, 72.50; H, 4.55; N, 8.71.

10-((1-(3,4-dinitrophenyl)-3-(2-oxo-2*H*-chromen-3-yl)-1*H*-pyrazol-4-yl)methylene)-3-methoxy-7-phenyl-7,10-dihydro-5*H*-benzo[*h*]thiazolo[2,3-*b*]quinazolin-9(6*H*)-one (6d)

Orange solid; Yield: 81 %; M.P.: 233-235; **IR** (KBr, cm^{-1}) υ_{max}: 1720 cm^{-1} (coumarin C=O), 1709 cm^{-1} (thiazole C=O), 1634 cm^{-1} (pyrimidine C=N), 1605 cm^{-1} (pyrazole C=N), 153 cm^{-1} (C=C of *α*, *β*-unsaturated carbonyl); **^{1}H NMR** (400 MHz, DMSO-d_6): δ 1.90-1.93 (m, 1H), 2.27-2.31 (m, 1H), 2.57-2.61 (m, 1H), 2.71-2.76 (m, 1H), 3.74 (s, 3H), 5.79 (s, 1H), 6.73-6.83 (m, 2H), 7.29-7.40 (m, 6H), 7.49 (t, 2H, *J* = 8.4 Hz), 7.67-7.84 (m, 3H), 8.21 (s, 1H), 8.38 (d, 1H, *J* = 8.8 Hz), 8.71-8.95 (m, 3H); **MS** (ESI) *m/z*: 785 $[M + H]^+$; Anal. calcd. for $C_{40}H_{26}N_6O_8S$: C, 63.99; H, 3.49; N, 11.19. Found: C, 64.29; H, 3.78; N, 11.32.

3-methoxy-7-(4-methoxyphenyl)-10-((3-(2-oxo-2*H*-chromen-3-yl)-1-phenyl-1*H*-pyrazol-4-yl)methylene)-7,10-dihydro-5*H*-benzo[h]thiazolo[2,3-*b*]quinazolin-9(6*H*)-one (6e)

Yellow solid; Yield: 86 %; M.P.: 254-256; **IR** (KBr, cm^{-1}) υ_{max}: 1729 cm^{-1} (coumarin C=O), 1704 cm^{-1} (thiazole C=O), 1634 cm^{-1} (pyrimidine C=N), 1598 cm^{-1} (pyrazole C=N), 1535 cm^{-1} (C=C of *α*, *β*-unsaturated carbonyl); **^{1}H NMR** (400 MHz, DMSO-d_6): δ 1.85-1.94 (m, 1H), 2.22-2.29 (m, 1H), 2.56-2.67 (m, 1H), 2.70-2.76 (m, 1H), 3.71 (s, 3H), 3.75 (s, 3H), 5.72 (s, 1H), 6.70 (s, 1H), 6.81-6.91 (m, 3H), 7.27 (d, 2H, *J* = 8.4 Hz), 7.39-

7.60 (m, 6H), 7.70 (t, 2H, *J* = 8.4 Hz), 7.84 (d, 1H, *J* = 7.6 Hz), 8.04 (d, 2H, *J* = 8.0 Hz), 8.40 (s, 1H), 8.75 (s, 1H); **MS** (ESI) *m/z*: 490 $[M + H]^+$; Anal. calcd. for $C_{41}H_{30}N_4O_5S$: C, 71.29; H, 4.38; N, 8.11. Found: C, 71.48; H, 4.69; N, 7.85.

7-(3,4-dimethoxyphenyl)-3-methoxy-10-((3-(2-oxo-2*H*-chromen-3-yl)-1-phenyl-1*H*-pyrazol-4-yl)methylene)-7,10-dihydro-5*H*-benzo[*h*]thiazolo[2,3-*b*]quinazolin-9(6*H*)-one (6f)

Yellow solid; Yield: 83 %; M.P.: 262-264; **IR** (KBr, cm^{-1}) υ_{max}: 1728 cm^{-1} (coumarin C=O), 1705 cm^{-1} (thiazole C=O), 1634 cm^{-1} (pyrimidine C=N), 1599 cm^{-1} (pyrazole C=N), 1533 cm^{-1} (C=C of *α*, *β*-unsaturated carbonyl); **^{1}H NMR** (400 MHz, DMSO-d_6): δ 1.94-2.32 (m, 2H), 2.60-2.74 (m, 2H), 3.70 (s, 3H), 3.75 (s, 3H), 3.79 (s, 3H), 5.73 (s, 1H), 6.75-6.97 (m, 5H), 7.42-7.59 (m, 6H), 7.71 (d, 2H, *J* = 7.6 Hz), 7.85 (d, 1H, *J* = 6.0 Hz), 8.05 (d, 2H, *J* = 7.2 Hz), 8.41 (s, 1H), 8.77 (s, 1H); **MS** (ESI) *m/z*: 721 $[M]^+$; Anal. calcd. for $C_{42}H_{32}N_4O_6S$: C, 69.99; H, 4.47; N, 7.77. Found: C, 70.23; H, 4.22; N, 7.58.

3-methoxy-10-((3-(2-oxo-2*H*-chromen-3-yl)-1-phenyl-1*H*-pyrazol-4-yl)methylene)-7-(2,3,4-trimethoxyphenyl)-7,10-dihydro-5*H*-benzo[*h*]thiazolo[2,3-*b*]quinazolin-9(6*H*)-one (6g)

Yellow solid; Yield: 80 %; M.P.: 266-268; **IR** (KBr, cm^{-1}) υ_{max}: 1728 cm^{-1} (coumarin C=O), 1703 cm^{-1} (thiazole C=O), 1633 cm^{-1} (pyrimidine C=N), 1597 cm^{-1} (pyrazole C=N), 1532 cm^{-1} (C=C of *α*, *β*-unsaturated carbonyl); **^{1}H NMR** (400 MHz, DMSO-d_6): δ 1.86-2.25 (m, 2H), 2.55-2.74 (m, 2H), 3.71 (s, 3H), 3.74 (s, 3H), 3.75 (s, 6H), 5.85 (s, 1H), 6.72-6.81 (m, 3H), 6.97 (d, 1H, *J* = 8.0 Hz), 7.41-7.49 (m, 4H), 7.59 (t, 2H, *J* = 8.0 Hz), 7.69 (t, 2H, *J* = 8.0 Hz), 7.84 (d, 1H, *J* = 7.6 Hz), 8.05 (d, 2H, *J* = 7.6 Hz), 8.40 (s, 1H), 8.76 (s, 1H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 165.09, 159.85, 159.49, 154.20, 150.08, 148.77, 144.05, 139.16, 137.15, 134.33, 132.54, 130.26, 130.18, 129.76, 128.47, 127.88, 127.20, 125.55, 124.78, 124.66, 121.37, 120.25, 119.78, 119.72, 118.90, 118.44, 116.78, 115.83, 115.61, 113.61, 113.37, 111.27, 60.07, 59.21, 55.32, 55.09, 52.46, 27.88, 24.98; **MS** (ESI) *m/z*: 751 $[M]^+$; Anal. calcd. for $C_{43}H_{34}N_4O_7S$: C, 68.79; H, 4.56; N, 7.46. Found: C, 68.48; H, 4.32; N, 7.22.

7-(3-hydroxy-4-methoxyphenyl)-3-methoxy-10-((3-(2-oxo-2*H*-chromen-3-yl)-1-phenyl-1*H*-pyrazol-4-yl)methylene)-7,10-dihydro-5*H*-benzo[*h*]thiazolo[2,3-*b*]quinazolin-9(6*H*)-one (6h)

Yellow solid; Yield: 79 %; M.P.: 244-246; **IR** (KBr, cm^{-1}) υ_{max}: 3516 cm^{-1} (OH), 1729 cm^{-1} (coumarin C=O), 1702 cm^{-1} (thiazole C=O), 1634 cm^{-1} (pyrimidine C=N), 1596 cm^{-1} (pyrazole C=N), 1535 cm^{-1} (C=C of *α*, *β*-unsaturated carbonyl); **^{1}H NMR** (400 MHz, DMSO-d_6): δ 1.90-1.99 (m, 1H), 2.23-2.31 (m, 1H), 2.58-2.66 (m, 1H), 2.70-2.79 (m, 1H), 3.71 (s, 3H), 3.75 (s, 3H), 5.66 (s, 1H), 6.73-6.83 (m, 4H), 6.93 (s, 1H), 7.39-7.71 (m, 8H), 7.84 (d, 1H, *J* = 7.6 Hz), 8.04 (d, 2H, *J* = 8.4 Hz), 8.40 (s, 1H), 8.75 (s, 1H), 9.09 (s, 1H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 165.15, 159.82, 159.40, 154.20, 150.05, 148.71, 146.57, 146.05, 144.02, 139.18, 137.21, 134.10, 132.49, 131.22, 129.74, 128.47, 127.84, 127.17, 125.73, 124.75, 124.56, 121.61, 121.07, 120.30, 120.11, 119.70, 118.92, 118.53, 116.76, 114.49, 113.98, 113.59, 111.24, 110.98, 59.80, 56.06, 55.32, 28.01, 24.98; **MS** (ESI) *m/z*: 707 $[M]^+$; Anal. calcd. for $C_{41}H_{30}N_4O_6S$: C, 69.68; H, 4.28; N, 7.93. Found: C, 69.48; H, 3.99; N, 8.27.

7-(4-fluorophenyl)-3-methoxy-10-((3-(2-oxo-2*H*-chromen-3-yl)-1-phenyl-1*H*-pyrazol-4-yl)methylene)-7,10-dihydro-5*H*-benzo[*h*]thiazolo[2,3-*b*]quinazolin-9(6*H*)-one (6i)

Yellow solid; Yield: 82 %; M.P.: 254-256; **IR** (KBr, cm^{-1}) υ_{max}: 1718 cm^{-1} (coumarin C=O), 1706 cm^{-1} (thiazole C=O), 1633 cm^{-1} (pyrimidine C=N), 1601 cm^{-1} (pyrazole C=N), 1539 cm^{-1} (C=C of *α*, *β*-unsaturated carbonyl); **^{1}H NMR** (400 MHz, DMSO-d_6): δ 1.86-1.92 (m, 1H), 2.26-2.32 (m, 1H), 2.59-2.75 (m, 2H), 3.75 (s, 3H), 5.83 (s, 1H), 6.75 (s, 1H), 6.82 (d, 1H, *J* = 8.4 Hz), 7.18 (t, 2H, *J* = 8.8 Hz), 7.40-7.61 (m, 8H), 7.70 (t, 2H, *J* = 8.8 Hz), 7.85 (d, 1H, *J* = 7.6 Hz), 8.05 (d, 2H, *J* = 8.4 Hz), 8.41 (s, 1H), 8.77 (s, 1H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 164.97, 159.86, 159.20, 154.18, 153.90, 151.83, 150.36, 148.69, 144.02, 139.22, 137.09, 133.14, 132.48, 129.76, 128.46, 127.82, 127.18, 126.01, 124.75, 124.34, 120.48, 120.34, 120.25, 119.71, 118.91, 118.62, 116.76, 113.50, 111.15, 107.34, 55.91, 55.29, 27.90, 24.51; **MS** (ESI) *m/z*: 679 $[M]^+$; Anal. calcd. for $C_{40}H_{27}FN_4O_4S$: C, 70.78; H, 4.01; N, 8.25. Found: C, 71.06; H, 3.87; N, 8.58.

7-(4-chlorophenyl)-3-methoxy-10-((3-(2-oxo-2*H*-chromen-3-yl)-1-phenyl-1*H*-pyrazol-4-yl)methylene)-7,10-dihydro-5*H*-benzo[*h*]thiazolo[2,3-*b*]quinazolin-9(6*H*)-one (6j)

Yellow solid; Yield: 80 %; M.P.: 251-253; **IR** (KBr, cm^{-1}) υ_{max}: 1720 cm^{-1} (coumarin C=O), 1709 cm^{-1} (thiazole C=O), 1631 cm^{-1} (pyrimidine C=N), 1596 cm^{-1} (pyrazole C=N), 1532 cm^{-1} (C=C of α, β-unsaturated carbonyl); **^{1}H NMR** (400 MHz, DMSO-d_6): δ 1.90-2.33 (m, 2H), 2.61-2.78 (m, 2H), 3.75 (s, 3H), 5.86 (s, 1H), 7.15-7.27 (m, 3H), 7.42-7.59 (m, 9H), 7.69 (s, 1H), 7.79-7.86 (m, 2H), 8.05 (d, 2H, *J* = 7.2 Hz), 8.41 (s, 1H), 8.78 (s, 1H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 165.04, 159.84, 154.20, 150.22, 148.79, 144.05, 139.15, 137.48, 135.28, 134.79, 134.63, 132.54, 132.42, 129.77, 129.04, 128.48, 128.01, 127.91, 127.37, 127.23, 126.76, 124.79, 123.31, 121.63, 120.23, 119.74, 119.62, 118.90, 118.40, 116.79, 115.65, 59.35, 59.10, 27.43, 24.99; **MS** (ESI) *m/z*: 695 $[M]^+$; Anal. calcd. for $C_{40}H_{27}ClN_4O_4S$: C, 69.11; H, 3.91; N, 8.06. Found: C, 69.41; H, 4.19; N, 8.31.

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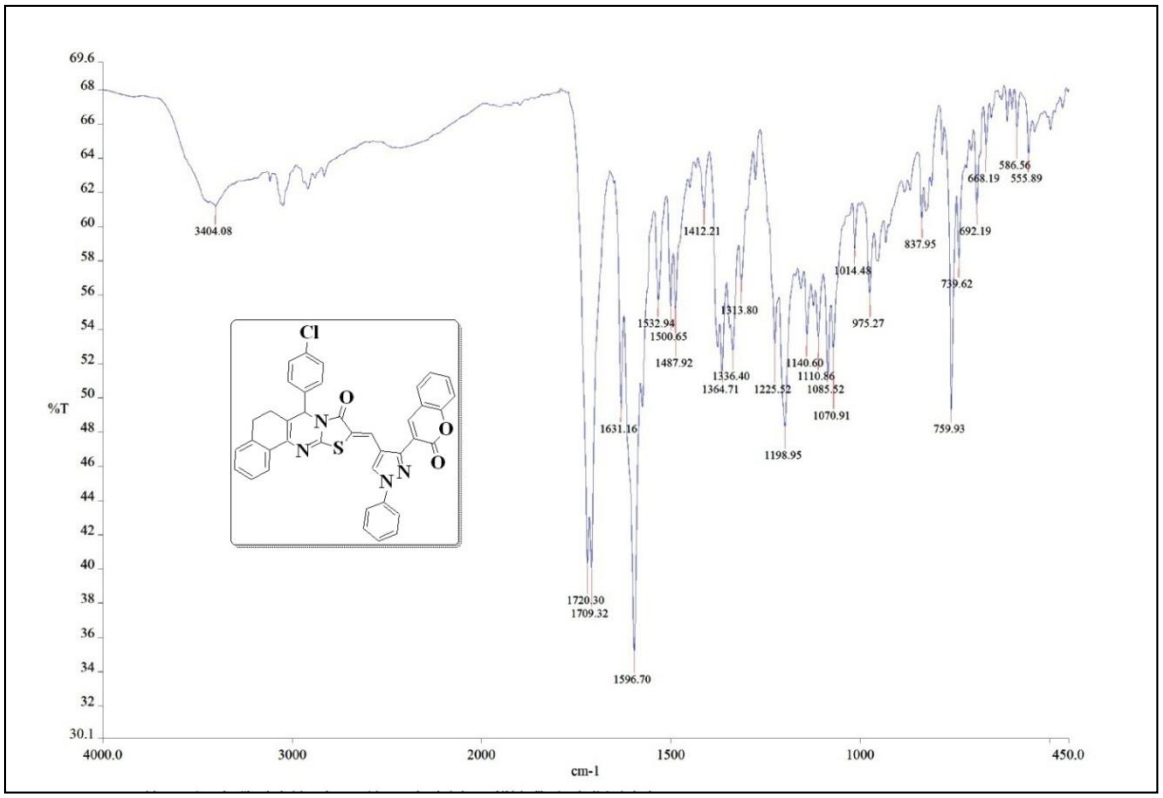
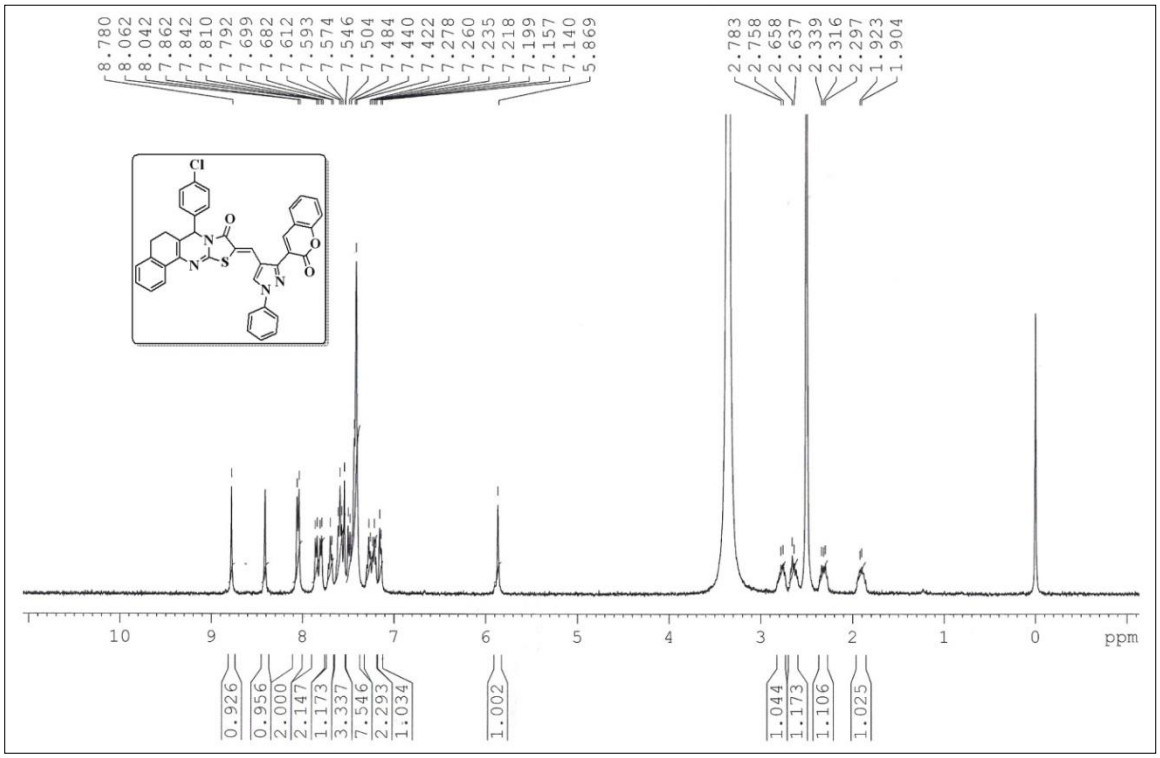
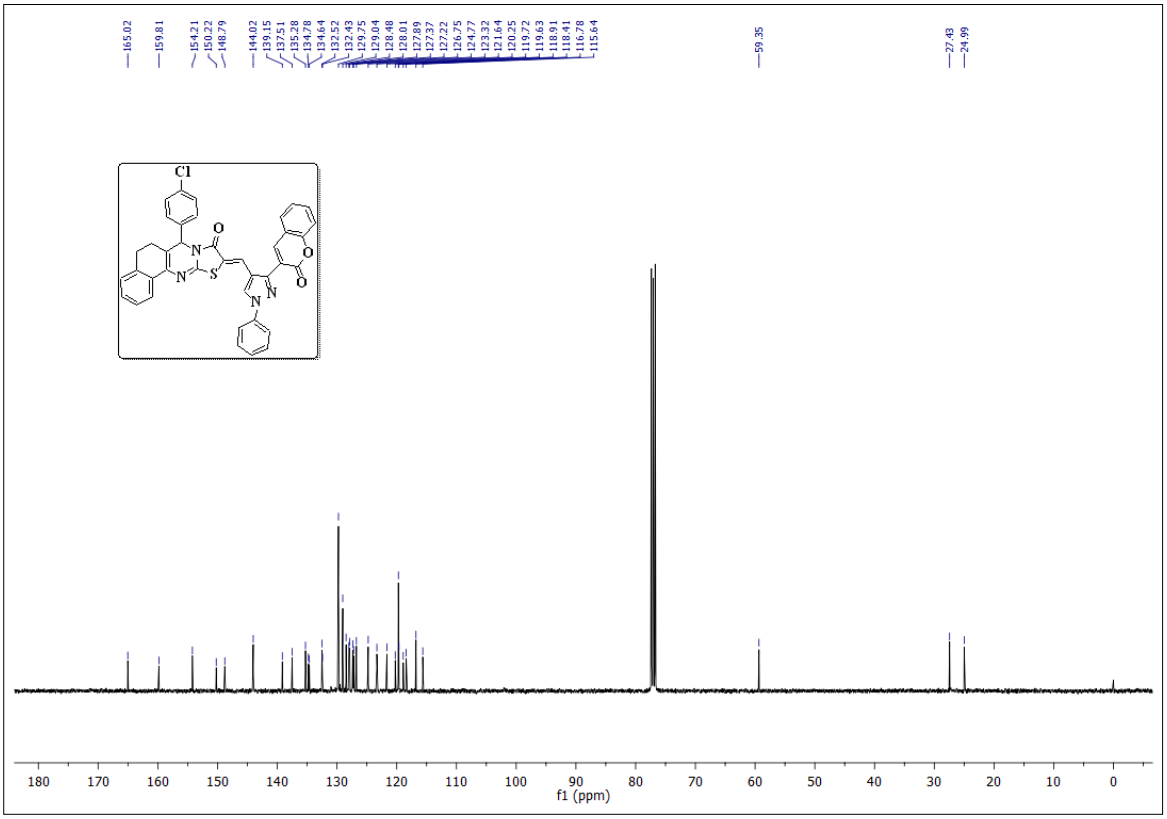


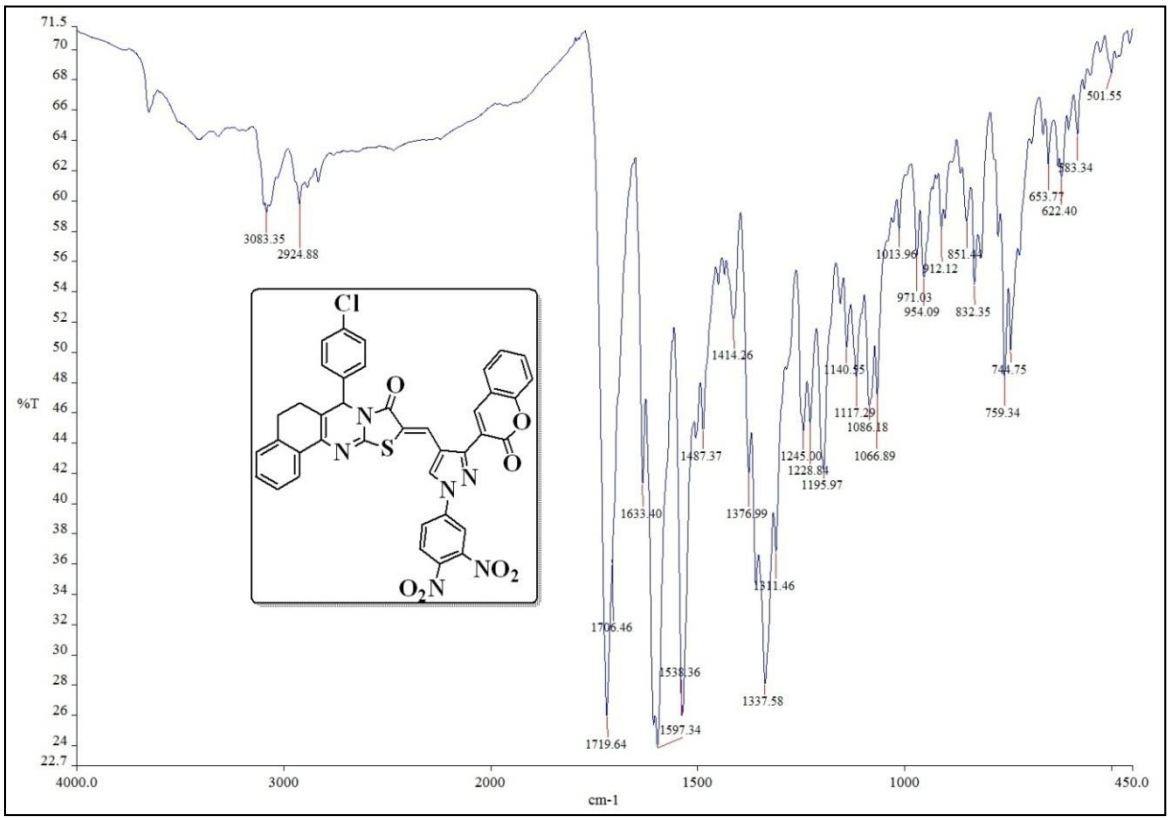
IR (KBr) spectrum of compound 6a



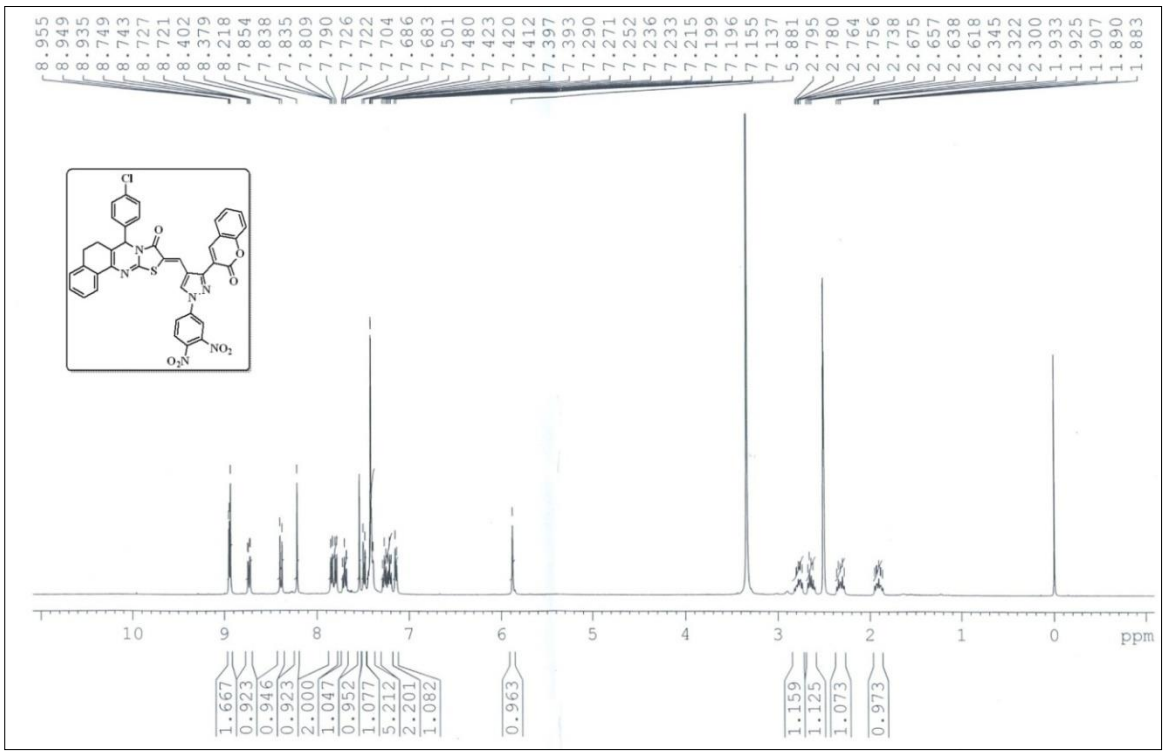
^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound 6a



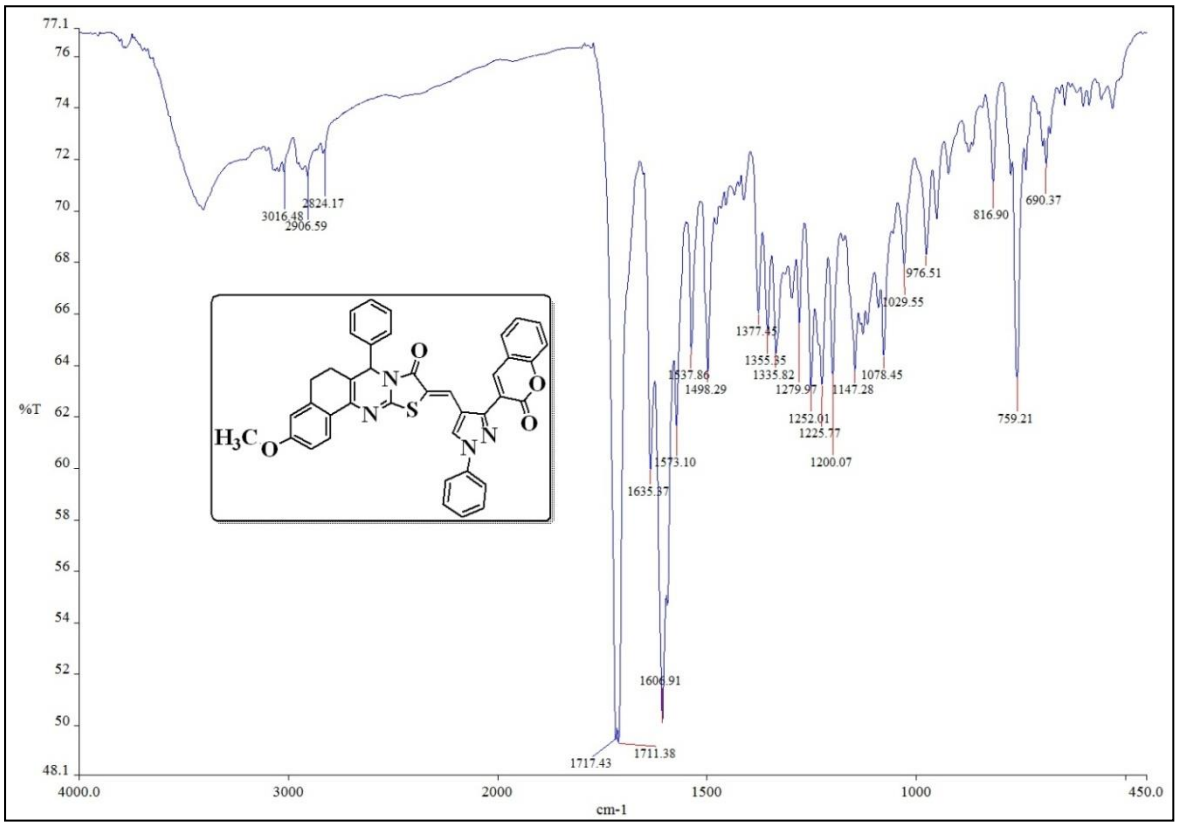
^{13}C NMR (100 MHz, $CDCl_3$) spectrum of compound 6a



IR (KBr) spectrum of compound 6b

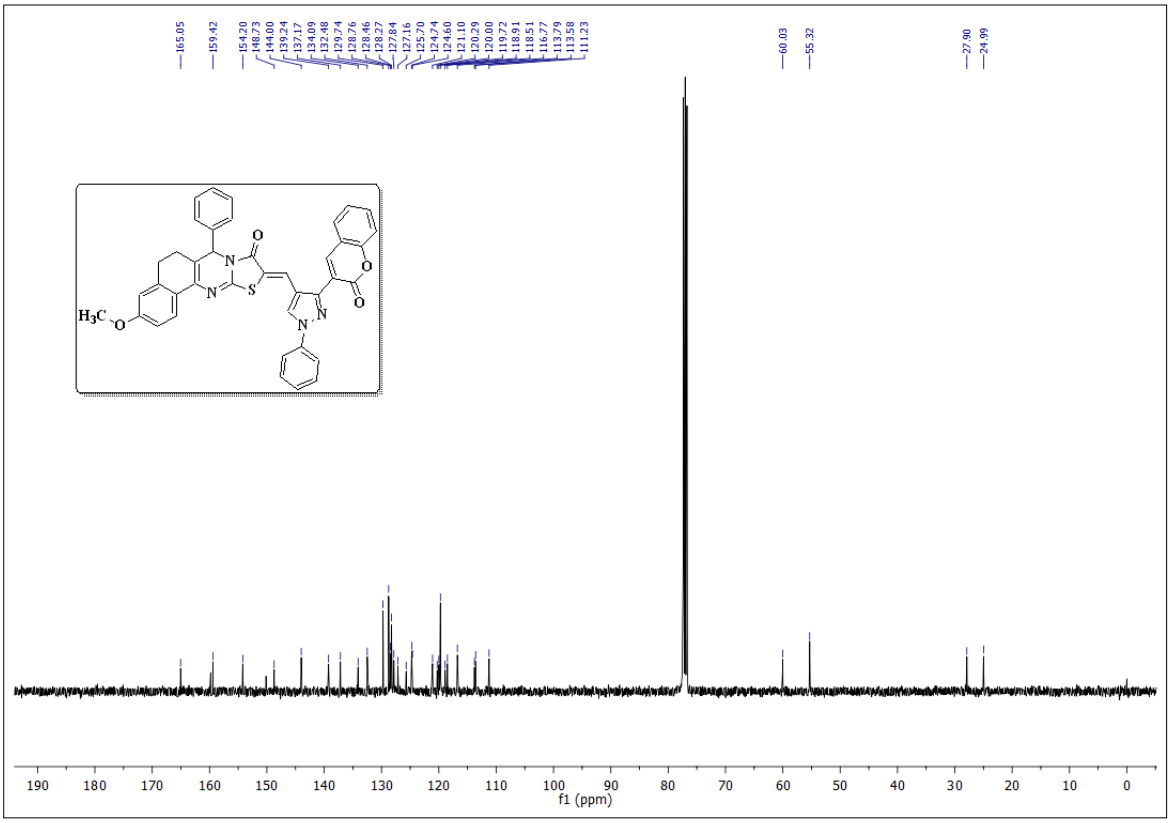


^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound 6b

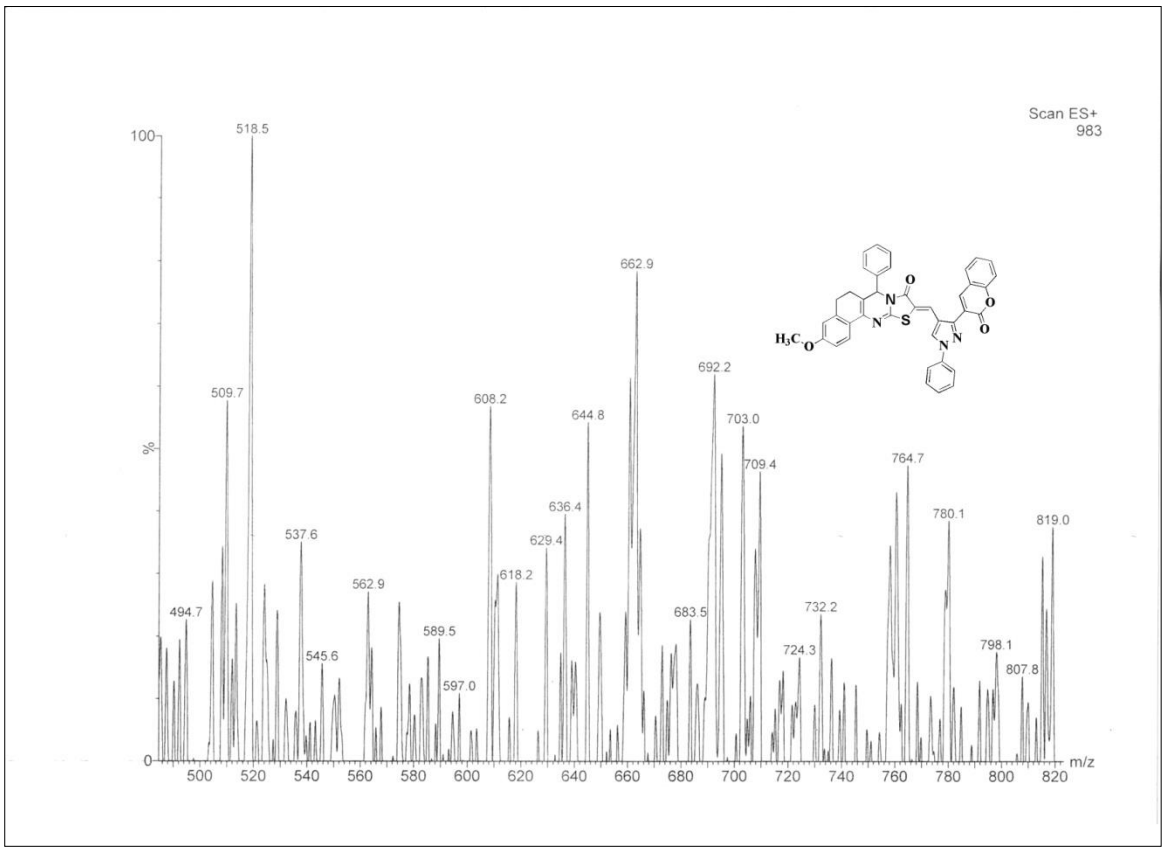


IR (KBr) spectrum of compound 6c

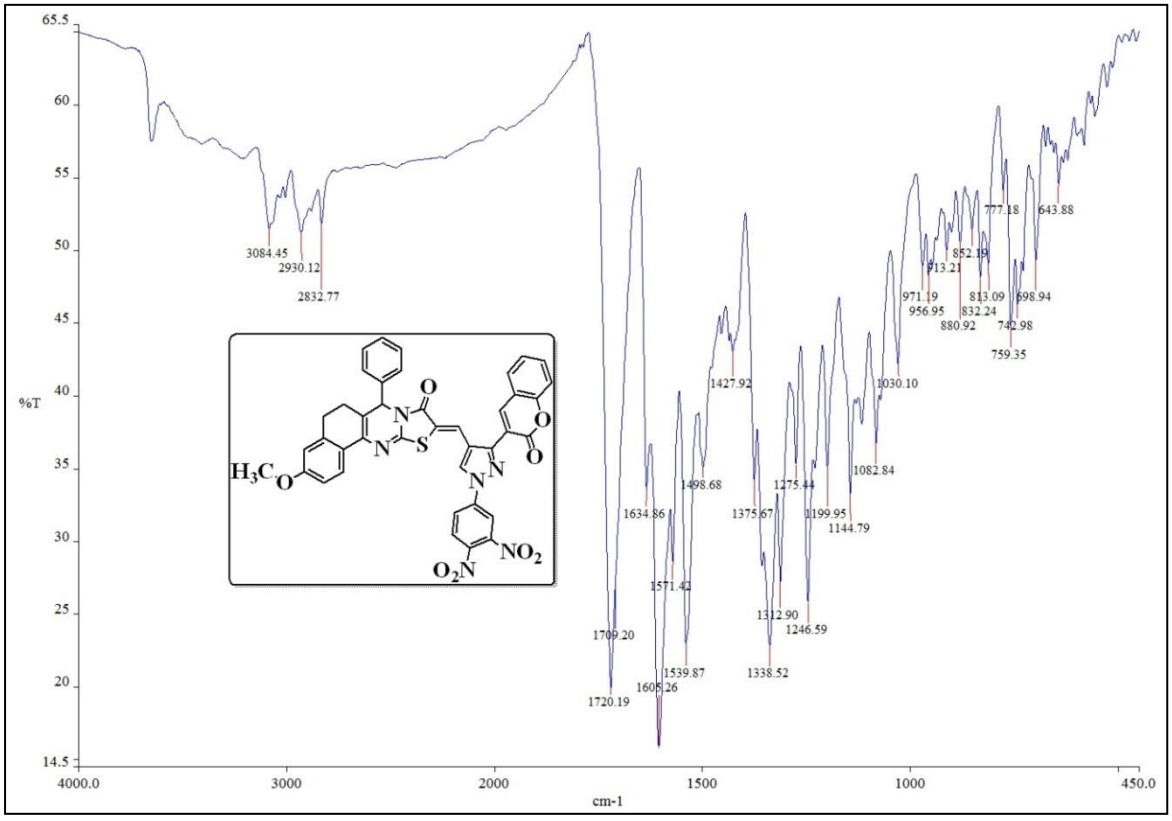
^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound 6c



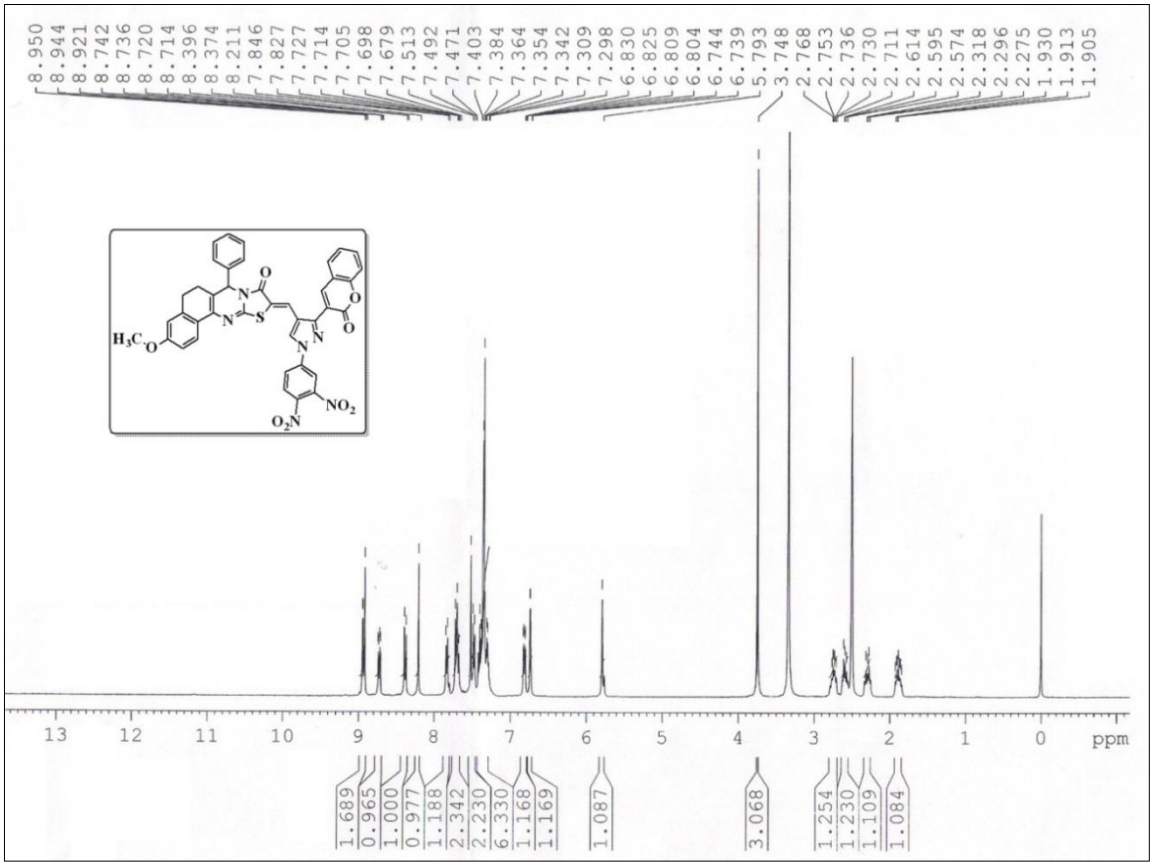
^{13}C NMR (100 MHz, $CDCl_3$) spectrum of compound 6c



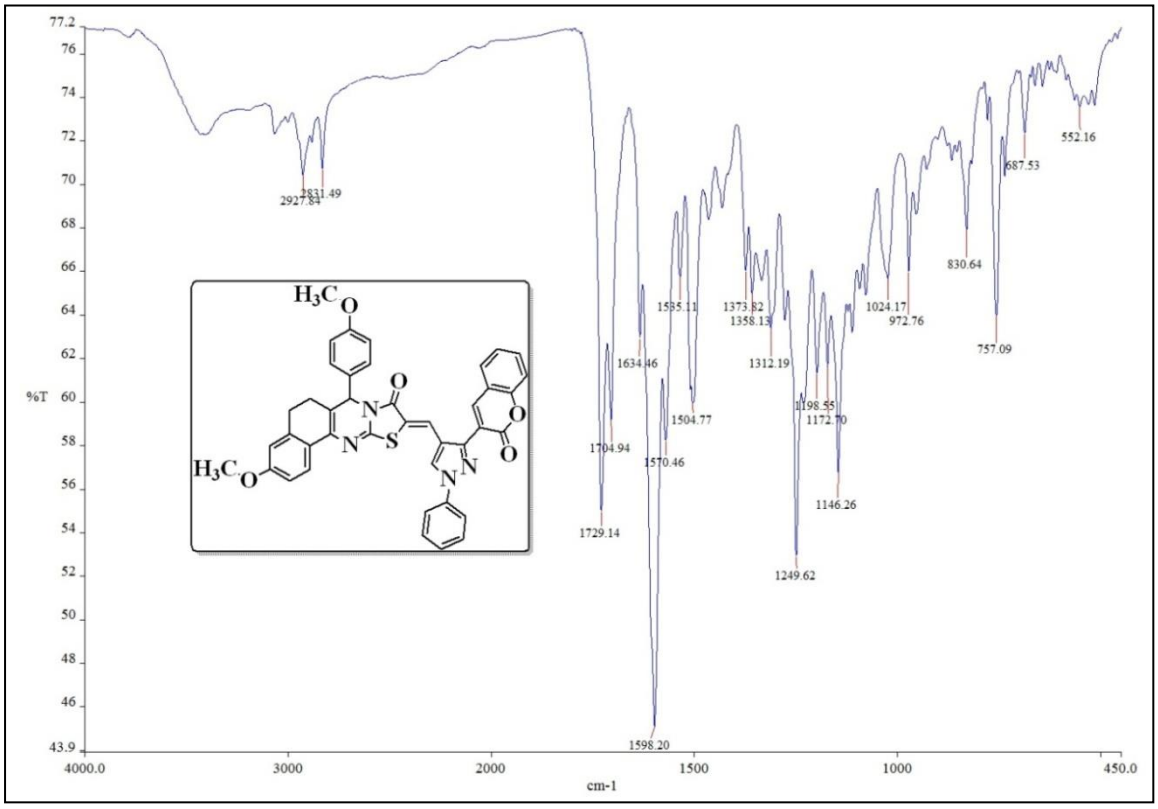
Mass spectrum of compound 6c (M.Wt: 661)



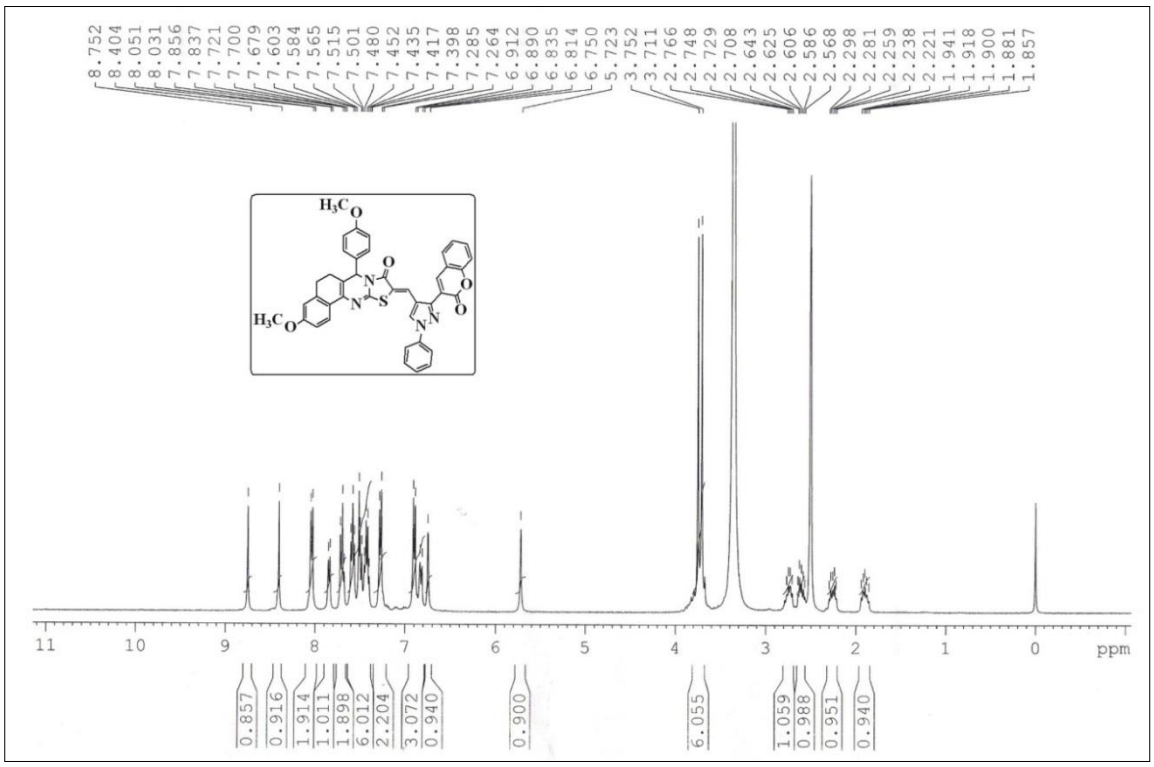
IR (KBr) spectrum of compound 6d



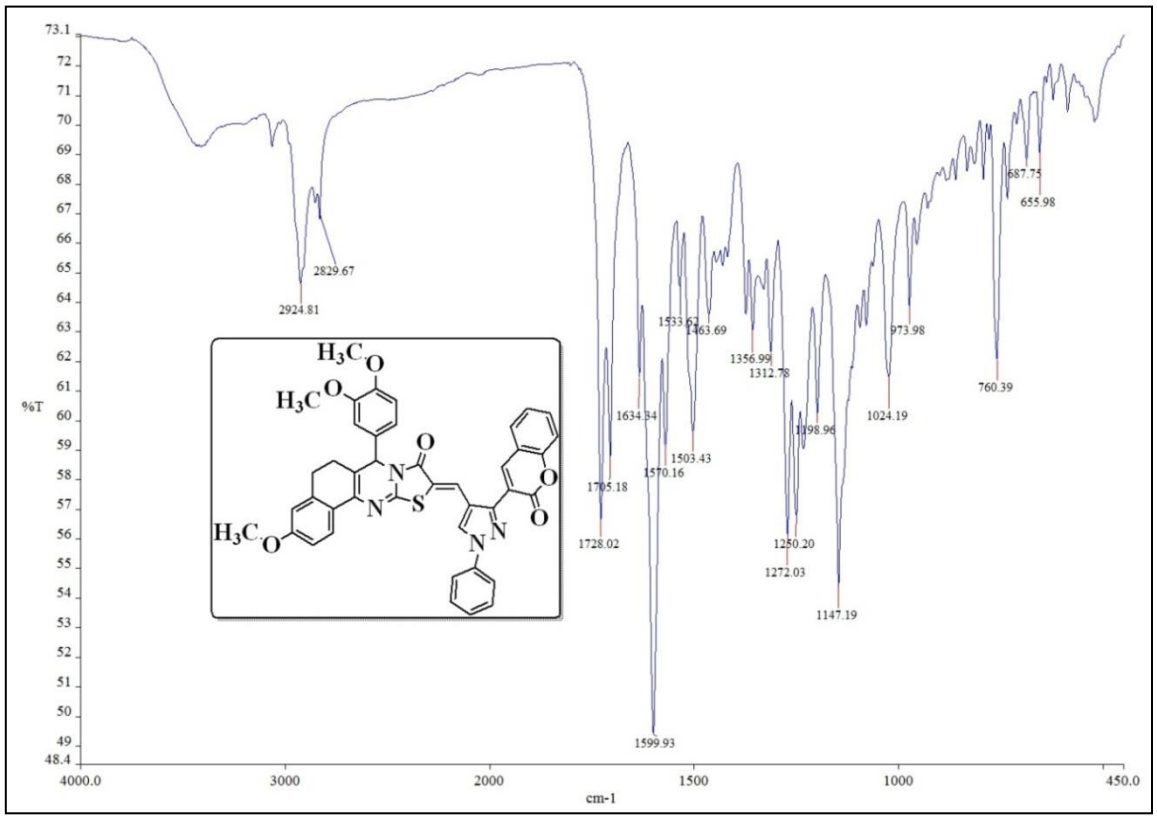
^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound 6d



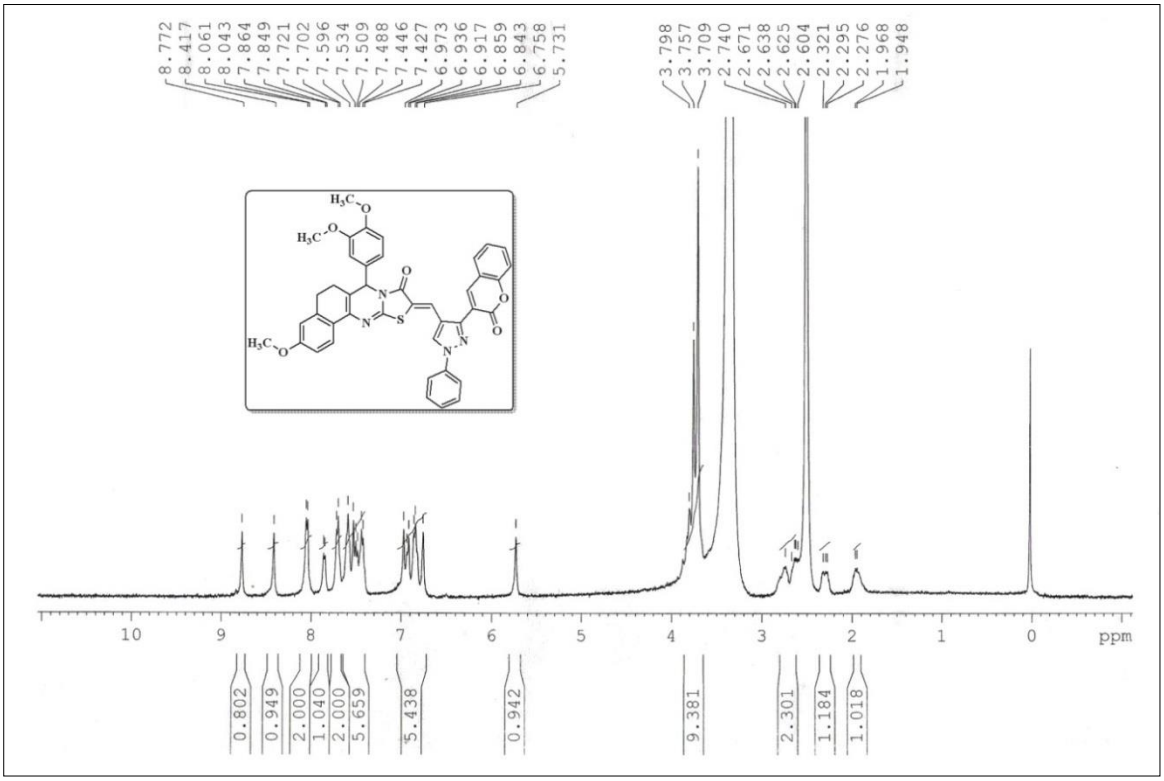
IR (KBr) spectrum of compound 6e



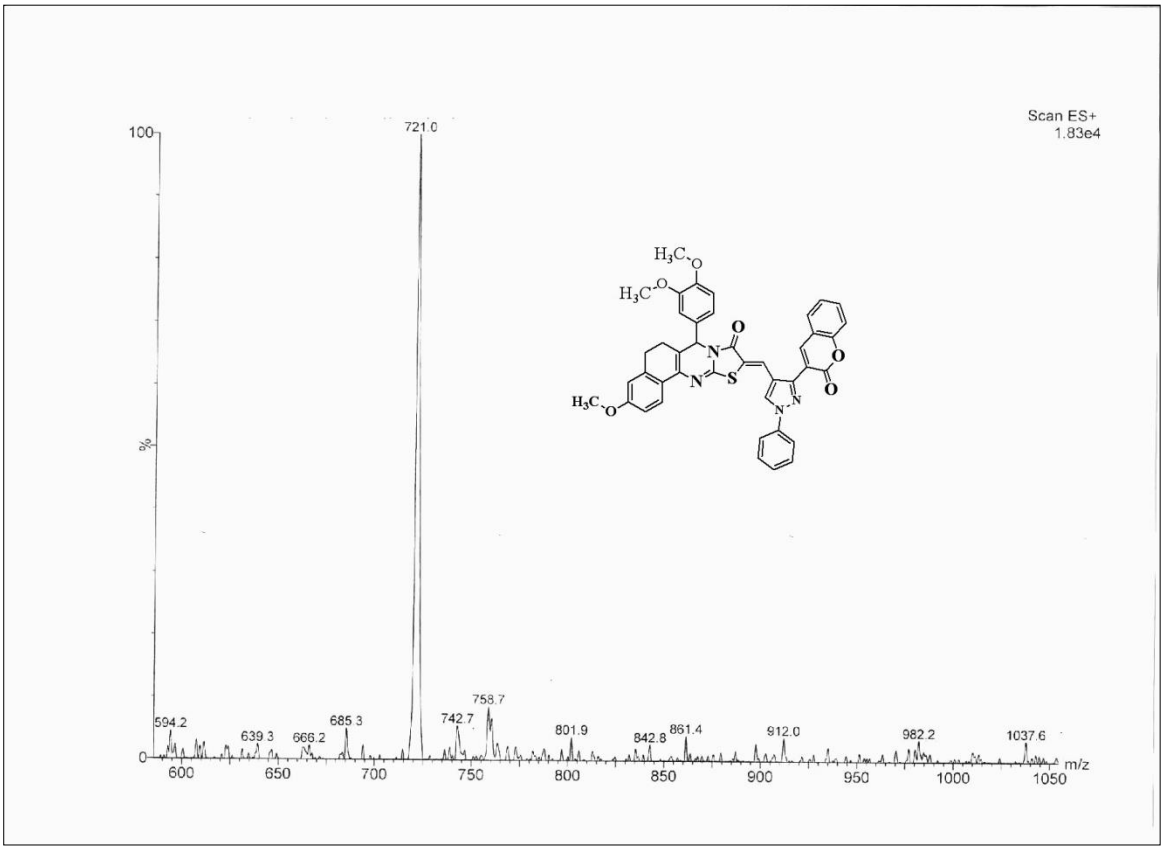
^{1}H NMR (400 MHz, DMSO-*d*$_6$) spectrum of compound 6e



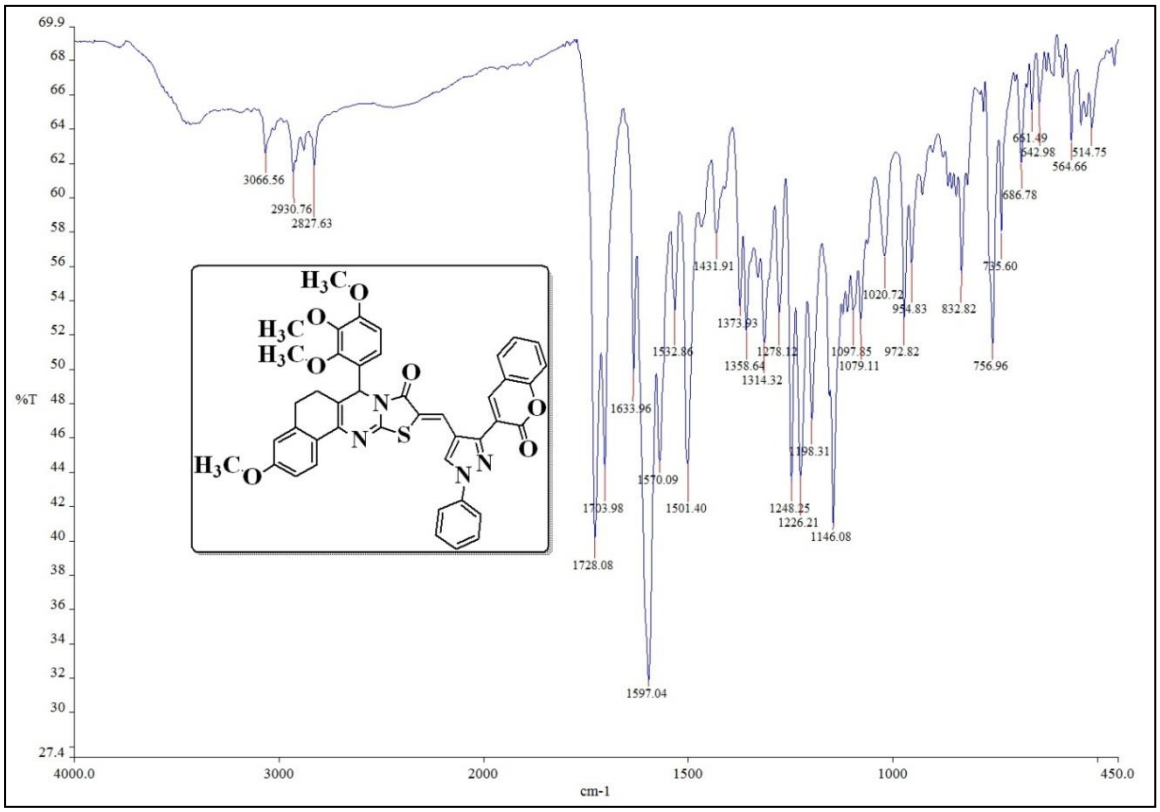
IR (KBr) spectrum of compound 6f



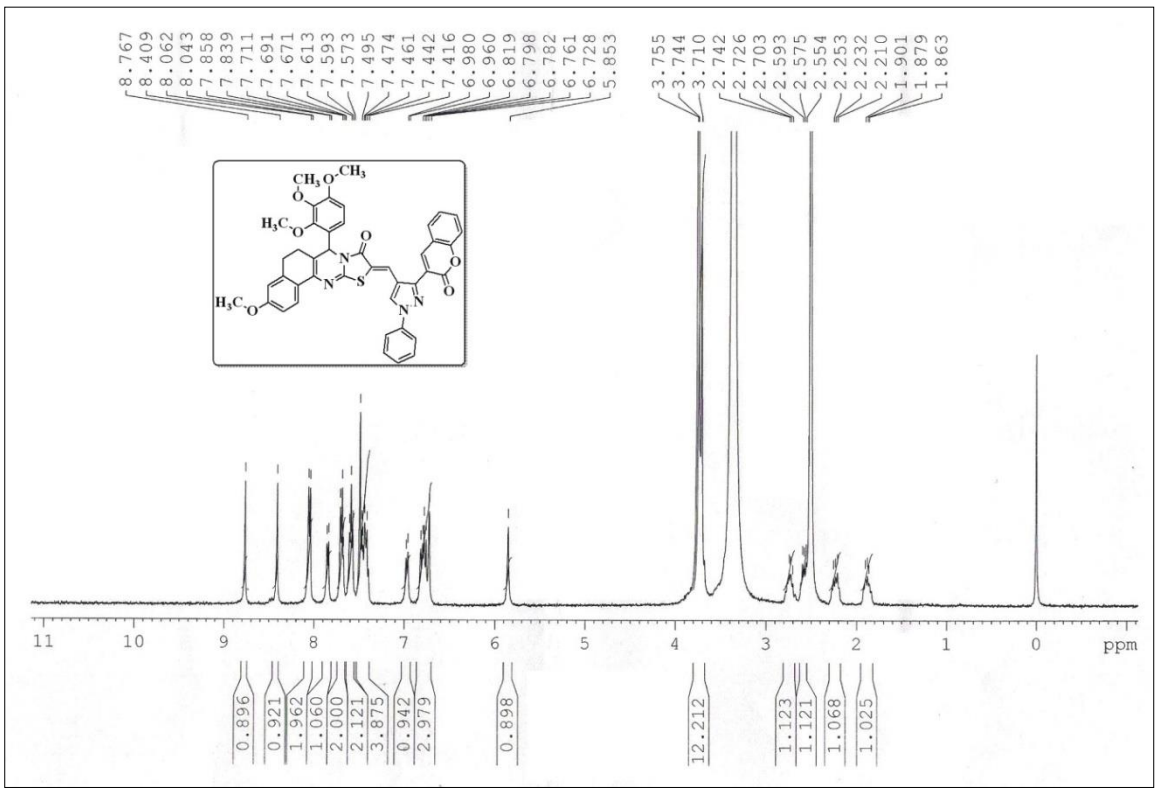
H NMR (400 MHz, DMSO-d_6) spectrum of compound 6f



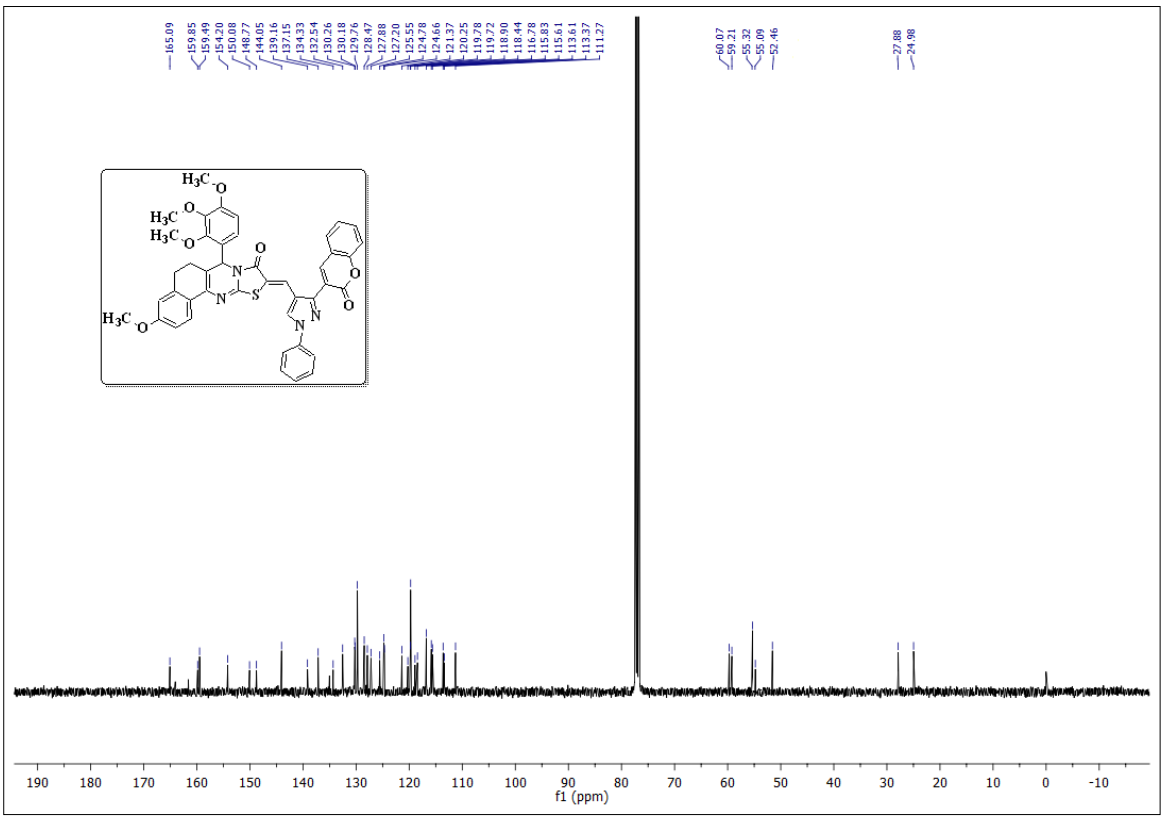
Mass spectrum of compound 6f (M.Wt: 721)



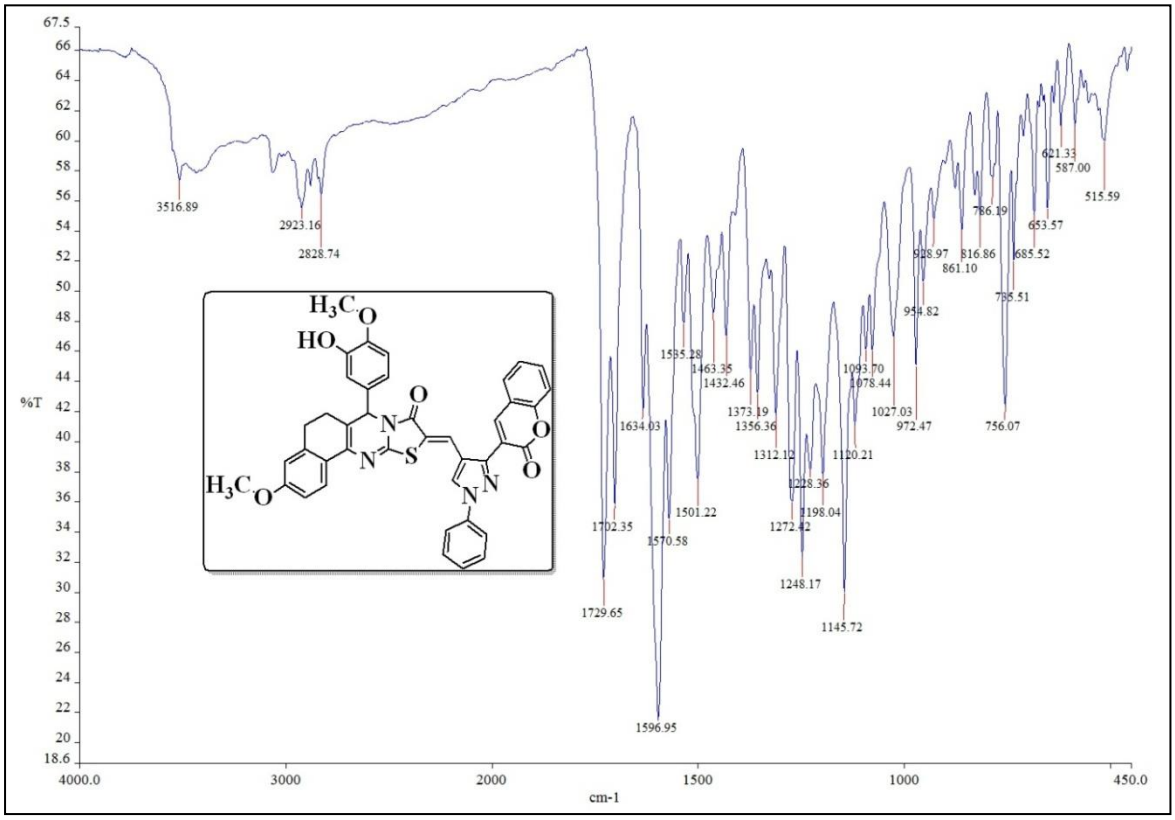
IR (KBr) spectrum of compound 6g



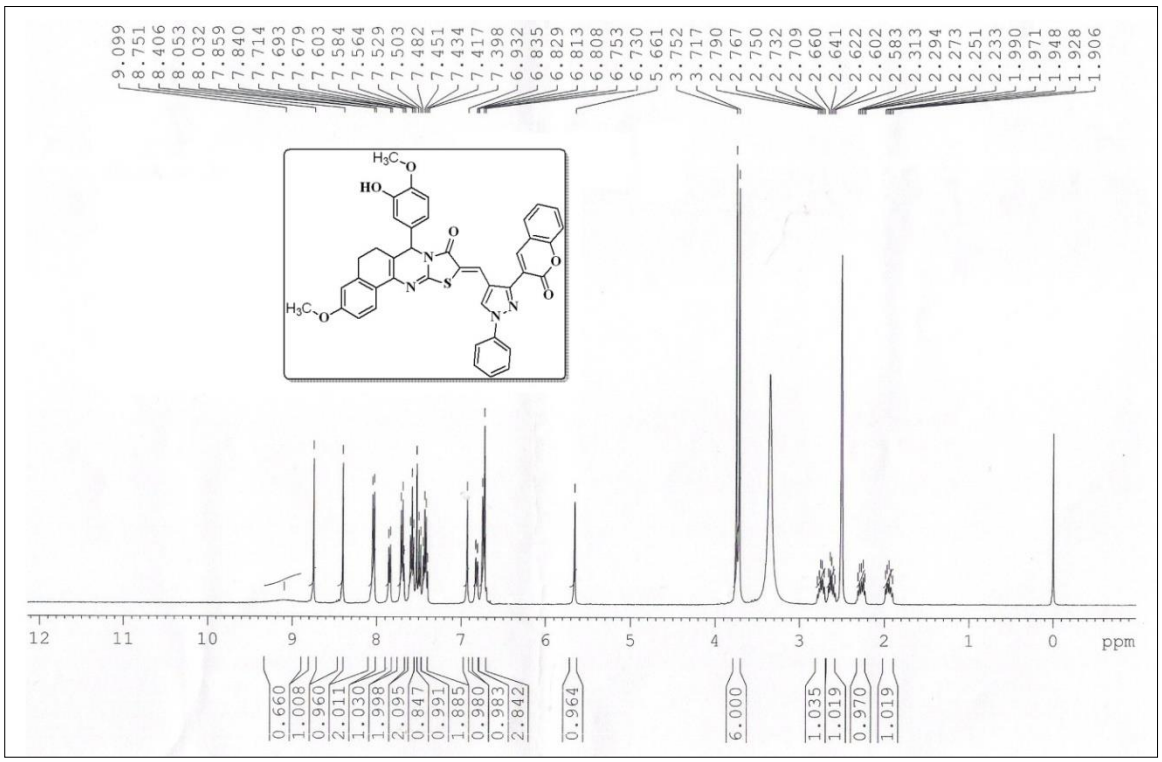
^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound 6g



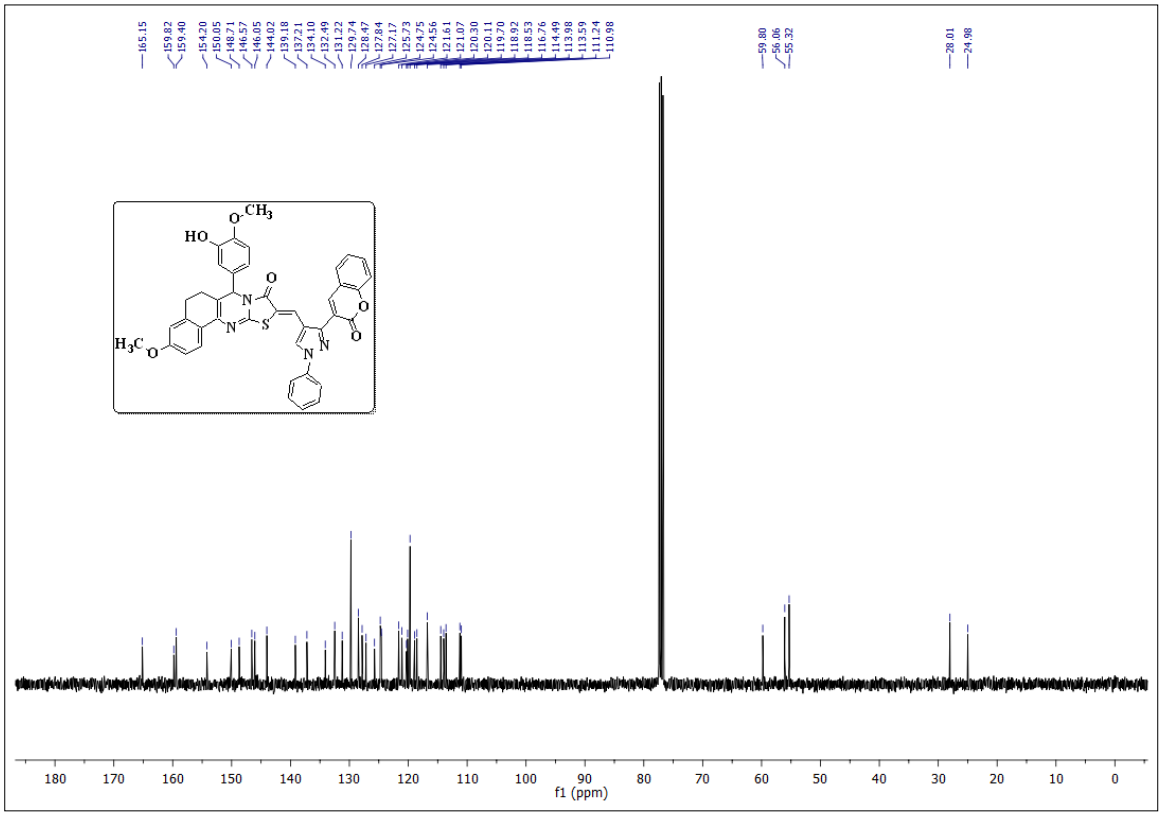
^{13}C NMR (100 MHz, $CDCl_3$) spectrum of compound 6g



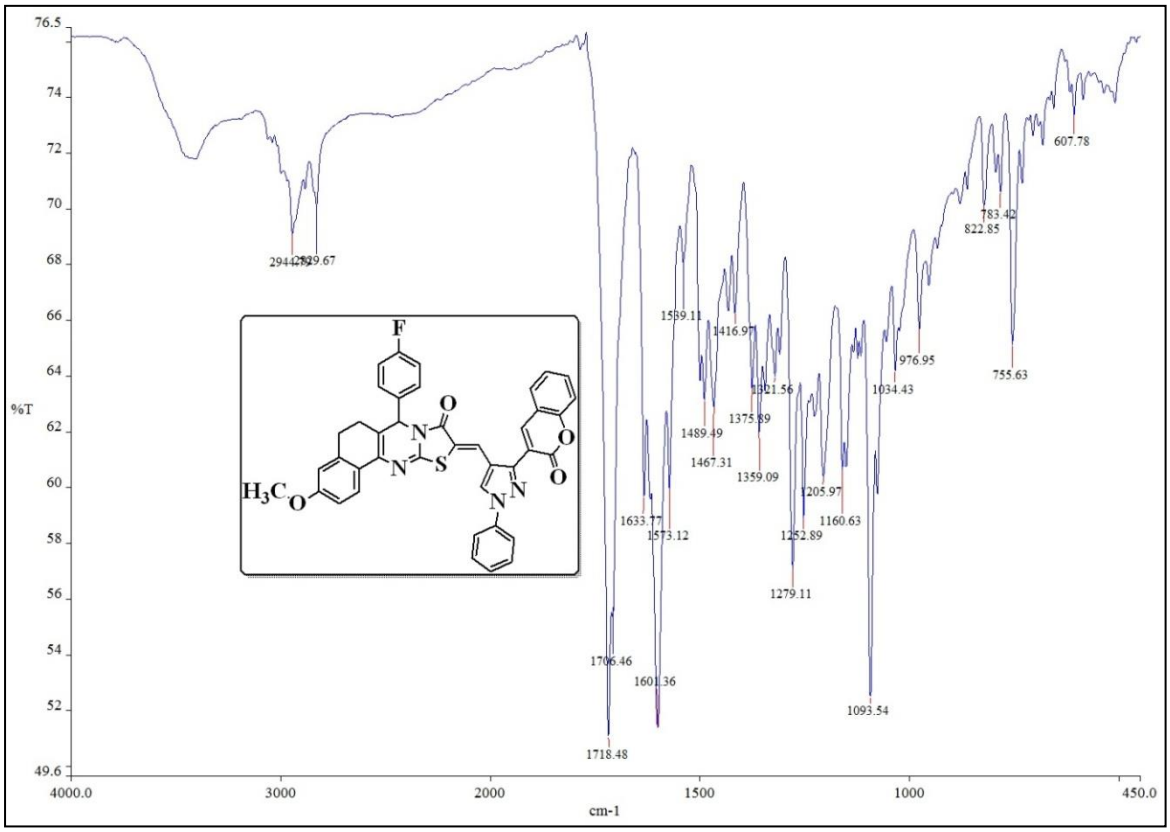
IR (KBr) spectrum of compound 6h



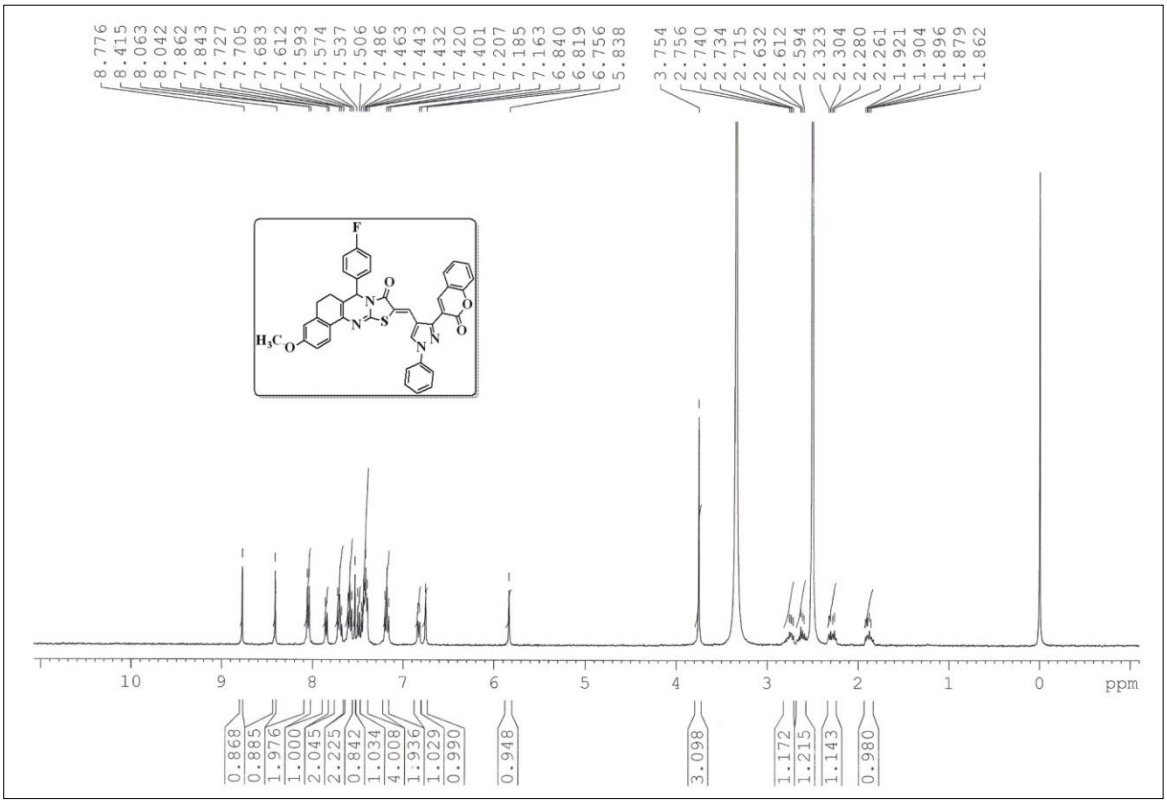
^{1}H NMR (400 MHz, DMSO-*d*$_6$) spectrum of compound 6h



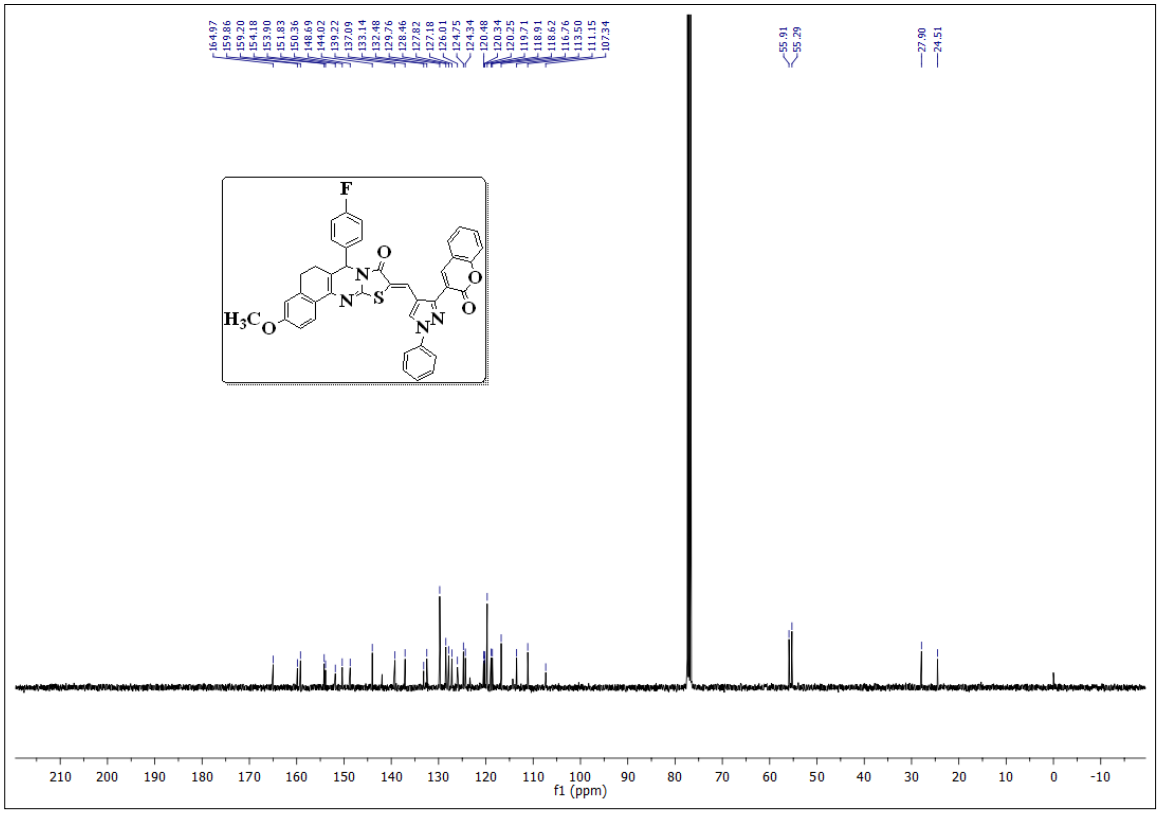
^{13}C NMR (100 MHz, CDCl$_3$) spectrum of compound 6h



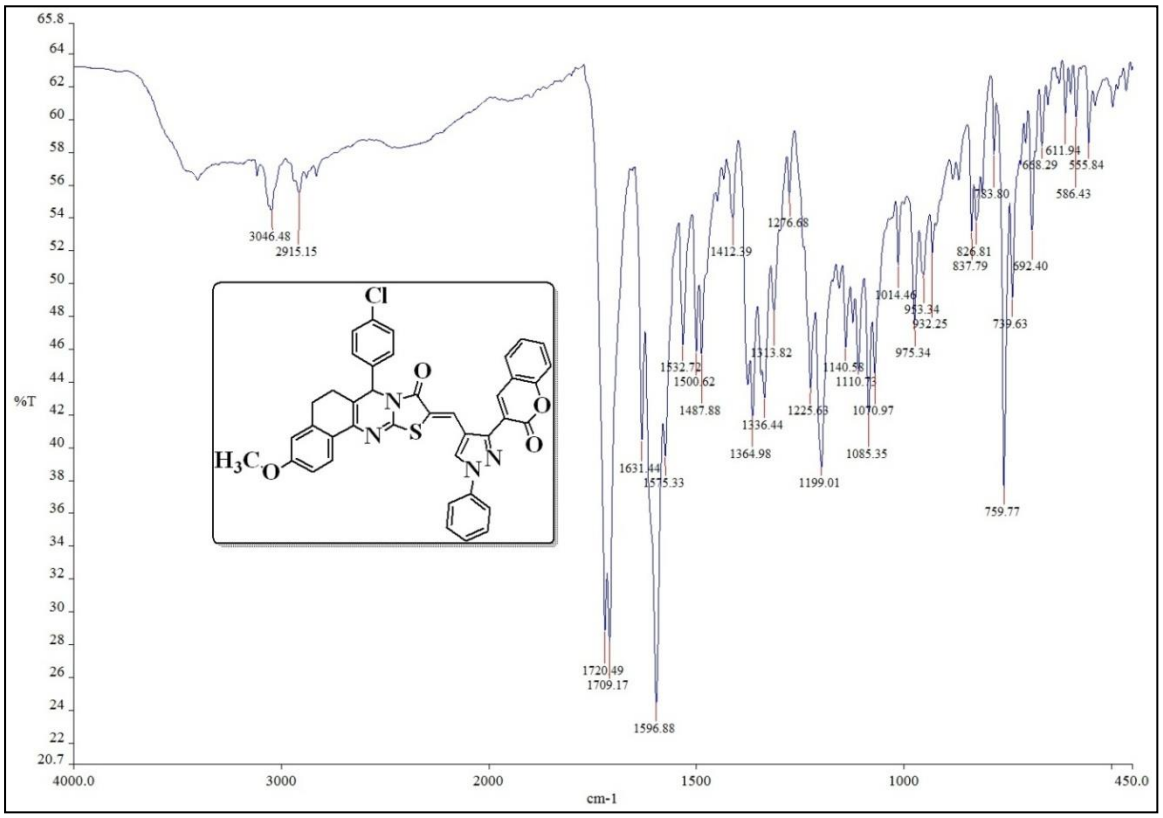
IR (KBr) spectrum of compound 6i



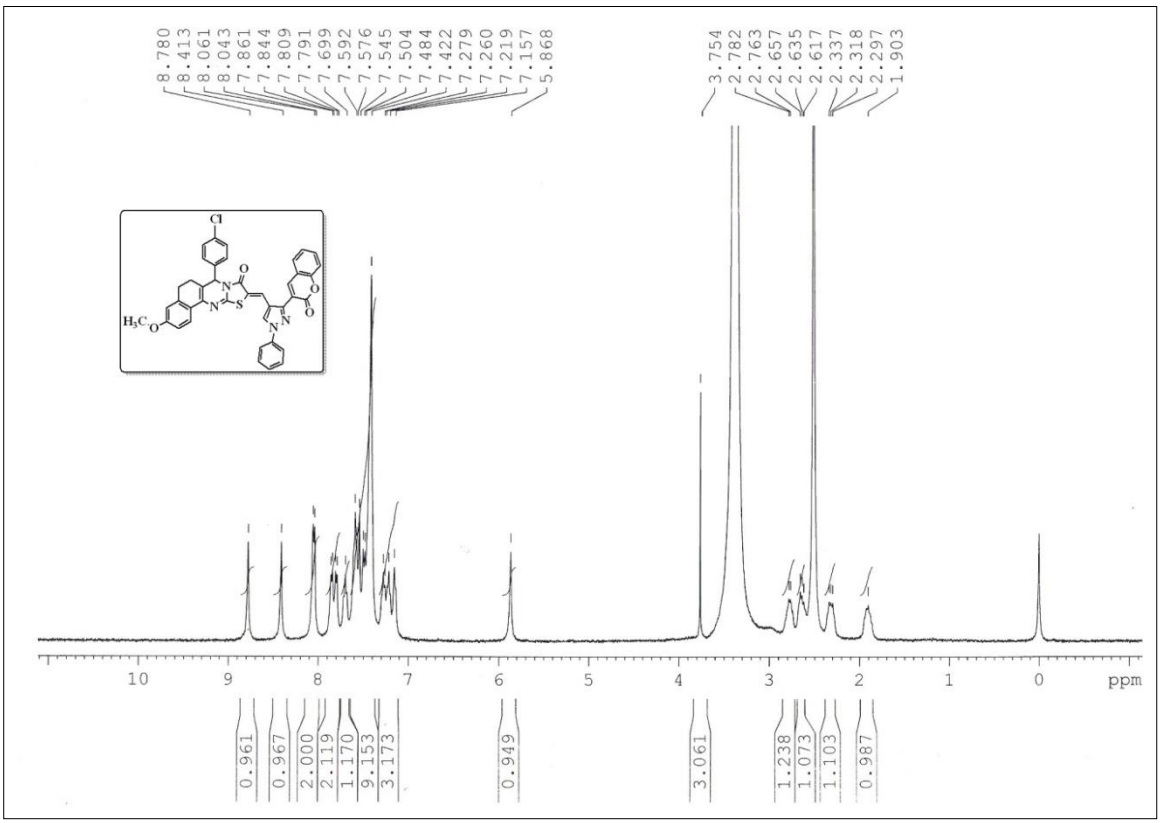
^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound 6i



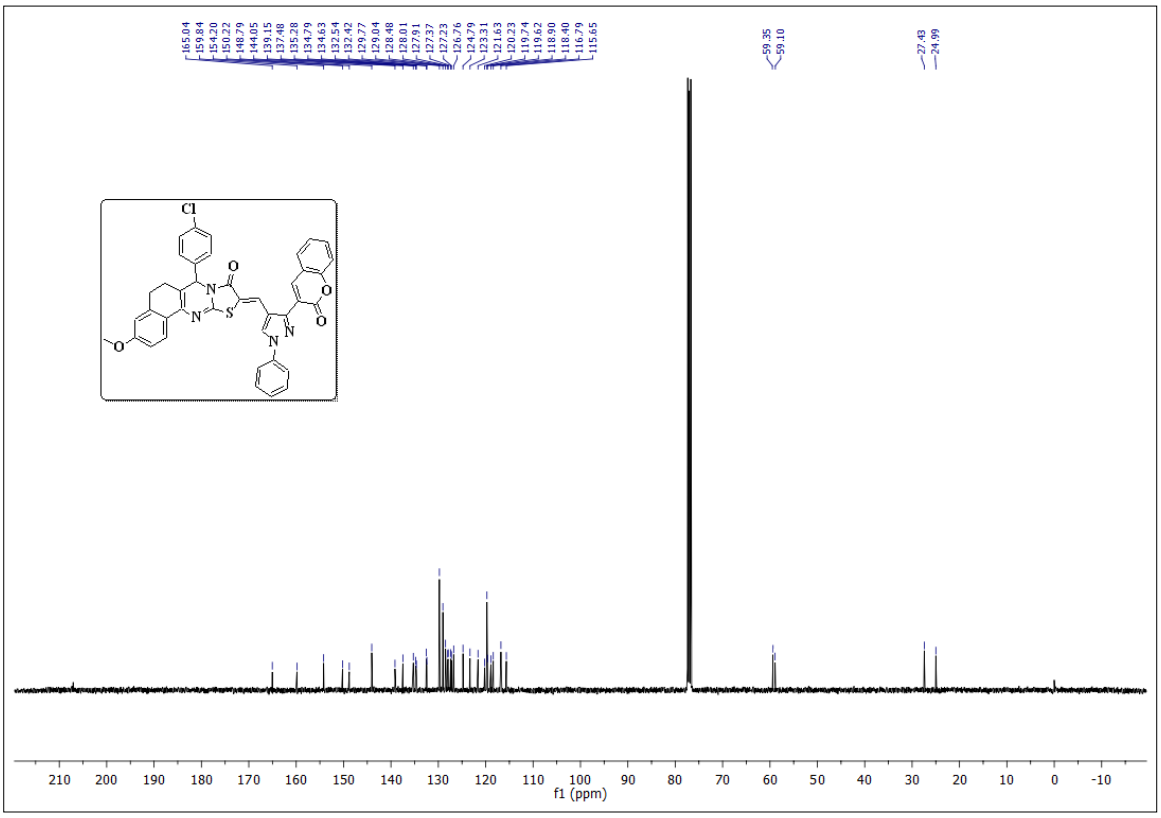
^{13}C NMR (100 MHz, DMSO-d_6) spectrum of compound 6i



IR (KBr) spectrum of compound 6j



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



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

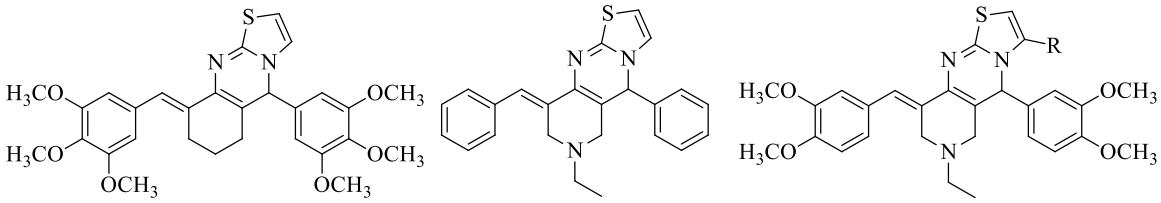
CHAPTER-IV (SECTION-B)

ONE POT THREE COMPONENT SYNTHESIS OF FUSED THIAZOLO[2,3-*b*]PYRIMIDINONE-MORPHOLINE BASED TRIAZOLYLE HYBRIDS

INTRODUCTION

Heterocycles are the largest class of organic compounds frequently observed in a variety of natural products, pharmaceutical active compounds, agro-chemicals. Many literature reports displayed the pharmacological importance of thiazolo[3,2-*a*]pyrimidines, hence the researchers have paid their attention towards their design and synthesis, because of their pharmacological properties such as anticancer,[1] anti-inflammatory,[2] antibiofilm,[3] antimicrobial,[4] antitubercular,[5] anti-HSV-1,[6] antitumour,[7] antimalarial, anti-HIV,[8] antifungal,[9] anti-viral,[10] antinociceptive,[11] antiallergic,[12] calcium antagonist[13] activities. They also reported to possess CDC25B phosphatase[14], xanthine oxidase enzymes,[15] and Bcl-2 family protein inhibitors.[16]

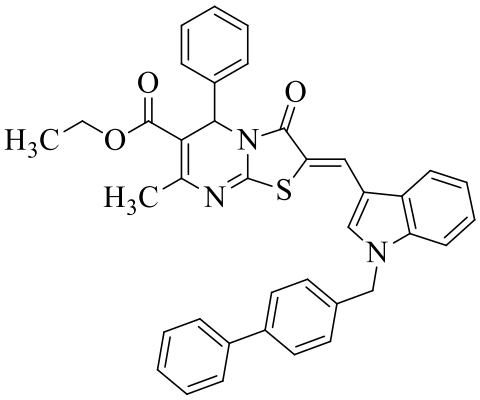
F. A. Al-Omary *et al*.[17] reported substituted thiazoles V. synthesis and antitumor activity of novel thiazolo[2,3-*b*]quinazoline and pyrido[4,3-*d*]thiazolo[3,2-*a*]pyrimidine analogues. All the synthesized compounds were assessed for their *in vitro* anticancer activity against 60 cancer cell-lines. Among the tested series of compounds **1**, **2**, **3** and **4** exhibited promising and broad spectrum activity. From the *in vitro* results, it was identified that the compounds **1** and **2** were ~9 fold more potent than the 5-FU, with GI_{50}, TGI, and LC_{50} values of 2.5, >100, >100; and 2.4, 9.1, 36.2 µM, respectively; while **3** and **4** were observed to be having almost ~7 fold more active than 5-FU, with GI_{50}, TGI, and LC_{50} values of 2.9, 12.4, 46.6 and 3.0, 16.3, 54.0 µM, respectively.



(1) **(2)** **(3): R = H** **(4): R = CH_3**

Fig. 1

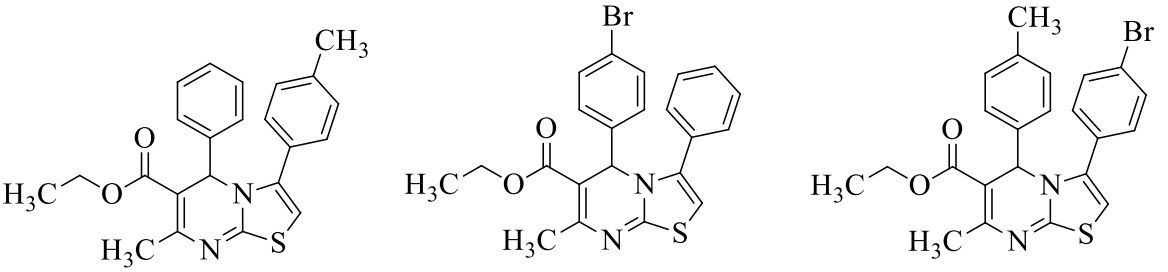
B. Zhou *et al*.[18] reported the discovery and development of thiazolo[3,2-*a*]pyrimidinone derivatives as general inhibitors of Bcl-2 family proteins. Synthesized compounds were tested *in vitro* against three Bcl-2 family proteins by binding assay. From the *in vitro* results BCL-LZH-40 (**5**) identified as a most potent among the tested series, which achieved tight binding to Bcl-x_L (K_i = 17 nm) comparable to ABT-737 (K_i=18 nm).



BCL-LZH-40 (5)

Fig. 2

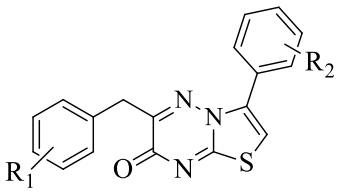
Samia G. Abdel Moty and co-workers[19] reported the design and synthesis of some substituted thiazolo[3,2-*a*]pyrimidine derivatives of potential biological activities. From the antibacterial and antifungal results, the compounds **6** and **7** against bacterial strain *B. cereu*s and **8** against the fungal strain *S. brevicaulis* exhibited good inhibiting activity.



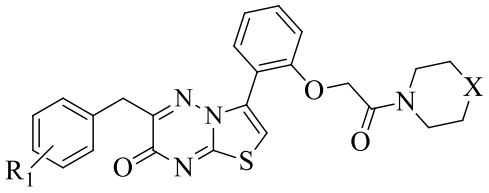
(**6**) (**7**) (**8**)

Fig. 3

Si-jie Liu *et al*.[20] published design, synthesis, and biological evaluation of 7*H*-thiazolo[3,2-*b*]-1,2,4-triazin-7-one derivatives as novel acetylcholinesterase inhibitors. Out of the synthesized compounds (**9** and **10**) some displayed greater than 50% AChE inhibition at 10 μM.



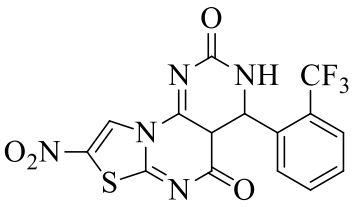
(**9**)



(**10**)

Fig. 4

V. U. Jean Kumar *et al.*[21] reported the discovery of novel inhibitors targeting the *Mycobacterium tuberculosis O*-acetylserine sulfhydrylase (CysK1) using virtual high-throughput screening. Synthesized compounds were assessed for their *in vitro* anti-tubercular activity against *Mycobacterium tuberculosis* H37Rv (ATCC27294) and investigated their cytotoxicity by MTT assay against HEK293T cells at 25 and 50 µM. Based on the experimental data compound **11** was identified as a most potent with an IC_{50} of 17.7 µM (CysK1) and MIC of 7.6 µM (antimycobacterial) and no cytotoxicity (>50 µM).

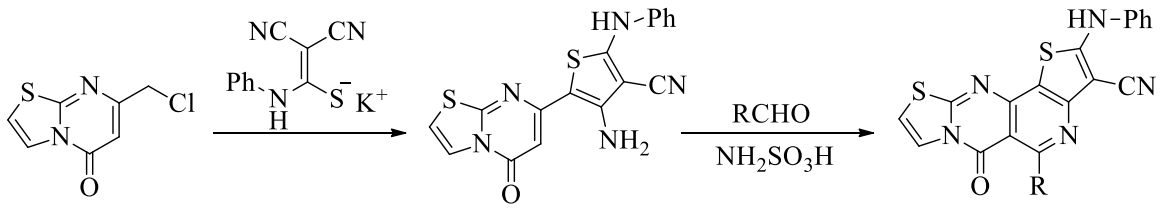


(**11**)

Fig. 5

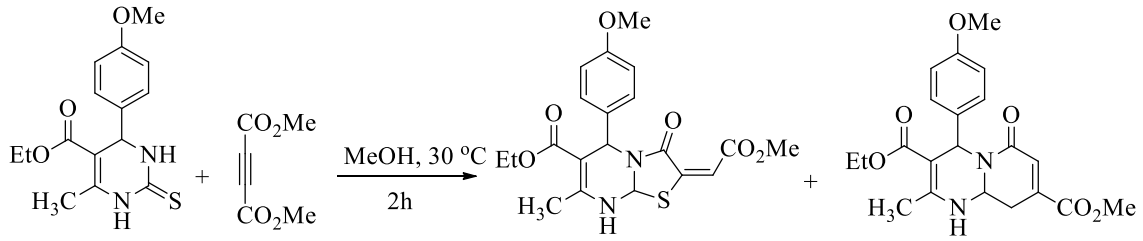
Various important approaches for the synthesis of thiazolo[3,2-*a*]pyrimidines

Dao-Lin Wang and co-workers[22] described an efficient method for the synthesis of thieno[3',2':2,3]pyrido-[4,5-*d*]- thiazolo[3,2-*a*]pyrimidinones.



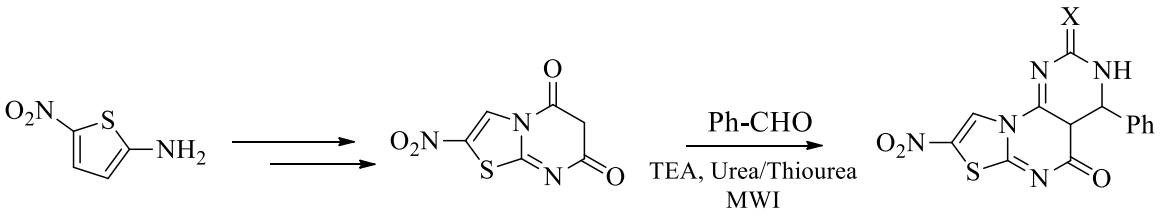
Scheme-1

Ali Darehkordi and Somayeh Ghazi[23] reported an An efficient ultrasonic-assisted synthesis of ethyl-5-(aryl)-2-(2-alkokxy-2-oxoethylidene)- 7-methyl-3-oxo-3, 5-dihydro-2*H*-thiazolo [3, 2-*a*] pyrimidine-6-carboxylate derivatives.



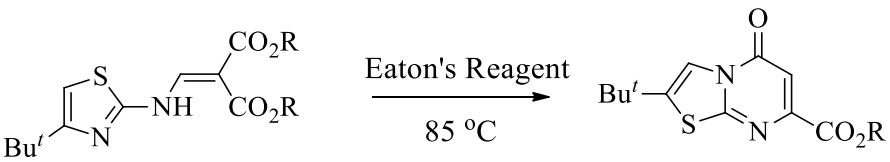
Scheme-2

Variam Ullas Jean kumar *et al.*[24] reported the discovery of novel inhibitors targeting the Mycobacterium tuberculosis *O*-acetylserine sulfhydrylase (CysK1) using virtual high-throughput screening.



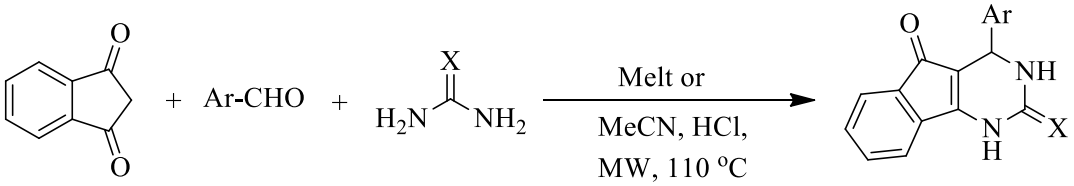
Scheme-3

Nadia M. Ahmad and Keith Jones[25] described an efficient synthesis of thiazolo[3,2-*a*]pyrimidinones.



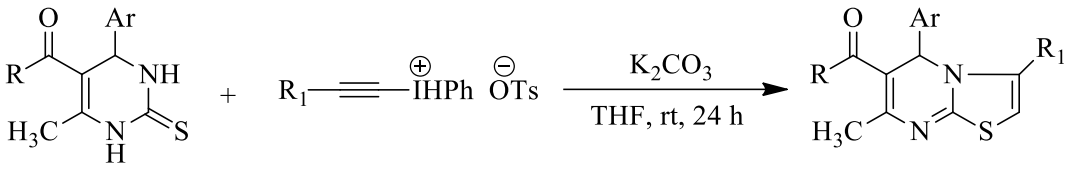
Scheme-4

H. J. M. Gijsen *et al*.[26] reported tricyclic 3,4-dihydropyrimidine-2-thione derivatives as potent TRPA1 antagonists.



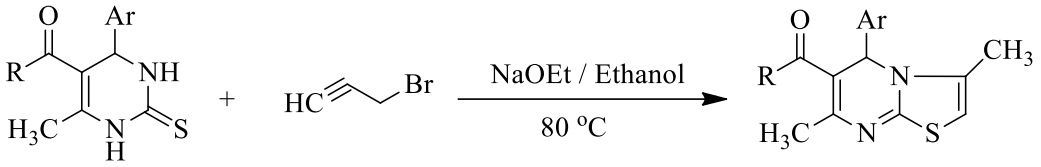
Scheme-5

A. V. Shelke *et al.*[27] reported a new synthesis of 3,5-disubstituted-5*H*-thiazolo[3,2-*a*]pyrimidine *via* ring annulation of 3,4-dihydropyrimidin-2(1*H*)-thione using alkynyl(aryl)iodonium salts**.**



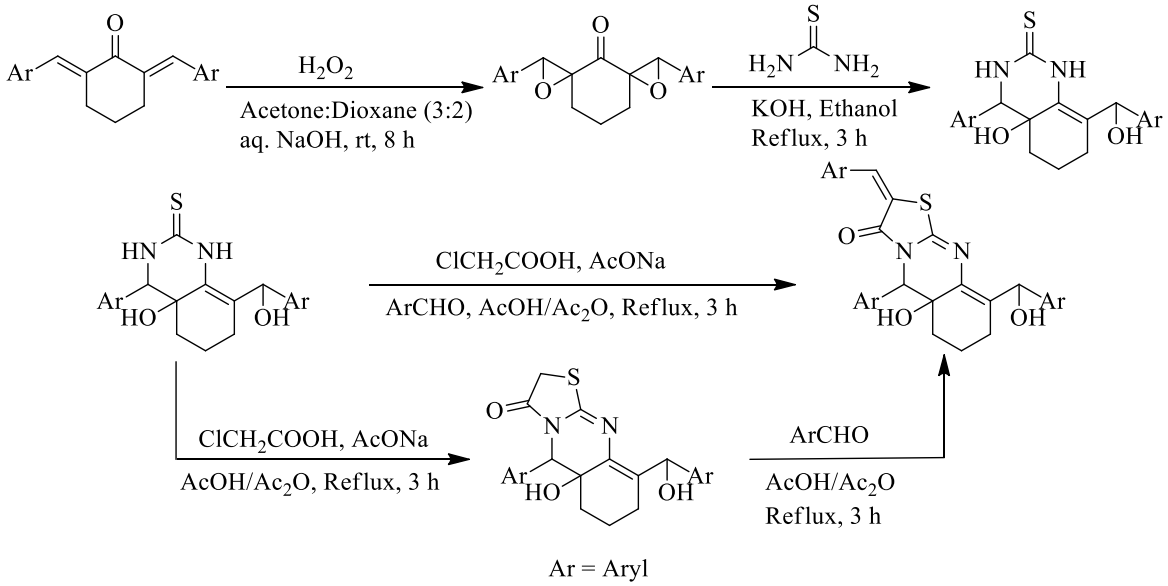
Scheme-6

S. Fatima *et al.*[28] reported a simple and efficient one-pot synthesis of multifunctional 5-Aryl-5*H*-thiazolo[3,2-*a*]pyrimidines.



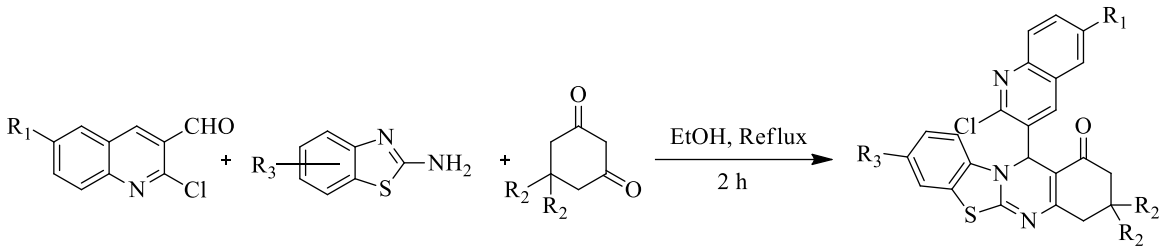
Scheme-7

Ael-G. Amr and **co-workers**[29] reported synthesis and reactions of some new substituted pyridine and pyrimidine derivatives as analgesic, anticonvulsant and antiparkinsonian agents.



Scheme-8

N. K. Shah *et al.*[30] described one-pot, multicomponent condensation reaction in neutral conditions: synthesis, characterization, and biological studies of fused thiazolo[2,3-*b*]quinazolinone derivatives.



Scheme-9

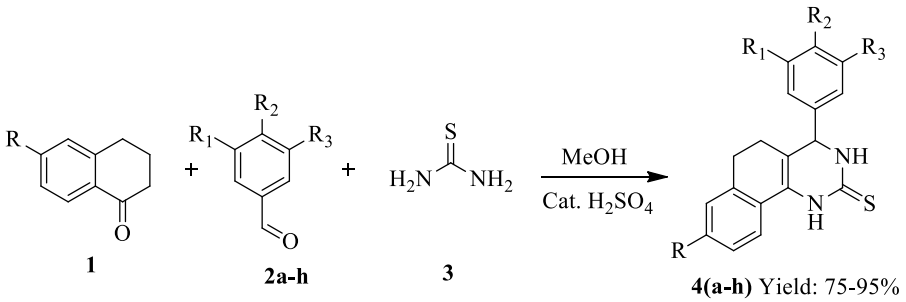
PRESENT WORK

Keeping in view of high degree profile of biological potency of thiazolo[3,2-*a*]pyrimidine and 1,2,3-triazoles, herein we designed novel structural framework embedding these two pharmacophores.

Preparation of starting materials

8-methoxy-4-aryl-3,4,5,6-tetrahydrobenzo[*h*]quinazoline-2(1*H*)-thiones (4a-f)[25]

Fused 3,4-Dihydropyrimidin-2(1*H*)-thiones **(4a-f)**, modified biginelli products were achieved by the three component condensation of substituted aromatic aldehydes **(2a-d)** with 6-methoxy-1-tetralone **(1)** and thiourea **(3)** in refluxing methanol in the presence of catalytic amount of conc. H_2SO_4 in excellent yields **(Scheme-10)**.



1 **2a-h** **3** **4(a-h)** Yield: 75-95%

1: R = OCH_3;

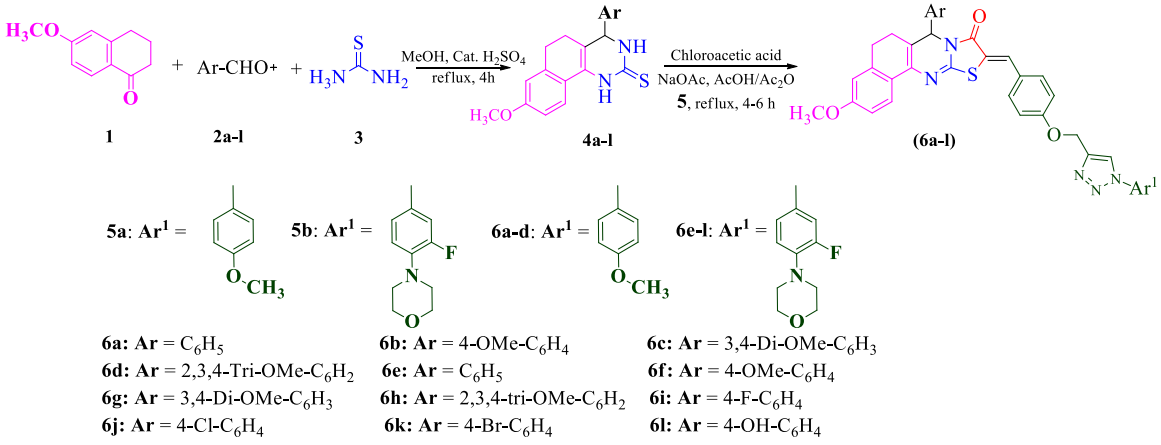
2a: R_1 = H; R_2 = H; R_3 = H
2b: R_1 = H; R_2 = OCH_3; R_3 = H
2c: R_1 = H; R_2 = OCH_3; R_3 = OCH_3
2d: R_1 = OCH_3; R_2 = OCH_3; R_3 = OCH_3
2e: R_1 = H; R_2 = F; R_3 = H
2f: R_1 = H; R_2 = Cl; R_3 = H
2g: R_1 = H; R_2 = Br; R_3 = H
2h: R_1 = H; R_2 = OH; R_3 = H

Scheme-10

Synthesis of starting materials 4-((1-(4-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)benzaldehyde (**5a**) and 4-((1-(3-fluoro-4-morpholinophenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)benzaldehyde (**5b**) were described in **Chapter-III, Section-A** and **Chapter-III, Section-B** respectively.

Synthesis of fused thiazolo[2,3-*b*]pyrimidinone-morpholine based triazolyle hybrids (6a–l)

Cyclization of modified Biginelli product (**4a-h**) using mono chloroacetic acid in refluxing AcOH in the presence of Ac_2O and NaOAc furnished the fused thiazolo[3,2-*a*]pyrimidinones. Further, knoevenagel condensation with corresponding aldehydes **(5a, b)** afforded the desired products (**6a-l**) in good to excellent yields (75-90 %).



Scheme-11

Results and discussion

The synthetic pathway to achieve the titled compounds (**6a-l**) was outlined in **Scheme 11**. The target compounds were achieved by the knoevenagel condensation of aldehydes (**5a, b**) with fused 3,4-dihydropyrimidin-2(1*H*)-thiones in methanol with catalytic amount of piperidine under reflux condition. The unrepeated intermediate fused 3,4-dihydropyrimidin-2(1*H*)-thiones obtained by the cyclization of modified Biginelli compound (**4a-h**) with mono chloroacetic acid in refluxing AcOH in the presence of Ac_2O and NaOAc furnished the desired products (**6a-l**) in good yields. The structures of the titled compounds were well established by the FTIR, NMR and mass spectral studies as well as elemental analyses (C, H and N).

Conclusion

A series of fused thiazolo[2,3-*b*]pyrimidinone-morpholine based triazolyle hybrids (**6a**–**l**) were designed by adopting molecular hybdridization and successfully synthesized by knoevenagel condensation of 4-((1-(4-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)benzaldehyde (**5a**) and 4-((1-(3-fluoro-4-morpholinophenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)benzaldehyde (**5b**) with fused thiazolo[2,3-*b*]pyrimidinones (**4a-h**) in acceptable yields.

Experimental

Synthesis of fused thiazolo[2,3-*b*]pyrimidinone-morpholine based triazolyle hybrids (6a–l)

Equmolar mixture of benzo[*h*]thiazolo[2,3-*b*]quinazolinone (**6a-h**, 1 mmol) and -((1-(4-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)benzaldehyde (**5a**)/4-((1-(3-fluoro-4-morpholinophenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)benzaldehyde (**5b**) (1 mmol) were taken in 10 mL of methanol, to this catalytic amount of piperidine was added and refluxed for 4-6 h. After the completion of the reaction (monitored by TLC). The solid separated out was filtered and washed with hot methanol which afforded the analytically pure product in good to excellent yields.

Spectral data

3-methoxy-10-(4-((1-(4-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)benzylidene)-7-phenyl-7,10-dihydro-5*H*-benzo[*h*]thiazolo[2,3-*b*]quinazolin-9(6*H*)-one (6a)

Yellow solid; 88 % yield; mp: 232-234 °C; **IR** (KBr) υ_{max} (cm^{-1}): 1705 (thiazolo C=O); **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.96 (s, 1H), 7.89 (d, *J* = 8.4 Hz, 1H), 7.63-7.60 (m, 3H), 7.44 (d, *J* = 8.4 Hz, 4H), 7.34-7.28 (m, 3H), 7.09 (d, *J* = 8.4 Hz, 2H), 7.01 (d, *J* = 8.8 Hz, 2H), 6.81 (d, *J* = 8.8 Hz, 1H), 6.67 (s, 1H), 5.68 (s, 1H), 5.32 (s, 2H), 3.86 (s, 3H), 3.81 (s, 3H), 2.81-2.75 (m, 1H), 2.72-2.64 (m, 1H), 2.27-2.19 (m, 1H); 2.12-2.03 (m, 1H); **Mass** (ESI) *m/z*: 655 (M + H); Anal. calcd. for $C_{38}H_{31}N_5O_4S$: C, 69.81; H, 4.78; N, 10.71; Found: C, 70.07; H, 4.48; N, 10.93.

3-methoxy-7-(4-methoxyphenyl)-10-(4-((1-(4-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)benzylidene)-7,10-dihydro-5*H*-benzo[*h*]thiazolo[2,3-*b*]quinazolin-9(6*H*)-one (6b)

Yellow solid; 90 % yield; mp: 228-230 °C; **IR** (KBr) υ_{max} (cm^{-1}): 1710 (thiazolo C=O); **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.96 (s, 1H), 7.88 (d, *J* = 8.4 Hz, 1H), 7.63-7.59 (m, 3H), 7.43 (d, *J* = 8.8 Hz, 2H), 7.37 (d, *J* = 8.8 Hz, 2H), 7.08 (d, *J* = 8.8 Hz, 2H), 7.00 (d, *J* = 8.8 Hz, 2H), 6.83 (d, *J* = 8.4 Hz, 3H), 6.67 (s, 1H), 5.63 (s, 1H), 5.31 (s, 2H), 3.85 (s, 3H), 3.81 (s, 3H), 3.76 (s, 3H), 2.80-2.67 (m, 2H), 2.25-2.16 (m, 1H), 2.12-2.04 (m, 1H); **Mass** (ESI) *m/z*: 685 (M + H); Anal. calcd. for $C_{39}H_{33}N_5O_5S$: C, 68.50; H, 4.86; N, 10.24; Found: C, 68.77; H, 4.62; N, 10.52.

7-(3,4-dimethoxyphenyl)-3-methoxy-10-(4-((1-(4-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)benzylidene)-7,10-dihydro-5*H*-benzo[*h*]thiazolo[2,3-*b*]quinazolin-9(6*H*)-one (6c)

Yellow solid; 85 % yield; mp: 240-242 °C; **IR** (KBr) υ_{max} (cm^{-1}): 1699 (thiazolo C=O); **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.97 (s, 1H), 7.88 (d, *J* = 8.4 Hz, 1H), 7.62 (d, *J* = 8.0 Hz, 3H), 7.44 (d, *J* = 8.4 Hz, 2H), 7.09 (d, *J* = 8.4 Hz, 2H), 7.01 (d, *J* = 8.4 Hz, 3H), 6.96 (s, 1H), 6.80 (d, *J* = 8.0 Hz, 2H), 6.69 (s, 1H), 5.63 (s, 1H), 5.32 (s, 2H), 3.86 (s, 3H), 3.83 (s, 6H), 3.81 (s, 3H), 2.82-2.66 (m, 2H), 2.29-2.21 (m, 1H), 2.14-2.06 (m, 1H); **Mass** (ESI) *m/z*: 715 (M + H); Anal. calcd. for $C_{40}H_{35}N_5O_6S$: C, 67.31; H, 4.94; N, 9.81; Found: C, 67.02; H, 4.72; N, 10.06.

3-methoxy-10-(4-((1-(4-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)benzylidene)-7-(2,3,4-trimethoxyphenyl)-7,10-dihydro-5*H*-benzo[*h*]thiazolo[2,3-*b*]quinazolin-9(6*H*)-one (6d)

Yellow solid; 85 % yield; mp: 237-239 °C; **IR** (KBr) υ_{max} (cm^{-1}): 1701 (thiazolo C=O); **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.97 (s, 1H), 7.88 (d, *J* = 8.4 Hz, 1H), 7.63 (d, *J* = 11.2 Hz, 3H), 7.46 (d, *J* = 8.8 Hz, 2H), 7.11 (d, *J* = 8.8 Hz, 2H), 7.02 (d, *J* = 8.8 Hz, 2H), 6.82 (d, *J* = 8.4 Hz, 1H), 6.68 (d, *J* = 9.6 Hz, 3H), 5.61 (s, 1H), 5.33 (s, 2H), 3.86 (s, 3H), 3.82 (s, 12H), 2.83-2.68 (m, 2H), 2.31-2.23 (m, 1H), 2.17-2.08 (m, 1H); **Mass** (ESI) *m/z*: 715 (M + H); Anal. calcd. for $C_{41}H_{37}N_5O_7S$: C, 66.20; H, 5.01; N, 9.42; Found: C, 66.46; H, 4.71; N, 9.17.

10-(4-((1-(3-fluoro-4-morpholinophenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)benzylidene)-3-methoxy-7-phenyl-7,10-dihydro-5*H*-benzo[*h*]thiazolo[2,3-*b*]quinazolin-9(6*H*)-one (6e)

Yellow solid; 89 % yield; mp: 253-255 °C; **IR** (KBr) υ_{max} (cm^{-1}): 1714 (thiazolo C=O); **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.98 (s, 1H), 7.88 (d, *J* = 8.8 Hz, 1H), 7.59 (s, 1H), 7.50-7.43 (m, 6H), 7.34-7.28 (m, 3H), 7.08 (d, *J* = 8.8 Hz, 2H), 7.02 (t, *J* = 8.4 Hz, 1H), 6.81 (d, *J* = 8.4 Hz, 1H), 6.67 (s, 1H), 5.67 (s, 1H), 5.31 (s, 2H), 3.88 (t, *J* = 4.4 Hz, 4H), 3.81 (s, 3H), 3.14 (t, *J* = 4.4 Hz, 4H), 2.77-2.67 (m, 2H), 2.27-2.17 (m, 1H), 2.11-2.03 (m, 1H); **Mass** (ESI) *m/z*: 728 (M + H); Anal. calcd. for $C_{41}H_{35}FN_6O_4S$: C, 67.75; H, 4.85; N, 11.56; Found: C, 67.50; H, 4.64; N, 11.25.

10-(4-((1-(3-fluoro-4-morpholinophenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)benzylidene)-3-methoxy-7-(4-methoxyphenyl)-7,10-dihydro-5*H*-benzo[*h*]thiazolo[2,3-*b*]quinazolin-9(6*H*)-one (6f)

Yellow solid; 86 % yield; mp: 222-224 °C; **IR** (KBr) υ_{max} (cm^{-1}): 1698 (thiazolo C=O); **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.00 (s, 1H), 7.90 (d, *J* = 8.8 Hz, 1H), 7.62 (s, 1H), 7.53-7.44 (m, 4H), 7.40 (d, *J* = 8.8 Hz, 2H), 7.10 (d, *J* = 8.8 Hz, 2H), 7.04 (t, *J* = 8.8 Hz, 1H), 6.86 (d, *J* = 8.8 Hz, 3H), 6.70 (s, 1H), 5.67 (s, 1H), 5.34 (s, 2H), 3.91 (t, *J* = 4.4 Hz, 4H), 3.84 (s, 3H), 3.78 (s, 3H), 3.16 (t, *J* = 4.4 Hz, 4H), 2.79-2.71 (m, 2H), 2.28-2.19 (m, 1H), 2.15-2.06 (m, 1H); **Mass** (ESI) *m/z*: 758 (M + H); Anal. calcd. for $C_{42}H_{37}FN_6O_5S$: C, 66.65; H, 4.93; N, 11.10; Found: C, 66.38; H, 4.73; N, 11.41.

7-(3,4-dimethoxyphenyl)-10-(4-((1-(3-fluoro-4-morpholinophenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)benzylidene)-3-methoxy-7,10-dihydro-5*H*-benzo[*h*]thiazolo[2,3-*b*]quinazolin-9(6*H*)-one (6g)

Yellow solid; 80 % yield; mp: 228-230 °C; **IR** (KBr) υ_{max} (cm^{-1}): 1700 (thiazolo C=O);; **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.97 (s, 1H), 7.88 (d, *J* = 8.4 Hz, 1H), 7.61 (s, 1H), 7.51-7.41 (m, 4H), 7.09 (d, *J* = 8.8 Hz, 2H), 7.04-7.00 (m, 2H), 6.96 (s, 1H), 6.83-6.79 (m, 2H), 6.68 (s, 1H), 5.64 (s, 1H), 5.32 (s, 2H), 3.88 (t, *J* = 4.4 Hz, 4H), 3.84 (s, 6H), 3.82 (s, 3H), 3.14 (t, *J* = 4.4 Hz, 4H), 2.77-2.70 (m, 2H), 2.29-2.21 (m, 1H), 2.14-2.06 (m, 1H); **Mass** (ESI) *m/z*: 788 (M + H); Anal. calcd. for $C_{43}H_{39}FN_6O_6S$: C, 65.63; H, 5.00; N, 10.68; Found: C, 65.85; H, 5.24; N, 10.88.

10-(4-((1-(3-fluoro-4-morpholinophenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)benzylidene)-3-methoxy-7-(2,3,4-trimethoxyphenyl)-7,10-dihydro-5*H*-benzo[*h*]thiazolo[2,3-*b*]quinazolin-9(6*H*)-one (6h)

Yellow solid; 75 % yield; mp: 236-238 °C; **IR** (KBr) υ_{max} (cm^{-1}): 1691 (thiazolo C=O); **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.99 (s, 1H), 7.87 (d, *J* = 8.4 Hz, 1H), 7.64 (s, 1H), 7.51-7.42 (m, 4H), 7.10 (d, *J* = 8.8 Hz, 2H), 7.02 (t, *J* = 8.8 Hz, 1H), 6.82 (d, *J* = 8.8 Hz, 1H), 6.66 (s, 3H), 5.61 (s, 1H), 5.32 (s, 2H), 3.89 (t, *J* = 4.4 Hz, 4H), 3.82 (s, 9H), 3.81 (s, 3H), 3.14 (t, *J* = 4.4 Hz, 4H), 2.77-2.74 (m, 2H), 2.31-2.23 (m, 1H), 2.17-2.08 (m, 1H); **Mass** (ESI) *m/z*: 818 (M + H); Anal. calcd. for $C_{44}H_{41}FN_6O_7S$: C, 64.69; H, 5.06; N, 10.29; Found: C, 64.89; H, 5.34; N, 10.58.

10-(4-((1-(3-fluoro-4-morpholinophenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)benzylidene)-7-(4-fluorophenyl)-3-methoxy-7,10-dihydro-5*H*-benzo[*h*]thiazolo[2,3-*b*]quinazolin-9(6*H*)-one (6i)

Yellow solid; 78 % yield; mp: 241-243 °C; **IR** (KBr) υ_{max} (cm^{-1}): 1695 (thiazolo C=O); **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.98 (s, 1H), 7.88 (d, *J* = 8.4 Hz, 1H), 7.61 (s, 1H), 7.51-7.42 (m, 6H), 7.09 (d, *J* = 8.4 Hz, 2H), 7.05-6.99 (m, 3H), 6.82 (d, *J* = 8.4 Hz, 1H), 6.68 (s, 1H), 5.68 (s, 1H), 5.33 (s, 2H), 3.89 (t, *J* = 4.0 Hz, 4H), 3.82 (s, 3H), 3.14 (t, *J* = 4.0 Hz, 4H), 2.80-2.69 (m, 2H), 2.26-2.18 (m, 1H), 2.10-2.02 (m, 1H); **Mass** (ESI) *m/z*: 746 (M + H); Anal. calcd. for $C_{41}H_{34}F_2N_6O_4S$: C, 66.12; H, 4.60; N, 11.28; Found: C, 65.78; H, 6.42; N, 7.15.

7-(4-chlorophenyl)-10-(4-((1-(3-fluoro-4-morpholinophenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)benzylidene)-3-methoxy-7,10-dihydro-5*H*-benzo[*h*]thiazolo[2,3-*b*]quinazolin-9(6*H*)-one (6j)

Yellow solid; 75 % yield; mp: 256-258 °C; **IR** (KBr) υ_{max} (cm^{-1}): 1694 (thiazolo C=O); **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.98 (s, 1H), 7.88 (d, *J* = 8.4 Hz, 1H), 7.61 (s, 1H), 7.51-7.38 (m, 6H), 7.30 (d, *J* = 8.0 Hz, 2H), 7.09 (d, *J* = 8.0 Hz, 2H), 7.02 (t, *J* = 8.8 Hz, 1H), 6.82 (d, *J* = 8.4 Hz, 1H), 6.68 (s, 1H), 5.66 (s, 1H), 5.32 (s, 2H), 3.89 (t, *J* = 4.4 Hz, 4H), 3.82 (s, 3H), 3.14 (t, *J* = 4.4 Hz, 4H), 2.80-2.68 (m, 2H), 2.26-2.18 (m, 1H), 2.09-2.01 (m, 1H); **Mass** (ESI) *m/z*: 762 (M + H); Anal. calcd. for $C_{41}H_{34}ClFN_6O_4S$: C, 64.69; H, 4.50; N, 11.04; Found: C, 64.72; H, 4.33; N, 10.79.

7-(4-bromophenyl)-10-(4-((1-(3-fluoro-4-morpholinophenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)benzylidene)-3-methoxy-7,10-dihydro-5*H*-benzo[*h*]thiazolo[2,3-*b*]quinazolin-9(6*H*)-one (6k)

Yellow solid; 76 % yield; mp: 263-265 °C; **IR** (KBr) υ_{max} (cm^{-1}): 1699 (thiazolo C=O); **Mass** (ESI) *m/z*: 807 (M + H); Anal. calcd. for $C_{41}H_{34}BrFN_6O_4S$: C, 61.12; H, 4.25; N, 10.43; Found: C, 61.45; H, 4.00; N, 10.69.

10-(4-((1-(3-fluoro-4-morpholinophenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)benzylidene)-7-(4-hydroxyphenyl)-3-methoxy-7,10-dihydro-5*H*-benzo[*h*]thiazolo[2,3-*b*]quinazolin-9(6*H*)-one (9l)

Yellow solid; 82 % yield; mp: 235-237 °C; **IR** (KBr) υ_{max} (cm^{-1}): 1689 (thiazolo C=O); **^{1}H NMR** (400 MHz, DMSO-d_6): δ 9.57 (s, 1H), 8.92 (s, 1H), 7.80 (d, *J* = 11.2 Hz, 1H), 7.71 (t, *J* = 8.8 Hz, 2H), 7.63 (s, 1H), 7.57 (d, *J* = 8.4 Hz, 2H), 7.23 (d, *J* = 8.8 Hz, 3H), 7.17 (d, *J* = 8.4 Hz, 2H), 6.81 (d, *J* = 8.4 Hz, 1H),), 6.73 (d, *J* = 8.0 Hz, 3H), 5.66 (s, 1H), 5.31 (s, 2H), 3.74 (s, 4H), 3.38 (s, 3H), 3.07 (s, 4H), 2.78-2.70 (m, 1H), 2.65-2.57 (m, 1H), 2.27-2.20 (m, 1H), 1.96-1.87 (m, 1H); **Mass** (ESI) *m/z*: 744 (M + H); Anal. calcd. for $C_{41}H_{35}FN_6O_5S$: C, 66.29; H, 4.75; N, 11.31; Found: C, 65.01; H, 4.48; N, 11.52.

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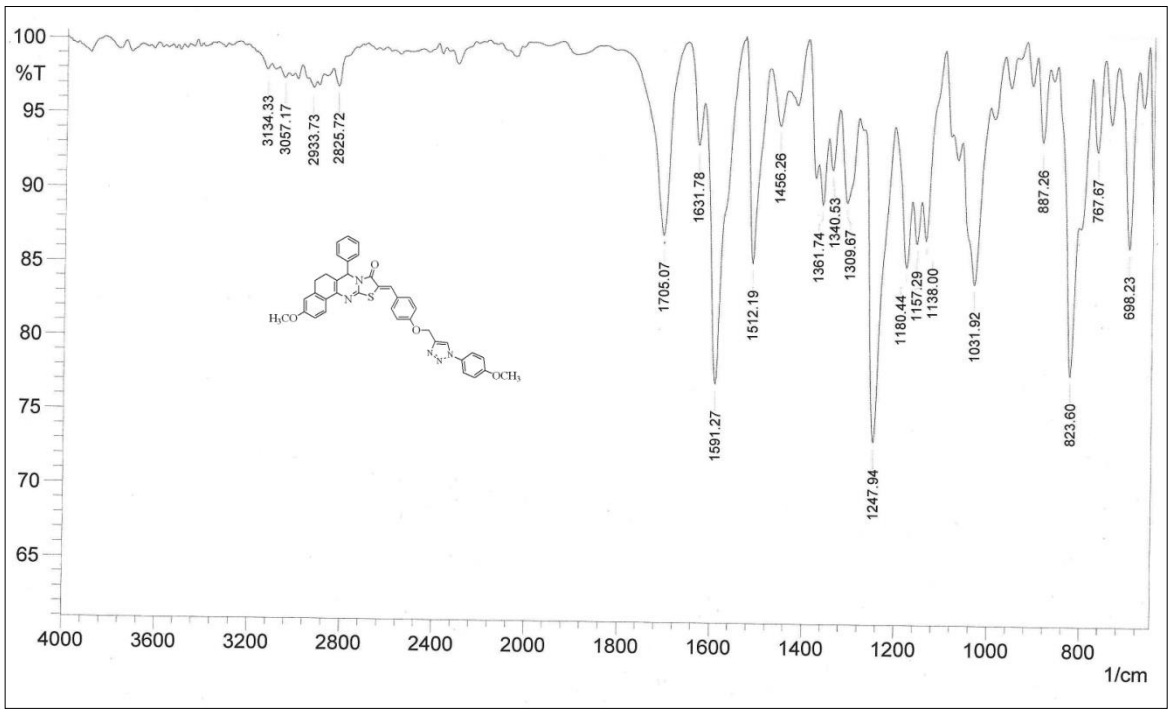
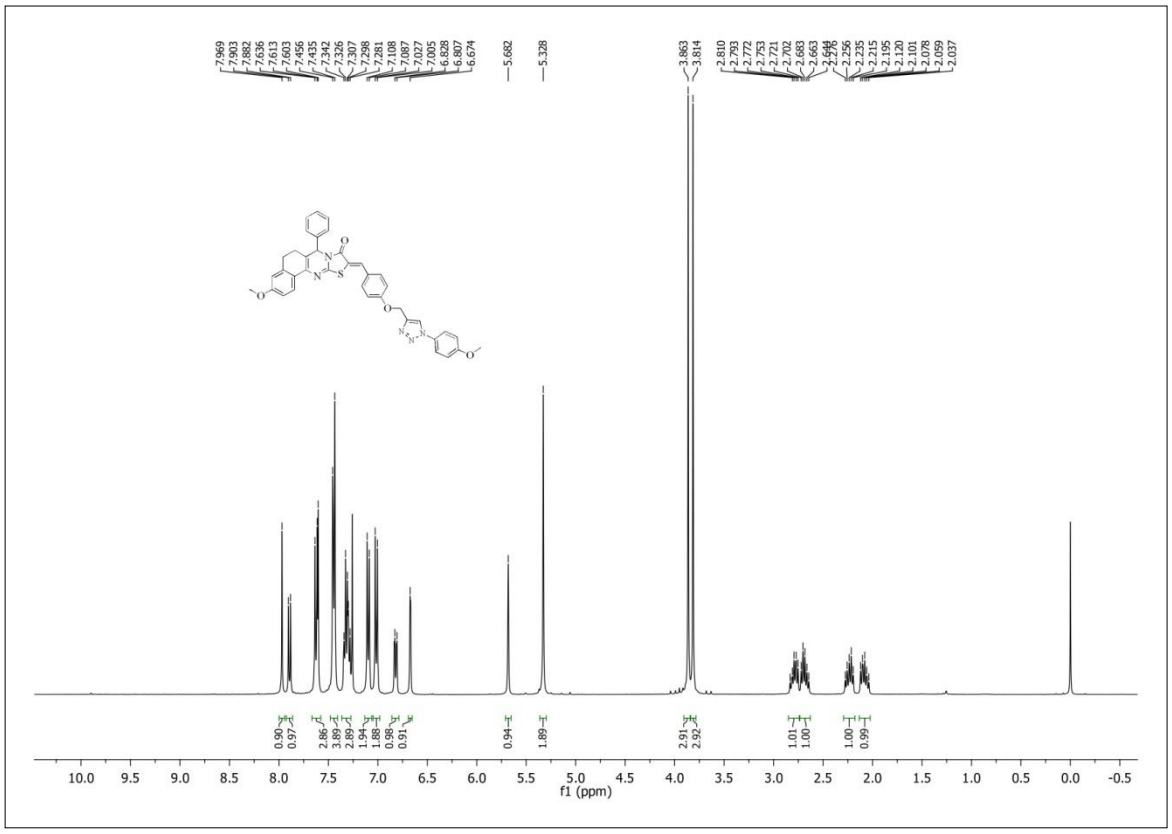
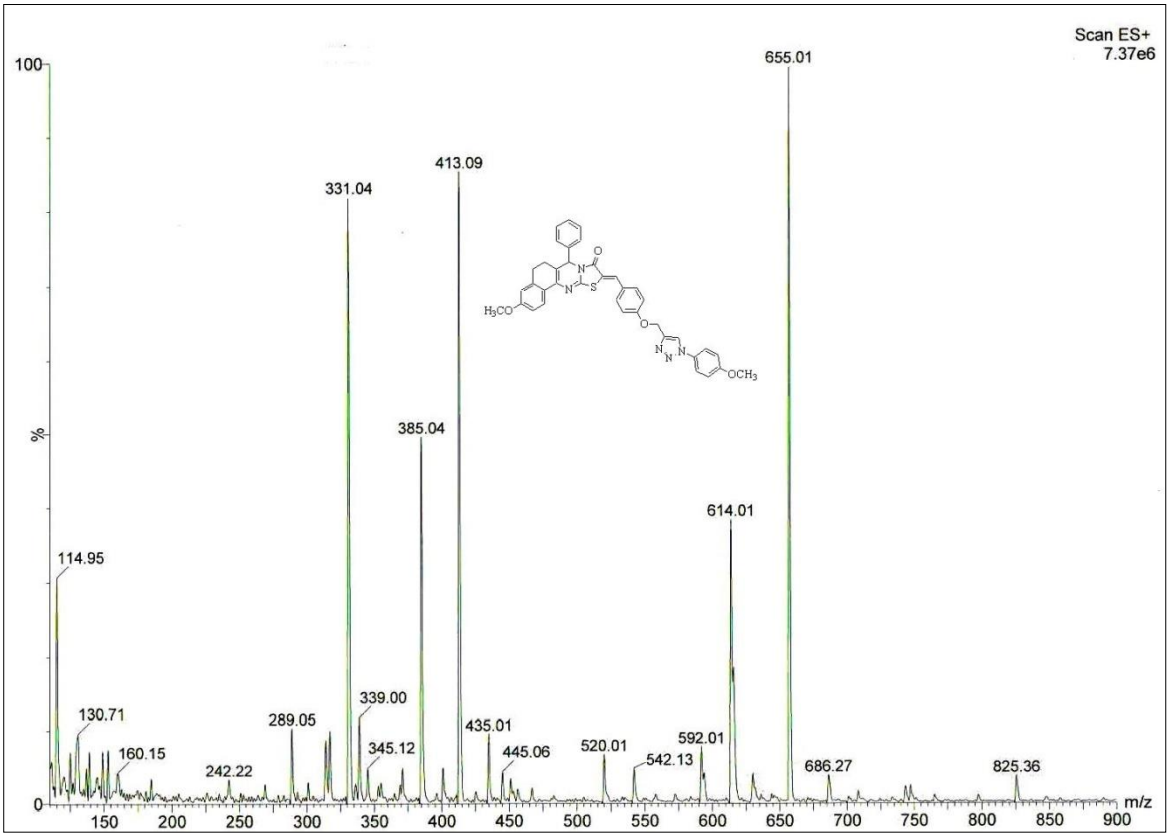


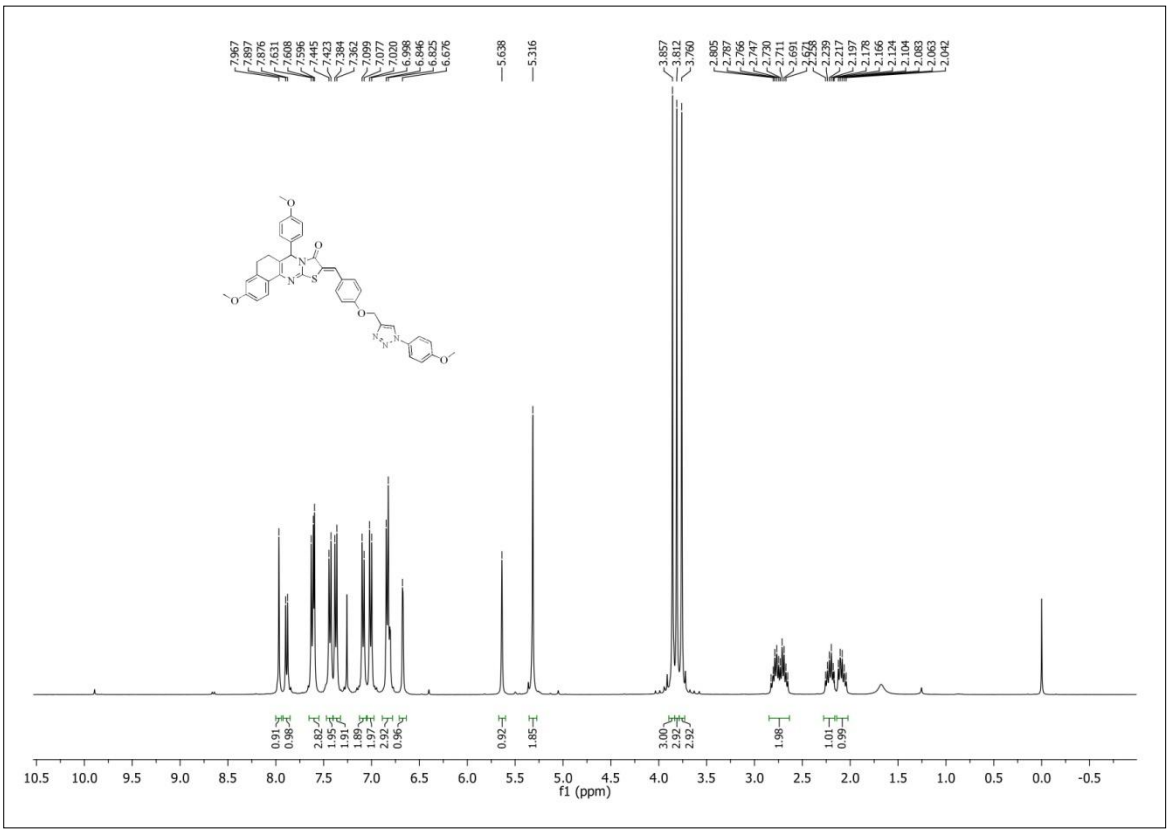
IR (KBr) spectrum of compound 6a



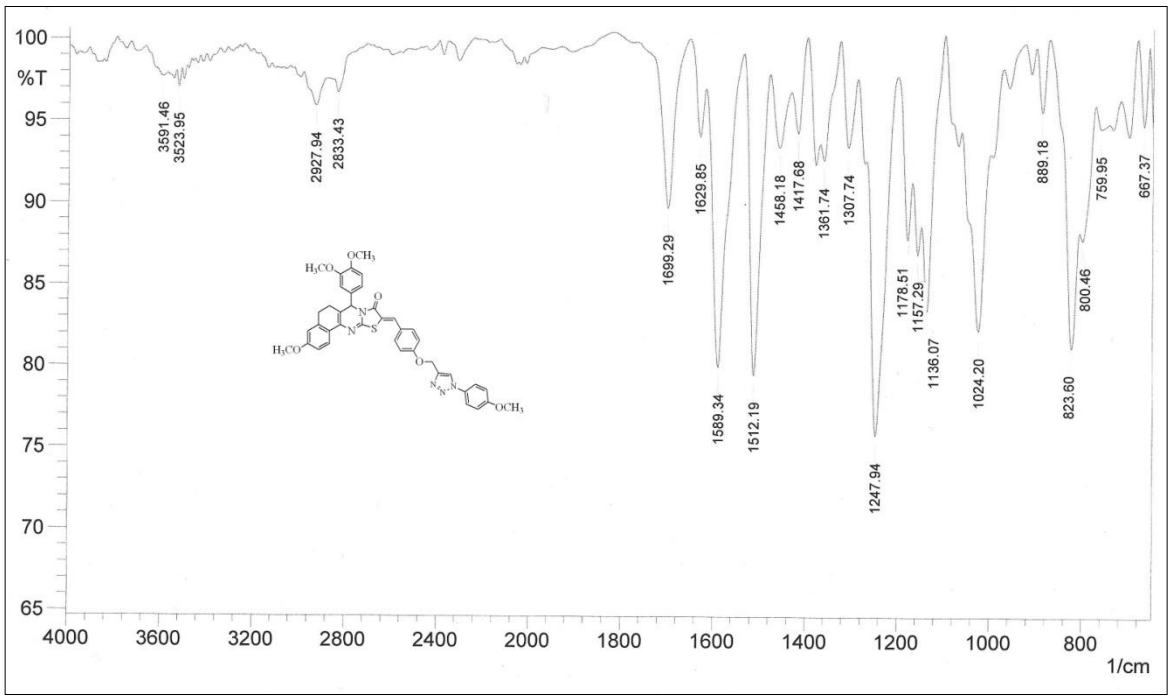
^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound 6a



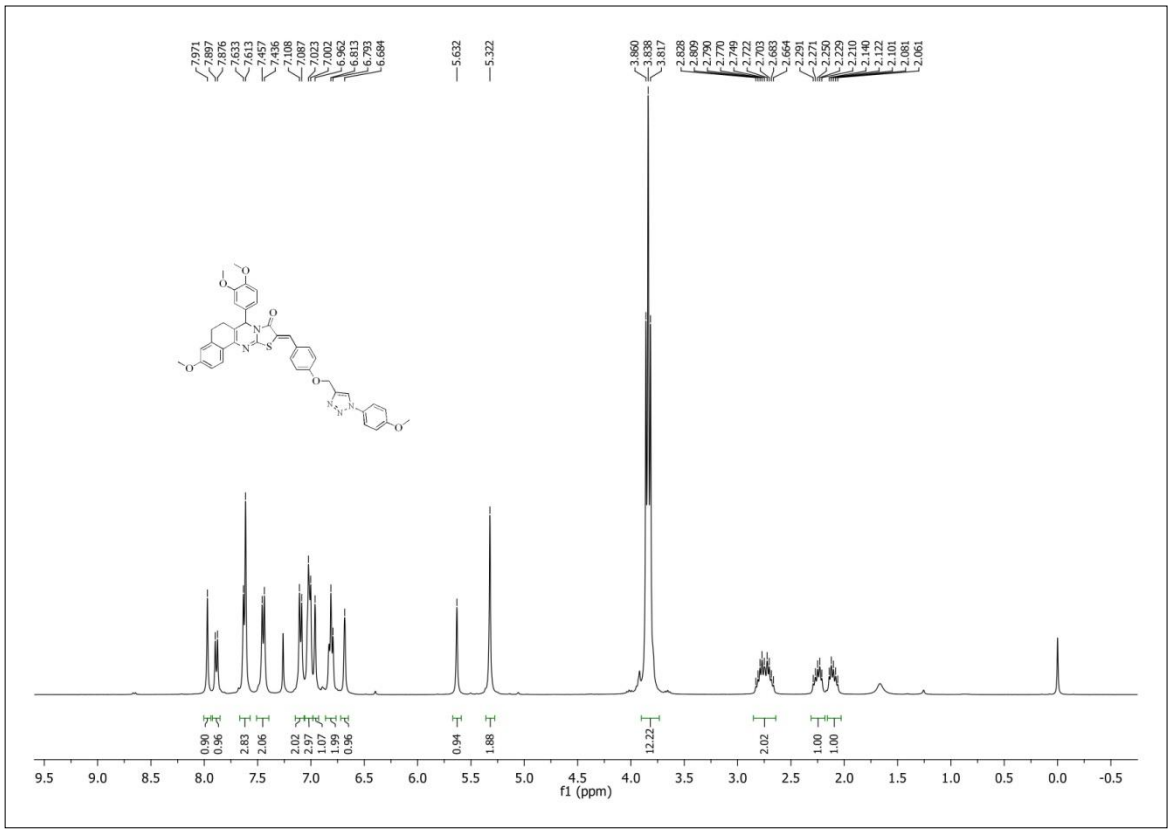
Mass spectrum of compound 6a (M.Wt: 654)



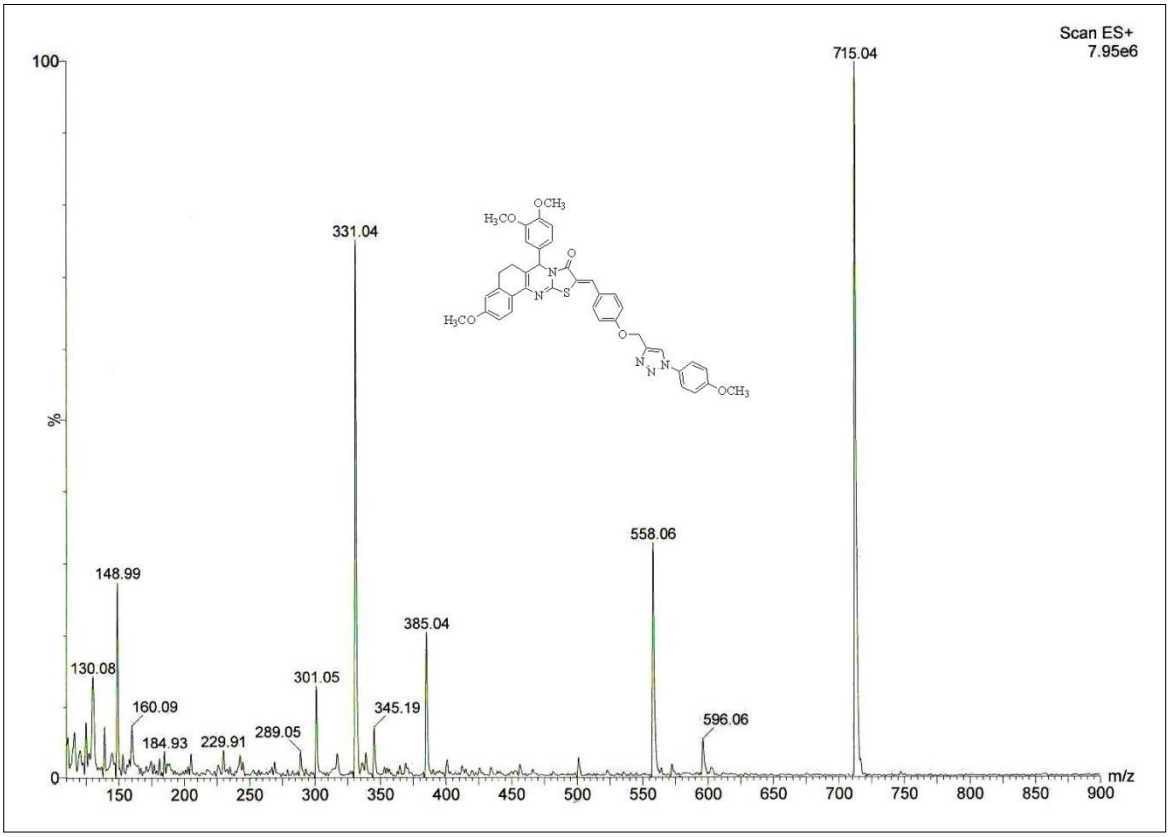
^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound 6b



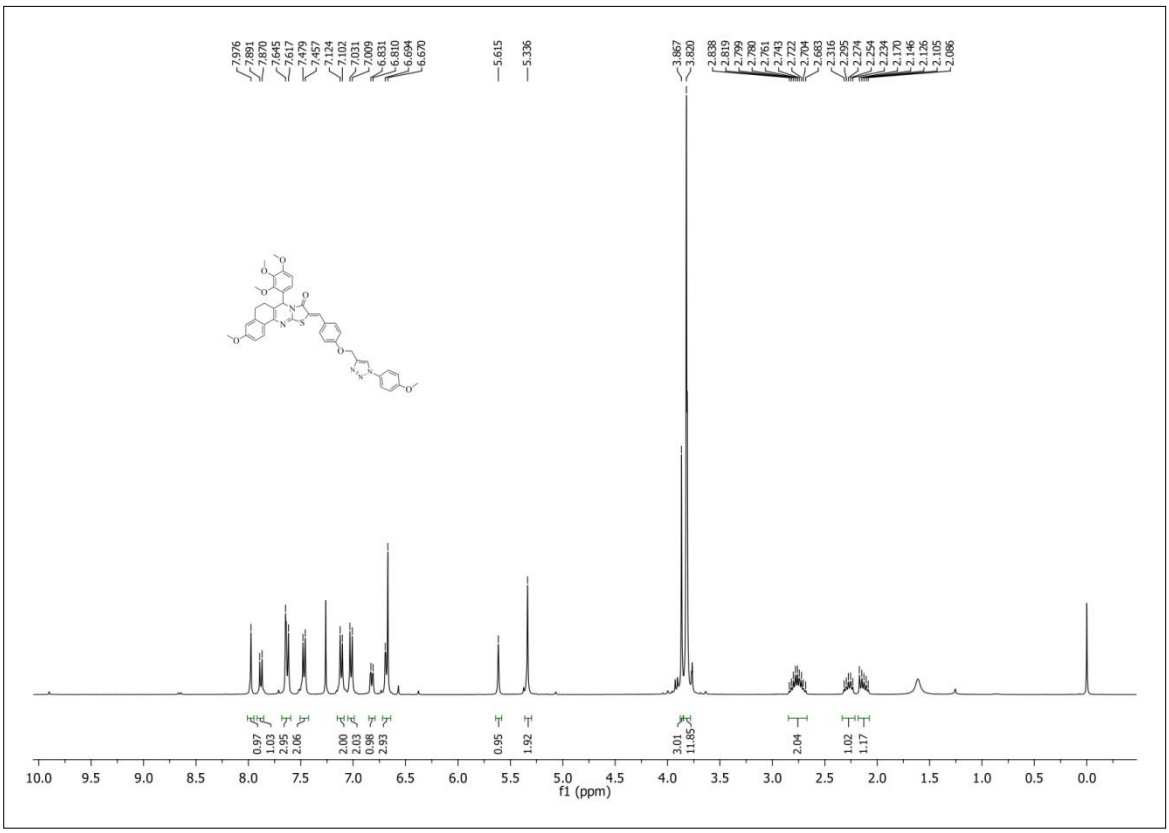
IR (KBr) spectrum of compound 6c



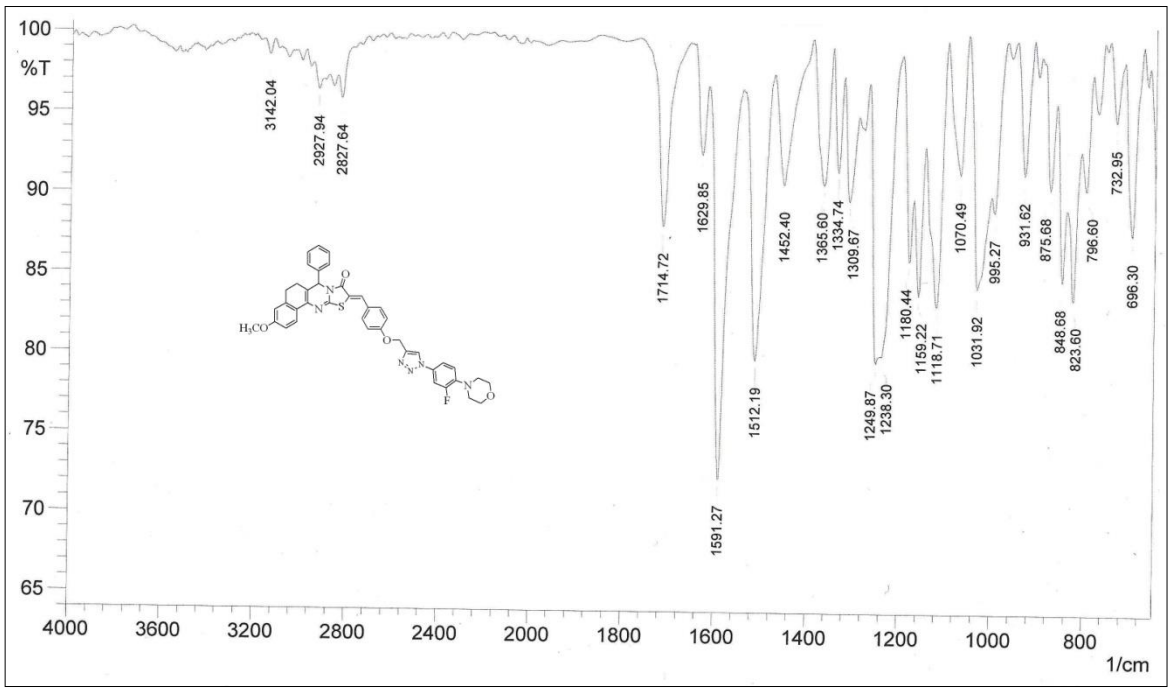
^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound 6c



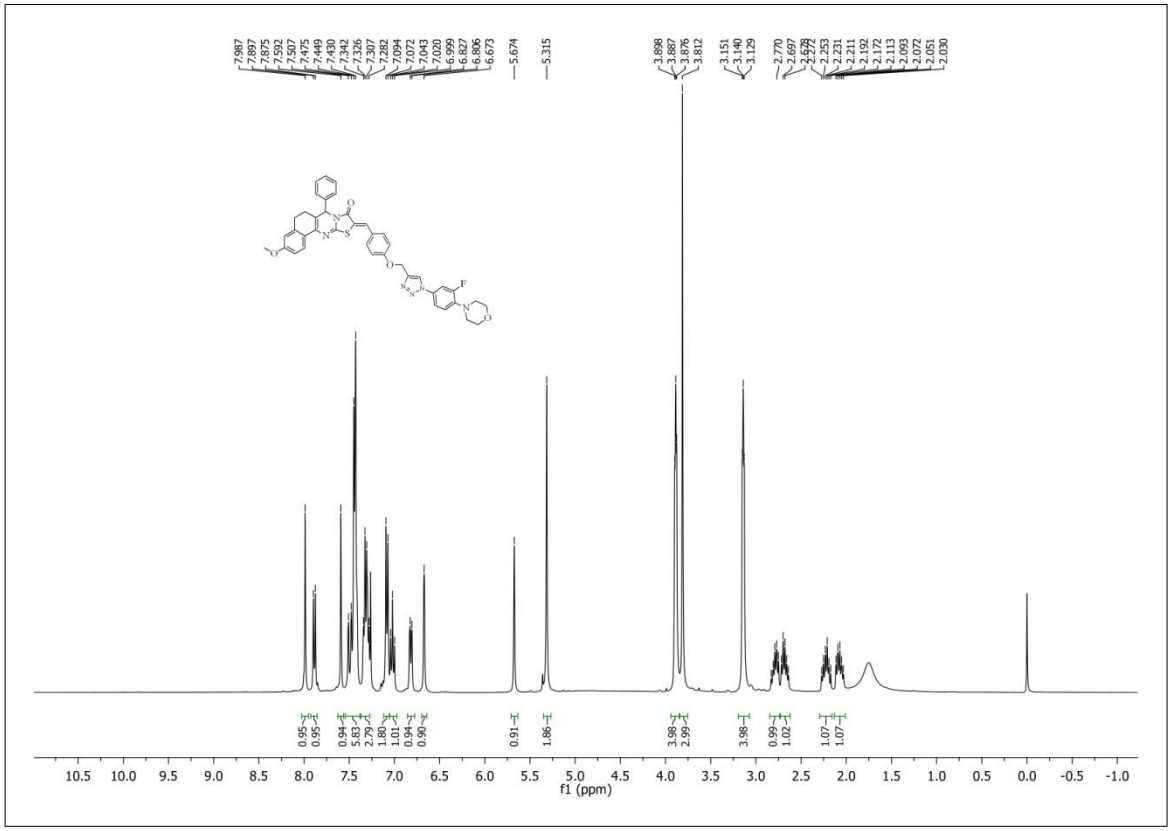
Mass spectrum of compound 6c (M.Wt: 714)



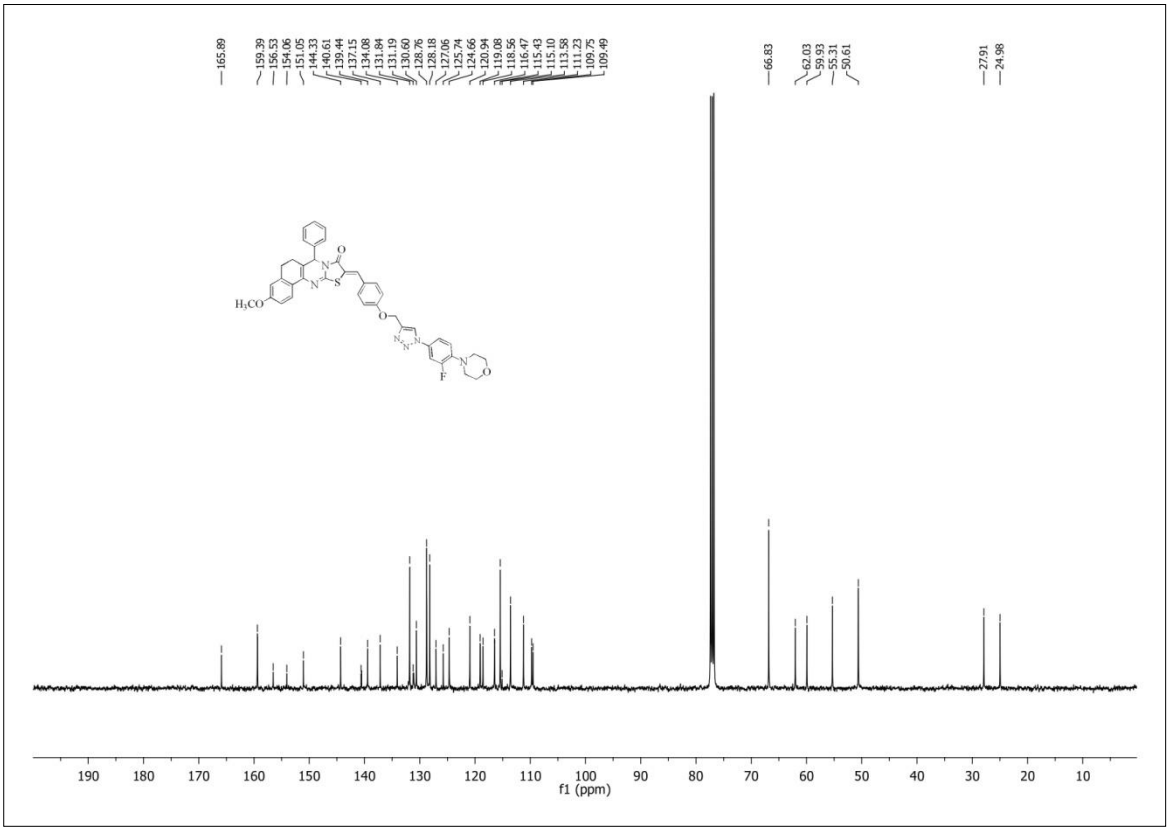
^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound 6d



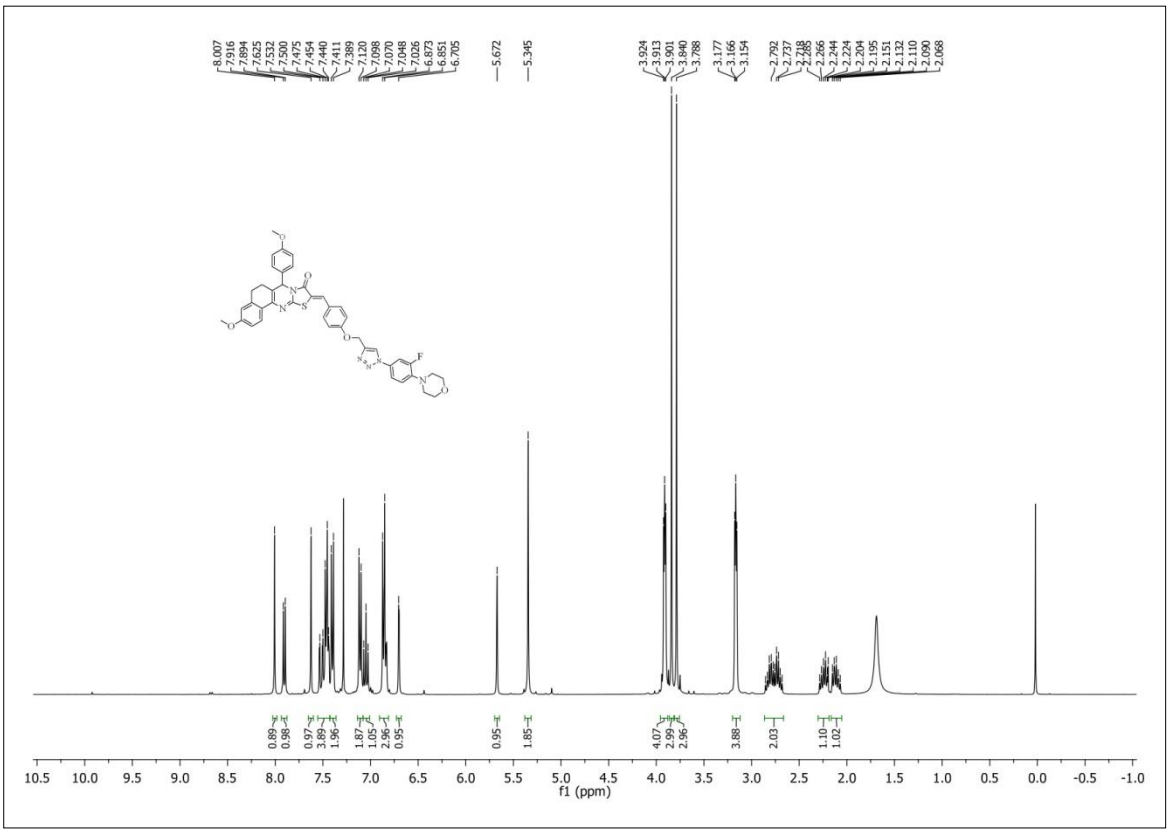
IR (KBr) spectrum of compound 6e



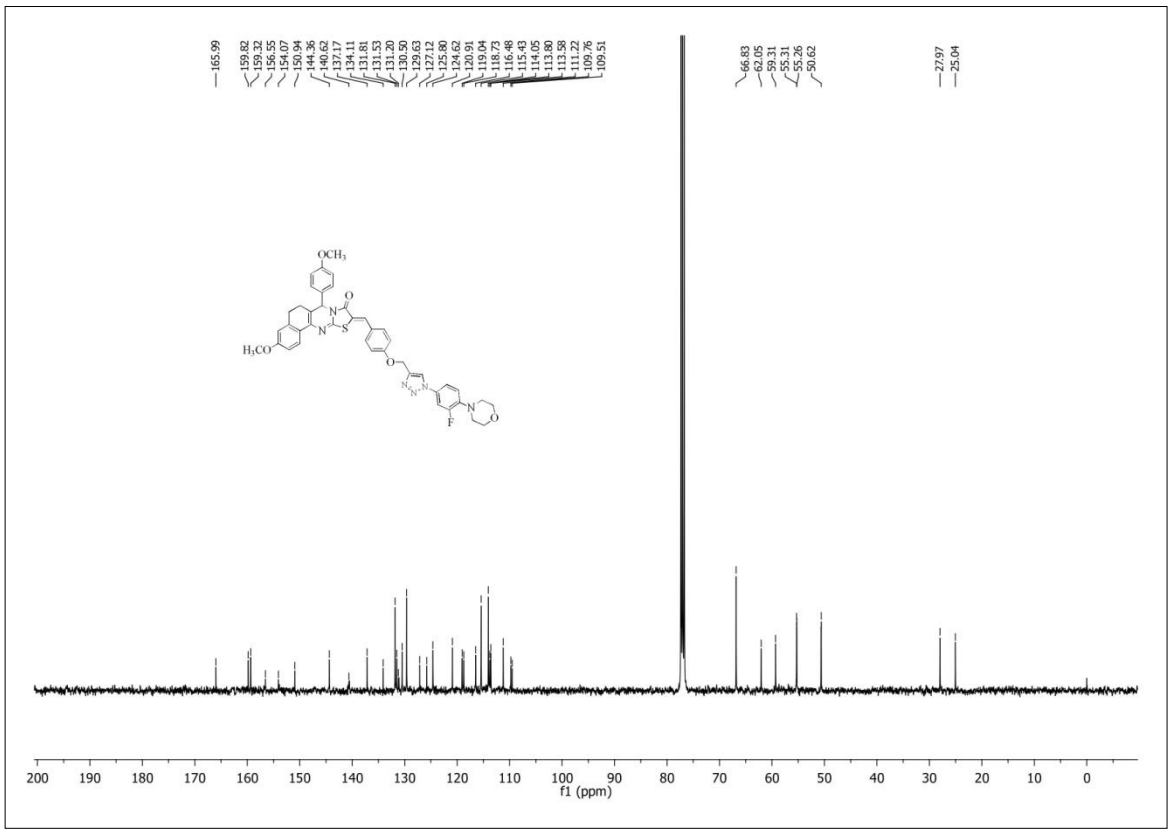
^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound 6e



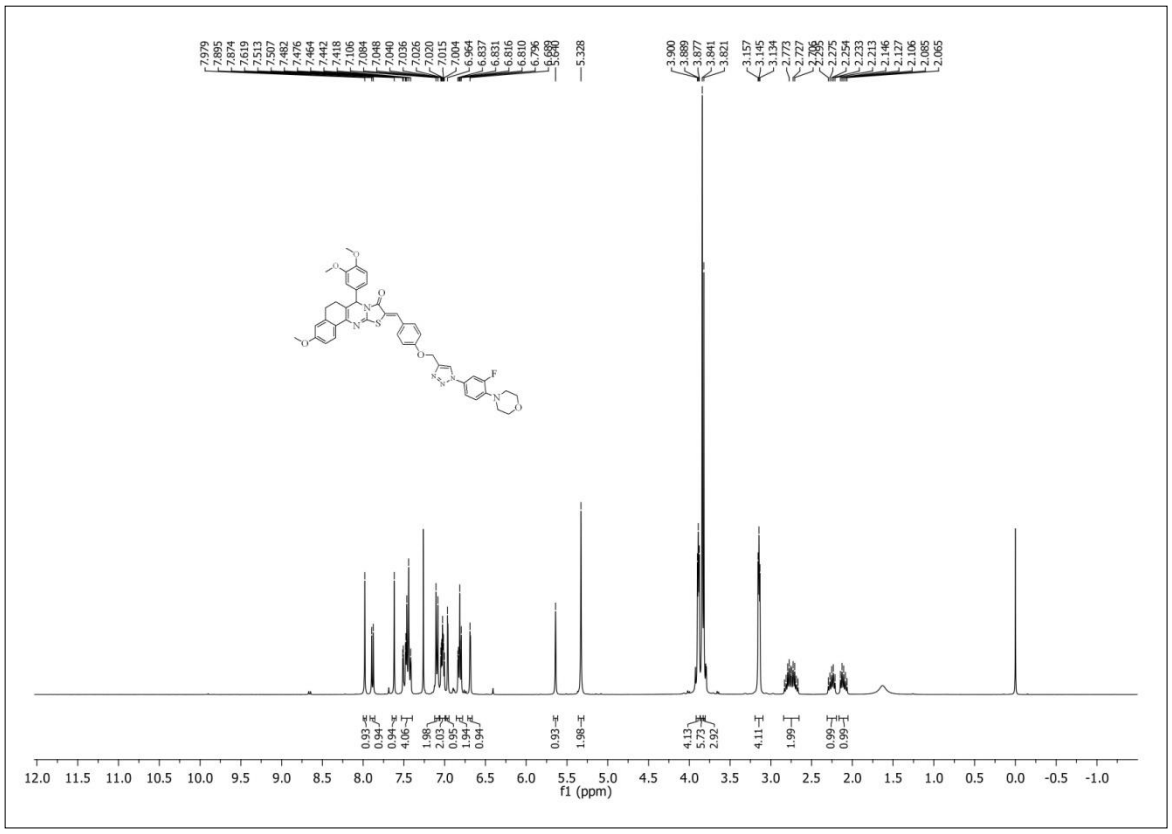
^{13}C NMR (100 MHz, $CDCl_3$) spectrum of compound 6e



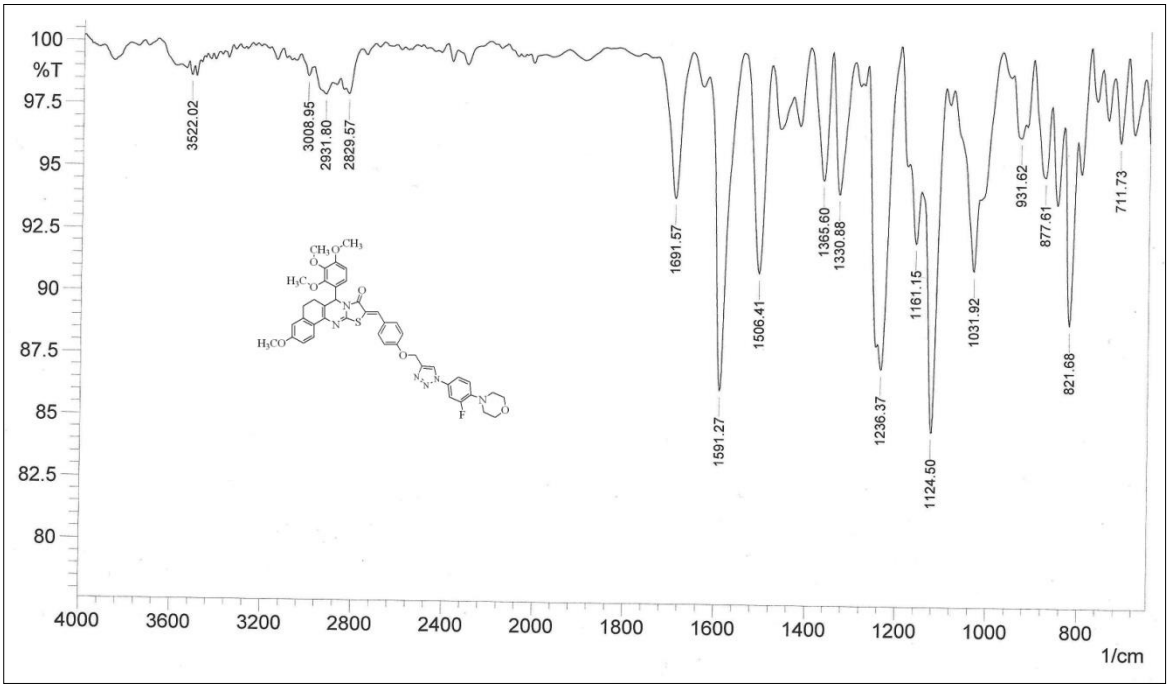
^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound 6f



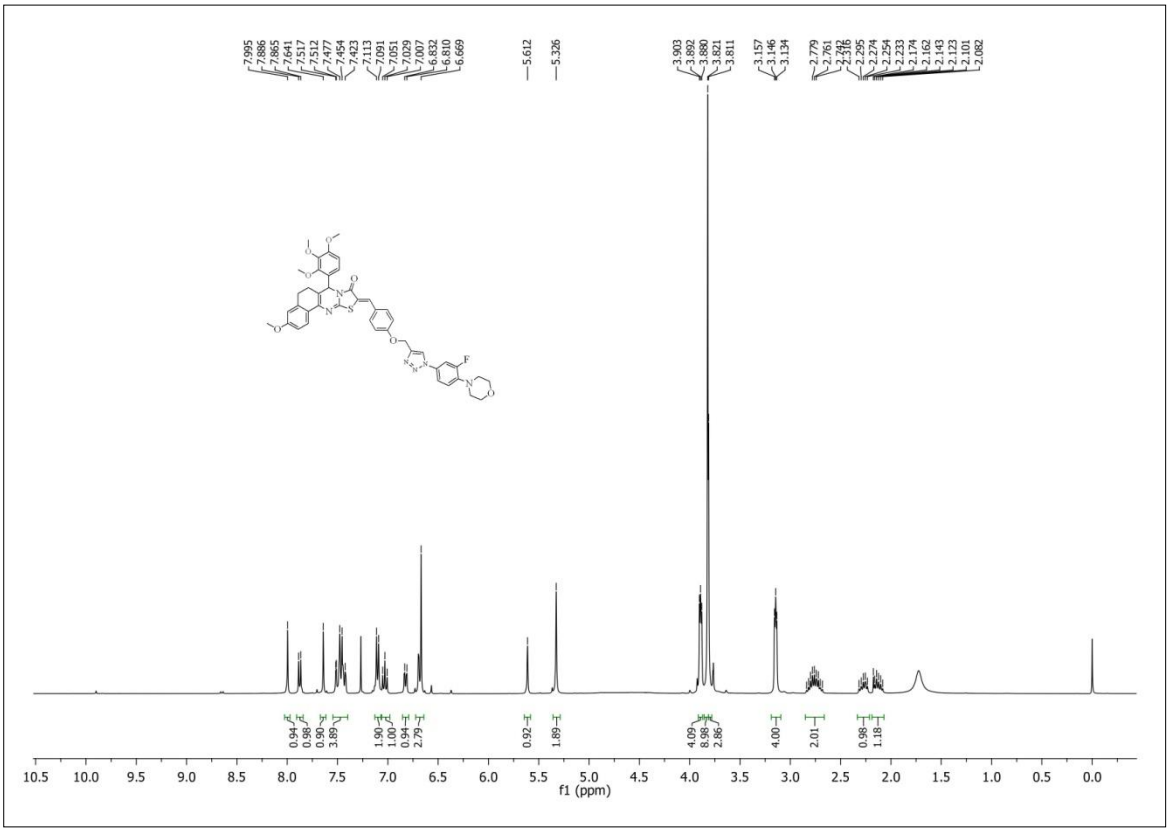
^{13}C NMR (100 MHz, $CDCl_3$) spectrum of compound 6f



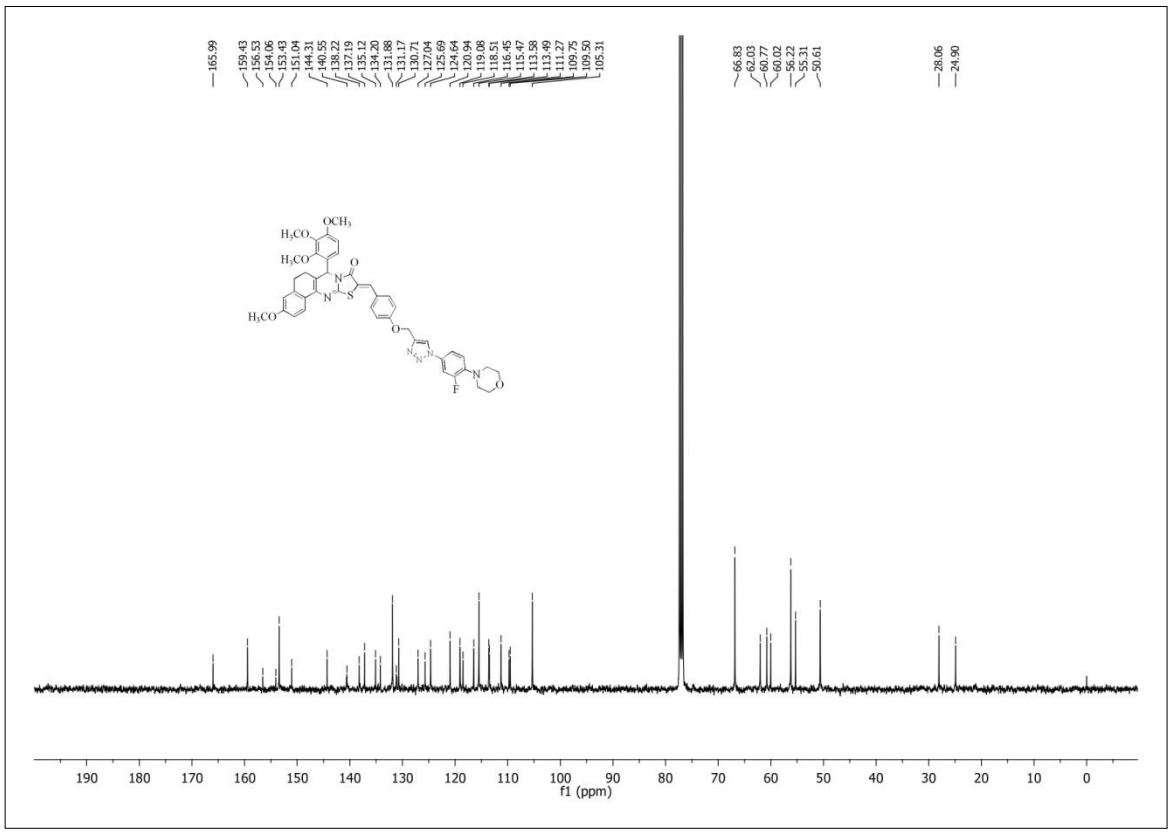
^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound 6g



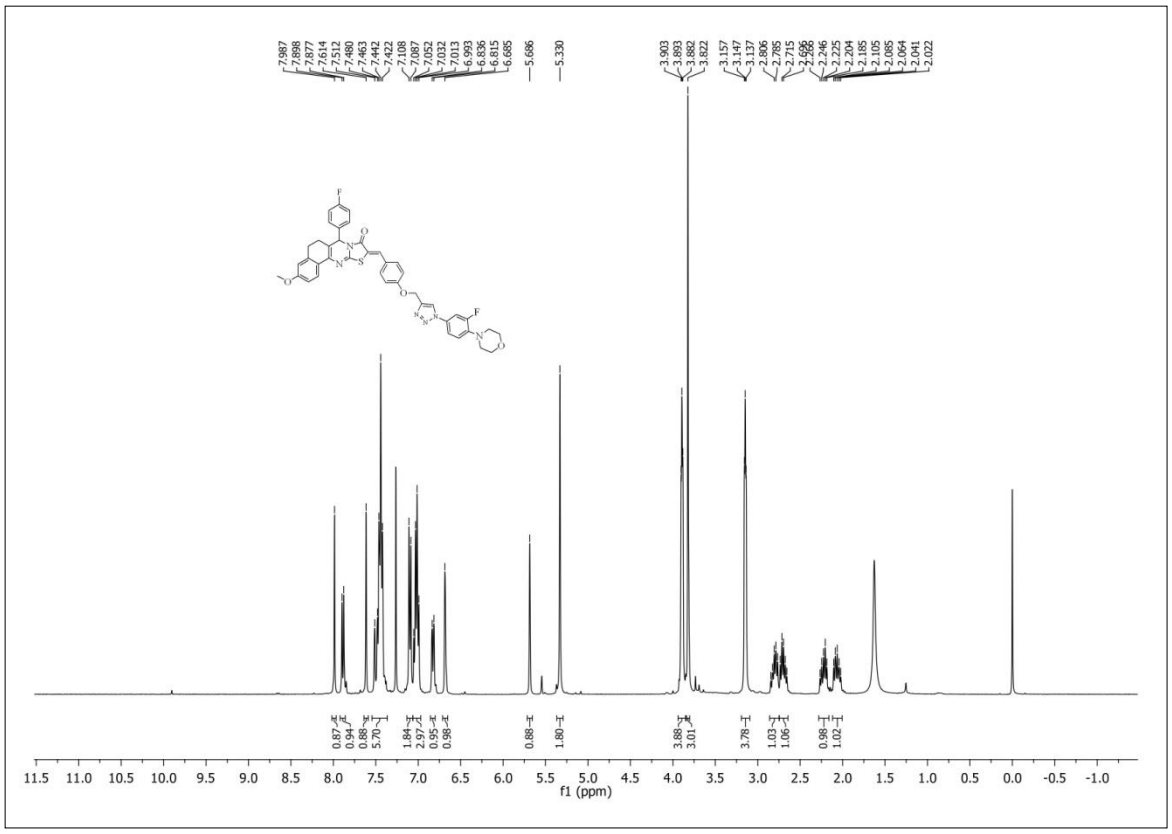
IR (KBr) spectrum of compound 6h



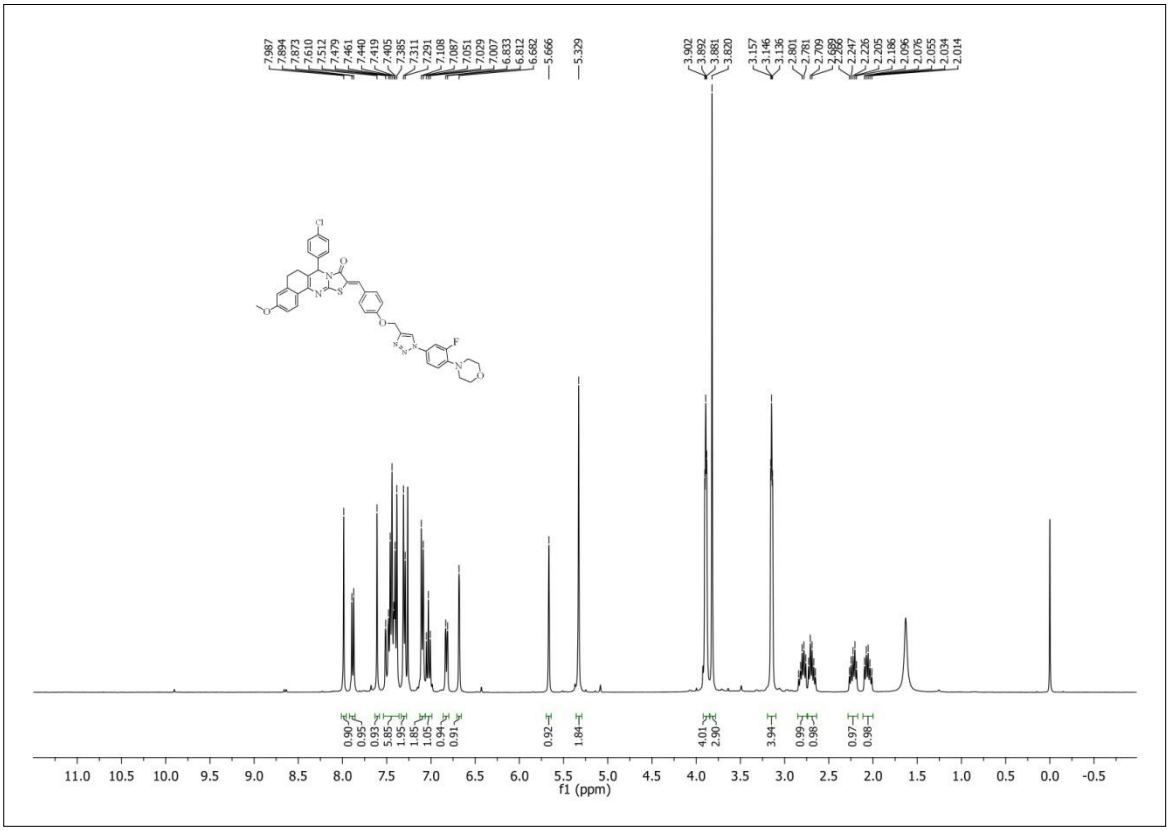
^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound 6h



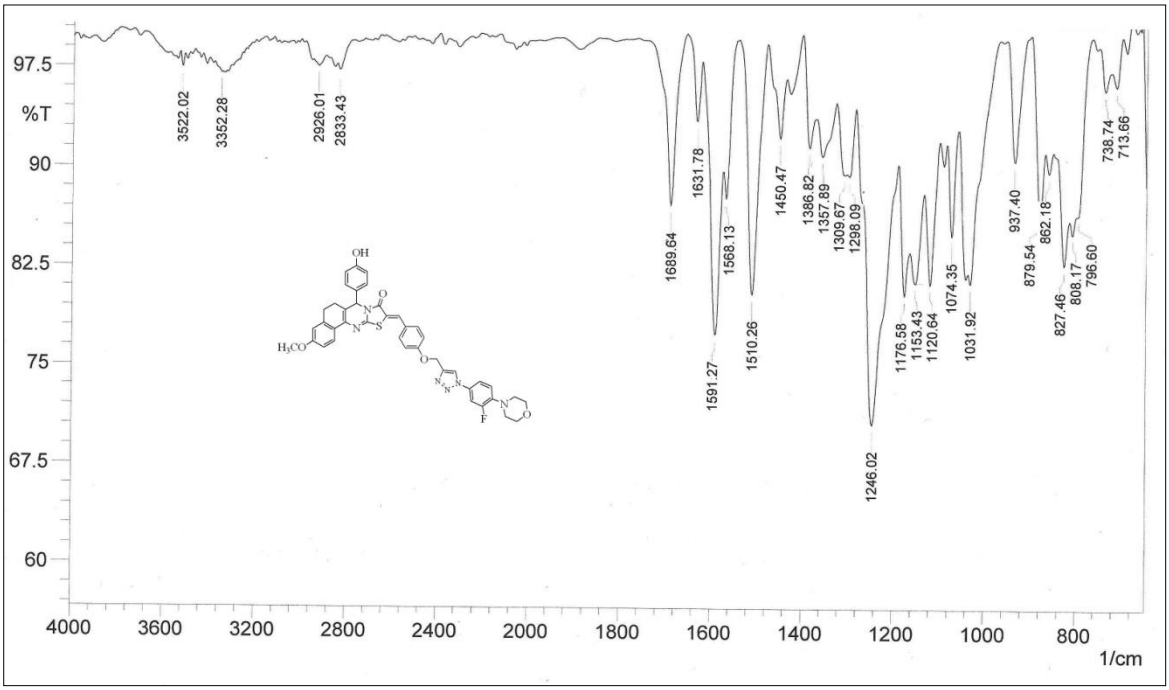
^{13}C NMR (100 MHz, $CDCl_3$) spectrum of compound 6h



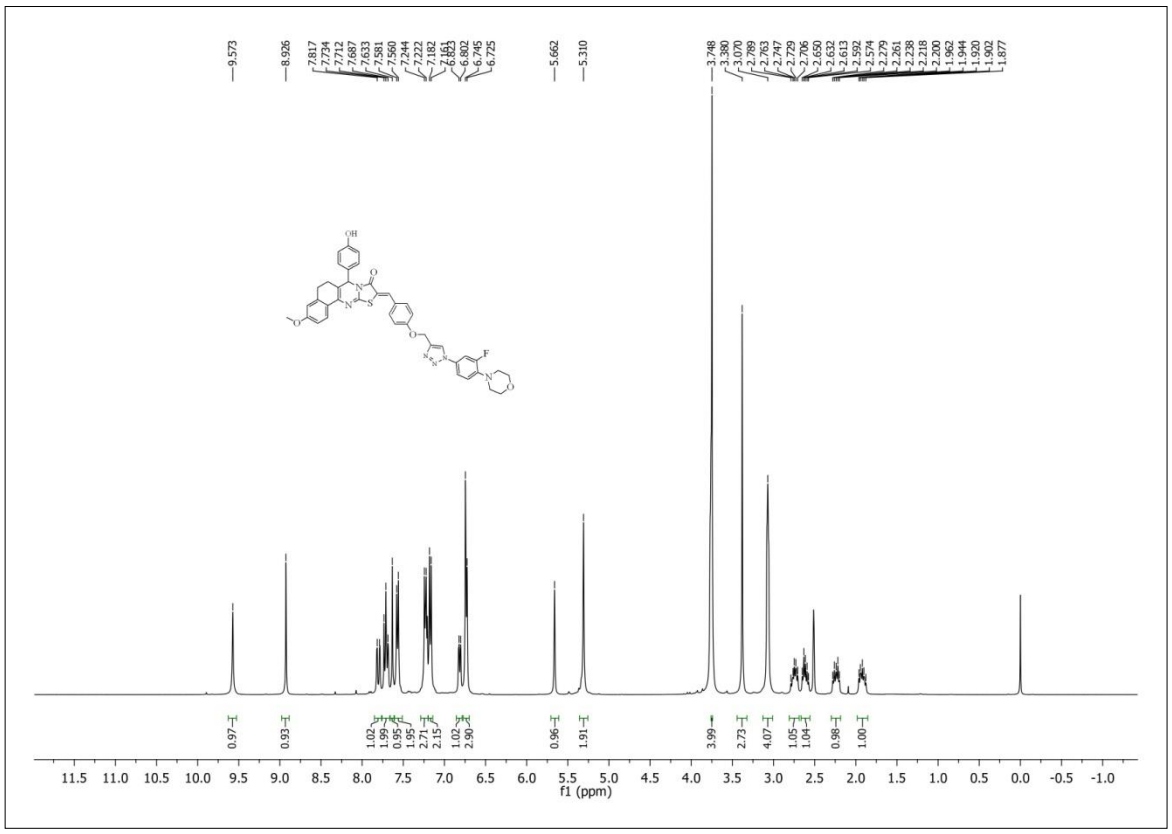
^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound 6i



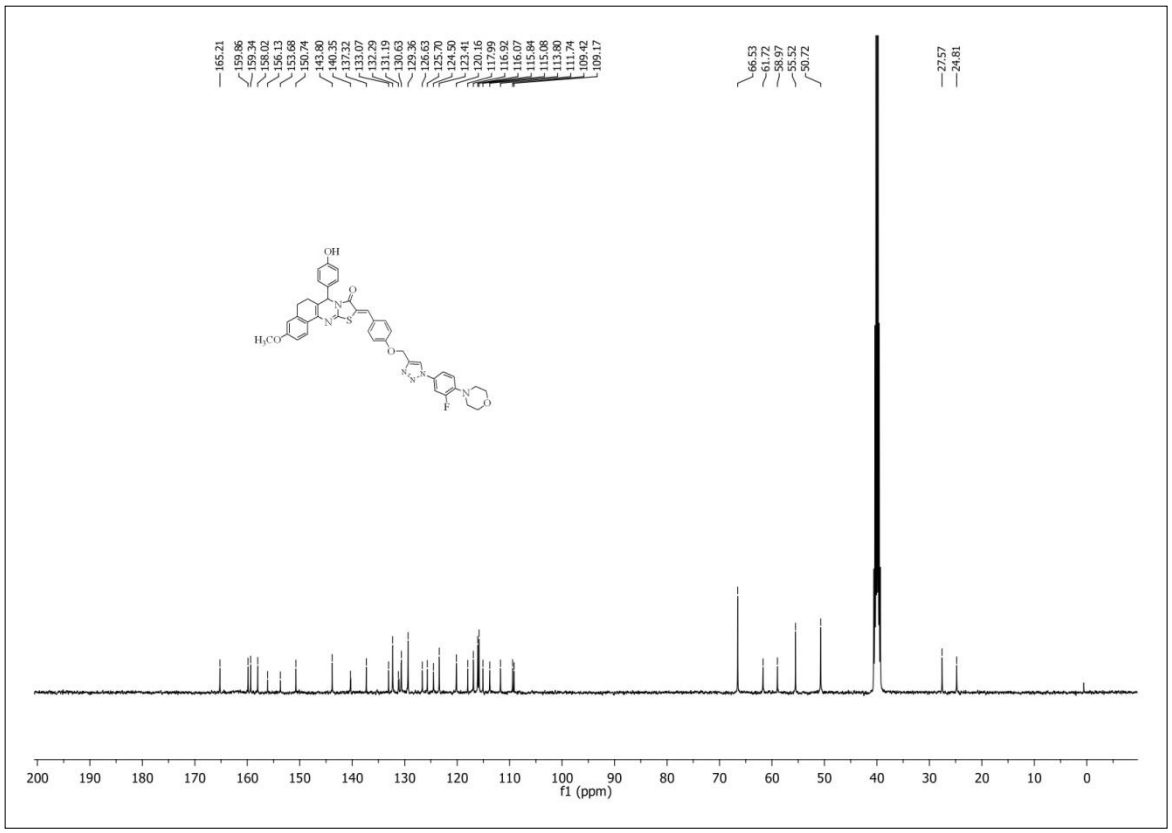
^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound 6j



IR (KBr) spectrum of compound 6l



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



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

CHAPTER-V

NOVEL DONEPEZIL LIKE FUSED CHALCONE-PYRIDINIUM BROMIDE HYBRIDS: SYNTHESIS, CHARACTERIZATION AND EVALUATION OF ANTICANCER, ANTIMICROBIAL ACTIVITY STUDIES

INTRODUCTION

In the recent year's pyridinium salts have attracted great attention as acetylcholinesterase (AChE) inhibitors[1-3] and AChE carbamate inhibitors[4] used to cure neurodegenerative disorders like Alzheimer's disease (AD) and autoimmune disorder Myasthenia gravis (MG) respectively. On the other hand, chalcones ((*E*)-1,3-diaryl-2-propene-1-one) and their synthetic analogs are reported to be precursor of flavonoids and iso-flavonoids, which have attracted great interest in medicinal chemistry owing to their diverse pharmacological properties including anticancer, antimicrobial,[5] anti-diabetic,[6] anti-oxidant,[7] anti-HIV,[8] antiinflammatory,[9] anti-protozoal,[10] antileishmanial,[11] antimalarial[12] and antitrypanosomal[13] activities (**Fig. 1**).

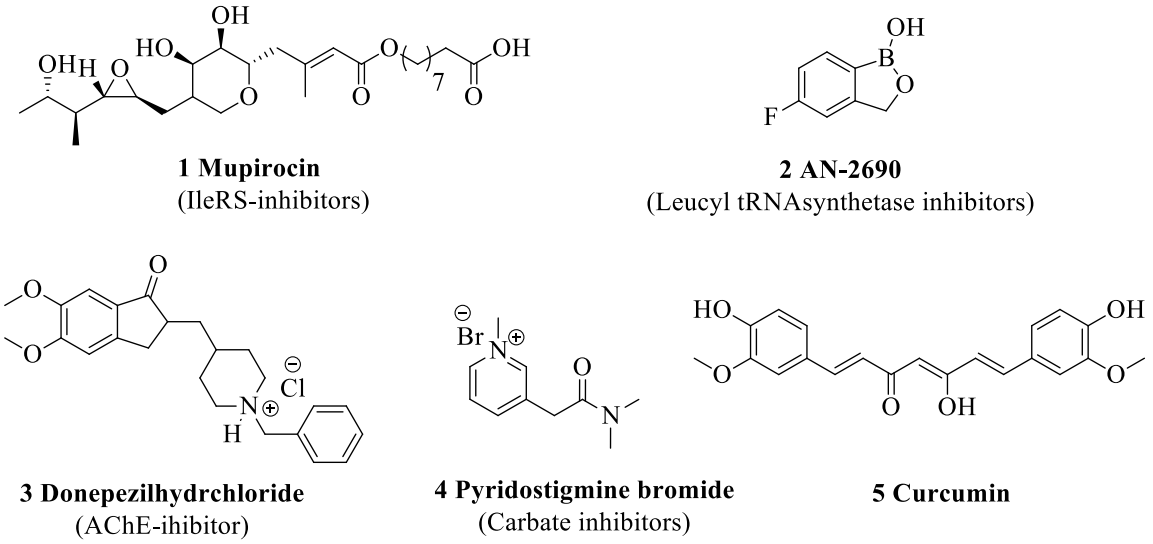


Fig. 1. Structures of **s**ome of the biologically potent antibacterial (**1** and **2**), anti-AChE (**3**), anti-AChE carbamate (**4**) and anticancer (**5**) agents.

Farzaneh Baharloo *et al.*[14] reported the benzofuran-derived benzylpyridinium bromides as potent acetylcholinesterase inhibitors. Among the tested series of compounds the derivatives **1**, **2** and **3** showed potent *in vitro* AChE activity than standard drug donepezil with IC_{50} values 4.1 nM, 4.9 nM and 5.8 nM respectively.

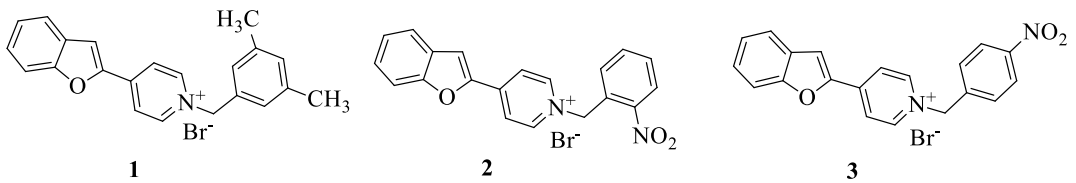


Fig. 2

Jichao Chen *et al.*[15] reported the design, synthesis and biological evaluation of novel nitric oxide-donating protoberberine derivatives as antitumor agents. All the synthesized compounds were evaluated for anti-hepatocellular carcinoma activities and from the results it was found that the compounds **4** (IC_{50} 1.36 μM), **5** (IC_{50} 1.98 μM) and **6** (IC_{50} 2.47 μM) exhibited more potent activity against HepG2 cells than parent compounds berberine and palmatine.

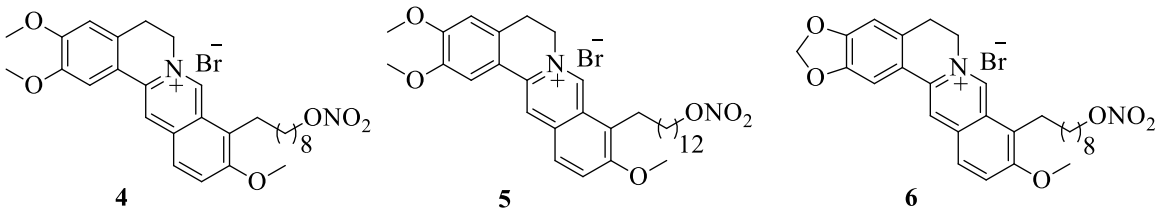


Fig. 3

Nisachon Khunnawutmanotham and co-workers[16] described the synthesis and anti-acetylcholinesterase activity of scopoletin derivatives. The synthesized compounds were assessed for their in vitro AchE inhibiting activity by the colorimetric Ellman's method. Among the tested compounds, the derivatives **7** and **8** found to be more potent than the remaining with IC_{50} values 0.215 μM and 0.340 μM respectively.

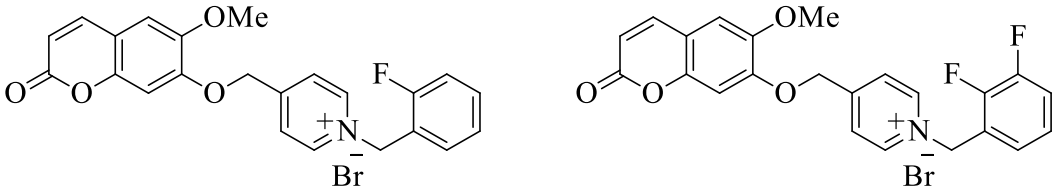


Fig. 4

Jiang-Kun Dai and his group[17] reported the synthesis, *in vitro* antibacterial activities of a series of 3-*N*-substituted canthin-6-ones. From the antibacterial results it was found that the compounds **9-13** were found to be the most potent compounds with minimal inhibitory concentration (MIC) values 0.98 μg/mL.

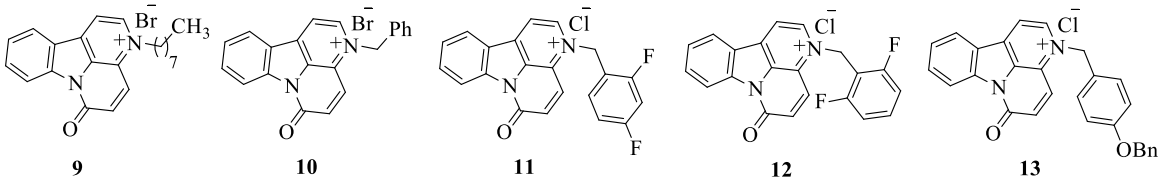
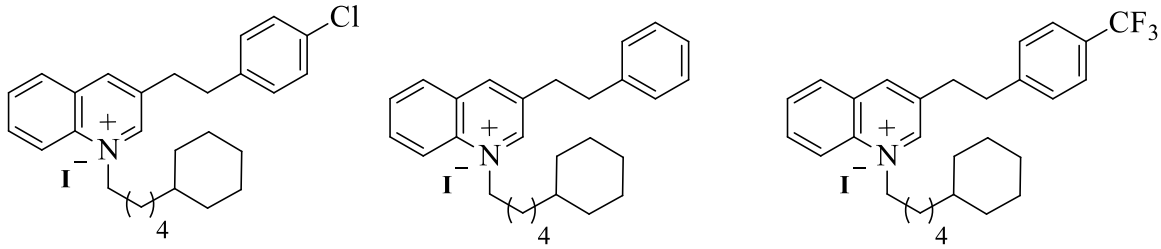


Fig.5

Sidney Bolden Jr and co-workers[18] reported the structure-activity relationship (SAR) and preliminary mode of action studies of 3-substituted benzylthioquinolinium iodide as anti-opportunistic infection agents. From the antifungal results it was found that, the compounds 14, 15 and 16 were more or equipotent than the standard drugs fluconazole and amphotericin B against *Cryptococcus neoformans*.

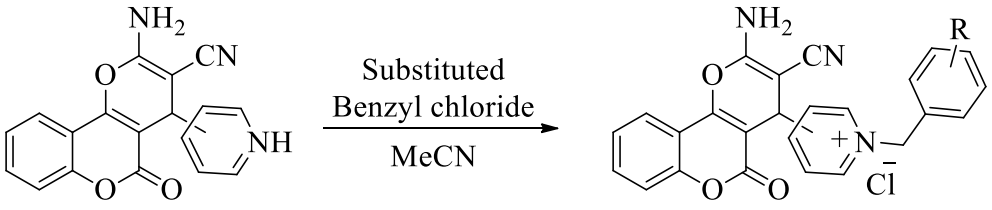


9 **10** **11**

Fig. 6

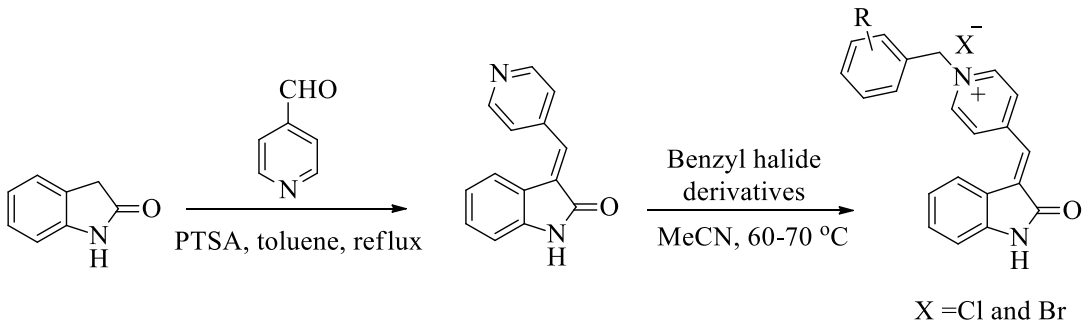
Various important approaches for the synthesis of pyridinium salts.

Mehdi Khoobi *et al.*[19] reported the design, synthesis, biological evaluation and docking study of 5-oxo-4,5-dihydropyrano[3,2-*c*]chromene derivatives as acetylcholinesterase and butyrylcholinesterase inhibitors.



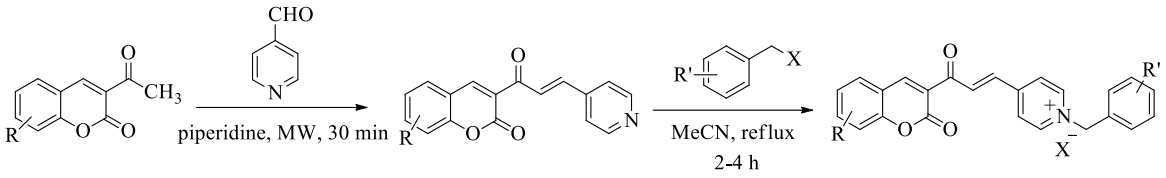
Scheme-1

Hamidreza Akrami and co-workers[20] reported Indolinone-based acetylcholinesterase inhibitors: Synthesis, biological activity and molecular modeling.



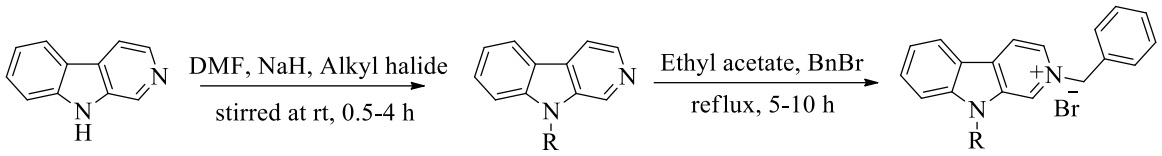
Scheme-2

Masoumeh Alipour *et al.*[21] reported novel coumarin derivatives bearing N-benzyl pyridinium moiety: Potent and dual binding site acetylcholinesterase inhibitors.



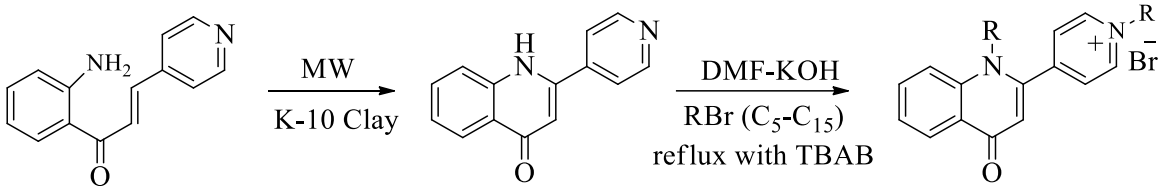
Scheme-3

Guoxian Zhang *et al.*[22] described the synthesis and structure-activity relationships of N^2-alkylated quaternary β-carbolines as novel antitumor agents.



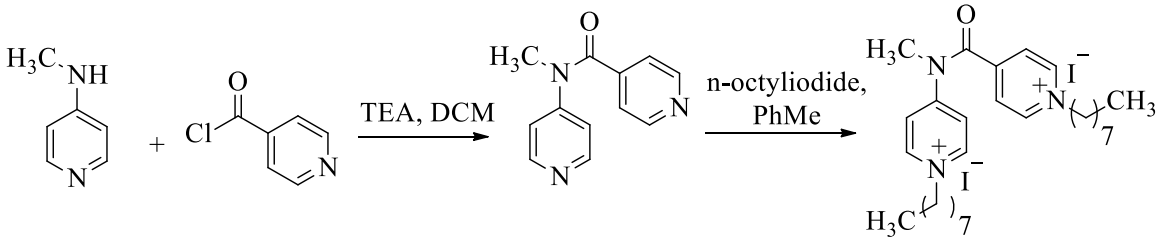
Scheme-4

Nuran Kahriman *et al.*[23] reported the synthesis, antibacterial and antioxidant activities of new 1-alkyl-4-(1-alkyl-4-oxo-1,4-dihydroquinolin-2-yl)pyridinium bromides.



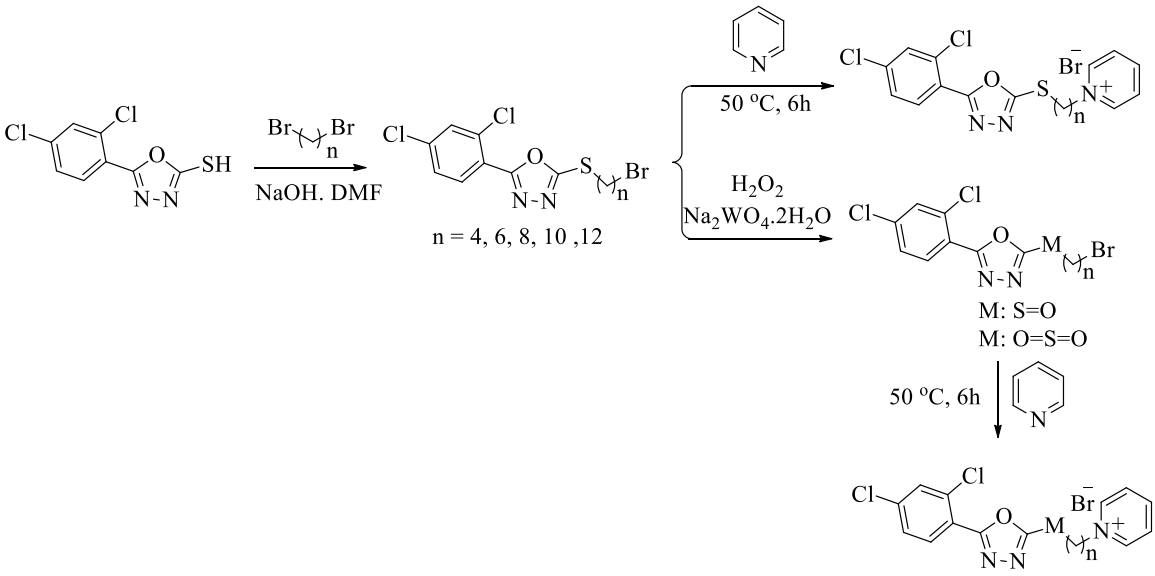
Scheme-5

Sara Fahs and co-workers[24] described the development of a novel, multifunctional, membrane-interactive pyridinium salt with potent anticancer activity.



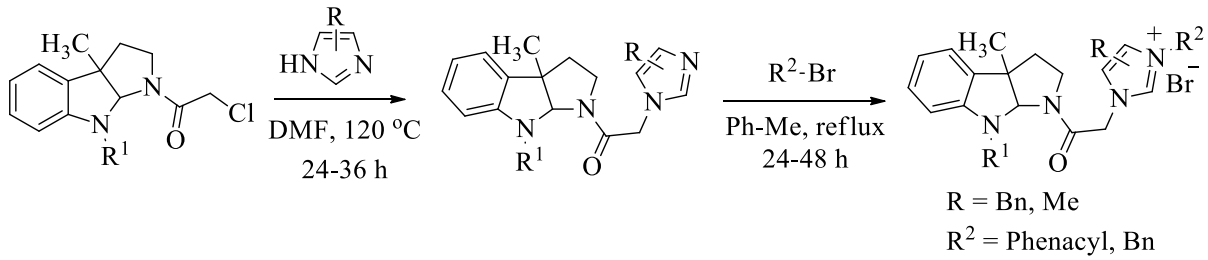
Scheme-6

Pei-Yi Wang and co-workers[25] reported the synthesis and antibacterial activity of pyridinium-tailored 2,5-substituted-1,3,4-oxadiazole thioether/sulfoxide/sulfone derivatives.



Scheme-7

Yunjing Zhou *et al.*[26] described the synthesis and cytotoxic activity of novel hexahydropyrrolo[2,3-*b*]indole imidazolium salts.



Scheme-8

PRESENT WORK

Cancer is the second most intractable diseases that leading to death across the world,[27,28] characterized by the abnormal cell growth. Genetic mutations, DNA damages and environmental exposure (exposure to UV-rays and tobacco consumption) are the primary reasons for the cause of malignancy. Besides, the existing anticancer drugs search for the new, potential and selective drugs to treat the cancer remains a major concern in cancer research. The molecules incorporating chalcone with variety of heterocyclic moieties

have been reported for their anticancer activity.[29] Most of these compounds incorporates chalcone with different heterocycles *viz* indole, triazole, thiazole, quinoline, quinoxaline, piperzine etc. Further, substituted naphthoquinones have been reported as potential anticancer agents.[30] In view of this, hybrid molecules incorporating unexplored heterocyclic pyridine with chalcone particularly substituted with naphthoquinones were envisaged for their synthesis and evaluation for their anticancer activity.

Prompted by the above facts chalcone-pyridinium hybrid molecules, substituted 6-methoxy-1-oxo-3,4-dihydronaphthalen-2(1*H*)-ylidene)methyl)pyridinium bromides have been synthesized for screening against different cancer cell lines. Since heterocyclic-chalcones, substituted napthoquinones[31,32] have also shown antimicrobial activity, these derivatives were also screened for their antimicrobial activity. It becomes more important because the anticancer drugs with antibacterial activity are not preferred as the expected long use of the anticancer drugs may lead to the development of resistance.[33] The molecules also have close resemblance with the anti-Alzheimer's drug donepezil,[34] which acts through acetylcholinesterase (AChE) enzyme. These synthesized molecules were also evaluated for their AChE inhibition. These studies may provide new leads for anticancer, antibacterial, antifungal and anti-Alzheimer's agents. Thus we herein, report the synthesis and structural elucidation of series of novel heterocyclic hybrids that fused chalcone and pyridinium bromides and *in vitro*, *in vivo* screening for their anticancer, *in vitro* antimicrobial and AChE inhibitory activities.

Preparation of starting materials

3-(2-Bromoacetyl)-2*H*-chromen-2-one and 2-(2-Bromoacetyl)-3*H*-benzo[*f*]chromen-3-one

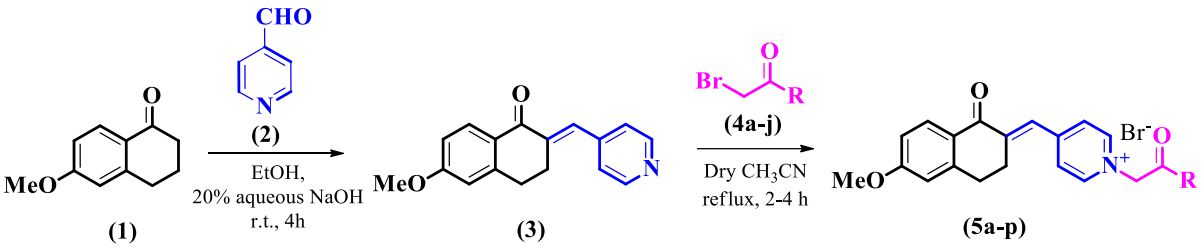
The above important starting compounds were prepared according to the literature procedure as described in **Chapter-II**, **Section-A**.

Synthesis of a chalcone (3)

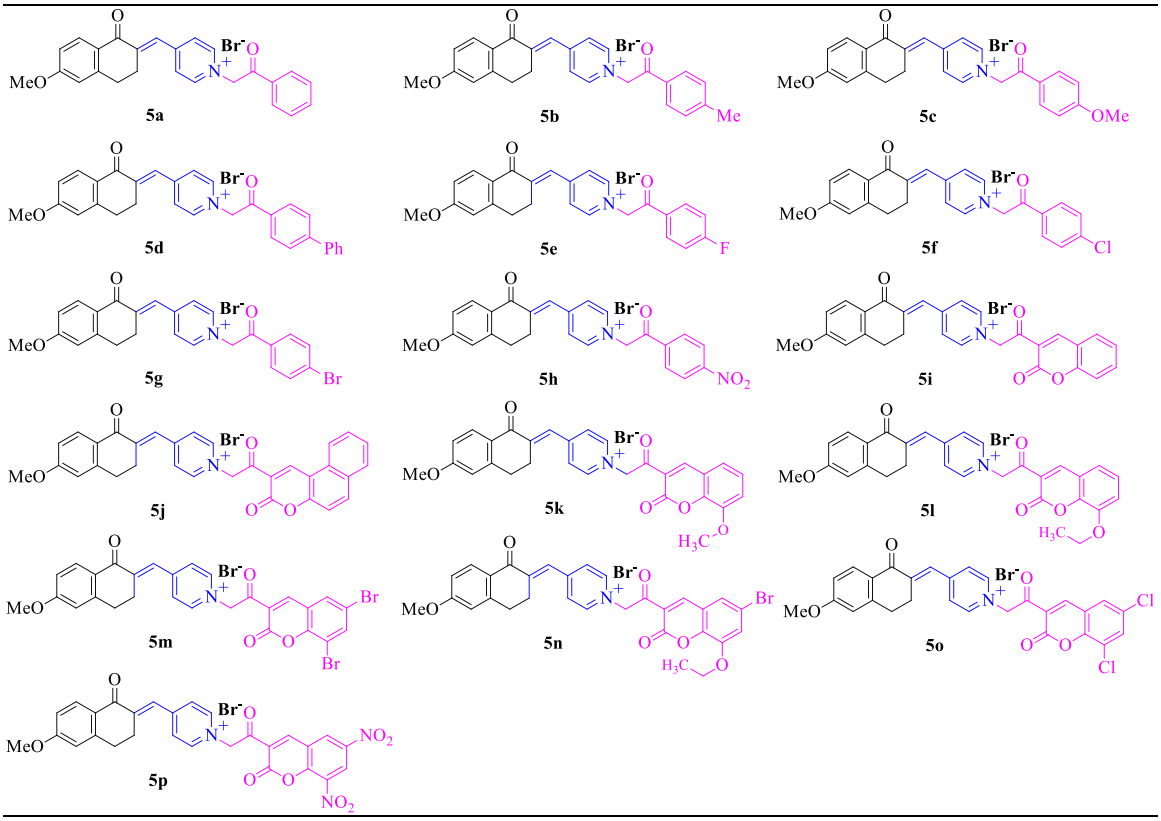
Equimolar concentration of ethanolic 6-methoxy-1-tetralone was added to 4-pyridinecarboxaldehyde in the presence of aqueous NaOH and stirred at room temperature. The precipitated solid was filtered and washed with a mixture of water and ethanol (1:1) yielded the desired product in good yields and was used for the next step without purification.

General procedure for synthesis of pyridinium bromide derivatives (5a-j)

To a solution of 6-methoxy-2-(pyridin-4-ylmethylene)-3,4-dihydronaphthalen-1(2*H*)-one (**3**, 1 mmol) in dry acetonitrile (3 mL) was added *α*-bromo ketone (**4a-j**, 2 mmol) and refluxed for about 2-4 h. After completion of the reaction (monitored by TLC), the mixture was cooled to room temperature. The solid separated out was filtered, washed with diethyl ether and purified by crystallization from (1:1) methanol-water.



Scheme-9



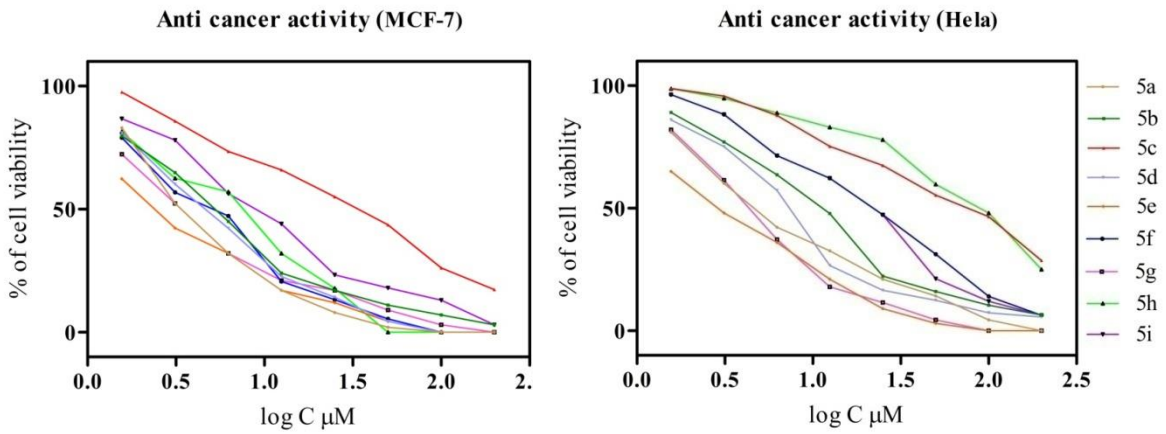
Results and discussion

The preparation of starting materials 3-(2-bromoacetyl)-2*H*-chromen-2-one (**4i**) and 2-(2-bromoacetyl)-3*H*-benzo[*f*]chromen-3-one (**4j**) were accomplished according to the earlier reported methods. The desired compounds (**5a-j**) were synthesized as detailed in Scheme 9. The aldol-condensation of 6-methoxy tetralone (**1**) with 4-pyridinecarboxaldehyde (**2**) in ethanolic sodium hydroxide at room temperature, furnished the corresponding chalcones (**3**) preferentially in thermodynamically favoured *E*-configuration.[35] The chalcone (**3**) on quaternization with the corresponding α-bromo ketones (**4a-j**) in refluxing dry acetonitrile furnished the title compounds (**5a-j**) in excellent yields (86-92%). The structures of all the newly synthesized compounds were well established by the spectroscopic techniques such as FTIR, ^{1}H NMR, ^{13}C NMR, mass spectral data and elemental analysis (C, H and N) and the results are presented in the experimental section.

Biological Evaluation

In vitro anti-proliferative assay

The antiproliferative potential of pyridinium bromides **5a-j**, was evaluated as *in vitro* cytotoxic activity against human MCF-7 (breast cancer), HeLa (cervical carcinoma) and U-87MG (human glioblastoma) cell lines by quantitative colorimetric method i.e. the (MTT) assay[36,37] using doxorubicin (DOX) as a positive control drug. The results are summarized in Table 1 where the cytotoxicity is expressed as IC_{50} (inhibitory concentration 50%) values. The survival curves of MCF-7, HeLa and U-87MG, plotted as surviving fraction *vs* concentration of drug (log μM) are illustrated in **figure 2**.



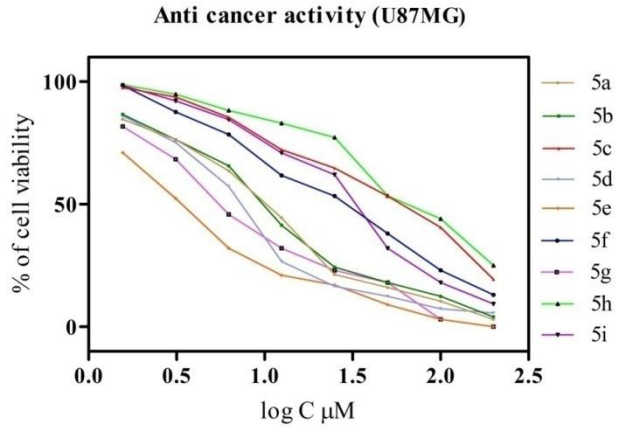


Figure 2. Survival curves of MCF-7, HeLa and U-87MG for pyridinium bromide derivatives (**5a-i**).

Based on the screening results of antiproliferative activity, the structure activity relationships (SAR) for some of the synthesized compounds **5a-j** are described as follows. The compound possessing electron donating group (methoxy) at 4th position of phenyl ring (**5c**) has exhibited, broad spectrum anti-proliferative activity with IC_{50} 10.86 ± 0.2 µM against MCF-7, IC_{50} 4.67 ± 0.5 µM against HeLa and IC_{50} 6.89 ± 0.3 µM against the U-87MG. Similarly, the compound bearing strong electron withdrawing group (nitro) on phenyl ring at 4th position (**5h**) was also found to be having potential cytotoxic activity with broad spectrum against MCF-7 with IC_{50} 7.82 ± 0.5 µM, HeLa with 3.88 ± 0.1 µM and U-87 MG with 4.53 ± 0.3 µM cell lines. Also the compounds **5f** and **5i** has moderate cytotoxic activity against against U-87MG cell line (IC50 12.19 ± 0.2 µM and 11.58 ± 0.4 µM respectively) and against HeLa with IC_{50} 15.13 ± 0.5 µM and 17.30 ± 0.6 µM respectively. And the remaining compounds were shown weak activity against all the tested cell lines.

Table 1. *In vitro* cytotoxic effects (IC_{50} in μM) of the pyridinium bromide derivatives (**5a-j**) on three different cancer cell lines, *viz*., MCF-7, HeLa and U-87 MG through Cell Viability (MTT) Assay.[a]

Analogs	MCF-7	HeLa	U-87 MG
5a	86.63 ± 0.3	53.90 ± 0.7	30.51 ± 0.2
5b	56.13 ± 0.4	29.65 ± 0.4	28.79 ± 0.2
5c	10.86 ± 0.2	4.67 ± 0.5	6.89 ± 0.3
5d	64.13 ± 0.6	38.90 ± 0.3	38.76 ± 0.3
5e	171.84 ± 0.8	126.64 ± 0.5	100.14 ± 0.7
5f	66.93 ± 0.5	15.13 ± 0.5	12.19 ± 0.2

5g	97.18 ± 0.3	6.31± 0.3	45.21 ± 0.4
5h	7.82 ± 0.5	3.88 ± 0.1	4.53 ± 0.3
5i	30.30 ± 0.1	17.30± 0.6	11.58 ± 0.4
5j	>200	>200	>200
5k	NT	NT	NT
5l	NT	NT	NT
5m	NT	NT	NT
5n	NT	NT	NT
5o	NT	NT	NT
5p	NT	NT	NT
Doxorubicin	2.55 ± 0.3	1.33 ± 0.1	1.76 ± 0.2

[a]IC_{50} values were expressed as mean ± SD of three independent experiments.

NT-not tested

Linear regression analysis

In order to understand the structural requirements in terms of hydrophobic, steric and electronic effects for the variation in anti-cancer activity, the quantitative structure activity relationship (QSAR) studies were performed using multiple linear regressions (MLR) analysis. In view of the major structural modifications in terms of aromatic substitution in 8 (**5a**-**5h**) out of 10 compounds were considered for studying these effects in different cancer cell lines.

Initially each parameter was correlated with anticancer activity where no significant correlations were obtained. However it was observed that individually electronic effect in terms of (σ) showed positive and almost similar influence on the three cell lines. The hydrophobic effects in terms of pi influenced anticancer activity negatively and the effects were more prominent for the cell lines MCF-7 and U87-MG, while steric effect in terms of MR influenced anticancer activity positively for all cell lines.

Since none of the physicochemical parameters showed more than 0.4 correlation and removal of one compound **5d** having a phenyl substitution at para position improved the correlation of anticancer activity of different cell lines with pi (excluding HeLa cell line) and MR. It also resulted in decreased intercorrelation between pi and MR (R^2= 0.818 to 0.237). Thus it appears that MR plays a key role in describing the variation of activity and

contributes positively followed by hydrophobicity which influences the activity negatively. Hence the effect of both noncollinear (R^2=0.237) parameters π and MR was studied knowing the limitation of dataset of 7 compounds for the three anticancer activities (Equations 1 to 3) where; n= number of compounds; R = multiple correlation coefficient; F = F-ratio; S = standard error of estimate.

For MCF-7 cell line

Log 1/C = -0.891 (± 0.201) π + 0.122 (± 0.027) MR-2.055

n = 7, R = 0.944, R2 = 0.89, S = 0.205, F = 16.254 (1)

For HeLa cell line

Log 1/C = -0.369 (± 0.192) π + 0.178 (± 0.026) MR-2.049

n = 7, R = 0.961, R2 = 0.923, S = 0.196, F = 23.84 (2)

For U-87 MG cell line

Log 1/C = -0.640 (± 0.353) π + 0.102 (± 0.047) MR-1.664

n = 7, R = 0.785, R2 = 0.616, S = 0.360, F = 3.21(3)

All the equations are statistically significant (>97 %) except equation 3 along with the relatively statistically significant regression coefficient values. Though both the parameters π and MR show similar positive and negative effects respectively for all the three cancer cell lines, but differ in terms of the magnitude which are as follows MCF-7>U-87MG>HeLa for π and HeLa>MCF-7> U-87MG for MR. Thus judicious structural substitution may result in both improvement and selectivity of anticancer activity towards these cell lines.

Table 2: The correlation matrix for the 2D-QSAR descriptors with biological activity.

	MCF-7	**HeLa**	**U 87-MG**	**π**	**σ**	**MR**
MCF-7	1/1					
HeLa	0.777/0.779	1/1				
U 87-MG	0.923/0.927	0.789/0.778	1/1			
π	-0.405/-0.574	-0.221/-0.042	-0.407/-0.417	1/1		
σ	0.304/0.292	0.386/0.361	0.367/0.344	-0.236/-0.179	1/1	
MR	0.128/0.592	0.1240.923	0.004/0.548	0.818/0.237	-0.068/0.225	1/1

***In vivo* cytotoxic activity**

Adult male Swiss albino mice (Sainath Enterprises, Hyderabad, India) of 8 weeks of age (mean weight in the range of 20-25 G) were selected and housed in polypropylene cages in a room, where the congenial temperature of 27±1 0C and 12 h light and dark cycles were maintained. The animals were allowed to acclimatize to the environment for 7 days supplied with a standard pellet diet and water ad libitum. All procedures using animals were reviewed and approved by the Institutional Animal Ethical Committee (IAEC/29/UCPSc/KU/2015) of Kakatiya University. The animals were divided into seven groups (n = 10). The normal group was not inoculated with tumor cells, while six other groups were injected with EAC cells (0.2 mL of 2x106 cells $mice^{-1}$) intraperitoneally. This was taken as a day '0' and the experimental treatment started 24 h later. From the first day 100 µLmouse-1 per day of sterile saline was administered intraperitoneally to the negative control group (EAC-bearing mice).The compounds **5c** and **5h** at doses of 5 mgkg-1 and 10 $mgkg^{-1}$ were administered each day to the treated groups, and the standard drug Cisplatin at a dose 5 $mgkg^{-1}$ was administered to each animal from the positive control group. The pharmacological treatment lasted for 9 days. Fourteen days after the treatment, five mice from each group were scarified for the study of antitumor activity. The rest of the animal groups were kept to checking the mean survival time of EAC tumor bearing hosts. The antitumor effects of the compounds were determined by the change in body weight, mean survival time (MST) and percentage of increased life span (%ILS). The MST of each group containing five mice was identified by recording mortality on a daily basis for 30 days, and the %ILS was calculated using the following equations MST = (day of first death + day of last death)/2, and %ILS = [(mean survival time of treated group/mean survival time of control group)-1] x 100. The effect of the compounds was also assessed by the determination of the body weight, tumor volume, packed cell volume and viable tumor cell count of EAC bearing mice by the Tryphan blue dye exclusion method.

The potent compounds (**5c** & **5h**) were examined for their *in vivo* anticancer activity in EAC bearing mice by using liquid tumor model.[38-40] The effect of the compounds in two different doses (5 mg kg^{-1} and 10 mg kg^{-1}) on body weight, mean survival time, % increase lifespan, tumor volume, packed cell volume and viable tumor cell count were studied (**Table 3**). The results indicated that the compounds **5c** and **5h** have shown

significant activity in both the doses and decreased the body weight of EAC- bearing mice. The both compounds have significantly increased the mean survival time, decreased the tumor volume, packed cell volume and viable cell count in both the doses. On day 14, the hematological and biochemical parameters, with regard to hemoglobin level, erythrocyte count, leukocytes count, SGPT, SGOT and total protein levels were compared with the EAC control group, standard drug Cisplatin treated groups and the groups injected with the compounds **5c** and **5h** (**Table 4**). From Table 4 it is clear that, the hematological and biochemical parameters, in the group treated with the compounds **5c** and **5h** have been recovered the normal values. The compound **5c** and **5h** have significantly decreased the ascetic fluid volume when compared with EAC control. The compounds **5c** and **5h** decreased the WBC levels at both the doses when compared to tumor control. Similarly, **5c** and **5h** have decreased the SGPT, SGOT and total protein levels when compared to tumor control. This indicates that these compounds possess protective action on hemopoietic system. The results suggest that these compounds **5c** and **5h** proved to possess remarkable activity. The potent anticancer drug can be developed using further investigations by effecting a simple modification in the structure.

Table 3: *In vivo* anticancer activity of **5c** and **5h** on EAC-bearing mice [a]

Parameter	EAC	Cisplatin (5mg/kg)	5c (5mg/kg)	5c (10mg/kg)	5h (5mg/kg)	5h (10mg/kg)
MST	12.59±0.45	25.34±0.57	18.62±0.73	22.80±0.17	19.17±0.16	23.14±0.66
%ILS	-	110.13	78.44	96.56	77.54	98.72
%IBW	10.57±0.34	4.27±0.15	7.22±0.17	6.11±0.72	6.87±0.28	5.83±0.47
Tumour volume	9.80±0.87	3.67±0.17	6.41±0.32	4.96±0.19	6.08±0.52	4.31±0.88
Packed cell volume	2.19±0.12	1.42±0.20	2.28±0.27	1.97±0.34	2.01±0.14	1.63±0.37
Viable cell count	6.34±0.38	0.31±0.03	2.26±0.17	1.34±0.86	2.07±0.23	1.22±0.21

[a] Values are expressed as mean ± SEM (n = 10)

Table 4: Effect of **5c** and **5h** on biochemical and hematological parameters in EAC-bearing mice [a]

Parameter	Haemglobin	RBC (million/mm^3)	WBC (10^3cells/mm^3)	Lymphocyte (%)	Neutrophils (%)	Monocyte (%)
Normal	13.17±0.28	4.62±0.21	7.14±0.33	66±0.47	28±0.71	1.12±0.25
EAC Control	5.52±0.13	2.78±0.28	19.08±0.43	23±0.72	71±0.48	2.37±0.02
Cisplatin[b]	11.34±0.84	4.18±0.32	9.14±0.03	61±0.87	32±0.17	1.32±0.17
5c[b]	8.05±0.38	3.24±0.22	13.11±0.56	42±0.25	51±0.65	1.88±0.14
5c[c]	8.53±0.26	3.98±0.24	10.23±0.84	54±0.37	39±0.32	1.61±0.77
5h[b]	8.16±0.19	3.18±0.11	13.22±0.26	38±0.23	58±0.14	1.93±0.54
5h[c]	8.86±0.32	3.83±0.14	10.94±0.12	52±0.84	42±0.32	1.64±0.26

[a] Values are expressed as mean ± SEM (n = 10) [b]5 mg/kg [c]10 mg/kg

In vitro antimicrobial activity

In order to explore the possible antimicrobial potential of the some of the newly synthesized compounds (**5a-j**), were screened *in vitro* against six pathogenic bacterial strains, *viz*. Gram-positive (G+) bacterial strains (*Bacillus subtilis* MTCC 96, *Staphylococcus aureus* MTCC 121, *Staphylococcus epidermidis* MTCC 2639) and Gram-negative (G-) bacteria (*Escherichia coli* MTCC 40, *Pseudomonas aeruginosa* MTCC 2453 and *Klebsiella pneumonia* MTCC 109) and the fungal strains (*Candida albicans*, *Aspergillus niger*, *Candida glabrata*, *Aspergillus parasiticus*). Antimicrobial screenings were conducted in triplicates by well-plate method using Mueller-Hinton (MH) agar.[41] Antimicrobial assay was performed at 100 μgmL^{-1} concentrations for the test compounds (**5a–j**) with respect to positive control drugs penicillin, streptomycin (for bacteria) and Fluconazole (for fungi) at 30 μgmL^{-1}. Zone of Inhibition (ZOI) values in mm for the tested compounds were measured at the end of the incubation period of about 24h for bacteria at 37±1 ˚C (**Table 5**) and 72 h for fungi at 27±1 ˚C given in **Table 6**.

The lowest concentration of antimicrobial agents required to inhibit the microbial growth is called minimum inhibitory concentration (MIC). The MICs of all the tested compounds as well as standards (**Table 4**) were also performed against all the bacterial strains, using a well-defined and standardized broth micro-dilution technique,[42] as one described by the European Committee for Antimicrobial Susceptibility Testing (EUCAST) of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID). Using

DMSO as a solvent control prepared different concentrations of test compounds and positive controls ranging from 150-0.1 μgmL^{-1}. Bacterial inoculums were also prepared. The bacterial suspensions were transferred (inoculums + sterile water) into a series of test tubes containing 1 mL of each of the derivative solutions (**5a-j**) to be tested at different concentrations. The test tubes were incubated at 37±1˚C for about 18h and determined the MIC values that completely inhibit the visible growth.

Table 5. *In vitro* antibacterial screening studies of the title compounds (**5a-j**)

Product	Antibacterial activity											
	B. s		***S. a***		***S. e***		***E. c***		***P. a***		***K. p***	
	ZOI	MIC	ZOI	MIC	ZOI	MIC	ZOI	MIC	ZOI	MIC	ZOI	MIC
5a	9	75	8	75	19	75	10	75	8	75	10	75
5b	9	75	21	3.12	6	>150	17	18.75	18	9.37	5	>150
5c	9	75	15	37.5	12	37.5	12	75	7	75	8	75
5d	14	37.5	17	18.75	13	37.5	10	37.5	15	37.5	10	37.5
5e	5	>150	7	>150	8	>150	8	>150	10	>150	8	75
5f	14	37.5	22	6.25	12	18.75	12	75	7	75	12	37.5
5g	9	75	8	75	8	150	9	75	8	150	7	75
5h	5	150	4	>150	9	75	8	>150	10	>150	5	>150
5i	5	>150	21	6.25	8	>150	12	75	21	6.25	15	18.75
5j	17	37.5	23	3.12	15	18.75	20	6.25	18	6.25	12	37.5
5a	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
5a	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
5a	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
5a	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
5a	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
5a	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Penicillin	22	1.56	21	1.56	20	3.12	20	12.5	19	12.5	20	6.25
Streptomycin	20	6.25	20	6.25	18	3.12	19	6.25	17	1.56	20	3.12

ZOI - Zone of inhibition in mm.
MIC - Minimum inhibitory concentration in μgmL^{-1}.
NT-not tested

In vitro antimicrobial screening (**Table 5**) results showed moderate to excellent inhibiting activities with (ZOI 4-23 mm and MIC 3.12- >150 μgmL^{-1} against all the tested bacterial strains (G+ and G- bacteria). Among all the tested compounds, the compounds **5b**, **5f**, **5i** and **5j** were found to be having an excellent inhibiting activity. The compound bearing benzo[*f*]coumarinyl (**5j**) moiety has shown excellent efficacy with broad spectrum of activity against, *S. aureus* (ZOI 23 mm, MIC 3.12 μgmL^{-1}), *E. coli* (ZOI 20 mm, MIC

6.25 μgmL^{-1}), *P. aeruginosa* (ZOI 18 mm, MIC 6.25 μgmL^{-1}), than that of positive control drugs penicillin and streptomycin. The compounds **5b** has shown excellent inhibiting activity against *S. aureus* (ZOI 21 mm, MIC 3.12 μgmL^{-1}), good activity against *P. aeruginosa* (ZOI 18 mm, MIC 9.37 μgmL^{-1}) and moderate activity against *E. coli* (ZOI 17 mm, MIC 18.5 μgmL^{-1}). Similarly, the compound **5f** exhibited excellent inhibiting activity against *S. aureus*, (ZOI 22 mm, MIC 6.25 μgmL^{-1}) and moderate activity against *S. epidermidis* (ZOI 12 mm, MIC 18.75 μgmL^{-1}). Also, the compound **5i** have shown an excellent bacterial inhibition against *S. aureus* (ZOI 21 mm, MIC 6.25 μgmL^{-1}) and *P. aeruginosa* (ZOI 21 mm, MIC 6.25 μgmL^{-1}) comparable to that of the standard drug streptomycin. The remaining test compounds **5a**, **5c**, **5d**, **5e**, **5g** and **5h** have shown moderate activity against all the bacterial strains with ZOI ranging from 4-15 mm and MIC values of 18.5- >150 μg mL^{-1}.

Table 6. *In vitro* antifungal activity of the pyridinium bromide derivatives (**5a-j**).

Product	**Antifungal activity**			
	C. albicans	***A. niger***	***C. glabrata***	***A. parasiticus***
	ZOI (mm)			
5a	10	8	8	7
5b	**24**	13	10	10
5c	NI	8	13	9
5d	NI	NI	NI	NI
5e	NI	**15**	NI	NI
5f	11	9	5	10
5g	8	10	10	3
5h	NI	8	6	NI
5i	NI	NI	NI	NI
5j	NI	NI	NI	NI
5k	NT	NT	NT	NT
5l	NT	NT	NT	NT
5m	NT	NT	NT	NT
5n	NT	NT	NT	NT
5o	NT	NT	NT	NT
5p	NT	NT	NT	NT
Fluconazole	**25**	**17**	**20**	**18**

NI- no inhibition.
NT-not tested

The *in vitro* antifungal activity results revealed that, out of all the tested compounds (**5a-j**), only compound **5b** against *C. albicans* with ZOI 24 mm and **5e** against *A. niger* with ZOI 15 mm have showed promising inhibiting activity comparable to that of the reference drug Fluconazole. The compounds (**5d**, **5i** and **5j**) possessing 4-phenyl, coumarinyl and benzo[*f*] coumarinyl, were completely inactive against all the tested fungal strains. The remaining compounds were shown week to moderate activity with ZOI ranging from 3-13 mm.

From the antimicrobial activity profile, we have observed structure activity relationship of the synthesized compounds (**5a**-**j**) as follows; the presence of (electron donating) methyl and (electron withdrawing) flouro and chloro substitutions at 4th position of phenyl ring, electron rich coumarinyl and benzo[*f*]coumarinyl rings, in the compounds **5b**, **5e**, **5f**, **5i** and **5j** respectively, had greatly influenced these compounds to exhibit antimicrobial inhibiting activity against the tested (bacterial and fungal) strains.

In vitro AchE activity

Ellman Method: The assay of AChE inhibition was performed according to method described by Ellman *et al*.[43] using the human AChE purified from red blood cells. The kinetic profile of the AChE enzyme activity was studied spectrophotometrically at a wavelength of 412 nM at an interval of 15s. The assay for each sample was run in duplicate and each experiment was performed thrice. The test substance was incubated with enzyme in the concentration of 100 µg/mL of reaction mixture for 30 min at 37 ^{o}C prior to obtaining the kinetic profile of AChE activity. Donepezil (1µg/mL) was used as standard AChE inhibitor (standard control). The AChE inhibitory activity was calculated on the basis of % decrease from control values i.e. AChE activity without incubation with any standard or test drug.

In vitro AChE inhibition

The structural similarity of these compounds with donepezil also prompted us to evaluate them for AChE activity. The compounds were evaluated using the *in vitro* assay method described by Ellman *et al*.[43] The compound **5c** having a 4-methoxy group which was the best among these did not show very promising activity (% inhibition = 39.6) and rest of the compounds had no significant AChE inhibition when compared with donepezil that showed 99.7 % AChE inhibition (**Table 7**).

Table 7: AChE Inhibition activity of the synthesized compounds.

Compound	Inhibition (%)	Compound	Inhibition (%)
Donepezil	99.7	**5i**	4.66
5a	15.5	**5j**	3.94
5b	4.9	**5k**	NT
5c	39.6	**5l**	NT
5d	2.4	**5m**	NT
5e	5.8	**5n**	NT
5f	5.2	**5o**	NT
5g	6.6	**5p**	NT
5h	2.9		

NT-not tested

Docking studies

In order to rationalize the structure–affinity relationships (SARs) and to authenticate the *in vitro* antimicrobial results and also to understand the possible key interactions of potent ligands with the receptor sites of an enzyme, docking simulations were performed using Lamarckian Genetic Algorithm (LGA),[44] inculcated in the docking program AutoDock Tools (1.5.6) and docking results were visualized using Maestro elements tutorial 1.8. Docking for the synthesized compounds (**5a**-**j**) was performed on the binding model based on the X-ray crystal structure of the tyrosyl-tRNA (TyrRS) synthetase complexed with the ligand SB-239629. The co-crystallized structure of target enzyme TyrRS (PDB code: 1JIJ)[45] from *S. aureus* was retrieved from RCSB Protein Data Bank (http://www.rcsb.org/pdb). Molecular docking studies revealed that, all the synthesized molecules exhibited excellent binding energies towards the receptor active pocket with binding energies ranging from -8.72 to -9.92 kcal mol^{-1} and depicted in **Table 8**. The binding profiles of most promising ligands (**5b**, **5f**, **5i** and **5j**) with the active pocket residues of TyrRS are depicted in **Figure 3** and **4**. After exhaustive analysis of docking results, best dock poses interms of binding energy, H-bondings and non-bonding interactions with receptor active pocket residues were chosen as best docked ligand orientations (**Table 8**). As shown in **Figure 3**, the ligand **5b** well fitted in the active pocket of tyrosyl-tRNA synthetase with four hydrogen bond interactions: observed the CH3O···H−N (2.46 Å) bond between 6-methoxy substituent on tetralone ring and Val224,

C=O···H− N (2.10 Å) bond between cyclic keto and Gly193, quaternary N^{+}···H− N between pyridinium ring and Gly38 and the keto carbonyl established C=O···H−N (2.33 Å) bond with Gly38. The 6-methoxy group of 5f exhibited H-bonding interactions with Val224 (2.07 Å), cyclic keto carbonyl oxygen with His47 (3.40 Å) and His50 (3.57 Å) and keto carbonyl oxygen with Gln174 (2.37 Å) amino acid residues in protein active site (PAS). The docked pose 5i established H-bond interactions with Tyr36 (2.65 Å), Gly38 (3.81 Å), Gly193 (2.68 Å), Val224 (3.53 Å) and His50. Besides, the H-bond interactions, also observed π-π stacking interaction between pyridinium ring in **5i** and the imidazole ring of His50 and formed salt bridge between quaternary-N^{+} and negatively charged amino acid residue Asp195. Whereas, the best docking orientation **5j**, held to the active site of protein with hydrogen-bonding interactions with Tyr36 (2.36 Å), Gly38 (3.85 Å), Gly193 (2.60 Å), His47 (3.74 Å) and His50. In addition, pyridinium ring of **5j** exhibited π-π stacking interaction with imidazole ring of His50 and also observed π-cation interaction with N3 of Asp195.

Our molecular modeling results revealed that, the cyclic keto carbonyl, 6-methoxy substituent on tetralone ring, pyridinium ring and cyclic ester (lactone) of coumarin moiety have great influence on the interactions of the protein-ligand complex and is crucial to the TyrRS inhibitory activity. Therefore removal of aforementioned groups may reduce the inhibitory activity. Also our modeling results revealed that, Tyr36, Gly38, His47, His50, Gly193 and Val224 are the key residues for the protein-ligand interactions to form the complex. Hence the above data can strongly rationalizes the potent antibacterial activity observed in biological assays for the compounds **5b**, **5f**, **5i** and **5j** and they can be considered as potential lead molecules for the development of antibacterial agents.

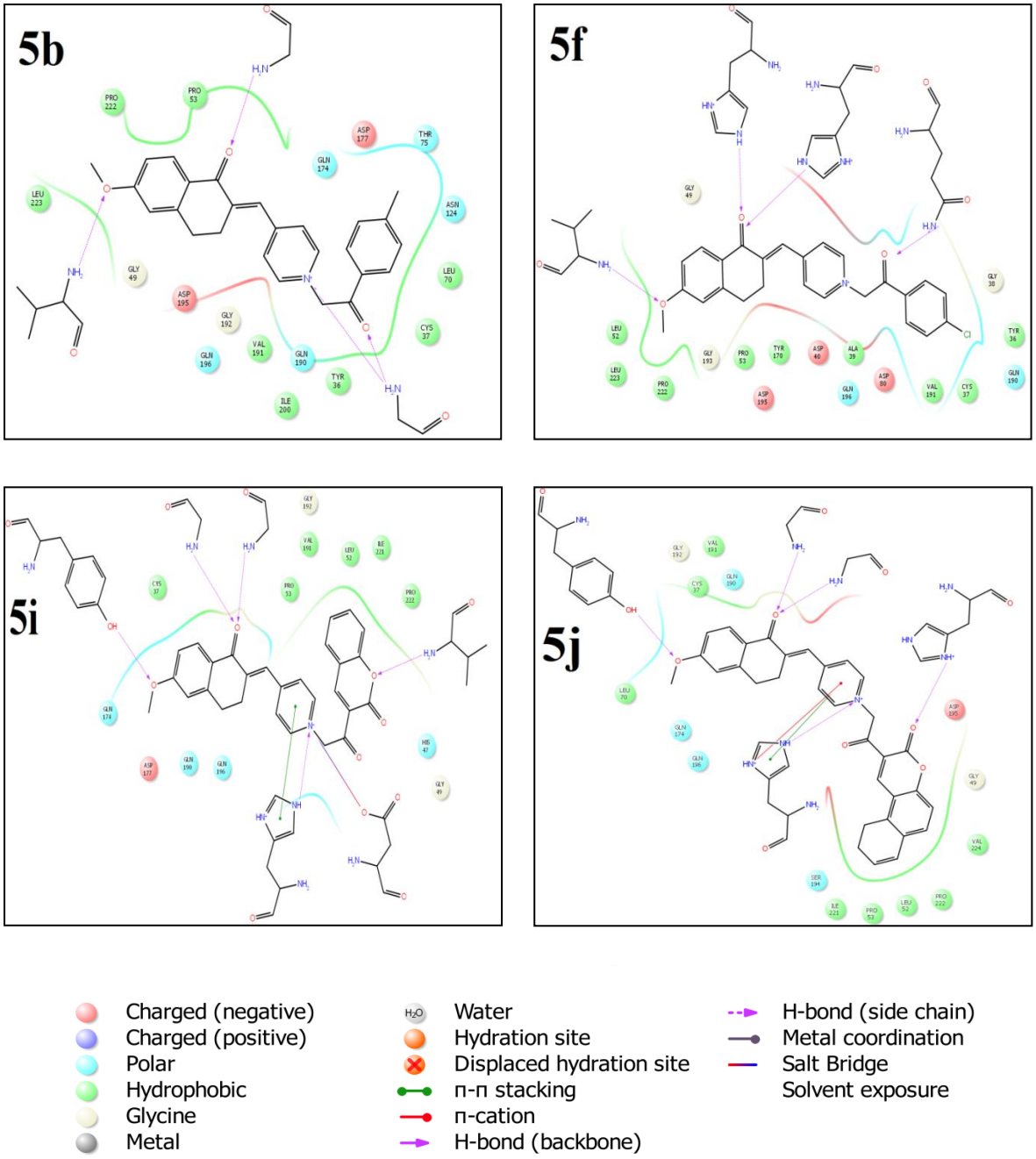


Figure 3. 2D visualizations depicting protein-ligands (most active compounds **5b**, **5f**, **5i** and **5j)** interactions with active pocket of tyrosyl-tRNA (TyrRS) synthetase.

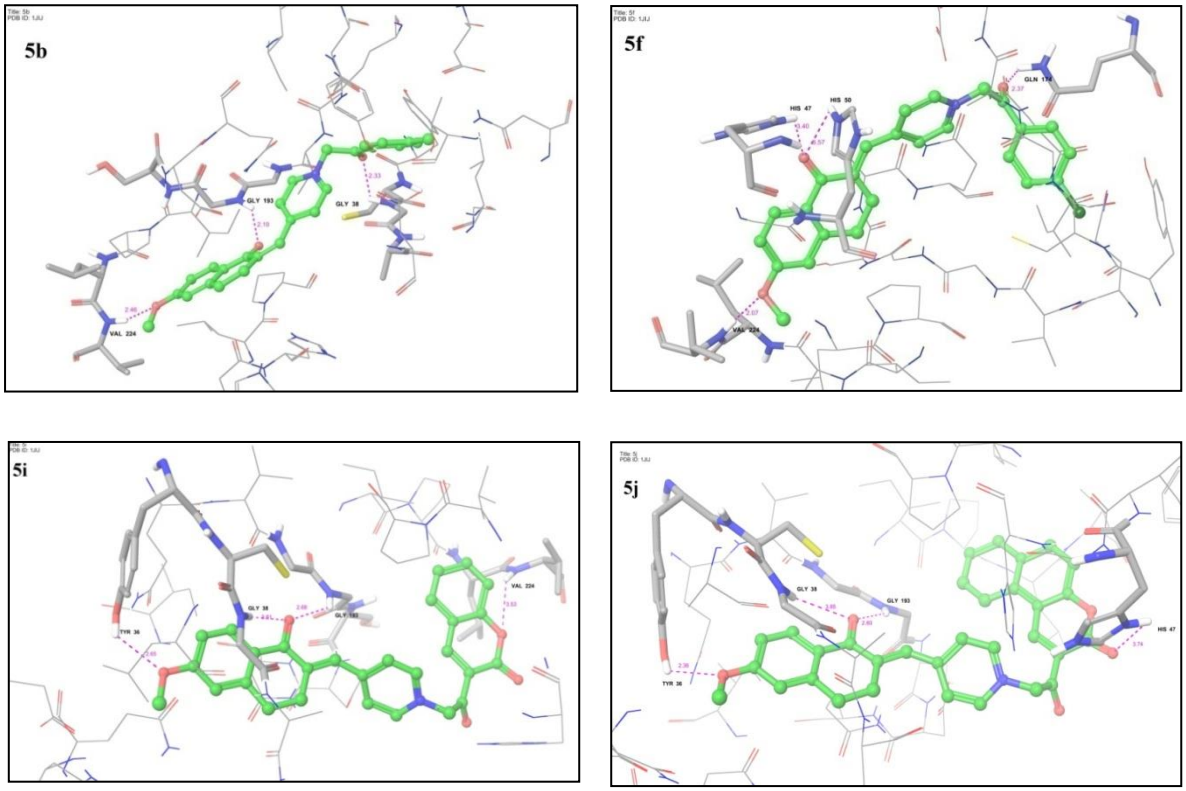


Figure 4. Binding interactions of the potent ligands **5b, 5f, 5i and 5j** (ball and stick) in the active site of tyrosyl-tRNA (TyrRS) synthetase (thin wire). In 3D-surface, the enzyme represented as a molecular cloud. Hydrogen bonding interactions are shown in pink dotted lines.

Table 8. Autodock binding energies, Type of Interactions, residues involved in hydrogen bonding interactions and no. of hydrogen bonds of ligands (**5a-j**) for *S. aureus* (PDB id: 1JIJ).

S.No.	Product	Binding energy (Kcal mol^{-1})	Inhibition Constant K_i (nM)	Type of Interactions	Residues involved in hydrogen-bonding interactions (no. of H-bonds)
1.	**5a**	-9.38	134.25	H-bonding	Val224 (1), Gly193 (1), Gly38 (2)
2.	**5b**	-9.58	94.53	H-bonding	Val224 (1), Gly193 (1), Gly38 (2)
3.	**5c**	-8.72	402.45	H-bonding, π-cation and π-π stacking	Tyr36 (1), Gly193 (1), Val224 (1)
4.	**5d**	-7.96	920.22	H-bonding and π-π stacking	Tyr36 (1), Gly193 (1), His50 (1)
5.	**5e**	-8.69	423.62	H-bonding, π-cation and π-π stacking	Tyr36 (1), Gly38 (1), Gly193 (1)

6.	**5f**	-9.28	157.43	H-bonding	Val224 (1), His47 (1), His50 (1), Gln174 (1)
7.	**5g**	-9.11	211.38	H-bonding	Val224 (1), His47 (1), His50 (1), Gln174 (1)
8.	**5h**	-8.38	718.16	H-bonding, π-cation and π-π stacking	Gly193 (1), Val224 (3)
9.	**5i**	-9.22	174.33	H-bonding and π-π stacking	Tyr36 (1), Gly38 (1), Gly193 (1), His (50), Val224 (1)
10.	**5j**	-9.92	53.92	H-bonding, π-cation and π-π stacking	Tyr36 (1), Gly38 (1), Gly193 (1), His50 (1), His47 (1)

Conclusion

In conclusion, a series of new tetralone based fused chalcone-pyridinium bromide hybrids (**5a-p**) has been synthesized and evaluated some of the compounds (**5a-j**) for their antiproliferative (*in vitro* and *in vivo*) against human cancer cell lines including MCF-7, HeLa and U-87MG and antimicrobial activity (bacterial and fungal). The *in vitro* anti-proliferative activity results revealed that, out of all the synthesized compounds (**5a-j**), the derivatives bearing 4-methoxy phenyl (**5c**) and 4-nitro phenyl (**5h**), showed significant anti-proliferative activity against all the tested cancer cell lines, than that of the remaining derivatives. The derivatives **5f** and **5i** exhibited moderate anticancer activity against tested strains. The *in vivo* results also suggests that, the compounds **5c** and **5h** distinctly exhibited inhibiting effect on tumour growth in mice bearing EAC. Further the QSAR studies revealed that the two physicochemical parameters hydrophobic (π) and steric (MR) were found to influence anticancer activity negatively and positively respectively. The antimicrobial studies on these compounds showed that, most of the synthesized compounds (**5a-j**) have promising inhibitory activity against all the bacterial strains where the derivatives **5b**, **5f**, **5i** and **5j** have shown broad and superior inhibiting activity, particularly against G+ methicillin resistant *S. aureus* (MRSA), than the positive controls. Antifungal results showed that, the compound **5b** and **5e** have comparable inhibiting activity to that of the standard drug Fluconazole against *C. albicans* and *A. niger* respectively. In order to authenticate the *in vitro* antibacterial results, molecular docking studies were performed for the synthesized compounds (**5a-j**). Based on the docking studies presence of keto group of tetralone ring, methoxy at 6^{th} position on tetralone and H-bond interaction with active site amino acid GLY 193 are key for the potency of the

derivatives. Thus the docking studies provide insights about the mode of binding and interactions of the compounds with the tyrosyl-tRNA synthetase. It is good that none of these compounds exhibited significant AchE inhibition. Hence these results provide the basis for further optimization and development of the lead molecules, to develop new cytotoxic agents as well as antimicrobial agents with better efficacy against methicillin-resistant *S. aureus*.

Experimental

Synthesis of (*E*)-6-methoxy-2-(pyridin-4-ylmethylene)-3,4-dihydronaphthalen-1(2*H*)-one (3)

To a solution of 6-methoxy-1-tetralone (**1**, 0.1 mol) in ethanol (10 mL) was added 20% aqueous NaOH (1mL) and 4-pyridinecarboxaldehyde (**2**, 0.1 mol). The mixture was stirred at room temperature for about 4 h. After completion of the reaction (ensured by TLC), the white precipitate formed was filtered and washed with a mixture of water and ethanol (1:1). The product (**3**) obtained was highly pure and used as such for further reactions.

General procedure for synthesis of pyridinium halide derivatives (5a-p)

To a solution of 6-methoxy-2-(pyridin-4-ylmethylene)-3,4-dihydronaphthalen-1(2*H*)-one (3, 1 mmol) in dry acetonitrile (3 mL) was added α-bromo ketone (**4a-j**, 2 mmol) and refluxed for about 2-4 h. After completion of the reaction (monitored by TLC), the mixture was cooled to room temperature. The solid separated out was filtered, washed with diethyl ether and purified by crystallization from (1:1) methanol-water.

Spectral data

(*E*)-4-((6-methoxy-1-oxo-3,4-dihydronaphthalen-2(1*H*)-ylidene)methyl)-1-(2-oxo-2-phenylethyl)pyridinium bromide (5a).

Colourless solid; Yield: 92%; mp. 211–213 ^{o}C; **IR** (KBr, cm^{-1}) υ_{max}: 1692, 1671 (C=O), 1643 (C=N), 1253 (C-O-C); **^{1}H NMR** (400 MHz, DMSO-d_6) δ: 8.95 (d, J = 6.8 Hz, 2H), 8.27 (d, J = 6.8 Hz, 2H), 8.08 (d, J = 7.2 Hz, 2H), 7.93 (t, J = 8.8 Hz, 1H), 7.80 (t, J = 7.2 Hz, 1H), 7.68 (t, J = 7.6 Hz, 1H), 6.95-6.87 (m, 2H), 6.49 (s, 2H), 6.16 (d, J = 5.2 Hz, 1H), 5.81 (s, 1H), 3.84 (s, 3H), 3.09-3.04 (m, 1H), 2.92-2.84 (m, 1H), 2.11-2.00 (m, 1H), 1.64-1.59 (m, 1H); **^{13}C NMR** (75 MHz, DMSO-d_6) δ: 194.88, 191.40, 166.22, 163.81,

147.46, 145.97, 135.21, 134.05, 129.75, 129.64, 128.75, 126.19, 125.37, 114.06, 112.98, 69.47, 56.05, 28.57, 21.96; **ESI-MS** *m/z*: 384 $[M]^+$; Anal. Calcd. for $C_{25}H_{22}BrNO_3$: C, 64.66; H, 4.78; N, 3.02. Found: C, 64.42; H, 4.58; N, 3.39.

(*E*)-4-((6-methoxy-1-oxo-3,4-dihydronaphthalen-2(1*H*)-ylidene)methyl)-1-(2-oxo-2-(p-tolyl)ethyl)pyridinium bromide (5b).

White crystalline solid; Yield: 90%; mp 216–218 ^oC; **IR** (KBr, cm^{-1}) υ_{max}: 1694, 1671 (C=O), 1643 (C=N), 1232 (C-O-C); **^{1}H NMR** (400 MHz, DMSO-d_6) δ: 8.92 (d, J = 6.8 Hz, 2H), 8.25 (d, J = 6.4 Hz, 2H), 7.97 (d, J = 8.0 Hz, 2H), 7.92 (t, J = 8.8 Hz, 1H), 7.48 (d, J = 8.4 Hz, 1H), 6.87-6.95 (m, 2H), 6.41 (s, 2H), 6.15 (d, J = 4.8 Hz, 1H), 5.81 (s, 1H), 3.84 (s, 3H), 2.87-3.07 (m, 2H), 2.45 (s, 3H), 2.03-2.07 (m, 1H), 1.59-1.63 (m, 1H); **^{13}C NMR** (75 MHz, $CDCl_3$+DMSO-d_6) δ: 199.37, 194.86, 171.18, 168.54, 151.73, 150.54, 136.09, 134.62, 134.50, 133.46, 130.83, 129.91, 118.46, 117.35, 70.51, 58.47, 33.59, 26.68, 22.52; **LC-MS**: *m/z*: 416 $[M+NH_4]^+$; Anal. Calcd for $C_{26}H_{24}BrNO_3$: C, 65.28; H, 5.06; N, 2.93. Found: C, 65.64; H, 5.30; N, 3.24.

(*E*)-4-((6-methoxy-1-oxo-3,4-dihydronaphthalen-2(1*H*)-ylidene)methyl)-1-(2-(4-methoxyphenyl)-2-oxoethyl)pyridinium bromide (5c).

White crystalline solid, yield: 91%, mp.: 213-215 ^oC; **IR** (KBr, cm^{-1}) υ_{max}: 1690, 1672 (C=O), 1643 (C=N), 1246 (C-O-C); **^{1}H NMR** (400 MHz, DMSO-d_6) δ: 8.92 (d, J = 6.8 Hz, 2H), 8.25 (d, J = 6.8 Hz, 2H), 8.05 (d, J = 8.8 Hz, 2H), 7.92 (d, J = 8.8 Hz, 1H), 7.19 (d, J = 8.8 Hz, 1H), 6.87-6.95 (m, 2H), 6.40 (s, 2H), 6.15 (d, J = 4.8 Hz, 1H), 5.81 (s, 1H), 3.90 (s, 3H), 3.84 (s, 3H), 2.87-3.07 (m, 2H), 2.02-2.07 (m, 1H), 1.58-1.62 (m, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6) δ 194.87, 189.62, 166.10, 164.76, 163.80, 147.45, 145.96, 131.70, 131.21, 129.74, 126.82, 126.18, 125.30, 114.90, 114.59, 114.05, 112.97, 69.44, 56.31, 56.05, 28.57, 21.94; **ESI-MS** *m/z*: 414 $[M]^+$; Anal. Calcd for $C_{26}H_{24}BrNO_4$: C, 63.17; H, 4.89; N, 2.83. Found: C, 62.90; H, 5.07; N, 3.18.

(*E*)-1-(2-([1,1'-biphenyl]-4-yl)-2-oxoethyl)-4-((6-methoxy-1-oxo-3,4-dihydronaphthalen-2(1*H*)-ylidene)methyl)pyridinium bromide (5d).

Light yellow crystalline solid; yield: 92%, mp.: 208-210 ^oC; **IR** (KBr, cm^{-1}) υ_{max}: 1694, 1672 (C=O), 1641 (C=N), 1242 (C-O-C); **^{1}H NMR** (400 MHz, DMSO-d_6) δ: 8.95 (d, J = 6.8 Hz, 2H), 8.28 (d, J = 6.4 Hz, 2H), 8.16 (d, J = 8.4 Hz, 2H), 8.00 (d, J = 8.4 Hz, 2H), 7.93 (d, J = 8.8 Hz, 1H), 7.84 (t, J = 7.2 Hz, 2H), 7.55 (t, J = 7.6 Hz, 1H), 7.48-7.50 (m,

1H), 6.88-6.96 (m, 2H), 6.50 (s, 2H), 6.20 (d, *J* = 4.8 Hz, 1H), 5.81 (s, 1H), 3.84 (s, 3H), 2.88-3.09 (m, 2H), 2.02-2.10 (m, 1H), 1.60-1.64 (m, 1H); **^{13}C NMR** (100 MHz, DMSO-*d*$_6$) δ 194.87, 190.95, 166.24, 163.82, 147.46, 146.37, 145.99, 138.96, 132.86, 129.69, 129.50, 129.28, 127.69, 127.60, 126.19, 125.38, 114.06, 112.99, 69.48, 56.05, 28.58, 21.97; **ESI-MS** *m/z*: 460 $[M]^+$; Anal. calcd. for $C_{31}H_{26}BrNO_3$: C, 68.89; H, 4.85; N, 2.59. Found: C, 68.59; H, 5.12; N, 2.79.

(*E*)-1-(2-(4-fluorophenyl)-2-oxoethyl)-4-((6-methoxy-1-oxo-3,4-dihydronaphthalen-2(1*H*)-ylidene)methyl)pyridinium bromide (5e).

White crystalline solid; yield: 90%, mp.: 210-212 ^oC; **IR** (KBr, cm^{-1}) υ_{max}: 1698, 1663 (C=O), 1643 (C=N), 1229 (C-O-C), 1158 (C-F); **^{1}H NMR** (400 MHz, DMSO-*d*$_6$) *δ*: 8.95 (d, *J* = 6.8 Hz, 2H), 8.27 (d, *J* = 6.4 Hz, 2H), 8.16-8.19 (m, 2H), 7.92 (d, *J* = 8.8 Hz, 1H), 7.53 (t, *J* = 8.8 Hz, 1H), 6.87-6.95 (m, 2H), 6.50 (s, 2H), 6.18 (d, *J* = 5.2 Hz, 1H), 5.81 (s, 1H), 3.84 (s, 3H), 2.88-3.09 (m, 2H), 2.03-2.07 (m, 1H), 1.59-1.63 (m, 1H); **^{13}C NMR** (100 MHz, DMSO-*d*$_6$) δ: 194.86, 190.12, 167.52, 166.27, 164.99, 163.81, 147.45, 145.95, 131.96, 131.86, 130.89, 129.74, 126.18, 125.38, 116.94, 116.72, 114.05, 112.98, 69.46, 56.05, 28.57, 21.94; **LC-MS**: *m/z*: 420 $[M+NH_4]^+$; Anal. calcd. for $C_{25}H_{21}BrFNO_3$: C, 62.25; H, 4.39; N, 2.90. Found: C, 62.51; H, 4.11; N, 3.15.

(*E*)-1-(2-(4-chlorophenyl)-2-oxoethyl)-4-((6-methoxy-1-oxo-3,4-dihydronaphthalen-2(1*H*)-ylidene)methyl)pyridinium bromide (5f).

White crystalline solid; yield: 90%, mp.: 235-237 ^oC; **IR** (KBr, cm^{-1}) υ_{max}: 1697, 1669 (C=O), 1644 (C=N), 1232 (C-O-C), 817 (C-Cl); **^{1}H NMR** (400 MHz, DMSO-*d*$_6$) *δ*: 8.92 (d, *J* = 6.8 Hz, 2H), 8.27 (d, *J* = 6.4 Hz, 2H), 8.09 (d, *J* = 8.4 Hz, 2H), 7.92 (d, *J* = 8.8 Hz, 1H), 7.76 (d, *J* = 8.4 Hz, 1H), 6.87-6.95 (m, 2H), 6.47 (s, 2H), 6.17 (d, *J* = 4.8 Hz, 1H), 5.81 (s, 1H), 3.84 (s, 3H), 2.88-3.09 (m, 2H), 2.03-2.09 (m, 1H), 1.59-1.63 (m, 1H); **^{13}C NMR** (100 MHz, DMSO) δ 194.86, 190.58, 166.30, 163.81, 147.45, 145.95, 140.03, 132.82, 130.64, 129.80, 126.18, 125.39, 114.05, 112.98, 69.46, 56.05, 28.57, 21.95; **ESI-MS** *m/z*: 420 $[M^+{+}2]$, 418 $[M]^+$; Anal. calcd. for $C_{25}H_{21}BrClNO_3$: C, 60.20; H, 4.24; N, 2.8. Found: C, 59.88; H, 4.46; N, 2.99.

(*E*)-1-(2-(4-bromophenyl)-2-oxoethyl)-4-((6-methoxy-1-oxo-3,4-dihydronaphthalen-2(1*H*)-ylidene)methyl)pyridinium bromide (5g).

White crystalline solid; yield: 87%, mp.: 243-245 ^{o}C; **IR** (KBr, cm^{-1}) υ_{max}: 1697, 1670 (C=O), 1644 (C=N), 1231 (C-O-C), 578 (C-Br); **^{1}H NMR** (400 MHz, DMSO-d_6) δ: 8.92 (d, J = 6.8 Hz, 2H), 8.26 (d, J = 6.8 Hz, 2H), 8.01 (d, J = 8.8 Hz, 2H), 7.90-7.93 (m, 2H), 6.87-6.95 (m, 2H), 6.45 (s, 2H), 6.15 (d, J = 4.8 Hz, 1H), 5.81 (s, 1H), 3.84 (s, 3H), 2.87-3.08 (m, 2H), 2.03-2.07 (m, 1H), 1.59-1.63 (m, 1H); **^{13}C NMR** (100 MHz, DMSO) δ 194.86, 190.79, 166.31, 163.82, 147.44, 145.96, 133.14, 132.75, 130.66, 129.75, 129.31, 126.18, 125.39, 114.04, 112.99, 69.48, 56.04, 28.57, 21.96; **ESI-MS** *m/z*: 465 [M^{+}+2], 463 [M]$^{+}$; Anal. calcd. for $C_{25}H_{21}Br_2NO_3$: C, 55.27; H, 3.90; N, 2.58. Found: C, 55.52; H, 4.22; N, 2.28.

(*E*)-4-((6-methoxy-1-oxo-3,4-dihydronaphthalen-2(1*H*)-ylidene)methyl)-1-(2-(4-nitrophenyl)-2-oxoethyl)pyridinium bromide (5h).

White crystalline solid; yield: 89%, mp.: 217-219 ^{o}C; **IR** (KBr, cm^{-1}) υ_{max}: 1703, 1669 (C=O), 1527, 1346 (NO_2), 1644 (C=N), 1223 (C-O-C); **^{1}H NMR** (400 MHz, DMSO-d_6) δ: 8.93 (d, J = 5.2 Hz, 2H), 8.49 (d, J = 8.8 Hz, 2H), 8.30 (t, J = 7.6 Hz, 3H), 7.92 (d, J = 8.8 Hz, 1H), 6.88-6.95 (m, 2H), 6.52 (s, 2H), 6.20 (d, J = 5.2 Hz, 1H), 5.82 (s, 1H), 3.84 (s, 3H), 2.88-3.09 (m, 2H), 2.03-2.08 (m, 1H), 1.60-1.64 (m, 1H); **^{13}C NMR** (100 MHz, DMSO) δ 194.86, 190.80, 166.46, 163.82, 151.12, 147.45, 145.97, 138.76, 130.22, 129.75, 126.18, 125.45, 124.68, 114.05, 112.99, 69.49, 56.05, 28.57, 21.97; **ESI-MS** *m/z*: 429 [M]$^{+}$; Anal. calcd. for $C_{25}H_{21}BrN_2O_5$: C, 58.95; H, 4.16; N, 5.50. Found: C, 58.72; H, 4.35; N, 5.29.

(*E*)-4-((6-methoxy-1-oxo-3,4-dihydronaphthalen-2(1*H*)-ylidene)methyl)-1-(2-oxo-2-(2-oxo-2*H*-chromen-3-yl)ethyl)pyridinium bromide (5i).

White crystalline solid; yield: 92%, mp.: 227-229 ^{o}C; **IR** (KBr, cm^{-1}) υ_{max}: 1731, 1701, 1678 (C=O), 1646 (C=N), 1246 (C-O-C); **^{1}H NMR** (400 MHz, DMSO-d_6) δ: 8.99 (s, 1H), 8.89 (d, J = 6.4 Hz, 2H), 8.25 (d, J = 6.4 Hz, 2H), 8.11 (t, J = 6.4 Hz, 1H), 7.85-7.93 (m, 1H), 7.60 (d, J = 8.4 Hz, 1H), 7.51 (t, J = 7.6 Hz, 1H), 6.87-6.95 (m, 2H), 6.29 (s, 2H), 6.19 (d, J = 5.2 Hz, 1H), 5.81 (s, 1H), 3.84 (s, 3H), 2.87-3.08 (m, 2H), 2.03-2.07 (m, 1H), 1.57-1.61 (m, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6) δ: 207.02, 194.86, 188.77, 166.20, 163.80, 159.12, 155.30, 150.02, 147.44, 145.91, 136.34, 132.02, 129.74, 126.18, 126.00, 125.23, 121.77, 118.43, 116.92, 114.05, 112.97, 69.46, 56.04, 28.55, 21.95; **LC-**

MS: *m/z*: 470 $[M+NH_4]^+$; Anal. calcd. for $C_{28}H_{22}BrNO_5$: C, 63.17; H, 4.17; N, 2.63. Found: C, 62.89; H, 4.34; N, 2.87.

(*E*)-4-((6-methoxy-1-oxo-3,4-dihydronaphthalen-2(1*H*)-ylidene)methyl)-1-(2-oxo-2-(3-oxo-3*H*-benzo[*f*]chromen-2-yl)ethyl)pyridinium bromide (5j).

Light yellow crystalline solid; yield: 91%, mp.: 251-253 ^oC; **IR** (KBr, cm^{-1}) υ_{max}: 1736, 1698, 1680 (C=O), 1646 (C=N), 1246 (C-O-C); **^{1}H NMR** (400 MHz, DMSO-d_6) δ: 9.58 (s, 1H), 8.91 (d, *J* = 5.6 Hz, 2H), 8.71 (d, *J* = 8.4 Hz, 1H), 8.48 (d, *J* = 8.8 Hz, 1H), 8.27 (d, *J* = 6.0 Hz, 2H), 8.17 (d, *J* = 8.4 Hz, 1H), 7.93 (d, *J* = 8.4 Hz, 1H), 7.85 (t, *J* = 7.2 Hz, 1H), 7.71-7.78 (m, 1H), 6.88-6.96 (m, 2H), 6.34 (s, 2H), 6.19 (d, *J* = 4.8 Hz, 1H), 5.83 (s, 1H), 3.84 (s, 3H), 2.88-3.08 (m, 2H), 2.04-2.08 (m, 1H), 1.58-1.61 (m, 1H); **ESI-MS** *m/z*: 502 $[M]^+$; Anal. calcd. for $C_{32}H_{24}BrNO_5$: C, 65.99; H, 4.15; N, 2.40. Found: C, 66.27; H, 4.38; N, 2.69.

((*E*)-4-((8-methoxy-1-oxo-3,4-dihydronaphthalen-2(1*H*)-ylidene)methyl)-1-(2-(6-methoxy-2-oxo-2*H*-chromen-3-yl)-2-oxoethyl)pyridin-1-ium bromide (5k).

Creamy white crystalline solid; yield: 80%, mp.: 265-267 ^oC; **IR** (KBr, cm^{-1}) υ_{max}: 1734, 1699, 1678 (C=O), 1645 (C=N), 1270 and 1248 (C-O-C); **^{1}H NMR** (400 MHz, DMSO-d_6) δ: 8.96 (s, 1H), 8.90 (d, *J* = 6.4 Hz, 2H), 8.26 (d, *J* = 6.8 Hz, 2H), 7.92 (d, *J* = 8.4 Hz, 1H), 7.64 (d, *J* = 7.2 Hz, 1H), 7.54 (s, 1H), 6.87-6.95 (m, 2H), 6.30 (s, 2H), 6.18 (d, *J* = 4.8 Hz, 1H), 5.81 (s, 1H), 3.98 (s, 3H), 3.84 (s, 3H), 2.88-3.08 (m, 2H), 2.03-2.07 (m, 1H), 1.57-1.61 (m, 1H); **^{13}C NMR** (101 MHz, DMSO) δ 194.86, 188.76, 166.20, 163.81, 158.84, 150.27, 147.44, 146.84, 145.91, 144.58, 129.74, 126.19, 125.95, 125.23, 122.74, 121.87, 118.98, 118.16, 114.05, 112.98, 69.47, 56.81, 56.05, 28.55, 21.95; **ESI-MS** *m/z*: 482 $[M]^+$; Anal. calcd. for $C_{29}H_{24}BrNO_6$: C, 61.93; H, 4.30; Br, 14.21; N, 2.49. Found: C, 62.27; H, 4.09; N, 2.73.

(*E*)-1-(2-(8-ethoxy-2-oxo-2*H*-chromen-3-yl)-2-oxoethyl)-4-((6-methoxy-1-oxo-3,4-dihydronaphthalen-2(1*H*)-ylidene)methyl)pyridinium bromide (5l).

Light Yellow crystalline solid; yield: 85%, mp.: 260-262 ^oC; **IR** (KBr, cm^{-1}) υ_{max}: 1733, 1704, 1661 (C=O), 1644 (C=N), 1271 and 1248 (C-O-C); **^{1}H NMR** (400 MHz, DMSO-d_6) δ: 8.95 (s, 1H), 8.90 (d, *J* = 6.4 Hz, 2H), 8.26 (d, *J* = 6.4 Hz, 2H), 7.92 (d, *J* = 8.8 Hz, 1H), 7.62 (d, *J* = 6.8 Hz, 1H), 7.53 (s, 1H), 6.87-6.95 (m, 2H), 6.30 (s, 2H), 6.18 (d, *J* = 4.8 Hz, 1H), 5.81 (s, 1H), 4.24 (q, *J* = 6.8 Hz, 2H), 3.84 (s, 3H), 2.88-3.08 (m, 2H), 2.03-

2.07 (m, 1H), 1.57-1.61 (m, 1H), 1.44 (t, *J* = 6.8 Hz, 3H); **^{13}C NMR** (100 MHz, DMSO) δ 194.86, 188.79, 166.19, 163.81, 158.93, 150.32, 147.44, 146.09, 145.92, 145.46, 144.64, 129.75, 126.18, 125.96, 125.21, 122.71, 121.83, 119.07, 114.06, 112.97, 69.46, 65.15, 56.04, 28.54, 21.94, 15.04; **ESI-MS** *m/z*: 496 $[M]^+$; Anal. calcd. for $C_{30}H_{26}BrNO_6$: C, 62.51; H, 4.55; N, 2.43; Found: C, 62.22; H, 4.83; N, 2.16.

(*E*)-1-(2-(6-bromo-8-ethoxy-2-oxo-2*H*-chromen-3-yl)-2-oxoethyl)-4-((6-methoxy-1-oxo-3,4-dihydronaphthalen-2(1*H*)-ylidene)methyl)pyridinium bromide (5m).

White crystalline solid; yield: 82%, mp.: 271-272 ^oC; **IR** (KBr, cm^{-1}) υ_{max}: 1743, 1709, 1697 (C=O), 1674 (C=N), 1271 and 1257 (C-O-C); **^{1}H NMR** (400 MHz, DMSO-d_6) δ: 8.89 (s, 1H), 8.25 (d, *J* = 5.6 Hz, 2H), 8.06 (d, *J* = 5.2 Hz, 1H), 7.91 (d, *J* = 11.2 Hz, 2H), 7.68 (s, 1H), 6.87-6.95 (m, 2H), 6.27 (s, 2H), 6.18 (s, 1H), 5.81 (s, 1H), 4.25 (q, *J* = 6.8 Hz, 2H), 3.84 (s, 3H), 2.83-3.07 (m, 2H), 1.99-2.08 (m, 1H), 1.57-1.59 (m, 1H), 1.43 (t, *J* = 6.8 Hz, 3H); **^{13}C NMR** (100 MHz, DMSO) δ 194.85, 188.63, 166.24, 163.81, 158.45, 148.97, 147.42, 147.03, 145.92, 144.05, 141.79, 129.74, 126.19, 125.22, 124.76, 124.30, 122.82, 121.12, 120.23, 117.33, 114.04, 112.97, 69.47, 65.83, 56.04, 28.55, 21.95, 14.90; **ESI-MS** *m/z*: 577 $[M^+ + 2]$, 575 $[M]^+$; Anal. calcd. for $C_{30}H_{25}Br_2NO_6$: C, 54.98; H, 3.85; N, 2.14; Found: C, 54.63; H, 4.13; N, 2.39.

(*E*)-1-(2-(6,8-dichloro-2-oxo-2*H*-chromen-3-yl)-2-oxoethyl)-4-((6-methoxy-1-oxo-3,4-dihydronaphthalen-2(1*H*)-ylidene)methyl)pyridinium bromide (5n).

White crystalline solid; yield: 80%, mp.: 286-288 ^oC; **IR** (KBr, cm^{-1}) υ_{max}: 1730, 1702, 1673 (C=O), 1646 (C=N), 1248 (C-O-C); **^{1}H NMR** (400 MHz, DMSO-d_6) δ: 8.92 (s, 1H), 8.87 (d, *J* = 6.4 Hz, 2H), 8.24-8.26 (m, 2H), 7.90-7.93 (m, 1H), 7.65 (d, *J* = 9.2 Hz, 1H), 6.87-6.96 (m, 2H), 6.27 (s, 2H), 6.18 (d, *J* = 4.8 Hz, 1H), 5.81 (s, 1H), 3.84 (s, 3H), 2.88-3.07 (m, 2H), 2.04-2.09 (m, 1H), 1.56-1.63 (m, 1H); **^{13}C NMR** (100 MHz, DMSO) δ 194.86, 188.66, 166.27, 163.82, 158.74, 153.92, 148.71, 147.44, 145.92, 135.64, 130.70, 129.76, 129.62, 126.18, 125.24, 122.78, 119.75, 119.02, 114.05, 112.99, 69.47, 56.04, 28.55, 21.93; **ESI-MS** *m/z*: 525 $[M^+ + 4]$, 523 $[M^+ + 2]$, 521 $[M]^+$; Anal. calcd. for $C_{28}H_{20}BrCl_2NO_5$: C, 55.93; H, 3.35; N, 2.33; Found: C, 55.69; H, 3.08; N, 2.60.

(*E*)-1-(2-(6,8-dibromo-2-oxo-2*H*-chromen-3-yl)-2-oxoethyl)-4-((6-methoxy-1-oxo-3,4-dihydronaphthalen-2(1*H*)-ylidene)methyl)pyridinium bromide (5o).

White crystalline solid; yield: 80%, mp.: 275-277 ^{o}C; **IR** (KBr, cm^{-1}) υ_{max}: 1744, 1735, 1673 (C=O), 1647 (C=N), 1248 (C-O-C); **^{1}H NMR** (400 MHz, DMSO-d_6) δ: 8.93 (s, 1H), 8.88-8.90 (m, 2H), 8.25 (d, J = 6.4 Hz, 2H), 8.00-8.03 (m, 1H), 7.92 (d, J = 8.4 Hz, 1H), 6.87-6.95 (m, 2H), 6.28 (s, 2H), 6.18 (s, 1H), 5.81 (s, 1H), 3.84 (s, 3H), 2.87-3.07 (m, 2H), 2.02-2.07 (m, 1H), 1.57-1.61 (m, 1H); **^{13}C NMR** (100 MHz, DMSO) δ 194.85, 188.63, 166.26, 163.82, 158.68, 154.32, 148.62, 147.43, 145.93, 138.37, 133.72, 129.75, 126.19, 125.24, 122.72, 120.24, 119.23, 117.44, 114.05, 112.98, 69.47, 56.05, 28.55, 21.95; **ESI-MS** *m/z*: 614 [M^{+}+4], 612 [M^{+}+2], 610 [M]$^{+}$; Anal. calcd. for $C_{28}H_{20}Br_2NO_5$: , 55.11; H, 3.30; N, 2.30; Found: C, 55.36; H, 3.59; N, 2.08.

(*E*)-1-(2-(6,8-dinitro-2-oxo-2*H*-chromen-3-yl)-2-oxoethyl)-4-((6-methoxy-1-oxo-3,4-dihydronaphthalen-2(1)-ylidene)methyl)pyridinium bromide (5p).

White crystalline solid; yield: 79%, mp.: 283-285 ^{o}C; **IR** (KBr, cm^{-1}) υ_{max}: 1737, 1703, 1669 (C=O), 1646 (C=N), 1347 (N-O), 1247 (C-O-C); **^{1}H NMR** (400 MHz, DMSO-d_6) δ: 9.14 (s, 1H), 8.89 (d, J = 6.4 Hz, 2H), 8.26 (d, J = 6.8 Hz, 2H), 7.92 (d, J = 8.8 Hz, 1H), 7.82 (d, J = 9.2 Hz, 1H), 6.88-6.96 (m, 2H), 6.29 (s, 2H), 6.18 (s, 1H), 5.81 (s, 1H), 3.84 (s, 3H), 2.88-2.92 (m, 1H), 3.04-3.08 (m, 1H), 2.05-2.09 (m, 1H), 1.58-1.62 (m, 1H); **^{13}C NMR** (100 MHz, DMSO) δ 194.86, 188.40, 166.31, 163.82, 158.58, 158.23, 148.86, 147.44, 145.93, 144.59, 130.12, 129.76, 127.70, 126.18, 125.26, 123.45, 118.72, 118.57, 114.05, 112.98, 69.47, 56.04, 28.55, 21.95; **ESI-MS** *m/z*: 542 [M]$^{+}$; Anal. calcd. for $C_{28}H_{20}BrN_3O_9$: C, 54.03; H, 3.24; N, 6.75; Found: C, 54.33; H, 3.01; N, 6.98.

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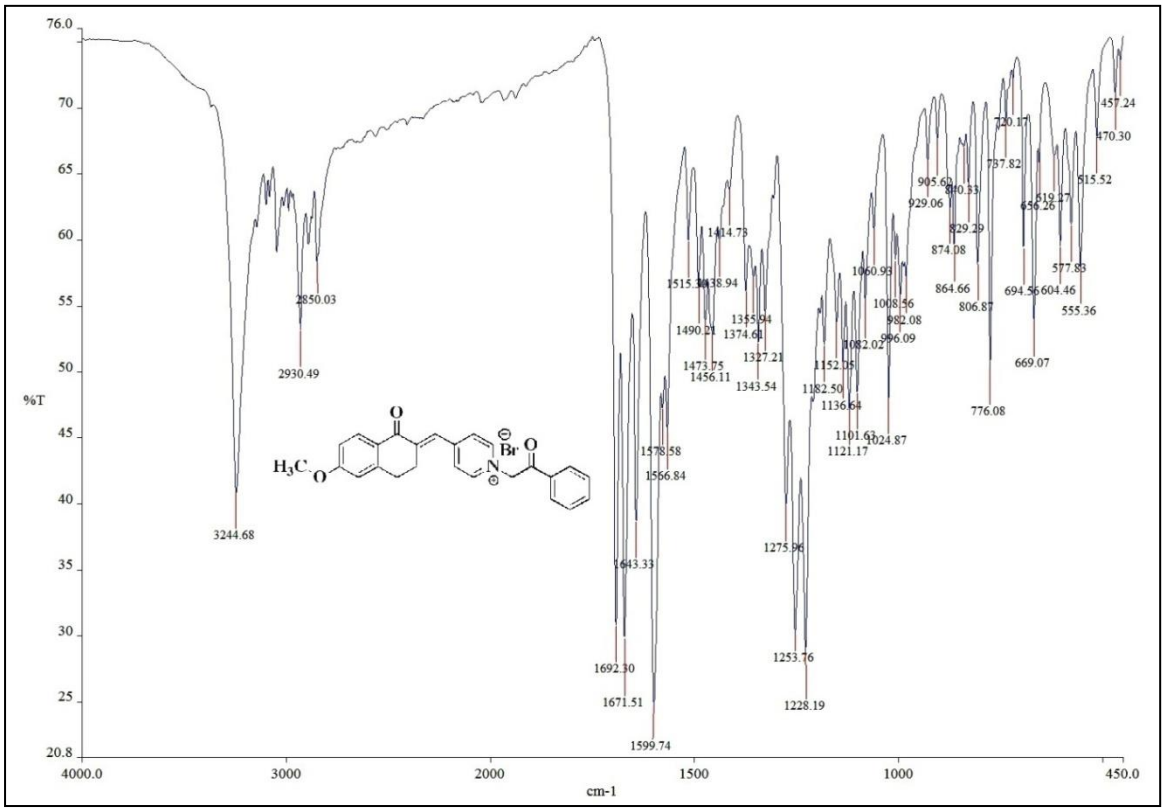
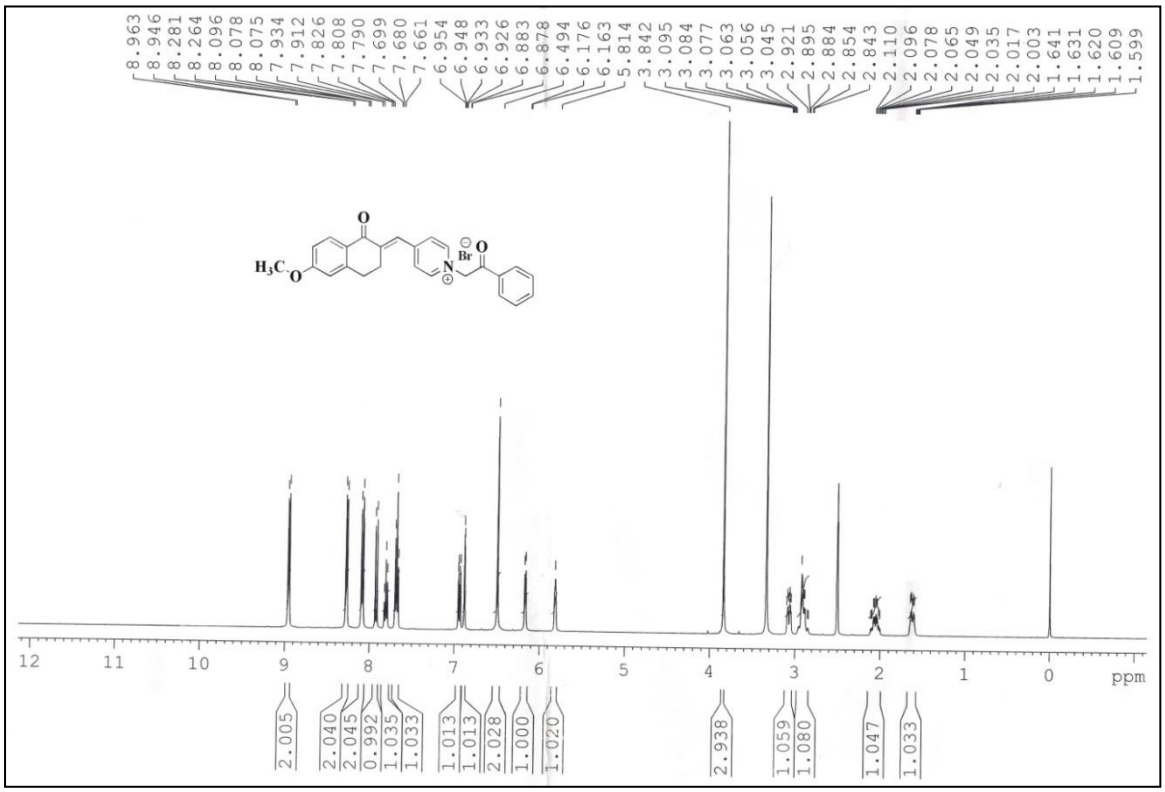
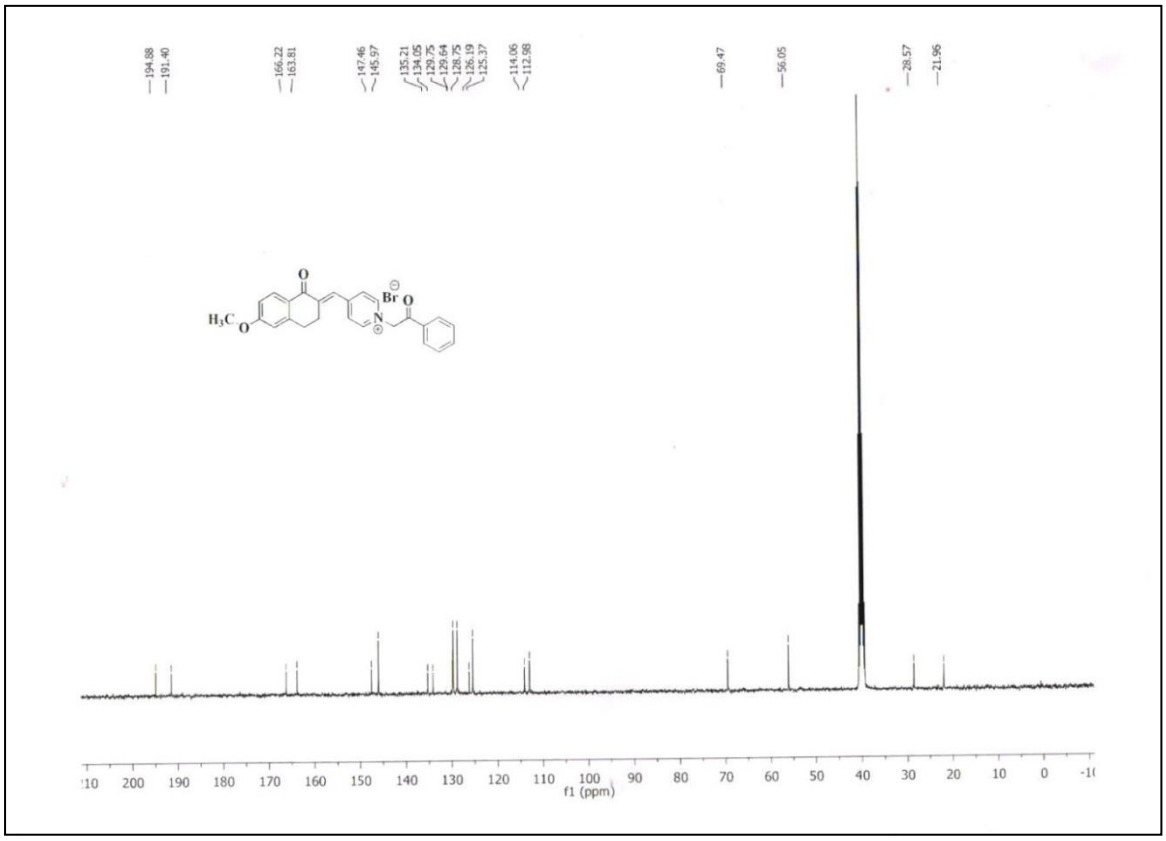


IR (KBr) spectrum of compound 5a



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



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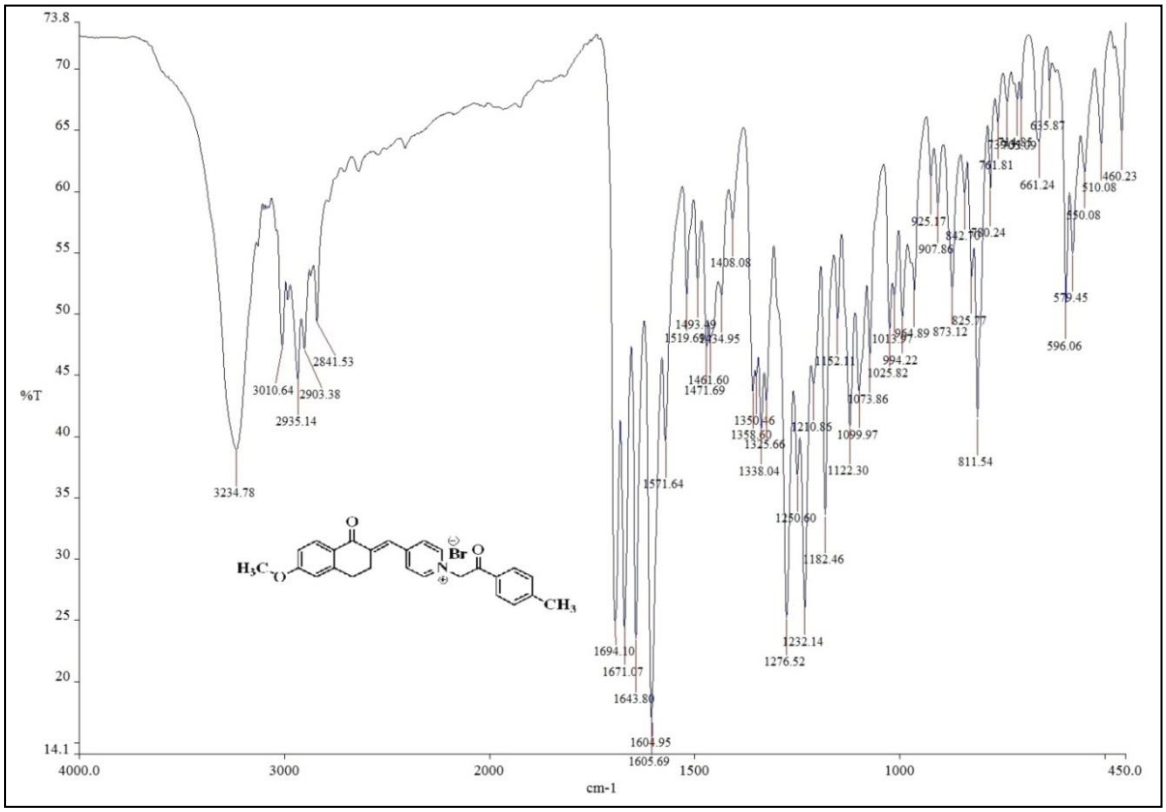
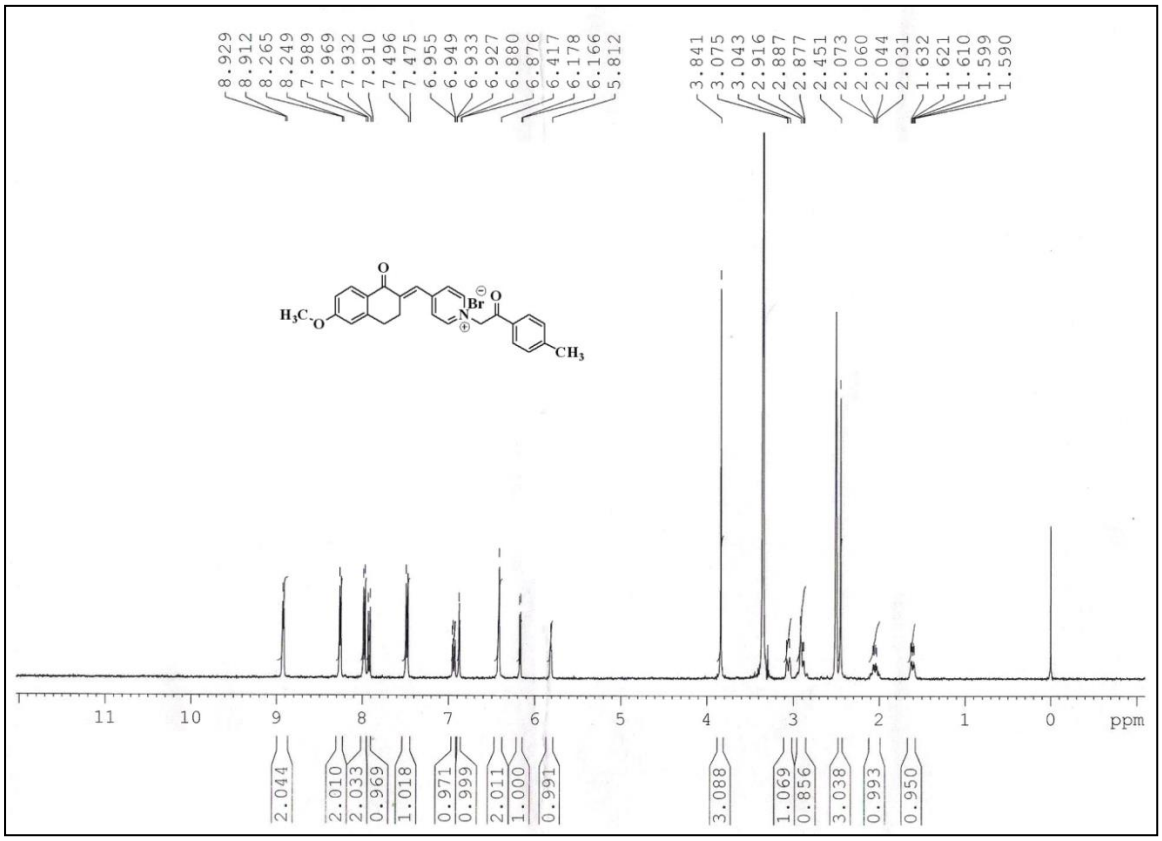
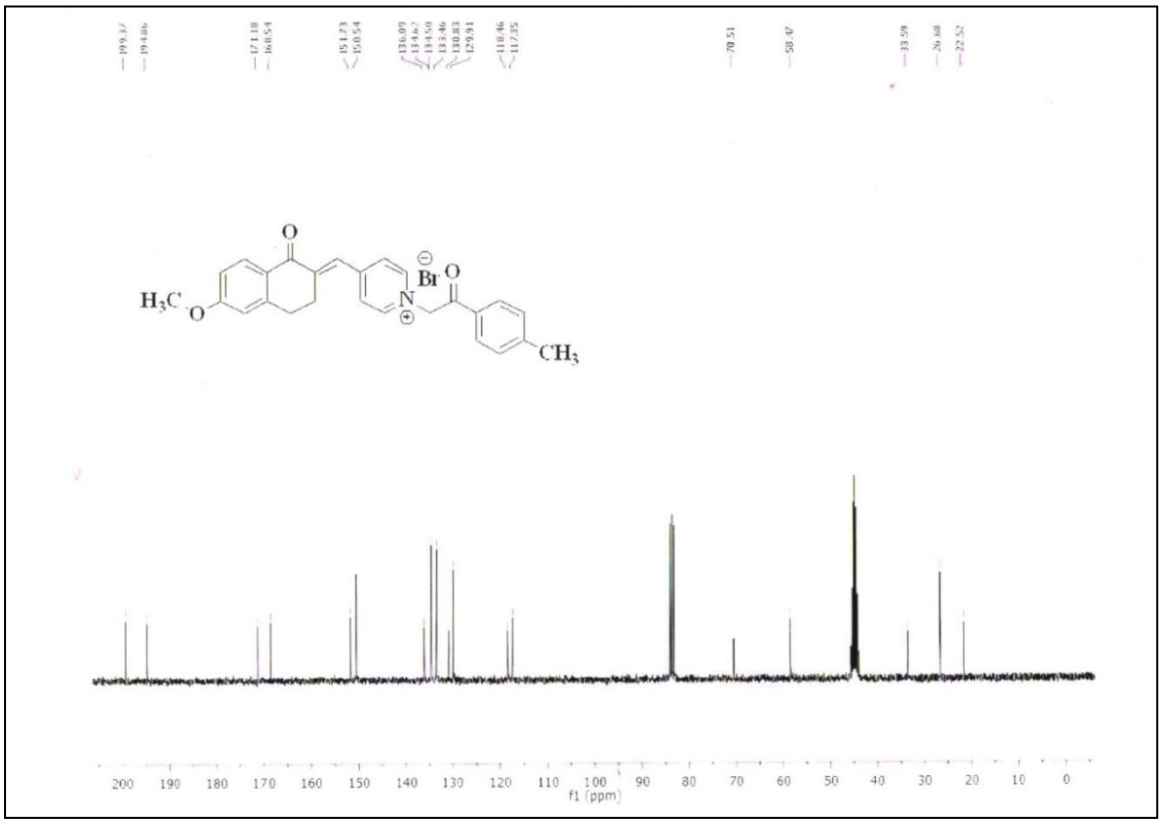


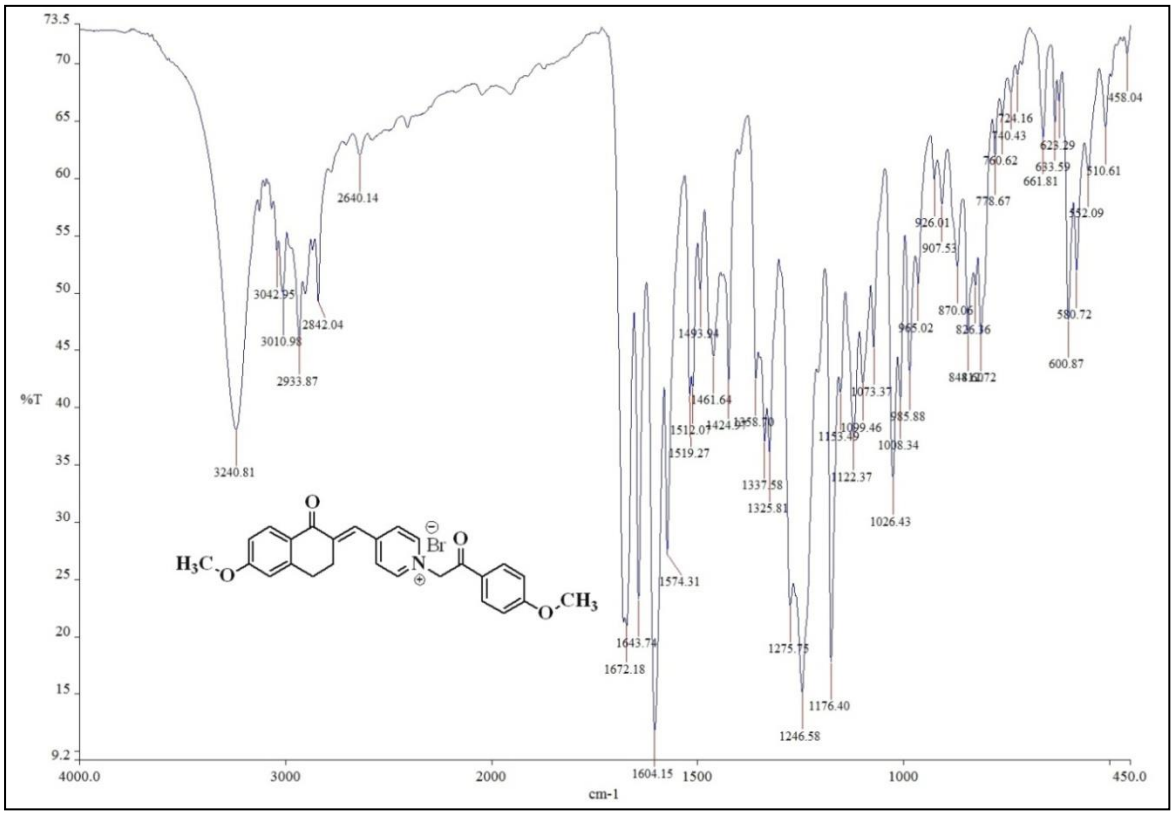
IR (KBr) spectrum of compound 5b



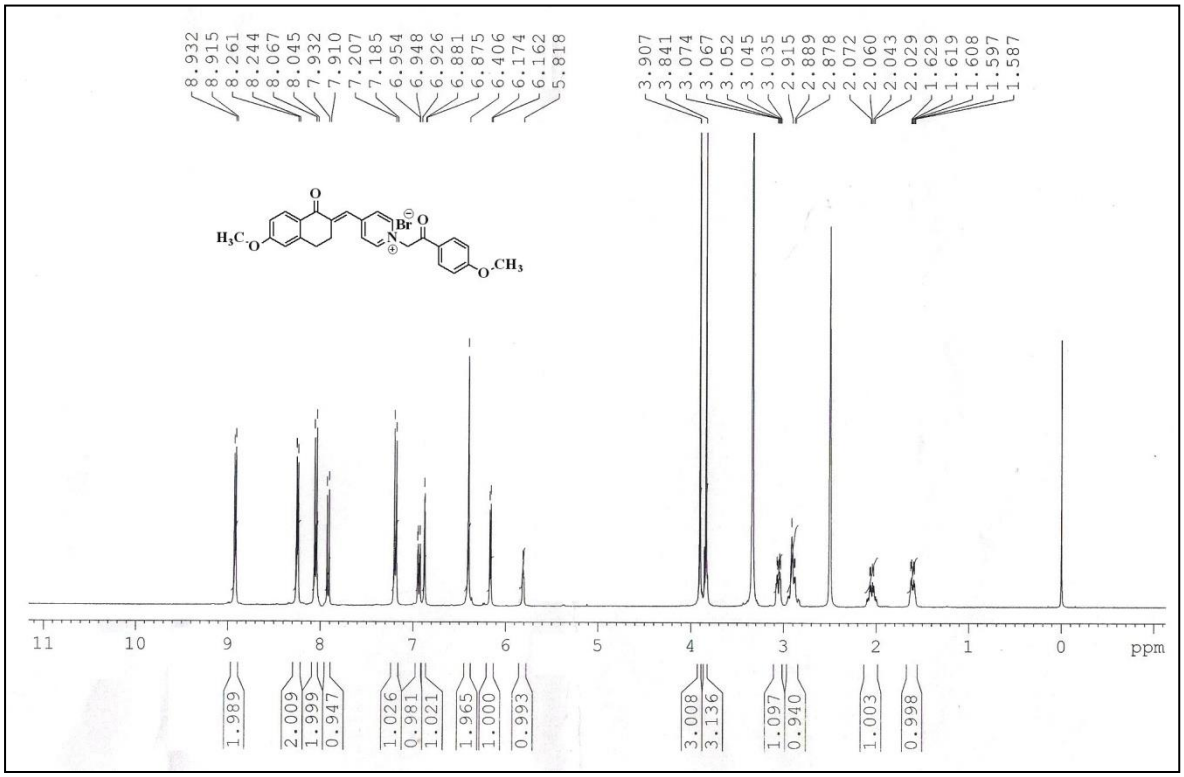
^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound 5b



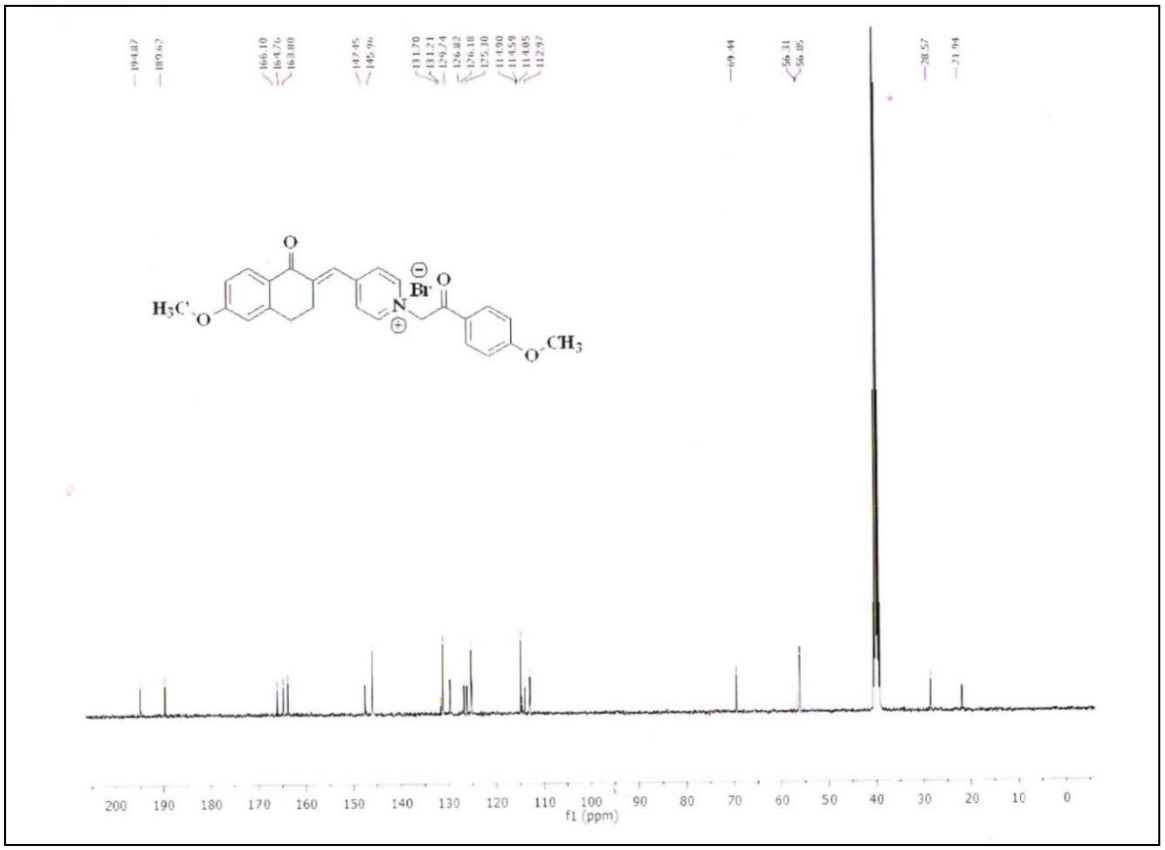
^{13}C NMR (75 MHz, CDCl$_3$+DMSO-d_6) spectrum of compound 5b



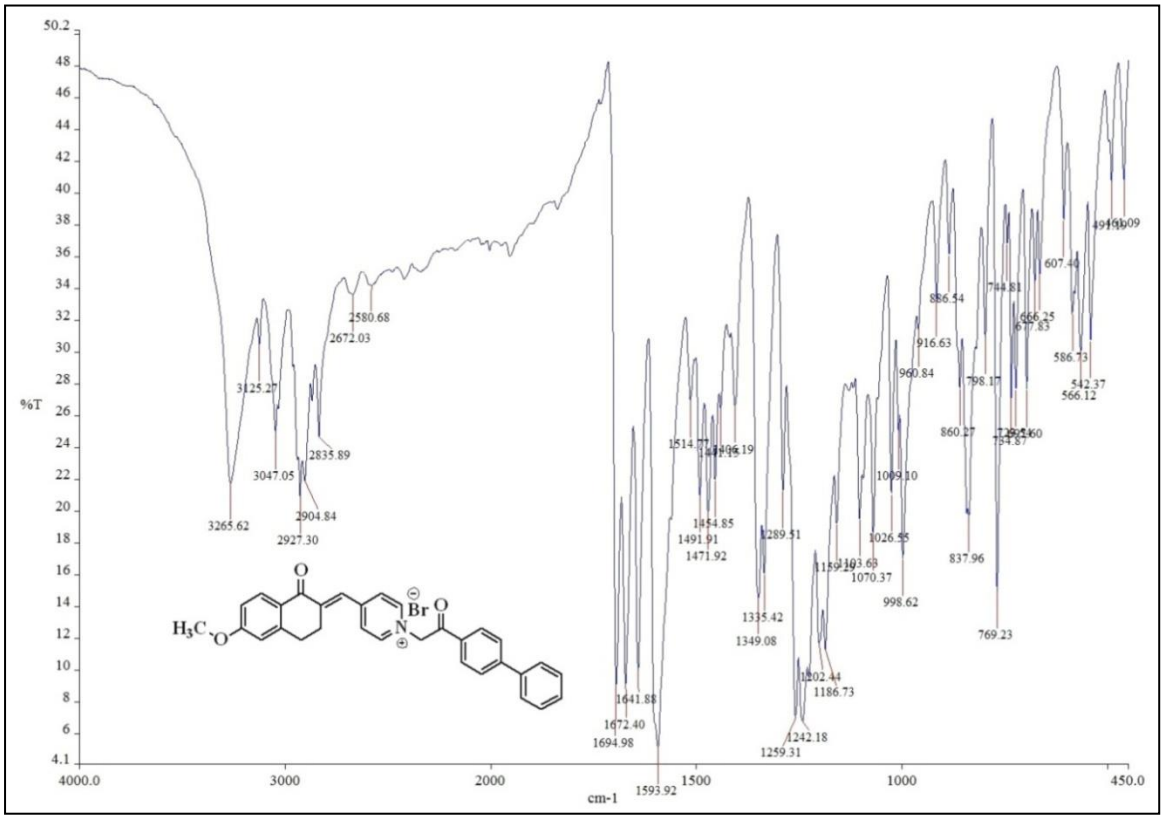
IR (KBr) spectrum of compound 5c



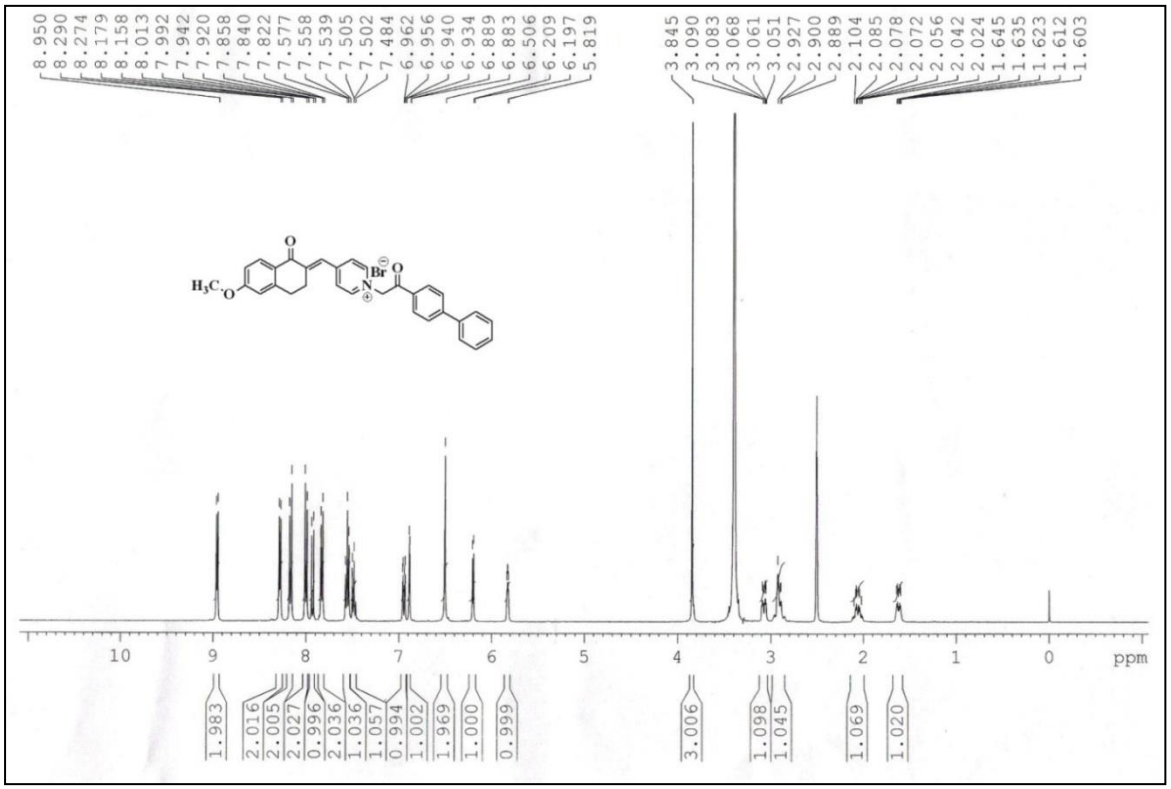
^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound 5c



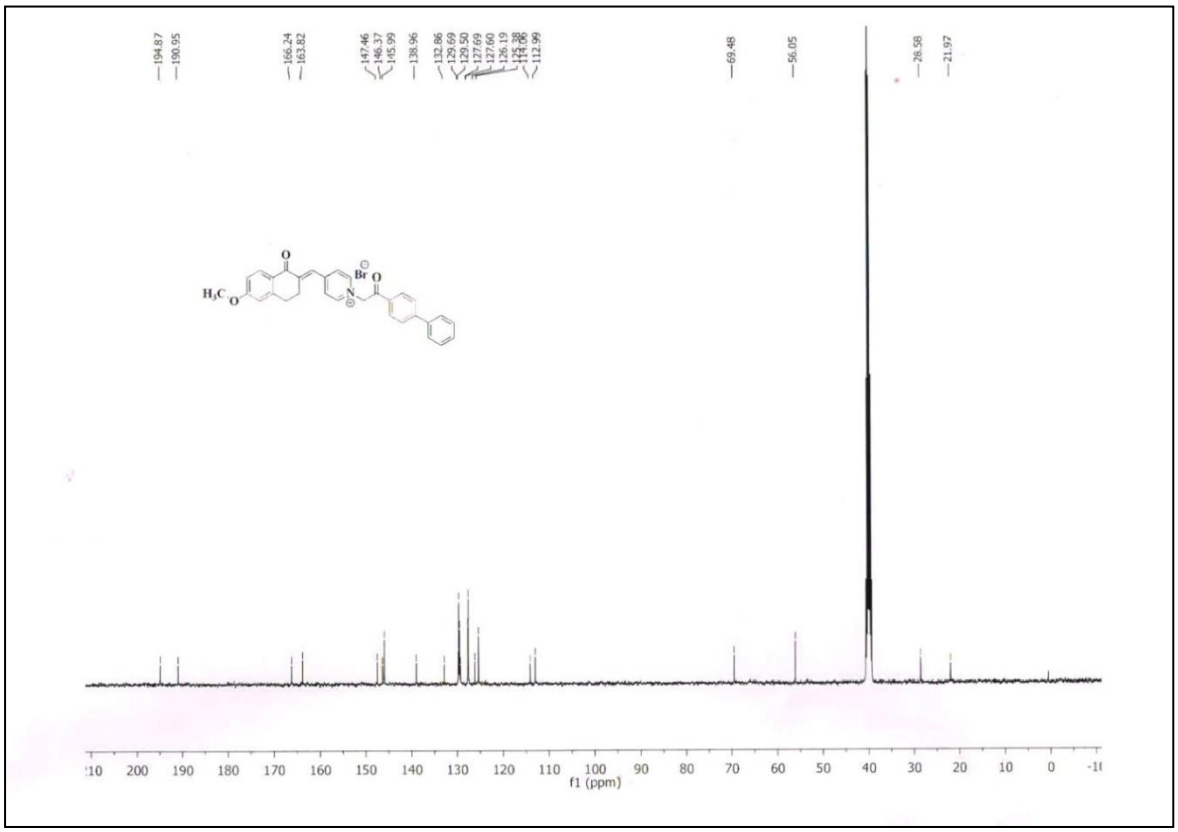
^{13}C NMR (100 MHz, DMSO-d_6) spectrum of compound 5c



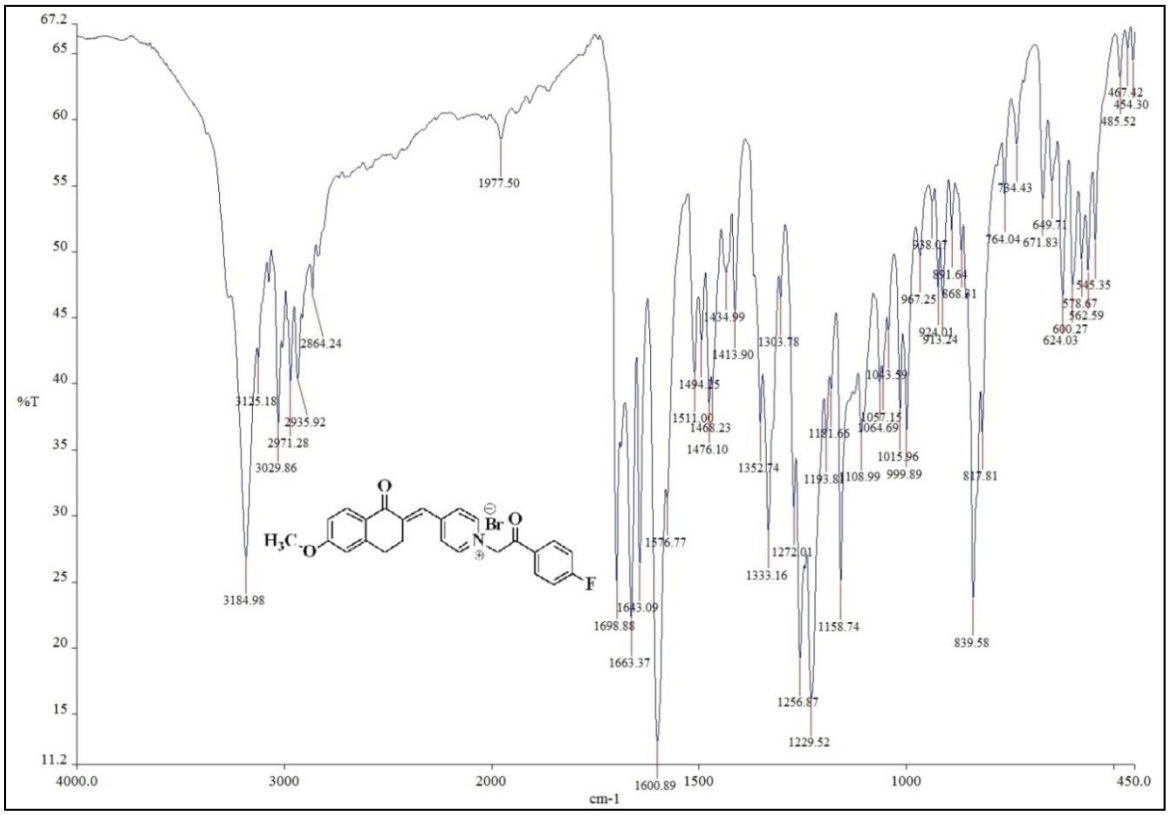
IR (KBr) spectrum of compound 5d



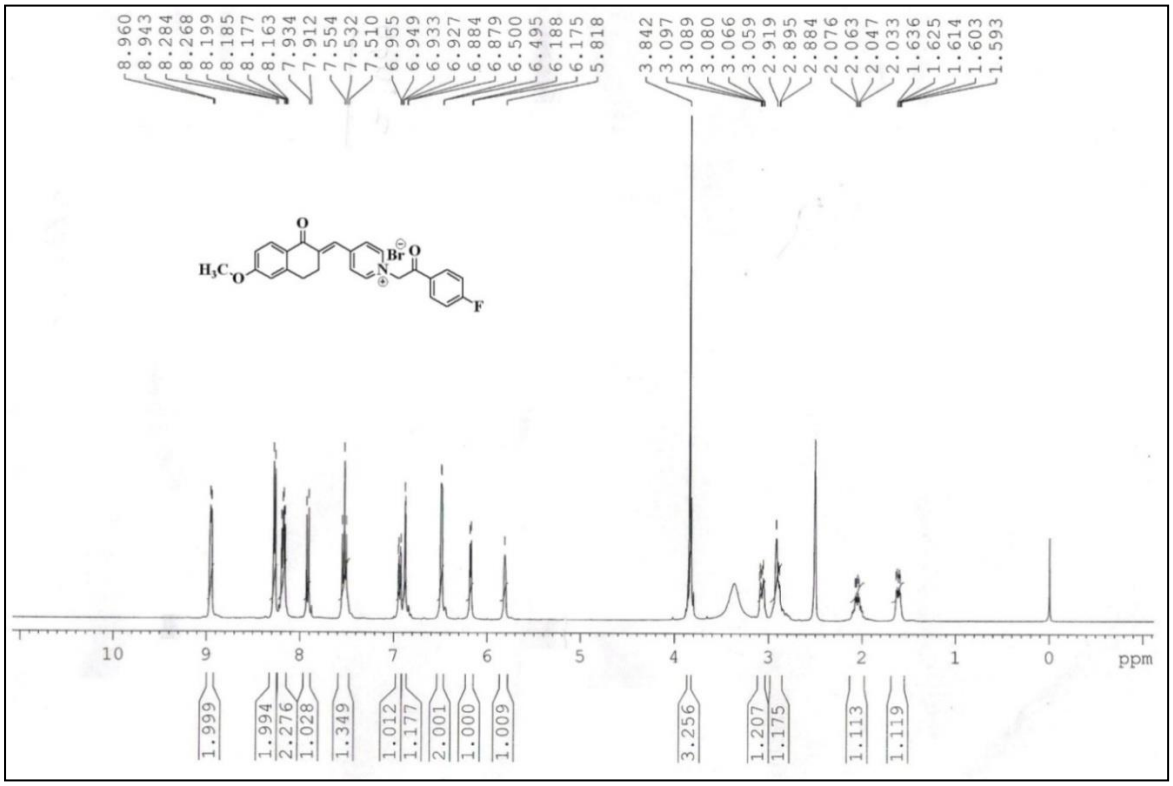
^{1}H NMR (400 MHz, DMSO-*d*$_6$) spectrum of compound 5d



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

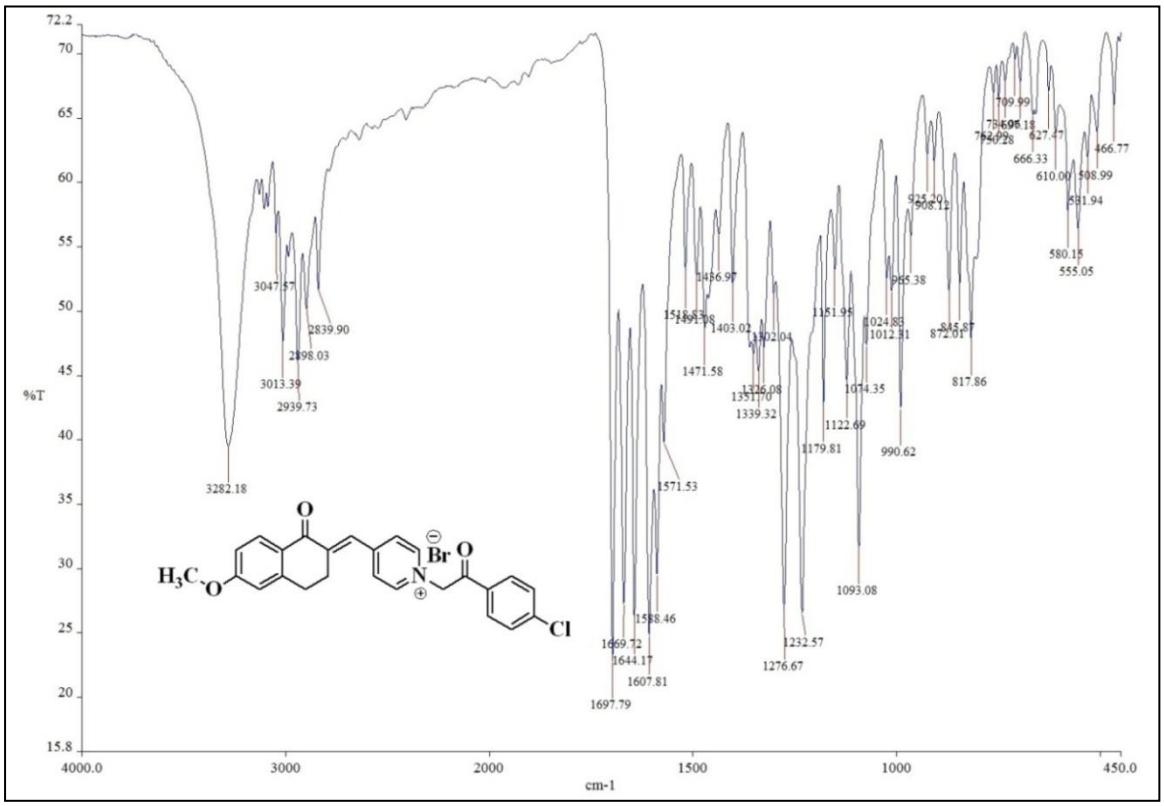


IR (KBr) spectrum of compound 5e

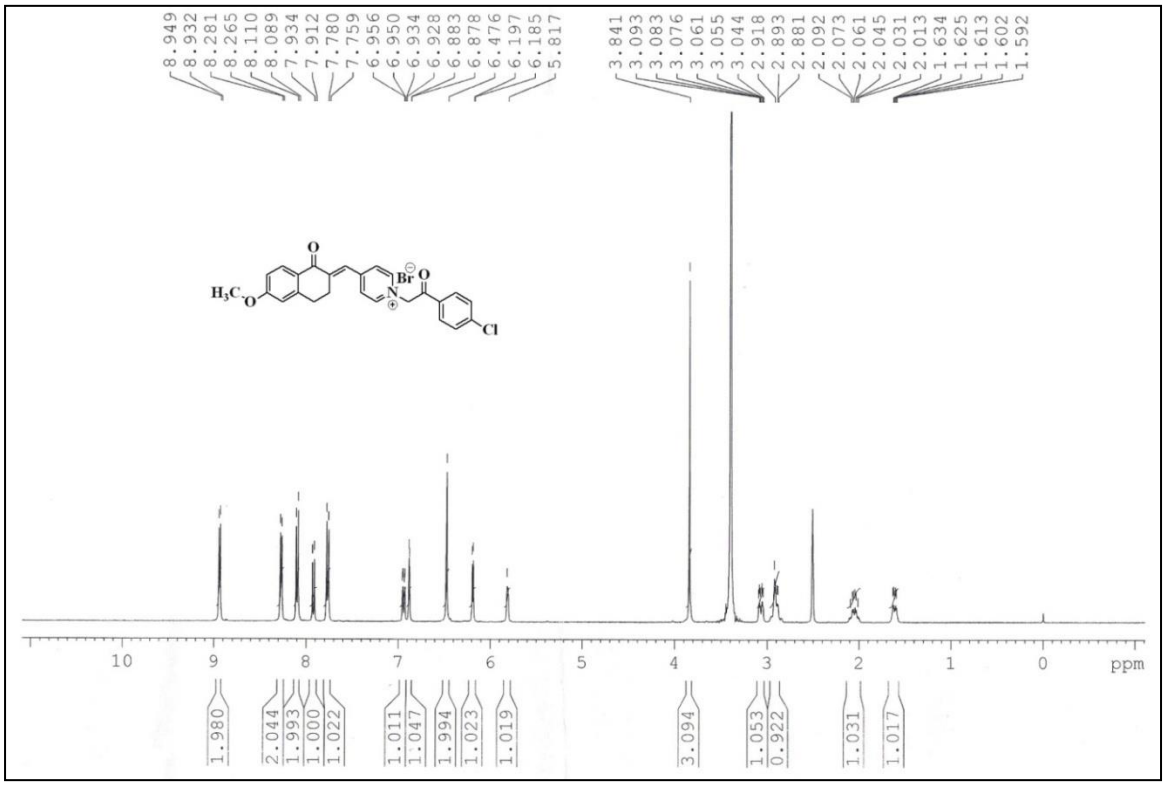


^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound 5e

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



IR (KBr) spectrum of compound 5f



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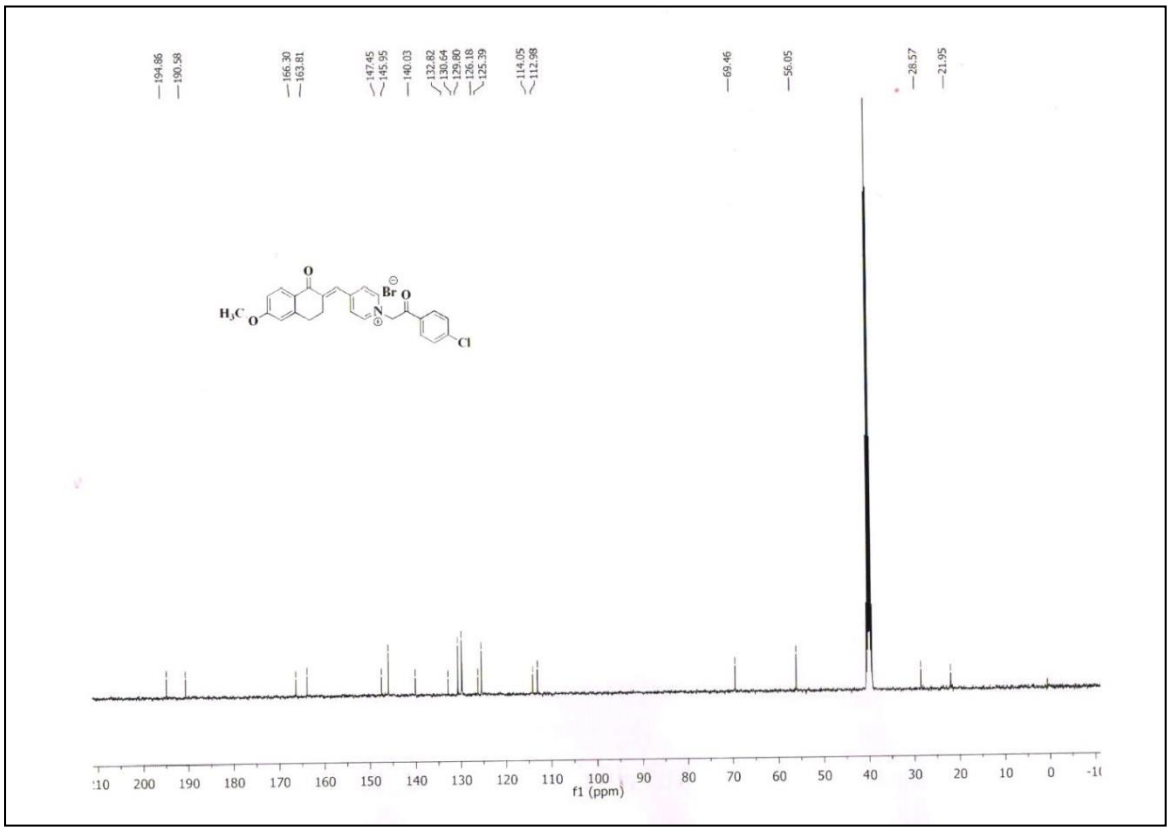
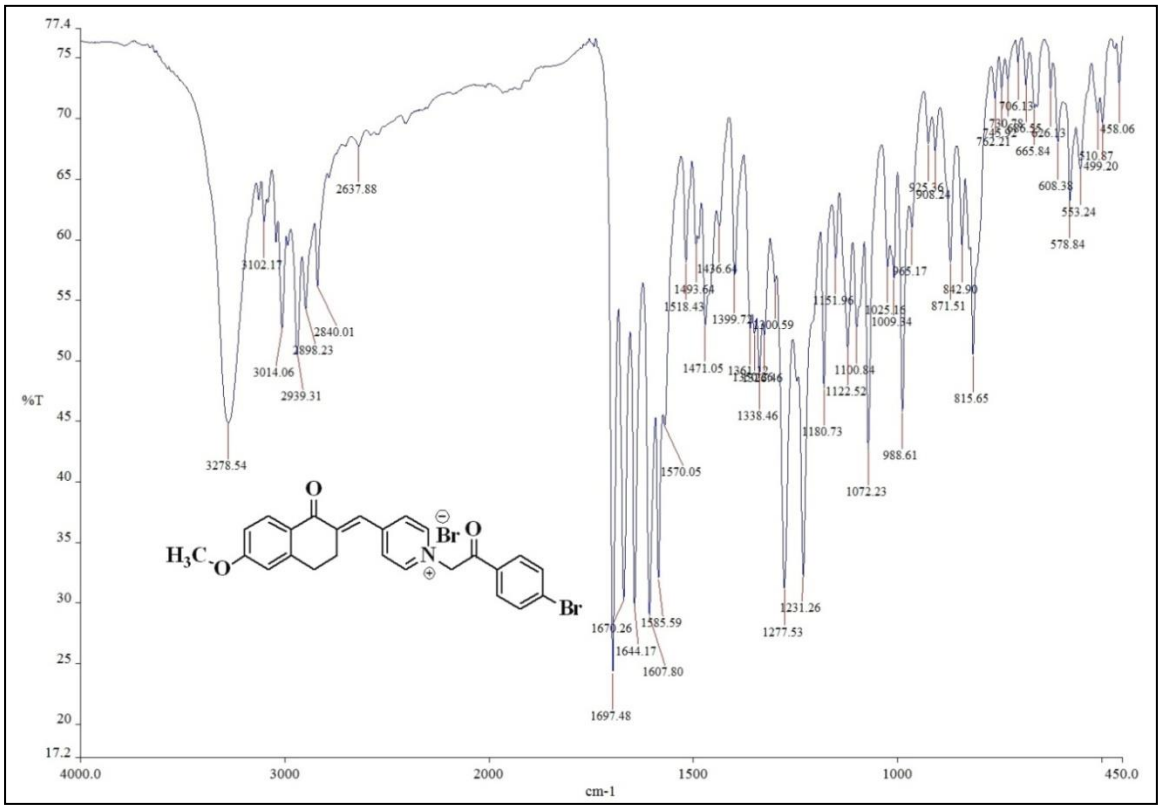
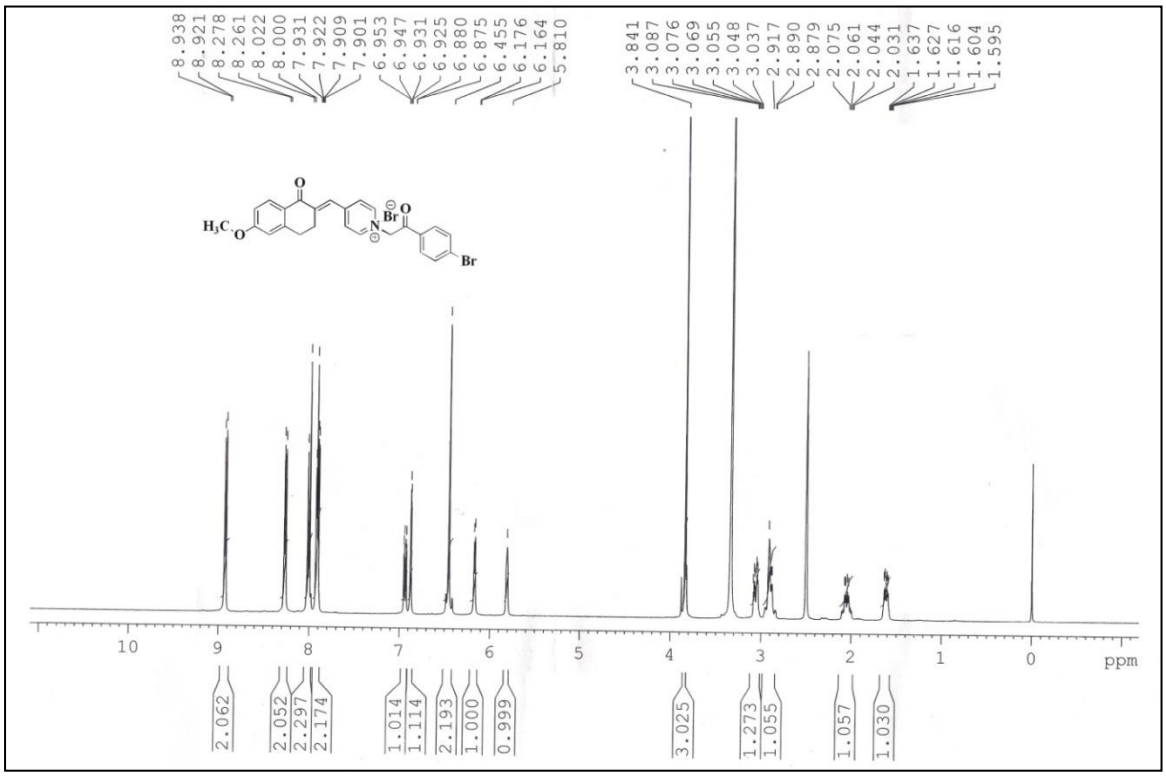


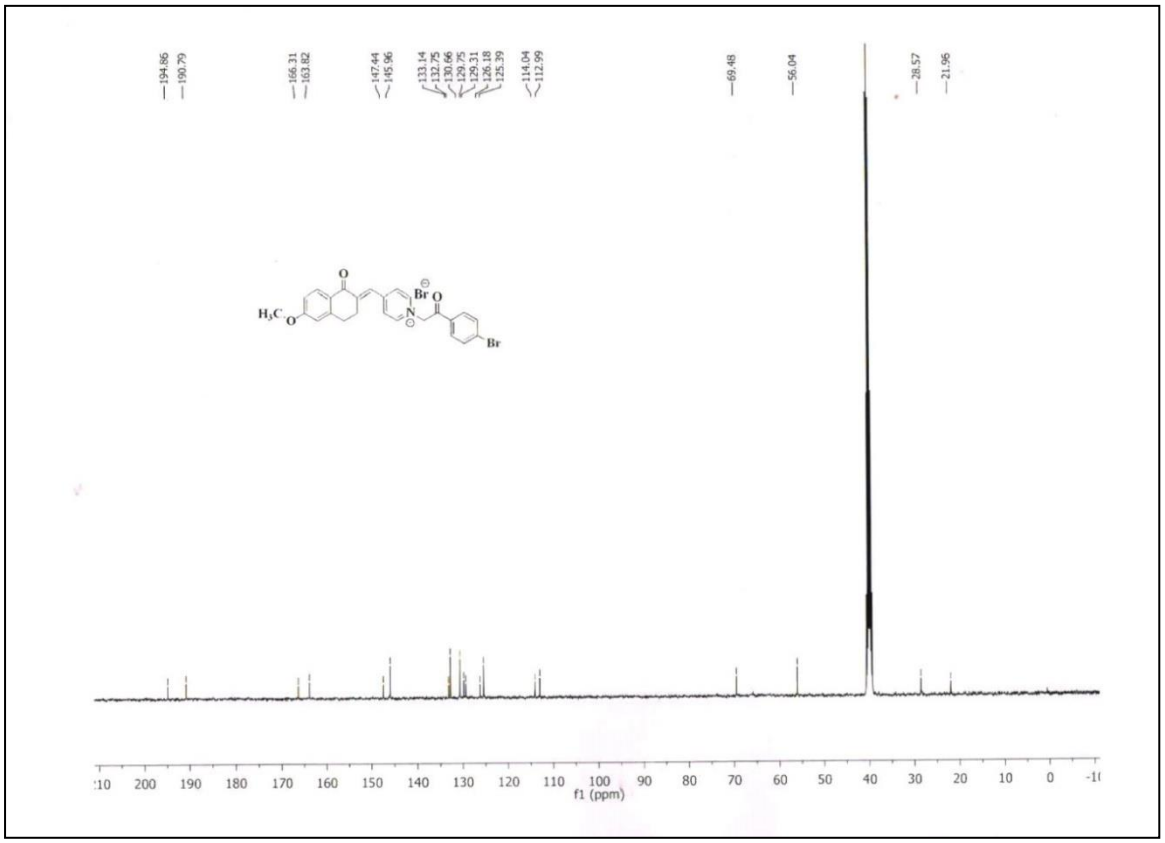
^{13}C NMR (100 MHz, DMSO-d_6) spectrum of compound 5f



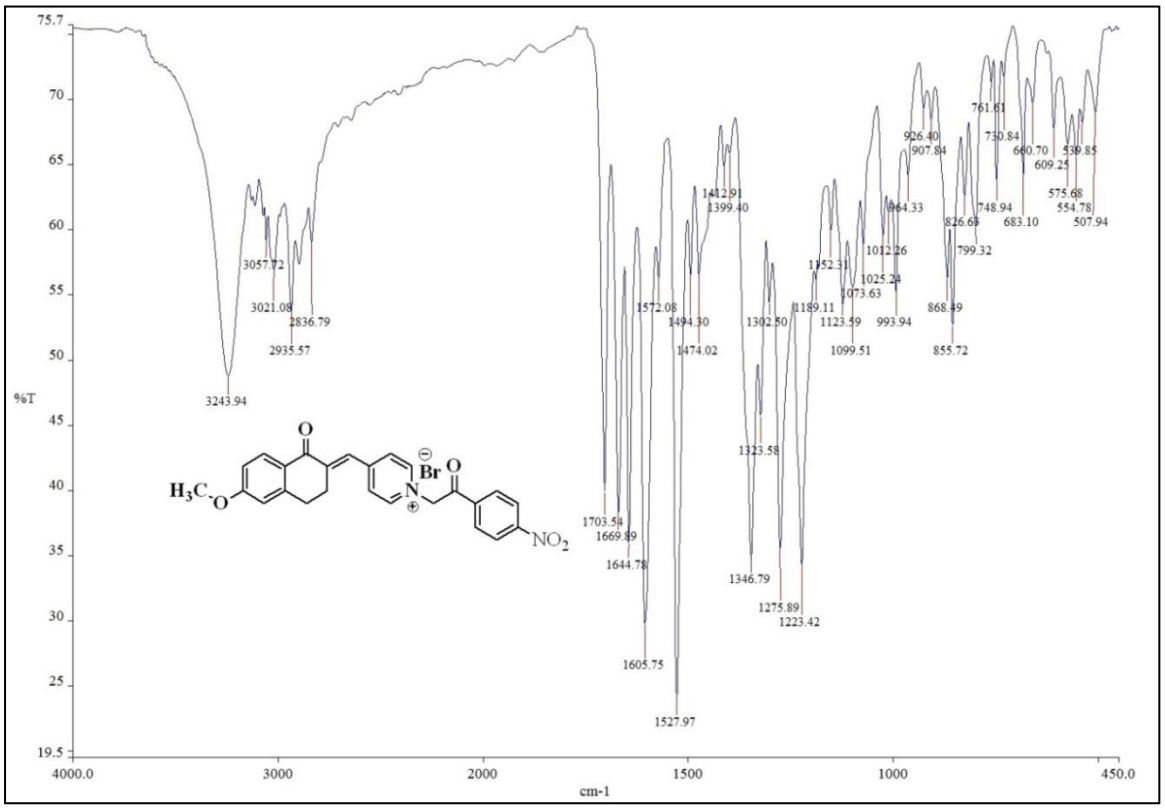
IR (KBr) spectrum of compound 5g



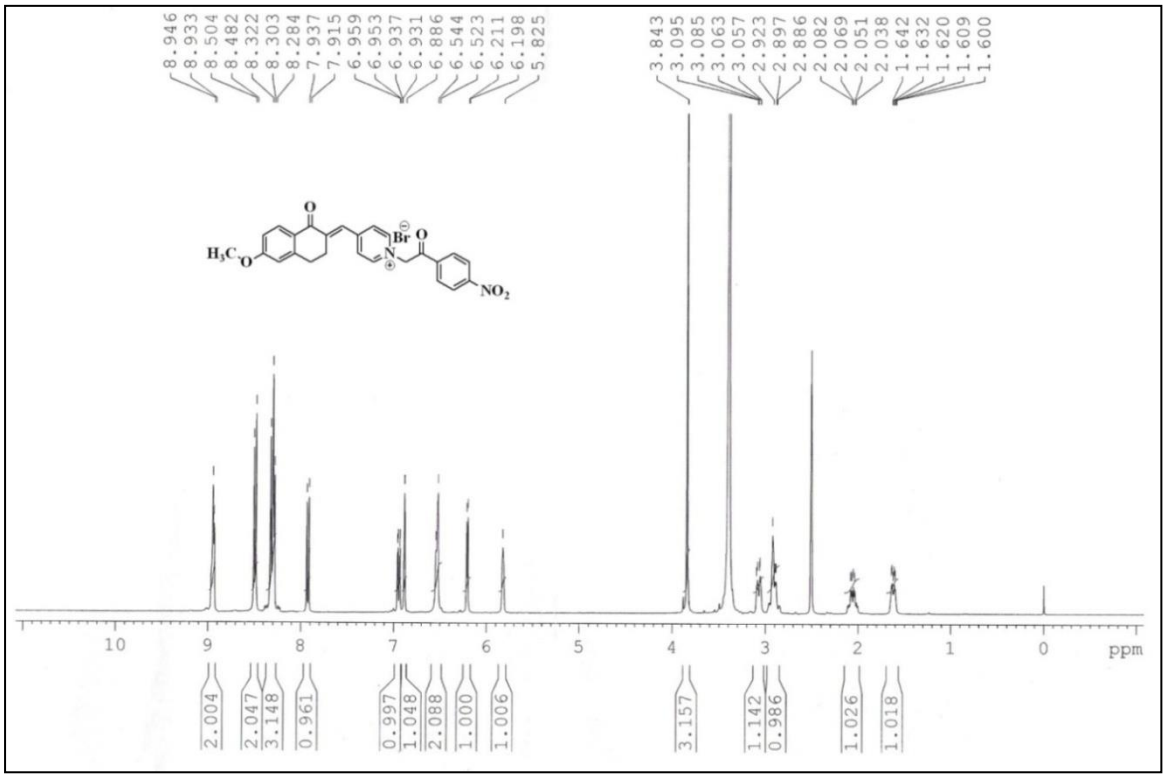
^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound 5g



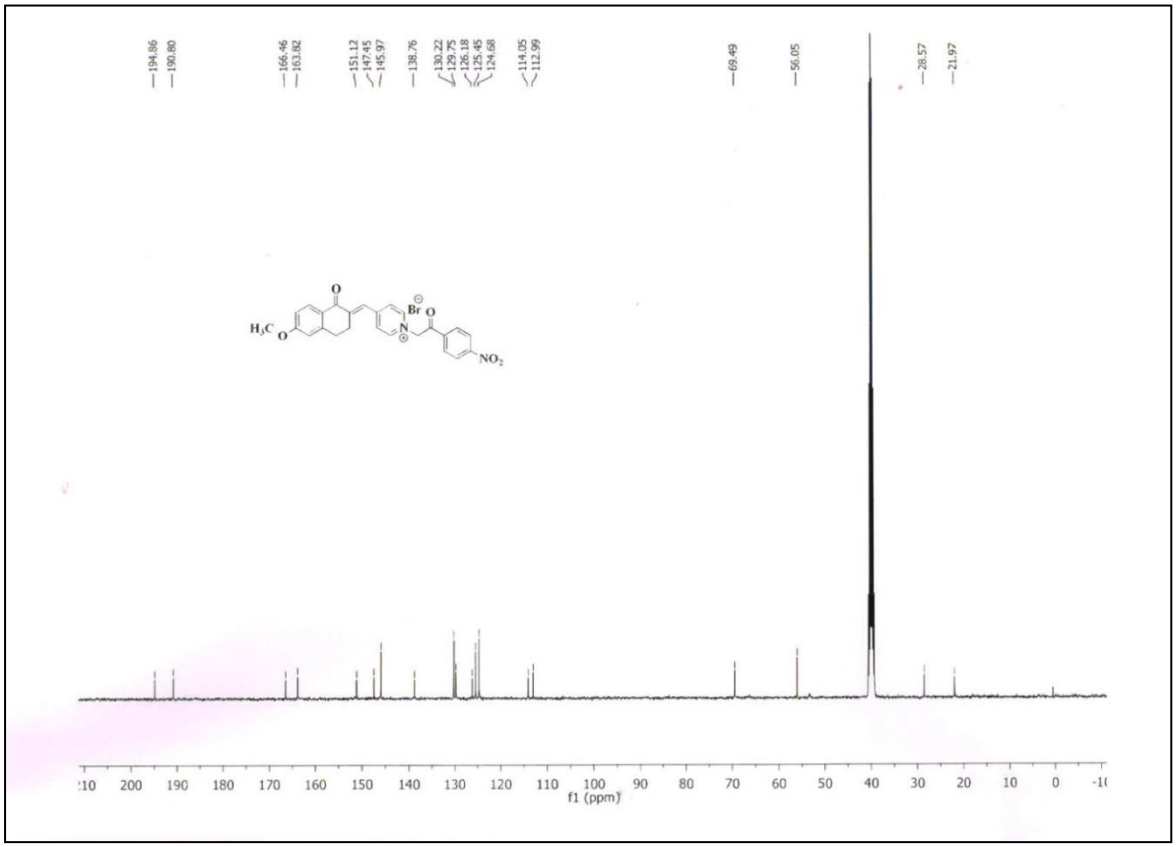
^{13}C NMR (100 MHz, DMSO-d_6) spectrum of compound 5g



IR (KBr) spectrum of compound 5h



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



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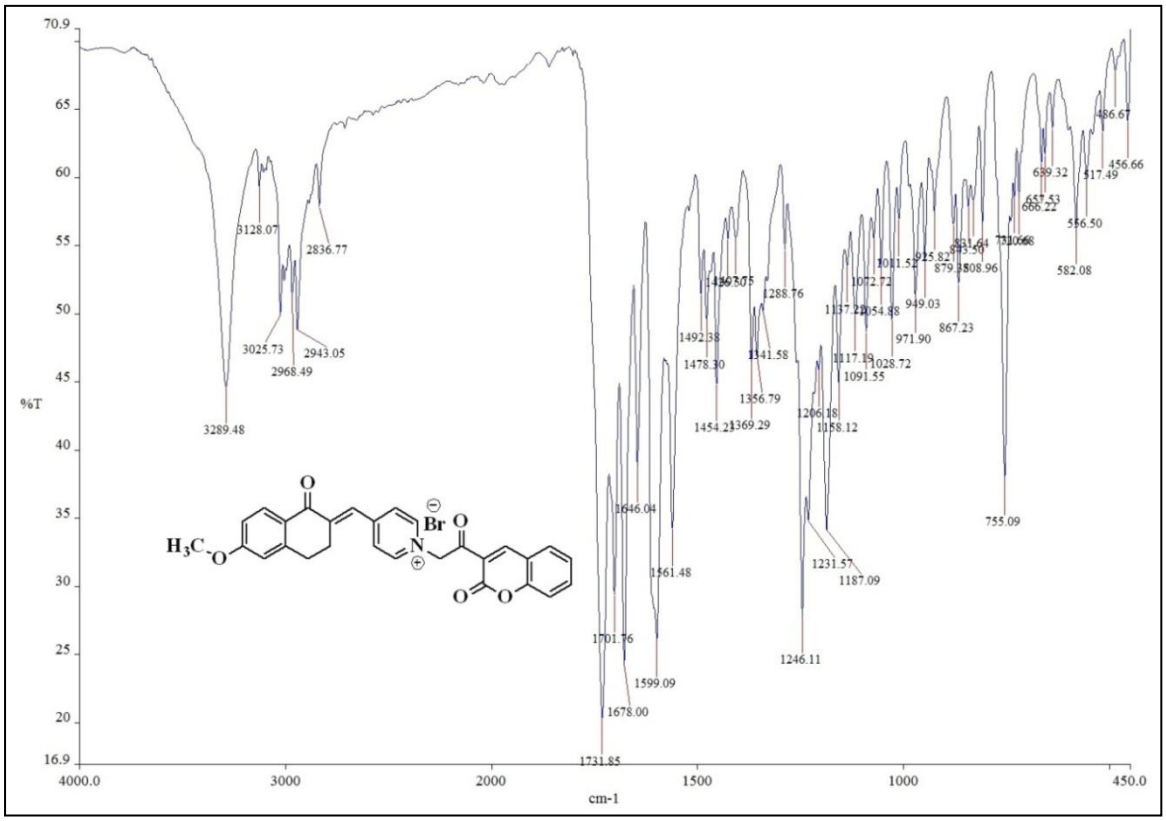
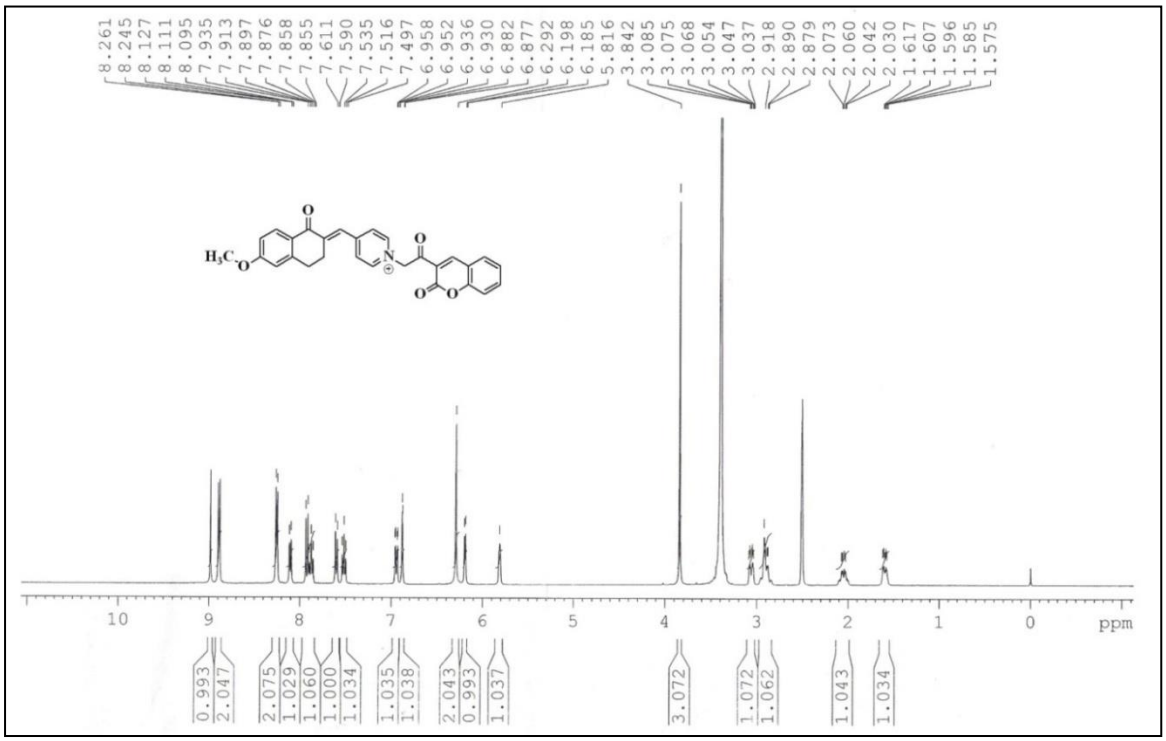
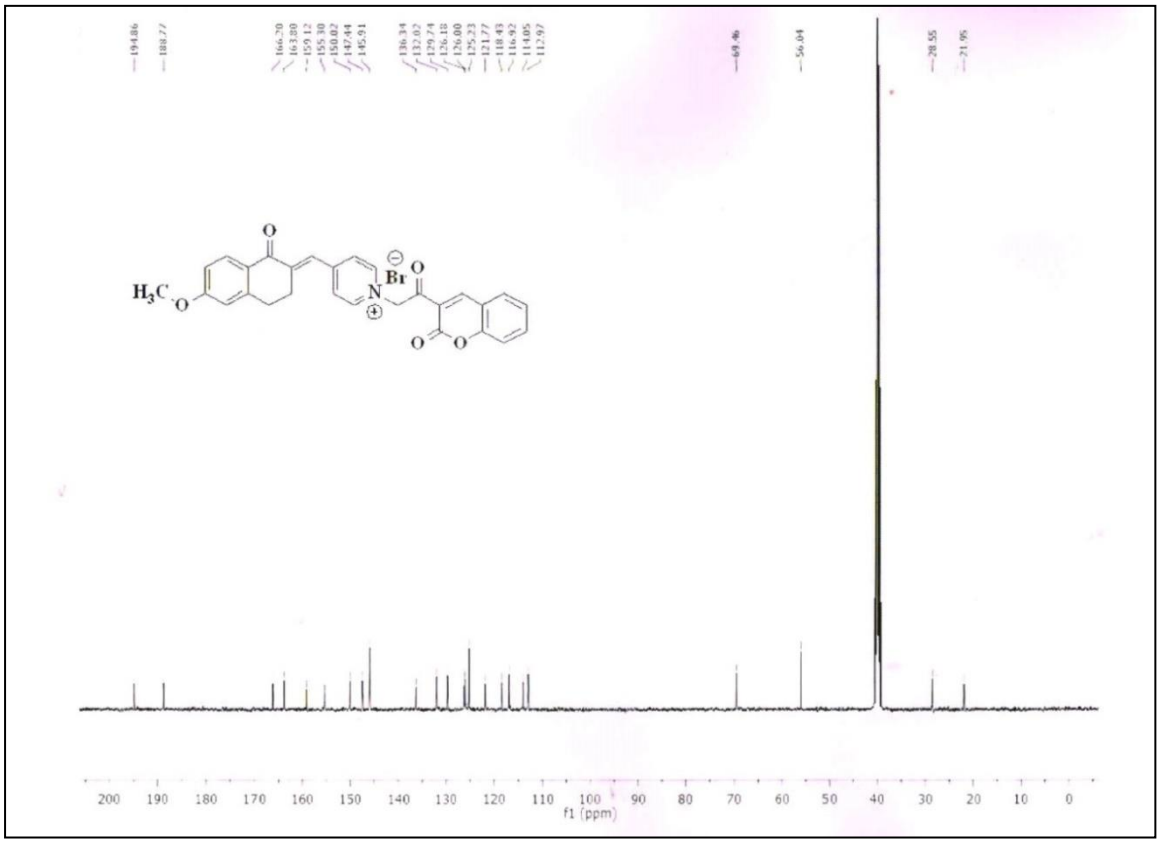


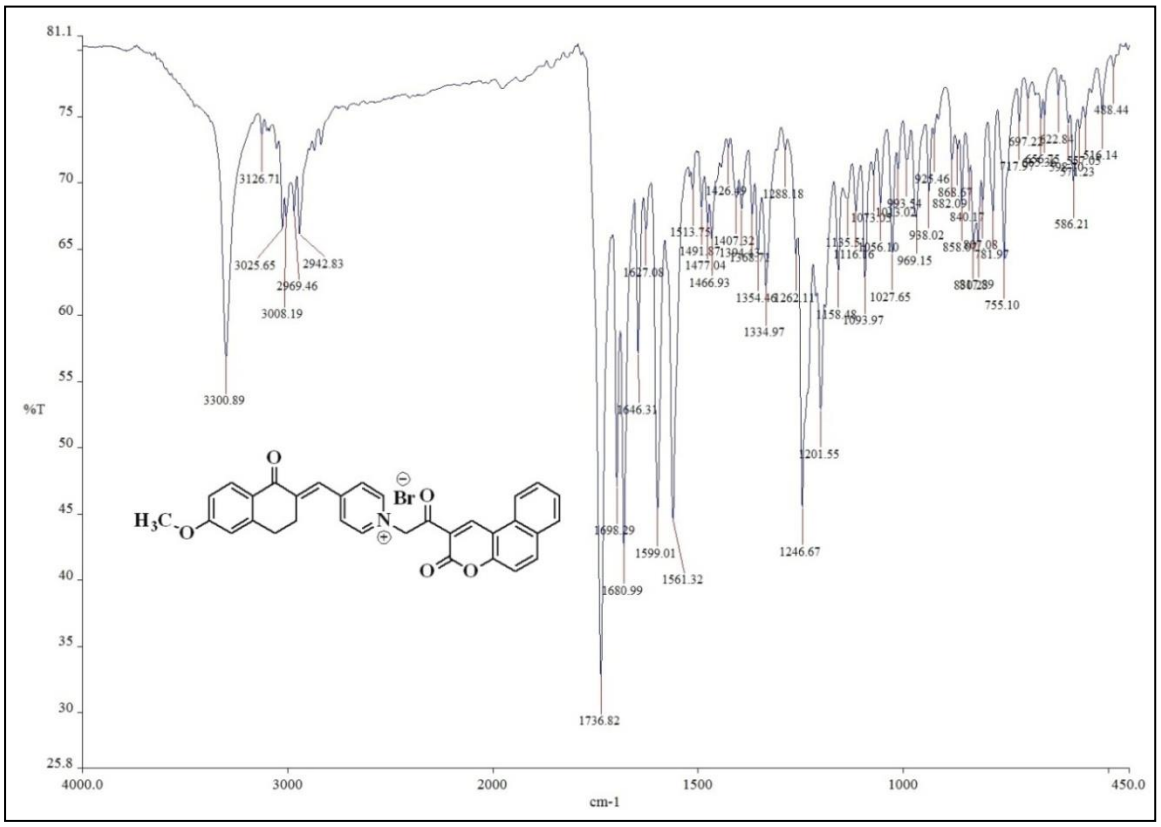
IR (KBr) spectrum of compound 5i



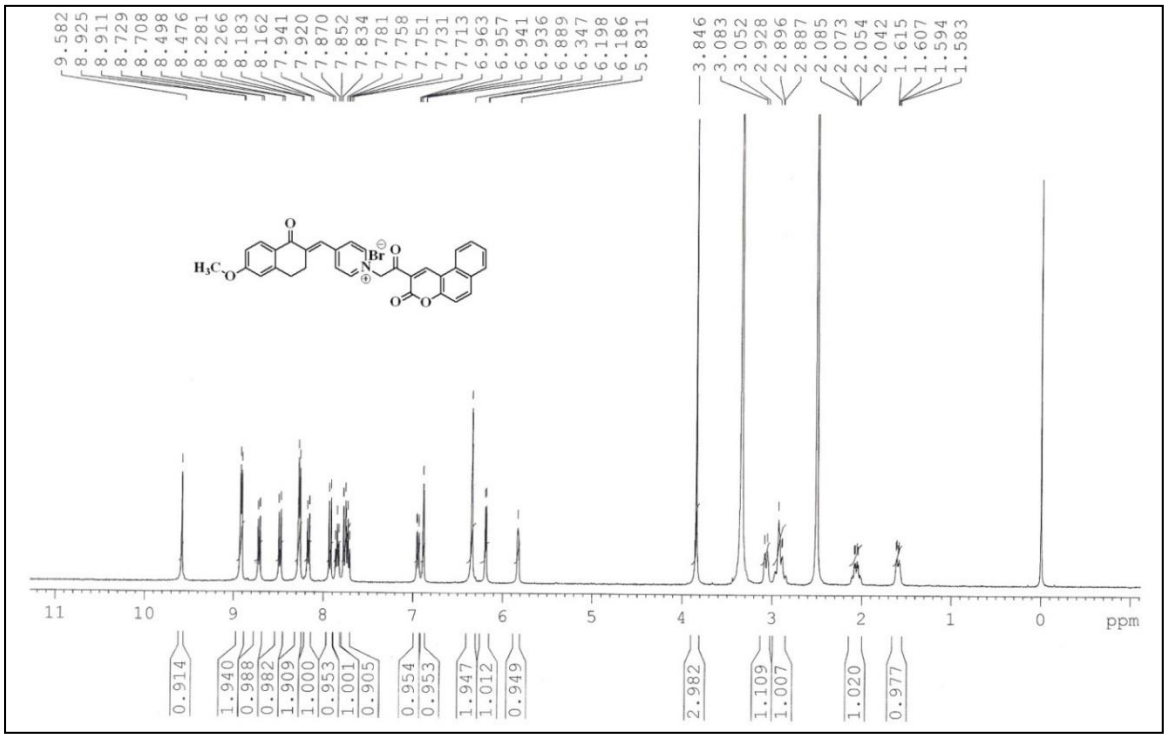
^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound 5i



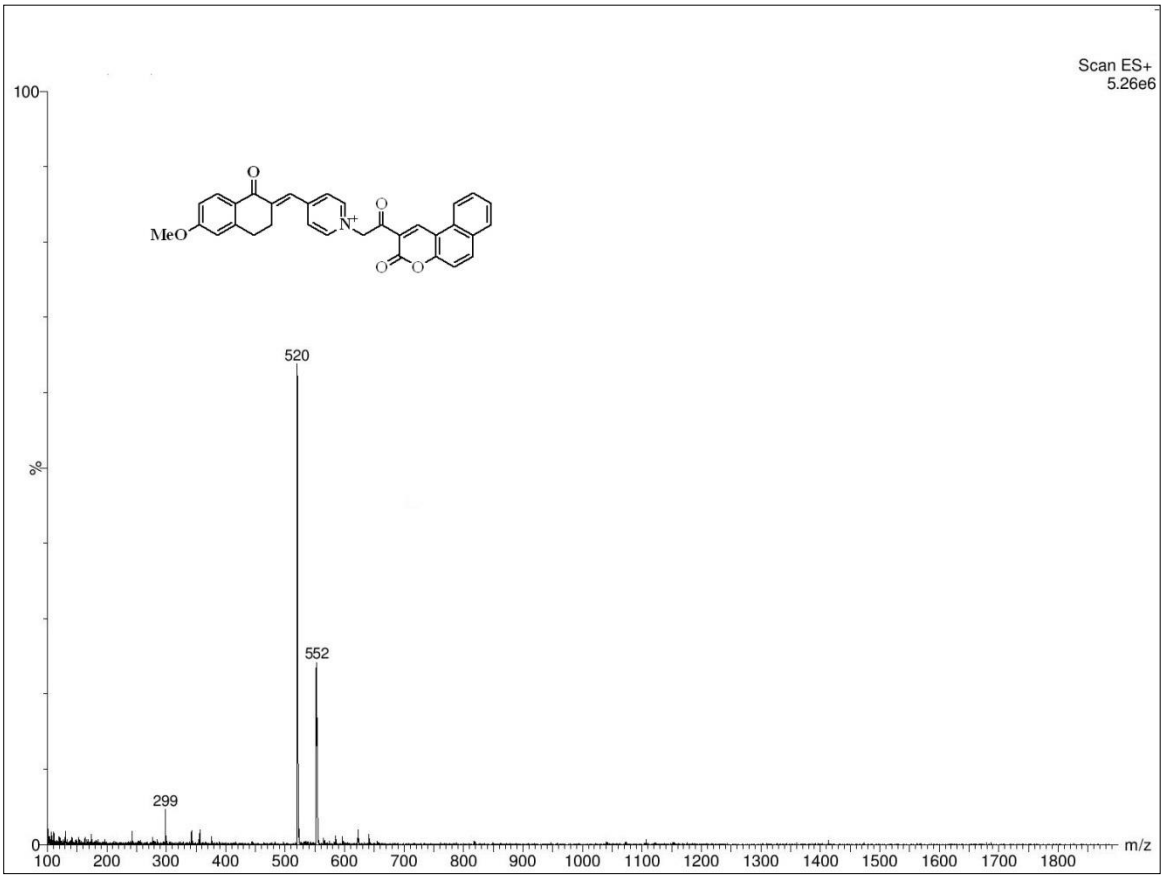
^{13}C NMR (100 MHz, DMSO-d_6) spectrum of compound 5i



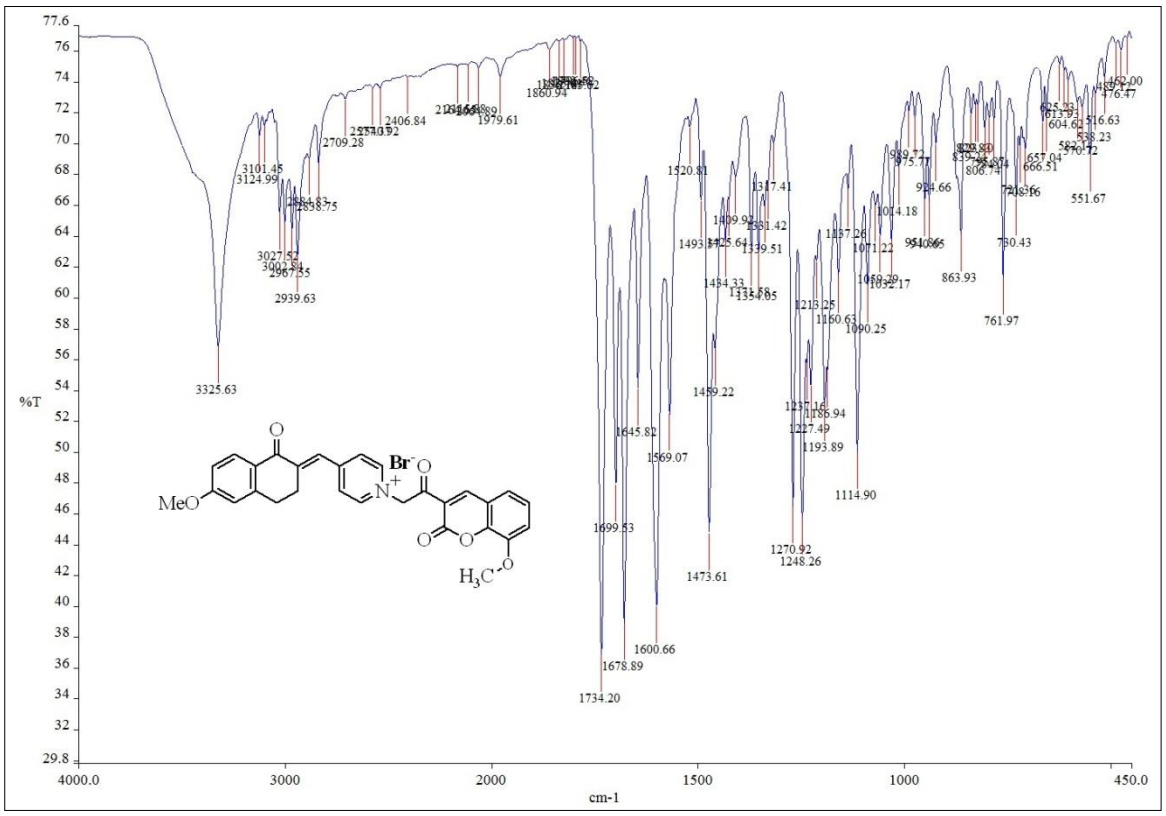
IR (KBr) spectrum of compound 5j



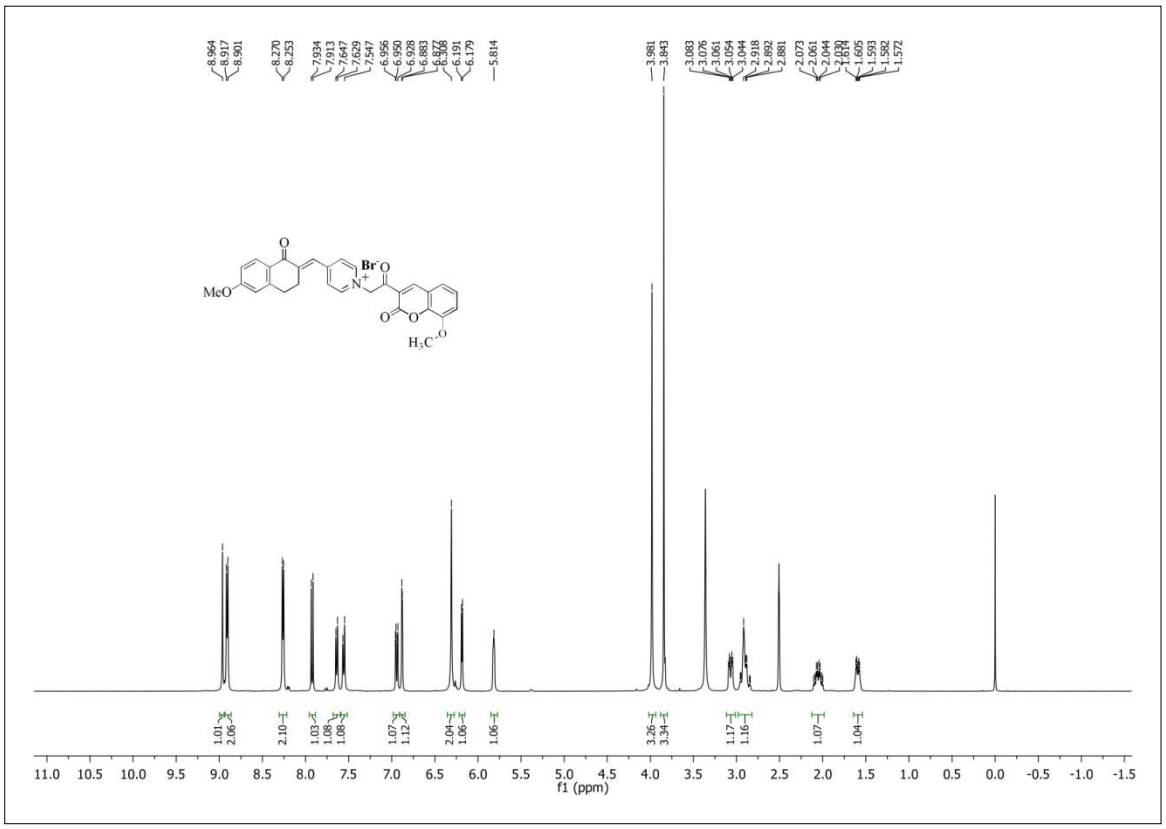
^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound 5j



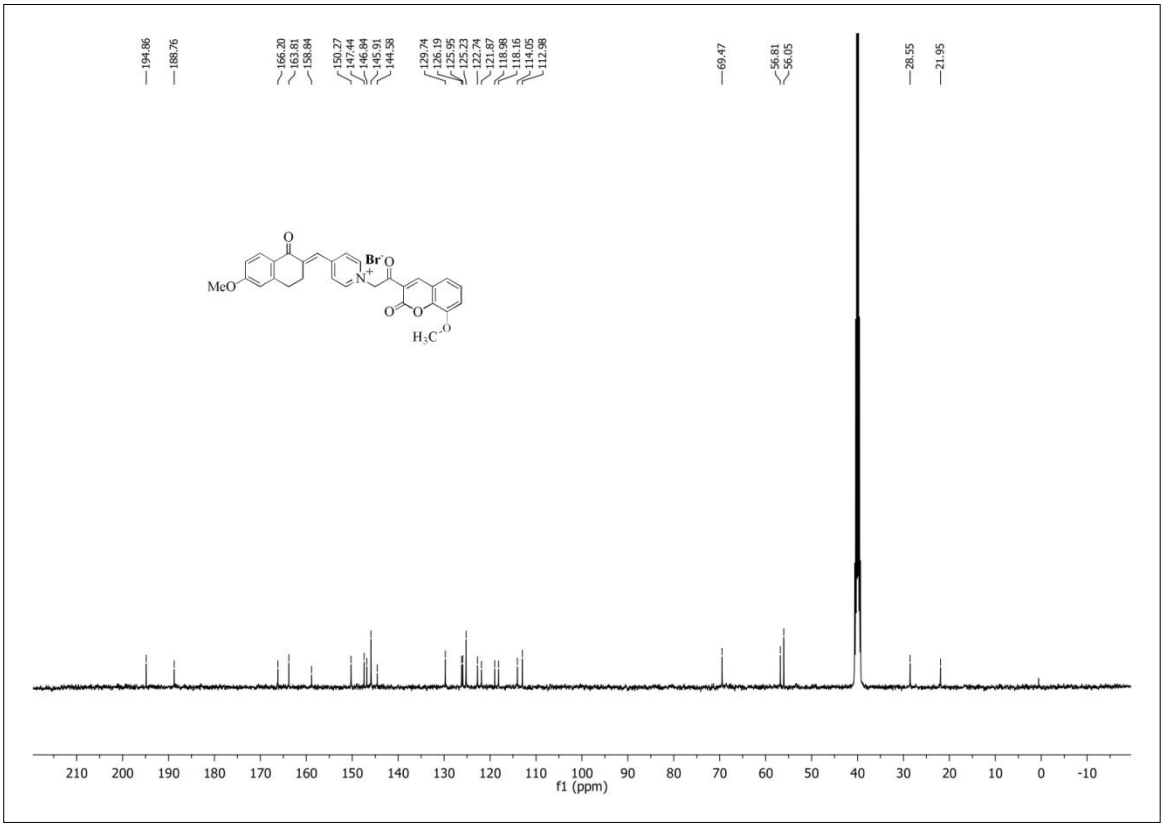
Mass spectrum of compound 5j (M.Wt: 502)



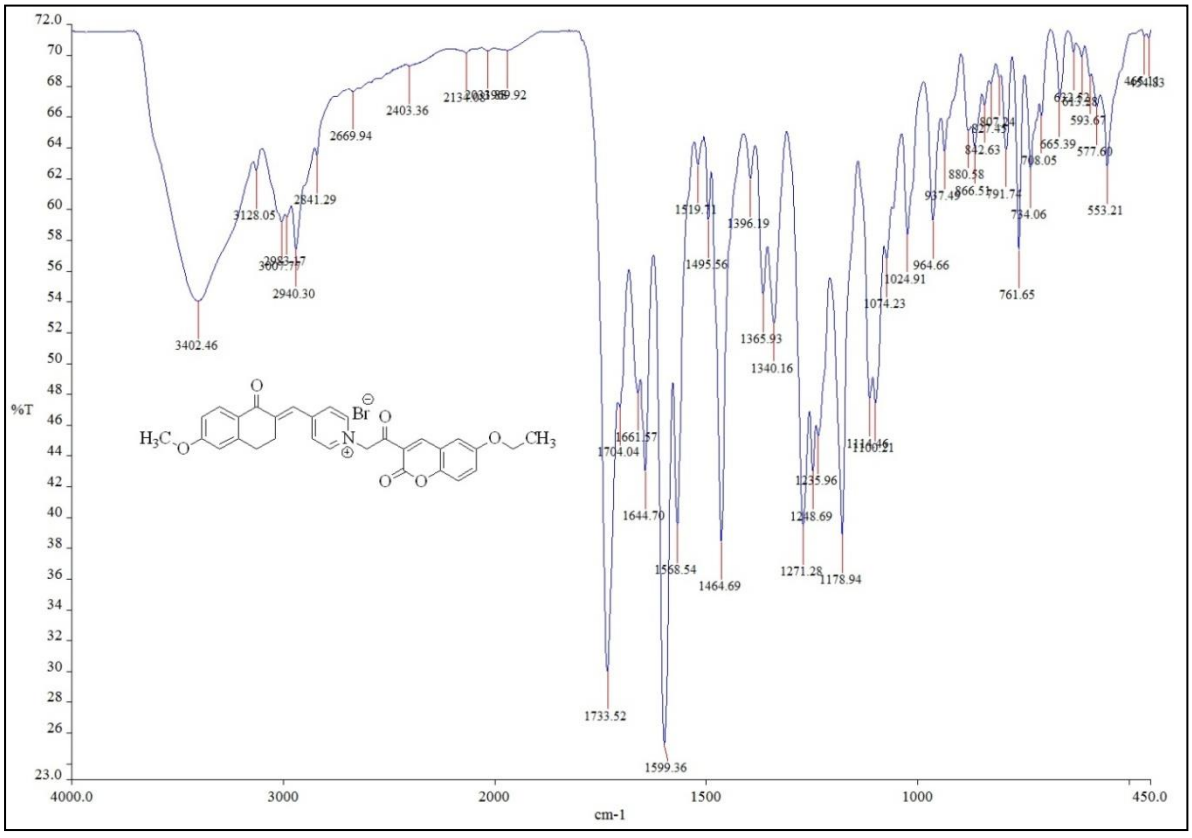
IR (KBr) spectrum of compound 5k



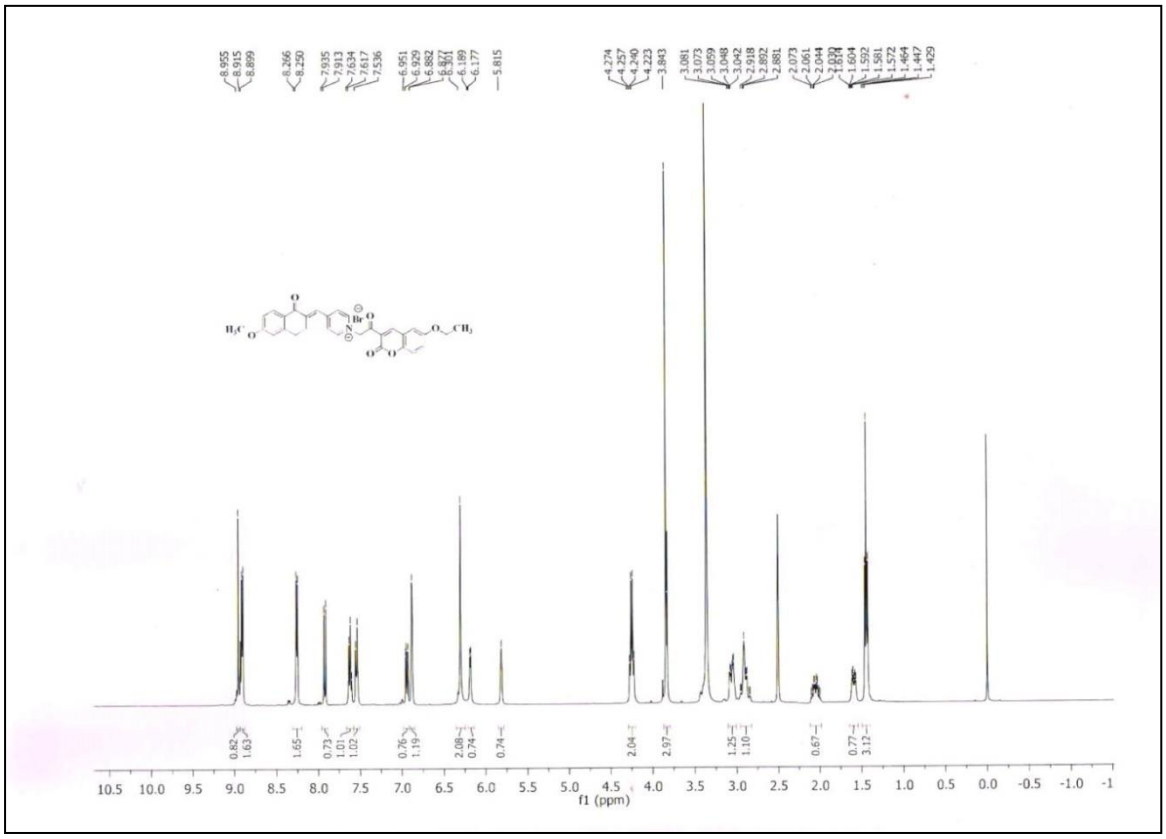
^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound 5k



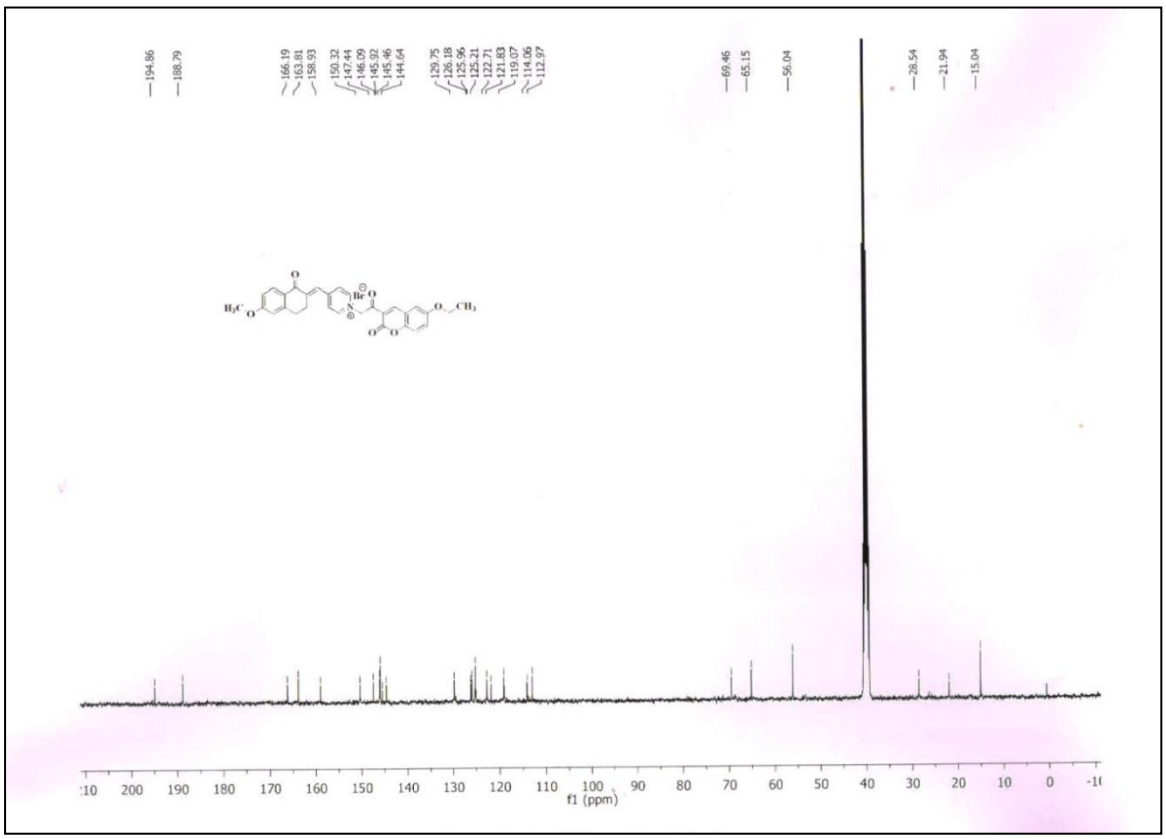
^{13}C NMR (100 MHz, DMSO-d_6) spectrum of compound 5k



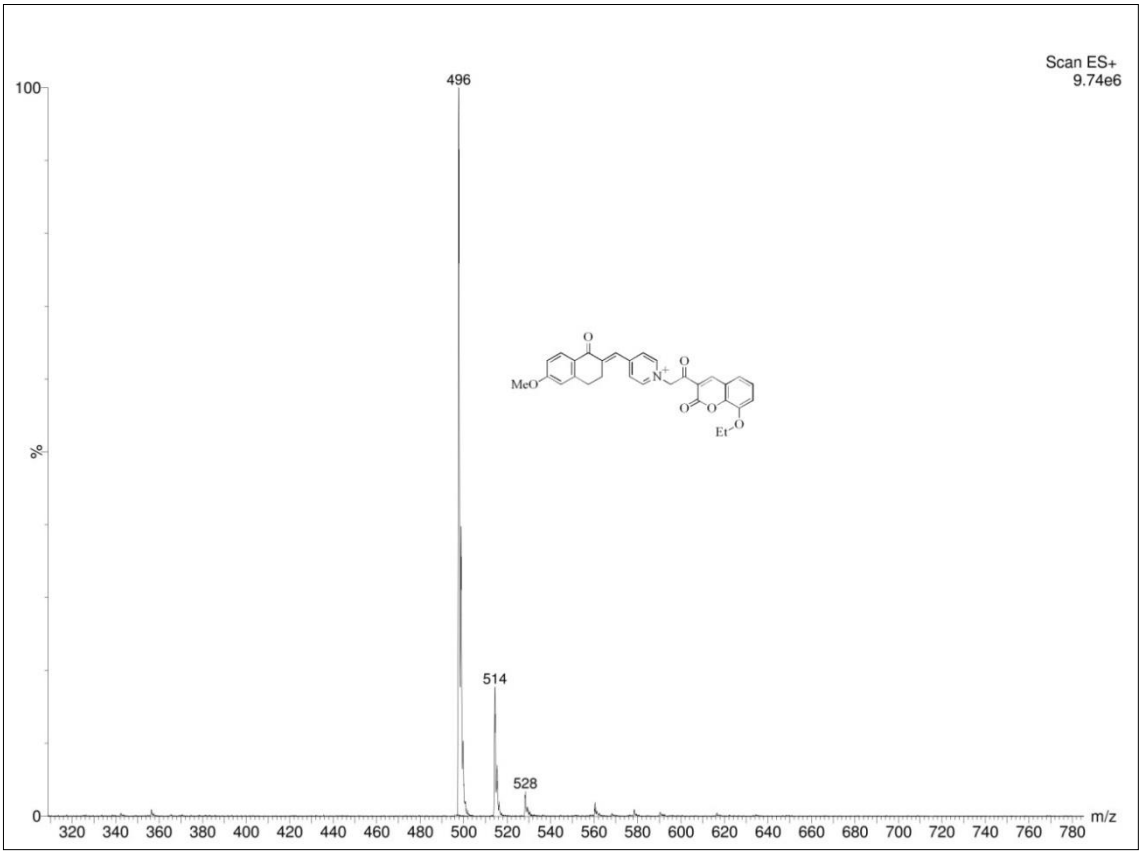
IR (KBr) spectrum of compound 5l



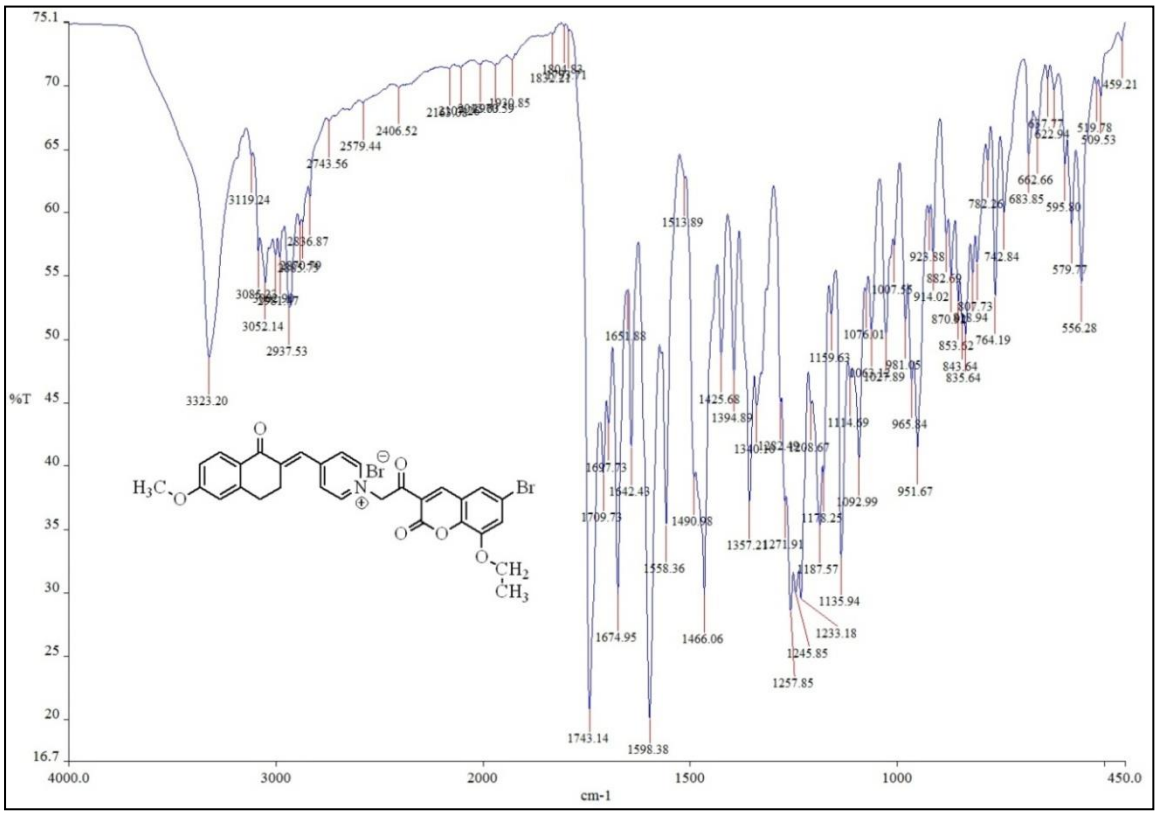
^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound 5l



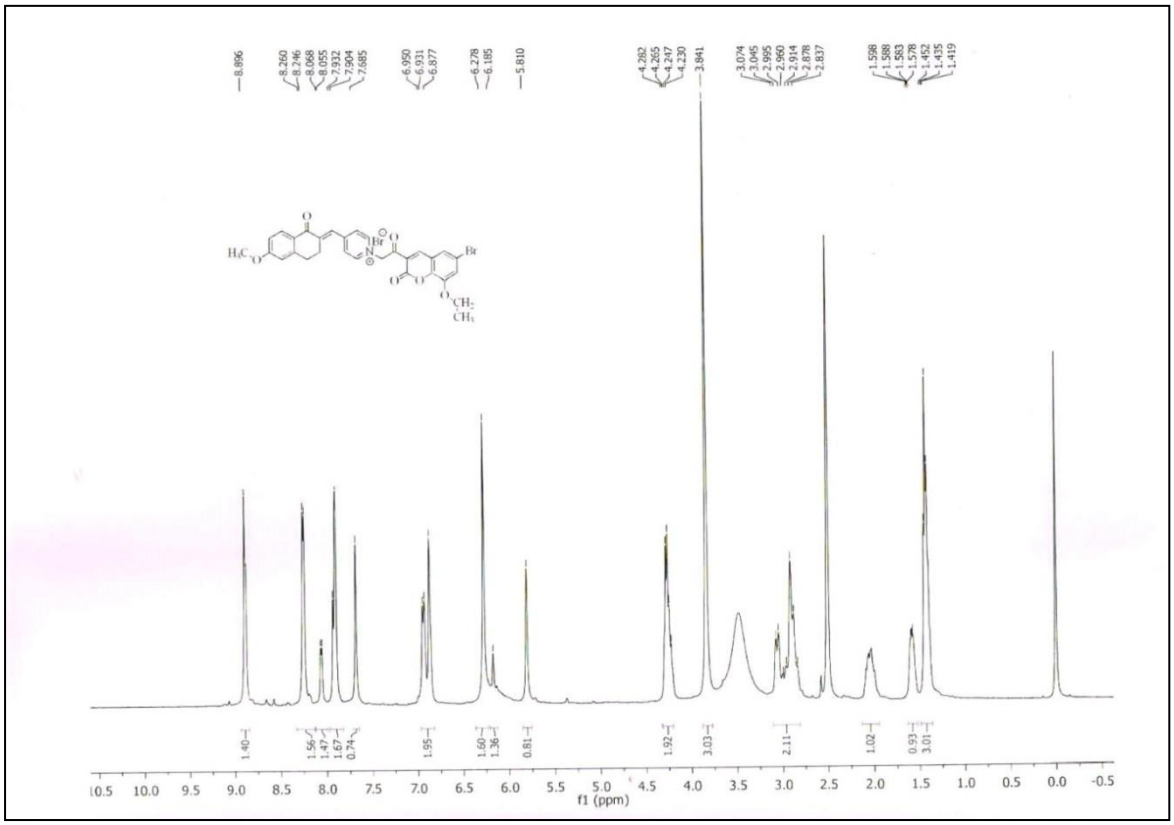
^{13}C NMR (100 MHz, DMSO-d_6) spectrum of compound 5l



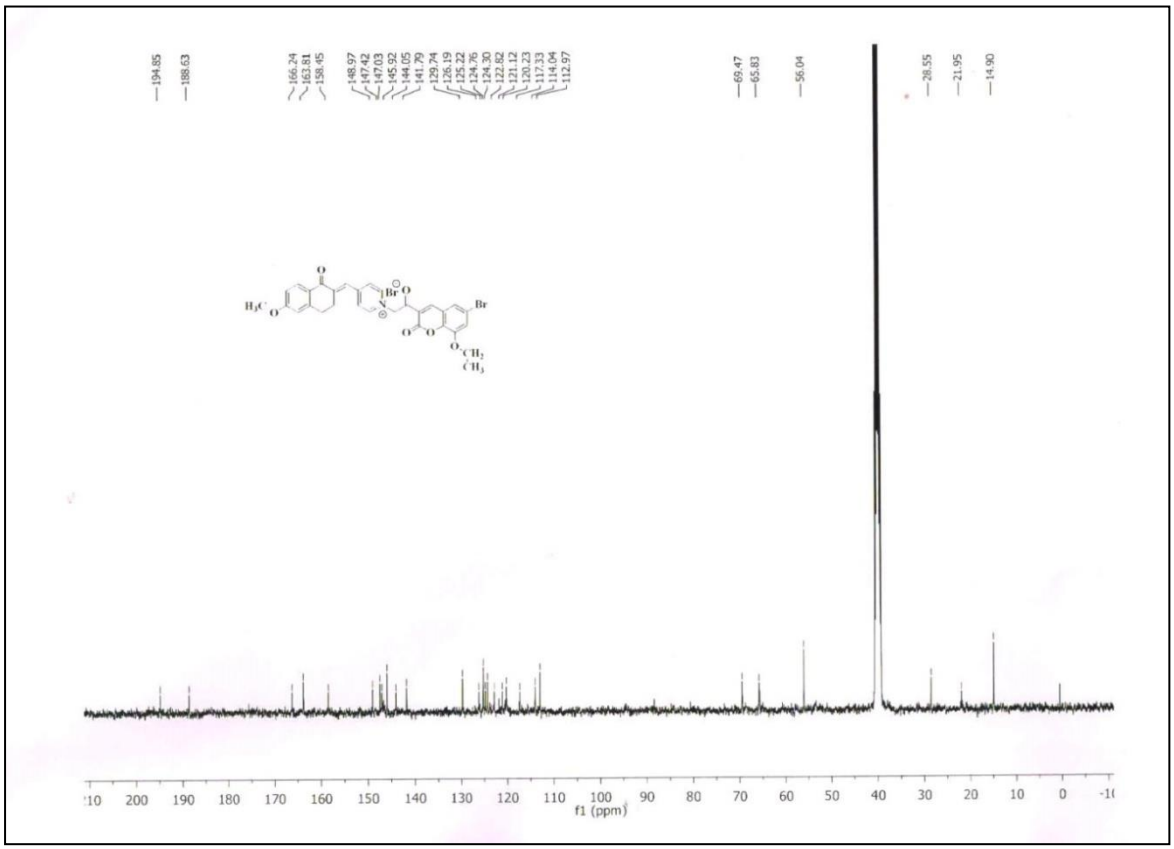
Mass spectrum of compound 5l (M.Wt: 496)



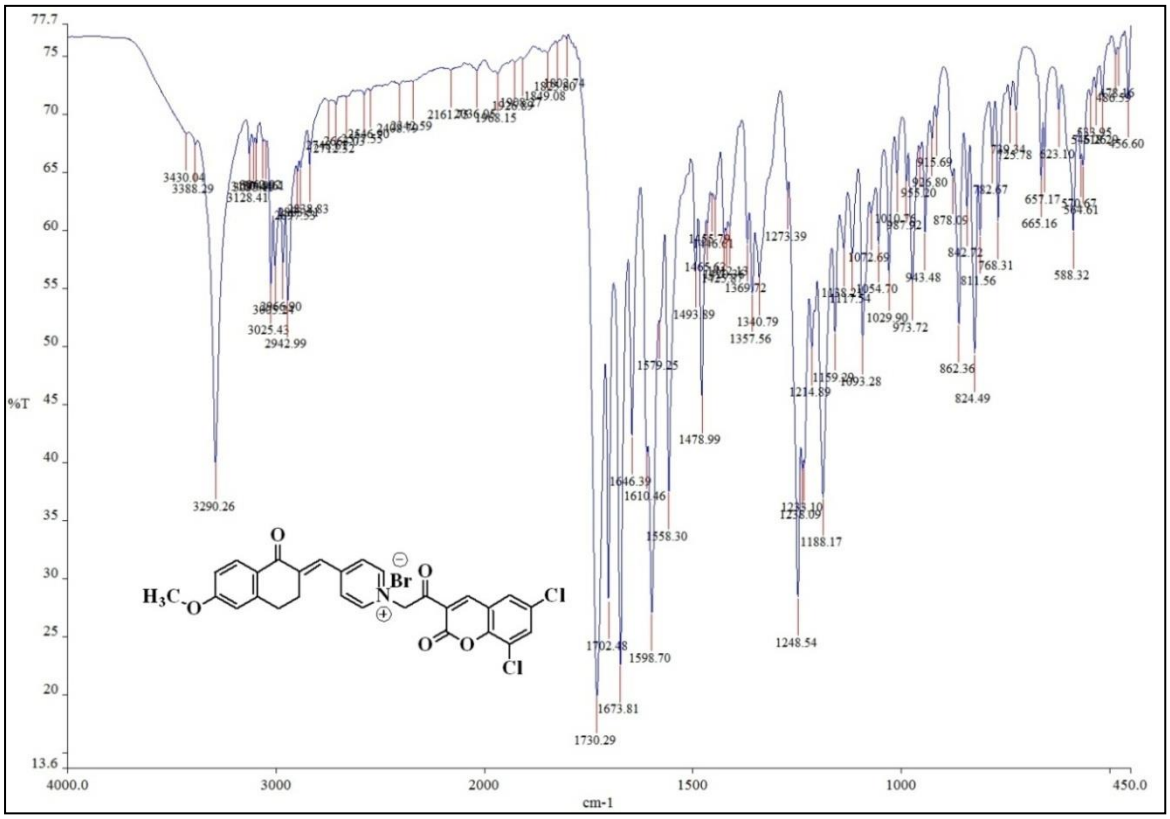
IR (KBr) spectrum of compound 5m



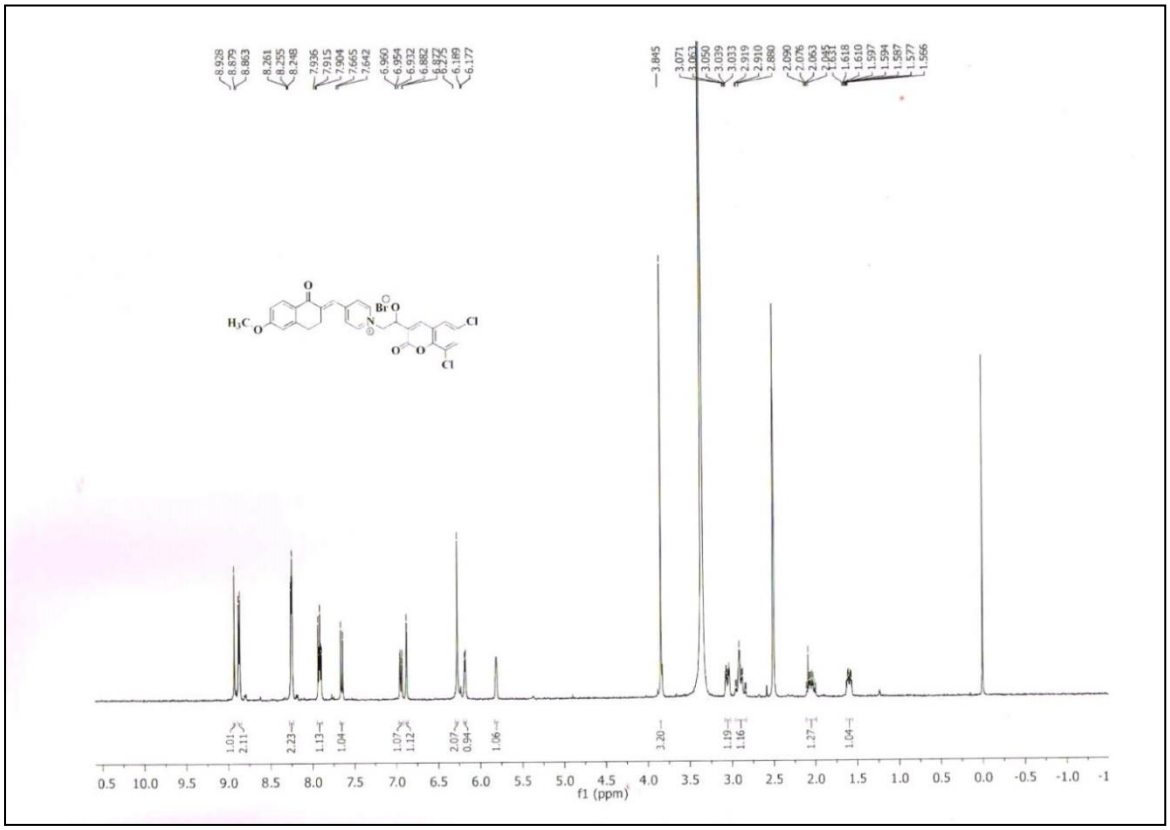
^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound 5m



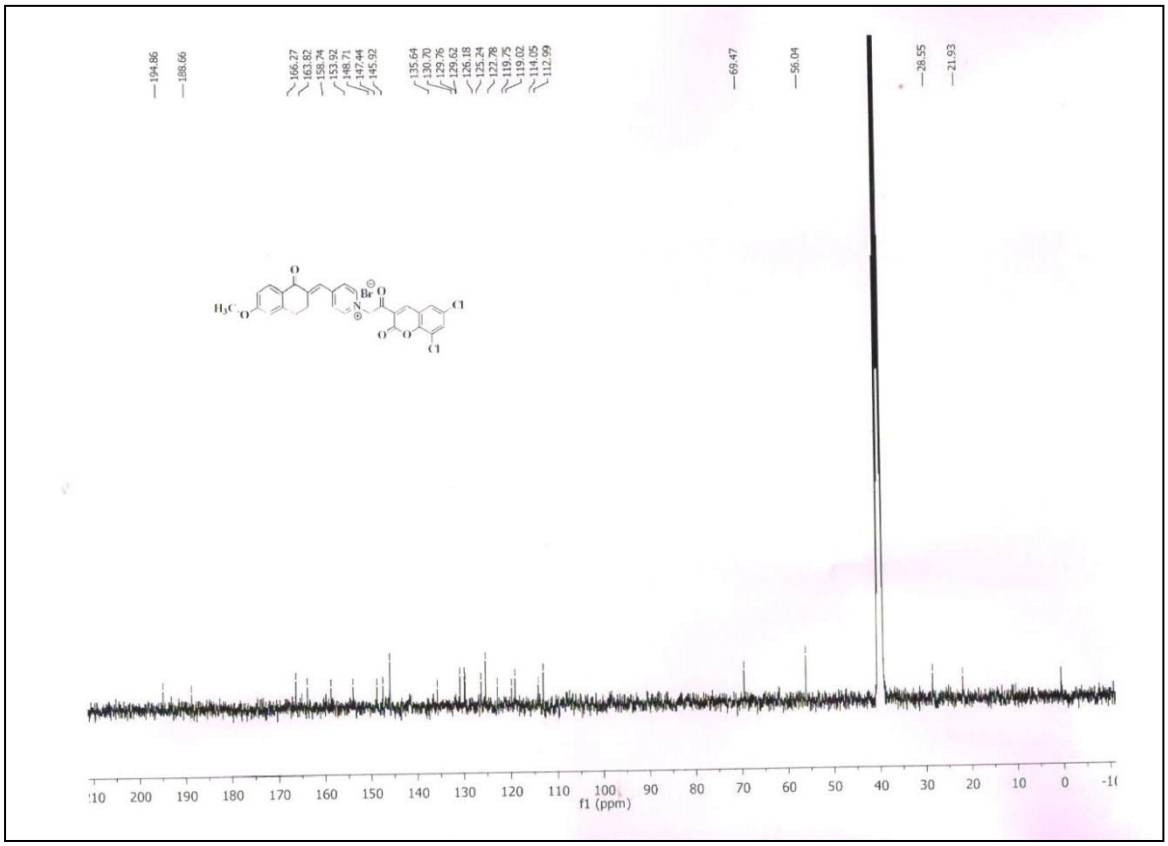
^{13}C NMR (100 MHz, DMSO-d_6) spectrum of compound 5m



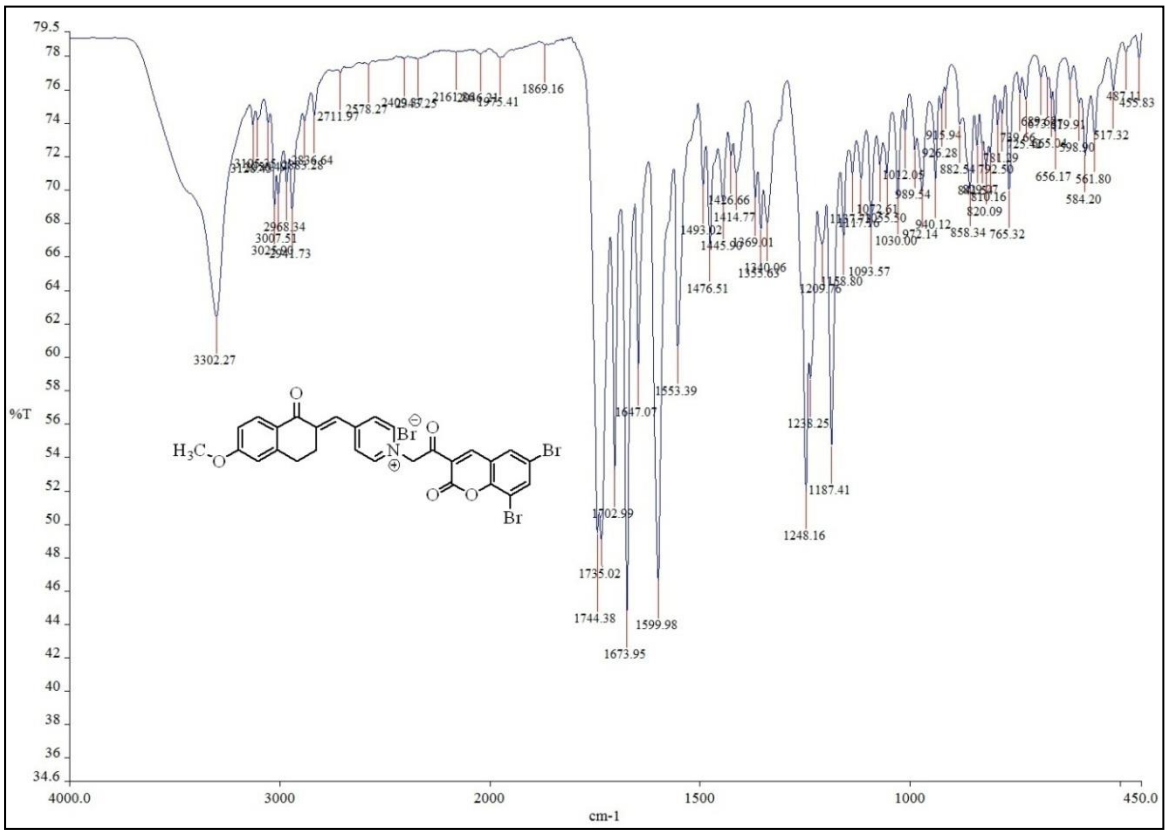
IR (KBr) spectrum of compound 5n



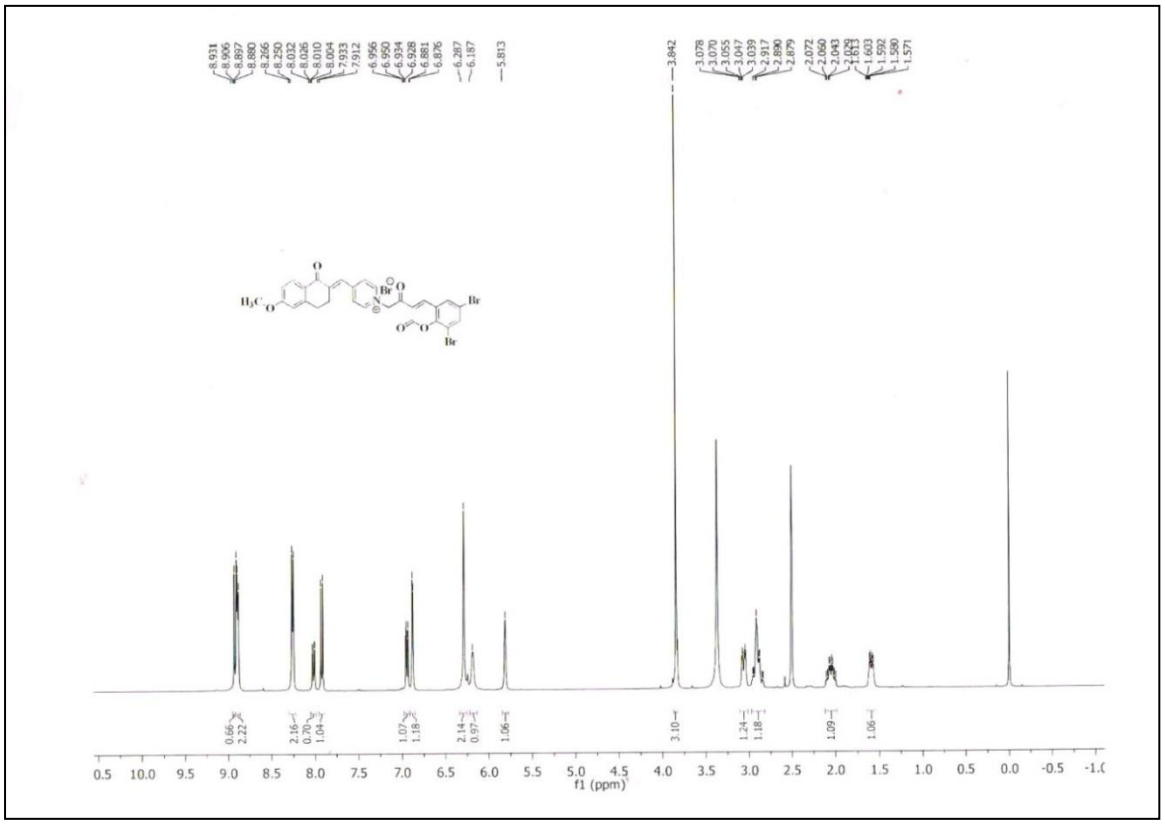
^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound 5n



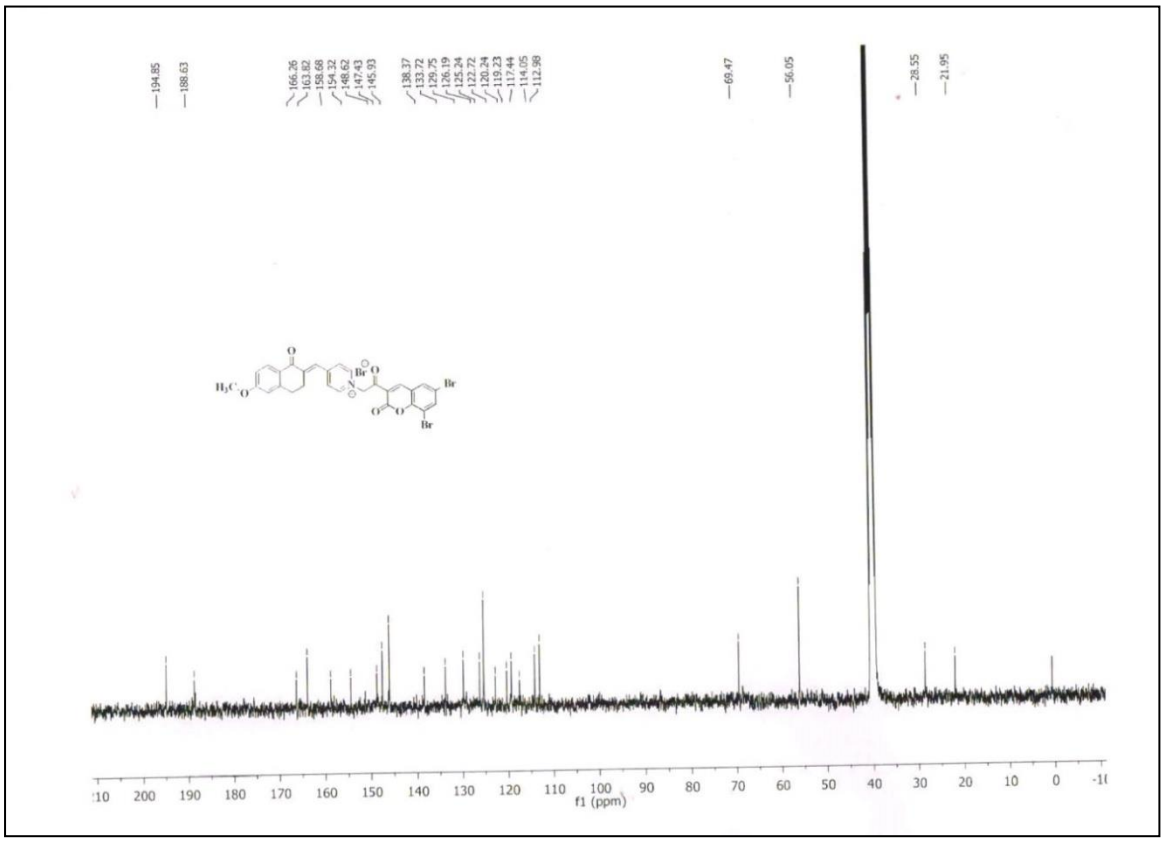
^{13}C NMR (100 MHz, DMSO-d_6) spectrum of compound 5n



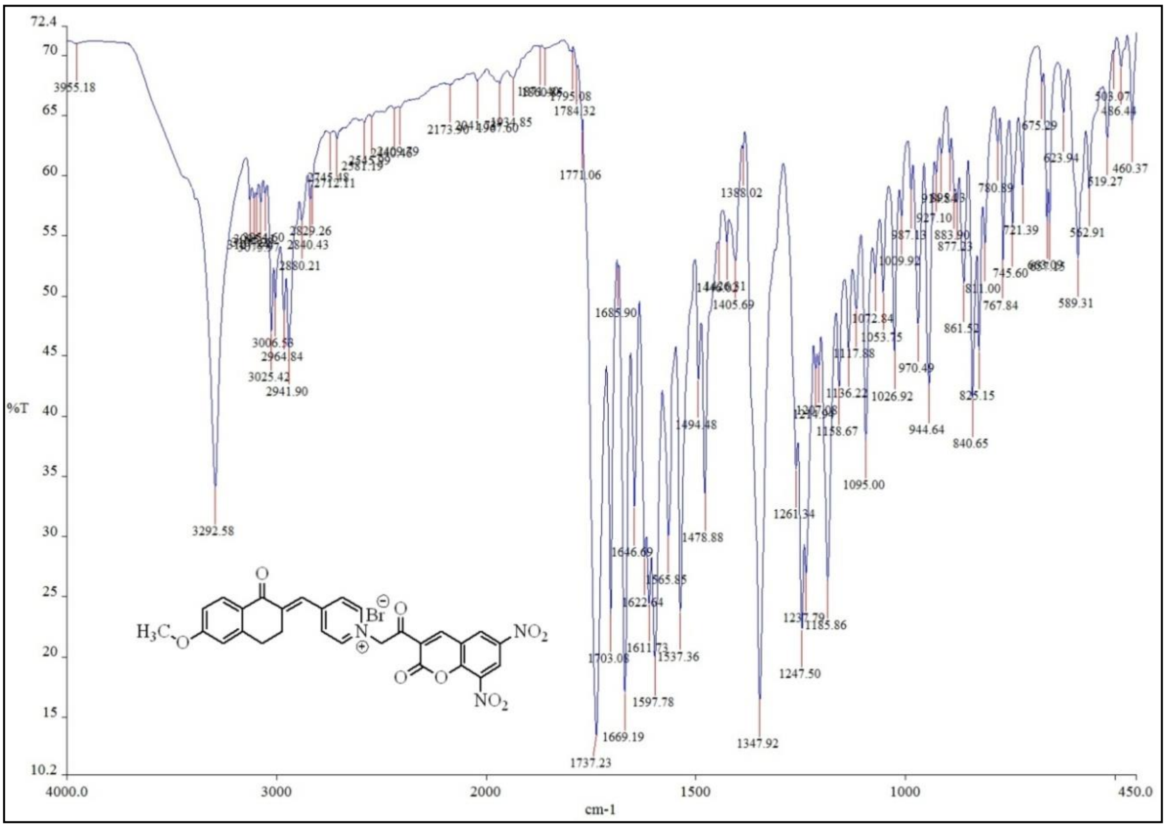
IR (KBr) spectrum of compound 5o



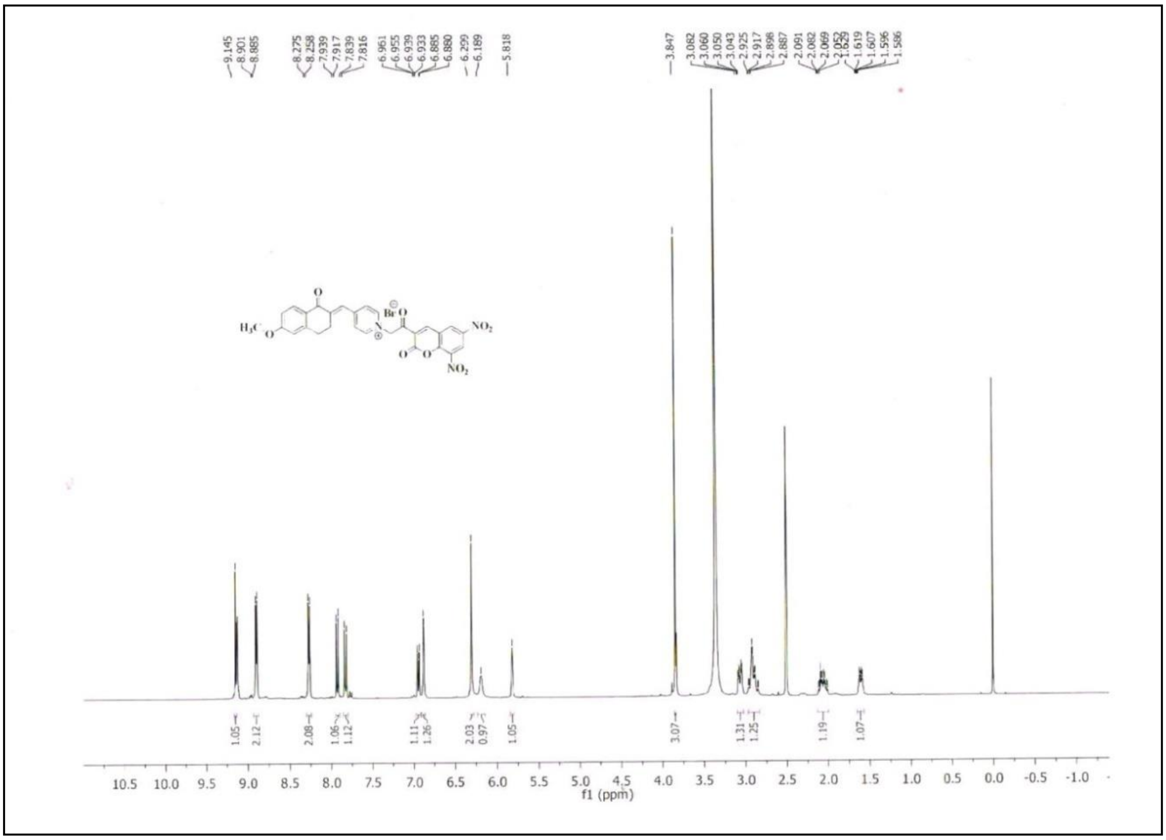
^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound 5o



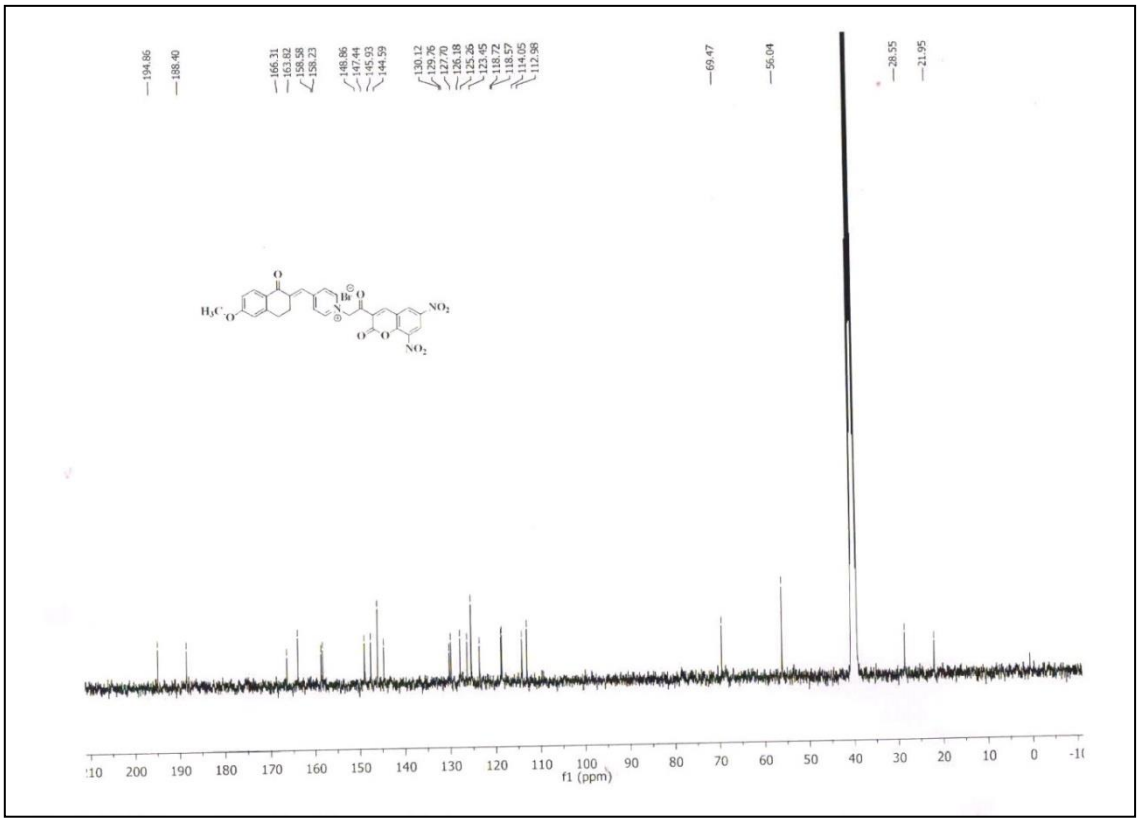
^{13}C NMR (100 MHz, DMSO-d_6) spectrum of compound 5o



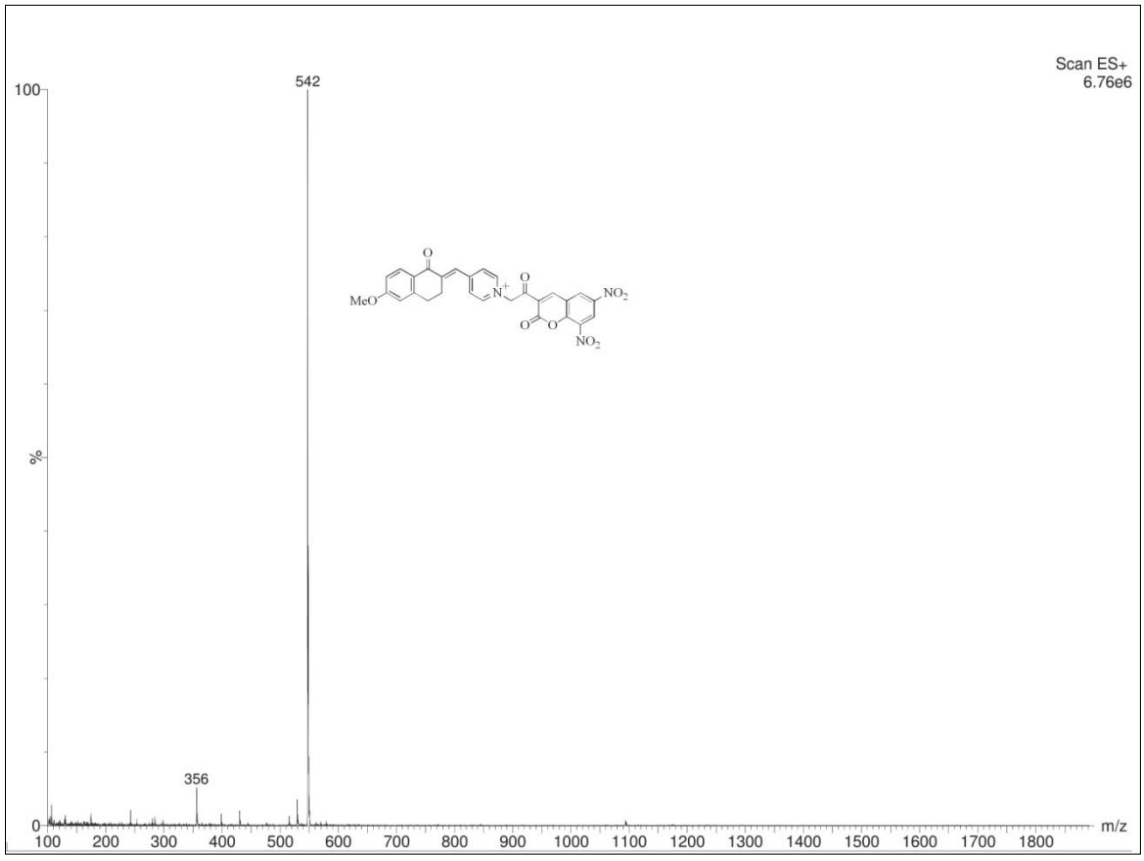
IR (KBr) spectrum of compound 5p



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



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



Mass spectrum of compound 5p (M.Wt: 542)

CHAPTER-VI (SECTION-A)

GREEN APPROACH: AN EFFICIENT SYNTHESIS OF 2,4-DISUBSTITUTED-1,3-THIAZOLES AND SELENAZOLES IN AQUEOUS MEDIUM UNDER ULTRASONIC IRRADIATION

INTRODUCTION

Thiazole ring is a core structural backbone of various natural and synthetic pharmacologically active compounds such as Vitamin B_1 (thiamine), Penicillin, Meloxicam **1**, Fanetizole **2**, Cefdinir **3** and Selenazofurin **4**. Thiazole derivatives have diverse applications in various fields of chemistry including medicinal and agricultural, such as anticancer,[1] antitumor,[2] antimalarial,[3] anti-inflammatory,[4] antimicrobial,[5] antihypertensive,[6] antiparasitic,[7] HIV-1 reverse transcriptase inhibitors[8] and herbicidal properties.[9] They were also reported as ligands at estrogen receptors[10] and novel class adenosine receptor antagonists,[11] and used as organic functional materials such as fluorescent dyes[12] and liquid crystals,[13] and also acts as good pharmacophores for the design of bioactive molecules as bioisoster of the imidazole ring.[14] Similarly, selenazoles were also reported to possess antibacterial,[15] antifilarial, antitumor,[16] superoxide anion scavenging [17] properties and acts as Akt, mitogen protein kinase activator,[18] and heavy metal detoxifying agents.[19]

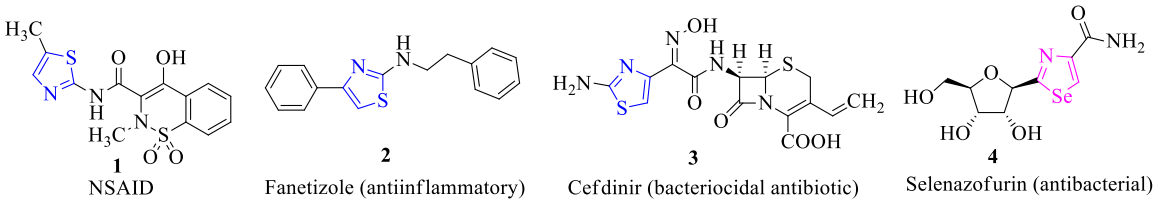


Fig. 1 Biologically active thiazole and selenazole derivatives

The element selenium (Se) is an essential antioxidant micronutrient with multiple roles in animals and humans in trace amounts, though it is toxic in high doses.[20] 1,3-selenazoles are also one of the important scaffolds in heterocyclic chemistry.[21] Particularly, functionalized 1,3-selenazole moieties have become hot spots and attained great attention by the medicinal chemists, as they found in many natural and synthetic pharmacologically active substances.[22]

Mirjana Popsavin and co-workers[23] reported the synthesis and *in vitro* antitumour activity of tiazofurin analogues with nitrogen functionalities at the C-2*'* position. Here they have synthesized six tiazofurin mimics (**5-10**) bearing the nitrogen functionalities at the C-2*'* starting from monoacetone D-glucose position in multistep method.

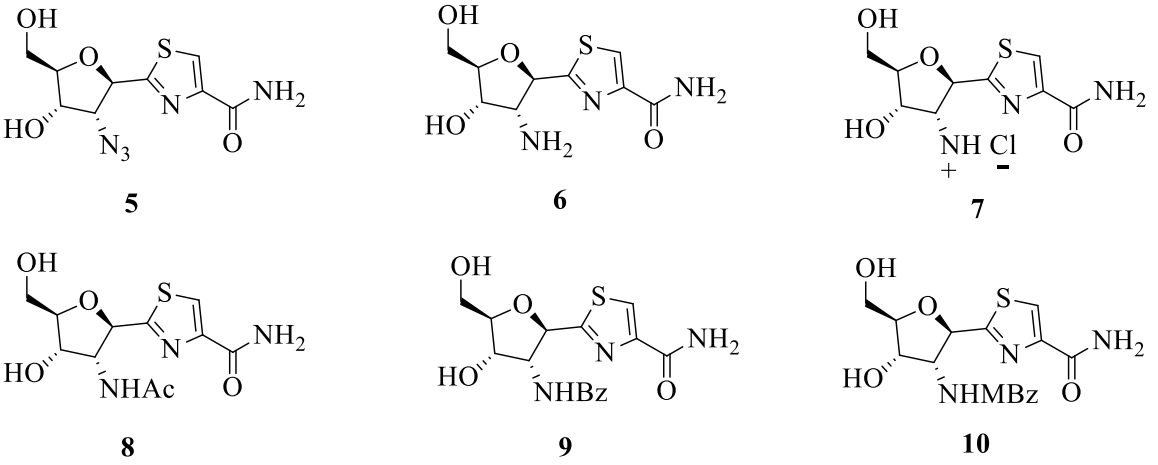


Fig.2

Zheng Li *et al.*[24] reported Design, synthesis and structure-activity relationship studies of new thiazole-based free fatty acid receptor 1 agonists for the treatment of type 2 diabetes. Among the synthesized compounds, the compound **11** found to be having potent free fatty acid receptor 1 (FFA1/GPR40) agonistic activity and produces a robustly hypoglycemic effect both in normal and type 2 diabetic mice, low risks of hypoglycaemia and liver toxicity even at the twice molar dose of TAK-875.

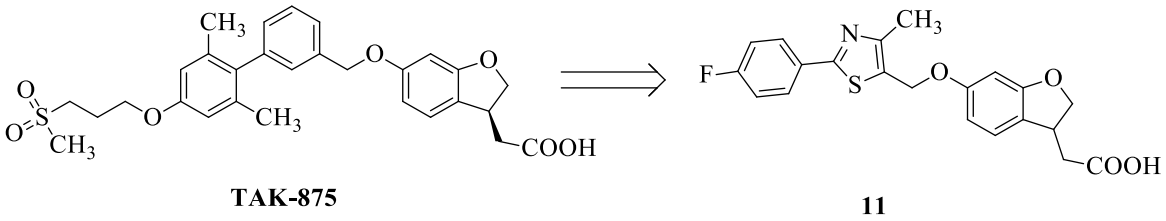
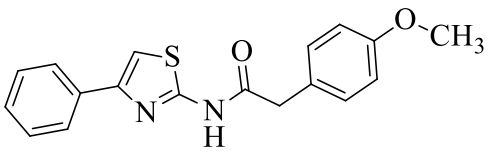


Fig. 3

Zhi-Qing Sun *et al.*[25] described Design and discovery of Novel Thiazole acetamide derivatives as anticholinesterase agent for possible role in the management of Alzheimer's. Among the synthesized compounds, **12** was identified as the most potent compound of acetyl-cholinesterase (AChE) (IC50= 3.14 ± 0.16lM) with a selectivity index (SI) of 2.94 against butyrylcholinesterase (BuChE).

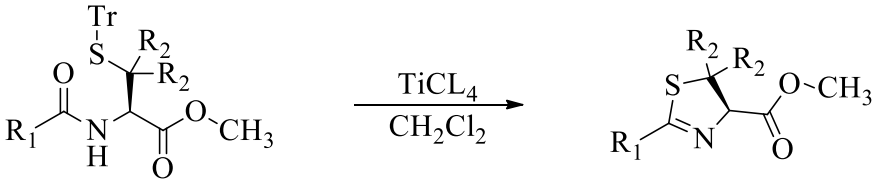


12

Fig. 4

Various important approaches for the synthesis of coumarin derivatives

Prakash Raman and co-workers[26] reported Titanium(IV)-mediated tandem deprotection-cyclodehydration of protected Cysteine *N*-Amides: Biomimetic syntheses of thiazoline-and thiazole-containing heterocycles.

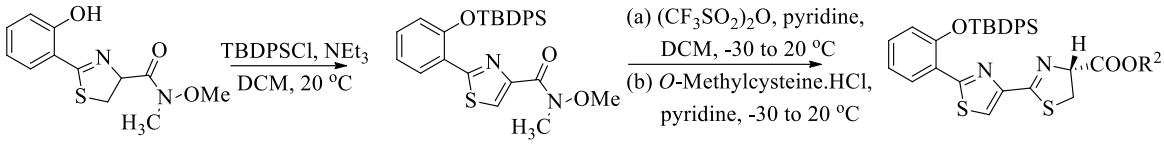


R_1 = Aryl or Alkyl; **R_2** = H or Me; **Tr** = trityl

53-96% Yield
22-99% ee

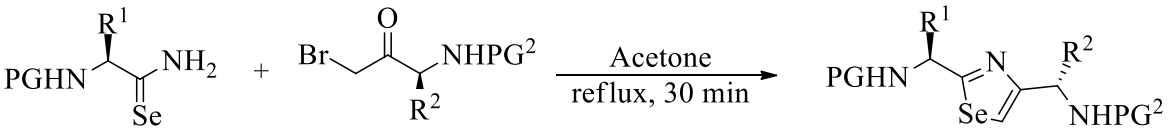
Scheme-1

Gaetan L. Mislin *et al.*[27] described the Synthesis of new thiazole analogues of pyochelin, a siderophore of *Pseudomonas aeruginosa* and *Burkholderia cepacia*. A new conversion of thiazolines into thiazoles.



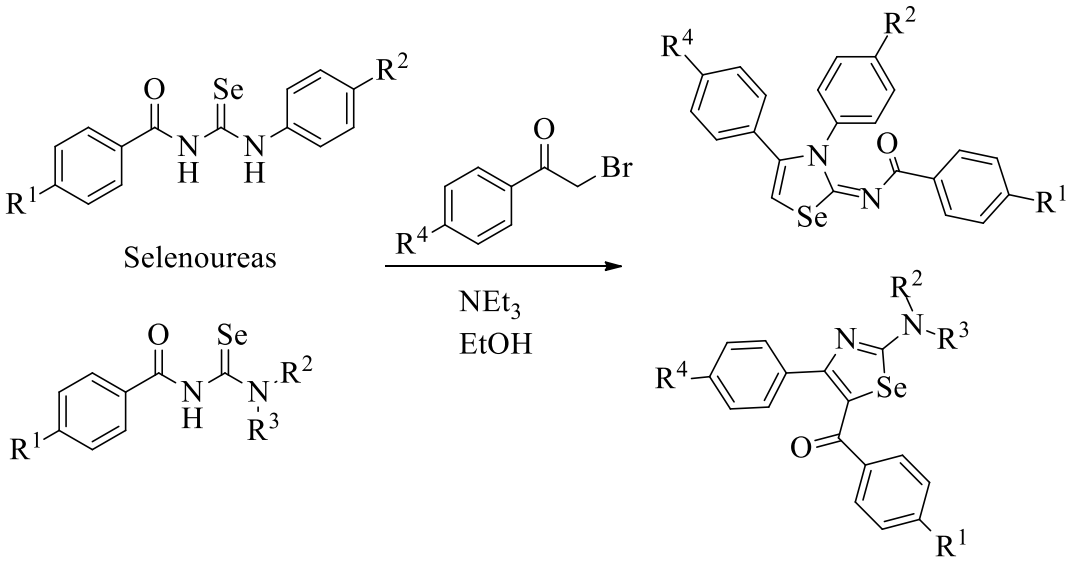
Scheme-2

Chilakapati Madhu *et al.*[28] reported one-pot synthesis of orthogonally protected dipeptide selenazoles employing N^{α}-amino selenocarboxamides and α-bromomethyl ketones.



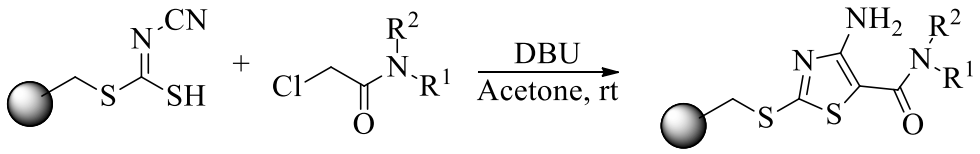
Scheme-3

Fabian Mohr[29] reported old Selenium heterocycles revisited: Synthesis, spectroscopic, and structural characterization of *N*-Acyl-1,3-selenazol-2(3*H*)-imines and 5-acyl-1,3-selenazol-2-aminesfrom acylselenourea derivatives.



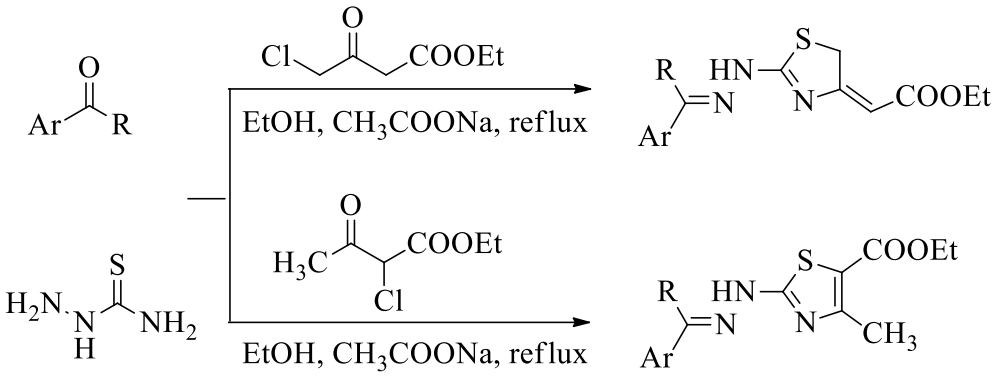
Scheme-4

Daehun Kim *et al.*[30] described efficient solid-phase synthesis of 2,4-disubstituted 5-carbamoyl-thiazole derivatives using a traceless support.



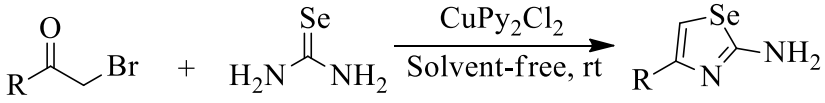
Scheme-5

Ma Xiabing *et al.*[31] reported facial one-pot, three-component synthesis of thiazole compounds by the reactions of aldehyde/ketone, thiosemicarbazide and chlorinated carboxylic ester derivatives.



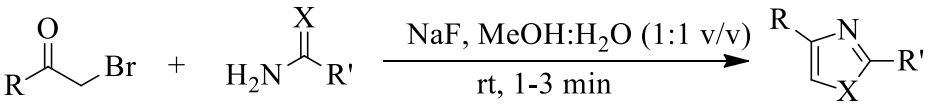
Scheme-6

From our Research group, **J. Venu Madhav *et al.***[32] and **B. Janardhan *et al.***[33] described solid-state synthesis of 1,3-Selenazoles Employing $CuPy_2Cl_2$ as a lewis acid catalyst and Sodium fluoride as an efficient catalyst for the synthesis of 2,4- disubstituted-1,3-thiazoles and selenazoles at ambient temperature respectively.



R = Aryl/coumaryl

Scheme-7



R = Aryl/coumarinyl; **R'** = NH_2/NH-Ph; **X** = S/Se

Scheme-8

PRESENT WORK

The applications of green chemistry principles have led to the development of cleaner and environmental benign chemical processes. In recent years, ultrasound irradiation has been extensively applied in organic reactions because of its special sonochemical property such

as cavitation effect that accelerates both catalytic and non-catalytic synthetic reactions.[34] More energy consumption for heating and producing hazardous bi-products are some disadvantageous factors of normal conventional techniques, which shows adverse effect on environment.

Moreover, when compared to the classical thermal methods of chemical processes, ultrasonic irradiation method has been attracting the modern synthetic chemist's due to their advantageous features *i.e.,* decreasing reaction times, increasing yields,[35] easy workup procedures, avoiding harsh reaction conditions,[36] better selectivity and high conversions.[37]

In view of the importance of thiazole and selenazole derivatives in various fields of chemistry, the classical Hantzsch synthesis as well as several methodologies have been reported utilizing various catalytic systems such as ammonium-12-molybdophosphate,[38] *β*-cyclodextrin,[39] NaCl,[40] HMCM-41,[41] iodine,[42] TiO_2,[43] $CuPy_2Cl_2$,[32] graphite oxide[44] and silica chloride,[45] and also reported in different solvent systems, such as ionic liquids,[46] PEG-400,[47] glycerin,[48] and water.[49] However, most of these reported methods have one or several draw backs such as lower yields, longer reaction times, complicated isolation procedures and use of hazardous, and expensive catalysts make them environmental non-friendly. To overcome the above limitations and as a part of our endeavour towards the development of novel eco-friendly methodologies for the synthesis of biologically potent heterocyclic compounds,[50] herein we proposed a versatile, simple, mild, environmental benign and highly efficient protocol for the synthesis of 2,4-disubstituted-1,3-thiazoles and 1,3-selenazoles in aqueous medium under ultrasonic irradiation.

Preparation of starting materials

3-Acetyl-2*H*-chromen-2-one

2-Acetyl-3*H*-benzo[*f*]chromen-3-one

Substituted 3-(2-Bromoacetyl)-2*H*-chromen-2-ones (1i-n)

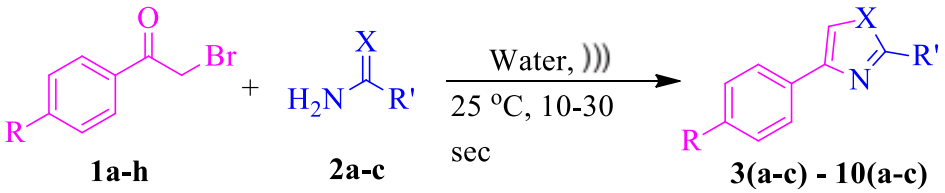
3-(2-bromoacetyl)-2*H*-chromen-2-one (**1i**), 3-(2-bromoacetyl)-6-chloro-2*H*-chromen-2-one (**1j**), 6-bromo-3-(2-bromoacetyl)-2*H*-chromen-2-one (**1k**), 6,8-dibromo-3-(2-bromoacetyl)-2*H*-chromen-2-one (**1l**), 3-(2-bromoacetyl)-6-methoxy-2*H*-chromen-2-one (**1m**) and 2-(2-Bromoacetyl)-3*H*-benzo[*f*]chromen-3-one (**1n**) were prepared according to the literature procedure as described in **Chapter-II**, **Section-A**.

1-Phenylthiourea[51]

Aqueous aniline was warmed with dil. HCl until a clear solution was obtained. To the above mixture was added aq. ammonium thiocyanate in portions. The reaction mixture was concentrated to less than half of its volume and upon cooling separated crystalline phenyl thiourea was filtered and washed with water.

Synthesis of 2,4-disubstituted-1,3-thiazoles and selenazoles (3-16)

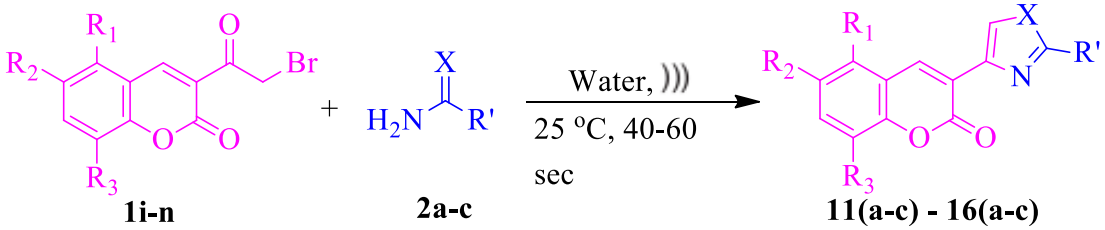
An equimolar mixture of phenacyl bromides/substituted 3(2-bromoacetyl)coumarins, thiourea/phenylthiourea/selenourea were sonicated at room temperature in the presence of water afforded the titled compounds in an excellent yields with analytical purity.



1a-h **2a-c** **3(a-c) - 10(a-c)**

R = H/F/Cl/Br/CH_3/OCH_3/NO_2/C_6H_5; **R'** = NH_2/NHPh; **X** = S/Se

Scheme-9



1i-n **2a-c** **11(a-c) - 16(a-c)**

R_1 = H; **R_2** = H/Cl/Br/OCH_3; **R_1 - R_2** = Benzo; **R_3** = H/Br
R' = NH_2/NHPh; X = S/Se

Scheme-10

Results and discussion

The schematic representation for the formation of 2,4-disubstituted-1,3-thiazoles and selenazoles (**3-16**) has shown in **Scheme 9 & 10**. Various phenacyl bromides (**1a-h**) and 3-(2-bromoacetyl)coumarins (**1i-n**) on condensation with thiourea (**2a)**, phenylthiourea

(**2b)** and selenourea **(2c)** in aqueous medium under ultrasonic irradiation at ambient temperature afforded the corresponding thiazoles and selenazoles (**3-16**) with excellent yields (90-99%) in short reaction times (10-60 sec).

In order to find the optimal conditions, a model reaction between equimolar quantities of phenacyl bromide (**1a**) and thiourea (**2a**) was performed in different solvents like water, methanol, acetic acid, acetonitrile and dimethylformamide at 25 ^{o}C bath temperature and 30 kHz ultrasonic frequency. We observed maximum yield (96%) of the product (**3a**) in aqueous medium within 10 sec. To improve the yield of the product in aqueous medium, the reaction was carried at 40 ^{o}C & 60 ^{o}C bath temperatures. But we observed slight decrease in yield of the product; this may due to the formation of unidentified impurities. The same reaction was also carried out at 50 kHz ultrasonic frequency by varying the bath temperature (25 ^{o}C, 40 ^{o}C & 60 ^{o}C), and observed maximum yield (98%) of the product at 25 ^{o}C bath temperature. We also observed the decrease in yield of the product as the temperature increases to 60 ^{o}C (**Table 1**). Recently, our group has reported the synthesis of 1,3-thiazoles and selenazoles in methanol-water (1:1 v/v) solvent system.[52] Therefore, we also tested the above reaction in methanol-water (1:1 v/v) at 50 kHz ultrasonic frequency and 25 ^{o}C bath temperature, but observed only 91% of the product yield even after 60 sec. The increase in yield of the product under ultrasonic irradiation has explained by the cavitations effect.

Cavitations effect

Cavitation is a physical phenomenon that creates localized hot spots in a reaction liquid core by the fast growth and implosions of micro-reactors like bubbles, that generates transient high temperatures and pressures and also leads to the formation of highly reactive radical intermediates at the localized hot spots. This rapid oscillation of formation, expansion and implosion of bubbles occurs beyond a certain threshold of pressure and constitutes acoustic cavitations. Such cavitations induce the reaction rapidly by accelerating the mixing of the reactants between two different phases by diffusion process at interfacial region in heterogeneous reactions. Generally ultrasounds generate the temperature in a reaction medium, which facilitates the acceleration of reaction. But beyond the optimum level, utilization efficiency of ultrasound considerably decreases due to the generation of large cavities with low oscillating energy and heavy crowding of active bubbles in a small space scatters the sound waves and lowers the focusing energy

on the reaction vessel. Thus the yield of the product formation increases in shorter reaction times.

Table 1. Optimizing the reaction conditions[a]

Entry	Solvent	Ultrasound frequency (kHz)	Bath temperature (°C)	Time (sec)	Yield[b] (%)
1	Water	30	25	10	96
2	Methanol	30	25	60	93
3	Acetic acid	30	25	120	86
4	Acetonitrile	30	25	120	88
5	Dimethylformamide	30	25	160	86
6	Water	30	40	10	95
7	Water	30	60	10	93
8	Water	50	25	10	98
9	Water	50	40	10	96
10	Water	50	60	10	93
11	Water : Methanol (1:1 v/v)	50	25	40	91

[a]Reaction conditions: Phenacyl bromide (**1a**, 1 mmol), thiourea (**2a**, 1 mmol), solvent (1 mL), ultrasonic irradiation.
[b]Isolated yields.

Utilizing these optimistic conditions (ultrasonication at 50 kHz frequency and 25 °C bath temperature in aqueous medium), a series of 1,3-thiazoles and selenazoles **(3-16)** have been synthesized by the reaction of phenacyl bromides (**1a-h**) and 3-(2-bromoacetyl)coumarins (**1i-n**) with thiourea (**2a)**, phenylthiourea (**2b)** and selenourea **(2c)** in excellent yields (**Table 2 & 3**). All the newly synthesized compounds were characterized by their spectral and analytical studies, and the known compounds were established by comparing their melting points with the reported values.

Table 2. Synthesis of 2,4-disubstituted-1,3-thiazoles and selenazoles (3-10) with phenacyl bromides.[a]

Product	R	R'	X	Time (sec)	Yield[b] (%)	Melting point (°C)	
						Observed	Lit. [Ref.]
3a	H	NH_2	S	10	98	152-153	150-152[33]
3b	H	NHPh	S	10	96	133-135	134-136[33]
3c	H	NH_2	Se	10	97	132-133	132[32]
4a	F	NH_2	S	25	95	117-118	119-120[46]
4b	F	NHPh	S	25	92	111-112	110-111[49]
4c	F	NH_2	Se	15	95	119-120	-
5a	Cl	NH_2	S	20	99	166-168	166–168[33]
5b	Cl	NHPh	S	25	96	144-146	145–146[33]
5c	Cl	NH_2	Se	20	97	156-157	155[32]
6a	Br	NH_2	S	25	95	182-183	182-184[33]
6b	Br	NHPh	S	30	93	230-232	230-232[33]

6c	Br	NH_2	Se	25	97	176	177-178[33]
7a	CH_3	NH_2	S	15	96	136-137	135-136[33]
7b	CH_3	NHPh	S	20	91	102-104	102-103[33]
7c	CH_3	NH_2	Se	15	92	167-168	166[32]
8a	OCH_3	NH_2	S	10	98	202-204	204-206[33]
8b	OCH_3	NHPh	S	15	94	136-138	137-138[33]
8c	OCH_3	NH_2	Se	10	97	194-196	173[32]
9a	NO_2	NH_2	S	30	91	284-285	284–286[33]
9b	NO_2	NHPh	S	30	91	204-206	206–207[33]
9c	NO_2	NH_2	Se	25	91	269-270	250[32]
10a	C_6H_5	NH_2	S	20	94	234-236	-
10b	C_6H_5	NHPh	S	25	90	222-224	-
10c	C_6H_5	NH_2	Se	20	94	135-136	-

[a]Reaction conditions: Phenacyl bromides (**1a-h**, 1 mmol), thiourea/phenylthiourea/selenourea (**2a-c**, 1 mmol), water (1 mL), ultrasonic irradiation at 50 kHz and 25 ^oC bath temperature.

[b]Isolated pure products yield.

Table 3. Synthesis of 2,4-disubstituted-1,3-thiazoles and selenazoles with 3-(2-bromoacetyl)coumarins (11-16).[a]

Product	**R_1**	**R_2**	**R_3**	**R'**	**X**	**Time (sec)**	**Yield[b] (%)**	**Melting point (^oC)**	
								Observed	**Lit. [Ref.]**
11a	H	H	H	NH_2	S	40	98	227-228	228-229[33]
11b	H	H	H	NHPh	S	45	94	188-190	188-190[33]
11c	H	H	H	NH_2	Se	40	96	218-220	217-218[33]
12a	H	Cl	H	NH_2	S	55	98	206-208	-
12b	H	Cl	H	NHPh	S	60	96	215-217	-
12c	H	Cl	H	NH_2	Se	50	99	305-307	-
13a	H	Br	H	NH_2	S	55	97	210-212	-
13b	H	Br	H	NHPh	S	55	95	229-231	-
13c	H	Br	H	NH_2	Se	50	94	346-348	-
14a	H	Br	Br	NH_2	S	60	92	277-279	-
14b	H	Br	Br	NHPh	S	60	92	225-227	-
14c	H	Br	Br	NH_2	Se	60	94	326-328	-
15a	H	OCH_3	H	NH_2	S	45	99	256-258	-
15b	H	OCH_3	H	NHPh	S	45	96	222-223	-
15c	H	OCH_3	H	NH_2	Se	40	97	237-239	-
16a	Benzo		H	NH_2	S	55	93	287-289	-
16b	Benzo		H	NHPh	S	60	93	262-264	-
16c	Benzo		H	NH_2	Se	55	93	358-360	-

[a]Reaction conditions: 3-(2-Bromoacetyl)coumarin (**1i-n**, 1 mmol), thiourea/phenylthiourea/selenourea (**2a-c**, 1 mmol), water (1 mL), ultrasonic irradiation at 50 kHz and 25 ^oC bath temperature.

[b]Isolated pure products yield.

Conclusion

In conclusion, we have efficiently synthesized a series of 2,4-disubstituted-1,3-thiazoles and selenazoles in aqueous medium under ultrasonic irradiation at ambient temperature. This non-conventional methodology has many advantages over conventional reported methods that include environmental friendly, rapid reaction completion, easy workup procedure and analytically pure products formation in excellent yields. This method can be effectively used for large scale production of thiazoles and selenazoles in shorter reaction times.

Experimental

General procedure for the synthesis of 2,4-disubstituted-1,3-thiazoles and selenazoles (3-16)

A 100 mL borosil test-tube was charged with phenacyl bromide (**1a-h**)/3(2-bromoacetyl)coumarin (**1i-n**) (1 mmol), thiourea (**2a**)/phenylthiourea (**2b**)/selenourea (**2c**) (1 mmol) and water (1 mL). The tube was kept in such a way that the surface of the reactants is just lower than the water level of ultrasonic bath, and sonicated with a frequency of 50 kHz at 25 ^{o}C for about 10 to 60 sec. The progress of the reaction was monitored by TLC. After completion of the reaction, the solid separated out was filtered and washed with water. Analytically pure products are formed without recrystallization.

Spectral data

4-(4-Fluorophenyl)-1,3-selenazol-2-amine (4c)

White solid; **IR** (KBr, ν_{max}, cm^{-1}): 3392, 3261 (NH_2), 1628 (C=N), 1098 (C-F); **^{1}H NMR** (400 MHz, DMSO-d_6): δ (ppm) 7.19 (s, 1H), 7.28 (s, 1H), 7.32 (d, *J* = 8.0 Hz, 1H), 7.77-7.81 (m, 3H), 8.44 (s, 1H); **MS** (ESI) *m/z*: 242 $[M + H]^+$; Anal. calcd. for $C_9H_7FN_2Se$: C, 44.83; H, 2.93; N, 11.62. Found: C, 44.61; H, 3.17; N, 11.83.

4-([1,1'-Biphenyl]-4-yl)thiazol-2-amine (10a)

White solid; **IR** (KBr, ν_{max}, cm^{-1}): 3370, 3258 (NH_2), 1625 (C=N); **^{1}H NMR** (400 MHz, DMSO-d_6): δ (ppm) 7.27 (s, 1H), 7.39 (t, *J* = 7.6 Hz, 1H), 7.49 (t, *J* = 7.2 Hz, 2H), 7.73 (d, *J* = 7.2 Hz, 2H), 7.79 (d, *J* = 8.4 Hz, 2H), 7.84 (d, *J* = 8.4 Hz, 2H), 8.48 (s, 2H); **MS** (ESI) *m/z*: 253 $[M + H]^+$; Anal. calcd. for $C_{15}H_{12}N_2S$: C, 71.40; H, 4.79; N, 11.10. Found: C, 71.16; H, 4.54; N, 10.85.

4-([1,1'-Biphenyl]-4-yl)-*N*-phenylthiazol-2-amine (10b)

White solid; **IR** (KBr, ν_{max}, cm^{-1}): 3332 (NH), 1698 (C=N); **^{1}H NMR** (400 MHz, DMSO-d_6): δ (ppm) 6.99 (t, *J* = 7.2 Hz, 1H), 7.31-7.43 (m, 6H), 7.61-7.69 (m, 6H), 7.93 (d, *J* = 8.4 Hz, 2H), 10.47(s, 1H); **MS** (ESI) *m/z*: 329 $[M + H]^+$; Anal. calcd. for $C_{21}H_{16}N_2S$: C, 76.80; H, 4.91; N, 8.53. Found: C, 76.47; H, 4.70; N, 8.27.

4-([1,1'-Biphenyl]-4-yl)-1,3-selenazol-2-amine (10c)

White solid; **IR** (KBr, ν_{max}, cm^{-1}): 3490, 3240 (NH_2), 1613 (C=N); **^{1}H NMR** (400 MHz, DMSO-d_6): δ (ppm) 7.38-7.42 (m, 1H), 7.50 (t, *J* = 7.2 Hz, 2H), 7.55 (s, 1H), 7.73-7.80 (m, 6H), 9.02 (s, 2H); **MS** (ESI) *m/z*: 300 $[M + H]^+$; Anal. calcd. for $C_{15}H_{12}N_2Se$: C, 60.21; H, 4.04; N, 9.36. Found: C, 60.56; H, 4.34; N, 9.12.

3-(2-Aminothiazol-4-yl)-6-chloro-2*H*-chromen-2-one (12a)

Yellow solid; **IR** (KBr, ν_{max}, cm^{-1}): 3315, 3248 (NH_2), 1735 (C=O), 1631 (C=N), 789 (C-Cl); **^{1}H NMR** (400 MHz, DMSO-d_6): δ (ppm) 7.77 (d, *J* = 7.2 Hz, 1H), 7.93 (d, *J* = 7.6 Hz, 1H), 8.08 (s, 1H), 8.45 (s, 1H), 8.61 (s, 1H), 8.80 (s, 2H); **MS** (ESI) *m/z*: 279 $[M + H]^+$; Anal. calcd. for $C_{12}H_7ClN_2O_2S$: C, 51.71; H, 2.53, N, 10.05. Found: C, 51.42; H, 2.75; N, 10.37.

6-Chloro-3-(2-(phenylamino)thiazol-4-yl)-2*H*-chromen-2-one (12b)

Yellow solid, **IR** (KBr, ν_{max}, cm^{-1}): 3359 (NH), 1719 (C=O), 1607 (C=N), 745 (C-Cl); **^{1}H NMR** (400 MHz, DMSO-d_6): δ (ppm) 7.01 (t, *J* = 7.2 Hz, 1H), 7.38 (t, *J* = 8.0 Hz, 2H), 7.48 (d, *J* = 8.8 Hz, 1H), 7.62-7.65 (m, 1H), 7.76 (d, *J* = 8.0 Hz, 2H), 7.80 (s, 1H), 8.13 (s, 1H), 8.66 (s, 1H), 10.36 (s, 1H); **MS** (ESI) *m/z*: 355 $[M + H]^+$; Anal. calcd. for $C_{18}H_{11}ClN_2O_2S$: C, 60.93; H, 3.12; N, 7.90. Found: C, 60.75; H, 3.28; N, 7.72.

3-(2-Amino-1,3-selenazol-4-yl)-6-chloro-2*H*-chromen-2-one (12c)

Yellow solid; **IR** (KBr, ν_{max}, cm^{-1}): 3297, 3441 (NH_2), 1728 (C=O), 1625 (C=N), 785 (C-Cl); **^{1}H NMR** (400 MHz, DMSO-d_6): δ (ppm) 7.89 (d, *J* = 6.8 Hz, 1H), 7.92 (d, *J* = 7.2 Hz, 1H), 8.09 (s, 1H), 8.46 (s, 1H), 8.61 (s, 1H), 8.79 (s, 2H); **MS** (ESI) *m/z*: 325 $[M]^+$; Anal. calcd. for $C_{12}H_7ClN_2O_2Se$: C, 44.26; H, 2.17; N, 8.60. Found: C, 44.55; H, 2.32; N, 8.36.

3-(2-Aminothiazol-4-yl)-6-bromo-2*H*-chromen-2-one (13a)

Yellow solid; **IR** (KBr, ν_{max}, cm^{-1}): 3340, 3186 (NH_2), 1717 (C=O), 1602 (C=N), 602 (C-Br); **^{1}H NMR** (400 MHz, DMSO-d_6): δ (ppm) 7.19 (s, 2H), 7.40 (d, *J* = 8.8 Hz, 1H), 7.54 (s, 1H), 7.72-7.75 (m, 1H), 8.11 (d, *J* = 8.0 Hz, 1H), 8.44 (s, 1H); **MS** (ESI) *m/z*: 324 [M + H]$^+$; Anal. calcd. for $C_{12}H_7BrN_2O_2S$: C, 44.60; H, 2.18; N, 8.67. Found: C, 44.45; H, 2.37; N, 8.46.

6-Bromo-3-(2-(phenylamino)thiazol-4-yl)-2*H*-chromen-2-one (13b)

Yellow solid; **IR** (KBr, ν_{max}, cm^{-1}): 3358 (NH), 1719 (C=O), 1605 (C=N), 558 (C-Br); **^{1}H NMR** (400 MHz, DMSO-d_6): δ (ppm) 7.01 (t, *J* = 7.6 Hz, 1H), 7.37-7.44 (m, 3H), 7.75-7.81 (m, 4H), 8.28 (d, *J* = 7.6 Hz, 1H), 8.67 (s, 1H), 10.36 (s, 1H); **MS** (ESI) *m/z*: 400 [M + H]$^+$; Anal. calcd. for $C_{18}H_{11}BrN_2O_2S$: C, 54.15; H, 2.78; N, 7.02. Found: C, 54.36; H, 2.56; N, 6.92.

3-(2-Amino-1,3-selenazol-4-yl)-6-bromo-2*H*-chromen-2-one (13c)

Yellow solid; **IR** (KBr, ν_{max}, cm^{-1}): 3483, 3372 (NH_2), 1735 (C=O), 1620 (C=N), 587 (C-Br); **^{1}H NMR** (400 MHz, DMSO-d_6): δ (ppm) 7.94 (d, *J* = 8.0 Hz, 1H), 8.03 (d, *J* = 7.2 Hz, 1H), 8.26 (s, 1H), 8.44 (s, 1H), 8.52 (s, 2H), 8.95 (s, 1H); **MS** (ESI) *m/z*: 371 [M + H]$^+$; Anal. calcd. for $C_{12}H_7BrN_2O_2Se$: C, 38.95; H, 1.91; N, 7.57. Found: C, 38.65; H, 1.67; N, 7.36.

3-(2-Aminothiazol-4-yl)-6,8-dibromo-2*H*-chromen-2-one (14a)

Yellow solid; **IR** (KBr, ν_{max}, cm^{-1}): 3492, 3389 (NH_2), 1740 (C=O), 1692 (C=N), 593, 608 (C-Br); **^{1}H NMR** (400 MHz, DMSO-d_6): δ (ppm) 7.23 (s, 2H), 7.56 (s, 1H), 8.09-8.14 (m, 2H), 8.41 (s, 1H); **MS** (ESI) *m/z*: 403 [M + H]$^+$; Anal. calcd. for $C_{12}H_6Br_2N_2O_2S$: C, 35.85; H, 1.50; N, 6.97. Found: C, 35.97; H, 1.39; N, 6.74.

6,8-Dibromo-3-(2-(phenylamino)thiazol-4-yl)-2*H*-chromen-2-one (14b)

Yellow solid; **IR** (KBr, ν_{max}, cm^{-1}): 3362 (NH), 1728 (C=O), 1603 (C=N), 501, 562 (C-Br); **^{1}H NMR** (400 MHz, DMSO-d_6): δ (ppm) 7.01 (t, *J* = 7.2 Hz, 1H), 7.38 (t, *J* = 8.0 Hz, 2H), 7.77 (d, *J* = 8.0 Hz, 2H), 7.82 (s, 1H), 8.12 (s, 1H), 8.32 (s, 1H), 8.64 (s,1H), 10.37 (s, 1H); **MS** (ESI) *m/z*: 479 [M + H]$^+$; Anal. calcd. for $C_{18}H_{10}Br_2N_2O_2S$: C, 45.21; H, 2.11; N, 5.86. Found: C, 45.43; H, 2.02; N, 5.67.

3-(2-Amino-1,3-selenazol-4-yl)-6,8-dibromo-2*H*-chromen-2-one (14c)

Yellow solid; **IR** (KBr, ν_{max}, cm^{-1}): 3450, 3310 (NH_2), 1723 (C=O), 1600 (C=N), 539, 558 (C-Br); **^{1}H NMR** (400 MHz, DMSO-d_6): δ (ppm) 8.01 (s, 1H), 8.08 (s, 1H), 8.15 (s, 1H), 8.42 (s, 1H), 8.75 (s, 2H); **MS** (ESI) *m/z*: 449 $[M + H]^+$; Anal. calcd. for $C_{12}H_6Br_2N_2O_2Se$: C, 32.10; H, 1.35; N, 6.24. Found: C, 32.32; H, 1.16; N, 6.42.

3-(2-Aminothiazol-4-yl)-6-methoxy-2*H*-chromen-2-one (15a)

Yellow solid; **IR** (KBr, ν_{max}, cm^{-1}): 3463, 3352 (NH_2), 1712 (C=O), 1616 (C=N), 1270 (C-O-C); **^{1}H NMR** (400 MHz, DMSO-d_6): δ (ppm) 3.90 (s, 3H), 7.27-7.35 (m, 5H), 7.49 (s, 1H), 8.45 (s, 1H); **MS** (ESI) *m/z*: 275 $[M + H]^+$; Anal. calcd. for $C_{13}H_{10}N_2O_3S$: C, 56.92; H, 3.67; N, 10.21. Found: C, 57.14; H, 3.45; N, 10.39.

6-Methoxy-3-(2-(phenylamino)thiazol-4-yl)-2*H*-chromen-2-one (15b)

Yellow solid; **IR** (KBr, ν_{max}, cm^{-1}): 3313 (NH), 1708 (C=O), 1606 (C=N), 1276 (C-O-C); **^{1}H NMR** (400 MHz, DMSO-d_6): δ (ppm) 3.94 (s, 3H), 7.00 (t, *J* = 7.2 Hz, 1H), 7.32-7.40 (m, 4H), 7.49-7.51 (m, 1H), 7.75 (d, *J* = 7.6 Hz, 2H), 7.79 (s, 1H), 8.66 (s, 1H), 10.35 (s, 1H); **MS** (ESI) *m/z*: 351 $[M + H]^+$; Anal. calcd. for $C_{19}H_{14}N_2O_3S$: C, 65.13; H, 4.03; N, 7.99. Found: C, 65.01; H, 4.19; N, 7.83.

3-(2-Amino-1,3-selenazol-4-yl)-6-methoxy-2*H*-chromen-2-one (15c)

Yellow solid; IR (KBr, ν_{max}, cm^{-1}): 3453, 3344 (NH_2), 1710 (C=O), 1617 (C=N), 1271 (C-O-C); ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 3.93 (s, 3H), 7.18 (d, *J* = 7.2 Hz, 1H), 7.70 (d, *J* = 7.2 Hz, 1H), 7.94 (s, 1H), 8.20 (s, 1H), 8.49 (s, 1H), 8.63 (s, 2H); MS (ESI) *m/z*: 321 $[M]^+$; Anal. calcd. for $C_{13}H_{10}N_2O_3Se$: C, 48.61; H, 3.14; N, 8.72. Found: C, 48.38; H, 3.29; N, 8.91.

2-(2-Aminothiazol-4-yl)-3*H*-benzo[*f*]chromen-3-one (16a)

Yellow solid; **IR** (KBr, ν_{max}, cm^{-1}): 3358, 3292 (NH_2), 1702 (C=O), 1632 (C=N); **^{1}H NMR** (400 MHz, DMSO-d_6): δ (ppm) 7.60-7.67 (m, 4H), 7.81 (t, *J* = 8.4 Hz, 1H), 8.08 (d, *J* = 8.0 Hz, 1H), 8.19-8.22 (m, 2H), 8.44 (d, *J* = 8.4 Hz, 1H), 9.24 (s, 1H); **MS** (ESI) *m/z*: 295 $[M + H]^+$; Anal. calcd. for $C_{16}H_{10}N_2O_2S$: C, 65.29; H, 3.42; N, 9.52. Found: C, 65.42; H, 3.25; N, 9.38.

2-(2-(Phenylamino)thiazol-4-yl)-3*H*-benzo[*f*]chromen-3-one (16b)

Yellow solid; **IR** (KBr, ν_{max}, cm^{-1}): 3303 (NH), 1726 (C=O), 1698 (C=N); **^{1}H NMR** (400 MHz, DMSO-d_6): δ (ppm) 7.04 (t, *J* = 7.2 Hz, 1H), 7.44 (t, *J* = 8.0 Hz, 2H), 7.62-7.69 (m, 2H), 7.78-7.87 (m, 4H), 8.10 (d, *J* = 8.0 Hz, 1H), 8.21 (d, *J* = 9.2 Hz, 1H), 8.42 (d, *J* = 8.4 Hz, 1H), 9.35 (s, 1H), 10.43 (s, 1H); **MS** (ESI) *m/z*: 371 $[M + H]^+$; Anal. calcd. for $C_{22}H_{14}N_2O_2S$: C, 71.33; H, 3.81; N, 7.56. Found: C, 71.50; H, 3.92; N, 7.34.

2-(2-Amino-1,3-selenazol-4-yl)-3*H*-benzo[*f*]chromen-3-one (16c)

Yellow solid; **IR** (KBr, ν_{max}, cm^{-1}): 3230, 3170 (NH_2), 1709 (C=O), 1620 (C=N); **^{1}H NMR** (400 MHz, DMSO-d_6): δ (ppm) 7.68 (s, 1H), 7.81 (t, *J* = 8 Hz, 2H), 8.09 (d, *J* = 8.8 Hz, 2H), 8.25 (d, *J* = 9.2 Hz, 1H), 8.66 (d, *J* = 8.4 Hz, 1H), 9.08 (s, 2H), 9.28 (s, 1H); **MS** (ESI) *m/z*: 341 $[M]^+$; Anal. calcd. for $C_{16}H_{10}N_2O_2Se$: C, 56.32; H, 2.95; N, 8.21. Found: C, 56.53; H, 2.65; N, 8.36.

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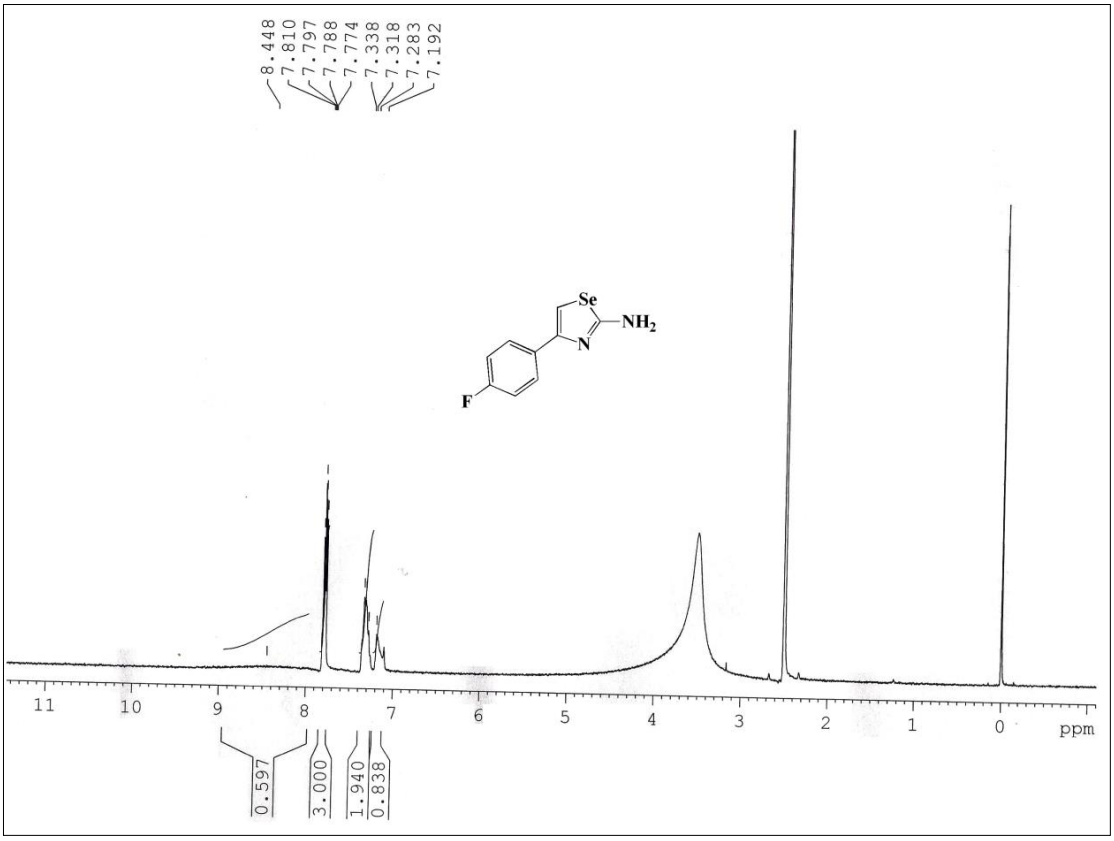
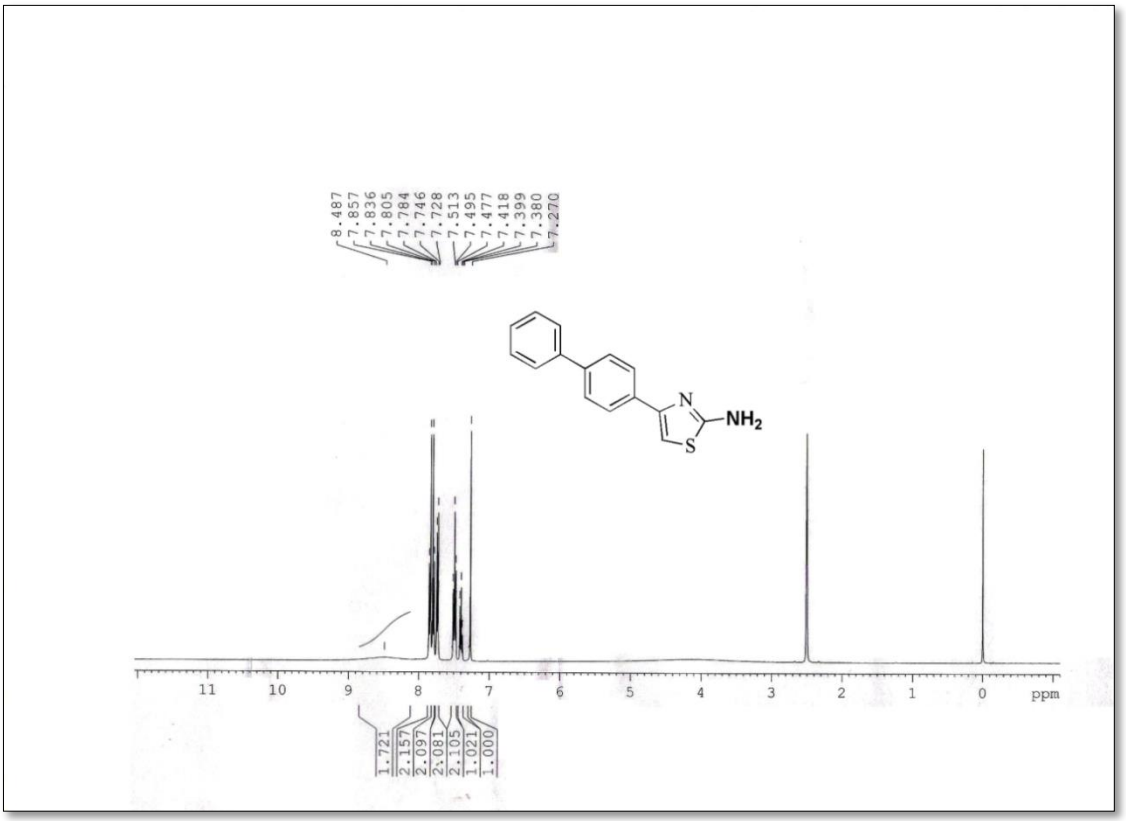
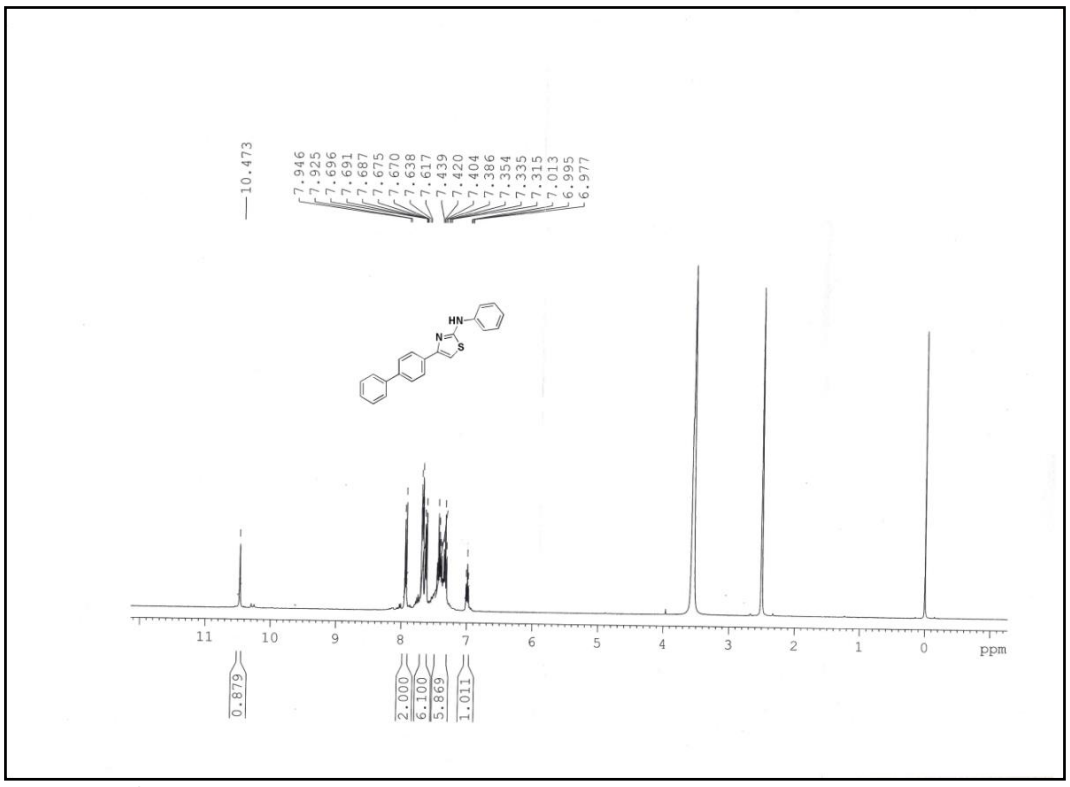


^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound 4c

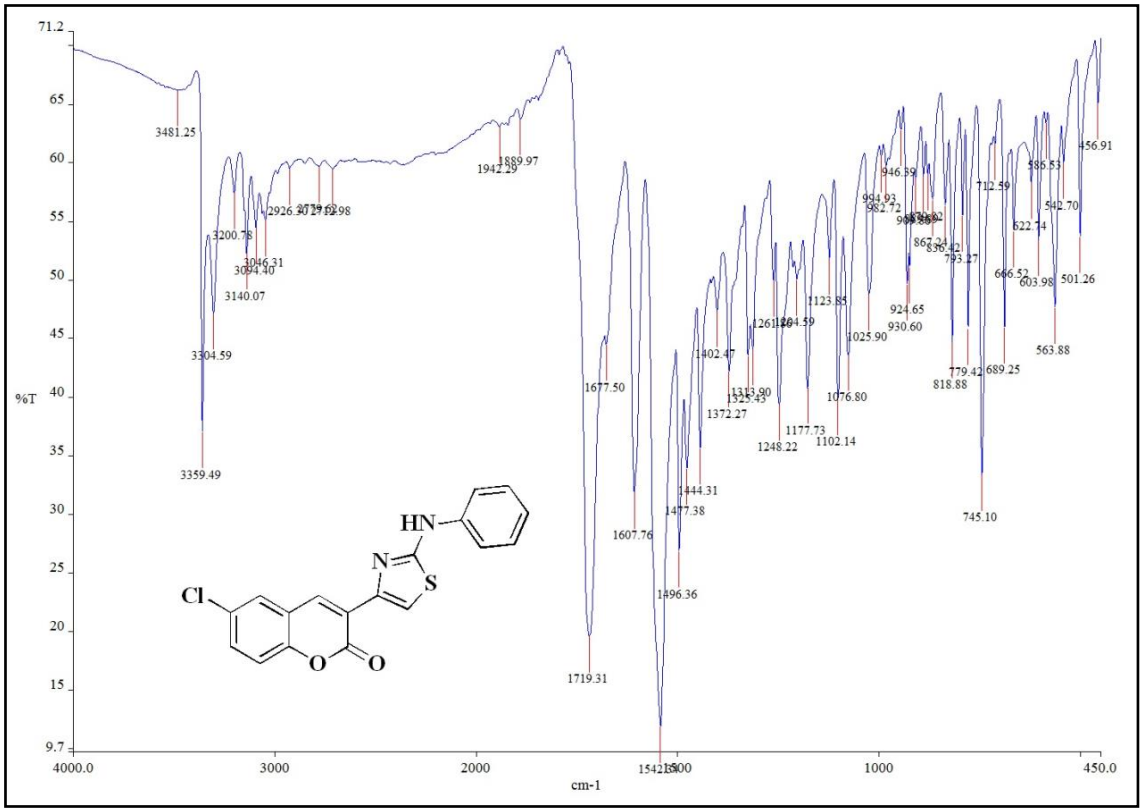


^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound 10a

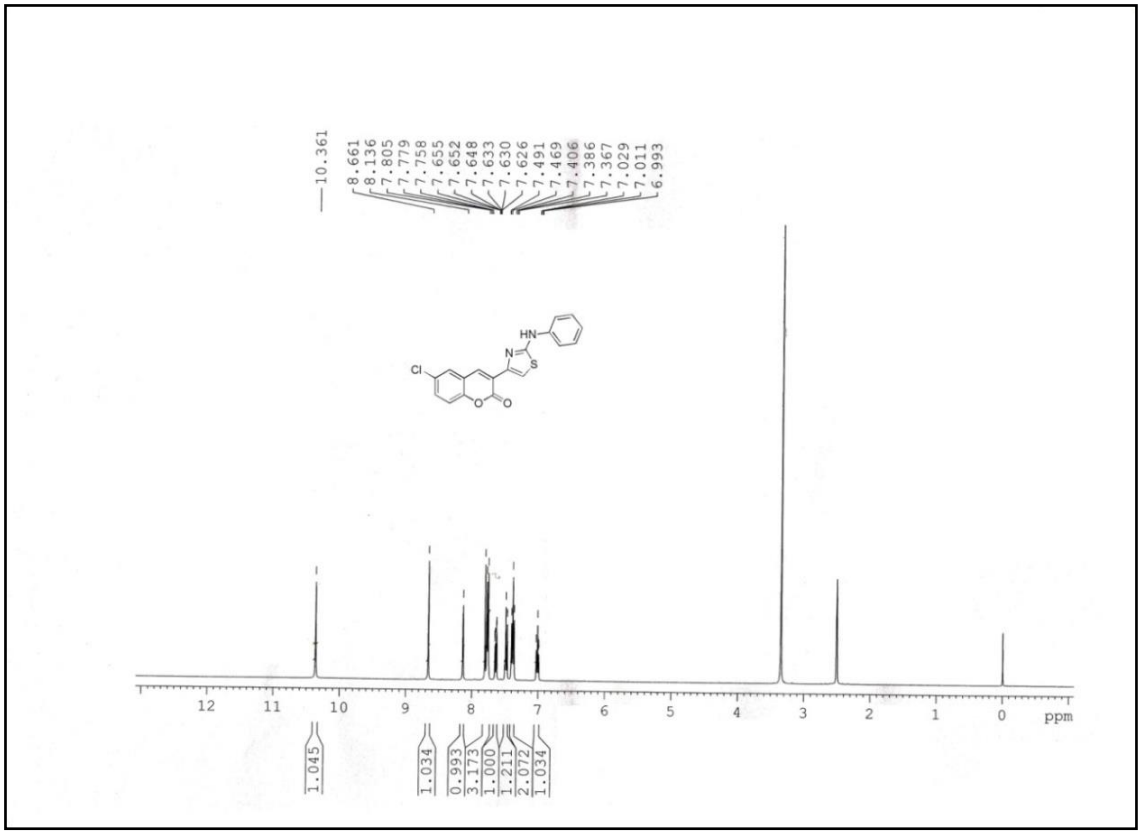


^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound 10b

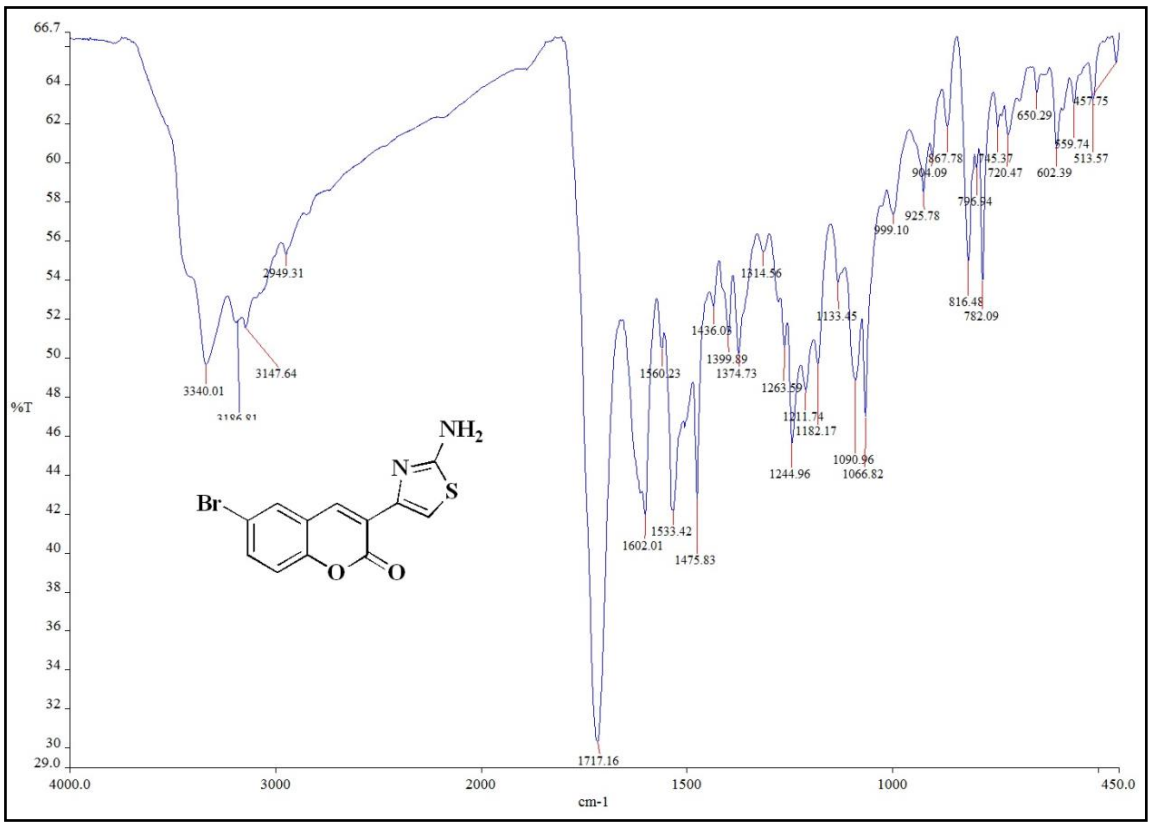
^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound 10c



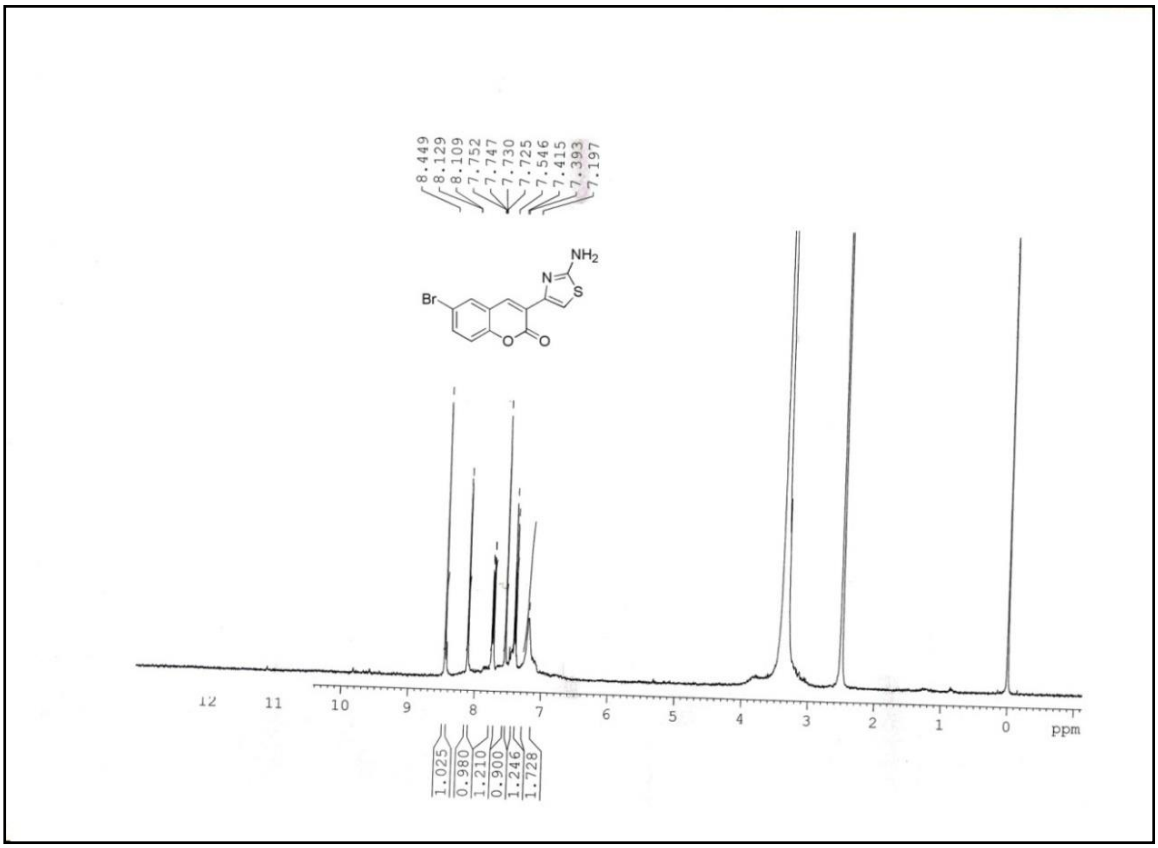
IR (KBr) spectrum of compound 12b



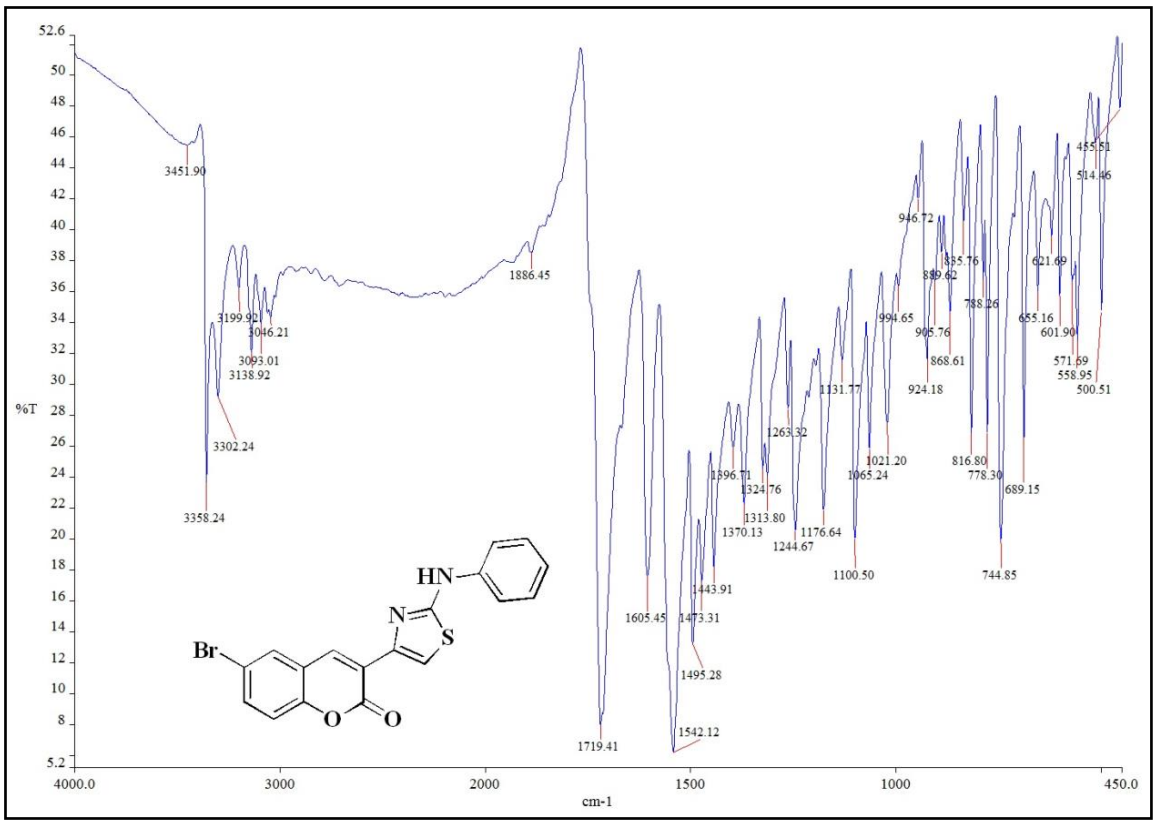
^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound 12b



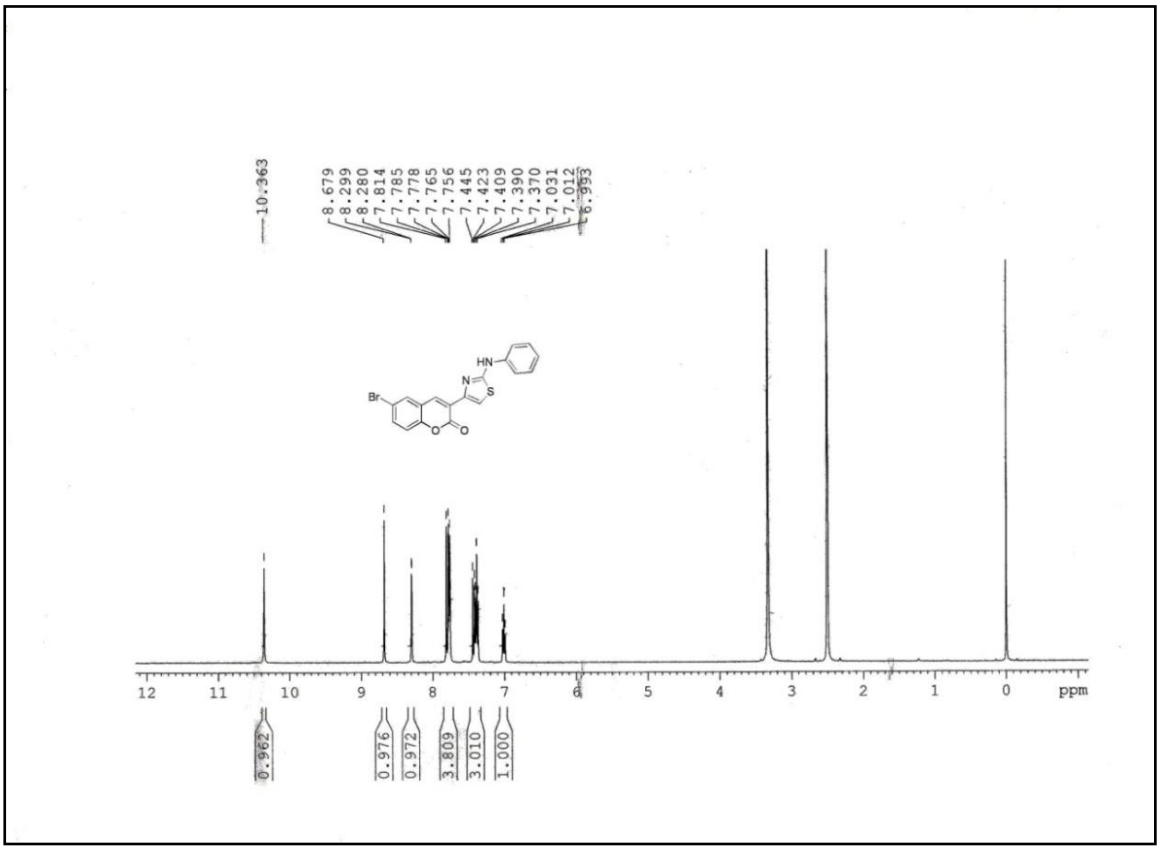
IR (KBr) spectrum of compound 13a



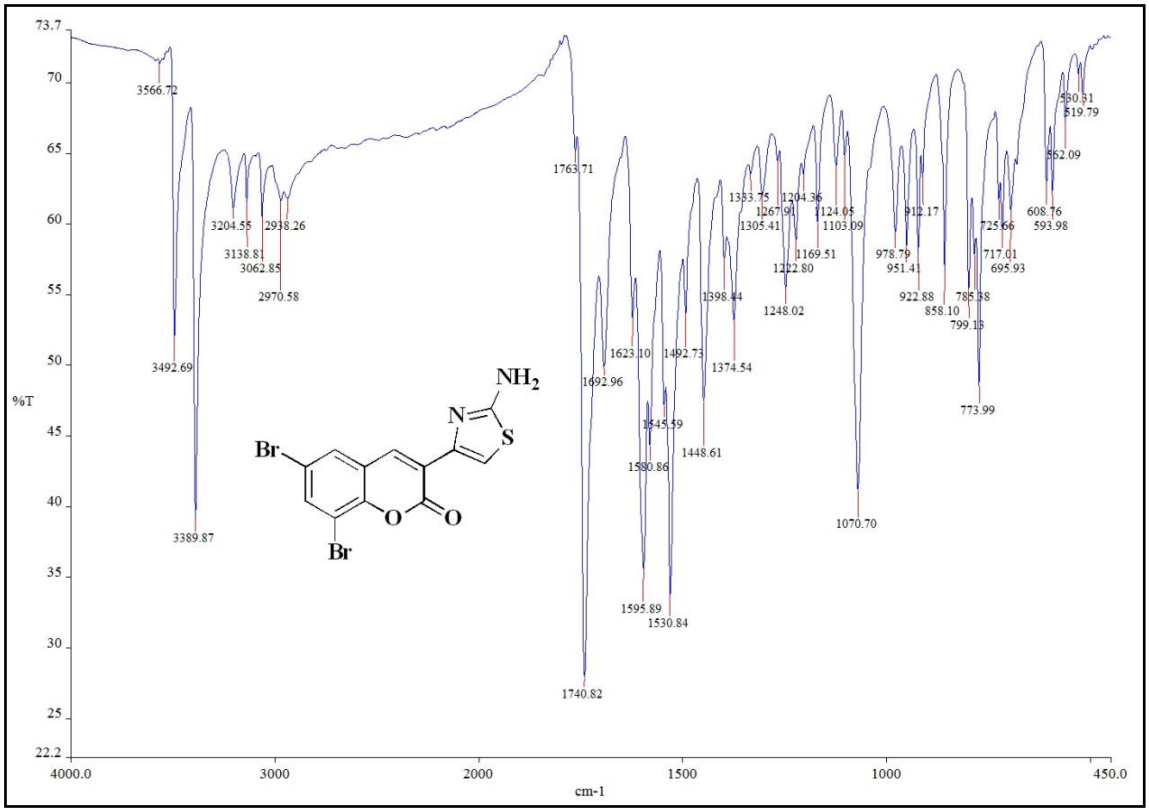
^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound 13a



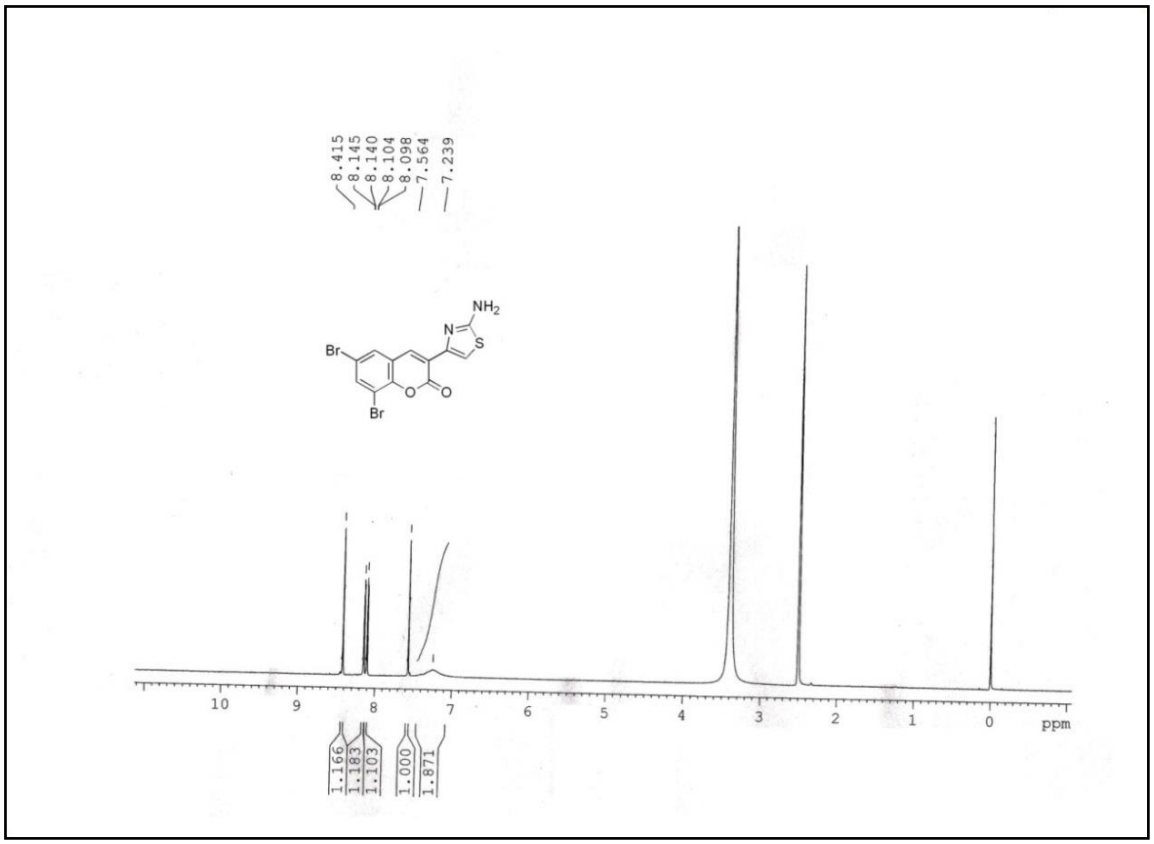
IR (KBr) spectrum of compound 13b



^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound 13b



IR (KBr) spectrum of compound 13b



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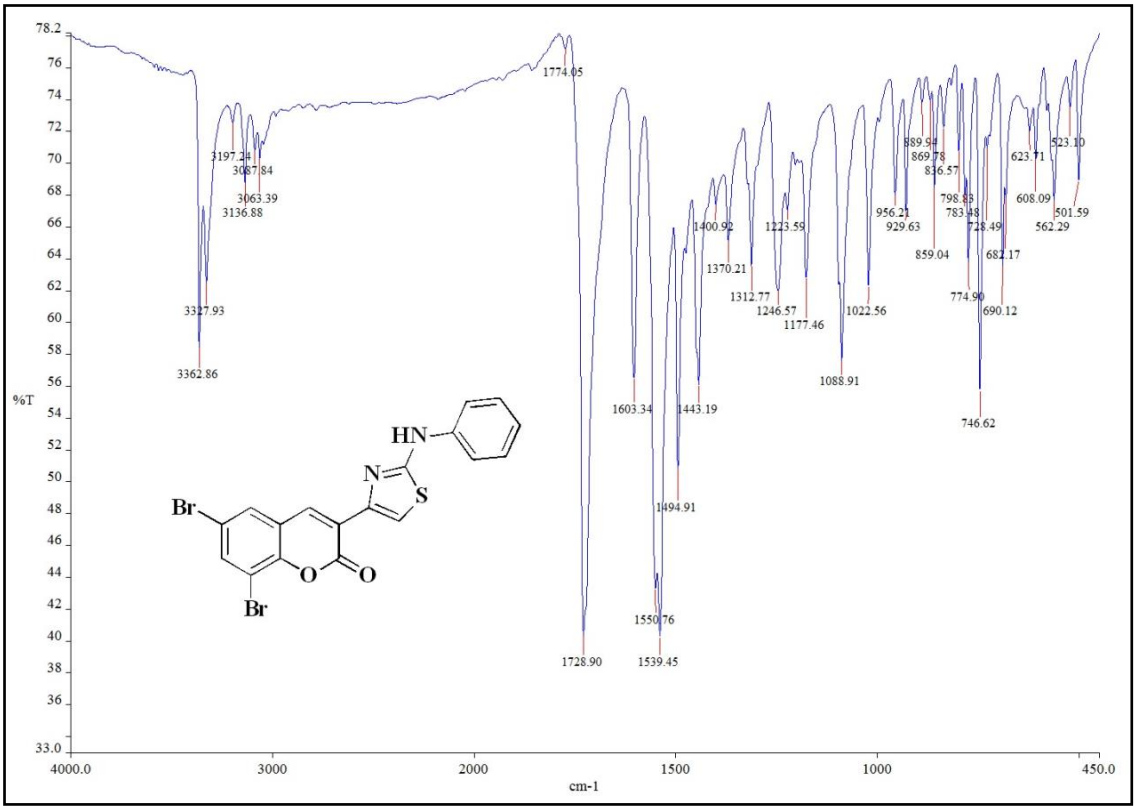
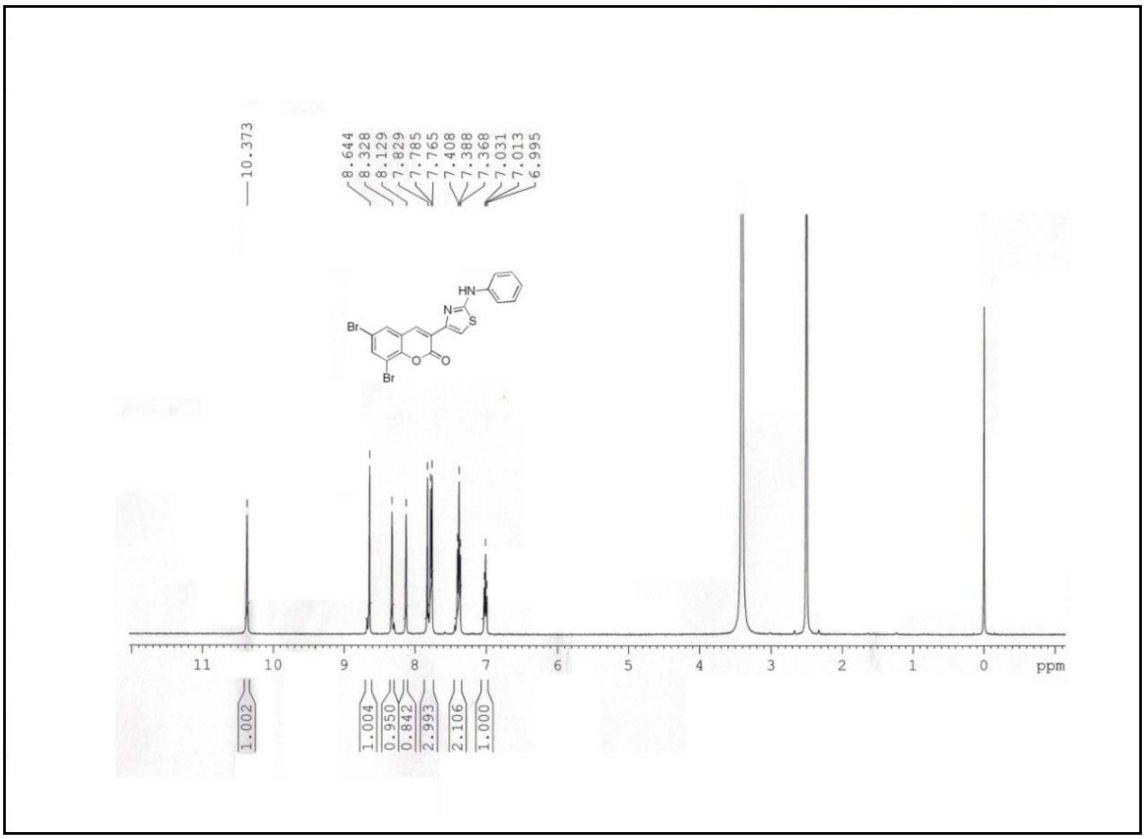


IR (KBr) spectrum of compound 14b



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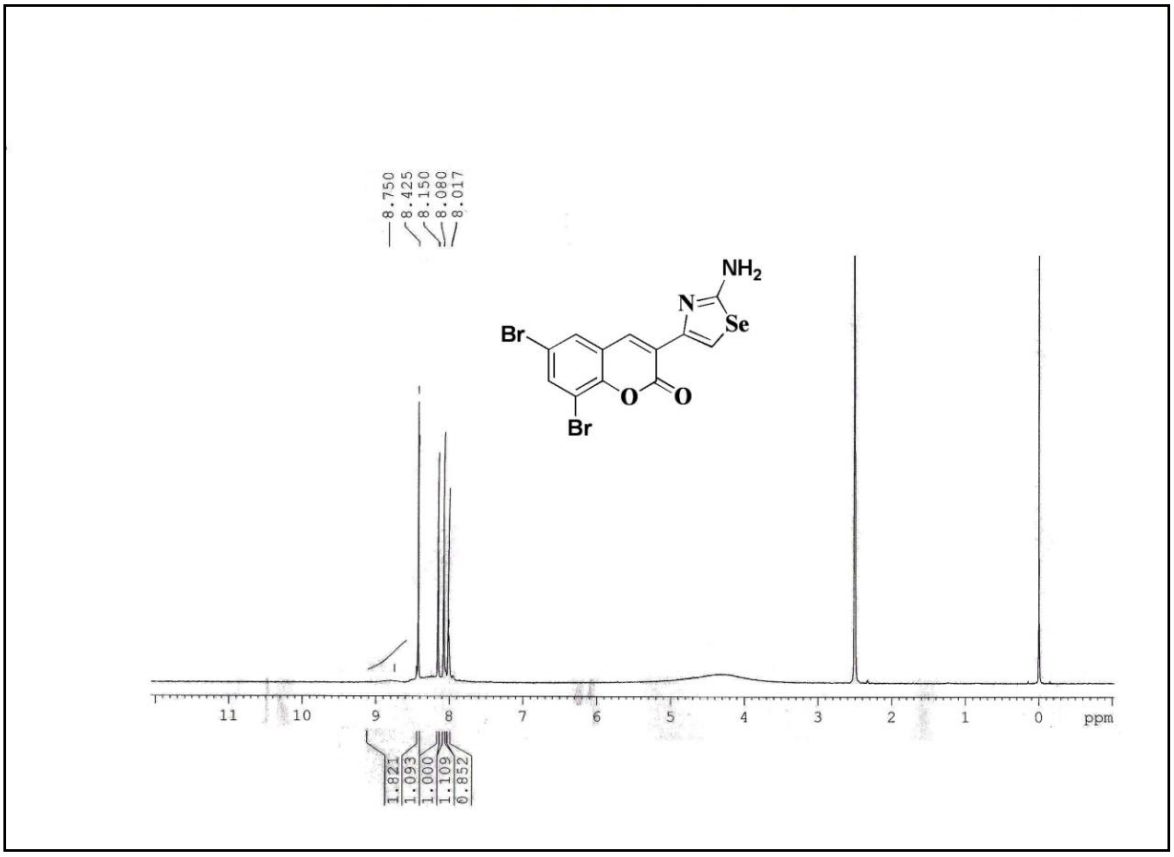
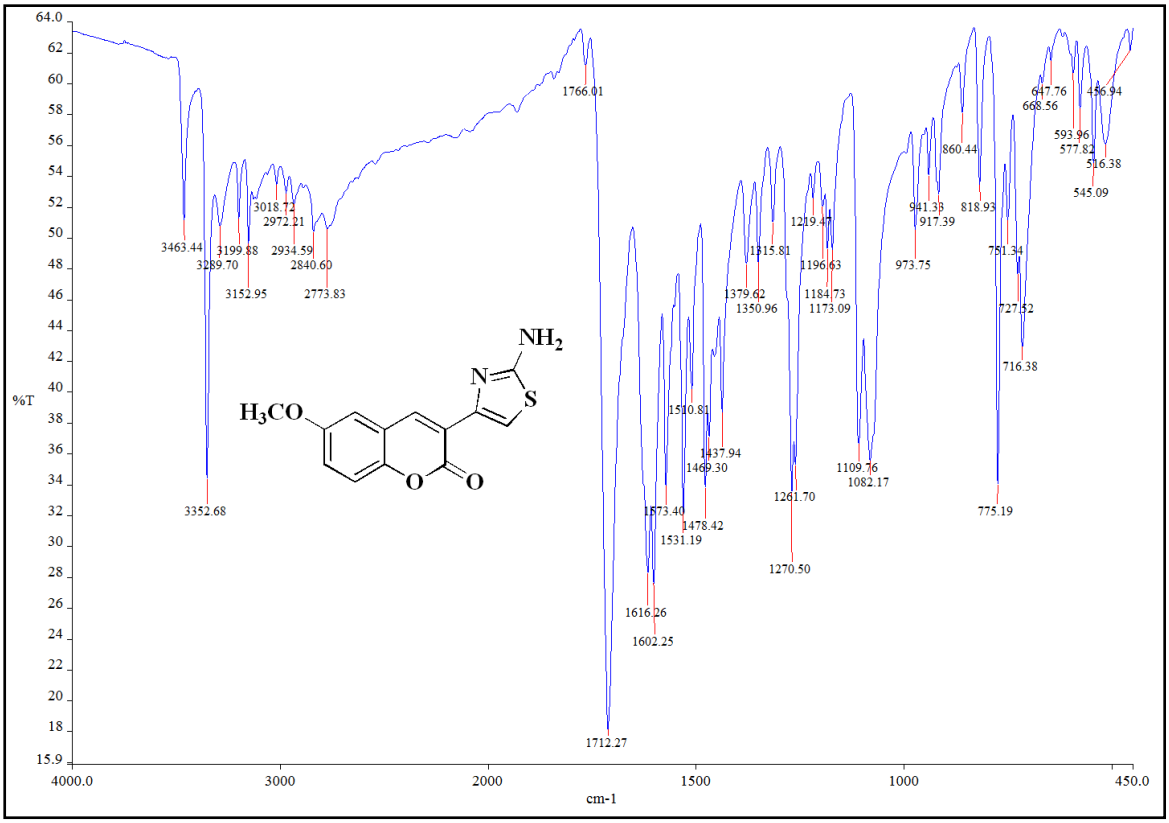
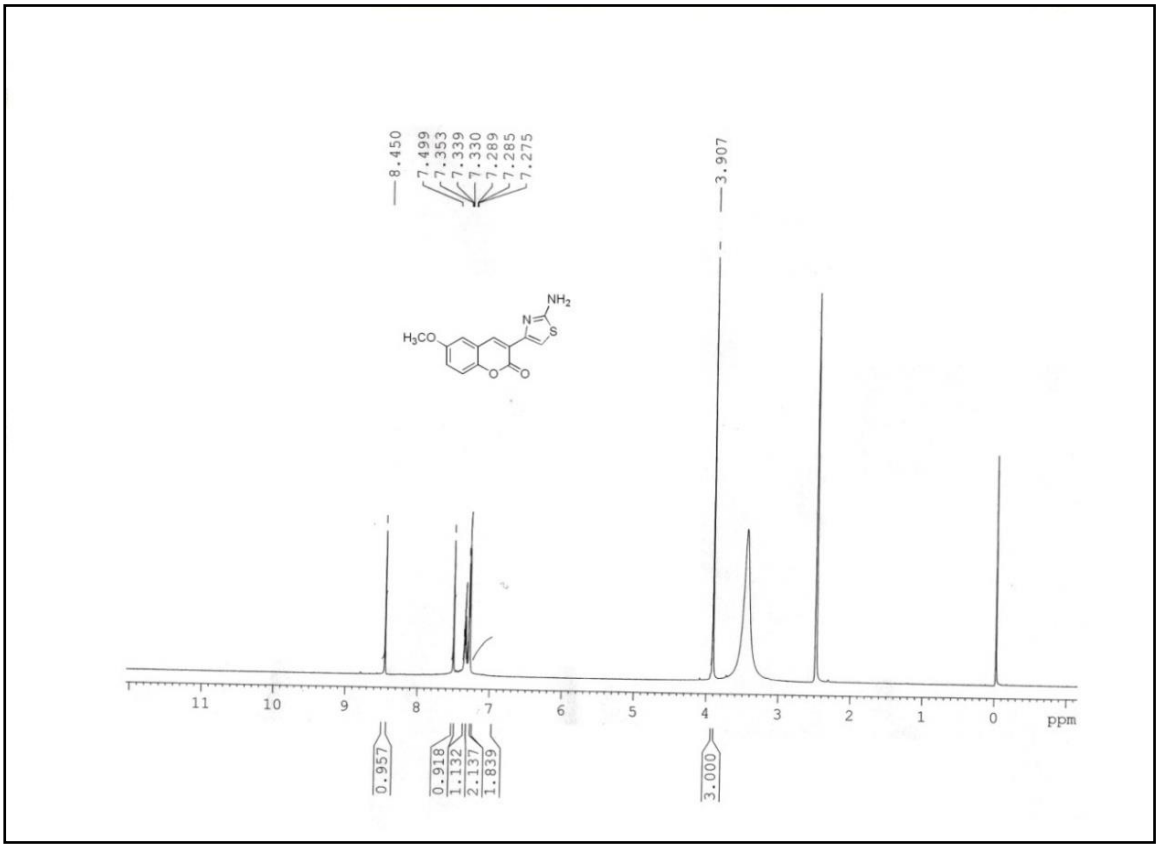


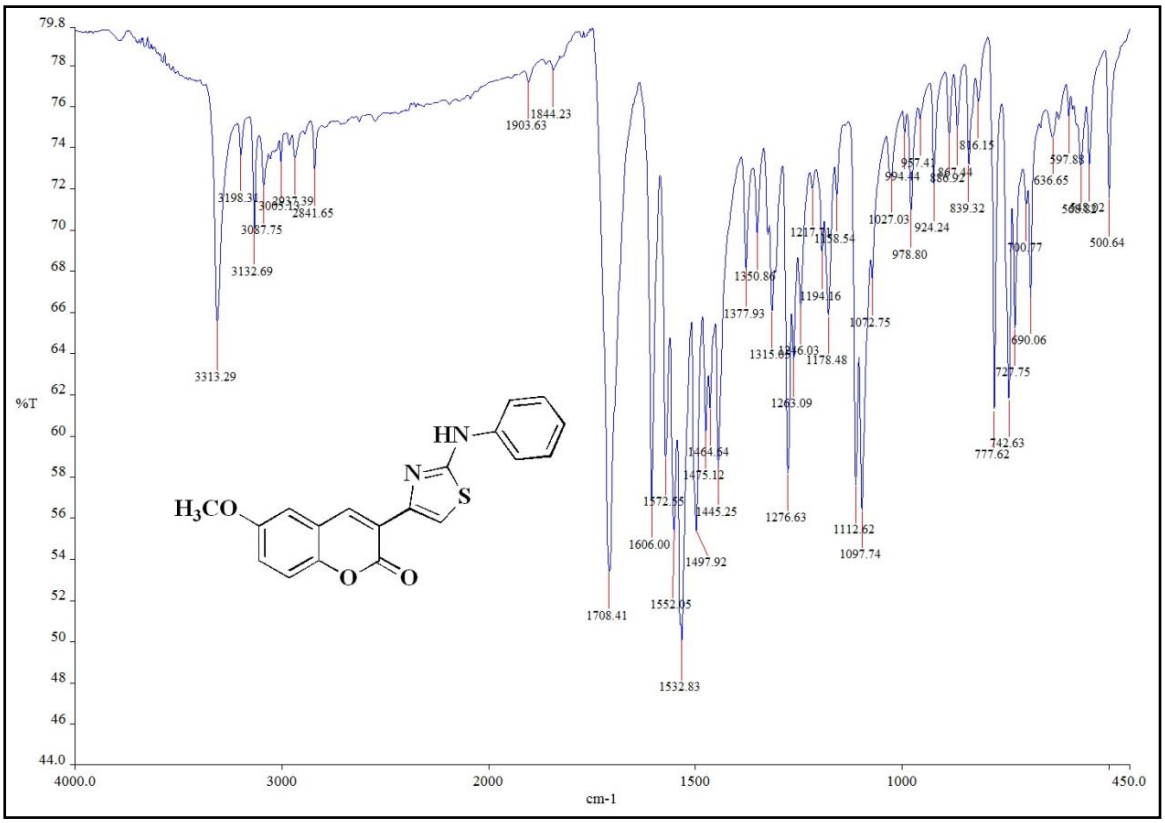
^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound 14c



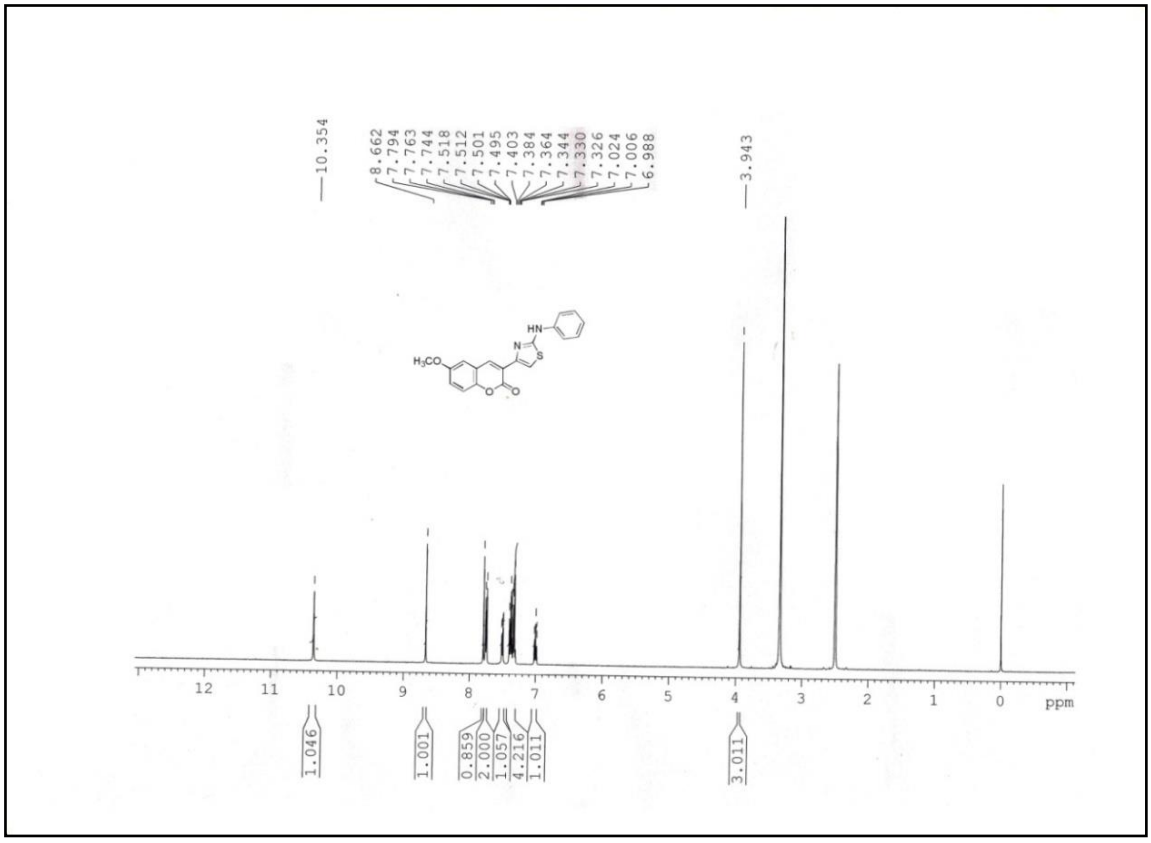
IR (KBr) spectrum of compound 15a



^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound 15a



IR (KBr) spectrum of compound 15b



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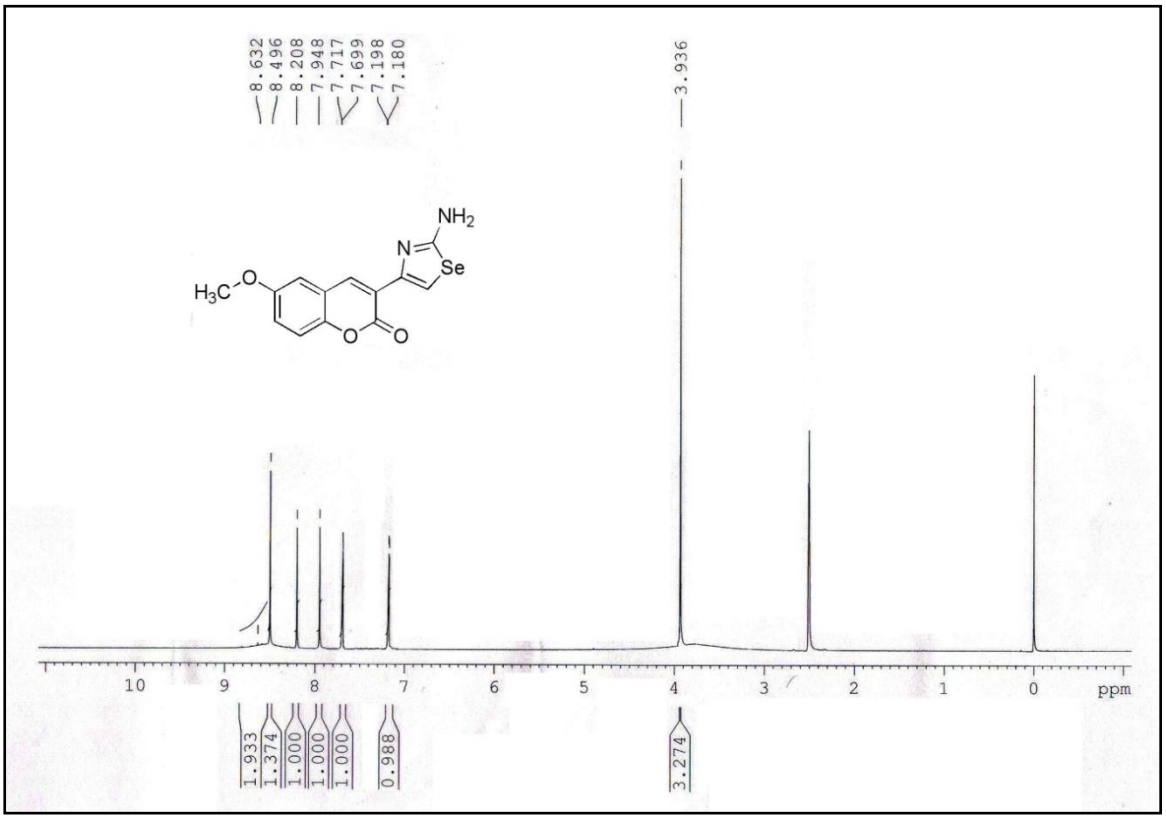

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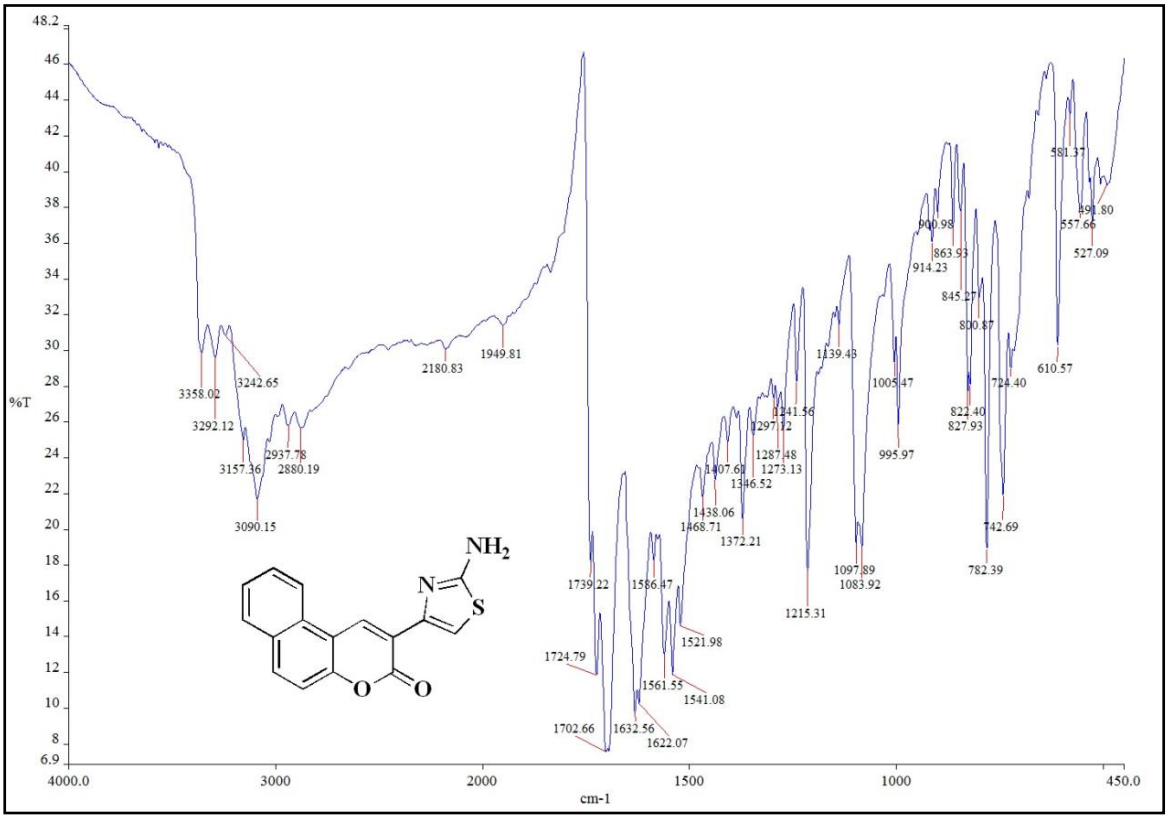
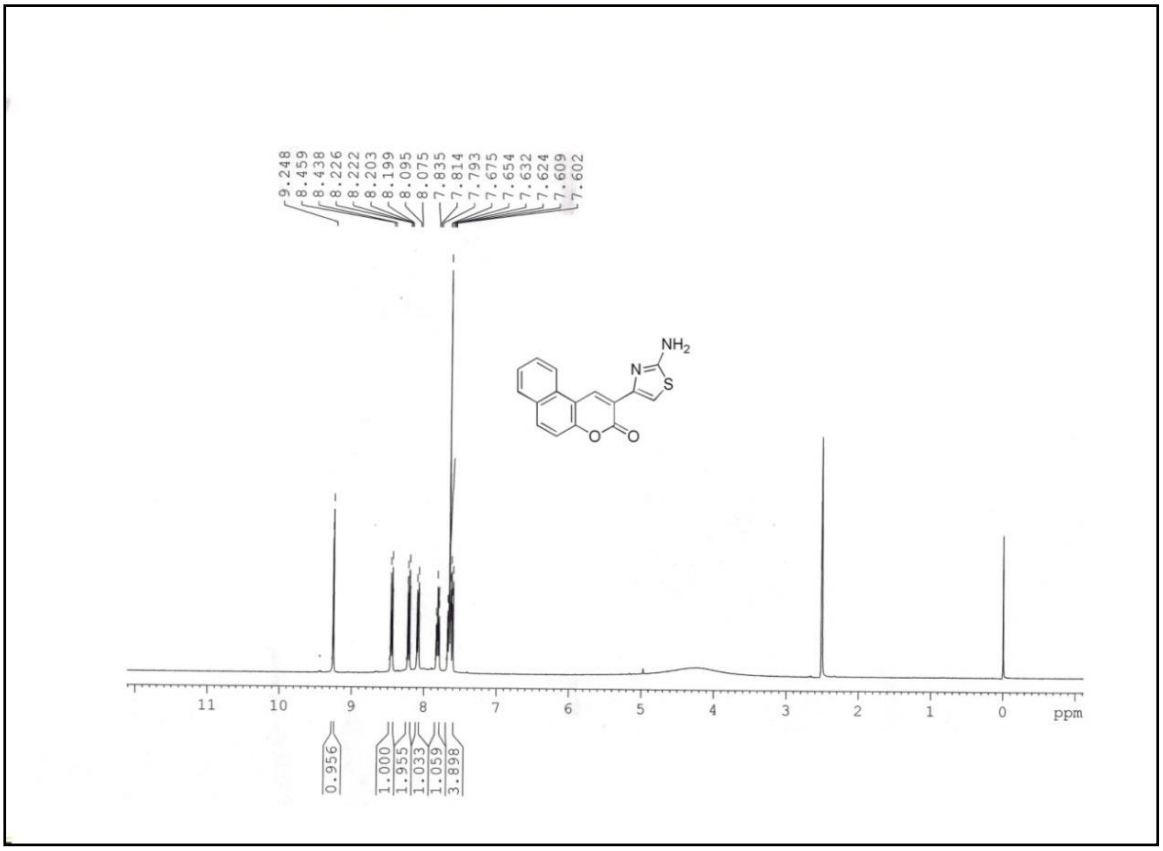
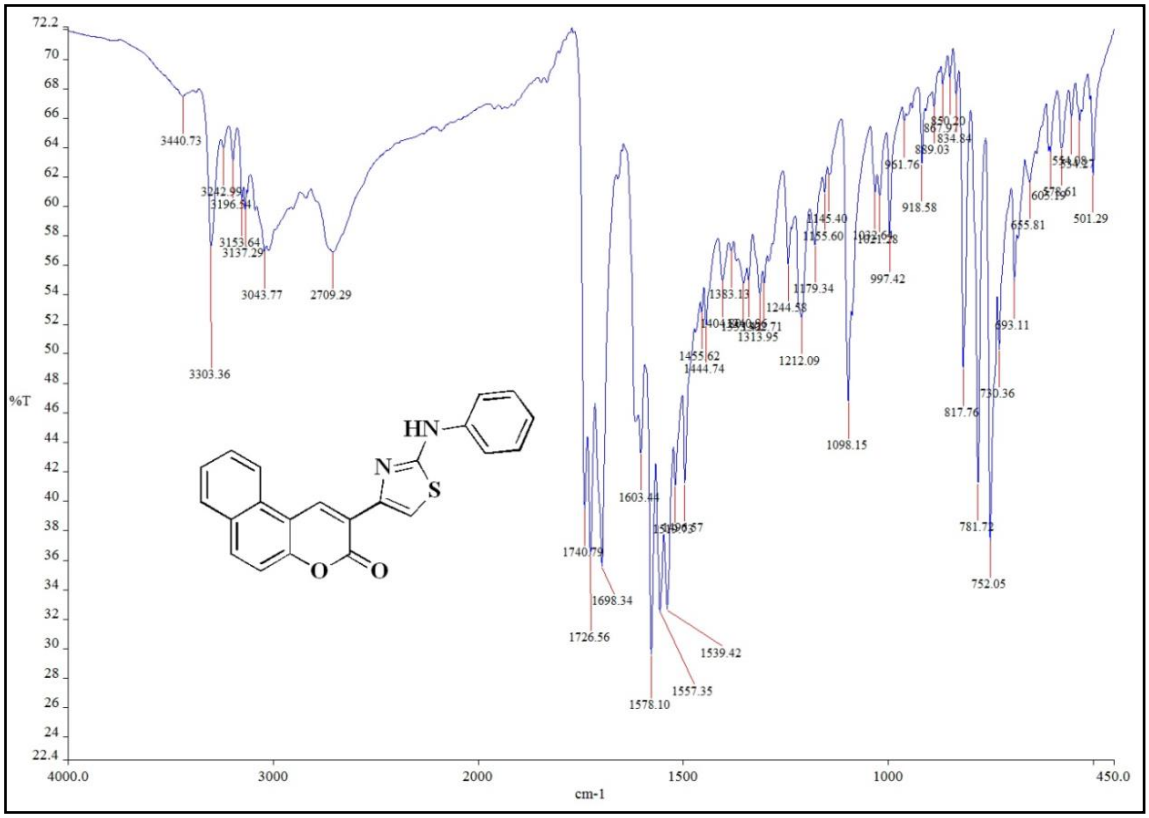


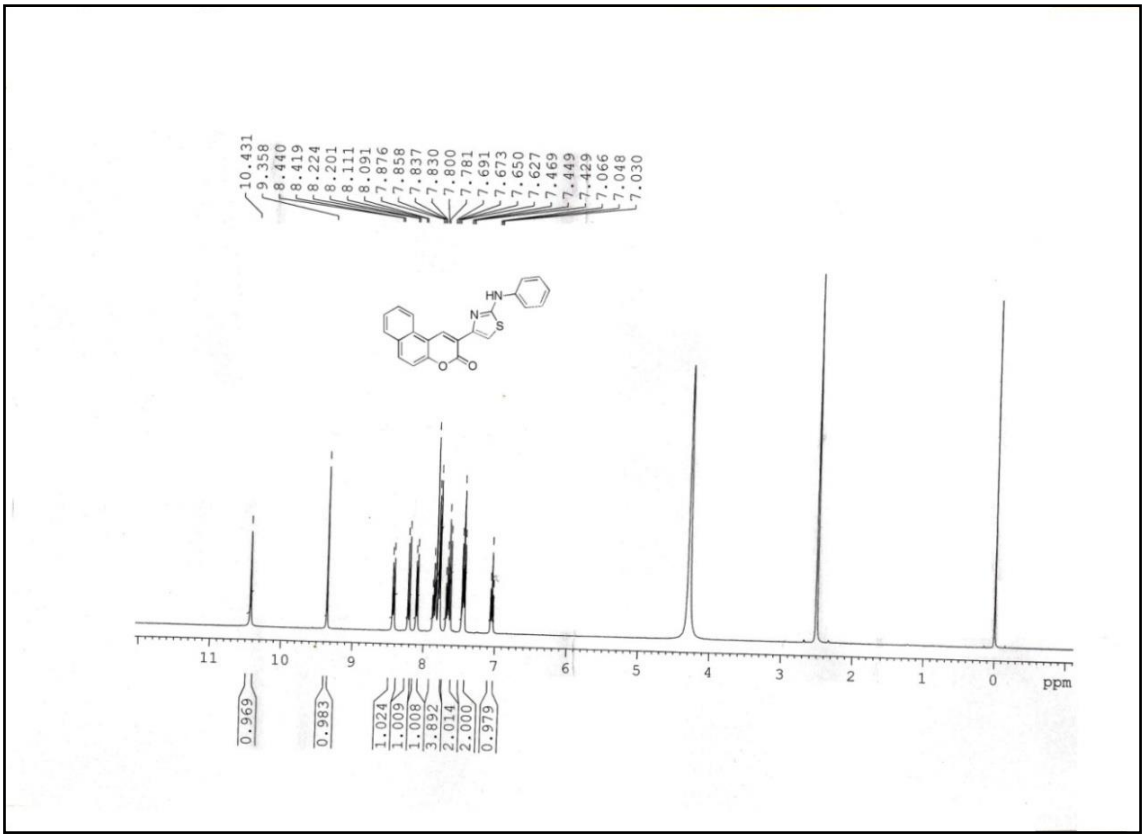
IR (KBr) spectrum of compound 16a



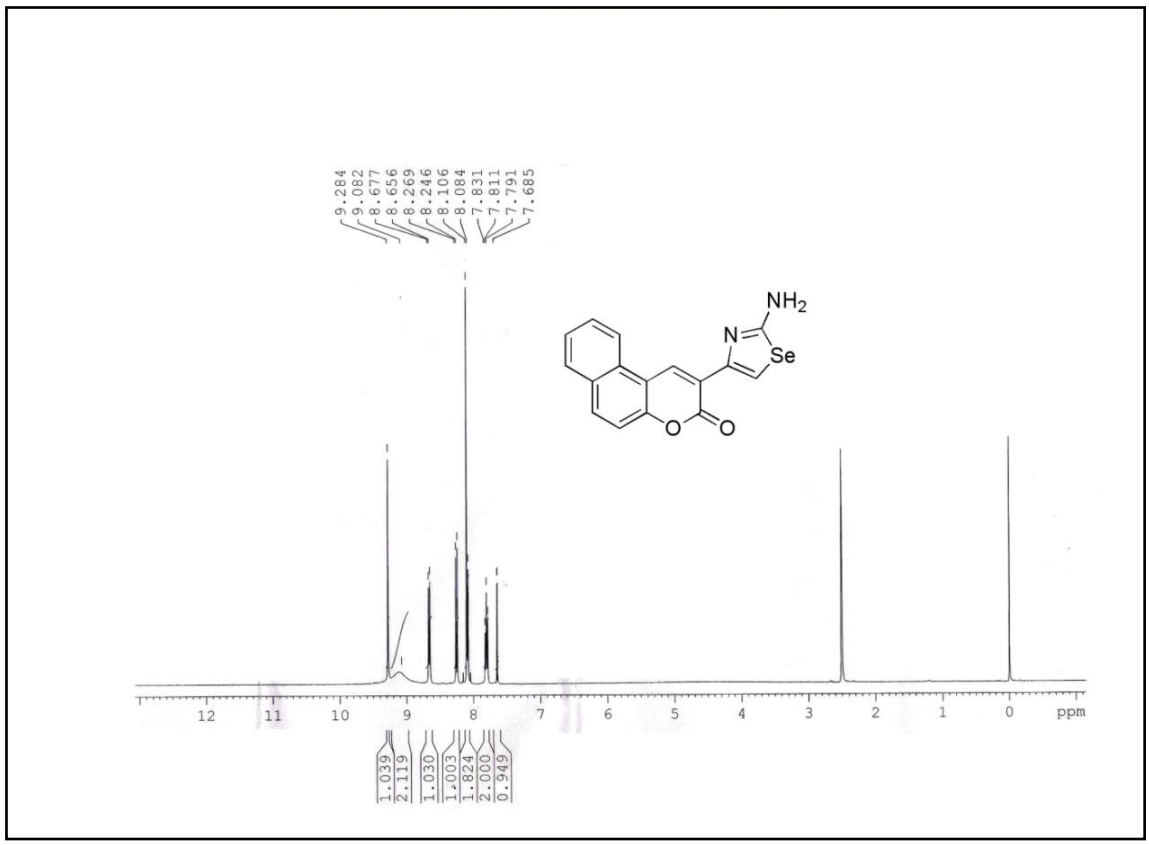
^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound 16a



IR (KBr) spectrum of compound 16b



^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound 16b



^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound 16c

CHAPTER-VI (SECTION-B)

RECYCLABLE TASK SPECIFIC ACIDIC IONIC LIQUID [NMP]H_2PO_4: MICROWAVE-ASSISTED EFFICIENT ONE-POT TWO STEP TANDEM SYNTHESIS OF FUSED THIAZOLO[2,3-*b*]QUINAZOLINONE AND THIAZOLO[2,3-*b*]QUINAZOLINE DERIVATIVES

INTRODUCTION

Fused thiazolo[2,3-*b*]quinazolinone and thiazolo[2,3-*b*]quinazoline derivatives represent an important class of heterocyclic compounds and emerged as an important lead molecules in a variety of therapeutic areas. Their synthesis have gained much attention and attracted the synthetic and medicinal chemists due to their versatile pharmacological properties. The diverse biological applications includes antibiofilm,[1] antimicrobial,[2] antifungal,[3] antiviral,[4] analgesic, antiulcer,[5] antioxidant,[6] anti-inflammatory,[7] antitubercular,[8] anticancer,[9] antitumor,[10] antihypertensive,[11] anticonvulsant,[12] antinociceptive,[13] and antiparkinsonian activities.[14] In addition they are also found to be 5-HT_{2A} receptor[15] and calcium antagonists,[16] (mGluR) metabotropic glutamate receptor (Group II) antagonists and the inhibitors of enzymes; acetylcholinesterase (AChE),[17] xanthine oxidase (XO),[18] diacylglycerol (DG) kinase, cell division cycle 25 (CDC25) phosphatase,[19] HIV-1 reverse transcriptase[20] and the inhibitors of Bcl-2 family of proteins.[21]

R. Gali *et al*.[22] Synthesized a series of novel 10-((1*H*-indol-3-yl)methylene)-7-aryl-7,10-dihydro-5*H*-benzo[*h*]thiazolo[2,3-*b*]quinaz-olin-9(6*H*)-ones and evaluated for their *in vitro* anticancer against human tumour cell lines MCF-7 and HepG2 and antimicrobial activities. Among the tested series, it was observed from the experimental data that, the compound **1** possessing 4-chlorophenyl on pyrimidine ring and 5-bromo indol-3-ylmethylene on thiazole ring was 1.15 fold potent than the positive control Doxorubicin (DOX) against breast cancer cell line-MCF-7. Compounds **2** and **3** bearing 4-fluoro phenyl on pyrimidine ring and 5-bromo indol-3-ylmethylene/indol-3-ylmethylene on thiazole ring were found to be having equipotent antimicrobial inhibition activity against *S. aureus* when compared with standard drug Streptomycin.

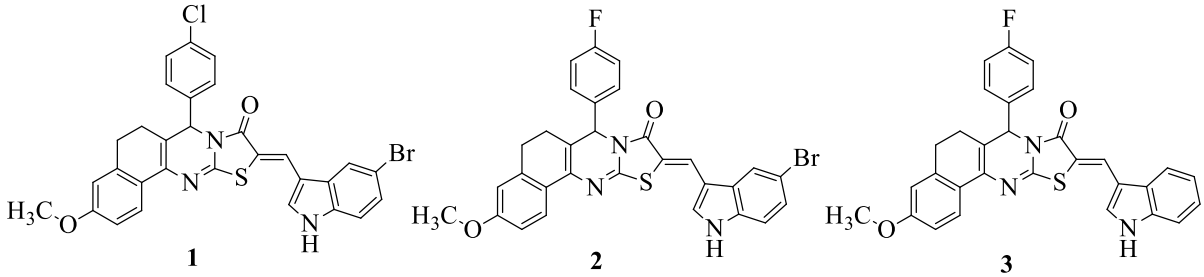


Fig. 1

Y. Feng *et al*.[23] designed and synthesized a new series of quinazoline-2(1*H*)-thione derivatives based on the chemical structure of the hit compound (DCBL55) **4** and reported as specific and potent inhibitors of antiapoptotic Bcl-2 family proteins Bcl-x_L, Bcl-2, and Mcl-1 inhibitor by computer virtual screening. Bcl-2 family members including antiapoptotic proteins (e.g., Bcl-x_L, Bcl-2, and Mcl-1) and proapoptotic proteins (e.g., Bax, Bad, Bid) regulates the mitochondria-mediated apoptotic pathway, the defects in this pathway leads to the Acute myeloid leukemia (AML). All the synthesized compounds were assessed for their Bcl-x_L inhibition activity by Fluorescence Polarization (FP) Assay. The compounds **5**, **6**, **7**, **8** and **9** showed a Bcl-x_L inhibitory activity with IC_{50} values of 1.9, 3.0, 5.2, 5.3 and 5.4 μM respectively.

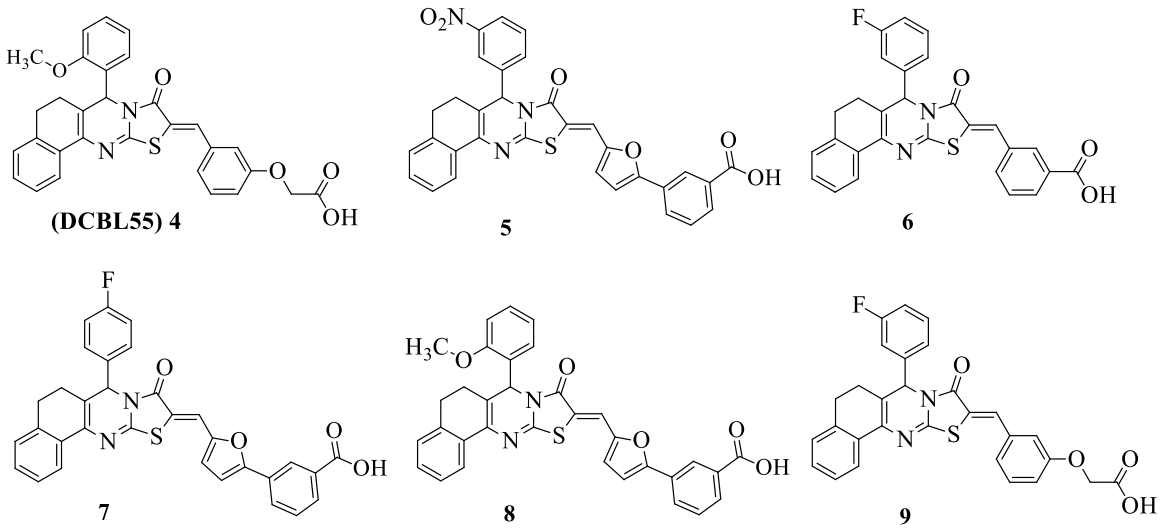
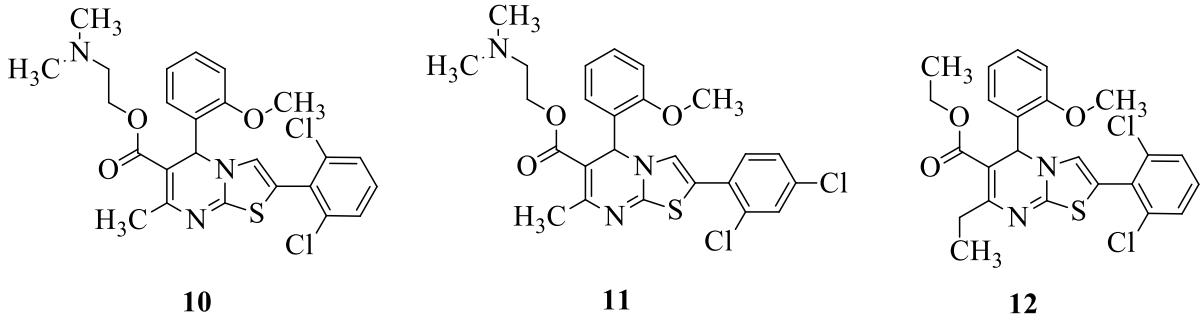


Fig. 2

J. Wichmann *et al*.[24] described the synthesis and reported the first non-amino acid subtype selective mGlu receptor antagonist at group 2 mGlu receptors described so far and explored the structure activity relationship of 5*H*-thiazolo[3,2-*a*]pyrimidine scaffolds. G-protein coupled, or metabotropic glutamate receptors (mGluRs) are one of the major classes of excitatory amino acid (EAA) receptors, which mediate the synaptic excitation in the mammalian central nervous system (CNS). By utilizing the GTPγ^{35}S binding model assessed the activities of the compounds at rat mGlu2 receptors. Out of the tested series, compounds **10**, **11** and **12** were found to be active with *Ki* (μM) values for the inhibition of 1S, 3R-ACPD (10μM) stimulated GTPγ^{35}S binding on rat mGlu2 receptor transfected cell membranes are 1.5, 1.0 and 1.0 μM respectively. Among them, the compound **12**

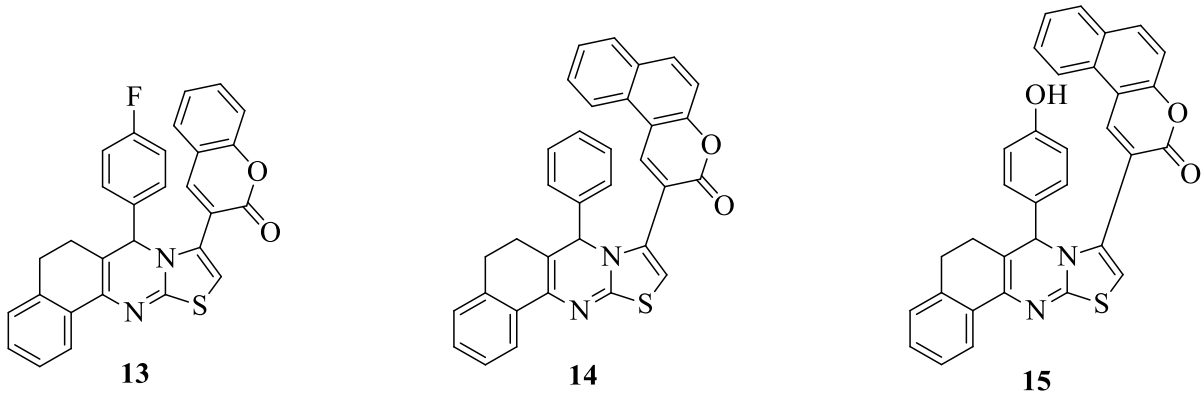
found to be most active and turned out to be selective against mGluR1 and mGluR4 as well as ionotropic receptors.



10 **11** **12**

Fig. 3

Janardhan Banothu *et al.*[25] described the synthesis, characterization and biological evaluation of fused thiazolo[3,2-*a*]pyrimidine derivatives. The *in vitro* antibacterial, antioxidant and DNA cleavage activities suggesting that, the synthesized compounds **13**, **14** and **15** can be better candidates for future investigations.

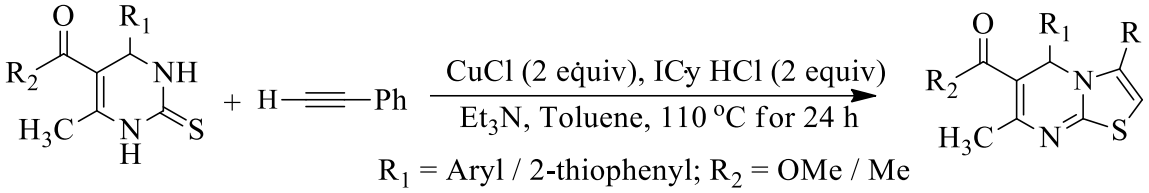


13 **14** **15**

Fig.4

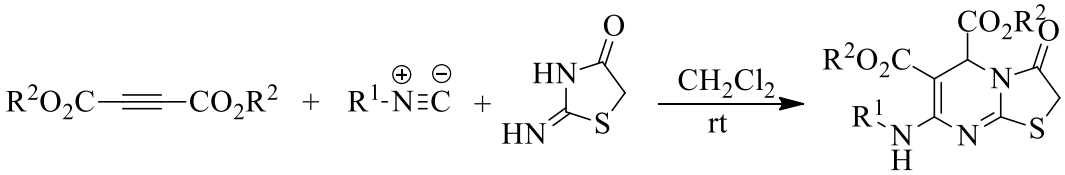
Various important approaches for the synthesis of fused thiazolo[2,3-*b*]quinazolinone and thiazolo[2,3-*b*]quinazolines derivatives

Dongmei Xiao and co-workers[26] described the Copper-mediated synthesis of *N*-fused heterocycles *via* C_{sp}–S coupling reactionand 5-*endo-dig* cyclization sequence.



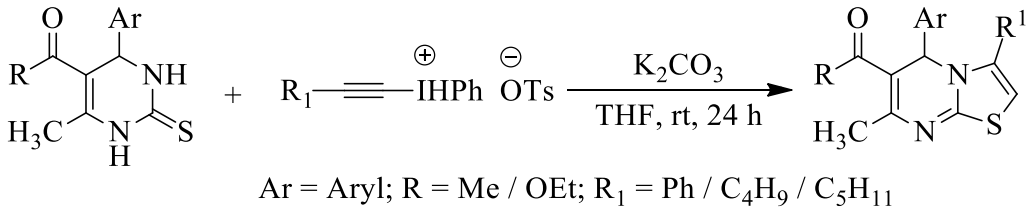
Scheme-1

A. E. Abbas and co-workers[27] described an An efficient regioselective synthesis of highly functionalized 3-oxo-2,3-dihydro-5*H*-thiazolo[3,2-*a*]pyrimidines via an isocyanide-based three-component reaction**.**



Scheme-2

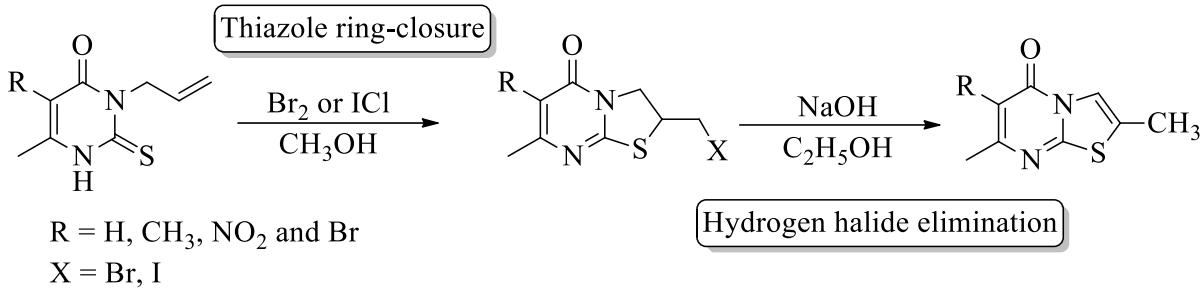
Amol V. Shelke[28] reported New synthesis of 3,5-disubstituted-5*H*-thiazolo[3,2-*a*]pyrimidine via ring annulation of 3,4-dihydropyrimidin-2(1*H*)-thione using alkynyl(aryl)iodonium salts.



Scheme-3

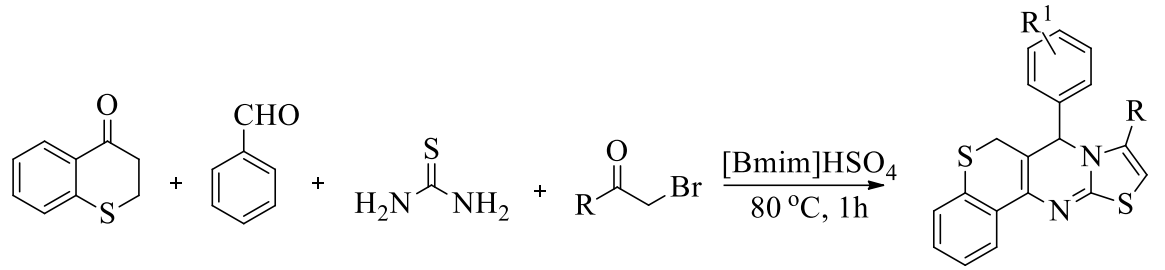
R. Studzinska *et al*.[29] reported the facile synthesis of novel thiazolo[3,2-*a*]pyrimidin-5-ones from dihydrothiazolo[3,2-*a*]pyrimidin-5-ones, the latter was synthesized by a simple

reaction: thiazole ring-closure of 3-allyl-2-thiouracil derivatives with bromine or iodine chloride followed by the halide elimination.



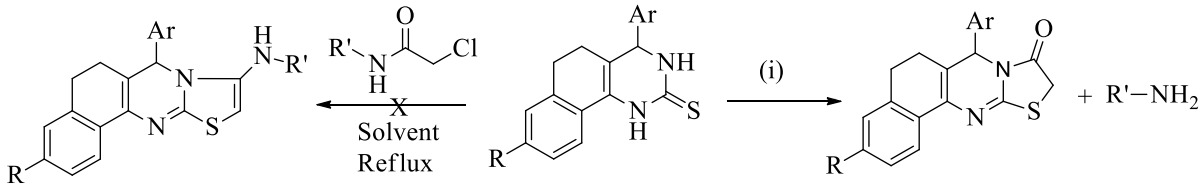
Scheme-4

Lingala Suresh *et al.*[30] have reported an expeditious four-component domino protocol for the synthesis of novel thiazolo[3,2-*a*]thiochromeno[4,3-*d*]pyrimidine derivatives as antibacterial and antibiofilm agents.



Scheme-5

Janardhan Banothu *et al.*[31] praposed the synthesis of fused thiazolo[3,2-*a*]pyrimidinones: *N*-aryl-2-chloroacetamides as doubly electrophilic building blocks.



Reaction conditions: (i) ***Method A*:** 2-Chloro-*N*-phenylacetamide (8), without or with Et_3N/AcONa/KOH (base), acetic acid, reflux, 4-6 h; ***Method B*:** *N*-(benzo[*d*]thiazol-2-yl)-2-chloroacetamide (9), without or with Et_3N/AcONa/KOH (base), 1,4-dioxane, reflux, 4-6 h.

Scheme-6

PRESENT WORK

Our nature provided a beautiful atmosphere for all living beings to lead their life healthier. But unfortunately, the most polluting species, the mankind, being damaging the atmosphere by exploiting and polluting the environment by conserving all the non-renewable energy resources and dumping hazardous waste materials. Hence, protecting, nurturing of our mother atmosphere, giving a healthy environment and energy resources to our future generation is emerging challenge to the scientific community by following the basic principles of Green chemistry[32]

In the direction of development of green approaches, over the past decades, many new synthetic methodologies (MCR approach, solid phase synthesis, ultrasonication, microwave irradiation, aqueous medium reactions, solvent-free reactions, using ionic liquids (ILs) as duel solvent-catalysts and phase transfer catalysis), analytical techniques (grinding and micellar catalysis) have been developed and employed in synthetic organic chemistry towards the designing of more environmentally sound and green procedures.

In this context, multi-component reactions (MCRs)[33] emerged as one of the most powerful strategies that involve more than two easily accessible reactants to give multi-functionalized complex structures in a single synthetic operation. This simple, atom economy and time-saving method became a major tool in the synthesis of diverse combinatorial libraries[34] and complex organic molecules of potential interest particularly in the area of drug discovery[35]. However, multi-component protocol generally advantageous over stepwise and divergent chemical processes in terms of lower reaction times, rapid reaction rates leading to higher yields and reproducibility[36].

Among the above mentioned various synthetic methodologies, the microwave-assisted organic synthesis[37] has gained much attention as one of the important alternative to conventional heating for promoting a variety of organic transformations[38] and also to make up the drawbacks raised in classical synthesis. Microwave irradiation technique has been preferred in scientific community due to its promising advantages like dramatic reduction in reaction times by choosing lower energy pathways, selecting suitable microwave energy and its uniform heat distribution, simple purification steps by reducing the side products, lower energy consumption and higher atom economy *via* optimized conditions.

From the view of green chemistry, using eco-friendly non-flammable recyclable task-specific dual solvent catalyst ionic liquids[39] have shown attractive alternative to polluting traditional homogeneous, heterogeneous catalysts and volatile organic solvents. The synergistic combination of MCR and task specific ILs (TSILs) already proven to be outstanding green protocol because of their unique properties, that includes, product distribution, negligible vapour pressure, recyclability, catalyst-immobilization, thermal stability, tunable viscosity, miscibility with water and ability to associate with reactants during activation with their solvent cavities[40].

Keeping in view of variety of biological applications of fused thiazolo[2,3-*b*]quinazolinone and thiazolo[2,3-*b*]quinazolines, emerged as an important lead molecules in a variety of therapeutic areas. Their efficient synthesis has attracted the synthetic and medicinal chemists due to their broad range of biological activities.

In our previous work[41], we have described the synthesis of compounds **4** and **5** and evaluated for their biological activity. However, the time and yields of the both of the title compounds were found to be good but, do not be satisfactory. Hence, in order to overcome the time, yield limitations and in search of synergistic and more sustainable synthetic protocols as earlier work[42], we aimed to develop highly efficient and rapid green method, we report herein eco-compatible highly efficient and simple protocol for the synthesis of thiazolo[2,3-*b*]quinazoline (**4a-f**) and thiazolo[2,3-*b*]quinazolinone (**5a-t**) derivatives by combined use of recyclable [NMP]H_2PO_4 as a dual green catalyst and microwave irradiation *via* MCR approach under solvent-free conditions.

Synthesis of starting materials

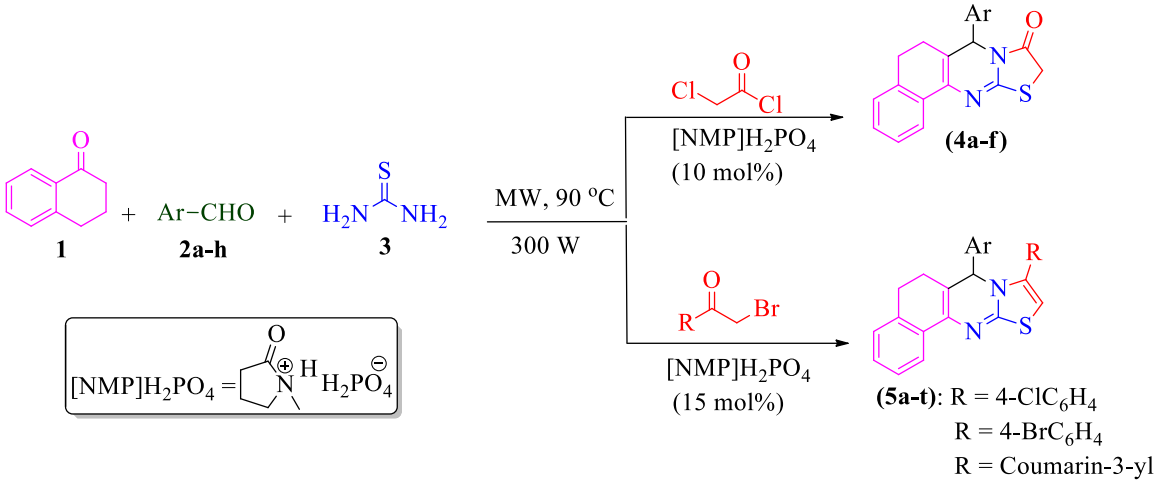
3-acetyl-2*H*-chromen-2-one and 3-(2-bromoacetyl)-2*H*-chromen-2-one

3-acetyl-2*H*-chromen-2-one and 3-(2-bromoacetyl)-2*H*-chromen-2-one were prepared according to the literature procedure as described in **Chapter-II**, **Section-A**.

Synthesis of thiazolo[2,3-*b*]quinazolinone (4a-f) and thiazolo[2,3-*b*]quinazoline (5a-t) derivatives

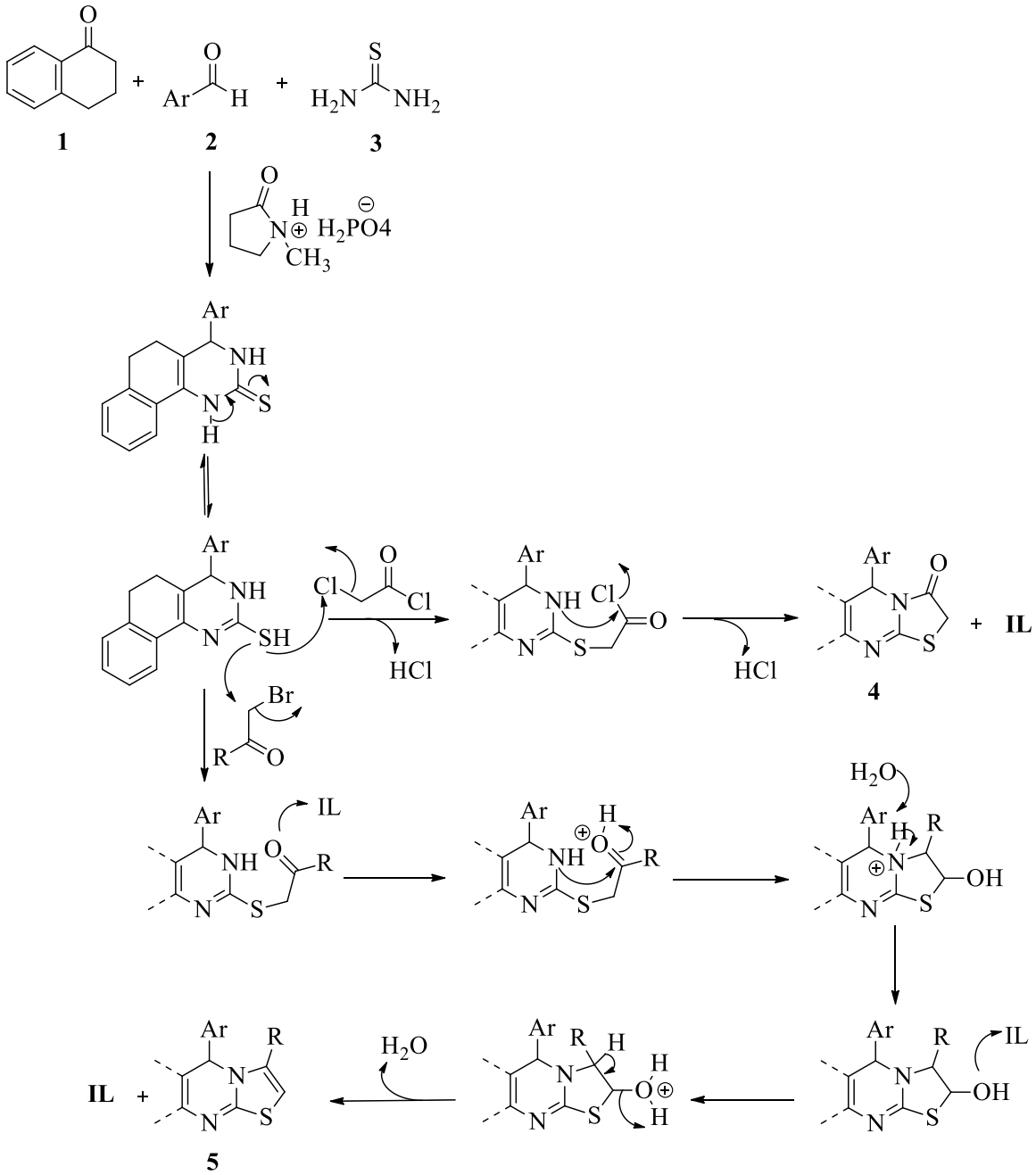
Condensation of an equimolar concentrations of 1-tetralone (**1**), substituted aromatic aldehydes (**2a-h**), thiourea (**3**) was carried out under MW irradiation in a 10 mL pressurized vial at 90 ^oC. After ensuring the completion of the reaction by TLC, was

added equimolar concentration of chloroacetyl chloride or substituted phenacyl bromides/3-(2-bromoacetyl)-2*H*-chromen-2-one and continued the process for the appropriate time as mentioned in **Table 3**. Cold water was added to the reaction mixture after the completion of the reaction, furnished the title compounds **(4a-f)** and **(5a-t)** respectively.



Scheme-7

Plausible Mechanism



Scheme-8

Results and discussion

Our aim to synthesize the title compounds **4** and **5** in excellent yields were achieved by following the steps outlined (Scheme 1). The reaction mixture was exposed to microwave irradiation at 300 W intermittently at 10s intervals *via* synergistic effect of ionic liquid at ambient temperature (90 ^{o}C) afforded the final compounds with in a very short time (2-8 min) in quantitative yields (92-98 %) (**Table 1**).

In order to find out an efficient and suitable catalytic medium, that can achieve the final proposed structures **4a** and **5a** was examined under different catalytic effects by conducting two model reactions (model-1 and model-2) between tetralone **1** (1 mmol), benzaldehyde **2a** (1 mmol), thiourea **3** (1 mmol), chloroacetic acid (1 mmol) and 4-chloro phenacyl bromide (1 mmol) respectively. From that it was clear that, when non-imidazolium ionic liquids were employed, the maximum and best yields of about 91 % and 80 % (entry 5) and 88 % and 73 % (entry 4) of conversion was observed in case of model-1 and model-2 reactions respectively (entry 4 & 5, **Table-1**). In case of conventional imidazolium ionic liquids (entry 2 & 3) the conversion was only up to 56 %, 72 % (model-1) and 60 %, 65 % (model-2), but the yields are not being satisfactory. Coming to the [BMIM]BF_4 was less efficient with conversion of 42 % (model-1) and 58 % (model-2). Before going to test the effect of various ionic liquids on the model reactions, initially, we have performed the same model reactions under catalytic-free conditions, from that it was clear that, for the transformation of above model reactions, the presence of catalysts should be necessary. Further, to investigate the optimized reaction conditions, we have conducted same model reactions at different MW-frequencies at different catalyst loadings under variety of solvents like, water, ethanol, acetic acid and acetonitrile. In order to highlight the role of MWs in these systems, also conducted the model reactions under controlled conditions (in the absence of MW-irradiation), from that we have noticed that, rapid heat transfer facilitated by MW heating allows the chemical transformations very rapidly compared to that of conventional heating, thereby limiting the formation of side products and considerable improvement in the product yield. High heating efficiency of microwaves providing, low catalyst loading, remarkable rate enhancement, dramatic reduction in reaction time. The above results revealed that, the best and optimized results were obtained at 90 ^{o}C under solvent free conditions at 10 mol% of [NMP]H_2PO_4 for the compound **4a** (98 %) and 15 mol% of

catalytic load for the compound **5a** (92 %) at microwave frequency of 300 W. The results were illustrated in **Table 2**.

Table 1. The effect of catalysts on a model reactions[a].

Entry	Catalyst	Yield[b] (%)/Time (min)	
		Model-1	**Model-2**
1	–	0/60	0/60
2	[BMIM]BF_4	42/60	58/60
3	[BMIM]HSO_4	56/60	60/60
4	[HMIM]HSO_4	72/60	65/60
5	[NMP]HSO_4	88/60	73/60
6	[NMP]H_2PO_4	91/60	80/60

[a]Reaction was carried on 1 mmol scale (entries 1-6, **Table 1**) by taking 10 mol % of the catalyst.
[b]Yields on isolated basis.

Table 2. Optimization of the reaction conditions for the synthesis of compounds **4a** (model-1) and **5a** (model-2)[a]

Entry	Catalyst Loading [NMP]H_2PO_4 (mol%)	Solvent	Temp (ºC)/Power (W)	Time (min)		Yield[b] (%)	
				Model-1	Model-2	Model-1	Model-2
1	5	–	–	60	60	40	38
2	10	–	–	30	30	91	80
3	5	–	70/200	15	17	65	54
4	10	–	70/200	9	15	91	70
5	15	–	70/200	12	10	73	83
6	20	–	70/200	10	12	88	62
7	30	–	70/200	20	22	85	72
8	10	–	90/300	2	9	98	85
9	15	–	90/300	10	5	81	92
10	15	Water	90/300	45	42	20	15
11	15	Ethanol	90/300	30	32	65	33
12	15	Acetic acid	90/300	17	22	85	72
13	15	Acetonitrile	90/300	20	21	70	62
14	15	–	100/300	10	11	80	69
15	15	–	120/300	10	13	78	71

[a]Reaction was carried on 1 mmol scale (entries 1-13, **Table 2**) by taking ionic liquid at different catalytic loads and at different reaction conditions.

Utilizing the above optimized conditions, the scope and efficiency of the procedure was explored and successfully synthesized structurally diverse thiazolo[2,3-*b*]quinazolinone **4** (**a-f**) and thiazolo[2,3-*b*]quinazoline **5** (**a-t**) derivatives in excellent yields (92-98%) with in a very short reaction time (2-8 min) and summarized in **Table 3**. All the synthesized compounds were well established by spectral and elemental analysis and also comparing with their literature melting points[41].

Table 3. Synthesis of benzo[*h*]thiazolo[2,3-*b*]quinazolinone (**4a-f**) and benzo[*h*]thiazolo[2,3-*b*]quinazoline (**5a-t**) derivatives[a].

Analogs[a]	Ar	R	Time (min)	Yield[b] (%)	Melting point (°C)	
					Observed	Lit value
4a	C_6H_5	–	2	98	191-193	192-194
4b	4-OHC_6H_4	–	4	96	286-288	285-287
4c	4-$OCH_3C_6H_4$	–	3	96	180-182	179-181
4d	3,4-$(OCH_3)_2C_6H_3$	–	4	95	121-123	122-124
4e	4-ClC_6H_5	–	6	93	210-212	208-210
4f	3-$NO_2C_6H_4$	–	6	92	212-214	214-216
5a	C_6H_5	4-ClC_6H_4	5	92	318-320	319-320
5b	4-OHC_6H_4	4-ClC_6H_4	6	94	282-294	292-294
5c	4-OH-3$OCH_3C_6H_3$	4-ClC_6H_4	5	95	271-272	271-273
5d	4-$OCH_3C_6H_4$	4-ClC_6H_4	5	94	259-260	260-262
5e	3,4-$(OCH_3)_2C_6H_3$	4-ClC_6H_4	5	94	231-233	233-236
5f	4-FC_6H_4	4-ClC_6H_4	8	92	300-301	301-303
5g	4-ClC_6H_4	4-ClC_6H_4	8	92	315-317	317-318
5h	C_6H_5	4-BrC_6H_4	7	96	322-323	322-324
5i	4-OHC_6H_4	4-BrC_6H_4	6	96	296-298	294-296
5j	4-OH-3$OCH_3C_6H_3$	4-BrC_6H_4	6	93	281-283	280-282
5k	4-$OCH_3C_6H_4$	4-BrC_6H_4	6	97	260-261	261-263
5l	3,4-$(OCH_3)_2C_6H_3$	4-BrC_6H_4	7	94	242-244	242-244
5m	4-FC_6H_4	4-BrC_6H_4	8	92	298-299	298-300
5n	C_6H_5	Coumarin-3-yl	8	96	289-291	290-291
5o	4-OHC_6H_4	Coumarin-3-yl	7	95	292-294	293-294
5p	4-OH-3$OCH_3C_6H_3$	Coumarin-3-yl	7	96	260-261	261-263
5q	4-$OCH_3C_6H_4$	Coumarin-3-yl	5	94	273-275	275-277
5r	3,4-$(OCH_3)_2C_6H_3$	Coumarin-3-yl	5	97	266-268	267-269
5s	4-FC_6H_4	Coumarin-3-yl	8	92	279-281	277-279
5t	4-ClC_6H_4	Coumarin-3-yl	8	94	280-281	280-282

[a]Reaction conditions: Tetralone (**1**, 1 mmol), substituted aromatic aldehydes (**2**, 1 mmol), thiourea (**3**, 1 mmol), chloroacetyl chloride (1 mmol) and substituted phenacyl bromides/3-(2-bromoacetyl)-2*H*-chromen-2-one (1 mmol), [NMP]H_2PO_4 (10 mol% & 15 mol% for the synthesis of compounds **4** and **5** respectively), MW irradiation (300 W), temperature (90 °C), 2-8 min, yield (92-98 %).

Recovery and recyclability of the catalyst

One of the important issues in green synthesis is catalyst recovery. Further to investigate the recyclability of the ionic liquid [NMP]H_2PO_4, distilled water (10 mL) was added to the cooled reaction mixture after completion of the reaction. The separated crude solid product was filtered and washed twice with distilled water (2 x 5mL). The residue (water) containing dissolved IL with halide impurities was collected by evaporating the solvent under reduced pressure and washed with dichloromethane (2 x 10 mL). Further, to the ionic liquid was added *tert*-butanol to strip out the halide impurities (Chloride and Bromide) as volatile *tert*-butyl halides, which can be pumped out away. Also water was added to the ionic liquid to drive off *tert*-butyl halides remains as azeotropes. Finally, the regenerated halide-free ionic liquid (orange colored viscous ionic liquid) was dried under reduced pressure and titrated against $AgNO_3$ to confirm the absence of halides. Then, the catalytic activity of recycled ionic liquid was checked for about 5 cycles at same optimistic conditions for the same model reactions (**Table 2**) and the results were summarized in **Table 4**. From the results it was confirmed that, the recycled IL showed considerable catalytic activity and can be reused effectively up to 4 cycles without loss of its catalytic activity (**Fig. 1**). But, after performing 5^{th} recyclable cycle there is a considerable decrease in the yields was observed.

Table 4. Reusability of [NMP]H_2PO_4 in the synthesis of title compounds **4a** and **5a**[a].

Run	Reaction Time (min)	Yield[b] (%)	
		Model-1	**Model-2**
1	2	98	88
2	2	97	82
3	2	94	81
4	2	91	83
5	2	76	65

[a]Reaction was carried on 1 mmol scale (entries 1-4, **Table 4**) by taking 10 mol % of the catalyst at optimized conditions
[b]Yields on isolated basis.

Conclusions

In summary, our newly developed protocol using task specific acidic ionic liquid [NMP]H_2PO_4 under microwave irradiation in one pot *via* MCR approach is an efficient,

simple, rapid and environmentally benign for the quantitative and qualitative synthesis of series of thiazolo[2,3-*b*]quinazolinone and thiazolo[2,3-*b*]quinazoline derivatives. This method is complementary to the classical method and offers advantages over conventional in terms of yield, time and operational simplicity. The non-imidazolium ionic liquid [NMP]H_2PO_4 can be recyclable and reusable as a catalyst, effective for about 4 cycles.

Experimental

General procedure for the preparation of synthesis of *N*-Methyl-2-pyrrolidonium dihydrogen phosphate [NMP]H_2PO_4 acidic ionic liquid[43]

N-methyl-2-pyrrolidinone (1 mmol) was slowly added drop-wise to the cooled equimolar concentration of phosphoric acid (1 mmol), and the resulting mixture was heated at 80 ^oC for 24h. The mixture was cooled to ambient temperature and washed with ether to remove any non-ionic residues. Then the residue is dried under high vacuum at 80 ^oC on a rotary evaporator until the weight of the residue remains constant. The obtained orange coloured, viscous ionic liquid was characterized by comparing the ^1H NMR of the authentic sample.

General procedure for the synthesis of thiazolo[2,3-*b*]quinazolinone derivatives (4a-f)

A equimolar mixture of tetralone, substituted aromatic aldehydes, thiourea and acidic task specific ionic liquid [NMP]H_2PO_4 (10 mol%) were placed in a 10 mL pressurized vial and subjected to MW irradiation [mono-mode CEM Discover microwave synthesis system at 300 W] at a temperature 90 ^oC for about 2-6 min, after that was added chloroacetyl chloride (1 mmol) and continued the process for the appropriate time as mentioned in Table 3. After ensuring the completion of the reaction (monitored by TLC) was added 10 mL of cold water to the cooled reaction mixture, the precipitated solid was filtered under vacuum and washed with distilled water furnished the compound (**4a-f**). The residue (water) containing dissolved IL was collected by evaporating under reduced pressure.

General procedure for the synthesis of thiazolo[2,3-b]quinazoline derivatives (5a-t)

A equimolar mixture of tetralone, substituted aromatic aldehyde, thiourea and acidic task specific ionic liquid [NMP]H_2PO_4 (15 mol%) was placed in a 10 mL pressurized vial and subjected to MW irradiation [mono-mode CEM Discover microwave synthesis

system at 300 W] at a temperature 90 ^{o}C for about 5-8 min, after that was added substituted Phenacyl bromides/3-(2-bromoacetyl)-2*H*-chromen-2-one (1 mmol) and continued the process for the appropriate time as mentioned in Table 3. After ensuring the completion of the reaction (monitored by TLC) was added 10 mL of cold water to the cooled reaction mixture, the precipitated solid was filtered under vacuum and washed with distilled water afforded (**5a-t**). The residue (water) containing dissolved IL was collected by evaporating under reduced pressure.

Spectral data

***7*-Phenyl-5,7-dihydro-6*H*-10-thia-7a,11-diaza-cyclopenta[*b*]phenanthren-8-one (4a)**

Orange solid; **IR** (KBr) υ_{max} (cm^{-1}): 1724 (C=O), 1635 (C=N), 1598 (C=C); **^{1}H NMR** (400 MHz, DMSO-d_6): δ 1.85-1.91 (m, 1H), 2.22-2.30 (m, 1H), 2.56-2.63 (m, 1H), 2.70-2.76 (m, 1H), 4.01-4.13 (m, 2H), 5.65 (s, 1H), 7.11 (d, *J* = 7.2 Hz, 1H), 7.16-7.26 (m, 2H), 7.32-7.37 (m, 5H), 7.78 (d, *J* = 7.6 Hz, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 170.9, 155.0, 140.0, 134.9, 132.3, 128.7, 128.3, 127.5, 127.3, 126.3, 122.6, 115.2, 58.8, 31.5, 26.7, 24.2; **MS** (ESI) *m/z*: 333 (M+H); Anal. Calcd. for $C_{20}H_{16}N_2OS$: C, 72.26; H, 4.85; N, 8.43; Found: C, 72.04; H, 4.62; N, 8.20.

7-(4-Hydroxy-phenyl)-5,7-dihydro-6*H*-10-thia-7a,11-diaza-cyclopenta[*b*]phenanthren-8-one (4b)

White solid; **IR** (KBr) υ_{max} (cm^{-1}): 3300 (OH), 1755 (C=O), 1674 (C=N), 1579 (C=C); **^{1}H NMR** (400 MHz, DMSO-d_6): δ 1.85-1.93 (m, 1H), 2.19-2.27 (m, 1H), 2.60-2.66 (m, 1H), 2.70-2.74 (m, 1H), 4.09 (s, 2H), 5.58 (s, 1H), 6.74 (d, *J* = 8.4 Hz, 2H), 7.14-7.26 (m, 5H), 7.84 (d, *J* = 6.8 Hz, 1H), 9.12 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 171.0, 170.0, 157.7, 135.1, 129.7, 129.0, 127.8, 127.4, 126.4, 122.6, 116.3, 115.4, 58.5, 32.2, 26.7, 24.1; **MS** (ESI) *m/z*: 349 (M+H); Anal. Calcd. for $C_{20}H_{16}N_2O_2S$: C, 68.94; H, 4.63; N, 8.04; Found: C, 68.87; H, 4.71; N, 7.89.

7-(4-Methoxy-phenyl)-5,7-dihydro-6*H*-10-thia-7a,11-diaza-cyclopenta[*b*]phenanthren-8-one (4c)

Orange solid; **IR** (KBr) υ_{max} (cm^{-1}): 1719 (C=O), 1636 (C=N), 1605 (C=C), 1248 (C-O-C); **^{1}H NMR** (400 MHz, DMSO-d_6): δ 1.85-1.93 (m, 1H), 2.21-2.27 (m, 1H), 2.58-2.64 (m, 1H), 2.69-2.74 (m, 1H), 3.72 (s, 3H), 3.98-4.11 (m, 2H), 5.59 (s, 1H), 6.91 (d, *J* = 8.8 Hz, 2H), 7.11-7.26 (m, 5H), 7.77 (d, *J* = 7.2 Hz, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ

170.9, 159.2, 154.8, 135.0, 132.4, 132.0, 128.8, 127.4, 127.2, 126.3, 122.6, 115.5, 114.0, 58.2, 55.0, 31.5, 26.7, 24.2; **MS** (ESI) *m/z*: 363 (M+H); Anal. Calcd. for $C_{21}H_{18}N_2O_2S$: C, 69.59; H, 5.01; N, 7.73; Found: C, 69.52; H, 5.19; N, 7.89.

7-(3,4-Dimethoxy-phenyl)-5,7-dihydro-6*H*-10-thia-7a,11-diaza-cyclopenta[*b*] phenanthren-8-one (4d)

Brown solid; **IR** (KBr) υ_{max} (cm^{-1}): 1720 (C=O), 1634 (C=N), 1598 (C=C), 1233, 1138 (C-O-C); **^{1}H NMR** (400 MHz, DMSO-d_6): δ 1.93-1.96 (m, 1H), 2.22-2.30 (m, 1H), 2.58-2.65 (m, 1H), 2.70-2.76 (m, 1H), 3.71 (s, 3H), 3.72 (s, 3H), 4.01-4.11 (m, 2H), 5.59 (s, 1H), 6.82-6.93 (m, 3H), 7.11-7.24 (m, 3H), 7.77 (d, *J* = 7.2 Hz, 1H); **MS** (ESI) *m/z*: 415 (M+Na); Anal. Calcd. for $C_{22}H_{20}N_2O_3S$: C, 67.33; H, 5.14; N, 7.14; Found: C, 67.52; H, 5.27; N, 7.01.

7-(4-Chloro-phenyl)-5,7-dihydro-6*H*-10-thia-7a,11-diaza-cyclopenta[*b*]phenanthren-8-one (4e)

Orange solid; **IR** (KBr) υ_{max} (cm^{-1}): 1732 (C=O), 1635 (C=N), 1596 (C=C), 737 (C-Cl); **^{1}H NMR** (400 MHz, DMSO-d_6): δ 1.82-1.90 (m, 1H), 2.21-2.29 (m, 1H), 2.57-2.65 (m, 1H), 2.70-2.77 (m, 1H), 4.01-4.12 (m, 2H), 5.69 (s, 1H), 7.12 (d, *J* = 7.2 Hz, 1H), 7.17-7.24 (m, 2H), 7.36 (d, *J* = 8.4 Hz, 2H), 7.43 (d, *J* = 8.4 Hz, 2H), 7.78 (d, *J* = 7.6 Hz, 1H); **MS** (ESI) *m/z*: 367 (M+H); Anal. Calcd. for $C_{20}H_{15}ClN_2OS$: C, 65.48; H, 4.12; N, 7.64; Found: C, 65.30; H, 4.29; N, 7.77.

7-(3-Nitro-phenyl)-5,7-dihydro-6*H*-10-thia-7a,11-diaza-cyclopenta[*b*]phenanthren-8-one (4f)

Pale yellow solid; **IR** (KBr) υ_{max} (cm^{-1}): 1719 (C=O), 1634 (C=N), 1606 (C=C), 1528, 1344 (NO_2); **^{1}H NMR** (400 MHz, DMSO-d_6): δ 1.81-1.90 (m, 1H), 2.26-2.34 (m, 1H), 2.57-2.65 (m, 1H), 2.71-2.79 (m, 1H), 4.02-4.13 (m, 2H), 5.93 (s, 1H), 7.12 (d, *J* = 7.2 Hz, 1H), 7.18-7.26 (m, 2H), 7.67-7.82 (m, 3H), 8.17-8.21 (m, 2H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 171.2, 155.2, 147.8, 141.9, 135.1, 134.0, 132.9, 132.1, 130.5, 127.7, 127.2, 126.4, 123.4, 122.8, 122.2, 114.0, 57.9, 31.6, 26.6, 23.8; **Mass** (ESI) *m/z*: 378 (M + H); **MS** (ESI) *m/z*: 378 (M+H); Anal. Calcd. for $C_{20}H_{15}N_3O_3S$: C, 63.65; H, 4.01; N, 11.13; Found: C, 63.50; H, 4.30; N, 11.37.

9-(4-Chlorophenyl)-7-phenyl-6,7-dihydro-5*H*-benzo[*h*]thiazolo[2,3-*b*]quinazoline (5a)

White solid; **IR** (KBr) υ_{max}(cm^{-1}): 1604 (C=N), 824 (C-Cl); **^{1}H NMR** (400 MHz, DMSO-d_6): δ 1.76 (t, *J* = 7.6 Hz, 1H), 2.33 (t, *J* = 8.0 Hz, 1H), 2.57-2.79 (m, 2H), 6.16 (s, 1H), 6.84 (d, *J* = 6.4 Hz, 2H), 7.17-7.46 (m, 11H), 7.65 (d, *J* = 6.8 Hz, 1H); **MS** (ESI) m/z: 427 $[M+H]^+$; Anal. Calcd. for $C_{26}H_{19}ClN_2S$: C, 73.14; H, 4.49; N, 6.56; Found: C, 73.01; H, 4.62; N, 6.78.

4-(9-(4-Chlorophenyl)-6,7-dihydro-5*H*-benzo[*h*]thiazolo[2,3-*b*]quinazolin-7-yl)phenol

(5b)

Yellow solid; **IR** (KBr) υ_{max}(cm^{-1}): 3244 (OH), 1609 (C=N), 836 (C-Cl); **^{1}H NMR** (400 MHz, DMSO-d_6): δ 1.75-1.82 (m, 1H), 2.26-2.32 (m, 1H), 2.61 (t, *J* = 8.0 Hz, 1H), 2.78 (t, *J* = 7.6 Hz, 1H), 6.04 (s, 1H), 6.52 (d, *J* = 8.4 Hz, 2H), 6.60 (d, *J* = 8.4 Hz, 2H), 7.25-7.40 (m, 6H), 7.50 (d, *J* = 8.0 Hz, 2H), 7.62 (d, *J* = 7.2 Hz, 1H), 9.63 (s, 1H); **MS** (ESI) m/z: 443 $[M+H]^+$; Anal. Calcd. for $C_{26}H_{19}ClN_2OS$: C, 70.50; H, 4.32; N, 6.32; Found: C, 70.31; H, 4.53; N, 6.12.

4-(9-(4-Chlorophenyl)-6,7-dihydro-5*H*-benzo[*h*]thiazolo[2,3-*b*]quinazolin-7-yl)-2-methoxyphenol (5c)

White solid; **IR** (KBr) υ_{max}(cm^{-1}): 3306 (OH), 1605 (C=N), 1242 (C-O-C), 821 (C-Cl); **^{1}H NMR** (400 MHz, DMSO-d_6): δ 1.78-1.84 (m, 1H), 2.26-2.34 (m, 1H), 2.59-2.65 (m, 1H), 2.75-2.81 (m, 1H), 3.47 (s, 3H), 6.09 (s, 1H), 6.42-6.45 (m, 1H), 6.63 (d, *J* = 8.4 Hz, 2H), 7.25-7.40 (m, 6H), 7.51 (d, *J* = 8.4 Hz, 2H), 7.66 (d, *J* = 7.6 Hz, 1H), 9.20 (s, 1H); **MS** (ESI) m/z: 473 $[M+H]^+$; Anal. Calcd. for $C_{27}H_{21}ClN_2O_2S$: C, 68.56; H, 4.48; N, 5.92; Found: C, 68.75; H, 4.28; N, 6.15.

9-(4-Chlorophenyl)-7-(4-methoxyphenyl)-6,7-dihydro-5*H*-benzo[*h*]thiazolo[2,3-*b*]quinazoline (5d)

Yellow solid; **IR** (KBr) υ_{max}(cm^{-1}): 1607 (C=N), 1246 (C-O-C), 822 (C-Cl); **^{1}H NMR** (400 MHz, DMSO-d_6): δ 1.75-1.82 (m, 1H), 2.28-2.35 (m, 1H), 2.60 (t, *J* = 8.4 Hz, 1H), 2.75-2.83 (m, 1H), 3.67 (s, 3H), 6.09 (s, 1H), 6.69-6.75 (m, 4H), 7.26-7.40 (m, 6H), 7.49 (d, *J* = 8.4 Hz, 2H), 7.63 (d, *J* = 7.6 Hz, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 161.3, 159.5, 138.5, 135.3, 134.9, 131.3, 130.6, 128.8, 128.5, 128.2, 128.1, 126.7, 123.7, 121.3,

114.0, 112.4, 110.1, 62.1, 55.1, 26.7, 23.2; **MS** (ESI) m/z: 457 $[M+H]^+$; Anal. Calcd. for $C_{27}H_{21}ClN_2OS$: C, 70.96; H, 4.63; N, 6.13; Found: C, 71.15; H, 4.85; N, 5.91.

9-(4-Chlorophenyl)-7-(3,4-dimethoxyphenyl)-6,7-dihydro-5*H*-benzo[*h*]thiazolo[2,3-*b*]quinazoline (5e)

Pale yellow solid; **IR** (KBr) $\upsilon_{max}(cm^{-1})$: 1609 (C=N), 1245 (C-O-C), 834 (C-Cl); **^{1}H NMR** (400 MHz, DMSO-d_6): δ 1.77-1.82 (m, 1H), 2.31 (t, *J* = 7.6 Hz, 1H), 2.59-2.79 (m, 2H), 3.47 (s, 3H), 3.67 (s, 3H), 6.13 (s, 1H), 6.56-6.58 (m, 1H), 6.81 (d, *J* = 8.4 Hz, 1H), 7.25-7.40 (m, 7H), 7.50 (d, *J* = 8.4 Hz, 2H), 7.63 (d, *J* = 7.6 Hz, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 161.1, 148.9, 147.9, 138.6, 135.3, 134.9, 131.5, 131.1, 128.8, 128.5, 128.1, 126.9, 126.7, 121.3, 119.5, 112.4, 112.1, 110.9, 109.8, 62.4, 55.4, 54.9, 26.7, 23.1; **MS** (ESI) m/z: 488 $[M+H]^+$; Anal. Calcd. for $C_{28}H_{23}ClN_2O_2S$: C, 69.05; H, 4.76; N, 5.75; Found: C, 69.27; H, 4.69; N, 5.92.

9-(4-Chlorophenyl)-7-(4-fluorophenyl)-6,7-dihydro-5*H*-benzo[*h*]thiazolo[2,3 *b*]quinazoline (5f)

White solid; **IR** (KBr) $\upsilon_{max}(cm^{-1})$: 1604 (C=N), 1236 (C-F), 837 (C-Cl); **^{1}H NMR** (400 MHz, DMSO-d_6): δ 1.70-1.77 (m, 1H), 2.31 (t, *J* = 8.4 Hz, 1H), 2.59-2.64 (m, 1H), 2.75-2.79 (m, 1H), 6.20 (s, 1H), 6.89-7.03 (m, 4H), 7.25 (d, *J* = 8.4 Hz, 3H), 7.32-7.48 (m, 5H), 7.64 (t, *J* = 6.8 Hz, 1H); **MS** (ESI) m/z: 445 $[M+H]^+$; Anal. Calcd. for $C_{26}H_{18}ClFN_2S$: C, 70.18; H, 4.08; N, 6.30; Found: C, 70.02; H, 4.26; N, 6.49.

7,9-Bis(4-chlorophenyl)-6,7-dihydro-5*H*-benzo[*h*]thiazolo[2,3-*b*]quinazoline (5g)

White solid; **IR** (KBr) $\upsilon_{max}(cm^{-1})$: 1611 (C=N), 826 (C-Cl); **^{1}H NMR** (400 MHz, DMSO-d_6): δ 1.72-1.79 (m, 1H), 2.31 (t, *J* = 8.0 Hz, 1H), 2.59-2.64 (m, 1H), 2.75-2.79 (m, 1H), 6.19 (s, 1H), 6.89 (d, *J* = 8.4 Hz, 2H), 7.23-7.35 (m, 7H), 7.39 (d, *J* = 7.6 Hz, 1H), 7.47 (d, *J* = 8.4 Hz, 2H), 7.62 (d, *J* = 7.6 Hz, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 161.5, 138.3, 137.5, 135.3, 135.0, 133.5, 131.4, 128.9, 128.7, 128.5, 128.1, 126.7, 121.4, 111.5, 110.1, 61.8, 26.6, 23.0; **MS** (ESI) m/z: 462 [M+H]+; Anal. Calcd. for $C_{26}H_{18}C_{l2}N_2S$: C, 67.68; H, 3.93; N, 6.07; Found: C, 67.77; H, 4.12; N, 6.28.

9-(4-Bromophenyl)-7-phenyl-6,7-dihydro-5*H*-benzo[*h*]thiazolo[2,3-*b*]quinazoline (5h)

White solid; **IR** (KBr) $\upsilon_{max}(cm^{-1})$: 1603 (C=N), 578 (C-Br); **^{1}H NMR** (400 MHz, DMSO-d_6): δ 1.72-1.80 (m, 1H), 2.28-2.34 (m, 1H), 2.59 (t, *J* = 8.0 Hz, 1H), 2.75-2.81 (m, 1H), 6.15 (s, 1H), 6.84 (d, *J* = 7.6 Hz, 2H), 7.15-7.40 (m, 9H), 7.58 (d, *J* = 8.4 Hz, 3H); **MS**

(ESI) m/z: 472 $[M+H]^+$; Anal. Calcd. for $C_{26}H_{19}BrN_2S$: C, 66.24; H, 4.06; N, 5.94; Found: C, 66.11; H, 4.28; N, 5.71.

4-(9-(4-Bromophenyl)-6,7-dihydro-5*H*-benzo[*h*]thiazolo[2,3-*b*]quinazolin-7-yl)phenol (5i)

Yellow solid; **IR** (KBr) $\upsilon_{max}(cm^{-1})$: 3253 (OH), 1609 (C=N), 631 (C-Br); **^{1}H NMR** (400 MHz, DMSO-d_6): δ 1.76-1.84 (m, 1H), 2.26-2.34 (m, 1H), 2.58-2.65 (m, 1H), 2.76-2.84 (m, 1H), 6.05 (s, 1H), 6.52 (d, *J* = 8.4 Hz, 2H), 6.58 (d, *J* = 8.4 Hz, 2H), 7.20-7.38 (m, 6H), 7.61-7.69 (m, 3H), 9.61 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 161.2, 157.8, 138.6, 135.3, 131.5, 131.4, 128.9, 128.8, 128.2, 127.1, 126.7, 126.6, 123.6, 123.4, 121.3, 115.3, 112.6, 110.0, 62.3, 26.7, 23.2; **MS** (ESI) m/z: 487 $[M]^+$; Anal. Calcd. for $C_{26}H_{19}BrN_2OS$: C, 64.07; H, 3.93; N, 5.75; Found: C, 64.31; H, 3.73; N, 5.94.

4-(9-(4-Bromophenyl)-6,7-dihydro-5*H*-benzo[*h*]thiazolo[2,3-*b*]quinazolin-7-yl)-2-methoxyphenol (5j)

White solid; **IR** (KBr) $\upsilon_{max}(cm^{-1})$: 3306 (OH), 1600 (C=N), 1277 (C-O-C), 634 (C-Br); **^{1}H NMR** (400 MHz, DMSO-d_6): δ 1.76-1.84 (m, 1H), 2.25-2.33 (m, 1H), 2.59-2.65 (m, 1H), 2.75-2.81 (m, 1H), 3.48 (s, 3H), 6.07 (s, 1H), 6.44-6.46 (m, 1H), 6.63 (d, *J* = 8.0 Hz, 2H), 7.22-7.40 (m, 6H), 7.60-7.66 (m, 3H), 9.21 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 160.9, 146.9, 146.8, 138.6, 135.3, 131.6, 131.4, 129.5, 128.7, 128.1, 127.3, 126.7, 123.6, 123.5, 121.3, 119.7, 116.1, 112.6, 111.5, 109.8, 62.5, 55.1, 26.7, 23.2; **MS** (ESI) m/z: 518 $[M+H]^+$ Anal. Calcd. for $C_{27}H_{21}BrN_2O_2S$: C, 62.67; H, 4.09; N, 5.41; Found: C, 62.42; H, 4.31; N, 5.60.

9-(4-Bromophenyl)-7-(4-methoxyphenyl)-6,7-dihydro-5*H*-benzo[*h*]thiazolo[2,3 *b*]quinazoline (5k)

White solid; **IR** (KBr) $\upsilon_{max}(cm^{-1})$: 1606 (C=N), 1245 (C-O-C), 634 (C-Br); **^{1}H NMR** (400 MHz, DMSO-d_6): δ 1.75-1.81 (m, 1H), 2.27-2.35 (m, 1H), 2.56-2.64 (m, 1H), 2.75-2.81 (m, 1H), 3.67 (s, 3H), 6.09 (s, 1H), 6.72 (d, *J* = 8.0 Hz, 4H), 7.19-7.40 (m, 7H), 7.63 (d, *J* = 8.4 Hz, 2H); **MS** (ESI) m/z: 502 $[M+H]^+$; Anal. Calcd. for $C_{27}H_{21}BrN_2OS$: C, 64.67; H, 4.22; N, 5.59; Found: C, 64.76; H, 4.48; N, 5.71.

9-(4-Bromophenyl)-7-(3,4-dimethoxyphenyl)-6,7-dihydro-5*H*-benzo[*h*]thiazolo[2,3-*b*]quinazoline (5l)

Pale yellow solid; **IR** (KBr) υ_{max}(cm^{-1}): 1598 (C=N), 1266 (C-O-C), 630 (C-Br); **^{1}H NMR** (400 MHz, DMSO-d_6): δ 1.77-1.83 (m, 1H), 2.31 (t, *J* = 8.0 Hz, 1H), 2.57-2.65 (m, 1H), 2.75-2.83 (m, 1H), 3.47 (s, 3H), 3.67 (s, 3H), 6.12(s, 1H), 6.57-6.59 (m, 1H), 6.81 (d, *J* = 8.4 Hz, 2H), 7.21-7.40 (m, 7H), 7.64 (d, *J* = 8.8 Hz, 2H); **MS** (ESI) m/z: 532 $[M+H]^+$; Anal. Calcd. for $C_{28}H_{23}BrN_2O_2S$: C, 63.28; H, 4.36; N, 5.27; Found: C, 63.39; H, 4.19; N, 5.04.

9-(4-Bromophenyl)-7-(4-fluorophenyl)-6,7-dihydro-5*H*-benzo[*h*]thiazolo[2,3-*b*]quinazoline (5m)

White solid; **IR** (KBr) υ_{max}(cm^{-1}): 1604 (C=N), 1227 (C-F), 632 (C-Br); **^{1}H NMR** (400 MHz, DMSO-d_6): δ 1.72-1.78 (m, 1H), 2.27-2.34 (m, 1H), 2.59-2.64 (m, 1H), 2.75-2.81 (m, 1H), 6.19 (s, 1H), 6.89-7.03 (m, 5H), 7.16-7.40 (m, 6H),7.61 (d, *J* = 8.4 Hz, 2H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 163.1, 161.5, 138.4, 135.3, 134.9, 131.5, 131.4, 129.2, 128.9, 128.1, 126.9, 126.7, 123.7, 121.2, 115.6, 115.4, 111.7, 61.8, 26.6, 23.0; **MS** (ESI) m/z: 490 $[M+H]^+$; Anal. Calcd. for $C_{26}H_{18}BrFN_2S$: C, 63.81; H, 3.71; N, 5.72; Found: C, 63.96; H, 3.88; N, 5.89.

3-(7-Phenyl-6,7-dihydro-5*H*-benzo[*h*]thiazolo[2,3-*b*]quinazolin-9-yl)-2*H*-chromen-2-one (5n)

Pale yellow solid; **IR** (KBr) υ_{max}(cm^{-1}): 1711 (C=O of lactone), 1630 (C=N); **^{1}H NMR** (400 MHz, DMSO-d_6): δ 1.76 (t, *J* = 6.4 Hz, 1H), 2.28-2.36 (m, 1H), 2.57-2.65 (m, 1H), 2.77-2.81 (m, 1H), 6.25 (s, 1H), 7.16-7.26 (m, 6H), 7.34-7.49(m, 5H), 7.64-7.89 (m, 4H); **MS** (ESI) m/z: 461 $[M+H]^+$; Anal. Calcd. for $C_{29}H_{20}N_2O_2S$: C, 75.63; H, 4.38; N, 6.08; Found: C, 75.91; H, 4.52; N, 6.27.

3-(7-(4-Hydroxyphenyl)-6,7-dihydro-5*H*-benzo[*h*]thiazolo[2,3-*b*]quinazolin-9-yl)-2*H*-chromen-2-one (5o)

Pale yellow solid; **IR** (KBr) υ_{max}(cm^{-1}): 3430 (OH), 1727 (C=O of lactone), 1633 (C=N); **^{1}H NMR** (400 MHz, DMSO-d_6): δ 1.74-1.79 (m, 1H), 2.26 (t, *J* = 8.0 Hz, 1H), 2.62 (t, *J* = 8.0 Hz, 1H), 2.76-2.80 (m, 1H), 6.10 (s, 1H), 6.52 (d, *J* = 8.4 Hz, 2H), 6.92 (d, *J* = 8.4 Hz, 2H), 7.24-7.51 (m, 6H), 7.68-7.74 (m, 3H), 7.92 (s, 1H),9.61 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 161.1, 158.5, 158.0, 153.5, 146.4, 135.4, 133.5, 133.4, 129.4, 129.1,

128.8, 128.5, 128.1, 126.6, 125.0, 121.4, 118.0, 116.2, 115.9, 115.5, 113.4, 112.0, 62.4, 26.7, 23.1; **MS** (ESI) m/z: 477 $[M+H]^+$; Anal. Calcd. for $C_{29}H_{20}N_2O_3S$: C, 73.09; H, 4.23; N, 5.88; Found: C, 73.32; H, 4.37; N, 5.71.

3-(7-(4-Hydroxy-3-methoxyphenyl)-6,7-dihydro-5*H*-benzo[*h*]thiazolo[2,3-*b*]quinazolin-9-yl)-2*H*-chromen-2-one (5p)

Pale yellow solid; **IR** (KBr) $\upsilon_{max}(cm^{-1})$: 3256 (OH), 1738 (C=O of lactone), 1638 (C=N), 1247 (C-O-C); **^{1}H NMR** (400 MHz, DMSO-d_6): δ 1.77-1.81 (m, 1H), 2.27 (t, *J* = 8.0 Hz, 1H), 2.60-2.66 (m, 1H), 2.77 (t, *J* = 7.6 Hz, 1H), 3.23 (s, 3H), 6.09 (s, 1H), 6.61 (d, *J* = 7.6 Hz, 3H), 7.24-7.50 (m, 6H), 7.61-7.74 (m, 3H), 7.93(s, 1H), 9.18 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 161.0, 158.5, 153.5, 147.2, 146.4, 135.4, 133.6, 129.7, 129.4, 128.8, 128.1, 126.7, 125.0, 123.7, 121.4, 120.0, 118.0, 116.2, 112.0, 111.8, 62.7, 54.9, 26.7, 23.0; **MS** (ESI) m/z: 507 $[M+H]^+$; Anal. Calcd. for $C_{30}H_{22}N_2O_4S$: C, 71.13; H, 4.38; N, 5.53; Found: C, 71.27; H, 4.52; N, 5.39.

3-(7-(4-Methoxyphenyl)-6,7-dihydro-5*H*-benzo[*h*]thiazolo[2,3-*b*]quinazolin-9-yl)-2*H*-chromen-2-one (5q)

Yellow solid; **IR** (KBr) υ_{max} (cm^{-1}): 1716 (C=O of lactone), 1608 (C=N), 1248 (C-O-C); **^{1}H NMR** (400 MHz, DMSO-d_6): δ 1.74-1.79 (m, 1H), 2.28 (t, *J* = 7.6 Hz, 1H), 2.61 (t, *J* = 8.0 Hz, 1H), 2.79 (t, *J* = 7.6 Hz, 1H), 3.58 (s, 3H), 6.18 (s, 1H), 6.69 (d, *J* = 8.4 Hz, 2H), 7.70 (d, *J* = 8.8 Hz, 2H), 7.24-7.51 (m, 6H), 7.66-7.87 (m, 4H); **MS** (ESI) m/z: 491 $[M+H]^+$; Anal. Calcd. for $C_{30}H_{22}N_2O_3S$: C, 73.45; H, 4.52; N, 5.71; Found: C, 73.25; H, 4.71; N, 5.52.

3-(7-(3,4-Dimethoxyphenyl)-6,7-dihydro-5*H*-benzo[*h*]thiazolo[2,3-*b*]quinazolin-9-yl)-2*H*-chromen-2-one (5r)

White solid; **IR** (KBr) $\upsilon_{max}(cm^{-1})$: 1734 (C=O of lactone), 1636 (C=N), 1261 (C-O-C); **^{1}H NMR** (400 MHz, DMSO-d_6): δ 1.76-1.83 (m, 1H), 2.29 (t, *J* = 8.0 Hz, 1H), 2.61-2.66 (m, 1H), 2.76-2.80 (m, 1H), 3.26 (s, 3H), 3.59 (s, 3H),6.15 (s, 1H), 6.68 (s, 1H), 6.75 (s, 2H), 7.25-7.50 (m, 6H), 7.66-7.65 (m, 3H), 7.88 (s, 1H); **MS** (ESI) m/z: 521 $[M+H]^+$; Anal. Calcd. for $C_{31}H_{24}N_2O_4S$: C, 71.52; H, 4.65; N, 5.38; Found: C, 71.41; H, 4.83; N, 5.50.

3-(7-(4-Fluorophenyl)-6,7-dihydro-5*H*-benzo[*h*]thiazolo[2,3-*b*]quinazolin-9-yl)-2*H*-chromen-2-one (5s)

White solid; **IR** (KBr) υ_{max}(cm^{-1}): 1740 (C=O of lactone), 1634 (C=N), 1226 (C-F); **^{1}H NMR** (400 MHz, DMSO-d_6): δ 1.76 (t, *J* = 6.4 Hz, 1H), 2.30 (t, *J* = 8.0 Hz, 1H), 2.60-2.66 (m, 1H), 2.76-2.83 (m, 1H), 6.27 (s, 1H), 7.02 (t, *J* = 8.4 Hz, 2H), 7.23-7.26 (m, 3H), 7.32-7.50 (m, 5H), 7.63-7.75 (m, 3H), 7.93 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 163.3, 161.4, 160.9, 158.4, 153.5, 146.9, 135.4, 135.2, 133.6, 133.1, 129.5, 129.4, 128.9, 128.1, 126.6, 125.0, 124.1, 121.5, 117.9, 116.2, 115.9, 115.7, 113.6, 111.3, 61.8, 26.6, 22.9; **MS** (ESI) m/z: 479 $[M+H]^+$; Anal. Calcd. for $C_{29}H_{19}FN_2O_2S$: C, 72.79; H, 4.00; N, 5.85; Found: C, 72.98; H, 3.87; N, 5.96.

3-(7-(4-Chlorophenyl)-6,7-dihydro-5*H*-benzo[*h*]thiazolo[2,3-*b*]quinazolin-9-yl)-2*H*-chromen-2-one (5t)

White solid; **IR** (KBr) υ_{max}(cm^{-1}): 1740 (C=O of lactone), 1636 (C=N), 840 (C-Cl); **^{1}H NMR** (400 MHz, DMSO-d_6): δ 1.73-1.78 (m, 1H), 2.27-2.35 (m, 1H), 2.60-2.66 (m, 1H), 2.76-2.80 (m, 1H), 6.27 (s, 1H), 7.22-7.26 (m, 5H), 7.34-7.51(m, 5H), 7.64-7.75 (m, 3H), 7.94 (s, 1H); **^{13}C NMR** (100 MHz, DMSO-d_6): δ 161.5, 158.4, 153.5, 147.0, 137.8, 135.4, 133.9, 133.7, 133.0, 129.4, 129.0, 128.9, 128.1, 126.7, 125.0, 121.5, 117.9, 116.3, 115.7, 113.6, 111.1, 61.8, 26.6, 22.9; **MS** (ESI) m/z: 495 $[M+H]^+$; Anal. Calcd. For $C_{29}H_{19}ClN_2O_2S$: C, 70.37; H, 3.87; N, 5.66; Found: C, 70.53; H, 3.76; N, 5.78.

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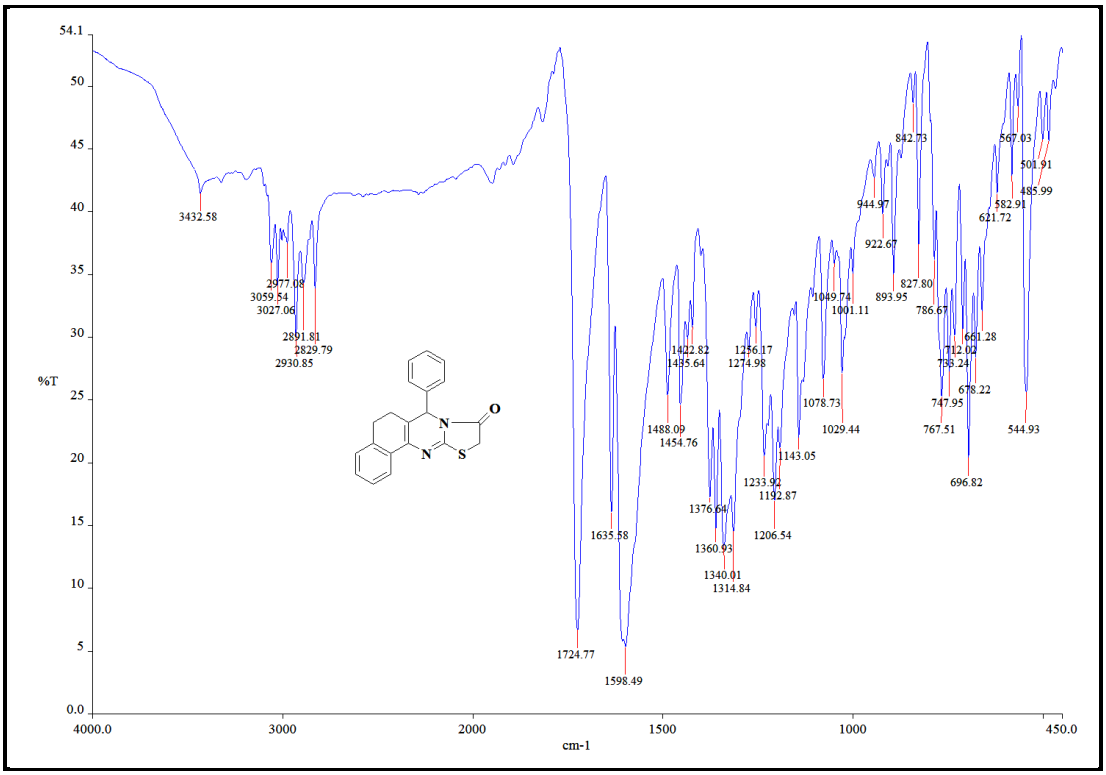
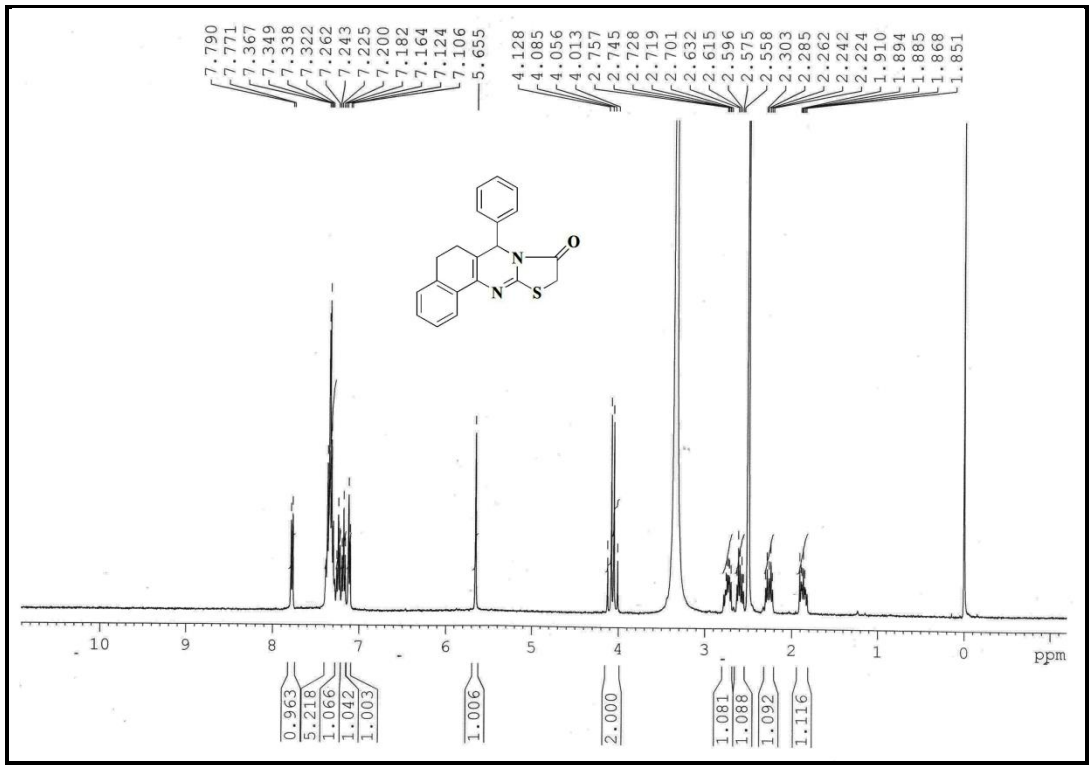
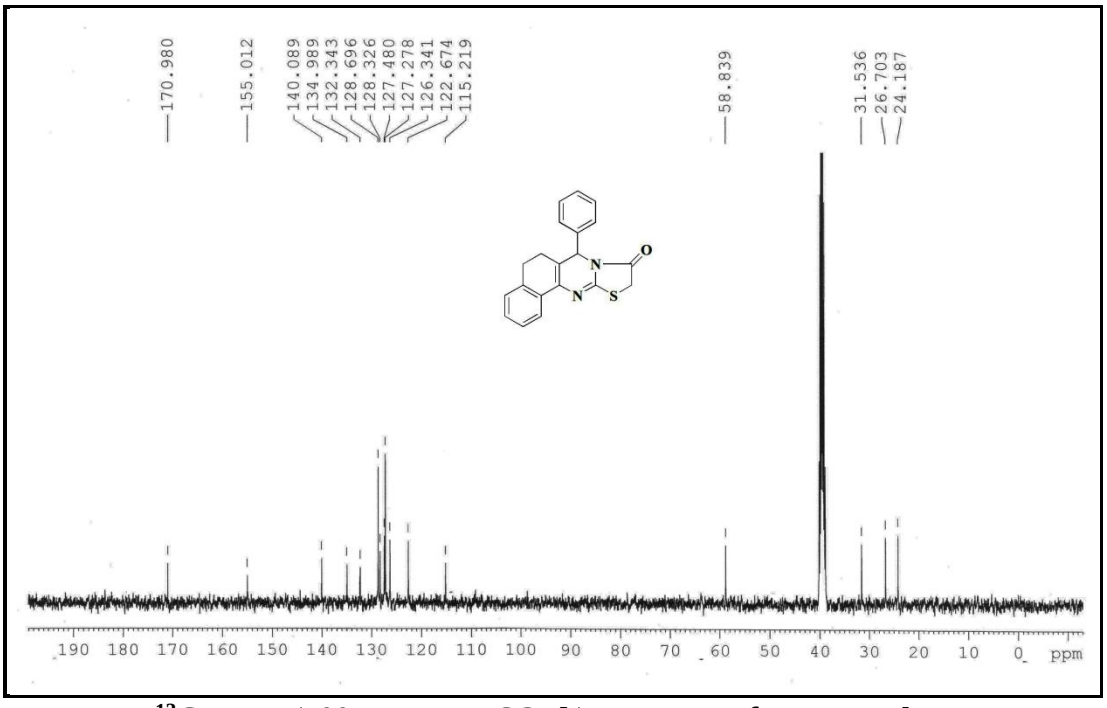


IR (KBr) spectrum of compound 4a



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



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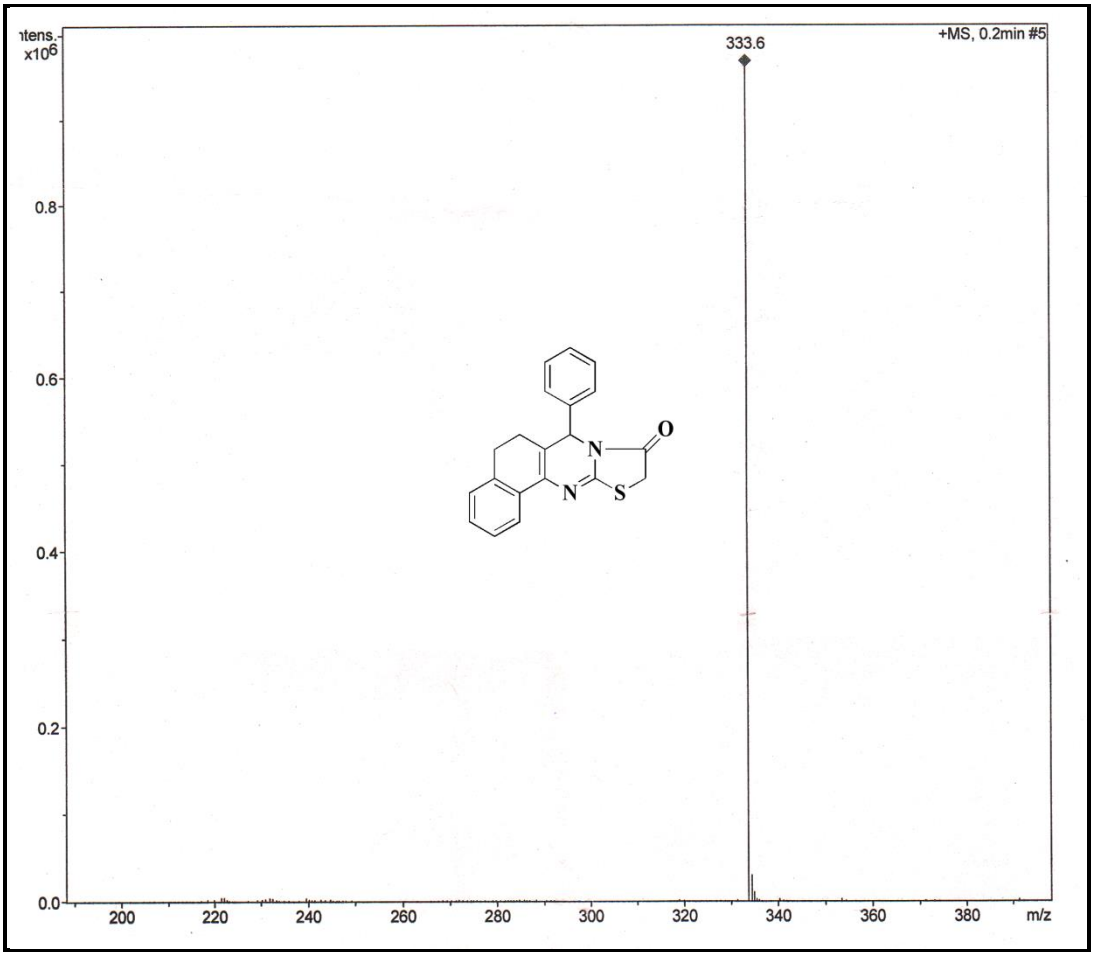
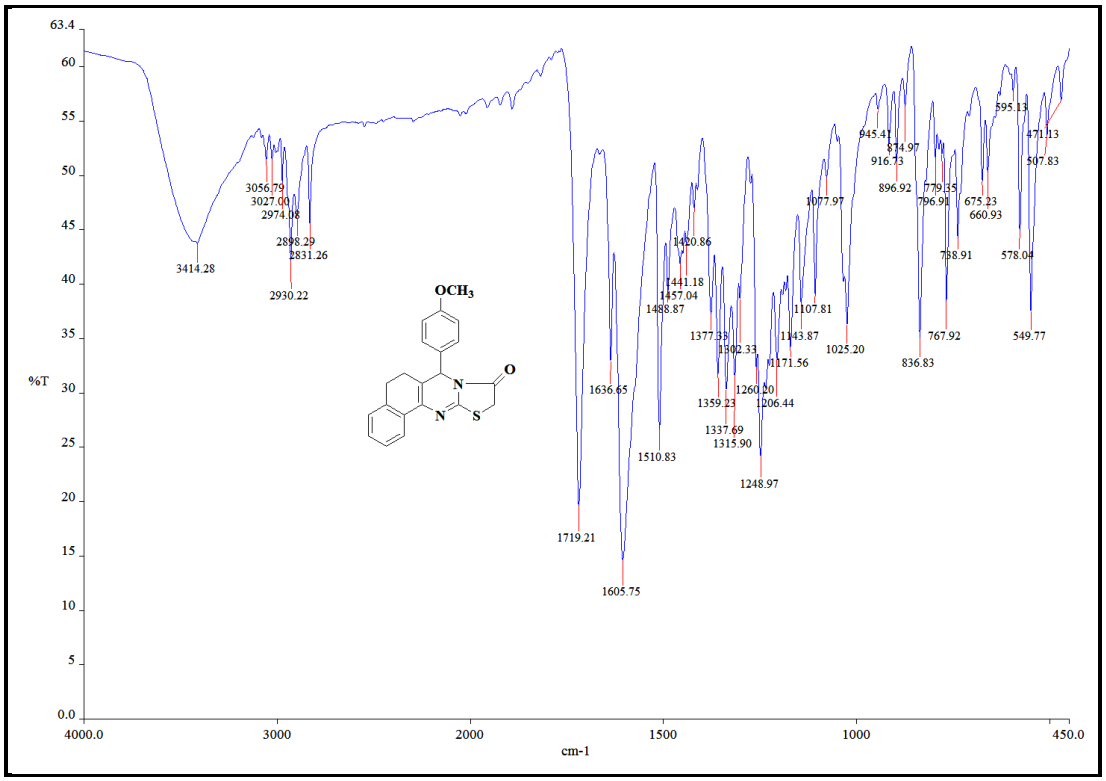
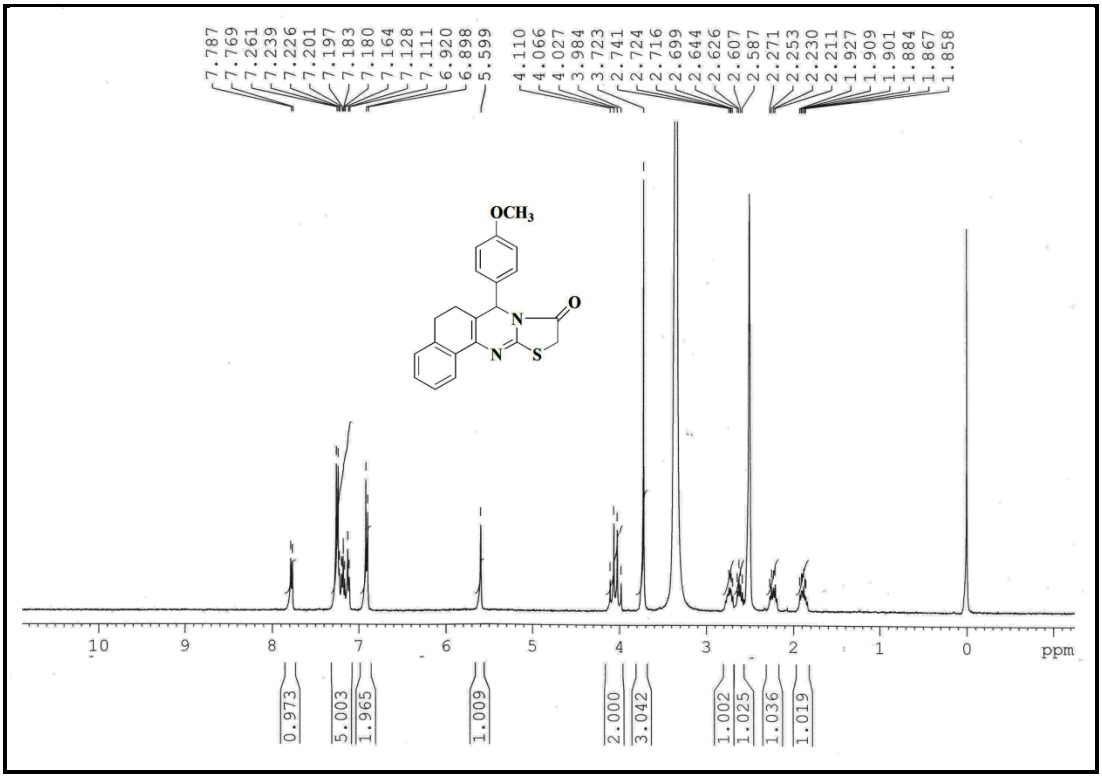


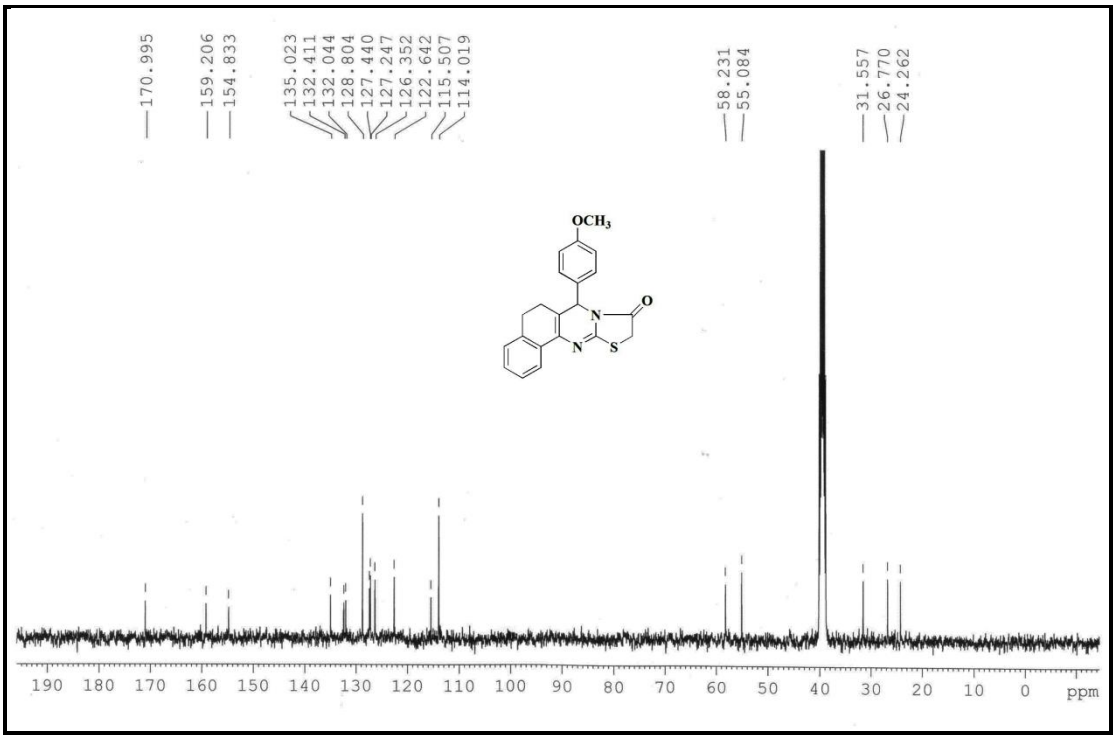
Mass (ESI) spectrum of compound 4a (M.Wt.: 332)



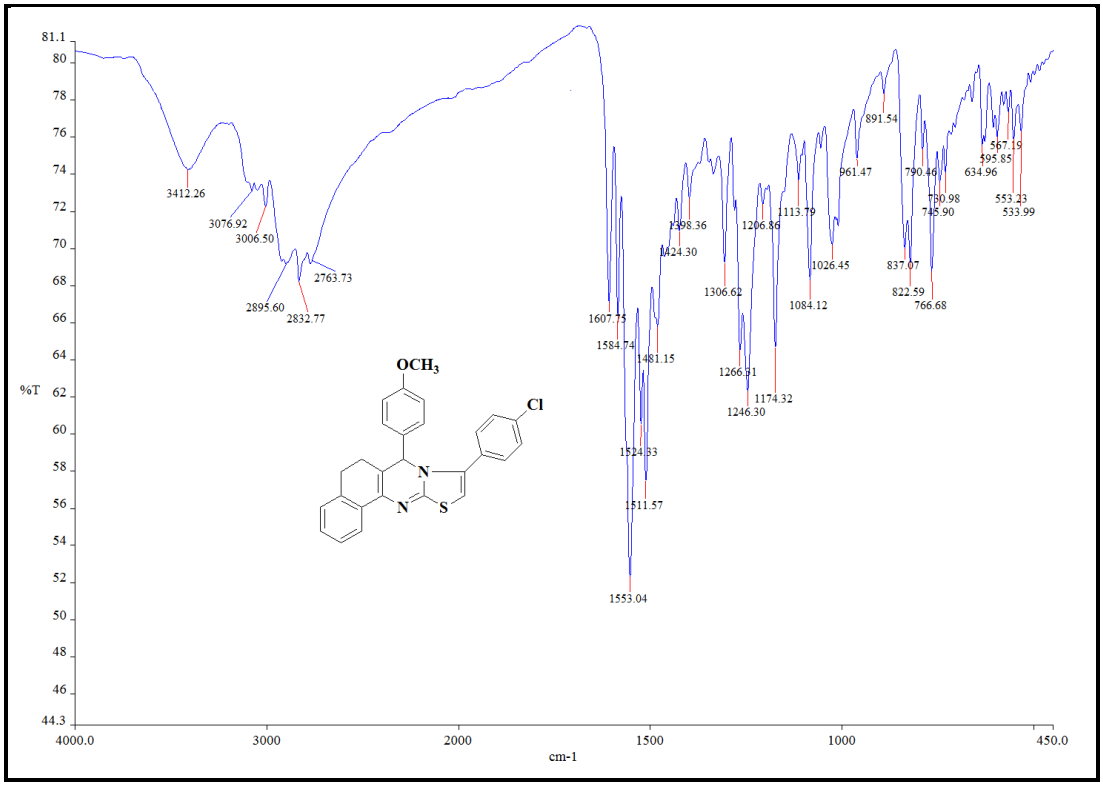
IR (KBr) spectrum of compound 4c



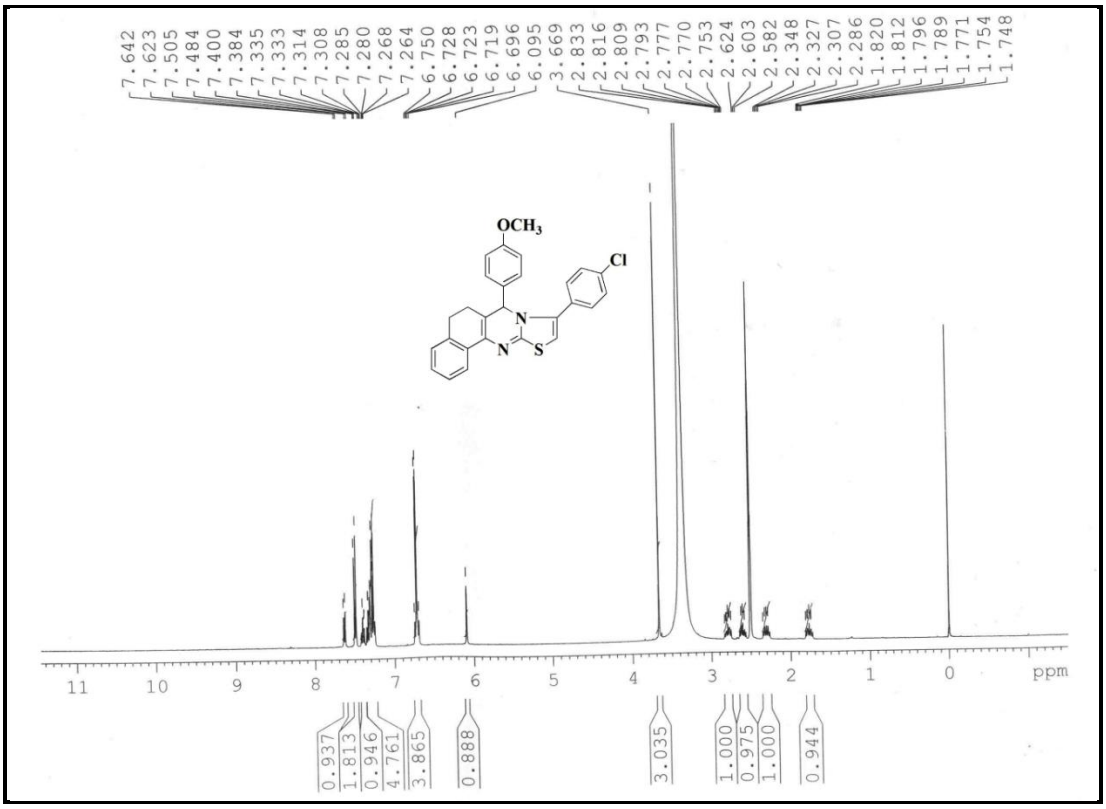
^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound 4c



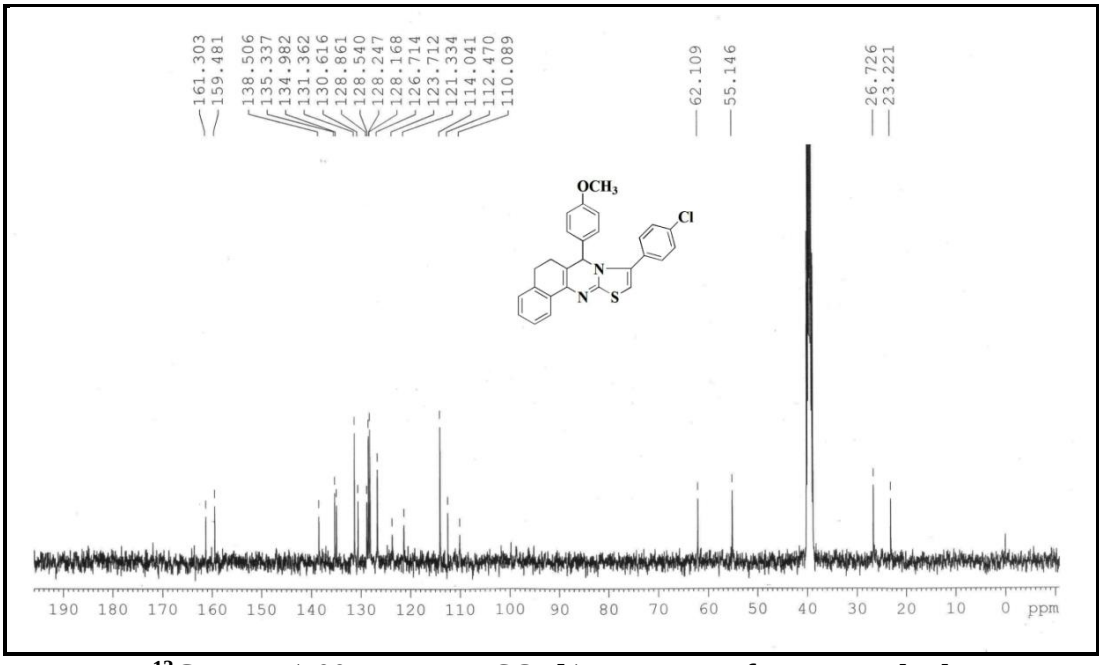
^{13}C NMR (100 MHz, DMSO-d_6) spectrum of compound 4c



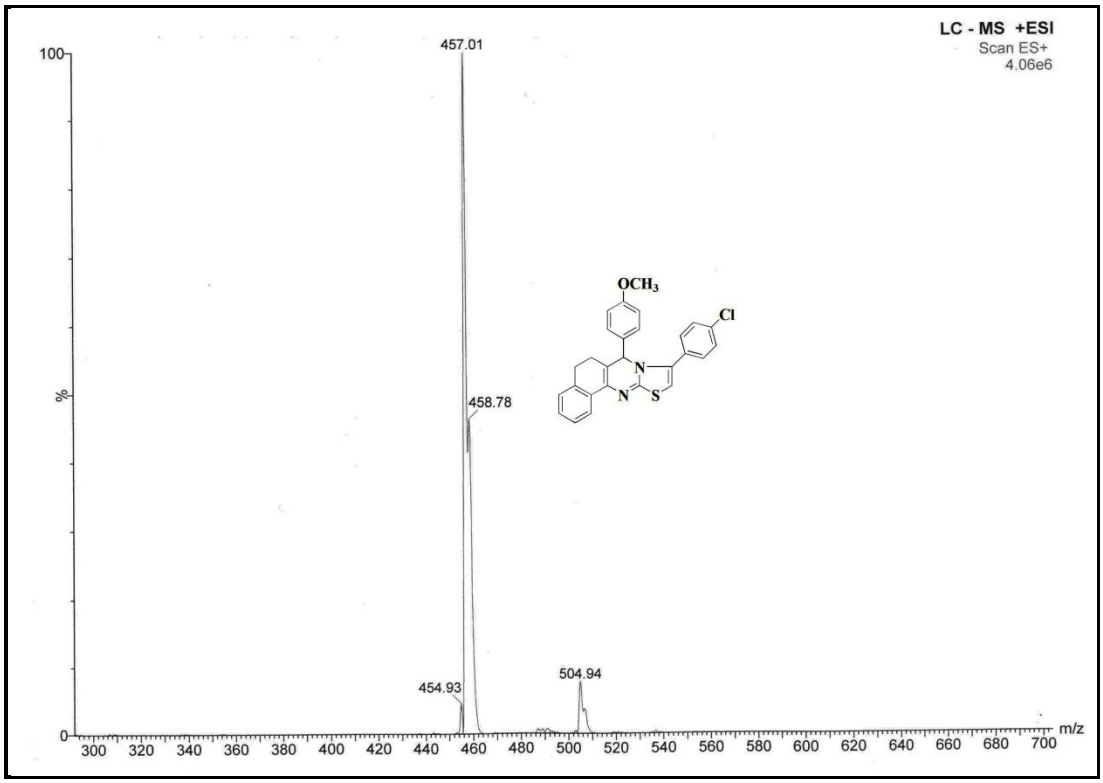
IR (KBr) spectrum of compound 5d



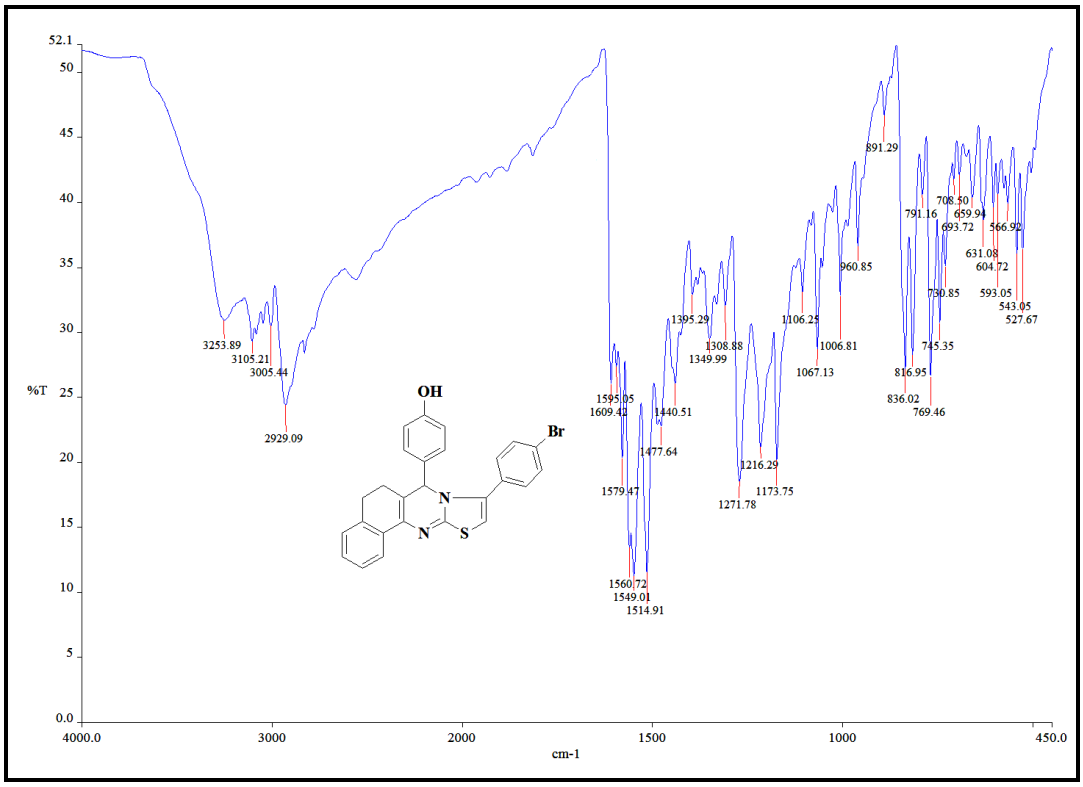
^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound 5d



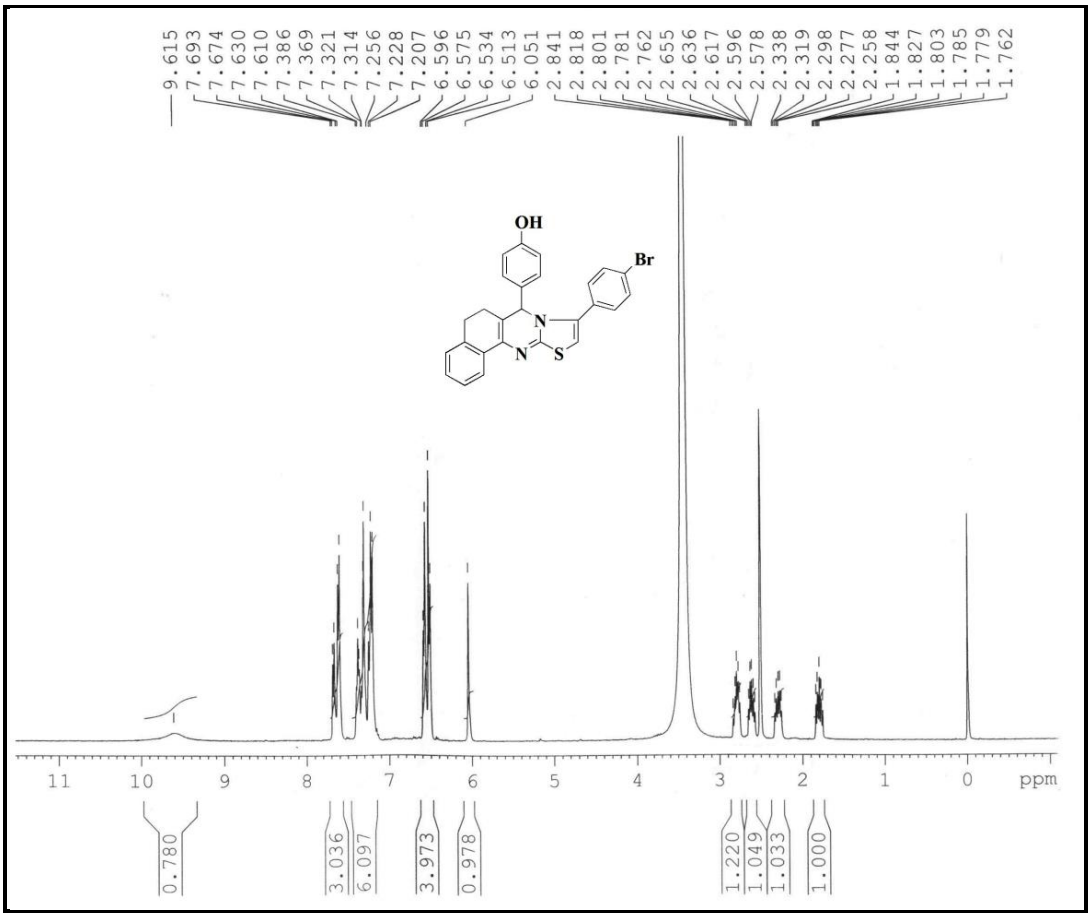
^{13}C NMR (100 MHz, DMSO-d_6) spectrum of compound 5d



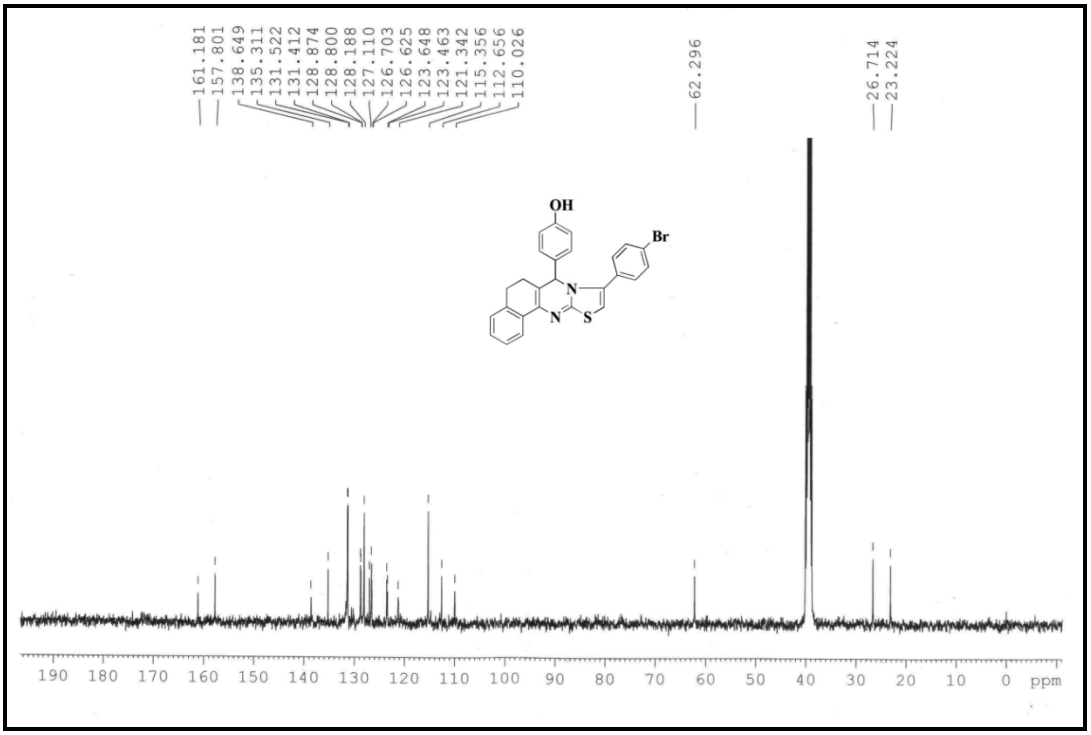
Mass spectrum of compound 5d (M.Wt.: 456)



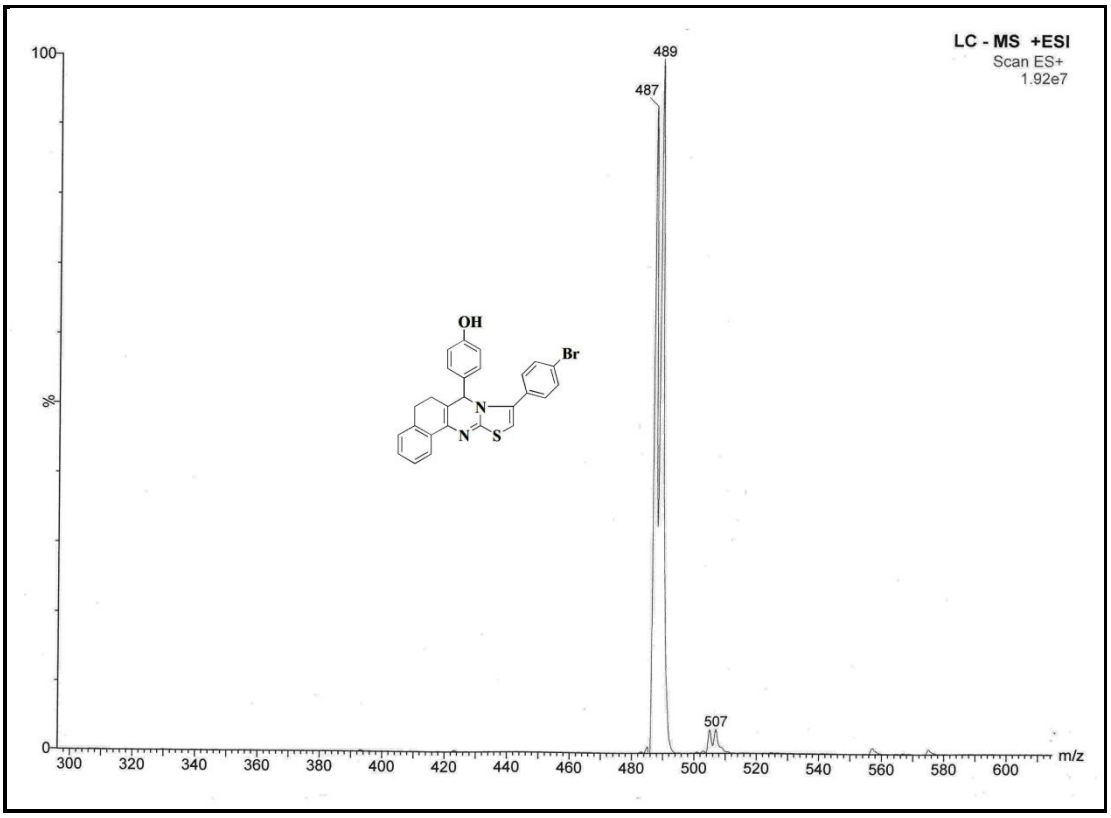
IR (KBr) spectrum of compound 5i



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



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



Mass spectrum of compound 5i (M.Wt.: 487)

SUMMARY

CHAPTER-I

Introduction to biologically potent heterocyclic compounds and reaction methods

In this chapter, a brief introduction and the pharmacological importance of various heterocyclic compounds like coumarins, benzothiazoles, pyrazoles, pyridinium salts, selenazoles, thiazoles, thiazdiazines, thiazolo[2,3-*b*]quinazolinones, thiazolo[2,3-*b*]quinazolines and triazoles is discussed. The above heterocyclic molecules have attracted much attention from the Researchers due to their broad spectrum of biological applications and in various fields of Chemistry. In addition, they also make the core structure of various bioactive natural and synthetic drugs **(Fig. 1)**. The aforementioned wide applications of the above heterocyclic scaffolds, prompted us to design and synthesize the new heterocyclic entities amalgamating one or more above pharmacophore units by employing either by solvent-free conditions or in aqueous medium or under conventional and unconventional method.

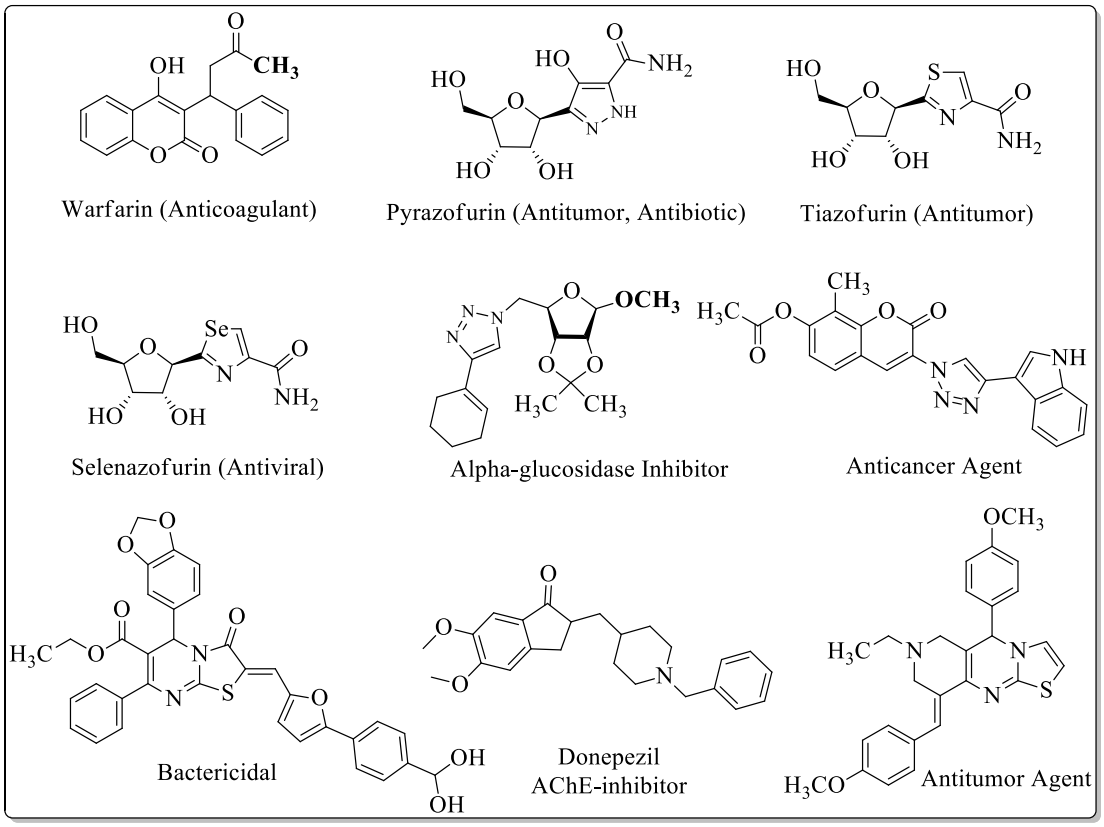
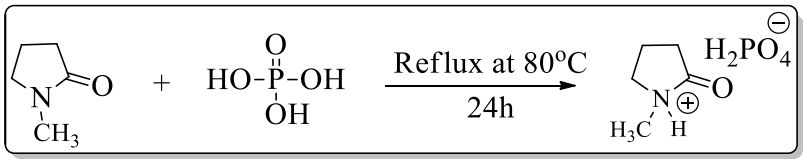


Fig. 1

The majority of the heterocyclic molecules described in this thesis were synthesized under conventional method. Only, the heterocyclic compounds included in the last chapter (**Chapter-VI**) were prepared by employing the non-traditional or non-conventional methods (ultrasonication and microwave irradiation) and by utilizing the [NMP]H_2PO_4 as an acidic ionic liquid **Chapter-VI** (**section-B**) and its preparation was illustrated below.

***N*-Methyl-2-pyrrolidonium dihydrogen phosphate [NMP]H_2PO_4 acidic ionic liquid**

NMP]H_2PO_4 was prepared according to the literature procedure[1] (s**cheme-1)**. *N*-methyl-2-pyrrolidinone was slowly added drop-wise to the cooled equimolar concentration of phosphoric acid, and the resulting mixture was heated at 80^{o}C for 24h. The mixture was cooled to ambient temperature and washed with ether to remove any non-ionic residues. Then the residue is dried under high vacuum at 80^{o}C on a rotary evaporator until the weight of the residue remains constant afforded the orange coloured, viscous ionic liquid.



Scheme-1

CHAPTER-II (SECTION-A)

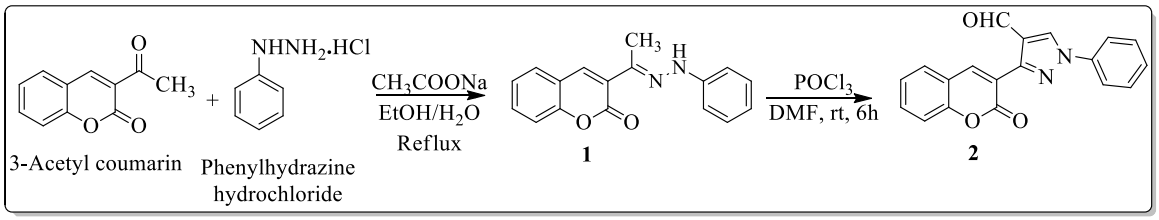
3-(1-Phenyl-4-((2-(4-arylthiazol-2-yl)hydrazono)-methyl)-1*H*-pyrazol-3-yl)-2*H*-chromen-2-ones: one-pot three component condensation, *in vitro* antimicrobial, antioxidant and molecular docking studies

Most of the literature studies revealed that, thiazoles, pyrazoles and coumarin are the key motifs in heterocyclic chemistry and are important structural backbone of various natural and synthetic biologically active molecules. They are known to possess a wide range of pharmacological activities that includes, antimicrobial, anticancer, anti-inflammatory, antitubercular, antihypertensive, antidepressant, anti-HIV, anti-parkinsonian, antiviral, antiallergenic, anticonvulsant, antipyretic and fibrinogen receptor antagonists with antithrombic activity.

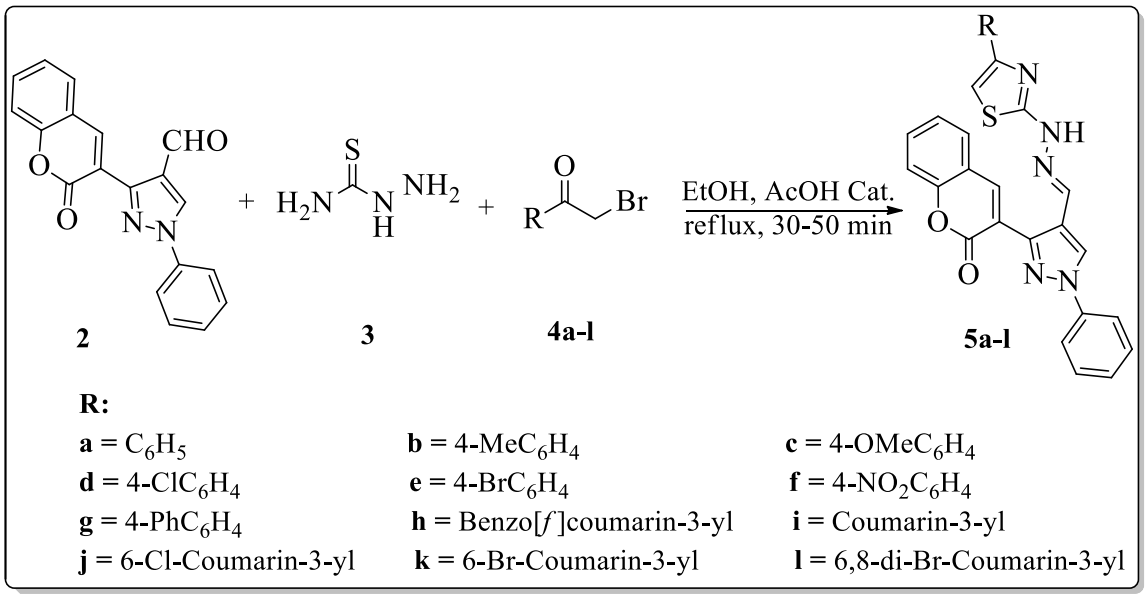
Further, extension to our earlier work and as a part of our endeavour towards the synthesis of biologically potent new heterocyclic scaffolds. Here in we report, the synthesis of novel

heterocyclic scaffold bearing a coumarin nucleus with a pyrazole and 4-functionalized thiazole rings.

The synthetic protocol for the title compounds, 3-(1-phenyl-4-((2-(4-aryl/heteryl-thiazol-2-yl)hydrazono)methyl)-1*H*-pyrazol-3-yl)-2*H*-chromen-2-ones (**5a-l**) has outlined in **Scheme-2** and **3**, and were synthesized by the one-pot three-component condensation reaction of 3-(2-oxo-2*H*-chromen-3-yl)-1-phenyl-1*H*-pyrazole-4-carbaldehyde (**2**), thiosemicarbazide (**3**) and phenacyl bromides (**4a-g**)/2-(2-bromoacetyl)-3*H*-benzo[*f*]chromen-3-one (**4h**)/3-(2-bromoacetyl)-2*H*-chromen-2-ones (**4i-l**) in ethanol in the presence of catalytic amount of acetic acid under reflux conditions with good yields (85-92%) in shorter reaction times (30-50 min).



Scheme-2



Scheme-3

Structures of all the synthesized compounds (**5a-l**) were established with the aid of their spectral (IR, NMR and Mass) and elemental (C, H and N) analyses. The title compounds were assessed for their *in vitro* antimicrobial, antioxidant activities and performed molecular docking studies.

Biological studies

***In vitro* antimicrobial activity**

All the synthesized compounds (**5a-l**) were screened for their *in vitro* antibacterial activity against four pathogenic microorganisms, including two Gram-positive bacteria, *Staphylococcus aureus* (MTCC 121) and *Bacillus subtilis* (MTCC 96), and two Gram-negative bacteria *Escherichia coli* (MTCC 40) and *Pseudomonas aeruginosa* (MTCC 2453). The standard pathogenic microbial cultures were procured from the Microbial Type Culture Collection (MTCC), Chandigarh, India. Antibacterial screenings were conducted in triplicates by well-plate method in Mueller-Hinton Agar[2] at 100 μg/mL concentration for the synthesized compounds (**5a-l**) with respect to positive control Streptomycin at 30 μg/mL. Zone of inhibition (ZOI) values were measured in mm and Minimum inhibitory concentration (MIC) for the tested compounds, as well as standards was measured in μg/mL by micro dilution method.[3] DMSO was used as a solvent control.

All the compounds (**5a-l**) were also screened for their *in vitro* antifungal activity against the fungal strains *Candida albicans*, *Aspergillus niger*, *Candida glabrata* and *Aspergillus parasiticus* using clotrimazole as a positive control.

Evaluation of antibacterial data revealed that, most of the tested compounds exhibited moderate to excellent antibacterial and good to moderate antifungal activity against all the tested microbial strains. Among them, the compound **5k** has exhibited excellent activity against *E. coli* (ZOI = 22 mm and MIC = 12.5 μg/mL), good activity against *S. aureus* (ZOI = 22 mm and MIC = 50 μg/mL) and moderate activity against *B. subtilis* (ZOI = 18 mm and MIC = 50 μg/mL), and *P. aeruginosa* (ZOI = 17 mm and MIC = 50 μg/mL). Similarly, the compound **5h** has shown good activity against *E. coli* (ZOI = 21 mm and MIC = 25 μg/mL) and *P. aeruginosa* (ZOI = 20 mm and MIC = 25 μg/mL), and moderate inhibiting activity against *S. aureus* (ZOI = 19 mm and MIC = 50 μg/mL). The compound **5a** has also exhibited good activity against *E. coli* (ZOI = 19 mm and MIC = 50 μg/mL) and moderate activity against *S. aureus* (ZOI = 17 mm and MIC = 50 μg/mL) and *P. aeruginosa* (ZOI = 17 mm and MIC = 50 μg/mL) with respect to the standard antibacterial drug Streptomycin. From the antifungal results we have observed that, the compounds **5b** (ZOI = 20 mm) and **5f** (ZOI = 19 mm) have shown good inhibiting activity against *A. niger* on comparing with the positive control drug Clotrimazole. Remaining all the

compounds have shown moderate activity against all the tested microbial strains with ZOI ranging from 7-16 mm and MIC 50-200 μg/mL for bacteria, and ZOI 8-18 mm for fungi.

Structure-activity relationship revealed marked antibacterial activity of the compounds (**5a-l**), when 6-bromo coumarinyl (**5k**), benzo[*f*]coumarinyl (**5h**) and simple phenyl groups (**5a**) introduced at the 4th position of thiazole moiety, while 4-methylphenyl (**5b**) and 4-flurophenyl (**5d**) enhanced antifungal activity.

***In vitro* antioxidant activity**

In order to investigate the possible biological studies for the synthesized compounds (**5a-l**), also screened *in vitro* antioxidant activity in terms of hydrogen donating or radical scavenging ability by rapid and convenient technique *i.e.* 1,1-diphenyl-2-pic-ryl-hydrazyl (DPPH) assay[4] using Trolox and Ascorbic acid as standard drugs. Methanol (95%), DPPH solution and standard drugs were used as blank, control and reference respectively. Absorbance was calculated at 517 nm (at absorption maximum of DPPH) after keeping the mixture of 100 μL of synthesized compounds of concentration 10 μg/mL (dissolved in DMSO) and 900 μL of DPPH radical solution (0.004% w/v of DPPH in methanol) in a dark for 30 min incubation period. Antioxidant activity was evaluated in IC_{50} in μM (the effective concentration at which 50% of the radicals were scavenged).

Evaluation of antioxidant activity revealed that, most of the tested compounds exhibited moderate to strong DPPH radical scavenging ability compared with the positive controls Trolox and Ascorbic acid. Among them, the compounds **5a** bearing phenyl, **5b** bearing 4-methyl phenyl and **5i** having 2*H*-chromen-2-one were found to be more effective and potent DPPH radical scavenging ability with ~1.11, ~1.09, ~1.02 folds than positive control drug Trolox. Remaining compounds have shown good to moderate radical scavenging activity with IC_{50} values in the range of 15.51-89.92 μM. It was noticed that, the compounds with electron donating groups on the phenyl ring were found to possess potent radical scavenging ability.

Molecular modelling studies

To explore and support the antibacterial mechanism, docking studies for the synthesized compounds (**5a-l**) was performed. This drug designing tool helpful to investigate and to gain a deep insight in to the mode of binding interactions of each of these ligands (**5a-l**) with the receptor sites of UDP-*N*-acetylenolpyruvoylglucosamine reductase, MurB (PDB id: 1MBT) and also to determine the best *in silico* conformation. Docking of the

synthesized ligands was employed by using Lamarckian Genetic Algorithm (LGA),[5] inculcated in the docking program AutoDock 4.2.

The docking studies revealed that, all the synthesized molecules exhibited excellent binding energies towards the receptor active pocket ranging from -9.02 to -11.15 kcal/mol. Among them, the conformations with lowest binding energies and those ligands exhibiting well established H-bonds with the closest range of 1.8-3.4 Å with one or more amino acids in the receptor active pocket were chosen as best docked ligand orientations (supporting file). Hence, the compounds **5a**, **5h** and **5k** were energetically favored for MurB active site and are exhibiting bonds with amino acids of active pocket of the receptor and considered as the best docking poses. The ligand **5a** exhibited H-bonding with SER229, ARG214, ARG159 amino acids, whereas 5h with ARG214, ARG159, SER50 amino acids and 5k with SER116, CYS113, SER50, ARG159, ARG214, SER229 amino acids. These results revealed a variety of binding modes that may provide a sufficient explanation and good compromise between docking scores and *in vitro* results of antibacterial activity.

CHAPTER-II (SECTION-B)

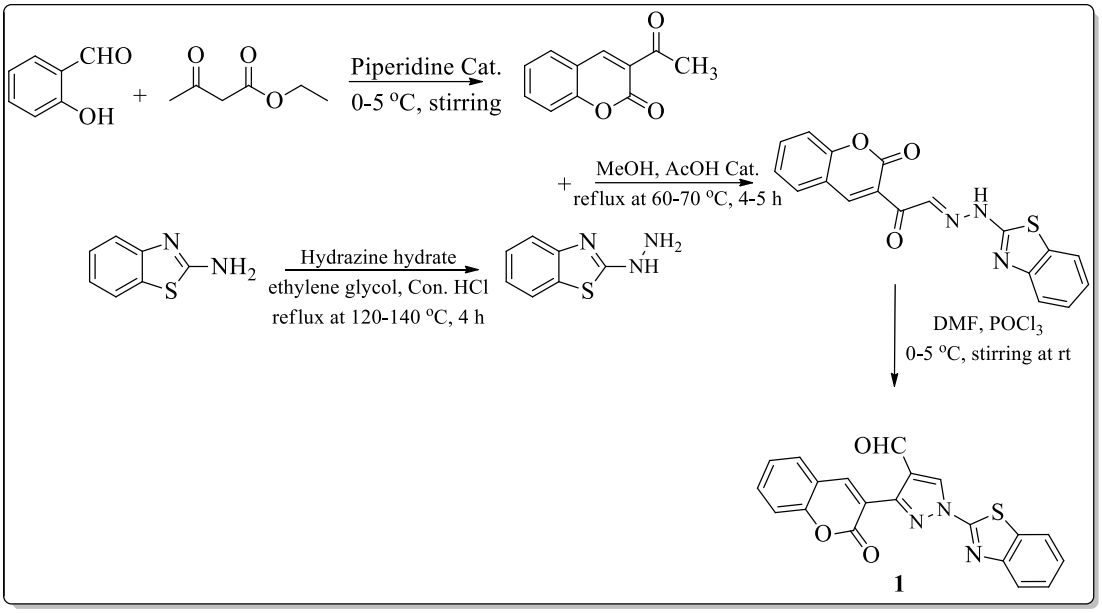
Design, three component one pot synthesis and *in vitro* biological evaluation of novel 1,3-disubstituted pyrazole-2,4-disubstituted thiazole hybrids embedding benzothiazole and coumarin moieties as antimicrobial, anti-biofilm agents

In this section, we have described the synthesis of new heterocyclic molecular hybrid framework and (**4a-l**) evaluated their antimicrobial activity.

Furthermore, literature survey indicates that benzothiazole, coumarin, 1,2-pyrazole and 1,3-thiazole scaffolds are the most versatile class of compounds attained a great attention in the field of drug design and discovery owing to their vast variety of biological activities. In addition, they also make the core structure of various bioactive natural and synthetic drugs.

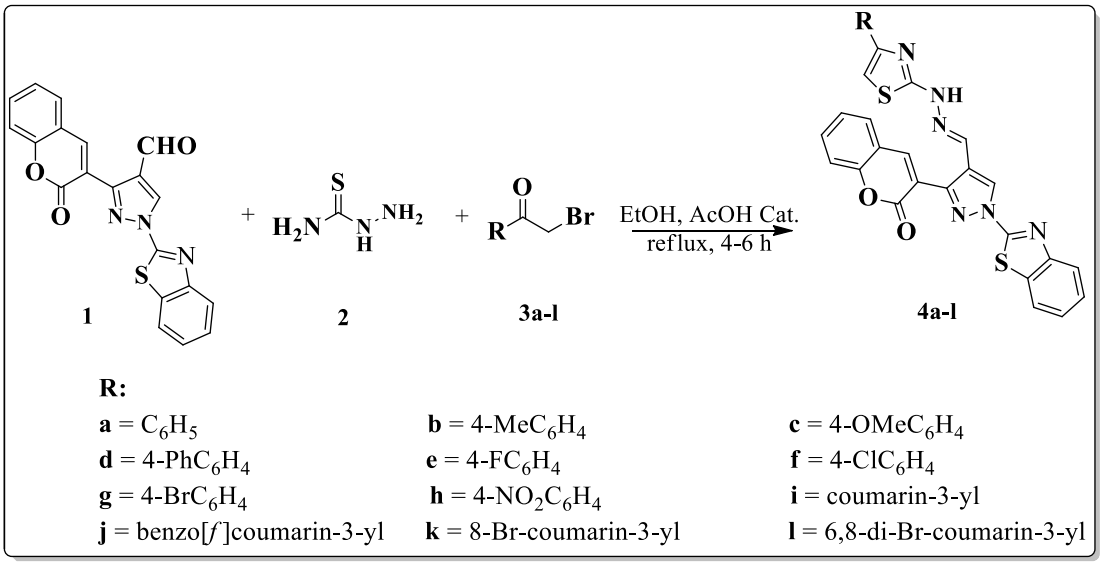
In light of above and also as a part of our endeavour in the search of novel heterocyclic hybrids, herein we, by adopting the hybridization approach[6], engendered a new molecular hybrid by amalgamated the four pharmacophores (Pyrazole, thiazole, coumarin and benzothiazole motifs) in one molecule.

The complete reaction conditions involved in the synthesis of titled compounds **4a-l** was illustrated in the **scheme-4** and **5**. The Vilsmeier-Haack formylation[7] of hydrazone achieved the key intermediate 1,3-disubstituted 4-functionalized pyrazole, which in turn afforded by the condensation of 3-acetyl coumarin and benzothiazole hydrazine. Further, the reaction of an aldehyde with thiosemicarbazide and various *α*-bromo ketones in refluxing ethanol in the presence of catalytic amount of glacial acetic acid furnished the target hybrid compounds **4a-l** in excellent yields (85-95%).



Scheme-4

All the synthesised compounds were well established using FTIR, NMR (^{1}H and ^{13}C), Mass spectrometry and CHN analysis data.



Scheme-5

Biological evaluation

To explore the antimicrobial potential, all the derivatives of the library were assessed for their *in vitro* antimicrobial activities such as minimum inhibitory concentration (MIC), minimum bactericidal concentration, and minimum fungicidal concentration (MFC), and anti-biofilm properties.

Antibacterial activity

In vitro antimicrobial activity of the novel series of synthesized molecular hybrids (**4a-l**) was screened against seven bacterial and one fungal strain by employing agar well diffusion method.[8] Gram positive pathogenic bacterial strains used in the present work were [*Staphylococcus aureus* (MTCC 96), *Bacillus subtilis* (MTCC 121), *Staphylococcus aureus* MLS-16 (MTCC 2940) *and Micrococcus luteus* (MTCC 2470)]. Gram-negative [*Klebsiella planticola* (MTCC 530), *Escherichia coli* (MTCC 739), *Pseudomonas aeruginosa* (MTCC 2453)] pathogenic bacterial strains.

In general, all of the tested compounds exhibited a certain degree of inhibiting activity. From the results, it was revealed that, out of the tested series of compounds, notably, **4b**, **4c**, **4g, 4k** and **4l** containing *p*-CH_3, *p*-OCH_3, *p*-Br on the phenyl ring and 8-bromo, 6,8-di-bromo coumarinyl substitutions respectively, have exhibited promising antibacterial activity towards the entire spectrum of gram positive and gram negative bacterial strains with MIC extending from 1.9 µg/mL to 7.8 µg/mL. In particular, the derivative **4b** has exhibited an excellent inhibitory activity with low MIC value (1.9 µg/mL) specifically against gram-positive *Bacillus subtilis* (MTCC 121) compared to that of remaining test compounds.

In general, all of the tested compounds exhibited a certain degree of inhibiting activity. From the results, it was revealed that, out of the tested series of compounds, notably, **4b**-**4d**, **4g**, and **4j**-**4l** exhibited a promising antimicrobial activity. The hybrid, **4b** with a tolyl substitution showed a very good to moderate activity with a MIC value of 1.9 µg/mL to 7.8 µg/mL against the tested pathogenic bacterial strains. The compound **4c** with a *p*-OCH_3 phenyl substitution showed a MIC of 7.8 µg/mL against all the test pathogens. The compound **4d** which is a biphenyl substituted thiazole derivative exhibited bacterial inhibiting activity with a MIC of 7.8 µg/mL against the test pathogens, except for *K. planticola* MTCC 530 and fungal strain *C. albicans* MTCC 3017. The compound **4g** a *p*-

Br phenyl substitution exhibited a good antimicrobial activity against the test pathogens MICs ranging from 3.9 µg/mL to 7.8 µg/mL except for *P. aeruginosa* MTCC 2453 and *C. albicans* MTCC 3017. The compound **4k** with 8-bromocoumarinyl substitution was observed as a lead compound with promising antimicrobial activity against all the test pathogens with MIC values ranging from 3.9 µg/mL to 7.8 µg/mL. The compound **4l** with 6,8-dibromocoumarinyl substitution showed a good antimicrobial activity with MIC of 3.9 µg/mL and 7.8 µg/mL against two Gram-positive strains *B. subtilis* MTCC 121 and *S. aureus* MLS 16 MTCC 2940 and MIC of 7.8 µg/mL against *K. planticola* MTCC 530 and *E. coli* MTCC 739. Whereas the compounds **4a**, **4e**, **4f**, **4h** and **4i** exhibited bacterial inhibition against some of the test strains at higher concentrations ranging from 15.6 µg/mL to 62.5 µg/mL

Antifungal activity

Candidiasis is the most common fungal infection in humans caused by any member of *Candida* spp. The chances of Candidiasis are propitious in infants and people with compromised immune system such as pregnant women, patients under medications for diabetes, chemo, HIV-AIDS. Statistics show around three out of every four women suffers from *Candida* infection for once at least in their life time. Also, *Candida* spp. has got the ability to form bio-films over medical devices such as cardiovascular catheters, contact lens, and voice prostheses etc. Keeping the aforementioned facts in mind, our compounds **4a-4l** were also screened for antifungal activity against 12 panel of fungal strains using Miconazole as a standard (NCCLS, Wayne, 2000). The screening showed that the compounds **4b** and **4c** were moderately effective against *C. albicans* MTCC 3017 with MICs of 7.8 µg/mL each. Whereas the compound **4j** was effective against *C. albicans* MTCC 3017, *C. albicans* MTCC 227, *C. albicans* MTCC 1637 with MICs ranging between 3.9 µg/mL to 7.8 µg/mL.

MIC (usually reported as mg/L or µg/mL) is the lowest concentration of an antimicrobial agent that will inhibit the visible growth of a microbe (at which it has bacteriostatic activity) after overnight incubation. Regardless, it didn't know from the MIC data that, the microorganisms are completely killed or not. That’s why once the MIC is determined; we have performed an extra set of steps in order to determine the lowest concentration at which an antimicrobial agent that reduces the viability of particular bacterium/fungi (MBC/MFC) by ≥99.9%.

Minimum bactericidal/fungicidal concentration (MBC/MFC)

The antimicrobials are normally regarded as bactericidal/fungicidal if the MBC/MFC is not more prominent than four circumstances the MIC.[9] Hence, considering the above MIC results of antibacterial and antifungal activity, using ciprofloxacin (MBC 0.9 µg/mL) and miconazole (MFC 7.8 µg/mL) as a reference drugs (positive controls) assayed for the MBC/MFC[2] for the compounds (**4b, 4c, 4d, 4g, 4j, 4k** and **4l)** which did not show any viability of the tested cells. It was observed from the results (**Table 1 and**) that, all the tested compounds exhibited good pattern of MBC (2xMIC = MBC) against the tested pathogenic strains. Especially, among them, the compound **4b**, **4c**, **4k** and **4l** has 2xMIC = MBC in case of all the tested bacterial strains except in case of *B. subtilis* for **4b**. However, the remaining other compounds **4d**, **4g**, **4j** and **4l** has also their bactericidal concentration below 2xMIC = MBC against some of the strains.

From the antifungal results, it was observed that the compounds **4b, 4c, 4j, 4k** showed fungicidal activity against *C. albicans* MTCC 3017 at MFC of 15.6 µg/mL. The compound **4j** showed MFC of 7.8 µg/mL against *C. albicans* MTCC 227 and *C. albicans* MTCC 1637 which equivalent to the standard. Further the compound **4k** showed a MFC of 7.8 µg/mL against *C. albicans* MTCC 227 which is equivalent to that of the positive control drug Miconazole.

Biofilm inhibition assay

Bio-films are self EPS (extra polymeric substance) based dynamic microbial communities with emerging characteristics that are different from single microbes. This collective matrix plays a role in increased resistance of microbial pathogens to antibiotic. Majority of the in-dwelling device associated and nosocomial microbial infections are associated with bio-film formation and enhanced resistance to available drugs. Bio-film formations are reported in contact lenses, intra uterine devices, prosthetic heart valves, breast implants, and dialysis catheters. Apart from bacteria, Candidiasis is the most common human infection involving bio-film formation thus posing the risk of resistance to current anti fungal compounds.

All these facts emphasize a great need for new compounds or hybrids of existing compounds that can destruct the bio-film matrix. Keeping in view of above facts, we explored the bio-film inhibiting property of the synthesized molecular hybrids. In the

current study, we tested compounds **4b, 4c, 4e, 4g, 4j** and **4k** against *M. luteus* MTCC 2470, *S. aureus* MTCC 96, *S. aureus* MLS16 MTCC2940, *B. subtilis* MTCC 121, *E. coli* and *K. planticola* MTCC 530, *Candida albicans* MTCC 3017, *P. aeruginosa* MTCC 2453. The results indicated that, the compound **4b** inhibits the biofilm formation of *S. aureus* MLS 16 MTCC 2940 and *M. luteus* MTCC 2470 with IC_{50} values of 28.5 µM and 19 µM respectively. While the compound **4c** showed the biofilm inhibition of *S. aureus* MTCC 96, *B. subtilis* MTCC 121, *S. aureus* MLS 16 MTCC 2940, *M. luteus* MTCC 2470, *E.* coli MTCC 739 with IC_{50} values of 28 µM, 19 µM, 24 µM, 22 µM, 27.8 µM respectively. The compound **4d** inhibited the biofilm formation of *S. aureus* MTCC 96, *M. luteus* MTCC 2470 and *K. planticola* MTCC 530 at IC_{50} values of 75 µM, 56 µM and 51 µM respectively. The compound **4j** inhibited the biofilm formation of *S. aureus* MTCC 96 with IC_{50} value of 11.8 µM. While the compound **4k** showed the antibiofilm property against *S. aureus* MTCC 96, *S. aureus* MLS 16 MTCC 2940, *K. planticola* MTCC 530, *E.* coli MTCC 739 and *C. albicans* MTCC 3017.

In conclusion, we have designed and synthesized a novel series of molecular hybrids (**4a-4l**) by amalgamating the Pyrazole, thiazole, coumarin and benzothiazole motifs for the first time. The scheme of the synthesis employed the Vilsmeier-Haack formylation for the one pot three component condensation reactions. The entire series of synthesized hybrids were screened for antibacterial, antifungal and anti biofilm properties. From the biological evaluation results, the compounds **4b** and **4k** showed promising antibacterial against the test pathogenic strains. The **4b** and **4k** also exhibited a very good antibiofilm property. Further the compound **4k** showed a good antifungal property against *Candida albicans* MTCC 3017 and *Candida albicans* MTCC 227.

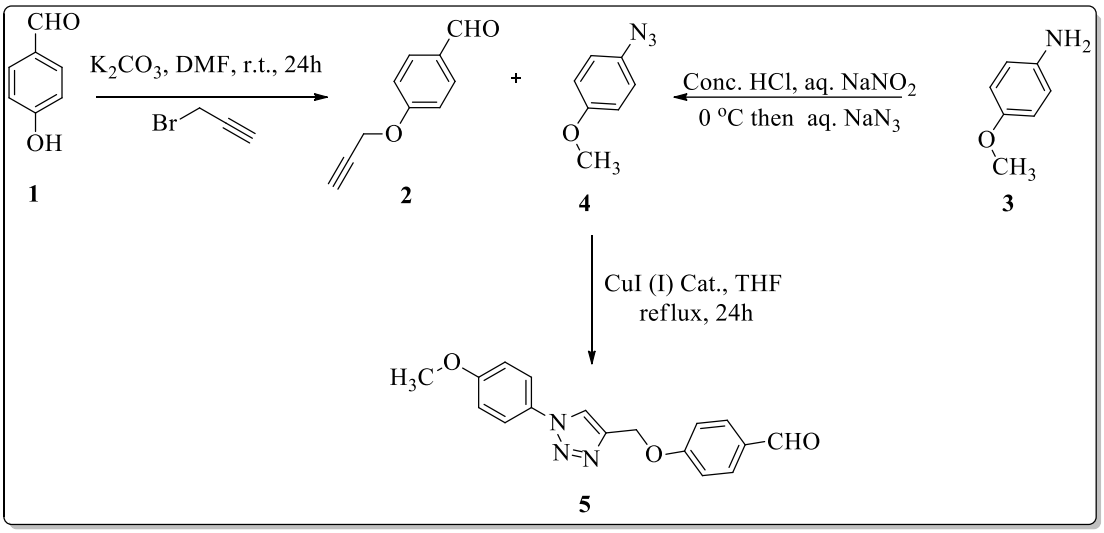
CHAPTER-III (SECTION-A)

Synthesis of new 4-substituted 1,2,3-triazole-hydrazinyl 1,3-thiazole hybrids by employing 'click' chemistry: *in vitro* antimicrobial and anti-biofilm studies

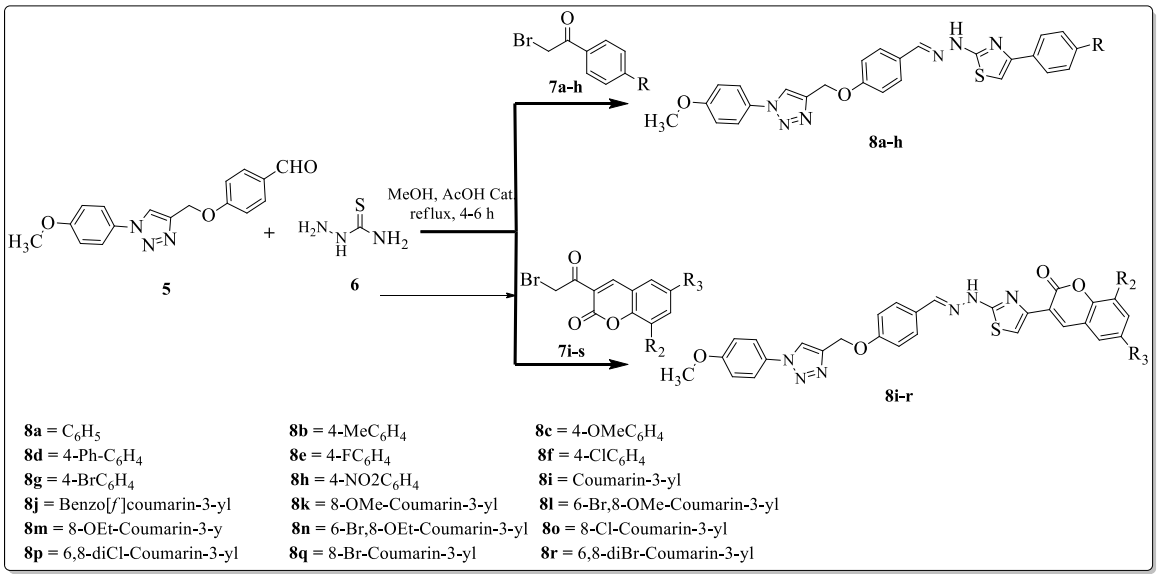
Embracing the hybridization approach, a series of novel hydrazinyl 1,3-thiazole-1,4-di-substituted 1,2,3-triazole hybrids (**8a-r**) were synthesized in quantitatively isolated yield by the three component condensation of aldehyde **5**, *N*-aminothiourea **6** and α-bromo ketones **7a-r** [4-substituted phenacyl bromides and substituted 3-(bromoacetyl)coumarins] under refluxing ethanol in the presence of catalytic amount of acetic acid. The synthetic

protocol for the title compounds has outlined in **scheme 7**. A key starting material in this work, 4-((1-(4-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)benzaldehyde **5** was achieved by the copper(I)-catalyzed region-selective Huisgen 1,3-dipolar cyclo-addition reaction[10] of a terminal alkyne **2** with an azide **4** *via* click chemistry approach. (**Scheme 6**).

With the key intermediate 1,4-disubstituted triazolyl aldehyde **5** in hand, all the title compounds **8a-r** were afforded in 82-95 % quantitative isolated yield. Structures of all the synthesized compounds were well established and they are in good agreement with their spectral (FTIR, ^{1}H NMR, ^{13}C NMR and ESI) and elemental analyses (C, H, and N).



Scheme-6



Scheme-7

Biological evaluation

Antimicrobial evaluation (MIC/MBC)

The *in vitro* antimicrobial activity of the novel series of synthesized molecular hybrids (**8a-r**) was screened against seven bacterial including four gram positive and three gram negative strains one fungal strain by employing agar well diffusion method.

In general, most of the synthesized compounds exhibited a certain degree of inhibiting activity. It was observed form the activity results that, out of the tested series of compounds, notably, **8c**, **8i-8l**, **8p** and **8q** displayed an appreciable antimicrobial activity. The hybrid, **8l** with a 6-bromo8-methoxycoumarinyl substitution and **8k** with 8-methoxycoumarinyl substitution exhibited excellent and broad spectrum of activity (8k, except against *P. aeruginosa*) against almost all the tested strains with MIC values ranging from 1.9 µg/mL to 3.9 µg/mL and MBC values ranging from 3.9 to 7.8 µg/mL. The remaining compounds (**8c**, **8i**, **8j**, **8p** and **8q**) exhibited good bacterial inhibiting activity with MICs of 3.9 µg/mL and MBC values ranging from 7.8 to 15.6 µg/mL against the test strains. Whereas the remaining compounds, exhibited moderate bacterial inhibition against the test strains with MIC values ranges from 7.8 to 15.6 µg/mL and MBC values ranging from 15.6 to 31.2 µg/mL.

Antifungal activity (MIC/MFC)

Keeping in mind about the aforementioned facts, our compounds **8a-4r** were also screened for *in vitro* antifungal activity against 12 panels of fungal strains using Miconazole as a standard. The screening results showed that the compounds **8i**, **8j**, **8l** and **8r** were effective against *C. albicans* strains with MICs of 3.9 µg/mL and MFCs 7.8 µg/mL.

Biofilm inhibition assay

Bio-films are self extra polymeric substance based dynamic microbial communities with emerging characteristics that are different from single microbes. This collective matrix plays a prominent role in protecting the microbial pathogens from antibiotics. Majority of the in-dwelling device associated and nosocomial microbial infections are associated with bio-film formation pathogens. Bio-film formations are reported in contact lenses, intra uterine devices, prosthetic heart valves, breast implants, and dialysis catheters. Apart from

bacteria, Candidiasis can also form bio-film, thus posing the risk of resistance to current anti fungal compounds.

Keeping in view of above facts, we explored the bio-film inhibiting property of the synthesized compounds (**8a**-**8r**). In the present work, the compounds **8i-8r** were tested against *M. luteus* MTCC 2470, *S. aureus* MTCC 96, *S. aureus* MLS16 MTCC2940, *B. subtilis* MTCC 121, *E. coli* MTCC 739 and *K. planticola* MTCC 530, *Candida albicans* MTCC 3017, *P. aeruginosa* MTCC 2453. The results indicated that, the compound **8i** against *B. subtilis* MTCC 121 with IC_{50} value 6.6 µM and **8l** and **8k** against *S. aureus* MTCC 96 with IC_{50} values 12.0 and 13.5 µM respectively, inhibited the biofilm formation.

In summary, inspired by the hybridization approach, herein we have designed a simple and efficient protocol for the synthesis of 1,2,3-triazole-hydrazinyl 1,3-thiazole hybrids *via* multicomponent strategy. All the compounds were confirmed by spectral data and screened for *in vitro* antimicrobial and bio-film activities. Compounds **8c**, **8i-8l**, **8p** and **8q** were identified as a potential lead compounds to develop antibacterial, antifungal and anti-biofilm agents.

CHAPTER-III (SECTION-B)

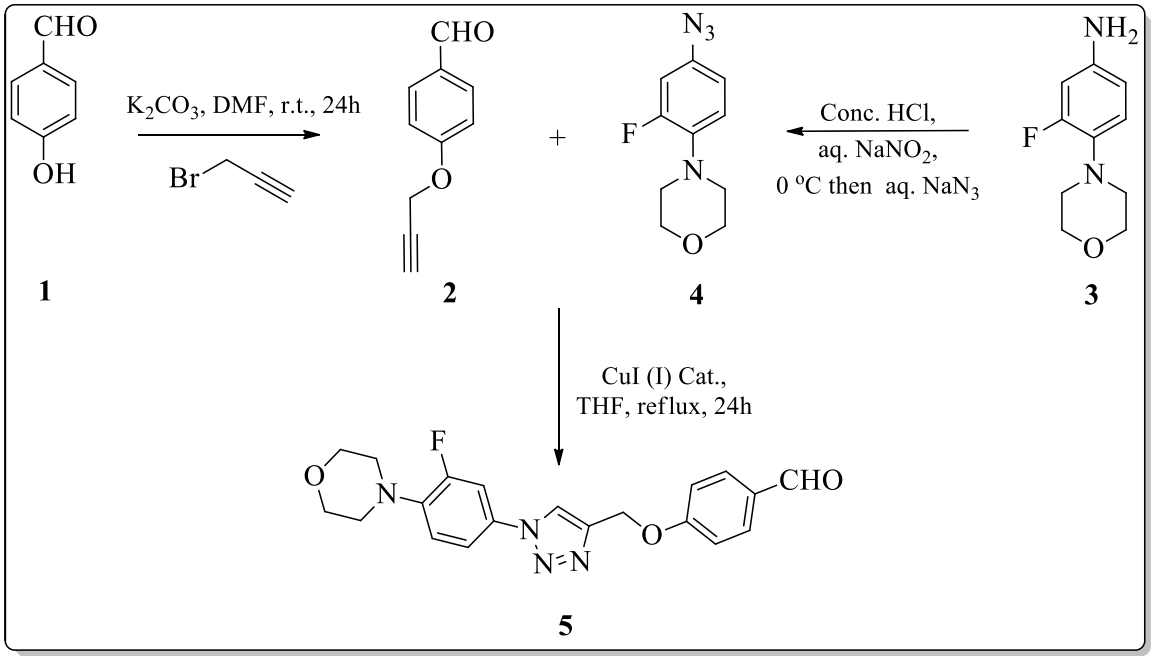
Synthesis of new morpholine based hydrazinyl 1,3,4-thiadiazin-1,2,3-triazole hybrids

This section deals with the synthesis and biological evaluation of morpholine based hydrazinyl 1,3,4-thiadiazin-1,2,3-triazole hybrids (**8a-j**) and the synthetic strategy employed in the synthesis of starting compound **5** and target compounds was illustrated in **scheme-8** and **9**.

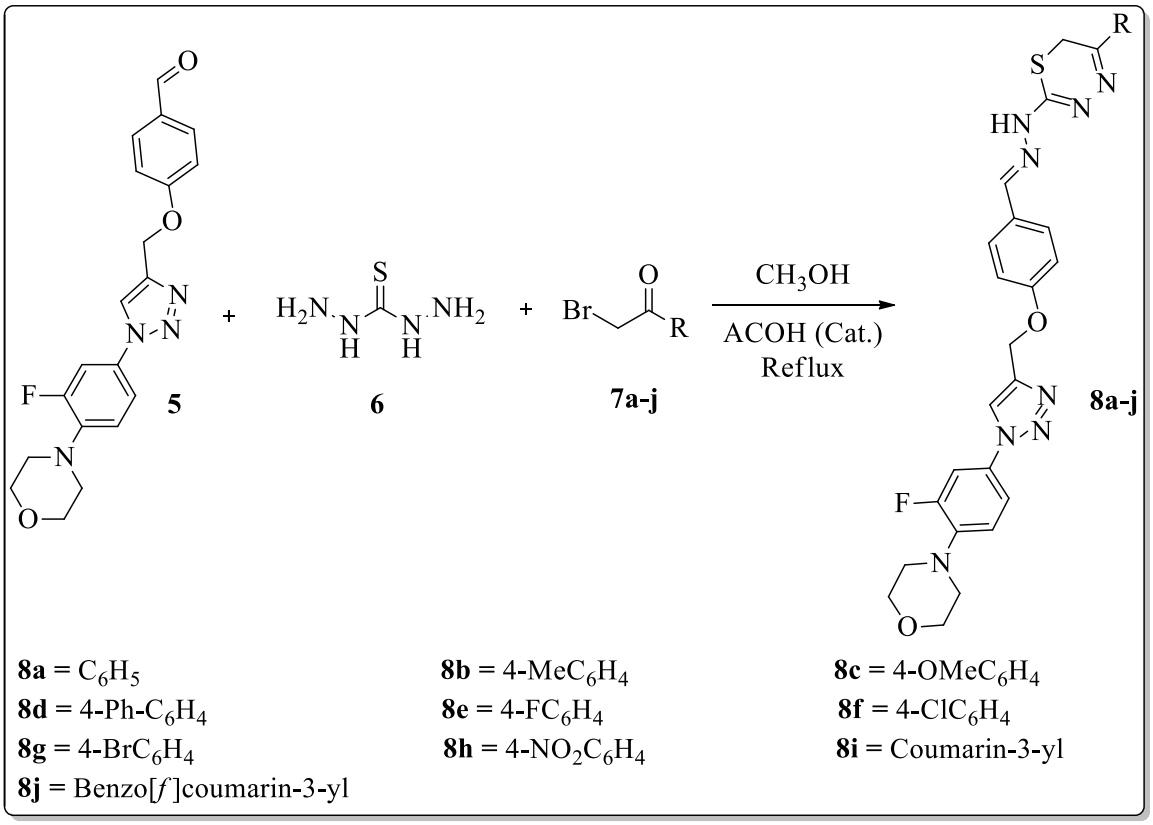
The key starting material in this work, **5** was achieved by the Cu(I)-catalyzed region-selective Huisgen 1,3-dipolar cyclo-addition reaction of a terminal alkyne **2** with an azide **3** *via* click chemistry approach (**Scheme-8)**.

One pot three component condensation reactions of aldehyde **5**, thiocarbohydrazide **6** and various *α*-bromo ketones (**7a-j)** in refluxing methanol in the presence of catalytic amount

of acetic acid (**Scheme-9)** furnished the title compounds (**8a-j**) in 80-90% isolated yield. Structures of all the synthesized compounds were well established and they are in good agreement with their spectral (FTIR, ^{1}H NMR, ^{13}C NMR and ESI) and elemental analyses (C, H, and N).



Scheme-8



Scheme-9

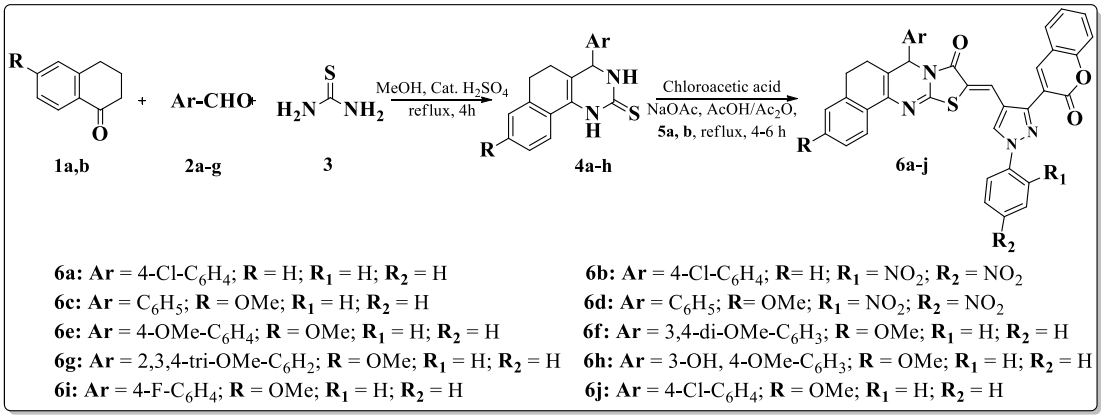
In conclusion, embracing the molecular hybridization approach, a series of hydrazinyl 1,3,4-thiadiazinyl-1,4-di-substituted 1,2,3-triazole hybrids (**8a-j**) were designed and synthesized successfully good to excellent yields by the one-pot three component condensation of an aldehyde **5**, TCH **6** and *α*-bromo ketones **7a-i** in refluxing methanol in the presence of catalytic amount of glacial acetic acid.

CHAPTER-IV (SECTION-A)

One pot synthesis of fused thiazolo[2,3-b]pyrimidinone-pyrazolylcoumarin hybrids and their anticancer, antibacterial activity and molecular docking studies

In the course of our longstanding research endeavour towards the development of new bioactive heterocycles, from the molecular design point of view and also by relying on the aforementioned biological data, here in we synthesized a series of novel heterocyclic hybrids (**6a-j**) embedding thiazolo[2,3-*b*]pyrimidinones and pyrazolyl chromenone moieties in their structural framework, hoping the synergistic influence of this combination on antibacterial and anticancer activity and to obtain new promising antibacterial and anticancer hits for further studies.

The target compounds (**6a-j**) were achieved by the one-pot three-component condensation of modified Biginelli product, fused 3,4-dihydropyrimidin-2(1*H*)-thiones (**4a-h**), mono chloroacetic acid and 3-(2-oxo-2*H*-chromen-3-yl)-1-aryl-1*H*-pyrazole-4-carbaldehyde (**5a**,**b**) in refluxing AcOH in the presence of Ac_2O and NaOAc furnished the desired products in good yields.



Scheme-10

All the synthesised compounds were well established by the spectral and analytical analyses and also assessed for their *in vitro* antibacterial studies against gram positive and gram negative bacteria and anticancer activity against diverse human cancer cell lines.

Biological Activity

Anti-proliferative activity

In vitro cytotoxic activity was carried out against human colorectal adenocarcinoma (Colo 205), chronic myelogenous leukaemia (K562), breast adenocarcinoma (MCF-7), breast adenocarcinoma (MDA-MB-231), hepatocellular Carcinoma Chronic Myelogenous (Hep G2), Human Embryonic Kidney 293 cells (HEK293). One of the most effective anticancer agents, Doxorubicin (DOX) was used as a positive control (reference). The cell lines were obtained from the National Centre for Cell Sciences, Pune, India, and were cultured at a seeding density of 0.2×106 in DMEM/RPMI medium supplemented with 10% FBS, 100 UmL^{-1} penicillin, and 100 μgmL^{-1} streptomycin, respectively, and maintained in a humidified atmosphere with 5% CO_2 at 37±1 °C. The samples were dissolved in dimethylsulfoxide (DMSO; not exceeding the final concentration of 0.01%) and further diluted in cell culture medium. The antiproliferative response of the extract was assessed by the quantitative colorimetric 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay[11] Cells (~10,000) were plated in 200 μL growth medium in the

presence or absence of the test sample (10 μM concentration) in 96-well culture plates for 24 h. Then the culture plates were centrifuged at 2000 rpm for 10 min at room temperature. 100 μL of supernatant was discarded and 20 μL of MTT (5 $mgmL^{-1}$ in PBS) was added to each well and incubated for 4h at 37±1 °C. The viability of the cells was determined using a spectrophotometer at 570 nm. HEK 293 cells were screened to evaluate toxicity of the sample. The response parameter was expressed in the average percentage of inhibition of samples at 10 μM concentrations and the experiment was performed in triplicate and the results were taken as a mean ± standard deviation (SD).

In vitro, cytotoxic activity results revealed that all of the tested samples exhibited moderate to weak inhibiting activity and most of the synthesised compounds were inactive. However, the derivatives **6a**, **6g**, **6h** and **6j** have shown moderate antiproliferative activity when compared with reference drug doxorubicin (DOX) against the Hep G2 and Colo 205 tumour cell lines with an average percentage of inhibition (Avg % inh.) ranging from 35.54 to 40.34. Among the moderately potent test compounds, **6a**, **6h** and **6j** exhibited good antiproliferative activity against the Hep G2 with an average percentage of inhibition values, 40.34, 39.69 and 36.31 respectively, and the compound **6g** have shown good antiproliferative activity against both Colo 205 and Hep G2 tumor cell lines with an average percentage of inhibition values, 35.54 and 36.17 respectively. On overall comparison, compounds derived from 4-chloro phenyl (**6a** and **6j**), 2,3,4-trimethoxy phenyl (**6g**) and 3-hydroxy4-methoxy phenyl (**6h**) have shown modest anticancer activity against the tested cell lines. Hence, further optimisations of the compounds may enhance their antiproliferative activity and they can be considered as a lead molecule for the development of new anticancer drugs.

Antibacterial Activity

All the synthesized compounds (**6a-j**) were assessed for their *in vitro* antibacterial activity against both Gram-positive (G^+) bacterial strains including *Staphylococcus aureus* (MTCC 121), *Bacillus subtilis* (MTCC 96) and *Staphylococcus epidermidis* (MTCC 2639) and Gram-negative (G^-) bacteria including *Escherichia coli* (MTCC 40), *Klebsiella pneumonia* (MTCC 109) and *Pseudomonas aeruginosa* (MTCC 2453). Standard pathogenic microbial cultures were procured from the Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, India, which was recognised by the World Intellectual Property Organization (WIPO). The experiments were carried out in triplicate

and the results were taken as a mean ± standard deviation (SD). The minimum inhibitory concentrations (MICs) for all the synthesised compounds after 24 h incubation in the darkness at 37±1 ^{o}C in a humid atmosphere were reported in μg ml^{-1} and the results were illustrated in **Table-2**. Antibacterial activity was assessed by the standard broth microdilution technique.[12] The antibiotics, penicillin and streptomycin were used as positive controls (standards) and DMSO was used as a negative control (solvent control) and they were also screened under identical conditions for the comparison of activity results.

It is evident from results that, the majority of the tested compounds (**6b**, **6c**, **6e**, **6f**, **6g**, **6h** and **6j)** exerted significant *in vitro* antibacterial inhibiting activity against all the tested bacterial strains with MICs ranging from 1.56 to 12.5 μg mL^{-1}. Among them, the compound **6g** with 2,3,4-trimethoxy substituent's on phenyl ring was found to be stronger and have shown equipotent inhibitory efficacies and broader antibacterial spectrum than that of the reference drugs. Compound **6g** exhibited excellent inhibiting activity than the standard streptomycin (MIC = 6.25 μg mL^{-1}) and equipotent to that of penicillin (MIC = 1.562 μg mL^{-1}) against *S. aureus* and *B. subtilis* with MIC values 1.56 μg mL^{-1}, exerted nearly as active as positive control drugs (MIC = 3.125 μg mL^{-1}) against gram positive *S. epidermidis*(MIC = 3.12 μg mL^{-1}) and effectively inhibited the gram-negative *E. coli* and *K. pneumonia* (MIC = 6.25 μg mL^{-1}) and also demonstrated inhibitory potency against *P. aeruginosa* (MIC = 12.5 μg mL^{-1}) equal to that of the standard penicillin. Compounds **6f** and **6h** could effectively inhibit the growth of *S. aureus* with MIC values, (MIC = 1.56 and 3.12 μg mL^{-1} respectively) and *P. aeruginosa* (MIC = 6.25 μg mL^{-1}). Compound **6c** and **6f** exhibited inhibition of *E. coli* (MIC = 12.5 μg mL^{-1}) nearly to that of penicillin. Compounds **6b**, **6e**, **6f** and **6h** have shown bioactivity against *P. aeruginosa* (MIC = 6.25 μg mL^{-1}) which was better than penicillin. Finally, the compounds **6h** and **6j** have shown equipotent activity than that of standards streptomycin and penicillin respectively against *B. subtilis* and *P. aeruginosa* ((MIC = 6.25 and 12.5 μg mL^{-1}).While, the rest of the compounds (**6a**, **6d** and **6i**) have shown modest activity against all the tested strains with MIC values ranging from 12.5 to 50 μg mL^{-1}.

Structure activity relationship (SAR)

Interestingly, it was observed from experimental data that, the most of the analogues displayed potent bioactivity against gram-positive pathogenic strains, *i.e.*, *B. subtilis*, *S.*

aureus and *S. epidermidis* with MIC values ranging from 1.56 to 3.12 μg $mL^{-1}$and displayed moderate to good activity against gram-negative microorganisms, *i.e*., *E. coli*, *P. aeruginosa* and *K. pneumonia* with MICs ranging from 6.25 to 12.5 μg mL^{-1}. From the above *in vitro* results, overall we can conclude that the derivatives **6e**, **6f**, **6g** and **6h** derived from 4-,3,4-di, 2,3,4-trimethoxyphenyl and 3-hydroxy-4-methoxyphenyl moieties on the thiazolo-quinazoline ring were found to be potent antibacterial agents.

Molecular docking study

Molecular docking studies were performed using Schrodinger suite 2010. Initially, the crystal structure of target enzyme MurB (PDB id: 1MBB) was obtained from protein data bank (http://www.rcsb.org/pdb). It was prepared, refined and minimised using protein preparation wizard available in the Schrodinger suite 2010. Later receptor grid was generated around the active site of the enzyme using GLIDE 5.6 (Schrödinger LLC, 2010), Glide, version 5.6. New York). During grid generation, the receptor Vander Waals scaling was set to 0.9.[13] Meanwhile, the ligands were drawn in Maestro build panel and prepared by LigPrep module available in the same suite. Finally, the low energy conformers of the prepared ligands were docked into the active site of the MurB enzyme. Docking results are tabulated in **Table 3**, energetically most favoured dock pose of each ligand was analyzed for interactions with the target receptor.

The docking studies clearly showed that the best active compound in the series (**6g**) can act as good anti-bacterial agent which is evident from its high dock score (-6.098 kcal mol^{-1}). It showed three hydrogen bond interactions with Tyr158, Lys 217, Lys 275 and a π-π stacking interaction with Arg 159. On the other hand compounds, **6c** and **6f** which possessed good activity values next to compound **6g** in the series showed dock scores of -5.005 and -5.248 kcal mol^{-1} respectively. Compound **6c** showed two hydrogen bonds with Lys 217 and Gln 288 whereas compound **6f** showed three hydrogen bonds with Ser 229, Lys 262, Tyr 254 and a π-cationic interaction with Lys 217. Compounds **6h** and **6e** which exhibited poor biological activities showed poor dock scores of -3.656 and -2.968 kcal mol^{-1} respectively. Further compound **6h** showed only one hydrogen interaction with Gln 287 and hydrogen bond interactions were completely absent in compound **6e**. This might be one of the reasons for their low biological activity. Compound **6i** which exhibited relatively better activity compared to **6h** and **6e** showed a dock score of -4.294 kcal mol^{-1} and formed a hydrogen bond with Gln 288. It also showed a π-cationic interaction with

Lys 262. The docking results well corroborated with *in vitro* antibacterial studies indicating that these compounds can be further optimised and developed as lead compounds.

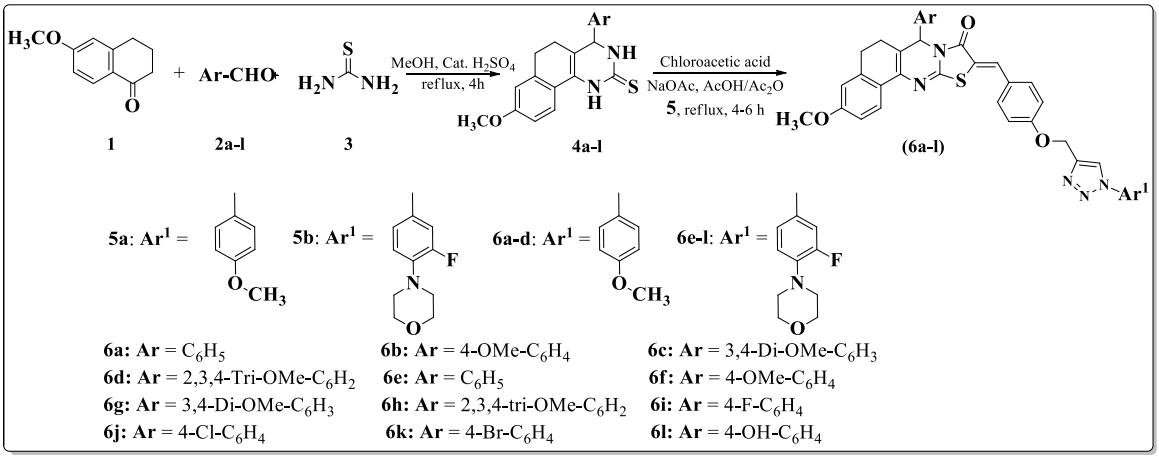
In summary, a series of novel 10-((3-(2-oxo-2*H*-chromen-3-yl)-1-aryl-1*H*-pyrazol-4-yl)methylene)-7-aryl-7,10-dihydro-5*H*-benzo[*h*]thiazolo[2,3-*b*]quinazolin-9(6*H*)-one derivatives (**6a-j**) were designed and synthesised by the one pot three-component approach with the hope of discovering new bioactive molecular hybrids with enhanced broad spectrum of pharmacological activities. All the newly synthesised compounds were well characterised by spectral and elemental analyses. And were investigated for their *in vitro* antiproliferative and antibacterial activities by MTT and broth microdilution technique respectively. From the experimental studies, it was revealed that, among the synthesized compounds (**6a-j**), the derivatives **6a**, **6b**, **6c**, **6e**, **6f**, **6g**, **6h** and **6j** were found to possess good biological activity. Among htem, compounds **6a**, **6g**, **6h** and **6j** were found to have moderate antiproliferative activity against Hep G2 and Colo 205 cell lines. From the antibacterial data, compounds **6f**, **6g** and **6h** showed broad and excellent antibacterial efficacy against both G^+ and G^- strains comparable to that of the standards. The compounds **6b**, **6c**, **6e** and **6j** exhibited excellent inhibiting activity against G^- strains. The rest of the compounds **6a**, **6d** and **6i** displayed no antibacterial activity and were found to be inactive. These *in vitro* antibacterial studies were further supported by molecular docking. Overall, from the *in vitro* anticancer and antibacterial studies, we can conclude that the presence of 4-chlorophenyl, mono, di and tri-methoxy phenyl and 3-hydroxy-4-methoxyphenyl moieties on the thiazolo-quinazoline scaffold has been suggested to be responsible for the *in vitro* antiproliferative and antibacterial activities of the title compounds. Based on the above results, the synthesised series of compounds could be potential candidates for further development of novel anticancer and antimicrobial agents.

CHAPTER-IV (SECTION-B)

One pot three component synthesis of fused thiazolo[2,3-*b*]pyrimidinone-morpholine based triazolyle hybrids

In this section we have designed and synthesized new molecular framework embedding biologically active pharmacophores thiazolo[2,3-*b*]pyrimidinone and triazole. The synthetic pathway to achieve the titled compounds (**6a-l**) was outlined in **Scheme 11**. The

target compounds were achieved by the knoevenagel condensation of aldehydes (**5a**, **b**) with fused 3,4-dihydropyrimidin-2(1*H*)-thiones in methanol with catalytic amount of piperidine under reflux condition. The unrepeated intermediate fused 3,4-dihydropyrimidin-2(1*H*)-thiones obtained by the cyclization of modified biginelli compound (**4a-h**) with mono chloroacetic acid in refluxing AcOH in the presence of Ac_2O and NaOAc furnished the desired products (**6a-l**) in good yields. The structures of the titled compounds were well established by the FTIR, NMR and mass spectral studies as well as elemental analyses (C, H and N).



Scheme-11

In conclusion, a series of fused thiazolo[2,3-*b*]pyrimidinone-morpholine based triazolyle hybrids (**6a**–**l**) were designed by adopting molecular hybdridization and successfully synthesized by knoevenagel condensation of 4-((1-(4-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)benzaldehyde (**5a**) and 4-((1-(3-fluoro-4-morpholinophenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)benzaldehyde (**5b**) with fused thiazolo[2,3-*b*]pyrimidinones (**4a**-**h**) in acceptable yields.

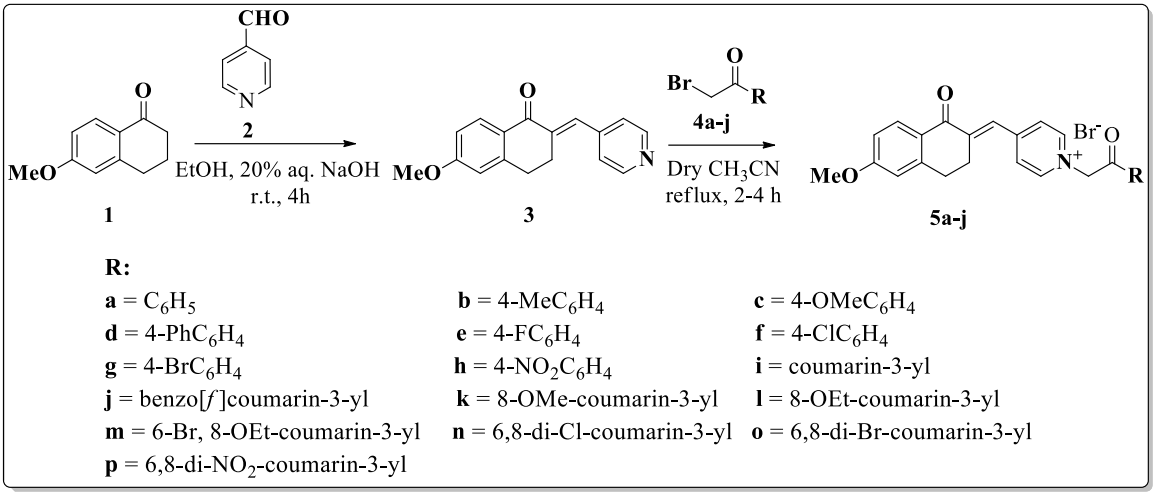
CHAPTER-V (SECTION-A)

Novel Donepezil like fused chalcone-pyridinium bromide hybrids: Synthesis, characterization and evaluation of anticancer, antimicrobial activity studies

Literature survey reveals the role of chalcones and pyridinium salts as an important pharmacophores with marked biological activity prompted us to undertake to develop a series of novel chalcone bearing new pyridinium bromide hybrids with an expectation to produce promising biologically active agents with diverse pharmacological activities. We herein, report the synthesis and structural elucidation of series of novel heterocyclic

hybrids that clubbed fused chalcone and pyridinium bromides and *in vitro*, *in vivo*[14] screening for their anticancer and *in vitro* antimicrobial activities.

The desired compounds (**5a-p)** were synthesized as detailed in **Scheme 12**, and were achieved by the aldol-condensation of 6-methoxy tetralone (**1**) with 4-pyridinecarboxaldehyde (**2**) in ethanolic sodium hydroxide at room temperature, furnished the corresponding chalcone **(3**) preferentially in *E*-configuration as thermodynamically favoured structure,[15] which on further quaternization with corresponding *α*-bromo ketones (**4a-p**) under refluxing dry acetonitrile, furnished the title compounds (**5a-p**) in an excellent yields (86-92%) with analytical purity.



Scheme-12

The structures of all the newly synthesized compounds were well established by the spectroscopic techniques such as FTIR, ^{1}H NMR, ^{13}C NMR, mass spectral data and elemental analysis (C, H and N) and the spectroscopic data were well in accordance with the proposed structures.

Biological Evaluation

In vitro anti-proliferative assay

The antiproliferative potential of pyridinium bromides **5a-j**, was evaluated as *in vitro* cytotoxic activity against human MCF-7 (breast cancer), HeLa (cervical carcinoma) and U-87MG (human glioblastoma) cell lines by quantitative colorimetric method i.e. the (MTT) assay[36,37] using doxorubicin (DOX) as a positive control drug. The results are summarized in Table 1 where the cytotoxicity is expressed as IC_{50} (inhibitory

concentration 50%) values. The survival curves of MCF-7, HeLa and U-87MG, plotted as surviving fraction *vs* concentration of drug (log μM).

Based on the screening results of antiproliferative activity, the structure activity relationships (SAR) for the some of the synthesized compounds **5a-j** are described as follows. The compound possessing electron donating group (methoxy) at 4th position of phenyl ring (**5c**) has exhibited, broad spectrum anti-proliferative activity with IC_{50} 10.86 ± 0.2 μM against MCF-7, IC_{50} 4.67 ± 0.5 μM against HeLa and IC_{50} 6.89 ± 0.3 μM against the U-87MG. Similarly, the compound bearing strong electron withdrawing group (nitro) on phenyl ring at 4th position (**5h**) was also found to be having potential cytotoxic activity with broad spectrum against MCF-7 with IC_{50} 7.82 ± 0.5 μM, HeLa with 3.88 ± 0.1 μM and U-87 MG with 4.53 ± 0.3 μM cell lines. Also the compounds **5f** and **5i** has moderate cytotoxic activity against against U-87MG cell line (IC50 12.19 ± 0.2 μM and 11.58 ± 0.4 μM respectively) and against HeLa with IC_{50} 15.13 ± 0.5 μM and 17.30 ± 0.6 μM respectively. And the remaining all the compounds were shown weak activity against all the tested cell lines.

Linear regression analysis

In order to understand the structural requirements in terms of hydrophobic, steric and electronic effects for the variation in anti-cancer activity, the quantitative structure activity relationship (QSAR) studies were performed using multiple linear regressions (MLR) analysis. In view of the major structural modifications in terms of aromatic substitution in 8 (**5a**-**5h**) out of 10 compounds were considered for studying these effects in different cancer cell lines.

Initially each parameter was correlated with anticancer activity where no significant correlations were obtained. However it was observed that individually electronic effect in terms of (σ) showed positive and almost similar influence on the three cell lines. The hydrophobic effects in terms of pi influenced anticancer activity negatively and the effects were more prominent for the cell lines MCF-7 and U87-MG, while steric effect in terms of MR influenced anticancer activity positively for all cell lines.

Since none of the physicochemical parameters showed more than 0.4 correlation and removal of one compound **5d** having a phenyl Substitution at para position improved the correlation of anticancer activity of different cell lines with pi (excluding Hela cell line)

and MR. It also resulted in decreased intercorrelation between pi and MR (R^2= 0.818 to 0.237). Thus it appears that MR plays a key role in describing the variation of activity and contributes positively followed by hydrophobicity which influences the activity negatively. Hence the effect of both noncollinear (R^2=0.237) parameters π and MR was studied knowing the limitation of dataset of 7 compounds for the three anticancer activities (Equations 1 to 3) where; n= number of compounds; R = multiple correlation coefficient; F = F-ratio; S = standard error of estimate.

For MCF-7 cell line

Log 1/C = -0.891 (± 0.201) π + 0.122 (± 0.027) MR-2.055

n = 7, R = 0.944, R2 = 0.89, S = 0.205, F = 16.254 (1)

For HeLa cell line

Log 1/C = -0.369 (± 0.192) π + 0.178 (± 0.026) MR-2.049

n = 7, R = 0.961, R2 = 0.923, S = 0.196, F = 23.84 (2)

For U-87 MG cell line

Log 1/C = -0.640 (± 0.353) π + 0.102 (± 0.047) MR-1.664

n = 7, R = 0.785, R2 = 0.616, S = 0.360, F = 3.21(3)

All the equations are statistically significant (>97 %) except equation 3 along with the relatively statistically significant regression coefficient values. Though both the parameters π and MR show similar positive and negative effects respectively for all the three cancer cell lines, but differ in terms of the magnitude which are as follows MCF-7>U-87MG>HeLa for π and HeLa>MCF-7> U-87MG for MR. Thus judicious structural substitution may result in both improvement and selectivity of anticancer activity towards these cell lines.

***In vivo* cytotoxic activity**

Adult male Swiss albino mice (Sainath Enterprises, Hyderabad, India) of 8 weeks of age (mean weight in the range of 20-25 G) were selected and housed in polypropylene cages in a room, where the congenial temperature of 27±1 0C and 12 h light and dark cycles were maintained. The animals were allowed to acclimatize to the environment for 7 days supplied with a standard pellet diet and water ad libitum. All procedures using animals were reviewed and approved by the Institutional Animal Ethical Committee (IAEC/29/UCPSc/KU/2015) of Kakatiya University. The animals were divided into seven groups (n = 10). The normal group was not inoculated with tumor cells, while six other

groups were injected with EAC cells (0.2 mL of 2x106 cells mice^{-1}) intraperitoneally. This was taken as a day '0' and the experimental treatment started 24 h later. From the first day 100 µLmouse-1 per day of sterile saline was administered intraperitoneally to the negative control group (EAC-bearing mice).The compounds **5c** and **5h** at doses of 5 mgkg-1 and 10 mgkg^{-1} were administered each day to the treated groups, and the standard drug Cisplatin at a dose 5 mgkg^{-1} was administered to each animal from the positive control group. The pharmacological treatment lasted for 9 days. Fourteen days after the treatment, five mice from each group were scarified for the study of antitumor activity. The rest of the animal groups were kept to checking the mean survival time of EAC tumor bearing hosts. The antitumor effects of the compounds were determined by the change in body weight, mean survival time (MST) and percentage of increased life span (%ILS). The MST of each group containing five mice was identified by recording mortality on a daily basis for 30 days, and the %ILS was calculated using the following equations MST = (day of first death + day of last death)/2, and %ILS = [(mean survival time of treated group/mean survival time of control group)-1] x 100. The effect of the compounds was also assessed by the determination of the body weight, tumor volume, packed cell volume and viable tumor cell count of EAC bearing mice by the Tryphan blue dye exclusion method.

The potent compounds (**5c** & **5h**) were examined for their *in vivo* anticancer activity in EAC bearing mice by using liquid tumor model.[16] The effect of the compounds in two different doses (5 mg kg^{-1} and 10 mg kg^{-1}) on body weight, mean survival time, % increase lifespan, tumor volume, packed cell volume and viable tumor cell count were studied. The results indicated that the compounds **5c** and **5h** have shown significant activity in both the doses and decreased the body weight of EAC- bearing mice. The both compounds have significantly increased the mean survival time, decreased the tumor volume, packed cell volume and viable cell count in both the doses. On day 14, the hematological and biochemical parameters, with regard to hemoglobin level, erythrocyte count, leukocytes count, SGPT, SGOT and total protein levels were compared with the EAC control group, standard drug Cisplatin treated groups and the groups injected with the compounds **5c** and **5h**. From results it is clear that, the hematological and biochemical parameters, in the group treated with the compounds **5c** and **5h** have been recovered the normal values. The compound **5c** and **5h** have significantly decreased the ascetic fluid volume when compared with EAC control. The compounds **5c** and **5h** decreased the WBC

levels at both the doses when compared to tumor control. Similarly, **5c** and **5h** have decreased the SGPT, SGOT and total protein levels when compared to tumor control. This indicates that these compounds possess protective action on hemopoietic system. The results suggest that these compounds **5c** and **5h** proved to possess remarkable activity. The potent anticancer drug can be developed using further investigations by effecting a simple modification in the structure.

***In vitro* antimicrobial activity**

In order to explore the possible antimicrobial potential of the some of the newly synthesized compounds (**5a-j**), were screened *in vitro* against six pathogenic bacterial strains, *viz*. Gram-positive (G+) bacterial strains (*Bacillus subtilis* MTCC 96, *Staphylococcus aureus* MTCC 121, *Staphylococcus epidermidis* MTCC 2639) and Gram-negative (G-) bacteria (*Escherichia coli* MTCC 40, *Pseudomonas aeruginosa* MTCC 2453 and *Klebsiella pneumonia* MTCC 109) and the fungal strains (*Candida albicans*, *Aspergillus niger*, *Candida glabrata*, *Aspergillus parasiticus*). Antimicrobial screenings were conducted in triplicates by well-plate method using Mueller-Hinton (MH) agar. Antimicrobial assay was performed at 100 μgmL^{-1} concentrations for the test compounds (**5a**–**j**) with respect to positive control drugs penicillin, streptomycin (for bacteria) and Fluconazole (for fungi) at 30 μgmL^{-1}. Zone of Inhibition (ZOI) values in mm for the tested compounds were measured at the end of the incubation period of about 24h for bacteria at 37±1 ˚C and 72 h for fungi at 27±1 ˚C.

The lowest concentration of antimicrobial agents required to inhibit the microbial growth is called minimum inhibitory concentration (MIC). The MICs of all the tested compounds as well as standards were also performed against all the bacterial strains, using a well-defined and standardized broth micro-dilution technique, as one described by the European Committee for Antimicrobial Susceptibility Testing (EUCAST) of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID). Using DMSO as a solvent control prepared different concentrations of test compounds and positive controls ranging from 150-0.1 μgmL^{-1}. Bacterial inoculums were also prepared. The bacterial suspensions were transferred (inoculums + sterile water) into a series of test tubes containing 1 mL of each of the derivative solutions (**5a-j**) to be tested at different concentrations. The test tubes were incubated at 37±1˚C for about 18h and determined the MIC values that completely inhibit the visible growth.

In vitro antimicrobial screening results showed moderate to excellent inhibiting activities with (ZOI 4-23 mm and MIC 3.12- >150 μgmL^{-1} against all the tested bacterial strains (G+ and G- bacteria). Among all the tested compounds, the compounds **5b**, **5f**, **5i** and **5j** were found to be having an excellent inhibiting activity. The compound bearing benzo[*f*]coumarinyl (**5j**) moiety have showed excellent efficacy with broad spectrum of activity against, *S. aureus* (ZOI 23 mm, MIC 3.12 μgmL^{-1}), *E. coli* (ZOI 20 mm, MIC 6.25 μgmL^{-1}), *P. aeruginosa* (ZOI 18 mm, MIC 6.25 μgmL^{-1}), than that of positive control drugs penicillin and streptomycin. The compounds **5b** have shown excellent inhibiting activity against *S. aureus* (ZOI 21 mm, MIC 3.12 μgmL^{-1}), good activity against *P. aeruginosa* (ZOI 18 mm, MIC 9.37 μgmL^{-1}) and moderate activity against *E. coli* (ZOI 17 mm, MIC 18.5 μgmL^{-1}). Similarly, the compound **5f** exhibited excellent inhibiting activity against *S. aureus*, (ZOI 22 mm, MIC 6.25 μgmL^{-1}) and moderate activity against *S. epidermidis* (ZOI 12 mm, MIC 18.75 μgmL^{-1}). Also, the compound **5i** have shown an excellent bacterial inhibition against *S. aureus* (ZOI 21 mm, MIC 6.25 μgmL^{-1}) and *P. aeruginosa* (ZOI 21 mm, MIC 6.25 μgmL^{-1}) comparable to that of the standard drug streptomycin. The remaining test compounds **5a**, **5c**, **5d**, **5e**, **5g** and **5h** have shown moderate activity against all the bacterial strains with ZOI ranging from 4-15 mm and MIC values of 18.5- >150 $\mu g\ mL^{-1}$.

The *in vitro* antifungal activity results revealed that, out of all the tested compounds (**5a-j**), only compound **5b** against *C. albicans* with ZOI 24 mm and **5e** against *A. niger* with ZOI 15 mm have showed promising inhibiting activity comparable to that of the reference drug Fluconazole. The compounds (**5d**, **5i** and **5j**) possessing 4-phenyl, coumarinyl and benzo[*f*] coumarinyl, were completely inactive against all the tested fungal strains. The remaining compounds were shown week to moderate activity with ZOI ranging from 3-13 mm.

From the antimicrobial activity profile, we have observed structure activity relationship of the synthesized compounds (**5a-j**) as follows; the presence of (electron donating) methyl and (electron withdrawing) flouro and chloro substitutions at 4^{th} position of phenyl ring, electron rich coumarinyl and benzo[*f*]coumarinyl rings, in the compounds **5b**, **5e**, **5f**, **5i** and **5j** respectively, had greatly influenced these compounds to exhibit antimicrobial inhibiting activity against the tested (bacterial and fungal) strains.

In vitro AchE activity

Ellman Method: The assay of AChE inhibition was performed according to method described by Ellman *et al*.[17] using the human AChE purified from red blood cells. The kinetic profile of the AChE enzyme activity was studied spectrophotometrically at a wavelength of 412 nM at an interval of 15s. The assay for each sample was run in duplicate and each experiment was performed thrice. The test substance was incubated with enzyme in the concentration of 100 µg/mL of reaction mixture for 30 min at 37 ^{o}C prior to obtaining the kinetic profile of AChE activity. Donepezil (1µg/mL) was used as standard AChE inhibitor (standard control). The AChE inhibitory activity was calculated on the basis of % decrease from control values i.e. AChE activity without incubation with any standard or test drug.

In vitro AChE inhibition

The structural similarity of these compounds with donepezil also prompted us to biologically evaluate them for AChE activity. The compounds were evaluated using the *in vitro* assay method described by Ellman *et al*. The compound **5c** having a 4-methoxy group which was the best among these did not show very promising activity (% inhibition = 39.6) and rest of the compounds had no significant AChE inhibition when compared with donepezil that showed 99.7 % AChE inhibition.

Docking studies

In order to rationalize the structure–affinity relationships (SARs) and to authenticate the *in vitro* antimicrobial results and also to understand the possible key interactions of potent ligands with the receptor sites of an enzyme, docking simulations were performed using Lamarckian Genetic Algorithm (LGA), inculcated in the docking program AutoDock Tools (1.5.6) and docking results were visualized using Maestro elements tutorial 1.8. Docking for the synthesized compounds (**5a-j**) was performed on the binding model based on the X-ray crystal structure of structure of the tyrosyl-tRNA (TyrRS) synthetase complexed with the ligand SB-239629. The co-crystallized Structure of target enzyme TyrRS (PDB code: 1JIJ) from *S. aureus* was retrieved from RCSB Protein Data Bank (http://www.rcsb.org/pdb). Molecular docking studies revealed that, all the synthesized molecules exhibited excellent binding energies towards the receptor active pocket with binding energies ranging from -8.72 to -9.92 kcal mol^{-1}. The binding profiles of most

promising ligands (**5b**, **5f**, **5i** and **5j**) with the active pocket residues of TyrRS. After exhaustive analysis of docking results, best dock poses interms of binding energy, H-bondings and non-bonding interactions with receptor active pocket residues were chosen as best docked ligand orientations. From the docking results it was identified that, they are well fitted in the active pocket of tyrosyl-tRNA synthetase with four hydrogen bond interactions: observed the CH3O···H−N (2.46 Å) bond between 6-methoxy substituent on tetralone ring and Val224, C=O···H−N (2.10 Å) bond between cyclic keto and Gly193, quaternary N^{+}···H− N between pyridinium ring and Gly38 and the keto carbonyl established C=O···H−N (2.33 Å) bond with Gly38. The 6-methoxy group of 5f exhibited H-bonding interactions with Val224 (2.07 Å), cyclic keto carbonyl oxygen with His47 (3.40 Å) and His50 (3.57 Å) and keto carbonyl oxygen with Gln174 (2.37 Å) amino acid residues in protein active site (PAS). The docked pose 5i established H-bond interactions with Tyr36 (2.65 Å), Gly38 (3.81 Å), Gly193 (2.68 Å), Val224 (3.53 Å) and His50. Besides, the H-bond interactions, also observed π-π stacking interaction between pyridinium ring in **5i** and the imidazole ring of His50 and formed salt bridge between quaternary-N^{+} and negatively charged amino acid residue Asp195. Whereas, the best docking orientation **5j**, held to the active site of protein with hydrogen-bonding interactions with Tyr36 (2.36 Å), Gly38 (3.85 Å), Gly193 (2.60 Å), His47 (3.74 Å) and His50. In addition, pyridinium ring of **5j** exhibited π-π stacking interaction with imidazole ring of His50 and also observed π-cation interaction with N3 of Asp195.

Our molecular modeling results revealed that, the cyclic keto carbonyl, 6-methoxy substituent on tetralone ring, pyridinium ring and cyclic ester (lactone) of coumarin moiety have great influence on the interactions of the protein-ligand complex and is crucial to the TyrRS inhibitory activity. Therefore removal of aforementioned groups may reduce the inhibitory activity. Also our modeling results revealed that, Tyr36, Gly38, His47, His50, Gly193 and Val224 are the key residues for the protein-ligand interactions to form the complex. Hence the above data can strongly rationalizes the potent antibacterial activity observed in biological assays for the compounds **5b**, **5f**, **5i** and **5j** and they can be considered as potential lead molecules for the development of antibacterial agents.

In conclusion, a series of new tetralone based fused chalcone-pyridinium bromide hybrids (**5a-p**) has been synthesized and evaluated some of the compounds (**5a-j**) for their antiproliferative (*in vitro* and *in vivo*) against human cancer cell lines including MCF-7,

HeLa and U-87MG and antimicrobial activity (bacterial and fungal). The *in vitro* anti-proliferative activity results revealed that, out of all the synthesized compounds (**5a-j**), the derivatives bearing 4-methoxy phenyl (**5c**) and 4-nitro phenyl (**5h**), showed significant anti-proliferative activity against all the tested cancer cell lines, than that of the remaining derivatives. The derivatives **5f** and **5i** exhibited moderate anticancer activity against tested strains. The *in vivo* results also suggests that, the compounds **5c** and **5h** distinctly exhibited inhibiting effect on tumour growth in mice bearing EAC. Further the QSAR studies revealed that the two physicochemical parameters hydrophobic (π) and steric (MR) were found to influence anticancer activity negatively and positively respectively. The antimicrobial studies on these compounds showed that, most of the synthesized compounds (**5a-j**) have promising inhibitory activity against all the bacterial strains where the derivatives **5b**, **5f**, **5i** and **5j** have shown broad and superior inhibiting activity, particularly against G+ methicillin resistant *S. aureus* (MRSA), than the positive controls. Antifungal results showed that, the compound **5b** and **5e** have comparable inhibiting activity to that of the standard drug Fluconazole against *C. albicans* and *A. niger* respectively. In order to authenticate the *in vitro* antibacterial results, molecular docking studies were performed for the synthesized compounds (**5a-j**). Based on the docking studies presence of keto group of tetralone ring, methoxy at 6^{th} position on tetralone and H-bond interaction with active site amino acid GLY 193 are key for the potency of the derivatives. Thus the docking studies provide insights about the mode of binding and interactions of the compounds with the tyrosyl-tRNA synthetase. It is good that none of these compounds exhibited significant AchE inhibition. Hence these results provide the basis for further optimization and development of the lead molecules, to develop new cytotoxic agents as well as antimicrobial agents with better efficacy against methicillin-resistant *S. aureus*.

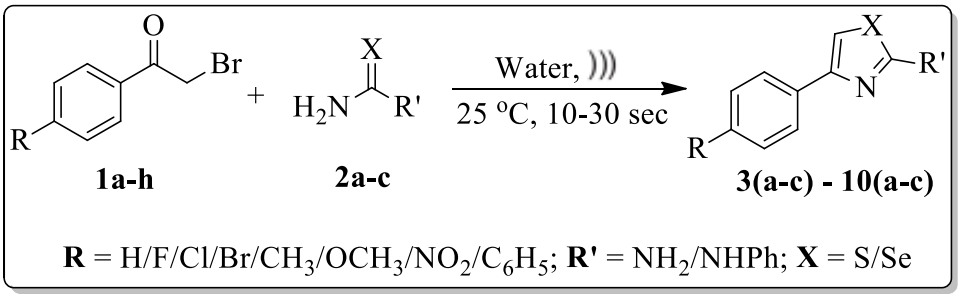
CHAPTER-VI (SECTION-A)

Green approach: An efficient synthesis of 2,4-disubstituted-1,3-thiazoles and selenazoles in aqueous medium under ultrasonic irradiation

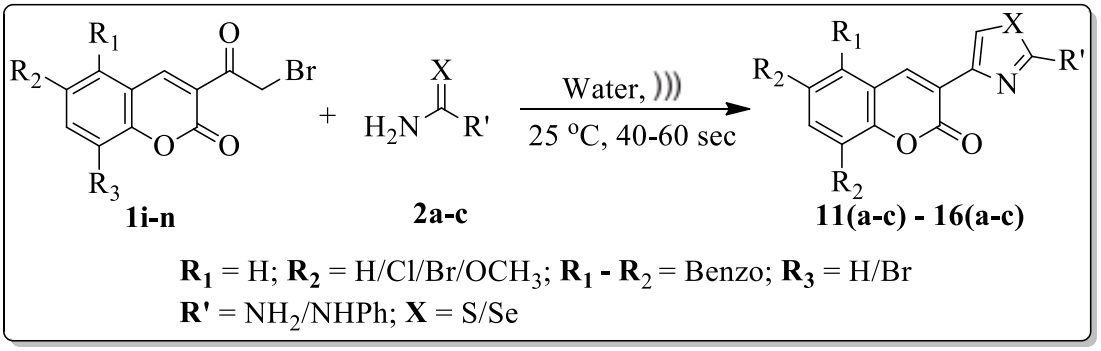
In this section, we described an efficient method for the synthesis of 2,4-disubstituted-1,3-thiazoles and selenazoles in aqueous medium under ultrasonic irradiation.[18]

In view of the importance of thiazole and selenazole derivatives in various fields of chemistry, the classical Hantzsch synthesis as well as several methodologies have been reported utilizing various catalytic systems such as ammonium-12-molybdophosphate, *β*-cyclodextrin, NaCl, HMCM-41, iodine, TiO_2, $CuPy_2Cl_2$, graphite oxide and silica chloride, and also reported in different solvent systems, such as ionic liquids, PEG-400, glycerine, and water. However, most of these reported methods have one or several draw backs such as lower yields, longer reaction times, complicated isolation procedures and use of hazardous, and expensive catalysts make them environmental non-friendly. To overcome the above limitations and as a part of our endeavour towards the development of novel eco-friendly methodologies for the synthesis of biologically potent heterocyclic compounds, herein we proposed a versatile, simple, mild, environmental benign and highly efficient protocol for the synthesis of 2,4-disubstituted-1,3-thiazoles and 1,3-selenazoles in aqueous medium under ultrasonic irradiation.

Utilizing the optimistic conditions (ultrasonication at 50 kHz frequency and 25 ^oC bath temperature in aqueous medium), a series of 1,3-thiazoles and selenazoles **(3-16)** have been synthesized by the reaction of phenacyl bromides (**1a-h**) and 3-(2-bromoacetyl)coumarins (**1i-n**) with thiourea (**2a)**, phenylthiourea (**2b)** and selenourea **(2c)** in excellent yields.



Scheme-13



Scheme-14

All the newly synthesized compounds were characterized by their spectral and analytical studies, and the known compounds were established by comparing their melting points with the reported values.

In conclusion, we have efficiently synthesized a series of 2,4-disubstituted-1,3-thiazoles and selenazoles in aqueous medium under ultrasonic irradiation at ambient temperature. This non-conventional methodology has many advantages over conventional reported methods that include environmental friendly, rapid reaction completion, easy workup procedure and analytically pure products formation in excellent yields. This method can be effectively used for large scale production of thiazoles and selenazoles in shorter reaction times.

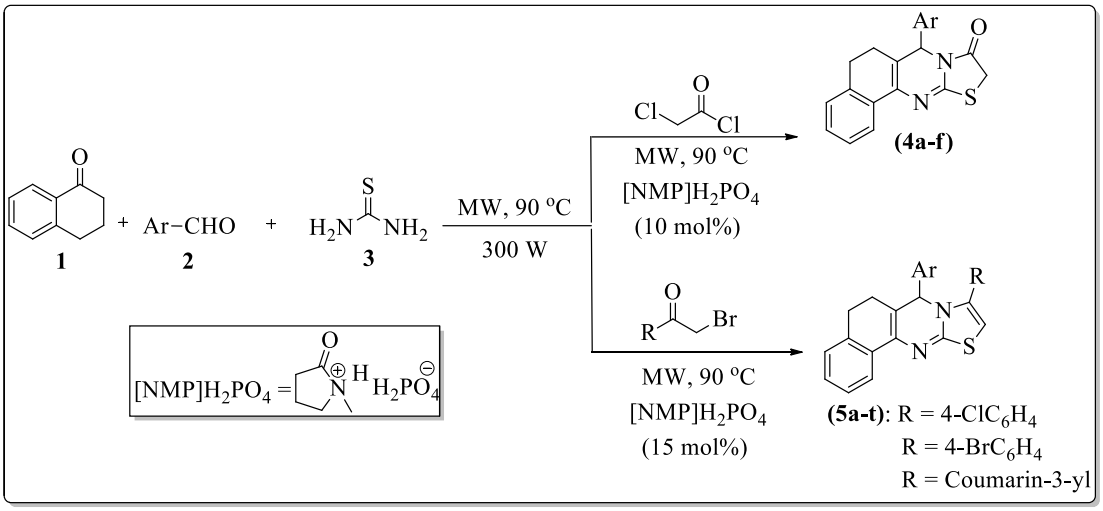
CHAPTER-VI (SECTION-B)

Recyclable task specific acidic ionic liquid [NMP]H_2PO_4: Microwave-assisted efficient one-pot two step tandem synthesis of fused thiazolo[2,3-*b*]quinazolinone and thiazolo[2,3-*b*]quinazoline derivatives

In our previous work,[19] we have described the synthesis of compounds **4** and **5** and evaluated for their biological activity. However, the time and yields of the both of the title compounds were found to be good but, do not be satisfactory. Hence, in order to overcome the time, yield limitations and in search of synergistic and more sustainable synthetic protocols as earlier work, we aimed to develop highly efficient and rapid green method, we report herein eco-compatible highly efficient and simple protocol for the synthesis of thiazolo[2,3-*b*]quinazoline (**4a-f**) and thiazolo[2,3-*b*]quinazolinone (**5a-t**) derivatives by combined use of recyclable [NMP]H_2PO_4 as a dual green catalyst and microwave irradiation *via* MCR approach under solvent-free conditions.

The best and optimized results were obtained at 90 ^oC under solvent free conditions at 10 mol% of [NMP]H_2PO_4 for the compound **4a** (98 %) and 15 mol% of catalytic load for the compound **5a** (92 %) at microwave frequency of 300 W.

Utilizing the optimized conditions, the scope and efficiency of the procedure was explored and successfully synthesized structurally diverse thiazolo[2,3-*b*]quinazolinone **4** (**a-f**) and thiazolo[2,3-*b*]quinazoline **5** (**a-t**) derivatives in excellent yields (92-98 %) with in a very short reaction time (2-8 min).



Scheme-15

All the synthesized compounds were well established by spectral and elemental analysis and also comparing with their literature melting points.

In summary, our newly developed protocol using task specific acidic ionic liquid $[NMP]H_2PO_4$ under microwave irradiation in one pot *via* MCR approach is an efficient, simple, rapid and environmentally benign for the quantitative and qualitative synthesis of series of thiazolo[2,3-*b*]quinazolinone and thiazolo[2,3-*b*]quinazoline derivatives. This method is complementary to the classical method and offers advantages over conventional in terms of yield, time and operational simplicity. The non-imidazolium ionic liquid $[NMP]H_2PO_4$ can be recyclable and reusable as a catalyst, effective for about 4 cycles.

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LIST OF PUBLICATIONS

1. 3-(1-Phenyl-4-((2-(4-arylthiazol-2-yl)hydrazono)-methyl)-1*H*-pyrazol-3-yl)-2*H*-chromen-2-ones: one-pot three component condensation, *in vitro* antimicrobial, antioxidant and molecular docking studies

Ramesh Gondru, Janardhan Banothu, Althaf Hussain Sk, Ranjith Thatipamula, Rajitha Bavantula*

RSC Advances 5 (2015) 33562-33569

2. Recyclable task-specific acidic ionic liquid [NMP]: Microwave-assisted, efficient one-pot two step tandem synthesis of fused thiazolo[2,3-*b*]quinazoline and thiazolo[2,3-*b*]quinazolinone derivatives

Ramesh Gondru, Rajitha Gali, Ravibabu Velpula, Bavantula Rajitha*

Research on Chemical Intermediates, 42 (2016) 3863–3873

3. Green approach: an efficient synthesis of 2,4-disubstituted-1,3-thiazoles and selenazoles in aqueous medium under ultrasonic irradiation

Ramesh Gondru, Banothu Janardhan, Bavantula Rajitha*

Research on Chemical Intermediates, 41 (2015) 8099–8109

4. One-pot multicomponent synthesis of indole incorporated thiazolylcoumarins and their antibacterial, anticancer and DNA cleavage studies

Rajitha Gali, Janardhan Banothu, **Ramesh Gondru**, Rajitha Bavantula*, Yashodhara Velivela, Peter A. Crooks

Bioorganic & Medicinal Chemistry Letters, 25 (2015) 106–112

5. One-pot multicomponent synthesis of novel thiazolylhydrazone derivatives and their antimicrobial activity

G. Rajitha, V. RaviBabu, **G. Ramesh**, B. Rajitha*, R. Jobina, B. Siddhardha, S. Vijaya laxmi

Research on Chemical Intermediates, 41 (2015) 9703–9713

6. Synthesis and antimicrobial activity of novel imidazo[1,2-*a*]pyridinopyrimidine-2,4,6(1*H*,3*H*,5*H*)-triones and thioxopyrimidine-4,6(1*H*,5*H*)diones.

G. Rajitha, V. Ravibabu, **G. Ramesh**, B. Rajitha*

Research on Chemical Intermediates, 42 (2016) 1989–1998

7. Synthesis and Antibacterial Evaluation of Novel 3,6-Disubstituted Coumarin Derivatives.

Ravibabu Velpula, **Ramesh Gondru**, Yashodhara Velivela, Rajitha Bavantula *

Synthetic Comunications, 45 (2015) 578-585

8. One-pot synthesis, biological evaluation and molecular docking studies of fused thiazolo[2,3-b]pyrimidinone-pyrazolylcoumarin hybrids as antibacterial agents

Communicated

9. Novel Donepezil like fused chalcone-pyridinium bromide hybrids: Synthesis, characterization, in vitro and in vivo anticancer, antimicrobial activity evaluation and molecular modeling studies

Communicated

10. Design, three component one pot synthesis and in vitro biological evaluation of novel 1,3-disubstituted pyrazole-2,4-disubstituted thiazole hybrids embedding benzothiazole and coumarin moieties as antimicrobial, anti-biofilm agents

Manuscript under preparation

11. Synthesis of new 4-substituted 1,2,3-triazole-hydrazinyl 1,3-thiazole hybrids by employing 'click' chemistry: *in vitro* anti-proliferative, antimicrobial and anti-biofilm studies

Manuscript under preparation

PAPERS PRESENTED IN INTERNATIONAL AND NATIONAL CONFERENCES

1. "Synthesis, *in vitro* biological evaluation and molecular docking studies of novel *N*-aroylpyridinium bromide derivatives" **6th International Symposium on "Current Trends in Drug Discovery and Research, (CTDDR-2016)"** organised by Central Drug Research Institute (CDRI), Lucknow, UP, India during 25th-28th February, 2016.

2. "3-(1-Phenyl-4-((2-(4-arylthiazol-2-yl)hydrazono)methyl)-1*H*-pyrazol-3-yl)-2*H*-chromen-2-ones: One-pot three component condensation, *in vitro* antimicrobial, antioxidant and molecular docking studies" **3rd International Conference and Exhibition on "Pharmacognosy, Phytochemistry and Natural Products (Pharmacognosy-2015)"** organised by OMICS-International, HICC, India during 26th-28th, October, 2015.

3. "One-pot three-component synthesis and antimicrobial activity of pyrazolyl coumarin bearing 2,4-disubstituted thiazole derivatives" **2nd International Conference on "Emerging Trends in Chemical and Pharmaceutical Sciences," (PharmaChem Expo-2014)"** organised by CSIR-Indian Institute of Chemical Technology, Hyderabad, India during 15th-17th October, 2014.

4. "Renewable task specific acidic ionic liquid [NMP]H2PO4: Microwave-assisted efficient one-pot two step tandem synthesis of fused thiazolo[2,3-*b*]quinazolinone and thiazolo[2,3-*b*]quinazoline derivatives" **National Conference on "Drug Discovery and Development in Chemistry Applications in Pharma Industry (DDDC-2015)"** organized by the Department of Chemistry, S V University, Tirupati, India, during 14th-15th September, 2015.

5. "Green approach: An efficient synthesis of 2,4-disubstituted-1,3-thiazoles and selenazoles in aqueous medium under ultrasonic irradiation" UGC sponsored National seminar on **"Recent Advances in Chemistry (RAC-2015)"**, organized by the Department of Chemistry, Kakatiya University, Warangal, Telangana State, India, during 30th-31st March, 2015.