Synthesis, Characterization and Biological Evaluation of Novel 1-(3-chloro-2-oxo-4-phenylazetidin-1-yl)-3-(2-oxo-2-(10*H*-phenothiazin-10-yl)ethyl)urea and thiourea derivatives

Dissertation Submitted to

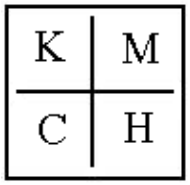
The Tamil Nadu Dr. M.G.R. Medical University, Chennai

In partial fulfillment for the requirement of the Degree of

MASTER OF PHARMACY

(Pharmaceutical Chemistry)

April-2012



DEPARTMENT OF PHARMACEUTICAL CHEMISTRY

KMCH COLLEGE OF PHARMACY,

KOVAI ESTATE, KALAPATTI ROAD,

COIMBATORE-641048.

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Submitted by

K. SHEEJA DEVI

Under the Guidance of

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CERTIFICATE

This is to certify that the dissertation work entitled **'Synthesis, characterization and biological evaluation of some Novel 1-(3-chloro-2-oxo-4-phenylazetidin-1-yl)-3-(2-oxo-2-(10H-phenothiazin-10-yl)ethyl)urea and thiourea derivatives'** is a bonafide research work carried out by **Ms. K. SHEEJA DEVI** (Reg. No. 26107139), student in Master of Pharmacy, Department of Pharmaceutical Chemistry, K.M.C.H College of Pharmacy, Coimbatore, under my supervision and guidance during the academic year 2011-12.

Dr. A. Rajasekaran M.Pharm.,Ph.D.,

Principal

DECLARATION

I do hereby declare that the dissertation work entitled **'Synthesis, characterization and biological evaluation of some Novel 1-(3-chloro-2-oxo-4-phenylazetidin-1-yl)-3-(2-oxo-2-(10*H*-phenothiazin-10-yl)ethyl)urea and thiourea derivatives'** submitted to The Tamil Nadu Dr. M.G.R. Medical University, Chennai, in partial fulfillment for the Degree of Master of Pharmacy in Pharmaceutical Chemistry**,** was carried out under the guidance of **Prof. Dr. A. Rajasekaran, M.Pharm., Ph.D., Principal, KMCH College of Pharmacy** during the academic year of 2011-2012.

K. SHEEJA DEVI

(26107139)

EVALUATION CERTIFICATE

This is to certify that the dissertation work entitled '**Synthesis, characterization and biological evaluation of some Novel 1-(3-chloro-2-oxo-4-phenylazetidin-1-yl)-3-(2-oxo-2-(10*H*-phenothiazin-10-yl)ethyl)urea and thiourea derivatives'** submitted by **K. SHEEJA DEVI** University **Reg. No: 26107139** to The Tamil Nadu Dr. M.G.R. Medical University, Chennai, in partial fulfillment for the Degree of Master of Pharmacy in Pharmaceutical Chemistry is a bonafide work carried out by the candidate in the Department of Pharmaceutical Chemistry, KMCH College of Pharmacy, was evaluated by us during the academic year 2011-2012.

Examination Center: KMCH College of Pharmacy, Coimbatore.

Date:

Internal Examiner **External Examiner**

Convener of Examinations

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Finally, words cannot suffice in thanking my dear family for the great support and sincere encouragement.

K. SHEEJA DEVI

ABBREVIATIONS

Abs	Absorbance
Conc	Concentration
Cnt	Control
DMF	Dimethyl formamide
DMSO	Dimethyl sulphoxide
DPPH	2, 2-Diphenyl-1-Picryl-hydrazyl
EC_{50}	Half maximal effective concentration
EIMS	Electron impact mass spectroscopy
$FeSO_4$	Ferrous sulphate
$FeCl_3$	Ferric chloride
Fig	Figure
FTIR	Fourier transform infrared
FRAP	Ferric Reducing Ability of Plasma
g	Gram
h	Hours
^{1}HNMR	Proton Nuclear Magnetic Resonance
ie	That is
IC_{50}	Half maximal inhibitory concentration
^{0}C	Degree Celsius
mg	Milligram
MIC	Minimum inhibitory concentration
min	Minutes
ml	Milliliter
mm	Millimeter
mM	Millimole
MTCC	Microbial Type Culture Collection
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5diphenyltetrazoliumbromide
NCIM	National Collection of Industrial Microorganisms
ppm	Parts per million
REMA	Resazurin microplate assay
TPTZ	2, 4, 6-tri(2-pyridyl)-s-triazine

CONTENTS

LIST OF TABLES

LIST OF FIGURES

Dedicated to

My Beloved Parents, My Brother

&

My Beloved Guru

Dr.A.Rajasekaran

Chapter 1

Introduction

1. INTRODUCTION

Medicinal chemistry is a science whose roots lie in all branches of chemistry and biology. It explains design and production of compounds that can be used for prevention, treatment or cure of human or animal diseases. It is concerned with the invention, discovery, design, identification and preparation of biologically active compounds, the study of their mode of action at the molecular level and construction of structure activity relationship (SAR). The primary objective of medicinal chemistry is the design and discovery of new moieties that are suitable for use as drugs.

Medicinal chemistry is the application of chemical research techniques to the synthesis of pharmaceuticals. During the early stages of medicinal chemistry development, scientists were primarily concerned with the isolation of medicinal agents found in plants. Today, scientists in this field are also equally concerned with the creation of new synthetic drug compounds. Medicinal chemistry received from the discovery made toward the end of nineteenth century by Paul Ehrlich (1854-1915), father of modern chemotheraphy, that certain compounds exhibited selective toxicity against particular infectious agents. On the other hand, during the same period, Emil Fisher's lock-and-key theory provided a rational explanation for the mechanism action of drugs. Further research work by Ehrlich and his successors resulted in the discovery of many new chemotherapeutical agents, outstanding among which are the antibiotics and the sulphonamides. Medicinal chemistry is almost always geared toward drug discovery and development. [1]

1.1 Drug Discovery[2,3,4,]

As the drug discovery process proceeds, the focus narrows as scientists develop specific chemicals and study their effects on identified disease targets. This requires sophisticated chemistry and testing on animal models of disease.

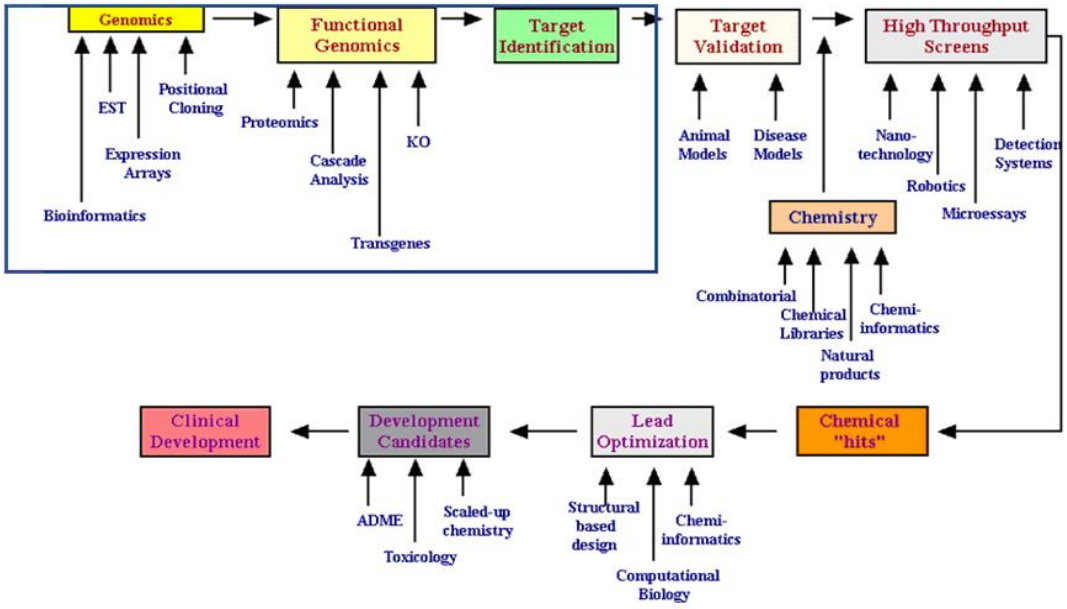


Fig.1 The Stages of Drug Discovery and Development

The steps commonly employed in the rational drug design are as follows:

1.1.1 Target identification

Once they have enough understanding of the underlying cause of a disease, pharmaceutical researchers select a "target" for a potential new medicine. A target is generally a single molecule, such as a gene or protein, which is involved in a particular disease. Even at this early stage in drug discovery it is critical that researchers pick a target that is "drugable," i.e., one that can potentially interact with and be affected by a drug molecule.

1.1.2 Target validation

After choosing a potential target, scientists must show that it actually is involved in the disease and can be acted upon by a drug. Target validation is crucial to help scientists avoid research paths that look promising, but ultimately lead to dead ends. Researchers demonstrate that a particular target is relevant to the disease being studied through complicated experiments in both living cells and in animal models of disease.

1.1.3 Lead compound identification

Armed with their understanding of the disease, scientists are ready to begin looking for a drug. They search for a molecule, or "lead compound," that may act on their target to alter the disease course. If successful over long odds and years of testing, the lead compound can ultimately become a new medicine.

There are a few ways to find a lead compound:

- ***Denova:***

Medicinal chemistry scientists can also create molecules from scratch. They can use sophisticated computer modelling to predict what type of molecule may work.

- ***High-throughput Screening*:**

This process is the most common way that leads are usually found. Advances in robotics and computational power allow researchers to test hundreds of thousands of compounds against the target to identify any that might be promising. Based on the results, several lead compounds are usually selected for further study.

- ***Structure based drug design:***

Three dimensional structures of compounds from virtual or physically existing libraries are docked into binding sites of target proteins with known or predicted structure.

1.1.4 Lead Optimization

Molecules are chemically modified and subsequently characterized in order to obtain compounds with suitable properties to become a drug. Leads are characterized with respect to pharmacodynamic properties such as efficacy and potency *in vitro* and *in vivo*, physiochemical properties, pharmacokinetic properties, and toxicological aspects.

1.1.5 Drug Development

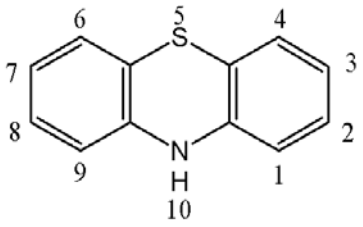
Finally, when Drug discovery occurs when a new compound is developed that addresses a specific disease target. After scientists identify a potentially viable drug candidate in the early drug discovery process, that compound proceeds to laboratory testing against specific targets to determine its effect. Most compounds are eliminated as they proceed from the discovery to the human development phase.

1.2 CHEMISTRY OF PHENOTHIAZINE [5]

A cyclic organic compound containing all carbon atoms in ring formation is referred to as carbocyclic compound. If at least one atom other than carbon forms a part of the ring system then it is a designated as a **heterocyclic compound.** A heterocyclic ring may contain more than one heteroatom which may be similar or dissimilar and it may be saturated or unsaturated. Example: O, N, S, Se, Te, P

Thiazines are heterocyclic compound containing a ring of four carbon, one nitrogen and one sulphur atom. Thiazines are used for dyes, tranquilizer and insecticide. Many compounds of 1, 4 thiazine are known, most of them derivatives of phenothiazine. ($C_{12}H_9NS$)

Phenothiazine is a **benzo** derivative of **thiazine** compounds that are tricyclic fused ring with nitrogen and sulphur being the heteroatoms. The phenothiazine nucleus contains a nitrogen that should be considered neutral. Two aromatic ring attached to a nitrogen, each withdrawing electrons reduce the basic property significantly. In most cases this nitrogen will not form a salt with acid.

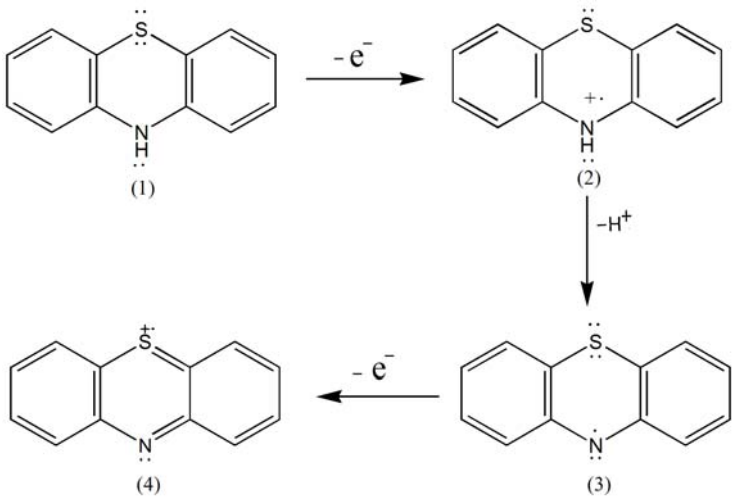


Phenothiazine

All phenothiazines are readily oxidised, particularly in presence of the sunlight and moisture and relatively inexpensive, widely available, well tolerated and nontoxic compounds. Phenothiazines are electron donar compounds with low oxidation potential and they can form easily radical cations. It is found as the redox active unit in donar acceptor systems but also in ligands used for functionalizing different surfaces.

1.2.1 MECHANISM

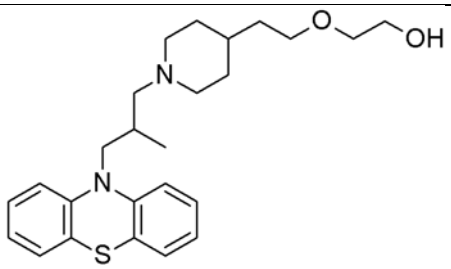
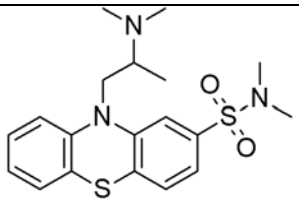
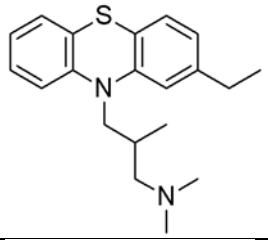
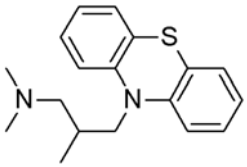
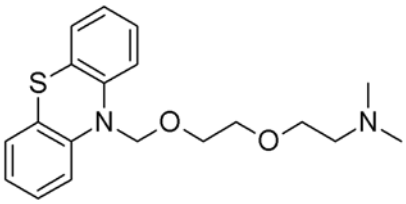
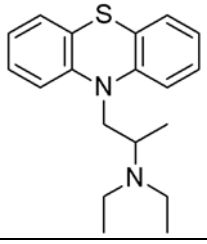
Craig et al[6] have proposed the following mechanism for the oxidation of phenothiazine. Abstraction of an electron occurs in the first step to give the semi oxidized or semiquinone form (2). Loss of a proton by (2) gives the uncharged free radical (3), Loss of a second electron from (3) gives a stable end product (4).

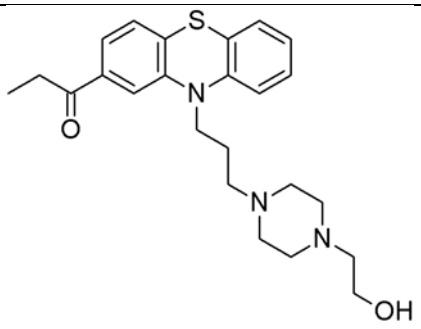
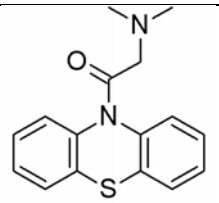
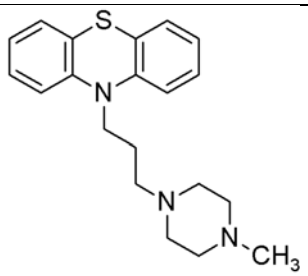
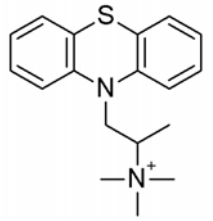
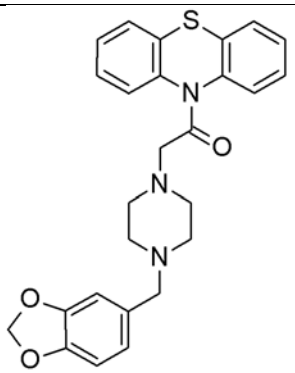
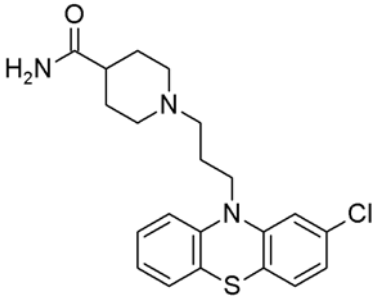


1.2.2 SYNTHETIC METHODS OF PHENOTHIAZINES

- Vilsmeier - Haack formylation
- Microwave assisted cyclo addition
- Polymerization
- Duff formylation
- Alkylation
- Smiles rearrangement

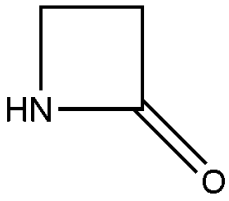
Table 1. Phenothiazines as medicinal agents

NAME	USE	STRUCTURE	IUPAC NAME
Dixyrazine	Neuroleptic, anxiolytic		2-{2-[4-(2-methyl-3-phenothiazin-10yl propel)piperazin-1-yl]ethoxy} ethanol
Dimetotiazine	Serotonin antagonist, migraine		10-(2-dimethylamino propel)-*N*,*N*-dimethyl phenothiazine-2-sulphonamide
Etymemazine	Anticholinergic, antihistamine		3-(2-ethyl phenothiazin-10yl)-*N*,*N*,2-trimethyl propan-1-amine
Almemazine	Antipruretic, eczema, motion sickness		*N*,*N*,2-trimethyl-3-phenothiazin-10-yl-propane-1-amine
Dimethoxanate	Used in cough suppressant		2-(2-dimethylamino ethoxy)ethyl phenothiazine -10-carboxylate
Ethopropazine	Antiparkinsonism, antiadrenergic		*N*,*N*-dimethyl-1-(10*H*-phenothiazine-10-yl)propan-2-amine

Carphenazine	Chronic schizopherenia		1-(10-{3-[4-(2-hydroxy ethyl)piperazin-1-ylpropyl}-10*H*-phenothiazin-2-yl)propane-1-one
Dacemacine	Histamine antagonisit (H1 suptype), antitussives		2-dimethylamino-1-phenothiazine-10-ylethanone
Perazine	Dopamine antagonist		10-[3-(4-methylpiperazin-1-yl)propel]-10*H*-phenothiazine
Thiazinam	Antihistamine		*N*,*N*,*N*-trimethyl-1-(10*H*-phenothiazin-10-yl)propane-2-aminium
Fenoverine	Antispasmodic		2-{4-[benzo(1,3)dioxol-5-ylmethyl]piperazin-1-yl}-1-(10*H*-phenothiazin-10-yl)ethanone
Pipamazine	Used in hepatotoxicity, morning sickness, and postoperative analgesia		1-[3-(2-chloro-10*H*-phenothiazin-10-yl)propyl]piperidine-4-carboxamide

1.3 CHEMISTRY OF AZETIDINONES [7, 8]

2-Azetidinone is a **ß-lactam cyclic amide** with four atoms in a ring. The ring ultimately proved to be the main component of the pharmacophore. So the term possesses medicinal as well as chemical significance. Cycloaddition of monochloro acetyl chloride with imines (Schiff base) result in formation of 2-azetidinone (ß-lactam).

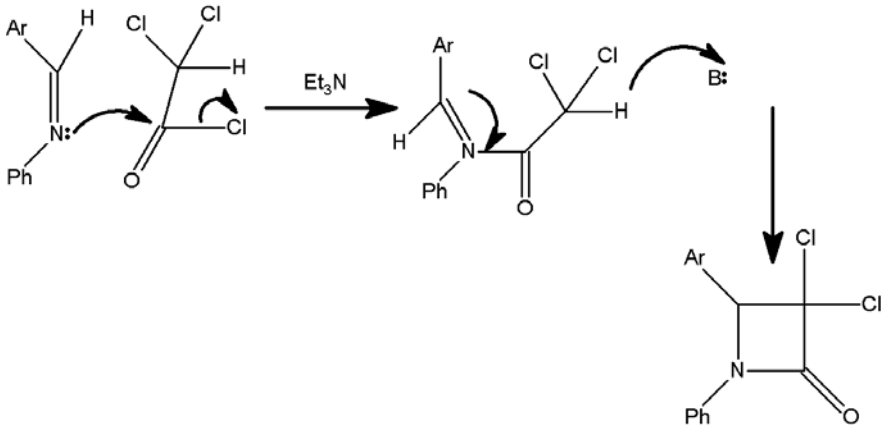


2-Azetidinone

Traditionally ß-lactam is a part of structure of broad spectrum antibiotic class of drugs penicillin's and cephalosporins. The ring ultimately is proven to be a generic descriptor for penicillin family. These molecules operate by forming a covalent adducts with membrane bound bacterial transpeptidases which are also known as penicillin binding proteins(PBPs), involved in the biosynthesis of cell walls. Azetidinone has been recognized as a useful building block for the synthesis of a large number of organic molecules by exploiting the strain energy associated with it.

1.3.1 MECHANISM

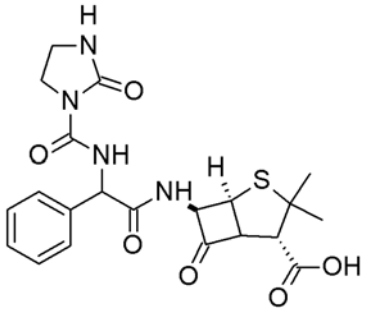
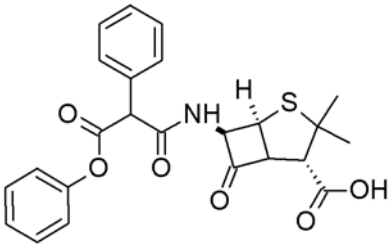
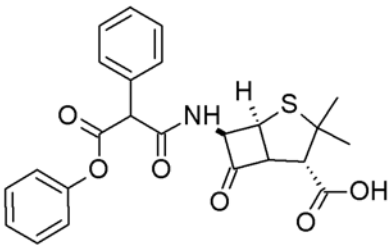
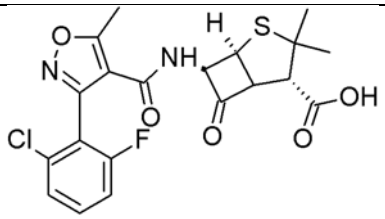
Synthesis of β-lactam of the addition of C - N and C = O component to form a ring substituted acetyl chloride with electron withdrawing substituents and atleast one hydrogen at α-carbon add to imines in the presence of amine bases. The mechanism take place by non concerted cycloaddition reaction as depicted in following reaction.

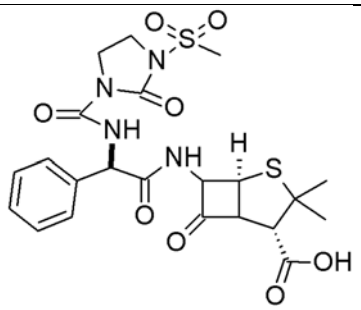
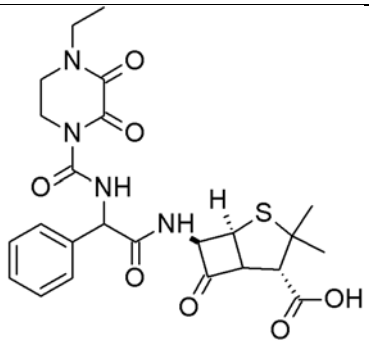
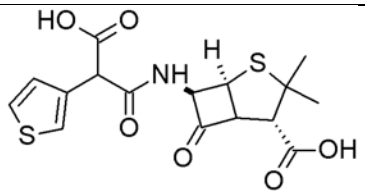
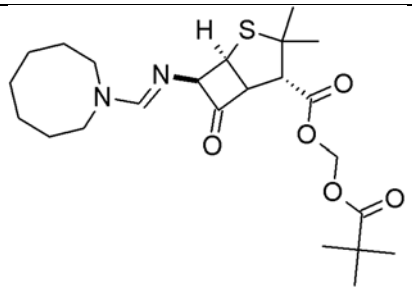


1.3.2 SYNTHETIC METHODS OF AZETIDINONES

- Ketene - imine Cycloaddition
- Wasserman Cyclization
- Staudinger Ketene - imine Cyclization
- Free Radical Cyclization
- Acid Chloride Addition Reaction
- Synthesis of beta - Lactams from Imidates
- Microwave irradiation

Table 2. Azetidinones as medicinal agents

NAME	USE	STRUCTURE	IUPAC NAME
Azlocilin	Acylampicillin antibiotic		(*2S,5R,6R*)-3,-dimethyl-7-oxo-6{[(2*R*)-2-{[(2-oxoimidazolidin-1-yl)carbonyl]amino}-2-phenylacetylamino}-4-thia-1-azabicyclo[3.2.0]heptanes-2-carboxyllic acid
Carfecillin	It is used as a prodrug		(*2S,5R,6R*)-3,3-dimethyl-7-oxo-6-[(3-oxo-3-(phenoxy)-2-phenylpropanoyl]amino]-4-thia-1-azabicyclo[3.2.0]heptanes-2-carboxyllic acid
Ciclacillin	Amino penicillin antibiotic		(*2S,5R,6R*)-6-{[(1-aminocyclohexyl)carbonyl]amino}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptanes-2-carboxyllic acid
Flucloxacillin	Narrow spectrum penicillin antibiotic		(*2S,5R,6R*)-6-({(3-(2-chloro-6-fluorophenyl)-5-methylisoxazole-4-yl)carbonyl}amino)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo(3.2.0)heptanes-2-carboxyllic acid

Melocillin	Broad spectrum penicillin antibiotic		(*2S,5S,6R*)-3,3-dimethyl-6-{[(2*R*)-2-[(3-methylsulphonyl-2-oxo-imidazolidin-1-yl)carbonyl)amino]-2-phenylacetyl]amino]-7-oxo-4-thia-1-azabicyclo(3.2.0)heptanes-2-carboxyllic acid
Piperacillin	Used in empirical theraphy of febrile neutropenia		(*2S,5R,6R*)-6-{[(*2R*)-2-[(4-ethyl-2,3-dioxo-piperazine-1-carbonyl)amino]-2-phenyl-acetyl)amino}-3,3-dimethyl-7-oxa-4-thia-1-azabicyclo[3.2.0]heptanes-2-carboxyllic acid
Ticarcillin	used in plant molecular biology		(*2S,5R.6R*)-6-{[(2*S*)-2-carboxy-2-(3-thienyl)acetyl]amino}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptanes-2-carboxyllic acid
Pivmecillinam	Used in empirical treatment of acute cystitis, and paratyphoid fever		2,2-dimethylpropanoyloxymethyl (*2S,5R,6R*)-6-[(azapen-1-ylmethylene)amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicylo(3.2.0)heptanes-2-carboxyllic acid

Chapter 2

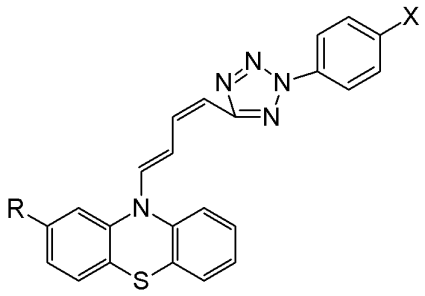
Literature Review

2.LITERATURE REVIEW

2.1 PHENOTHIAZINES AS PROMISING MEDICINAL AGENTS

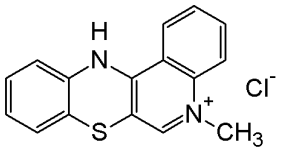
2.1.1 Phenothiazines as anticancer agents

1.Nagy et al[9] synthesized at a series of new tetrazolyldienyl phenothiazines derivatives showed *in vitro* anticancer activity against reversal of MDR of tumor cells. 2-Chloro-10{(1*E*,3*Z*)-4-[2-(4-methoxyphenyl)-2*H*-tetrazol-5-yl]buta-1,3-dien-1-yl10*H*phenothiazine was subjected to investigations and its reversal of MDR was studied in human MDR1 gene transfected mouse lymphoma cells by measuring increased the rhodamine accumulation by 2.5 fold in our in vitro flow cytometric studies.

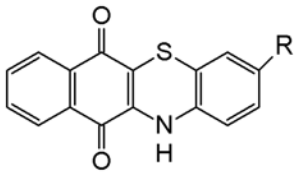


R= Cl, X=OCH_3

2. Zieba et al[10] reported antiproliferative activity *in vitro* of the compounds alkyl-12*(H)*-quino[3,4-b] [1,4] benzothiazinium salts was assessed using two cancer cell lines (Hct116 and LLC) and doxorubicin as a reference (2.2-19.6 mg/ml concentration range).

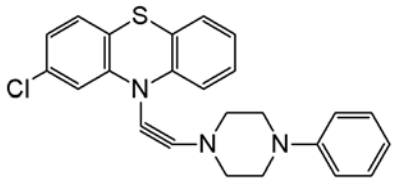


3. Tandon et al[11] reported a series of 2-chloro-3arylsulfanyl[1,4]naphthoquinones , 2,3-bis-arylsulfanyl-[1,4]naphthoquinones and 12*H*-benzo[b]phenothiazine-6,11-diones and their analogues were evaluated for their anti proliferative activity against human cervical cancer (HeLa) cells. Compound 3-Methoxy-6*H*-benzo[b]phenothiazine-6,11(12*H*)- dione were found to possess most potent anti proliferative and cell killing ability and antifungal activity.

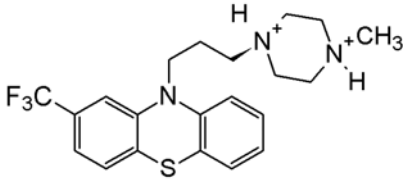


R=OCH_3

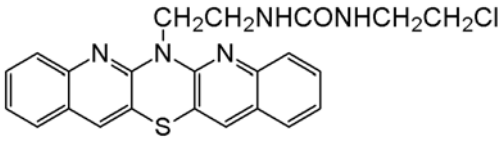
4. Bisi et al[12] demonstrated a series of phenothiazine derivatives bearing a rigid but-2-ynyl amino side chain were synthesized and tested to evaluate the two haematological tumour cell lines, the HL60 and the CCRF/CEM, and their MDR variants HL60R and CEM/ VBL300. The compound 2-Chloro-10-[4-(4-phenylpiperazin-1-yl)but-2-ynyl]- 10*H*-phenothiazine was shown to increase doxorubicin retention in multidrug resistant cells, suggesting a direct interaction with P-glycoprotein and induce antiproliferative effects on resistant cell lines and to interfere with the G1 phase cell cycle.



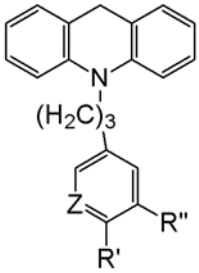
5.Sovilj et al[13] reported the new ruthenium(II) complexes with N alkyl phenothiazines tested *in vitro* cytotoxicity assay aganist four human carcinoma cell lines MCF7, MDA-MB-453 (breast carcinoma), SW-480 (colon adenocarcinoma) and IM9 (myeloma multiple cells). Complex 10-[3-(4-Methyl-1-piperazinyl)propyl]-2-trifluoromethyl phenothiazine dihydrochloride is the most sensitive against human breast cancer cell line (MDA-MB-453) and human colon adenocarcinoma cell line (SW-480) than other two in low concentration and induced almost total cell death with IC_{50} value in the range of (15-25 mM).



6. Pluta et al[14] synthesized azaphenothiazines: tricyclic 10-substituted dipyrido thiazines , pentacyclic-6-substituted-diquinothiazines and hexacyclic-diquino thiazinium salt was tested on 55–60 *in vitro* cell lines. The cell lines included nine types of cancer: leukaemia, non-small cell lung cancer, colon cancer, CNS cancer, melanoma, ovarian cancer, renal cancer, prostate cancer and breast cancer. The most cytotoxic compound was the half-mustard derivative. The GI_{50} value of this compound was 40 ng/ml.

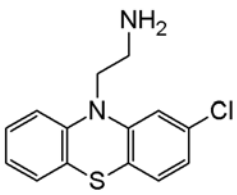


7. Andreanil et al [15] reported the synthesis of phenothiazine derivatives such as the substituent at position 2 is a trifluoromethyl group, of phenothiazine most cytotoxic active compound with the value of the IC_{50} 1 µg/ml.

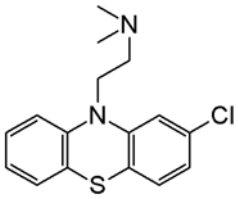


X=S, R=CF_3, Z=CH, R^1=OCH_3, R^{11}=OCH_3

8.Motohashi et al[16] demonstrated the synthesis and biological activity of several N-acyl phenothiazines showed higher cytotoxic activity against human leukemic and squamous carcinoma cell lines than phenothiazine, the parent compound. 10-(3 aminopropyl)-2-chloro-10*H*-phenothiazine maleate, Chlorpromazine hydrochloride showed the greatest cytotoxic activity (CC_{50}< 0.031 mM).

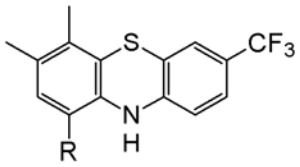


10-(3 Aminopropyl)-2-chloro-10*H*-phenothiazine maleate

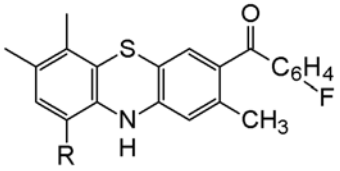


Chlorpromazine HCL

9. Gupta et al[17] reported Synthetic and spectral investigation of fluorinated phenothiazines and 4*H*-l ,4-benzothiazines as potent anticancer agents.



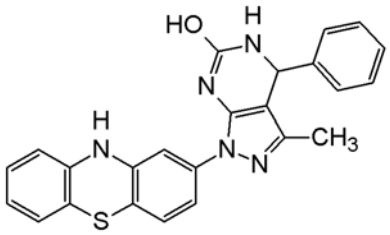
Fluorinated phenothiazine



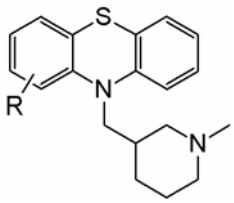
4*H*-1,4-benzothiazine

2.1.2 Phenothiazines as antitubercular agents

1. Shah et al [18] synthesized 2-heterocycle-substituted phenothiazines having a pyrazolo [3,4-*d*] pyrimidinenucleus by using the Biginelli multi-component cyclocondensation reaction and evaluated antitubercular activity against *Mycobacterium tuberculosis* H37 Rv by broth dilution method, the MIC values in the range of > 6.25.



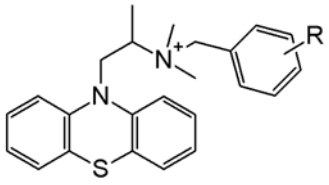
2. Madrid et al[19] reported phenothiazines with reduced binding to dopamine and serotonin receptors examined as antitubercular agents against *Mycobacterium tuberculosis* H37Rv by microalamar blue assay. The following compounds were proven to be the most active, with MIC values ranging from compounds 2 to 4 µg/ml.



Compound 1 R=2-Ph

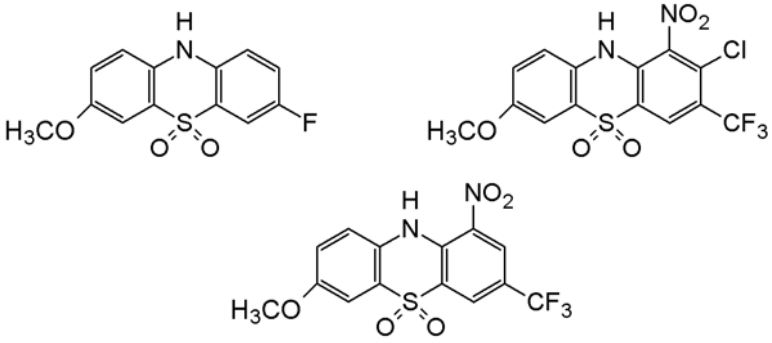
Compound2 R=3-Ph

3. Mitchell et al[20] synthesized quaternized promazine and promethazine derivatives examined as antitubercular agents against both actively growing and non replicating Mycobacterium tuberculosis H37Rv by Microalamar blue assay. Impressively, several compounds inhibited non-replicating *M. tuberculosis* at concentrations equal to or double their MICs against the actively growing strain. N–benzyl substitution in quaternized promazine and promethazine is a requirement for significant antitubercular activity.

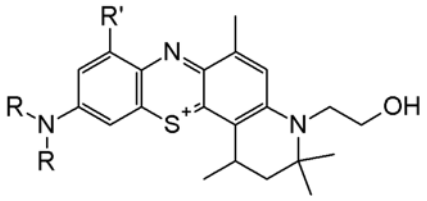


2.1.3 Phenothiazines as antimicrobial agents

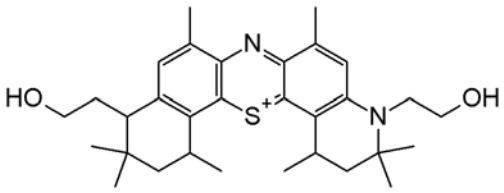
1. Gautam et al[21] synthesized a series of fluorinated 10*H*-phenothiazines by Smiles rearrangement were screened *in vitro* for their antibacterial and antifungal activities using broth microdilution method. Three compounds were found to be the most potent compounds against all the tested strains.



2. Wainwright et al[22] reported synthesis and biological testing of a new class of phenothiazinium derivatives, having either one or two tetrahydropyridine rings fused to the phenothiazinium chromophore. The derivatives exhibited extended absorption wavelengths, increased amphiphilic character and much greater photo antimicrobial efficacies compared to methylene blue. The high activities of this class of photosensitiser recommend its use in infection control, both locally and in blood product decontamination.

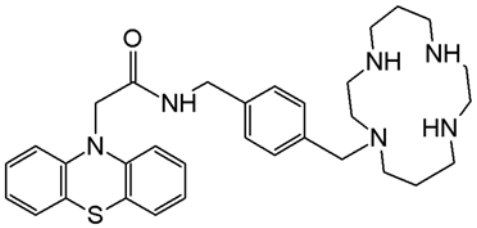


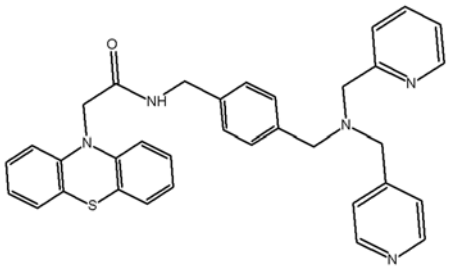
Tetracyclic derivatives



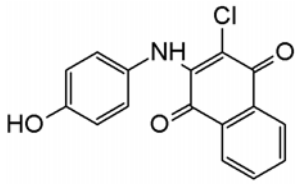
Pentacyclic derivatives

3. Wang et al[23] synthesized a series of conjugates of metal chelators and efflux transporter substrates showed *in vitro* antibacterial activity against two gram positive and gram negative strains. Two compounds were found to be more potent antibacterial activity with an MIC value of 7.8 µg/ml against Gram positive *Bacillus subtilis.*



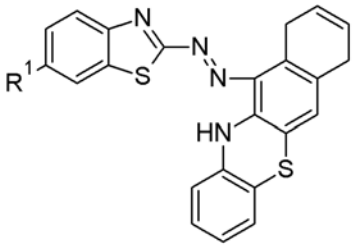


4. Tandon et al[24] synthesized a series biological evaluation of novel nitrogen and sulphur containing hetero-1,4-naphthoquinones tested for *in vitro* antifungal and antibacterial activity. Most of the compounds showed moderate to strong antibacterial activity and compound 2-Chloro-3-(4-hydroxyphenylamino)naphthalene-1,4-dione better antifungal activity than standard drug Fluconazole against *Sporothrix schenckii Candida albicans, Cryptococcus neoformans* and *Trichophyton mentagraphytes* (MIC=0.78 µg/ml).

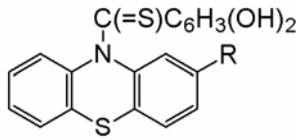


2-chloro-3-(4-hydroxyphenylamino)naphthalene-1,4-dione

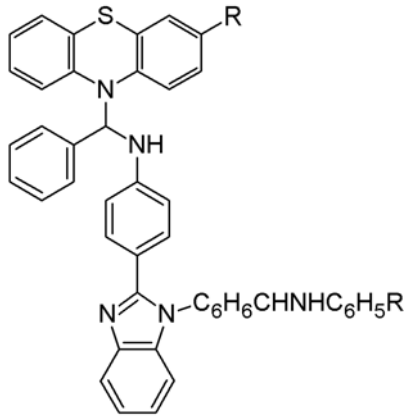
5. Ojha et al[25] reported a series of some new phenothiazine derivatives were synthesized and evaluated for *in vitro* antibacterial activity against gram positive and gram negative bacterial strains. Preliminary results indicated that most of the compounds demonstrated very good antibacterial activity, comparable to standard drug.



6. Kostecka et al[26] synthesized a series of benzo[a]phenothiazine derivatives and evaluated in their *in vitro* antifungal activity against pathogenic strains. Preliminary results indicated that most of the compounds demonstrated very good antifungal activity, comparable to standard drugs. The most effective compounds have exhibited activity at MIC of 6.25 mg/ml.

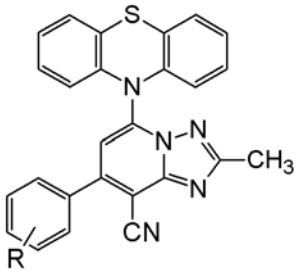


7. Kumar et al[27] reported Benzimidazolyl-Phenothiazine Derivatives showed *in vitro* antibacterial activity against pathogenic strains by disc diffusion method . Three compounds were found to be more potent antibacterial activity against *E.Coli, B.subtilis*, *S.aureus.*

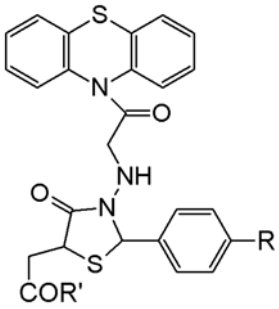


Compound1 R=P-Cl
Compound2 R= M-OH
Compound3 R=M-NO_2

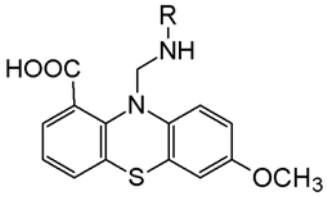
8. Raval et al[28] demonstrated a variety of *N* (2-methyl-7-aryl-8-cyano-[1,2,4] triazolo [1,5-*a*] pyridin-5-yl) phenothiazines were synthesized by using chalcones of *N* – acetylphenothiazine showed antimicrobial activity by streak plate and cup plate method. some of these compounds have shown significant antibacterial and antifungal activities.



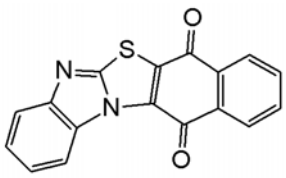
9. Talesara et al[29] reported phthalimido or succinimido-[2-aryl-4-oxo-3-[{2-oxo-2-(phenothiazin-10-yl)ethyl}amino]-1,3-thiazolidin-5-yl]ethanoate evaluated *in vitro* antifungal and antibacterial activity against pathogenic strains. All the compounds have shown significant inhibition of bacterial and fungal growth.



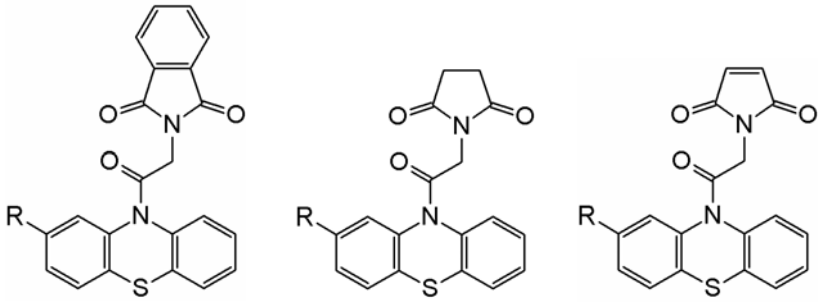
10. Radadiya et al[30] synthesized a series of 10*N*-{[Aryl}-amino]-methyl}-3-methoxy-10,10a-dihydro-4a-*H*-phenothiazine-9-carboxyllic acid in presence of Mannich reaction. The biological activity of these compounds have been determined against various gram positive, gram negative bacteria and fungi by cup-plate method, which revealed that all tested compounds of this investigation were moderate to highly active against all the tested pathogenic bacteria and fungi.



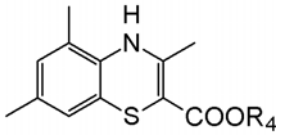
11. Loiseau et al[31] evaluated Phenothiazines and related polycyclics as new lead structures determined for *in vitro* trypanocidal activity against *Trypanosoma brucei* and antifilarial activity against *Molinema dessetae* infective larvae. Lead compound naphtha (2'3': 4,5) thiazoio (3,2-a) benzimidazole -7,12-dione was the most antifilarial with IC_{50}, of 8 μM. A slight effect was observed against the trypanosomes maintained and minimum inhibitory concentrations of active compounds were 50 μM.



12. Dongre et al[32] synthesized a series of 2-substituted N-acylphenothiazines were synthesized by using imides, N-carboxymethyl imides and the structures of these newly synthesized compounds were confirmed by spectral and elemental analyses. All new compounds were tested for their antibacterial and antifungal activities against pathogenic strains. The preliminary screening results indicated that most of the compounds demonstrated moderate to very good antibacterial and antifungal activities compared to the first-line drugs.

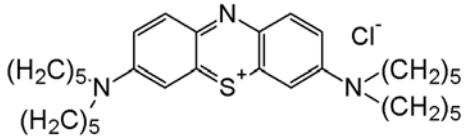


13. Rathore et al[33] reported 7-Chloro-5-trifluoromethyl-7-fluoro-7-trifluoromethyl-4*H*-1,4-benzothiazines have been synthesized by 2-amino-5-fluoro/5-trifluoromethyl/5-chloro-3-trifluoromethyl benzenethiols condensed with b-diketone/b-ketoesters in the presence of DMSO involving oxidative cyclization and evaluated biological activity of antibacterial and antifungal against their pathogenic strains. The following were the most potent compounds reported.

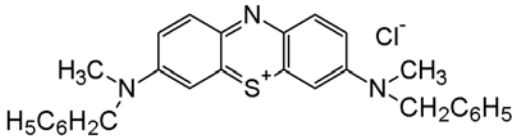


Compound1 R^1= H, R^2=F, R^3=CH_3,
Compound2 R^1= H, R^2=F, R^3=CH_3, R^4=$C_6H_4C_2H_5$(P)
Compound3 R^1= H, R^2=F, R^3=CH_3, R^4=C_6H_4Cl(P)

14. Ihara et al[34] evaluated a series of phenothiazinium chlorides were synthesized and evaluated for *in vitro* antiprotozoal activities against *Plasmodium falciparum, Trypanosoma cruzi Trypanosoma brucei rhodesiense, and Leishmania donovani.* The compound 3,7 bis(piperidinyl)phenothiazinium chloride showed IC_{50} of 0.097 µmol L^{-1} against *T. cruzi* and 3,7-bis(benzyl(methyl)amino)phenothiazinium chloride exhibited IC_{50} of 0.081 µmol L^{-1} against *L. donovani*, although the cytotoxicities of these compounds against L-6 cells were observed at low concentration.

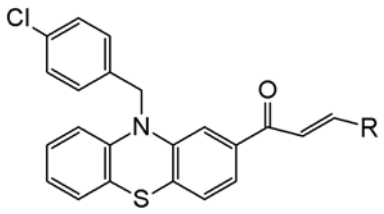


3,7bis(piperidinyl)phenothiazinium chloride



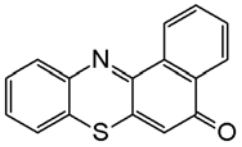
3,7bis(benzyl(methyl)amino)phenolthiazinium chloride

15. Upadhyay et al[35] synthesized a series of 1-[2-(10-p-Chlorobenzyl) pheno- thiazinyl]-3-(substituted aryl)-2-propen-1-ones evaluated antimicrobial activity by Filter Paper Disc method. The preliminary screening results indicated that most of the compounds demonstrated moderate to very good antibacterial and antifungal activities compared to standard chloramphenical.

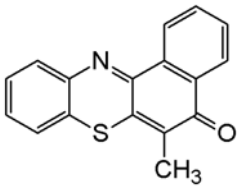


2.1.4 Phenothiazines an antiviral agent

1. Mucsi et al[36] reported combination of some benzo[*a*]phenothiazines and 9-[2-hydroxy (ethoxy) methyl]guanine (acycloguanosine, acyclovir, ACV) and evaluated their *in vitro* antiviral activity against *herps simplex virus* (*HSV-2).* Two most effective derivatives of 5-oxo-5*H*-benzo[*a*]phenothiazine and 6-methyl-5-oxo-5*H*-benzo[*a*]phenothiazine were used with ACV against a wild type *HSV-2* strain and enhance their antiviral activity.



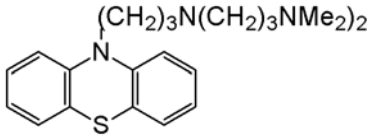
5-oxo-5*H*-benzo[*a*]phenothiazine



6-methyl-5-oxo-5*H*-benzo[*a*]phenothiazine

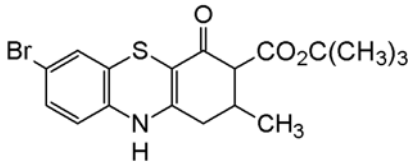
2.1.5 Phenothiazines as antimalarial agent

1. Tilley et al[37] synthesized a series of novel phenothiazine compounds showed *in vitro* antimalarial activity that inhibit the growth of both chloroquine-sensitive and chloroquine resistant strains of *plasmodium falcifarum.* Each phenothiazine that possess an alkyl amino side chain linked to the nitrogen atom showed inhibitory activity. The potent compound 2-Chloro-10-[3-(*N*,*N*-bis(*N*,*N*-(dimethyl amino)-propyl)amino)propyl}-10*H*-phenothiazine which has a branched tribasic side chain showed good antimalarial activity as a inhibition of β- heamatin.



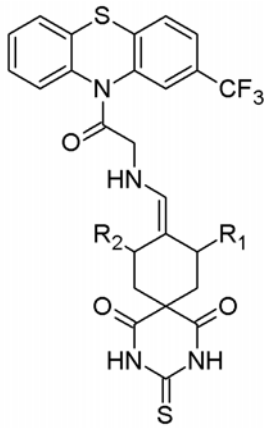
2.1.6 Phenothiazines as anticonvulsant agents

1. Scotte et al[38] reported a series of 3-Carboalkoxy-2-methyl-2,3-dihydro-1*H*-phenothiazin-4[10*H*)-one Derivatives showed for their *in vivo* anticonvulsant activity. The compound trans-7-Bromo-lcarbo-tert-butoxy-2-methydihydro-1*H*-phenothiazin-4[10*H*)-one showed better anticonvulsant activity.



Trans-7-Bromo-lcarbo-tert-butoxy-2-methydihydro-1H-phenothiazin-4[10*H*)-one

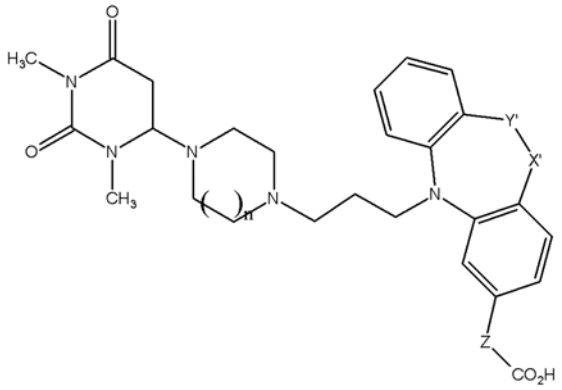
2. Kumar et al[39] synthesized a series of 10-[7,11-(2,4 di substituted phenyl)-3-oxo-9-aminoimino-2,4-diazaspiro[5,5] phenothiazine derivatives evaluated *in vivo* anticonvulsant activity against electrically (MES) and chemically (PTZ, picrotoxin and bicuculline) induced seizures and compared with the standard drugs phenytoin, and sodium valproate. The compound 10-[7,11-(3,5disubstituteddiphenyl)-3-thia-9-aminoimino-2,4-diazaspiro[5,5] undecane 1,5-dione]acetyl phenothiazine was found to be most potent compound of this series.



R=4-OCH_3,C_6H_5

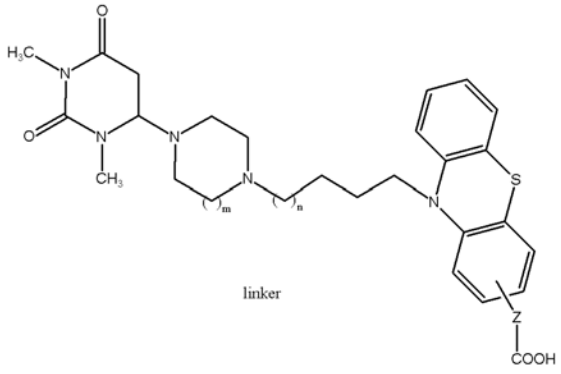
2.1.7 Phenothiazines as antihistaminic and anti-inflammatory agents

1. Isobe et al[40] synthesized a series of tricyclic carboxylic acids having 6-amino-pyrimidine-2,4(1*H*,3*H*)-dione with piperazino or homopiperazino moiety linked by propylene, and evaluated for their *in vivo* anti-histaminic activity and in mice, bioavailability in rats, and their anti-inflammatory activity in mice OVA-induced biphasic cutaneous reaction model. Among the compounds tested, dibenzoxazepine carboxylic acid showed both histamine H1 receptor antagonistic activity and anti-inflammatory activity.

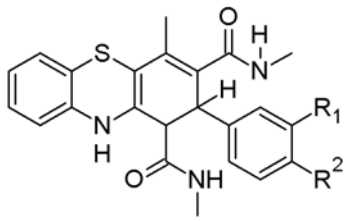


n=2, X1, Y1 = O, CH2, Z = bond

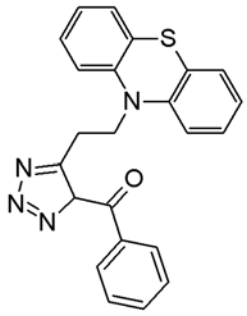
2. Isobe et al[41] synthesized a series of phenothiazine carboxylic acid derivatives, having 6-amino-pyrimidine-2,4(1*H*,3*H*)-dione moiety were evaluated for their *in vivo* oral antihistaminic activity in mice and bioavailability in rats. Finlly promising compounds were examined for their anti-inflammatory potential in mice OVA-induced biphasic cutaneous reaction model. Among the compounds tested, phenothiazine acetic acid compound showed both histamine H_1-receptor antagonistic activity and anti-inflammatory activity.

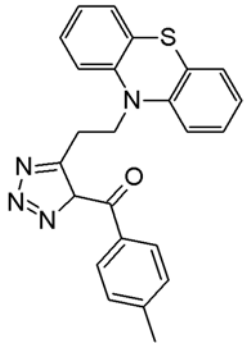


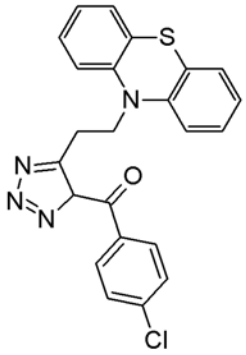
3. Rao et al[42] reported a number of substituted phenothiazines were synthesized and screened for their *in vitro* enzyme inhibitory activity against the regulatory enzymes involved in inflammatory diseases. The compounds 2,10-dihydro-1*H*-phenothiazine and 2-aryl-10H-phenothiazine derivatives exhibited promising *in vitro* enzyme inhibitory activity and further structural variation of these compounds could result in designing a potent lead molecules in the therapy of many inflammatory diseases.

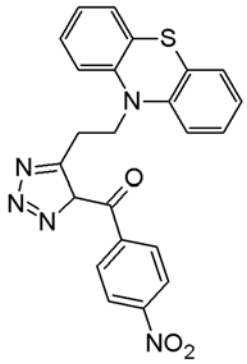


4. Rajasekaran et al[43] synthesized a series of 5[b-(phenothiazinyl-10-yl)ethyl]- 1-(acyl)-1,2,3,4-tetrazoles were *in vitro* screened for analgesic activity tested both by acetic acid induced writhing method and hot plate method and anti-inflammatory activity tested by carrageen in induced paw oedema method. Out of the 12 compounds synthesized, compound 5[b-(phenothiazinyl-10-yl)ethyl]- 1-(benzoyl)-1,2,3,4-tetrazole, compound 5[b (phenothiazinyl-10-yl)ethyl]-1-(*p*-tolyl)-1,2,3,4-tetrazole showed promising analgesic activity and compound 5[b-(phenothiazinyl-10-yl)ethyl]-1-(*p*-chlorobenzoyl)-1,2,3,4-tetrazole and compound 5[b-(phenothiazinyl-10- yl)ethyl]-1-(*p*-nitrobenzoyl)-1,2,3,4-tetrazole showed promising anti-inflammatory activity.



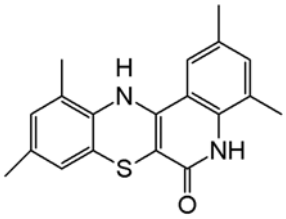






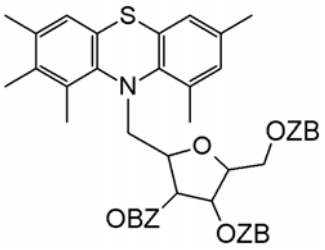
2.1.8 Phenothiazines as antioxidant agents

1. Kumar et al[44] synthesized quinolinobenzothiazinones have been evaluated for their antioxidant (LPO & GSH) and radical scavenging activities by DPPH and ABTS assays. The synthesized compounds have also shown antioxidant activity as measured by estimating reduced glutathione (GSH) and lipid peroxidation (LPO) in liver of Swiss albino mice. The following compounds possessed excellent antioxidant activity.



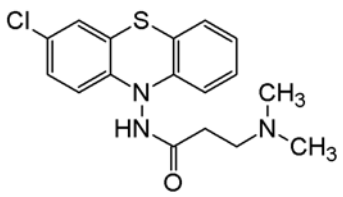
Compound1: R_1=OCH_3, R_2=H, R_3=OCH_3, R_4
Compound2: R_1=H, R_2=Br, R_3=OCH_3, R_4=H
Compound3: R_1=CF_3, R_2=H, R_3=H, R_4=CH_3

2. Dixita et al[45] reported 10*H*-phenothiazines are prepared by Smiles rearrangement. These prepared phenothiazines are used as base to prepare ribofuranosides by treatment with β Dribofuranosyl- 1-acetate-2,3,5-tribenzoate and evaluated in their *in vitro* antioxidant activity and antimicrobial activity. Some compounds were found to be good antioxidant activity and all the compounds exhibited moderately active against various bacteria, such as *Staphylococcus aureus* and *Pseudomonas flueroscense* and fungi *Aspergillus niger, Aspergillus flavus.*



2.1.9 Phenothiazines as antialzheimer agent

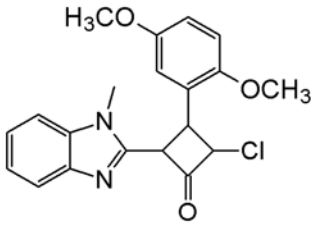
1. Conde et al[46] reported that N-acyl amino phenothiazines showm antialzheimer's activity in brain of alzehimer's disease model mice, mechanism involved was proposed as inhibition of butyrylcholinesterase (BuChE), protected neurons against damage caused by both exogenous and mitochondrial free radicals, showed low toxicity, and could penetrate into the CNS.



2.2 AZETIDINONES AS PROMISING MEDICINAL AGENTS

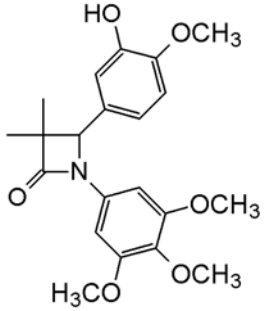
2.2.1 Azetidinones as anticancer agents

1. Noolvi et al[47] synthesized a series of 1-methyl-N-[(substituted phenylmethy lidene)-1*H*-benzimidazol-2-amines and evaluated *in vitro* cytotoxicity and antibacterial activity aganist MCF-7 cell line by MTT assay. All compounds exhibited moderately antibacterial activity and 3-Chloro-4-(2,5-dimethoxyphenyl)-1-(1-methyl-1*H*benzimidazol- 2-yl) azetidin-2-one shown good cytotoxicity activity with IC_{50} value in the range of 6.0 μM.



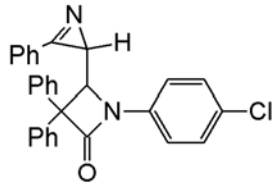
3-Chloro-4-(2,5-dimethoxyphenyl)-1-(1-methyl-1*H*benzimidazol- 2-yl) azetidin-2-one

2. Meegan et al[48] investigated synthesis and study of the structure activity relationships of a series of rigid analogues of combretastatin A-4 are described which contain the 1,4-diaryl-2-azetidinone (β-lactam) ring system and evaluated in their antiproliferative activity and tubulin targeting effects against MCF-7 and MDA-MB-231 human breast carcinoma cell lines. Two compounds display antiproliferative activity and antimitotic effects through an inhibition of tubulin polymerisation and subsequent G2/M arrest of the cell cycle in human MDA-MB-231 breast cancer cells, with similar activity to that of CA-4.



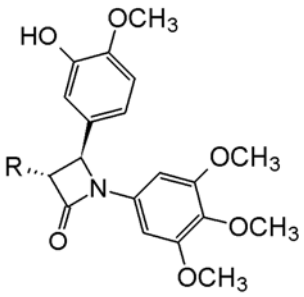
Compound1: R_1=H, R_2=H
Compound 2: R_1=CH_3, R_2=H

3. Lotufo et al[49] 2-Azetidinones and 2*H*-azirines were assayed for antibacterial and cytotoxic activities. None of them showed antibacterial activity on the tested strains, but both 2H-azirine-2-azetidinones showed cytotoxicity against four tumour cell lines (HL-60, leukaemia, HCT-8, colon cancer, MDA-MB-435, melanoma and SF-295, CNS). The IC_{50} values ranged from 1.1 to 10.5 μM.

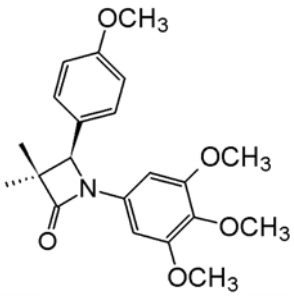


1-(4-chloride)-3,3-diphenyl-4-(3-phenyl-2*H*-azirinyl)-2-azetidinone

4. Meegan et al[50] synthesized a series azetidin-2-ones derivatives and evaluated for antiproliferative, cytotoxic and tubulin-binding activity. Heterocyclic derivatives, 3-(2-thienyl) analogue 28 and 3-(3-thienyl) analogue displayed the highest potency in human MCF-7 breast cancer cells with IC_{50} values of 7 nM and 10 nM,. No significant toxicity was observed in normal murine breast epithelial cells. 3-naphthyl derivative and 3-benzothienyl derivative , resulted in relatively lower antiproliferative activity in the micromolar range. Tubulin-binding studies of 3-(thienyl) β-lactam confirmed that the molecular target of this series of compounds is tubulin.

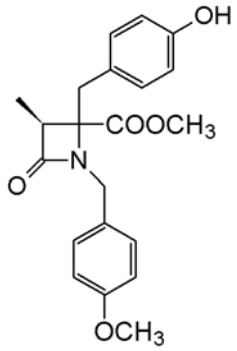


Compound1:R=2-thienyl
Compound2:R=3-thienyl

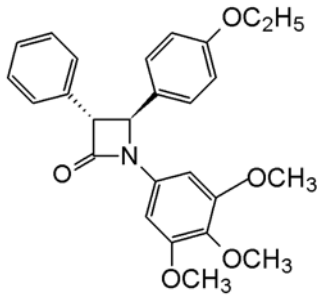


Compound 3:R_1=2-naphthyl
Compound4:R_1=benzothienyl

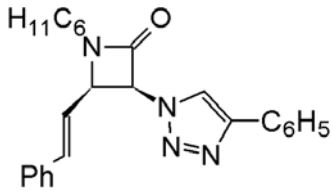
5. Muniz et al[51] reported *in vitro* cytotoxictity assay several enantio pure (3*S*,4*S*)- and (3*R*,4*R*)-1,3,4,4-tetrasubstituted β-lactams derived from amino acids against HT29 cell lines. The compound (3*S*,4*S*)-4-benzyl-1-p-methoxybenzyl-3- methyl-4-methoxycarbonyl derivative showed significant activity compared to standard drug Doxorubicin.



6. Meegan et al[52] reported that structure-activity relationships and synthesis of azetidinone series of compounds was achieved utilizing the Staudinger and Reformatsky reactions. The antiproliferative activity was assessed in MCF-7 cells, where the 4-(4-ethoxy)phenyl substituted compound displayed the most potent activity with an IC_{50} value of 0.22 μM and arrest of MCF-7 cells in the G2/M phase of the cell cycle and induction of apoptosis.

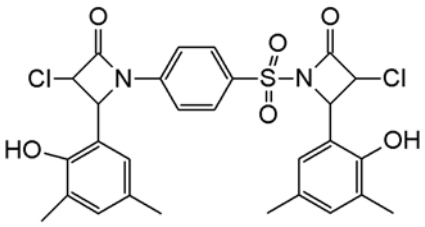


7.Kumar et al[53] 3-Azido-3-amino and 3-(1,2,3-triazol-1-yl)-β-lactams were synthesized and evaluated for their antiplasmodial activity against four strains of *Plasmodium falciparum* and KB cells for their cytotoxicity profiles. The presence of a cyclohexyl substituent at N-1 and a phenyl group on the triazole ring markedly improved the activity profiles of triazole-tethered β-lactam exhibiting IC_{50} values of 6.25 μM against 3D7, K1 and W2 strains respectively.

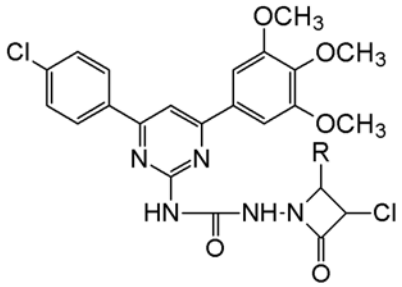


2.2.2 Azetidinones as antitubercular agents

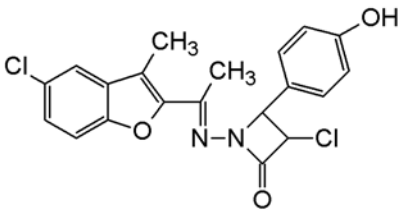
1. Omprakash et al[54] synthesized a series of azetidinones and 4-thiozolidinones of 4,4' sulphonyl aniline were screened for their *in vitro* antifungal ,antibacterial and antitubercular activity against mycobacterium tuberculosis H37RV by Lowenstain-Jension medium method. All the synthesized compounds exhibited moderate to potent activities of antifungal, antibacterial and antitubercular activity.



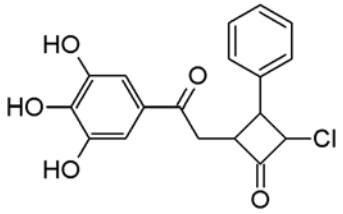
2. Chikalia et al[55] reported pyrimidine based azetidinones derivatives evaluated in their antitubercular activity against *Mycobacterium tuberculosis* H37RV. The β-lactam ring connected to ureido linkage showed moderate to excellent antitubercular activity.



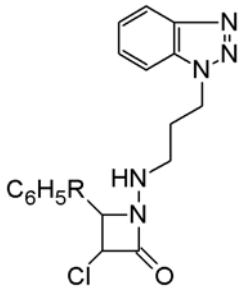
3. Basawaraj et al[56] synthesized a series of azetidinone and thiazolidinones derivatives from 5-chloro-3-methyl benzofuran and evaluated their antitconvulsion and antitubercular activity against *Mycobacterium tuberculosis* H37RV strain by broth dilution method. Results of the antitubercular screening showed two compounds with good antitubercular activity , when compared with streptomycin and None of the tested compounds abolished pentylene tetrazol induced convulsions in test animals.



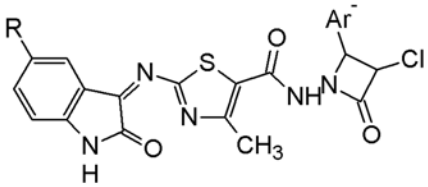
4. Ilango et al[57] reported the synthesis of novel trihydroxy benzamido azetidin-2-one derivatives and evaluated *in vitro* antibacterial, antitubercular activity aganist *Mycobacterium tuberculosis* H37RV strain by microplate alamarblue assay. Some compounds exhibited very good antibacterial and antitubercular activities.



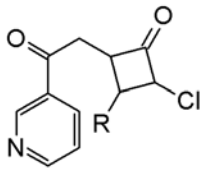
5. Dubey et al[58] carried out the synthesis of 2-oxo-4-substituted aryl-azetidine derivatives of benzotriazole and screening for their *in vitro* antitubercular activity against *Mycobacterium tuberculosis* H37RV strain by agar micro dilution method. Some compounds exhibited excellent antitubercular activity.



6. Dighe et al[59] investigated synthesis and evaluation of isatinyl thiazole derivatives as anti-*Mycobacterium tuberculosis* agents by broth micro dilution method and MIC value in the range of 6.25 µg/ml.

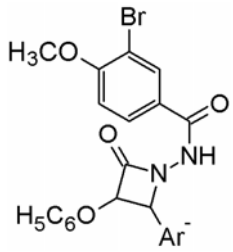


7. Sharma[60] et al reported 1-(nicotinylamino)-2 substituted azetidin 4-ones have been synthesized. They have exhibited significant activity against the bacteria and fungi and also anti-tubercular activity.

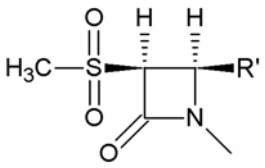


2.2.3 Azetidinones as antimicrobial agents

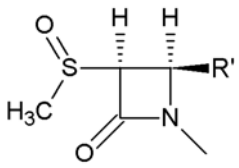
1. Sahoo et al[61] synthesized novel azetidinone derivatives showed *in vitro* antibacterial and antifungal activity against pathogenic strains. The preliminary screening results indicated that most of the compounds demonstrated moderate to very good antibacterial and antifungal activities.



2. Zarei et al[62] reported a series of 3-thiolated β-lactams were synthesized by [2+2] ketene imine cycloaddition reaction from S-substituted mercaptoacetic acids and Schiff bases. All the compounds were characterized by spectral data and elemental analyses and were evaluated for their *in vitro* antibacterial and antifungal activities against pathogenic strains including *Staphylococcus aureus* (Methicillin resistant strain). The preliminary screening results indicated that some of these compounds demonstrated moderate to very good antibacterial and antifungal activities.

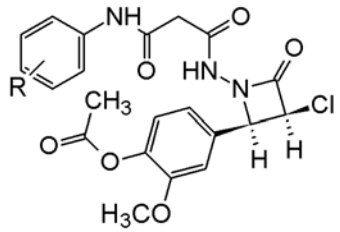


3-(methylsulfonyl) b-lactams

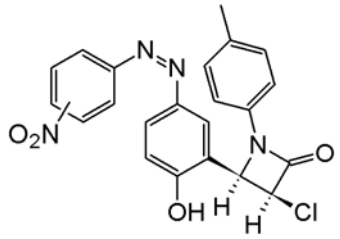


(methylsulfinyl) b-lactams

3. Halve et al[63] synthesized a series of 3-chloro-4-(3-methoxy-4 acetyloxyphenyl)-1-[3-oxo-3-(phenylamino)propanamido] azetidin-2-ones and 3-chloro-4-[2-hydroxy-5-(nitro substituted phenylazo)phenyl]-1-phenylazetidin-2-ones were established on the basis of elemental analysis and spectroscopic data. The antimicrobial activity of the synthesized compounds was screened against several microbes. Several of these molecules showed potent antimicrobial activity against *Bacillus anthracis, Staphyococcus aureus* and *Candida albicans* and significant structure–activity relationship (SAR) trends.

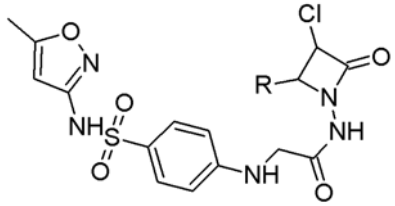


3-chloro-4-(3-methoxy-4 acetyloxyphenyl)-1-[3-oxo-3-(phenylamino)propanamido] azetidin-2-ones

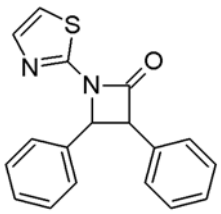


3-chloro-4-[2-hydroxy-5-(nitro substituted phenylazo)phenyl]-1-phenyl azetidin-2-ones

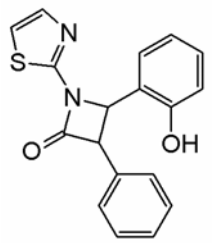
4. James et al[64] reported a series of novel Azetidinone derivatives have been synthesized from the intermediate Schiff bases. Cyclocondensation of schiff's bases with acetylchloride resulted in the formation of azetidinone derivatives. The compounds, characterized on the basis of satisfactory spectral data (IR, NMR and Mass spectroscopy) have shown moderate to good antimicrobial activity against some bacteria and fungi.



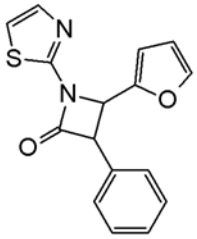
5. Meshram et al[65] synthesized novel N-thiazole, 3-phenyl, 4-substituted phenyl azetidin-2-ones were screened for their *in vitro* antibacterial activity against four microorganisms: *Staphylococcus aureus* (Gram positive), *Pseudomonas vulgaris* (Gram positive), *Pseudomonas Aeruginosa* (Gram negative) and *Escherichia coli* (Gram negative).The antibacterial screening data shows three compounds are highly active against the used strains.



2,3-diphenyl-4-(1,3-thiazol-2-yl)cyclobutanone

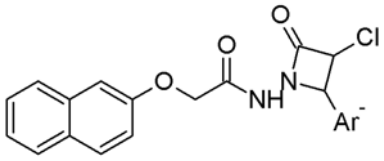


3-(2-hydroxyphenyl)-2-phenyl l-4-(1,3-thiazol-2- yl)cyclobutanone

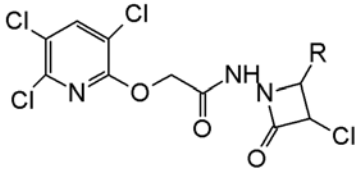


3-(2-furyl)-2-phenyl-4-(1,3-thiazol-2- yl)cyclobutanone

6. Rokade et al[66]] reported azetidinone derivatives were synthesized from β-naphthol and screened for their anti-bacterial and antifungal activities.



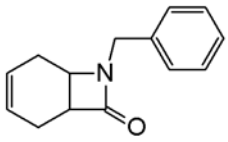
7. Synthesis and herbicidal activity of different azetidinones were reported by Gajari et al[67]. some of these compounds showed good herbicidal activity.



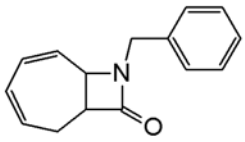
R=Alkyl, Aryl, Hetero

2.2.4 Azetidinones as antimalarial agents

1. Kaushik et al[68] reported a series of bicyclic *N*-substituted and unsubstituted β-lactams were synthesized and evaluated as targeted potential antimalarials. The compounds MNR4 and MNR5 were found to have highest potency *against Plasmodium falciparum in vitro*.

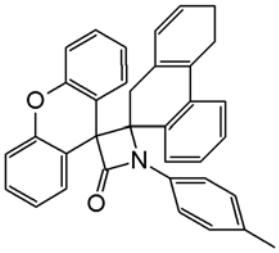


MNR4



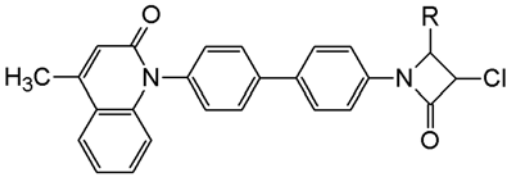
MNR5

2. Jarrahpour et al[69] demonstrated Some new mono-, bis-spiro- and dispiro-β lactams have been synthesized from imines derived from 9*H*-fluoren- 9-one and ketene by [2+2] cycloaddition reaction. The *in vitro* antimalarial activity of these monocyclic β-lactams was successfully investigated against *Plasmodium falciparum* K14 resistant strain with excellent EC_{50} values up to 5 mM.



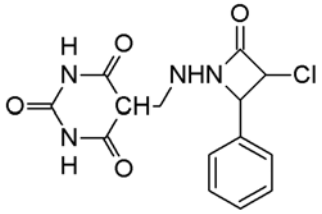
2.2.5 Azetidinones as anticonvulsant agents

1. Pawar et al[70] synthesized a series of ***N***-Substituted-7-hydroxy-4-methyl-2-oxoquinolines evaluated *in vivo* for their anticonvulsant activity by MES and two compounds showed significant anticonvulsant activity.



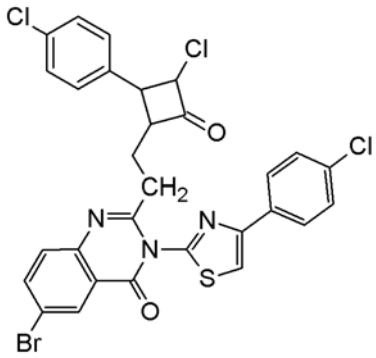
Compound1: R=C_4H_3O
Compound2: R=C_6H_5

2. Soodet al[71] reported a series of 5-[(3' chloro-4'-substituted phenyl -2'-oxo-azetidin-1'-yl)amino] barbituric acid have been synthesized and it shows better anticonvulsant activity.

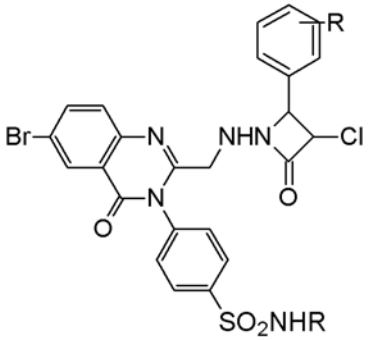


2.2.6 Azetidinones as anti-inflammatory agents

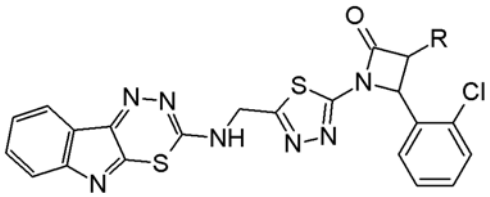
1.Synthesis of 3-[40-(p-chlorophenyl)-thiazol-20-yl]-2-[(substituted azetidinone /thiazolidinone)-aminomethyl]-6-bromoquinazolin-4- ones as anti-inflammatory agent were reported by Kumar et al[72]. 2-[(40-Oxo-30-chloro-20-{o-chlorophenyl}-azetidin- 10-yl)aminomethyl]-3-[400-(p-chlorophenyl)thiazol-200-yl]-6-bromoquinazolin-4-one showed promising anti-inflammatory and analgesic activity.



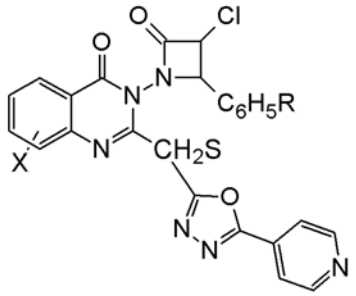
2. Kumar et al[73] synthesized a series of Indole derivatives were screened for their *in vivo* anti-inflammatory and analgesic activity. All the compounds exhibited moderate to good anti inflammatory and analgesic activity.



3. Kumar et al[74] synthesized a series of substituted azetidinoyl and thiazolidinoyl-1,3,4-thiadiazino (6,5-b) indoles were evaluated for their *in vivo* anti-inflammatory, ulcerogenic and analgesic activities. Compound 2-3-chloro-4-aryl-1-{5 [{[1,3,4]thiadiazino(6,5-b) indol-3-ylamino]methyl]-1,3,4-thiadiazol-2-yl}azetidin-2-one has shown most active anti-inflammatory and analgesic activities with better ulcerogenic activity than phenylbutazone.

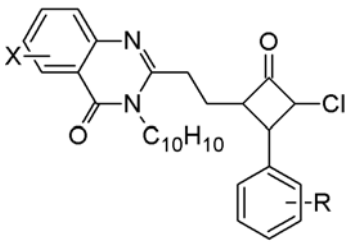


4. Synthesis and anti-inflammatory activity of newer quinazolin-4-one derivatives as anti-inflammatory agent were reported by Kumar et al [75]. All the compounds showed promising anti-inflammatory activity.



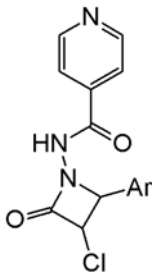
2.2.7 Azetidinones as antiparkinsonism agent

1. Kumar et al[76] synthesized a series of azetidinonyl/thiazolidinonyl quinazolinone derivatives were screened for their antiparkinsonism activity. All azetidinone derivative compounds shown mild activity compared to thiazolidinone derivatives.



2.2.8 Azetidinones as antidepressant agent

1. Thomas et al [77] reported synthesis and biological evaluation of Schiff's bases and 2-azetidinones of isonocotinyl hydrazone were screened for their antidepressant and nootropic activity. All synthesised Schiff's bases and azetidinone analogues exhibited antidepressant and nootropic activity. The results confirmed the fact that the 2-azetidinone skeleton has potential as a CNS activity.



Chapter 3

Objectives of the Present study

3.OBJECTIVES OF THE PRESENT STUDY

Phenothiazine and its derivatives reported to possess antipsychotic, antiemetic, antihistamine, antiinflammatory, anthelmintic, antitubercular, antimicrobial, antitumour, antiparkinsonism and anticancer agents. A slight variation in the substitution pattern on the phenothiazine nucleus often resulted in a marked difference in activities and therefore phenothiazines with various substituents are being synthesized and tested for various activities in search of better medicinal agents.

Azetidin-2-ones also reported to possess wide therapeutic activity viz. sedative, hypnotics, anticonvulsant, antimicrobial, antitubercular, antidiabetic, anti-inflammatory, analgesic activity, anticancer and antiparkinsonism activities.

An attempt was made to synthesize some new congeners by linking azetidinones with phenothiazine pharmacophore to evaluate their possible synergic activity as there were no reports on phenothiazine derivatives containing azetidinone moiety. β-lactam ring connected to ureido linkage possess significant antitubercular activity and tricyclic compounds containing NH and thio group with rigid side chain reported to possess significant antimicrobial, antioxidant and anticancer activity. With this background, a series novel phenothiazine derivatives flanked with azetidinones were synthesized and evaluated for *in vitro* antimicrobial, antitubercular, antioxidant and anticancer activity.

The objectives of the present work are to:

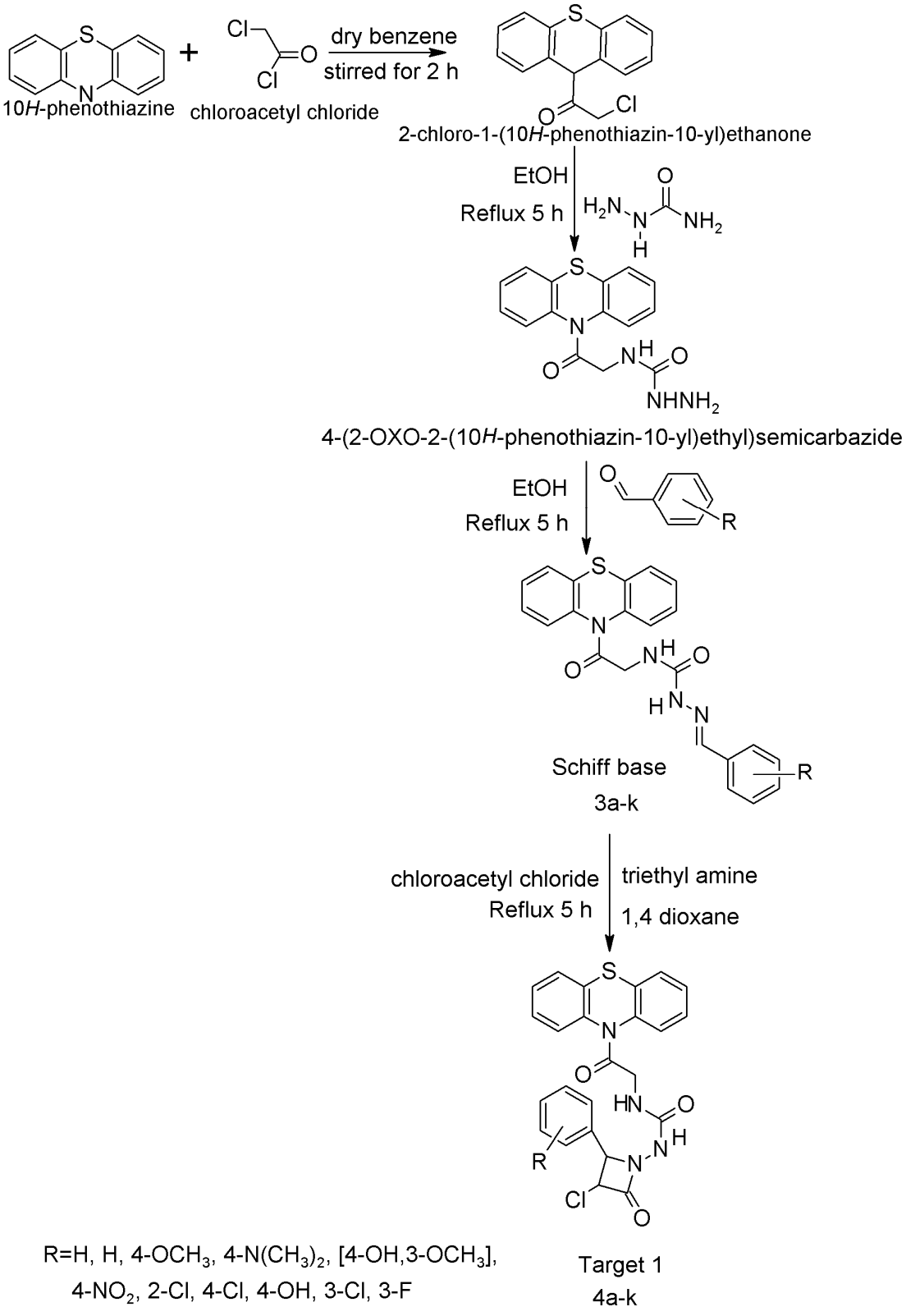
- synthesize various substituted phenothiazine derivatives
- characterize the synthesized compounds using FT-IR, ^{1}H-NMR and mass spectroscopy
- carry out *in vitro* antimicrobial, antitubercular, antioxidant and anticancer evaluation of the synthesized derivatives
- explain the possible SAR of the synthesized derivatives.

Chapter 4

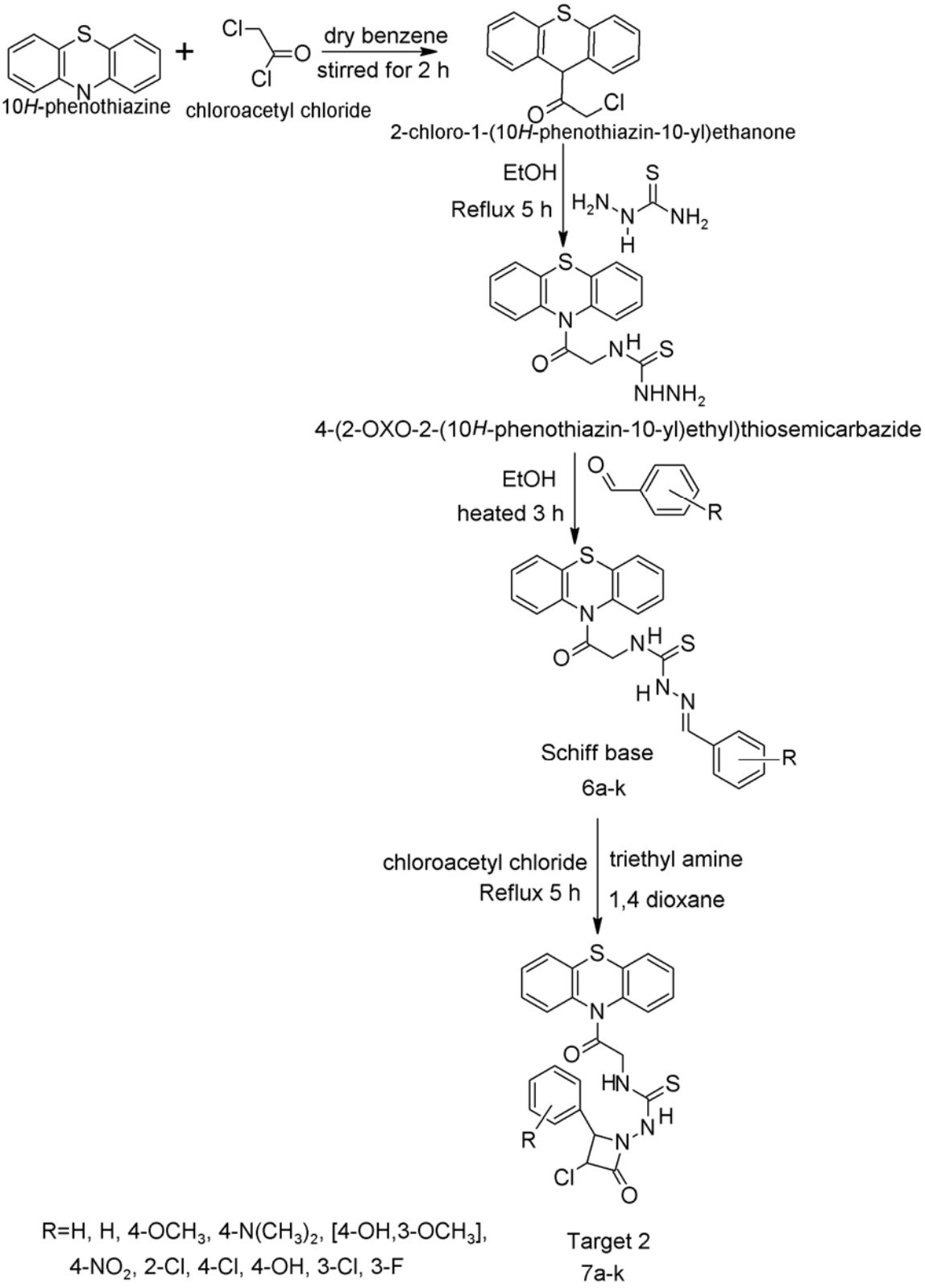
Experimental Work

4.EXPERIMENTAL WORK

4.1 SCHEME 1



4.2 SCHEME 2

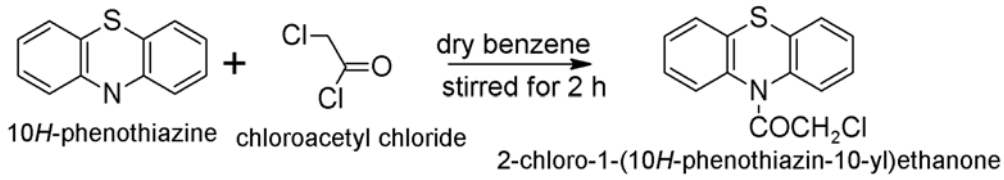


R=H, H, 4-OCH_3, 4-$N(CH_3)_2$, [4-OH,3-OCH_3], 4-NO_2, 2-Cl, 4-Cl, 4-OH, 3-Cl, 3-F

SYNTHESIS OF PHENOTHIAZINE DERIVATIVES

4.1 SCHEME 1

4.1.1 STEP 1: Synthesis of 2-chloro-1-(10*H*-phenothiazin-10-yl)ethanone from phenothiazine

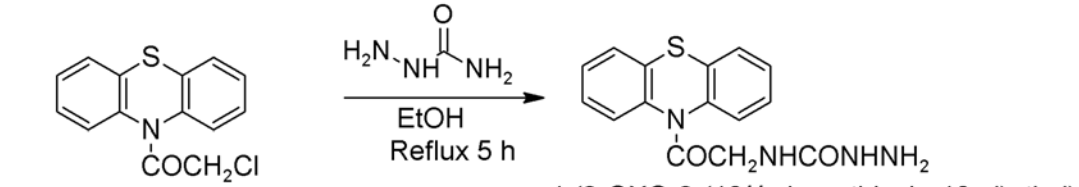


10*H*-phenothiazine chloroacetyl chloride 2-chloro-1-(10*H*-phenothiazin-10-yl)ethanone

Procedure

Chloroacetyl chloride (0.02 mol, 1.68 ml) was added drop by drop at 0-5^{0}C to phenothiazine (0.01 mol, 1.99 g) in dry benzene (50 ml) and stirred for 2 h. Solution was kept at room temperature where the solid separated was filtered and washed with petroleum ether and recrystallized from chloroform.[33,40]

4.1.2 STEP 2: Synthesis of 4-(2-oxo-2-(10*H*-phenothiazin-10yl)ethyl) semicarbazide

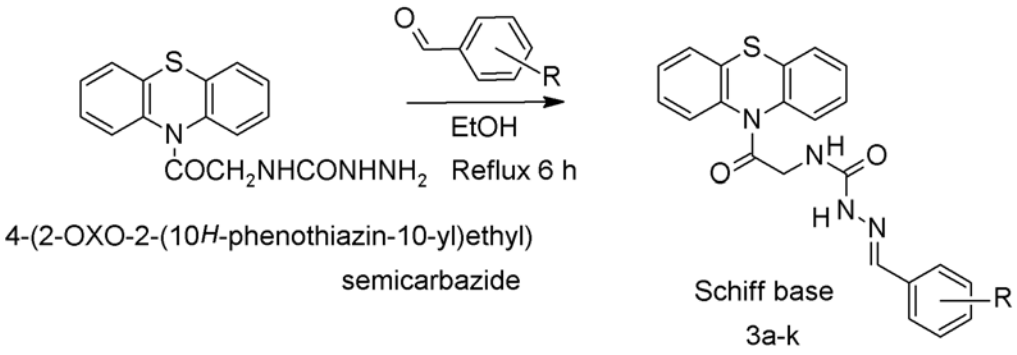


2-chloro-1-(10*H*-phenothiazin-10-yl)ethanone 4-(2-OXO-2-(10*H*-phenothiazin-10-yl)ethyl) semicarbazide

Procedure

A mixture of 2-chloro-1-(10*H*-phenothiazin-10-yl) ethanone (0.01 mol, 2.75 g) and semicarbazide (0.04 mol, 3 g) in absolute ethanol was refluxed for 5 h. Contents were poured into ice cold water. The resulting solid was filtered, dried and recrystallised from chloroform.[30,78]

4.1.3 STEP 3: **Synthesis of schiff' base**

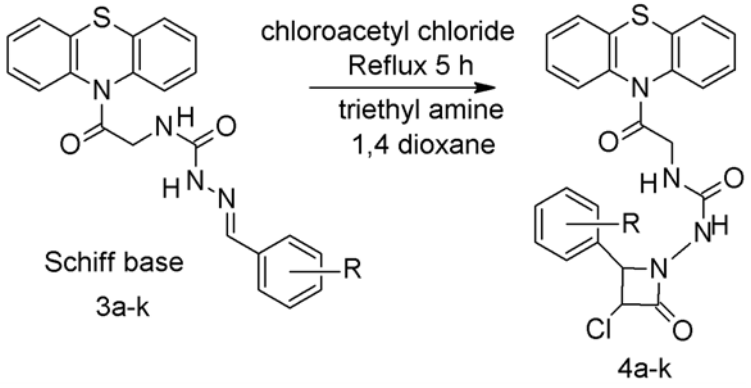


R= H, H, 4-OCH_3, 4-$N(CH_3)_2$, [4-OH, 3-OCH_3], 4-NO_2, 2-Cl, 4-Cl, 4-OH, 3-Cl, 3-F

Procedure:

Mixture of 4-(2-oxo-2-(10*H*-phenothiazin-10-yl)ethyl)semicarbazide (0.01 mol, 3.14 g) and substituted benzaldehyde (0.01 mol, 1.06 ml) in ethanol containing acetic acid (0.5 ml) was refluxed for 6 h. Excess of solvent was distilled off, concentrated and cooled. The solid thus separated was filtered, washed and recrystallised from ethanol.[79,80]

4.1.4 STEP4: 1-(3-chloro-2-oxo-4-phenylazetidin-1-yl)-3-(2-oxo-2-(10*H*-pheno thiazin-10-yl)ethyl)urea Derivatives.



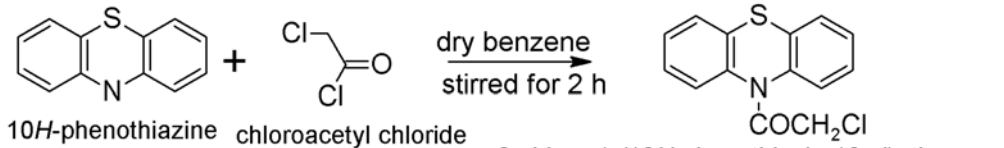
R= H, H, 4-OCH_3, 4-$N(CH_3)_2$, [4-OH, 3-OCH_3], 4-NO_2, 2-Cl, 4-Cl, 4-OH, 3-Cl, 3-F

Procedure

Chloroacetyl chloride (0.01 mol, 1.12 ml) was added dropwise to substituted Schiff's base (0.005 mol, 1.93 g) and triethylamine (0.01 mol, 1.01 ml) in 1,4 dioxane (12.5 ml) over a period of 30 minutes. The reaction mixture was refluxed for 5 h and filtered. The filterate is concentrated half its volume, then poured into crushed ice. The product obtained was filtered and washed with water and recrystallised from ethanol.[81]

4.2 SCHEME-2

4.2.1 STEP 1: Synthesis of 2-chloro-1-(10*H*-phenothiazin-10-yl)ethanone from phenothiazine

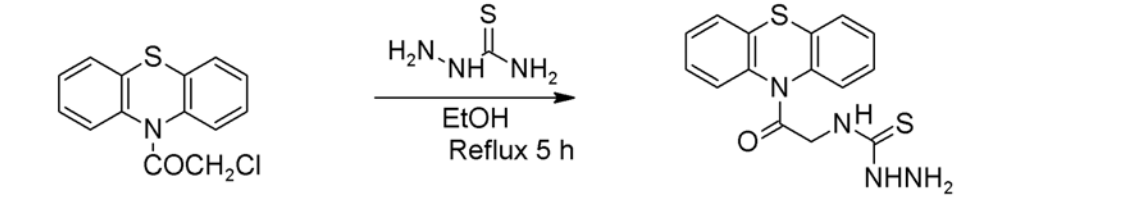


10*H*-phenothiazine chloroacetyl chloride 2-chloro-1-(10*H*-phenothiazin-10-yl)ethanone

Procedure

Chloroacetyl chloride (0.02 mol, 1.68 ml) was added drop by drop at 0-5^{0}C to phenothiazine (0.01 mol, 1.99 g) in dry benzene (50 ml) and stirred for 2 h. Solution was kept at room temperature where the solid separated was filtered and washed with petroleum ether and recrystallized from chloroform.[33,40]

4.2.2 STEP 2: Synthesis of 4-(2-oxo-2-(10*H* phenothiazin 10yl)ethyl)thio semicarbazide

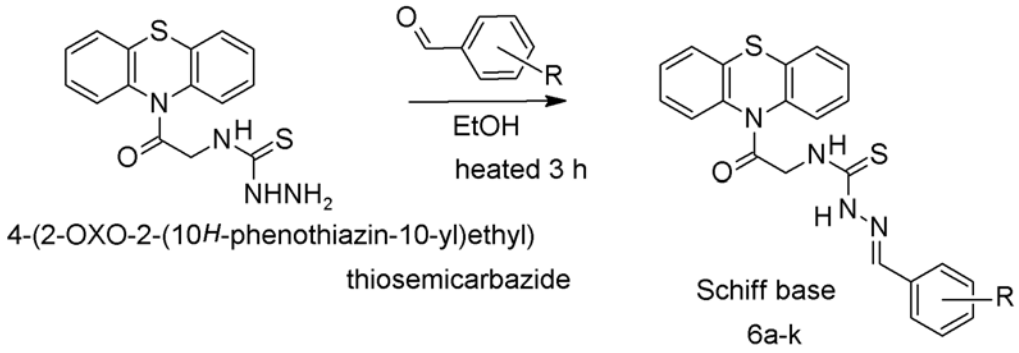


2-chloro-1-(10*H*-phenothiazin-10-yl)ethanone 4-(2-OXO-2-(10*H*-phenothiazin-10-yl)ethyl) thiosemicarbazide

Procedure:

A mixture of 2-chloro-1-(10*H*-phenothiazin-10-yl)ethanone (0.01 mol, 2.75 g) and thiosemicarbazide (0.04 mol, 3.4 g) in absolute ethanol was refluxed for 5 h. Contents were poured into ice cold water. The resulting solid was filtered, dried and recrystallised from chloroform.

4.2.3 STEP 3: Synthesis of schiff' base

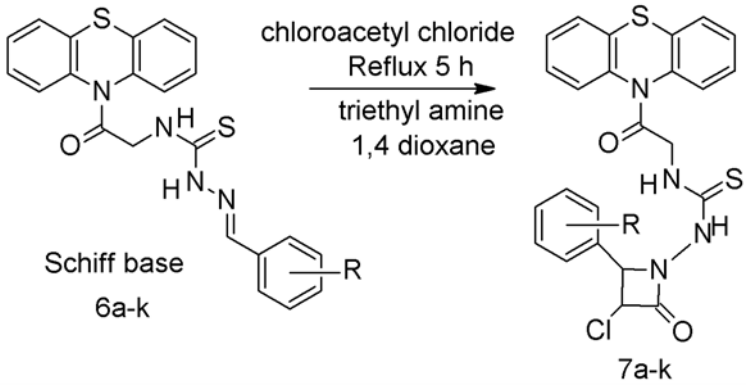


R= H, H, 4-OCH_3, 4-$N(CH_3)_2$, [4-OH, 3-OCH_3], 4-NO_2, 2-Cl, 4-Cl, 4-OH, 3-Cl, 3-F

Procedure:

Mixture of 4-(2-oxo-2-(10*H*-phenothiazin-10-yl)ethyl)thiosemicarbazide (0.01 mol, 3.30 g) and substituted benzaldehyde (0.01 mol, 1.06 ml) in ethanol (20 ml) were taken in a beaker. The mixture was heated on a water bath for 3 h, until a clear solution was obtained. The clear solution was kept overnight when respective Schiff's base fall out which was filtered, washed by petroleum ether and air dried.[82]

4.2.4 STEP4: 1-(3-chloro-2-oxo-4-phenylazetidin-1-yl)-3-(2-oxo-2-(10*H*-pheno thiazin-10-yl)ethyl)thiourea derivatives



R= H, H, 4-OCH_3, 4-$N(CH_3)_2$, [4-OH, 3-OCH_3], 4-NO_2, 2-Cl, 4-Cl, 4-OH, 3-Cl, 3-F

Procedure

Chloro acetylchloride (0.01 mol, 1.12 ml) was added drop wise to the mixture of triethylamine (0.01 mol, 1.01 ml in 12.5 ml of dry 1,4 dioxane) and solution of substituted Schiff bases (0.005 mol, 2.01 g). The reaction mixture was stirred for 30 min and then refluxed for 5 h. A solid was obtained was recrystallized from a mixture of ethanol and water.[83]

4.3 CHARACTERIZATION

4.3.1 Melting point

The melting points of all the synthesized compounds were determined using capillary tubes with Thermonic model C-LMP-1-Campbell melting point apparatus and are uncorrected **(Table 5).**

4.3.2 Thin layer chromatography

The synthesized compounds were tested for their purity by performing TLC over glass plates coated with silica gel with suitable mobile phase system and detected by iodine vapour **(Table 5)**.

4.3.3 Infra red spectroscopy

The structures of the synthesized compounds were elucidated by JASCO FTIR-4100 spectrophotometer in KBr disc **(Table 6)**.

4.3.4 NMR spectroscopy

^{1}HNMR spectral study was done using AV-III 400 Fourier Transform NMR spectrophotometer in TMS as standard **(Table 6)**.

4.3.5 Mass spectroscopy

Mass spectra of selected compounds were determined on JOEL SX 102 -GC MATE 700 EV instrument employing electron impact ionization technique **(Table 6)**.

Chapter 5

Biological Screening

5. BIOLOGICAL SCREENING

5.1 ANTIMICROBIAL SUSCEPTIBILITY TESTING

Antimicrobial susceptibility testing is indicated for any organism that contributes to an infectious disease warranting antimicrobial chemotherapy, if its susceptibility cannot be reliably predicted from pre-existing antibiograms. The introduction of a variety of antimicrobials today makes it a necessity to perform the antimicrobial susceptibility test, the results of which can guide us to the selection of appropriate antimicrobial drug with unquestionable benefit and least problem of antimicrobial resistance (AMR). Two methods were used for the antimicrobial susceptibility testing.[84,85,86]

- **DISC DIFFUSION METHOD (Zone of inhibition)**
- **SERIAL DILUTION METHOD (Minimum inhibitory concentration)**

5.1.1 ANTIBACTERIAL SCREENING (Disc diffusion method)

The antibacterial activities of synthesized compounds were screened in the concentration of 1 µg/µl in dimethyl sulphoxide (DMSO) against the listed microorganisms, obtained from National Collection of Industrial Microorganisms (NCIM), Pune and Microbial Type Culture Collection (MTCC), Chandigarh, in the Muller Hinton agar medium by disc diffusion method using ciprofloxacin (0.5 µg/µl) as standard. The antibacterial activity was evaluated by measuring zone of inhibition in mm.

Table 3. List of bacterial strains used in the study

GRAM POSITIVE	GRAM NEGATIVE
Micrococcus luteus NCIM 2169	*Escherichia coli* NCIM 2068
Staphylococcus aureus NCIM 2079	*Pseudomonas aeruginosa* NCIM 2206
Bacillus subtilis NCIM 2063	*Salmonella Paratyphii* NCIM 2075
Staphylococcus albus NCIM 2178	*Vibreo cholera* MTCC 1738

5.1.1.1 PREPARATION OF INOCULUMS

Preparation of inoculums of bacteria was carried out by Muller Hinton Broth and transferred to test tube and kept it for sterilization in autoclave at 120^0 C for 15 min. Then added culture of each bacteria to each tube (this step was carried out in aseptic room near laminar air flow) then kept it for incubation in incubator for 18-24 h at 37^0 C.

5.1.1.2 PROCEDURE

Muller Hinton agar medium was prepared by dissolving 21 g of Muller Hinton agar in 1000 ml of distilled water and agar-agar 1-2 g for solublization. Then kept it for sterilization in autoclave for 121^0C for 15 min. The petri plates were cleaned, sterilized and marked. These medium (Muller Hinton agar) were poured into petri-plates under aseptic conditions and allowed to solidify. Standardized bacterial inoculum was spread uniformly over the surface of medium by using a sterile non-absorbent cotton swab and finally the swab was passed around the edge of the medium. The inoculated petri plates were closed with the lid and allowed to dry at room temperature. The sample impregnated discs and standard discs were placed on the inoculated agar medium. All petriplates were incubated at 37^0C for 24 h. After the incubation, diameter of zone of inhibition produced by the sample and standard was measured. The details are tabulated in **Table 7 and Fig 32- 40**

5.1.2 ANTIFUNGAL SCREENING (Disc diffusion method)

The antifungal activities of synthesized compounds were screened in the concentration of 1µg/µl in dimethyl sulphoxide (DMSO) against the listed fungal strains, obtained from National Collection of Industrial Microorganisms (NCIM), Pune and Microbial Type Culture Collection (MTCC), Chandigarh, in the Sabourand's Dextrose Broth by disc diffusion method using Clotrimazole (10 µg disc) as standard. The antifungal activity was evaluated by measuring zone of inhibition in mm.

Table 4. List of fungal strains used in the study
Candida albicans MTCC 3100
Monascus purpureus MTC 1090
Aspergillus niger NCIM 1207
Trichophyton rubrum MTCC 3272

5.1.2.1 PREPARATION OF INOCULUMS

Preparation of inoculums of fungus was carried out by Sabourand's Dextrose Broth and transferred to test tube and kept it for sterilization in autoclave at 120^0 C for 15 min. Then added culture of each fungal to each tube (this step was carried out in aseptic room near laminar air flow) then kept it for incubation in incubator for 24-48 h at 29^0 C.

5.1.2.2 PROCEDURE

Sabourand's Dextrose agar medium was prepared by dissolving 1 gm of peptone and 4 gm of dextrose in 100 ml of distilled water and agar-agar 1-2 gm for solublization. Then kept it for sterilization in autoclave for 121^0 C for 15 min. The petri plates were cleaned, sterilized and marked. These medium (Sabourand's Dextrose agar) were poured into petri-plates under aseptic conditions and allowed to solidify. Standardized fungal inoculum was spread uniformly over the surface of medium by using a sterile non-absorbent cotton swab and finally the swab was passed around the edge of the medium. The inoculated petri plates were closed with the lid and allowed to dry at room temperature. The sample impregnated discs and standard discs were placed on the inoculated agar medium. All petriplates were incubated at 29^0C for 24 - 48 h. After the incubation, diameter of zone of inhibition produced by the sample and standard was measured. The details are tabulated in **Table 8** and **Fig. 41- 49.**

5.1.3 MIC STUDIES

5.1.3.1 PROCEDURE FOR DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION FOR SYNTHESIZED COMPOUNDS AGAINST BACTERIA BY BROTH DILUTION METHOD

The serial dilution of compound solutions were made from the stock (1000 μg/ml) by using Muller Hinton broth using the method described below.

1. The tubes were labeled 1 to 8 and 1 ml of Muller Hinton broth was added to the first 5 tubes and 8th tube, and then added 0.5 ml Muller Hinton broth to 6th and 7th tubes.
2. One ml of different synthesized compounds was added to the 1st tube, mixed and transferred 1 ml serially up to tube 6. Mixed and transferred 0.5 ml to the 7th tube so that each tube, 1 to 7 contained 1 ml diluted extract. The 8th tube served as the control.
3. With a standardized micro pipette, added a drop of the diluted broth culture approximately 0.01 ml of the test organism to all tubes, including the control, gently mixed and incubated at 37^{0} C for 18 h.
4. After incubation the turbidity was observed. The highest dilution of particular compounds showing no turbidity and recorded. This was taken as the end point, and this dilution was considered to contain the concentration of drug equivalent to MIC. (**Table 9** and **Fig. 50- 52**)

5.1.3.2 PROCEDURE FOR DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION FOR SYNTHESIZED COMPOUNDS AGAINST FUNGI BY BROTH DILUTION METHOD

The serial dilution of compound solutions were made from the stock (1000 µg/ml) by using Sabourand's dextrose broth using the method described below.

1. The tubes were labeled 1 to 8 and 1 ml of Sabourand's dextrose broth was added to the first 5 tubes and 8th tube, and then added 0.5 ml Muller Hinton broth to 6th and 7th tubes.
2. One ml of different synthesized compounds was added to the 1st tube, mixed and transferred 1 ml serially up to tube 6. Mixed and transferred 0.5 ml to the 7th tube so that each tube, 1 to 7 contained 1 ml diluted extract. The 8th tube served as the control.
3. With a standardized micro pipette, added a drop of the diluted broth culture approximately 0.01ml of the test organism to all tubes, including the control, gently mixed and incubated at 27^0 C for 24 - 48 h.
4. The highest dilution of particular compounds showing no turbidity was observed and recorded. This was taken as the end point, and this dilution was considered to contain the concentration of drug equivalent to MIC. (**Table 10** and **Fig. 53- 55**)

5.2 ANTITUBERCULAR SCREENING

Conventional methods like agar diffusion technique for susceptibility tests, which were based on the size of zone of inhibition surrounding a drug-containing disc, are not suitable for the slowly growing *mycobacterium* species because the drug diffuses throughout the medium before the organism grows.

Susceptibility testing: Direct method and indirect method

Direct method

This was done in case where acid-fast bacilli are seen on the smear of the concentrated clinical specimen. Dilutions are made and inoculated.

Indirect method

In this method bacterial mass is suspend in middle Brook 7H9 broth containing 3 or 4 small sterile glass beads. Mixture is placed on a vortex mixer and precautions are taken to prevent aerosol production. Tube was allowed to stand for 15min. The stock suspensions were diluted and 0.1 ml was inoculated onto control and drug containing media.[87]

5.2.1 RESAZURIN MICROTITRE PLATE ASSAY (REMA)

Resazurin (7-Hydroxy-3*H*-phenoxazin-3-one 10-oxide) is a blue dye, itself nonfluorescent until it is reduced to the pink colored and highly red fluorescent resorufin by oxidoreductases within viable cells. It is used mainly as an oxidation-reduction indicator in cell viability assays for bacteria and mammalian cells, the principle being that resazurin does not fluoresce when exposed to green light while resorufin fluoresces.

5.2.2 PROCEDURE

The anti-TB activity of the compounds was tested by resazurin microplate assay as per Martin *et al*[88] with slight modification. *M. tuberculosis* H37Rv was grown in Middlebrook 7H9 broth (Difco BBL, Sparks, MD, USA) supplemented with 10 % OADC (Becton Dickinson, Sparks, MD, USA) and 0.5% glycerol. The optical density of the bacterial culture was adjusted to McFarland 1.0 unit and 50 µl from this suspension was used as the inoculums. Stock solutions of the test compounds were prepared in dimethyl formamide (DMF) and were added to fresh medium in the wells of a 96-well microplate to which 50 µl inoculum was added making the total assay volume 200 µl. The final concentrations of the

test molecules were 1, 10 and 100 µg/ml. Growth control wells contained medium and *M. tuberculosis* H37Rv alone. Rifampicin (1.0 µg/ml) served as positive control for inhibition of growth. Negative control wells contained the highest volume of DMF used in test wells without any compound. After incubation at 37ºC for 7 days, 15 µl of 0.01% resazurin (Sigma, St. Louis. MO, USA) solution in sterile water was added to the first growth control wells and incubated for 24 h. Once the first set of growth controls turned pink, the dye solution was added to the second set of growth controls and the test wells, and incubated for 24 h at 37ºC. Blue color in the wells containing the test compounds indicated inhibition of growth and pink indicated lack of inhibition of growth of *M. tuberculosis*. The details are tabulated in **Table 11** and **Fig 56**.

5.3 *IN VITRO* ANTIOXIDANT STUDIES

An antioxidant is the one capable of slowing or preventing the oxidation where it protects the cell damage by reactive oxygen species that are produced during redox reactions in the cell. Antioxidants found in the body can be small molecules such as glutathione, vitamins, or macromolecules such as catalase (CAT), glutathione peroxidase (GPx). As oxidative stress contributes to the development of many diseases including Alzheimer's disease, Parkinson's disease, diabetes, rheumatoid arthritis and neurodegeneration, the use of antioxidants in pharmacology is intensively studied.[89] The antioxidant assays can roughly be classified into two types:

A) Assays based on hydrogen atom transfer (HAT) reactions

The majority of HAT-based assays apply a competitive reaction scheme, in which antioxidant and substrate compete for thermally generated peroxyl radicals through the decomposition of azo compounds. Some examples of such assays include inhibition of induced low-density lipoprotein autoxidation, oxygen radical absorbance capacity (ORAC), total radical trapping antioxidant parameter (TRAP) and crocin bleaching assays.

B) Assays based on electron transfer (ET)

These assays measure the capacity of an antioxidant in the reduction of an oxidant, which changes color when reduced. The sample's antioxidant concentration is correlated with the degree of its color change. Examples of such assays include the total phenols assay by Folin-Ciocalteu reagent (FCR), Trolox equivalence antioxidant capacity (TEAC), ferric ion

reducing antioxidant power (FRAP), "total antioxidant potential" assay using a Cu(II) complex as an oxidant and DPPH radical scavenging assay.

In addition, there are also other assays intended to measure a sample's scavenging capacity of biologically relevant oxidants such as singlet oxygen, superoxide anion, peroxynitrite etc.[90]

In-vitro Anti-oxidant screening of synthesized compounds were done by using two methods

- ✓ DPPH method
- ✓ FRAP method

5.3.1 DPPH RADICAL SCAVENGING METHOD

The free radical scavenging activity of a compound determined by this method is based on a reaction between a chomogen compound and antioxidant, and the concentration of the chomogen which is unreacted determined spectrophotometrically or colorimetrically. Most often used chomogen compound is DPPH (2,2-Diphenyl-1-Picryl-hydrazyl).

5.3.1.1 REAGENTS USED

Radical : DDPH

Solvent : Ethanol

Standard : Ascorbic acid

5.3.1.2 PREPARATION OF 0.3 mM DPPH SOLUTION

It was prepared by dissolving DPPH (5.91 mg) in 50 ml of ethanol. This stock solution was prepared freshly and kept in the dark at ambient temperature when not used.

5.3.1.3 PREPARATION OF SAMPLE STOCK SOLUTION

The sample stock solution was prepared by dissolving the compound in suitable solvent (ethanol) with a final concentration of 1 mg/ml.

5.3.1.4 PREPARATION OF STANDARD STOCK SOLUTION

The standard stock solution was prepared by dissolving the Ascorbic acid in suitable solvent (ethanol) with a final concentration of 1 mg/ml.

5.3.1.5 PROCEDURE

The effect of compound on DPPH radical was assayed using the method of Mensor *et al*[91]. Sample stock solutions (1.0 mg/ml) were diluted to appropriate final concentrations in ethanol. An ethanolic solution of 1 ml of DPPH (0.3 mM) was added to 0.5 ml of the compound and allowed to react at room temperature in a dark place for 30 minutes. After 30 minutes the absorbance values were measured at 518 nm. All the measurements were taken as a triplicate values. From the average of the absorbance values, lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The DPPH radical scavenging capability was calculated using the following equation:

$$\% \text{ inhibition} = (ABS_{control} - ABS_{test}) / ABS_{control} \times 100$$

Where,

$ABS_{Control}$=Absorbance of ethanol+DPPH

ABS_{Test} =Absorbance of DPPH + Compound /Standard

The percentage antioxidant activity (% inhibition) was extrapolated against concentration of the compound and EC_{50} was determined graphically. The results are tabulated in **Table 12** and **Fig. 57- 61.**

5.3.2 FRAP RADICAL SCAVENGING METHOD

FRAP (Ferric Reducing Ability of Plasma) is one of the most rapid test and very useful for routine analysis. The antioxidative activity is estimated by measuring the increase in absorbance caused by the formation of ferrous ions from FRAP reagent containing TPTZ (2, 4, 6 – tri (2 – pyridyl) – s – triazine) and $FeCl_3 6H_2O$.[92]

5.3.2.1 REAGENTS USED

Radical : FRAP

Solvent : Ethanol

Standard : Ferrous sulphate

5.3.2.2 PREPARATION OF FRAP REAGENT

FRAP reagent was prepared by mixing10 parts of 300 mM sodium acetate buffer at pH 3.6, 1 part of 10.0 mM TPTZ solution and 1 part of 20.0 mM $FeCl_3 . 6H_2O$ solution.

5.3.2.3 PREPARATION OF TEST SOLUTION

10 mg of each of the drug samples were accurately weighed separately and dissolved in 1 ml of ethanol. These solutions were serially diluted with ethanol to obtain the lower dilutions**.**

5.3.2.4 PREPARATION OF STANDARD SOLUTION

10 mg of ferrous sulphate were accurately weighed separately and dissolved in suitable solvent(water, ethanol). These solutions were serially diluted with ethanol to obtain the lower dilutions**.**

5.3.2.5 PROCEDURE

FRAP assay was carried out 0.2 ml of the compound is added to 3.8 ml of FRAP reagent and the reaction mixture was incubated at 37°C for 30 min and the increase in absorbance at 593 nm is measured. $FeSO_4$ is used for calibration. Quantitative calculation for each sample was done by plotting linear calibration curve. The antioxidant activities were expressed as the concentration of antioxidants having a ferric reducing ability equivalent to that of 1 mM / l $FeSO_4$. The equation used for Absorbance was as **(y) = 0.274 x + 0.114 [r^2 = 0.974]**. The results are tabulated in **Table 13** and **Fig. 62- 65.**

5.4. *IN VITRO* ANTICANCER SCREENING

Several approaches have been used in the past for the measurement of cell viability and growth. Though trypan blue staining is a simple way to evaluate cell membrane integrity (and thus assume cell proliferation or death), the method is not sensitive and cannot be adapted for high-throughput screening. The uptake of radioactive substances, usually tritium-labeled thymidine, can be measured. This method is accurate but is also time-consuming and involves handling of radioactive substances.

5.4.1. MTT ASSAY

MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a yellow tetrazole), is reduced to purple formazan by mitochondrial succinate dehydrogenase in living cells. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, coloured (dark purple) formazan product. The insoluble purple formazan product is converted to a colored solution using a solubilization agent (usually either DMSO, an acidified ethanol solution, or a solution of the detergent sodium dodecyl sulfate in diluted hydrochloric acid). The absorbance of this colored solution is then quantified by measuring at a certain wavelength (usually between 500 and 600 nm) by a spectrophotometer. Since reduction of MTT can only occur in metabolically active cells, this assay gives a direct measure of the viability of cells.

5.4.2 PROCEDURE

The human cervical cancer cell line (HeLa) was obtained from National Centre for Cell Science (NCCS), Pune. The cells were grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS).

For screening experiment, the cells were seeded into 96-well plates in 100 µl of medium containing 5% FBS, at plating density of 10,000 cells/well and incubated at 37^0C, 5% CO_2, 95% air and 100% relative humidity for 24 h prior to addition of samples. The samples were solubilized in Dimethylsulfoxide and diluted in serum free medium. After 24 h, 100 µl of the medium containing the samples at various concentration (6.25, 12.5, 25, 50, and 100 µM) were added and incubated at 37^0C, 5% CO_2, 95% air and 100% relative humidity for 48 h. Triplicate was maintained and the medium containing without extracts were served as control.

After 48 h, 15 µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37^0C for 4 h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100 µl of DMSO and then measured the absorbance at 570 nm using micro plate reader. The % cell inhibition was determined using the following formula.

% cell Inhibition = 100- Abs (sample)/Abs (control) x100

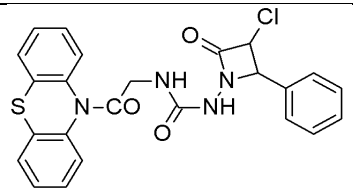
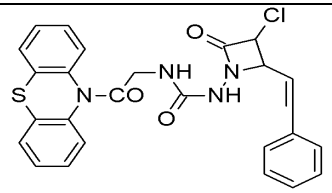
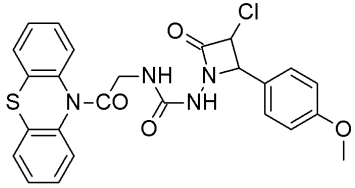
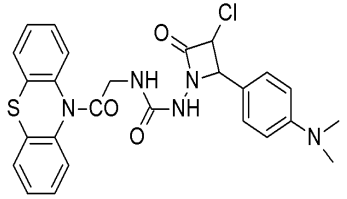
Non linear regression graph was plotted between percentage cell inhibition and Log_{10} concentration and IC_{50} was determined using GraphPad Prism software.[93,94]. The results are tabulated in **Table 14 – 16** and **Fig. 66- 81.**

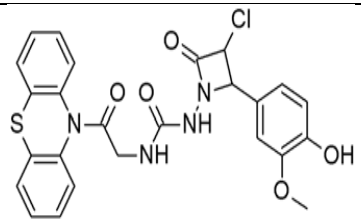
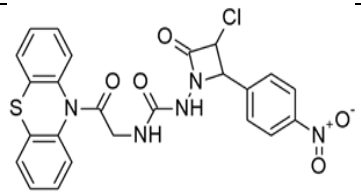
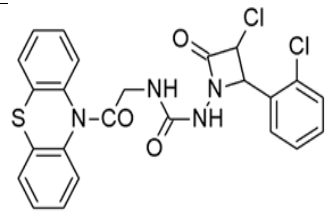
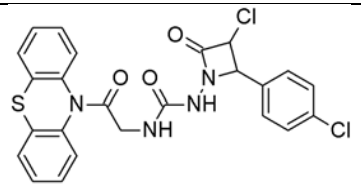
Chapter 6

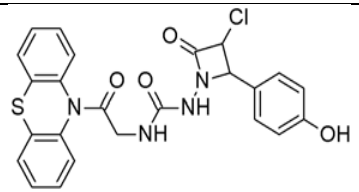
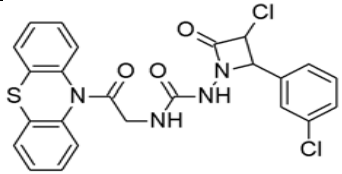
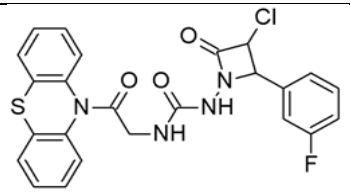
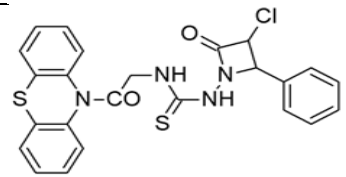
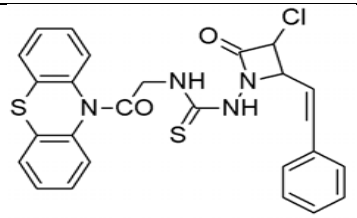
Results

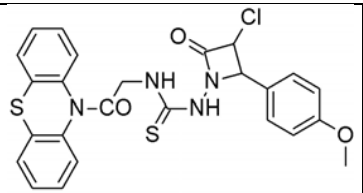
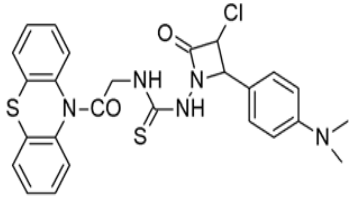
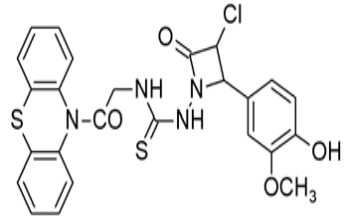
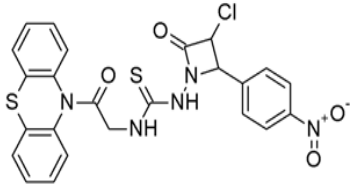
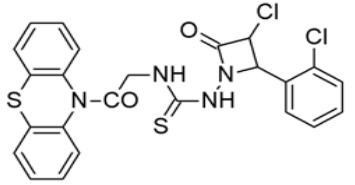
6.RESULTS

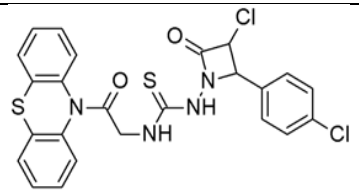
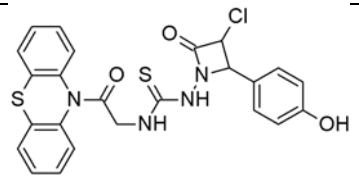
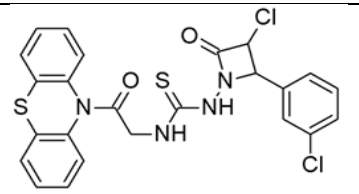
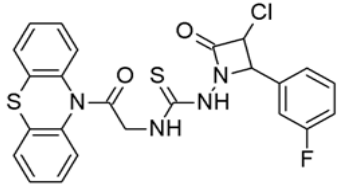
Table 5 . Physical and analytical data of synthesized compounds

Code no	Structure	IUPAC Name	Melting range (^{o}c)	*Rf	Molecular Formula	Mol.wt	Colour	% Yield	Solublity
4a		1-(3-chloro-2-oxo-4-phenylazetidin-1-yl)-3-(2-oxo-2-(10*H*-phenothiazin-10-yl)ethyl)urea	106-110	0.66	$C_{24}H_{19}ClN_4O_3S$	478.95	Pale pink	65.50	DMSO, DMF, Acetone,
4b		1-(3-chloro-2-oxo-4-styrylazetidin-1-yl)-3-(2-oxo-2-(10*H*-phenothiazin-10-yl)ethyl)urea	159-162	0.69	$C_{26}H_{21}ClN_4O_3S$	504.99	Green	85.38	DMSO, DMF, Dichloromethane
4c		1-(3-chloro-2-(4-methoxyphenyl)-4-oxoazetidin-1-yl)-3-(2-oxo-2-(10*H*-phenothiazin-10-yl)ethyl)urea	141-144	0.49	$C_{25}H_{21}ClN_4O_4S$	508.98	Bright green	82.72	DMSO, DMF, Ethyl acetate
4d		1-(3-chloro-2-(4-(dimethylamino)phenyl)-4-oxozetidin-1-yl)-3-(2-oxo-2-(*10*H-phenothiazin-10-yl)ethyl)urea	170-173	0.58	$C_{26}H_{24}ClN_5O_3S$	522.02	Blackish brown	71.11	DMSO, DMF, Dichloromethane

4e		1-(3-chloro-2-(4-hydroxy-3-methoxyphenyl)-4-oxoazetidin-1-yl)-3-(2-oxo-2-(10*H*-phenothiazin-10-yl)ethyl)urea	150-155	0.72	$C_{25}H_{21}ClN_4O_5S$	524.98	Bluish green	67.71	DMSO, DMF, Ethyl acetate, Acetone
4f		1-(3-chloro-2-(4-nitrophenyl)-4-oxoazetidin-1-yl)-3-(2-oxo-2-(10*H*-phenothiazin-10-yl)ethyl)urea	186-190	0.65	$C_{24}H_{18}ClN_5O_5S$	523.95	Black	55.33	DMSO, DMF, Dichloromethane Acetone
4g		1-(3-chloro-2-(2-chlorophenyl)-4-oxoazetidin-1-yl)-3-(2-oxo-2-(10*H*-phenothiazin-10-yl)ethyl)urea	165-169	0.63	$C_{24}H_{18}Cl_2N_4O_3S$	513.4	Pink	76.44	DMSO, DMF, Acetone, Dichloromethane, Ethyl acetate
4h		1-(3-chloro-2-(4-chlorophenyl)-4-oxoazetidin-1-yl)-3-(2-oxo-2-(10*H*-phenothiazin-10-yl)ethyl)urea	165-167	0.54	$C_{24}H_{18}Cl_2N_4O_3S$	513.4	Pale brown	79	DMSO, DMF, Acetone, Dichloromethane

4i		1-(3-chloro-2-(4-hydroxyphenyl)-4-oxoazetidin-1-yl)-3-(2-oxo-2-(10*H*-phenothiazin-10-yl)ethyl)urea	149-152	0.76	$C_{24}H_{19}ClN_4O_4S$	494.95	Pale green	66.07	DMSO, DMF, Dichloromethane
4j		1-(3-chloro-2-(3-chlorophenyl)-4-oxoazetidin-1-yl)-3-(2-oxo-2-(10*H*-phenothiazin-10-yl)ethyl)urea	155-160	0.55	$C_{24}H_{18}Cl_2N_4O_3S$	513.4	Green	69.33	DMSO, DMF, Dichloromethane
4k		1-(3-chloro-2-(3-fluorophenyl)-4-oxoazetidin-1-yl)-3-(2-oxo-2-(10*H*-phenothiazin-10-yl)ethyl)urea	168-172	0.58	$C_{24}H_{18}ClFN_4O_3S$	496.94	Pale pink	72.86	DMSO, DMF, Acetone
7a		1-(3-chloro-2-oxo-4-phenylazetidin-1-yl)-3-(2-oxo-2-(10*H*-phenothiazin-10-yl)ethyl)thiourea	106-110	0.66	$C_{24}H_{19}ClN_4O_2S_2$	495.02	Green	82	DMSO, DMF, Ethyl acetate, Acetone
7b		1-(3-chloro-2-oxo-4-styrylazetidin-1-yl)-3-(2-oxo-2-(10*H*-phenothiazin-10-yl)ethyl)thiourea	116-120	0.5	$C_{26}H_{21}ClN_4O_2S_2$	521.05	yellow	63.87	DMSO, DMF, Dichloromethane

7c		1-(3-chloro-2-(4-methoxyphenyl)-4-oxoazetidin-1-yl)-3-(2-oxo-2-(10*H*-phenothiazin-10-yl)ethyl)thiourea	136-140	0.63	$C_{25}H_{21}ClN_4O_3S_2$	525.04	Brown	59.82	DMSO, DMF, Ethyl acetate
7d		1-(3-chloro-2-(4-(dimethylamino)phenyl)-4-oxoazetidin-1-yl)-3-(2-oxo-2-(10*H*-phenothiazin-10-yl)ethyl)thiourea	160-163	0.79	$C_{26}H_{24}ClN_5O_2S_2$	538.08	Brown	82.1	DMSO, DMF, Dichloromethane
7e		1-(3-chloro-2-(4-(dimethylamino)phenyl)-4-oxoazetidin-1-yl)-3-(2-oxo-2-(10*H*-phenothiazin-10-yl)ethyl)thiourea	156-162	0.71	$C_{26}H_{24}ClN_5O_2S_2$	541.04	Yellow	55.60	DMSO, DMF, Acetone, Dichloromethane
7f		1-(3-chloro-2-(4-nitrophenyl)-4-oxoazetidin-1-yl)-3-(2-oxo-2-(10*H* phenothiazin-10-yl)ethyl)thiourea	115-119	0.68	$C_{24}H_{18}ClN_5O_4S_2$	540.01	Greyish green	80.7	DMSO, DMF, Dichloromethane, Acetone
7g		1-(3-chloro-2-(2-chlorophenyl)-4-oxoazetidin-1-yl)-3-(2-oxo-2-(10*H*-phenothiazin-10-yl)ethyl)thiourea	118-123	0.66	$C_{24}H_{18}Cl_2N_4O_2S_2$	529.46	Cream yellow	81.53	DMSO, DMF, Dichloromethane, Ethyl acetate

7h		1-(3-chloro-2-(4-chlorophenyl)-4-oxoazetidin-1-yl)-3-(2-oxo-2-(10*H*-phenothiazin-10-yl)ethyl)thiourea	143-149	0.71	$C_{24}H_{18}Cl_2N_4O_2S_2$	529.46	Green	83.75	DMSO, DMF, Dichloromethane, Acetone
7i		1-(3-chloro-2-(4-hydroxyphenyl)-4-oxoazetidin-1-yl)-3-(2-oxo-2-(10*H*-phenothiazin-10-yl)ethyl)thiourea	145-153	0.72	$C_{24}H_{19}ClN_4O_3S_2$	511.02	Green	65.06	DMSO, DMF, Ethyl acetate, Acetone
7j		1-(3-chloro-2-(3-chlorophenyl)-4-oxoazetidin-1-yl)-3-(2-oxo-2-(10*H*-phenothiazin-10-yl)ethyl)thiourea	108-112	0.56	$C_{24}H_{18}Cl_2N_4O_2S_2$	529.46	Grayish green	54.01	DMSO, DMF, Dichloromethane, Acetone
7k		1-(3-chloro-2-(3-fluorophenyl)-4-oxoazetidin-1-yl)-3-(2-oxo-2-(10*H*-phenothiazin-10-yl)ethyl)thiourea	118-125	0.62	$C_{24}H_{18}ClFN_4O_2S_2$	513.01	Yellow	52.53	DMSO, DMF, Dichloromethane, Ethyl acetate

***MOBILE PHASE: 4a-k, 7a-k** - benzene:chloroform (1:9)

DETECTING AGENT: 4a-k, 7a-k- iodine vapours.

Table 6. Spectral data of synthesized compounds

CODE	(DMSO, λ_{max}, nm) IR (KBr, ν, cm^{-1})	^{1}H NMR (DMSO, δ, ppm)	EIMS (M+)
4a	3340 (NH stretching) 3167,1596,736, (aromatic stretching) 1718 (C=O stretching) 1472 (C-N stretching), 811(C-Cl) 700(C-S-C stretching)	-	-
4b	3135 (NH stretching) 2921,1599,697 (aromatic stretching) 1695 (C=O stretching) 1596 and 1570(phenothiazine ring), 1444 (C-N stretching), 919 (CH=CH stretching) 811 (C-Cl) 756 (C-S-C stretching)	-	-
4c	3340(NH stretching) 3133, 736 (aromatic stretching) 1597 (C=O stretching 1596 and 1570 (phenothiazine ring), 1401 (C-N stretching) 1253 (C-O-C), 832(C-Cl) 686(C-S-C stretching)	-	-
4d	3410 (NH stretching) 3150,1542,737 (aromatic stretching) 1620 (C=O stretching), 1596 and 1570 (phenothiazine ring),1470 (C-N stretching), 1312 (CN of $N(CH_3)_2$ 827 (C-Cl stretching), 686 (C-S-C stretching)	6.59-7.0 (m,12H, Ar-H) 2.9 (s,6H,$N(CH_3)_2$) 3.4 (s,2H,CH_2) 2.5(d,1H,C-CH-Cl) azetidinone ring 8.5 (s, 1H,NH) NH_2 not detectable	-
4e	3340 (OH stretching), 3154 (NH stretching), 2932, 1595,736 (aromatic stretching), 1750(C=O stretching) 1596 and 1570(phenothiazine ring), 1120 (C-O-C stretching) 1400 (C-N stretching) 821 (C-Cl), 686(C-S-C stretching)	-	524.9
4f	3490 (NH stretching) 3134,1578,736 (aromatic stretching 1699 (C=O stretching), 1596 and 1570 (phenothiazine ring), 1517,1341 (N=O stretching)1467 (C-N stretching) 816 (C-Cl),688 (C-S-C stretching)	-	-

4g	3346 (NH stretching) 3127,1578,737 (aromatic stretching) 1721 (C=O stretching) 1596 and 1570 (phenothiazine ring), 1463 (C-N stretching) 816 (C-Cl stretching) 688 (C-S-C stretching)	6.52-7.26 (m,12H,Ar-H), 3.32 (s,2H,CH_2) 2.5 (d,1H,C-CH-Cl) azetidinone ring 7.4-7.8 (m,2H,+2H,Ar-H) o-chloro phenyl ring, 8.5(s,1H,NH), NH_2 not detectable	-
4h	3340 (NH stretching) 3133,1517,737 (aromatic stretching) 1596 and 1570(phenothiazine ring), 1596 (C=O stretching) 1470 (C-N stretching) 821(C-Cl) 655 (C-S-C stretching)	-	-
4i	3340 (OH stretching) 3132(NH stretching) 2920,1512,737,(aromatic stretching) 1596 and 1570 (phenothiazine ring), 1596 (C=O stretching) 1470 (C-N stretching) 827 (C-Cl), 686 (C-S-C stretching)	-	-
4j	3346 (NH stretching) 3127,1595,736 (aromatic stretching) 1671(C=O stretching) 1596 and 1570(phenothiazine ring) 1460 (C-N stretching) 837(C-Cl) 656(C-S-C stretching)	-	-
4k	3346 (NH stretching) 2920,1596,736(aromatic stretching) 1718 (C=O stretching) 1596 and 1570 (phenothiazine ring), 1472 (C-F stretching) 1401(C-N stretching) 876(C-Cl) 656(C-S-C stretching)	-	-

7a	3340(NH stretching) 3134,737 (aromatic stretching) 1596 (C=O stretching) 1472 (C-N stretching) 1112 (C=S attached NH) 827(C-Cl), 689 (C-S-C stretching)	-	-
7b	3340(NH stretching) 3132,738(aromatic stretching) 1596 (C=O stretching) 1400(C-N) 1132 (C=S attached NH 968(CH=CH stretching), 827(C-Cl), 689 (C-S-C stretching)	-	-
7c	3340(NH stretching) 3111,1533,754 (aromatic stretching) 1670 (C=O stretching) 1596 and 1570(phenothiazine ring), 1518,1151(C=S linked to NH) 1400 (C-N stretching), 1188 (C-O-C) 836 (C-Cl), 690 (C-S-C stretching)	6.68-7.14 (m,12H,Ar-H) 3.3 (s,3H,OCH_3) 3.7 (s,2H,CH_2) 2.5 (d,1H,C-CH-Cl) azetidinone ring, NH and NH_2 not detectable	-
7d	3340(NH stretching) 3160,1524,736 (aromatic stretching) 1599 (C=O stretching) 1596 and 1570(phenothiazine ring), 1512,1089 (C=S linked to NH) 1472 (C-N stretching),1311 (CN of $N(CH_3)_2$, 817 (C-Cl) 716(C-S-C stretching)	-	-
7e	3340 (OH stretching) 3135 (NH stretching) 2918,1596,736 (aromatic stretching) 1600(C=O) 1596 and 1570(phenothiazine ring), 1512,1122 (C=S attached NH) 1083 (C-O-C)) 1400(C-N stretching) 827(C-Cl) 716(C-S-C stretching)	6.68-7.0 (m,11H,Ar-H) 3.3 (s,3H,OCH_3) 3.4 (s,1H,OH) 2.5 (d,1H,C-CH-Cl) azetidinone ring 8.5(s,1H, NH) NH_2 not detectable	-

7f	3128 (NH stretching) 2918,1595,748 (aromatic stretching) 1600 (C=O stretching) 1596 and 1570(phenothiazine ring), 1515,1342(N=Ostretching),1468,1117 (C=S linked to NH) 1401 (C-N stretching) 837(C-Cl), 656(C-S-C stretching)	-	541.11
7g	3340(NH stretching) 3148,1594,736 (aromatic stretching) 1694 (C=O stretching) 1596 and 1570(phenothiazine ring), 1532,1105 (C=S linked to NH) 1401(C-N stretching) 821(C-Cl), 686(C-S-C stretching)	-	-
7h	3340 (OH stretching) 3135 (NH stretching) 2918,1596,736(aromatic stretching) 1600 (C=O stretching) 1596 and 1570(phenothiazine ring), 1512,1122 (C=S linked to NH) 1400 (C-N stretching) 716 (C-Cl), 686 (C-S-C stretching)	6.-7.4 (m,12H,Ar-H) 3.3 (s,2H, CH_2) 7.7-7.8 (m,2H,+2H,Ar-H) P-chloro phenyl ring. 2.5 (d,1H,C-CH-Cl) azetidinone ring 8.6 (s1H, NH) NH_2 not detectable.	-
7i	3341 (OH stretching) 3147 (NH stretching) 2918,1596,738 (aromatic stretching) 1596 and 1570(phenothiazine ring), 1650 (C=O stretching) 1510,1136 (C=S linked to NH) 1401(C-N stretching),716 (C-Cl) 655 (C-S-C stretching)	-	-
7j	3339 (NH stretching) 3131,1570,730 (aromatic stretching) 1595 (C=O stretching) 1596 and 1570(phenothiazine ring), 1472,1185 (C=S linked to NH) 1401 (C-N stretching) 876 (C-Cl),655 (C-S-C stretching)	-	-
7k	3340 (NH stretching) 3113,1582,754 (aromatic stretching) 1670 (C=O stretching) 1596 and 1570(phenothiazine ring), 1519, 1151(C=S linked to NH) 1471 (C-F stretcting) 3336 (C-N stretching) 837 (C-Cl) 626 (C-S-C stretching)	6.6-7.0(m,12H,Ar-H) 7.3-7.5 (m,4H,Ar-H) flouro substituted phenyl ring. 3.5(s,2H,CH_2) 2.5(d,1H,C-CH-Cl) azetidinone ring 8.7(s,1H, NH) NH_2 not detectable.	-

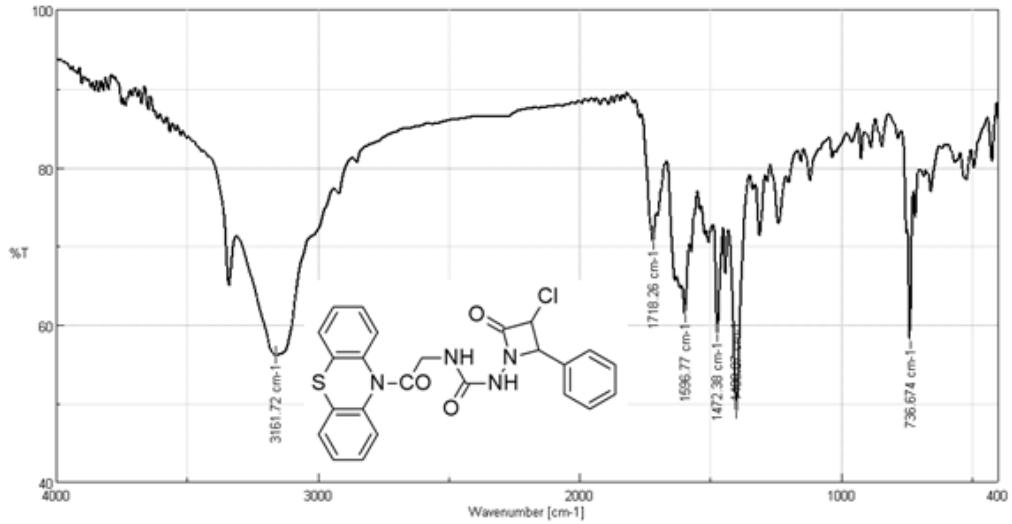


Fig 2. IR spectrum of compound **4a**

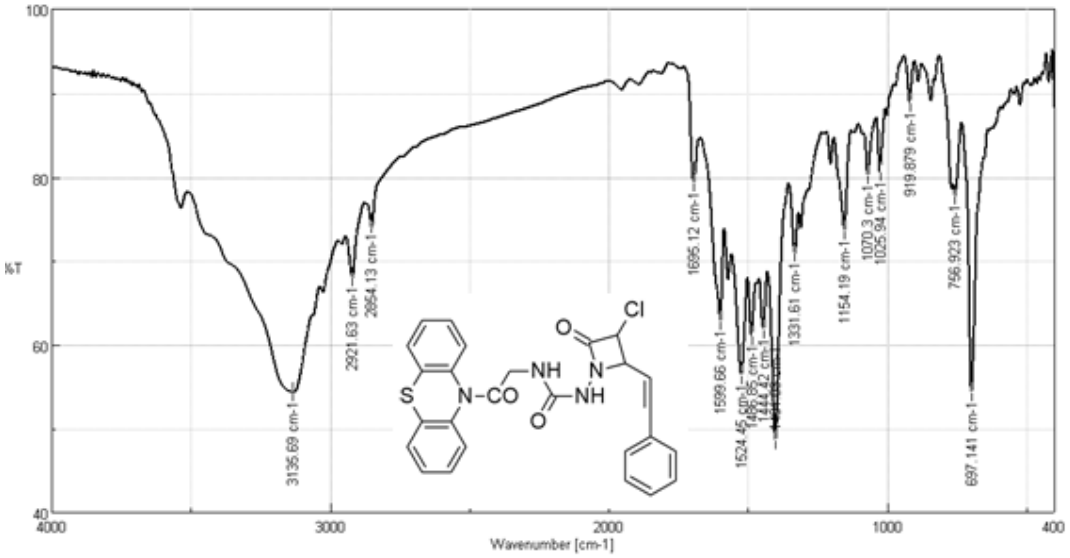


Fig 3. IR spectrum of compound **4b**

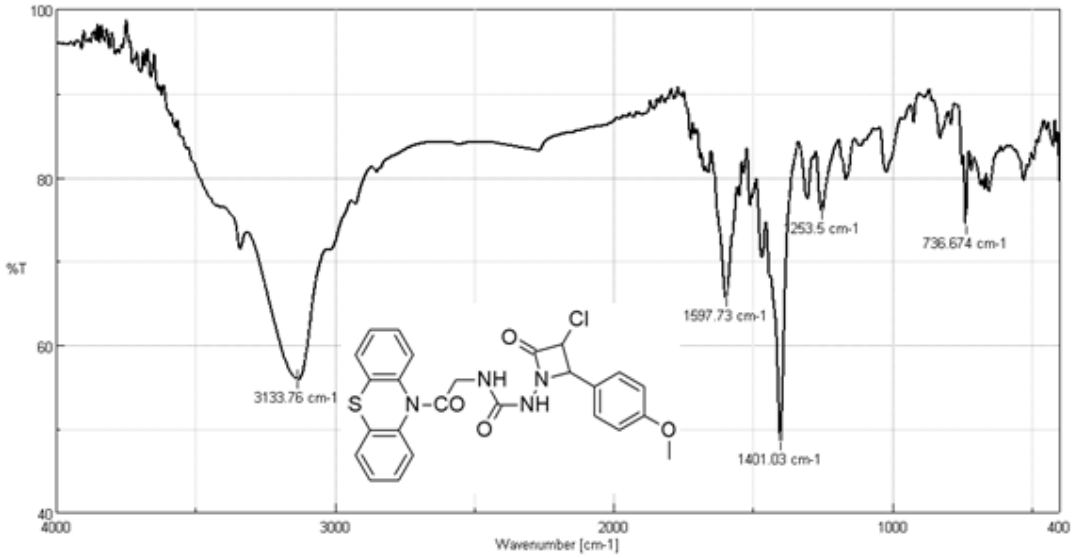


Fig 4. IR spectrum of compound **4c**

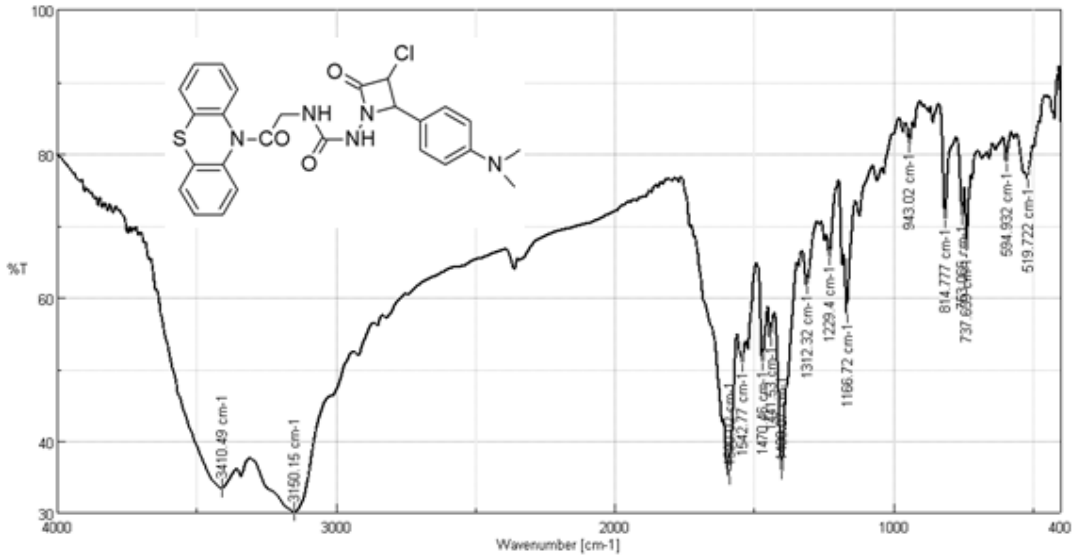


Fig 5. IR spectrum of compound **4d**

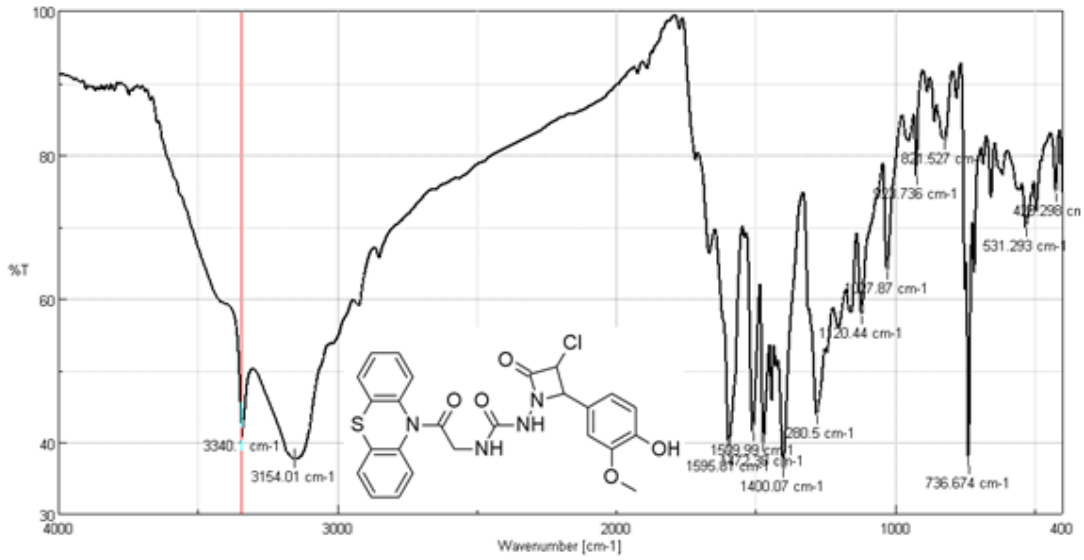


Fig 6. IR spectrum of compound **4e**

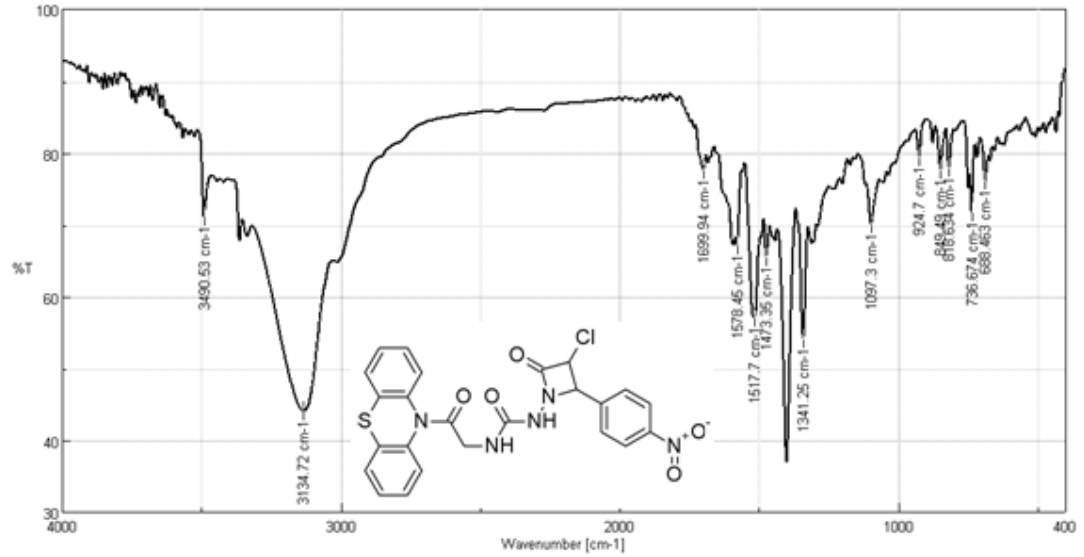


Fig 7. IR spectrum of compound **4f**

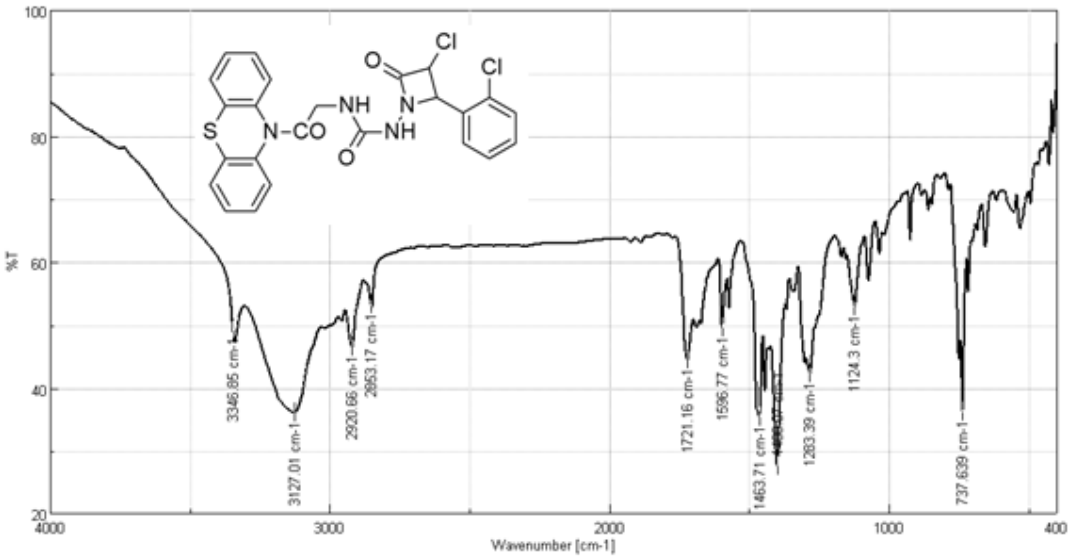


Fig 8. IR spectrum of compound **4g**

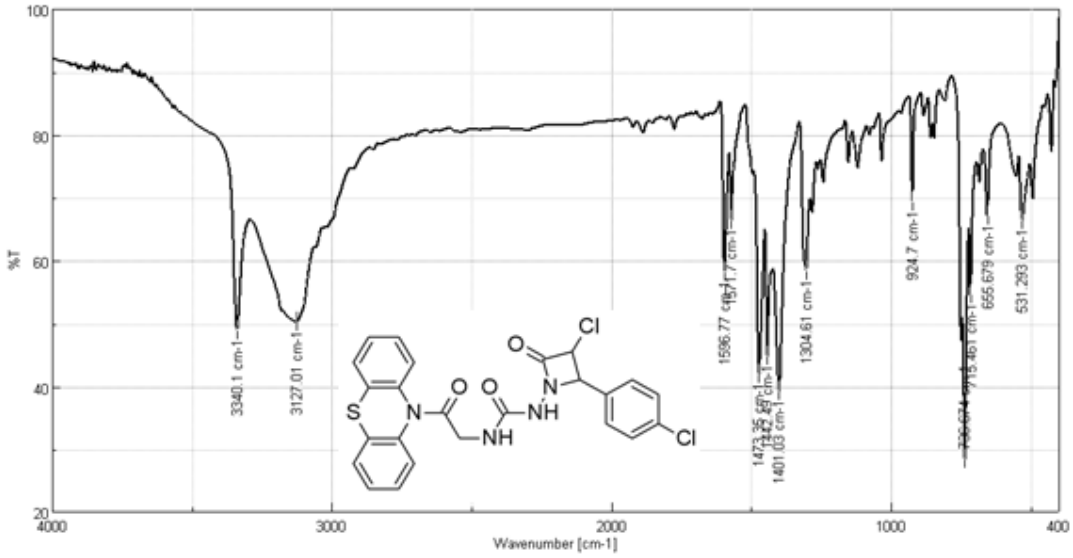


Fig 9. IR spectrum of compound **4h**

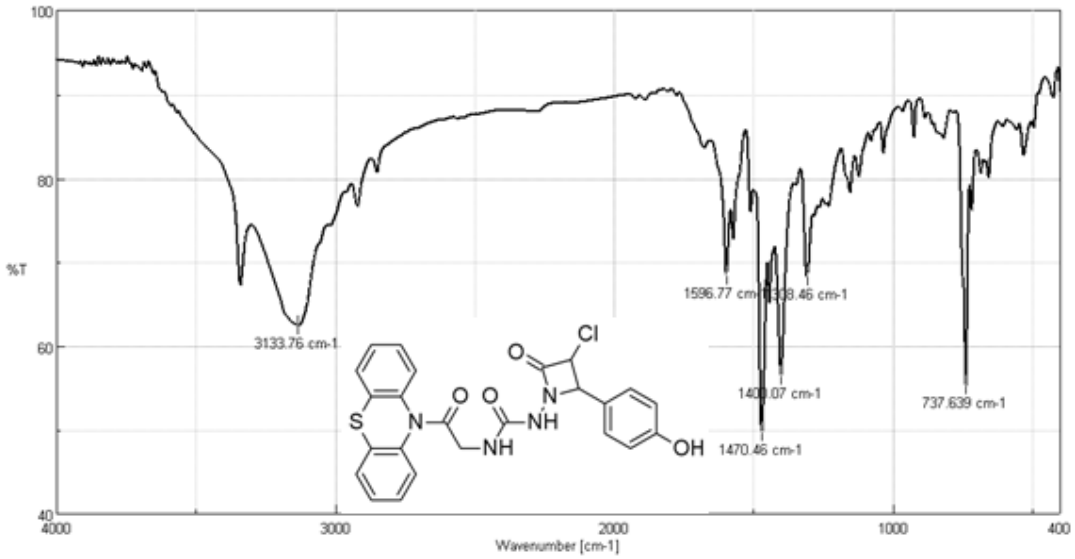


Fig 10. IR spectrum of compound **4i**

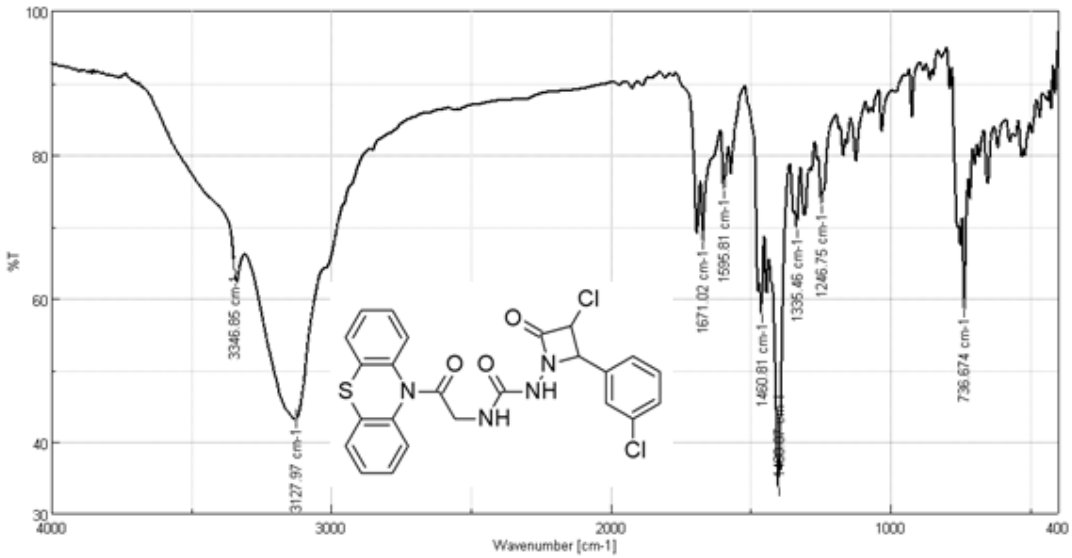


Fig 11. IR spectrum of compound **4j**

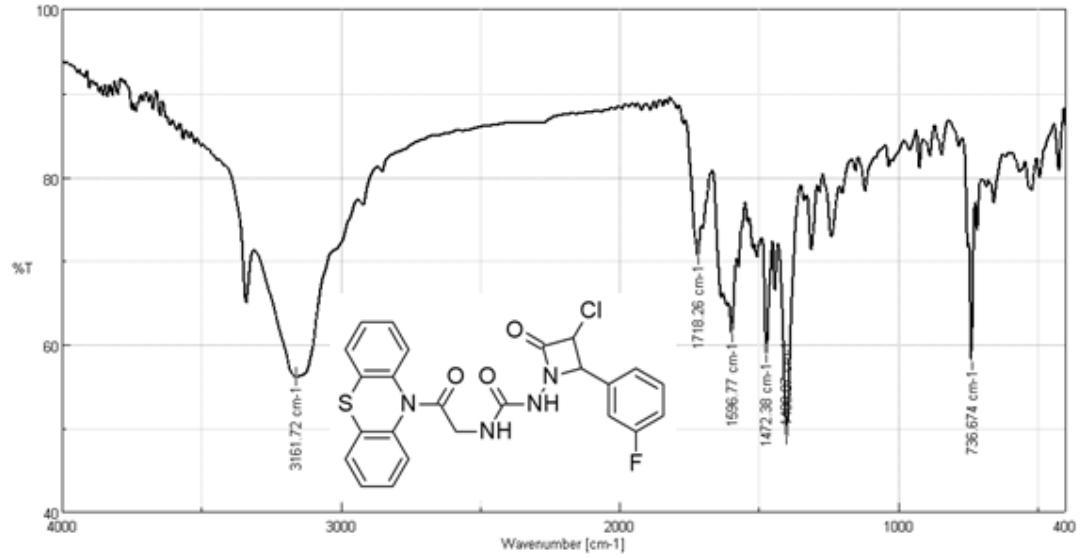


Fig 12. IR spectrum of compound **4k**

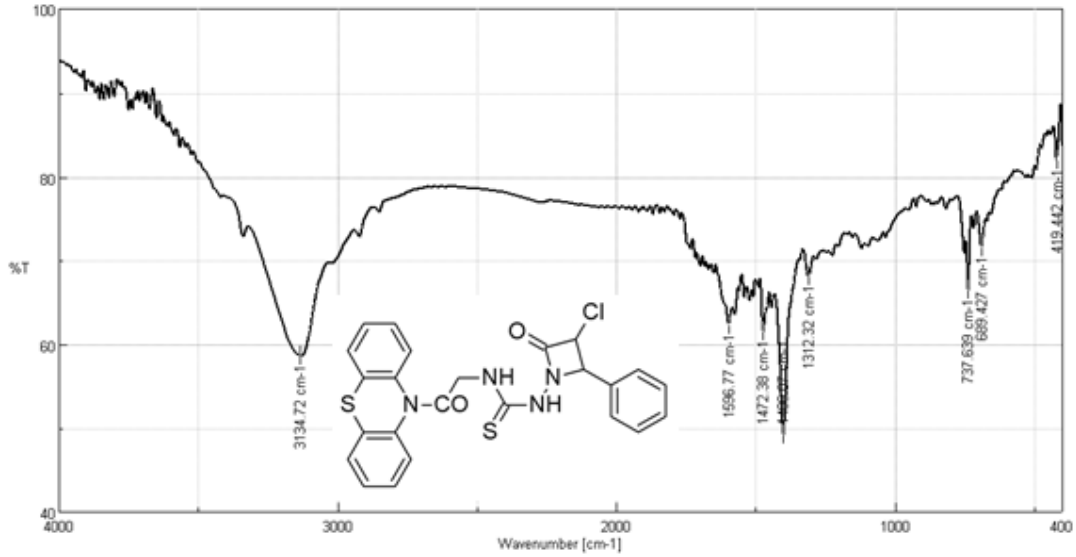


Fig 13. IR spectrum of compound **7a**

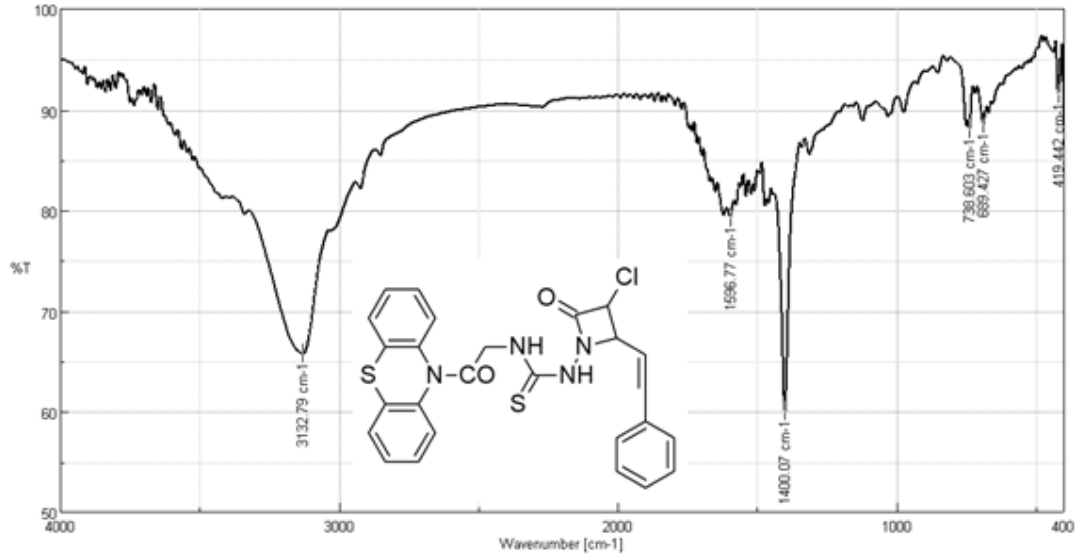


Fig 14. IR spectrum of compound **7b**

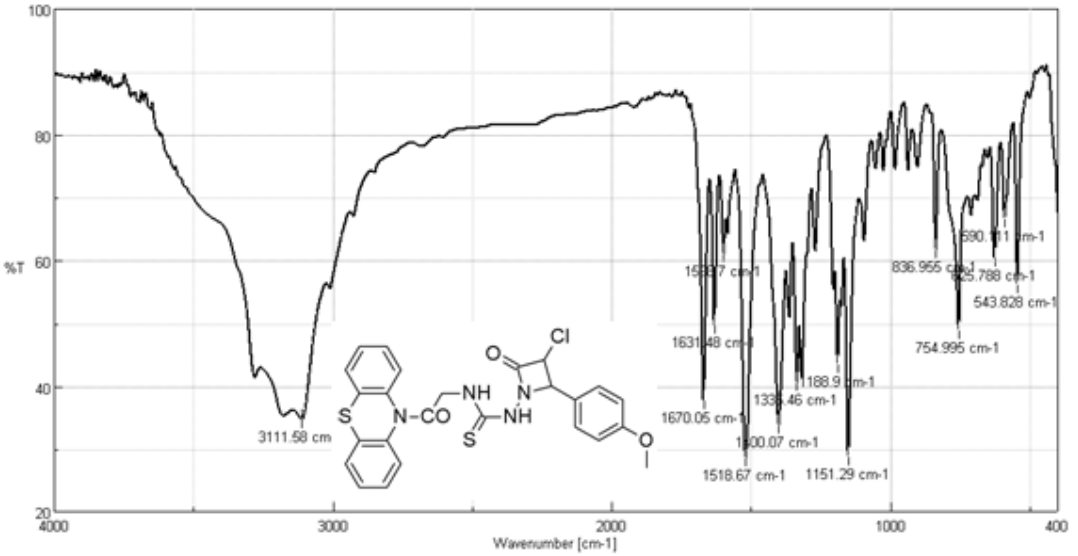


Fig 15. IR spectrum of compound **7c**

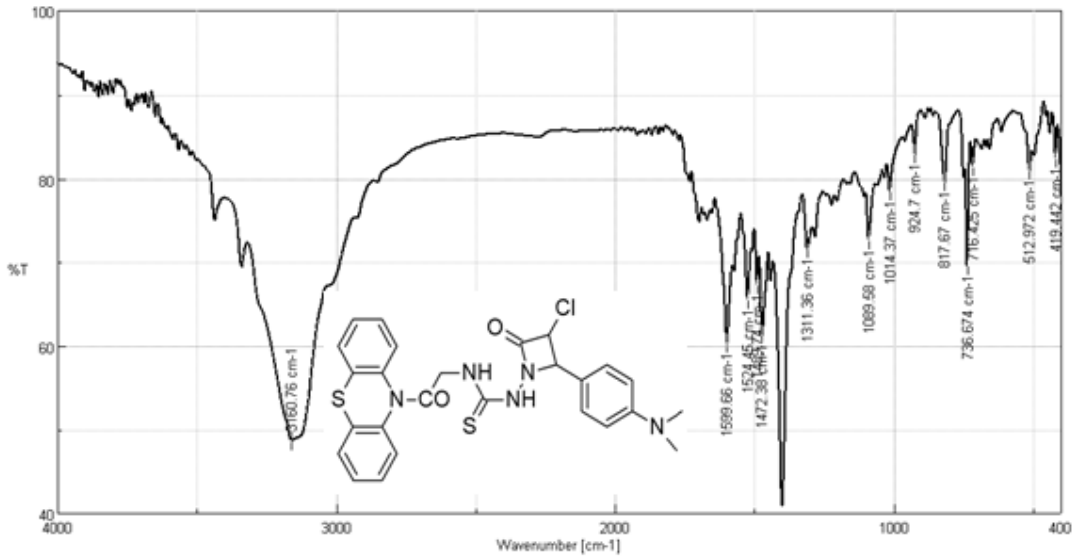


Fig 16. IR spectrum of compound **7d**

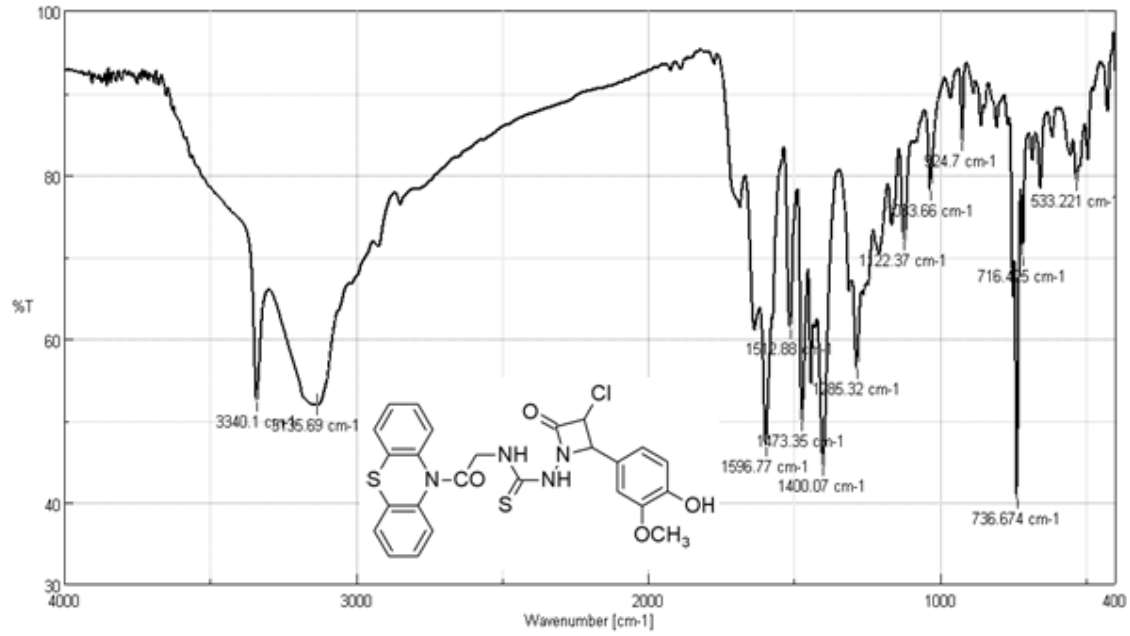


Fig 17. IR spectrum of compound **7e**

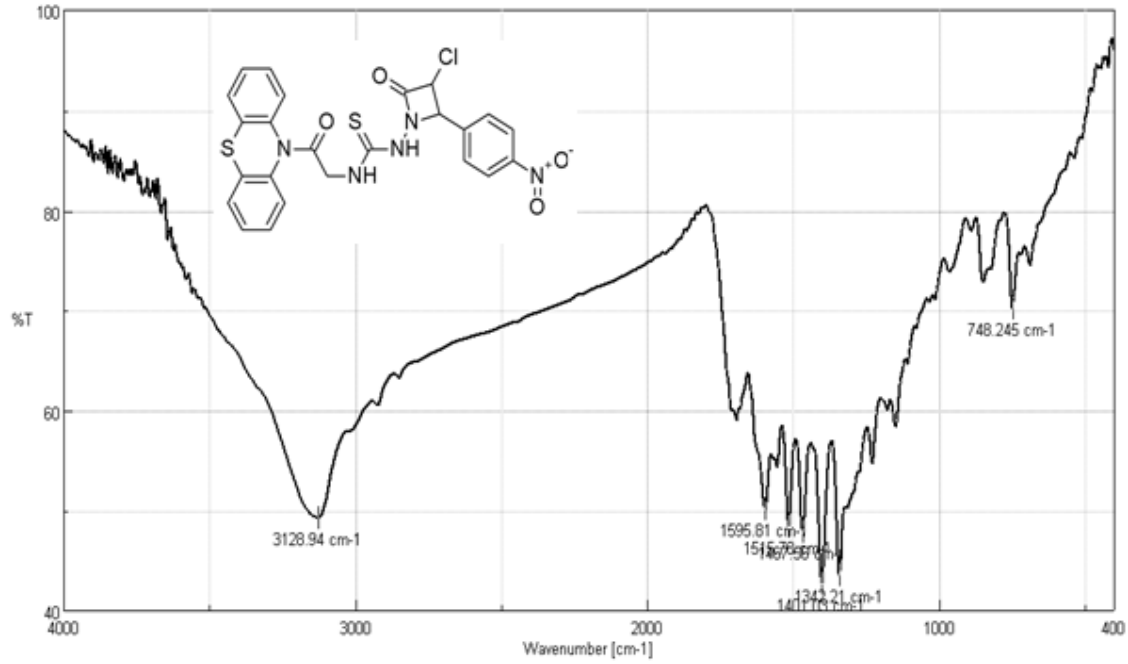


Fig 18. IR spectrum of compound **7f**

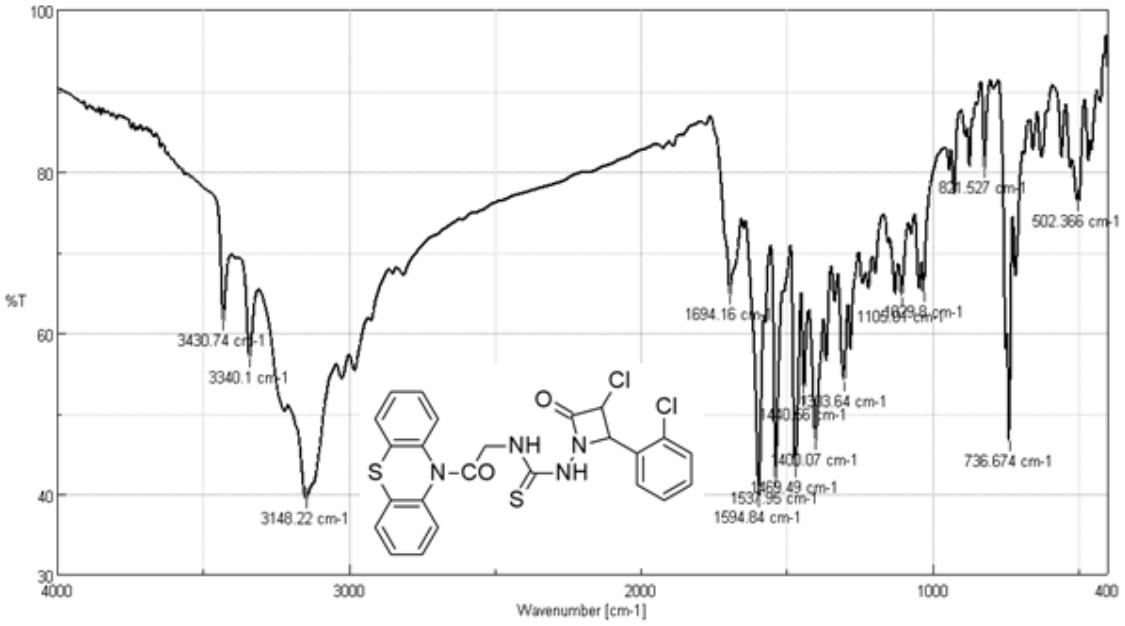


Fig 19. IR spectrum of compound **7g**

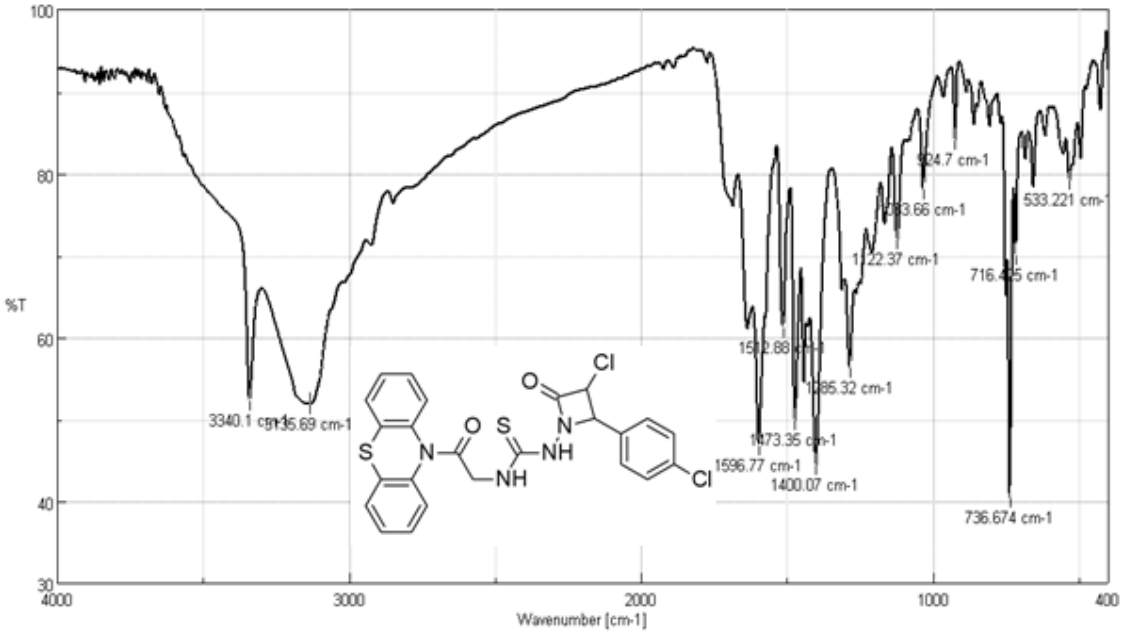


Fig 20. IR spectrum of compound **7h**

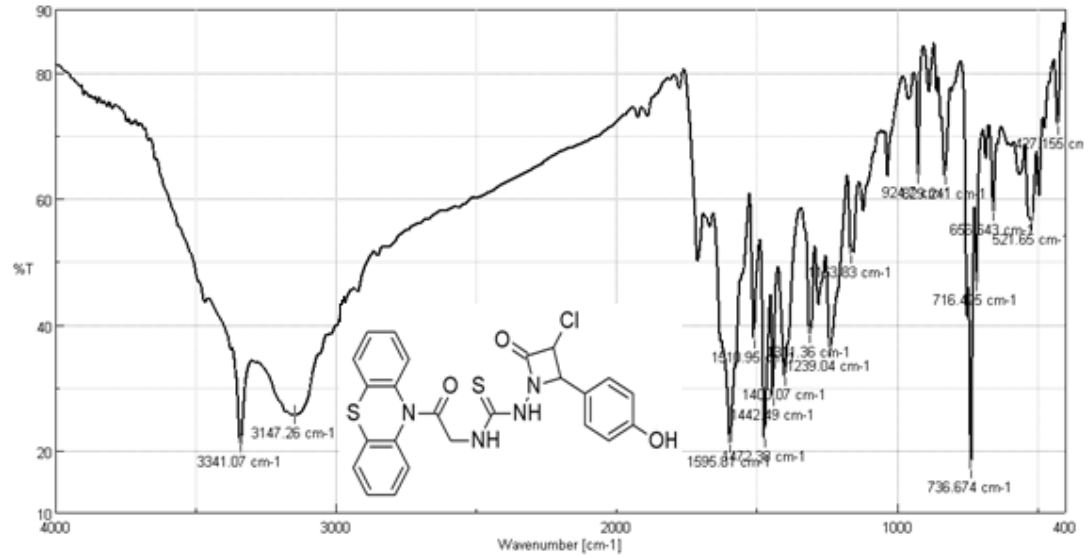


Fig 21. IR spectrum of compound **7i**

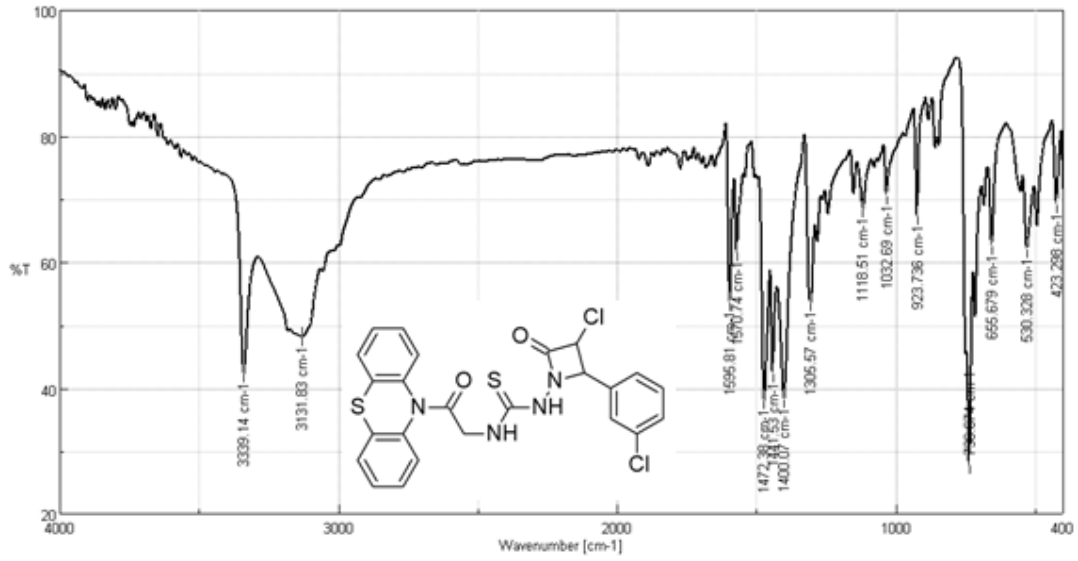


Fig 22. IR spectrum of compound **7j**

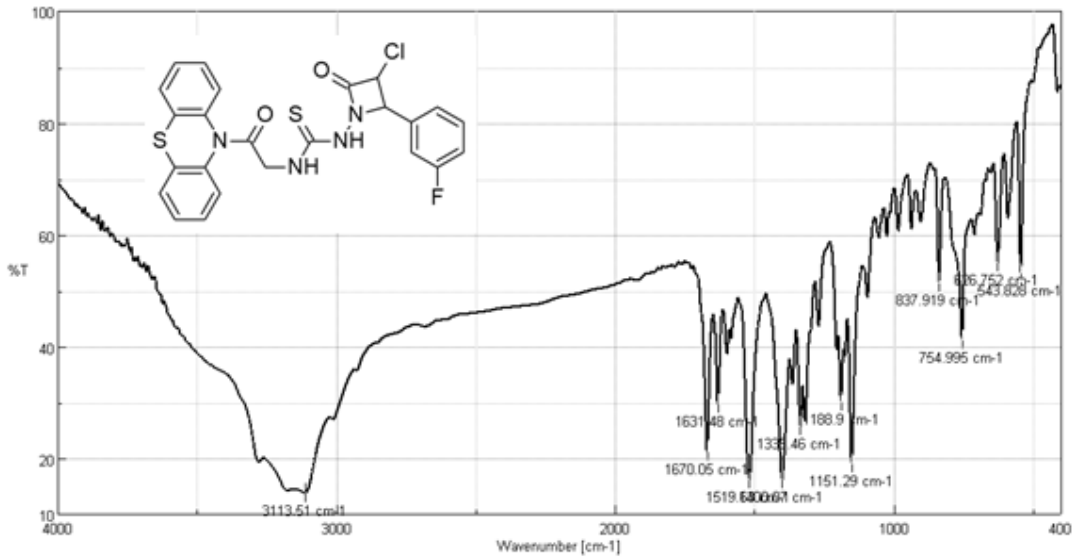
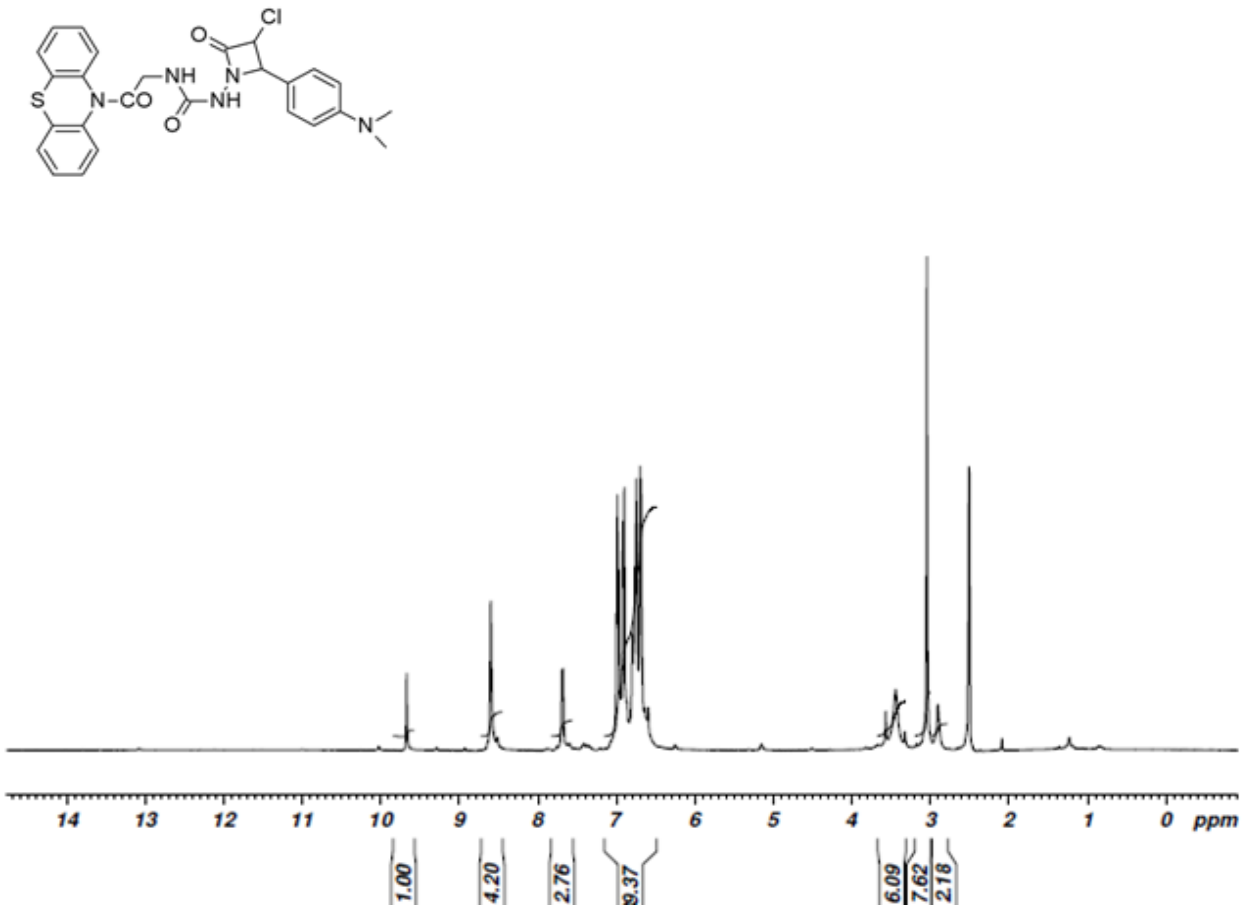


Fig 23. IR spectrum of compound **7k**

4D……..Sheeja Devi.

Fig 24. ^{1}H NMR spectrum of compound **4d**

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EXPNO                 22
PROCNO                 1

F2 - Acquisition Parameters
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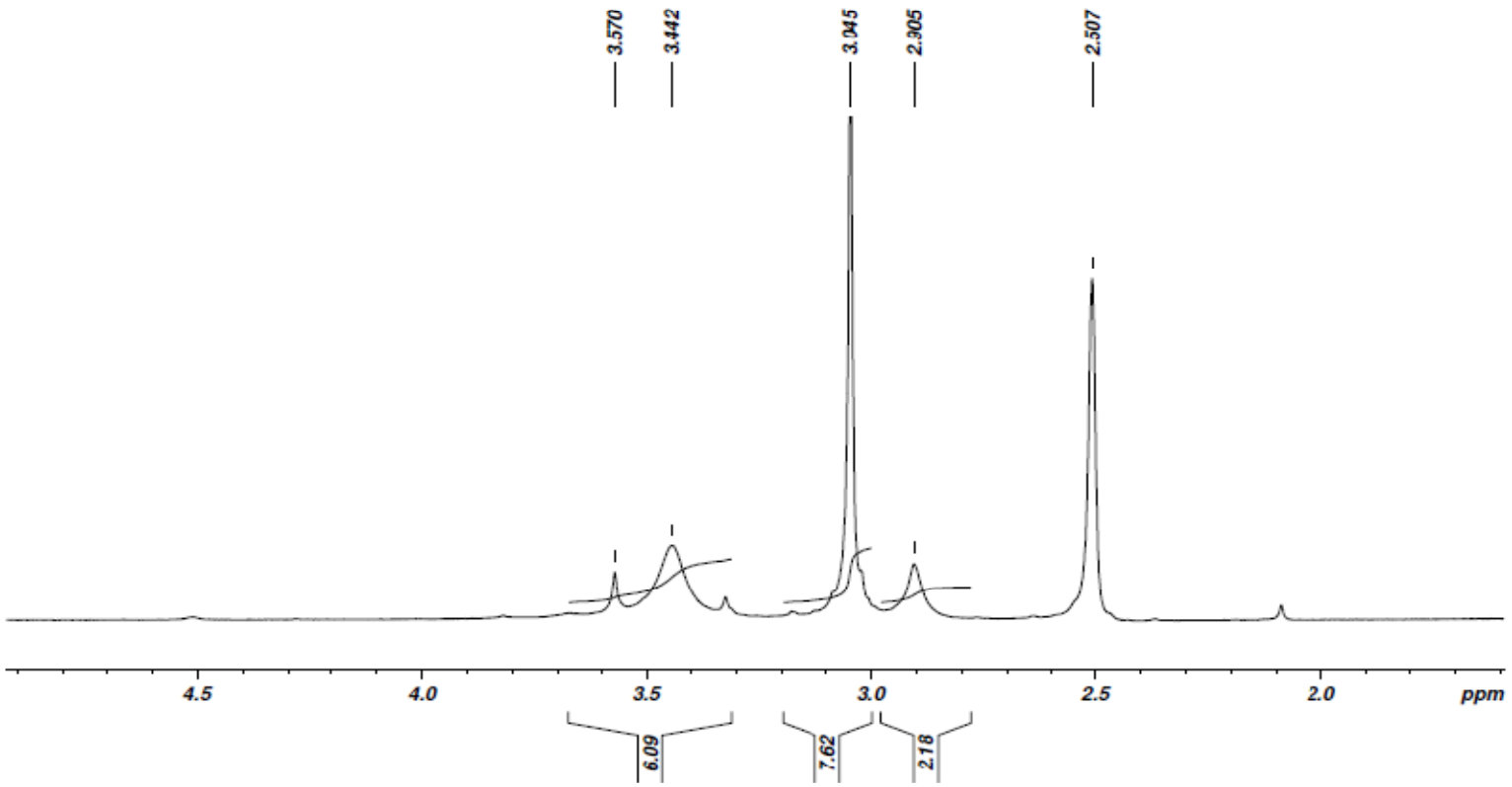
Time               13.58
INSTRUM             spect
PROBHD   5 mm PABBO BB-
PULPROG             zg30
TD                 32768
SOLVENT             DMSO
NS                    32
DS                     2
SWH            10330.578 Hz
FIDRES         0.315264 Hz
AQ             1.5860212 sec
RG                   203
DW                48.400 usec
DE                 6.50 usec
TE                295.7 K
D1             1.00000000 sec
TD0                    1

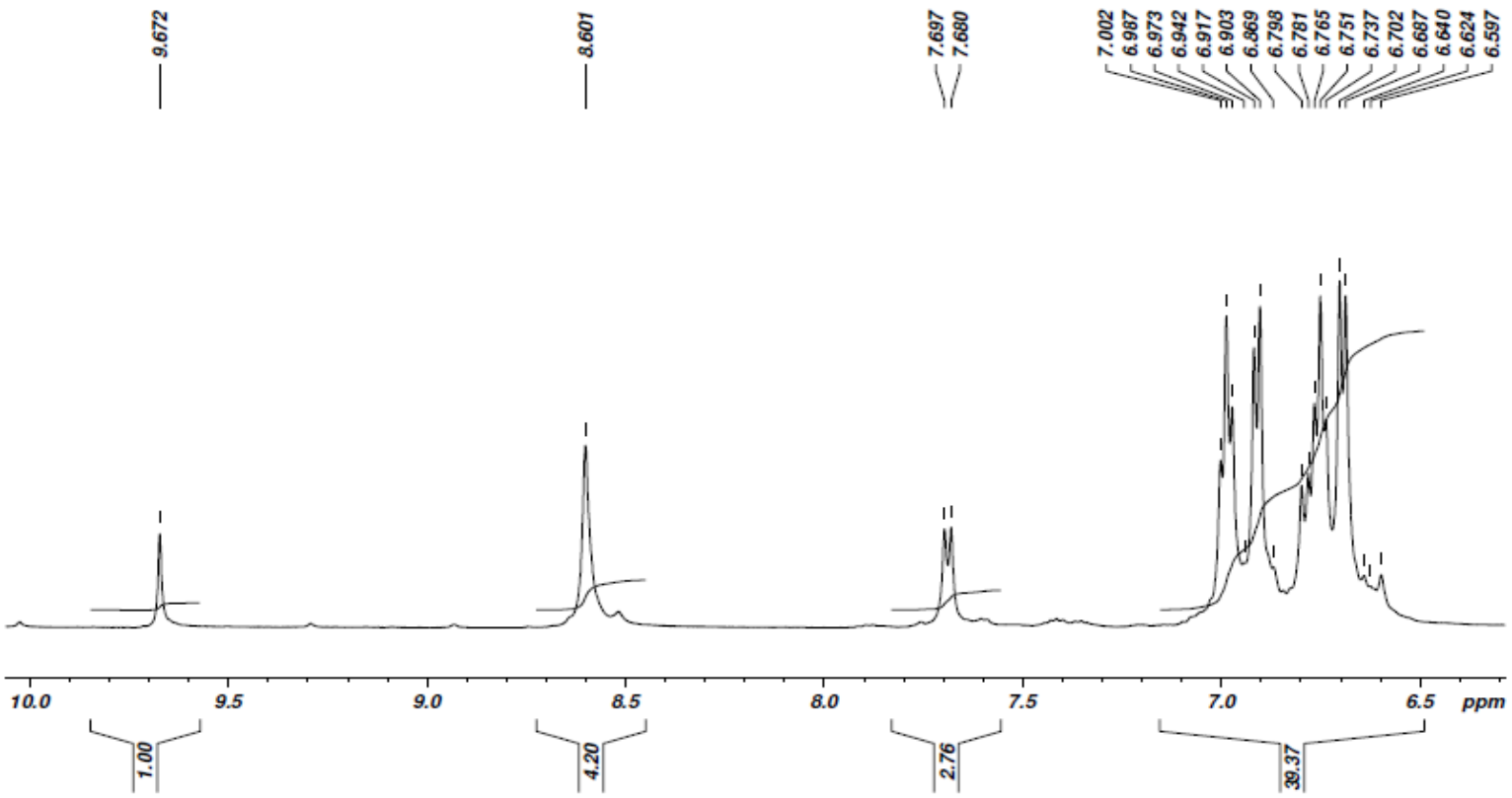
======== CHANNEL f1 ========
NUC1                  1H
P1                10.65 usec
PL1                0.00 dB
PL1W         23.53637505 W
SFO1         500.1330885 MHz

F2 - Processing parameters
SI                 32768
SF           500.1300000 MHz
WDW                   EM
SSB                    0
LB                 0.30 Hz
GB                     0
PC                  1.00
```

4D.....Sheeja Devi.

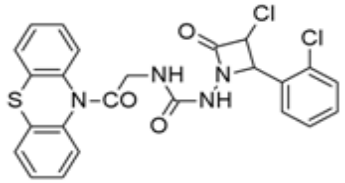


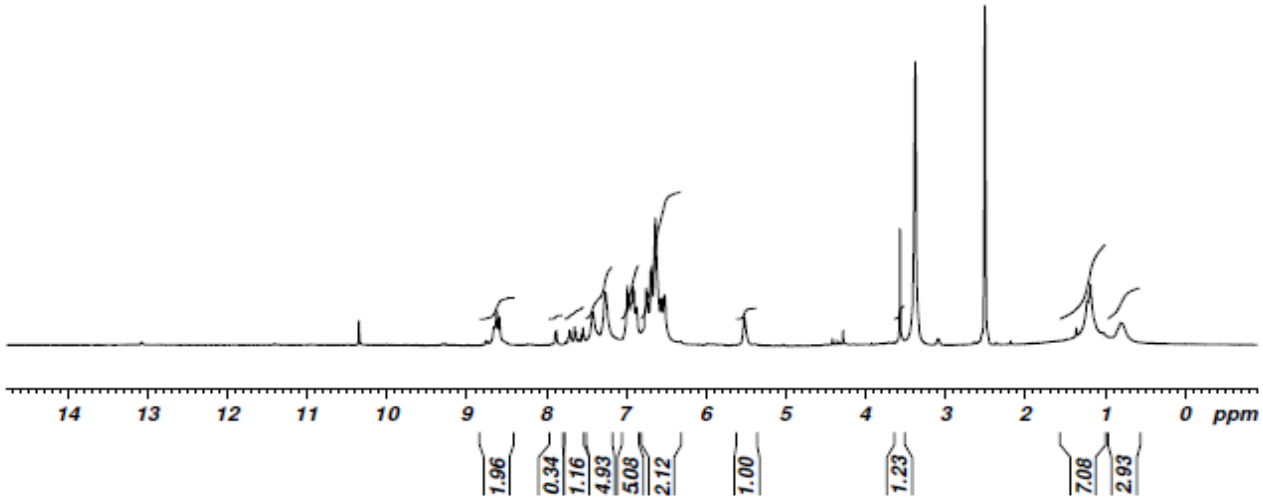
4D.....Sheeja Devi.



4G.....Sheeja Devi.

Fig 25. ^{1}H NMR spectrum of compound **4g**



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EXPNO                         21
PROCNO                         1

F2 - Acquisition Parameters
Date_                   20111212
Time                       13.53
INSTRUM                    spect
PROBHD    5 mm PABBO BB-
PULPROG                     zg30
TD                         32768
SOLVENT                     DMSO
NS                             32
DS                              2
SWH                    10330.578 Hz
FIDRES                 0.315264 Hz
AQ                     1.5860212 sec
RG                           203
DW                        48.400 usec
DE                         6.50 usec
TE                        295.7 K
D1                     1.00000000 sec
TD0                            1

====== CHANNEL f1 ======
NUC1                          1H
P1                        10.65 usec
PL1                        0.00 dB
PL1W                23.53637505 W
SFO1                500.1330885 MHz

F2 - Processing parameters
SI                         32768
SF                  500.1300000 MHz
WDW                           EM
SSB                            0
LB                         0.30 Hz
GB                             0
PC                         1.00
```
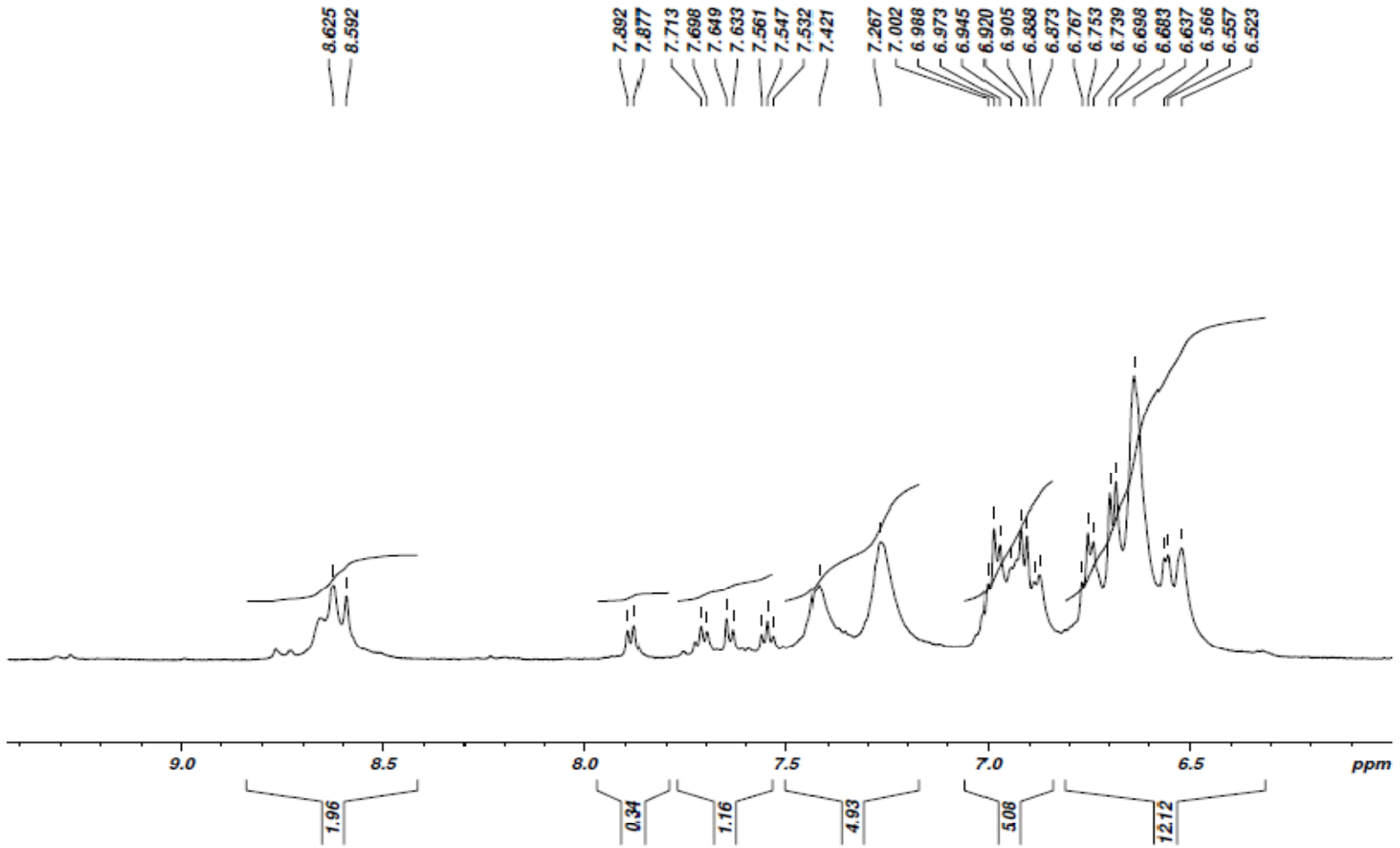
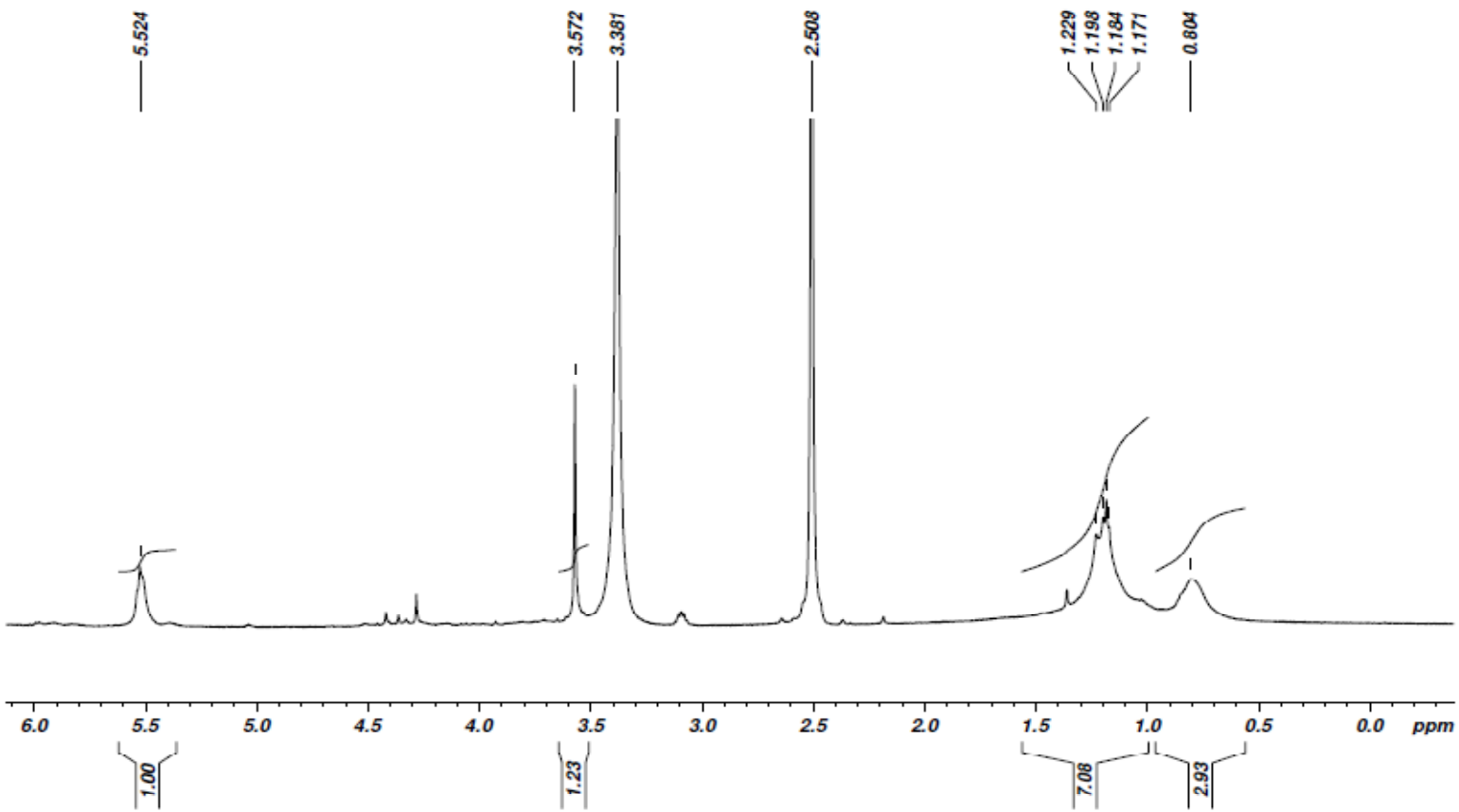

4G.....Sheeja Devi.

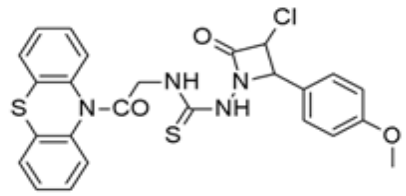


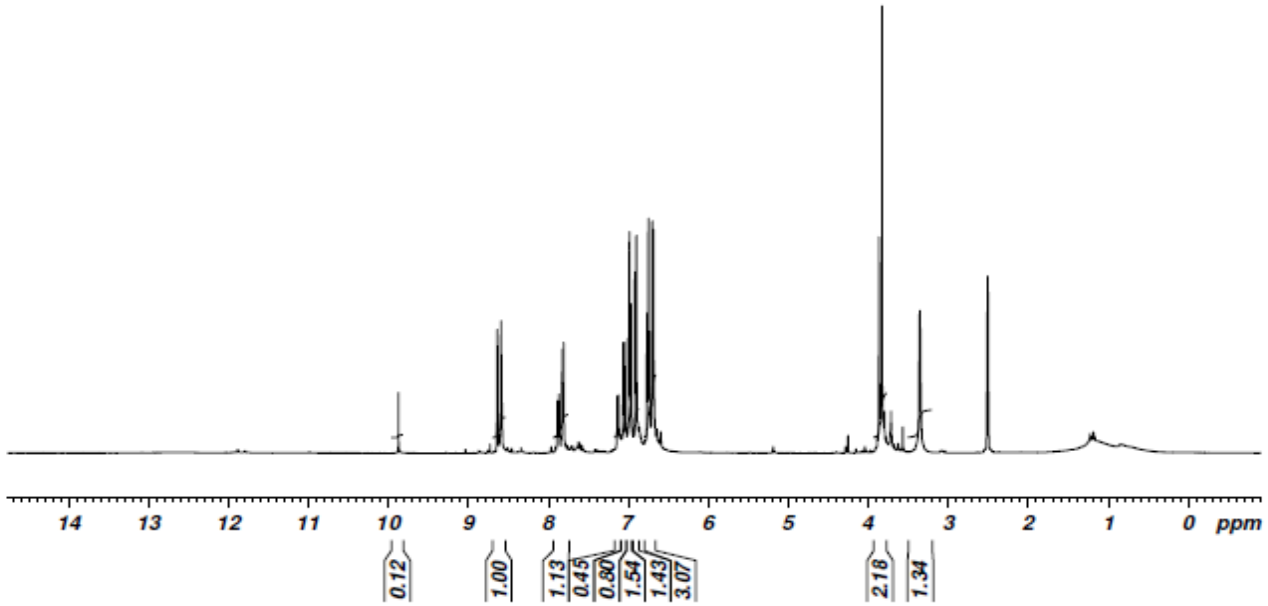
4G.....Sheeja Devi.



7C……Sheejadevi.

Fig 26. ^{1}H NMR spectrum of compound **7c**



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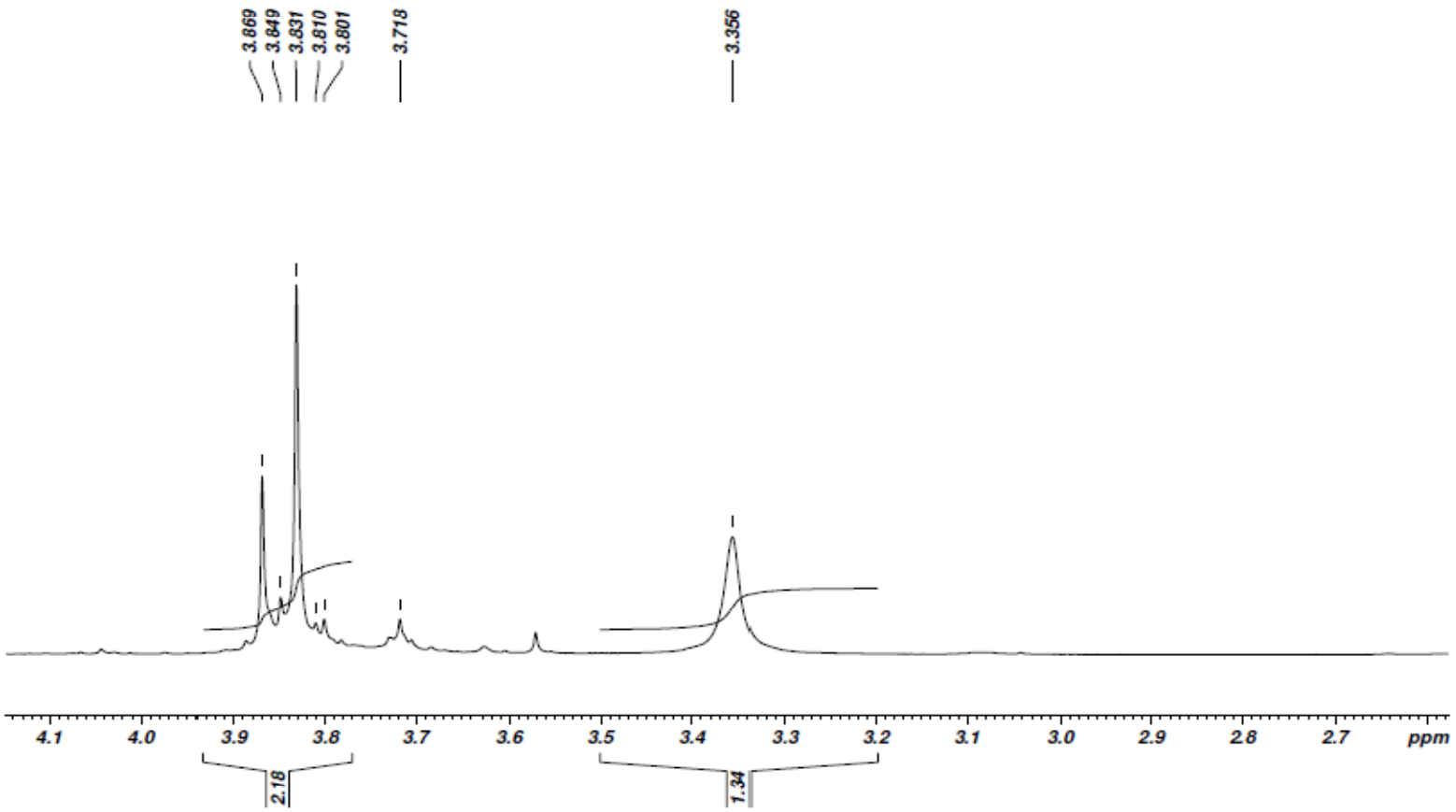
EXPNO                 29
PROCNO                 1

F2 - Acquisition Parameters
Date_           20111212
Time               15.51
INSTRUM            spect
PROBHD   5 mm PABBO BB-
PULPROG             zg30
TD                 32768
SOLVENT             DMSO
NS                    32
DS                     2
SWH            10330.578 Hz
FIDRES         0.315264 Hz
AQ             1.5860212 sec
RG                   161
DW                48.400 usec
DE                 6.50 usec
TE                296.7 K
D1             1.00000000 sec
TD0                    1

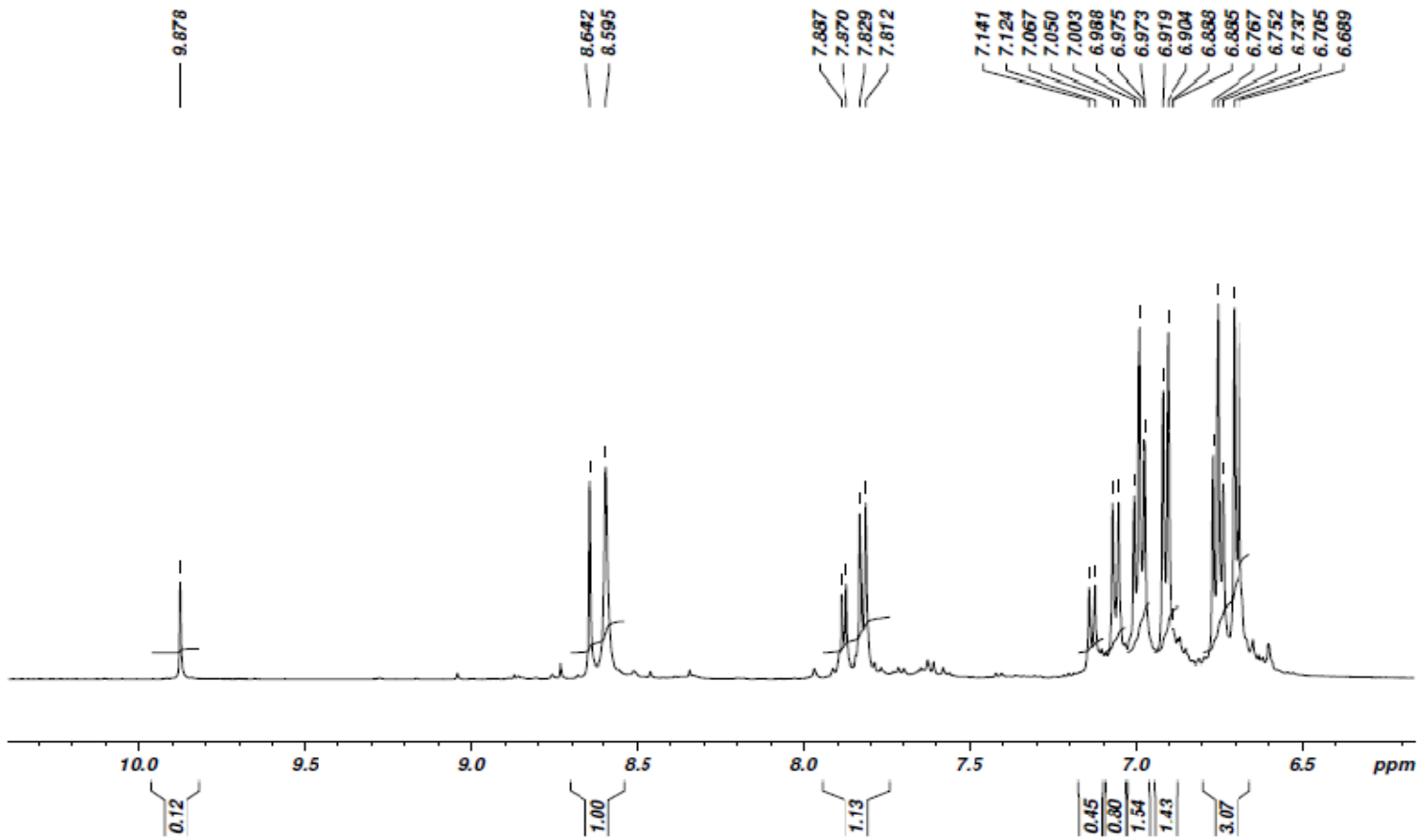
====== CHANNEL f1 ======
NUC1                  1H
P1                10.65 usec
PL1                0.00 dB
PL1W        23.53637505 W
SFO1       500.1330885 MHz

F2 - Processing parameters
SI                 32768
SF         500.1300000 MHz
WDW                   EM
SSB                    0
LB                 0.30 Hz
GB                     0
PC                  1.00
```

7C.....Sheejadevi.

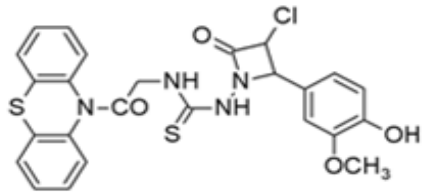


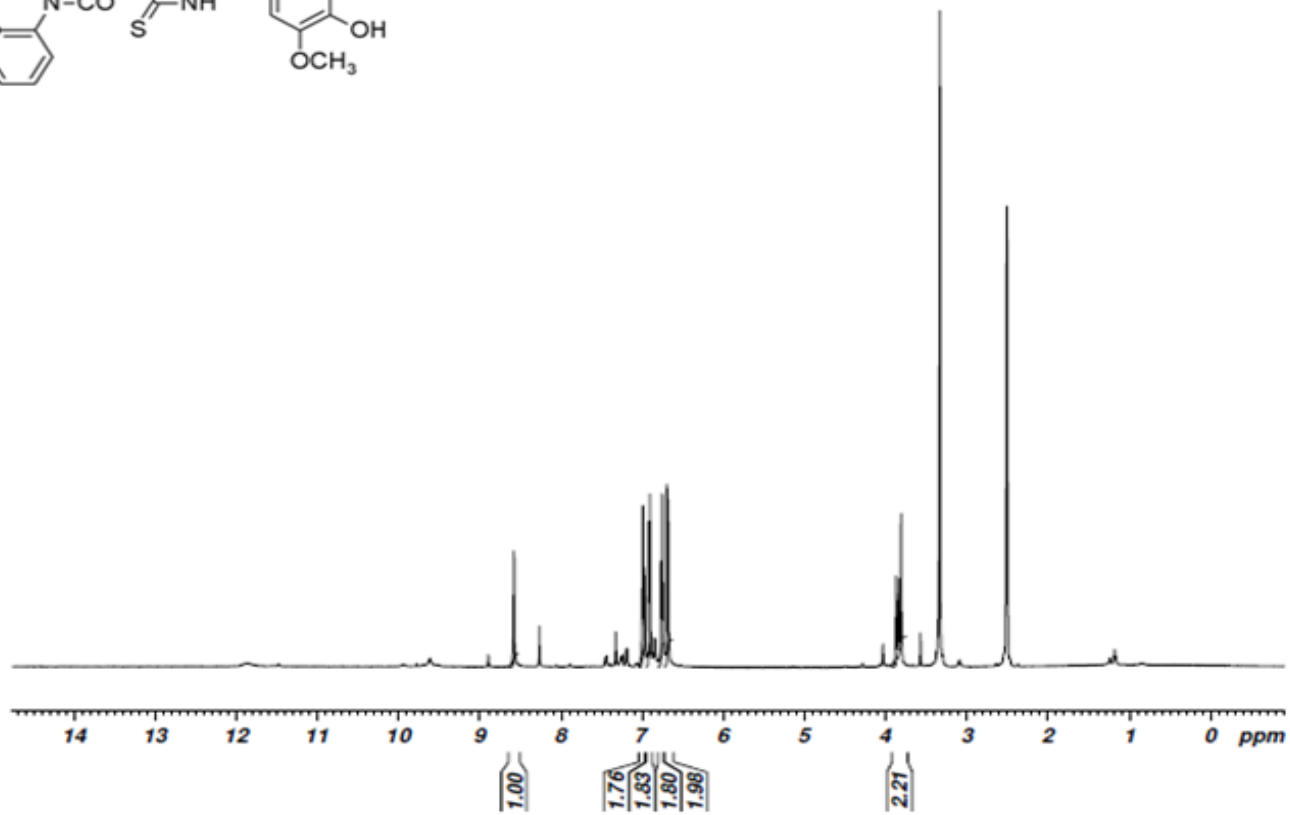
7C……Sheejadevi.



7E.....Sheejadevi.

Fig 27. ^{1}H NMR spectrum of compound **7e**



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Current Data Parameters
NAME          Dec12-2011
EXPNO                 27
PROCNO                 1

F2 - Acquisition Parameters
Date_           20111212
Time               15.01
INSTRUM             spect
PROBHD   5 mm PABBO BB-
PULPROG             zg30
TD                 32768
SOLVENT             DMSO
NS                    32
DS                     2
SWH           10330.578 Hz
FIDRES         0.315264 Hz
AQ            1.5860212 sec
RG                   203
DW               48.400 usec
DE                6.50 usec
TE               296.5 K
D1            1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                  1H
P1                10.65 usec
PL1                0.00 dB
PL1W        23.53637505 W
SFO1       500.1330885 MHz

F2 - Processing parameters
SI                 32768
SF          500.1300000 MHz
WDW                   EM
SSB                    0
LB                0.30 Hz
GB                     0
PC                  1.00
```
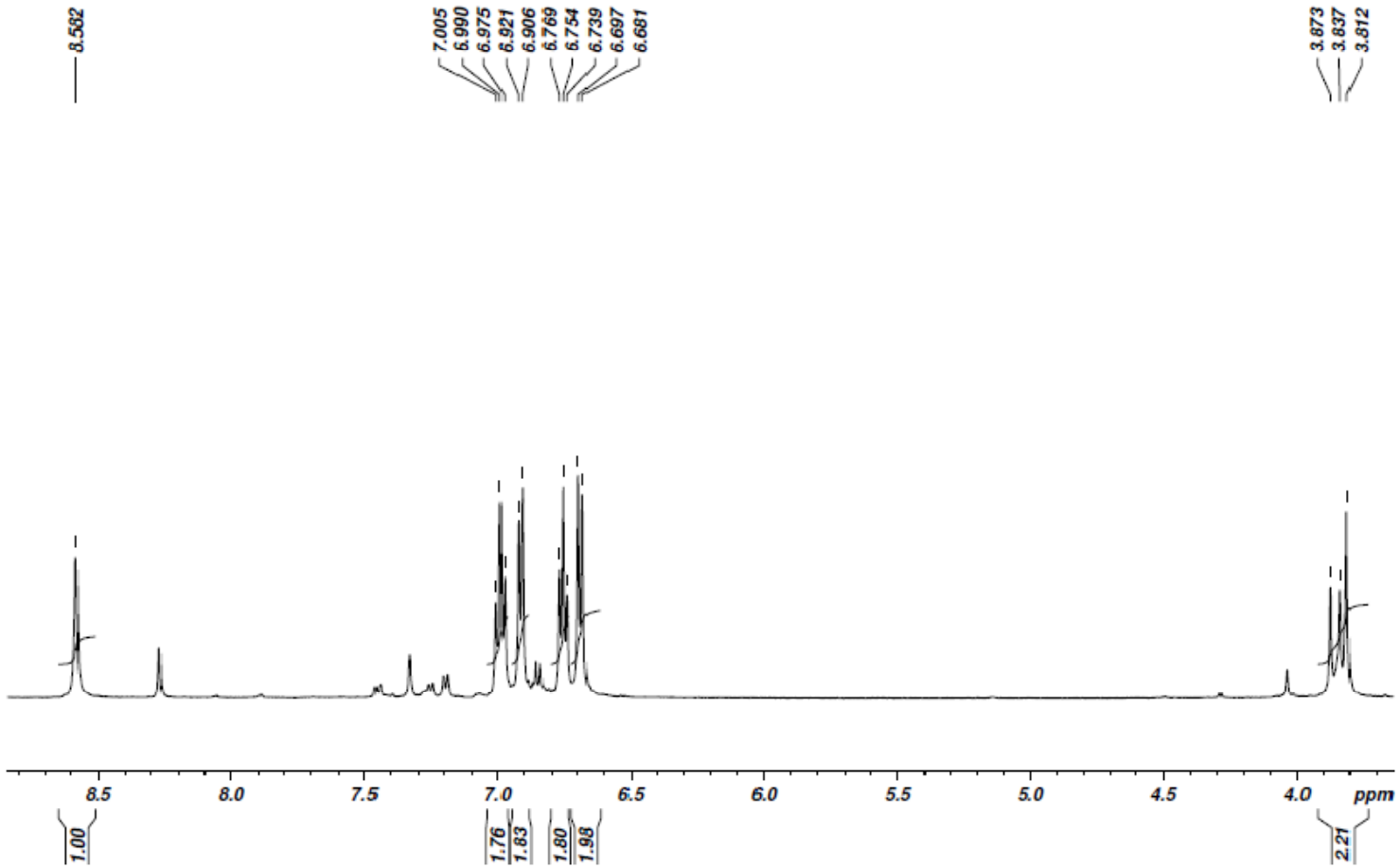
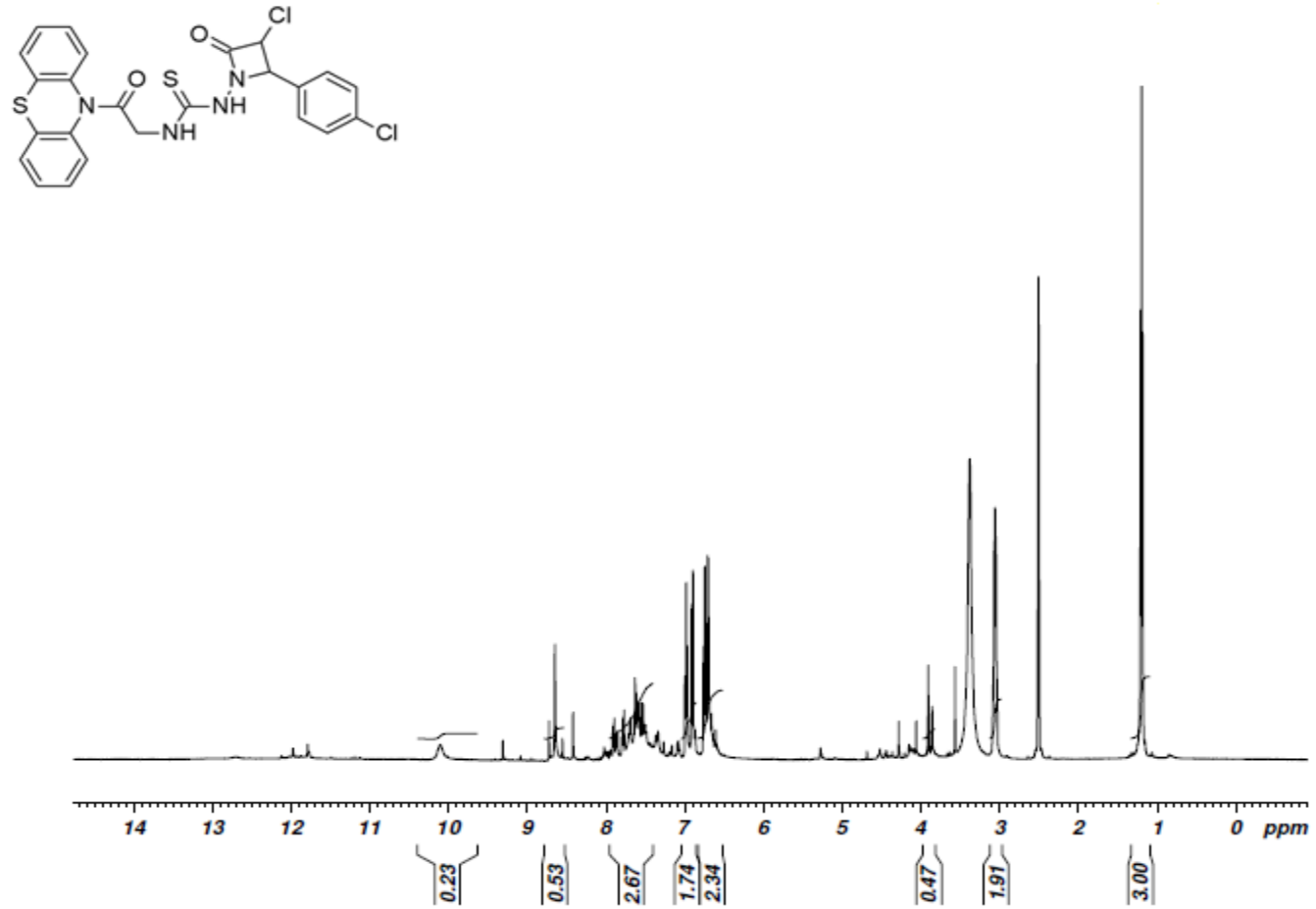

7E.....Sheejadevi.



7H.....Sheeja Devi.

Fig 28. ^{1}H NMR spectrum of compound **7h**

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Current Data Parameters
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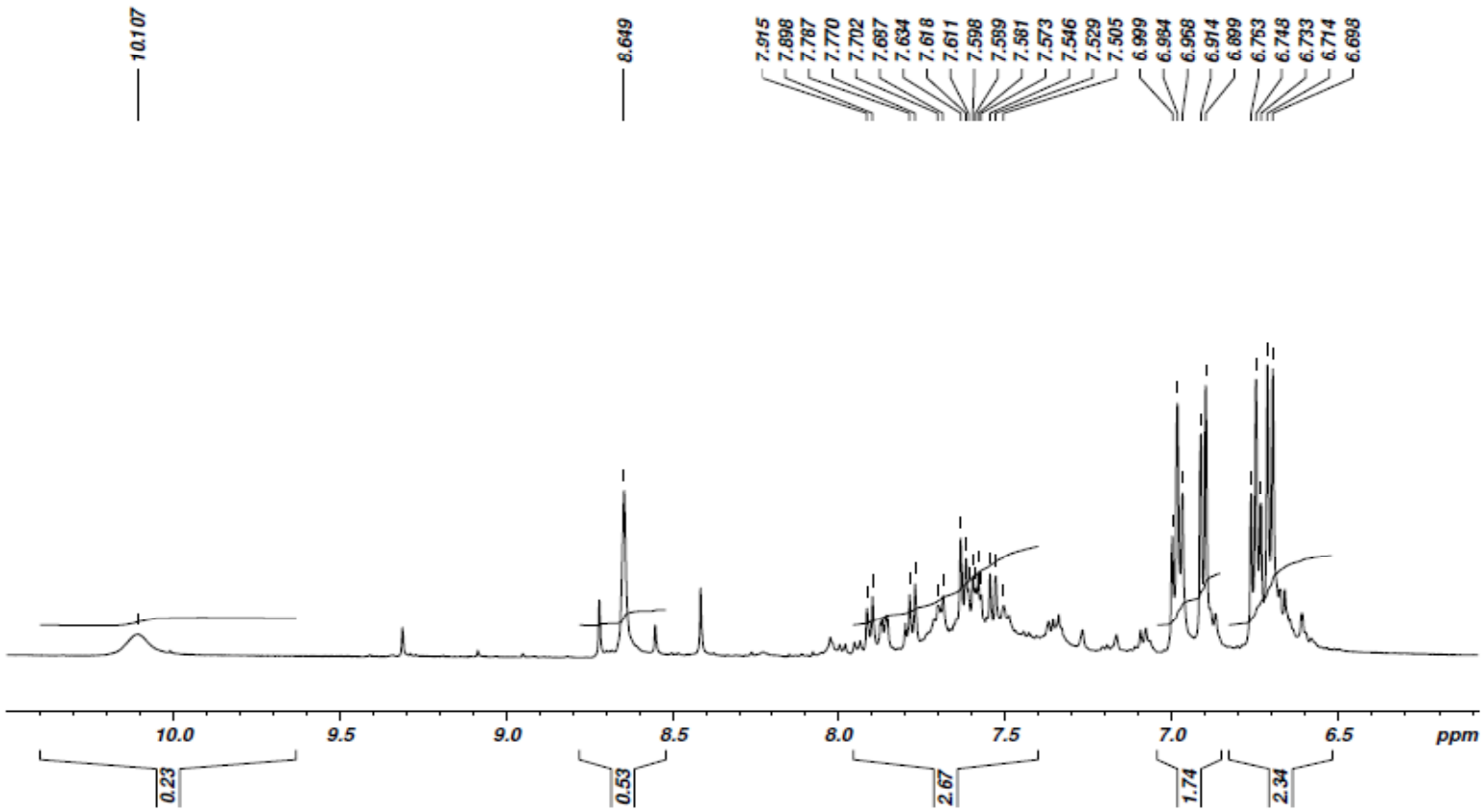
EXPNO                        19
PROCNO                        1

F2 - Acquisition Parameters
Date_                 20111212
Time                      13.44
INSTRUM                   spect
PROBHD     5 mm PABBO BB-
PULPROG                   zg30
TD                       32768
SOLVENT                   DMSO
NS                           32
DS                            2
SWH                  10330.578 Hz
FIDRES                0.315264 Hz
AQ                   1.5860212 sec
RG                          203
DW                     48.400 usec
DE                      6.50 usec
TE                      295.7 K
D1                   1.00000000 sec
TD0                           1

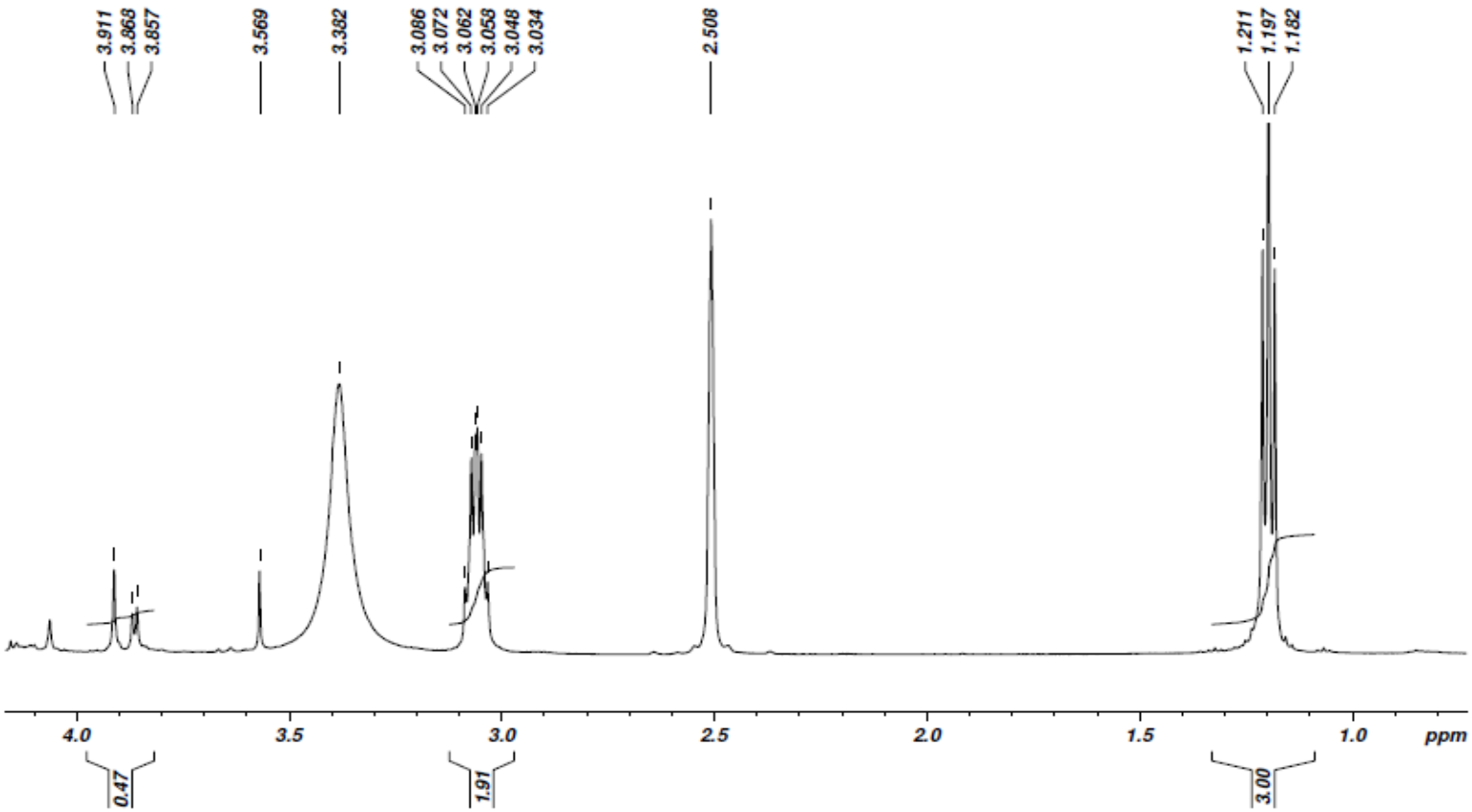
====== CHANNEL f1 ======
NUC1                         1H
P1                      10.65 usec
PL1                      0.00 dB
PL1W                23.53637505 W
SFO1                500.1330885 MHz

F2 - Processing parameters
SI                           32768
SF                  500.1300000 MHz
WDW                          EM
SSB                           0
LB                       0.30 Hz
GB                            0
PC                         1.00
```

7H.....Sheeja Devi.

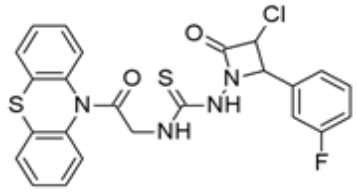


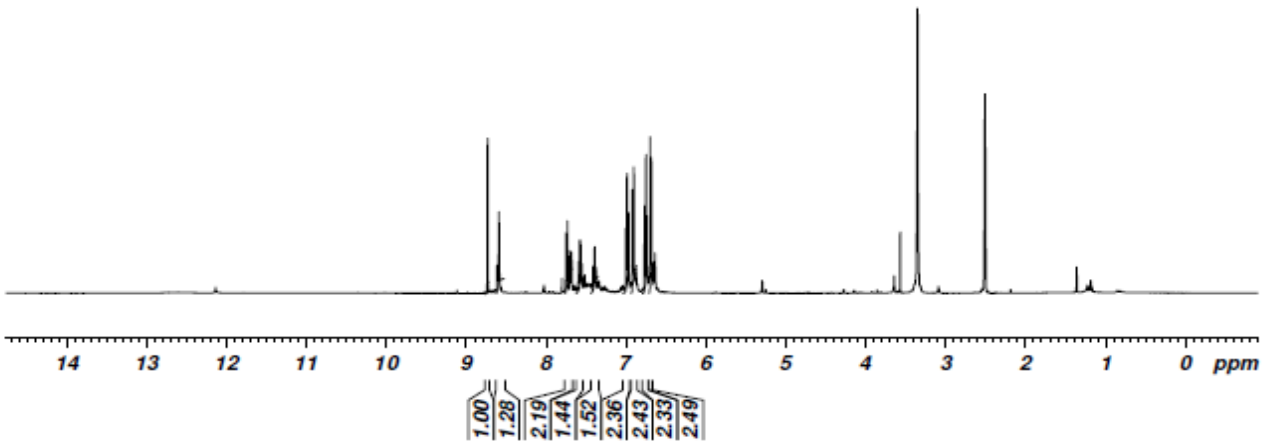
7H.....Sheeja Devi.



7K.....Sheeja Devi.

Fig 29. ^{1}H NMR spectrum of compound **7k**



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EXPNO                 20
PROCNO                 1

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Time               13.49
INSTRUM             spect
PROBHD   5 mm PABBO BB-
PULPROG             zg30
TD                 32768
SOLVENT             DMSO
NS                    32
DS                     2
SWH            10330.578 Hz
FIDRES          0.315264 Hz
AQ             1.5860212 sec
RG                   203
DW                48.400 usec
DE                 6.50 usec
TE                295.7 K
D1             1.00000000 sec
TD0                    1

====== CHANNEL f1 ======
NUC1                  1H
P1                 10.65 usec
PL1                 0.00 dB
PL1W        23.53637505 W
SFO1        500.1330885 MHz

F2 - Processing parameters
SI                 32768
SF          500.1300000 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00
```
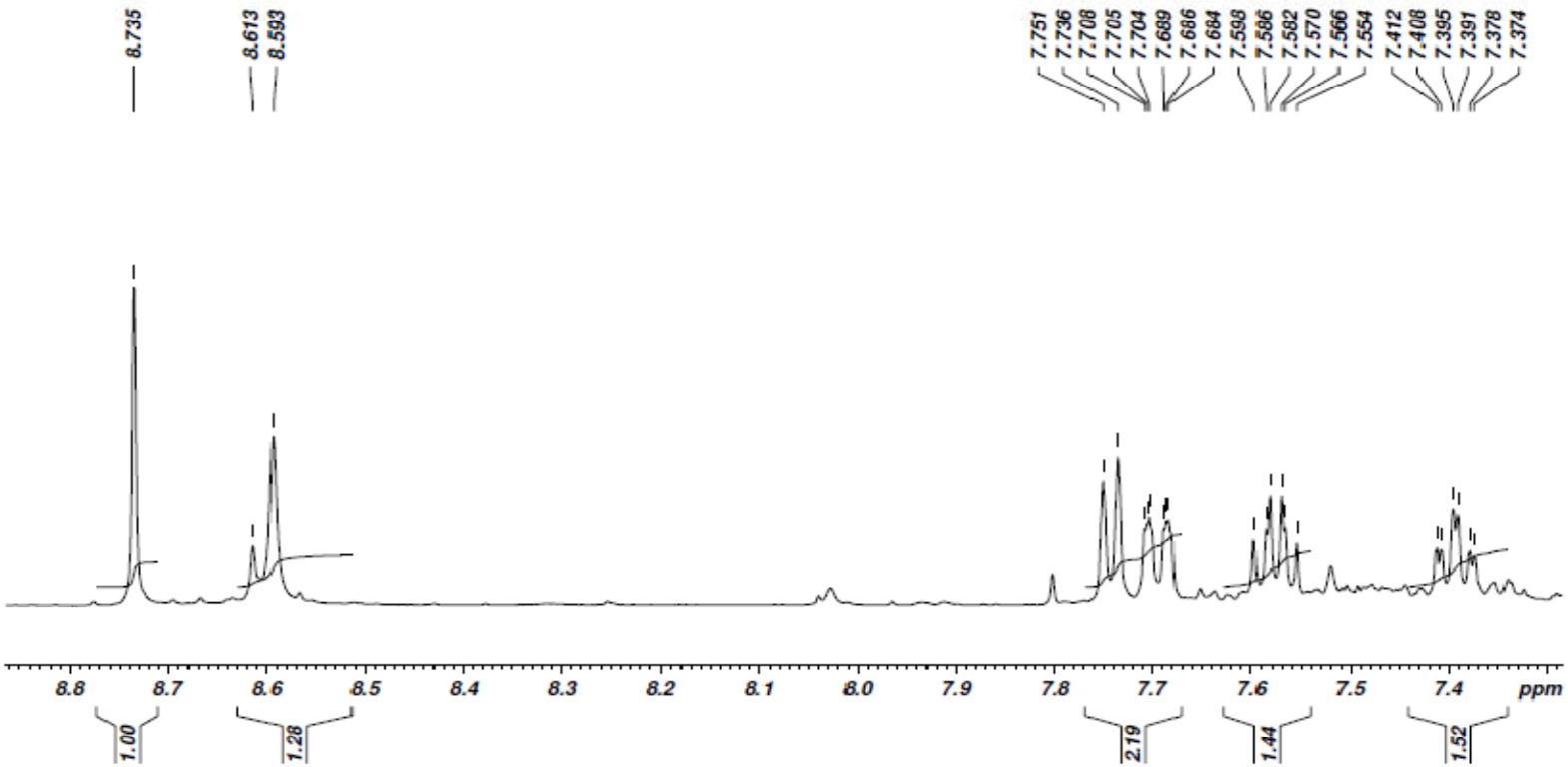
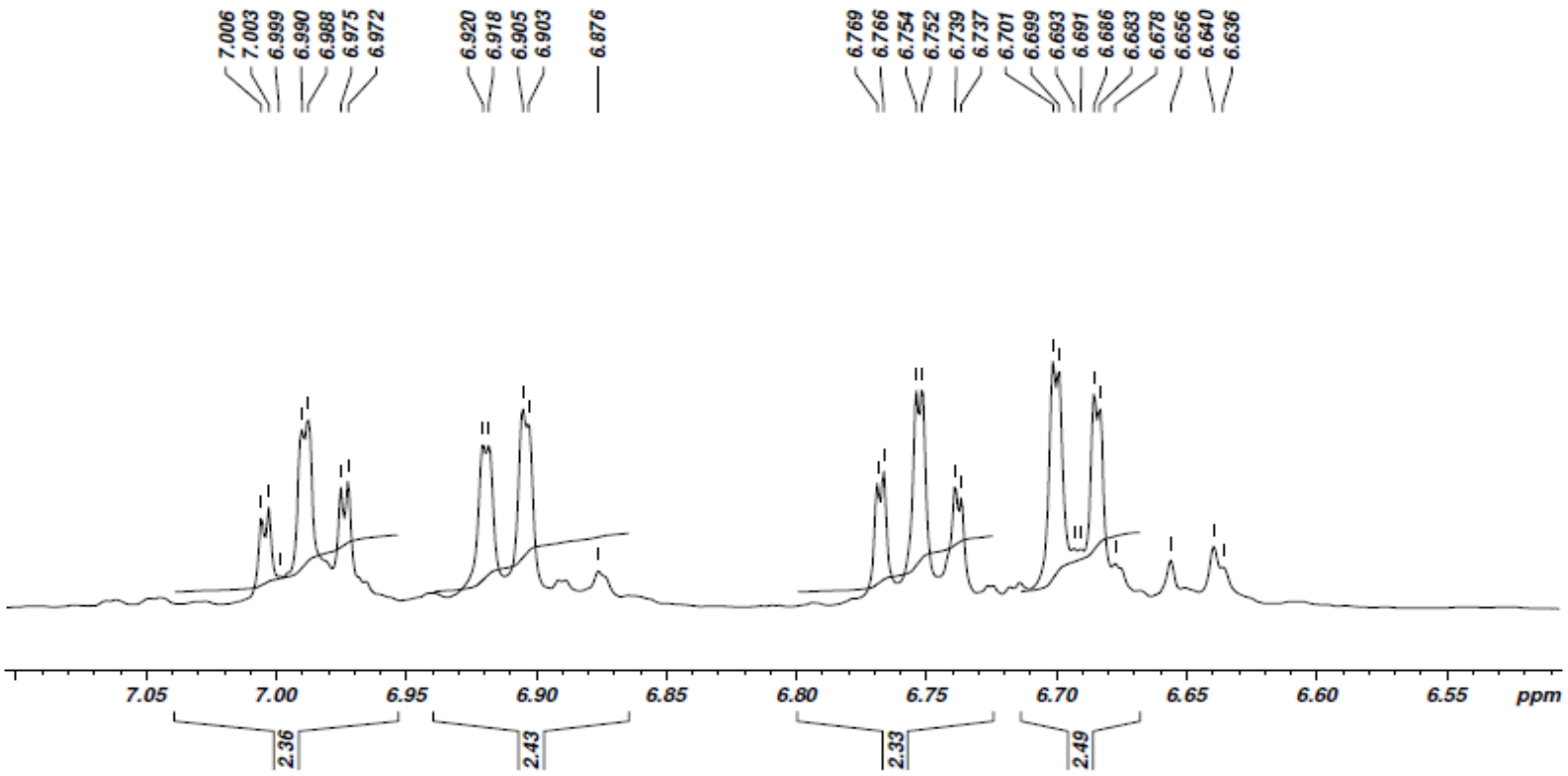

7K.....Sheeja Devi.



7K.....Sheeja Devi.



File: SHEEJA DEVI **4e** Date Run: 09-30-2011 (Time Run: 10:23:10)
Sample:
Instrument: JEOL GCmate
Inlet: Direct Probe Ionization mode: EI+

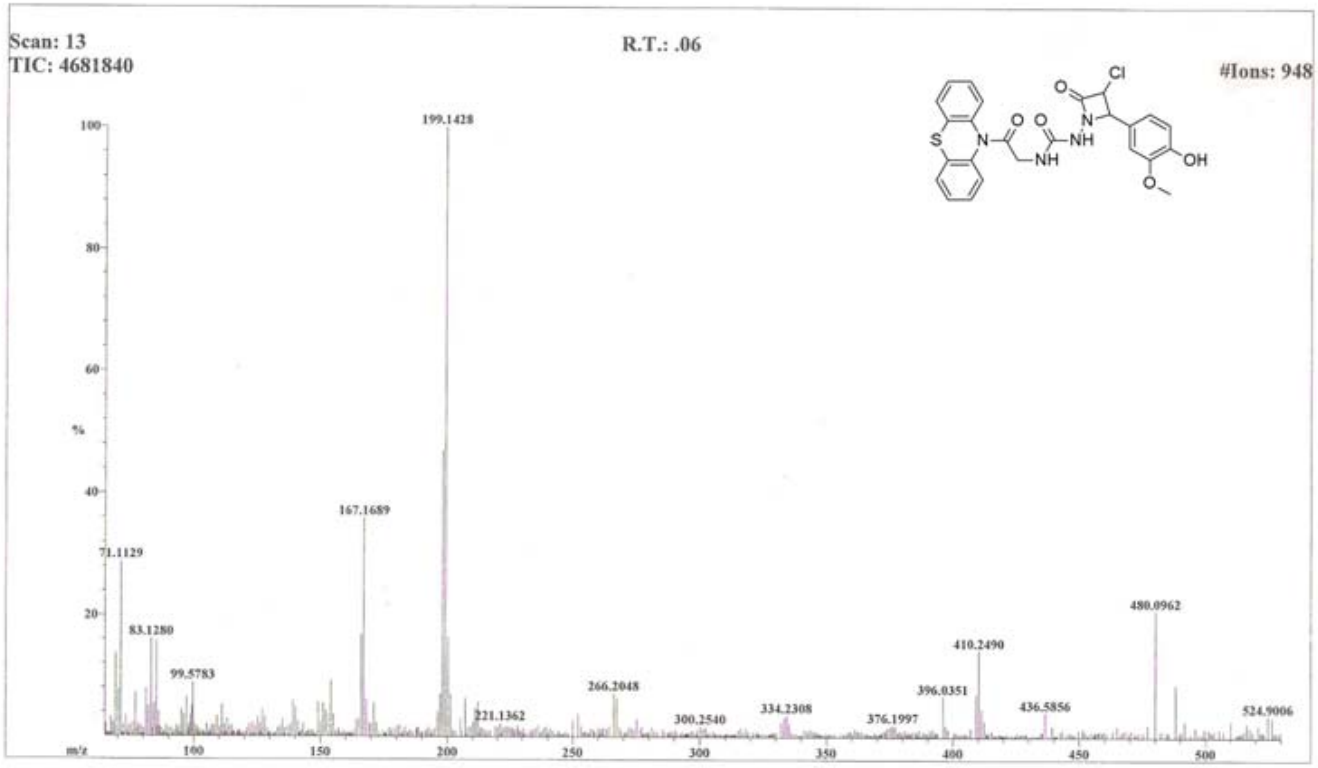


Fig.30 Mass spectrum of compound 4e

File: SHEEJA DEVI **7f**
Sample:
Instrument: JEOL GCmate
Inlet: Direct Probe

Date Run: 09-30-2011 (Time Run: 10:29:16)

Ionization mode: EI+

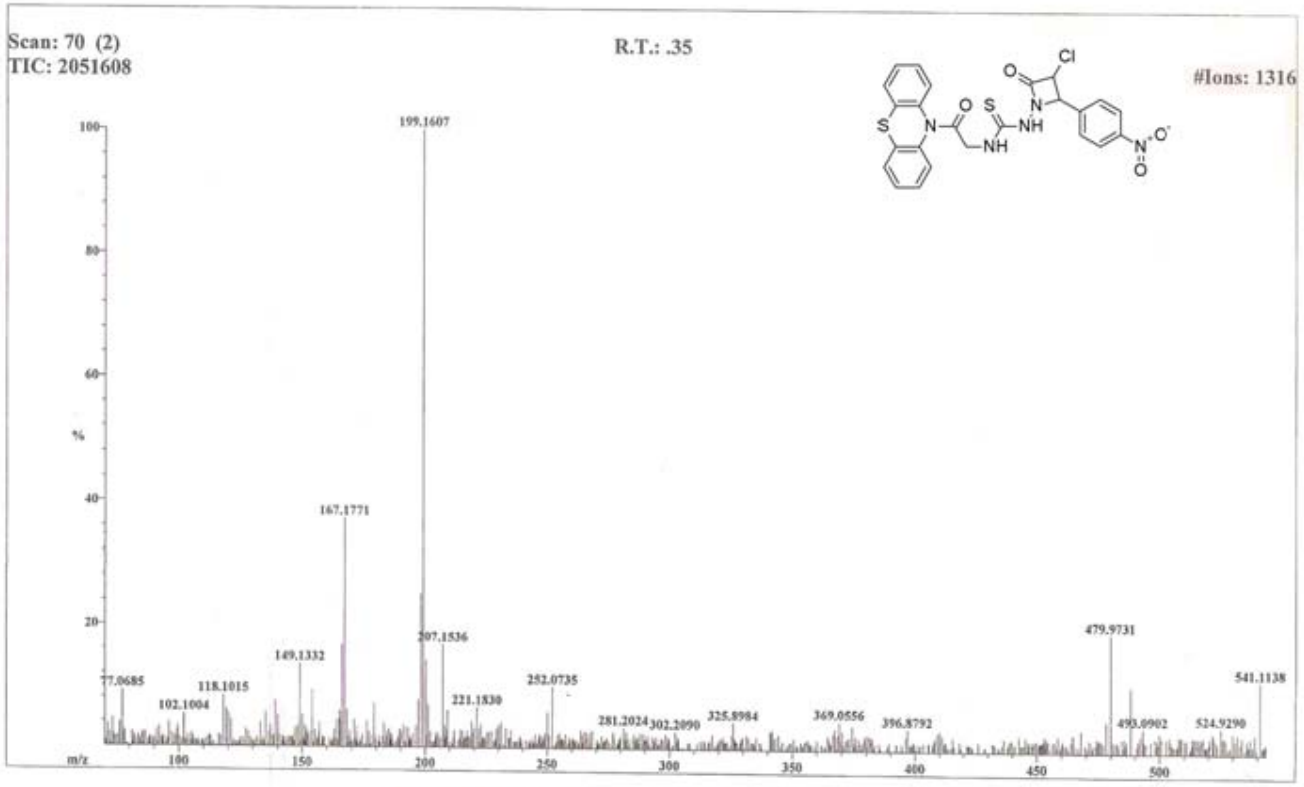


Fig.31 Mass apectrum of compound 7f

ANTIBACTERIAL STUDIES

Table 7. Antibacterial screening of titled compounds by disc diffusion method

Code	ZONE OF INHIBITION in mm							
	E.coli	***P.auroginosa***	***S.paratyphyi***	***V.chlorea***	***M.luteus***	***S.aureus***	***B.subtilis***	***S.albus***
4a	7	5	5	7	4	-	6	7
4b	5	7	7	6	5	3	5	8
4c	7	8	8	9	7	5	7	10
4d	12	7	6	8	5	8	8	8
4e	10	10	10	10	8	7	6	5
4f	-	15	7	5	3	4	5	4
4g	7	8	12	6	7	4	7	5
4h	5	19	5	18	5	4	7	5
4i	18	5	12	9	12	9	10	13
4j	10	5	10	8	5	7	11	11
4k	11	6	10	9	7	7	11	11
7a	12	8	11	10	14	10	12	10
7b	7	5	14	9	6	10	8	7
7c	9	7	6	12	4	14	15	8
7d	5	6	7	12	6	12	6	8
7e	8	9	5	8	6	7	12	9
7f	7	12	7	5	10	20	13	6
7g	12	10	10	8	10	10	12	10
7h	14	11	9	7	11	7	12	15
7i	7	12	11	7	8	7	11	7
7j	18	12	13	10	8	12	10	11
7k	19	15	11	9	7	11	11	13
Ciprofloxacin	22	20	18	15	16	20	25	23

4a-k, 7a-k = synthesized compounds in the concentration of 10 µg/disc, Ciprofloxacin in the concentration of 5 µg/disc

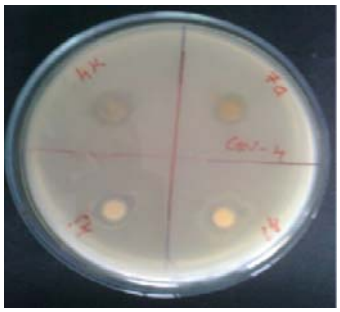


Fig 32 . Antibacterial evaluation of compounds **4i-k,7a** against *E.coli*

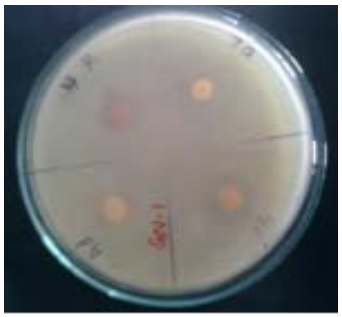


Fig 33 . Antibacterial evaluation of compounds **7b-e** against *P.auroginosa*

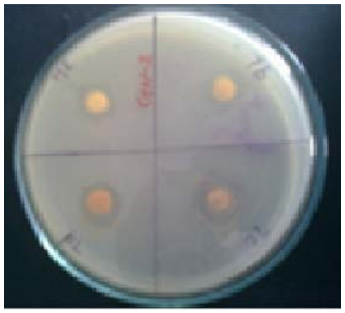


Fig 34 . Antibacterial evaluation of compounds **7e-h** against *S.paratyphyi*

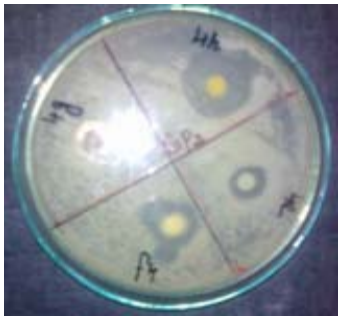


Fig 35. Antibacterial evaluation of compounds **4i-k, 7a** against *V.chlorea*

Fig 36 . Antibacterial evaluation of compounds **4i-k, 7a** against *M.luteus*

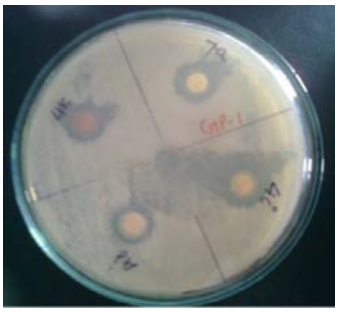


Fig 37 . Antibacterial evaluation of compounds **7i-j** against *M.luteus*

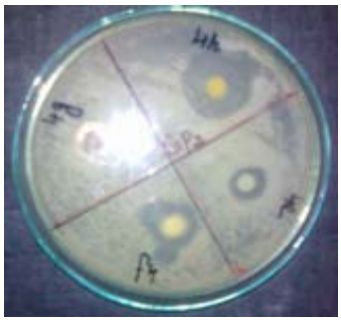


Fig 38. Antibacterial evaluation of compounds **4e-h** against *S.aureus*

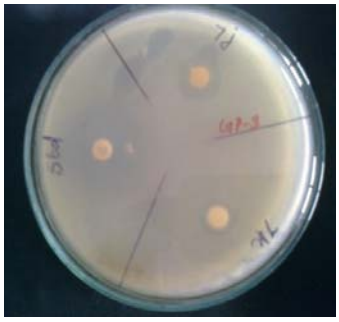


Fig 39 . Antibacterial evaluation of compounds **7i-j** against *B.subtilis*

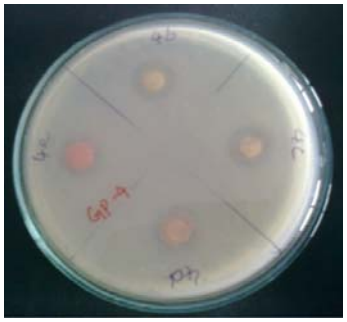


Fig 40. Antibacterial evaluation of compounds **4a-d** against *S.albus*

ANTIFUNGAL SCREENING

Table 8. Antifungal screening of titled compounds by disc diffusion method

Code	ZONE OF INHIBITION in mm			
	C.albicans	***M.purpurea***	***A.niger***	***T.rubrum***
4a	6	3	4	4
4b	8	6	6	8
4c	7	6	10	6
4d	7	7	8	7
4e	10	7	5	4
4f	14	13	9	5
4g	12	10	8	10
4h	7	12	12	6
4i	13	10	8	8
4j	12	7	7	6
4k	11	10	7	7
7a	12	10	7	4
7b	4	5	4	4
7c	8	8	8	4
7d	8	12	4	16
7e	9	10	8	8
7f	5	5	6	6
7g	8	10	8	6
7h	6	13	14	7
7i	5	10	6	6
7j	10	8	10	5
7k	13	6	4	6
std	-	20	4	22

4a-k, 7a-k= synthesized compounds in the concentration of 10 µg/disc

std = Clotrimazole in the concentration of 10 µg/disc

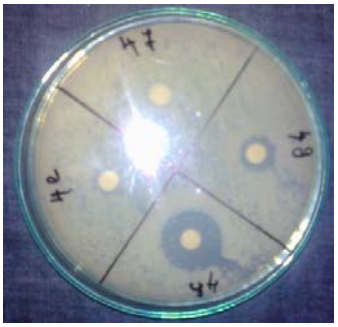


Fig 41 . Antifungal evaluationof compounds **4e-h** against *C.albicans*

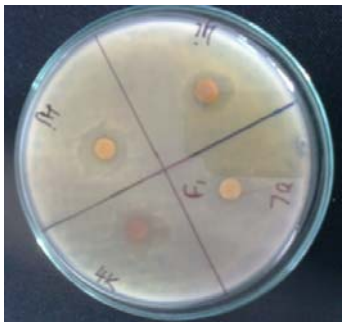


Fig 42 . Antifungal evaluation of compounds **4i-k,7a** against *C.albicans*

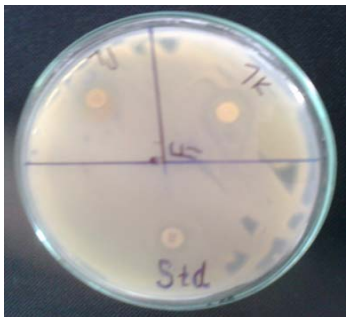


Fig 43. Antifungal evaluationof compounds **7j-k** against *C.albicans*

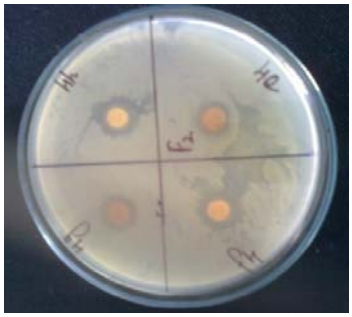


Fig 44. Antifungal evaluation of compounds **4e-h** against *M.purpurea*

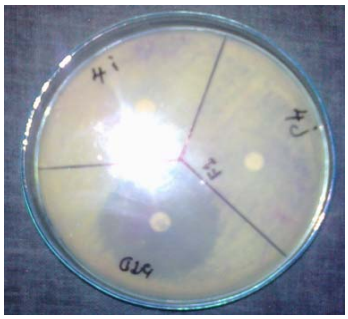


Fig 45. Antifungal evaluation of compounds **4i-j** against *M.purpurea*

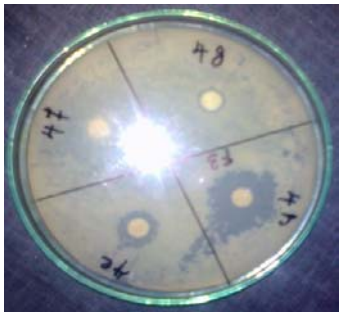


Fig 46. Antifungal evaluation of compounds **4e-h** against *A.niger*

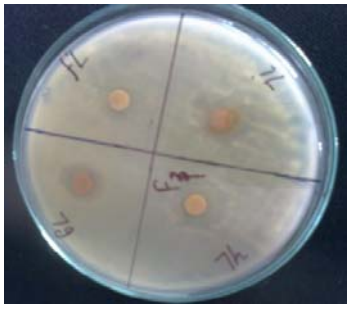


Fig 47. Antifungal Evaluation of compounds **7f-i** against *A.niger*

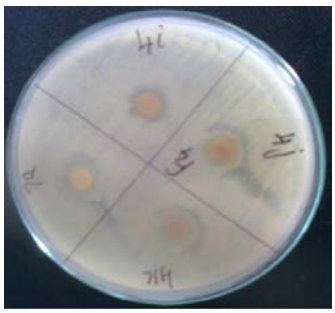


Fig 48. Antifungal Evaluation of compounds **4i-k,7a** against *T.rubrum*

Fig 49. Antifungal Evaluation of compounds **7b-e** against *T.rubrum*

MIC STUDIES

Table 9 . Minimum inhibitory concentration of synthesized compounds against bacteria by broth dilution method

Code	MIC (µg/ml)							
	E.coli	***P.auroginosa***	***S.paratyphyi***	***V.chlorea***	***M.luteus***	***S.aureus***	***B.substilis***	***S.albus***
4a	250	500	500	250	250	250	250	250
4b	125	250	125	125	250	500	250	125
4c	125	125	125	125	250	250	250	62.5
4d	62.5	250	250	125	250	125	125	125
4e	125	125	125	125	250	250	250	250
4f	500	62.5	125	250	250	250	250	250
4g	250	250	125	250	250	500	250	250
4h	250	62.5	250	62.5	250	62.5	125	250
4i	62.5	250	62.5	125	62.5	125	125	125
4j	125	500	125	250	250	250	125	125
4k	125	250	125	125	250	250	62.5	125
7a	62.5	250	250	250	250	250	250	250
7b	250	500	62.5	250	250	125	125	125
7c	125	250	250	125	500	62.5	62.5	125
7d	250	250	250	125	250	125	250	250
7e	125	125	500	125	250	250	125	125
7f	250	125	250	250	125	62.5	125	250
7g	125	125	125	125	125	125	125	125
7h	62.5	125	125	125	125	125	125	62.5
7i	250	125	250	250	250	250	125	250
7j	62.5	125	125	250	250	250	125	125
7k	62.5	62.5	125	250	250	125	125	125
Std	62.5	62.5	125	125	125	62.5	62.5	62.5

Std- Ciprofloxacin

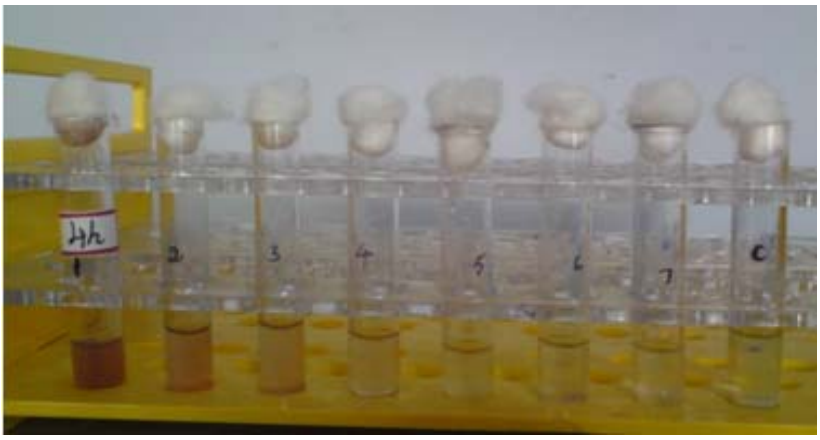


Fig 50. Antibacterial actvity of compound **4h** showed MIC at 62.5µg/ml against *E.coli*

Fig 51. Antibacterial activity of compound **7f** showed MIC at 62.5µg/ml against *B.substilis*

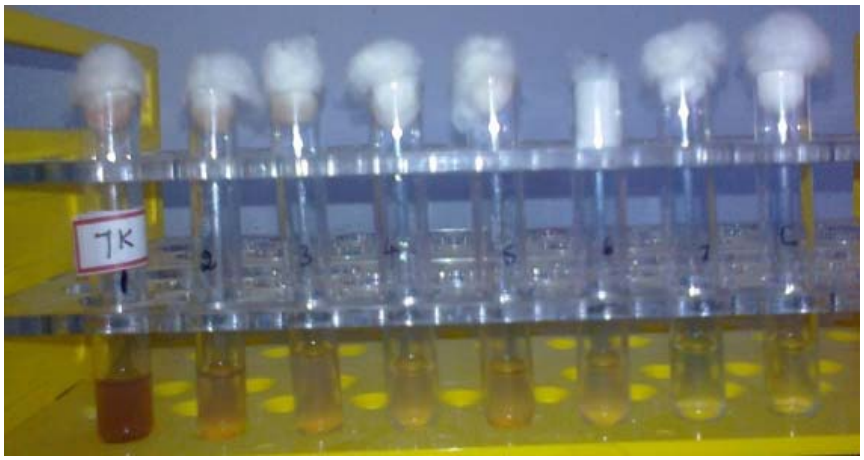


Fig 52. Antibacterial activity of compound **7k** showed MIC at 62.5µg/ml against *S.aureus*

Table 10 . Minimum inhibitory concentration of synthesized compounds against fungi by broth dilution method

Code	MIC (µg/ml)			
	C.albicans	***M.purpurea***	***A.niger***	***T.rubrum***
4a	500	250	250	250
4b	250	250	250	125
4c	250	250	125	250
4d	250	250	125	250
4e	125	250	250	250
4f	62.5	62.5	500	250
4g	62.5	125	125	125
4h	125	62.5	125	250
4i	62.5	500	62.5	250
4j	62.5	250	250	250
4k	125	125	250	125
7a	125	125	250	250
7b	250	250	500	250
7c	250	250	250	250
7d	250	62.5	250	62.5
7e	250	125	250	125
7f	500	250	250	125
7g	250	125	250	125
7h	500	62.5	62.5	62.5
7i	250	125	250	250
7j	125	250	125	250
7k	125	250	125	250
Std	62.5	62.5	125	62.5

Std- Clotrimazole

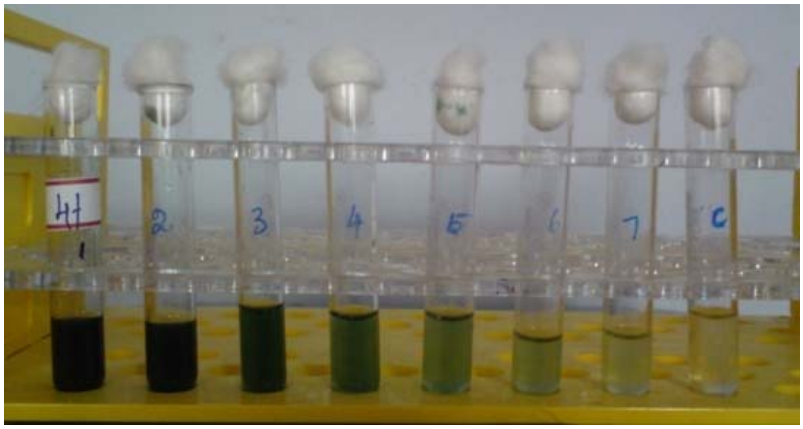


Fig 53. Antifungal activity of compound **4f** showed MIC at 62.5µg/ml against *C.albicans*

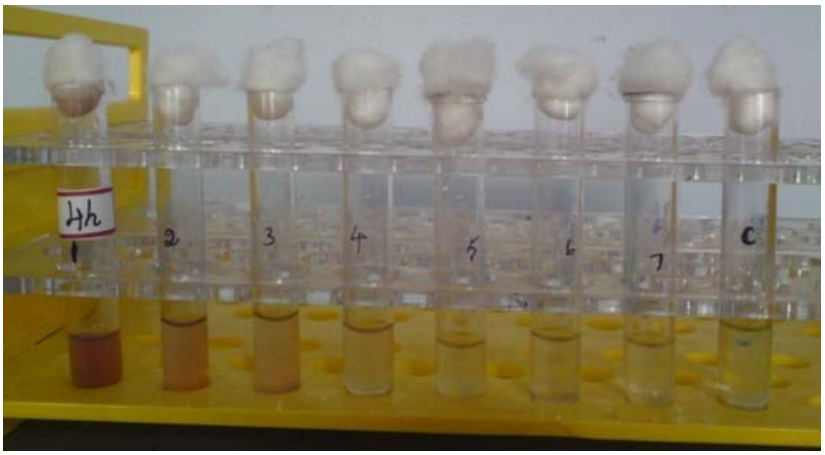


Fig 54. Antifungal activity of comound **4h** showed MIC at 62.5µg/ml against *M.purpurea*

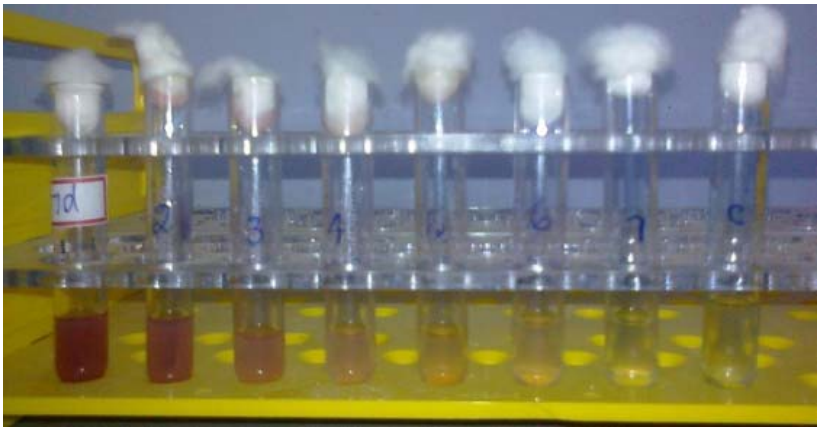


Fig 55. Antifungal activity of compound **7d** showed MIC at 62.5µg/ml against *T.rubrum*

ANTITUBERCULAR STUDIES

Table 11. ***In vitro*** **antitubercular screening by REMA**

No	Compound	Concentration µg/ml		
		1	10	100
1	**4d**	**N**	**N**	**P**
2	**4f**	**N**	**N**	**P**
3	**4j**	**N**	**P**	**P**
4	**7h**	**N**	**P**	**P**
5	**7i**	**N**	**N**	**N**
6	**7k**	**N**	**P**	**P**
7	**std**	**P**	**P**	
8	**control**	**N**	**N**	

N = no inhibition

P = inhibition

Std= Rifampicin

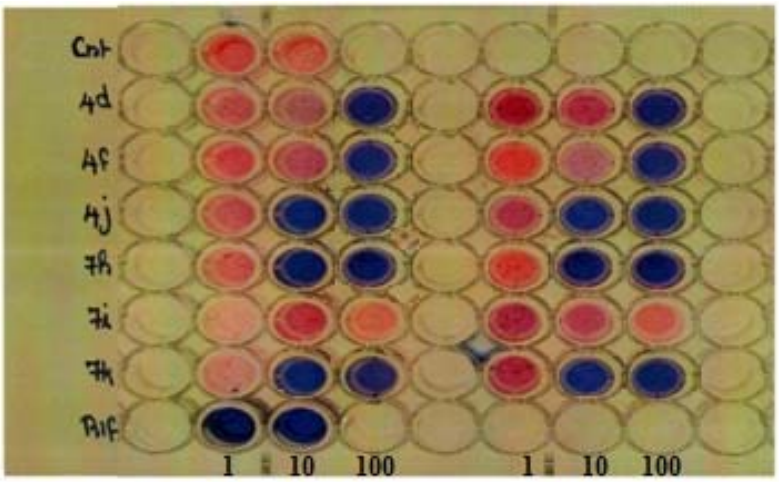


Fig 56. *In vitro* antitubercular screening by REMA.

ANTIOXIDANT STUDIES

Table 12. DPPH radical scavenging activity of titled compounds

Code	Concentration	% inhibition	EC_{50} (µg/ml)
4d	25	40.01	55
	50	48.26	
	75	69.71	
	100	86.62	
4e	25	68.93	-
	50	85.78	
	75	79.34	
	100	80.83	
4g	25	26.81	63
	50	43.08	
	75	79.12	
	100	88.76	
7c	25	82.46	-
	50	86.68	
	75	87.70	
	100	91.59	
7e	25	87.93	-
	50	92.10	
	75	90.86	
	100	84.17	
7f	25	91.71	-
	50	82.48	
	75	89.32	
	100	79.97	
7g	25	33.34	57
	50	42.65	
	75	74.82	
	100	91.53	
7h	25	21.20	66
	50	39.08	
	75	64.10	
	100	83.02	
7i	25	85.54	-
	50	82.67	
	75	72.67	
	100	80.29	
7k	25	25.1	47
	50	56.02	
	75	70	
	100	92.36	
STANDARD (Ascorbic acid)	25	45.7	40
	50	58.9	
	75	81	
	100	96	

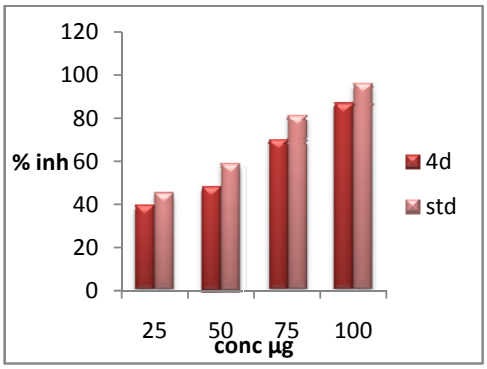


Fig 57. DPPH radical scavenging activity of compound **4d**, measured at 517 nm

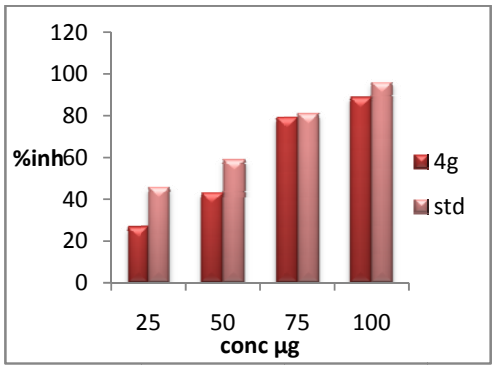


Fig 58.DPPH radical scavenging activity of compound **4g**, measured at 517 nm

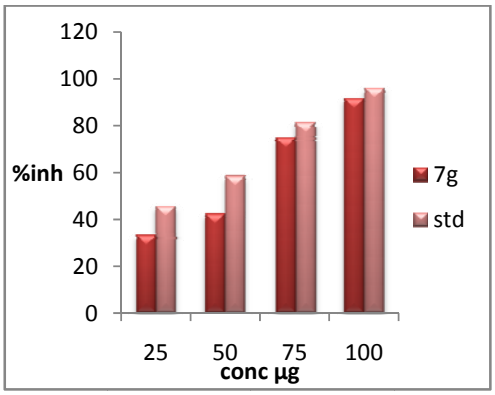


Fig 59. DPPH radical scavenging activity of compound **7g**, measured at 517 nm

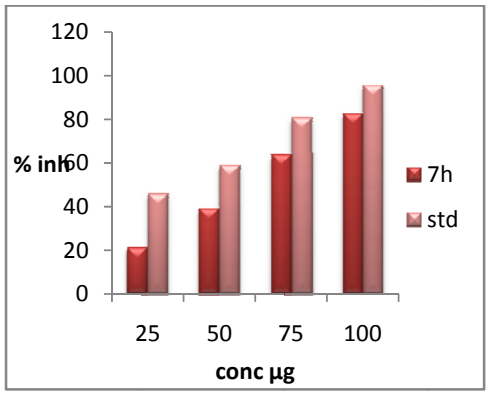


Fig 60 .DPPH radical scavenging activity of compound **7h,** measured at 517 nm

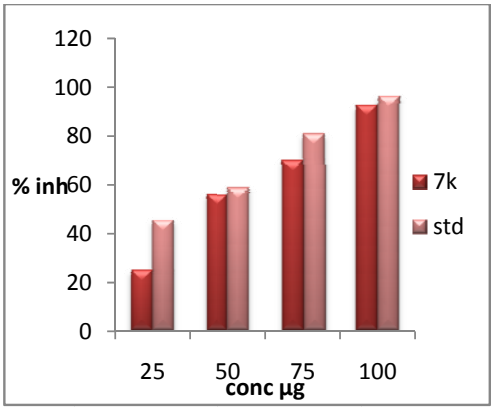


Fig 61. DPPH radical scavenging activity of compound **7k**, measured at 517 nm

Table 13. FRAB radical scavenging activity of titled compounds

Code	Concentration	Absorption	R^2
4d	25	0.3005	0.956
	50	0.3269	
	75	0.3368	
	100	0.3732	
4e	25	0.1481	0.664
	50	0.2983	
	75	0.2081	
	100	0.9089	
4g	25	0.2751	0.957
	50	0.3393	
	75	0.3639	
	100	0.3981	
7c	25	0.1832	0.512
	50	0.5312	
	75	0.3982	
	100	0.5301	
7e	25	0.0321	0.637
	50	0.0891	
	75	0.0899	
	100	1.2910	
7f	25	0.0184	0.444
	50	0.3265	
	75	0.1037	
	100	0.6493	
7g	25	0.2751	0.586
	50	0.3393	
	75	0.4119	
	100	0.4723	
7h	25	0.1533	0.466
	50	0.4736	
	75	0.3147	
	100	1.8951	
7i	25	0.0691	0.945
	50	0.1031	
	75	0.1143	
	100	0.1582	
7k	25	0.9163	0.967
	50	1.0568	
	75	1.1043	
	100	1.2076	
STANDARD (Ferrous sulphate)	25	0.9509	0.981
	50	1.0988	
	75	1.1843	
	100	1.2743	

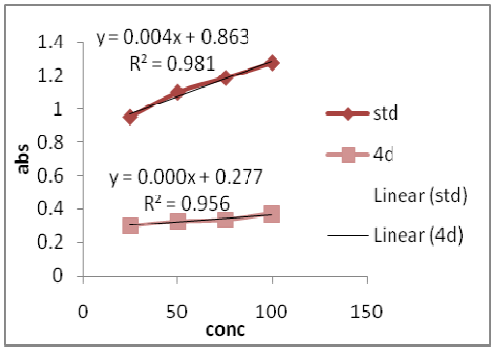


Fig 62. FRAP radical scavenging activity of compound **4d**, measured at 593 nm

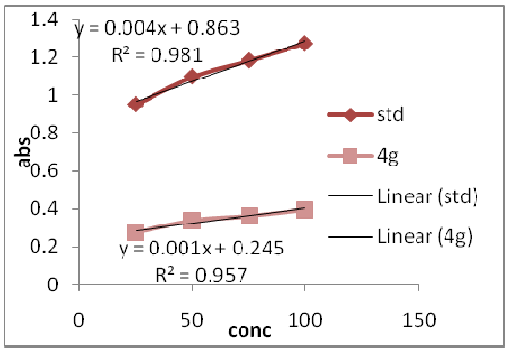


Fig 63.FRAP radical scavenging activity of compound **4g**, measured at 593 nm

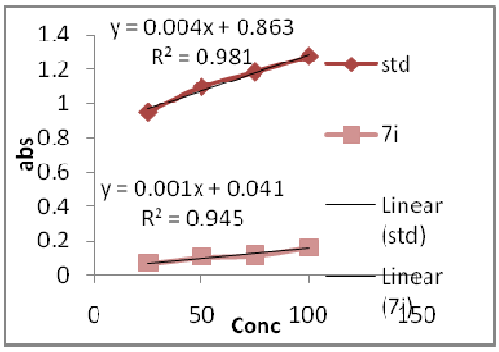


Fig 64. FRAP radical scavenging activity of compound **7i,** measured at 593 nm

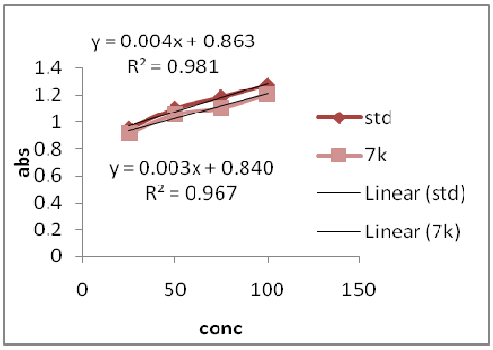


Fig 65 .FRAP radical scavenging activity of compound **7k**, measured at 593 nm

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Table 14. Absorbance of various concentrations of samples at 570 nm

Code	Conc (µM)	6.25	12.5	25	50	100	Cont
4f	ABS	0.209	0.155	0.121	0.077	0.032	0.274
		0.222	0.152	0.117	0.075	0.026	0.268
		0.19	0.166	0.116	0.066	0.027	0.271
	Avg	0.207	0.157	0.118	0.074	0.028	0.271
4h	ABS	0.269	0.243	0.228	0.187	0.158	0.274
		0.279	0.256	0.227	0.174	0.154	0.268
		0.271	0.266	0.228	0.179	0.141	0.271
	Avg	0.270	0.255	0.227	0.180	0.151	0.271
4i	ABS	0.270	0.259	0.226	0.176	0.115	0.274
		0.272	0.253	0.212	0.170	0.131	0.268
		0.270	0.26	0.22	0.163	0.131	0.271
	Avg	0.270	0.257	0.219	0.169	0.121	0.271

Table 15. Absorbance of various concentrations of samples at 570 nm

Code	Conc (µM)	0.1	1	10	100	Cont
7d	ABS	0.387	0.392	0.102	0.053	0.371
		0.408	0.405	0.113	0.060	0.417
		0.393	0.386	0.117	0.048	0.407
	Avg	0.396	0.394	0.111	0.054	0.398
7j	ABS	0.39	0.362	0.307	0.132	0.371
		0.40	0.361	0.311	0.143	0.417
		0.376	0.372	0.277	0.121	0.407
	Avg	0.389	0.365	0.298	0.132	0.398
7k	ABS	0.336	0.318	0.20	0.105	0.371
		0.346	0.304	0.179	0.107	0.417
		0.361	0.305	0.216	0.113	0.407
	Avg	0.347	0.305	0.198	0.108	0.398

Table 16 . Percentage cell inhibition produced by titled compounds at varying concentrations

Code	Conc (µM)	% Cell Inhibition	IC_{50} (µM)
4f	6.25	23.61624	18.26
	12.5	41.82042	
	25	56.45756	
	50	73.18573	
	100	89.5449	
4h	6.25	0.123001	111.2
	12.5	5.904059	
	25	15.91016	
	50	33.57934	
	100	44.28044	
4i	6.25	0.123001	84.11
	12.5	5.04305	
	25	19.06519	
	50	37.39237	
	100	53.01353	
7d	0.1	0.585774	6.13
	1	1.004184	
	10	72.21754	
	100	86.5257	
7j	0.1	2.426778	40
	1	8.368201	
	10	25.1046	
	100	66.86192	
7k	0.1	12.71967	11.09
	1	22.42678	
	10	50.20921	
	100	72.80335	

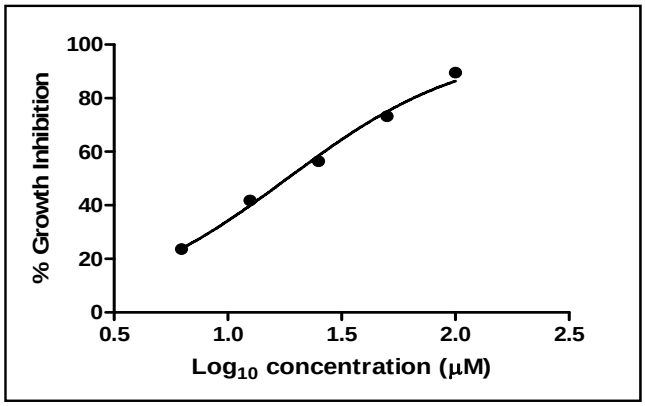


Fig 66. Graph plotted between the percentage growth inhibition and log concentration of compound **4f** (R^2=0.9922)

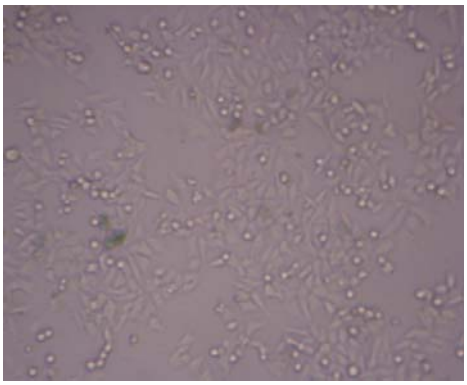


Fig 67. Inhibition of HeLa by Compound **4f** (6.5 μM)

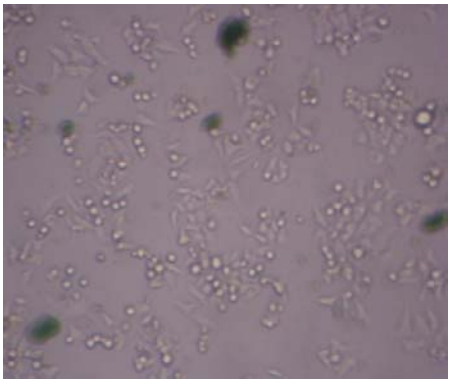


Fig 68. Inhibition of HeLa by Compound **4f** (12.5 μM)

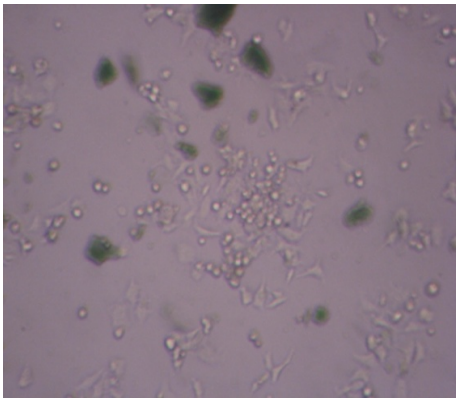


Fig 69. Inhibition of HeLa by Compound **4f** (25 μM)

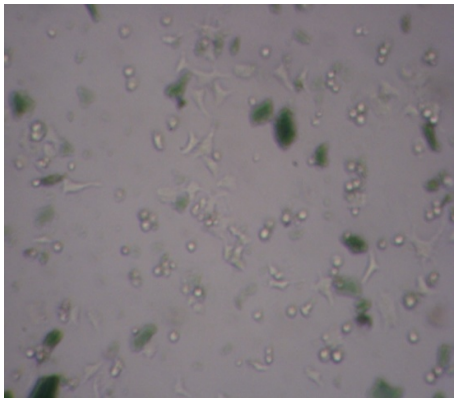


Fig 70. Inhibition of HeLa by Compound **4f** (50 μM)

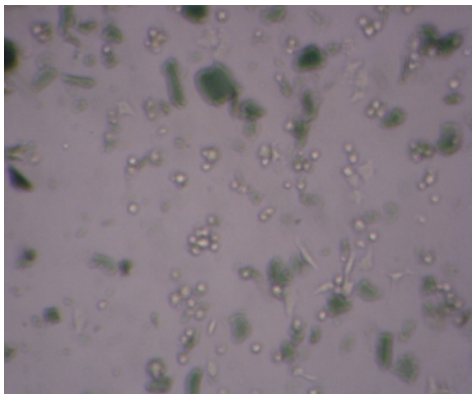


Fig 71. Inhibition of HeLa by Compound **4f** (100 μM)

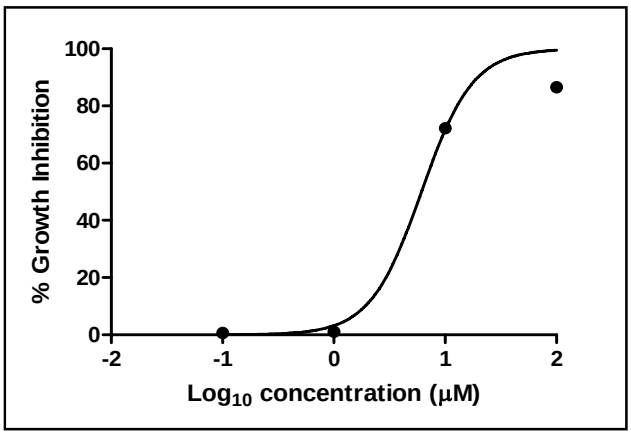


Fig 72. Graph plotted between the percentage growth inhibition and log concentration of compound **7d** (R^2=0.9724)

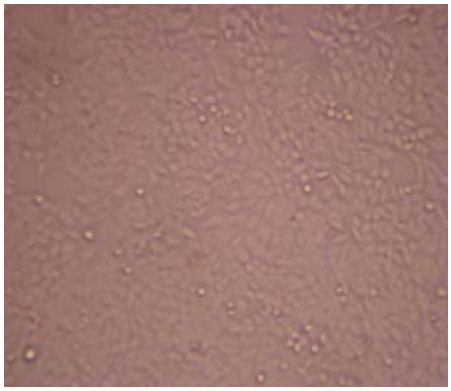


Fig 73 . Inhibition of HeLa by Compound **7d** (0.1 μM)

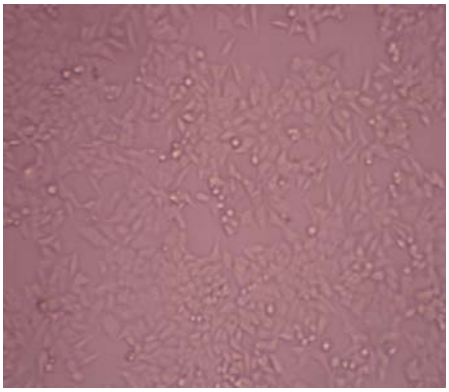


Fig 74. Inhibition of HeLa by compound **7d** (1 μM)

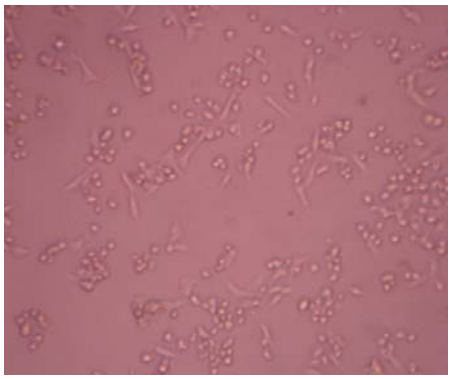


Fig 75. Inhibition of HeLa by compound**7d** (10 μM)

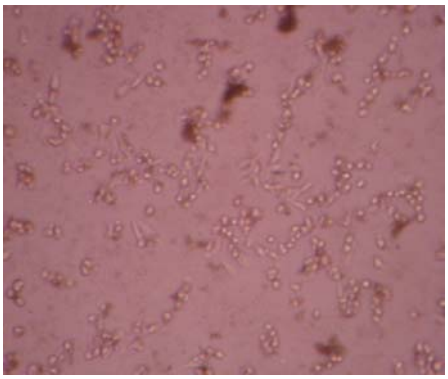


Fig 76. Inhibition of HeLa by compound **7d** (100 μM)

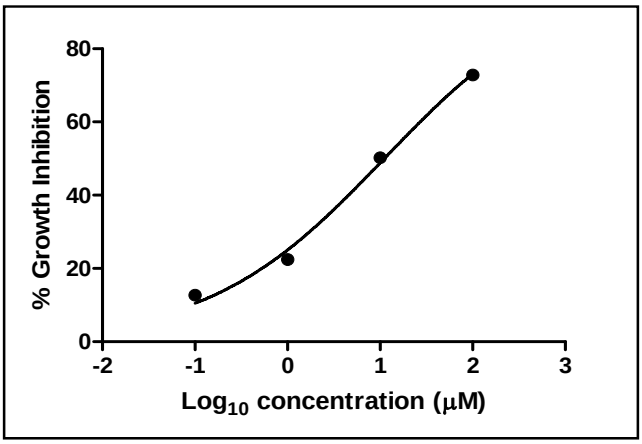


Fig 77 . Graph plotted between the percentage growth inhibition and log concentration of compound **7k** (R^2=0.9938)

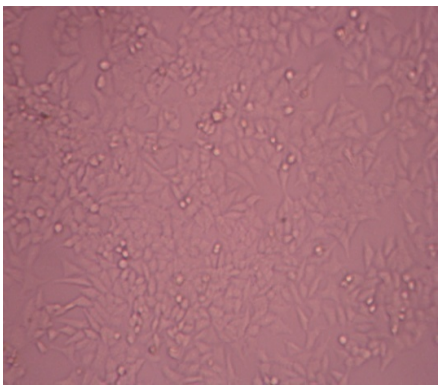


Fig 78. Inhibition of HeLa by compound**7k** (0.1 μM)

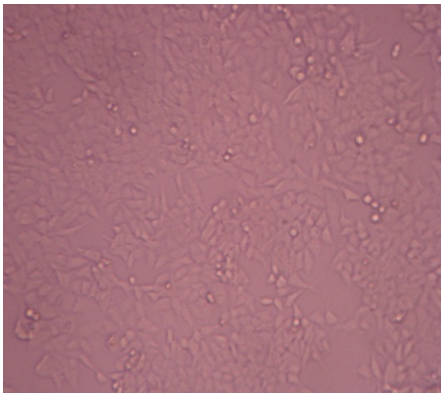


Fig 79. Inhibition of HeLa by compound**7k** (1 μM)

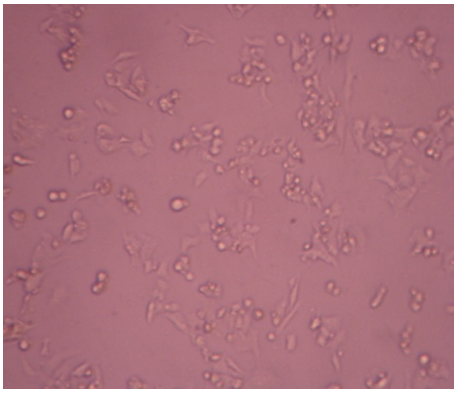


Fig 80. Inhibition of HeLa by compound**7k** (10 μM)

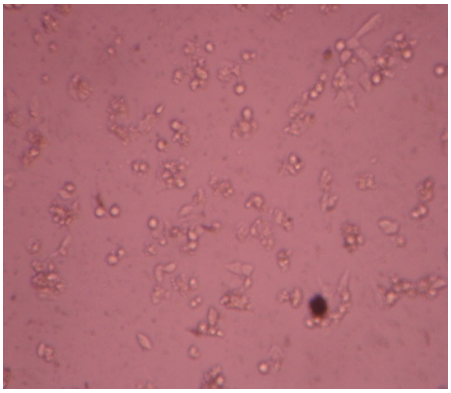


Fig 81. Inhibition of HeLa by compound **7k** (100 μM)

Chapter 7

Discussion

7.DISCUSSION

7.1 Chemistry

Two different series with a total of 22 compounds of 1-(3-chloro-2-oxo-4-phenylazetidin-1-yl)-3-(2-oxo-2-(10*H*-phenothiazin-10-yl)ethyl)urea and thiourea derivatives have been synthesized.

The derivatives were synthesized by a four step reaction as described in Scheme-I and Scheme-2. In the first step, 2-chloro-1-(10*H*-phenothiazin-10-yl)ethanone has been prepared from phenothiazine and chloroacetyl chloride using dry benzene as solvent followed by the treatment with semicarbazide/thiosemicarbazide to form 4-(2-oxo-2-(10*H*-phenothiazin-10yl)ethyl) semicarbazide/thiosemicarbazide (step 2, 5). Schiff bases of phenothiazines were then synthesized by the condensation of 4-(2-oxo-2(10*H*-phenothiazin-10yl) semicarbazide/thiosemicarbazide with various substituted aromatic aldehydes (step 3, 6). Finally a series of substituted azetidinones have been synthesized by cyclocondensation of various Schiff bases of phenothiazine with chloroacetyl chloride in presence of triethyl amine (step 4, 7).

All the titled compounds yielded the products in the range of 52-83%. The melting points of the compounds **4a-k, 7a-k** were observed in the range of 110-190^{0}C. All compounds showed only one spot of migration from the origin on TLC plates, thereby confirming their purity.

7.2 Characterization

All the newly synthesized compounds were characterized by FTIR and six selected compounds were characterized by ^{1}HNMR and two selected compounds by Mass spectroscopic method.

The structure of intermediate compounds **3a-k** and **6a-k** were confirmed by the presence of characteristic peaks in the regions of 1690 cm^{-1} for C=O stretching, 3350 cm^{-1} for NH stretching, 3080,1600 and 790 cm^{-1} for aromatic stretching and1569 cm^{-1} for N=CH stretching . The compounds **5a-k, 6a-k,** and **7a-k** showed characteristic bands for C=S linked to NH at 1512, 1165 cm^{-1}. Compounds **4b-k** and **7c-k** confirmed the presence of phenothiazine ring due to the IR absorption bands in the region 1596 and 1570 cm^{-1}. The compounds of the both series **4a-k** and **7a-k** showed characteristic peaks in the regions of

1657 cm^{-1} for C=O stretching for azetidinone, 3340 cm^{-1} for NH stretching, 3129 cm^{-1}, 1592 cm^{-1} and 737 cm^{-1} for aromatic stretching, 1467 cm^{-1} for C-N stretching and 827 cm^{-1} for C-Cl stretching. Compounds containing NO_2 group showed absorption bands at 1518, 1343 cm^{-1} for the N=O stretching and the peaks at 650-740 cm^{-1} could be assigned to C-S-C stretching. Presence of hydroxyl group was confirmed by the appearance of broad peak at 3430-3161 cm^{-1}.

Six synthesized compounds were characterized using ^{1}HNMR. The spectrum of **4d** revealed a singlet at δ 2.9 corresponding to six protons of $N(CH_3)_2$ group. The compound **7c** showed singlet at δ 3.3 for three protons of OCH_3 group. The spectra of compound **7e** showed two singlets at δ 3.4 broad and δ 3.8 for one and three protons of OH and OCH_3 groups respectively. The compound **7h** revealed multiplet signals in the range of δ 7.7-7.8 for Ar-H of *p*-chloro phenyl ring. The compounds **4d, 4g, 7c, 7e, 7h,** and **7k** showed multiplet signals in the range of δ 6.52 - 7.5 for the protons of aromatic ring, doublets in the range of δ 2.5 for one proton of -CH-Cl group of azetidinone, singlet at δ 3.35 for two protons of CH_2 group and a singlet at δ 8.5 for one NH proton. The NH_2 proton was however, not detectable.

Electron impact mass spectral analysis was carried out on two randomly selected compounds **4e** and **7f**. The molecular mass of their corresponding molecular ion peaks (M+.) were found to be 524.90 and 541.11 respectively, which is in correlation with the synthesized molecules. The base peak of **4e** and **7f** was observed at 199.14 which representing the phenothiazine fragment.

7.3 Biological evaluation

7.3.1 Antibacterial screening

All the titled compounds were investigated for antibacterial activity against NCIM and MTCC bacterial strains (four Gram positive and four Gram negative) by disc diffusion method to determine the zone of inhibition and broth dilution method to determine the minimum inhibitory concentration.

Among the first series of compounds, 1-(3-chloro-2-(4-chlorophenyl)-4-oxoazetidin-1-yl)-3-(2-oxo-2-(10*H*-phenothiazin-10-yl)ethyl)urea (**4h**) showed significant antibacterial activity against Gram negative organisms *P.aeruginosa* and *V.cholerae* (zone of inhibition 19 mm and 18 mm respectively, MIC 62.5 µg/ml). This may be due to the presence of electron withdrawing chloro phenyl group on ring nitrogen of azetidinone moiety, which is in

correlation with reported azetidinone moiety possess antimicrobial action[95] or the presence of phenothiazine could be another important reason for its antibacterial activity.[35] The compounds 1-(3-chloro-2-oxo-4-phenylazetidin-1-yl)-3-(2-oxo-2-(10*H*-phenothiazin-10-yl)ethyl)urea **(4a)** and 1-(3-chloro-2-oxo-4-styrylazetidin-1-yl)-3-(2-oxo-2-(10*H*-phenothiazin-10-yl)ethyl)urea **(4b)**, showed mild activity against all the Gram positive and Gram negative microbes.1-(3-chloro-2-(4-hydroxyphenyl)-4-oxoazetidin-1-yl)-3-(2-oxo-2-(10*H*-phenothiazin-10-yl) ethyl) urea **(4i)** showed comparable activity as that of standard ciprrofloxacin against *E.coli* (zone of inhibition 18 mm, MIC 62.5 µg/ml) and this activity may be due to the presence of electron donating phenolic hydroxy group on the azetidinone nucleus [96].

Among the second series of compounds, 1-(3-chloro-2-(4-nitrophenyl)-4-oxoazetidin-1-yl)-3-(2-oxo-2-(10*H* phenothiazin-10-yl)ethyl)thiourea **(7f)** could be considered an effective antibacterial agent which showed almost equal activity compared to that of standard ciprofloxacin against Gram positive microbe *S.aureus* (zone of inhibition 20 mm, MIC 62.5 µg/ml) which may be explained on the basis of nitro substituted aromatic ring on azetidinone.[64] The compounds 1-(3-chloro-2-(3-chlorophenyl)-4-oxoazetidin-1-yl)-3-(2-oxo-2-(10*H*-phenothiazin-10-yl)ethyl)thiourea **(7j)**, 1-(3-chloro-2-(3-fluorophenyl)-4-oxoazetidin-1-yl)-3-(2-oxo-2-(10*H*-phenothiazin-10-yl) ethyl)thiourea **(7k)** showed significant antibacterial activity against Gram negative microbe *E.coli* (18 mm and 19 mm zone of inhibition respectively, MIC 62.5 µg/ml). This may be due to the presence of chloro and flouro substituted phenyl ring on azetidinone moiety which has been reported to possess effective antimicrobial activity and may be due to increased lipophilicity of flouro substituted compounds.[98] Compounds 1-(3-chloro-2-(4-methoxyphenyl)-4-oxoazetidin-1-yl)-3-(2-oxo-2-(10*H*-phenothiazin-10-yl)ethyl)thiourea **(7c),** 1-(3-chloro-2-(4-chlorophenyl)-4-oxoazetidin-1-yl)-3-(2-oxo-2-(10*H*-phenothiazin-10-yl)ethyl)thiourea **(7h)** substituent were found to be equally effective against Gram positive microbes *B.subtilis* and *S.albus* (zone of inhibition 15 mm, MIC 62.5 µg/ml) and the methoxy substituted aromatic moiety reported that it inhibits DNA Gyrase of Gram positive microbes[97].

7.3.2 Antifungal screening

All the titled compounds were investigated for antifungal activity against four MTCC fungal strains by disc diffusion method to determine the zone of inhibition and broth dilution method to determine the minimum inhibitory concentration.

Among the first series of compounds, 1-(3-chloro-2-(4-nitrophenyl)-4-oxoazetidin-1-yl)-3-(2-oxo-2-(10*H*-phenothiazin-10-yl)ethyl)urea **(4f)** was found to show excellent activity against *C. albicans* and *M. purpurea* (14 mm, 13 mm zone of inhibition respectively, MIC 62.5 µg/ml). The compound is expected to be highly active due to the presence highly electronegative nitro group in addition to the presence of azetidinone linked with urea moiety. The compounds 1-(3-chloro-2-(2-chlorophenyl)-4-oxoazetidin-1-yl)-3-(2-oxo-2-(10*H*phenothiazin -10-yl)ethyl)urea **(4g)**, 1-(3-chloro-2-(4-chlorophenyl)-4-oxoazetidin-1-yl)-3-(2-oxo-2-(10*H*-phenothiazin-10-yl) ethyl) urea **(4h)** and 1-(3-chloro-2-(3-chlorophenyl)-4-oxoazetidin-1-yl)-3-(2-oxo-2-(10*H*-phenothiazin-10-yl)ethyl)urea **(4j)** which showed equipotent activity against *C.albicans M.purpurea and A.niger* (10 mm zone of inhibition respectively, MIC 62.5 µg/ml) which may be due to the ortho, para and meta substitution of chloro substituted phenyl group.[27] The activity of 1-(3-chloro-2-(4-hydroxyphenyl)-4-oxoazetidin-1-yl)-3-(2-oxo-2-(10*H*-phenothiazin-10-yl)ethyl)urea **(4i)** showed inhibition against *C. albicans* (zone of inhibition 13 mm respectively, MIC 125 µg/ml) may be attributed due to the presence of phenolic hydroxyl group in its structure. Also the compounds 1-(3-chloro-2-oxo-4-phenylazetidin-1-yl)-3-(2-oxo-2-(10*H*-phenothiazin-10-yl)ethyl)urea **(4a)** and 1-(3-chloro-2-oxo-4-styrylazetidin-1-yl)-3-(2-oxo-2-(10*H* phenothiazin -10-yl)ethyl)urea **(4b)** showed mild activity against the organisms tested.

Among the second series of compounds, 1-(3-chloro-2-(4-(dimethylamino)phenyl)-4-oxoazetidin-1-yl)-3-(2-oxo-2-(10*H*-phenothiazin-10-yl)ethyl)thiourea **(7d)** and 1-(3-chloro-2-(4-chlorophenyl)-4-oxoazetidin-1-yl)-3-(2-oxo-2-(10*H*-phenothiazin-10-yl)ethyl)thiourea**(7h)** was found to be highly active against *T.rubrum, M.purpurea and A.niger* (16 mm, 14 mm,13 mm, 12 mm zone of inhibition respectively, MIC 62.5 µg/ml). This activity may be due to the presence of thiouridyl linkage of azetidinone moiety. The compounds 1-(3-chloro-2-(3-chlorophenyl)-4-oxoazetidin-1-yl)-3-(2-oxo-2-(10*H*-phenothiazin-10-yl)ethyl)thiourea **(7j)** and 1-(3-chloro-2-(3-fluorophenyl)-4-oxoazetidin-1-yl)-3-(2-oxo-2-(10*H*-phenothiazin-10-yl)ethyl)thiourea **(7k)** were found to be active against *C.albicans* and *A.niger* (13 mm and 10 mm zone of inhibition respectively, MIC 125 µg/ml). Compound **(7b)** 1-(3-chloro-2-oxo-4-styrylazetidin-1-yl)-3-(2-oxo-2-(10*H*-phenothiazin-10-yl)ethyl)thiourea showed very less antifungal activity against the fungal strains.

7.3.3 Antitubercular screening

In vitro antitubercular screening of six compounds which showed significant antibacterial and antifungal activity were studied by REMA which revealed the compounds 1-(3-chloro-2-(3-chlorophenyl)-4-oxoazetidin-1-yl)-3-(2-oxo-2-(10*H*-phenothiazin-10-yl)ethyl)urea **(4j),** 1-(3-chloro-2-(4-chlorophenyl)-4-oxoazetidin-1-yl)-3-(2-oxo-2-(10*H*-phenothiazin-10-yl) ethyl)thiourea**(7h)** and 1-(3-chloro-2-(3-fluorophenyl)-4-oxoazetidin-1-yl)-3-(2-oxo-2-(10*H*-phenothiazin-10-yl) ethyl thiourea **(7k)** showed significant antimycobacterial activity at a concentration of 10 µg/ml. The activity may be due to electon withdrawing group of meta and para chloro substitution and highly electronegative group of meta flouro substitution of phenyl ring at the fourth position of azetidinone nucleus[57,59] and electron-withdrawing substituentson the phenothiazine ring and various halogen substitutions on the benzyl group.[20] The bulkier group of compound **(4d)** and electron withdrawing group of compound **(4f)** possessed antimycobacterial activity at a concentration of 100 µg/ml.[58] The electron donating group of hydroxyl phenyl substitution **(7i)** showed lack of antimycobacterial activity against *Mycobacterium tuberculosis* H37Rv strain.

7 .3.4 Antioxidant screening

The antioxidant studies performed on 10 randomly selected samples against the standard ascorbic acid which revealed that 5 compounds (**4d, 4g, 7g, 7h** and **7k**) showed appreciable DPPH radical scavenging activity with EC_{50} values of 55, 63, 57, 66, and 47 µg/ml respectively.

FRAP radical scavenging method performed on 10 selected samples against the standard ferrous sulphate which revealed that 4 compounds **(4d, 4g, 7i** and **7k)** showed excellent activity with R^2 values of 0.956, 0.957, 0.945, and 0.967 respectively. Compound **(7k)** 1-(3-chloro-2-(3-fluorophenyl)-4-oxoazetidin-1-yl)-3-(2-oxo-2-(10*H*-phenothiazin-10-yl)ethylthio urea showed significant DPPH and FRAP radical scavenging activity compared to standard, because fluorine in a specified position of a molecule instead of hydrogen blocks an essential biochemical path and leads to inhibits the growth of disease.[45]

7.3.5 Anticancer screening

The *in vitro* anticancer studies were performed on 6 randomly selected compounds using MTT assay against HeLa cell line (NCCS). The results indicated that among the six compounds tested, the compounds **4f, 7d** and **7k** were found to have significant cytotoxic activity against Hela cell line and then IC_{50} values were found to be 18.26 μM, 6.13 μM and 11.09 μM.

The bulkier group of dimethylamino aryl substituents on the thiazine nitrogen atom of phenothiazine **(7d)** possess excellent cytotoxic activity against nine types of cancer lines.[14] The SAR studies of phenothiazine reported flourine substituted phenyl ring **(7k)** showed good cytotoxic activity and also the highest log P value[98].

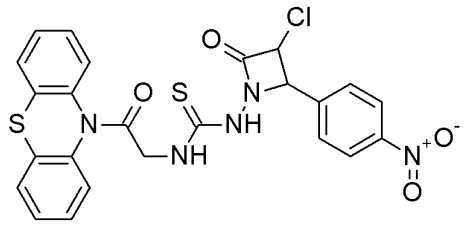
Chapter 8

Conclusion

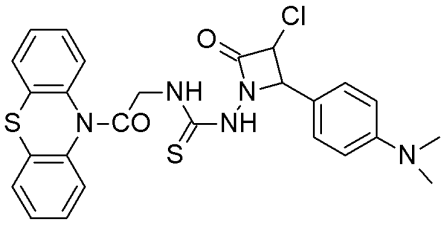
8.CONCLUSION

Twenty two novel 1-(3-chloro-2-oxo-4-phenylazetidin-1-yl)-3-(2-oxo-2-(10*H*-phenothiazin-10-yl)ethyl)urea and thiourea derivatives were synthesized, characterized and evaluated for *in vitro* antibacterial, antifungal, antioxidant and anticancer activities.

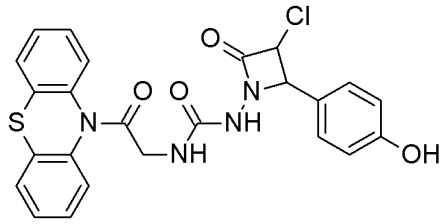
1.Compound (**7f)** 1-(3-chloro-2-(4-nitrophenyl)-4-oxoazetidin-1-yl)-3-(2-oxo-2-(10*H*-phenothiazin-10-yl)ethyl)urea showed could serve as novel template for bacterial infection chemotherapy.

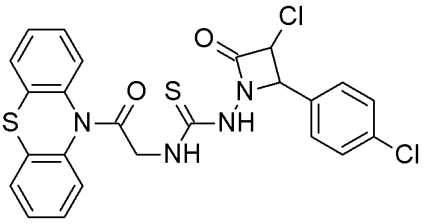


2. Compound **(7d)** 1-(3-chloro-2-(4-(dimethylamino) phenyl)-4-oxoazetidin-1-yl)-3-(2-oxo-2-(10*H*-phenothiazin-10-yl) ethyl) thiourea may be potential candidate for new antifungal agent.

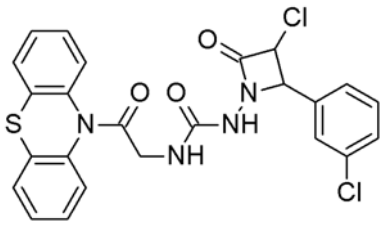


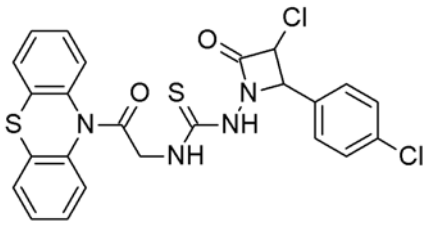
3. Compound (**4i)** 1-(3-chloro-2-(4-hydroxyphenyl)-4-oxoazetidin-1-yl)-3-(2-oxo-2-(10*H*-phenothiazin10yl)ethyl) urea and (**7h)** 1-(3-chloro-2-(4-chlorophenyl)-4-oxo azetidin-1-yl)-3-(2-oxo-2-(10*H*-phenothiazin-10-yl)ethyl) thiourea exhibited significant antibacterial and antifungal properties.

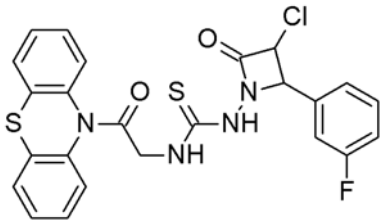




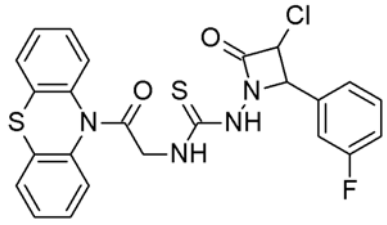
4.The compounds 1-(3-chloro-2-(3-chlorophenyl)-4-oxoazetidin-1-yl)-3-(2-oxo-2-(10*H*-phenothiazin-10-yl)ethyl)urea **(4j),** 1-(3-chloro-2-(4-chlorophenyl)-4 oxo azetidin -1-yl)-3-(2-oxo-2-(10*H*-phenothiazin-10-yl)ethyl) thiourea **(7h)** and 1-(3-chloro-2-(3-fluorophenyl)-4-oxoazetidin-1-yl)-3-(2-oxo-2-(10*H*-phenothiazin-10yl) ethyl thiourea (**7k**) exhibited significant antimycobacterial activity at a concentration of 10 µg/ml.



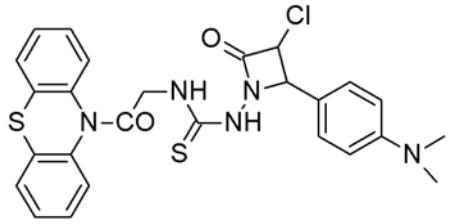




5.Compound (**7k**) 1-(3-chloro-2-(3-fluorophenyl)-4-oxoazetidin-1-yl)-3-(2-oxo-2-(10*H*-phenothiazin-10-yl)ethylthiourea showed significant antioxidant activity with EC_{50} value of 47 µg/ml respectively.



6. Compound **(7d)** 1-(3-chloro-2-(4-(dimethylamino)phenyl)-4-oxoazetidin-1-yl)-3-(2-oxo-2-(10*H*-phenothiazin-10-yl)ethyl)thiourea exhibited significant cytotoxicity was observed against HeLa cell line with IC_{50} value in the range of 6.13 μM respectively.



The results obtained, encouraged us to pursue further research in the synthesis of many derivatives of titled compounds to perform *in vivo* trials in experimental animals to broaden their pharmacological assessment and receptor interactions.

Chapter 9

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ABSTRACT

A new series of twenty two novel 1-(3-chloro-2-oxo-4-phenylazetidin-1yl)-3-(2-oxo-2-(10*H*-phenothiazin-10-yl)ethyl)urea and thiourea derivatives **4a-k, 7a-k** were synthesized. The synthesized compounds were characterized by IR, MASS and ^{1}H NMR spectral data and evaluated for *in vitro* anti-tubercular, anti-cancer, anti-bacterial, anti-fungal and antioxidant activity by REMA, MTT assay, disc diffusion method, MIC method, DPPH and FRAP method respectively. The compounds **4j, 7h** and **7k** at a concentration of 10 µg/ml, **4d** and **4f** at a concentration of 100 µg/ml showed inhibition against the growth of *Mycobacterium tuberculosis.* The compounds **4f, 7d** and **7k** (18.26 µM, 6.13 µM and 11.09 µM) showed significant activity aganist human cervical cancer cell line (HELA). All synthesized compounds showed moderate to significant anti-bacterial and anti-fungal activity and compounds **4d, 4g, 7i** and **7k** showed good antioxidant activity. The synthesized compound **7k** showed broad spectrum of action for *in vitro* anti-tubercular, anti-cancer, anti-bacterial, anti-fungal and anti-oxidant activity.

Keywords: Phenothiazines, 2-azetidinones, MTT Assay, REMA