

**SYNTHESIS OF ORGANIC COMPOUNDS OF POSSIBLE
THERAPEUTIC VALUES AND PREPARATION OF A DRUG
INTERMEDIATE**

**THESIS SUBMITTED
UNIVERSITY OF MUMBAI**

**FOR THE DEGREE OF
MASTER OF PHARMACEUTICAL SCIENCES**

**SUBMITTED BY
SHARVANI K. VINGKAR**

**UNDER THE GUIDANCE OF
DR. B.G. KHADSE**

**HAFFKINE INSTITUTE FOR TRAINING, RESEARCH & TESTING
MUMBAI – 400 012**

Disclaimer

This research work belongs to

Sharvani K. Vingkar

No content of this work can be
circulated or used for any
commercial purpose without the
consent of respective researcher

**Synthesis of Organic Compounds of Possible Therapeutic
Values and Preparation of A Drug Intermediate.**

**A
Thesis Submitted
to
The University of Mumbai
for The Degree of
Master of Pharmaceutical Sciences
in The
Faculty of Technology**

**by
Sharvani K. Vingkar
B. Pharm.**

**Department of Chemotherapy,
Haffkine Institute for Training, Research and Testing,
Acharya Donde Marg, Parel, Mumbai - 400012.
2000**

HAFFKINE INSTITUTE LIBRARY,
MUMBAI.

Call No.
Price

Acc No.
Source Purchased.
Donated

438

Statement

Statement submitted under rule 0.412.

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

Statement submitted under rule 0.413.

Statement I:

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

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

Statement II:

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

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

Dr. B.G. Khadse



Signature of Research Guide

Ms. Sharvani Vingkar



Signature of Research Student:

:Contents:

	Page no.
Acknowledgement Statement	
Part I-A:	
Introduction	1-4
Review of Literature	5-39
Plan of Work	40-51
Part I-B:	
Chemistry of Diazonium salts	53-57
Hydrazones	58-61
Friedel-Craft's acylation to form ketone, Cinnoline and cinnlone	62-67
Halogenation of ketones	68-71
Nitriles	72-79
Thioamides	80-85
Thiazoles	86-91
Part I-C:	
Schematic Representation	92-99
Part II: Synthesis of 3-(2-alkyl/ aryl,4-substituted thiazolo) -6-chloro-cinnoline-4-ones:	
Synthesis of ethyl 2(4-chlorophenyl hydrazono)-3-oxybutyrate	102
Synthesis of 3-acetyl-6-chloro cinnoline-4-one	102
Synthesis of 3-bromoacetyl-6-chloro cinnoline-4-one	103
Synthesis of 4-amino methyl benzonitrile HCl	103
Synthesis of N,N-dimethyl amino benzonitrile	104
Synthesis of thioamides	105
Synthesis of thiazoles	107
Part III: Synthesis of 3-(2-alkyl/ aryl,4-substituted thiazolo) -6-fluoro-cinnoline-4-ones:	
Synthesis of ethyl 2(4-fluorophenyl hydrazono)-3-oxybutyrate	110
Synthesis of 3-acetyl-6-fluoro cinnoline-4-one	110
Synthesis of 3-bromoacetyl-6-fluoro cinnoline-4-one	111
Synthesis of thiazoles	111-112
Part IV: Synthesis of Intermmediate for Letrozole	
Synthesis of 1-formyl-3-thiosemicarbazide	115
Synthesis of 1H-1,2,4-triazolo-3(5)-thiol	115
Synthesis of 1H-1,2,4-triazole	115

Synthesis of 1,2,4-triazolyl-methyl benzonitrile	116
--	-----

Part V:

Biological screening and Results	117-124
---	---------

Conclusion	125-126
-------------------	---------

References	127-139
-------------------	---------

Appendix

Synopsis

: ACKNOWLEDGEMENT :

I take this opportunity to express my immense sense of gratitude to my guide, Respected Dr. B.G.Khadse, Director, Haffkine Institute, whose valuable guidance and encouragement made me carry out my research work. I have always been benefitted from his teaching and constant counselling. It gives me great pleasure to recollect my association with him as his student.

I am thankful to Dr. S.V.Gadre, Asst. Director, Haffkine Institute, for his valuable assistance and help.

I am profoundly grateful to Dr. A.S.Bobade, Department of Chemotherapy, for his ever-helping attitude and kind co-operation during this research work.

I express my very special and sincere thanks to Ms. Bharati Kulkarni, Senior Scientific Officer, Department of Chemotherapy, for reviewing the manuscript and important discussions and encouragement.

I owe special thanks to Ms.P.A.Kirtikar, Mr.L.G.Mistry and Mr.Pramod Andhere, for carrying out elemental analysis and Mr.K.R.Pawar for his help during antimicrobial screening of the compounds.

With great pleasure I acknowledge the entire staff of the Department of Chemotherapy, for their help.

I am grateful to Respected Dr. Ambaye, Department of Biochemistry, for reviewing the manuscript and his critical suggestions which enabled me to make this thesis more acceptable.

I extend my appreciation to Dr.S.B.Dholam, Department of Bioavailability, for his kindness and help during the typing of the thesis. I also thank Ms.Preeti Lali and Ms.Sarita Chaudhari for their help.

I sincerely thank Ms.M.V.Joshi and the entire library staff for maintaining excellent library facilities.

I also thank the authorities of 'Animal House', HBPCCL, for providing material as per the requirements during the antimicrobial screening of the compounds.

I am grateful to the NMR section, RSIC, IIT, Powai for their prompt assistance in providing the NMR spectras. I am also thankful to Dr. Aravind Manian for his help for the interpretation of the spectras.

Last but not the least, I would like to acknowledge my senior colleagues, Pallavi Kharkar, Sonali Wayangankar, Chandan Jagtap, Sandeep Paranjape, Hemant Narkhede, Ravi Chambhare, for their valuable suggestions and guidance during the practical work.

I experience a feeling of great delight to be associated with my colleagues, Sagar Agnihotri, Chandrakant Bonde, Vinod Rane, Yogesh Kulkarni, Tushar Lokhande, Sunil Narkhede, Anagha Samant, Mukta Bohra and Jaya John who always maintained a healthy working atmosphere and helping attitude, making my task easier. I'll always appreciate Ms. Rucha Sharma, Department of Virology, for her moral support and encouragement.

I am indebted to my parents and brother, whose patience, inspiration and support enabled me to carry out this entire work.

Above all, I thank God Almighty for His blessings and providing me the strength to survive through the most difficult times.

- Sharvani Vingkar.

Introduction

INTRODUCTION:

'No scientific discipline is currently as hotly pursued as chemistry; if one not compelled, therefore, to apply its astounding results to man?..... Chemists concentrate the fragrance of cinnamon into a few drops and distill from treacherous poison and innocent plants---- the amazing medications. Yet there is more to come. If chemistry keeps its promises.... whatever is alive, will be perfect; and, in the end man will live for centuries, free from pain and sufferings, in indestructible health, until he dissolves serenely, in peace.'

-- JOHANNES GAUB (1734).

Drug discovery has its roots in the beginning of mankind. Even our polygenetic cousins, the chimpanzees have been observed to chew certain leaves to alleviate gastrointestinal distress. Likewise, in the evolution of early hunter gatherer communities, it was only a short step from gathering vegetation to be eaten to assuage the hunger pangs, to gathering material to be ingested to diminish the pain and discomfort from illness and injury.

Medicinal chemistry concerns the discovery, the development, the identification and the interpretation of mode of action of biologically active compounds at molecular level. Thus medicinal chemistry covers the following three critical steps.¹

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

To identify the lead molecule, several approaches are undertaken.

1) Random Approach:

Although the search for new entities is being increasingly governed by rational and high throughput techniques, serendipitous discoveries do still occur, as in old days of medieval herbs.

2) Non-Random Approach:

Compounds uncovered under random screening or compounds containing functional groups other than the lead molecule are tested selectively.

a) Drug Metabolism studies:

Drug metabolites are isolated and screened in order to determine if the activity observed is derived from the drug molecule or from a metabolite.

b) Clinical Observations:

When a drug exhibits more than one pharmacological action i.e. side-effects, then the compound can be used as a lead for secondary activity.

c) Lead Modification:

Synthetic derivatives and structural analogues of biologically interesting substances are tested for activities associated with the 'lead'. Such programs include the branching, lengthening or shortening of chain structure, the variation of the kind and position of substituents and other empirical molecular modifications within the framework of reasonably close analogy.

d) Structure Activity Relationship(SAR):

Structurally specific drugs act on specific receptor sites. Their activity and potency are very susceptible to small changes in chemical structure. Compounds with each type of activity eventually possess certain structural features in common.

Quantitative Structure Activity Relationship(QSAR): The concept of Quantitative drug design is based on the fact that biological properties of a compound are a function of its

physicochemical properties such as solubility, lipophilicity, electronic effect, ionization and stereochemistry etc.

Different methods used to correlate physicochemical properties with biological activity include

- 1)Hansch Analysis or Linear Multiple Regression Analysis.
 - 2)Free Wilson or *de Novo* method.
 - 3)Topliss orientation scheme.
 - 4)Enhancement factor.
 - 5)Batch selection method, Batchwise Topliss Operation Scheme, Cluster analysis etc.
- e)*Computer based QSAR:*

Free binding energy of compounds with the receptor is determined experimentally. Distance between various atoms in the molecule, its conformation and rotation about a single bond causing change in conformation and in distance are studied. Possible binding sites in terms of geometry and chemical characteristics of the site are deduced by computerised method with the help of above studies.

f)*Molecular Graphics:*

The rationale for identifying a lead compound has been based on a molecular understanding of the drug and its receptor. This is frequently accomplished by the use of three dimensional computer graphics representation to model the drug itself or the drug receptor complex.

Review of Literature

Antibacterial agents:

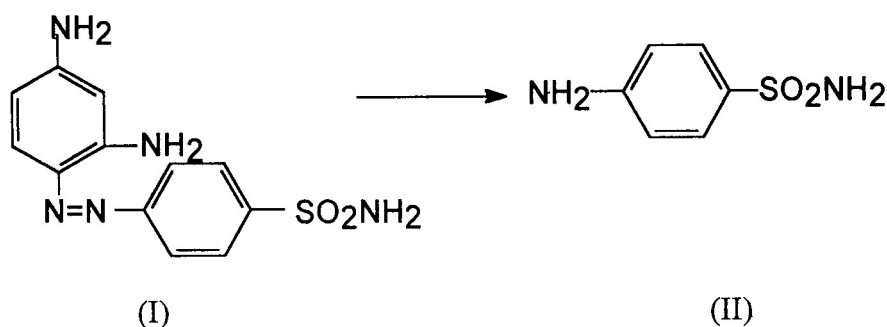
Bacterial infections have always remained a serious problem worldwide. The use of antimicrobial agents to control these infections is almost entirely a development of this century.

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

Systemic antibacterials include a number of classes as described below.

Sulfonamides:

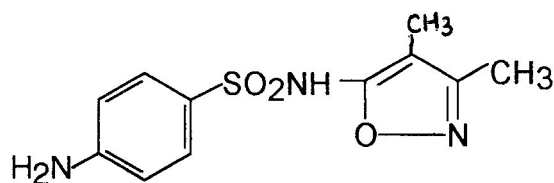
Sulfonamides were discovered in the middle 1930s. Protonsil rubrum(I), a red dye was observed by Domagk² to be taken up selectively by bacterial cells in case of streptococcal infection in mice and staphylococcal infection in rabbit. The dye killed the bacteria. Fractionation led to identification of an active substance known as amino benzene sulphonic acid(sulfanilamide)(II)³, a colourless cleavage product formed by liver metabolism of the drug which is responsible for the activity.



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

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 are safe for human cells.

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



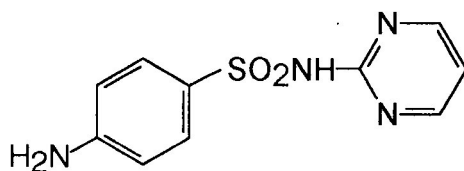
(III)

Sulfisoxazole(III)⁴ is one of the most popular sulfonamide 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 to other more preferred agents.

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

Plasmid mediated resistance is commonly developed, particularly among gram negative organisms, due to decreased sensitivity to dihydropteroate synthetase.

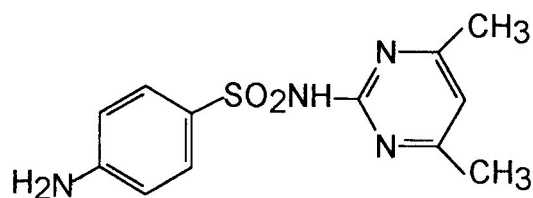
Superinfections, allergic rashes, photosensitivity and drug fever are common side effects. Hemolytic anaemia, liver and kidney damage, Steven-Johnson syndrome and ulcer of mucous membrane of eye, mouth, ureter occur occasionally.



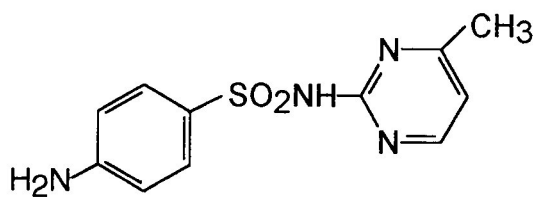
(IV)

Sulphadiazine(IV) has retained the preeminence 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 meningitis* involved is sulfonamide sensitive⁵.

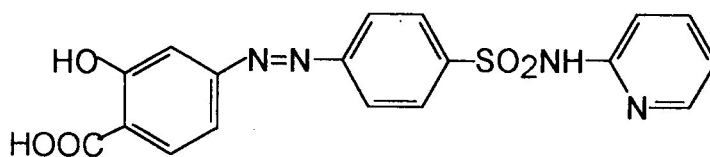


(V)



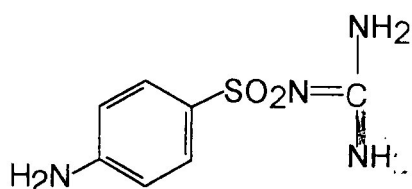
(VI)

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



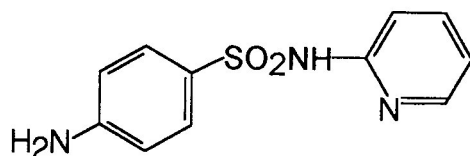
(VII)

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

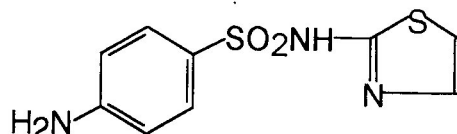


(VIII)

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

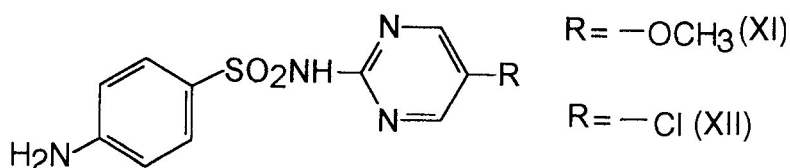


(IX)



(X)

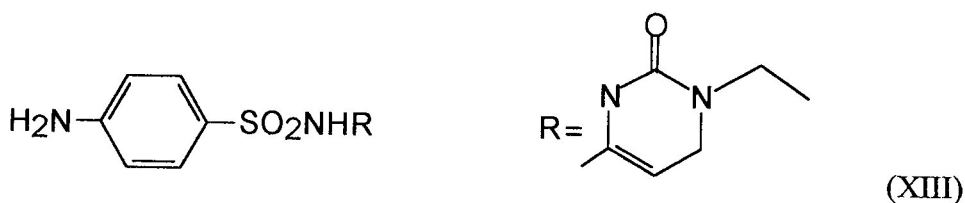
Sulphapyridine(IX)¹², reported in 1938, was in use for the treatment of pneumonia and remained as a drug of choice until it was replaced by sulphathiazole(X)¹³.

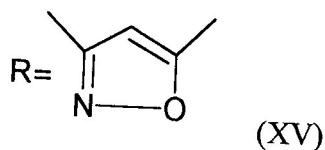
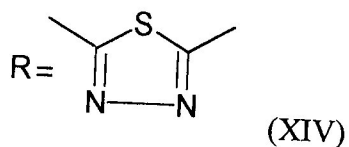


Long acting sulfonamides include Sulphamethoxypyridazine(XI)¹³, which has a half life of about 37 hours. Thus, it needs to be administered only once a day.

Sulphachlorpyridazine(XII) has a half life of 7 hours and is useful for urinary tract infection (UTI).

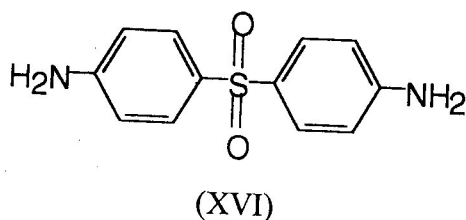
Other sulfonamides include Sulphacytin(XIII), Sulphamethizole(XIV), Sulphamethoxazole(XV) etc.



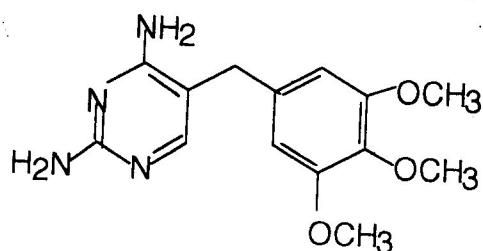


Sulphones:

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



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



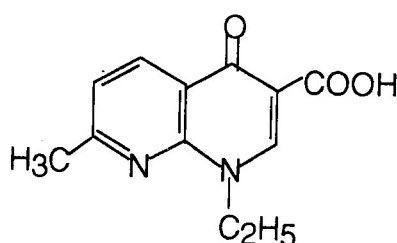
(XVII)

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

Side effects include megaloblastosis, leukopenia, thrombocytopenia, etc.

Quinolones:

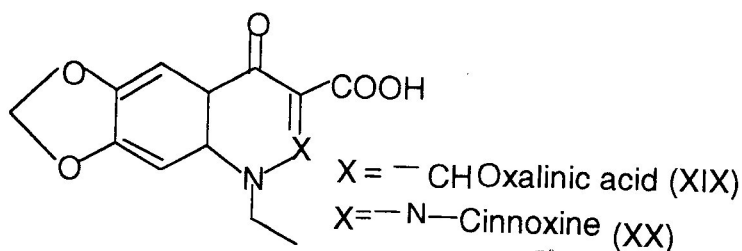
Quinolone antimicrobials comprise of a group of synthetic substances possessing in common an N-alkylated-3-carboxypyrid-4-one ring, fused with a second aromatic ring that carries other substituents.



(XVIII)

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

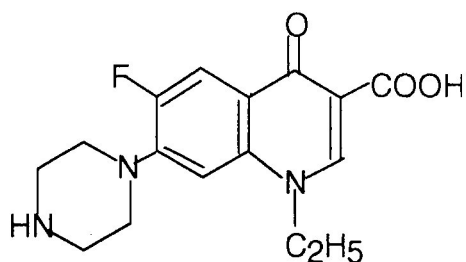
Over the following years, the remaining first generation quinolones were introduced¹⁷. These compounds consist of more potent agents with a better spectrum of activity, such as oxalinic acid (XX), cinnoxin (XXI) etc.



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

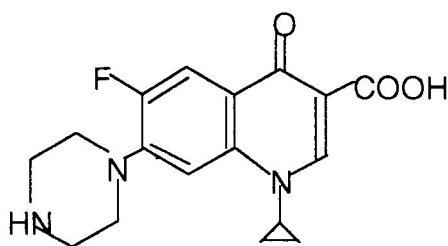
Gastro intestinal upset, skin rashes, visual disturbances and occasionally convulsions and photosensitivity are the adverse reactions observed.

Fluoroquinolones:



(XXI)

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



(XXII)

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

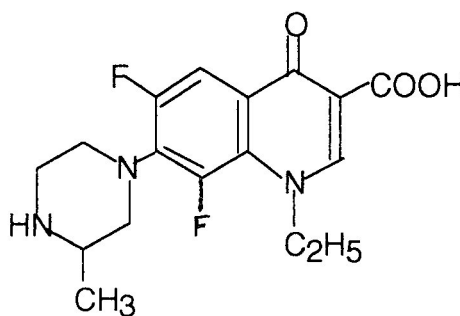
These drugs cause inhibition of enzyme DNA-gyrase that unwinds the supercoiled DNA helix prior to replication and transcription. This inhibition makes the cell DNA inaccessible and leads to cell death.

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

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

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

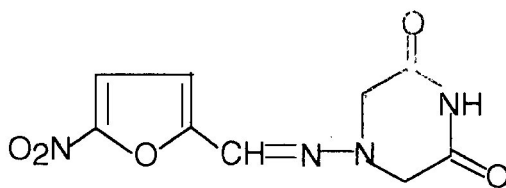
Lomefloxacin(XXIII), Ofloxacin etc. are the other agents in use.



(XXIII)

Nitrofurans:

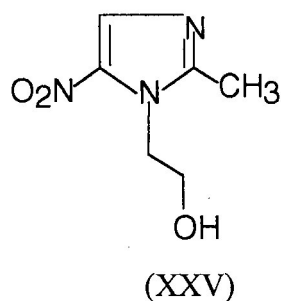
The first nitroheterocyclic compound to be introduced in chemotherapy were the nitrofurans. Nitrofurantoin(XXIV) is used widely for urinary tract infections(UTI).



(XXIV)

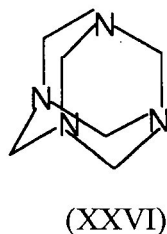
Nitrofurazine²⁰ and Furazolidone²¹ are other broad spectrum nitrofurans in clinical

use for prophylactic treatment of UTI, and they impair kidney stone growth. Nausea and vomiting are common side effects.



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

Methanamine:



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

The Antibiotics:

β- lactum 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-lactum antibiotics alone.

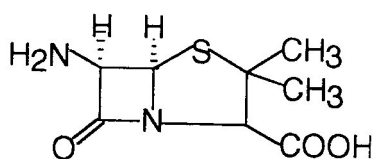
Mode of action:

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

Penicillins:

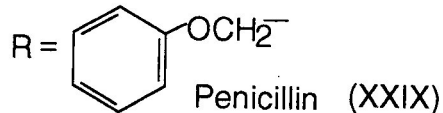
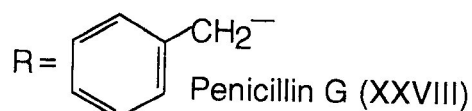
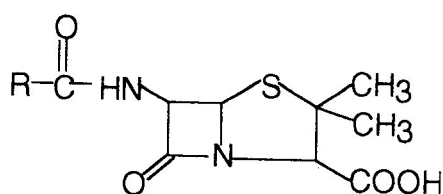
In 1929, Alexander Fleming for the first time discovered *Penicillium notatum* causing partial lysis of *Staphylococcal* colonies. Later on, Penicillin was isolated and gradually large scale production also took place. The penicillin field was then expanded to include orally active penicillins, broad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

6-Amino penicillanic acid (XXVII), i.e. the fundamental nucleus, is prepared by using naturally occurring fungal enzyme that causes selective side chain cleavage of penicillins without breaking the β -lactum 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 V) (XXIX) were synthesized by fermentation.

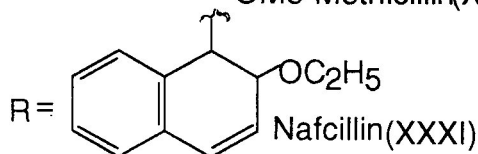
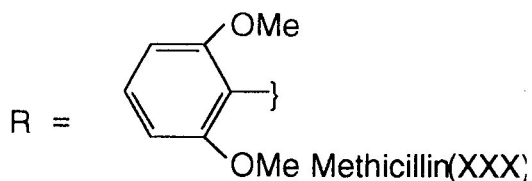
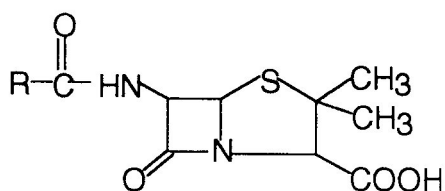


Resistance:

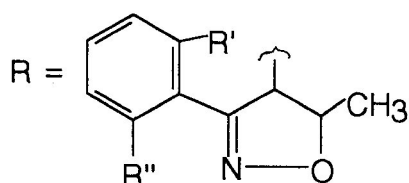
Resistance develop in the bacteria that produce the enzyme which deactivates penicillin, namely β -lactamase and acylase. β -lactamase catalyses hydrolysis of β -lactum ring whereas acylase is responsible for hydrolysis of acylamino side chain. In gram negative organisms, the resistance develops due to poor penetrability.

Penicillinase resistant Penicillins:

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



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



R' = R'' = H Oxacillin (XXXII)

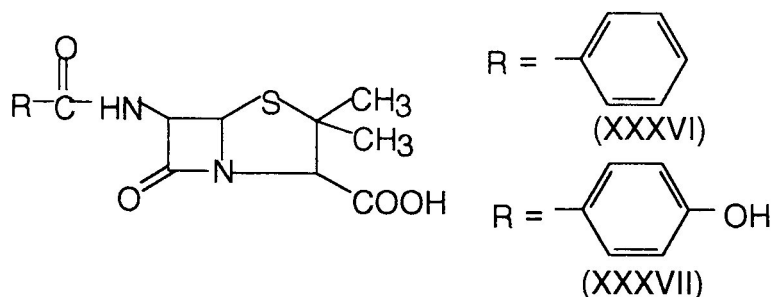
R' = H, R'' = Cl Cloxacillin (XXXIII)

R' = R'' = Cl Dicloxacillin (XXXIV)

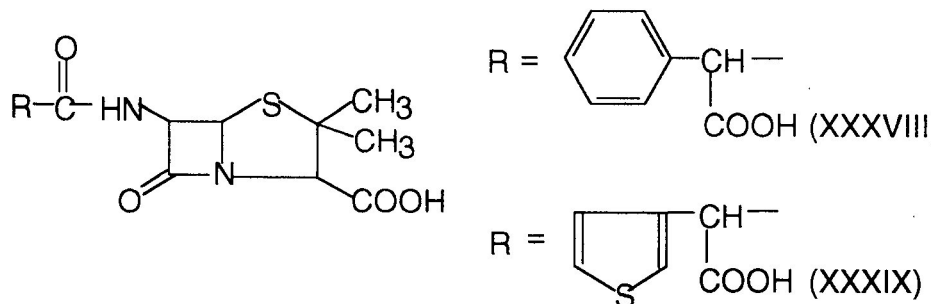
R' = F, R'' = Cl Flucloxacillin (XXXV)

Extended Spectrum Pencillins:

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)³², Amoxicillin (XXXVII)³³ etc.



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

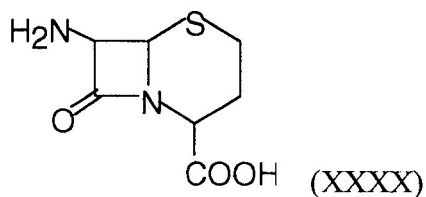


Allergic reactions include from skin rashes to drug fever and anaphylaxis, cross sensitivity³⁶. Allergy occurs due to production of antigenic penicilloyl proteins³⁷.

Cephalosporins:

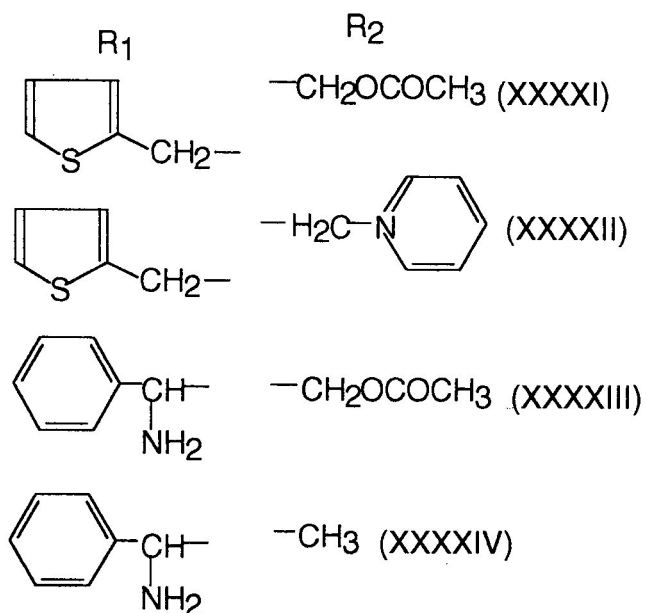
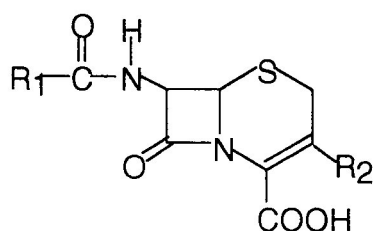
Cephalosporins were discovered by Brotuz and were isolated by Abraham and Newton. They have a wide range of activity against gram positive and gram negative organisms.

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



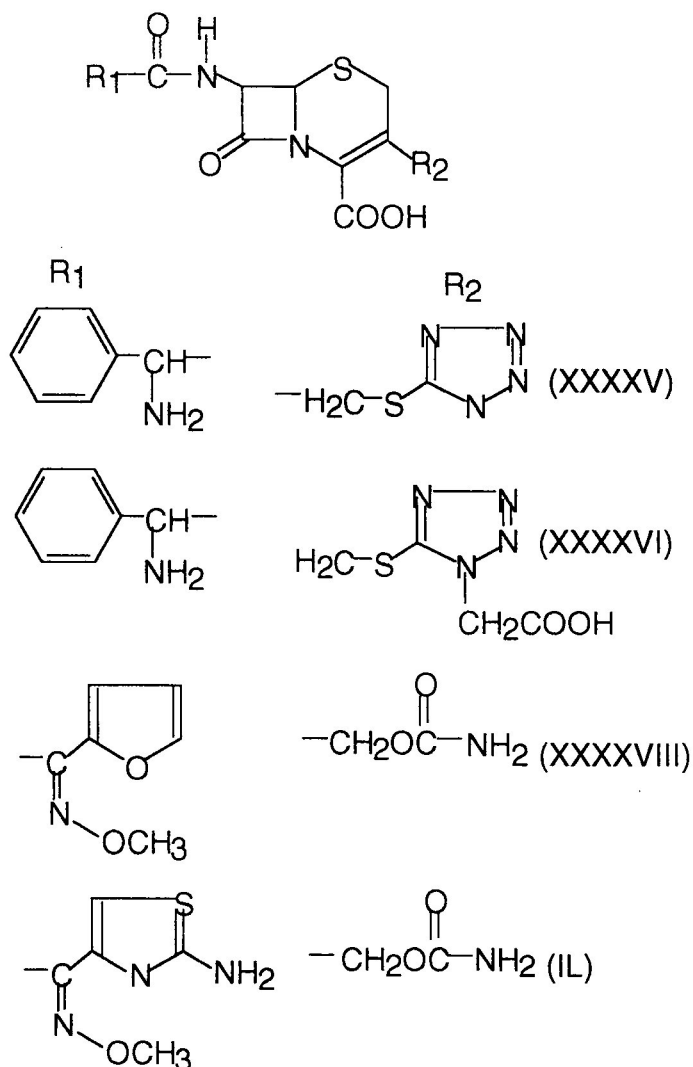
Cephalosporins have a broad spectrum of activity with high potency against gram-positive organisms and moderate potency against gram negative organisms.

Cephalothin(XXXXXI)³⁸ and Cephaloridine (XXXXXII) are administered parenterally, whereas Cephaloglycin (XXXXXIII) and Cephalexin (XXXXXIV)³⁹ are active orally.



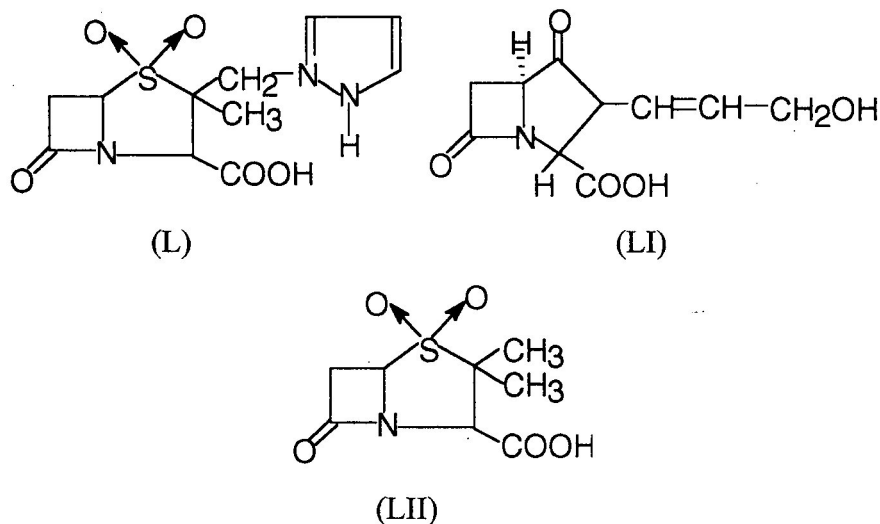
Resistance:

With consistent use resistance develops among several bacteria which produce cephalosporinase and thus hydrolyse the β -lactum ring. The semisynthetic agents with plasmid mediated cephalosporinase resistance⁴⁰ and acid stability were developed later on. These include Cephamandol(XXXXV)⁴¹, Cephonacid(XXXXVI), Cephuroxime (XXXXVII)⁴², Cefataxime(XXXXVIII)^{43,44}.



β -lactamase inhibitors:

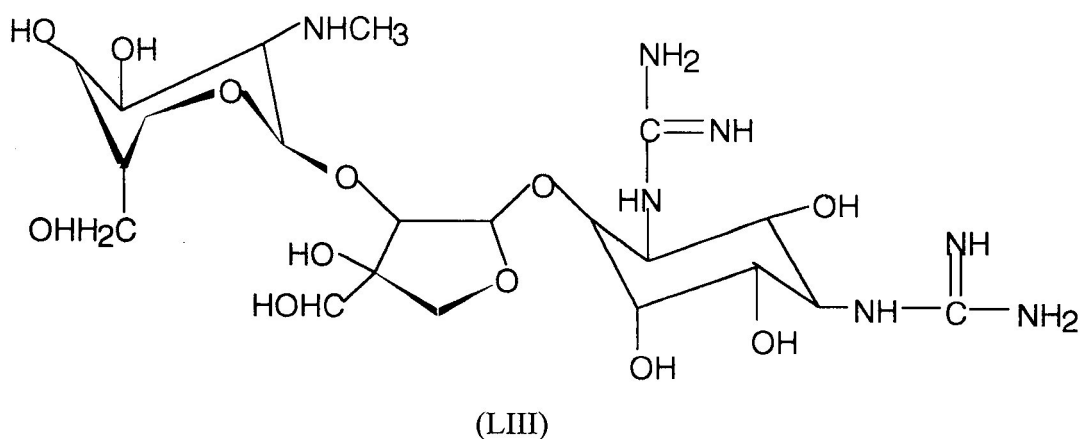
Many substances have been found to inhibit the β -lactamase enzyme, directly or by acting synergistically with some of the antibiotics. UTR-830 (L), clavulonic acid (LI) and sulbactam (LII) are the examples.



Aminoglycosides:

Aminoglycosides contain 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. Kanamycin⁴⁵, Gentamycin⁴⁶, Tobramycin⁴⁷, Netilmicin⁴⁸, Amikacin⁴⁹.

Streptomycin^{49,50,51}, (LIII) is the most effective of the group for the chemotherapy of tuberculosis, brucellosis, tularemia, *Yersinia* infections.



Paramomycin^{52,53} is used primarily for amoebic dysentery.

Mode of action:

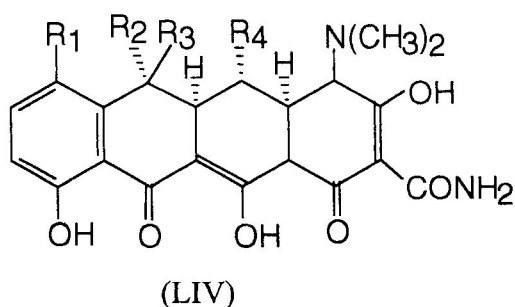
Aminoglycosides bind to the 30S ribosomal subunit to form a complex and thus inhibit proper amino acid polymerization. The binding causes misreading leading to mutation of genetic code, This causes incorporation of improper amino acids into the peptide chain. Thus, they inhibit initiation of protein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

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

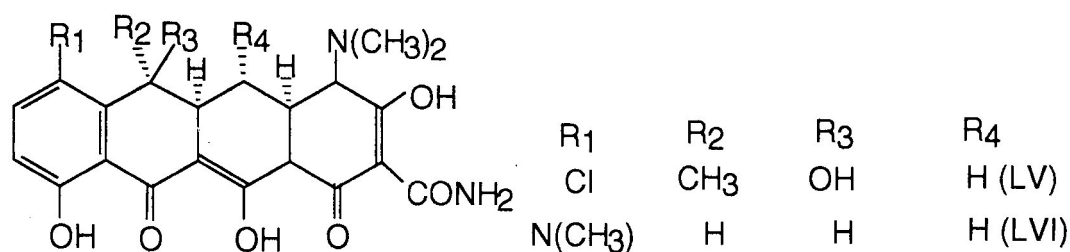
Tetracyclines:

Tetracyclines(LIV)⁵⁴ are derived from *Streptomyces* species or semisynthetically. The basic nucleus is octahydrophenanthrene. Tetracyclines have a broad spectrum of any known antibacterial agent. They are active against a wide range of gram positive and gram- negative bacteria, Spirochetes, Mycoplasmas, Rickettsiae, Chlamydiae.

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

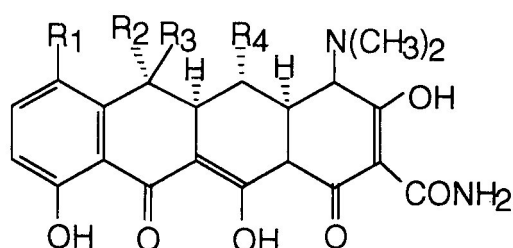


In 1938, Duggar⁵⁵ isolated Chlortetracycline. Electron withdrawing groups, e.g. in chlortetracycline⁵⁶(LV) as well as electron donating groups as in Minocycline(LVI) enhance the activity of the parent molecule.



Oxytetracycline⁵⁷(LVII) has similar activity as that of Chlortetracycline. Doxycycline⁵⁸ (LVIII) and Methacycline⁵⁹(LIX) are more active *in vitro* than Oxytetracycline.

In 1957, MaCormik⁶⁰*et. al.* isolated Demechlorycline(LX) with similar spectrum of activity as that of other tetracyclines, but slightly greater activity.



R ₁	R ₂	R ₃	R ₄
H	CH ₃	OH	OH (LVII)
H	H	CH ₃	OH (LVIII)
H	CH ₂	—	H (LIX)
Cl	H	OH	H (LX)

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

Macrolides:

Macrolide compounds have been derived from the *Actinomyces* species. They contain highly substituted lactone ring⁶¹ aglycon to which amino and neutral deoxy sugars are attached. In 1950, Picromycin was reported. McGuire⁶² in 1952, first reported the isolation of Erythromycin from *Strptococcus erythreus*.

It is well tolerated and useful for infections of upper respiratory tract, skin, soft tissue and genital tract. More recently, its activity against *Mycoplasma pneumoniae*⁶³ has been shown.

Erythromycin acts by binding selectively to 50S ribosomal subunit and thus prevents translocation in protein synthesis⁶⁴.

Non-specific resistance among gram negative bacilli is related to poor penetrability⁶⁵.

Modifications in Erythromycin gave Cclarithromycin, Flurithromycin etc. Clarithromycin is effective against *Mycobacterium avium - intercellulariae* complex^{66,67}. Olendomycin is one more important agent of the macrolide family.

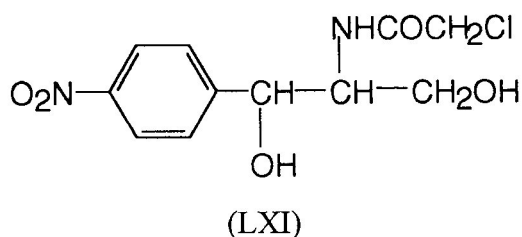
Lincomycins:

Lincomycins are sulphur containing antibiotics isolated from *S. lincilnensis*. Spectrum and mode of action is similar to that of macrolides. Lincomycin, which was isolated by Manson *et. al.*⁶⁸, is useful for the treatment of infection by gram positive organisms notably Staphylococci, β -haemolytic Streptococci and Pneumococci. Clindamycin⁶⁹ 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.*⁷⁰ in 1947, isolated Chloramphenicol, a broad spectrum antibiotic, from *Streptomyces venezuelae*. Its structure was established in 1949 by Constroulis, Rebstock and Crooks⁷¹.



Chloramphenicol(LXI) is active against Penicillin G and Ampicillin resistant species of *Salmonella*, *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Bacillus fragilis*, *N.meningitidis*. 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 ring gives rise to the 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 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. It acts by inhibiting the cell-wall synthesis. No cross resistance to β -lactum, Cycloserin, Bacitracin etc. is

observed. It is used in systemic infections such as *Staphylococcal* enterocolitis and pseudomembranous colitis associated with Clindamycin therapy.

Polypeptides:

Polypeptide class of antibiotics include Gramicidine, Polymixin, Bacitracin, Colistine etc. Severe renal toxicity limits their use in therapeutics.

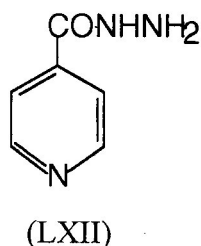
Antitubercular agents:

Mycobacterium tuberculosis was identified by Koch in 1882 as a causative bacteria for chronic infections like tuberculosis. It is estimated that 1.7 billion people harbour the pathogen and every year 8 million individuals develop new clinical disease⁷². One third to half of the world's population is infected by *M. tuberculosis*. 6% of the deaths occurring worldwide^{73,74}. Patients with Human Immunodeficiency Virus(HIV) are highly susceptible to tubercular infection.

Introduction of chemotherapy of Mycobacterial infections over the past five decades has brought about a dramatic decrease in mortality and morbidity due to the illness. Multiple drug resistance within the organisms makes the treatment more challenging. Drug toxicity and patient compliance are the other problems. Research is needed to be carried out to find the best therapeutic regimen.

Antitubercular drugs are classified majorly as 'primary' or frontline drugs, and secondary or second line drugs. Frontline drugs are used in standard therapeutic regimen due to their high level of efficacy and acceptable degree of toxicity. These drugs include Isoniazide, Rifampicin, Ethambutol and Streptomycin. 'Secondary' or second line drugs are used occasionally because of bacterial resistance or certain patient related factors. These drugs include Pyrizinamide, Ethionamide, Para amino salicylic acid, Cycloserin, Viomycin, Kanamycin, Amikacin, Capreomycin, Thioacetazone, Thiocarlide etc.

Isoniazide(INH):



Isonicotinyl hydrazide(Isoniazid)(LXII), described in 1912⁷⁵ was prepared as an intermediate in the synthesis of isonicotinyl aldehyde. Outstanding activity of INH in experimental infection was also confirmed by clinical trials. N-alkylated derivative (Iproniazide) was also found to be active.

INH has both bacteriostatic and bactericidal activity against various strains of *Mycobacterium tuberculosis* which are resistant to other drugs. The MIC is about 0.04 µg/ml. Bactericidal action is exhibited against replicating organisms, whereas in case of non-replicating organisms, it is only bacteriostatic⁷⁶.

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

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

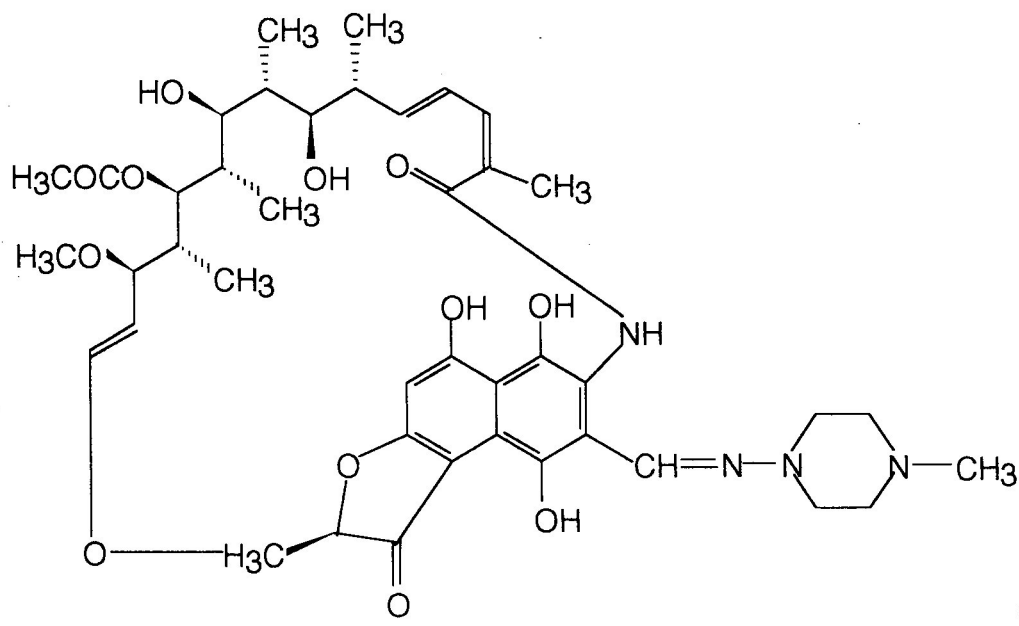
More recently it has been shown that the activity of INH results from peroxidative reaction catalysed by peroxidase which is encoded by Kat G Gene⁷⁷. *M. tuberculosis* which lacks this gene are resistant to INH⁷⁸.

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

Rifampicin:

The Rifampicin antibiotics were discovered as metabolites of *Streptomyces mediterranei*. Recently it has been stated to be *Amycolatopsis mediterranei*⁷⁹.

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)⁸⁰ was the most active *in vivo* at concentration 1 µg/ml and is useful clinically.



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

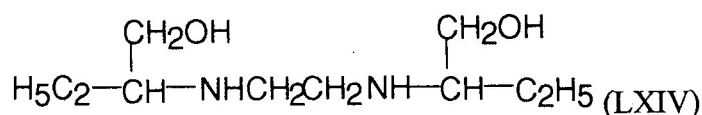
Resistance is rare and occurs spontaneously in strains not exposed previously to the antibiotic. The mechanism of resistance is through mutation in *rpoL* gene which encodes β -subunit of RNA-polymerase.

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

The therapeutic efficacy of Rifampicin is comparable to that of INH and is superior to all other drugs.

It possesses low toxicity with gastrointestinal disturbances and cutaneous reaction as the side effects.

Ethambutol:



Among the randomly screened compounds, Ethambutol(+)(LXIV) is found to be very active, both *in vitro* and *in vivo* against *M. tuberculosis* at 1µg/ml. Organisms resistant to other drugs are 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^{81,82}. Another hypothesis states that it causes an inhibitory effect on phosphorylation⁸³.

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

Streptomycin:

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

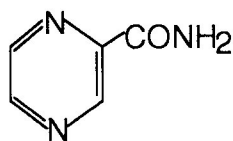
In mycobacteria, mutation in the *rspL* gene which encodes the ribosomal protein S12, have been shown to confer resistance to Streptomycin⁸⁴.

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

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

Other aminoglycosides such as Kanamycin, Amikacin are used secondarily.

Pyrazinamide:



(LXV)

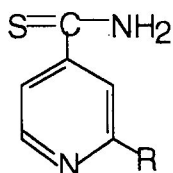
The best activity of pyrazinamide(LXV) is against intracellular *Mycobacteria* causing sterilizing effect in acidic conditions. Susceptible bacteria produce deaminase which converts Pyrazinamide to pyrazinoic acid, an active form.

It gets readily absorbed orally and is metabolized to pyrazinoic acid which gets oxidised to its 3-hydroxy derivative.

Hepatotoxicity is the most common and serious side effect. Other effects include artralgia.

It is useful in short term chemotherapeutic regimen. Treatment for longer than two months is not recommended⁸⁵.

Ethionamide:



R = -C₂H₅ (LXVI)

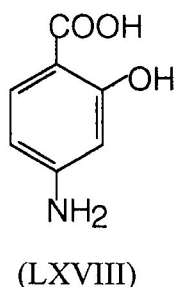
R = -n-C₃H₇ (LXVII)

2-ethyl thioisonicotinamide(Ethionamide)(LXVI) and 2-n-propyl analog (Prothionamide) (LXVII) 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 large dose(>500mg) is not well tolerated. It gets metabolized to give the sulfoxide.

Side effects include GI irritation, hepatitis, CNS effects and hypersensitivity.

Para amino salicylic acid(PAS):

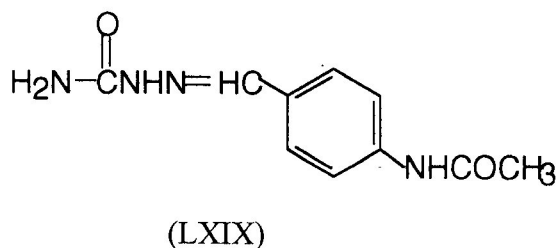


Among the various compounds related to benzoic acid, PAS(LXVIII) is believed to be the most active agent⁸⁶ against growing cells of *M. tuberculosis*.

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, which impairs iron uptake⁸⁷.

10-12gm/day is required to be taken orally in 2-3 divided doses. It is no longer in use. GI toxicity is a major side effect.

Thioacetazone:



Thioacetazone(LXIX) is the most active thiosemicarbazone against *M. tuberculosis*. Lack of cross resistance to INH and Streptomycin indicate the use of thioacetazone in combination with these drugs to delay bacterial resistance.

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

Drug	Initial phase(2months)	Continuation phase(4months)
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)
Pyrizinamide	30mg/kg/day(1.5-2gm/day)	—
Ethambutol*	15-25mg/kg/day	
Intermittent regimen:		
Rifampicin	10mg/kg(3times weekly)	10mg/kg(3times weekly)
INH	15mg/kg(3times weekly)	15mg/kg(3times weekly)
Pyrazinamide	50mg/kg(3times weekly)	50mg/kg(3times weekly)

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

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

Antifungal agents:

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

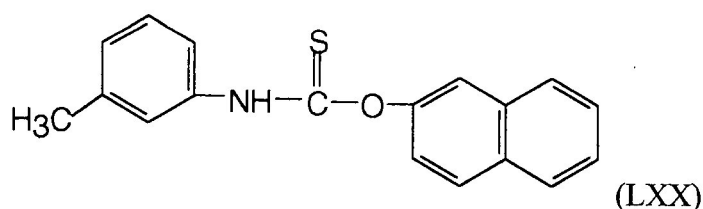
1)Dermatophytosis(Tinea infections), which are contagious superficial epidermal infections caused by various *Epidermophyton*, *Microsporum* and *Trichophyton* species; and

2)Mycosis, caused by pathogenic saprophytic yeasts, which are contagious involving 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:



The o-2-naphthyl ester of N-dimethyl thiocarbanilic acid was found in 1960 to have good activity against dermatophytes when applied topically. Poor permeability of Tolnaftate (LXIV) limits its use. 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.

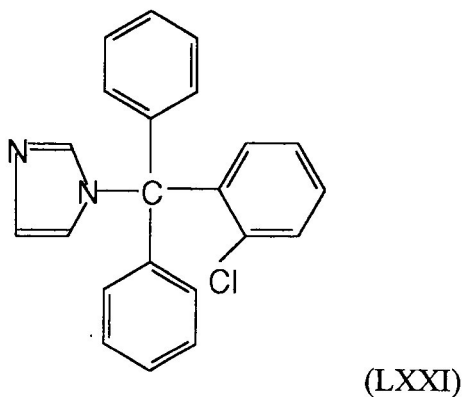
Substituted azoles:

Substituted imidazoles represent a versatile group of antiungal agents with a unique mechanism of action. The early members reported were Cotrimazole(LXV)⁸⁸ and Miconazole (LXVI)⁸⁹ 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*.

The mechanism of action of the drug is elucidated⁹⁰ extensively. At high concentration (micromolar), they are fungicidal; at low concentration (nanomolar), they are fungistatic. The fungicidal effect is associated with damage to the cell wall with the

loss of essential cell components such as potassium ion and amino acids. Imidazole inhibits the cytochrome P-450 oxidase enzyme that catalyses the 14- α -demethylation of lanosterol to ergosterol.

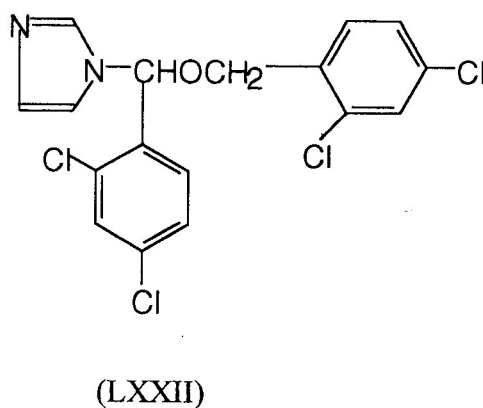
Cotrimazole:



Cotrimazole (LXXI) is a broad spectrum antifungal agent used topically for the treatment of tinea infection and Candidiasis.

As it causes severe gastric disturbances, it is not suitable for systemic use.

Miconazole:

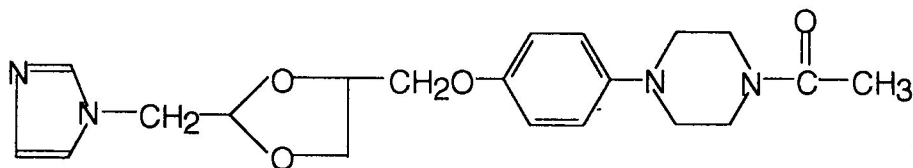


Miconazole is intended for the treatment of serious systemic fungal infections like Candidiasis, Cryptococcosis, Petriellidiasis and Paracoccididomycosis.

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:



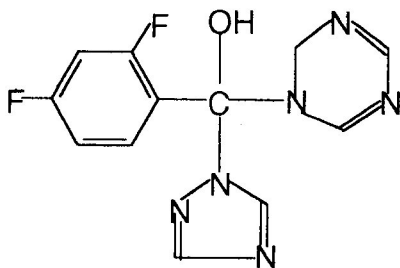
(LXXIII)

Ketoconazole(LXXIII) is administered orally for the treatment of systemic mycosis. It requires gastric acidity for absorption. Antacids and drugs like H₂-histamine antagonists, anticholinergics, that inhibit gastric secretion, interfere with its absorption. It is metabolised to the inactive form and is excreted by the enterohepatic route.

Ketoconazole is recommended for systemic mycosis such as Coccidiomycosis, Blastomycosis, Histoplasmosis, chromomycosis, paracoccidiomycosis and severe cutaneous infections.

Hepatotoxicity is a major adverse effect. Also, levels of testosterone and corticosterone are lowered at high doses of Ketoconazole.

Fluconazole:

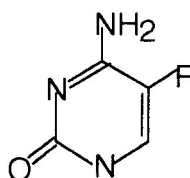


(LXXIV)

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

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

Flucytosine:



(LXXV)

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

Incorporation of fluorinated pyrimidine into RNA, following selective deamination to 5-fluoro uracil in the fungus appears to be required for activity. 5-fluoro uracil gets metabolised to 5-fluoro-2'-deoxyuridilic acid which suppresses DNA synthesis by inhibiting thymidylate synthetase.

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

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

Antifungal antibiotics:

Polyene antibiotics:

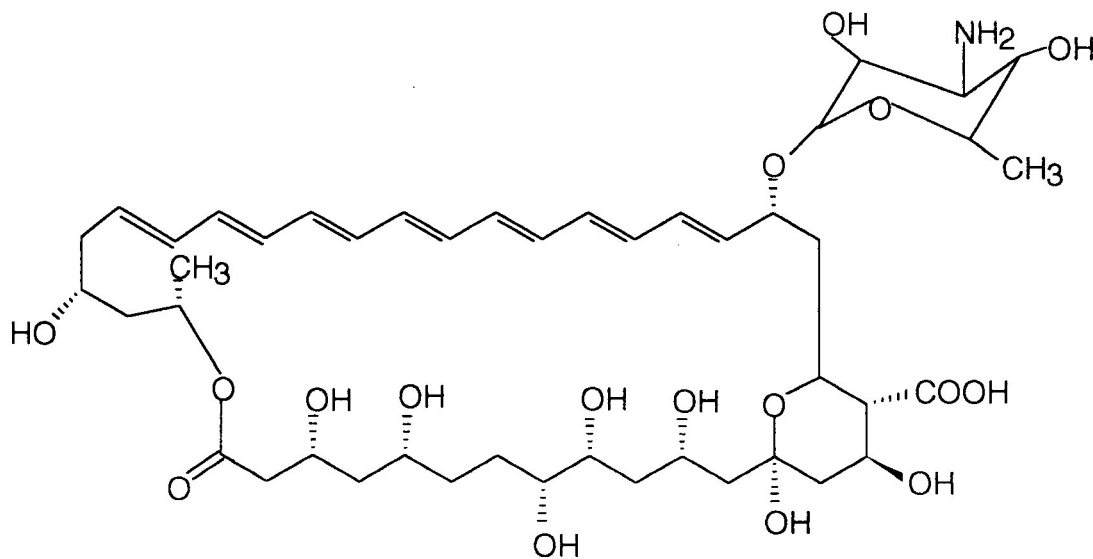
The polyene antibiotics contain conjugate double bonds in large lactone rings. They differ from macrolides in the size of the lactone ring and the presence of conjugated-‘ene’ system.

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

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

Amphoterecin B:

A potent antifungal agent with a polyene structure was isolated in 1956 by Gold *et.al.*⁹¹ from *Streptomyces nodosum*, found in the soil from Venezuela. The antibiotic material was a mixture of two closely related compounds, Amphoterecin A and B of which B is more active.

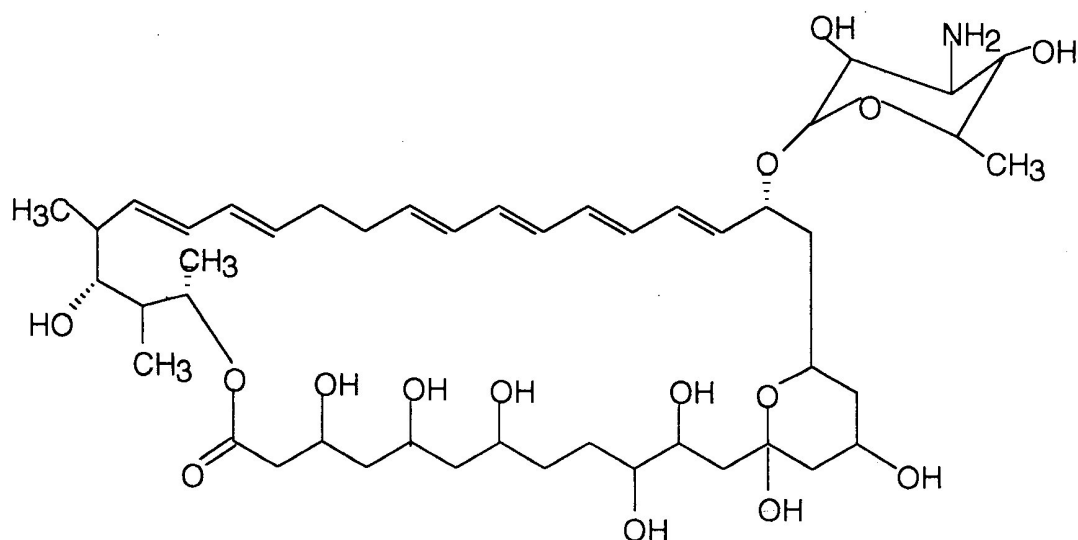


Amphoterecin B (LXXVI) contains primary amino group in the sugar moiety, mycosamine and a carboxyl group attached to the macrolide ring.

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

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

Nystatin:



(LXXVII)

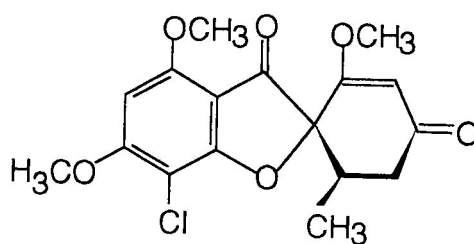
A polyene antibiotic first isolated in 1951 from a strain of *Streptomyces noursei* by Hazen and Brown⁹², Nystatin (LXXVII) is a valuable agent for the treatment of local and

gastrointestinal monolial infections caused by *Candida albicans* and other *Candida* species. It is also recommended for thrush or esophagitis by *C. albicans* in patients with AIDS. It is available in cream, ointment or powder form for topical use, whereas tablets or troches are used systemically.

Griseofulvin:

Griseofulvin (LXXVIII) was first reported in 1939 by Oxford *et.al.*⁹³ as an antibiotic obtained from the mold *Penicillium griseofulvum*. Dierckx. Its structure has been determined by Grove *et.al.*⁹⁴

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



(LXXVIII)

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 assembly⁹⁵.

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

Several other antibiotics in use include Candidicin, Natamycin etc.

Novel approaches:

Out of the novel approaches to a fungal chemotherapy, one approach might be to persist the infected host to withhold growth essential iron from the fungi and not the host.⁹⁶ Poorly soluble iron chelators such as hydroxy pyridon, rilopyrox are being developed for topical use in dermatophytosis.

Diamidines, hydroxy stildamide and isothionate have been found to be possess activity against Pneumocytosis, but have severe side-effects.



Plan of Work

:PLAN OF WORK:

Pharmaceutical research particularly in medicinal chemistry provides a basis for the development of new approaches to combat human diseases. The biomedical research can be basic (seeking an understanding of biological phenomenon which are unknown) or applied (using principles which are known to produce desired new product or effect). In either case the research has the effect of obviating the established therapies in favour of new modalities which would be safer and more effective.

As the abilities of the chemist to synthesize, isolate and characterize new compounds grew and as biologists learnt more about the actions of drugs and about biological mechanisms in health and diseases, the search for new drugs gradually began on a more rational basis. Today, useful drugs arise from natural products, chemical synthesis or combination of both sources by means of approaches that vary in a complete spectrum between serendipitous discovery and rational synthesis.

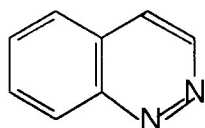
Organic chemists in pharma industry synthesize hundreds of new compounds every week. In most cases, the chemist has a specific reason for synthesizing a particular compound; usually based on theoretical considerations, medicinal chemistry, biological mechanisms or a combination of all these three. In the past three decades, the biological sciences have undergone a major redirection towards molecular understanding of biological systems.

The search for chemical structures that which exhibit pharmacological activity is a difficult goal for the organic chemists. Compounds are submitted for screening for numerous types of biological and pharmacological action. Observations on interesting and repeatable biological activity opens new pathways for additional chemical research efforts in the expansion of the series and often leads to significant new medicinal products. Organic chemist has succeeded in the deliberate chemical alteration of the natural drugs and produces derivatives which are either more potent or superior in some other respect. Such modification of known pharmacodynamic compounds, both natural and synthetic, constitutes one of the main kind of research efforts in the field of chemotherapy.

An important aspect of medicinal chemistry has been to establish a relationship between chemical structure and biological action. An increased consideration in recent years has been to correlate the chemical structure with reactivity or physical properties and these correlations in turn, be related to therapeutic action.

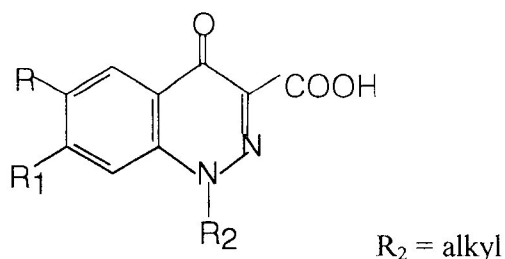
The logic supporting the work presented in this thesis was formulated bearing in mind the biological activities of known moieties and attempting certain structural modifications or adaptation in light of the recent trends in drug research in incorporating newly emerged pharmacophores on existing moieties.

Chapter II: Synthesis of 1H-3(2-alkyl/aryl, 4-substituted thiazolo)-6-chloro-cinnoline-4-ones:



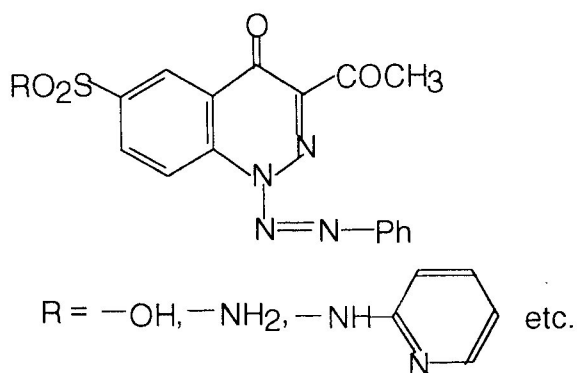
(I)

Cinnoline (I), a member of the heterocyclic family, has been documented in the literature for its appreciable antibacterial activity²⁰³.



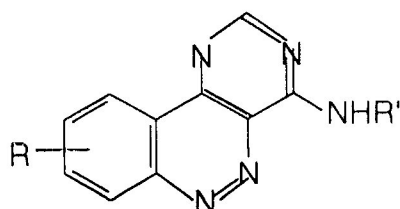
(II)

1-alkyl-3-carboxy-4-cinnolones (II) also show good antibacterial activity²⁰⁴.



(III)

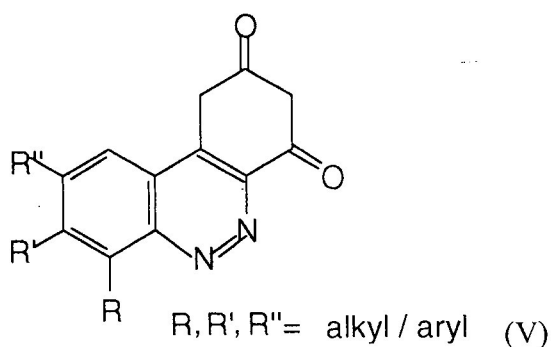
3-acetyl-phenylazo-6-substituted cinnoline-4-ones (III) have been reported to exhibit potential antibacterial, but low antifungal activity²⁰⁵.



$R, R' = \text{alkyl / aryl}$

(IV)

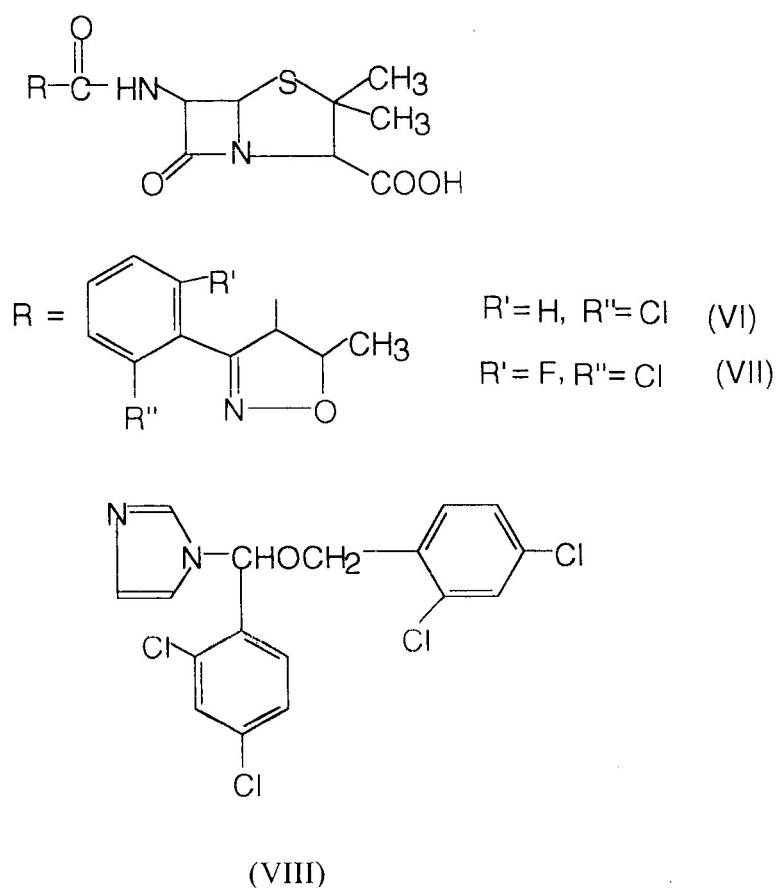
Similarly, 4-arylamino pyrimido(5,4-c) cinnolines²⁰⁶ (IV) have been reported to possess activity from 0.3 $\mu\text{g/ml}$ to 0.05 $\mu\text{g/ml}$ against gram positive *Bacillus cirroflagelus*, from 0.21 $\mu\text{g/ml}$ to 0.025 $\mu\text{g/ml}$ against gram positive *Staphylococcus aureus*, and 3.16 $\mu\text{g/ml}$ against gram negative *Escherichia coli*.



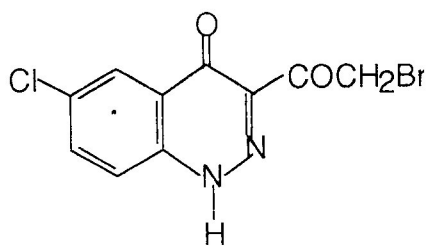
Pyrimido (5,4-c)cinnolines-2,4(1H,3H) diones²⁰⁷(V), are known to possess moderate activity, i.e. upto 100 µg/ml against a number of gram positive and gram negative organisms and fungi like *Aspergillus niger* and *Candida albicans*.

Relatively less work has been carried out on the cinnoline ring. Hence, we decided to emphasize on this ring system and carry out studies on compounds bearing a combination of cinnoline and other heterocyclic systems, in the hope of developing a prospective antimicrobial agent.

Electron-withdrawing substituents like halogens and nitro are believed to impart stability to the ring and thereby contribute to the activity. e.g. Cloxacillin (Antibacterial) (VI), Flucloxacillin (antibacterial) (VII), Miconazole (antifungal) (VIII) etc.



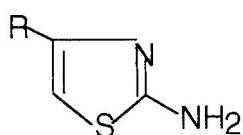
Hence, we selected 6-chloro-(3-halocetyl)-1H-cinnoline-4-one (IX) as our parent moiety.



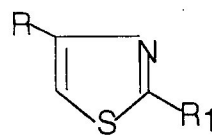
(IX)

Thiazole:

Thiazole moiety is well established as a biologically active moiety, especially for its antibacterial effect²⁰⁸. Russian research workers²⁰⁹ showed that amino substitution at 2-position of thiazole ring along with alkyl/aryl group at 4-position (X) impart bacteriostatic activity to the molecule.



(X)



(XI)

Similarly, vast synthetic work carried out in our laboratory on 2,4-disubstitute thiazoles (XI) also revealed²¹⁰ that many thiazoles of such types are active against H₃₇R strain of *Mycobacterium tuberculosis* at concentrations ranging from 5 µg/ml to 0.0 µg/ml.

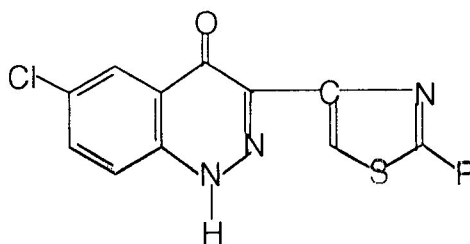
These properties of thiazole nucleus as antitubercular agent strongly suggest that this nucleus should be explored further in order to achieve good antitubercular activity.

Keeping in mind the good antibacterial activity of 2,4-disubstituted thiazole, we decided to combine cinnoline with thiazole.

In order to form a thiazole, we decided to condense the 3-bromoacetyl-6-chloro cinnoline-4-one with various substituted thioamides.

The thioamides were synthesized using established literature procedure.

The final compounds in this series will have the following common structure (XII).



(XII)

R = aminomethyl phenyl, pyridyl, benzyl, phenyl etc.

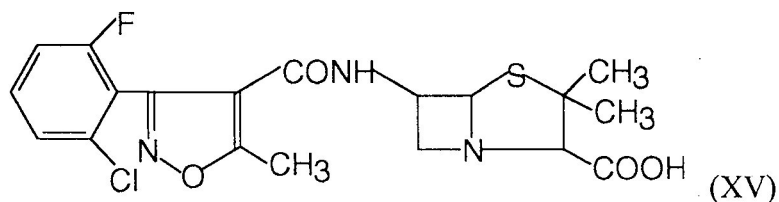
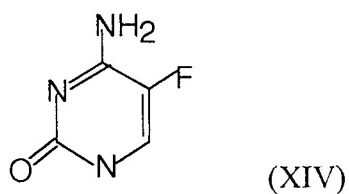
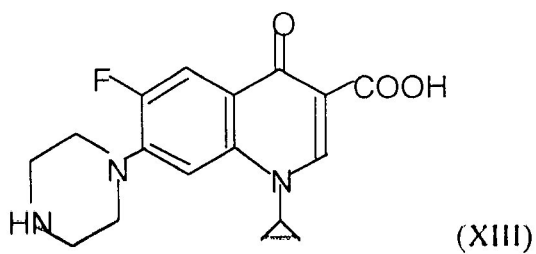
A total of ten compounds were synthesized in this series which were confirmed by elemental and spectral analysis.

Chapter II: Synthesis of 1H-3(2-alkyl/aryl, 4-substituted thiazolo)-6-fluoro-cinnoline-4-ones:

Extensive work has been reported on the advantages of organo fluorine compounds. Fluorine molecule itself has numerous advantages, like-

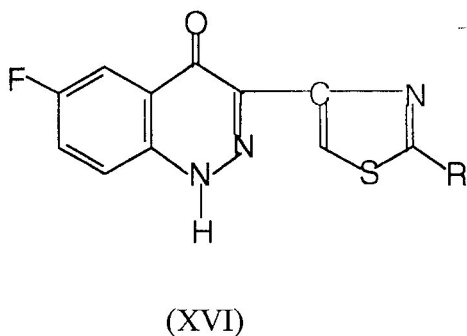
- 1) Fluorine, the second smallest atom as measured by atomic radius and internuclear distance to carbon, would most closely resemble bioactive hydrogen analogues with respect to steric requirements at enzyme receptor sites.
- 2) Fluorine atom imparts increased oxidative and thermal stability because of its high binding energy which exceeds that of chlorine by 17 Kcal/mole.
- 3) Fluorine atom leads to increased lipid solubility which enhances the rate of absorption and *in vivo* transport.

Numerous fluorinated compounds are known e.g. Ciprofloxacin (antibacterial) (XIII), Flucytosine (antifungal) (XIV), Flucloxacillin (antibacterial) (XV) etc.



Thus, we decided to replace chlorine at the 6-position with fluorine to check its effect on activity. The fluorinated compounds were synthesized by a similar procedure using 4-fluoro aniline as the starting compound.

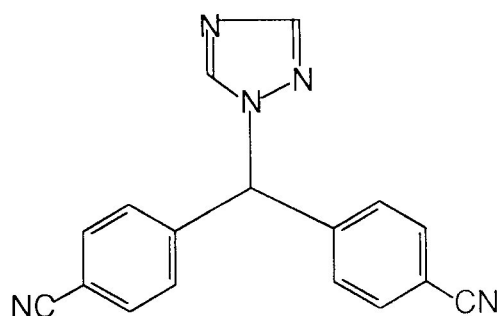
The final structure is as follows-



R = alkyl/ aryl/ substituted aryl etc.

Ten compounds with similar substitutions as that in series I were synthesized and confirmed by elemental and spectroscopic analysis. Comparison will be carried out between the activity of both the series in order to establish a structure activity relationship.

Chapter III: Synthesis of Intermediate for Letrozole:



(XVII)

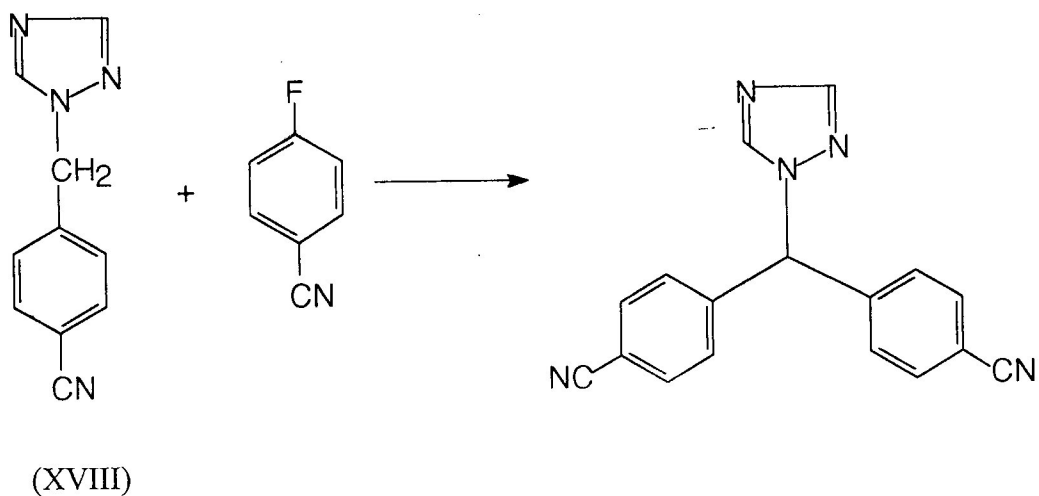
Letrozole (XVII) is a relatively new drug, used as an antineoplastic agent. Using modified nude mouse model of post-menopausal breast cancer, CGS(20267) was shown to have a marked antitumor effect and to significantly suppress plasma estrogen²¹¹.

Letrozole is currently undergoing phase II and phase III clinical trials for breast cancer in U.S.A.

Pharmacological action:

Letrozole is a highly potent and selective non-steroidal aromatase inhibitor. Aromatase catalyses the conversion of androgens to estrogens which leads to an increase in plasma estrogen level. Letrozole was shown to inhibit aromatase in peripheral tissues at doses of 0.5 to 2.5 mg/day for 6 weeks²¹¹.

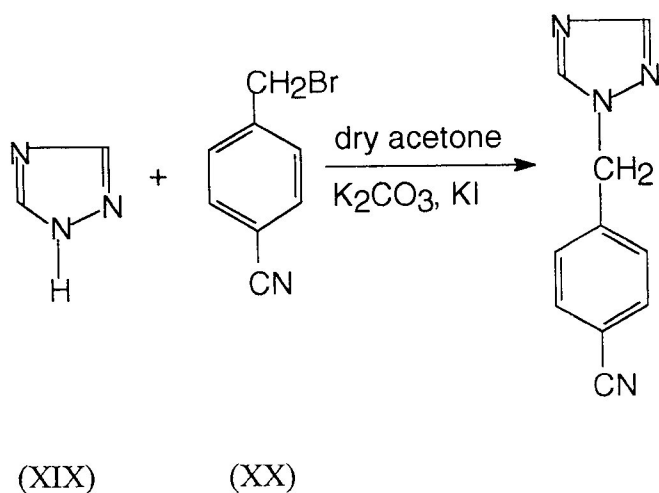
The synthetic route of Letrozole is as follows²¹²:



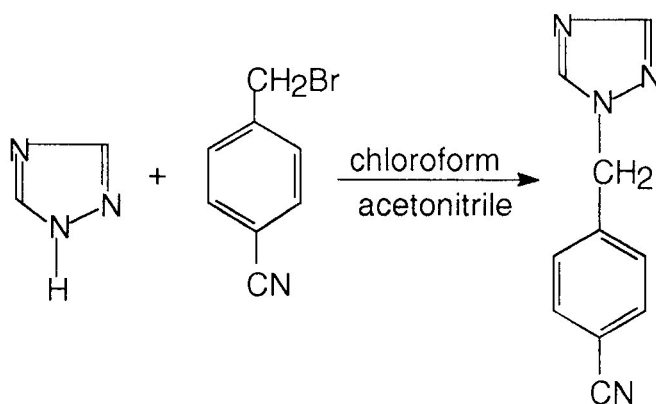
A number of antineoplastic drugs can be synthesized using 4-(1,2,4-triazolyl methyl) benzonitrile (XVIII) e.g. MPV-2213ad²¹³, Letrozole etc.

The intermediate 4-(1,2,4-triazolyl methyl) benzonitrile is prepared by condensation of 1H-1,2,4- triazole (XIX) with 4-cyano benzyl bromide (XX) by different routes enumerated as follows:

1) 1H-1,2,4-triazole and 4-cyano benzyl bromide are dissolved in dry acetone. To the mixture is then added potassium carbonate and potassium iodide and refluxed for 8 hours²¹⁴.



2) 4-cyano benzyl bromide was dissolved in a mixture of chloroform and acetonitrile. 1H-1,2,4-triazole dissolved in the same system is added to the former and refluxed for 15 hours. Then it is neutralized with 3% sodium bicarbonate and passed on a silica gel column for separation of the product²¹⁴.



Thus, 1H-1,2,4-triazole is a key intermediate for the synthesis of Letrozole. 1H-1,2,4-triazole can be prepared by various routes²¹⁵ which are summarized as follows:

- 1) By oxidation of substituted 1,2,4-triazoles.
- 2) By treating urazole with phosphorus pentasulphide.
- 3) By heating equimolar quantities of formyl hydrazine and formamide.
- 4) By removal of amino function of 4-amino-1,2,4-triazole.
- 5) By oxidation of 1,2,4-triazole-3(5)-thiol with hydrogen peroxide.
- 6) By decarboxylation of 1,2,4-triazole-3(5)-carboxylic acid.
- 7) By heating hydrazide salt with formamide²¹⁶.
- 8) By rapid distillation of hydrazine hydrate with 2M equivalent of formamide²¹⁷.
- 9) By heating N,N' diformyl hydrazine with excess ammonia in an autoclave at 200°C for 2-4 hours²¹⁸.

10) By the reaction of 1,3,5-triazine and hydrazine monohydrochloride²¹⁹.

11) By oxidation of 1,2,4-triazole-3(5)-thiol in conc. HNO_3 ²¹⁵.

We planned to carry out the oxidation of 1,2,4-triazole-3(5)-thiol for a better yield applying certain modifications to the literature procedure.

Modifications:

- 1) During work up of oxidation of 1,2,4-triazole-3(5)-thiol, the mixture will be neutralised with liquid ammonia in order to minimise inorganic bulk.
- 2) Instead of hectic and multiple extraction procedure, the triazole will be extracted in ethyl acetate with recovery of the solvent to maximum.
- 3) The use of large quantity of ethyl alcohol will be avoided which will make the procedure cost effective.
- 4) Also, use of benzene will be avoided. Benzene, being highly carcinogenic may cause health hazards to the workers, when used on industrial scale.

The triazole and benzonitrile will be subjected to elemental and spectroscopic analysis for confirmation.

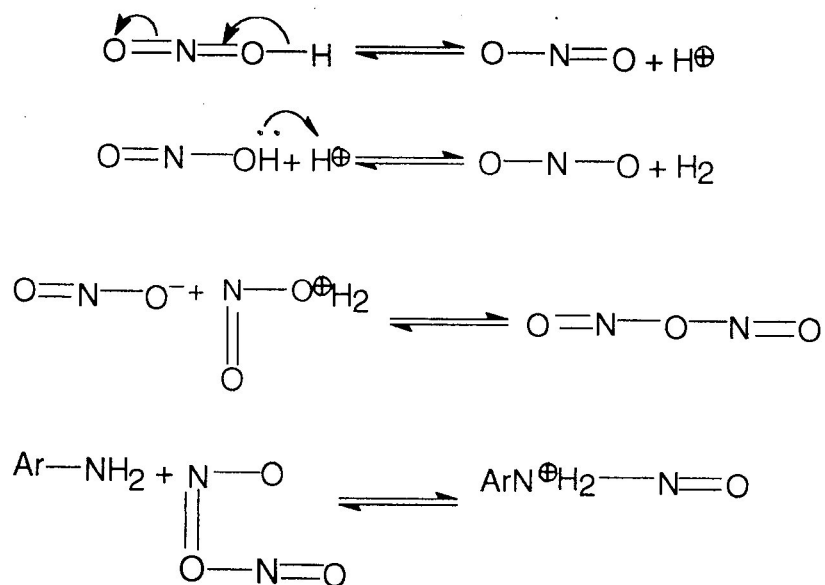
Chemistry

Diazonium salts

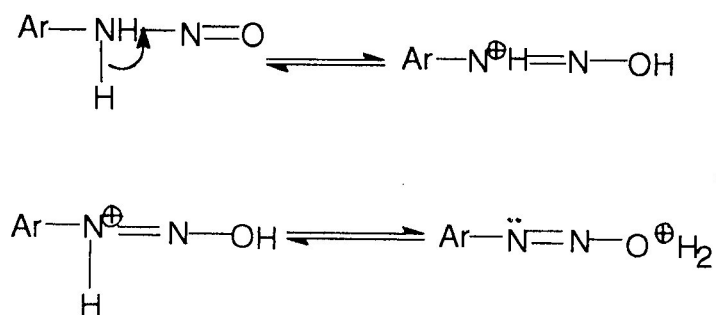
Diazotization of amines:

The reaction of amino salt with nitrous acid is known as diazotization. Pelercriss in 1858, carried out this reaction for the first time with an aromatic amine.

The kinetics of diazotization involve a nucleophilic attack by amine on dinitroxide; which is in equilibrium with nitrous acid .



The free amine, although present in a very small concentration, is highly reactive in the acidic solution used. The conjugate acid of the primary nitroso amine, by a series of proton transfer and loss of water, gives a diazonium salt.

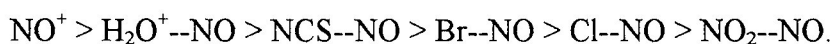




Recently, the intermediate step of a primary amine has been shown to be isolated⁹⁷ in the course of the diazotization of an appropriate amine.

Since most of the diazotizations are performed in aqueous solutions, protonated nitrous acid (from sodium nitrate and hydrochloric acid)⁹⁸ is usually used. Free NO^+ should only appear in concentrated acid and in the course of conversion with complex NO^+ salts.

The sequence of reactivity is shown as below-



I] Aqueous solution:

Normal diazotization of aromatic amines with nitrous acid is carried out in dilute aqueous mineral acid and usually yields the diazonium salt in solution. The direct method starts from solution or suspension of the amines in dilute hydrochloric acid (approximately 2.5 mole acid / mole amine), which is treated with the molar quantity of concentrated aqueous sodium nitrite solution at $0^\circ\text{--}10^\circ\text{C}$. The conversion proceeds rapidly and almost quantitatively⁹⁹. 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 acid¹⁰⁰.

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

II] Concentrated acids¹⁰¹.

Weakly basic amines bearing strongly electron-attracting substituents on the aromatic or heteroaromatic nucleus are diazotized preferentially in concentrated acids,

since hydrolysis¹⁰² of the generated diazonium compound occurs in aqueous solution with increasing dilution. Good results have been obtained with conc. sulphuric acid¹⁰³.

III] Organic solvents¹⁰⁴.

In order to prepare a solid diazonium salt, diazotization of aromatic amines is carried out in organic solvents (glacial acetic acid¹⁰⁵, methanol, ethanol, formamide, DMF, acetone) by means of nitrous acid esters (preferably pentyl nitrile).

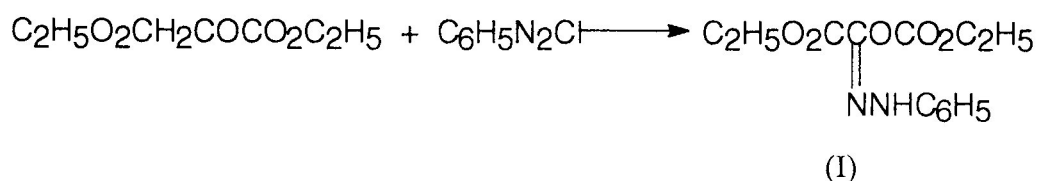
N-diazonium salt will couple with an aliphatic compound containing an activated carbon-hydrogen bond. This is limited to those reactions in which both nitrogen atoms of the diazonium salt are retained in the resulting molecule.

Victor Meyer reported the coupling of diazonium salt with an activated aliphatic carbon atom.

It is generally assumed that the hydrazone is a stable form when coupling occurs at a methyl or methylene carbon and azo structure is formed when coupling occurs at a methinyl carbon without elimination of the group.

The reaction of β -keto ester with a diazonium salt has been studied extensively. Products from ethyl acetoacetate and over fifty different diazonium salts have been reported. Good yields of the hydrazones of α,β -esters are obtained if 1mole of the diazonium salt is employed.

Diethyl oxaloacetate reacts with 1mole of benzene diazonium chloride. If only 1mole of the salt is used, the product obtained is dioxosuccinate phenyl hydrazone(I)^{106,107,108}.



For diazotization, aqueous solution of sodium nitrite is added to mineral acid salt of aryl amine at temperature 0° - 5°C . If the reaction is carried out in aqueous solution, sodium acetate is used as a buffer. Occasionally, alcohol, pyridine or acetic acid are also used as solvents for water- insoluble reactants.

Reactant ratio:

Equivalent amount of diazonium salt and reactant are commonly employed. Excess of diazonium salt should be avoided as it can couple again with the product such as hydrazone to form a formazan derivative. This reaction is favoured by strongly acidic solution.

Reactions are usually rapid and the product can soon be isolated after the addition of diazonium salt. However, the reactions that involve intramolecular coupling need more time to complete.

Preparation of ethyl 2-(aryl phenyl hydrazone)-3-oxybutyrate:

To the aryl amine (0.039mole) dissolved in a 1:1 mixture of conc. HCl and water (10ml) and cooled at 0° - 5°C on an ice bath. A cold aqueous solution of sodium nitrite (0.078mole) was added. The diazonium salt solution was filtered into a cooled solution of ethyl acetoacetate (0.039mole) and sodium acetate (0.58mole) in ethanol (30ml). The resulting solid was filtered, washed with water and recrystallised in ethanol.

M.P. = 94°C .

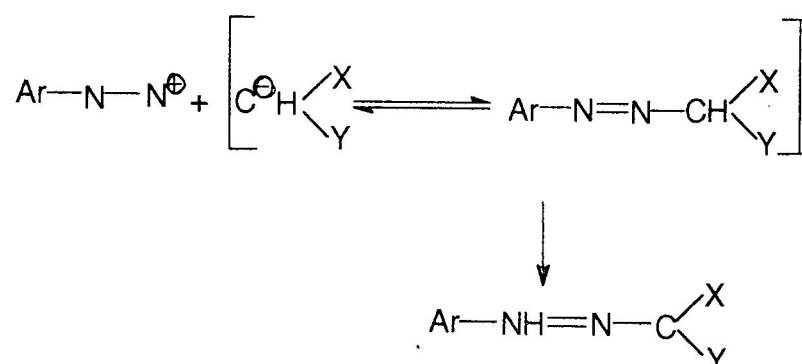
Lit. M.P. = 94°C

Yield = 83%.

Hydrazones

Chemistry of hydrazones:

Aliphatic compounds containing an activated methylene group couple with diazonium salt to form aryl hydrazone^{109,110}. The reaction is an electrophilic attack of the diazonium ion on the carbanion derivative from the methylene compound, forming an unstable azo compound which spontaneously tautomerises to a hydrazone.

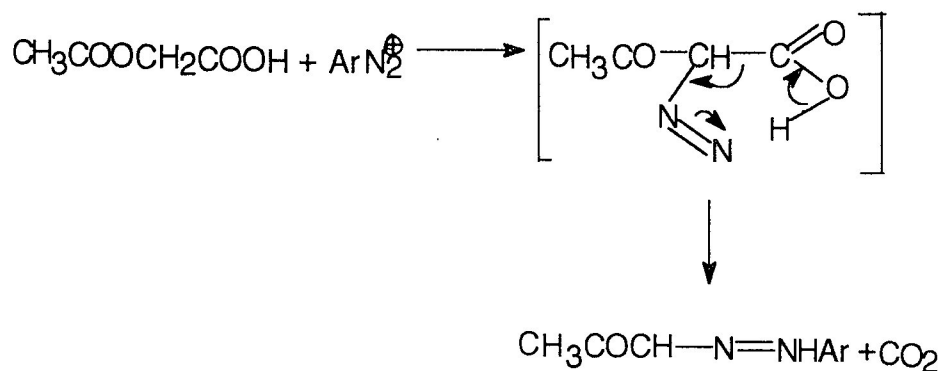


The reaction is usually carried out in cold aqueous solution buffered with sodium acetate. The pH of the medium can be lowered for strongly activated methylene compounds. Hunig and Boes¹¹¹ suggested the sequence of activating effect of the substituents (X and Y) as follows.



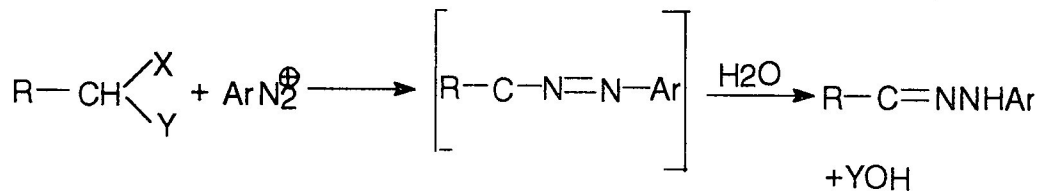
Other activating groups include the tertiary sulphonium and quaternary pyridinium ions and heterocyclics such as 2-pyridyl and 2-quinolyl.

When one of the activating group is a carboxyl, coupling is accompanied by decarboxylation.

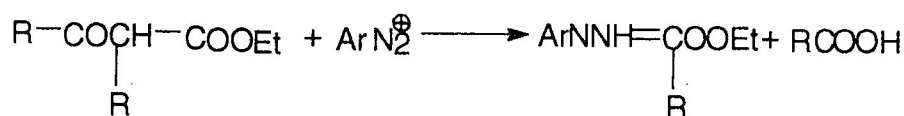
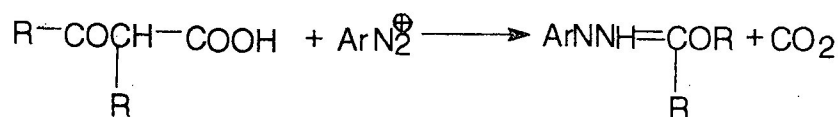


Compounds carrying active methyl group at the 2 or 4-position to the ring nitrogen such as 2-picoline¹¹² and 9-methyl acridine¹¹³, yield a tetrahydrazone on coupling with a diazonium salt. The activity of methyl group increases if the hetero atom is quaternized to onium salt prior to the attack of diazonium ion.

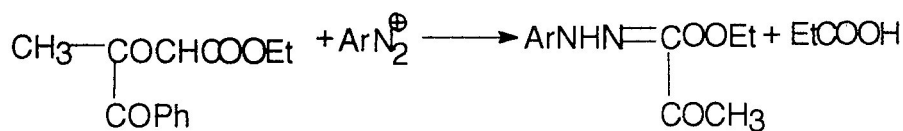
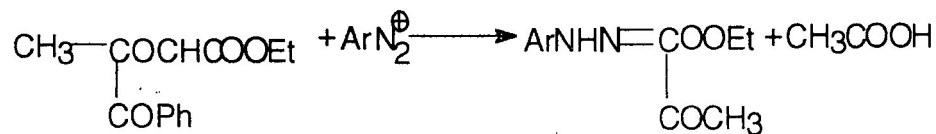
A methinyl group bearing at least two activating groups reacts with diazonium salt to give an aryl hydrazone by hydrolytic cleavage of one of the electron attracting groups on the intermediate unstable azo compound.



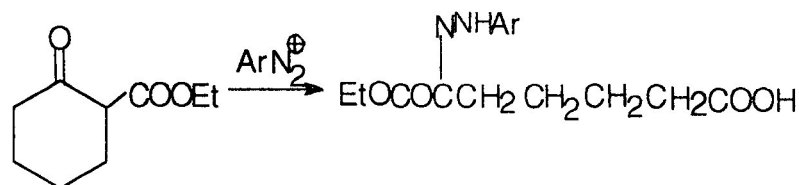
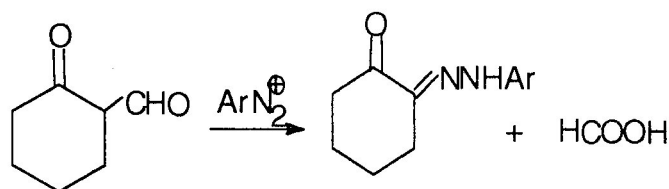
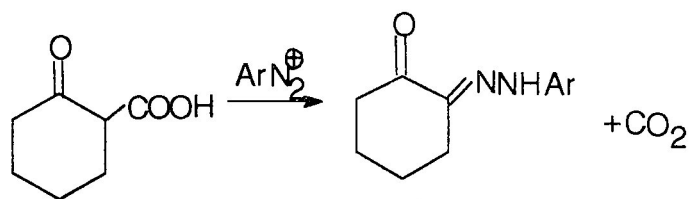
The reaction is described as Japp- Klingemann reaction^{110,114}. Groups that usually undergo splitting are carboxyl, acyl and alkoxy carbonyl. etc. e.g.



In diacylacetic ester, the acyl group is more rapidly cleaved which corresponds to the weaker acid¹¹⁵ e.g.



In case of 2-substituted cyclohexanones, various activating groups undergo splitting relative to the ring carbonyl. It is either side chain splitting, e.g. 2-carboxyl and 2-formyl or is a ring opening, e.g. ethoxycarbonyl and acetyl, which are bound more strongly^{114,116}.



*Friedel-Crafts acylation to form ketone,
Cinnoline and Cinnolone.*

Friedel-Crafts acylation:

Many acid chlorides can easily be induced to eliminate hydrogen chloride intramolecularly.

The cyclization is carried out in a suitable solvent alongwith a cyclizing agent. The cyclization agents or catalysts include¹¹⁷ AlCl_3 , Stannic chloride, hydrogen fluoride etc. The yield of the reactions varies from 40-90 % depending upon the reaction conditions like temperature, pressure and the reactants¹¹⁸.

The catalyst which is employed most frequently is AlCl_3 .

The most common solvents used are benzene and carbon disulfide. Others such as petroleum ether, sym- tetrachloroethane, monochlorobenzene etc. are also employed.

Stannic chloride:

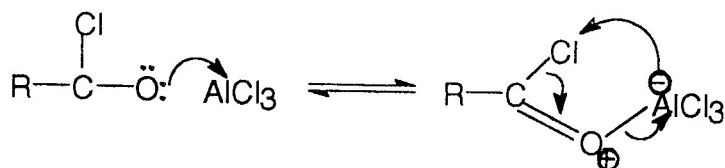
Cyclization with stannic chloride is generally effective in bringing about ring closure into polycyclic nuclei.

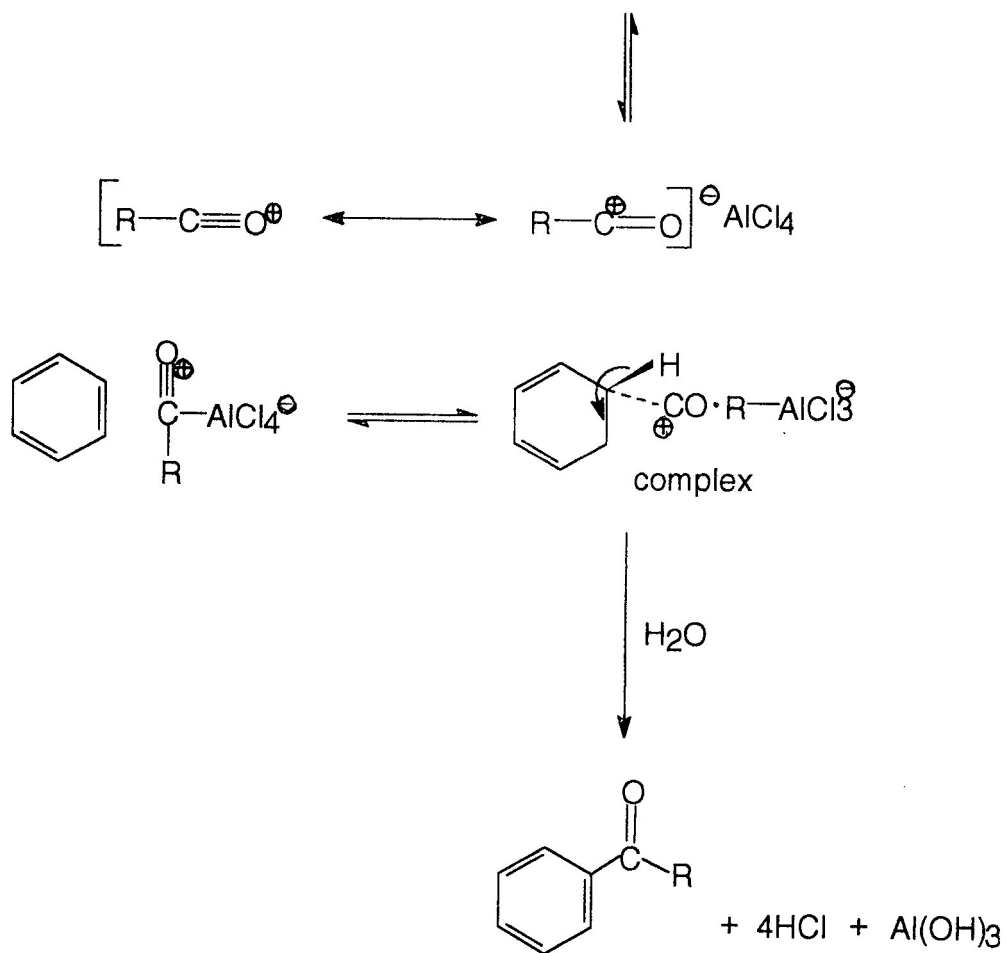
The acid is reacted first with reagents like phosphorus pentachloride¹¹⁹, thionyl chloride etc. to form acid chloride, which is then reacted with stannic chloride under anhydrous conditions.

Aluminium chloride:

The most widely used agent for cyclization is aluminium chloride. Several types of aryl phosphoric acids and aryl butyric acids have been cyclized by this method. The reagents used include phosphorus pentachloride, thionyl chloride etc. and the reaction occurs under anhydrous conditions.

The mechanism of the reaction is same as that of acid chloride and can be explained as follows¹²⁰:





Formation of cinnolone:

To ethyl-2(4-chlorophenyl hydrazono)-3-oxybutyrate (30gm,0.112mole) was added anhydrous AlCl_3 (29.83gm,0.223mole). Chlorobenzene (360ml) was added in order to dissolve the solids and then the mixture was refluxed for 12 hours. It was cooled and dilute HCl (200ml) was added to it and was heated on a water bath. Further it was cooled, filtered and washed twice with dil. NaOH soln. and filtered. Then the product was recrystallized from methanol.

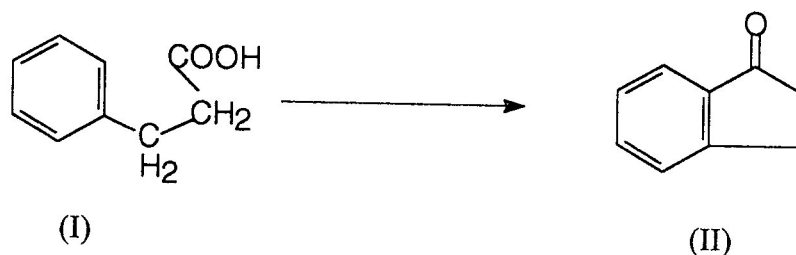
M.P.=197°C

Yield =19.05gm,63.5%

Other methods for cyclic ketones:

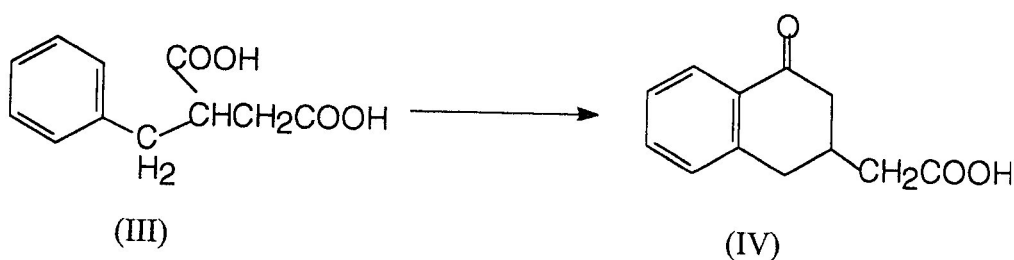
1) From hydrogen fluoride:

In 1939, Fieser and Hershberg¹²¹, cyclized β -phenyl propionic acid (I) using anhydrous liquid hydrogen fluoride to yield 73 % of ketone by intramolecular acylation.



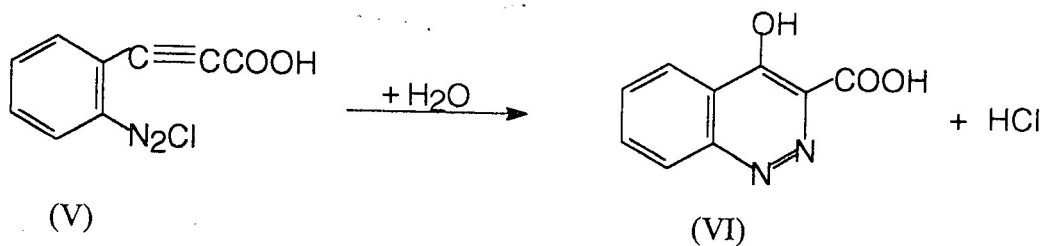
2) From sulphuric acid:

γ -aryl butyric and β -aryl propionic acid (III) get cyclized to ketone (IV) in the presence of sulphuric acid¹²².

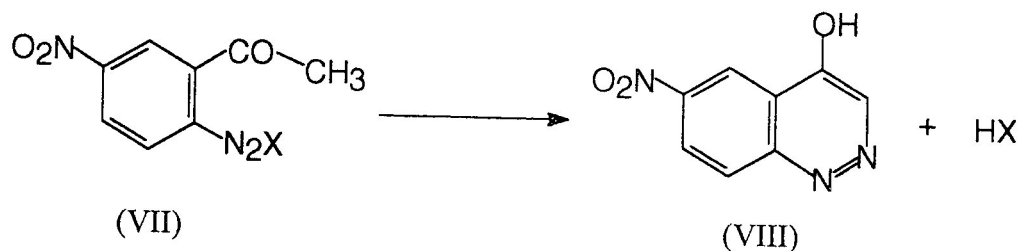


Methods for cinnoline:

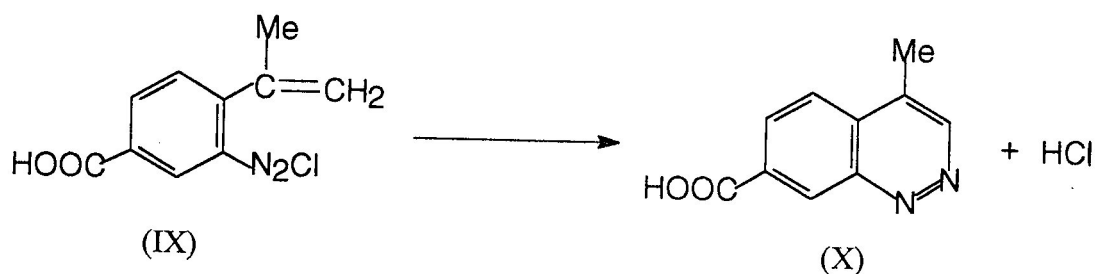
1) The cinnoline ring system was discovered in 1883 by Richter¹²³, who converted diazotized o-nitro phenyl propionic acid (V) into 4-hydroxycinnoline-3-carboxylic acid (VI).



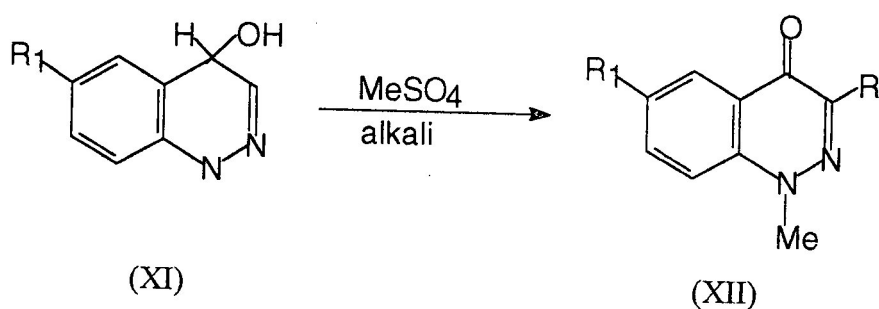
2) Brosche and Herbert¹²⁴ found that diazotized 5-nitro-2-amino acetophenone (VII) slowly on standing, yields 6-nitro-4-hydroxy cinnoline (VIII).



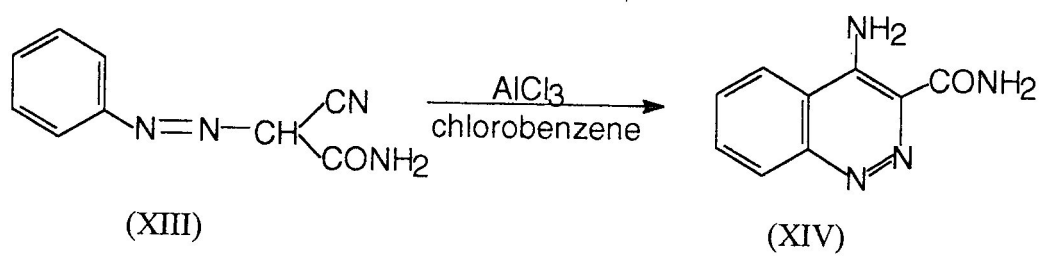
3) Widman¹²⁵ prepared 4-methyl cinnoline-7-carboxylic acid (X) by diazotization of 1-methyl-1,2'-amino-4'-carboxy phenyl ethylene (IX).



4) Methylation of 4-hydroxy cinnoline (XI) with methyl sulphate and alkali gives 1-methyl-1,4-dehydro cinnoline-4-one (XII)¹²⁶.



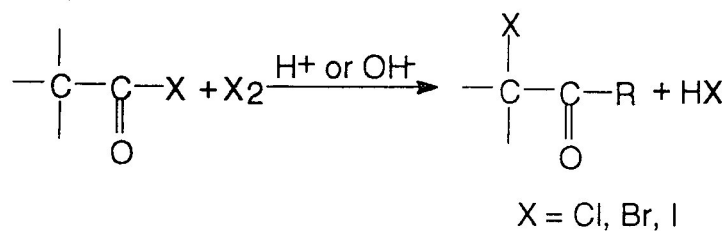
5) Aminocinnoline carboxamide (XIII)¹²⁷ was prepared by cyclization of aryl hydrazono (cyano) acetamide (XIV) with anhydrous AlCl_3 in chlorobenzene.



Halogenation of ketones

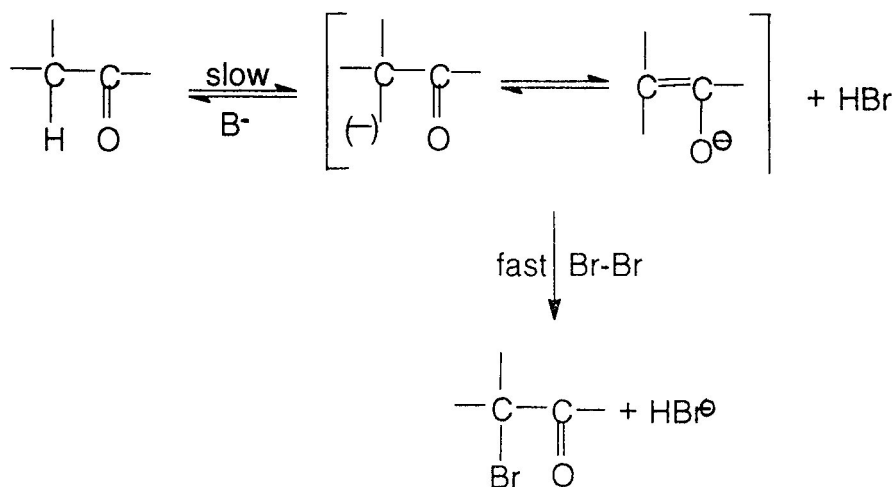
Halogenation of ketones:

Ketones that possess α -hydrogen can readily be replaced by halogen in the presence of acidic as well as basic medium.



Base-promoted halogenation of ketones:

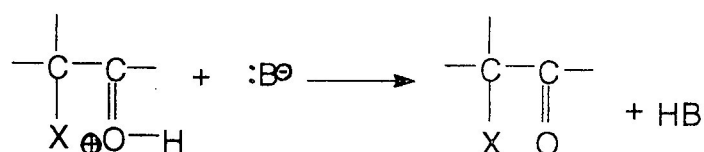
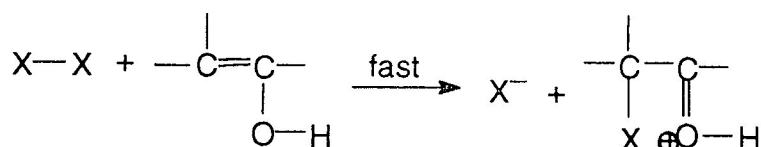
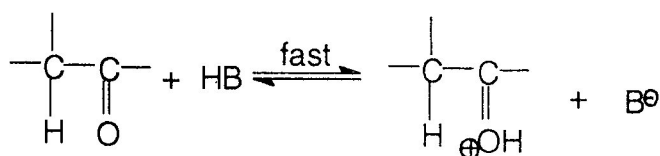
A base strongly abstracts a proton from a ketone to form carbanion (I) which then rapidly reacts to yield bromoketone(II).



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

Acid-catalysed halogenation:

Acids also catalyse the halogenation of ketones.



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

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

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

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

Bromination:

In case of bromination, 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 favours the attack of polarized Br₂ molecule or Br⁻ on the enol form of the ketone.

Synthesis of 6-chloro-3bromoacetyl-1H-cinnoline-4-one:

6-chloro-3-acetyl-1H-cinnoline-4-one (25gm, 0.112 mole) was dissolved in acetic acid(150 ml) and bromine (13.48 gm, 0.16 mole) dissolved in acetic acid(15 ml) was added dropwise. The reaction was provided with slight heating (60° - 65° C) and a source of light. The reaction was continued for 6-7 hours with constant stirring.

Dry air was passed to remove HBr vapours. It was then dumped on ice cold water and the solid was filtered, dried and recrystallized from methanol.

M.P.= 115° C.

Yield = 63.91%

Nitriles

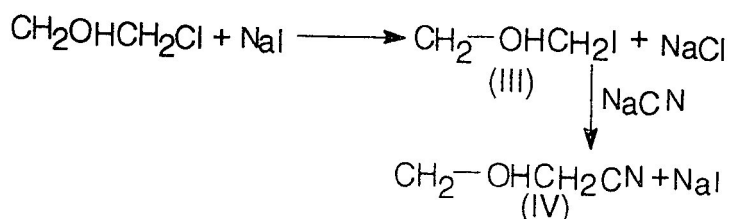
Chemistry of nitriles:

Synthesis:

1] From halides:

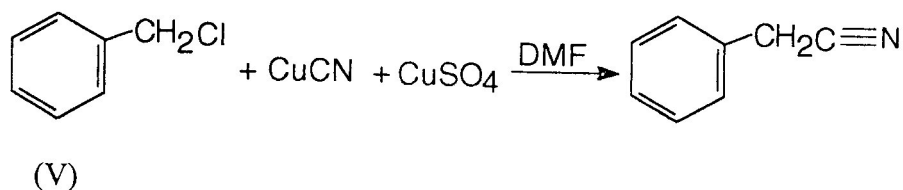
Aliphatic halides:

Metal cyanide reacts with alkyl or aryl halide (III) to form aliphatic nitrile(IV). The order of reactivity of the halide is $-I > -Br > -Cl$. Sodium iodide, when used as a promoter, converts chloride to iodide in the primary halide by exchange and this in turn gives nitrile with metal cyanide.

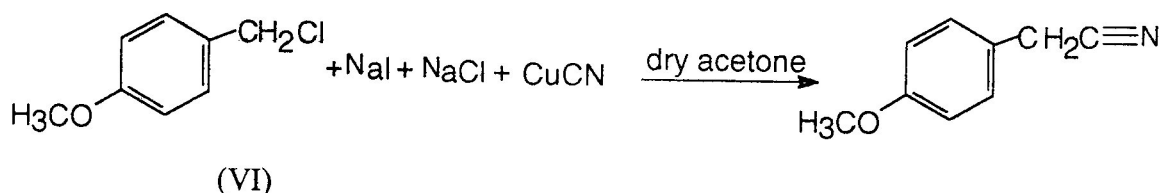


Other metal cyanides which form nitriles are potassium and cuprous cyanide.

The yield varies from 75-90%. The solvents like ethylene glycol¹²⁸, methyl cellosolve¹²⁹, tetrahydrofurfuryl alcohol¹³⁰ and DMF improve the yield as compared to alcohol which causes slow reaction. Secondary halides give lower yield and tertiary halides give a little or no nitrile. The conversion of alkyl halide(V) into nitrile is accomplished best with sodium or cuprous nitrile with pyridine, quinoline, DMF or N-methyl pyrrolidine as solvent¹³¹. The reaction can be accelerated by addition of small amount of a nitrile and traces of copper sulphate as a catalyst.



Halide, like anisyl chloride (VI) yields 75% of nitrile¹³².

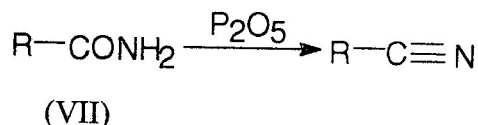


B) From heterocyclic halides:

Halides of pyridine¹³³, quinoline¹³⁴ give nitrile in satisfactory yields with cuprous cyanide¹³⁰.

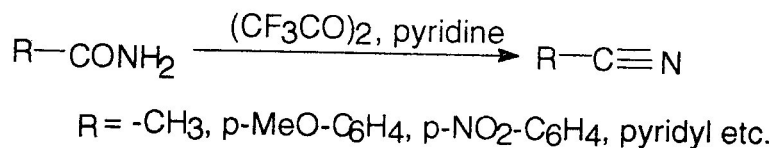
2] From amide:

Amide (VII) gives nitrile in 60-70% yields with a dehydrating agent like phosphorous pentoxide¹³⁵ or phosphorous pentachloride¹³⁶.



Sometimes dehydrating agents like carbonyl chloride¹³⁷, benzene, p-toluene sulphonyl chloride^{138,139}, phosphorous oxychloride are used in the nucleophilic solvent such as pyridine or tertiary amine. Even addition of salt like sodium chloride¹⁴⁰ or sodium metabisulphite¹⁴¹ to phosphorous oxychloride enhances the effectiveness of the reagent.

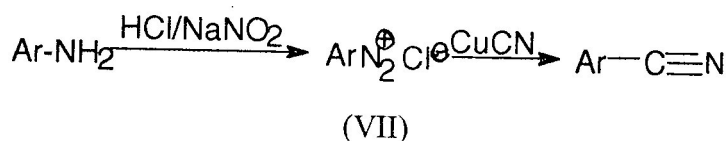
Recently, a more convenient method employing trifluoroacetic anhydride/pyridine system¹⁴² was found to lead a rapid reaction with 85-95% yield.



With excellent yield and mild reaction conditions, the method is especially suitable for thermolabile nitriles.

3] From diazonium salts: (Sandmeyer's reaction)

Diazonium compounds (VII) with cuprous cyanide give 65-85% of nitrile.



Complex salt of cuprous cyanide such as $\text{Na}_3\text{Cu}(\text{CN})_5\text{NH}_3$ ¹⁴³ has also been used to a great extent. Prior neutralization of diazonium salt is necessary in order to avoid escape of halogen cyanide o-tolunitrile¹⁴⁴.

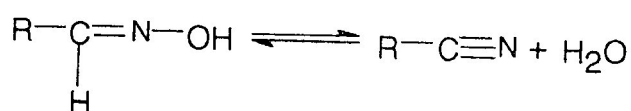
4] From aldehyde:

Several methods have been reported for conversion of an aldehyde to nitrile. e.g.
A) dehydration of aldoxime using carbodimide¹⁴⁵, carbonyl biimidazole¹⁴⁶ or phosgene imino chloride¹⁴⁷.

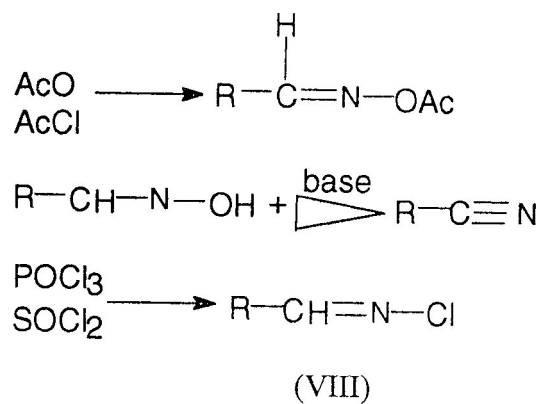
B) 1,2 elimination of o-substituted aldoxime^{148,149} and

C) 1,2 elimination of $\text{R-CH=N-N}-(\text{CH}_3)_3$ and R-CH=N-tosyl by base¹⁵⁰.

A] Dehydration of aldoxime:

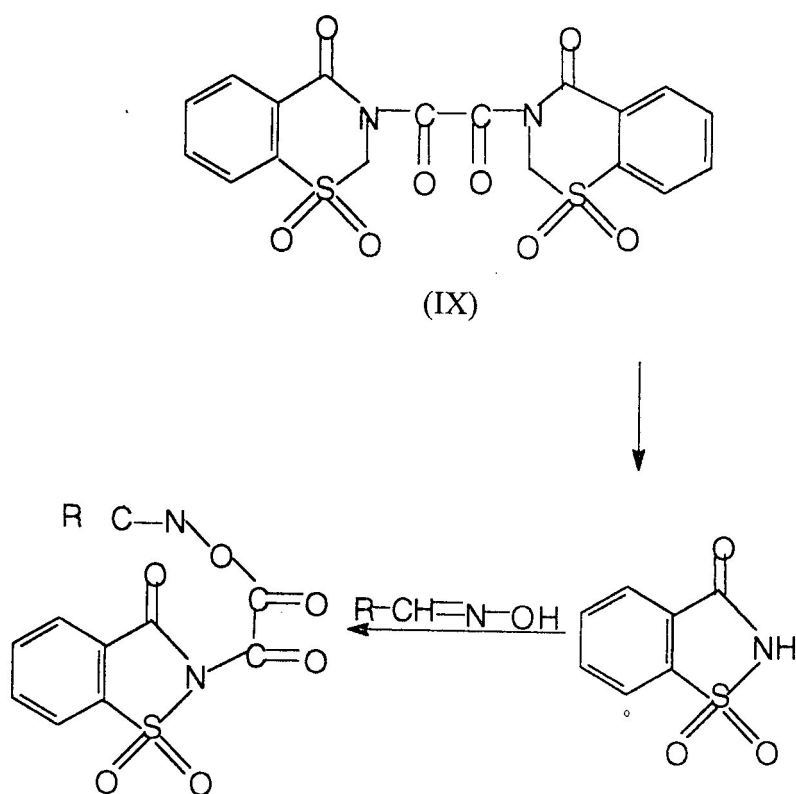


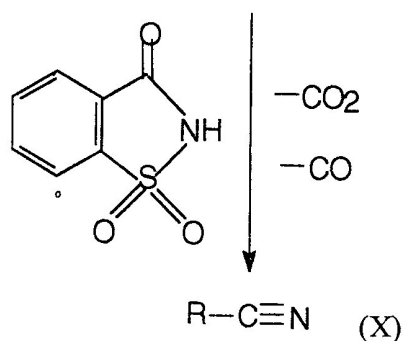
A large number of dehydrating agents have been employed for this preparative method¹⁵¹. e.g. acetic anhydride, phosphorous oxide, thionyl chloride, acetyl chloride etc. The dehydrating agent forms a reactive complex (VIII), which renders the aldoxime easily with the loss of water molecule.



Relatively new dehydrating agents have been introduced. e.g. dicyclohexyl carbonyl biimidazole¹⁴⁵ and phosgene immonium chloride¹⁴⁷.

Kilagawa, T. *et.al.*¹⁵² described a new dehydrating agent, 2-2'-oxalyldi (o-sulfobenzimide) (ODS)(IX), for convenient synthesis. The possible mechanism proposed is as follows-



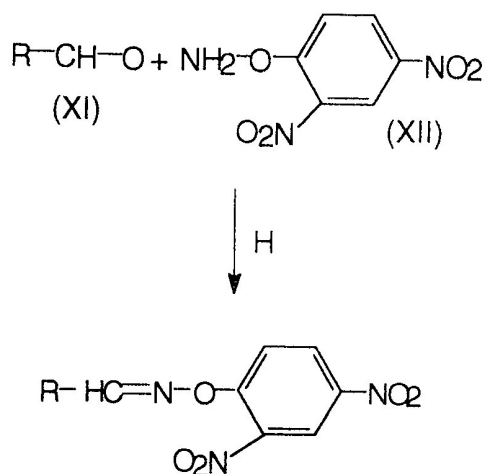


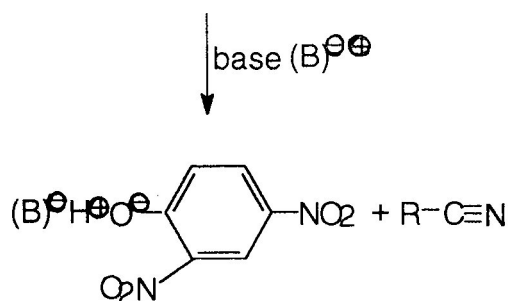
The reaction is carried out at room temperature or in cold.

B] 1,2-elimination of o-substituted aldoxime:

Miller *et.al.*¹⁴⁹ described this method which does not need to isolate the intermediate oxime.

The development of efficient amino protecting groups has facilitated the synthesis of a variety of sensitive compounds including o-substituted hydroxyl amine¹⁵³. Although a number of these are unstable¹⁵⁴, others have significant synthetic utility. Their electrophilic character has been exploited in their increasing use as aminating agents¹⁵⁵⁻¹⁵⁷. Their nucleophilicity has been demonstrated by their reaction with ketones to form substituted oximes. This 2,4-dinitrophenyl hydroxyl amine (XI) can be reacted with aromatic or aryl aldehyde (XII) to form corresponding o-substituted aldoxime.

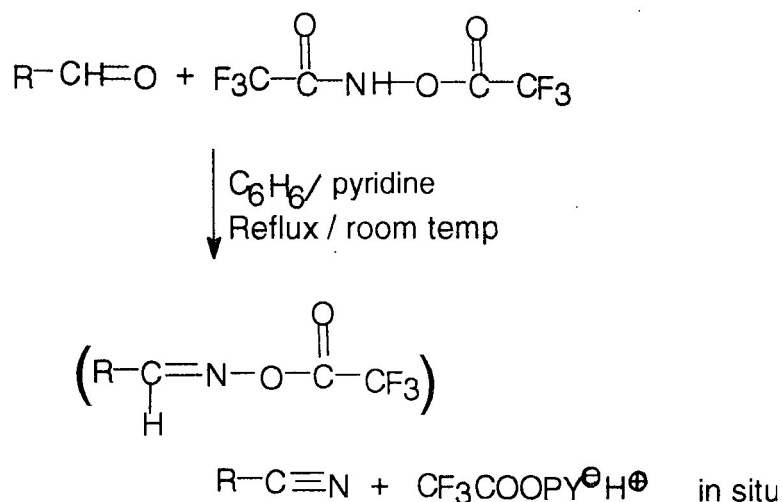




Nitriles can be prepared from these o-substituted aldoximes by 1,2-elimination reaction using excess base as shown above. The development of deep yellow to red colour of the 2,4-dinitrophenolate ion indicates the extent of completion of the reaction.

C] Method C:

This is a one step reaction involving the use of o-N-bis(trifluoroacetyl) hydroxyl amine (XII) as one of the reagents.



The reaction conditions are very mild and directly afford the nitrile, eliminating the intermediate step¹⁵⁸. The yields are in the range of 70-90%. The reagent could be obtained by reacting trifluoro acetic anhydride with hydroxyl amine hydrochloride in suitable solvent. The aldoxime is unstable and hence, cannot be isolated. It instantly decomposes to furnish the nitrile.

Synthesis of 4-N,N-dimethyl amino phenyl benzonitrile:

4-N,N-dimethyl amino benzaldehyde (10gm,0.067mole) was dissolved in warm ethanol (30ml) and stirred for 15 min. To it was added hydroxylamine hydrochloride (5.59gm,0.0804mole) in water (20ml) and stirred for 20 min. at room temp. Sodium hydroxide (4.13gm,0.1mole) dissolved in water (20ml) was added dropwise to this soln. and it was stirred for 4 hrs. Ethanol was removed to half the quantity under reduced pressure and the reaction mixture was dumped on crushed ice. The solid obtained after filtration was oxime.

Aldoxime (6gm,0.036mole) was dissolved in acetic anhydride (7.5gm,6.93ml) and refluxed for 4 hrs. using dry conditions. It was then cooled and poured on crushed ice. The solid was filtered and washed with cold water. It was recrystallized from methanol.

M.P.=212⁰-213⁰C

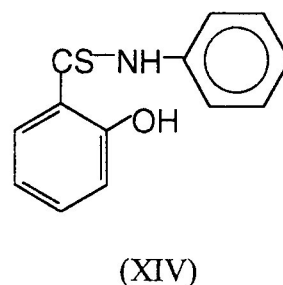
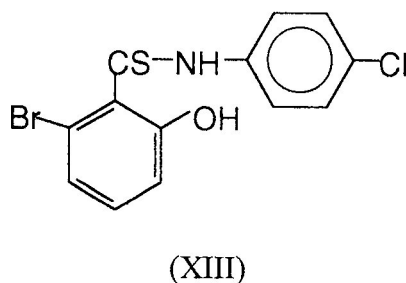
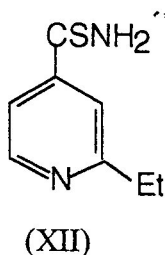
Lit.M.P.=215⁰-216⁰C²³¹

Yield = 77.48%.

Thioamides

Chemistry of Thioamides:

Liberman and coworkers in 1955, observed the *in vivo* activity of Ethionamide (XII) against *M. tuberculosis* and it exhibited low toxicity. Later on therapeutically effective agents such as substituted thiobenzamide (XIII), salicylic acid thioamide (XIV) were found.



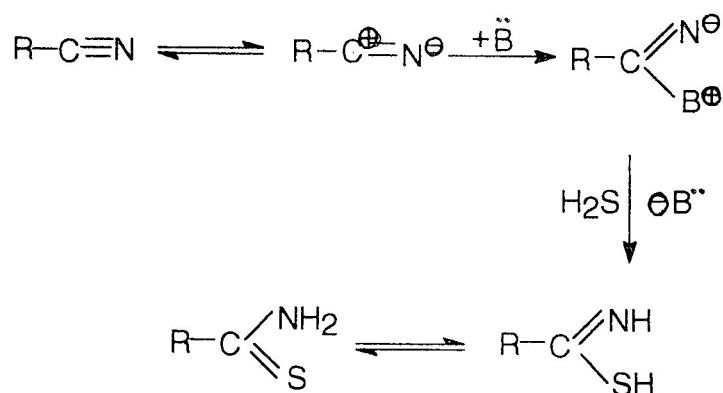
Synthetic methods:

1] From nitriles:

Nitriles in the presence of excess of hydrogen sulphide and a catalyst base yield thiocarboxide^{159,160}.

Water soluble sulphides or polysulphides can be used instead of hydrogen sulphide¹⁶¹

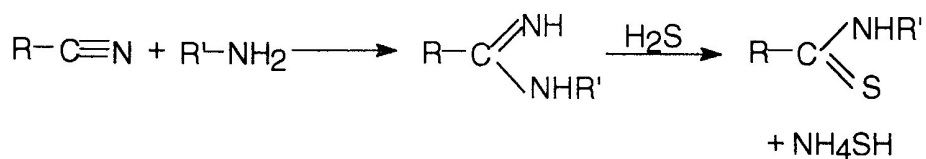
The reaction occurs as follows-



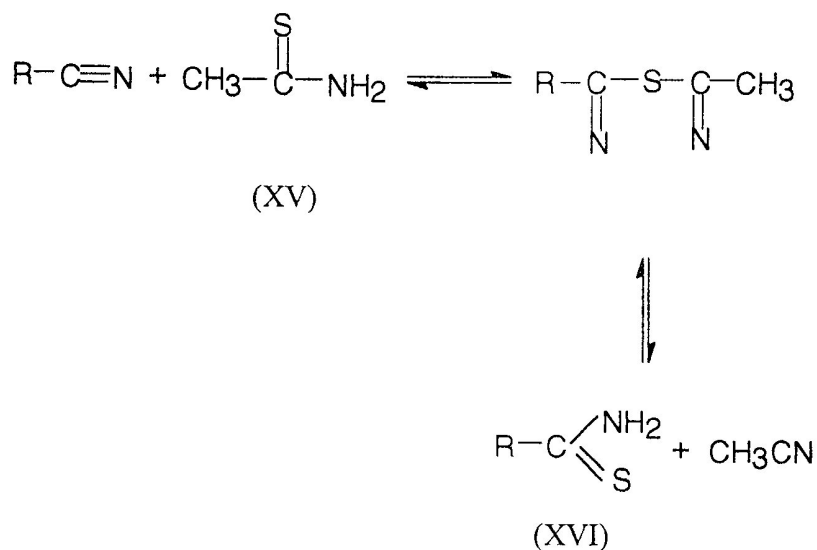
Catalysts used include ammonia, aliphatic amine, pyridine, alkoxide, potassium hydrogen sulphide^{162,163} etc. Fairfull *et.al.*¹⁶⁴ obtained very good yields by addition of H₂S to nitrile in presence of pyridine and triethylamine.

Aliphatic nitriles need elevated temperature and pressure¹⁶⁵.

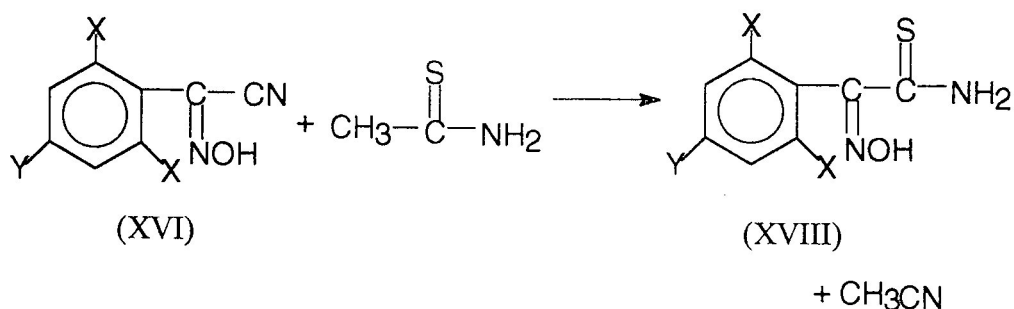
Kindler¹⁶² and Hanford¹⁶⁶ extended the method by addition of primary amine.



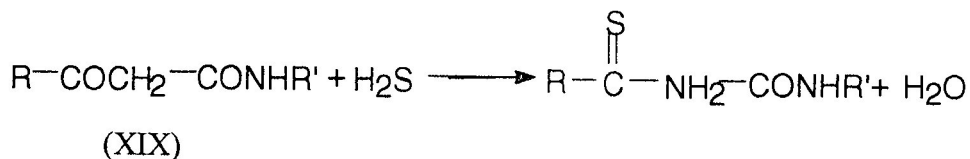
Reactions of nitriles with thioacetamide (XV) appeared more frequently in literature than with H₂S^{167,168}.



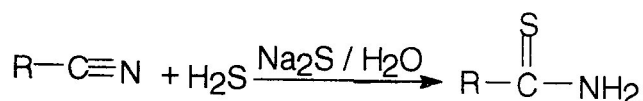
Removal of acetonitrile leads to the formation of thiocarboxamide (XVI) and thus is important for preparation of thiocarboxamide from α-cyanobenzaldoximes (XVII).



If the nitrile contains hydroxyl group, it must be protected by acylation^{169,170}. The keto group such as that in (XIX) if present is readily converted to thioxo in acidic solution¹⁷¹.



Mayer *et.al.* explained the base-catalyzed conversion of ketone to thiones¹⁷². Recently Casser *et.al.*¹⁷³, introduced aqueous sodium sulphide as a phase transfer catalyst in the general procedure to get high yields of thioamide.



This system consists of three phases such as aqueous, organic and H₂S gas.

2] From amides:

Replacement of oxygen of carboxamide by sulphur is mostly used to obtain N-monosubstituted and N-disubstituted compounds in presence of P₂S₅¹⁷⁴. Potassium thiophosphate alongwith P₂S₅ enhances the yield further¹⁷⁵.

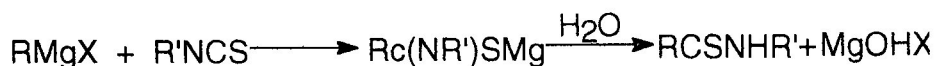


Solvents such as benzene, xylene, toluene, tetrahydrofuran, dioxane, diethylene glycol and diethyl ether¹⁷⁶⁻¹⁷⁹ are commonly used and these should be dry and inert in order to avoid undesirable side reactions and to reduce time.

Reactions in pyridine give pure thioamide in higher yields^{180,181}. Reactions of liquid amides are carried out in an excess of amide as solvent^{182,183}. Thermally unstable amides are not suitable for this reaction. Hydroxyl group must be protected by acylation before sulphurization^{184,185}.

3] From isothiocyanates:

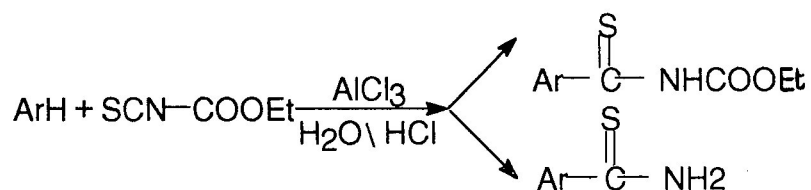
Sachs and Loevy¹⁸⁶ described the reaction of isothiocyanates with Grignard's reagent and subsequent hydrolysis under mild conditions to lead thiocarboxamide.



4] Single step synthesis of aromatic thioamides:

a) From ethoxy carbonyl isothiocyanate:

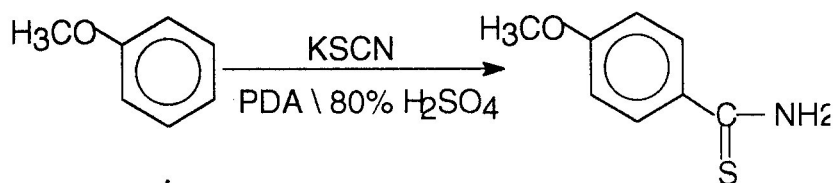
Ethoxy carbonyl isothiocyanate¹⁸⁷ reacts with aromatic compounds in the presence of anhydrous AlCl_3 (Fridel-Craft isothiocyanation). However, depending upon the conditions, it either yields the N-ethoxy carboxy thioamide or thioamide itself.



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

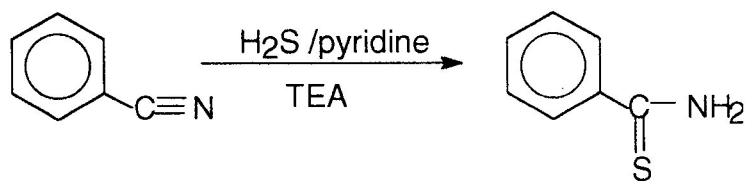
Reactions at a low temperature lead to N-ethoxy carbonyl thioamide.

B)Thioamides can also be prepared by heating anise (0.01N) or veratrol at 60°C in the presence of polyphosphoric acid or 80% H₂SO₄ with potassium thiocyanate (0.01N)¹⁸⁸.



50%yield in PPA can be increased by 5-10% with 80%H₂SO₄.

Solution of aromatic nitrile (6gm,0.0582 mole) in pyridine (30 ml) was taken in a three necked round bottom flask. Dry gaseous H₂S was bubbled through the reaction mixture containing triethylamine (4ml) as catalyst. H₂S was generated by the action of conc.HCl on ferrous sulphide. The reaction took 8-12 hours to complete, with yields of 70-80% at alkaline pH.



M.P. =116°C

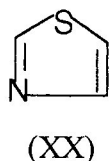
Lit. M.P. = 116⁰-118⁰C²²¹

Yield=78%.

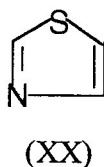
Thiazoles

Chemistry of Thiazoles:

The thiazole heterocycle (XX) was first delineated by Hantzsch and Weber¹⁸⁹. Further, they have described it as 'the pyrrole of the thiophene series'.



The thiazole moiety exists in two different configurations as (XX) and (XXI).



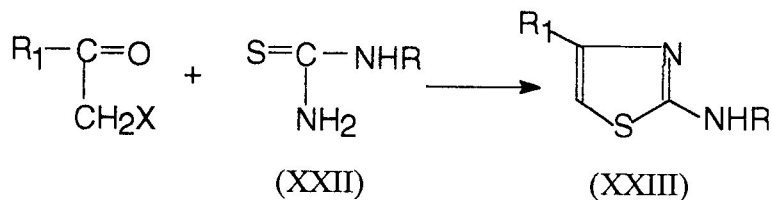
The high reactivity of the thiazole molecule is due to the group attached to the carbon between sulphur and nitrogen.

Large number of thiazoles occur naturally. They include Vitamin B (thiamine), Penicillin etc. Their origin has often been related to that of a peptide chain at cysteine residue with formation of a thiazole.

General methods for synthesis:

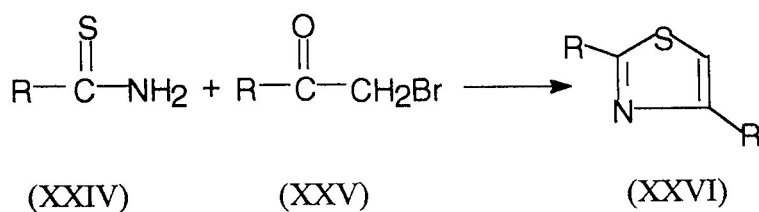
1] Synthesis of 2-substituted amino thiazoles:

The cyclization of N-monosubstituted thiourea (XXII) with halocarbonyl compound gives 2-monosubstituted (XXIII) thiazole^{191,192}.



2] 2,4-disubstituted thiazoles:

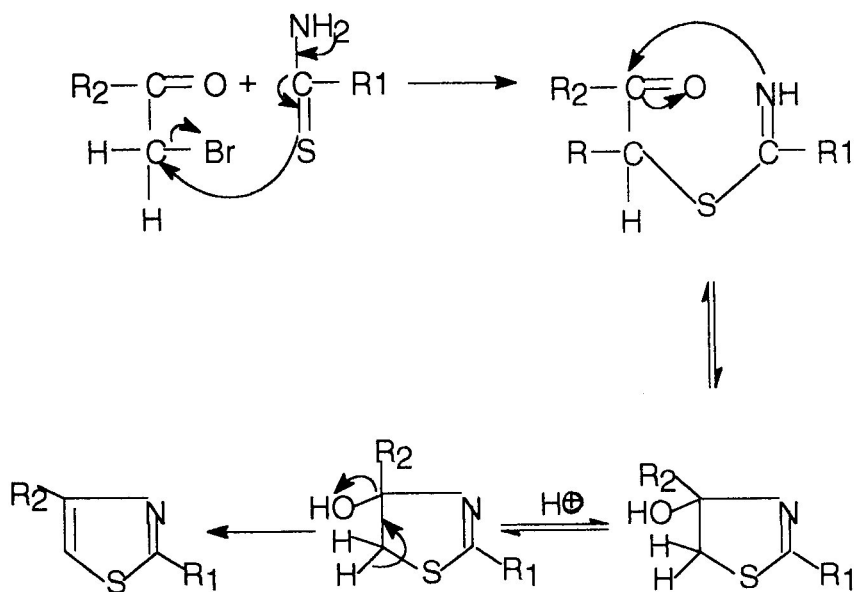
These were prepared in 1950 by Erylenmeyer *et. al.*¹⁹³ from thiobenzamide (XXIV) and bromoacetophenone (XXV) in alcohol as a solvent which yielded 50-80% of thiazole (XXVI).



Mechanism:

The first step involves 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 step II, the lone electron pair of N-atom attacks the carbon carrying the carbonyl function, giving rise to a new cyclic intermediate that reacts with acid leading to the thiazole molecule^{194,195}.



Synthesis of 3(2-alkyl/ aryl-4-substituted)-6-chloro-cinnoline-4-one:

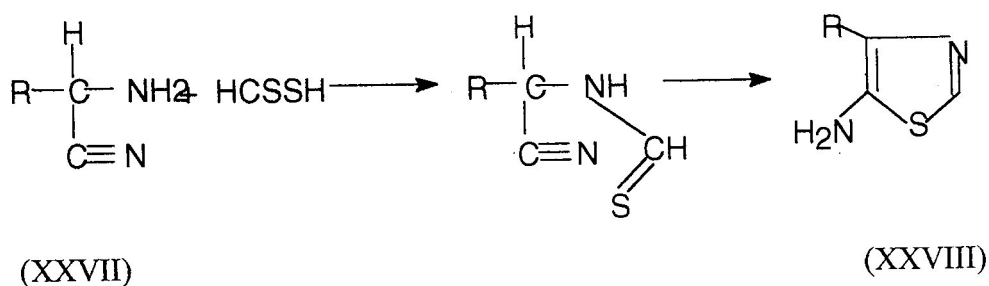
3-Bromoacetyl-6-chloro cinnoline 4-one (2 gm, 0.066 mole) and thiobenzamide (1.49 gm, 0.099 mole) were dissolved separately in ethyl alcohol and then mixed. This mixture was then refluxed for 48-50 hours to yield a thiazole with a characteristic odour. Alcohol was then removed under reduced pressure and the product was recrystallized using methanol as the solvent.

M.P.= 110°C

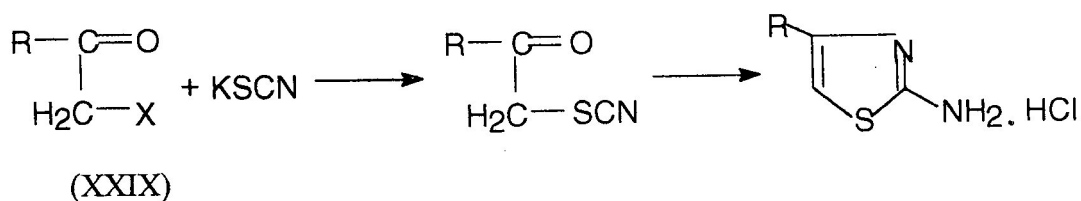
Yield= 70.66%

Other synthetic methods for thiazole:

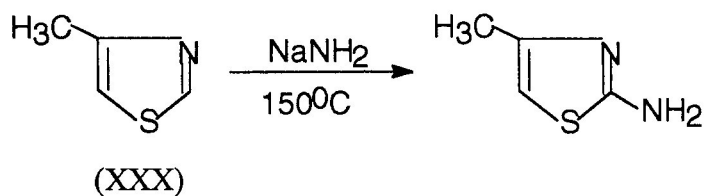
1] α -amino nitrile (XXVII) reacts with dithioformic acid (XXVIII) at room temperature to give an intermediate which cyclizes to yield 5-amino thiazole derivative¹⁹⁶.



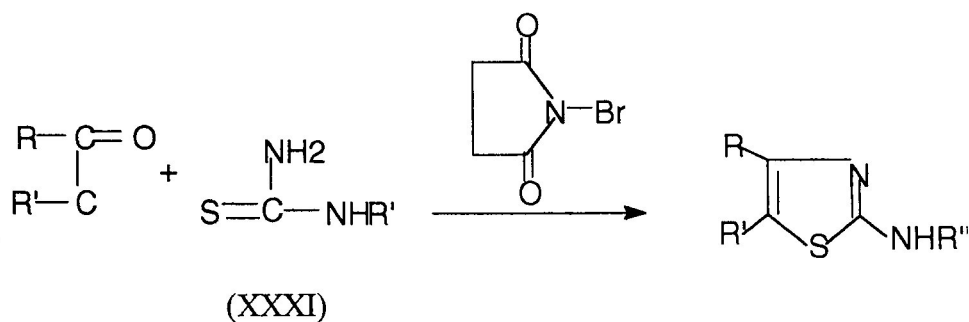
2] Potassium thiocyanate reacts with halocarbonyl compound (XXIX) to give 2-amino thiazole hydrochloride¹⁹⁷.



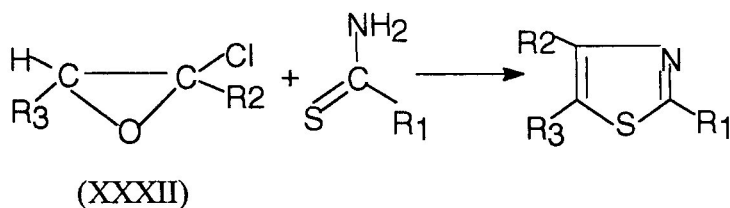
3] 4-Methyl thiazole (XXX) on heating with sodamide for 15 hours at 150°C yields 2-amino-4-methyl thiazole¹⁹⁸.



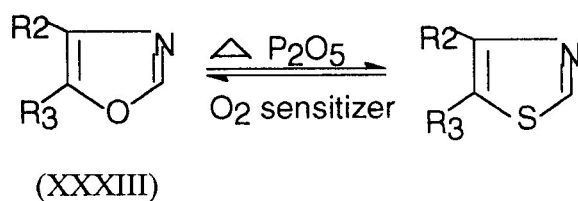
4]Reaction of substituted thiourea (XXXI) with ketone in the presence of N-bromosuccinimide furnishes 2-aminothiazole derivative¹⁹⁹.



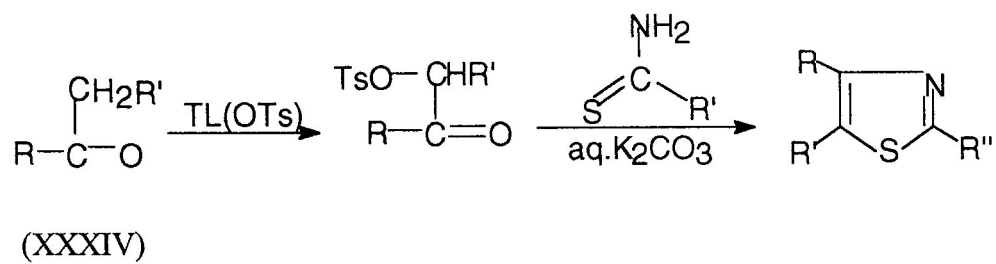
5]1-chloro 1,2 epoxide (XXXII) when condensed with thioamide leads to 2,4,5-trisubstituted thiazole²⁰⁰.



6]Oxazole (XXXIII) when heated with P_2S_5 gives a thiazole²⁰¹.



7]Enolized ketone (XXXIV) reacts with thioamide or substituted thioamide in acetonitrile in the presence of thallium p-tolyl sulphonate (TTS) to give 2,4,6-trisubstituted thiazole²⁰².

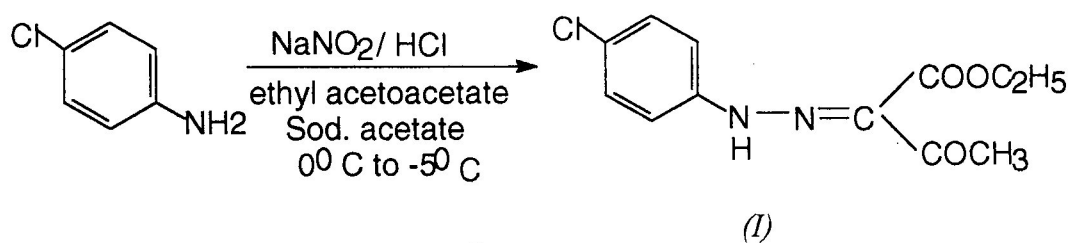


Schematic representation

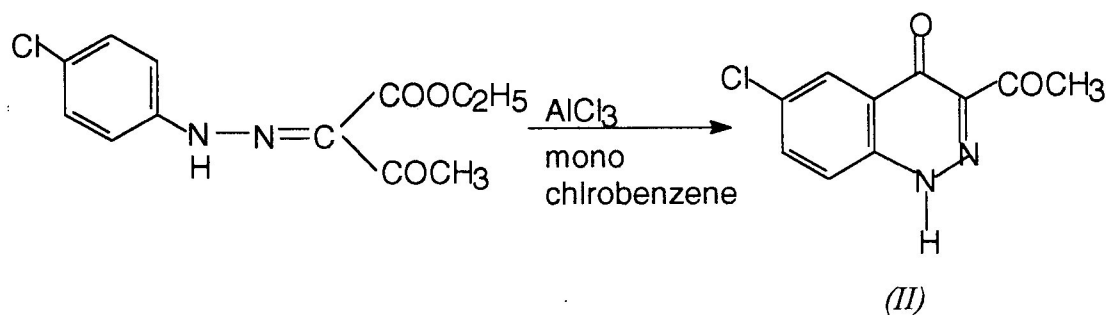
Schematic representation of the compounds synthesised:

Scheme I: Synthesis of 3(2-alkyl/ aryl,4-substituted thiazolo)-6-chloro-cinnoline-4-ones:

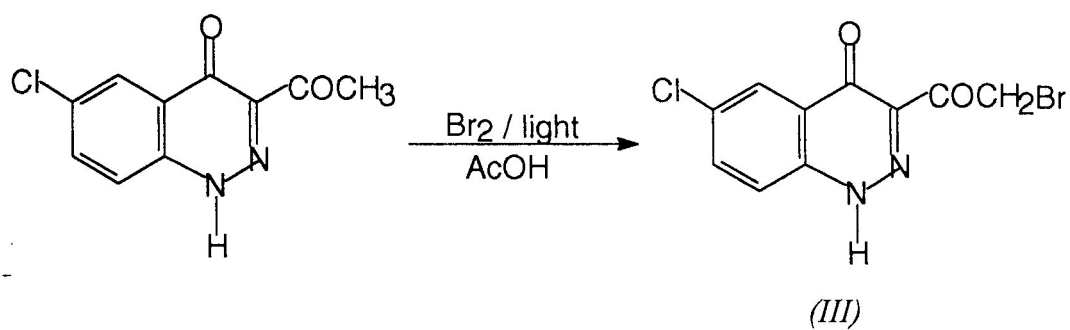
Step I: Synthesis of ethyl-2(4-chlorophenyl hydrazono)-3-oxybutyrate(I):



Step II: Synthesis of 3-acetyl-6-chloro-cinnoline-4-one(II):

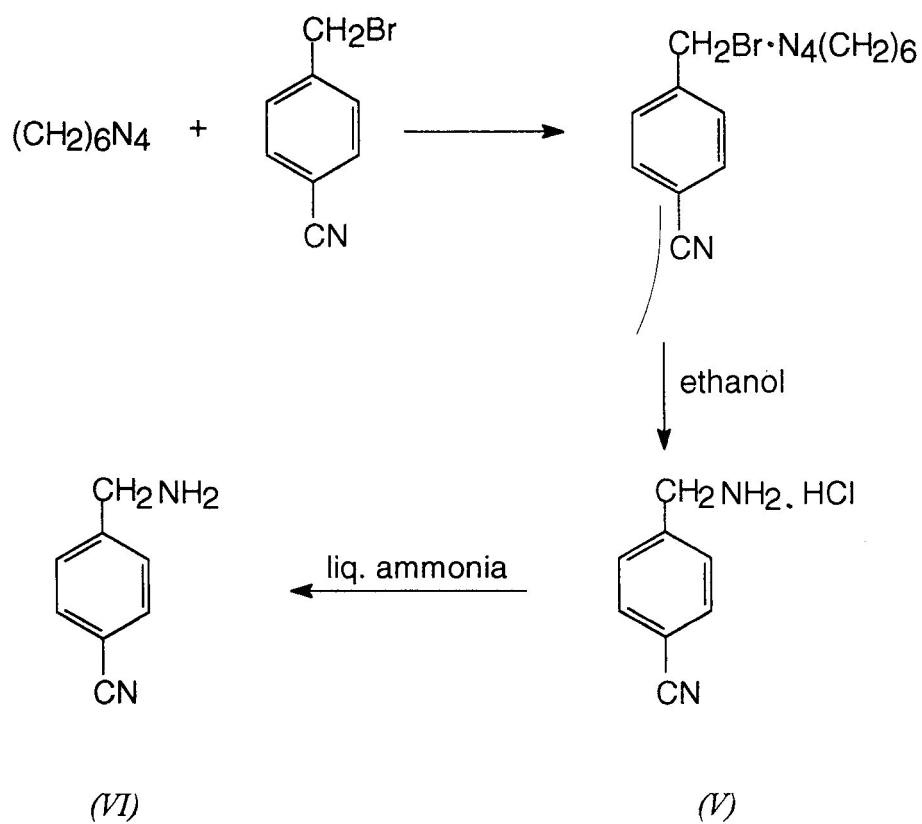


Step III: Synthesis of 3-bromoacetyl-6-chloro-cinnoline-4-one(III):

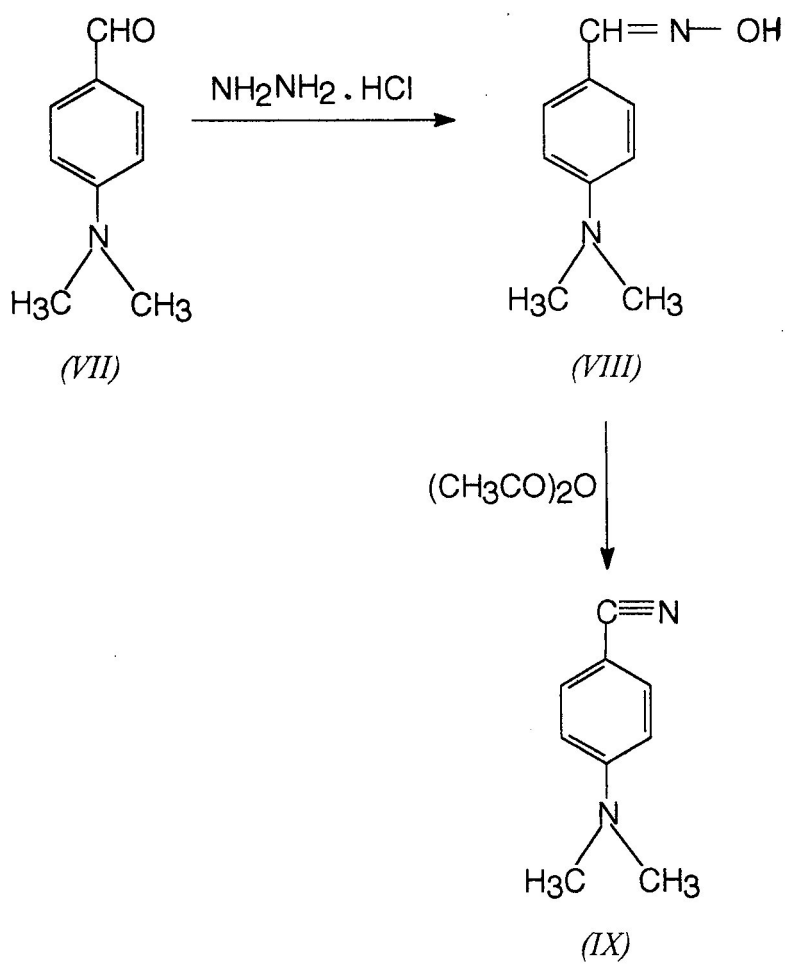


Scheme II: Synthesis of nitriles:

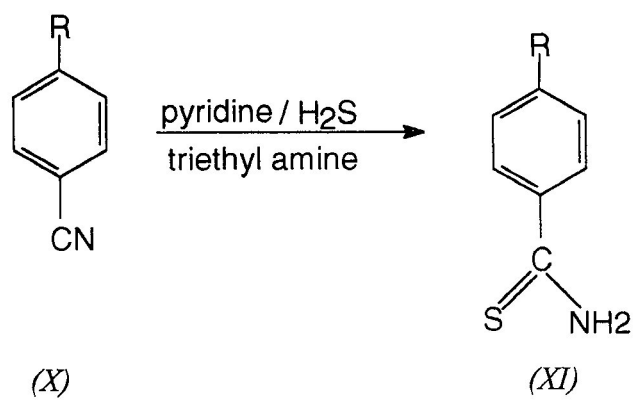
I) Synthesis of 4-amino benzonitrile(VI):



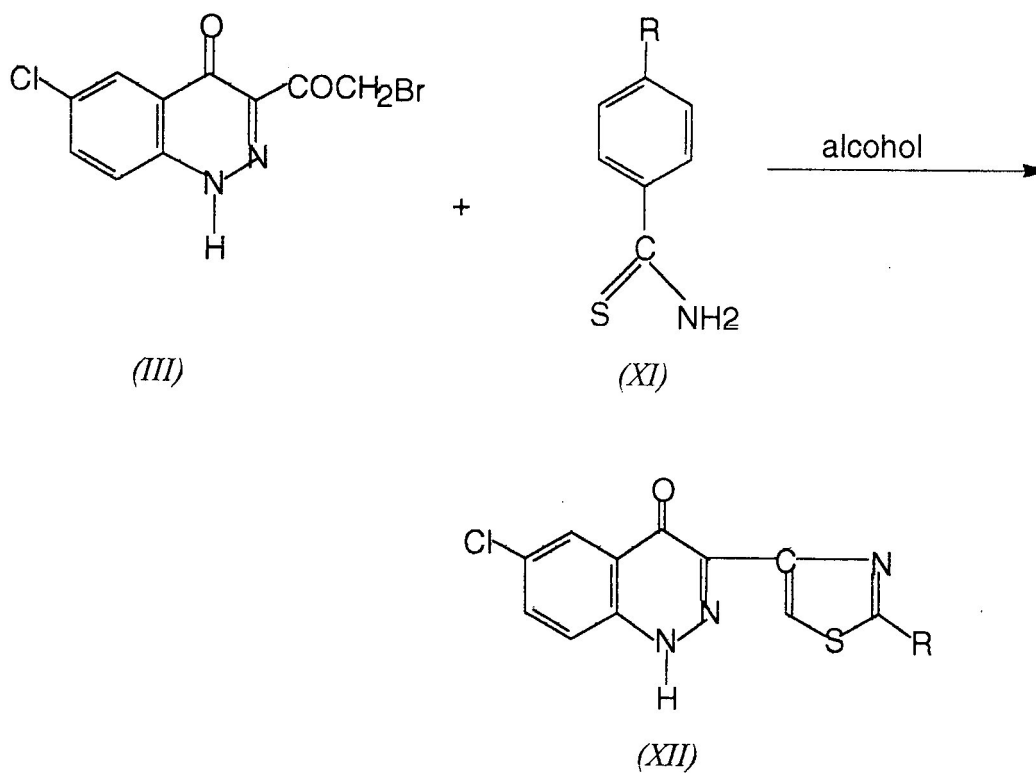
II) Synthesis of *N,N*-dimethyl amino benzonitrile(IX):



Scheme III: Synthesis of thioamides (XI):

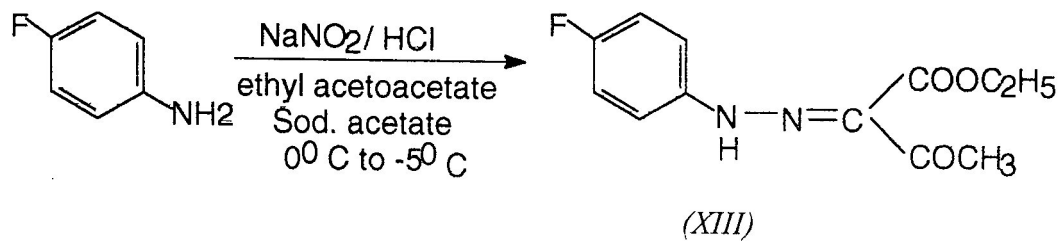


Scheme IV: Synthesis of thiazoles (XII):

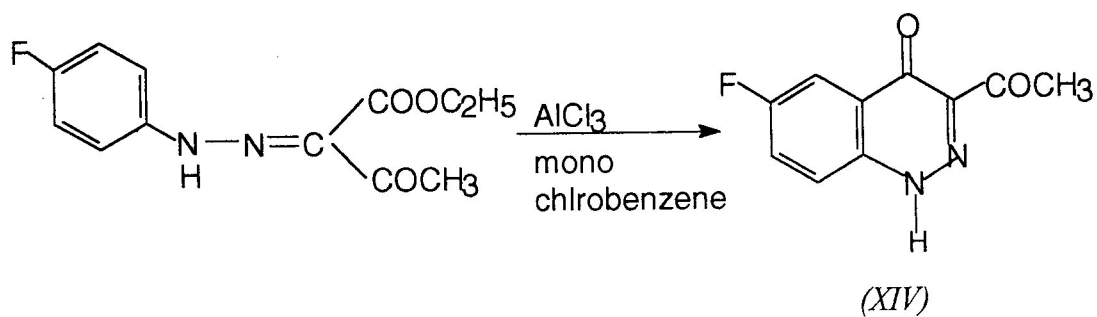


Scheme V: Synthesis of 3(2-alkyl/ aryl,4-substituted thiazolo)-6-fluoro-cinnoline-4-ones:

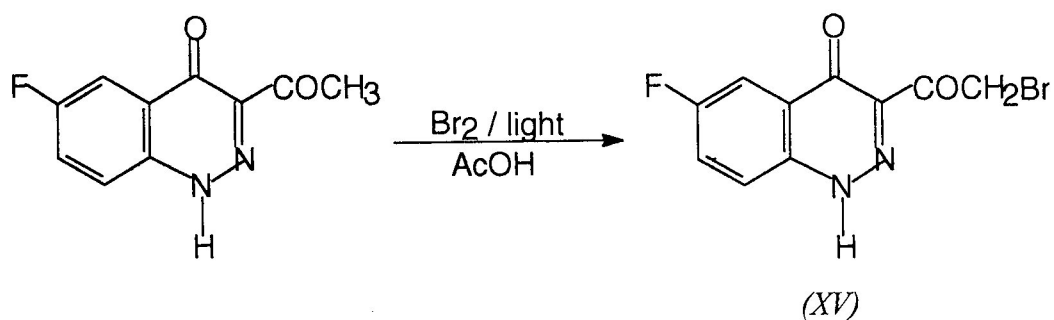
Step I: Synthesis of ethyl 2(4-fluorophenyl hydrazono)-3-oxobutrate (XIII):



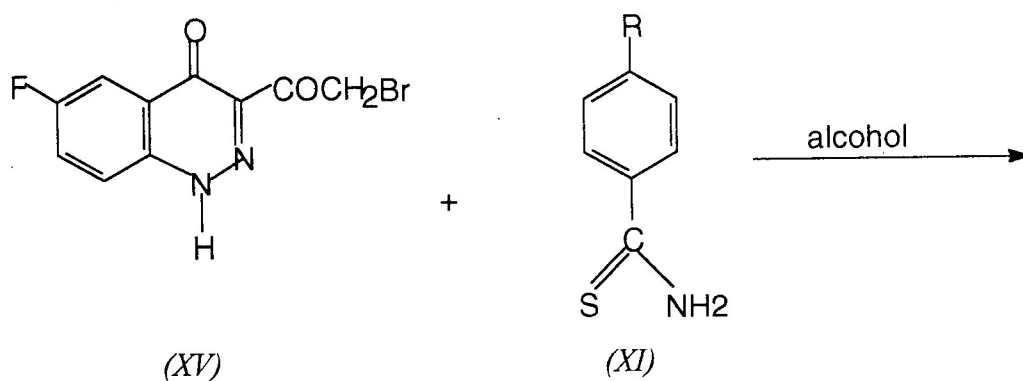
Step II: Synthesis of 3-acetyl-6-fluoro-cinnoline-4-ones(XIX):

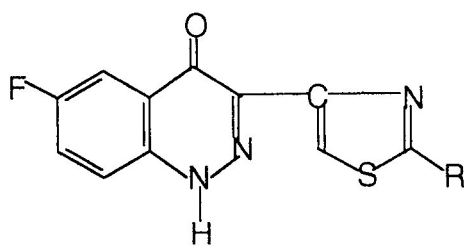


Step III: Synthesis of 3-bromoacetyl-6-fluoro-cinnoline-4-ones(XV):



Step IV: Synthesis of thiazoles(XVI):



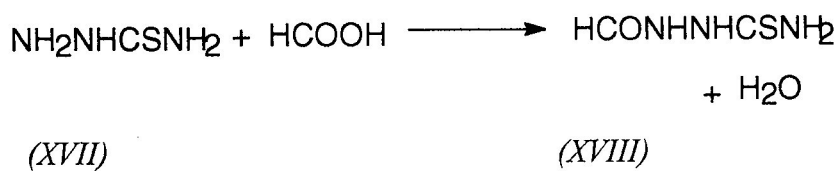


(XVI)

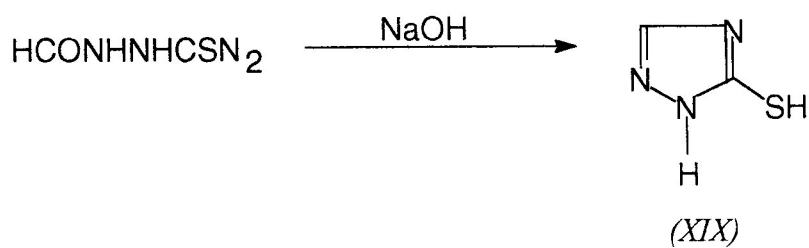
R = alkyl / aryl/ substituted aryl etc.

Scheme VI: Synthesis of intermediate for Letrozole:

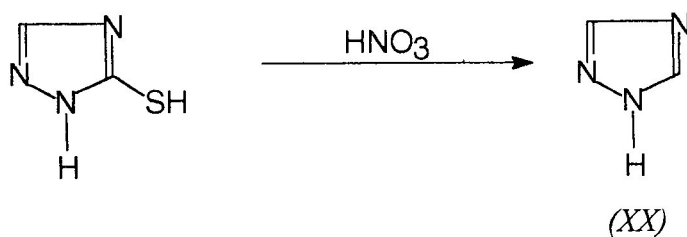
Step I: Synthesis of formyl thiosemicarbazide (XVIII):



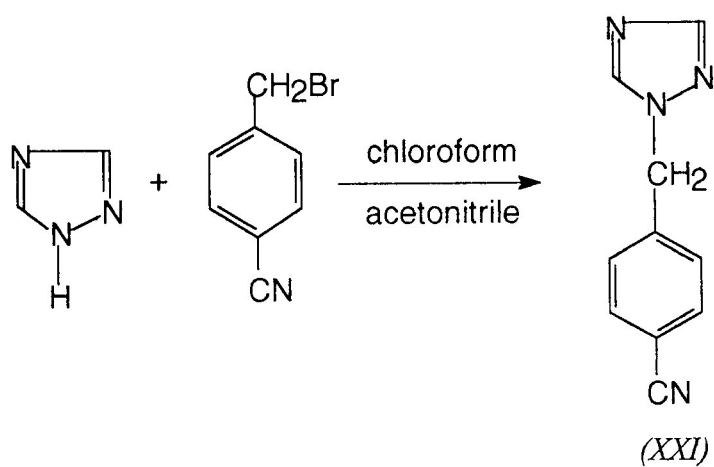
Step II: Cyclisation to 1,2,4-triazole-3(5)-thiol (XIX):



Step III: Synthesis of 1H-1,2,4-triazole (XX):



Step IV: Synthesis of 4(1H-1,2,4-triazolyl methyl) benzonitrile(XXI):



Experimental

Part -9: Synthesis of 3 [2-alkyl/aryl, 4-substituted thiazolo]-6-chloro cinnoline-4-ones.

Part -I: Synthesis of 3[2-alkyl/aryl,4-substituted thiazolo]-6-chloro cinnoline-4-ones:

Synthesis of ethyl 2(4-chlorophenyl hydrazono)-3-oxobutyrates:

To 4-chloro aniline (50gm,0.39mole) dissolved in a mixture of conc. HCl (15ml), water (15ml) and cooled at 0° to -5°C in an ice bath, a cold solution of sodium nitrite (40.02gm,0.58mole) was added with stirring. The diazonium salt was filtered into a cooled solution of ethyl acetoacetate (50.78gm,0.39mole) in ethanol (60ml) and sodium acetate (640gm,7.8mole) in water (700ml). The solid was collected and recrystallised from methanol.

M.P.=94°C.

Lit. M.P.=94°C

Yield =32.66gm,65.32%

Elemental analysis:

	Calculated	Found
C	53.63 %	54.03 %
H	4.84 %	5.16 %
N	10.42 %	10.73 %

Synthesis of 3-acetyl-6-chloro cinnoline-4-one:

To ethyl-2(4-chlorophenyl hydrazono)-3-oxobutyrates (30gm,0.112mole) was added anhydrous AlCl₃ (29.83gm,0.223mole). Chlorobenzene (360ml) was added in order to dissolve the solids and then refluxed for 12 hours. The mixture was cooled and dilute HCl (200ml) was added to it. It was then heated on a water bath. It was cooled, filtered and washed twice with dil. NaOH soln. and filtered. The product was recrystallised from methanol.

M.P.=197°C

Yield =19.05gm,63.5%

Elemental analysis

	Calculated	Found
C	53.93 %	53.85 %
H	3.14 %	3.39 %
N	12.58 %	12.44 %

Synthesis of 3-bromoacetyl-6-chloro cinnoline-4-one:

3-acetyl-6-chloro-cinnoline-4-one (25gm,0.112mole) was dissolved in chloroform (40ml). Bromine (13.48gm,0.16mole) dissolved in 15ml of chloroform was added dropwise with stirring in the presence of light. The mixture was stirred at 70⁰-75⁰C for 10 hrs. Dry air was passed through the solution and then the mixture was dumped on ice cold water. The solid was filtered and dried. It was recrystallized from methanol.

M.P.= 115⁰C

Yield = 15.97gm,63.91%

Elemental analysis:

	Calculated	Found
C	39.86 %	40.04 %
H	1.99 %	2.28 %
N	9.28 %	9.58 %

Synthesis of nitriles:

1]Synthesis of 4-amino methyl benzonitrile HCl.[Delepine reaction]:

Hexamine (12.5gm,0.0892 mole) was dissolved in 25ml of chloroform. To it was added 4-cyanobenzyl bromide (15gm,0.0765 mole) and the mixture was stirred for 6 hrs. to get a solid adduct. It was then filtered, washed with chloroform and dried.

To this adduct a 1:1 mixture of ethanol (20ml) and conc.HCl (20ml) was added and refluxed for 3 hrs. And cooled. Needles of amine HCl, obtained were filtered and dried.

M.P.=220⁰C

Lit.M.P.= 222⁰C

Yield = 82.5%

2]4-N,N-dimethyl amino benzonitrile:

Step-I: Synthesis of aldoxime of N,N-dimethyl amino benzaldehyde-

4-N,N-dimethyl amino benzaldehyde (10gm,0.067mole) was dissolved in warm ethanol (30ml) and stirred for 15 min. To it was added hydroxylamine hydrochloride (5.59gm,0.0804mole) in water (20ml) and stirred for 20 min. at room temperature. Sodium hydroxide (4.13gm,0.1mole) dissolved in water (20ml) was added to this solution dropwise. It was stirred for 4 hrs. Ethanol was removed under reduced pressure to half the quantity and the reaction mixture was dumped on ice. The solid obtained filtered was oxime.

Step-II: Synthesis of 4-N,N-dimethyl amino benzonitrile:

Aldoxime (6gm,0.036mole) was dissolved in acetic anhydride (7.5gm,6.93ml) and refluxed for 4 hrs. under dry conditions. It was then cooled and poured on crushed ice. The solid was filtered and washed with cold water and recrystallized from methanol.

M.P.=212⁰-213⁰C

Lit.M.P.=215⁰-216⁰C²²⁰

Yield = 77.48%.

Synthesis of thioamides:

1] Thiobenzamide:

Benzonitrile (6gm, 0.0582mole) was dissolved in pyridine (30ml) in a three necked round bottom flask. Triethylamine (4ml) was added to it and placed in an ice bath. H₂S gas was bubbled through this solution continuously for 10 hrs. Then the reaction mixture was poured on ice bath and the solid was filtered. It was then recrystallised from benzene.

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

M.P. = 116⁰C

Lit. M.P. = 116⁰-118⁰C²²¹

Yield = 78%

2] 4-pyridyl thioamide:

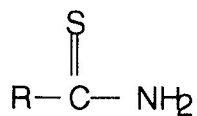
4-cyanopyridine (6gm, 0.0576mole) was dissolved in pyridine (20ml) and triethylamine (4ml) was added to it. It was then placed in an ice bath and H₂S gas was bubbled through the solution for 8 hrs. Then the reaction mixture was poured on ice and solid was collected. It was recrystallized from benzene.

M.P. = 202⁰-205⁰C

Lit. M.P. = 203⁰-205⁰C²²²

Yield = 73%.

Substituted Thioamides:



R = alkyl/aryl/substituted aryl etc

Sr.No	Substitution	M.P. °C (obtained)	M.P. °C (reported)	Yield %	Reference
1.	Methyl	112-114	113-115	74	221
2.	Phenyl	116	116	82	223
3.	Benzyl	98	97	89	223
4.	Amio methyl phenyl.HCl	222	222	74	-
5.	4-pyridyl	202-205	203-204	69	222
6.	2-Amino-5-nitro phenyl	164	165-166	77	224
7.	N,N-dimethyl amino phenyl	215-217	215-216	80	225
8.	4-Amino phenyl	185-186	182	83	226
9.	4-Chloro phenyl anilino	204-205	201-201.5	77	227

Synthesis of thiazoles:

1]2-benzyl-4[(6-chloro,3-substituted) cinnoline-4-one] thiazole.HBr:

3-bromoacetyl-6-chloro cinnoline-4-one (2gm,0.0066mole) and benzyl thioamide (1.49 gm,0.0099mole) were dissolved separately, each in 20ml of ethanol. Then both the solutions were mixed and refluxed for 20hrs. Alcohol was removed under reduced pressure. It was then recrystallized from methanol.

M.P.= 110°C

Yield = 70.66%

Elemental analysis:

	Calculated	Found
C	61.10 %	61.42 %
H	3.39 %	3.64 %
N	11.88 %	12.08 %

2]Synthesis of 2[(4-aminomethyl phenyl), 4(3-substituted,6-chloro cinnoline-4-one] thiazole HBr.

3-Bromoacetyl-6chloro cinnoline-4-one (1.5gm,0.0049mole) was dissolved in 15ml of benzene. 4-Aminomethyl benzothioamide (0.82gm,0.0099mole) was dissolved in 20ml of benzene. Both the reactants were mixed and refluxed for 22hrs. Benzene was removed and recrystallised from methanol.

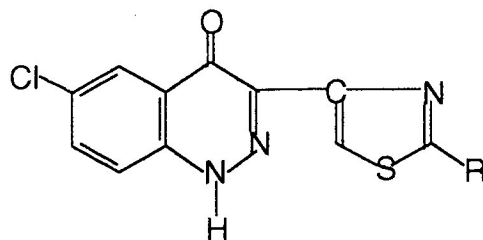
M.P.= 98°C

Yield = 68.29%

Elemental analysis:

	Calculated	Found
C	48.05 %	48.04 %
H	3.11 %	3.19 %
N	12.45 %	12.33 %

3-(2alkyl/aryl-4-substituted thiazolo)-6-chloro-cinnoline-4-ones:



Sr.No.	Substitution	Molecular formula	M.P. ^o C	Yield %	Elemental analysis% (obtd)			Elemental analysis%(Calc)		
					C	H	N	C	H	N
1.	amino	C ₁₀ H ₈ ClN ₄ OS.HBr	98	66	51.08	2.71	23.0	52.11	2.82	22.63
2.	methyl	C ₁₂ H ₈ ClN ₃ OS	111	74	38.08	2.29	11.28	39.11	2.41	11.61
3.	phenyl	C ₁₇ H ₁₀ ClN ₃ OS.HBr	115	79	59.0	2.7	13.0	59.18	2.94	12.57
4.	benzyl	C ₁₈ H ₁₂ ClN ₃ OS.HBr	110	85	61.42	3.64	12.08	61.1	3.39	11.88
5.	4-pyridyl	C ₁₆ H ₉ ClN ₄ OS.HBr	112	68	55.18	2.64	17.04	56.38	2.64	16.64
6.	Amino methyl phenyl	C ₁₈ H ₁₃ ClN ₄ OS.HBr	98	88	48.04	3.19	12.33	48.05	3.11	12.45
7.	Amino phenyl	C ₁₇ H ₁₀ ClN ₄ OS.HBr	116	82	56.07	3.14	15.94	57.34	3.10	15.79
8.	2-Amino-5-nitro phenyl	C ₁₆ H ₁₁ ClN ₅ OS.HBr	78	91	50.76	2.38	17.41	51.06	2.50	17.52
9.	N,N-dimethylamino phenyl	C ₁₉ H ₁₅ ClN ₄ OS.HBr	129	82	57.2	3.5	13.92	57.6	3.92	14.34
10.	4-Chloro phenyl anilino	C ₁₇ H ₁₀ Cl ₂ N ₄ OS.HBr	104	63	43.95	2.87	12.9	43.5	2.34	12.22

Spectral studies of 3[2(4-pyridyl), 4-substituted thiazolo]-6-chloro-cinnoline-4-one.(Compound no. 5)

IR (KBr) cm^{-1} 1676 cyclised ketone (C = O), 3234 -NH-, 1587pyridine, 1457, 1024 thiazole, 736 C-Cl.

Spectral studies of 3[2(4-amino phenyl), 4-substituted thiazolo]-6-chloro-cinnoline-4-one.(Compound no. 7)

IR (KBr) cm^{-1} 1681 (C = O), 3301-NH-, 1600, 1417, 1018 thiazole, 830 aromatic

PMR spectral analysis of compound no.7

PMR (CDCl_3) : δ 6.74 (s, 2H, NH₂)

δ 7.11 - 8.22 (s, H, NH)

(s, H, thiazole C₅ - H)

(thiazole, 7H, Ar - H).

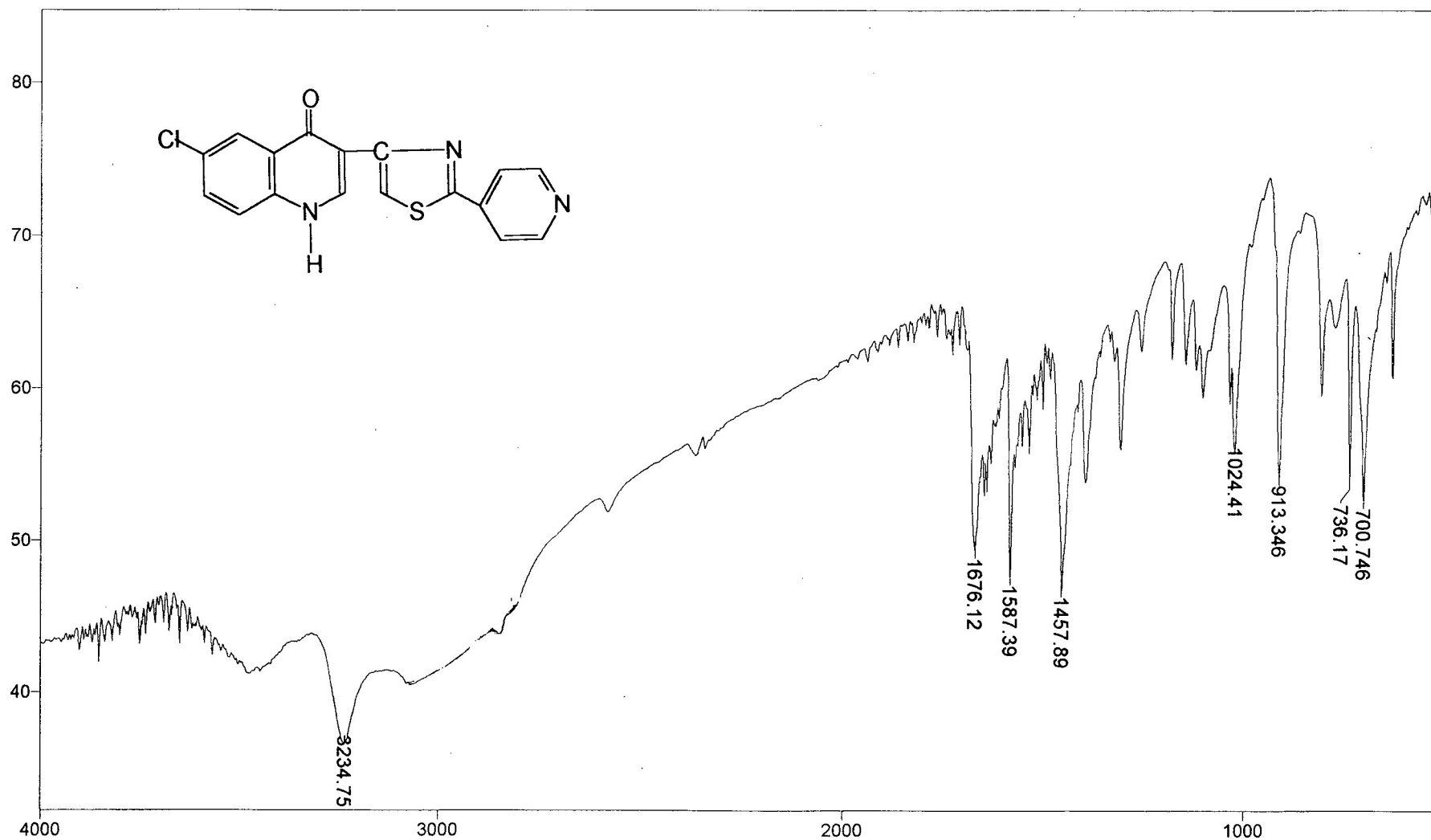
Sample Description: ptat

Scans= 10 Slow

Res=4 cm⁻¹

Apod= Cosine

Zero Filling= 1 x



File # 1 = C-AMP

Mode= 2 (Mid-IR)

1/4/80 11:08 AM

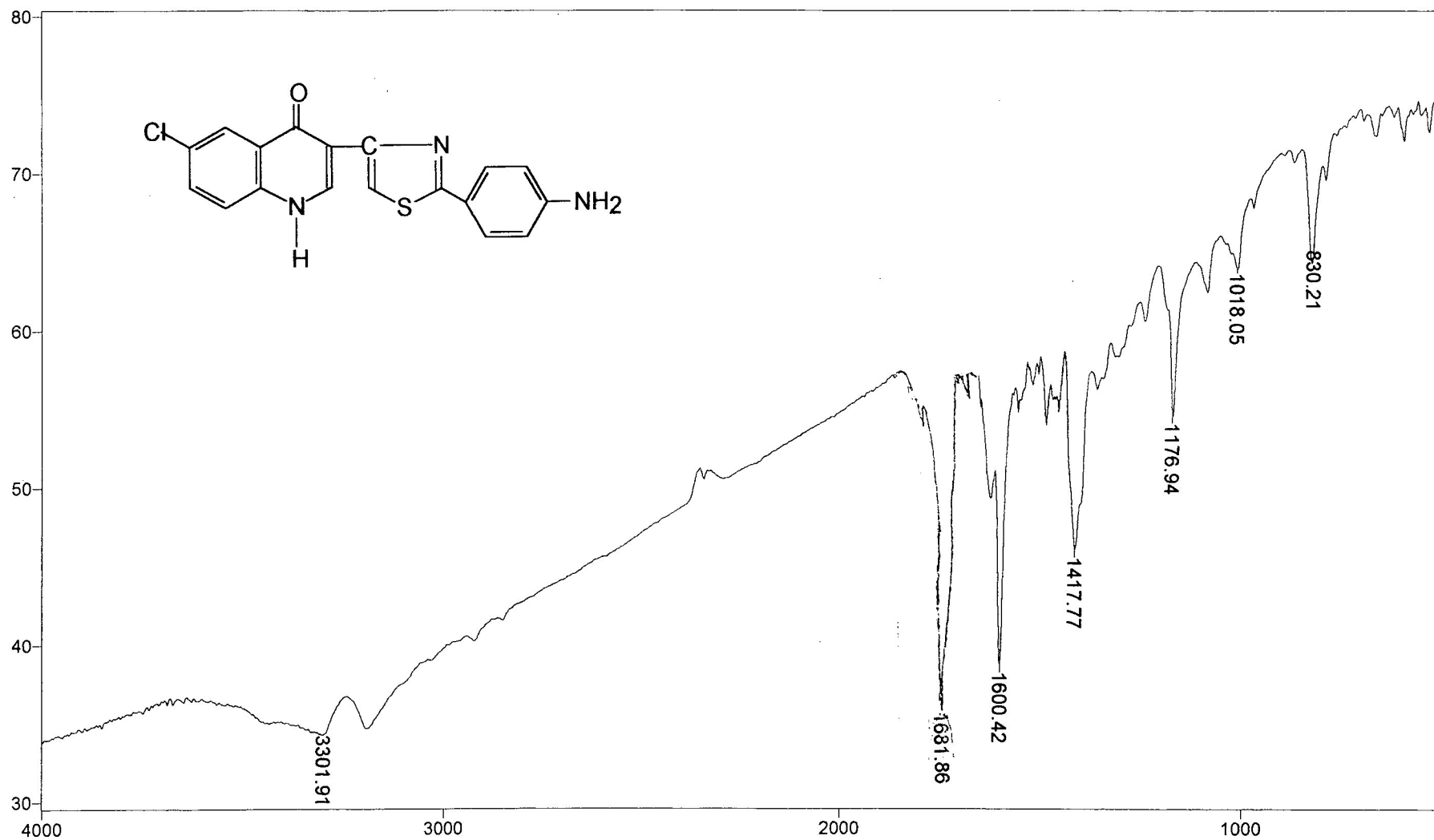
Sample Description: s-9

Scans= 10 Slow

Res=4 cm-1

Apod= Cosine

Zero Filling= 1 x



OBSERVE

Nucleus H1
Spec Width 9998.8 Hz
Acq Time 2.000 sec
Pulse Width 10.0 sec

Freq 299.9486 MHz
Offset 1700.0 Hz
Delay 0 sec
Transmits 400

DECUPLE

Nucleus H1
Mode 0
Modulation Mode 0
Pulse Width 17.0 sec
Power Mode not used

F101 PROCESSING

FN not used sec CD sec
LB 0.100 not used sec CD sec
Width 4560.5 Hz from Scan 54.2 Hz from

EXPERIMENT

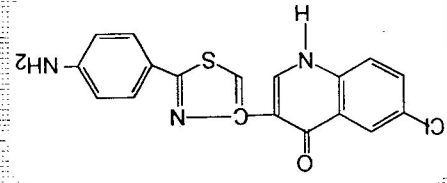
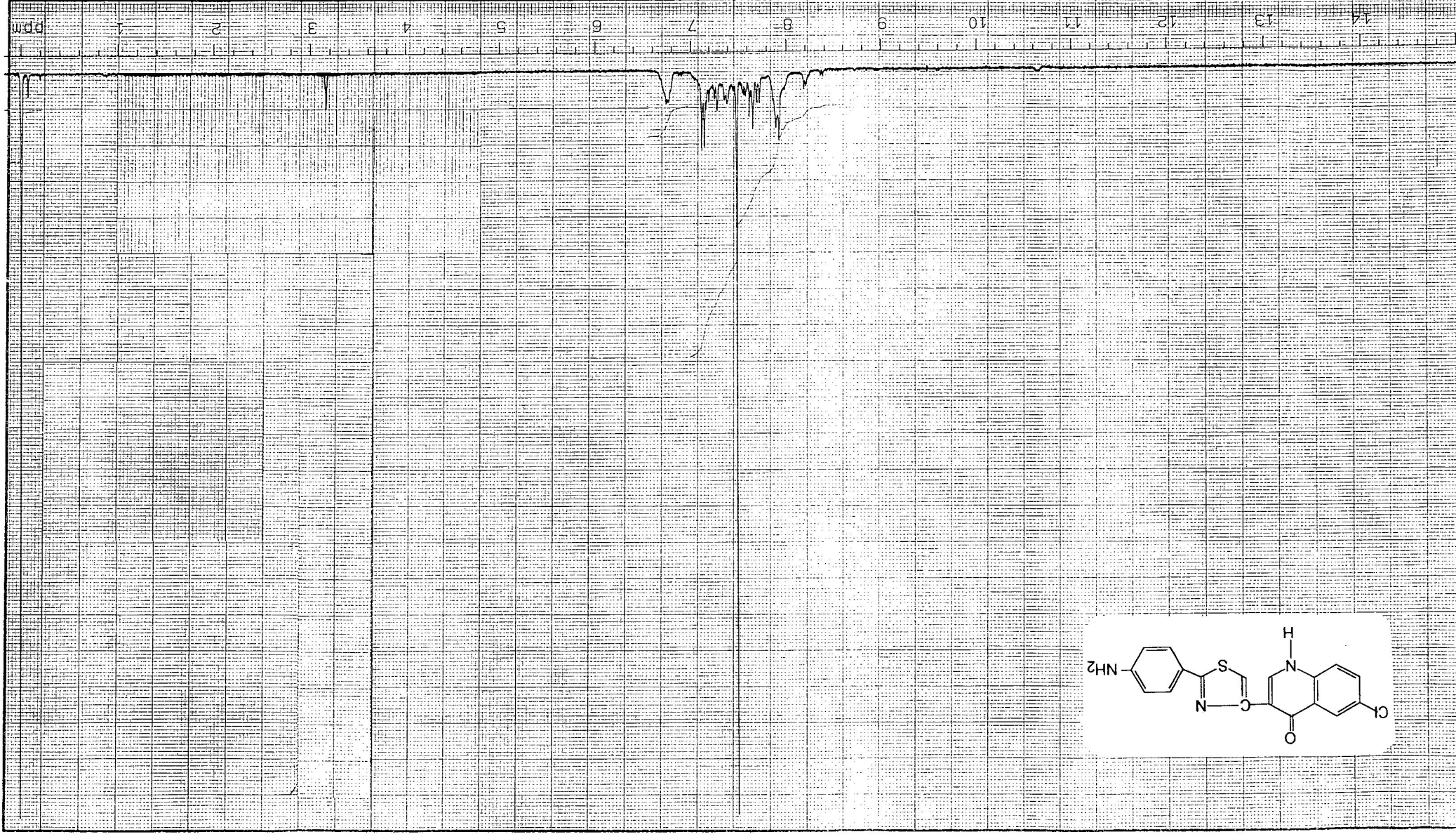
Pulse Sequence s2pu1
Tube OD mm
Temp not used °C
Solvent CDCl3

SAMPLE

J-2

A 1010 07.1010 10000

Number exp
File exp
Date Dec 30 98
VXR0 300



Part-77: Synthesis of 3(2-alkyl/aryl, 4-substituted thiazolo)-6-fluoro cinnoline-4-ones:

Part-II: Synthesis of 3(2-alkyl/aryl,4-substituted thiazolo)-6-fluoro cinnoline-4-ones:

1]Synthesis of ethyl-2-[4-fluorophenyl hydrazono)-3-oxybutyrate:

To 4-fluoro aniline (50gm,42.62ml,0.45mole), a mixture of conc.HCl (17ml) and water (17ml) was added. This was then kept in an ice bath and the temperature was maintained at 0° to -5°C. A cold aqueous solution of sodium nitrite (62.1gm,0.9mole) was added to form the diazonium salt. The salt was then filtered into a cooled solution of ethyl acetoacetate (58.56gm,57.41ml,0.45mole) in ethanol (60ml) and a large excess of sodium acetate was added. The solid was filtered and recrystallised from methanol.

M.P.= 170°C

Lit.M.P.=172°C

Yield =35.16gm,70.32%

Elemental analysis:

	Calculated	Found
C	57.14 %	57.48 %
H	3.15 %	3.48 %
N	11.11 %	10.76 %

2]Synthesis of 3-acetyl-6-fluoro cinnoline-4-one:

To ethyl-2 (4-fluorophenyl hydrazono)-3-oxybutyrate (32gm,0.1333mole) dissolved in monochlorobenzene (350ml) was added. AlCl₃ (35.59gm,0.266mole) was added to it and dissolved. The mixture was then refluxed for 14 hrs. It was cooled and dil.HCl (250ml) was added. The solid obtained was then filtered and washed with dil.NaOH soln. It was recrystallised from methanol.

M.P.= 158°C

Yield =20.24gm,63.28%

Elemental analysis:

	Calculated	Found
C	58.25 %	58.68 %
H	3.39 %	3.59 %
N	13.59 %	13.18 %

3] Synthesis of 3-bromoacetyl-6-fluoro cinnoline-4-one:

3-acetyl-6-fluoro cinnoline-4-one (20gm, 0.097mole) was dissolved in chloroform (300ml). Bromine (9.6gm, 0.12mole) was dissolved in CHCl_3 (40ml) was added to it at temperature 60°C . The mixture was then stirred in the presence of light for 10 hrs. Dry air was passed through the solution and then it was dumped on ice. The solid was filtered and recrystallised from methanol.

M.P. = 89°C

Yield = 15.2gm, 76.0%

Elemental analysis:

	Calculated	Found
C	42.10 %	42.45 %
H	2.10 %	2.42 %
N	9.82 %	9.58 %

Synthesis of thiazoles:

1] 2-phenyl-4-[(6-fluoro-3-substituted) cinnoline-4-one] thiazole:

3-bromoacetyl-6-fluoro cinnoline-4-one (2gm, 0.0071mole) and thiobenzamide (1.44 gm, 0.0105mole) were dissolved separately in alcohol (each in 20ml) and mixed. Then this mixture was refluxed for 19 hrs. Alcohol was removed under reduced pressure and the solid was recrystallised from methanol.

M.P.= 183⁰C

Yield = 83.39 %

Elemental analysis:

	Calculated	Found
C	50.49 %	50.40 %
H	2.72 %	2.71 %
N	10.39 %	9.96 %

2] Synthesis of 2(5-nitro-2-amino phenyl), 4-[(6-fluoro,3-substituted) cinnoline-4-one] thiazole:

3-bromoacetyl-6-fluoro cinnoline-4-one (2gm.0.0071mole) was dissolved in 20ml of ethanol. 5-nitro-2-amino phenyl thioamide (2.098 gm,0.0106 mole)was dissolved in 30ml of ethanol.

Both the reactants were mixed and refluxed for 20 hrs. Alcohol was then removed and the solid was recrystallised from methanol.

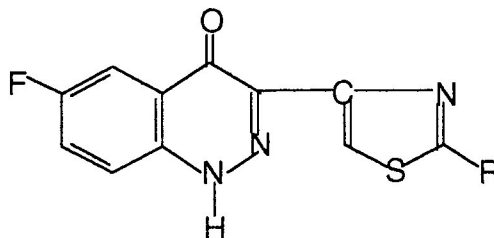
M.P.= 205⁰C

Yield = 78.78 %

Elemental analysis:

	Calculated	Found
C	43.99 %	44.04 %
H	2.37 %	2.42 %
N	15.08 %	15.35 %

3-2(alkyl/aryl,-4-substituted thiazolo)-6-fluoro-cinnoline-4-ones:



Sr.No.	Substitution	Molecular formula	M.P. ^o C	Yield %	Elemental analysis%(obtd)			Elemental analysis%(Calc)		
					C	H	N	C	H	N
1.	amino	C ₁₁ H ₇ FN ₄ OS.HBr	98	62	39.56	2.43	17.1	39.48	2.33	16.82
2.	methyl	C ₁₂ H ₈ FN ₃ OS	207-209	75	53.7	3.17	16.6	54.17	3.17	16.49
3.	phenyl	C ₁₇ H ₁₀ FN ₃ OS.HBr	183	81	50.40	2.71	9.96	50.49	2.71	10.39
4.	benzyl	C ₁₈ H ₁₂ FN ₃ OS.HBr	87	89	48.72	2.62	9.28	49.07	2.74	9.65
5.	4-pyridyl	C ₁₆ H ₉ FN ₄ OS.HBr	117	64	49.11	0.34	14.66	48.60	0.24	13.82
6.	Amino methyl phenyl	C ₁₈ H ₁₃ FN ₄ OS.HBr	90-93	64	50.72	3.34	12.68	49.88	3.23	12.93
7.	Amino phenyl	C ₁₇ H ₁₁ FN ₄ OS.HBr	133	80	60.95	3.29	17.06	60.35	3.25	16.56
8.	2-Amino-5-nitro phenyl	C ₁₇ H ₁₀ FN ₅ O ₃ S.HBr	205	77	44.04	2.42	15.35	43.99	2.37	15.08
9.	N,N-dimethylamino phenyl	C ₁₉ H ₁₅ FN ₄ OS.HBr	157-161	68	52.89	3.29	12.08	53.19	3.58	12.52
10.	4-Chloro phenyl anilino	C ₁₇ H ₁₀ ClFN ₄ OS.HBr	124-126	71	45.27	2.74	13.03	44.98	2.42	12.74

Spectral studies of 3[2(phenyl), 4-substituted thiazolo]-6-fluoro-cinnoline-4-one.(Compound no. 3)

IR (KBr) cm^{-1} 1685 cyclised ketone ($\text{C} = \text{O}$), 3229 -NH-, 1479, 1019 thiazole, 1361, 1323 C - F

Spectral studies of 3[2(2-nitro, 5-amino phenyl), 4-substituted thiazolo]-6-fluoro-cinnoline-4-one.(Compound no. 3).

IR (KBr) cm^{-1} 1650 cyclised ketone ($\text{C} = \text{O}$), 3240-NH-, 3334, primary amine - NH_2 , 1500, 1321 - NO_2 , 1606, 1569, 1024 thiazole, 1262, 1149 C - F.

PMR spectral analysis of compound no.3

PMR (CDCl_3) : δ 6.38 (s, 2H, - NH_2)

6.84 (s, H, - NH)

6.88 (s, H, thiazole C_5 -H)

δ 11-8.27 (thiazole, 6H, Ar -H).

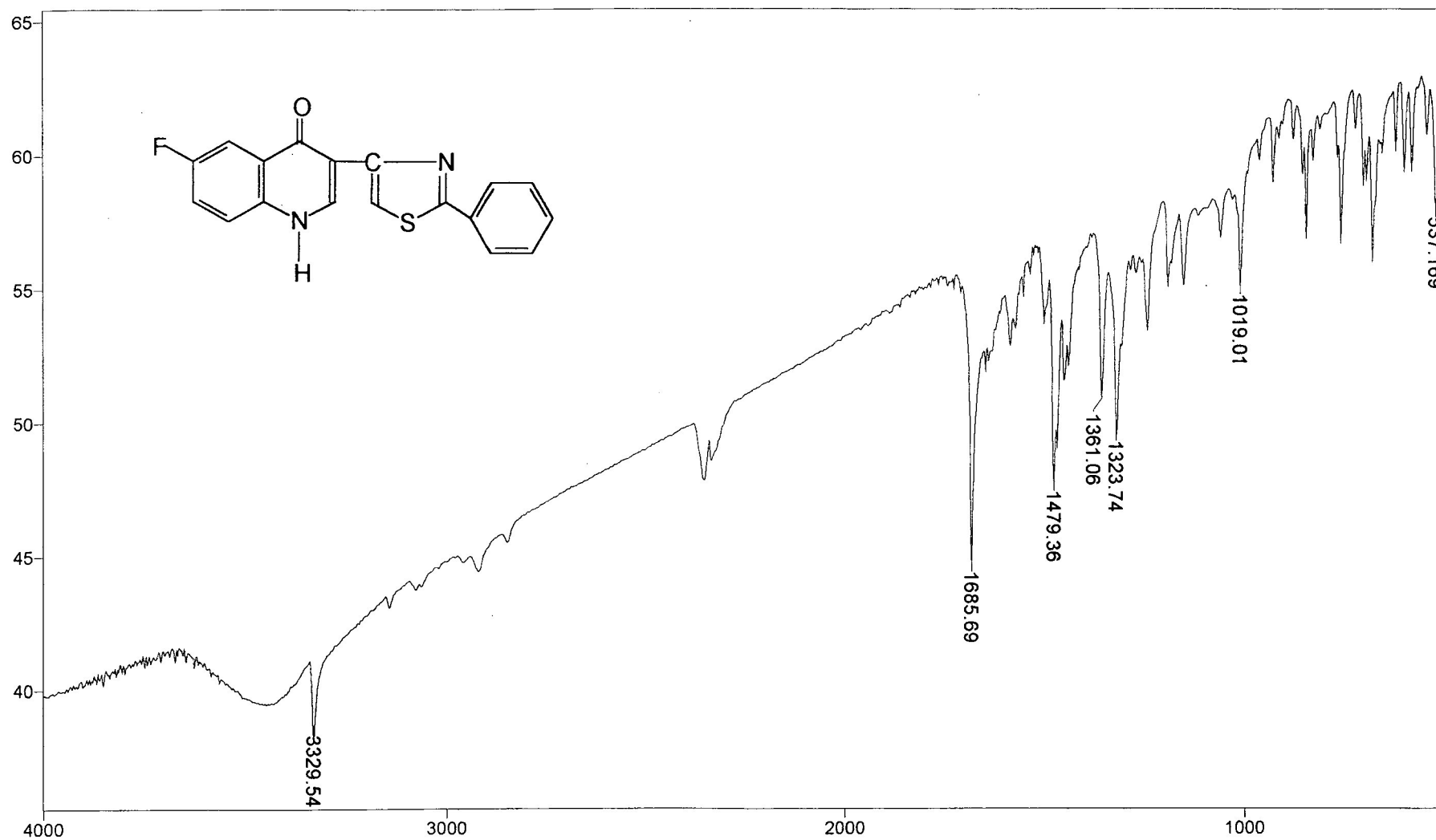
Sample Description: f-b

Scans= 10 Slow

Res=4 cm⁻¹

Apod= Cosine

Zero Filling= 1 x



File # 1 = F-AN1

Mode= 2 (Mid-IR)

1/4/80 12:14 PM

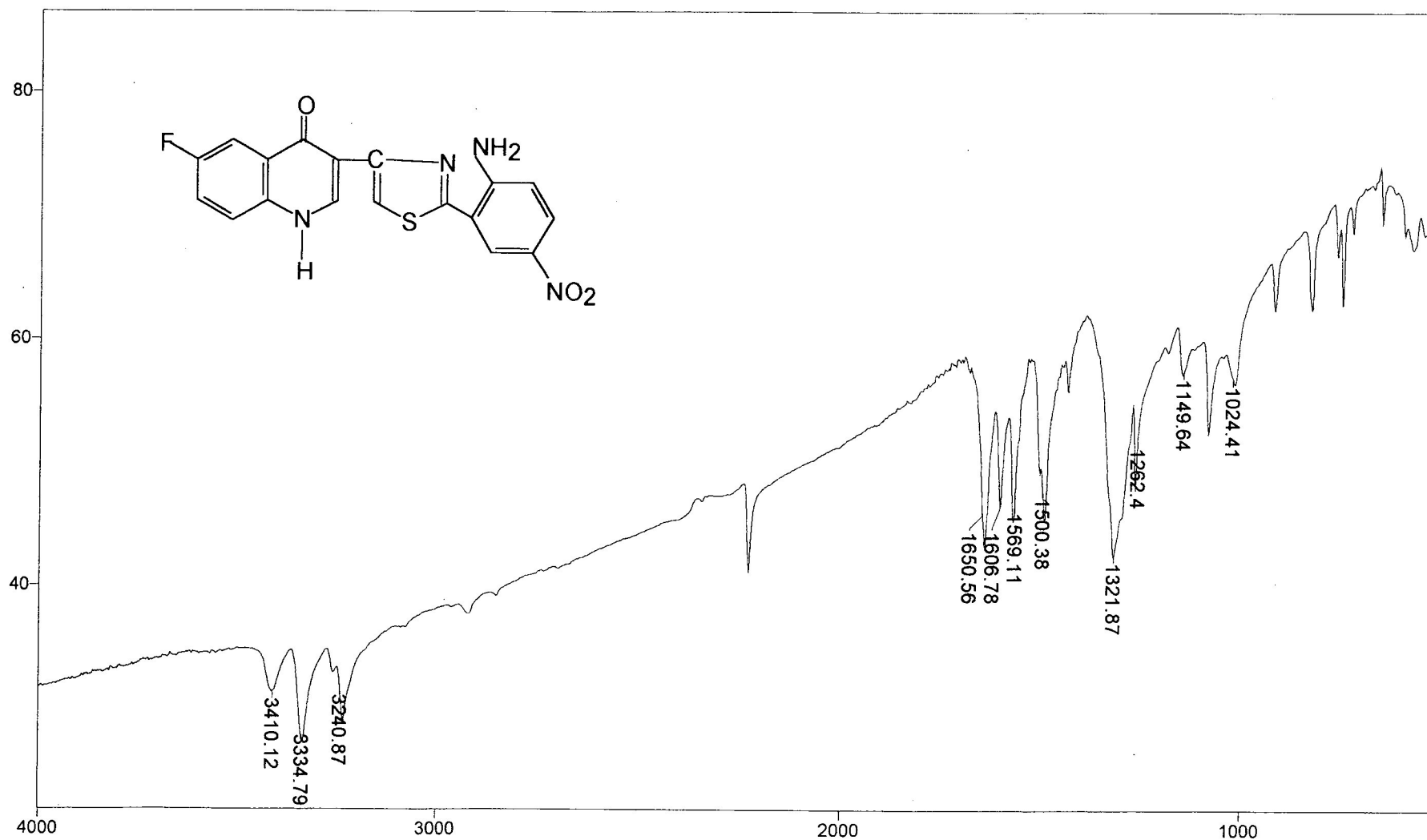
Sample Description: f-an1

Scans= 10 Slow

Res=4 cm-1

Apod= Cosine

Zero Filling= 1 x



*Part -999: Synthesis of 1,2,4-
triazolyl- methyl benzonitrile as an
intermediate for Letrozole:*

Part -III: Synthesis of 1,2,4-triazolyl-methyl benzonitrile as an intermediate for Letrozole:

Step-I: Synthesis of 1-formyl-3-thiosemicarbazide:

90% formic acid (25.97 ml, 0.689mole) was taken in a round bottom flask and heated for 15 min. To it was added thiosemicarbazide (12 gm,0.13 mole) and dissolved. It was heated for 30 min. and cooled. To this mixture 100 ml of boiling water was added. This milky solution was then filtered cooled on an ice bath for 2 hours and filtered to get 1-formyl-3-thiosemicarbazide.

M.P.=175⁰-177⁰C

Lit.M.P.=177⁰-178⁰C²¹⁴

Yield = 72.28%

Step-II: Synthesis of 1-H,1,2,4-triazole-3(5)-thiol:

A solution of 1-formyl-3-thiosemicarbazide (10gm,0.0952 mole) and sodium hydroxide (3.80 gm,0.0952 mole) in water (120ml) was heated for 1 hour. Then it was cooled on an ice bath for 30 min. Conc. HCl (50 ml) was added with stirring. Then the reaction mixture was cooled in an ice bath for 2hrs. and filtered to get crud 1-H,1,2,4-triazole-3(5)-thiol. It was then dissolved in boiling water, filtered and cooled on ice.

M.P.=222⁰-224⁰C

Lit. M.P.=220⁰-222⁰C²¹⁴

Yield =66%

Step-III: Synthesis of 1-H,1,2,4-triazole:

A mixture of water (11ml), conc. nitric acid (22 ml) and sodium nitrite (0.014 gm) was taken in a three necked flask and stirred at 45-50⁰C. To it a small portion of 1-H,1,2,4-triazole-3(5)-thiol was added. Brown fumes of NO₂ were observed which evolved during oxidation. 1H,1,2,4-triazole-3(5)-thiol (7gm,0.0693 mole) was added in small portions over 30 min. During the addition, the temperature was maintained between 45-50⁰C with occasional cooling in a water bath. Then the water bath was removed and the mixture was stirred for 2 hrs. until the reaction mixture comes to room temperature

Then it was neutralised with liquid ammonia and the solvent was removed under reduced pressure. Solid was extracted in ethyl acetate. The solvent was removed to get crude 1-H,1,2,4-triazole. It was then recrystallised from ethanol.

M.P.=121⁰-123⁰C

Lit.M.P.=119⁰-120⁰C²¹⁴

Yield =52.08%

Elemental analysis:

	Calculated	Found
C	34.78 %	34.46 %
H	4.34 %	4.61 %
N	60.86 %	60.76 %

Step-IV: Synthesis of 1,2,4-triazolyl methyl benzonitrile:

4-cyano benzyl bromide (6 gm,0.0306 mole) was dissolved in a mixture of chloroform (20ml) and acetonitrile (4ml). 1H-1,2,4-triazole (9.27 gm,0.1344 mole) was dissolved in a mixture of CHCl₃ (160 ml) and acetonitrile (32ml) and added to the 4-cyano benzyl bromide solution with stirring. Then it was refluxed for 12 hours and it was neutralized with 3% sodium bicarbonate solution. The organic content was then extracted in chloroform and the solvent was reduced to half the quantity. It was then passed through silica gel column in order to separate the product. The system used was CHCl₃-isopropanol (9:1). It was then recrystalliaed from methanol.

M.P.=77⁰-79⁰C

Lit.M.P.=77⁰-79⁰C²²⁸

Yield =57.58%

Elemental analysis:

	Calculated	Found
C	65.21 %	65.51 %
H	4.34 %	4.70 %
N	30.43 %	30.35 %

Spectral studies of 1H, 1,2,4- triazole.

IR (KBr) cm^{-1} 3120 N - H, 980 1,2,4 - triazole.

Spectral studies of 4-(1,2,4- triazolyl methyl) benzonitrile.

IR (KBr) cm^{-1} 3103 N - H, 961 1,2,4 - triazole, 2229 nitrile, 1010 1,4- disubstituted aromatic ring.

PMR spectral analysis of 4-(1,2,4- triazolyl methyl) benzonitrile.

PMR (CDCl_3) : d 5.42 (s, 2H, $-\text{CH}_2-$)

7.30 - 7.38 (δ , 2H, Ar-H / ortho to nitrile)

7.65 - 7.73 (δ , 2H, Ar-H / ortho to $-\text{CH}_2-$)

8.0 (s, H, triazole-H)

8.15 (s,H, triazole-H)

File # 1 = TRZ-S

Mode= 2 (Mid-IR)

10/8/99 10:43 AM

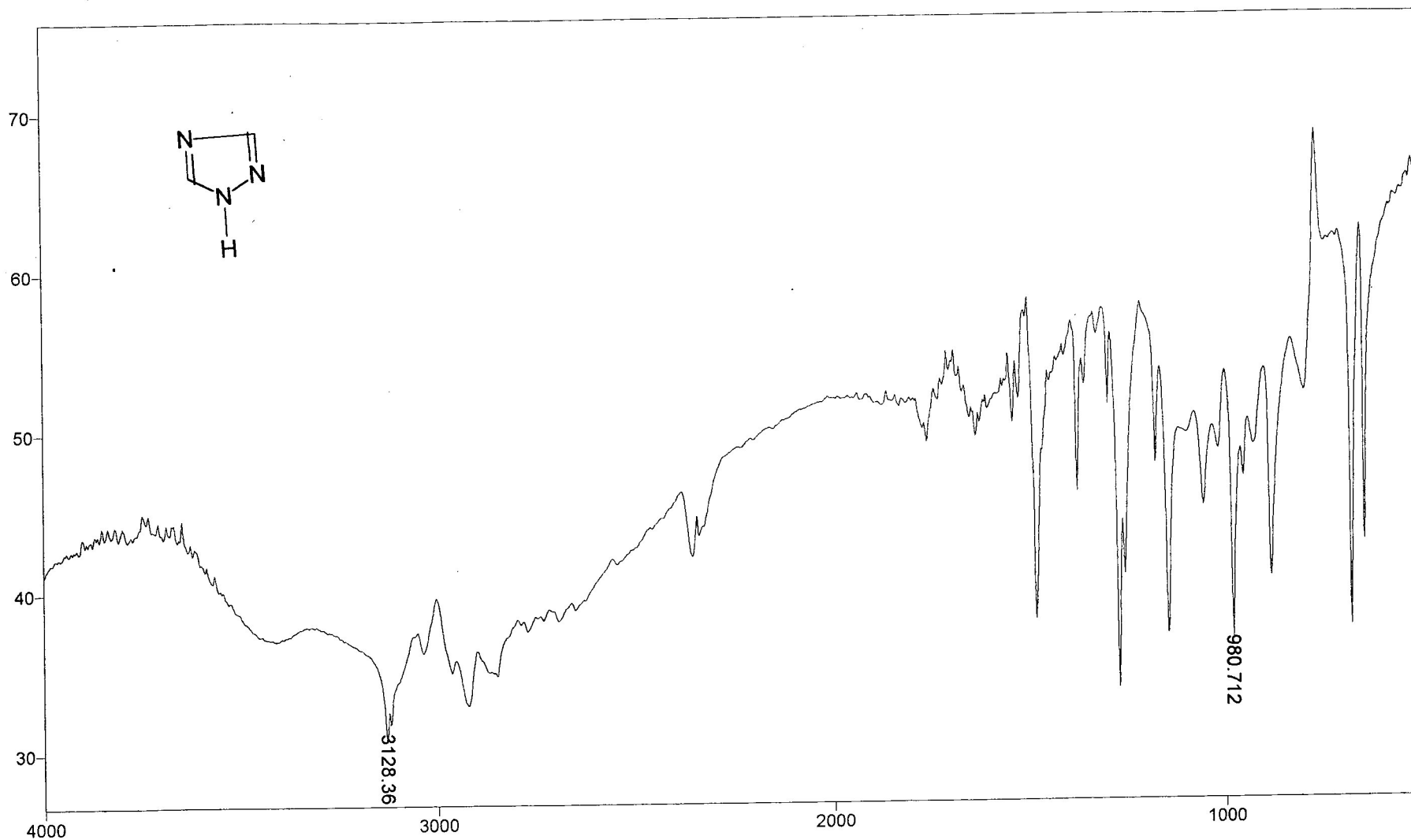
Sample Description: trz-s

Scans= 10 Slow

Res=4 cm⁻¹

Apod= Cosine

Zero Filling= 1 x



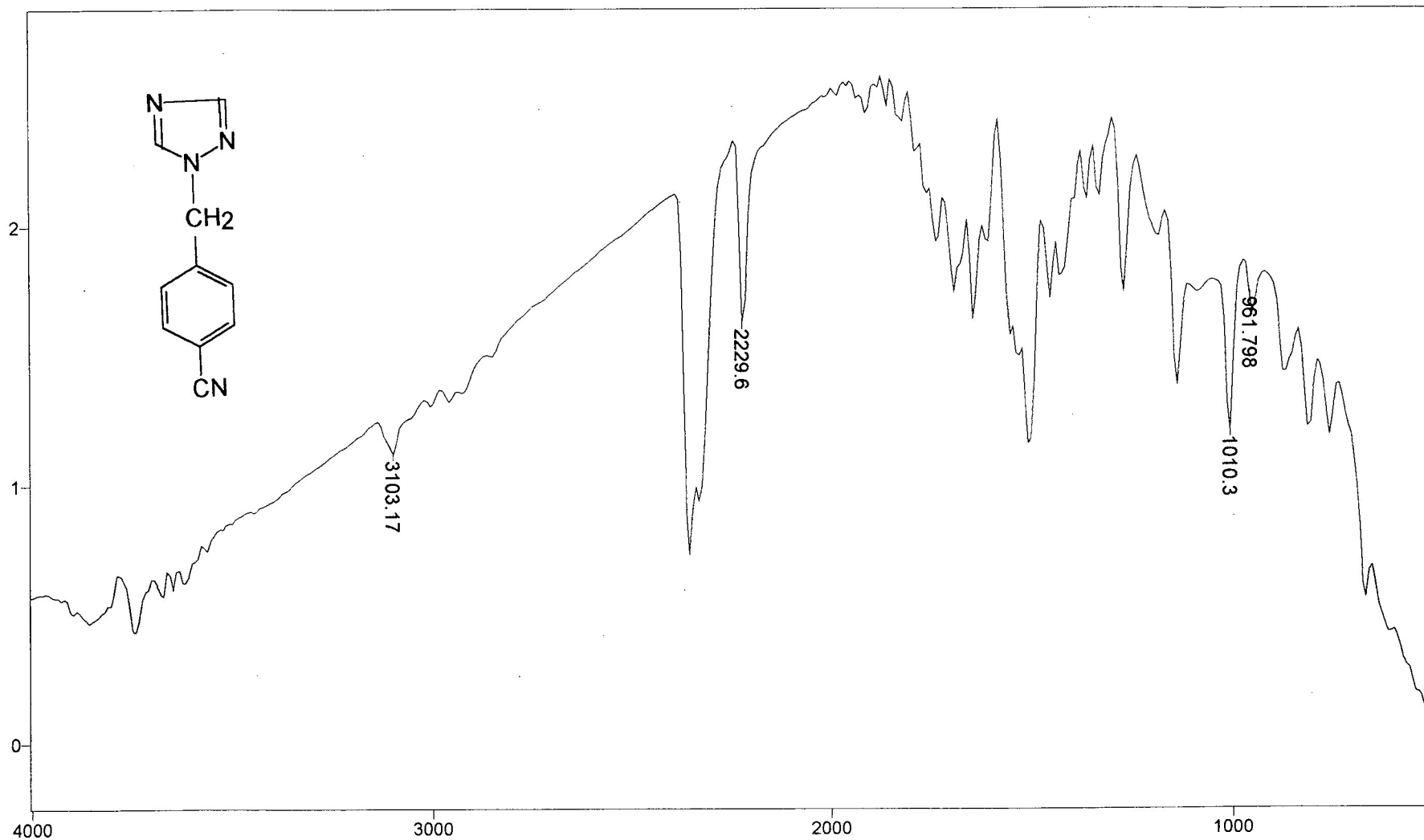
Sample Description: i-bn

Scans= 10 Slow

Res=16 cm⁻¹

Apod= Cosine

Zero Filling= 1 x



OBSERVE

Nucleus H1
 Spec Width 5998.8 Hz
 Acq Time 2.00 sec
 Delay 0 sec
 Transients 200
 Freq 299.9486 MHz
 Offset 1200.0 Hz

DECOUPLE

Nucleus H1
 Mode 0
 Modulation Mode 0
 Pulse Width 12.5 sec
 Power Mode not used
 Offset 3115.8 Hz
 Power 20 dB
 Freq 200 Hz

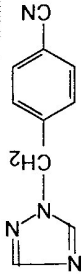
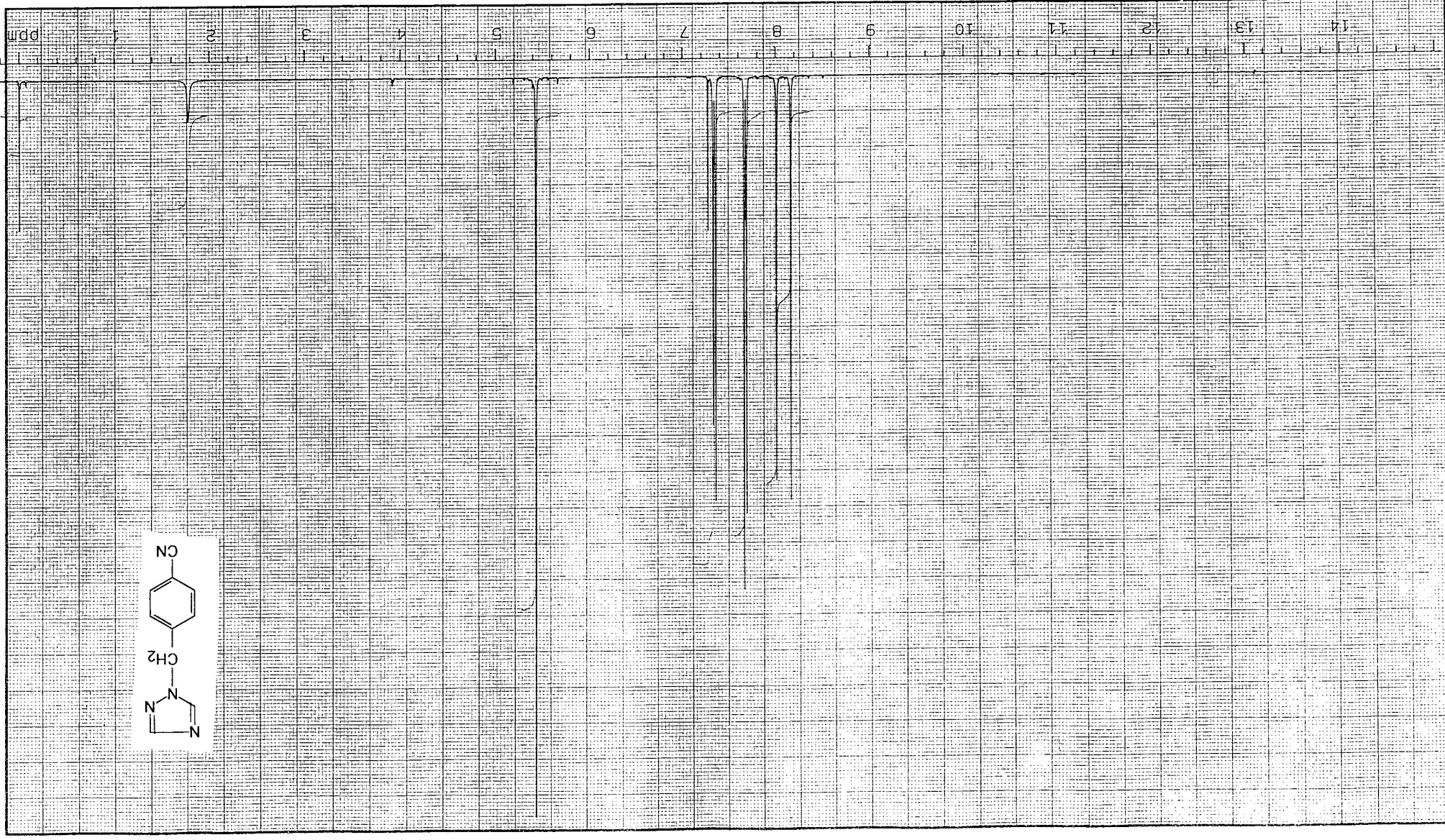
PLOT/PROCESSING

Reference not used
 FN not used
 LB 0.100
 Width 4580.3 Hz/ppm
 Scan -60.8 Hz/ppm
 Tube OD mm
 Temp not used
 Solvent CDCl3
 Pulse Sequence s2pu1

EXPERIMENT

SAMPLE
 I-bn

Number _____
 File exp
 Date Dec 30 96
 VXRO 300



Biological Screening and Conclusions

Biological Screening and Conclusions:

All the compounds synthesized in the present work were subjected to antimicrobial screening programs.

The various screening programs carried out include the *in vitro* study of against Gram positive *Staphylococcus aureus* ATCC 3750 and Gram negative *Salmonella typhi* NCTC 786 and *in vitro* study of activity against H₃₇ Rv strain of *Mycobacterium tuberculosis*. Also, *in vitro* study against fungal strains such as *Candida albicans*, *Trichophyton mentagrophytes* and *Trichophyton rubrum* was carried out.

Drug Stock Solution :

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

Controls:

1. Media Control: Sterilized medium was kept as a control so as to assure sterility of the medium. If this control shows growth, it shows that the medium is not properly sterilized and hence in that case results are not valid.
2. Culture Control: It contains sterilised 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 quality of solvent used to prepare dry stock solution. If the growth is not observed it can be concluded that the solvent inhibits the growth of organisms and the results are not valid.

4. Turbidity Control: It contains medium and 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 similar order of standard drug 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.

Meuller Hinton broth was used as a culture medium. Sterilized medium was dispensed in each borosilicate glass test tube (150+20mm). The drug solution was added in order to attain final drug concentrations as 200,100,40,20,10,5,2,1 µg/ml etc.

Innoculum of standard suspension, (0.1ml of the test organism strain which contains 10^6 bacilli/ml) was added. The tubes were incubated at 37°C for 48 hours and then examined for the presence or absence of growth of the organism. The lowest concentration which showed no visible growth was taken as an end point (MIC). Ampicillin (MIC=0.01µg/ml against gram positive *S. aureus*) and Trimethoprim (MIC=1µg/ml against gram negative *S. typhi*) were used as the standard drugs.

Antitubercular Screening:

The test compounds were subjected to *in vitro* screening by tube dilution method employing the human virulent H₃₇Rv strain of *Mycobacterium tuberculosis*.

Kirchner's medium containing Tween 80 as described by Rake *et.al.*²²⁸ was used.

The sterile medium was dispensed in each borosilicate test tube to attain final dilutions as 200,100,40,20,10,5,2,1,0.5µg/ml. Inoculum of standard suspension, i.e.0.1ml of H₃₇Rv strain of *M.tuberculosis* containing 10⁶ bacilli/ml, was added. Tubes were incubated at 37⁰C for ten days and then examined for the presence or absence of growth of the test organism. The lowest concentration which showed no visible growth was taken as the end point. Isoniazide (MIC=0.04µg/ml) was used as a standard.

Antifungal Screening:

The organisms employed for test were *Candida albicans*, *Trichophyton mentagrophytes* and *Trichophyton rubrum*.

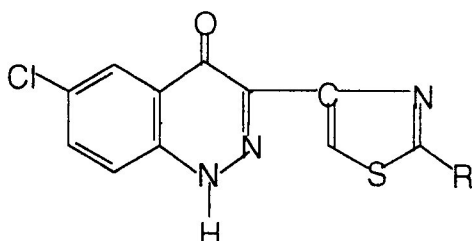
Seraufataller's medium was employed for growth and the pH of the medium was adjusted to 5.2. The sterile medium was dispensed in each borosilicate test tube. The drug solution was added in order to get final drug concentrations of 200,100,40,20, 10, 5,2 etc. µg/ml etc.

The tubes were incubated at room temperature (28-30⁰C) in a dark place. *Candida albicans* was incubated for 7 days while *T.mentagrophytes* and *T.rubrum* were incubated for 14 days. The presence or absence of growth was observed visually. The lowest conc. which showed no visible growth was taken as an end point.

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

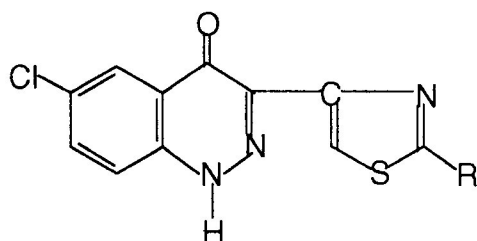
Results and conclusion:

Table I: 3(2-alkyl/ aryl, 4-substituted thiazolo)-6-chloro cinnoline-4-ones:



Sr.No	Substitution (R)	Antibacterial activity in $\mu\text{g/ml}$		
		H ₃₇ Rv strain of <i>M.tuberculosis</i>	<i>S.aureus</i>	<i>S.typhi</i>
1.	methyl	100	100	100
2.	phenyl	100	40	10
3.	benzyl	40	4	4
4.	Pyridyl	40	10	10
5.	Amino	100	100	10
6.	Amino phenyl	100	4	10
7.	Amino methhyl phenyl	40	4	100
8.	2-Amino-5-nitro phenyl	100	10	100
9.	N,N-dimethyl amino phenyl	40	100	4
10.	4-Chloro phenyl anilino	100	100	100

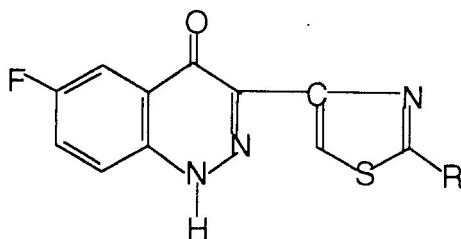
Table II: 3(2-alkyl/ aryl, 4-substituted thiazolo)-6-chloro-cinnoline-4-ones:



Sr.No	Substitution (R)	Antifungal activity in $\mu\text{g/ml}$.		
		<i>C.albicans</i>	<i>T.mentagrophytes</i>	<i>T.rubrum</i>
1.	methyl	N.A.	N.A.	N.A.
2.	phenyl	N.A.	100	N.A.
3.	benzyl	N.A.	100	200
4.	Pyridyl	200	100	N.A.
5.	Amino	N.A.	N.A.	40
6.	Amino phenyl	N.A.	40	100
7.	Amino methyl phenyl	20	40	40
8.	2-Amino-5-nitro phenyl	N.A.	N.A.	40
9.	N,N-dimethyl amino phenyl	100	40	200
10.	4-Chloro phenyl anilino	N.A.	40	100

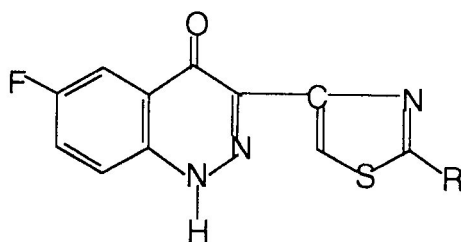
where N.A. = inactive at 200 $\mu\text{g/ml}$.

Table III: 3(2-alkyl/ aryl, 4-substituted thiazolo)-6-fluoro-cinnoline-4-ones:



Sr.No	Substitution (R)	Antibacterial activity in $\mu\text{g/ml}$		
		<i>H₃₇Rv</i> strain of <i>M.tuberculosis</i>	<i>S.aureus</i>	<i>S.typhi</i>
1.	methyl	100	100	100
2.	phenyl	100	100	100
3.	benzyl	100	10	10
4.	Pyridyl	100	10	100
5.	Amino	100	10	10
6.	Amino phenyl	100	10	10
7.	Amino methyl phenyl	40	100	100
8.	2-Amino-5-nitro phenyl	100	40	40
9.	N,N-dimethyl amino phenyl	40	100	100
10.	4-Chloro phenyl anilino	100	40	100

Table IV: 3(2-alkyl/ aryl, 4-substituted thiazolo)-6-fluoro-cinnoline-4-ones:



Sr.No	Substitution (R)	Antifungal activity in $\mu\text{g/ml}$.		
		<i>C.albicans</i>	<i>T.mentagrophytes</i>	<i>T.rubrum</i>
1.	methyl	N.A.	40	100
2.	phenyl	N.A.	40	40
3.	benzyl	N.A.	200	20
4.	Pyridyl	N.A.	N.A.	N.A.
5.	Amino	N.A.	40	100
6.	Amino phenyl	100	40	100
7.	Amino methyl phenyl	N.A.	40	N.A.
8.	2-Amino-5-nitro phenyl	N.A.	40	40
9.	N,N-dimethyl amino phenyl	N.A.	40	200
10.	4-Chloro phenyl anilino	N.A.	40	100

where N.A. = not active at 200 $\mu\text{g/ml}$.

Conclusion:

- 1) Out of all the ten substitutions made, 3(2-benzyl,4-substituted thiazolo)-6-chloro-cinnoline-4-one is found to be the most active against both the organisms, *Staphylococcus aureus* and *Salmonella typhi* i.e. 4 µg/ml. The activity was retained for the fluorinated compound and is 10 µg/ml against both *S.aureus* and *S.typhi* respectively.
- 2) Amino phenyl substituted compound have shown very good activity against *S.aureus* and *S.typhi* i.e. 4 µg/ml and 10 µg/ml respectively, which is retained for the fluorinated compounds as 10 µg/ml and 10 µg/ml respectively.
- 3) N,N-dimethyl amino phenyl and amino methyl phenyl substituted compounds have shown good activity i.e. upto 4 µg/ml against *S.aureus* and *S.typhi*. Corresponding fluorinated compounds have shown moderate activity.
- 4) Phenyl substituted compound is moderately active i.e. 40µg/ml and 10 µg/ml respectively against *S.aureus* and *S.typhi*. Fluorinated compound is equipotent.
- 5) 4-chloro anilino, methyl, 4-pyridyl substituted compounds have shown moderate activity upto 100µg/ml in both the series.
- 6) In case of 5-nitro-2-amino phenyl substitution, improved antibacterial activity upto 40 µg/ml is observed for fluorinated compound than 100µg/ml of chloro analogue.
- 7) Moderate antitubercular activity upto 40 µg/ml is observed for amino methyl phenyl, benzyl, N,N-dimethyl amino phenyl, phenyl and 4-pyridyl substituted compounds from both the series. It is concluded that cinnoline does not have any markable effect on antitubercular activity of thiazole.
- 8) Overall markable improvement in antifungal activity is observed in case of fluoro compounds. This may be because of the contribution of fluorine atom as many of the standard antifungal agents are contain fluorine atom which enhances the activity. e.g. fluconazole, flucytosine etc.
- 9) Against *Candida albicans* amino methyl phenyl compound is very active upto 20 µg/ml. Activity is moderate upto 100µg/ml and 200 µg/ml for different compounds. N,N-dimethyl amino phenyl compound from the chlorinated series and amino phenyl compound

from the fluorinated series are active upto 100 µg/ml. All the remaining compounds are active at 200 µg/ml.

10) In case of *Trichophyton mentagrophytes*, eight of the ten fluorinated compounds have shown good activity upto 40 µg/ml. Chloro compounds like N,N-dimethyl amino phenyl, amino methyl phenyl and amino phenyl are active upto 40 µg/ml and compounds with 4-pyridyl, phenyl, benzyl, 4-chloro anilino substitutions are moderately active upto 100 µg/ml.

11) Also, increase in activity for fluoro compounds is observed against *Trichophyton rubrum*. Compound with benzyl substitution is the most active with MIC of 20 µg/ml.

Amino, 5-nitro-2-amino phenyl substituted compounds have good activity upto 40 µg/ml. From the chlorinated series, 5-nitro-2-amino phenyl, amino methyl phenyl, amino substituted compounds are active upto 40 µg/ml. All the remaining compounds are active at 100 µg/ml and 200 µg/ml.

References

- 1) Silverman, Richard B; 'The Organic Chemistry of Drug Design and Drug Action'; 4 (1992).
- 2) 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).
- 3) Fuller, A.T.; *Lancet*, 1, 194 (1937).
- 4) Walsh, C.T.; *Annu. Rev. Biochem.*; 55, 493 (1984).
- 5) Greenwood, D.; 'Antimicrobial Chemotherapy'; 3rd edn., ed. by Greenwood, D., Oxford Univ. Press, Oxford, 50 (1995).
- 6) Robin, R.O. Jr., Williams, J.H., Winnek, P.S. and English, J.P.; *J. Am. Chem. Soc.*; 62, 2002 (1940).
- 7) Caldewell, W.T. and Kime, H.B.; *J. Am. Chem. Soc.*; 62, 2365 (1940).
- 8) Caldewell, W.T., Cornfeld, E.C. and Donnel C.K.; *J. Am. Chem. Soc.*; 63, 3028 (1941).
- 9) Sprague, J.M., Kissinger, L.W., Lincoln, R.M.; *J. Am. Chem. Soc.*; 63, 3038 (1941).
- 10) 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).
- 11) Whithy, L.H.E.; *Lancet*, 2, 1210 (1938).
- 12) Nicoles, R.L., Jones, W.H. Jr. and Finland, M.; *Proc. Soc. Exp. Bio. Med.*; 92, 637 (1956).
- 13) Lester, M.M. and English, J.P.; *U.S.Pat*, 2, 790, 798, (1957) cited from C.A., 51, 15610 (1957).
- 14) Fullerton, D.S.; in 'Wilson and Giswold's Textbook of Organic Medicinal and Pharmaceutical Chemistry'; 9th edn, J.B. Lippincot Company, Philadelphia.
- 15) 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).
- 16) Herbert, V.; *J.Infect. Dis.*; 128, (Nov. suppl.), 432 (1973).

- 17) Reimer, W.A. and Bear, S.; in '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).
- 18) Martin, A.R.; in 'Wilson and Giswold's Textbook of Organic Medicinal and Pharmaceutical Chemistry'; 10th edn. Lippincot-Raven Publishers, Philadelphia, New York, p. 201.
- 19) 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).
- 20) Drake, G.D. and Haynes, K.J.; *U.S.Pat.*, 2759, 931 (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*, 271 (1997).
- 21) Greenwood, D.; *Antimicrobial Chemotherapy*; 3rd edn., d. by Greenwood, D., Oxford Univ. Press, Oxford, 57-58 (1995).
- 22) 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, p.230 (1991).
- 23) Waxman, 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, p.230 (1991).
- 24) 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.
- 25) Doyle, F.P., Hardy, K.D., Nayler, J.H.C., Soulal, M.J., Stove, E.R. and Waddington, H.R.J.; *J. Chem. Soc.*, 1453 (1962).
- 26) Yurchenco, J.A., Hopper, M.W. and Warren, G.H.; *Antimicrobial Chemotherapy*; 12, 534 (1962).
- 27) Nayler, J.H.C.; *Adv. Drug Res.*, 7, 52 (1973).
- 28) Doyle F.L., Long, A.A.W., Naylor, J.H.C. and Stove, E.R.; *Nature*; 192, 1183 (1961).

- 29) 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).
- 30) Bennet, J.V., Gravenkemper, C.F., Bordie, J.I. and Kirby, W.M.M.; *Antimicrob. Agents Chemother.*; 257, (1965).
- 31) Sutherland, R., Croydon, E.A.P. and Rolinson, G.N.; *Brit. Med. J.*; 4, 455 (1970).
- 32) Rolinson, G.N. and Stevens, S.; *Brit. Med. J.*; 2, 191 (1969).
- 33) Broden, R.N., Speight, T.M. and Avery, G.S.; *Drugs*; 9, 88 (1975).
- 34) Acred P., Brown, D.M., Kundson, E.T., Rolinson, G.N. and Sutherland, R.; *Nature*; 215, 25 (1967).
- 35) Morimoto, S. , Nomura, H., Fugono,T., Azuma , T.; Minami, I., Hori, M. and Masuad , T.; *J. Med. Chem* ; 15, 1108 (1972).
- 36) Stewart,G.W.; *The Penicillin Group of Drugs*, Amsterdam, Elsevier; (1965) cited from 'Wilson and Giswold's Textbook of Orgnic Medicinal and Pharmeceutical Chemistry', 9th edn. J.B. Lippincot and Company, Philadelphia.
- 37) Batchlor , F.R. *et.al.*; *Nature*; 206 , 362 (1962).
- 38) 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).
- 39) Ryan, C.W., Simpson, R. L., and Heyningen, E.M.; *J. Med. Chem.*; 12, 310 (1969).
- 40) Durkhelmer, W., Adam, F., Fischer, G. And Kirrstetter, R.(B. Testa, Ed); *Advances in Drug Research*, Academic Press; 17, 61-234 (1988); 'Burger's Medicinal Chemistry and Drug Discovery', 5th edn., ed. by Wolf, M.E., A Wiley-Interscience Publication, John-Wiley & Sons., 4, *Therapeutic Agents*, 299 (1997).
- 41) Adams, H.G., Stilwell, G.A. and Turk, M.; *Antimicrob. Agents Chemother.*; 9,1019 (1976).
- 42) Brogden, R.N., Heel, R.C., Speight, T.M. and Avery, G.S.; *Drugs*; 17, 233 (1979).
- 43) Muytens, H.L., Vander Ros-van, J., Repe.; *Antimicrob. Agents Chemother.*; 21, 925 (1982).

- 44) Symposium; *Review of Infectious Diseases*; 4, Supplement 281-488 (1988) cited from 'Burger's Medicinal Chemistry and Drug Discovery', 5th edn., ed. by Wolf, M.E., A Wiley-Interscience Publication, John-Wiley & Sons., 4, *Therapeutic Agents*, (1997).
- 45) Umezwa H. *et.al.*; *J. Antibit.*; 10, 181 (1957) cited from 'Wilson and Giswold's Textbook of Orgnic Medicinal and Pharmeceutical Chemistry', 9th edn., J.B. Lippincot and Company, Philadelphia, p.277 (1991).
- 46) Weinstein, M.J. *et. al.*; *J. Med. Chem.*; 6, 463 (1963).
- 47) Koch, K.F. *et.al.*; *J. Antibiot.*; 26, 783, (1963) cited from 'Wilson and Giswold's Textbook of Orgnic Medicinal and Pharmeceutical Chemistry', 9th edn., J.B. Lippincot and Company, Philadelphia, p. 279.
- 48) Wrihght, J.J.; *J. Chem. Soc; Chem. Commun*; 206 (1976).
- 49) Dyer, J.R., Todd, A.W.; *J. Am. Chem. Soc.*; 85, 3896 (1963).
- 50) Dyer, J.R. *et.al.*; *J. Am. Chem. Soc.*; 87, 654 (1965).
- 51) Umezawa, S. *et.al.*; *J. Antibiot.*; 27, 997 (1974) cited from 'Wilson and Giswold's Textbook of Orgnic Medicinal and Pharmeceutical Chemistry', 9th edn., J.B. Lippincot and Company, Philadelphia, p.279.
- 52) Haskell, J.H. *et.al.*; *J. Am. Chem. Soc.*; 81, 3482 (1974).
- 53) De Jongh, D.C. *et.al.*; *J. Am. Chem. Soc.*; 89, 3364 (1967).
- 54) Conover, L.H.; *U.S.Pat.*; 2, 693, 054, Jan. 11, 1955 cited from
- 55) Duggar, B.B.; *Ann. N.Y. Acad. Sci.*; 51, 177 (1948).
- 56) Mrtell, M.J. Jr., Booth, J.H.; *J.Med.Chem.*; 10, 44 (1967).
- 57) Finley, A.C. *et. al.*; *Science*; 111, 85 (1950).
- 58) Stephens, C.R. *et.al.* *J. Am. Chem. Soc.*; 80, 5324 (1958).
- 59) Blackwood, R.R. *et.al.*; *J. Am. Chem. Soc.*; 83, 2773 (1961).
- 60) Minuth, J.N.; *Antimicrob. Agents Chemother.*; 6, 411 (1964).
- 61) Woodward, R.B.; *Angrew. Chem.*; 69,50-58 (1957).
- 62) Mc Guire, J.M. *et.al.*; *Antimicrob. Agents Chemother.*; 2, 821 (1952).
- 63) Malmborg, A.S.; *J. Antimicrob. Chemother.*; 18, 293 (1986).
- 64) Wilhelm, J.M. *et.al.*; *Antimicrob. Agents Chemother.*; 236 (1967).

- 65) Gutman, L.T. *et.al.*; *Lancet*; 1, 464 (1967).
- 66) Barradell, L.B., Plosker, G.L. and Mc Tavison, D.; *Drugs*; 46, 289-313 (1993).
- 67) St. Georgiev, V.; *Int. J. Antimicrob. Agents*; 4, 247-270 (1994).
- 68) Manson, D.J. *et.al.*; *Antimicrob.-Agents Chemother.*; 544 (1962).
- 69) Magerlein, B.J. *et.al.*; *J. Med. Chem.*; 10, 355 (1967).
- 70) Erlich, J. *et. al.*; *Science*; 106, 417 (1963).
- 71) Controlis, J. *et.al.*; *J. Am. Chem. Soc.*; 71, 2463 (1949).
- 72) Emmerson, A.E.; David Greenwood's 'Antimicrobial Chemotherapy'; 3rd edn., ed. by Greenwood, D., Oxford Univ. Press, Oxford, 301 (1995).
- 73) Marwick, C.; *JAMA*; 257, 1174 (1992).
- 74) Daniel, T.M.; 'Tuberculosis' in 'Harison's Principles of Internal Medicine'; 12th edn. (Jean D. Wilson ed.) New York, Mc Graw-Hill (1991).
- 75) Meyer, H. And Mally, J.; *Monatsh eftr fiir chemie Monatsh Chem*; 33, 392 (1912)
cited from 'Burger's Medicinal Chemistry and Drug Discovery'; 5th edn., ed. by Wolf, M.E., A Wiley-Interscience Publication, John-Wiley & Sons., 4, *Therapeutic Agents*, p.596 (1997).
- 76) Lemke, Thomas L., Foye, W.O.; 'Principles of Medicinal Chemistry'; 4th edn., ed. By B.I. Waverly, New Delhi, 748 (1995).
- 77) Young, D.B.; *Carr. Biol.*; 4, 351 (1994) cited from 'Burger's Medicinal Chemistry and Drug Discovery'; 5th edn., ed. by Wolf, M.E., A Wiley-Interscience Publication, John-Wiley & Sons., 4, *Therapeutic Agents*, p.597 (1997).
- 78) Zang, Y., Heym, B., Allen, B., Young, D. And Cole, S.; *Nature*; 358, 591 (1992).
- 79) Sensi, P., Grassi, G.G.; in 'Burger's Medicinal Chemistry and Drug Discovery'; 5th edn., ed. by Wolf, M.E., A Wiley-Interscience Publication, John-Wiley & Sons., 4, *Therapeutic Agents*, p.610 (1997).
- 80) Sensi, P., Maggi, N., Furesz, S. And Maffii, G.; *Antimicrob. Agents Chemother.*; 1966, 699 (1967).
- 81) Forbes, M., Kuck, N.A. and Peets, E.A.; *J. Bacteriol.*; 89, 1099 (1962).
- 82) Forbes, M., Kuck, N.A. and Peets, E.A.; *J. Bacteriol.*; 89, 1299 (1965).

- 83) Reutgen, H. And Iwainsky, Z.; *Naturforsch*; 27b, 1405 (1972) cited from 'Burger's Medicinal Chemistry and Drug Discovery'; 5th edn., ed. by Wolf, M.E., A Wiley-Interscience Publication, John-Wiley & Sons., 4, *Therapeutic Agents*, p.602(1997).
- 84) Honore, N. and Cole, S.T.; *Antimicrob. Agents Chemother.*; 38, 238 (1994).
- 85) Emmerson, A.M.; in 'David Greenwood's Antimicrobial Chemotherapy'; 3rd edn. ed. by Greenwood, D., Oxford Univ. Press, Oxford, 306-307 (1995).
- 86) Lehmann, J.; *Lancet*, 1, 15 (1946).
- 87) Sensi, P and Grassi, G.G.; in 'Burger's Medicinal Chemistry and Drug Discovery'; 5th edn., ed. by Wolf, M.E., A Wiley-Interscience Publication, John-Wiley & Sons., 4, *Therapeutic Agents*, p.602(1997).
- 88) Plempel, M., *et.al.*; *Dtsch. Med. Wochenschr.*; 94 , 1356 (1969) cited from 'Wilson and Giswold's Textbook of Orgnic Medicinal and Pharmeceutical Chemistry'; 9th edn., J .B. Lippincot and Company, Philadelphia p. 150 (1991).
- 89) Godefroi, E. F. , *et.al.*; *J. Med. Chem.*; 12, 784 (1968).
- 90) Thomas , A.H. ; *J. Antimicrob. Chemother.*; 17, 269 (1986).
- 91) Gold, W., *et.al.*; *Antibiotics Annual*; (1955-56) p.576, New York Medical Encyclopedia (1956) cited from 'Wilson and Giswold's Textbook of Organic Medicinal and Pharmeceutical Chemistry'; 9th edn. J.B. Lippincot and Company, Philadelphia. p-151.
- 92) Hazen, E. L ., Brown, R.; *Pract. Soc. Exp. Biol. Med.*; 76, 93 (1951) cited from 'Wilson and Giswold's Textbook of Organic Medicinal and Pharmeceutical Chemistry', 9th edn. J.B. Lippincot and Company, Philadelphia. p-151.
- 93) Oxford, A. E. *Et.al.*; *Biochem. J.* ; 33, 240 (1939)
- 94) Grove, J. F. *et.al.*; *J. Chem. Soc.*; 3977 (1952)
- 95) Sloboda, R. D., *et.al.*; *Biochem Biophys Res Commune*; 107, 882_(1982)
- 96) Kruse,R., *et.al.*; *Pharmacology*; 43, 247 (1991) cited from Foye, W.E., Lemke, T.L.; ' Principles of Medicinal Chemistry'; 4th edn. by B.I. Waverly, New Delhi, 811 (1995).
- 97) Dorofienko, G.N.; Andreichikov and Turkloan, Get. Khim., Geterotki, Soedin, 1344, (1974) cited from Squi and Patai; 'The Chemistry of Diazonium and Diazo Group'; Part II, 655.
- 98) Willox, T.H.; *J. Am. Dyest. Rept.*; 55, 891 (1966).

- 99) Putter, R.; 'Methodender Organic Chemie'; Vol. X, Part III, 4th edn., Georg Thieme Verlag Stuttgart, Hauber-Weyl-Muller, p.16 (1965).
- 100) Schank, K.; *Methodicum Chemicum*; Vol. 6 (Edn. Zymalkowsky, F.) Academic press, New York, San Fransisco, London, p. 164 (1975) George Thieme Publishers Stuttgart, (1965).
- 101) Putter, R.; 'Methodender Organic Chemie'; Vol. X, Part III, 4th edn., Georg Thieme Verlag Stuttgart, Hauber-Weyl-Muller, p.22 (1965).
- 102) Putter, R.; 'Methodender Organic Chemie'; Vol. X, Part III, 4th edn., Georg Thieme Verlag Stuttgart, Hauber-Weyl-Muller, p.12 (1965).
- 103) Putter, R.; 'Methodender Organic Chemie'; Vol. X, Part III, 4th edn., Georg Thieme Verlag Stuttgart, Hauber-Weyl-Muller, p.22 (1965).
- 104) *Brit. Pat.*; 741, 228 cited from C.A.; 50, 9913 (1956), *Brit. Pat.*; 736, 568 cited from C.A.; 50, 13097 (1956).
- 105) Putter, R.; 'Methodender Organic Chemie'; Vol. X, Part III, 4th edn., Georg Thieme Verlag Stuttgart, Hauber-Weyl-Muller, p.28 (1965).
- 106) Coonna, M., Greci, L., Bruni, P.; *Gazz. Chim. Ital.*; 527 (1972).
- 107) Wislicenus and Jensen; *Ber.*; 25, 3448 (1892).
- 108) Rabischong; *Bull. Soc. Chim. France*; (3) 31, 76 (1904).
- 109) Rotheburg, Von; *Ber.*; 27, 685 (1894).
- 110) Parmeter, S.M.; *Org. Reactions*; 10, 1 (1959).
- 111) Enders, E. in 'Methodden der Organischen Chemie'; (Houben-Weyl), 4th edn., Vol. X/3, 467 (1965).
- 112) Hunig, S. And Boes, O.; *Ann. Chem.*; 57, 928 (1953).
- 113) Kharkharov, A.A.; *Zh. Obshch Khim.*; 23, 1175 (1955) cited from C.A. 42, 12396 (1953).
- 114) Porai, A.E., Koshits and Kharkharov, A.A.; *Bull. Acad. Sci.; URSS*, 79 (1944) cited from C.A. 39, 1631 (1945).
- 115) Philips, R.R.; *Org. Reactions*; 10, 143 (1959).
- 116) Bulow and Hailer, E.; *Ber.*; 35, 915 (1902).
- 117) 'Organic Reactions'; Vol. 2, John Wiley and Sons, New York, 130 (1946).

- 118) 'Organic Reactions'; Vol. 2, John Wiley and Sons, New York, 132(1946).
- 119) 'Organic Reactions'; Vol. 2, John Wiley and Sons, New York, 136-144 (1946).
- 120) 'Vogel's Textbook of Practical Organic Chemistry'; 4th edn., 771 (1978).
- 121) Fieser and Hershberg; *J. Am. Chem. Soc.*; 61, 1272 (1939)
- 122) Reimer, *Ber.*, 14, 1802 (1881) and Roser; *Ann.*; 247, 152 (1882) cited from 'Organic Reactions'; Vol. 2, John Wiley and Sons, New York, 162-169 (1946).
- 123) Richter; *Ber.*; 16, 677 (1883) cited from Simpson, J.C.E.; 'Chemistry of Heterocyclic compounds'; Interscience Publishers, INC, New York, 5, 46 (1953).
- 124) Borsche and Herbert; *Ann.*; 546, 293 (1941) cited from Simpson, J.C.E.; 'Chemistry of Heterocyclic compounds'; Interscience Publishers, INC, New York, 5, 47 (1953).
- 125) Widman; *Ber.*; 17, 722 (1884) cited from Simpson, J.C.E.; 'Chemistry of Heterocyclic compounds'; Interscience Publishers, INC, New York, 5, 26 (1953).
- 126) Schofield and Simpson; *J. Chem. Soc.*; 1945, 512.
- 127) Gewald, K., Calderon, O., Schaffer, H. And Hein, V.; *Justus Liebigs Ann. Chem.*; 7,
- 128) (1984) cited from C.A.; 101, 130651 (1984).
- 129) Lewis, R.N. and Susim, P.V.; *J. Am. Chem. Soc.*; 4, 840 (1952).
- 130) Corpin, H.B.; (1956) *U.S. Pat.*; 2, 715, 137 (1955) cited from C.A.; 50, 7160 (1956).
- 131) Rogees, A.O.; *U.S. Pat.*; 2, 415, 267 (1947) cited from C.A.; 41, 3119 (1941).
- 132) Newman, M.S. and Boden, H.; *J. Org. Chem.*; 26, 2525 (1963).
- 133) Rorig, K. *et al.*; *Org. Synthesis*; Coll. Vol. 4, 575 (1963).
- 134) McElvein, S.M. and Goese, M.A.; *J. Am. Chem. Soc.*; 63, 2283 (1941).
- 135) Gilman, H. and Spatz, S.M.; *J. Am. Chem. Soc.*; 63, 1553 (1941).
- 136) Kent, R.E. and McElvein, S.M.; *Org. Synthesis*; Coll. Vol. 3, 493 (1955)
- 137) Corsow, B.B. *et. al.*; *Org. Synthesis*; Coll. Vol. 2, 379 (1943).
- 138) Brown, P.M. *et. al.*; *J. Chem. Soc.*; 2882 (1957).
- 139) Stephens, C.R. *et.al.*; *J. Am. Chem. Soc.*; 77, 1701 (1955).
- 140) Surrey, A.R.; *Org. Synthesis*; Coll. Vol. 3, 535 (1955).
- 141) Herbst, R.M. and Wilson, K.R.; *J. Org. Chem.*; 22, 1142 (1957).
- 142) Campagna, F., Carotti, A. And Casini, G.; *Tetrahedron Letters*; 21, 1813-16 (1977).

- 143) Haganest, H. And Stauf, F.W.; *U.S.Pat.*; 1, 879, 209 (1932) cited from C.A.; 28, 4848 (1934).
- 144) Carke, H.T. and Read, R.R.; *Org. Synthesis*; Coll. Vol. 1, 514 (1941).
- 145) Vohwinkel, E. and Bartel; *J. Chem. Soc.*; 107, 1221 (1974).
- 146) Farey, H.G. and Dalton, D.R.; *J. Chem. Soc. Chem. Commun.*; 628 (1973).
- 147) Kukhar, V.P. and Pasternak, V.I.; *Synthese*; 563 (1974).
- 148) Streith, J. and Fizet, C.; *Helv. Chim. Acta.*; 59, 2788 (1976).
- 149) Miller, M.J. and Zoudon, G.M.; *J.Org. Chem.*; 40, 126 (1975).
- 150) Glass, R.S. and Hoy, C.; *Tetrahedron Letters*; 20, 1781 (1976).
- 151) Houben-Weyl 'Methoden der Organischen Chemie'; 4th edn., vol.3, 325 (1952).
- 152) Kitagawa, T. *et.al.*; *Chem. Phar. Bull.*; 38, 2583 (1990).
- 153) Carpino, L.A.; *Accounts Chem Res.*; 6, 191 (1973).
- 154) Carpino, L.A. Giza, C.A. and Carpino, B.A.; *J.Am. Chem. Soc.*; 81, 955 (1959).
- 155) Sheradsky, A.; *J.Het. Chem.*; 4, 413 (1967).
- 156) Tamare, Y., Minamikawa, I., Kita, Y., Kim, N.H. and Ikeda, M.; *Tetrahedron Letters*; 29, 1063 (1973).
- 157) Tamare Y. *et.al.*; *J.Org. Chem.*; 38, 1239 (1973).
- 158) Pomeroy, J.H. and Creig, C.A.; *J.Am. Chem. Soc.*; 81, 6340 (1959).
- 159) Volchel, C., Liebigs; *Ann. Chem.*; 38, 314 (1841).
- 160) Roumanian Ministry for Petro and Chem. Industry; *Brit. Pat.*; 980, 997 (1965) cited from C.A.; 63, 13221f (1965).
- 161) Fuches, O., Senkaiak, V., Nemes, A., Lazar, A. and Somgyl, T.; *Austrelian Pat.*; 241461 (1965).
- 162) Kindler, K.; Liebigs *Ann. Chem.*; 431, 187 (1923).
- 163) Singer, F.A., Schafer, W. And Chrwitte, E.; *Agrew. Chem.*; 76, 273 (1964).
- 164) Fairful, A.E.S., Lowe, J.L. and Peak, D.A.; *J. Chem. Soc.*; 742 (1952).
- 165) Cairns, T.L., Larchar, A.W., McKusicj, B.C.; *J. Org. Chem.*; 18, 748 (1953).
- 166) Von Esch, A.M. and Shermann, H.R.; *U.S.Pat.*; 3158623 (1964) cited from C.A.; 62, 4008 (1965).
- 167) Hanford, W.E.; *U.S.Pat.*; 2201170 (1940) cited from C.A.; 34, 6388² (1946).

- 168) Taylor, E.G. and Zaltewicz, J.A.; *J.Am. Chem. Soc.*; 182, 2656 (1960).
- 169) Albert, A.; *Ber. Dtsch. Chem. Ger.*; 48, 470 (1915).
- 170) Olin, J.F. and Johnson, T.B.; *Recueil. Trav. Chim. Pay. Bas.*; 50, 72 (1931).
- 171) Goerdeler, J. and Mitter, W.; *Chem. Ber.*; 96, 944 (1963).
- 172) Mayer, r., Hiller, G., Nitzchke, M. And Jentz, J.; *Agrew. Chem.*; 75, 1011 (1963).
- 173) Casser, L., Panossian, S. And Giordana, C.; *Synthesis*; 917 (1978).
- 174) Hofmann, H.W.; *Ber. Dtsch. Chem. Ger.*; 11, 340 (1878).
- 175) Zaiden, Hogin, Seisan, Kaihatsu, Kagku, Kenyusho; *Jap. Pat.*; 9002/65 (1965).
- 176) Cerecedo, L.R. and Tolpin, J.G.; *J.Am. Chem. Soc.*; 59, 1660 (1937).
- 177) Schmitz, W.R.; *U.S.Pat.*; 2682558 (1954) cited from C.A.; 49, 9029c (1955).
- 178) Westphal, K. and Andersag, H.; *Canad. Pat.*; 392513 (1940) cited from C.A.; 35, 1413⁵ (1940).
- 179) Behringer, H. Ruff, M. And Wiedmann, R.; *Chem. Ber.*; 97, 1732 (1964).
- 180) Pesina, A.G.; *Zh. Obshch. Khim.*; 9, 804 (1939).
- 181) Kingsberg, E. and Papa, D.; *J.Am. Chem. Soc.*; 73, 4988 (1951).
- 182) Wagner, G. and Singer, D.; *Z.Chem.*; 2, 306 (1962).
- 183) Willstaller, R. and Wirth, T.; *Ber. Dtsch. Chem. Ger.*; 42, 1911 (1909).
- 184) Kikuchi, K.; *Jap. Pat.*; 1710 (1951) cited from C.A.; 47, 4908 (1953).
- 185) Jancevska, M., Jacopicin, K. And Hahu, V.; *Croat. Chem. Acta.*; 37, 67, (1965).
- 186) Sachs, F. and Loevy, H.; *Ber. Dtsch. Chem. Ger.*; 36, 585 (1976).
- 187) Eleftherious, Papadopoulos, P.; *J.Org.Chem.*; 41(6), 962 (1976).
- 188) Sastry.S. and Kudav, V.A.; *Ind. J.Chem.*; 18B(5), 455 (1971).
- 189) Hantzch, A.R. and Weber, H.J.; *Ber.*; 20, 3118 (1887).
- 190) Jones, E.R.H., Robinson, F.A. and Strachan, M.N.; *J. Chem Soc.*; 87 (1946).
- 191) Vernim, G. and Metzger, J.; *Bull. Soc. Chim. France*; 2498 (1963).
- 192) Sarkis, G.V. and Agawe, S.A.; *J. Chem. Eng. Data*; 18, 99 (1973).
- 193) Erylenmeyer, H., Erne, M.; *Helv. Chem. Acta.*; 31, 652 (1948).
- 194) Hantzch, A.R. and Weber, H.J.; *Ber.*; 21, 938 (1888).
- 195) Vogel, A.I.; 'Element Pract. Org. Chem.'; 4th edn., 848 (1984).
- 196) Mathes, R.A., Gregory, J.T.; *J.Am. Chem. Soc.*; 74, 3867 (1952).

- 197) Cook, A.H., Downer, J.D. and Heilbron, I.; *J. Am. Chem. Soc.*; 1202 (1948).
- 198) Ochiai, E. and Nagasawa, F.; *J. Pharm. Soc. Japan*; 59, 43 (1939).
- 199) Dahiya, R. And Pujari, H.K.; *Ind. J. Chem.*; 25B, 966 (1986).
- 200) 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).
- 201) *U.S. Pat.*; 2, 652, 396.
- 202) Khanna, M.S., Chandra, P., Garg and Kapoor, R.P.; *Ind. J. Chem.*; 32B, 364 (1993).
- 203) Simpson, J.C.E.; 'Chemistry of Heterocyclic Compounds'; Interscience Publishers, INC, New York, 5, 5 (1953).
- 204) Fuscho, R., Piselli, F.L. and Boshi, P.M.; *Eur. Pat. Appl.* EP 287 (1988) cited from C. A.; 110, 75540 (1989).
- 205) Abbady, M.S., Radhwan, Sh. M., Bhakite, E.A.; *Ind. J. Chem.*; 32B, 1281-1284 (1993).
- 206) Nargund, L.V.G., Nargund, K.S.; *Arzneimittel Forchung Drug Research*; 1284 (1993).
- 207) Gopikumar Menon, C.R., Purushotthaman, E.; *J. Ind. Chem. Soc.*; 72, 713-734 (1995).
- 208) Gardener, T.S., Wenis, E., Lee, J.; *J. Org. Chem.*; 20, 976-980 (1955).
- 209) Carp. Eugenis and Toma, A.; C.A.; 63, 14483d (1965).
- 210) Choudhari, D.T., Sabnis, S.S., Patel, M.R. Deliwala, C.V.; *Bull. Haff. Inst.*; 4(1), 8-15(Eng.) (1976).
- 211) Ciba Geigy; Chugai; *Drugs Fut.*; 21(4), 434 (1996).
- 212) Ledniser, D.; 'Strategies in Drug Synthesis'; 6, 86.
- 213) Bowman, R.M., Steele, R.E., Browne, L. (Ciba Geigy Corp.) U.S. US 4,978, 672 (Cl. 514-383; A 61K31/ 41); (1990), C.A.; 114, 247887y and 247288 (1991).
- 214) *Drugs Fut.*; 23(10), 1071-1074 (1998).
- 215) 'Organic Synthesis'; John Wiely and Sons., 40, 99-102 (1965).
- 216) Strain, H.H.; *J. Am. Chem. Soc.*; 49, 1995 (1927).
- 217) Ainsworth, C. And Jones, R.G.; *J. Am. Chem. Soc.*; 77, 621 (1955).
- 218) Grudman, C. And Ratz, R.; *J. Org. Chem.*; 21, 1037 (1956).

- 219) Hoggarth, E.; *J. Chem. Soc.*; 1949, 1163.
- 220) Blatter, Kukasazewski, Stevens; *J. Am. Chem. Soc.*; 83, 2203 (1961).
- 221) Hafmann, H.W.; *Ber. Dtsch. Chem. Ges.*; 11, 340 (1878).
- 222) John, Lee; U.S. Pat.; 2, 653, 942 (1953), C.A.; 48, 11500d (1954).
- 223) Fairfull, A.E.S., Lowe, J.L. and Peak, D.A.; *J. Chem. Soc.*; 1952, 742.
- 224) Martiz, K.L., Taube, Carl (Farbentadriken Bayer A.G.) Brit. Pat.; C.A.; 71, 14164j (1969).
- 225) Prabhu, S.R., 'Chemotherapy of Tuberculosis', Ph. D. Thesis, Haff. Inst., 113 (1982).
- 226) Kokimoto S., Seydel, J. And Wempe, E.; (Hokkaido Univ.; Sapporo, Japan) *Arzneimittel Forsch*; 12, 127-33 (1962). C.A.; 57, 9813a (1962).
- 227) Cited from C.A., 64, 8061 (1966).
- 228) Bowman, R.M., Steele, R.E., Browne, L. (Ciba Geigy Corp.) *U.S. Pat.*; US 4,978, 672 (Cl. 514-383; A 61K31/ 41); (1990).
- 229) Rake, G., Jambor, W.P., McKee, C.M., Pansy, F., Wiselodge, E.Y., Donobic, R.; *Am. Rev. Tubercle.*; 60, 121 (1949).

:APPENDIX:

1. The melting points have been determined in Open capillaries in a veego VMP-1 melting point apparatus.
2. The TLC of the compounds have been run of on Eastman Kodak precoated silica gel plates of type K301 R and E.Merck precoated silica gel plates of type 60F 254.
3. The Infrared spectras were recorded on a BOMEN MB-104 Fourier Transmission Infra Spectrometer.
4. The PMR spectras were recorded on Varian 300MHz Spectrometer using TMS as internal reference at RSIC, IIT, Powai.

Synopsis

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

TITLE OF THE THESIS : Synthesis of organic compounds ~~having~~ of *juvai* possible therapeutic values and preparation of a drug intermediate.

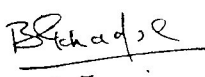
NAME OF THE CANDIDATE : Ms. Sharvani Kusumakar Vingkar.

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

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

NUMBER AND DATE OF REGISTRATION : 33 / Dec. 9, 1997

SIGNATURE OF THE STUDENT : 

SIGNATURE OF THE GUIDE : 

:SYNOPSIS:

Among the several characteristics unique to *Homo sapiens* is our propensity to treat ailments, physical and mental, with medicines. From archaeological evidence, this urge to sooth the burdens of disease is as old as humanity's search for other tools. Drugs have been used to gain increased control over our lives and to make them longer, healthier and happier.

In the 19th century, the developments in synthetic and analytical chemistry led to the isolation and structural elucidation of medicinally active compounds such as, alkaloids, flavones, steroids etc. from natural origin. Today, useful drugs arise from natural products, fermentation broth, chemical synthesis or a combination of both the sources using rational/ empirical approaches. However, there is a constant need to develop better drugs with higher efficacy and lower toxicity than those currently in use. A successful chemotherapeutic agent should have following properties.

- 1) It must exert an antimicrobial action at high dilution and in the complex chemical environment of the body.
- 2) It must assure higher safety to the body tissues and yet be highly efficacious.
- 3) It should overcome the drug resistance of various invading microorganisms.

A medicinal chemist deals with the incorporation of newly emerged pharmacophores on an established biologically active moiety. One such genuine effort has been made and will be documented in the thesis.

The thesis will consist of the two parts- Part I and Part II and will be divided into five chapters.

PART-I

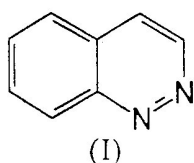
- i) Introduction, Review of Literature and Plan of Work.

PART-II

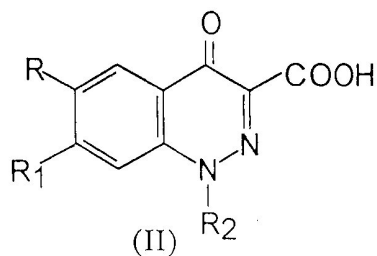
- ii) Synthesis of 1H-3(2-alkyl\aryl,4-substituted thiazolo)-6-chloro-cinnoline-4-ones.
- iii) Synthesis of 1H-3(2-alkyl\aryl,4-substituted thiazolo)-6-fluoro-cinnoline-4-ones.
- iv) Synthesis of intermediate for Letrozole..
- v) Biological screening and results.

PART-II: Chapter-ii;

Synthesis of 1H-3(2-alkyl\aryl,4-substituted thiazolo)-6-chloro-cinnoline-4-ones.



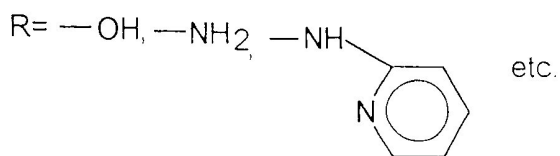
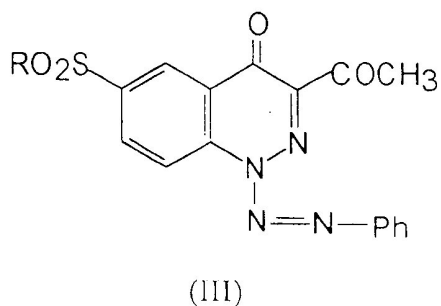
Cinnoline(I), a member of the heterocyclic family, has been documented in the literature for its appreciable antibacterial activity¹.



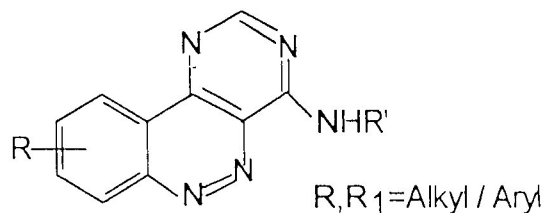
R/R1= H, electron donor grp.

R2= C₁₋₆alkyl

1-alkyl-3-carboxy-4-cinnolones(II) also show good antibacterial activity².

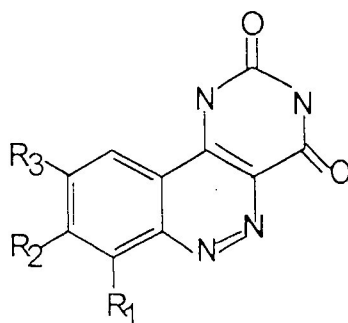


3-acetyl-phenylazo-6-substituted cinnoline-4-ones(III) have been reported to exhibit potential antibacterial, but low antifungal activity³.



(IV)

Similarly, 4-arylamino pyrimido(5,4-c) cinnolines⁴(IV) have been reported to possess activity from 0.3μg/ml to 0.05μg/ml against gram positive *Bacillus cirroflagelus*, from 0.21μg/ml to 0.025 μg/ml against gram positive *Staphylococcus aureus*, and 3.16μg/ml against gram negative *Escherichia coli*.



R₁, R₂, R₃ = Alkyl / Aryl

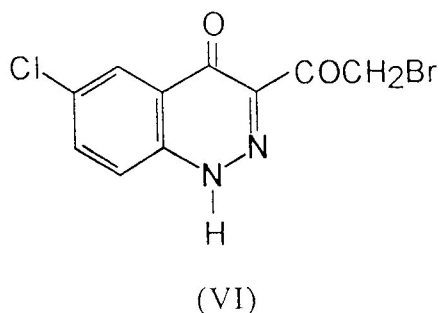
(V)

Pyrimido (5,4-c) cinnoline-2,4(1H,3H) diones⁵ (V) are known to possess moderate activity i.e. upto 100μg/ml against a number of gram positive and Gram-negative organisms and fungi like *Aspergillus niger* and *Candida albicans*.

Relatively less work has been carried out on the cinnoline ring. Hence, we decided to emphasize on this ring system and carry out studies on compounds

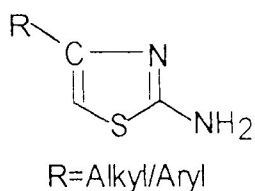
bearing a combination of cinnoline and other heterocyclic systems, in the hope of developing a prospective antimicrobial agent.

Electron withdrawing substituents like halogens and nitro are believed to impart stability to the ring and thereby contribute to the activity. Hence, we selected 6-chloro-(3-haloacetyl)-1H-cinnoline-4-one(VI) as our parent moiety.

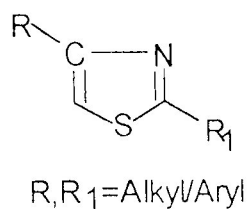


Thiazole:

Thiazole moiety is well established as a biologically active moiety especially for its antibacterial activity⁶. Russian research workers⁷ showed that amino substitution at 2-position of thiazole ring along with alkyl/aryl substitution at 4-position(VII) imparts bacteriostatic activity to the molecule.



(VII)



(VIII)

Similarly, vast synthetic work carried out in our laboratory on 2,4-disubstituted thiazoles (VIII) also revealed⁸ that many thiazoles of such types are active against H₃₇Rv strain of *Mycobacterium tuberculosis* at concentrations

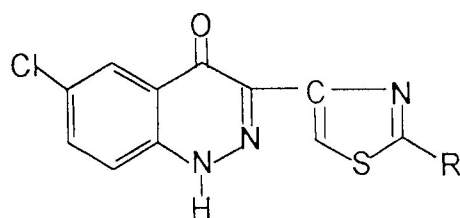
ranging from 5 μ g/ml to 0.01 μ g/ml.

These properties of thiazole nucleus as antitubercular agent strongly suggest that this nucleus should be explored further in order to achieve good anti tubercular activity.

Keeping in mind the good antibacterial activity of 2,4-disubstituted thiazole, we decided to combine cinnoline with thiazole.

In order to obtain the thiazole, the 3-bromoacetyl-6-chloro-1H-cinnoline-4-one was condensed with various substituted thioamides.

The final compounds in this series have the following substitutions-



(IX)

R= aminomethylphenyl, pyridyl, benzyl etc.

All the ten compounds synthesised in this series were confirmed by elemental and spectroscopic analysis.

Chapter-ii:

Synthesis of 1H-3(2-alkyl/aryl,4-substituted thiazolo)-6-fluoro-cinnoline-4-ones.

Extensive work has been reported on the advantages of organo fluorine compounds. Fluorine atom itself has numerous advantages, like-

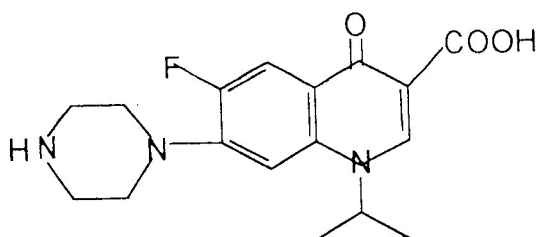
- 1) Fluorine, the second smallest atom as measured by atomic radius and internuclear distance to carbon, would most closely resemble bioactive hydrogen analogues with respect to steric requirements at enzyme receptor

sites.

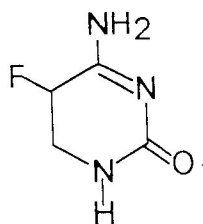
2.) Fluorine atom imparts increased oxidative and thermal stability because of high binding energy.

3.) Fluorine atom leads to increased lipid solubility which enhances the rate of absorption and *in vivo* transport.

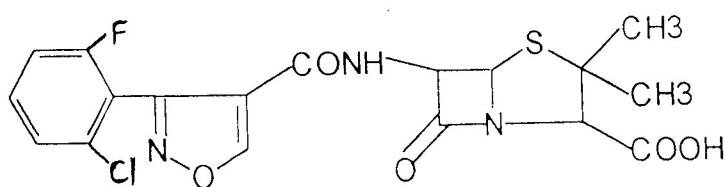
Numerous fluorinated compounds are known e.g. Ciprofloxacin(X) (antibacterial), Flucytosine(XI)(antifungal), Flucloxacillin (XII)(antibacterial), etc.



Ciprofloxacin (X)



Flucytosine (XI)

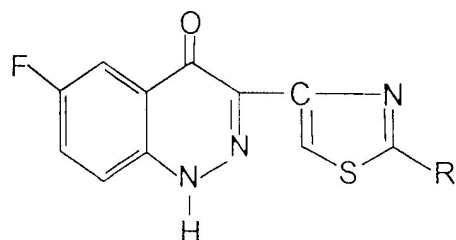


Flucloxacillin

(XII)

Thus, we decided to replace chlorine at 6-position by fluorine to check its effect on activity. The fluorinated compounds were synthesized by a similar procedure using 4-fluoro aniline as the starting compound.

The final structure is as follows-



R = Alkyl/Aryl.

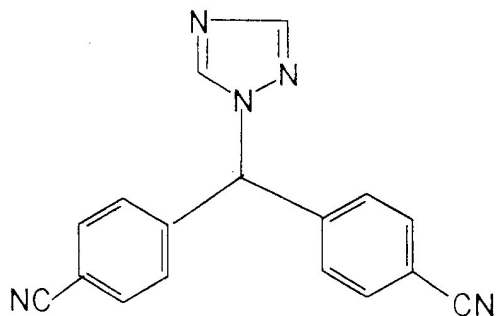
(XIII)

R= alkyl /aryl/ substituted aryl

All the ten compounds synthesised in series II were confirmed by elemental and spectroscopic analysis.

Chapter-iii;

Synthesis of intermediate for Letrozole:



Letrozole

CGS(20267) (XIV)

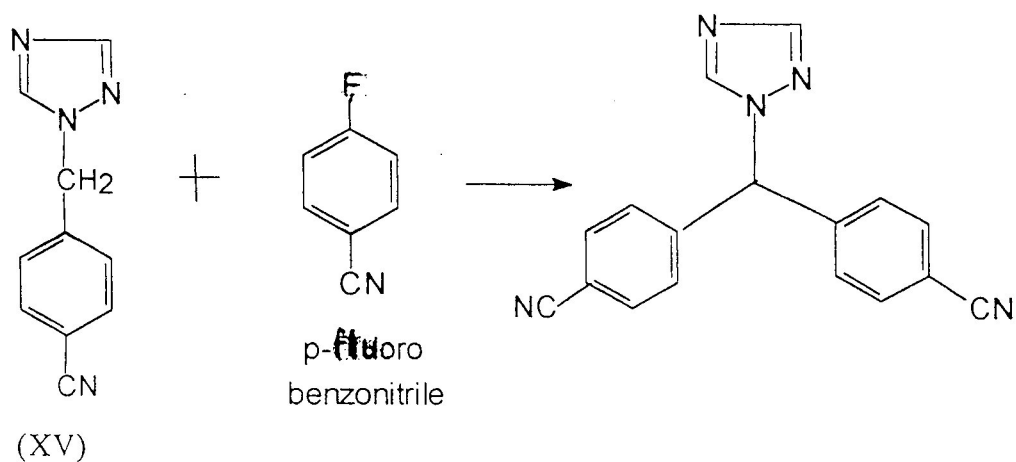
Letrozole(XIV) is a relatively new drug used as an antineoplastic agent. Using a modified nude mouse model of post-menopausal breast cancer, CGS(20267) was shown to have a marked antitumor effect and to significantly suppress plasma estrogen⁹.

Letrozole is currently undergoing phase II and phase III clinical trials for breast cancer in U.S.A.

Pharmacological action:

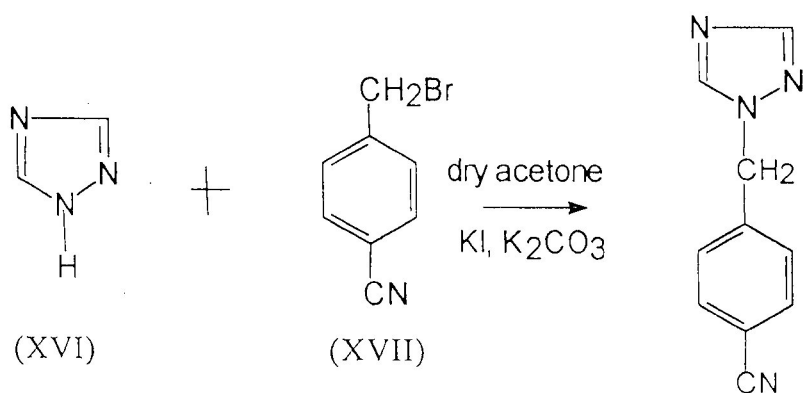
Letrozole is a highly potent and selective non-steroidal aromatase inhibitor. Aromatase catalyses the conversion of androgens to estrogens which leads to an increase in plasma estrogen level. Letrozole was shown to inhibit aromatase in peripheral tissues at concentrations of 0.5 –2.5 mg/day for 6 weeks⁹.

The synthetic route of Letrozole is as follows¹⁰:



Number of antineoplastic drugs can be synthesized using 4(1,2,4 triazolyl methyl) benzonitrile(XV) e.g. MPV-2213ad¹¹, Letrozole etc.

The intermediate 4(1,2,4 -triazolyl methyl) benzonitrile is prepared by condensation of 1 H-1,2,4 –triazole(XVI) with cyanobenzyl bromide(XVII)¹².



Thus, 1,2,4 -triazole is a key intermediate for the synthesis of Letrozole 1,2,4 -triazole can be prepared by various routes¹³ which are summarized as follows:

- 1.) By the oxidation of substituted 1,2,4 -triazoles.
- 2.) By treating urazole with phosphorus pentasulfide.
- 3.) By heating equimolar quantities of formyl hydrazine and formamide.
- 4.) By removal of the amino functions of 4--amino- 1,2,4 -triazole.
- 5.) By oxidation of 1,2,4 -triazole-3(5) thiol with hydrogen peroxide.
- 6.) By decarboxylation of 1,2,4 -triazole-3(5) carboxylic acid.
- 7.) By heating hydrazide salt with formamide¹⁴.
- 8.) By rapid distillation of hydrazine hydrate with 2M equivalent of formamide¹⁵.
- 9.) By heating N,N' diformyl hydrazine with excess ammonia in an autoclave at 200 °C for 2-4 hrs.¹⁶.
- 10.) By the reaction of 1,3,5-triazine and hydrazine monohydrochloride¹⁷.
- 11.) By oxidation of 1,2,4 -triazole-3(5) thiol in conc. HNO₃¹³.

We've carried out the oxidation of 1,2,4-triazole-3(5) thiol for better yield by applying certain modifications to the literature procedure.

MODIFICATIONS:

- 1.) During the work up of oxidation of 1,2,4 -triazole -3(5) thiol ,we neutralized the reaction mixture with liquid ammonia in order to minimize the inorganic bulk.
- 2.) Instead of hectic and multiple extraction procedure , we extracted the triazole in ethyl acetate and recovered the solvent upto 90%.
- 3.) The use of large quantity of ethyl alcohol was avoided and this makes the process cost effective.
- 4.) Also, use of benzene was avoided. Benzene being highly carcinogenic may cause health hazards to the workers, when used at industrial scale.

The triazole and the benzonitrile were subjected to elemental analysis and spectroscopic studies for confirmation.

Chapter-v:

Biological Screening:

All the compounds synthesised were subjected to antibacterial, antifungal and antitubercular screening.

For antibacterial screening, the organisms used were *Staphylococcus aureus* ATCC3750 and *Salmonella typhi* NCTC786. The medium used was Mueller Hinton Broth. Standard drugs used were Trimethoprim(MIC1µg/ml) against *S. typhi* and Ampicillin(MIC0.01µg/ml) against *S. aureus*.

Six compounds have shown good antibacterial activity upto 4µg/ml while five compounds have shown activity upto 10µg/ml.

For antifungal screening, the organisms used were *Candida albicans*, *Tricophyton mentagrophytes* and *Tricophyton rubrum*. The medium used for growth was Sraufataller medium. Miconazole(MIC1.56µg/ml) was used as the standard drug.

Four compounds have exhibited activity upto 100µg/ml.

For antitubercular screening, H₃₇Rv strain of *Micobacterium tuberculosis* was grown on Kirchner medium and the activity was compared with standard drug Isoniazide(MIC0.04µg/ml).

Five compounds have shown activity upto 40µg/ml while one compound is below 20µg/ml.

REFERENCES:

- 1.) "Chemistry of Heterocyclic Compounds", Simpson, J.C.E.; Interscience Publishers, INC, New York; 5,5,(1953).
- 2.) Fuscho,R., Piselli,F.L. & Boshi,P.M.; *Eur. Pat. Appl.* EP 287(1988); C.A.; 110(1989)75540.
- 3.) Abbady, M.S., Radhwan, Sh.M., Bhakite, E.A.; *Ind. J. Chem.*; 32B;1281-1284(1993).
- 4.) Nargund L.V.G., Nargund, K.S.; *Arzneimittel Forchung Drug Research*; 45;113,(1995).
- 5.) Gopikumar Menon, C.R., Purushotthaman, E.; *J. Ind. Chem. Soc.*; 72;731-734,(1995).
- 6.) Gardener, T.S., Wenis, E., Lee, J.; *J. Org. Chem.*; 20;976-980(1955).
- 7.) Carp. Eugenis & Toma A.; C.A.; 63,14483d(1965).
- 8.) Choudhari, D.T., Sabnis, S.S., Patel, M.R., Deliwala, C.V.; *Bull. Haff. Inst.*; 4(1); 8-15(Eng.)(1976).
- 9.) Ciba Geigy; Chugai; *Drugs Fut.*; 21(4);434,(1996).
- 10.) "Strategies in Drug Synthesis"; by Daniel Ledniser; 6;86.
- 11.) Bowman, R.M., Steele, R.E., Browne, L; C.A.; 114;247287y and 247288(1991).
- 12.) *Drugs Fut.*; 23(10); 1071-1074.
- 13.) "Organic Synthesis", ed. by John Wiley & Sons.; 40; 99-102.,(1965).
- 14.) Strain, H.H.; *J. Ame. Chem. Soc.*; 49,1995,(1927).
- 15.) Ainsworth, C. & Jones, R.G.; *J. Am. Chem. Soc.*; 77; 621,(1955).
- 16.) Grudman, C. & Ratz, R.; *J. Org. Chem.*; 21; 1037,(1956).
- 17.) Hoggarth, E.; *J. Chem. Soc.*; 1949. 1163.

Disclaimer

This research work belongs to

Sharvani K. Vingkar

No content of this work can be
circulated or used for any
commercial purpose without the
consent of respective researcher

**Synthesis of Organic Compounds of Possible Therapeutic
Values and Preparation of A Drug Intermediate.**

**A
Thesis Submitted
to
The University of Mumbai
for The Degree of
Master of Pharmaceutical Sciences
in The
Faculty of Technology**

**by
Sharvani K. Vingkar
B. Pharm.**

**Department of Chemotherapy,
Haffkine Institute for Training, Research and Testing,
Acharya Donde Marg, Parel, Mumbai - 400012.
2000**

HAFFKINE INSTITUTE LIBRARY,
MUMBAI.

Call No.
Price

Acc No.
Source Purchased.
Donated

438

Statement

Statement submitted under rule 0.412.

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

Statement submitted under rule 0.413.

Statement I:

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

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

Statement II:

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

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

Dr. B.G. Khadse



Signature of Research Guide

Ms. Sharvani Vingkar



Signature of Research Student:

:Contents:

	Page no.
Acknowledgement Statement	
Part I-A:	
Introduction	1-4
Review of Literature	5-39
Plan of Work	40-51
Part I-B:	
Chemistry of	
Diazonium salts	53-57
Hydrazones	58-61
Friedel-Craft's acylation to form ketone,	62-67
Cinnoline and cinnlone	
Halogenation of ketones	68-71
Nitriles	72-79
Thioamides	80-85
Thiazoles	86-91
Part I-C:	
Schematic Representation	92-99
Part II: Synthesis of 3-(2-alkyl/ aryl,4-substituted thiazolo)-6-chloro-cinnoline-4-ones:	
Synthesis of ethyl 2(4-chlorophenyl hydrazono)-3-oxybutyrate	102
Synthesis of 3-acetyl-6-chloro cinnoline-4-one	102
Synthesis of 3-bromoacetyl-6-chloro cinnoline-4-one	103
Synthesis of 4-amino methyl benzonitrile HCl	103
Synthesis of N,N-dimethyl amino benzonitrile	104
Synthesis of thioamides	105
Synthesis of thiazoles	107
Part III: Synthesis of 3-(2-alkyl/ aryl,4-substituted thiazolo)-6-fluoro-cinnoline-4-ones:	
Synthesis of ethyl 2(4-fluorophenyl hydrazono)-3-oxybutyrate	110
Synthesis of 3-acetyl-6-fluoro cinnoline-4-one	110
Synthesis of 3-bromoacetyl-6-fluoro cinnoline-4-one	111
Synthesis of thiazoles	111-112
Part IV: Synthesis of Intermmediate for Letrozole	
Synthesis of 1-formyl-3-thiosemicarbazide	115
Synthesis of 1H-1,2,4-triazolo-3(5)-thiol	115
Synthesis of 1H-1,2,4-triazole	115

Synthesis of 1,2,4-triazolyl-methyl benzonitrile	116
--	-----

Part V:

Biological screening and Results	117-124
---	---------

Conclusion	125-126
-------------------	---------

References	127-139
-------------------	---------

Appendix

Synopsis

: ACKNOWLEDGEMENT :

I take this opportunity to express my immense sense of gratitude to my guide, Respected Dr. B.G.Khadse, Director, Haffkine Institute, whose valuable guidance and encouragement made me carry out my research work. I have always been benefitted from his teaching and constant counselling. It gives me great pleasure to recollect my association with him as his student.

I am thankful to Dr. S.V.Gadre, Asst. Director, Haffkine Institute, for his valuable assistance and help.

I am profoundly grateful to Dr. A.S.Bobade, Department of Chemotherapy, for his ever-helping attitude and kind co-operation during this research work.

I express my very special and sincere thanks to Ms. Bharati Kulkarni, Senior Scientific Officer, Department of Chemotherapy, for reviewing the manuscript and important discussions and encouragement.

I owe special thanks to Ms.P.A.Kirtikar, Mr.L.G.Mistry and Mr.Pramod Andhere, for carrying out elemental analysis and Mr.K.R.Pawar for his help during antimicrobial screening of the compounds.

With great pleasure I acknowledge the entire staff of the Department of Chemotherapy, for their help.

I am grateful to Respected Dr. Ambaye, Department of Biochemistry, for reviewing the manuscript and his critical suggestions which enabled me to make this thesis more acceptable.

I extend my appreciation to Dr.S.B.Dholam, Department of Bioavailability, for his kindness and help during the typing of the thesis. I also thank Ms.Preeti Lali and Ms.Sarita Chaudhari for their help.

I sincerely thank Ms.M.V.Joshi and the entire library staff for maintaining excellent library facilities.

I also thank the authorities of 'Animal House', HBPCCL, for providing material as per the requirements during the antimicrobial screening of the compounds.

I am grateful to the NMR section, RSIC, IIT, Powai for their prompt assistance in providing the NMR spectras. I am also thankful to Dr. Aravind Manian for his help for the interpretation of the spectras.

Last but not the least, I would like to acknowledge my senior colleagues, Pallavi Kharkar, Sonali Wayangankar, Chandan Jagtap, Sandeep Paranjape, Hemant Narkhede, Ravi Chambhare, for their valuable suggestions and guidance during the practical work.

I experience a feeling of great delight to be associated with my colleagues, Sagar Agnihotri, Chandrakant Bonde, Vinod Rane, Yogesh Kulkarni, Tushar Lokhande, Sunil Narkhede, Anagha Samant, Mukta Bohra and Jaya John who always maintained a healthy working atmosphere and helping attitude, making my task easier. I'll always appreciate Ms. Rucha Sharma, Department of Virology, for her moral support and encouragement.

I am indebted to my parents and brother, whose patience, inspiration and support enabled me to carry out this entire work.

Above all, I thank God Almighty for His blessings and providing me the strength to survive through the most difficult times.

- Sharvani Vingkar.

Introduction

INTRODUCTION:

'No scientific discipline is currently as hotly pursued as chemistry; if one not compelled, therefore, to apply its astounding results to man?..... Chemists concentrate the fragrance of cinnamon into a few drops and distill from treacherous poison and innocent plants---- the amazing medications. Yet there is more to come. If chemistry keeps its promises.... whatever is alive, will be perfect; and, in the end man will live for centuries, free from pain and sufferings, in indestructible health, until he dissolves serenely, in peace.'

-- JOHANNES GAUB (1734).

Drug discovery has its roots in the beginning of mankind. Even our polygenetic cousins, the chimpanzees have been observed to chew certain leaves to alleviate gastrointestinal distress. Likewise, in the evolution of early hunter gatherer communities, it was only a short step from gathering vegetation to be eaten to assuage the hunger pangs, to gathering material to be ingested to diminish the pain and discomfort from illness and injury.

Medicinal chemistry concerns the discovery, the development, the identification and the interpretation of mode of action of biologically active compounds at molecular level. Thus medicinal chemistry covers the following three critical steps.¹

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

To identify the lead molecule, several approaches are undertaken.

1) Random Approach:

Although the search for new entities is being increasingly governed by rational and high throughput techniques, serendipitous discoveries do still occur, as in old days of medieval herbs.

2) Non-Random Approach:

Compounds uncovered under random screening or compounds containing functional groups other than the lead molecule are tested selectively.

a) Drug Metabolism studies:

Drug metabolites are isolated and screened in order to determine if the activity observed is derived from the drug molecule or from a metabolite.

b) Clinical Observations:

When a drug exhibits more than one pharmacological action i.e. side-effects, then the compound can be used as a lead for secondary activity.

c) Lead Modification:

Synthetic derivatives and structural analogues of biologically interesting substances are tested for activities associated with the 'lead'. Such programs include the branching, lengthening or shortening of chain structure, the variation of the kind and position of substituents and other empirical molecular modifications within the framework of reasonably close analogy.

d) Structure Activity Relationship(SAR):

Structurally specific drugs act on specific receptor sites. Their activity and potency are very susceptible to small changes in chemical structure. Compounds with each type of activity eventually possess certain structural features in common.

Quantitative Structure Activity Relationship(QSAR): The concept of Quantitative drug design is based on the fact that biological properties of a compound are a function of its

physicochemical properties such as solubility, lipophilicity, electronic effect, ionization and stereochemistry etc.

Different methods used to correlate physicochemical properties with biological activity include

- 1)Hansch Analysis or Linear Multiple Regression Analysis.
 - 2)Free Wilson or *de Novo* method.
 - 3)Topliss orientation scheme.
 - 4)Enhancement factor.
 - 5)Batch selection method, Batchwise Topliss Operation Scheme, Cluster analysis etc.
- e)*Computer based QSAR:*

Free binding energy of compounds with the receptor is determined experimentally. Distance between various atoms in the molecule, its conformation and rotation about a single bond causing change in conformation and in distance are studied. Possible binding sites in terms of geometry and chemical characteristics of the site are deduced by computerised method with the help of above studies.

f)*Molecular Graphics:*

The rationale for identifying a lead compound has been based on a molecular understanding of the drug and its receptor. This is frequently accomplished by the use of three dimensional computer graphics representation to model the drug itself or the drug receptor complex.

Review of Literature

Antibacterial agents:

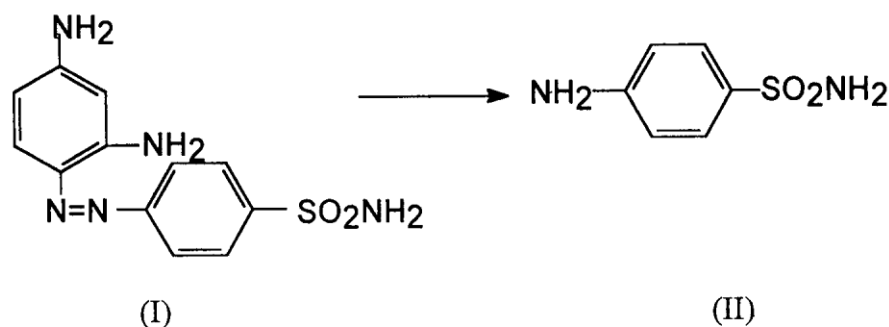
Bacterial infections have always remained a serious problem worldwide. The use of antimicrobial agents to control these infections is almost entirely a development of this century.

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

Systemic antibacterials include a number of classes as described below.

Sulfonamides:

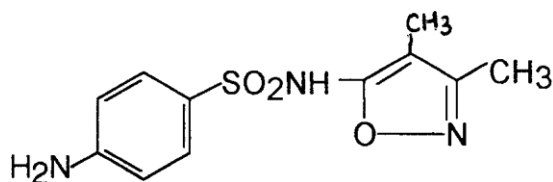
Sulfonamides were discovered in the middle 1930s. Protonsil rubrum(I), a red dye was observed by Domagk² to be taken up selectively by bacterial cells in case of streptococcal infection in mice and staphylococcal infection in rabbit. The dye killed the bacteria. Fractionation led to identification of an active substance known as amino benzene sulphonic acid(sulfanilamide)(II)³, a colourless cleavage product formed by liver metabolism of the drug which is responsible for the activity.



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

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 are safe for human cells.

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



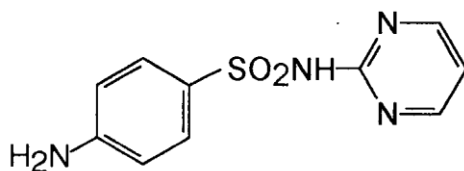
(III)

Sulfisoxazole(III)⁴ is one of the most popular sulfonamide 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 to other more preferred agents.

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

Plasmid mediated resistance is commonly developed, particularly among gram negative organisms, due to decreased sensitivity to dihydropteroate synthetase.

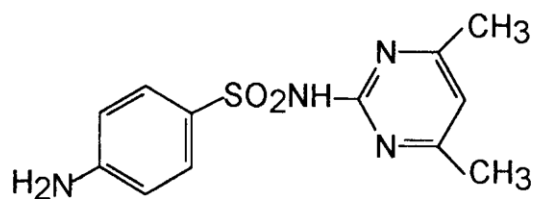
Superinfections, allergic rashes, photosensitivity and drug fever are common side effects. Hemolytic anaemia, liver and kidney damage, Steven-Johnson syndrome and ulcer of mucous membrane of eye, mouth, ureter occur occasionally.



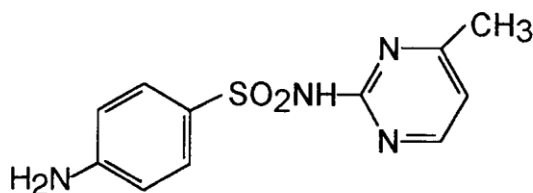
(IV)

Sulphadiazine(IV) has retained the preeminence 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 meningitis* involved is sulfonamide sensitive⁵.

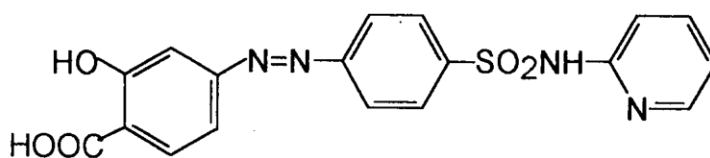


(V)



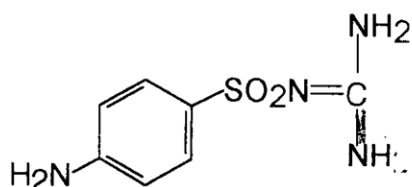
(VI)

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



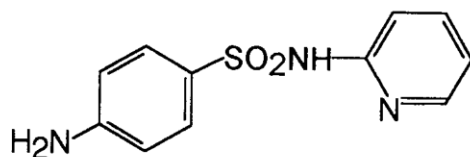
(VII)

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

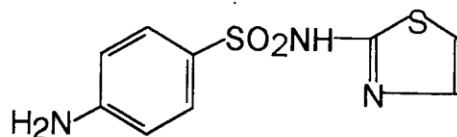


(VIII)

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

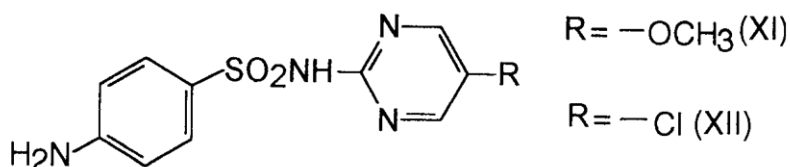


(IX)



(X)

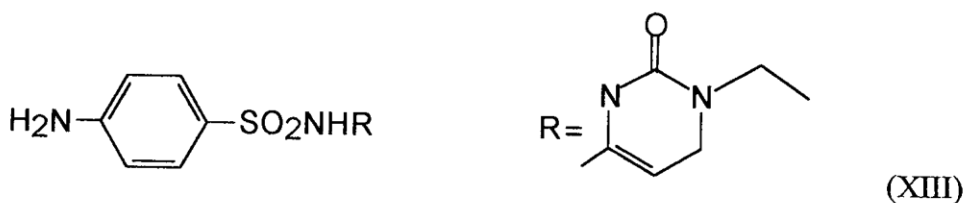
Sulphapyridine(IX)¹², reported in 1938, was in use for the treatment of pneumonia and remained as a drug of choice until it was replaced by sulphathiazole(X)¹³.

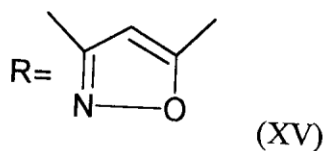
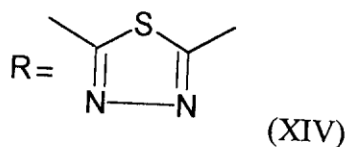


Long acting sulfonamides include Sulphamethoxypyridazine(XI)¹³, which has a half life of about 37 hours. Thus, it needs to be administered only once a day.

Sulphachlorpyridazine(XII) has a half life of 7 hours and is useful for urinary tract infection (UTI).

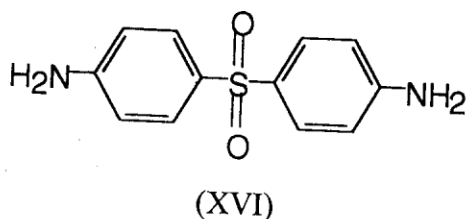
Other sulfonamides include Sulphacytin(XIII), Sulphamethizole(XIV), Sulphamethoxazole(XV) etc.



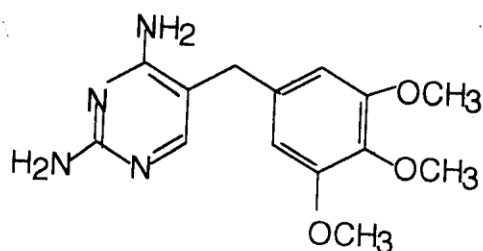


Sulphones:

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



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



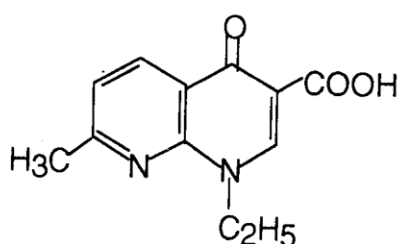
(XVII)

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

Side effects include megaloblastosis, leukopenia, thrombocytopenia, etc.

Quinolones:

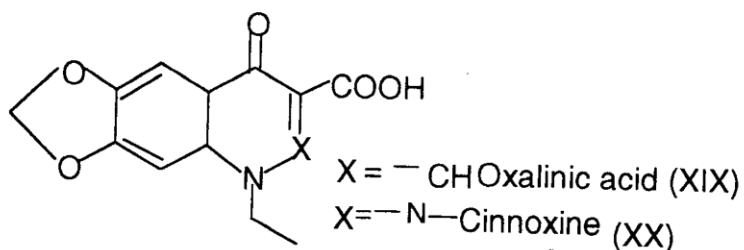
Quinolone antimicrobials comprise of a group of synthetic substances possessing in common an N-alkylated-3-carboxypyrid-4-one ring, fused with a second aromatic ring that carries other substituents.



(XVIII)

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

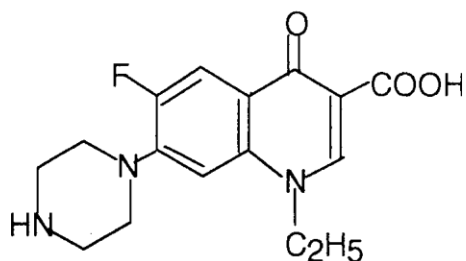
Over the following years, the remaining first generation quinolones were introduced¹⁷. These compounds consist of more potent agents with a better spectrum of activity, such as oxalinic acid (XX), cinnoxin (XXI) etc.



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

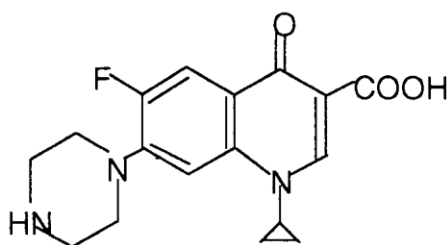
Gastro intestinal upset, skin rashes, visual disturbances and occasionally convulsions and photosensitivity are the adverse reactions observed.

Fluoroquinolones:



(XXI)

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



(XXII)

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

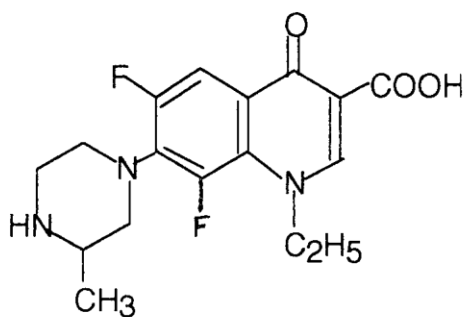
These drugs cause inhibition of enzyme DNA-gyrase that unwinds the supercoiled DNA helix prior to replication and transcription. This inhibition makes the cell DNA inaccessible and leads to cell death.

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

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

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

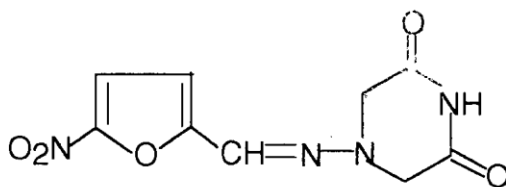
Lomefloxacin(XXIII), Ofloxacin etc. are the other agents in use.



(XXIII)

Nitrofurans:

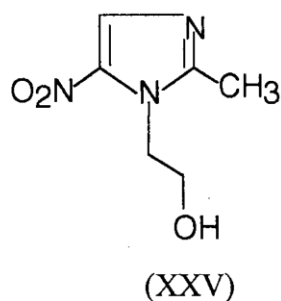
The first nitroheterocyclic compound to be introduced in chemotherapy were the nitrofurans. Nitrofurantoin(XXIV) is used widely for urinary tract infections(UTI).



(XXIV)

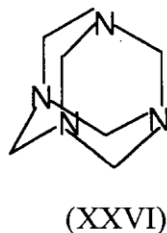
Nitrofurazine²⁰ and Furazolidone²¹ are other broad spectrum nitrofurans in clinical

use for prophylactic treatment of UTI, and they impair kidney stone growth. Nausea and vomiting are common side effects.



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

Methanamine:



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

The Antibiotics:

β- lactum 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-lactum antibiotics alone.

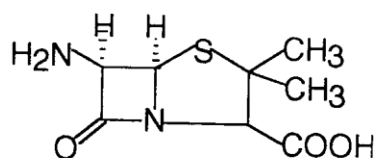
Mode of action:

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

Penicillins:

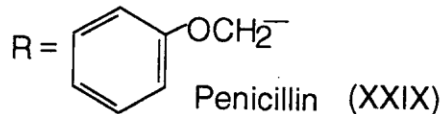
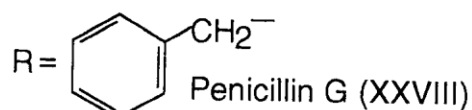
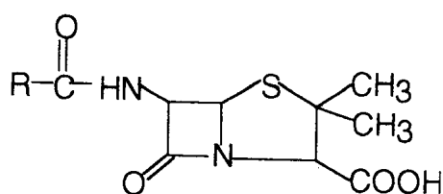
In 1929, Alexander Fleming for the first time discovered *Penicillium notatum* causing partial lysis of *Staphylococcal* colonies. Later on, Penicillin was isolated and gradually large scale production also took place. The penicillin field was then expanded to include orally active penicillins, broad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

6-Amino penicillanic acid (XXVII), i.e. the fundamental nucleus, is prepared by using naturally occurring fungal enzyme that causes selective side chain cleavage of penicillins without breaking the β -lactum 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 V) (XXIX) were synthesized by fermentation.

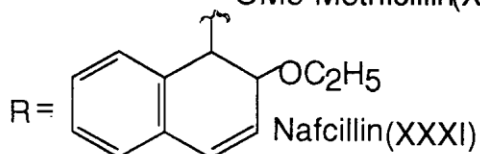
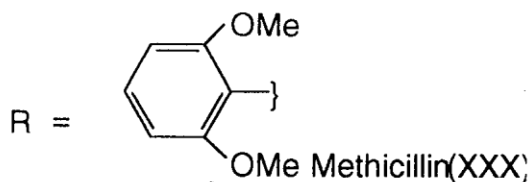
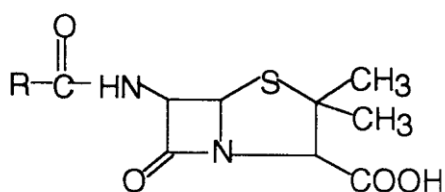


Resistance:

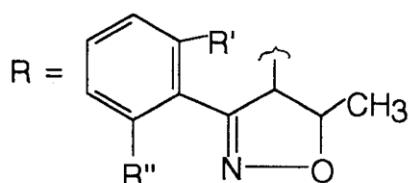
Resistance develop in the bacteria that produce the enzyme which deactivates penicillin, namely β -lactamase and acylase. β -lactamase catalyses hydrolysis of β -lactum ring whereas acylase is responsible for hydrolysis of acylamino side chain. In gram negative organisms, the resistance develops due to poor penetrability.

Penicillinase resistant Penicillins:

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



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



R' = R'' = H Oxacillin (XXXII)

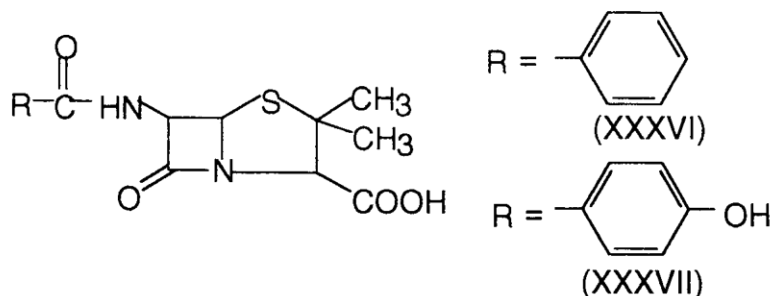
R' = H, R'' = Cl Cloxacillin (XXXIII)

R' = R'' = Cl Dicloxacillin (XXXIV)

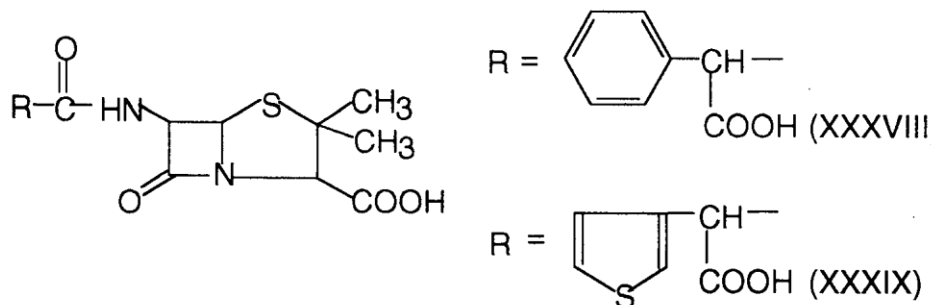
R' = F, R'' = Cl Flucloxacillin (XXXV)

Extended Spectrum Pencillins:

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



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

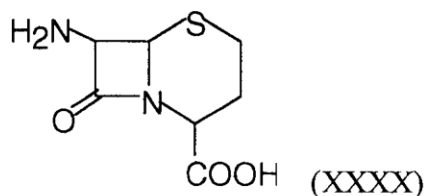


Allergic reactions include from skin rashes to drug fever and anaphylaxis, cross sensitivity³⁶. Allergy occurs due to production of antigenic penicilloyl proteins³⁷.

Cephalosporins:

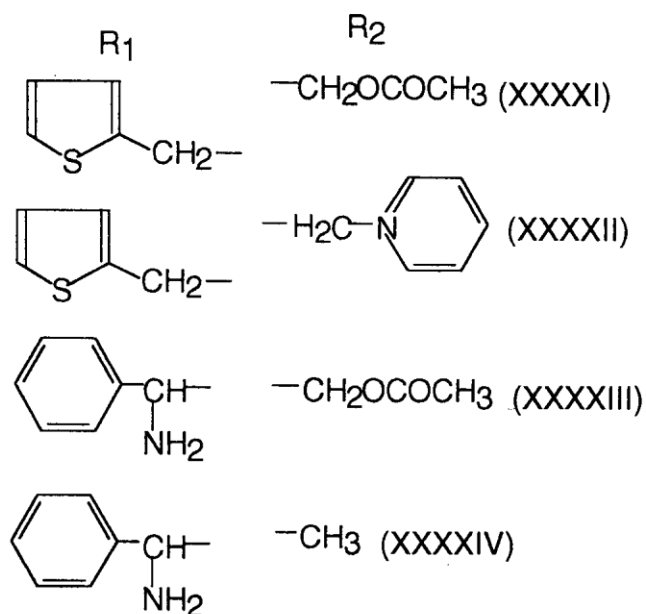
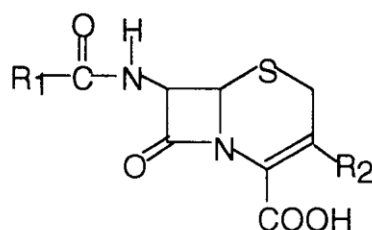
Cephalosporins were discovered by Brotuz and were isolated by Abraham and Newton. They have a wide range of activity against gram positive and gram negative organisms.

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



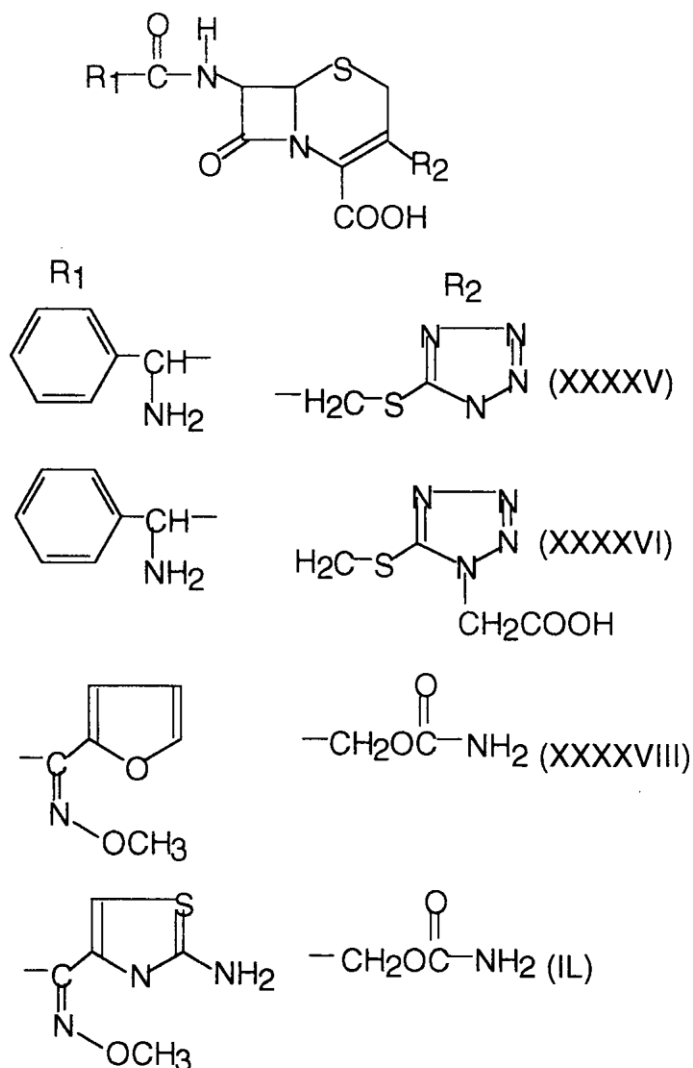
Cephalosporins have a broad spectrum of activity with high potency against gram-positive organisms and moderate potency against gram negative organisms.

Cephalothin(XXXXXI)³⁸ and Cephaloridine (XXXXXII) are administered parenterally, whereas Cephaloglycin (XXXXXIII) and Cephalexin (XXXXXIV)³⁹ are active orally.



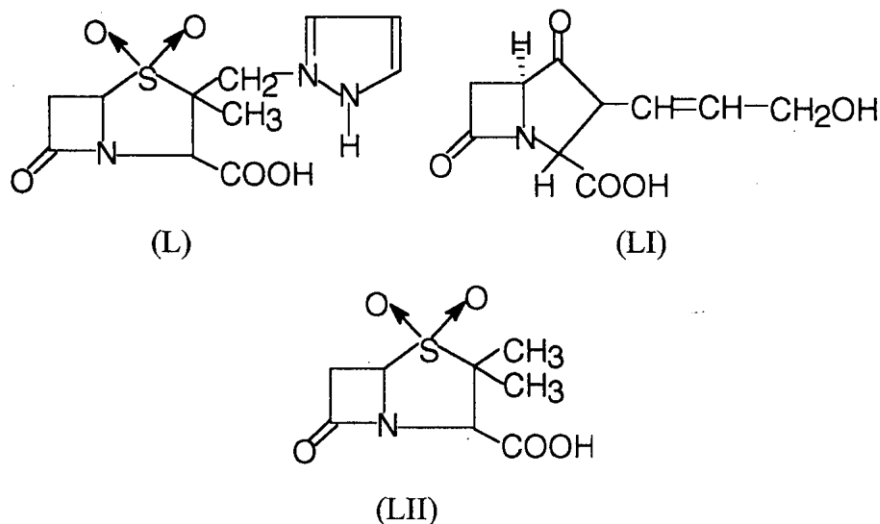
Resistance:

With consistent use resistance develops among several bacteria which produce cephalosporinase and thus hydrolyse the β -lactum ring. The semisynthetic agents with plasmid mediated cephalosporinase resistance⁴⁰ and acid stability were developed later on. These include Cephamandol(XXXXV)⁴¹, Cephonacid(XXXXVI), Cephuroxime (XXXXVII)⁴², Cefataxime(XXXXVIII)^{43,44}.



β -lactamase inhibitors:

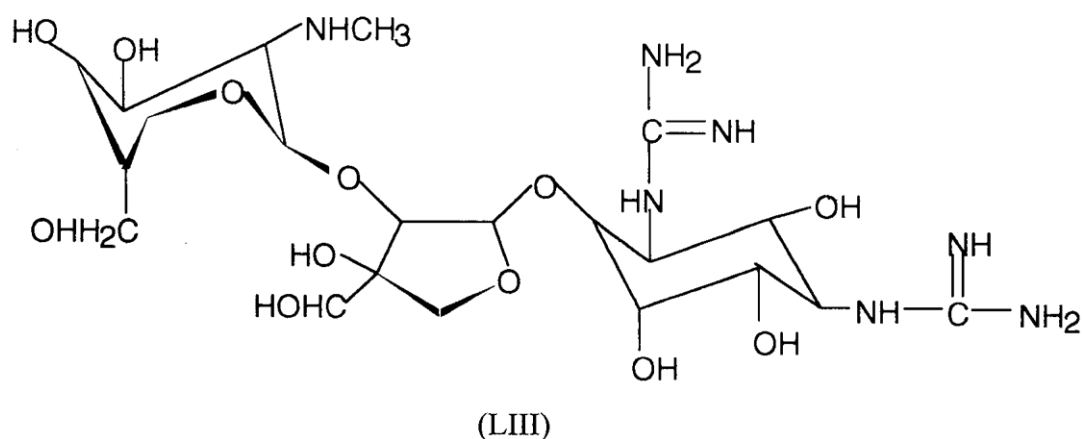
Many substances have been found to inhibit the β -lactamase enzyme, directly or by acting synergistically with some of the antibiotics. UTR-830 (L), clavulonic acid (LI) and sulbactam (LII) are the examples.



Aminoglycosides:

Aminoglycosides contain 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. Kanamycin⁴⁵, Gentamycin⁴⁶, Tobramycin⁴⁷, Netilmicin⁴⁸, Amikacin⁴⁹.

Streptomycin^{49,50,51}, (LIII) is the most effective of the group for the chemotherapy of tuberculosis, brucellosis, tularemia, *yersinia* infections.



Paramomycin^{52,53} is used primarily for amoebic dysentery.

Mode of action:

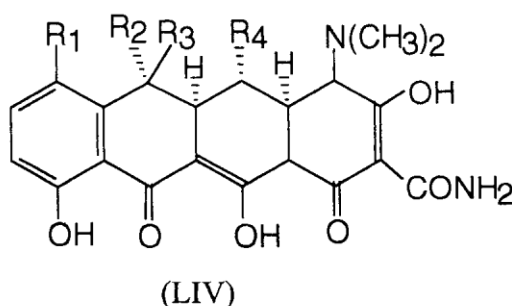
Aminoglycosides bind to the 30S ribosomal subunit to form a complex and thus inhibit proper amino acid polymerization. The binding causes misreading leading to mutation of genetic code, This causes incorporation of improper amino acids into the peptide chain. Thus, they inhibit initiation of protein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

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

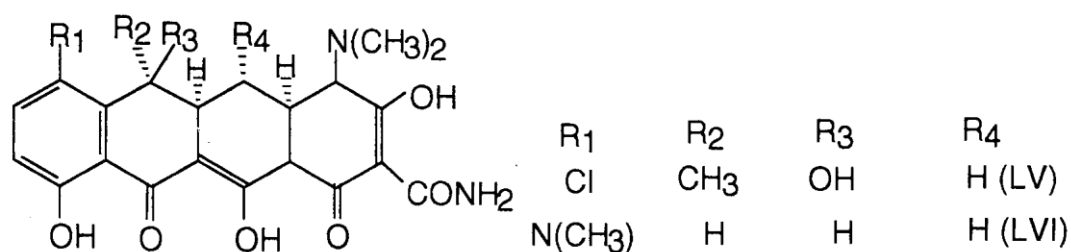
Tetracyclines:

Tetracyclines(LIV)⁵⁴ are derived from *Streptomyces* species or semisynthetically. The basic nucleus is octahydrophenanthrene. Tetracyclines have a broad spectrum of any known antibacterial agent. They are active against a wide range of gram positive and gram- negative bacteria, Spirochetes, Mycoplasmas, Rickettsiae, Chlamydiae.

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

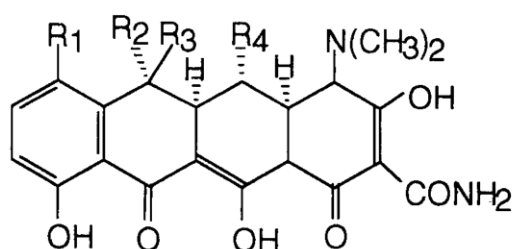


In 1938, Duggar⁵⁵ isolated Chlortetracycline. Electron withdrawing groups, e.g. in chlortetracycline⁵⁶(LV) as well as electron donating groups as in Minocycline(LVI) enhance the activity of the parent molecule.



Oxytetracycline⁵⁷(LVII) has similar activity as that of Chlortetracycline. Doxycycline⁵⁸ (LVIII) and Methacycline⁵⁹(LIX) are more active *in vitro* than Oxytetracycline.

In 1957, MaCormik⁶⁰*et. al.* isolated Demechlorycline(LX) with similar spectrum of activity as that of other tetracyclines, but slightly greater activity.



R ₁	R ₂	R ₃	R ₄
H	CH ₃	OH	OH (LVII)
H	H	CH ₃	OH (LVIII)
H	CH ₂	—	H (LIX)
Cl	H	OH	H (LX)

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

Macrolides:

Macrolide compounds have been derived from the *Actinomyces* species. They contain highly substituted lactone ring⁶¹ aglycon to which amino and neutral deoxy sugars are attached. In 1950, Picromycin was reported. McGuire⁶² in 1952, first reported the isolation of Erythromycin from *Strptococcus erythreus*.

It is well tolerated and useful for infections of upper respiratory tract, skin, soft tissue and genital tract. More recently, its activity against *Mycoplasma pneumoniae*⁶³ has been shown.

Erythromycin acts by binding selectively to 50S ribosomal subunit and thus prevents translocation in protein synthesis⁶⁴.

Non-specific resistance among gram negative bacilli is related to poor penetrability⁶⁵.

Modifications in Erythromycin gave Cclarithromycin, Flurithromycin etc. Clarithromycin is effective against *Mycobacterium avium - intercellulariae* complex^{66,67}. Olendomycin is one more important agent of the macrolide family.

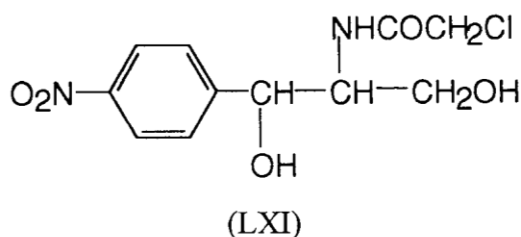
Lincomycins:

Lincomycins are sulphur containing antibiotics isolated from *S. lincilnensis*. Spectrum and mode of action is similar to that of macrolides. Lincomycin, which was isolated by Manson *et. al.*⁶⁸, is useful for the treatment of infection by gram positive organisms notably Staphylococci, β -haemolytic Streptococci and Pneumococci. Clindamycin⁶⁹ 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.*⁷⁰ in 1947, isolated Chloramphenicol, a broad spectrum antibiotic, from *Streptomyces venezuelae*. Its structure was established in 1949 by Constroulis, Rebstock and Crooks⁷¹.



Chloramphenicol(LXI) is active against Penicillin G and Ampicillin resistant species of *Salmonella*, *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Bacillus fragilis*, *N.meningitidis*. 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 ring gives rise to the 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 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. It acts by inhibiting the cell-wall synthesis. No cross resistance to β -lactum, Cycloserin, Bacitracin etc. is

observed. It is used in systemic infections such as *Staphylococcal* enterocolitis and pseudomembranous colitis associated with Clindamycin therapy.

Polypeptides:

Polypeptide class of antibiotics include Gramicidine, Polymixin, Bacitracin, Colistine etc. Severe renal toxicity limits their use in therapeutics.

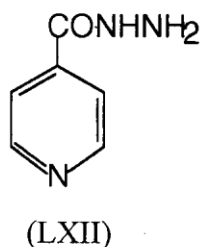
Antitubercular agents:

Mycobacterium tuberculosis was identified by Koch in 1882 as a causative bacteria for chronic infections like tuberculosis. It is estimated that 1.7 billion people harbour the pathogen and every year 8 million individuals develop new clinical disease⁷². One third to half of the world's population is infected by *M. tuberculosis*. 6% of the deaths occurring worldwide^{73,74}. Patients with Human Immunodeficiency Virus(HIV) are highly susceptible to tubercular infection.

Introduction of chemotherapy of Mycobacterial infections over the past five decades has brought about a dramatic decrease in mortality and morbidity due to the illness. Multiple drug resistance within the organisms makes the treatment more challenging. Drug toxicity and patient compliance are the other problems. Research is needed to be carried out to find the best therapeutic regimen.

Antitubercular drugs are classified majorly as 'primary' or frontline drugs, and secondary or second line drugs. Frontline drugs are used in standard therapeutic regimen due to their high level of efficacy and acceptable degree of toxicity. These drugs include Isoniazide, Rifampicin, Ethambutol and Streptomycin. 'Secondary' or second line drugs are used occasionally because of bacterial resistance or certain patient related factors. These drugs include Pyrizinamide, Ethionamide, Para amino salicylic acid, Cycloserin, Viomycin, Kanamycin, Amikacin, Capreomycin, Thioacetazone, Thiocarlide etc.

Isoniazide(INH):



Isonicotinyl hydrazide(Isoniazid)(LXII), described in 1912⁷⁵ was prepared as an intermediate in the synthesis of isonicotinyl aldehyde. Outstanding activity of INH in experimental infection was also confirmed by clinical trials. N-alkylated derivative (Iproniazide) was also found to be active.

INH has both bacteriostatic and bactericidal activity against various strains of *Mycobacterium tuberculosis* which are resistant to other drugs. The MIC is about 0.04 µg/ml. Bactericidal action is exhibited against replicating organisms, whereas in case of non-replicating organisms, it is only bacteriostatic⁷⁶.

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

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

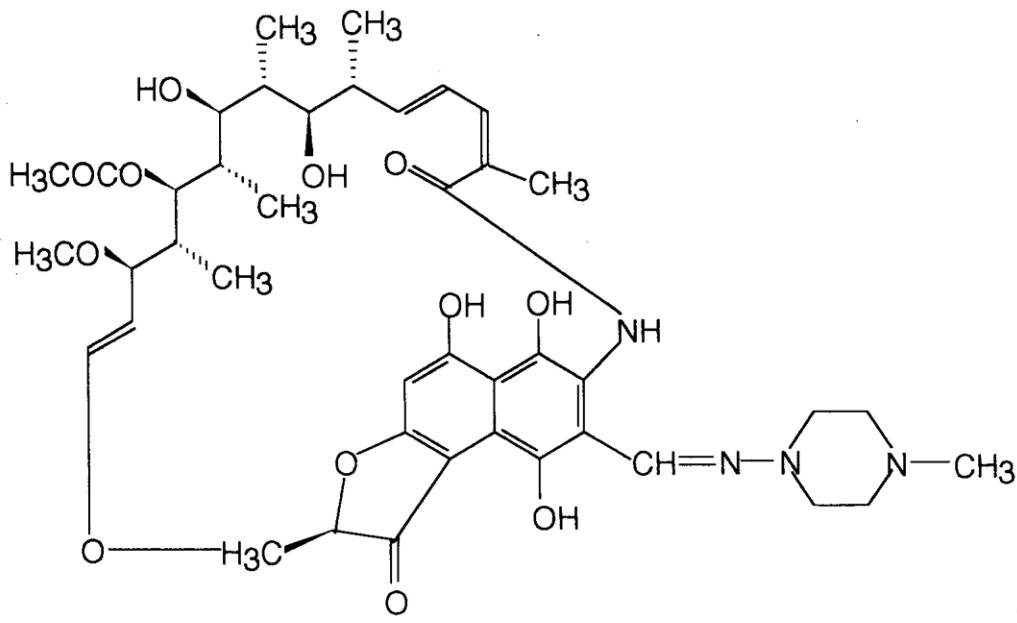
More recently it has been shown that the activity of INH results from peroxidative reaction catalysed by peroxidase which is encoded by Kat G Gene⁷⁷. *M. tuberculosis* which lacks this gene are resistant to INH⁷⁸.

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

Rifampicin:

The Rifampicin antibiotics were discovered as metabolites of *Streptomyces mediterranei*. Recently it has been stated to be *Amicolatopsis mediterranei*⁷⁹.

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)⁸⁰ was the most active *in vivo* at concentration 1 µg/ml and is useful clinically.



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

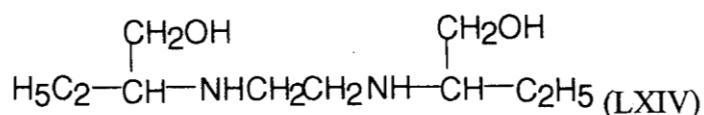
Resistance is rare and occurs spontaneously in strains not exposed previously to the antibiotic. The mechanism of resistance is through mutation in rpoL gene which encodes β -subunit of RNA-polymerase.

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

The therapeutic efficacy of Rifampicin is comparable to that of INH and is superior to all other drugs.

It possesses low toxicity with gastrointestinal disturbances and cutaneous reaction as the side effects.

Ethambutol:



Among the randomly screened compounds, Ethambutol(+)(LXIV) is found to be very active, both *in vitro* and *in vivo* against *M. tuberculosis* at 1µg/ml. Organisms resistant to other drugs are 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^{81,82}. Another hypothesis states that it causes an inhibitory effect on phosphorylation⁸³.

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

Streptomycin:

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

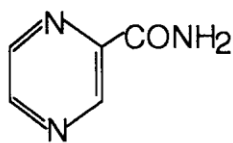
In mycobacteria, mutation in the *rspL* gene which encodes the ribosomal protein S12, have been shown to confer resistance to Streptomycin⁸⁴.

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

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

Other aminoglycosides such as Kanamycin, Amikacin are used secondarily.

Pyrazinamide:



(LXV)

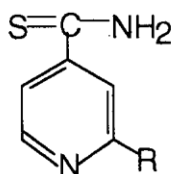
The best activity of pyrazinamide(LXV) is against intracellular *Mycobacteria* causing sterilizing effect in acidic conditions. Susceptible bacteria produce deaminase which converts Pyrazinamide to pyrazinoic acid, an active form.

It gets readily absorbed orally and is metabolized to pyrazinoic acid which gets oxidised to its 3-hydroxy derivative.

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

It is useful in short term chemotherapeutic regimen. Treatment for longer than two months is not recommended⁸⁵.

Ethionamide:



R = -C₂H₅ (LXVI)

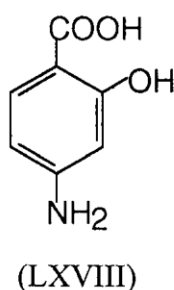
R = -n-C₃H₇ (LXVII)

2-ethyl thioisonicotinamide(Ethionamide)(LXVI) and 2-n-propyl analog (Prothionamide) (LXVII) 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 large dose(>500mg) is not well tolerated. It gets metabolized to give the sulfoxide.

Side effects include GI irritation, hepatitis, CNS effects and hypersensitivity.

Para amino salicylic acid(PAS):

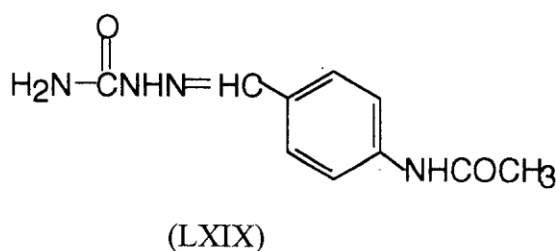


Among the various compounds related to benzoic acid, PAS(LXVIII) is believed to be the most active agent⁸⁶ against growing cells of *M. tuberculosis*.

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, which impairs iron uptake⁸⁷.

10-12gm/day is required to be taken orally in 2-3 divided doses. It is no longer in use. GI toxicity is a major side effect.

Thioacetazone:



Thioacetazone(LXIX) is the most active thiosemicarbazone against *M. tuberculosis*. Lack of cross resistance to INH and Streptomycin indicate the use of thioacetazone in combination with these drugs to delay bacterial resistance.

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

Drug	Initial phase(2months)	Continuation phase(4months)
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)
Pyrizinamide	30mg/kg/day(1.5-2gm/day)	—
Ethambutol*	15-25mg/kg/day	
Intermittent regimen:		
Rifampicin	10mg/kg(3times weekly)	10mg/kg(3times weekly)
INH	15mg/kg(3times weekly)	15mg/kg(3times weekly)
Pyrazinamide	50mg/kg(3times weekly)	50mg/kg(3times weekly)

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

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

Antifungal agents:

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

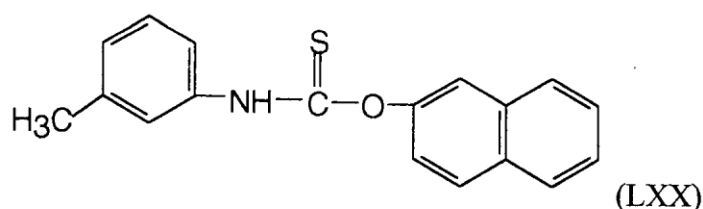
1)Dermatophytosis(Tinea infections), which are contagious superficial epidermal infections caused by various *Epidermophyton*, *Microsporum* and *Trichophyton* species; and

2)Mycosis, caused by pathogenic saprophytic yeasts, which are contagious involving 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:



The o-2-naphthyl ester of N-dimethyl thiocarbanilic acid was found in 1960 to have good activity against dermatophytes when applied topically. Poor permeability of Tolnaftate (LXIV) limits its use. 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.

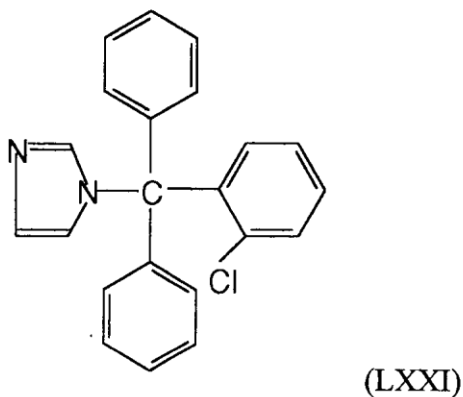
Substituted azoles:

Substituted imidazoles represent a versatile group of antiungal agents with a unique mechanism of action. The early members reported were Cotrimazole(LXV)⁸⁸ and Miconazole (LXVI)⁸⁹ 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*.

The mechanism of action of the drug is elucidated⁹⁰ extensively. At high concentration (micromolar), they are fungicidal; at low concentration (nanomolar), they are fungistatic. The fungicidal effect is associated with damage to the cell wall with the

loss of essential cell components such as potassium ion and amino acids. Imidazole inhibits the cytochrome P-450 oxidase enzyme that catalyses the 14- α -demethylation of lanosterol to ergosterol.

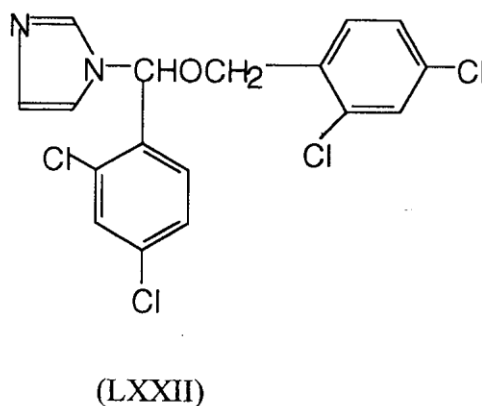
Cotrimazole:



Cotrimazole (LXXI) is a broad spectrum antifungal agent used topically for the treatment of tinea infection and Candidiasis.

As it causes severe gastric disturbances, it is not suitable for systemic use.

Miconazole:

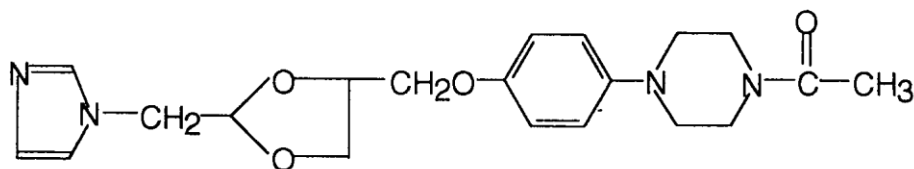


Miconazole is intended for the treatment of serious systemic fungal infections like Candidiasis, Cryptococcosis, Petriellidiasis and Paracoccididomycosis.

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:



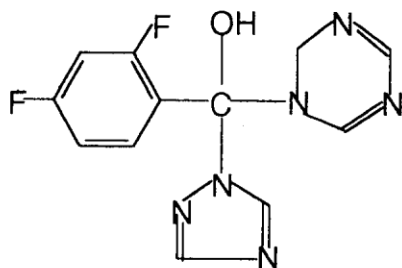
(LXXIII)

Ketoconazole(LXXIII) is administered orally for the treatment of systemic mycosis. It requires gastric acidity for absorption. Antacids and drugs like H₂-histamine antagonists, anticholinergics, that inhibit gastric secretion, interfere with its absorption. It is metabolised to the inactive form and is excreted by the enterohepatic route.

Ketoconazole is recommended for systemic mycosis such as Coccidiomycosis, Blastomycosis, Histoplasmosis, chromomycosis, paracoccidiomycosis and severe cutaneous infections.

Hepatotoxicity is a major adverse effect. Also, levels of testosterone and corticosterone are lowered at high doses of Ketoconazole.

Fluconazole:

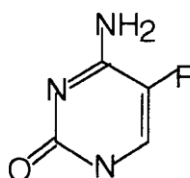


(LXXIV)

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

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

Flucytosine:



(LXXV)

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

Incorporation of fluorinated pyrimidine into RNA, following selective deamination to 5-fluoro uracil in the fungus appears to be required for activity. 5-fluoro uracil gets metabolised to 5-fluoro-2'-deoxyuridic acid which suppresses DNA synthesis by inhibiting thymidylate synthetase.

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

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

Antifungal antibiotics:

Polyene antibiotics:

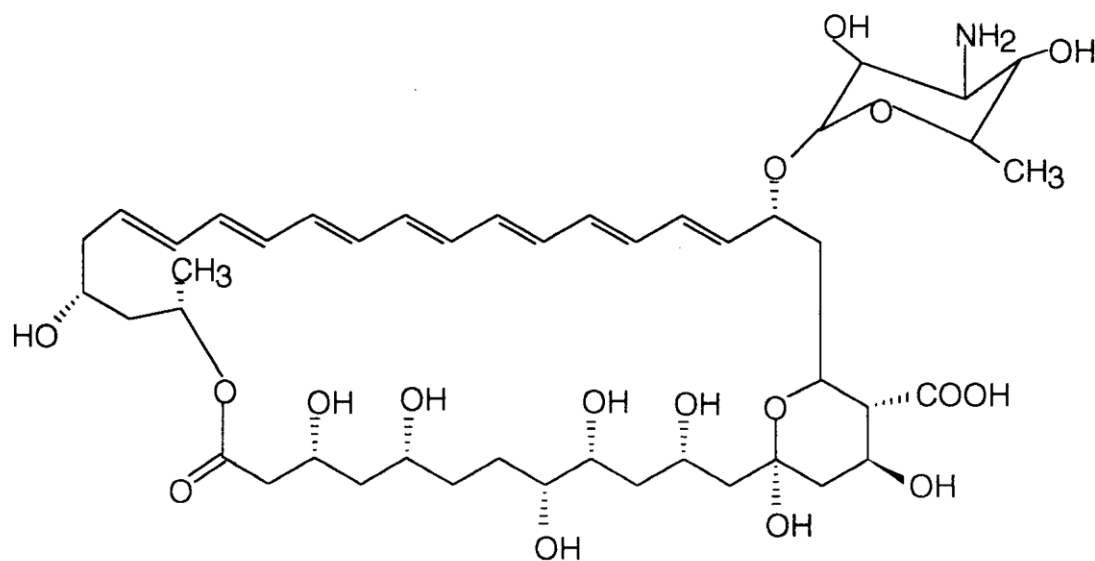
The polyene antibiotics contain conjugate double bonds in large lactone rings. They differ from macrolides in the size of the lactone ring and the presence of conjugated- 'ene' system.

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

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

Amphoterecin B:

A potent antifungal agent with a polyene structure was isolated in 1956 by Gold *et.al.*⁹¹ from *Streptomyces nodosum*, found in the soil from Venezuela. The antibiotic material was a mixture of two closely related compounds, Amphoterecin A and B of which B is more active.



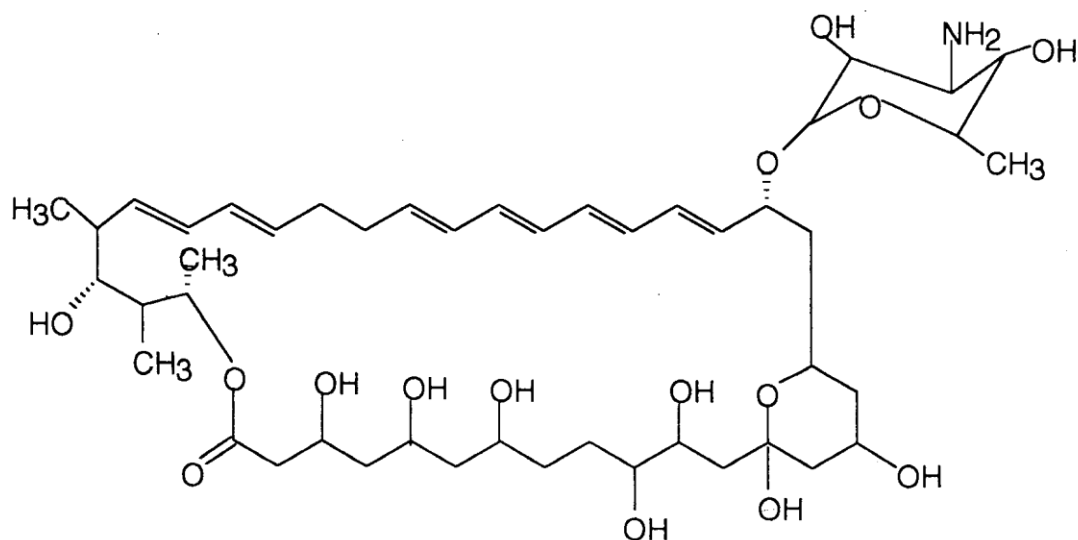
(LXXVI)

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

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

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

Nystatin:



(LXXVII)

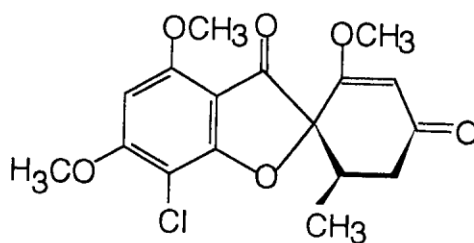
A polyene antibiotic first isolated in 1951 from a strain of *Streptomyces noursei* by Hazen and Brown⁹², Nystatin (LXXVII) is a valuable agent for the treatment of local and

gastrointestinal monolial infections caused by *Candida albicans* and other *Candida* species. It is also recommended for thrush or esophagitis by *C. albicans* in patients with AIDS. It is available in cream, ointment or powder form for topical use, whereas tablets or troches are used systemically.

Griseofulvin:

Griseofulvin (LXXVIII) was first reported in 1939 by Oxford *et.al.*⁹³ as an antibiotic obtained from the mold *Penicillium griseofulvum*. Dierckx. Its structure has been determined by Grove *et.al.*⁹⁴

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



(LXXVIII)

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 assembly⁹⁵.

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

Several other antibiotics in use include Candidicin, Natamycin etc.

Novel approaches:

Out of the novel approaches to a fungal chemotherapy, one approach might be to persist the infected host to withhold growth essential iron from the fungi and not the host.⁹⁶ Poorly soluble iron chelators such as hydroxy pyridon, rilopyrox are being developed for topical use in dermatophytosis.

Diamidines, hydroxy stildamide and isothionate have been found to be possess activity against Pneumocytosis, but have severe side-effects.



Plan of Work

:PLAN OF WORK:

Pharmaceutical research particularly in medicinal chemistry provides a basis for the development of new approaches to combat human diseases. The biomedical research can be basic (seeking an understanding of biological phenomenon which are unknown) or applied (using principles which are known to produce desired new product or effect). In either case the research has the effect of obviating the established therapies in favour of new modalities which would be safer and more effective.

As the abilities of the chemist to synthesize, isolate and characterize new compounds grew and as biologists learnt more about the actions of drugs and about biological mechanisms in health and diseases, the search for new drugs gradually began on a more rational basis. Today, useful drugs arise from natural products, chemical synthesis or combination of both sources by means of approaches that vary in a complete spectrum between serendipitous discovery and rational synthesis.

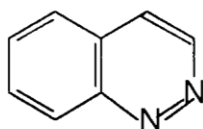
Organic chemists in pharma industry synthesize hundreds of new compounds every week. In most cases, the chemist has a specific reason for synthesizing a particular compound; usually based on theoretical considerations, medicinal chemistry, biological mechanisms or a combination of all these three. In the past three decades, the biological sciences have undergone a major redirection towards molecular understanding of biological systems.

The search for chemical structures that which exhibit pharmacological activity is a difficult goal for the organic chemists. Compounds are submitted for screening for numerous types of biological and pharmacological action. Observations on interesting and repeatable biological activity opens new pathways for additional chemical research efforts in the expansion of the series and often leads to significant new medicinal products. Organic chemist has succeeded in the deliberate chemical alteration of the natural drugs and produces derivatives which are either more potent or superior in some other respect. Such modification of known pharmacodynamic compounds, both natural and synthetic, constitutes one of the main kind of research efforts in the field of chemotherapy.

An important aspect of medicinal chemistry has been to establish a relationship between chemical structure and biological action. An increased consideration in recent years has been to correlate the chemical structure with reactivity or physical properties and these correlations in turn, be related to therapeutic action.

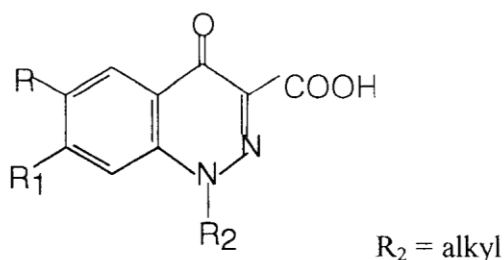
The logic supporting the work presented in this thesis was formulated bearing in mind the biological activities of known moieties and attempting certain structural modifications or adaptation in light of the recent trends in drug research in incorporating newly emerged pharmacophores on existing moieties.

Chapter II: Synthesis of 1H-3(2-alkyl/aryl, 4-substituted thiazolo)-6-chloro-cinnoline-4-ones:



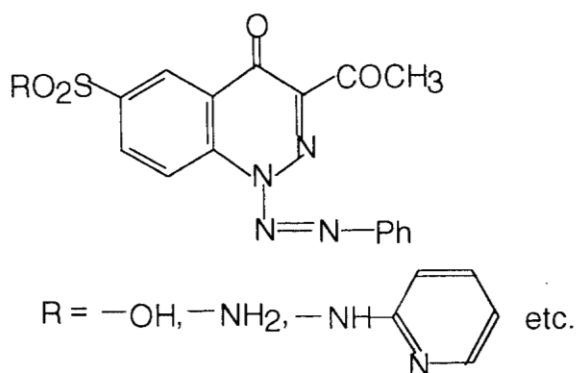
(I)

Cinnoline (I), a member of the heterocyclic family, has been documented in the literature for its appreciable antibacterial activity²⁰³.



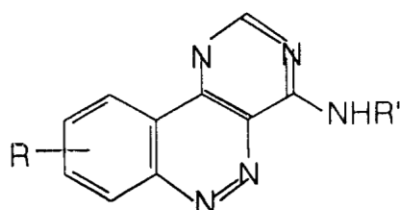
(II)

1-alkyl-3-carboxy-4-cinnolones (II) also show good antibacterial activity²⁰⁴.



(III)

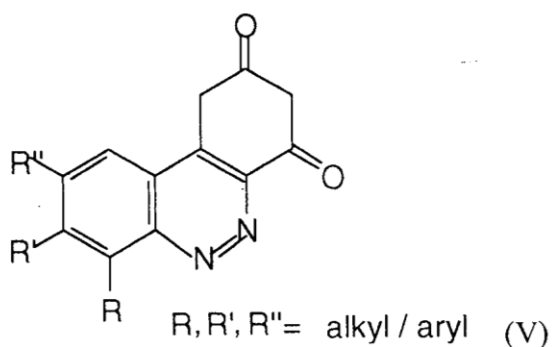
3-acetyl-phenylazo-6-substituted cinnoline-4-ones (III) have been reported to exhibit potential antibacterial, but low antifungal activity²⁰⁵.



$R, R' = \text{alkyl / aryl}$

(IV)

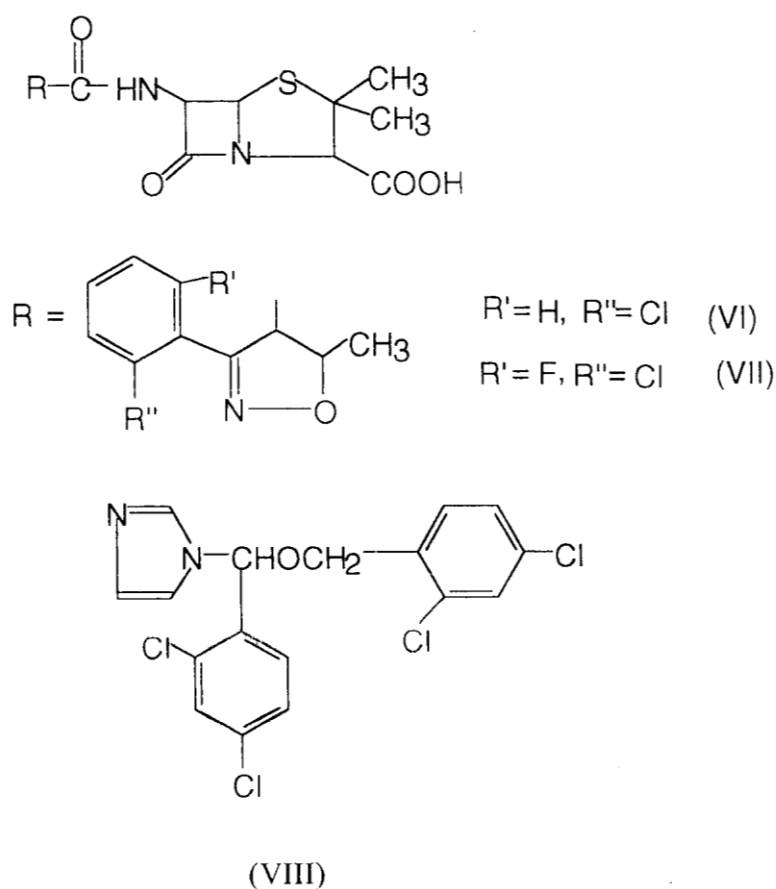
Similarly, 4-arylamino pyrimido(5,4-c) cinnolines²⁰⁶ (IV) have been reported to possess activity from 0.3 $\mu\text{g/ml}$ to 0.05 $\mu\text{g/ml}$ against gram positive *Bacillus cirroflagelus*, from 0.21 $\mu\text{g/ml}$ to 0.025 $\mu\text{g/ml}$ against gram positive *Staphylococcus aureus*, and 3.16 $\mu\text{g/ml}$ against gram negative *Escherichia coli*.



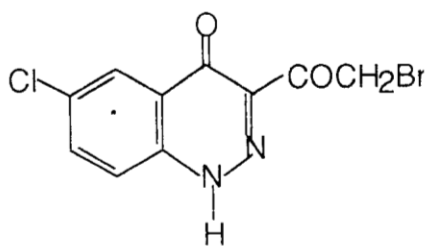
Pyrimido (5,4-c)cinnolines-2,4(1H,3H) diones²⁰⁷(V), are known to possess moderate activity, i.e. upto 100 µg/ml against a number of gram positive and gram negative organisms and fungi like *Aspergillus niger* and *Candida albicans*.

Relatively less work has been carried out on the cinnoline ring. Hence, we decided to emphasize on this ring system and carry out studies on compounds bearing a combination of cinnoline and other heterocyclic systems, in the hope of developing a prospective antimicrobial agent.

Electron-withdrawing substituents like halogens and nitro are believed to impart stability to the ring and thereby contribute to the activity. e.g. Cloxacillin (Antibacterial) (VI), Flucloxacillin (antibacterial) (VII), Miconazole (antifungal) (VIII) etc.



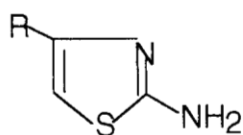
Hence, we selected 6-chloro-(3-halocetyl)-1H-cinnoline-4-one (IX) as our parent moiety.



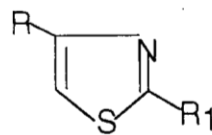
(IX)

Thiazole:

Thiazole moiety is well established as a biologically active moiety, especially for its antibacterial effect²⁰⁸. Russian research workers²⁰⁹ showed that amino substitution at 2-position of thiazole ring along with alkyl/aryl group at 4-position (X) impart bacteriostatic activity to the molecule.



(X)



(XI)

Similarly, vast synthetic work carried out in our laboratory on 2,4-disubstitute thiazoles (XI) also revealed²¹⁰ that many thiazoles of such types are active against H₃₇R strain of *Mycobacterium tuberculosis* at concentrations ranging from 5 µg/ml to 0.0 µg/ml.

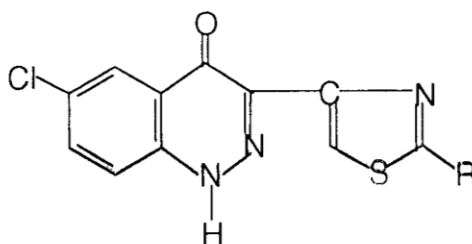
These properties of thiazole nucleus as antitubercular agent strongly suggest that this nucleus should be explored further in order to achieve good antitubercular activity.

Keeping in mind the good antibacterial activity of 2,4-disubstituted thiazole, we decided to combine cinnoline with thiazole.

In order to form a thiazole, we decided to condense the 3-bromoacetyl-6-chloro cinnoline-4-one with various substituted thioamides.

The thioamides were synthesized using established literature procedure.

The final compounds in this series will have the following common structure (XII).



(XII)

R = aminomethyl phenyl, pyridyl, benzyl, phenyl etc.

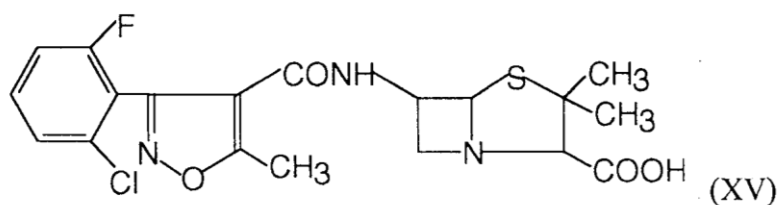
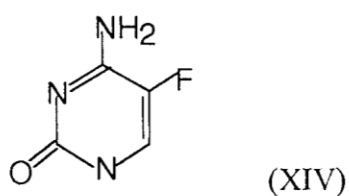
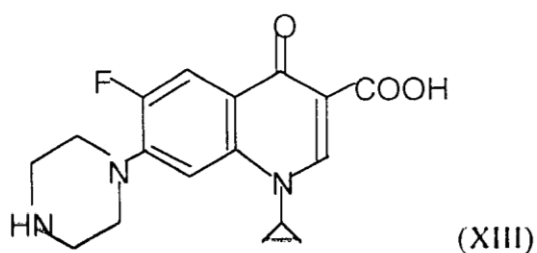
A total of ten compounds were synthesized in this series which were confirmed by elemental and spectral analysis.

Chapter II: Synthesis of 1H-3(2-alkyl/aryl, 4-substituted thiazolo)-6-fluoro-cinnoline-4-ones:

Extensive work has been reported on the advantages of organo fluorine compounds. Fluorine molecule itself has numerous advantages, like-

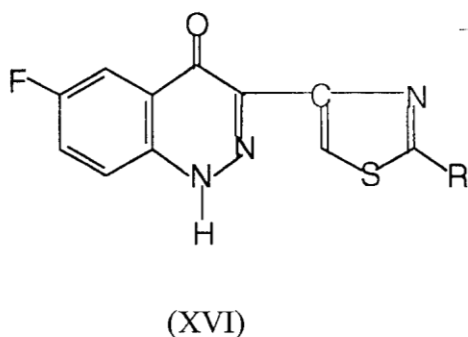
- 1) Fluorine, the second smallest atom as measured by atomic radius and internuclear distance to carbon, would most closely resemble bioactive hydrogen analogues with respect to steric requirements at enzyme receptor sites.
- 2) Fluorine atom imparts increased oxidative and thermal stability because of its high binding energy which exceeds that of chlorine by 17 Kcal/mole.
- 3) Fluorine atom leads to increased lipid solubility which enhances the rate of absorption and *in vivo* transport.

Numerous fluorinated compounds are known e.g. Ciprofloxacin (antibacterial) (XIII), Flucytosine (antifungal) (XIV), Flucloxacillin (antibacterial) (XV) etc.



Thus, we decided to replace chlorine at the 6-position with fluorine to check its effect on activity. The fluorinated compounds were synthesized by a similar procedure using 4-fluoro aniline as the starting compound.

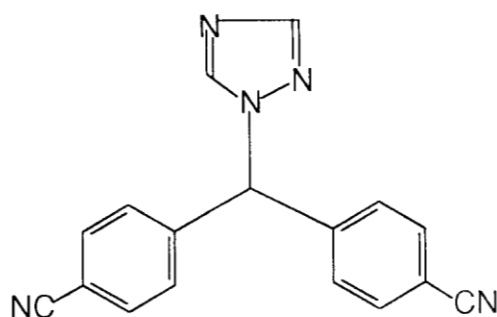
The final structure is as follows-



R = alkyl/ aryl/ substituted aryl etc.

Ten compounds with similar substitutions as that in series I were synthesized and confirmed by elemental and spectroscopic analysis. Comparison will be carried out between the activity of both the series in order to establish a structure activity relationship.

Chapter III: Synthesis of Intermediate for Letrozole:



(XVII)

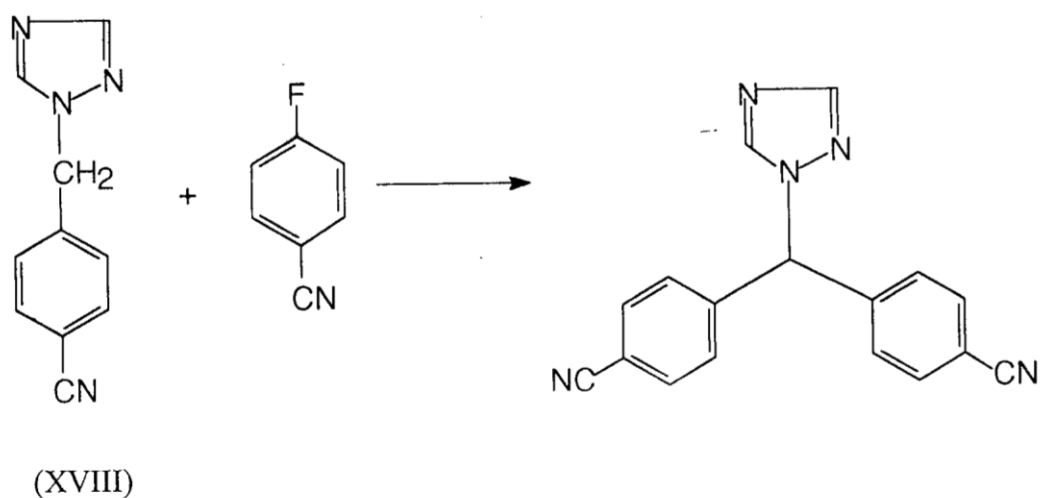
Letrozole (XVII) is a relatively new drug, used as an antineoplastic agent. Using modified nude mouse model of post-menopausal breast cancer, CGS(20267) was shown to have a marked antitumor effect and to significantly suppress plasma estrogen²¹¹.

Letrozole is currently undergoing phase II and phase III clinical trials for breast cancer in U.S.A.

Pharmacological action:

Letrozole is a highly potent and selective non-steroidal aromatase inhibitor. Aromatase catalyses the conversion of androgens to estrogens which leads to an increase in plasma estrogen level. Letrozole was shown to inhibit aromatase in peripheral tissues at doses of 0.5 to 2.5 mg/day for 6 weeks²¹¹.

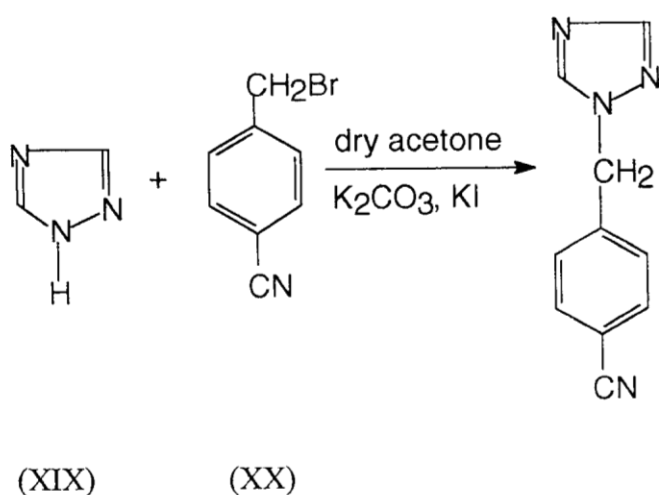
The synthetic route of Letrozole is as follows²¹²:



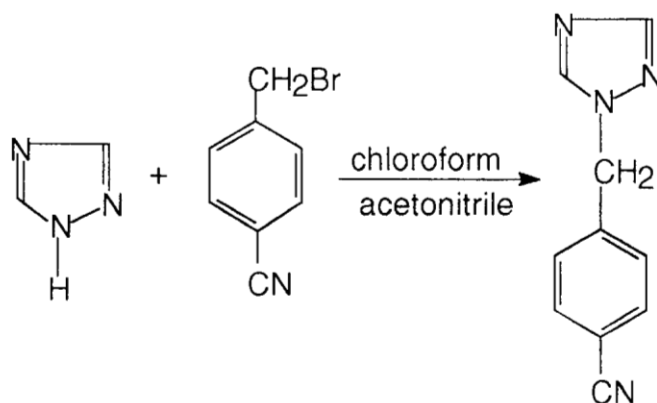
A number of antineoplastic drugs can be synthesized using 4-(1,2,4-triazolyl methyl) benzonitrile (XVIII) e.g. MPV-2213ad²¹³, Letrozole etc.

The intermediate 4-(1,2,4-triazolyl methyl) benzonitrile is prepared by condensation of 1H-1,2,4- triazole (XIX) with 4-cyano benzyl bromide (XX) by different routes enumerated as follows:

1) 1H-1,2,4-triazole and 4-cyano benzyl bromide are dissolved in dry acetone. To the mixture is then added potassium carbonate and potassium iodide and refluxed for 8 hours²¹⁴.



2) 4-cyano benzyl bromide was dissolved in a mixture of chloroform and acetonitrile. 1H-1,2,4-triazole dissolved in the same system is added to the former and refluxed for 15 hours. Then it is neutralized with 3% sodium bicarbonate and passed on a silica gel column for separation of the product²¹⁴.



Thus, 1H-1,2,4-triazole is a key intermediate for the synthesis of Letrozole. 1H-1,2,4-triazole can be prepared by various routes²¹⁵ which are summarized as follows:

- 1) By oxidation of substituted 1,2,4-triazoles.
- 2) By treating urazole with phosphorus pentasulphide.
- 3) By heating equimolar quantities of formyl hydrazine and formamide.
- 4) By removal of amino function of 4-amino-1,2,4-triazole.
- 5) By oxidation of 1,2,4-triazole-3(5)-thiol with hydrogen peroxide.
- 6) By decarboxylation of 1,2,4-triazole-3(5)-carboxylic acid.
- 7) By heating hydrazide salt with formamide²¹⁶.
- 8) By rapid distillation of hydrazine hydrate with 2M equivalent of formamide²¹⁷.
- 9) By heating N,N' diformyl hydrazine with excess ammonia in an autoclave at 200°C for 2-4 hours²¹⁸.

10) By the reaction of 1,3,5-triazine and hydrazine monohydrochloride²¹⁹.

11) By oxidation of 1,2,4-triazole-3(5)-thiol in conc. HNO_3 ²¹⁵.

We planned to carry out the oxidation of 1,2,4-triazole-3(5)-thiol for a better yield applying certain modifications to the literature procedure.

Modifications:

- 1) During work up of oxidation of 1,2,4-triazole-3(5)-thiol, the mixture will be neutralised with liquid ammonia in order to minimise inorganic bulk.
- 2) Instead of hectic and multiple extraction procedure, the triazole will be extracted in ethyl acetate with recovery of the solvent to maximum.
- 3) The use of large quantity of ethyl alcohol will be avoided which will make the procedure cost effective.
- 4) Also, use of benzene will be avoided. Benzene, being highly carcinogenic may cause health hazards to the workers, when used on industrial scale.

The triazole and benzonitrile will be subjected to elemental and spectroscopic analysis for confirmation.

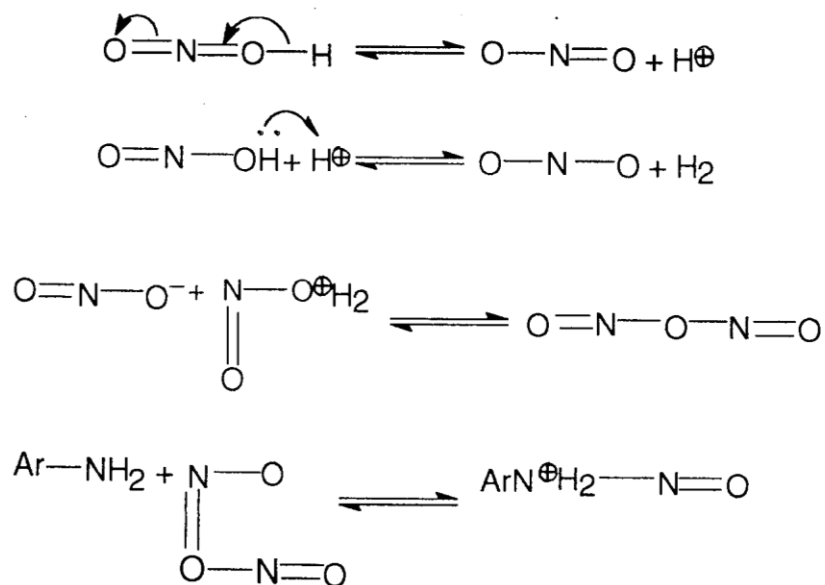
Chemistry

Diazonium salts

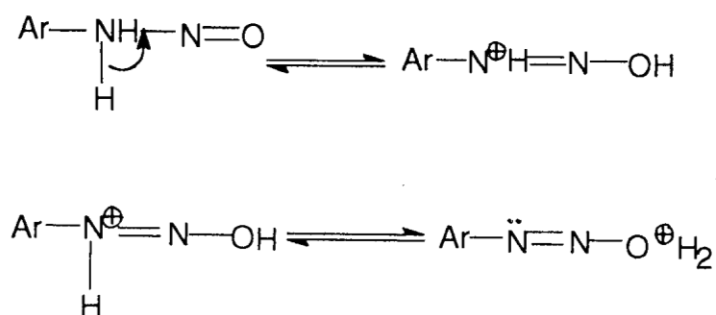
Diazotization of amines:

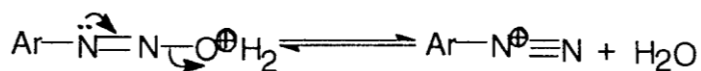
The reaction of amino salt with nitrous acid is known as diazotization. Pelierciess in 1858, carried out this reaction for the first time with an aromatic amine.

The kinetics of diazotization involve a nucleophilic attack by amine on dinitroxide; which is in equilibrium with nitrous acid .



The free amine, although present in a very small concentration, is highly reactive in the acidic solution used. The conjugate acid of the primary nitroso amine, by a series of proton transfer and loss of water, gives a diazonium salt.

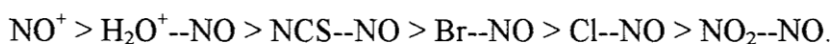




Recently, the intermediate step of a primary amine has been shown to be isolated⁹⁷ in the course of the diazotization of an appropriate amine.

Since most of the diazotizations are performed in aqueous solutions, protonated nitrous acid (from sodium nitrate and hydrochloric acid)⁹⁸ is usually used. Free NO^+ should only appear in concentrated acid and in the course of conversion with complex NO^+ salts.

The sequence of reactivity is shown as below-



I] Aqueous solution:

Normal diazotization of aromatic amines with nitrous acid is carried out in dilute aqueous mineral acid and usually yields the diazonium salt in solution. The direct method starts from solution or suspension of the amines in dilute hydrochloric acid (approximately 2.5 mole acid / mole amine), which is treated with the molar quantity of concentrated aqueous sodium nitrite solution at $0^\circ\text{--}10^\circ\text{C}$. The conversion proceeds rapidly and almost quantitatively⁹⁹. 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 acid¹⁰⁰.

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

II] Concentrated acids¹⁰¹:

Weakly basic amines bearing strongly electron-attracting substituents on the aromatic or heteroaromatic nucleus are diazotized preferentially in concentrated acids,

since hydrolysis¹⁰² of the generated diazonium compound occurs in aqueous solution with increasing dilution. Good results have been obtained with conc. sulphuric acid¹⁰³.

III] Organic solvents¹⁰⁴.

In order to prepare a solid diazonium salt, diazotization of aromatic amines is carried out in organic solvents (glacial acetic acid¹⁰⁵, methanol, ethanol, formamide, DMF, acetone) by means of nitrous acid esters (preferably pentyl nitrile).

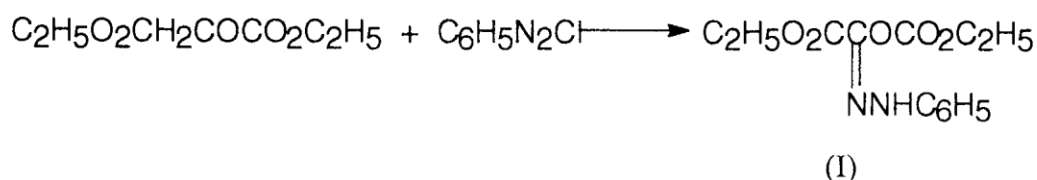
N-diazonium salt will couple with an aliphatic compound containing an activated carbon-hydrogen bond. This is limited to those reactions in which both nitrogen atoms of the diazonium salt are retained in the resulting molecule.

Victor Meyer reported the coupling of diazonium salt with an activated aliphatic carbon atom.

It is generally assumed that the hydrazone is a stable form when coupling occurs at a methyl or methylene carbon and azo structure is formed when coupling occurs at a methinyl carbon without elimination of the group.

The reaction of β -keto ester with a diazonium salt has been studied extensively. Products from ethyl acetoacetate and over fifty different diazonium salts have been reported. Good yields of the hydrazones of α,β -esters are obtained if 1mole of the diazonium salt is employed.

Diethyl oxaloacetate reacts with 1mole of benzene diazonium chloride. If only 1mole of the salt is used, the product obtained is dioxosuccinate phenyl hydrazone(I)^{106,107,108}.



For diazotization, aqueous solution of sodium nitrite is added to mineral acid salt of aryl amine at temperature 0° - 5°C . If the reaction is carried out in aqueous solution, sodium acetate is used as a buffer. Occasionally, alcohol, pyridine or acetic acid are also used as solvents for water- insoluble reactants.

Reactant ratio:

Equivalent amount of diazonium salt and reactant are commonly employed. Excess of diazonium salt should be avoided as it can couple again with the product such as hydrazone to form a formazan derivative. This reaction is favoured by strongly acidic solution.

Reactions are usually rapid and the product can soon be isolated after the addition of diazonium salt. However, the reactions that involve intramolecular coupling need more time to complete.

Preparation of ethyl 2-(aryl phenyl hydrazone)-3-oxybutyrate:

To the aryl amine (0.039mole) dissolved in a 1:1 mixture of conc. HCl and water (10ml) and cooled at 0° - 5°C on an ice bath. A cold aqueous solution of sodium nitrite (0.078mole) was added. The diazonium salt solution was filtered into a cooled solution of ethyl acetoacetate (0.039mole) and sodium acetate (0.58mole) in ethanol (30ml). The resulting solid was filtered, washed with water and recrystallised in ethanol.

M.P. = 94°C .

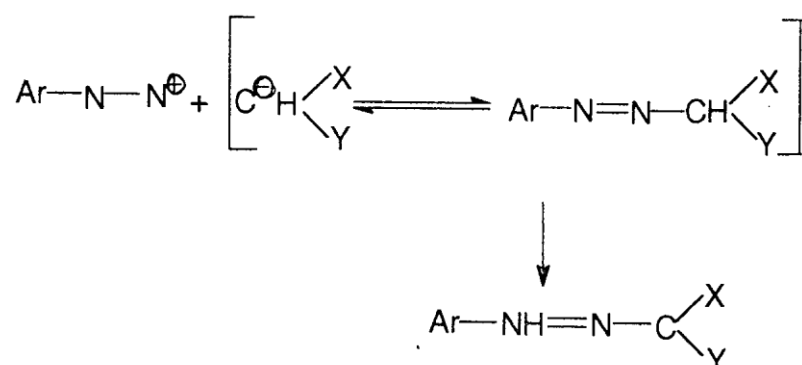
Lit. M.P. = 94°C

Yield = 83%.

Hydrazones

Chemistry of hydrazones:

Aliphatic compounds containing an activated methylene group couple with diazonium salt to form aryl hydrazone^{109,110}. The reaction is an electrophilic attack of the diazonium ion on the carbanion derivative from the methylene compound, forming an unstable azo compound which spontaneously tautomerises to a hydrazone.

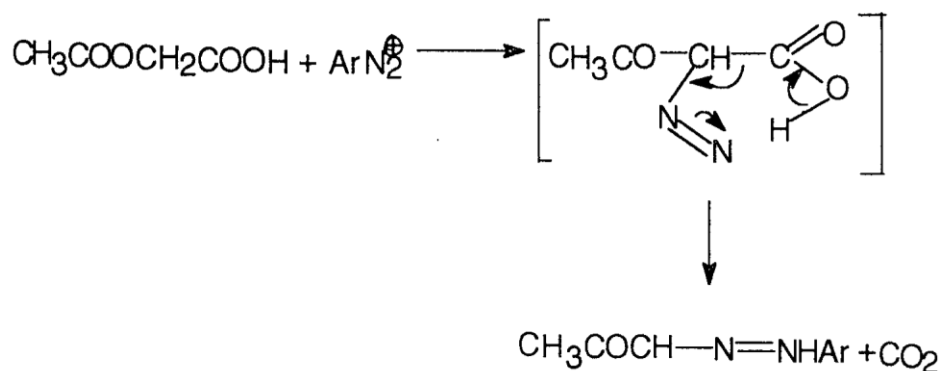


The reaction is usually carried out in cold aqueous solution buffered with sodium acetate. The pH of the medium can be lowered for strongly activated methylene compounds. Hunig and Boes¹¹¹ suggested the sequence of activating effect of the substituents (X and Y) as follows.



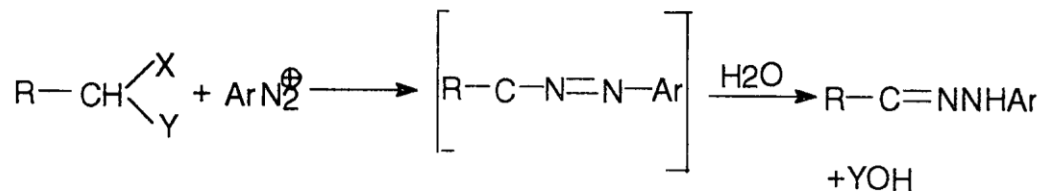
Other activating groups include the tertiary sulphonium and quaternary pyridinium ions and heterocyclics such as 2-pyridyl and 2-quinolyl.

When one of the activating group is a carboxyl, coupling is accompanied by decarboxylation.

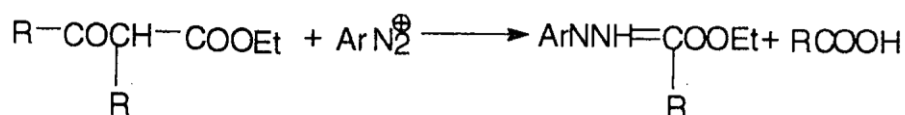
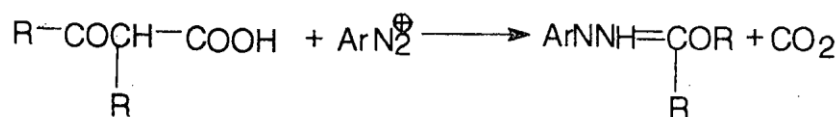


Compounds carrying active methyl group at the 2 or 4-position to the ring nitrogen such as 2-picoline¹¹² and 9-methyl acridine¹¹³, yield a tetrahydrazone on coupling with a diazonium salt. The activity of methyl group increases if the hetero atom is quaternized to onium salt prior to the attack of diazonium ion.

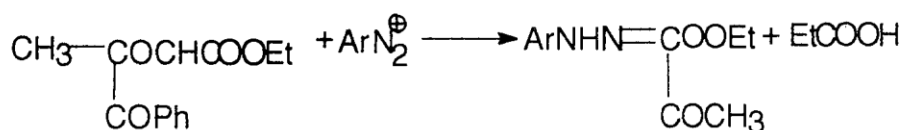
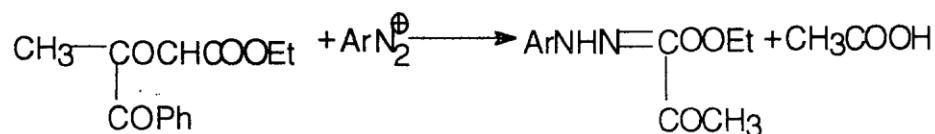
A methinyl group bearing at least two activating groups reacts with diazonium salt to give an aryl hydrazone by hydrolytic cleavage of one of the electron attracting groups on the intermediate unstable azo compound.



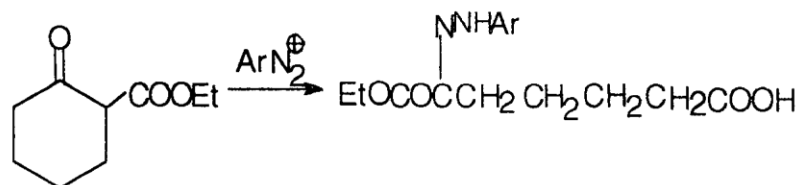
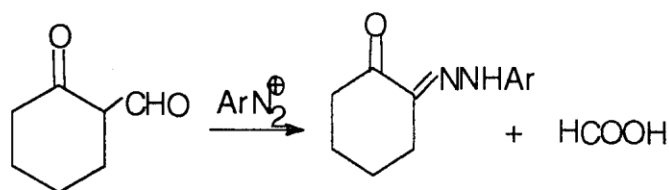
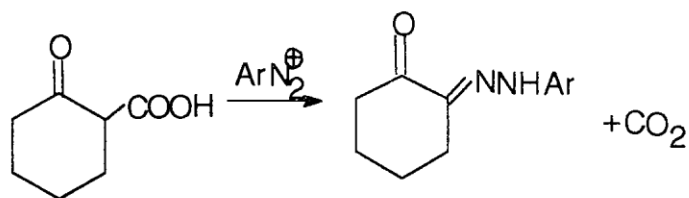
The reaction is described as Japp- Klingemann reaction^{110,114}. Groups that usually undergo splitting are carboxyl, acyl and alkoxy carbonyl. etc. e.g.



In diacylacetic ester, the acyl group is more rapidly cleaved which corresponds to the weaker acid¹¹⁵ e.g.



In case of 2-substituted cyclohexanones, various activating groups undergo splitting relative to the ring carbonyl. It is either side chain splitting, e.g. 2-carboxyl and 2-formyl or is a ring opening, e.g. ethoxycarbonyl and acetyl, which are bound more strongly^{114,116}.



*Friedel-Crafts acylation to form ketone,
Cinnoline and Cinnolone.*

Friedel-Crafts acylation:

Many acid chlorides can easily be induced to eliminate hydrogen chloride intramolecularly.

The cyclization is carried out in a suitable solvent alongwith a cyclizing agent. The cyclization agents or catalysts include¹¹⁷ AlCl_3 , Stannic chloride, hydrogen fluoride etc. The yield of the reactions varies from 40-90 % depending upon the reaction conditions like temperature, pressure and the reactants¹¹⁸.

The catalyst which is employed most frequently is AlCl_3 .

The most common solvents used are benzene and carbon disulfide. Others such as petroleum ether, sym- tetrachloroethane, monochlorobenzene etc. are also employed.

Stannic chloride:

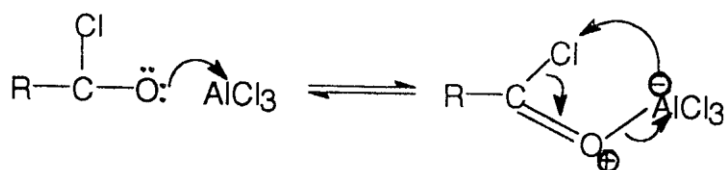
Cyclization with stannic chloride is generally effective in bringing about ring closure into polycyclic nuclei.

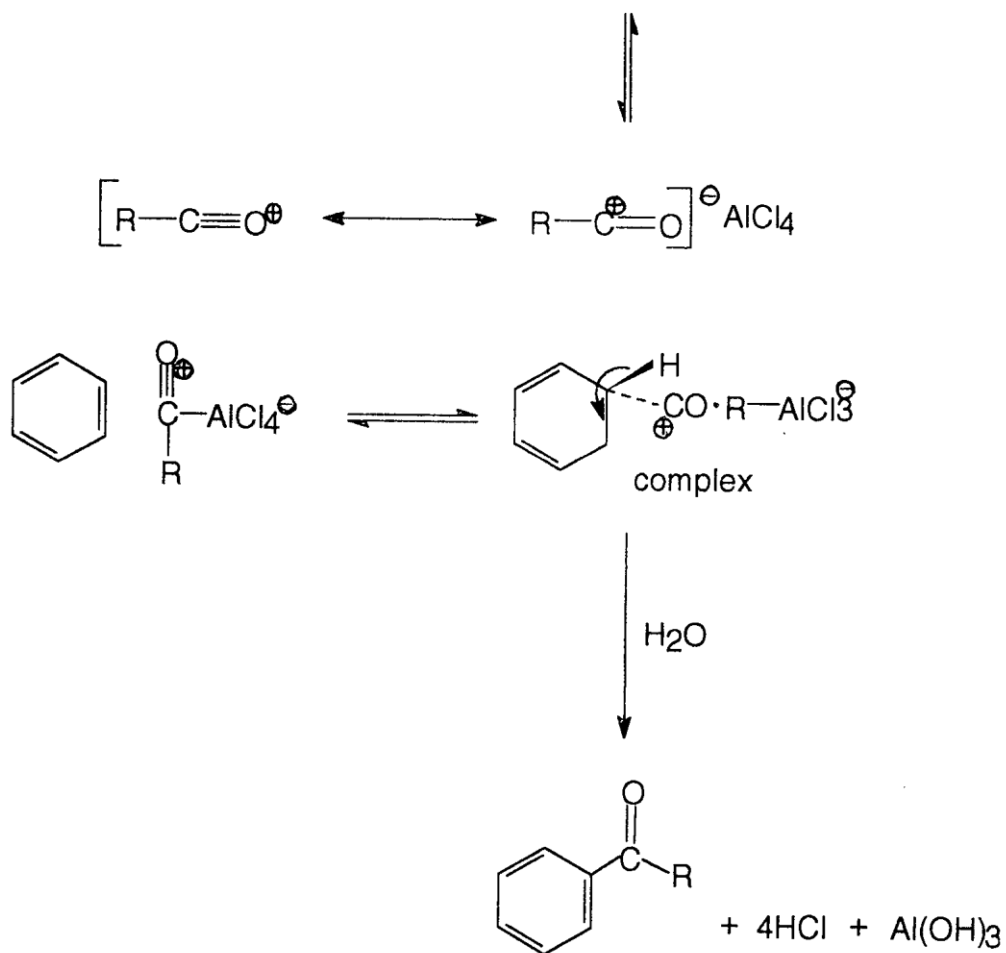
The acid is reacted first with reagents like phosphorus pentachloride¹¹⁹, thionyl chloride etc. to form acid chloride, which is then reacted with stannic chloride under anhydrous conditions.

Aluminium chloride:

The most widely used agent for cyclization is aluminium chloride. Several types of aryl phosphoric acids and aryl butyric acids have been cyclized by this method. The reagents used include phosphorus pentachloride, thionyl chloride etc. and the reaction occurs under anhydrous conditions.

The mechanism of the reaction is same as that of acid chloride and can be explained as follows¹²⁰:





Formation of cinnolone:

To ethyl-2(4-chlorophenyl hydrazono)-3-oxybutyrate (30gm,0.112mole) was added anhydrous AlCl_3 (29.83gm,0.223mole). Chlorobenzene (360ml) was added in order to dissolve the solids and then the mixture was refluxed for 12 hours. It was cooled and dilute HCl (200ml) was added to it and was heated on a water bath. Further it was cooled, filtered and washed twice with dil. NaOH soln. and filtered. Then the product was recrystallized from methanol.

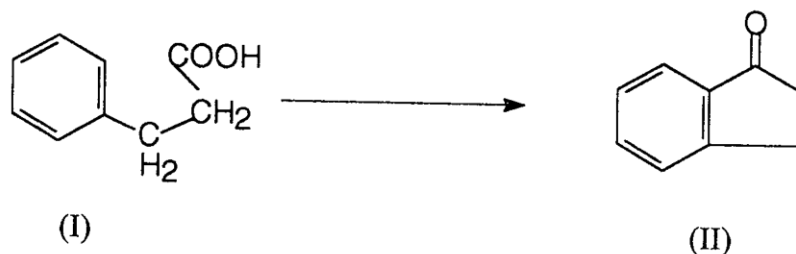
M.P.=197°C

Yield =19.05gm,63.5%

Other methods for cyclic ketones:

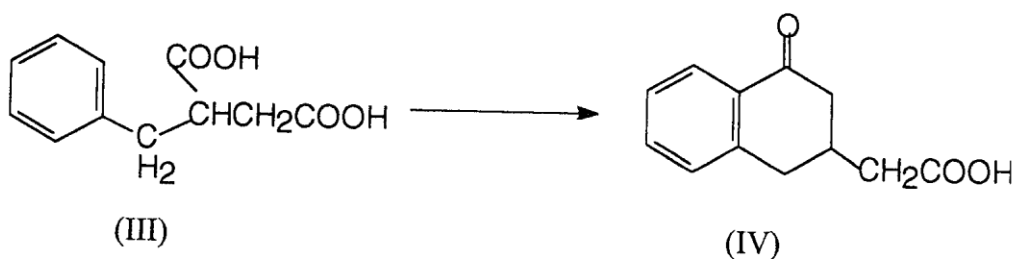
1) From hydrogen fluoride:

In 1939, Fieser and Hershberg¹²¹, cyclized β -phenyl propionic acid (I) using anhydrous liquid hydrogen fluoride to yield 73 % of ketone by intramolecular acylation.



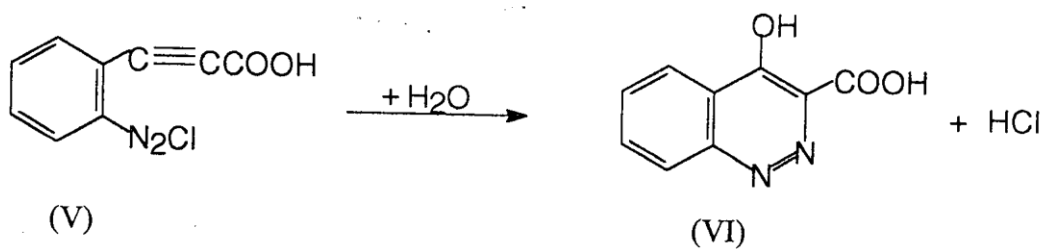
2) From sulphuric acid:

γ -aryl butyric and β -aryl propionic acid (III) get cyclized to ketone (IV) in the presence of sulphuric acid¹²².

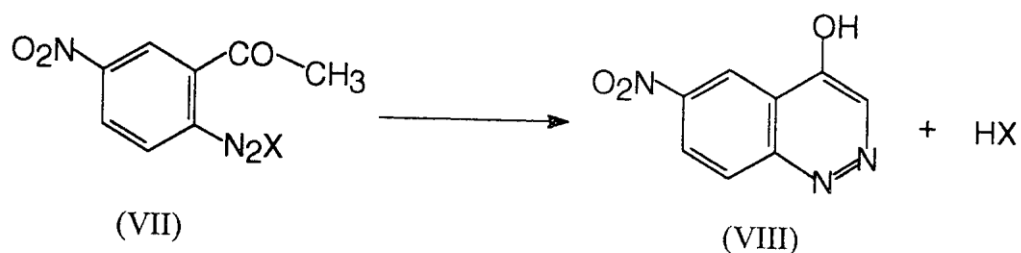


Methods for cinnoline:

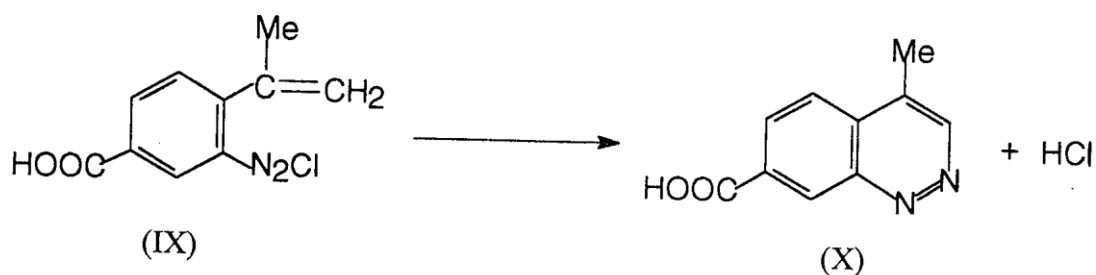
1) The cinnoline ring system was discovered in 1883 by Richter¹²³, who converted diazotized o-nitro phenyl propionic acid (V) into 4-hydroxycinnoline-3-carboxylic acid (VI).



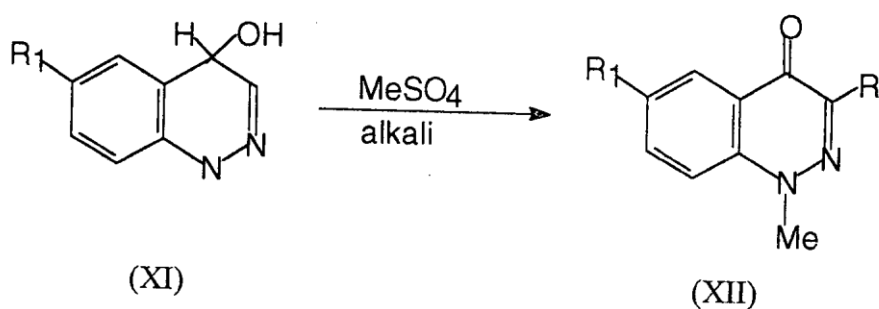
2) Brosche and Herbert¹²⁴ found that diazotized 5-nitro-2-amino acetophenone (VII) slowly on standing, yields 6-nitro-4-hydroxy cinnoline (VIII).



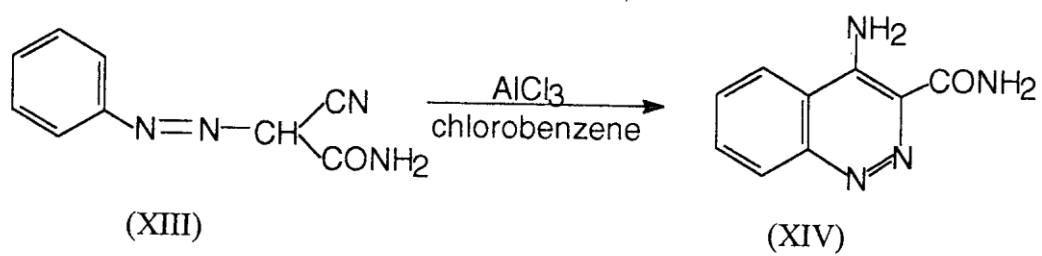
3) Widman¹²⁵ prepared 4-methyl cinnoline-7-carboxylic acid (X) by diazotization of 1-methyl-1,2'-amino-4'-carboxy phenyl ethylene (IX).



4) Methylation of 4-hydroxy cinnoline (XI) with methyl sulphate and alkali gives 1-methyl-1,4-dehydro cinnoline-4-one (XII)¹²⁶.



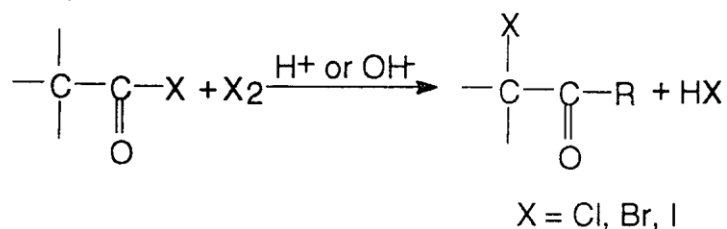
5) Aminocinnoline carboxamide (XIII)¹²⁷ was prepared by cyclization of aryl hydrazono (cyano) acetamide (XIV) with anhydrous AlCl_3 in chlorobenzene.



Halogenation of ketones

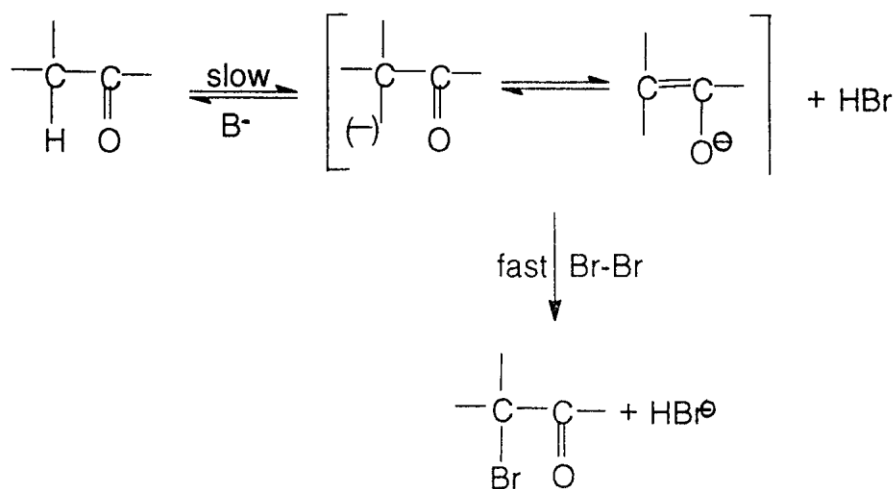
Halogenation of ketones:

Ketones that possess α -hydrogen can readily be replaced by halogen in the presence of acidic as well as basic medium.



Base-promoted halogenation of ketones:

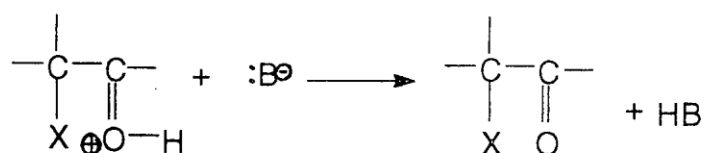
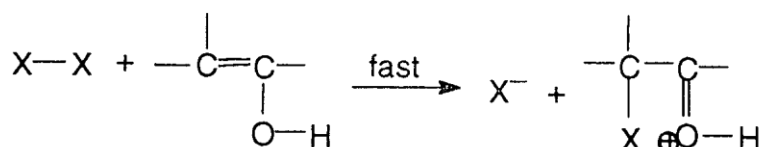
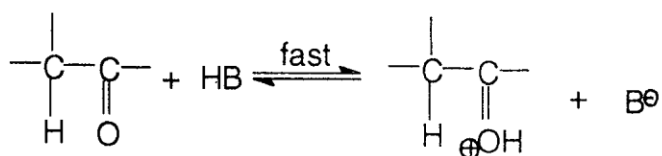
A base strongly abstracts a proton from a ketone to form carbanion (I) which then rapidly reacts to yield bromoketone(II).



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

Acid-catalysed halogenation:

Acids also catalyse the halogenation of ketones.



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

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

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

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

Bromination:

In case of bromination, 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 favours the attack of polarized Br₂ molecule or Br⁻ on the enol form of the ketone.

Synthesis of 6-chloro-3bromoacetyl-1H-cinnoline-4-one:

6-chloro-3-acetyl-1H-cinnoline-4-one (25gm, 0.112 mole) was dissolved in acetic acid(150 ml) and bromine (13.48 gm, 0.16 mole) dissolved in acetic acid(15 ml) was added dropwise. The reaction was provided with slight heating (60° - 65° C) and a source of light. The reaction was continued for 6-7 hours with constant stirring.

Dry air was passed to remove HBr vapours. It was then dumped on ice cold water and the solid was filtered, dried and recrystallized from methanol.

M.P.= 115° C.

Yield = 63.91%

Nitriles

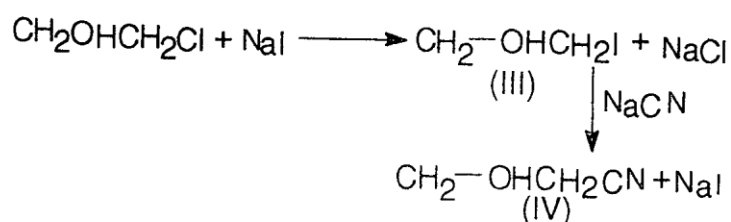
Chemistry of nitriles:

Synthesis:

1] From halides:

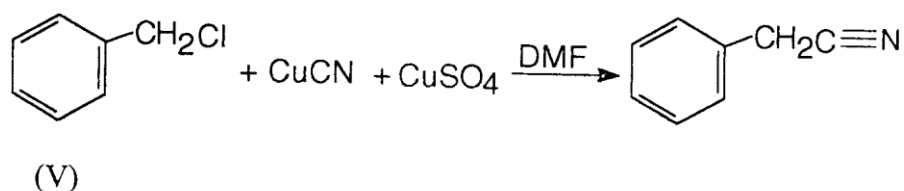
Aliphatic halides:

Metal cyanide reacts with alkyl or aryl halide (III) to form aliphatic nitrile(IV). The order of reactivity of the halide is $-I > -Br > -Cl$. Sodium iodide, when used as a promoter, converts chloride to iodide in the primary halide by exchange and this in turn gives nitrile with metal cyanide.

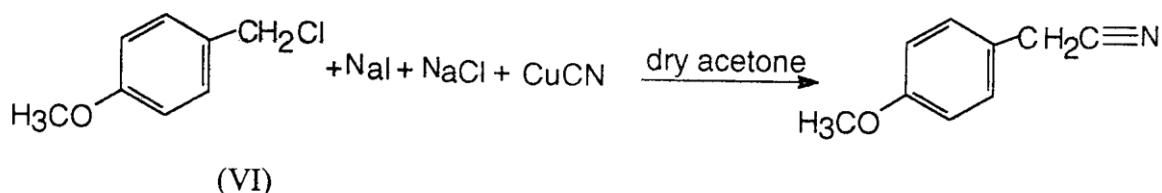


Other metal cyanides which form nitriles are potassium and cuprous cyanide.

The yield varies from 75-90%. The solvents like ethylene glycol¹²⁸, methyl cellosolve¹²⁹, tetrahydrofurfuryl alcohol¹³⁰ and DMF improve the yield as compared to alcohol which causes slow reaction. Secondary halides give lower yield and tertiary halides give a little or no nitrile. The conversion of alkyl halide(V) into nitrile is accomplished best with sodium or cuprous nitrile with pyridine, quinoline, DMF or N-methyl pyrrolidine as solvent¹³¹. The reaction can be accelerated by addition of small amount of a nitrile and traces of copper sulphate as a catalyst.



Halide, like anisyl chloride (VI) yields 75% of nitrile¹³².

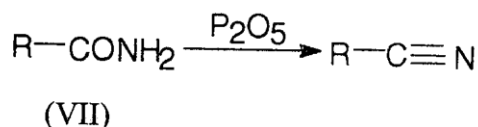


B) From heterocyclic halides:

Halides of pyridine¹³³, quinoline¹³⁴ give nitrile in satisfactory yields with cuprous cyanide¹³⁰.

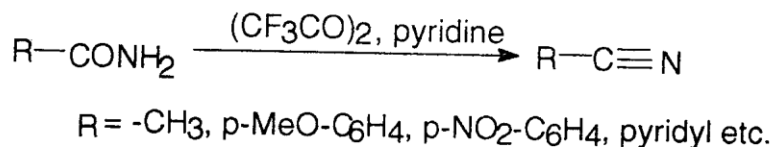
2] From amide:

Amide (VII) gives nitrile in 60-70% yields with a dehydrating agent like phosphorous pentoxide¹³⁵ or phosphorous pentachloride¹³⁶.



Sometimes dehydrating agents like carbonyl chloride¹³⁷, benzene, p-toluene sulphonyl chloride^{138,139}, phosphorous oxychloride are used in the nucleophilic solvent such as pyridine or tertiary amine. Even addition of salt like sodium chloride¹⁴⁰ or sodium metabisulphite¹⁴¹ to phosphorous oxychloride enhances the effectiveness of the reagent.

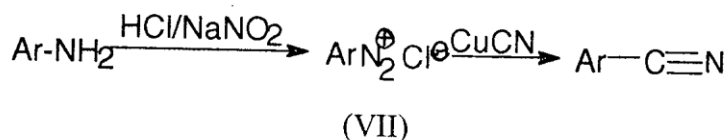
Recently, a more convenient method employing trifluoroacetic anhydride/pyridine system¹⁴² was found to lead a rapid reaction with 85-95% yield.



With excellent yield and mild reaction conditions, the method is especially suitable for thermolabile nitriles.

3] From diazonium salts: (Sandmeyer's reaction)

Diazonium compounds (VII) with cuprous cyanide give 65-85% of nitrile.



Complex salt of cuprous cyanide such as $\text{Na}_3\text{Cu}(\text{CN})_5\text{NH}_3$ ¹⁴³ has also been used to a great extent. Prior neutralization of diazonium salt is necessary in order to avoid escape of halogen cyanide o-tolunitrile¹⁴⁴.

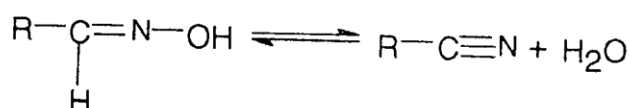
4] From aldehyde:

Several methods have been reported for conversion of an aldehyde to nitrile. e.g.
A) dehydration of aldoxime using carbodimide¹⁴⁵, carbonyl biimidazole¹⁴⁶ or phosgene imino chloride¹⁴⁷.

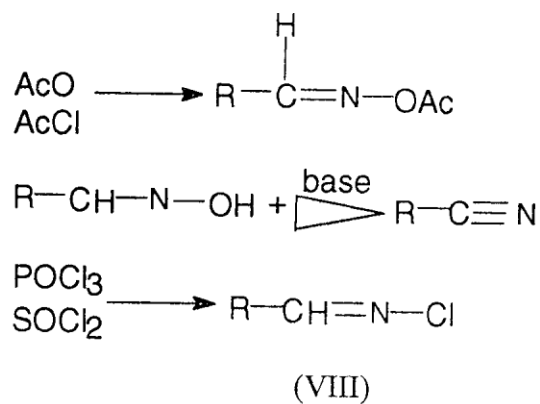
B) 1,2 elimination of o-substituted aldoxime^{148,149} and

C) 1,2 elimination of $\text{R-CH=N-N}-(\text{CH}_3)_3$ and R-CH=N-tosyl by base¹⁵⁰.

A] Dehydration of aldoxime:

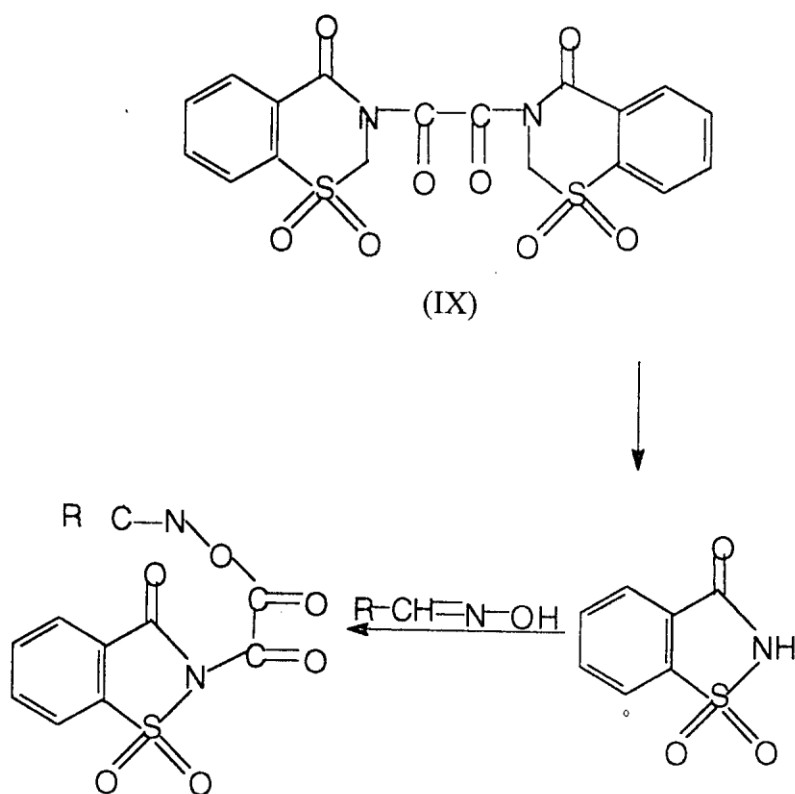


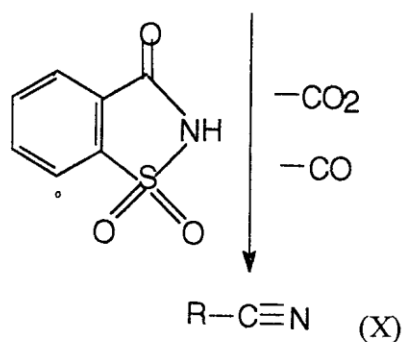
A large number of dehydrating agents have been employed for this preparative method¹⁵¹. e.g. acetic anhydride, phosphorous oxide, thionyl chloride, acetyl chloride etc. The dehydrating agent forms a reactive complex (VIII), which renders the aldoxime easily with the loss of water molecule.



Relatively new dehydrating agents have been introduced. e.g. dicyclohexyl carbonyl biimidazole¹⁴⁵ and phosgene immonium chloride¹⁴⁷.

Kilagawa, T. *et.al.*¹⁵² described a new dehydrating agent, 2-2'-oxalyldi (o-sulfobenzimide) (ODS)(IX), for convenient synthesis. The possible mechanism proposed is as follows-



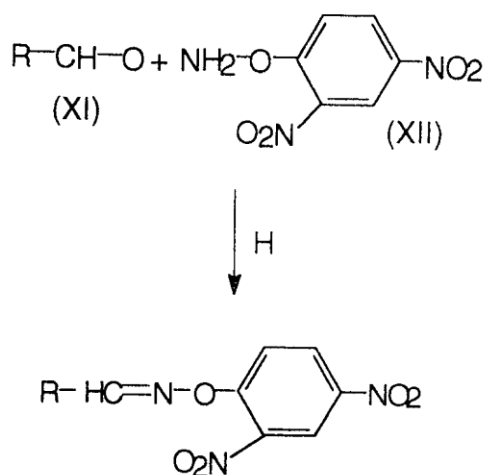


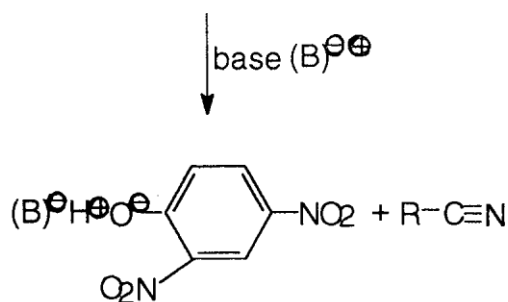
The reaction is carried out at room temperature or in cold.

B] 1,2-elimination of o-substituted aldoxime:

Miller *et.al.*¹⁴⁹ described this method which does not need to isolate the intermediate oxime.

The development of efficient amino protecting groups has facilitated the synthesis of a variety of sensitive compounds including o-substituted hydroxyl amine¹⁵³. Although a number of these are unstable¹⁵⁴, others have significant synthetic utility. Their electrophilic character has been exploited in their increasing use as aminating agents¹⁵⁵⁻¹⁵⁷. Their nucleophilicity has been demonstrated by their reaction with ketones to form substituted oximes. This 2,4-dinitrophenyl hydroxyl amine (XI) can be reacted with aromatic or aryl aldehyde (XII) to form corresponding o-substituted aldoxime.

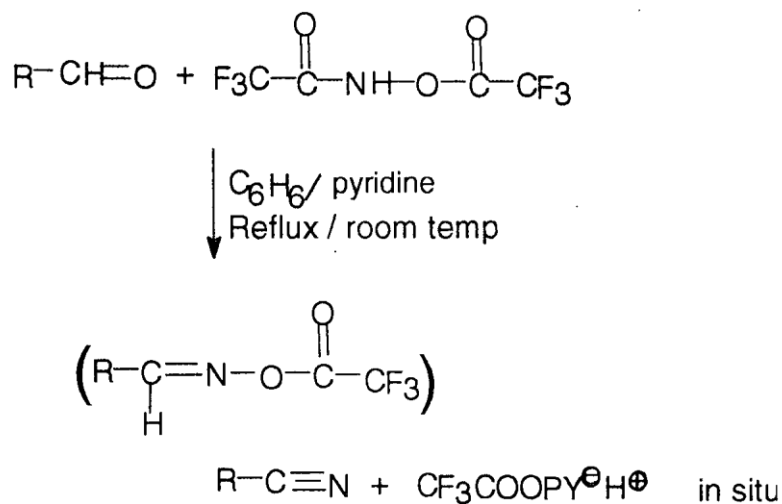




Nitriles can be prepared from these o-substituted aldoximes by 1,2-elimination reaction using excess base as shown above. The development of deep yellow to red colour of the 2,4-dinitrophenolate ion indicates the extent of completion of the reaction.

C] Method C:

This is a one step reaction involving the use of o-N-bis(trifluoroacetyl) hydroxyl amine (XII) as one of the reagents.



The reaction conditions are very mild and directly afford the nitrile, eliminating the intermediate step¹⁵⁸. The yields are in the range of 70-90%. The reagent could be obtained by reacting trifluoro acetic anhydride with hydroxyl amine hydrochloride in suitable solvent. The aldoxime is unstable and hence, cannot be isolated. It instantly decomposes to furnish the nitrile.

Synthesis of 4-N,N-dimethyl amino phenyl benzonitrile:

4-N,N-dimethyl amino benzaldehyde (10gm,0.067mole) was dissolved in warm ethanol (30ml) and stirred for 15 min. To it was added hydroxylamine hydrochloride (5.59gm,0.0804mole) in water (20ml) and stirred for 20 min. at room temp. Sodium hydroxide (4.13gm,0.1mole) dissolved in water (20ml) was added dropwise to this soln. and it was stirred for 4 hrs. Ethanol was removed to half the quantity under reduced pressure and the reaction mixture was dumped on crushed ice. The solid obtained after filtration was oxime.

Aldoxime (6gm,0.036mole) was dissolved in acetic anhydride (7.5gm,6.93ml) and refluxed for 4 hrs. using dry conditions. It was then cooled and poured on crushed ice. The solid was filtered and washed with cold water. It was recrystallized from methanol.

M.P.=212⁰-213⁰C

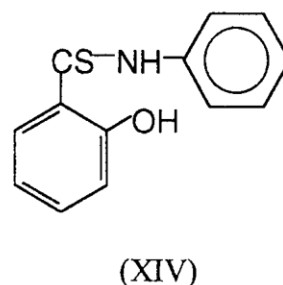
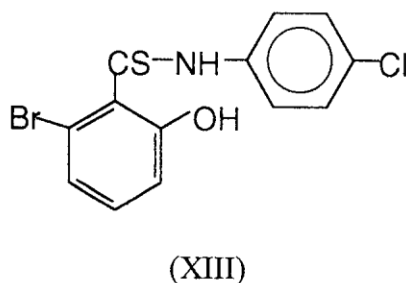
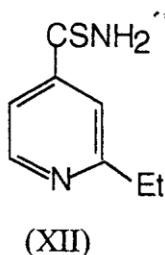
Lit.M.P.=215⁰-216⁰C²³¹

Yield = 77.48%.

Thioamides

Chemistry of Thioamides:

Liberman and coworkers in 1955, observed the *in vivo* activity of Ethionamide (XII) against *M. tuberculosis* and it exhibited low toxicity. Later on therapeutically effective agents such as substituted thiobenzamide (XIII), salicylic acid thioamide (XIV) were found.



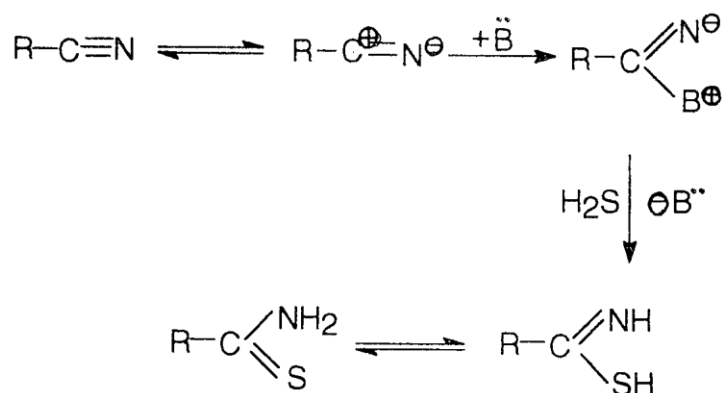
Synthetic methods:

1] From nitriles:

Nitriles in the presence of excess of hydrogen sulphide and a catalyst base yield thiocarboxide^{159,160}.

Water soluble sulphides or polysulphides can be used instead of hydrogen sulphide¹⁶¹

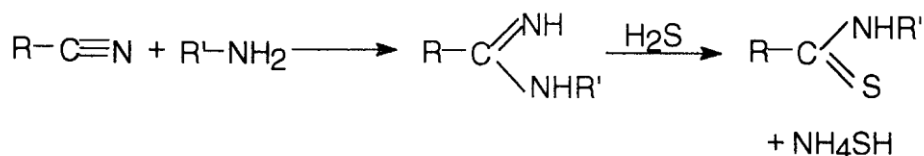
The reaction occurs as follows-



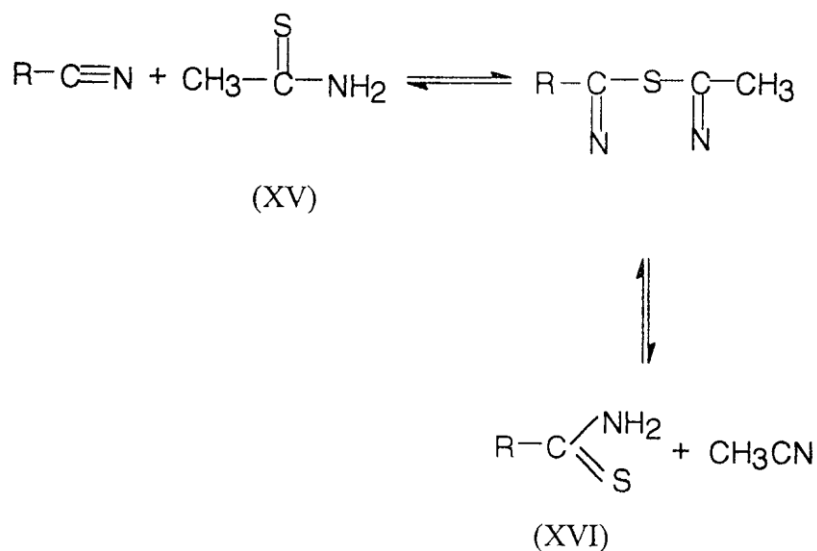
Catalysts used include ammonia, aliphatic amine, pyridine, alkoxide, potassium hydrogen sulphide^{162,163} etc. Fairfull *et.al.*¹⁶⁴ obtained very good yields by addition of H₂S to nitrile in presence of pyridine and triethylamine.

Aliphatic nitriles need elevated temperature and pressure¹⁶⁵.

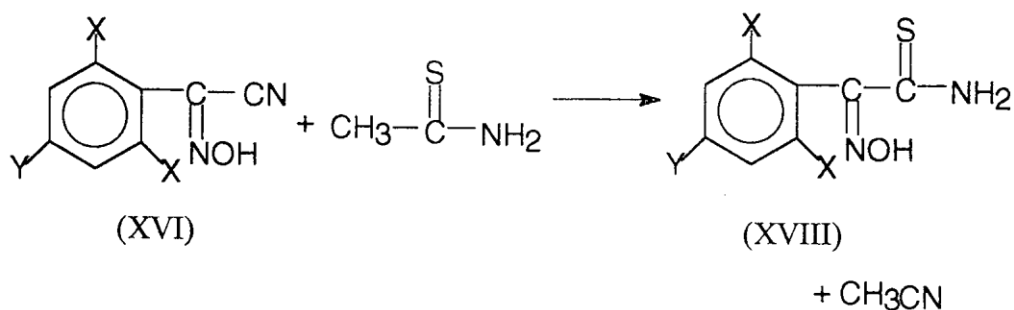
Kindler¹⁶² and Hanford¹⁶⁶ extended the method by addition of primary amine.



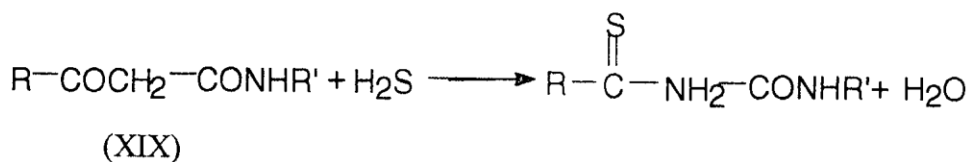
Reactions of nitriles with thioacetamide (XV) appeared more frequently in literature than with H₂S^{167,168}.



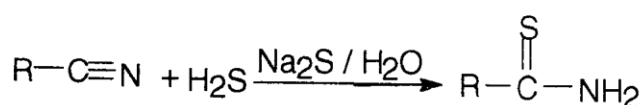
Removal of acetonitrile leads to the formation of thiocarboxamide (XVI) and thus is important for preparation of thiocarboxamide from α-cyanobenzaldoximes (XVII).



If the nitrile contains hydroxyl group, it must be protected by acylation^{169,170}. The keto group such as that in (XIX) if present is readily converted to thioxo in acidic solution¹⁷¹.



Mayer *et.al.* explained the base-catalyzed conversion of ketone to thiones¹⁷². Recently Casser *et.al.*¹⁷³, introduced aqueous sodium sulphide as a phase transfer catalyst in the general procedure to get high yields of thioamide.



This system consists of three phases such as aqueous, organic and H₂S gas.

2] From amides:

Replacement of oxygen of carboxamide by sulphur is mostly used to obtain N-monosubstituted and N-disubstituted compounds in presence of P₂S₅¹⁷⁴. Potassium thiophosphate along with P₂S₅ enhances the yield further¹⁷⁵.

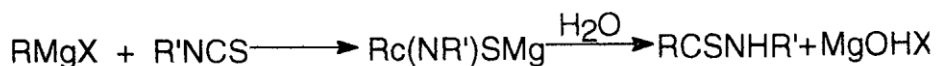


Solvents such as benzene, xylene, toluene, tetrahydrofuran, dioxane, diethylene glycol and diethyl ether¹⁷⁶⁻¹⁷⁹ are commonly used and these should be dry and inert in order to avoid undesirable side reactions and to reduce time.

Reactions in pyridine give pure thioamide in higher yields^{180,181}. Reactions of liquid amides are carried out in an excess of amide as solvent^{182,183}. Thermally unstable amides are not suitable for this reaction. Hydroxyl group must be protected by acylation before sulphurization^{184,185}.

3] From isothiocyanates:

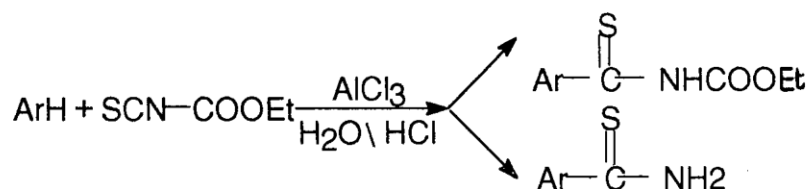
Sachs and Loevy¹⁸⁶ described the reaction of isothiocyanates with Grignard's reagent and subsequent hydrolysis under mild conditions to lead thiocarboxamide.



4] Single step synthesis of aromatic thioamides:

a) From ethoxy carbonyl isothiocyanate:

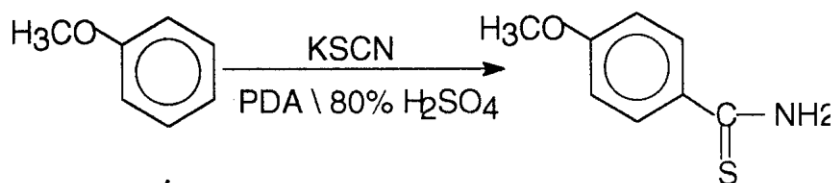
Ethoxy carbonyl isothiocyanate¹⁸⁷ reacts with aromatic compounds in the presence of anhydrous AlCl_3 (Friedel-Craft isothiocyanation). However, depending upon the conditions, it either yields the N-ethoxy carboxy thioamide or thioamide itself.



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

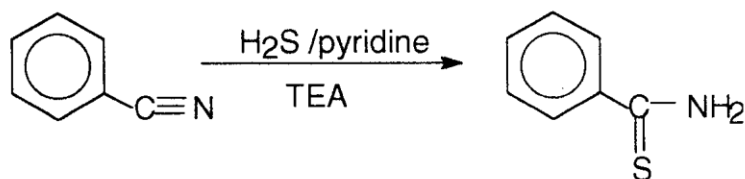
Reactions at a low temperature lead to N-ethoxy carbonyl thioamide.

B)Thioamides can also be prepared by heating anise (0.01N) or veratrol at 60°C in the presence of polyphosphoric acid or 80% H₂SO₄ with potassium thiocyanate (0.01N)¹⁸⁸.



50%yield in PPA can be increased by 5-10% with 80%H₂SO₄.

Solution of aromatic nitrile (6gm,0.0582 mole) in pyridine (30 ml) was taken in a three necked round bottom flask. Dry gaseous H₂S was bubbled through the reaction mixture containing triethylamine (4ml) as catalyst. H₂S was generated by the action of conc.HCl on ferrous sulphide. The reaction took 8-12 hours to complete, with yields of 70-80% at alkaline pH.



M.P. =116°C

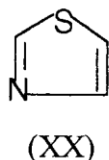
Lit. M.P. = 116⁰-118⁰C²²¹

Yield=78%.

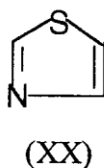
Thiazoles

Chemistry of Thiazoles:

The thiazole heterocycle (XX) was first delineated by Hantzsch and Weber¹⁸⁹. Further, they have described it as 'the pyrrole of the thiophene series'.



The thiazole moiety exists in two different configurations as (XX) and (XXI).



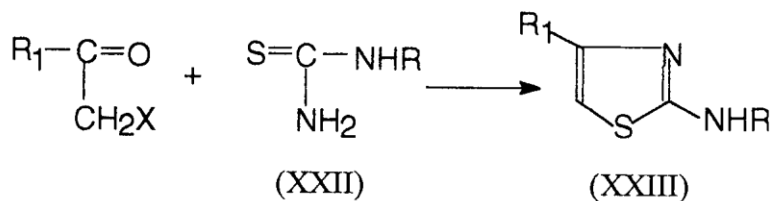
The high reactivity of the thiazole molecule is due to the group attached to the carbon between sulphur and nitrogen.

Large number of thiazoles occur naturally. They include Vitamin B (thiamine), Penicillin etc. Their origin has often been related to that of a peptide chain at cysteine residue with formation of a thiazole.

General methods for synthesis:

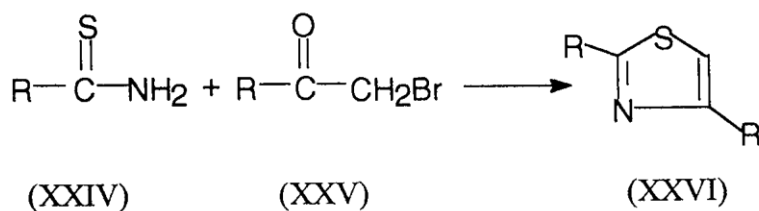
1] Synthesis of 2-substituted amino thiazoles:

The cyclization of N-monosubstituted thiourea (XXII) with halocarbonyl compound gives 2-monosubstituted (XXIII) thiazole^{191,192}.



2] 2,4-disubstituted thiazoles:

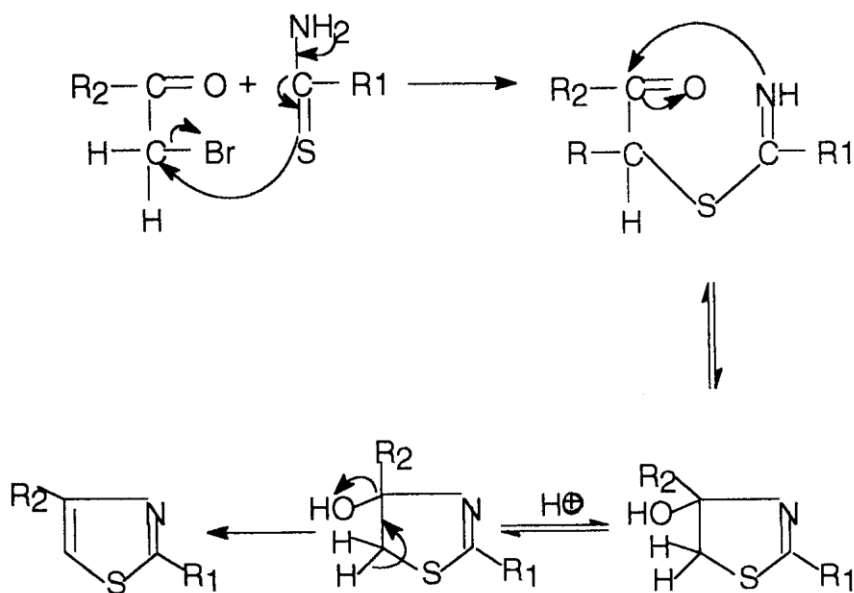
These were prepared in 1950 by Erylenmeyer *et. al.*¹⁹³ from thiobenzamide (XXIV) and bromoacetophenone (XXV) in alcohol as a solvent which yielded 50-80% of thiazole (XXVI).



Mechanism:

The first step involves 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 step II, the lone electron pair of N-atom attacks the carbon carrying the carbonyl function, giving rise to a new cyclic intermediate that reacts with acid leading to the thiazole molecule^{194,195}.



Synthesis of 3(2-alkyl/ aryl-4-substituted)-6-chloro-cinnoline-4-one:

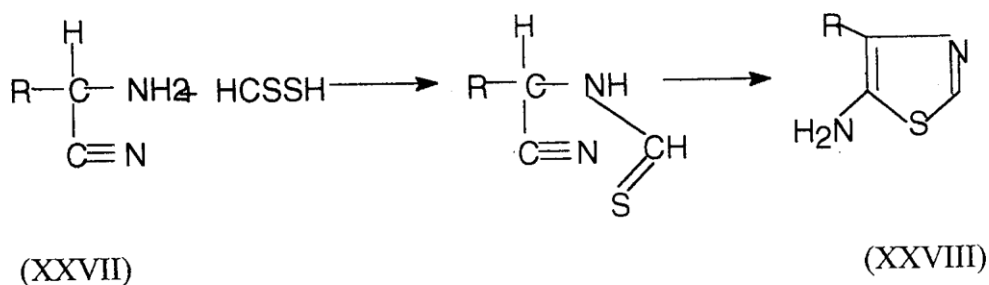
3-Bromoacetyl-6-chloro cinnoline 4-one (2 gm, 0.066 mole) and thiobenzamide (1.49 gm,0.099 mole) were dissolved separately in ethyl alcohol and then mixed. This mixture was then refluxed for 48-50 hours to yield a thiazole with a characteristic odour. Alcohol was then removed under reduced pressure and the product was recrystallized using methanol as the solvent.

M.P.= 110°C

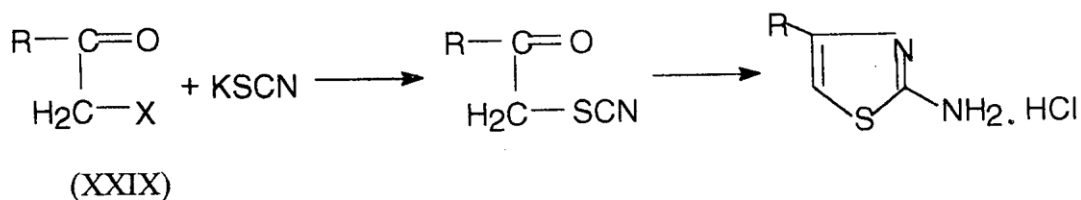
Yield= 70.66%

Other synthetic methods for thiazole:

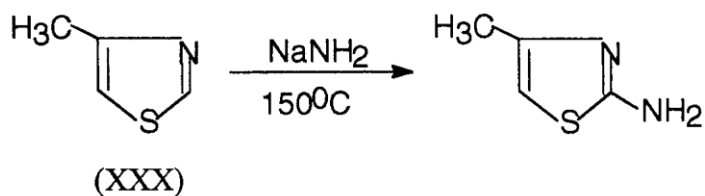
1]α-amino nitrile (XXVII) reacts with dithioformic acid (XXVIII) at room temperature to give an intermediate which cyclizes to yield 5-amino thiazole derivative¹⁹⁶.



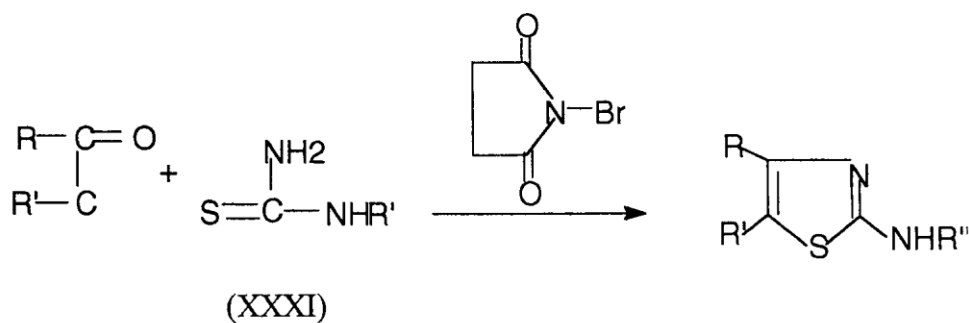
2]Potassium thiocyanate reacts with halocarbonyl compound (XXIX) to give 2-amino thiazole hydrochloride¹⁹⁷.



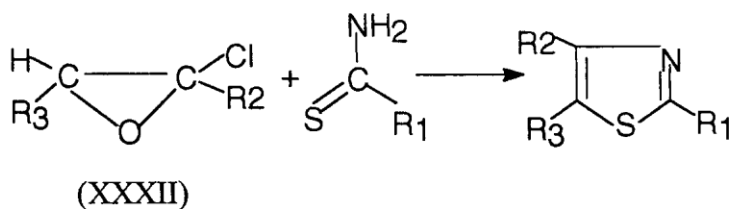
3]4-Methyl thiazole (XXX) on heating with sodamide for 15 hours at 150°C yields 2-amino-4-methyl thiazole¹⁹⁸.



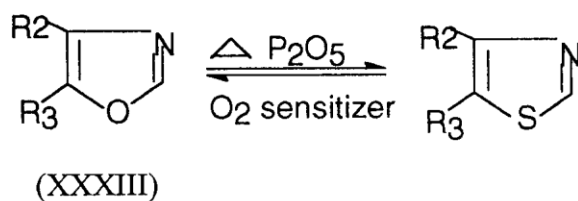
4]Reaction of substituted thiourea (XXXI) with ketone in the presence of N-bromosuccinimide furnishes 2-aminothiazole derivative¹⁹⁹.



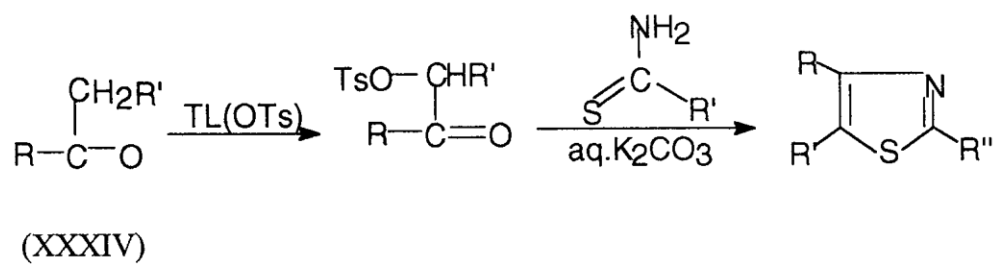
5]1-chloro 1,2 epoxide (XXXII) when condensed with thioamide leads to 2,4,5-trisubstituted thiazole²⁰⁰.



6]Oxazole (XXXIII) when heated with P_2S_5 gives a thiazole²⁰¹.



7]Enolized ketone (XXXIV) reacts with thioamide or substituted thioamide in acetonitrile in the presence of thallium p-tolyl sulphonate (TTS) to give 2,4,6-trisubstituted thiazole²⁰².

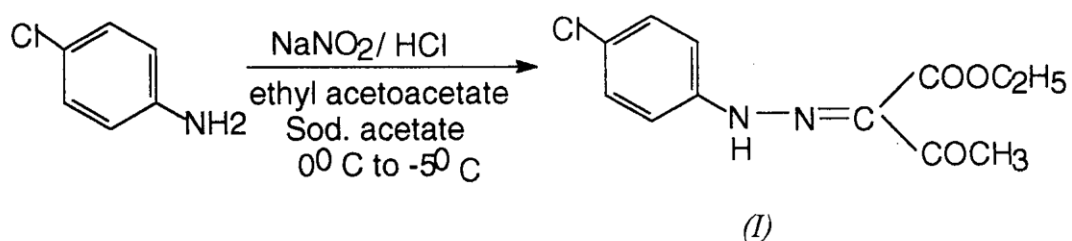


Schematic representation

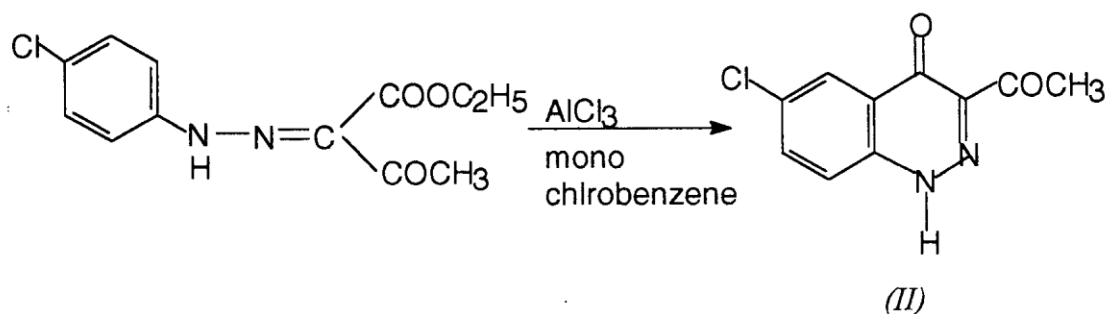
Schematic representation of the compounds synthesised:

Scheme I: Synthesis of 3(2-alkyl/ aryl,4-substituted thiazolo)-6-chloro-cinnoline-4-ones:

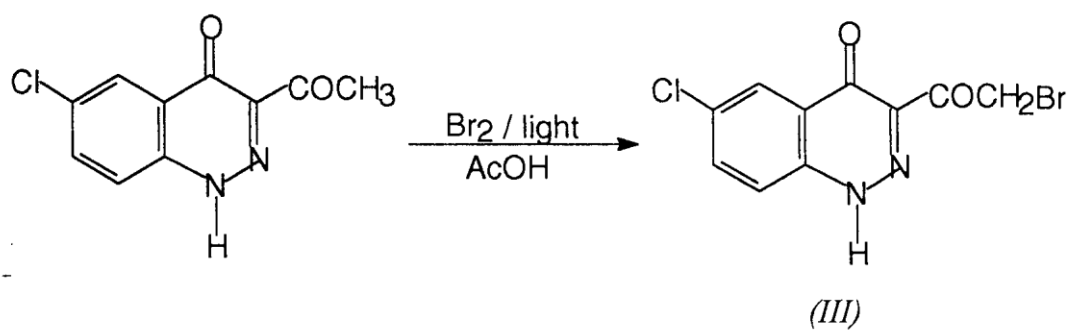
Step I: Synthesis of ethyl-2(4-chlorophenyl hydrazono)-3-oxybutyrate(I):



Step II: Synthesis of 3-acetyl-6-chloro-cinnoline-4-one(II):

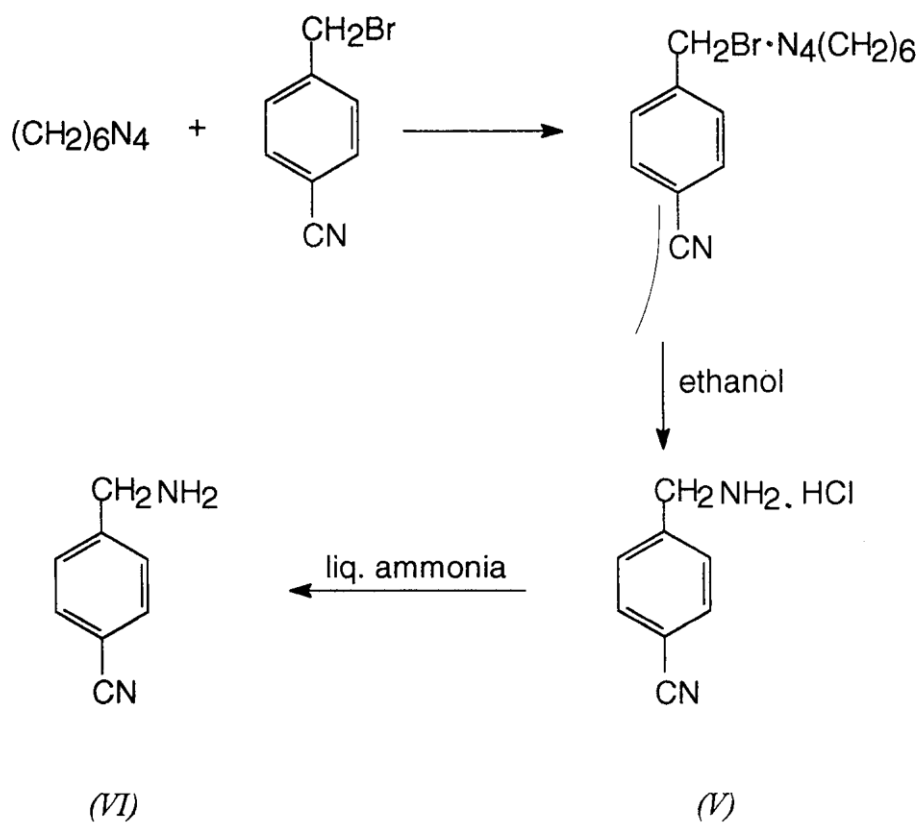


Step III: Synthesis of 3-bromoacetyl-6-chloro-cinnoline-4-one(III):

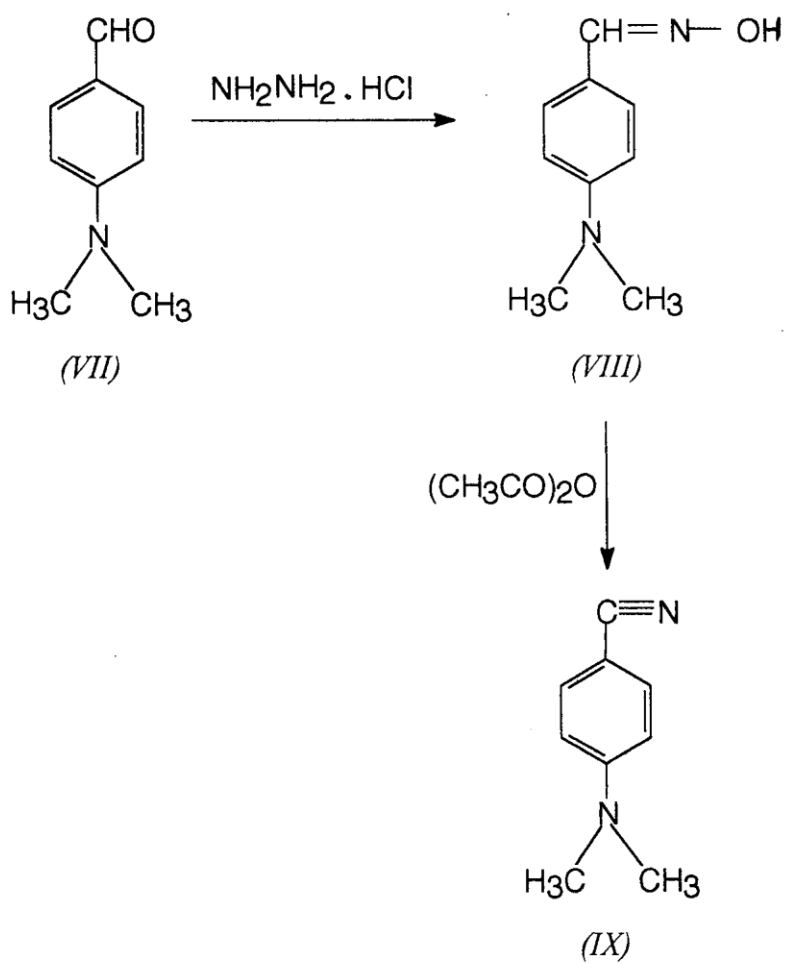


Scheme II: Synthesis of nitriles:

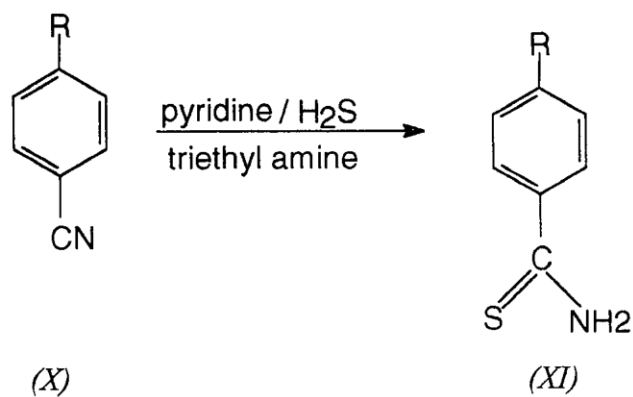
I) Synthesis of 4-amino benzonitrile(VI):



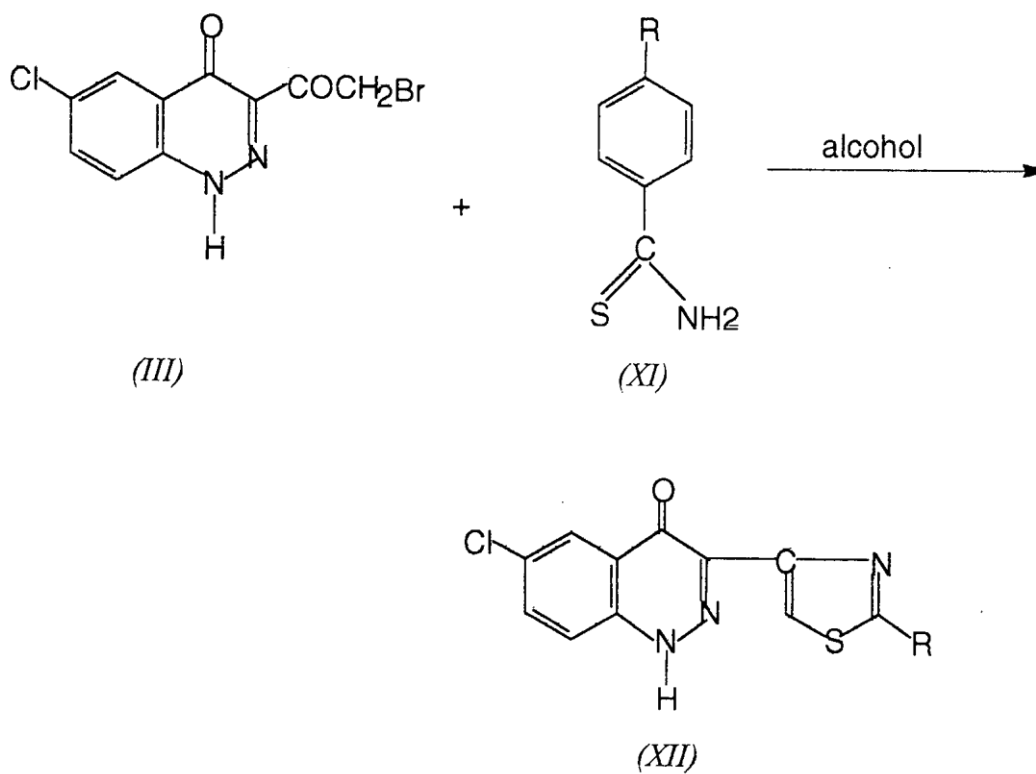
II) Synthesis of *N,N*-dimethyl amino benzonitrile (IX):



Scheme III: Synthesis of thioamides (XI):



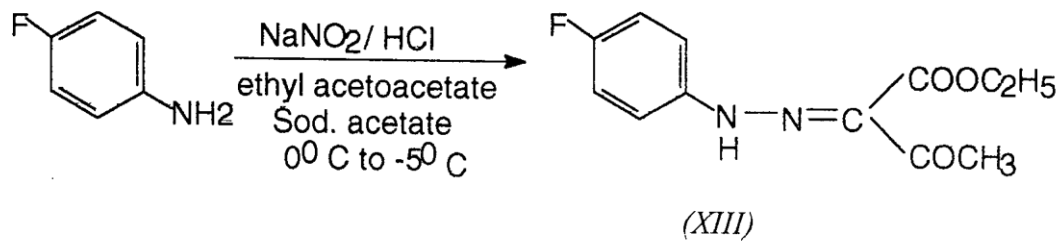
Scheme IV: Synthesis of thiazoles (XII):



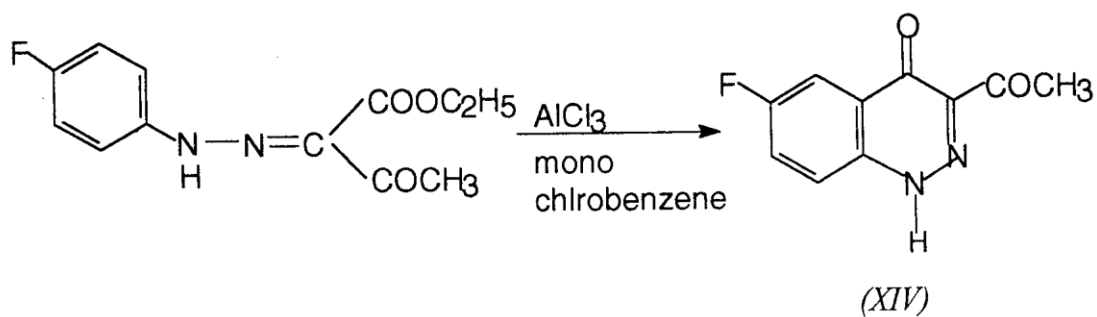
R = phenyl, benzyl, pyridyl, aminomethyl phenyl etc.

Scheme V: Synthesis of 3(2-alkyl/ aryl,4-substituted thiazolo)-6-fluoro-cinnoline-4-ones:

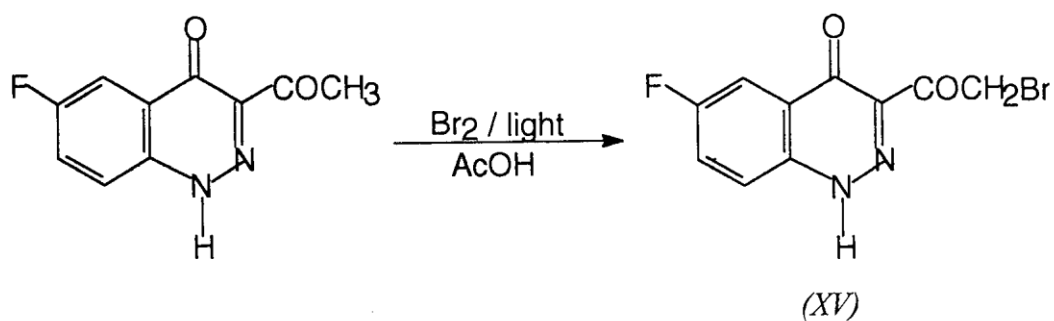
Step I: Synthesis of ethyl 2(4-fluorophenyl hydrazono)-3-oxobutrate (XIII):



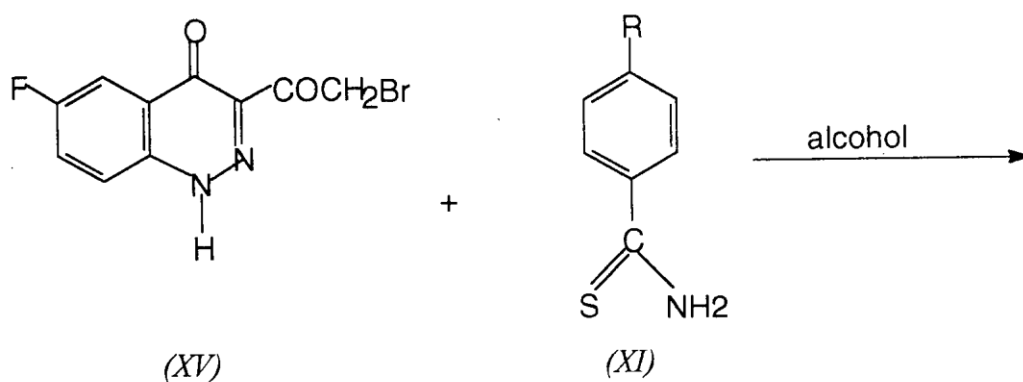
Step II: Synthesis of 3-acetyl-6-fluoro-cinnoline-4-ones(XIX):

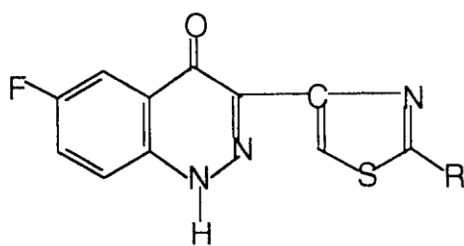


Step III: Synthesis of 3-bromoacetyl-6-fluoro-cinnoline-4-ones(XV):



Step IV: Synthesis of thiazoles(XVI):



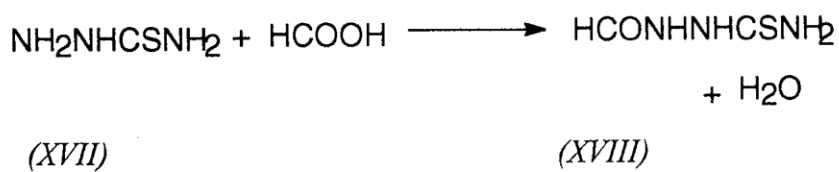


(XVI)

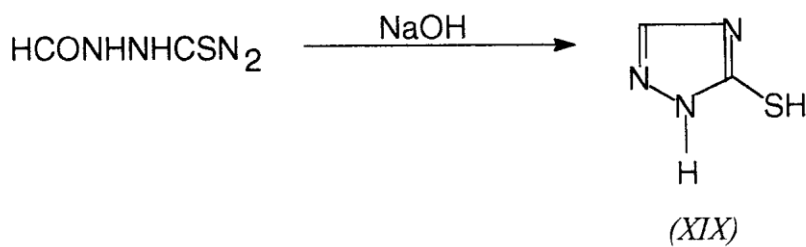
R = alkyl / aryl/ substituted aryl etc.

Scheme VI: Synthesis of intermediate for Letrozole:

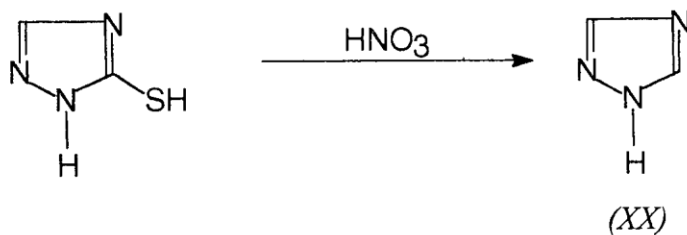
Step I: Synthesis of formyl thiosemicarbazide (XVIII):



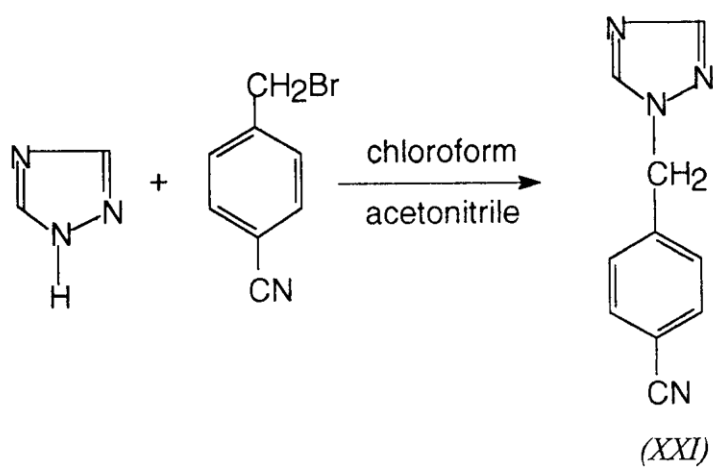
Step II: Cyclisation to 1,2,4-triazole-3(5)-thiol (XIX):



Step III: Synthesis of 1H-1,2,4-triazole (XX):



Step IV: Synthesis of 4(1H-1,2,4-triazolyl methyl) benzonitrile(XXI):



Experimental

Part -9: Synthesis of 3 [2-alkyl/aryl, 4-substituted thiazolo]-6-chloro cinnoline-4-ones.

Part -I: Synthesis of 3[2-alkyl/aryl,4-substituted thiazolo]-6-chloro cinnoline-4-ones:

Synthesis of ethyl 2(4-chlorophenyl hydrazono)-3-oxobutyrates:

To 4-chloro aniline (50gm,0.39mole) dissolved in a mixture of conc. HCl (15ml), water (15ml) and cooled at 0° to -5°C in an ice bath, a cold solution of sodium nitrite (40.02gm,0.58mole) was added with stirring. The diazonium salt was filtered into a cooled solution of ethyl acetoacetate (50.78gm,0.39mole) in ethanol (60ml) and sodium acetate (640gm,7.8mole) in water (700ml). The solid was collected and recrystallised from methanol.

M.P.=94°C.

Lit. M.P.=94°C

Yield =32.66gm,65.32%

Elemental analysis:

	Calculated	Found
C	53.63 %	54.03 %
H	4.84 %	5.16 %
N	10.42 %	10.73 %

Synthesis of 3-acetyl-6-chloro cinnoline-4-one:

To ethyl-2(4-chlorophenyl hydrazono)-3-oxobutyrates (30gm,0.112mole) was added anhydrous AlCl₃ (29.83gm,0.223mole). Chlorobenzene (360ml) was added in order to dissolve the solids and then refluxed for 12 hours. The mixture was cooled and dilute HCl (200ml) was added to it. It was then heated on a water bath. It was cooled, filtered and washed twice with dil. NaOH soln. and filtered. The product was recrystallised from methanol.

M.P.=197°C

Yield =19.05gm,63.5%

Elemental analysis

	Calculated	Found
C	53.93 %	53.85 %
H	3.14 %	3.39 %
N	12.58 %	12.44 %

Synthesis of 3-bromoacetyl-6-chloro cinnoline-4-one:

3-acetyl-6-chloro-cinnoline-4-one (25gm,0.112mole) was dissolved in chloroform (40ml). Bromine (13.48gm,0.16mole) dissolved in 15ml of chloroform was added dropwise with stirring in the presence of light. The mixture was stirred at 70⁰-75⁰C for 10 hrs. Dry air was passed through the solution and then the mixture was dumped on ice cold water. The solid was filtered and dried. It was recrystallized from methanol.

M.P.= 115⁰C

Yield = 15.97gm,63.91%

Elemental analysis:

	Calculated	Found
C	39.86 %	40.04 %
H	1.99 %	2.28 %
N	9.28 %	9.58 %

Synthesis of nitriles:

1]Synthesis of 4-amino methyl benzonitrile HCl.[Delepine reaction]:

Hexamine (12.5gm,0.0892 mole) was dissolved in 25ml of chloroform. To it was added 4-cyanobenzyl bromide (15gm,0.0765 mole) and the mixture was stirred for 6 hrs. to get a solid adduct. It was then filtered, washed with chloroform and dried.

To this adduct a 1:1 mixture of ethanol (20ml) and conc.HCl (20ml) was added and refluxed for 3 hrs. And cooled. Needles of amine HCl, obtained were filtered and dried.

M.P.=220⁰C

Lit.M.P.= 222⁰C

Yield = 82.5%

2]4-N,N-dimethyl amino benzonitrile:

Step-I: Synthesis of aldoxime of N,N-dimethyl amino benzaldehyde-

4-N,N-dimethyl amino benzaldehyde (10gm,0.067mole) was dissolved in warm ethanol (30ml) and stirred for 15 min. To it was added hydroxylamine hydrochloride (5.59gm,0.0804mole) in water (20ml) and stirred for 20 min. at room temperature. Sodium hydroxide (4.13gm,0.1mole) dissolved in water (20ml) was added to this solution dropwise. It was stirred for 4 hrs. Ethanol was removed under reduced pressure to half the quantity and the reaction mixture was dumped on ice. The solid obtained filtered was oxime.

Step-II: Synthesis of 4-N,N-dimethyl amino benzonitrile:

Aldoxime (6gm,0.036mole) was dissolved in acetic anhydride (7.5gm,6.93ml) and refluxed for 4 hrs. under dry conditions. It was then cooled and poured on crushed ice. The solid was filtered and washed with cold water and recrystallized from methanol.

M.P.=212⁰-213⁰C

Lit.M.P.=215⁰-216⁰C²²⁰

Yield = 77.48%.

Synthesis of thioamides:

1] Thiobenzamide:

Benzonitrile (6gm, 0.0582mole) was dissolved in pyridine (30ml) in a three necked round bottom flask. Triethylamine (4ml) was added to it and placed in an ice bath. H₂S gas was bubbled through this solution continuously for 10 hrs. Then the reaction mixture was poured on ice bath and the solid was filtered. It was then recrystallised from benzene.

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

M.P. = 116⁰C

Lit. M.P. = 116⁰-118⁰C²²¹

Yield = 78%

2] 4-pyridyl thioamide:

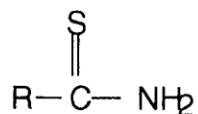
4-cyanopyridine (6gm, 0.0576mole) was dissolved in pyridine (20ml) and triethylamine (4ml) was added to it. It was then placed in an ice bath and H₂S gas was bubbled through the solution for 8 hrs. Then the reaction mixture was poured on ice and solid was collected. It was recrystallized from benzene.

M.P. = 202⁰-205⁰C

Lit. M.P. = 203⁰-205⁰C²²²

Yield = 73%.

Substituted Thioamides:



R = alkyl/aryl/substituted aryl etc

Sr.No	Substitution	M.P. °C (obtained)	M.P. °C (reported)	Yield %	Reference
1.	Methyl	112-114	113-115	74	221
2.	Phenyl	116	116	82	223
3.	Benzyl	98	97	89	223
4.	Amio methyl phenyl.HCl	222	222	74	-
5.	4-pyridyl	202-205	203-204	69	222
6.	2-Amino-5-nitro phenyl	164	165-166	77	224
7.	N,N-dimethyl amino phenyl	215-217	215-216	80	225
8.	4-Amino phenyl	185-186	182	83	226
9.	4-Chloro phenyl anilino	204-205	201-201.5	77	227

Synthesis of thiazoles:

1]2-benzyl-4[(6-chloro,3-substituted) cinnoline-4-one] thiazole.HBr:

3-bromoacetyl-6-chloro cinnoline-4-one (2gm,0.0066mole) and benzyl thioamide (1.49 gm,0.0099mole) were dissolved separately, each in 20ml of ethanol. Then both the solutions were mixed and refluxed for 20hrs. Alcohol was removed under reduced pressure. It was then recrystallized from methanol.

M.P.= 110°C

Yield = 70.66%

Elemental analysis:

	Calculated	Found
C	61.10 %	61.42 %
H	3.39 %	3.64 %
N	11.88 %	12.08 %

2]Synthesis of 2[(4-aminomethyl phenyl), 4(3-substituted,6-chloro cinnoline-4-one] thiazole HBr.

3-Bromoacetyl-6chloro cinnoline-4-one (1.5gm,0.0049mole) was dissolved in 15ml of benzene. 4-Aminomethyl benzothioamide (0.82gm,0.0099mole) was dissolved in 20ml of benzene. Both the reactants were mixed and refluxed for 22hrs. Benzene was removed and recrystallised from methanol.

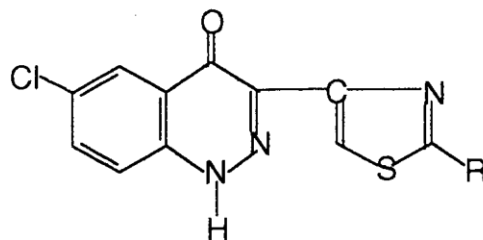
M.P.= 98°C

Yield = 68.29%

Elemental analysis:

	Calculated	Found
C	48.05 %	48.04 %
H	3.11 %	3.19 %
N	12.45 %	12.33 %

3-(2alkyl/aryl-4-substituted thiazolo)-6-chloro-cinnoline-4-ones:



Sr.No.	Substitution	Molecular formula	M.P. ^o C	Yield %	Elemental analysis% (obtd)			Elemental analysis%(Calc)		
					C	H	N	C	H	N
1.	amino	C ₁₀ H ₈ ClN ₄ OS.HBr	98	66	51.08	2.71	23.0	52.11	2.82	22.63
2.	methyl	C ₁₂ H ₈ ClN ₃ OS	111	74	38.08	2.29	11.28	39.11	2.41	11.61
3.	phenyl	C ₁₇ H ₁₀ ClN ₃ OS.HBr	115	79	59.0	2.7	13.0	59.18	2.94	12.57
4.	benzyl	C ₁₈ H ₁₂ ClN ₃ OS.HBr	110	85	61.42	3.64	12.08	61.1	3.39	11.88
5.	4-pyridyl	C ₁₆ H ₉ ClN ₄ OS.HBr	112	68	55.18	2.64	17.04	56.38	2.64	16.64
6.	Amino methyl phenyl	C ₁₈ H ₁₃ ClN ₄ OS.HBr	98	88	48.04	3.19	12.33	48.05	3.11	12.45
7.	Amino phenyl	C ₁₇ H ₁₀ ClN ₄ OS.HBr	116	82	56.07	3.14	15.94	57.34	3.10	15.79
8.	2-Amino-5-nitro phenyl	C ₁₆ H ₁₁ ClN ₅ OS.HBr	78	91	50.76	2.38	17.41	51.06	2.50	17.52
9.	N,N-dimethylamino phenyl	C ₁₉ H ₁₅ ClN ₄ OS.HBr	129	82	57.2	3.5	13.92	57.6	3.92	14.34
10.	4-Chloro phenyl anilino	C ₁₇ H ₁₀ Cl ₂ N ₄ OS.HBr	104	63	43.95	2.87	12.9	43.5	2.34	12.22

Spectral studies of 3[2(4-pyridyl), 4-substituted thiazolo]-6-chloro-cinnoline-4-one.(Compound no. 5)

IR (KBr) cm^{-1} 1676 cyclised ketone (C = O), 3234 -NH-, 1587pyridine, 1457, 1024 thiazole, 736 C-Cl.

Spectral studies of 3[2(4-amino phenyl), 4-substituted thiazolo]-6-chloro-cinnoline-4-one.(Compound no. 7)

IR (KBr) cm^{-1} 1681 (C = O), 3301-NH-, 1600, 1417, 1018 thiazole, 830 aromatic

PMR spectral analysis of compound no.7

PMR (CDCl_3) : δ 6.74 (s, 2H, NH₂)

δ 7.11 - 8.22 (s, H, NH)

(s, H, thiazole C₅ - H)

(thiazole, 7H, Ar - H).

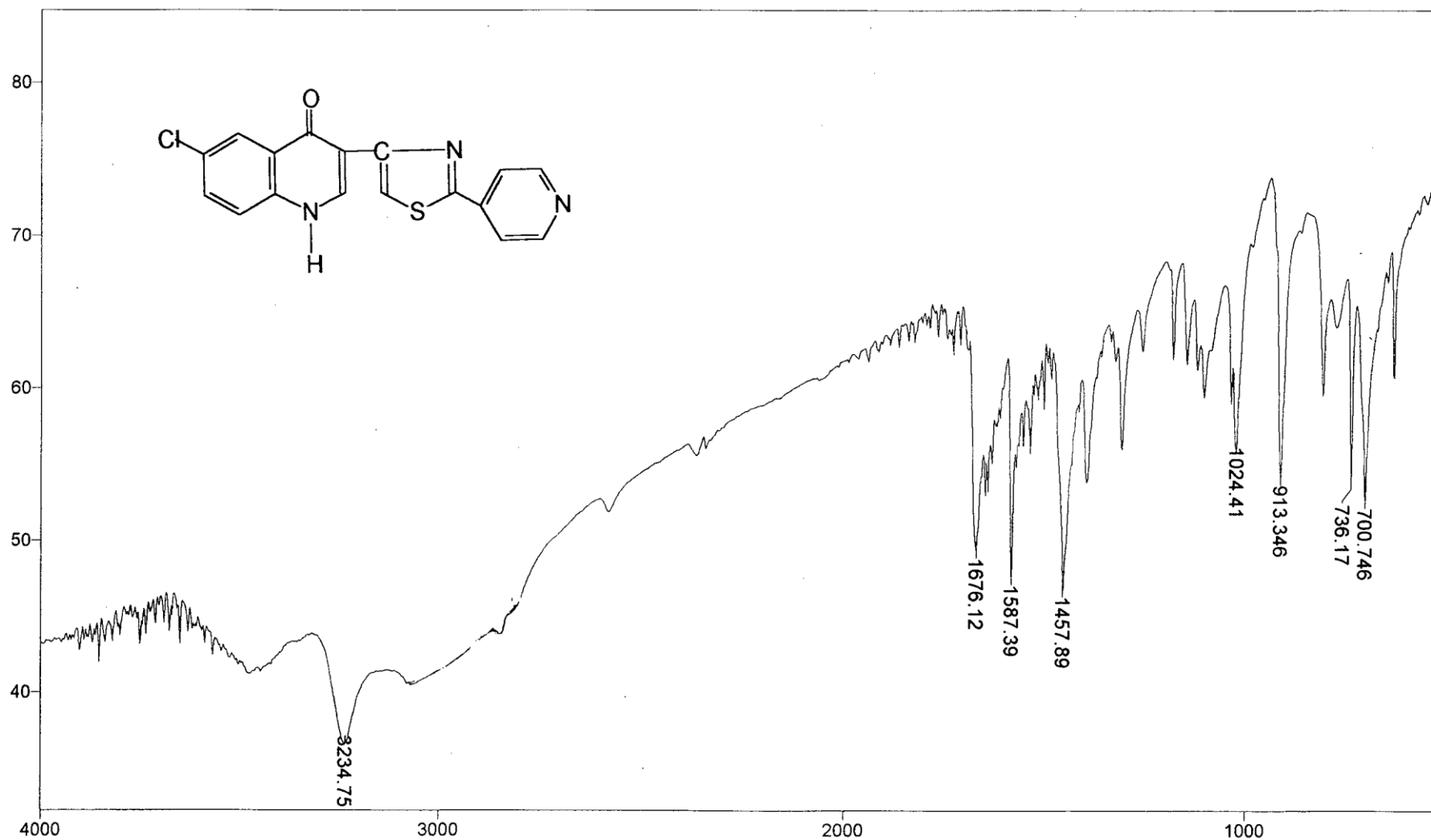
Sample Description: ptat

Scans= 10 Slow

Res=4 cm⁻¹

Apod= Cosine

Zero Filling= 1 x



File # 1 = C-AMP

Mode= 2 (Mid-IR)

1/4/80 11:08 AM

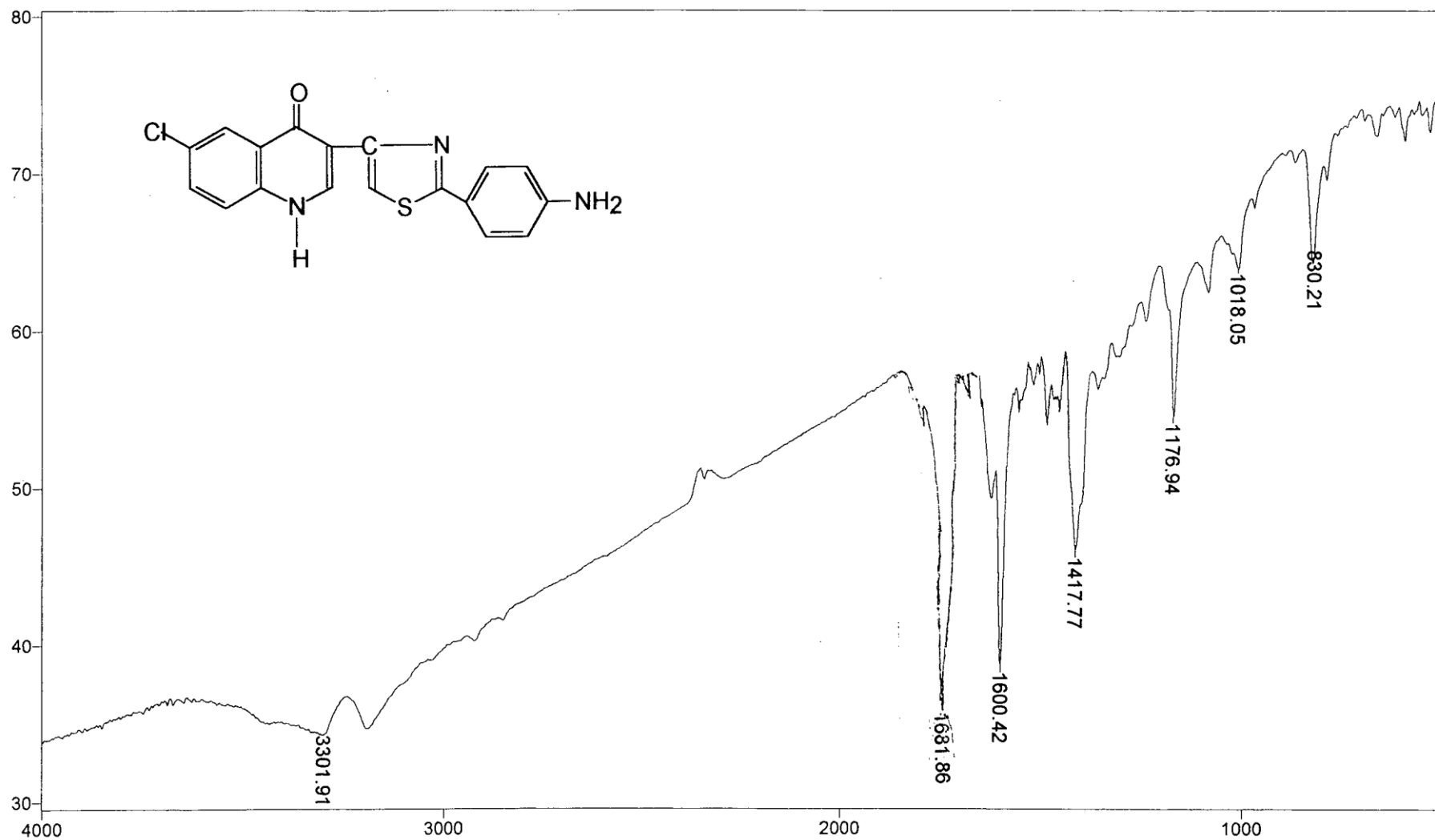
Sample Description: s-9

Scans= 10 Slow

Res=4 cm⁻¹

Apod= Cosine

Zero Filling= 1 x



Transmittance / Wavenumber (cm⁻¹)

OBSERVE

Pulse Width 10-0 sec
Acq. Time 2-00 sec
Spec. Width 9998-8 Hz
Nucleus H1

Freq 299-9486-MHz
Offset 1700-0 Hz
Delay 0 sec
Transients 400

DECOUPLE

Nucleus H1
Mode n
Power 20 dB
Offset 3145-8 Hz
Pulse Width 17-5 sec
Power Mode not-used

F101 PROCESSING

FN not-used sec CD
LB 0.100 not-used CCD
Width 4560.5 Hz/gain Scan 54.2 Hz/gain
Sensitivity not-used

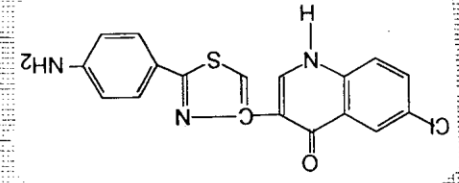
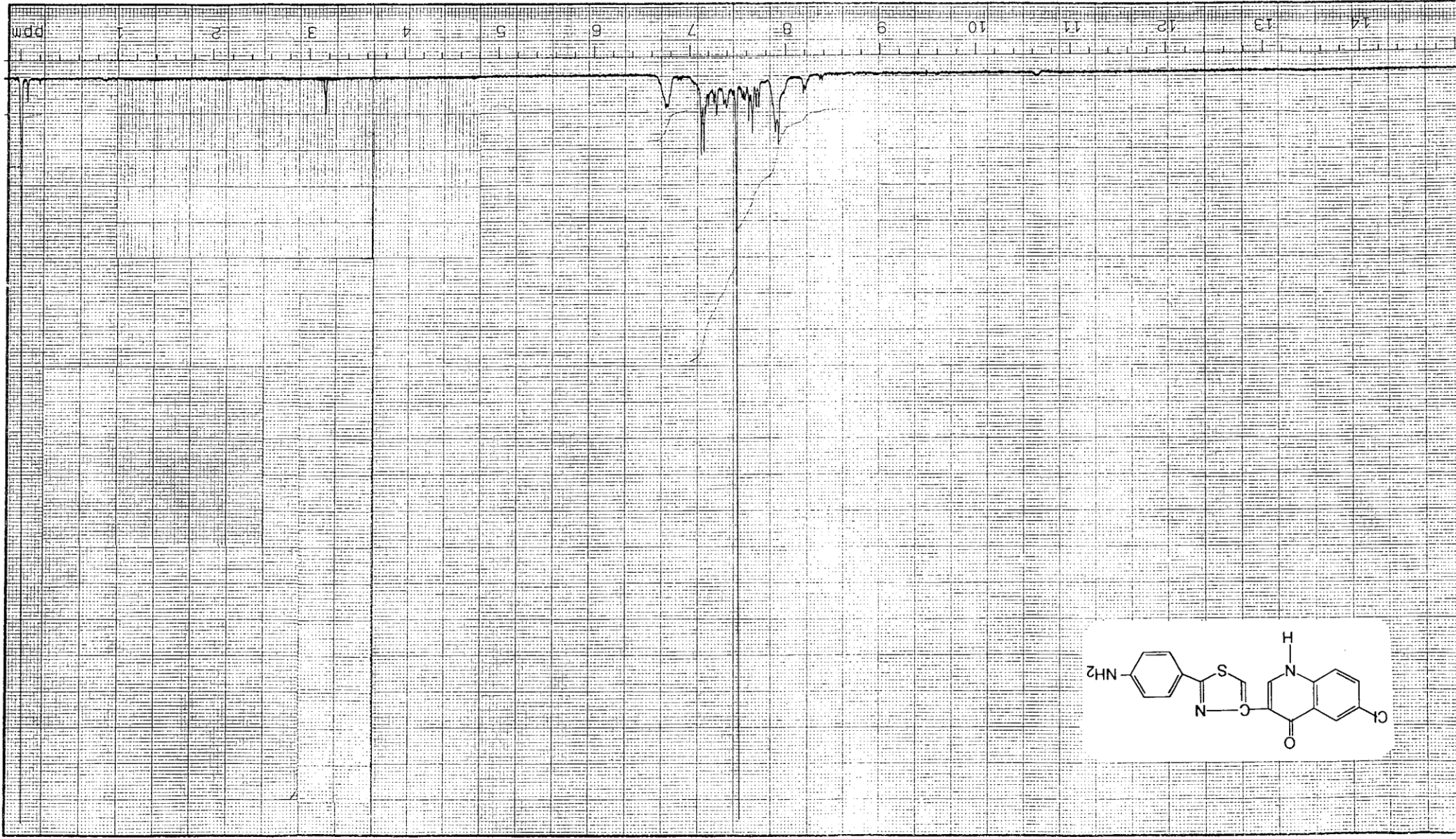
EXPERIMENT

Pulse Sequence 52pul
Tube OD mm
Temp not-used°C
Solvent CDCl3

SAMPLE

J-2
A 1010 J4 1476 10000

Number
File exp
Date Dec 30 96
VXR0 300



Part-77: Synthesis of 3(2-alkyl/aryl, 4-substituted thiazolo)-6-fluoro cinnoline-4-ones:

Part-II: Synthesis of 3(2-alkyl/aryl,4-substituted thiazolo)-6-fluoro cinnoline-4-ones:

1]Synthesis of ethyl-2-[4-fluorophenyl hydrazono)-3-oxybutyrate:

To 4-fluoro aniline (50gm,42.62ml,0.45mole), a mixture of conc.HCl (17ml) and water (17ml) was added. This was then kept in an ice bath and the temperature was maintained at 0° to -5°C. A cold aqueous solution of sodium nitrite (62.1gm,0.9mole) was added to form the diazonium salt. The salt was then filtered into a cooled solution of ethyl acetoacetate (58.56gm,57.41ml,0.45mole) in ethanol (60ml) and a large excess of sodium acetate was added. The solid was filtered and recrystallised from methanol.

M.P.= 170°C

Lit.M.P.=172°C

Yield =35.16gm,70.32%

Elemental analysis:

	Calculated	Found
C	57.14 %	57.48 %
H	3.15 %	3.48 %
N	11.11 %	10.76 %

2]Synthesis of 3-acetyl-6-fluoro cinnoline-4-one:

To ethyl-2 (4-fluorophenyl hydrazono)-3-oxybutyrate (32gm,0.1333mole) dissolved in monochlorobenzene (350ml) was added. AlCl₃ (35.59gm,0.266mole) was added to it and dissolved. The mixture was then refluxed for 14 hrs. It was cooled and dil.HCl (250ml) was added. The solid obtained was then filtered and washed with dil.NaOH soln. It was recrystallised from methanol.

M.P.= 158°C

Yield =20.24gm,63.28%

Elemental analysis:

	Calculated	Found
C	58.25 %	58.68 %
H	3.39 %	3.59 %
N	13.59 %	13.18 %

3] Synthesis of 3-bromoacetyl-6-fluoro cinnoline-4-one:

3-acetyl-6-fluoro cinnoline-4-one (20gm, 0.097mole) was dissolved in chloroform (300ml). Bromine (9.6gm, 0.12mole) was dissolved in CHCl_3 (40ml) was added to it at temperature 60°C . The mixture was then stirred in the presence of light for 10 hrs. Dry air was passed through the solution and then it was dumped on ice. The solid was filtered and recrystallised from methanol.

M.P. = 89°C

Yield = 15.2gm, 76.0%

Elemental analysis:

	Calculated	Found
C	42.10 %	42.45 %
H	2.10 %	2.42 %
N	9.82 %	9.58 %

Synthesis of thiazoles:

1] 2-phenyl-4-[(6-fluoro-3-substituted) cinnoline-4-one] thiazole:

3-bromoacetyl-6-fluoro cinnoline-4-one (2gm, 0.0071mole) and thiobenzamide (1.44 gm, 0.0105mole) were dissolved separately in alcohol (each in 20ml) and mixed. Then this mixture was refluxed for 19 hrs. Alcohol was removed under reduced pressure and the solid was recrystallised from methanol.

M.P.= 183⁰C

Yield = 83.39 %

Elemental analysis:

	Calculated	Found
C	50.49 %	50.40 %
H	2.72 %	2.71 %
N	10.39 %	9.96 %

2] Synthesis of 2(5-nitro-2-amino phenyl), 4-[(6-fluoro,3-substituted) cinnoline-4-one] thiazole:

3-bromoacetyl-6-fluoro cinnoline-4-one (2gm.0.0071mole) was dissolved in 20ml of ethanol. 5-nitro-2-amino phenyl thioamide (2.098 gm,0.0106 mole)was dissolved in 30ml of ethanol.

Both the reactants were mixed and refluxed for 20 hrs. Alcohol was then removed and the solid was recrystallised from methanol.

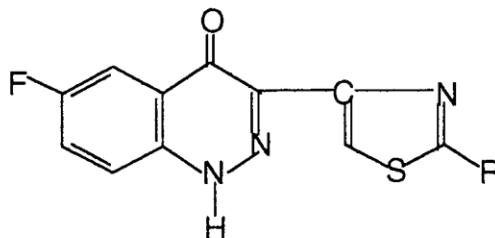
M.P.= 205⁰C

Yield = 78.78 %

Elemental analysis:

	Calculated	Found
C	43.99 %	44.04 %
H	2.37 %	2.42 %
N	15.08 %	15.35 %

3-2(alkyl/aryl,-4-substituted thiazolo)-6-fluoro-cinnoline-4-ones:



Sr.No.	Substitution	Molecular formula	M.P. ^o C	Yield %	Elemental analysis%(obtd)			Elemental analysis%(Calc)		
					C	H	N	C	H	N
1.	amino	C ₁₁ H ₇ FN ₄ OS.HBr	98	62	39.56	2.43	17.1	39.48	2.33	16.82
2.	methyl	C ₁₂ H ₈ FN ₃ OS	207-209	75	53.7	3.17	16.6	54.17	3.17	16.49
3.	phenyl	C ₁₇ H ₁₀ FN ₃ OS.HBr	183	81	50.40	2.71	9.96	50.49	2.71	10.39
4.	benzyl	C ₁₈ H ₁₂ FN ₃ OS.HBr	87	89	48.72	2.62	9.28	49.07	2.74	9.65
5.	4-pyridyl	C ₁₆ H ₉ FN ₄ OS.HBr	117	64	49.11	0.34	14.66	48.60	0.24	13.82
6.	Amino methyl phenyl	C ₁₈ H ₁₃ FN ₄ OS.HBr	90-93	64	50.72	3.34	12.68	49.88	3.23	12.93
7.	Amino phenyl	C ₁₇ H ₁₁ FN ₄ OS.HBr	133	80	60.95	3.29	17.06	60.35	3.25	16.56
8.	2-Amino-5-nitro phenyl	C ₁₇ H ₁₀ FN ₅ O ₃ S.HBr	205	77	44.04	2.42	15.35	43.99	2.37	15.08
9.	N,N-dimethylamino phenyl	C ₁₉ H ₁₅ FN ₄ OS.HBr	157-161	68	52.89	3.29	12.08	53.19	3.58	12.52
10.	4-Chloro phenyl anilino	C ₁₇ H ₁₀ ClFN ₄ OS.HBr	124-126	71	45.27	2.74	13.03	44.98	2.42	12.74

Spectral studies of 3[2(phenyl), 4-substituted thiazolo]-6-fluoro-cinnoline-4-one. (Compound no. 3)

IR (KBr) cm^{-1} 1685 cyclised ketone ($\text{C} = \text{O}$), 3229 -NH-, 1479, 1019 thiazole, 1361, 1323 C - F

Spectral studies of 3[2(2-nitro, 5-amino phenyl), 4-substituted thiazolo]-6-fluoro-cinnoline-4-one. (Compound no. 3).

IR (KBr) cm^{-1} 1650 cyclised ketone ($\text{C} = \text{O}$), 3240-NH-, 3334, primary amine - NH_2 , 1500, 1321 - NO_2 , 1606, 1569, 1024 thiazole, 1262, 1149 C - F.

PMR spectral analysis of compound no.3

PMR (CDCl_3) : δ 6.38 (s, 2H, - NH_2)

6.84 (s, H, - NH)

6.88 (s, H, thiazole C_5 -H)

δ 11-8.27 (thiazole, 6H, Ar -H).

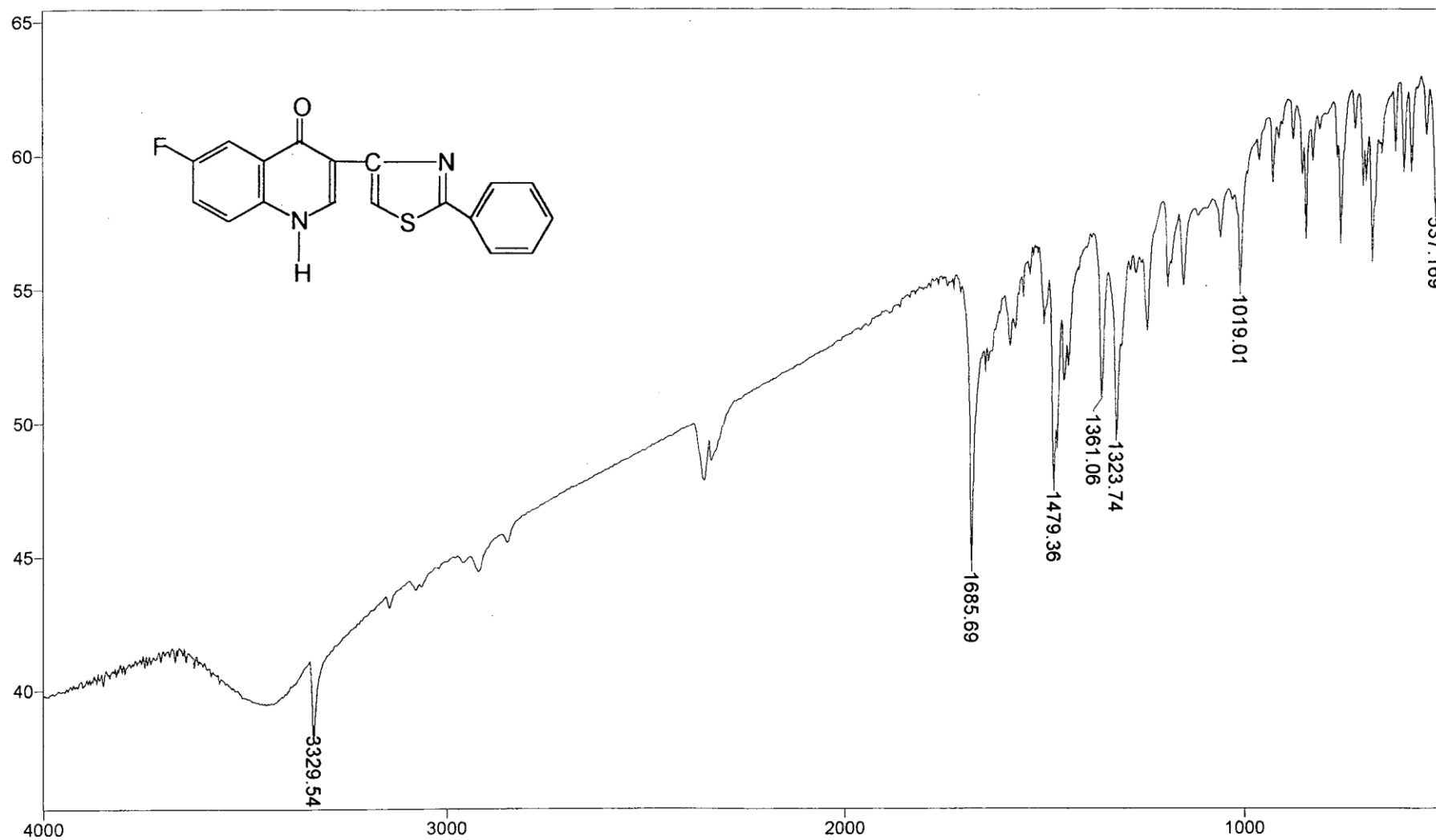
Sample Description: f-b

Scans= 10 Slow

Res=4 cm-1

Apod= Cosine

Zero Filling= 1 x



File # 1 = F-AN1

Mode= 2 (Mid-IR)

1/4/80 12:14 PM

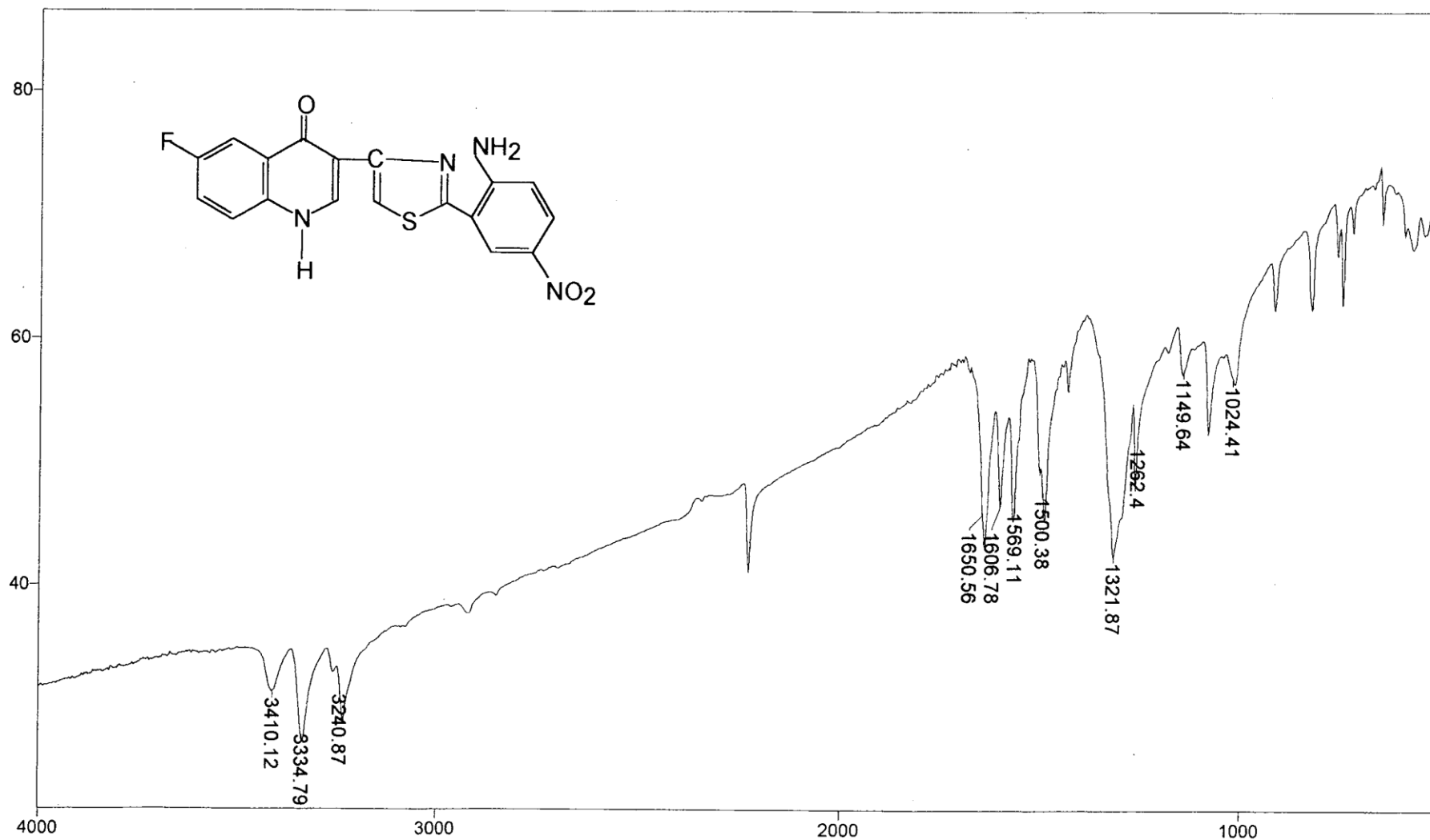
Sample Description: f-an1

Scans= 10 Slow

Res=4 cm-1

Apod= Cosine

Zero Filling= 1 x



*Part -999: Synthesis of 1,2,4-
triazolyl- methyl benzonitrile as an
intermediate for Letrozole:*

Part -III: Synthesis of 1,2,4-triazolyl-methyl benzonitrile as an intermediate for Letrozole:

Step-I: Synthesis of 1-formyl-3-thiosemicarbazide:

90% formic acid (25.97 ml, 0.689mole) was taken in a round bottom flask and heated for 15 min. To it was added thiosemicarbazide (12 gm,0.13 mole) and dissolved. It was heated for 30 min. and cooled. To this mixture 100 ml of boiling water was added. This milky solution was then filtered cooled on an ice bath for 2 hours and filtered to get 1-formyl-3-thiosemicarbazide.

M.P.=175⁰-177⁰C

Lit.M.P.=177⁰-178⁰C²¹⁴

Yield = 72.28%

Step-II: Synthesis of 1-H,1,2,4-triazole-3(5)-thiol:

A solution of 1-formyl-3-thiosemicarbazide (10gm,0.0952 mole) and sodium hydroxide (3.80 gm,0.0952 mole) in water (120ml) was heated for 1 hour. Then it was cooled on an ice bath for 30 min. Conc. HCl (50 ml) was added with stirring. Then the reaction mixture was cooled in an ice bath for 2hrs. and filtered to get crud 1-H,1,2,4-triazole-3(5)-thiol. It was then dissolved in boiling water, filtered and cooled on ice.

M.P.=222⁰-224⁰C

Lit. M.P.=220⁰-222⁰C²¹⁴

Yield =66%

Step-III: Synthesis of 1-H,1,2,4-triazole:

A mixture of water (11ml), conc. nitric acid (22 ml) and sodium nitrite (0.014 gm) was taken in a three necked flask and stirred at 45-50⁰C. To it a small portion of 1-H,1,2,4-triazole-3(5)-thiol was added. Brown fumes of NO₂ were observed which evolved during oxidation. 1H,1,2,4-triazole-3(5)-thiol (7gm,0.0693 mole) was added in small portions over 30 min. During the addition, the temperature was maintained between 45-50⁰C with occasional cooling in a water bath. Then the water bath was removed and the mixture was stirred for 2 hrs. until the reaction mixture comes to room temperature

Then it was neutralised with liquid ammonia and the solvent was removed under reduced pressure. Solid was extracted in ethyl acetate. The solvent was removed to get crude 1-H,1,2,4-triazole. It was then recrystallised from ethanol.

M.P.=121⁰-123⁰C

Lit.M.P.=119⁰-120⁰C²¹⁴

Yield =52.08%

Elemental analysis:

	Calculated	Found
C	34.78 %	34.46 %
H	4.34 %	4.61 %
N	60.86 %	60.76 %

Step-IV: Synthesis of 1,2,4-triazolyl methyl benzonitrile:

4-cyano benzyl bromide (6 gm,0.0306 mole) was dissolved in a mixture of chloroform (20ml) and acetonitrile (4ml). 1H-1,2,4-triazole (9.27 gm,0.1344 mole) was dissolved in a mixture of CHCl₃ (160 ml) and acetonitrile (32ml) and added to the 4-cyano benzyl bromide solution with stirring. Then it was refluxed for 12 hours and it was neutralized with 3% sodium bicarbonate solution. The organic content was then extracted in chloroform and the solvent was reduced to half the quantity. It was then passed through silica gel column in order to separate the product. The system used was CHCl₃-isopropanol (9:1). It was then recrystalliaed from methanol.

M.P.=77⁰-79⁰C

Lit.M.P.=77⁰-79⁰C²²⁸

Yield =57.58%

Elemental analysis:

	Calculated	Found
C	65.21 %	65.51 %
H	4.34 %	4.70 %
N	30.43 %	30.35 %

Spectral studies of 1H, 1,2,4- triazole.

IR (KBr) cm^{-1} 3120 N - H, 980 1,2,4 - triazole.

Spectral studies of 4-(1,2,4- triazolyl methyl) benzonitrile.

IR (KBr) cm^{-1} 3103 N - H, 961 1,2,4 - triazole, 2229 nitrile, 1010 1,4- disubstituted aromatic ring.

PMR spectral analysis of 4-(1,2,4- triazolyl methyl) benzonitrile.

PMR (CDCl_3) : d 5.42 (s, 2H, $-\text{CH}_2-$)

7.30 - 7.38 (δ , 2H, Ar-H / ortho to nitrile)

7.65 - 7.73 (δ , 2H, Ar-H / ortho to $-\text{CH}_2-$)

8.0 (s, H, triazole-H)

8.15 (s,H, triazole-H)

File # 1 = TRZ-S

Mode= 2 (Mid-IR)

10/8/99 10:43 AM

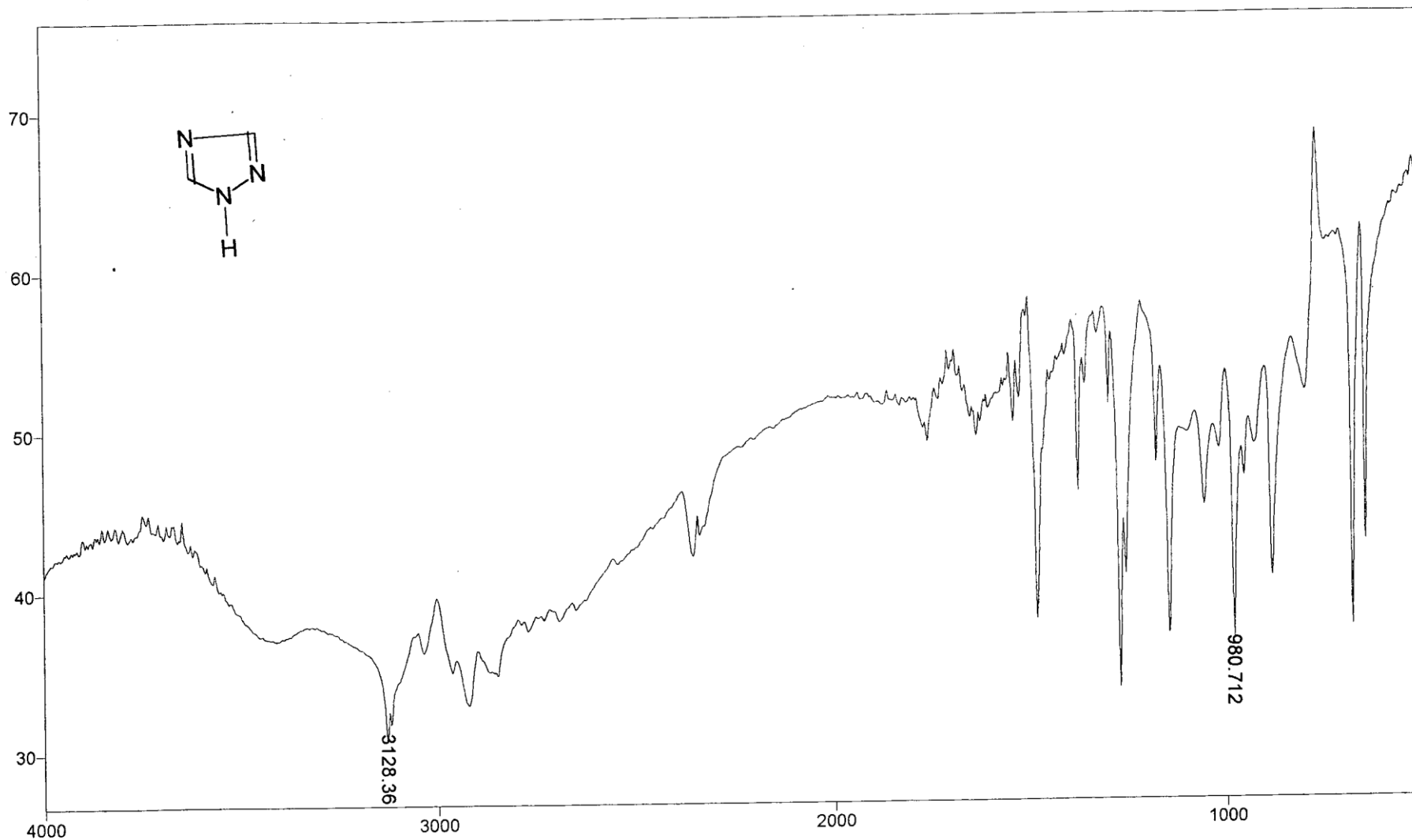
Sample Description: trz-s

Scans= 10 Slow

Res=4 cm⁻¹

Apod= Cosine

Zero Filling= 1 x



Transmittance / Wavenumber (cm⁻¹)

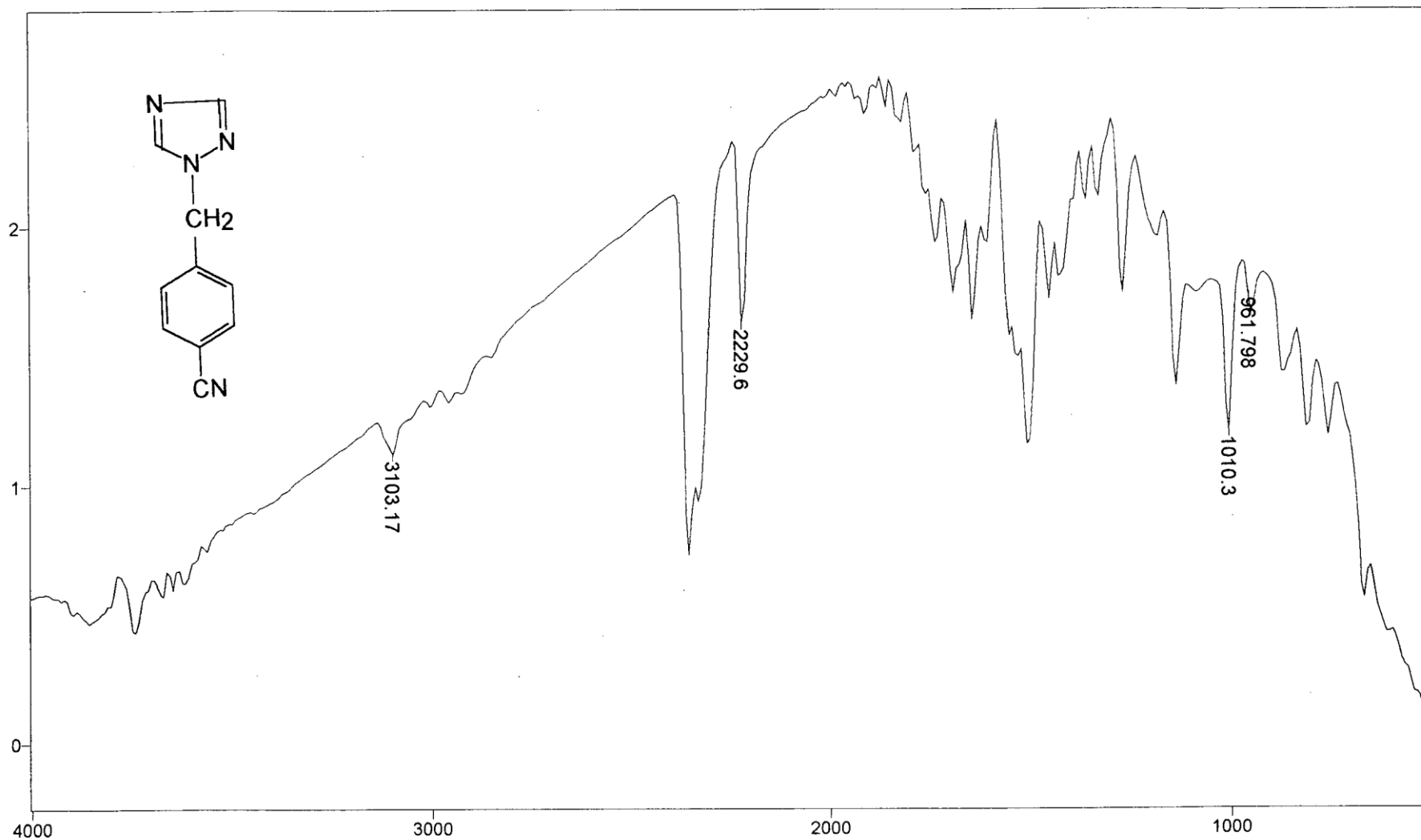
Sample Description: i-bn

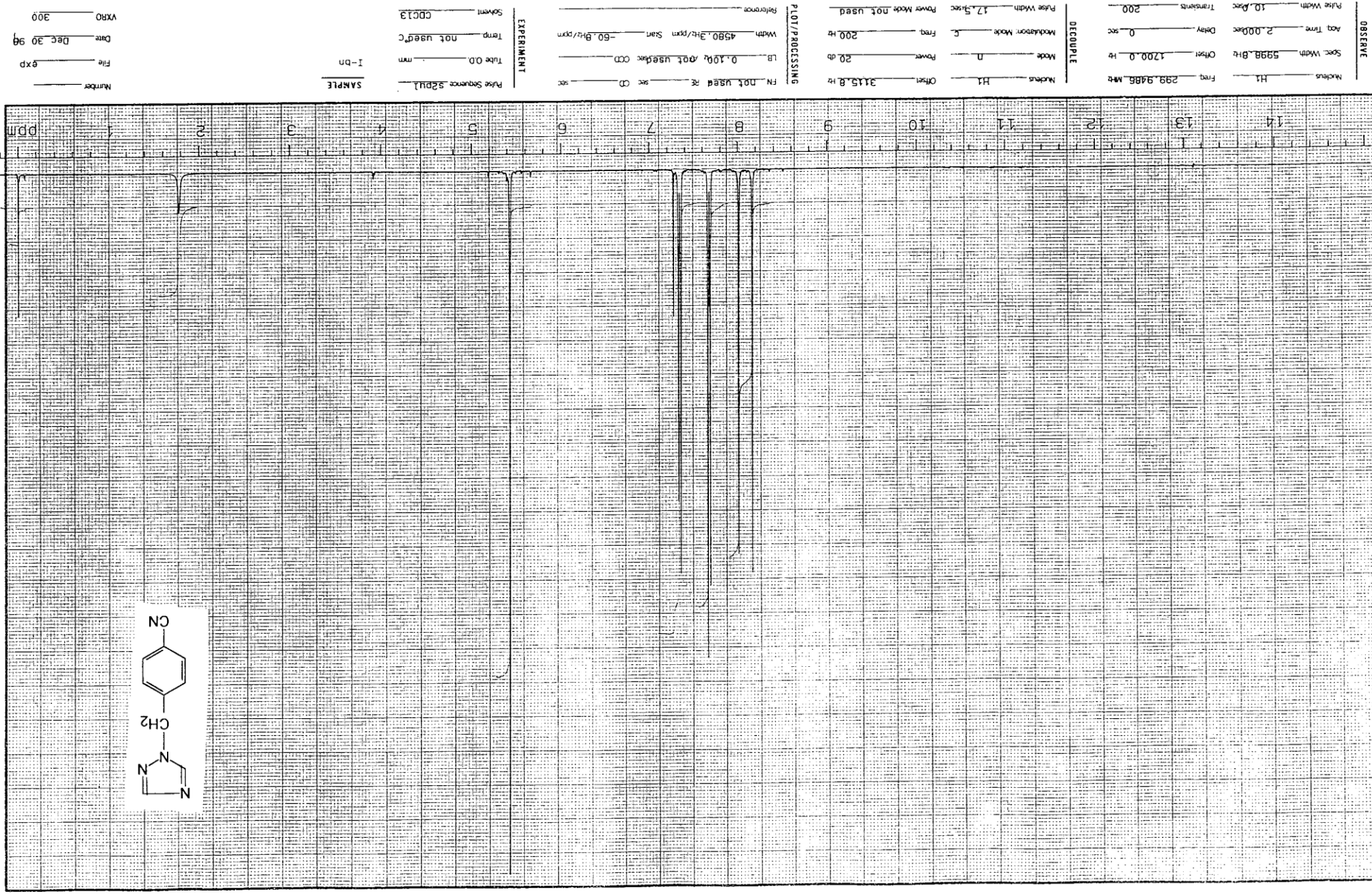
Scans= 10 Slow

Res=16 cm⁻¹

Apod= Cosine

Zero Filling= 1 x





Biological Screening and Conclusions

Biological Screening and Conclusions:

All the compounds synthesized in the present work were subjected to antimicrobial screening programs.

The various screening programs carried out include the *in vitro* study of against Gram positive *Staphylococcus aureus* ATCC 3750 and Gram negative *Salmonella typhi* NCTC 786 and *in vitro* study of activity against H₃₇ Rv strain of *Mycobacterium tuberculosis*. Also, *in vitro* study against fungal strains such as *Candida albicans*, *Trichophyton mentagrophytes* and *Trichophyton rubrum* was carried out.

Drug Stock Solution :

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

Controls:

1. Media Control: Sterilized medium was kept as a control so as to assure sterility of the medium. If this control shows growth, it shows that the medium is not properly sterilized and hence in that case results are not valid.
2. Culture Control: It contains sterilised 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 quality of solvent used to prepare dry stock solution. If the growth is not observed it can be concluded that the solvent inhibits the growth of organisms and the results are not valid.

4. Turbidity Control: It contains medium and 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 similar order of standard drug 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.

Meuller Hinton broth was used as a culture medium. Sterilized medium was dispensed in each borosilicate glass test tube (150+20mm). The drug solution was added in order to attain final drug concentrations as 200,100,40,20,10,5,2,1 µg/ml etc.

Innoculum of standard suspension, (0.1ml of the test organism strain which contains 10^6 bacilli/ml) was added. The tubes were incubated at 37°C for 48 hours and then examined for the presence or absence of growth of the organism. The lowest concentration which showed no visible growth was taken as an end point (MIC). Ampicillin (MIC=0.01µg/ml against gram positive *S. aureus*) and Trimethoprim (MIC=1µg/ml against gram negative *S. typhi*) were used as the standard drugs.

Antitubercular Screening:

The test compounds were subjected to *in vitro* screening by tube dilution method employing the human virulent H₃₇Rv strain of *Mycobacterium tuberculosis*.

Kirchner's medium containing Tween 80 as described by Rake *et.al.*²²⁸ was used.

The sterile medium was dispensed in each borosilicate test tube to attain final dilutions as 200,100,40,20,10,5,2,1,0.5µg/ml. Inoculum of standard suspension, i.e.0.1ml of H₃₇Rv strain of *M.tuberculosis* containing 10⁶ bacilli/ml, was added. Tubes were incubated at 37⁰C for ten days and then examined for the presence or absence of growth of the test organism. The lowest concentration which showed no visible growth was taken as the end point. Isoniazide (MIC=0.04µg/ml) was used as a standard.

Antifungal Screening:

The organisms employed for test were *Candida albicans*, *Trichophyton mentagrophytes* and *Trichophyton rubrum*.

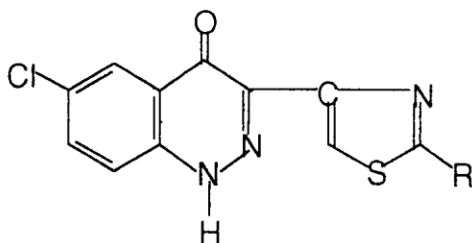
Serafataller's medium was employed for growth and the pH of the medium was adjusted to 5.2. The sterile medium was dispensed in each borosilicate test tube. The drug solution was added in order to get final drug concentrations of 200,100,40,20, 10, 5,2 etc. µg/ml etc.

The tubes were incubated at room temperature (28-30⁰C) in a dark place. *Candida albicans* was incubated for 7 days while *T.mentagrophytes* and *T.rubrum* were incubated for 14 days. The presence or absence of growth was observed visually. The lowest conc. which showed no visible growth was taken as an end point.

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

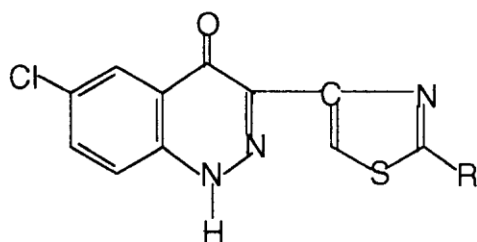
Results and conclusion:

Table I: 3(2-alkyl/ aryl, 4-substituted thiazolo)-6-chloro cinnoline-4-ones:



Sr.No	Substitution (R)	Antibacterial activity in $\mu\text{g/ml}$		
		H ₃₇ Rv strain of <i>M.tuberculosis</i>	<i>S.aureus</i>	<i>S.typhi</i>
1.	methyl	100	100	100
2.	phenyl	100	40	10
3.	benzyl	40	4	4
4.	Pyridyl	40	10	10
5.	Amino	100	100	10
6.	Amino phenyl	100	4	10
7.	Amino methhyl phenyl	40	4	100
8.	2-Amino-5-nitro phenyl	100	10	100
9.	N,N-dimethyl amino phenyl	40	100	4
10.	4-Chloro phenyl anilino	100	100	100

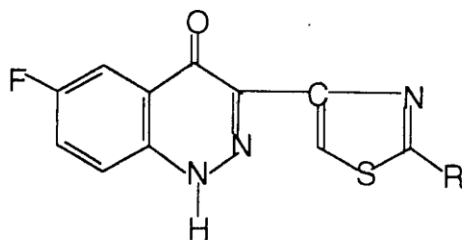
Table II: 3(2-alkyl/ aryl, 4-substituted thiazolo)-6-chloro-cinnoline-4-ones:



Sr.No	Substitution (R)	Antifungal activity in $\mu\text{g/ml}$.		
		<i>C.albicans</i>	<i>T.mentagrophytes</i>	<i>T.rubrum</i>
1.	methyl	N.A.	N.A.	N.A.
2.	phenyl	N.A.	100	N.A.
3.	benzyl	N.A.	100	200
4.	Pyridyl	200	100	N.A.
5.	Amino	N.A.	N.A.	40
6.	Amino phenyl	N.A.	40	100
7.	Amino methyl phenyl	20	40	40
8.	2-Amino-5-nitro phenyl	N.A.	N.A.	40
9.	N,N-dimethyl amino phenyl	100	40	200
10.	4-Chloro phenyl anilino	N.A.	40	100

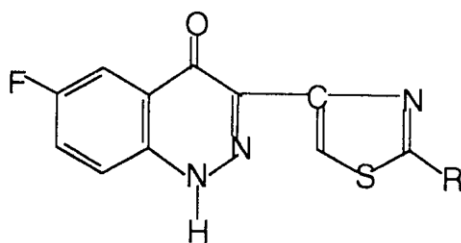
where N.A. = inactive at 200 $\mu\text{g/ml}$.

Table III: 3(2-alkyl/ aryl, 4-substituted thiazolo)-6-fluoro-cinnoline-4-ones:



Sr.No	Substitution (R)	Antibacterial activity in µg/ml		
		H ₃₇ Rv strain of <i>M.tuberculosis</i>	<i>S.aureus</i>	<i>S.typhi</i>
1.	methyl	100	100	100
2.	phenyl	100	100	100
3.	benzyl	100	10	10
4.	Pyridyl	100	10	100
5.	Amino	100	10	10
6.	Amino phenyl	100	10	10
7.	Amino methyl phenyl	40	100	100
8.	2-Amino-5-nitro phenyl	100	40	40
9.	N,N-dimethyl amino phenyl	40	100	100
10.	4-Chloro phenyl anilino	100	40	100

Table IV: 3(2-alkyl/ aryl, 4-substituted thiazolo)-6-fluoro-cinnoline-4-ones:



Sr.No	Substitution (R)	Antifungal activity in $\mu\text{g/ml}$.		
		<i>C.albicans</i>	<i>T.mentagrophytes</i>	<i>T.rubrum</i>
1.	methyl	N.A.	40	100
2.	phenyl	N.A.	40	40
3.	benzyl	N.A.	200	20
4.	Pyridyl	N.A.	N.A.	N.A.
5.	Amino	N.A.	40	100
6.	Amino phenyl	100	40	100
7.	Amino methyl phenyl	N.A.	40	N.A.
8.	2-Amino-5-nitro phenyl	N.A.	40	40
9.	N,N-dimethyl amino phenyl	N.A.	40	200
10.	4-Chloro phenyl anilino	N.A.	40	100

where N.A. = not active at 200 $\mu\text{g/ml}$.

Conclusion:

- 1) Out of all the ten substitutions made, 3(2-benzyl,4-substituted thiazolo)-6-chloro-cinnoline-4-one is found to be the most active against both the organisms, *Staphylococcus aureus* and *Salmonella typhi* i.e. 4 µg/ml. The activity was retained for the fluorinated compound and is 10 µg/ml against both *S.aureus* and *S.typhi* respectively.
- 2) Amino phenyl substituted compound have shown very good activity against *S.aureus* and *S.typhi* i.e. 4 µg/ml and 10 µg/ml respectively, which is retained for the fluorinated compounds as 10 µg/ml and 10 µg/ml respectively.
- 3) N,N-dimethyl amino phenyl and amino methyl phenyl substituted compounds have shown good activity i.e. upto 4 µg/ml against *S.aureus* and *S.typhi*. Corresponding fluorinated compounds have shown moderate activity.
- 4) Phenyl substituted compound is moderately active i.e. 40µg/ml and 10 µg/ml respectively against *S.aureus* and *S.typhi*. Fluorinated compound is equipotent.
- 5) 4-chloro anilino, methyl, 4-pyridyl substituted compounds have shown moderate activity upto 100µg/ml in both the series.
- 6) In case of 5-nitro-2-amino phenyl substitution, improved antibacterial activity upto 40 µg/ml is observed for fluorinated compound than 100µg/ml of chloro analogue.
- 7) Moderate antitubercular activity upto 40 µg/ml is observed for amino methyl phenyl, benzyl, N,N-dimethyl amino phenyl, phenyl and 4-pyridyl substituted compounds from both the series. It is concluded that cinnoline does not have any markable effect on antitubercular activity of thiazole.
- 8) Overall markable improvement in antifungal activity is observed in case of fluoro compounds. This may be because of the contribution of fluorine atom as many of the standard antifungal agents are contain fluorine atom which enhances the activity. e.g. fluconazole, flucytosine etc.
- 9) Against *Candida albicans* amino methyl phenyl compound is very active upto 20 µg/ml. Activity is moderate upto 100µg/ml and 200 µg/ml for different compounds. N,N-dimethyl amino phenyl compound from the chlorinated series and amino phenyl compound

from the fluorinated series are active upto 100 µg/ml. All the remaining compounds are active at 200 µg/ml.

10) In case of *Trichophyton mentagrophytes*, eight of the ten fluorinated compounds have shown good activity upto 40 µg/ml. Chloro compounds like N,N-dimethyl amino phenyl, amino methyl phenyl and amino phenyl are active upto 40 µg/ml and compounds with 4-pyridyl, phenyl, benzyl, 4-chloro anilino substitutions are moderately active upto 100 µg/ml.

11) Also, increase in activity for fluoro compounds is observed against *Trichophyton rubrum*. Compound with benzyl substitution is the most active with MIC of 20 µg/ml.

Amino, 5-nitro-2-amino phenyl substituted compounds have good activity upto 40 µg/ml. From the chlorinated series, 5-nitro-2-amino phenyl, amino methyl phenyl, amino substituted compounds are active upto 40 µg/ml. All the remaining compounds are active at 100 µg/ml and 200 µg/ml.

References

- 1) Silverman, Richard B; 'The Organic Chemistry of Drug Design and Drug Action'; 4 (1992).
- 2) 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).
- 3) Fuller, A.T.; *Lancet*; 1, 194 (1937).
- 4) Walsh, C.T.; *Annu. Rev. Biochem.*; 55, 493 (1984).
- 5) Greenwood, D.; 'Antimicrobial Chemotherapy'; 3rd edn., ed. by Greenwood, D., Oxford Univ. Press, Oxford, 50 (1995).
- 6) Robin, R.O. Jr., Williams, J.H., Winnek, P.S. and English, J.P.; *J. Am. Chem. Soc.*; 62, 2002 (1940).
- 7) Caldewell, W.T. and Kime, H.B.; *J. Am. Chem. Soc.*; 62, 2365 (1940).
- 8) Caldewell, W.T., Cornfeld, E.C. and Donnel C.K.; *J. Am. Chem. Soc.*; 63, 3028 (1941).
- 9) Sprague, J.M., Kissinger, L.W., Lincoln, R.M.; *J. Am. Chem. Soc.*; 63, 3038 (1941).
- 10) 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).
- 11) Whithy, L.H.E.; *Lancet*; 2, 1210 (1938).
- 12) Nicoles, R.L., Jones, W.H. Jr. and Finland, M.; *Proc. Soc. Exp. Bio. Med.*; 92, 637 (1956).
- 13) Lester, M.M. and English, J.P.; *U.S. Pat.*; 2, 790, 798, (1957) cited from C.A., 51, 15610 (1957).
- 14) Fullerton, D.S.; in 'Wilson and Giswold's Textbook of Organic Medicinal and Pharmaceutical Chemistry'; 9th edn, J.B. Lippincot Company, Philadelphia.
- 15) 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).
- 16) Herbert, V.; *J. Infect. Dis.*; 128, (Nov. suppl.), 432 (1973).

- 17) Reimer, W.A. and Bear, S.; in '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).
- 18) Martin, A.R.; in 'Wilson and Giswold's Textbook of Organic Medicinal and Pharmaceutical Chemistry'; 10th edn. Lippincot-Raven Publishers, Philadelphia, New York, p. 201.
- 19) 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).
- 20) Drake, G.D. and Haynes, K.J.; *U.S.Pat.*, 2759, 931 (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*, 271 (1997).
- 21) Greenwood, D.; *Antimicrobial Chemotherapy*; 3rd edn., d. by Greenwood, D., Oxford Univ. Press, Oxford, 57-58 (1995).
- 22) 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, p.230 (1991).
- 23) Waxman, 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, p.230 (1991).
- 24) 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.
- 25) Doyle, F.P., Hardy, K.D., Nayler, J.H.C., Soulal, M.J., Stove, E.R. and Waddington, H.R.J.; *J. Chem. Soc.*, 1453 (1962).
- 26) Yurchenco, J.A., Hopper, M.W. and Warren, G.H.; *Antimicrobial Chemotherapy*; 12, 534 (1962).
- 27) Nayler, J.H.C.; *Adv. Drug Res.*, 7, 52 (1973).
- 28) Doyle F.L., Long, A.A.W., Naylor, J.H.C. and Stove, E.R.; *Nature*; 192, 1183 (1961).

- 29) 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).
- 30) Bennet, J.V., Gravenkemper, C.F., Bordie, J.I. and Kirby, W.M.M.; *Antimicrob. Agents Chemother.*; 257, (1965).
- 31) Sutherland, R., Croydon, E.A.P. and Rolinson, G.N.; *Brit. Med. J.*; 4, 455 (1970).
- 32) Rolinson, G.N. and Stevens, S.; *Brit. Med. J.*; 2, 191 (1969).
- 33) Broden, R.N., Speight, T.M. and Avery, G.S.; *Drugs*; 9, 88 (1975).
- 34) Acred P., Brown, D.M., Kundson, E.T., Rolinson, G.N. and Sutherland, R.; *Nature*; 215, 25 (1967).
- 35) Morimoto, S. , Nomura, H., Fugono,T., Azuma , T.; Minami, I., Hori, M. and Masuad , T.; *J. Med. Chem* ; 15, 1108 (1972).
- 36) Stewart,G.W.; *The Penicillin Group of Drugs*, Amsterdam, Elsevier; (1965) cited from 'Wilson and Giswold's Textbook of Orgnic Medicinal and Pharmeceutical Chemistry', 9th edn. J.B. Lippincot and Company, Philadelphia.
- 37) Batchlor , F.R. *et.al.*; *Nature*; 206 , 362 (1962).
- 38) 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).
- 39) Ryan, C.W., Simpson, R. L., and Heyningen, E.M.; *J. Med. Chem.*; 12, 310 (1969).
- 40) Durkheimer, W., Adam, F., Fischer, G. And Kirrstetter, R.(B. Testa, Ed); *Advances in Drug Research*, Academic Press; 17, 61-234 (1988); 'Burger's Medicinal Chemistry and Drug Discovery', 5th edn., ed. by Wolf, M.E., A Wiley-Interscience Publication, John-Wiley & Sons., 4, *Therapeutic Agents*, 299 (1997).
- 41) Adams, H.G., Stilwell, G.A. and Turk, M.; *Antimicrob. Agents Chemother.*; 9,1019 (1976).
- 42) Brogden, R.N., Heel, R.C., Speight, T.M. and Avery, G.S.; *Drugs*; 17, 233 (1979).
- 43) Muytens, H.L., Vander Ros-van, J., Repe.; *Antimicrob. Agents Chemother.*; 21, 925 (1982).

- 44) Symposium; *Review of Infectious Diseases*; 4, Supplement 281-488 (1988) cited from 'Burger's Medicinal Chemistry and Drug Discovery', 5th edn., ed. by Wolf, M.E., A Wiley-Interscience Publication, John-Wiley & Sons., 4, *Therapeutic Agents*, (1997).
- 45) Umezwa H. *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, p.277 (1991).
- 46) Weinstein, M.J. *et. al.*; *J. Med. Chem.*; 6, 463 (1963).
- 47) 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, p. 279.
- 48) Wrihght, J.J.; *J. Chem. Soc; Chem. Commun*; 206 (1976).
- 49) Dyer, J.R., Todd, A.W.; *J. Am. Chem. Soc.*; 85, 3896 (1963).
- 50) Dyer, J.R. *et.al.*; *J. Am. Chem. Soc.*; 87, 654 (1965).
- 51) 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, p.279.
- 52) Haskell, J.H. *et.al.*; *J. Am. Chem. Soc.*; 81, 3482 (1974).
- 53) De Jongh, D.C. *et.al.*; *J. Am. Chem. Soc.*; 89, 3364 (1967).
- 54) Conover, L.H.; *U.S.Pat.*; 2, 693, 054, Jan. 11, 1955 cited from
- 55) Duggar, B.B.; *Ann. N.Y. Acad. Sci.*; 51, 177 (1948).
- 56) Mrtell, M.J. Jr., Booth, J.H.; *J.Med.Chem.*; 10, 44 (1967).
- 57) Finley, A.C. *et. al.*; *Science*; 111, 85 (1950).
- 58) Stephens, C.R. *et.al.* *J. Am. Chem. Soc.*; 80, 5324 (1958).
- 59) Blackwood, R.R. *et.al.*; *J. Am. Chem. Soc.*; 83, 2773 (1961).
- 60) Minuth, J.N.; *Antimicrob. Agents Chemother.*; 6, 411 (1964).
- 61) Woodward, R.B.; *Angrew. Chem.*; 69,50-58 (1957).
- 62) Mc Guire, J.M. *et.al.*; *Antimicrob. Agents Chemother.*; 2, 821 (1952).
- 63) Malmborg, A.S.; *J. Antimicrob. Chemother.*; 18, 293 (1986).
- 64) Wilhelm, J.M. *et.al.*; *Antimicrob. Agents Chemother.*; 236 (1967).

- 65) Gutman, L.T. *et.al.*; *Lancet*; 1, 464 (1967).
- 66) Barradell, L.B., Plosker, G.L. and Mc Tavison, D.; *Drugs*; 46, 289-313 (1993).
- 67) St. Georgiev, V.; *Int. J. Antimicrob. Agents*; 4, 247-270 (1994).
- 68) Manson, D.J. *et.al.*; *Antimicrob.-Agents Chemother.*; 544 (1962).
- 69) Magerlein, B.J. *et.al.*; *J. Med. Chem.*; 10, 355 (1967).
- 70) Erlich, J. *et. al.*; *Science*; 106, 417 (1963).
- 71) Controlis, J. *et.al.*; *J. Am. Chem. Soc.*; 71, 2463 (1949).
- 72) Emmerson, A.E.; David Greenwood's 'Antimicrobial Chemotherapy'; 3rd edn., ed. by Greenwood, D., Oxford Univ. Press, Oxford, 301 (1995).
- 73) Marwick, C.; *JAMA*; 257, 1174 (1992).
- 74) Daniel, T.M.; 'Tuberculosis' in 'Harison's Principles of Internal Medicine'; 12th edn. (Jean D. Wilson ed.) New York, Mc Graw-Hill (1991).
- 75) Meyer, H. And Mally, J.; *Monatsh eftr fiir chemie Monatsh Chem*; 33, 392 (1912)
cited from 'Burger's Medicinal Chemistry and Drug Discovery'; 5th edn., ed. by Wolf, M.E., A Wiley-Interscience Publication, John-Wiley & Sons., 4, *Therapeutic Agents*, p.596 (1997).
- 76) Lemke, Thomas L., Foye, W.O.; 'Principles of Medicinal Chemistry'; 4th edn., ed. By B.I. Waverly, New Delhi, 748 (1995).
- 77) Young, D.B.; *Carr. Biol.*; 4, 351 (1994) cited from 'Burger's Medicinal Chemistry and Drug Discovery'; 5th edn., ed. by Wolf, M.E., A Wiley-Interscience Publication, John-Wiley & Sons., 4, *Therapeutic Agents*, p.597 (1997).
- 78) Zang, Y., Heym, B., Allen, B., Young, D. And Cole, S.; *Nature*; 358, 591 (1992).
- 79) Sensi, P., Grassi, G.G.; in 'Burger's Medicinal Chemistry and Drug Discovery'; 5th edn., ed. by Wolf, M.E., A Wiley-Interscience Publication, John-Wiley & Sons., 4, *Therapeutic Agents*, p.610 (1997).
- 80) Sensi, P., Maggi, N., Furesz, S. And Maffii, G.; *Antimicrob. Agents Chemother.*; 1966, 699 (1967).
- 81) Forbes, M., Kuck, N.A. and Peets, E.A.; *J. Bacteriol.*; 89, 1099 (1962).
- 82) Forbes, M., Kuck, N.A. and Peets, E.A.; *J. Bacteriol.*; 89, 1299 (1965).

- 83) Reutgen, H. And Iwainsky, Z.; *Naturforsch*; 27b, 1405 (1972) cited from 'Burger's Medicinal Chemistry and Drug Discovery'; 5th edn., ed. by Wolf, M.E., A Wiley-Interscience Publication, John-Wiley & Sons., 4, *Therapeutic Agents*, p.602(1997).
- 84) Honore, N. and Cole, S.T.; *Antimicrob. Agents Chemother.*; 38, 238 (1994).
- 85) Emmerson, A.M.; in 'David Greenwood's Antimicrobial Chemotherapy'; 3rd edn. ed. by Greenwood, D., Oxford Univ. Press, Oxford, 306-307 (1995).
- 86) Lehmann, J.; *Lancet*; 1, 15 (1946).
- 87) Sensi, P and Grassi, G.G.; in 'Burger's Medicinal Chemistry and Drug Discovery'; 5th edn., ed. by Wolf, M.E., A Wiley-Interscience Publication, John-Wiley & Sons., 4, *Therapeutic Agents*, p.602(1997).
- 88) Plempel, M., *et.al.*; *Dtsch. Med. Wochenschr.*; 94 , 1356 (1969) cited from 'Wilson and Giswold's Textbook of Orgnic Medicinal and Pharmeceutical Chemistry'; 9th edn., J .B. Lippincot and Company, Philadelphia p. 150 (1991).
- 89) Godefroi, E. F. , *et.al.*; *J. Med. Chem.*; 12, 784 (1968).
- 90) Thomas , A.H. ; *J. Antimicrob. Chemother.*; 17, 269 (1986).
- 91) Gold, W., *et.al.*; *Antibiotics Annual*; (1955-56) p.576, New York Medical Encyclopedia (1956) cited from 'Wilson and Giswold's Textbook of Organic Medicinal and Pharmeceutical Chemistry'; 9th edn. J.B. Lippincot and Company, Philadelphia. p-151.
- 92) Hazen, E. L ., Brown, R.; *Pract. Soc. Exp. Biol. Med.*; 76, 93 (1951) cited from 'Wilson and Giswold's Textbook of Organic Medicinal and Pharmeceutical Chemistry', 9th edn. J.B. Lippincot and Company, Philadelphia. p-151.
- 93) Oxford, A. E. *Et.al.*; *Biochem. J.* ; 33, 240 (1939)
- 94) Grove, J. F. *et.al.*; *J. Chem. Soc.*; 3977 (1952)
- 95) Sloboda, R. D., *et.al.*; *Biochem Biophys Res Commune*; 107, 882_(1982)
- 96) Kruse,R., *et.al.*; *Pharmacology*; 43, 247 (1991) cited from Foye, W.E., Lemke, T.L.; ' Principles of Medicinal Chemistry'; 4th edn. by B.I. Waverly, New Delhi, 811 (1995).
- 97) Dorofienko, G.N.; Andreichikov and Turkloan, Get. Khim., Geterotki, Soedin, 1344, (1974) cited from Squi and Patai; 'The Chemistry of Diazonium and Diazo Group'; Part II, 655.
- 98) Willox, T.H.; *J. Am. Dyest. Rept.*; 55, 891 (1966).

- 99) Putter, R.; 'Methodender Organic Chemie'; Vol. X, Part III, 4th edn., Georg Thieme Verlag Stuttgart, Hauber-Weyl-Muller, p.16 (1965).
- 100) Schank, K.; *Methodicum Chemicum*; Vol. 6 (Edn. Zymalkowsky, F.) Academic press, New York, San Fransisco, London, p. 164 (1975) George Thieme Publishers Stuttgart, (1965).
- 101) Putter, R.; 'Methodender Organic Chemie'; Vol. X, Part III, 4th edn., Georg Thieme Verlag Stuttgart, Hauber-Weyl-Muller, p.22 (1965).
- 102) Putter, R.; 'Methodender Organic Chemie'; Vol. X, Part III, 4th edn., Georg Thieme Verlag Stuttgart, Hauber-Weyl-Muller, p.12 (1965).
- 103) Putter, R.; 'Methodender Organic Chemie'; Vol. X, Part III, 4th edn., Georg Thieme Verlag Stuttgart, Hauber-Weyl-Muller, p.22 (1965).
- 104) *Brit. Pat.*; 741, 228 cited from C.A.; 50, 9913 (1956), *Brit. Pat.*; 736, 568 cited from C.A.; 50, 13097 (1956).
- 105) Putter, R.; 'Methodender Organic Chemie'; Vol. X, Part III, 4th edn., Georg Thieme Verlag Stuttgart, Hauber-Weyl-Muller, p.28 (1965).
- 106) Coonna, M., Greci, L., Bruni, P.; *Gazz. Chim. Ital.*; 527 (1972).
- 107) Wislicenus and Jensen; *Ber.*; 25, 3448 (1892).
- 108) Rabischong; *Bull. Soc. Chim. France*; (3) 31, 76 (1904).
- 109) Rotheburg, Von; *Ber.*; 27, 685 (1894).
- 110) Parmeter, S.M.; *Org. Reactions*; 10, 1 (1959).
- 111) Enders, E. in 'Methodden der Organischen Chemie'; (Houben-Weyl), 4th edn., Vol. X/3, 467 (1965).
- 112) Hunig, S. And Boes, O.; *Ann. Chem.*; 57, 928 (1953).
- 113) Kharkharov, A.A.; *Zh. Obshch Khim.*; 23, 1175 (1955) cited from C.A. 42, 12396 (1953).
- 114) Porai, A.E., Koshits and Kharkharov, A.A.; *Bull. Acad. Sci.; URSS*, 79 (1944) cited from C.A. 39, 1631 (1945).
- 115) Philips, R.R.; *Org. Reactions*; 10, 143 (1959).
- 116) Bulow and Hailer, E.; *Ber.*; 35, 915 (1902).
- 117) 'Organic Reactions'; Vol. 2, John Wiley and Sons, New York, 130 (1946).

- 118) 'Organic Reactions'; Vol. 2, John Wiley and Sons, New York, 132(1946).
- 119) 'Organic Reactions'; Vol. 2, John Wiley and Sons, New York, 136-144 (1946).
- 120) 'Vogel's Textbook of Practical Organic Chemistry'; 4th edn., 771 (1978).
- 121) Fieser and Hershberg; *J.Am. Chem. Soc.*; 61, 1272 (1939)
- 122) Reimer, *Ber.*, 14, 1802 (1881) and Roser; *Ann.*; 247, 152 (1882) cited from 'Organic Reactions'; Vol. 2, John Wiley and Sons, New York, 162-169 (1946).
- 123) Richter; *Ber.*; 16, 677 (1883) cited from Simpson, J.C.E.; 'Chemistry of Heterocyclic compounds'; Interscience Publishers, INC, New York, 5, 46 (1953).
- 124) Borsche and Herbert; *Ann.*; 546, 293 (1941) cited from Simpson, J.C.E.; 'Chemistry of Heterocyclic compounds'; Interscience Publishers, INC, New York, 5, 47 (1953).
- 125) Widman; *Ber.*; 17, 722 (1884) cited from Simpson, J.C.E.; 'Chemistry of Heterocyclic compounds'; Interscience Publishers, INC, New York, 5, 26 (1953).
- 126) Schofield and Simpson; *J. Chem. Soc.*; 1945, 512.
- 127) Gewald, K., Calderon, O., Schaffer, H. And Hein, V.; *Justus Liebigs Ann. Chem.*; 7,
- 128) (1984) cited from C.A.; 101, 130651 (1984).
- 129) Lewis, R.N. and Susim, P.V.; *J.Am. Chem. Soc.*; 4, 840 (1952).
- 130) Corpin, H.B.; (1956) *U.S.Pat.*; 2, 715, 137 (1955) cited from C.A.; 50, 7160 (1956).
- 131) Rogees, A.O.; *U.S.Pat.*; 2, 415, 267 (1947) cited from C.A.; 41, 3119 (1941).
- 132) Newman, M.S. and Boden, H.; *J.Org. Chem.*; 26, 2525 (1963).
- 133) Rorig, K. *et al.*; *Org. Synthesis*; Coll. Vol. 4, 575 (1963).
- 134) McElvein, S.M. and Goese, M.A.; *J.Am. Chem. Soc.*; 63, 2283 (1941).
- 135) Gilman, H. and Spatz, S.M.; *J.Am. Chem. Soc.*; 63, 1553 (1941).
- 136) Kent, R.E. and McElvein, S.M.; *Org. Synthesis*; Coll. Vol. 3, 493 (1955)
- 137) Corsow, B.B. *et. al.*; *Org. Synthesis*; Coll. Vol. 2, 379 (1943).
- 138) Brown, P.M. *et. al.*; *J. Chem. Soc.*; 2882 (1957).
- 139) Stephens, C.R. *et.al.*; *J.Am. Chem. Soc.*; 77, 1701 (1955).
- 140) Surrey, A.R.; *Org. Synthesis*; Coll. Vol. 3, 535 (1955).
- 141) Herbst, R.M. and Wilson, K.R.; *J.Org. Chem.*; 22, 1142 (1957).
- 142) Campagna, F., Carotti, A. And Casini, G.; *Tetrahedron Letters*; 21, 1813-16 (1977).

- 143) Haganest, H. And Stauf, F.W.; *U.S.Pat.*; 1, 879, 209 (1932) cited from C.A.; 28, 4848 (1934).
- 144) Carke, H.T. and Read, R.R.; *Org. Synthesis*; Coll. Vol. 1, 514 (1941).
- 145) Vohwinkel, E. and Bartel; *J. Chem. Soc.*; 107, 1221 (1974).
- 146) Farey, H.G. and Dalton, D.R.; *J. Chem. Soc. Chem. Commun.*; 628 (1973).
- 147) Kukhar, V.P. and Pasternak, V.I.; *Syntheis*; 563 (1974).
- 148) Streith, J. and Fizet, C.; *Helv. Chim. Acta.*; 59, 2788 (1976).
- 149) Miller, M.J. and Zoudon, G.M.; *J.Org. Chem.*; 40, 126 (1975).
- 150) Glass, R.S. and Hoy, C.; *Tetrahydron Letters*; 20, 1781 (1976).
- 151) Houben-Weyl 'Methoden der Organischen Chemie'; 4th edn., vol.3, 325 (1952).
- 152) Kitagawa, T. *et.al.*; *Chem. Phar. Bull.*; 38, 2583 (1990).
- 153) Carpino, L.A.; *Accounts Chem Res.*; 6, 191 (1973).
- 154) Carpino, L.A. Giza, C.A. and Carpino, B.A.; *J.Am. Chem. Soc.*; 81, 955 (1959).
- 155) Sheradsky, A.; *J.Het. Chem.*; 4, 413 (1967).
- 156) Tamare, Y., Minamikawa, I., Kita, Y., Kim, N.H. and Ikeda, M.; *Tetrahydron Letters*; 29, 1063 (1973).
- 157) Tamare Y. *et.al.*; *J.Org. Chem.*; 38, 1239 (1973).
- 158) Pomeroy, J.H. and Creig, C.A.; *J.Am. Chem. Soc.*; 81, 6340 (1959).
- 159) Volchel, C., Liebigs; *Ann. Chem.*; 38, 314 (1841).
- 160) Roumanian Ministry for Petro and Chem. Industry; *Brit. Pat.*; 980, 997 (1965) cited from C.A.; 63, 13221f (1965).
- 161) Fuches, O., Senkaiak, V., Nemes, A., Lazar, A. and Somgyl, T.; *Austrelian Pat.*; 241461 (1965).
- 162) Kindler, K.; Liebigs *Ann. Chem.*; 431, 187 (1923).
- 163) Singer, F.A., Schafer, W. And Chrwitte, E.; *Agrew. Chem.*; 76, 273 (1964).
- 164) Fairful, A.E.S., Lowe, J.L. and Peak, D.A.; *J. Chem. Soc.*; 742 (1952).
- 165) Cairns, T.L., Larchar, A.W., McKusicj, B.C.; *J. Org. Chem.*; 18, 748 (1953).
- 166) Von Esch, A.M. and Shermann, H.R.; *U.S.Pat.*; 3158623 (1964) cited from C.A.; 62, 4008 (1965).
- 167) Hanford, W.E.; *U.S.Pat.*; 2201170 (1940) cited from C.A.; 34, 6388² (1946).

- 168) Taylor, E.G. and Zaltewicz, J.A.; *J.Am. Chem. Soc.*; 182, 2656 (1960).
- 169) Albert, A.; *Ber. Dtsch. Chem. Ger.*; 48, 470 (1915).
- 170) Olin, J.F. and Johnson, T.B.; *Recueil. Trav. Chim. Pay. Bas.*; 50, 72 (1931).
- 171) Goerdeler, J. and Mitter, W.; *Chem. Ber.*; 96, 944 (1963).
- 172) Mayer, r., Hiller, G., Nitzchke, M. And Jentz, J.; *Agrew. Chem.*; 75, 1011 (1963).
- 173) Casser, L., Panossian, S. And Giordana, C.; *Synthesis*; 917 (1978).
- 174) Hofmann, H.W.; *Ber. Dtsch. Chem. Ger.*; 11, 340 (1878).
- 175) Zaiden, Hogin, Seisan, Kaihatsu, Kagku, Kenyusho; *Jap. Pat.*; 9002/65 (1965).
- 176) Cerecedo, L.R. and Tolpin, J.G.; *J.Am. Chem. Soc.*; 59, 1660 (1937).
- 177) Schmitz, W.R.; *U.S.Pat.*; 2682558 (1954) cited from C.A.; 49, 9029c (1955).
- 178) Westphal, K. and Andersag, H.; *Canad. Pat.*; 392513 (1940) cited from C.A.; 35, 1413⁵ (1940).
- 179) Behringer, H. Ruff, M. And Wiedmann, R.; *Chem. Ber.*; 97, 1732 (1964).
- 180) Pesina, A.G.; *Zh. Obshch. Khim.*; 9, 804 (1939).
- 181) Kingsberg, E. and Papa, D.; *J.Am. Chem. Soc.*; 73, 4988 (1951).
- 182) Wagner, G. and Singer, D.; *Z.Chem.*; 2, 306 (1962).
- 183) Willstaller, R. and Wirth, T.; *Ber. Dtsch. Chem. Ger.*; 42, 1911 (1909).
- 184) Kikuchi, K.; *Jap. Pat.*; 1710 (1951) cited from C.A.; 47, 4908 (1953).
- 185) Jancevska, M., Jacopicin, K. And Hahu, V.; *Croat. Chem. Acta.*; 37, 67, (1965).
- 186) Sachs, F. and Loevy, H.; *Ber. Dtsch. Chem. Ger.*; 36, 585 (1976).
- 187) Eleftherious, Papadopoulos, P.; *J.Org.Chem.*; 41(6), 962 (1976).
- 188) Sastry.S. and Kudav, V.A.; *Ind. J.Chem.*; 18B(5), 455 (1971).
- 189) Hantzch, A.R. and Weber, H.J.; *Ber.*; 20, 3118 (1887).
- 190) Jones, E.R.H., Robinson, F.A. and Strachan, M.N.; *J. Chem Soc.*; 87 (1946).
- 191) Vernim, G. and Metzger, J.; *Bull. Soc. Chim. France*; 2498 (1963).
- 192) Sarkis, G.V. and Agawe, S.A.; *J. Chem. Eng. Data*; 18, 99 (1973).
- 193) Erylenmeyer, H., Erne, M.; *Helv. Chem. Acta.*; 31, 652 (1948).
- 194) Hantzch, A.R. and Weber, H.J.; *Ber.*; 21, 938 (1888).
- 195) Vogel, A.I.; 'Element Pract. Org. Chem.'; 4th edn., 848 (1984).
- 196) Mathes, R.A., Gregory, J.T.; *J.Am. Chem. Soc.*; 74, 3867 (1952).

- 197) Cook, A.H., Downer, J.D. and Heilbron, I.; *J. Am. Chem. Soc.*; 1202 (1948).
- 198) Ochiai, E. and Nagasawa, F.; *J. Pharm. Soc. Japan*; 59, 43 (1939).
- 199) Dahiya, R. And Pujari, H.K.; *Ind. J. Chem.*; 25B, 966 (1986).
- 200) 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).
- 201) *U.S. Pat.*; 2, 652, 396.
- 202) Khanna, M.S., Chandra, P., Garg and Kapoor, R.P.; *Ind. J. Chem.*; 32B, 364 (1993).
- 203) Simpson, J.C.E.; 'Chemistry of Heterocyclic Compounds'; Interscience Publishers, INC, New York, 5, 5 (1953).
- 204) Fuscho, R., Piselli, F.L. and Boshi, P.M.; *Eur. Pat. Appl.* EP 287 (1988) cited from C. A.; 110, 75540 (1989).
- 205) Abbady, M.S., Radhwan, Sh. M., Bhakite, E.A.; *Ind. J. Chem.*; 32B, 1281-1284 (1993).
- 206) Nargund, L.V.G., Nargund, K.S.; *Arzneimittel Forchung Drug Research*; 1284 (1993).
- 207) Gopikumar Menon, C.R., Purushotthaman, E.; *J. Ind. Chem. Soc.*; 72, 713-734 (1995).
- 208) Gardener, T.S., Wenis, E., Lee, J.; *J. Org. Chem.*; 20, 976-980 (1955).
- 209) Carp. Eugenis and Toma, A.; C.A.; 63, 14483d (1965).
- 210) Choudhari, D.T., Sabnis, S.S., Patel, M.R. Deliwala, C.V.; *Bull. Haff. Inst.*; 4(1), 8-15(Eng.) (1976).
- 211) Ciba Geigy; Chugai; *Drugs Fut.*; 21(4), 434 (1996).
- 212) Ledniser, D.; 'Strategies in Drug Synthesis'; 6, 86.
- 213) Bowman, R.M., Steele, R.E., Browne, L. (Ciba Geigy Corp.) U.S. US 4,978, 672 (Cl. 514-383; A 61K31/ 41); (1990), C.A.; 114, 247887y and 247288 (1991).
- 214) *Drugs Fut.*; 23(10), 1071-1074 (1998).
- 215) 'Organic Synthesis'; John Wiely and Sons., 40, 99-102 (1965).
- 216) Strain, H.H.; *J. Am. Chem. Soc.*; 49, 1995 (1927).
- 217) Ainsworth, C. And Jones, R.G.; *J. Am. Chem. Soc.*; 77, 621 (1955).
- 218) Grudman, C. And Ratz, R.; *J. Org. Chem.*; 21, 1037 (1956).

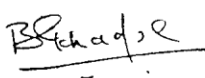
- 219) Hoggarth, E.; *J. Chem. Soc.*; 1949, 1163.
- 220) Blatter, Kukasazewski, Stevens; *J. Am. Chem. Soc.*; 83, 2203 (1961).
- 221) Hafmann, H.W.; *Ber. Dtsch. Chem. Ges.*; 11, 340 (1878).
- 222) John, Lee; U.S. Pat.; 2, 653, 942 (1953), C.A.; 48, 11500d (1954).
- 223) Fairfull, A.E.S., Lowe, J.L. and Peak, D.A.; *J. Chem. Soc.*; 1952, 742.
- 224) Martiz, K.L., Taube, Carl (Farbentadriken Bayer A.G.) Brit. Pat.; C.A.; 71, 14164j (1969).
- 225) Prabhu, S.R.; 'Chemotherapy of Tuberculosis', Ph. D. Thesis, Haff. Inst., 113 (1982).
- 226) Kokimoto S., Seydel, J. And Wempe, E.; (Hokkaido Univ.; Sapporo, Japan) *Arzneimittel Forsch*; 12, 127-33 (1962). C.A.; 57, 9813a (1962).
- 227) Cited from C.A., 64, 8061 (1966).
- 228) Bowman, R.M., Steele, R.E., Browne, L. (Ciba Geigy Corp.) *U.S. Pat.*; US 4,978, 672 (Cl. 514-383; A 61K31/ 41); (1990).
- 229) Rake, G., Jambor, W.P., McKee, C.M., Pansy, F., Wiselodge, E.Y., Donobic, R.; *Am. Rev. Tubercle.*; 60, 121 (1949).

:APPENDIX:

1. The melting points have been determined in Open capillaries in a veego VMP-1 melting point apparatus.
2. The TLC of the compounds have been run of on Eastman Kodak precoated silica gel plates of type K301 R and E.Merck precoated silica gel plates of type 60F 254.
3. The Infrared spectras were recorded on a BOMEN MB-104 Fourier Transmission Infra Spectrometer.
4. The PMR spectras were recorded on Varian 300MHz Spectrometer using TMS as internal reference at RSIC, IIT, Powai.

Synopsis

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

TITLE OF THE THESIS	Synthesis of organic compounds having of <u>juva</u> possible therapeutic values and preparation of a drug intermediate.
NAME OF THE CANDIDATE	Ms. Sharvani Kusumakar Vingkar.
NAME AND DESIGNATION OF THE GUIDE	Dr.B.G.Khadse Director and Head of Dept. of Chemotherapy , Haffkine Institute, Parel, Mumbai -12.
PLACE OF RESEARCH	Department of Chemotherapy, Haffkine Institute, Parel, Mumbai -12.
NUMBER AND DATE OF REGISTRATION	33 / Dec. 9, 1997
SIGNATURE OF THE STUDENT:	
SIGNATURE OF THE GUIDE	

:SYNOPSIS:

Among the several characteristics unique to *Homo sapiens* is our propensity to treat ailments, physical and mental, with medicines. From archaeological evidence, this urge to sooth the burdens of disease is as old as humanity's search for other tools. Drugs have been used to gain increased control over our lives and to make them longer, healthier and happier.

In the 19th century, the developments in synthetic and analytical chemistry led to the isolation and structural elucidation of medicinally active compounds such as, alkaloids, flavones, steroids etc. from natural origin. Today, useful drugs arise from natural products, fermentation broth, chemical synthesis or a combination of both the sources using rational/ empirical approaches. However, there is a constant need to develop better drugs with higher efficacy and lower toxicity than those currently in use. A successful chemotherapeutic agent should have following properties.

- 1) It must exert an antimicrobial action at high dilution and in the complex chemical environment of the body.
- 2) It must assure higher safety to the body tissues and yet be highly efficacious.
- 3) It should overcome the drug resistance of various invading microorganisms.

A medicinal chemist deals with the incorporation of newly emerged pharmacophores on an established biologically active moiety. One such genuine effort has been made and will be documented in the thesis.

The thesis will consist of the two parts- Part I and Part II and will be divided into five chapters.

PART-I

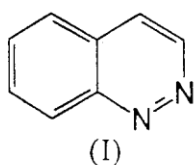
- i) Introduction, Review of Literature and Plan of Work.

PART-II

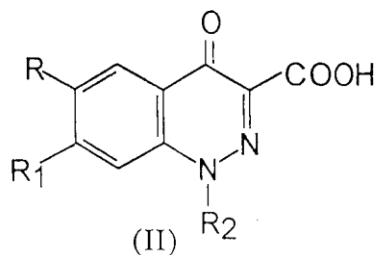
- ii) Synthesis of 1H-3(2-alkyl\aryl,4-substituted thiazolo)-6-chloro-cinnoline-4-ones.
- iii) Synthesis of 1H-3(2-alkyl\aryl,4-substituted thiazolo)-6-fluoro-cinnoline-4-ones.
- iv) Synthesis of intermediate for Letrozole..
- v) Biological screening and results.

PART-II: Chapter-ii;

Synthesis of 1H-3(2-alkyl\aryl,4-substituted thiazolo)-6-chloro-cinnoline-4-ones.



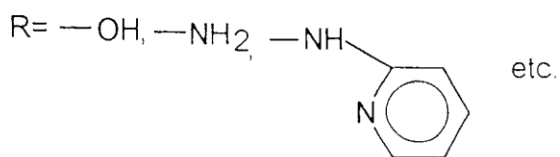
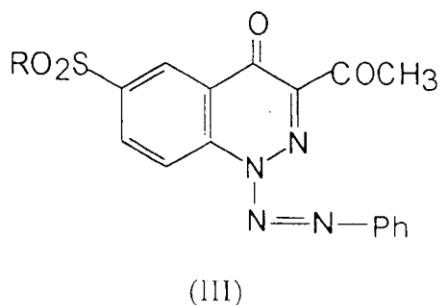
Cinnoline(I), a member of the heterocyclic family, has been documented in the literature for its appreciable antibacterial activity¹.



R/R1 = H, electron donor grp.

R2 = C₁₋₆alkyl

1-alkyl-3-carboxy-4-cinnolones(II) also show good antibacterial activity².

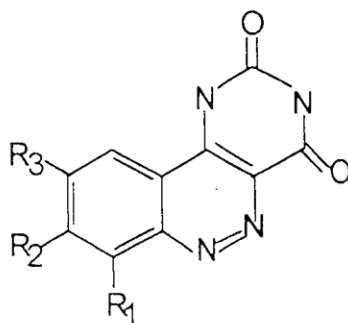


3-acetyl-phenylazo-6-substituted cinnoline-4-ones(III) have been reported to exhibit potential antibacterial, but low antifungal activity³.



(IV)

Similarly, 4-arylamino pyrimido(5,4-c) cinnolines⁴(IV) have been reported to possess activity from 0.3µg/ml to 0.05µg/ml against gram positive *Bacillus cirroflagelus*, from 0.21µg/ml to 0.025 µg/ml against gram positive *Staphylococcus aureus*, and 3.16µg/ml against gram negative *Escherichia coli*.



R₁, R₂, R₃ = Alkyl / Aryl

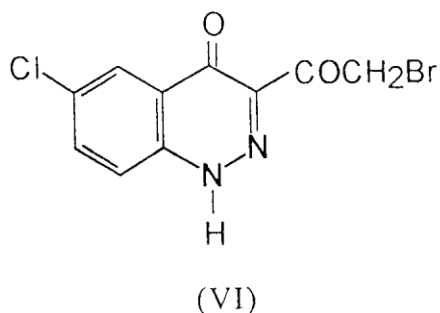
(V)

Pyrimido (5,4-c) cinnoline-2,4(1H,3H) diones⁵ (V) are known to possess moderate activity i.e. upto 100µg/ml against a number of gram positive and Gram-negative organisms and fungi like *Aspergillus niger* and *Candida albicans*.

Relatively less work has been carried out on the cinnoline ring. Hence, we decided to emphasize on this ring system and carry out studies on compounds

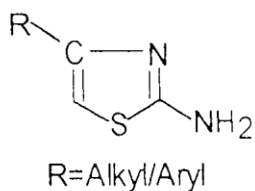
bearing a combination of cinnoline and other heterocyclic systems, in the hope of developing a prospective antimicrobial agent.

Electron withdrawing substituents like halogens and nitro are believed to impart stability to the ring and thereby contribute to the activity. Hence, we selected 6-chloro-(3-haloacetyl)-1H-cinnoline-4-one(VI) as our parent moiety.

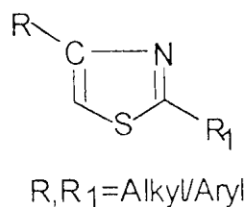


Thiazole:

Thiazole moiety is well established as a biologically active moiety especially for its antibacterial activity⁶. Russian research workers⁷ showed that amino substitution at 2-position of thiazole ring along with alkyl/aryl substitution at 4-position(VII) imparts bacteriostatic activity to the molecule.



(VII)



(VIII)

Similarly, vast synthetic work carried out in our laboratory on 2,4-disubstituted thiazoles (VIII) also revealed⁸ that many thiazoles of such types are active against H₃₇Rv strain of *Mycobacterium tuberculosis* at concentrations

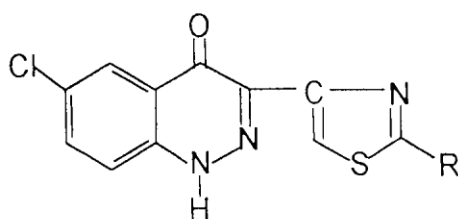
ranging from 5 μ g/ml to 0.01 μ g/ml.

These properties of thiazole nucleus as antitubercular agent strongly suggest that this nucleus should be explored further in order to achieve good anti tubercular activity.

Keeping in mind the good antibacterial activity of 2,4-disubstituted thiazole, we decided to combine cinnoline with thiazole.

In order to obtain the thiazole, the 3-bromoacetyl-6-chloro-1H-cinnoline-4-one was condensed with various substituted thioamides.

The final compounds in this series have the following substitutions-



(IX)

R= aminomethylphenyl, pyridyl, benzyl etc.

All the ten compounds synthesised in this series were confirmed by elemental and spectroscopic analysis.

Chapter-ii:

Synthesis of 1H-3(2-alkyl/aryl,4-substituted thiazolo)-6-fluoro-cinnoline-4-ones.

Extensive work has been reported on the advantages of organo fluorine compounds. Fluorine atom itself has numerous advantages, like-

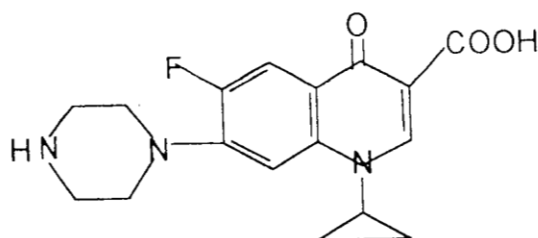
- 1) Fluorine, the second smallest atom as measured by atomic radius and internuclear distance to carbon, would most closely resemble bioactive hydrogen analogues with respect to steric requirements at enzyme receptor

sites.

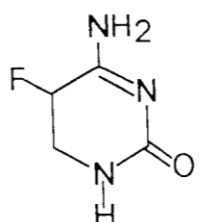
2.) Fluorine atom imparts increased oxidative and thermal stability because of high binding energy.

3.) Fluorine atom leads to increased lipid solubility which enhances the rate of absorption and *in vivo* transport.

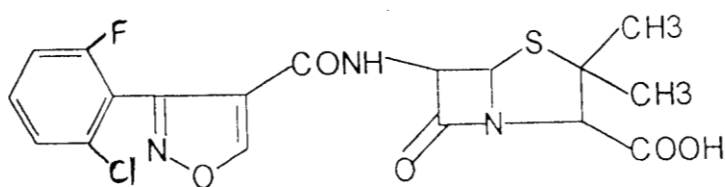
Numerous fluorinated compounds are known e.g. Ciprofloxacin(X) (antibacterial), Flucytosine(XI)(antifungal), Flucloxacillin (XII)(antibacterial), etc.



Ciprofloxacin (X)



Flucytosine (XI)

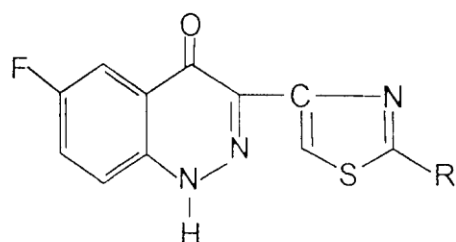


Flucloxacillin

(XII)

Thus, we decided to replace chlorine at 6-position by fluorine to check its effect on activity. The fluorinated compounds were synthesized by a similar procedure using 4-fluoro aniline as the starting compound.

The final structure is as follows-



R = Alkyl/Aryl.

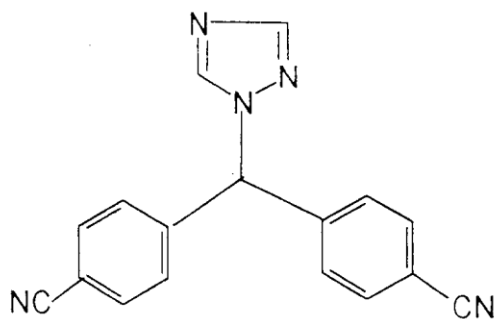
(XIII)

R= alkyl /aryl/ substituted aryl

All the ten compounds synthesised in series II were confirmed by elemental and spectroscopic analysis.

Chapter-iii;

Synthesis of intermediate for Letrozole:



Letrozole

CGS(20267) (XIV)

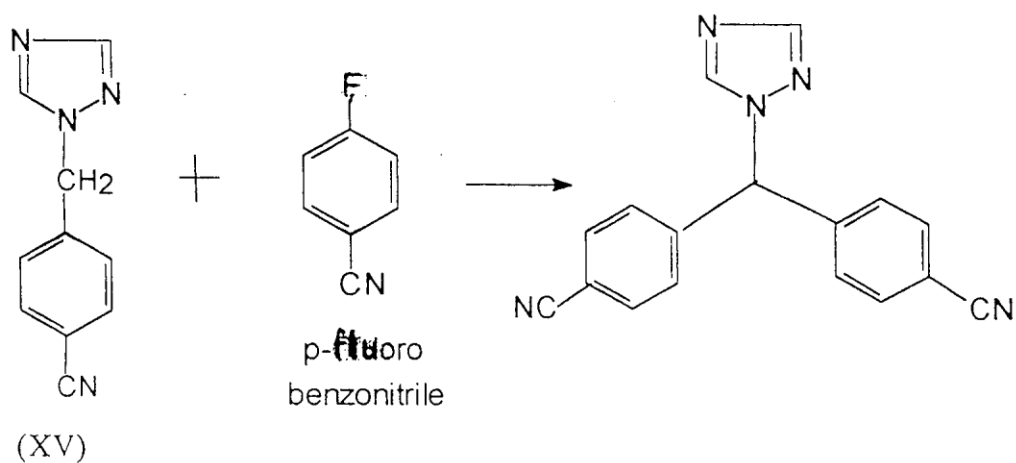
Letrozole(XIV) is a relatively new drug used as an antineoplastic agent. Using a modified nude mouse model of post-menopausal breast cancer, CGS(20267) was shown to have a marked antitumor effect and to significantly suppress plasma estrogen⁹.

Letrozole is currently undergoing phase II and phase III clinical trials for breast cancer in U.S.A.

Pharmacological action:

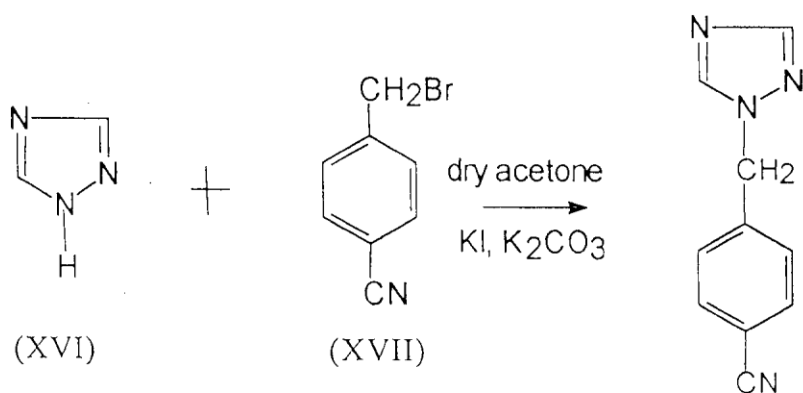
Letrozole is a highly potent and selective non-steroidal aromatase inhibitor. Aromatase catalyses the conversion of androgens to estrogens which leads to an increase in plasma estrogen level. Letrozole was shown to inhibit aromatase in peripheral tissues at concentrations of 0.5 –2.5 mg/day for 6 weeks⁹.

The synthetic route of Letrozole is as follows¹⁰:



Number of antineoplastic drugs can be synthesized using 4(1,2,4 triazolyl methyl) benzonitrile(XV) e.g. MPV-2213ad¹¹, Letrozole etc.

The intermediate 4(1,2,4 -triazolyl methyl) benzonitrile is prepared by condensation of 1 H-1,2,4 –triazole(XVI) with cyanobenzyl bromide(XVII)¹².



Thus, 1,2,4 -triazole is a key intermediate for the synthesis of Letrozole 1,2,4 -triazole can be prepared by various routes¹³ which are summarized as follows:

- 1.) By the oxidation of substituted 1,2,4 -triazoles.
- 2.) By treating urazole with phosphorus pentasulfide.
- 3.) By heating equimolar quantities of formyl hydrazine and formamide.
- 4.) By removal of the amino functions of 4--amino- 1,2,4 -triazole.
- 5.) By oxidation of 1,2,4 -triazole-3(5) thiol with hydrogen peroxide.
- 6.) By decarboxylation of 1,2,4 -triazole-3(5) carboxylic acid.
- 7.) By heating hydrazide salt with formamide¹⁴.
- 8.) By rapid distillation of hydrazine hydrate with 2M equivalent of formamide¹⁵.
- 9.) By heating N,N' diformyl hydrazine with excess ammonia in an autoclave at 200 °C for 2-4 hrs.¹⁶.
- 10.) By the reaction of 1,3,5-triazine and hydrazine monohydrochloride¹⁷.
- 11.) By oxidation of 1,2,4 -triazole-3(5) thiol in conc. HNO₃¹³.

We've carried out the oxidation of 1,2,4-triazole-3(5) thiol for better yield by applying certain modifications to the literature procedure.

MODIFICATIONS:

- 1.) During the work up of oxidation of 1,2,4 -triazole -3(5) thiol ,we neutralized the reaction mixture with liquid ammonia in order to minimize the inorganic bulk.
- 2.) Instead of hectic and multiple extraction procedure , we extracted the triazole in ethyl acetate and recovered the solvent upto 90%.
- 3.) The use of large quantity of ethyl alcohol was avoided and this makes the process cost effective.
- 4.) Also, use of benzene was avoided. Benzene being highly carcinogenic may cause health hazards to the workers, when used at industrial scale.

The triazole and the benzonitrile were subjected to elemental analysis and spectroscopic studies for confirmation.

Chapter-v:

Biological Screening:

All the compounds synthesised were subjected to antibacterial, antifungal and antitubercular screening.

For antibacterial screening, the organisms used were *Staphylococcus aureus* ATCC3750 and *Salmonella typhi* NCTC786. The medium used was Mueller Hinton Broth. Standard drugs used were Trimethoprim(MIC1µg/ml) against *S. typhi* and Ampicillin(MIC0.01µg/ml) against *S. aureus*.

Six compounds have shown good antibacterial activity upto 4µg/ml while five compounds have shown activity upto 10µg/ml.

For antifungal screening, the organisms used were *Candida albicans*, *Tricophyton mentagrophytes* and *Tricophyton rubrum*. The medium used for growth was Sraufataller medium. Miconazole(MIC1.56µg/ml) was used as the standard drug.

Four compounds have exhibited activity upto 100µg/ml.

For antitubercular screening, H₃₇Rv strain of *Micobacterium tuberculosis* was grown on Kirchner medium and the activity was compared with standard drug Isoniazide(MIC0.04µg/ml).

Five compounds have shown activity upto 40µg/ml while one compound is below 20µg/ml.

REFERENCES:

- 1.) "Chemistry of Heterocyclic Compounds", Simpson, J.C.E.; Interscience Publishers, INC, New York; 5,5,(1953).
- 2.) Fuscho,R., Piselli,F.L. & Boshi,P.M.; *Eur. Pat. Appl.* EP 287(1988); C.A.; 110(1989)75540.
- 3.) Abbady, M.S., Radhwan, Sh.M., Bhakite, E.A.; *Ind. J. Chem.*; 32B;1281-1284(1993).
- 4.) Nargund L.V.G., Nargund, K.S.; *Arzneimittel Forchung Drug Research*; 45;113,(1995).
- 5.) Gopikumar Menon, C.R., Purushotthaman, E.; *J. Ind. Chem. Soc.*; 72;731-734,(1995).
- 6.) Gardener, T.S., Wenis, E., Lee, J.; *J. Org. Chem.*; 20;976-980(1955).
- 7.) Carp. Eugenis & Toma A.; C.A.; 63,14483d(1965).
- 8.) Choudhari, D.T., Sabnis, S.S., Patel, M.R., Deliwala, C.V.; *Bull. Haff. Inst.*; 4(1); 8-15(Eng.)(1976).
- 9.) Ciba Geigy; Chugai; *Drugs Fut.*; 21(4);434,(1996).
- 10.) "Strategies in Drug Synthesis"; by Daniel Ledniser; 6;86.
- 11.) Bowman, R.M., Steele, R.E., Browne, L; C.A.; 114;247287y and 247288(1991).
- 12.) *Drugs Fut.*; 23(10); 1071-1074.
- 13.) "Organic Synthesis", ed. by John Wiley & Sons.; 40; 99-102.,(1965).
- 14.) Strain, H.H.; *J. Ame. Chem. Soc.*; 49,1995,(1927).
- 15.) Ainsworth, C. & Jones, R G.; *J. Am. Chem. Soc.*; 77; 621,(1955).
- 16.) Grudman, C. & Ratz, R.; *J. Org. Chem.*; 21; 1037,(1956).
- 17.) Hoggarth, E.; *J. Chem. Soc.*; 1949. 1163.

