

SYNTHESIS AND STUDY OF BIOLOGICAL ACTIVITY OF CHEMOTHERAPEUTIC AGENTS

THESIS SUBMITTED
UNIVERSITY OF MUMBAI

FOR THE DEGREE OF
MASTER OF PHARMACEUTICAL SCIENCES
IN
PHARMACEUTICAL CHEMISTRY

SUBMITTED BY
JAYA JOHN

UNDER THE GUIDANCE OF
DR. B.G. KHADSE

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HAFFKINE INSTITUTE FOR TRAINING, RESEARCH & TESTING
MUMBAI - 400 012

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TO
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MASTER OF PHARMACEUTICAL
SCIENCES IN THE
FACULTY OF TECHNOLOGY
(PHARMACEUTICAL CHEMISTRY)

BY
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2001

448

STATEMENT

Statement submitted under rule 0.412.

For the Thesis which I intend to submit, no degree or diploma or distinction has been conferred on me before, either in this or any other university.

Statement submitted under rule 0.413

Statement I:

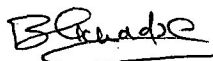
The work embodied in the Thesis to be submitted is original, based on the discovery of new facts viz. the synthesis and characterization of new and unknown compounds of chemotherapeutic value. Also, included in this Thesis, are new relation of facts, observed on the basis of in vitro testing.

The assessment of the compounds has been made at the Department of Microbiology and Department of Chemotherapy, Haffkine Institute, Mumbai-12.

Statement II:

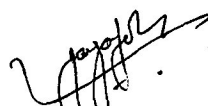
During the study, all the available information of related nature published in scientific journals by previous workers in allied fields, has been used as a source of information, as indicated in the "References".

The entire work has been planned and carried out by the candidate.



Dr. B.G. Khadse

Signature of Research Guide



Ms. Jaya John

Signature of Research Student

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- *Jaya.*

INDEX

	Page
PART I - Introduction	1
Review of Literature	2
Plan of Work	34
Chemistry of Reaction Intermediates	41
Schematic Representation	62
PART II - Synthesis of 4-{1-[(1,2,4-triazolyl)methyl]phenyl}-2 (substituted) thiazol.	65
PART III - Synthesis of 4-methyl-2-(3-n-butyl-4-substituted-2,3 dihydrothiazol-2-ylidene) aminopyridine.	69
PART IV - Synthesis of 6-(1-imidazolyl) quinoxaline(1H, 4H)-dione hydrochloride-A drug intermediate of YM-90K [6-(1-imidazolyl)-7-nitroquinoxaline-(1H,4H)-dione hydrochloride.	72
PART V - Biological Results and Conclusions.	73
RERERENCES	82
APPENDIX	
SYNOPSIS	

Introduction

INTRODUCTION

Drug discovery has its roots since the beginning of mankind. The earliest drug discoveries were probably made by random sampling of various plant sources and their therapeutic values were established serendipitiously. For instance, fox glove used in the treatment of failing heart, peppermint for aching tooth etc. serve as examples. With the passage of time and increase in knowledge, the approach towards drug discovery has taken a revolutionary turn. Drug discovery, today, involves more of a rational approach rather than completely relying on serendipitous findings.

Medicinal chemistry is a stream of science that has been devoted to the field of drug discovery. The principal goal of medicinal chemistry lies in the discovery of lead compounds, their molecular modifications, preparation of drug and explanation of their molecular mode of action. Thus, medicinal chemistry can be seen as an extension of organic chemistry, existing in the boundary between chemistry and biology.

Medicinal chemistry covers three critical steps¹.

- **A discovery step**, consisting of identification and production of new active substances usually called “lead” compounds. The lead compound can originate from synthetic organic chemistry, from natural sources or from biotechnological processes.
- **An optimisation step**, that deals with the synthetic modifications of the lead compound in order to improve potency, selectivity and to decrease toxicity. Its characteristics are the establishments of Structure Activity Relationship (SAR).
- **A development step**, which involves the optimisation of the synthetic route for bulk production, and the modification of pharmacokinetic and pharmaceutical properties of the active substance to render it suitable for clinical use.

Considerable development in this field has provided mankind with several magical moieties called “drugs”, which have cured and managed to check various diseases and disorders. But the battle against infections is not fully won due to the emergence of multi-drug resistant strains of microorganisms, which has rendered many treatments ineffective. Hence the battle continues.

The present work is a positive effort in this direction.

Review of Literature

REVIEW OF LITERATURE

Chemotherapy is the practise of administering chemical preparations to a diseased or an infected person with the aim of relieving the disease symptoms or eliminating the infection. This particular section, review of literature specifically deals with various chemotherapeutic agents used in the treatment of infectious diseases. They are listed as below.

Antibacterial agents:

The essence of antimicrobial chemotherapy is selective toxicity to kill or inhibit the microbes without harming the patient. The early 1940's saw the appearance of potent, non-toxic chemotherapeutic agents selectively active against bacteria, and thus revolutionized the treatment of bacterial infections. The Discovery of "miracle drugs"- Sulfonamide, Penicillin and Streptomycin provided respite from many infections, thus reducing the morbidity and mortality among humans and their domestic hands. But the advantage was short-lived because of the quiet emergence of multidrug resistant bacteria. Hence, intense research in this field has become all the more important.

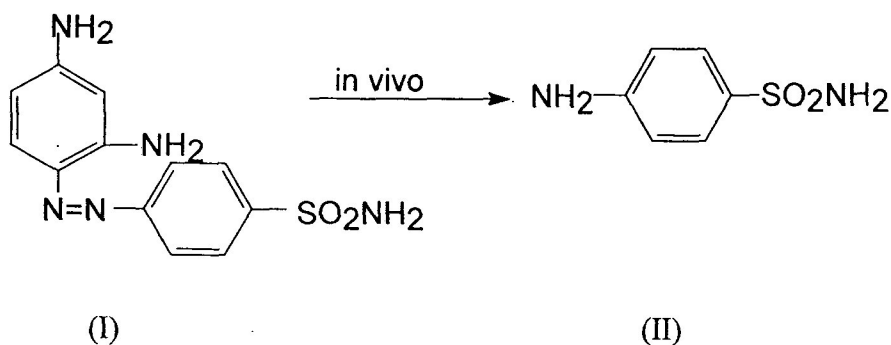
Review of literature gives the major developments in the field of antimicrobial chemotherapy.

Topical antibacterial agents include disinfectants, antiseptics and preservatives. Topical agents show a little selective toxicity between the microbes and the host.

Systemic antibacterials include a number of classes which are enlisted below-

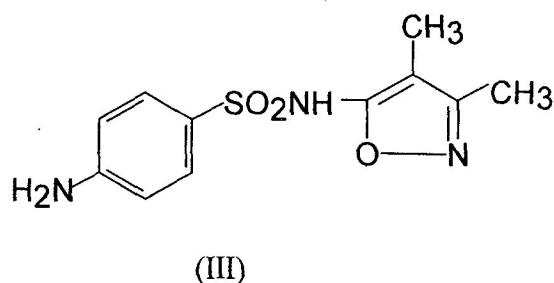
Sulfonamides:

Sulfonamides were discovered in the mid 1930's. Prontosil rubrum (I), a red dye was observed by Domagk² to be taken up selectively by bacterial cells in case of *Streptococcal* infections in mice and *Staphylococcal* infections in rabbits. The dye killed the bacteria. Fractionation led to identification of an active substance known as amino benzene sulphonamide (sulfanilamide) (II)³, a colourless cleavage product formed by liver metabolism of the drug , which is responsible for the activity.



Sulfonamides are predominantly bacteriostatic in nature. They inhibit the enzyme dihydropteroate synthetase, an important biocatalyst needed for the biosynthesis of folic acid derivatives and ultimately DNA. They do this by competitive inhibition of p- amino benzoic acid (PABA) at the active site, due to structural resemblance. Sulfonamide is incorporated into tetrahydrofolic acid molecule by condensation with a dihydropteroate diphosphate precursor under the influence of dihydropteroate synthetase. Sulphonamides also act as antimetabolites by preventing the synthesis of purine nucleotide and thymidine. In human beings, folic acid is directly supplied in diet and is synthesized. Thus, sulfonamides may be considered safe for human cells.

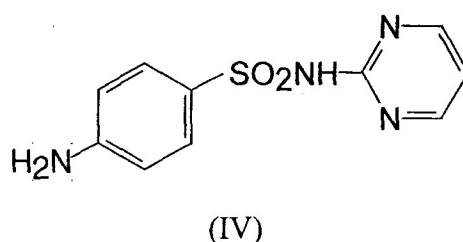
Bacteria which are capable of taking up preformed folic acid are intrinsically resistant to sulfonamides.



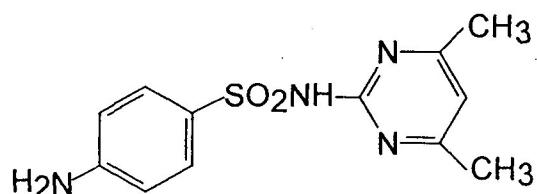
Sulfisoxazole (III)⁴ is one of the most popular sulfonamides in present use which has a pK_a of 5.0. It has a broad antimicrobial spectrum and is clinically used for urinary tract infection and as a backup for other more preferred agents.

It is well absorbed after oral administration, widely distributed and excreted by the kidneys. It maintains adequate blood levels for prolonged period.

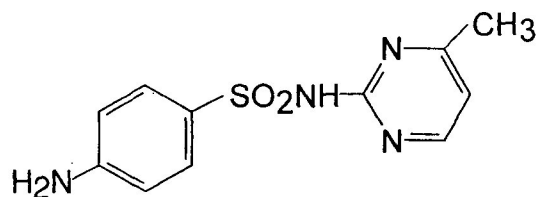
Plasmid-mediated resistance is commonly developed, particularly among Gram-negative organisms, due to decreased sensitivity to dihydropteroate synthetase. Superinfections, allergic rashes, photosensitivity and drug fever are some of the common side effects of the drug.



Sulphadiazine (IV) has retained its pre-eminence because of its broad spectrum of activity, low toxicity and high tissue penetration. It can be used for meningococcal meningitis provided the strains of *Neisseria meningitidis* involved are sulfonamide sensitive⁵.

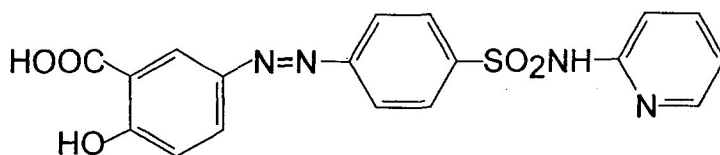


(V)



(VI)

Sulphamethazine (V) and Sulphamerazine (VI)⁶⁻¹⁰ have greater tolerance and high solubility. Multiple(triple) sulfas, a 1:1:1 combination of three sulphapyrimidines, namely, sulphadiazine, sulphamethazine and sulphamerazine, is a very potent combination.



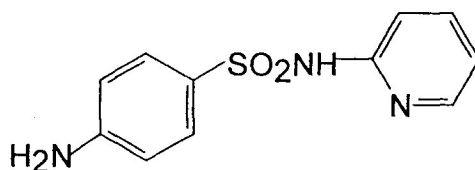
(VII)

Sulphasalazine (VII) is absorbed in the duodenum and is converted to para amino salicylic acid (PAS) which is used for ulcerative colitis and Crohn's disease because of its anti-inflammatory effect.

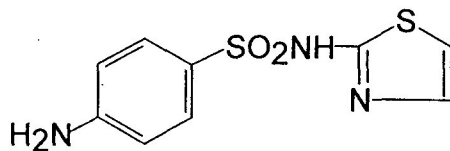


(VIII)

Sulphaguanidine (VIII) was extensively used for intestinal infections. Now, it has been withdrawn in many countries¹¹ due to poor absorption and toxicity.

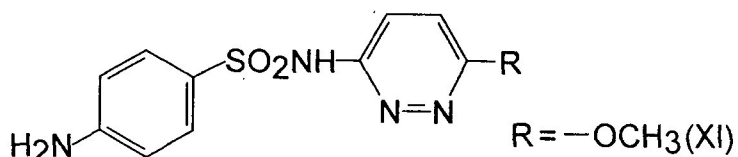


(IX)



(X)

Sulphapyridine(IX)¹², reported in 1938, was in use for the treatment of pneumonia and remained as a drug of choice until it was replaced by sulphathiazole(X)¹³.

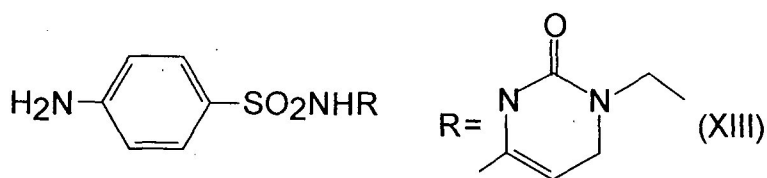


R = -OCH₃ (XI)

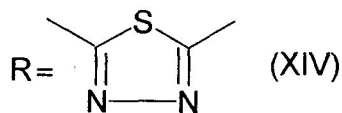
R = -Cl (XII)

Long-acting sulfonamides include Sulphamethoxypyridazine(XI)¹³, which has a half life of about 37 hours. Thus, it needs to be administered only once a day. Sulphachloropyridazine(XII), a related sulphaonamide, has a half-life of 7 hours and is useful for urinary tract infections (UTI).

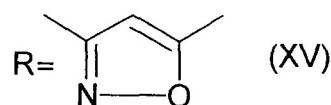
Other sulfonamides include Sulphacytine(XIII), Sulphamethizole(XIV), Sulphamethoxazole(XV) etc.



R = (XIII)



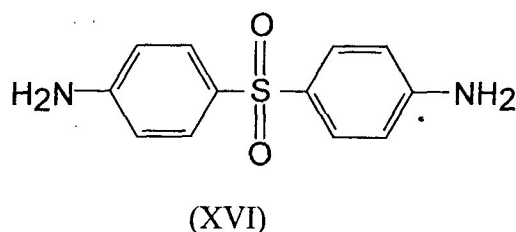
R = (XIV)



R = (XV)

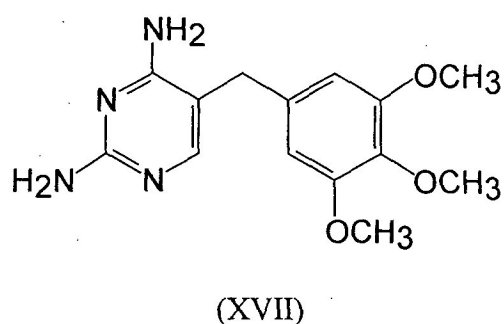
Sulphones:

The parent sulphone, Dapsone (4,4'-sulphonyl dianiline)(XVI), is a prototype for a variety of analogues that have been studied widely. Dapsone¹⁴ is effective against *Mycobacterium leprae* at low concentrations. Its mode of action is similar to that of sulfonamides. p,p'-diamino diphenyl sulphone (DDS) has unique beneficial effects on some dermal inflammatory conditions, especially dermatologic, and it is likely that this activity may contribute to its activity against *M. leprae*.



Synergism with Dihydrofolate Reductase Inhibitors:

Blocking the biosynthesis of folate coenzyme at more than one point, i.e. using two drugs, will result in a synergistic antimicrobial effect¹⁵ and microbes will not be able to develop resistance¹⁶ as quickly as that with a single agent. This approach has been used widely in antibacterial therapy and has resulted in the combination of sulphamethoxazole and trimethoprim (XVII), i.e. Cotrimoxazole, which is widely used for several indications.

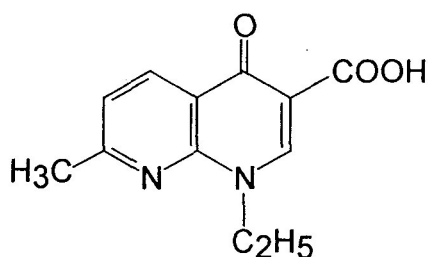


It can be used for urinary tract infections, gonorrhoea, chest infections caused by *Pneumococci*, *Haemophilis influenzae* and enteric *Salmonella* infections.

Side effects include megaloblastosis, leukopenia, thrombocytopenia, etc.

Quinolones:

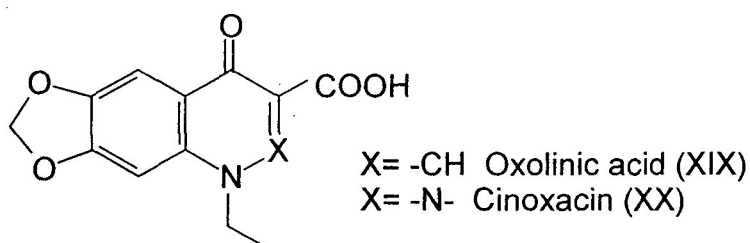
Quinolone antimicrobials comprise of a group of synthetic substances possessing in common an N-alkylated-3-carboxypyrid-4-one ring, fused with a second aromatic ring that carries other substituents.



(XVIII)

The first quinolone to be marketed in 1965 was Nalidixic acid(XVIII). It is primarily effective against Gram-negative organisms and its clinical spectrum is reserved for oral treatment of noncomplicated urinary tract infections caused by susceptible microbes, usually *Escherichia coli*.

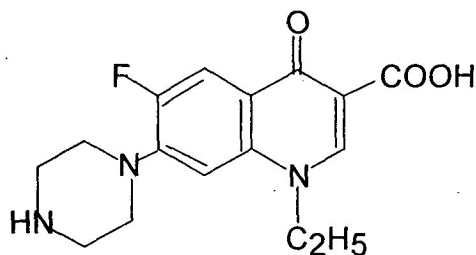
Over the years, the remaining first generation quinolones were introduced¹⁷. These compounds consisted of more potent agents with a better spectrum of activity, such as oxolinic acid (XIX), cinoxacin(XX) etc.



Although well absorbed on oral administration, they get highly bound to serum proteins and hence, are less potent.

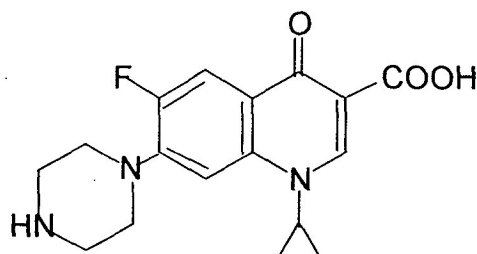
Gastrointestinal upset, skin rashes, visual disturbances and occasionally convulsions and photosensitivity are the adverse reactions observed.

Fluoroquinolones:



(XXI)

Norfloxacin(XXI) was the first fluoroquinolone to be introduced in 1986. It has a broad spectrum of activity with potency equivalent to many of the fermentation-derived antibiotics. It is usually used for the treatment of urinary tract infections.



(XXII)

Ciprofloxacin(XXII)¹⁸ is the most widely used fluoroquinolone because of its improved pharmacokinetic properties and a broader spectrum of activity.

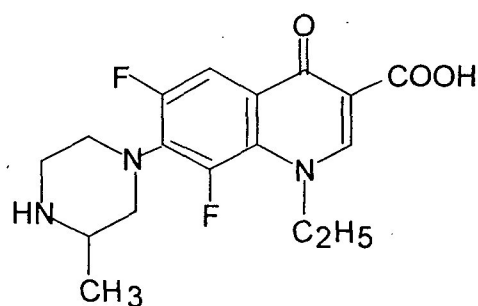
These drugs cause inhibition of the enzyme DNA-gyrase that unwinds the supercoiled DNA helix prior to replication and transcription. This inhibition makes the cell DNA inaccessible and leads to cell death.

Resistance is observed more frequently and it develops due to lesser cellular uptake or lesser sensitivity to DNA-gyrase.

Convulsions, especially in epileptics, CNS problems like hallucinations, insomnia, visual disturbances, diarrhoea, vomiting, abdominal pain, anorexia etc. are among the adverse reactions observed.

Ciprofloxacin is used in prostatitis, upper respiratory tract infections, bone infections, septicemia, *Staphylococcal* and *Pseudomonas* endocarditis, meningitis, chronic ear infections, purulent osteoarthritis and in gonorrhoea, in combination with Cephalosporins¹⁹.

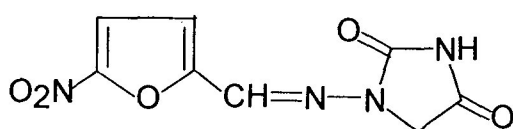
Lomefloxacin(XXIII), Ofloxacin etc. are the other agents in use.



(XXIII)

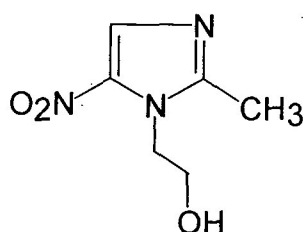
Nitrofurans:

The first nitroheterocyclic compounds to be introduced in chemotherapy were the nitrofurans. Nitrofurantoin(XXIV) is used widely for urinary tract infections(UTI).



(XXIV)

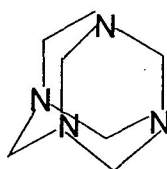
Nitrofurazone²⁰ and Furazolidone²¹ are other broad spectrum nitrofurans in clinical use for the prophylactic treatment of UTI, and they also impair kidney stone growth. Nausea and vomiting are common side effects.



(XXV)

Nitroimidazoles include Metronidazole(XXV) (antiamoebic), Nifurtimox (antiprotozoal) etc. Metronidazole²² is a drug of choice for the treatment of anaerobic infections.

Methenamine:



(XXVI)

Methenamine(XXVI) is internally used as an urinary antiseptic for chronic UTI. Structurally, it is a polymer of ammonia and formaldehyde. Formaldehyde which is strongly bactericidal, is responsible for the activity.

The Antibiotics:

β -lactam antibiotics:

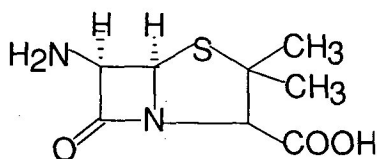
The 1990 worldwide commerce in antibiotics was measured in tonnages and its value is in excess of seven billion. About half of this is associated with β -lactam antibiotics alone.

β -lactam antibiotics are believed to act by selectively inhibiting the cell wall synthesis²³, specifically, by inhibiting the biosynthesis of the dipeptidoglycan that is needed to provide strength and rigidity to the cell wall. Penicillin and Cephalosporin acylate specific bacterial D-alanine transpeptidase²⁴, thereby rendering it inactive for cross-linking of peptides.

Penicillins:

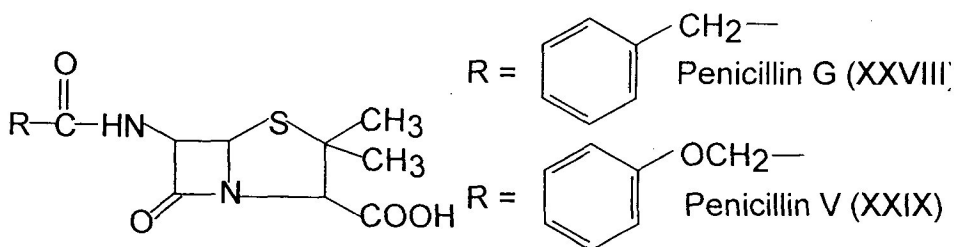
In 1929, Alexander Fleming for the first time discovered *Penicillium notatum* causing partial lysis of *Staphylococcal* colonies. Later on, Penicillin was isolated and gradually large scale production also took place. The penicillin field was then expanded to include orally active penicillins, broad spectrum and enzymatically stable penicillins.

6-amino penicillamic acid(XXVII), i.e. the fundamental nucleus, is prepared by using naturally occurring fungal enzyme that causes selective side chain cleavage of penicillins without breaking the β -lactam bond. More recently, selective chemical processes have been devised for accomplishing biosynthetic analogues.



(XXVII)

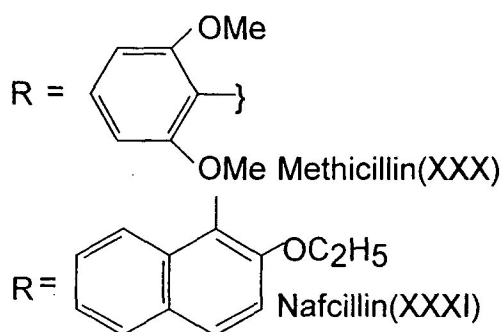
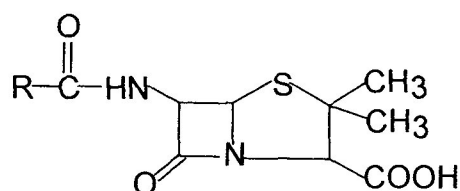
In the presence of phenyl acetic acid, benzyl penicillin (penicillin G)(XXVIII) and with phenoxy acetic acid, phenoxy methyl penicillin (penicillin V)(XXIX) were synthesized by fermentation.



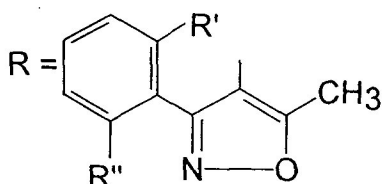
Resistance develops in the bacteria by the deactivation of penicillin by the enzymes namely β -lactamase and acylase. β -lactamase catalyses hydrolysis of β -lactam ring whereas acylase is responsible for hydrolysis of acylamino side chain. In Gram- negative organisms, the resistance develops due to poor penetrability.

Penicillinase- resistant Penicillins:

Substitution at ortho-position of a phenyl ring, i.e. Methicillin(XXX)²⁵ or at 2-position of 4-naphthyl system, i.e. Nafcillin(XXXI)²⁶, increases steric hinderance of the acyl group and confers increased β -lactamase resistance.



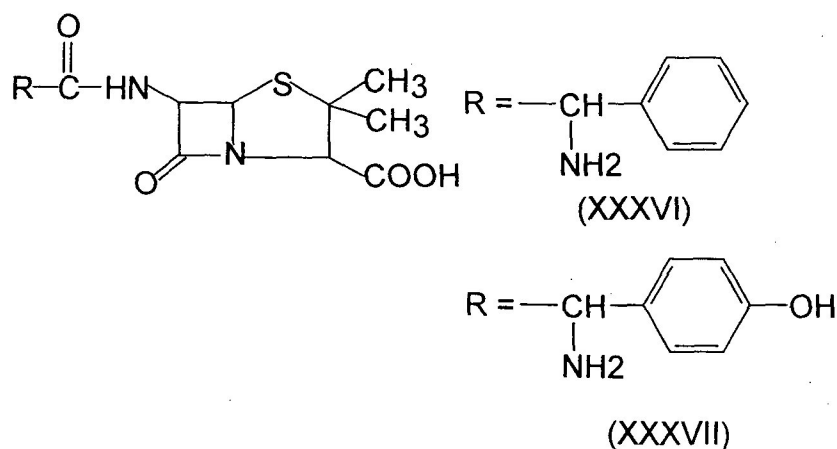
Bulkier substituents are more beneficial among five membered heterocyclic ring derivatives²⁷ e.g. Oxacillin(XXXII)²⁸, Cloxacillin(XXXIII)²⁹, Dicloxacillin(XXXIV)³⁰ and Flucloxacillin(XXXV)³¹.



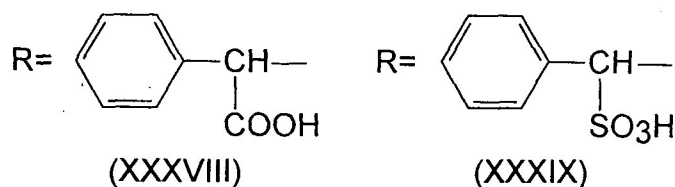
$R'=R''=H$	Oxacillin (XXXII)
$R'=H, R''=Cl$	Cloxacillin(XXXIII)
$R'=R''=Cl$	Dicloxacillin(XXXIV)
$R'=F, R''=Cl$	Flucloxacillin(XXXV)

Extended Spectrum Pencillins:

The introduction of highly polar substituents, e.g. amino group at α -carbon of benzyl side chain group, confers activity against Gram -negative genera such as *Klebsiella*, *Escherichia*, *Haemophylis*, *Salmonella*, *Shigella* and *Proteus*. e.g. Ampicillin(XXXVI)³², Amoxicillin (XXXVII)³³ etc.



The introduction of acidic substituents at α -carbon of benzyl group of Penicillin G, extends the spectrum of activity against Ampicillin resistant strains in addition to the sensitive ones such as *Pseudomonas*, *Enterobacter*, *Serratia*, *Providencia* and also Penicillin-G sensitive Gram-positive organisms e.g. Carbenicillin(XXXVIII)³⁴, Sulbenicillin(XXXIX)³⁵.

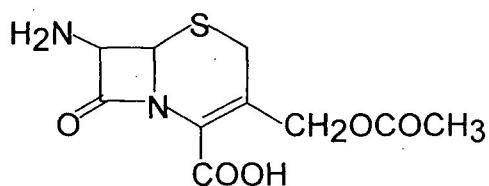


The Allergic reactions include from skin rashes to drug fever, anaphylaxis and cross sensitivity³⁶. Allergy occurs due to production of antigenic penicilloyl proteins³⁷

Cephalosporins:

Cephalosporins were discovered by Brotuz and were isolated and characterized by Abraham and Newton³⁸. They have a wide range of activity against Gram-positive and Gram-negative organisms.

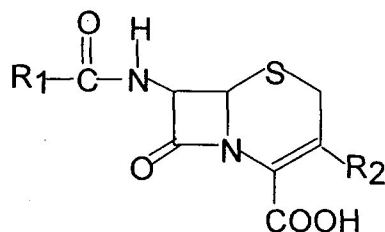
7-amino cephalosporinic acid (XXXX) is a key intermediate for the synthesis of a number of synthetic and semisynthetic Cephalosporins.



(XXXX)

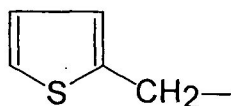
Cephalosporins have broad spectrum of activity with high potency against Gram-positive organisms and moderate potency against Gram-negative organisms.

Cephalothin(XXXXI)³⁹ and Cephaloridine (XXXXII) are administered parenterally, whereas Cephaloglycin (XXXXIII) and Cephalexin (XXXXIV)⁴⁰ are active orally.

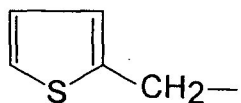


R₁

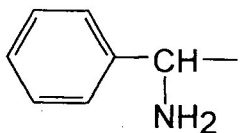
R₂



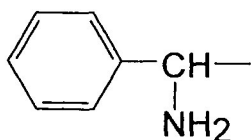
—CH₂OCOCH₃ (XXXXI)



—H₂C—N⁺(C₆H₅) (XXXXII)

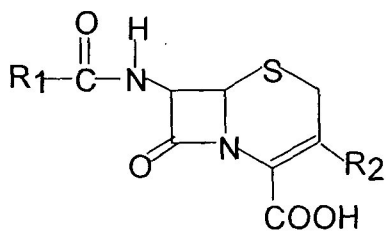


—CH₂OCOCH₃ (XXXXIII)



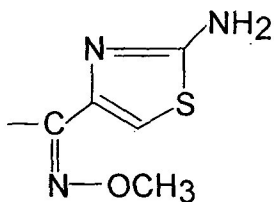
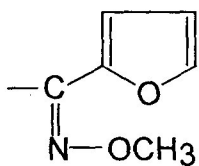
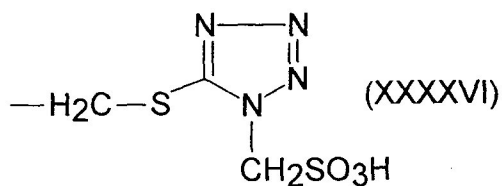
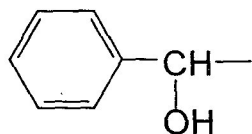
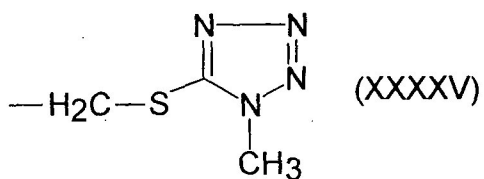
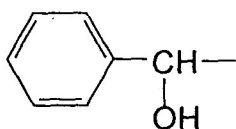
—H₃C (XXXXIV)

With consistent use, resistance develops among several bacteria which produce cephalosporinase and thus hydrolyses the β -lactam ring. The semisynthetic agents with plasmid-mediated cephalosporinase resistance⁴¹ and acid stability were developed later on. These include Cephamandol(XXXXV)⁴², Cephonacid(XXXXVI), Cephuroxime (XXXXVII)⁴³, Cefatoxime(XXXXVIII)^{44, 45} etc.



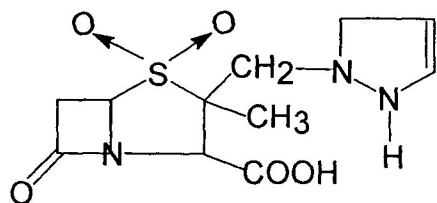
R₁

R₂

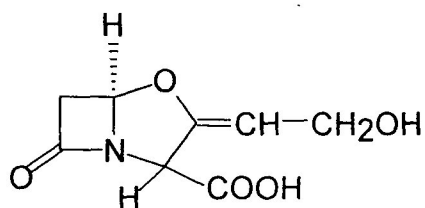


β -lactamase inhibitors:

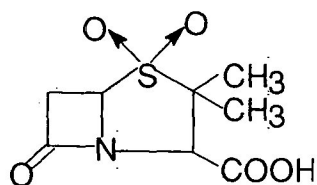
Many substances have been found to inhibit the β -lactamase enzyme, directly or by synergistic action along with some of the antibiotics. UTR-830 (IL), Clavulanic acid (L) and Sulbactam (LI) are some of the examples.



(IL)



(L)



(LI)

Aminoglycosides:

Aminoglycosides contain an amino sugar linked glycosidally to a highly substituted 1,3-diaminocyclohexane central ring. They are broad spectrum antibiotics with special usefulness in serious systemic infections caused by aerobic Gram-negative organisms. e.g. Kanamycin⁴⁶, Gentamycin⁴⁷, Tobramycin⁴⁸, Netilmicin⁴⁹, Amikacin⁵⁰ etc.

Streptomycin^{50, 51, 52} (LII) is the most effective of the group for the chemotherapy of tuberculosis, brucellosis, tularemia, *Yersinia* infections etc.

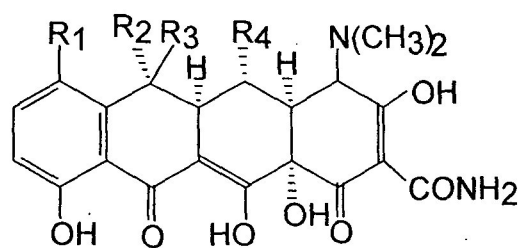


Aminoglycosides bind to the 30S ribosomal subunit to form a complex and thus inhibit proper amino acid polymerization. The binding causes misreading leading to mutation of the genetic code. This causes incorporation of improper amino acids into the peptide chain. Thus, they inhibit initiation of protein synthesis.

The resistance gets transferred from one bacterium to another and is attributed to the extrachromosomal R-factor (DNA), which is self-replicating and transferable by conjugation. These strains synthesize enzymes that are capable of acetylating, phosphorylating or adenylating key amino hydroxy groups of the amino glycosides. The most significant antibiotic resistant to bacterial enzymes is Amikacin.

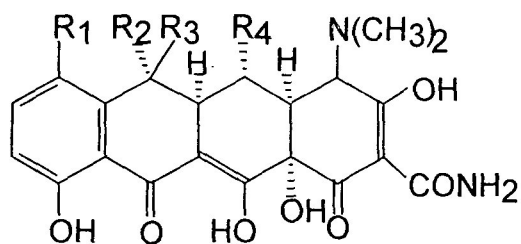
Tetracyclines(LIII)⁵⁵ are derived semisynthetically or from *Streptomyces* species. The basic nucleus is octahydrophenanthrene. Tetracyclines have a broad spectrum of activity as compared to any known antibacterial agent. They are active against a wide range of Gram-positive and Gram-negative bacteria, Spirochetes, Mycoplasmas, Rickettsiae, Chlamydiae etc.

16



(LIII)

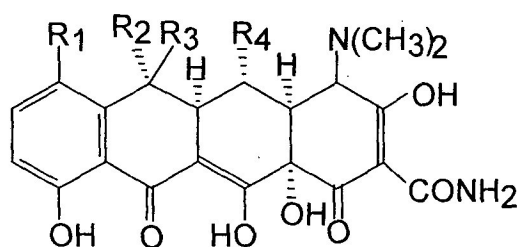
In 1938, Duggar⁵⁶ isolated Chlortetracycline. Electron-withdrawing groups, e.g. in chlortetracycline⁵⁷ (LIV), as well as electron-donating groups, as in Minocycline(LV), enhance the activity of the parent molecule.



R ₁	R ₂	R ₃	R ₄
Cl	CH ₃	OH	H (LIV)
N(CH ₃) ₂	H	H	H (LV)

Oxytetracycline⁵⁸(LVI) has similar activity as that of Chlortetracycline. Doxycycline⁵⁹(LVII) and Methacycline⁶⁰(LVIII) are more active *in vitro* than Oxytetracycline.

In 1957, MaCormik⁶¹ *et al* isolated Demeclocycline(LIX) with similar spectrum of activity as that of other tetracyclines, but of slightly greater activity.



R ₁	R ₂	R ₃	R ₄	
H	CH ₃	OH	OH	(LVI)
H	H	CH ₃	OH	(LVII)
H	CH ₂	-	OH	(LVIII)
Cl	H	OH	H	(LIX)

Tetracycline and chlortetracycline are available as topical ointments for *Acne vulgaris* and are also used in ophthalmic solutions. Oxytetracycline is used parenterally; while Doxycycline is available in capsules and aqueous suspension forms. Minocycline is active against bacteria resistant to Methacycline and other Tetracyclines.

Macrolides:

Macrolide antibiotics have been derived from the *Actinomyces* species. They contain highly substituted lactone ring⁶² aglycon, to which amino and neutral deoxy sugars are attached. In 1950, Picromycin was the first to be reported. McGuire⁶³, in 1952, first reported the isolation of Erythromycin from *Streptococcus erythreus*.

It is well tolerated and useful for infections of the upper respiratory tract, skin, soft tissue and genital tract. More recently, its activity against *Mycoplasma pneumoniae*⁶⁴ has been reported.

Erythromycin acts by binding selectively to 50S ribosomal subunit and thus prevents translocation in protein synthesis⁶⁵.

Non-specific resistance among Gram-negative bacilli is related to poor penetrability⁶⁶ of the drug.

The structural modifications in Erythromycin gave Clarithromycin, Flurithromycin etc. Clarithromycin is effective against *Mycobacterium avium - intercellulariae* complex^{67, 68}. Olendomycin is one more important agent of the macrolide family.

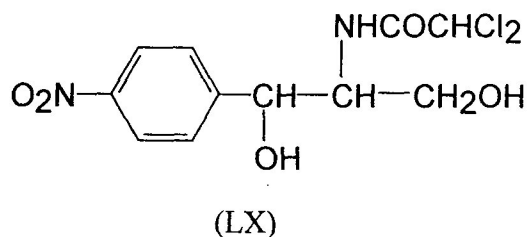
Lincomycins:

Lincomycins are sulphur-containing antibiotics isolated from *S. lincolnensis*. Its spectrum and mode of action are similar to that of the macrolides. Lincomycin, which was isolated by Manson *et al*⁶⁹, is useful for the treatment of infection by Gram-positive organisms, notably Staphylococci, β -haemolytic Streptococci and Pneumococci. Clindamycin⁷⁰ is superior to Lincomycin because of greater penetrability and is used in upper respiratory tract, skin and soft tissue infections.

Adverse effects include gastrointestinal toxicity from distress to pseudomembranous colitis.

Chloramphenicol:

Ehrlich *et al*⁷¹, in 1947, isolated Chloramphenicol (LX), a broad spectrum antibiotic, from *Streptomyces venezuelae*. Its structure was established in 1949 by Constroulis, Rebstock and Crooks⁷².



Chloramphenicol is active against Penicillin G and Ampicillin-resistant species of *Salmonella*, *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Bacillus fragilis*, *N. meningitidis* etc. It is used in urinary tract infections and rickettsial infections. It is administered orally as Chloramphenicol capsule or in the form of the palmitate ester.

Bacteria, producing Chloramphenicol acetyl transferase enzyme, are resistant to the drug. Adverse effects include serious blood dyscrasias and toxic reactions.

Any substitution on the phenyl ring gives rise to compounds that are active but are less potent than Chloramphenicol.

Novobiosin:

Novobiosin was isolated from *Streptomyces* species and was first reported in 1955.

It acts by binding to DNA-gyrase subunit and interfering with DNA supercoiling and energy transduction in bacteria. It is active against Gram-positive bacteria and a few strains of *P. vulgaris*. Low activity against Gram-negative organisms is due to poor absorption.

Cross resistance is not developed, especially in *S. aureus* infections. Therefore, it is useful in *Staphylococcal* infections resistant to other antibiotics and sulfas, and patients allergic to these drugs.

Urticaria, allergic rashes, hepatotoxicity and blood dyscrasias are the major side effects.

Vancomycin:

Vancomycin, isolated from *S. orientalis*, is a glycopeptide which acts by inhibiting the cell-wall synthesis. No cross resistance to β -lactam, Cycloserin, Bacitracin etc. has been observed. It is used in systemic infections such as *Staphylococcal* enterocolitis and pseudomembranous colitis associated with Clindamycin therapy.

Polypeptides:

The polypeptide class of antibiotics includes Gramicidine, Polymixin, Bacitracin, Colistine etc. Severe renal toxicity limits their use in therapeutics.

Antitubercular agents:

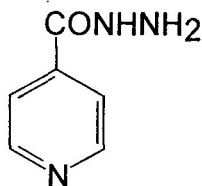
Tuberculosis is a disease largely caused by *Mycobacterium tuberculosis*, an acid fast bacilli. Today, as it has been for centuries, tuberculosis remains the leading cause of deaths in the world from infectious diseases. Globally, tuberculosis accounts for almost 3 million deaths and 8 million new cases emerging each year^{73,74,75}. Tuberculosis is a reemerging problem in many industrialised countries. In the modern world of global interdependancy, wide network of communication and changes in social and cultural patterns, tuberculosis in any country is a threat to the people in every other country. The present problem is further aggravated by the emergence of multidrug resistant strain of bacteria and HIV pandemic. This is further compounded by, drug toxicity and patient in compliance. Thus, the chemotherapy in this field has become more challenging, leading to further research in this particular field.

Antitubercular drugs that are currently used are generally classified as-

- i) Primary or Frontline drugs.
- ii) Secondary or Second line drugs.

'Primary' or frontline drugs are used as a standard therapeutic regimen due to their high level of efficacy and acceptable degree of toxicity. Front-line drugs include Isoniazide, Rifampicin, Ethambutol and Streptomycin. 'Secondary' or second line drugs are used occasionally in case of bacterial resistance or certain patient related factors. These drugs include Pyrazinamide, Ethionamide, Para amino salicylic acid, Cycloserin, Viomycin, Kanamycin, Amikacin, Capreomycin, Thioacetazone, Thiocarlide, etc.

Isoniazide(INH):



(LXI)

Isonicotinyl hydrazide(Isoniazid)(LXI), described in 1912⁷⁶, was prepared as an intermediate in the synthesis of isonicotinyl aldehyde. Outstanding activity of INH in experimental infection was also confirmed by clinical trials. The N-alkylated derivative (Iproniazide) was also found to be active.

INH has both bacteriostatic and bactericidal activity against various strains of *Mycobacterium tuberculosis*, which are resistant to other drugs. The MIC is about 0.04 µg/ml. Bactericidal action is exhibited against replicating organisms, whereas in case of non-replicating organisms, it is only bacteriostatic⁷⁷ in nature.

The mode of action of INH is believed to be the inhibition of the synthesis of mycolic acid, a vital component of the cell wall. Another hypothesis suggests that isonicotinic acid is formed enzymatically by oxidation of INH and it competes with the natural coenzyme, nicotinic acid, to alter the lipid metabolism.

INH is rapidly absorbed orally. Food and antacids, containing aluminium, interfere with its absorption. The drug is to be taken on empty stomach. It gets metabolized by acetylation.

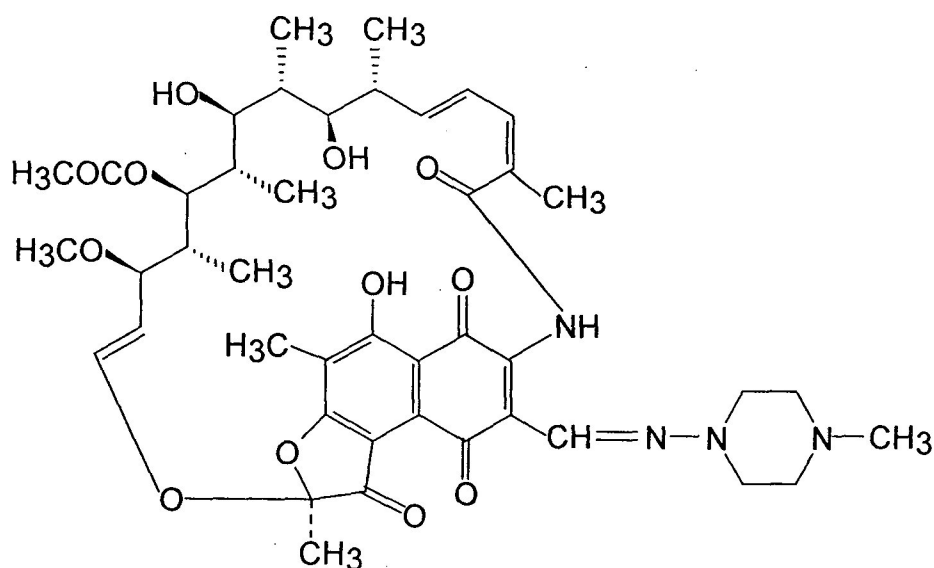
More recently it has been shown that the activity of INH results from peroxidative reaction catalysed by peroxidase, which is encoded by Kat G Gene⁷⁸. *M. tuberculosis* which lacks this gene is resistant to INH⁷⁹.

INH is used in combination with other agents to delay the development of resistance. It is generally suitable for long term treatment. The side effects range from hepatic abnormalities to severe hepatitis. The interference with pyridoxin metabolism inhibits formation of the active form and causes peripheral neuritis.

Rifampicin:

The Rifampicin antibiotics were discovered as metabolites of *Streptomyces mediterranei*. Recently, it has been stated to be *Amycolatopsis mediterranei*⁸⁰.

Several chemical modifications have been carried out on the parent moiety. The hydrazone of 3-formyl rifamycin with N-amino-N-methyl piperazine(Rifampicin)(LXIII)⁸¹ was the most active form *in vivo*, at a concentration of 1 µg/ml, and is useful clinically.



(LXII)

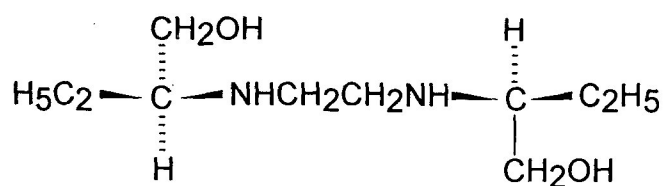
Rifampicin inhibits specifically the activity of the enzyme DNA-dependent RNA polymerase and is active against rapidly dividing intra and extracellular organisms.

Resistance is rare and occurs spontaneously in strains exposed previously to the antibiotic. The mechanism of resistance is through mutation in *rpoL* gene, which encodes the β -subunit of the RNA-polymerase.

Rifampicin is readily absorbed from the intestine. Food interferes with its absorption, and, therefore, it is preferred to be taken on empty stomach. It gets metabolized by deacetylation and desacetylation.

The therapeutic efficacy of Rifampicin is comparable to that of INH and is superior to all other drugs. It possesses low toxicity, with gastrointestinal disturbances and cutaneous reaction as side effects.

Ethambutol:



(LXIII)

Among the randomly screened compounds, (+)-2,2'-(Ethylenediamino)-di-1-butanol (Ethambutol) (LXIII) is found to be very active, both *in vitro* and *in vivo* against *M. tuberculosis* at 1 µg/ml. The organisms resistant to other drugs are found to be susceptible to Ethambutol.

The bacteriostatic action of Ethambutol is not understood and is independent of concentration, being more related to the time of exposure.

Ethambutol is believed to interfere with the role of polymerase and divalent cations in ribonucleic acid metabolism^{82,83}. Another hypothesis states that it causes an inhibitory effect on phosphorylation⁸⁴.

It is absorbed 75-80% when given orally. The side effects mainly include ocular toxicity, i.e. retrobulbar neuritis, causing visual impairment.

Streptomycin:

Streptomycin is both bacteriostatic and bactericidal for the tubercle bacilli *in vitro*, depending upon its concentration. Its MIC is 1 µg/ml against H₃₇Rv strain of *M. tuberculosis*.

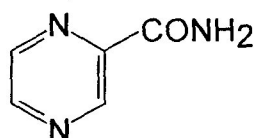
In mycobacteria, mutation in the *rspL* gene, which encodes the ribosomal protein S12, has been shown to confer resistance to Streptomycin⁸⁵.

It gets moderately absorbed on intramuscular administration, which is painful.

The most important toxic effect of Streptomycin is damage to the 8th cranial (vestibulo-cochlear) nerve, particularly in the vestibular portion, causing equilibrium disturbances. Renal damage and hypersensitivity are the other side effects.

Other aminoglycosides such as Kanamycin, Amikacin are used secondarily.

Pyrazinamide:



(LXIV)

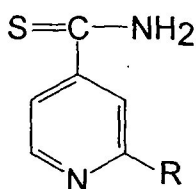
The best activity of Pyrazinamide(LXIV) is against intracellular *Mycobacteria* causing sterilizing effect in acidic conditions. The susceptible bacteria produces deaminase which converts Pyrazinamide to Pyrazinoic acid, an active form.

Pyrazinamide gets readily absorbed orally and is metabolized to Pyrazinoic acid, which gets oxidised to its 3-hydroxy derivative.

Hepatotoxicity is the most common and serious side effect of this drug. Other effects include arthralgia.

It is useful in short term chemotherapeutic regimens. Treatment for longer than two months is not recommended⁸⁶.

Ethionamide:



R = —C₂H₅ (LXV)

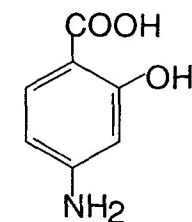
R = —n—C₃H₇ (LXVI)

2-ethylthioisonicotinamide(Ethionamide)(LXV) and its 2-n-propyl analog (Prothionamide)(LXVI) were selected for clinical use against tubercular infections. Ethionamide is active at a concentration of 0.6-2.5 µg/ml *in vitro*, against strains of *M. tuberculosis* which are resistant to INH, Streptomycin and PAS.

Ethionamide inhibits protein synthesis by blocking the incorporation of amino acids, Cysteine and Methionine. It is active orally, but a large dose(>500mg) is not well tolerated. It gets metabolized to give the sulfoxide.

The side effects include GI irritation, hepatitis, CNS effects and hypersensitivity.

Para amino salicylic acid (PAS):



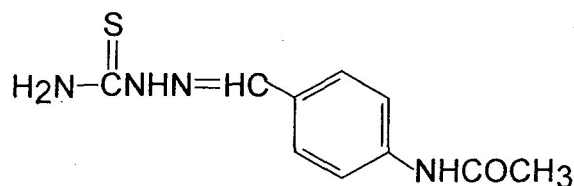
(LXVII)

Among the various compounds related to benzoic acid, PAS(LXVII) is believed to be the most active agent⁸⁷ against growing cells of *M. tuberculosis*.

The mode of action of PAS is believed to be competitive blocking of the synthesis of dihydrofolic acid. On the other hand, the formation of mycobactin, an ionophore for iron transport, is also blocked, thus bacteriostatic activity may be due to the blocking of metabolic pathway of iron uptake⁸⁸.

10-12gm/day of PAS is required to be taken orally in 2-3 divided doses. However, it is no longer in use due to GI toxicity, which is a major side effect.

Thioacetazone:



(LXVIII)

Thioacetazone(LXVIII) is the most active thiosemicarbazone effective against *M. tuberculosis*. A lack of cross resistance to INH and Streptomycin indicates the use of thioacetazone in combination with these drugs to delay bacterial resistance.

Severe side effects such as GI disturbances, liver damage and anemia limits its use in therapy.

Drug Regimens followed in the treatment of Tuberculosis:

<u>Drug</u>	<u>Initial phase(2months)</u>	<u>Continuation phase(4months)</u>
<u>Standard regimen:</u>		
Rifampicin	10mg/kg/day(450-600mg/day)	10mg/kg/day(450-600mg/day)
INH	5mg/kg/day(200-300mg/day)	5mg/kg/day(200-300mg/day)
Pyrazinamide	30mg/kg/day(1.5-2gm/day)	—
Ethambutol*	15-25mg/kg/day	

Intermittent regimen:

Rifampicin	10mg/kg(3times weekly)	10mg/kg(3times weekly)
INH	15mg/kg(3times weekly)	15mg/kg(3times weekly)
Pyrazinamide	50mg/kg(3times weekly)	50mg/kg(3times weekly)

*or Streptomycin(15mg/kg or 0.71-1gm/day).

Clofazimine, Capreomycin, Cycloserin and Viomycin are the other second line drugs.

Antifungal agents:

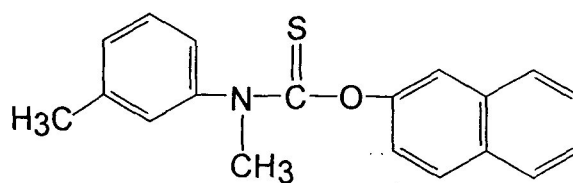
Most of the fungal infections (mycoses) involve superficial invasion of the skin or the mucous membranes of body orifices. These diseases, which can usually be controlled by local application of an antifungal agent, are conveniently divided into two etiological groups.

1) Dermatophytosis(Tinea infections), which are contagious superficial epidermal infections caused by various *Epidermophyton*, *Microsporum* and *Trichophyton* species;

2) Mycosis, caused by pathogenic saprophytic yeasts, which are contagious and include Aspergillosis, Blastomycosis, Candidiasis, Cryptococcosis, Histoplasmosis etc. Under certain conditions, some of these species are capable of invading deep body cavities and causing systemic mycosis, which are less contagious. Such infections may become serious and occasionally life- threatening and are difficult to treat.

During the earlier decades of the 20th century, the synthetic agents employed topically included fatty acids such as zinc undecylenate, salicylates such as N-phenyl salicyl amide, aniline dyes such as malachite green, gentian violet, quinolones such as 5-chloro-7-iodo-8-quinolinol, phenols like cresol, phenyl mercuric nitrate, chelating agents like biguanides etc. These were later replaced by Amphotericin B, Flucytosin and then more recently by two substituted imidazoles, Miconazole and Ketoconazole.

Tolnaftate:



(LXIX)

In 1960, the o-2-naphthyl ester of N-dimethyl thiocarbanilic acid (Tolnaftate), was found to have good activity against dermatophytes when applied topically. Poor permeability of Tolnaftate (LXIX) limits its use in therapy. It is available in a number of dosage forms for the treatment of ringworm, athletes foot etc.

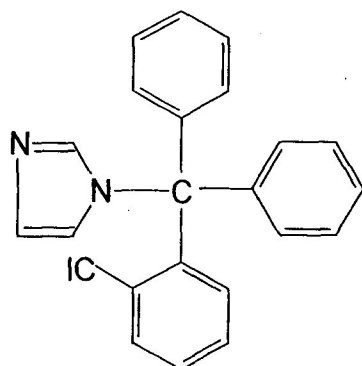
In susceptible fungi, Tolnaftate suppresses squalene epoxidase which converts squalene to ergosterol and thereby affects cell wall formation.

Substituted azoles:

The substituted imidazoles represent a versatile group of antifungal agents with a unique mechanism of action. The earlier members reported were Clotrimazole(LX)⁸⁹ and Miconazole (LXXI)⁹⁰, in 1969. These agents are active against dermatophytes such as *Trichophyton*, *Epi-dermophyton*, *Microsporum* and yeasts like *Candida albicans*. They are also active systemically in infections caused by *Cryptococci*, *Blastomyces*, *Histoplasma*, *Paracoccidioides*.etc.

The mechanism of action of the drug is elucidated⁹¹ extensively. At high concentrations (micromolar), they are fungicidal; while at low concentrations (nanomolar), they are fungistatic. The fungicidal effect is associated with damage to the cell wall with the loss of essential cell components, such as potassium ion and amino acids. The imidazole inhibits the cytochrome P-450 oxidase enzyme that catalyses the 14- α -demethylation of lanosterol to ergosterol.

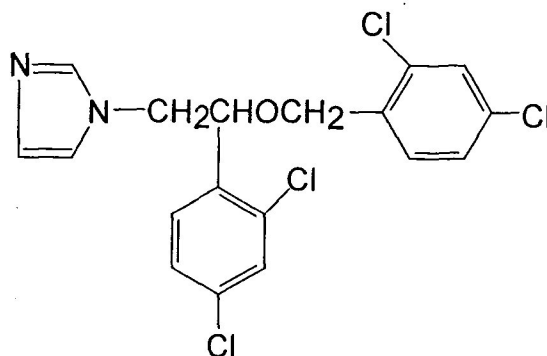
Clotrimazole:



(LXX)

Clotrimazole (LXX) is a broad spectrum antifungal agent used topically for the treatment of tinea infection and Candidiasis. As it causes severe gastric disturbances, it is not suitable for systemic use.

Miconazole:

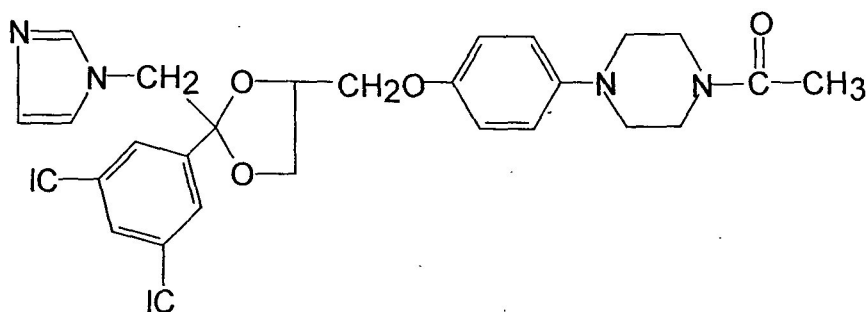


(LXXII)

Miconazole(LXXII) is intended for the treatment of serious systemic fungal infections like Candidiasis, Cryptococcosis, Petriellidiasis and Paracoccididomycosis. The adverse reactions include thrombophlebitis, pruritis, fever and G.I. disturbances.

Miconazole is solubilised with polyethylene glycol and castor oil to facilitate adequate intestinal absorption.

Ketoconazole:

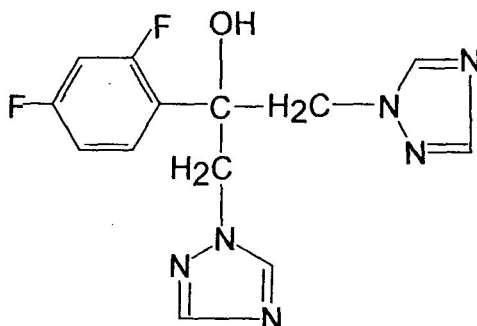


(LXXII)

Ketoconazole(LXXII) is administered orally for the treatment of systemic mycosis. It requires gastric acidity for absorption. Antacids and drugs like H_2 -histamine antagonists and anticholinergics that inhibit gastric secretion, interfere with its absorption. It is metabolised to the inactive form and is excreted by the enterohepatic route.

Ketoconazole is recommended for systemic mycosis such as Coccidiomycosis, Blastomycosis, Histoplasmosis, Chromomycosis, Paracoccidiomycosis and severe cutaneous infections. Hepatotoxicity is a major adverse effect. Also, levels of testosterone and corticosterone are lowered at high doses of Ketoconazole.

Fluconazole:

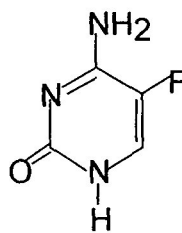


(LXXIII)

The absorption of Fluconazole(LXXIII) does not depend upon gastric acidity; moreover, the drug can be administered intravenously. It readily crosses the blood-brain barrier, and thus, is useful for fungal meningitis.

Other azoles include Econazole, Butaconazole, Fenticonazole, Oxiconazole, Sulconazole, Tioconazole, Itraconazole and Terconazole.

Flucytosine:



(LXXIV)

This fluorinated pyrimidine was first synthesized in the 1950s as an antileukemic drug. Flucytosine(LXXIV) has limited antifungal activity and sensitive strains of *Aspergillus*, *Candida*, *Cladosporium* and *Cryptococcus* are inhibited.

The incorporation of fluorinated pyrimidine into RNA, following selective deamination to 5-fluorouracil in the fungus, appears to be required for activity. 5-fluoro uracil (5-Fu) gets metabolised to 5-fluoro-2'-deoxyuridilic acid, which suppresses DNA synthesis by inhibiting thymidylate synthetase. 5-Fu is extensively used in the treatment of various human cancers.

Due to the chance of development of resistance, it is used in combination with Amphotericin B(polyene antibiotic), which lowers the risk.

Other active compounds include alkyl amines like Naftifine and Terbinafine, Triacetin, Acrisorcin, Haloprogin etc.

Antifungal antibiotics:

Polyene antibiotics:

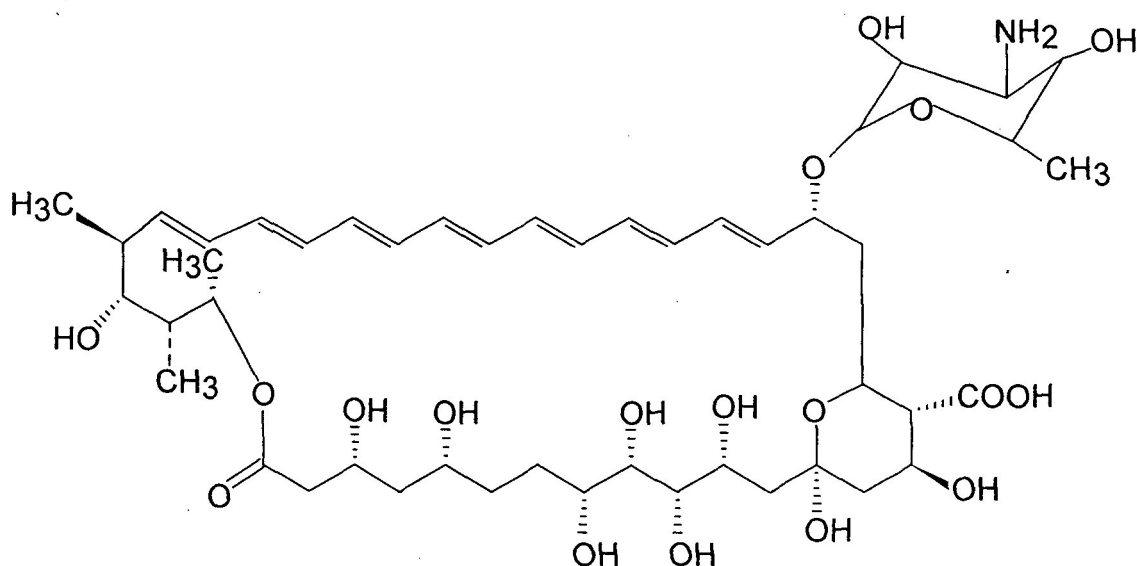
The polyene antibiotics contain conjugate double bonds in large lactone rings. They differ from macrolides in the size of the lactone ring and the presence of conjugated- 'ene' system.

They have a broad spectrum of activity against a number of species of pathogenic yeasts, molds and dermatophytes. Their low water solubility, low stability and toxicity are the major problems.

The mode of action involves binding with sterols in cell membrane to cause disorganisation and loss of cell constituents, especially potassium ion. The action is bacterio-static at low concentrations and bactericidal at the higher doses.

Amphotericin B:

A potent antifungal agent with a polyene structure was isolated in 1956 by Gold *et al*⁹² from *Streptomyces nodosum*, found in the soil from Venezuela. The antibiotic material was a mixture of two closely related compounds, Amphotericin A and B, of which B is more active.



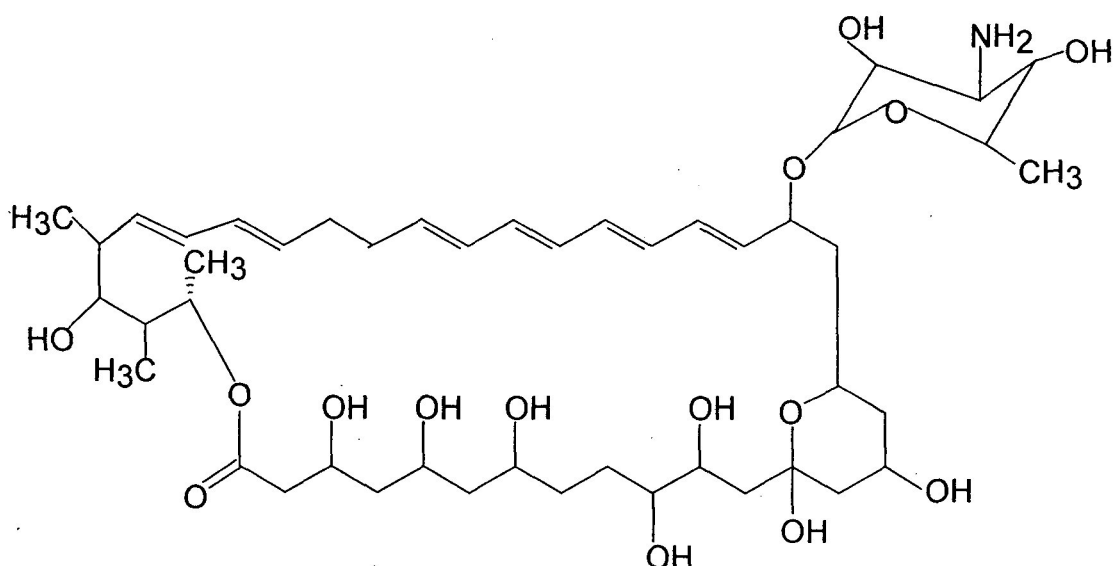
(LXXV)

Amphotericin B (LXXV) contains a primary amino group in the sugar moiety, mycosamine and a carboxyl group attached to the macrolide ring.

Parenterally, Amphotericin B is indicated for the treatment of serious, potentially life-threatening fungal infections, including disseminated forms of coccidioidomycosis, mucormycosis, aspergillosis, North American blastomycosis and cryptococcosis.

A high prevalence of adverse reactions limits its use. Nephrotoxicity occurs in 80% of the patients. General toxicity includes hypokalemia, distal tubular acidosis, fever, headache, anorexia, G.I. disturbances, muscle and joint pain, malaise etc. Pain at the site of injection and thrombophlebitis occurs on intravenous administration. It should not be administered intramuscularly. Amphotericin is also used topically to treat cutaneous and mucocutaneous infections.

Nystatin:



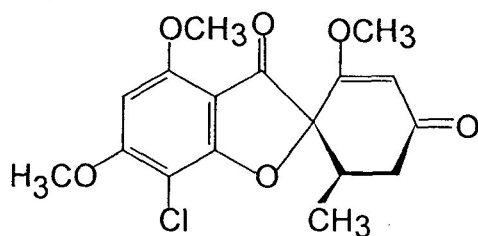
(LXXVI)

A polyene antibiotic first isolated in 1951 from a strain of *Streptomyces noursei* by Hazen and Brown⁹³, Nystatin (LXXVI) is a valuable agent for the treatment of local and gastrointestinal monolial infections caused by *Candida albicans* and other *Candida* species. It is also recommended for thrush or esophagitis caused by *C. albicans* in patients with AIDS. It is available in cream, ointment or powder form for topical use, whereas tablets or troches are used systemically.

Griseofulvin:

Griseofulvin (LXXVII) was first reported in 1939 by Oxford *et al*⁹⁴ as an antibiotic obtained from the mould *Penicillium griseofulvum* Dierckx. Its structure has been determined by Grove *et al*⁹⁵.

Griseofulvin is recommended for systemic treatment of refractory ring worm infections of the body, nails, hair and feet caused by various species of *Epidermophyton*, *Trichophyton* and *Microsporum*.



(LXXVII)

Relatively few adverse reactions have been reported. The most common are mild allergic reactions such as skin rashes and urticaria. Occasionally, G.I. upset, headache, dizziness or insomnia may occur.

Griseofulvin causes rapid, reversible dissolution of the mitotic spindle apparatus, apparently by binding with the tubulin dimer required for microtubular assembly⁹⁶.

The bioavailability of Griseofulvin may be enhanced by administration with fatty meal. It gets metabolised by methylation.

Several other antibiotics in use include Candidicin, Natamycin etc.

Novel approaches:

Out of the novel approaches to a fungal chemotherapy, one approach might be to persist the infected host to withhold growth essential iron from the fungi⁹⁷. Poorly soluble iron chelators such as hydroxy pyridon, rilopyrox are being developed for topical use in dermatophytosis.

Diamidines, hydroxy stilbamide and isethionate have been found to possess activity against Pneumocytosis, but have severe side-effects.

Plan of Work

PLAN OF WORK

Tropical diseases such as bacterial infections, fungal infections, helminthiasis etc. affect hundreds of millions of people every year, causing immense suffering and hindering development. This has been a hard fact even though considerable advances have been made in the field of chemotherapy of microbial diseases. The major problems such as fast emergence of resistance among the microorganisms and adverse effects that prevail along with the drug, further hampers the development in the field. Hence, the quest for ideal novel drug becomes mandatory. The requisite for the ideal drug being-

- i) Broad spectrum of activity.
- ii) Increased potency and decreased side effects.

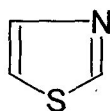
The present work is an effort directed towards the discovery and development of newer and better molecules as chemotherapeutic agents. It involves the synthesis of novel heterocyclic molecules and their evaluation as possible chemotherapeutic agents.

This research project involves the synthesis of 2 series of compounds, the details of which will be discussed in Part II and Part III of the thesis and also synthesis of a drug intermediate has been attempted in this project Part IV.

PART II :-

Synthesis of 2{4'-[1-(1,2,4-triazolyl) methyl] phenyl}-4-(substituted) thiazoles.

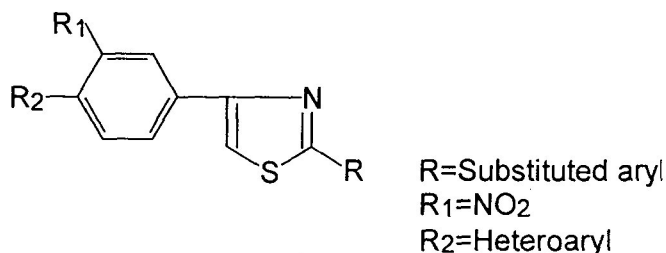
Thiazole derivatives have been reported to exhibit significant chemotherapeutic activities⁹⁸. Various therapeutically established compounds viz. Aminitrazole, Tetramisole, Thiazobenzazole, Niridazole, Sulfathiazole etc. all contain thiazole ring (I), in one form or another which is the pharmacophore responsible for the activity.



(I)

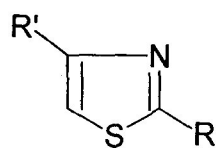
Several, literature reports further strengthened this observation.

Burmistov *et al*⁹⁹ reported the synthesis of substituted thiazole derivatives (II), possessing antibacterial and antifungal activities.



(II)

Prabhu *et al*¹⁰⁰ further reported good activity of 2,4-disubstituted thiazoles (III) against tubercle bacilli.

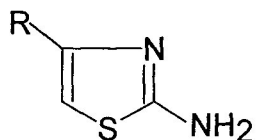


R= alkyl

R'= 2-pyrazinyl; 5-nitro-2'-fury

(III)

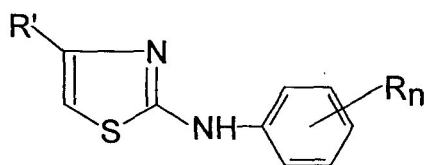
In 1965, Romanian research workers, Carp *et al*¹⁰¹, showed that an alkyl or substituted aryl group at the 4-position and amino group at 2-position of the thiazole imparts bacteriostatic activity to the thiazole molecule (IV).



R=— C₆H₅CH₃, — C₆H₅Cl, etc.

(IV)

Likewise, Gupta *et al*¹⁰² reported that the presence of phenyl group at the 4- position of thiazole ring (V) system increases the fungitoxicity in comparison to the methyl group. This activity is further enhanced by the presence of both halogen and nitro group at ortho or para position of the phenyl ring.

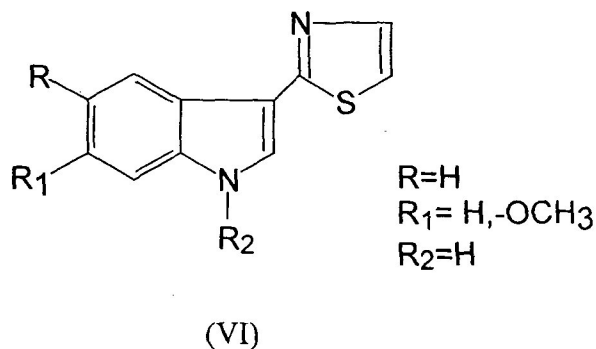


R'=methyl, phenyl.

R= halogen, nitro, methyl (n=2,3 or 4)

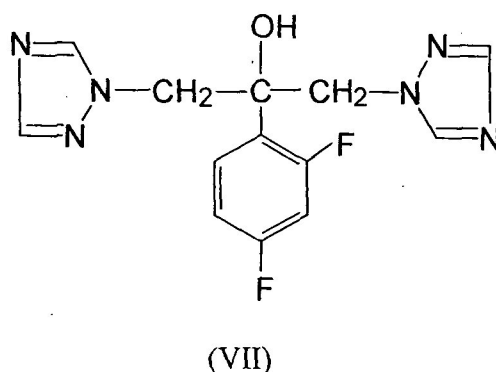
(V)

It has also been reported that thiazoles (VI) bearing other ring systems possess good antifungal activity¹⁰³.



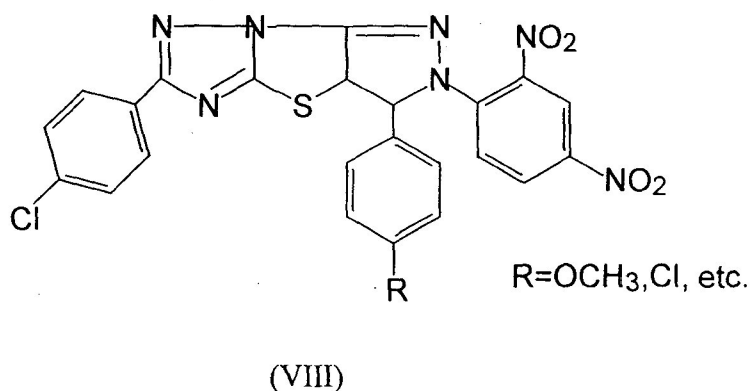
The above mentioned reports encouraged us to choose 2,4-disubstituted thiazole as the lead molecule of the series and further modifications were planned.

Introduction of 1,2,4- triazole was considered in the thiazole moiety as regards to its good antifungal activity . The fact ascertained by its presence in the recently developed broad spectrum antifungal drug, Fluconazole¹⁰⁴ (VII).

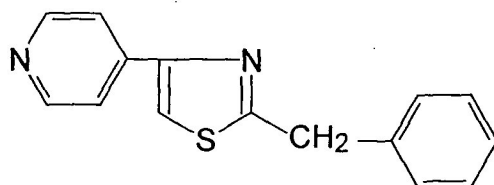


Several references indicating the activity of thiazolo triazoles were also obtained from the literature survey.

Jagmohan *et al*¹⁰⁵ have synthesized pyrazolo [3',4':4,5] thiazolo [3,2-b]-s-triazoles (IX), which were found to exhibit both antibacterial and antifungal activity.

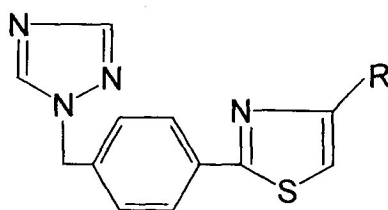


Boulton *et al*¹⁰⁶ synthesized 2-benzyl-4-(4'-pyridyl) thiazole (IX) with methyl phenyl substituent at 2-position of the thiazole ring and reported its immunoregulatory activity. Hence, we decided to introduce the methyl phenyl group at 1- position of 1,2,4- triazole and check for its pharmacological activity.



(IX)

Thus it was planned to select 2,4-disubstituted thiazole as the parent moiety with 1,2,4-triazolyl-1-benzyl- substitution at its 2-position and various halo substituted phenyl /heterocyclic substituents at its 4-position. Thus, a series of 2-{4'-[1-(1,2,4-triazolyl) -methyl] phenyl}-4-(substituted) thiazoles (X) synthesized and screened for possible antibacterial, antitubercular and antifungal activity.



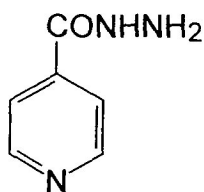
R=substituted phenyl /
heterocycle

(X)

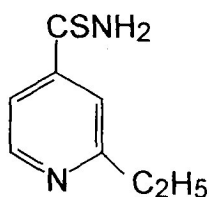
PART III :-

Synthesis of 4-methyl -2-(3-n-butyl-4- substituted-2,3-dihydrothiazole-2-ylidene) amino pyridines.

Pyridine, a well known moiety for its broad spectrum of biological activity¹⁰⁷, forms the pharmacophore of several important antitubercular drugs such as Isoniazide(XI), Ethionamide(XII) etc.

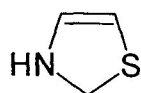


(XI)



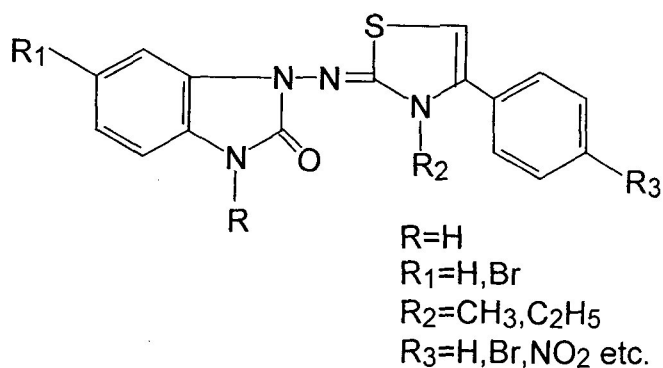
(XII)

Similarly, thiazoline (XIII) and its derivatives were also found to exhibit a wide variety of biological activities such as antimicrobial¹⁰⁸, antiinflammatory, antihistaminic etc.



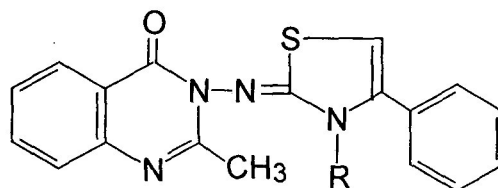
(XIII)

For instance, Karali *et al*¹⁰⁹ synthesized derivatives of 3-[3,4-disubstituted-4-thiazoline - 2-ylidene hydrazono]-1H-2-indolinones (XIV) as potent antitubercular agents having MIC <12.5µg /ml against H₃₇ Rv strain *M. tuberculosis*.



(XIV)

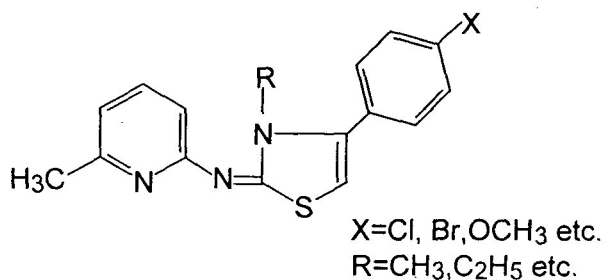
Ahmad and Fraglay¹¹⁰ synthesized 4-(3H)-quinoxolinone derivatives having thiazoline moiety at the 3-position (XV), which exhibited good antibacterial and antifungal activities.



R= Allyl, Benzyl, Phenyl etc.

(XV)

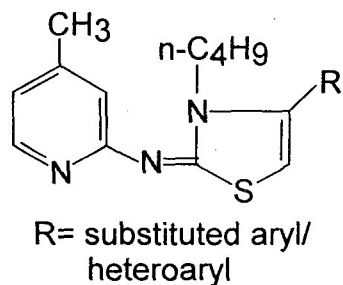
Recently, Hassan *et al*¹¹¹ have synthesized 6-methyl-2-(3,4-disubstituted-2,3-dihydrothiazole-2-ylidene)aminopyridine (XVI), which showed moderate activity against Gram positive bacteria.



(XVI)

The presence of an alkyl group in a compound is found to increase its lipophilicity, in turn leading to a better biological activity. With this fact in mind, the alkyl group, $n\text{-C}_4\text{H}_9$, was introduced on the parent thiazoline ring system.

Thus, considering the wide range of biological activities of both thiazoline as well as pyridine ring system, an effort was made to synthesize compounds combining both the ring system. Thus a series of compounds namely, 4-methyl-2-(3- n -butyl-4-substituted-2,3-dihydrothiazol-2-ylidene)aminopyridine (XVII) was synthesized, with probable antibacterial and antitubercular activity.



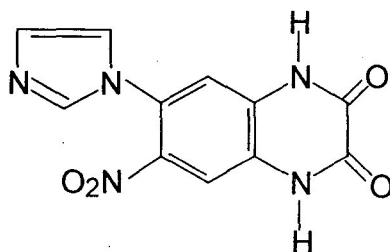
(XVII)

The current research venture has also been directed towards the modification of synthetic procedures of fine chemicals to improve upon the quality of the product, as regards economy, time and purity. Accordingly, the synthesis of an intermediate of YM-90K was attempted. The details are recorded in Part IV.

PART-IV:-

Synthesis of 6-(1-imidazolyl) quinoxaline-2,3(1H,4H)-dione. hydrochloride-An intermediate of YM-90K.

YM-90K [6-(1-imidazolyl)-7-nitro quinoxaline-2,3-(1H,4H)-dione hydrochloride] (XVIII), is a potent and selective AMPA receptor antagonist with neuroprotective action.

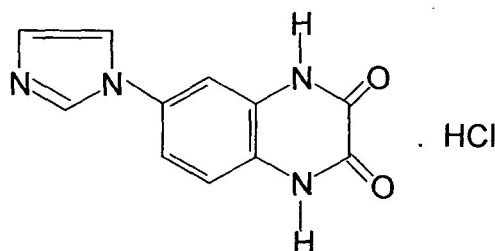


(XVIII)

AMPA or Alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid, a subtype of glutaminergic receptors, is known to mediate glutaminergic neurotransmission. In cases of cerebral ischemia, there is an excessive stimulation of AMPA receptors leading to neuronal cell death¹¹². Hence, there has been considerable interest in the design of an AMPA antagonist.

YM-90K is currently under phase-II clinical studies ¹¹³.

Considering the presence of fewer drugs of this particular class in the market, the synthesis of an intermediate for this drug, 6-(1-imidazolyl)quinoxaline-2,3-(1H,4H)-dione.hydrochloride(XIX), was planned.



(XIX)

The synthesis of this moiety involves three steps. Several modifications have been carried out in the literature procedure ¹¹⁴ in order to make the process more feasible and economical at industrial scale. The process is summarized in the following three steps:

Step-1:

Reaction of 5-chloro-2-nitroaniline with imidazole using Dimethyl sulfoxide (DMSO) in presence of KOH as solvent.

In the modified step, DMSO which is a costlier solvent, was replaced by Dimethyl formamide (DMF), a cheaper solvent in comparison, thus making the process more economical, industrially.

Step-2:

5-imidazolyl-2-nitroaniline obtained was then reduced by H₂ gas in the presence of Pd/C catalyst in 1N HCl, to give 5-imidazolyl phenylene diamine.

The modified step involved the replacement of the costlier Pd/C catalyst by the very cheap and easily available Sodium sulphide (Na₂S.XH₂O). Also, it did not involve the passing of hydrogen gas, thereby avoiding the hazards involved in the process.

Step-3:

5-imidazolyl phenylenediamine was cyclised with oxalic acid in 4N HCl giving 6-(1-imidazolyl)quinoxaline -2,3-(1H,4H)-dione hydrochloride.

These modifications are depicted in scheme-III.

Chemistry

CHEMISTRY

This part deals with the chemistry of the following compounds / intermediates involved in the project.

- * Thioamide**

- * Halogenation of ketones**

- * Thiourea**

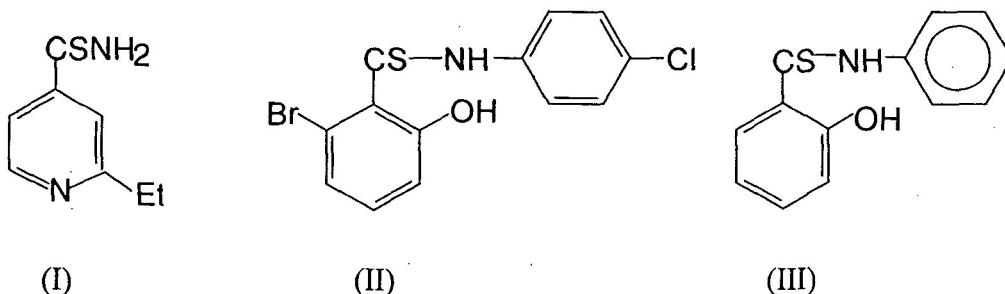
- * Thiazole / Thiazoline**

- * Reduction of Nitro Compounds**

- * Quinoxaline**

Chemistry of thioamides:

Liberman and coworkers, in 1955, observed the *in vivo* activity of Ethionamide (I) against *M. tuberculosis* and it was found to exhibit low toxicity. Later on, therapeutically effective agents such as substituted thiobenzamide (II), salicylic acid thioamide (III) were found.



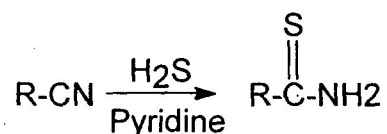
General synthetic methods:

1. From nitriles:

Nitriles in the presence of excess of hydrogen sulphide and a catalyst base yield thiocarboxamides^{115,116}.

Water-soluble sulphides or polysulphides can be used instead of hydrogen sulphide¹¹⁷.

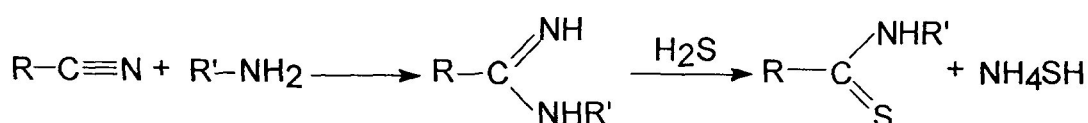
The reaction occurs as follows-



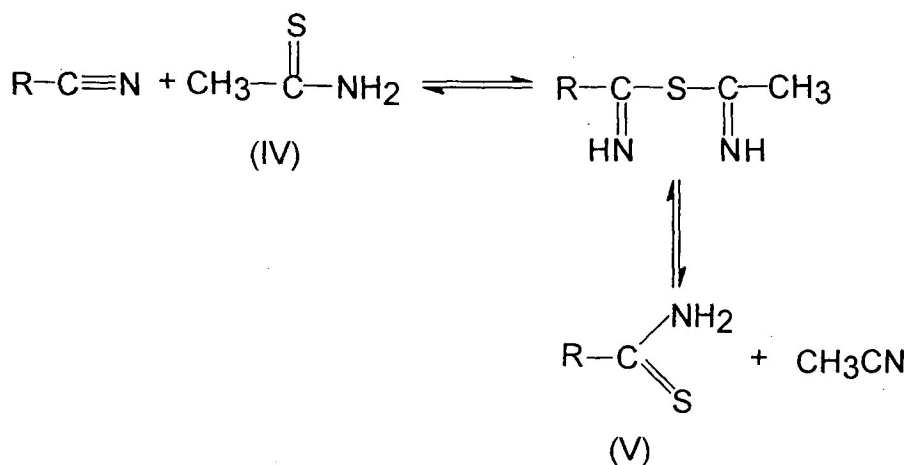
Catalysts used include ammonia, aliphatic amine, pyridine, alkoxide and potassium hydrogen sulphide^{118,119}. Fairfull *et al*¹²⁰ obtained very good yield by addition of H₂S to the nitrile in the presence of pyridine and triethylamine.

Aliphatic nitriles need elevated temperature and pressure¹²¹, as they react slowly and give poor yield.

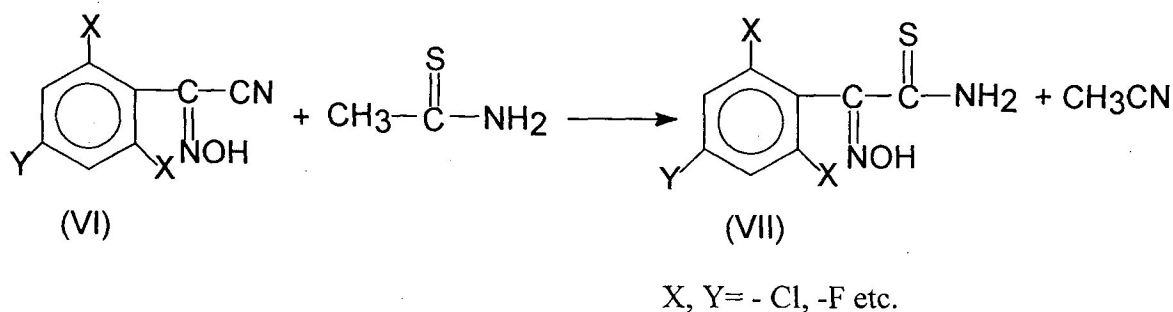
Kindler¹¹⁸ and Hanford¹²² extended the method by addition of primary amine.



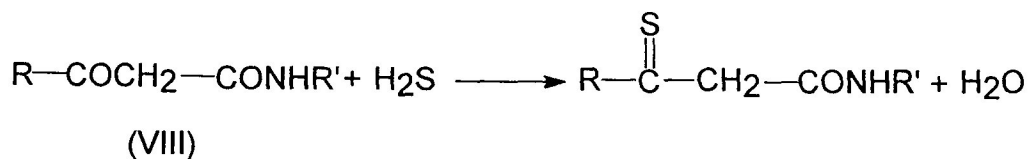
The reactions of nitriles with thioacetamide (IV) have appeared more frequently in literature, than with H_2S ^{123,124}.



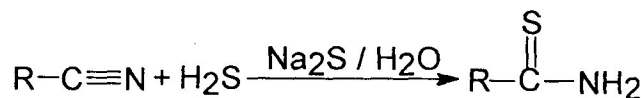
The removal of acetonitrile leads to the formation of thiocarboxamide (V) and thus is an important method for preparation of thiocarboxamide(VII) from α -cyanobenzaldoximes (VI).



If the nitrile contains a hydroxyl group, it must be protected by acylation^{125,126}. The keto group, such as that in (VIII), if present, is readily converted to thioxo in acidic solution¹²⁷.



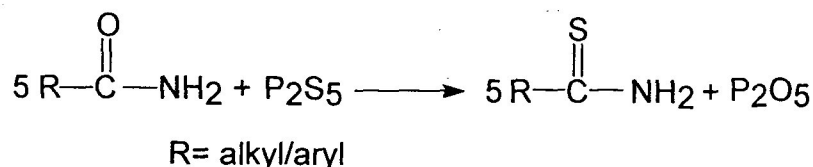
Mayer *et al*¹²⁸ explained the base-catalyzed conversion of ketone to thiones. Recently, Casser *et al*.¹²⁹, modified it and introduced aqueous sodium sulphide as a phase transfer catalyst in the general procedure to get high yields of thioamide.



This system consists of three phases, aqueous, organic and H_2S gas, providing higher yield of the aromatic thioamide.

2. Sulphurization of amides:

The replacement of oxygen of the carboxamide by sulphur is mostly used to obtain N-monosubstituted and N-disubstituted compounds in the presence of P_2S_5 ¹³⁰. Potassium thiophosphate, along with P_2S_5 , enhances the yield further¹³¹.

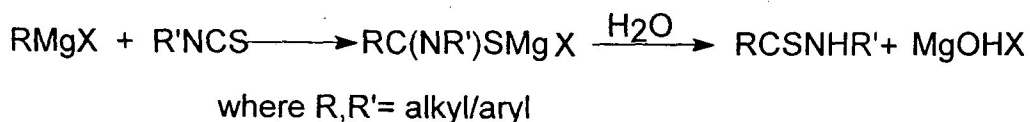


Solvents such as benzene, xylene, toluene, tetrahydrofuran, dioxane, diethylene glycol and diethyl ether¹³²⁻¹³⁵ are commonly used. They should be dry and inert in nature to avoid undesirable side reactions and to reduce the time.

The reactions in pyridine are reported to give pure thioamide in higher yields^{136,137}. The reactions of liquid amides are carried out in an excess of amide as solvent^{138,139}. Thermally unstable amides are not suitable for this reaction. Hydroxyl group must be protected by acylation before sulphurization^{140,141}.

3. From isothiocyanates:

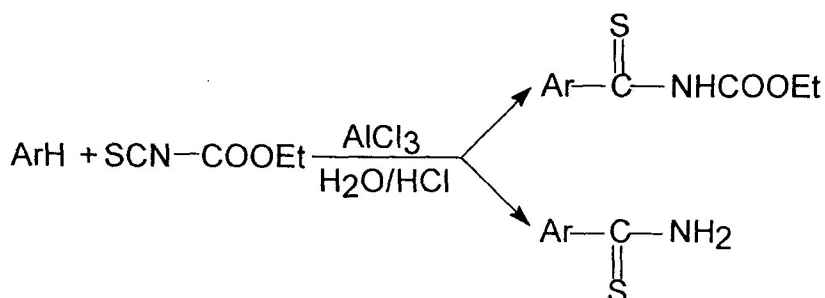
Sachs and Loevy¹⁴² described the reaction of isothiocyanates with Grignard's reagent, and subsequent hydrolysis under mild conditions, to yield the thiocarboxamide.



4. Single step synthesis of aromatic thioamides:

a) From ethoxy carbonyl isothiocyanate:

Ethoxy carbonyl isothiocyanate¹⁴³ reacts with aromatic compounds in the presence of anhydrous AlCl_3 (Fridel-Craft isothiocyanation). However, depending upon the conditions, it either yields the N-ethoxy carbonyl thioamide or thioamide itself.

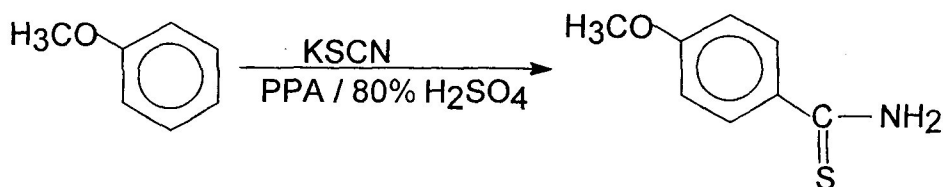


For thioamide to be a major product, the molar ratio of AlCl_3 to isothiocyanate should be 2:1. Thioamide takes a long period to form.

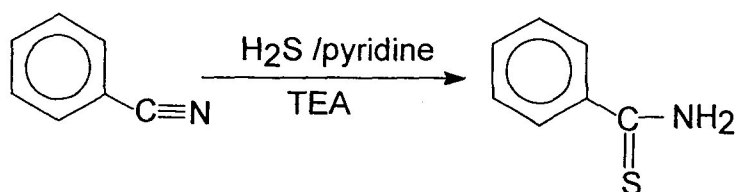
The reactions at a low temperature lead to N-ethoxy carbonyl thioamide.

b) Thioamides can also be prepared by heating anisole (0.01M) or veratrol at 60°C in the presence of polyphosphoric acid or 80% H_2SO_4 with potassium thiocyanate (0.01M)¹⁴⁴.

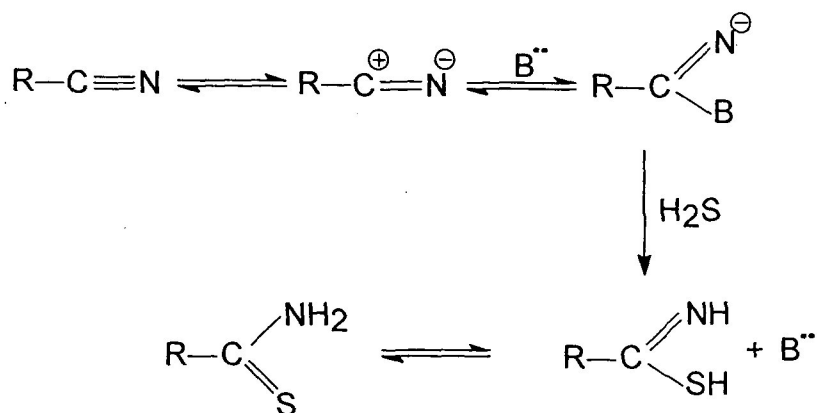
50% yield in PPA can be increased by 5-10% with 80% H_2SO_4 .



In the present work, thioamide was prepared by dissolving aromatic nitrile in pyridine and H_2S gas was bubbled into the reaction mixture, and triethylamine used as a catalyst.

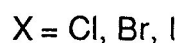
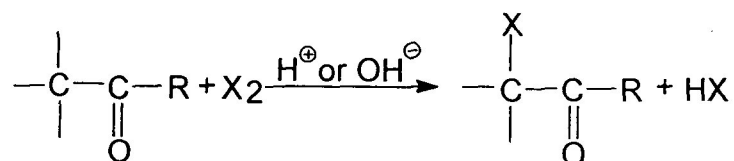


Mechanism:



Halogenation of ketones:

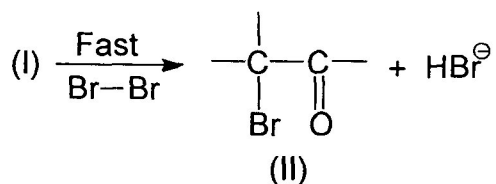
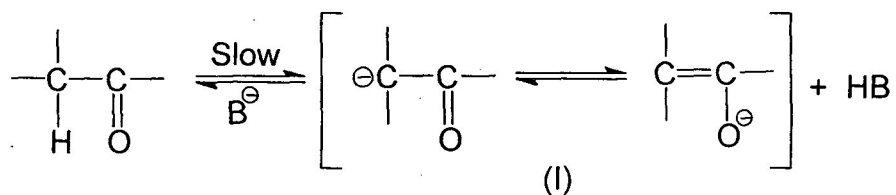
Ketones that possess an α -hydrogen can readily be converted to the corresponding halides by replacing the hydrogen with halogen, in the presence of acidic as well as basic medium.



General methods for synthesis:

1. Base-promoted halogenation of ketones:

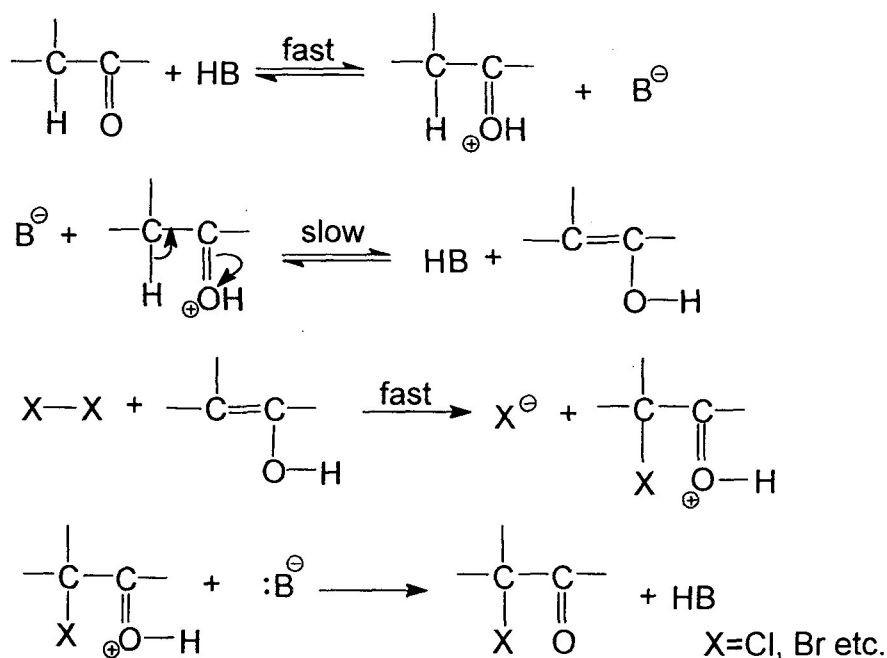
A base strongly abstracts a proton from a ketone to form the carbanion (I), which then rapidly reacts to yield the bromoketone (II).



The generation of carbanion determines the overall rate of the reaction. As soon as the carbanions are generated, they are snapped by the bromine molecule.

2. Acid-catalysed halogenation:

Acids also catalyses the halogenation of ketones.



The rate determining step is the formation of enol, which involves two steps-

- i) Rapid reversible protonation step of the carboxyl oxygen, followed by
- ii) Slow loss of an α -hydrogen. Once formed, the enol rapidly reacts with halogen to give the haloketone.

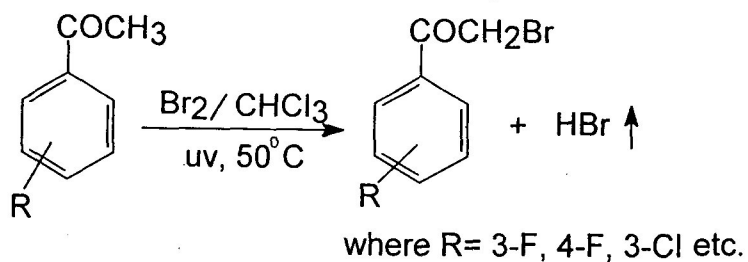
Solvents used include chloroform, acetic acid, carbon tetrachloride, diethyl ether, etc. When diethyl ether is used as the solvent, aluminium chloride is used to promote enolization.

Cupric halides have occasionally been used to halogenate aromatic ketones, particularly if the use of free halogen causes nuclear halogenation, e.g. with phenolic ketones. The copper (II) halide presumably catalyses enol formation and then the halogen gets transferred to the enol to give the α -haloketone.

3. Bromination:

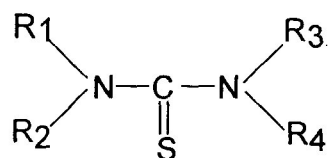
In case of bromination, the presence of sunlight (UV) or slight heating (60° - 65°C) facilitates the initiation of the reaction. It helps bromine to get polarized as the $\text{Br}^{\cdot}$ radical, which favours the attack of polarized Br_2 molecule or Br^- on the enol form of the ketone.

Bromination of various substituted acetophenones in the present work was carried out by dissolving them in chloroform and adding equimolar quantity of bromine dropwise with constant stirring. The reaction was carried out in the presence of light and the temperature was maintained at 50°C.



Chemistry of thiourea:

Thioureas are commonly represented by the general structure:



where $R^n = H$, alkyl, aryl, etc
($n=1,2,3,4$)

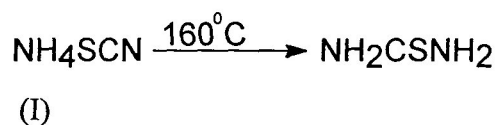
Thioureas are valuable synthons in organic synthesis especially in the field of heterocyclic chemistry.

The chemistry of thioureas deals with the reactions promoted by the nucleophilic reactivity of these compounds, the centre of reaction being isolated either at the S-atom or at the N-atom. The formation of thiazole ring with a heteroacetyl group is the pharmaceutically important feature of thioureas.

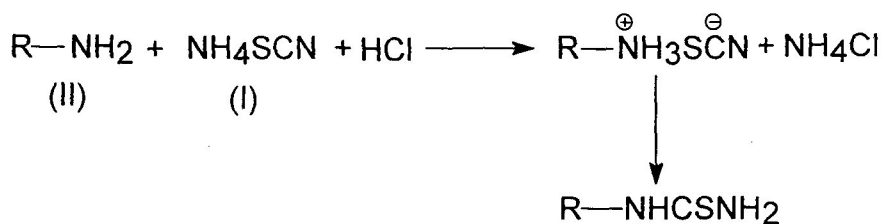
General methods for synthesis:

1. Action of alkali thiocyanate on amine hydrochloride:

It has long been known that heating ammonium thiocyanate (I), at 160° C for several hours, causes it to rearrange to thiourea.

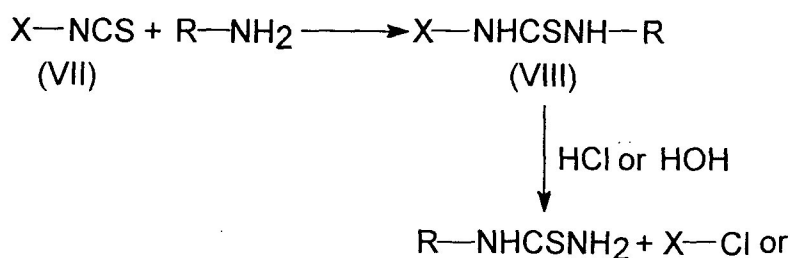


A mixture of a primary amine (II) and ammonium thiocyanate (I) is simply heated in the presence of hydrochloric acid in an inert solvent or aqueous medium to obtain the corresponding thiourea.



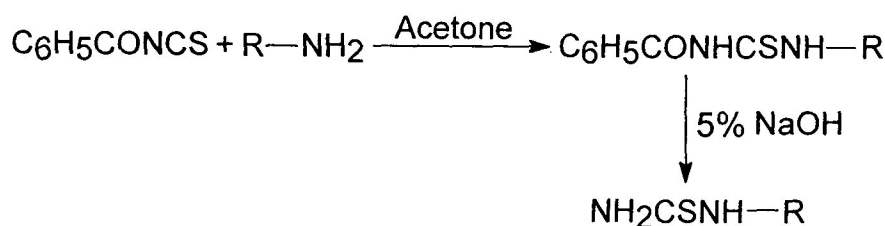
R= alkyl/substituted aryl

stituted and 1,1,3- trisubstituted thioureas ¹⁵¹. To achieve monosubstitution, the unwanted substitution is removed by alkaline hydrolysis.



where, X= -COPh, -COCH₃, -CO₂C₂H₅, t- butyl, -t-amyl etc
R= aryl/ heteroarylsubstituent

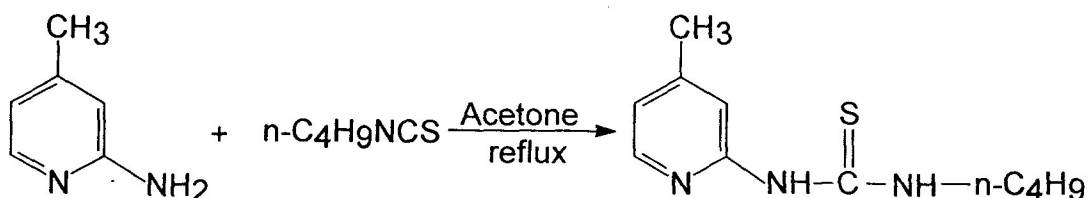
Rasmussen *et al* ¹⁵² reported improved procedures for the preparation of cycloalkyl-, arylalkyl-, arylthioureas by the reaction of benzoyl isothiocyanate with anilines in acetone, and debenzoylation of the resultant N-aryl-N'- benzoyl thioureas with 5% aqueous NaOH.



R = substituted aryl/ heteroaryl

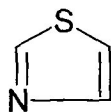
In the present synthetic work, 1,3-disubstituted thioureas have been synthesised .

The synthesis has been carried out by dissolving both 2- amino-4- methyl pyridine and n-butyliothiocyanate (1:2) in acetone separately. These were then mixed and refluxed for 8 hours. On cooling, a solid separated out which was then filtered and given a wash with hexane. Recrystallization was carried out using alcohol.



Chemistry of Thiazoles and Thiazolines:

The thiazole heterocycle (I) was first delineated by Hantzsch and Weber¹⁵³. Further, they described it as 'the pyrrole of the thiophene series'.

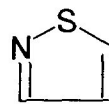


(I)

The thiazole moiety exists in two different configurations, (II) and (III).



(II)



(III)

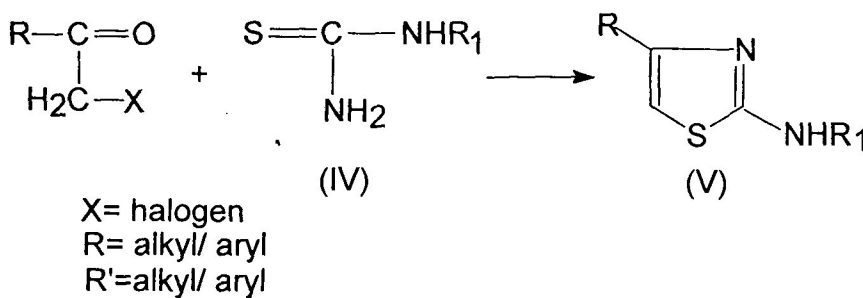
The high reactivity of the thiazole molecule is due to the group attached to the carbon between sulphur and nitrogen.

Large number of thiazoles occur naturally. They include Vitamin B (thiamine), Penicillin etc.

General methods for synthesis:

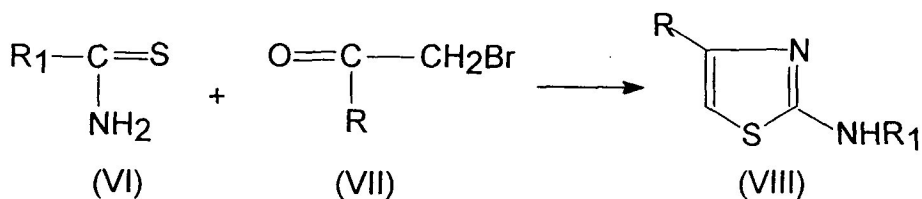
1. Synthesis of 2-substituted amino thiazoles:

The cyclization of N-monosubstituted thiourea (IV) with halocarbonyl compound gives 2-monosubstituted (V) thiazole^{154,155,156}.



2. 2,4-disubstituted thiazoles:

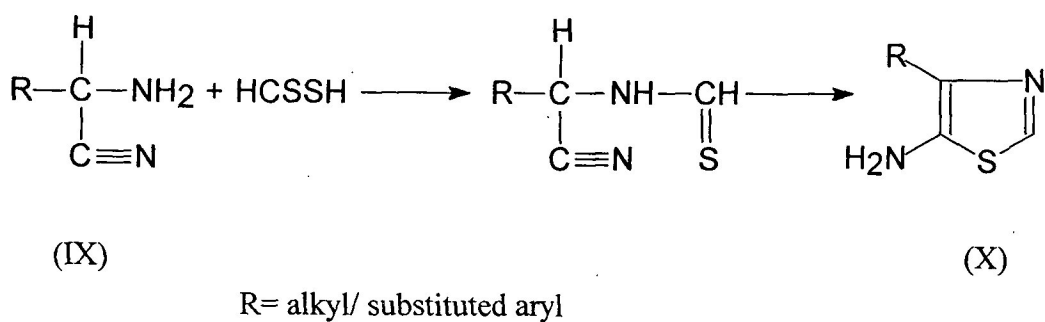
These were prepared in 1950 by Erylenmeyer *et al.*¹⁵⁷ from thiobenzamide (VI) and bromoacetophenone (VII) in alcohol as a solvent, which yielded 50-80% of the thiazole (VIII).



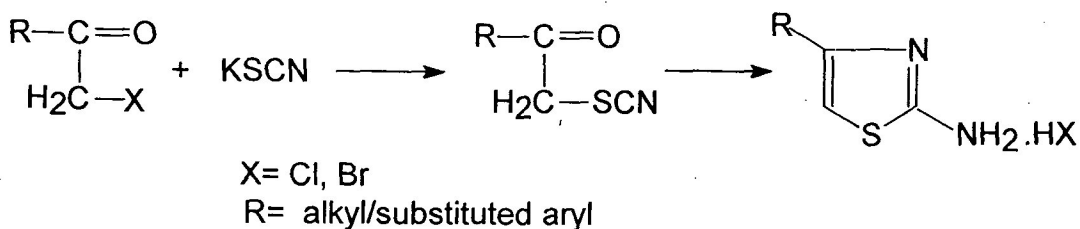
R= substituted aryl, R'= substituted aryl

Other synthetic methods for thiazole:

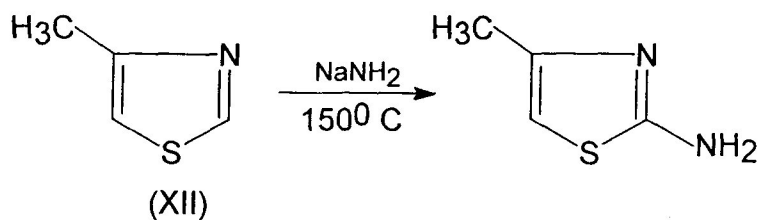
1. α -amino nitrile (IX) reacts with dithioformic acid (X) at room temperature to give an intermediate, which cyclizes to yield 5-amino thiazole derivative¹⁵⁸.



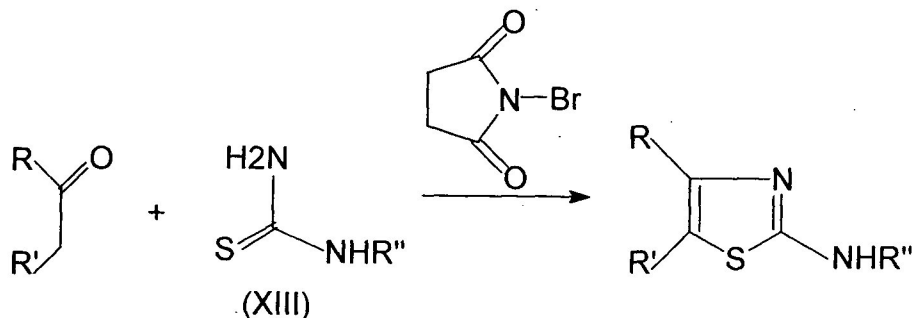
2. Potassium thiocyanate reacts with halocarbonyl compound (XI) to give 2-amino thiazole hydrochloride¹⁵⁹.



3. 4-methyl thiazole (XII) on heating with sodamide for 15 hours at 150°C yields 2-amino-4-methyl thiazole¹⁶⁰.

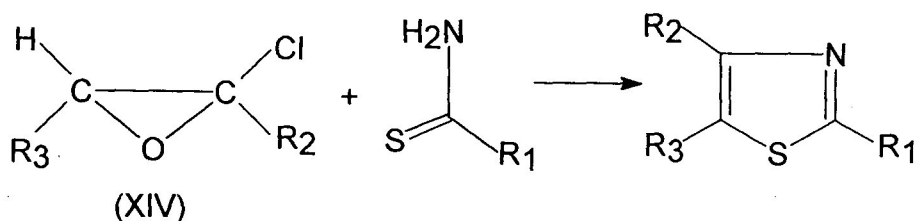


4. The reaction of substituted thiourea (XIII) with ketone in the presence of N-bromosuccinimide furnishes 2-aminothiazole derivative¹⁶¹.



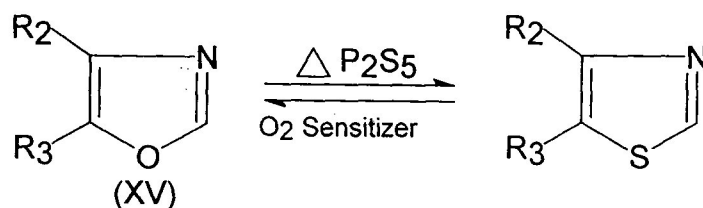
$R_1 = R_2 = R_3 = \text{substituted aryl/alkyl}$

5. 1-chloro-1,2 epoxide (XIV), when condensed with thioamide, leads to 2,4,5-trisubstituted thiazole¹⁶².



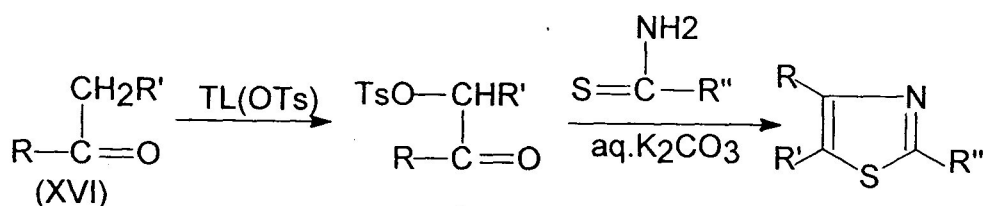
$R_1 = R_2 = R_3 = \text{substituted alkyl/aryl}$

6. Oxazole (XV), when heated with P_2S_5 , gives a thiazole¹⁶³.

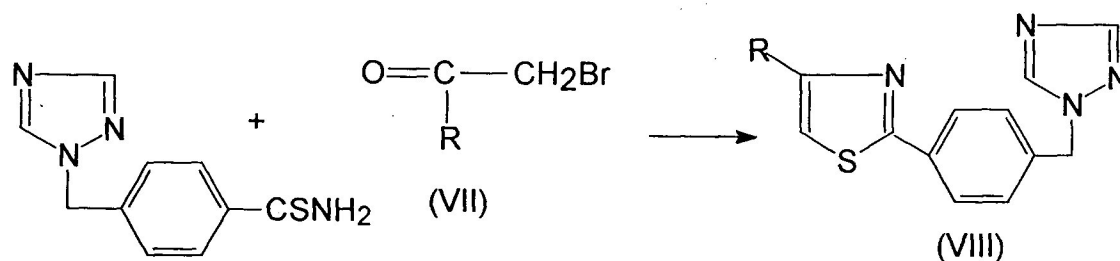


$R_2 = R_3 = \text{alkyl/ substituted aryl}$

7. Enolized ketone (XVI) reacts with thioamide or substituted thioamide in acetonitrile, in the presence of thallium p-tolyl sulphonate (TTS), to give 2,4,5-trisubstituted thiazole¹⁶⁴.



In the present work, 2,4-disubstituted thiazole has been prepared by refluxing a mixture of 1H-1,2,4-triazolyl methyl benzothioamide with various alpha bromo substituted phenyl / heterocyclic acetophenones (1:1) in dry alcohol.

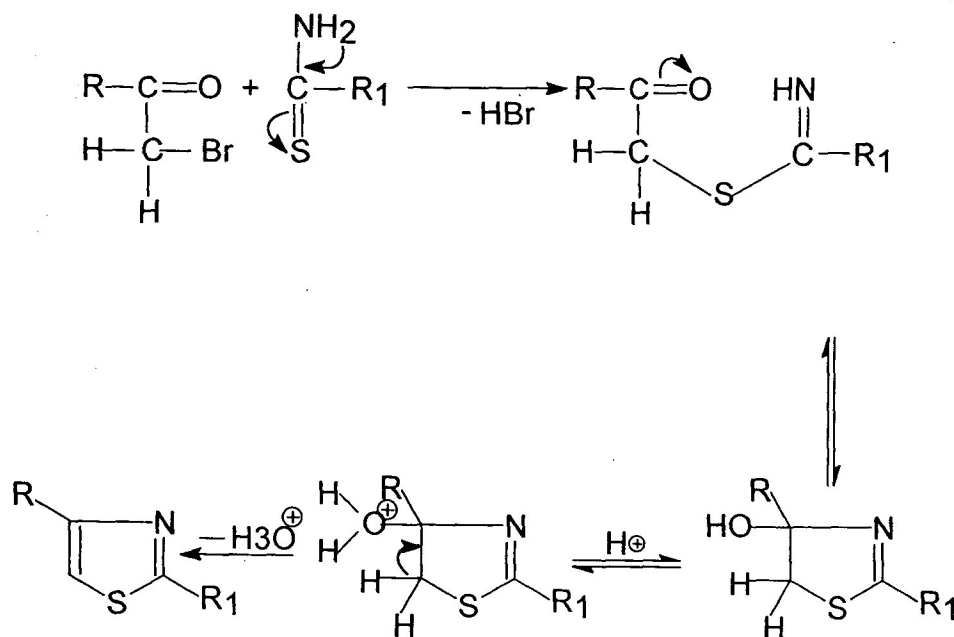


R= alkyl/substituted aryl

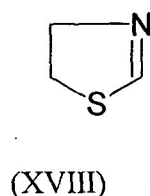
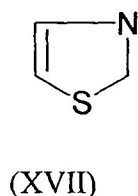
Mechanism:

The first step involves the formation of an acyclic intermediate by nucleophilic attack of the S-atom, by one of its lone pair of electrons, on the halogen carrying C-atom to form C-S-C bond.

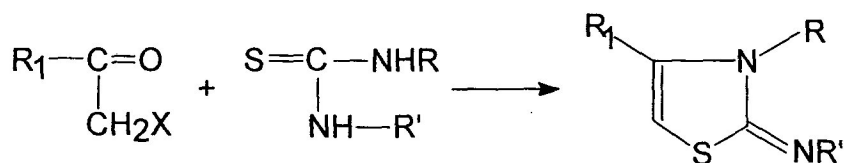
In the second step, the lone electron pair of N-atom attacks the carbon carrying the carbonyl function, giving rise to a new cyclic intermediate that reacts with acid leading to the thiazole molecule^{165,166}.



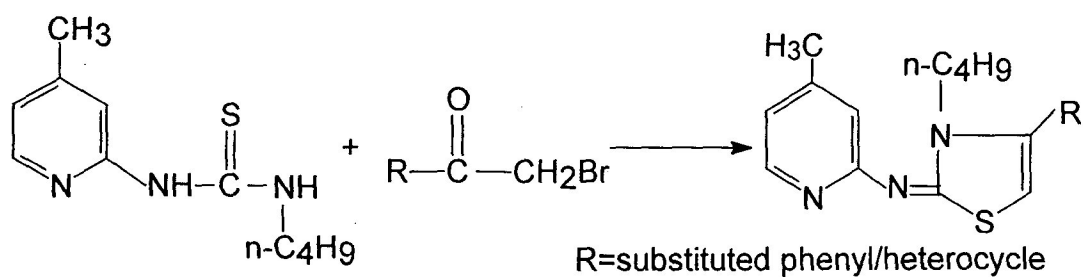
Thiazoline, another thiazole derivative, is present as 4,5-dihydrothiazole (XVII) and 2,3-dihydrothiazole (XVIII).



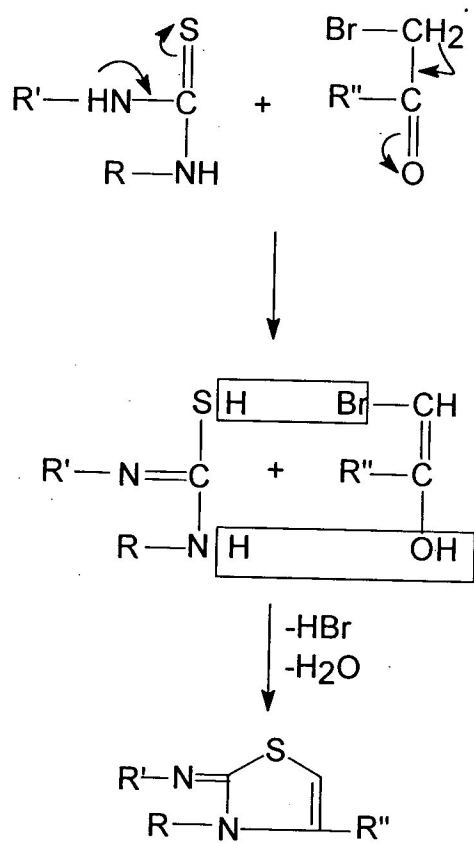
The symmetrical and unsymmetrical disubstituted thioureas do not give thiazole ring, but give thiazoline ring which is derived from the tautomeric imino form of the 2-aminothiazole¹⁶⁷. Mechanism of formation thiazoline is similar to that of thiazole ring



In the present work, 3,4- disubstituted thiazolines have been synthesized by reaction of N-n-butyl - N'- 4-methylpyridyl thiourea with various substituted phenacyl bromides in dry alcohol.



Mechanism :



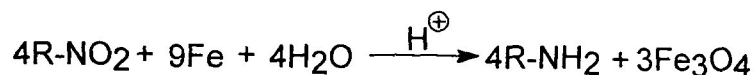
Chemistry of reduction of nitro compounds:

Various methods have been reported in the literature for the reduction of the nitro group, viz. reduction using metals such as iron, tin, zinc (in acidic condition), catalysts such as ferric chloride, platinum dioxide, Raney nickel, and also by using sodium sulphide, ammonium polysulphide (alkaline medium) etc.

The reduction of nitro compounds involves the progressive replacement of oxygen in the $-\text{NO}_2$ group by hydrogen. Under suitable conditions, aromatic nitro compounds are readily reduced to amino compounds by means of acidic, reducing system such as metals as iron and tin. In the solution of the metal in acetic acid, the nitro compound is reduced and the amine may be recovered from the reaction mixture by making it alkaline, by steam distillation or by filtration.

Metals used in reduction:

If the catalytic methods are avoided, iron and zinc are the metals of choice for reductive procedures, in the research laboratory. The advantage of iron is that only a catalytic amount of acid is required to be added and the iron oxide precipitates out to permit simple separation.



The disadvantage is that the quality of iron powder from various commercial sources is variable. Some powders give spontaneous exothermic reduction. Reduction with this reagent must be carried out in benzene or aqueous alcohol. Furthermore, iron powder in acetic acid is capable of reducing aliphatic optically active nitro compounds to the amines, with the retention of configuration. On the other hand, basic reducing agents such as lithium aluminium hydride lead to racemization.

Zinc is a powerful reducing agent, but the success rate is very less by this method. For efficient reduction, a constant amount of zinc is required to be supplied during the entire reaction period. If fortunate enough, the amine hydrochloride will separate out. If not, then the entire reaction mixture should be made strongly alkaline and extracted with a suitable solvent. During this process problem of emulsion formation is faced very often.

Though there is a smooth generation of hydrogen from mossy tin and acid, the difficulty in work up makes this method less suitable for large scale reductions.

On the other hand, stannous chloride is a useful and selective reducing agent for nitro groups when carbonyl groups are present, such as in reduction of *m*-nitrobenzaldehyde. Amal-

amalgamated aluminium is another reducing agent best adopted in neutral or basic reducing conditions. For instance, 2-nitronaphthene suspended in boiling ethanol, when treated with amalgamated aluminium and then portionwise with boiling water, is reduced in 85 % yield to the corresponding amino compound.

Catalysts used in reduction:

The most commonly used catalysts for the reduction of nitro compounds are platinum (low pressure) and Raney nickel (high pressure). Supporting catalysts such as platinum on carbon^{168,169} and palladium on carbon¹⁷⁰ are also used frequently.

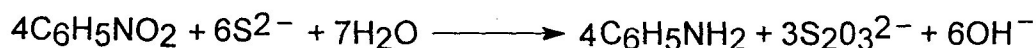
The solvent used in the reaction should be pure and inert in nature. Water is the safest solvent but many organic compounds remain insoluble in it. The most commonly used solvents are methyl- and ethyl- alcohol. Benzene may be used (only if thiophene free), although it attacks the rubber stoppers in the parr hydrogenator apparatus and is itself strongly bound to the surface of most catalysts, resulting in a decrease in the reaction rate. Solvents that form peroxide should be avoided. For example, dioxane should be used after purification and distillation.

Reduction, using catalysts, provides product with lesser impurities and thus enables clean and easy workup^{171, 172}. There are many disadvantages associated with the usage of catalysts. Highly inflammable hydrogen gas has to be passed in the reaction, which makes the process hazardous, industrially¹⁷³. Further, there are chances of poisoning of the catalyst if not handled or discarded properly. Again, costly apparatus is required to carry out this process.

Reduction using Sulphides and Polysulphides:

The reduction of nitroarenes by negative divalent sulfur (sulfide, sulfhydrylate and polysulfides) is referred to as Zinin reduction¹⁷⁴. This is a rather simple and versatile reaction that can be carried out using standard laboratory equipments and has been used for the plant scale manufacture of aromatic amines, when other reduction media are destructive or result in undesired side reactions.

The stoichiometry of Zinin reduction is as follows:



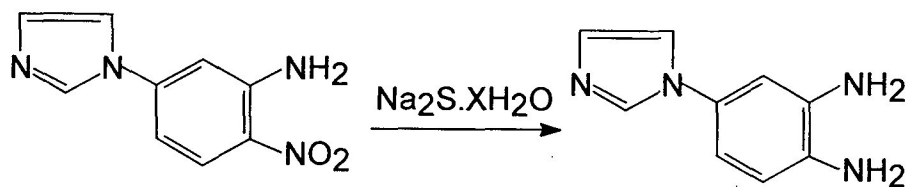
The reagents used generally are ammonium, sodium or potassium sulfides. Manganous and Ferrous sulfide are also used. Commonly used solvents are methyl and ethyl alcohol.

Another unique aspect of this reaction is its ability to selectively reduce one nitro group in a dinitro or trinitro arene.

Considering the simplicity, economy and safe features of the reaction, reduction in the present work was carried out using Sodium sulfide.

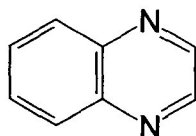
Synthesis of 5-(1-imidazolyl)-o-phenylenediamine:

5-(1-imidazolyl)-2-nitroaniline was dissolved in methanol and to it was added sodium sulfide, portionwise with continuous stirring. The reaction mixture was refluxed for 4 hours and then methanol removed under reduced pressure. The residue obtained was extracted using chloroform. The chloroform layer was then concentrated to obtain the diamine.



Chemistry of Quinoxaline:

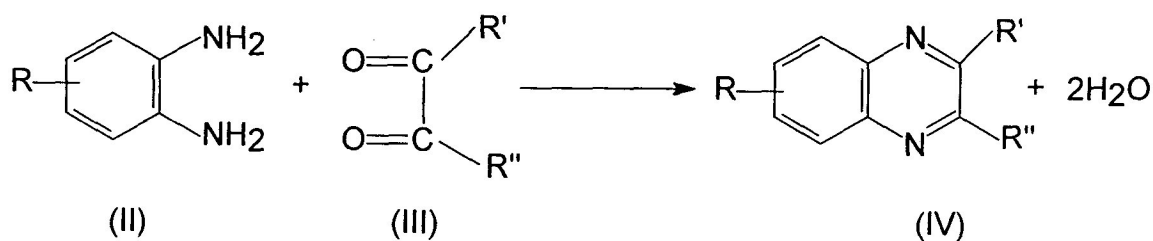
Quinoxaline (I) is an eight membered heterocyclic ring system.



• (I)

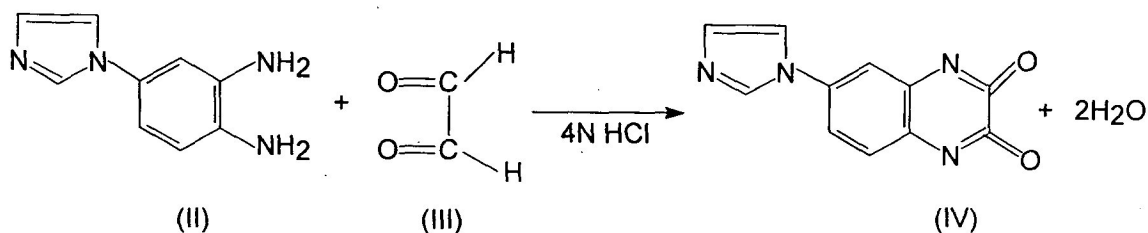
Vast majority of quinoxalines are of synthetic origin, and with very few exceptions, the synthetic method used is to condense an o-disubstituted benzene with a two carbon synthon.

Thus, condensation of o-phenylenediamines (II) with aliphatic dicarbonyl compounds (III) results in quinoxaline (IV), as shown in the Scheme below.



By using suitable substituted o-phenylenediamine and dicarbonyl component, various types of substituted quinoxaline derivatives have been synthesised ¹⁷⁵.

In the present work, the required quinoxalinone derivative was prepared by dissolving both 5-(1-imidazolyl)-o-phenylenediamine and oxalic acid in 4N HCl and refluxing it overnight. The solid obtained was filtered and then recrystallised from water to obtain the final product.

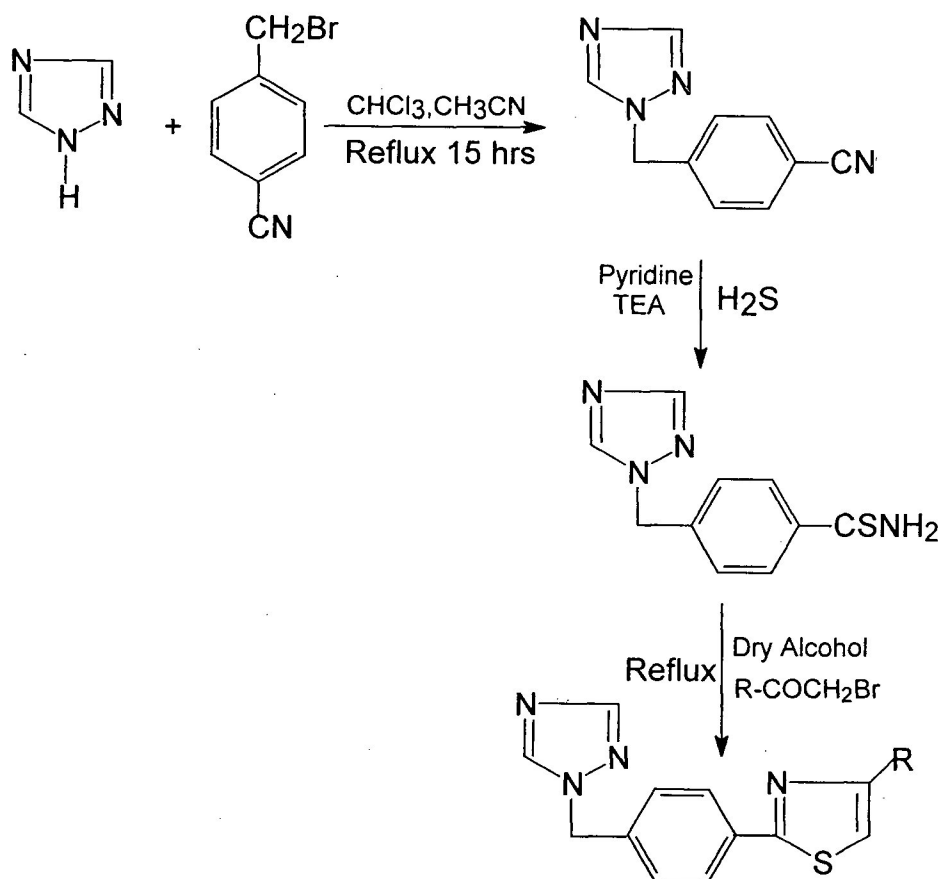


Schematic Representation

SCHEMATIC REPRESENTATION OF THE COMPOUNDS SYNTHESIZED:

Scheme I :

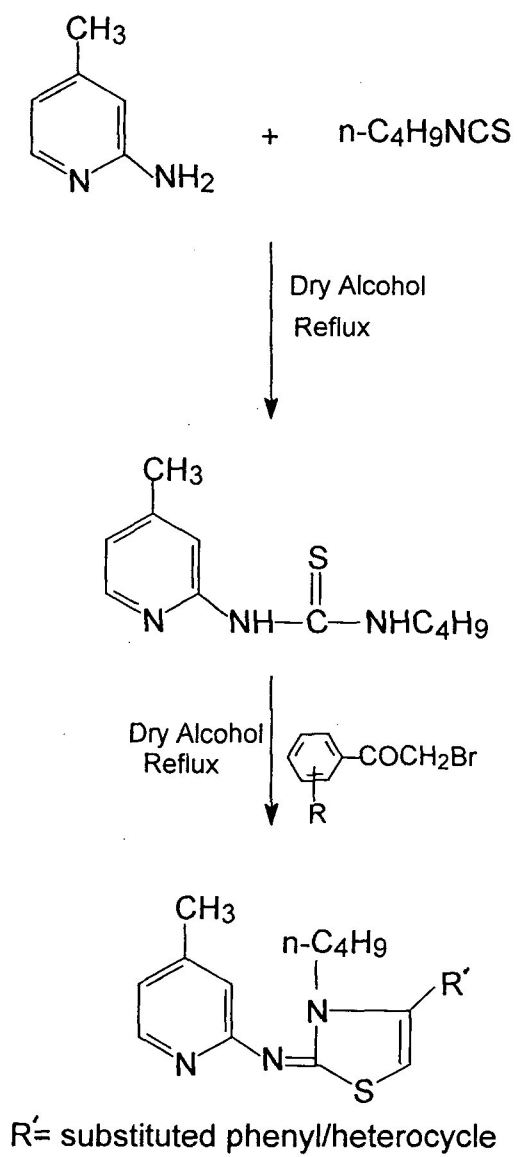
Synthesis of 2-{4'-[1-(1,2,4- triazolyl) methyl] phenyl }-4-(substituted) thiazoles.



R= substituted phenyl/ heterocycle

Scheme II :

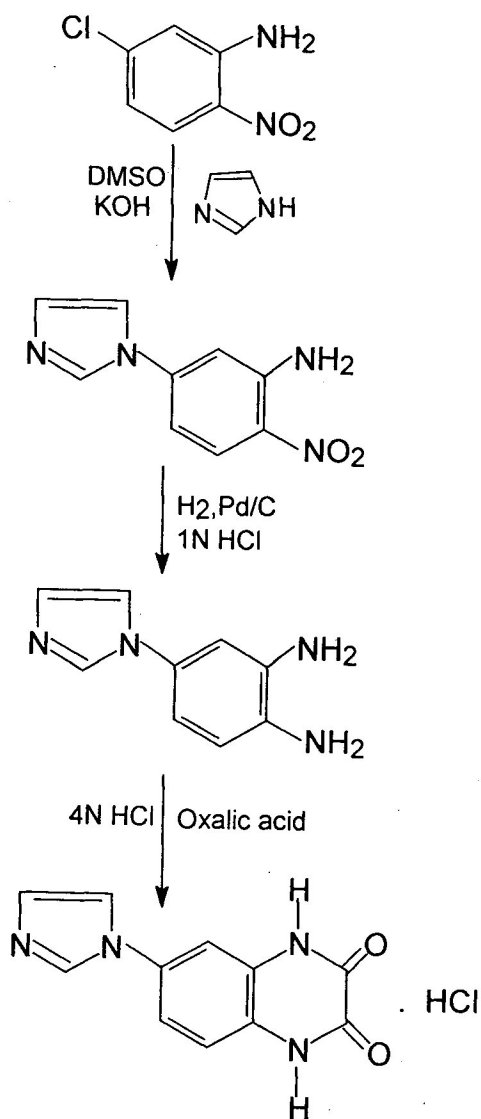
Synthesis of 4-methyl-2-(3-n-butyl-4-substituted-2,3-dihydrothiazol- 2-ylidene) aminopyridine.



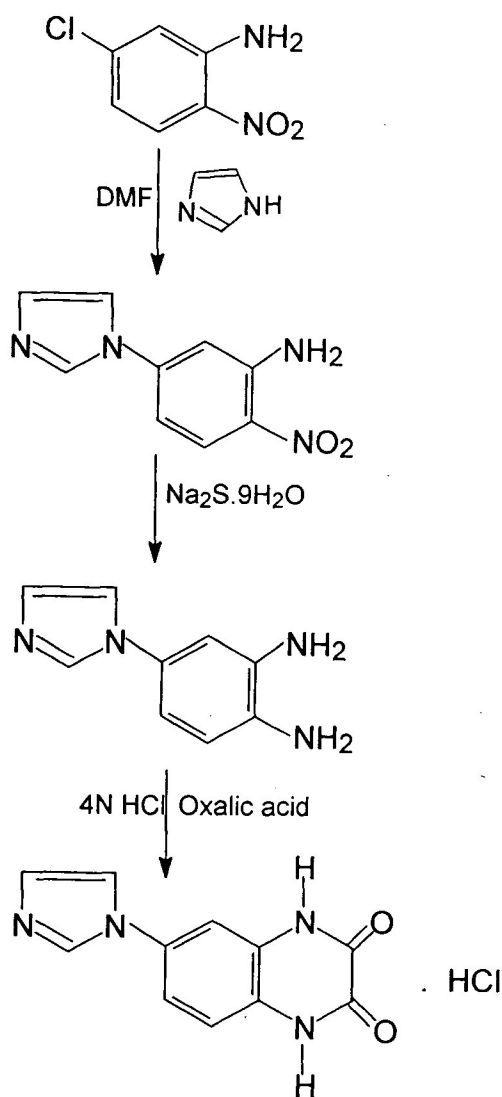
Scheme III :

Synthesis of 6-(1-imidazolyl) quinoxaline-2,3(1H,4H)-dione hydrochloride.

Literature scheme



Modified scheme



Experimental

EXPERIMENTAL

This section of the thesis deals with the synthetic details of the compounds synthesized. The purity of the raw materials used in various synthetic reactions were ascertained by melting point (Confirmed by the literature M.P.) and TLC. The completion of the reaction was monitored by TLC and reaction continued until both the reactants were consumed or largely consumed. Product isolated, purified and recrystallized using suitable solvent system. The product obtained was then characterized by elemental and spectral analysis.

Part II :

Synthesis of 2-{4'-[1-(1,2,4- triazolyl) methyl] phenyl }-4-(substituted) thiazoles.

Step-I: Synthesis of 4-[1-(1,2,4- triazolyl) methyl] benzonitrile:

4- cyanobenzylbromide (6g, 0.0306 M) and 1,2,4- triazole (9.27g, 0.1344 M), both dissolved in a mixture of chloroform (180 ml) and acetonitrile (36 ml), were stirred and refluxed for 15 hours. The solution was cooled and neutralised with 3% aqueous Sodium bicarbonate solution. The organic solution dried (over anhydrous Sodium sulphate) and evaporated. The residue was then chromatographed on silica gel (80-120 mesh). Elution with chloroform / isopropanol (9:1) gave the product, which was then recrystallised from methanol.

M.P = 77^o- 79^o C.

Lit. M.P.¹⁷⁶ = 77- 79^o C.

Yield = 57.6 %.

Elemental analysis: C₁₀H₈N₄

	C%	H%	N%
Calculated	65.21	4.34	30.34
Found	65.51	4.70	30.35

Step-II: Synthesis of 4'-[1-(1,2,4- triazolyl) methyl] benzothioamide:

4-[1-(1,2,4- triazolyl) methyl] benzonitrile (5g, 0.0271M) was dissolved in pyridine (25 ml) in a three-necked round bottomed flask. Triethylamine (4 ml) was added to it and the reaction mixture was placed in an icebath. H₂S gas was bubbled through the solution continuously for 10 hours. The reaction mixture was then poured on crushed ice and the solid obtained was filtered and recrystallised from ethanol.

H₂S gas was generated by the action of conc.HCl on ferrous sulphide.

M.P =187-189° C.

Yield = 68.5%.

Elemental analysis: C₁₀H₁₀N₄S

	C%	H%	N%
Calculated	55.04	4.58	25.68
Found	54.64	4.31	25.96

Synthesis of p-fluorophenacylbromide:

4- fluoroacetophenone (3g, 0.0218 M) dissolved in dry chloroform in a 100 ml 3- necked round bottomed flask, equipped with a dropping funnel and a water condenser fitted with a guard tube (anhydrous CaCl₂). The reaction mixture was then exposed to a light source of 200 watts. Bromine (3.48g, 0.0218 M) dissolved in 10 ml dry chloroform was added dropwise with continuous stirring into the reaction mixture. Addition of the next drop of bromine was done only after the previous drop was consumed, marked by the disappearance of the brown color. The reaction mixture was warmed to 50° C for half an hour. Then a stream of dry air was passed through the reaction mixture to sweep out the HBr. The reaction mixture was diluted with petroleum ether and refluxed to dissolve the syrupy mass. The solution was then purified and concentrated to obtain an oily liquid, which crystallised on cooling. The product was further recrystallised from ethanol.

M.P = 48-50° C

Lit. M.P.¹⁷⁷ = 47- 49° C.

Yield = 61.7%

Rest of the phenacyl bromides used in the reactions were synthesized in similar manner and obtained from the laboratory.

Step III: *Synthesis of 2-{4'-[1-(1,2,4- triazolyl) methyl] phenyl}-4-(4'- chlorophenyl) thiazole.*

4- [1- (1,2,4-triazolyl) methyl] benzothioamide (0.5g,0.0023M) and 4- fluorophenacylbromide (0.5g, 0.0023M) were both dissolved seperately in dry alcohol (25 ml), mixed in a 50ml round bottom flask fitted with a reflux condenser and refluxed for 8 hours. The excess alcohol was removed under reduced pressure and the solid product isolated and recrystallized from methanol.

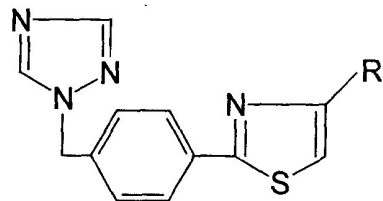
M.P = 155- 156° C.

Yield =52.5%

Elemental analysis: $C_{18}H_{13}ClN_4S.HBr$

	C%	H%	N%
Calculated	49.82	3.22	12.91
Found	50.20	2.85	12.59

Table I : Physical constants of 2-{4'-[1-(1,2,4- triazolyl) methyl] phenyl }-4-(substituted)-thiazole.



R=substituted phenyl /
heterocycle

Sr.No.	R	Molecular Formula	M.P(^o C)	Yield (%)	Elemental Analysis					
					Calculated(%)			Obtained(%)		
					C	H	N	C	H	N
1.	2-fluorophenyl	C ₁₈ H ₁₃ FN ₄ S.HBr	213-215	59.2	54.67	3.11	13.42	54.32	2.87	13.11
2.	3-fluorophenyl	C ₁₈ H ₁₃ FN ₄ S.HBr	212-214	55.6	54.67	3.11	13.42	54.98	3.26	13.13
3.	4-fluorophenyl	C ₁₈ H ₁₃ FN ₄ S.H ₂ O	155-156	52.5	64.40	3.95	15.81	64.79	3.70	15.53
4.	3-chlorophenyl	C ₁₈ H ₁₃ ClN ₄ S.HBr	151-153	49.3	49.82	3.22	12.91	49.52	2.98	12.98
5.	4-chlorophenyl	C ₁₈ H ₁₃ ClN ₄ S.HBr	211-213	48.4	49.82	3.22	12.81	50.20	2.85	12.59
6.	2,4-dichlorophenyl	C ₁₈ H ₁₂ Cl ₂ N ₄ S.HBr	230-232	60.5	46.15	2.77	11.96	46.49	2.98	12.01
7.	2,3-dichlorophenyl	C ₁₈ H ₁₂ Cl ₂ N ₄ S.HBr	229-231	52.5	46.15	2.77	11.96	45.85	2.39	12.34
8.	3,4-dichlorophenyl	C ₁₈ H ₁₂ Cl ₂ N ₄ S.HBr	229-231	59.6	46.15	2.77	11.96	46.55	3.01	12.30
9.	5-chlorothiophene	C ₁₆ H ₁₁ ClN ₄ S ₂ .HBr	220-222	57.9	47.11	2.94	13.74	47.52	3.11	14.09
10.	2,4-dichloro- 5-fluorophenyl	C ₁₈ H ₁₁ Cl ₂ FN ₄ S.HBr	260-262	53.87	44.44	2.26	11.52	44.05	2.21	11.49
11.	3-trifluoromethyl phenyl	C ₁₉ H ₁₃ F ₃ N ₄ S.HBr	219-221	45.0	48.8	2.99	11.99	48.45	2.65	12.35
12.	2-hydroxy-4- methoxyphenyl	C ₁₉ H ₁₆ Cl ₂ ON ₄ S.HBr	180-182	61.01	51.23	3.59	12.58	50.91	3.25	12.22

Spectral Characterization:

1) 2-{4'-[1-(1,2,4-triazolyl) methyl] phenyl}-4-(2'-fluorophenyl)thiazole (Compd. No.1)

IR(KBr) cm^{-1} : 1575.47, 1488.17, 1057.05 (thiazole); 3078.2, 1443.02 (- CH_2);
976.94 (1H-1,2,4-triazole).

2) 2-{4'-[1-(1,2,4-triazolyl) methyl] phenyl}-4-(4'-chlorophenyl) thiazole (Compd.No.5)

IR(KBr) cm^{-1} : 1587.94, 1475.19, 1037.36 (thiazole); 3015.6, 1450.04 (- CH_2);
977.95 (1H-1,2,4-triazole).

PMR (DMSO) : δ 5.5 (s, - CH_2),
 δ 7.4 - 8.75 [m, 11H(8H, ArH), (2H, triazole-H) (1H, thiazole -5H)]

File # 1 : 2-FTHTZ

Mode= 2 (Mid-IR)

1/4/80 10:38 AM

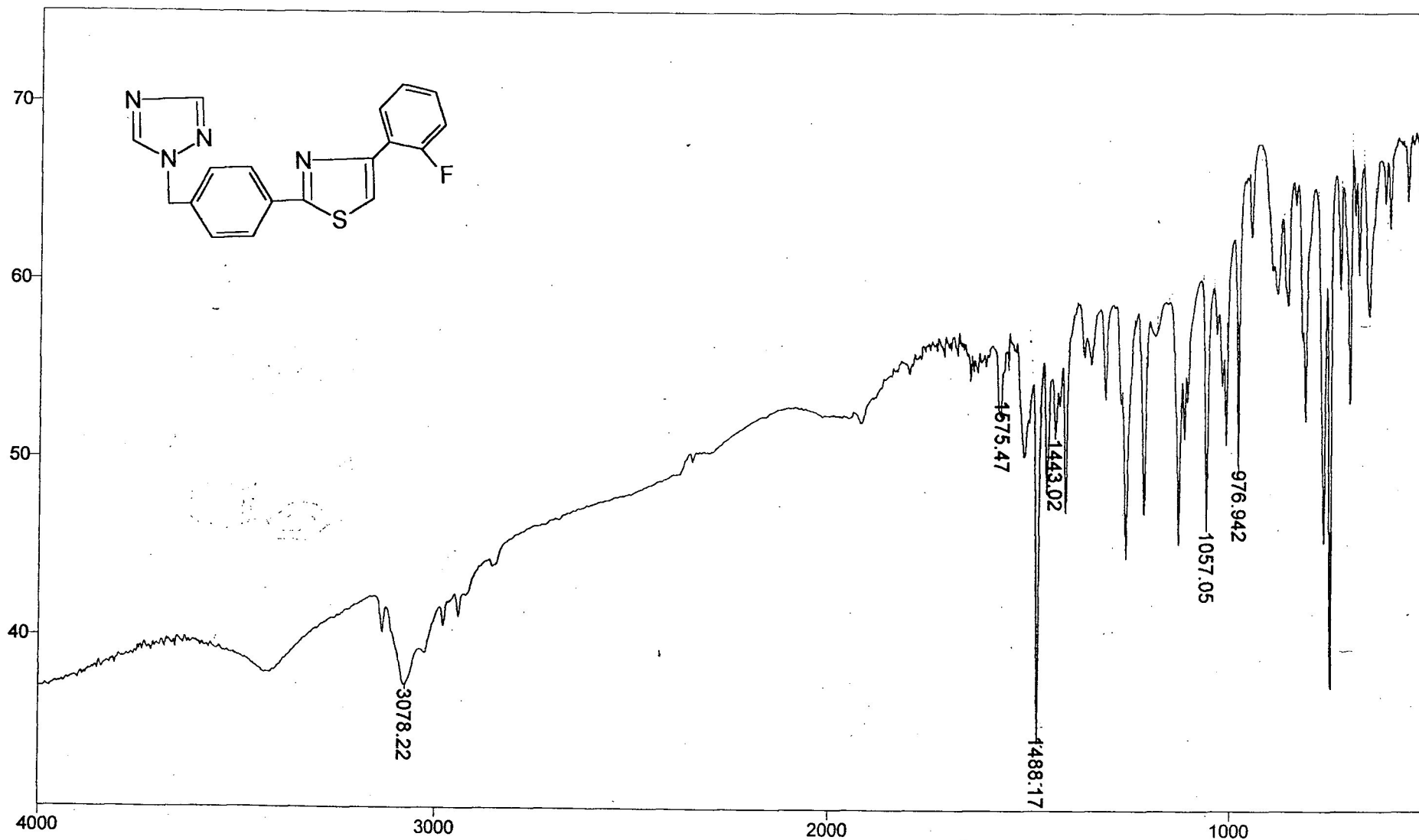
Sample Description:

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Res=4 cm-1

Apod= Cosine

Zero Filling= 1 x



Transmittance / Wavenumber (cm-1)

File # 1 = DTHTZ

Mode= 2 (Mid-IR)

3/22/00 2:20 PM

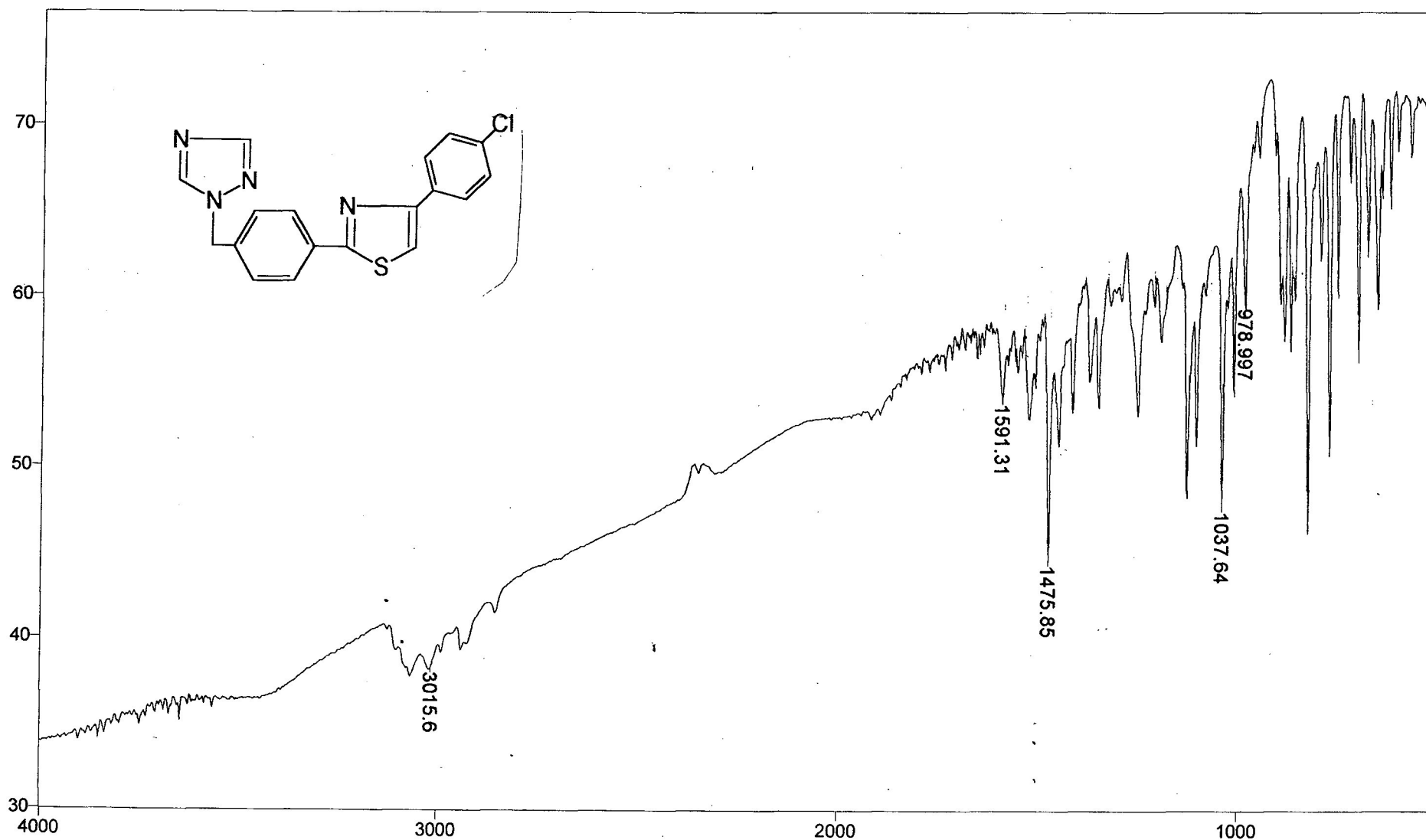
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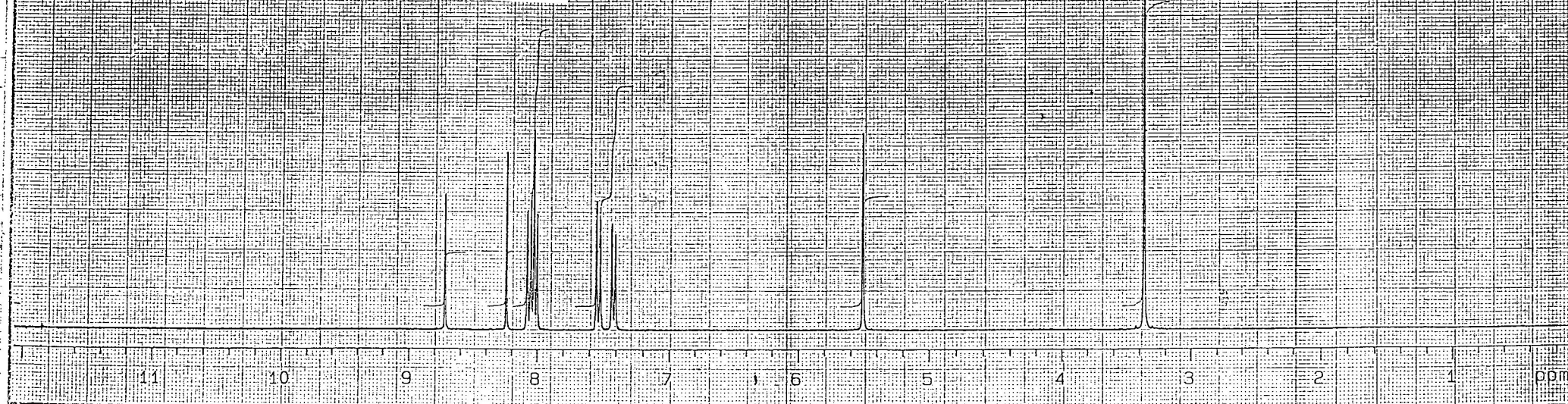
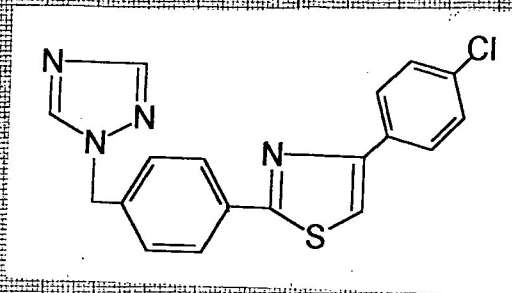
Scans= 10 Slow

Res=4 cm-1

Apod= Cosine

Zero Filling= 1 x





OBSERVE	Nucleus <u>H1</u>	Freq <u>299.9486</u> MHz	DECOUPLE	Nucleus <u>H1</u>	Offset <u>-1769.8</u> Hz	PLOT/PROCESSING	FN <u>not used</u> RE <u> </u> sec CD <u> </u> sec	EXPERIMENT	Pulse Sequence <u>s2pul</u>	SAMPLE	Number <u> </u>	
	Spec. Width <u>5998.8</u> Hz	Offset <u>1200.0</u> Hz		Mode <u>n</u>	Power <u>20</u> dB		LB <u>0.100</u> not used		CCD <u> </u>	Take O.D. <u> </u> mm	JTH	File <u>JTH.f1d</u>
	Acq. Time <u>4.00</u> sec	Delay <u>0</u> sec		Modulation Mode <u>C</u>	Freq <u>200</u> Hz		Width <u>3605.1</u> Hz/ppm		Start <u>11.2</u> Hz/ppm	Temp <u>25.0</u> °C	Date <u>Mar 17 99</u>	
	Pulse Width <u>10.0</u> sec	Transients <u>80</u>		Pulse Width <u>17.5</u> sec	Power Mode <u>not used</u>		Reference <u> </u>		Solvent <u>DMSO</u>	VXHO <u>300</u>		

Part III:

Synthesis of 4-methyl-2-(3-n-butyl,4-substituted-2,3-dihydrothiazol-2-ylidene)aminopyridine.

Step-I: Synthesis of N-4-methyl pyridyl-N'-n-butylthiourea.

In a 50 ml round bottomed flask equipped with a reflux condenser was placed 4-methyl-2-aminopyridine (5.0g, 0.046M) and n-butyl isothiocyanate (5.0g, 0.092M), both dissolved in acetone (15 ml). The reaction mixture was refluxed for 6-8 hours. After cooling, white solid separated out, which was filtered and recrystallized from ethanol.

M.P = 108- 110° C.

Yield =70.3%

Elemental analysis: $C_{11}H_{13}N_3S$

	C%	H%	N%
Calculated	59.19	7.62	18.83
Found	58.84	7.25	18.70

Step II: Synthesis of 4-methyl-2-(3-n-butyl-4-p-fluorophenyl-2,3-dihydrothiazol-2-ylidene)aminopyridine.

In a 50 ml round bottomed flask, N-4-methylpyridyl-N'-n-butylthiourea (0.5g, 0.002M), 4-fluorophenacyl bromide (0.5g, 0.002M) and sodium acetate (0.328g, 0.004M) was dissolved in dry alcohol (25 ml) and refluxed for 12 hours. The excess alcohol was removed under reduced pressure and the solid obtained was filtered, washed with water and recrystallized using methanol.

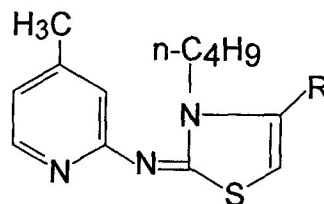
M.P = 95- 96° C.

Yield = 58 %.

Elemental analysis: $C_{19}H_{20}FN_3S \cdot H_2O$

	C%	H%	N%
Calculated	66.86	5.86	12.82
Found	67.25	5.56	13.21

Table II: Physical constants of 4- methyl-2-(3,4-disubstituted-2,3-dihydrothiazol-2-ylidene) aminopyridine.



Sr.No	R	Mol. Formula	M.P(°C)	Yield(%)	Elemental Analysis					
					(calculated) %			(obtained) %		
					C	H	N	C	H	N
1.	3-fluorophenyl	$C_{19}H_{20}FN_3S$	201-203	55.6	54.02	4.13	9.95	54.41	3.84	9.64
2.	4-fluoro phenyl	$C_{19}H_{20}FN_3S.H_2O$	95-96	58.0	66.86	5.86	12.82	67.25	5.56	13.21
3.	4-methoxyphenyl	$C_{20}H_{23}N_3OS.HBr$	108-110	49.8	55.29	5.29	9.67	54.90	4.92	9.28
4.	5-chlorothiophene	$C_{17}H_{18}ClN_3S_2.HBr$	108-110	49.8	55.29	5.29	9.67	54.90	4.92	9.30
5.	4-chlorophenyl.	$C_{19}H_{20}ClN_3S$	87-88	48.8	63.77	5.59	11.75	64.13	5.26	12.01
6.	3-Trifluoromethyl phenyl	$C_{20}H_{20}F_3N_3S$	99-100	59.5	61.48	5.11	10.74	61.68	4.84	10.40

Spectral Characterization:

**1) 4-methyl-2-(3-n-butyl-4-m-fluorophenyl-2,3-dihydrothiazol-2-ylidene)aminopyridine
(Compd. No. 1).**

IR (KBr) cm^{-1} : 2965.46, 2936.3 (- CH_3 , -n- C_4H_9); 1608.39 (-CN); 1556.8, 1245.4
1076.4 (thiazoline); 1573.87, 1512.85, 1425.05 (pyridine).

**2) 4-methyl-2-(3-n-butyl-4-p-fluorophenyl-2,3-dihydrothiazol-2-ylidene)aminopyridine
(Compd. No. 2)**

IR (KBr) cm^{-1} : 2976.23, 2934.75 (- CH_3 , -n- C_4H_9); 1606.78(-CN); 1550.28, 1219.31,
1098.47 (thiazoline); 1569.11, 1506.49, 1428.06 (pyridine).

PMR (CDCl_3) : δ 0.77- 0.82 (t, 3H, - CH_2 - CH_2 - CH_2 - $\underline{\text{CH}_3}$)
 δ 1.128 -1.256 (sextet, 2H, - CH_2 - CH_2 - $\underline{\text{CH}_2}$ - CH_3)
 δ 1.538 -1.66 (pentet, 2H, - CH_2 - $\underline{\text{CH}_2}$ - CH_2 - CH_3)
 δ 2.30 (s, 3H, Ar- CH_3)
 δ 4.0 - 4.05 (t, 2H, - $\underline{\text{CH}_2}$ - CH_2 - CH_2 - CH_3)
 δ 6.66 - 8.30 [m, 8H, (4-ArH)(3-Pyridine H)]
 δ 6.15 (s, H, (thiazoline -5H))

File # 1 = JPB-3

Mode= 2 (Mid-IR)

9/4/99 5:18 PM

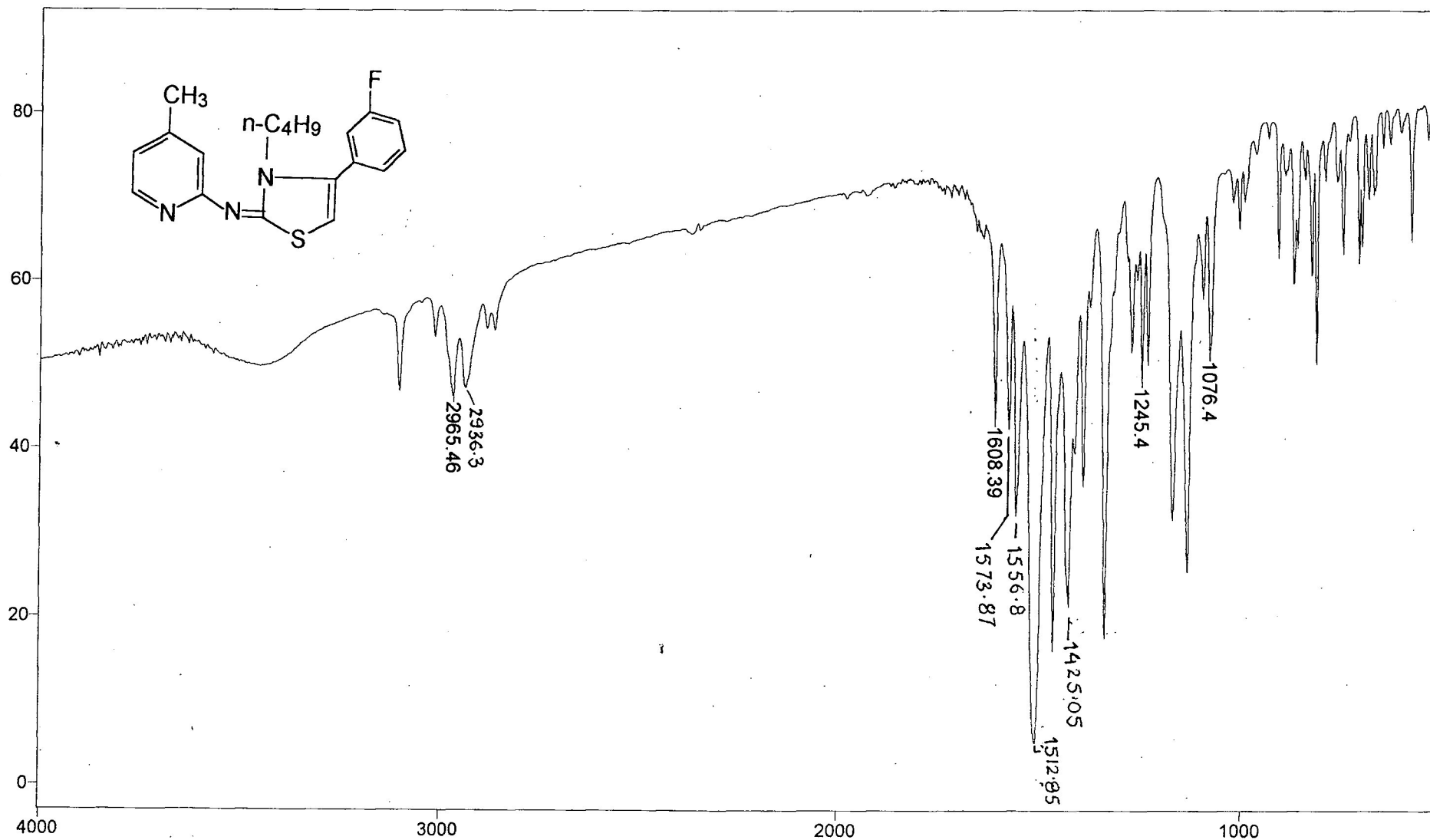
Sample Description:

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Res=4 cm⁻¹

Apod= Cosine

Zero Filling= 1 x



Transmittance / Wavenumber (cm⁻¹)

File # 1 = JPB-2

Mode= 2 (Mid-IR)

7/31/99 12:05 PM

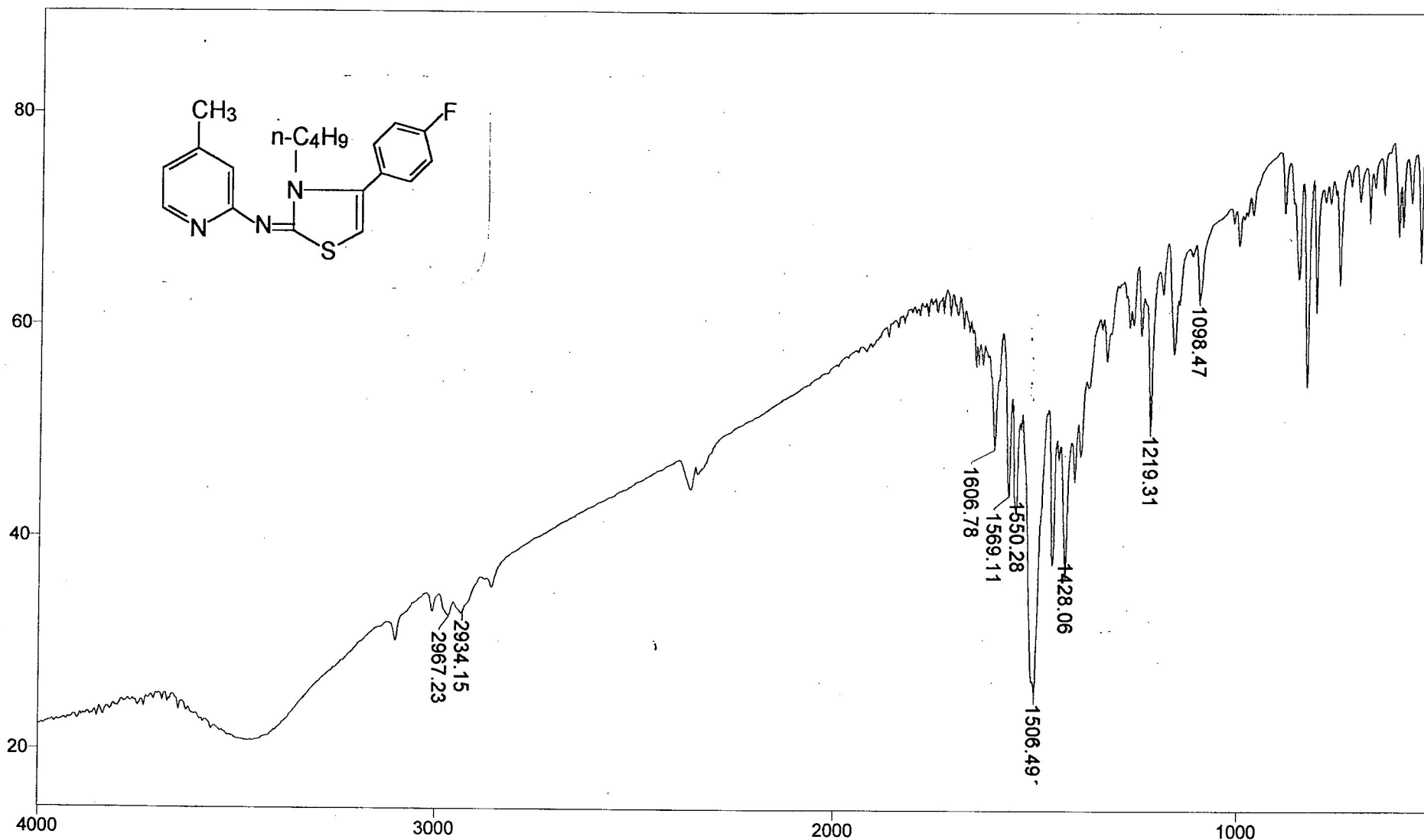
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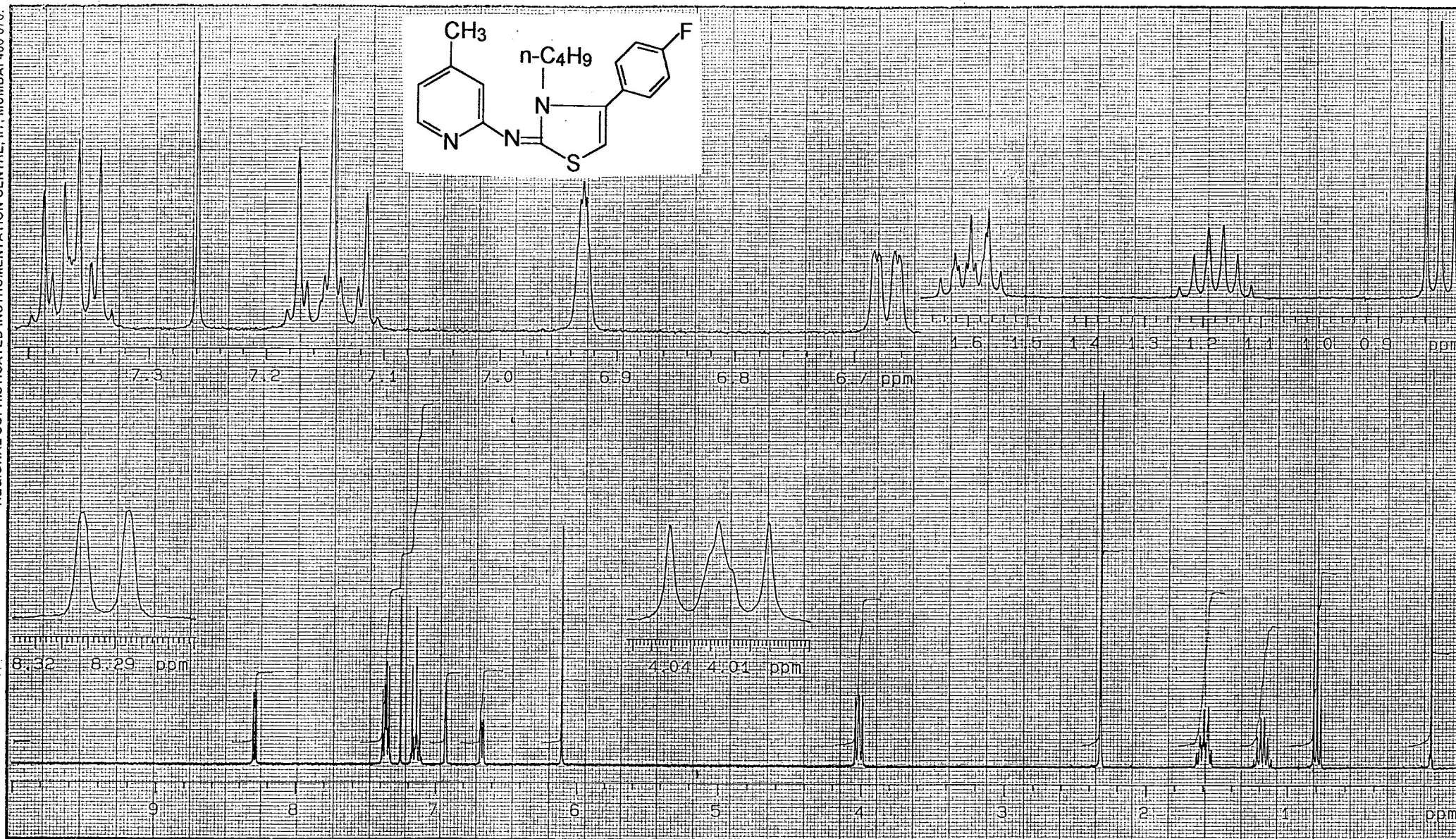
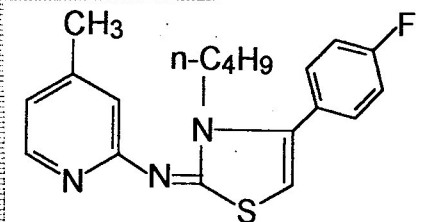
Scans= 10 Slow

Res=4 cm⁻¹

Apod= Cosine

Zero Filling= 1 x





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 LB 0.100 sec APT not used sec CCD sec
 Width 3073.3 Hz/ppm Start -72.5 Hz/ppm
 Reference sec

EXPERIMENT Pulse Sequence g2pu1 SAMPLE
 Tube O.D. mm JPB
 Temp not used °C
 Solvent CDCl3

Number sec
 File JPB.fid
 Date Mar 17 99
 VXRO 300

Part IV:

Synthesis of 6-(1-imidazolyl) quinoxaline-2,3(1H,4H)-dione hydrochloride- An intermediate of YM-90K.

Step-I: Synthesis of 5-(1-imidazolyl)-2-nitroaniline.

In a 50 ml round bottomed flask, 5-chloro-2-nitroaniline (5.0g, 0.0285M) and imidazole (7.752g, 0.114 M) were both dissolved in dry DMF (20 ml) and refluxed for 6 hours. Then the reaction mixture was poured over crushed ice and the yellow solid precipitated out was filtered, washed with water and recrystallized from alcohol.

M.P = 186-188° C.

Yield = 73.2 %.

Elemental analysis: $C_6H_5ClN_2O_2$

	C%	H%	N%
Calculated	52.94	3.92	27.45
Found	52.65	3.68	27.25

Step-II: Synthesis of 5-(1-imidazolyl) o-phenylenediamine.

In a 150 ml round bottomed flask, 5-(1-imidazolyl)-2-nitroaniline (5.0g, 0.024M) was dissolved in methanol (75 ml). Then sodium sulfide (20g) was added to it portionwise with constant stirring. The reaction mixture was refluxed for 4 hours. The excess methanol was removed under reduced pressure and the resulting residue was extracted using chloroform. The extracted layer was further concentrated to obtain the final product.

M.P = 162- 163° C.

Yield = 56.6%.

Elemental analysis: $C_6H_7ClN_2$

	C%	H%	N%
Calculated	62.06	5.74	32.18
Found	62.39	5.56	31.79

Step-III: *Synthesis of 6-(1-imidazolyl)quinoxaline-2,3-(1H,4H)-dione monohydrochloride.*

In a 50 ml round bottomed flask, 5-(1-imidazolyl)-o-phenylenediamine (1.0g, 0.0056M) and oxalic acid (0.76g, 0.0085M) were both dissolved in 4N HCl (30 ml) and refluxed overnight. The solid obtained was filtered, washed with water and recrystallized from water.

M.P = > 300° C.

Lit. M.P.¹⁷⁸ = >300° C.

Yield = 67.5 %

Elemental analysis: C₁₁H₈N₄O₂.HCl

	C%	H%	N%
Calculated	49.90	3.40	21.17
Found	49.61	3.17	21.40

Spectral Characterization:

6-(1-imidazolyl) quinoxaline-2,3(1H, 4H)- dione hydrochloride.

IR (KBr) cm^{-1} : 3392.54 (-NH stretching), 1695.77 (-CO), 1623.6 (-CN), 1544.16,
1500.38, 1387.62 (imidazole).

File # 1 = IQD

Mode= 2 (Mid-IR)

1/4/80 5:18 PM

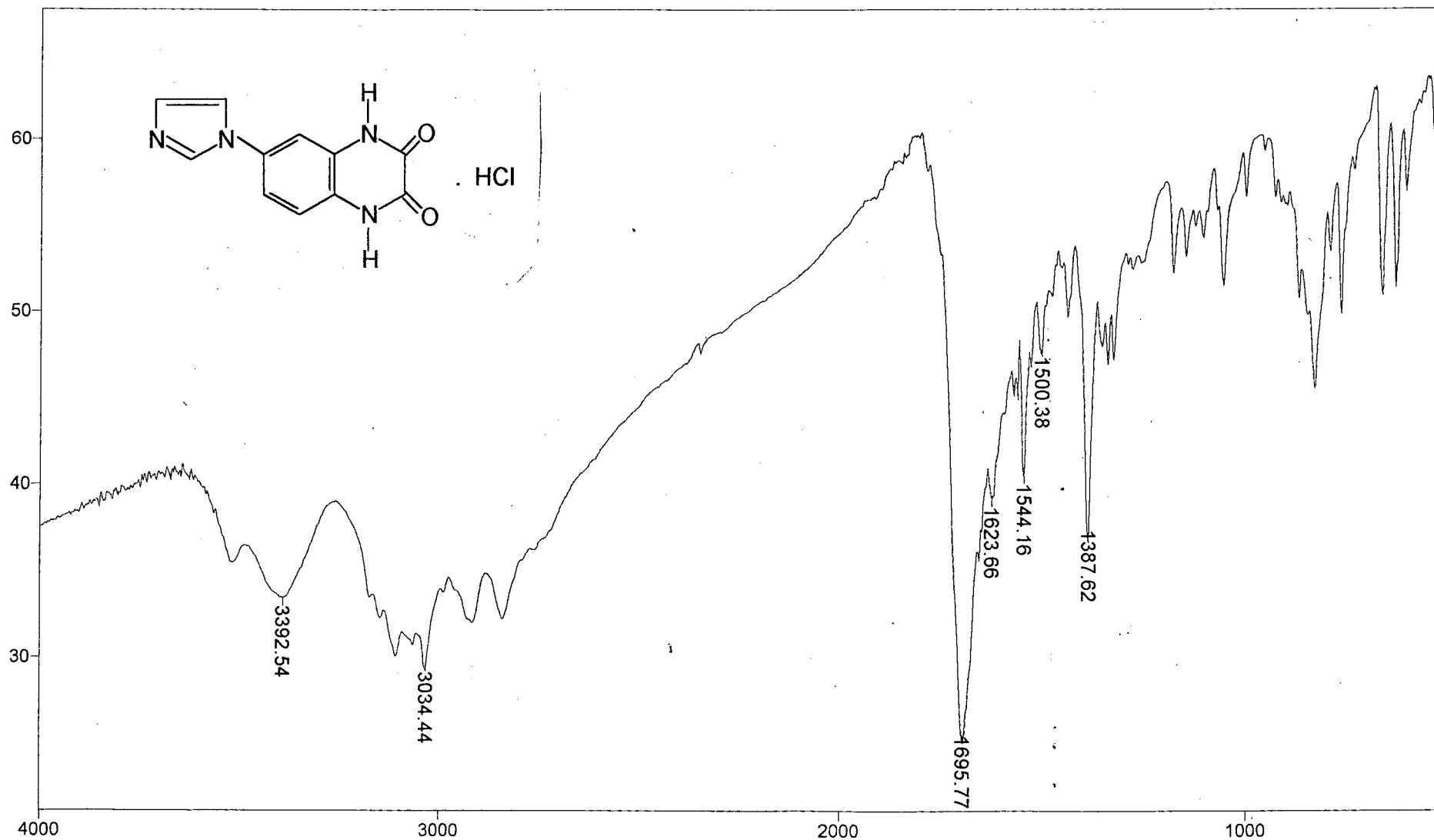
Sample Description:

Scans= 10 Slow

Res=4 cm⁻¹

Apod= Cosine

Zero Filling= 1 x



***Biological Screening
and
ConlucSIONs***

BIOLOGICAL SCREENING

All the compounds synthesized during the course of work were subjected to a biological screening program in order to determine their antibacterial, antifungal and antitubercular activities and thus to establish its therapeutic value.

The screening program involved an *in vitro* study against Gram-positive *Staphylococcus aureus* ATCC 3750 and Gram-negative *Salmonella typhi* NCTC 786 for antibacterial activity and H37Rv strain of *Mycobacterium tuberculosis* for establishing antitubercular activity. Also, *in vitro* study against fungal strains such as *Candida albicans*, *Trichophyton mentagrophytes* and *Trichophyton rubrum* was carried out.

Details involved in the screening program is elaborated in the following section:

Drug Stock Solution:

The test compound (10 mg) was dissolved in a suitable solvent (10 ml) such as DMF, ethanol, dil. HCl as the case may be, so as to prepare a stock solution of 1000 µg/ml. From this solution, the drug concentration viz. 200, 100, 40 and 20 µg/ml were made. For lower dilutions the stock solution was diluted to prepare concentrations such as 20, 10, 5, 2 µg/ml.

Controls:

1. Media Control : Sterilized medium was kept as a control so as to assure the sterility of the medium. If this control shows growth of any type then results are considered invalid.

2. Culture Control : It contains sterilized medium and culture of the organism. It should show growth in the medium which assures optimum pH and sufficient nutrients to promote growth. If no growth is observed, then the medium is considered to be faulty and results are not reliable.

3. Solvent Control : It contains sterilized medium, solvent used to dissolve the drug and the suspended culture in it. Organism should show growth in the solvent used for preparation of the drug stock solution. If the growth is not observed, it can be concluded that solvent inhibits the growth of the organism and hence in that case results are considered invalid.

4. Turbidity Control : It contains medium and the drug stock solution. The turbidity is observed against the growth in different drug dilutions. It assures reliability against misinterpretation between growth and turbidity due to the drug.

4. Standard Control : It contains medium, organism culture and dilution of standard drug stock solution corresponding to that of the drugs under test. The inhibition of growth was observed against that in standard.

Antibacterial Screening :

The test compounds have been subjected to in vitro screening against Gram- positive *S. aureus* and Gram-negative *S. typhi* using tube dilution technique.

Mueller Hinton broth was used as a culture medium. DMF was used as solvent for the preparation of drug stock solution. Sterilized medium was dispensed in each borosilicate glass test tube (150+ 20mm). The drug solution was added in-order to attain final drug concentrations as 200, 100, 40, 20, 10, 4, 2, 1 µg/ml etc.

Innoculum of standard suspension (0.1ml of the test organism strain which contains 10^6 bacilli/ml) was added. The tubes were incubated at 37° C for 48 hours and then examined for the presence or absence of the growth of the organism. The lowest concentration which showed no visible growth was taken as the end point (MIC). Ampicillin (MIC = 0.01 µg/ml against Gram- positive *S. aureus*) and Trimethoprim (MIC = 1.0 µg/ml against Gram- negative *S. typhi*) were used as the standard drugs.

Antitubercular Screening :

H37Rv strain of *Mycobacterium tuberculosis* was used for the in vitro screening of the compounds and the method employed was tube dilution method.

Middle Brook with ADC growth supplement was used as the medium for the growth of the culture.

The sterile medium was dispensed in a borosilicate test tube. Horse serum (0.5ml) was added in all the tubes and then to it, drug stock solution was added to attain the different dilutions such as 200, 100, 40, 20, 10, 4, 2 µg/ml etc. Innoculum of standard suspension i.e. 0.1ml of H37 Rv strain of *M. tuberculosis* containing 10^6 bacilli/ml, was added. Tubes were incubated for ten days and then examined for the presence or absence of growth of the test organism. The lowest concentration which showed no visible growth was taken as the end point. Isoniazide (MIC= 0.04 µg/ml) was used as the standard.

Antifungal Screening :

The organisms used for the test were *Candida albicans*, *Trichophyton mentagrophytes* and *Trichophyton rubrum*. Again, the method employed was the tube dilution method.

Serau fataller's medium was used as the growth media (pH=5.2). The sterile media was dispensed in a sterile borosilicate test tube. The drug solution was added in order to get the final

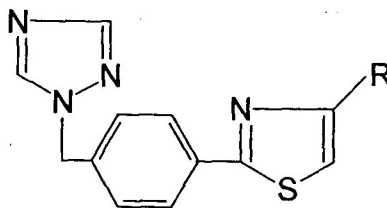
drug concentrations of 200, 100, 40, 20, 10, 4, 2 µg/ml etc.

The tubes were incubated at room temperature (28-30° C) in a dark place. *C.albicans* was incubated for 7 days while *T. mentagrophytes* and *T. rubrum* were incubated for 14 days. The presence or absence of growth was observed visually. The lowest concentration at which no growth was observed was taken as the end point (MIC).

Miconazole (MIC= 6.25 µg/ml against *C.albicans*, 1.56 µg/ml against *T. mentagrophytes* and *T. rubrum*.) was used as a standard.

RESULTS:

Table I : Antimicrobial activity of 2-{4'-[1-(1,2,4-triazolyl) methyl] phenyl}-4-(substituted) thiazoles.



S.No.	Substitution(R)	Antibacterial activity (µg/ml)		
		H37Rv strain of <i>M. tuberculosis</i>	<i>S. aureus</i>	<i>S. typhi</i>
1.	2-fluorophenyl	40	100	100
2.	3-fluorophenyl	100	100	100
3.	4-fluorophenyl	N.A*	100	100
4.	3-chlorophenyl	N.A	100	100
5.	4-chlorophenyl	100	100	100
6.	2,4-dichlorophenyl	100	100	100
7.	2,3-dichlorophenyl	40	100	100
8.	3,4-dichlorophenyl	N.A	100	100
9.	5-chlorothiophene	N.A	100	100
10.	2,4-dichloro-5-fluorophenyl	200	100	100
11.	3-trifluoromethylphenyl	100	100	100
12.	2-hydroxy-4-methoxyphenyl	N.A	100	100

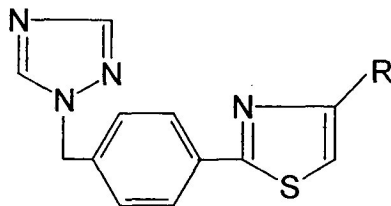
* N.A- Inactive at 200 µg/ml.

* Isoniazide (MIC= 0.04 µg/ml) as standard against H37Rv strain of *M. tuberculosis*.

* Ampicillin (MIC= 0.01 µg/ml) as standard against *S.aureus*.

* Trimethoprim (MIC= 1.0 µg/ml) as standard against *S.typhi*.

Table II: Antifungal activity of 2-{4'-[1-(1,2,4-triazolyl) methyl] phenyl}-4-(substituted) thiazoles.

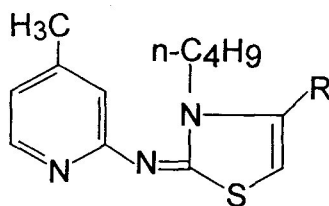


S.No.	Substitutions(R)	Antifungal activity (µg/ml)		
		<i>C.albicans</i>	<i>T. mentagrophytes</i>	<i>T. rubrum</i>
1.	2-fluorophenyl	N.A	40	40
2.	3-fluorophenyl	N.A	N.A	N.A
3.	4-fluorophenyl	N.A	100	40
4.	3-chlorophenyl	N.A	N.A	N.A
5.	4-chlorophenyl	N.A	N.A	N.A
6.	2,4-dichlorophenyl	N.A	N.A	N.A
7.	2,3-dichlorophenyl	N.A	N.A	N.A
8.	3,4-dichlorophenyl	N.A	N.A	N.A
9.	5-chlorothiophene	N.A	N.A	N.A
10.	2,4-dichloro-5-fluorophenyl	N.A	N.A	N.A
11.	3-trifluoromethylphenyl	N.A	100	40
12.	2-hydroxy-4-methoxyphenyl	N.A	N.A	N.A

* Miconazole (MIC= 6.25 µg/ml) as standard for *C. albicans*.

* Miconazole (MIC= 1.56 µg/ml) as standard for *T.mentagrophytes* and *T.rubrum*.

Table III : Antimicrobial activity of 4-methyl -2-(3-n-butyl-4-substituted-2,3-dihydrothiazol-2-ylidene) aminopyridines.



S.No.	Substitutions(R)	Antibacterial activity (µg/ml)		
		H37Rv strain of <i>M. tuberculosis</i>	<i>S. aureus</i>	<i>S. typhi</i>
1.	3-fluorophenyl	N.A	100	100
2.	4-fluorophenyl	N.A	40	100
3.	4-methoxyphenyl	100	100	100
4.	5-chlorothiophene	100	100	100
5.	4-chlorophenyl	N.A	40	100
6.	3-trifluoromethylphenyl	100	100	100

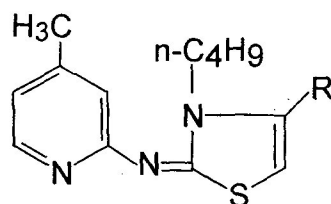
* N.A- Inactive at 200 µg/ml.

* Isoniazide (MIC= 0.04 µg/ml) as standard for H37Rv strain of *M. tuberculosis*.

* Ampicillin (MIC= 0.01 µg/ml) as standard for *S.aureus*.

* Trimethoprim (MIC= 1.0 µg/ml) as standard for *S.typhi*.

Table IV : Antifungal activity of 4-methyl -2-(3-n-butyl-4-substituted-2,3-dihydro-thiazole-2-ylidene) aminopyridines.



S.No.	Substitutions(R)	Antifungal activity (µg/ml)		
		<i>C.albicans</i>	<i>T. mentagrophytes</i>	<i>T. rubrum</i>
1.	3-fluorophenyl	N.A	N.A	N.A
2.	4-fluorophenyl	N.A	N.A	N.A
3.	4-methoxyphenyl	N.A	N.A	N.A
4.	5-chlorothiophene	N.A	N.A	N.A
5.	4-chlorophenyl	N.A	N.A	N.A
6.	3-trifluoromethylphenyl	N.A	N.A	N.A

* Miconazole (MIC= 6.25 µg/ml) as standard for *C. albicans*.

* Miconazole (MIC= 1.56 µg/ml) as standard for *T.mentagrophytes* and *T.rubrum*.

CONCLUSIONS:

The present research project deals with the synthesis of several novel heterocyclic moieties and assessment of their possible therapeutic value. In order to evaluate their efficacy and relate structural aspects with biological activity, these compounds were screened for in vitro antibacterial, antitubercular and antifungal activities.

Part II of the thesis involves with the synthesis of 2-{4'-[1-(1,2,4-triazolyl)-methyl]phenyl}-4- (substituted) thiazoles. In this series, twelve compounds had been synthesised and screened for their biological activities (Tables I & II).

1. All the twelve compounds screened for antibacterial activity against *S. aureus* and *S. typhi*, exhibited moderate activity upto 100 µg/ml.

2. The same series of compounds, when subjected to antifungal testing exhibited variations in biological activity.

i.) 4-fluorophenyl, 2-fluorophenyl and 3-trifluoromethylphenyl derivatives in this series exhibited activity upto 40 µg/ml against *T. rubrum*. This improvement in activity may be due to the presence of the fluorine atom as can be seen by its presence in various standard drugs such as Flucytosine, Fluconazole etc. While rest of the compounds in the series were found to be inactive against the organism.

ii.) 4-fluorophenyl and trifluoromethylphenyl substituted derivatives exhibited moderate activity upto 100 µg/ml against *T. mentagrophytes*. Rest of the compounds were found to be inactive with the exception of o-fluorophenyl derivative which exhibited activity upto 40 µg/ml. Again, the activity might be due to the presence of fluorine atom.

iii.) On the contrary, all the compounds in the series were found to be inactive against *C. albicans*.

3. 2-fluorophenyl and 2,3-dichlorophenyl derivatives possessed activity upto 40 µg/ml against H37Rv strain of *M. tuberculosis*. 3-fluorophenyl, 3-trifluoromethylphenyl, 4-chlorophenyl, 2,4-dichlorophenyl substituted compounds exhibited activity upto 100 µg/ml. 2,4-dichloro-5-fluorophenyl derivative exhibited activity upto 200 µg/ml. All other compounds in the series were found to be inactive. The results indicate the influence of halophenyl group in exhibiting activity against *M. tuberculosis*.

It could be concluded from the above data that the activity which was expected to be achieved by combination of the two biologically significant moieties 1H-1,2,4-triazole and substituted thiazole was, however, not satisfactorily observed. As can be seen, haloaryl substitution failed to give significant activity against *S. aureus* and *S. typhi*. While few fluoro substituted

derivatives exhibited activity against *T. rubrum* and *T. mentagrophytes*. In case of *M. tuberculosis* both chloro and fluoro substitutions exhibited significant activity. By introducing suitable substituents on the thiazole ring further investigations could be pursued on the triazolo-thiazolyl moiety to reach up on a therapeutically potent agent which could be at par with standard drugs.

Part III involves the synthesis of 4-methyl-2-(3-n-butyl-4- substituted-2,3-dihydrothiazol-2-yliidine) aminopyridine (Table III & IV).

1. All the compounds in the series exhibited moderate activity with 100 µg/ml, against *S. aureus* and *S. typhi* with the exception of p-fluorophenyl substituted compound which possessed activity about 40 µg/ml against *S. aureus*.
2. 3-trifluorophenylmethyl, 4-methoxy, 5-chlorothiophene substituted derivatives showed activity upto 100 µg/ml against H37Rv strain of *M. tuberculosis*, while the rest of compounds were found to be inactive.
3. All the compounds the series, screened for antifungal activity against all the three organisms *C. albicans*, *T. rubrum* and *T. mentagrophytes*, were found to be inactive.

It could be concluded from the above data that 2,3,4-trisubstituted thiazoline molecule did not exhibit encouraging results as expected from the combination of thiazoline ring with biologically active pyridine moiety. The molecule shows moderate activity against *S. aureus* and *S. typhi* which leads us to believe that by making suitable changes on the thiazoline moiety possibly a molecule with promising activity could be arrived at.

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Appendix

APPENDIX

1. The melting points have been determined in open capillaries in a veego VMP-1 melting point apparatus and are uncorrected.
2. Thin Layer Chromatography of the compounds have been run on E. Merck precoated silica gel plates of type 60 F 254.
3. Elemental Analysis of the compounds was carried out on Hareus CHN elemental analyzer (Hareus, Hanau, Germany) rapid instrument.
4. Infrared spectra were recorded on a BOMEM MB- 104 Fourier Transform Infrared Spectrometer.
5. PMR spectra was recorded on Varian 300 MHz spectrometer usind TMS as internal reference.

Synopsis

SYNOPSIS
OF THE THESIS TO BE SUBMITTED TO
THE UNIVERSITY OF MUMBAI
FOR THE DEGREE OF
MASTER OF PHARMACEUTICAL SCIENCES
IN THE FACULTY OF TECHNOLOGY.

TITLE OF THE THESIS : Synthesis and study of biological activity of chemotherapeutic agents.

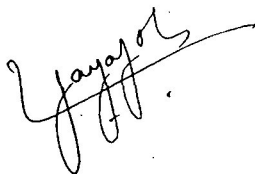
NAME OF THE CANDIDATE : Ms. Jaya John.

NAME AND DESIGNATION OF THE GUIDE : Dr. B.G. Khadse
Ex-Director and head of Dept. of Chemotherapy
Haffkine Institute, Parel, Mumbai-12.

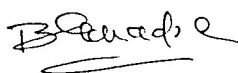
PLACE OF RESEARCH : Department of Chemotherapy,
Haffkine Institute,
Parel, Mumbai-12.

NUMBER AND DATE OF REGISTRATION : 40, Feb. 01, 1999.

SIGNATURE OF THE STUDENT :



SIGNATURE OF THE GUIDE :



SYNOPSIS

The battle between humans and microbes has been waged since time immemorial. For a brief period following the discovery and development of the main groups of antibiotics antibacterials, mankind appeared to gain an upperhand against the legion of bacteria responsible for much morbidity and mortality among human and their domestic animals. But the advantage was illusory because the bacteria were quietly and efficiently evolving and acquiring resistance. Hence, the quest for newer antimicrobials with greater efficacy, lesser side effects and broad spectrum of activity continues.

Medicinal chemistry, an extension of organic chemistry, not only deals with the synthetic aspects of the drug, but also the biological aspect of it. Thus, it occupies a strategic position at interface of chemistry and biology. Based on this aspect a rational approach has been made in the thesis to synthesise several new molecules involving various active moieties like thiazole, triazole etc.

A total of eighteen compounds have been synthesised and characterised by TLC, MP (recorded in open capillaries and are uncorrected), FT-IR (recorded on Hartmann-Braun, MB-104 series), NMR (recorded at RSIC, IIT, Mumbai), and Carbon, Hydrogen, Nitrogen analysis (recorded on Heraeus C,H,N Elemental Analyzer).

The thesis will consist of the following parts:-

PART 1 :- Introduction, Review of Literature and Plan of Work.

PART 2 :- Synthesis of 2-{4'-[1-(1,2,4-triazolyl)methyl]phenyl}-4-(substituted) thiazoles.

PART 3:- Synthesis of 4-methyl-2-(3-n-butyl-4-substituted-2,3-dihydrothiazol-2-ylidene) aminopyridine.

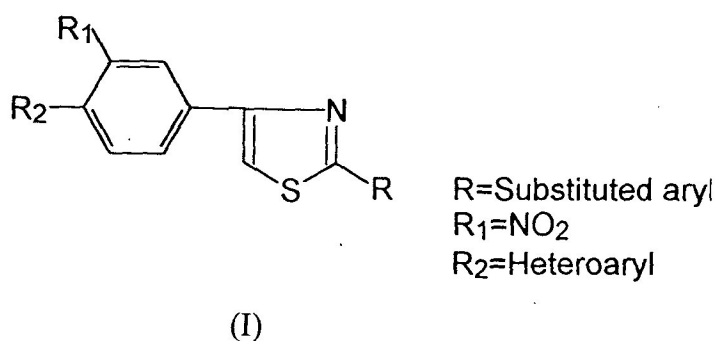
PART 4 :- Synthesis of 6-(1-imidazolyl) quinoxaline-2,3(1H,4H)-dione hydrochloride-An intermediate of 6-(1-imidazolyl)-7-nitroquinoxaline-2,3-(1H,4H)-dione monohydrochloride (YM-90K).

PART 5 :- Biological screening, Results and Discussion.

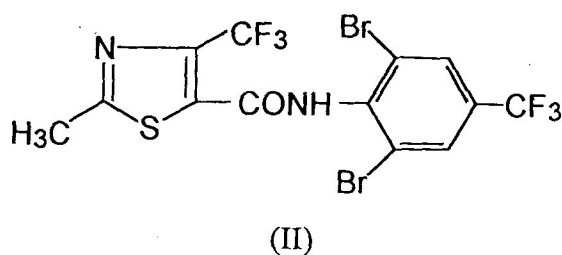
PART 1 :- This part will give general introduction covering salient aspects of the research project and will also include Review of Literature. Plan of Work will highlight the objectives involved in the synthetic work.

PART 2 :- Synthesis of 2-{4'-[1-(1,2,4-triazolyl)methyl]phenyl}-4-(substituted) thiazoles.

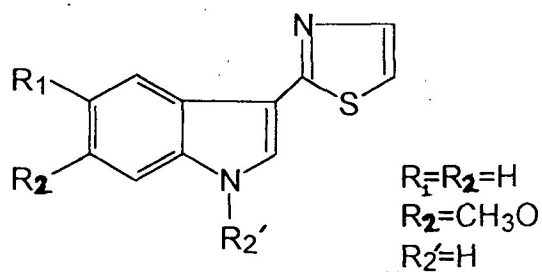
Burmistov *et al* have reported substituted thiazole derivatives with antibacterial and antifungal activities¹. Also, 2,4 -disubstituted thiazoles (I) are known for their antitubercular activity².



In 1992, Shell Research Centre³ reported thiazole derivative (II) with a fluoro substituent possessing excellent antifungal activity.



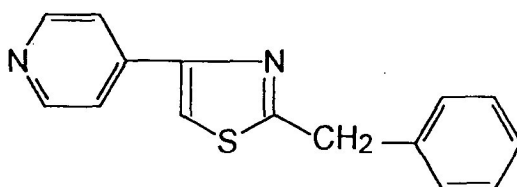
It has also been reported that thiazoles (III) bearing other heterocyclic ring systems possess good antifungal activity⁴.



(III)

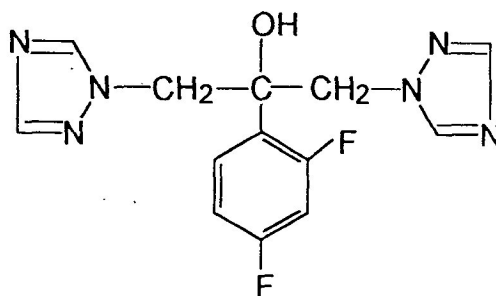
These reports were reason enough to include the thiazole nucleus as part of the series to be designed with the hope of achieving antimicrobial activity.

Boulton ,D. *et al*⁵ synthesized 2-benzyl-4-(4'-pyridyl) thiazole (IV) with methyl phenyl substituent at 2-position of the thiazole and reported its pharmacological activity. Hence we decided to introduce the methyl phenyl group at 1- position of 1,2,4- triazole and check for its pharmacological activity.



(IV)

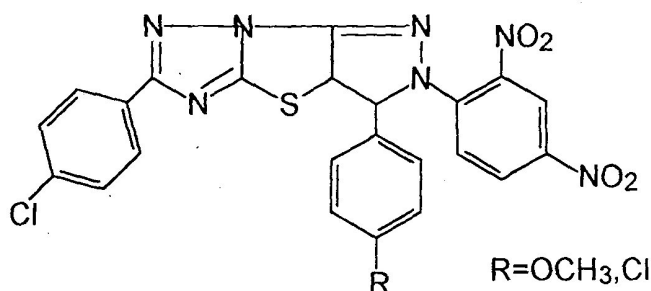
1,2,4- triazole is a well known moiety exhibiting antifungal activity the facts ascertained by its presence in the recently developed broad spectrum antifungal drug, Fluconazole ⁶ (V).



(V)

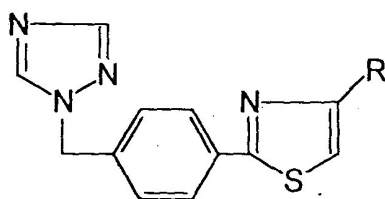
Taking into view the above reports a fusion of the thiazole and the 1,2,4-triazole † was thus planned. Several references indicating the activity of thiazolo triazoles were also found which further encouraged the selection of two moieties.

Jagmohan *et al* have synthesized pyrazolo [3',4':4,5] thiazolo [3,2-b]-s-triazoles (VI) which were found to exhibit both antibacterial and antifungal activity.



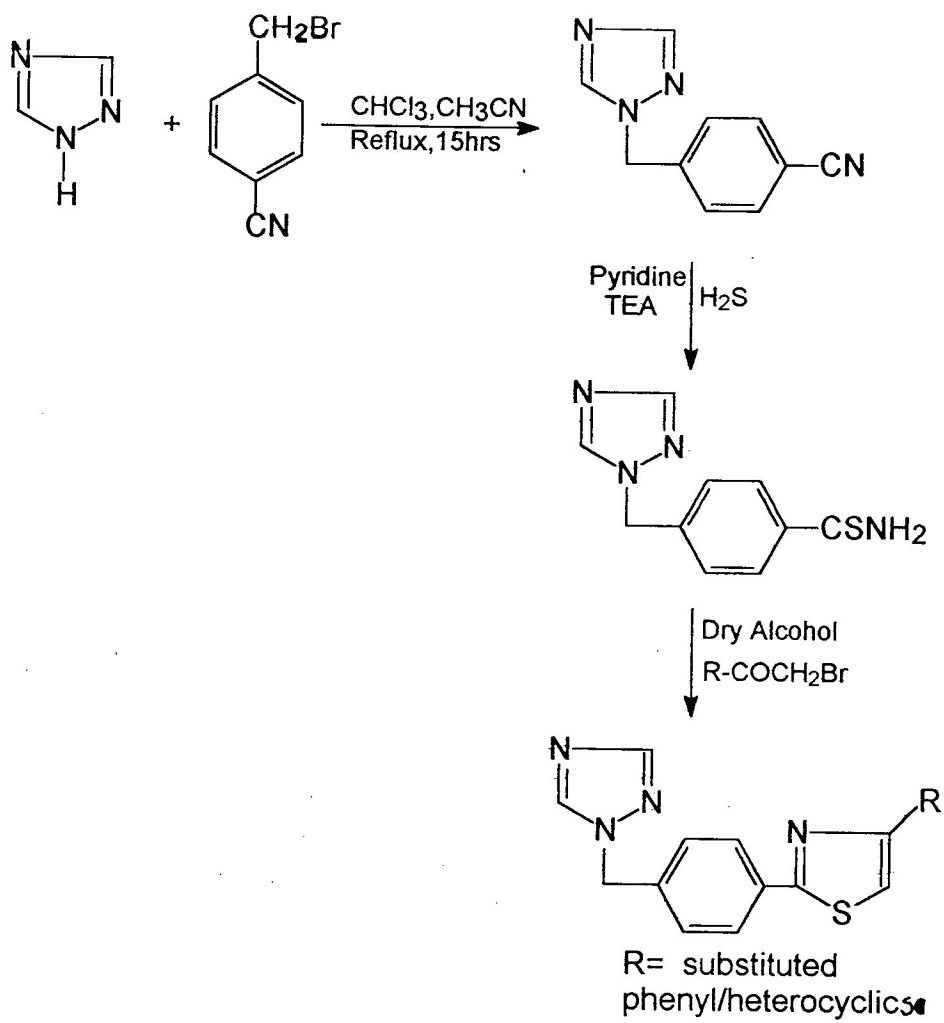
(VI)

Hence, taking into consideration all the above given facts it was planned to synthesize 2,4-disubstituted thiazole as the parent moiety with 1,2,4-triazolyl-1-benzyl-substitution at its 2-position and various halo substituted phenyl /heterocyclic substituents would be introduced at its 4-position. A total of twelve compounds were synthesized as per the method outlined in scheme -1. The final compounds in the series has the following structure.



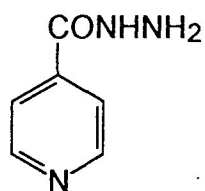
R = Substituted phenyl/heterocycles etc.

SCHEME I

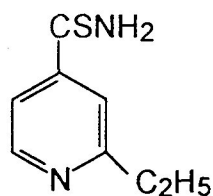


**PART 3 :- Synthesis of 3-methyl -2-(3-n-butyl-4-substituted -2,3-dihydrothiazole-
-2-ylidene) amino pyridines.**

Pyridine , a well known moiety for its broad spectrum of biological activity ⁸, forms the pharmacophores several important drugs such Isoniazide (VIII), Ethionamide (IX) etc.

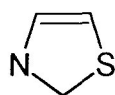


(VIII)



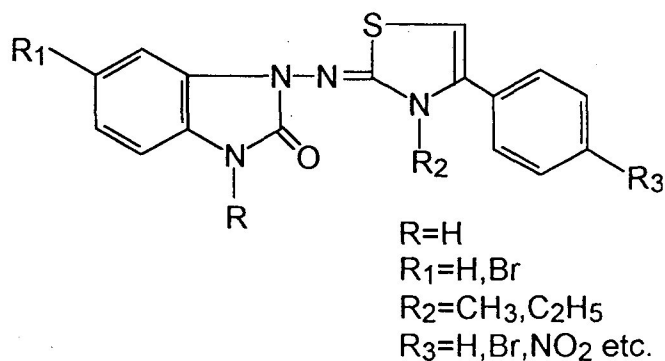
(IX)

Similarly, thiazoline (X) and its derivatives also found to exhibit a wide variety of biological activities such as antimicrobial ⁹, antiinflammatory, antihistaminic etc.



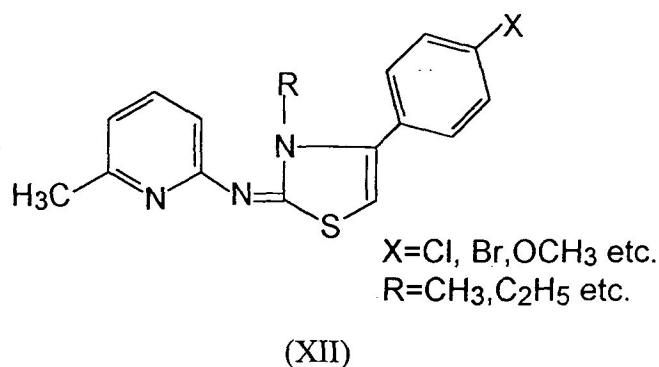
(X)

Karali *et al* ¹⁰ synthesized derivatives of 3-[3,4-disubstituted-4-thiazoline - 2-ylidene hydrazono]-1H-2-indolinones (XI) as potent antitubercular agents having MIC <12.5 µg /ml against *M. tuberculosis* H₃₇ RV strain .



(XI)

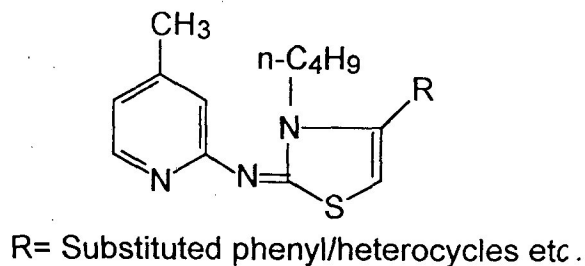
Recently, Hassan *et al*¹¹ have synthesized 6-methyl-2-(3,4-disubstituted -2,3-dihydrothiazole-2-ylidene)aminopyridine (XII) which showed moderate activity against Gram -positive bacteria.



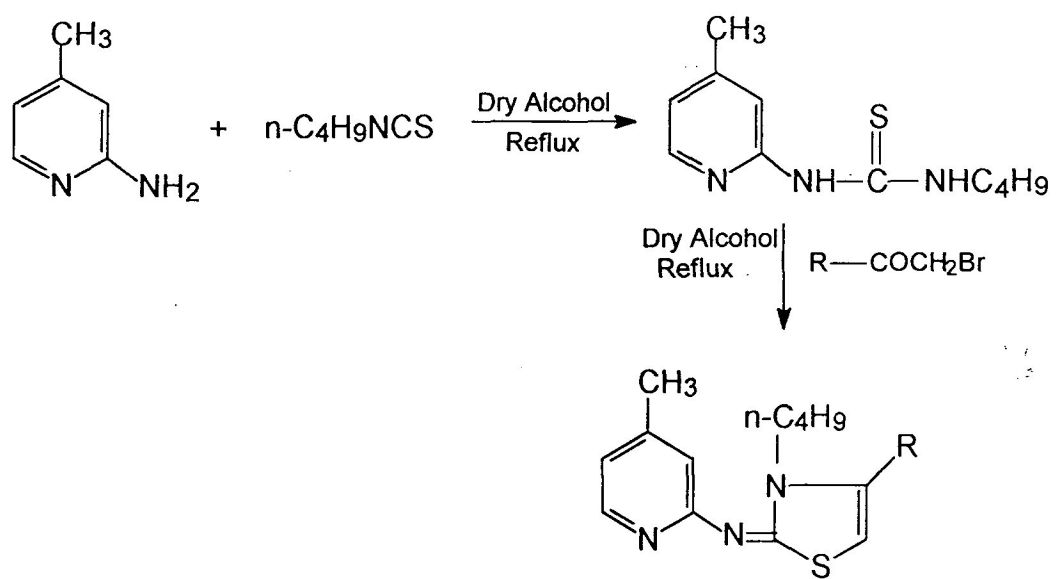
The above reports lead us to reach upon a moiety including both the thiazoline as well as pyridine.

Further, it was found the presence of an alkyl group in a compound increases its lipophilicity and thus leading to an improvement in activity, hence an alkyl group, *n*-C₄H₉ was introduced in the parent thiazoline ring system.

Considering the above facts an attempt has been made to synthesise a series of six compounds namely 4-methyl-2-(3-*n*-butyl-4-disubstituted-2,3-dihydro thiazole-2-ylidene)aminopyridine (XIII) with probable antibacterial and anti tubercular activity.



SCHEME II

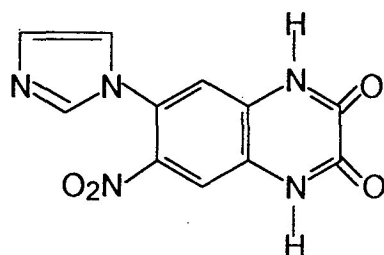


R= Substituted phenyl/heterocycles etc

PART 4:- Synthesis of 6-(1-imidazolyl)quinoxaline-2,3(1H,4H)-dione hydrochloride-An intermediate of YM-90K.

Alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptors involved in mediating fast glutaminergic neurotransmission are also known to be involved in neuronal damage following cerebral ischemia. Hence, in the recent past there has been considerable interest in the design of an AMPA antagonist.

YM-90K(XIV) is a selective and potent AMPA receptor antagonist.

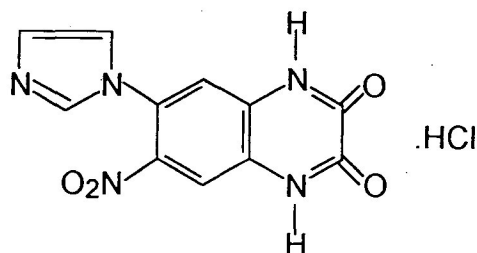


(XIV)

The pharmacological role of YM-90K as AMPA receptor antagonist cerebral ischemia has been studied. It has been shown to have neuroprotective effects in focal ischemia models¹².

YM-90K is currently under phase (ii) clinical studies¹³.

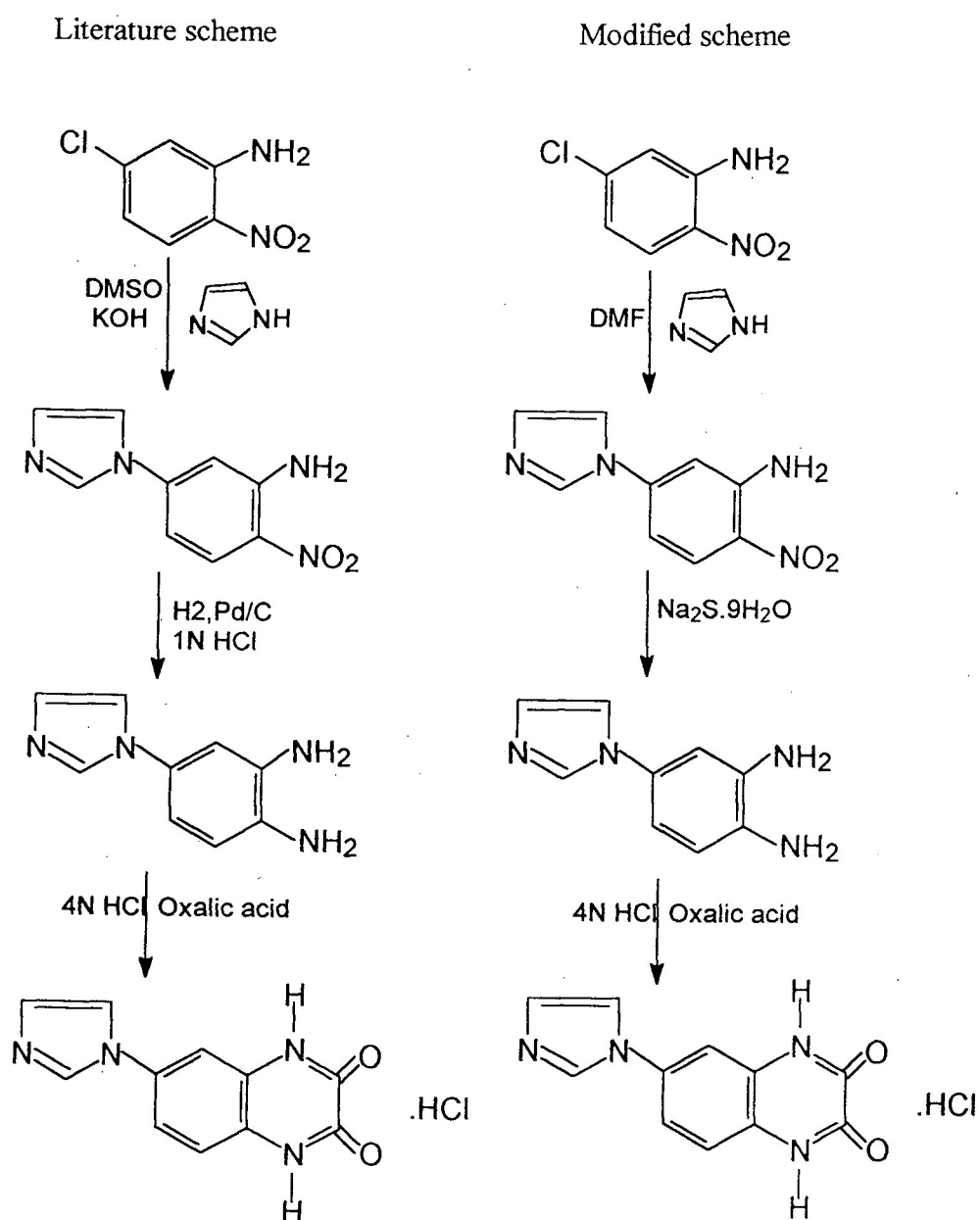
Considering the importance of YM-90K it was planned to synthesise the intermediate for this drug, 6-(1-imidazolyl)quinoxaline-2,3-(1H,4H)-dione.hydrochloride(xv).



(XV)

The synthesis of this moiety involves three steps. Several modifications have been carried out in order to make the process easier and economical at industrial scale, the process is summarized in the Scheme-3.

SCHEME III



PART-5:-Biological Screening ,Results and Conclusions.

All the compounds synthesized were subjected to microbiological screening which involves antitubercular, antibacterial and antifungal screening.

Antitubercular Screening:-

The *in vitro* antitubercular screening of the compounds was carried out by the tube dilution technique employing H₃₇RV strain of *Mycobacterium tuberculosis*. This method uses Kirschner's medium and Middle Brook 7H9 broth base with ADC Growth supplement. The standard drug used was Isoniazide(MIC 0.04 µg /ml).

Antibacterial Screening:

The compounds synthesised were also subjected to *invitro* test against *Staphylococcus aureus* ATCC 3750 and Salmonella typhi NCTC 786. The method used was that of tube dilution and the medium employed in the screening was Mueller-Hinton Broth. The standard drugs used were Ampicillin (MIC 0.01 mg/ml) against *S.aureus* and Trimethoprim (MIC 0.1 µg /ml) against *S.typhi*.

Antifungal Screening:-

For antifungal screening organisms used were *Candida albicans*, *Trichophyton mentagrophytes* and *Trichophyton rubrum*. The medium used for growth was Scaufataller medium. Miconazole (MIC 6.25 µg /ml) was used as the standard drug against all the three organisms.

An attempt has been made to obtain some promising leads for antimicrobial compounds. The synthetic and microbiological evaluation of the compounds will be dealt in the thesis.

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