

SYNTHESIS AND STUDY OF SUBSTITUTED PYRAZOLES
AND TRIAZOLE THIAZOLES AS POSSIBLE ANTIMICROBIAL
AGENTS

THESIS SUBMITTED
UNIVERSITY OF MUMBAI

FOR THE DEGREE OF
MASTER OF SCIENCE
IN
ORGANIC CHEMISTRY

SUBMITTED BY
AMEYA GAJANAN YADAV

UNDER THE GUIDANCE OF
DR. ANIL BOBADE

DEPARTMENT OF CHEMOTHERAPY
HAFFKINE INSTITUTE FOR TRAINING, RESEARCH & TESTING
MUMBAI

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Mr. Ameya Gajanan Yadav

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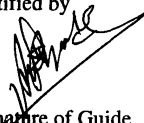
Thank you Lord for helping me throughout.

- Ameya

STATEMENT BY THE CANDIDATE

As required by the University Regulation No. R. 2190, I wish to state that the work embodied in this thesis titled “ Synthesis and study of substituted pyrazoles and triazolo thiazoles as possible antimicrobial agents” forms my own contribution to the research work carried out under the guidance of Dr. Anil S. Bobade at the Department of Chemotherapy, Haffkine Institute, Parel, this work has not been submitted for any other degree of this or any other University. Whenever references have been made to previous works of others, it has been clearly indicated as such and included in the Bibliography.

Certified by



Signature of Guide

Dr. Anil S. Bobade



Signature of Candidate

Ameya Gajanan Yadav

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Introduction

Introduction

“Medicinal Chemistry remains a challenging science which provides profound satisfaction to its practitioners. It intrigues those of us who like to solve problems posed by nature. It verges increasingly on biochemistry and on all the physical, genetic and chemical riddles in animal physiology, which bear on medicine. Medicinal chemists have a chance to participate in the fundamentals of prevention, therapy and understanding of diseases and thereby to contribute to a healthier and happier life.”

ALFRED BURGER¹

Pasteur and Joubert were among the first to recognize the potential of microbial products as therapeutic agents. In 1877, they published their observations that common microorganisms could inhibit growth of anthrax bacilli in urine. The modern era of antimicrobial chemotherapy dates to 1936, with the introduction of sulfanilamide into clinical practice. Penicillin became available in quantities sufficient for clinical use in 1941. Streptomycin, chloramphenicol and chlortetracycline were identified towards the end of or soon after World War II. Since then, numerous classes of antimicrobial agents have been discovered and are most commonly used. For example, 30% or more of all hospitalized patients are treated with one or more course of antimicrobial therapy. The inevitable consequence of the widespread use of antimicrobial agents has been the emergence of antibiotic-resistant pathogens, fueling an ever-increasing need for new drugs and contributing to the rising cost of medical care. Moreover, the pace of antimicrobial drug development has dramatically slowed during the last decade, with only a handful of new agents, few of which are really novel, being introduced in clinical practice each year.

In the strictest sense, antibiotics are substances produced by various species of microorganisms (bacteria, fungi, actinomycetes) that suppress the growth of other microorganisms. Common usage often extends the term antibiotics to include synthetic antimicrobial agents, such as sulfonamides and quinolones. Antibiotics differ markedly in physical, chemical and pharmacological properties, in antibacterial spectrum and in mechanisms of action. Knowledge of molecular mechanisms of bacterial, fungal and viral replication has greatly facilitated rational development of compounds that can interfere with the life cycles of microorganisms.

Historically, the most common classification of antimicrobial agents has been based on chemical structure and proposed mechanism of action², as follows: 1) agents that inhibit synthesis of bacterial cell walls; these includes the *penicillins* and *cephalosporins*, which are structurally similar, and dissimilar agents such as *cycloserine*, *vancomycin*, *bacitracin* and the *azole* antifungal agents (*e.g. clotrimazole*, *fluconazole* and *itraconazole*); 2) agents that act directly on the cell membrane of the microorganisms, affecting permeability and leading to leakage of intracellular compounds; these include the detergents such as *polymyxin* and the polyene antifungal agents *nystatin* and *amphotericin B*, which bind to cell-wall sterols; 3) agents that affect the function of 30S or 50S ribosomal subunits to cause a reversible inhibition of protein synthesis; these bacteriostatic drugs include *chloramphenicol*, the *tetracyclines*, *erythromycin*, *clindamycin* and *pristinamycins*; 4) agents that bind to the 30S ribosomal subunit and alter protein synthesis, which eventually leads to cell death; these includes the *aminoglycosides*; 5) agents that affect bacterial nucleic acid metabolism, such as *rifamycins* (*e.g. rifampin*) which inhibit RNA polymerase, and the *quinolones*, which inhibit topoisomerases; 6) the antimetabolites including *trimethoprim* and the *sulfonamides*, which block essential enzymes of folate metabolism; and 7) antiviral agents, which are of several classes including: a) nucleic acid analogs, such as *acyclovir* or *ganciclovir*, that selectively inhibit viral DNA polymerase, and *zidovudine* or *lamivudine*, which inhibit reverse transcriptase; b) nonnucleoside reverse transcriptase inhibitors, such as *nevirapine* or *efavirenz*; and c) inhibitors of other essential viral enzymes, *e.g.* inhibitors of HIV protease or influenza neuraminidase. Additional categories likely will emerge as more complex mechanisms are elucidated. The precise mechanism of action of some antimicrobial agents is unknown. The achievable concentration for an antibiotic in serum typically guides selection of the breakpoint for designating a microorganism as either susceptible or resistant by *in vitro* susceptibility testing. If an inhibitory or bactericidal concentration cannot be achieved safely, then the microorganism is considered resistant to that drug. Thus, the drug may be only marginally effective or ineffective in such cases even though standardized *in vitro* test would likely report the microorganism as “sensitive”. For an antibiotic to be effective, it must reach its target, bind to it, and interfere with its function. Bacterial resistance to an antimicrobial agents falls into three general categories: 1) the drug does not reach its target; 2) the drug is not active; or 3) the target is altered.

Azole antifungal agents have dominated drug development and clinical use for nearly three decades. While the antifungal spectrum, physical properties and pharmacology differ among compounds; azoles are remarkable as a drug class for their broad spectrum, oral bioavailability and low toxicity. New compounds are still being developed, though the innovations are becoming more marginal, and resistance to azoles is slowly emerging in species that formerly were susceptible, particularly *Candida albicans*³. Determination of blood concentrations of antifungal drugs remains a research procedure except in the case of flucytosine. The toxicity of flucytosine clearly depends on drug concentration; azotemia readily increases flucytosine to toxic levels. For the azoles and amphotericin B formulations, blood levels have not predicted either toxic or efficacy. The advance of systemic antifungal agents into common medicinal practice has made it important to define their efficacy, toxicity and interaction with other drugs.

Medicinal chemistry is a stream of science that has been devoted to field of drug discovery. Medicinal chemistry can be seen as an extension of organic chemistry, existing in the boundary between chemistry and biology. Taken in the prospective sense Medicinal Chemistry relates to the design and production of compounds that can be used in medicine for prevention, treatment or cure of human and animal diseases. Taken into retrospective sense, Medicinal Chemistry covers three critical steps⁴:

- **A discovery step**, consisting of identification and production of new active substances usually called “Lead” compounds, which can originate from synthetic organic chemistry, from natural sources or from biotechnological processes.
- **An optimization step**, that deals with the synthetic modification 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 optimization of the synthetic route for bulk production, and the modification of pharmacokinetic and pharmaceutical properties of the active substances to render it suitable for clinical use.

The battle against infections is not fully won and continues due to the emergence of multi-drug resistant strains of microorganisms, which has rendered many treatments ineffective.

The present work is a positive effort in this direction.

Review of Literature

REVIEW OF LITERATURE

Chemotherapy is the practice 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 quite 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.

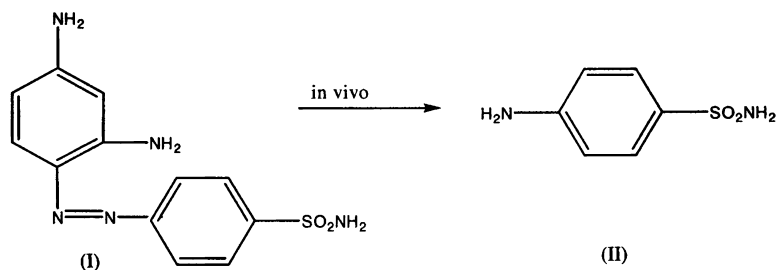
Topical antibacterial agents include disinfectants, antiseptics and preservatives. Topical agents show a little selective toxicity between the microbes and the host.

Systematic antibacterials include a number of classes, which are enlisted below-

Sulfonamides:

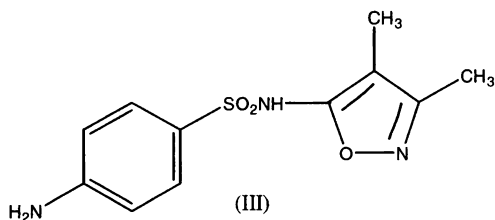
Sulfonamides were discovered in the mid 1930's. Protonsil rubrum (I), a red dye was observed by Domagk⁵ to be taken up selectively by bacterial cells in case of *Streptococcal* infections in mice and *Staphylococcal* infections in rabbits. The dye killed the bacteria. Fractionation led to identification of an active substance known as

amino benzene sulfonamide (sulfanilamide) (II)⁶, a colorless 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. Sulfonamides 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.

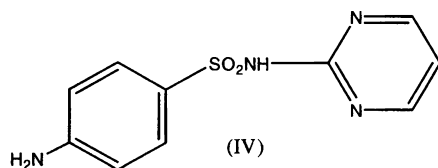
Bacteria, which are capable of taking up preformed folic acid, are intrinsically resistant to sulfonamides.



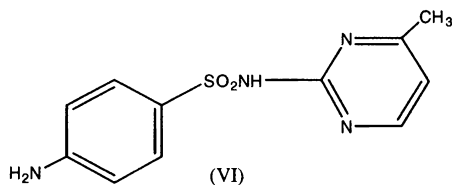
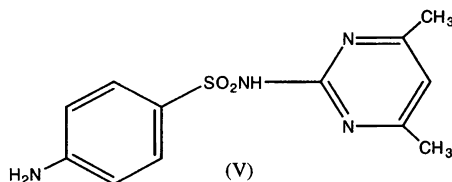
Sulfisoxazole (III)⁷ is one of the most popular sulfonamides in present use, which has a pKa of 5.0. It has a broad antimicrobial spectrum and is clinically used for urinary tract infection and as a backup for other more preferred agents.

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

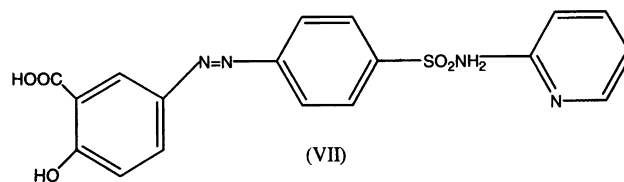
Plasmid-mediated resistance is commonly developed, particularly among Gram-negative organisms, due to decreased sensitivity to dihydropteroate synthetase. Super infections, allergic rashes, photosensitivity and drug fever are some of the common side effects of the drug.



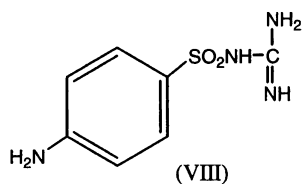
Sulphadiazine (IV) has retained pre-eminence because of its broad spectrum of activity, low toxicity and high tissue penetration. It can be used for meningococcal meningitis provided the strains of *Neisseria meningitides* involved are sulfonamide sensitive⁸.



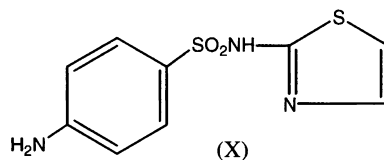
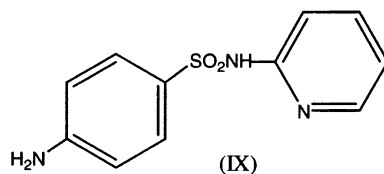
Sulphamethazine (V) and Sulphamerazine (VI)⁹⁻¹³ have greater tolerance and high solubility. Multiple (triple) sulfas, a 1:1:1 combination of three sulphapyrimidines, namely, sulphadiazine, sulphamethazine and sulphamerazine, is a very potent combination.



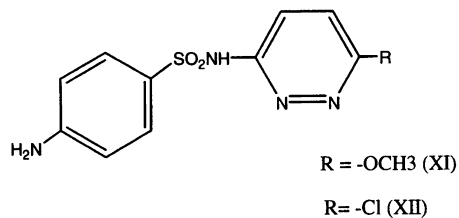
Sulphasalazine (VII) is absorbed in the duodenum and is converted to Para amino salicylic acid (PAS) which is used for ulcerative colitis and Crohn's disease because of its anti-inflammatory effect.



Sulphaguanidine (VIII) was extensively used for intestinal infections. Now, it has been withdrawn in many countries¹⁴ due to poor absorption and toxicity.

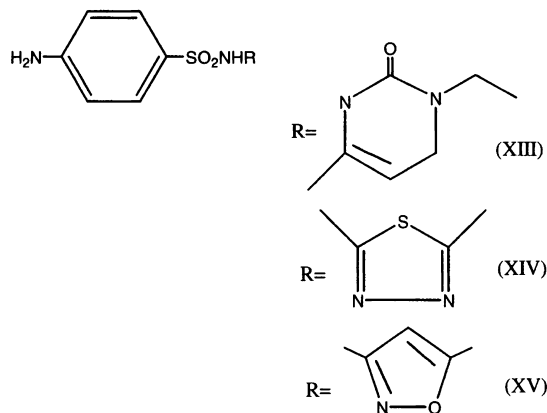


Sulphapyridine (IX)¹⁵, reported in 1938, was in use for the treatment of pneumonia and remained as a drug of choice until it was replaced by sulphathiazole (X)¹⁶.



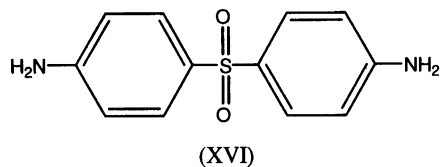
Long-acting sulfonamides include Sulphamethoxypyridazine (XI)¹⁶, which has a half-life of about 37 hours. Thus, it needs to be administered only once a day. Sulphachloropyridazine (XII), a related sulphonamide, has a half-life of 7 hours and is useful for urinary tract infections (UTI).

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



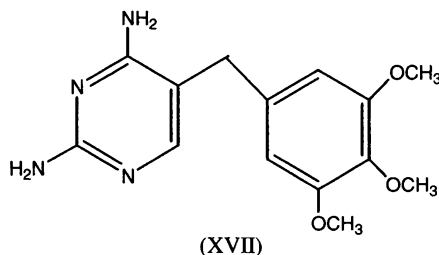
Sulphones:

The parent sulphone, Dapsone (4,4'-sulphonyl dianiline) (XVI), is a prototype for a variety of analogues that have been studied widely. Dapsone¹⁷ 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 effect¹⁸ and microbes will not be able to develop resistance¹⁹ 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.

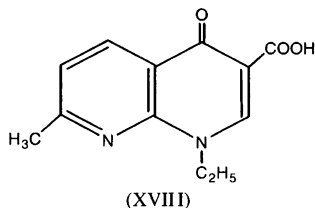


It can be used for urinary tract infections, gonorrhoea, chest infections caused by *Pneumococci*, *Haemophilis influenzae* and enteric *Salmonella* infections.

Side effects include megaloblastosis, leukopenia, thrombocytopenia, etc.

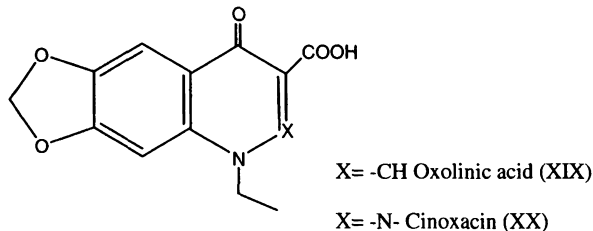
Quinolones:

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 substituent.



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*.

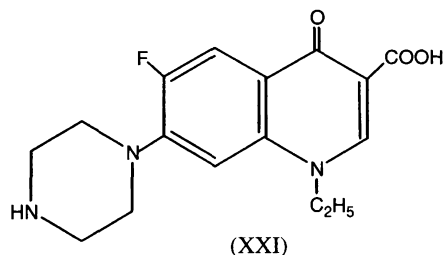
Over the years, the remaining first generation quinolones were introduced²⁰. These compounds consisted of more potent agents with a better spectrum of activity, such as oxolinic acid (XIX), cinoxacin (XX) etc.



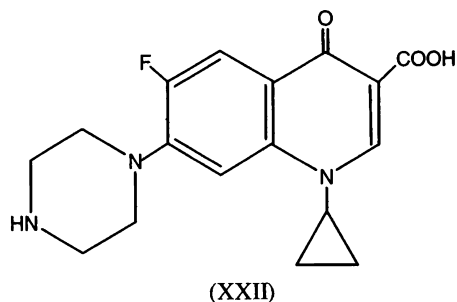
Although well absorbed on oral administration, they get highly bound to serum proteins and hence, are less potent.

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

Fluoroquinolones:



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.



Ciprofloxacin (XXII)²¹ is the most widely used fluoroquinolone because of its improved pharmacokinetic properties and a broader spectrum of activity.

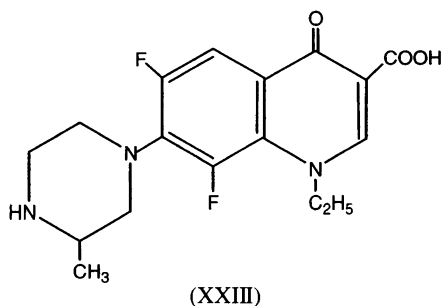
These drugs cause inhibition of the enzyme DNA-gyrase that unwinds the supercoiled DNA helix prior to replication and transcription. This inhibition makes the cell DNA inaccessible and leads to the cell death.

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.

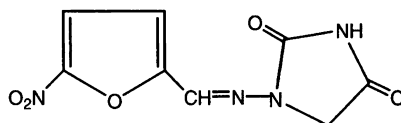
Ciprofloxacin is used in prostatic, upper respiratory tract infections, bone infections, septicemia, *Staphylococcal* and *Pseudomonas* endocarditis, meningitis, chronic ear infections, purulent osteoarthritis and in gonorrhoea, in combination with Cephalosporins²².

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



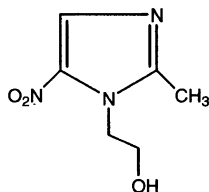
Nitrofurans:

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



(XXIV)

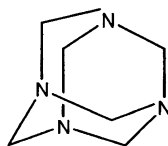
Nitrofurazone²³ and furazolidone²⁴ are other broad-spectrum nitrofurans in clinical use for the prophylactic treatment of UTI, and they also impair kidney stone growth. Nausea and vomiting are common side effects.



(XXV)

Nitroimidazoles include Metronidazole (XXV) (antiamoebic), Nifurtimox (antiprotozoal) etc. Metronidazole²⁵ is a drug of choice for the treatment of anaerobic infections.

Methenamine:



(XXVI)

Methenamine (XXVI) is internally used as a 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:

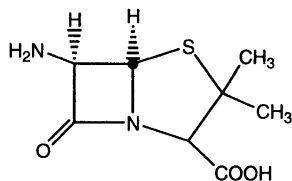
The 1990 worldwide commerce in antibiotics was measured in tonnages and its value is in excess of seven billion. About half of this is associated with β -lactam antibiotics alone.

β -lactam antibiotics are believed to act by selectively inhibiting the cell wall synthesis²⁶, 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 transpeptidase²⁷, thereby rendering it inactive for cross-linking of peptides.

Penicillins:

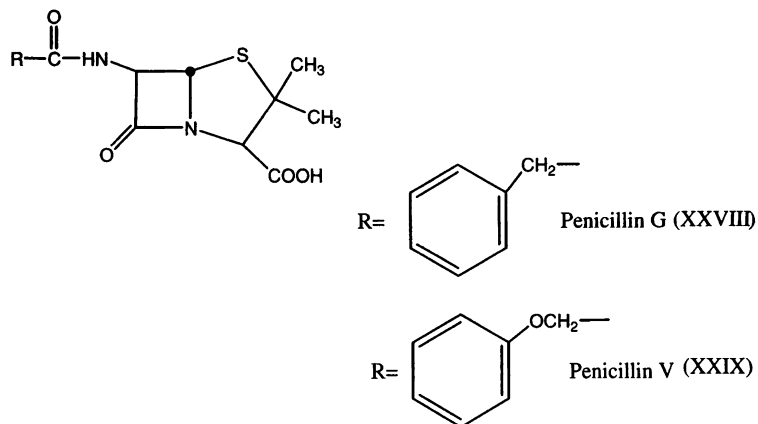
In 1929, Alexander Fleming for the first time discovered *Penicillium notatum* causing partial lysis of *Staphylococcal* colonies. Later on, Penicillin was isolated and gradually large-scale production also took place. The penicillin field was then expanded to include orally active penicillins, broad 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 with out breaking the β -lactam bond. More recently, selective chemical processes have been devised for accomplishing biosynthetic analogues.



(XXVII)

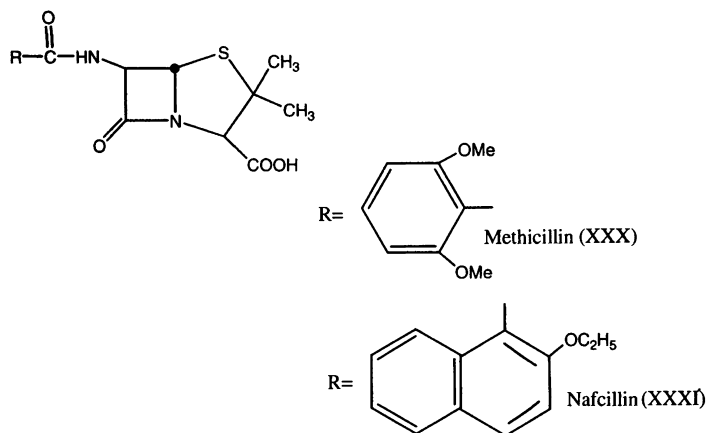
In the presence of phenyl acetic acid, benzyl penicillin (penicillin G) (XXVIII) and with phenoxy acetic acid, phenoxy methyl penicillin (penicillin V) (XXIX) was synthesized by fermentation.



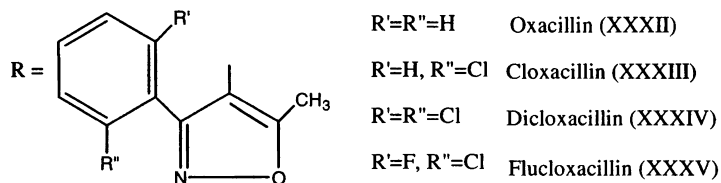
Resistance develops in the bacteria by the deactivation of the penicillin by the enzymes namely β -lactamase catalyses hydrolysis of β -lactam ring whereas acylase is responsible for hydrolysis of acylamino side chain. In Gram-negative organisms, the resistance develops due to poor penetrability.

Penicillinase-resistant Penicillins:

Substitution at ortho-position of a phenyl ring, i.e. Methicillin (XXX)²⁸ or at 2-position of 4- naphthyl system, i.e. Naficillin (XXXI)²⁹, increases steric hindrance of the acyl group and confers increased β -lactamase resistance.

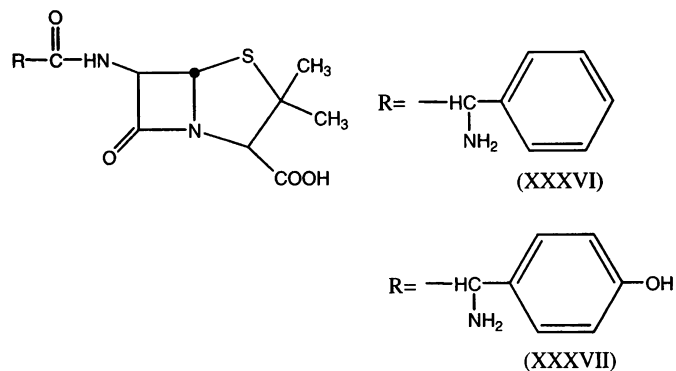


Bulkier substituents are more beneficial among five membered heterocyclic ring derivatives³⁰ e.g. Oxacillin (XXXII)³¹, Cloxacillin (XXXIII)³², Dicloxacillin (XXXIV)³³ and Flucloxacillin (XXXV)³⁴.

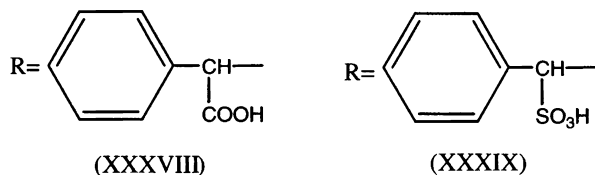


Extended Spectrum Penicillins:

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*, *Haemophilis*, *Salmonella*, *Shigella* and *Proteus*. e.g. Ampicillin (XXXVI)³⁵, Amoxicillin (XXXVII)³⁶ etc.



The introduction of acidic substituent 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 organism e.g. Carbenicillin (XXXVIII)³⁷, Sulbenicillin (XXXIX)³⁸.

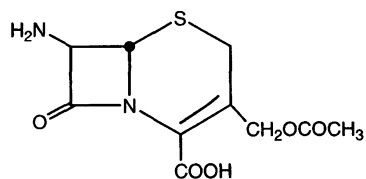


The allergic reactions include from skin rashes to drug fever, anaphylaxis and cross sensitivity³⁹. Allergy occurs due to production of antigenic penicilloyl protiens⁴⁰.

Cephalosporins:

Cephalosporins were discovered by Brotuz and were isolated and characterized by Abraham and Newton⁴¹. They have a wide range of activity against Gram-positive and Gram-negative organisms.

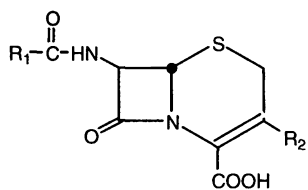
7-amino cephalosporinic acid (XXXX) is a key intermediate for the synthesis of a number of synthetic and semi-synthetic Cephalosporins.



(XXXX)

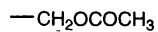
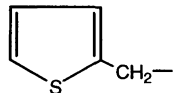
Cephalosporins have broad spectrum of activity with high potency against Gram-positive organisms and moderate potency against Gram-negative organisms.

Cephalothin (XXXXI)⁴² and Cephaloridine (XXXXII) are administered parenterally, whereas Cephaloglycin (XXXXIII) and Cephalexin (XXXXIV)⁴³ are active orally.

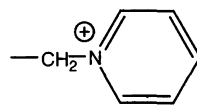
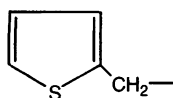


R₁

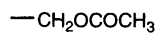
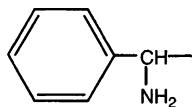
R₂



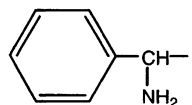
(XXXXI)



(XXXXII)

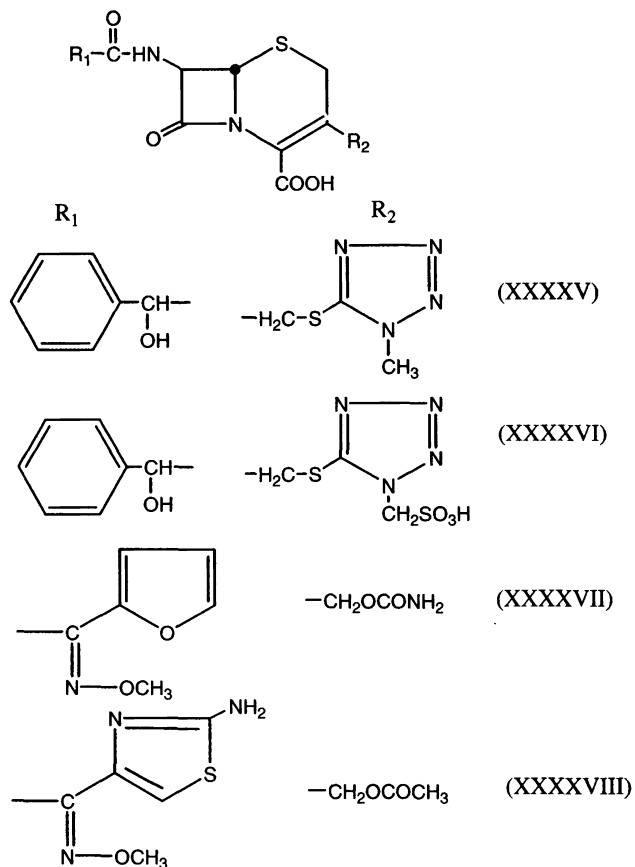


(XXXXIII)



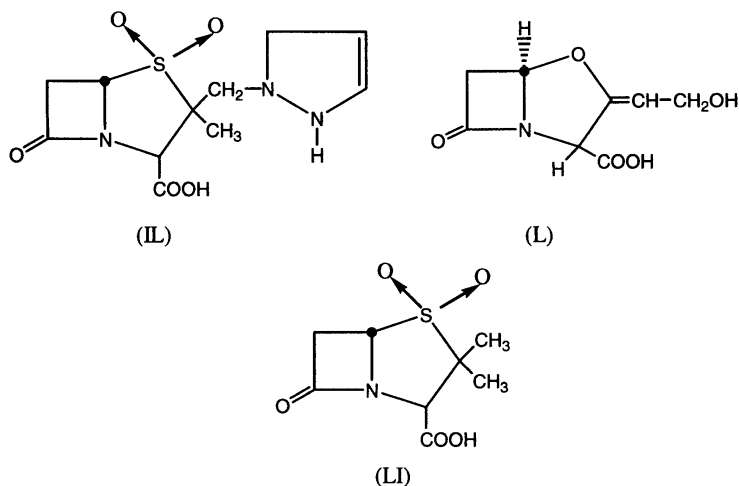
(XXXXIV)

With consistent use, resistance develops among several bacteria, which produce cephalosporinase, and thus hydrolyses the β -lactam ring. The semi-synthetic agents with plasmid-mediated cephalosporinase resistance⁴⁴ and acid stability were developed later on. These include Cephamandol (XXXXV)⁴⁵, Cephonacid (XXXXVI), Cephiroxime (XXXXVII)⁴⁶, Cefatoxime (XXXXVIII)^{47,48} etc.



β-lactamase inhibitors:

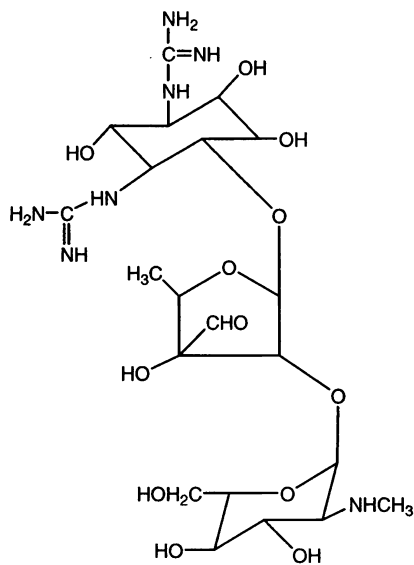
Many substances have been found to inhibit the β-lactamase enzyme, directly or by synergistic action along with some of the antibiotics. UTR-830 (IL), Clavulanic acid (L) and Sulbactam (LI) are some of the examples.



Aminoglycosides:

Aminoglycosides contain an amino sugar linked glycosidically 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. Kanamycin⁴⁹, Gentamycin⁵⁰, Tobramycin⁵¹, Netilmicin⁵², Amikacin⁵³ etc.

Streptomycin^{53,54,55} (LII) is the most effective of the group for the chemotherapy of tuberculosis, brucellosis, tularemia, *Yersinia* infections etc.



(LII)

Parmomycin^{56,57} is used primarily for amoebic dysentery.

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.

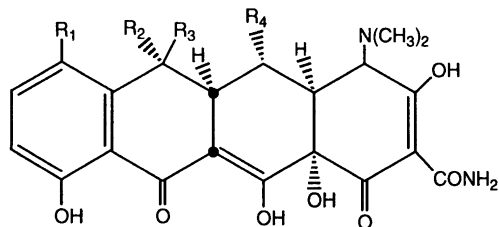
The resistance gets transferred from one bacterium to another and is attributed to the extrachromosomal R-factor (DNA), which is self-replicating and transferable by conjugation. These strains synthesize enzymes that are capable of acetylating, phosphorylating or adenylyating key amino hydroxy groups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

Tetracyclines:

Tetracyclines (LIII)⁵⁸ 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

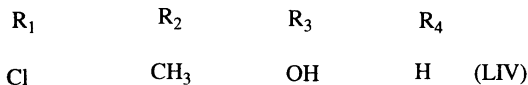
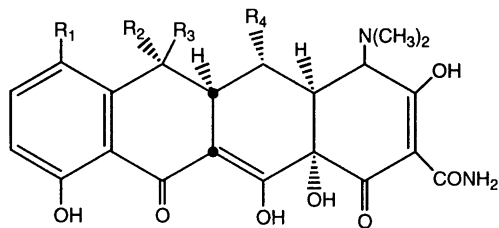
against a wide range of Gram-positive and Gram-negative bacteria, Spirochetes, Mycoplasmas, Rickettsiae, Chlamydiae etc.

But incomplete absorption and activity against natural intestinal flora causes superinfections. The development of resistance on persistent use is common. Parenteral Tetracyclines may cause liver damage, especially in pregnancy, or impaired renal function.



(LIII)

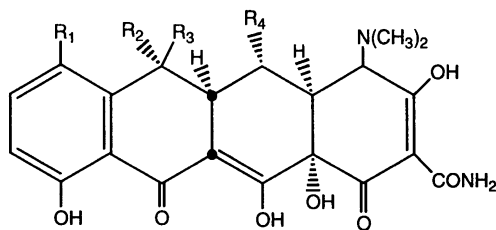
In 1938, Duggar⁵⁹ isolated Chlortetracycline. Electron withdrawing groups, e.g. in chlortetracycline⁶⁰ (LIV), as well as electron-donating groups, as in Minocycline (LV), enhance the activity of the parent molecule.



Oxytetracycline⁶¹ (LVI) has similar activity as that of Chlortetracycline. Doxycycline⁶² (LVII) and Methacycline⁶³ (LVIII) are more active *in vitro* than Oxytetracycline.

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In 1957, McCormik⁶⁴ *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 ring⁶⁵ aglycon, to which amino and neutral deoxy sugars are attached. In 1950, Picromycin was the first to be reported. McGuire⁶⁶, 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*⁶⁷ has been reported.

Erythromycin acts by binding selectively to 50S ribosomal subunit and thus prevents translocation in protein synthesis⁶⁸.

Non-specific resistance among Gram-negative bacilli is related to poor penetrability⁶⁹ of the drug.

The structural modifications in Erythromycin gave Clarithromycin, Flurithromycin etc. Clarithromycin is effective against *Mycobacterium avium – intercellulariae* complex^{70,71}. Olendomycin is one more important agent of the macrolide family.

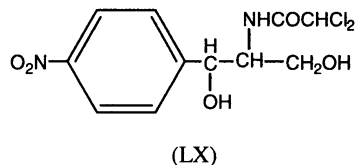
Lincomycins:

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*⁷², is useful for the treatment of infection by Gram-positive organisms, notably Staphylococci, β -haemolytic Streptococci and Pneumococci. Clindamycin⁷³ 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*⁷⁴, in 1974, isolated Chloramphenicol (LX), a broad spectrum antibiotic, from *Streptomyces venezuelae*. Constroulis, Rebstock and Crooks⁷⁵ established its structure in 1949.



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:

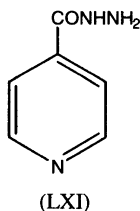
Tuberculosis is a disease largely caused by *Mycobacterium tuberculosis*, acid-fast bacilli. Today, as it has been for centuries, tuberculosis remains the leading cause of deaths in the world from infectious diseases. Globally, tuberculosis accounts for almost 3 million deaths and 8 million new cases emerging each year^{76,77,78}. Tuberculosis is a reemerging problem in many industrialized countries. In the modern world of global interdependency, 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, Rifampacin, 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, Tiocarlide, etc.

Isoniazide (INH):



Isonicotinyl hydrazide (Isoniazid) (LXI), described in 1912⁷⁹, 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 (Isoniazide) 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; it is only bacteriostatic⁸⁰ 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 suggest 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. Acetylation metabolizes it.

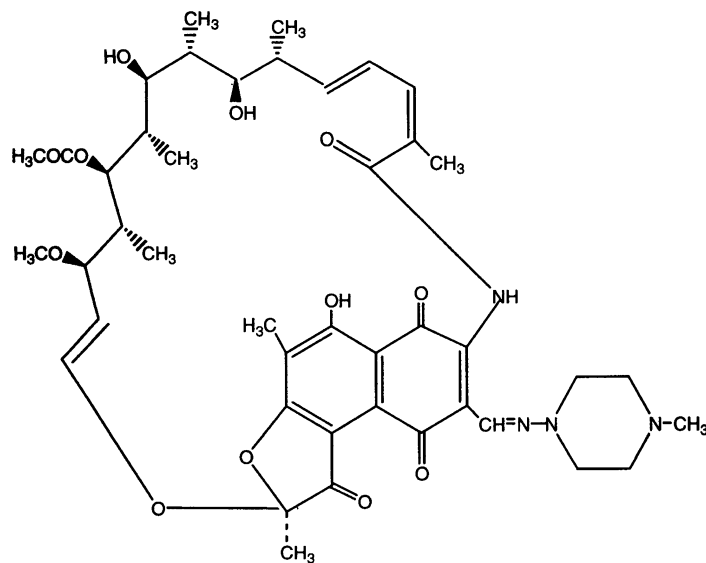
More recently it has been shown that the activity of INH results from peroxidative reaction catalyzed by peroxidase, which is encoded by Kat G Gene⁸¹. *M. tuberculosis*, which lacks this gene, is resistant to INH⁸².

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 *Amycolaptopsis mediterranei*⁸³.

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)⁸⁴ was the most active form *in vivo*, at a concentration of 1 µg/ml, and is useful clinically.



(LXII)

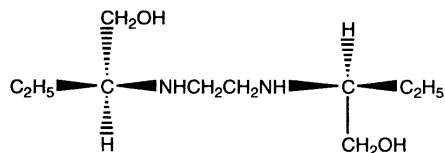
Rifampicin inhibits specifically the activity of the enzyme DNA-dependent RNA polymerase and is active against rapidly dividing intra and extra cellular organisms.

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. Deacetylation and desacetylation metabolize it.

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 organism 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^{85,86}. Another hypothesis states that it causes an inhibitory effect on phosphorylation⁸⁷.

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*.

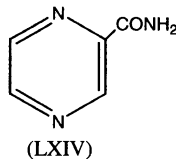
In mycobacteria, mutation in the *rspL* gene, which encodes the ribosomal protein S12, has been shown to confer resistance to Streptomycin⁸⁸.

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

The most important toxic effect of Streptomycin is damage to the 8th cranial (vestibulocochlear) 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:



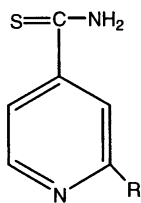
The best activity of Pyrazinamide (LXIV) is against intracellular Mycobacteria causing sterilizing effect in acidic conditions. The susceptible bacteria produce deaminase, which converts Pyrazinamide to Pyrazinoic acid, an active form.

Pyrazinamide gets readily absorbed orally and is metabolized to Pyrazinoic acid, which gets oxidized 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 recommended⁸⁹.

Ethionamide:



R = -C₂H₅ (LXV)

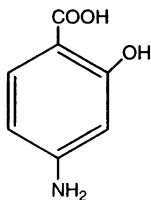
R = -N-C₃H₇ (LXVI)

2-ethylthioisonicotinamide (Ethionamide) (LXV) and its 2-N-propyl analogue (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):



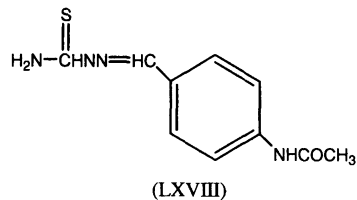
(LXVII)

Among the various compounds related to benzoic acid, PAS (LXVII) is believed to be the most active agent⁹⁰ 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 uptake⁹¹.

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:



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 limit its use in therapy.

Drug Regimens followed in the treatment of Tuberculosis:

<u>Drug</u>	<u>Initial phase(2 months)</u>	<u>Continuation phase(4 months)</u>
-------------	--------------------------------	-------------------------------------

Standard regimen:

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*	15-20mg/kg/day	–

Intermittent regimen:

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

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

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

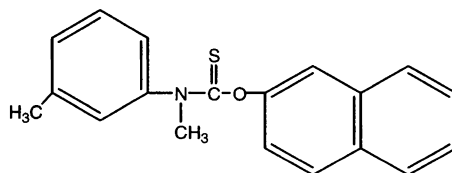
Antifungal agents:

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 is 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.

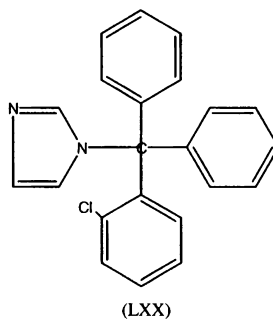
In susceptible fungi, Tolnaftate suppresses squalene epoxidase, which converts squalene to ergosterol and thereby affects cell wall formation.

Substituted azoles:

The substituted imidazoles represent a versatile group of antifungal agents with a unique mechanism of action. The earlier members reported were Clotrimazole (LXX)⁹² and Miconazole (LXXI)⁹³, in 1969. These agents are active against dermatophytes such as *Trichophyton*, *Epidermophyton*, *Microsporum* and yeasts like *Candida albicans*. They are also active systemically in infections caused by *Cryptococci*, *Blastomyces*, *Histoplasma*, *Paracoccidioides* etc.

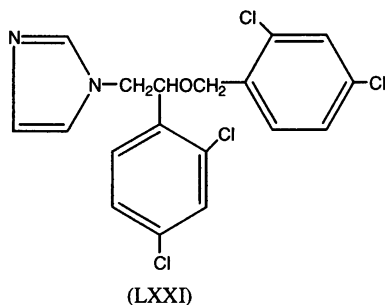
The mechanism of action of the drug is elucidated⁹⁴ extensively. At high concentrations (micromolar), they are fungicidal, while at low concentrations (nanomolar), they are fungistatic. The fungicidal effect is associated with damage to the cell wall with the loss of essential cell components, such as potassium ions and amino acids. The imidazole inhibits the cytochrome P-450 oxidase enzyme that catalyses the 14- α -demethylation of lanosterol to ergosterol.

Clotrimazole:



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

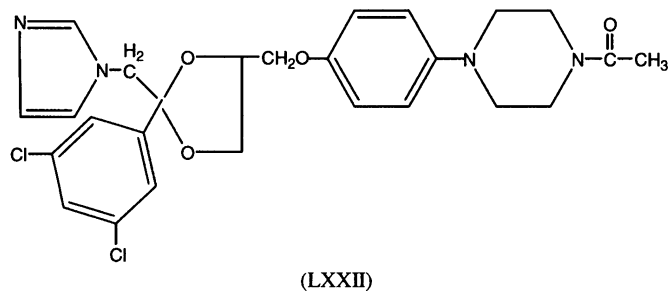
Miconazole:



Miconazole (LXXI) 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:

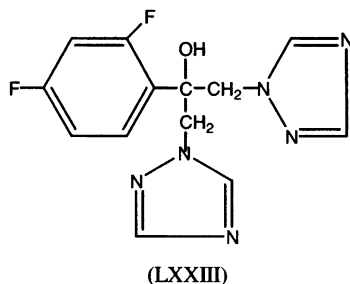


Ketoconazole (LXXII) is administered orally for the treatment of systemic mycosis. It requires gastric acidity for absorption. Antacids and drugs like H₂-histamine antagonists and anticholinergics that inhibit gastric secretion, interfere with

its absorption. It is metabolized 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.

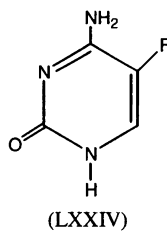
Fluconazole:



The absorption of Fluconazole (LXXIII) does not depend upon gastric acidity; more over, 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, Ticonazole, Itraconazole and Terconazole.

Flucytosine:



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 inhibited³.

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

Due to 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:

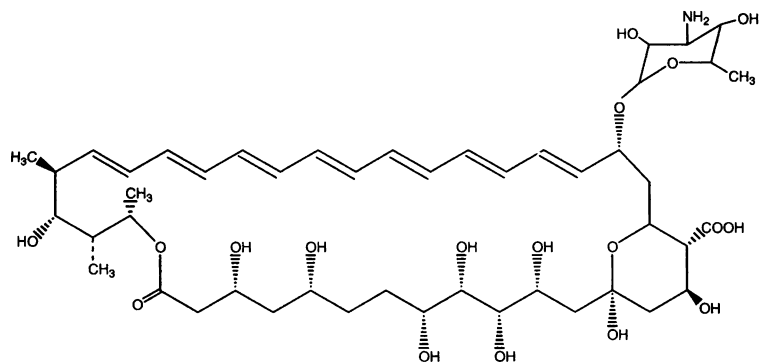
Polyene antibiotics:

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, moulds 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 disorganization and loss of cell constituents, especially potassium ions. The action is bacteriostatic at low concentrations and bactericidal at the higher doses.

Amphotericin B:



(LXXV)

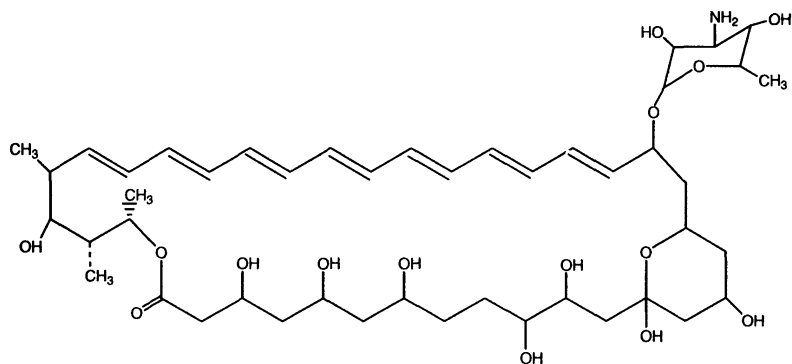
A potent antifungal agent with a polyene structure was isolated in 1956 by Gold *et al*⁹⁵ from *Streptomyces nodosum*, found in the soil from Venezuela. The antibiotic material was a mixture of two closely related compounds, Amphotericin A and B, of which B is more active.

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 the injection and thrombophlebitis occurs on intravenous administration. It should not be administered intramuscularly. Amphotrocin is also used topically to treat cutaneous and mucocutaneous infections.

Nystatin:



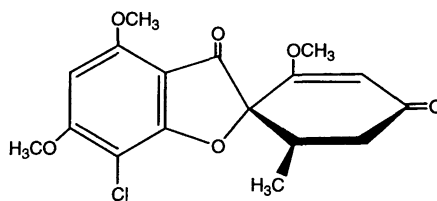
(LXXVI)

A polyene antibiotic first isolated in 1951 from a strain of *Streptomyces noursei* by Hazen and Brown⁹⁶, 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 the patients with AIDS. It is available in cream, ointment or powder form of topical use, whereas tablets or troches are used systemically.

Griseofulvin:

Griseofulvin (LXXVII) was first reported in 1939 by Oxford *et al*⁹⁷ as an antibiotic obtained from the mould *Penicillium griseofulvum* Direckx. Its structure has been determined by Grove *et al*⁹⁸.

Griseofulvin is recommended for systemic treatment of refractory ringworm 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 assembly⁹⁹.

The bioavailability of Griseofulvin may be enhanced by administration with fatty meal. Methylation metabolizes it.

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 fungi¹⁰⁰. 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, helminthiasis, etc. affects hundreds of millions of people every year, causing immense suffering and hindering development. This has been 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 4 series of compounds, the details of which will be discussed in Part II, Part III, Part IV and Part V of the thesis.

PART II :-

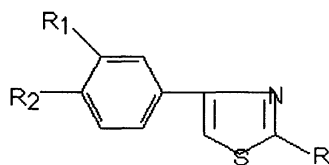
Synthesis of 2{4'-[1-(1,2,4-triazolyl) methyl] phenyl}-4-(substituted) thiazoles.

Thiazole derivatives have been reported to exhibit significant chemotherapeutic activities¹⁰¹. Various therapeutically established compounds viz. Aminitrazole, Tetramisole, Thiabendazole, Niridazole, Sulphathiazole, etc. all contain thiazole ring (I), in one form or another which is the pharmacophore responsible for the activity.



Several, literature reports further strengthened this observation.

Burmistov *et al*¹⁰², reported the synthesis of substituted thiazole derivatives (II), possessing antibacterial and antifungal activities.



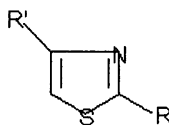
(II)

R= Substituted aryl

R₁= NO₂

R₂= Heteroaryl

Prabhu *et al*¹⁰³ further reported good activity of 2,4-disubstituted thiazoles (III) against *tubercle bacilli*.

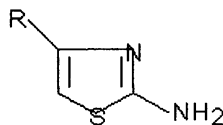


(III)

R= alkyl

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

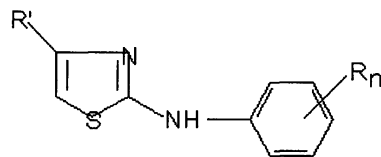
In 1965, Romanian-research workers, Carp *et al*¹⁰⁴, showed that an alkyl or substituted aryl group at the 4-position and amino group at 2-position of the thiazole imparts bacteriostatic activity to the thiazole molecule (IV).



(IV)

R= -C₆H₅CH₃, -C₆H₅Cl, etc.

Likewise, Gupta *et al*¹⁰⁵ reported that the presence of phenyl group at the 4-position of thiazole ring (V) system increases the fungi toxicity 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.

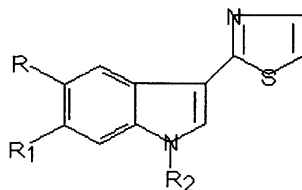


(V)

R' = methyl, phenyl

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

It has also been reported that thiazoles (VI) bearing other ring systems possess good antifungal activity¹⁰⁶.



(VI)

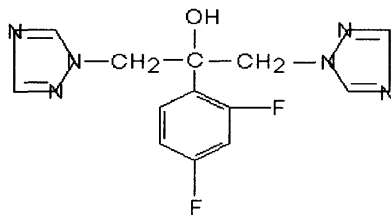
R = H

R₁ = H, -OCH₃

R₂ = H

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

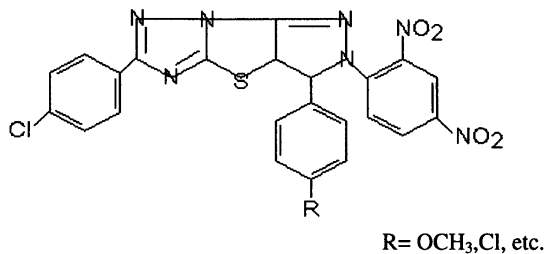
Introduction of 1,2,4-triazole was considered in the thiazole moiety as regards to its good antifungal activity. The fact ascertained by its presence in the recently developed broad-spectrum antifungal drug, Fluconazole¹⁰⁷ (VII).



(VII)

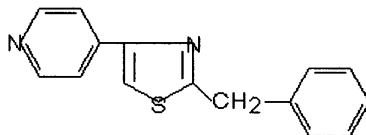
Several references indicating the activity of thiazolo triazoles were also obtained from the literature survey.

Jagmohan *et al*¹⁰⁸ have synthesized pyrazolo [3',4':4,5] thiazolo [3,2-b]-s-triazoles (VIII), which were found to exhibit both antibacterial and antifungal activity.



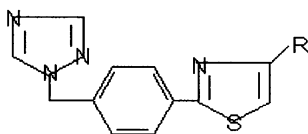
(VIII)

Boulton *et al*¹⁰⁹ 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 substituted phenyl/heterocyclic substituent 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 and antifungal activity.



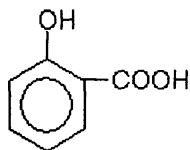
(X)

R= Substituted phenyl / heterocycle

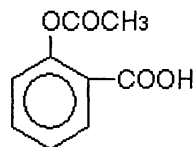
PART 3 :- Synthesis of 2-(1H- Benzimidazol-2-yl mercapto) -N- (1,5-Dimethyl-2- substituted phenyl-3- oxo-2,3- dihydro-1H-Pyrazol-4-yl) acyl amine.

Pyrazoles are an important class of heteroaromatic ring systems that find extensive use in the agrochemical industry¹¹⁰ and pharmaceutical industries¹¹¹. After the discovery of the antipyretic action of salicylic acid in 1875, many phenol and aniline derivatives were tried. Antipyrine (antifebrine) was among first of this group, later came acetanilide, acetophenetidin (phenacetin), acetyl salicylic acid (aspirin), aminopyrine (pyramidon), cinchophen (atophan) and neocinchophen (tolysin). The formulas disclose the structure of the important compounds.

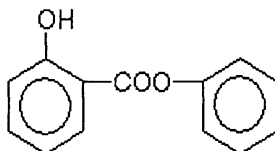
Salicylates



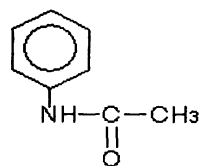
Salicylic acid



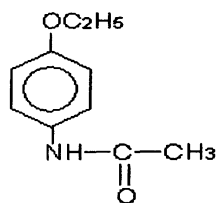
Acetyl salicylic acid



Phenyl salicylate (Salol)

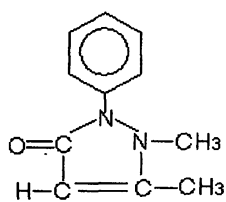


Acetanilide

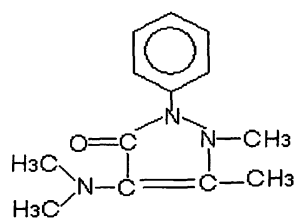


Acetophenetidin

Pyrazolon derivatives

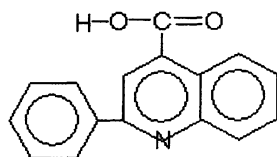


Antipyrine

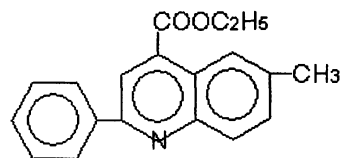


Aminopyrine

Quinoline derivatives



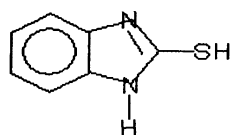
Cinchophen



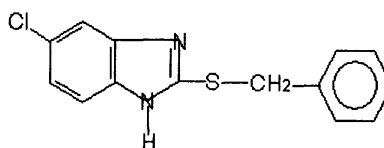
Neocinchophen

Benzimidazole is one of the oldest and chemically well studied nitrogen heterocycles, it has quietly recognized as the versatile nucleus for building molecules possessing broad spectrum of biological activities such as powerful antihelmintic, antimicrobial, pesticidal, antimalarial, anticancer and pharmacological activities.

During the literature survey, it was found that 2-mercapto benzimidazole (XI) itself is a bacteriostatic and antifungal agent¹¹², also its derivatives¹¹³ namely 5(6)-chloro-2-(benzylthio) benzimidazole (XII) posses antifungal activity against *Trichophyton interdigitale*, at a dilution 1:290,900 or 3.4µg/ml. Similarly, bacteriostatic activity was also reported for 2-mercapto benzimidazole against *S. aureus*¹¹⁴.

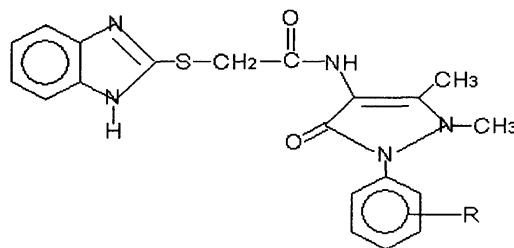


(XI)



(XII)

Hence, taking into considerations above all facts mentioned above the fusion of 2-mercapto benzimidazole and pyrazolone derivative ie. Antipyrine containing different substituents in phenyl ring of it was planned and synthesized. The compounds were synthesized as per the method outlined in Scheme-II (page: 76). The final compound from this series will have the following structure (XIII).

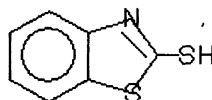


R= 3-chloro, 2,4-dichloro, etc.

(XIII)

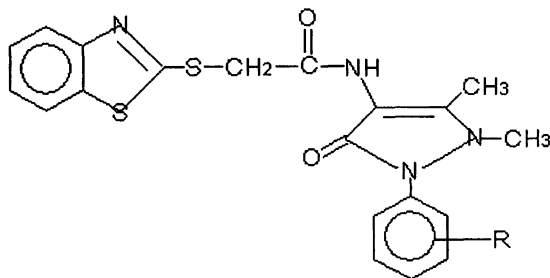
PART 4 :- Synthesis of 2-(1,3- Benzothiazol-2-yl mercapto) -N- (1,5-Dimethyl-2- substituted phenyl-3- oxo-2,3- dihydro-1H-Pyrazol-4-yl) acyl amine.

Variety of benzothiazole derivatives are known in the literature for their prodigious range of activities, particularly they are more valuable as antiparasitic¹¹⁵, anti-inflammatory¹¹⁶, fungicidal¹¹⁷, herbicidal¹¹⁸ and antibacterial¹¹⁹ agents. A mercapto group is an important toxophore responsible for pesticidal activity¹²⁰. Therefore, in this series a combination of 2-mercapto benzothiazole (XIV) with pyrazolone derivative mentioned in part-3 was planned.



(XIV)

Thus the final compound (XV) from this series will have the structure mentioned below and the method used for synthesis is outlined in Scheme-III (page: 77).



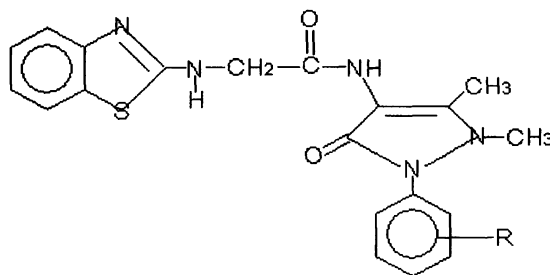
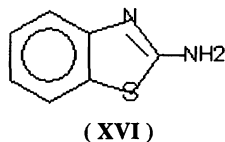
R= 3-chloro, 2,4-dichloro, etc.

(XV)

PART 5 :- Synthesis of 2-(1,3- Benzothiazolyl-2-amino) -N- (1,5-Dimethyl-2- substituted phenyl-3- oxo-2,3- dihydro-1H-Pyrazol-4-yl) acyl amine.

The literature survey revealed that 2-amino-6- substituted benzothiazole are known to exhibit excellent antitubercular activity. For example, some 6- substituted-2- amino benzothiazoles show antitubercular activity at the dose level of 0.025 to 1.0 $\mu\text{g/ml}$ *in vitro* testing¹²¹.

The fact mentioned in part-3 about pyrazolone derivative and as discussed above the activity of substituted 2- amino benzothiazole the fusion of 2-amino benzothiazole (XVI) and pyrazolone derivative was planned and compounds were synthesized. The final compound was synthesized using the method outlined in Scheme IV (page: 78). The final compound (XVII) synthesized will have the following structure.



R= 3-chloro, 2,4-dichloro, etc. (XVII)

Chemistry

CHEMISTRY

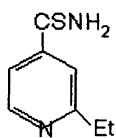
This part deals with the chemistry of the following compounds / intermediates involved in the project.

- ♦ **Thioamide**
- ♦ **Halogenation of ketones**
- ♦ **Thiazoles**
- ♦ **Benzimidazoles**
- ♦ **Benzothiazoles**
- ♦ **Diazonium salts**
- ♦ **2-Pyrazolin-5-ones**
- ♦ **Nitration**

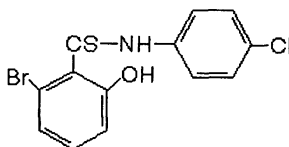
Thioamide

Chemistry of thioamides:

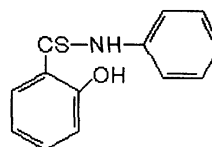
Liberman and co-workers, in 1955, observed the in vivo activity of Ethioinamide (I) against *M. tuberculosis* and it was found to exhibit low toxicity. Later on, therapeutically effective agents such as substituted thiobenzamide (II), salicylic acid thioamide (III) were found.



(I)



(II)



(III)

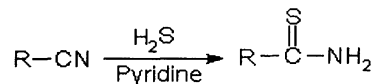
General synthetic methods:

1. From nitriles:

Nitriles in the presence of excess of hydrogen sulphide and a catalyst based yields thiocarboxamides¹²²⁻¹²³.

Water-soluble sulphides or polysulphides can be used instead of hydrogen sulphide¹²⁴.

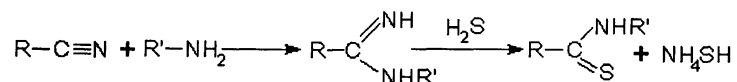
The reaction occurs as follows –



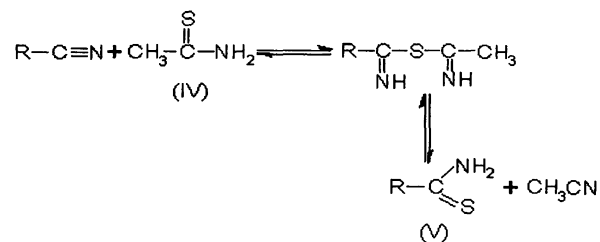
Catalyst used include ammonia, aliphatic amine, pyridine, alkoxide and potassium hydrogen sulphide¹²⁵⁻¹²⁶. Fairfull et al¹²⁷ obtained very good yield by addition of H₂S to the nitrile in the presence of pyridine and triethylamine.

Aliphatic nitriles need elevated temperature and pressure¹²⁸, as they react slowly and give poor yield.

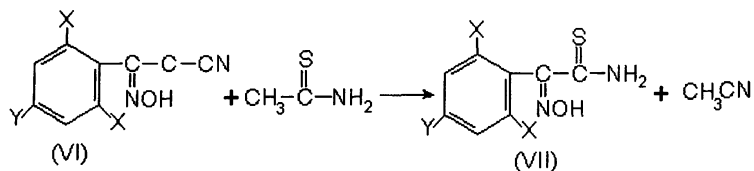
Kindler¹²⁵ and Hanford¹²⁹ extended the method by addition of primary amine.



The reaction of nitriles with thioacetamide(IV) have appeared more frequently in literature, than with H_2S ¹³⁰⁻¹³¹.

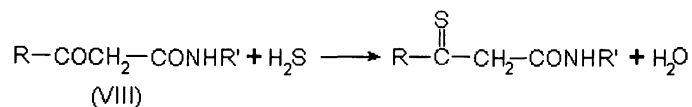


The removal of acetonitrile leads to the formation of thiocarboxamide(V) and thus is an important method for preparation of thiocarboxamide(VII) from α -cyanobenzaldoximes(VI).

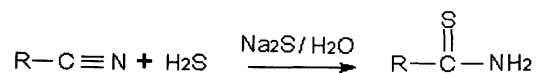


X, Y = -Cl, -F, etc.

If the nitrile contains a hydroxyl group, it must be protected by acylation¹³²⁻¹³³. The ketogroup, such as that in (VIII) if present, is readily converted to thioxo in acidic solution¹³⁴.



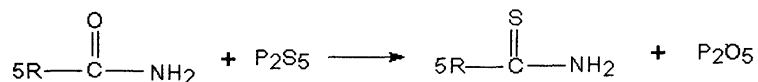
Mayer *et al*¹³⁵ explained the base catalysed conversion of ketone to thiones. Recently, Casser *et al*¹³⁶ modified it and introduced aqueous sodium sulphide as phase transfer catalyst in the general procedure to get high yields of thioamide.



This system consists of three phases, aqueous, organic and H₂S 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 P₂S₅¹³⁷. Potassium thiophosphate, along with P₂S₅, enhances the yield further¹³⁸.



R = alkyl/aryl.

Solvents such as benzene, xylene, toluene, tetrahydrofuran, dioxane, diethylene glycol and diethyl ether¹³⁹⁻¹⁴² 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 yields¹⁴³⁻¹⁴⁴. The reactions of liquid amides are carried out in an excess of amide as a solvent¹⁴⁵⁻¹⁴⁶. Thermally unstable amides are not suitable for this reaction. Hydroxyl group must be protected by acylation before sulphurization¹⁴⁷⁻¹⁴⁸.

3. From isothiocyanates:

Sach and Loevy¹⁴⁹ described the reaction of isothiocyanates with Grignard's reagent and subsequently hydrolysis under mild conditions, to yield the thiocarboxamide.

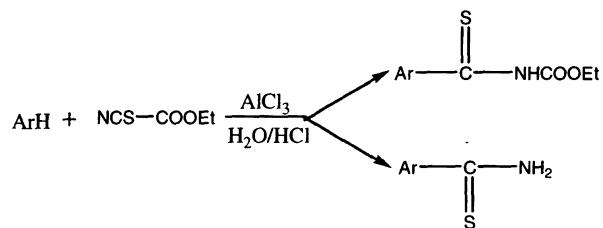


Where R, R' = alkyl/aryl.

4. Single step synthesis of aromatic thioamides:

a) From ethoxy carbonyl isothiocyanate:

Ethoxy carbonyl isothiocyanate¹⁵⁰ reacts with aromatic compounds in the presence of anhydrous AlCl_3 (Fridel- Crafts isothiocynylation). However, depending upon the conditions, it either yields the N- ethoxy carbonyl thioamide or thioamide itself.

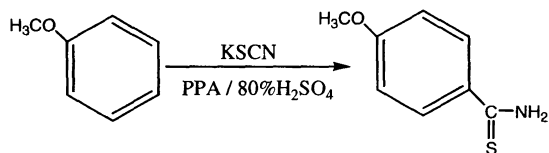


For thioamide to be a major product, the major ratio of AlCl_3 to isothiocyanate should be 2:1. Thioamide takes a long period to form.

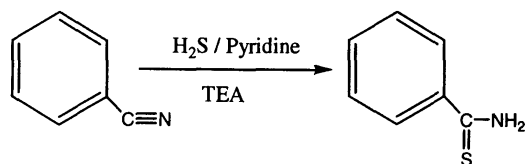
The reaction at a low temperature leads to N-ethoxy carbonyl thioamide.

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)¹⁵¹.

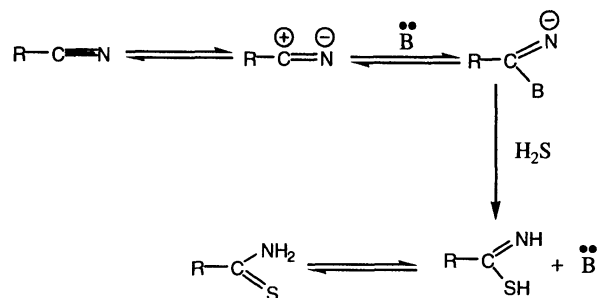
50% yield in PPA can be increased by 5-10% with 80% H_2SO_4 .



In the present work, thioamide was prepared by dissolving aromatic nitrile in pyridine and H₂S gas was bubbled in the reaction mixture using triethylamine as a catalyst.

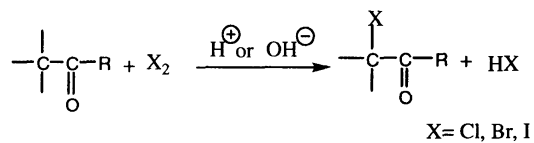


Mechanism:



Halogenation of ketones

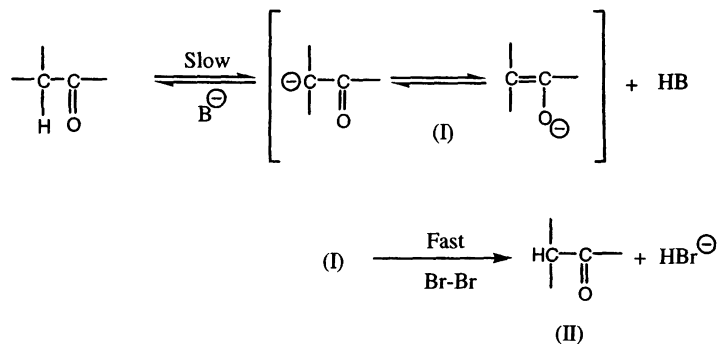
Ketones that possess an α -hydrogen can readily be converted to the corresponding halides by replacing the hydrogen with halogen, in the presence of acidic as well as basic medium.



General methods for synthesis:

1) Base-promoted halogenation of ketones:

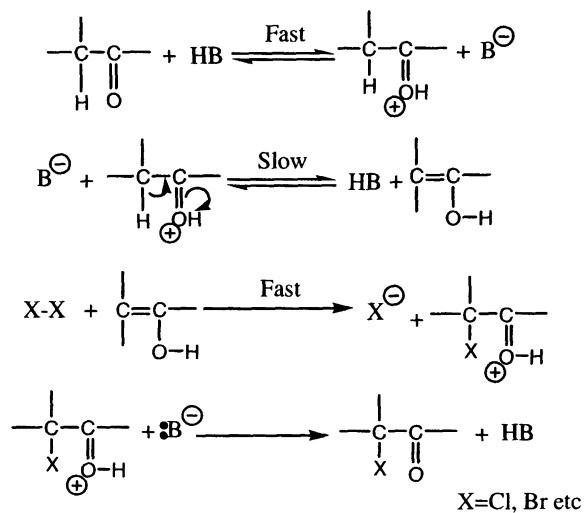
A base strongly abstracts a proton from a ketone to form the carbanion (I), which then rapidly reacts to yield the bromoketone (II).



The generation of carbanion determines the overall rate of the reaction. As soon as the carbanions are generated, the bromine molecule snaps them.

2) Acid catalysed halogenation:

Acids also catalyses the halogenation of ketones.



X=Cl, Br etc

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

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

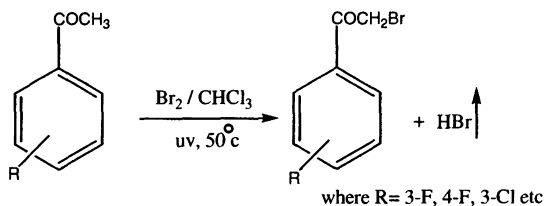
Solvents used include chloroform, acetic acid, carbon tetrachloride, diethyl ether, etc. When diethyl ether was used as a solvent, aluminium chloride is used to promote enolization.

Cupric halides have occasionally been used to halogenate aromatic ketones, particularly is the use of free halogen causes nuclear halogenation, eg. 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:

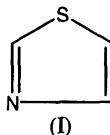
In the case of bromination, the presence of sunlight (UV) or slight heating (60° - 65°C) facilitates the initiation of the reaction. It helps bromine to get polarized as the Br radical, which favors the attack of polarized Br_2 molecule or Br on the enol form of the ketone.

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

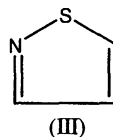
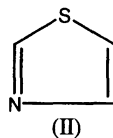


Chemistry of thiazoles

The thiazole heterocycle (I) was first delineated by Hantzsch and Weber¹⁵². Further, they described it as “the pyrrole of the thiophene series”.



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



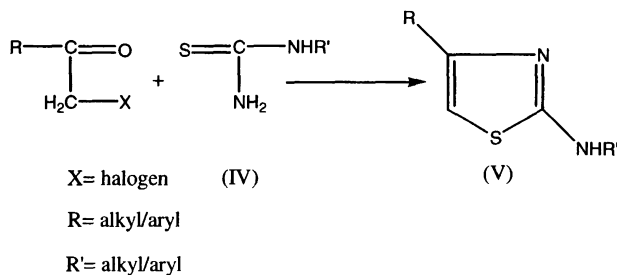
The reactivity of the thiazole molecule is due to the group attached to the carbon between Sulphur and nitrogen.

Large number of thiazoles occurs naturally. They include vitamin B (thiamine), Penicillin, etc.

General methods for synthesis:

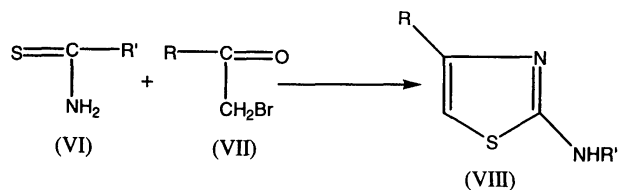
1) Synthesis of 2-substituted amino thiazoles:

The cyclization of N- monosubstituted thiourea (IV) with halo carbonyl compound gives 2- monosubstituted (V) thiazoles¹⁵³⁻¹⁵⁵.



2) 2,4-disubstituted thiazoles:

These were prepared in 1950 by Erylenmeyer *et al*¹⁵⁶ from thiobenzamide (VI) and bromoacetophenone (VII) in alcohol as a solvent, which yielded 50-80% of the thiazole (VIII).

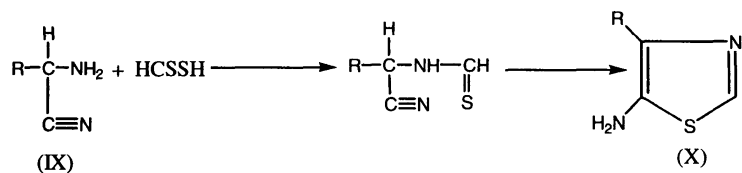


R= substituted aryl

R'= substituted aryl

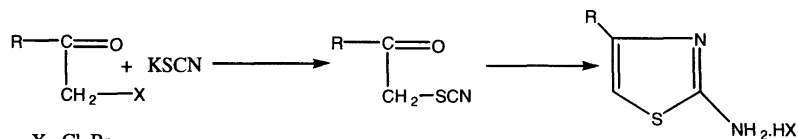
Other synthetic methods for thiazole:

- 1) α - Amino nitrile (IX) reacts with dithioformic acid (X) at room temperature to give an intermediate, which cyclizes to yield 5- amino thiazole derivative¹⁵⁷.



R= alkyl/ substituted aryl

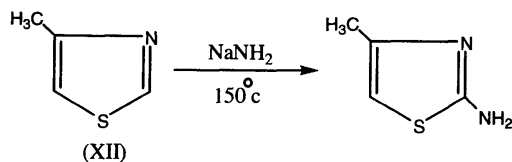
- 2) Potassium thiocyanate reacts with halocarbonyl compound (XI) to give 2-



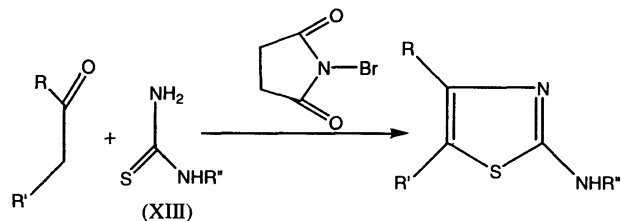
X= Cl, Br

R= alkyl/ substituted aryl
amino thiazole hydrochloride¹⁵⁸.

- 3) 4-methyl thiazole (XII) on heating with sodamide for 15hrs. at 150°C yields 2-amino-4-methyl thiazole¹⁵⁹.

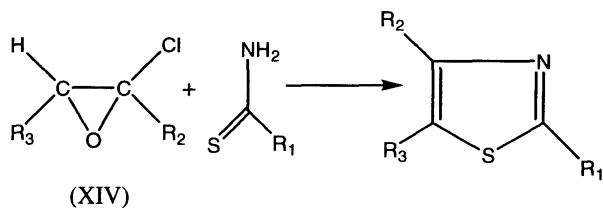


- 4) The reaction of substituted thiourea (XIII) with ketone in the presence of N-bromosuccinimide furnishes 2-amino thiazole derivative¹⁶⁰.



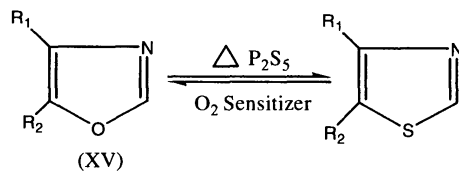
R=R'=R''= Substituted aryl / alkyl

- 5) 1-chloro-1,2-epoxide(XIV), when condensed with thioamide leads to 2,4,5-trisubstituted thiazole¹⁶¹.



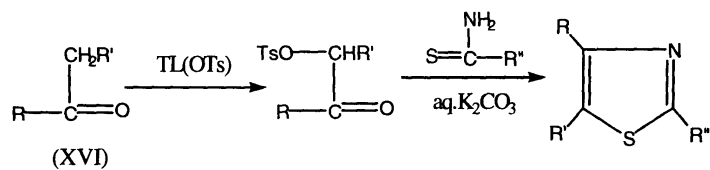
R₁=R₂=R₃= Substituted aryl / alkyl

- 6) Oxazole (XV) when heated with P₂S₅, gives thiazole¹⁶².

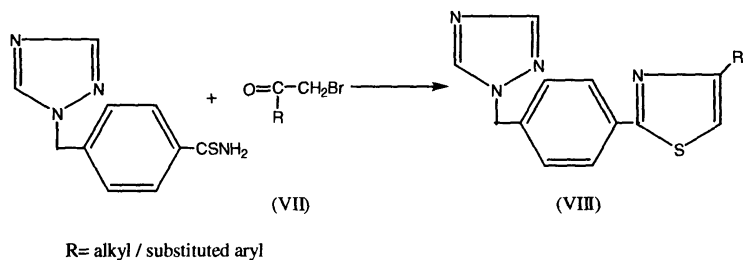


R₁= R₂= alkyl / substituted aryl

- 7) Enolized ketone (XVI) reacts with thioamide or substituted thioamide in the presence of thallium p-tolyl sulphonate (TTS) to give 2,4,5-trisubstituted thiazole¹⁶³.



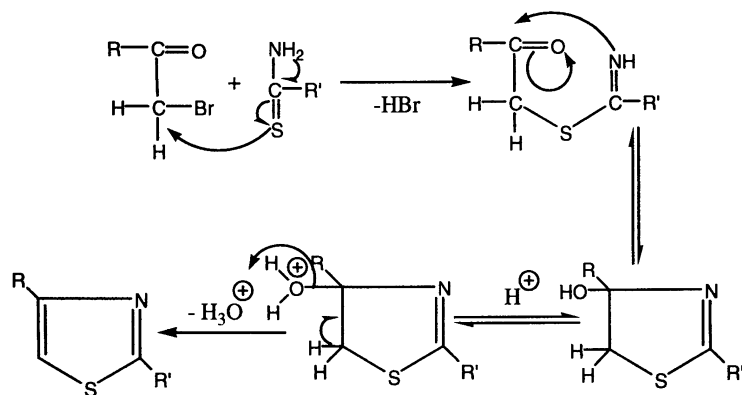
In the present work, 2,4-disubstituted thiazole has been prepared by refluxing a mixture of 1H-1,2,4-triazolyl methyl benzonitrile with various α -bromo phenyl/heterocyclic acetaphenones (1:1) in dry alcohol.



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 molecule¹⁶⁴⁻¹⁶⁵.



Chemistry of benzimidazoles

Many benzimidazole derivatives possess antiparasitic¹⁶⁶, antibacterial¹⁶⁷ and antiviral¹⁶⁸ activities.

These are relatively non-toxic and have fewer side effects.

General methods for synthesis:

In general all synthetic routes for benzimidazole derivatives start from o-phenylene diamine.

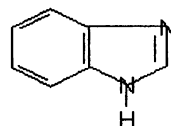
Following are the various methods reported in the literature for the synthesis of benzimidazole.

- By reacting o-phenylene diamine with carboxylic acid¹⁶⁹⁻¹⁷⁰.
- By reacting o-phenylene diamine with acid chlorides¹⁷¹⁻¹⁷², esters¹⁷³ or acid anhydride¹⁷⁴.
- By reacting o-phenylene diamine with aldehyde¹⁷⁵ or ketone¹⁷⁶.
- By reacting o-phenylene diamine with urea¹⁷⁷ or thiourea¹⁷⁸.
- By reacting o-phenylene diamine with isothiocyanate¹⁷⁹.

The benzimidazole nucleus is very stable in character. It does not get easily oxidized or reduced. Benzimidazole is weakly basic, but somewhat less than imidazoles. They are in general soluble in dilute acids. Benzimidazole is sufficiently acidic to be soluble in aqueous alkali and form N-metallic compounds.

The acidic properties of benzimidazole like those of imidazoles¹⁸⁰ seem to be due to stabilization of ions by resonance. The solubility of benzimidazole in alkaline solution depends on the particular compound in reaction. The more acidic benzimidazole may be soluble in less basic solution, such as K_2CO_3 solution. Benzimidazole form salt with acid readily.

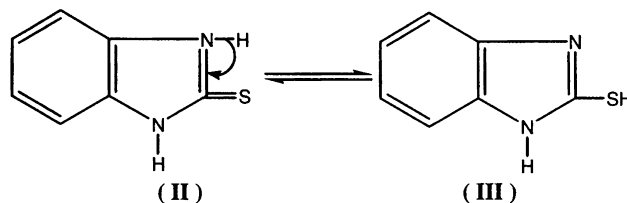
The benzimidazole contains a phenyl ring fused to an imidazole ring indicated in the structure (I).



(I)

2-Mercapto benzimidazole

2-(3H)-benzimidazole thione (II) and 2-mercapto benzimidazole (III) are tautomeric forms, analogous to 2-(3H)-benzimidazolone and 2-hydroxy benzimidazole.



(II)

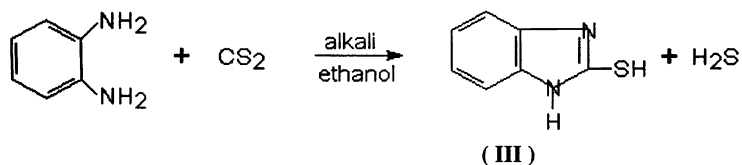
(III)

Methods of preparation of 2-mercapto benzimidazole:

a) Synthesis from o-phenylene diamine and carbon disulphide:

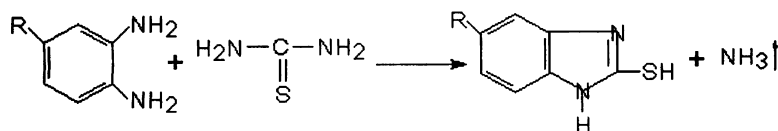
2-mercapto benzimidazole, in general may be prepared very conveniently by this method. The reaction is carried usually by heating the reactant in alcoholic solution with or without the addition of alkali to the reaction mixture.

2-mercapto benzimidazole (III) itself has been prepared by this method¹⁸¹⁻¹⁸². o-phenylene diamine having substituent in the phenyl ring have been used, including those with alkyl¹⁸³, halogen¹⁸⁴, phenyl¹⁸⁵, aniline¹⁸⁶ and sulphonamido¹⁸⁷ group.



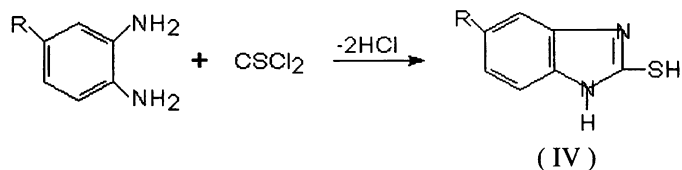
b) From o-phenylene diamine and thiourea:

The reaction is analogous to the preparation of 2-hydroxy benzimidazole from o-phenylene diamine and urea. 2-mercapto benzimidazoles may be prepared by this method by heating o-phenylene diamine dihydrochloride and thiourea to 170-180°C¹⁸⁸. Excellent yield have been obtained by carrying out the reaction in amyl alcohol and heating under reflux until solution of ammonia becomes negligible.



c) From o-phenylene diamine and thiophosgene:

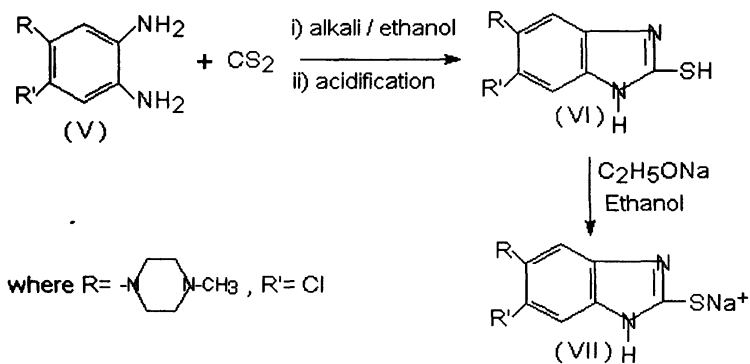
Billester and Steiner¹⁸⁹ obtained 2-mercapto benzimidazole and 5- methyl-2-mercapto benzimidazole by action of thiophosgene on o-phenylene diamine and 3,4-diamino toluene respectively. 3,4-diamino benzene arsenic acid and thiophosgene gave 70per cent 2-mercapto-5-arsenic acid benzimidazole¹⁹⁰.



d) Miscellaneous methods:

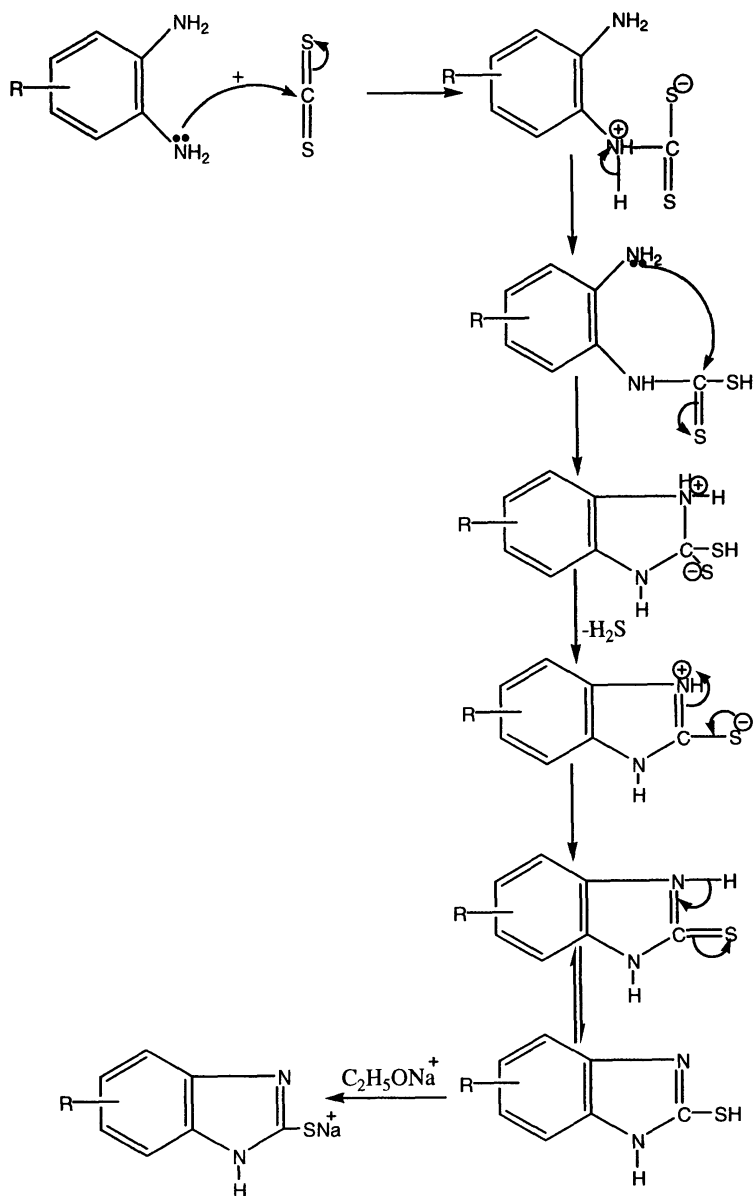
2-mercapto benzimidazole can be prepared in 84-86% yield by the action of potassium ethyl xanthate on o-phenylene diamine¹⁹¹.

2-mercapto benzimidazole have also been prepared from o-phenylene diamine dihydrochloride and ammonium thiocyanate. A solution of two substances is usually evaporated to dryness on steam bath and then heated for short time. By this method, 2-mercapto benzimidazole and 5-chloro¹⁸⁴, 4-methyl¹⁹², 5-methyl derivatives of 2-mercapto benzimidazole have been prepared.



Probable reaction mechanism:

Simple nucleophilic addition to the carbon atom of reactive carbon disulphide.



Chemistry of Benzothiazoles

General methods for the synthesis of 2-amino benzothiazole:

1. Thiocyanation reaction:

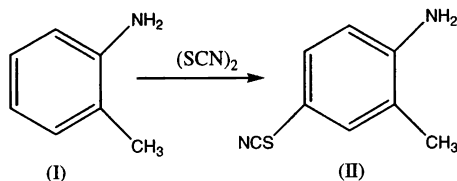
Thiocyanation is one of the many organic reactions useful to synthetic organic chemist. It is a simple electrophilic reaction showing close similarity with halogenation. Therefore, the reaction is often classified as pseudohalogenation and the reagent as pseudohalogen. As far as chemical reactivity is concerned the thiocyanogen behaves exactly similar as halogen.

The direct substitution of hydrogen atom by a thiocyano group (-SCN) through the use of thiocyanogen ($(\text{SCN})_2$) is commonly referred to as thiocyanation.

Since the reagent is extremely unstable, certain precautions must be observed for its use in the reactions. Thiocyanogen is a liquid which on cooling forms a colorless crystalline solid melting between -3°C to -2°C . At room temperature it polymerizes rapidly to a reddish orange amorphous mass of indefinite composition known as pseudocyanogen. Although, relatively stable in inert dry solvents, thiocyanogen may polymerize in solution, especially under the catalytic effect, influence of heat, light and moisture.

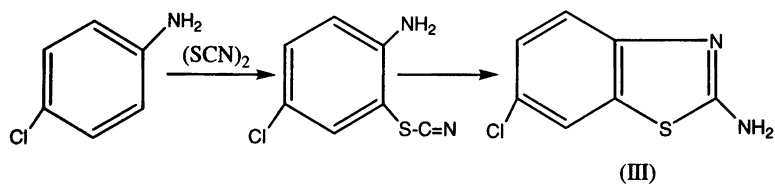
Thiocyanation of aromatic amines:

The thiocyano group (-SCN) is introduced into aromatic amines with rapidity. It enters preferentially at a free para position if available otherwise at ortho position. For example, o-toluidine (I) is converted to 4-thiocyano-o-toluidine (II) (80% yield).



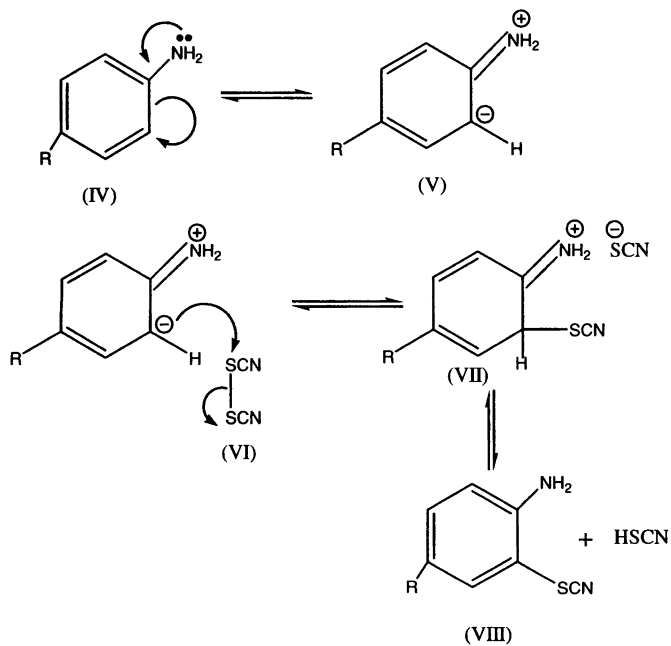
When thiocyanation takes place in the position ortho to the primary amino group as in p-toluidine, p-chloro aniline, p-nitro aniline, the final product in such cases is often an amino benzothiazole (III) formed by a secondary reaction between the amino group and the adjacent thiocyano group. In this secondary reaction, since

the amino group is having lone pair of electrons, it will be added to the C=N bond of -SCN group to give resultant 2-amino benzothiazole.



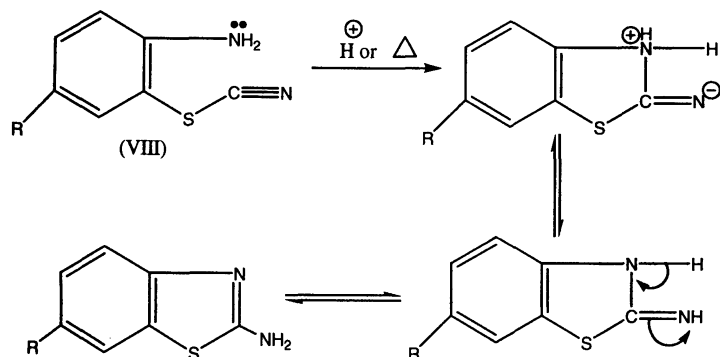
Mechanism:

The mechanism of thiocyanation reaction probably consists of the initial attack of thiocyanogen (VI) to the activated site of 4-substituted aniline (IV) where due to mesomeric effect of amino group; the ortho position (V) contains a surplus electrons which renders the site to act as a good electron rich centre, so as to form an intermediate (V) as shown below:

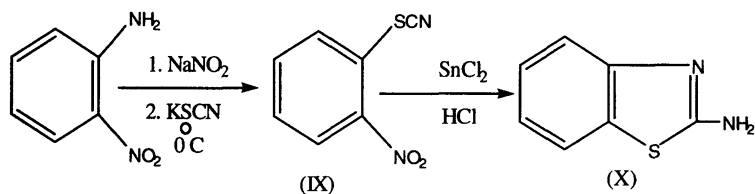


The unstable intermediate species (VII) immediately loses a proton to retain the aromatic character of the molecule (VIII) which gives higher stability.

(VIII) Under the influence of acid and heat, add the NH_2 function to the adjacent thiocyno group as shown below:



2. 2-amino benzothiazoles (X) have been prepared also from the o-thiocyano nitro compounds of the type of (IX) by chemical reduction (stannous chloride in HCl and FeSO_4 in ammonia). The yields are 60-85%.

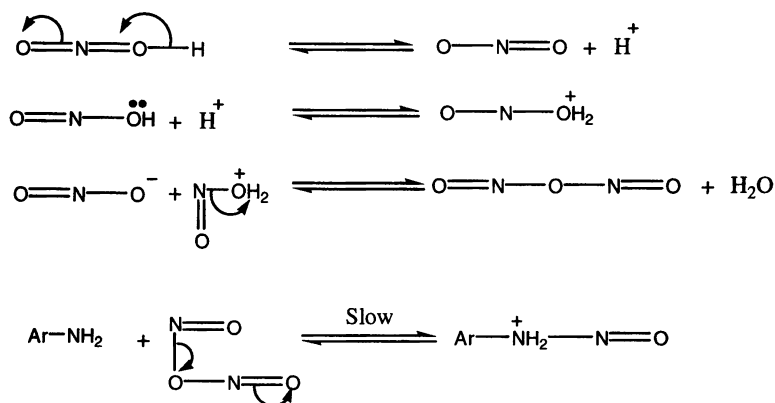


Chemistry of Diazonium salts

Formation of aromatic diazonium salts from the corresponding amines by diazotization.

The reaction of amino salt with nitrous acid is known as diazotization. Diazonium salts were prepared first by Petercriess in 1858 by the action of nitrous acid on the salt of aromatic amine.

A detail study of kinetics of diazotization has shown that it involves nucleophilic attack by amine on dinitrotrioxide, which is in equilibrium with nitrous acid.



Normally, the free amine is the reactive species even though its concentration may be very small in the acidic solutions used. The conjugate acids of the primary nitroso amine by a series of proton transfer and loss of water, gives this diazonium ion.



Recently, the intermediate step of primary nitroso amine could be isolated in the course¹⁹³ of the diazotization of an appropriate amine.

Since most diazotizations are performed in aqueous medium, protonated nitrous acid (from sodium nitrite and acid)¹⁹⁴ is usually used, free NO^+ should only appear in concentrated acid and in the course of conversions with complex NO^+ salts.

Reactivity decreases under normal conditions in the following sequence:
 $\text{NO}^+ > \text{H}_2\text{O}^+-\text{NO} > \text{NCS}-\text{NO} > \text{Br}-\text{NO} > \text{Cl}-\text{NO} > \text{O}_2\text{N}-\text{NO}$.

1. In aqueous medium:

Normal diazotization of aromatic amines with nitrous acid is carried out in dilute aqueous mineral acids and usually yields the diazonium salts in the solution. The direct method starts from solution or suspension of amines in dil. HCl (approx. 2.5 mol. acid per mol. amine), which are treated with molar amount of conc. aqueous

sodium nitrite solution at 0-10°C. The conversion proceeds rapidly and almost quantitatively¹⁹⁵, the end point can be determined by numerous methods and an excess of nitrous acid can easily be removed by adding urea which reacts with nitrous acid to form nitrogen, carbon dioxide and water or sulphamic acid¹⁹⁶.

Since nitrous acid is an unstable compound, it is generated in the presence of the amine by reaction between sodium nitrite and a mineral acid, usually hydrochloric acid or sulphuric acid. Excess acids serve to keep the mixture strongly acidic, which is necessary to prevent undesirable side reactions.

2. In concentrated acids¹⁹⁷:

Weakly basic amines bearing strongly electron attracting substituents on the aromatic (or hetero aromatic) nucleus are diazotized preferentially in conc. acids, since hydrolysis¹⁹⁸ of the generated diazonium compounds occurs in aqueous solutions with rising dilution. Good results have been described with conc. sulphuric acid¹⁹⁹.

3. In organic solvents²⁰⁰:

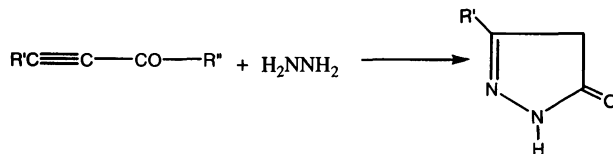
Usually, in order to prepare solid diazonium salts, diazotization of aromatic amines is carried out in organic solvents (glacial acetic acid²⁰¹, methanol, ethanol, formamide, DMF, acetone) by means of nitrous acid esters (preferentially pentyl nitrile).

Diazotization of substituted aniline:

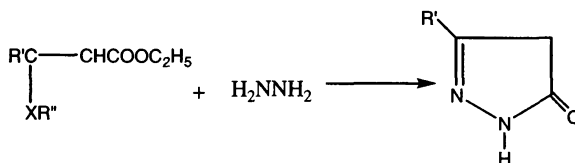
100ml of concentrated HCl was stirred into a solution of 10ml/gm of substituted aniline in 40ml of glacial acetic acid at room temperature and then was cooled at 0°C. The mixture was then treated with the solution of 5.38gms of sodium nitrite in 15ml of cold water. After completion of the diazotization, the diazonium salt solution gives a positive starch iodide paper test. The cold solution of diazonium salt was treated rapidly with a cold solution of stannous chloride (i.e. 44.368gms of SnCl₂ in 50ml conc. HCl). The insoluble complex salt is collected by filtration. The salt is dried and dissolved in aqueous NaOH and toluene is added to the same solution. Two layers of toluene and aqueous NaOH separates. Separate the upper layer of organic solvent (i.e. toluene) in large evaporating dish and keep overnight.

Chemistry of 2-Pyrazolin-5-ones

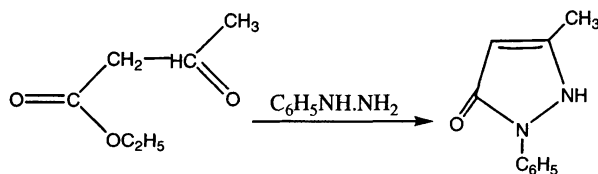
A number of syntheses of 2-pyrazolin-5-ones depend on the reaction of compounds other than β -ketoesters, substituted in the β -position of α,β -unsaturated acids, esters and amides. The most frequently used unsaturated compounds are those having a triple bond in the α,β -position^{202, 203}.



The reaction of ketone with aryl hydrazines forms 2-pyrazolin-5-ones²⁰⁴⁻²⁰⁵.



Synthesis of 3-methyl-1-phenyl pyrazol-5-one:



Mix together 50gm (49ml, 0.384mol.) of redistilled ethyl aceto acetate and 40gm (36.5ml, 0.37mol.) of phenyl hydrazine in a large evaporating dish. Heat the mixture on a boiling water bath in a fume cupboard for about 2hrs. and stir from time to time with glass rod. Allow the heavy reddish syrup to cool somewhat, add about 100ml of ether and stir vigorously. The syrup, which is insoluble in ether, will solidify within 15mins. Filter the solid at the pump and wash it thoroughly with ether to remove colored impurities. Recrystallize it from hot water or from a mixture of equal volumes of ethanol and water. The yield of methyl phenyl pyrazolone is 80%²⁰⁶.

In the similar manner different substituted phenyl hydrazines were prepared.

Chemistry of nitration

Nitration of organic substances, a reaction discovered in 1834 (Mitcherlich, synthesis of nitrobenzene) is one of the most common and important reaction in organic chemistry. The aim of nitration is to substitute one or several hydrogen atoms in the aromatic or heteroaromatic nucleus by one or several nitro groups.

The number of nitro groups entering the organic molecules depends on the conc. of the acid used, on temperature and the duration of the process, on the nature of the substance being nitrated and on other factors. In the course of nitration, the main reaction is accompanied by side reactions, which diminish the yield of main product i.e. the nitro compound. The oxidizing effect of nitric acid is responsible for one of these undesirable side reactions. The oxidizing effect of nitric acid is enhanced by rise in temperature due to water content of reaction mixture and in the amount of nitrogen oxides in the nitric acid. The introduction of nitro group into an organic molecule is accompanied by formation of molecule of water. The accumulation of water in the reaction mixture lowers the concentration of nitric acid. If nitric acid alone is used as a nitrating agent, the lowering of its conc. below a certain limit leads to practical ceasing of the reaction and complete utilization of the nitric acid becomes impossible. Hence in practice a mixture of nitric acid and sulphuric acid is used for nitration. This is the so called the nitrating mixture.

The temperature at which nitration is carried out is another very important factor affecting the course of process. Nitration is an exothermic reaction and when a mixture of nitric acid and sulphuric acid is used in the nitration of aromatic compounds a large amount of additional heat is given off by the dilution of sulphuric acid with water formed in reaction. Hence it is usually necessary to use external cooling and to mix the reagents gradually. Variation in temperature not only affects the number of nitro groups entering the molecule but also the position, which the nitro group enters. It is self-evident therefore, that for each particular nitration there is an optimal temperature. The introduction of nitro groups into aromatic or heterocyclic compounds exhibiting aromatic properties is always affected by means of nitric acid or nitrating mixture. It has also been possible to obtain nitro compounds by the action of nitric acid alone, although better results are commonly obtained by using nitrating mixtures and other nitrating agents. Even when anhydrous nitric acid is used for

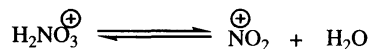
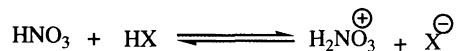
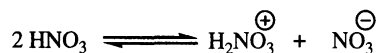
nitrating aromatic and heterocyclic compounds its dilution by the water formed during the reaction raises its oxidizing properties at the expense of its nitrating properties.

Nitration with a mixture of nitric acid and sulphuric acid.

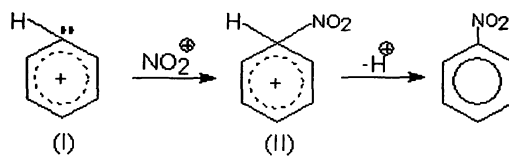
The most wide spread method for nitrating aromatic compound, uses a mixture of nitric acid with sulphuric acid (nitrating mixture). The mixture is more powerful nitrating agent than nitric acid alone. The presence of sulphuric acid not only contributes its dehydrating properties but also enhances the nitrating action of nitric acid, whilst diminishing its oxidizing properties. However sulphuric acid serves as a good solvent for many compounds, which is another favorable factor in nitration processes.

Mechanism:

It is now accepted that nitration is due to the action of positively charged ion (i.e. a cation) NO_2^+ . Therefore a nitration by ionic substitution (or displacement) is an electrophilic substitution, according to the well-known nomenclature introduced by Ingold²⁰⁷. There was overwhelming evidence that in all of the nitrating systems (i.e. HNO_3 in mineral acids) the nitronium ion (NO_2^+) was effective electrophile. The process involved could be written as follows, in the first case heterolysis of nitric acid being dependent upon autoprotection and the second being dependant upon the action of stronger acid (HX).



In electrophilic substitution, the two electrons which form the new covalent bond (i.e. the bond between the aromatic compound and the electrophilic reagent) are both supplied by the aromatic compound (I).

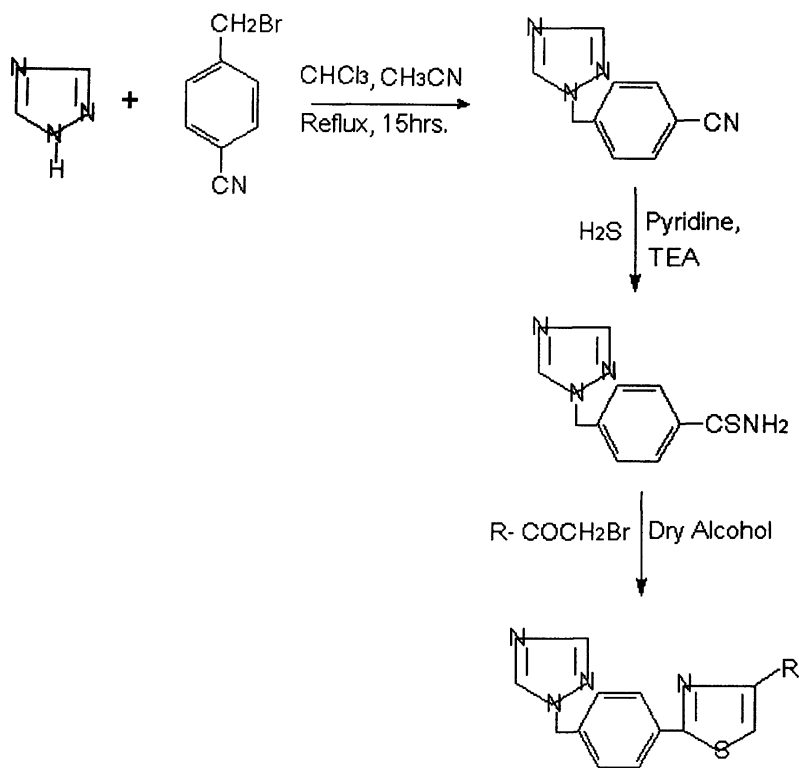


At first, two free electrons are supplied by the aromatic system (Electron sextet). The addition of the electrophilic agent NO_2^+ results in the formation of the intermediate compounds (II) (Whel and intermediate)²⁰⁸.

According to Melander²⁰⁹ the formation of II constitutes the rate-determining step. The overall reaction is of second order and follows the sp^2 mechanism i.e. bimolecular electrophilic substitution.

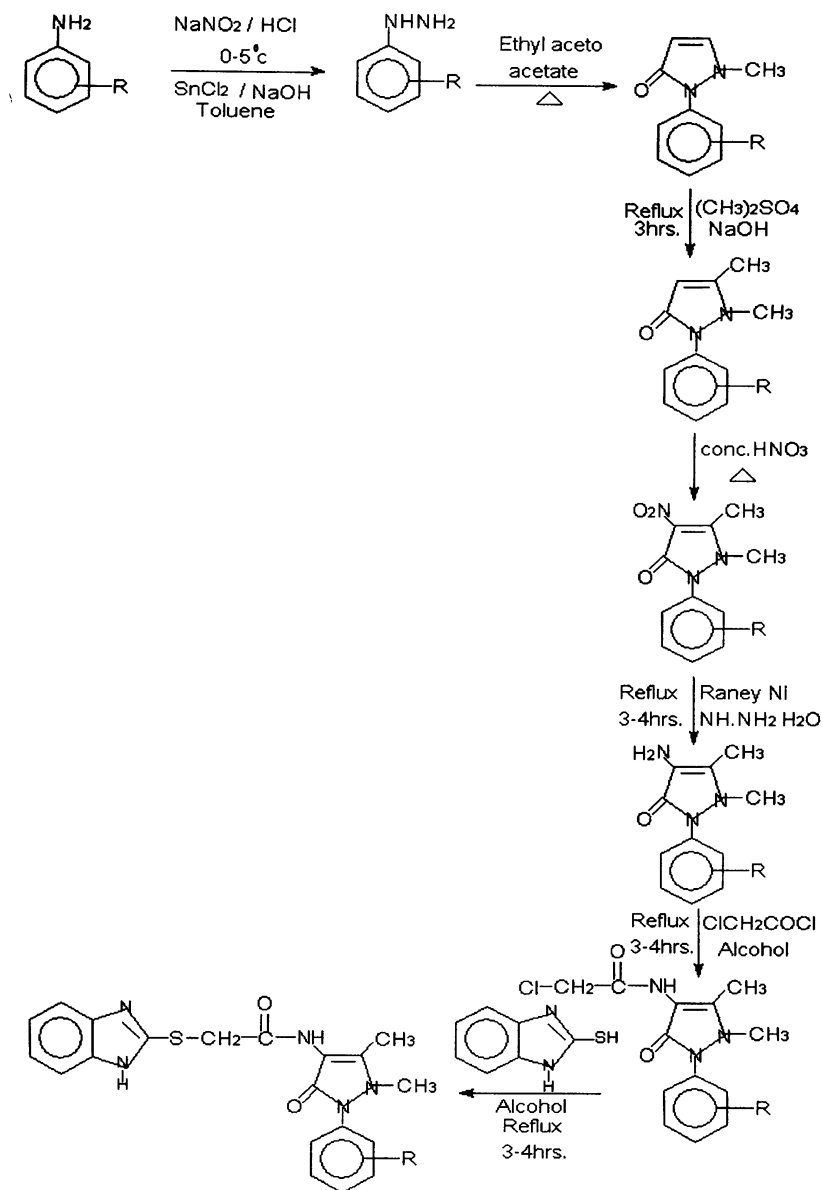
*Schematic
Representation*

Scheme I



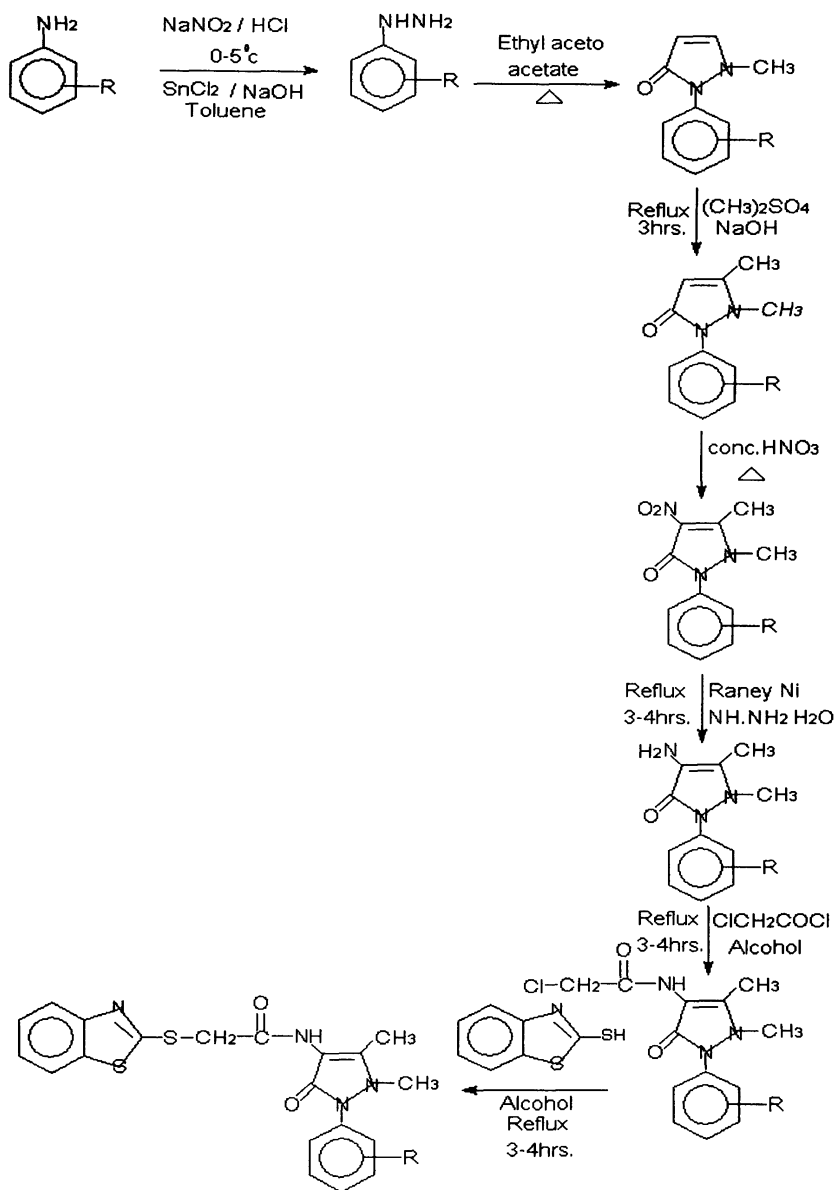
R=Substituted phenyl/heterocycle.

Scheme II



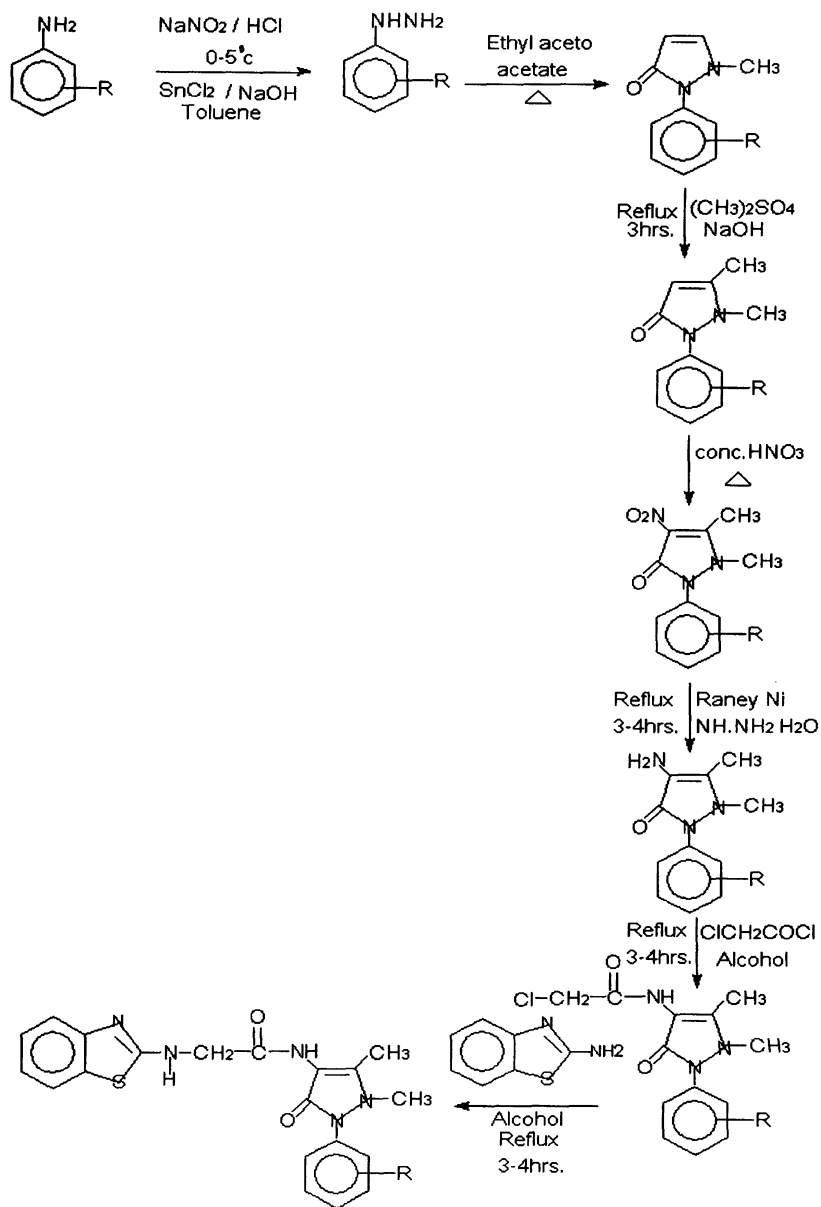
$\text{R} = 3\text{-chloro}, 2,4\text{-dichloro}, \text{etc.}$

Scheme III



R= 3-chloro, 2,4-dichloro, etc.

Scheme IV



R= 3-chloro, 2,4-dichloro, etc.

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 was 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 recrystallised 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 (V).

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

4- cyanobenzylbromide (6g, 0.0306 M) and 1,2,4- triazole (9.27g, 0.1344 M), both dissolved in a mixture of chloroform (180ml) and acetonitrile (36ml), were stirred and refluxed for 15 hours. The solution was cooled and neutralized with 3% aqueous Sodium bicarbonate solution. The organic solution was 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 = 77-79°C.

Lit. M.P.²¹⁰ = 77-79°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 (IV):

4-[1-(1,2,4-triazolyl) methyl] benzonitrile (5g, 0.0271M) was dissolved in pyridine (25ml) in a three-necked round-bottomed flask. Triethylamine (4ml) was added to it and the reaction mixture was placed in an ice bath. 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 2,4-dihydroxy phenacyl bromide:

2,4-dihydroxy acetophenone (3g, 0.022 M) dissolved in a dry chloroform in a 100ml 3-necked round bottom 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 (3.5g, 0.022M) dissolved in 10ml 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 a dry air was passed through the reaction mixture to sweep out 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 solidify on cooling. The product was further recrystallised from ethanol.

M.P = 172-173°C.

Yield = 82%

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-(2,4-dihydroxy phenyl) thiazole (V).*

4-[1-(1,2,4-triazolyl) methyl] benzothioamide (0.5g, 0.0023M) and 2,4-dihydroxy phenacyl bromide (0.53g, 0.0023M) were both dissolved separately in a dry alcohol (25ml), 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 was isolated and recrystallised from methanol.

M.P = 122-124°C.

Yield = 50.29%

Elemental analysis: C₁₈H₁₄N₄O₂S.HBr

	C%	H%	N%
Calculated	61.71	4.0	16.0
Found	62.02	4.31	15.78

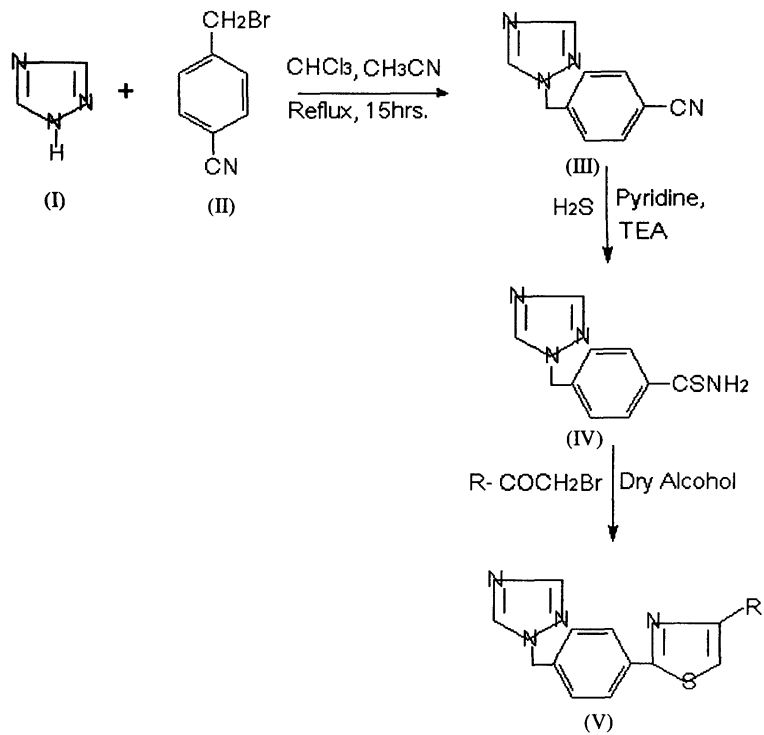
Spectral Characterization:

Serries I (2) i.e. 1B:

IR (KBr) cm⁻¹: 1522, 1414, 1144 (thiazole); 3149, 1450 (-CH₂); 1040 (1H, -1,2,4-triazole); 3252, 3384 (OH).

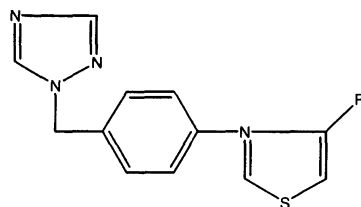
PMR (DMSO): δ5.7 (s, -CH₂), δ6.9-8.9 [m, 10H (7H, ArH) (2H, triazoleH) (1H, thiazole 3H)].

Scheme I



R=Substituted phenyl/heterocycle.

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



R= Substituted phenyl / heterocycle

Sr.No.	R	Molecular Formula	M.P (°C)	Yield (%)	Elemental Analysis					
					Calculated (%)			Obtained (%)		
					C	H	N	C	H	N
1.	4-amino phenyl	C ₁₈ H ₁₅ N ₅ S.HBr	255-256	56	52.17	3.62	16.9	52.36	3.44	16.72
2.	2,4-dihydroxy phenyl	C ₁₈ H ₁₄ N ₄ O ₂ S.HBr	122-124	52	50.12	3.45	12.99	50.26	3.64	12.71
3.	2,6-dihydroxy phenyl	C ₁₈ H ₁₄ N ₄ O ₂ S.HBr	133-135	59	50.12	3.45	12.99	50.31	3.52	12.68
4.	2,5-dihydroxy phenyl	C ₁₈ H ₁₄ N ₄ O ₂ S.HBr	162-164	60	50.12	3.45	12.99	50.21	3.58	12.80
5.	2-hydroxy phenyl	C ₁₈ H ₁₄ N ₄ OS.HBr	124-126	58	52.05	3.37	13.49	52.22	3.17	13.66
6.	2,4,6-trihydroxy phenyl	C ₁₈ H ₁₄ N ₄ O ₃ S.HBr	235-238	58	48.32	3.13	12.53	48.02	3.25	12.46
7.	6-chloro cinnolin-4-one	C ₂₀ H ₁₃ N ₆ OS.HBr	159-160	55	47.86	2.59	16.75	47.94	2.48	16.49

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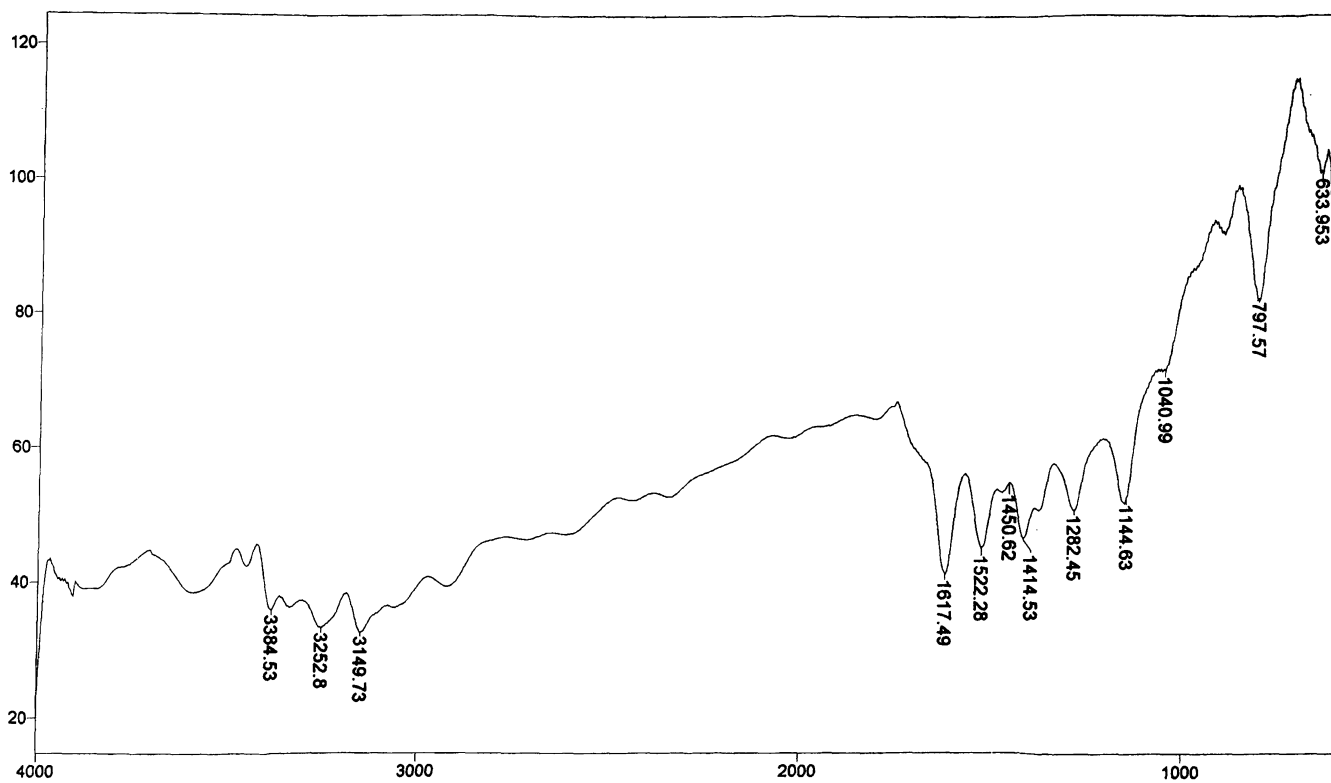
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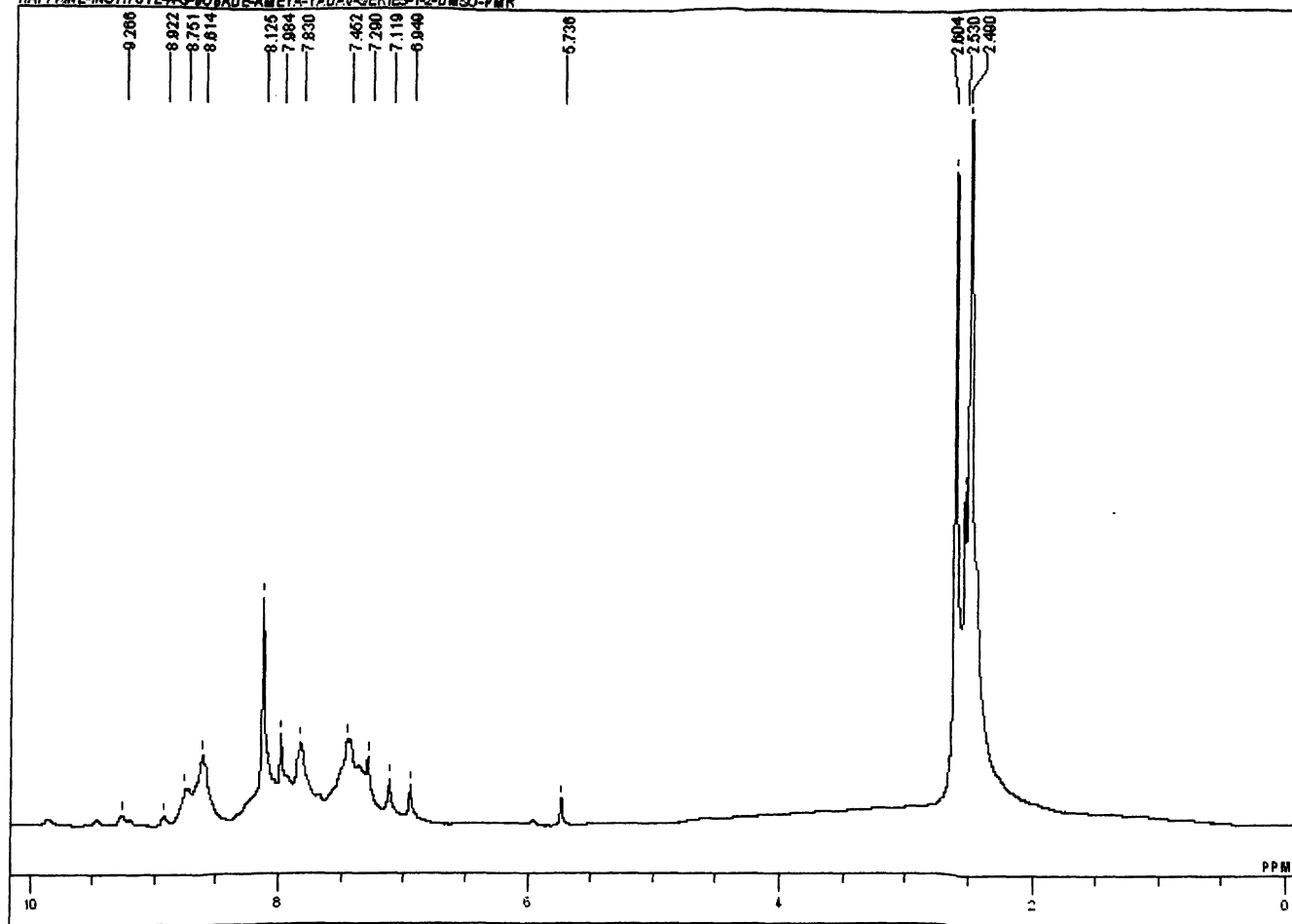
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Zero Filling= 1 x



Transmittance / Wavenumber (cm-1)

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Part III:

Synthesis of 2-(1H-Benzimidazol-2-yl mercapto)-N- [1,5-dimethyl-2- (substituted) phenyl-3-oxo-2,3-dihydro-1H-pyrazol-4-yl] acyl amine (IX).

Step I: Synthesis of 3-chloro phenyl hydrazine²¹¹ (II).

100ml of concentrated HCl was stirred into a solution of 3-chloro aniline (10ml, 0.078M) in 40ml of glacial acetic acid at room temperature and then was cooled at 0-5°C in 250ml round bottom flask equipped with mechanical stirrer. To the cold mixture Sodium nitrite (5gm) dissolved in 15ml cold water was added with continuous stirring. The cold mixture of diazonium salt was treated rapidly with the cold solution of Stannous chloride (i.e. 35gm of SnCl₂ dissolved in 50ml HCl). The insoluble complex salt was collected by filtration. The salt is dried and dissolved in aqueous NaOH solution. Toluene is added to the same solution. Two layers of NaOH and Toluene separate out. The upper layer in separating funnel is organic layer of toluene and is collected in large evaporating dish and kept at room temperature. After few hours toluene gets evaporated and oily liquid of 3-chloro phenyl hydrazine remains. The residue was then chromatographed on silica gel (80-120 mesh). Elution with chloroform/isopropanol (9:1) gave the product. Product is purified by distillation.

B.P.= 165°C

Lit. B.P.²¹¹= 165°C

Yield = 72.8%

Elemental Analysis: C₆H₇N₂Cl

	C%	H%	N%
Calculated	50.53	4.91	19.65
Found	50.40	4.61	19.78

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Step II: Synthesis of 3-methyl-1- (3-chloro) phenyl pyrazol-5-one (III).

Mix together redistilled ethyl aceto acetate (5ml, 0.035M) and 3-chloro phenyl hydrazine (5ml, 0.035M) in a large evaporating dish. Heat the mixture on a water bath in fume cupboard for 2hrs. and stir from time to time with a glass rod. Allow the heavy reddish liquid to cool somewhat. Add about 25ml of ether and stir vigorously. The syrup, which is insoluble in ether, will solidify within 15mins. Filter at the pump and wash with ether to remove colored impurities. The residue was then chromatographed on silica gel (80-120 mesh). Elution with ethyl acetate/pet. ether (1:3) gave the product. Recrystallise the product from 50% alcohol.

M.P.= 128°C

Yield = 73.14%

Elemental Analysis: C₁₀H₉N₂OCl

	C%	H%	N%
Calculated	57.55	4.32	13.43
Found	57.44	4.51	13.28

Step III: Synthesis of 2,3-dimethyl-1-(3-chloro) phenyl pyrazol-5-one (IV).

In a 100ml three necked round bottom flask equipped with a dropping funnel, sealed stirrer and a condenser mix a solution of 7gm of NaOH in a little water and a solution of 3-methyl-1-(3-chloro) phenyl pyrazol-5-one (2gm, 0.0096M) in 10ml methanol. Warm the mixture on water bath and add Dimethyl sulphate (0.92ml, 0.0096M). Reflux the mixture for 1hour with continuous stirring. Distill off methanol. Add hot water to the residue and filter from impurities. Extract the product with benzene and evaporate the solvent. The residue was then chromatographed on silica gel (80-120 mesh). Elution with carbon tetrachloride/ethyl acetate (3:2) gave the product. Recrystallise from benzene or benzene light petroleum.

M.P.= 151°C

Yield = 52.1%

Elemental Analysis: $C_{11}H_{11}N_2OCl$

	C%	H%	N%
Calculated	59.33	4.94	12.58
Found	59.49	4.71	12.37

Step IV: *Synthesis of 4-amino-2,3-dimethyl-1-(3-chloro) phenyl pyrazol-5-one (VI).*

2,3-Dimethyl-1-(3-chloro) phenyl pyrazol-5-one (3gm, 0.014M) was dissolved in an alcohol in conical flask. The solution was warmed to dissolve the compound completely. Then slowly 7-8ml of concentrated HNO_3 was added to the solution with constant stirring. Heat the flask till no more brown fumes evolve from it. Cool the flask by keeping in ice bath. Filter the product obtained and dry it.

In a single necked round bottom flask in an alcohol a paste of Raney Nickel in small quantity and excess of hydrazine hydrate solution was refluxed for 1hr. To this solution while refluxing add slowly solution of nitrated compound dissolved in alcohol through the condenser and reflux for 4-5hrs. Filter the Raney Nickel and collect the filtrate in an evaporating dish and evaporate the solvent. The solid obtained is dried and purified from alcohol.

M.P.= 172°C

Yield = 59.8%

Elemental Analysis: $C_{11}H_{12}N_3OCl$

	C%	H%	N%
Calculated	55.58	5.05	17.68
Found	55.39	4.91	17.57

Preparation of 2-mercapto benzimidazole (VIII) has been discussed in the **Part I Chemistry** section under the title '*Chemistry of Benzimidazoles*' page no – 61.

Step V: Synthesis of 2-(1H-Benzimidazol-2-yl mercapto)-N- [1,5-dimethyl-2-(3-chloro) phenyl-3-oxo-2,3-dihydro-1H-pyrazol-4-yl] acyl amine (IX).

4-amino-2,3-dimethyl-1-(3-chloro) phenyl pyrazol-5-one (0.005M) dissolved in alcohol in an round bottom flask was placed in ice bath. Then slowly equimolar quantity of chloro acetyl chloride (0.005M) was added which resulted in the solid product. The product obtained is unstable and treated rapidly for further reaction.

To an ethanolic solution of 4-(α/β -halogenoacyl amino)-1,5-dimethyl-2-(3-chloro) phenyl-2,3-dihydro-1H-pyrazol-3-one (0.005M), 2-mercapto-1H-benzimidazole (0.005M) was added and the reaction mixture was refluxed for 4hrs. After cooling the solution was evaporated to dryness under reduced pressure. The product was precipitated with sat. NaHCO₃ and purified by recrystallisation from ethanol.

M.P.= 150-151°C

Yield = 59.2%

Elemental Analysis: C₂₀H₁₈N₅O₂SCl

	C%	H%	N%
Calculated	56.14	4.21	16.37
Found	56.19	4.42	16.14

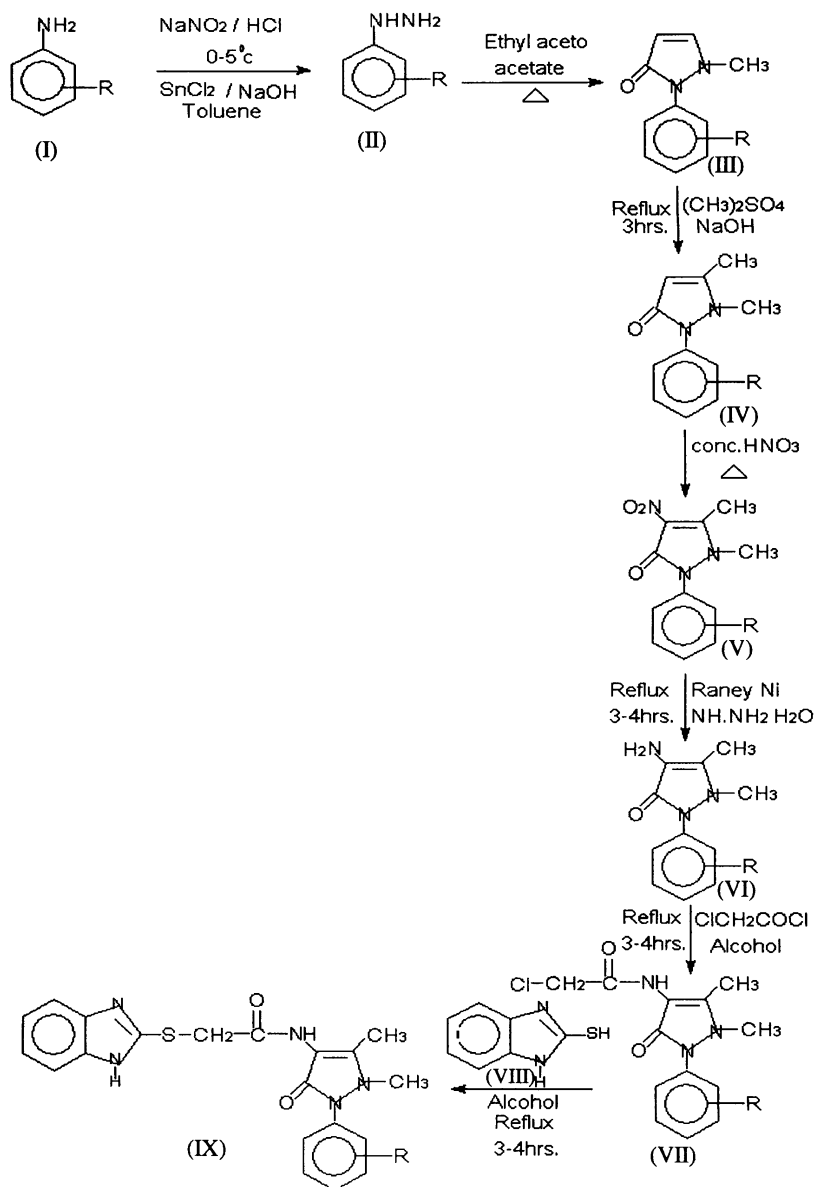
Spectral Characterization:

Serries II (3) i.e. 2C:

IR (KBr) cm⁻¹: 3415(-NH), 2943(-CH₃), 1719(C=O), 1624 (CONH); 1445(-Cl), 773 (-CH₂-S-)

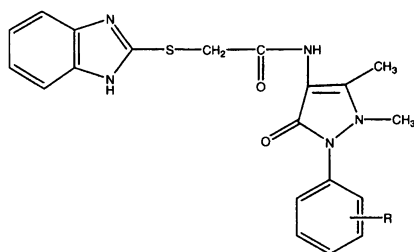
PMR (DMSO): δ 2.9 (s, 3H, C-CH₃), δ 7.1-7.6 [m, 9H (8H, ArH) (1H, NH), δ 8.3(s, 1H, -CONH), δ 3.6(s, 3H, N-CH₃)

Scheme II



R= 3-chloro, 2,4-dichloro, etc.

Table II: Physical constants of 2-[1H-Benzimidazol-2-yl mercapto]-N- [1,5-dimethyl-2- (substituted phenyl)-3-oxo-2, 3-dihydro-1H-pyrazol-4-yl] acyl amine.



R= 3-chloro, 2,4-dichloro etc.

Sr.No.	R	Molecular Formula	M.P (°C)	Yield (%)	Elemental Analysis					
					Calculated (%)			Obtained (%)		
					C	H	N	C	H	N
1.	4-chloro	C ₂₀ H ₁₈ N ₅ O ₂ SCl	117-118	56	56.14	4.21	16.37	56.23	4.36	16.19
2.	2,4-difluoro	C ₂₀ H ₁₇ N ₅ O ₂ SF ₂	126-128	52	55.94	3.96	16.32	55.83	3.87	16.44
3.	3-chloro	C ₂₀ H ₁₈ N ₅ O ₂ SCl	150-151	59	56.14	4.21	16.37	56.19	4.42	16.14
4.	2,4-dichloro	C ₂₀ H ₁₇ N ₅ O ₂ SCl ₂	186-188	60	51.95	3.68	15.15	51.73	3.44	15.38
5.	3,4-dichloro	C ₂₀ H ₁₇ N ₅ O ₂ SCl ₂	82-84	58	51.95	3.68	15.15	51.82	3.51	15.27
6.	3-chloro-4-fluoro	C ₂₀ H ₁₇ N ₅ O ₂ SClF	60-61	58	53.87	3.82	15.71	53.67	3.78	15.60
7.	2,5-dimethoxy	C ₂₂ H ₂₃ N ₅ O ₄ S	175-176	55	58.28	5.07	15.45	58.39	4.93	15.66
8.	2-methoxy	C ₂₁ H ₂₁ N ₅ O ₃ S	170-171	54	59.57	4.96	16.55	59.24	4.71	16.38

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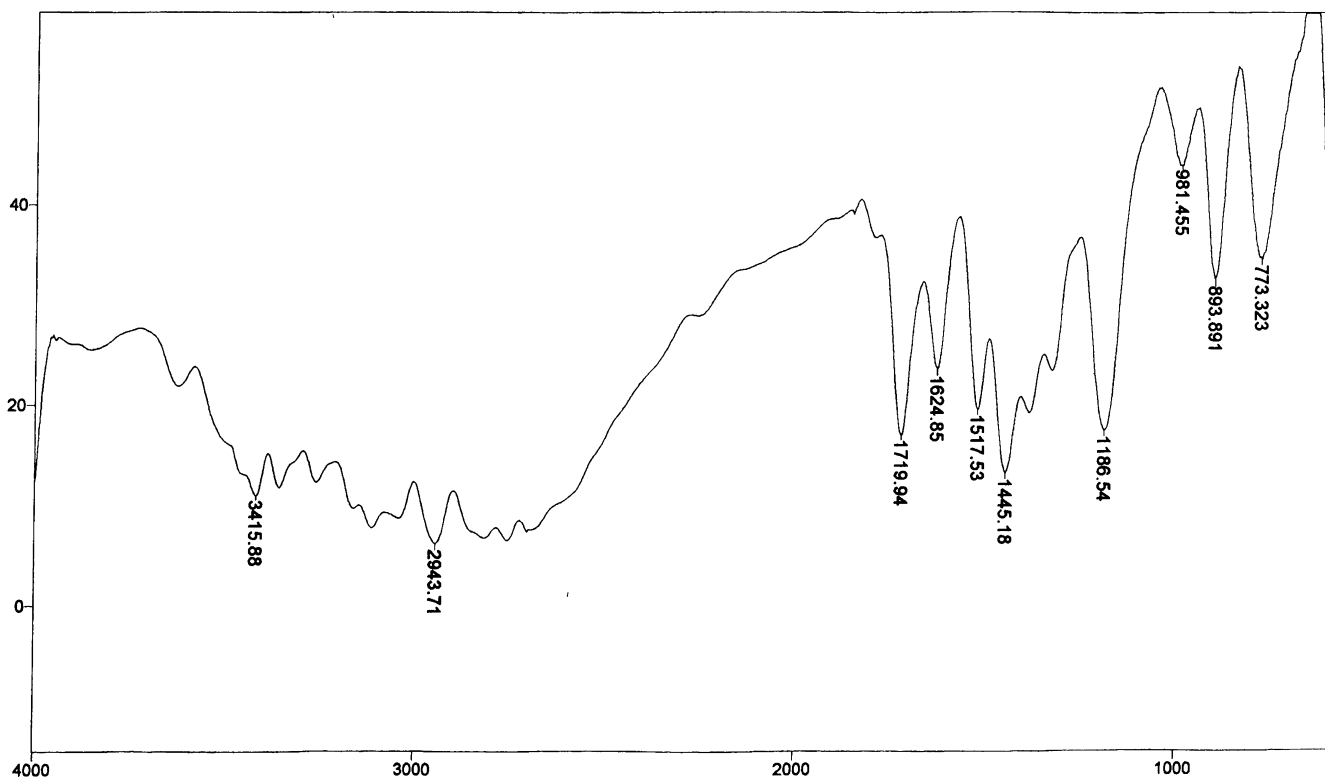
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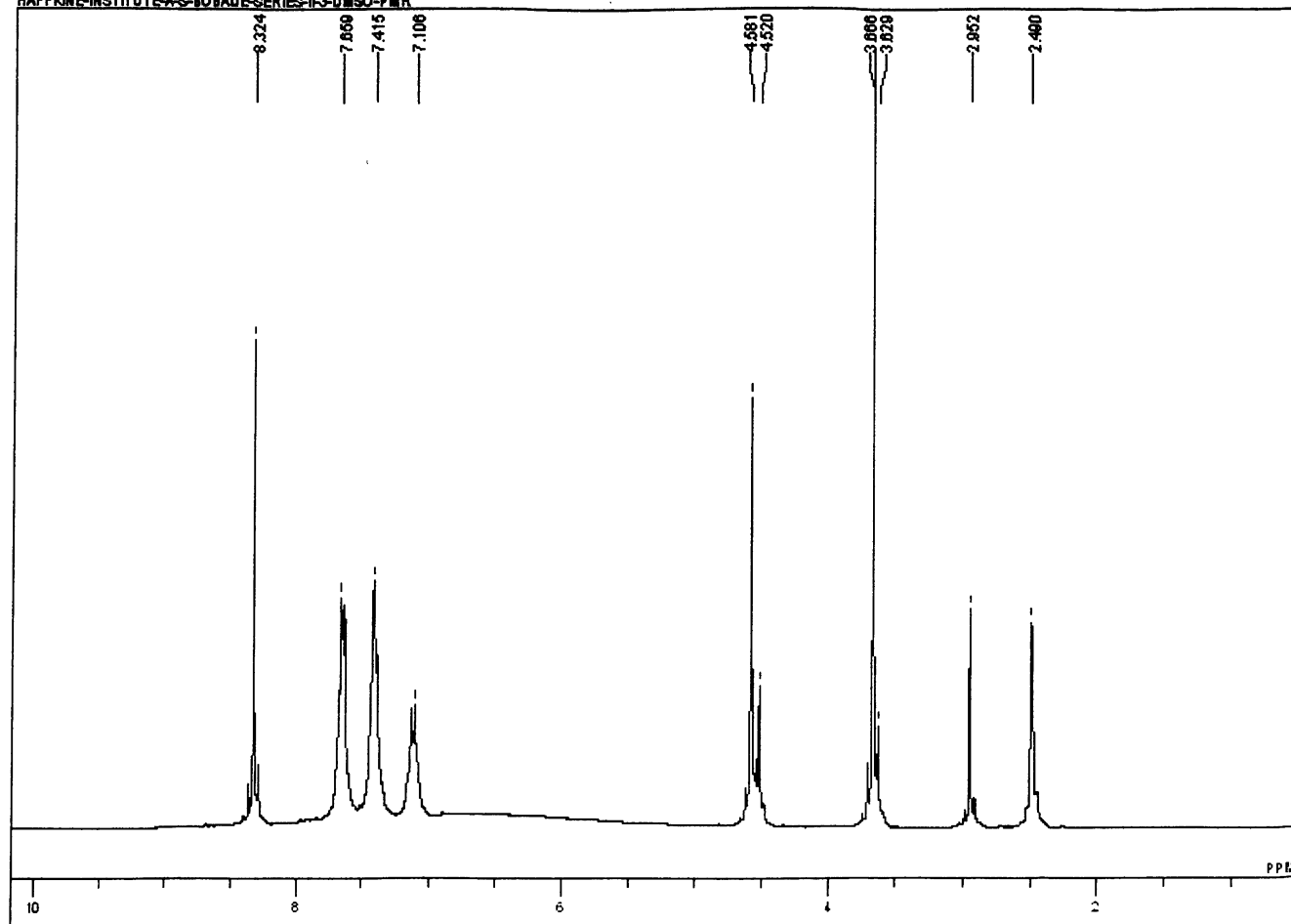
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Apod= Cosine

Zero Filling= 1 x



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Part IV:

Synthesis of 2-[1,3-Benzothiazol-2-yl mercapto]-N- [1,5-dimethyl-2- (substituted phenyl)-3-oxo-2, 3-dihydro-1H-pyrazol-4-yl] acyl amine (IX).

Step I: Synthesis of 3-chloro phenyl hydrazine²¹¹ (II).

100ml of concentrated HCl was stirred into a solution of 3-chloro aniline (10ml, 0.078M) in 40ml of glacial acetic acid at room temperature and then was cooled at 0-5°C in 250ml round bottom flask equipped with mechanical stirrer. To the cold mixture Sodium nitrite (5gm) dissolved in 15ml cold water was added with continuous stirring. The cold mixture of diazonium salt was treated rapidly with the cold solution of Stannous chloride (i.e. 35gm of SnCl₂ dissolved in 50ml HCl). The insoluble complex salt was collected by filtration. The salt is dried and dissolved in aqueous NaOH solution. Toluene is added to the same solution. Two layers of NaOH and Toluene separate out. The upper layer in separating funnel is organic layer of toluene and is collected in large evaporating dish and kept at room temperature. After few hours toluene gets evaporated and oily liquid of 3-chloro phenyl hydrazine remains. The residue was then chromatographed on silica gel (80-120 mesh). Elution with chloroform/isopropanol (9:1) gave the product. Product is purified by distillation.

B.P.= 165°C

Lit. B.P.²¹¹= 165°C

Yield = 72.8%

Elemental Analysis: C₆H₇N₂Cl

	C%	H%	N%
Calculated	50.53	4.91	19.65
Found	50.40	4.61	19.78

Step II: Synthesis of 3-methyl-1- (3-chloro) phenyl pyrazol-5-one (III).

Mix together redistilled ethyl aceto acetate (5ml, 0.035M) and 3-chloro phenyl hydrazine (5ml, 0.035M) in a large evaporating dish. Heat the mixture on a water bath in fume cupboard for 2hrs. and stir from time to time with a glass rod. Allow the heavy reddish liquid to cool somewhat. Add about 25ml of ether and stir vigorously. The syrup, which is insoluble in ether, will solidify within 15mins. Filter at the pump and wash with ether to remove colored impurities. The residue was then chromatographed on silica gel (80-120 mesh). Elution with ethyl acetate/pet. ether (1:3) gave the product. Recrystallise the product from 50% alcohol.

M.P.= 128°C

Yield = 73.14%

Elemental Analysis: C₁₀H₉N₂OCl

	C%	H%	N%
Calculated	57.55	4.32	13.43
Found	57.44	4.51	13.28

Step III: Synthesis of 2,3-dimethyl-1-(3-chloro) phenyl pyrazol-5-one (IV).

In a 100ml three necked round bottom flask equipped with a dropping funnel, sealed stirrer and a condenser mix a solution of 7gm of NaOH in a little water and a solution of 3-methyl-1-(3-chloro) phenyl pyrazol-5-one (2gm, 0.0096M) in 10ml methanol. Warm the mixture on water bath and add Dimethyl sulphate (0.92ml, 0.0096M). Reflux the mixture for 1hour with continuous stirring. Distill off methanol. Add hot water to the residue and filter from impurities. Extract the product with benzene and evaporate the solvent. The residue was then chromatographed on silica gel (80-120 mesh). Elution with carbon tetrachloride/ethyl acetate (3:2) gave the product. Recrystallise from benzene or benzene light petroleum.

M.P.= 151°C

Yield = 52.1%

Elemental Analysis: $C_{11}H_{11}N_2OCl$

	C%	H%	N%
Calculated	59.33	4.94	12.58
Found	59.49	4.71	12.37

Step IV: *Synthesis of 4-amino-2,3-dimethyl-1-(3-chloro) phenyl pyrazol-5-one (VI).*

2,3-Dimethyl-1-(3-chloro) phenyl pyrazol-5-one (3gm, 0.014M) was dissolved in an alcohol in conical flask. The solution was warmed to dissolve the compound completely. Then slowly 7-8ml of concentrated HNO_3 was added to the solution with constant stirring. Heat the flask till no more brown fumes evolve from it. Cool the flask by keeping in it ice bath. Filter the product obtained and dry it.

In a single necked round bottom flask in an alcohol a paste of Raney Nickel in small quantity and excess of hydrazine hydrate solution was refluxed for 1hr. To this solution while refluxing add slowly solution of nitrated compound dissolved in alcohol through the condenser and reflux for 4-5hrs. Filter the Raney Nickel and collect the filtrate in an evaporating dish and evaporate the solvent. The solid obtained is dried and purified from alcohol.

M.P.= 172°C

Yield = 59.8%

Elemental Analysis: $C_{11}H_{12}N_3OCl$

	C%	H%	N%
Calculated	55.58	5.05	17.68
Found	55.39	4.91	17.57

Step V: Synthesis of 2-mercapto benzothiazole (VIII).

Potassium hydroxide (9.6gm, 0.17 mole) was dissolved in the mixture of 150ml ethanol (95%) and 30ml water. To this, 13.8gm CS₂ (10.71ml, 0.17 mole) was added with stirring. This solution at boiling temperature was added dropwise to a solution of 26gm (0.17 mole) of 2-aminothiophenol in 100ml of ethanol, after 6hrs of reflux the ethanol was distilled off, the white residue was dissolved in water and the product was precipitated by addition of 50ml dilute acetic acid (50%) to get 86% yield of 2-mercapto benzothiazole.

M.P.= 177-179°C

Yield = 86%

Step VI: Synthesis of 2-(1,3-Benzothiazol-2-yl mercapto)-N- [1,5-dimethyl-2-(3-chloro) phenyl-3-oxo-2,3-dihydro-1H-pyrazol-4-yl] acyl amine (IX).

4-amino-2,3-dimethyl-1-(3-chloro) phenyl pyrazol-5-one (0.005M) dissolved in alcohol in an round bottom flask was placed in ice bath. Then slowly equimolar quantity of chloro acetyl chloride (0.005M) was added which resulted in the solid product. The product obtained is unstable and treated rapidly for further reaction.

To an ethanolic solution of 4-(α/β -halogenoacyl amino)-1,5-dimethyl-2-(3-chloro) phenyl-2,3-dihydro-1H-pyrazol-3-one (0.005M), 2-mercapto-1,3-benzothiazole (0.005M) was added and the reaction mixture was refluxed for 4hrs. After cooling the solution was evaporated to dryness under reduced pressure. The product was precipitated with sat. NaHCO₃ and purified by recrystallisation from ethanol.

M.P.= 129-130°C

Yield = 60.0%

Elemental Analysis: C₂₀H₁₇N₄O₂S₂Cl

	C%	H%	N%
Calculated	53.99	3.82	12.60
Found	53.81	3.77	12.71

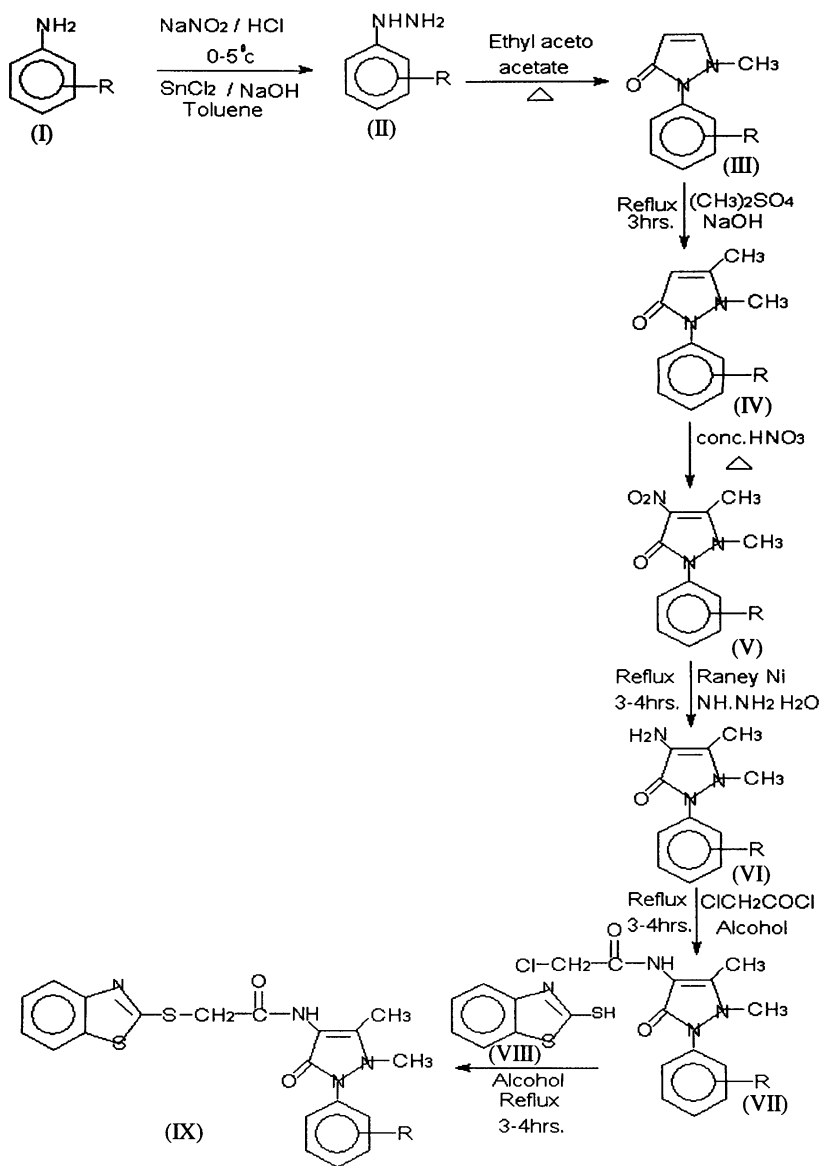
Spectral Characterization:

Series III (4) i.e. 3D:

IR (KBr) cm^{-1} : 3268(-NH), 3118(-CH₃), 1706(C=O), 1660 (CONH); 1417(-Cl), 745 (-CH₂-S-)

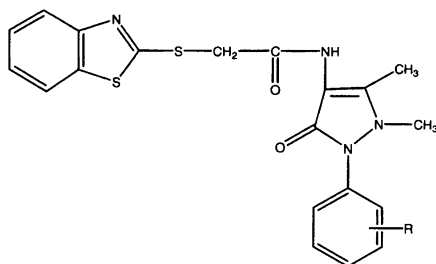
PMR (DMSO): δ 2.76 (s, 3H, C-CH₃), δ 7.11-7.98[m, 8H ArH] (1H, NH), δ 8.29(s, 1H, -CONH), δ 4.22(s, 2H, S-CH₂).

Scheme III



R = 3-chloro, 2,4-dichloro, etc.

Table III: Physical constants of 2-[1,3-Benzothiazol-2-yl mercapto]-N- [1,5-dimethyl-2- (substituted phenyl)-3-oxo-2, 3-dihydro-1H-pyrazol-4-yl] acyl amine.



R= 3-chloro, 2,4-dichloro etc.

Sr.No.	R	Molecular Formula	M.P (°C)	Yield (%)	Elemental Analysis					
					Calculated (%)			Obtained (%)		
					C	H	N	C	H	N
1.	4-fluoro	C ₂₀ H ₁₇ N ₄ O ₂ S ₂ F	166-168	56	56.07	3.97	13.08	55.87	3.84	13.18
2.	4-chloro	C ₂₀ H ₁₇ N ₄ O ₂ S ₂ Cl	160-162	52	53.99	3.82	12.60	53.69	3.75	12.88
3.	2,4-difluoro	C ₂₀ H ₁₆ N ₄ O ₂ S ₂ F ₂	132-134	59	53.81	3.59	12.56	53.97	3.34	12.72
4.	3-chloro	C ₂₀ H ₁₇ N ₄ O ₂ S ₂ Cl	129-130	60	53.99	3.82	12.60	53.81	3.77	12.71
5.	3,4-dichloro	C ₂₀ H ₁₆ N ₄ O ₂ S ₂ Cl ₂	90-91	58	50.10	3.34	11.69	50.22	3.08	11.53
6.	3-chloro-4-fluoro	C ₂₀ H ₁₆ N ₄ O ₂ S ₂ ClF	126-127	58	51.89	3.46	12.11	51.98	3.33	12.36
7.	2,5-dimethoxy	C ₂₂ H ₂₂ N ₄ O ₄ S ₂	144-145	55	56.17	4.68	11.91	55.89	4.55	11.83
8.	2,4-dichloro	C ₂₀ H ₁₆ N ₄ O ₂ S ₂ Cl ₂	138-139	51	50.10	3.34	11.69	50.34	3.10	11.77

File # 1 : SERIES3D

Mode= 2 (Mid-IR)

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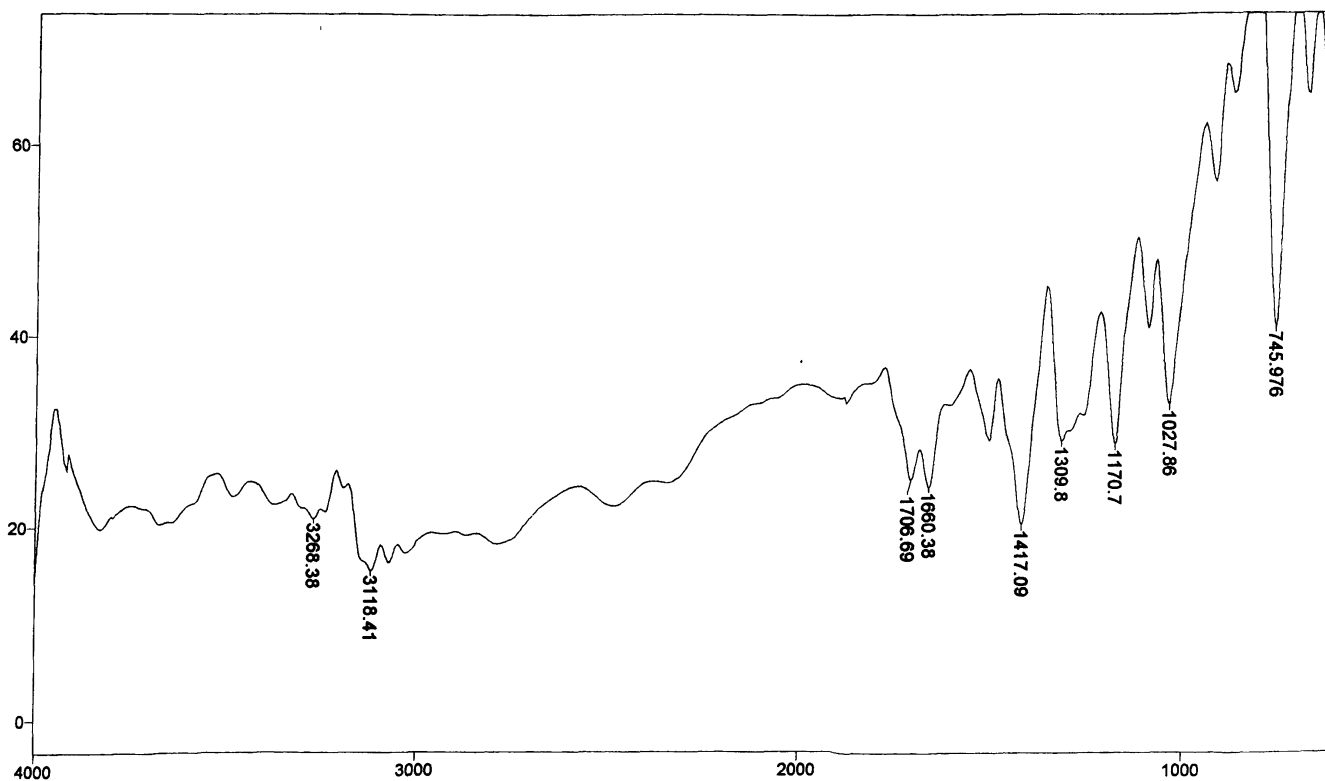
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Scans= 20 Slow

Res=8 cm-1

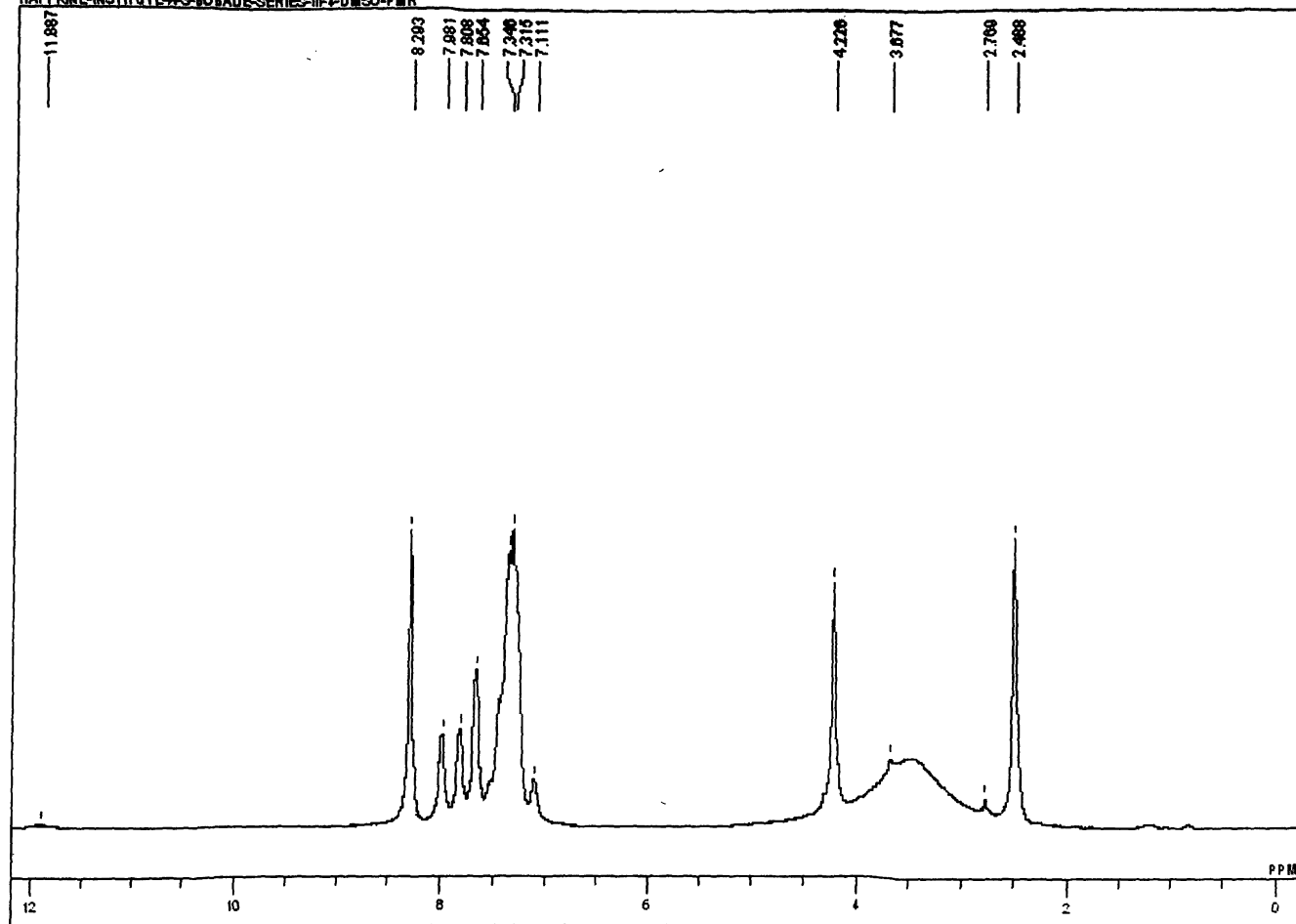
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Transmittance / Wavenumber (cm-1)

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Part V:

Synthesis of 2-[1,3-Benzothiazol-2-yl amino]-N- [1,5-dimethyl-2- (substituted phenyl)-3-oxo-2, 3-dihydro-1H-pyrazol-4-yl] acyl amine (IX).

Step I: Synthesis of 2,4-dichloro phenyl hydrazine²¹² (II).

100ml of concentrated HCl was stirred into a solution of 2,4-dichloro aniline (10gm, 0.06M) in 40ml of glacial acetic acid at room temperature and then was cooled at 0-5°C in 250ml round bottom flask equipped with mechanical stirrer. To the cold mixture Sodium nitrite (5gm) dissolved in 15ml cold water was added with continuous stirring. The cold mixture of diazonium salt was treated rapidly with the cold solution of Stannous chloride (i.e. 35gm of SnCl₂ dissolved in 50ml HCl). The insoluble complex salt was collected by filtration. The salt is dried and dissolved in aqueous NaOH solution. Toluene is added to the same solution. Two layers of NaOH and Toluene separate out. The upper layer in separating funnel is organic layer of toluene and is collected in large evaporating dish and kept at room temperature. After few hours toluene gets evaporated and oily liquid of 3-chloro phenyl hydrazine remains. The residue was then chromatographed on silica gel (80-120 mesh). Elution with chloroform/isopropanol (9:1) gave the product. Product is purified by distillation.

M.P.= 94°C

Lit. M.P.²¹²= 93-94.5°C

Yield = 77.48%

Elemental Analysis: C₆H₆N₂Cl₂

	C%	H%	N%
Calculated	40.68	3.39	15.82
Found	40.40	3.61	15.58

Step II: Synthesis of 3-methyl-1- (2,4-dichloro) phenyl pyrazol-5-one (III).

Mix together redistilled ethyl aceto acetate (5ml, 0.035M) and 2,4-dichloro phenyl hydrazine (6.2gm, 0.035M) in a large evaporating dish. Heat the mixture on a water bath in fume cupboard for 2hrs. and stir from time to time with a glass rod. Allow the heavy reddish liquid to cool somewhat. Add about 25ml of ether and stir vigorously. The syrup, which is insoluble in ether, will solidify within 15mins. Filter at the pump and wash with ether to remove colored impurities. The residue was then chromatographed on silica gel (80-120 mesh). Elution with ethyl acetate/pet. ether (1:3) gave the product. Recrystallise the product from 50% alcohol.

M.P.= 124°C

Yield = 78.19%

Elemental Analysis: $C_{10}H_8N_2OCl_2$

	C%	H%	N%
Calculated	49.38	3.29	11.52
Found	49.64	3.47	11.28

Step III: Synthesis of 2,3-dimethyl-1-(2,4-dichloro) phenyl pyrazol-5-one (IV).

In a 100ml three necked round bottom flask equipped with a dropping funnel, sealed stirrer and a condenser mix a solution of 7gm of NaOH in a little water and a solution of 3-methyl-1-(2,4-dichloro) phenyl pyrazol-5-one (6gm, 0.025M) in 10ml methanol. Warm the mixture on water bath and add Dimethyl sulphate 4.16gm(3.15ml, 0.025M). Reflux the mixture for 1hour with continuous stirring. Distill off methanol. Add hot water to the residue and filter from impurities. Extract the product with benzene and evaporate the solvent. The residue was then chromatographed on silica gel (80-120 mesh). Elution with carbon tetrachloride/ethyl acetate (3:2) gave the product. Recrystallise from benzene or benzene light petroleum.

M.P.= 146°C

Yield = 57.04%

Elemental Analysis: $C_{11}H_{10}N_2OCl_2$

	C%	H%	N%
Calculated	51.36	3.89	10.89
Found	51.49	3.61	10.65

Step IV: Synthesis of 4-amino-2,3-dimethyl-1-(2,4-dichloro) phenyl pyrazol-5-one (VI).

2,3-Dimethyl-1-(2,4-dichloro) phenyl pyrazol-5-one (3gm, 0.012M) was dissolved in an alcohol in conical flask. The solution was warmed to dissolve the compound completely. Then slowly 7-8ml of concentrated HNO_3 was added to the solution with constant stirring. Heat the flask till no more brown fumes evolve from it. Cool the flask by keeping in it ice bath. Filter the product obtained and dry it.

In a single necked round bottom flask in an alcohol a paste of Raney Nickel in small quantity and excess of hydrazine hydrate solution was refluxed for 1hr. To this solution while refluxing add slowly solution of nitrated compound dissolved in alcohol through the condenser and reflux for 4-5hrs. Filter the Raney Nickel and collect the filtrate in an evaporating dish and evaporate the solvent. The solid obtained is dried and purified from alcohol.

M.P.= 158°C

Yield = 65.7%

Elemental Analysis: $C_{11}H_{11}N_3OCl_2$

	C%	H%	N%
Calculated	48.53	4.04	15.44
Found	48.39	3.88	15.67

Preparation of 2-amino benzothiazole (VIII) has been discussed in the **Part I Chemistry** section under the title '*Chemistry of Benzothiazoles*' page no – **66** as per the procedure mentioned in literature²¹³⁻²¹⁴.

Step V: *Synthesis of 2-[1,3-Benzothiazol-2-yl amino]-N- [1,5-dimethyl-2- (2,4-dichloro) phenyl-3-oxo-2, 3-dihydro-1H-pyrazol-4-yl] acyl amine (IX).*

4-amino-2,3-dimethyl-1-(2,4-dichloro) phenyl pyrazol-5-one (0.005M) dissolved in alcohol in an round bottom flask was placed in ice bath. Then slowly equimolar quantity of chloro acetyl chloride (0.005M) was added which resulted in the solid product. The product obtained is unstable and treated rapidly for further reaction.

To an ethanolic solution of 4-(α/β -halogenoacyl amino)-1,5-dimethyl-2-(2,4-dichloro) phenyl-2,3-dihydro-1H-pyrazol-3-one (0.005M), 2-amino-1,3-benzothiazole (0.005M) was added and the reaction mixture was refluxed for 4hrs. After cooling the solution was evaporated to dryness under reduced pressure. The product was precipitated with sat. NaHCO₃ and purified by recrystallisation from ethanol.

M.P.= 235-236°C

Yield = 72.4%

Elemental Analysis: C₂₀H₁₇N₅O₂SCl₂

	C%	H%	N%
Calculated	51.95	3.68	15.15
Found	51.69	3.79	14.97

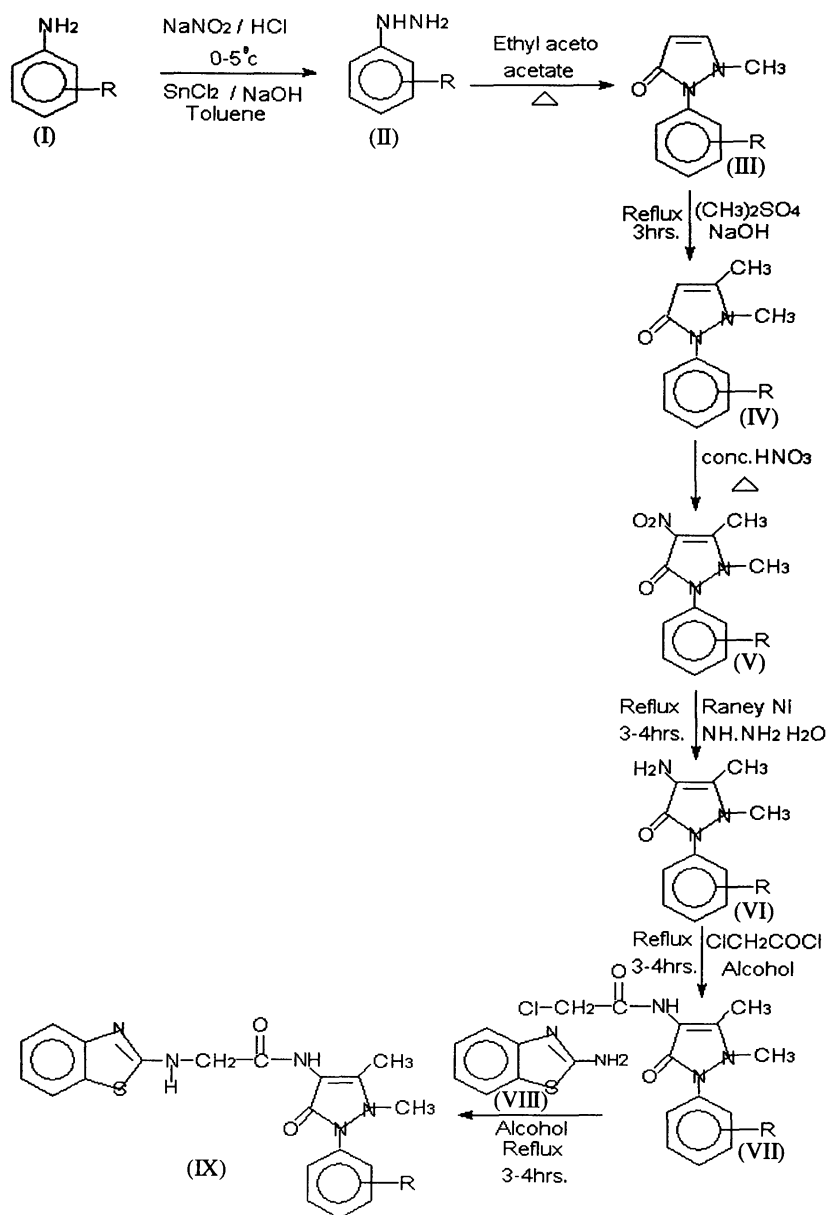
Spectral Characterization:

Series IV (4) i.e. 4D:

IR (KBr) cm⁻¹: 3414(-NH), 2988(-CH₃), 1783(C=O), 1649(CONH); 1461(-Cl)

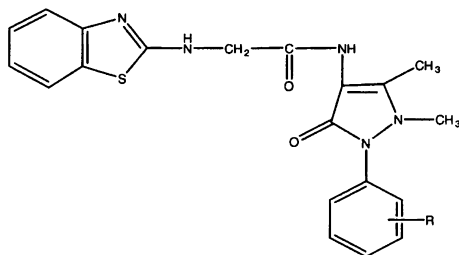
PMR (DMSO): δ 4.79(d, 2H, CH₂), δ 7.25-7.88[m, 7H ArH], δ 9.89(s, 1H, -CONH)

Scheme IV



R= 3-chloro, 2,4-dichloro, etc.

Table IV: Physical constants of 2-[1,3-Benzothiazol-2-yl amino]-N- [1,5-dimethyl-2- (substituted phenyl)-3-oxo-2, 3-dihydro-1H-pyrazol-4-yl] acyl amine.



R= 3-chloro, 2,4-dichloro etc.

Sr.No.	R	Molecular Formula	M.P (°C)	Yield (%)	Elemental Analysis					
					Calculated (%)			Obtained (%)		
					C	H	N	C	H	N
1.	4-chloro	C ₂₀ H ₁₈ N ₅ O ₂ SCl	230-232	64	56.14	4.21	16.37	55.89	4.04	16.26
2.	2,4-difluoro	C ₂₀ H ₁₇ N ₅ O ₂ SF ₂	218-220	60	55.94	3.96	16.32	55.68	3.76	16.12
3.	3-chloro	C ₂₀ H ₁₈ N ₅ O ₂ SCl	195-197	68	56.14	4.21	16.37	56.01	4.35	16.49
4.	2,4-dichloro	C ₂₀ H ₁₇ N ₅ O ₂ SCl ₂	235-236	72	51.95	3.68	15.15	51.69	3.79	14.97
5.	3,4-dichloro	C ₂₀ H ₁₇ N ₅ O ₂ SCl ₂	198-200	58	51.95	3.68	15.15	52.15	3.60	15.20
6.	3-chloro-4-fluoro	C ₂₀ H ₁₇ N ₅ O ₂ SClF	97-98	55	53.87	3.82	15.71	53.80	3.75	16.04
7.	2,5-dimethoxy	C ₂₂ H ₂₃ N ₅ O ₄ S	202-203	56	58.28	5.07	15.45	58.13	4.87	15.33
8.	4-fluoro	C ₂₀ H ₁₈ N ₅ O ₂ SF	122-123	70	58.39	4.38	17.03	58.26	4.16	16.84

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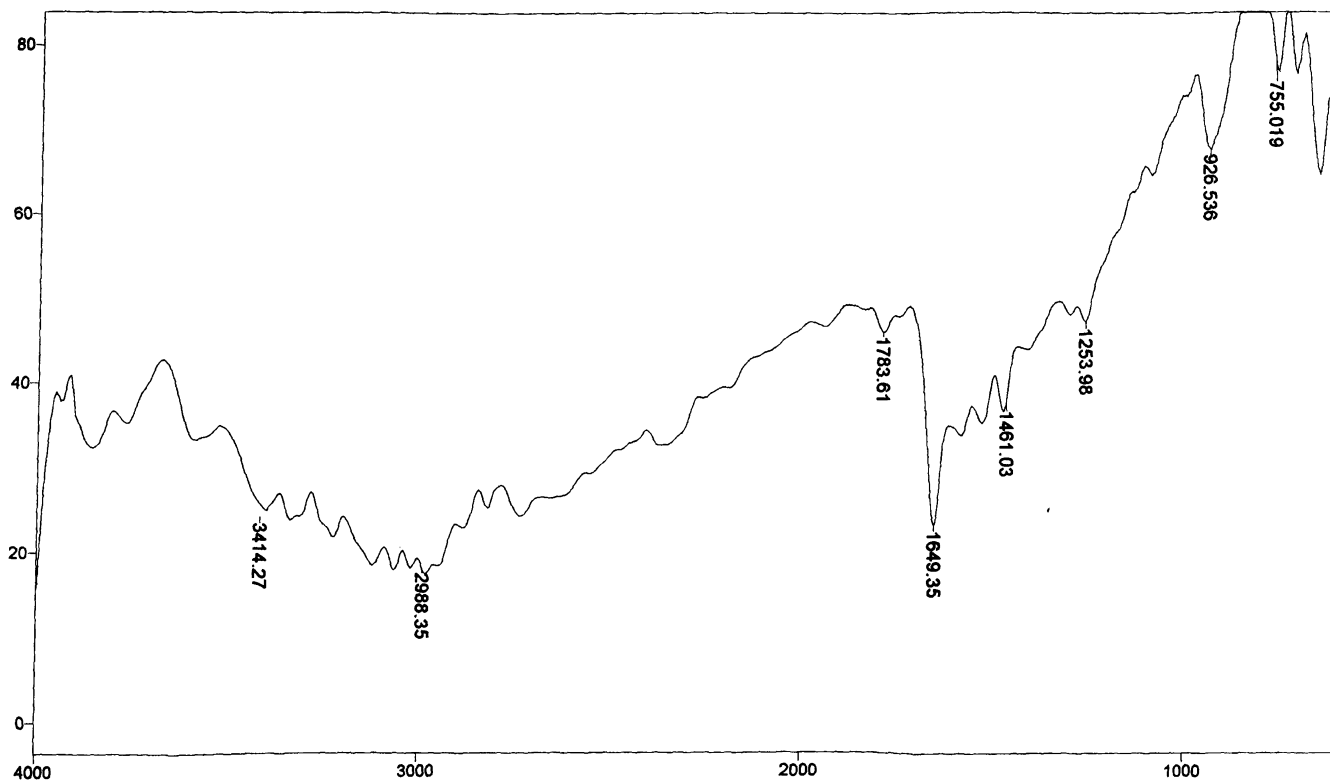
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Res=8 cm-1

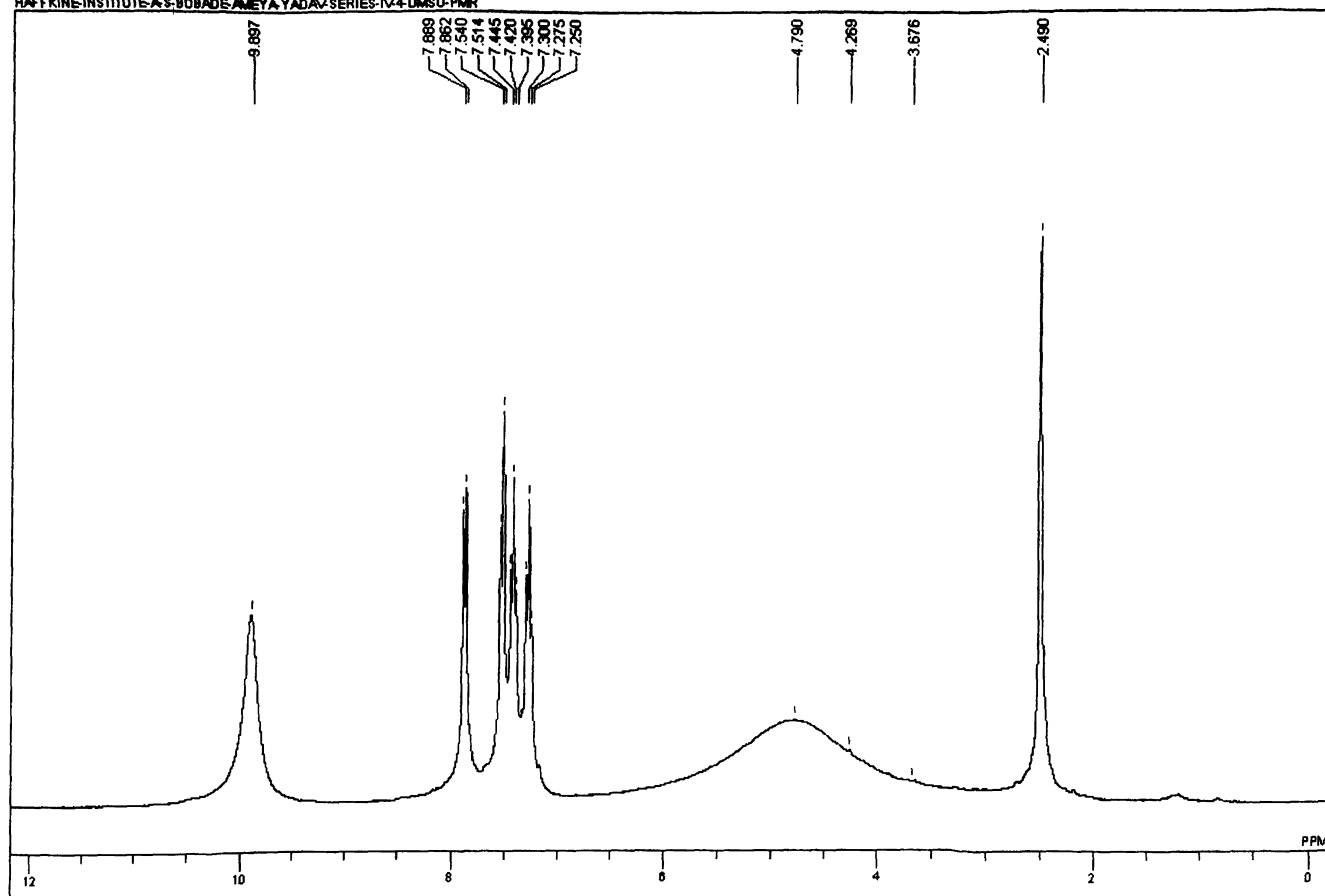
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Zero Filling= 1 x



Transmittance / Wavenumber (cm-1)

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***Biological Screening, Results
and
Conclusions***

BIOLOGICAL SCREENING

All the compounds synthesized during the course of work were subjected to a biological screening program in order to determine their antibacterial and antifungal activities and thus to establish their 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. Also, *in vitro* study against fungal strains such as *Candida albicans* ATCC 10231 and *Aspergillus niger* ATCC 16404 was carried out.

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

Drug Stock Solution:

The test compound (10mg) was dissolved in a suitable solvent (10ml) such as DMF, ethanol, dil. HCl, DMSO as the case may be, so as to prepare a stock solution of 1000µg/ml. From this solution, the drug concentration viz. 200, 100, 50 and 20µg/ml were made. For lower dilutions the stock solution was diluted to prepare concentrations such as 20, 10, 5, 2µg/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.
5. **Standard Control:** It contains medium, organism culture and dilution of the 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.

Muller Hinton broth was used as a culture medium. DMSO was used as a 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 200,100,50,20,10,5,2,1µg/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 Minimum Inhibitory Coefficient (MIC). Ampicillin (MIC= 0.01µg/ml against Gram-positive *S.aureus*) and Trimethoprim (MIC= 1.0µg/ml against Gram-negative *S.typhi*) were used as the standard drugs.

Antifungal Screening:

The organisms used for the test were *Candida albicans* and *Aspergillus niger*. Again the method employed was the tube dilution method.

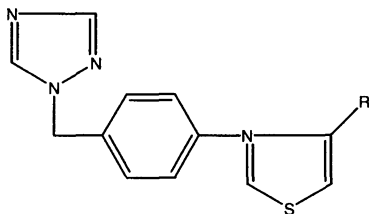
Sabraoud's medium was used as the growth media (pH=5.6). The sterile media was dispensed in a sterile borosilicate test tube. The drug solution was added in order to get the final drug concentrations of 200, 100, 50, 20, 10, 5, 2 µg/ml etc.

The tubes were incubated at room temperature (28-30°C) in a dark place. *C. albicans* and *A. niger* were incubated for 7 days. The presence or absence of the 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* and *A. niger*) was used as a standard drug.

RESULTS:

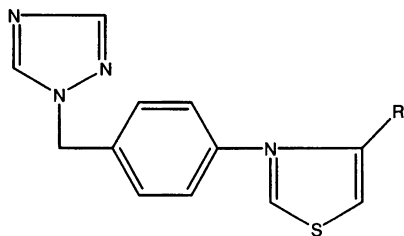
Table I: Antibacterial activity of 2-[4'-[1-(1,2,4-triazolyl) methyl] phenyl]-4-(substituted) thiazoles.



Sr.No.	Substitutions (R)	MIC (µg/ml)	
		<i>S. aureus</i>	<i>S. typhi</i>
1.	4-amino phenyl	200	200
2.	2,4-dihydroxy phenyl	10	200
3.	2,6-dihydroxy phenyl	10	200
4.	2,5-dihydroxy phenyl	50	100
5.	2-hydroxy phenyl	200	N.A
6.	2,4,6-trihydroxy phenyl	200	200
7.	6-chloro cinnolin-4-one	100	100

- Ampicillin (MIC-0.04 µg/ml) used as standard against *S. aureus*.
- Trimethoprim (MIC 0.01 µg/ml) used as standard against *S. typhi*.
- Miconazole (MIC 6.25 µg/ml) as standard against *C. albicans* and *A. niger*.
- N.A.- Inactive at 200 µg/ml.

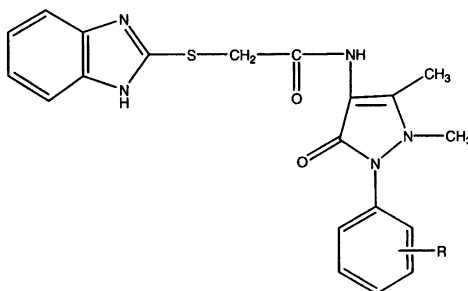
Table II: Antifungal activity of 2-{4'-[1-(1,2,4-triazolyl) methyl] phenyl}-4-(substituted) thiazoles.



Sr.No.	Substitutions (R)	MIC (µg/ml)	
		<i>C. albicans</i>	<i>A. niger</i>
1.	4-amino phenyl	50	100
2.	2,4-dihydroxy phenyl	50	100
3.	2,6-dihydroxy phenyl	50	100
4.	2,5-dihydroxy phenyl	50	100
5.	2-hydroxy phenyl	50	100
6.	2,4,6-trihydroxy phenyl	50	100
7.	6-chloro cinnolin-4-one	50	50

- Ampicillin (MIC-0.04 µg/ml) used as standard against *S. aureus*.
- Trimethoprim (MIC 0.01 µg/ml) used as standard against *S. typhi*.
- Miconazole (MIC 6.25 µg/ml) as standard against *C. albicans* and *A. niger*.

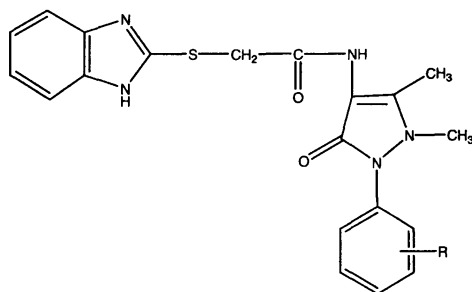
Table III: Antibacterial activity of 2-[1H-Benzimidazol-2-yl mercapto]-N-[1,5-dimethyl-2-(substituted phenyl)-3-oxo-2,3-dihydro-1H-pyrazol-4-yl] acyl amine.



Sr.No.	Substitutions (R)	MIC (µg/ml)	
		<i>S. aureus</i>	<i>S. typhi</i>
1.	4-chloro	200	100
2.	2,4-difluoro	200	200
3.	3-chloro	200	200
4.	2,4-dichloro	100	200
5.	3,4-dichloro	100	200
6.	3-chloro-4-fluoro	100	100
7.	2,5-dimethoxy	100	100
8.	2-methoxy	200	200

- Ampicillin (MIC-0.04 µg/ml) used as standard against *S. aureus*.
- Trimethoprim (MIC 0.01 µg/ml) used as standard against *S. typhi*.
- Miconazole (MIC 6.25 µg/ml) as standard against *C. albicans* and *A. niger*.

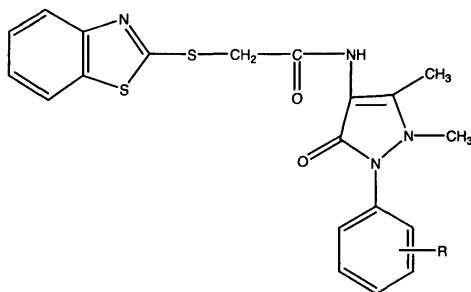
Table IV: Antifungal activity of 2-[1H-Benzimidazol-2-yl mercapto]-N-[1,5-dimethyl-2-(substituted phenyl)-3-oxo-2,3-dihydro-1H-pyrazol-4-yl] acyl amine.



Sr.No.	Substitutions (R)	MIC (µg/ml)	
		<i>C. albicans</i>	<i>A. niger</i>
1.	4-chloro	100	200
2.	2,4-difluoro	100	100
3.	3-chloro	100	200
4.	2,4-dichloro	100	200
5.	3,4-dichloro	100	200
6.	3-chloro-4-fluoro	100	100
7.	2,5-dimethoxy	100	200
8.	2-methoxy	200	200

- Ampicillin (MIC-0.04 µg/ml) used as standard against *S. aureus*.
- Trimethoprim (MIC 0.01 µg/ml) used as standard against *S. typhi*.
- Miconazole (MIC 6.25 µg/ml) as standard against *C. albicans* and *A. niger*.

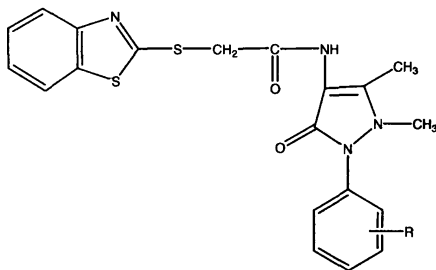
Table V: Antibacterial activity of 2-[1,3-Benzothiazol-2-yl mercapto]-N-[1,5-dimethyl-2-(substituted phenyl)-3-oxo-2,3-dihydro-1H-pyrazol-4-yl] acyl amine.



Sr.No.	Substitutions (R)	MIC (µg/ml)	
		<i>S. aureus</i>	<i>S. typhi</i>
1.	4-fluoro	200	100
2.	4-chloro	200	100
3.	2,4-difluoro	200	100
4.	3-chloro	100	100
5.	3,4-dichloro	200	100
6.	3-chloro-4-fluoro	200	200
7.	2,5-dimethoxy	100	200
8.	2,4-dichloro	200	100

- Ampicillin (MIC-0.04 µg/ml) used as standard against *S. aureus*.
- Trimethoprim (MIC 0.01 µg/ml) used as standard against *S. typhi*.
- Miconazole (MIC 6.25 µg/ml) as standard against *C. albicans* and *A. niger*.

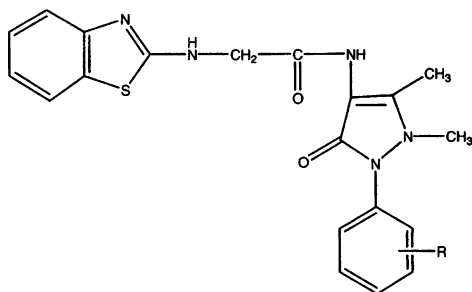
Table VI: Antifungal activity of 2-[1,3-Benzothiazol-2-yl mercapto]-N-[1,5-dimethyl-2-(substituted phenyl)-3-oxo-2,3-dihydro-1H-pyrazol-4-yl] acyl amine.



Sr.No.	Substitutions (R)	MIC (µg/ml)	
		<i>C. albicans</i>	<i>A. niger</i>
1.	4-fluoro	100	100
2.	4-chloro	100	100
3.	2,4-difluoro	100	100
4.	3-chloro	50	50
5.	3,4-dichloro	200	200
6.	3-chloro-4-fluoro	100	100
7.	2,5-dimethoxy	100	200
8.	2,4-dichloro	100	100

- Ampicillin (MIC-0.04 µg/ml) used as standard against *S. aureus*.
- Trimethoprim (MIC 0.01 µg/ml) used as standard against *S. typhi*.
- Miconazole (MIC 6.25 µg/ml) as standard against *C. albicans* and *A. niger*.

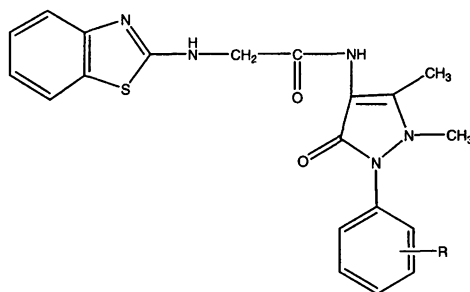
Table VII: Antibacterial activity of 2-[1,3-Benzothiazol-2-yl amino]-N-[1,5-dimethyl-2-(substituted phenyl)-3-oxo-2,3-dihydro-1H-pyrazol-4-yl] acyl amine.



Sr.No.	Substitutions (R)	MIC (µg/ml)	
		<i>S. aureus</i>	<i>S. typhi</i>
1.	4-chloro	200	100
2.	2,4-difluoro	200	200
3.	3-chloro	200	100
4.	2,4-dichloro	200	100
5.	3,4-dichloro	200	200
6.	3-chloro-4-fluoro	200	100
7.	2,5-dimethoxy	200	100
8.	4-fluoro	200	100

- Ampicillin (MIC-0.04 µg/ml) used as standard against *S. aureus*.
- Trimethoprim (MIC 0.01 µg/ml) used as standard against *S. typhi*.
- Miconazole (MIC 6.25 µg/ml) as standard against *C. albicans* and *A. niger*.

Table VIII: Antifungal activity of 2-[1,3-Benzothiazol-2-yl amino]-N-[1,5- dimethyl-2-(substituted phenyl)-3-oxo-2,3-dihydro-1H-pyrazol-4-yl] acyl amine.



Sr.No.	Substitutions (R)	MIC (µg/ml)	
		<i>C. albicans</i>	<i>A. niger</i>
1.	4-chloro	100	100
2.	2,4-difluoro	100	100
3.	3-chloro	N.A	100
4.	2,4-dichloro	100	200
5.	3,4-dichloro	100	200
6.	3-chloro-4-fluoro	100	100
7.	2,5-dimethoxy	100	100
8.	4-fluoro	100	100

- Ampicillin (MIC-0.04 µg/ml) used as standard against *S. aureus*.
- Trimethoprim (MIC 0.01 µg/ml) used as standard against *S. typhi*.
- Miconazole (MIC 6.25 µg/ml) as standard against *C. albicans* and *A. niger*.
- N.A.- Inactive at 200 µg/ml.

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 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 seven compounds had been synthesized and screened for their biological activities (Table I & II).

1. All the seven compounds screened for antibacterial activity against *S.aureus* and *S.typhi*, exhibited moderate to good activity. The compounds 2,4-dihydroxy phenyl, 2,6-dihydroxy phenyl and 2,5-dihydroxy phenyl substitutions exhibited good activity upto 10µg/ml, 10µg/ml and 50µg/ml respectively against *S.aureus* and moderate activity upto 200µg/ml, 200µg/ml and 100µg/ml against *S.typhi* respectively. The compounds 4-amino phenyl, 2-hydroxy phenyl and 2,4,6-trihydroxy phenyl substitutions showed activity upto 200µg/ml against *S.aureus* and upto 200µg/ml against *S.typhi* with exception of 2-hydroxy phenyl substitution which was found to be inactive at 200µg/ml against *S.typhi*. The 6-chloro cinnolin-4-one showed moderate activity upto 100µg/ml against *S.aureus* and *S.typhi*.
2. All the compounds synthesized exhibited good antifungal activity upto 50µg/ml against *C.albicans* and moderate activity upto 100µg/ml against *A.niger* except for 6-chloro cinnolin-4-one phenyl substitutions, which exhibited good activity upto 50µg/ml.

It could be concluded from the above data that the activity which was expected to be achieved by combination of two biologically significant moieties 1H-1,2,4-triazole and substituted thiazole was however not satisfactorily observed. As few dihydroxy substituted derivatives exhibited activity against *S.aureus*. All the seven compounds exhibited significant activity against the fungal species. Therefore,

by introducing suitable substitutions on the thiazole ring, further investigations could be pursued on the triazolo thiazolyl moiety to reach upon a therapeutically potent agent, which could be at par with standard drugs.

Part III involves the synthesis of 2-[1H-Benzimidazol-2-yl mercapto]-N-[1,5-dimethyl-2- (substituted phenyl)-3-oxo-2, 3-dihydro-1H-pyrazol-4-yl] acyl amine. In this series seven compounds had been synthesized and screened for their biological activities (Table III & IV).

1. All the eight compounds in the series exhibited moderate activity against *S.aureus*. The compound with 4-chloro, 2,4-difluoro, 3-chloro and 2-methoxy substitution exhibited activity upto 200µg/ml, while the compound with substitutions 2,4-dichloro, 3,4-dichloro, 3-chloro-4-fluoro and 2,5-dimethoxy exhibited activity upto 100µg/ml against *S.aureus*. The compounds with substitution 4-chloro, 3-chloro-4-fluoro and 2,5-dimethoxy exhibited activity upto 100µg/ml, while the compounds 2,4-difluoro, 3-chloro, 2,4-dichloro, 3,4-dichloro and 2-methoxy substitution exhibited activity upto 200µg/ml against *S.typhi*.
2. All the compounds showed moderate activity upto 100µg/ml against fungal species of *C.albicans* upto 100µg/ml exceptional of 2-methoxy substitutions with activity at 200µg/ml. The compounds 2,4-difluoro and 3-chloro-4-fluoro substitution showed activity at 100µg/ml while remaining others at 200µg/ml against *A.niger* species.

It could be concluded from the above data that substituted pyrazolin derivatives did not exhibit encouraging results as expected from the combination of biologically active moieties of benzimidazolyl ring with pyrazolin ring system. The molecule shows moderate activity against bacterial and fungal strains, which lead us to believe, that by making suitable changes on the pyrazolin moiety or benzimidazolyl moiety possibly a molecule with promising activity could be arrived at.

Part IV involves the synthesis of 2-[1,3-Benzothiazol-2-yl mercapto]-N- [1,5-dimethyl-2- (substituted phenyl)-3-oxo-2, 3-dihydro-1H-pyrazol-4-yl] acyl amine. In this series seven compounds had been synthesized and screened for their biological activities (Table V & VI).

1. All the eight synthesized compounds showed moderate activity upto 200µg/ml among which 3-chloro and 2,5-dimethoxy showed activity upto 100µg/ml against *S.aureus*. The compounds 3-chloro-4-fluoro and exhibited activity upto 200µg/ml while the other remaining compounds exhibited activity upto 100µg/ml against *S.typhi*.
2. All the compounds exhibited moderate antifungal activity upto 100µg/ml against *C.albicans* except the 3-chloro substituted compounds, which exhibited good antifungal activity upto 50µg/ml against both *C.albicans* and *A.niger*. The compounds 3,4-dichloro and 2,5-dimethoxy substituted exhibited activity upto 200µg/ml while rest of the compounds exhibited activity against *A.niger* at 100µg/ml.

From the above data it can be concluded that all the compounds showed moderate activity against bacterial strains and the fungal strains. The 3-chloro substituted compounds showed significant antifungal activity. Therefore, by introducing certain suitable substitutions in possibly both the moieties may lead to the compounds with promising activity.

Part V involves the synthesis of 2-[1,3-Benzothiazol-2-yl amino]-N- [1,5-dimethyl-2- (substituted phenyl)-3-oxo-2, 3-dihydro-1H-pyrazol-4-yl] acyl amine. In this series seven compounds had been synthesized and screened for their biological activities (Table VII & VIII).

1. All the eight synthesized compounds exhibited activity upto 200µg/ml against *S.aureus*. The compounds with 2,4-difluoro and 3,4-difluoro substitutions exhibited activity upto 200µg/ml whereas rest of the compounds exhibited moderate activity upto 100µg/ml against *S.typhi*.
2. All the eight synthesized compounds exhibited moderate activity upto 100µg/ml against *C.albicans* except the 2,4-dichloro and 3,4-dichloro which

showed activity upto 200µg/ml. Rest all the compounds showed activity upto 100µg/ml against *A.niger*.

It could be concluded from the above data that the activity, which was expected to achieve by combining two moieties pyrazolin and 2-amino benzothiazole was not observed against *S.aureus*. As can be seen the substitutions gave moderate activity against fungal species, introducing a suitable substitution in any one of the moiety or possibly both the moieties can enhance the activity.

References

References

1. Burger, A.; (1990) Preface in Hansch, C., Sammes, P. G. and Taylor, J. B. (eds.). *Comprehensive Medicinal Chemistry*. Vol-1, pg-1, Pergamon Press Oxford.
2. Henry F. Chambers. "Antimicrobial Agents: General Considerations", cited from Goodman & Gilman's "The Pharmacological Basis of Therapeutics". 10th edn. Mc'Graw Hill. pp 1143.
3. John E. Bennett. "Antimicrobial agents: Antifungal Agents", cited from Goodman & Gilman's "The Pharmacological Basis of Therapeutics". 10th edn. Mc'Graw Hill. pp 1295.
4. Wermuth, C. G., 'The Practice of Medicinal Chemistry', 4, (1992) ed. by laboratories de Pharmacochimie Moléculaire, Faculté de Pharmacie, Université Louis Pasteur, 74, Route du Rhin, 67401, Illkirch Cedex, France.
5. Domagk, G., Deut. Med. Wochenschr; 61, 250 (1935) cited from "Burger's Medicinal Chemistry and Drug Discovery", 5th edn., ed. By Wolf, M.E., A Wiley-Interscience Publication, John-Wiley & sons., 2, *Therapeutic Agents*, 527-566 (1997).
6. Fuller, A.T., *Lancet*, 1, 194 (1937).
7. Walsh, C.T., *Annu. Rev. Biochem.*, 55, 493 (1984).
8. Greenwood, D.; 'Antimicro. Chemother.' 3rd edn., ed. by Greenwood, D., Oxford Univ. Press, Oxford, 50 (1995).
9. Robin, R.O. Jr, Williams, J.H., Winnek, P.S. and English, J.P., *J. Am. Chem. Soc.*, 62, 2002 (1940).
10. Caldwell, W.T. and Kime, H.B., *J. Am. Chem. Soc.*, 62, 2365 (1940).
11. Caldwell, W.T., Cornfeld, E.C. and Donnel, C.K., *J. Am. Chem. Soc.*, 63, 3028 (1941).
12. Sprague, J.M., Kissinger, L.W., Lincoln, R.M., *J. Am. Chem. Soc.*, 63, 3038 (1941).
13. Nitya Anand, 'Burger's Medicinal Chemistry and Drug Discovery', 5th edn., ed. by Wolf, M.E., A Wiley-Interscience Publication, John-Wiley & Sons., 2, *Therapeutic Agents*, 533 (1997).
14. Whithy, L.H.E., *Lancet*, 2, 1210 (1938).

15. Nicoles, R.L., Jones, W.H. Jr. and Finland, M., *Proc. Soc. Exp. Bio. Med.*, 92, 637 (1956).
16. Lester, M.M. and English, J.P., *U.S. Pat. 2*, 790, 798 (1957) cited from C.A., 51, (1957).
17. Fullerton, D.S., 'Wilson and Giswold's Textbook of Organic Medicinal and Pharmaceutical Chemistry'; 9th edn., J.B. Lippincot Company, Philadelphia, 203 (1991).
18. Hitchings, G.H. and Bushby, S.R.M., 5th *Int. Congr. Biochem.*, 165 (1961) cited from 'Burger's Med. Chem. and Drug Discovery'; 5th edn., ed. by Wolf, M.E., A Wiley-Interscience Publication, John-Wiley & Sons., 4, *Therapeutic Agents*, 273 (1997).
19. Herbert, V., *J. Infect. Dis.*, 128, (Nov. suppl.), 432 (1973).
20. Reimer, W.A. and Bear, S., 'Burger's Med. Chem. and Drug Discovery'; 5th edn., ed. by Wolf, M.E., A Wiley-Interscience Publication, John-Wiley & Sons., 4, *Therapeutic Agents*, 267-271 (1997).
21. Martin, A.R., 'Wilson and Giswold's Textbook of Organic Medicinal and Pharmaceutical Chemistry'; 10th edn., Lippincot-Raven Publishers, Philadelphia, New York, 201 (1998).
22. Stillman, W.B. and Scott, A.B., *U.S.Pat. 2*, 416, 234 (1947) cited from 'Burger's Med. Chem. and Drug Discovery'; 5th edn., ed. by Wolf, M.E., A Wiley-Interscience Publication, John-Wiley & Sons., 4, *Therapeutic Agents*, 271 (1997).
23. Drake, G.D. and Haynes, K.J., *U.S.Pat. 2*, 759, 931 (1995) cited from 'Burger's Med. Chem. and Drug Discovery'; 5th edn., ed. by Wolf, M.E., A Wiley-Interscience Publication, John-Wiley & Sons., 4, *Therapeutic Agents*, 267-271 (1997).
24. Greenwood, D.; 'Antimicro. Chemother.' 3rd edn., ed. by Greenwood, D., Oxford Univ. Press, Oxford, 57-58 (1995).
25. Yocum, R.R. *et al.*, *J. Biol. Chem.*, 255, 3977 (1986) cited from 'Wilson and Giswold's Textbook of Organic Medicinal and Pharmaceutical Chemistry'; 9th edn., J.B. Lippincot Company, Philadelphia, 230 (1991).
26. Waxman, D.J., Strominger, J.L., *Annu. Rev. Pharmaceutical*, 52, 825 (1983) cited from 'Wilson and Giswold's Textbook of Organic Medicinal and

- Pharmaceutical Chemistry'; 9th edn., J.B. Lippincot Company, Philadelphia, 203 (1991).
27. Nikaido, H., Nakae, T., Adv. Microb. Physiol., 20, 163 (1976) cited from 'Wilson and Giswold's Textbook of Organic Medicinal and Pharmaceutical Chemistry'; 9th edn., J.B. Lippincot Company, Philadelphia, 203 (1991).
 28. Doyle, F.P., Hardy, K.D., Nayler, J.H.C., Soulal, M.J., Stove, E.R. and Waddington, J. *Chem. Soc.*, 1453 (1962).
 29. Yurchenco, J.A., Hopper, M.W. and Warren, G.H.; *Antimico. Chemother.*, 12, 534 (1962).
 30. Nayler, J.H.C., *Adv. Drug Res.*, 7, 52 (1973).
 31. Doyle, F.L., Long, A.A.W., Nayler, J.H.C. and Stove, E.R., *Nature*, 192, 1183 (1961).
 32. Nayler, J.H.C., Long, A.A.W., Brown, D.M., Acred, P., Rolison, G.N., Batchelor, F.R., Stevens, H. and Sutherland, R., *Nature*, 195, 1264 (1962).
 33. Bennet, J.V., Gravenkemper, C.F., Bordie, J.I. and Kirby, W.M.M., *Antimicrob. Agents Chemother.*, 257. (1965).
 34. Sutherland, R., Croydon, E.A.P. and Rolinson, G.N., *Brit. Med. J.*, 4, 455 (1970).
 35. Rolinson, G.N. and Stevens, S., *Brit. Med. J.*, 2, 191 (1969).
 36. Broden, R.N., Speight, T.M. and Avery, G.S., *Drugs*, 9, 88 (1975).
 37. Acred, P., Brown, D.M., Kundson, E.T., Rolinson, G.N. and Sutherland, R., *Nature*, 215, 25 (1967).
 38. Morimoto, S., Nomura, H., Fugono, T., Azuma, T., Minami, I., Hori, M. and Masuad, T., *J. Med. Chem.*, 15, 1108 (1972).
 39. Stewart, G.W., The Penicillin Group of Drugs, Amsterdam, Elsevier, (1965) cited from 'Wilson and Giswold's Textbook of Organic Medicinal and Pharmaceutical Chemistry'; 9th edn., J.B. Lippincot and Company, Philadelphia, 233 (1991).
 40. Batchlor, F.R. et al., *Nature*, 206, 362 (1962).
 41. Abraham, E.P., Chain, E., Gardner, A.D., Heatley, N.G., Jennings, M.A., and Florey, H.W., *Lancet*, 2, 177 (1941).
 42. Chauvette, R.R., Jakson, B.G., Lavergnino, E.R., Morr, R.R., Mueller, R.A., Pioch, R.P., Roeske, R.W., Ryan, C.W., Spencer, J.L., Van Heyningen, E., *J. Am. Chem. Soc.*, 84, 3401 (1962).

43. Ryan, C.W., Simpson, R.L. and Heyningen, E.M., *J. Med. Chem.*, 12, 310 (1969).
44. Durkheimer, W., Adam, F., Fischer, G. and Kirrstetter, R. (B. Testa, Ed.) Advances in Drug Research, *Academic Press*, 17, 61-234 (1988) cited from 'Burger's Med. Chem. and Drug Discovery'; 5th edn., ed. by Wolf, M.E., A Wiley-Interscience Publication, John-Wiley & Sons., 4, *Therapeutic Agents*, 299 (1997).
45. Adams, H.G., Stilwell, G.A. and Turk, M.; *Antimicrob. Agents Chemother.*, 9, 1019 (1976).
46. Brogden, R.N., Heel, R.C., Speight, T.M. and Avery, G.S., *Drugs*, 17, 233 (1979).
47. Muytens, H.L., Vander Ros-van, J., Repe, *Antimicrob. Agents Chemother.*, 21, 925 (1982).
48. Symposium, *Review of Infectious Diseases*, 4, Supplement 281-488 (1988) cited from 'Burger's Med. Chem. and Drug Discovery'; 5th edn., ed. by Wolf, M.E., A Wiley-Interscience Publication, John-Wiley & Sons., 4, *Therapeutic Agents*, 299 (1997).
49. Umezwa, H.Et. *et al.*, *J. Antibiot.*, 10, 181 (1957) cited from 'Wilson and Giswold's Textbook of Organic Medicinal and Pharmaceutical Chemistry'; 9th edn., J.B. Lippincot and Company, Philadelphia, 277 (1991).
50. Weinstein, M.J. *et al.*, *J. Med. Chem.*, 6, 463 (1963).
51. Koch, K.F. *et al.*, *J. Antibiot.*, 26, 783 (1963) cited from 'Wilson and Giswold's Textbook of Organic Medicinal and Pharmaceutical Chemistry'; 9th edn., J.B. Lippincot and Company, Philadelphia, 279 (1991).
52. Wrihght, J.J., *J. Chem. Soc., Chem. Commun.*, 206 (1976).
53. Dyer, J.R., Todd, A.W., *J. Am. Chem. Soc.*, 85, 3896 (1963).
54. Dyer, J.R. *et al.*, *J. Am. Chem. Soc.*, 87, 654 (1965).
55. Umezawa, S. *et al.*, *J. Antibiot.*, 27, 997 (1974) cited from 'Wilson and Giswold's Textbook of Organic Medicinal and Pharmaceutical Chemistry'; 9th edn., J.B. Lippincot and Company, Philadelphia, 279 (1991).
56. Haskell, J.H. *et al.*, *J. Am. Chem. Soc.*, 81, 3482 (1974).
57. De Jongh, D.C. *et al.*, *J. Am. Chem. Soc.*, 89, 3364 (1964).

58. Conover, L.H., *U.S.Pat.* 2, 693, 054, Jan. 11, 1955 cited from 'Burger's Med. Chem. and Drug Discovery'; 5th edn., ed. by Wolf, M.E., A Wiley-Interscience Publication, John-Wiley & Sons., 4, *Therapeutic Agents*, (1997).
59. Duggar, B.B., *Ann. N.Y. Acad. Sci.*, 51, 177 (1948).
60. Mrtell, M.J. Jr., Booth, J.H., *J. Med. Chem.*, 10, 44 (1967).
61. Finley, A.C. *et al.*, *Science*, 111, 85 (1950).
62. Stephens, C.R. *et al.*, *J. Am. Chem. Soc.*, 80, 5324 (1958).
63. Blackwood, R.R. *et al.*, *J. Am. Chem. Soc.*, 83, 2773 (1961).
64. Minuth, J.N., *Antimicrob. Agents Chemother.*, 6, 411 (1964).
65. Woodward, R.B., *Angrew. Chem.*, 69, 50-58 (1957).
66. Mc Guire, J.M. *et al.*, *Antimicrob. Agents Chemother.*, 2, 821 (1952).
67. Malmborg, A.S., *J. Antimicrob. Chemother.*, 18, 293 (1986).
68. Wilhelm, J.M. *et al.*, *Antimicrob. Agents Chemother.*, 236 (1967).
69. Gutman, L.T. *et al.*, *Lancet*, 1, 464 (1967).
70. Barradell, L.B., Polsker, G.L. and Mc Tavison, D., *Drugs*, 46, 289-313 (1993).
71. St. Georgiev, V., *Int. J. Antimicrob. Agents*, 4, 247-270 (1994).
72. Manson, D.J. *et al.*, *Antimicrob. Agents Chemother.*, 544 (1962).
73. Magerlein, B.J. *et al.*, *J. Med. Chem.*, 10, 355 (1967).
74. Erlich, J. *et al.*, *Science*, 106, 417 (1963).
75. Controlis, J. *et al.*, *J. Am. Chem. Soc.*, 71, 2463 (1949).
76. Emmerson, A.E., David Greenwood's, *Antimicrob. Chemother.*, 3rd edn., ed. by Greenwood, D., Oxford Univ. Press, Oxford, 301 (1995).
77. Marwick, C., *JAMA*, 257, 1174 (1992).
78. Daniel, T.M., 'Tuberculosis' in 'Harison's Principles of Internal Medicine', 12th edn. (Jean D. Wilson ed.) New York, Mc Graw-Hill (1991).
79. Meyer, H. and Mally, J.; *Montash eftr fiir chemie Montash Chem.*, 33, 392 (1912) cited from 'Burger's Med. Chem. and Drug Discovery'; 5th edn., ed. by Wolf, M.E., A Wiley-Interscience Publication, John-Wiley & Sons., 4, *Therapeutic Agents*, (1997).
80. Lemke, Thomas L., Foye, W.O. and Williams D.A., 'Principles of Medicinal Chemistry', 4th edn., A B.I. Wavrely Pvt. Ltd., New Delhi Publication, India, 748 (1995).

81. Young, D.B., *Carr. Biol.*, 4, 351 (1994) cited from 'Burger's Med. Chem. And Drug Discovery'; 5th edn., ed. by Wolf, M.E., A Wiley-Interscience Publication, John-Wiley & Sons., 4, *Therapeutic Agents*, 597 (1997).
82. Zang, Y., Heym, B., Allen, B., Young, D. and Cole, S., *Nature*, 358, 591 (1992).
83. Sensi, P., Grassi, G.G., 'Burger's Med. Chem. and Drug Discovery'; 5th edn., ed. by Wolf, M.E., A Wiley-Interscience Publication, John-Wiley & Sons., 4, *Therapeutic Agents*, 610 (1997).
84. Sensi, P., Maggi, N., Furesz, S. and Maffii, G., *Antimicrob. Agents Chemother.*, 1966, 699 (1967).
85. Forbes, M., Kuck, N.A. and Peets, E.A., *J. Bacteriol.*, 89, 1099 (1962).
86. Forbes, M., Kuck, N.A. and Peets, E.A., *J. Bacteriol.*, 89, 1299 (1965).
87. Reutgen, H. and Iwainsky, Z., *Naturforsch.*, 27b, 1405 (1972) cited from 'Burger's Med. Chem. and Drug Discovery'; 5th edn., ed. by Wolf, M.E., A Wiley-Interscience Publication, John-Wiley & Sons., 4, *Therapeutic Agents*, 602 (1997).
88. Honore, N. and Cole, S.T., *Antimicrob. Agents Chemother.*, 38, 238 (1994).
89. Emmerson, A.M., 'David Greenwood's Antimicrobial Chemotherapy', 3rd edn., ed. by Greenwood, D., Oxford Univ. Press, Oxford, 306-307 (1995).
90. Lehmann, J., *Lancet*, 1, 15 (1946).
91. Ratledge, C. and Brown, K.A., *Am. Rev. Respir. Dis.*, 106, 774 (1972) cited from 'Burger's Med. Chem. and Drug Discovery'; 5th edn., ed. by Wolf, M.E., A Wiley-Interscience Publication, John-Wiley & Sons., 4, *Therapeutic Agents*, 602 (1997).
92. Plempel, M., *et al.*, *Dtsch. Med. Wochenschr.*, 94, 1356 (1969) cited from 'Wilson and Giswold's Textbook of Organic Medicinal and Pharmaceutical Chemistry'; 9th edn., J.B. Lippincot and Company, Philadelphia, 150 (1991).
93. Godefroi, E.F., *et al.*, *J. Med. Chem.*, 12, 784 (1968).
94. Thomas, A.H., *J. Antimicrob. Chemother.*, 17, 269 (1986).
95. Gold, W., *et al.*, *Antibiotics Annual*, 576 (1955-56), New York Medical Encyclopedia (1956) cited from 'Wilson and Giswold's Textbook of Organic Medicinal and Pharmaceutical Chemistry'; 9th edn., J.B. Lippincot and Company, Philadelphia, 151 (1991).

96. Hazen, E.L., Brown, R., *Pract. Soc. Exp. Biol. Med.*, 76, 93 (1951) cited from 'Wilson and Giswold's Textbook of Organic Medicinal and Pharmaceutical Chemistry'; 9th edn., J.B. Lippincot and Company, Philadelphia, 279 (1991).
97. Oxford, A.E., *et al.*, *Biochem. J.*, 33, 240 (1939).
98. Grove, J.F., *et al.*, *J. Chem. Soc.*, 3977 (1952).
99. Sloboda, R.D., *et al.*, *Biochem. Biophys. Res. Commun.*, 107, 882 (1982).
100. Kruse, R., *et al.*, *Pharmacology*, 43, 247 (1991) cited from William Foye's 'Principles of Medicinal Chemistry', 4th edn., A B.I. Wavrely Pvt. Ltd., New Delhi Publication, India, 811 (1995).
101. Ahluwalia, V.K., Singh, R.P. and Rishi Singh., *Indian J. Chem.*, 26(B), 297 (1987).
102. Burmistov and Tabovasky, R.G (To Ben Venue Lab Inc.) *U.S Patent* 3,278,372, Feb. 26, (1965); cited through C.A: vol: 65, 20068e (1966).
103. Prabhu, S.R., Lokhande, S.R., Bhamaria, R.P. and Khadse, B.G., *Indian Journal of Chemistry*, 26(B), 856 (1987).
104. Carp, E., Toma, A., 'Al I Cuza' Iasi. Sect; (1) 67 (1965), cited from CA., 105, 363,14843 (1965).
105. Gupta, S., Rani, S., *J. Ind. Chem. Soc.*; 55, 730 (1978).
106. Ayer, William, A. *et al*; *Tetrahedron*, 48(14), 2929-34 (1992).
107. Richardson, K., Brammer, K. W., Harriot, M. S. and Troke, P. F.; *Antimicrobial Agents Chemotherapy*, 27, 832 (1985).
108. Mohan, J., Kataria, S. and Anupama; *Indian Journal Of Heterocyclic Chemistry*, 8, 237-238 (1999).
109. Boulton, D. *et al*; *Eur. Pat. Appl. EP*, 1,69,502 (1986) cited through C.A: vol. 105, 60595a (1986).
110. Elguero, J. in *Comprehensive Heterocyclic Chemistry*; Katritzky, A. Ed.; Pergamon Press: Oxford 1984; vol. 5 pg. 291.
111. Elguero, J. in *Comprehensive Heterocyclic Chemistry*; Katritzky, A., Rees, C. W., Scriven, E. F. V. Eds.; Elsevier Science Ltd.: Oxford, 1996; vol. 3 pp 1-75.
112. Allan, V.; Deacon; *Organic Synthesis* vol. 2, 569 (1963).
113. Nakajima, Shotaro; Tonak Ichivo; Seki Teniya; Anmo Toxhlo; *Yakugaka Zasshi* 78, 1378-82 (1958), cited from C.A: vol. 53, 8124b (1959).
114. Balio; Alessandro; *Ricerca Sci.*; 20, 1268 (1950).

115. Dunn, B. G., Actor, P. and Dipasquo, V. J. *Journal of Medicinal Chemistry*, 19, 751-53 (1966).
116. Wada, Jin, Suzuki, Tadayuki, Miyamatsu, Hiroki, Ueno, Shinji, Shimizu, Mitsuhiro. *Ger. Offen Japan Pat.* 2,238,761 (1973) cited through C. A: vol. 78, 136265h (1973).
117. Popoff, Ivanc. , Buchholz, Bernard. , Miller. , Harold J. , *U.S Pat.* 3,519,630 (Jul. 1970) cited through C.A: vol. 73, 76154f (1970).
118. Tsoi, Z. I., Loi, N. P., Umarov, A. A., Ananeva, K. V., Zavyalova, L. V., C.A: vol. 85, 172661n (1976).
119. Sutoris, V., Foltinova, P. and Blockinger, G., *Chem. Zvesty.* , 1977, 31(1), 92-7 (Eng.); Cited through C.A: vol. 88, 89573b (1978).
120. Cited through C.A: vol. 94, 12152f (1981).
121. Freelanders, B. L. and French, F. A., *Proc. Soc. Exptl. Bio. Med.*, 66, 362-5(1947).
122. Volchel, C., Liebig's; *Ann. Chem.*, 38, 314 (1941).
123. Roumanian Ministry for Petro and Chem. Industry; *Brit. Pat.*, 980,997 (1965) cited from C.A., 63, 13221f (1965).
124. Fuches, O., Senkaiak, V., Nemes, A., Lazar, A. and Somgyl, T., *Australian Pat.*, 241461 (1965).
125. Kindler, K., Liebig's, *Ann. Chem.*, 431, 187 (1923).
126. Singer, F.A., Schafer, W. and Chrwitte, E., *Agrw. Chem.*, 76, 273 (1964).
127. Fairful, A.E.S., Lowe, J.L. and Peak, D.A., *J. Chem. Soc.*, 742 (1952).
128. Cairns, T.L., Larchar, A.W., McKusicj, B.C., *J. Org. Chem.*, 18, 748 (1953).
129. Hanford, W.E., *U.S. Pat.*, 2201170 (1940) cited from C.A., 34, 6388² (1946).
130. Von Esch, A.M. and Shermann, H.R., *U.S. Pat.*, 3158623 (1964) cited from C.A., 62, 4008 (1965).
131. Taylor, E.G. and Zaltewicz, J.A., *J. Am. Chem. Soc.*, 182, 2656 (1960).
132. Albert, A., *Ber. Dtsch. Chem. Ger.*, 48, 470 (1915).
133. Olin, J.F. and Johnson, T.B., *Recueil. Trav. Chim. Pay. Bas.*, 50, 72 (1931).
134. Goerdeler, J. and Mitter, W., *Chem. Ber.*, 96, 944 (1963).
135. Mayer, R., Hiller, G., Nitzchke, M. and Jentz, J., *Agrw. Chem.*, 75, 1011 (1963).
136. Casser, L., Panossian, S. and Giordana, C., *Synthesis*, 917 (1978).
137. Hofmann, H.W., *Ber. Dtsch. Chem. Ger.*, 11, 340 (1878).

138. Zaiden, Hogin, Seisan, Kaihatsu, Kagku, Kenyusho, *Jap. Pat.*, 9002 / 65 (1965).
139. Cerecedo, L.R. and Tolpin, J.G., *J. Am. Chem. Soc.*, 59, 1660 (1937).
140. Schmitz, W.R., *U.S. Pat.*, 2682558 (1954) cited from C.A., 49, 9029c (1955).
141. Westphal, K. and Andersag, H., *Canad. Pat.*, 392513 (1940) cited from C.A., 35, 1413⁵ (1940).
142. Behringer, H., Ruff, M. and Wiedmann, R., *Chem. Ber.*, 97, 1732 (1964).
143. Pesina, A.G., *Zh. Obshch. Khim.*, 9, 804 (1939).
144. Kingsberg, E. and Papa, D., *J. Am. Chem. Soc.*, 73, 4988 (1951).
145. Wagner, G. and Singer, D., *Z. Chem.*, 2, 306 (1962).
146. Willstaller, R. and Wirth, T., *Ber. Dtsch. Chem. Ger.*, 42, 1911 (1909).
147. Kikuchi, K., *Jap. Pat.*, 1710 (1951) cited from C.A., 47, 4908 (1953).
148. Jancevska, M., Jacopicin, K. and Hahu, V., *Croat. Chem. Acta.*, 37, 67 (1965).
149. Sachs, F. and Loevy, H., *Ber. Dtsch. Chem. Ger.*, 36, 585 (1976).
150. Eleftherious, Papadopoulos, P., *J. Org. Chem.*, 41(6), 962 (1976).
151. Sastry, S. and Kudav, V.A., *Ind. J. Chem.*, 18B(5), 455 (1971).
152. Hantzsch, A.R. and Weber, H.J., *Ber.*, 20, 3118 (1887).
153. Jones, E.R.H., Robinson, F.A. and Strachan, M.N., *J. Chem. Soc.*, 87 (1946).
154. Vernim, G. and Metzger, J., *Bull. Soc. Chim. France*, 2498 (1963).
155. Sarkis, G.V. and Agawe, S.A., *J. Chem. Eng. Data*, 18, 99 (1973).
156. Erlyenmeyer, H., Erne, M., *Helv. Chem. Acta.*, 31, 652 (1948).
157. Mathes, R.A., Gregory, J.T., *J. Am. Chem. Soc.*, 74, 3867 (1952).
158. Cook, A.H., Downer, J.D. and Heilbron, I., *J. Am. Chem. Soc.*, 1202 (1948).
159. Ochiai, E. and Nagasawa, F., *J. Pharm. Soc. Japan*, 59, 43 (1939).
160. Dahiya, R. and Pujari, H.K., *Ind. J. Chem.*, 25B, 966 (1986).
161. Durgayan, A.A., Titanyan, S.A. and Kazaryan, R., *Izvest Akad. Nauk. Armyarm SSR Khim Nauki*, 14, 165 (1961) cited from C.A., 56, 4741 (1962).
162. *U.S. Pat.*, 2,652,396.
163. Khanna, M.S., Chandra, P., Garg and Kapoor, R.P., *Ind. J. Chem.*, 32B, 364 (1993).
164. Hantzsch, A.R. and Weber, H.J., *Ber.*, 21, 938 (1888).
165. Vogel, A.I., '*Element Pract. Org. Chem.*', 4th edn., 848 (1984).
166. Robert Asies (1966), New Benzimidazoles Belg., 666,351, Jan. 3, 1966; cited from C.A., 65, 5465f (1966).

167. Helweg, A. (1962), Microbial breakdown of the fungicide Benomyl; *Soil Biol. Biochem.*, 4(3), 377-378 (1962).
168. a) Akinama, Sumiyuki, Dkude, Msayashi, Sato, Katsuo, Iwarbushi, Shizue., *Yakugaki Zasshi*, 88(6), 684-689 (1968).
b) Guwatieri, F., Brody, G., Fieldslell, A.H. and Skinner, W.A., *J. Med. Chem.*, 15(A), 420 (1972).
169. Fischer, O. and Voiel, O., *Ber.*, 38 (1905).
170. Philips, M.A., *J. Chem. Soc.*, pp 2820-2828 (1929).
171. Green, H. and Day, A.R., *J. Am. Chem. Soc.*, 64, 1167 (1942).
172. McKee, R. L. and Bost, R. W., *J. Am. Chem. Soc.*, 69, 471 (1947).
173. Sexton, W.A., *J. Chem. Soc.*, 303 (1942).
174. Simonor, A.N. and Izmailiskii, V.A., cited from C.A., 35, 2870 (1941).
175. Wright, J.B., *J. Am. Chem. Soc.*, 71, 2035 (1949).
176. Elderfield, R.C. and Kreysa, F.J., *J. Am. Chem. Soc.*, 70, 44 (1948).
177. Kym, O., *J. Prakt. Chem.*, 75, 323 (1907).
178. Minstry, S.M. and Guha, P.C., *J. Ind. Chem. Soc.*, 7, 793 (1930).
179. Omar, A., monsen, M.E., Habib, H.S. and Aboulwafa Omaina, M., *Synthesis*, 864-865 (1977), cited from C.A., 88, 89559b (1978).
180. Remick, A., 'Electronic Interpretation of Organic Chemistry', John Wiley & Sons Inc., New York, pg. 155 (1949).
181. Bywater, W.G., McGiverty, D.A. and Jense, M.D., *J. Pharmacol.*, 85, 14 (1945).
182. Kawaoka, Y., cited from C.A., 35, 2368 (1941).
183. Jacobson, P., *Ann.*, 287, 150-196 (1895).
184. Fischer, O. and Limmer, F., *J. Prakt. Chem.*, 74, 60 (1906).
185. Dziurzynski, M., *Chem. Zentr.*, 79, 948 (1908).
186. James, A.J. and Turner, E.E., *J. Chem. Soc.*, p 1515 (1950).
187. Allen, C.F.H., Bell, A. and Wilson, C.V., *J. Am. Chem. Soc.*, 66, 835 (1944).
188. Kym, O., *J. Prakt. Chem.*, 75(2), 324 (1907).
189. Billester, O. and Steiner, A., *Ber.*, 20, 229-231 (1887).
190. Everett, J.G., *J. Chem. Soc.*, 677 (1929).
191. Cope, A.C., *Org. Synth.*, 30; John Wiley & Sons Inc., New York, 56 (1950).
192. Lellmann, E., *Ann.*, 228, 244 (1885).

193. Dorofienko, G.N., Andreichikov and Trukhan; *G eE khim.*, Geterotki, Soedin, 1344 (1974), quoted from Squil & Patai, '*The Chemistry of Diazonium & Diazo group*' Part II, p 655.
194. Wilcox, T.H., *J. Am. Dyest. Rep.*, 55, 891 (1966).
195. Putler, R., George Thieme Verlag Stuttgart, Hauber-Weyl-Muller, *Methodender Organischem Chemie*, Vol X, Part III, 4th edn., p 16 (1965).
196. Schank, K., *Methodicum Chemicum*, Vol 6 (Ed. Zymalkowski, F), Academic Press, New York, San Fransisco, London, p 164 (1975), George Thieme publishers, Stuttgart (1975).
197. Putler, R., George Thieme Verlag Stuttgart, Hauber-Weyl-Muller, *Methodender Organischem Chemie*, Vol X, Part III, 4th edn., p 22 (1965).
198. a) Matrka, Z.M., Sagner, Z., Chmatal, V., Sterba, V. and Vesley, Mcoll, *Czech., Commun.*, 32, 1462 (1967).
 b) Putler, R., George Thieme Verlag Stuttgart, Hauber-Weyl-Muller, *Methodender Organischem Chemie*, Vol X, Part III, 4th edn., p 12 (1965).
199. a) Fierz-David, H.E. and Blangley, L., *Grundlegende Operationen der Farbenchemie*, 8th edn., Springer Verlag Wien, p 224 (1952).
 b) Putler, R., George Thieme Verlag Stuttgart, Hauber-Weyl-Muller, *Methodender Organischem Chemie*, Vol X, Part III, 4th edn., p 22 (1965).
200. *Brit. Pat.* 741,228 [C.A., 50, 9913 (1956)]; *Brit. Pat.* 736,568 [C.A., 50, 13097 (1956)].
201. Putler, R., George Thieme Verlag Stuttgart, Hauber-Weyl-Muller, *Methodender Organischem Chemie*, Vol X, Part III, 4th edn., p 28 (1965).
202. Teuber, H.J., *Chem. Ber.*, 98, 2111 (1965).
203. Uddo, *Gazz. Chim. Ital.*, 50, 11,258 (1920).
204. De and Dutt, *Indian Chem. Soc.*, 7, 473 (1930).
205. Backer and Meyer, *Rec. Trav. Chim.*, 45, 82 (1926).
206. Vol VI, 6. Practical Organic Chemistry, pg 882.
207. Ingold, C.K., *Chem. Rev.*, 15, 265 (1934).
208. Whel and C.W., *J. Am. Chem. Soc.*, 64, 900 (1949).
209. Melander, L., *Nature*, 163, 599 (1949).
210. Bowman, R.M., Steele, R.E., Browne, L. (Ciba Geigy Corp.) *U.S. Pat.* 4,978,672 (Cl. 514-383, A 61K31/41), (1990).
211. a) Brown, F.C., *J. Org. Chem.*, 1959, 24, 1056.

- b) Bullock, M.W., *J. Am. Chem. Soc.*, 1956, 78, 5854.
- c) Moyer Hundsberger, *J. Chem. Soc.*, 1956, 21, 394.
- 212. a) Chattaway, F.D. *et al*, *J. Chem. Soc.*, 1915, 107, 32.
- b) Carlin, R.B. *et al*, *J. Am. Chem. Soc.*, 1948, 70, 3421.
- d) Bullock, M.W. *et al*, *J. Am. Chem. Soc.*, 1956, 78, 5854.
- 213. Ochiai and Katada, *J. Pharm. Soc.*, Japan, (1940) 60, 211, 543.
- 214. Boggust and Cocker, *J. Chem. Soc.* (1949) 355.

Appendix

APPENDIX

1. The melting points have been determined in open capillaries on a Thermo-nik Melting Point Apparatus (Campbell Electronics, Mumbai, India) and are uncorrected.
2. Thin Layer Chromatography of the compounds have been run on E. Merck per coated silica gel plates of type 60 F 254.
3. Elemental Analysis of the compounds was carried out at SAIF (Sophisticated Analytical Instruments Facility), IIT, Powai, Mumbai.
4. Infrared spectra were recorded on a BOMEM MB-104 Fourier Transform Infrared Spectrometer.
5. PMR was recorded on JEOL 300 MHz spectrometer using TMS as internal reference at Institute of Science, Mumbai.

Synopsis

SYNOPSIS
OF THE THESIS TO BE SUBMITTED TO
THE UNIVERSITY OF MUMBAI
FOR THE DEGREE OF
MASTERS OF SCIENCE
IN THE FACULTY OF ORGANIC CHEMISTRY.

TITLE OF THE THESIS	: Synthesis and study of substituted pyrazoles and triazolo thiazoles as possible antimicrobial agents.
NAME OF THE CANDIDATE	: Mr. Ameya Gajanan Yadav.
QUALIFICATION	: B.Sc. Chemistry (March 2005).
NAME AND DESIGNATION OF THE RESEARCH SUPERVISOR	: Dr. Anil S. Bobade. (Scientific Officer)
PLACE OF RESEARCH	: Department of Chemotherapy Haffkine Institute for Training, Research and Testing. Parel, Mumbai-12.
NUMBER AND DATE OF REGISTRATION	: 19, Dec. 07, 2006.
DATE OF SUBMISSION	: 5/12/2008


**SIGNATURE OF
STUDENT**


**SIGNATURE OF THE
RESEARCH SUPERVISOR**

Introduction

Van Leeuwen Hoek, following his experiments with the microscope, first identified bacteria in the 1870s. However, it was not until the 19th century that their link with disease was appreciated. During the later half of the 19th century scientist such as Koch were able to identify the microorganisms responsible for communicable disease such as tuberculosis, cholera and typhoid. However, Paul Ehrlich switched the direction and entered the field, which he defined as “Chemotherapy”. This change introduced a number of antitubercular and antimicrobial agents, which are being used today. However, there is a constant need for new drugs due to resistance exhibited by various organisms.

Bacteria are evolving and acquiring resistance hence, antimicrobials with greater efficacy, lesser side effects and broad spectrum of activities is a challenge. Medicinal chemistry, an extension of organic chemistry, not only deals with the synthetic aspect of drug but also the biological aspect of it. One aspect of medicinal chemistry deals with the incorporation of newly emerged pharmacophore on already existing biologically active moieties to produce newer molecules with higher efficacy. These and other reasons inspired the present research work to synthesize certain novel heterocyclic compounds.

Heterocyclic compounds have been proved as invaluable in the field of medicinal chemistry. Several heterocyclic derivatives find important applications in the compounds of therapeutic value. The benzimidazoles are synonymous with antihelmintic agents; thiazoles are useful for several purposes ranging from antibacterial to immunosuppressive agents. Pyridines are found in antiulcer and antitubercular compounds, while isoxazoles find application in penicillin's and semi synthetic antibiotics. The semi synthetic pharmaceutical product such as antibiotics of the

cephalosporin series like ceftizoxime contains thiazole molecule. The list could be endless.

Microbial infections have tormented mankind in various forms since ancient times. Tuberculosis and leprosy are well documented since the biblical era. As discussed above heterocyclic derivatives play an important role in control of various microbial diseases and hence, in this present study a new series of heterocyclic agents have been synthesized and studied for their properties as antibacterial agents.

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 2-(1H- Benzimidazol-2-yl mercapto) -N- (1,5-Dimethyl-2- substituted phenyl-3- oxo-2,3- dihydro-1H-Pyrazol-4-yl) acyl amine.

PART 4 :- Synthesis of 2-(1,3- Benzothiazol-2-yl mercapto) -N- (1,5-Dimethyl-2- substituted phenyl-3- oxo-2,3- dihydro-1H-Pyrazol-4-yl) acyl amine.

PART 5 :- Synthesis of 2-(1,3- Benzothiazolyl-2-amino) -N- (1,5-Dimethyl-2- substituted phenyl-3- oxo-2,3- dihydro-1H-Pyrazol-4-yl) acyl amine.

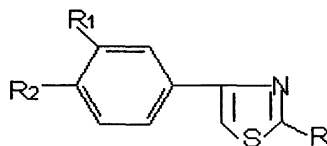
PART 6 :- Biological screening, Results and Discussion.

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** and 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.

Sulphur and nitrogen containing heterocycles in general are shown to possess various biological activities ¹. The thiazole ring was first described by Hantzsch and Waber has been recognized as an important pharmacophore for its various applications in the field of medicinal chemistry. The potentialities of the thiazole molecule as well as versatile use of it in therapeutically important compounds have resulted in several outstanding pharmaceutical products, such as sulphathiazole, tetramisole, promizole and semi-synthetic antibiotics of the cephalosporin series like ceftizoxime.

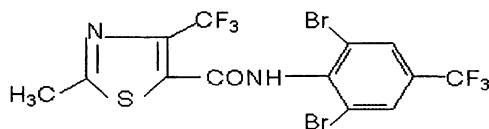
Burmistov et al have reported substituted thiazole derivatives with antibacterial and antifungal activities ². Also, 2,4-disubstituted thiazoles (I) are known for their antitubercular activity ³.



(I)

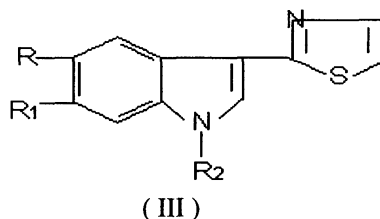
R= Subs. Aryl, R₁= NO₂, R₃= Heteroaryl.

In 1992, Shell Research Centre ⁴ reported thiazole derivative (II) with a fluoro substituent possessing excellent anti fungal activity.



(II)

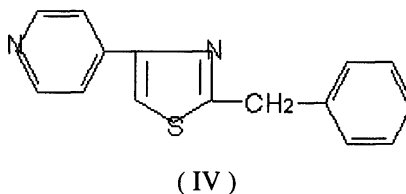
It has also been reported that thiazoles (III) bearing other heterocyclic ring systems possess good antifungal activity ⁵.



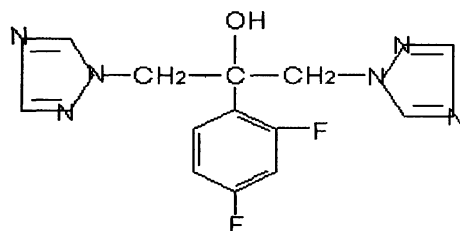
R=R₂= H, R₁= CH₃O.

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

Boulton, D *et al* ⁶ synthesized 2-(benzyl)-4-(4'-pyridyl) thiazole (IV) with methyl phenyl substituent at 2-position of 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.



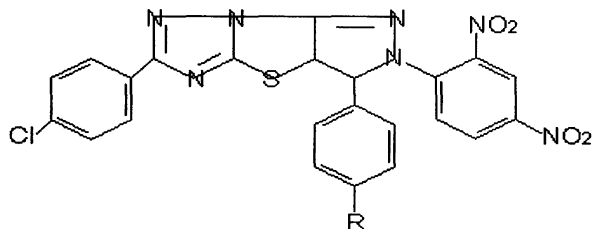
1,2,4-triazole is well known moiety exhibiting antifungal activity the facts ascertained by its presence in the recently developed broad-spectrum antifungal drug, Fluconazole ⁷ (V).



(V)

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

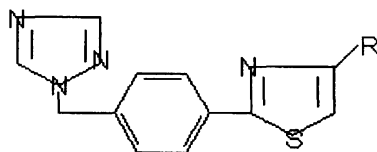
Jagmohan et al have synthesized pyrazolo [3',4' : 4,5] thiazolo [3,2-b]-s-triazoles (VI) which were found to exhibit both antibacterial and antifungal activity⁸.



R= OCH₃, Cl

(VI)

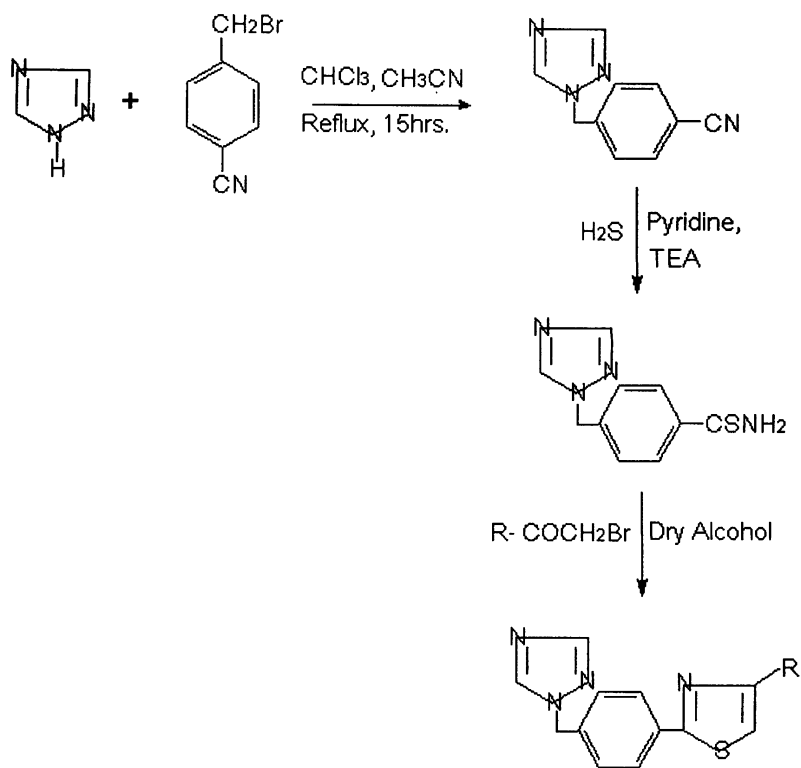
Hence, taking into considerations above all given facts it was planned to synthesize, 2,4-disubstituted thiazole as the parent moiety with 1,2,4-triazolyl-1-benzyl-substitution at its 2-position and various substituted phenyl substituents would be introduced at its 4-position. A total of seven compounds were synthesized as per the method outlined in scheme I. The final compounds in the series will have the following structure (VII).



(VII)

R= Substituted phenyl / heterocycle.

Scheme I

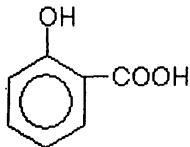


R=Substituted phenyl/heterocycle.

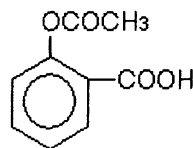
PART 3 :- Synthesis of 2-(1H- Benzimidazol-2-yl mercapto) -N- (1,5-Dimethyl-2- substituted phenyl-3- oxo-2,3- dihydro-1H- Pyrazol-4-yl) acyl amine.

Pyrazoles are an important class of heteroaromatic ring systems that find extensive use in the agrochemical industry⁹ and pharmaceutical industries¹⁰. After the discovery of the antipyretic action of salicylic acid in 1875, many phenol and aniline derivatives were tried. *Antipyrine* (antifebrine) was among first of this group, later came acetanilide, acetophenetidin (phenacetin), acetyl salicylic acid (aspirin), aminopyrine (pyramidon), cinchophen (atophan) and neocinchophen (tolysin). The formulas disclose the structure of the important compounds.

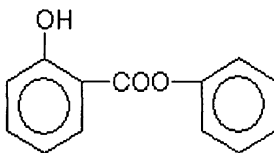
Salicylates



Salicylic acid

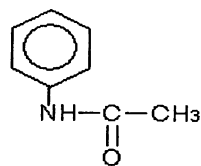


Acetyl salicylic acid

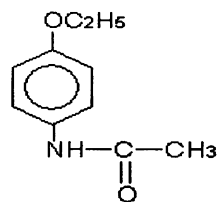


Phenyl salicylate (Salol)

Aniline derivatives

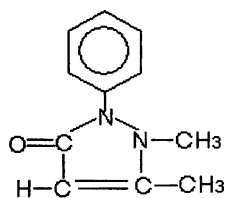


Acetanilide

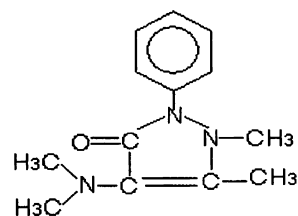


Acetophenetidin

Pyrazolon derivatives

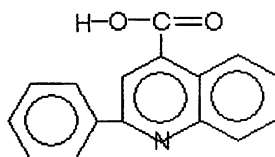


Antipyrine

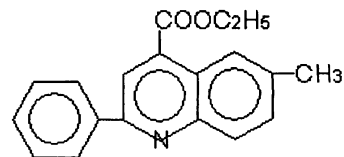


Aminopyrine

Quinoline derivatives



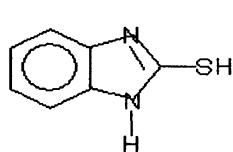
Cinchophen



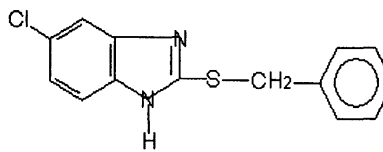
Neocinchophen

Benzimidazole is one of the oldest and chemically well studied nitrogen heterocycles, it has quietly recognized as the versatile nucleus for building molecules possessing broad spectrum of biological activities such as powerful antihelmintic, antimicrobial, pesticidal, antimalarial, anticancer and pharmacological activities.

During the literature survey, it was found that 2-mercapto benzimidazole (VIII) itself is a bacteriostatic and antifungal agent¹¹, also its derivatives¹² namely 5(6)-chloro-2-(benzylthio) benzimidazole (IX) posses antifungal activity against *Trichophyton interdigitale*, at a dilution 1:290,900 or 3.4µg/ml. Similarly, bacteriostatic activity was also reported for 2-mercapto benzimidazole against *S. aureus*¹³.

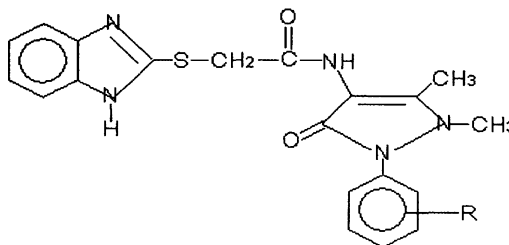


(VIII)



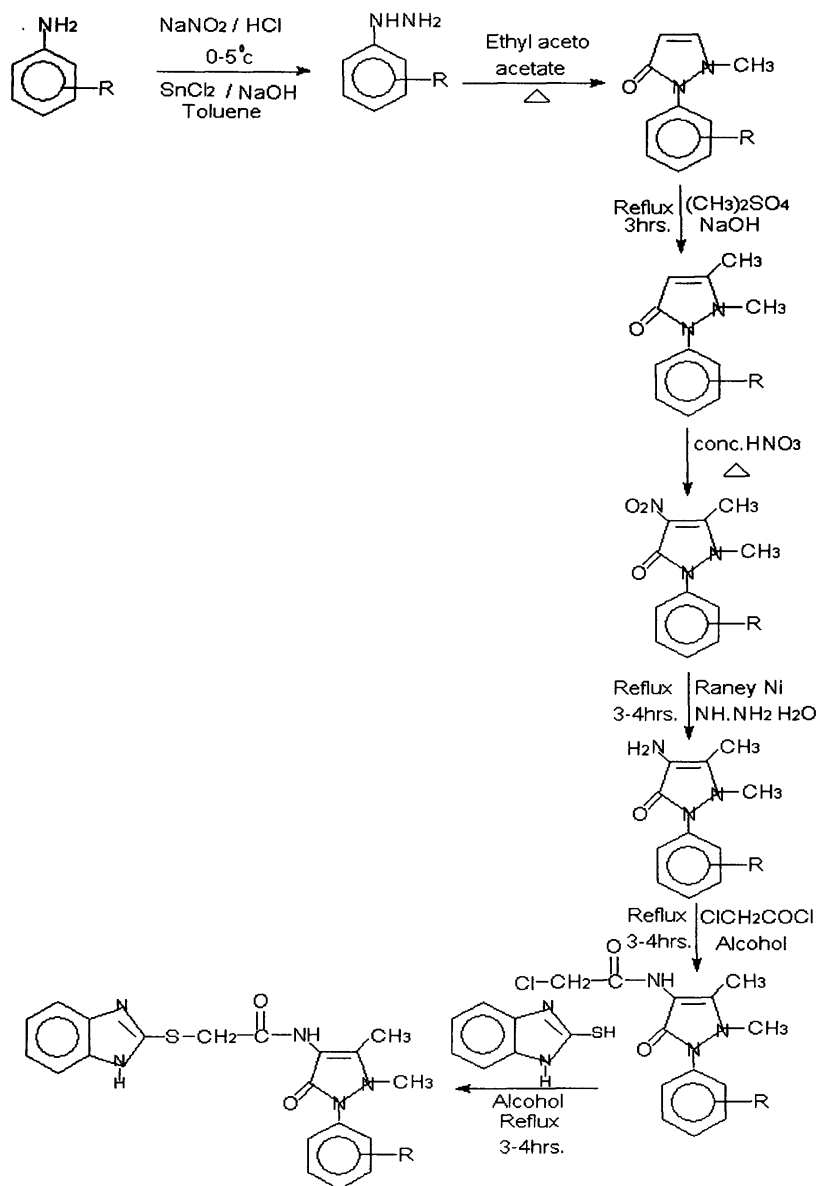
(IX)

Hence, taking into considerations above all facts mentioned above the fusion of 2-mercapto benzimidazole and pyrazolone derivative ie. Antipyrine containing different substituents in phenyl ring of it was planned and synthesized. The compounds were synthesized as per the method outlined in Scheme-II. The final compound from this series will have the following structure (X).



R= 3-chloro, 2,4-dichloro, etc. (X)

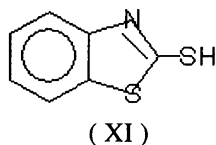
Scheme II



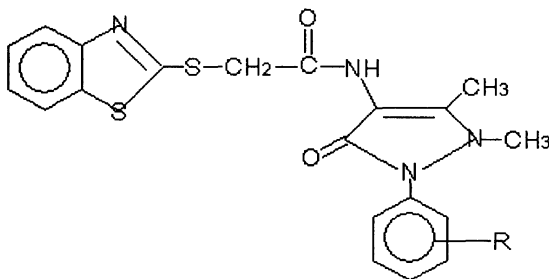
R = 3-chloro, 2,4-dichloro, etc.

PART 4 :- Synthesis of 2-(1,3- Benzothiazol-2-yl mercapto) -N- (1,5-Dimethyl-2- substituted phenyl-3- oxo-2,3- dihydro-1H-Pyrazol-4-yl) acyl amine.

Variety of benzothiazole derivatives are known in the literature for their prodigious range of activities, particularly they are more valuable as antiparasitic¹⁴, anti-inflammatory¹⁵, fungicidal¹⁶, herbicidal¹⁷ and antibacterial¹⁸ agents. A mercapto group is an important toxophore responsible for pesticidal activity¹⁹. Therefore, in this series a combination of 2-mercapto benzothiazole (XI) with pyrazolone derivative mentioned in part-3 was planned.

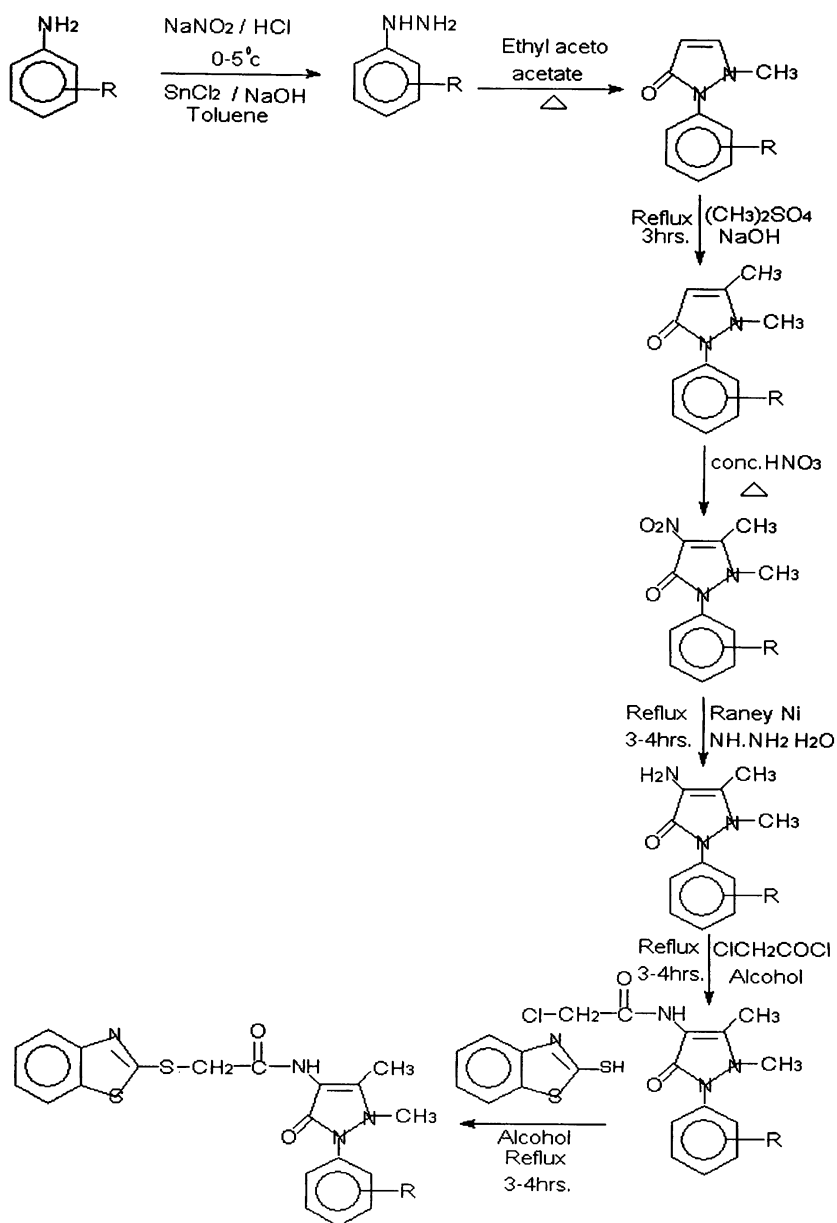


Thus the final compound (XII) from this series will have the structure mentioned below and the method used for synthesis is outlined in Scheme-III.



R= 3-chloro, 2,4-dichloro, etc. (XII)

Scheme III

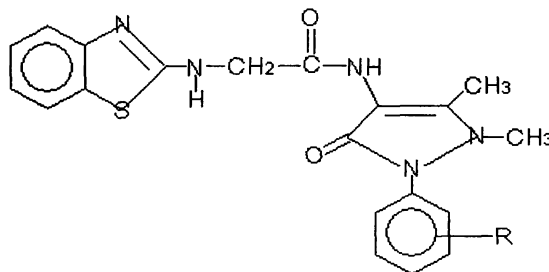
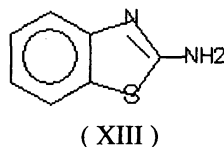


R= 3-chloro, 2,4-dichloro, etc.

PART 5 :- Synthesis of 2-(1,3- Benzothiazolyl-2-amino) -N- (1,5-Dimethyl-2- substituted phenyl-3- oxo-2,3- dihydro-1H-Pyrazol-4-yl) acyl amine.

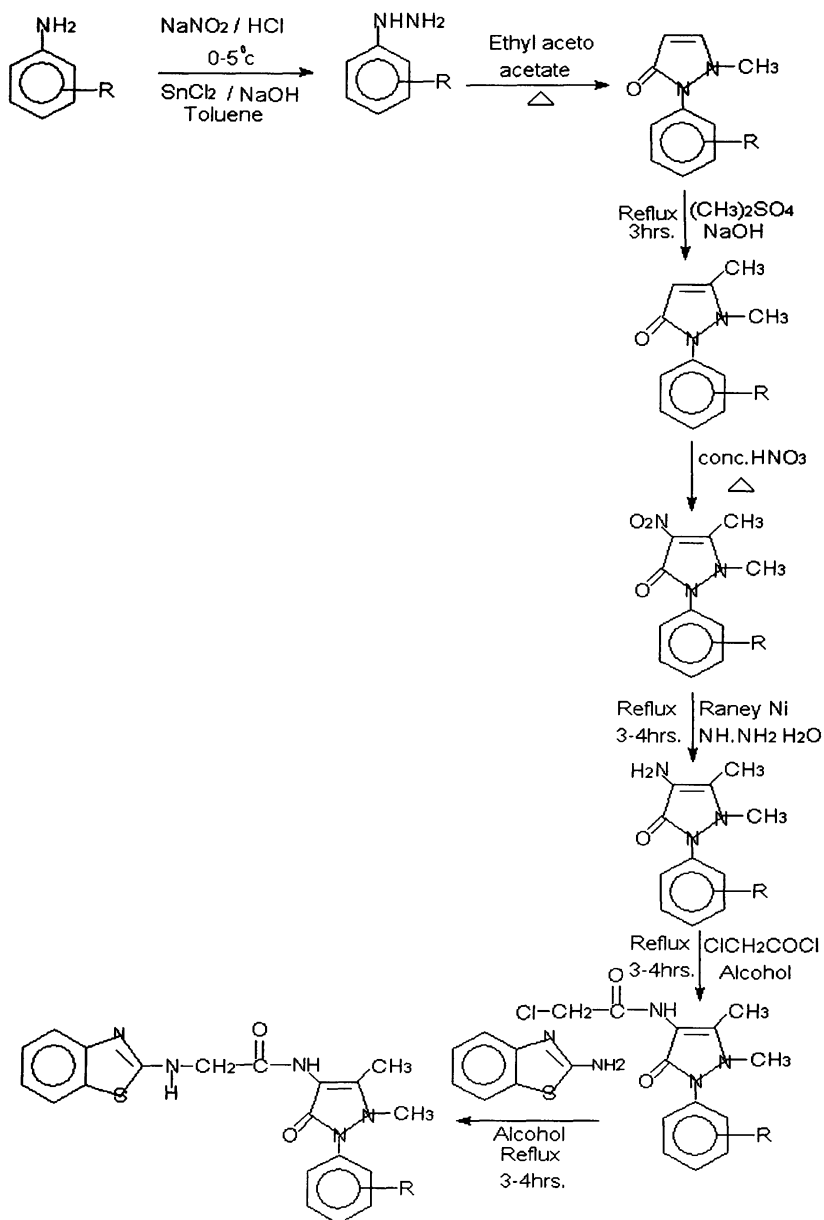
The literature survey revealed that 2-amino-6- substituted benzothiazole are known to exhibit excellent antitubercular activity. For example, some 6- substituted- 2- amino benzothiazoles show antitubercular activity at the dose level of 0.025 to 1.0 µg/ml *in vitro* testing²⁰.

The fact mentioned in part-3 about pyrazolone derivative and as discussed above the activity of substituted 2- amino benzothiazole the fusion of 2-amino benzothiazole (XIII) and pyrazolone derivative was planned and compounds were synthesized. The final compound was synthesized using the method outlined in Scheme IV. The final compound (XIV) synthesized will have the following structure.



R= 3-chloro, 2,4-dichloro, etc. (XIV)

Scheme IV



R= 3-chloro, 2,4-dichloro, etc.

PART 6 :- Biological screening, Results and Discussion.

All the compounds from series I, II, III and IV were synthesized and were confirmed by Thin Layer Chromatography (TLC), Carbon Hydrogen Nitrogen (CHN) analysis, Nuclear Magnetic Resonance (NMR) Spectroscopy, Infra-Red (IR) Spectroscopy and same compounds were subjected to microbiological screening, which involves anti-bacterial and anti-fungal screening.

Anti-bacterial screening:

The compounds synthesized were 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 Broth²¹. The standard drugs used were Ampicillin (MIC 0.1µg /ml) against *S. aureus* and Trimethoprim (MIC 0.1µg/ ml) against *S. typhi*.

Anti-fungal screening:

Compounds reported in series I, II, III and IV were also screened for their anti-fungal activity by tube dilution technique²² against fungal strains viz. *Candida albicans*.

In different screening methods, the standard drugs employed were trimethoprim as general anti-bacterial agent and miconazole as an anti-fungal agent.

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

REFERENCES

1. S.S.Tiwari, K.Sengupta and Kumar.J. Indian Pharm. 1970, 32, 91.
cited from C.A: vol: 44, 59191 (1950).
2. Burmistov and Tabovasky, R.G (To Ben Venue Lab Inc.) U.S
Patent 3,278,372, Feb. 26, (1965); cited through C.A: vol: 65,
20068e (1966).
3. Prabhu, S.R., Lokhande, S.R., Bhamaria, R.P. and Khadse, B.G.,
Indian Journal of Chemistry 26(B) 856 (1987).
4. Shell Research; Chem. and Ind., 7, 893 (1992).
5. Ayer, William, A. et al; Tetrahedron, 48(14), 2929-34 (1992).
6. Boulton, D. et al; Eur. Pat. Appl. EP, 1,69,502 (1986) cited through
C.A: vol. 105, 60595a (1986).
7. Richardson, K., Brammer, K. W., Harriot, M. S. and Troke, P. F.;
antimicrobial Agents Chemotherapy, 27, 832 (1985).
8. Mohan, J., Kataria, S. and Anupama; Indian Journal Of
Heterocyclic Chemistry, 8, 237-238 (1999).
9. Elguero, J. in Comprehensive Heterocyclic Chemistry; Katritzky,
A. Ed.; Pergamon Press: Oxford 1984; vol. 5 pg. 291.
- 10.Elguero, J. in Comprehensive Heterocyclic Chemistry; Katritzky,
A., Rees, C. W., Scriven, E. F. V. Eds.; Elsevier Science Ltd.:
Oxford, 1996; vol. 3 pp 1-75.
- 11.Allan, V.; Deacon; Organic Synthesis vol. 9, 569 (1963).
- 12.Nakajima, Shotaro; Tonak Ichivo; Seki Teniya; Anmo Toxhlo;
Yakugaka Zasshi 78, 1378-82 (1958), cited from C.A: vol. 53,
8124b (1959).
- 13.Balio; Alessandro; Ricerca Sci.; 20, 1268 (1950).
- 14.Dunn, B. G., Actor, P. and Dipasquo, V. J. Journal of Medicinal
Chemistry, 19, 751-53 (1966).

- 15.Wada, Jin, Suzuki, Tadayuki, Miyamatsu, Hiroki, Ueno, Shinji, Shimizu, Mitsuhiro. Ger. Offen Japan Pat. 2,238,761 (1973) cited through C. A: vol. 78, 136265h (1973).
- 16.Popoff, Ivanc. , Buchholz, Bernard. , Miller. , Harold J. , U.S Pat. 3,519,630 (Jul. 1970) cited through C.A: vol. 73, 76154f (1970).
- 17.Tsoi, Z. I., Loi, N. P., Umarov, A. A., Ananeva, K. V., Zavyalova, L. V., C.A: vol. 85, 172661n (1976).
- 18.Sutoris, V., Foltinova, P. and Blockinger, G., Chem. Zvesty. , 1977, 31(1), 92-7 (Eng.); Cited through C.A: vol. 88, 89573b (1978).
- 19.Cited through C.A: vol. 94, 12152f (1981).
- 20.Freelanders, B. L. and French, F. A. Proc. Soc. Exptl. Bio. Med., 66, 362-5(1947).
- 21.Konemann E.V; Janda W.M; Allen S. D; Sommers K. M; Dowell V. R; Winn W. C; 'Color Atlas and Textbook of Diagnostic Microbiology'. J.B. Lippincott company, Third Edtn. 487 (1988).
- 22.E. Joan Stokes, Clinical Bacteriology Fourth Edtn. 226 (1955).
