

**SYNTHESIS OF HETEROCYCLIC COMPOUNDS AS
ANTIBACTERIAL, ANTIFUNGAL AND
ANTITUBERCULAR AGENTS**

**THESIS SUBMITTED
UNIVERSITY OF MUMBAI**

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

**SUBMITTED BY
CHANDRAKANT BONDE**

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

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

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Mr. Chandrakant G. Bonde

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TO
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FACULTY OF TECHNOLOGY
(PHARMACEUTICAL CHEMISTRY)

BY
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Haffkine Institute,
Mumbai-12

Bonde C.G.
Chandrkant Bonde.

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

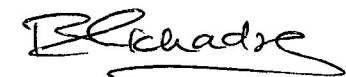
The work embodied in the thesis to be submitted is original, based on the discovery of new facts viz. The synthesis and characterisation of new and unknown compounds of possible chemotherapeutic value. also included in this thesis, are new relation of facts, observed on the basis of in vitro testing.

The assessment of the compounds has been made at the dept of microbiology and the dept of chemotherapy. Haffkine institute, Mumbai-12

Statement-II:

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

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



Dr. B. G. Khadse.,

Signature of Research guide

Mr. Chandrakant Bonde.



Signature of Research Student

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INTRODUCTION
AND
REVIEW OF LITERATURE

The discovery of effective agents to prevent and treat infections caused by bacteria and other pathogenic microorganisms is one of the most important developments of modern medicines, the use of such agents has not been limited to the present era. Substances with antiinfective potential have been applied medically for thousands of years. Indeed, more than 2500 years ago the Chinese were aware of the therapeutic properties of moldy soyabean curd applied to carbuncles, boils, and other infections¹, and the ancient Greek physicians, including Hyppocrates, routinely used substances with antimicrobial activity including wine, myrrh and inorganic salts in the treatment of wounds². Until the discovery of the microbiologic basis of infections in the nineteenth century, however, the therapy for infections remained strictly empiric. Heavy metals such as arsenic and bismuth were found to be useful against a number of infections, including *syphilis*, in the early 1900s, but the modern era of chemotherapy did not really begin until the discovery and initial clinical use of the sulfonamides in 1936¹. This was followed in the 1940s by the discovery of the therapeutic value of Penicillin and Streptomycin and by 1950 the "Golden age" of antimicrobial chemotherapy was well underway. Though the battle against various infections has largely been won, infectious diseases are still the major health hazards to mankind.

The growing prevalence of cancer, cardiovascular diseases and other chronic conditions in addition to the long standing problem of communicable diseases such as cholera, malaria and tuberculosis impose a double burden on healthcare systems in the developing countries. In addition, there is the pandemic of Human Immuno Deficiency Virus (HIV) infections and Acquired Immuno Deficiency Syndrome (AIDS), which impose a particularly heavy burden on developing countries.

To add to this, there is emergence of bacterial resistance to a number of antimicrobial agents such as macrolides, quinolones, vancomycin and β -lactam antibiotics. Many of these strains are also resistant to macrolides, tetracyclines,

aminoglycosides and fluoroquinolones. Multidrug resistant strains of *Mycobacterium tuberculosis* have emerged in several countries including United States³. The resistance of *Bacterioides fragilis* to β -lactum ranges from 3 to 30%, nearly all of them being resistant to tetracyclines. This rapid and extensive emergence of resistant bacteria has resulted in a clear-cut need to discover new chemotherapeutic agents.

The present investigation is an attempt in this direction for obtaining possible solutions to some of the persistent health problems that affect human life, especially bacterial and fungal infections.

Tuberculosis :

Tuberculosis is one of the most widespread infections known to man. This disease is largely caused by *Mycobacterium tuberculosis*, an 'acid fast' bacilli. It has been estimated that 1.7 billion people or a third of the world's population, harbor the pathogen and every year 8 million individuals develop new clinical disease, causing 3 million deaths⁴.

It attacks both humans and animals, affects all ages and every organ in the body, and ranges in its stage from latent to hyperacute. Infection with human immunodeficiency virus (HIV) is the most potent risk factor for development of tuberculosis (TB) and by 1995, 3.1 million people were expected to be co-infected with HIV and TB according to the World Health Organisation.

Tuberculosis is sometimes an acute but more frequently a chronic communicable disease that derives its character from several properties of tubercle bacilli. Like other chronic infections, tuberculosis is a rather difficult disease to treat. The major reasons for this are

1. Caseation necrosis, a unique feature of tuberculosis, allows the bacilli to remain in a dormant condition by sealing off the communication of the necrotic area with the communicating channels like the bronchus and

ureter. When the communication channel opens, the solid caseous material is autolysed and the liquid contents are expelled, resulting in the development of a cavity with a caseous lining. When the dormant bacilli get an access to the atmospheric oxygen, they multiply rapidly, producing a spread of the infection and an increase in the size of the cavity. Bronchial stenosis or ureteric constriction, which may occur as sequelae, prevent adequate drainage of the caseous materials⁵.

2. *Mycobacterial* infections such as tuberculosis are chronic diseases, which do not elicit a rapid response from the host. After initial infection and multiplication, the bacilli may lie dormant for a long time without giving rise to any of the clinical symptoms. Often, however, the bacilli may be reactivated due to malnutrition, immunosuppressive drugs and diseases including HIV infection.
3. The cavity formation tends to block the blood vessels supplying the necrotic area making penetration by antitubercular drugs difficult. The caseated material itself is also impenetrable to majority of the chemotherapeutic agents.
4. Tubercle bacilli remain viable and multiply even when ingested by macrophages. Also the chemotherapeutic agents, with few exceptions, penetrate poorly into the macrophages.
5. The tubercle bacilli are vulnerable to bactericidal drugs only when they are metabolically active and replicating. Here, the problem lies in the fact that a small subpopulation of the bacilli can remain dormant indefinitely, except for short periods when they become transiently active, thus evading chemotherapeutic agents. Another difficulty is in the existence of drug resistant mutants in populations of bacilli hitherto unexposed to antibiotics.

A truly effective drug should, therefore, be capable of penetrating the tubercle and the cavities, enter the phagocytes within which the bacilli are growing and finally destroy them. It should possess potent bactericidal/sterilizing activity against metabolically active and semi dormant bacilli while also preventing the emergence of resistant strains throughout the normally prolonged period of treatment.

No effective chemotherapeutic agent was available for treating till as late as 1940. The treatment then mainly consisted of rest, fresh air and good food. In addition, surgical methods like phrenic nerve avulsion; artificial pneumothorax and pneumoperitoneum were widely employed⁵. The discovery of effective para-aminosalicylic acid by Bernheim in 1941 was the first step in the development of effective therapeutic agents. The other two commonly used agents, Streptomycin and Isoniazid, were discovered in 1944 and 1952, respectively.

After the fast progress in the understanding of the epidemiology, prevention and treatment of TB, although far from eradication, this age-old killer of man has become a preventable and curable disease. Disturbing new problems have risen with the treatment of co-existing infection of HIV and TB.

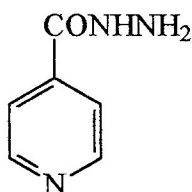
Following are the currently used drugs in the treatment of tuberculosis.

1. Standard drugs – (used in the initial treatment)
Streptomycin, Simonized, Rifampicin, Ethambutol, Thiacetazone
2. Rescue drugs – (used in resistant cases)
Pyrazinamide, p-amino salicylic acid (PAS), Kanamycin, Cycloserine, Capreomycin, Viomycin and Ethionamide.

There is no known individually self-sufficient drug, possessing all the required antitubercular properties. Hence, a combination of two or more drugs is used for the treatment, which may stretch to several months before a reasonable cure is affected. A combination of drugs also helps to prevent the development of resistance.

Isoniazid (INH)

Isoniazid (I), the hydrazide of isonicotinic acid, was first described in 1912, but its action against the tubercle bacilli was only discovered in 1952. It is unrivalled in its bactericidal action and ensures the destruction of the bacilli within a few days of starting the treatment. It is highly active against replicating tubercle bacilli, but does not affect the semidormant species⁶, which resume multiplication after removal of contact with the drug.



(I) Isoniazid

Though the exact mechanism of action of Isoniazid is not known, it has been suggested that the drug inhibits phospholipid synthesis and damages cell membrane of the tubercle bacilli.

INH is rapidly absorbed when administered either orally or parenterally and diffuses readily into all fluids and tissues. INH is the member of all antitubercular chemotherapeutic combinations currently recommended by the World Health Organisation (WHO).

INH is generally well tolerated at the recommended dose, which is approximately 5 mg/kg (maximum 300mg) daily for adults and children. Hypersensitivity to Isoniazid is rare. The most important side effects of the drug are related to its toxicity, especially affecting the peripheral and central nervous system. Peripheral neuritis is the most common reaction and is said to occur in 17% of the patients receiving 6mg/kg of Isoniazid daily. Administering 100mg of Pyridoxine, a naturally occurring form of Vitamin B₆. Hepatitis is a not so common but

nevertheless a serious side effect associated with INH therapy. This is mostly prevented by a prompt withdrawal of the treatment. A sharp rise in the serum concentrations of hepatic transaminases at the onset of treatment is usually not clinically significant. If the enzyme levels normalise on suspension of dosage, then there is less chance of them rising once again when treatment is resumed⁷.

Rifampicin :

Rifampicin is a semi-synthetic derivative of Rifamycin B, isolated from *Streptomyces mediterranei*. It is one of the most exciting and potentially valuable antitubercular drugs. Active against a wide range of other bacteria, it has also proved to be the most potent drug against human tuberculosis, both in primary treatment and treatment of relapses, given daily or intermittently. However, it has not yet become a universally used standard drug in developing countries because it is expensive, and it has troublesome side effects requiring special supervision and care. Once a week regimen shows more toxicity than twice weekly, and with daily regimen side effects are also uncommon and trivial. The side effects are normally mild, and can usually be controlled by reducing either the dose size or interval between the doses⁷. However the occurrence of purpura is an indication to stop the drug and should it not be given again.

Primary resistance of tubercle bacilli to Rifampin is rare (less than 1 percent). It is believed to act by inhibiting DNA-dependent RNA polymerase, thus stopping the expression of bacterial genes. It is bactericidal and acts against both intra-cellular and extra-cellular organisms.

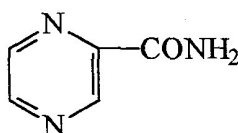
Streptomycin :

This was the first effective drug against tuberculosis in humans. This aminoglycoside antibiotic was isolated from a culture of *Streptomyces griseus* by Schatz and Waksman in 1944. Like all aminoglycosides, it is not absorbed when given orally, and must be administered as an intramuscular injection. Once administered, it diffuses readily into various tissues of the body and attains

bactericidal concentrations in tuberculous cavities. The drug is of particular use when primary resistance to other drugs is suspected.

It is more actively bactericidal in proliferative than in the resting phase of bacterial growth. The normal dosage for adults and children is 15 mg/kg daily, while in patients over 60 years of age it should be used with caution. It should be avoided during pregnancy as it crosses the placenta, thus causing the same effect in the foetus. The injections are very painful and must be administered by deep intramuscular route; One injection with approximate dosage per day is sufficient but barely tolerable. The duration of treatment is 1-2 months⁸.

Pyrazinamide :



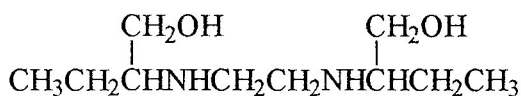
(II) Pyrazinamide

Pyrazinamide (II), a synthetic analogue of nicotinamide, is only weakly bactericidal against *M. tuberculosis*, but it has a special sterilizing effect on intracellular tubercle bacilli in acidic condition. It has, therefore, found an important place in short course chemotherapy, in which the important and troublesome factor is the incidence of relapse, after cessation of treatment⁷. It is administered in the dose of 30mg/kg daily, in both adults and children.

When given orally, it produces high serum levels, and penetrates freely into the CSF. It is usually well tolerated but there have been reports of slight flushing of skin. The dose of Pyrazinamide should be reduced in cases of renal impairment and avoided in patients with gout and severe liver damage.

Ethambutol :

Ethambutol (IIa), developed as a synthetic congener of related ethanediamines, primarily has a bacteriostatic action on proliferating bacteria. It is now accepted as a replacement for PAS. In combination with other antituberculous drugs, it is active against *M. tuberculosis* and many of the atypical mycobacteria⁷.



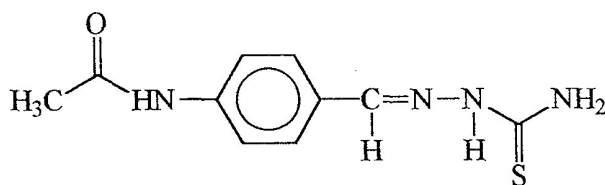
(IIa) Ethambutol

Approximately 75% of strains of the human type are sensitive to Ethambutol at a concentration of 1µg/ml. It is administered orally and is readily absorbed from the gastrointestinal tract. The normal dosage for adults and children ranges from 15-25 mg/kg daily or 40mg/kg three times weekly. The dosage is carefully calculated on a weight basis to avoid toxicity, and is reduced in patients with impaired renal function. Resistance develops slowly during therapy, with primary resistance occurring in less than 4 percent strains of *M. tuberculosis*⁷.

Ethambutol can bring about dose dependent optic neuritis, readily resulting in impairment of the use of the drug. This is usually reversible, but blindness can occur if treatment is not discontinued promptly.

Thiacetazone :

Thiacetazone (III), a thiosemicarbazone derivative, is one of the oldest antitubercular drugs known since before 1950. Thiacetazone is bacteriostatic against *M. tuberculosis* and is often used in combination with Isoniazid to inhibit the emergence of resistance to Isoniazid, particularly in the continuation phase of the long-term regimens⁹.



(III) Thiacetazone

The drug is satisfactorily absorbed from the gastrointestinal tract. It rapidly diffuses into various body tissues and also crosses the placental barrier. The side effects of the drug include anorexia, nausea and vomiting, while drug fever, skin rashes, jaundice, bone marrow depression etc. appear rarely.

Other bacterial infections:

Man's search for effective remedies is as old as mankind itself. But before the aetiological agents of infectious disease were identified and made amenable to laboratory investigation, progress had to rely entirely on the vagaries of chance and empirical observation.

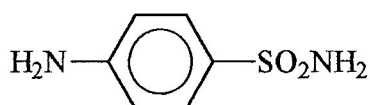
Not surprisingly, therefore, mankind's earliest therapeutic successes against infecting organisms are in the form of plant extracts. Observations that natural substances controlled the spectacular symptoms of certain diseases led to the initial recognition of two other ancient remedies : Quinine, obtained from the bark of the Cinchona tree, and Emetine, an alkaloid obtained from Ipecacuanha root. Both of these are active against protozoa (the parasites of malaria and amoebic dysentery, respectively).

The history of antibacterials has been dynamic, characterised by the constant emergence of new challenges, followed by investigation, discovery and the production of new drugs. A complete review of the various agents employed as antibacterials would be beyond the scope of this work and hence is not attempted.

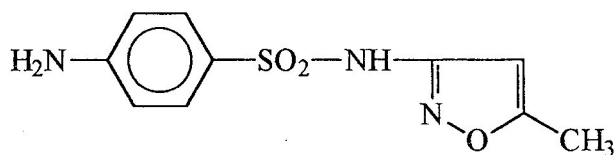
The following pages present a brief summary of the important classes of compounds used for this purpose.

Sulfonamides :

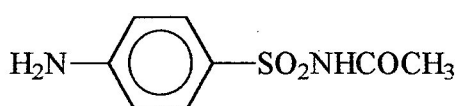
The term sulfonamide is usually employed as a generic name for derivatives of p-aminobenzenesulfonamide. Domagk¹⁰, Mietzsch and Klarer¹¹ in 1935 discovered Prontosil, and reported that this compound cures *Streptococcal* infections in mice and *Staphylococcal* infections in rabbits. After this success, sulfonamides were the first synthetic chemotherapeutic agents employed to prevent and cure bacterial infections in man and were quite effective in bringing about a sharp decline in the morbidity and mortality figures for the treatable infectious diseases. Important members of this class include Sulfanilamide (IV), Sulfamethoxazole (V), Sulfacetamide (VI), Sulfadiazine (VII) and Sulfisoxazole (VIII).



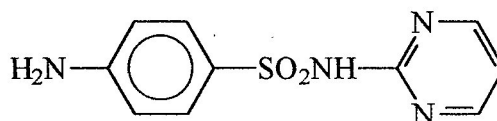
(IV) Sulfanilamide



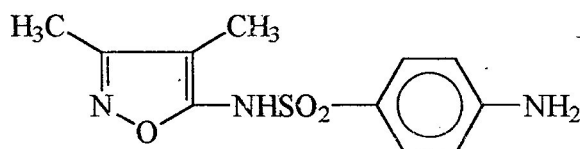
(V) Sulfamethoxazole



(VI) Sulfacetamide



(VII) Sulfadiazine



(VIII) Sulfisoxazole

The sulfonamides have a wide range of antibacterial activity against both gram-positive and gram-negative bacteria. They are usually rapidly absorbed from the gastrointestinal tract, except in the bowel. Approximately about 70-100% of an oral dose is absorbed and finally eliminated from body, partly as metabolic products; the largest fraction being excreted in the urine¹².

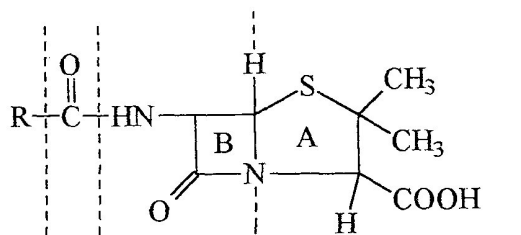
Strains of bacteria resistant to the sulfonamides have become common during recent years. Also, the advent of Penicillin and other antibiotics has diminished the role of sulfonamides as antibacterial agents. However, the introduction of the combination of Trimethoprim and Sulfamethoxazole, known popularly as Cotrimoxazole, has resulted in increased use of Sulfonamides for the treatment of specific bacterial infections.

Sulfonamides normally act by inhibiting the enzyme folic acid synthetase, which is involved in the conversion of p-aminobenzoic acid to folic acid. This causes folic acid deficiency, which is important in bacterial metabolism¹³. This results in injury to the bacterial cell and such cells can be easily phagocytosed.

Penicillins and Cephalosporins :

Since Alexander Fleming's serendipitous discovery of Penicillin in 1928, it has remained one of the most important group of antibiotics. Despite the discovery of several other bacterial agents, the Penicillins are widely used, with new derivatives being produced every year. Due to their unique advantages, members of this group of antibiotics are presently the drugs of choice for the treatment of a large number of infectious diseases.

The basic structure of Penicillin (IX), also known as 6-amino penicillanic acid (6-APA), consists of thiazolidine ring (A) connected to β -lactum ring (B) which is attached to a side chain(R) through amide carbonyl. The penicillin nucleus is an important requirement for biological activity, alteration of which causes loss of all significant activity. Many of the bacterial and pharmacological characteristics of penicillins are determined by the side chain attached, which can also alter the susceptibility of the resultant compounds to inactivating enzymes (β -lactamases)¹⁴.



(IX)

The penicillins are classified on the basis of their spectrum of antibacterial activity. While the pharmacological properties of the individual drug in the various classes would constitute a lengthy discussion, certain generalisations can, however, be made. Following absorption, penicillins are widely distributed throughout the body. The therapeutic concentrations of these agents are quite rapidly achieved in tissues but comparatively much lower levels are found in brain tissues and in intra-ocular fluids.

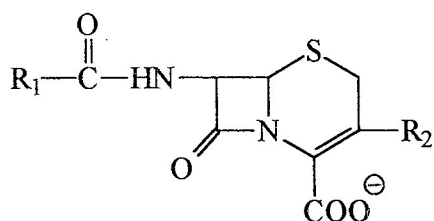
The penicillins do not penetrate the living phagocytic cells to a great extent. This class of compounds is quite rapidly eliminated, particularly by glomerular filtration and renal tubular filtration, as a result of which high concentrations of the drug are found in the urine. Therefore, they exhibit short half-lives, approximately 30-60 minutes¹⁵.

No sooner was the fact established that penicillins are capable of eradicating microorganisms, than it was practically administered in every situation where there was a risk of bacterial infection. But after the investigations of prophylaxis under controlled conditions, it is now understood that penicillin is highly efficient in some situations, quite the contrary in others and of questionable value in still others.

The commonly cited adverse effects in the usage of penicillin, which are probably the most common causes of drug allergy, have been the hypersensitivity reactions. Such reactions could occur with any dosage form of penicillin, but as the drug is rapidly eliminated, the allergic reactions usually subside. In some cases, the symptoms may persist for one to two weeks after withdrawal of treatment,

while in other cases the reaction is mild and disappears during therapy with the drug. At the worst of circumstances, an extreme allergic reaction may even result in death of the patient.

The Cephalosporins (X) have shown tremendous growth over the past decade and are usually classified by the 'generations' system, which is based on the general features of antibacterial activity.



(X)

The first generation Cephalosporins exhibit good activity against gram-positive, and relatively lower activity against gram-negative bacteria. The second generation Cephalosporins exhibit better activity against gram-negative microorganisms, but are inferior to the third generation cephalosporins. The third generation cephalosporins are generally less active against gram-positive bacteria as compared to first generation agents, but are much more active against β -lactamase producing strains¹⁶.

The Cephalosporins are widely used as antibiotics, and perhaps overused for conditions where much less expensive antibiotic could not provide relief. Nevertheless, they are indeed useful as alternatives to penicillins for a variety of infections in patients who suffer allergic reactions to penicillins.

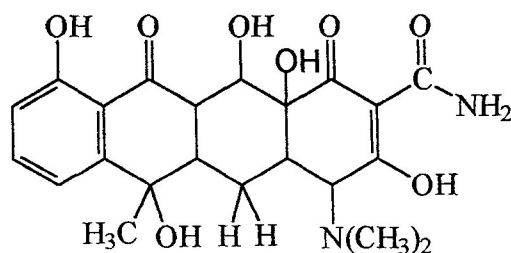
The Cephalosporins also find wide use in the prophylaxis during and after surgery, wherein the first generation agents have been shown to be safe and effective. A few of the third generation Cephalosporins are the drugs of choice for meningitis and are in some cases superior to a combination of Ampicillin and Chloramphenicol.

The β -lactam antibiotics (Penicillins and Cephalosporins) suffer bacterial resistance due to a variety of factors. It may be caused by the inability of the agents to enter the site of action¹⁷. Also, it is possible for a sensitive strain to acquire resistance by mutation and has been described for gram-positive cocci¹⁸.

Bacteria can also destroy the β -lactam antibiotics enzymatically. β -lactamases are capable of inactivating certain of these antibiotics and is the major term of this enzyme although different microorganisms synthesise a number of distinct β -lactamases. Depending on substrate specifications these are often described as either penicillinases or cephalosporinases. Individual penicillins and cephalosporins vary in their susceptibility to these enzymes¹⁷.

Tetracyclines :

Tetracyclines (XI) exhibit a wide range of activity against gram-positive and gram-negative bacteria. In general, gram-positive bacteria are affected by lower concentrations of Tetracycline than the gram-negative species. However, they are rarely recommended for infections caused by gram-positive bacteria due to problems of resistance and availability of superior antibacterial agents¹⁸.



(XI)

Microorganisms that have become resistant to one Tetracycline frequently exhibit resistance to other members of the family. The bacterial species possibly exhibit resistance only after exposure to the drug. The Tetracyclines were initially useful for treatment of infections with aerobic gram-negative bacteria, but most species

are now relatively resistant. Nevertheless, more than 90% strains of *Haemophilus influenzae* may still be sensitive to either Tetracycline or Doxycycline.

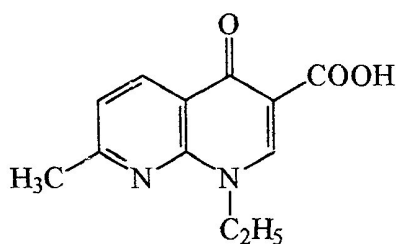
All Tetracyclines produce gastrointestinal irritation to varying degrees in some individuals and this is more common after oral administration. Diarrhoea may also be a side effect, which though frequent and fluid, does not contain blood or leukocytes. Doxycycline, and to a lesser extent other derivatives, may produce mild to severe reactions in the skin of treated individuals exposed to sunlight.

Other important antibacterial agents include Chloramphenicol and furan derivatives such as Nitrofurantoin, Nitrofurazones, Furazolidone etc. Several of these drugs are active against various species of gram-positive and gram-negative microorganisms besides mycobacteria.

Quinolones :

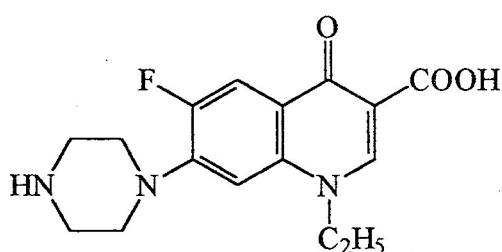
The quinolone carboxylic acids, (or) 4-quinolones are a group of synthetic antibacterial agents structurally related to Nalidixic acid (XII). Nalidixic acid is active against gram-negative bacteria but has little activity against *Pseudomonas* and gram-positive organisms, because of which bactericidal use of this class has generally been limited to the treatment of urinary tract infections.

Modifications in the structure of Nalidixic acid has produced related antibacterial agents such as oxolinic acid, piromidic acid, cinoxacin and acrosoxacin. None has been considered to represent a significant clinical advance over Nalidixic acid¹⁹.

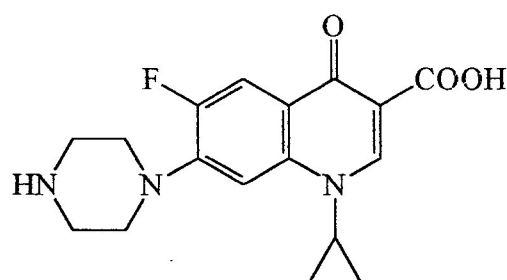


(XII) Nalidixic acid

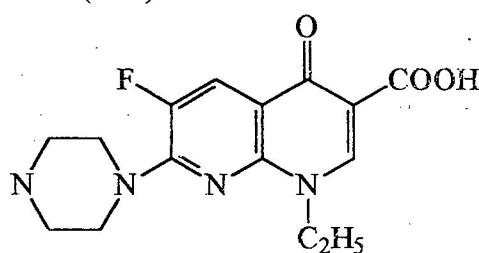
Addition of the 7-piperazinyl group and a fluorine atom at position 6 has produced a group of fluoroquinolones with a broad spectrum of activity as compared to Nalidixic acid, and are more useful for the treatment of systemic infections. They include Norfloxacin (XIII), Ciprofloxacin (XIV), Enoxacin (XV), Ofloxacin (XVI) and Lomefloxacin (XVII). One disadvantage of all the quinolone antibacterial agents is that they are generally not recommended for use in children, adolescents, pregnant and breast feeding women, because of their propensity to cause joint erosions in immature animals.



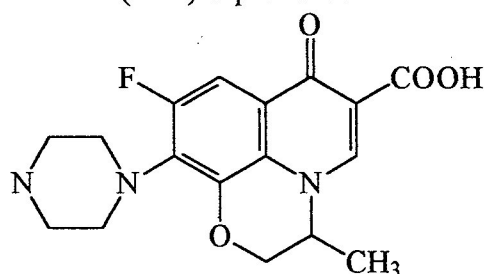
(XIII) Norfloxacin



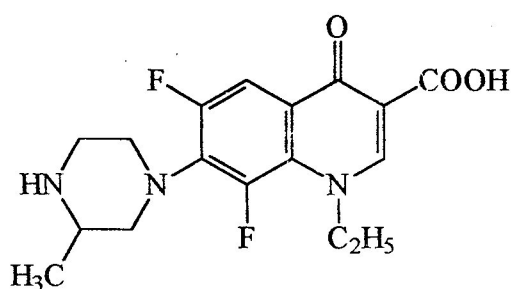
(XIV) Ciprofloxacin



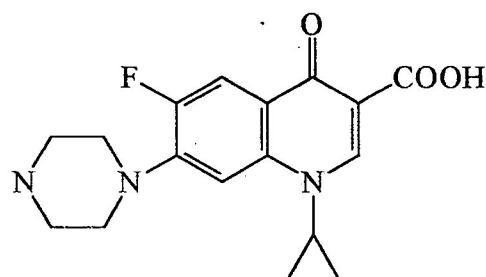
(XV) Enoxacin



(XVI) Ofloxacin



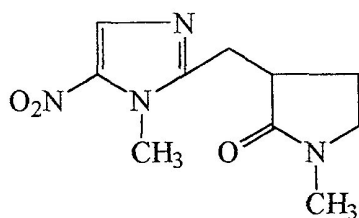
(XVII) Lomefloxacin



(XVIII) Tefloxacin

Tefloxacin (XVIII) was demonstrated by J. L. Chen *et al*²⁰ to possess activity against penicillin resistant *Staphylococcus*, *Streptococcus* and *Haemophilus* species. It was itself a β -lactamase inhibitor and displayed synergy with Ampicillin.

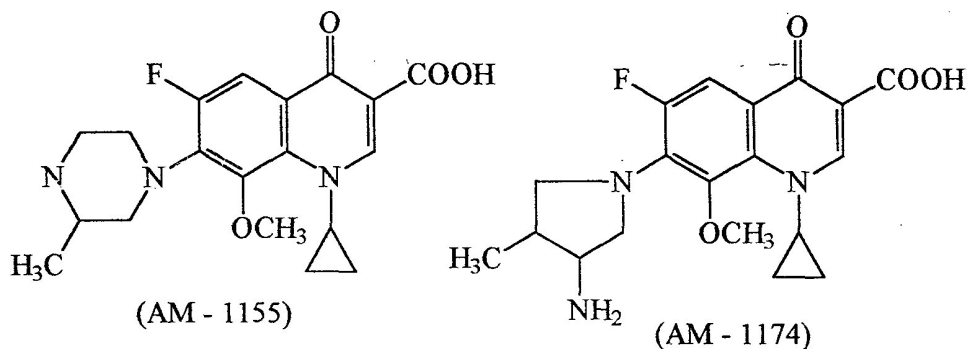
Miscellaneous : Activity against anaerobic organisms was reported for a series of 2-substituted-5-nitroimidazoles²¹. Compound (XIX) was unique in this series, and was found to be more potent both *in vitro* and *in vivo* than Metronidazole.



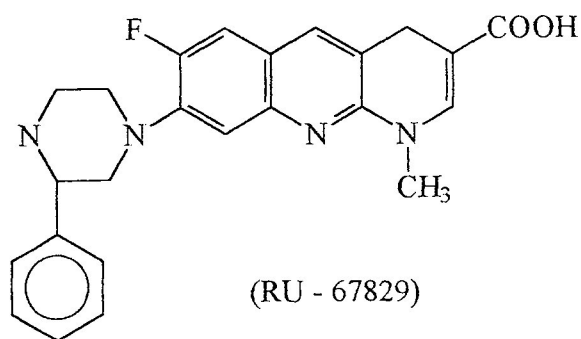
(XIX)

Quinolones :

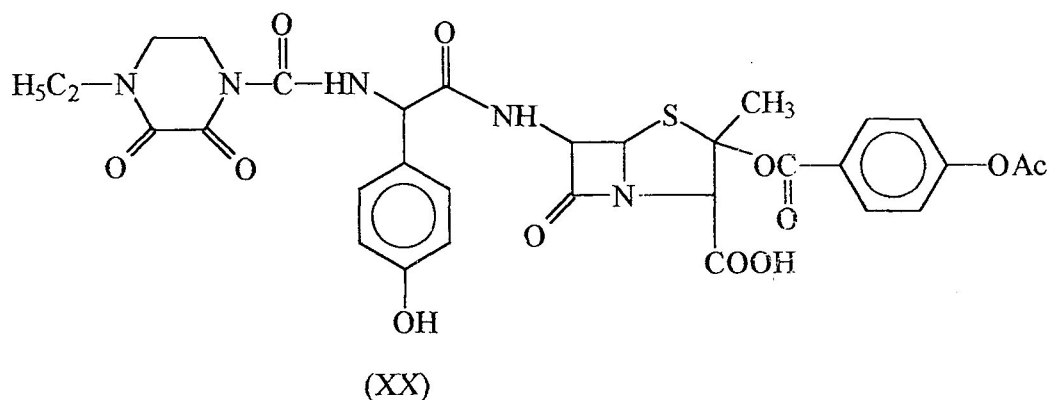
Use of N-cyclopropyl and C-8 hetero atom substituents continued to provide potent antibacterial agents. AM – 1155 and AM – 1174, new quinolones bearing 8-methoxy group, outperformed Ciprofloxacin against anaerobes and a range of gram-positive bacteria with potency upto 16 to 62 – fold greater than Ciprofloxacin²².



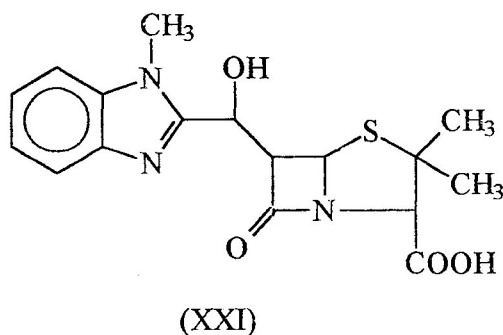
RU –67829 (a compound in benzonaphthyridine series) was found to be more active against gram-positive organisms than Ciprofloxacin²³.



In penicillins, the catechol containing compounds (XX) had outstanding activity against *Pseudomonas*, and stability against gram-negative β -lactamases, but not against the lactamases of *S. aureus*²⁴.

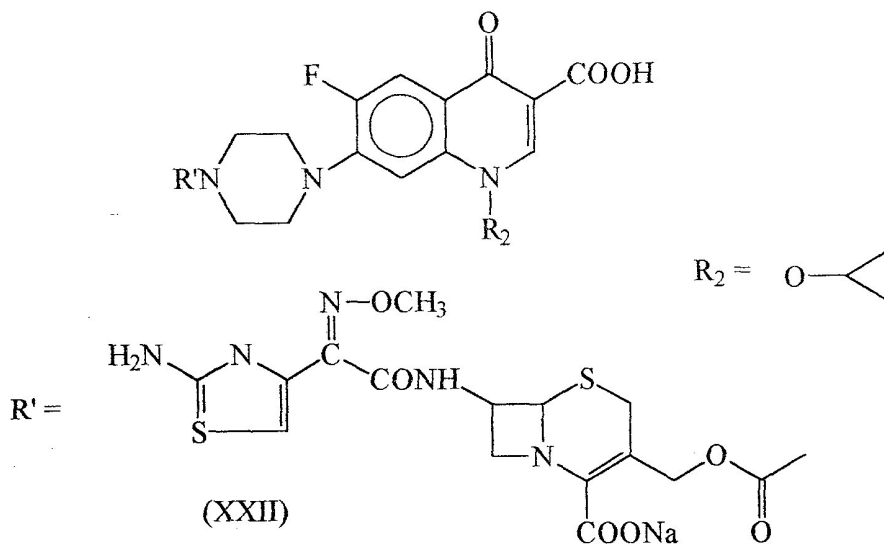


The stability to gram-positive β -lactamases could be achieved through the use of a (benzimidazole-2-yl) hydroxymethyl group at R-position (XXI)²⁴.



AM - 1174 exhibited activity 128-fold that of Ciprofloxacin against Ciprofloxacin-resistant strains of *S. aureus*²⁵.

A class of new β -lactam quinolone hybrids have been reported. The carbamate linked R0-24-4383 (XXII) showed broad spectrum activity and exhibited aqueous solubility much greater than that of the quinolone component alone²⁶.



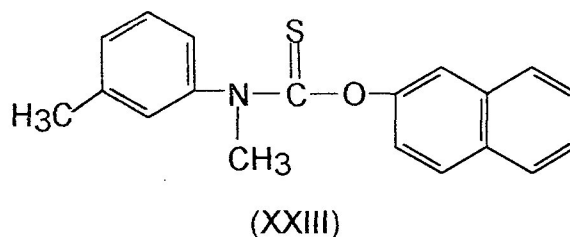
Antifungal agents:

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, Histoplasmosis²⁷ etc. Under certain conditions, some of these species are capable of invading deep body cavities and causing systemic mycoses, 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 Ketoconazole²⁸.

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 (XXIII) limits its use. It is available in a number of dosage forms for the treatment of ringworm, athletes foot etc.

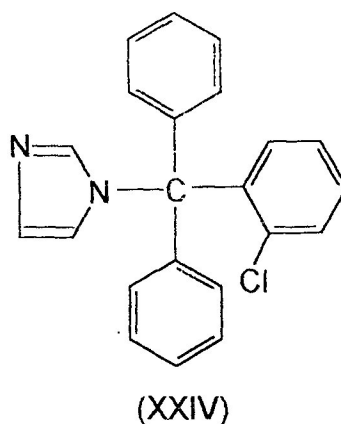
In susceptible fungi, Tolnaftate suppresses squalene epoxidase, which converts squalene to ergosterol, thereby preventing cell-wall formation. __

Substituted azoles:

Substituted imidazoles represent a versatile group of antifungal agents with a unique mechanism of action. The early members reported were Cotrimazole (XXIV)²⁹ and Miconazole (XXV)³⁰ 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*, and *Paracoccidioidomycosis*..

The mechanism of action of the drug is elucidated³¹ extensively. At high concentrations (micromolar), they are fungicidal; at low concentrations (nanomolar), they are fungistatic. The fungicidal effect is associated with damage to the cell wall leading to a loss of essential cell components such as potassium ions and amino acids. Imidazole inhibits the cytochrome P-450 oxidase enzyme that catalyses the 14- α -demethylation of lanosterol to ergosterol and thus interferes in the formation of cell wall.

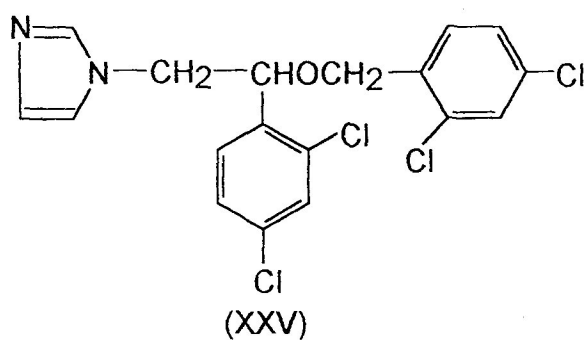
Cotrimazole:



Cotrimazole (XXIV) 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:

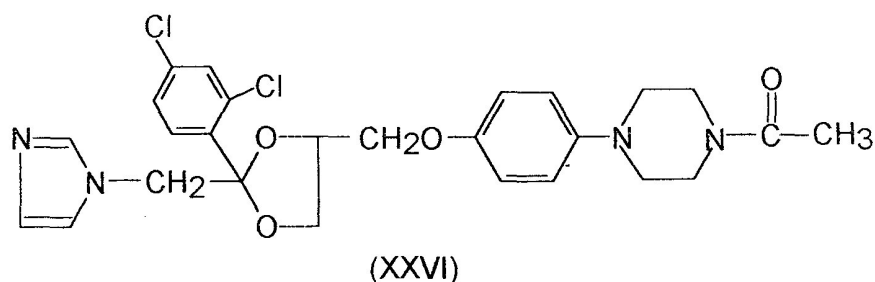


Miconazole(XXV) is intended for the treatment of serious systemic fungal infections like Candidiasis, Cryptococcosis, Petriellidiasis and Paracoccididomycosis.

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:

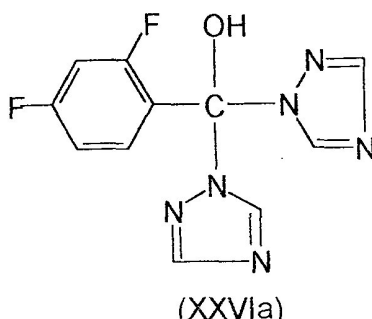


Ketoconazole³²(XXVI) is administered orally for the treatment of systemic mycoses. It requires gastric acidity for absorption. Antacids and drugs like H₂-histamine antagonists, anticholinergics, that inhibit gastric secretion, interfere with its absorption. It is metabolised to the inactive form and is excreted by the enterohepatic route.

Ketoconazole is recommended for systemic mycoses such as Coccididomycosis, Blastomycosis, Histoplasmosis, chromomycosis, paracoccidiodomycosis and severe cutaneous infections.

Hepatotoxicity is a major adverse effect of the drug. Also, levels of testosterone and corticosterone are lowered at high doses of Ketoconazole³².

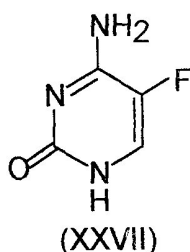
Fluconazole:



The absorption of Fluconazole³³ (XXVIa) 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:



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

The 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 metabolized 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 Amphotericin B (polyene antibiotic), which lowers the risk.

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

Antifungal antibiotics:

Polyene antibiotics:

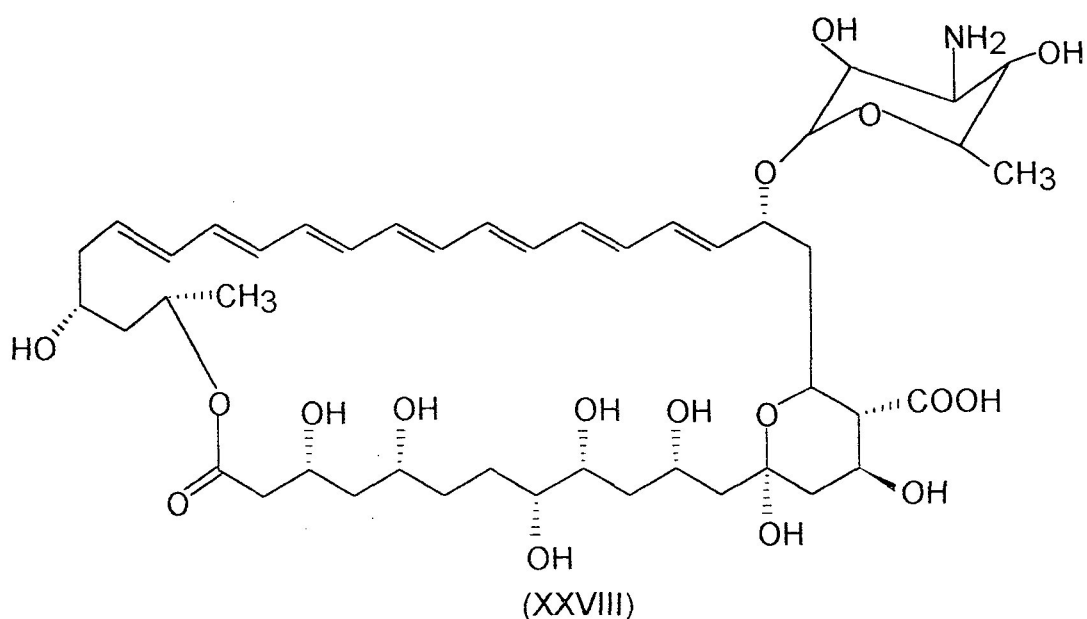
The polyene antibiotics contain conjugate double bonds in large lactone rings. They differ from macrolides in the size of the lactone ring and the presence of conjugated- 'ene' system.

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

The mode of action of this class involves binding with sterols in cell membrane to cause disorganisation and loss of cell constituents, especially potassium ions³⁴. The action is bacteriostatic at low concentrations, and bactericidal at higher doses.

Amphotericin B:

A potent antifungal agent with a polyene structure was isolated in 1956 by Gold *et al*³⁵ from *Streptomyces nodosum*, found in the soil from Venezuela. The antibiotic material was a mixture of two closely related compounds, Amphotericin A and B of which B is more active.

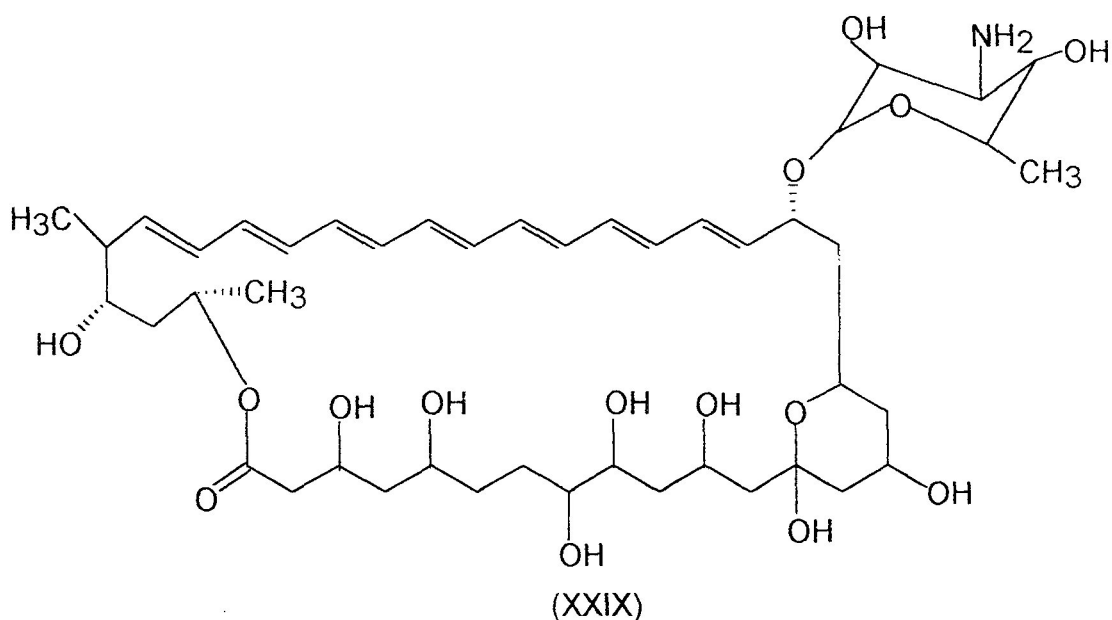


Amphotericin B (XXVIII) contains a primary amino group in the sugar moiety, mycosamine and a carboxyl group attached to the macrolide ring.

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

A high prevalence of adverse reactions limits the use of Amphotericin B. Nephrotoxicity occurs in 80% of the patients. General toxicity includes hypokalemia, distal tubular acidosis, fever, headache, anorexia, G.I. disturbances, muscle and joint pain, malaise etc. Pain at the site of injection and thrombophlebitis occurs on intravenous administration. It should not be administered intramuscularly. Amphotericin is also used topically to treat cutaneous and mucocutaneous infections³⁵.

Nystatin:

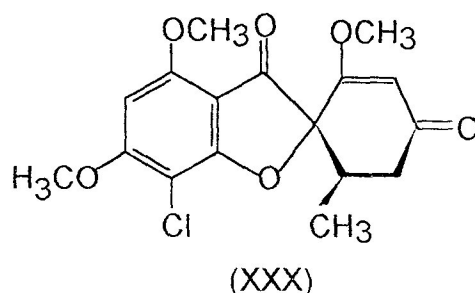


A polyene antibiotic first isolated in 1951 from a strain of *Streptomyces noursei* by Hazen and Brown³⁶, Nystatin (XXIX) is a valuable agent for the treatment of local and gastrointestinal monolial infections caused by *Candida albicans* and other *Candida* species. It is also recommended for thrush 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 (XXX) was first reported in 1939 by Oxford *et.al.*³⁷ as an antibiotic obtained from the mold *Penicillium griseofulvum*. Dierckx. Its structure has been determined by Grove *et al*³⁸.

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 *Microsporium*³⁸.



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 assembly³⁹.

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:

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.⁴⁰ Poorly soluble iron chelators such as hydroxy pyridon, rilopyrox are being developed for topical use in dermatophytosis.

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

PLAN OF WORK

Medicinal chemistry is the science that deals with the discovery and design of new therapeutic chemicals and their development into useful medicines. It may involve synthesis of new compounds, investigations of the relationships between the structure of natural and/or synthetic compounds and their biological activities, elucidation of their interactions with receptors of various kinds, including enzymes and DNA, determination of their absorption, transportation and distribution properties and studies of the metabolic transformations of these chemicals into other chemicals.

Voltaire (1694-1774) stated, "Therapeutics is the pouring of a drug of which one knows nothing, into a patient of whom one knows less."

Successive discoveries in the field of medicinal chemistry have added to the list of infections, which can be cured or curbed by chemotherapy. Still some of the drugs, which are necessary to use, are far from being ideal, either because of toxicity or unsatisfactory pharmacological properties, and some forms of treatment being consequently less often successful than others. Moreover, microbial resistance remains a constant threat to the future usefulness of almost any drug.

All successful chemotherapeutic agents should have certain properties in common:

They must exert antimicrobial action, whether inhibitory or lethal, in high dilutions and in the complex chemical environment, which they encounter in the body.

Also, since they are brought into contact with every tissue in the body, they must exert their action without any disrupting or harmful effects on the normal functions of any organ.

To these essential qualities may be added the others, viz., stability, slow rate of excretion, diffusibility into remote areas etc.

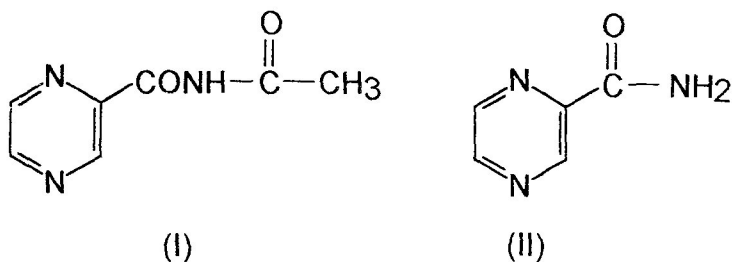
Over the years, there have been immense advances in the knowledge of the anatomy, chemical composition and metabolism of various microbial cells. This new knowledge has been helpful in understanding what drugs do to the infecting organism, i.e. the mechanism of action of the drug.

A line of advance, other than the discovery of new drugs, is the adaptation of old ones. An outstanding example of what can be achieved in this way is presented by the sulfonamides. Similar efforts in the field of antibiotics have given fruitful and encouraging results. Most of these changes are produced by carrying out additions to the drug molecule. But it is observed that modifications in the essential structure of the drug molecule alters its antimicrobial properties usually for the worse, and rarely to an advantage.

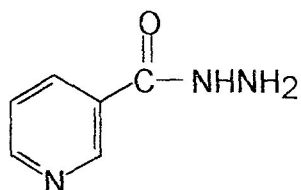
The logic supporting the work presented in this thesis was formulated, bearing in mind the biological activities of known moieties and attempting certain structural modifications or adaptations in the light of the recent trends in drug research, in incorporating newly emerged pharmacophores on existing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

Synthesis of N-[acetamido(2-hydroxy pyrazine)]-N'-(substituted aryl/alkyl) thiourea:

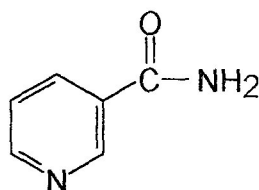
The literature search shows that 2-substituted pyrazine derivatives(I) exhibit antitubercular activity⁴¹, of which Pyrazinamide (II) is the clinically used, first line antitubercular agent.



Isoniazide (III) is the most commonly used drug for the treatment of tuberculosis and it contains nicotinic acid as the pharmacophore. Also, Nicotinamide(IV) contains nicotinic acid group, which is responsible for its anti-tubercular activity. As a result, Pyrazinamide and Nicotinic acid are considered bioisosters of each other⁴². This was the reason for selecting the pyrazine moiety as the lead structure in our work

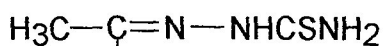


(III)

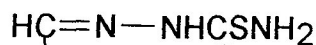


(IV)

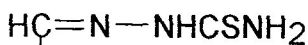
Thiosemicarbazones are reported to exhibit antitubercular activity Which is retained even if the benzene nucleus is substituted by a pyridine ring^{42a}. The thiosemicarbazone of 4-acetyl pyridine and 3- acetyl pyridine are also found to be active against *M. tuberculosis*. Eilhauer and co-workers have synthesized a series of 2- alkyl-4-acylpyridine thiosemicarbazone having pronounced antitubercular activity.



4-Acetylpyridine
thiosemicarbazone



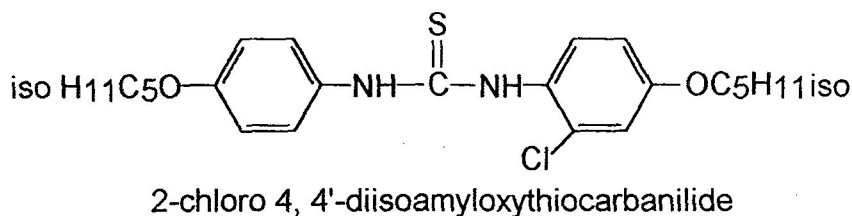
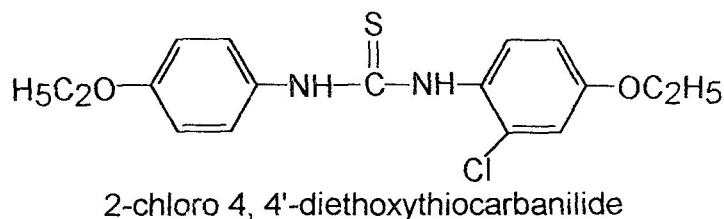
Isonicotinaldehyde
thiosemicarbazone



Nicotinaldehyde
thiosemicarbazone

In an attempt to achieve anti-tubercular activity it was decided to introduce a thiosemicarbazone on the selected pyrazine moiety.

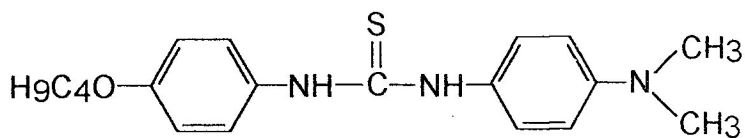
Cuong *et al* have reported the antitubercular activity of halogenated derivatives of alkoxythiocarbanilides⁴³ (V). These compounds have exhibited antitubercular activity in the range of 5µg/ml.



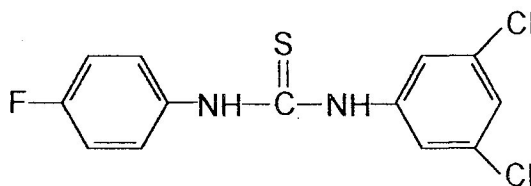
(V)

Buu-Hoi^{43a} and associates claim that the tuberculostatic activity of thiocarbanilides is due to their ability to form stable complexes with heavy metals. The thiourea portion is responsible for this ability. To achieve this activity the compound must have physical characteristics such that the partition coefficient between aqueous and fatty phases is favorable for the penetration of compound into the tissues of the host and into the bacilli.

These compounds contain (-NH-C=S) group, which acts as the pharmacophore. Also, 1,3-disubstituted thioureas are well documented in literature for their biological activity. e.g. thiambutasine⁴⁴ (VI), Fluonilide⁴⁵ (VII) is a well-known antifungal agent which is a simple disubstituted thiourea.

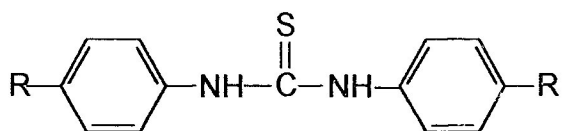


(VI)



(VII)

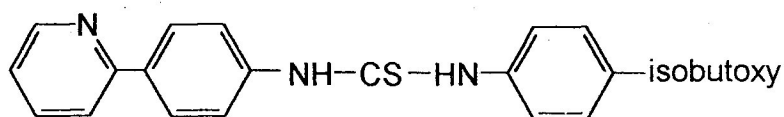
Fox *et. al.* showed that 4,4'-disubstituted diphenyl thioureas (VIII) have *in vitro* antitubercular activity.⁴⁶



R=Cl, F, OCH₃ etc.

(VIII)

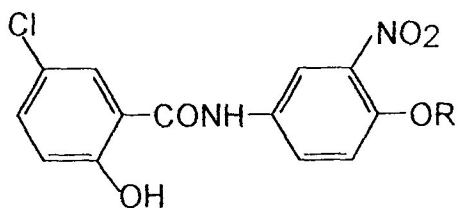
One of the most effective compounds encountered among the thiocarbanilides is p-isobutoxy-p'-(2-pyridyl) thiocarbanilide(thiocarbanidin). But in clinical trials it was not found to be very effective due to poor absorption from the human intestinal tract.



Thiocarbanilides

These references reveled the significance of the thiourea group and prompted us to include it.

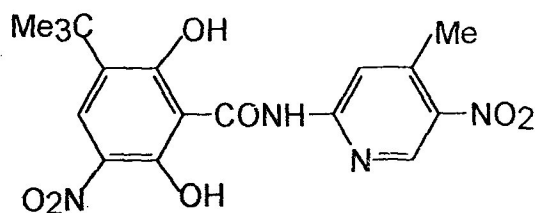
The presence of -CONH- linkage also plays an important role in imparting antifungal and antitubercular activity⁴⁷ to the molecules, which is reiterated by the following compounds (IX).



(IX)

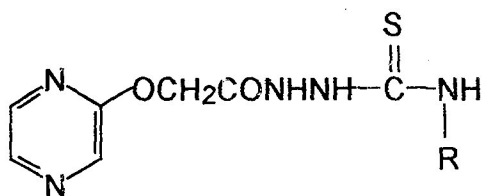
R=C₆H₅, C₂H₅ etc.

The interesting example of substituted pyridylamide (X) showed activity against *F. hepatica* and the contribution of -CONH- linkage to the activity is good.



(X)

Considering all these factors, a (-NH-C=S) group and a (-CONH-) group was incorporated on the pyrazine nucleus to obtain the title compounds (Xa). A total of 13 such compounds were synthesized.

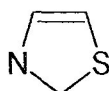


R=Substituted aryl/alkyl etc

(Xa)

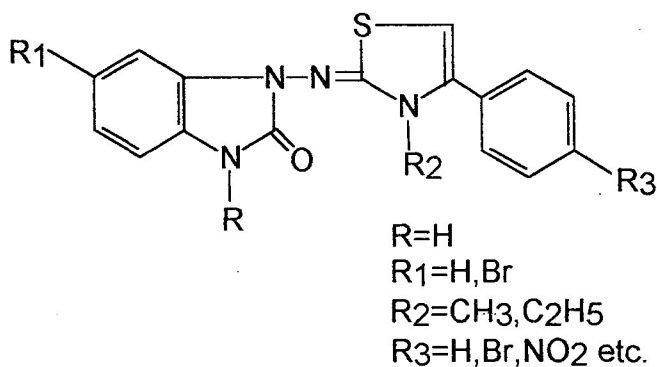
Synthesis of [3-(4'-chlorophenyl)-4-(aryl substituted)]thiazoline-(2-methoxy carbonyl pyrazine) hydrazones.

Thiazoline (XI) and its derivatives were found to exhibit a wide variety of biological activities such as antimicrobial⁴⁸ antiinflammatory, antihistaminic etc. This prompted us to include the pyridine and the thiazoline nucleus.



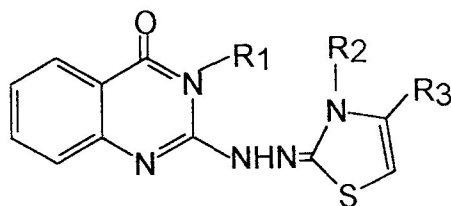
(XI)

Karali *et al*⁴⁹ synthesized derivatives of 3-[3,4-disubstituted-4-thiazoline - 2-ylidene hydrazino]-1H-2-indolinones(XII) as potent antitubercular agents having MIC <12.5 /ml against *M. tuberculosis* H₃₇ RV strain .



(XII)

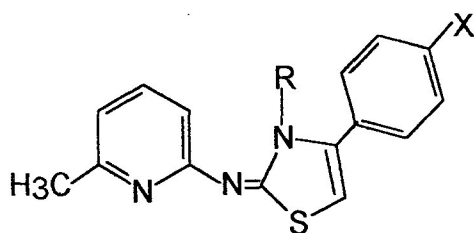
Thiazoline ring prepared by cyclization of thiourea was found to impart antimicrobial activity to the compounds.



R₁ = Allyl, C₆H₅NH₂ etc.
 R₂ = Allyl, C₆H₅NH₂ etc.
 R₃ = C₆H₅, 4-C₆H₅Cl etc.

In the above structure, the activity of the parent quinazoline was enhanced on converting it to its thiazoline derivative. These derivatives exhibited significant *in vitro* antibacterial activity against gram-positive organisms. The activity of some of the products was found to be equivalent to that of Penicillin.⁵⁰

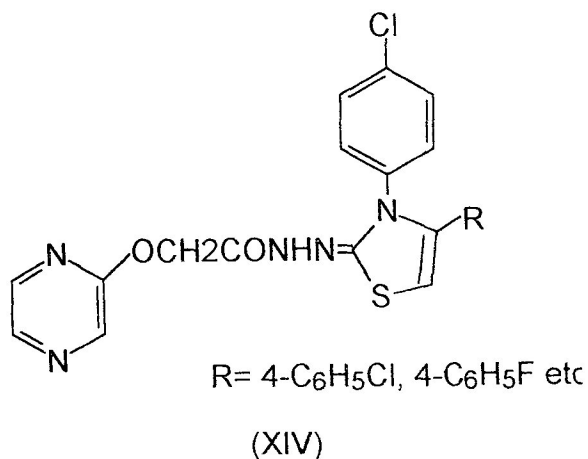
Hassan *et al*⁵¹ observed moderate antimicrobial activity in the compounds(XIII) bearing a thiazoline ring on the parent pyridine moiety.



X=Br, Cl, OCH₃ etc.
 R=CH₃, C₂H₅, C₆H₅ etc.

(XIII)

The activity of the thiazoline ring prompted us to introduce this moiety on the parent pyrazine molecule (significance discussed in part I) in an attempt to achieve anti-microbial activity for the title compounds (XIV).



All the compounds synthesized in series-1 and series-2 are subjected to antifungal, antitubercular and antibacterial screening

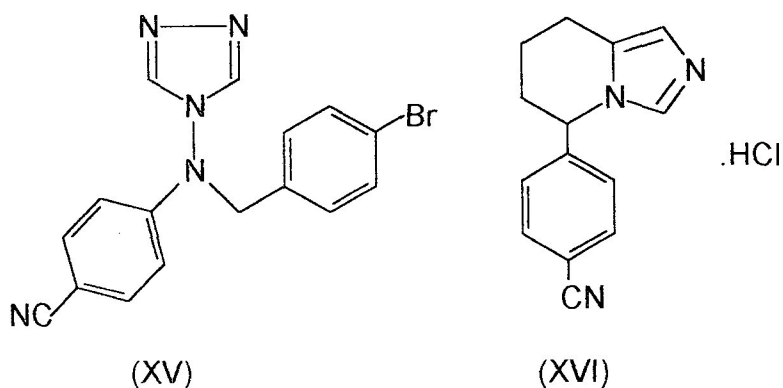
Synthesis of 4-[N-(4-Bromobenzyl)-N-(1,2,4-triazole-4-yl)benzonitrile (YM-511)]⁵².

At present there is no drug in the market with good anticancer (breast and ovarian cancer) activity. Hence we decided to prepare the drug YM-511, which is in phase 2 clinical trial and has shown some promising results.

The enzymes required for the formation of biologically active estrogens (aromatase, estrone, sulfatase and estradiol dehydrogenase) are present in both normal and malignant tissues. These enzymes are rational targets for the development of drugs to treat breast cancer and other diseases in which female hormones are known to be a contributing factor.

YM-511 (XV) is a new non-steroidal aromatase inhibitor with high potency and selectivity. It inhibits the enzyme from ovarian microsomes and human placenta with IC₅₀ of 0.4nM and 0.12 nM, respectively. It is found to be more potent than

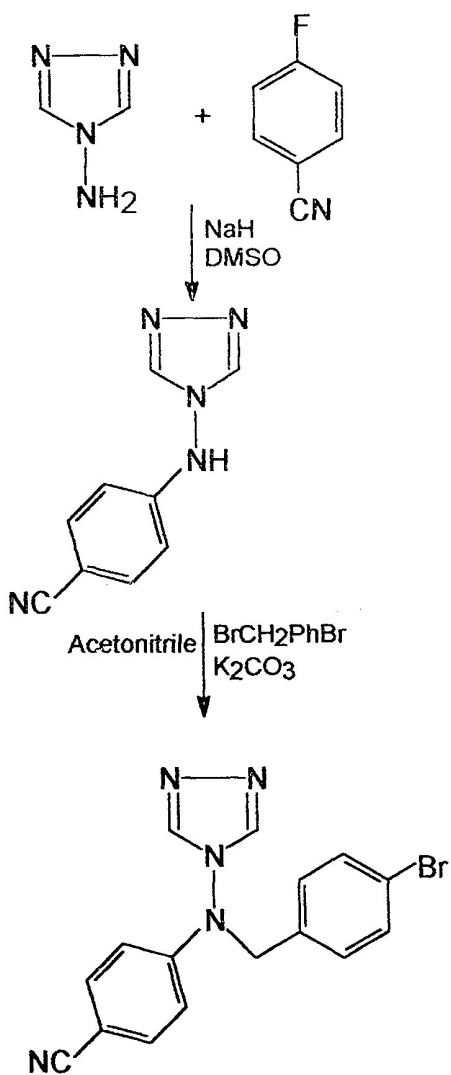
Fadrazole hydrochloride (XVI)(IC₅₀ 1.5nM and 0.37 nM, respectively)⁵², which is a new drug developed by Ciba-Geigy for similar indications.



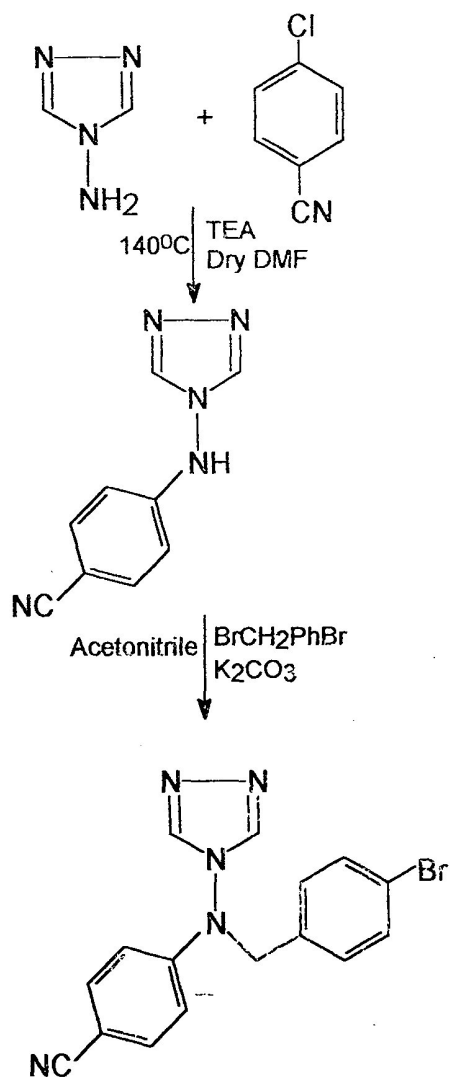
In addition, YM-511 was found to be more selective than Fadrazole. The route of synthesis of YM-511 has been reported in literature⁵².

However, we thought of synthesizing this compound by alternate route in an attempt to avoid use of toxic and hazardous chemicals like NaH and also to make the process cost effective.

Literature scheme



Modified scheme



As shown in the above scheme, we avoided the use of p- fluoro benzonitrile which is expensive as compared to p- chloro benzonitrile. We also avoided the use of NaH , which is a very toxic and explosive chemical.

CHEMISTRY

CHEMISTRY OF ISOTHIOCYANATES

In the past century, many sulfur compounds of plant origin attracted attention due to their pungent and irritant properties and their abilities to prevent the spoilage of food and fermentation of fruit juices. Two of these substances were found to be allyl isothiocyanate and benzyl isothiocyanate. These discoveries marked the beginning of an important class of compounds having the general formula R-NCS. It goes without saying that our knowledge of isothiocyanates has progressed rapidly, both in the chemistry of isothiocyanates and in specialized fields, such as the knowledge of interesting biological effects of naturally occurring and synthetic isothiocyanates.

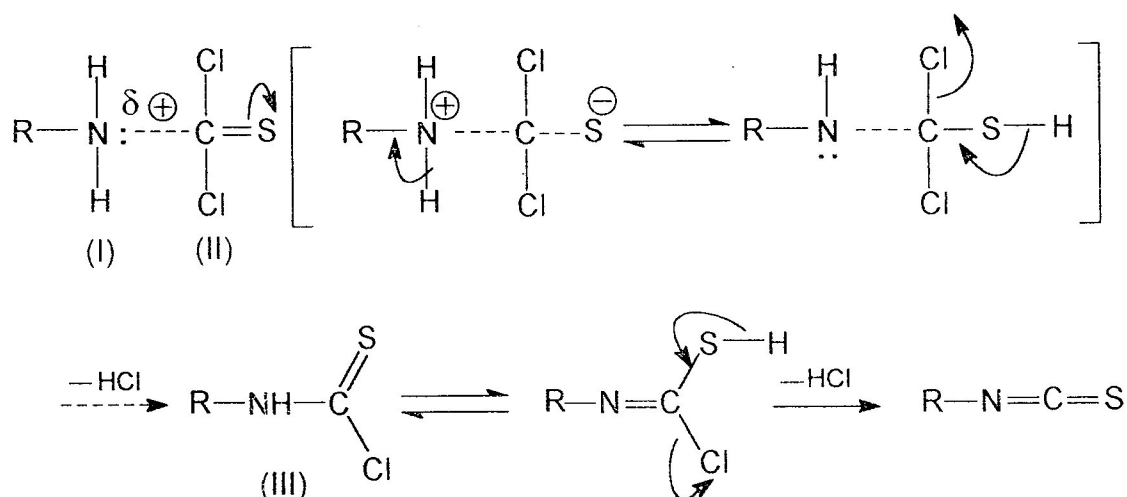
At present many methods are available for the preparation of the isothiocyanates but none of them are generally applicable. The choice of a method for the preparation of the isothiocyanate will depend upon the starting compound which is selected for the required type of isothiocyanates. Organohalides with a reactive halogen and primary amines serve most frequently as the starting substances. In the case of the organohalides, a substitution reaction with inorganic thiocyanates is followed by rearrangements of the resulting thiocyanates into corresponding isothiocyanates. These can be obtained either directly by the action of some sulphur compounds (e.g., thiophosgene, dithiocarbamoyl chloride, trichloromethyl sulphide, etc.) on amines or by converting them into suitable intermediates (dithiocarbamates, dithiocarbazates, thiourea, etc.), which are then decomposed into isothiocyanates by reaction with various agents.

1) Reaction of amines with sulfur compounds:

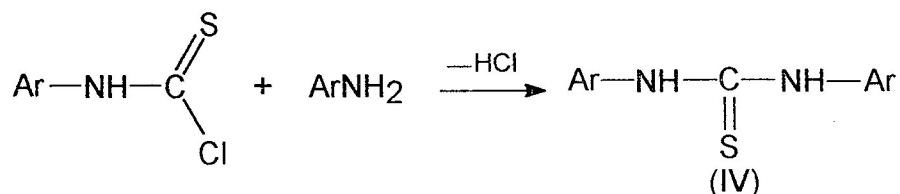
a) Thiophosgene:

The most generally used method for the preparation of isothiocyanates is based on the direct treatment of primary amine⁵³ (I) with thiophosgene (II). The first product of the reaction is an unstable thiocarbamoyl chloride (III), which

releases hydrogen chloride, yielding isothiocyanates⁵³. The course of the reaction can be illustrated by the following addition-elimination mechanism.



In the case of aromatic amines, especially with electron accepting substituents, N,N -disubstituted thioureas(IV) may be formed as by-products⁵⁴.



The formation of thioureas can be prevented by using a small excess of thiophosgene. Since isothiocyanates as well as thiophosgene are relatively insensitive to water, they can be prepared in an aqueous medium⁵⁵. Not only free amines but also their salts, particularly hydrochlorides, react with thiophosgene to produce isothiocyanates⁵⁶.

The free amine is frequently reacted by adding its chloroform⁵⁷, dichloromethane⁵⁸ or toluene⁵⁹ solution to the water emulsion of thiophosgene. Hydrogen chloride released in the course of the reaction combines with the strongly basic amine hydrochloride, which is very difficult to hydrolyze in water and thus complicates the

reaction of thiophosgene with the amine. For this reason, bases are frequently added to the reaction mixture for the binding of hydrochlorides e.g., calcium carbonate⁵⁸, sodium carbonate^{60, 61}, and triethylamine⁶². It is not advisable to use a strong base for this purpose since isothiocyanates are easily hydrolyzed in an alkaline medium.

During the preparation of isothiocyanates from the hydrochlorides of primary amines, an aqueous solution of amine hydrochloride is used, maintaining similar reaction conditions as employed with free amines⁵⁸. The addition of CaCO_3 or alkali carbonates keeps the reaction mixture neutral and assists in the slight hydrolysis of the hydrochloride. This modified thiophosgene method is used primarily for the preparation of aliphatic isothiocyanates and for amines that are sensitive to atmospheric carbon dioxide and oxidation (benzylamine, some diamines, etc.)

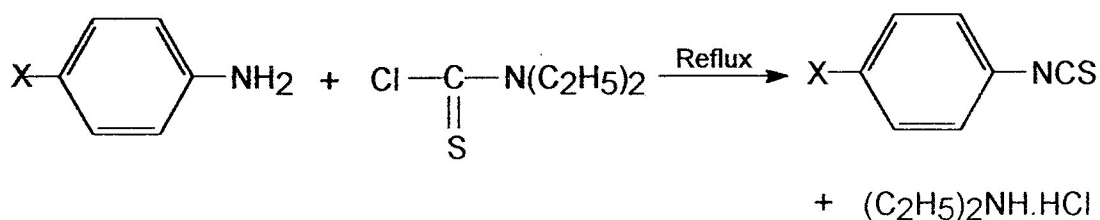
The thiophosgene method, thanks to its versatility and mild reaction conditions, is being used in the synthesis of isothiocyanates of very varied structures.

This is the simplest and most universal method for the preparation of isothiocyanates. It gives high yield and is suitable for the preparation of aliphatic as well as aromatic isothiocyanates. Its importance has increased due to the fact that thiophosgene has become a commercially available reagent, though some authors object its unpleasant odor and toxicity.

b) Thiocarbamoyl derivatives:

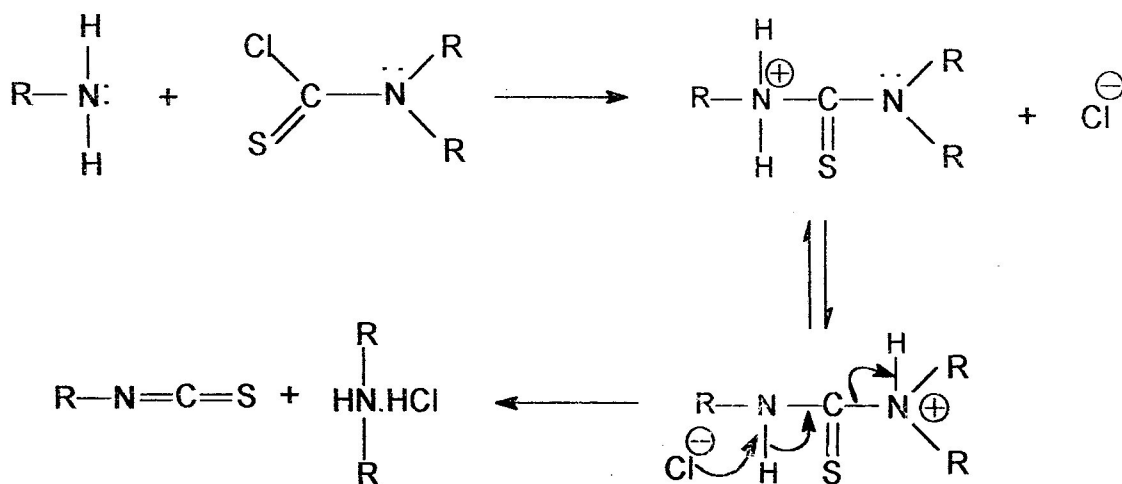
Sayigh and co-workers⁶³ have proposed the use of N,N- diethyl thiocarbamoyl chloride (V) as a new reagent which could replace the toxic thiophosgene. It is easily available and is suitable for the preparation of aromatic isothiocyanates with electron-withdrawing substituents. It is a one-step method and involves the heating of the aromatic amine with N,N- diethyl thiocarbamoyl chloride(V) in an inert

solvent such as chlorobenzene, benzene, toluene, or ethylene dichloride.



X = NO₂, CN, Cl

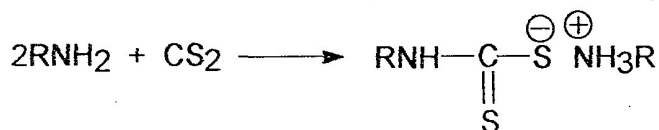
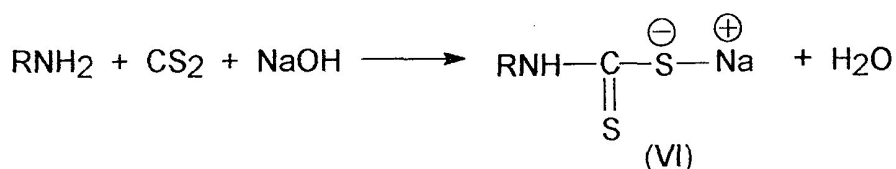
The yield decreases with the increasing basicity of amines. The limiting pK_a value of the reaction medium is 4.6. Under these conditions, phenyl isothiocyanates is obtained from aniline in a very poor yield. Reaction takes place via S_N² mechanism.



2) Decomposition of dithiocarbamic acid and its salts or esters:

a) Decomposition of heavy metal salts:

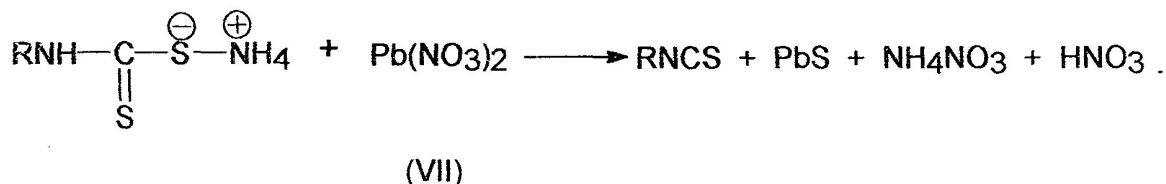
This method of synthesis involves two steps. In the first step the corresponding salts of dithiocarbamic acid (VI) are prepared by the action of carbon disulphide and alkali^{64, 65} (or ammonia⁶⁶) on the aqueous or organic (alcohol, methylene chloride, toluene, etc.) solution of the primary amine. Aliphatic amines react with carbon disulphide in the absence of other bases yielding alkyl ammonium dithiocarbamates⁶⁷.



Where R = alkyl.

Obviously, amines whose basicity is so low that they do not form dithiocarbamates cannot be used (e.g., nitroanilines).

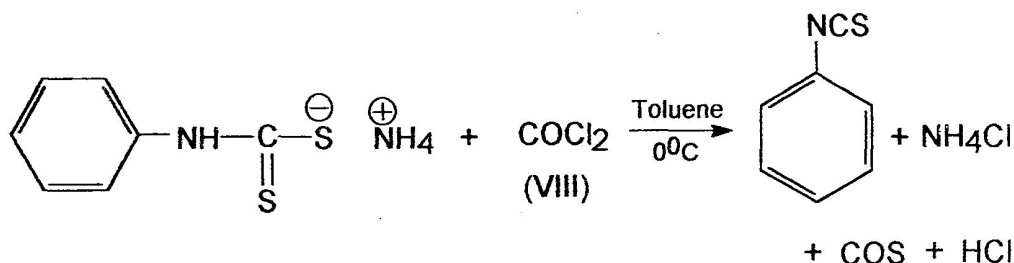
In second step, heating of alkaline dithiocarbamates with heavy metal salts (VII) in an aqueous medium produces unstable heavy metal dithiocarbamates, which decompose easily into isothiocyanates and metal sulphides.



For the decomposition of dithiocarbamates, mercury salts^{67, 68}, lead nitrate^{69, 70, 71}, cupric sulphate⁶⁸, ferric chloride⁷², etc. have been used.

b) Decomposition of chlorine containing compounds:

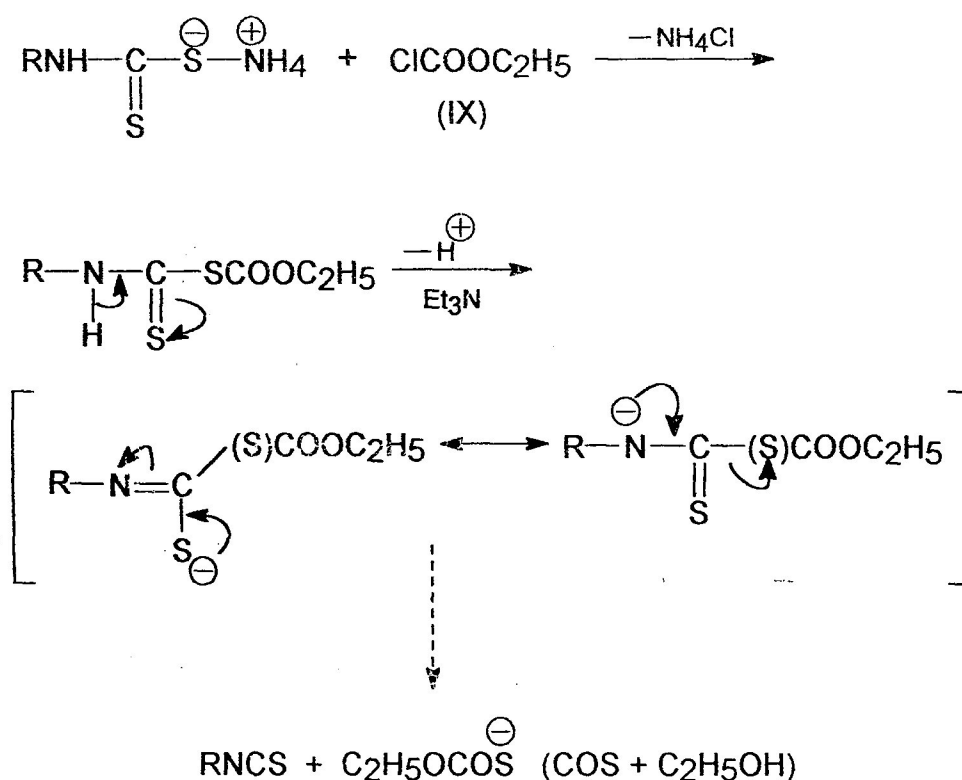
Some aromatic isothiocyanates are advantageously prepared by the decomposition of dithiocarbamates with phosgene (VIII)⁷³.



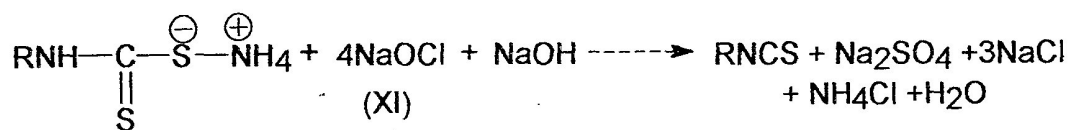
The phosgene method failed to succeed in the preparation of some

isothiocyanates with electron withdrawing substituents (4-bromophenyl isothiocyanates, nitro phenyl isothiocyanates etc). It has furnished very good yield in the case of 4-hydroxyphenyl isothiocyanate, which could be managed by thiophosgene method.

The decomposition of dithiocarbamates with ethyl⁷⁴⁻⁷⁸ or methyl chloroformates⁷⁹ (IX) produces aliphatic isothiocyanates in very good yields. The reaction takes place via the unstable carbethoxy dithiocarbamate (X), which decomposes into isothiocyanate, carbonyl sulfide and ethanol. Bases, like alkali and triethylamine, have a catalytic effect on the decomposition of carbethoxy dithiocarbamate^{80, 81, 82}.

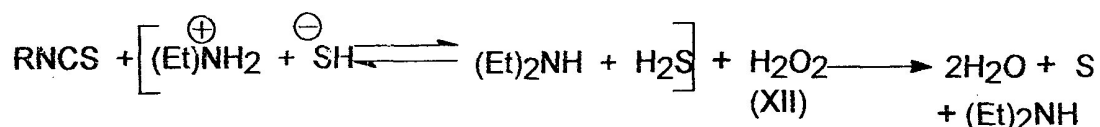
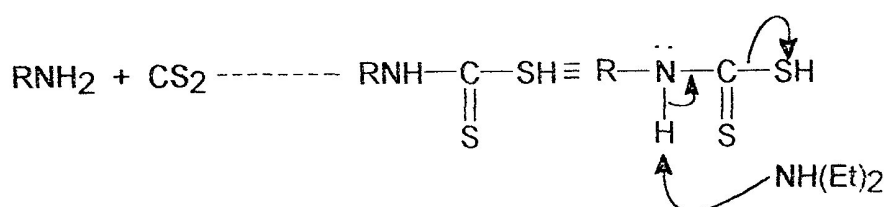


Aliphatic and aromatic isothiocyanates can also be prepared by the oxidative decomposition of dithiocarbamates using sodium hypochloride (XI) in an alkaline medium^{83, 84}.



c) Decomposition by hydrogen peroxide:

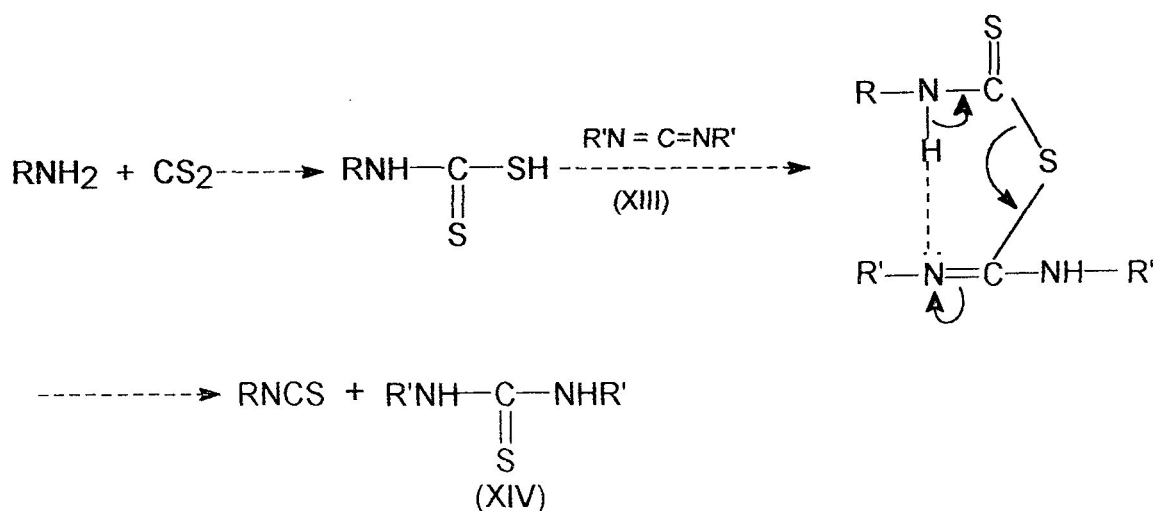
The treatment of a mixture of primary amine and carbon disulphide with hydrogen peroxide (XII) in the presence of a secondary aliphatic amine produces a rapid exothermic reaction, giving rise to isothiocyanates⁸⁵.



This one-step reaction is suitable for the preparation of aliphatic isothiocyanates. Thioureas are produced as by-products.

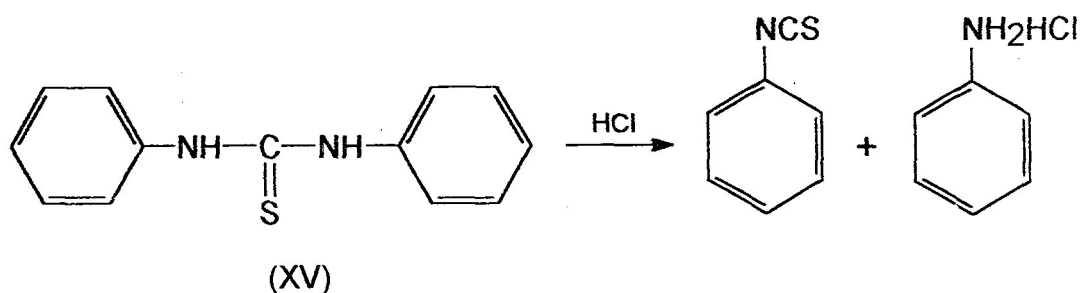
d) Decomposition involving carbodiimides:

Primary amines react with carbon disulphide and dicyclohexyl carbodiimides (XIII) in a suitable organic solvent at a temperature below 0°C, forming isothiocyanate and 1,3-dicyclohexyl thioureas (XIV). For the preparation of aliphatic isothiocyanates, ether or tetrahydrofuran⁸⁶ proved to be good solvents. Aromatic amines react under identical conditions only with a one half molar equivalent of carbodiimide yielding quantitatively, symmetrical diaryl dithioureas. If, however, the reaction is carried out in pyridine, and in some cases in the presence of a molar equivalent of triethylamine, then isothiocyanates are also formed from aromatic primary amines in very good yields⁸⁷.



3) Decomposition of thioureas:

Aromatic amines can be prepared by the decomposition of sym-diaryl thioureas (XV) with acids such as hydrochloric acid⁸⁸⁻⁹⁰, sulfuric acid^{91, 92} and phosphoric acid. The salt of the corresponding aryl amine is formed as a by-product⁹³.



Isothiocyanates are also obtained by the decomposition of N-monoaryl thioureas during prolonged heating in chlorobenzene^{94, 95}.

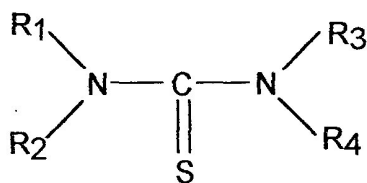
In the present work for the preparation of isothiocyanates, thiophosgene was used in the final reaction so as to minimize the side reactions, and to effect a clean workup of reactions. This gives a desired product of high purity.

CHEMISTRY OF THIOUREAS

Since the first synthesis of thiourea and its derivatives was made in about 1870, a vast literature on these compounds has accumulated. In fact an extensive knowledge of the properties and principles of synthesis and reactivity of thioureas was available at a relatively early stage in the history of thiocarbonyl chemistry. A main reason for this situation is that thioureas in general are well-defined, crystalline compounds of high stability which can be easily obtained by relatively simple synthetic procedures.

Thioureas have found wide application as pharmaceutical, insecticides, preservatives, and rodenticides. They are also of commercial value use in dyes, photographic films, elastic, textiles etc.

Thioureas in common practice are represented by the following structure (I).



(I)

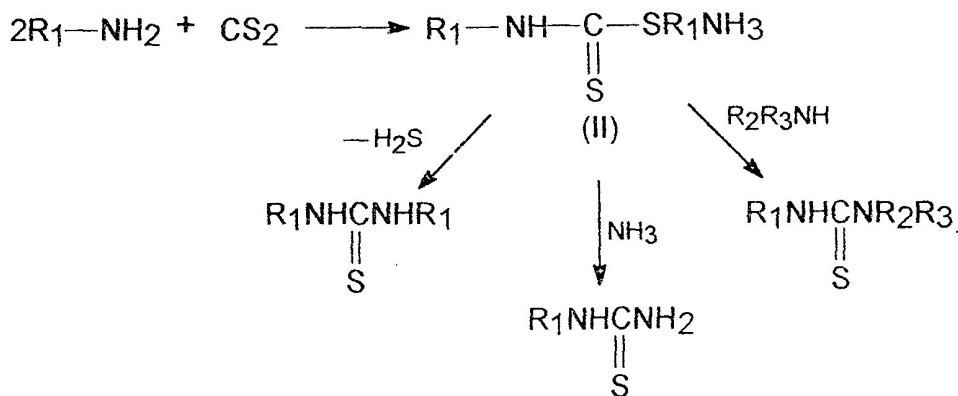
R_n = H, Alkyl, Aryl, etc.

Summary of general synthetic methods:

As a synthetic route to thioureas, the direct thiocyanation of the carbonyl group of urea by means of phosphorous pentasulphide, is of minor significance. Apparently, it applies to the synthesis of tetra-substituted thioureas and certain heterocycles having a thiourea moiety^{96, 97} incorporated in it.

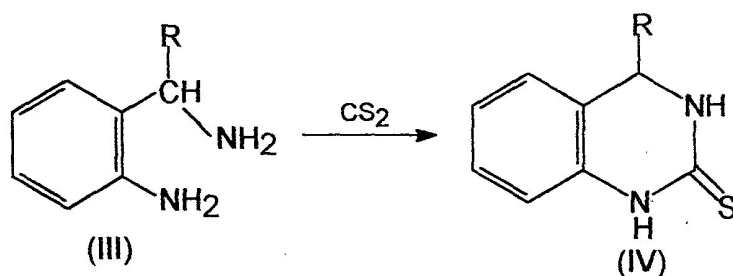
However, several other well-established methods are available for the synthesis of thioureas. One of the cheapest method of preparing symmetrical thioureas is based on the reaction of primary amines (I) with carbon disulphide^{96, 97, 98}.

The mechanism of this is not quite clear, but apparently an intermediate ammonium dithiocarbamate (II) is involved in the reaction, when carried out in the presence of ammonia or another amine, afforded by products derived from the latter.



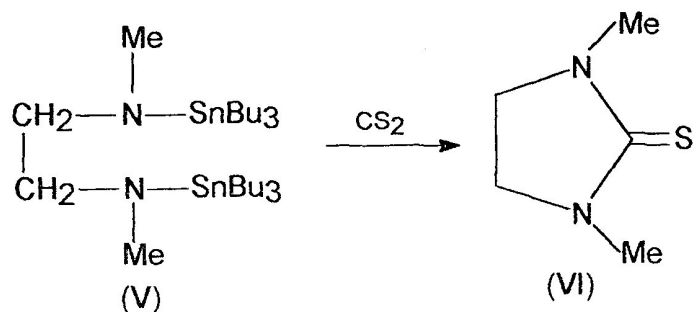
R_n= Alkyl, aryl etc.

The reaction is influenced by catalysts such as elemental sulphur, hydrogen peroxide, hydrogen ion, iodine and pyridine, and is also applicable to the synthesis of cyclic thioureas as exemplified by the preparation of (IV) from (III)⁸⁹.

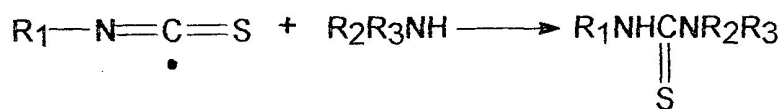


R = H, Me etc.

An interesting variation involves the reaction of the tributyl tin- substituted ethylene diamine (**V**) with carbon disulphide affording the cyclic thiourea (**VI**)¹⁰⁰.



Perhaps the most important standard method of synthesizing thiourea is based on the reaction of alkyl and aryl isothiocyanates with ammonia or amines¹⁰¹. By this reaction, monosubstituted, 1,3-disubstituted and 1,1,3-tri substituted thioureas can be obtained.

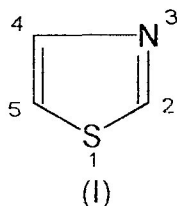


$\text{R}_1, \text{R}_2, \text{R}_3 = \text{H, alkyl, aryl, etc.}$

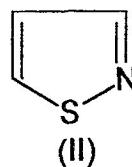
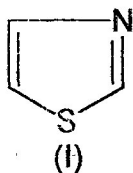
The broad scope of the reaction is most clearly demonstrated by the large variety of thioureas that have been synthesized on the basis of active participation of aliphatic, aromatic, open chain and cyclic amines, as well as of amine derivatives such as ketoamines, hydroxylamine, imino esters and amidines^{96,97,98}. The same reaction is used to synthesize the various thioureas and have been tabulated in table no-

CHEMISTRY OF THIAZOLE

The thiazole heterocycle (I) was first delineated by Hantzsch and Weber¹⁰². Further they have rightly described it as "pyridine of the thiophene series" since it closely resembles both the heterocycles, viz. pyridine and thiophene. Despite this the reactivity of the thiazole, by and large, is similar to that of pyridine.



The thiazole moiety exists in two different configurations as shown in (I) and (II), as the thiazole and isothiazole, respectively.



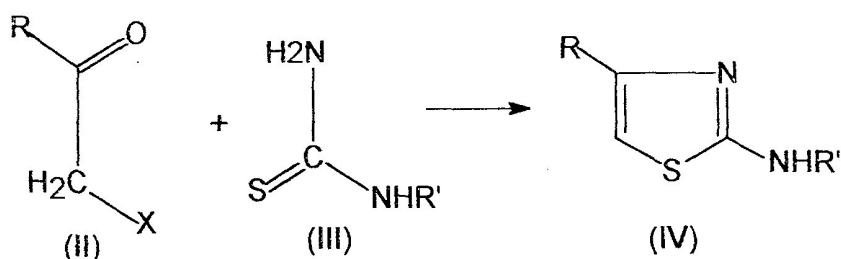
The thiazole derivatives have been more extensively studied as compared to the isothiazole derivatives, since they appear as an important structural requirement in innumerable naturally occurring compounds possessing diverse properties.

The thiazole derivatives appear in the composition of vitamin B1 (thiamine) and in the penicillins. Many fungicides, pesticides and bactericides are derivatives of 2-amino thiazole. Other thiazole derivatives possess mito-depressive and mito-static properties¹⁰³. Certain Schiff bases derived from 2-amino/2-phenyl thiazoles and their derivatives show diuretic properties¹⁰⁴. Mercapto thiazole derivatives are used to accelerate the vulcanization of rubber, while alkyl and acyl thiazoles are known to be flavoring agents. Several thiazole derivatives have been isolated from products.

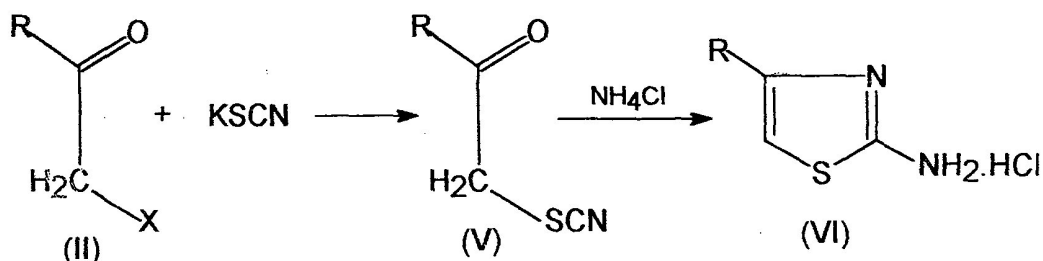
General Methods For The Synthesis Of Thiazole Derivatives:

Methods of preparation of amino thiazoles and its derivatives:

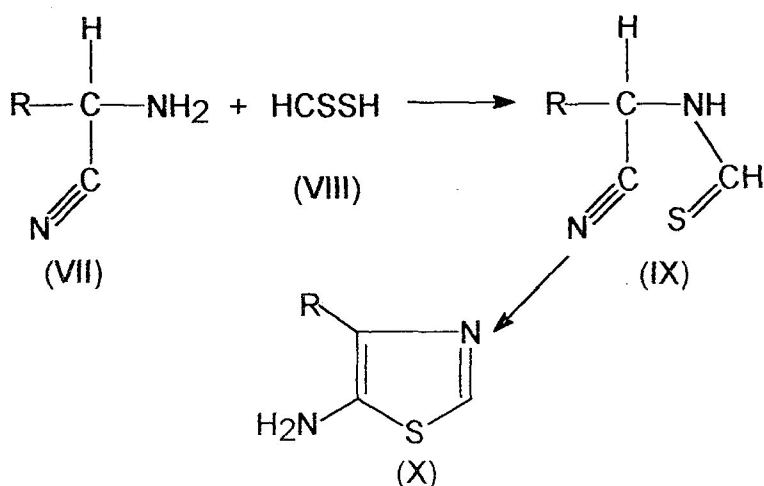
i) The most common method employed for the synthesis of substituted thiazole has been the one described by Hantzsch¹⁰⁵. The reaction between α -halo carbonyl compound (II) and thiourea /substituted thiourea (III) yields the respective 2-amino thiazole or its derivative (IV).



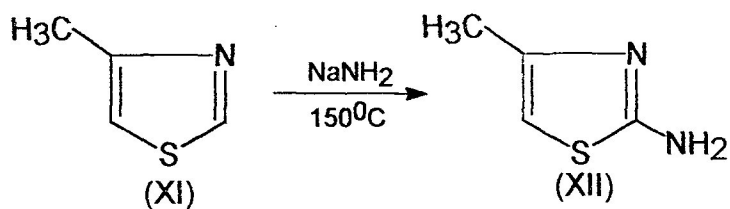
ii) Potassium thiocyanate reacts with α -halo carbonyl compound (II) to yield α -thiocyanato ketone (V), which on treatment with ammonium chloride yields the hydrochloride of 2-amino thiazole (VI)¹⁰⁶.



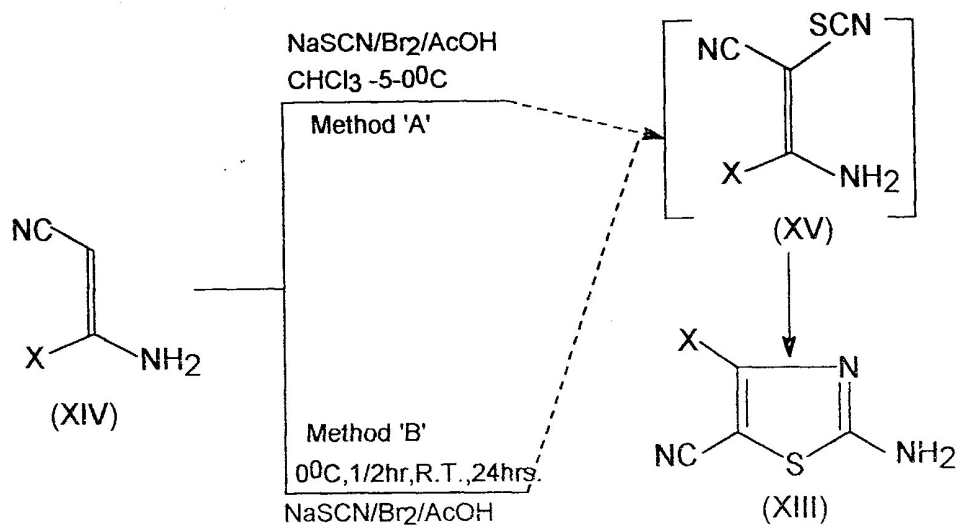
iii) α -aminonitrile (VII) reacts with dithioformic acid (VIII) at room temperature to give an intermediate (IX), which cyclises to yield the 5-amino thiazole (X) derivative¹⁰⁷.



iv) 2- amino 4- methyl thiazole (XII) is formed by heating 4- methyl thiazole (XI) with sodamide for 15 hours at 150°C^{108} . This reaction has been extensively studied for the preparation of 2- amino pyridine, but has received less attention in the case of thiazole nucleus.

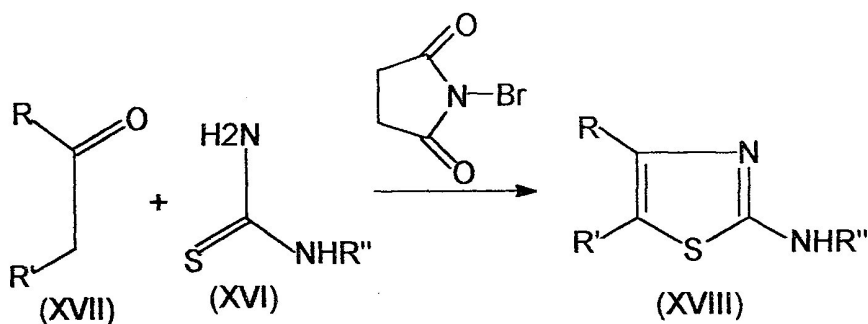


v) Recently, Maria, T.C. *et al* have reported¹⁰⁹ an improved procedure for the preparation of 2,4 diamino thiazoles(XIII) by reacting 3-amino 3-(dialkylamino)-propene nitriles (XIV) with sodium thiocyanate in the presence of bromine and acetic acid. The intermediate (XV) formed cyclises rapidly to form the thiazole (XIII) derivative.

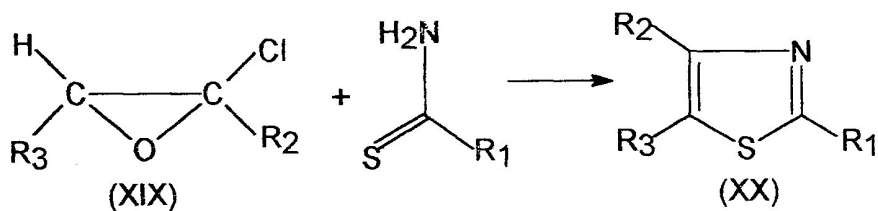


X = Pyrrolidino, piperidino, morpholino, 4-methyl piperazino, benzyl amine etc.

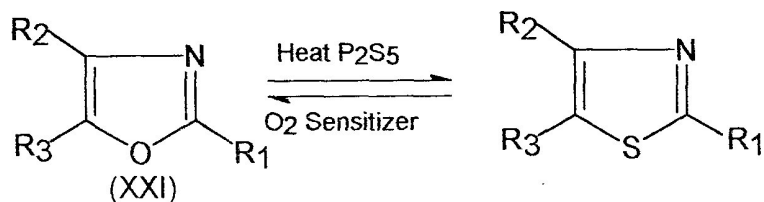
vi) The reaction of substituted thiourea (XVI) with ketones (XVII) in presence of N-bromosuccinimide furnishes the 2-amino thiazole derivative (XVIII)¹¹⁰.



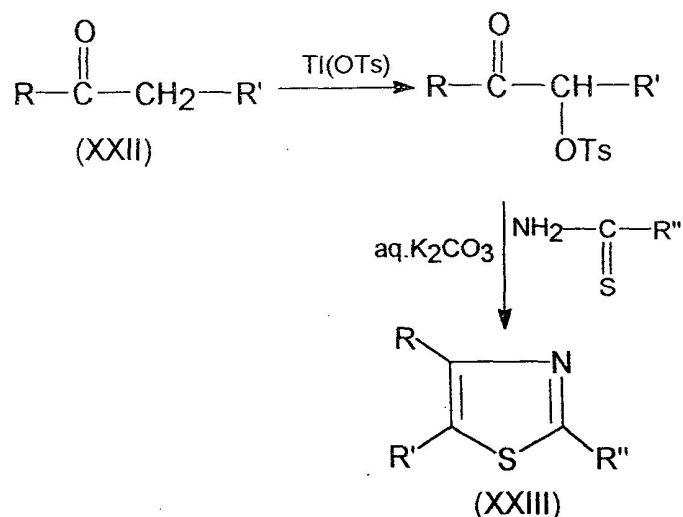
vii) 1-chloro-1,2-epoxides (XIX) condensed with thioamides leads to 2,4,5-trisubstituted thiazoles (XX)¹¹¹.



viii) Thiazoles from oxazole have also been reported¹¹². Oxazole (XXI) heated with P_2S_5 can be converted to thiazoles.

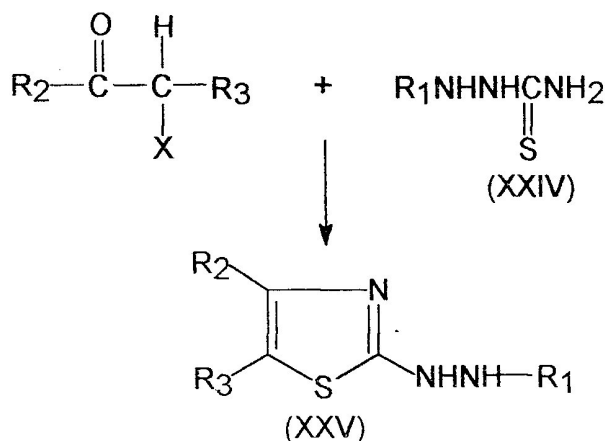


ix) Enolisable ketones (XXII) react with thioamides / substituted thioamides in acetonitrile, in the presence of thallium (III) p-tolyl sulphonate (TTS), to yield 2-substituted thiazoles (XXIII)¹¹³.

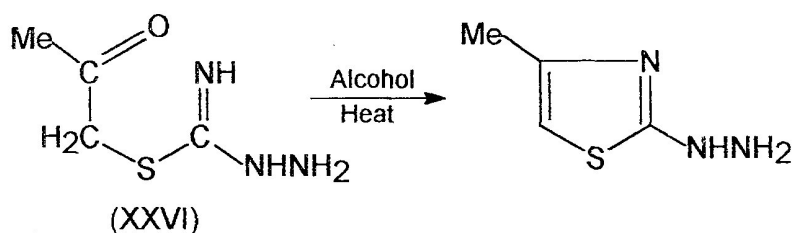


B) Methods for the synthesis of 2- hydrazino thiazole and its derivatives.

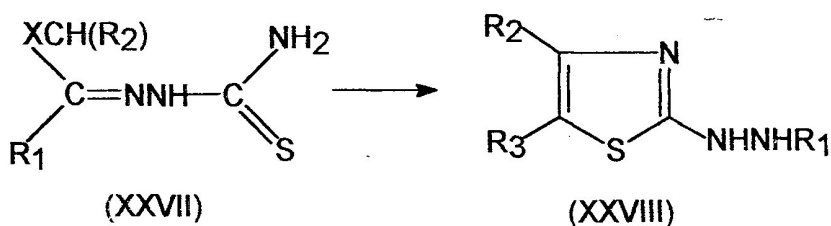
i) Thiosemicarbazides (XXIV) $R_1=H$, yield the 2-hydrazino thiazoles(XXV) on reaction with α -halo-carbonyl compounds and several derivatives of this type have been reported with^{114,115} $R_1=R_3=H$ and $R_2= CH_3, C_6H_5$.



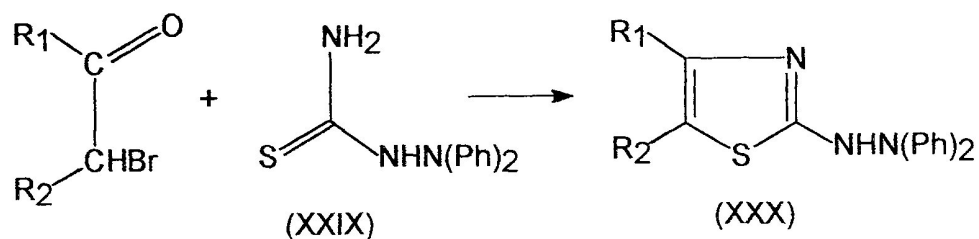
ii) With chloroacetone in neutral medium, acyclic intermediate (XXVI) gives thiazoles analogous to those obtained^{116,117} with thiourea and thioamides.



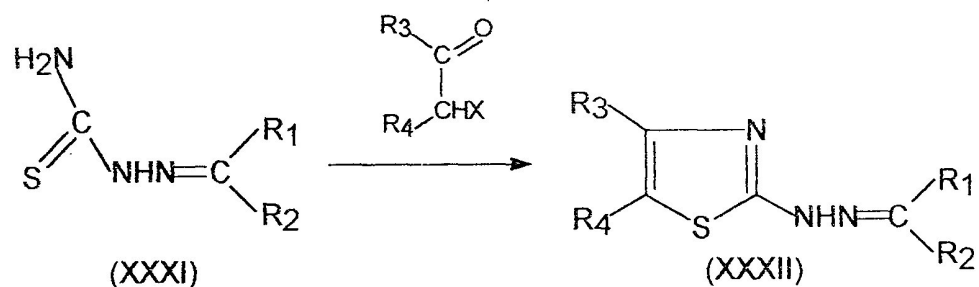
iii) Under acidic conditions, thiosemicarbazone intermediate (XXVII) has been isolated, and cyclised in alcohol to the corresponding 2-hydrazino thiazole (XXVIII) in the presence of benzaldehyde^{118,119,120}.



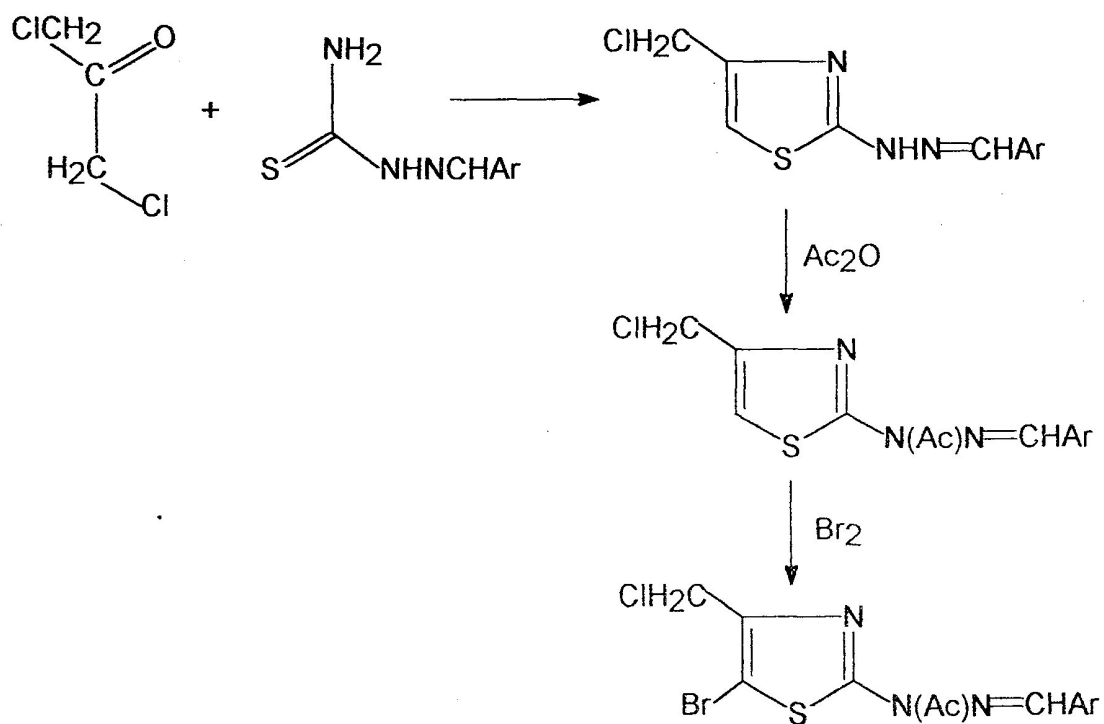
iv) Disubstituted thiosemicarbazides such as (XXIX) gave α -hydrazino derivatives (XXX) $\text{R}_1=\text{CH}_3$ and C_6H_5 and $\text{R}_2=\text{H}$, CH_3 , C_6H_5 the yield ranges between 60-90%¹²¹



v) Thiosemicarbazones of general formula (XXXI) react with α -halo carbonyl compounds to yield the corresponding 2-hydrazinothiazole (XXXII)^{122,123,124}.

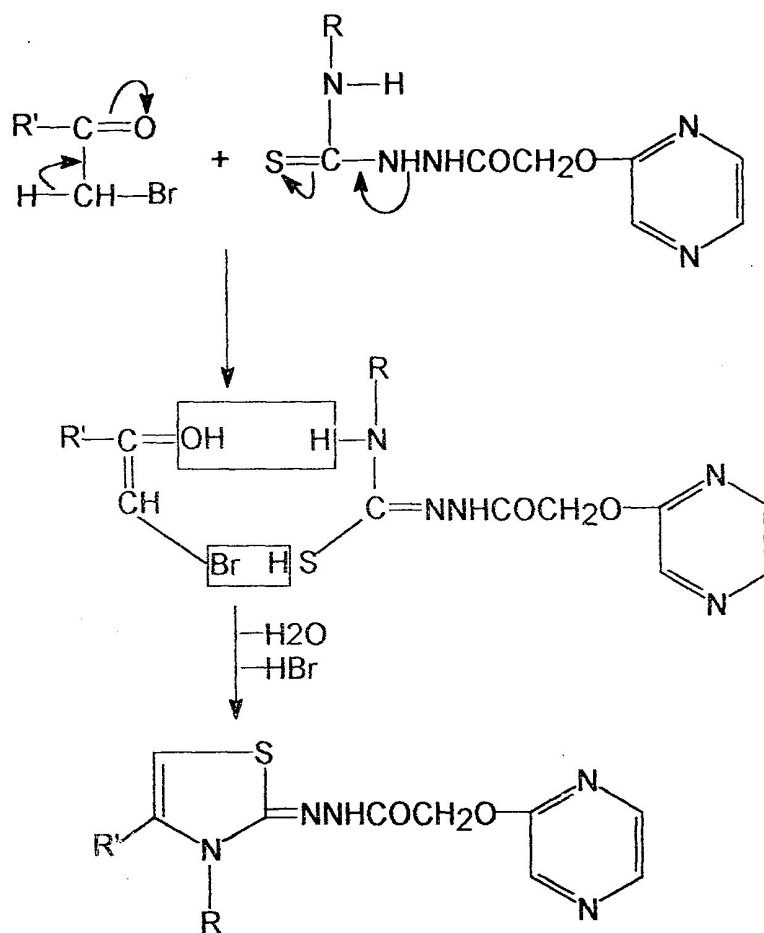


vi) The interaction of sym-dichloroacetone with thiosemicarbazones similarly yields 2-arylidene hydrazine-4-chloro methyl thiazoles¹²⁵, which can be further acylated and brominated



In our course of synthetic work, we have condensed the α - haloketones with N[acetamido(2-hydroxy pyrazine)]-N'-(p-chloro phenyl) thiourea.

MECHANISM

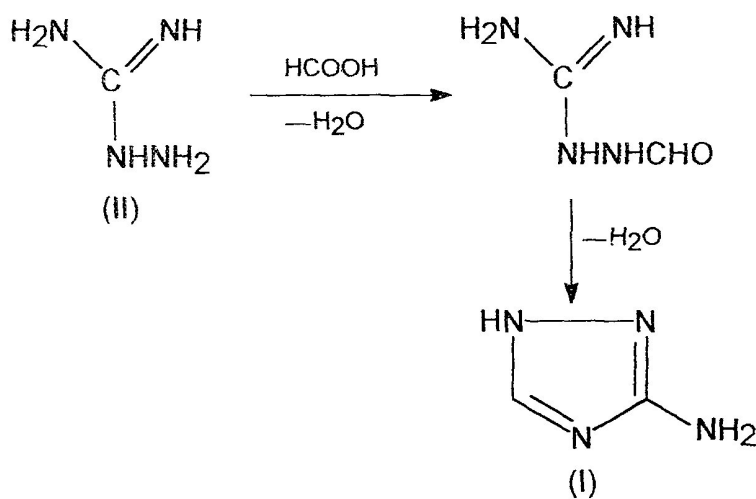


CHEMISTRY OF TRIAZOLES

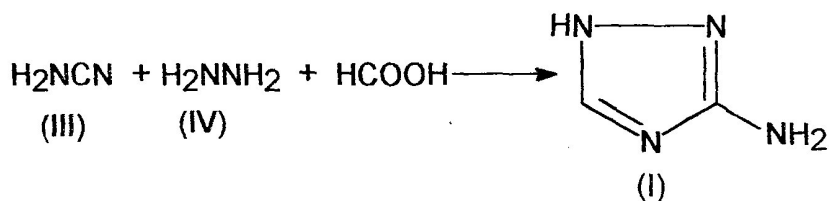
General Method for the synthesis of amino triazole derivatives:

A) Method for the preparation of 3-amino triazole and its derivatives.

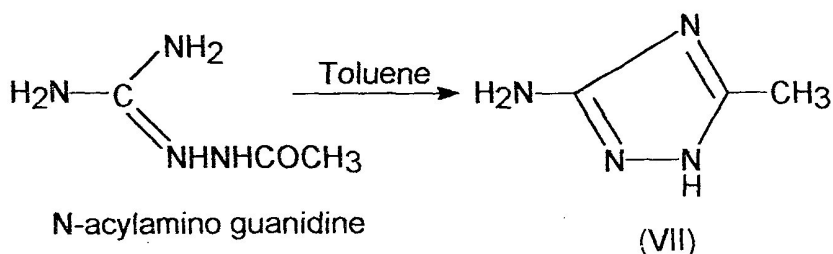
i) The preparation of 3-amino triazole (I) is readily accomplished by heating a mixture of an amino guanidine (II) salt of ($\text{H}_2\text{CO}_3, \text{HCl}, \text{HNO}_3, \text{H}_2\text{SO}_4$) and formic acid in toluene and separating the water formed in the reaction ^{126,127}



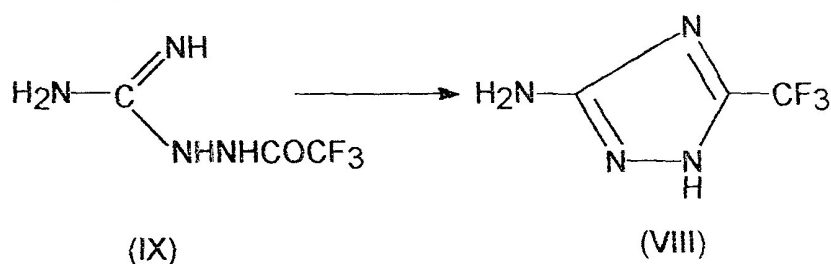
ii) Pure 3-amino triazole has been claimed to result from the interaction of an equimolar mixture of cyanamide (III) and hydrazine (IV) with formic acid ¹²⁸.



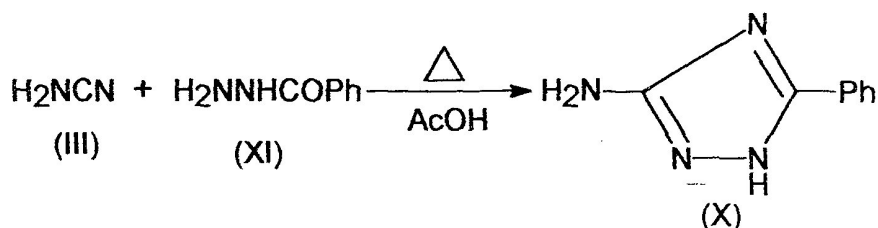
iii) The conversion of N-acylamino guanidines (VI) to 3-amino -5-alkyl triazoles (VII) has been effected by azeotropic removal of water in hot toluene ¹²⁹ and by treatment with dilute base, either at room temperature ^{130,131} or at reflux, ^{132,133}



iv) Excellent yields of perfluoroalkyl triazole (VIII) were obtained by the thermal dehydration of the hydrazides of perfluoro carboxylic acids (IX). The cyclisation of this type of hydride in basic medium was unsuccessful.

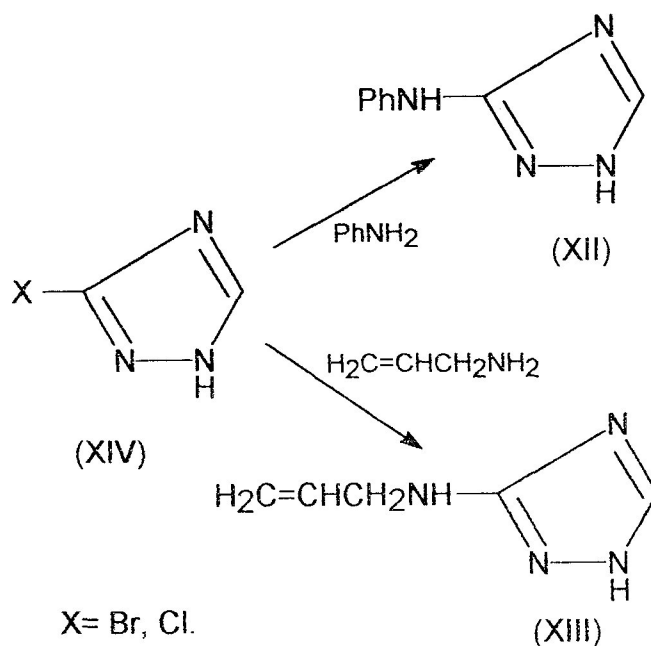


v) The 3-amino-5-aryl triazoles (X) are prepared by the treatment of aryl hydrazines (XI) with cyanamide (III) in acetic acid under reflux¹³².

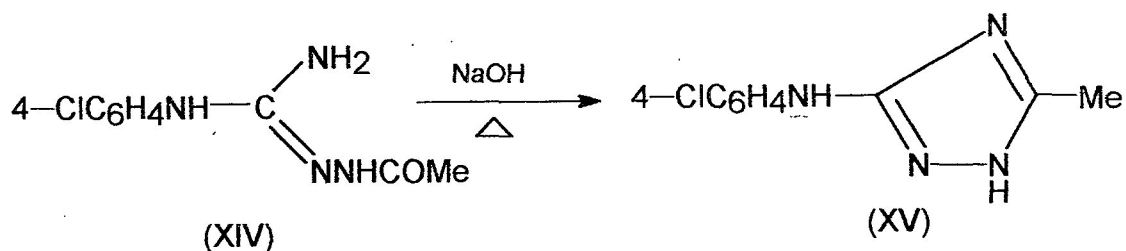


Various 3- amino-5-aryl triazoles are reported to be prepared by various methods including the cyclisation of N-arylamino guanidines either in hot base or fusion of solid at temperatures greater than 100 °C^{132,134-136}.

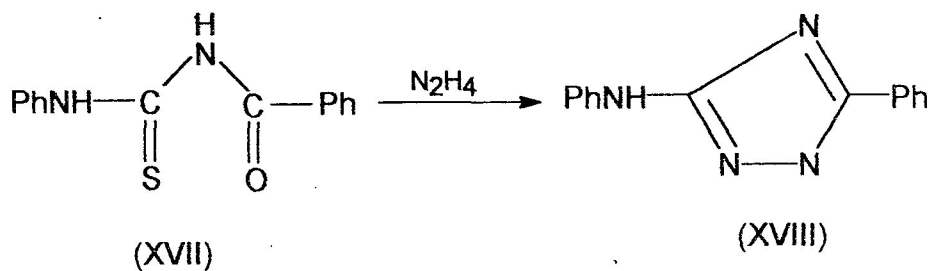
vi) The simple 3-substituted amino triazoles (XII) and (XIII) are easily prepared in high yields by amination of 3-bromo triazoles (XIV) with alkyl and aryl amines at temperatures greater than 130 °C¹³⁷⁻¹³⁹.



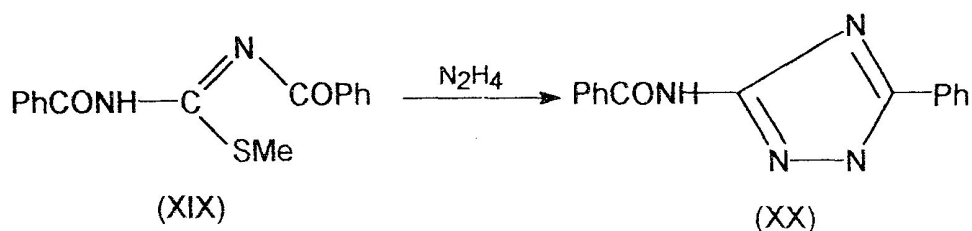
vii) Excellent yields of 3- arylamino-5-alkyl triazoles (XV) were produced by the cyclisation of N-aryl-N'-acylamino guanidine (XIV) with aqueous sodium hydroxide^{136,140}. This method is illustrated by the conversion of (XIV) to (XV) in which cyclisation occurs between the carbonyl function and the unsubstituted amino group.



viii) 3-aryl-1-benzoylthiourea (XVII) reacts with hydrazine in hot toluene to give 3- arylamino-5-phenyl triazole (XVIII)¹⁴¹.

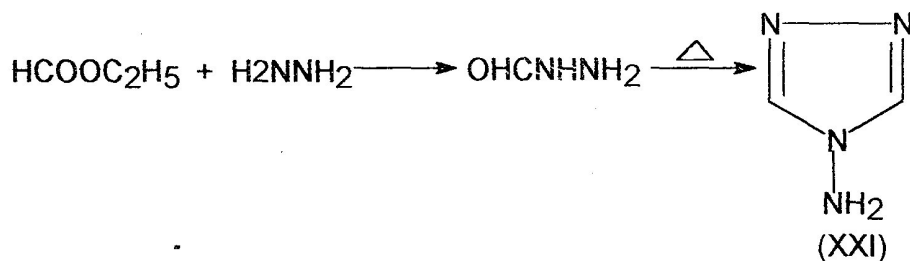


Under similar conditions the conversion of 3-alkyl-1-benzoyl thiourea to the triazole was unsuccessful. In contrast, the 1,3-dibenzoyl 5-methyl isothiuronium compound (XIX) and hydrazine hydrate in refluxing ethanol gave 85% yield of 3-benzamide 5-phenyl triazole (XX)¹⁴²

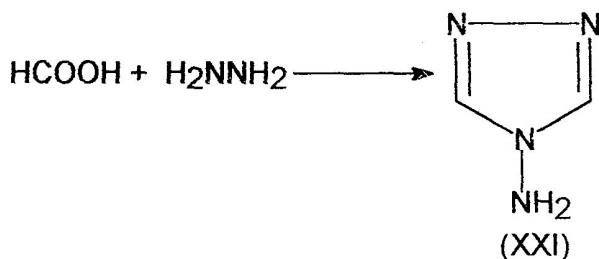


B) Methods for the preparation of 4-amino triazole and its derivatives:

i) 4-amino triazole (XXI) has been prepared^{143,144} by the reaction of ethyl formate (XXII) with hydrazine (IV) to give formhydrazide, which was heated at 150 to 200°C to effect self condensation to give (XXI).

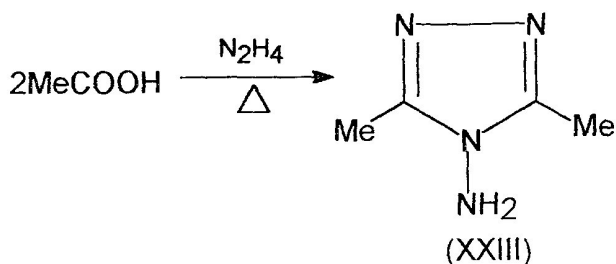


Similar interaction of formic acid and hydrazine gave good yield of 4-amino triazole(XXI)¹⁴⁵⁻¹⁴⁷.

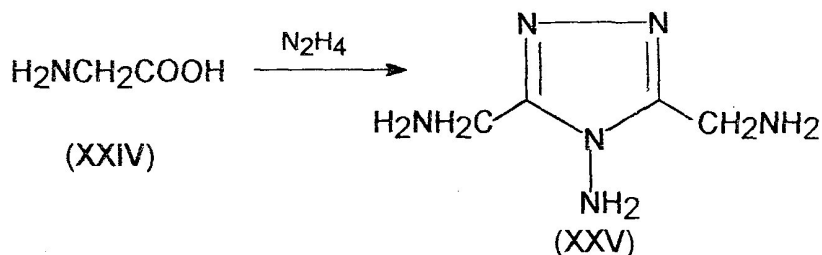


ii) Other carboxylic acids and their functional derivatives have also been used to prepare alkyl and substituted alkyl-4-amino triazoles e.g. acetic acid and hydrazine

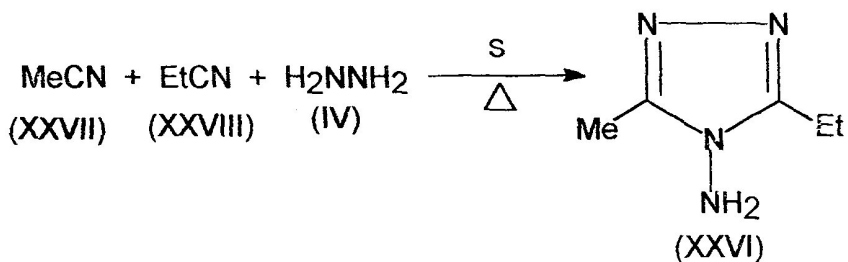
hydrate at high temperature gave 3,5-dimethyl-4-amino triazole (XXIII)^{145,146,148}



iii) Similar reactions have been reported for the preparation of 4-amino-3,5-bis(amino alkyl) triazoles^{149,-151} which are prepared by the reaction of glycine (XXIV) with hydrazine to give (XXV).

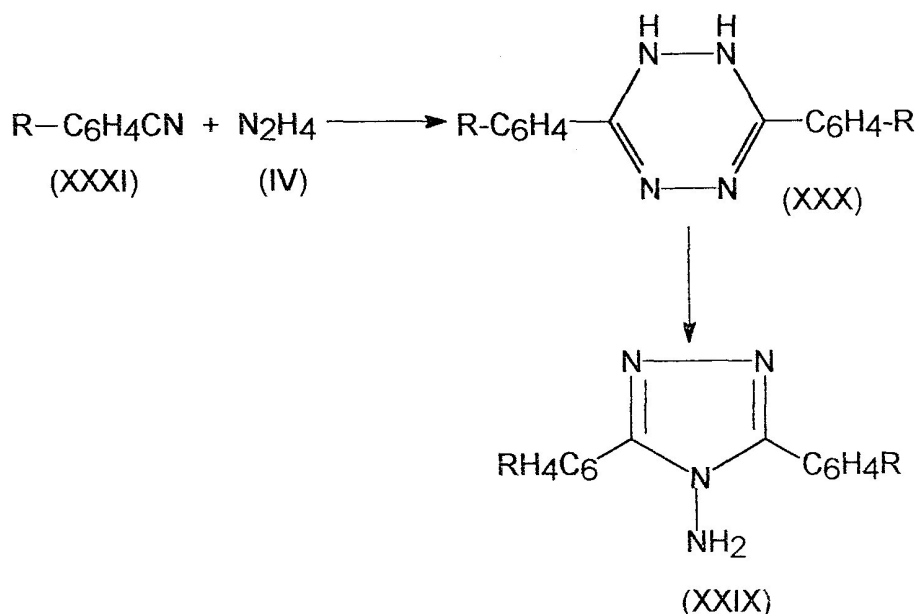


iv) Sulphur or sulphur containing compounds catalyze the formation of 4-amino triazoles e.g. 4-amino triazole (XXVI) substituted at 3- and 5- position with different substituents was prepared by the reaction of equimolar amount of acetonitrile(XXVII) and propionitrile (XXVIII) with a mixture of hydrazine (IV) and sulfur¹⁵².

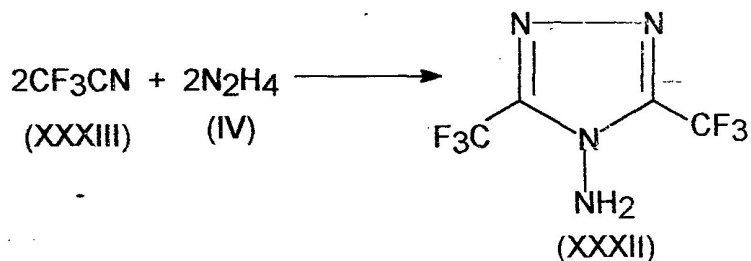


v)The preparation of 4-amino- 3,5-diaryltriazoles(XXIX) is usually effected by the

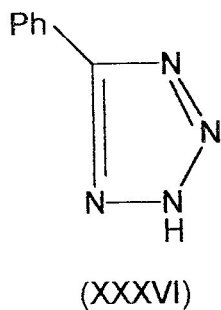
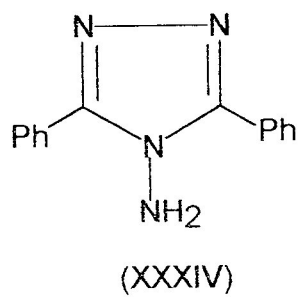
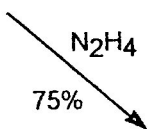
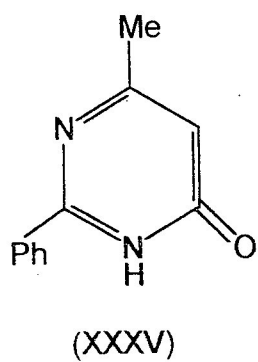
acidic rearrangement of 1,2-dihydrotetrazines(XXIX) which are obtained by the reaction of equimolar amounts of aryl nitriles(XXXI) and hydrazine, refluxed in benzene/ toluene¹⁵³.



vi) Perfluoroalkyl triazoles (XXXII) are prepared by various methods e.g. perfluoro acetonitrile (XXXIII) with hydrazine (IV) in sealed tube at room temperature gave (XXXII)¹⁵⁴.



vii) 4-amino-3,5-diphenyl triazoles (XXXIV) are also formed from the decomposition of other hetero aromatic ring systems, e.g. reaction of oxo pyrimidine (XXXV) with hydrazine¹⁵⁵. And also, the thermolysis of 5-phenyl tetrazole (XXXVI) in various solvents¹⁵⁶ gives (XXXIV).



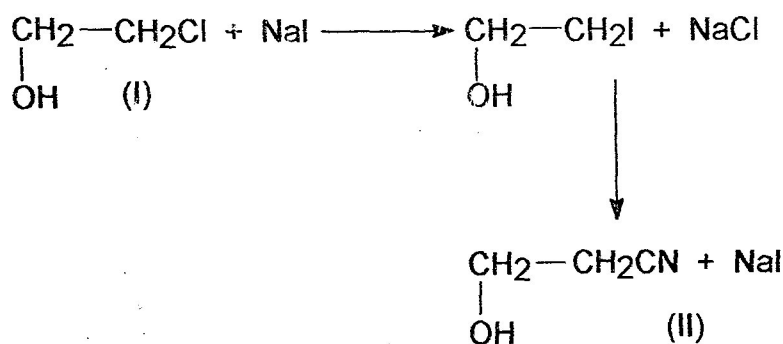
CHEMISTRY OF NITRILE

Nitriles are prepared by the classical methods described in literature and are listed below.

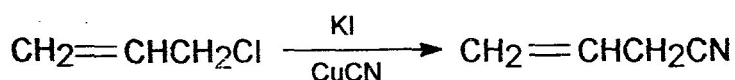
1) From halides:

a) From aliphatic halides:

The metal cyanides react with alkyl (I) or aryl halides to provide mainly aliphatic nitriles (II). The reactivity of the halides in decreasing order is $-I > -Br > -Cl$. Therefore, the yields of nitriles obtained from primary halides are increased by the introduction of promoters such as sodium iodide, which by exchange, converts the chloride first into the iodide. This in turn, reacts with the metallic cyanide to form the nitrile.

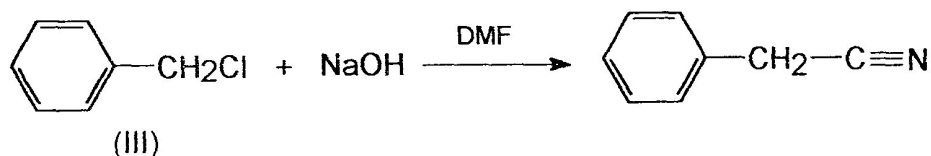


Other metallic cyanides used to form nitriles are potassium cyanide or cuprous cyanide.

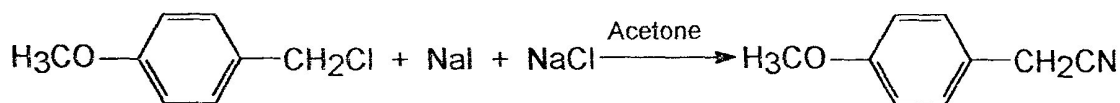


The yields in such cases vary from 75-90%. The solvent alcohol accelerates the reaction very slowly and higher solvents like ethylene glycol¹⁵⁷, methylcellulose¹⁵⁸, tetrahydrofuryl alcohol¹⁵⁹ and DMF improve yields; but with secondary halides the yields are low and tertiary halides give little or no nitriles. The conversion of alkyl halides (III) into nitriles is accomplished best with sodium or cuprous cyanide with

pyridine, quinoline, dimethyl formamide¹⁶⁰ or N-methyl pyrrolidine¹⁶¹ as solvents. The reaction time can be shortened by the addition of small amount of nitrile and traces of copper sulphate as catalyst.



Anisyl chloride (IV) gives its nitrile in 75% yield when it is treated with sodium iodide and sodium cyanide in dry acetone¹⁶².

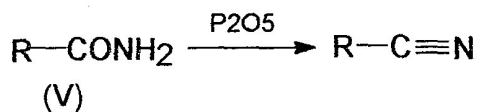


b) From heterocyclic halides:

The halides of pyridine¹⁶³, quinoline¹⁶⁴ yield nitriles satisfactorily with cuprous cyanide alone¹⁶¹.

2) From amides:

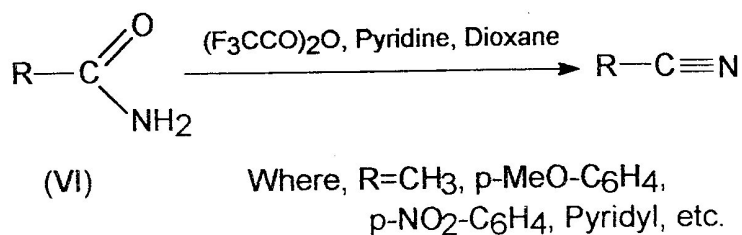
The preparation of nitrile from the amide (V) can be performed in 60-70% yield. The water is removed by using usual dehydrating agent, like phosphorous pentoxide¹⁶⁵ or phosphorous pentachloride¹⁶⁶.



Sometimes, dehydrating agents like carbonyl chloride¹⁶⁷, benzene, p-toluene sulphonyl chloride¹⁶⁵⁻¹⁶⁷, phosphorous oxychloride are used in the nucleophilic solvents such as pyridine and tertiary amines. Even addition of salts like sodium chloride¹⁶⁸ or sodium metabisulphite¹⁶⁹ to phosphorous oxychloride, enhances the effectiveness of the reagents.

The recently reported convenient synthesis of nitriles¹⁷⁰ under mild

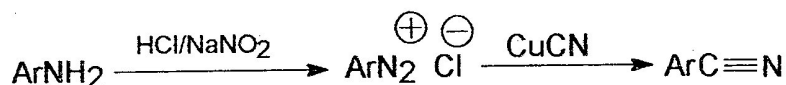
conditions, makes use of the trifluoroacetic anhydride/ pyridine system, which leads to rapid conversion of amide (VI) to nitrile with 85-95% yields.



The excellent yields and mild reaction conditions of the new method are especially useful for thermally labile nitriles.

3) From diazonium salts(Sandmeyer's reaction)

The replacement of aromatic amino groups with cyanide is obtained in 65-85% yields by the action of cuprous cyanide diazonium compounds (VII)



The double or complex salt of cuprous cyanide such as Na₃Cu(CN)₅NH₃¹⁷¹ has also been employed to a great extent. While carrying out the reaction, it is important to neutralize the diazonium salt before addition to the cyanide solution, in order to avoid the escape of hydrogen cyanide. o-tolunitrile¹⁷² can be obtained by this procedure in 64-70% yield.

4) From aldehydes:

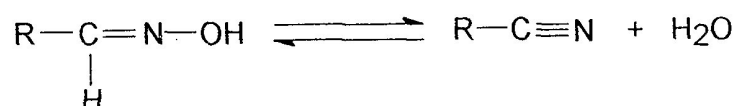
Many methods have been reported for the conversion of aldehydes to nitriles, e.g.

(a) dehydration of aldoximes using dicyclohexyl carbodiimides¹⁷³, carbonyl biimidazole¹⁷⁴, or phosgene immonium chloride¹⁷⁵, (b) 1,2-elimination of o-substituted aldoxime^{176,177} and (c) 1,2-elimination of R-CH=N-N-(CH₃)₃ and R-CH=N- OTs by base¹⁷⁸.

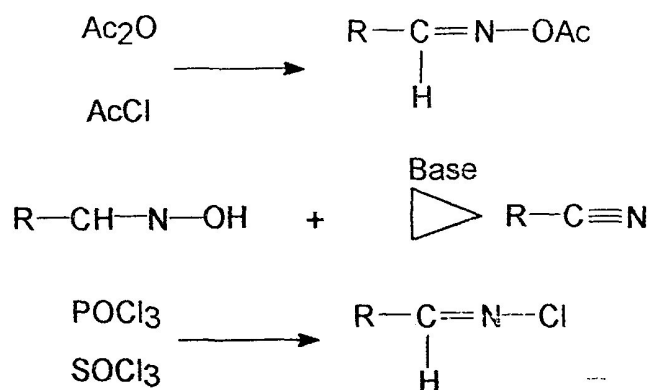
Method "A" is discussed under the title "dehydration of aldoximes" and Method "B"

and "C" are discussed under the headings " 1,2-elimination of o-substituted aldoximes.

Method-A (Dehydration of aldoximes)

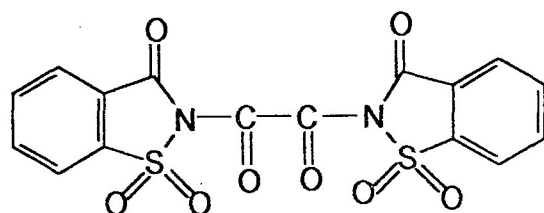


The dehydration of aldoximes represents one of the method for preparation of nitriles from corresponding aldehydes. A large number of dehydrating agents are employed to accomplish the dehydration of aldoximes¹⁷⁹. e.g. acetic anhydride, phosphorous oxides, thionyl chloride, acetyl chloride, etc. In all these cases, the dehydrating agent forms a reactive complex (VIII), which renders the aldoximes to lose a water molecule easily.

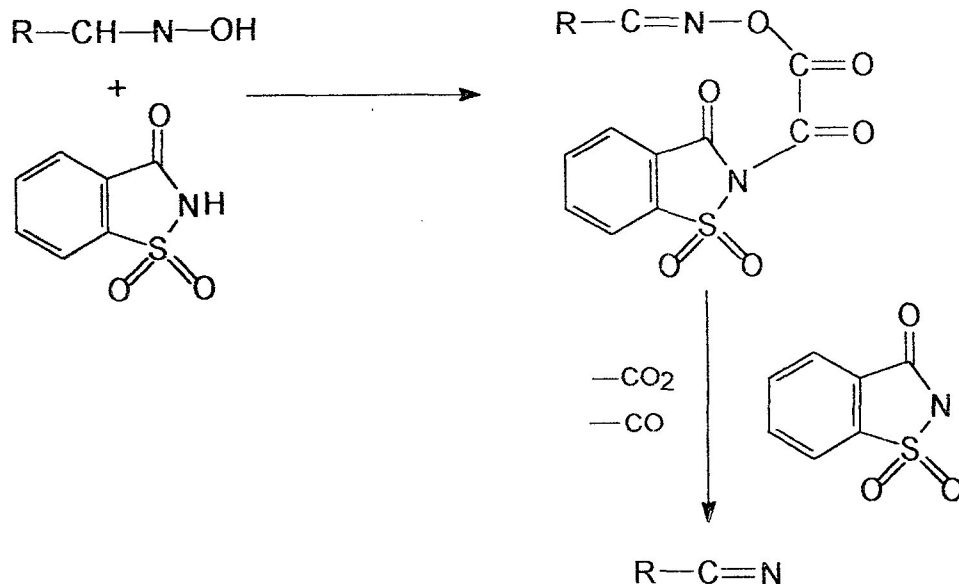


Recently, new dehydrating agents have been introduced, e.g. dicyclohexyl carbodiimide¹⁷³, carbonyl biimidazole¹⁷⁴ and phosgene immonium chloride¹⁷⁵.

Very recently, Kitagawa, T. *et al*¹⁸⁰ described a new dehydrating agent, 2-2'-oxalyldi(o-sulphobenzimide)(ODS)(IX) for a convenient one-pot synthesis of nitriles from aldoximes. The possible mechanism proposed was as follows-



ODS (IX)



These reagents are highly reactive and involve a very clean reaction at room temperature or cold temperature.

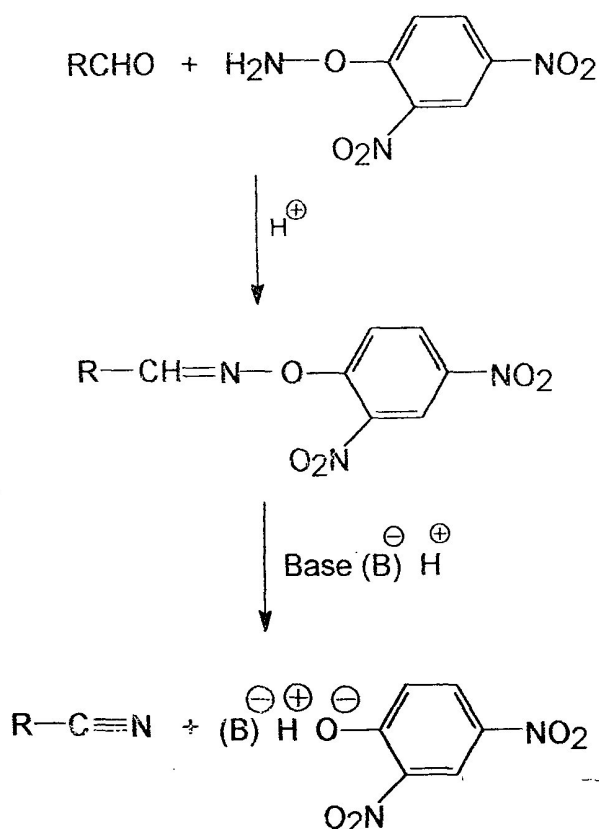
Method-B & C (1,2-Elimination of o-substituted aldoximes)

Method B:

This is the method recently developed method by Miller *et. al*¹⁷⁷. for accomplishing the conversion of aldehyde to nitriles. This method differs from other routine methods in the fact that the chemist need not isolate and purify the intermediate oximes or related aldehyde derivatives, although he may do so, if he wishes. This method, therefore, saves one step in the aldehyde to nitrile conversion.

The development of efficient amino protecting groups as facilitate the synthesis of a variety of sensitive compounds, including o-substituted hydroxylamines¹⁸¹.

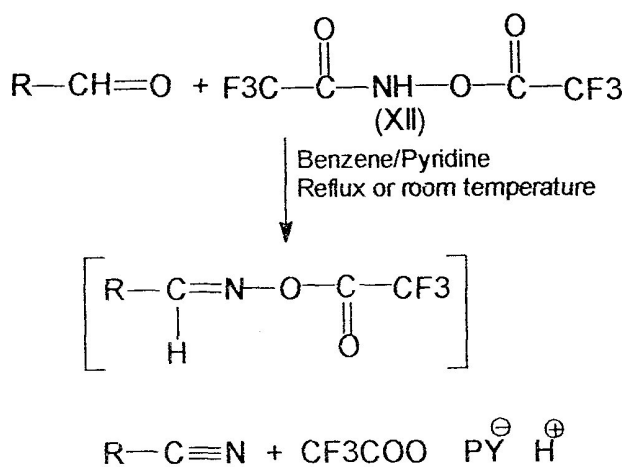
Although a number of these are highly unstable¹⁸², others have significant synthetic utility. Their electrophilic character has been exploited in their increasing use as aminating agents¹⁸³⁻¹⁸⁵. Their nucleophilicity has been demonstrated by their reaction with ketones to form substituted oximes. Thus, 2,4-dinitrophenyl hydroxyl amine (X) can be reacted with aromatic or alkyl aldehydes(XI) to form corresponding o-substituted aldoximes.



Nitriles can be prepared from these o-substituted aldoximes by 1,2- elimination reaction using excess base [KOH or N(C₂H₅)₃], as shown above. The development of deep yellow to red color of the 2,4-dinitrophenolate ion indicates the extent of completion of the reaction.

Method C:

This method is one-step reaction involving the use of O-N-bis (trifluoroacetyl) hydroxyl amine (XII) as one of the reagent as follows-

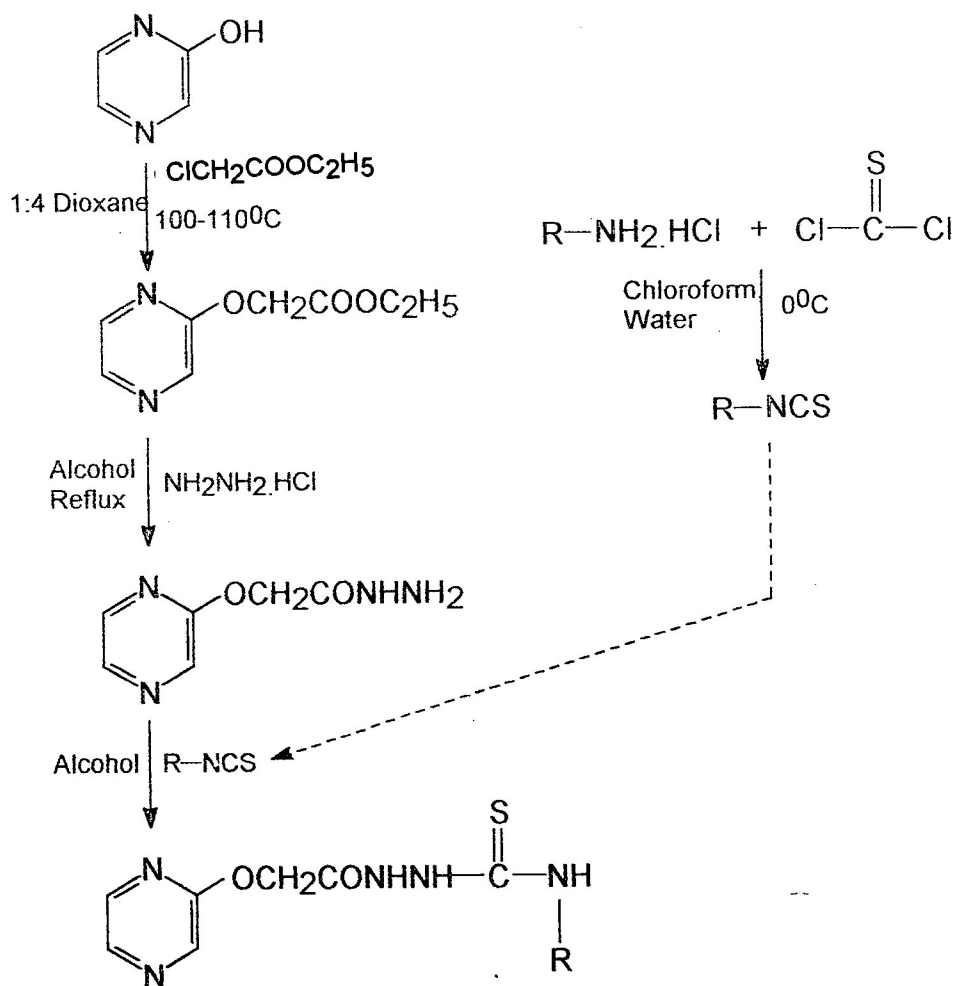


The reaction conditions are very mild and directly afford nitrile elimination, the intermediate step¹⁸⁶. The yields are in the range of 70-90 %.

The reagent could be obtained by reacting trifluoroacetic anhydride with hydroxyl amine hydrochloride in suitable solvents. The intermediate aldoxime is so unstable that it could not be isolated and instantly decomposed to furnish nitrile; thus, eliminating the intermediate step.

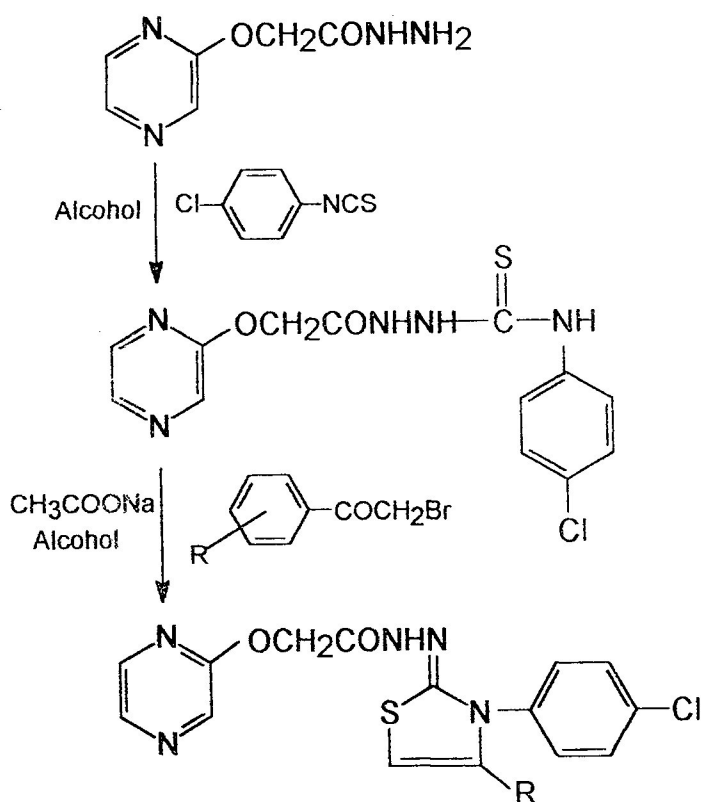
SCHEMATIC PRESENTATION

SCHEME-I



R=Substituted aryl/alkyl

SCHEME-II



$R = \text{Substituted Aryl}$

EXPERIMENTAL

Preparation of ethyl ester of 2- methoxy Pyrazine:

In a 500 ml three necked round bottomed flask equipped with a reflux condenser, dropping funnel and guard tube, was placed a mixture of triethylamine (26.35g; 0.26M), hydroxy pyrazine (20g; 0.21M) and 200 ml dry 1,4-dioxane. The reaction mixture was stirred with a mechanical stirrer for 10-15 min. To this was added drop wise a solution of chloroethyl acetate (43.48g, 0.26M). During addition the temp was maintained at 90⁰c for one hour. Then the reaction mixture was stirred for 7-8 hrs. at 100⁰C. The excess dioxane was then removed under vacuum. The sticky solid mass thus obtained was dumped in ice-cold water and extracted with chloroform. The chloroform layer was evaporated to dryness under vacuum and the product obtained.

Yield: 72%

M.P. :68-69⁰C

Elemental analysis of C₈H₁₀N₂O₃

	% C	% H	% N
Calculated	52.74	5.49	15.38
Found	52.61	5.31	15.26

Synthesis of 2- methoxy hydrazino pyrazine:

A mixture of ethyl ester of 2- hydroxy pyrazine (15g; 0.08M), hydrazine hydrate (94.12g, 0.08M) and absolute alcohol (100ml) was refluxed for 12 hrs. The excess solvent was distilled off. The reaction mixture was cooled to 4-5⁰C and solid crystals that separated were filtered, washed with cold water, dried and recrystallized from ethanol.

Yield: 62%

M.P. : 170-171⁰C

Elemental analysis of C₆H₈N₄O₂

	% C	% H	% N
Calculated	42.85	4.76	33.33
Found	42.62	4.51	33.28

Synthesis of 2,5- dimethoxy phenyl isothiocyanate:

In a 200 ml three necked flask equipped with mercury sealed mechanical stirrer, water condenser and dropping funnel, was taken thiophosgene (4.24g; 0.04M) in 100 ml chloroform. To it was added a solution of 2,5 dimethoxy aniline hydrochloride (7.58. 0.04M) in water, drop wise, maintaining the temperature at 0°C. The reaction mixture was stirred further at this temperature for 4 hrs. The chloroform layer was separated and washed with water till it was neutral. The chloroform layer was dried over sodium sulphate. The chloroform was removed under vacuum. The solid obtained was recrystallized from hexane.

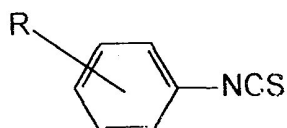
Yield: 63%

M.P. (Lit)^{186a} 33-36°C

M.P. : 33-35°C

All the required isothiocyanates were similarly prepared and are listed in table- I

TABLE NO.I



S.N	R	Molecular formula	Found (0°C)		Reported (0°C)		Ref.
			M.P.	B.P.	M.P.	B.P.	
1	2,4-Cl ₂	C ₇ H ₃ NCl ₂ S	36-38	-	36-37	-	187
2	4-F	C ₇ H ₄ FNS	27-29	-	26-28	-	188
3	2-NO ₂ , 4-Cl	C ₇ H ₃ N ₂ ClSO ₂	102	-	103	-	189
4	4-Cl	C ₇ H ₄ CINS	45	-	46-47	-	190
5	3,5-Cl ₂ ,4-OH	C ₇ H ₃ NSCl ₂ O	97-98	-	98	-	191

Synthesis of N- [acetamido (2-hydroxy pyrazine)]-N- [p-chlorophenyl] thiourea:

In a 50 ml round bottomed flask was taken p-chlorophenyl isothiocyanate (0.6g; 0.0035M) in alcohol (20ml). To it was added 2-methoxy hydrazino pyrazine (0.3g; 0.004M). The mixture was refluxed it on water bath for 3 hrs. The alcohol was removed under vacuum. The solid thus obtained was recrystallized using a solvent system containing alcohol: acetonitrile (1:1).

Yield: 77.55%

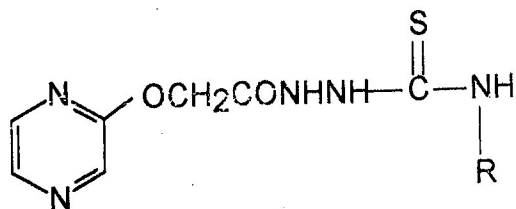
M.P. :220-222⁰C

Elemental analysis of C₁₃H₁₂N₅O₂SCl

	% C	% H	% N
Calculated	46.22	3.55	20.74
Found	45.85	3.68	21.38

All the other thioureas were similarly prepared and are listed in table- II.

TABLE NO-II



SR. NO.	R	Molecular Formula	Mol. Wt.	M.P. ($^{\circ}$ C)	Yield (%)	Elemental Analysis (%)					
						Calculated			Found		
						C	H	N	C	H	N
1	4-Fluoro phenyl	$C_{13}H_{12}N_5O_2SF$	321	>260	68.58	48.59	3.73	21.80	48.32	3.56	21.73
2	4-Chloro phenyl	$C_{13}H_{12}N_5O_2SCl$	337.5	220-222	77.55	46.22	3.55	20.74	46.02	3.39	20.54
3	Allyl	$C_{10}H_{14}N_5O_2S$	268	>250	58.57	44.77	5.22	26.12	44.92	5.45	26.53
4	Isopropyl	$C_{11}H_{17}N_5O_2S$	283	233-234	55.43	46.64	6.00	24.73	46.45	6.25	24.93
5	n-Butyl	$C_{11}H_{17}N_5O_2S$	283	152-154	63.34	46.64	6.00	24.73	46.47	5.91	24.86
6	Ethyl	$C_{19}H_{13}N_5O_2S$	255	182-184	48.91	42.35	5.09	27.45	42.59	5.32	27.37
7	2,4-Dichloro phenyl	$C_{13}H_{11}N_5O_2Cl_2S$	372	214-215	59.32	41.93	2.95	18.82	41.73	2.89	18.55
8	2,5-Dimethoxy phenyl	$C_{15}H_{17}N_5O_4S$	363	245	62.66	49.58	4.68	19.28	49.35	4.43	19.45
9	2-Nitro,4-chloro phenyl	$C_{13}H_{13}N_6O_4SCl$	382.5	221-222	51.29	40.78	2.87	21.96	40.39	2.72	21.79
10	4-carboxyethyl phenyl	$C_{16}H_{17}N_5O_4S$	375	225-227	65.21	51.20	4.53	18.66	51.42	4.76	18.45
11	4-Carboxy, 3-ethyl phenyl	$C_{16}H_{17}N_5O_4S$	375	215-216	68.21	51.20	4.53	18.67	51.39	4.76	18.86
12	2-Fluoro phenyl	$C_{13}H_{12}N_5O_2SCl$	337.5	192-194	59.78	48.59	3.73	21.80	48.75	3.57	21.68
13	2-Chloro phenyl	$C_{13}H_{12}N_5O_2SF$	321	210-212	64.27	46.22	3.55	20.74	46.43	3.76	20.56

Synthesis of [3-(4'-chlorophenyl)-4-(p-chlorophenyl)] thiazoline-(2-methoxy carbonyl pyrazine) hydrazones.

A mixture of N- [acetamido (2-hydroxy pyrazine)]-N- [p-chlorophenyl] thiourea (0.6g; 0.002M), p-chloro phenacyl bromide (0.41g; 0.002M) and sodium acetate (0.6g; 0.01M) was refluxed for 4-5 hrs. The solvent was evaporated under vacuum and the product obtained was washed with water. It was then recrystallized it from methanol.

Yield: 67.32 %

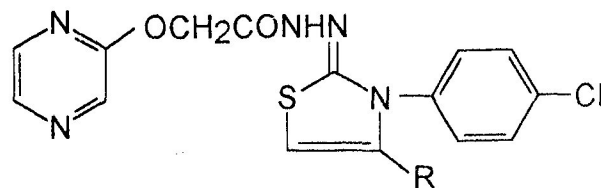
M.P.: 205-207⁰C

Elemental analysis of $C_{21}H_{15}N_5O_2SCl_2$

	% C	% H	% N
Calculated	53.38	3.17	14.83
Found	53.59	3.25	14.64

All the other compounds were similarly prepared and are listed in table- III.

TABLE--III



S. N.	R	Molecular Formula	Mol. Wt.	M.P. (°C)	Yield (%)	Elemental Analysis (%)					
						Calculated			Found		
						C	H	N	C	H	N
1	4-Fluoro phenyl	C ₂₁ H ₁₅ N ₅ O ₂ SClF	455.5	210-211	66.43	55.32	3.29	15.36	55.45	3.36	15.57
2	4-Chloro phenyl	C ₂₁ H ₁₅ N ₅ O ₂ SCl ₂	472	205-207	67.32	53.38	3.17	14.83	53.59	3.25	14.64
3	2,4-Dichloro, 5-Fluro phenyl	C ₂₁ H ₁₃ N ₅ O ₂ SCl ₃ F	524.5	233-234	62.43	48.05	2.47	13.34	48.23	2.61	13.73
4	2-Fluoro phenyl	C ₂₁ H ₁₅ N ₅ O ₂ SClF	455.5	185-186	70.19	55.32	3.29	15.36	55.65	3.32	15.54
5	2-Chloro phenyl	C ₂₁ H ₁₅ N ₅ O ₂ SCl ₂	472	>245	61.89	53.38	3.17	14.83	53.18	3.26	14.62
6	2,4-Dichloro, 6-Fluro phenyl	C ₂₁ H ₁₃ N ₅ O ₂ SCl ₃ F	524.5	217-218	59.72	48.05	2.47	13.34	48.39	2.52	13.42

Synthesis of 4-chloro benzonitrile

STEP-1:

Synthesis of aldoxime of 4-chlorobenzaldehyde.

4-chlorobenzaldehyde (10g; 0.07M) was dissolved in warm ethanol (30 ml) and stirred for 15 min. To it was added hydroxylamine hydrochloride (4.90g; 0.07M) in water (30ml) and stirred for 20 min at room temperature. Sodium hydroxide (2.8g; 0.07M) dissolved in water (30ml) 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 was filtered off to obtain the oxime.

STEP-2:

Synthesis of 4- chlorobenzonitrile:

Aldoxime (5g; 0.032M) was dissolved in acetic anhydride (6.95g; 0.064M) and refluxed for 4hrs under anhydrous condition. It was then cooled and poured on crushed ice. The solid was thus obtained was filtered, washed with cold water dried and recrystallized from methanol.

Yield: 65%

M.P. (Lit)¹⁹²: 91-93°C

M.P. : 90-91°C.

Synthesis of 4-amino-1,2,4 triazole:

In a 500 ml round bottomed flask equipped with an efficient water condenser, were placed (14.8g; 0.2M) of ethyl formate and 15ml of 95% alcohol. To this was added (12g; 0.2M) hydrazine hydrate with shaking, over a period of 10 min. After the reaction subsided, the solution was refluxed on a steam bath for 18 hrs. The bulk of the water and alcohol were then removed by evaporation under reduced pressure until the volume in the flask was about 15 ml. The resulting syrup, crude formhydrazide was heated at atmospheric pressure for 3 hrs, during which time the temperature of the bath was raised from 150-200°C. After cooling to about 100°C, the oil was taken up in 5 ml of 95% ethanol and 0.5g of Norite was

The filtered solution was then diluted with 7.5 ml of ether and placed in an icebox to cool. The crystalline product was filtered, washed with 5 ml of 1:2 alcohol-ether mixture and dried.

Yield: 65%

M.P. (Lit)¹⁸⁹: 76-78°C

M.P. : 77-78°C.

Synthesis of 4(N-1,2,4 triazole-4-yl) benzonitrile:

4- amino.1,2,4 triazole (7g; 0.083M) was dissolved in minimum quantity of dry DMF and stirred for 5 min. To it was added TEA (8.41g; 0.083M) and again stirred for 5 min. Then by using dropping funnel, 4- chloro benzonitrile(11.45g; 0.083M) was added and refluxed and reflux it at 160°C for 8 hrs. The mixture was then filtered to separate TEA hydrochloride crystals. The mother liquor was dumped in ice-cold water, filtered and recrystallized from alcohol to obtain the title compound.

Yield: 60.2%

M.P. : 107-109°C

Elemental analysis of C₉H₇N₅

	% C	% H	% N
Calculated	58.37	3.78	37.83
Found	58.09	3.63	37.77

Synthesis of 4-[N- (4-bromobenzyl)-N- (1,2,4- triazole-4-yl) benzonitrile.

4-(N-1,2,4 triazole-4-yl) benzonitrile (5g; 0.027M) was dissolved in dry 50ml acetonitrile. To it was added potassium carbonate (3.72g; 0.027M) and stirred it for 1 hr. Then bromobenzyl bromide(6.75g; 0.027M) solution in 20ml acetonitrile was added by using dropping funnel. The mixture was refluxed for 8-

refluxed for 8-9 hrs. The reaction mixture was dumped in ice-cold water to get a white product, which was filtered and recrystallized from acetonitrile.

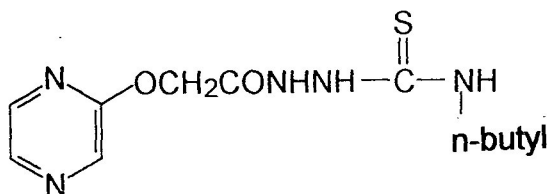
Yield: 68%

M.P. (Lit)⁵²: 200°C

M.P. : 200-201°C.

SPECTRAL ANALYSIS

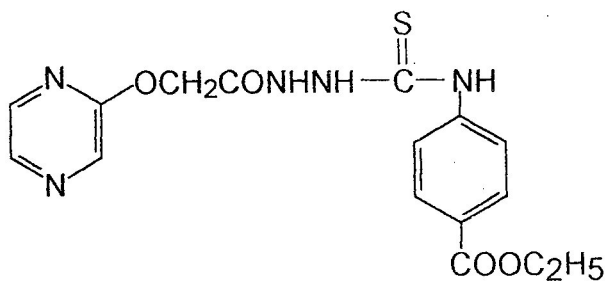
N-{acetamido(2-hydroxy pyrazine)-N'-(n-butyl) Thiourea:



IR (Kbr) cm^{-1}

3328.68, 3203.45	(NH stretching,).
2965.46, 2927.8, 2871.54	(alkyl groups).
1706.81	(C=O stretching)
1338.27	(C=S stretching)
1187.31	(C-O stretching of ether).

N-{acetamido(2-hydroxy pyrazine)-N'-(carboxy ethyl phenyl) Thiourea:



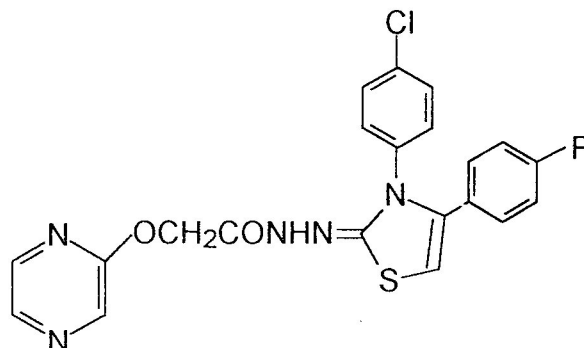
IR (Kbr) cm^{-1}

3228.39	(NH stretching,).
2984.30, 2921.68	(alkyl groups).
1698.97	(C=O stretching)
1355.04	(C=S stretching)
1188.88	(C-O stretching of ether)

^1H -nmr (DMSO) δ :

(7.3-8.2)	(7H, phenyl & het protons).
(9.60)	(s, 1H, -CONH).
(10.04, 10.639)	(s, 2H, 2NH of thiourea).
(4.314)	(q, 2H, -CH ₂).
(1.317)	(t, 3H, -CH ₃).
(4.702)	(s, 2H, -O CH ₂).

[3-(4 -chlorophenyl)-4'-(4-fluoro phenyl)]thiazoline-2-(methoxy carbonyl pyrazine)hydrazones:



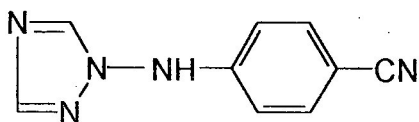
IR (KBr) cm^{-1}

3247.23	(NH stretching).
3065.74	(alkyl group).
1663.03	(C=O stretching).
1119.53	(C-O stretching of ether).
1619.25	(C-N stretching of thiazoline).
1042.39, 1286.73	(thiazoline ring).

^1H -nmr (DMSO) δ :

(7.34-8.12)	(8H, 3H phenyl & het. protons).
(6.4)	(1H, thiazoline protons).
(10.59)	(s, 1H, -CONH).
(4.69)	(s, 2H, -OCH ₂).

4-(N-1,2,4 triazole-4-yl) benzonitrile



IR (KBr) cm^{-1}

3103.17	(NH- stretching).
2229.6	(-CN group).
1010.3, 961.798	(C-N stretching of triazole).

1 : THIO2

Mode= 2 (Mid-IR)

1/4/80 3:08 PM

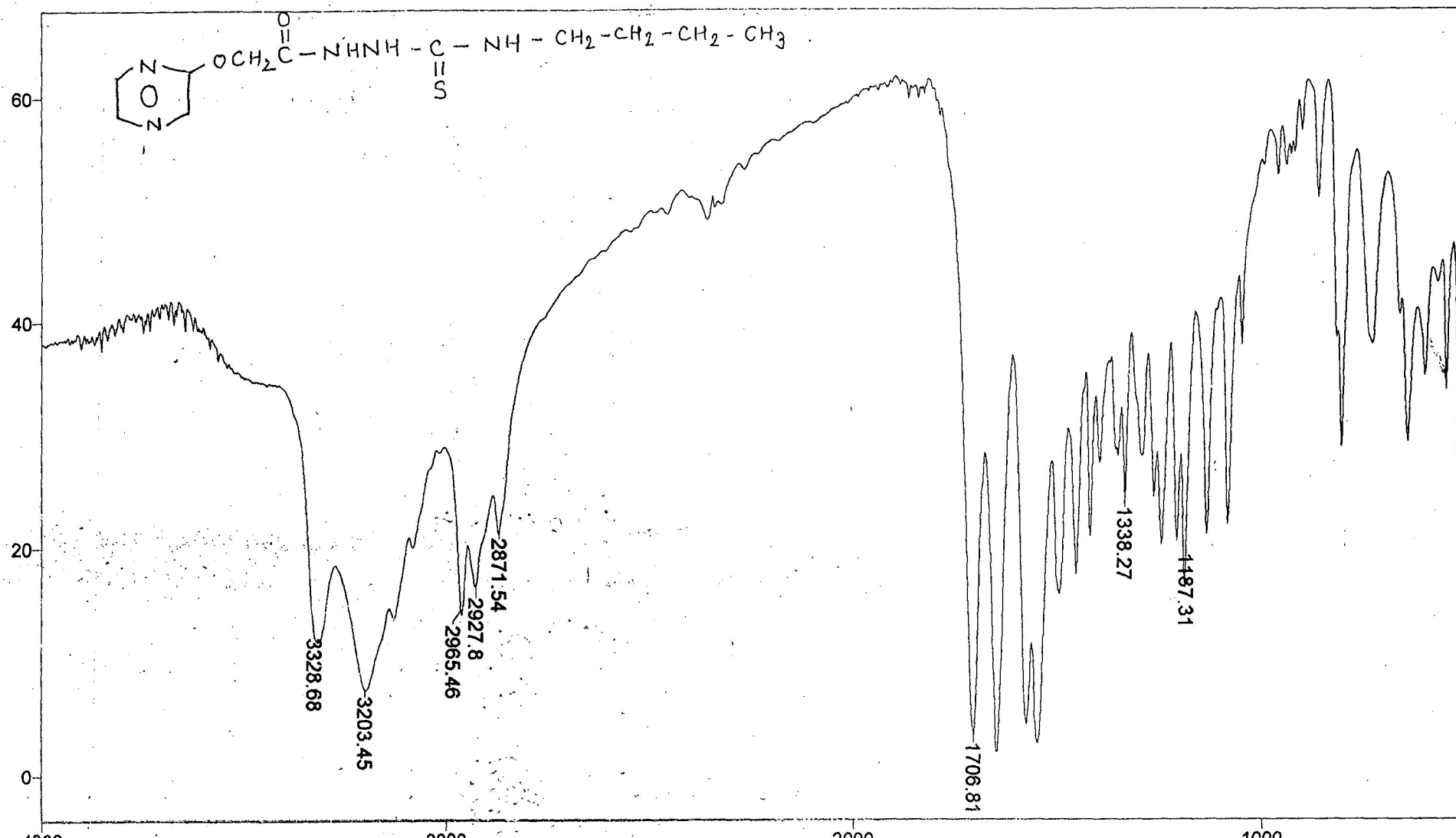
Sample Description: N{Acetamido(2-Hydroxy pyrazine)}-N'-(n-butyl)Thiourea

Scan= 10" Slow

Res=4 cm-1

Apod= Cosine

Zero Filling= 1 x



1 : THIO7

Mode= 2 (Mid-IR)

1/4/80 3:35 PM

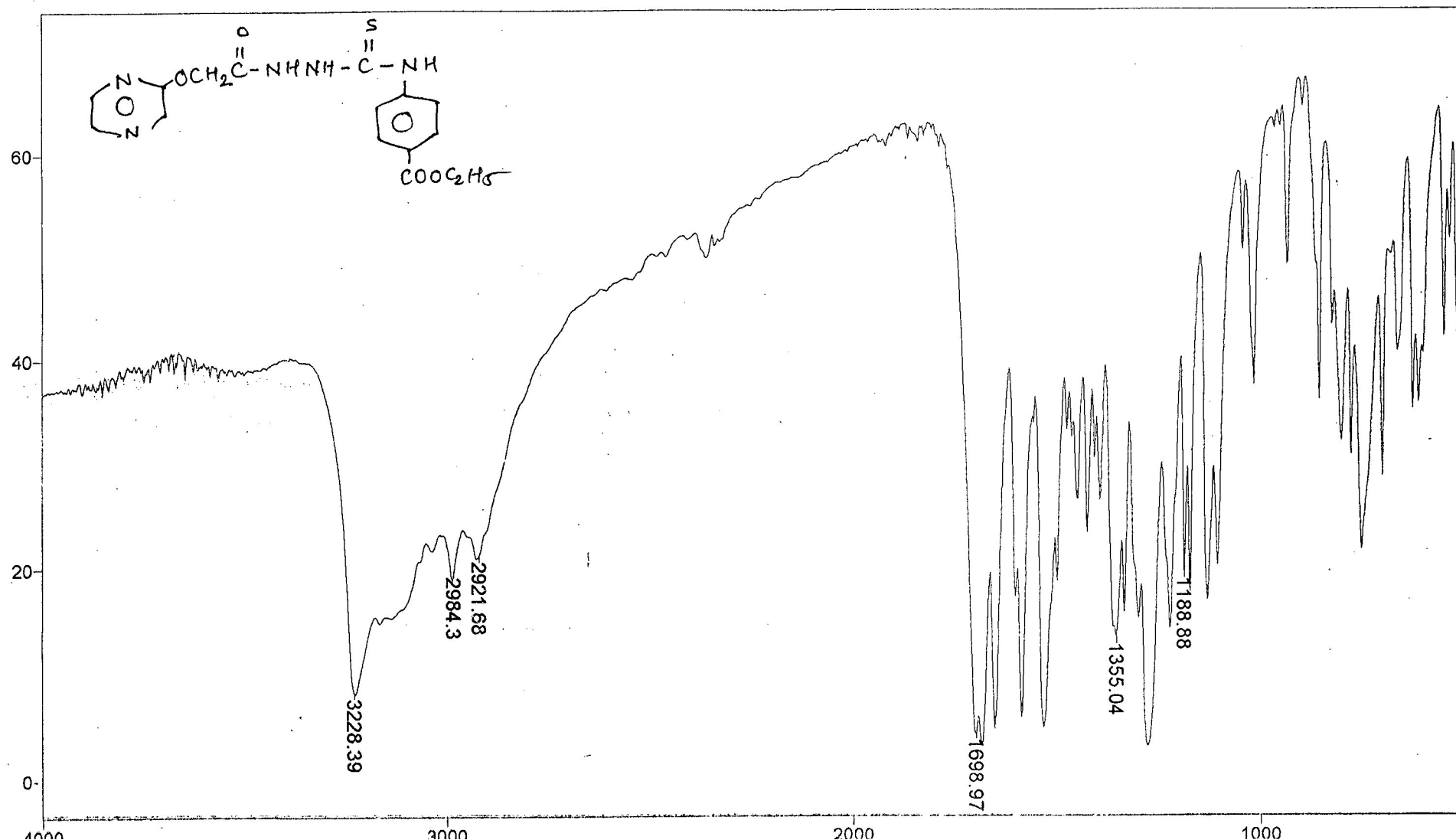
Sample Description: N{Acetamido(2-Hydroxy pyrazine))-N'-(p-ethyl ester)Thiourea

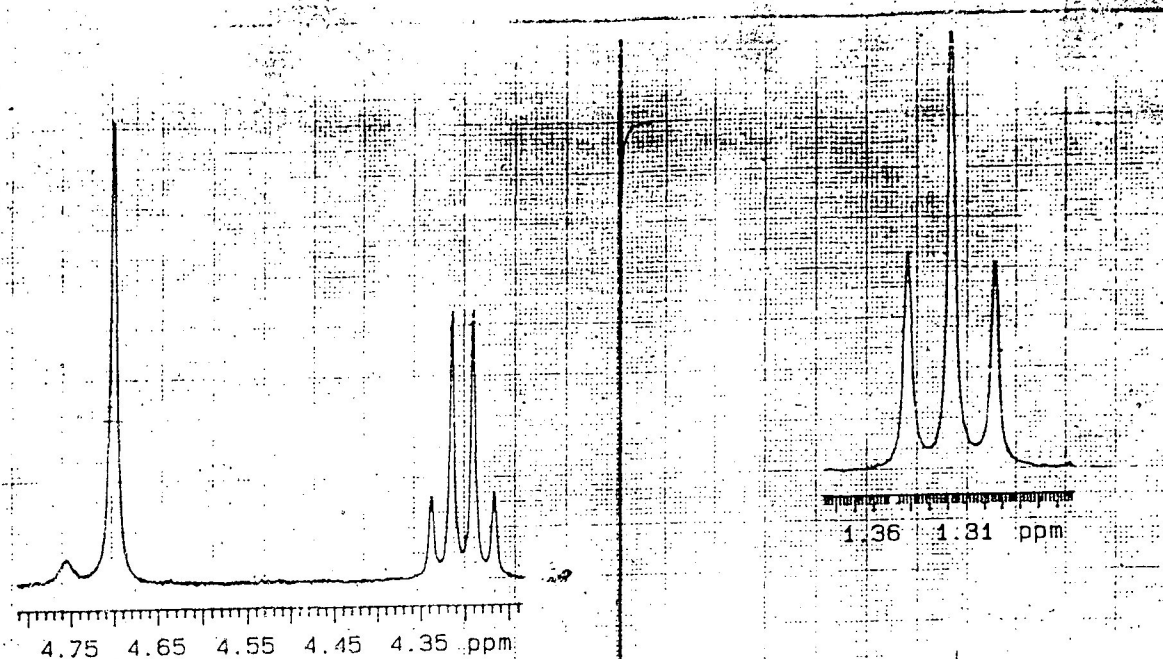
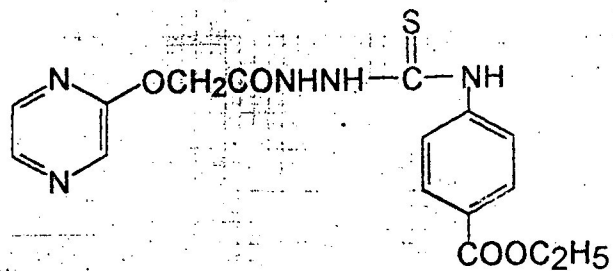
ans= 10 Slow

Res=4 cm-1

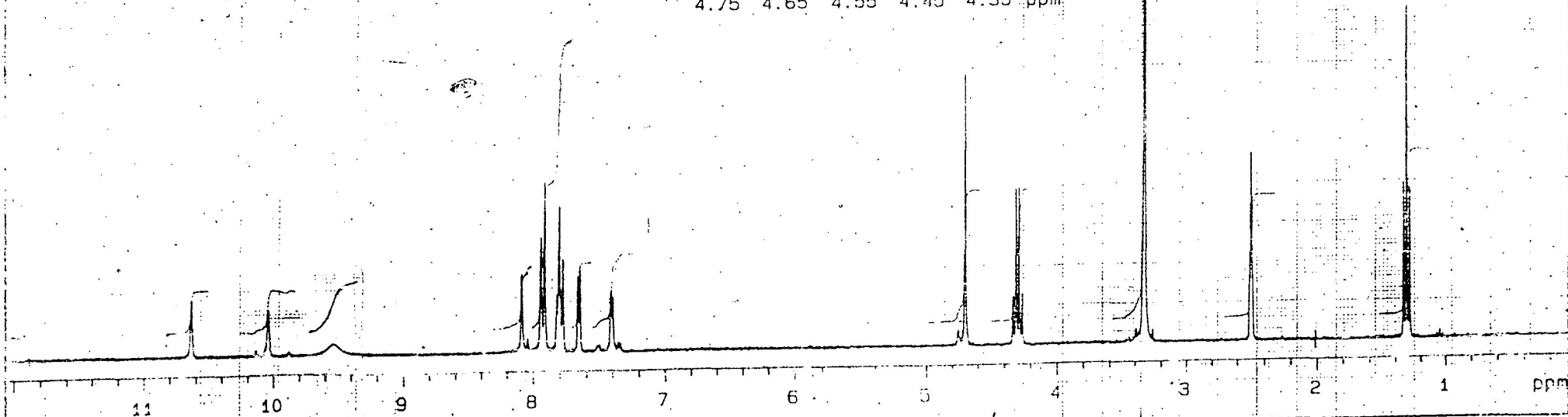
Apod= Cosine

Zero Filling= 1 x





1.36 1.81 ppm



H1

299.9486

H1

-1769.8

not used

a2pu1

100

5000.0

1200.0

20

0.130 not used

3600.1

25.0

Jan 17 2011

e # 1 = THIAZO-2

Mode= 2 (Mid-IR)

4/5/00 4:58 PM

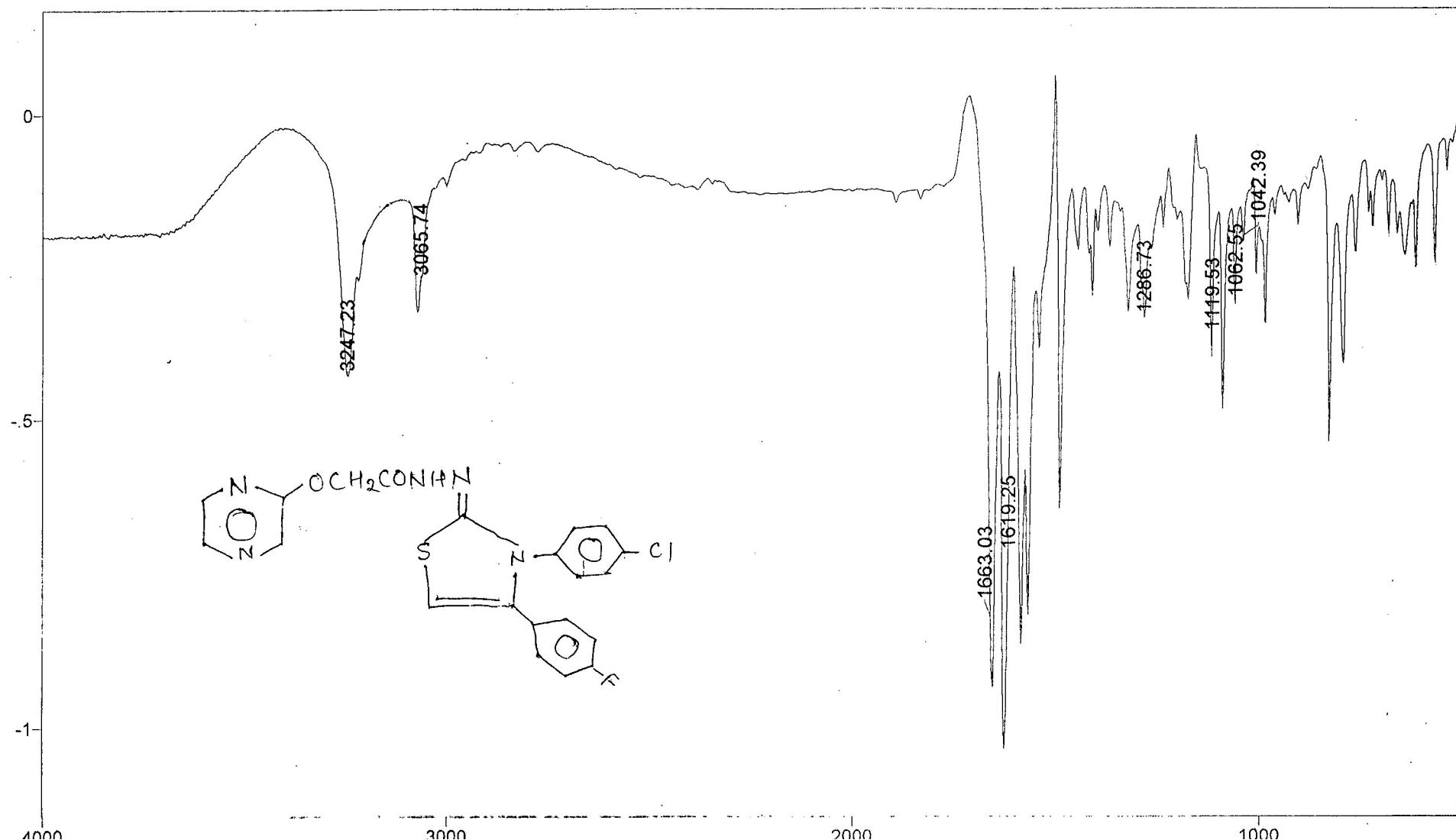
Sample Description: 3-(4'-chlorophenyl)-4-(4-fluorophenyl) thiazoline-(2-methoxy carbonyl pyrazine)hydrazone

Scans= 10 Slow

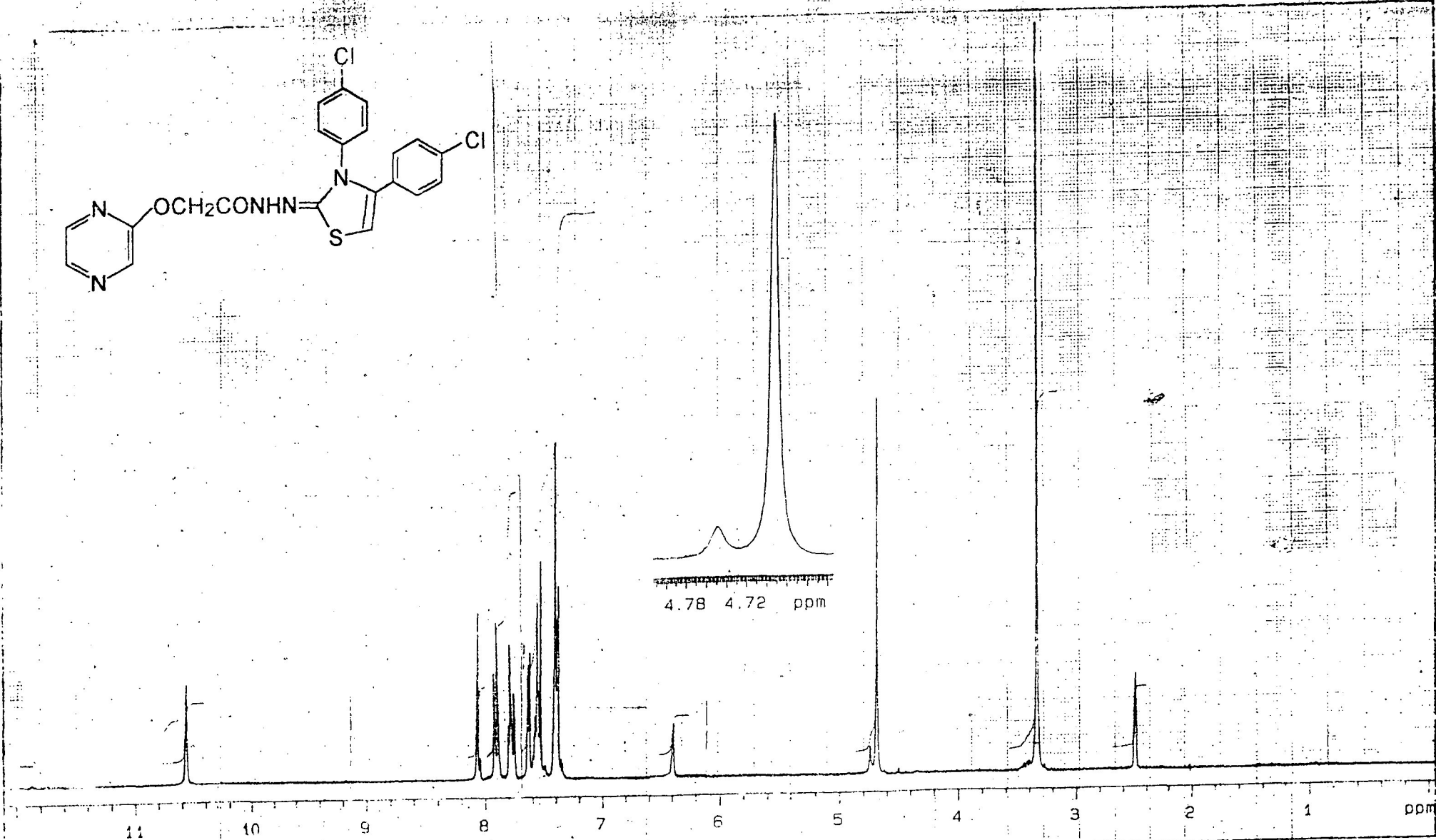
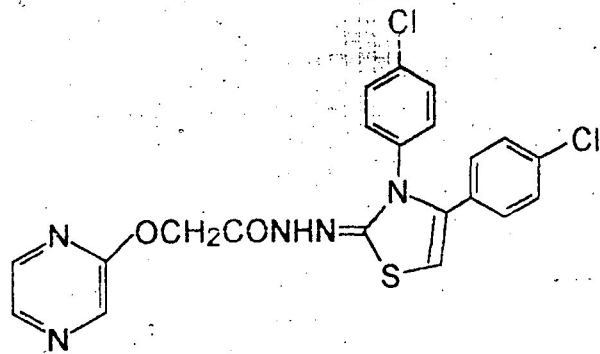
Res=4 cm-1

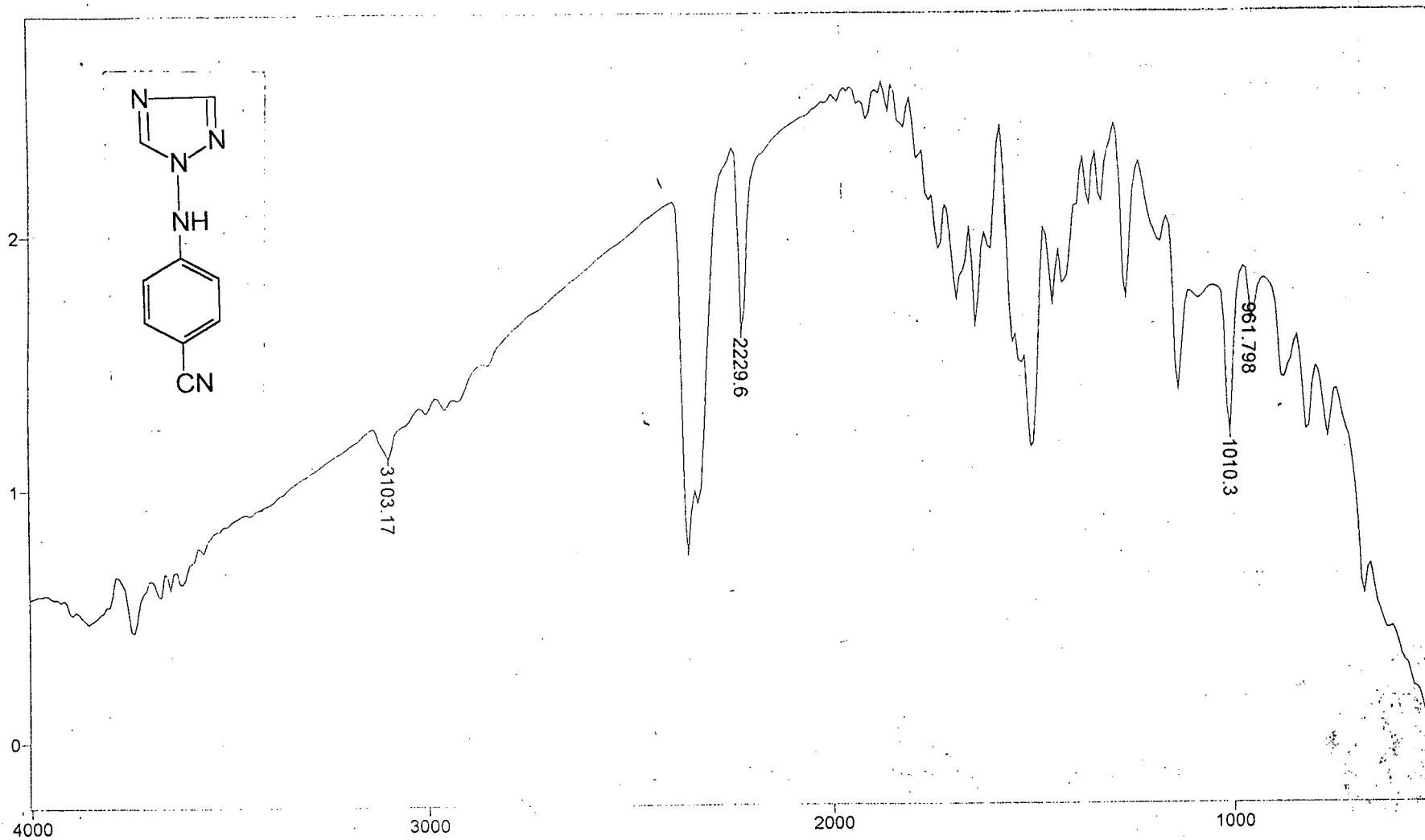
Apod= Cosine

Zero Filling= 1 x



OH





Arbitrary SB / Wavenumber (cm-1)

**BIOLOGICAL SCREENING,
RESULTS AND
DISCUSSION**

Biological Screening, Results and Discussion:

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

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

Details involved in the screening are recorded as below:

Drug Stock Solution:

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

Controls:

1. Media Control : Sterilized medium was kept as a control so as to assure the sterility of the medium. If this control shows growth of any type then results are considered invalid.
2. Culture Control : It contains sterilized medium and culture of the organism. It should

nutrients to promote growth. If no growth is observed, then the medium is considered to be faulty and results are not reliable.

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

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

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

Antibacterial Screening :

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

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

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

S.aureus) and Trimethoprim (MIC = 1.0 µg/ml against Gram- negative *S.typhi*) were used as the standard drugs.

Antitubercular Screening :

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

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

The sterile medium was dispensed in a borosilicate test tube. Horse serum (0.5ml) was added in all the tubes and then to it, drug stock solution was added to attain the different dilutions such as 200, 100, 40, 20, 10, 4, 2 µg/ml etc. Inoculum of standard suspension i.e. 0.1ml of H₃₇ Rv strain of *M. tuberculosis* containing 10⁶ bacilli/ml, was added. Tubes were incubated for ten days and then examined for the presence or absence of growth of the test organism. The lowest concentration which showed no visible growth was taken as the end point. Isoniazide (MIC= 0.04 µg/ml) was used as the standard.

Antifungal Screening :

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

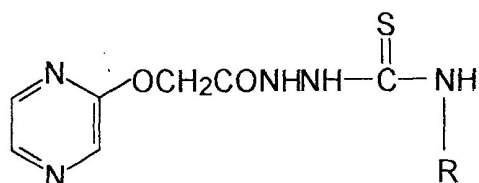
Serafataller's medium was used as the growth media (pH=5.2). The sterile media was dispensed in a sterile borosilicate test tube. The drug solution was added in order to get the final drug concentrations of 200, 100, 40, 20, 10, 4, 2 µg/ml etc.

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

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

Results:

Table I : Antimicrobial activity of N-[acetamido(2-hydroxy pyrazine)]-N-(substituted alkyl/aryl) thiourea.



S.No.	Substitutions (R)	Antibacterial activity (µg/ml)		
		H ₃₇ Rv strain of <i>M. tuberculosis</i>	<i>S. aureus</i>	<i>S. typhi</i>
1.	4-fluorophenyl	40	100	40
2.	4-chlorophenyl	100	N.A.	N.A.
3.	allyl	40	N.A.	100
4.	isopropyl	40	N.A.	100
5.	n-butyl	40	N.A.	40
6.	ethyl	N.A.	N.A.	100
7.	2,4-dichlorophenyl	100	100	N.A.
8.	2,5-dimethoxy phenyl	20	40	20
9.	2-nitro, 4-chloro phenyl	20	100	40
10.	4-carboxyethyl phenyl	N.A.	100	100
11.	4-carboxy, 3-ethyl phenyl	N.A.	N.A.	N.A.
12.	2-fluoro phenyl	40	100	100
13.	2-chloro phenyl	N.A.	N.A.	N.A.

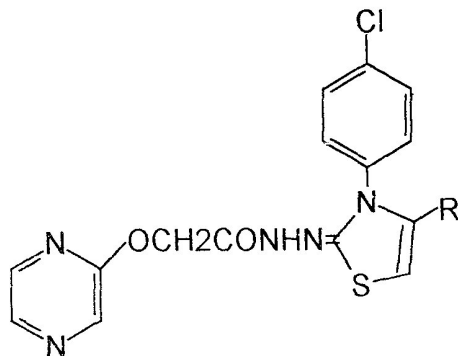
* N.A.- Inactive at 200 µg/ml.

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

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

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

**Table-II : Antimicrobial activity of 3-(4'-chlorophenyl)-4-(aryl substituted)]
thiazoline-(2- methoxy carbonyl pyrazine)hydrazones**



Sr.No.	Substitutions (R)	Antibacterial activity (µg/ml)		
		H ₃₇ Rv strain of <i>M. tuberculosis</i>	<i>S. aureus</i>	<i>S. typhi</i>
1.	4-fluorophenyl	20	100	100
2.	4-chlorophenyl	100	N.A.	N.A.
3.	2,4-dichloro, 5-fluro phenyl	40	100	40
4.	2-fluorophenyl	40	100	100
5.	2-chlorophenyl	N.A.	N.A.	N.A.
6.	2,4-dichloro, 6-fluro phenyl	40	40	40

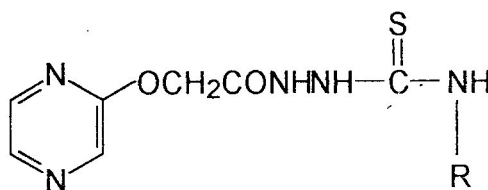
* N.A. - Inactive at 200 µg/ml.

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

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

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

Table III : Antifungal activity of N-[acetamido(2-hydroxy pyrazine)]-N-(substituted alkyl/aryl) thiourea.

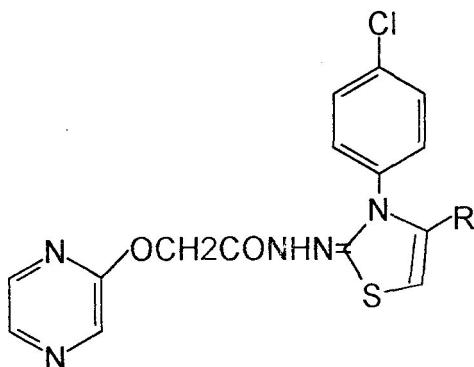


S.No.	Substitutions (R)	Antifungal activity (µg/ml)		
		<i>C.albicans</i>	<i>T. mentagrophytes</i>	<i>T. rubrum</i>
1.	4-fluorophenyl	100	100	100
2.	4-chlorophenyl	N.A.	40	N.A.
3.	allyl	N.A.	N.A.	N.A.
4.	isopropyl	N.A.	N.A.	N.A.
5.	n-butyl	N.A.	N.A.	N.A.
6.	ethyl	N.A.	N.A.	N.A.
7.	2,4-dichlorophenyl	100	100	100
8.	2,5-dimethoxy phenyl	40	100	100
9.	2-nitro, 4-chloro phenyl	100	40	100
10.	4-carboxyethyl phenyl	100	N.A.	40
11.	4-carboxy, 3-ethyl phenyl	100	100	100
12.	2-fluoro phenyl	100	40	100
13.	2-chloro phenyl	100	100	N.A.

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

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

**Table-IV: Antifungal activity of 3-(4'-chlorophenyl)-4-(aryl substituted)]
thiazoline-(2- methoxy carbonyl pyrazine)hydrazones**



Sr.No.	Substitutions (R)	Antifungal activity (µg/ml)		
		<i>C.albicans</i>	<i>T. mentagrophytes</i>	<i>T. rubrum</i>
1.	4-fluorophenyl	100	100	N.A.
2.	4-chlorophenyl	N.A.	N.A.	100
3.	2,4-dichloro, 5-fluro phenyl	100	N.A.	N.A.
4.	2-fluorophenyl	100	100	100
5.	2-chlorophenyl	N.A.	100	N.A.
6.	2,4-dichloro, 6-fluro phenyl	N.A.	N.A.	100

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

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

Discussion:

Part-II : N-[acetamido(2-hydroxy pyrazine)]-N-(substituted alkyl/aryl) thiourea:

All the 13 compounds in this series were screened for their antimicrobial and antifungal activity. (Table No.1 and 3)

Of the 13 compounds screened for antibacterial activity, one compound (i.e. compound no.8) exhibited activity at 40 µg/ml against gram positive bacteria, five compounds exhibited activity at 100 µg/ml against *gram positive bacteria*, while others are inactive.

Also one compound (i.e compound no. 8) exhibited activity at 20 µg/ml, three compounds (i.e. compound no. 1,5,9) exhibited activity at 40 µg/ml, five compounds (i.e. compound no. 3,4,6,10,12) exhibited activity of 100 µg/ml against *gram negative bacteria*, while others are inactive.

In antitubercular screening two compounds (i.e compound no. 8,9) exhibited antitubercular activity at 20 µg/ml while five compounds (i.e. compound no. 1,3,4,5,12) exhibited antitubercular activity at 40 µg/ml and two compounds (i.e. compound no. 2,7) exhibited antitubercular activity at 100 µg/ml, against *tubercular bacilli*, while rest are inactive.

In antifungal screening one compound was active at conc. of 40µg/ml, seven compounds were active at conc. of 100µg/ml against *C. albicans* while rest were found inactive (Table no. III).

Three compounds were active at conc. of 40µg/ml, five compounds were active at conc. of 100µg/ml against *T. mentagrophytes* while others are inactive (Table no. III).

one compound was active at conc. of 40 µg/ml, six compounds were active at conc. of 100 µg/ml against *T. rubrum*, while others are inactive (Table no. III).

Part-III: 3-(4'-chlorophenyl)-4-(aryl substituted)] thiazoline-(2- methoxy carbonyl pyrazine)hydrazones.

All the 6 compounds in this series were screened for their antimicrobial and antifungal activity (Table No.2 and 4).

Of the 6 compounds screened for antibacterial activity, one compound (i.e. compound no.6) exhibited activity at 40 µg/ml, three compounds exhibited activity at 100 µg/ml against *gram positive bacteria*, while others were found inactive.

Also two compounds (i.e compound no. 3,6) exhibited activity at 40 µg/ml, two compounds (i.e. compound no. 1,4) exhibited activity at 100 µg/ml against *gram negative bacteria*, while the rest were found inactive.

In case of, antitubercular screening one compounds (i.e compound no. 1) exhibited antitubercular activity at 20 µg/ml, three compounds (i.e. compound no. 3,4,6) exhibited antitubercular activity at 40 µg/ml, one compound (i.e. compound no.2) exhibited antitubercular activity at 100 µg/ml, against H₃₇Rv strain of *tubercular bacilli* while compound no. 5 was inactive.

None of the compounds from this series exhibited antifungal activity at conc.of 20 or 40 µg/ml against all the three fungal strains.(Table no.IV)

CONCLUSION

CONCLUSION:

The present investigation was focused on the synthesis of novel heterocyclic compounds of possible therapeutic value. To evaluate their efficacy, they were screened for their *in vitro* antibacterial, antifungal and antitubercular activity.

In experimental section, synthesis of N-[acetamido(2-hydroxy pyrazine)]-N-(substituted alkyl/aryl) thiourea has been described. Antimicrobial activity of the series reveals the following points.

1. Electronegative groups like methoxy, nitro on the phenyl ring imparted the antitubercular activity to the compound (20 µg/ml).
2. Alkyl substitution on the one side of the thiourea (more than two carbon atoms) shows antitubercular activity at 40 µg/ml.
3. Alkyl substitution on the one side of the thiourea gives inactive compounds against *gram positive organism*.
4. Electronegative groups like methoxy, nitro on the phenyl ring impart the antibacterial activity to the compound.
5. Alkyl substitution on the one side of the thiourea gives inactive antifungal compound.
6. Substituted phenyl on one side of thiourea shows moderate antifungal activity.

In the experimental section, 3-(4'-chlorophenyl)-4-(aryl substituted)] thiazoline - (2- methoxy carbonyl pyrazine)hydrazones has been described. Antimicrobial activity of the series reveals the following points.

1. Compound with 4-fluoro group at the phenyl ring gives the activity at 20 µg/ml against *M. tuberculosis*.

2. Halogenated phenyl ring were not given the satisfactory result against gram positive and gram negative organism.
3. Halogenated phenyl ring were not given the satisfactory result against all three strains of fungus.

Though it may be concluded that the present investigation is not successful in rendering any new organic compound more active than the existing drugs, in the class of infection for which these compounds were screened. It may be worth while to pursue further investigation with related compounds bearing in mind certain trends observed in the present investigation as a part of efforts to discover novel and potent drugs.

REFERENCES

REFERENCES

1. Weinstein L.' General considerations in Goodman, L.S., and Gilman, A.,(eds). **The Pharmacological Basis Of Therapeutics**, New York, The Macmillan Company, 1970, p-1154.
2. Majno, G.,**The Healing Hand: Man and Wound in the Ancient World**. Cambridge, Mass, Harvard University Press, 1975, pp 154 &215.
3. **Annual Reports in Medicinal Chemistry, Vol.32, pg 121**, ed: James A. Bristol Academic Press.
4. **Antimicrobial Chemotherapy, 3rd edn., p 301**, edited by: David Greenwood, Published: Oxford University press.
5. Satoskar, R.S., Bhandarkar, S.D., **Pharmacology & Pharmacotherapeutics, , 8th edn, P 588**, Published: Popular Prakashan Pvt Ltd.
6. Mackaness, G.B., Smith, N., **Amer.Rev.Tubercl., 67, 322 (1953)**.
7. **Antimicrobial Chemotherapy, 3rd edn., p 305**, edited by: David Greenwood, Published: Oxford University press.
8. Dogmagk, G.G., **Deut.Med. Wochenschr, 61, 250 (1931)**.
9. Meitzsch, F., and Klarer, H., **J. Ger.Pat.607537 (1935)**, Cited from **C.A. 29, 4135 (1935)**.
10. Jaffe, A., Chabbert, Y.A., Semonin, O., **Antimicrobial agents and Chemotherapy, 22, 942 (1982)**.
11. Malousin, F., Bryan, L.E., **Antimicrobial agents and Chemotherapy, 30, 1 (1986)**.
12. Chen, Y.L, et.al., **J. Antibiot., 44, 970 (1991)**.
13. Mark, J., Suto, J., Domagala, M. and Paul F, **Miller, Annual Reports in Medicinal Chemistry, 27:119**, Academic Press, WC, California (1992).
14. Barreau, M., Antoine, M., Benichon, J.L., Desconclousis, J.F., Girand, P., Robin, M., Wentzler, S., Picault, G., Densnotter, J.F., Rotin, O. and Bouanchaud, D.H., **31st Interscience Conference on Antimicrobial agents and Chemotherapy, 369 (1991)**.
15. Barlkovitz, D., Corraz, A., Dietcher, K., Georjopapadakou, N., Keith, D., Mook, R.,

- Pruess, D., and Wei, C.C., **31st Interscience Conference on Antimicrobial agents and Chemotherapy, 824 (1991).**
16. Hirai, K., Hosaka, J., Niwata, Y., Yasue, T., Fukuda, H., Ishizaki, T., Suzue, S., and Nishino, K., **Interscience Conference on Antimicrobial agents and Chemotherapy, 385 (1990).**
 17. Beskid, G., Albrecht, H.A., Fallat, V., Keith, D.D., Luipschitz, E.R., McGarry, C.M., McGarry, D.H., Rossman, P., and Siebelist, J., **Interscience Conference on Antimicrobial agents and Chemotherapy, 403 (1990).**
 18. **Burger's Medicinal Chemistry and Drug Discovery, 5th edn., vol 4, Therapeutic agents, P:406**, edited by: Manfred E. Wolff, Published: A Wiley Interscience Publication.
 19. Hart, R.J., and Lee, R.M., **Exp. Parasitol, 18, 332(1966).**
 20. Hass, D.K., and Collons, J.A., **Am. J. Vet. Res., 35, 103(1974).**
 21. Seidel, E.E., Kilbourn, D.L., Peardon, R.D., Tetzlaff, E.D., Weiler H. and Weir W.D., in **20th World Veterinary Congress Summaries, vol 2, Thessalonki, Greece, 1975, P:805; through Vet. Bull.(London), 45,904(1975).**
 22. Daus, A., **Drug Treatment in Intestinal Helminthiasis, World Health Organisation, Geneva, 1973, P:13.**
 23. Brown, H.D., Matzuk, A.R., Ilves, I.R., Peterson, L.H., Harris, S.A., Saret L.H., Egerton, J.R., Yakstis, J.J., Campbell, W.C., Cuckler, A.C., **J. Am. Chem.Soc.,83, 1764(1961).**
 24. Tocco, D.J., Buhs, R.P., Brown, H.D., Matzuk, A. R., Mertel, H.E., Harman, R.E., Turner, N.R., **J. Med. Chem.7,399(1964).**
 25. Brugmans, J.P., Thienpont, D.C., Wijngaarden, O.F., Vanparijs, V.L., Schuermans, and H.L., Lauwers, G., **J. Am. Med Assoc. 217, 313(1971).**
 26. Wolfe, M.S., and Wershing, J.M., **J. Am. Med Assoc.,230, 1408(1974).**
 27. Panitz, E., **J. Helminthol., 51, 23(1977).**
 28. Khadse, B.G., Chaudhari, D.T., Patil, A.M., Kamat, V.R., **Bull. Haffkin Inst., 5, 56(1977).**
 29. Khadse, B.G., Shah, M.H., Deliwala, C.V., **Bull. Haffkin Inst., 3, 27(1975).**

30. Khadse, B.G., Shah, M.H., Deliwala, C.V., Bhide, M.B., Mahajan, S.S., Bhat M.V.,
Bull. Haffkin Inst., 5, 9(1977).
31. Raeymaekers, A.H.M., Allewijn, F.T.N., Vandenberg, J., Dmoe, P.J.A.,
VanOffenwert, T.T.T., Jansen, P.A.J., **J. Med. Chem., 9, 545(1966).**
32. Plempel, M., et. al., **Dtsch. Med. Wochenschr., 94, 1356(1969)** cited from '**Wilson and Giswold's Textbook of Organic Medicinal and Pharmaceutical Chemistry**',
9th edn., J.B. Lippincot and Company, Philadelphia **P:150(1991).**
33. Godefroi, E.F., et. al., **J. Med. Chem., 12, 784(1968).**
34. Thomas, A.H., **J. Antimicrob. Chemother., 17, 269(1986).**
35. 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 Pharmaceutical Chemestry., 9th edn** J.B. Lippincot and Company,
Philadelphia.**P:151.**
36. Hazen, E. L., Brown, R., **Pract. Soc. Exp. Biol. Med., 76, 93(1951)** cited from
'**Wilson and Giswold's Textbook of Organic Medicinal and Phamaceutical
Chemistry**', **9th edn** J.B. Lippincot and Company, Philadelphia.**P:151.**
37. Oxford, A.E., et. al., **Biochem. J., 33, 240(1939).**
38. Grove, J.F., et. al., **J. Chem. Soc., 3977(1952).**
39. Sloboda, R.D., **Biochem Biophys Res Commune, 107, 882(1982).**
40. Kruse, R., et. al., **J. Chem. Soc., 3977(1952).**
41. Kushner, S., Dalalian, H., *et al.*, **J.Am.Chem.Soc., 74, 3617(1952).**
42. Cuong, D.Q., Buu-Hoi, N.P. and Xuong, N.D.; **J. Med. Chem., 6(4), 452(1963).**
43. Mayor, R.L.; **Chem. Abstr., 36; 5199(1942).**
44. Davy, T.F.; **Leprosy Rev., 29 ;25(1958).**
45. Madan, A.G., **Belg. Pat. 613, 154(1962)** cited from **Chem.Abstr., 58:474f.**
46. Fox, H.H.; **J.Org.Chem., 17, 542(1952).**
47. Singh, H., Singh, A.K., *et al* ; **J. Med. Chem, 20(6) 826-829(1977).**
48. Omar, A.M, Shamas, M.E., El-Din, S.A., Ghabashy, A.A., Khan, M.A., **Eur. J. Med.
Chem., 16 ,77-80(1981).**
49. Nilgun, K., Nalan, T. and Aysel Gursoy ;**Arzneim-Forsch Drug Res., 48(11),**

7,758(1998).

50. Mohsen, M.E., Shams. S.A., *et al*; **Eur. J . Med. Chem**, **16**,77-80 (1981).
51. Hassan, H.Y., El-Koussi, N.A., *et al*; **Chem. Pharm. Bull** , **46(5)**, 863-866(1998).
52. **Drugs Fut.**, **21(7)**, 716-718(1996).
53. Dyson, G.M., George, H.J., **J. Am. Chem. Soc.**, **125**, 2013(1939).
54. Dyson, G.M., George, H.J., and Hunter R.F., **J. Chem. Soc.**, 436(1927).
55. Mekee, H.L. and Bost, R.W., **J. Am. Chem. Soc.**, **68**, 2506(1946).
56. Siefken, W., **Ann. Chem.**, **562**, 75(1947).
57. Dyson, G.M., and Hunter R.F., **J. Ind. Chem. Soc.**, **45**, 81(1926).
58. **"Methoden der Organischen Chemie"** (Houben-Weyl)(1955) IX, Thieme Verlag, Stuttgart, **pp 875-877**.
59. Beaver, D.J., Roman, D.O. and Stoffel, L.J., **J. Am. Chem. Soc.**, **79**, 1236(1957)
60. Schutz, O.E. and Gauri, K.K., **Arch. Pharm.** **295**, 146(1962).
61. Brintziner, H., Pfannstiel, K., and Koddenbusch, H., **Chem. Ber.** **82**, 393(1949).
62. Friis, D., **Acta Chem Scand**, **19**, 766(1965).
63. Sayigh, A.A.R., Ulrich, H and Potta, J.S., **J. Org. Chem** **30**, 2405(1965).
64. Jale, A.L., **J. Am. Chem Soc.** **75**, 675(1953).
65. Saccardi, P., **Gazz. Chem. Ital.**, **75**, 87(1945).
66. Losanitsch, S.M., **Chem. Ber** **24**, 3021(1891).
67. Hoffmann, A.W., **Chem. Ber** **8**, 107(1875).
68. Loose, G., and Weddige, H., Liebigs, Y., **Ann Chem** **636**, 144(1960).
69. Hoffmann, A.W., **Ann Chem** **373**, 201(1909).
70. Anschutz, R., **Ann. Chem.** **359**,202(1908).
71. Dains, F.B., Brenster, R.C. and Olander, C.P., **Org Stntheses Coll.**, **I(1932)447**, John Wiley, New York.
72. Goldschmidt, S. and Martin, K., **Patent Ger.** **700**, 436.(1936) Houben-Weyl, Vol. IX, George Thieme Verlag, Stuttgart, **p 869 (1955)**.
73. Hartke, K., **Arch. Pharm.** **299**, 174(1966).
74. Andreasch, R., **Monatsh** **27**, 1221(1906).
75. Andreasch, R., **Monatsh** **31**, 788(1906).

76. Kaluza, L., **Monatsh**, **30**, 716(1909).
77. Nagele, M., **Montash**, **33**, 948(1912).
78. Neil, G.J.O. and Pattison, F.L.M., **J. Am. Chem. Soc.** **79**, 1956(1957).
79. Schering, A.G., **Brit. Pat.** **892,790(1962)**, Mar.28 Cited through **Chem. Abstr.** **57:4552d (1962)**.
80. Hodgkins, J.E., and Ettlinger, M.G., **J.Org Chem** **21**, 404(1956).
81. Hodgkins, J.E., Reeves, W.P. and Lui, Y.T., **J. Am. Chem. Soc.** **83**, 2532(1961).
82. Hodgkins, J.E., Reeves, W.P., **J. Org. Chem.** **29**, 3098(1964).
83. Schimdt, E., Zaller, F., Moosmuler, F., and Kammerl, E., **Ann. Chem.** **585,230,233(1954)**.
84. Schimdt, E., Ross, D., Kittle J., Von Dusel, H.H. and Wamster, K., **Ann Chem** **636**, 144(1960).
85. Johar, G.S., Agarwal, U. and Rao, P.B. **Ind. J. Chem.** **8**, 759(1970).
86. Jochims, J.C. and Seeliger, A., **Angew. Chem.** **79**, 151(1967).
87. Jochims, J.C. **Chem. Ber.** **101**, 1746(1968).
88. Mentz, W. and Weith, W., **Z. Chem.** **589(1969)**.
89. Loconte, J.N. and Chence, L.H., **J. Am. Chem. Soc.** **71**, 2240(1922).
90. Volinkin, N.I., Obshch, S., **Z. Khim.** **37**, 483(1957).
91. Lieberman, C.I. and Natanson, S., **Chem. Ber.** **13**, 1576(1880)
92. Bly, R.S., perkins, G.A. and Lewis, W.I., **J. Am. Chem. Soc.** **44**, 2897(1922).
93. Hoffmann, A.W., **Chem. Ber.**, **15**, 985(1888).
94. Baxter, J.N., Cymerman-Craig, J., Moyle, M., and White, R.A., **Chem and Ind.****785(1954)**.
95. Baxter, J.N., Cymerman-Craig, J., Moyle, M., and White, R.A., **Chem and Ind.****659(1954)**.
96. Dous, F., 'Organic Compounds of Sulphur, Selenium and Tellurium' ed, D.H. Reid, **The Chemical Society, London**, **2**, Chapter 4 (1973).
97. Dous, F., 'Organic Compounds of Sulphur, Selenium and Tellurium' ed, D.H. Reid, **The Chemical Society, London**, **3**, Chapter 5 (1975).
98. S. Mc Kenzie in Dous, F., 'Organic Compounds of Sulphur, Selenium and

- Tellurium'** ed, D.H. Reid, **The Chemical Society, London, 1, Chapter 5 (1970).**
99. Kempter, G., Ziegner, H.L., and Moser, G., **Z. Chem., 11, 12(1971).**
 100. Sakai, S., Asai, Y., Kiyohara, Y., Itoh, K., and Ishii, Y., **Organometallic Chem. Synth. 1, 45(1970-1971).**
 101. Schroeder, D.C., **Chem. Rev., 55, 181(1955).**
 102. Hantzsch, A.R., and Weber, H.J., **Ber. 20, 3113(1887).**
 103. Gagui, F., Suci, T., Henegar, O., and Gyorifi, Z., **Arch. Pharm. Weinheim, 303, 102(1970), Cited from Chem. Abstr. 72:129446(1970).**
 104. Bissin, P., Tetu, O. and Selim, M., **Chim. Ther., 4, 220(1969), Cited from Chem. Abstr 71:101762**
 105. Hantzsch, A.R., and Weber, H.J., **Ber. 21, 930(1888).**
 106. Mathes, R.A., Gregory, J.T., **J. Am. Chem. Soc. 74, 3867(1952).**
 107. Cook, A.H., Downer, J.D. and Heilbron, I., **J. Am. Chem. Soc. 1262(1948).**
 108. Ochiai, E. and Nagsawa, F., **J. Pharm. Soc. Japan. 59, 43(1939).**
 109. Maria, T.C., Onnis, V., **Synthesis 199(1993).**
 110. Dahiya, R. and Pujari, H.K. **Ind. J. Chem., 25B, 966(1986).**
 111. Durgayan, A.A., Titany, S.A. and Kazaryan, R., **Izvest Akad Nauk Armyam SSR Khim Nauki 14, 165(1961) Cited from Chem. Abstr. 56:4741.**
 112. **U.S. Pat. 2,652,396.**
 113. Khanna, M.S., Chandra, P., Garg and Ram P. Kapoor, **Ind. J. Chem. 32B: 364(1993).**
 114. Bhagwat, V.S. and Nargund, **J.Ind. Chem Soc. 45, 270(1968). Cited from Chem. Abstr. 69, 106602.**
 115. Bilinski, S. and Bielak, L., **Ann. Univ. M. Curie. Sklodowska Swit. 21, 263(1966).**
 116. Smith, J.L.B., and Sapiro, R.H., **Trans. Roy. Soc. S. Africa, 18, 229(1929).**
 117. Fodor, G. and Wilhelm., **Acta. Chim. Asad. Sci. Hung. 2, 189(1952).**
 118. Beyer, H., Hohem, H. and Laessig, W., **Chem. Ber. 85, 1122(1952). Cited from Chem. Abstr. 47, 11183.**
 119. Beyer, H., Laessig, W., Rulhig, G., **Chem. Ber. 86, 764(1953). Cited from Chem. Abstr. 48, 10736.**

120. Beyer, H., Laessig, W., Bulka, E. and Behrens, D., **Chem. Ber. 87, 1392(1954).**
Cited from **Chem. Abstr. 49, 15868.**
121. Beyer, H., Kroger, C. and Zander, M., **Chem. Ber. 87, 1392(1954).** Cited from
Chem. Abstr. 49, 15868.
122. Smiti, I. and Coman, M., **Ann. Chim., France. 7, 33(1972).** Cited from **Chem.**
Abstr. 77, 75156.
123. Solanki, M.S. and Trivedi, J.P., **J. Ind. Chem. Soc. 49, 37(1972).**
124. Gursoy, A., **Istanbul Uni. Beezaulik Fak Meemuasi 10, 87(1974),** Cited from
Chem. Abstr. 82:12512.
125. Smiti, I. and Coman, M., **Bull. Soc. Chim., France. 9, 3276(1969).** Cited from
Chem. Abstr. 72, 21635.
126. Zweidler R. and Keller E, **US Pat. 2784, 184 (1957),** cited through **Chem. Abstr.**
53: 13934h.
127. Niese M. and Delfis D. **US Pat. 2875, 209(1959),** cited through **Chem. Abstr.**
53: 17151e.
128. Oshima, T., Hata, S., Takamatsu, H., Okuda, M. and. Yokota, M **Jap. Patent, 72,**
08, 820(1972) cited through **Chem. Abstr. 76: 153796h.**
129. Ythomas, K., **British Patent 1053085(1966)** cited through **Chem. Abstr. 66:**
46427k.
130. Bangal, L. I., Pevzner, M.S., Lopyrev V.A., **USSR Patent 176,913(1965)** cited
through **Chem. Abstr. 64: 12685d.**
131. Boots, S.G. and Onen, C.C. , **J. Heterocyclic Chem. 4,(1967), 272.** cited through
Chem. Abstr. 32651n.
132. Grinstein V. and Chipin G.I., **Zh. Obshch Khim 31, 886(1961)** cited through
Chem. Abstr. 55:23507a.
133. Bangal, L. I., Pevzner, M.S., **Lopyrev Khim geterotsiki Soedin Sb 1:**
azotsoderzhashchie geterotskly 184 (1967. cited through **Chem. Abstr.**
70:77877u.
134. Chambus, V.C., **J Am. Chem .Soc. 82,(1960), 605.** cited through **Chem. Abstr.**
54:9937a.

135. Shepardd, K.L., Mason, J.W., Woltersdorf, O. W., Jones, J.H., James H. and Crageol, E.J. **J. med. Chem.** **12**,280(1969) cited through **Chem. Abstr.** **70:96755u.**
136. Russo, F., Santagati M. and Poppalardo, G., **Ann. Chim. (Rome)** **62**, (1972), 351. cited through **Chem. Abstr.** **77:126518h.**
137. Becker, H.G.O. and Eisenshimidt, V., **Z. Chem.** **8**, 105(1968). cited through **Chem. Abstr.** **68:95761q.**
138. Becker, H., and Wehner, K., **Brit. Pat.** **1157256(1969).** cited through **Chem. Abstr.** **71:81375s.**
139. Barascut, J.L., Viblafont P. and Dauni, J., **Bull. Soc. Chim. France(1975)** 1649. cited through **Chem. Abstr.** **84:17241m.**
140. Genhlen, H., Dost J. and Cemak, J., **Justus Liebig Ann. Chem.** **643**,(1961),116. cited through **Chem. Abstr.** **55:24728c.**
141. Sgarbi, R., **Chim. Ind(Milan)** **48**, 1966),18. cited through **Chem. Abstr.** **64:9727d.**
142. Dowidson, J.S., **Chem. Ind.(London)**,11(1972),464. cited through **Chem. Abstr.** **77:48344g.**
143. Allen, C.F.H.,and Bell, A., **Org. Synthesis** **24(1944)**,12. cited through **Chem. Abstr.** **38:58283a.**
144. Kato, Y. and Hirao, I., **Kyushu Kogyo Daigaku Kenkyu Hokoku, No** **14(1964)**,35. cited through **Chem. Abstr.** **61:14662g.**
145. Herbst, R.M. and Garrison, J.A. **J Org, Chem.** **18**,(1953),872. cited through **Chem. Abstr.** **48:7006c.**
146. Navikov, S. S., Rudenko V.A. and Brusnikina, V. M., **Izv. Akad. Nauk USSR, Otdel. Khom. Nauk** **1961**,1148. cited through **Chem. Abstr.** **55:27282f.**
147. Mizushima, K., Saheki, M., Shillna, H.,and Hamada, A., **Japanese Pat.** **7135045(1971)** cited through **Chem. Abstr.** **76: p3867t.**
148. Boyalkovskii- Krupin, K., and Lopyrev V.A. ,**Matody Paluch. Khim. Reaktiv Prep** **22**, (1970) **92** cited through **Chem. Abstr.** **77: 5410h.**
149. Boulton, A.J., and Lartritzky, A.R., **J. Chem . Soc.(1962)**2083. cited through

Chem. Abstr. 57:5911g.

150. Wiloth, F. , **Ger. Pat . 1135920(1962)**. cited through **Chem. Abstr. 58:8071d.**
151. Taniyama, M., Nagaoka, T., Takada, T., **Japanese Pat. 9221(1962)**. cited through **Chem. Abstr. 59:10065h.**
152. Krysin, E.P., Levchenko, S.N., Andronova, L.G., and Nakenina, M.M., **USSR Pat. 491636(1975)** cited through **Chem. Abstr. 84:90156s**
153. Bowie, R.A., Gardner, M.D., Neilson, D.G., Watson, K.M., Mahmood S.,and Ridd V., **J. Chem . Soc., Parin Trans 1,(1972),2395**. cited through **Chem. Abstr. 77:152130v.**
154. Grammaticakis, P. **C R Acad. Sci. Paris 258, (1964), 1262**. cited through **Chem. Abstr. 60:14508e.**
155. Brown H.C. and Pilipovich, D., **J Amer. Chem. Soc. 82,(1960),4700**. cited through **Chem. Abstr. 55:6474c.**
156. Baumabach, F., Henning H.G., and Hilgetag, R., **Z. Chem. 4, (1964), 67**. cited through **Chem. Abstr. 60:12006c.**
157. Lewis, R. N. and Susim, P.V., **J. Amer. Chem. Soc., 74:840(1952)**.
158. Copelin, H.B.(1956); **U.S. Pat. 2,715,137(1955)**, cited through **Chem. Abstr. 50:7126(1956).**
159. Rogers, A.O., **U.S. Pat. 2,415,267(1947)**; cited through **Chem. Abstr. 41:3119(1947).**
160. Friedman, L. and Schchter, H., **J. Org. Chem., 26:2525(1961).**
161. Newman, M.S. and Boden, H., **J. Org. Chem 26:2525(1961).**
162. Rorig, K. et.al. **Org. Synth. Coll. Vol.4:575(1963).**
163. Mc Elvein, S.M.and Goese, M.A., **J. Am. Chem. Soc.,63:2283(1941).**
164. Gilman, H and Spatz, S.M., **J. Am. Chem. Soc 63:1553(1941).**
165. Kent, R.E. and McElvain, S.M., **Org. Syn. Coll. Vol.3, 493(1955).**
166. Corson,B.B.et.al., **Org. Syn. Coll. Vol.2 , 2,379(1943).**
167. Brown, P.M. et. Al., **J. Chem. Soc., 3, 2882(1957).**
168. Stephens, C.R. et.al., **J. Am. Chem. Soc, 77, 1701(1955).**
169. Surry,A.R. **Org. Syn. Coll. Vol. 3, 535(1955).**

170. Herbst, R.M. and Wilson , K.R., **J. Org .Chem.,22,1142(1957).**
171. Campagna, F., Carotti,A., and Casini,G., **Tetrahedran Letters, 21, 1813-16(1977).**
172. Hagenest, H., and Stauf, F.W., **U.S. Pat. 1,879,209(1932); cited from Chem abstr.,28, 4848(1934).**
173. Carke, H.T. and Read ,S., **Org. syn. Coll. Vol.1, 514(1941).**
174. Vohwinkel, E. and Bartel, G., **J. Chem Ber.,107,1221(1974)**
175. Farey, H.G. and Dalton, D.R., **J. Chem. Soc. Chem. Commun.,628(1973).**
176. Kukhar, V.P. and Pasternak, V.I., **Synthesis, 563(1974).**
177. Streith, J. and Fizet, C., **Helv. Chim. Acta.,59, 126(1975).**
178. Miller, M.J. and Zoudon,G.M., **J. Org. Chem, 40, 126(1975).**
179. Glass, R.S. and Hoy, C., **Tetrahedran Letters, 1781(1976).**
180. Houben-Wcyl., **Methodender Organischen Chemie, 4th edn., 3,325(1952).**
181. Kitagawa, T. et,al., **Chem. Pharm. Bull., 38,2583(1990).**
182. Carpino, L.A., **Accounts Chem. Res., 6, 191(1973).**
183. Carpino, L.A., Giza, C.A. and Carpino, B.A., **J. Amer. Chem. Soc., 81, 955(1959).**
184. Sheradsky, A., **J. Het. Chem., 4, 413(1967).**
185. Tamare, Y., Minamikawa,I., Kita, Y., Kim, N.H. and Ikeda, M., **Tetrahedran Letters, 29,1063(1973).**
186. Tamare, Y., et.al., **J. Org. Chem., 38,1239(1973).**
- 186a Hodgkins, J. E. and Reeves H., **J. Org. Chem. 29, 3098(1964).**
187. Eugene Lieber and J. Ramchandran **Can. J Chem. 37, 101(1959).**
188. Buu-Hoi, N.P., et. al., **J.C.S.,1573(1955).**
189. Galstukhova, N.B. and Shchukina, M.N., **Zhur. Obscher. Khim., 31, 1090-2, (1961), Cited through C.A. 55:24778d.**
190. Connolly, J.M., et.al., **J.C.S., 679, (1935).**
191. Reifschneider, Walter (Dow Chem Co.), **U.S. 36,55,773 (Cl. 260-609;C07c), 11 Apr 1972, 15,86,402 Mar 1970. 3pp. Cited through C.A. 77:P5171f.**
192. Bowman, R.M., Steele, R.E., Browne, L., (Ciba- Giegy Corp.) **U.S. Pat; US**

SYNOPSIS

SYNOPSIS

Bacteria were first identified in the 1870s by Van Leeuwen Hoek, following his experiments with the microscope. However, it was not until the 19th century that their link with disease was appreciated. During the latter half of the 19th century scientists such as Koch were able to identify the microorganisms responsible for communicable diseases such as tuberculosis, cholera and typhoid. However, Paul Ehrlich switched the direction and entered a field which he defined as "chemotherapy". This change introduced a number of anti-tubercular and anti-microbial agents, which are being used to date. However, there is a constant need for new drugs due to the resistance exhibited by various organisms.

The WHO has estimated that within the next ten years 30 million people worldwide will die of tuberculosis¹. At present Isoniazide, Streptomycin, Rifampin etc. are the drugs of choice and are used in different combinations in the treatment of tuberculosis. Now a days because of multidrug resistant strain of *Mycobacterium tuberculosis* and also due to AIDS, tuberculosis has once again become a major health problem worldwide. Therefore, there is an urgent need for new approaches to combat the spread of tuberculosis and other microbial infections.

Medicinal chemistry is the study of those chemicals which are expected to have beneficial effects on the infected living systems. At its heart is synthetic organic chemistry. But no medicinal chemist is merely interested in the chemical properties of the molecule under investigation. The entire logic and all efforts towards the synthesis of any new compound are for an anticipated biological activity. But it happens most frequently that the joint efforts result in compounds with a lack of activity.

Amidst the frequent, but much needed failures and seldom occurring breakthroughs, the strife in medicinal chemistry continues for various reasons; more silently for -

1. Obtaining compounds with higher efficacy and lower toxicity.
2. To hit upon moieties that tackle drug resistance of various microorganisms.

One aspect of Medicinal chemistry deals with the incorporation of newly emerged pharmacophores on already existing biologically active moieties to produce newer molecules with higher efficacy. These and other reasons inspired the present research work to synthesize certain novel heterocyclic compounds.

A total of 20 compounds have been synthesized and characterized by TLC, M.P. (recorded in open capillaries and are uncorrected), FT-IR (recorded on Hartmann-Braun, MB-104 series), NMR (recorded at RSIC, IIT, Mumbai), and carbon, nitrogen and hydrogen analysis (recorded on Heraeus C,H,N Elemental Analyzer, Haffkine Institute).

The thesis will consist of the following parts:

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

PART 2: Synthesis of N-[acetamido(2-hydroxy pyrazine)]-N'-(substituted aryl/alkyl) thiourea.

PART 3: Synthesis of [3-(4 -chlorophenyl)-4'-(substituted aryl)]thiazoline-2-(methoxy carbonyl pyrazine) hydrazones.

PART 4: Synthesis of 4-[N-(4-Bromobenzyl)-N-(1,2,4-triazole-4-yl) benzonitrile.(YM511)

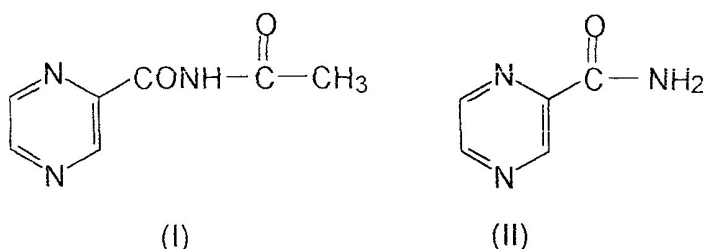
PART 5: Biological screening, results and discussion.

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

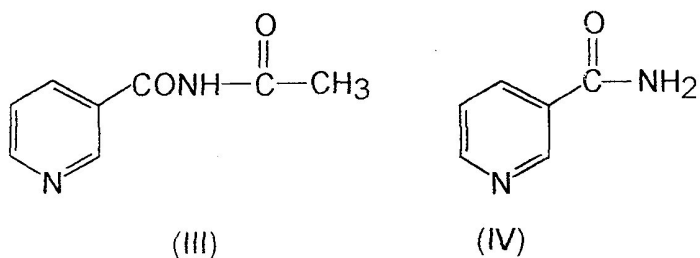
This part of the thesis will be devoted to the general Introduction which will briefly highlight the background of current research project. This will also cover a modestest review of literature with reference to the selected heterocycles and the plan of work executed during the period.

PART-2 : Synthesis of N-[acetamido(2-hydroxy pyrazine)]-N'-(substituted aryl/alkyl) thioureas:

The literature search shows that 2-substituted pyrazine derivatives(I) exhibit antitubercular activity², of which Pyrazinamide (II) is the clinically used, first line antitubercular agent.

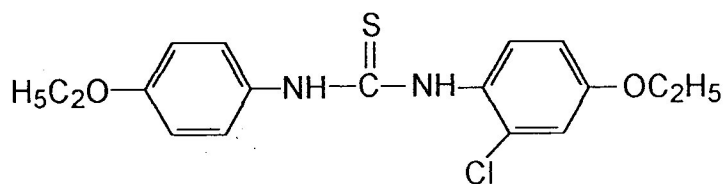


Isoniazide (III) is the most commonly used drug for the treatment of tuberculosis and it contains nicotinic acid as the pharmacophore. Also, Nicotinamide(IV) contains nicotinic acid group, which is responsible for its anti-tubercular activity³. However, Pyrazinamide and Nicotinic acid are considered bioisosters of each other.

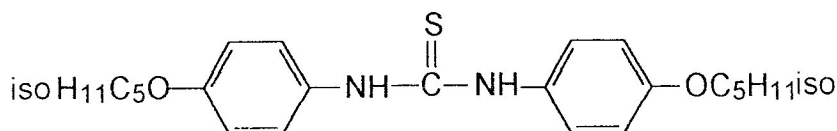


Hence, it was decided to use pyrazine as the parent moiety.

Cuong *et al.* have reported the antitubercular activity of halogenated derivatives of alkoxythiocarbanilides⁴(V). These compounds have exhibited promising antitubercular activity in the range of 5µg/ml..



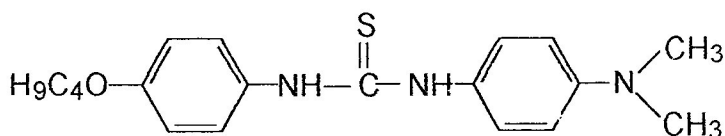
2-Chloro 4, 4'-diethoxythiocarbanilide



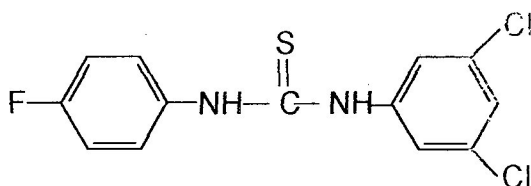
2-Chloro 4, 4'-diisoamyloxythiocarbanilide

(V)

These compounds contain (-NH-C=S) group which acts as the pharmacophore. Also, 1,3-disubstituted thioureas are well documented in literature for their biological activity. e.g. thiambutasine⁵(VI). Fluonilide⁶(VII) is a well-known antifungal agent which is a simple disubstituted thiourea.

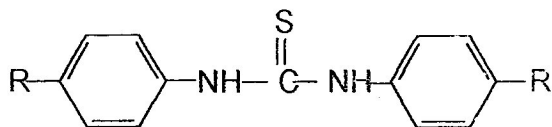


(VI)



(VII)

Fox *et al* showed that 4,4'-disubstituted diphenyl thioureas(VIII) have *in vitro* antitubercular activity.⁷

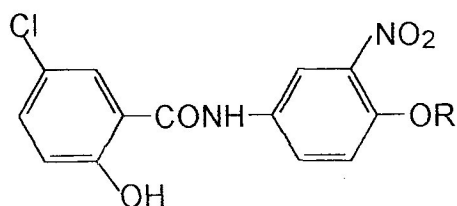


R=Cl, F, OCH₃ etc.

(VIII)

These references prompted us to include the thiourea group in our study.

The presence of -CONH- linkage also plays an important role in imparting antifungal and antitubercular activity⁸ to the molecules, which is reiterated by the following compounds(IX).

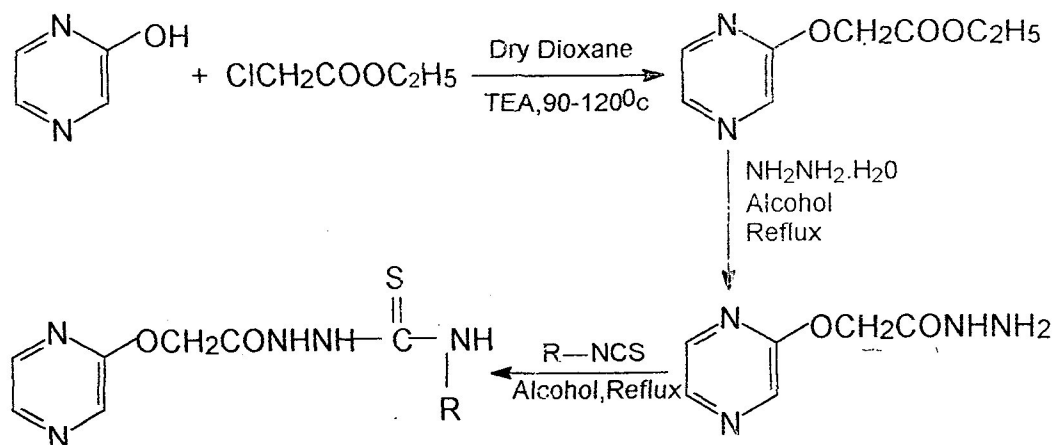


R=C₆H₅, C₂H₅ etc.

(IX)

Considering all these factors, a (-NH-C=S) group and a (-CONH-) group was incorporated on the pyrazine nucleus to obtain the title compounds. A total of 13 compounds were synthesized as per the method outlined in scheme I.

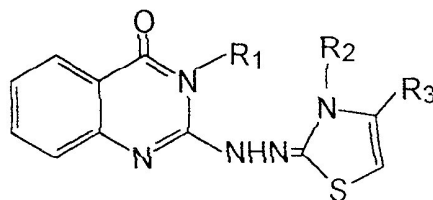
SCHEME-I



R=Substituted aryl/alkyl

PART- 3 : Synthesis of [3-(4'-chlorophenyl)-4-(aryl substituted)]thiazoline-(2- methoxy carbonyl pyrazine) hydrazones.

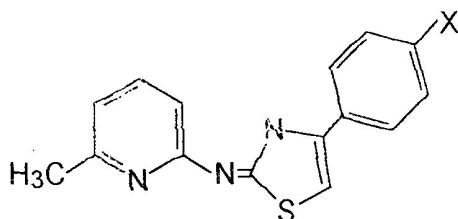
Thiazoline ring prepared by cyclization of thiourea was found to impart antimicrobial activity to the compounds.



R1=Allyl, C₆H₅NH₂ etc.
R2=Allyl, C₆H₅NH₂ etc.
R3=C₆H₅, 4-C₆H₅Cl etc.

In the above structure, the activity of the parent quinazoline was enhanced on converting it to its thiazoline derivatives. These derivatives exhibited significant *in vitro* antibacterial activity against gram-positive organisms. The activity of some of the products was found to be equivalent to that of Penicillin.⁹

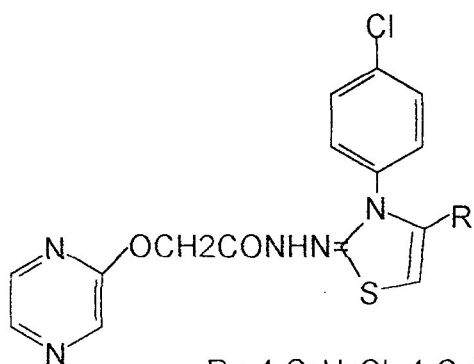
Hassan *et al*¹⁰ observed moderate antimicrobial activity in the compounds(X) bearing a thiazoline ring on the parent pyridine moiety.



X=Br, Cl, OCH₃ etc.
R=CH₃, C₂H₅, C₆H₅ etc.

(X)

The activity of thiazoline ring prompted us to introduce this moiety on the parent pyrazine molecule (significance discussed in part I) in an attempt to achieve anti-microbial activity for the title compounds(XI).

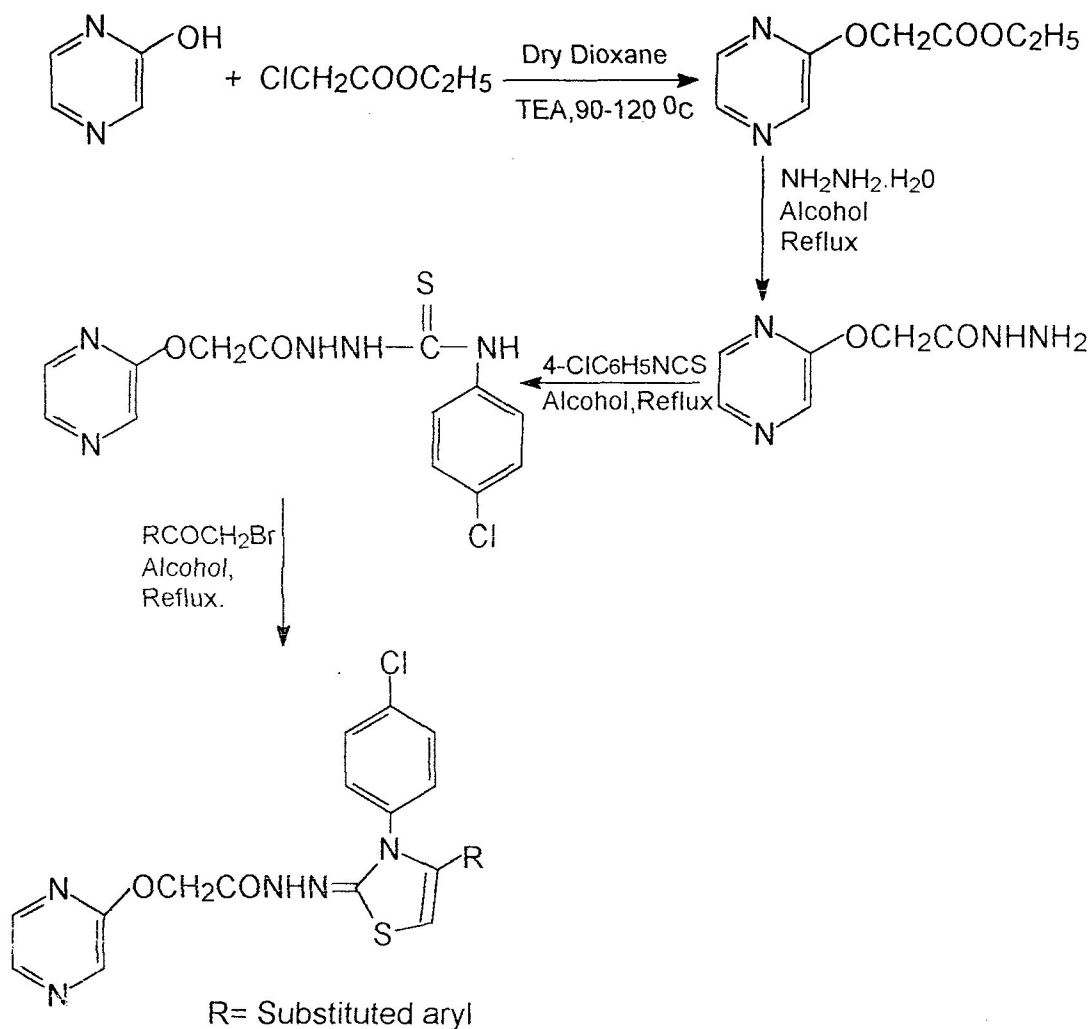


R = 4-C₆H₅Cl, 4-C₆H₅F etc

(XI)

This was achieved by cyclising a halosubstituted thiourea with various substituted phenacyl bromides, as mentioned in scheme II. A total of seven such compounds were obtained.

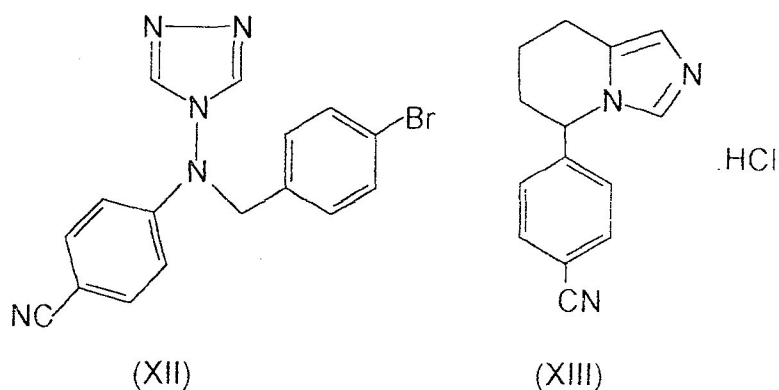
SCHEME-II



PART-4 :Synthesis of 4-[N-(4-Bromobenzyl)-N-(1,2,4-triazole-4-yl) benzonitrile.(YM-511).

The enzymes required for the formation of biologically active estrogens (aromatase, estrone, sulfatase and estradiol dehydrogenase) are present in both normal and malignant tissues. These enzymes are rational targets for the development of drugs to treat breast cancer and other related diseases in which female hormones are known to be a contributing factor.

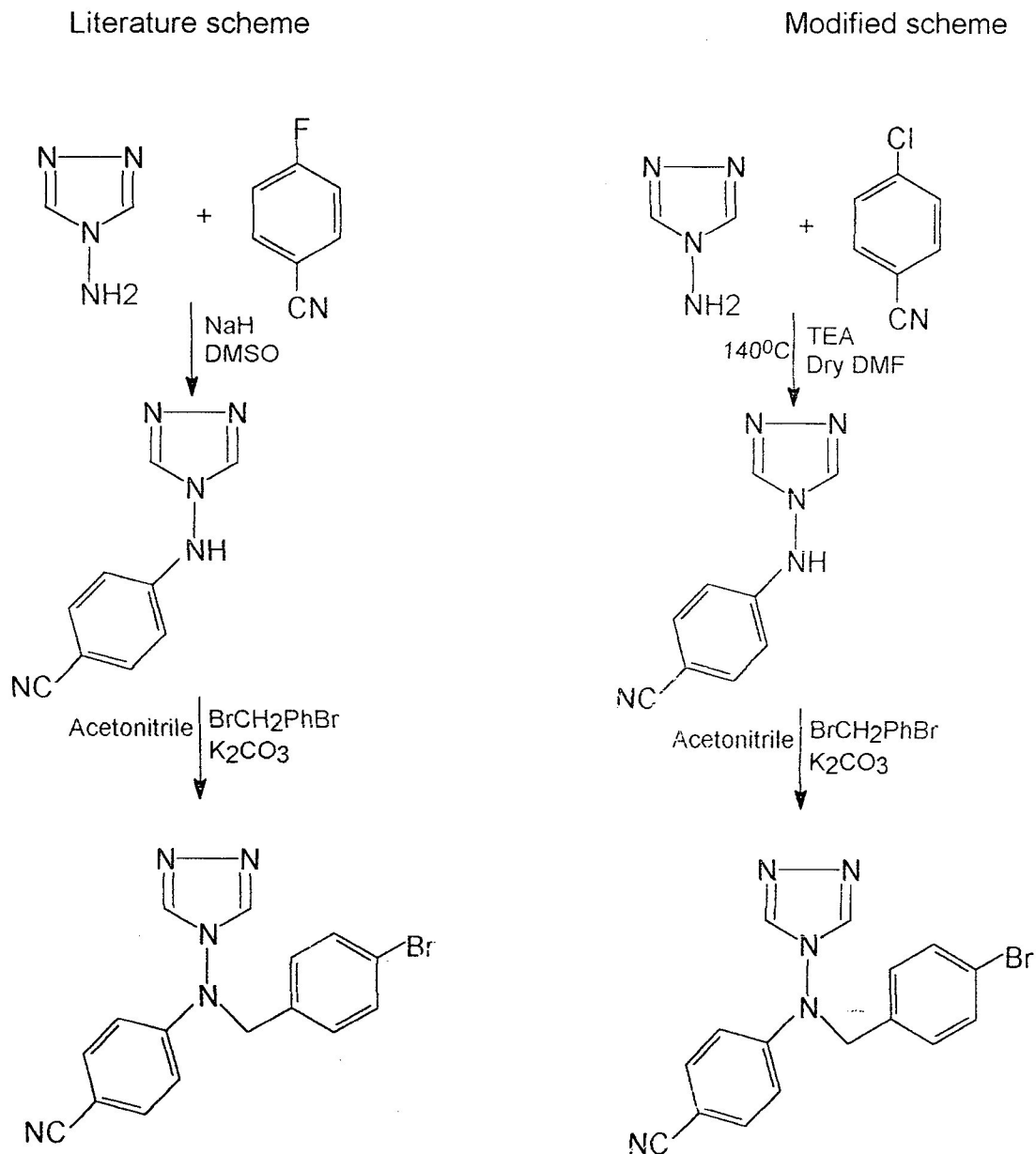
YM-511(XII) is a new non-steroidal aromatase inhibitor having high potency and selectivity. It inhibits the enzyme from ovarian microsomes and human placenta with IC_{50} of 0.4nM and 0.12 nM, respectively. It is found to be more potent than Fadrazole hydrochloride(XIII)(IC_{50} 1.5nM and 0.37 nM, respectively)¹¹, which is a new drug developed by Ciba-Geigy for similar indications.



In addition, YM-511 was found to be more selective than Fadrazole. The route of synthesis of YM-511 has been reported in literature¹¹.

However, we thought of synthesizing this compound by alternate route in an attempt to avoid use of toxic and hazardous chemicals like NaH and also to make it cost effective.

SCHEME III



Part - 5 : Biological screening:

All the 20 compounds synthesized herein have been screened for their *in vitro* antitubercular, antibacterial and antifungal activities.

Antitubercular screening:- The *in vitro* antitubercular screening of the compounds was carried out by the tube dilution technique employing H₃₇RV strain of *Mycobacterium tuberculosis*. This method uses Kirchner's medium containing Tween 80 as described by Rake *et al*¹². The standard drug used

was Isoniazide. The MIC of Isoniazide is 0.04 µg /ml against H₃₇RV strain of *M. tuberculosis*.

Antibacterial screening:- The compounds synthesized were also subjected to *in vitro* test against *Staphylococcus aureus* ATCC 3750 and *Salmonella typhi* NCTC 786 by using the tube dilution method. The medium employed in the screening was Mueller Hinton Broth. The standard drugs used were Ampicillin Sodium(MIC=0.01µg/ml) against *S. aureus* and Trimethoprim(MIC=0.1µg/ml) against *S. typhi*.

Antifungal screening:- The compounds have also been screened for their antifungal activity against *Candida albicans*, *Trichophyton mentagrophytes* and *Trichophyton rubrum*. The standard drug used was Fluconazole. The MIC of Miconazole is 6.25 µg /ml against *C.albicans* and 1.56 µg /ml against *T.mentagrophytes* and *T. rubrum*.

REFERENCES:

1. Moran, N.; **Nature medicine**; **2**; 337(1996).
2. Kushner, S., Dalalian, H. *et al*.; **J.Am.Chem.Soc.**, **74**; 3617(1952).
3. Chorine V., **C.R. Soc. Biol. (Paris)**,220,150(1945).
4. Cuong, D.Q., Buu-Hoi, N.P. and Xuong, N.D.; **J. Med. Chem.**, **6(4)**; 452(1963).
5. Mayor, R.L.; **Chem. Abstr.**, **36**; 5199(1942).
6. Davy, T.F.; **Leprosy Rev.**, **29** ;25(1958).
7. Fox , H.H.; **J.Org.Chem.**, **17**, 542(1952).
8. Singh, H., Singh, A.K. *et al* ; **J. Med. Chem**, **20(6)** 826-829(1977).
9. Mohsen, M.E., Shams, S.A. *et al*; **Eur. J . Med. Chem**, **16**,77-80 (1981).
- 10.Hassan, H.Y., El-Koussi, N.A. *et al*; **Chem. Pharm. Bull** , **46(5)**, 863-866(1998).
- 11.**Drugs Fut.**, **21(7)**, 716-718(1996).
- 12.Rake, G., Jambor, W.P., Mckee, C.M.,Pancy, F., Wiselogle, E.Y., Donobic, R., **Am Rev. Tubercl.**, **60**,121(1949).

