

SYNTHESIS OF HETEROCYCLIC COMPOUNDS AS  
POSSIBLE ANTIBACTERIAL ANTIFUNGAL AND  
ANTITUBERCULAR AGENTS

THESIS SUBMITTED  
UNIVERSITY OF BOMBAY

FOR THE DEGREE OF  
MASTER OF PHARMACEUTICAL SCIENCES  
IN  
PHARMACEUTICAL CHEMISTRY

SUBMITTED BY  
PALLAVI S. KHARKAR,

UNDER THE GUIDANCE  
DR. B. G. KHADSE

HAFFKINE INSTITUTE FOR TRAINING, RESEARCH & TESTING  
BOMBAY

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FOR THE DEGREE OF  
MASTER OF PHARMACEUTICAL SCIENCES  
IN THE  
FACULTY OF TECHNOLOGY  
(PHARMACEUTICAL CHEMISTRY)

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1998

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Haffkine Institute  
Mumbai - 400 012.

..... Pallavi S. Kharkar

## 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 :

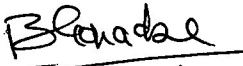
The work embodied in the Thesis to be submitted is original, based on the discovery of new facts, viz. the synthesis and characterization of new and unknown compounds of possible chemotherapeutic value. Also included in the Thesis, are new relation of facts, observed on the basis of in vitro testing.

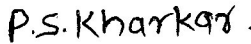
The assessment of the compounds has been made at the Department of Microbiology and Department of Chemotherapy, Haffkine Institute, Mumbai 400 -012.

### 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**  
Signature of Research Guide

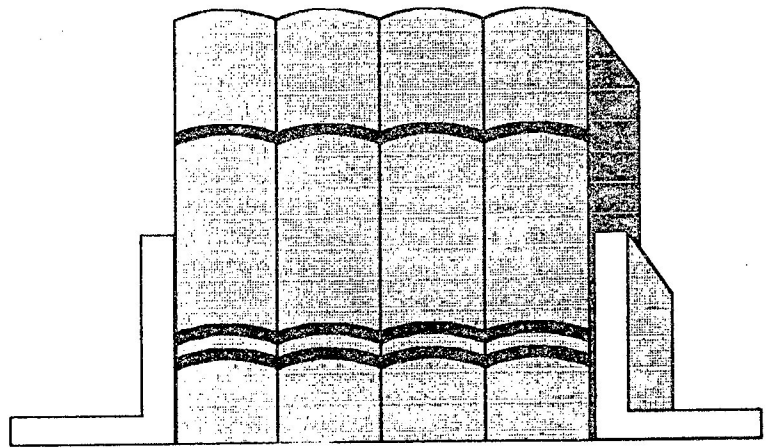
  
**Pallavi S. Kharkar**  
Signature of the Research Student

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*INTRODUCTION  
AND  
REVIEW OF LITERATURE*





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

Thus wrote Alfred Burger, one of the great figures of medicinal chemistry in the opening chapter of Comprehensive Medicinal Chemistry.

Medicinal Chemistry is the study of those chemicals which have potential beneficial effects on disordered living system. Chemists play a key role in pharmaceutical research, they are required to synthesize, purify and analyze novel compounds for biological testing. The real difficulty is in deciding what to make which fundamental molecular framework will have the desired biological action? What are the optimum positions for substituents on the basic skeleton to maximize activity?

The process of drug design comprises two important and distinct processes.

- 1) Discovery of new molecule as a lead.
- 2) Optimization process i.e. structural modification of lead till desired pharmacological profile has been obtained.

To identify the lead molecule, several approaches can be undertaken.

**1) Random screening :**

All compounds irrespective of their structures are tested in the bioassay.

Today, this is used to a lesser degree but still it is very important in order to discover drugs or lead of unexpected and unusual structures for various targets.

**2) Non random screening :**

Compound uncovered in random screen or compounds containing different functional groups than leads may be tested selectively.

**3) Drug metabolism studies :**

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

**4) Clinical observation :**

When a drug candidate exhibits more than one pharmacological activity i.e. side effect then the compound can be used as a lead for secondary activity. None of the above approaches to lead discovery involves a major rational component. Rational approach includes identification of the cause for the disease state.

e.g. At Merck, serotonin was believed to be possible mediator of inflammation and hence was chosen as a lead from which drug indomethacin was developed.<sup>2</sup>

**Lead Modification :**

Only small part of the lead compound may be involved in the interaction with receptors. The relevant groups on a molecule that interact with a receptor and are responsible for the activity are collectively known as pharmacophore. The additional group on lead may be not essential or superfluous and the approach is to cut away these groups.

**Functional Group Modification :**

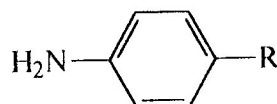
Discovery of tolbutamide antidiabetic from carbutamide possessing antidiabetic and antibacterial action.

**Structure Activity Relationship :**

Structurally specific drugs act at specific sites. Their activity and potency are very susceptible to small changes in chemical structure.

Molecules with similar biological activities tend to have common structural features.

It was found that compounds of the following general structure (I) exhibited antimicrobial, diuretic and antidiabetic actions. Compounds with each type of activity eventually were shown to possess certain structural features in common.



(I)

### Structure Modification :

Number of structural modification methodologies are as follows.

- 1) Homologation
- 2) Chain branching
- 3) Ring chain transformation
- 4) Bioisosterism

### Quantitative Structure Activity Relationship :

The concept of quantitative drug design is based on the fact that biological properties of a compound are a function of its physicochemical parameters, that is, physical properties, such as solubility, lipophilicity, electronic effect, ionization and stereochemistry, that have a profound influence on the chemistry of compound.

Different methods used to correlate physicochemical parameters with biological activity include -

- 1) Hansch Analysis or Linear Multiple Regression Analysis.
- 2) Free Wilson or de Novomethod.
- 3) Topliss orientation scheme.
- 4) Enhancement factor.
- 5) Batch selection method. Batchwise Topliss operation scheme, cluster analysis and others.

### Computer based methods of QSAR related to receptor binding :

Several computer based methods that have been used to correlate molecular structure with receptor binding and therefore activity.

Free energy of binding of compound with receptor is determined experientially. Distance between various atoms in a molecule, its conformation and rotation about single bond causing change in conformation and therefore in distances etc. have been studied. Possible binding sites, in terms of geometry and chemical character of site are deduced by computerized method with the help of above studies.

### **Molecular Graphics :**

It was realized that drug design could be aided if structures of receptors and drugs could be displayed on a terminal and molecular processes could be visualized. The three dimensional computer Graphics representation allows operator to visualize the interactions of small molecules with biologically important macromolecules. Better the complementary fit of the drug to the receptor, more potent the drug will be. This is lock and key hypothesis in which the receptor is the lock into which the key ( i.e. the drug ) fits.

Here pharmacokinetics i.e. absorption, transport, degradation, excretion etc. are ignored not involved. Calculations are carried out in absense of solvent lowest energy conformer is determined but molecule during binding process need not be in ground state. These are two major problems of the method.

Thus Medicinal Chemistry covers three critical steps<sup>3</sup> -

- 1) Lead identification.
- 2) Lead modification to produce drug.
- 3) Development step where there is optimization of the synthetic route for bulk production.

Drugs acting directly on the casual agent of disease are called aetiological drugs and represent true medicament. Presently most of them belong to the class of chemotherapeutic agents. i.e. to compounds used to treat infections diseases which include tuberculosis, bacterial and fungal infections and parasitic diseases.

The choice and subsequent management of antimicrobial chemotherapy is governed by clinical, microbial and pharmacological considerations.

**Clinical :**

- 1) Does the patient have an infection?
- 2) If so, will it benefit from specific antimicrobial therapy?
- 3) If it will, how urgent is treatment?
- 4) Are there any focal features?

**Microbiological :**

- 5) What pathogen(s) are likely?
- 6) Are there any epidemiological clues?

**Pharmacological :**

- 7) What spectrum dose and dosing frequencies are required?
- 8) Will the site of infection be reached?
- 9) Does anything about the patient limit the choice of agent?
- 10) Does treatment need monitoring?
- 11) What duration of treatment is required?

**Tuberculosis :**

Tuberculosis is one of the most important widespread infections known to man, an estimated 1.7 billion people harbour the pathogen, Mycobacterium tuberculosis and every year 8 million individuals develop new clinical diseases.<sup>4</sup> It is the infectious disease with highest world wide death rate. It is estimated that third to half of the world's population is infected with M. tuberculosis with tuberculosis causing 6 % of all death worldwide.<sup>5,6</sup> In 1991, center for Disease control reported 25,701 cases of T.B.<sup>7</sup> Today, the press and professional publications announce the "epidemic"<sup>7</sup> spread of T.B.

Infection with Human Immunodeficiency Virus (H.I.V.) is the most potent risk factor for development of tuberculosis. The treatment of mycobacterial infections has become more important and challenging problem because of the emergence of multiple drug resistant organism.

Because the micro-organisms grow slowly and the diseases often are chronic, development of microbial resistance, drug toxicity and patient compliance present special therapeutic problems. Prior to 1984, only 10 % of the organisms isolated from the T.B. patients were resistant to any drug. In 1984, 52 % of the organism were resistant to at least one drug and 32% were resistant to more than one drug.<sup>7</sup>

Tuberculosis has been described as 'King of Diseases' in the Vedas and was mentioned by Charaka and Sushruta about 600 B.C.<sup>8</sup> With the discovery of tubercle bacillus, the causative organism by Robert Koch in 1892, the disease became known as Tuberculosis.

M. tuberculosis is transmitted primarily via the respiratory route. The organism appears in water droplets expelled during coughing, sneezing or talking.

Tuberculosis mainly affects lungs (80-85% of the cases), but M. tuberculosis can spread through the blood stream and the lymphatic system to brain, bones, eyes and skin (extrapulmonary T.B).<sup>7</sup>

In Pulmonary tuberculosis, primary infection follows airborne transmission from a case of pulmonary tuberculosis. Given an adequate host defenses, exposure results in formation of primary complex. The thick lipid containing cell wall of mycobacteria renders the organism resistant to phagocytosis. The inhaled bacteria are then walled off by fibrosis to form a granuloma with central caseating necrosis. This necrosis allows the bacilli to remain in a dormant condition by sealing off the communication of the necrotic area with the communicating channel like bronchus and ureter. When the communication opens, the solid caseous material is autolyzed and the liquid contents are expelled, resulting in the development of cavity with a caseous lining when the dormant bacilli get an access to the atmospheric oxygen, they multiply rapidly, producing spread of an infection and an increase in the size of the cavity.

Extrapulmonary TB is much more common in HIV infected patients (40 to 75%).<sup>9</sup> Approximately half of the all AIDS patients develop an infection caused by M. avium and M. intracellulare. These organisms are difficult to distinguish and are considered a complex, thus the acronym MAC.<sup>9</sup> In AIDS patient, infection may involve bone marrow, lymph nodes, liver and blood.

### **Drug therapy for tuberculosis :**

Before the advent of chemotherapy during the 1940s treatment of tuberculosis involved,<sup>10</sup>

- 1) Increase in the patient's resistance to the disease.
- 2) Prolonged rests in hospitals and sanatoria.
- 3) Special diets and avoidance of physical activity also.
- 4) Immobilization of lung tissue by artificial pneumotherapy or removal of affected lung tissue.
- 5) Amputation of affected limbs.

The first chemotherapeutic agent used against tuberculosis was sulphonamides in 1940 in guinea pigs infected with tubercle bacilli which was found to have little if any activity against human tuberculosis.<sup>10</sup> The main landmarks in the treatment of tuberculosis are as follows.

| Year | Treatment                                |
|------|------------------------------------------|
| 1944 | Streptomycin                             |
| 1949 | Combination therapy streptomycin and PAS |
| 1952 | Isoniazid                                |
| 1956 | Ambulatory outpatient treatment          |
| 1964 | Intermittent regimens, fully supervised  |
| 1967 | Rifampicin                               |
| 1972 | Short course chemotherapy                |

The drug therapy divides these agents into

- 1) First line agents :

Which are normally used in the treatment.

Isoniazid, Rifampin, Pyrazinamide, Ethambutol, Streptomycin.

2) Second line agents :

Although these agents are active antibacterials, they usually are less well tolerated or have high incidence of adverse effects. So they are used in cases of high resistance, retreatment or intolerance to first line drugs.

Isoniazid (INH) :

This is considered as a primary drug for treatment of M tuberculosis. Its action is bactericidal against replicating organisms but appears to be only bacteriostatic against nonreplicating organism.<sup>9</sup>

Mode of action :

Though extensively investigated remains unknown. According to one theory, INH(4) is converted to isonicotinic acid (III) which by acting as an antimetabolite of nicotinic acid (II), gets incorporated into  $\text{NAD}^+$  in place of nicotinic acid. This fraudulent  $\text{NAD}^+$  is not able to catalyze the normal oxidation/reduction reaction. Secondly it is said to be inhibitor of the conversion of  $\text{C}_{24}$  and  $\text{C}_{26}$  saturated fatty acids to  $\text{C}_{24}$  and  $\text{C}_{26}$  unsaturated acids by blocking desaturase. These acids appear to serve as precursors to mycolic acid, a key component in bacterial cell wall.

Isoniazid is rapidly absorbed following oral administration. Food and antacid containing aluminium may interfere or delay absorption. So it is recommended that the drug be taken on an empty stomach. Isoniazid is mainly metabolized by acetylation.

Adverse reactions :

Hepatitis is an uncommon (0.1 %) but potentially serious reaction that can be averted by prompt withdrawal of treatment.<sup>11</sup>

Side effects are frequently seen in slow acetylators like insomnia, restlessness and twitching.





Major side effects involve influenza like symptoms, skin reactions, GI disturbances. Patients should be warned that it causes an orange discolouration of urine and other body secretions.

### Pyrazinamide :

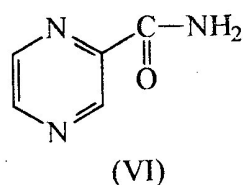
Pyrazinecarboxamide was discovered during investigations of analogs of nicotinamide. It is bioester of nicotinamide possessing bactericidal against M. tuberculosis.

Pyrazinamide(VI) has a special sterilizing effect on intracellular tubercle bacilli in acid conditions and has therefore found application in short course chemotherapy. Compound shows good activity at pH 5.5 but is nearly inactive at neutral pH.

Susceptible organisms produce deaminidase, which is responsible for conversion to pyrazinoic acid. Resistant strains do not produce this enzyme and thereby acid. This suggests that acid is an active form and that this acid may lower the pH of the immediate surrounding of M. tuberculosis to an extent that organism is unable to grow.

Pyrazinamide is readily absorbed when given orally. Major metabolite is pyrazinoic acid which may be oxidized to 5-hydroxy pyrazinoic acid.

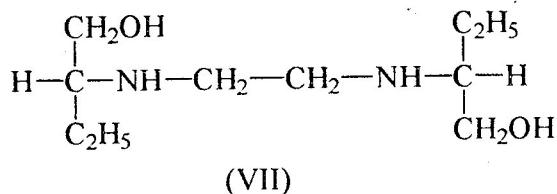
Adverse reactions are rare, but hepatic toxicity has been a problem. Hypersensitivity and photosensitivity of the skin also occur. Treatment for longer than two months is not recommended.<sup>11</sup> The dose should be reduced in renal impairment and avoided in patients with severe liver damage and gout.



### Ethambutol :

Owing to the presence of two asymmetric centers in the molecule, a (-) form, a (+) form, and a mesoform of above structure can occur in addition to the (+) isomer. Study of all these forms revealed that (+) form is the most active. The generic name of this isomer is Ethambutol (VII).

Ethambutol is utilized in its d-isomer, which is 200-500 times more active than l isomer. This difference in activity suggests a specific receptor site.



In combination with other drugs, it is active against M. tuberculosis and many of the atypical mycobacteria. It has bacteriostatic action on proliferating bacteria.

There is some evidence suggesting that ethambutol may interfere with cell wall synthesis in M. tuberculosis. Ethambutol is readily absorbed (75-80 %) following oral administration.

Ethambutol can give rise to dose-dependent optic neuritis. This can result in impairment of visual activity and colour vision. Early changes are reversible, but blindness can occur if treatment is not discontinued promptly.<sup>11</sup>

### Streptomycin :

Streptomycin was isolated by Waksman and coworkers and represented the first biologically active aminoglycoside. This was the first effective drug against tuberculosis in humans. It is more actively bactericidal in the proliferative than in the resting phase of bacterial growth.

Streptomycin inhibits protein synthesis, but additional effects on misreading of a m-RNA template and membrane damage may contribute to the bactericidal action of streptomycin. Binding of streptomycin to the 30S ribosomal subunit appears to be the primary site of action. As a result of this binding, disruption of normal protein synthesis occurs, leading to inhibition of protein synthesis.

Unlike all aminoglycosides, it is not absorbed when given orally and must be administered as an intramuscular injection. The injections are painful and must be administered by deep intramuscular route, 1 per day is sufficient but barely tolerable. Duration of treatment is 1-2 months.

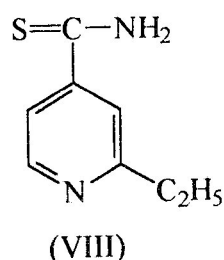
**Ethionamide :**

The discovery of Ethionamide (VIII) is a result of synthesis of nicotinamide analogs. This compound is bactericidal against M. tuberculosis and M. leprae.

Ethionamide inhibits protein synthesis by blocking the incorporation of cysteine and methionine into proteins.

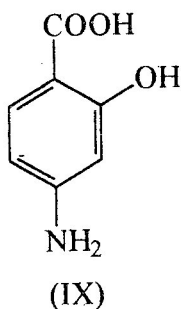
It is orally active but large dose (>500mg) is not well tolerated. One of the metabolite is ethionamide sulfoxide.

Side effects include GI irritation, hepatitis, CNS effects, hypersensitivities.

**Para Amino Salicylic Acid (PAS) :**

This was formerly used in combination regimens to prevent drug resistant organisms. The PAS (IX) is thought to interfere with p- amino benzoic acid incorporation into folic acid.

Upto 10-12 g a day was given orally, in two or three doses. The drug is unpleasant in taste. GI intolerance was common. PAS is no longer used and is included for historic reasons only.

**Cycloserine, Viomycin, Kanamycin and Capreomycin :**

These are weak antitubercular drugs used only in three drug regimens for the treatment of tuberculosis resistant to the major antituberculous drugs. All four need to be given by injection.

| Drug                        | Initial phase<br>(2 months) | Continuation phase<br>(4 months) |
|-----------------------------|-----------------------------|----------------------------------|
| <b>Std. Regimen</b>         |                             |                                  |
| Rifampicin                  | 10mg /kg/d (450-600mg/d)    | 10mg /kg/d (450-600mg /d)        |
| Isoniazid                   | 5mg /kg/d (200-300 mg/d)    | 5mg /kg /d (200-300mg /d)        |
| Pyrazinamide                | 30mg /kg/d (1.5gm-2gm/d)    |                                  |
| Ethambutol*                 | 15-25mg /kg/d               |                                  |
| <b>Intermittent Regimen</b> |                             |                                  |
| Rifampicin                  | 10mg /kg (3 times weekly)   | 10mg /kg (3 times weekly)        |
| Isoniazid                   | 15mg /kg (3 times weekly)   | 15mg /kg (3 times weekly)        |
| Pyrazinamide                | 50mg /kg (3 times weekly)   | 50mg /kg (3 times weekly)        |

\* or Streptomycin ( 15 mg /kg/d or 0.75 -1 g/d for 2 months).

**Antibacterial Chemotherapy :**

The essence of antibacterial chemotherapy is selective toxicity to kill or inhibit the bacillus without harming the patient.

Synthetic antibacterials include two major classes :

1) Topical agents, which include disinfectants, antiseptics and preservatives. Antiseptics and disinfectants differ from systemic agents in that they show little selective toxicity between the microbes and the host. Most of these agents do not aid wound healing.

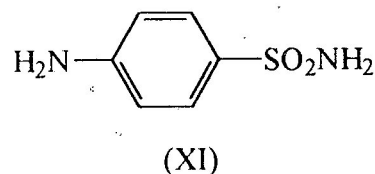
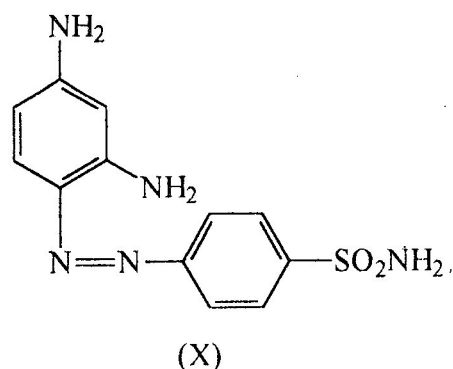
2) Systemic antibacterials, which include :

- 1) Sulphonamides
- 2) Antimycobacterial agents
- 3) Quinolones
- 4) Nitrofurans
- 5) Methanamine
- 6) Cotrimoxazole

**Sulphonamides :**

Domagk<sup>13</sup> observed in 1932 that Prontosil protected mice against streptococcal infections and rabbits against staphylococcal infections.

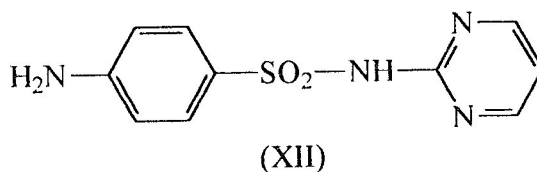
Fuller<sup>14</sup> demonstrated the presence of sulfanilamide in blood and urine of patients under treatment with Prontosil (X) and firmly established the reduction of Pronosil in the body to form sulfanilamide (XI) and thus concluded that therapeutic action of Prontosil was very likely due to its reduction *in vivo* to sulfanilamide.



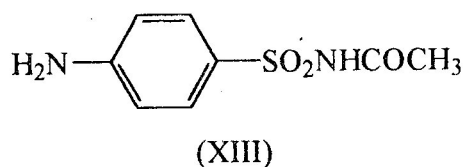
Sulphonamides are broad spectrum, predominantly bacteriostatic and relatively slow to act. Sulphonamide, an analogue of para aminobenzoic acid (PABA), is essential for bacterial folate synthesis. Most bacteria synthesize folic acid and cannot take it up preformed from the environment. Sulphonamides block an early stage in folate synthesis: The condensation of PABA with dihydropteridine to form dihydropteroic acid. Depletion of folate leads to failure to synthesize purine nucleotides and thymidine.

Resistance emerges readily, and bacteria resistant to one sulfonamide are also resistant to the others. The emergence of resistant strains and the appearance of more potent anti- microbial agents has relegated the sulphonamide group to a minor therapeutic role, chiefly as agents for use in uncomplicated urinary infections.

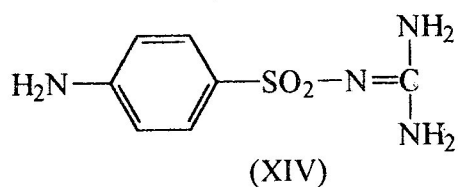
Sulphadiazine (XII) has retained preeminence because of its broad spectrum antibacterial activity, low toxicity, high tissue penetration and use for protection of close contacts of cases of meningococcal meningitis, provided the strain of Neisseria meningitis involved is sulfonamide sensitive.<sup>15</sup>



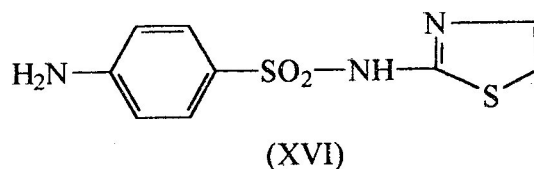
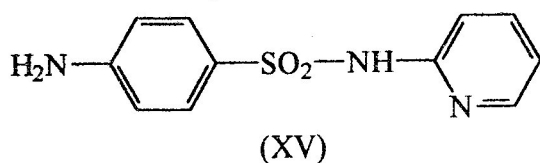
Sulfacetamide(XIII) is widely used in ophthalmic practice.



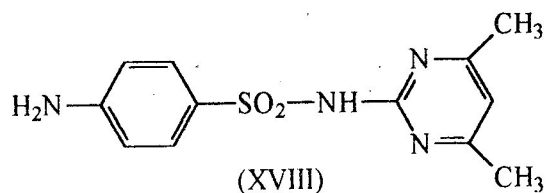
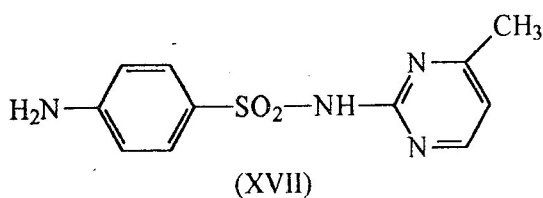
Sulfaguanidine(XIV) was extensively used for intestinal infections. Now it has been withdrawn in many countries<sup>16</sup> due to poor absorption and toxicity problems.



Sulfapyridine<sup>17</sup> (XV), reported in 1938, was in use for the treatment of pneumonia and remained the drug of choice until it was replaced by sulfathiazole<sup>16</sup>(XVI).



Two methylated derivatives of sulfadiazine (XII), namely sulfamerazine (XVII) and sulfamethazine (XVIII), were soon introduced in therapeutics.<sup>18-22</sup> Sulfamethazine, though less active than the other two, because of its greater tolerance and higher solubility, possesses advantage over the other two.



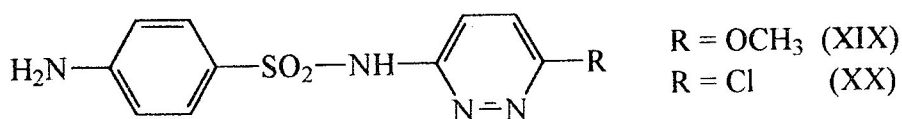
Sulfadiazine and sulfamerazine had a long half life (17 and 24 hr. resp.) and required less frequent administration than was prescribed.



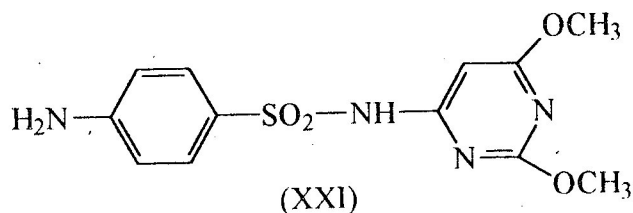
Clinically used sulfas have five-membered heterocycles; thiazoles, oxazoles, isoxazole, pyrazole are amongst more active molecules. Sulfathiazole<sup>23</sup> (XVI) was the first member of this group.

The era of new long-acting sulfonamides started in 1956 with the introduction of sulfamethoxypyridazine<sup>24</sup> (XIX), having half life of around 37 hours, which was needed to be administered only once a day.

Sulfachlorpyridazine<sup>25</sup> (XX) has half life of about 7 hours and is useful for urinary tract infections.

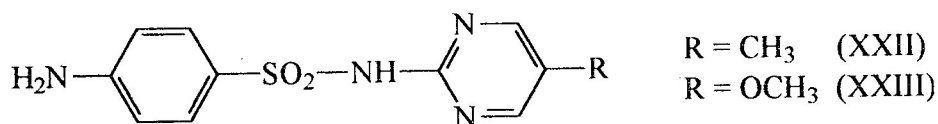


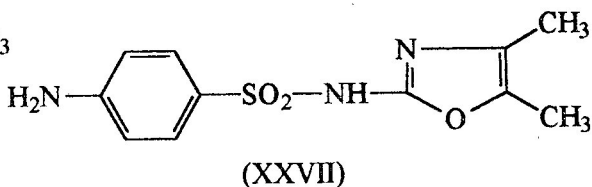
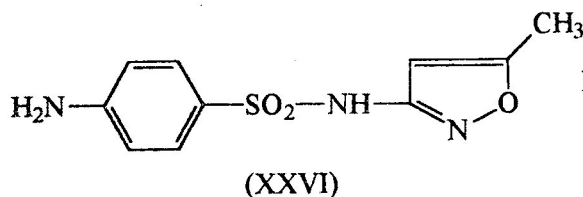
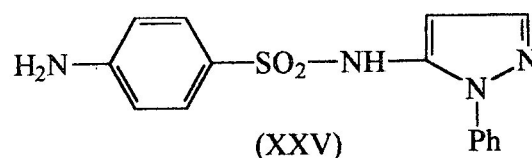
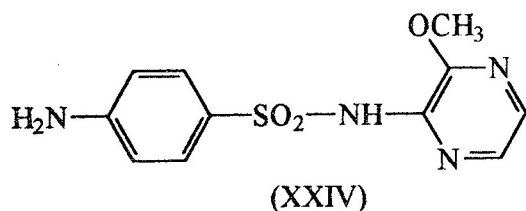
In 1959, sulfadimethoxine (XXI) was introduced. Sulphomethoxine<sup>26,27</sup> has the longest half life, i.e. about 150 hours, and needs administration once a week.



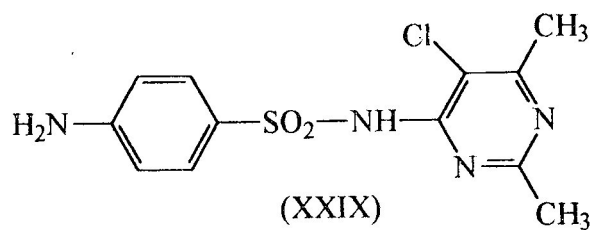
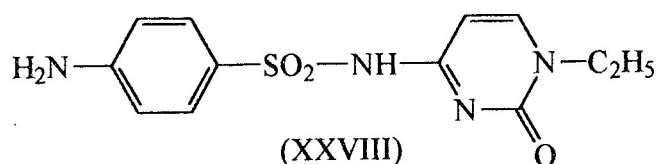
Other long acting sulfonamides include -

Sulfamethyldiazine(XXII), Sulfamethoxydiazine(XXIII), sulfamethoxypyrazine(XXIV), sulfaphenazole(XXV), sulfamethoxazole(XXVI) and sulfamoxol (XXVII).





N-sulfanilyl-1 ethylcytosine<sup>28</sup> (XXVIII), reported in 1970, is 3-10 times more potent than sulfisoxazole, sulfachlorpyridazine (XX) and sulfisoimidine. Sulfacloamide (XXIX) also possessed a broad spectrum of activity.

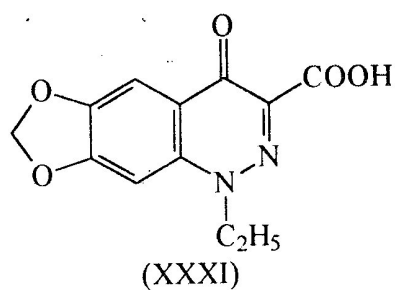
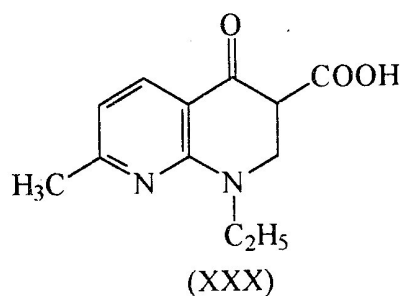


### Quinolones :

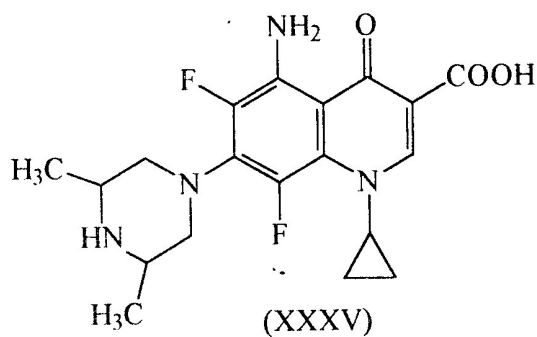
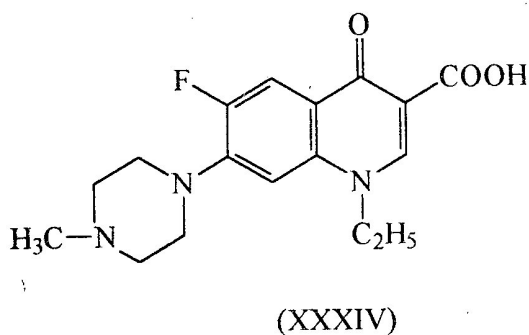
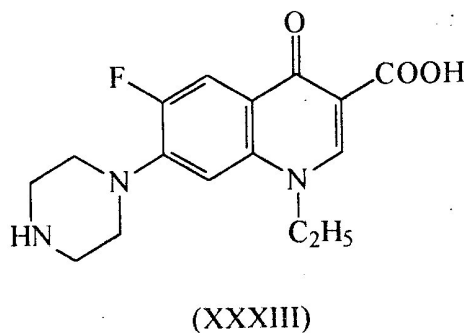
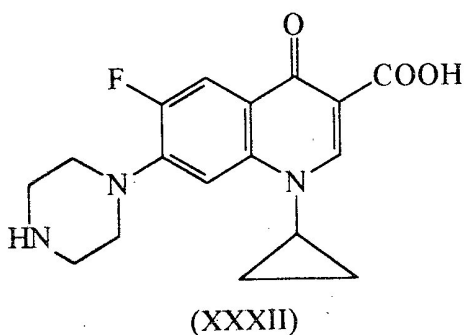
4- quinolone compounds have a number of advantages over other antibacterials.

They are effective against many organisms, are well-absorbed orally, are well-distributed in tissues, and have relatively long serum half-lives.

Nalidixic acid (XXX) was the first representative of this family. Early members of the group, developed in 1960s and 1970s include Cinoxacin (XXXI), Oxolinic acid, Pipemidic acid, Acrosoxacin<sup>29</sup> and several others.

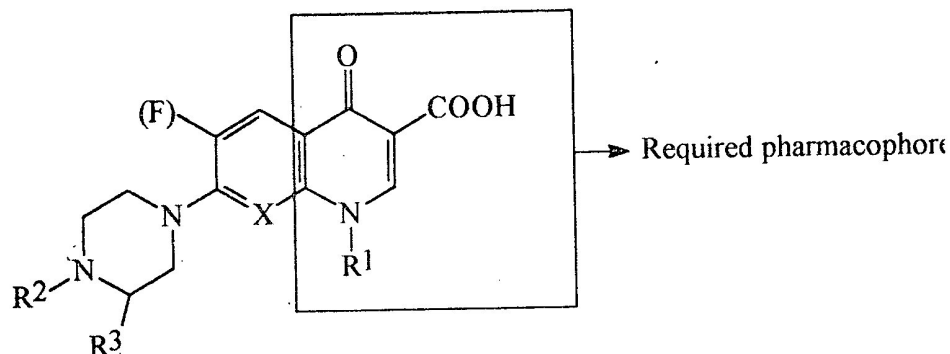


Chemical modification of structure led to thousands of new derivatives and include Ciprofloxacin (XXXII), Norfloxacin (XXXIII), Pefloxacin (XXXIV) and Sparfloxacin (XXXV).



The site of action of these agents is subunit of DNA gyrase enzyme, which unwinds the supercoiled DNA helix prior to replication and transcription.

The minimum pharmacophore consists of the 4-pyridone ring with 3-carboxylic acid group.<sup>30</sup>



#### Other features<sup>30</sup> :

- 1) Fluorine group present in most of the quinolones increases lipophilicity and facilitates penetration into tissues and cells.
- 2) Piperazine group broadens the spectrum by including Pseudomonas aeruginosa but also increases CNS side-effects. This can be reduced by adding methyl ethyl group to piperazine ring.
- 3) Isosteric replacement of 'N' provides (XXXI), having good antibacterial action.

#### **Antibacterial action :**

Most Gram-negative organisms, with the exception of Pseudomonas aeruginosa, are susceptible to Nalidixic acid and its early congeners, but Gram-positive organisms are usually resistant. These drugs are normally used in only urinary infection.

Fluoroquinolones exhibit greatly enhanced activity against enterobacteria and expand the spectrum of earlier quinolones to include Gram-positive cocci and Pseudomonas aeruginosa.

#### Absorption :

Nalidixic acid congeners are all well-absorbed orally. Major metabolite is hydroxy nalidixic acid.

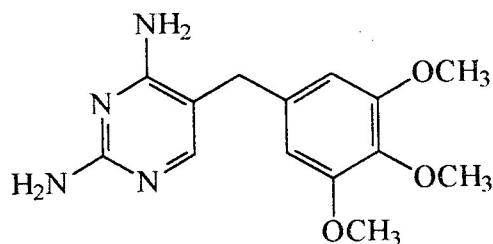
Fluoroquinolones are administered by oral route. Ciprofloxacin and Ofloxacin can also be given intravenously.

The antibacterial spectrum and ease of administration of fluoroquinolones make them attractive candidates for 'blind' therapy in hospitals and domiciliary practice. Fluoroquinolones are well-tolerated but rashes, GI disturbances, photophobia etc. may occur.

These agents cannot be recommended for respiratory tract infections since the antipneumococcal activity is unreliable.<sup>29</sup> These compounds also affect the deposition of cartilage in experimental animals and licensing authorities have cautioned against their use in children and pregnant women.<sup>29</sup>

Cotrimoxazole: Inhibitors of the enzyme dihydrofolate reductase prevent formation of important tetrahydrofolates which results in decreased nucleotide synthesis and cell death. There are species differences in dihydrofolate reductase and these differences have been used to discover compounds that are lethal to bacteria but relatively innocuous to mammals. The selective inhibitor of bacterial dihydrofolate reductase used clinically is Trimethoprim (XXXVI), i.e. 5(3,4,5-trimethoxybenzyl)-2,4-diaminopyrimidine.

It has broad spectrum of activity against gram-positive and gram-negative bacteria, but Pseudomonas aeruginosa and certain strains of E. coli are resistant.



(XXXVI)

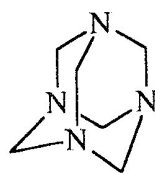
Trimethoprim, in combination with sulfamethoxazole, a sulfonamide with similar half life, is known as co-trimoxazole. It has a synergistic effect that prevents the development of bacterial resistance<sup>31</sup> and is used mainly in urinary tract infections. It is effective against gonorrhoea, chest infection caused by pneumococci and Haemophilis influenza and enteric salmonella infections.

Side effects include megaloblastosis, leukopenia, thrombocytopenia.<sup>32</sup>

**Methanamine :**

The free base has no antibacterial action but acidification results in liberation of formaldehyde which is strongly bactericidal.

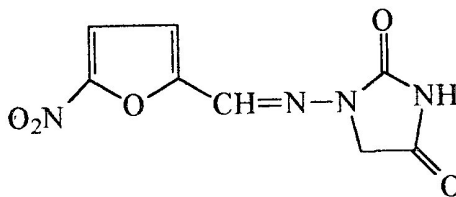
Methanamine (XXXVII) is used sometimes in long term suppression of bacterial urinary tract infections .



(XXXVII)

**Nitrofurans :**

A number of nitrofurantoin derivatives have attracted attention over the years. But only one, Nitrofurantoin (XXXVIII), has found widespread use and is restricted to treatment of urinary infections.



(XXXVIII)

Nitrofurantoin is active against Gram-positive and Gram-negative bacteria at conc. of 5-10 mg/ml. It is active against most urinary tract pathogens, but Proteus spp and Pseudomonas aeruginosa are usually resistant . The activity is affected by pH, acid conditions favouring the activity.

Other agents whose use is approved for human use include Nitrofurazone<sup>33</sup> (XXXIX) and Furazolidone<sup>34</sup> (XXXX).

Nitrofurazone has a broad spectrum of antibacterial action in the range of 1: 100000 to 1 : 20000, but is not effective against Pseudomonas aeruginosa or fungi. It is used topically in the treatment of burns and in prevention of bacterial infection in skin graft procedures.

Furazolidone has broad spectrum of bactericidal activity against intestinal pathogens like various species of Salmonella, Shigella, Proteus and Enterobacter, E. coli and Vibrio cholerae. It is useful for treatment of bacterial or protozoal diarrhoea.

It has been suggested that nucleic acids, especially t-RNA are the primary targets in nitrofurantoin mutagenesis and carcinogenesis.

### Nitroimidazoles :

They have exhibited variety of antimicrobial spectra. Metronidazole was originally used for the treatment of protozoal infections, first for trichomoniasis then later for amoebiasis and giardiasis.

Metronidazole is so effective against anaerobic bacteria and resistance is so uncommon that it is now widely considered the drug of choice for the treatment of anaerobic infections.<sup>35</sup>

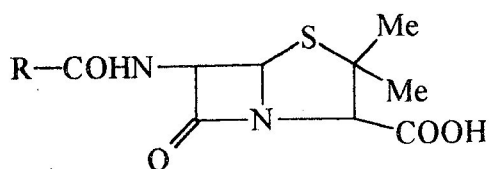
### Use :

It is used as prophylactic drug in some surgical procedures in which post operative anaerobic infection is a frequent complication and as an alternative to Vancomycin in the treatment of antibiotic associated colitis.<sup>35</sup> The basis of selective activity against anaerobes resides in the fact that the antibacterially active form of the drug is a reduced derivative produced intracellularly at the low redox values attainable by anaerobes but not by aerobes. This reduced form of Metronidazole is thought to act by inducing strand breakage in DNA by a mechanism that has not been precisely determined.<sup>35</sup>

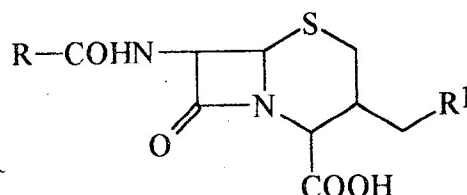
### $\beta$ -Lactam Antibiotics :

With the advent of the antibiotics, the use of sulfonamide drugs began to decline in a measure, but the usefulness of both types of agents was extended by combination therapy. Among the most frequently used preparation of this nature now available to the clinician are formulations combining crystalline benzylpenicillin, erythromycin, chloramphenicol or the tetracycline antibiotics with 'triple sulfonamides'.

The  $\beta$ -lactam antibiotics, Penicillins (XXXXI) and subsequently Cephalosporine (XXXXII) family of antibiotics, marked the beginning of an era that has since produced a wide range of  $\beta$ -lactam structural types.

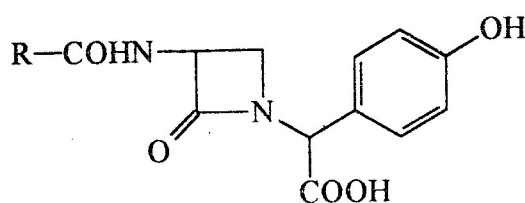


(XXXXI)

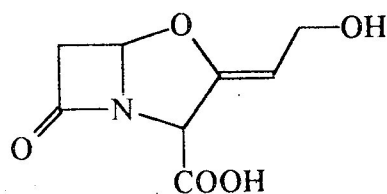


(XXXXII)

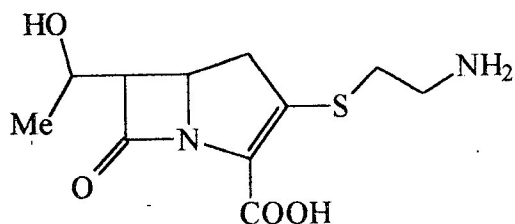
In 1970, the isolation of the  $\beta$ -lactamase stable cephamycins was quickly followed by the discovery from natural sources of a variety of new  $\beta$ -lactams, structurally distinct from the penicillins and cephalosporins. These include Nocardicins<sup>36</sup> (XXXXIII), Clavulanic acid<sup>37</sup> (XXXXIV), and the Carbapenems such as Thienamycin<sup>38</sup> (XXXXV), and Olivanic acid<sup>39</sup> (XXXXVI) from various *Streptomyces* strains.



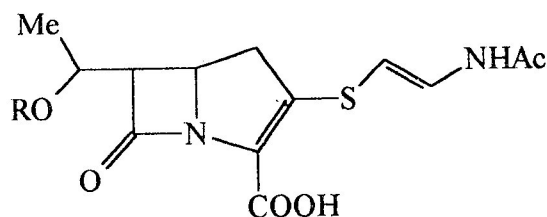
(XXXXIII)



(XXXXIV)

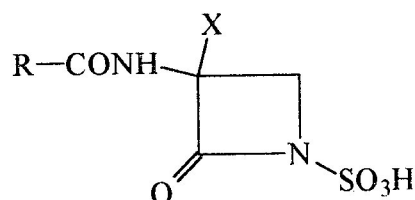


(XXXXV)



(XXXXVI)

Later, monobactams<sup>40,41</sup> (XXXXVII) were isolated from bacteria.



X = H, OMe

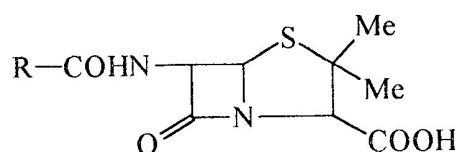


**Penicillins :**

The observation of partial lysis of staphylococcal colonies on plates contaminated with Penicillium notatum by Alexander Fleming was made in 1928.

But life saving capacity of Penicillin was first demonstrated by Florey, Chain, Abraham and their colleagues<sup>42</sup> at Oxford University in animals in 1940 and in humans the following year.<sup>43</sup>

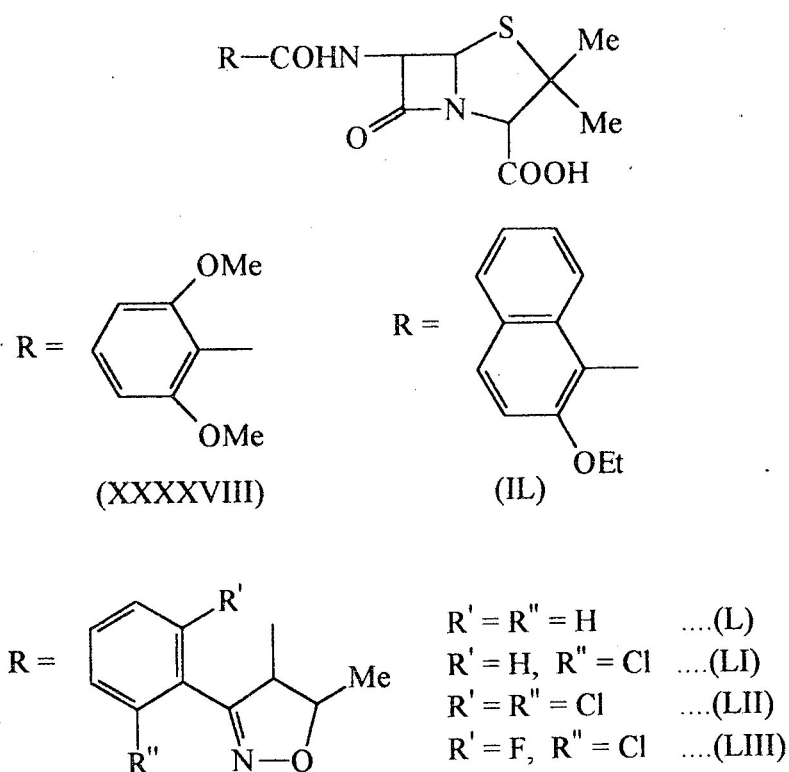
The surface cultures of low yielding Penicillium notatum were replaced by high yielding strain of Penicillium chrysogenum.

**Biosynthetic Penicillin :**

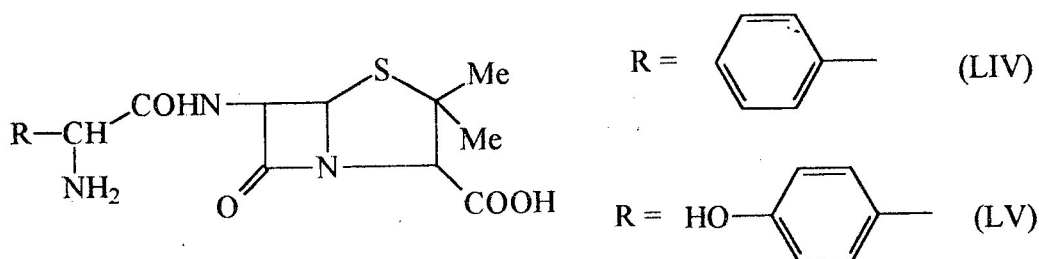
| Name                                  | R                                                           | US Name   |
|---------------------------------------|-------------------------------------------------------------|-----------|
| 2- Pentenyl penicillin                | CH <sub>3</sub> -CH <sub>2</sub> -CH=CH-CH <sub>2</sub> -   | F         |
| Pentylpenicillin                      | CH <sub>3</sub> -(CH <sub>2</sub> ) <sub>4</sub> -          | Dihydro F |
| Heptyl penicillin                     | CH <sub>3</sub> -(CH <sub>2</sub> ) <sub>5</sub> -          | K         |
| p-Aminobenzylpenicillin               | 4H <sub>2</sub> N-PhCH <sub>2</sub> -                       | T         |
| p-Nitrobenzyl penicillin              | 4O <sub>2</sub> NPhCH <sub>2</sub> -                        |           |
| p-Hydroxybenzyl penicillin            | 4HO-PhCH <sub>2</sub> -                                     | X         |
| Benzyl penicillin                     | Ph-CH <sub>2</sub> -                                        | G         |
| Phenoxymethyl penicillin              | Ph-O-CH <sub>2</sub> -                                      | V         |
| Allythiomethyl penicillin             | CH <sub>2</sub> =CH-CH <sub>2</sub> -S-CH <sub>2</sub> -    | O         |
| D-4- Amino- 4-carboxybutyl penicillin | D HOOC-CH(NH <sub>2</sub> )-(CH <sub>2</sub> ) <sub>3</sub> | N         |

The widespread use of Penicillin G had already resulted in an increase in the occurrence of penicillinase producing strains of Staphylococcus aureus. Much early research was therefore directed at identifying new penicillins resistant to inactivation by these penicillinases.

Methicillin<sup>44</sup> (XXXXVIII) was introduced into clinical practice in 1960 followed by Nafcillin<sup>45</sup> (XXXXIX) and then isoxazole penicillins Oxacillin<sup>46</sup> (L), Cloxacillin<sup>47</sup> (LI), Dicloxacillin<sup>48</sup> (LII) and Flucloxacillin<sup>49</sup> (LIII).

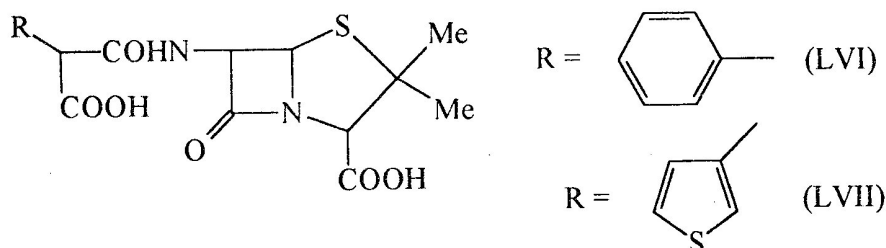


The aminopenicillins like Ampicillin<sup>50</sup> (LIV), Amoxycillin<sup>51</sup> (LV) and others were introduced into the clinic between 1961-1972. These have antibacterial activity against both Gram-positive and Gram-negative bacteria and have good oral absorption.



Prodrug esters like Talampicillin,<sup>52</sup> Bacampicillin,<sup>53</sup> Pivampicillin,<sup>54</sup> which release Ampicillin once absorbed, have been developed to achieve excellent levels of absorption of Ampicillin.

In order to extend the spectrum of action to cover the opportunistic pathogen Pseudomonas aeruginosa, agents like Carbenicillin<sup>55</sup> (LVI), Sulbénicillin,<sup>56</sup> Ticarcillin (LVII)<sup>57</sup> have been developed.



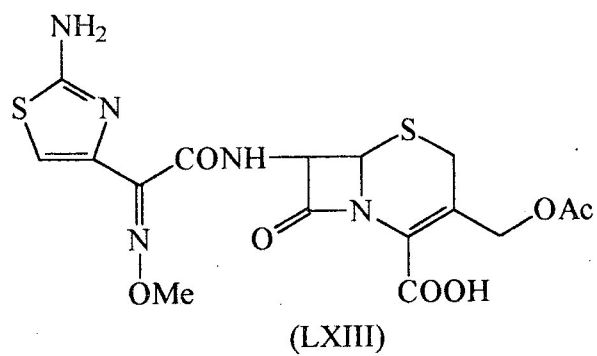
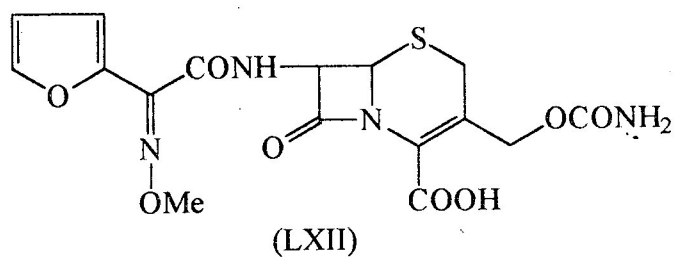
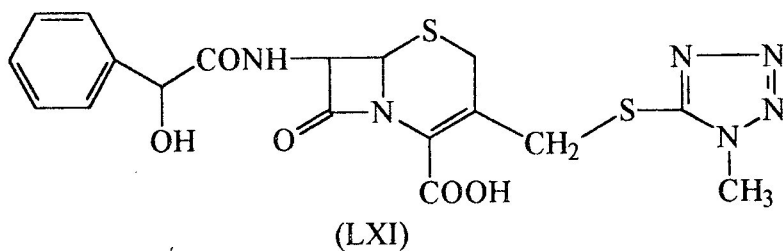
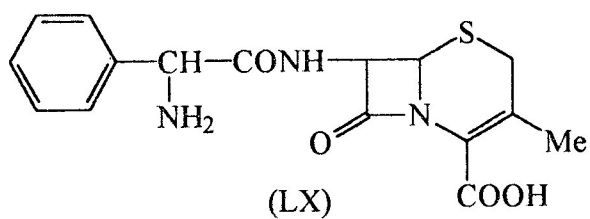
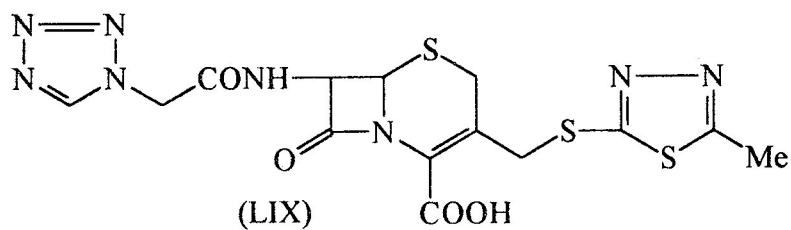
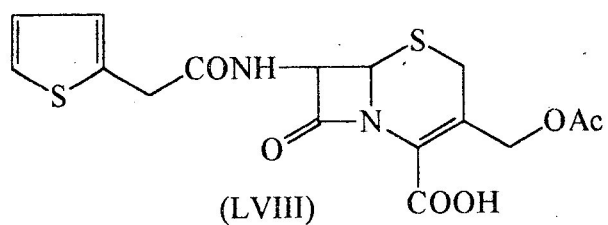
### Cephalosporins :

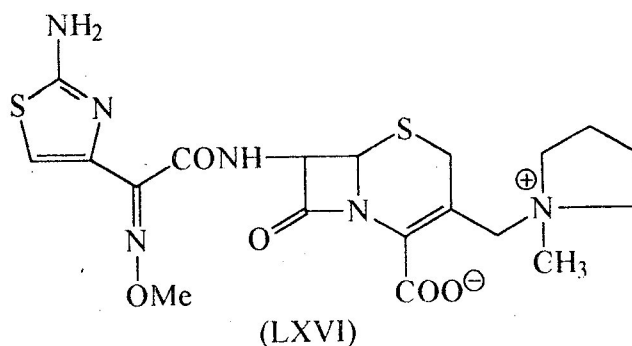
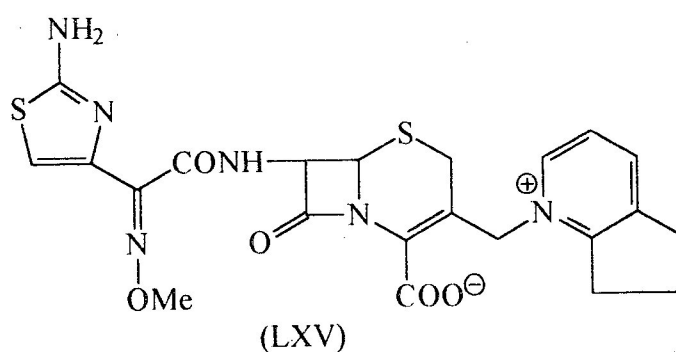
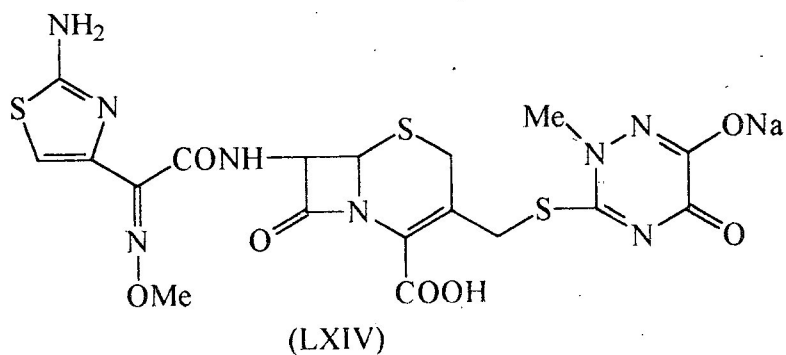
Here  $\beta$ -lactam is fused to a six membered dihydrothiazine ring. The antibacterial activity of Cephalosporium species was demonstrated by Brotzu in 1945. About fifty different Cephalosporins are in clinical use or at advanced stage of development. They are divided into “generations” depending on their antibacterial spectrum.

| Generation        | Agents                                                                                                    | Antibacterial activity                                                                                                                                         |
|-------------------|-----------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------|
| First generation  | Cephalothin <sup>58</sup> (LVIII)<br>Cefazoline <sup>59</sup> (LIX)<br>Cephalexin <sup>60</sup> (LX)      | Good activity against Gram-positive bacteria but narrow spectrum against Gram-negative strains.                                                                |
| Second generation | Cephmandole <sup>61</sup> (LXI)<br>Cephuroxime <sup>62</sup> (LXII)<br>Cefaclor <sup>63</sup>             | Broader Spectrum of action and enhanced stability to $\beta$ -lactamase & hence enhanced activity against <u>Haemophilus influenzae</u> and enterobacteriaceae |
| Third generation  | Cefotaxime <sup>64,65</sup> (LXIII)<br>Ceftriaxone <sup>64,66</sup> (LXIV)<br>Cefsulodin <sup>64,67</sup> | Good activity against Enterobacteriaceae, potent Gram negative activity and moderate Gram positive activity.                                                   |
| Fourth generation | Cefpirome <sup>68</sup> (LXV)<br>Cefepime <sup>69</sup> (LXVI)                                            | Still undergoing clinical development.                                                                                                                         |

Third generation Cephalosporins have increased resistance to many plasmid and chromosomally mediated  $\beta$ -lactamases.<sup>70</sup>

Compounds with prolonged half life, e.g. Ceftriaxone, make it suitable for once or twice daily administration.





#### Applications :

- 1) Benzylpenicillin still, after forty years, continues to be an important antibiotic.
- 2) Amoxycillin and Augmentin (amoxycillin + clavulanic acid) are widely used for respiratory tract infections, urinary tract infections and infections of skin and soft tissues.
- 3) Cefazolin is used for surgical wound prophylaxis.
- 4) First and second generation agents show good activity against Streptococci and Staphylococci.
- 5) Third generation compounds like Ceftazidime and Cefoperazone have expanded Gram-negative activity with some activity against Pseudomonas species.

### Aminoglycosides :

They contain amino sugars and /or aminocyclitol as principal components. The common features are multiple amino and hydroxyl groups that render strongly basic molecules and confer good water solubility. Streptomycin (described in details earlier) was the first aminoglycoside to be discovered. Other important members include Neomycin, Paramomycin, Lividomycin, Kanamycin, Tobramycin, Netilmycin etc.

Aminoglycoside inactivating enzymes are well-studied and are known to destroy antibiotic activity via N-acetylation, O-phosphorylation, O-adenylation.<sup>71,72</sup> Major clinical concern about them is their potential toxicity, principally nephrotoxicity and ototoxicity.

### Macrolide antibiotics :

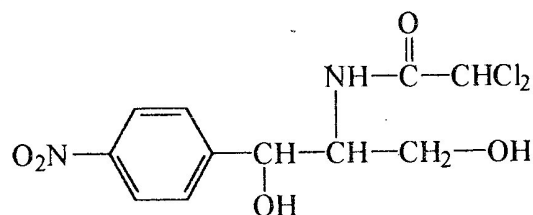
They contain macrocyclic, highly substituted lactone ring,<sup>73</sup> aglycone to which a variety of amino and neutral deoxy sugars are attached. Erythromycin is the most widely used macrolide antibiotic consisting of 14-membered aglycone.

It is used to treat infections of respiratory tract, skin and soft tissues and genital tract.

Modification of Erythromycin gave useful molecules such as Clarithromycin, Flurithromycin etc. Clarithromycin is effective against Mycobacterium avium-intracellulairiae complex.<sup>74,75</sup>

Erythromycin is orally absorbed and relatively safe. Its degradation in stomach is a major problem and is hence given in enteric coated form.

**Chloramphenicol:** Chloramphenicol (LXVII) is an antibiotic produced by Streptomyces venezualae, an organism first isolated in 1947 and was successfully used in epidemic typhus in Bolivia.



(LXVII)

By 1950, it became evident that the drug could cause serious and fatal blood dyscrasias. The drug is therefore reserved for patients with serious infections, such as meningitis, typhus and typhoid fever, who cannot take safer alternatives because of resistance or allergies.

#### Therapeutic uses :

- 1) In typhoid fever
  - 2) In bacterial meningitis caused by M. influenzae.
  - 3) Effective against most anaerobic bacteria.
  - 4) Rickettsial Diseases: In patients sensitive to Tetracycline or in pregnant woman and in children below 8 years of age, Chloramphenicol is the drug of choice for this disease.
- The same is true for brucellosis.

#### Tetracyclines :

7-chlortetracycline was the first Tetracycline discovered in 1947. Other molecules belonging to this group that have shown clinical value are Minocycline, Doxycycline, Methacycline, 6-demethyl- 7-chlortetracycline, Tetracycline and 5- hydroxy Tetracycline.

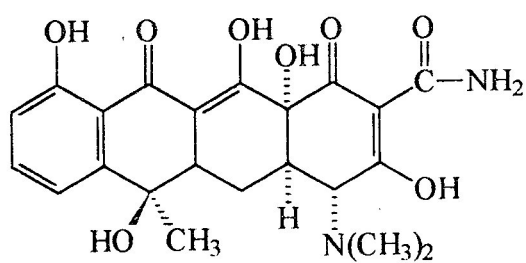
The Tetracyclines are bactericidal in high concentrations and bacteriostatic in clinically used concentrations. They are specially useful in diseases caused by Chlamydia i.e. pneumonia, bronchitis, sinusitis, rickettsiae and mycoplasmas.

#### Side-effects :

Discolouration of teeth in children has been observed. Treatment of pregnant patient may produce discolouration of teeth in their children.

Tetracyclines are deposited in the skeleton during gestation and throughout childhood. A 40 % depression of bone growth has been demonstrated:

Therefore, they should not be given to pregnant patients and should not be employed for the treatment of common infections in children under the age of 8 years.



(LXVIII)



### Antifungal Chemotherapy :

There are perhaps over 10,000 species of fungi, but less than 100 cause diseases in human.<sup>76</sup> Fungi may cause benign, but unsightly infections of the skin, nail or hair, relatively trivial infection of mucous membranes ( thrush) or systemic infection causing progressive, often fatal diseases.

#### Classification of medically important fungi<sup>77</sup> :

- 1) True yeasts ( eg. Cryptococcus neoformans).
- 2) Yeast like fungi that produce a pseudomycelium (eg. Candida albicans).
- 3) Filamentous fungi that produce a true mycelium (Aspergillus fumigatus).
- 4) Dimorphic fungi that grow as yeast or filamentous fungi depending on the cultural conditions (e.g. Histoplasma capsulatum).

There is now evidence<sup>77</sup> that Pneumocystis carinii, an organism of uncertain affiliation that is an important opportunist pathogen of patients with AIDS, may be a fungus. If so, it is unclear to which group it belongs. This organism has morphological features resembling protozoa and it is not susceptible to antifungal agents.

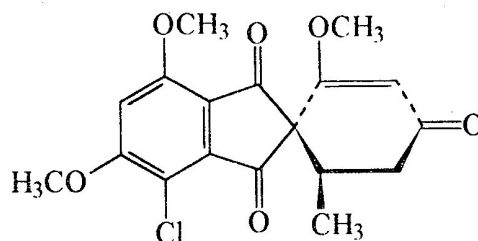
Fungi are eukaryotic organisms, and antibacterial agents have no effects on them. Specialized antifungal agents must be used and some are quite toxic. In order to minimize problems of toxicity, superficial lesions are usually treated by topical application. But deep mycoses, which are serious life-threatening infections, need vigorous systemic therapy.

**Mycotic Infectious Diseases of Humans :**

| Diseases                                 | Etiological agents                                                       | Main tissue affected                                         |
|------------------------------------------|--------------------------------------------------------------------------|--------------------------------------------------------------|
| <b>Contagious, superficial agent</b>     |                                                                          |                                                              |
| Dermatophytoses                          | <u>Epidermophyton</u> , <u>Microsporum</u> ,<br><u>Trichophyton</u> spp. | Skin, hair, nails                                            |
| <b>Non-contagious, systemic diseases</b> |                                                                          |                                                              |
| Aspergillosis                            | <u>Aspergillus</u> spp.                                                  | External ear, lungs, eye, brain                              |
| Blastomycosis                            | <u>Blastomyces dermatitis</u>                                            | Lungs, skin, bone, testes                                    |
| Candidiasis                              | <u>Candida</u> spp.                                                      | Respiratory, Gastrointestinal<br>and urogenital tracts, skin |
| Chromomycosis                            | <u>Cladosporium fonsecaea</u> and<br><u>Phidlophora</u> spp.             | Skin                                                         |
| Coccidioidomycosis                       | <u>Coccidioides immitis</u>                                              | Lungs, skin, joints,<br>meninges                             |
| Cryptococcosis                           | <u>Cryptococcus neoformans</u>                                           | Lungs, meninges                                              |
| Histoplasmosis                           | <u>Histoplasma capsulatum</u>                                            | Lungs, spleen, liver, adrenals,<br>lymph nodes.              |
| Mucormycosis                             | <u>Absidia</u> , <u>Mucor</u> , <u>Rhizopus</u> spp.                     | Nasal mucosa, lungs, blood<br>vessels, brain                 |
| Paracoccidioidomycosis                   | <u>Paracoccidioides brasiliensis</u>                                     | Skin, nasal mucosa, lungs,<br>liver, adrenals, lymph nodes   |
| Pneumocystosis                           | <u>Pneumocystis carinii</u>                                              | Lungs                                                        |
| Pseudallescheriasis                      | <u>Pseudallescheria boydii</u>                                           | External ear, lungs, eye                                     |
| Sporodrichosis                           | <u>Sporothrix schenkii</u>                                               | Skin, joints, lungs                                          |

**Potentially Effective Antifungal compounds :**

| Diseases               | Compounds                                                                                                                                   |
|------------------------|---------------------------------------------------------------------------------------------------------------------------------------------|
| Dermatophytoses        | Azoles( Itraconazole, Miconazole, Clotrimazole),<br>Griseofulvin, Tolnaftate, Naftifine, Terbinatine                                        |
| Aspergillosis          | Amphotericin B ± 5-Fluorocytosine, Itraconazole                                                                                             |
| Blastomycosis          | Amphotericin B, Itraconazole, Ketoconazole                                                                                                  |
| Candidiasis            | Amphotericin B ± 5-Fluorocytosine, Nystatin, Azoles<br>(Fluconazole, Ibaconazole, Ketoconazole, Clotrimazole,<br>Miconazole, Econazole etc) |
| Chromomycosis          | 5- Fluorocytosine, Itraconazole, Ketoconazole                                                                                               |
| Coccidioidomycosis     | Amphotericin B, Fluconazole, Ketoconazole, Itraconazole                                                                                     |
| Cryptococcosis         | Amphotericin B ± 5-Fluorocytosine, Fluconazole                                                                                              |
| Histoplasmosis         | Amphotericin B, Itraconazole, Ketoconazole                                                                                                  |
| Mucormycosis           | Amphotericin B                                                                                                                              |
| Paracoccidioidomycosis | Itraconazole, Ketoconazole                                                                                                                  |
| Pneumocystosis         | Trimethoprim/Sulfamethoxazole, Pentamidine isethionate,<br>LY-303, 366, Deferoxamine.                                                       |
| Pseudallescheriasis    | Amphotericin B, Miconazole.                                                                                                                 |
| Sporotrichosis         | Amphotericin B, Itraconazole, Potassium iodide.                                                                                             |

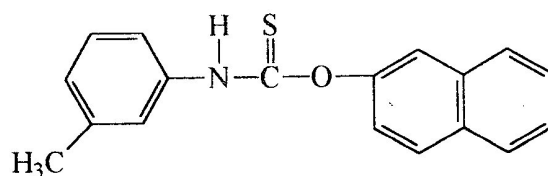
**Griseofulvin :**

(LXIX)

1) This antibiotic, a product of Penicillium griseofulvum was discovered in 1939. But antifungal activity became known in 1951.

Griseofulvin(LXIX) is neither active against bacteria nor systemic fungal diseases in humans. However, in 1958, the antibiotic was found to be effective against dermatophytosis when administered orally.<sup>78</sup> The drug has demonstrated teratogenic and carcinogenic activities in animal models<sup>78</sup> and therefore it should be reserved for treatment of dermatophytes that are known to be resistant to topical antidermatophytic compounds.

It binds to RNA and microtubule associated protein to result in inhibition of nucleic acid synthesis and of microtubule function.

**Tolnaftate :**

(LXX)

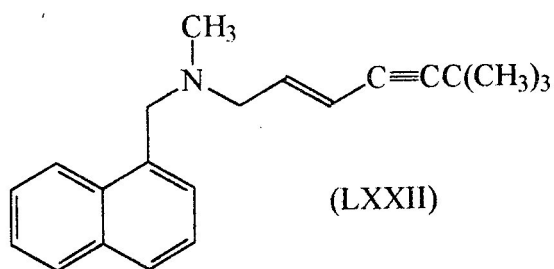
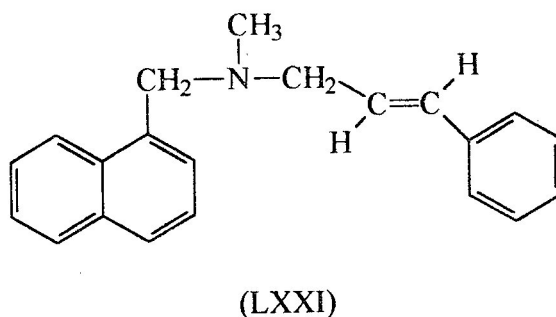
This drug was found in 1960 to have good activity against dermatophytes when applied topically.<sup>78</sup> In susceptible fungi, the drug suppresses squalene epoxidase to result in accumulation of squalene with consequent decrease in ergosterol synthesis.

It is effective in most cutaneous mycoses caused by T. rubrum, T. mentagrophytes, T. tonsurans, Microsporum canis, Microsporum audouinii, Microsporum gypsum but ineffective against Candida.<sup>80</sup>

### Allylamines :

The two most important drugs belonging to this group are a) Naftifine(LXXI) and b) Terbinafine(LXXII). Mechanism of action is inhibition of squalene - 2,3 - epoxidase and thus inhibition of fungal biosynthesis of ergosterol.

Naftifine shows broad spectrum fungicidal activity *in vitro*. Its hydrochloride salt is used for treating infections of Tinea cruris and Tinea corporis.<sup>80</sup>



Terbinafine cream can be used in cutaneous candidiasis and Tinea versicolour.<sup>80</sup>

### Azoles :

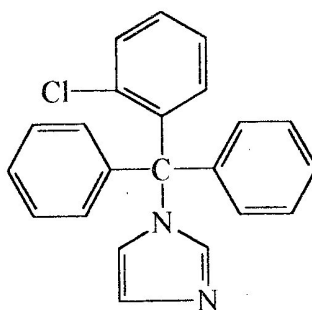
During the past quarter century, various azoles, i.e. imidazoles, triazoles, have been screened for antifungal action and exhibited broad spectrum of activity against pathogenic fungi.

Azoles can be used both topically and systemically.<sup>78</sup> Resistance to imidazoles or triazoles among the fungi that cause superficial mycoses is very rare.<sup>80</sup>

Azoles exert antifungal activity by inhibiting a cytochrome P-450 that catalyzes the 14  $\alpha$ -demethylation of lanosterol to ergosterol. The N-3 atom of azole ring binds to the ferric ion atom in the heme prosthetic group to prevent activation of oxygen for insertion into lanosterol.<sup>78</sup>

They are available for cutaneous applications and vaginal applications, i.e vaginal creams, suppositories and tablets for vaginal candidiasis.<sup>80</sup> None of them is useful for trichomoniasis.<sup>80</sup>

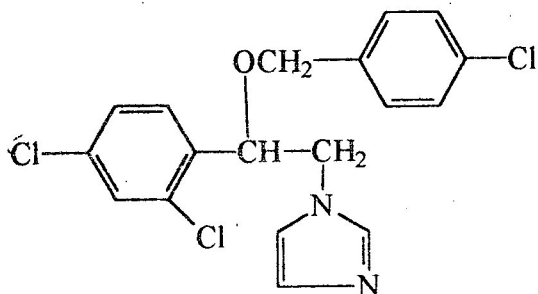
Clotrimazole (LXXIII) has been reported to cure dermatophyte infections in 60 to 100 % of cases.<sup>80</sup> Cure rates in cutaneous candidiasis are 80 to 100 % and above 80 % in vaginal candidiasis when 7 day regimen is used.<sup>80</sup>



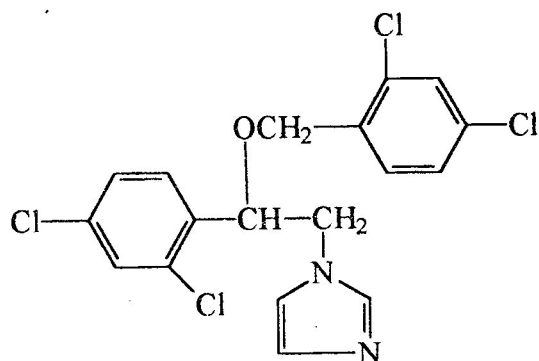
(LXXIII)

Econazole (LXXIV) readily penetrates the stratum corneum and is found in effective concentrations down to the mid-dermis.<sup>80</sup> Miconazole (LXXV) readily penetrates the stratum corneum and persists there for more than 4 days after application.<sup>80</sup>

Miconazole is considered safe for use during pregnancy but some authors believe that its vaginal use should be avoided during first trimester.<sup>80</sup> It is used in the treatment of Tinea pedis, Tinea cruris, Tinea versicolour, where cure rate may be over 90% and in vaginal candidiasis cure rate at the end of one month is about 80-95%.<sup>80</sup>



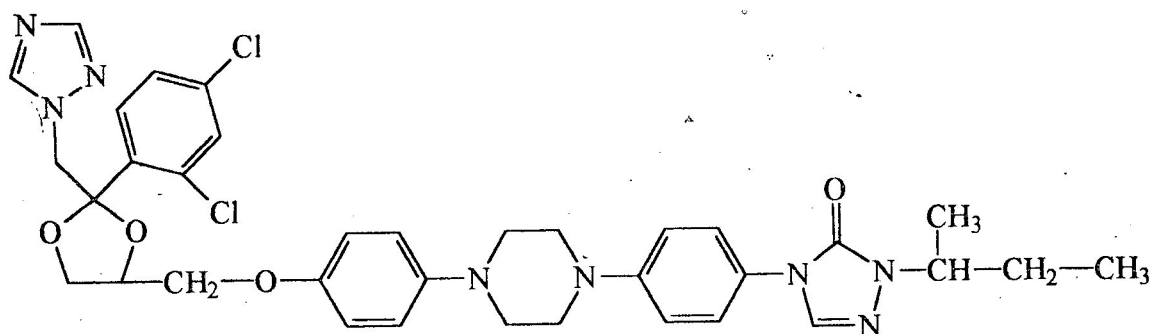
(LXXIV)



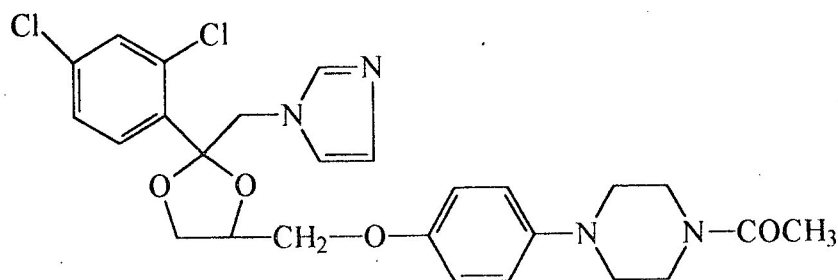
(LXXV)

Itraconazole (LXXVI) is more active than Ketoconazole (LXXVII) against aspergillosis and sporotrichosis.<sup>78</sup>

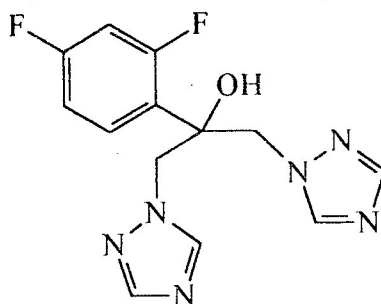
Fluconazole (LXXVIII) is active against *Candida tropicalis*, whereas Ketoconazole (LXXVII) is not. Conversely, Ketoconazole is active against *Candida krusei*, an agent resistant to Fluconazole.<sup>78</sup> Inhibition of gonadal and adrenal steroidogenesis has been observed with Ketoconazole. Some loss of male libido and sexual potency, as well as development of gynecomastia, has been reported.<sup>78</sup> Ketoconazole has been used clinically to reduce steroidogenesis in such conditions as femal acne, hirsutism, precocious puberty, prostatic and male breast carcinoma and Cushing's syndrome.<sup>78</sup>



(LXXVI)



(LXXVII)



(LXXVIII)

### Amphotericin B:

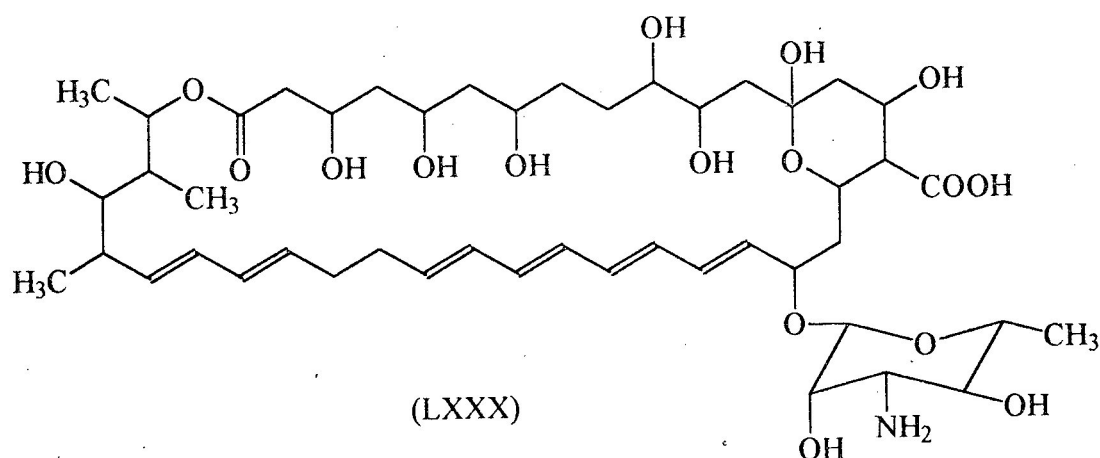
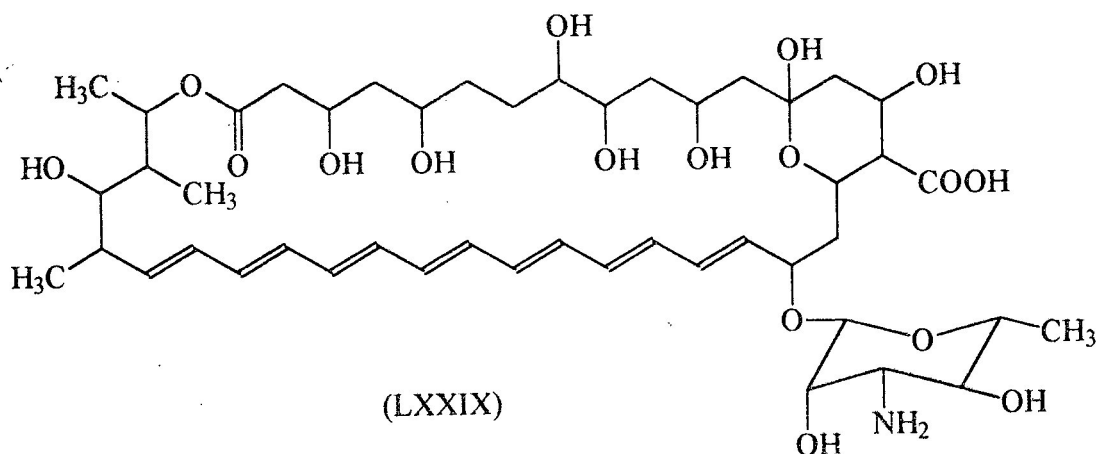
This antibiotic (LXXIX) produced by *Streptomyces nodosus* was discovered in 1955. It became the first systemic antifungal drug. It is a polyene macrolide and contains seven conjugated double bonds, an ester group, a three carboxy group, glycoside side-chain with a primary amino group.

Approximately 60 polyene antifungal compounds have been described.<sup>78</sup> Nystatin (LXXX) and Pimaricin also belong to the same class. Former is used for cutaneous, oral, vaginal candidiasis and the latter for mycotic keratoses.

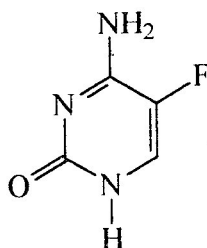
Amphotericin B binds approximately ten-fold more strongly to fungal cell membrane ergosterol than to animal cell membrane cholesterol.

Although resistance to polyene antibiotics develops rarely, not all fungal pathogens are susceptible. The drug may not penetrate readily into all infected tissues.

Adverse side-effects primarily include nephrotoxicity, renal tubular acidosis, hypokalemia, hypomagnesemia, nephrocalcinosis.<sup>78</sup>



**Flucytosin (5- Fluorocytosine) :**



The basic nucleus is pyrimidine. This fluorinated pyrimidine was synthesised as antileukemic drug and is not effective against neoplastic cells but inhibits sensitive strains of Aspergillus, Candida, Cladosporium and Cryptococcus. For Aspergillus, Candida and Cryptococcus



infections, the drug is combined with Amphotericin B. This suppresses emergence of resistance to 5- fluorocytosine (LXXXI).

Susceptible fungal cells convert the drug to 5-fluorouracil which inhibits thymidylate synthetase and hence suppresses DNA synthesis.

Other antifungal agents include antifolates such as 1:6 combination of Trimethoprim and Sulfamethoxazole, Isethionate of 2-hydroxy stilbamidine, Echinocandins, Iron chelators such as Deferoxamine etc.

# *PLAN OF WORK*



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

Voltaire<sup>81</sup> (1694-1778) stated "Therapeutics is the pouring of a drug of which one knows nothing into a patient of whom one knows less".

The medical and pharmaceutical sciences have been working diligently to ameliorate both aspects of the problem.

Man has searched for cures of illness by chewing herbs, berries, roots and barks. However, not until last 100-150 years has knowledge of the active constituents of natural sources been known.

Two of the earliest medicines were described about 5100 years ago by the Chinese Emperor Shen Nung in his book of herbs called Pentsao.<sup>82</sup> One of these is Ch'ang Shan, the root, *Dichroa febrifuga*, which was prescribed for fevers. This plant contains alkaloids which are used in the treatment of malaria today. Another plant called Ma Huang (now known as *Ephedra sinica*) was used as a heart stimulant, a diaphoretic agent (perspiration producer), and to allay coughing. It contains ephedrine, a drug that raises blood pressure and relieves bronchial spasms.

Theophrastus in third century B.C mentioned opium poppy juice as an analgetic, and in the tenth century, A.D. Rhazes (Persia) introduced opium pills for coughs, mental disorders, aches and pains. The opium poppy *Papaver somniferum* contains morphine, a potent analgetic, and codeine, prescribed today as cough suppressant.

The antihypertensive drug reserpine was extracted by ancient Hindus from snakelike root of *Rauwolfia serpentina* plant and used to treat hypertension, insomnia and insanity.

Modern therapeutics is considered to have begun with an extract of foxglove plant, which was cited by Welsh physicians in 1250, named by Fuchsius in 1542, and introduced for the treatment of dropsy (now congestive heart failure) in 1785 by Withering.<sup>83,84</sup> The active constituents are digitoxin and digoxin.

Today, digitalis which refers to all of the cardiac glycosides, is still manufactured by extraction of foxglove and related plants.

Natural products make up a small percentage of drugs on the current market. Typically, when a natural product is found to be active, it is chemically modified in order to improve its properties.

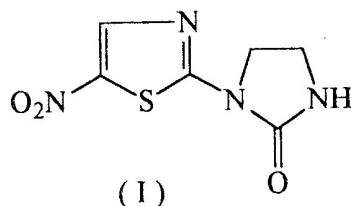
Clinically used drugs are not discovered. What is more likely discovered is lead compound. The lead is prototype compound that has the desired biological or pharmacological activity but may have other undesirable characteristics. The structure of the lead compound is then modified by synthesis to amplify the desired activity and to minimize or eliminate the unwanted properties.

The logic supporting the work presented in this thesis was formulated, bearing in mind the biological activities of known moieties and attempting certain structural modifications or adaptations in light of the recent trends in drug research in incorporating newly emerged pharmacophores on existing moieties. Benzisothiazoles are amongst these newly emerged pharmacophores and have exhibited variety of biological actions. In present work various linkages of benzisothiazoles have been synthesized and studied for their antibacterial, antifungal and antitubercular actions.

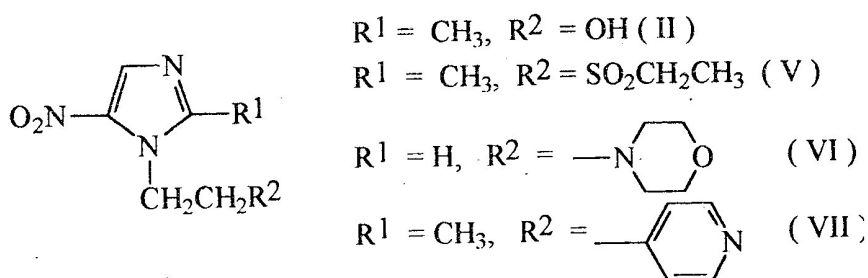
## PART II

### Synthesis of 3-substituted amino-5-nitro- 2,1- benzisothiazole

Nitroheterocyclic compounds have been studied extensively in literature for various types of biological activities. This led to the development of very important molecules, such as Niridazole (I), Metronidazole (II), Flunidazole (III), Nitrofurantoin (IV)

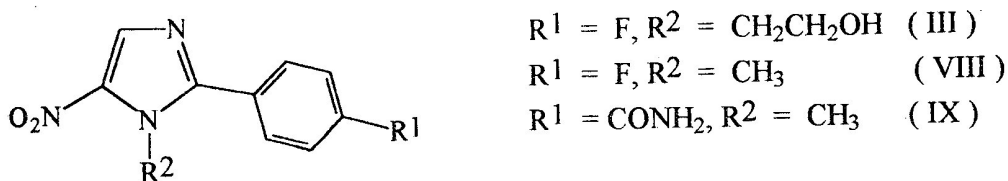


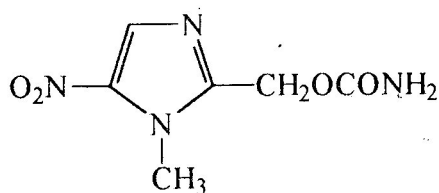
In 1964, Ciba Laboratories announced<sup>85-89</sup> the discovery of novel schistosomicide, nitridazole<sup>85</sup>(I). Niridazole was synthesized as a part of systemic investigation of heterocyclic nitro compounds as potential antiparasitic agents. One requirement for activity in all niridazole congeners appeared to be presence of 5-nitro group<sup>90</sup>.



Amongst the nitroimidazoles, metronidazole (II) is established as a drug of choice in the treatment of all forms of amoebiasis.<sup>91-95</sup> In order to improve trichomonocidal activity and metabolic stability it led to the discovery of tinidazole (V), nimorazole (VI), panidazole (VIII) with retention of nitro group at fifth position.

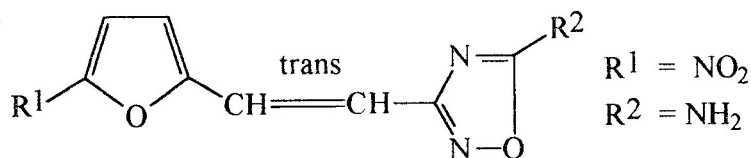
Most interesting nitroimidazoles include flunidazole (III), MF-nitroimidazoles (VIII), MCA-nitroimidazole (IX), Ronidazole(X).





(X)

Newer, more interesting experimental compounds include oxadiazole derivative (SQ 18506) (XI).<sup>98-100</sup>



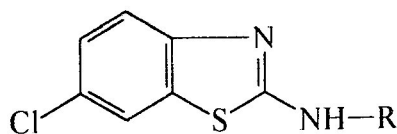
(XI)

This was consistently active at high dose levels against *S. mansoni* and *S. japonicum* in mice and monkeys. The nonnitrated compound lacked activity.

Nitropyrroles<sup>101</sup> and nitropyridines<sup>102,103</sup> have been investigated for their antiamoebic properties.

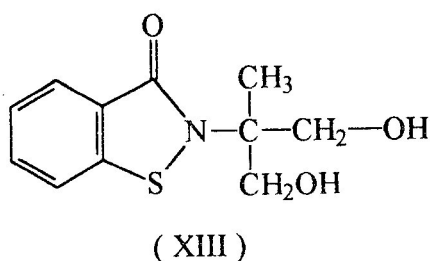
Because of the wide variety of biological activities nitroheterocycles have possessed such as schistosomicidal, trichomonocidal and amoebicidal activities, it was proposed to prepare nitroheterocyclic molecules.

Benzimidazoles and benzothiazoles have drawn considerable attention due to their excellent antiparasitic and anthelmintic activities. Apart from this, substituted amino benzothiazoles (XII) synthesized in our laboratory<sup>104</sup> have shown to possess promising antitubercular activity.



(XII)

Recently, considerable attention has been drawn by newer benzisothiazoles due to their excellent antibacterial activity especially against Gram-positive bacteria.<sup>105</sup> They also possess excellent antifungal activity.<sup>105</sup>

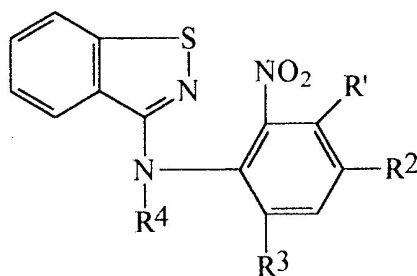


N-hydroxyalkyl derivative of 1,2 benzisothiazol -3- ( 2 H) one (XIII) showed excellent antifungal and antibacterial activity which is comparable with that of clotrimazole, cefotaxime and gentamycin. MIC of all given below in  $\mu\text{g/ml}$ .<sup>105</sup>

|              | <u>T. Mentagrophytes</u> | <u>C. albicans</u> | <u>S. aureus</u> | <u>S. albus</u> | <u>B. subtilis</u> |
|--------------|--------------------------|--------------------|------------------|-----------------|--------------------|
| (XIII)       | 1                        | 5                  | 2                | 1.5             | 3                  |
| Clotrimazole | 0.5                      | 1.25               | -                | -               | -                  |
| Cefotaxime   | -                        | -                  | 1.5              | 0.5             | 0.5                |
| Gentamycin   | -                        | -                  | 2.5              | 1.5             | 0.5                |

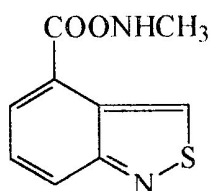
Apart from antifungal and antibacterial activities benzisothiazoles also possess herbicidal<sup>177</sup> algicidal<sup>178</sup>, trichomonocidal<sup>179</sup>, insecticidal<sup>180</sup>, pesticidal<sup>107</sup> activities.

Nitro derivative of 3-anilinobenzisothiazoles (XIV) proved themselves<sup>106</sup> as agrochem fungicides giving 100 % control of Plasmopara viticola.



(XIV)

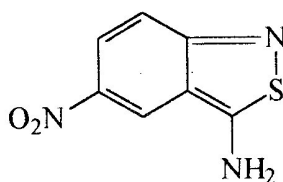
In spite of such interesting activities of substituted benzisothiazoles, marginal attention has been paid to 2,1, - benzisothiazoles.



(XV)

2,1- benzisothiazol -4-ol - methyl carbamate ester (XV) is found to possess pesticidal activity against Mexican bean beetle larvae and houseflies.<sup>107</sup>

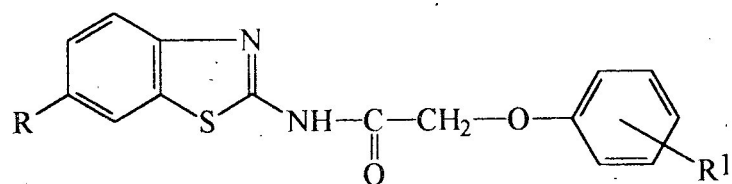
Taking into consideration importance of nitroheterocycles as well as increasing interest in benzisothiazoles, it was proposed to prepare 3-amino-5-nitro-2,1- benisothiazole (XVI)



(XVI)

Lacova M *et al*<sup>108</sup> have reported compounds of following type (XVII) having good biological activity.





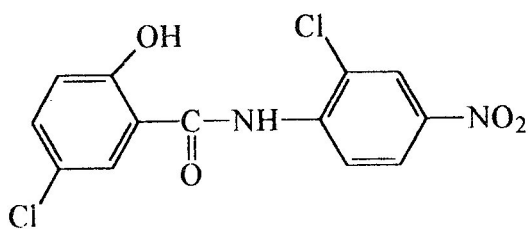
(XVII)

R<sup>1</sup> = H or Cl

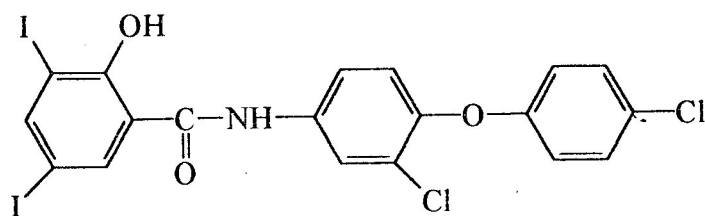
R = Me, Cl

Aromatic acid amides have proved to be useful in the field of medicinal chemistry.

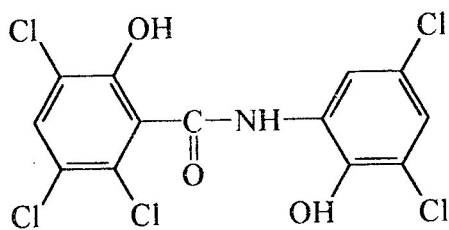
Niclosamide<sup>109,110</sup> (XVIII), rafoxanide<sup>111</sup> (XIX), oxyclozanide<sup>112</sup> (XX) are well known for their anthelmintic activity.



(XVIII)



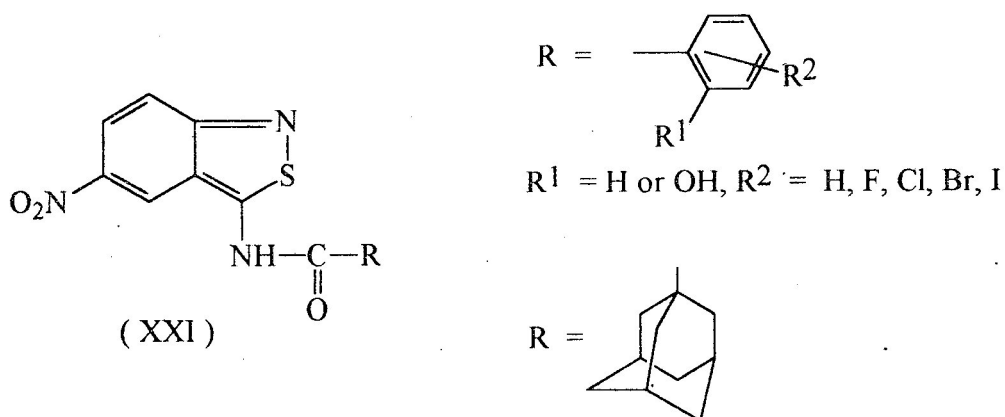
(XIX)



(XX)

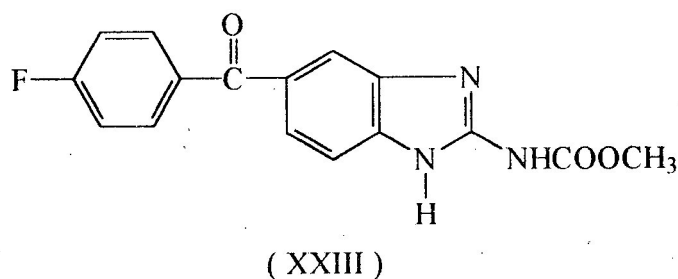
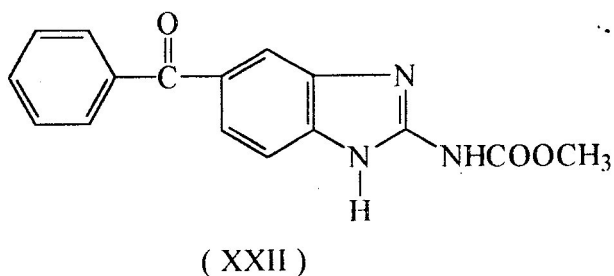
The above drugs indicate the significance of halogenated salicylic acid amides.

From all above literature search, it was proposed to prepare amide linkage of 3 - amino-5 - nitro-2,1-benzisothiazole (XVI) with halogenated salicyloyl chlorides, halogenated benzoyl chlorides and also with adamantyl carbonyl chloride to check if there is any enhancement of activity of amine as a result of amide linkage (XXI).

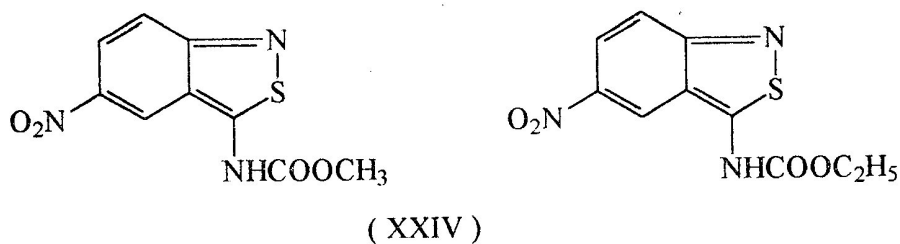


All compounds prepared above were subjected to antifungal, antibacterial and antitubercular screening.

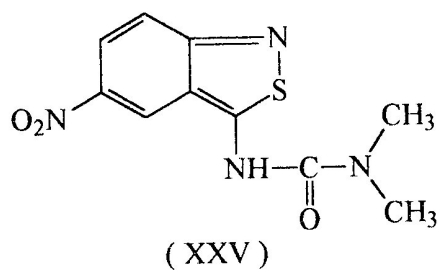
Several benzimidazole analogs such as mebendazole<sup>113</sup> (XXII), flubendazole (XXIII) are well known anthelmintics. Carbamate group ' $\text{NHCOOCH}_3$ ' appeared to be essential for their action.



Thus it was proposed to prepare carbamate linkage of 3-amino-5-nitro-2,1- benzisothiazole (XXIV) and test the same for antimicrobial action.



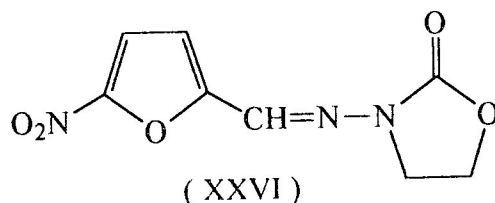
Hetrazan<sup>114</sup>, a diethyl carbamoyl derivative of N - methyl piperazine is the drug of choice in filariasis. Taking into consideration significance of this linkage, it was proposed to prepare dimethyl carbamido linkage of 3 - amino - 5 - nitro -2,1- benzisothiazole (XXV) by reacting it with N,N dimethyl carbamoyl chloride and to test the same for antimicrobial action.



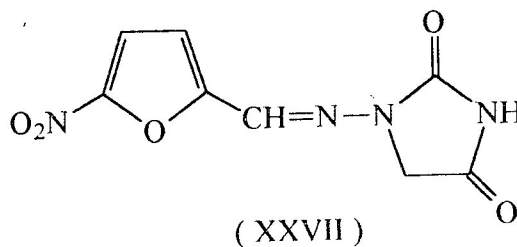
### PART III

#### Synthesis of 3 - amine - N - (substituted phenyl / toluyl / heterocyclic) methylene - 5 - nitro-2,1- benzisothiazole.

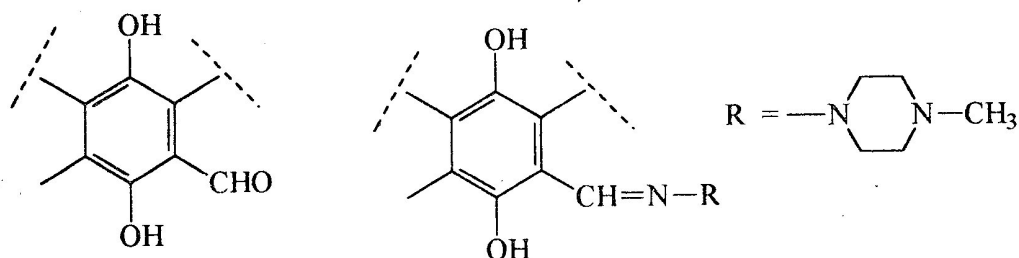
Importance of nitroheterocyclic compounds is well documented earlier . Amongst the nitroheterocyclic compounds most common in use are nitrofurans e.g. 1) nitrofurazone 2) nitrofurazolidone and 3) nitrofurantoin. All these compounds apart from nitrofuran structure share one common aspect i.e. presence of azomethine linkage.



Nitrofurazolidone<sup>34</sup> (XXVI) has broad spectrum bactericidal activity in intestinal pathogens including various species of salmonolla, shigella, proteus, enterobacter as well as E. coli and Vibrio cholerae.

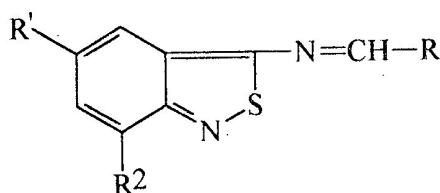


Nitrofurantoin<sup>96</sup> is active against many Gram-positive and Gram-negative bacteria at concentration 5-12 µg/ml. It accumulates in urine in sufficient concentration for treatment of urinary tract infection.



(XXVIII)

When 3-formyl rifamycin SV was prepared and it was found that many of its N, N-disubstituted hydrazones<sup>115,116</sup> (XXVIII) had high activity both *in vitro* and *in vivo* against M. tuberculosis and Gram-positive bacteria.



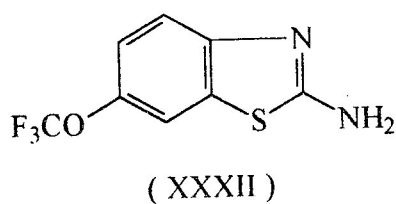
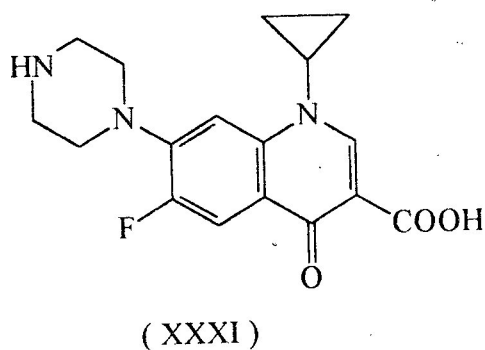
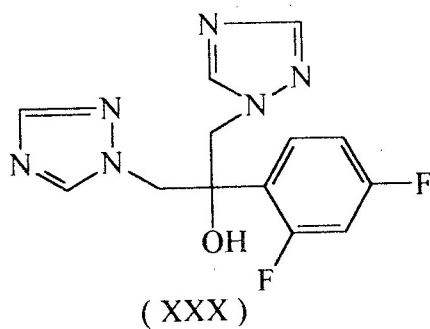
(XXIX)

Bellotti A, Coghi A, Sgorbati O<sup>117</sup> recently revealed antimycotic and antistaphylococcal activities of 3- azomethine linkage of 2,1- benzisothiazole (XXIX). Nitrofurfural derivative showed very good activity against S. aureus.

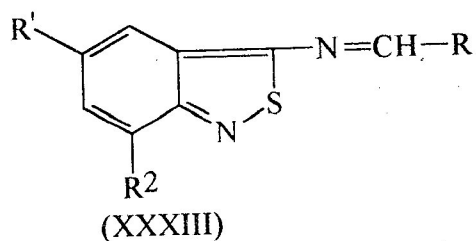
Schiff bases from 5 - chloro salicylaldehyde, 5 - bromo salicylaldehyde and N,N dimethyl-aminobenzaldelyde have been reported to have encouraging tuberculostatic action<sup>118</sup>. The presence of azomethine linkage in compounds has also been known to decrease toxicity considerably.<sup>119</sup>

Thus it was proposed to prepare azomethine linkage of 3 - amino - 5 - nitro 2,1 - benzisothiazole by condensing it with various aldehydes.

Fluorine containing drugs such as fluconazole (XXX), ciprofloxacin (XXXI), riluzole (XXXII) have been proved to be very specific drugs for the treatment of particular diseases.



Taking into consideration importance of azometmine linkage as well as fluorinated compounds Schiff bases of 3 - amino- 5 -nitro - 2,1- benzisothiazole with various fluorinated aldehydes were prepared (XXXIII) and subjected to antifungal, antibacterial and antitubercular screening.



R = substituted phenyl / toluyl / heterocyclic ring

## PART IV

### Synthesis of Tioxidazole:

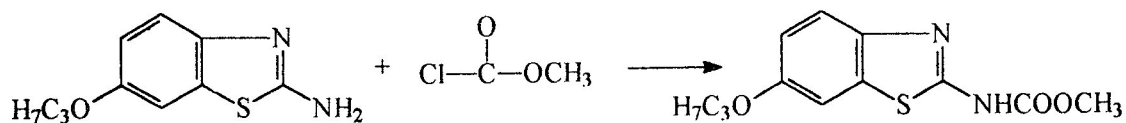
The spectrum of anthelmintic activity displayed by tioxidazole, a benzothiazole type of compound related to benzimidazoles is, in general, typical of benzimidazoles in the horse. Thus efficacious removals of large strongyles, S. vulgaris and S. edentatus, small strogyles, P. equorum and O. equi recorded for tioxidazole at the dose level of 11 mg/ kg compare favourably with mebendazole at 8.8 mg/ kg, cambendazole at 20 mg/ kg, fenbendazole at 5 or 10 mg/ kg, oxibendazole at 10 mg / kg and oxfendazole at 10 mg / kg.<sup>120</sup>

Taking into consideration broad spectrum activity of tioxidazole against gastrointestinal round worms in the orally infected horses, it was thought of interest to prepare this molecule from basic stage.

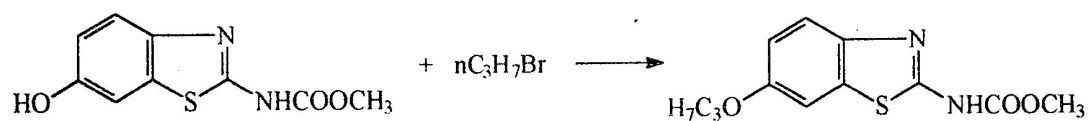
Thus tioxidazole was proposed to prepare by two different routes -

- 1) Condensation of 2 - amino- 6 - propoxy benzothiazole with methyl chloroformate.
- 2) Propylation of methyl -6- hydroxy benzothiazole -2 - carbamate

- 1) p- Propoxyaniline prepared in our laboratory was subjected to thiocyanation to give 2-amino-6-propoxy-benzothiazole which was further condensed with methyl chloroformate to give methyl-6-propoxy benzothiazole-2-carbamate or tioxidazole.



- 2) In second route, benzoquinone was reacted with thiourea to give 2-amino-6-hydroxy benzothiazole which was selectively converted into methyl 6-hydroxy benzothiazole-2-carbamate with an aid of methyl chloroformate. Methyl - 6 - hydroxybenzothiazole - 2- carbamate was further treated with n-propylbromide in propanol to obtain tioxidazole.



From our experimental experiences, first route for the synthesis of tioxidazole i.e. preparation of carbamate derivative of 2 - amino - 6 - propoxy benzothiazole was found to be much better than second route.



## PART V

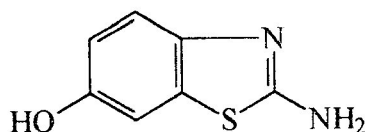
### Synthesis of an intermediate for Riluzole

Rhone- Poulenc Rorer's glutamate antagonist, Rilutek (Riluzole) has been launched in U.S. Rilutek is the first drug shown to extend survival in amyotrophic lateral sclerosis (ALS).<sup>121</sup>

ALS is characterized by a progressive degenerative disorder of motor neurons in spinal cord, brain stem and motor cortex, although sensory nerve, bladder and bowel functions are unaffected. The patients die because of respiratory failure usually within 4-5 yrs of diagnosis.

Chemically, riluzole (XXXII ) is 2-amino-6- trifluoromethoxy benzothiazole. The route of synthesis of this compound has been reported in literature as thiocyanation of 4-trifluoromethoxy aniline.

However we thought of an alternative route for synthesis of riluzole i.e. trifluoromethylation of 2 - amino - 6 - hydroxy benzothiazole (XXXIII).



(XXXIII)

Thus 2-amino- 6- hydroxy benzothiazole may serve as an intermediate for riluzole. With this idea, attempt was made to synthesize 2-amino-6-hydroxy benzothiazole by different routes.

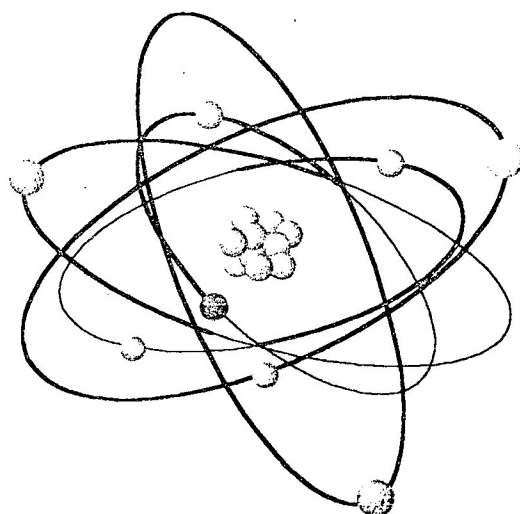
- 1) Thiocyanation of p- amino phenol had been tried but was unsuccessful.
- 2) 2-amino-6-methoxy benzothiazole prepared in our laboratory was subjected to demethylation by 1) 47% HBr 2) Aluminium trichloride

Demethylation reaction of 2-amino-6-methoxy benzothiazole using above two demethylating agents was a failure.

- 3) p- benzoquinone (2 mole) was reacted with thiourea (1 mole) in Ethanol to give 2-amino- 6 - hydroxy benzothiazole (XXXIII) in quantitative yield.

2 amino-6-hydroxy benzothiazole (XXXIII) can be subjected to trifluoromethylation using fluorinating agent. Amino group can be protected in this reaction. Due to non-availability of special type of apparatus for fluorination, this step was not carried out. Cost effectiveness has to be taken into consideration while carrying out trifluoromethylation as several fluorinating agents are very costly.

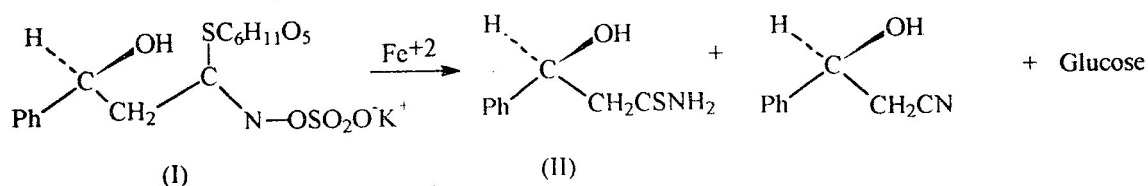
# *CHEMISTRY*



*CHEMISTRY OF  
THIOAMIDE*

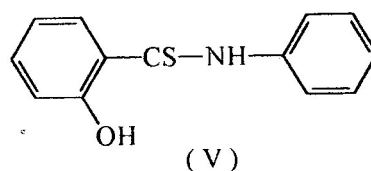
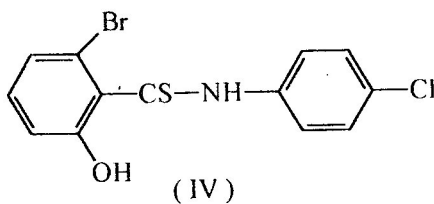
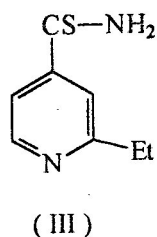
### Natural occurrence of Thioamides :

Real thioamides have not yet been found in biological material. The non-enzymatic degradation of some sulfur containing glycosides yields, real open chain thioamides e.g.  $\beta$ -hydroxy  $\beta$ -phenyl propionic acid thioamide (II) is obtained from glucobarbarine(I).



### Applications :

Early investigation by the groups of Bavin, Rogers, Gardner and Meltzer have dealt with the activity of several thioamides against M. tuberculosis. It was Liberman and coworkers who succeeded in finding the first thioamide of sufficient *in vivo* activity and low toxicity (1956), namely 'Ethionamide' (III). It seems, however that no thioamide of increased suitability with respect to (III) has been obtained so far.



Activity of thioamides against bacteria other than M. tuberculosis has been found by Weuffen and coworkers, the substituted thiobenzamide (IV) being especially effective. Salicylic acid thioanilide (V) has fungistatic activity. Secondary dithiooxamides are amoebicides.

### Miscellaneous Applications :

Thioamides may be used as herbicides especially polychlorothiobenzamides and cyclopropane and cyclobutane derivatives have been proposed.

Thioamides are theoretically used as antioxidants, corrosion inhibitors and also vulcanization promoters.

Thioamides are technically used as generator of hydrogen sulphide. Pyridinethiocarbo-xamide and thiocaprolactum are suitable for photometric determination of  $\text{Fe}^{+2}$  or  $\text{Bi}^{+3}$  respectively.

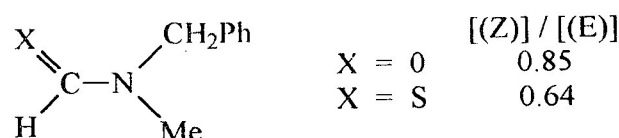
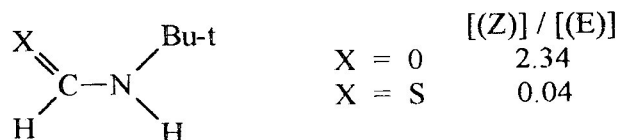
### Comparison between amide and thioamide

The most important element of the geometrical structure, namely the planar arrangement of the functional group, occurs in thioamides as well as in amides. The differences in physical properties of these 'isologous' species are gradual, but this does not hold for their chemical behaviour. The higher atomic number of the 'S' atom accounts for the enlarged kernel of electrons which for its part causes an increased radius of covalency. (O :  $0.74\text{\AA}$ ; S :  $1.04\text{\AA}$ ), decreased tendency to form  $\text{P}_\pi - \text{P}_\pi$  double bonds, etc.

- 1) Energy of activation,  $E_a$ , for rotating the  $\text{NR}_2$  group out of plane of the  $\text{R-X-NR}_2$  molecule, i.e. the double bond character of the central C-N bond, increases in going from  $\text{X=O}$  to  $\text{X=S}$  (no significant changes in bond lengths). This is likely to be due to non-bonded repulsion forces e.g.

| $\text{PhCXNMe}_2$ | $\text{X = O}$ | $\text{X = S}$ |
|--------------------|----------------|----------------|
| $E_a$ (Kcal/mole)  | 7.5            | 15.4           |

- 2) The preference for the (Z) configuration is less marked in secondary and tertiary thioamides than in the corresponding amides on account of the enlarged steric requirement of S atom.



- 3) The thioamide molecule is more polar and more polarizable.

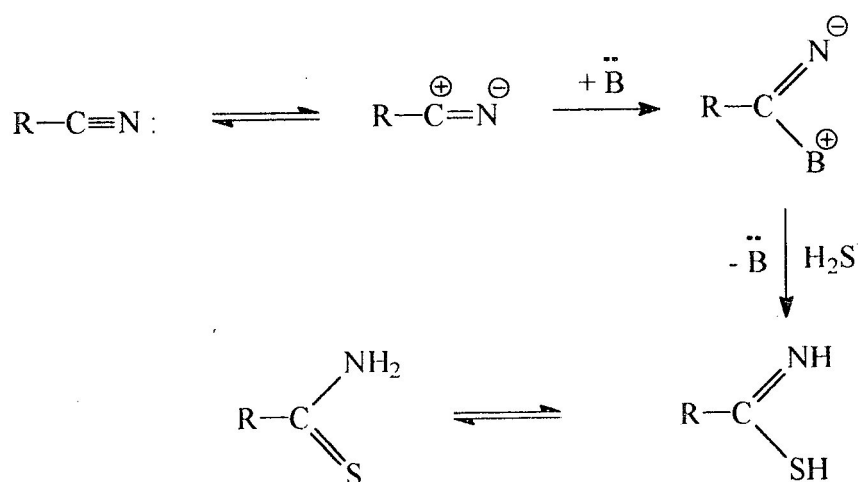
### Synthetic methods of thioamides :

#### 1) Reaction of nitrile with hydrogen sulfides and thiols :

The synthesis of thiocarboxamides from nitriles is one of the oldest one. The reaction requires high hydrogen sulphide concentration and is catalyzed by bases. It is favoured by exclusion of water.<sup>122,123</sup>

Water soluble sulphides or polysulphides can be used instead of hydrogen sulphide.<sup>124</sup> Ammonium and calcium hydrosulphides have been described for synthesis of thioamides.<sup>123</sup>

The base catalyzed reaction can be written as follows :



Suitable catalysts apart from ammonia, include aliphatic amines, pyridine, alkoxides and potassium hydrogen sulphide.<sup>125,126</sup> Fairfull *et al*<sup>127</sup> obtained very good yields by addition of hydrogen sulphide to aromatic nitriles in presence of pyridine and triethylamine.

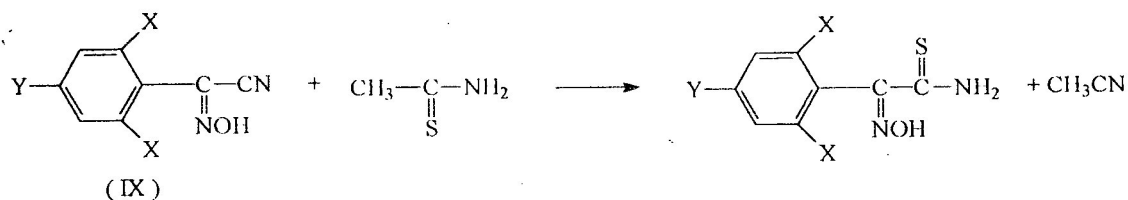
Aliphatic nitriles often react very slowly and give poor yields unless the reaction is carried out at elevated temperature and pressure.<sup>128</sup>

Kindler<sup>125</sup> and Hanford<sup>129</sup> extended the method, by addition of primary amine as follows





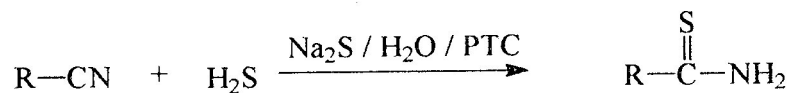
Removal of acetonitrile favours the formation of thiocarboxamide (VIII). The method is advantageous as it can be used for the preparation of thiocarboxamide from nitriles and reducible substance e.g.  $\alpha$ -cyanobenzaldoximes (IX).



If the nitrile contains hydroxyl group it must be protected by acylation<sup>133,134</sup>. The keto group if present is readily converted into thioxo groups in acidic solutions.<sup>135</sup>



Another method developed by Mayer *et al* involves base catalyzed conversion of ketones into thiones.<sup>136</sup> Recently, the general procedure of thioamide i.e. the base catalyzed reaction of nitriles with  $\text{H}_2\text{S}$  in a polar organic solvent, was modified by Casser *et al*.<sup>137</sup> They have introduced a phase transfer catalyst known as aq. solution of sodium sulphide. The system which contains three phases namely, aqueous, organic and  $\text{H}_2\text{S}$  gas, providing aromatic thioamides in high yields.



Here,  $\text{SH}^-$  are transferred from aqueous to the organic phase to give respective thioamides.

## 2) Sulphurization of amides :

This involves replacement of oxygen in carboxamides by sulphur. It permits synthesis of N-monosubstituted and N-disubstituted compounds from the readily obtainable amides. The most useful reagent for such replacement is phosphorous pentasulfides.<sup>138</sup> Potassium thiophosphate along with phosphorous pentasulfide enhances the yield of thioamides.<sup>139</sup>



Generally, low yields are obtained on fusion of amides with phosphorus pentasulfide.<sup>140</sup> Better results are obtained in dry, inert solvents. In such cases the reaction time is reduced and undesirable side reactions are avoided. Solvents used are benzene, xylene, toluene, tetrahydrofuran dioxane, diethylene glycol and diethyl ether.<sup>141-144</sup>

The sulphurization of liquid amides is also possible in an excess of amide as solvents.<sup>145,146</sup>

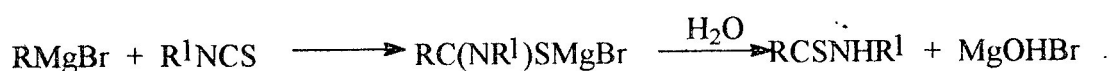
Pure thiocarboxamides are obtained in high yields in pyridine as solvents.<sup>147,148</sup>

This reaction can not be used with thermally unstable amides. Hydroxyl group must be protected by acetylation before sulphurization.<sup>149,150</sup>

### 3) Addition of nucleophiles to isothiocyanates :

#### Organometallic compounds :

Isothiocyanates react with Grignard reagents and subsequent hydrolysis under mild conditions leads to thiocarboxamides.

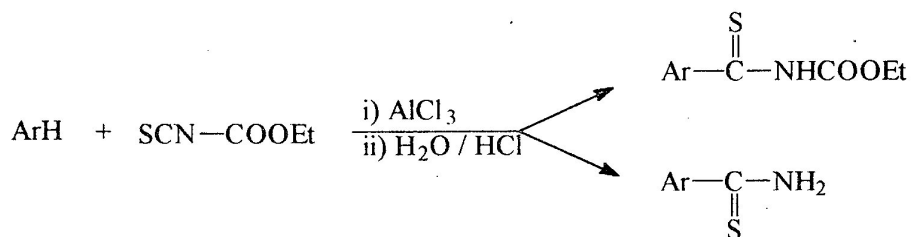


This was first used by Sachs and Loevy.<sup>151</sup>

### 4) One step synthesis of aromatic thioamides :

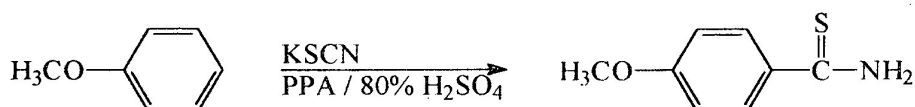
#### a) Fridal Craft's thiocyanation with ethoxy carbonyl isothiocyanate :

It has been found<sup>152</sup> that ethoxy carbonyl isothiocyanate reacts readily with various aromatic compounds in presence of anhydrous  $\text{AlCl}_3$ . However, depending upon the condition it either yields the expected N-ethoxy carboxythioamide or thioamide itself.



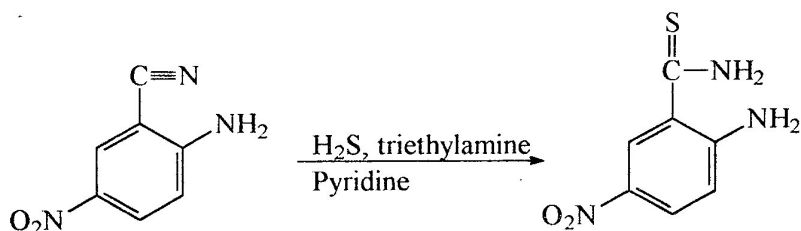
It appears that nature of the product is controlled by reaction temperature and quantity of catalyst. For thioamide to be the major product, molar ratio of  $\text{AlCl}_3$  to isothiocyanate equal to at least 2 : 1 must be maintained. Low reaction temperature favours the N-ethoxy carbonylthioamide. Large period of time favours the synthesis of thioamide.

b) Thioamides can be also be prepared by heating anise (0.01N) or veratrole at  $60^\circ\text{C}$  in presence of polyphosphoric acid (PPA) or 80%  $\text{H}_2\text{SO}_4$  with potassium thiocyanate (0.01N).<sup>153</sup>



In case of PPA, thioamides were obtained in 50% yields whereas yield is increased by 5 - 10% if 80%  $\text{H}_2\text{SO}_4$  is used instead of PPA.

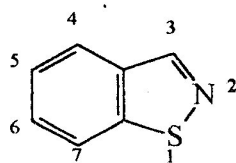
In our present research work, we have prepared thioamide from 5-nitroanthranilonitrile and reaction can be written as follows :



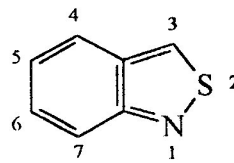
Thus, 2-amino-5-nitrobenzenecarbothioamide is prepared by reacting 5-nitroanthranilonitrile with dry gaseous hydrogen sulphide in pyridine, in presence of triethylamine as base. Hydrogen sulphide is generated by action of conc. hydrochloric acid on ferrous sulfide. Yield upto 80% is obtained if basic characteristic of reaction is maintained.

*CHEMISTRY OF  
BENZISOTHIAZOLE*

There are two series of fused bicyclic compounds incorporating both an isothiazole and a benzene ring. These two ring systems are 1,2- benzisothiazole(I) and 2,1- benzisothiazole(II).



(I)

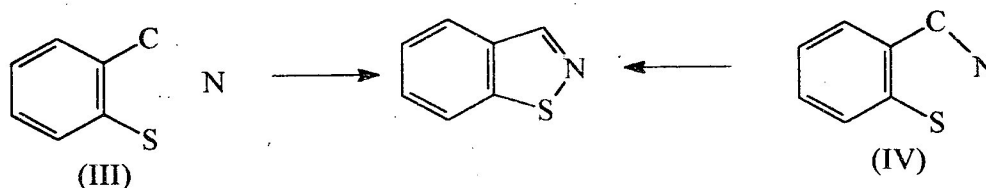


(II)

Other names for 1,2 - benzisothiazole and 2,1- benzisothiazole are benz (d) isothiazole or thioindoxazene and benz (c) isothiazole or thioanthranil respectively. Both the ring systems were reviewed by Bambas in 1952 in the Interscience series of monographs on 'The Chemistry of Heterocyclic compounds'.<sup>154</sup>

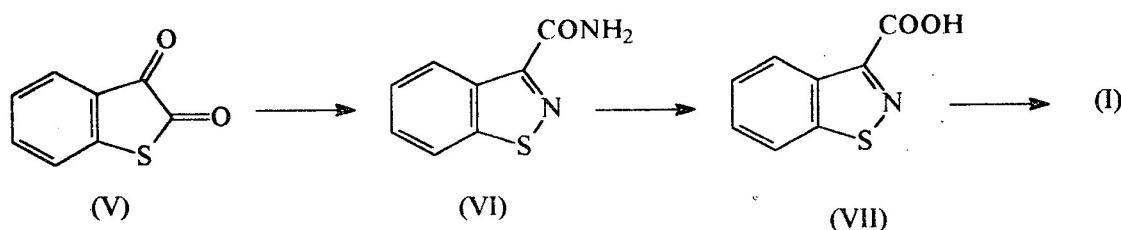
#### Formation of 1,2 - benzisothiazole

Synthesis of 1,2- benzisothiazole requires an aromatic sulfur containing compound as a precursor. The pattern of this synthesis is either from 'III' or 'IV'.

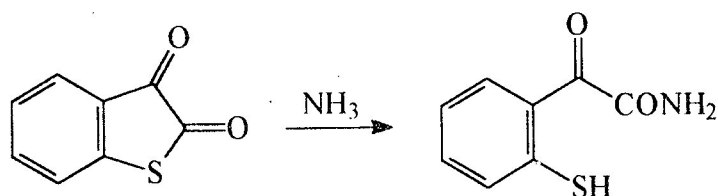


This limits the general availability of 1,2 - benzisothiazole as sulfur containing precursors are prepared often only with some difficulty.

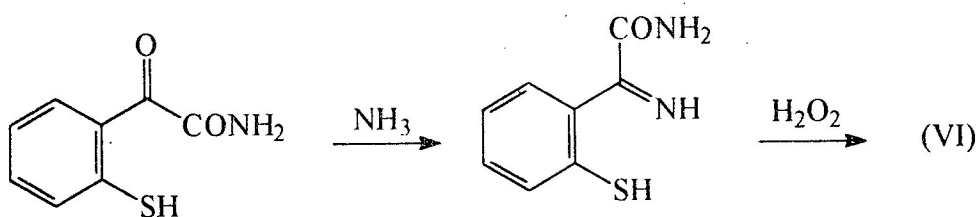
The 1,2-benzisothiazole was first prepared in 1923 by Stolle and coworkers<sup>155,156</sup>. Treatment of thianaphthenequinone(V) with aqueous ammonia and hydrogen peroxide afforded 1,2-benzisothiazole-3-carboxamide(VI) which was hydrolyzed to the acid and latter decarboxylated.



Here probably ammonia breaks the sulfur- carbonyl bond first.



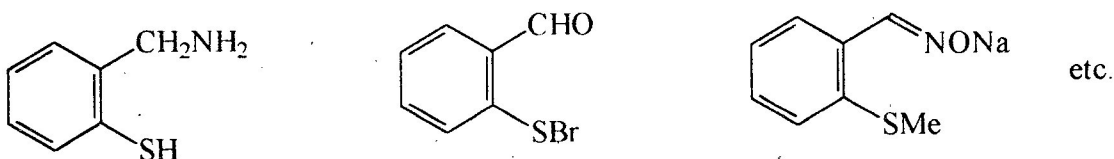
Secondly adds to the second carbonyl group, giving an imine function.



Sulfur-nitrogen bonds are readily formed by oxidation of imino-thiols such as (VIII).

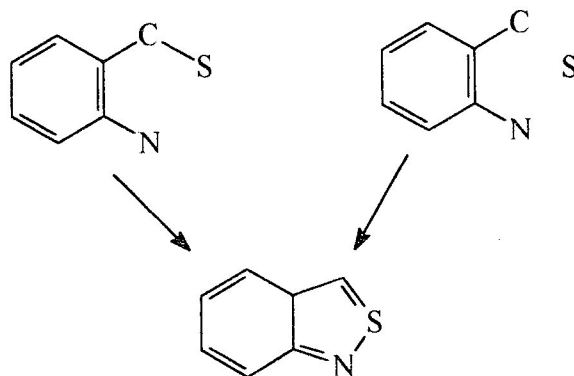
This method is a useful one in the preparation of isothiazoles generally.

Few examples of molecules which act as sulfur containing precursor for synthesis of 1,2-benzisothiazole include,



#### Formation of 2,1 - Benzisothiazoles.

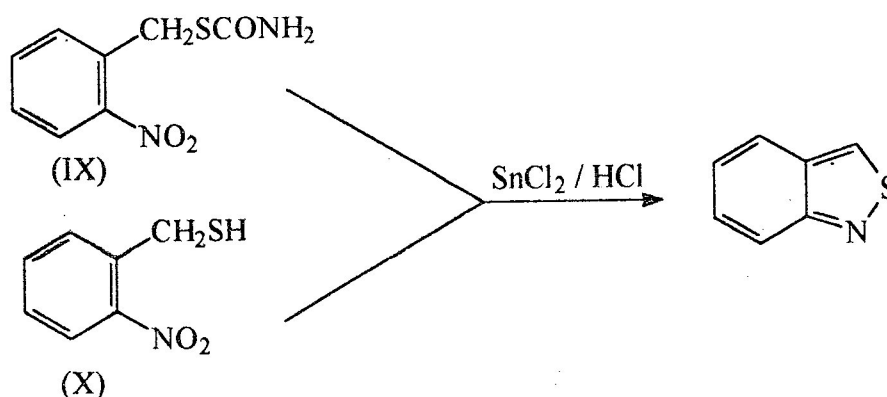
Two precursors for the construction of 2,1-benzisothiazole are of following type :-



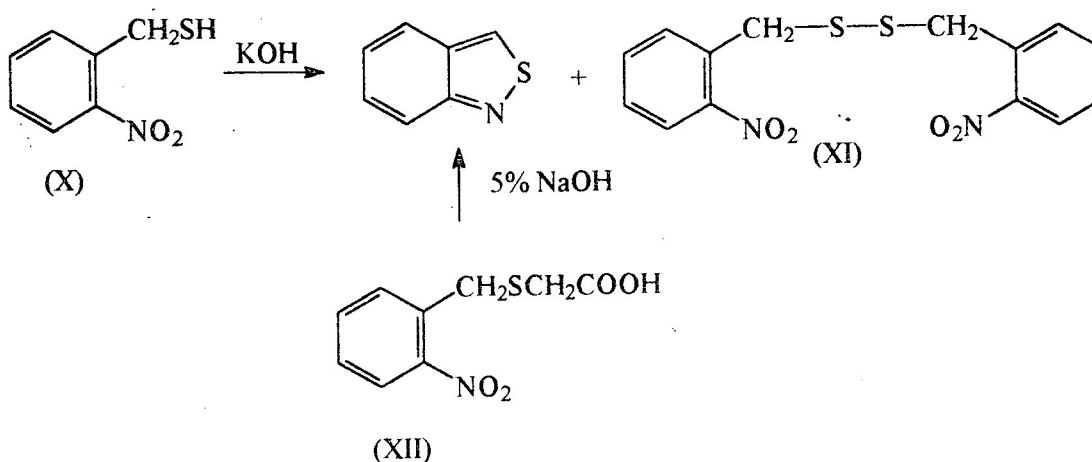
Many procedures for the synthesis of 2,1-benzisothiazole have been reported in the literature.

1) From o-nitro- $\alpha$ -toluenethiol and Related Compounds : -

Gabriel and co-workers<sup>157,158</sup> first prepared 2,1-benzisothiazole in 1890's. Reduction of o-nitro- $\alpha$ -toluenethiol carbamate (IX) or o-nitro- $\alpha$ -toluenethiol (X) with stannous chloride and hydrochloric acid yielded a crystalline solid. This solid is stannic chloride complex of the free base. This on addition of water and steam distillation afforded 2,1-benzisothiazole as the complex is decomposed by steam and the base is liberated.



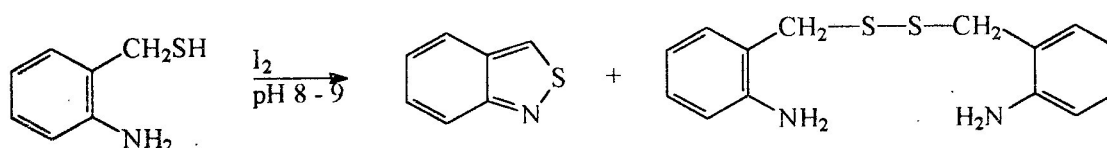
The thiol (X) also yields 2,1-benzisothiazole on treatment with conc. KOH soln. Here most of the thiol is oxidized to disulfide (XI) and yield of product is low.<sup>159</sup>



Later on, it was found that o-nitro toluenethiol acetic acid (XII) undergoes a similar reaction with NaOH solution, the yield of product is however very small<sup>160</sup>, i.e. less than 10 %.

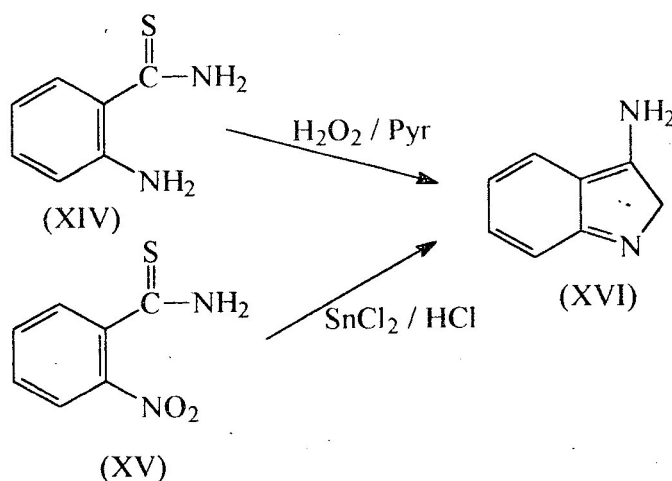
## 2) From o-amino- $\alpha$ -toluenethiol :

This is oxidative cyclization of aminothiols<sup>161</sup> and is generally useful for the synthesis of nitrogen-sulfur heterocycles. o-Amino- $\alpha$ -toluene-thiol (XIII); treated with iodine and dilute base, affords 2,1-benzisothiazole. Control of pH of the reaction is very essential to control the percentage of disulfide formation. The yield of product is also lowered by further substitution in the benzene ring of the thiol.<sup>162</sup>



## 3) From o-aminothiobenzamides and o-nitrothiobenzamides : -

This method can be applied for the synthesis of amino substituted 2,1-benzisothiazoles. But no such attempt was made before 1963, when Parke Davis company filed a patent application<sup>163</sup> in which preparation of a number of 3-substituted- 2,1-benzisothiazoles was described. This was followed by a definite paper<sup>164</sup>, indicating the preparation of 2,1-benzisothiazoles from o-aminothiobenzamide (XIV) and o-nitrothiobenzamide (XV) by oxidative and reductive cyclization respectively.



This method is very important for the synthesis of 3-amino-2,1-benzisothiazoles (XVI).

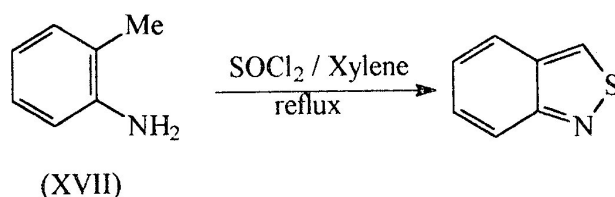
Independently and almost simultaneously two other groups filed<sup>165,166</sup> patent application describing similar oxidative ring closure.



In present work, 3-amino-5-nitro-2,1-benzisothiazole was prepared from 2-amino-5-nitro benzene carbothioamide by oxidative ring closure method as mentioned above.

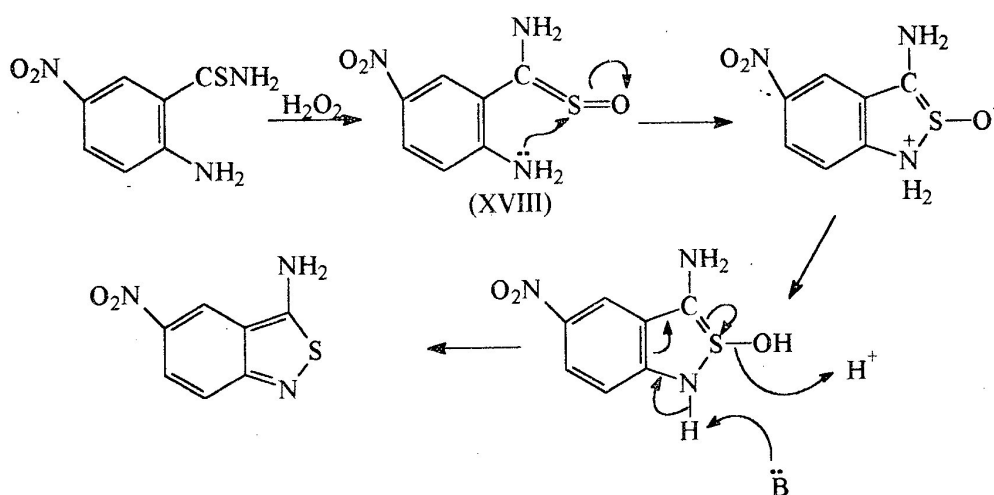
#### 4) From o- toluidines and thionyl chloride : -

Reaction between o- toluidine (XVII) and thionyl chloride in a high boiling inert solvent (such as xylene or bromobenzene) affords 2,1-benzisothiazoles.<sup>167,168</sup>

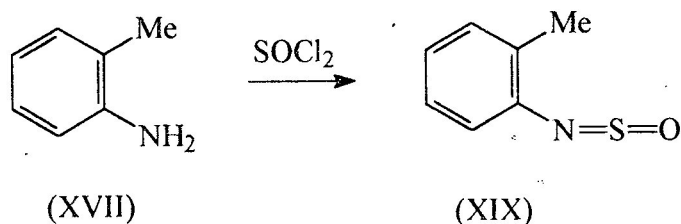


#### MECHANISM OF REACTION

Mechanism of the oxidative cyclization, which is the more useful amongst the two described in method 3 is not known with certainty, but s-oxide (XVIII) intermediate is probable and such is known to be formed by the action of hydrogen peroxide on thioamides.<sup>169</sup> The sulfur atom in the s-oxide is likely to be more susceptible to intramolecular nucleophilic attack by adjacent nitrogen atom.

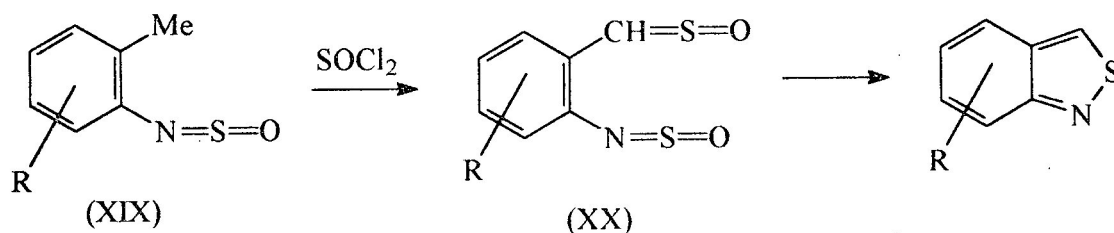


Mechanism of action in which o-toluidine is converted into 2,1-benzisothiazole is not known with certainty but N- sulfinylamine (XIX) is a definite intermediate.



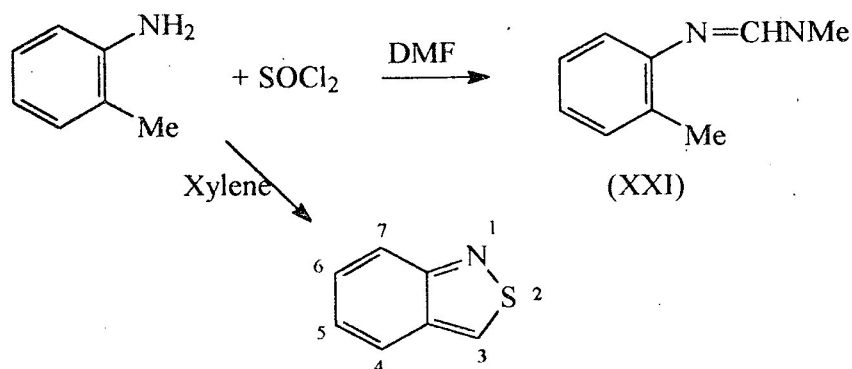
A second equivalent of thionyl chloride is required before cyclization of N-sulfinylamine occurs.

Here it is suggested<sup>168</sup> that N sulfinylamino group is an electron acceptor and this renders methyl group sufficiently acidic to react with second molecule of thionyl chloride. If this is true then one might expect a sulfine (XX) or related compound to be a further intermediate. An analogous sulfine intermediate has been suggested in a related synthesis of thiazoles.<sup>170</sup>



Recently Naito & coworkers<sup>171</sup> studied the use of thionyl chloride and sulfur monochloride for construction of the isothiazole ring in DMF from compound containing an amino group and a relatively activated methylene group.

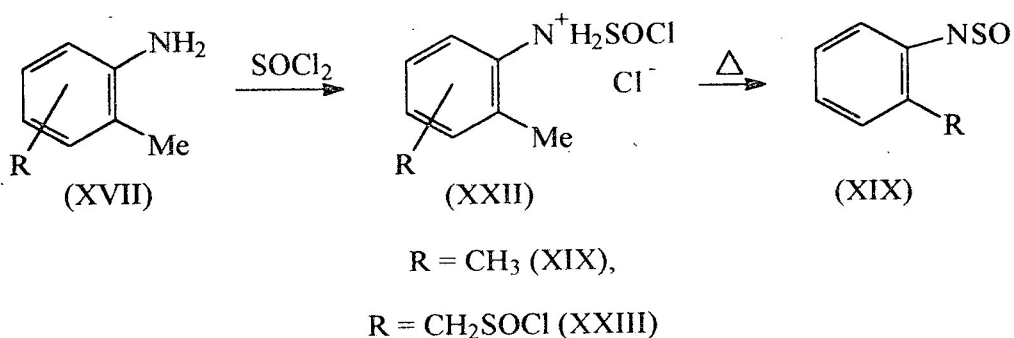
Reaction between (XVII) and thionyl chloride in DMF under general conditions used by the Japanese workers<sup>171</sup> afforded amidine(XXI) as the main product no. 2,1-benzisothiazole could be detected. However Michael Davis and Alex White found<sup>168</sup> during their study that if xylene were used as a solvent, 2,1- benzisothiazole was formed in reasonable yield.



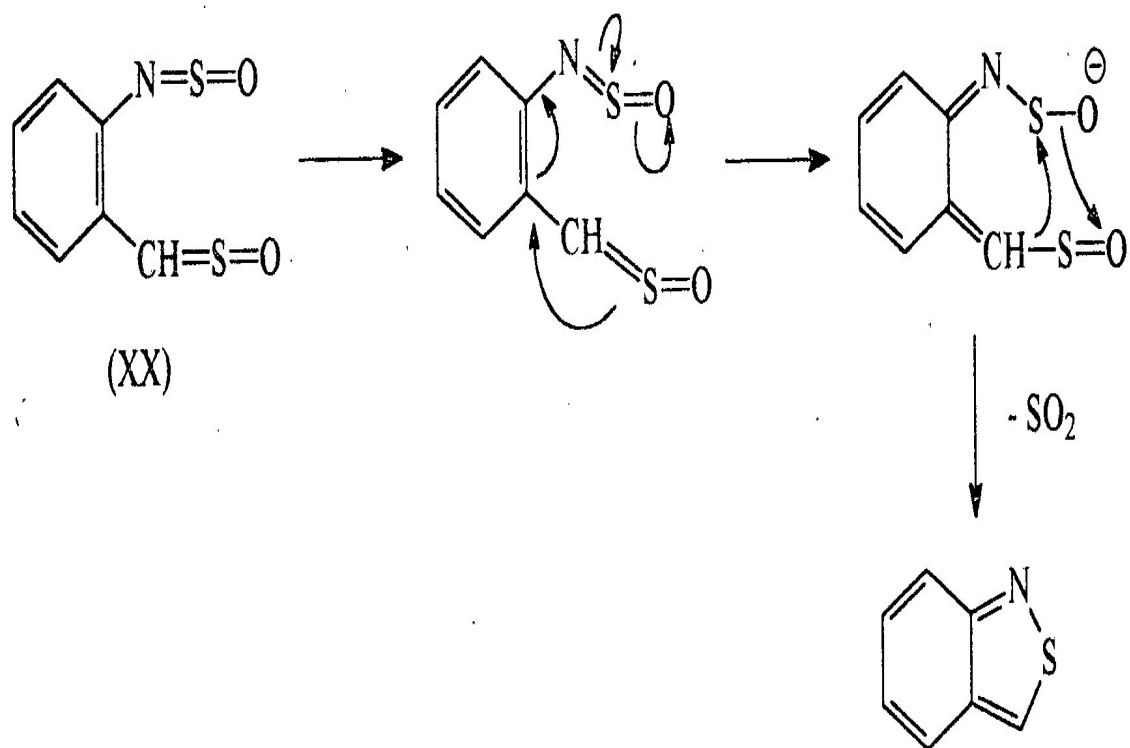
Now we know that substituted 2,1 benzisothiazoles may be prepared from the corresponding substituted 2-aminotoluenes in yield as high as 80 %. So far only 2-amino-5-nitro toluene has failed to yield the desired product.

It is found by Davis and White<sup>168</sup> that molecules such as (XIX) when heated on their own or in high boiling inert solvents do not yield 2,1-benzisothiazole but when heated in presence of excess of thionyl chloride, 2,1-benzisothiazoles are formed, indicating that thionyl chloride or a product of thermal decomposition of thionyl chloride is implicated in the cyclodehydration reaction which generates the heterocyclic ring.

The initial vigorous reaction between (XVII) and  $\text{SOCl}_2$  produces a yellow crystalline compound which is probably the sulfinyl chloride (XXII). This on heating leave HCl forming N-sulfinylamine (XIX). Continued heating with excess thionyl chloride yields sulfinyl chloride<sup>168</sup> (XXIII).



This sulfinyl chloride (XXIII) similarly loses a molecule of HCl resulting in benzyldenesulfinyl (XX) which eliminating molecule of  $\text{SO}_2$ , generating 2,1-benzisothiazole.<sup>168</sup>

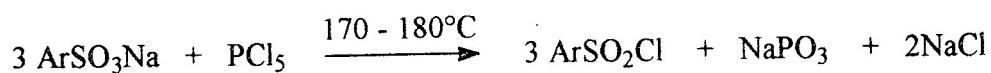


*CHEMISTRY OF  
ACID CHLORIDES*

Acid chlorides are used as intermediates in synthesis. They have good reactivity and are mostly liquids with few exceptions. Acid chloride of adamantyl carboxylic acid is solid. Acid chlorides readily react with ROH and NH<sub>3</sub> to yield esters and amides respectively.

Conversion of acid to acid chloride is a reaction in which there is replacement of hydroxyl group by chloride through the use of inorganic chlorine compounds. Amongst there, thionyl chloride, phosphorous pentachloride, sulphuryl chloride and phosgene gas are most important.

Phosphorous pentachloride is highly reactive and often used for the preparation of acid chlorides. It is commonly used for conversion of aryl sulphonic acids as well as their sodium salts to corresponding acid chlorides.<sup>172</sup>

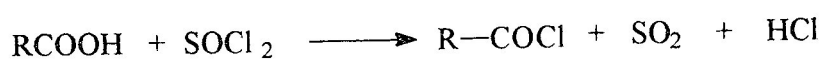


The products of the reaction are phosphorous oxychloride and hydrogen chloride.



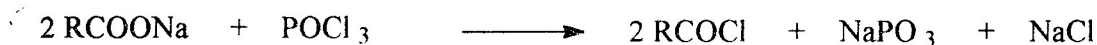
Phosphorous oxychloride can be removed by distillation under reduced pressure (25°C / 120 mm Hg).<sup>173</sup>

The use of thionyl chloride<sup>174</sup> for the preparation of acid chlorides presents many advantages. The reaction results in the formation of sulfur dioxide and hydrogen chloride in addition to acid chloride and hence isolation of acid chloride is simple.

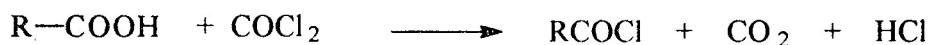


Thionyl chloride must be pure, as impure product has been known to cause explosive decomposition in some instances. The procedure involves heating the acid in an inert solvent with thionyl chloride. The excess of thionyl chloride can be distilled off.

Phosphorous oxychloride, reacting with the sodium salt of carboxylic acid gives acid chlorides. This method is of importance with acids which undergo decomposition with other chlorides of phosphorous.

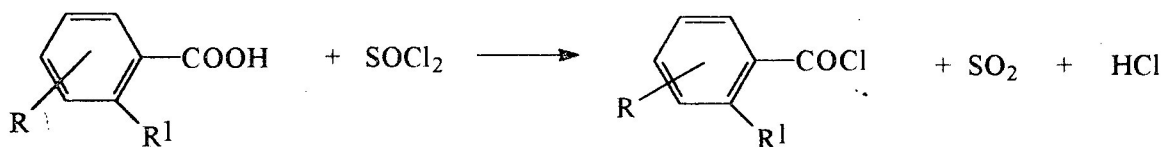


The reaction of phosgene<sup>176</sup> with carboxylic acids and p-toluene sulfochloride or of sodium chlorosulfonate with salts of carboxylic acids are also used for the preparation of acid chlorides.

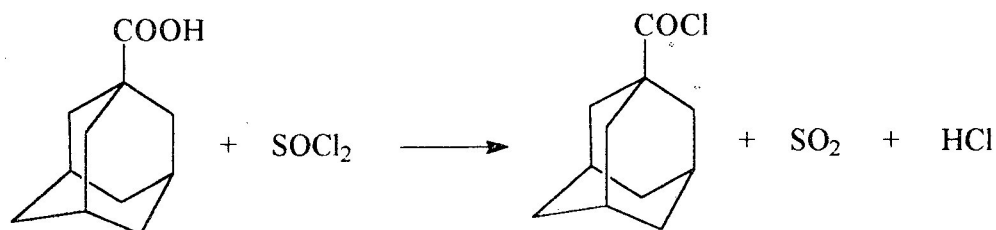


The acid chlorides can also be prepared by action of sulfur chloride with anhydrous salts of organic acids.

In present work, salicyloyl chlorides, benzoyl chlorides and adamantyl carboxylic acid chloride were prepared by reacting these acids with thionyl chloride in benzene.



$\text{R} = \text{F}, \text{Cl}, \text{Br}, \text{I} \text{ etc.}, \text{H}, \text{R}^1 = \text{H} \text{ or } \text{OH}$



Catalytic amount of tertiary base is used in the preparation of acid chloride. Pyridine is an essential catalyst for conversion of phthalic or succinic acids into the corresponding diacid

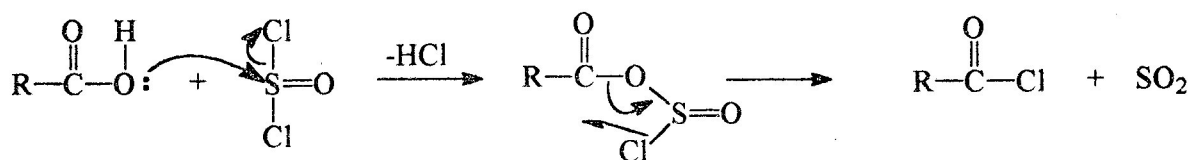
chloride. In the absence of catalyst anhydride is obtained. Pyridine is also necessary for conversion of aromatic hydroxy acids into corresponding acyl chlorides.

In present work, pyridine in catalytic amount was added to trap HCl liberated during reaction.

### REACTION MECHANISM



Hydroxyl group cannot be displaced directly by  $\text{Cl}^-$  as it is a very poor leaving group i.e. a strong base. With thionyl chloride, reaction of the following type occurs to provide better leaving group in place of hydroxyl group.

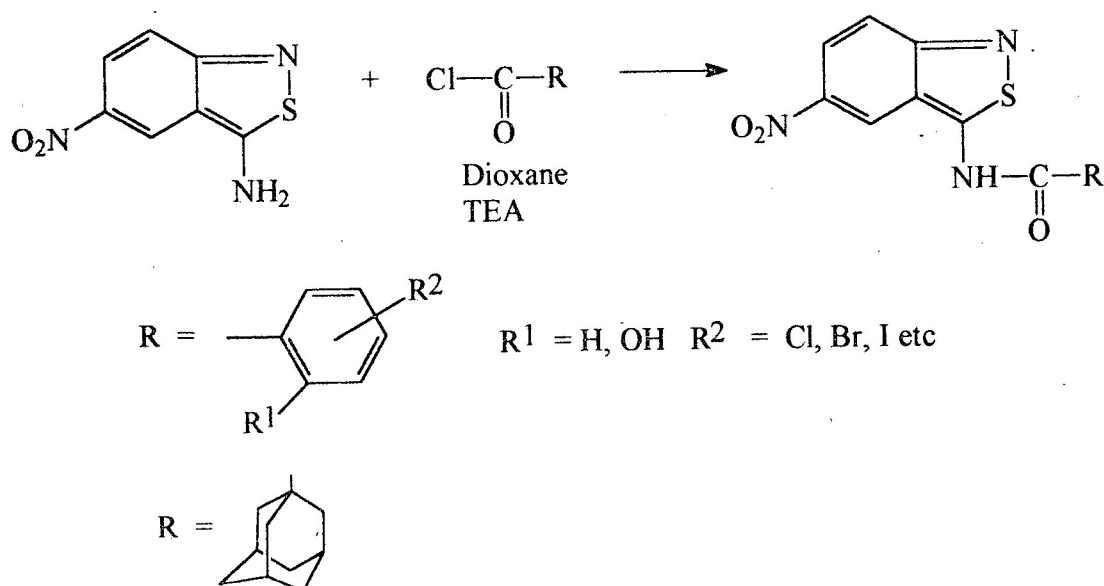




*CHEMISTRY OF  
3-SUBSTITUTED AMINO  
-5-NITRO-2,1-  
BENZISOTHIAZOLE*

Benzisothiazoles have been found to possess variety of biological activity such as antifungal,<sup>105</sup> antibacterial,<sup>105</sup> herbicidal,<sup>177</sup> insecticidal<sup>180</sup> pesticidal<sup>107</sup> and trichomonocidal<sup>179</sup>. Nitroheterocycles have exhibited antiparasitic, antiprotozoal, antibacterial action. Amide linkage in oxyclozanide,<sup>112</sup> clioxanide<sup>181</sup> has played essential role in their anthelmintic action.

In view of this, few 3-substituted amino-5-nitro-2,1-benzisothiazoles were synthesized by condensing various acid chlorides with 3-amino-5-nitro-2,1-benzisothiazole in nonpolar solvent at elevated temperature. The synthetic route can be represented as below :-



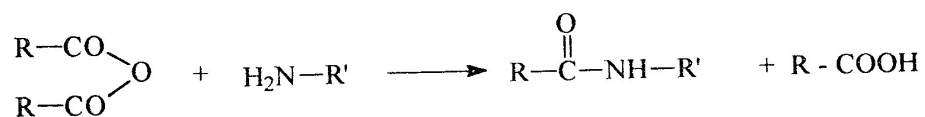
Heterocyclic amines are in general less reactive than aromatic amines. 3-amino-5-nitro-2,1-benzisothiazole has less solubility in most of the organic solvents. Refluxing of amine with acid chloride in dioxane was found to be the best method for interaction of amine with acid chloride.

Hydrogen chloride liberated in the reaction was trapped by triethylamine forming hydrochloride salt. Triethylamine hydrochloride  $[(\text{C}_2\text{H}_5)_3\text{N}^+\text{HCl}^-]$  is highly soluble in water. Product being insoluble in water can be isolated easily by dumping the reaction in water.

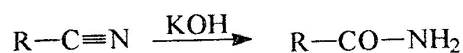
3-amino-5-nitro-2,1-benzisothiazole is red in colour whereas its amides are yellow, orange or brown in colour.

Use of pyridine in place of triethylamine is also very common. Other methods of synthesis of amide include -

- 1) Action of amine on ester.
- 2) Action of acid anhydride on amine

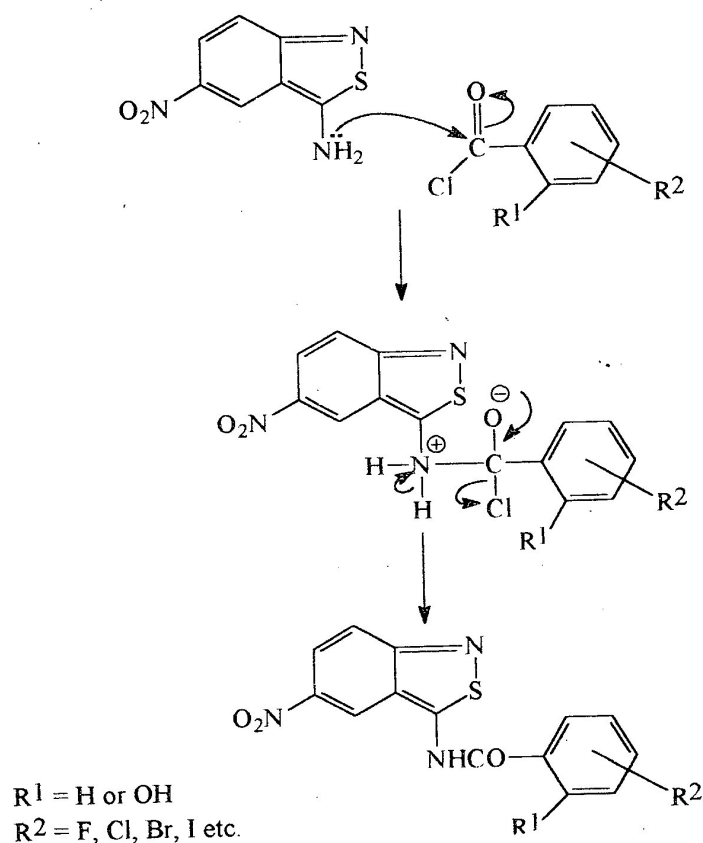


- 3) Hydrolysis of nitriles in alkali

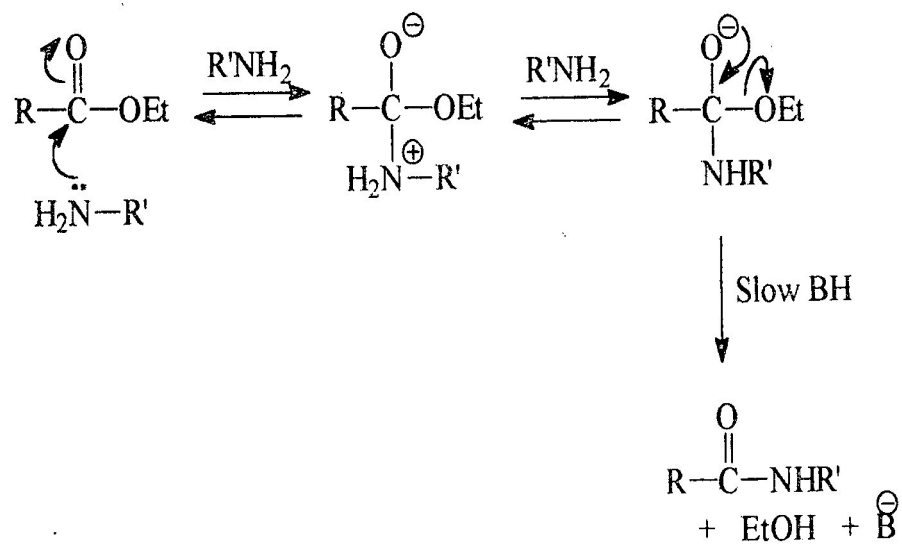


#### Mechanism of Reaction :

- 1) Acid chlorides are readily attacked at carbonyl carbon by nucleophile leading to formation of an amide linkage and elimination of hydrogen chloride gas.



2) Reaction between an amine and ester is as follows : -



*CHEMISTRY OF  
SCHIFF BASES*

Schiff bases are formed by the condensation of amine and carbonyl group of aldehyde.



Schiff,<sup>182</sup> for the first time reported condensation of this kind and hence the condensation products are referred to as 'Schiff bases'

Pelton et al had shown<sup>183</sup> the antifungal and antibacterial activity of some substituted salicylaldehydes. Schiff bases from different aldehydes have shown encouraging antituberculosis activity *in vitro* in our laboratory.<sup>118</sup> The presence of azomethine linkage in compounds has been shown to decrease their toxicity considerably.<sup>119</sup>

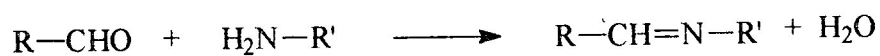
Many procedures have been described in literature for the synthesis of Schiff bases. Important methods are listed as below :

- 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 Clapp,<sup>184</sup> obtained Schiff bases by refluxing the amine and aldehyde in toluene with a few drops of piperidine. The water formed during the course of reaction was removed by employing a Dean Stark trap.
- 3) In 1950, Hayes et al<sup>185</sup> prepared Schiff bases by heating a mixture of an aldehyde and an amine in benzene and removing water formed during the reaction using Dean Stark apparatus.
- 4) In 1953, Giovambettista and Rabassa<sup>186</sup> suggested acetic acid as a catalyst for the synthesis of Schiff bases in good yield.
- 5) In 1971, Condensation of hydrazine and an aldehyde using glacial acetic acid as a solvent has been described.

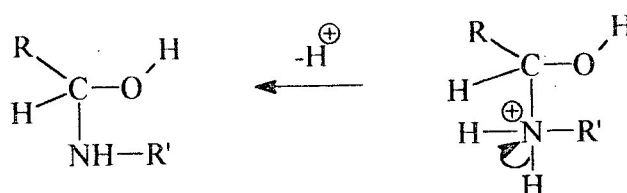
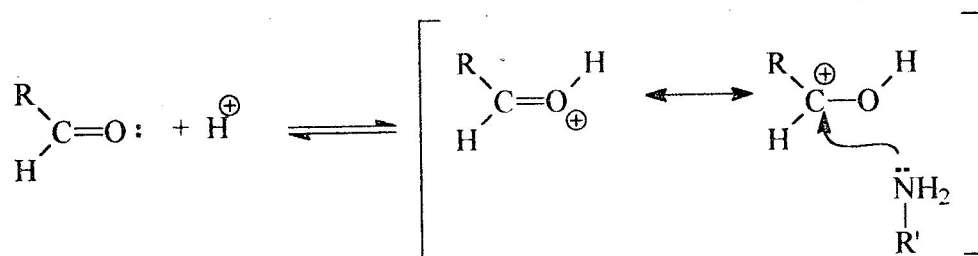
As the first method was found to give good yields, it was followed initially. Addition of few drops of acetic acid improved the yield. Later on, during the course of investigation last method using acetic acid as a solvent was found to be the best for following reasons.

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

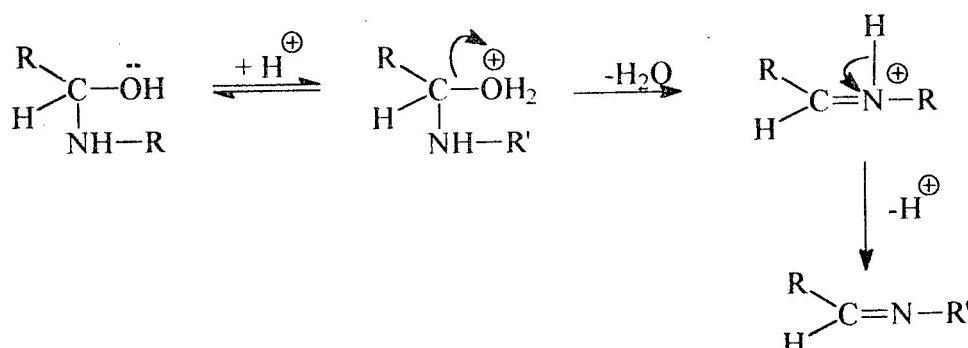
### Reaction Mechanism



Heating amine and an aldehyde in presence of acid. Acid protonates carbonyl oxygen and promotes the attack of nucleophilic amine.



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

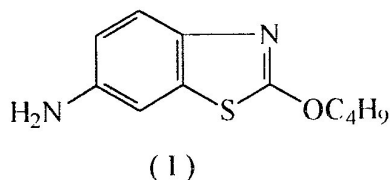


*CHEMISTRY OF  
2-AMINO-6-  
PROPOXYBENZOTHAZOLE*

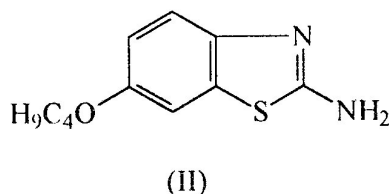


Benzothiazole derivatives are known for their antiparasitic,<sup>187,188</sup> antibacterial,<sup>189,190</sup> antiviral,<sup>191</sup> antifungal<sup>192</sup> and antituberculostatic<sup>193,194</sup> activity.

2-Butoxy-6-amino benzothiazole (I) showed *in vitro* antituberculosis activity<sup>196</sup> at 0.02 µg/ml.

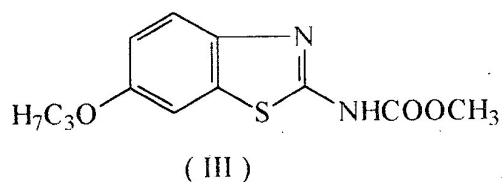


2-Amino-6-substituted benzothiazole of the following type (II) showed<sup>196</sup> minimum inhibitory concentration ( *in vitro* ) at 0.025 mg %.



2-Amino-6-propoxy benzothiazole has been reported in the literature<sup>197</sup> as an intermediate of a well known anthelmintic drug, tioxidazole (III). Tioxidazole is chemically methyl-6- propoxy benzothiazole-2-carbamate and is prepared by various standard procedures. In present work, Tioxidazole was prepared by two different routes.

- 1) From 2-amino-6-propoxy benzothiazole.
- 2) From methyl-6-hydroxy benzothiazole-2-carbamate.

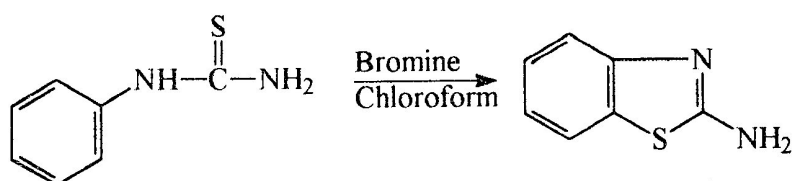


#### Various procedures for the preparation of 2-amino-6-substituted benzothiazole

- 1) Thiocyanation
- 2) Hugerschhoff synthesis<sup>198,199</sup>:- This synthesis is restricted to the preparation of 2 amino derivatives of the benzothiazole and involves use of oxidizing agent such as bromine. Bromine

reacts with arylthiourea in an inert solvent, such as chlorobenzene, chloroform, ethylenedichloride or acetic acid. Sulfuryl chloride can be used as a substitute for bromine.<sup>200-202</sup>

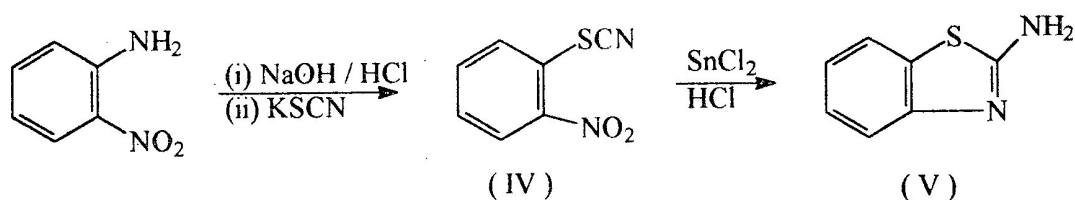
However, potassium ferricyanide in alkaline media, the reagent for the Jacobson ring closure, gives no thiazole from the arylthioureas.<sup>198,199</sup>



Yields in this reaction are quantitative

3) 2-Aminobenzothiazole(V) has been prepared also from o-thiocyanonitrobenzene compounds of the type (IV) by chemical reduction (stannous chloride in hydrochloric acid and ferrous sulfate in ammonia).<sup>203,204</sup>

The yield was 60 - 85 %.



1) Thiocyanation : Thiocyanation is a simple electrophilic reaction showing close similarity with halogenation. Therefore reaction is often called pseudohalogenation and the reagent as pseudohalogen.

The direct substitution of hydrogen atom by a thiocyano group (-SCN) through the use of thiocyanogen (SCN)<sub>2</sub> is commonly referred as thiocyanation.

Thiocyanogen is extremely unstable liquid which on cooling forms a colourless crystal solid melting between -3°C and -2°C. At room temperature, it polymerizes readily to reddish orange mass of indefinite composition known as pseudothiocyangen. Although stable in inert dry

solvents, thiocyanogen may polymerize in solution, especially under the catalytic effect, influence of heat, light and moisture.

Due to the extreme sensitivity of thiocyanogen towards hydrolysis and polymerization, it is employed in solution and more commonly is produced in situ.

#### **Methods for the use of thiocyanogen :**

Two general methods, which can be practically applicable, have been described for the use of thiocyanogen in substitution reaction.

- 1) Free thiocyanogen in organic solvents.
- 2) Thiocyanogen liberated gradually in an organic solvent from a metal thiocyanate by various reagents.

#### **1) Free thiocyanogen :**

This method was the first to be employed and is still a useful procedure. Usually the only contaminant of the product other than starting material is polythiocyanogen which is entirely insoluble in water and organic solvents.

The reagent is prepared by the action of an oxidizing agent upon thiocyanic acid or a metal thiocyanate. The oxidation of thiocyanic acid in an organic solvent is accomplished by means of such reagents as lead tetraacetate, lead peroxide or manganese dioxide<sup>205</sup> but the yield is so low that the preparation of metal thiocyanate is much to be preferred. Lead thiocyanate reacts rapidly and quantitatively with bromine to form thiocyanogen and lead bromide which is removed readily by filtration. Halogen carriers such as phenyliodo chlorides,<sup>206</sup> sulfuryl chloride<sup>207</sup> or certain N - chloroamides<sup>208-210</sup> can also be used but do not appear to possess any advantage over bromine or chlorine.

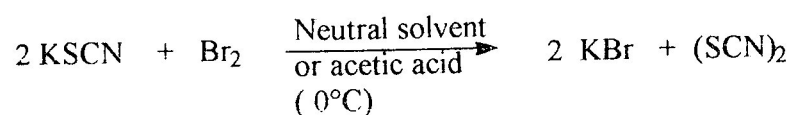
Solvents that have been used with thiocyanogen include benzene, bromobenzene, carbon tetrachloride, chloroform, ether, ethylene bromide, carbon disulfide, petroleum ether, methyl acetate, nitromethane, anhydrous formic acid and acetic acid.

The yield of thiocyanation of amine is 20-30 % higher when reaction is carried out in a neutral medium like methanol rather than in a acetic acid. The formation of thiazole is also inhibited in a neutral solvent. Ether is usually not satisfactory because the solvent is attacked and because some of the amine is precipitated as the thiocyanate.<sup>211,212</sup>

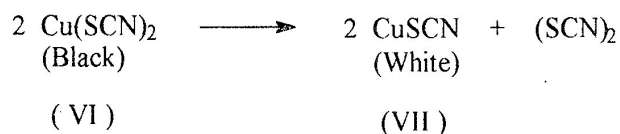
Moisture must be excluded from thiocyanation solution in order to prevent hydrolysis. Another troublesome side reaction particularly in concentrated solutions is polymerization, which is induced by light, heat and the presence of hydrolysis products. Polymerization is reported to be dependent upon dielectric constant of the solvent.<sup>211,213,214</sup> Stirring is said to retard polymerization. For the usual synthetic reaction the preferred procedure is to generate thiocyanogen in a solution of the substance to be thiocyanated at a rate equal to the rate of removal by reaction. The low concentration of reagent maintained in this way, minimizes polymerization. The reagent is generated from the thiocyanate salts by a chemical reaction.

## 2) Thiocyanogen generated from salts by chemical reaction :

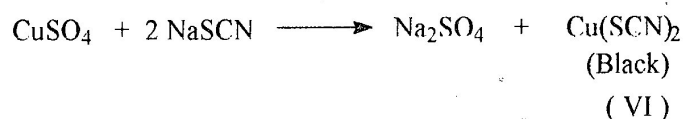
In this method the alkali metal thiocyanate is dissolved in acetic acid or formic acid<sup>212,215</sup> or better in neutral solvent like methyl acetate, acetone or methanol.<sup>216,217</sup> The compound to be thiocyanated is introduced to the above solution. Bromine or chlorine is added to the cooled solution at such a rate that, thiocyanogen reacts as fast as it is liberated. The neutral solvent that is susceptible to attack by halogen is protected by saturating the solution with an appropriate alkali metal halide or by using large excess of an alkali metal thiocyanate in the reaction mixture. Other reagents for producing thiocyanogen from ammonium thiocyanate have been described and include N, N - dichloro urea,<sup>218</sup> N- chloroacetamide<sup>208</sup> etc. The chemical transformation can be written as below.



Cupric thiocyanate,<sup>219</sup> the use of which may be considered still another modification of this general procedure shows promise of being very effective. It releases thiocyanogen merely by the dissociation of the cupric (VI) to cuprous salt (VII)



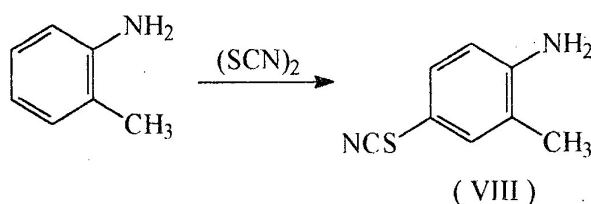
Cupric thiocyanate (VI), prepared in advance, or a paste of copper sulfate and sodium thiocyanate in equivalent proportions is added to a solution of the compound in methanol or acetic acid and the mixture is warmed to 35-80°C until black cupric thiocyanate has changed completely to the white cuprous thiocyanate.



This procedure has the advantage over the others of permitting higher temperature for thiocyanation.

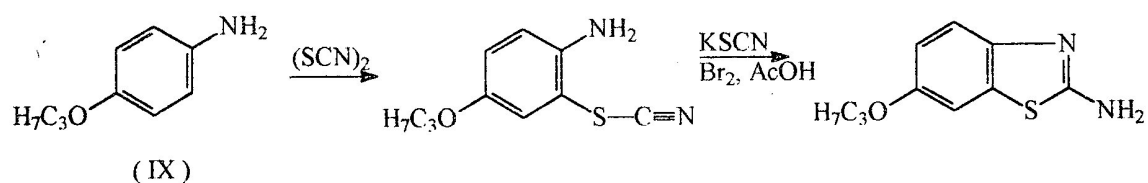
#### Thiocyanation of aromatic amines :

The thiocyano group (-SCN) is introduced into aromatic amines with rapidity. It enters preferentially at a free para position if available, otherwise at ortho position. For example, o-toluidine is converted to 4- thiocyano-o-toluidine (VIII) ( 80 % yield).<sup>208</sup>



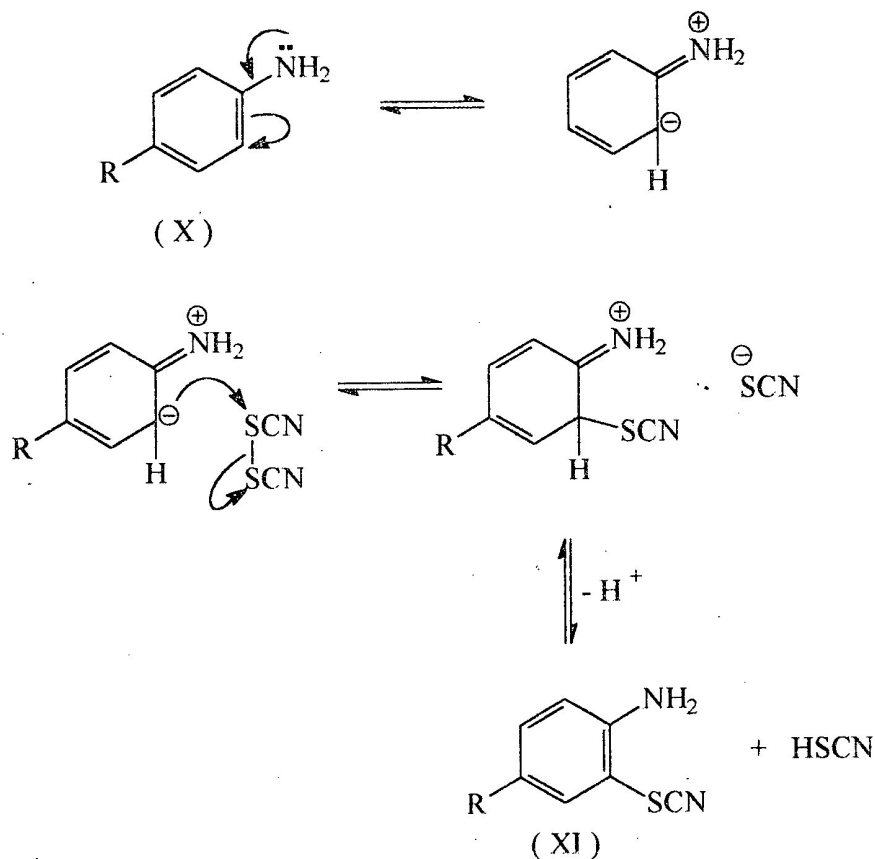
When thiocyanation takes place in position ortho to a primary amino group as in p-toluidine, p-chloroaniline etc., the final product is often a benzothiazole formed by a secondary reaction between the amino group and the adjacent thiocyano group.

In the present work, p-propoxy aniline (IX) is thiocyanated by means of thiocyanogen generated from the action of KSCN and bromine in acetic acid at cold temperature.



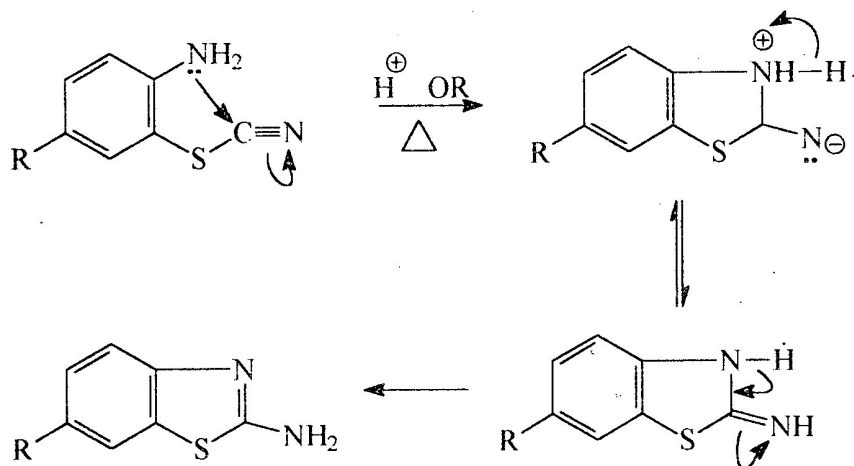
### Reaction Mechanism :

The mechanism of thiocyanation reaction probably consists the initial attack of thiocyanogen to the activated site of 4-substituted aniline (IX) where, due to the mesomeric effect of amino group, the ortho position is having surplus of electrons which rendered the site to act as good electron rich center, to give an intermediate species as shown below.



The unstable intermediate immediately loses a proton to retain the aromatic character of the molecule which gives higher stability.

(XI) under the influence of acid and heat, add a  $\text{NH}_2$  function to the adjacent thiocyano group as shown below.

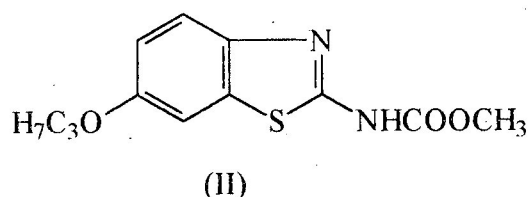
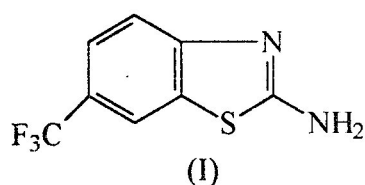


*CHEMISTRY OF  
2-AMINO-6-  
HYDROXYBENZOTHIAZOLE*



Benzothiazoles are known for their manifold medicinal activity. In our laboratory it has been reported earlier<sup>104</sup> that 6 - chloro- 2 -substituted amino benzothiazoles have promising antitubercular activity. Some 2 - substituted amino benzothiazoles have also been reported by Mikulasek S. *et al*<sup>220</sup> as antimycobacterial agents.

2- Amino - 6- hydroxy benzothiazole is a special one which may serve as an intermediate for two very well known drugs 1) Riluzole (I) and 2) Tioxidazole (II).



With this view, synthesis of 2 amino-6-hydroxy benzothiazole was carried out successfully in our laboratory.

#### Methods of preparation of substituted amino hydroxy benzothiazoles :-

- 1) 2- Dimethylamino-6-hydroxy benzothiazole is prepared<sup>221</sup> by heating bis (p-ethoxyphenyl) thiourea with HCl and subjecting the resulting p-ethoxy phenylthiocyanate to condensation with dimethylamine. The N,N'-dimethyl-N'-(p-ethoxy phenyl) thiourea formed is cyclized into 2-dimethylamino-6-ethoxy benzothiazole by treatment with bromine with subsequent cleavage of latter by heating with HBr.
- 2) 6  $\overline{\text{RONC}_6\text{H}_3\text{SC(NMe}_2\text{)}}$  (R = Me) and HI are refluxed for 10 hrs and then cooled. Water is added (twice the volume of above mixture). SO<sub>2</sub> gas is passed with cooling. Ammonium hydroxide is added and precipitate is filtered off (R = H) to give 2-dimethylamino-6-hydroxy benzothiazole.<sup>222</sup>

Methods one and two deal with dealkylation of alkoxy benzothiazoles to give hydroxy benzothiazole. Apart from above mentioned demethylating agents, some other demethylating agents have been reported in literature<sup>225</sup> such as 1) Aluminium trichloride 2) Boron trichloride 3) Chloroformic acid esters 4) Hydrochloric acid 5) Iron (III) Chloride 6) Lead tetraacetate 7) Lithium t-butylthiolate 8) Magnesium iodide 9) Piperidine 10) Pyridine-phosgene 11) Quinuclidine 12) Thiophenol etc.

Demethylation reactions of 2-amino-6-methoxy benzothiazole prepared in our laboratory by 47% HBr and  $\text{AlCl}_3$  were found to be unsuccessful.

#### **Methods of preparation of aminohydroxy benzothiazole :-**

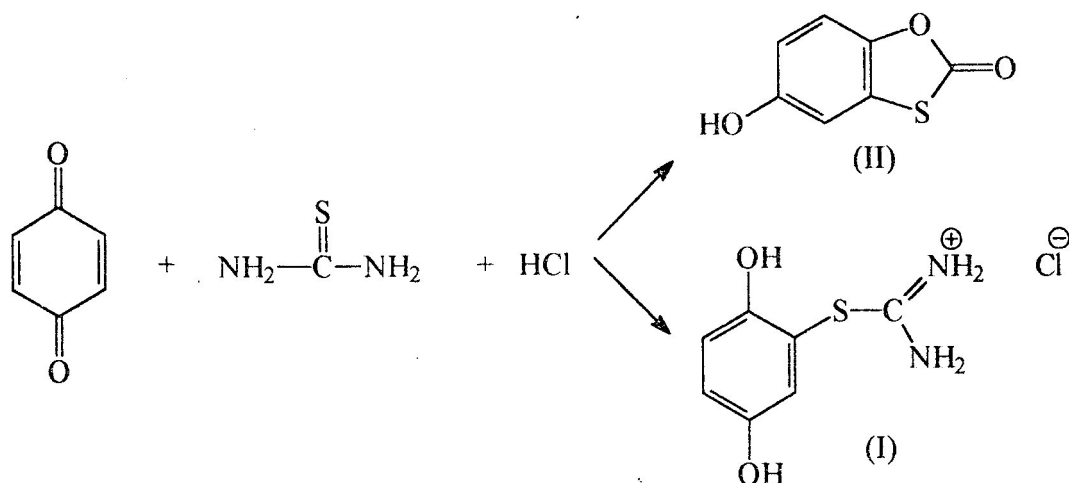
- 1) Cyclization of hydroxyphenylthioureas with bromine gives<sup>223</sup> corresponding 2- amino-6-hydroxy bromobenzothiazoles and not 2-amino-6-hydroxy benzothiazoles. Synthesis of 2-amino- 6 - hydroxy benzothiazole is described in literature in details.<sup>223</sup>
- 2) The reaction of thiourea with excess of 1,4 benzoquinone in presence of conc. HCl offers a convenient route for the synthesis of variety of 2- amino-6-hydroxy benzothiazole.<sup>224</sup>

The last method gives one pot synthesis of 2-amino-6-hydroxy benzothiazole. No special apparatus is needed for the synthesis and hence it is found to be the best amongst all.

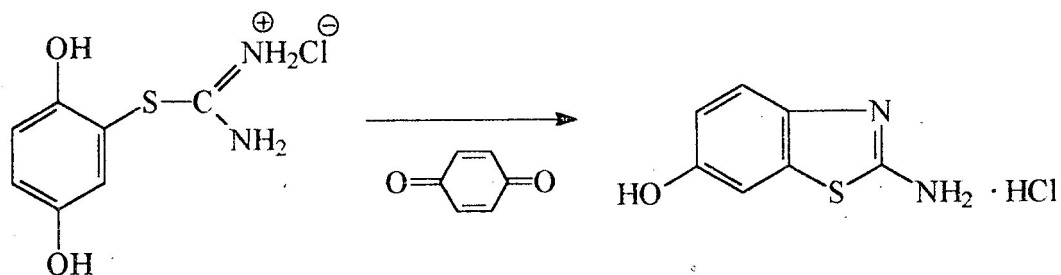
#### **Reactions of benzoquinone with thiourea**

Very interesting data<sup>224</sup> is available for the reactions of benzoquinone with thiourea.

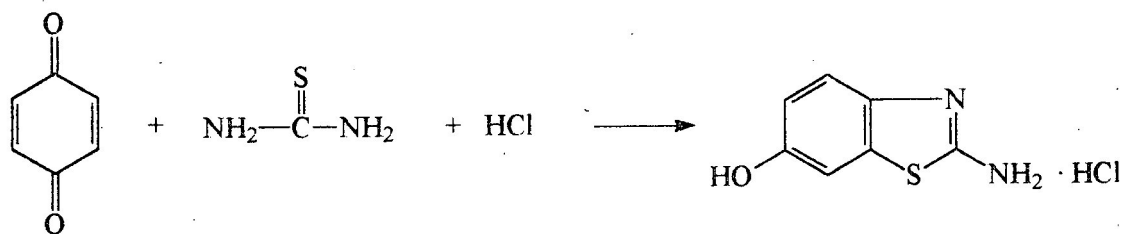
The reaction of benzoquinone with excess of thiourea in presence of aq. mineral acid affords, depending upon the reaction conditions, a wide variety of S-( 2,5-dihydroxyaryl) thiouronium chlorides (I) and 5 - hydroxy -1,3- benzoxathiol-2-ones (II)



When thiourea is treated with 1 molar excess of benzoquinone in presence of mineral acid, none of the compound I or II was formed. Instead, a new heterocyclic compound, 2- amino-6-hydroxy benzothiazole was isolated in yield greater than 70%. The same compound is obtained when thiouronium salt (I) in ethanol is treated with equimolar solution of benzoquinone which suggests that the thiouronium salt is an intermediate in this reaction.



So, the overall reaction can be written as,



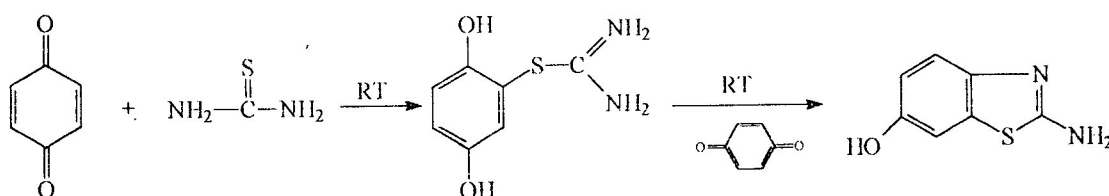
Change in the molar ratio of the reactants has been reported to affect yield to a great extent.<sup>224</sup>

| Quinone mole | Thiourea mole | HCl mole | Yield %        |
|--------------|---------------|----------|----------------|
| 2.5          | 1.0           | 1.0      | 73             |
| 2.0          | 1.0           | 1.0      | 76             |
| 1.5          | 1.0           | 1.0      | 75             |
| 1.0          | 1.0           | 1.0      | 0 <sup>a</sup> |
| 1.5          | 1.0           | 0.0      | 0 <sup>b</sup> |
| 1.5          | 1.0           | 0.5      | 31             |
| 1.5          | 1.0           | 2.0      | 75             |

a = S-(2,5- dihydroxy phenyl) thiouronium chloride.

b = black insoluble amorphous material.

The reaction between thiourea and benzoquinone proceeds via an intermediate i.e. S-(2,5 dihydroxyary) thiouronium chloride and hence the overall reaction can be written as :



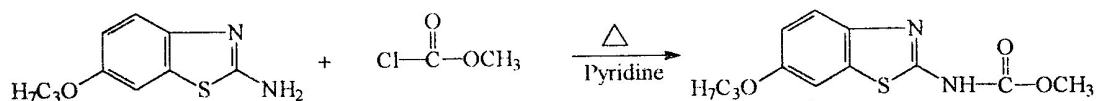
Experimentally it was observed that best product was obtained with freshly prepared benzoquinone.

*CHEMISTRY OF  
METHYL-6-  
PROPOXYBENZOTHIAZOLE-  
2-CARBAMATE*

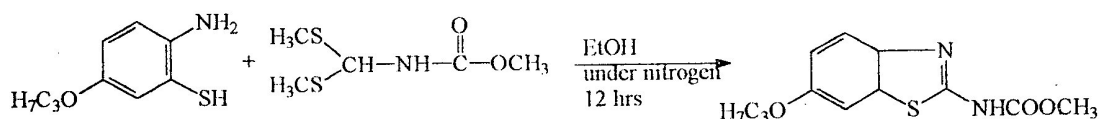
Tioxidazole is chemically methyl-6-propoxy benzothiazole-2-carbamate having broad spectrum activity against gastrointestinal round worms.<sup>197</sup> It is found to be effective against *Trichinella spiralis* and *Strongyloides ratti* in mice and against hookworms in dogs. Single oral doses of 5-25 mg / kg were effective in eliminating over 90 % of *Parascaris equorum*, *Oxyuris equi*, *Strongylus vulgaris* etc. in horse. Dose levels upto 100 mg/kg have had no adverse effects in the horse.<sup>197</sup>

Tioxidazole is prepared by various standard procedures :-

1) 2- Amino- 6- propoxy benzothiazole is reacted with methyl chlorocarbonate in pyridine as solvent.<sup>197</sup>



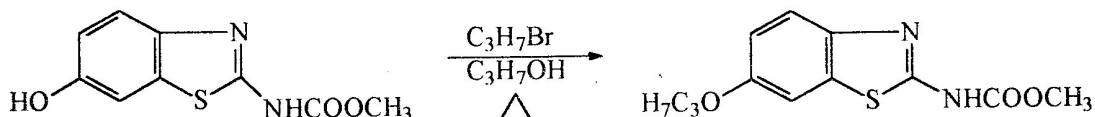
2) Cyclocondensation of 2-mercapto-4-propoxyaniline with methyl-N-dimethyldithiomethyl carbamate in refluxing ethanol under nitrogen gives methyl-6-propoxy benzothiazole-2-carbamate after twelve hours.<sup>226</sup>



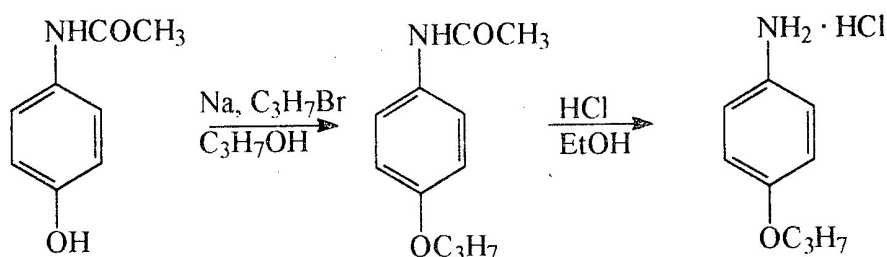
In present work, tioxidazole was prepared by two different routes :-

A) First route : Reaction of 2-amino-6-propoxy benzothiazole with methyl chloroformate as mentioned earlier.

B) Second route : Propylation of 6- hydroxy benzothiazole - 2- carbamate.

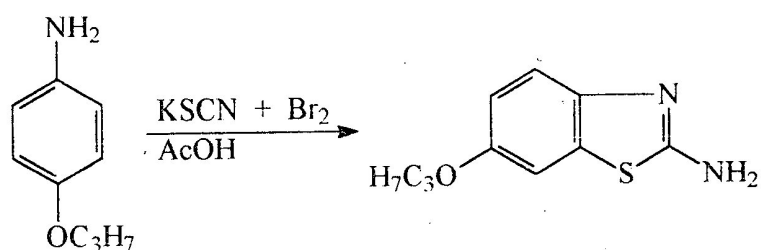


In the first route p-propoxyaniline was synthesized from paracetamol.

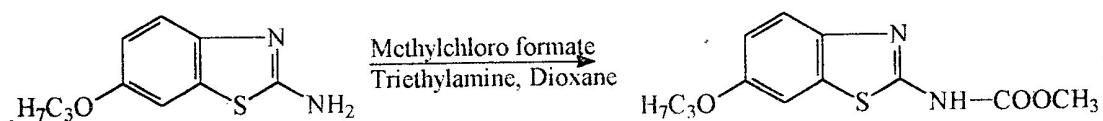


Paracetamol in propanol was reacted with n-propylbromide in presence of reactive sodium to give p-propoxy acetanilide. Acid hydrolysis of p-propoxy acetanilide was carried out to give hydrochloride salt of p-propoxyaniline.

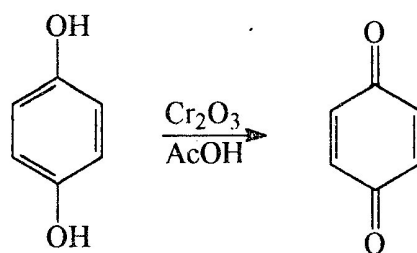
p-Propoxyaniline was thiocyanated by means of potassium thiocyanate and bromine in acetic acid to give 2-amino-6-propoxy benzothiazole.



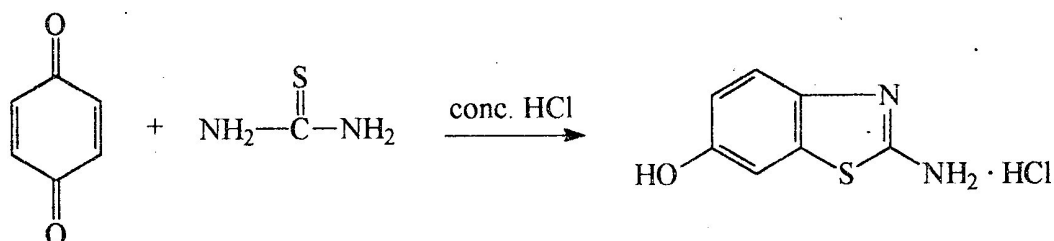
2-Amino-6-propoxy benzothiazole was reacted with methyl chloroformate in dioxane in presence of triethylamine to give methyl-6-propoxy benzothiazole 2-carbamate or tioxidazole.



In the second route, hydroquinone was oxidized by means of chromium trioxide in aq. acetic acid to benzoquinone.<sup>227</sup> Such freshly prepared benzoquinone is yellow in colour and turns dark when exposed to atmosphere.



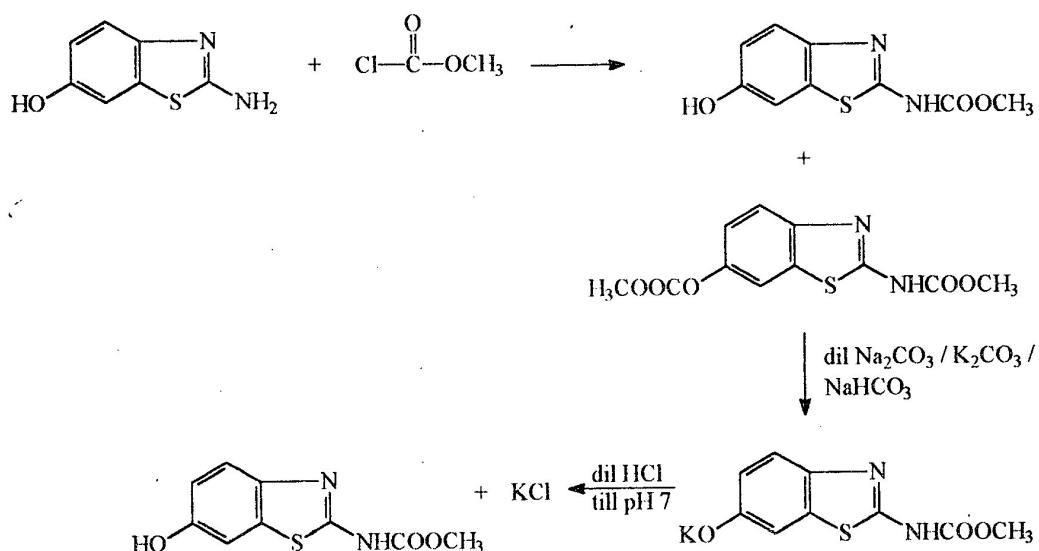
Two moles of benzoquinone were reacted with one mole of thiourea in presence of conc. HCl to form hydrochloride salt of 2- amino- 6- hydroxy benzothiazole.<sup>224</sup>



2-Amino- 6 -hydroxy benzothiazole in dioxane and triethylamine was refluxed with methyl chloroformate.

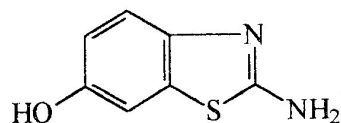
Hydrogen from hydroxyl group is also likely to get displaced by alkyl chloroformate. The product obtained was therefore treated with dilute alkali in cold to hydrolyze such type of bond. The carbamate linkage was not affected by dilute alkali. Then the compound was reprecipitated by addition of acid to the neutralization point.





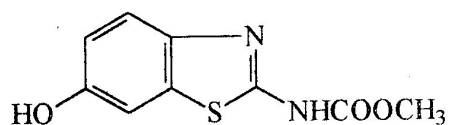
Evidence for disubstitution on 2-amino-6-hydroxy benzothiazole.

1)



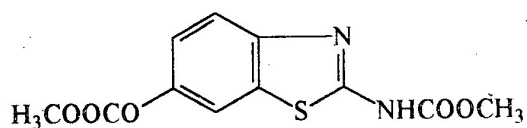
IR Spectra (KBr)  $\text{cm}^{-1}$   
 3450 (-OH), 3300 (-NH<sub>2</sub>)

2)



Expected IR spectra (KBr)  $\text{cm}^{-1}$   
 3450 (-OH), 1738 (-NHCOOCH<sub>3</sub>)

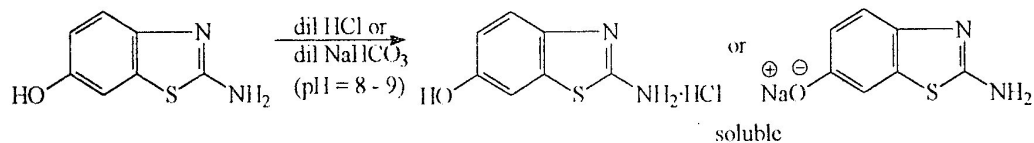
3) Actual product



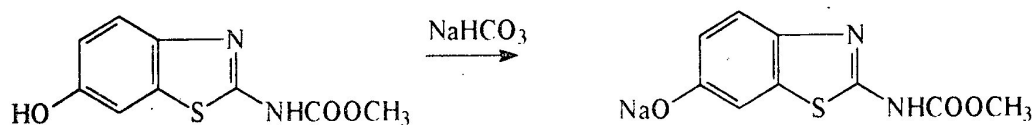
IR spectra(KBr)  $\text{cm}^{-1}$   
 1720 and 1750 indicating presence of dicarbonyl group. Absence of 3450 (-OH) is also indicated the disubstitution.

Above spectral data indicated the presence of disubstitution on 2-amino-6-hydroxy benzothiazole which was supported by solubility tests as follows.

- 1) 2-Amino-6-hydroxy benzothiazole is soluble in dil alkaline or dil. acidic solutions.

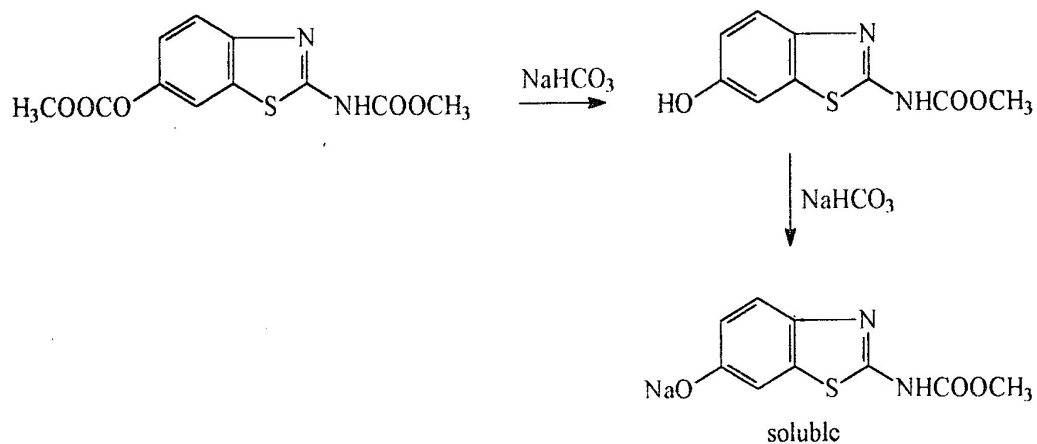


- 2) Expected product methyl -6 - hydroxy benzothiazole -2- carbamate was expected to go in solution with ease in presence of dilute alkali



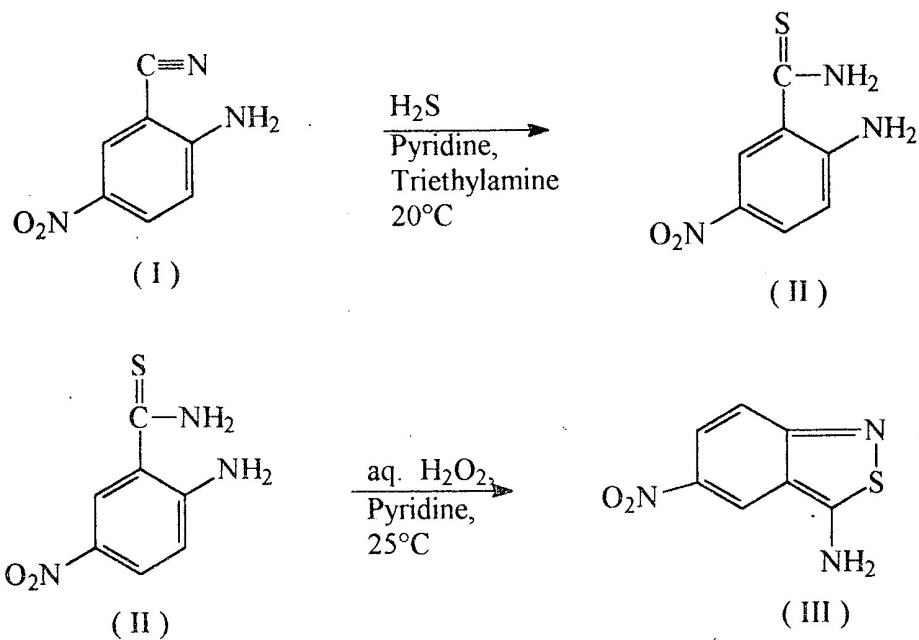
But this was not the case with the product.

- 3) The product obtained was found to take sometime to go in solution in presence of alkali at pH >10 in cold condition. So the time taken can be considered as time required for hydrolysis.



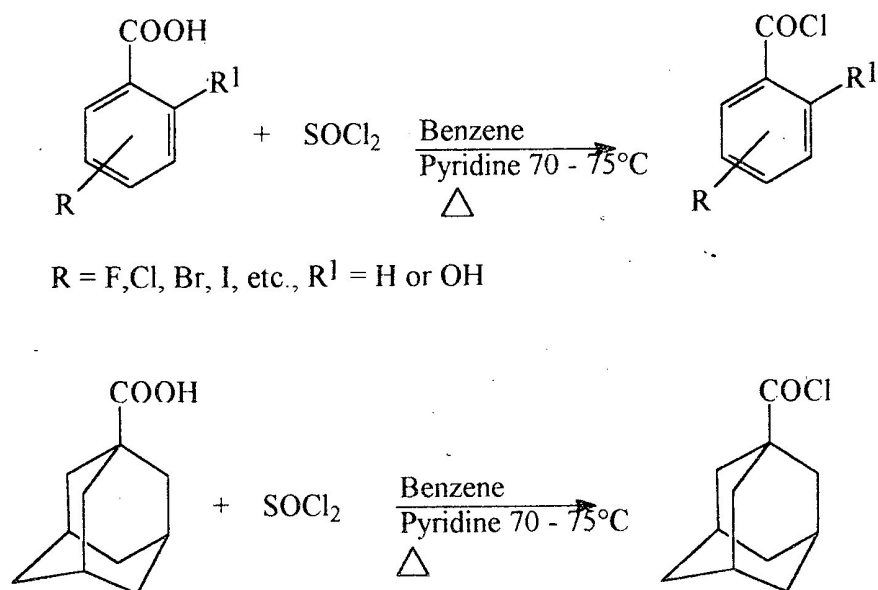
### SCHEME I

Synthesis of 3-amino-5-nitro-2,1-benzisothiazole



### SCHEME II

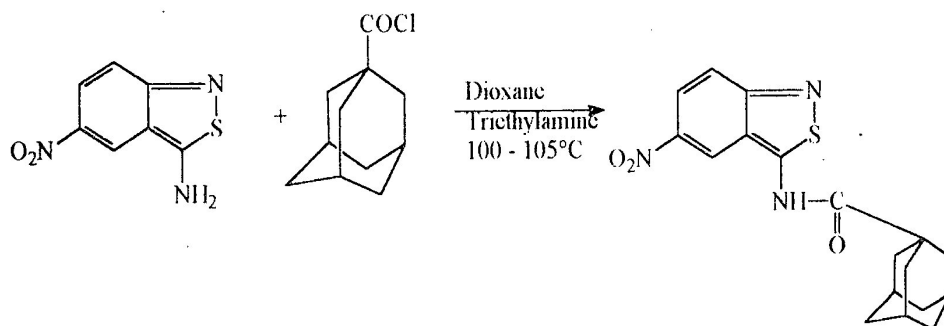
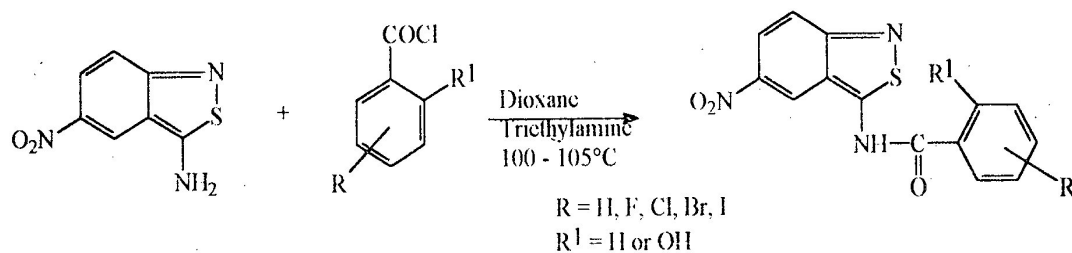
Synthesis of acid chlorides of unsubstituted and substituted salicylic acids, substituted benzoic acids and adamantyl carboxylic acids



$R = \text{F, Cl, Br, I, etc.}, R^I = \text{H or OH}$

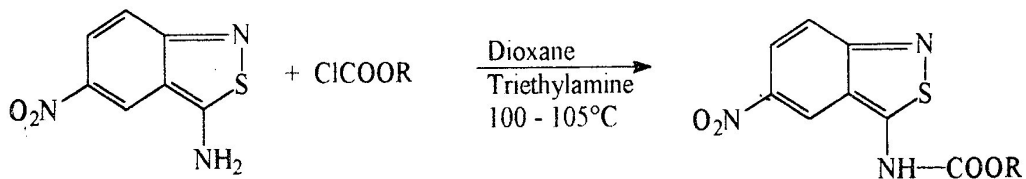
### SCHEME III

Synthesis of 3-(substituted phenyl, adamantyl carboxamido)-5-nitro-2,1-benzisothiazole

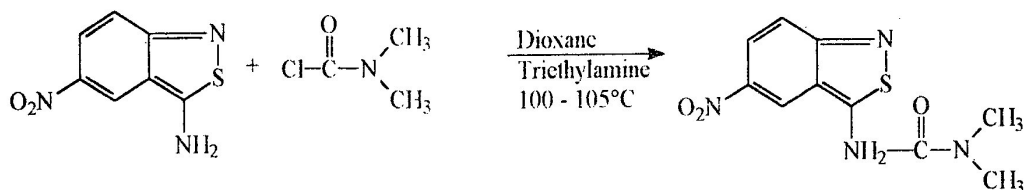


### SCHEME IV

Synthesis of 5-nitro-2,1-benzisothiazole-3-carbamate

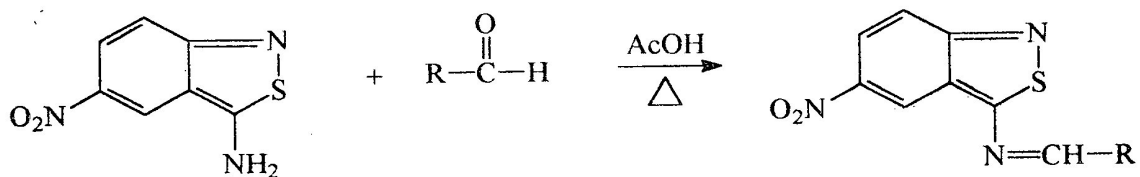


Synthesis of 3-dimethyl carbamido-5-nitro-2,1-benzisothiazole



### SCHEME V

Synthesis of 2,1-benzisothiazole-3-amine-N-(substituted phenyl/heterocyclic)  
methylene-5-nitro

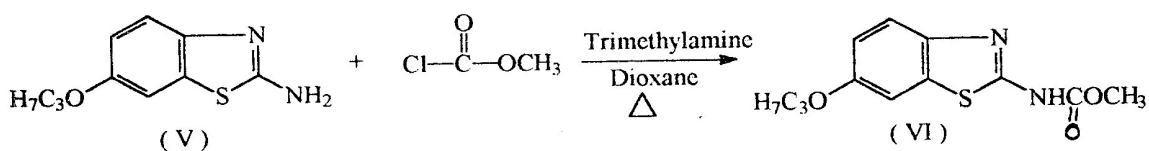
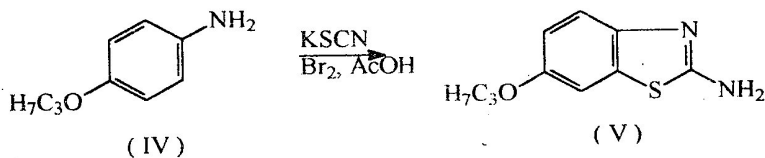
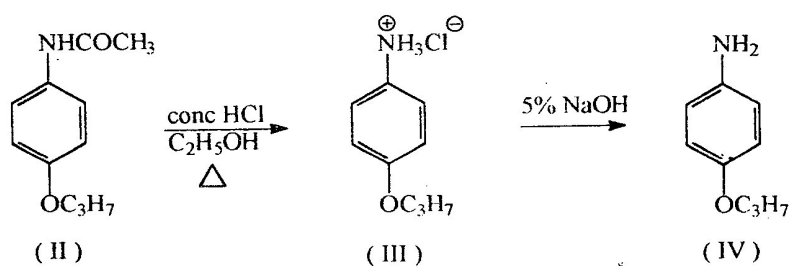
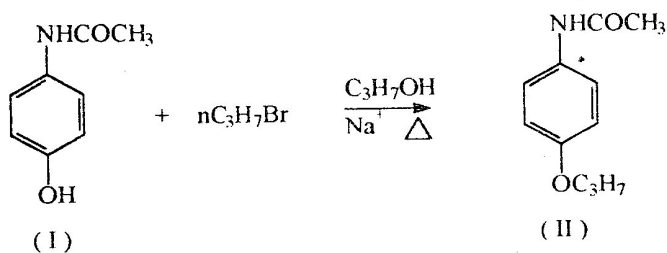


R = substituted phenyl / substituted toluyl / thiophenyl / substituted naphthyl

### SCHEME VI

Synthesis of methyl-6-propoxy benzothiazole-2-carbamate

Route 1 :

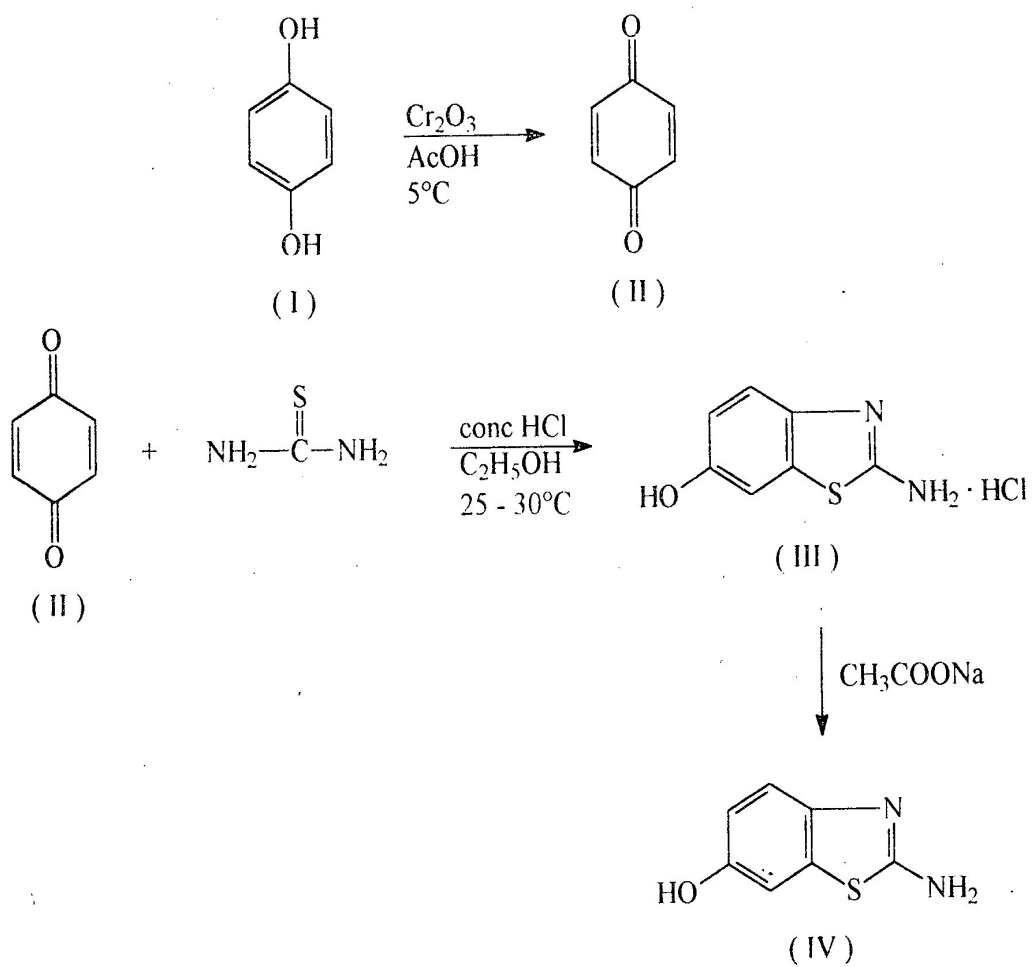


## SCHEME VII

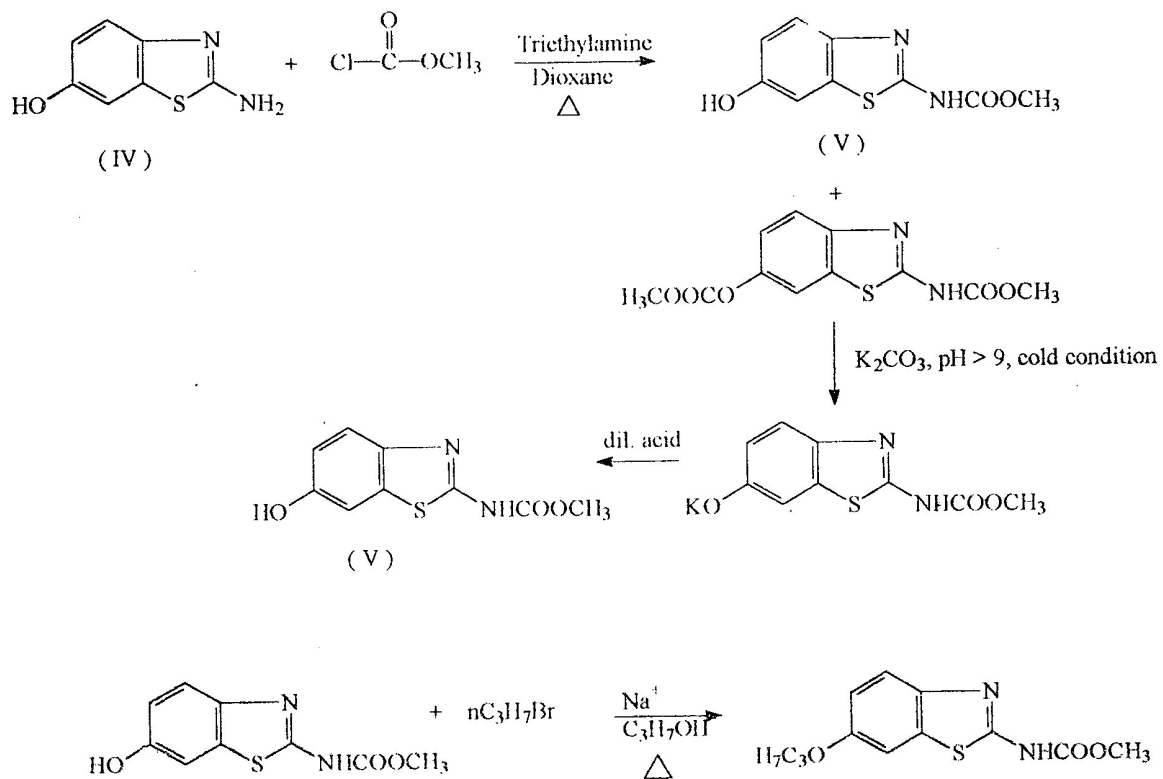
### Synthesis of methyl-6-n-propoxy benzothiazole-2-carbamate

#### Route 2:

Synthesis of 2-amino-6-hydroxy benzothiazole



# Synthesis of methyl-6-propoxy benzothiazole-2-carbamate

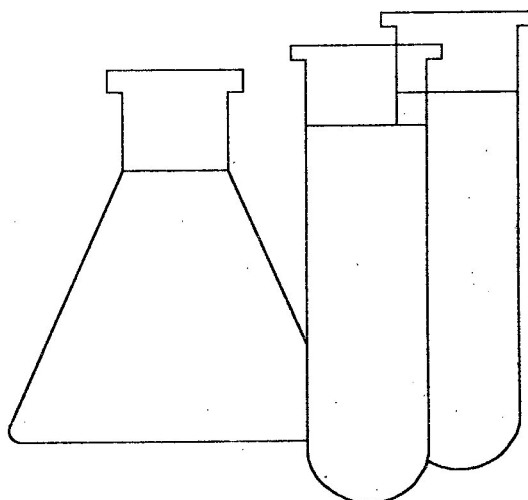


This part deals with the synthesis of

- 1) 3-substituted amino - 5-nitro, 2,1-benzisothiazole which involves the following steps.
  - a) Synthesis of 2-amino - 5-nitro benzenecarbothioamide.
  - b) Synthesis of 3-amino - 5-nitro - 2,1-benzisothiazole.
  - c) Synthesis of acid chlorides.
  - d) Synthesis of title compounds by condensation of 3-amino-5-nitro-2,1-benzisothiazole with various acid chlorides, alkyl chloroformates and N,N-dimethyl carbamoyl chloride.
2. Synthesis of 3-amine-N-(substituted phenyl / tolyl / heterocyclic) methylene-5-nitro-2,1-benzisothiazole.



# *EXPERIMENTAL*



### Synthesis of 2-amino-5-nitro benzenecarbothioamide

In a 500 ml conical flask was placed 5-nitroanthranilonitrile (50 g, 0.3M) in 250 ml pyridine and triethylamine (30g, 0.3 M). This solution was treated with H<sub>2</sub>S at 20°C for 8-9 hrs and the mixture was poured with stirring into 2.5 litre water to give 2-amino-5-nitro benzenecarbothioamide which was purified from ethanol as reddish yellow leaflets.

Yield - 48.34 g ( 80 %)

M.P. 164°C

Lit <sup>228</sup> M.P. 165 -166°C

### Synthesis of 3 - amino - 5 - nitro - 2,1-benzisothiazole

In a 250 ml three necked flask attached to an air condenser and dropping funnel was placed (29.3g, 0.148 M) 2-amino-5-nitro benzenecarbothioamide in 80 ml pyridine and was treated dropwise with 18 ml 30% H<sub>2</sub>O<sub>2</sub> (approx. 0.16 m) at 25°C and the mixture was stirred for 6 hrs to give 3 amino-5-nitro-2,1 -benzisothiazole which was purified from DMF as red coloured product

Yield - 21.5 g ( 74.13 %)

M.P > 230° (dec.)

Lit <sup>228</sup> M.P. > 230° (dec.)

### Synthesis of substituted benzoyl chlorides, substituted salicyloyl chlorides and adamantyl carbonyl chloride.

#### Synthesis of 4- chloro benzoyl chloride

A mixture of 4-chloro benzoic acid (3.13 g, 0.02 M) and freshly distilled thionyl chloride (3.56 g, 0.03 M) in 25 ml dry benzene and 2-3 drops of pyridine was taken in 100 ml round bottomed flask equipped with reflux condenser and guard tube. The reaction mixture was heated with occasional shaking till evolution of HCl gas ceases. Excess of thionyl chloride was distilled out along with benzene. The residual oil was distilled under vacuum at 20 mm.

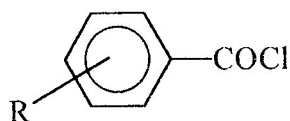
Yield - 2.8 g (80%)

BP 20mm 113° C

Lit <sup>231</sup> BP<sub>18mm</sub> 111° C

Similarly fluoro substituted benzoyl chlorides have been prepared and their physical constants have been given in Table No: 1. In a similar manner halo substituted salicylic acids and adamantyl carboxylic acid are converted into corresponding acid chlorides.

**TABLE 1**  
**Substituted Benzoyl Chlorides**



| Sr. No | R   | Yield | Boiling Point (°) |               | Reference |
|--------|-----|-------|-------------------|---------------|-----------|
|        |     |       | Observed          | Reported      |           |
| 1      | 2F  | 80    | 74 (20 mm)        | 66.67 (15mm)  | 230       |
| 2      | 3F  | 81    | 76 (25 mm)        | 68.69 (15 mm) | 230       |
| 3      | 4F  | 83    | 72 (20 mm)        | 69.70 (15 mm) | 230       |
| 4      | 4Cl | 80    | 113 (20 mm)       | 111 (18 mm)   | 231       |

### Synthesis of 3-substituted amino - 5 - nitro - 2,1-benzisothiazole.

#### Synthesis of 3-[-N -(4- chloro phenyl carboxamido)] - 5 - nitro- 2,1 benzisothiazole.

In a 100 ml three necked round bottomed flask equipped with a reflux condenser and dropping funnel and guard tube was placed a mixture of triethylamine (0.101 g, 0.001M) and 3 - amino - 5 - nitro benzisothiazole (0.195 g, 0.001 M) and 20 ml dry 1,4-dioxane. The reaction mixture was stirred on a magnetic stirrer for 10-15 min. To this was added dropwise a solution of 4-chloro benzoyl chloride (0.175 g, 0.001 M) in 5 ml dry 1,4-dioxane and heated at 70°C for 1 hr. Then the reaction mixture was stirred for 7-8 hrs at 100°C. It was then poured into crushed ice. The solid separated out was filtered and washed with 1% potassium bicarbonate and water. It was then recrystallised from ethanol and subjected to elemental analysis.

Yield = 0.139 g ( 77 %)

M. P. 195°C

#### Elemental analysis of $C_{14}H_8ClN_3O_3S$

|            | C%    | H%  | N%    |
|------------|-------|-----|-------|
| Calculated | 50.37 | 2.4 | 12.59 |
| Found      | 49.92 | 2.2 | 12.9  |

In a similar manner halo substituted benzoyl chlorides, salicyloyl chlorides, adamantyl carbonyl chloride, alkyl chloroformates and dimethyl carbamoyl chloride were reacted with 3-amino-5-nitro-2,1 benzisothiazole to get corresponding amides, carbamates and carbamido compounds respectively whose physical data has been represented in Table No : 2.

**Synthesis of 3 - amine - N - (substituted phenyl / toluyl / heterocyclic) methylene-5-nitro-2,1 benzisothiazole**

In a 50 ml round bottomed flask fitted with reflux condenser was taken 3-amino-5-nitro-2,1 benzisothiazole (0.487 g 0.0025 M) in 15 ml glacial acetic acid. To this was added  $\alpha,\alpha,\alpha$ -trifluoro-m-tolualdehyde (0.43 g, 0.0025 M) in 5 ml glacial acetic acid. This mixture was refluxed for four hours and then pour onto crushed ice. The yellow solid separated out was filtered and recrystallised from ethanol.

Yield - 0.736 g ( 77.5 %)

M.P. 245° C

**Elemental analysis of  $C_{15}H_8FN_3O_2S$**

|            | C%    | H%   | N%    |
|------------|-------|------|-------|
| Calculated | 51.28 | 2.28 | 11.96 |
| Found      | 51.56 | 2.2  | 11.71 |

In similar manner various fluorinated and chlorinated benzaldehydes, tolualdehydes, 5-bromo salicylaldehyde, 2-hydroxynaphthaldehyde and thiophene-2-carboxaldehyde were reacted with 3-amino-5-nitro-2,1 benzisothiazole to obtain corresponding Schiff bases whose physical data has been summarized in Table No. 3.

All the aldehydes used in the synthetic work were obtained from FLUKA chemie AG

Table 2

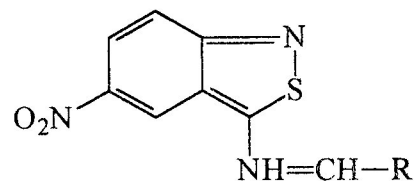
## 3-substituted amino-5-nitro-2,1 benzisothiazole



| Sr. No | R                           | Molecular Formula                                                             | Molecular Weight | MP (°C) | Yield % | Elemental Analysis |      |       |       |      |       |
|--------|-----------------------------|-------------------------------------------------------------------------------|------------------|---------|---------|--------------------|------|-------|-------|------|-------|
|        |                             |                                                                               |                  |         |         | Calculated         |      |       | found |      |       |
|        |                             |                                                                               |                  |         |         | C                  | H    | N     | C     | H    | N     |
| 1      | 2-Hydroxy phenyl            | C <sub>14</sub> H <sub>9</sub> N <sub>3</sub> O <sub>4</sub> S                | 315              | 220     | 72.0    | 53.33              | 2.86 | 13.33 | 53.73 | 2.4  | 13.1  |
| 2      | 5-Chloro-2-hydroxy phenyl   | C <sub>14</sub> H <sub>8</sub> ClN <sub>3</sub> O <sub>4</sub> S              | 349.5            | 225     | 77.0    | 48.06              | 2.28 | 12.01 | 48.5  | 2.46 | 11.96 |
| 3      | 5-Bromo-2-hydroxy phenyl    | C <sub>14</sub> H <sub>8</sub> BrN <sub>3</sub> O <sub>4</sub> S              | 394              | >250    | 69.13   | 42.63              | 2.03 | 10.65 | 42.12 | 1.97 | 11.1  |
| 4      | 3,5-Diiodo-2-hydroxy phenyl | C <sub>14</sub> H <sub>7</sub> I <sub>2</sub> N <sub>3</sub> O <sub>4</sub> S | 566.8            | 165     | 84.02   | 29.64              | 1.24 | 7.41  | 30.01 | 1.32 | 7.8   |
| 5      | Adamantyl                   | C <sub>18</sub> H <sub>19</sub> N <sub>3</sub> O <sub>3</sub> S               | 357.19           | 227     | 75.06   | 60.47              | 5.32 | 11.75 | 59.8  | 5.2  | 11.9  |
| 6      | 4-Chlorophenyl              | C <sub>14</sub> H <sub>8</sub> ClN <sub>3</sub> O <sub>3</sub> S              | 333.5            | 195     | 74.0    | 50.37              | 2.4  | 12.59 | 49.92 | 2.2  | 12.9  |
| 7      | 4-Fluorophenyl              | C <sub>14</sub> H <sub>8</sub> FN <sub>3</sub> O <sub>3</sub> S               | 317              | >250    | 78.0    | 52.99              | 2.52 | 13.25 | 53.35 | 2.81 | 12.85 |
| 8      | 3-Fluorophenyl              | C <sub>14</sub> H <sub>8</sub> FN <sub>3</sub> O <sub>3</sub> S               | 317              | >250    | 74.05   | 52.99              | 2.52 | 13.25 | 53.3  | 2.76 | 12.75 |
| 9      | 2-Fluorophenyl              | C <sub>14</sub> H <sub>8</sub> FN <sub>3</sub> O <sub>3</sub> S               | 317              | 187     | 77.2    | 52.99              | 2.52 | 13.25 | 53.42 | 2.86 | 12.7  |
| 10     | Methoxy                     | C <sub>9</sub> H <sub>7</sub> N <sub>3</sub> O <sub>4</sub> S                 | 253              | 185     | 67.0    | 42.69              | 2.76 | 16.60 | 43.01 | 2.94 | 17.3  |
| 11     | Ethoxy                      | C <sub>10</sub> H <sub>9</sub> N <sub>3</sub> O <sub>4</sub> S                | 267              | 210     | 64.7    | 44.94              | 3.37 | 15.72 | 45.20 | 3.09 | 16.2  |
| 12     | Dimethylamino               | C <sub>10</sub> H <sub>10</sub> N <sub>4</sub> O <sub>4</sub> S               | 266              | 210     | 70.59   | 45.11              | 3.76 | 21.05 | 44.79 | 3.35 | 21.55 |

Table 3

3-amine-N-(substituted phenyl / tolyl / heterocyclic) methylene-5-nitro- 2,1-benzisothiazole.



| Sr. No | R                                | Molecular Formula                                                             | Molecular Weight | MP (°C) | Yield % | Elemental Analysis |      |       |       |      |       |
|--------|----------------------------------|-------------------------------------------------------------------------------|------------------|---------|---------|--------------------|------|-------|-------|------|-------|
|        |                                  |                                                                               |                  |         |         | Calculated         |      |       | found |      |       |
|        |                                  |                                                                               |                  |         |         | C                  | H    | N     | C     | H    | N     |
| 1      | 2-Fluorophenyl                   | C <sub>14</sub> H <sub>8</sub> FN <sub>3</sub> O <sub>2</sub> S               | 301              | 193     | 74.5    | 55.81              | 2.65 | 13.95 | 55.21 | 2.31 | 14.4  |
| 2      | 4-Fluorophenyl                   | C <sub>14</sub> H <sub>8</sub> FN <sub>3</sub> O <sub>2</sub> S               | 301              | 230     | 77.5    | 55.81              | 2.65 | 13.95 | 55.36 | 2.42 | 14.45 |
| 3      | 3-Chlorophenyl                   | C <sub>14</sub> H <sub>8</sub> ClN <sub>3</sub> O <sub>2</sub> S              | 317.5            | 235     | 70.3    | 52.91              | 2.51 | 13.22 | 53.30 | 2.8  | 12.89 |
| 4      | 4-Chlorophenyl                   | C <sub>14</sub> H <sub>8</sub> ClN <sub>3</sub> O <sub>2</sub> S              | 317.5            | 178     | 72.0    | 52.91              | 2.51 | 13.22 | 53.25 | 2.76 | 12.75 |
| 5      | α,α,α-Trifluoro-2-tolyl          | C <sub>15</sub> H <sub>8</sub> F <sub>3</sub> N <sub>3</sub> O <sub>2</sub> S | 351              | 240     | 66.2    | 51.28              | 2.28 | 11.96 | 51.48 | 2.21 | 11.86 |
| 6      | α,α,α-Trifluoro-3-tolyl          | C <sub>15</sub> H <sub>8</sub> F <sub>3</sub> N <sub>3</sub> O <sub>2</sub> S | 351              | 245     | 77.5    | 51.28              | 2.28 | 11.96 | 51.56 | 2.2  | 11.71 |
| 7      | α,α,α-Trifluoro-4-tolyl          | C <sub>15</sub> H <sub>8</sub> F <sub>3</sub> N <sub>3</sub> O <sub>2</sub> S | 351              | 183     | 69.7    | 51.28              | 2.28 | 11.96 | 51.51 | 2.36 | 11.59 |
| 8      | 2-Fluoro-α,α,α-trifluoro-4-tolyl | C <sub>15</sub> H <sub>7</sub> F <sub>4</sub> N <sub>3</sub> O <sub>2</sub> S | 369              | 238     | 68.0    | 48.78              | 1.9  | 11.38 | 48.4  | 2.1  | 11.89 |
| 9      | 5-Bromo-2-hydroxy phenyl         | C <sub>14</sub> H <sub>8</sub> BrN <sub>3</sub> O <sub>3</sub> S              | 378              | 240     | 71.7    | 44.44              | 2.11 | 11.11 | 43.99 | 2.01 | 11.20 |
| 10     | 2-Hydroxy naphthyl               | C <sub>18</sub> H <sub>11</sub> N <sub>3</sub> O <sub>3</sub> S               | 349              | 176     | 72.6    | 61.89              | 3.51 | 12.03 | 62.0  | 3.71 | 11.96 |
| 11     | 2-Thiophenyl                     | C <sub>12</sub> H <sub>7</sub> N <sub>3</sub> O <sub>2</sub> S <sub>2</sub>   | 289              | 245     | 70.1    | 49.82              | 2.42 | 14.53 | 49.62 | 2.22 | 14.79 |

**Synthesis of methyl-6-propoxy benzothiazole-2-carbamate.**

Methyl - 6 -propoxy benzothiazole-2-carbamate was prepared by two routes.

First route :

- 1) Synthesis of p-propoxyaniline.
- 2) Synthesis of 2-amino-6-propoxy benzothiazole.
- 3) Synthesis of methyl-6- propoxy benzothiazole -2 carbamate.

Second route :

- 1) Synthesis of 1,4 -benzoquinone.
- 2) Synthesis of 2-amino-6-hydroxy benzothiazole.
- 3) Synthesis of methyl-6 hydroxy benzothiazole -2-carbamate.
- 4) Synthesis of methyl- 6- propoxy benzothiazole-2-carbamate.

Synthesis of p-propoxyaniline was carried out in two steps -

- 1) Synthesis of p-propoxy acetanilide from p- hydroxy acetanilide.
- 2) Hydrolysis of p-propoxy acetanilide to p-propoxyaniline hydrochloride which was subsequently converted into free base.

Synthesis of an intermediate for Riluzole.

2-Amino-6-hydroxy benzothiazole was synthesised as probable intermediate for Riluzole.

Synthesis of 2-amino-6-hydroxy benzothiazole is described in details under 'Synthesis of methyl-6-propoxy benzothiazole-2-carbamate'.



### Synthesis of p-propoxy acetanilide

Clean sodium (1.55g, 0.0675 M) was placed in a 250 ml round-bottomed flask equipped with a reflux condenser. To this was added 40 ml propanol and kept until solution was complete. The above mixture was cooled and p-hydroxy acetanilide (10 g, 0.066 M) was added to it. Then through the condenser n-propylbromide (12.3 g, 0.1 M) was introduced slowly at such a rate that crystalline product did not separate. After addition of propyl bromide separation of some crystalline product was observed. The mixture was refluxed for two hours. Then the flask was cooled in ice bath and product was collected by suction, washed with cold water and purified from about 80 ml rectified spirit.

Yield = 10.2 g (80 %)

M.P. 117°C

Lit<sup>232</sup> M.P 118-120°C

### Synthesis of p-propoxyaniline

p-Propoxy acetanilide (16.2 g, 0.084 M) dissolved in 80 ml boiling ethanol contained in a 250 ml round-bottomed flask equipped with a reflux condenser. To this was added 22 ml of concentrated hydrochloric acid in small portions with an aid of dropping funnel. This mixture was refluxed for one and half hour. Small amount of mixture was removed and diluted with water and no turbidity was observed indicating hydrolysis of p-propoxy acetanilide. The above solution was then poured on 200 ml ice. cold water and with vigorous stirring. 5% sodium hydroxide solution was added until just alkaline. The p-propoxy aniline separated as oil. This oil was extracted with ethyl acetate, the latter was distilled off. The p-propoxy aniline was distilled at reduced pressure.

Yield = 12 g (95 %)

B.P<sub>20 mm</sub> 146°C

Lit<sup>233</sup> B.P<sub>11 mm</sub> 137°C

### Synthesis of 2 - amino - 6 - propoxy benzothiazole

In a 500 ml three necked flask equipped with water condenser, mechanical stirrer and dropping funnel was placed 4-propoxy aniline (5 g, 0.33 m), potassium thiocyanate (6.8g, 0.07 m) and 40 ml acetic acid. To this bromine (8 g, 0.05 m) in 50 ml acetic acid was added dropwise maintaining the temperature between 5-10°C. The addition took one and half hour. During the addition, the reaction mixture was well stirred. After the addition was complete, reaction mixture was stirred at 25°C for 18-22 hours. Then the reaction mixture was warmed

on water bath, diluted with 150 ml of hot water and filtered. Filtrate was cooled and neutralized with saturated sodium carbonate solution. Crude 2-amino-6-propoxy benzothiazole was precipitated out, which was recrystallised from ethanol.

Yield = 4.95 g ( 72 %)

M.P. 137°C

Lit.<sup>234</sup> M.P. 137 - 139°C

#### Synthesis of methyl-6-propoxy benzothiazole-2-carbamate

2-amino-6-propoxy benzothiazole ( 2.08 g, 0.01m) was placed in 25 ml 1,4-dioxane in 50 ml round bottomed flask equipped with water condenser. To this was added triethylamine (1.01 g, 0.01 m) and stirred on a magnetic stirrer at 60°C for 30 min. Then methyl chloroformate (0.945 g, 0.01 m) was added and stirred at 60°C for one hour and then refluxed for four hours. The reaction mixture was poured onto 150 ml ice cold water. White product separated out. The product was collected by suction and recrystallised from ethanol.

Yield = 1.78 g ( 67 %)

M.P. 177- 180°C

Lit.<sup>197</sup> M.P. 178-180°C

#### Synthesis of 1,4 benzoquinone

Hydroquinone (33 g, 0.33 m) in 150 ml of 60 % acetic acid was placed in 500 ml three necked round-bottomed flask equipped with dropping funnel, mechanical stirrer and air condenser. This mixture was cooled to below 5°C in an ice bath. The solution of chromium trioxide (42 g, 0.42 m) in 70 ml water and 30 ml glacial acetic acid was added dropwise with an aid of dropping funnel to the mechanically stirred solution of hydroquinone. The addition took two hours. After the addition, the reaction mixture was stirred at 5°C for 30 minutes.

The temperature throughout the reaction did not rise above 7°C. Filtered the mixture at once and quinone was washed several times with 10 ml portions of ice-cold water and dried on filter paper. After drying bright yellow crystalline solid was collected.

Yield= 21 g ( 66 %)

M.P. 115°C

Lit.<sup>227</sup> M.P. 115°C

### Synthesis of 2-amino- 6- hydroxy benzothiazole

In a one litre three necked round-bottomed flask equipped with dropping funnel, mechanical stirrer and air condenser was placed thiourea (7.6 g, 0.1M) in 200 ml ethanol and 9 ml(0.1 mol) of concentrated hydrochloric acid. To this was added dropwise the solution of freshly prepared 1,4- benzoquinone (21.6 g, 0.2 M) in 400 ml hot ethanol at room temperature. The reaction mixture was stirred for 24 hrs at room temperature. The solvent was removed under reduced pressure. The brownish mass was obtained. This mass was triturated well in mortar and pestle with hot acetonitrile and then washed with cold ethanol. The colour of brownish mass changed dramatically to whitish crystalline solid. This solid was recrystallised from water.

Yield = 15.2 g ( 75 %)

M.P. 263°C

Lit<sup>224</sup> M.P. 263°C

Lit<sup>223</sup> M.P. 303°C,

The free base was obtained by dissolving the salt in water and carefully neutralizing it with sodium acetate. This base i.e. 2-amino-6-hydroxy benzothiazole was recrystallised from aq. ethanol yielded straw coloured product.

M.P. 246°C

Lit<sup>223</sup> M.P. 247° C

Lit<sup>224</sup> M.P. 249 -251°C

### Synthesis of methyl - 6 - hydroxy benzothiazole -2- carbamate

2-amino -6- hydroxy benzothiazole (1.66 g, 0.01 m) was placed in 25 ml 1,4- dioxane in 50 ml round- bottomed flask equipped with water condenser. To this was added triethylamine (1.01 g, 0.01 m) and stirred on a magnetic stirrer at 60°C for 30 min. Then methyl chloroformate (0.945 g, 0.01 m) was added and stirred at 60°C for one hour and then refluxed for four hours. The reaction mixture was poured onto 150 ml ice-cold water. White product separated out. The product was collected by suction. IR spectrum of this product showed disappearance of "OH" peak, possibly due to formation of "OCOOCH<sub>3</sub>" linkage. So the product was treated with dilute alkali in the cold to eliminate traces of "OCOOCH<sub>3</sub>" linkage if present. IR spectrum of this product showed presence of 'OH' group. The product was recrystallised from acetone.

Yield = 1.59 g ( 70.98 %)

M.P.> 240°C

**Synthesis of methyl-6-propoxy benzothiazole-2-carbamate**

Clean sodium (0.1 g, 0.0043 M) was placed in a 50 ml round bottomed flask equipped with a reflux condenser. To this was added 20 ml propanol and kept until the solution was complete. The above mixture was cooled and 1-methyl-6-hydroxy benzothiazole-2- carbamate (10.672 g, 0.003 M ) was added to it.

Then through the condenser n-propyl bromide (0.37 g, 0.003 M) was introduced slowly. Separation of some product was observed during and after the addition. Above reaction mixture was refluxed for six hours. During this period, the reaction was monitored on TLC. Then the flask was cooled in ice bath and product was collected by suction, washed with cold water and purified from rectified spirit. The yield of product was found to be very low. The unreacted reactant did not interfere as it was removed as 'ONa' salt

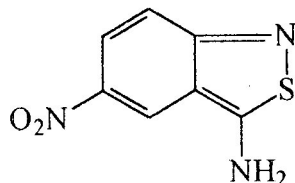
Yield = 0.16 g ( 20 %)

M.P. 177°C

Lit<sup>197</sup> M.P. 178-180°C

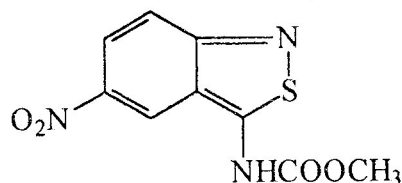
## Spectral analysis of 3-substituted amino-5-nitro-2,1-benzisothiazole

## 3-amino-5-nitro-2,1-benzisothiazole

IR (KBr)  $\text{cm}^{-1}$ 

|               |                                              |
|---------------|----------------------------------------------|
| 3379.95       | (N-H stretching of amino group)              |
| 3063.79       | (C-H stretching, heterocyclic ring)          |
| 1648.28       | (C=N stretching of benzisothiazole)          |
| 1598          | (N-H deformation of amino group)             |
| 1512.66       | (aromatic, C-NO <sub>2</sub> , asym.)        |
| 1476.0        | (C=C skeletal vibration of aromatic nucleus) |
| 1305.01       | (aromatic, C-NO <sub>2</sub> , sym.)         |
| 1134.99, 1067 | (benzisothiazole group)                      |

## Methyl-5-nitro-2,1-benzisothiazole-3-carbamate

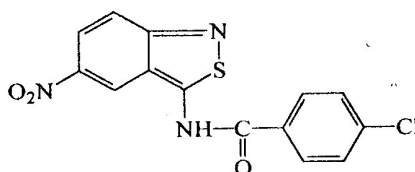
IR (KBr)  $\text{cm}^{-1}$ 

|            |                                               |
|------------|-----------------------------------------------|
| 3300       | (N-H stretching)                              |
| 2950       | (C-H stretching, CH <sub>3</sub> , asym.)     |
| 1748.88    | (C=O stretching of carbamate)                 |
| 1619.0     | (C=N stretching of benzisothiazole)           |
| 1511.75    | (aromatic C-NO <sub>2</sub> , asym.)          |
| 1434.16    | (C-H deformation, CH <sub>3</sub> asym.)      |
| 1300.71    | (aromatic C-NO <sub>2</sub> , asym.)          |
| 1450.0     | (C=C, skeletal vibration, heterocyclic group) |
| 1185.0     | (C-O stretching ester)                        |
| 1045, 1000 | (Benzisothiazole group)                       |

PMR(CDCl<sub>3</sub>)  $\delta$  : 3.9 (s, 3H, -OCH<sub>3</sub>)  
 8.59-9.2 (m, 3H, aromatic protons)  
 10.9 (s, 1H, -NHCO) (D<sub>2</sub>O exchangeable).

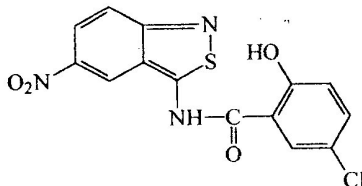
<sup>13</sup>C NMR  $\delta$  : 54.945 (OCH<sub>3</sub>)

117.507, 120.709, 124.611, 129.299, 143.641, 148.286  
 (heterocyclic carbons + C-3 carbon)  
 166.783 (C=N)  
 178.373 (C=O)

**3-(4-chlorophenyl carboxamido)-5-nitro-2,1-benzisothiazole**

 IR (KBr)  $\text{cm}^{-1}$ 

|                          |                                       |
|--------------------------|---------------------------------------|
| 3299.3                   | (N-H stretching)                      |
| 3086.85                  | (C-H Stretching, heterocyclic ring)   |
| 1694                     | (C=O stretching of -CONH-)            |
| 1665.63                  | (C=N stretching of benzisothiazole)   |
| 1587.4                   | (N-H deformation, -CONH-)             |
| 1510.8                   | (aromatic, C-NO <sub>2</sub> , asym.) |
| 1354.36                  | (aromatic, C-NO <sub>2</sub> , sym.)  |
| 1095.05, 1000.73, 900.34 | (benzisothiazole group)               |
| 845.73                   | (two adjacent H atom)                 |
| 739.0                    | (C-Cl stretching)                     |

PMR(DMSO)  $\delta$  : 7.2 - 8.9 (m, 7 aromatic and heterocyclic protons)  
 11.1 (s, 1H, -NHCO)

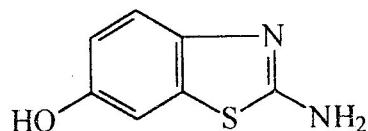
**3-(5-chloro-2-hydroxy phenyl carboxamido)-5-nitro-2,1-benzisothiazole**

 IR (KBr)  $\text{cm}^{-1}$ 

|                          |                                                            |
|--------------------------|------------------------------------------------------------|
| 3442.97                  | (O-H stretching)                                           |
| 3336.62, 3224.42         | (N-H stretching, -CONH)                                    |
| 1730.33                  | (C=O stretching, -CONH)                                    |
| 1659.86                  | (C=N stretching of benzisothiazole)                        |
| 1607.03                  | (N-H deformation -CONH)                                    |
| 1520.54                  | (aromatic, C-NO <sub>2</sub> , asym.)                      |
| 1471.23                  | (C=C, skeletal vibration, heterocyclic and aromatic group) |
| 1399.97                  | (O-H deforming, tertiary alcohol)                          |
| 1319.37                  | (aromatic C-NO <sub>2</sub> , sym.)                        |
| 1190.00                  | (C-O stretching)                                           |
| 1128.98, 1090.21, 920.41 | (Benzisothiazole group)                                    |
| 897.38                   | ( one hydrogen atom)                                       |
| 827.37                   | (two adjacent hydrogen atom)                               |
| 762.54                   | ( C-Cl stretching)                                         |

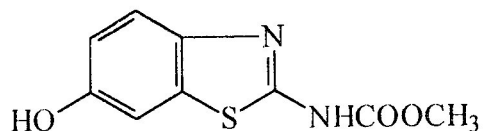
PMR(DMSO)  $\delta$  : 7.06 - 8.22 (m, 6H aromatic and heterocyclic protons)

9.35 (s, 1H, -NHCO)

3.42 (-OH phenolic)

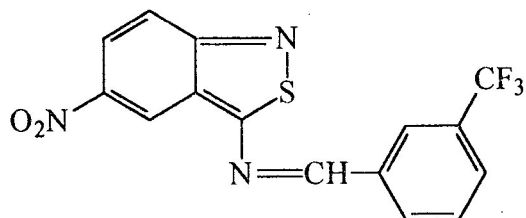
**2-amino-6-hydroxy benzothiazole**IR (KBr)  $\text{cm}^{-1}$ 

|                  |                                                 |
|------------------|-------------------------------------------------|
| 3437.56          | (O-H stretching)                                |
| 3314.36          | (N-H stretching)                                |
| 1613.42          | (C=N stretching of benzothiazole)               |
| 1569             | (N-H deformation of amine)                      |
| 1434.77          | (C=C, skeletal vibration of heterocyclic group) |
| 1363.6           | (O-H deformation of tertiary alcohol)           |
| 1211             | (C-O stretching of tertiary alcohol)            |
| 1113.95, 1052.73 | (Benzothiazole ring skeletal vibration)         |

**Methyl-6-hydroxy benzothiazole-2-carbamate**IR (KBr)  $\text{cm}^{-1}$ 

|                |                                                 |
|----------------|-------------------------------------------------|
| 3501.0         | (O-H stretching)                                |
| 3178.0         | (N-H stretching of NHCOOCH <sub>3</sub> )       |
| 2927.0         | (C-H stretching, CH <sub>3</sub> , asym.)       |
| 1713.25        | (C=O stretching of NHCOOCH <sub>3</sub> )       |
| 1619.25        | (C=N, stretching of benzothiazole)              |
| 1575.0         | (N-H deformation of NHCOOCH <sub>3</sub> )      |
| 1463.0         | (C=C, skeletal vibration of heterocyclic group) |
| 1303.0         | (C-O stretching of tertiary alcohol)            |
| 1208.0         | (O-H deformation of tertiary alcohol)           |
| 1081.0, 1058.0 | (Benzothiazole)                                 |
| 855            | (one hydrogen atom)                             |
| 826            | (two adjacent hydrogen atoms)                   |

**3-amine-N-(3-trifluorotoluy) methylene-5-nitro-2,1-benzisothiazole**

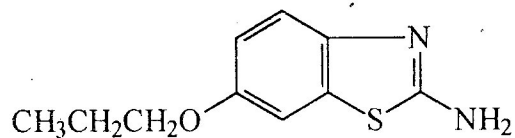


IR (KBr)  $\text{cm}^{-1}$

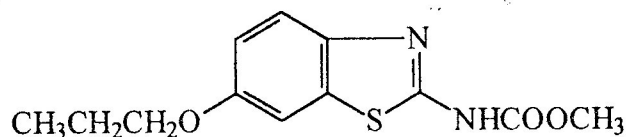
|                  |                                                           |
|------------------|-----------------------------------------------------------|
| 1681.86          | (-C=N stretching of schiff base)                          |
| 1656.92          | (C=C, stretching of benisothiazole)                       |
| 1608.41          | (C=C skeletal vibration, heterocyclic and aromatic group) |
| 1533.55          | (aromatic C-NO <sub>2</sub> , sym.)                       |
| 1327.56          | (aromatic C-NO <sub>2</sub> , sym.)                       |
| 1139.01, 1086.22 | (benzisothiazole group)                                   |
| 1027.81, 924.29  | (C-F stretching)                                          |
| 848.557          | (One hydrogen atom)                                       |
| 820.805          | (2 adjacent hyrogen atoms)                                |

PMR (DMSO)  $\delta$  : 7.283 - 9.411 (m, 7 H aromaic and heterocyalic protons).  
12.86 (s, 1H, aldehydic proton).



**2-Amino-6-propoxy benzothiazole**IR (KBr)  $\text{cm}^{-1}$ 

|                 |                                                 |
|-----------------|-------------------------------------------------|
| 3428            | (N-H stretching)                                |
| 3291            | (N-H stretching)                                |
| 3078            | (C-H stretching, heterocyclic ring)             |
| 2965            | (C-H stretching, propyl)                        |
| 1636            | (C=N of benzothiazole)                          |
| 1600            | (N-H deformation of amine)                      |
| 1537            | (C=C, skeletal vibration of heterocyclic group) |
| 1458            | (C-H deformation of propyl group)               |
| 1272            | (C-N stretching of amine)                       |
| 1204            | (C-N stretching of aralkyl ether)               |
| 1105, 1070, 990 | (Benzothiazole group)                           |
| 846             | (one hydrogen atom)                             |
| 807             | (two adjacent hydrogen atoms)                   |

**Methyl-6-propoxy benzothiazole-2-carbamate**IR (KBr)  $\text{cm}^{-1}$ 

|                 |                                           |
|-----------------|-------------------------------------------|
| 3178            | (N-H stretching, <chem>NHCOOCH3</chem> )  |
| 2965            | (C-H stretching, alkyl group)             |
| 1726            | (C=O stretching, <chem>NHCOOCH3</chem> )  |
| 1609            | (C=N stretching of benzothiazole)         |
| 1562            | (N-H deformation, <chem>NHCOOCH3</chem> ) |
| 1459            | (C-H deformation of alkyl group)          |
| 1274            | (C-O stretching of ether)                 |
| 1218            | (C-O stretching of ester)                 |
| 1059, 1024, 993 | (Benzothiazole group)                     |
| 805             | (two adjacent hydrogen atoms)             |

PMR( $\text{CDCl}_3$ )  $\delta$  :

|                                                                          |
|--------------------------------------------------------------------------|
| 1.05 (t, 3H, $-\text{CH}_2-\text{CH}_3$ )                                |
| 1.8 - 1.9 (sextet, 2H, $-\text{O}-\text{CH}_2-\text{CH}_2-\text{CH}_3$ ) |
| 3.9 (s, 3H, $\text{OCH}_3$ )                                             |
| 4.1 (t, 2H, $-\text{OCH}_2\text{CH}_2\text{CH}_3$ )                      |
| 7.0 - 8.0 (m, 3H, heterocyclic ring)                                     |
| 11.4 ( $-\text{NHCOOCH}_3$ )                                             |

 $^{13}\text{C}$  NMR  $\delta$  : 10.5548 ( $\text{O}-\text{CH}_2-\text{CH}_2-\text{CH}_3$ )22.66614 ( $-\text{O}-\text{CH}_2-\text{CH}_2-\text{CH}_3$ )53.2261 ( $-\text{CO}-\text{O}-\text{CH}_3$ )70.2843 ( $-\text{O}-\text{CH}_2$ )

105 - 142 (6 aromatic carbons)

159.1710 ( $-\text{C}=\text{N}$ )179.0110 ( $-\text{C}=\text{O}$ )

File # 1 : BIT

Mode= 2 (Mid-IR)

4/29/97 12:20 PM

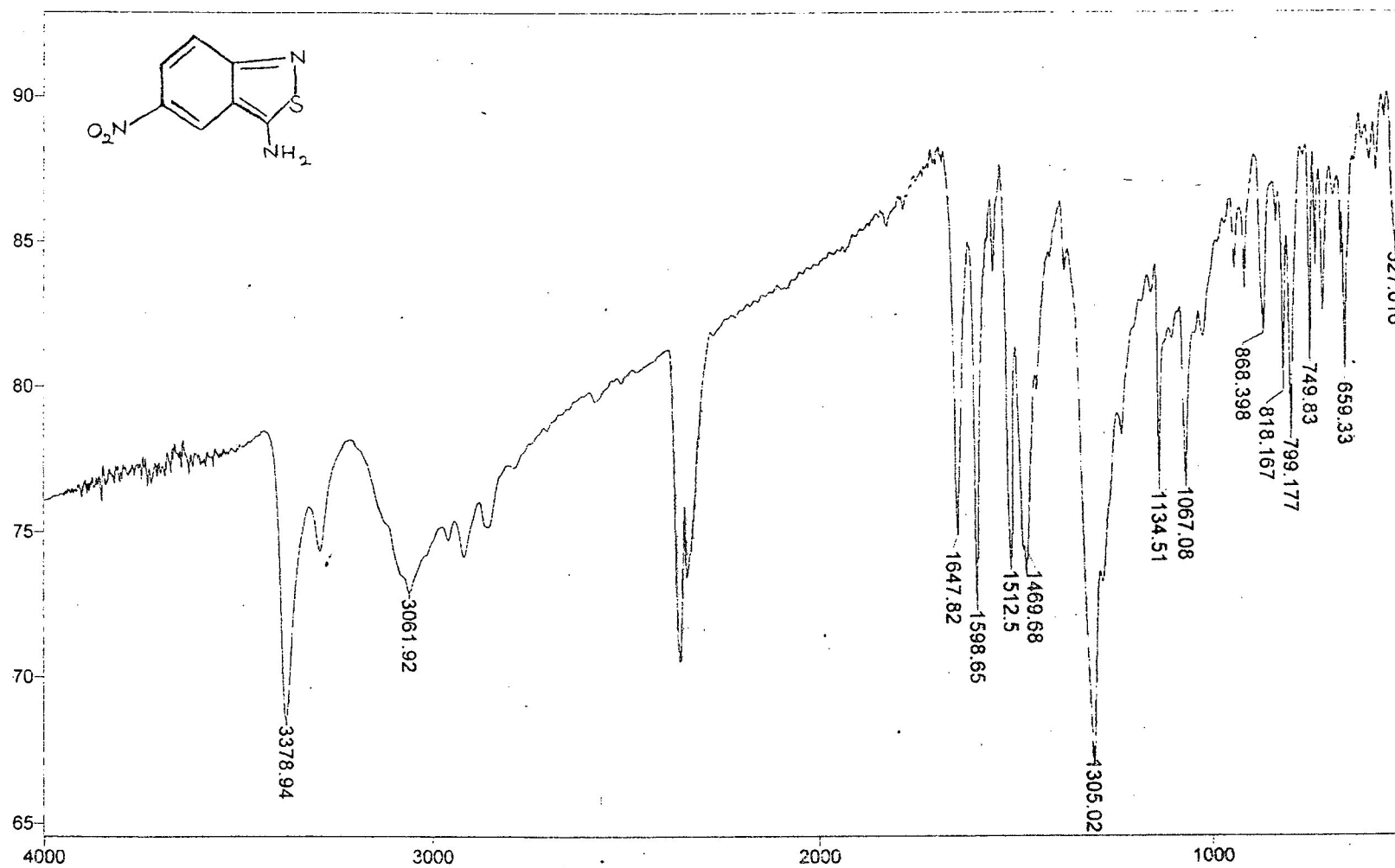
Sample Description: aminobenzisothiazole

Scans= 10 Slow

Res=4 cm-1

Apod= Cosine

Zero Filling= 1 x



File # 2 : BMF2

Mode= 2 (Mid-IR)

1/3/97 12:34 PM

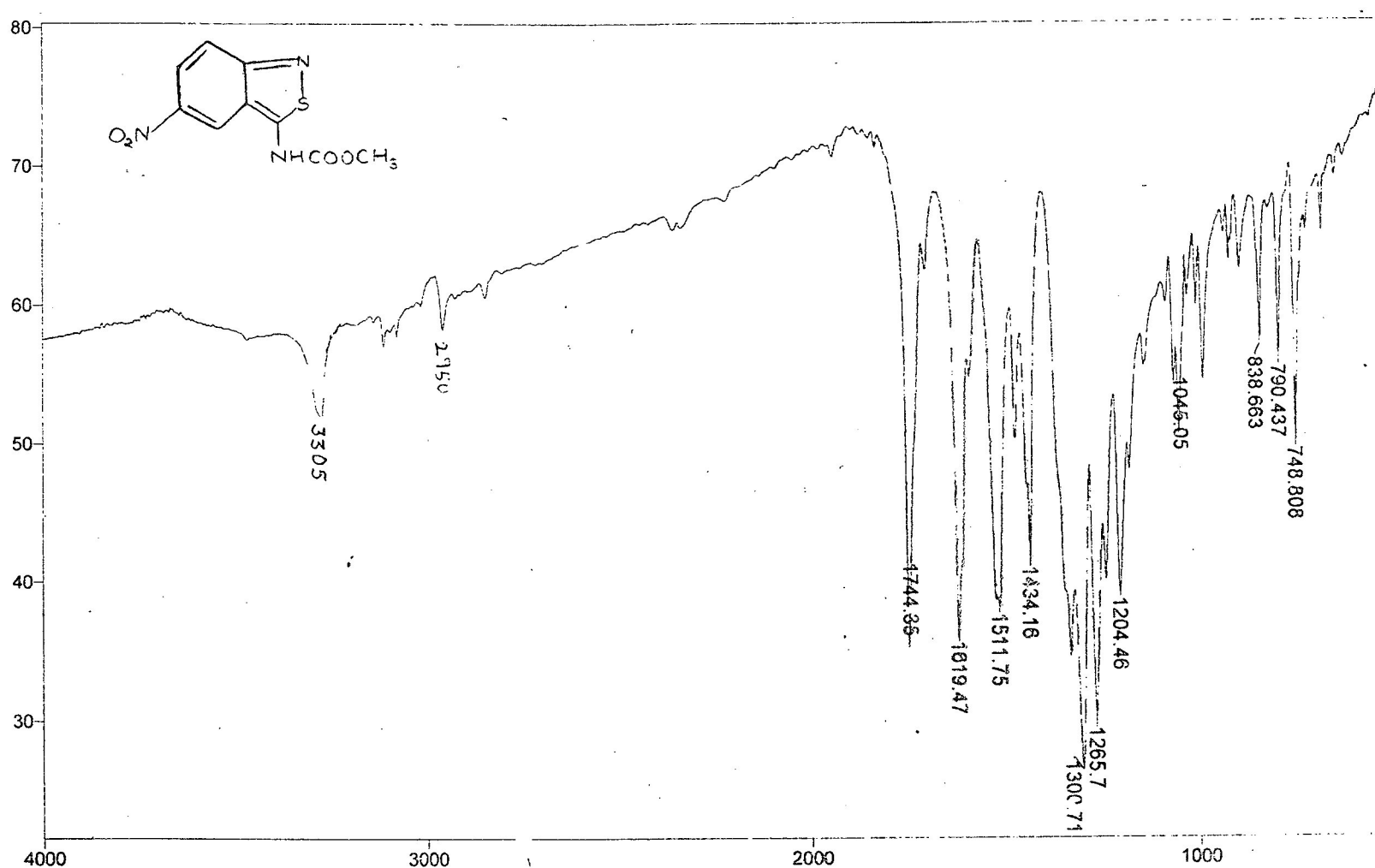
Sample Description: methoxyamidobenzisothiazole

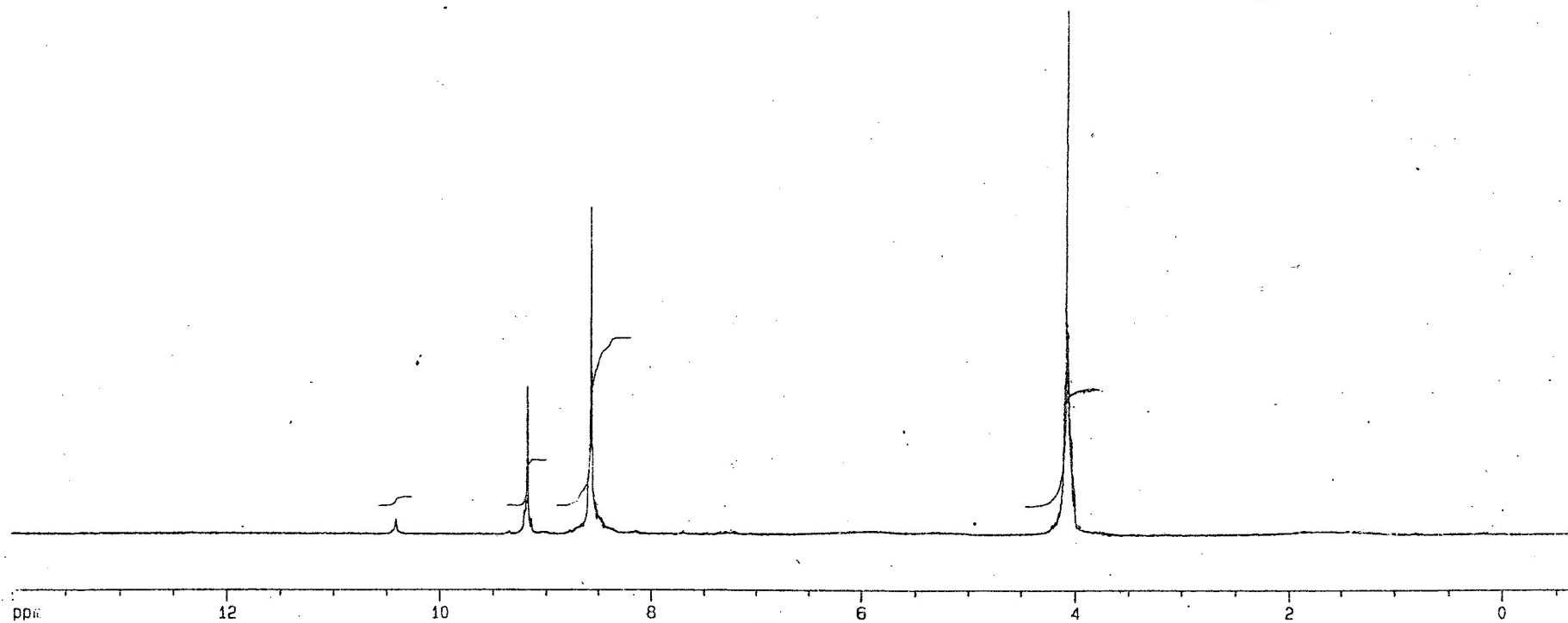
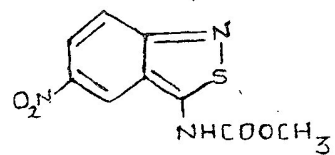
Scans= 10 Slow

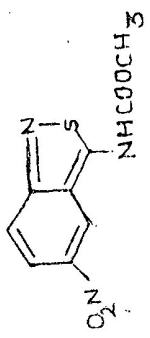
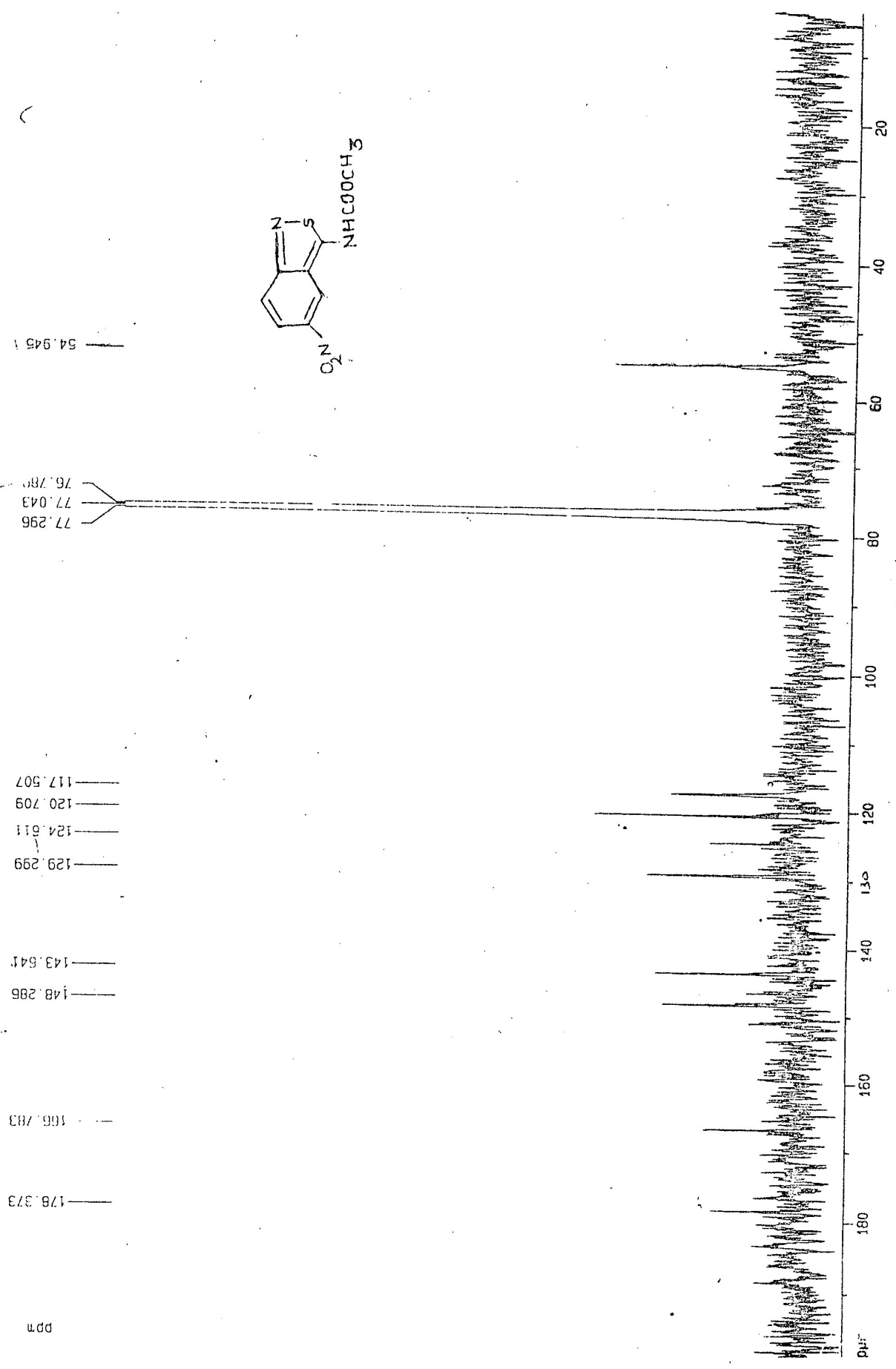
Res=4 cm-1

Apod= Cosine

Zero Filling= 1 x







File # 1 = BBC1R

Mode= 2 (Mid-IR)

1/9/97 4:57 PM

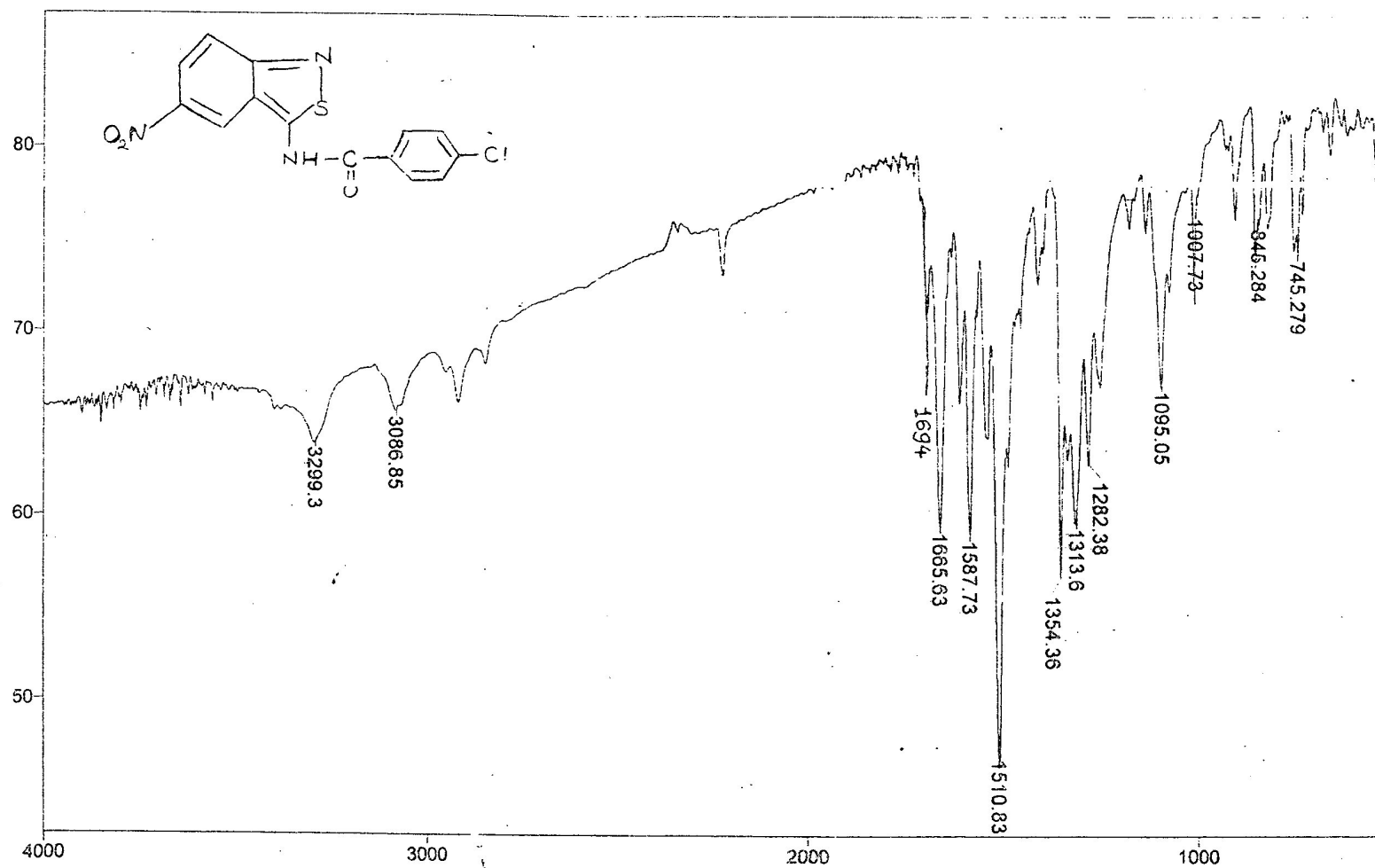
Sample Description: phenylamidobenzisothiazole

Scans= 10 Slow

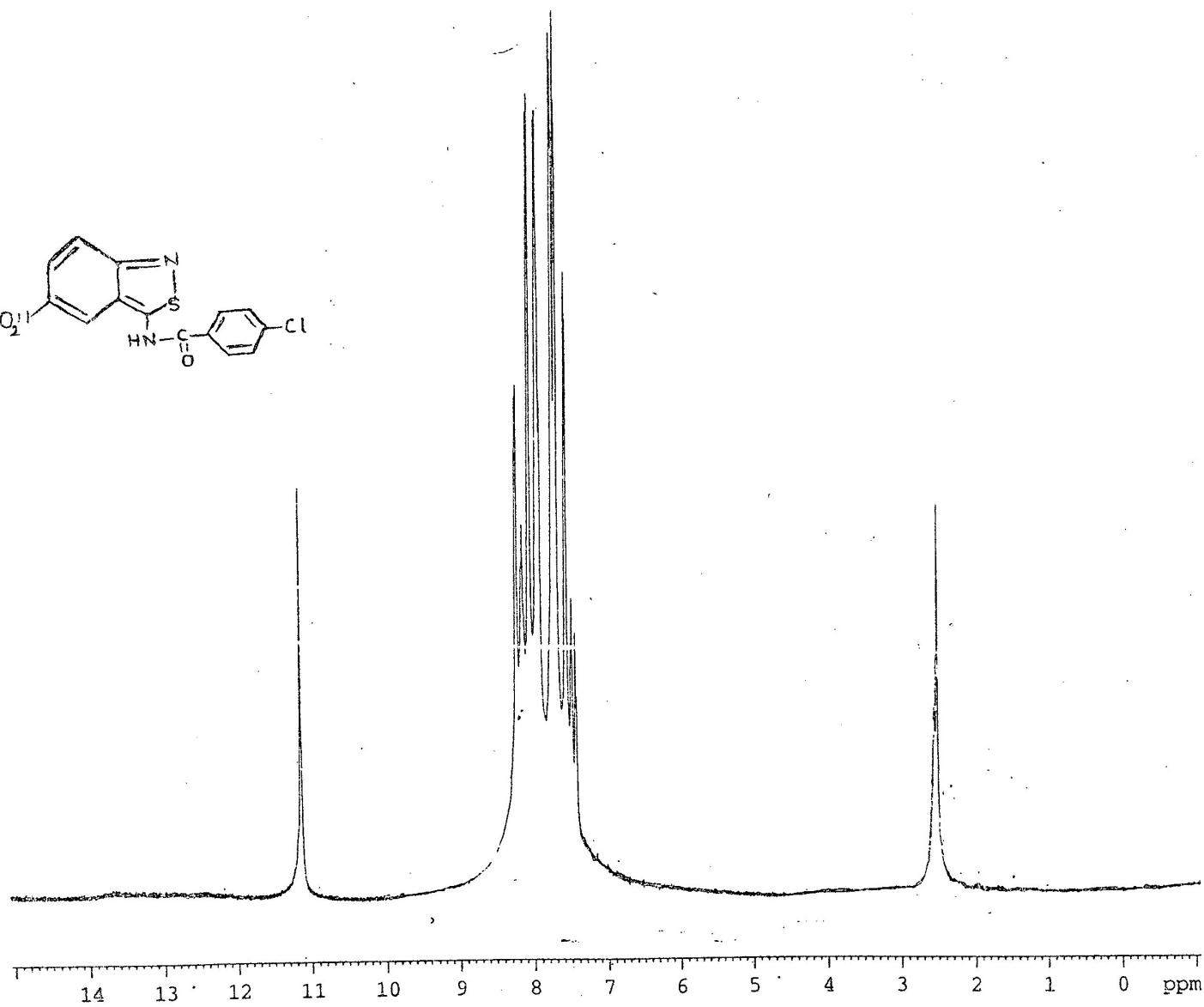
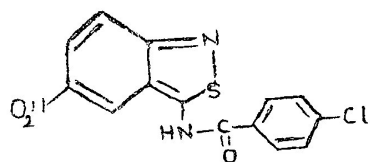
Res=4 cm-1

Apod= Cosine

Zero Filling= 1 x



Transmittance / Wavenumber (cm-1)





File # 1 : BCSA5R4

Mode = Mid-IR

3/14/97 10:31 AM

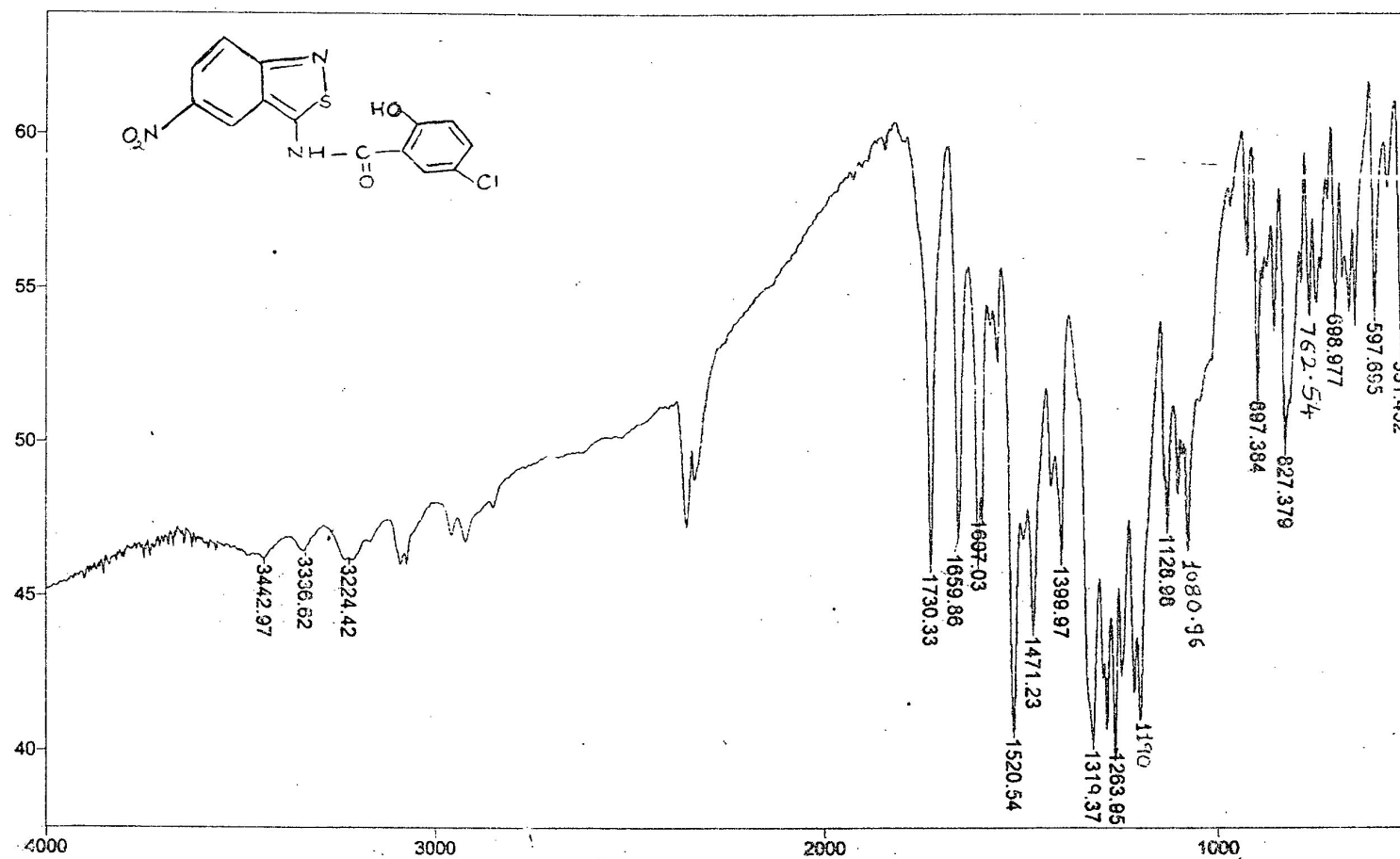
Sample Description: chlorophenolicamidobenzisothiazole

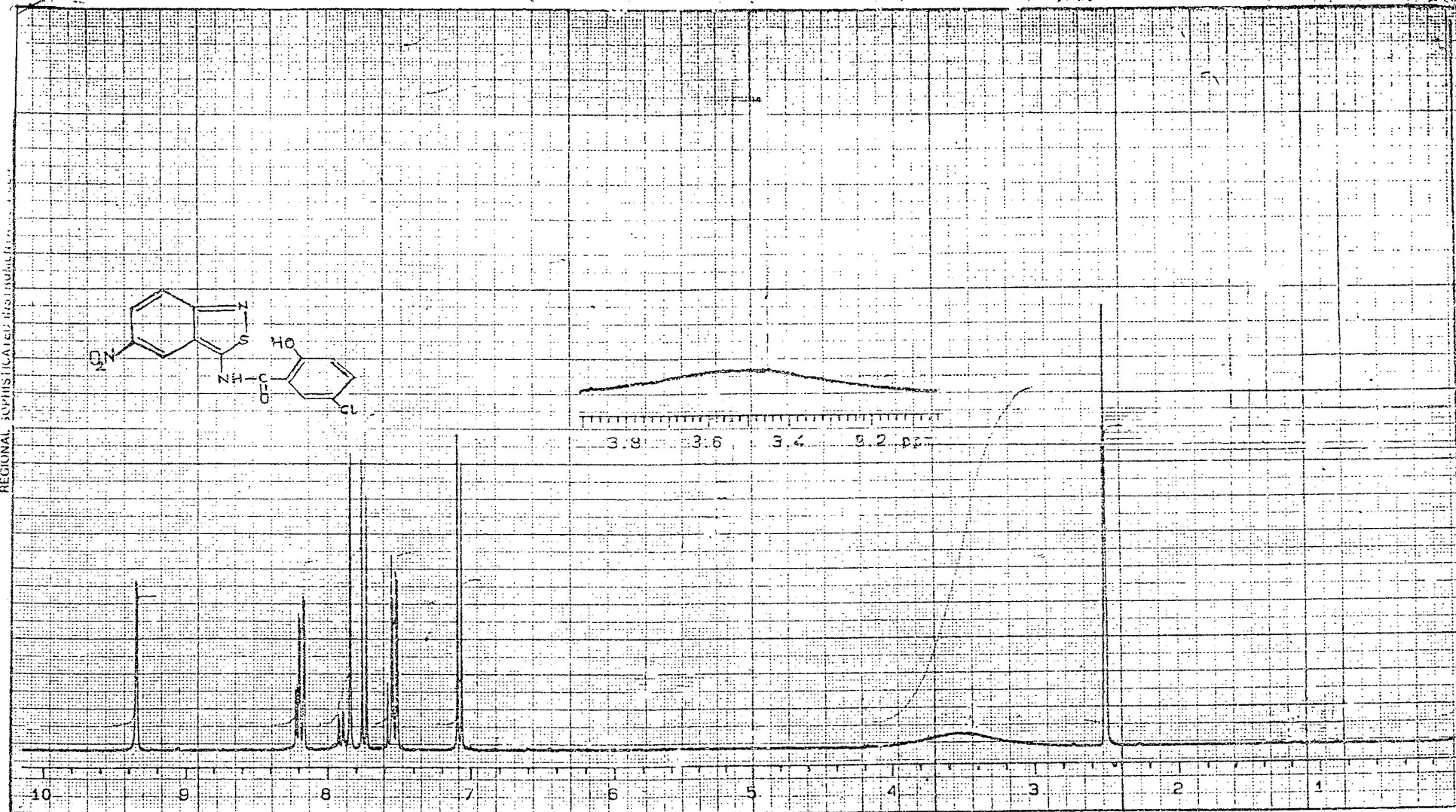
Scans= 10 Slow

Res=4 cm-1

Apod= Cosine

Zero Filling= 1 x





Nucleus — H1      Freq — 299.9485 MHz  
 Spec. Width — 5938.6 Hz      Offset — 900.0 Hz  
 Acq. Time — 4.00 sec      Delay — 0 sec  
 Pulse Width — 12.000 sec      Transmittance — 138

Nucleus — H1      Offset — 1323.8 Hz  
 Mode — n      Power — 20 dB  
 Modulation Mode — c      Freq — 200 Hz  
 Pulse Width — 12.000 sec      Power Mode — not used

F1 — not used      F2 — not used      CD — not used  
 LD — 0.100 not used      CCD — not used  
 VWH — 3123.5 Hz      Start — -60.4 Hz  
 Reference —

Tube Sequence — 02pu1  
 Tube OD — mm      5.27 mm  
 Temp — 25.0°C  
 Solvent — DMSO

Sample —  
 Date — 8/2/81  
 Vial — 304

File # 1 : AHBTS

Mode= 2 (Mid-IR)

7/8/97 4:41 PM

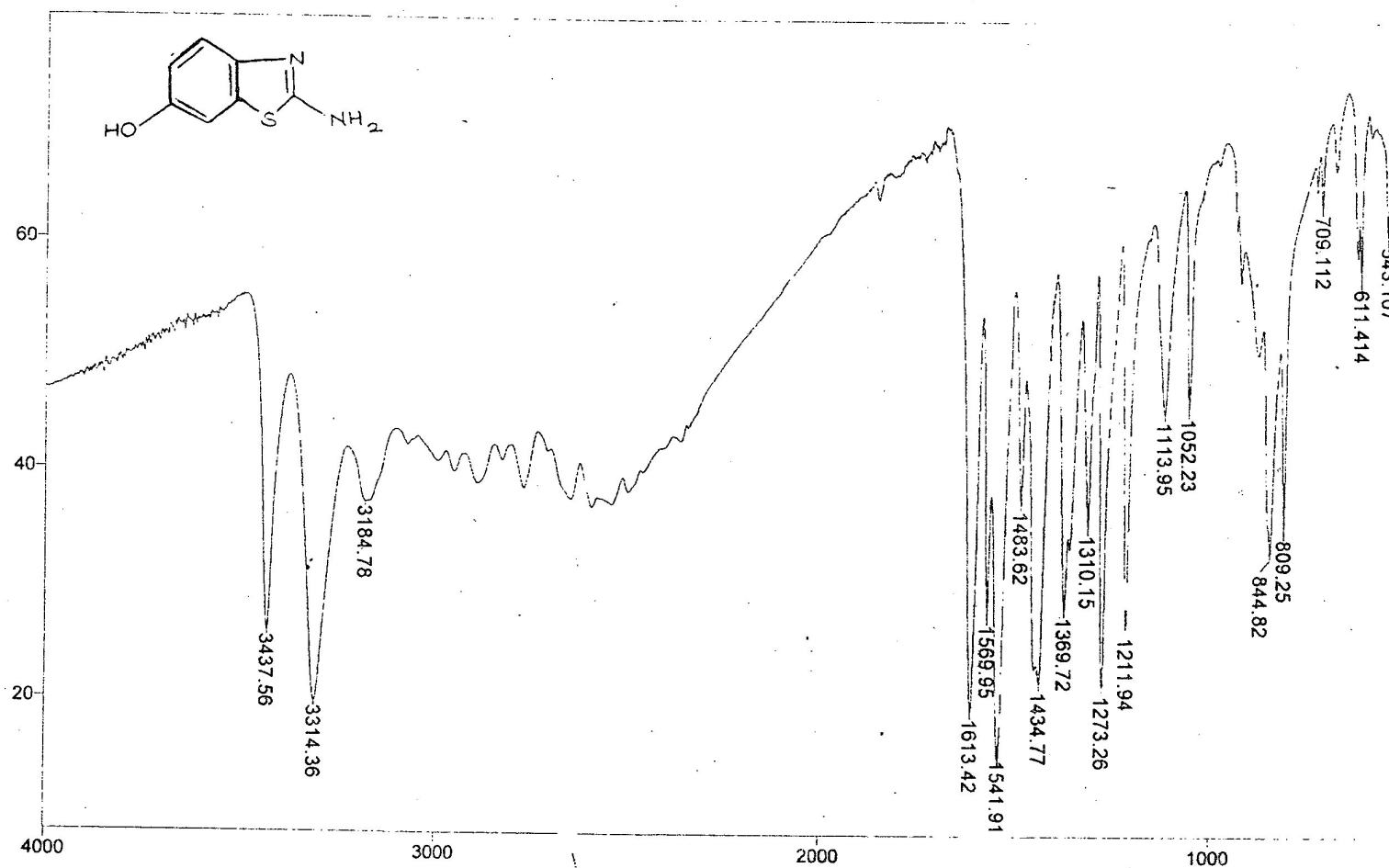
Sample Description: aminohydroxybenzothiazole

Scans= 10 Slow

Res=4 cm-1

Apod= Cosine

Zero Filling= 1 x



File # 1 : CAR

Mode= 2 (Mid-IR)

6/25/97 7:12 PM

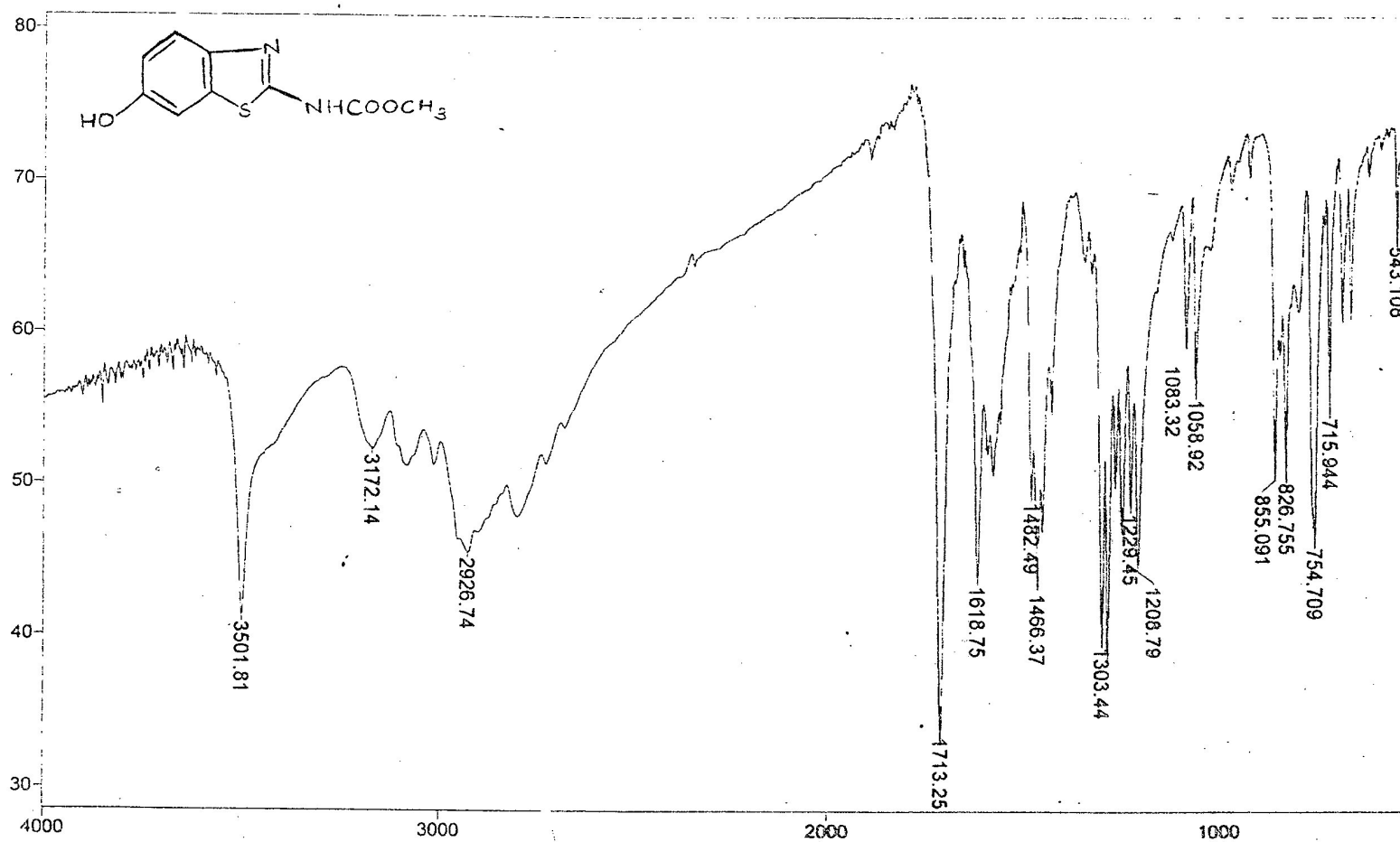
Sample Description: methylhydroxybenzothiazolecarbamate

Scans= 10 Slow

Res=4 cm<sup>-1</sup>

Apod= Cosine

Zero Filling= 1 x



Transmittance / Wavenumber (cm<sup>-1</sup>)

File # 1 = BTTFT

Mode= 2 (Mid-IR)

5/20/98 9:59 PM

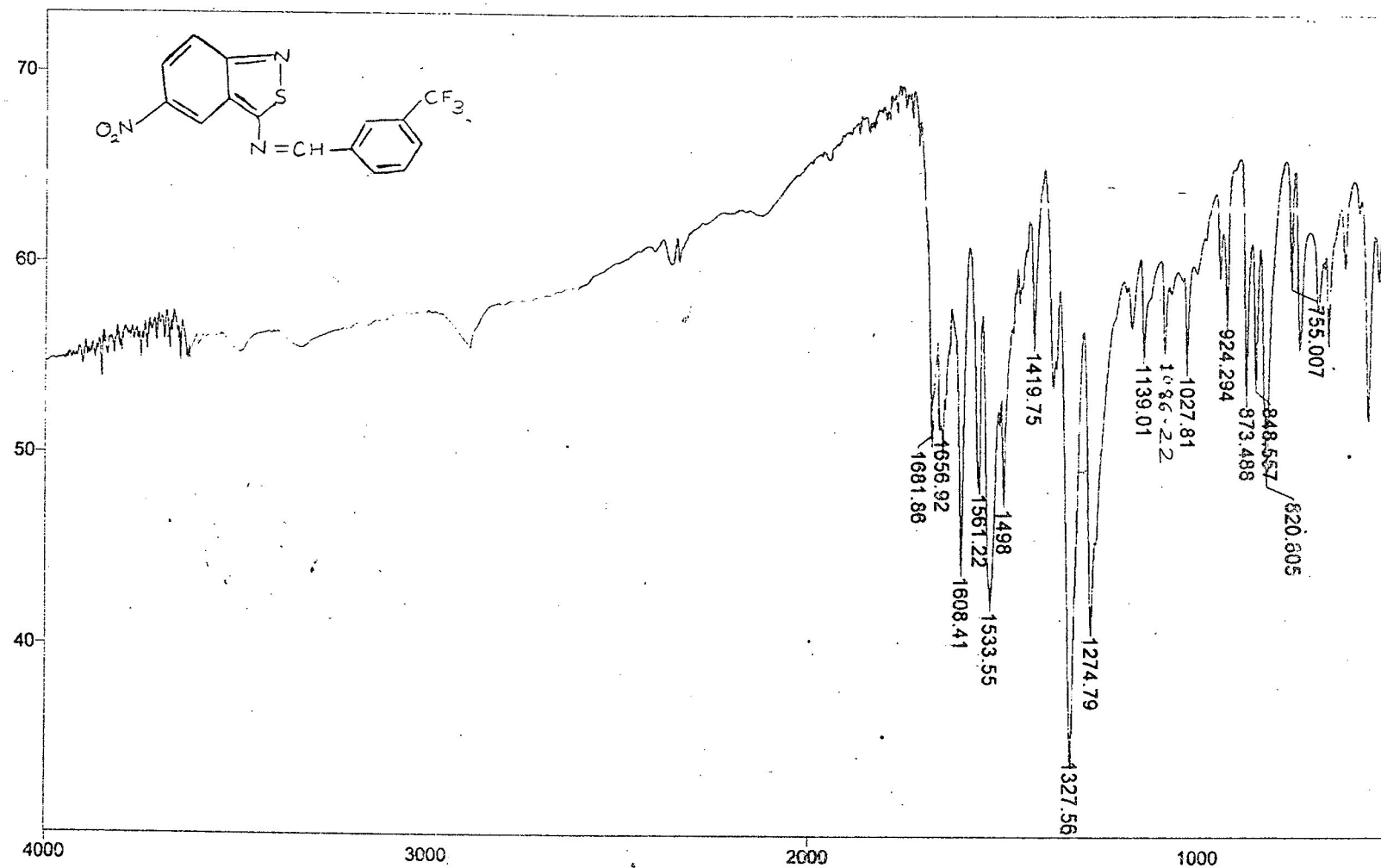
Sample Description: schiffbase

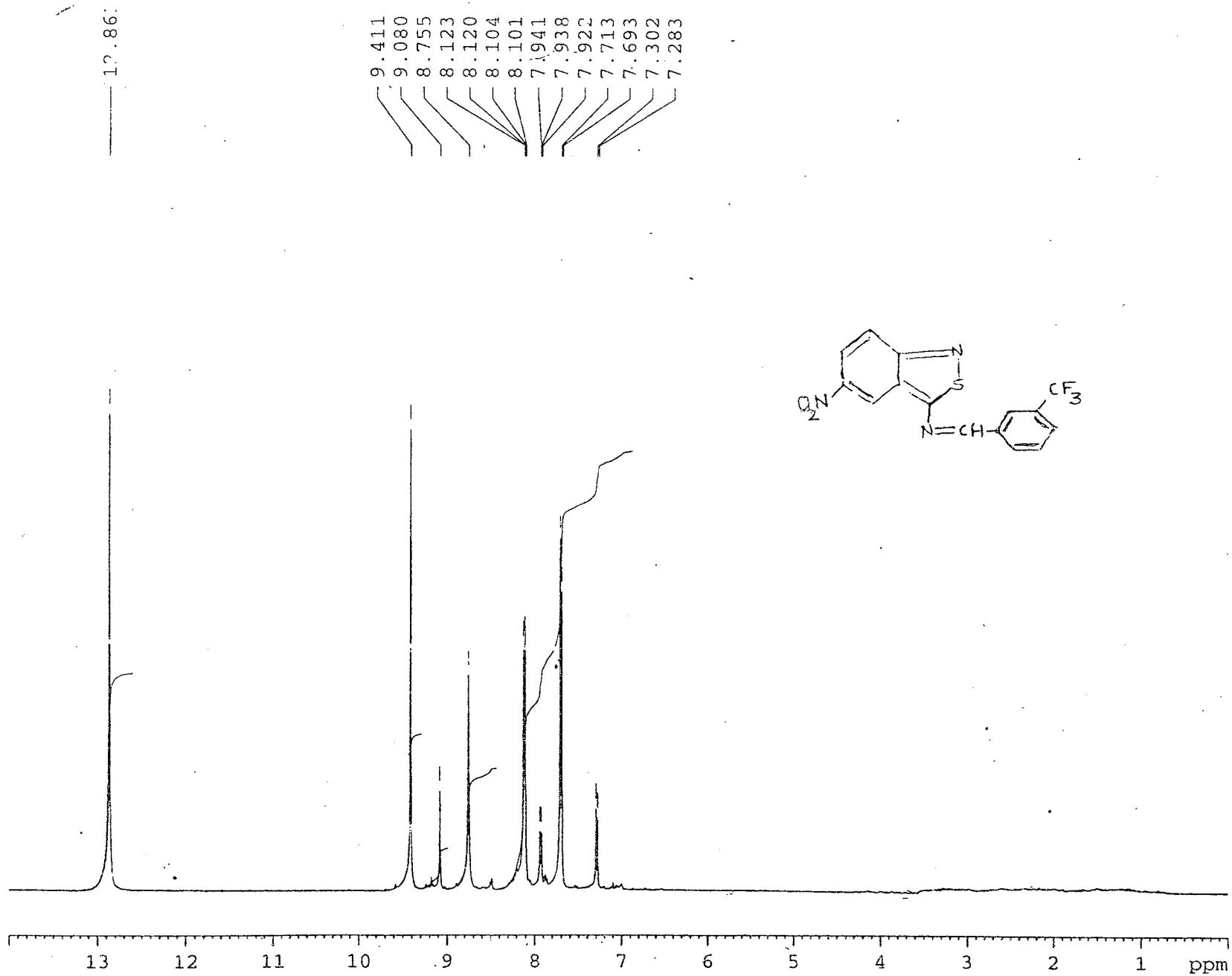
Scans= 10 Slow

Res=4 cm-1

Apod= Cosine

Zero Filling= 1 x





File # 1 : APBTH

Mode= 2 (Mid-IR)

6/25/97 6:25 PM

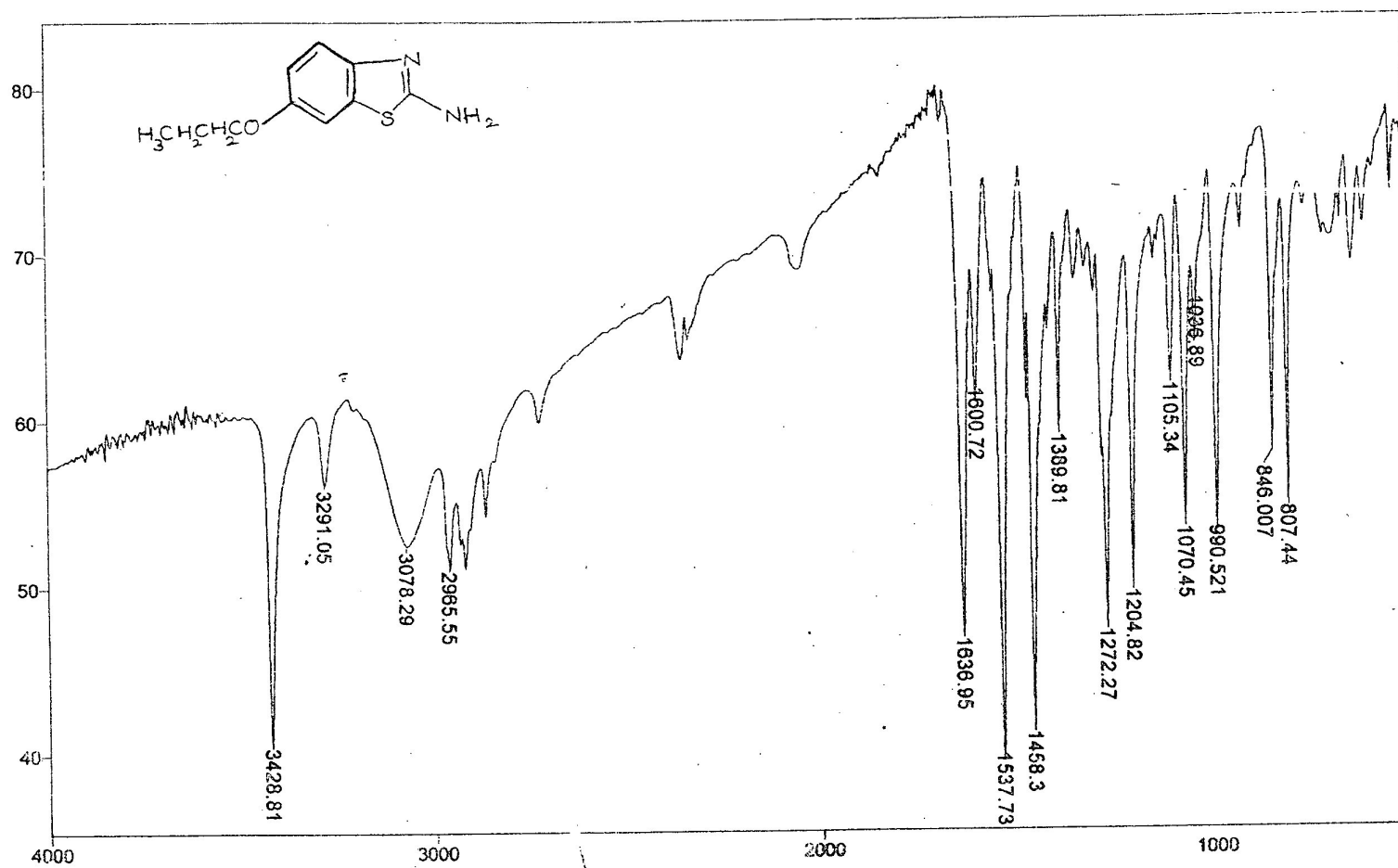
Sample Description: aminopropoxybenzothiazole

Scans= 10 Slow

Res=4 cm-1

Apod= Cosine

Zero Filling= 1 x



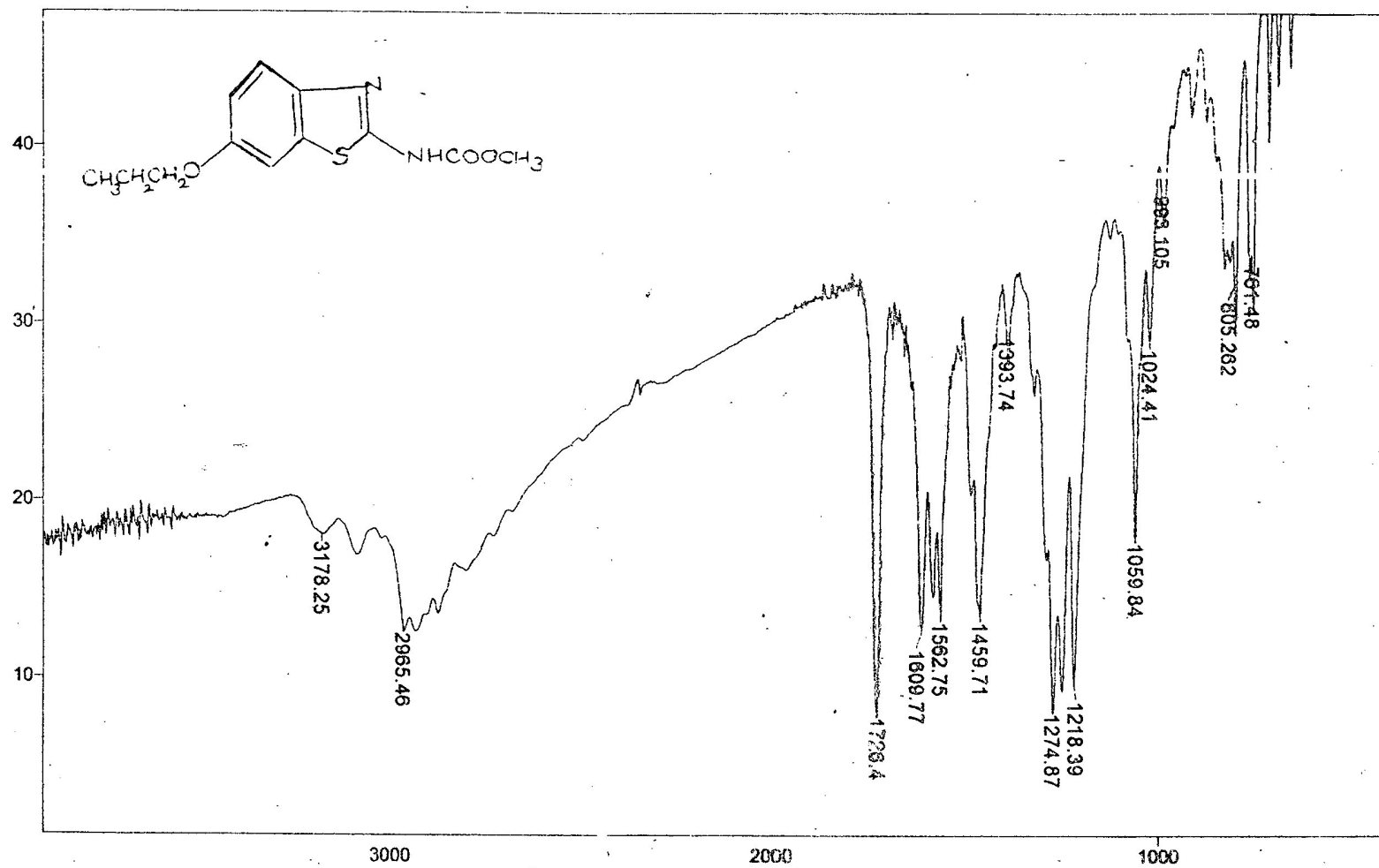
Sample Description: methylpropoxybenzothiazolecarbamate

Scans= 10 Slow

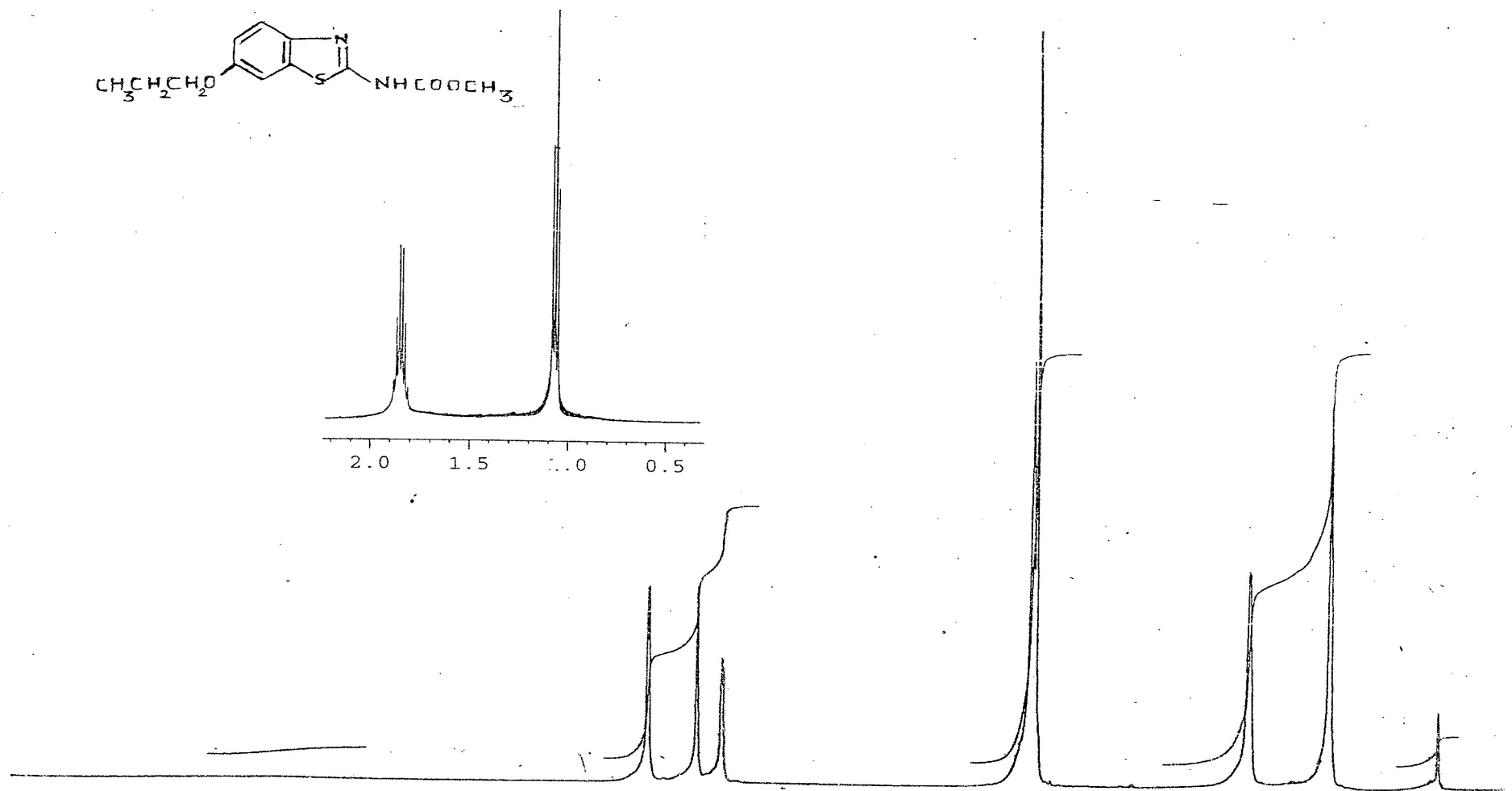
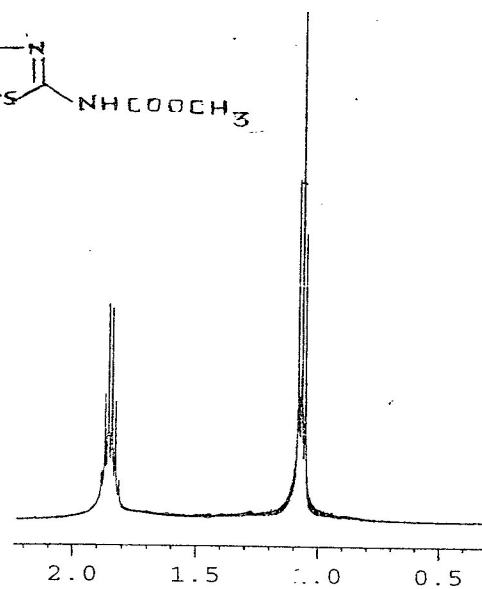
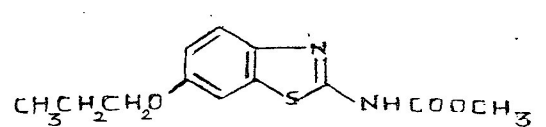
Res=4 cm-1

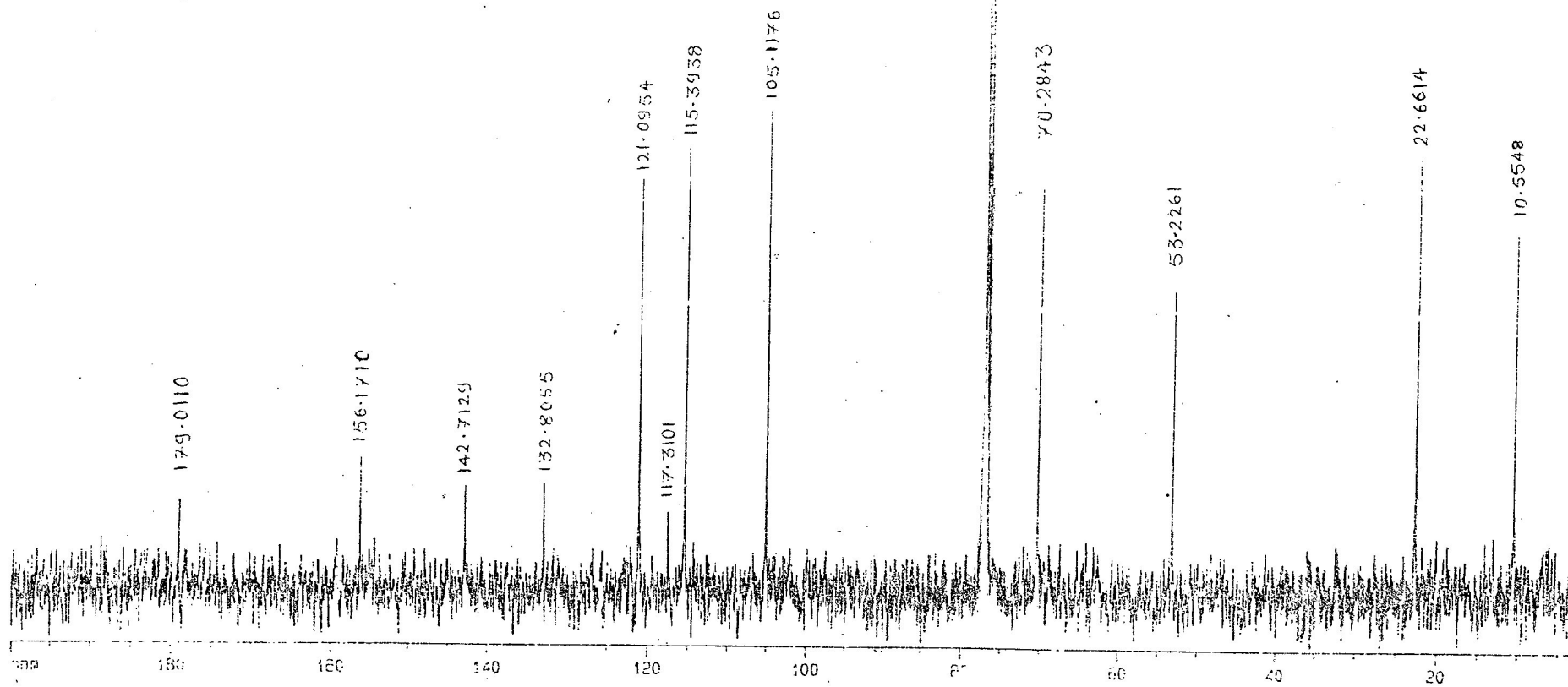
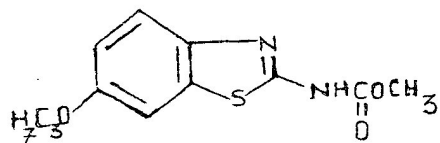
Apod= Cosine

Zero Filling= 1 x









*BIOLOGICAL RESULTS  
AND  
DISCUSSION*



The compounds synthesized in the present work have been subjected to various biological screening programs.

The various screening programs carried out include the *in vitro* study against H<sub>37</sub>RV strain of Mycobacterium tuberculosis, *in vitro* study against Gram-positive and Gram-negative bacteria viz. Staphylococcus aureus ATCC 3750 and Salmonella typhi NCTC 786 and also *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 suitable solvent (10 ml) such as dimethylformamide, ethanol, dil HCl as the case may be, so as to prepare drug stock solution 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 50 µg/ml, 2.5 µg/ml etc.

### Controls

- 1) Media control : Sterilized medium was kept as control so as to assure sterility of 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 and organism. Organism should grow in the medium. If no growth is observed, it can be concluded that medium is unable to support the growth.
- 3) Solvent Control : This includes sterilized medium, solvent (used to dissolve the drug) and culture. Organism should be able to grow in the quantity of solvent used in testing. If no growth is observed, it can be concluded that solvent inhibits the growth of organism and in that case results of the test are not valid.

### **ANTITUBERCULAR SCREENING :**

The test compounds have been subjected to *in vitro* screening by tube dilution technique employing the human virulent H<sub>37</sub>RV strain of M. tuberculosis. In this method, Kirchner's medium containing tween -80 as described by Rake *et al*<sup>175</sup> was used.

Kirchner's medium (sterilized) was dispensed in each borosilicate tube and to this 0.5 ml of sterile horse serum was added. The drug solution was added in each tube so as to attain final drug concentrations as 200, 100, 40, 20, 10, 5, 2, 1, 0.5, 0.25 µg/ml. Finally, the inoculum of standard suspension i.e. 0.1 ml of M. tuberculosis (H<sub>37</sub>RV strain) 10<sup>6</sup> bacilli per ml was added. The tubes were incubated at 37°C for ten days and then examined for the presence or absence of growth of the test organism. The lowest concentration which showed no visible growth was taken as the end point. i.e. Minimum Inhibitory Concentration. Isoniazid was used as a standard drug and was also subjected to same screening pattern. Minimum Inhibitory concentration of isoniazid is 0.04 µg/ml

### **ANTIBACTERIAL SCREENING :**

The test compounds have been subjected to *in vitro* screening by the tube dilution technique against Gram-positive and Gram-negative bacteria viz. S. aureus and S. typhi using Mueller Hinton broth as the culture medium.

Mueller Hinton broth (sterilized) was dispensed in each borosilicate test tube (150 x 20 mm). The drug solution was added in order to attain final drug concentrations as 200, 100, 40, 20, 10, 5, 2, 1, 0.5, 0.25 etc. µg/ml

At the end, inoculum of standard suspension, 0.1ml of the desired bacterial strain (S. aureus or S. typhi) containing 10<sup>6</sup> 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 test organisms.

The lowest concentration which showed no visible growth was taken as an end point (MIC).

Ampicillin and Trimethoprim were used as standard drugs against Gram-positive and Gram-negative bacteria respectively and were subjected to similar screening programs.

MIC of ampicillin is 0.01  $\mu\text{g}$  / ml against S. aureus and that of trimethoprim is 1  $\mu\text{g}$ /ml against S. typhi.

#### **ANTIFUNGAL SCREENING :**

The organisms employed in the test were

1) Candida albicans 2) Trichophyton mentagrophytes and 3) Trichophyton rubrum.

These organisms were maintained on Sabouraud's agar slants by regular subculturing C. albicans was cultured every week and the rest of the organism at the interval of 12 days.

Serafataller medium was employed for the testing and pH of medium was adjusted to 5.2. The medium (sterilized) was dispensed in each borosilicate tube. The drug solution was added in order to attain final drug concentrations as 200, 100, 40, 20, 10, 5, 2, 1, 0.5  $\mu\text{g}$  /ml.

The tubes were incubated at room temperature ( 28-30°C) in a dark place.

Incubation period for C. albicans is 7 days whereas that of T. mentagrophytes and T. rubrum is 14 days.

The presence or absence of growth was noted visually . The lowest concentration which showed no visible growth was taken as end point (MIC).

Miconazole, was used as a standard drug and was subjected to same screening pattern .

MIC of miconazole is 6.25  $\mu\text{g}$ /ml for C. albicans and 1.56  $\mu\text{g}$ /ml for T. mentagrophytes and T. rubrum.

## RESULT AND DISCUSSION :

### PART II

In this series 12 compounds were synthesized. All these compounds along with starting 3-amino- 5 nitro- 2,1- benzisothiazole were subjected to the various screening programs. This series contains three types of chemical linkages, carboxamido linkage, carbamate linkage, dialkyl carbamido linkage.

3 - amino - 5 - nitro - 2,1- benzisothiazole showed moderate activity of 100 µg/ml against S. aureus, S. typhi and M. tuberculosis. It was not active at 200 µg/ml against C. albicans and T. mentagrophytes but active against T. rubrum at 40 µg/ml. In case of carbamate esters as well as carbamido linkage no much deviation of activity from the starting compound has been observed.

#### Antibacterial activity

Amide linkage showed unexpected increase in the biological activity.

Four derivatives namely,

- 1) 3-(5-chloro- 2- hydroxy phenyl) carboxamido- 5 nitro 2,1- benzisothiazole
- 2) 3-(4 - fluorophenyl) carboxamido - 5 - nitro- 2,1- benzisothiazole .
- 3) 3-( 4 -fluorophenyl) carboxamido - 5-nitro- 2,1 benzisothiazole and
- 4) 3-(3-fluorophenyl) carboxamido-5-nitro 2,1- benzisothiazole

(compounds 2,6,8 and 9 of table 5 ) have exhibited excellent activity against S. aureus i.e. 0.25 µg /ml.

The same four compounds showed good activity against S. typhi i.e. 10 - 40 µg/ml.

In case of antitubercular screening, no deviation in the activity from activity of starting material was observed except for 3-(3-fluorophenyl) carboxamido-5-nitro-2,1- benzisothiazole which was active at 40 µg/ml.

In antifungal screening :

3-(5-bromo-2-hydroxy) carboxamido-2,1-benzisothiazole had shown good activity against T. rubrum i.e. 5 µg/ml and T. mentagrophytes i.e. 10 µg/ml. The biological activity of each compound against all above mentioned organism was described in Table 6.

Azomethine linkage: Out of eleven compounds synthesized, five showed very good activity against S. aureus i.e. 1-5 µg/ml and four against S. typhi i.e. 5 µg/ml. All these compounds were the halo substituted derivatives.

3-amine-N-(4-trifluoromethylphenyl) methylene-5-nitro 2,1-benzisothiazole has exhibited excellent antifungal activity against C. albicans, T. mentagrophytes and T. rubrum i.e. 1-10 µg/ml.

Out of eleven compounds synthesized, eight were active against T. mentagrophytes at concentration of 1-20 µg/ml and five were active against T. rubrum at 10 -20 µg/ml.

Antimycobacterial activity :

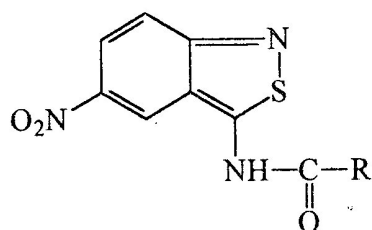
Out of eleven compounds synthesized five have exhibited good activity i.e. 5-10µg/ml against H<sub>37</sub>R<sub>V</sub> strain of M. tuberculosis.

Schiff's bases in which halo substituent is present at para position of phenyl ring are more active than those in which halogen is at ortho or meta position.

3-amine - N - (5 -bromo - 2 - hydroxy phenyl) methylene - 5 - nitro - 2,1 - Benzisothiazole (compound no. 9 of table 7) is found to be most active amongst the series i.e. at 0.25µg/ml. All the compounds have exhibited enhancement in antimycobacterial activity compared to parent compound 3-amino-5-nitro-2,1-benzisothiazole.

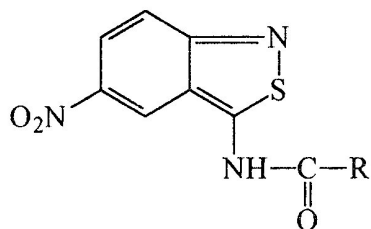


**Table 5**  
**3-substituted amino-5-nitro-2,1-benzisothiazole**



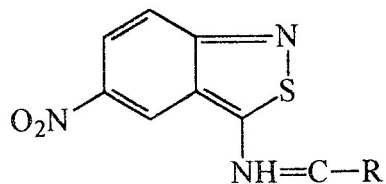
| Sr. No. | R                           | Mycobacterium tuberculosis<br>( $\mu\text{g} / \text{ml}$ ) | Staphylococcus aureus<br>( $\mu\text{g} / \text{ml}$ ) | Salmonella typhi<br>( $\mu\text{g} / \text{ml}$ ) |
|---------|-----------------------------|-------------------------------------------------------------|--------------------------------------------------------|---------------------------------------------------|
| 1       | 2-hydroxy phenyl            | 100                                                         | 40                                                     | 40                                                |
| 2       | 5-chloro-2-hydroxy phenyl   | 100                                                         | 0.25                                                   | 10                                                |
| 3       | 5-bromo-2-hydroxy phenyl    | 100                                                         | 40                                                     | 40                                                |
| 4       | 3,5-diiodo-2-hydroxy phenyl | 100                                                         | 100                                                    | 100                                               |
| 5       | adamantyl                   | 100                                                         | 100                                                    | 100                                               |
| 6       | 4-chlorophenyl              | 100                                                         | 0.25                                                   | 10                                                |
| 7       | 2-fluorophenyl              | 100                                                         | 5                                                      | 100                                               |
| 8       | 3-fluorophenyl              | 100                                                         | 0.25                                                   | 40                                                |
| 9       | 4-fluorophenyl              | 100                                                         | 0.25                                                   | 40                                                |
| 10      | methoxy                     | 100                                                         | 200                                                    | 200                                               |
| 11      | ethoxy                      | 100                                                         | 100                                                    | 100                                               |
| 12      | dimethylamino               | 100                                                         | 100                                                    | 100                                               |

**Table 6**  
**3-substituted amino-5-nitro-2,1-benzisothiazide**



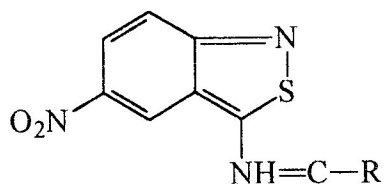
| Sr. No. | R                           | Candida albicans<br>( $\mu\text{g} / \text{ml}$ ) | Trichophyton mentagrophytes<br>( $\mu\text{g} / \text{ml}$ ) | Trichophyton rubrum<br>( $\mu\text{g} / \text{ml}$ ) |
|---------|-----------------------------|---------------------------------------------------|--------------------------------------------------------------|------------------------------------------------------|
| 1       | 2-hydroxy phenyl            | 200                                               | 10                                                           | 10                                                   |
| 2       | 5-chloro-2-hydroxy phenyl   | 200                                               | 40                                                           | 40                                                   |
| 3       | 5-bromo-2-hydroxy phenyl    | 100                                               | 10                                                           | 5                                                    |
| 4       | 3,5-diiodo-2-hydroxy phenyl | -                                                 | -                                                            | -                                                    |
| 5       | adamantyl                   | -                                                 | -                                                            | 40                                                   |
| 6       | 4-chlorophenyl              | 200                                               | 40                                                           | 40                                                   |
| 7       | 2-fluorophenyl              | -                                                 | -                                                            | -                                                    |
| 8       | 3-fluorophenyl              | -                                                 | -                                                            | -                                                    |
| 9       | 4-fluorophenyl              | -                                                 | -                                                            | -                                                    |
| 10      | methoxy                     | -                                                 | 100                                                          | 40                                                   |
| 11      | ethoxy                      | 200                                               | 40                                                           | 40                                                   |
| 12      | dimethylamino               | -                                                 | 40                                                           | 40                                                   |

**Table 7**  
**3-amine-(N-substituted phenyl / tolyl / heterocyclic) methylene-5-nitro-2,1-**  
**benzisothiazole**



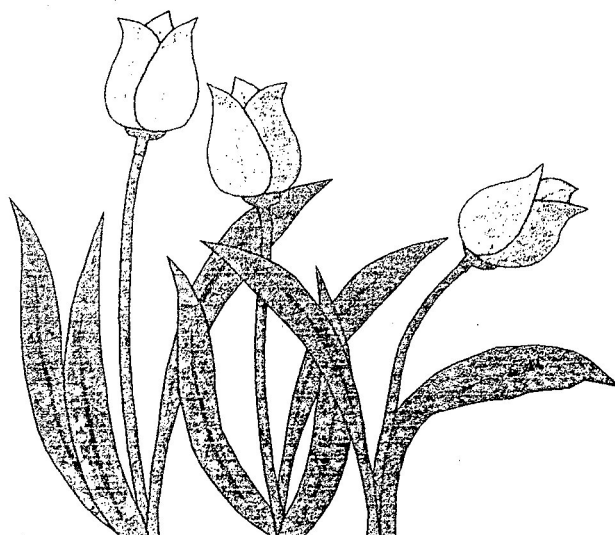
| Sr. No. | R                                | Mycobacterium tuberculosis (µg / ml) | Staphylococcus aureus (µg / ml) | Salmonella typhi (µg / ml) |
|---------|----------------------------------|--------------------------------------|---------------------------------|----------------------------|
| 1       | 2-fluorophenyl                   | 40                                   | 100                             | 5                          |
| 2       | 4-fluorophenyl                   | 5                                    | 5                               | 5                          |
| 3       | 3-chlorophenyl                   | 40                                   | 5                               | 5                          |
| 4       | 4-chlorophenyl                   | 10                                   | 5                               | 5                          |
| 5       | α, α, α-trifluoro-2-tolyl        | 40                                   | 100                             | 100                        |
| 6       | α, α, α-trifluoro-3-tolyl        | 40                                   | 100                             | 100                        |
| 7       | α, α, α-trifluoro-4-tolyl        | 5                                    | 40                              | 40                         |
| 8       | 2-fluoro-α,α,α-trifluoro-4-tolyl | 5                                    | 1                               | 40                         |
| 9       | 5-bromo-2-hydroxy-phenyl         | 0.25                                 | 5                               | 100                        |
| 10      | 2-hydroxy naphthyl               | 5                                    | 100                             | 100                        |
| 11      | 2-thiophenyl                     | 40                                   | 100                             | 100                        |

**Table 8**  
**3-amine-(N-substituted phenyl / toluyl / heterocyclic) methylene-5-nitro-2,1-**  
**benzisothiazole**



| Sr. No. | R                                                     | <i>Candida albicans</i><br>( $\mu\text{g} / \text{ml}$ ) | <i>Trichophyton mentagrophyte</i><br>( $\mu\text{g} / \text{ml}$ ) | <i>Trichophyton rubrum</i><br>( $\mu\text{g} / \text{ml}$ ) |
|---------|-------------------------------------------------------|----------------------------------------------------------|--------------------------------------------------------------------|-------------------------------------------------------------|
| 1       | 2-fluorophenyl                                        | 200                                                      | 20                                                                 | 20                                                          |
| 2       | 4-fluorophenyl                                        | 200                                                      | 20                                                                 | 40                                                          |
| 3       | 3-chlorophenyl                                        | 100                                                      | 5                                                                  | 40                                                          |
| 4       | 4-chlorophenyl                                        | 200                                                      | 1                                                                  | 20                                                          |
| 5       | $\alpha, \alpha, \alpha$ -trifluoro-2-tolyl           | 40                                                       | 40                                                                 | 100                                                         |
| 6       | $\alpha, \alpha, \alpha$ -trifluoro-3-tolyl           | 40                                                       | -                                                                  | -                                                           |
| 7       | $\alpha, \alpha, \alpha$ -trifluoro-4-tolyl           | 10                                                       | 1                                                                  | 10                                                          |
| 8       | 2-fluoro- $\alpha, \alpha, \alpha$ -trifluoro-4-tolyl | 40                                                       | 10                                                                 | 20                                                          |
| 9       | 5-bromo-2-hydroxy-phenyl                              | 20                                                       | 10                                                                 | 10                                                          |
| 10      | 2-hydroxy naphthyl                                    | 100                                                      | 5                                                                  | 40                                                          |
| 11      | 2-thiophenyl                                          | 40                                                       | 40                                                                 | 100                                                         |

# CONCLUSION



The present work was focused on the synthesis of novel heterocyclic compounds of possible antitubercular, antibacterial and antifungal activity.

In Part II the synthesis of 3 - substituted amino- 5 - nitro 2,1- benzisothiazoles has been described. The antimicrobial activity of the series reveals following points.

- 1) The presence of amide linkage and halogen substituent such as fluorine, chlorine, bromine on benzisothiazole moiety has shown excellent increase in antibacterial activity of the parent compound, 3- amino- 5 - nitro - 2,1- benzisothiazole . But amide linkage has not proved beneficial as far as antimycobacterial activity is concerned. In case of antifungal screening 5-bromosalicylamide derivative has shown best activity against T. mentagrophytes and T. rubrum.
- 2) Carbamate esters have not shown any significant increase in antibacterial antifungal and antitubercular activity. The same is observed in case of dimethyl carbamido derivative [compound no 10,11,12 respectively of Table 5].

In Part III, the synthesis of 3 amine- ( N- substituted phenyl / toluy / heterocyclic) methylene - 5-nitro-2,1benzisothiazole has been described. From various screening programs, following observations are made.

- 1) The presence of azomethine linkage on 3- amino - 5 - nitro -2,1- benzisothiazole has definitely improved its antibacterial action. Mono halo substituents have exhibited better action ( compound number 1,2,3,4,8 and 9 from Table 7).
- 2) The parent compound has not shown activity against T. mentagrophytes at 200 µg/ml and has exhibited moderate activity against T. rubrum i.e. at 40 µg/ml. But all azomethine derivatives have exhibited excellent activity against T. mentagrophytes i.e. 1-20 µg/ml and good to moderate activity against T. rubrum i.e. 10-100 µg/ml

Thus from above discussion it may be concluded that it is worth while to pursue further investigation on benzisothiazoles.

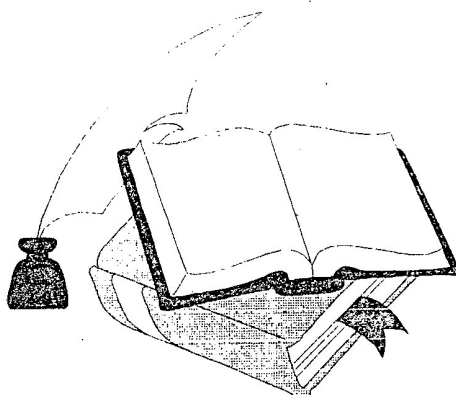
In Part II, nine amide linkages have been synthesized. Four amides have exhibited excellent antibacterial action against S. aureus i.e. 0.25 µg/ml which is comparable to most of the existing antibacterials. [ Cefotaxime, MIC for S. aureus is 1.5 µg/ml]

Nitrofurantoin as a representative of nitroheterocycles containing azomethine linkage is active against Gram-positive and Gram-negative bacteria at conc. of 5- 10 µg /ml.

In part III we have synthesized more active nitro substituted azomethine analogues Activities of most of the derivatives against T. mentagrophytes were comparable to that of Miconazole, Fluconazole, [compound No 1,2,3,4,8 of Table No 8 ]. Compounds synthesized in part III were all active against H<sub>37</sub>Rv strain of M. tuberculosis. Out of 11 compounds synthesized 5 have exhibited good activity i.e. 5-10µg/ml. Schiff base prepared from 5-bromo salicylaldehyde has exhibited excellent activity i.e. 0.25µg/ml.

The above activities are quite interesting and further study on the molecule is essential.

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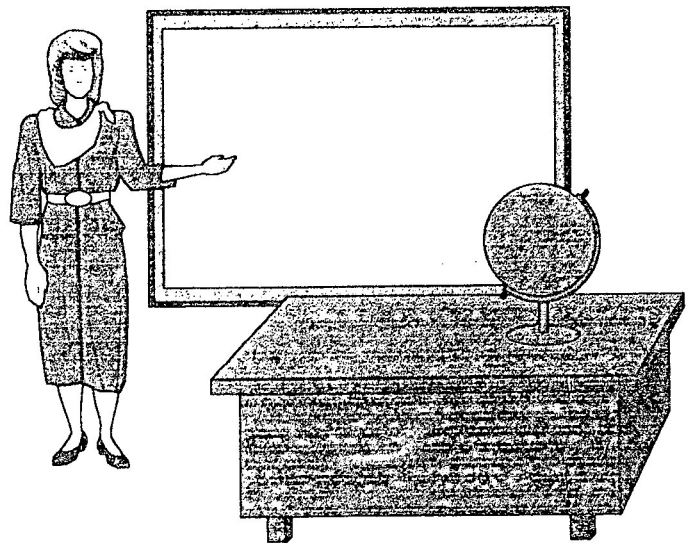
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## APPENDIX

1. The melting points have been determined in Open capillaries in a veego VMP - 1 melting point apparatus.
2. The TLC of the compounds have been run on Eastman Kodak precoated silica gel plates of type K 301 R and E. Merck pre -coated silica gel plates of type 60 F 254.
3. The Infrared spectra was recorded on a BOMEM MB - 104 Fourier Transform Infrared Spectrometer.
4. The PMR spectra and  $^{13}\text{C}$  NMR were recorded on Brucker AC 500 MHz Spectrometer and Varian 300 MHz spectrometer using TMS as internal reference.

# *SYNOPSIS*



**A SYNOPSIS  
OF THE THESIS TO BE SUBMITTED TO  
THE UNIVERSITY OF MUMBAI  
FOR THE DEGREE OF MASTER OF PHARMACY  
IN THE FACULTY OF TECHNOLOGY**

**TITLE OF THE THESIS** : SYNTHESIS OF HETEROCYCLIC  
COMPOUNDS AS POSSIBLE  
ANTIBACTERIAL, ANTIFUNGAL AND  
ANTITUBERCULAR AGENTS.

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OF THE GUIDE** : DR. B.G. KHADSE  
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**PLACE OF RESEARCH** : DEPARTMENT OF CHEMOTHERAPY  
HAFFKINE INSTITUTE,  
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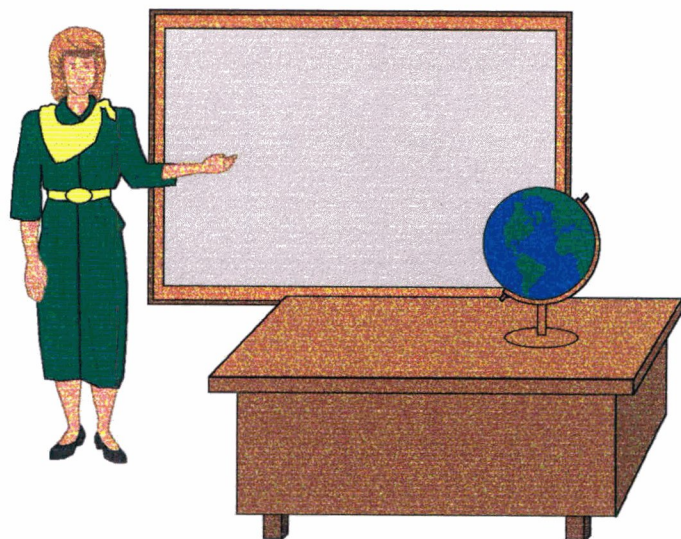
**NUMBER AND DATE OF  
REGISTRATION** : 17 / 4th DEC. 1995.

**SIGNATURE OF THE STUDENT** : P.S. Kharkar

**SIGNATURE OF THE GUIDE** :



# *SYNOPSIS*



Man's search for effective remedies is as old as mankind itself, but before the aetiological agents of infectious diseases were identified and made amenable to laboratory investigation, progress had to rely on the vagaries of chance and empirical observation. Not surprisingly therefore mankind's earlier therapeutic successes against infecting organisms came in the form of plant extracts that expelled worms visible to naked eye. Herbal anthelmintics since antiquity include extracts of male fern and oil of chenopodium. Two other ancient remedies include quinine obtained from cinchona bark, emetine obtained from ipecacuanha root, active against parasites of malaria and amoebic dysentery respectively.

So far as antibacterial remedies were concerned mercury was used for Syphilis since sixteenth century, Chaulmoogra oil from *Hydnocarpus* in Leprosy and mercury salts and phenolic substances as antiseptics.

The word 'Chemotherapy' was coined by Paul Ehrlich to articulate the concept of selective inhibition of microbes by chemicals in the early years of this century. He reasoned that since parasites of Malaria and African sleeping sickness could be differentiated from tissue by dyes, therefore dyes might display preferential affinity for parasites i.e. they might exhibit selective toxicity. This idea finally led to the discovery of wide variety of therapeutically useful antimicrobial compounds which though uncoloured themselves were derived from dyes.

The progression from dye to antimalarial was accomplished finally by scientists at Bayer. Information gained on modification of dye prompted scientists to try similar substitution on heterocycles. This idea led to the discovery of very important molecules then as antimalarial drugs, pamaquine and mepacrine. Further the discovery of primaquine and chloroquin also is the fruit of structural modification in the field of heterocyclic compounds. These drugs are still in use as the most effective and useful antimalarial drugs.

One of the more significant achievements of this century has been discovery and commercial development of numerous therapeutic agents that provide reliable effective treatments for many infectious diseases that had previously caused mortality, morbidity and fear.

Based on this aspect, efforts have been made to synthesise heterocyclic molecules such as benzisothiazoles and will be documented in the thesis. The thesis will also contain synthesis of Tioxidazole. This drug has been proved to be very important Veterinary Anthelmintic for naturally infected horses. Synthesis of an intermediate for recently developed drug 'Riluzole' is also involved in the thesis.

The thesis will also include synthesis of Tioxidazole starting from 2-amino-6-hydroxy benzothiazole. This compound is obtained in one step by the reaction of p-benzoquinone and thiourea. 2-amino-6-hydroxy benzothiazole could also act as an intermediate for the synthesis of very recently developed drug 'Riluzole', which is present in several anticonvulsant, anxiolytic and hypnotic preparations.

The thesis will consist of the following parts :

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

**PART 2 :** Synthesis of 5-nitro-3-substituted amino-2,1-Benzisothiazole.

**PART 3 :** Synthesis of 2,1-benzisothiazole-3-amine-N-(Substituted phenyl/heterocyclic) methylene-5-nitro.

**PART 4 :** Synthesis of Tioxidazole

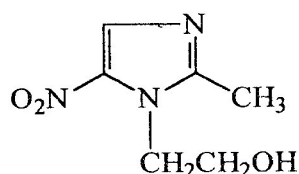
**PART 5 :** Synthesis of an intermediate for Riluzole.

**PART 6 :** Biological Screening.

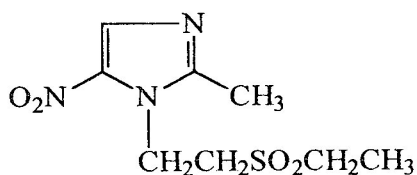
**PART 2 : Synthesis of 5-nitro-3-substituted amino-2,1-benzisothiazole.**

This consists of synthesis of 5-nitro-3-amino-2,1-benzisothiazole in the first place.

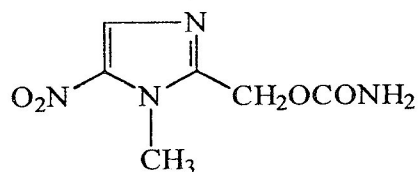
Nitroheterocycles have been proved in the field of medicinal chemistry as very important group of biologically active compounds. The most promising compounds are (1) Metronidazole (i) (2) Tinidazole (ii)<sup>1</sup> (3) Ronidazole (iii) (4) Nitrofurantoin, Furazolidone (iv) etc.



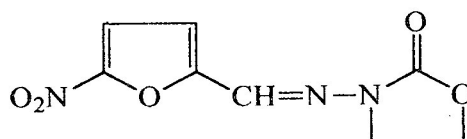
(i)



(ii)

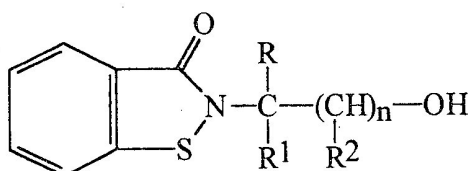


(iii)



(iv)

In all the above drugs, nitro group at a particular position has been found to be essential for antiparasitic and antiprotozoal activity. It was also proved experimentally that if the position of 'NO<sub>2</sub>' group in the molecule of a particular drug is changed or the group is removed from the molecule, there is total loss of biological activity. Taking these facts into consideration, it was proposed to synthesise 5-nitro-2,1-benzisothiazole derivative because from the literature search it was also revealed that 1,2-benzisothiazole-3(2H)-one(v) has exhibited excellent antibacterial activity against Gram positive bacteria such as *S. aureus* and *S. albus*.<sup>2</sup>



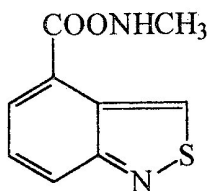
(v)

Apart from antibacterial and antifungal actions these types of compounds have also been proved to possess herbicidal<sup>3</sup>, algicidal<sup>4</sup>, trichomonicidal<sup>5</sup>, insecticidal<sup>6</sup> and pesticidal activities<sup>7</sup>.

They also proved as excellent preservatives<sup>4</sup>, agrochemical fungicides<sup>8</sup>, and anthelmintic compounds in animals<sup>9</sup>.

In spite of such interesting activities, marginal attention has been paid to 2,1-Benzisothiazoles.

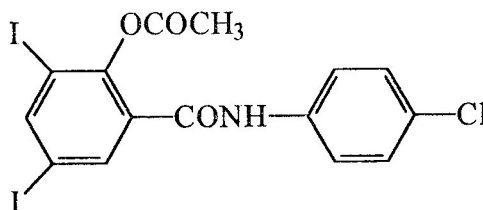
Singerman *et al.* and Garry M. *et al.* described pesticidal activity of 2,1-Benzisothiazole-4-ol-methylcarbamate ester (vi) against Mexican bean beetle larvae and houseflies<sup>7</sup>.



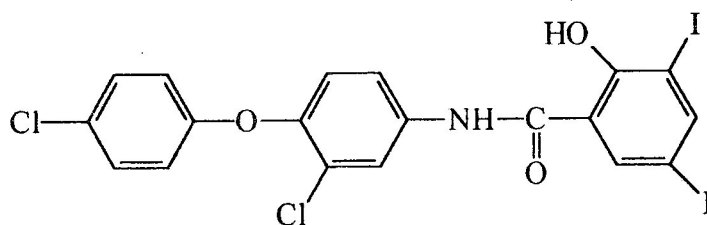
(vi)

2,1-Benzisothiazolamine has been found to have activity against several plant pathogenic fungi *in vitro* and in vegetables, and also activity against gram positive bacteria<sup>10</sup>.

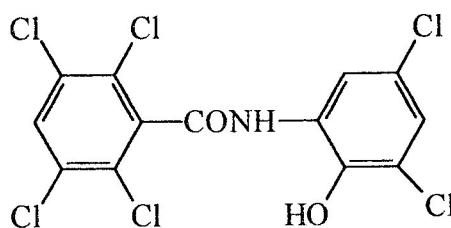
In the synthesis of important anthelmintic drugs such as Clioquinide (vii), Rafoxanide (viii), Oxylozanide (ix), amido linkage has played very essential role.



(vii)

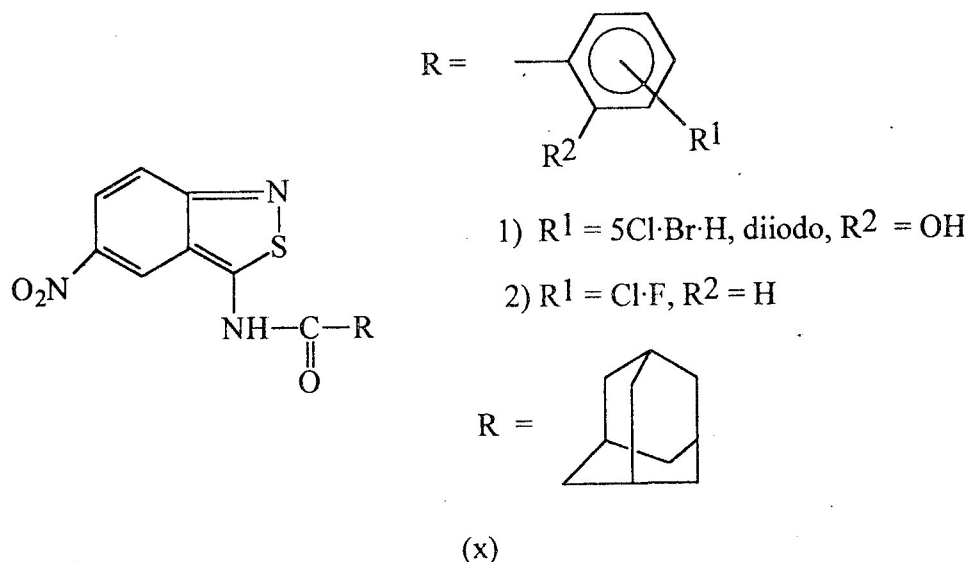


(viii)

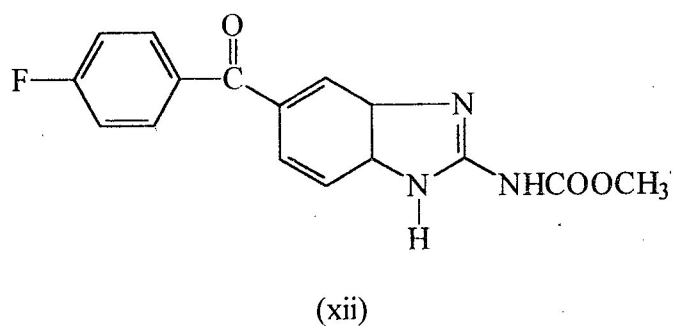
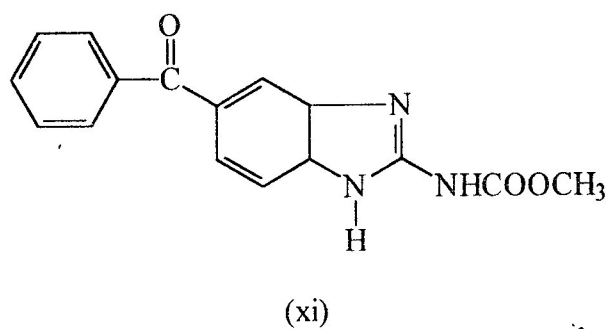


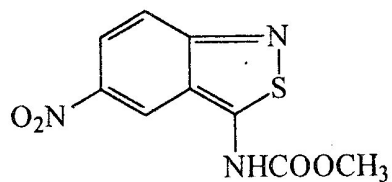
(ix)

The above drugs indicate the significance of halogenated salicylic acid amides. Hence it was thought vital to prepare different amido derivatives of 5-nitro-3-amino-2,1-benzisothiazole (x) using halogenated salicyloyl chlorides, substituted benzoyl chlorides and adamantyl acid chloride. The general structure of these compounds is shown as below.



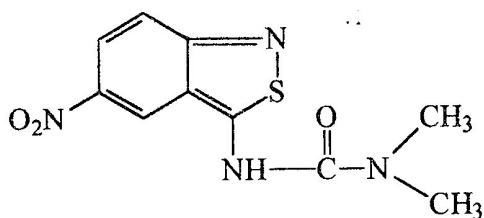
The biological importance of N-methyl carbamate linkage in the field of anthelmintics such as Mebendazole (xi), Flubendazole (xii), Albendazole is well documented. Hence N-carbmethoxy derivative of 5-nitro-3-amino-2,1-benzisothiazole in the analogy with the above mentioned anthelmintic compounds was prepared (xiii)





(xiii)

Hetrazan, a diethyl carbamoyl derivative of N-methyl piperazine is an excellent and only drug in the treatment of filarial infection since 1950. With the structural analogy of this compound, the 2,1-benzisothiazolamine was reacted with diethyl carbamoyl chloride to obtain the compound of following type (xiv)



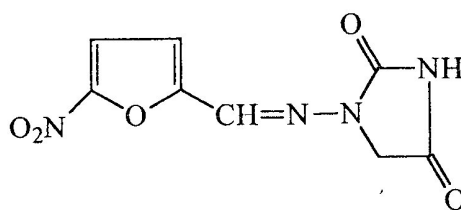
(xiv)



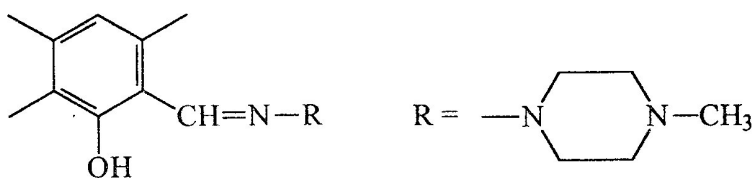
**PART 3 : Synthesis of 2,1-Benzisothiazole-3-amine-N-(substituted phenyl/heterocyclic) methylene-5-nitro**

Schiff bases from 5-chlorosalicylaldehyde, 5-bromo-salicylaldehyde and 4-N,N-dimethylaminobenzaldehyde have been reported to have encouraging tuberculostatic action<sup>11</sup>. The presence of azomethine linkage in compounds has also been known to decrease the toxicity considerably<sup>12</sup>.

The presence of azomethine linkage in the variety of biologically active compounds such as Nitrofurantoin (xv) Furazolidone (iv) and also in the field of antibiotics such as Rifampin (xvi) has been proved to be very essential.

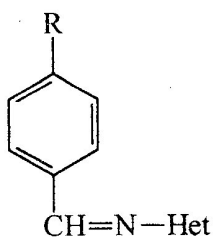


(xv)



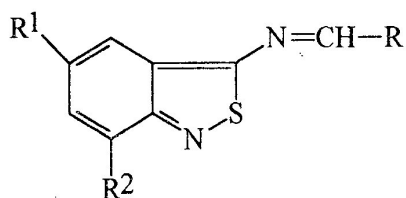
(xvi)

Recently R. Fioravanti *et al.* have reported antimicrobial activity of some new heteroaryl benzylamines and their Schiff bases<sup>13</sup> (xvii).

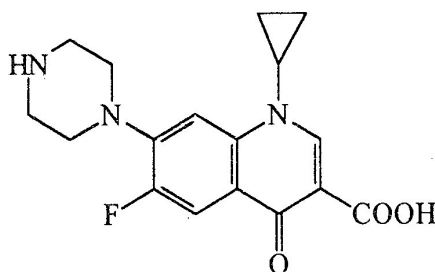


(xvii)

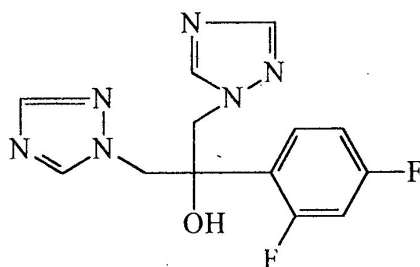
Bellotti A.*et al.*, Coghi *et al.* and Sgnorbati O. *et al.* recently revealed antimycotic and antistaphylococcal activities of 3-azomethine linkage of 2,1-benzisothiazoles



Fluorine containing drugs such as Ciprofloxacin (xviii), Fluconazole (xix), Riluzole have been proved to be very specific drugs for the treatment of particular diseases.

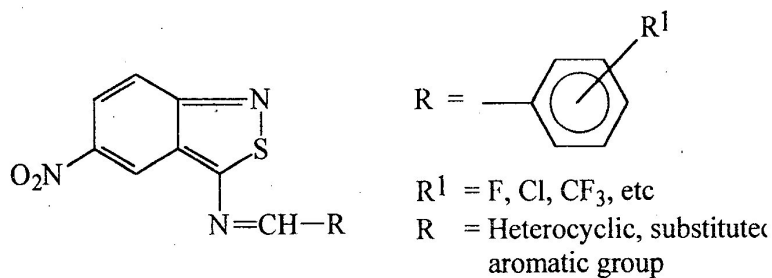


(xviii)



(xix)

Considering this special property of fluorinated compounds, Schiff bases of 5-nitro-2,1-benzisothiazolamine with various fluorinated aldehydes were prepared and tested for their biological activity (xx).



#### **PART 4 :     Synthesis of Tioxidazole**

It was already mentioned in the previous discussion that N-carbomethoxy derivative of benzimidazole such as Mebendazole (xi), Flubendazole (xii), Oxfendazole, Albendazole and Fenbendazole proved to be excellent, broad spectrum anthelmintic compounds. The latest drug, Tioxidazole, has been reported to have broad spectrum activity against gastrointestinal roundworms in naturally infected horses. It was also found to be effective in mice against *Nematospiroides dubius* and *Syphacia obvelvata* when administered at dietary levels of 0.018% to 0.03% or by gavage at 50 mg/kg for 5 days. A single 75 mg/kg oral dose was over 99% effective against *Trichinella spiralis* infection in mice. It was also active against *Strongyloides ratti* by gavage as a single oral dose of 200 mg/kg.

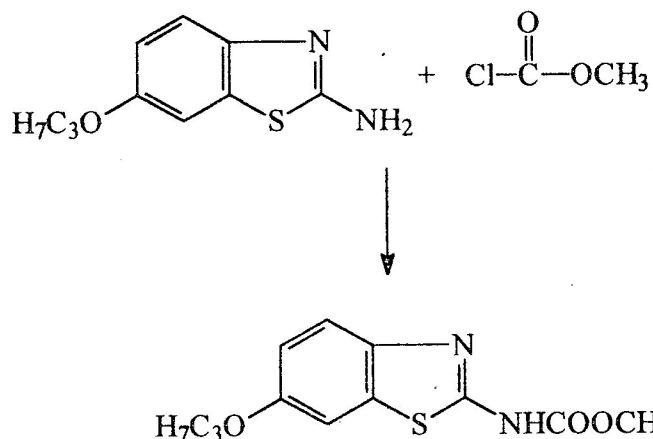
In dogs, a single oral dose of 200 mg/kg of this drug is active against *Toxascaris leonina* and also was effective against hookworms and *Trichuris vulpis*.

Tioxidazole in horses was found to be effective against *Parascaris equorum*, *Oxyuris equi*, *Strongylus vulgaris*. Dose levels upto 100 mg/kg have had no adverse effect in the horse. Currently Tioxidazole is used as an anthelmintic for naturally infected horses.

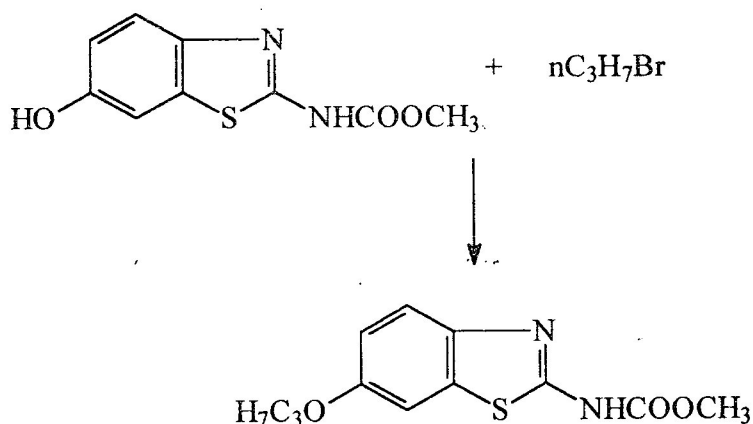
Thus, Tioxidazole has been proved to be very important veterinary anthelmintic.

This being an important drug which is not at all manufactured in India, the synthesis of this compound was taken up from the basic stage. It was synthesised in our laboratory by two routes.

- (I) 6-n-propoxy-2-amino-benzothiazole was obtained by thiocyanation of *p*-propoxyaniline as an important intermediate. This was further condensed with methyl chloroformate to obtain carbomethoxy, 6-propoxy-benzothiazole-2-carbamate or Tioxidazole.



- (II) In the second route, 2-amino-6-hydroxy benzothiazole was first synthesised by the reaction of two moles of benzoquinone with one mole of thiourea. This was then selectively converted into 6-hydroxy benzothiazole-2-carbamate by using methyl chloroformate. This compound was further treated with n-propylbromide in propanol to obtain Tioxidazole.



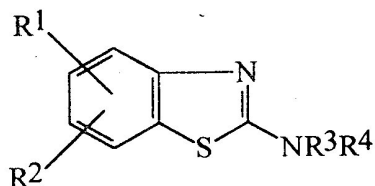
Alternatively, 2-amino, 6-hydroxy benzothiazole was first reacted with n-propyl bromide assuming to get 6-n-propoxy-2-amino benzothiazole. However, it led to formation of complex mixture of compounds which were very difficult to obtain in pure form. Thus, to obtain drug with the third route was not proved to be possible.

From our experimental experiences we recommend that the best method for preparation of Tioxidazole(xxi) is preparation of carbamate derivative of 6-n-propoxy-2-amino benzothiazole.



**PART 5 : Synthesis of an intermediate for Riluzole.**

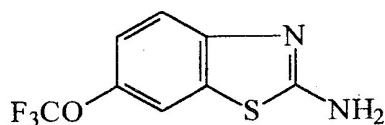
Johnson *et al.*, Graham *et al.* and Pavia *et al.* have described substituted 2-aminobenzothiazole and derivatives<sup>14</sup> as useful cerebrovascular agents, of the following type (xxii).



$R^1, R^2 = \text{H, Alkyl, Alkenyl, Phenyl, CF}_3, \text{Halo, Nitro, etc}$

$R^3, R^4 = \text{H, Alkyl, Alkyl heterocyclic etc.}$

The above compounds are prepared as drugs for treatment of cerebrovascular disorders or as anaesthetics in surgery where risk of cerebrovascular damage exists. In rats, it controlled collateral zone infarct by 90% and hemispheric infarct by 88%. Recently Riluzole a benzothiazole derivative of the following structure has been reported to have very broad type of biological activity. It has been found to act as 1) Glutamate antagonist, 2) Anti-ischemic agent, 3) An agent in familial amyotrophic lateral sclerosis.

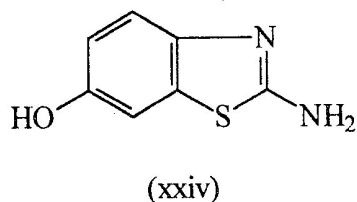


(xxii)

This compound has a very simple structure with trifluoromethoxy group as a substituent at sixth position of 2-amino benzothiazole (xxiii). Several anticonvulsant, anxiolytic, hypnotic formulations were prepared containing 2-amino-6-trifluoromethoxy benzothiazole as an active ingredient. LD50 values in mice are 46 mg/kg (intraperitoneal) and 67mg/kg(oral). Tablets, capsules, suppositories were prepared containing 1-50mg of (xxiii).

The route of synthesis of this compound has been reported in literature<sup>15</sup> as thiocyanation of 4-trifluoromethoxy aniline.

However, we thought of synthesising this compound by alternate route, that is trifluoromethylation of 2-amino-6-hydroxy benzothiazole. With this idea, 2-amino-6-hydroxy benzothiazole (xxiv) was synthesised from 2 moles of *p*-benzoquinone and 1 mole of thiourea.



The above compound can be subjected to trifluoromethylation using a fluorinating agent, but due to non-availability of special type of apparatus for fluorination, this step was not carried out. Cost effectiveness has to be taken into consideration while carrying out trifluoromethylation as several fluorinating agents are very costly.

**PART 6 : Biological Screening**

The compounds reported herein have been subjected to elemental and spectroscopic analysis, and the results were found to be satisfactory.

The compounds reported in parts 2 & 3 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 a human virulent H37Rv strain of *M. tuberculosis*. By this method using Kirchners medium containing Tween80 as described by Rake *et al.*, dispersed growth of *M. tuberculosis* was obtained. About 10 compounds have shown moderate activity i.e. 100 mg/ml. One compound has exhibited good antitubercular activity i.e. 50 mg/ml. The standard drug used for antitubercular screening was Isoniazide. The standard dose of Isoniazide is 0.04 mg/ml for H37Rv strain of *M. tuberculosis*.

**ANTIBACTERIAL SCREENING**

The compounds described in parts 2 & 3 were also subjected to *in vitro* tests against *S. aureus* and *S. typhi*. The culture medium employed in the screening was Mueller-Hinton broth. Five compounds have shown excellent activity against *S. aureus* i.e. 1.56 mg/ml - 6.25 mg/ml. Six compounds have shown moderate activity against *S. aureus* i.e. 100 mg/ml. Four compounds have shown good activity i.e. 12.5 mg/ml - 50 mg/ml against *S. typhi*. Seven compounds have exhibited moderate activity i.e. 100mg/ml against *S. typhi*. The standard drugs used for antibacterial screening were AMPICILIN SODIUM against *S. aureus* and TRIMETHOPRIM against *S. typhi*. The standard dose of AMPICILIN SODIUM is 0.01 mg/ml and that of TRIMETHOPRIM is 1 mg/ml.

**ANTIFUNGAL SCREENING**

The compounds have also been screened for their antifungal activity *in vitro* by the Tube Dilution technique against fungal strains viz. *C. albicans*, *T. rubrum* and *T. mentagrophytes*. Five compounds have shown good activity against *T. mentagrophytes* i.e. 50 mg/ml. Seven compounds have exhibited good activity against *T. rubrum* i.e. 50 mg/ml. The standard drug used was MICONAZOLE for antifungal screening. The standard dose of MICONAZOLE is 6.25 mg/ml for *C. albicans*, 1.56 mg/ml for *T. mentagrophytes* and *T. rubrum*.

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