

**SYNTHESIS AND MICROBIOLOGICAL EVALUATION OF
ORGANIC COMPOUNDS OF POSSIBLE THERAPEUTIC
VALUE**

**THESIS SUBMITTED
UNIVERSITY OF MUMBAI**

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

**SUBMITTED BY
YOGESH KULKARNI**

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

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

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**SYNTHESIS AND MICROBIOLOGICAL EVALUATION
OF ORGANIC COMPOUNDS OF POSSIBLE
THERAPEUTIC VALUE**

**A
Thesis Submitted
To The
University Of Mumbai
For The Degree of
Master of Pharmaceutical Sciences
In
The Faculty Of Technology
(Pharmaceutical Chemistry)**

**By
Mr. Kulkarni Yogesh P.
B.Pharm.**

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**DEPARTMENT OF CHEMOTHERAPY
HAFFKINE INSTITUTE FOR TRAINING, RESEARCH AND TESTING
A.D. MARG, PAREL, MUMBAI – 400 012**

2001

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Haffkine Institute
Mumbai – 400 012

Yogesh P. Kulkarni

STATEMENT

Statement submitted under rule 0.412

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

Statement submitted under rule 0.413

Statement I :

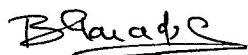
The work embodied in the Thesis to be submitted is original, based on the discovery of new facts, viz. the synthesis and characterization of new and unknown compounds of possible chemotherapeutic value. Also included in the Thesis, are new relations of facts, observed on the basis of in vitro testing.

The assessment of the compounds has been made at the Department of Microbiology and Department of Chemotherapy, Haffkine Institute, Mumbai 400 – 012.

Statement II :

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

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



Dr. B. G. Khadse
Research Guide



Kulkarni Yogesh P.
Research Student

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Part IA

***Introduction
and
Review of Literature***

For centuries, man has observed that natural substances can be used for their nutritional value and for the treatment of diseases, while being aware of their possible toxic and lethal effects.

The field of medicinal chemistry has evolved from an emphasis on the synthesis, isolation and characterization of drugs, to an increased awareness of the biochemistry of diseased states and the design of drugs for the prevention of diseases. An important aspect of the medicinal chemistry has been to establish a relationship between chemical structure and biological activity.

An increased consideration in recent years has been to correlate the chemical structure with chemical reactivity or physical properties and these correlations can, in turn, be related to their therapeutic actions.

Although a great deal of success in understanding the relationship between chemical structure and biological activity has been observed in numerous areas, especially for antibacterial drugs, there are still many human sufferings which require new and improved drugs.

Cancer, viral infections, cardiovascular diseases and mental diseases need new agents and approaches for treating and preventing these maladies. In developing drugs with specific activities, several approaches are used. The effect of natural products or synthetic drugs is determined on various biological systems (or screens) for "leads" to agent specific against various diseases.

Once the effect of the drug is known, the medicinal chemist and the pharmacologist work together to improve the activity of the known active molecule or the "lead molecule". This process normally goes through a synthesis - biological test - synthesis - biological test cycle until a drug with a desired activity is obtained.

Today, the structures of receptors and functions of enzymes, which may be involved in the pathogenesis of a disease, is clearly understood.

These molecules, in turn, are used as targets for the design of drugs which act as agonist or antagonist of receptors or inhibitors of the enzymes. Thus, this information adds a new phase to the cycle-which is now drug design-synthesis-biological activity-drug design etc.

The most frequent approach to obtaining drugs to be used for treating a particular disease is to synthesize analogs of drugs that are known to be effective in the treatment of the disease. The pharmacophore is a chemical segment of a molecule that is responsible for its biological actions. Normally, it is found that a specific type of biological activity of the molecule is dependent upon more than just one functional group. Hence, it becomes important to find such pharmacophores and join them together to obtain a moiety that acts as 'lead'. Various substitutions are then carried out on this moiety to obtain active molecules.

BACTERIAL INFECTIONS :

The development of resistance to current antibacterial therapy continues to drive the search for more effective agents. Bacteria are commonly responsible for many diseases, which were considered until recently to be resistant to chemotherapy. The bacteria were thought to be primitive in structure to be influenced by chemicals, which are not highly toxic to the host. Many attempts have been made to combat bacterial infections in animals by the use of the drugs, but most of them have met with little or no success with the exception of Morgenroth and Levy's partial success in the year 1911, in combating bacterial infections of the blood stream. They found that ethyldihydrocuprein (optochin), a quinine derivative would cure *Pneumococcal* infection in mice. Heidelberger and Jacobs, coupled diazotised p-aminobenzene sulfonamide with dihydrocuprein, and found that the resultant compound had only mild activity, and was not further investigated.

However, Domagk¹, Mietzsch and Klarer² in 1935, first dispelled the failure of earlier scientists by discovering Prontosil, and reported that this compound cures *Streptococcal* infection in mice and *Staphylococcal* infection in rabbits. This period was considered to be a brilliant era in the chemotherapy of microbial infections.

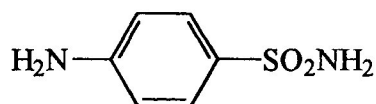
The history of antibacterials has been dynamic, characterized by the constant emergence of new challenges, followed by investigation, discovery and the production of new drugs. A complete review of the various agents would be beyond the scope of this work and hence, is not attempted. The following pages present a brief summary of the important classes of compounds used for this purpose.

Synthetic Antibacterial agents :

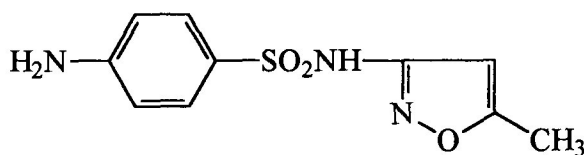
Sulphonamides

Sulphonamides were the first, systemic 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. The Sulfonamides are bacteriostatics when used in achievable doses and, inhibit the enzyme dihydropteroate synthetase (an important biocatalyst needed for the biosynthesis of folic acid derivatives) and ultimately DNA synthesis. They do it by competing at the active site with p-aminobenzoic acid (PABA), a normal structural component of folic acid derivatives.

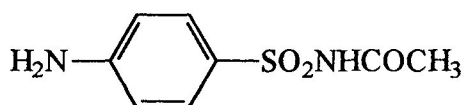
The important members of this class include Sulphanilamide (I), Sulphamethoxazole (II), Sulphacetamide (III), Sulphadiazine (IV) and Sulphisoxazole (V).



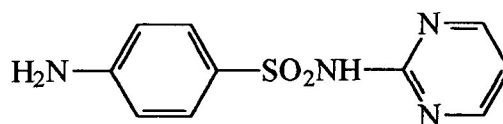
(I)



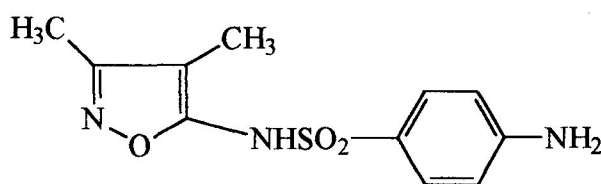
(II)



(III)



(IV)



(V)

The sulphonamides 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 is finally eliminated from the body partly, as the unchanged drug and partly as metabolic products, the largest fraction being excreted in the urine.

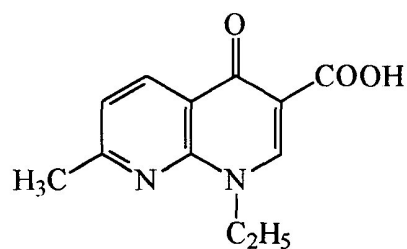
The strains of bacteria resistant to the sulphonamides have become common during recent years. Also, the advent of Penicillins and other antibiotics has diminished the role of sulphonamides as antibacterial agents. However, the introduction of the combination of Trimethoprim and Sulphamethoxazole, known popularly as Cotrimoxazole, has resulted in an increased use of the sulphonamides for the treatment of specific bacterial infections.

Quinolones

The 4-quinolone antibacterial compounds have a number of advantages over other classes of antibacterials. They are effective against many organisms, well absorbed orally, well distributed in tissues, have a relatively long serum half-life and minimum toxicity. Because of deep tissue and cell penetration, they are especially useful for urinary tract infections, prostatic infections of skin and bones and Penicillin resistant sexually transmitted diseases.

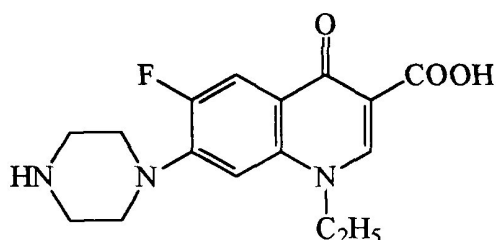
The quinolone carboxylic acids or 4-quinolones are a group of synthetic antibacterial agents structurally related to Nalidixic acid (VI). Nalidixic acid is active against gram-negative bacteria but has little activity against *Pseudomonas* and gram-positive organisms, because of which its bactericidal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

A modification of the structure of Nalidixic acid has produced related antibacterial agents such as Oxolinic acid, Pyromidic acid, Cinoxacin and Acrosoxacin. None has been considered to represent a significant clinical advantages over Nalidixic acid.

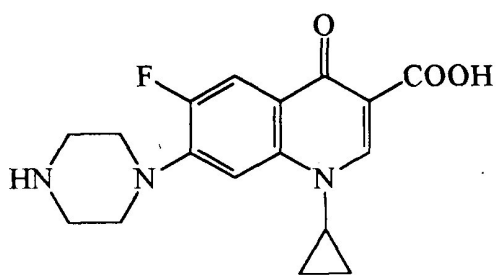


(VI)

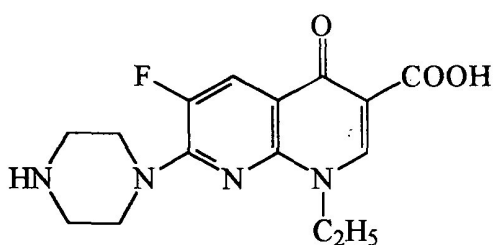
The addition of the 7-piperazinyl group and a fluorine atom at position 6 of the 4-quinolones has produced a group of fluoroquinolones with a broad spectrum of activity as compared to Nalidixic acid and are most useful for the treatment of systemic infections. They include Norfloxacin (VII), Ciprofloxacin (VIII), Enoxacin (IX), Ofloxacin (X) and Lomefloxacin (XI). One disadvantage of all 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 erosion in immature animals.



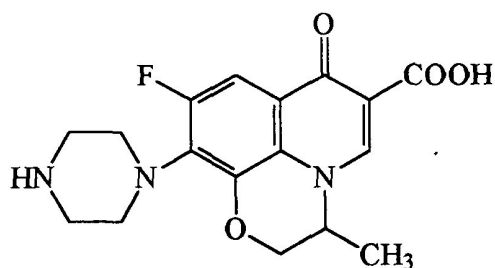
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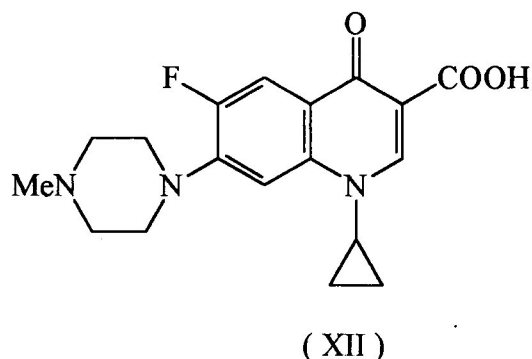
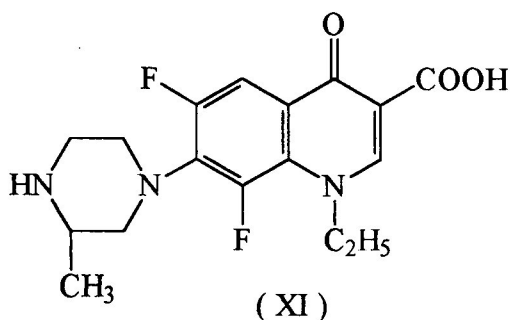
(VIII)



(IX)



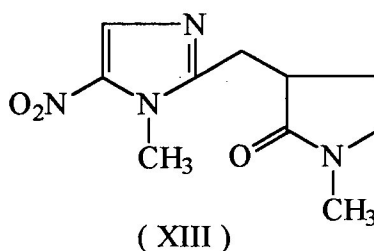
(X)



Tefloxacin (XII) was demonstrated by Y. L. Chen³ to bear activity against Penicillin resistant *Staphylococcus*, *Streptococcus* and *Haemophilus* species. It was itself a β -lactamase inhibitor and displayed synergism with Ampicillin.

Miscellaneous

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

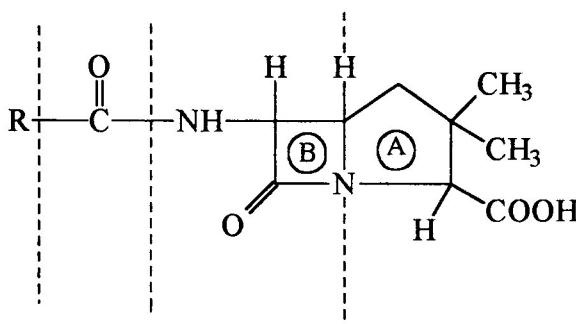


The minimum chemical requirements for significant antibacterial activity include presence of the 4-pyridine ring with a 3-carboxylic acid group attached to it. The mechanism of action of the quinolones is prevention of the detachment of gyrase from DNA.

Penicillins and Cephalosporins

Since Alexander Fleming's serendipitous discovery of Penicillin in 1928, it has remained one of the most important groups of antibiotics. Despite the discovery of several other antibacterial 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 a large number of infectious diseases.

The basic structure of Penicillin (XIV) consists of a thiazolidine ring (A) connected to a beta-lactam ring (B), to which is attached a side chain (R). The Penicillin nucleus is an important requirement for biological activity, alteration of which causes loss of all significant activity. The side chain, which can alter the susceptibility of the resultant compound to inactivating enzymes (beta-lactamases), determines many of the antibacterial and pharmacological characteristics of Penicillin.

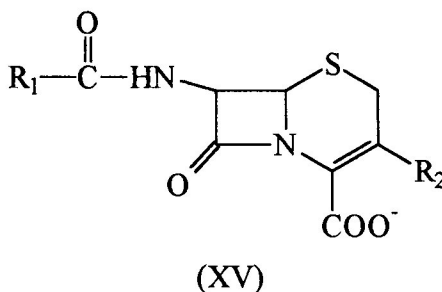


(XIV)

The Penicillins are classified according to their spectrum of antibacterial activity. While the pharmacological properties of the individual drugs in the various classes would constitute a lengthy discussion, certain generalizations can be made. Following absorption, Penicillins are widely distributed throughout the body. Therapeutic concentrations of these agents are quite rapidly achieved in tissues, but comparatively much lower levels are found in prostatic secretions, brain tissue and intraocular fluid. The Penicillins do not penetrate the living phagocytic cells to a great extent. Glomerular filtration and renal tubular filtration quite rapidly eliminate this class of compounds, as a result of which high concentrations of the drug are found in the urine. Therefore, they exhibit short half-lives, approximately in the range of 30-60 minutes.

No sooner was the fact established that Penicillins are capable of eradicating microorganisms, it was practically administered in every situation where there was a risk of bacterial infection. But after investigation 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 effect in the usage of Penicillins, which was probably the most common cause of drug allergy, has 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. Under the worst of circumstances, an extreme allergic reaction may even result in death of the patient.



The Cephalosporins (XV) 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. The first generation Cephalosporins exhibit good activity against gram-positive bacteria and relatively low activity against gram-negative bacteria. The second generation Cephalosporins exhibit better activity against gram-negative microorganisms but are inferior to 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 beta lactamase producing strains.

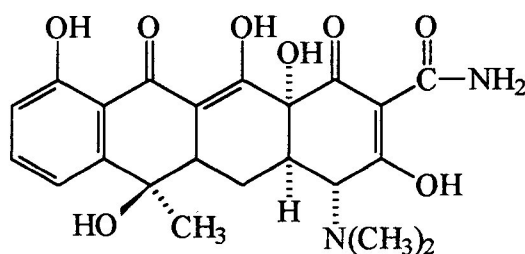
The Cephalosporins are the most widely used antibiotics, perhaps overused for conditions where much less expensive antibiotics could provide relief. Nevertheless, they are indeed useful as alternatives to Penicillins for a variety of infections, in patients who suffer allergic reactions to Penicillins. The Cephalosporins also find wide use in 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 in some cases superior to a combination of Ampicillin and Chloramphenicol.

The beta-lactam antibiotics (Penicillins and Cephalosporins) suffer bacterial resistance due to a variety of factors. It may be caused by the inability of the agent to enter the site of action⁴. Also, it is possible for a sensitive strain to acquire resistance by mutation and has been described for gram-positive cocci⁵. Bacteria can also

destroy the beta-lactam antibiotic enzymatically. Beta lactamases are capable of inactivating certain of these antibiotics and is the major form of this enzyme, although different microorganisms synthesize a number of distinct beta-lactamases. Depending on substrate specificities, these are often described as either penicillinases or cephalosporinases. Individual Penicillins and Cephalosporins vary in their susceptibility to these enzymes.

Tetracycline

The Tetracyclines (XVI) 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 agents.



(XVI)

The microorganisms that have become resistant to one type of Tetracycline, frequently exhibit resistance to the other members of the family. The bacterial species possibly exhibit resistance only after exposure to the drug. The Tetracyclines are useful for treatment of infections with aerobic gram-negative bacteria, but most species are now relatively resistant. Nevertheless, more than 90% of strains of *Haemophilus influenza* may still be sensitive to either Tetracycline or Doxycycline.

All the Tetracyclines produce gastrointestinal irritation to varying degrees in some individuals and 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 reaction in the skin of treated individuals exposed to sunlight. Other important antibacterial agents include Chloramphenicol and the furan derivatives such as Nitrofurantoin, Furazolidone etc. Several of the drugs active against *M. tuberculosis*

discussed later are also active against various species of gram-positive and gram-negative microorganisms besides mycobacteria.

FUNGAL INFECTIONS

Fungi are heterotrophic, eukaryotic microorganisms that are distinguished from algae by their lack of photosynthetic ability. Fungi differ from bacteria in their greater size and possession of such intracellular structures as nuclear membranes and mitochondria. Cells of fungi pathogenic to animals are nonmotile and generally have a rigid cell wall containing chitin and polysaccharides.

Fungi cause many infections of plants. The lesions produced include various types of blights, mildews, leafspots, and galls, and such systemic diseases as rusts and smuts. In contrast to plants, fungi cause far fewer infectious diseases of animals and humans than are initiated by bacteria or viruses. Mycotic infections of humans are conveniently divided into two groups

1. The dermatophytes, which consist of contagious superficial skin infections.
2. The systemic mycoses, which are not contagious, except in the case of neonates.

Severe toxic and haemorrhagic diseases can occur in animals and humans after ingestion of harvested grains and nuts in which saprophytic fungi have grown and produced toxins. Likewise, hemolytic, neurotoxic, and gastrointestinal diseases are associated with ingestion of certain types of saprophytic mushrooms. Antifungal drugs are powerless in the treatment of mycotoxic diseases.

The discovery that specific fungi are the etiologic agents of specific infectious diseases preceded the work of Pasteur and Koch on pathogenic bacteria by three decades. Nevertheless, medical mycology has not received as much attention as have other areas of medical microbiology and at present there exist neither clinically available vaccines nor useful antisera for mycotic diseases. The incidence of systemic mycoses continues to rise steadily. This increase is due in part to the prolonged survival of patients with defects in their host defence mechanism, including patients with neoplastic diseases, organ transplant recipients, diabetics and patients with AIDS. These patients are susceptible to an ever-growing list of opportunistic fungi. From the data collected by the Center for Disease Control, it is

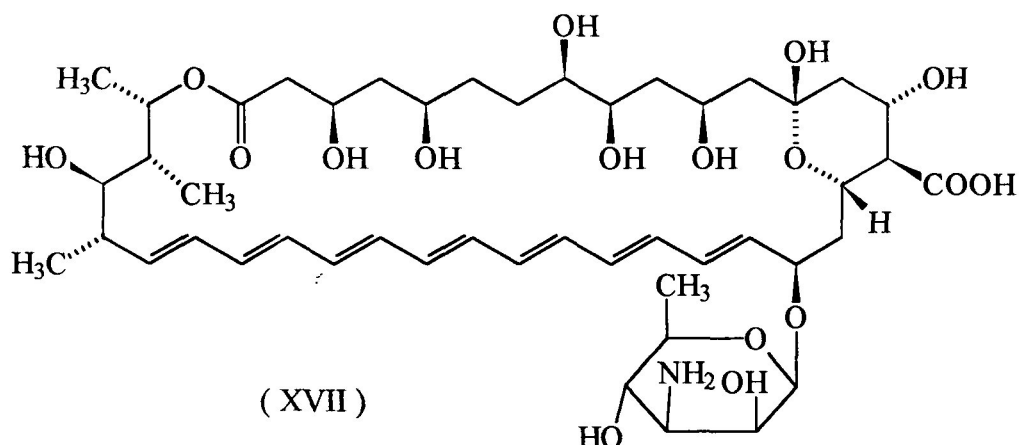
known that among the 1 and 2 million healthy Americans who have already been infected with AIDS, a significant number of these cases are associated with various fungal infections⁶. One yeast-like fungus, *Cryptococcus neoformans*, is reported to be the most common debilitating and life-threatening infections in AIDS patients⁷.

The following pages present a brief description of the major / principal drugs that are used in treating fungal infections.

Amphotericin B

This antibiotic produced by *Streptomyces nodusus*, was discovered in 1955. It became the first systemic antifungal drug. Amphotericin B (XVII) is a polyene macrolide with seven conjugated double bonds (a heptene), an internal ester, a free carboxyl group and a glycoside side chain with a primary amino group. The drug is amphoteric, insoluble in water and is not absorbed via oral or intramuscular routes and is very active against pathogenic yeasts⁸.

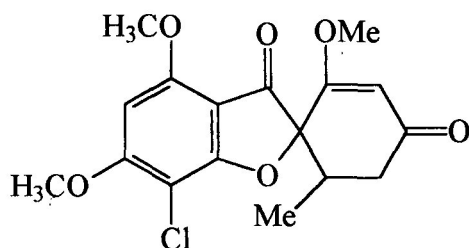
The primary mechanism of action of the polyenes in general, and amphotericin B in particular, is binding to ergosterol, the principal sterol present in the cell membrane of sensitive fungi⁹.



The most significant adverse effect of Amphotericin B is nephrotoxicity. In spite of the adverse effects, it is still the drug of choice for treating most deeply invasive mycoses, including both endemic mycoses and opportunistic mycoses.

Griseofulvin

Although Oxford A. E. *et al* discovered this antibiotic, a product of *Penicillium griseofulvum* (XVIII), in 1939, its antifungal activity was not utilized until 1951. Groove predicted its chemical structure in 1950¹⁰. However, in 1958, the antibiotic was found to be effective against dermatophytes when administered orally. The fluoro analogues retain the potency of the original drug but the bromo and dichloro analogues are ineffective. The replacement of the methoxy substituent on the cyclohexane ring either with propoxy or butoxy, increased invert activity, whereas substitution of an amino acid group at this site eliminates activity.

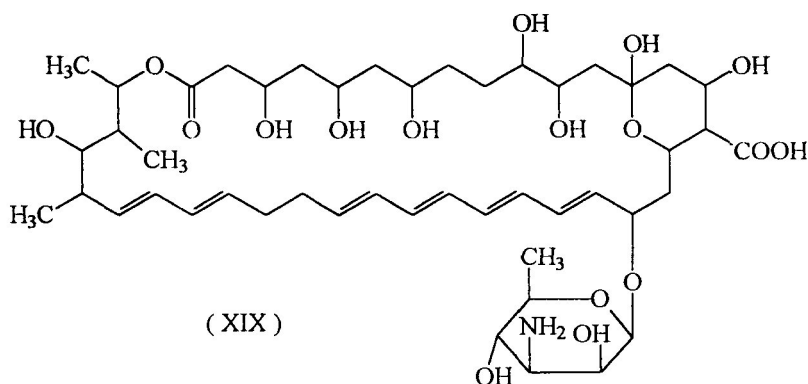


(XVIII)

In susceptible fungi, Griseofulvin binds to RNA and to microtubule-associated proteins to result in inhibition of nucleic acid synthesis and microtubule function. In addition to causing the formation of abnormal fungal cells, the drug can inhibit mitosis of animal and plant cells to cause multipolar mitosis and production of abnormal nuclei.

Nystatin

Nystatin (XIX) is produced by *Streptomyces noursei*. It was discovered by Hazen and Brown¹¹ and possesses good antifungal activity. Toxicity after parenteral administration to animals has led to its topical or oral use.

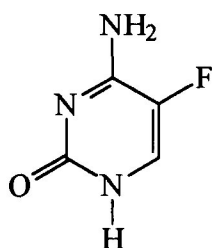


(XIX)

The minimum inhibitory concentration of this drug for *Candida* species, *Cryptococcus neoformans* and the dimorphic fungi causing systemic mycoses is in the range of 1.56-12.6 µg/ml.

5-Fluorocytosine

This fluorinated pyrimidine (XX) was first synthesized in the 1950s as a potential antileukemic drug. It was found to have *in vitro* and *in vivo* antifungal activity¹². It is being increasingly used as an adjunct to Amphotericin B therapy. Accordingly, in *Aspergillus*, *Candida* and *Cryptococcus* infections, the drug is combined with Amphotericin B.



(XX)

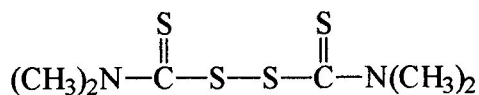
The susceptible fungal cells deaminate 5-fluorocytosine to 5-fluorouracil. The latter is metabolized to 5-fluoro-2'-deoxyuridylic acid, which suppresses DNA synthesis, by inhibition of thymidylate synthetase. The drug can also be incorporated into RNA to cause inhibition of protein synthesis. 5-fluorocytosine is extensively used in a number of human malignancies.

Sulphur containing molecules as antifungal agents

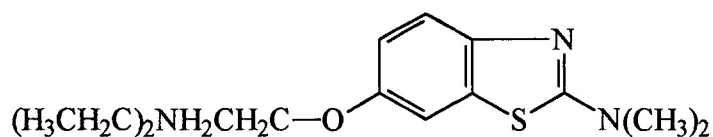
A variety of organic compounds that contain divalent sulphur attached to carbon atom have *in vitro* fungistatic action, and many of these have been employed in topical therapy of dermatophytoses. Among these are dithiocarbamates (XXI), tetraethylthiuram disulphide (XXII) and benzothiazoles such as 6-diethylaminoethoxy-2-dimethyl amino derivatives (XXIII)^{13,14}.



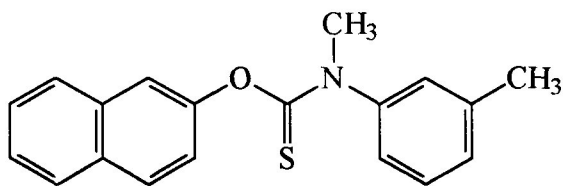
(XXI)



(XXII)



(XXIII)

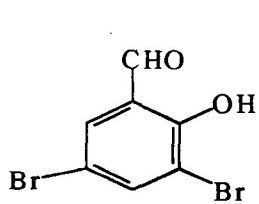


(XXIV)

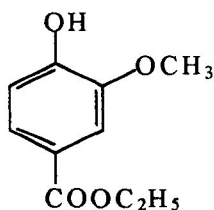
A widely used compound with unusually high *in vitro* and *in vivo* activity against the dermatophytes is o-2-naphthyl ester of *m*-N-dimethylthiocarbanilic acid (XXIV) (Tolnaftate). It is also effective against *Candidal* lesions¹⁵.

Phenolics and Diamides

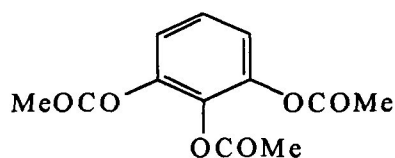
Phenolic compounds were known for their antifungal potency. Among the numerous phenolic compounds that have been used externally for mycotic infections are phenol, phenyl mercuric nitrate, cresol, *m*-cresyl acetate, benzoic acid, 4-hydroxybenzoic acid, salicylic acid, salicylanilide, 3,5-dibromo salicylaldehyde (XXV), ethyl vanillate (XXVI), pyrogallol triacetate (XXVII), 3-iodo-2-propyl-2', 4', 5'-trichlorophenyl ether (XXVIII), haloprogin 1,8-hydroxyanthranol (XXIX) and tetrachlorobenzoquinone (XXX.).



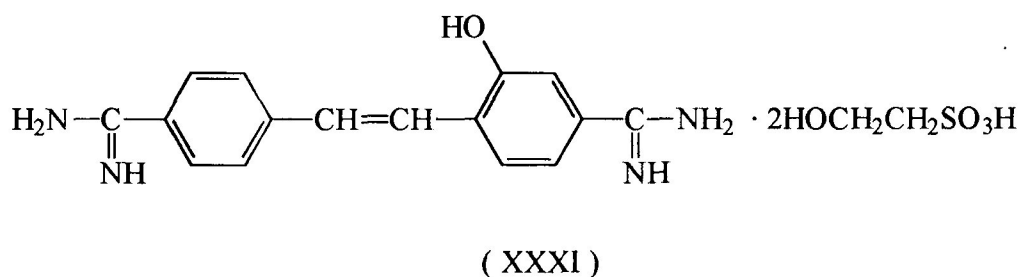
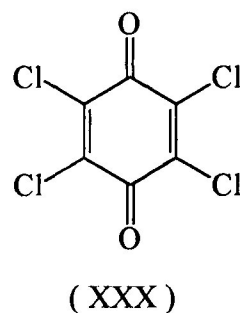
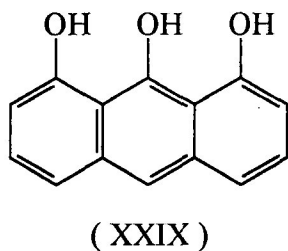
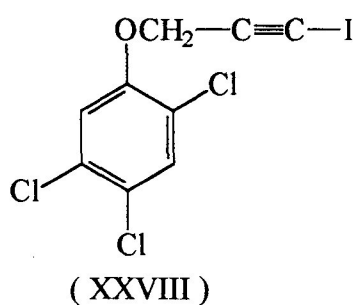
(XXV)



(XXVI)



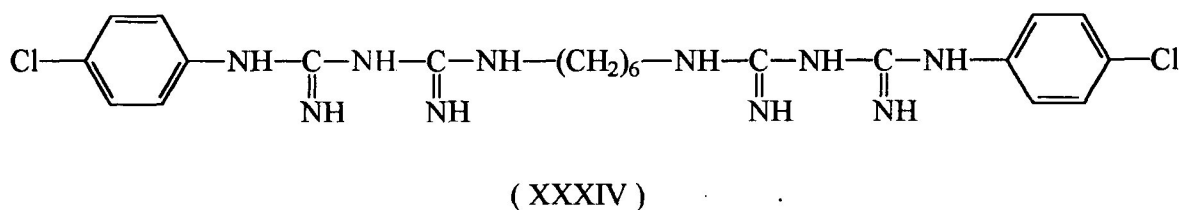
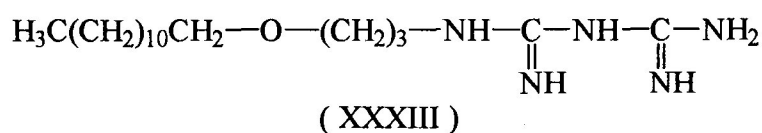
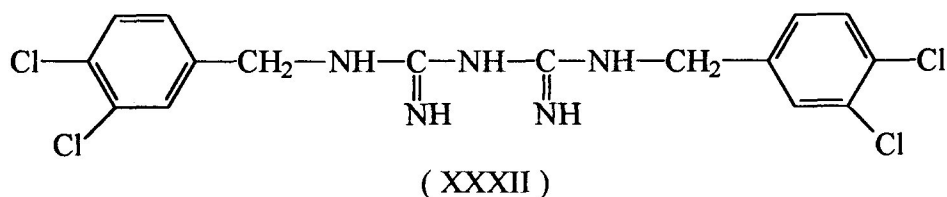
(XXVII)



Several biamidines (e.g. stibamidine, propamidine, pentamidine) are antifungal *in vitro*. 2-hydroxy stilbamidine diisothionate (XXXI) has been successfully employed in patients with blastomycosis. It is less toxic than Amphotericin B¹⁶.

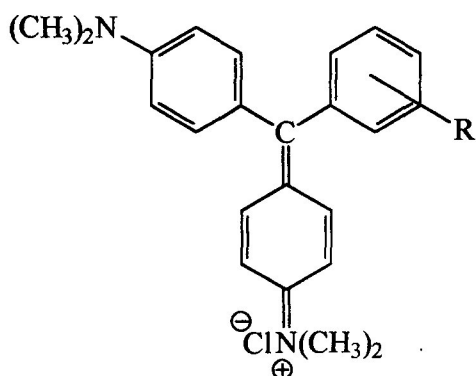
Chelating Agents

Among chelating agents with considerable *in vitro* antifungal potency are substituted biguanides like N',N⁵-bis-(3,4-dichlorobenzyl) biguanide (XXXII), N'-Y-laurooxypropyl biguanide (XXXIII), 1,6-bis- (5-p-chlorophenyl biguanidine) hexane (XXXIV)¹⁷.



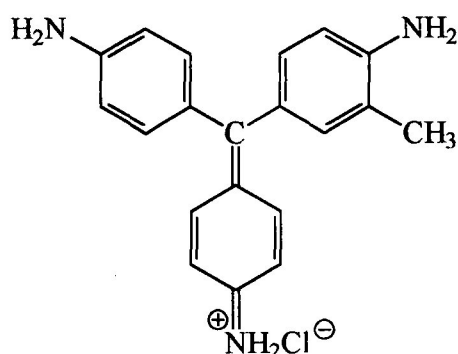
Aniline Dyes

Aniline dyes such as malachite green (XXXV), gentian violet (XXXVI), methyl rosaniline chloride (XXXVII) etc have been employed in topical therapy of dermatophytoses as well as Candidiasis of skin and mucous membranes.



(XXXV) R = H

(XXXVI) R = $\text{N}(\text{CH}_3)_2$



(XXXVII)

Azoles

During the past quarter century, a series of imidazoles and triazoles have been synthesised and examined for antifungal activity. Various members of this series inhibit a broad spectrum of fungal pathogens. Some of the drugs are employed topically, for cutaneous yeasts as well as dermatophytic lesions. Imidazoles (two nitrogen atoms in a 5-membered ring) include Butaconazole, Cloconazole, Clotrimazole, Econazole, Fenticonazole, Ketoconazole, Miconazole, Oxiconazole, Sulconazole and Tioconazole. Triazoles (three nitrogen atoms in a 5-membered ring) include Fluconazole, Itraconazole and Terconazole.

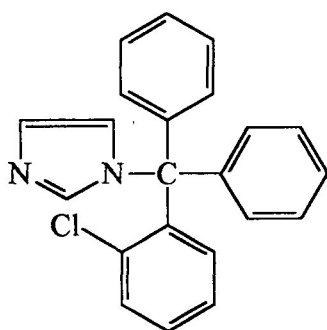
Clotrimazole (XXXVIII) was developed by investigators of the Bayer Laboratories in 1969, and is utilized topically in superficial mycoses or orally in clinical trials for deep mycoses¹⁸.

Miconazole (XXXIX) is a well-known antifungal drug developed by the investigators of Janssen Pharmaceutica in 1969. It is utilized topically in superficial mycoses and by oral and intravenous routes in deep mycoses in clinical experimental trials¹⁹.

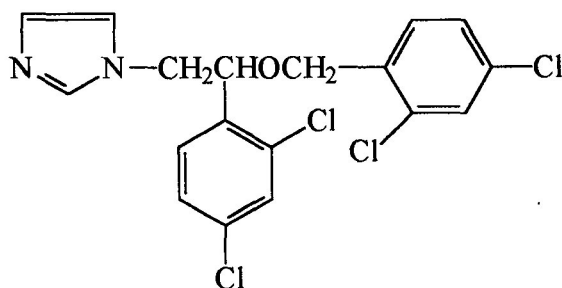
Econazole (XXXX) is a phenethyl imidazole derivative. Like Miconazole, it is utilized as a topical agent in superficial mycoses and as a systemic antifungal by oral and intravenous routes in deep mycoses²⁰.

Fluconazole (XXXXI) is a water-soluble fluorine substituted bis-triazole. It has comparable activity to that of Ketoconazole in inhibition of fungal C-14 demethylase. It is also a potential candidate for prevention and early treatment of disseminated candidiasis in granulocytopenic patients²¹.

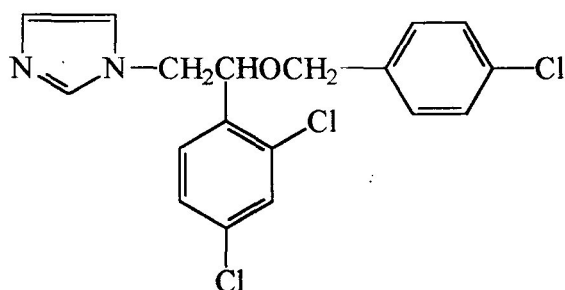
Itraconazole (XXXXII) is a water-soluble, lipophilic triazole analogue that exhibits excellent activity against most fungal pathogens. When compared with Ketoconazole, its potential advantages include a broader spectrum of activity (including *Aspergillus* sp. and *Sporothrix schenckii*), a more desirable pharmacokinetic profile and less toxicity²².



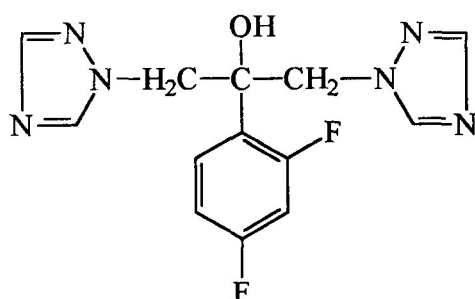
(XXXVIII)



(XXXIX)



(XXXX)



(XXXXI)

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 *Mycobacterium tuberculosis*, and every year 8 million individuals develop the disease, causing 3 million deaths²³.

It attacks both humans and animals, affects all ages and every organ in the body, and ranges 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 World Health Organisation.

Tuberculosis is sometimes an acute, but more frequently a chronic communicable disease that derives its character from several properties of the 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 necrotic area with the communicating channel like bronchus and ureter. When the communication opens, the solid caseous material is autolysed and the liquid contents are expelled, resulting in the development of cavity with a caseous lining. When the dormant bacilli gets 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 materials²⁴.
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 a majority of the chemotherapeutic agents.
4. Tubercle bacilli remain viable and multiply even when ingested by macrophages. The chemotherapeutic agents, with few exceptions, penetrate poorly into the macrophages.
5. Also, 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 semidormant 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 employed²⁴. 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.

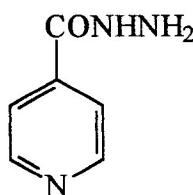
The drugs used currently in the treatment of tuberculosis are as follows:

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

There is no known drug individually self-sufficient, 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 effected. A combination of drugs also helps to prevent the development of resistance.

Isoniazid (INH)

Isoniazid (XXXXIII), 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 treatment. It is highly active against replicating tubercle bacilli but does not affect the semidormant species²⁵ which resume multiplication after removal of contact with the drug.



(XXXXIII)

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 (W.H.O.)

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 to 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. This can be prevented by 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 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 again when the treatment is resumed.

Rifampicin :

Rifampicin is a semi-synthetic derivative of Rifamycin B, isolated from *Streptomyces mediteranei*. 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 has troublesome side effects, requiring special supervision and care. Once weekly regimen shows more toxicity than twice weekly, and with daily regimen side effects are uncommon and trivial. The side effects are normally mild, and can usually be controlled by reducing either the dose size or interval between the doses. However occurrence of purpura is an indication to stop the drug and should not be given again.

Primary resistance to tubercle bacilli 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.

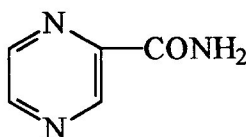
Streptomycin :

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 months²⁶.

Pyrazinamide :

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



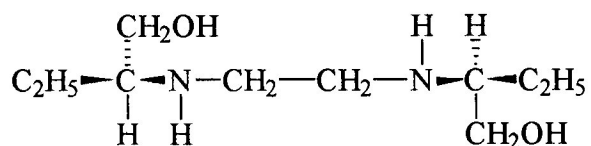
(XXXXIV) Pyrazinamide

When given orally, it produces high serum levels, and penetrates freely into cerebrospinal fluid. It is usually well tolerated but there have been reports of slight

flushing of skin. The dose of Pyrazinamide should be reduced in renal impairment and avoided in patients with severe liver damage and gout.

Ethambutol :

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

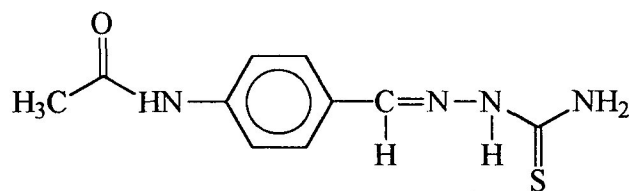


Approximately 75% of the strains of the human type are sensitive to Ethambutol at a concentration of 1µg/ml. It is administered orally and is readily absorbed from the gastrointestinal tract. The normal dosage for adults and children ranges from 15-25 mg/kg daily or 40mg/kg three times weekly. The dosage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

Ethambutol can bring about dose-dependent optic neuritis readily resulting in impairment after use, which is usually reversible, but blindness can occur if treatment is not discontinued promptly.

Thiacetazone :

Thiacetazone (XXXXVI), a thiosemicarbazone derivative, is one of the oldest antitubercular drug 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 regimens.



(XXXXVI) Thiacetazone

The drug is satisfactorily absorbed from 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 appear rarely.

HELMINTHIASIS :

Since prehistoric times, helminthiasis - a disease caused by worms (helminths) has posed severe hazards to man and animals. It is a problem of considerable importance, both to the medical practitioners and the veterinary surgeons. The helminths as parasites of human and livestock, constitute the single most important group of infections, although many separate species are involved.

Dr. Stoll's estimate of global parasitism in 1947^{27a} appears to have altered little till the middle of the last decade^{27b}. The various forms of helminth infections merely seem to have grown in proportion with respect to the growth of the population (Table 1).

Table 1 Global intestinal helminth prevalence

	1947 (in million)	1989 (in billion)
Ascariasis	650	1.26
Hookworm	450	932
Trichuris	350	687

About 4.5 billion people are affected by different kinds of helminths.

The wide prevalence of parasitic diseases is essentially due to distinctive climatic conditions in the tropics, suitable for the helminthes, poor environmental sanitation, low socio-economic status, lack of health education and other related factors.

TYPE OF INFECTIONS

Among the various helminth parasites which infect man, livestock and poultry, the intestinal helminths pose the most serious threat. The hookworm is considered to be an important intestinal nematode whose presence causes severe disorders in the body functions. It is responsible for continued blood loss and the vitality of the patient is seriously impaired long before the disease is diagnosed. Infections due to two species of hookworms, *Ancylostoma duodenale* and *Necator americanus* continue to be major causes of iron deficiency anaemia and associated ill health.

Filariasis or elephantiasis (a common term describing its final stage) is a group of infections that cause disability and severe disfigurement of legs. It is transmitted by the bite of a mosquito. The filariae migrate to the lymphatic system wherein they mature. The pathology is caused essentially by continuous blockade of lymph flow. The resultant damming up of this fluid stretches and distorts the tissue into horrendous disfigurement.

Among soil-transmitted parasitic infections, ascariasis is a major public health problem in the developing countries in the world, primarily affecting children. It contributes significantly to the widespread menace of malnutrition and remains undiminished.

Strongyloides stercoralis has a direct effect in causing intractable diarrhoea. Schistosomiasis is another serious malady, which causes liver, gut and bladder fibrosis from schistosome eggs, deposited by worms.

Agents effective against Helminth infection

Halogenated hydrocarbons

The anthelmintic activity of halogenated hydrocarbons has been known for quite some time now^{28,29}. Several chlorinated hydrocarbons such as carbon tetrachloride, ethyl chloride, n-butyl chloride and 2-chlorobutane are reported to possess varying degrees of antihookworm activity. However, very few of these compounds find use in human clinical treatment due to side effects.

Tetrachloroethylene ($\text{Cl}_2\text{C}=\text{CCl}_2$) has been used in some quarters. It is reported to induce nausea, vomiting, headache, dizziness and abdominal cramps. Patients with severe anaemia have been known to collapse during therapy.

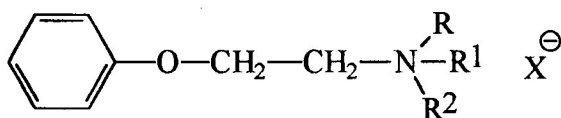
At a dose of 2 ml/adult, carbon tetrachloride (CCl_4) is capable of eliminating hookworms. However, prolonged use may lead to jaundice and cirrhosis, and thus it was replaced by the more active tetrachloroethylene.

Chloroform (CHCl_3) too has been used in the treatment of human hookworm infections³⁰ and has shown good response against *N. americanus* and *A. duodenale*.

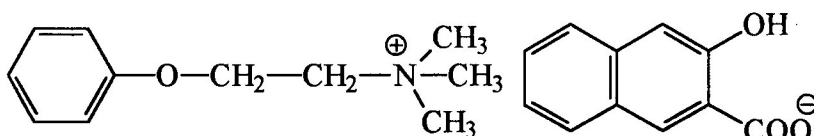
Nevertheless, the drug should be given under strict medical supervision, while children and pregnant women are recommended to abstain from it.

Quarternary ammonium compounds

Copp and his co-workers at Wellcome Laboratories reported in 1958, the potent anthelmintic activity associated with phenoxyethylammonium salts (XXXXVII) of which bephenium hydroxynaphthoate (XXXXVIII) (Alcopar®) had the highest activity.



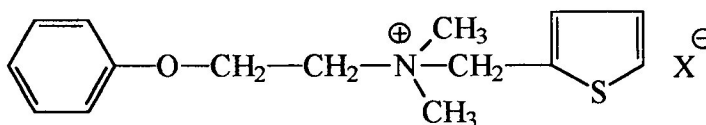
(XXXXVII)



(XXXXVIII)

Bephenium is effective against *N. americanus* and *A. duodenale* in man. It is also found to give 95-100% cure rates in dogs and cats carrying hookworms. The main side effects accompanying this compound are nausea and vomiting.

Thenium (IL) (Canopar®) is a thiopenium used extensively to control canine and feline hookworm diseases due to its lesser property to cause vomiting than bephenium.

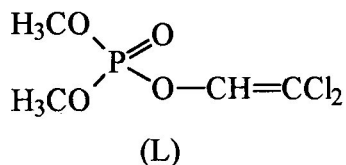


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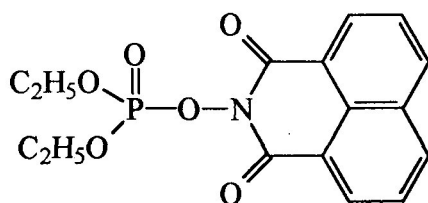
Organophosphorous compounds

A large number of organophosphates have been claimed to possess activity against different helminths, but few have found use in human clinical treatment, essentially due to their high toxicity and narrow margin of safety.

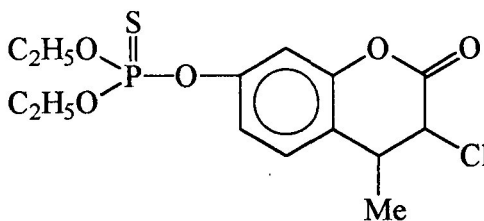
Largely due to its unique formulation, dichlorovos (L) has a broader margin of safety and high efficiency compared to other organophosphates. It is given as polyvinyl chloride resin pellets which release the drug slowly by evaporation, thus saving the patient from sudden toxicity of concentrated dose. The body detoxifies the low concentrations released from the pellets, which are ultimately excreted in 2-3 days.



Naphthalophos (LI) and Coumaphos (LII) are other organophosphates, which are highly active against helminth infection. The former is capable of removing 90-95% of the hookworms at a dose of 50µg/kg, while the latter clears *Bunostomum* in calves at a dose of 2mg/kg.



(LI) Naphthalophos

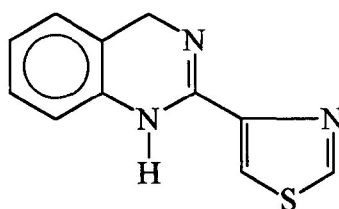


(LII) Coumaphos

Organophosphates are potent inhibitors of acetylcholinesterases and it is believed that they exert their toxic effects in worms in a fashion more or less similar to that in insects and vertebrates.

3) Benzimidazoles :

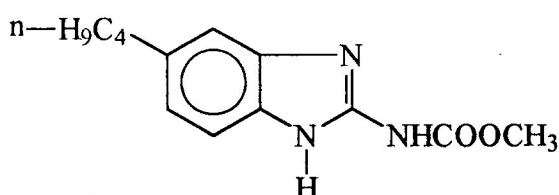
Modern parasitologic research has given birth to a series of benzimidazoles exhibiting marked activity against different intestinal nematodes. Thiabendazole (LIII), the first truly modern anthelmintic, was introduced in 1961 by Merck³¹. This was quickly followed by a generation of various benzimidazoles, several of which are currently used in the treatment of human helminth infections



(LIII) Thiabendazole

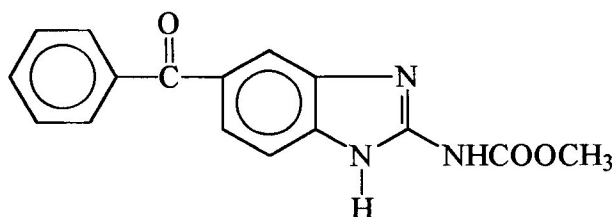
Thiabendazole is a very good agent for the treatment of hookworm infections and is most effective in multiple dose therapy, achieving cure rates to the order of 75-83%. It also shows anti-inflammatory activity, which may be advantageous in the treatment of human hookworm infections. However, it suffers a disadvantage of being readily deactivated in sheep due to hydroxylation at its 5-position³² which could be the cause for development of resistance to this class of compounds.

Parbendazole(LIV) was introduced by Smith-Kline and French Laboratories and it exhibited significant activity against different helminth parasites, although its action is rather limited against hookworms. It shows about 66-88% clearance of *A. caninum* infections in dogs at an oral dose of 20 mg/kg. It represents the first of the benzimidazole anthelmintic agents which contain the methyl carbamate moiety in the 2-position.



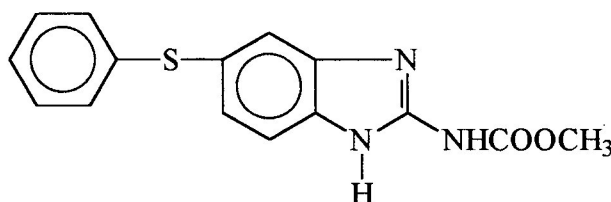
(LIV) Parbendazole

Parbendazole was soon followed by Mebendazole (LV), marketed by Janssen Pharmaceutica³³, and it has been confirmed as a remarkably safe, broad spectrum anthelmintic that is especially useful in the treatment of human whipworm (*Trichuris trichiura*) and pinworm (*Enterobius vermicularis*) infections³⁴.



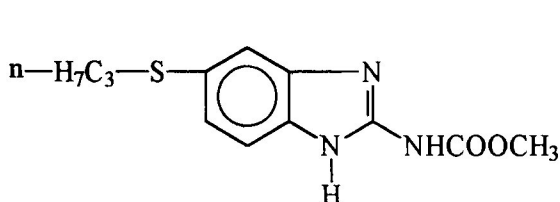
(LV) Mebendazole

Fenbendazole (LVI) introduced by Hoechst, exhibits marked activity against different species of lung worms and intestinal nematodes.

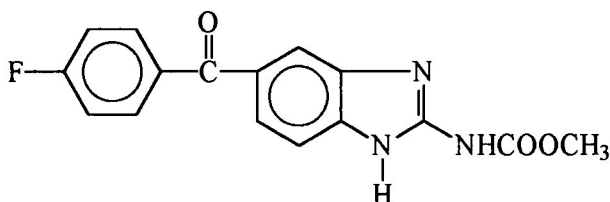


(LVI) Fenbendazole

Two more compounds in this class were introduced by Janssen Pharmaceutica – Albendazole (LVII) and Flubendazole (LVIII).



(LVII) Albendazole



(LVIII) Flubendazole

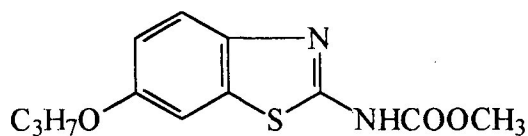
Albendazole exhibits high efficacy against *A. caninum* in dogs at a dose of 50mg/kg given for three days, but it shows relatively weak activity if a single dose of 250 mg/kg is administered.

Flubendazole shows a high level of activity against a variety of nematode and cestode infections, either natural or induced, in experimental animals. It exhibits excellent activity at a dose of 10mg/kg against a number of intestinal nematodes.

Thus it can be seen that benzimidazoles represent the only class of truly broad spectrum anthelmintics. However, poor drug absorption and lack of water solubility are problems characteristic of these compounds, which limit their usage.

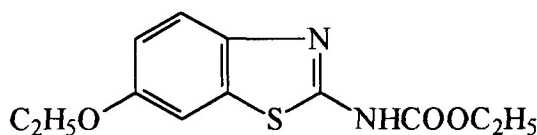
Benzothiazoles and related compounds

Attempts to extend the functional characteristics of the benzimidazole class of compounds to the benzothiazoles with a view to develop possible anthelmintics have met with some success. Several substituted benzothiazoles have been synthesised of which methyl-6-propoxybenzothiazole-2-carbamate (LIX) (Tioxidazole) has emerged as a potent anthelmintic compound for intestinal worms³⁵. At a dose of 50mg/kg for five days it shows excellent activity against *N. dubius* in mice. However, the drug has been restricted to veterinary use so far.



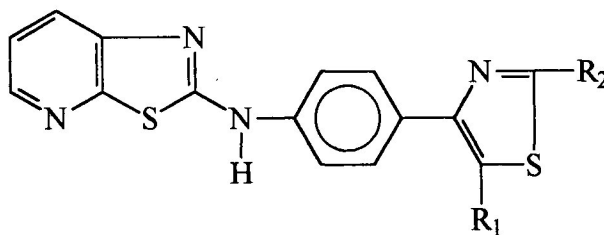
(LIX) Tioxidazole

Another benzothiazole of similar structure, ethyl-6-ethoxybenzothiazole-2-carbamate (LX), exhibits over 90% clearance of *H. contortus* and *B. trigonocephalum* in lambs at a dose of 200mg/kg³⁵.

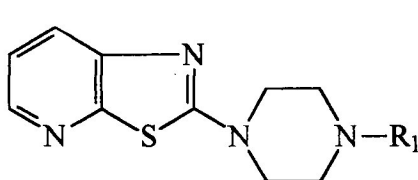


(LX)

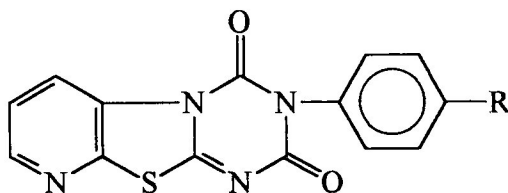
A few pyridothiazoles (LXI-LXIII) have been reported to possess anthelmintic activity³⁶⁻³⁸ of which (LXI) caused 90% mortality of adult *N. dubius* worms at a dose of 1mg/kg. The other two compounds, (LXII) and (LXIII), exhibited moderate activity against *N. dubius* in mice.



(LXI)



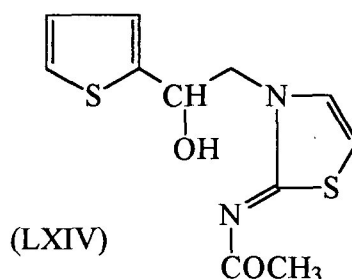
(LXII)



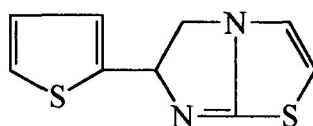
(LXIII)

Imidazothiazoles :

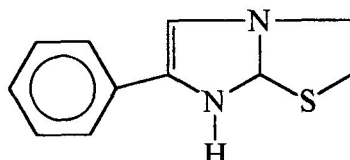
In the 1960s, Janssen Pharmaceutica, while engaged in the search for a broad spectrum anthelmintic, discovered that thiazothienol (LXIV) showed promising anthelmintic activity in chickens³⁹.



While (LXIV) also exhibited activity against sheep nematodes, it was ineffective in rats or mice suggesting that the active principle was probably a metabolite. Thereafter, chicken were dosed with (LXIV) and thiazothieliet(LXV) isolated from the faeces was found to be far more potent than its precursor and was also active in both mice and rats³⁹.



With the Imidazothiazole as the lead compound, other such molecules were synthesised of which Tetramisole (LXVI) is an ideal representative. The anthelmintic activity is only due to its S(-) isomer, termed Levamisole.



Tetramisole is also used for veterinary purpose and exhibits high activity against various gastrointestinal nematodes of sheep, dogs, fowl, cattle and horses. In humans, it gives a 72-75% cure rate against hookworm infections at a dose of 2.5 - 5.0 mg/kg, given for a period of three days.

Part IB

Plan of Work

Medicinal chemists work to devise efficient methods for optimizing the potency and the profile of a given active substance. These methods may consist of more or less intuitive approaches such as the synthesis of analogue isomers and isosters or the modification of existing ring systems.

They may also rest on computer-assisted designs, such as identifying the pharmacophore by molecular modeling or optimizing activity by means of quantitative structure-activity relationships. The medicinal chemist is responsible for developing as rapidly as possible more active molecules that are both, selective and less toxic. Up to the 1970s the discovery of lead compounds depended essentially upon random occurrences such as accidental observations, fortuitous findings, hearsay or laborious screening of a large number of molecules.

Since then, more rational approaches have become available, based on the knowledge of the structures of the endogenous metabolites, the enzymes and the receptors, or on the nature of the biochemical disorder implied in the disease. The analysis of ways discoveries are made allows one to distinguish four strategies leading to new chemical entities useable as lead compounds.

The first is based on the modification and improvement of already existing active molecules.

The second strategy consists of the systematic screening of sets of arbitrarily chosen compounds in selected biological assays. The third approach involves the retroactive exploitation of various pieces of biological information that sometimes result from new discoveries made in biology and medicine and sometimes are simply the fruits of more or less fortuitous observation.

Finally, the fourth route to new active compounds is a rational design based on the knowledge of the molecular cause of the pathological dysfunction.

Medicinal chemistry covers three critical steps: -

1. A discovery step, consisting of the identification and production of new active substances, usually called 'lead' compounds. The lead compounds can originate from synthetic organic chemistry, from natural sources or from biotechnological processes.
2. An optimization step, that deals mainly with the synthetic modification of the lead structure in order to improve potency, selectivity and to lessen toxicity. Its characteristics are the establishment and analysis of structure-activity relationships.
3. A development step, consisting of the optimization of the synthetic route for bulk production and the modification of the pharmacokinetic and the pharmaceutical properties of the active substance, to render it suitable for clinical use. This process also involves the preparation of chemical formulations that are better absorbed or are more water-soluble or have sustained release properties.

Additionally, it may result in the elimination of properties, which may compromise patient compliance, such as an unpleasant taste, irritation or pain at the site of injection.

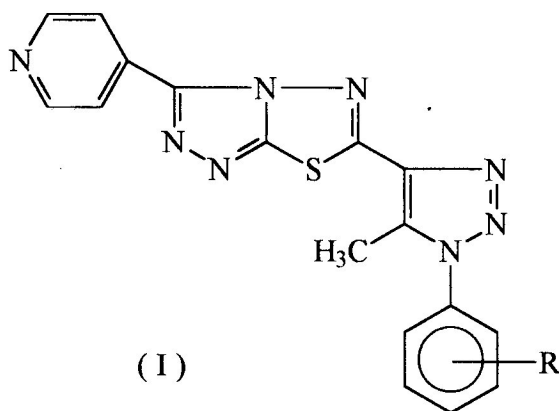
Thus, keeping in mind the broad-spectrum of activity, as far as eradication of infectious toxicity of the drug is concerned, it is necessary to build up new chemotherapeutic agent.

Part II:

Synthesis of 3,6-disubstituted-1,2,4-triazolo [3, 4-b] [1,3,4] thiadiazoles :

3,6-disubstituted-1, 2, 4-triazolo [3,4-b] [1,3,4] thiadiazole derivatives have anti-bacterial⁴⁰⁻⁴² and anti-inflammatory activity⁴³ as well as interesting CNS depressant action⁴⁴.

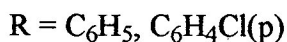
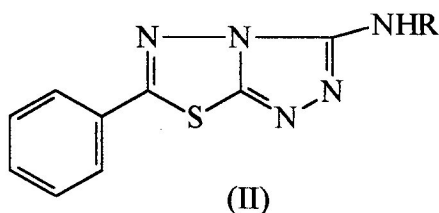
The survey of literature revealed that s-triazolo [3,4-b]-1,3,4-thiadiazole (I), an interesting fused system of s-triazole and 1,3,4-thiadiazole rings, has received much attention during recent years on account of its prominent utilization as antifungal, anti-inflammatory, anti-viral, analgesic and anthelmintic agent⁴⁵⁻⁴⁸.



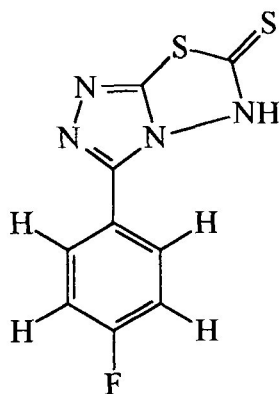
During the past years considerable evidence has been accumulated to demonstrate efficacy of 1,3,4-thiadiazole in inducing antibacterial⁴⁹⁻⁵³, antifungal⁵⁴⁻⁵⁶, amebicidal⁵⁷, antiviral⁵⁸, insecticidal⁵⁹, CNS depressant⁶⁰ and antitumor⁶¹ activities when fused to an s-triazolo ring system.

A novel series of 2-aryl thiocarbamoyl-hydrazino-5-phenyl-1,3,4-thiadiazoles with DCCD (II).

Some of the products were found to be only moderately active against *S. aureus* and *Candida albicans*.

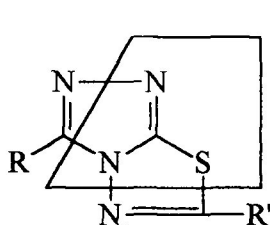


Also, Jagmohan *et al*⁶² reported the synthesis of condensed bridge head nitrogen heterocyclic systems, i.e. s-triazolo thiadiazoles derived from 3-p-fluorophenyl-4-amino-5-mercapto-s-triazole (III) and showed activity against *P. aeruginosa* and *C. albicans*.

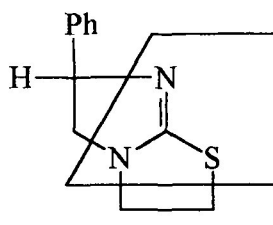


(III)

El-Khawass *et al*⁶² have reported the anthelmintic activity of a series of triazolo thiadiazoles (IV) which are structurally and isosterically related to Tetramisole (IV), the established anthelmintic agent, against third stage larvae of *Acylostoma caninum*.

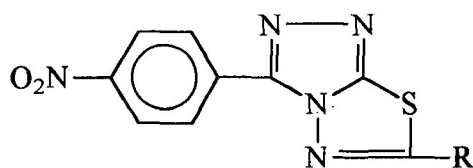


(IV)



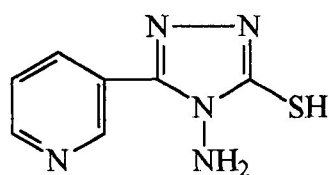
(V)

It has also been reported by Shi-Hai-Jian *et al*⁶⁴, that some of the 3,6- disubstituted triazolo thiadiazoles possess strong antibacterial activity (VI).

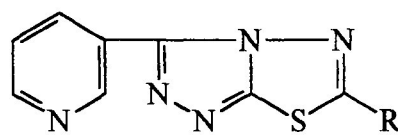


(VI)

Further, Zhang, Ziyi, Wang *et al*⁶⁵ reported that some triazolo thiadiazoles (VIII) prepared by refluxing amino triazolothiol (VII) with different aromatic acids in POCl_3 for 7hr., have bactericidal activity at 0.01% MIC.

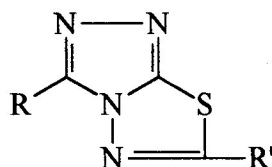


(VII)



(VIII)

Taking lead from these known facts, it was proposed to synthesize, 3,6-disubstituted triazolo thiadiazoles, which were studied for their antifungal, antibacterial antituberculosis and anthelmintic activity. These compounds were synthesized by refluxing the 3-substituted-4-amino-5-mercapto 1,2,4-triazole with substituted acid chlorides in phosphorous oxychloride. They have the following general structure :



Part III:

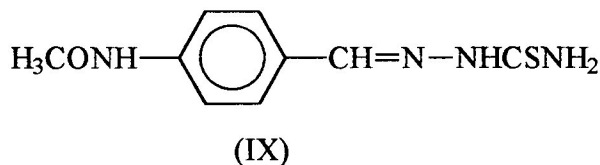
Synthesis of 3-[(substituted phenyl)-1-methylene]-amino-5-mercapto-1,2,4-triazole

4-amino-5-mercapto-1,2,4-triazoles are the starting materials for the synthesis of a wide variety of heterocyclic derivatives, which are of great importance in medicinal chemistry^{66,67}.

The presence of azomethine linkage in compounds has been known to decrease their toxicity considerably^{68,69}.

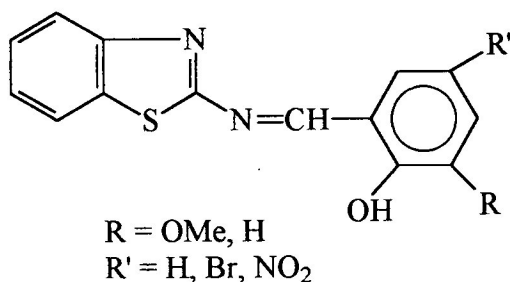
Schiff bases from 5-chloro salicylaldehyde, 5-bromosalicyl aldehyde and 4-N,N-dimethyl amino benzaldehyde have shown encouraging antituberculosis activity *in vitro*^{70,71}.

The thiosemicarbazone of 4-acetamido benzaldehyde, Thiacetazone (IX), was introduced for the treatment of tuberculosis in 1950.

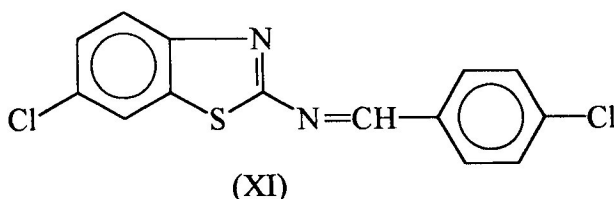


This was the first synthesized drug that approached the activity of Streptomycin in experimental infection in animals^{72,73,74}.

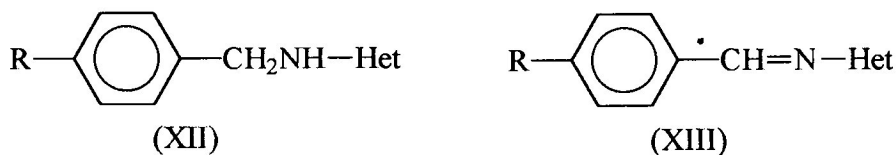
Some Schiff bases in the benzothiazole series (X) have been synthesized and their physicochemical properties have been studied⁷⁵.



Takahiro Yabuchi⁷⁶ reported the synthesis of N-(p-chloro benzylidene)-amino-6-chloro benzothiazole [XI] with antimicrobial activity.

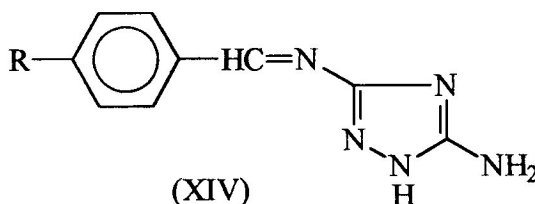


Recently, R. Fiorvanti *et al*⁷⁷ have reported the antimicrobial properties of some new heteroaryl benzyl amines (XII) and their Schiff bases (XIII).



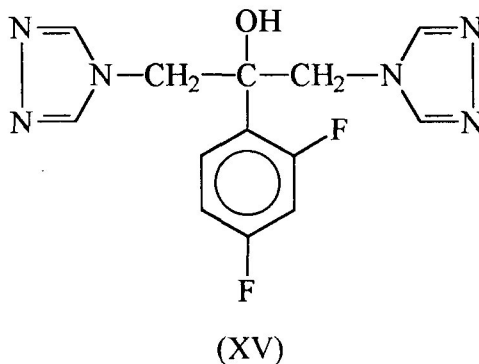
$R = \text{H, CH}_3, \text{Br, etc}$

The azomethine linkage is known to impart biological activity to various heterocyclic moieties, e.g. Straukas J. *et al*⁷⁸ have reported the anti-inflammatory activity of triazole Schiff bases (XIV)

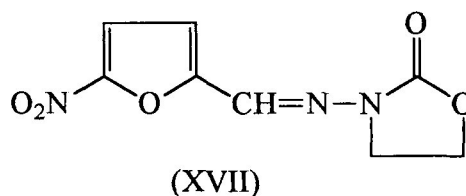
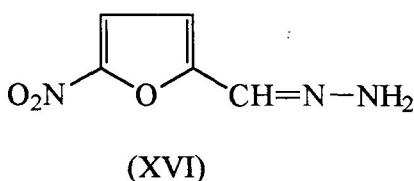


$R = 2\text{-Cl, 4-F, 4-OMe, etc}$

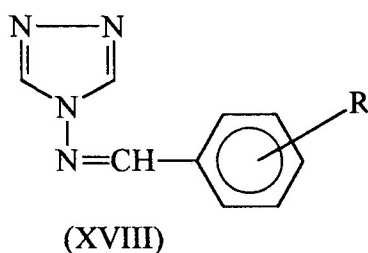
The importance of 1,2,4-triazole moiety as a major pharmacophoric requirement to exhibit enhanced antifungal activity is well manifested by its inevitable presence in the most recent antifungal drugs, viz. Fluconazole⁷⁹ (XV).



Many of the biologically active compounds such as Nitrofurantoin (XVI), Nitrofurazone (XVIII)⁸⁰ are used as antibacterial and anti-infective agents. These compounds also contain the azomethine linkage, which enhances the anti-microbial activity.



Taking into account the pharmacophoric importance of triazole in various newly introduced antifungal drugs like Fluconazole, Itraconazole, Saperconazole etc., and the importance of azomethine linkage that imparts antimicrobial activity, Schiff bases of 3-substituted-4-amino-5-mercapto-1,2,4-triazole were synthesized which have the following general structure (XVIII). They were tested for their antifungal, antibacterial, and antitubercular activity.



R = 4F, 4-CF₃, etc

Part IV

Synthesis of 2-chloro-1-(benzyl)-benzimidazole-the intermediate for Lerisetron.

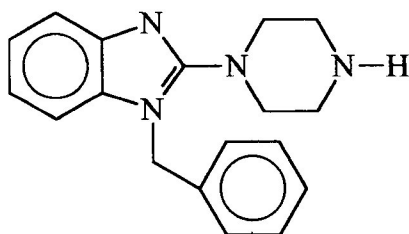
Pharmacologically, the most important pain receptor in the irritable bowel syndrome is serotonin, which acts on sensory nerve endings through 5-HT receptors. 5-HT³ receptors are distributed in the hippocampus, substantia nigra, globus pallidus,

postganglionic sympathetic neurons and mediate a number of functions, such as sympathetic and parasympathetic neuro excitation, Vogel excitation and emesis. They are coupled to an intrinsic transmitted sodium channel that promotes increased intracellular calcium concentration via depolarization induced calcium mobilization mechanisms. Other receptor types involved in intestinal sensory terminals include bradykinin B², prostaglandin EP² and adenosine receptors.

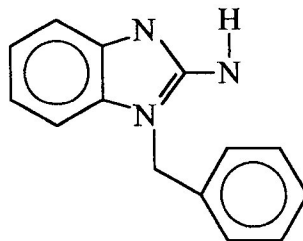
Irritable bowel syndrome (IBS) is a functional digestive disorder, in the pathophysiology of which visceral sensitivity plays an important role. Patients commonly present alterations of the visceral sensitivity, including hypersensitivity to visceral stimuli (rectal distention), associated with motor disturbances. Therefore, the sensitive pathways innervating the colon have emerged as possible pharmacological targets to relieve pain or modify altered reflexes in patients. 5-HT³ receptor antagonists are currently being used for a variety of conditions, including nausea/vomiting and IBS.

A series of 5-HT³ receptor antagonists has been synthesized, and one such compound is Lerisetron(XIX)⁸¹, which is under Phase II clinical trials. Another drug of similar category is Cilanstron(XXI)⁸².

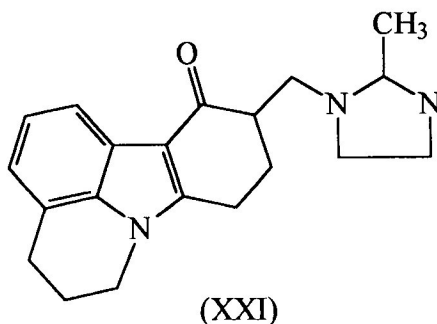
Another drug having a similar type of intermediate is Norastemizole (XX), which is a H₁-receptor antagonist. It has been manufactured by Sepracor Inc. (US) and is currently under clinical investigations⁸³.



(XIX)



(XX)



For synthesis of both drugs, i.e. Lerisetron and Norastemizole, 2-chloro-5-(benzyl)-benzimidazole is used as the starting material.

The synthesis of this raw material involves the following steps :

1. Preparation of 2-mercaptobenzimidazole
2. Preparation of 2-chlorobenzimidazole

The 2-chlorobenzimidazole is mixed with benzyl chloride to obtain the raw material for Lerisetron.

Modifications

In literature, it is reported that the reaction is carried out in sodium hydride (NaH) and Dimethyl formamide. Sodium hydride is a costly, inflammable and moisture sensitive solid. Also, work with DMF is fairly messy and time-consuming. In our laboratory, we carried out the reaction in a mixed solvent system containing dioxane and dimethyl formamide (9 : 1). This allows the reaction to occur at a faster rate and it was also found that the final product obtained was relatively free of impurities.

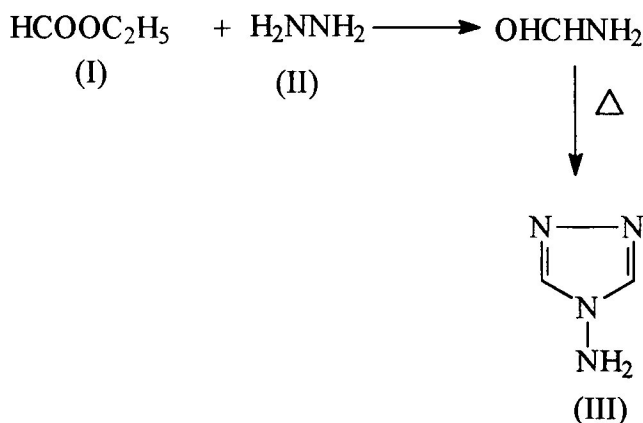
Part 1C

Chemistry

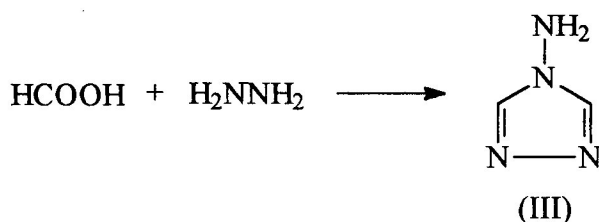
CHEMISTRY OF TRIAZOLES: -

Methods of preparation of 4-amino triazole and its derivatives:

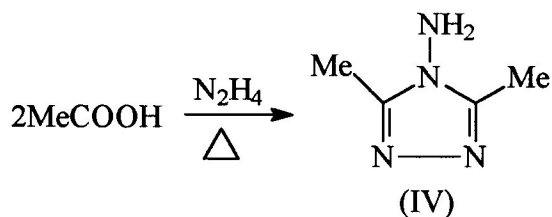
i) 4-amino triazole (III) has been prepared^{84,85} by the reaction of ethyl formate (I) with hydrazine (II) to give formhydrazide, which was heated at 150 to 200°C to effect self condensation to give (III)



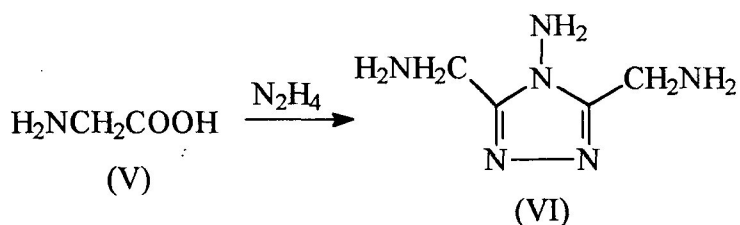
Similar interaction of formic acid and hydrazine gave good yield of 4-amino triazole (III)^{86,87,88}.



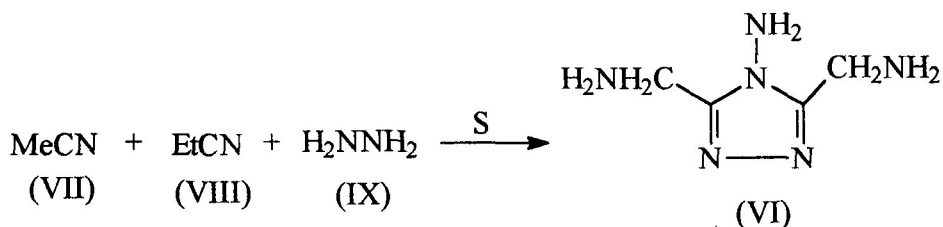
ii) Other carboxylic acids and their functional derivatives have also been used to prepare alkyl and substituted alkyl-4-amino triazoles, e.g. acetic acid and hydrazine hydrate at a high temperature give 3,5-dimethyl-4-amino triazole (IV)^{86,87,89}.



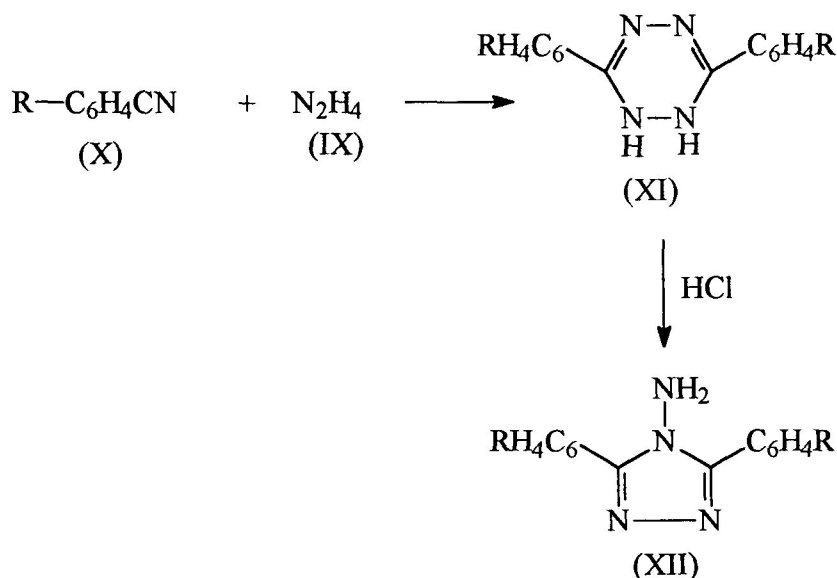
iii) Similar reactions have been reported for the preparation of 4-amino-3,5-bis (amino alkyl) triazole(VI)⁹⁰⁻⁹³, which is prepared by the reaction of glycine (V) with hydrazine to give (VI).



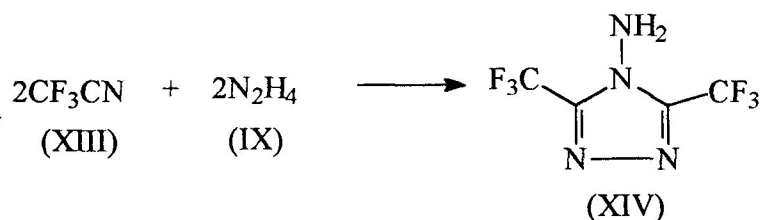
iv) Sulfur or sulfur containing compounds catalyze the formation of 4-amino triazoles e.g. 4-amino triazole(VI) substituted at 3,5-position with different substituent was prepared by the reaction of equimolar amounts of acetonitrile (VII) and propionitrile (VIII) with a mixture of hydrazine (IX) and sulfur⁹⁴.



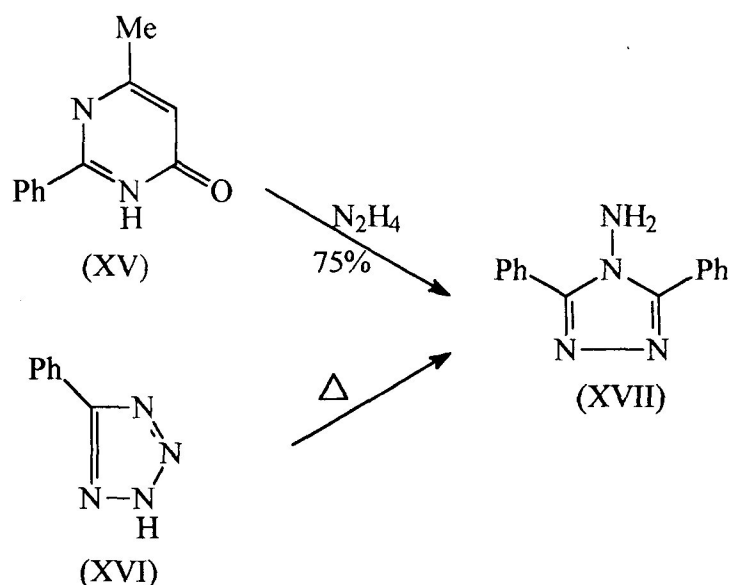
v) The preparation of 4-amino-3,5-diaryl triazoles (XII) are usually effected by the acidic rearrangement of 1,2-dihydro tetrazines (XI), which are obtained by the reactions of equimolar amounts of aryl nitriles (XIII) and hydrazine (IX) in refluxing benzene or toluene⁵⁶.



vi) Perfluoro alkyl triazoles (XIV) are prepared by various methods e.g. per fluoro acetonitrile (XII) with hydrazine (IX) in a sealed tube at room temperature gave (XIV)⁹⁶.

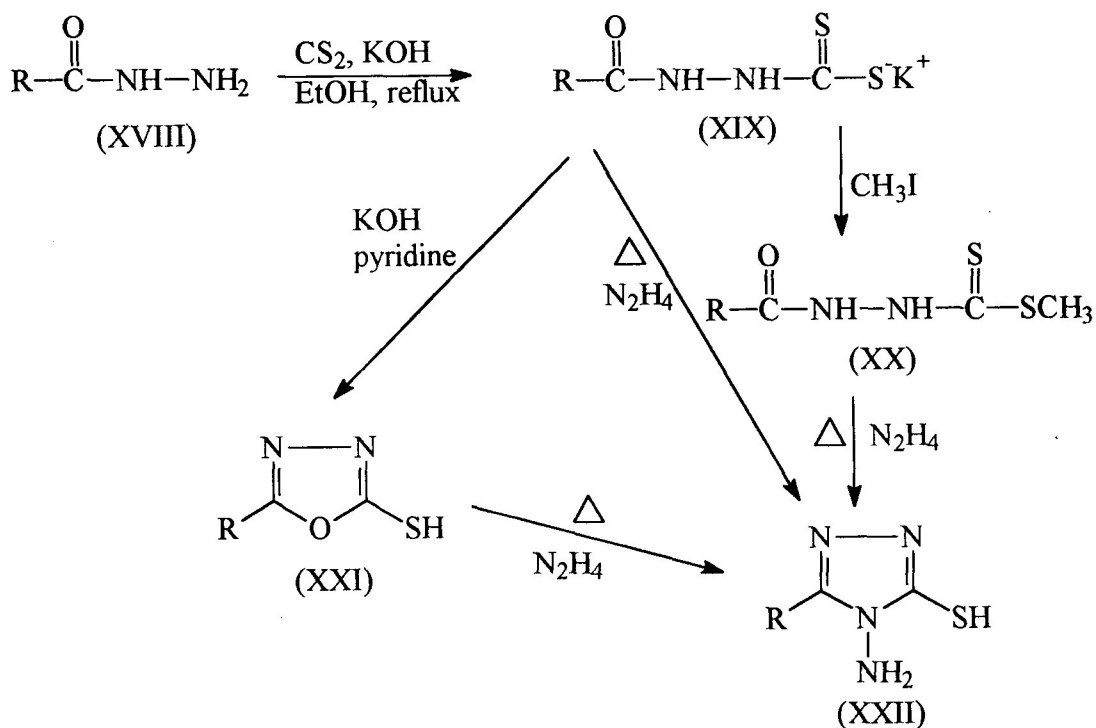


vii) 4-amino-3,5-diphenyl triazoles (XVII) are also formed by the decomposition of other hetero aromatic ring systems e.g. reaction of pyrimidine (XV) with hydrazine⁹⁷. Also the thermolysis of 5-phenyl tetrazole (XVI) in various solvents⁹⁸ gives (XVII).



Methods of preparation of 3-(substituted phenyl)-4-amino-5-mercapto-1,2,4-triazole:

i) One of the methods for the synthesis of triazole is Hoggarth synthesis⁹⁹. This methodology involves the condensation of carboxylic acid hydrazides with carbon disulphide and potassium hydroxide to yield the potassium dithiocarbazates (XIX) which, after s-alkylation with methyl iodide, undergo ring closure with an excess of hydrazine, to produce the amino thiols (XXII) in good yields.

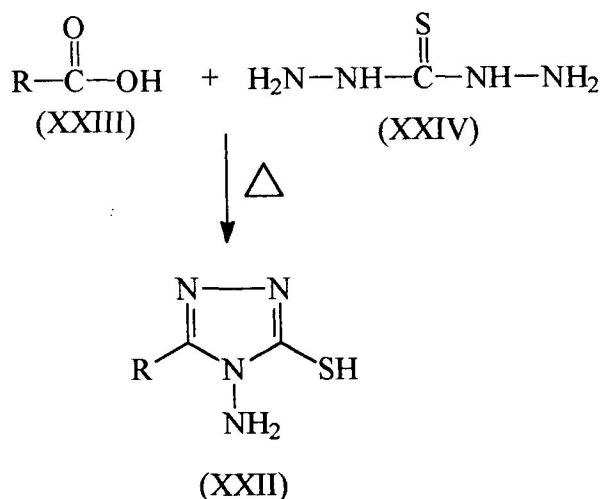


ii) More recently, Heindel and Reid have reported the direct conversion of the carbazate salts by reaction with an excess of hydrazine; this avoids 5-methylation, resulting in a higher yield and a shorter reaction time¹⁰⁰.

iii) Alternatively, oxadiazoles (XXI) can be converted to triazoles(XXII) by reaction with hydrazine.

iv) It has also been reported that the same aliphatic carboxylic acids react with thio carbo-hydrazide to afford directly the 3-substituted-4-amino-5-mercapto-1,2, 4-triazoles(V).

It has also been found that the reaction of thiocarbonylhydrazide with carboxylic acids can be conveniently applied to both aliphatic and aromatic acid derivatives¹⁰¹⁻¹⁰².



CHEMISTRY OF ESTERS

Various methods used for ester formation are as follow¹⁰³.

a) Direct esterification procedure:

The interaction between a carboxylic acid and an alcohol is a reversible process and proceeds very slowly.



Equilibrium is attained after refluxing for several days. If, however about 5% (of the weight of the alcohol) of either concentrated sulphuric acid or of dry hydrogen chloride is added to the mixture, the same point of equilibrium can be reached within a few hours.

This method of esterification gives good yields with primary alcohols and fairly good yield, with secondary alcohols. The method is unsatisfactory for use with tertiary alcohols.

b) Fischer-speier method:

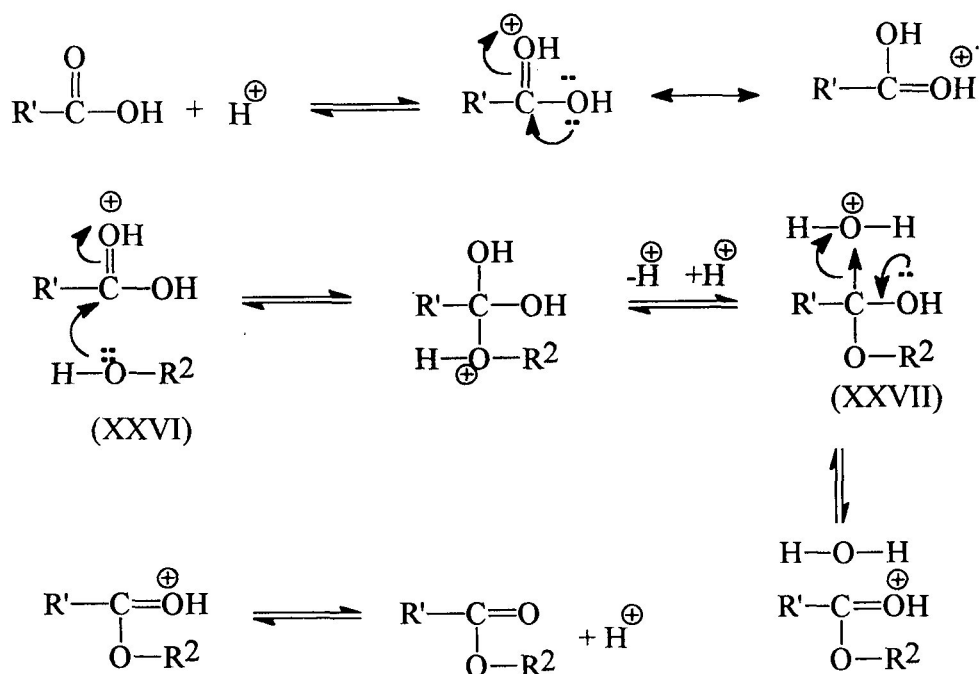
Esterification with alicyclic alcohols proceeds best when the alcohol is saturated with hydrogen chloride and treated with an excess of the carboxylic acid; a very impure ester results if sulphuric acid is used as the catalyst.

c) Esters of formic acid are most simply prepared from the alcohol and an excess of formic acid, which being a comparatively strong acid, does not require the use of

added mineral acid to catalyze the esterification reaction. Sulphuric acid, in any case, should not be added since it causes the decomposition of formic acid to carbon monoxide.

Reaction Mechanism

The acid-catalyzed esterification reaction usually proceeds via an acyl-oxygen fission process. This involves the cleavage of the bond between the original carbonyl-carbon atom and an oxygen of an hydroxyl group in the intermediate (XXVII) arising from nucleophilic attack by an alcohol molecule on the protonated carboxylic acid group (XXVI).



Several modifications of the direct esterification process have been developed.

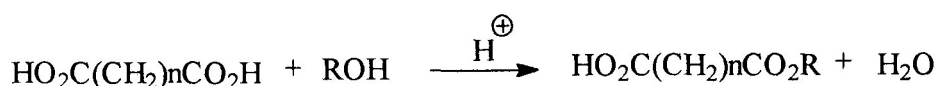
For example, it is sometimes convenient to prepare an ester by heating the organic acid, the alcohol, and sulphuric acid in a solvent such as benzene upon the addition of water, followed by separation and distillation of the benzene layer (after washing and drying). Benzene and alcohol pass over first, followed by the ester.

The process of acid-catalyzed esterification in the presence of benzene, or better still toluene, is greatly facilitated if the water produced in the reaction is removed by distillation as an azeotrope.

When the reaction mixture is slowly fractionated a ternary azeotrope of the alcohol, toluene and water will pass over first followed by a binary toluene alcohol azeotrope. Continued distillation affords the required ester in good yield.

b) Preparation of esters of dicarboxylic acids: -

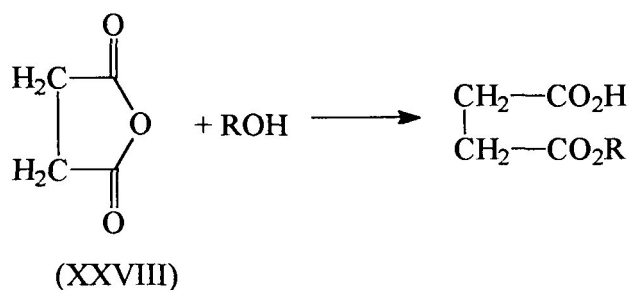
The acid-catalyzed reaction of a dicarboxylic acid with an excess of alcohol yields the diester. However, the process may be adapted to prepare acid esters of dicarboxylic acids by using molar proportions of the diacid and alcohol.



Alternatively, the acid ester may be prepared by subjecting the diester to controlled partial hydrolysis with one molar proportion of potassium hydroxide.



The acid esters of 1,2-dicarboxylic esters are conveniently prepared by heating the corresponding cyclic anhydride (XXVIII) with one molar proportion of the alcohol.

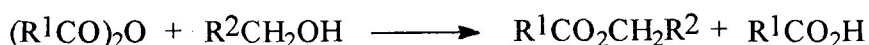


c) Use of acyl chlorides and acid anhydrides:

Acyl chlorides react readily with primary and secondary alcohols to give esters in very good yields. With tertiary alcohols the presence of a base is essential to prevent acid-catalyzed side reactions, such as dehydration or formation of the alkyl chloride.



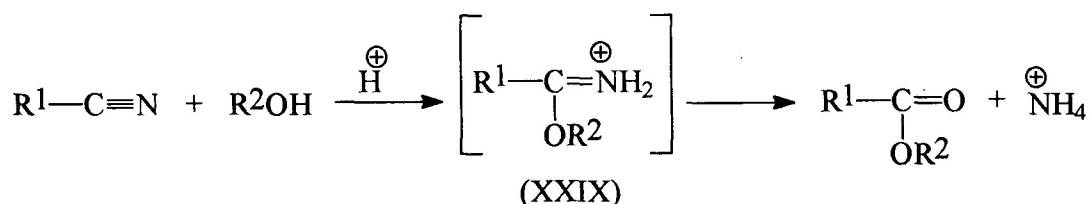
Acylation may also be carried out with acid anhydrides in the presence of a suitable catalyst; an acid catalyst such as sulfuric acid or zinc chloride, or a basic catalyst such as pyridine, may be used.



The use of acyl chlorides or acid anhydride is the method of choice for the synthesis of phenyl esters.

d) Alcoholysis of nitriles:

An ester is formed when a nitrile is heated with an alcohol in the presence of conc. sulphuric acid, thus providing a two-step synthesis of an ester from an alkyl halide. The reaction proceeds by way of an intermediate imino-ester (XXIX), which is not usually isolated.



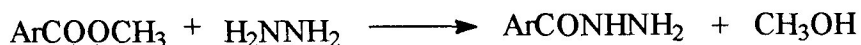
The method has been widely used for the preparation of poly functional compounds.

e) Ortho-esters:

These have the general formula $R^1-C(OR^2)_3$ and are stable derivatives of the unstable ortho acids $R-C(OH)_3$.

CHEMISTRY OF HYDRAZIDE

Acid hydrazides are obtained by reaction of the methyl or ethyl ester with hydrazine hydrate.

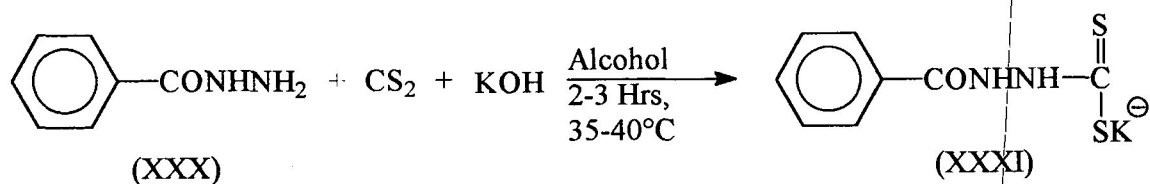


The general procedure followed for the synthesis of acid hydrazides in the present work is analogous to that reported by Vogel¹⁰⁴.

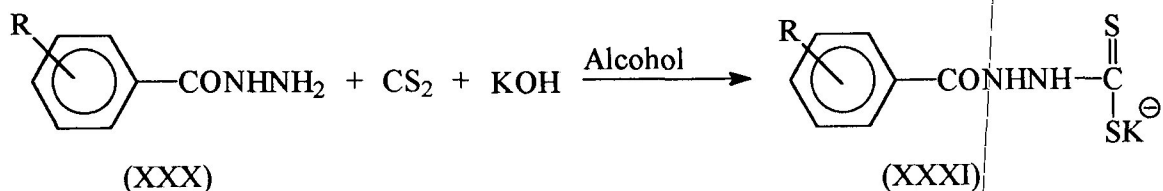
CHEMISTRY OF DITHIOCARBAMATE SALTS:

i) Busch and Starke¹⁰⁵ obtained crystalline potassium salt of 2-benzylthio carbazinic acid (XXXI) by stirring together benzhydrazide (XXX), carbon disulphide and a solution of KOH in absolute alcohol for 2-3 hrs. at 35-40°C and then for 12-18

hrs. at room temperature. Later Hoggarth synthesized the potassium salts of aryl dithiocarbamate using the same procedure¹⁰⁶.



ii) Reid¹⁰⁷ *et al* prepared the potassium salts by stirring together KOH, CS₂ and aryl hydrazide in absolute alcohol for 12-16 hrs and the product was washed with dry ether.



In present investigation, the method employed for the synthesis of potassium salt of substituted aryl dithiocarbamate is that of Reid *et al*. The advantage of this method is the better yield obtained. However, the reaction mixture was stirred for 4-5 hours only, as it was seen that yields obtained after stirring for 4-5 hours or more were the same.

CHEMISTRY OF ACID CHLORIDES

Acid chlorides are used primarily as intermediates in synthesis. They are fairly reactive compounds and are usually liquids. Acid chlorides are obtained from acids through the replacement of the hydroxyl group with chloride. This exchange may be accomplished by the use of a number of inorganic chlorine compounds, among these thionyl chloride, phosphorous pentachloride, sulphuryl chloride and phosgene gas are most important.

Phosphorous pentachloride is a highly reactive compound and its use offers a general method for the preparation of acid chlorides. The products of the reaction are phosphorous oxychloride and hydrogen chloride, in addition to the acid chloride.

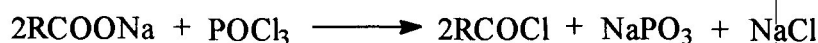


The use of thionyl chloride¹⁰⁸ for the preparation of acid chlorides presents many advantages in a large number of cases. The reaction results in the formation of sulphur dioxide and hydrogen chloride in addition to the acid chloride and the isolation of the chloride is for this reason a simple matter.

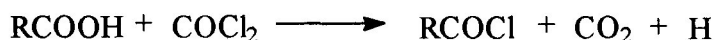


The thionyl chloride must be pure, as impure product has been known to cause a somewhat explosive decomposition in some instances. The procedure consists of mixing the chlorinating agent with acid and heating the mixture, if necessary. The product may be isolated by fractional distillation or if solid, by crystallization.

Phosphorous oxy-chloride reacts with the sodium salt of carboxylic acid to give acid chloride. This method is of importance with acids which undergo decomposition with other chlorides of phosphorous.

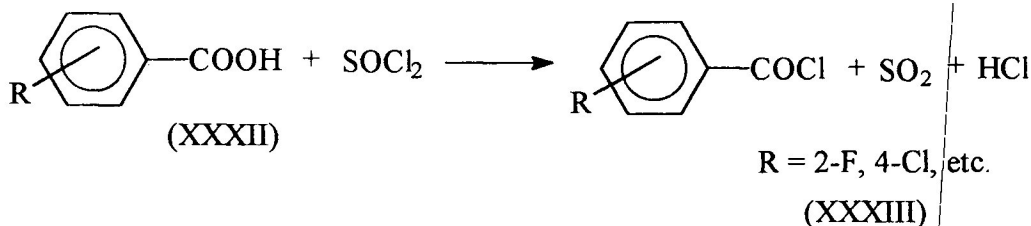


The reaction of phosgene¹⁰⁹ with carboxylic acids, and p-toluene sulphonylchloride or of sodium chlorosulphonate with salts of carboxylic acids is also used for the preparation of acid chlorides.

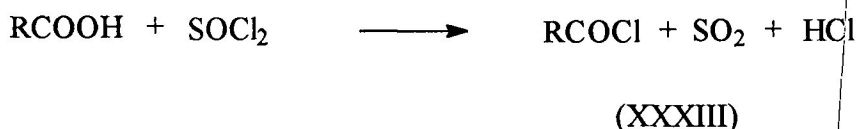


The acid chlorides are obtained readily through the reaction of sulfonyl chlorides with anhydrous salts of organic acids. The reagents are well mixed and subjected to distillation.

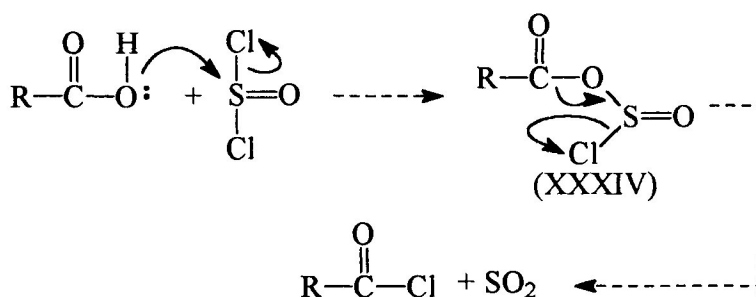
Thus, in the present work all the acid chlorides were prepared by reacting thionyl chloride with the corresponding acids.



Reaction Mechanism :



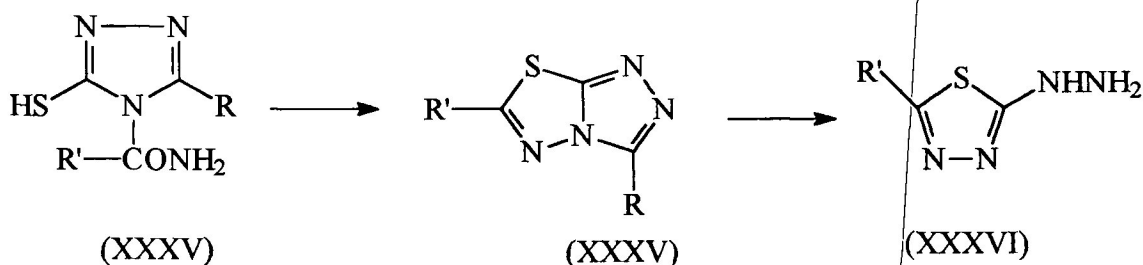
Acids and their salts are the only common starting materials of acid chlorides and several reagents are utilized as a source of nucleophilic chloride. All the reagents form reactive intermediate (XXXIV), so that the leaving group in the substitution is not hydroxyl group.



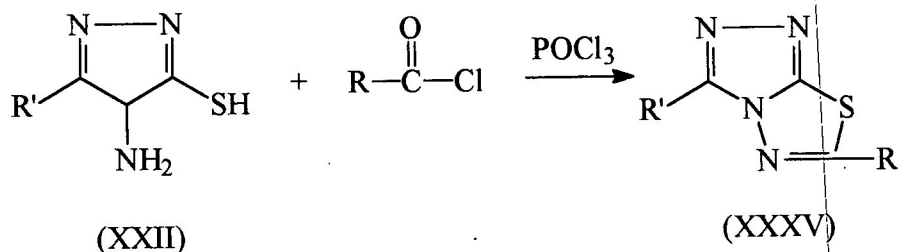
CHEMISTRY OF TRIAZOLOTHIA DIAZOLES:

Methods for the preparation of 1,2,4-triazolo [3, 4-b] [1,3,4] thiadiazoles:

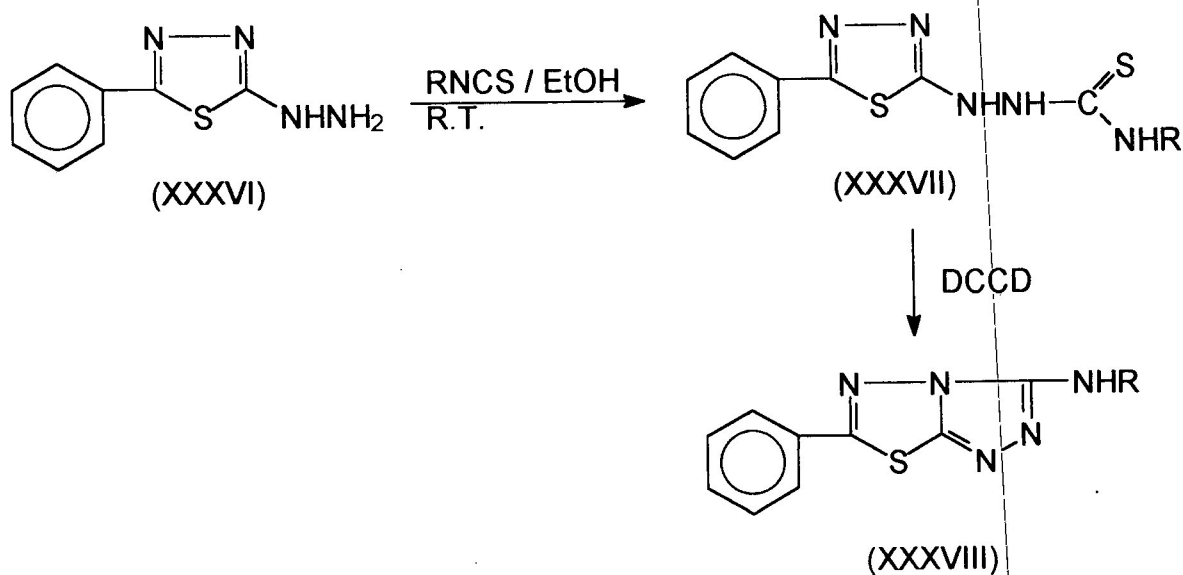
i) Alkyl and aryl-substituted derivatives of the 1,2,4-triazolo-[3,4-b] [1,2,4] thiadiazoles (XXXVI) were first obtained by Kanaoka¹¹⁰, both through ring closure of 4-acyl amino-s-triazole-5-thiol and by reaction of 1,3,4-thiadiazol-2-yl hydrazines (XXXVI) with ortho esters.



ii) More recently, Potts and Huseby prepared similar compounds¹¹¹ by ring closure of 3-substituted-4-amino-5-mercapto-(4H)-1,2,4 triazoles using suitable acyl chlorides in the presence of phosphoryl chloride.

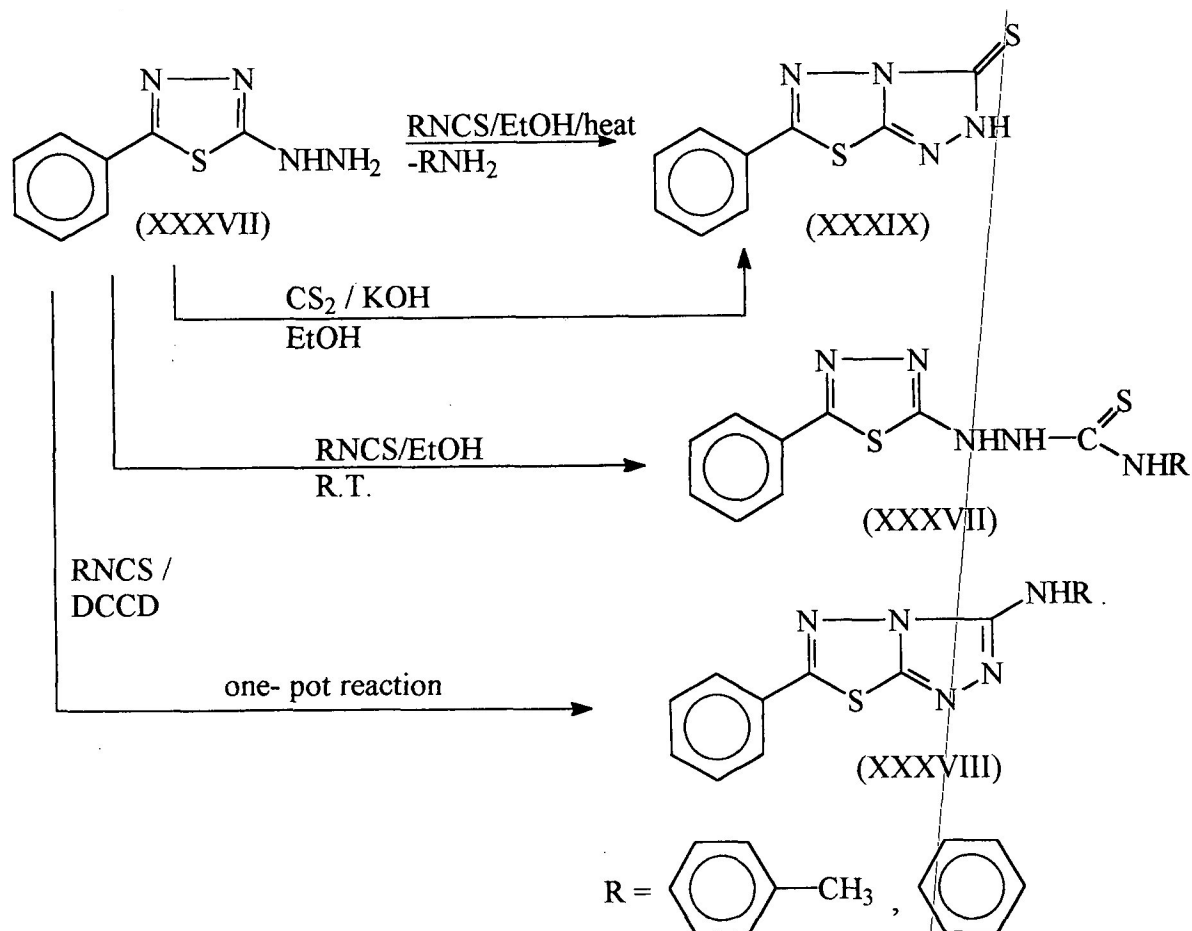


iii) A similar type of heterocyclic system was synthesized by cyclodesulfurization of 2-aryl thiocarbamoyl-hydrazino-5-phenyl-1,3,4-thiadiazoles (XXXVI) into the corresponding 3-arylamino-6-phenyl-s-triazolo [3, 4-b] [1,3,4] thiadiazoles (XXXVIII) with DCCD. (Dicyclohexyl carbodiimide)



The reactions proceeded smoothly with 1-5 molar equivalent of DCCD in a boiling mixture of ethanol and benzene and gave the corresponding products.

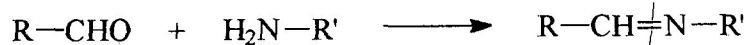
The treatment of a mixture of equimolar amounts of the hydrazino thiadiazole with 1.5 molar equivalent of DCCD, according to the one-pot cyclodesulfurization reaction conditions, produced corresponding s-triazolo [3,4-b] [1,3,4] thiadiazole derivatives^{112, 113,114}.



All s-triazolo [3,4-b] [1,3,4]-thiadiazoles in which the two substituents were identical were also prepared by a one step synthesis through heating of an excess of the appropriate carboxylic acid with thiocarbonylhydrazide in the presence of phosphorous oxychloride¹¹⁵.

CHEMISTRY OF SCHIFF BASES:

Schiff bases are formed by condensation of an amine and the carboxyl group of aldehydes.



Schiff¹¹⁶ for the first time reported condensation of this kind and hence the condensation products are referred to as "Schiff Bases".

Pelton *et al* had shown¹¹⁷ the antifungal and antibacterial activity of some substituted salicylaldehydes. Schiff bases from different aldehydes have shown encouraging anti-tuberculosis activity *in vitro* in our laboratory^{68,69}. Also, the presence of an

azomethine linkage in compounds has been shown to decrease their toxicity considerably^{70,71}.

Many procedures have been described in literature for the synthesis of Schiff bases. The important methods are listed below:

1. The simplest method appears to be the one in which equimolar quantities of an amine and an aldehyde are condensed by boiling in methanol or ethanol.
2. In 1946, Tipson and Clapp¹¹⁸ obtained Schiff bases by refluxing the amine and the aldehyde in toluene, with a few drops of piperidine. The water formed during the course of the reaction was removed by employing a Dean-Stark trap.
3. In 1950, Hayes *et al*¹¹⁹ prepared Schiff bases by heating a mixture of an aldehyde and an amine in benzene and removing the water formed during the reaction using Dean-Stark apparatus.
4. In 1953, Giovambettista and Rabassa¹²⁰ suggested the use of acetic acid as a catalyst for the synthesis of schiff bases in good yield.
5. In 1971, the condensation of hydrazine and an aldehyde using glacial acetic acid as a solvent was described.

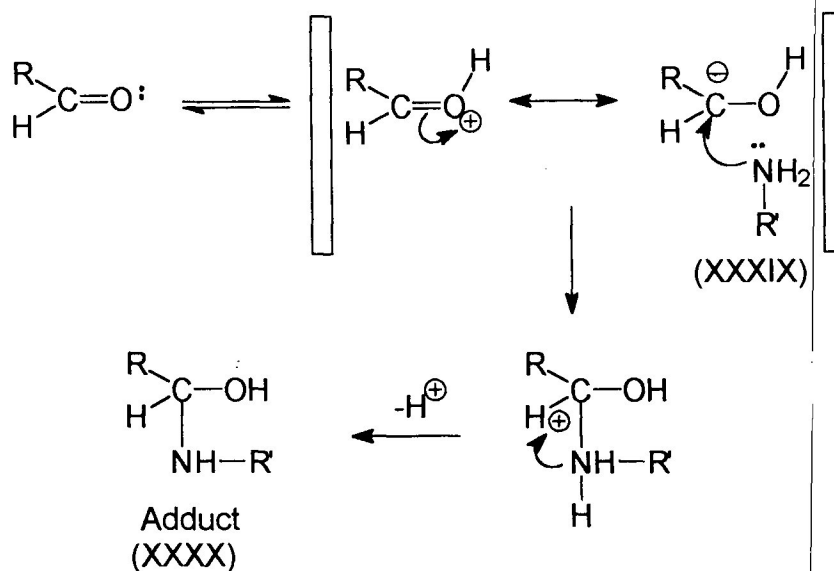
As the first method was found to give good yields it was followed initially. The addition of few drops of acetic acid was found to improve the yield. Later on, during the course of investigation the last method using acetic acid as a solvent, was found to be the best for the following reasons.

1. Simple apparatus and no need of Dean Stark Trap.
2. Good yield
3. Time course of the reaction was reduced.

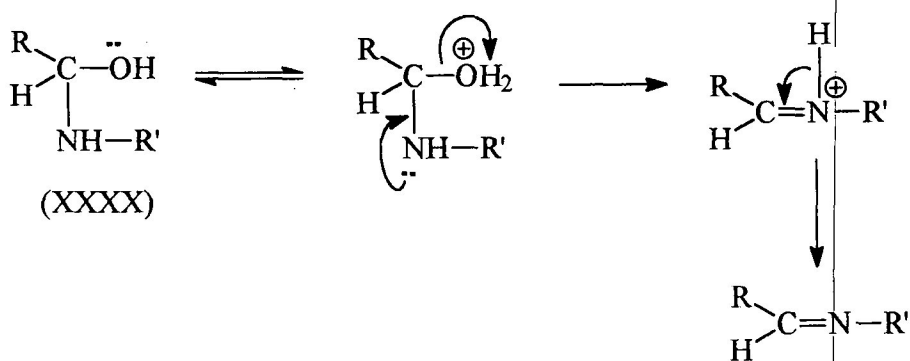
Reaction mechanism:



The amine and the aldehyde is heated in the presence of an acid. The acid protonates the carboxyl oxygen and promotes the attack of nucleophilic amine.

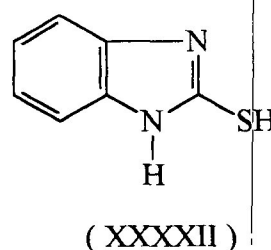
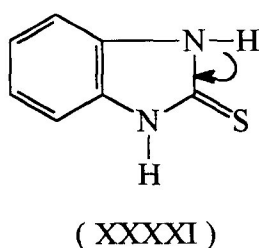


The adduct (XXXX) thus formed undergoes a further reaction to eliminate water and form a carbon-nitrogen double bond.



CHEMISTRY OF 2- MERCAPTOBENZIMIDAZOLE

2-(3H) benzimidazole thione (XXXXI) and 2-mercapto benzimidazole (XXXXII) are tautomeric substances analogous to 2-(3H) benzimidazolone and 2-hydroxy benzimidazole.

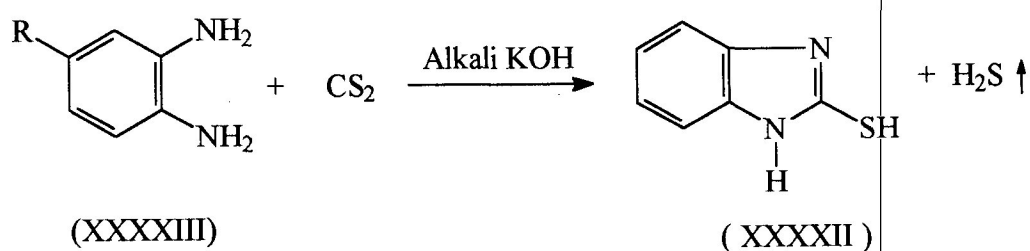


Different methods of preparation of 2 – mercapto benzimidazole

a) Synthesis from o-phenylene diamine and carbon disulphide

2-mercapto benzimidazole, in general, may be prepared very conveniently by this method. The reaction is carried out usually by heating the reactants in alcoholic

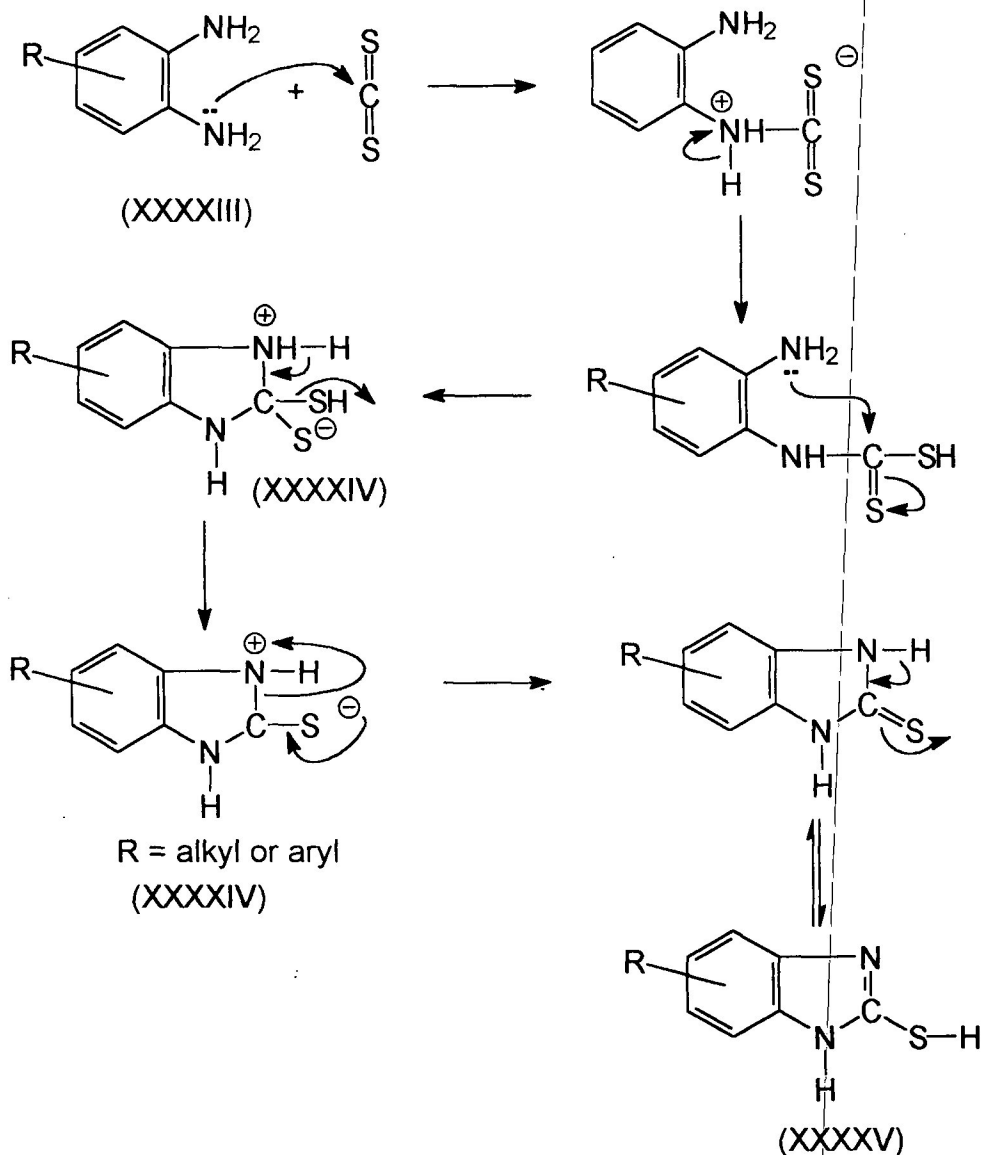
solution, with or without the addition of alkali to the reaction mixture. 2-mercapto benzimidazole itself has been prepared by this method^{121,122}.



R = Alkyl or aryl group

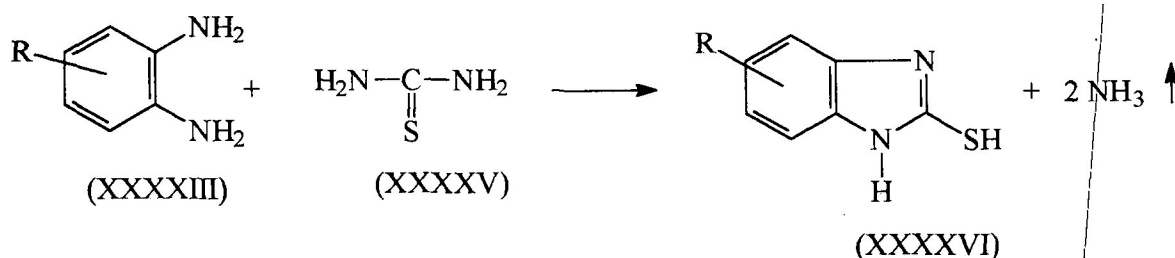
o-phenylene diamines containing substituents on the phenyl ring have been used, including those containing alkyl¹²³, halogen¹²⁴, phenyl¹²⁵, aniline¹²⁶ and sulphonamido¹²⁷ groups.

Probable reaction mechanism -



b) Synthesis from o-phenylene diamine and thiourea

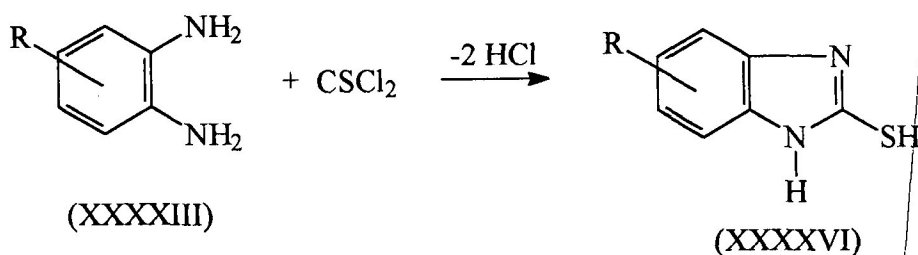
This reaction is analogous to the preparation of 2-hydroxy benzimidazole from *o*-phenylene diamine and urea. The substituted-2-mercapto benzimidazole (V) may be prepared by this method by heating substituted *o*-phenylene diamine dihydrochloride(XXXXIII) and thiourea (XXXXV) to 170-180°C¹²⁸. Excellent yields have been obtained by carrying out the reaction in amyl alcohol and heating under reflux until the evolution of ammonia becomes negligible.



R = Alkyl or aryl

c) Synthesis from o-phenylene diamine and thiophosgene

Billeter and Steiner¹²⁹ obtained 2-mercapto benzimidazole and 5-methyl-2-mercapto benzimidazole by the action of thiophosgene on o-phenylene diamine and 3,4-diamino toluene, respectively. 3,4-diamino benzene arsenic acid and thiophosgene gave 78% yield of 2-mercapto-s-arsenic acid benzimidazole¹³⁰.



R = Alkyl or aryl

d) Miscellaneous methods

2-mercapto benzimidazole can be prepared in 84-86.5% yield by the action of potassium ethyl xanthate on O-phenylene diamine¹³¹. 2-mercapto benzimidazole has also been prepared from O-phenylene diamine dihydrochloride and ammonium thiocyanate. A solution of the two substances is usually evaporated to dryness on a steam bath and then heated for a short time. 2-mercapto benzimidazole¹³² and 5-chloro,4-methyl¹³³ and 5-methyl¹³⁴ derivatives of 2-mercapto benzimidazole have been prepared by this method.

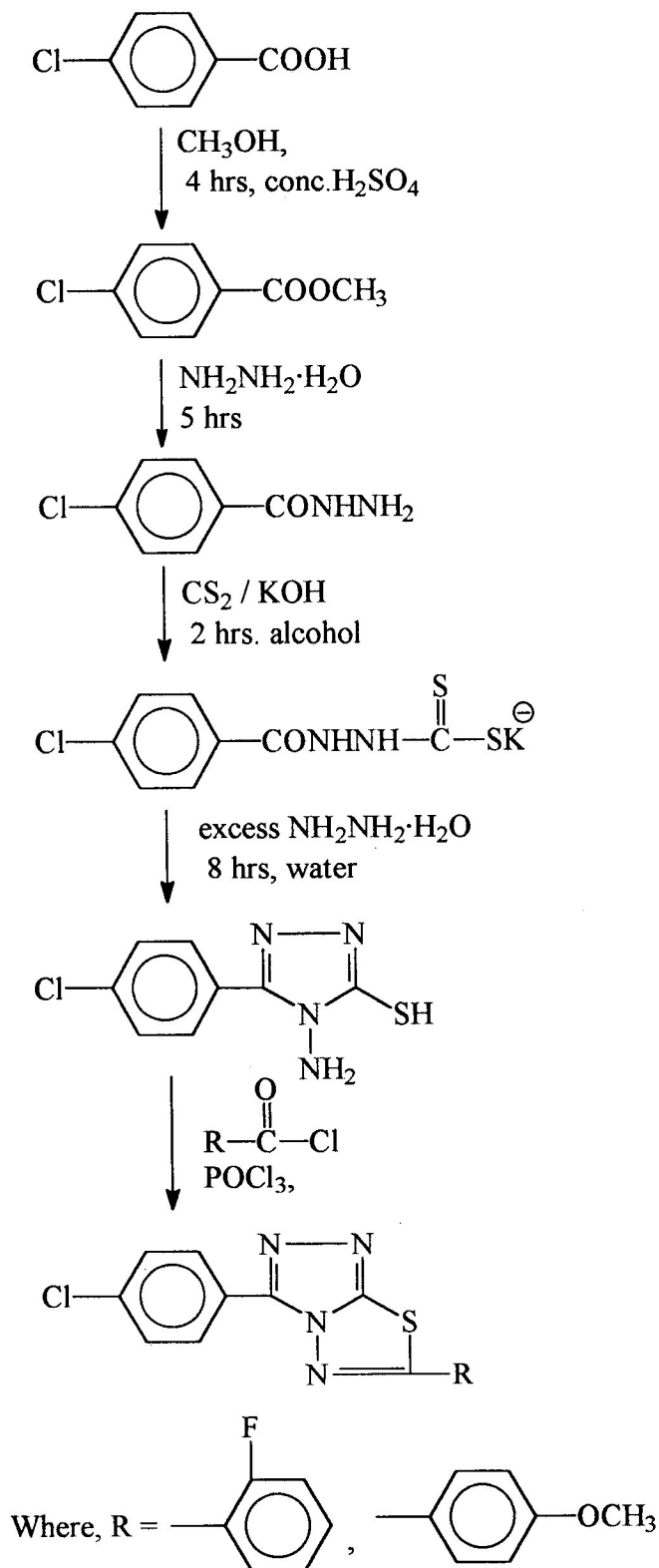
Part ID

***Schematic Representation
of series of compounds
synthesized and drug
intermediate***

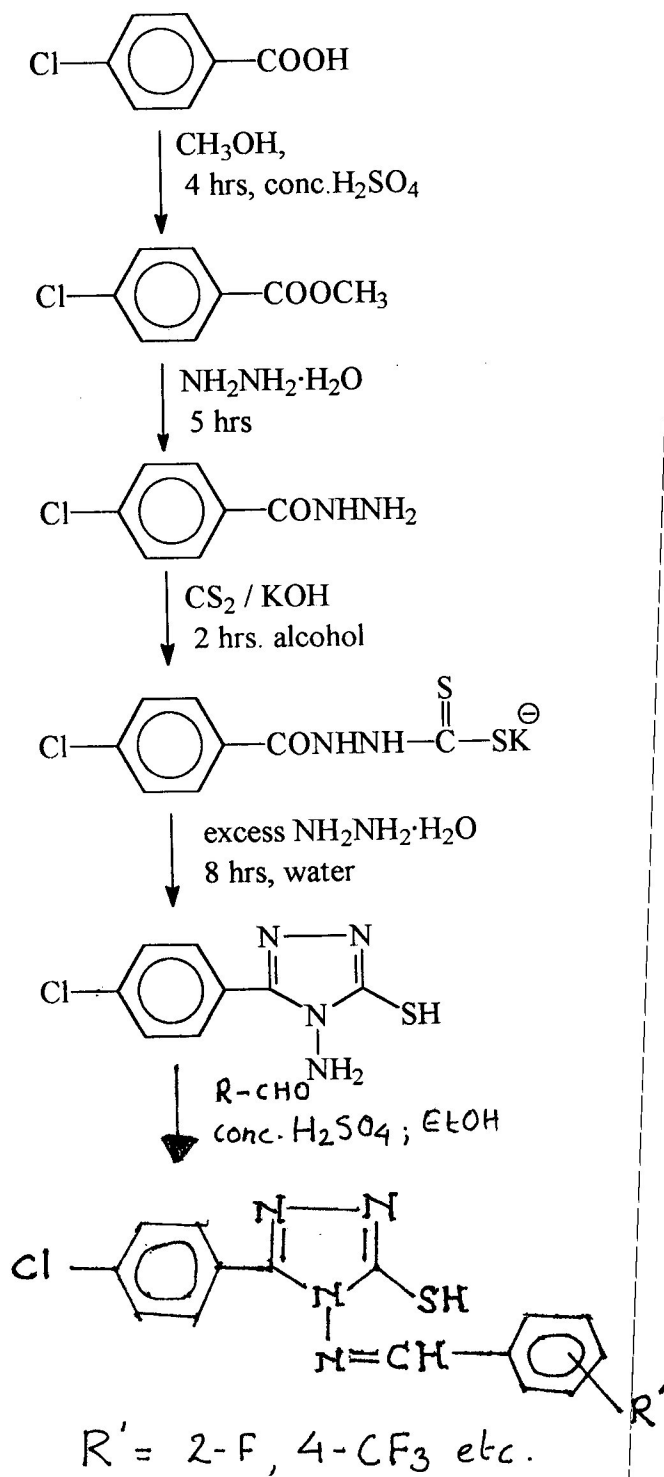
SCHEMATIC REPRESENTATION

Scheme I

Synthesis of 3-(p-chlorophenyl)-1,2,4-triazolo-6-(substituted phenyl)- [3-4-b][1,3,4] thiadiazoles

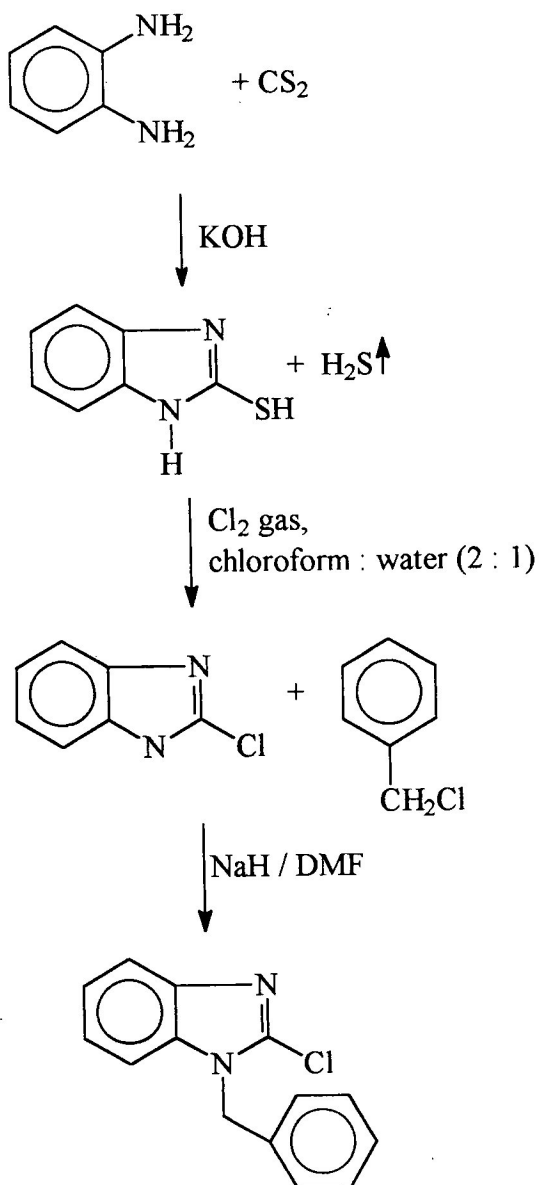


Scheme II

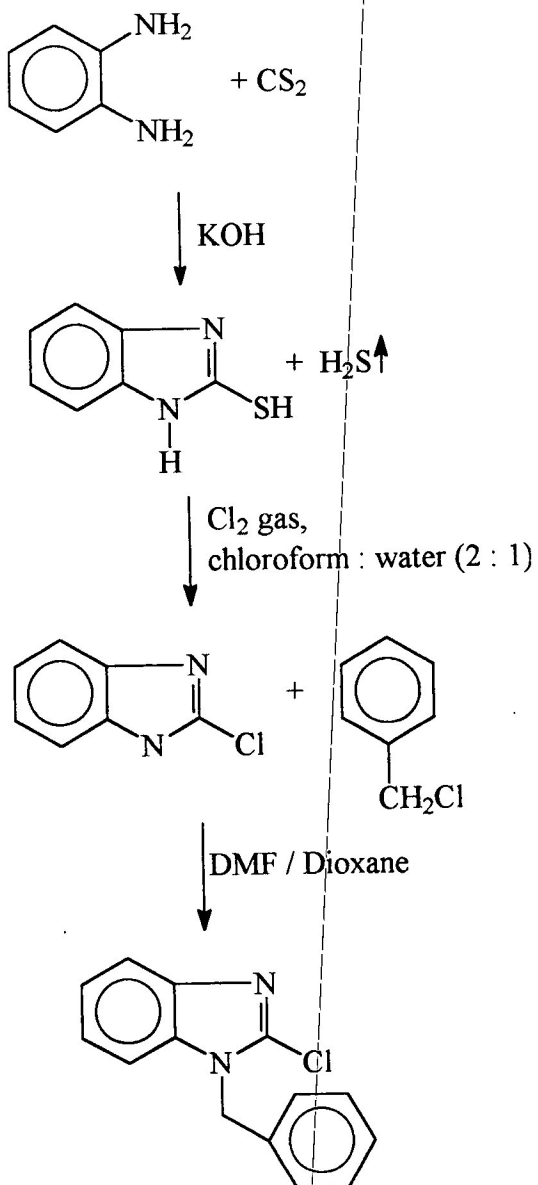


Scheme III

Literature Scheme



Modified Scheme



Experimental

Part II

Synthesis of 3,6- disubstituted-1,2,4- triazolo [3, 4-b] [1,3,4] thiadiazoles

Part II

3,6-disubstituted-1,2,4-triazolo [3,4-b] [1,3,4] thiadiazoles can be synthesized by two methods.

1. Condensation of 3-substituted-4-amino-(4H)-1,2,4-triazole with appropriately substituted acid chloride. This method has been successfully carried out by many researchers¹³⁵.
2. s-triazolo [3,4-b] [1,3,4]-thiadiazoles in which two substituents are identical, were also prepared by a one step synthesis through heating of an excess of the appropriate carboxylic acid with thiocarbohydrazide in the presence of phosphorous oxychloride¹³⁶.

In the present research work, method (1) was used because of lesser number of steps involved, starting from the easily available material.

3-substituted-4-amino-s-mercapto-(4H)-1,2,4-triazole was synthesized by cyclization of the potassium salt of s-substituted dithiocarbazate with hydrazine hydrate. The potassium salt of 3-substituted dithiocarbazate was in turn prepared by the reaction of substituted aryl hydrazide with carbon disulphide and potassium hydroxide, as detailed in the general procedure.

In this research project, 3-(4'-chlorophenyl)-4-amino-s-mercapto-(4H)-1,2,4-triazole was condensed with (i) various acid chlorides to obtain a series of triazolo thiadiazoles, and (ii) with various aromatic aldehydes to obtain Schiff bases. p-chloro benzoic acid was used as the starting material for the synthesis of the triazole. It includes the following steps (Scheme I).

Experimental

Synthesis of p-chloro methyl benzoate

In 500 ml round-bottomed flask, a mixture of 0.246 mole of p-chloro benzoic acid, 80gm. (101 ml., 2.5 mole) of absolute methanol and 5gm. (2.7 ml.) of conc. H_2SO_4 was placed. A few small porcelain chips were added and the mixture was refluxed gently on a water-bath for 4hrs. The formation of the ester was confirmed by TLC and quantitative test for ester¹⁰³.

The excess of methanol was removed under reduced pressure and the reaction mixture was cooled. The residue was poured into about 50 ml. of water contained in a separating funnel and the flask was rinsed with some more distilled water.

The lower layer of the ester was carefully separated. A strong solution of sodium bicarbonate was added to the aqueous layer in the separating funnel until all free acid is removed, which is evidenced by no further evolution of carbon dioxide. Carbon tetrachloride was added to the solution and shaken vigorously, releasing the pressure occasionally. After separation of the two layers, the aqueous layer was rejected. The organic layer was dried by passing it over anhydrous magnesium sulphate. The carbon tetrachloride was boiled off and the ester passed over anhydrous calcium chloride.

Yield = 37.73 gm (90%)

Synthesis of p-chloro-benzohydrazide:

0.1 ml of hydrazine hydrate (5gm., 4.9 ml.) was placed in a 100 ml round bottomed flask connected to a reflux condenser. The flask was warmed on a water-bath and 0.1 mole of methyl ester of p-chloro benzoic acid was added drop-wise with intermittent shaking. After the addition was complete, the reaction mixture was refluxed for further a one-hour period. The reaction mixture was then poured into a 250 ml beaker, where-in the product separated as a solid. It was filtered under reduced pressure and recrystallized from rectified spirit¹³⁷.

Yield = 35 gm. (70%)

M.P. = 161-162°C

M.P._{lit} = 163°C

Synthesis of potassium salt of p-chloro-benzohydrazide :

In a 500 ml conical flask, 8.4 gm. (0.15 M) of KOH was placed in 200 ml of absolute alcohol. The solution was stirred on a magnetic stirrer at room temperature. To the stirred solution was added 0.1 mole of aryl hydrazide in small portions, ensuring that the earlier portion was dissolved completely. A separating funnel was clamped over the conical flask, containing 11.4 gm of carbon disulphide (9ml., 0.15 mole)⁹⁹.

After all the aryl hydrazide was dissolved, carbon disulphide was added drop-wise to the flask over 2 minute duration, ensuring no rise in temperature of the reaction mixture. As the addition of carbon disulphide reached completion, a yellowish-white solid separated from the solution and finally the whole solution thickened up. 150 ml of absolute alcohol was further added and stirred for the next 4-5 hrs. It was then diluted with 200 ml of dry ether and the solid was filtered. Further washings of dry ether (2 x 50 ml.) were given to the filtered solid. The solid was further dried in a vacuum over at 65-70°C for 4 hrs. The salt prepared had a sharp melting point and was employed without further purification.

Synthesis of 3-(p-chloro-phenyl)-5-Thiol-1,2,4-Triazole

A mixture of potassium p-chloro benzoyldithiocarboxinate (28.45 gm., 0.1 M), 80% hydrazine hydrate (12 ml., 0.2 mole) and water (10 ml.) was heated under reflux for 4hrs. The colour of the solution became green during heating. The reaction mixture was cooled, diluted with water (500 ml.) and neutralized with conc. hydrochloric acid. The white solid obtained was filtered, washed thoroughly with water and finally crystallized from aqueous ethanol¹⁰⁰.

Yield : 17.3 gms (76.4%)

M.P. : 150-152°C

M.P. lit¹³⁶ : 150-155°C

Synthesis of 3-(p-chloro-phenyl)-1,2,4-triazolo-6-N,N-Diethyl-[3,4-b] [1,3,4] thiadiazoles: (Table No.-1,Compound No. 10)

A mixture of crude aminothirole (2.23 gms., 0.01 mole) and the appropriate N,N-diethyl carbamoyl chloride (1.349 ml., 0.01 mole) in phosphoryl chloride (20 ml.) was refluxed for 5 hours. The excess phosphoryl chloride was removed under reduced pressure and the residue was added to crushed ice. The mixture was stirred at room temperature for one hour, during which time the solution was gradually neutralized with solid sodium bicarbonate. The precipitated solid product was filtered, washed with diluted aqueous solution of sodium bicarbonate (20 %) and water. The collected solid was dried and crystallized¹³⁸.

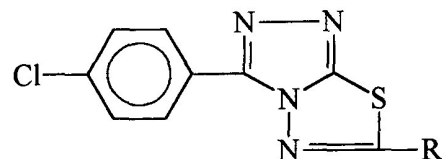
M.P. = 148-150°C

Yield % = 1.18 gms. (53%)

Elemental Analysis of C₁₄H₁₇ClN₄S

	C(%)	H(%)	N(%)
Calculated	50.73	4.55	22.76
Found	50.43	4.35	22.36

Table No. 1 : 3-(p-chlorophenyl)-1,2,4-triazolo-6-(substituted phenyl)-[3-4-b][1,3,4] thiadiazoles



Sr No	R	M.P.(°C) (uncorrected)	Yield (%)	Elemental Analysis (%)					
				Calculated			Found		
				C	H	N	C	H	N
1	4-fluorophenyl	> 250°C	50	54.46	2.45	16.94	54.16	2.15	16.64
2	2-fluorophenyl	183-185°C	55	54.46	2.45	16.94	54.06	2.05	16.54
3	3,4-dichlorophenyl	82-84°C	56	43.06	1.91	13.33	42.96	1.61	13.19
4	Cyclopropyl	98-100°C	45	45.99	3.19	17.88	45.6	2.89	17.58
5	4-methoxyphenyl	196-198°C	59	56.05	3.21	16.35	55.65	2.91	16.05
6	Cinnamoyl	193-195°C	57	60.26	3.24	16.54	59.86	2.94	16.34
7	Cyclohexyl	132-134°C	60	56.69	4.40	17.63	56.33	4.40	17.23
8	Thiophenyl	98-101°C	61	48.97	2.19	17.58	48.57	1.99	17.28
9	3,4dimethoxy phenyl	217-219°C	52	54.76	3.48	15.03	54.36	3.18	14.83
10	N,N-diethyl	148-150°C	53	50.73	4.55	22.76	50.43	4.35	22.36
11	N,N-dimethyl	168-170 °C	60	49.71	3.76	21.09	49.51	3.46	20.99
12	4-trifluoromethyl phenyl	228-230°C	61	50.45	2.10	14.71	50.05	1.7	14.31

Part III :

Synthesis of 3-(4'-chlorophenyl)-4-[(4-fluoro)-1-methylene]-amino-5-mercapto-1,2,4- triazole (Compound No. 5, Table No. 2)

3-(substituted phenyl) 4-amino-5-mercapto-1,2,4-triazole is the starting material which was synthesized as mentioned in part II (Scheme II).

The 4-amino triazole (0.42g, 0.005M) was placed in a 50 ml round bottomed flask equipped with a reflux condenser and 5 ml of dry methanol was added. It was slightly warmed in order to obtain a clear solution. To this was added 4-fluoro benzaldehyde (0.960 gm, 0.005 M) dissolved in 5 ml of methanol and the mixture was refluxed on a water bath for 3-4 hrs. It was then cooled to room temperature. The solid separated was filtered and recrystallized from ethanol¹³⁹.

Yield: 78%

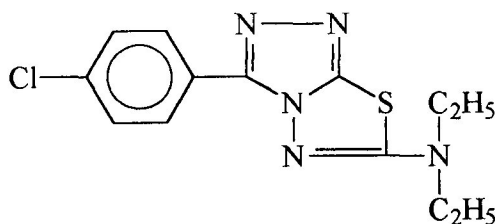
M.P. = 179-181°C

Elemental analysis of C₁₆H₉ClF₄N₄S

	C(%)	H(%)	N(%)
Calculated	60.10	3.00	18.69
Found	59.80	2.60	18.29

SPECTRAL ANALYSIS

Spectral Studies of 3-(p-chloro-phenyl)-1,2,4-triazolo-6-N,N-Diethyl-[3,4-b]
[1,3,4] thiadiazoles (Table No. 1 Compound No. 10)



IR (KBr) cm^{-1}

Wave number

2971.82

1581.6

1456.3

968.16

Assignment

ethyl group

C=N Stretching

C=N and C=C Stretching

NCS

PMR (DMSO - d_6) δ :

1.30-1.40

(t, 6H, $-\text{CH}_2\text{CH}_3$)

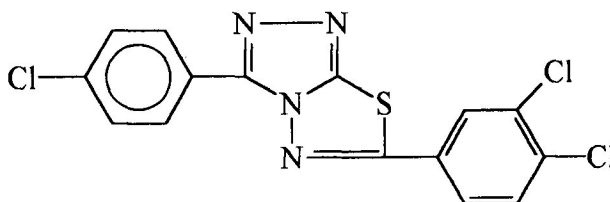
3.49-3.56

(q, 4H, $-\text{CH}_2\text{CH}_3$)

7.40-8.25

(2d, 4H, ArH)

Spectral Studies of 3-(p-chloro-phenyl)-1,2,4-triazolo-6-(3,4-dichloro)-[3,4-b]
[1,3,4] thiadiazoles (Table No. 1 Compound No. 3)



IR (KBr) cm^{-1}

Wave number

1606-1487

1087

1011

Assignment

C=N and C=C Stretching

NCS Stretching

NCS Stretching

File # 1 = CTDET-1

Mode= 2 (Mid-IR)

1/4/80 5:47 PM

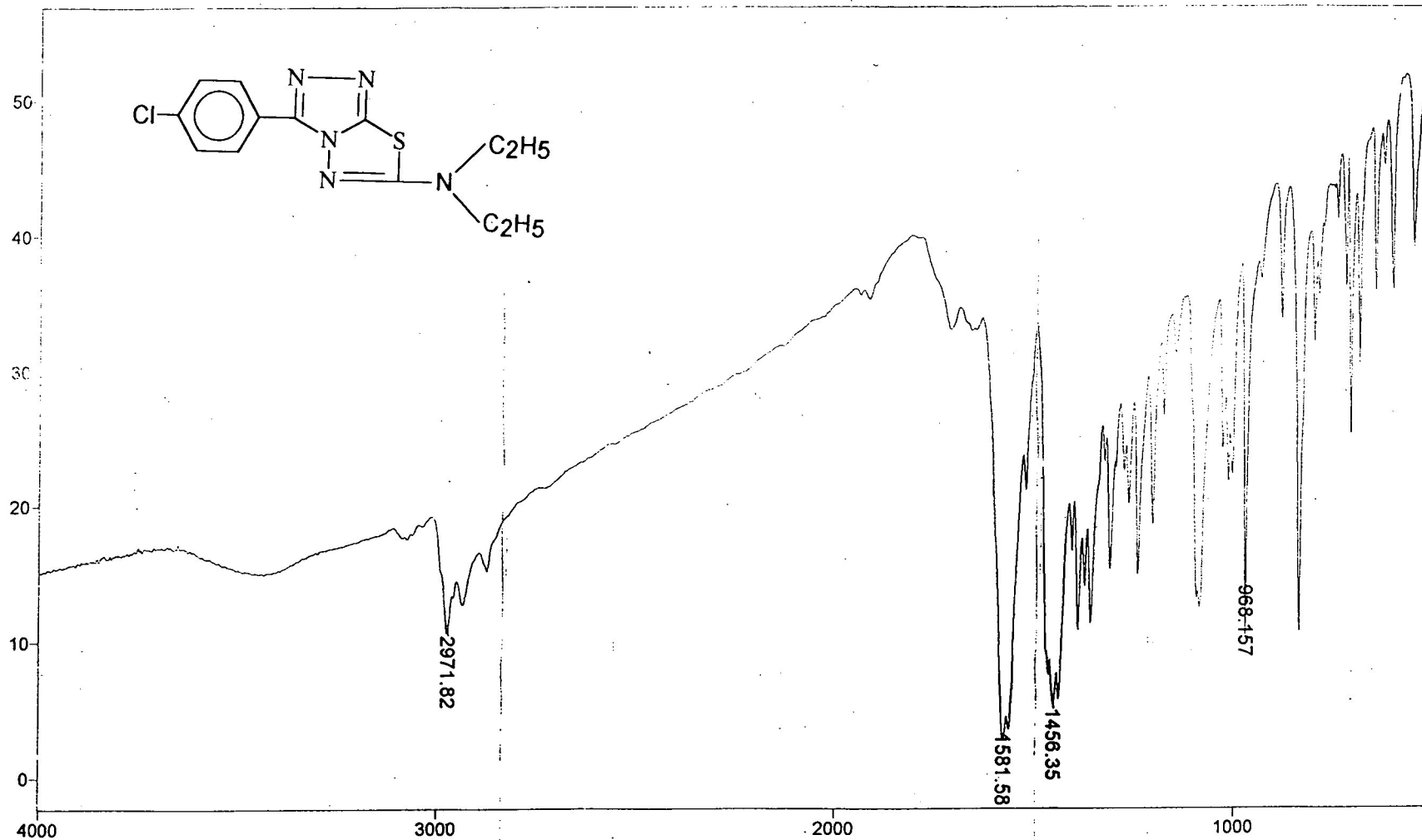
Sample Description: chloro triazole diethyl thiadiazole

Scans= 10 Slow

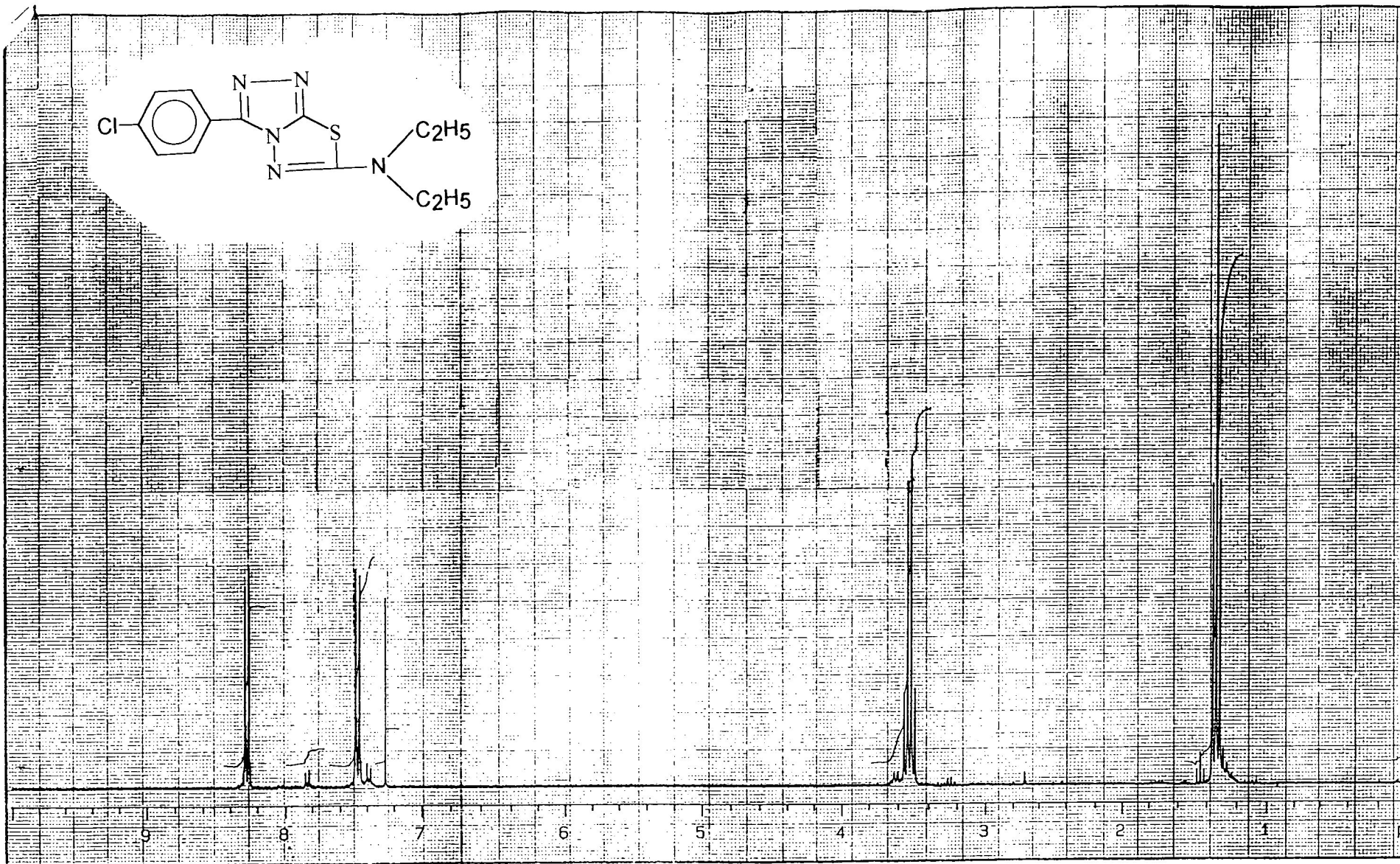
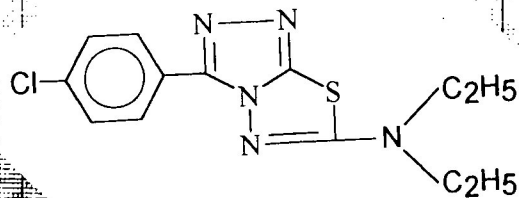
Res=4 cm-1

Apod= Cosine

Zero Filling= 1 x



Transmittance / Wavenumber (cm-1)



OBSERVE	Nucleus	H1	Freq	299.9486 MHz	DISCOUPLE	Nucleus	H1	Offset	-1769.8 Hz	F1, Det used	1H	sec	CD	sec	Pulse Sequence	s2p41	SAMPLE	Number	
	Spec Width	5998.8 Hz	Offset	1200.0 Hz		Mass	0	Power	20 dB		0.100	not used	100	Tube OD		CTDET		File	CTDET
	Acq Time	4.00 sec	Lock	0 Hz		Wavelength	0.10157 m	Frequency	299.9486 MHz		3063.4	Hz	Start	-61.0 Hz/ppm	Temp	not used	Date	MAR 17	
	Pulse Width	10.00 sec	Transmit	104		Relax. W.	17.5 sec	Power	not used					Solvent	CDCl3	Vis	300		

File # 1 = CTRIDCTH

Mode= 2 (Mid-IR)

1/4/80 11:22 AM

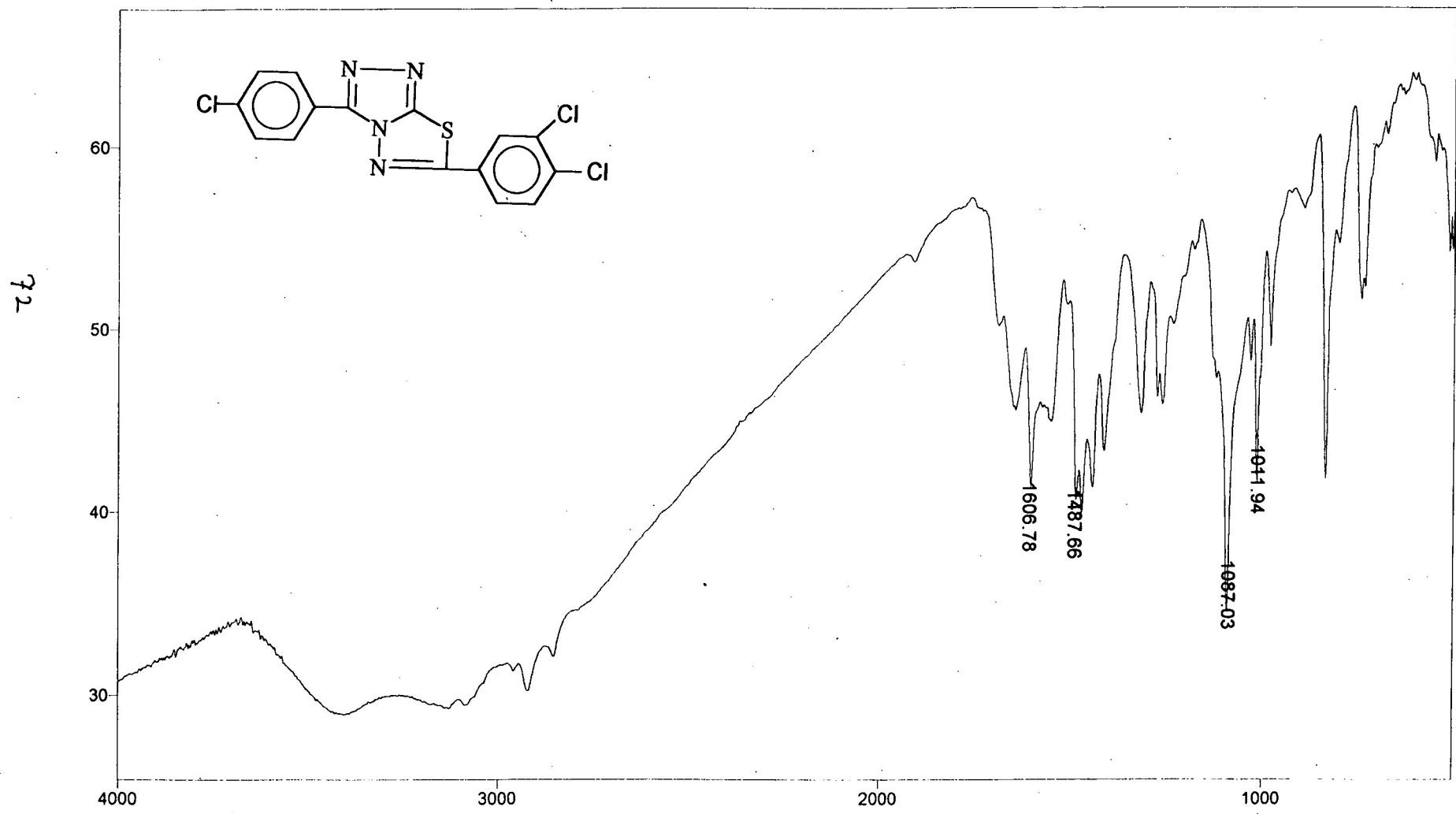
Sample Description: p-chloro triazole dichloro thiadiazole

Scans= 10 Slow

Res=4 cm-1

Apod= Cosine

Zero Filling= 1 x



Part III :

Synthesis of 3-(4'-chlorophenyl)-4-[(4-fluoro)-1-methylene]-amino-5-mercapto-1,2,4- triazole (Compound No. 5, Table No. 2)

3-(substituted phenyl) 4-amino-5-mercapto-1,2,4-triazole is the starting material which was synthesized as mentioned in part II (Scheme II).

The 4-amino triazole (0.42g, 0.005M) was placed in a 50 ml round bottomed flask equipped with a reflux condenser and 5 ml of dry methanol was added. It was slightly warmed in order to obtain a clear solution. To this was added 4-fluoro benzaldehyde (0.960 gm, 0.005 M) dissolved in 5 ml of methanol and the mixture was refluxed on a water bath for 3-4 hrs. It was then cooled to room temperature. The solid separated was filtered and recrystallized from ethanol¹³⁹.

Yield: 78%

M.P. = 179-181°C

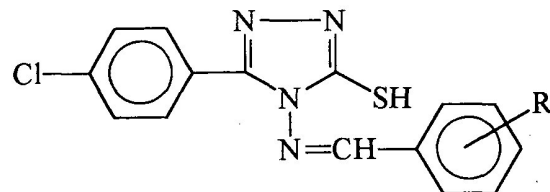
Elemental analysis of C₁₆H₉ClF₄N₄S

	C(%)	H(%)	N(%)
Calculated	60.10	3.00	18.69
Found	59.80	2.60	18.29

Part III

***Synthesis of 3-(4'-
chlorophenyl)-4-[(4-fluoro)-1-
methylene]-amino-5-mercapto-
1,2,4- triazole***

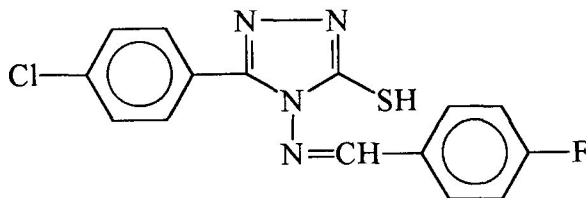
Table 2 : 3-(4'-chlorophenyl)-4-[(2-substituted phenyl)-1-methylene]-amino-5-mercapto-1,2,4- triazole



Sr No	R	M.P.(°C) (uncorrected)	Yield (%)	Elemental Analysis (%)					
				Found			Calculated		
				C	H	N	C	H	N
1	2-fluoro-4-trifluoromethyl phenyl	179-181°C	78%	47.94	2.24	13.98	47.75	2.05	13.79
2	2-trifluoromethyl phenyl	234°C	65%	50.19	2.61	14.64	50.09	2.41	14.24
3	3-trifluoromethyl phenyl	205-207°C	69%	50.19	2.61	14.64	49.81	2.51	14.35
4	4-trifluoromethyl phenyl	160-162°C	61%	50.19	2.61	14.64	49.89	2.21	14.44
5	4-fluoro phenyl	219-220°C	75%	60.10	3.00	18.69	59.7	2.8	18.39

SPECTRAL ANALYSIS

Spectral Studies 3-(4'-chlorophenyl)-4-[(4-fluoro)-1-methylene]-amino-5-mercapto-1,2,4- triazole (Table No. 2, Compound No. 5)



IR (KBr) cm^{-1}

Wave number

1601.49

1548.72

884.64

Assignment

Schiff base, C=N

N-heterocycles conjugated, C=C and C=N

C-N

PMR (DMSO - d_6) δ :

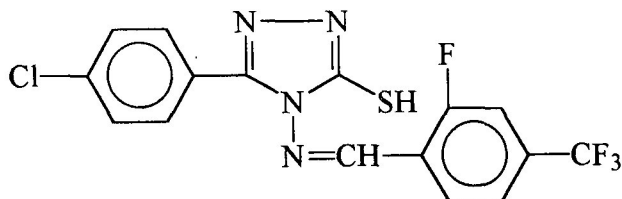
7.37-8

[m, 9H(8H, ArH) (1H, -SH)]

9.75

(s, 1H, -N=CH)

Spectral Studies 3-(4'-chlorophenyl)-4-[(2-fluoro-4-trifluoro methyl phenyl)-1-methylene]-amino-5-mercapto-1,2,4- triazole (Table No. 2, Compound No. 1)



IR (KBr) cm^{-1}

Wave number

1063.29

1603.1

1539.6

911.65

Assignment

polyfluorination

Schiff base

N-heteroaryl conjugated (C=N)

SH Stretching

File # 1 = YA-1

Mode= 2 (Mid-IR)

1/4/80 7:19 PM

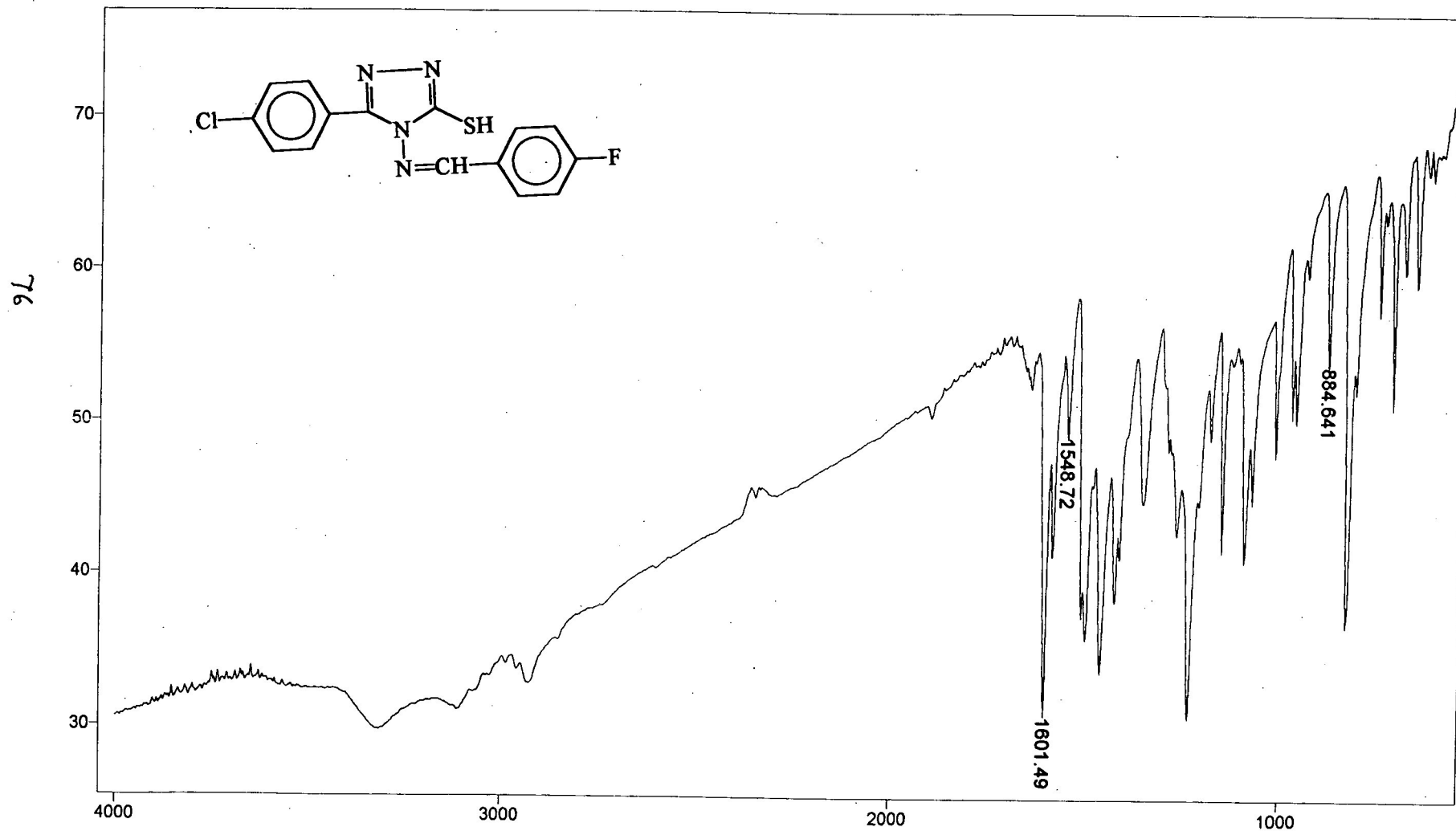
Sample Description: 4-fluoro benzaldehyde -1

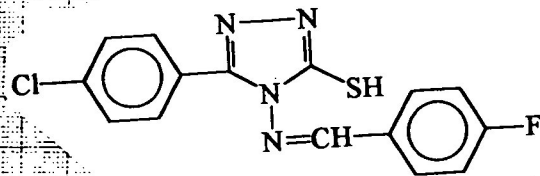
Scans= 10 Slow

Res=4 cm-1

Apod= Cosine

Zero Filling= 1 x





OBSERVE	Nucleus	<u>H1</u>	Freq	<u>299.9486</u> MHz	Nucleus	<u>H1</u>	Offset	<u>-1769.8</u> Hz
	Spec Width	<u>5998.8</u> Hz	Offset	<u>1200.0</u> Hz	Wave	<u>n</u>	Time	<u>20</u> sec
	Acq Time	<u>4.00</u> sec	Delay	<u>0</u> sec	Waveform	<u>C</u>	Time	<u>200</u> sec
	Pulse Width	<u>10.0</u> sec	Transmit	<u>80</u>	Spec Width	<u>17.5</u> MHz	Pulse Mass	<u>not used</u>

FID PROCESSING	PN	<u>not used</u>	IE	<u>sec</u>	CU	<u>sec</u>
	Id	<u>0.100</u>	not used	SD	<u>sec</u>	
	Wave	<u>3634.6</u> Hz	Time	<u>17.9</u> sec	sec	

EXPERIMENT	Pulse Sequence	<u>g2p11</u>
	Tube OD	<u>mm</u>
	Temp	<u>25.0</u> °C
	Solvent	<u>DMSO</u>

SAMPLE
YA-1

Number _____
File YA1.f1d
Date Mar 17
VXRO 300

File # 1 : YA-2SPC

Mode= 2 (Mid-IR)

4/24/00 10:00 AM

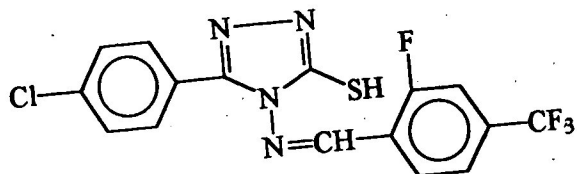
Sample Description:

Scans= 10 Slow

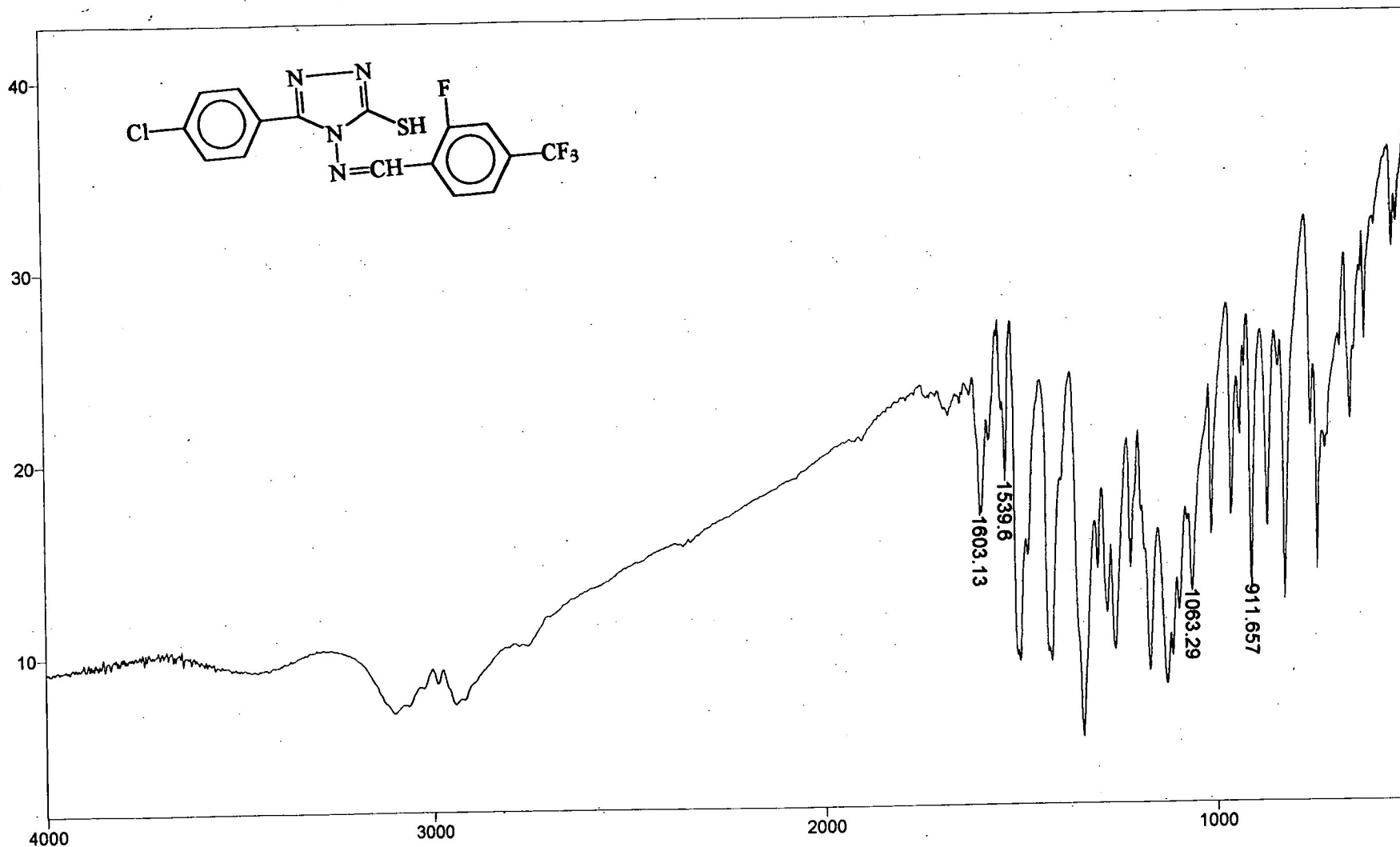
Res=4 cm-1

Apod= Cosine

Zero Filling= 1 x



78



Transmittance / Wavenumber (cm-1)

Part IV

***Drug intermediate for
Lerisetron***

***Synthesis of 2-chloro-1-
(benzyl)-benzimidazole***

Part IV:

Synthesis of 2-chloro-1-(benzyl)-benzimidazole:

Synthesis of this drug intermediate involves the following steps (Scheme III) :

Step I: Preparation of 2-mercapto benzimidazole: -

In a 100 ml round bottom flask equipped with a water condenser was placed 7.125 gm. (0.05 M) of 4-chloro-1,2-phenylenediamine. It was dissolved in 40 ml. of ethanol. To this was added 3.1 gm (0.055 M) of KOH and 4.18 gm. (3.3 ml., 0.055 M) of CS₂. The reaction mixture was refluxed on a water-bath for 4 to 5 hours and the excess ethanol was removed under reduced pressure. The residue obtained was dumped in cold water and acidified with dil HCl till pH of the solution became acidic. The solid separated was filtered and dried¹³².

Yield = 67.2 %

M.P. = 289 – 290°C

Reported¹⁴⁹ M.P. = 290-292°C

Step II: Preparation of 2-chloro-benzimidazole:

In a 100 ml three-necked flask equipped with an air condenser, 2-mercapto-benzimidazole (2g, 0.01 M) was taken in a mixture of chloroform: water (2:1) and cooled to 5°C. A slow stream of chlorine gas was bubbled through the solution for 1 hour while maintaining the temperature below 10°C. At the end of this, the reaction mixture was transferred to an evaporating dish to expel the excess chlorine. The evaporation of the solvent afforded a sticky mass. The passage of this material through a silica gel column and elution with chloroform gave the purified product.

Yield = 45.77%

M.P. = 180-182°C

M.P.(lit) = 180°C

Step III: Addition of benzoyl chloride to 2-chloro-benzimidazole:

In a 50 ml round-bottomed flask was taken a mixture of 2-chloro-benzimidazole (6 gm, 0.03 M) and of triethylamine (0.7 ml, 0.01 M) in 20 ml dioxane and 2 ml DMF. The mixture and was stirred at 60°C for 1hr and 8.4 gms. (0.058 M) of benzyl

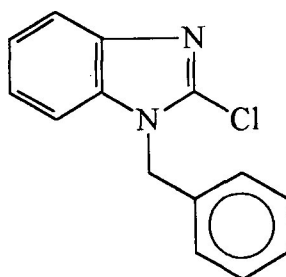
chloride was then added. This was refluxed for 4hr. After completion of the reaction, the mixture was poured into 70ml of cold water. The precipitate formed was filtered and recrystallised from methanol.

Yield = 59.2 %

M.P. = 104-106°C

M.P. literature⁸¹ = 106-108°C

Spectral Studies 2-chloro-1-(benzyl)-benzimidazole



IR (KBr) cm^{-1}

Wave number

2952, 3040

1600

1506

665

Assignment

CH₂, alkyl group stretching

C=N stretching of heterocyclic group

C=C skeletal vibration of aromatic ring

C-Cl stretching

Part V

Biological Screening, Results and Discussions

File # 1 : FBCBI

Mode= 2 (Mid-IR)

7/15/99 3:55 PM

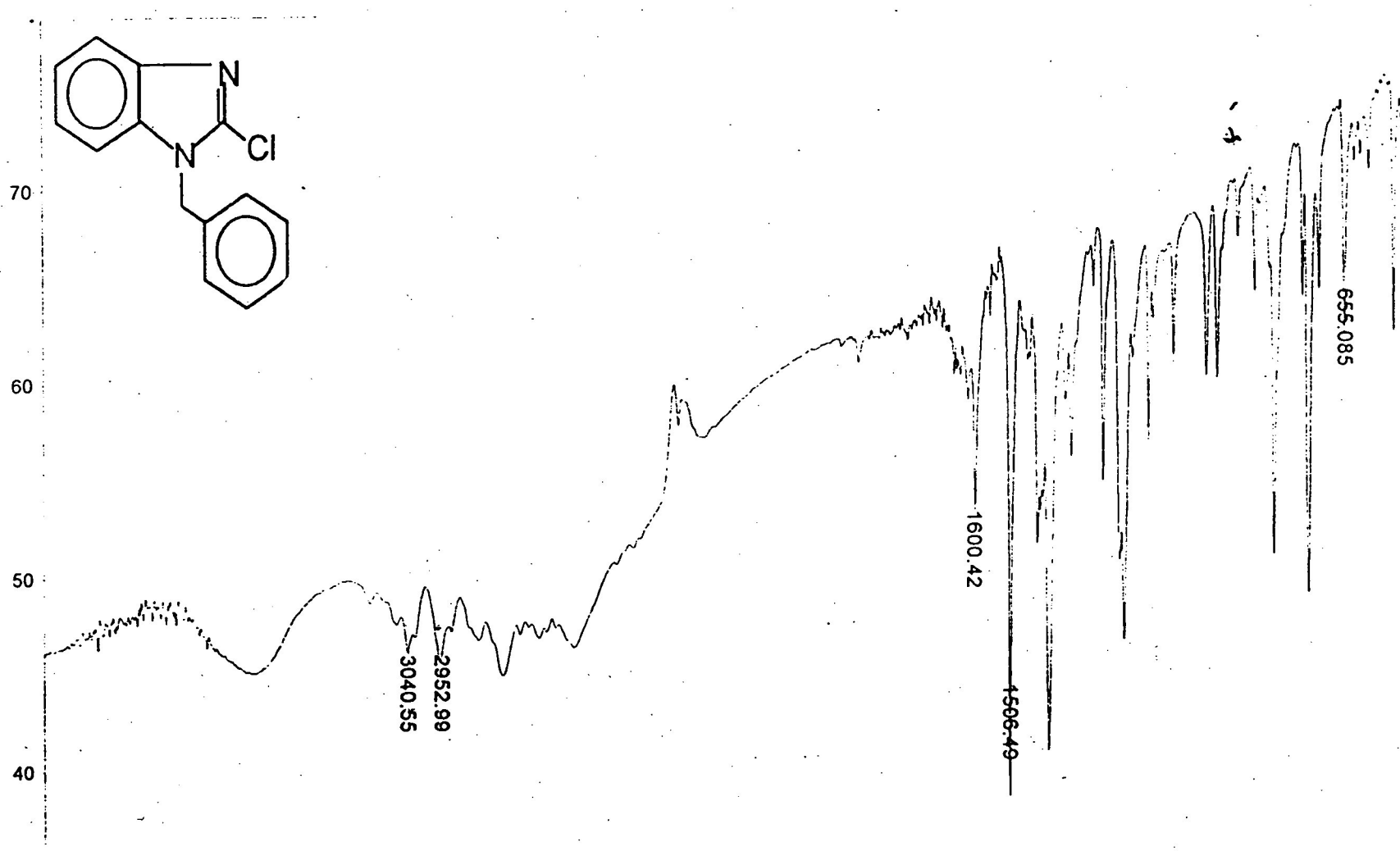
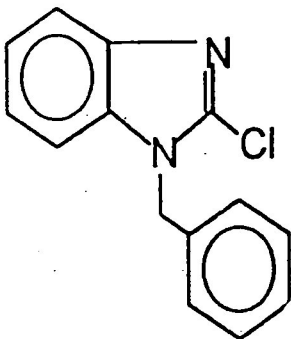
Sample Description: fbcbi

Scans= 10 Slow

Res=4 cm-1

Apod= Cosine

Zero Filling= 1 x



Part V

BIOLOGICAL RESULTS AND DISCUSSION

The compounds synthesized in the present investigation have been subjected to various biological screening programmes based on their structural features so as to ascertain their suitability as potential chemotherapeutic agents. All compounds have been screened for antimicrobial activity, while compounds from part II have also been screened for *in vivo* anthelmintic activity.

Various *in vitro* screening programmes have been carried out against various species of gram-positive and gram-negative bacteria, viz. *Staphylococcus aureus* ATCC 3750 (*S. aureus*) and *Salmonella typhi* NCTC 789 (*S. typhi*) for determining antimicrobial activity.

It also includes *in vitro* study against various fungal strains, viz. *Candida albicans*, *Trichophyton mentagrophytes* and *Trichophyton rubrum*. The methodology adopted for the various biological screening programs undertaken in this study has been described in the following section.

The test compounds (10 mg) were dissolved in a suitable solvent (10 ml) such as dimethyl formamide, ethanol, dil. HCl, as the case may be, so as to prepare a drug stock solution of concentration 1000 µg/ml. From this stock solution, following final drug concentrations were made 200, 100, 40, 20 µg/ml. For lower final drug concentrations, the drug stock solution was diluted to prepare various stock solutions such as 10, 5, 2 and 1 µg/ml.

Controls

1. Media Control

Sterilized medium was kept as control so as to assure sterility of the medium. If this control shows growth, medium is not properly sterilized and in that case results are not valid.

2. Organism Control

It contains sterilized medium inoculated with the organism. Organism should grow in the medium. If no growth is observed, it can be concluded that the medium is unable to support the growth.

3. Solvent control

This includes sterilized medium, solvent (used to dissolve the drug) and the culture. The organism should be able to grow in the quantity of solvent used in testing. If no growth is observed, it can be concluded that the solvent inhibits the growth of the organism and in that case results of the test are not valid.

ANTIBACTERIAL SCREENING

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

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

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

ANTITUBERCULAR SCREENING

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

Kirchner's medium containing Tween 80 as described by Rake *et al*¹⁴¹ was used. The sterile medium was dispensed in each borosilicate test tube to attain final dilutions of 200, 100, 40, 20, 10, 5, 2, 1, 0.5 µg/ml.

Innoculum of standard suspension, i.e. 0.1 ml of H₃₇Rv strain of *M. tuberculosis* containing 10⁶ bacilli/ml, was added. The tubes were incubated at 37°C for 11 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. Isoniazid (MIC = 0.04 µg/ml) was used as a standard.

ANTIFUNGAL SCREENING

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

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

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

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

ANTHELMINTIC SCREENING

Compounds synthesized in Part II were screened for their *in vivo* anthelmintic activity against *Hymenolepis nana* in albino mice. The method used is the one described by Agarwal Alka *et al*¹⁴².

SCREENING PROCEDURE

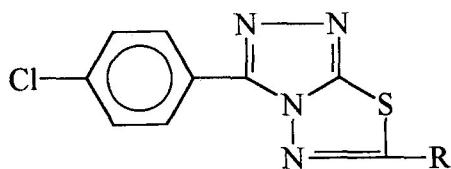
The *H. nana* infection was obtained from the intestine of bandicoots, it was crushed in 0.85% physiological saline and passaged to a group of control albino mice. These were male albino mice weighing 18-20 gms. After 21 days the animals were autopsied and the intestines checked for the presence of *H. nana*. This confirmed infection was then passaged into a new group of mice after adjusting the titre to 100 eggs/ml. The mice were divided into groups of five, with each set of 5 for each individual drug and one group of 5 as the control group. On day 14-16 post infection (p.i.), suspensions of drugs in Tween 80(1%) were given by gavage to the animals. A concentration of 250 mg/kg was used for the test compounds as well as for tetramisole and Albendazole (standard drugs). The animals were then autopsied on day 20-21 p.i. and the intestine checked for the worms. A count of the surviving worms per mouse was taken and the average number of surviving worms (in the set of 5 mice for each drug) was calculated.

The calculations for the % efficacy were done as follows

$$\% \text{ efficacy} = \frac{\text{number of eggs injected} - \text{average no. of surviving worms}}{\text{number of eggs injected}} \times 100$$

The anthelmintic activity of the synthesized compounds was determined as the % efficacy and is graded as per the degree of activity defined in Steward *et al*¹⁴³.

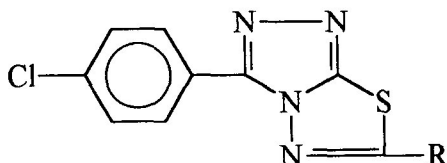
Table 3 : Antibacterial activity of 3-(p-chlorophenyl)-1,2,4-triazolo-6-(substituted phenyl)-[3-4-b][1,3,4] thiadiazoles



Sr. No.	Substitution R	Antibacterial activity in µg/ml		
		H ₃₇ Rv strain of <i>M. tuberculosis</i>	<i>S. aureus</i>	<i>S. typhi</i>
1	N,N diethyl	100	10	10
2	N,N dimethyl	100	4	10
3	Cinnamoyl	100	NA	10
4	4-methoxy phenyl	NA	10	10
5	4-trifluoromethyl phenyl	40	100	100
6	3,4-dimethoxy phenyl	100	100	100
7	2-fluorophenyl	40	10	10
8	4-fluorophenyl	40	100	100
9	3,4-dichlorophenyl	400	10	10
10	Cyclopropyl	NA	100	100
11	Thienyl	NA	100	100
12	Cyclohexyl	NA	100	100

NA – Inactive at 200µg/ml

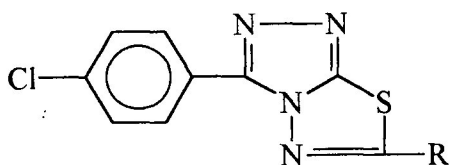
Table 4 : Antifungal activity of 3-(p-chlorophenyl)-1,2,4-triazolo-6-(substituted phenyl)-[3-4-b][1,3,4] thiadiazoles



Sr. No.	Substitution R	Antifungal activity in µg/ml		
		<i>Candida albicans</i>	<i>Trichothyton mentagrophytes</i>	<i>Trichothyton rubrum.</i>
1	N,N diethyl	NA	40	40
2	N,N dimethyl	NA	40	40
3	Cinnamoyl	NA	NA	40
4	4-methoxy phenyl	NA	NA	100
5	4-trifluoromethyl phenyl	NA	NA	NA
6	3,4-dimethoxy phenyl	NA	NA	NA
7	2-fluorophenyl	NA	NA	NA
8	4-fluorophenyl	NA	NA	NA
9	3,4-dichlorophenyl	NA	40	40
10	Cyclopropyl	NA	NA	NA
11	Thienyl	NA	NA	NA
12	Cyclohexyl	NA	NA	NA

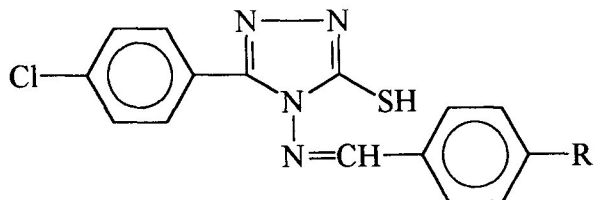
NA – Inactive at 200µg/ml

Table 5 : Anthelmintic activity of 3-(p-chlorophenyl)-1,2,4-triazolo-6-(substituted phenyl)-[3-4-b][1,3,4] thiadiazoles



Sr. No.	Substitution R	(Single dose 250mg/kg) % efficacy
1	N,N diethyl	58.18
2	N,N dimethyl	50.00
3	Cinnamoyl	73.63
4	4-methoxy phenyl	61.81
5	4-trifluoromethyl phenyl	75.45
6	3,4-dimethoxy phenyl	60.90
7	2-fluorophenyl	61.81
8	4-fluorophenyl	60.0
9	3,4-dichlorophenyl	72.72
10	Cyclopropyl	64.54
11	Thienyl	65.45
12	Cyclohexyl	62.72

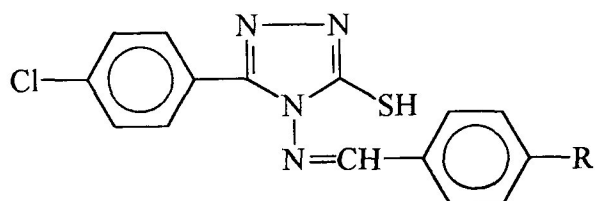
Table 6 : Antibacterial activity of Synthesis of 3-(4'-chlorophenyl)-4-[(substituted phenyl)- 1-methylene]-amino-5-mercapto-1,2,4- triazole



Sr. No.	Substitution R	Antibacterial activity in µg/ml		
		H ₃₇ Rv strain of <i>M. tuberculosis</i>	<i>S. aureus</i>	<i>S. typhi</i>
1	4-fluoro	100	100	100
2	2-fluoro, 4-trifluoro methyl	100	100	100
3	3-trifluoromethyl	NA	100	100
4	2-trifluoromethyl	NA	100	100
5	4-trifluoromethyl	100	100	100

NA – Inactive at 200µg/ml

Table 7 : Antifungal activity of Synthesis of 3-(4'-chlorophenyl)-4-[(substituted phenyl)- 1-methylene]-amino-5-mercapto-1,2,4- triazole



Sr. No.	Substitution R	Antifungal activity in µg/ml		
		<i>Candida albicans</i>	<i>Trichothyton mentagrophytes</i>	<i>Trichothyton rubrum.</i>
1	4-fluoro	NA	NA	NA
2	2-fluoro, 4-trifluoro methyl	100	NA	NA
3	3-trifluoromethyl	NA	NA	NA
4	2-trifluoromethyl	NA	NA	NA
5	4-trifluoromethyl	NA	NA	NA

NA – Inactive at 200µg/ml

Conclusion

CONCLUSIONS

Part II : 3,6-disubstituted-1,2,4-triazole [3,4-b] [1,3,4] thiadiazoles

All twelve compounds in this series have been screened for their antibacterial, antitubercular and anthelmintic activity (Table No. 3,4,5, pg. 77,78)

1. Of all the twelve substitutions made compound with N,N-dimethyl substituent (Table No.3, pg. 77, Compound No. 2) was found to be the most active against *S. aureus* with MIC of 4 µg/ml and 10 µg/ml against *S. typhi*.
2. N,N-diethyl, 4-methoxy phenyl, 2-fluorophenyl, 3,4-dichlorophenyl (Table No. 3, pg. 77, Compound Nos 1,4,7 and 9) substituted compounds have shown good activity against *S. aureus* and *S. typhi* with MIC of 10 µg/ml for both organisms.
3. Also, all other six substitutions (Table No. 3, pg. 77, Compound Nos. 5,6,8,10,11 and 12) have shown moderate activity against *S. aureus* and *S. typhi* i.e. 100µg/ml for both organisms. An exception is the compound with Cinnamoyl substitution (Compound No. 3), which was shown good activity against *S. typhi*, but is inactive against *S. aureus*.
4. Moderate antitubercular activity upto 40µg/ml was observed for 4-trifluoromethyl, 2-fluorophenyl and 4-fluorophenyl substitutions and other substitutions like N,N-dimethyl, Cinnamoyl, 3,4-dimethoxy phenyl, 3,4-dichlorophenyl (Table No. 3, pg. 77, Compound Nos. 5,7,8 and Compound Nos. 2,3,6,9 respectively), have given compounds with activity upto 100µg/ml. The remaining 4-methoxy phenyl, cyclopropyl, thienyl and cyclohexyl substitutions (Table No. 3, pg. 77, Compound Nos. 4,10,11 and 12) have no activity.
5. Anthelmintic screening showed variable results. Three substitutions, i.e. Cinnamoyl (Table No. 5, pg. 78, Compound Nos. 3,5 and 9), 4-trifluoromethyl, 3,4-dichlorophenyl, seem to be very active, i.e. percent efficacy is more than 70%. All other compounds showed clearance from 50-70%, which indicates that these are active.

6. All the synthesized compounds were also subjected to the antifungal screening. All compounds were found to be inactive against *Candida albicans*.
7. Also, some substitutions like 4-trifluoromethyl, 3,4-dimethoxy, 2-fluoromethyl, 4-fluoromethyl, cyclopropyl, thienyl, cyclohexyl (Table No. 4, pg. 77, Compound Nos. 5,6,7,10,11 and 12) are inactive against all three fungal organisms i.e. *C. albicans*, *T. mentagrophytes*, *T. rubrum*.
8. Three substituted compounds, i.e. N,N-diethyl, N,N-dimethyl and 3,4-dichlorophenyl (Table No. 4, pg. 78, Compound Nos. 1,2 and 9) have shown moderate activity against both *T. mentagrophytes* and *T. rubrum* whereas cinnamoyl substitution (Compound No. 3) has activity only against *T. rubrum*.

Various other substitutions will have to be carried out to obtain active analogs.

Part III : 3-[p-chlorophenyl]-4-[(substituted phenyl)-1-methylene] amino 1,2,4-triazole

1. The compounds synthesized in this series have not shown any encouraging antibacterial activity. Most compounds have exhibited activity in the range of 100 µg/ml against both *S. aureus* and *S. typhi*.
2. In antifungal screening only 2-fluoro 4-trifluoromethyl substitution (Table No. 7, pg. 79, Compound No. 2) have shown moderate activity against *C. albicans*. No significant or promising activity was observed in spite of the presence of the triazole nucleus. Hence, various other substituents will probably have to be introduced to obtain active compounds.

SUMMARY

A total of 17 compounds have been synthesized as part of this research project and have been screened for their anti-bacterial, antifungal, antitubercular and anthelmintic activities. Although no single compound has exhibited significant biological activity an attempt should be made towards exploiting these 2 series of compounds for antimicrobial and anthelmintic agents. These series have provided 'leads' which can be substituted with different groups or moieties to obtain potent analogs.

The modified method for the synthesis of the drug intermediate suggested in this project should be scaled up to achieve benefits commercially and towards a better quality of the product.

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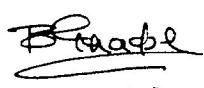
Appendix

APPENDIX

1. The Melting points have been determined in open capillaries in a Veego VMP-1 melting point apparatus, and are uncorrected.
2. The TLC of the compounds have been run on Eastman Kodak precoated Silica gel plates of type K301 R and E. Merck precoated silica gel plates of type 60 F 254.
3. The Infrared Spectra were recorded on BOMEM MB-104 Fourier Transform Infrared Spectrometer.
4. The PMR Spectras were recorded on Varian 300 MHz spectrometer using TMS as the internal reference, at RSIC, IIT, Powai.
5. Carbon, nitrogen and hydrogen analyses were carried out on Heraeus C, H, N, Elemental Analyzer at the Haffkine Institute.

Synopsis

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

TITLE OF THE THESIS	:	Synthesis and microbiological evaluation of organic compounds of possible therapeutic value.
NAME OF THE CANDIDATE	:	Mr. Kulkarni Yogesh P.
NAME AND DESIGNATION OF THE GUIDE	:	Dr. B.G. Khadse Director and head of Dept. of Chemotherapy Haffkine Institute, Parel, Mumbai-12.
PLACE OF RESEARCH	:	Department of Chemotherapy, Haffkine Institute, Parel, Mumbai-12.
NUMBER AND DATE OF REGISTRATION	:	43, Feb 01, 1999.
SIGNATURE OF THE STUDENT	:	
SIGNATURE OF THE GUIDE	:	

SYNOPSIS

Parasites have been identified as a major animal and human health problem for many decades. Substantial resources have been allocated to classify this multitude of life forms and to understand their complex and often fascinating life styles, resulting in a large body of literature dedicated to this subject. Even so the surface has only been scratched. One of the major challenges that remains is to discover a broad-spectrum, effective, non - toxic and cheap anthelmintic agent.

The discipline of medicinal chemistry is devoted to the discovery and development of new agents for treating diseases. Most of this activity is directed towards new natural or synthetic organic compounds. Hundreds and thousands of new organic chemicals are prepared annually throughout the world and many of them are entered into the pharmacological screens to determine whether they have useful biological activity.

This process of random screening has been considered insufficient, but it has resulted in the identification of new lead compounds. These structures have been optimized to produce clinical agents.

Based on this aspect, efforts have been made to synthesize heterocyclic molecules such as triazolothiadiazoles and these will be documented in the thesis. An interesting addition in the thesis is the synthesis of an intermediate for the drug Lerisetron, which is a 5-HT₃ antagonist and it will also include azomethine linkages prepared from the parent triazole moiety.

All synthesized compounds have been 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 analyses (recorded on Heraeus C,H,N Elemental Analyzer) Haffkine Institute.

The thesis will consist of the following parts -

PART -I : Introduction , Review of Literature and Plan of work.

PART-II : Synthesis of 3,6-disubstituted-1,2,4-triazolo-[3,4-b][1,3,4]thiadiazoles .

PART-III : Synthesis of 3-[(4-substituted phenyl)-1-methylene]amino-5-mercapto-1,2,4 -triazole .

PART-IV : Synthesis of 2-chloro-1-(benzyl)-benzimidazole.

PART-V : Biological Screening and conclusions.

PART-I

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 modest review of literature with reference to the selected heterocycles and the plan of work executed during the period.

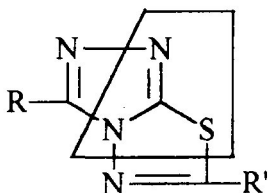
PART-II

Synthesis of 3,6-disubstituted-1,2,4-triazolo[3,4-b][1,3,4]thiadiazole.

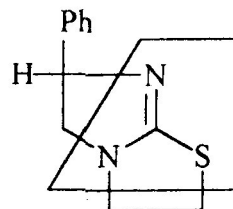
The most fruitful basis for the discovery of new drugs is to start with an old drug. We decided to follow this time-tested methodology of research and selected an established anthelmintic agent, Tetramisole, as the lead molecule.

Almost all anthelmintic agents exert their action by penetrating the cutaneous-muscular layer of the helminths and thereby causing paralysis or death of the worm. This effect can be enhanced by increasing the lipophilicity of the molecule, which in turn can be achieved by introducing halogen atoms on the parent moiety¹. Taking this fact into consideration, it was decided to synthesize moieties such that the final analogue contains at least one halogen atom.

El-Khawass *et al*² have reported the anthelmintic activity of a series of triazolothiadiazoles (I) which are structurally and isosterically related to Tetramisole (II), the established anthelmintic agent, against third stage larvae of *Acyclostoma caninum*.

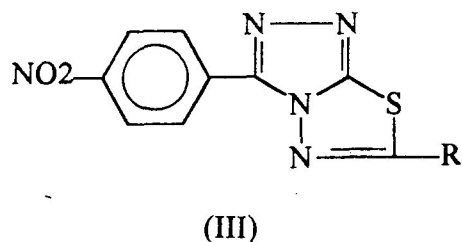


(I)

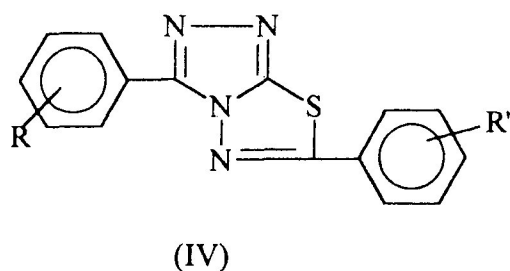


(II)

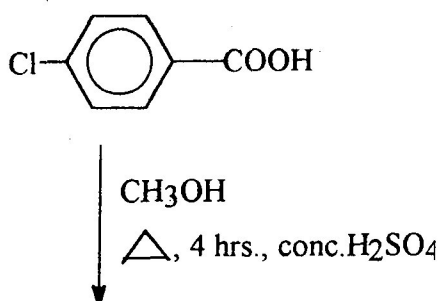
It has also been reported by Shi Hai-Jian *et al*³ that some 3,6-disubstituted triazolothiadiazoles(III) possess strong anti-bacterial activity.

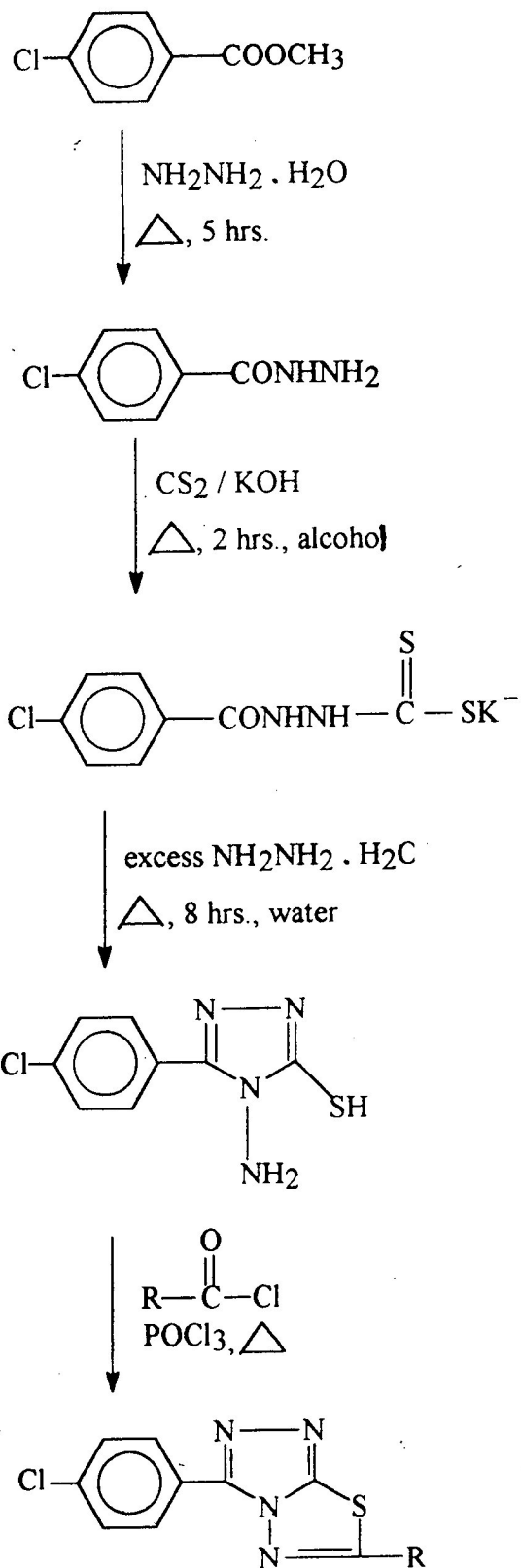


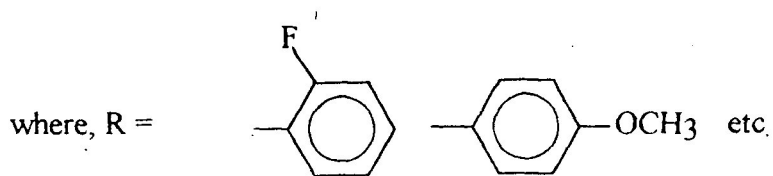
Hence, a series of halogenated triazolothiadiazoles(IV) was synthesized, with the hope of achieving anthelmintic and anti-microbial activity.



SCHEME I:



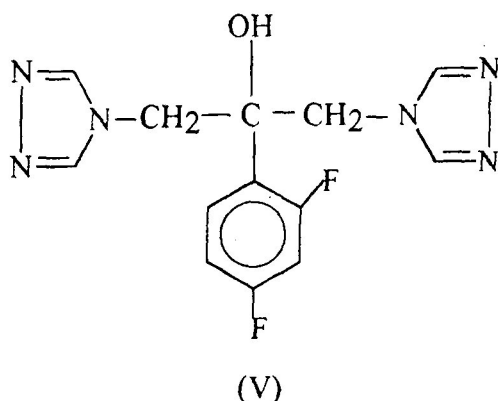




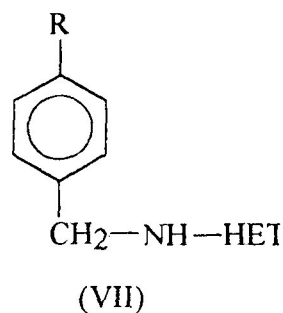
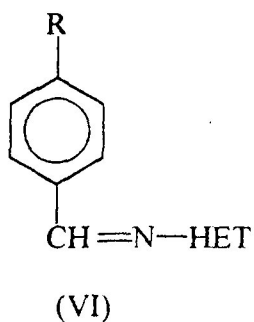
PART - III:

Synthesis of 3[(4-substituted phenyl)-1-methylene]amino-5-mercapto-1,2,4-triazole.

The importance of 1,2,4 -triazole moiety as a major pharmacophoric requirement to exhibit enhanced antifungal activity is well manifested by its inevitable presence in the most recent antifungal drugs, viz. Fluconazole⁴ (V).

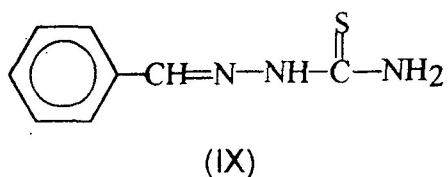
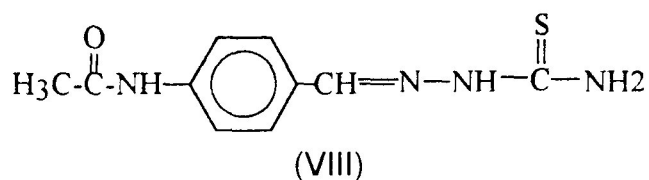


Recently, R. Fioravanti *et al*⁵ have reported the antimicrobial activity of some new heteroaryl benzylamines (VI) and their Schiff bases (VII).

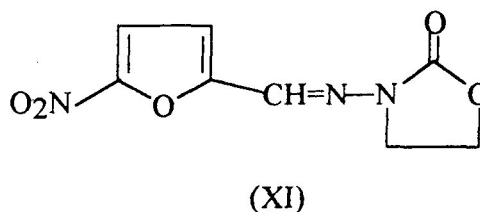
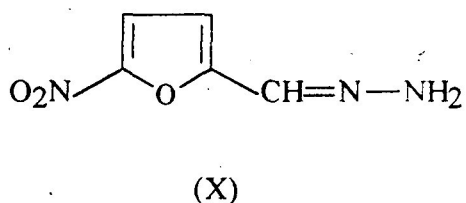


R = H, CH₃, Br, F etc.

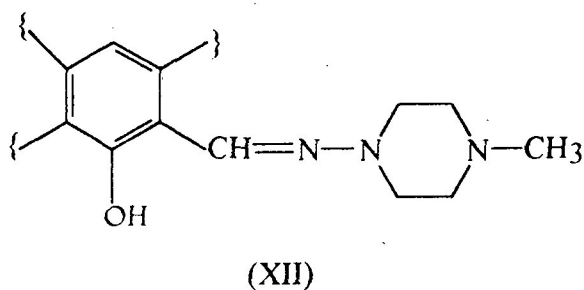
Also, during the screening of intermediates for the synthesis of sulfathiadiazoles, benzaldehyde thiosemicarbazone (VIII) was shown to be active against tuberculosis⁶. Structural modifications in this compound produced the azomethine derivative, amithiozone (IX), an orally active anti-tubercular compound.



Many of the biologically active compounds such as synthetic antibacterials [Nitrofurantoin (X), Nitrofurazone(XI)]⁷ are used as antibacterial and antiinfective agents. These compounds also contain the azomethine linkage which enhances the antimicrobial activity.



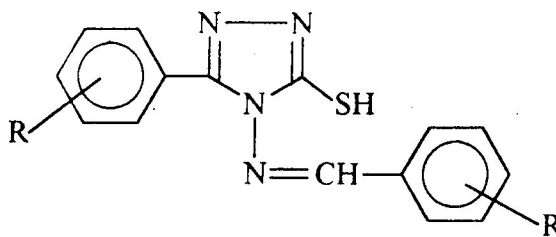
Antibiotics such as Rifampicin(XII) prove that azomethine linkage plays a vital role as regards biological activity. Rifampicin is an antibiotic prepared from Rifamycin B and it contains the azomethine linkage, which is responsible for its enhanced activity as compared to the parent moiety, Rifamycin B.



Schiff bases from 5-bromosalicylaldehyde and 5-chloro salicylaldehyde, have been reported to have encouraging tuberculostatic activity⁸. The presence of azomethine linkage in the compound has also been known to decrease toxicity considerably⁹.

Thus, taking into consideration the importance of the triazole nucleus and the pharmacophoric importance of the azomethine linkage, Schiff bases of 3-substituted-4-

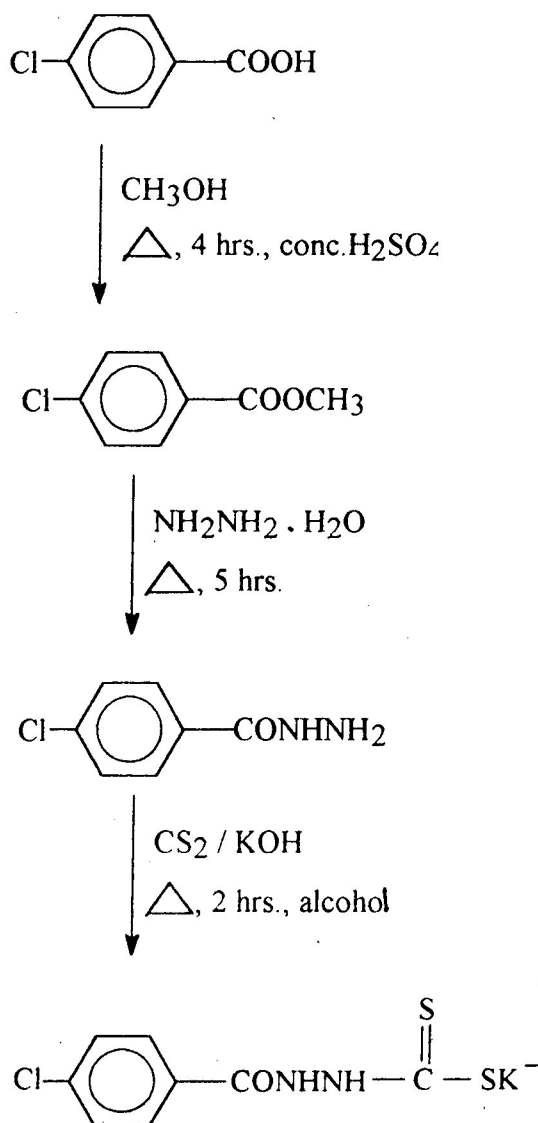
amino-1,2,4- triazole were prepared to obtain the title compounds(XIII). These were obtained by condensing the triazole with various substituted benzaldehydes.

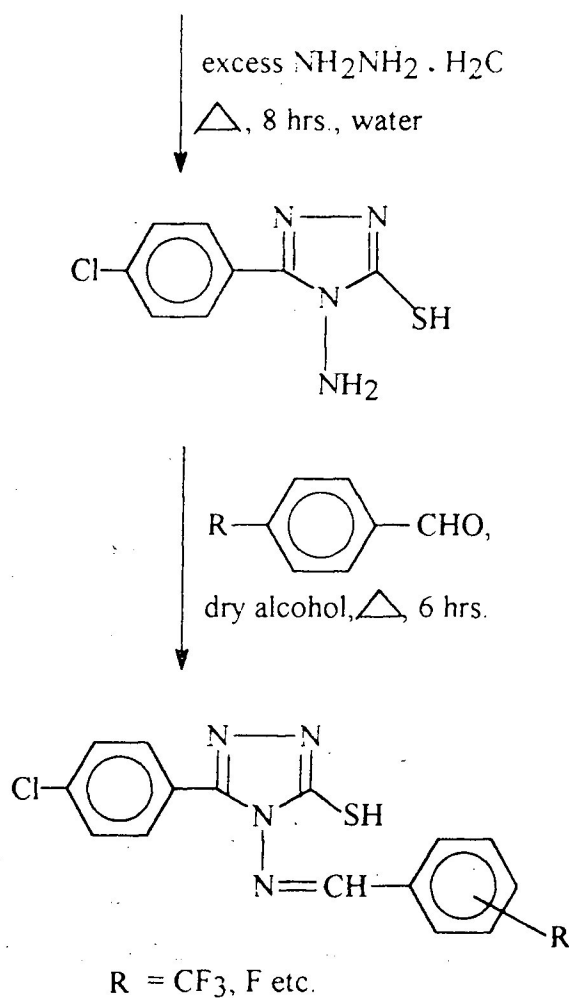


R = 2-F, 3-F, 4-CF₃ etc.

(XIII)

SCHEME:II





PART IV:

Synthesis of 2-chloro-1-(benzyl)-benzimidazole - the intermediate for Lerisetron.

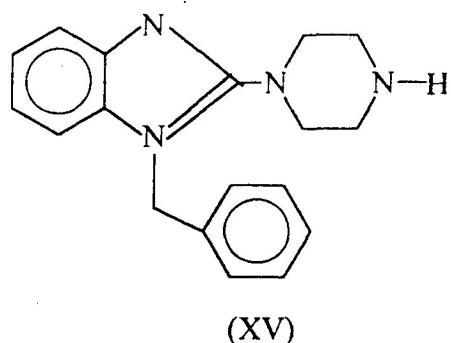
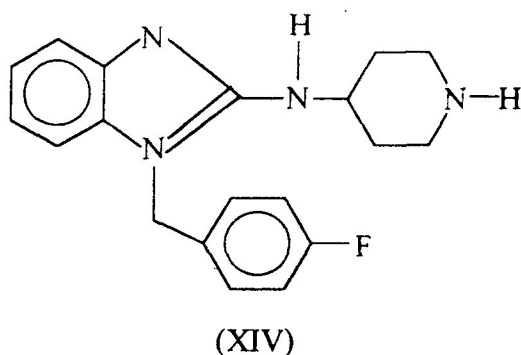
Irritable bowel syndrome (IBS) is a functional digestive disorder in the pathophysiology of which visceral sensitivity plays an important role. Pharmacologically, the most important pain mediator in irritable bowel syndrome is serotonin, which acts on sensory endings through 5-HT₃ receptors.

5-HT₃ receptors are distributed in the striatum, hippocampus, sensory neurons and post-ganglionic sympathetic neurons.

5-HT₃ receptor antagonists are currently being used for a variety of conditions, including nausea/ vomiting and IBS.

A series of 5-HT₃ receptor antagonists has been synthesized and one such compound is Lerisetron (XV), which is under Phase II clinical trials. Another compound of

the similar category, Norastemizole (XIV), has been manufactured by Sepracor, Inc. (U.S.), and is currently under clinical investigations¹⁰.



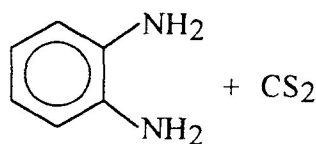
For synthesis of both these drugs 2-chloro-1- (benzyl)-benzimidazole is used as the starting material. Synthesis of this intermediate involves the following steps :

- 1) preparation of 2-mercaptobenzimidazole
- 2) preparation of 2- chloro benzimidazole
- 3) Addition of benzyl chloride to 2-chlorobenzimidazole.

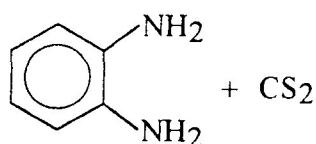
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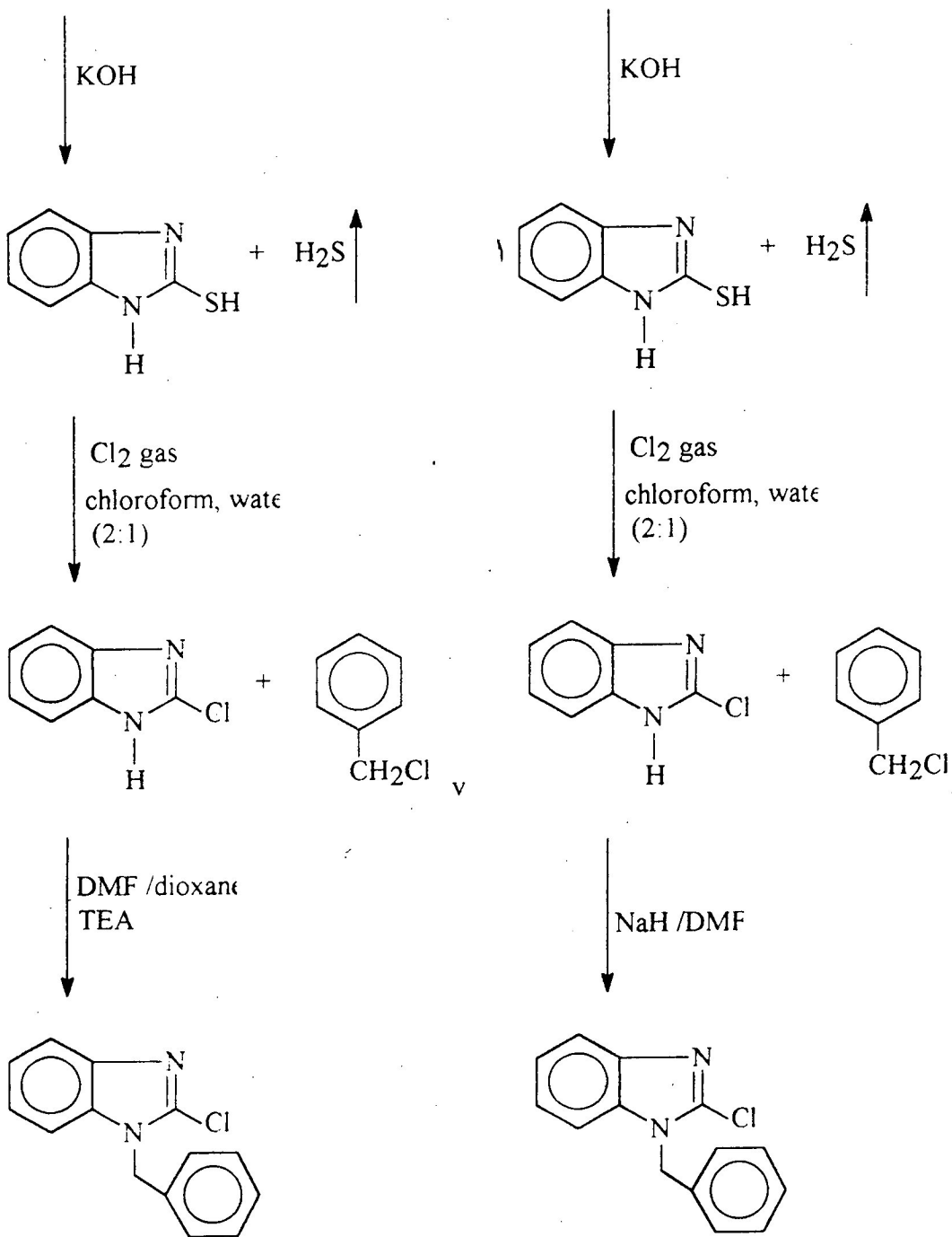
In literature, it is reported that the reaction is carried out in Sodium Hydride (NaH) and Dimethyl Formamide. Sodium Hydride (NaH) is a costly, flammable and moisture sensitive solid. Also, work with DMF is fairly messy and time - consuming. This increases handling and the cost of production at industrial scale. In our laboratory we carried out the reaction in a mixed solvent system containing Dioxane and Dimethyl formamide (9: 1). This change in the solvent system allowed the reaction to occur at a slightly faster rate. it was also found that the final product which was obtained by this method relatively free of impurities and had a sharp melting point. This effectively helps to reduce the cost as regards the time of production and amount of solvent needed for purification.

Method I



Method II





Part - 5 : Biological screening:

All the 17 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*. 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*.

Anthelmintic screening : The compounds were subjected to *in vivo* anthelmintic testing against *Hymenolepis nana* in albino mice. The method used was that of Standen *et al*. The standard drug used was Tetramisole.

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- 2) El -Khawass, S.M., Khalil, M.A., Hazza, A.A.B., Bassiony, H.A., Lontfy, N.F.; (Fac. Pharm.Univ. Alexandria Egypt) **Farmaco**, **44(7-8)** , 703-9 (Eng.) (1989)
- 3) Shi, Hai- Jian, Shi Hao -Xin, Wang. Zhang-Yi; (Dept. of Chemistry, Anhui Normal University, Wuhu, Peop. Rep. China 241000) **Youji Hauxue**, **19(2)**, 195-199(Ch), Kexue Chubanshe (1999) cited from **C.A.**, **131**, 44776k (1999).
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