

**SYNTHESIS OF ORGANIC COMPOUNDS OF POSSIBLE
BIOLOGICAL ACTIVITY**

**THESIS SUBMITTED
UNIVERSITY OF MUMBAI**

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

**SUBMITTED BY
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**UNDER THE GUIDANCE OF
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**DEPARTMENT OF MUMBAI
HAFFKINE INSTITUTE FOR TRAINING, RESEARCH & TESTING
MUMBAI – 400 012**

1999

**Synthesis of
Organic Compounds of Possible
Biological Activity**

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

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1999

STATEMENT

Statement submitted under rule 0.412

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

Statement submitted under rule 0.413

Statement I :

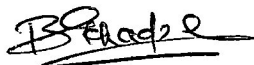
The work embodied in the Thesis to be submitted is original, based on the discovery of new facts, viz. the synthesis and characterization of new and unknown compounds of possible chemotherapeutic value. Also included in the Thesis, are new relations of facts, observed on the basis of in vitro testing.

The assessment of the compounds has been made at the Department of Microbiology and Department of Chemotherapy, Haffkine Institute, Mumbai 400012.

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



Sandeep Paranjape
Signature of the Research Student

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Synopsis

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Sandeep Paranjape

Introduction

INTRODUCTION

“Medicinal chemistry remains a challenging science which provides profound satisfaction to its practitioners. Medicinal chemists have chance to participate in the fundamental of preventing therapy and understanding of diseases to contribute to healthier and happier life”¹ -A. Burger.

In medicinal chemistry, the chemist attempts to design and synthesize a medicine or a pharmaceutical agent which will benefit humanity. Such a compound is also called as “drug”.

The development of new medicine takes places mainly in modern pharmaceutical industry and most of the new drugs are also discovered in research laboratories of pharmaceutical companies.

For several thousand years man has used herbs and potions as medicines, but it was only in the mid-nineteenth century that concerted efforts were made to isolate and purify active principles of these remedies. These natural products became the lead compounds for major synthetic efforts, where chemists made literally thousands of analogues in an attempt to improve on what nature had provided.

Now a days the concept of ‘drug research’ has changed totally and modern concepts are now available for designing the drugs or pharmaceutical agents. Broadly speaking, one can discern the following four main sources on which new drug could be developed.

- 1) Natural products : Plants, fermentation broths, marine product.
- 2) Existing drugs : Promising leads for new structural moiety.
- 3) Biological screens : Interpretation of screening data.
- 4) Physiological mechanisms : Drug action gives clue for new derivatives.

Natural products as sources for new drugs :-

Historically, natural products provide the oldest sources for new medicines. Natural selection during evolution and competition between the species, has produced powerful biologically active natural products which can serve as chemical leads, to be refined by the chemist to give more specifically acting drugs, for eg., moulds and bacteria produce substances that prevent other organisms from growing in their vicinity. The famous *Penicillium* mould led to the considerable range of semisynthetic penicillins and gave rise to the concept of seeking naturally occurring antibiotics, which have provided some very potent life saving drugs. eg. Streptomycin, Chloramphenicol, Chlortetracycline, Erythromycin,

Neomycin, Polymixin etc. Also many plants have provided pharmacologically active natural products which are mostly alkaloids like Morphine, Atropine, Reserpine Vincristine, Vinblastine Hyoscyamine Theophylline etc.

Existing drugs as a base for new drug discovery :-

The most fruitful basis for discovery of a drug is to start with an old drug. This has been the most common and reliable route to a new product. Thus, existing drugs may need to be improved, e.g. to obtain better dosage forms, to increase absorption, to increase potency, to reduce the daily dose or to avoid toxic side effects. For example, antihistaminics used for treating hay fever often make people sleepy which is a nuisance when they need to stay alert, particularly when driving car. This stimulated the development of new antihistaminic agents that have a much lower tendency to cause sedation. e.g. Terfenadine, Astemizole.

Disease models as screens for new drug :-

The screening of natural products or synthetic chemicals has been the third main source of new drugs. The background of this approach lies in the dye-stuff industry. Paul Ehrlich, founder and prophet of chemotherapy, discovered that synthetic dyes were absorbed differentially into the tissues and they could kill parasites and bacteria without affecting the host cells. From then on, a key to new drug discovery was meant to be the examination of hundreds of synthetic chemicals. The success of this discovery in chemotherapy dominated research thinking in pharmaceutical industry for many years and screens were also established for non-infectious diseases e.g. anticonvulsant, analgesic, antihypertensive and antiulcer drugs. However, the major drawback was how to generate new compounds. The approach is now mainly historical since the outlook for the future is based on more rational grounds as indicated below.

Physiological mechanisms, the modern rational approach :-

This is a modern approach to drug design, which is becoming increasingly important with the development of information in the cell biochemistry and cell biology, especially where this is understood at the molecular level. This approach of drug discovery requires very close collaboration between chemist and biologist and realising this, most of the large pharmaceutical companies are now organizing their research project on these lines instead of by scientific disciplines.

In modern era of medicinal chemistry, computer based methods of Quantitative Structure Activity Relationship (QSAR) related to receptor binding are used to correlate molecular structure with receptor binding and, therefore, activity. Free energy of binding of compound with receptor is determined theoretically. Distance between various atoms in the molecule, its conformation and rotation about single bonds causing change in conformation and, therefore, distance 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 structure of receptors and drugs could be displayed on terminal and molecular processes could be visualized. Three dimensional computer graphics allow the operator to visualize the interaction of small molecules with biologically important macromolecules. Better the complementary fit of the drug to the receptor, more potent will be the drug. This is the lock and key hypothesis in which the receptor is the lock and drug is the key.

Thus, discovery of new lead compounds, possibly leading to new drugs, can be schematically classified into four approaches -

- 1) Lead identification
- 2) Lead modification
- 3) Systematic screening of retroactive
- 4) Attempt towards rational design.

It would be imprudent to compare hastily the merits of each of these characteristics. Therefore, the highest importance is given to random character of discovery and planned invention of new active principal. Thus, decision makers in pharmaceutical companies appealed for all four strategies that have been described above. In conclusion, all strategies result in identification of lead compounds, that are equally good, provided the research they subsequently induce is done in a rational manner.

REVIEW OF LITERATURE

Infectious diseases are a major health hazard of living beings. They take a serious toll of life and in many cases leave behind a saga of suffering. The damage in terms of morbidity and mortality caused by these diseases is incomparable.

Tuberculosis

Mycobacterium tuberculosis, an acid fast pathogen, has since early days been exacting a substantial toll of morbidity and mortality on humanity. Suspected tubercular disease has been found in bony remains of a stone age individual who lived about 6000 years ago².

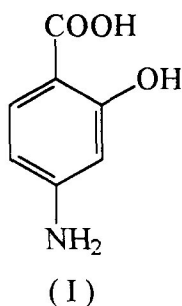
Indeed, the disease is mentioned in the scriptures, describing plagues that could afflict humanity. The industrial revolution, bringing an increasing population into crowded cities caused infection epidemic. The London Bill of mortality recorded that one in five deaths was caused by tuberculosis (TB) in mid 1600s. By the turn of the 19th century, the world wide death rate was 7 million, of the 50 million people openly infected, India, unfortunately, contributes about 25% to the global tuberculosis burden.

HIV infection is the strongest risk factor yet identified for progression to active TB³. Persons who are co-infected with *M. tuberculosis* and HIV develop active TB at the rate of 8-10 % compared with estimated 5-10 %, as developed by HIV negative individuals. In addition, HIV infected individuals who acquired new infection with *M. tuberculosis* have an even higher risk of rapidly developing TB. Tuberculosis remains a major public health problem because of several multiple factors, acting independently and in combination, contributing to the increase of the disease. The concurrent epidemic of HIV infection and AIDS, increases the immigration from countries where TB is endemic and primary transmission among some populations such as children and the homeless. Cases of drug - resistant TB have been described since the introduction of effective anti-TB therapy. However, in recent years, cases of both single drug and multiple drug resistance in TB have been increasingly reported throughout the world.

The chemotherapy of TB includes the following drugs :

P- AMINOSALICYLIC ACID

p-Aminosalicylic acid (I) (PAS) is a structural analog of Para Amino Benzoic acid (PABA). As with other salicylates, the drug deteriorates rapidly when exposed to moisture, heat or light. Owing to the high incidence of adverse effects, its use is reserved for patients with multiple drug resistant tuberculosis.



Activity and Mechanism of action of PAS

PAS inhibits the synthesis of folic acid by preventing the conversion of aminobenzoic acid to dihydrofolic acid and is active only against *M. tuberculosis*. Susceptible strains are inhibited by 0.5-2.0 µg/ml. This limited activity is unlike sulfonamides or other structural analogs of para-aminobenzoic acid, which are broad spectrum antibacterials. As with other anti-TB agents, resistance develops rapidly when the drug is used alone, but when used in combination with other agents, resistance is delayed or prevented.

Pharmacokinetics

PAS, available as a sodium salt, is readily absorbed orally. Peak serum levels of 40-75 µg/ml are attained after a 4gm dose. The half-life of PAS is about 1 hour, the drug undergoing acetylation to inactive metabolites in the intestine and liver.

Adverse effects

The drug is unpleasant in taste. GI intolerance was common. PAS is no longer used and is included for historic reasons only.

ISONIAZID

Isoniazid (IV) (isonicotinic acid hydrazine,INH) was found to have activity against *Mycobacterium* species in 1945. In 1952 INH was reported to be a useful potent anti-TB drug in both animal and human studies⁴.

Activity and Mechanism of action of INH

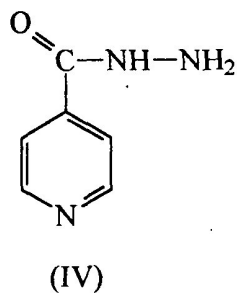
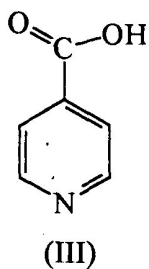
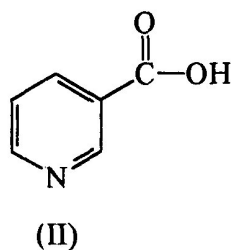
INH is bactericidal for TB. Its mechanism of action, however, remains unclear. According to theory, INH is converted to isonicotinic acid (III), which acts as an antimetabolite of nicotinic acid (II). One step inhibited is the desaturation of the C24 and C26 long chain fatty acid precursors of mycolic acid, a unique part of the mycobacterial cell wall. This effect may explain the narrow spectrum of INH. The drug also affects NAD⁺ and pyridoxal phosphate metabolism and produces a cuprous complex toxic to the bacillus. INH appears to be inhibitory to resting bacilli and bactericidal to actively replicating ones. It has been found to be more active than other first line agents in early bactericidal activity against TB. The minimal inhibitory concentration for INH is 0.025-0.5 µg/ml for the tubercle bacillus. Resistance can develop when therapy is inadequate.

Pharmacokinetics

INH is readily absorbed from the gut with peak levels of 3-7 µg/ml, 1-2 hrs, after an oral dose of 300mg. Food and antacids containing aluminium may interfere or delay absorption. So it is recommended that the drug be taken on an empty stomach. INH is mainly metabolized by acetylation giving rise to acetyl derivative, acetyl isonicotinic acid hydrazide.

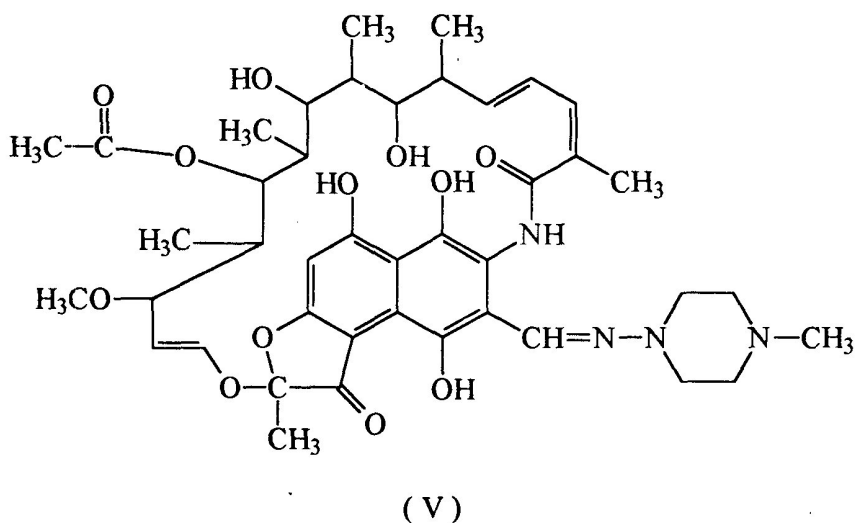
Adverse effects

Hepatitis is an uncommon (0.1%) but potentially serious reaction that is averted by prompt withdrawal of treatment⁵. Side effects like insomnia, restlessness, twitching are frequently seen in slow acetylators. INH interferes completely with pyridoxine metabolism by inhibiting formation of active form of the vitamin. Hence, it results in peripheral neuropathy, which can be prevented by co-administration of pyridoxine.



RIFAMPIN

Rifampin (V) is a semisynthetic derivative of Rifamycin B. It is a water-soluble, reddish-brown crystalline powder. The drug is a first line anti-TB agent which, in combination with INH, has made TB therapy more effective.



Mechanism of action and activity

Rifampin (V) can be inhibitory against *M. tuberculosis* at levels as low as 0.25 µg/ml. The drug achieves its antibacterial effect by binding with high affinity to DNA dependent RNA polymerase. The binding inhibits the initiation of RNA chain formation. It does not have its effect on mammalian cells.

This commonly used agent is active in vitro and in vivo against *M. tuberculosis*, *M. kansasii*, *M. marinum*, *M. leprae* and some strains of *M. avium-intracellulare* complex. Its cidal effect against the tubercle bacillus is more efficient than INH because it is better at killing bacilli that are predominantly metabolically dormant. Widespread use of rifampin for bacterial infection could result in increasing amounts of resistance to TB. Resistance

development has been seen in vitro and in vivo. In vitro, it is a one step process, which is likely to be related to a modification in the beta subunit of the RNA polymerase.

Pharmacokinetics

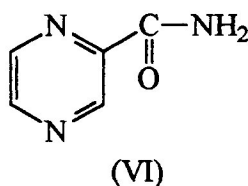
A 600 mg oral dose of rifampin will achieve a peak level of about 7µg/ml, with a range of 4-32 µg/ml. Peak concentrations may be slightly reduced or delayed if taken with food and therefore the drug should be taken on an empty stomach. Rifampin is metabolised by the liver by deacetylation into an active metabolite, desacetyl rifampin. The drug absorption is reduced in the presence of PAS or Clofazimine. Therefore, these drugs should not be given with rifampin.

Adverse effects

Major side-effects involved are influenza like symptoms, skin reaction and GI disturbances. Patient should be warned that it causes an orange discoloration of urine and other body secretions.

PYRAZINAMIDE

Pyrazinamide (VI) (PZA) is a synthetic pyrazine analog of nicotinamide. It was synthesized and found to be active against *M. tuberculosis* in 1952. PZA has a unique ability to act within the macrophage and in acidic environments. PZA is an integral part of short course therapy, reducing the time to sterilize the sputum. It appears to be the most effective in the first two months of therapy with much less effect afterwards. Therefore, it is often discontinued after two months of therapy, while continuing the other agent.



Mechanism of action and activity

The exact mechanism of action of PZA is not clear. The drug is bactericidal at an acid pH (5.0-5.5), but has little activity at higher pH. Susceptible organisms produce deaminidase which is responsible for its conversion to pyrazinoic acid. Resistant stains do not produce this

enzyme and thereby no acid is synthesised. This acid may lower the pH of the immediate surroundings of *M. tuberculosis* to such an extent that the organism is unable to grow.

Pharmacokinetics

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

Adverse effects

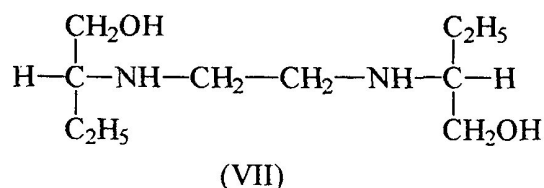
Adverse reactions are rare, but hepatic toxicity has been a problem. Hypersensitivity and photosensitivity of the skin also occur. Treatment for longer than two months is not recommended⁵. The dose should be reduced in case of patients with renal impairment and avoided, in patients with severe liver damage and gout.

ETHAMBUTOL

Ethambutol (EMB) is bacteriostatic in nature. EMB (VII) is considered to be a first line therapy in patients with TB along with INH, rifampin and PZA. In EMB, it consists of two asymmetric centers in the molecule, a (-) form, a (+) form. The study of all these forms revealed that (+) form is the most active. EMB is utilized in its d-isomer, which is 200 to 500 times more active than the other isomer.

Activity and Mechanism of action

Although the mechanism of action is not known, it appears to have effect on RNA synthesis. EMB also inhibits the incorporation of mycolic acid into the cell wall. The drug diffuses only into actively growing mycobacterial cells. EMB is a highly specific agent with activity only against mycobacteria. As many as 90% strains of *M. tuberculosis* are inhibited by a dose of 2 µg/ml or less.



Pharmacokinetics

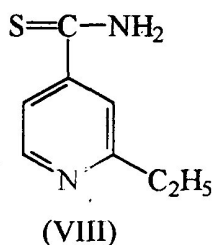
75-80% of EMB is absorbed from the gut and the rate is not affected by food. Aluminum-containing salts, however, can reduce the absorption. The drug is widely distributed with the highest concentrations in kidney and lungs. It is metabolized in the liver to two inactive forms.

Adverse effects

EMB can give rise to dose-dependent optic neuritis. This can result in impairment of visual activity and color vision. Early changes are reversible, but blindness can occur if treatment is not discontinued promptly⁵.

ETHIONAMIDE

Soon after the discovery of INH, several independent groups discovered the anti-TB activity of thionicotinamide. The α -ethyl derivative of the compound, ethionamide, was synthesized in France in 1956 and was found to be more effective than the parent drug. It is a bright yellow powder with a sulfide odor. Ethionamide (VIII) is a second line agent that is not recommended for treatment regimens in previously untreated patients because of its frequent and potentially serious toxicity.



Activity and Mechanism of action

The drug is thought to inhibit peptide synthesis. The precise mode of action is unclear⁶. The similarity in structure to INH could offer some answers about the activity of ethionamide, but lack of cross resistance between INH and Ethionamide suggests that this may not be the case. The report of gene encoding as a target for both INH and Ethionamide, however suggests cross resistance can occur.

Active only against some mycobacteria, ethionamide inhibits the TB bacillus in vitro at 0.6-1.2 $\mu\text{g/ml}$.

Pharmacokinetics

Administered orally, Ethionamide is rapidly absorbed with a bioavailability approaching 100%. The drug is widely distributed in most body tissues and fluids with tissue concentrations approximating blood levels. Levels of drug in lung tissue were found to be 85-90% of those in the serum⁷. CSF levels are similar to serum levels in both inflamed and normal meninges. Although the risk in pregnancy is not well defined the drug crosses the placenta and its use during pregnancy should be avoided, if possible. The concentration in breast milk is unknown. Ethionamide has a serum half-life of 2 hours, and is extensively metabolized. One of the metabolites is ethionamide sulfoxide. The sulfoxide derivative retains activity and may be converted back to the parent drug *in vivo*⁸. Most elimination is via the renal route, with 1% being excreted as the unchanged drug, 5% as active metabolites and the remainder as inactive metabolites⁹. It is orally active but large dose (>500mg) is not well tolerated.

Adverse effects

Adverse effects of Ethionamide include GI irritation, hepatitis, CNS effects, hypersensitivities etc.

STREPTOMYCIN

Aminoglycosides are made up of two or more amino sugars attached to a hexose nucleus via glycosidic linkages. Streptomycin (IX) was the first effective anti-TB agent when it was introduced by Waksman et al. in 1944¹⁰. 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 mechanism of action. As a result of this binding, disruption of normal protein synthesis occurs, leading to inhibition of protein synthesis.

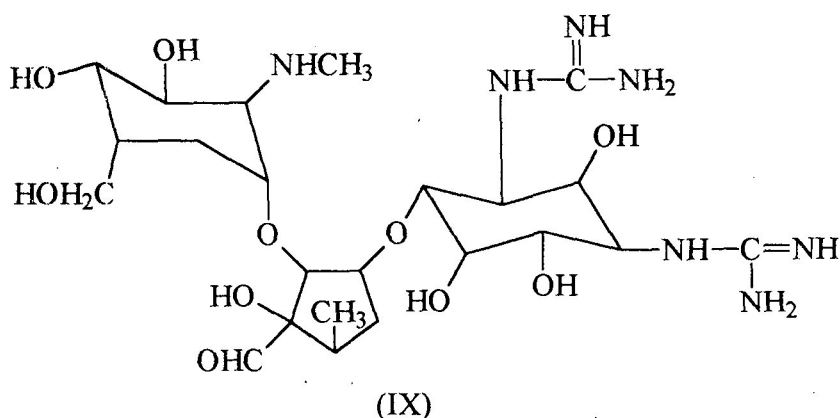
Pharmacokinetics

Unlike all aminoglycosides, it is not absorbed when given orally and must be administered as an intramuscular injection. However, these are painful and so the drug must be administered by deep intramuscular route. Duration of treatment is 1-2 months.

Adverse effects

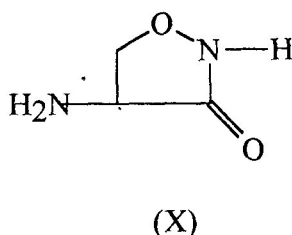
Aminoglycosides can cause nephrotoxicity, ototoxicity and neuromuscular blockade.

Streptomycin can cause as many as 20% of patients to develop vestibular damage.



CYCLOSERINE

It is a structural analog of D-alanine. It can now be prepared synthetically from serine. The use of the drug is limited due to its severe toxicity.



Activity and Mechanism of action

The enzyme l-alanine synthetase is necessary to produce D-alanine. Since the drug is a structural analog of D-alanine, there is a competitive inhibition of D-alanine, leading to death of the bacterial cell. The antibacterial effects of cycloserine are antagonized by the presence of D-alanine. The drug is active against *M. tuberculosis* as well as some strains of *M. bovis*, *M. avium intracellulare*. The MIC for the tubercule bacillus is 5-20 µg/ml and slightly higher for non TB mycobacteria¹¹.

Pharmacokinetics

After an oral dose, 70-90 % of the drug is rapidly absorbed. A serum level of 10-20µg/ml is produced by a dose of 250 mg twice daily.

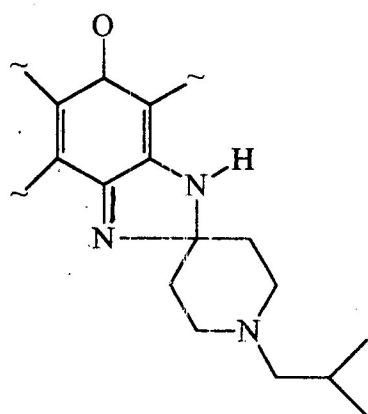
Adverse reactions

Cycloserine (X) links with neuropsychiatric symptoms, and should be avoided in patients with anxiety and/or depression. Since the toxic/therapeutic ratio is quite narrow and drug is retained in patients with renal insufficiency, extreme caution must be utilized in such patients and the drug should be avoided, if possible, in those with significant renal disease.

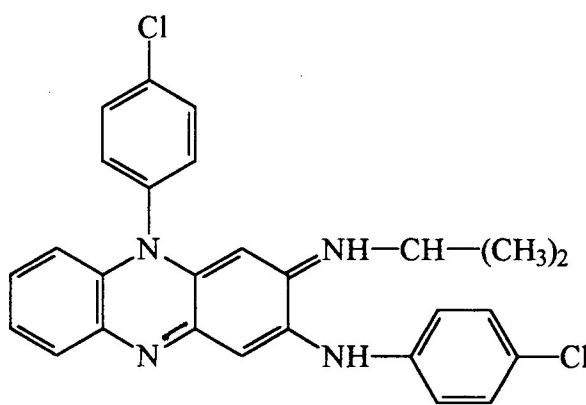
MISCELLANEOUS ANTIMYCOBACTERIAL DRUGS

Rifabutin(XI), is a rifamycin derivative which has been shown to be useful in preventing or delaying *M. avium-intracellulare* complex bacteremia in AIDS patients¹².

Clofazimine(XII), is a hydrophobic phenazine quinoneimine. It acts by inhibiting certain mycobacterial energy yielding reactions¹³.

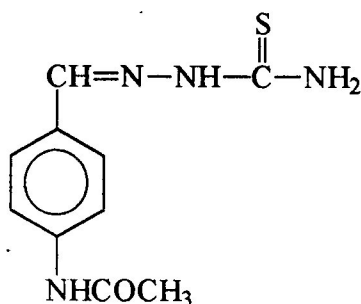


(XI)



(XII)

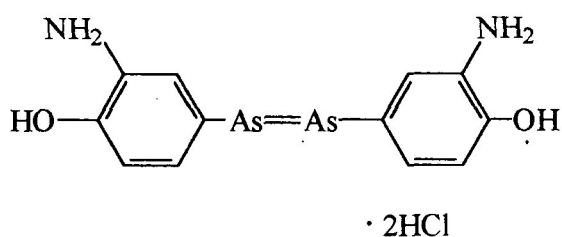
Thiacetazone (XIII), because of its toxicity and low potency, is rarely used in industrialized countries. However, it is used in developing countries in combination with INH¹⁴.



(XIII)

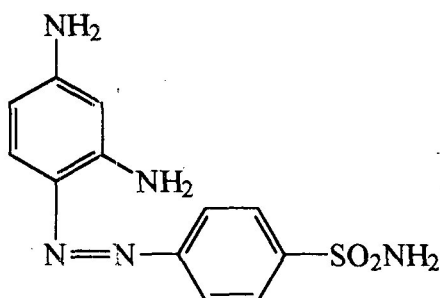
Anti-bacterial agents

The fight against bacterial infection is one of the greatest success stories of medicinal chemistry and runs parallel to the introduction of sulfonamide and penicillin in anti-bacterial therapy. In 1910, Ehrlich had successfully demonstrated the first example of a purely synthetic anti-microbial agent. This was an arsenic containing compound, Salvarsan (XIV).

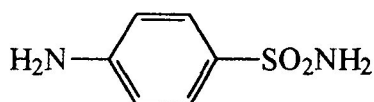


(XIV)

Although it was not effective against a wide range of bacteria, it did prove effective against the protozoal diseases and the spirochete disease of syphilis. In 1935, with the discovery of the red dye, Prontosil, which was eventually recognized as the prodrug for a new class of antibacterial agents - the sulfa drugs, a new chapter was written in the history of chemotherapy.



(XV)



(XVI)

The discovery of this drug was a real breakthrough, since it represented the first drug to be effective against bacterial infections present in the blood stream. Both of the above drugs were used until the penicillins became available in the early 1940s.

Introduction of penicillin revolutionized the fight against bacterial infections and a number of semisynthetic penicillins and other antibiotics were then available for the treatment of bacterial infections.

Thus, the known antibacterial actions, have been mainly classified in the following classes :

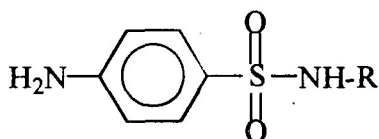
1. Inhibition of cell metabolism.
2. Inhibition of bacterial cell wall synthesis.
3. Interaction with the plasma membrane.
4. Disruption of protein synthesis.
5. Inhibition of nucleic acid transcription and replication.

Inhibition of cell metabolism

Antibacterial agents which inhibit cell metabolism are called antimetabolites. These compounds inhibit the metabolism of microorganisms, but not the metabolism of the host cells. They do this by inhibiting an enzyme-catalyst reaction, which is present in the bacterial cell, but not in the animal cells. The best example of antibacterial agents acting as antimetabolites is the sulfonamides.

The story of sulfonamide began in 1935 with the discovery of Prontosil (XV), which was later recognized as the prodrug of sulfanilamide (XVI). Thus, sulfanilamide was synthesized in the laboratory and became the first synthetic antibacterial agent active against a wide range of organisms. Further development led to a range of sulfonamides which proved effective against Gram-positive organisms, especially *Pneumococci* and *Meningococci*.

Sulfanilamide analogues.



R : Heterocyclic or aromatic moieties.

'R' can be varied by incorporating a large range of heterocyclic or aromatic moiety which affect the extent to which the drug binds to plasma proteins. This in turn controls the blood level of the drug such that it is either short acting or long acting.

Well absorbed, short and intermediate acting sulfonamides are sulfamethazole, sulfapyridine, sulfamethoxazole, sulfadiazine. The examples of well absorbed long lasting sulfa drugs are sulfamerazine and sulfameter.

Structure activity relationship (SAR) of sulfonamides

1. The para amino group is essential for activity and must be unsubstituted.
2. Amides, by themselves, are inactive but can be metabolized in the body to regenerate the active compounds.
3. Aromatic ring and sulfonamide functional group are both required.
4. In sulfonamide, nitrogen must be secondary.

Mechanism of action

The sulfonamides act as competitive enzyme inhibitors and block the biosynthesis of folic acid in bacterial cells. They do this by inhibiting the enzyme responsible for linking together the component part of folic acid. As a consequence, the cell dies. Folic acid is a precursor for tetrahydrofolate, which is responsible for the carbon source in many biosynthetic pathways. Sulfonamides do not actively kill bacterial cells. They do, however, prevent cell division and spread.

Sulfonamides act as inhibitors by mimicking p-amino benzoic acid (PABA), one of the normal constituents of folic acid pathway, because of their structural similarity with PABA.

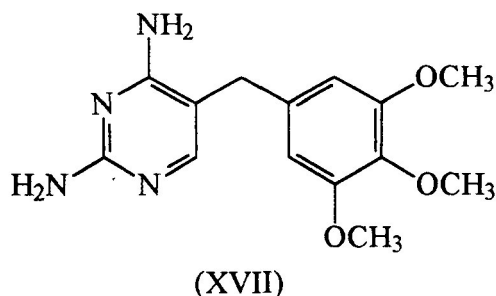
Adverse effects

A variety of serious toxic and hypersensitivity problems have been reported with the sulfonamides. Weinstein¹⁵ has noted that these occur in about 5% of patients. Hypersensitivity reactions include drug fever, skin eruptions, allergies, myocarditis, photosensitization and related conditions.

Examples of other anti metabolites

There are other anti-metabolites in medical use apart from the sulfonamides. Two examples are Trimethoprim and a group of compounds called sulphones, e.g. Dapsone, which is used in the treatment of leprosy.

Trimethoprim



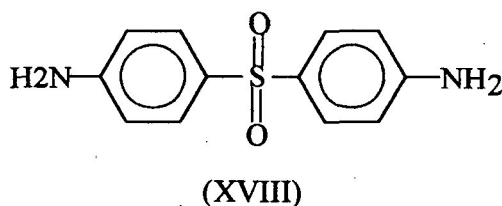
In an attempt to synergize the action of sulfonamides and to avoid the development of resistance to them, the most logical approach is to combine them with agents that block the same metabolic pathway as that blocked by sulfonamides, but at one or more different sites. The elucidation of the folic acid pathway and demonstration by both sulfonamides and dihydrofolate reductase inhibitors provided this possibility¹⁶.

The synergism of Trimethoprim and sulfonamide has added a new dimension to the treatment of plasmodial, toxoplasma and coccidial infections¹⁷.

Trimethoprim(XVII) is chemically a diaminopyrimidine, and it has proved to be a highly selective orally active antibacterial and antimalarial agent. Unlike sulfonamides, it acts against dihydrofolate reductase - the enzyme which carries out the conversion of folic acid to tetrahydrofolate. The overall effect, however, is the same as with sulfonamide -the inhibition of cell synthesis and cell growth.

Sulfones

The sulphones are most important drugs used in the treatment of leprosy. It is believed that they inhibit the same bacterial enzyme inhibited by sulfonamide, that is dihydropteroate synthetase. The best example of this is Dapsone (XVIII).



Antibacterial agents which inhibit cell wall synthesis

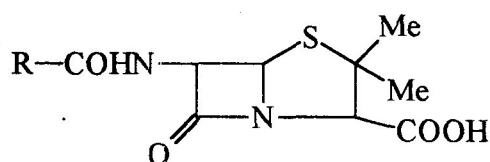
There are two major classes of drugs which act in this fashion, penicillins(XIX) and cephalosporins(XX).

Penicillin

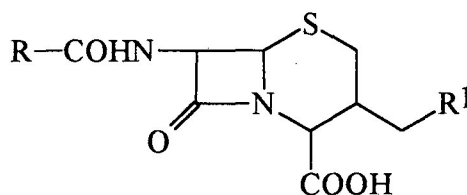
The accidental discovery of penicillin by Sir Alexander Fleming¹⁸ in 1929 was the prime factor in starting the fascinating and fruitful research activity that has produced the amazingly effective anti-infective agent commonly known as 'antibiotic'.

In 1928 Fleming found that the bacterial culture which had been left over for several weeks open to the air had become infected by fungus. The bacterial colonies in the areas surrounding the fungal colonies were found to be dead. It was then found that this was due to penicillin.

Penicillin contains a highly unstable-looking bicyclic system consisting of a four membered β -lactam ring fused with a five-membered thiazolidine ring. The skeleton of the molecule suggests that it is derived from the amino acid cysteine and valine and this has been established.



(XIX)



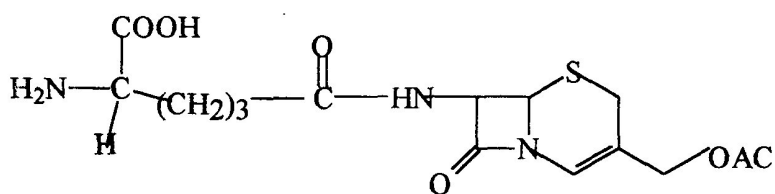
(XX)

But the life saving capacity was first demonstrated by Florey and Chain²⁰ and their colleagues at Oxford University in animals in 1940 and in humans the following year^{20,21}.

Those penicillins having useful activity against gram-positive and gram-negative bacteria are known as broad spectrum antibiotics. Ampicillin²² and Amoxicillin²³ are class I broad-spectrum antibiotics, active against gram-positive and against those gram-negative bacteria which do not produce penicillinase. Carbenicillin has an activity against a wider range of gram-negative bacteria as compared to Ampicillin. It is resistant to most penicillinases and also active against the stubborn *Pseudomonas aeruginosa*.

Cephalosporins

The cephalosporins (XX) are antibiotics obtained from a species of fungus namely *Cephalosporium* and from semisynthetic processes. The chemistry and antibacterial properties of cephalosporins and their relation with the penicillins have been revived by Hou and Poole¹⁹ and Van Heyningen²⁴. The structure of cephalosporin C (XXI) is similar to that of penicillin. It has a bicyclic system containing a four membered β -lactam ring. However, the β -lactam ring is fused with the six-membered dihydrothiazine ring. This larger ring relieves the strain in the bicyclic system to some extent, but it is still a reactive system.



(XXI)

Third generation Cephalosporins have increased the resistance to many plasmid and chromosomally mediated β -lactamases²⁵.

Antibacterial agents which act on plasma membrane structure

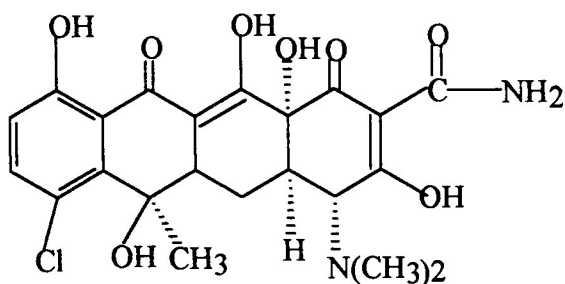
Some antibacterial agents interact with the plasma membrane of bacterial cells to affect membrane permeability. This has fatal results for the cell. Polymyxins and tyrothricin operate in this manner. The peptides, Valinomycin and Gramicidin, both act as ion conducting antibiotics and allow the uncontrolled movement of ions across the cell membrane. Unfortunately both these agents show no selective toxicity to the bacterial cells over mammalian cells and are hence useless as therapeutic agents.

Antibacterial agents which impair protein synthesis

Disruption of protein synthesis means that essential enzymes required for the survival of the cell can no longer be synthesised. Agents which disrupt protein synthesis include the rifamycins, aminoglycosides, tetracyclines and chloramphenicol. Rifamycin and aminoglycosides are previously documented in anti-mycobacterial drugs. In this part we will discuss only tetracycline and chloramphenicol.

The tetracyclines have a broad spectrum of activity and are the most widely prescribed form of antibiotics after the penicillins. They are also capable of attacking the

malarial parasites. One of the best known tetracyclines is Chlortetracycline (XXII), which



(XXII)

was discovered in 1948. It is a broad spectrum antibiotic, active against both gram-positive and gram-negative bacteria. Unfortunately, it does have side effects due to the fact that it kills the intestinal flora that make vitamin K - a vitamin, which is needed as a part of the clotting process.

Mechanism of action

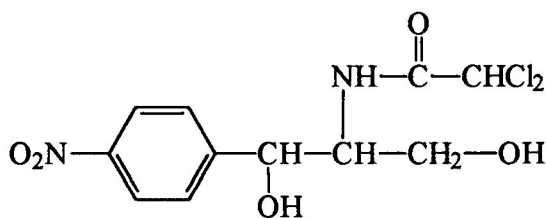
Chlortetracycline inhibits protein synthesis by binding to the 30s subunit of ribosome and prevents the aminoacyl t-RNA binding to the site on the ribosome²⁶. This prevents the codon-anticodon interaction, and hence, protein release is also inhibited.

Adverse effects

The drug has poor absorption coupled with biliary excretion. It exhibits higher incidences of superinfection.

Chloramphenicol

Chloramphenicol (XXIII) was originally isolated from *Streptomyces venezuela*, but it is now prepared synthetically. It has two chiral centers, but only the R-R isomer is active.



(XXIII)

Chloramphenicol

SAR studies have demonstrated that there must be a substituent on aromatic ring which can resonate with it (NO_2). The R-R propanediol is essential. The -OH group must be free and presumably be involved in hydrogen bonding. The dichloroacetamide group is important but can be replaced by other electronegative groups.

Mechanism of action

Chloramphenicol binds to the 50s subunit of ribosomes and appears to act by inhibiting the movement of ribosomes along with the m-RNA, probably by inhibiting the peptidyl transferase reaction by which the peptide chain is extended. Chloramphenicol is the drug of choice in typhoid and is used in severe bacterial infections, which are insensitive to other antibacterial agents. It has also found widespread use against eye infections. Adverse effects include acute toxicity, especially of the bone marrow.

Macrolides

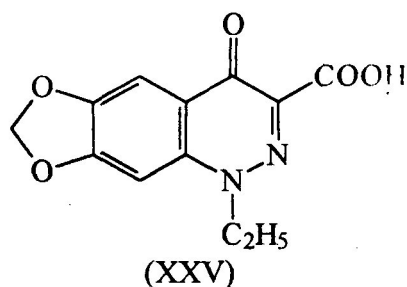
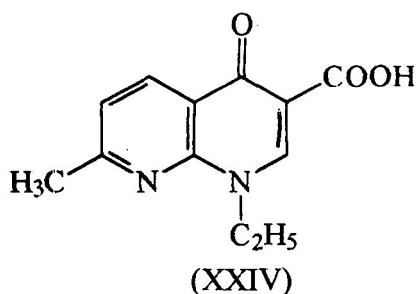
The best known example of this class is Erythromycin - a metabolite produced by the organism *Streptomyces erythreus*. The structure consists of a macrocyclic lactone ring with sugar and an amino sugar attached to it. The sugar residue is important for activity. Erythromycin acts on the ribosomal 50s subunit by an unknown mechanism. It works the same way as Chloramphenicol by inhibiting translocation, where the elongated peptide chain attached to t-RNA is shifted back from the aminoacyl site to the peptidyl site. Modification of erythromycin resulted in useful molecules such as clarithromycin, fluorithromycin etc. which are effective against **ma-intraction**^{27,28}. Erythromycin was used against penicillin resistant *staphylococci*. It is however, the drug of choice against legionnaires disease.

Agents which act on nucleic acid transcription and replication.

The inhibition of nucleic acid function prevents cell division and/or synthesis of essential enzymes. Agents acting in this manner include Nalidixic acid and proflavin, quinolones and fluoroquinolones.

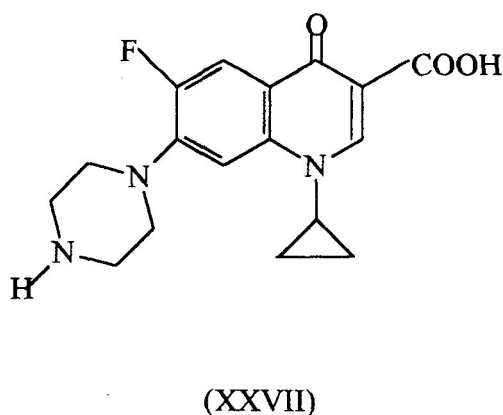
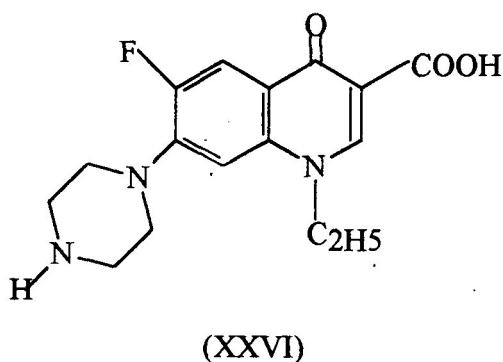
Nalidixic acid

Nalidixic acid (XIV) was the first therapeutically useful agent in this class of compounds. Early members of this group developed in 1960s and 1970s include Cinoxacin (XXV)²⁹.



It is active against gram (-) bacteria and is useful in short term therapy of urinary tract infection (UTI). It can be taken orally but bacteria can develop resistance to it. Various analogues have been synthesized which have the same properties of Nalidixic acid. A major break through was made, however when fluorine atom was introduced at the 6-position and piperazine residue at 7-position of the heteroatomic skeleton which has a greatly increased spectrum of activity against gram-positive and gram-negative bacteria.

Further adjustment leads to Norfloxacin(XXVI) and Ciprofloxacin (XXVII), currently the agents of choice in treating traveller's diarrhoea.



It is used in the treatment of a large range of infections, involving the urinary tract, respiratory tract and the gastro-intestinal tract, as well as infection of skin, bone and joints. It

has been claimed that Ciprofloxacin may be the most active broad spectrum antibacterial agent on the market.

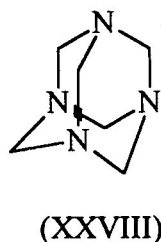
Mechanism of action

The quinolones and fluoroquinolones act on the bacterial enzyme, DNA gyrase. This enzyme catalyses the supercoiling of chromosomal DNA into its tertiary structure. A consequence of this is that replication and transcription are inhibited and the genetic code of the bacterial cells remains unread.

Some other examples of synthetic heterocyclic antibacterials.

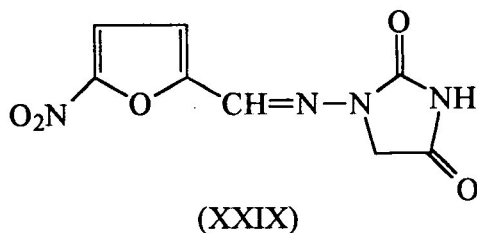
Methanamine :

The free base has no antibacterial action but acidification results in the liberation of formaldehyde, which is strongly bactericidal. Methanamine (XXVIII) is used sometimes in long term suppression of bacterial urinary tract infections.



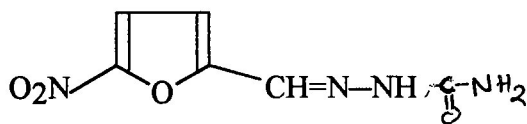
Nitrofurans :

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

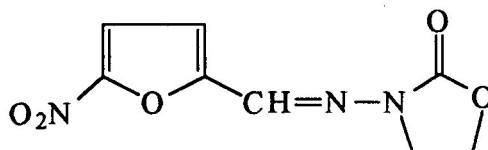


Nitrofurantoin is active against Gram-positive and Gram-negative bacteria at a concentration of 5-10 mg/ml. It is active against most urinary tract pathogens, but *Proteus* species and *Pseudomonas aeruginosa* are usually resistant. The activity is affected by pH; acid conditions favouring the activity.

Nitrofurazone³⁰ (XXX) and Furazolidone³¹ (XXXI) are the other approved agents used in humans.



(XXX)



(XXXI)

Nitrofurazone has a broad spectrum of antibacterial action in the range of $1:10^5$ 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 a 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 nitrofuran mutagenesis and carcinogenesis.

Nitroimidazoles :

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

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.³² 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.³²

Plan of work

PLAN OF WORK

In the last 30 years and following the discovery of penicillin by Fleming over 60 years ago, various antibiotics and synthetic antibiotics have been developed. Successive discoveries in the field of medicinal chemistry have been added to the list of the new chemical agents used for chemotherapy of various infections. Still this is not enough because many cases of microbial resistance remain a constant threat to the future utility of almost any drug.

Increasing clinical importance of drug-resistant bacterial pathogens has lent additional urgency to microbiological and antibacterial research. As pointed out in a number of reviews, there have been relatively rapid and dramatic changes in antibiotic resistance patterns in both, community and institutional setting, which is likely to have considerable impact on the therapeutic strategies for treatment of bacterial infections. Most worrisome as a public health threat, is the recent increase in the incidence of tuberculosis caused by multidrug resistant strains of *Mycobacterium tuberculosis*³³.

The WHO has estimated that within the next ten years, 30 million people worldwide will die of tuberculosis³⁴. At present INH, Streptomycin, Rifampin etc. are the drugs of choice in different combinations in the treatment of tuberculosis. Now a days because of multidrug resistant strains of *M. tuberculosis* and also due to AIDS, tuberculosis has once again become a major health problem in India as well as the world over. Therefore, there is an equally urgent need for new research approaches to help combat the spread of tuberculosis and other microbial infections.

Hence, considering the importance of above, a plan was prepared to synthesise new antitubercular agent as well as antibacterial agents. 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 recent trends in drug research in incorporating newly emerged pharmacophores on existing moieties. Triazolo benzothiazole derivatives are among these newly emerged pharmacophores and have exhibited a variety of biological activities. In the present work, various linkages of triazolo benzothiazole have been synthesised and studied for their antibacterial and antitubercular action. The plan also proposed the synthesis of an intermediate of Famotidine. Famotidine is a H₂ antagonist and has the potential to substitute Cimetidine and Ranitidine.

PART 1

This includes Introduction, Review of literature and plan of work.

PART 2

Synthesis of sulfonamide and s-benzyl derivatives of substituted / unsubstituted s-triazolo-(3,4-b)-benzothiazole.

This part deals with.

Section A

Synthesis of 3- N{[substituted heterocyclic/ phenyl]-s-triazolo-(3,4-b)-benzothiazole}-sulfonamide.

Section B

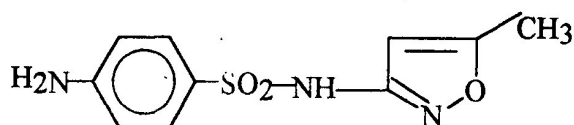
Synthesis of 5/6-Nitro-3-{[substituted phenyl]-s- triazolo-(3,4-b)-benzothiazole}thione.

Section A

Synthesis of 3- N{[substituted heterocyclic/ phenyl]-s-triazolo-(3,4-b)-benzothiazole}-sulfonamide.

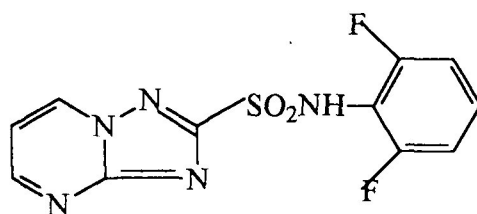
In 1932 Domagk³⁵ observed that Prontosil protected mice against *Streptococcal* infection and rabbits against *Staphylococcal* infection. The effect of Prontosil was most probably due to its in vivo conversion to the active metabolite, Sulfanilamide. Hence, sulfonamides were introduced in the treatment of bacterial infections. They still continue to be an important class of anti-microbial agents. These drugs are commonly used in preventing *Streptococcal* infections and rheumatic fever. Sulfonamides are broad spectrum, predominantly bacteriostatic and relatively slow acting analogs of p-aminobenzoic acid (PABA).

A combination of Sulphamethoxazole (I) and Trimethoprim has been found to be useful, both for prophylaxis and treatment of *Pneumocystis carinii* pneumonia in patients with AIDS.



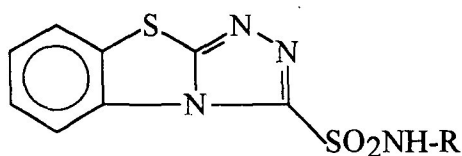
(I)

In agriculture, Flumetsulam³⁶ (II) is used as a herbicide. It is a triazolo-pyrimidine sulfonamide.



(II)

Hence, considering the importance of sulfonamido linkages directly attached to the heterocyclic ring system, it was proposed to synthesise the sulfonamide of triazolo benzothiazole.(III)



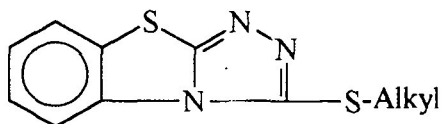
(III)

R : Heterocyclic /Substituted phenyl

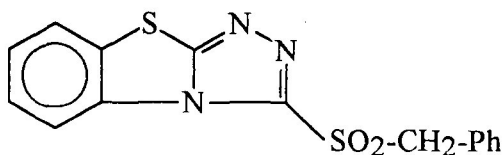
Section B

Synthesis of 5/6-Nitro-3-[[substituted phenyl]-s- triazolo-(3,4-b)-benzothiazole}thione.

In literature, s-alkyl (IV) and sulphone (V) derivatives of triazolobenzothiazole³⁷ were found to be more active anti-tubercular agents as compared to the derivatives synthesised by Gvozdiakova, A. *et al* ³⁸. This is thought to be due to the s-alkyl linkage introduced into the molecule.



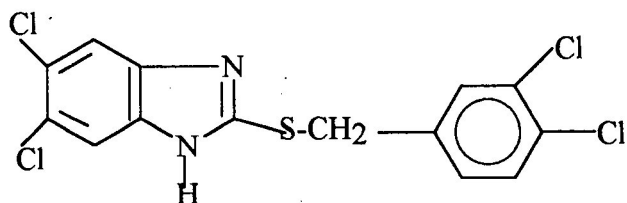
(IV)



(V)

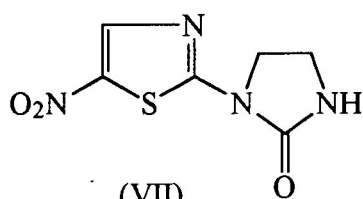
Ph : Phenyl

In our laboratory a number of 2-thiobenzimidazole derivatives were prepared and tested for their antitubercular activity, of which 5,6-dichloro-2-(3,4-dichlorobenzyl) thiobenzimidazole (VI) showed antitubercular activity at the concentration of 3.12 μ g/ml³⁹.



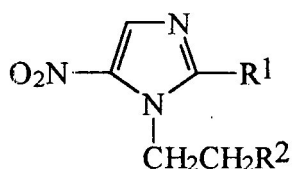
(VI)

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 (VII), Metronidazole (VIII), Flunidazole (IX).



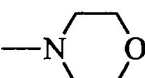
(VII)

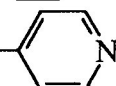
In 1964, Ciba Laboratories announced⁴⁰⁻⁴⁴ the discovery of novel schistosomicide, niridazole⁴⁴⁻⁴⁸ (VII). Niridazole was synthesized as a part of systemic investigation of heterocyclic nitro compounds as potential antiparasitic agents. One requirement for activity in all niridazole congeners appeared to be the presence of 5-nitro group⁴⁵.



R¹ = CH₃, R² = OH (VIII)

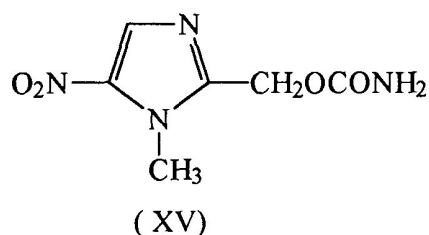
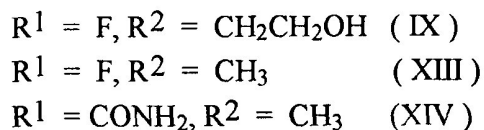
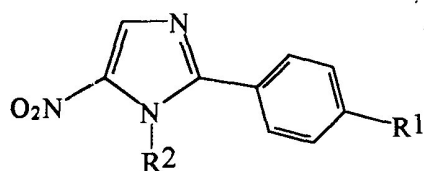
R¹ = CH₃, R² = SO₂CH₂CH₃ (X)

R¹ = H, R² =  (XI)

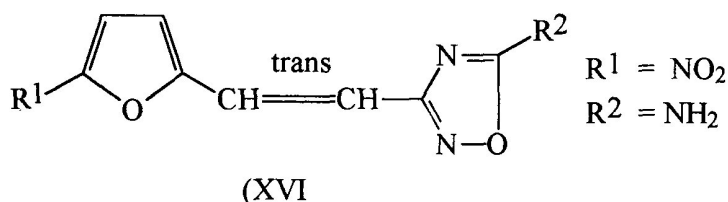
R¹ = CH₃, R² =  (XII)

Amongst the nitroimidazoles, metronidazole (VIII) is established as a drug of choice in the treatment of all forms of amoebiasis.⁴⁶⁻⁵⁰ The quest to improve trichomonocidal activity and metabolic stability led to the discovery of tinidazole (X), nimorazole (XI), panidazole (XIII) with retention of nitro group at fifth position.

Most interesting nitroimidazoles include flunidazole (IX), MF-nitroimidazoles (XIII), MCA-nitroimidazole (XIV), Ronidazole (XV).



Newer and more interesting experimental compounds include oxadiazole derivative (SQ 18506) (XVI) ⁵¹⁻⁵³

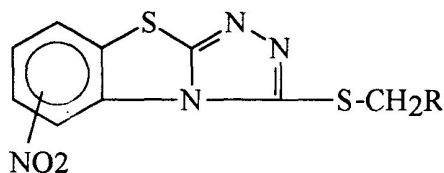


This was consistently active at high dose levels against *S. mansoni* and *S. japonicum* in mice and monkeys. The non-nitrated compound lacked activity.

Nitropyrroles¹⁰¹ and nitropyridines^{102,103} have been investigated for their antiamoebic properties.

The presence of -NO₂ group in heterocyclic compounds plays a vital role for biological activity such as schistosomicidal sp., trichomonocidal and amoebicidal activities. Hence, it was proposed to introduce -NO₂ group at 5 or 6 position of -s-triazolo-(3,4-b)-benzothiazole.

Thus, from the above literature search, it was proposed to condense 5/6 nitro-3-[[substituted phenyl]-s-triazolo-(3,4-b)-benzothiazole}-thione (XVII). These compounds were then tested to find out if there is any enhancement in the activity of triazolobenzothiazole.



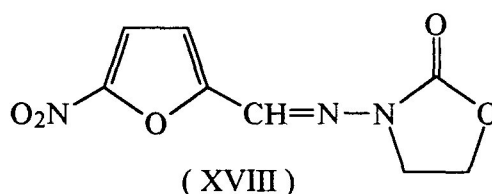
R : Substituted phenyl.

(XVII)

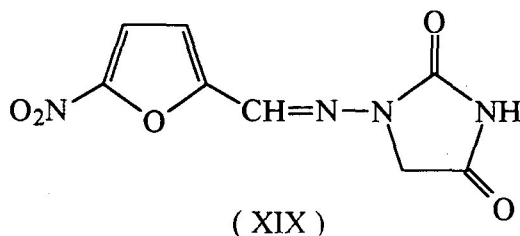
PART 3

Synthesis of 3-hydrazino-N-(substituted / heterocyclic) methylene-s-triazolo-(3,4-b)-benzothiazole.

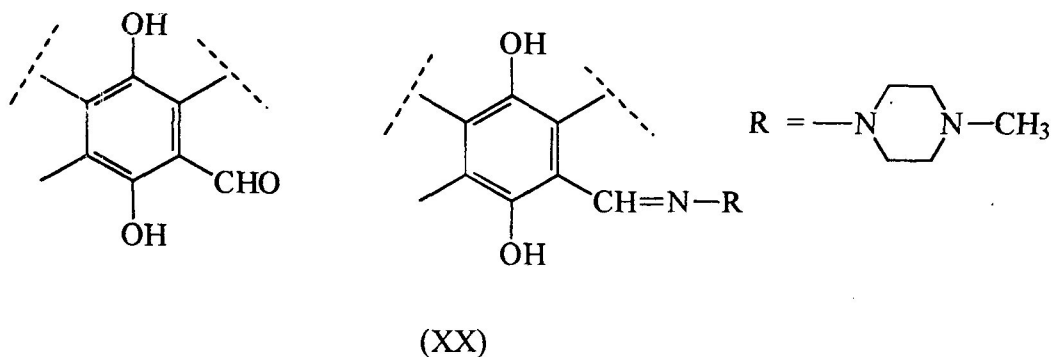
Importance of nitroheterocyclic compounds is well documented earlier. Amongst the nitroheterocyclic compounds most common in use are nitrofurans e.g. 1) nitrofurazone, 2) nitrofurazolidone and 3) nitrofurantoin. All these compounds apart from nitrofuran structure share one common aspect, i.e. presence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



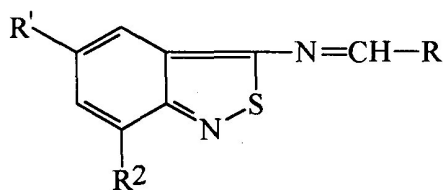
Nitrofurazolidone⁵⁷ (XIX) has broad spectrum bactericidal activity in intestinal pathogens including various species of *Salmonella*, *Shigella*, *Proteus*, *Enterobacter* as well as *E. coli* and *Vibrio cholerae*.



Nitrofurantoin⁵⁸ 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.



When 3-formyl rifamycin SV was prepared it was found that many of its N, N-disubstituted hydrazones⁵⁹⁻⁶⁰ (XX) had high activity both in vitro and in vivo against M. tuberculosis and Gram-positive bacteria.



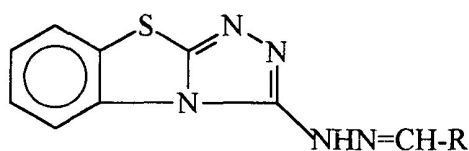
(XXI)

R₁ R₂ = H, R = phenyl

Bellotti A, *et al*⁶¹ recently revealed antimycotic and *antistaphylococcal* activities of 3-azomethine linkage of 2,1- benzisothiazole (XXI). 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 action⁶². The presence of azomethine linkage in compounds has also been known to decrease toxicity considerably.⁶³

Considering this activity and the importance of azomethine and hydrazino-methine linkage in Nitrofurazone, it was proposed to synthesise hydrazino-methine linkage of 3-hydrazino-s-triazolo-(3,4-b)-benzothiazole.(XXII), by condensing it with different halogenated benzaldehydes. Structures of these compounds were conformed by elemental analysis, IR and NMR spectra and finally, they were subjected to biological screening.



(XXII)

R : Chlorophenyl, Fluorophenyl, Trifluoromethyl phenyl, N-methyl pyrrole etc.

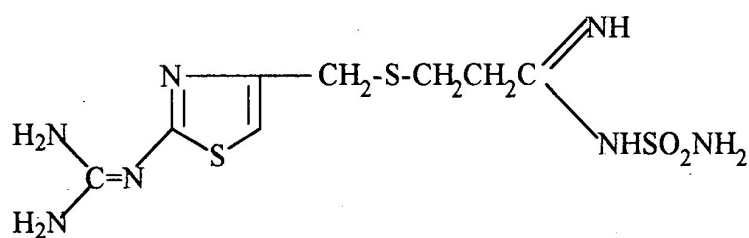
PART 4

It consists of

A : Synthesis of 3{[(2-thioureido-4-thiazolyl)methyl] thio}- propionitrile as an intermediate of Famotidine.

B :Synthesis of sulfamide as an intermediate of Famotidine as well as a material for resin, dye, and reducing agent.

In 1986, Famotidine (XXIII), which is 2-guanidinothiazole isoster, was introduced as an H₂-antagonist. This drug contains N-amino sulphonylimidamide, which represents polar moiety on side chain and seems to be responsible for the higher potency of the drug. The drug showed a high potency in animals (dogs) and so the studies were extended to human beings. The clinical trials proved conclusively that a single dose of 40mg of Famotidine was sufficient to prevent gastric acid secretion.



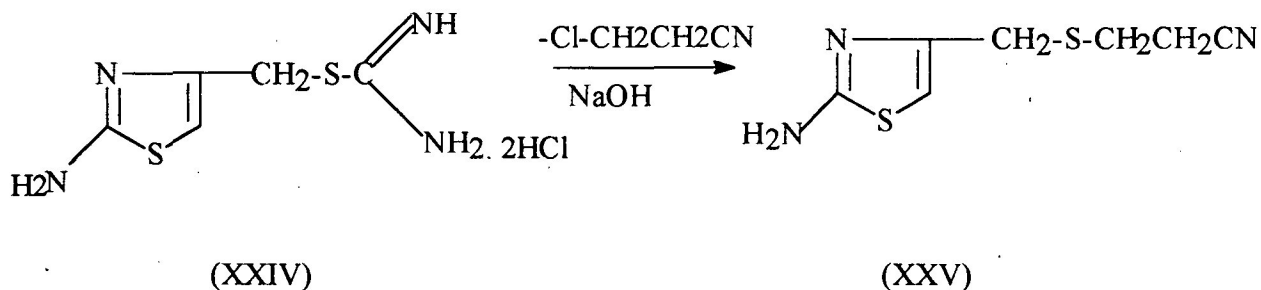
(XXIII)

Famotidine is 39 times more potent than Cimetidine in histamine-stimulated Heidenhain pouch dog⁶⁵. It also proved to be a well absorbed drug. When administered intraduodenally to the pylorus-ligated rat, Famotidine was found to be 50 times more active than Cimetidine in inhibiting acid output⁶⁵. Thus, Famotidine is a potent histamine H₂-antagonist and a powerful inhibitor of gastric acid secretion. Extensive clinical studies showed its efficacy in the treatment of peptic ulcer and related conditions¹². Yanagisawa, I. *et al* carried out the synthesis of Famotidine⁶⁴⁻⁶⁵ with a of yield 54.7%.

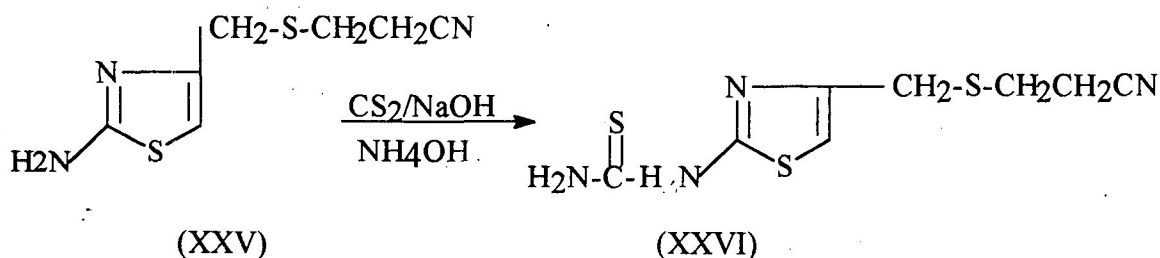
Hence, it was decided to synthesise an intermediate of Famotidine with improved yield. A modification was carried out at different steps to improve yield at every step, to make the process cost-effective and to avoid the use of costly fine chemicals like benzoyl isothiocyanate.

The basic intermediate, Thiouronium salt of thiazole (XXIV), was prepared by reaction of 1,3-dichloroacetone with thiourea, 3-{[(2-aminothiazolyl) methyl]thio} propionitrile (XXV) was prepared from thiouronium salt and chloropropionitrile. At this stage, the process was

modified by using water : 1,4-dioxane mixture (50:50) in presence of KOH. This improved the yield by about 15% over the reported method, which uses water : ethanol as the solvent.



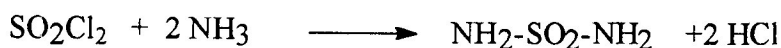
In 1967 Yamamoto Yuichi⁶⁶ et al, prepared thiourea of aminothiazole using CS₂ and NaOH. Hence it was proposed to synthesise thioureidothiazole (XXVI) from (XXV) by utilising the same method.



These modifications avoid one complete step from the established procedure. Also, the process becomes economical as the use of benzoyl isothiocyanate, a hazardous and costly fine chemical, is completely avoided. From our experimental experience, the original route of synthesise of Thioureidothiazole was found to be better than the method utilized by us.

B :Synthesis of sulfamide as an intermediate of Famotidine

Sulfamide is used in many dyes, material for resin and also reducing agent. But most importantly it is used for the synthesise of Famotidine. There are a number of ways to prepare sulfamide. Hence, we planned to synthesise sulfamide by the existing⁶⁷ method. Sulfamide was prepared from sulfuryl chloride and gaseous ammonia as well as with liquid ammonia at a temperature of -50 to 70°C.



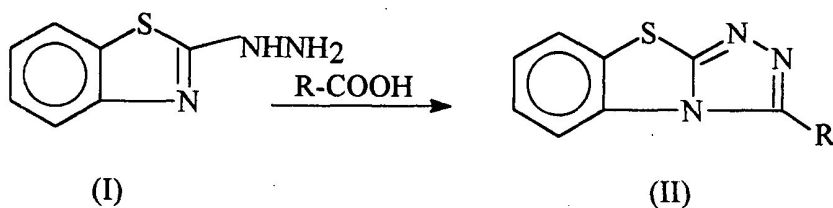
Chemistry

CHEMISTRY

Chemistry of s-triazolo-(3,4-b)benzothiazole.

The s-triazolo-(3,4-b)benzothiazole derivatives showed good antifungal⁶⁸, and antitubercular⁶⁹ and antibacterial activities⁷⁰. Synthesis of s-triazolo-(3,4-b) benzothiazole has been reported by Reynolds and van Allan⁷¹.

When 2-hydrazinobenzothiazole(I) is refluxed with formic acid or triethylorthoformate, the cyclization gives s-triazolo-(3,4-b)benzothiazole(II), where R is H. Refluxing 2-hydrazinobenzothiazole with acetic acid and propionic acid leads to (IIa) and (IIB), respectively.

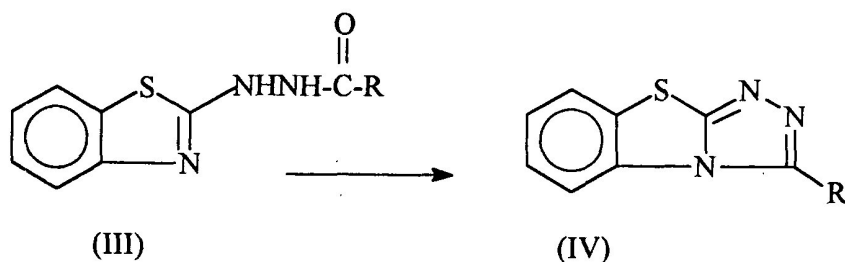


R: H

II a R= -CH₃

II b R= -C₂H₅

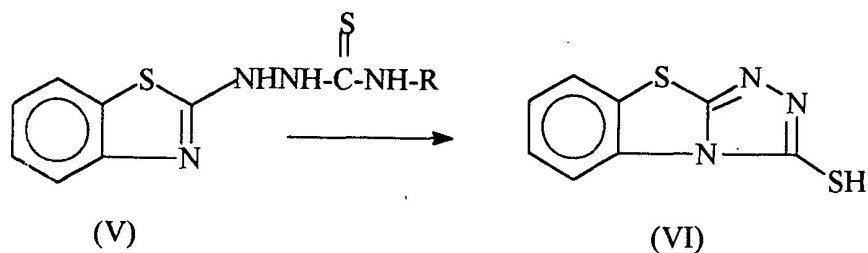
This led to the conclusion that any hydrazide could be cyclized by refluxing it in phenol.



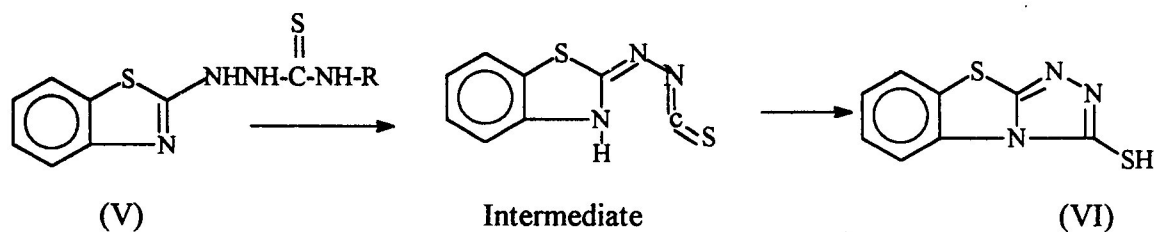
R : C₃H₇

This reaction affords a convenient route of synthesis of higher alkyl derivatives.

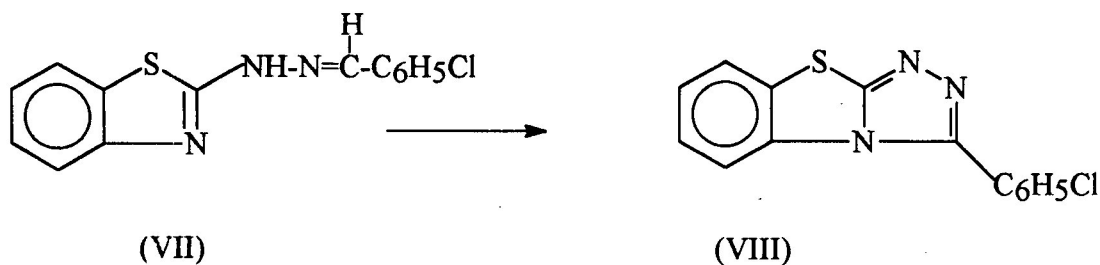
When heated either with or without a solvent, 1-(2-benzothiazoyl)-4phenyl thiosemicarbazide(V) readily undergoes ring closure with the loss of aniline to give 3-mercapto-s-triazolobenzothiazole(VI).



Mechanism

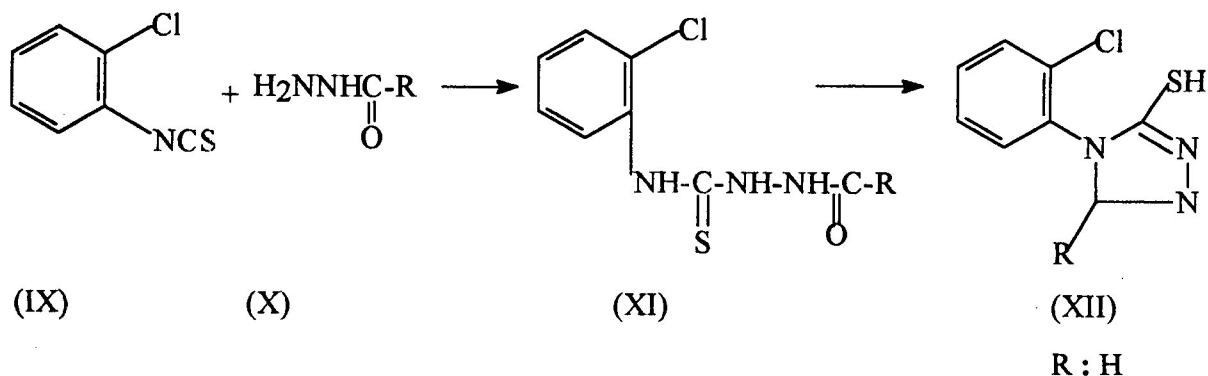


Butlter R. N. et al⁷²⁻⁷³ reported the oxidative cyclization of the substituted benzothiazolyl hydrazone(VII). Cyclization of the hydrazonyl bromide is a single step cyclization of the parent hydrazones with bromine which gives substituted s-triazolo-(3,4-b) benzothiazole(VIII).

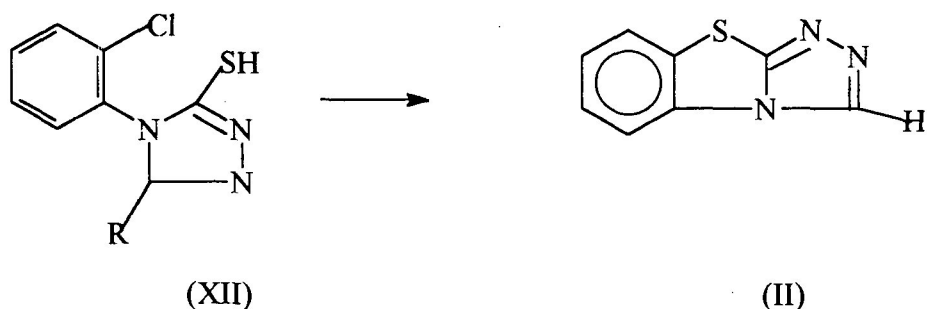


In another literature procedure⁷⁴, a novel ring forming reaction for the synthesis of s-triazolo-(3,4-b) benzothiazole is reported. This method allows a variety of substituted s-triazolo-(3,4-b) benzothiazoles to be obtained in a series of facile reactions.

Thus, 2-chlorophenyl isothiocyanate (IX) when reacted with different substituted hydrazides (X) resulted in compounds (XI) and (XII).

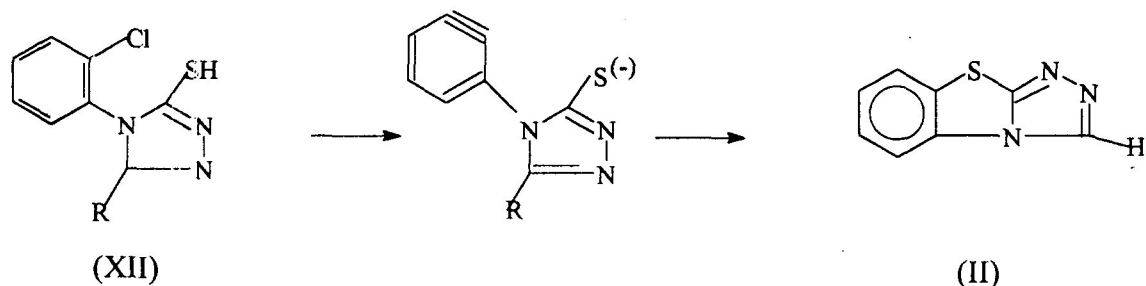


Heating potassium salt of (XII) in refluxing dimethylformamide(DMF) yielded s-triazolo-(3,4-b) benzothiazole (II).



R : H

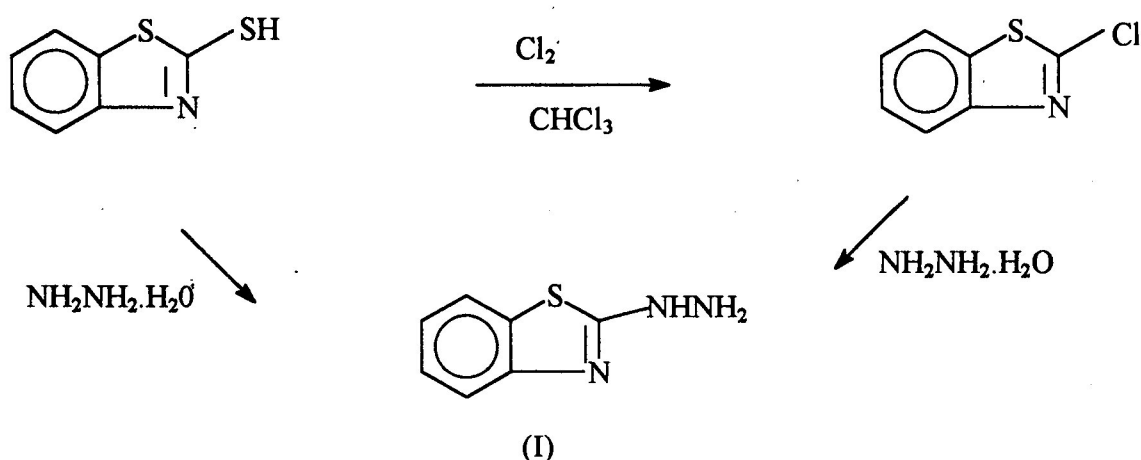
However, simply treating compound (XI) or (XII) with sodium hydride followed by refluxing DMF gave compound(II). Compound (IX) is also directly converted into (II), but the product thus obtained is difficult to purify. A possible mechanism for the formation of s-triazolo(3,4-b)benzothiazole from (XII) would be a nucleophilic displacement by thiolate anion. Alternatively, the reaction mechanism could involve elimination of halogen to yield a benzyne followed by thiolate addition to close the ring.



R : H

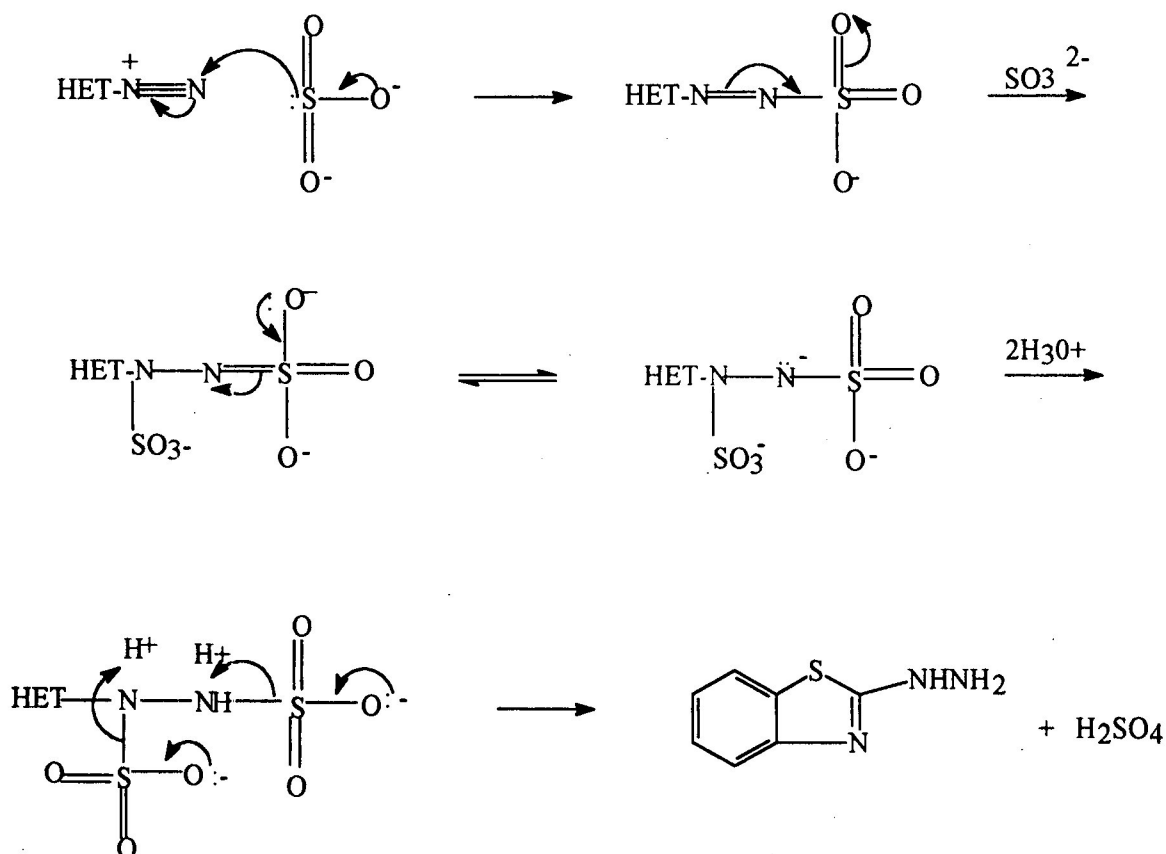
The starting 2-hydrazinobenzothiazole (I) can be prepared from 2-chlorobenzothiazole⁷³, 2-mercaptobenzothiazole⁷⁵ and 2-aminobenzothiazole⁷⁶.

Thus, when 2-chlorobenzothiazole is condensed with hydrazine hydrate (80%) to form 2-hydrazinobenzothiazole, and when 2-mercaptobenzothiazole and hydrazine hydrate (80%) are heated to reflux in dioxane, 2-hydrazinobenzothiazole is obtained. The 2-chlorobenzothiazole is prepared from 2-mercaptobenzothiazole by oxidative chlorination.



2-hydrazinobenzothiazole can also be prepared from 2-aminobenzothiazole by reacting it either with hydrazine hydrate in presence of ethylene glycol and HCl at 150°C, or reducing the diazonium salt⁷⁷ of 2-aminobenzothiazole with an excess of warm sodium sulfide solution, followed by acidification with hydrochloric acid. The hydrochloride usually crystallizes out on cooling and treatment of the latter with excess sodium hydroxide solution liberates the free base. The mechanism of this reduction probably involves the initial addition of a sulfite ion to the diazonium group to give an azosulphonate which undergoes further conjugate (1,4) addition of the nucleophilic sulfite ion. The resulting intermediate is protonolytically cleaved on heating under acidic condition.

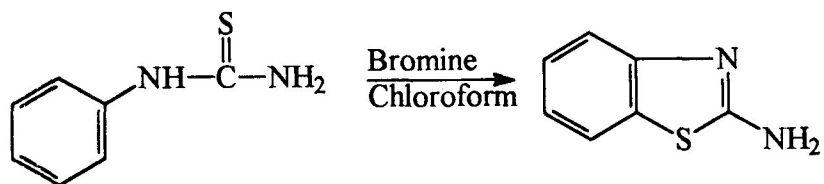
Mechanism of reduction.



2-aminobenzothiazole is conveniently prepared from aniline by thiocyanation. Various procedures for the preparation of 2-amino-6-substituted benzothiazole are

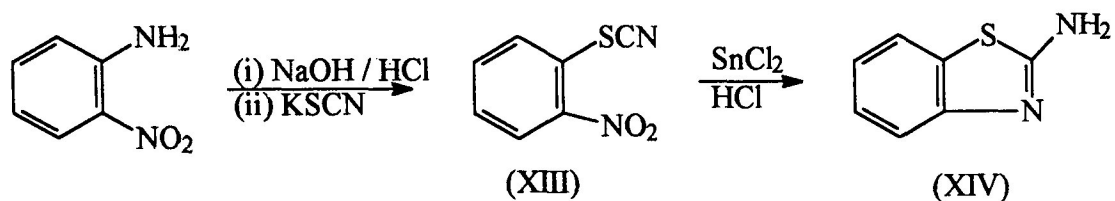
1. Thiocyanation
2. Hugerschoff synthesis^{78,79} - This synthesis is restricted to the preparation of 2-amino derivatives of the benzothiazole and involves the use of oxidizing agents such as bromine. Bromine reacts with arylthiourea in an inert solvent, such as chlorobenzene, chloroform, ethylene dichloride or acetic acid. Sulfuryl chloride can be used as a substitute for bromine.⁸⁰⁻⁸²

However, potassium ferricyanide in alkaline media, the reagent for the Jacobson ring closure, gives no thiazole from the arylthioureas.^{78,79}



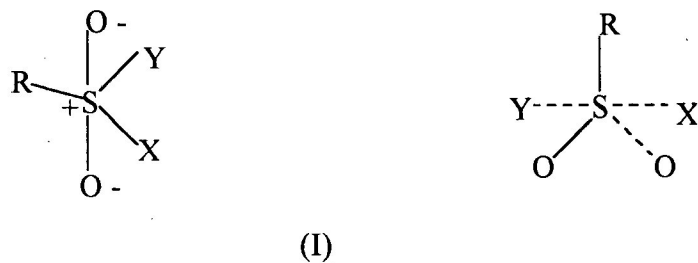
Yields in this reaction are quantitative.

3. 2-Aminobenzothiazole(XIV) has also been prepared from o-thiocyanonitrobenzene compounds of the type (XIII) by chemical reduction (stannous chloride in hydrochloric acid and ferrous sulfate in ammonia).^{83,84} The yield was 60-85 %.

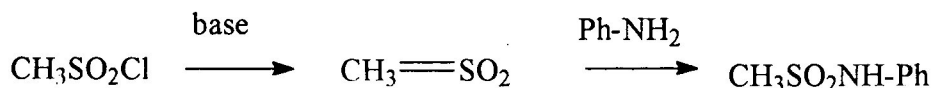


Chemistry of s-triazolo -(3,4-b) benzothiazole-3- sulfonamide

The sulfonamides are highly crystalline, particularly the derivative from sulfonyl chloride. The preparation of sulfonamide from sulfonyl chloride is a nucleophilic substitution at $R-SO_2Cl$ in a manner similar to the attack of $R-COCl$, but the mechanism is not same because a 'tetrahydal' intermediate in this case (I) would have five groups on the central atom⁸⁵



This mechanism more closely resembles the SN_2 mechanism, but the reaction of the substrate, which carries a α -hydrogen, is a strong evidence that the reaction takes place by an elimination-addition mechanism going through sulfene intermediate. e.g. reaction of methane sulfonyl chloride and aniline gives sulfonamide as follows -



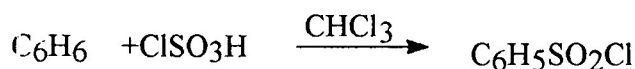
Preparation of sulfonyl chloride

The sulfonyl chlorides are prepared by various methods.

1. Sulfonyl chloride is made by reacting sodium sulfonate with PCl_5 or $POCl_3$ at $180^\circ C$ ⁸⁵. In this reaction, benzene sulfonate reacts with the PCl_5 or $POCl_3$ to yield benzene sulfonyl chloride.



Chlorosulfonic acid is utilized for the conversion of aromatic hydrocarbons to sulfonyl derivatives and can be used in the characterization of unknown hydrocarbons⁸⁶. In this, chlorosulfonyl chloride is used to provide the sulfonyl chloride ($-SO_2Cl$) group to the aromatic hydrocarbon. Thus, the sulfonyl chloride group is introduced to the aromatic nucleus by treatment with an excess of chlorosulfonic acid in chloroform.⁸⁷



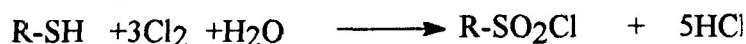
Sulfonyl chlorides have also been prepared by the reaction of Grignard reagent first with SO₂ and then with chlorine gas.⁸⁸



R : Aliphatic, aromatic compounds

Aliphatic hydrocarbons can also be chlorosulfonated with sulfonyl chloride derivatives.⁸⁹ eg. 2,3-dimethyl butane yields 20% of 2,3-dimethyl butane-1-sulfonyl chloride⁸⁹. More recently, photolytic chlorosulfonation of alkane has been reported to yield alkanesulfonyl halides^{90,91}. In this mixture C₁₀-C₂₀ alkane sulfonyl chlorides were prepared by photolytic chlorosulfonation of a paraffin mixture with chlorine and a large excess of SO₂. The advantage of this method is that it minimizes the chlorination and degradation of sulfonyl chloride to give a high yield.

Mercaptans or disulfides on reaction with chlorine in aqueous hydrochloric acid can be converted to sulfonyl chlorides⁹². Thus, methane mercaptan is converted into methane sulfonyl chloride by simultaneous hydrolysis and chlorination at 40-70°C in saturated HCl.



Disulfide can also be converted to sulfonylchloride by simultaneous hydrolysis and chlorination.



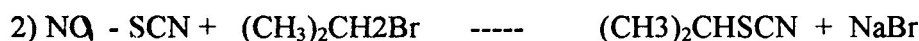
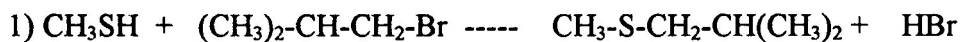
There are relatively few heterocyclic sulfonamides in which the sulfur atom is attached directly to a carbon of heterocyclic ring. In such a cases, sulfonyl chloride (sulfonamide) are usually prepared by chlorosulfonation or conversion to sulfonic acid and acid chloride, followed by the amidation to get sulfonamide. The oxidative chlorination at low temperature of such heterocycles has been found to be quite widely applicable to the preparation of these compounds.

Some exceptions have been found in general application of the method to the preparation of sulfonyl chlorides. The instability of many sulfonyl chlorides may account for the exception. On long standing of oxidative chlorination the decomposition of the product was found to the corresponding chloro derivative .eg 2-mercaptobenzothiazole is converted into 2-chlorobenzothiazole when the reaction is carried out for a long period at room temperature⁹³.

Chemistry of 5/6-Nitro-3-[[substituted phenyl]-s-triazolo-(3,4-b)-benzothiazole] -thione

In general, sulfur compounds show greater nucleophilic reactivity than their oxygen counterparts and substitution by sulfur containing nucleophiles often occurs under very mild conditions giving high yields. The common leaving groups include halide, sulfonate ions and water [from conjugate acid of alcohols].

e.g.



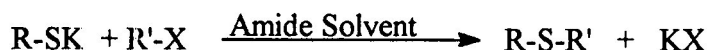
Alkylation occurs readily with replacement of the 2-mercapto hydrogen to yield s-alkylated derivatives.^{94,95}

2-mercaptobenzimidazole may be alkylated with substituted benzyl halides by refluxing with 2N NaOH solution.⁹⁶

Preparation of sulfides

In the preparation of sulfides, the required sodium mercaptans are prepared from mercaptans and aqueous or alcoholic solution of sodium hydroxide or sodium ethoxide.

The sodium mercaptide reacts with halides⁹⁷⁻⁹⁹, chlorohydrin esters of sulfinic acid¹⁰⁰ or alkylsulfonate¹⁰¹ giving sulfides in 70% yield. Aryl halides when reacted with mercaptan or thiolate ion also give sulfides¹⁰². A general procedure for synthesizing arylthioethers via nucleophilic displacement of aryl halides by thiolate ion are reported. This method involves simply heating an alkali metal thiolate with halide together in an amide solvent.

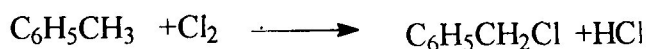


R and R': Substituted phenyl

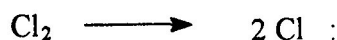
Preparation of benzyl halides

The substituted benzylhalides required for the synthetic work were prepared by the methods described in the literature. The general procedures are discussed as below.

Side chain substitution is favored by high temperature and light, and the absence of halogen carrier. e.g. Toluene is converted to benzyl chloride, when chlorinated at its boiling point in presence of light.



It is generally believed that side chain substitution proceeds by chain reaction.



Similarly, p-chlorotoluene at 120°C in the presence of light gives p-chloro benzylbromide.

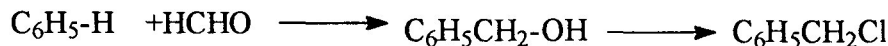


Another method for the preparation of benzyl halides is treatment with formaldehyde and HCl; the CH₂Cl group is introduced into the ring in a reaction called Chloromethylation.

The reaction was also carried out with other aldehydes and with HBr or HI.¹⁰³ The more general term haloalkylation covers these cases. The reaction is successful for benzene. It is greatly hindered by m-directing group which decreases yield or completely prevents the reaction. The compound with lower yield can often be chloromethylated with chloromethyl methyl ether, Bis (Chloromethyl methyl) ether,¹⁰⁴ methoxy acetyl chloride¹⁰⁵. ZnCl₂ is the most common catalyst used in such reactions but other Friedel Craft catalysts are also employed.

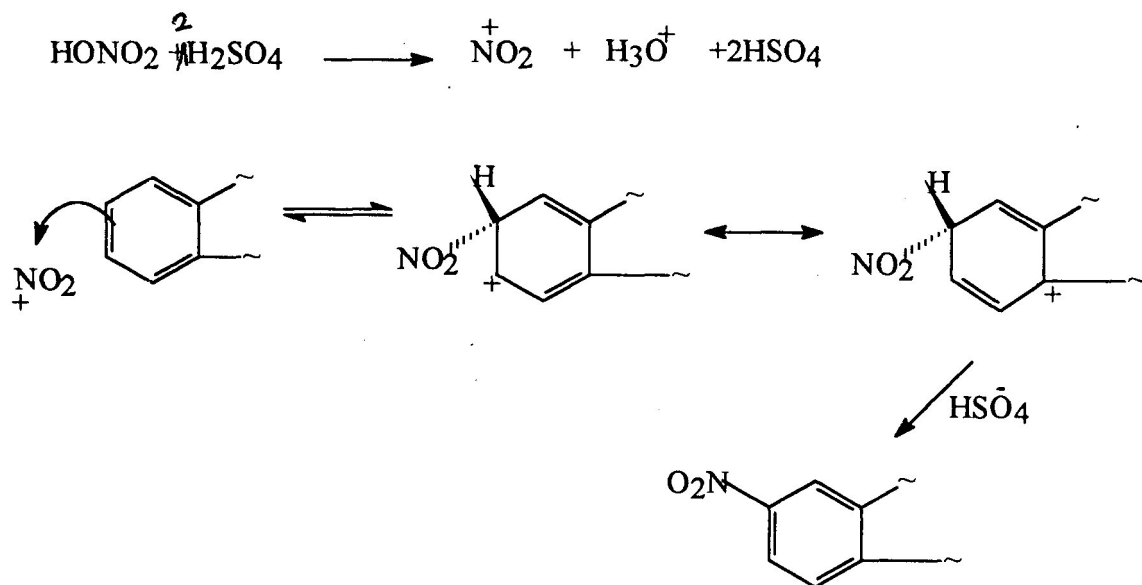
Mechanism of Chloromethylation.

The initial step involves the reaction of the aromatic compound with aldehyde to form hydroxy alkyl compound, then HCl converts this to the chloroalkyl compound.¹⁰⁶

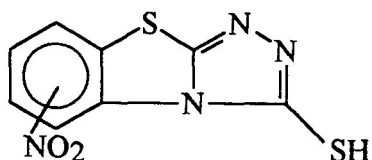


The acceleration of reaction by ZnCl₂ has been attributed to the rising of the acidity of medium causing increase in concentration of HOCH₂⁺ ion.¹⁰⁷

Mechanism for nitration of 5/6-Nitro-3-[[substituted phenyl]-s-triazolo-(3,4-b)-benzothiazole}thione.



Thus, nitration of s-triazolo-(3,4-b)-benzothiazole gives 5/6-nitro-s-triazolo-(3,4-b)-benzothiazole. Position of the nitro group is not confirmed.



Chemistry of Schiff bases.

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



R and R' :Substituted phenyl

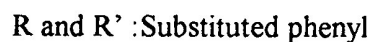
Schiff,¹⁰⁸ for the first time reported condensation of this kind and hence, the condensation products are referred to as 'Schiff bases'. Pelton *et al* have shown¹⁰⁹ the antifungal and antibacterial activity of some substituted salicylaldehydes. Schiff bases from different aldehydes have shown encouraging antituberculosis activity in vitro in our laboratory.⁶² The presence of azomethine linkage in compounds has been shown to decrease their toxicity considerably.⁶³

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

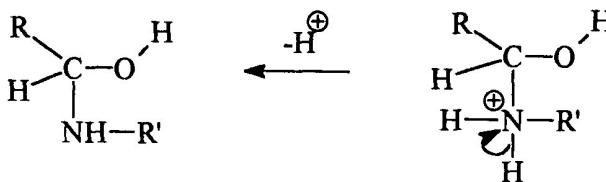
1. The simplest method appears to be the one in which equimolar quantities of an amine and an aldehyde are condensed by boiling in methanol or ethanol.
2. In 1946, Tipson and Clapp,¹¹⁰ obtained Schiff bases by refluxing the amine and aldehyde in toluene with a few drops of piperidine. The water formed during the course of reaction was removed by employing a Dean Stark trap.
3. In 1950, Hayes *et al*¹¹¹ prepared Schiff bases by heating a mixture of an aldehyde and an amine in benzene and removing the water formed during the reaction using Dean Stark apparatus.
4. In 1953, Giovambettista and Rabassa¹¹² 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 a few drops of acetic acid improved the yield. Later on, during the course of investigation, the last method using acetic acid as a solvent was found to be the best for following reasons.

- ### Reaction Mechanism


$$\begin{array}{c} \text{R} \\ \diagdown \\ \text{C}=\text{O}: \\ \diagup \\ \text{H} \end{array} + \text{H}^{\oplus} \rightleftharpoons \left[\begin{array}{c} \text{R} \\ \diagdown \\ \text{C}=\text{O}-\text{H} \\ \diagup \\ \text{H} \end{array} \right] \longleftrightarrow \begin{array}{c} \text{R} \\ \diagdown \\ \text{C}^{\oplus}-\text{O}-\text{H} \\ \diagup \\ \text{H} \end{array}$$

$\begin{array}{c} \text{NH}_2 \\ | \\ \text{R}' \end{array}$


$$\begin{array}{ccccccc}
 \begin{array}{c} \text{R} \\ \diagup \\ \text{C} - \ddot{\text{O}}\text{H} \\ \diagdown \\ \text{H} \end{array} & \begin{array}{c} \text{NH-R} \\ | \\ \text{C} \end{array} & \xrightleftharpoons{+\text{H}^{\oplus}} & \begin{array}{c} \text{R} \\ \diagup \\ \text{C} - \text{OH}_2^{\oplus} \\ \diagdown \\ \text{H} \end{array} & \begin{array}{c} \text{NH-R'} \\ | \\ \text{C} \end{array} & \xrightarrow{-\text{H}_2\text{O}} & \begin{array}{c} \text{H} \\ | \\ \text{R} \text{---} \text{C} = \text{N}^{\oplus} \text{---} \text{R} \\ \diagdown \\ \text{H} \end{array} \\
 & & & & & & \downarrow -\text{H}^{\oplus} \\
 & & & & & & \begin{array}{c} \text{R} \\ \diagup \\ \text{C} = \text{N-R'} \\ \diagdown \\ \text{H} \end{array}
 \end{array}$$

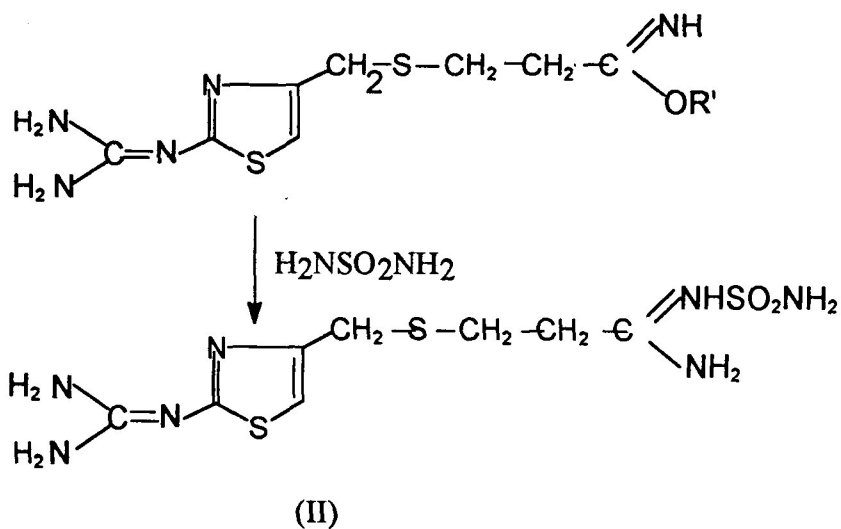
46

Chemistry of Famotidine.

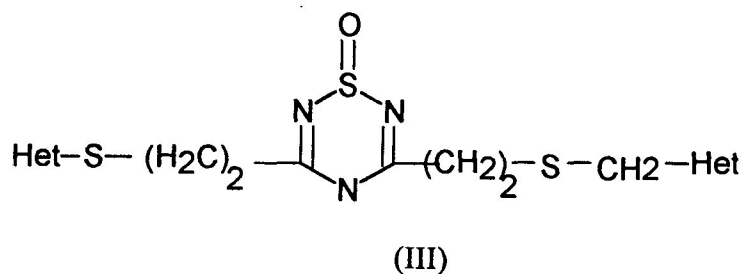
It includes the chemistry of Famotidine, 3-[[[(thiouredio thiazolyl) methyl] thio]-propionitrile and the chemistry of sulfamide.

Famotidine is an anti-ulcer drug now available in the market due to the efforts of Yanagisawa and coworkers⁶⁵, who showed that the N-sulfamoyl and sulfonyl amidine derivatives are more active than the previously reported N-cyano and N-carbamoylamidine derivatives⁶⁴ with respect to their H₂ antagonist property.

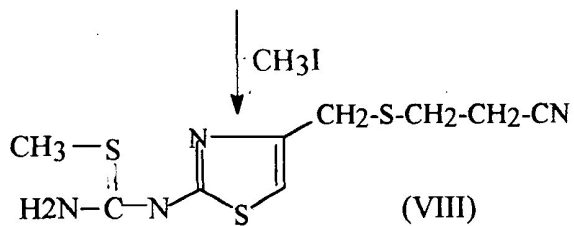
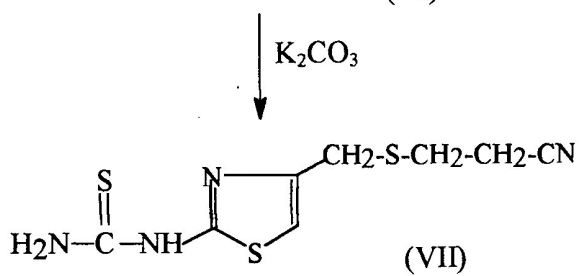
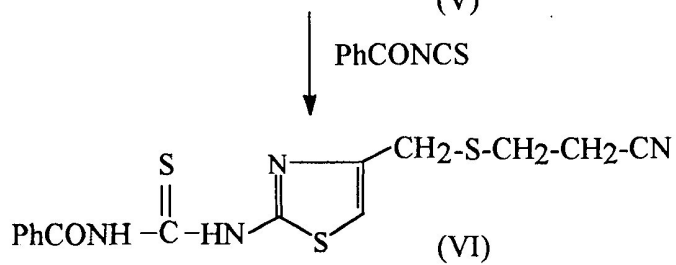
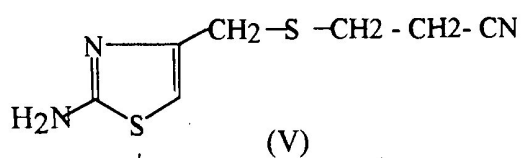
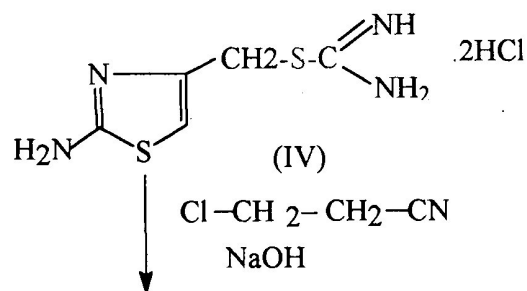
The synthesis of Famotidine involves the reaction of imidates (I) R=H, with the sulfamide. In this reaction sulfamide dimer is also obtained as a byproduct (described later).

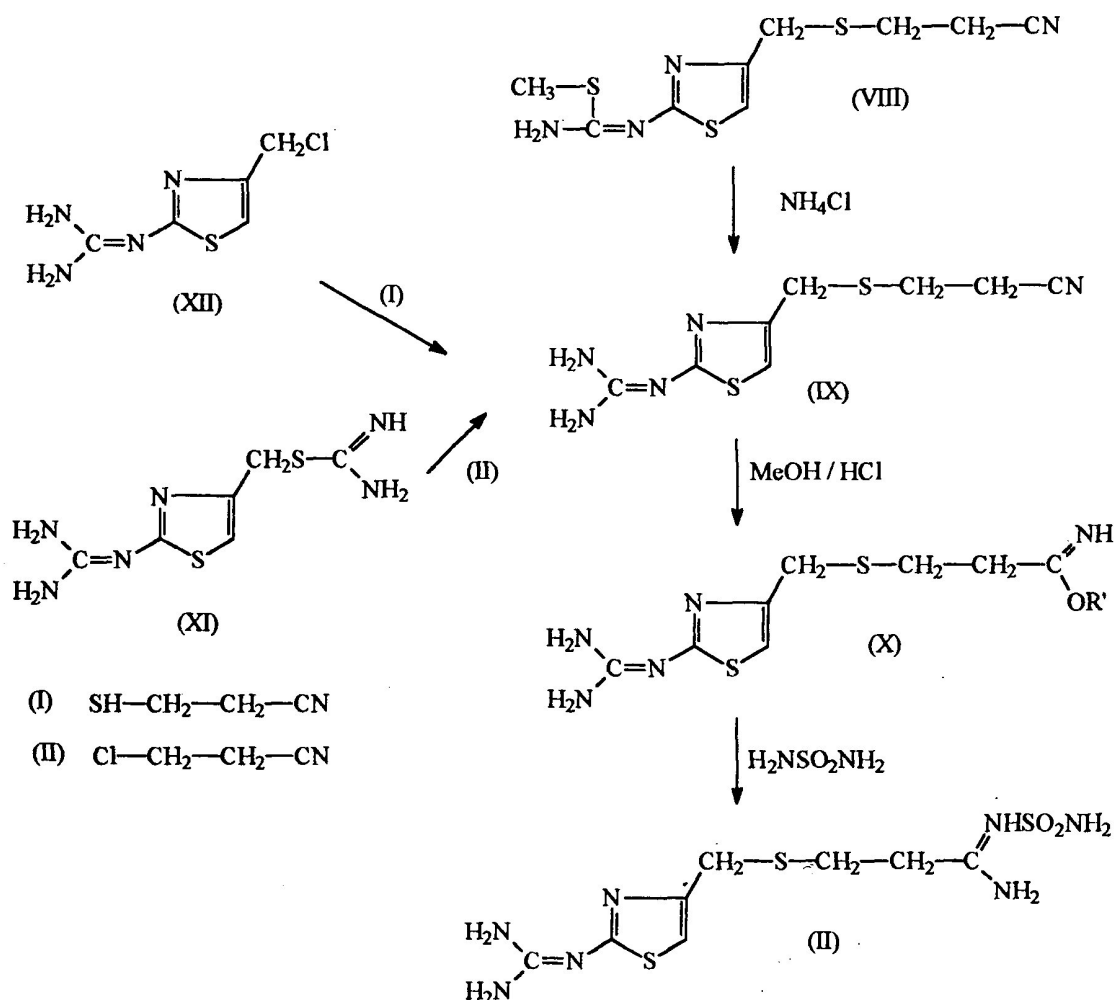


R' : CH₃



Imidates were prepared by the following steps. The following scheme shows the preparation of thiazole derivative which results into the synthesis of Famotidine, finally.





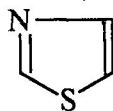
3-[[[(2-amino thiazolyl)methyl]thio]-propionitrile(V) was prepared from thiouronium salt (IV) and 3-chloropropionitrile under an atmosphere of nitrogen. The thioureidothiazole (VII) was made from aminothiazole (V) and benzoyl isothiocyanate, followed by hydrolysis of the intermediate (benzylthioureido)thiazole (VI). The (VII) is reacted with methyl iodide to provide s-methyl isothioureidothiazole (VIII) which was allowed to react with ammonium chloride or methyl amine to give [[[(diamino methylene) aminothiazoyl] methyl thio] propionitrile(IX).

The (IX) can also be prepared from analogous route either by reacting (XI) with 3-chloropropionitrile or by reacting compound (XII) with 3-mercapto propionitrile.

The nitrile (IX) was then treated with alcohol and hydrogen chloride gas in methanol at a low temperature to offer imidate hydrochloride (X), which was basified and converted to Famotidine (II), by reacting it with sulfamide.

Chemistry of 3-[(thiouredio thiazolyl) methyl thio]-propionitrile

The thiazole ring (I) was first described by Hantzsch and Weber¹²⁰ in 1887 as the pyridine of the thiophene series.



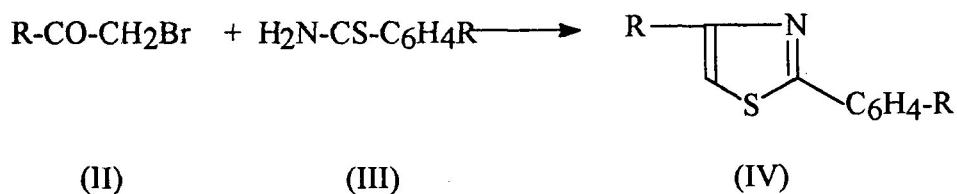
(I)

The high reactivity of the thiazole molecule is due to the group attached to the carbon atom placed between sulfur and nitrogen. They proposed the prefix meso for this position in the normal azole series.

Although unsubstituted thiazole is not found in nature, naturally occurring thiazoles are numerous. This origin has often been related to the cyclization of the peptide chain at cystein residue with formation of thiazolidine ring.¹¹³ Several antibiotics such as Althromycine¹¹⁴ and Micromycine¹¹⁵ contain thiazole ring as do many metabolites of living organisms, numerous natural aromas such as tomato¹¹⁶ contain thiazole.

General method of synthesis of thiazole

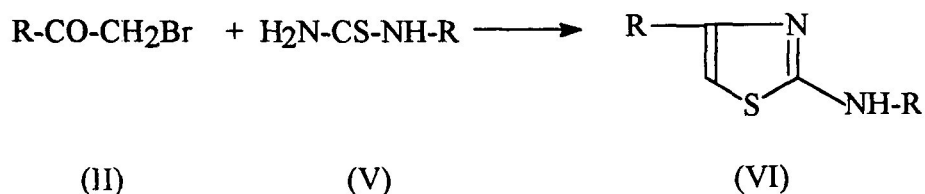
They were prepared in 1950 by Erylenmeyer et al¹¹⁷ from thiobenzamide III and ω-bromo acetophenone (II) in alcoholic solution with a yield of 50-80%.



R Substituted phenyl

General method for the synthesis of substituted 2-amino thiazole.

The cyclization of N-monosubstituted thioureas (V) with halocarbonyl compounds gives 2-monosubstituted aminothiazoles(VI).^{118,119}



R :Substituted phenyl

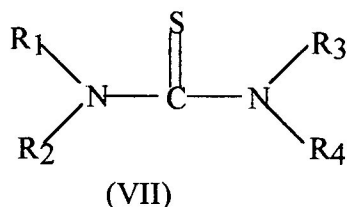
Mechanism of synthesis (Hantzsch's synthesis)

The first step in the reaction is the formation of an alicyclic intermediate by nucleophilic attack of 'S' atom by one of its lone pair of electrons on the halogen carrying 'C' atom to form C-S-C bond.

In the second step, the lone pair of electrons of 'N' attack the 'C' carrying the carbonyl function, giving rise to a new cyclic intermediate. This intermediate upon treatment with acid leads to the thiazole molecule.^{120,121}

Chemistry of thiourea / Substituted thiourea.

The thioureas are generally represented by the structure (VII)



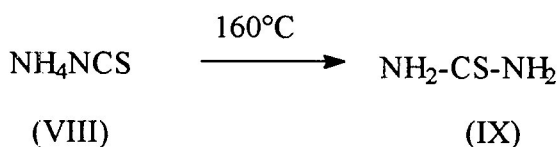
R : H

Their chemistry essentially deals with the reaction product obtained by the nucleophilic reactivity of these compounds. The center of the reaction is either -S- atom or -N- atom. Formation of the thiazole ring with haloacetyl group is of pharmaceutical importance.

General method of synthesis

A. Action of alkylthiocyanate on amine hydrochloride.

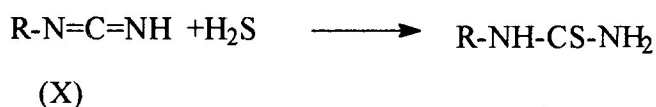
When ammonium thiocyanate (VIII) is heated at 160°C for several hours, thiourea (IX) is obtained.



A mixture of primary amine and ammonium thiocyanate is heated in the presence of HCl in an inert solvent or an aqueous medium to give substituted thioureas.

B. Thio hydrolysis of carbodiamides and cyanamides.

On passing H₂S gas into the substituted carbodi-imides (X) and cyanamides (XI) corresponding thioureas (XII) are formed.



The reaction is mostly dependent on the ability of carbodi-cyanamide to add to H₂S.

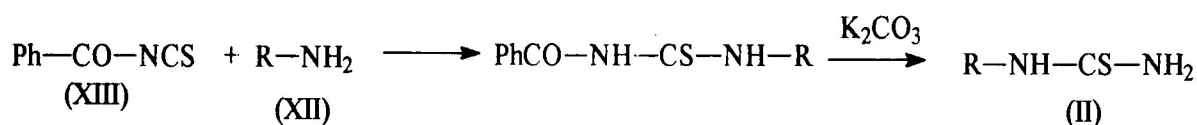
C. Action of isothiocyanate on ammonia or primary amine.

I. Primary amine (XII) reacts with thiophosgene to give corresponding thiocyanate, which further reacts with ammonia to yield the corresponding thiourea.



R : Substituted /unsubstituted phenyl

Benzoyl isothiocyanate (XIII) reacts with primary amine (XII) to give asymmetric 1,3-disubstituted thioureas. Substituted isothiocyanates are chosen such that after disubstituted product is formed the substituted group can be easily removed by hydrolysis or heterolysis to obtain monosubstituted thioureas or corresponding primary amines.

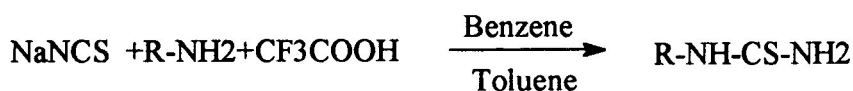


R : Substituted /unsubstituted phenyl

Neville *et al*¹²² have reported a method using silicon tetrathiocyanate as a thiocarbonylating agent. It is prepared by the action of ammonium thiocyanate and silicon tetrachloride in benzene which on treatment with primary amine yields a silicon complex, which further undergoes hydrolysis to give the monosubstituted thiourea.

Vijaykumar Nair¹²³ has reported the synthesis of tetrabutyl isothiocyanate which substitutes aromatic aliphatic and heterocyclic primary amines to give disubstituted unsymmetric thioureas. These on hydrolysis give the respective monosubstituted thioureas.

Loev *et al*¹²⁴ have reported the synthesis monosubstituted thioureas by the action of isothiocyanate on appropriate aromatic amines in the presence of trifluoroacetic acid using benzene or toluene as the solvent.



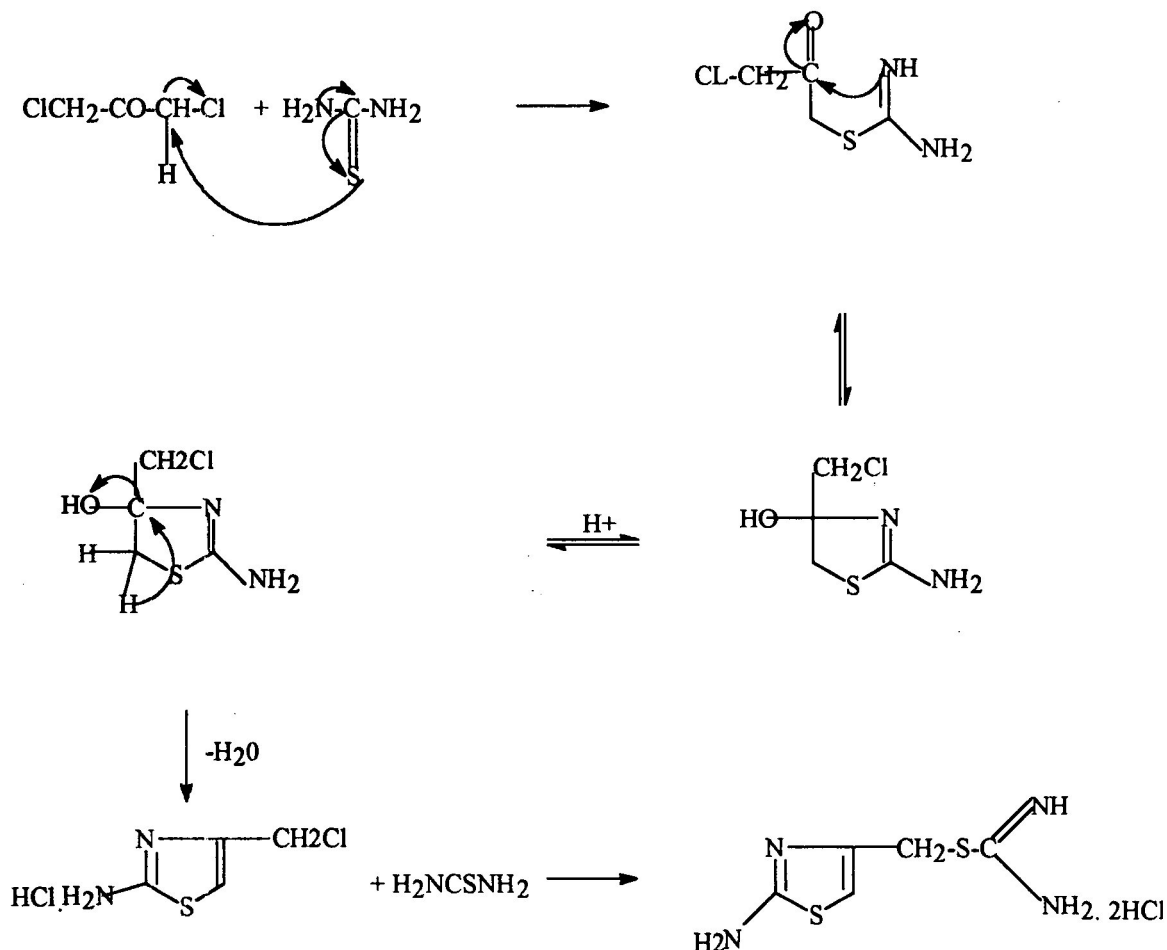
Rasmussen et al.¹²⁵ have reported an improved procedure for the preparation of cycloalkyl, aryl thioureas by the action of benzoyl isothiocyanate on aniline in acetone and debenzoylation of the resultant N-aryl, N-benzoylthioureas with 5% aq. NaOH.

In the present work, it is proposed to synthesize the intermediate of Famotidine, eg. Thioureidothiazole from 2-aminothiouronium salt of thiazole by employing Hantzsch synthesis^{118,119} and Yamamoto Yuichi's⁶⁶ method for synthesis of thioureidothiazole and 2-aminothiouronium salt of thiazole, respectively.

1-3 dichloroacetone was treated with unimolar quantity of thiourea in water and ethanol as solvent at 80°C to obtain the 2-amino-4-methyl chlorothiazole.

Treating this 2-amino-4-methyl chlorothiazole with one mole thiourea gives thiouronium salt of thiazole. Instead of using thiourea in one mole quantity, the use of 2 moles of thiourea also gives the same product in one step and hence, the procedure was modified accordingly.

The probable mechanism of formation of thiouronium salt is as below.



Earlier Yamamoto Yuichi ⁶⁶ has reported the use of CS₂, NaOH and ammonia in the preparation of thiourea of the thiazole.

Thus, in the present work the same method is proposed and employed. Unfortunately, the result of this experimental trial was not satisfactory over the reported method utilized in synthesis the of Famotidine

Chemistry of sulfamide.

Sulfamide is useful as a flammability, reducing agent, a material for resins, dyes and medicines. The importance of sulfamide has increased now because of its role in Famotidine synthesis. ^{Sulfamide} is prepared by various methods and these methods are enlisted below. A comparison of their economy for laboratory scale preparation is also made.

In 1964 Yukio and coworkers ¹²⁶ reported the production of sulfamide using NH₃, SO₂ and chlorine. A "sulfamide condensate" was obtained as the by product. Interaction of the sulfamide and the sulfamide condensate also takes place, but upon hydrolysis it gives more sulfamide.

Another procedure for the synthesis of sulfamide is the reaction of sulfuryl chloride and liquid ammonia. ¹²⁷ In this reaction vigorous stirring is required and also the use of diluent is necessary to increase the yield of sulfamide. Thus, 150ml of SO₂Cl₂ in 500ml petroleum ether was added dropwise to 600ml of liquid ammonia at -60°C under vigorous stirring. The reaction mixture was then allowed to reach room temperature. The hydrolysis of the crude product with HCl and extraction with acetone gave 80.20% yield of sulfamide ¹²⁷.

In one more method ¹²⁸ of preparation of sulfamide, finely pulverized SO₂Cl₂ is introduced into a stream of liquid ammonia at -60 to -70°C and SO₂(NH₂)₂ is separated from NH₄Cl, washed with NH₃ and after evaporation of ammonia purified with ethyl acetate.

Sulfamide can also be prepared from SO₂Cl₂ and ammonia, and upon extraction in dioxane ¹²⁹ the pure sulfamide is obtained. Use of dioxane avoids inorganic impurities and pure sulfamide can be obtained. Though all these methods of synthesis are from SO₂Cl₂ and ammonia, some other methods are also employed to synthesise sulfamide. ¹³⁰ This method of synthesizing sulfamide comprises of hydrolysis of N,N bis- (tetramethyl silyl)sulfamide, which has been prepared by reacting hexamethyldisilazane with sulfuryl chloride in dichloroethane. The residue is hydrolyzed with water. The crude sulfamide is filtered and washed to obtain pure sulfamide in quantitative yield.

Experimental Work

EXPERIMENTAL SECTION

PART II

Synthesis of sulfonamide and s-benzyl derivative of substituted/unsubstituted s-triazolo-(3,4-b)-benzothiazole.

This part consists of two sections.

Section I Synthesis of 3-N{[substituted heterocyclic/phenyl]-s-triazolo-(3,4-b)-benzothiazole }-sulfonamide.

Section II Synthesis of 5/6-Nitro-3-{{substituted phenyl]-s-triazolo-(3,4-b)-benzothiazole} thione.

Section I : Synthesis of 3-N{[substituted heterocyclic/phenyl]-s-triazolo-(3,4-b)-benzothiazole }-sulfonamide.

It involves -

- A. Preparation of 2-hydrazino benzothiazole
- B. Preparation of s-triazolo-(3,4-b)-benzothiazole-3-thione.
- C. Preparation of s-triazolo-(3,4-b)-benzothiazole-3-sulfonyl chloride
- D. Preparation of s-triazolo-(3,4-b)-benzothiazole-3-sulfonamide.
- E. Synthesis of title compounds 3-N{[substituted heterocyclic/ phenyl]-s-triazolo-(3,4-b)-benzothiazole }-sulfonamide.

A. Preparation of 2-hydrazino benzothiazole.

In a round bottomed flask, 1.67gm (0.01mole) of 2-mercapto benzothiazole was dissolved in 50 ml dry 1,4-dioxane and 2.85g(0.057mole) hydrazine hydrate, was added slowly (color changes from yellow to brown). The reaction mixture was then refluxed for 4 hours at 110⁰C, and then cooled to room temperature. The crude mass was filtered, dried and washed with dilute NaOH solution. It was then recrystallised from ethanol.

Yield : 1.12 gm (67.87%)

M. P. 198⁰C .

(Lit.¹³² M.P. 197-199⁰C)

B. Preparation of s-triazolo-(3,4-b)-benzothiazole-3-thione.

In a 50ml round bottomed flask, 1.65gm.(0.01mole) of 2-hydrazinobenzothiazole was dissolved in 50 ml of hot methanol. In another flask 0.56 gm.(0.01mole) KOH was mixed with 5ml (excess) of CS₂ with stirring, while maintaining the temperature below 15⁰C. This mixture was then introduced into the solution of 2-hydrazinobenzothiazole. The mixture was then refluxed at 50⁰c for 6hr. After completion of reaction, the solvent and excess of CS₂ were removed and the solid thus obtained was dissolved in cold water and filtered. The filtrate was then acidified with dil. HCl (pH 6.5) to obtain the product.

Yield = 1.82gm (88%)

MP : 255⁰C

Lit.¹³³ M.P. : 255⁰C

C. Preparation of s-triazolo-(3,4-b)-benzothiazole-3-sulfonyl chloride

In a three-necked round bottomed flask equipped with air condenser was placed 1 gm.(0.0048mole) of s-triazolo-(3,4-b)-benzothiazole-3-thione in 10 ml dil. HCl (10%) and the mixture was stirred rapidly in an ice bath, while chlorine was being introduced through a capillary tube at a moderately rapid rate for 1-2 hours. Care was taken to keep the temperature below 7-8⁰C, in order to minimize decomposition of the product. The product, thus obtained was filtered under suction, washed well with ice cold water and dried to yield 0.6gm (46%) of s-triazolo-(3,4-b)-benzothiazole-3-sulfonyl chloride

M.P. : 167⁰C

D. Preparation of s-triazolo-(3,4-b)-benzothiazole-3-sulfonamide.

In a 100 ml beaker, 1.08 gm.(0.00146mole) of s-triazolo-(3,4-b)-benzothiazole-3-sulfonyl chloride was placed and liquor ammonia (10ml) was added to it. Excess ammonia was evaporated on water bath. The solid obtained was washed with acetone to remove unreacted sulfonyl chloride, if any. It was then recrystallised in water to obtain the final product.

Yield : 0.6gm (60%)

M.P. : 250⁰C

Elemental analysis of $C_8H_6N_4O_2S_2$

	C%	H%	N%
Calculated	37.79	2.36	22.04
Found	37.58	2.33	22.25

E. Synthesis of title compound 3-N{[substituted heterocyclic/ phenyl]-s-triazolo-(3,4-b)-benzothiazole }-sulfonamide.

In a 50ml flask, 0.282 gm (0.003 mole) of 2-aminopyridine was dissolved in 10 ml anhydrous acetone and 1ml of pyridine was added to it. In another 50ml flask, 0.85 gm. of s-triazolo (3,4-b) -benzothiazole-3-sulfonyl chloride was dissolved in 10 ml anhydrous acetone. Both the mixtures were mixed at room temperature. The reaction mixture was set aside overnight and 0.55 gm (almost pure) sulfonamide was filtered off. Dilution of the filtrate with water yielded some more compound. It was recrystallised in water.

Yield : 0.72gm (70%)

M.P. : 230°C

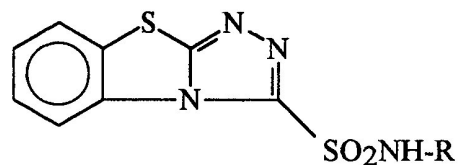
Elemental analysis of $C_{13}H_9N_5O_2S_2$

	C%	H%	N%
Calculated	47.41	2.73	21.27
Found	47.01	2.91	21.60

Similarly, various substituted sulfonamides were prepared and their physical data has been summarized in Table no.1

TABLE NO. 1

3-N{[substituted heterocyclic/ phenyl]-s-triazolo-(3,4-b)-benzothiazole }sulfonamide.



Sr. No	R	Molecular Formula	Molecular Weight	MP (°C)	Yield %	Elemental Analysis					
						Calculated			Found		
						C	H	N	C	H	N
1	2-pyridine	C ₁₃ H ₉ N ₅ O ₂ S ₂	329	230	74.5	47.41	2.73	21.27	47.01	2.91	21.60
2	4-methyl-2-pyridine	C ₁₄ H ₁₁ N ₅ O ₂ S ₂	345	250	77.5	48.69	3.18	20.28	48.28	3.1	20.68
3	5-chloro-2-pyridine	C ₁₃ H ₈ ClN ₅ O ₂ S ₂	365.5	245	70.3	42.68	2.18	19.15	42.3	2.22	19.38
4	4,6-dimethyl- 2-pyrimidine	C ₁₄ H ₁₂ N ₆ O ₂ S ₂	360	245	72.0	46.66	3.33	23.33	46.32	3.12	22.10
5	4,6-dihydroxy-2-pyrimidine	C ₁₂ H ₈ N ₆ O ₄ S ₂	364	>260	66.2	39.56	2.19	23.07	39.16	2.21	23.4
6	2-pyrimidine	C ₁₂ H ₈ N ₆ O ₂ S ₂	318	260	77.5	45.28	2.57	26.4	45.5	2.2	26.7
7	2-thiazole	C ₁₁ H ₇ N ₅ O ₂ S ₃	337	>280	69.7	39.16	2.07	20.77	39.40	2.2	20.96
8	5-nitro-2-thiazole	C ₁₁ H ₆ N ₆ O ₄ S ₃	368	255	68.0	35.8	1.6	22.82	35.77	1.75	22.67
9	4-fluorophenyl	C ₁₄ H ₉ FN ₄ O ₂ S ₂	348	220	71.7	48.27	2.58	16.09	48.66	2.41	16.4
10	2,5-dimethylphenyl	C ₁₆ H ₁₄ N ₄ O ₄ S ₂	364	210	72.6	52.74	3.84	15.38	52.4	3.71	15.57
11	3-chloro-4-fluorophenyl	C ₁₄ H ₈ ClFN ₄ O ₂ S ₂	382.5	205	70.1	43.92	2.09	14.64	44.08	2.22	14.78

Section II : Synthesis of 5/6-Nitro-3-{[substituted phenyl]-s-triazolo-(3,4-b)-benzothiazole} thione.

This part includes the preparation of 5/6-nitro-s-triazolo-(3,4-b)-benzothiazole-3-thione and synthesis of the title compounds.

Preparation of 5/6-nitro-s-triazolo-(3,4-b)-benzothiazole-3-thione

In a 500 ml conical flask was placed 5gm(0.05mole) conc. nitric acid (sp. gr.=1.42) and 7.4gm(4ml) of conc. sulphuric acid was added in portions with stirring. The mixture was kept cool during the addition by immersing the flask in ice bath. 11.34gm(0.045mole) of 3-mercapto-s-triazolo-(3,4-b)-benzothiazole was placed in 10ml conc. sulphuric acid and the above nitrating mixture was introduced in small quantities with constant stirring. The temperature was maintained between 0-10°C. After complete addition, the reaction mixture was allowed to stand for 1 hour. The reaction mixture was placed in 100 ml cold water to obtain the 5/6-nitro-s-triazolo-(3,4-b)-benzothiazole-3-thione. The liquid was decanted and the solid was boiled with 200 ml water. It was then filtered and dried. The product was recrystallised in ethanol.

Yield : 10.82(78%)

M.P. : 275°C

Elemental analysis of C₈H₄N₄S₂O₂

	C%	H%	N%
Calculated	38.09	1.58	22.22
Found	38.45	1.63	22.54

Synthesis of 5/6-Nitro-3-{[substituted phenyl]-s-triazolo-(3,4-b)-benzothiazole} -thione.

In a 50ml round bottomed flask, 0.504gm(0.002mole) of 5/6-nitro-s-triazolo-(3,4-b)-benzothiazole-3-thione was dissolved in 10 ml of dry 1,4-dioxane. A dilute solution of p-fluorobenzyl chloride, 0.289gm(0.002mole) in dry 1,4-dioxane was prepared and added to the above solution in presence of KOH. The mixture was heated under reflux on an oil bath for 3 hours. The reaction mixture was cooled and poured in water. The solid obtained was filtered and dried. It was recrystallised from methanol.

Yield : 0.504(70 %)

M.P. : 180°C

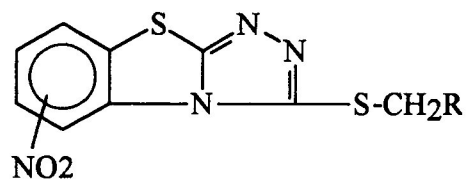
C,H,N analysis of compound $C_{15}H_9FN_4O_2S_2$

	C%	H%	N%
Calculated	50	2.5	15.5
Found	49.95	2.13	15.87

Similarly all other compounds were prepared and their physical data has been recorded in Table no. 2

TABLE NO. 2

5/6-Nitro-3-{{substituted phenyl }-s-triazolo-(3,4-b)-benzothiazole}thione



Sr. No	R	Molecular Formula	Molecular Weight	MP (°C)	Yield %	Elemental Analysis					
						Calculated			found		
						C	H	N	C	H	N
1	4-fluorophenyl	C ₁₅ H ₉ FN ₄ O ₂ S ₂	360	180	74.5	50.00	2.50	15.5	49.95	2.13	15.87
2	2-fluorophenyl	C ₁₅ H ₉ FN ₄ O ₂ S ₂	360	170	77.5	50.00	2.50	15.5	50.2	2.33	15.3
3	3,4-dichlorophenyl	C ₁₅ H ₈ Cl ₂ N ₄ O ₂ S ₂	410	240	70.3	43.9	1.95	13.6	43.6	1.87	13.93
4	4-trifluoromethylphenyl	C ₁₆ H ₉ F ₃ N ₄ O ₂ S ₂	353	175	72.0	54.39	2.54	15.86	54.1	2.39	15.48

PART III

Synthesis of 3-Hydrazino-N (substituted/ heterocyclic) methylene -s-triazolo-(3,4-b)-benzothiazole .

This part consists of the following sections.

- A. Preparation of s-triazolo-(3,4-b)-benzothiazole.
- B. Preparation of 3-chloro-s-triazolo-(3,4-b)-benzothiazole.
- C. Preparation of 3 hydrazino-s- triazolo-(3,4-b)-benzothiazole .
- D. Synthesis of the title compounds by condensation with substituted benzaldehydes.

A. Preparation of s- triazolo benzothiazole

In a round bottomed flask equipped with water condenser, 0.334 gm.(0.002mole) of 2-hydrazinobenzothiazole and excess of triethyl orthoformate were refluxed in an oil bath at 105°C for 4 hours. The reaction mixture was then cooled and dumped in ice-cold water. The product separated was filtered, dried and recrystallised from ethanol.

Yield : :0.28(80%)

M.P. :175⁰ C

(Lit⁷⁰. M.P. :175⁰ C).

B. Preparation of 3-chloro-s- triazolo benzothiazole.

In a 100ml flask, s-triazolo benzothiazole(1.75gm) was taken in chloroform: water mixture (2:1) and cooled to 5°C. A slow stream of chlorine gas was bubbled through the solution for one hour, while maintaining the temperature below 10°C. The excess solvent was then evaporated and the material was passed through silica gel column. It was eluted with chloroform.

Yield : 0.75gm (45.73%)

M.P. :164⁰ C.

(Lit. ⁷⁰M.P. :164⁰ C).

C. Preparation of 3- hydrazino s- triazolo benzothiazole.

In a 50 ml round bottomed flask equipped with a reflux condenser, 1.1 gm (0.05mole) of 3-chloro-s- triazolo benzothiazole and 0.8 ml (0.017mole) hydrazine hydrate were taken in ethanol and refluxed for 8 hours, using triethylamine. The excess ethanol was then removed under vacuum and the resultant mass was dumped in water. It was filtered, dried and recrystallised from methanol.

Yield : 0.64 gm.(60%)

M.P. : 205⁰ C.

Elemental analysis of C₈H₇N₅S

	C%	H%	N%
Calculated	46.82	3.41	34.14
Found	46.60	3.21	34.42

D. Synthesis of 3- hydrazino – N - (substituted / Heterocyclic) methylene- s- triazolo (3,4-b)-benzothiazole.

In a 50 ml round bottomed flask, a solution of 0.41gm,(0.002mole) of 3-hydrazino-s-triazolo-(3,4-b)- benzothiazole in warm acetic acid (10ml) was taken and a solution of 0.280gm (0.002 mole) of p-chlorobenzaldehyde in acetic acid was slowly introduced into it. This mixture was stirred at 100-110°C for one hour. The mixture was then cooled to separate the crude product. The product was filtered and dried. Addition of cold water to the mother liquor yielded more product. Both the solids thus obtained were recrystallised from ethanol.

Yield : 0.5 gm. (74.62%)

M.P. : 250⁰ C

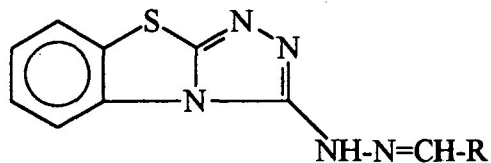
Elemental analysis of C₁₅H₁₀ClN₅S

	C%	H%	N%
Calculated	54.96	3.05	21.37
Found	55.21	3.34	21.58

In a similar manner, various substituted benzaldehydes and N-methyl pyrrole-2-carboxaldehyde were reacted with 3-hydrazino-s- triazolo-(3,4-b) benzothiazole to obtained the corresponding Schiff bases. The physical data of these compounds has been summarized in Table No. 3. (All the aldehydes used in the synthetic work were obtained from FLUKA Chemie AG.)

Table No. 3

3- hydrazino-N-(substituted/Heterocyclic)methylene-s-triazolo(3,4-b)-benzothiazole.



Sr. No	R	Molecular Formula	Molecular Weight	MP (°C)	Yield %	Elemental Analysis					
						Calculated			Found		
						C	H	N	C	H	N
1	4-chlorophenyl	C ₁₅ H ₁₀ ClN ₅ S	327.5	250	74.5	54.96	3.05	21.37	54.66	2.91	21.60
2	Phenyl,H:CH ₃	C ₁₆ H ₁₃ N ₅ S	322	236	77.5	66.33	4.54	22.72	66.56	4.31	22.68
3	2,4-dimethoxyphenyl	C ₁₇ H ₁₅ N ₅ O ₂ S	353	230	70.3	57.68	4.2	19.83	57.48	4.11	19.58
4	3,4,5-trimethoxyphenyl	C ₁₈ H ₁₇ N ₅ O ₃ S	383	230	72.0	56.39	4.43	18.27	56.71	4.56	18.56
5	4-trifluoromethylphenyl	C ₁₆ H ₁₀ F ₃ N ₅ S	361	244	66.2	53.11	2.07	19.39	53.35	2.21	19.46
6	N-Methyl pyrrole	C ₁₄ H ₁₂ N ₆ S	296	216	77.5	56.75	4.05	28.37	56.5	4.17	28.48

PART IV

This includes

- A. Synthesis of 3{[(2-thioureido-4-thiazolyl)methyl]thio}propionitrile as intermediate of Famotidine.
- B. Synthesis of sulfamide .

A. Synthesis of 3{[(2 – thioureido – 4 - thiazolyl) methyl] thio} propionitrile as intermediate of Famotidine.

The synthesis of 3{[(2-thioureido-4-thiazolyl)methyl]thio}propionitrile consists of the following steps -

- 1. Preparation of thiuronium salt of thiazole.
- 2. Preparation of 3{[(2-aminothiazolyl)methyl]thio}propionitrile.
- 3. Preparation of 3{[(2-thioureido-4-thiazolyl)methyl]thio}propionitrile .

1. Preparation of thiuronium salt of thiazole.

In a 250 ml round bottomed flask equipped with reflux condenser and dropping funnel, 15.2gm (0.2mole) of thiourea was suspended in 40 ml water : alcohol (9:1) mixture. The mixture was stirred and 12.7gm (0.1mole) of 1,3-dichloroacetone (previously dissolved in alcohol) was added to it over a period of 15min. The thiourea dissolved as the temperature increased. The yellow solution was refluxed for 6 hr. The excess solvent was evaporated and the solid obtained was washed with hot acetone. The white solid was obtained as the thiuronium salt of thiazole.

Yield : 18.64 gm(71.42%).

C,H,N analysis was carried out for confirmation of the compound.

C,H,N analysis of $C_5H_{10}Cl_2N_4S_2$.

	C%	H%	N%
Calculated	22.98	3.83	21.45
Found	23.29	3.43	21.63

2. Preparation of 3{[(2-aminothiazolyl)methyl]thio}propionitrile

In a round bottomed flask, 6.5 gm (0.025mole) of thiouronium salt of thiazole was placed in 20 ml water : dioxane mixture (50:50). A solution of 7gm(0.0125mole) of KOH was added dropwise in cold condition. To this, 3ml (0.03mole) of 3-chloropropionitrile was added and the reaction mixture was stirred for 4hr at room temperature. It was then evaporated to half the volume of the reaction mixture and extracted in chloroform. Chloroform layer was evaporated to yield the crude product, which on recrystallisation yielded 3.54gm (71.42%) of 3{[(aminothiazolyl)methyl]thio}propionitrile.

M.P. : 104⁰C

Reported⁶⁴ M.P. :104-106⁰C.

3. Synthesis of 3{[(2-thioureido-4-thiazolyl)methyl]thio}propionitrile

In a 50ml round bottomed flask, ^{10.73 ml}CS₂ (0.012mole) and 0.1gm NaOH (0.0025) mole were added to 1.99gm(0.01mole) of 3{[(aminothiazolyl)methyl]thio}propionitrile, dissolved in 50 ml ethanol. The reaction mixture was stirred at room temperature to dissolve NaOH. 5ml of ammonia solution was then added and the mixture was stirred for 5hr. and refluxed for 15hr. The excess ethanol was then removed under vacuum and the solid recrystallised from ethyl acetate.

Yield : 0.55gm (21.31%)

M.P. : 133 ⁰C

Lit⁶⁴. M.P. : 135⁰C

B. Synthesis of sulfamide.

In a two-necked round bottomed flask, 15ml of SO_2Cl_2 in 500ml of dried petroleum ether, was added dropwise to liquid ammonia at -30°C under vigorous stirring over a period of 10hrs. The mixture was allowed to reach room temperature and evaporated at 60°C to give the crude product. A solution of the crude product in 50 ml water was adjusted to pH 2.5 with HCl. This solution was then evaporated under vacuum at 40°C and the residue was extracted in acetone to give 70.0% sulfamide. (The yield was 35% without the acid treatment.) Recrystallisation from methanol offered pure sulfamide.

M.P. : 93°C

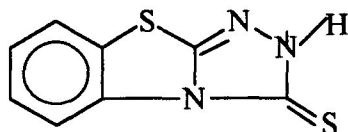
Lit¹²⁹. M.P. : 93°C

* All melting points ($^\circ\text{C}$) in experimental section were determined with open capillary method and were uncorrected

Spectral analysis of synthesised compounds

The IR spectra were recorded on a BOMEM Hartmann and braum FTIR in KBr pellets The ^1H -NMR were recorded with TMS as the internal standard.

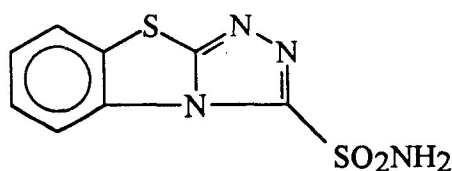
s-triazolo-(3,4-b)-benzothiazole-3-thione.



IR (KBr) cm^{-1}

3374	(N-H stretching)
1650	(C=N stretching of benzothiazole)
1502	(Triazolobenzothiazole)
1455	(C=C skeletal vibration of aromatic nucleus)
1068	(benzothiazole group)

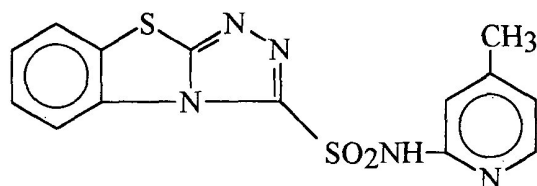
s-triazolo-(3,4-b)-benzothiazole-3-sulfonamide.



IR (KBr) cm^{-1}

3341	(N-H stretching)
1562	(N-H deformation of amino group)
1487	(Triazolobenzothiazole)
1440	(C=C skeletal vibration of aromatic nucleus)
1354	(sulfonamide S=O)
1172	(sulfonamide N-H)

3-N{[4-methyl 2-pyridenyl]-s-triazolo-(3,4-b)-benzothiazole }sulfonamide.



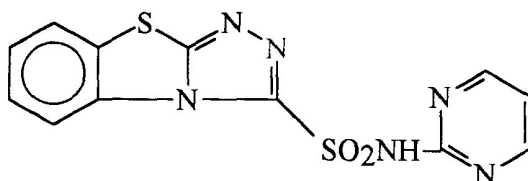
IR (KBr) cm^{-1}

3309	(N-H stretching)
1481	(Triazolobenzothiazole)
1631	(pyridine group)
2965	(C-H of methyl)
1462	(C=C skeletal vibration of aromatic nucleus)
1354	(sulfonamide S=O)

PMR(DMSO) δ :

2.5	(S,3H,-CH ₃),
8.5,6.4,	(3H-Pyridine)
13.1	(N-H)
7.68-8.05,	(4H-Aromatic)

3-N{[2-pyrimidenyl]-s-triazolo-(3,4-b)-benzothiazole }sulfonamide.



IR (KBr) cm^{-1}

3288	(N-H stretching)
1487	(Triazolobenzothiazole)
1680	(pyrimidine group)
1462	(C=C skeletal vibration of aromatic nucleus)
1372	(sulfonamide S=O)
1631	(C=N stretching)

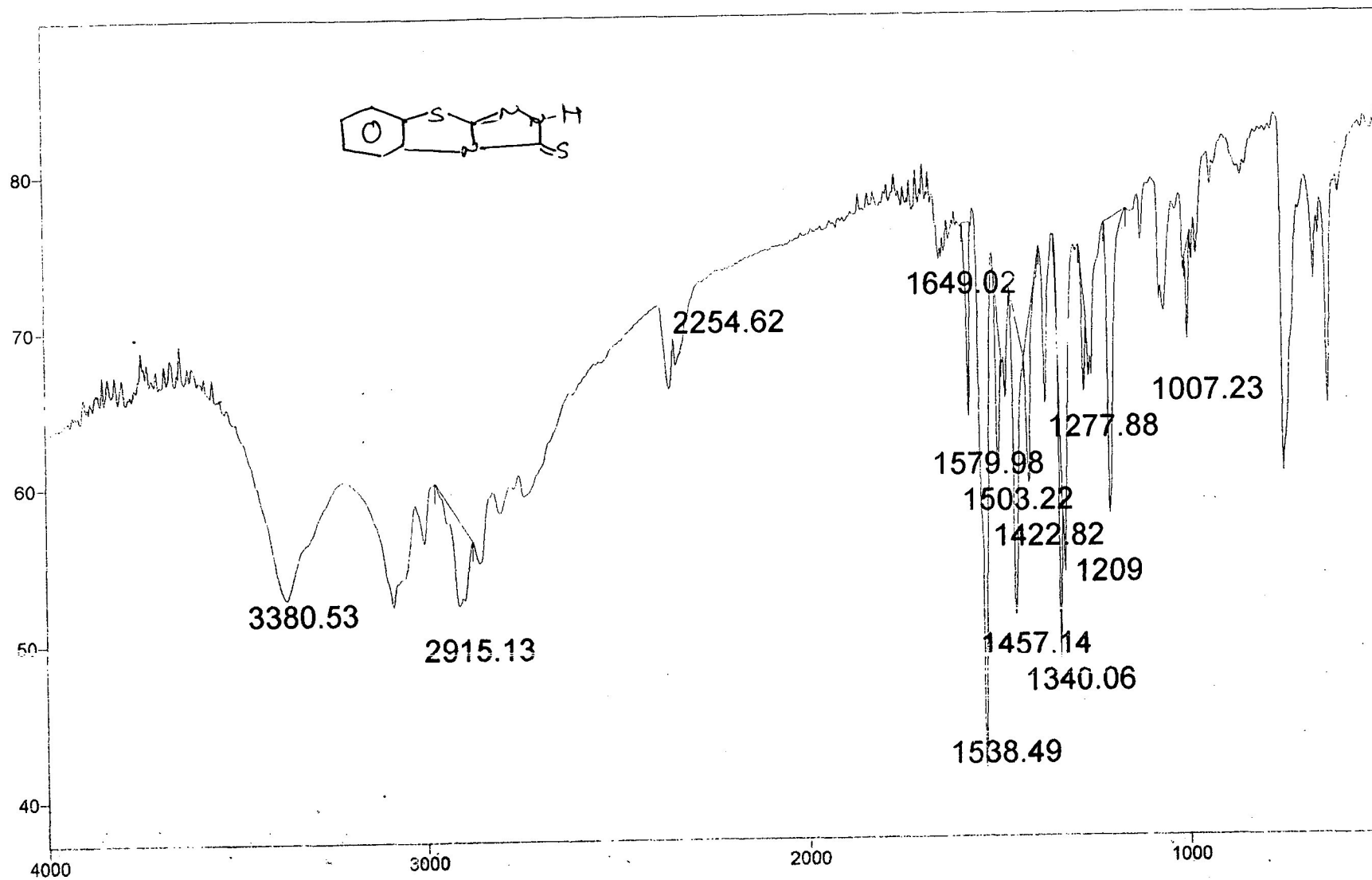
PMR(DMSO) δ :

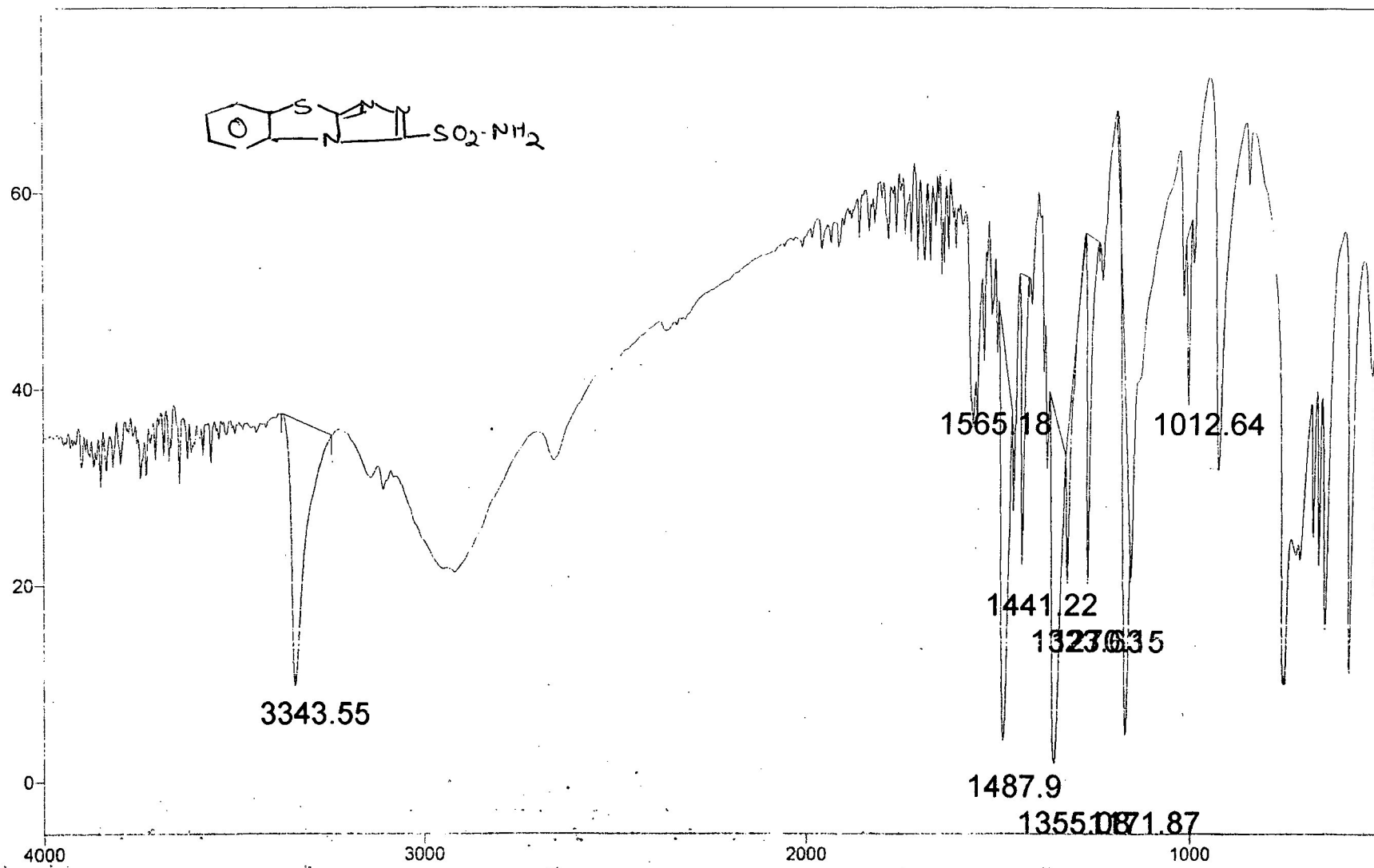
8.65,7.6,	(3H-Pyrimidine),
6.95-8.05,	(4H-Aromatic)

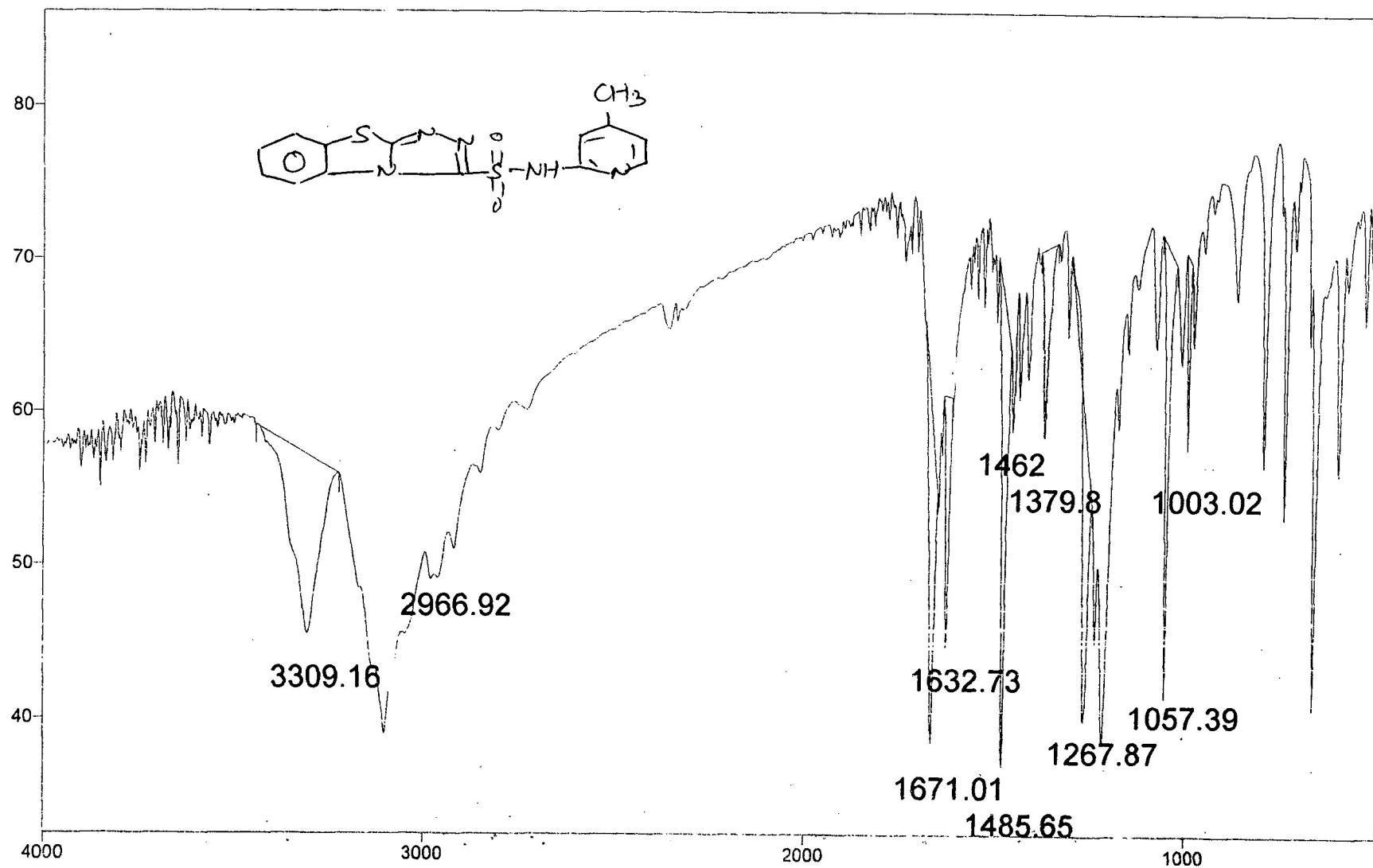
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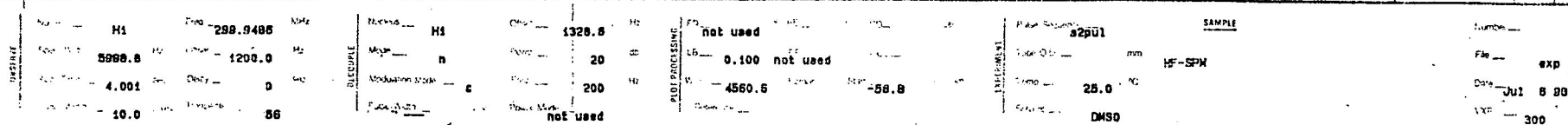
Res=4 cm-1

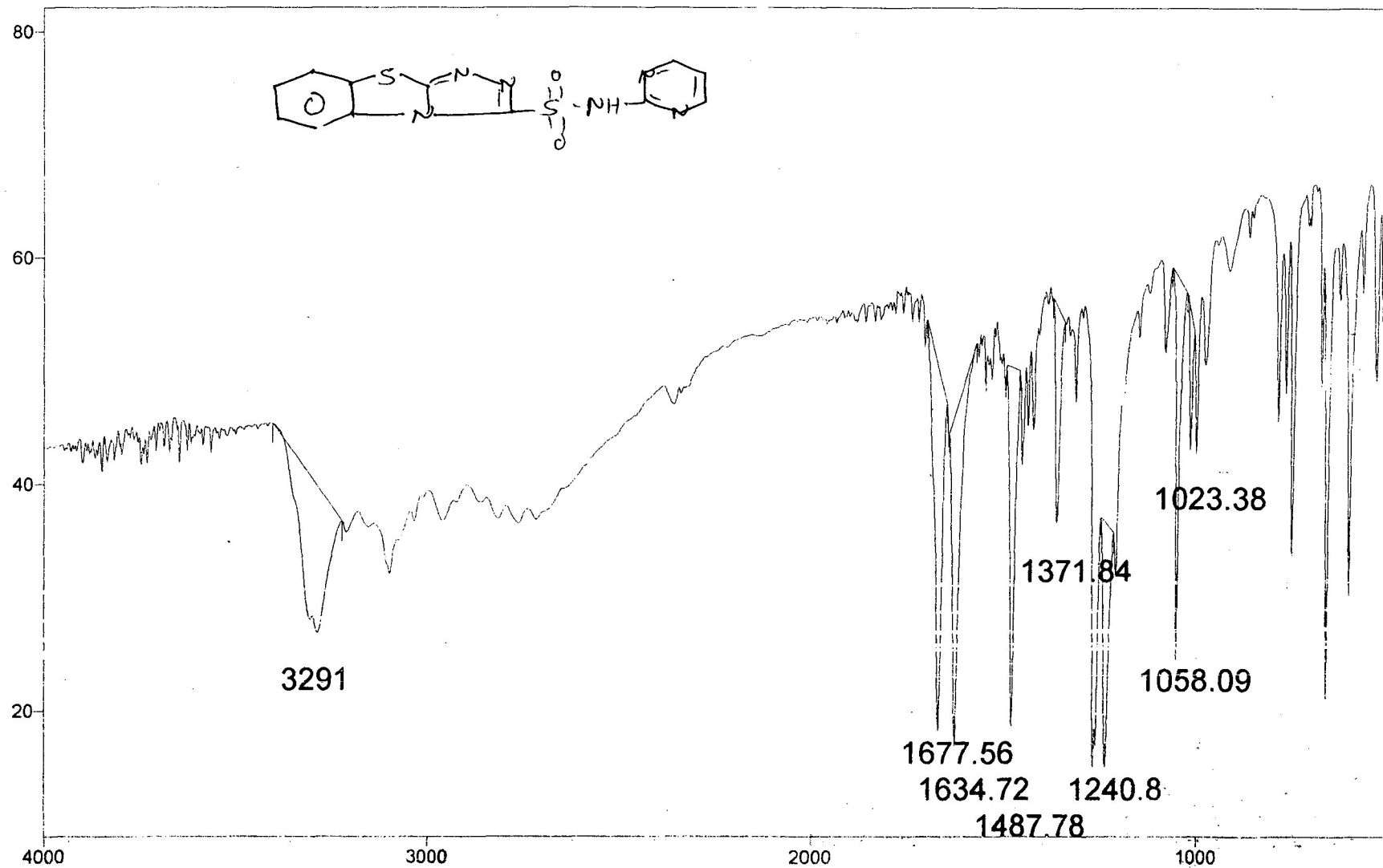
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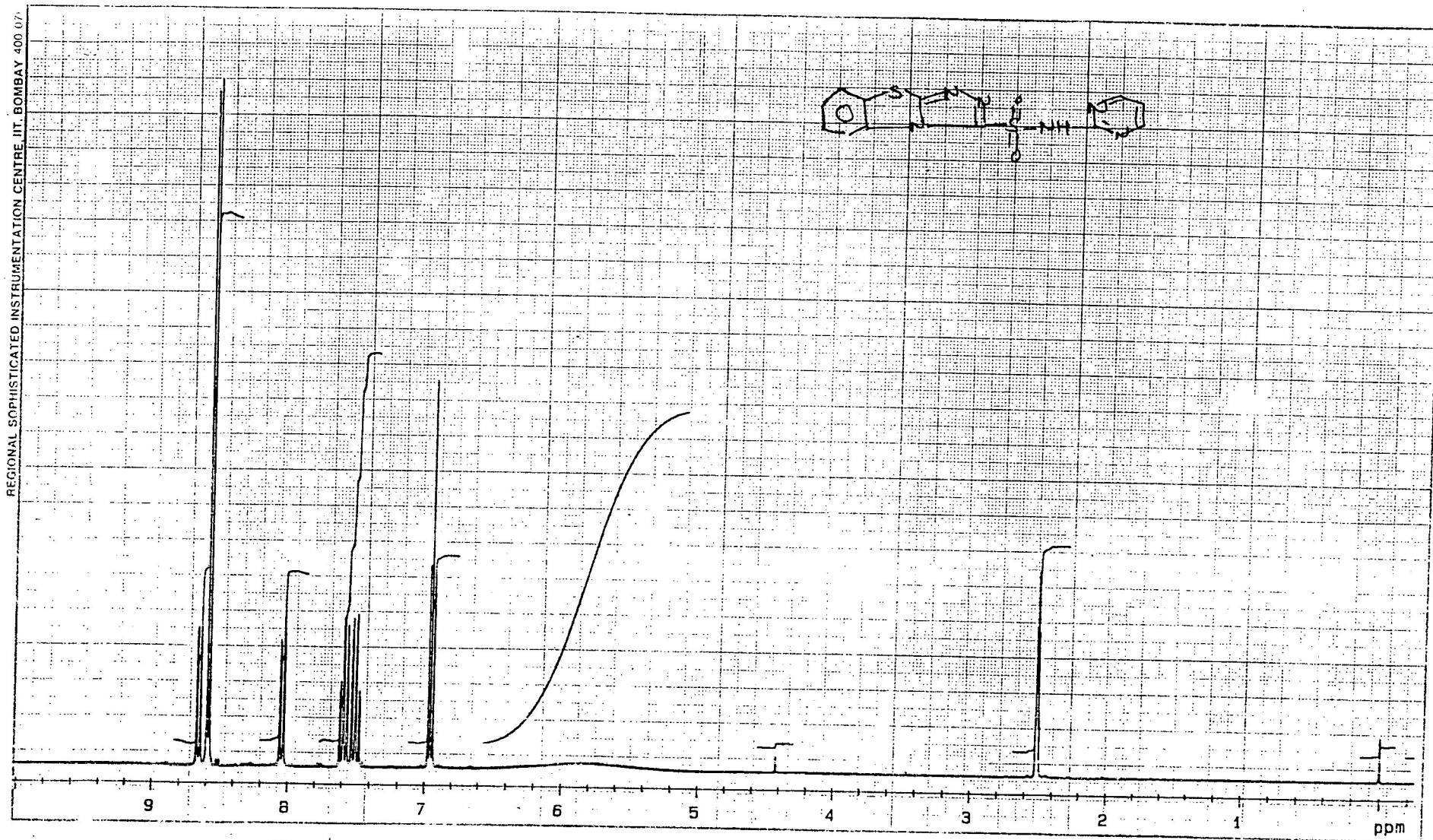












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H1
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 not used

not used
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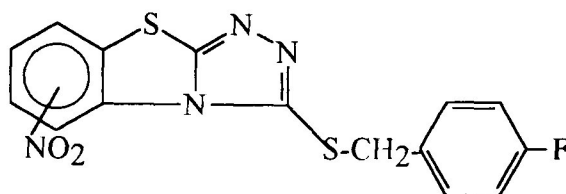
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820U1
 25.0
 DMSO

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 HF-SPY

exp
 Jul 5 88
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5/6-Nitro-3-{{4-fluorophenyl }-s-triazolo-(3,4-b)-benzothiazole}thione



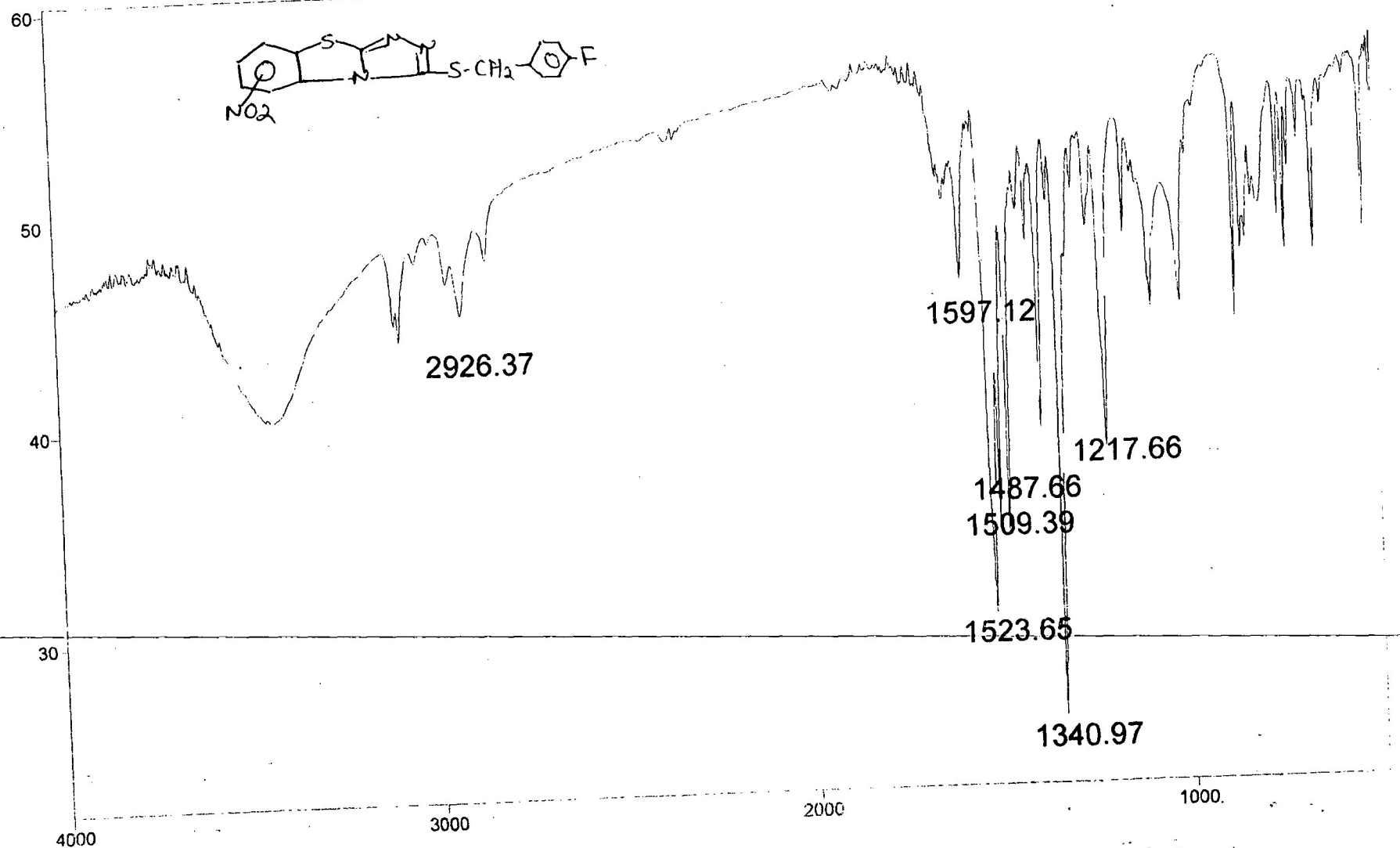
IR (KBr) cm^{-1}

1487	(Triazolobenzothiazole
1462	(C=C skeletal vibration of aromatic nucleus)
2926	(CH ₂ of benzyl group)
1518	(NO ₂ group)
1509	(Triazolobenzothiazole)
1598	(C=N stretching)

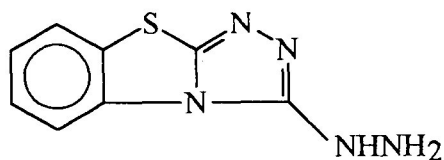
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Res=4 cm-1

1 : 4FSTBZ



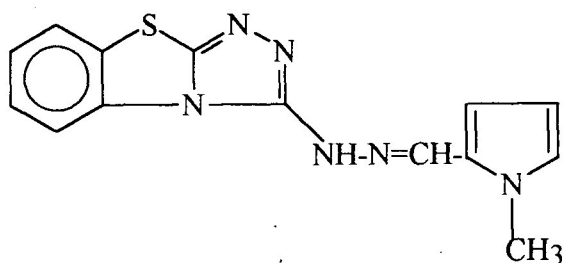
3- hydrazino -s-triazolo(3,4-b)-benzothiazole.



IR (KBr) cm^{-1}

3286,3388	(N-H deformation)
1507	(Triazolobenzothiazole)
1639	(NH ₂ deformation)
1566	(C=N, Triazolobenzothiazole)
1455	(C=C skeletal vibration of aromatic nucleus)
1313	(C-N,Stretching)

3- hydrazino-N-(N-methyl-pyrrole)methylene-s-triazolo(3,4-b)-benzothiazole.



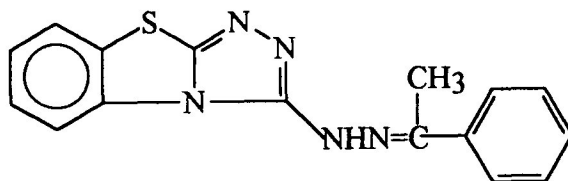
IR (KBr) cm^{-1}

3400	(N-H deformation)
1504	(Triazolobenzothiazole)
2924	(CH ₃ pyrrole)
1380	(C=N, pyrrolic)
1657	(C=N,azomethene)
1447	(C=C skeletal vibration of aromatic nucleus)
1307	(C-N,Stretching)

PMR(DMSO) δ :

- 1.92 , (CH₃),
- 6.07-6.43,- (3H-pyrrol),
- 8.2 (1-H,C-H)
- 7.4-8.05, (4H-Aromatic)
- 10.63(1H-N-H)

3- hydrazino-N-(1-methyl, 1-phenyl)methylene-s-triazolo(3,4-b)-benzothiazole.



PMR(CDCl₃) δ :

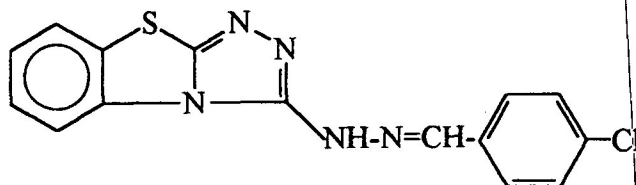
2.5 , (3H,CH₃),

7.6(1-H,C-H)

7.4,7.6,8.1, (4H,5H-Aromatic),

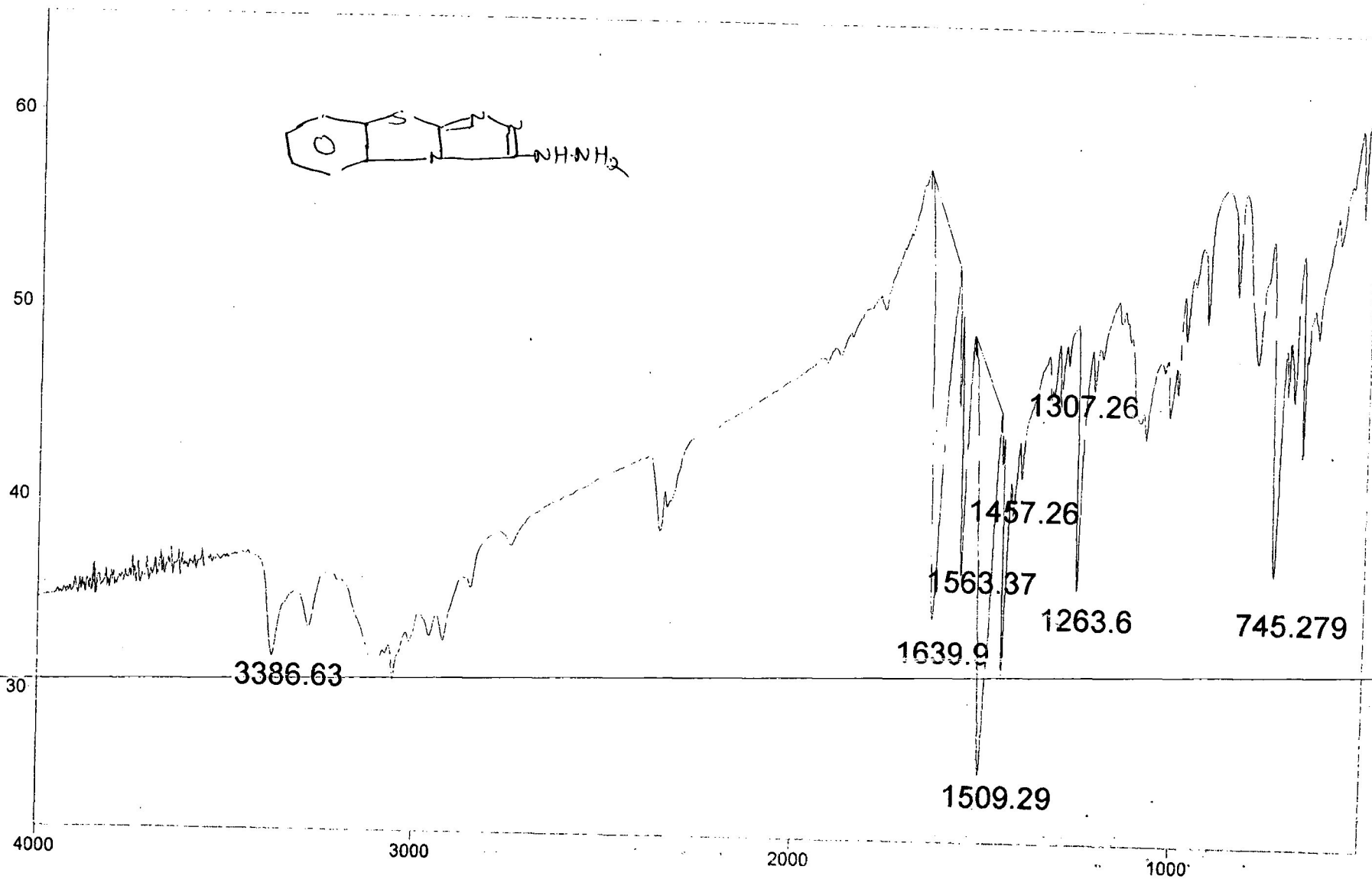
5.7(1H-N-H)

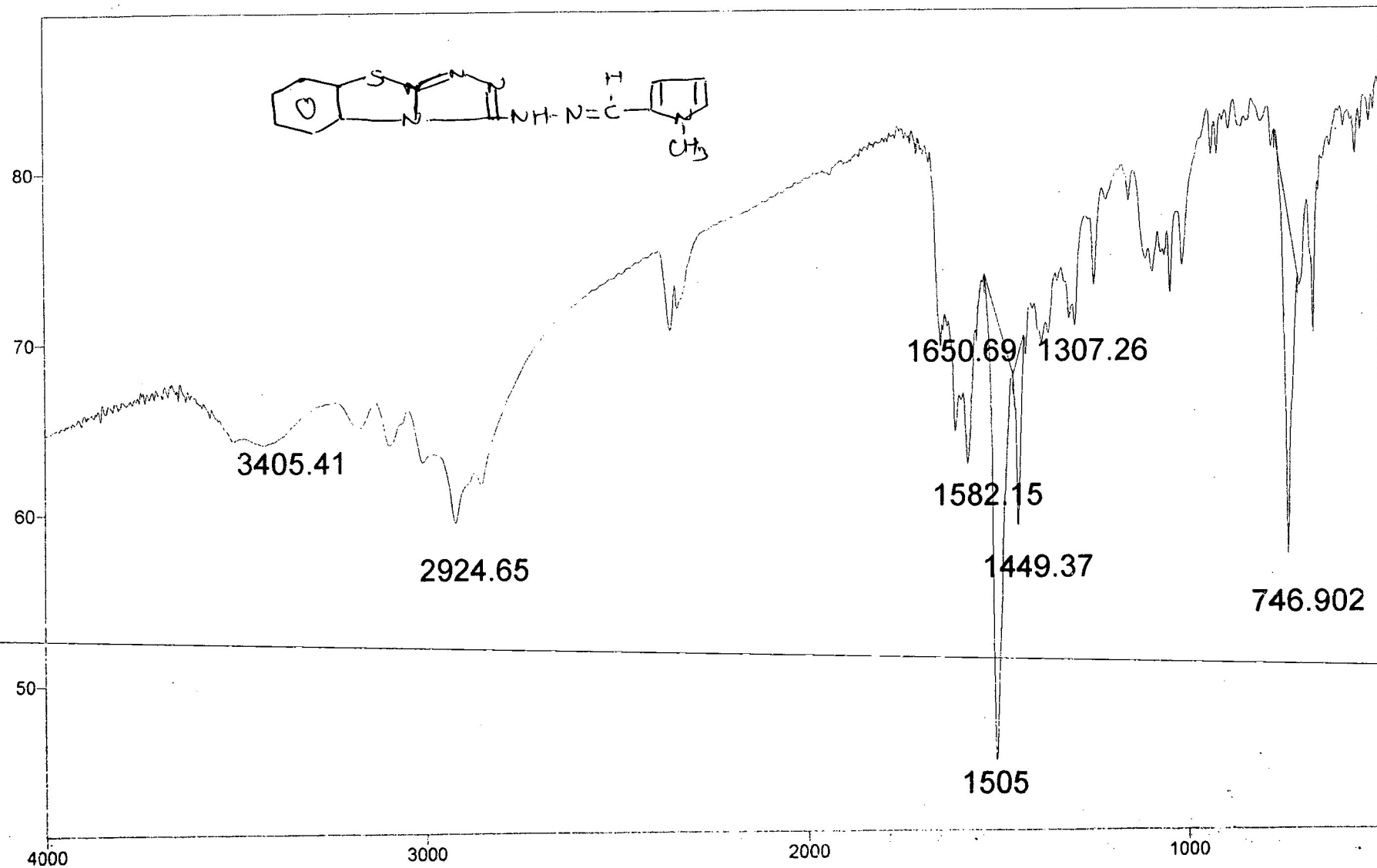
3- hydrazino-N-(4-chloro phenyl)methylene-s-triazolo(3,4-b)-benzothiazole.



IR (KBr) cm⁻¹

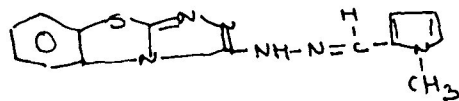
3411	(N-H deformation)
1501	(Triazolobenzothiazole)
1618	(C=N,azomethene)
1453	(C=C skeletal vibration of aromatic nucleus)
1318	(C-N,Stretching)





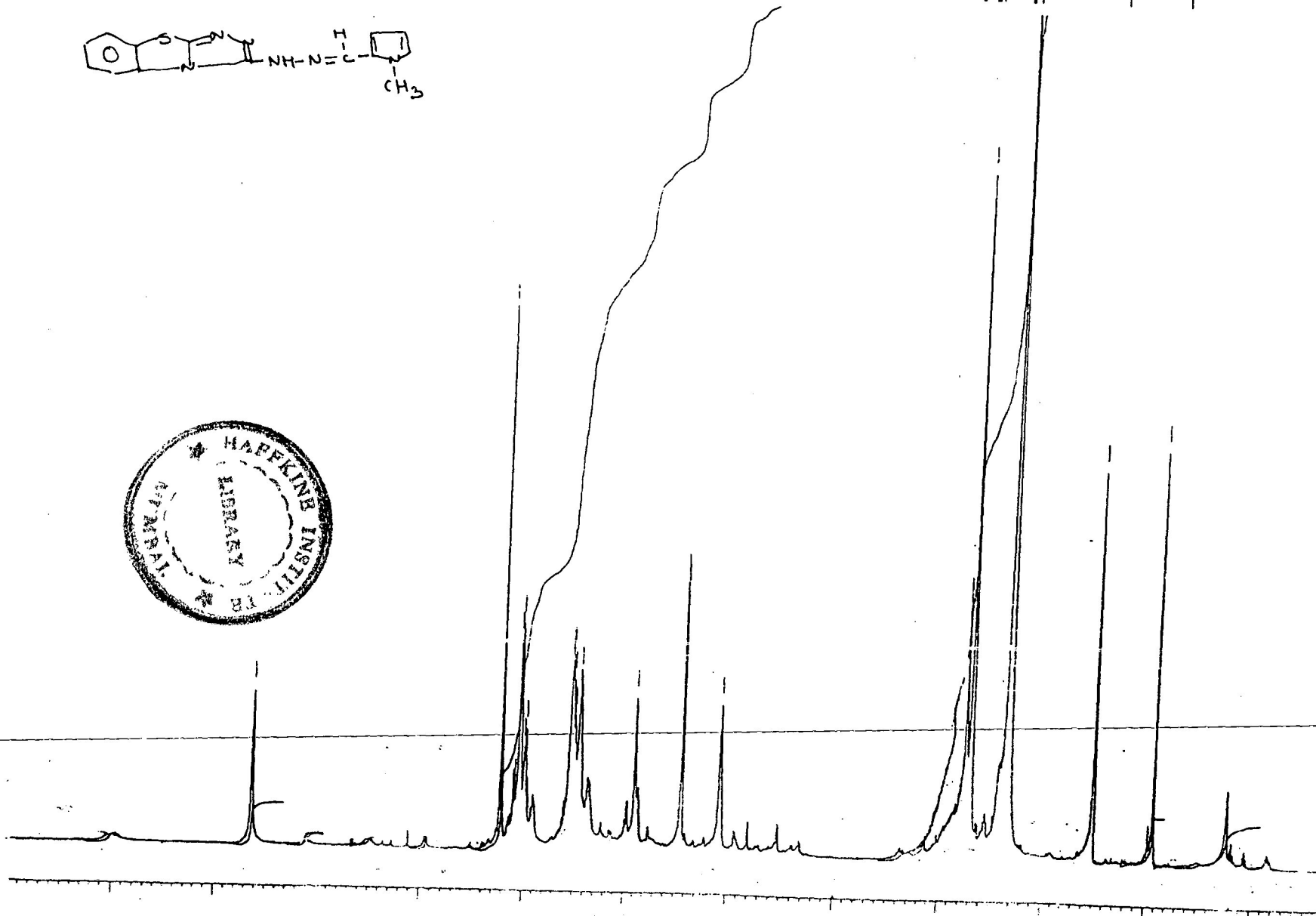
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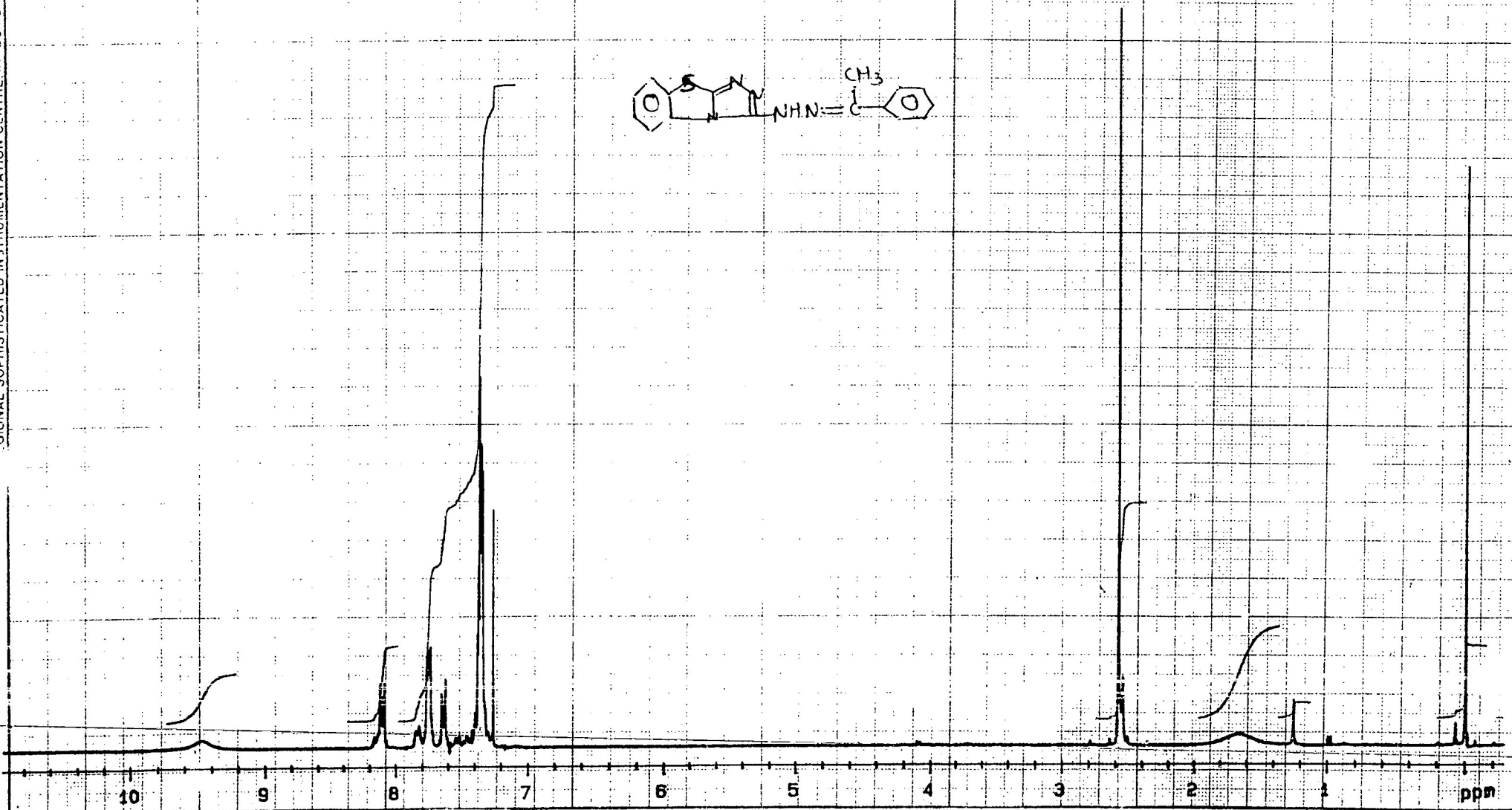
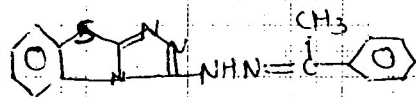
— 10.6



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8.05
8.03
8.00
7.98
7.56
7.53
7.51
7.50
7.46
7.45
6.90
6.44
6.43
6.07

3.89
3.88
3.77
3.76
3.75
3.73
3.70
3.42
3.42
3.40
3.34
2.50
1.92





100 MHz

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 Acq. Time **4.001**
 Pulse Width **10.0**

Freq **200.9480**
 Offset **1200.0**
 Delay **0**
 Transvers **104**

Decouple **H1**
 Modulation **n**
 Pulse **not used**

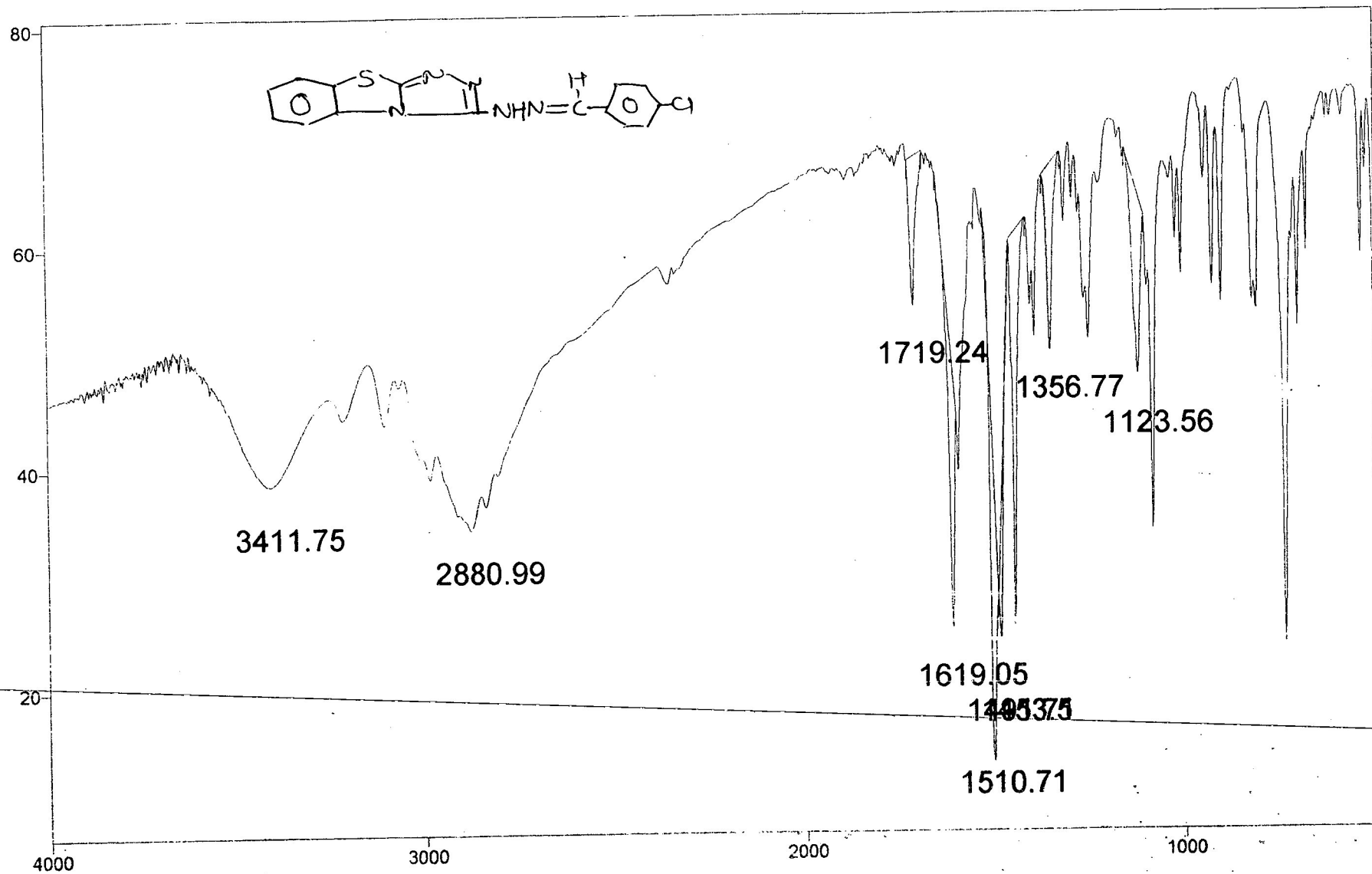
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not used
 not used
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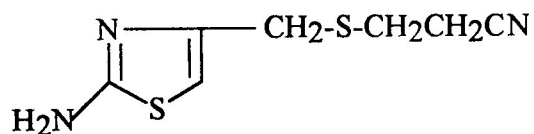
SAMPLE

SAP

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 21 2 83
 303



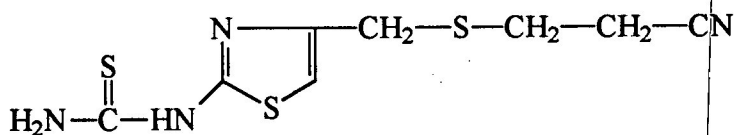
3-[(2-aminothiazolyl)methyl]thio]propionitrile



IR (KBr) cm^{-1}

3403	(N-H Stretching)
1619	(NH ₂ deformation)
2915	(CH ₂ deformation)
2250	(C≡N nitrile)
1533	(C=N thiazole)

3-[[[(thiouredothiazolyl) methyl]thio]propionitrile



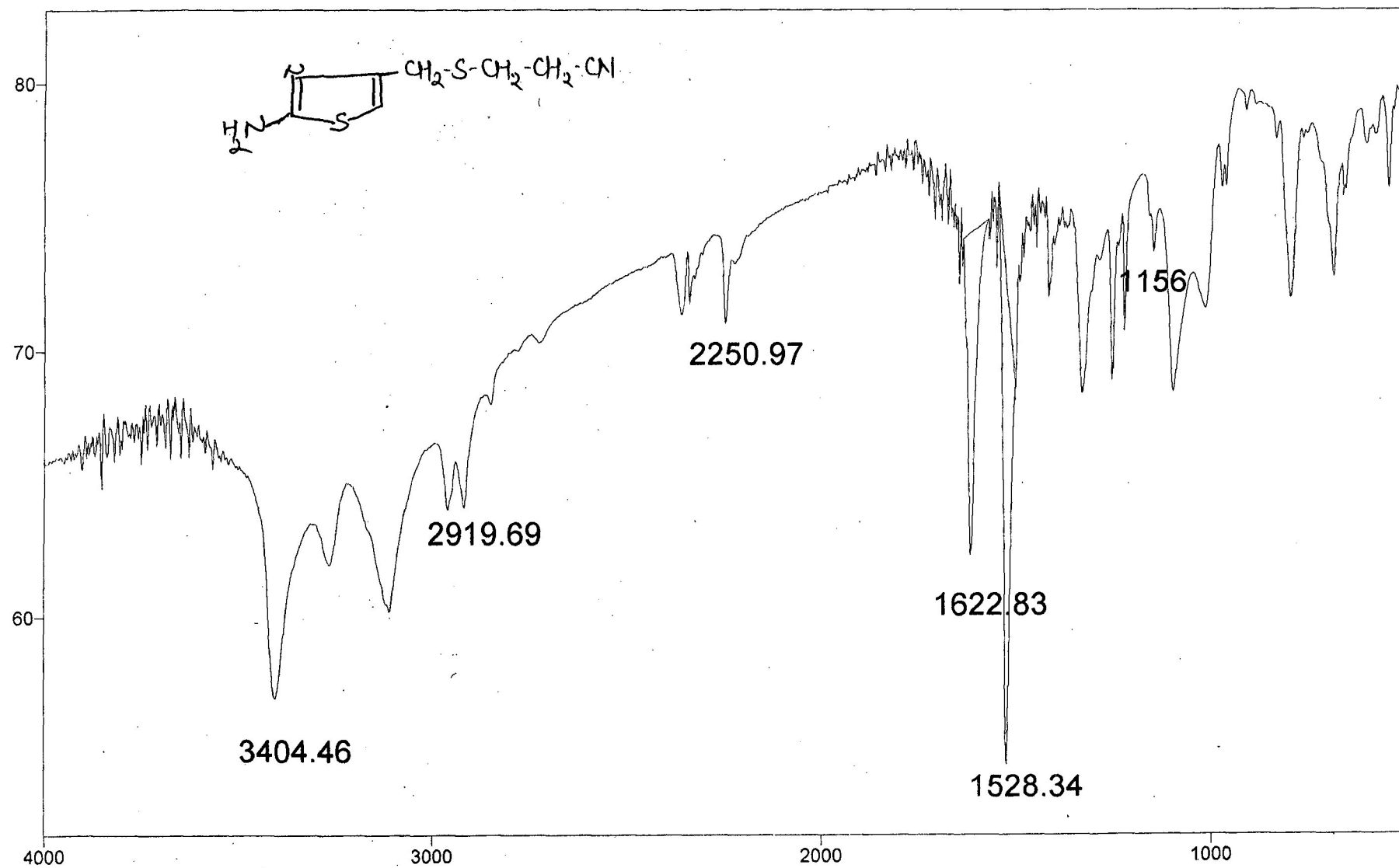
IR (KBr) cm^{-1}

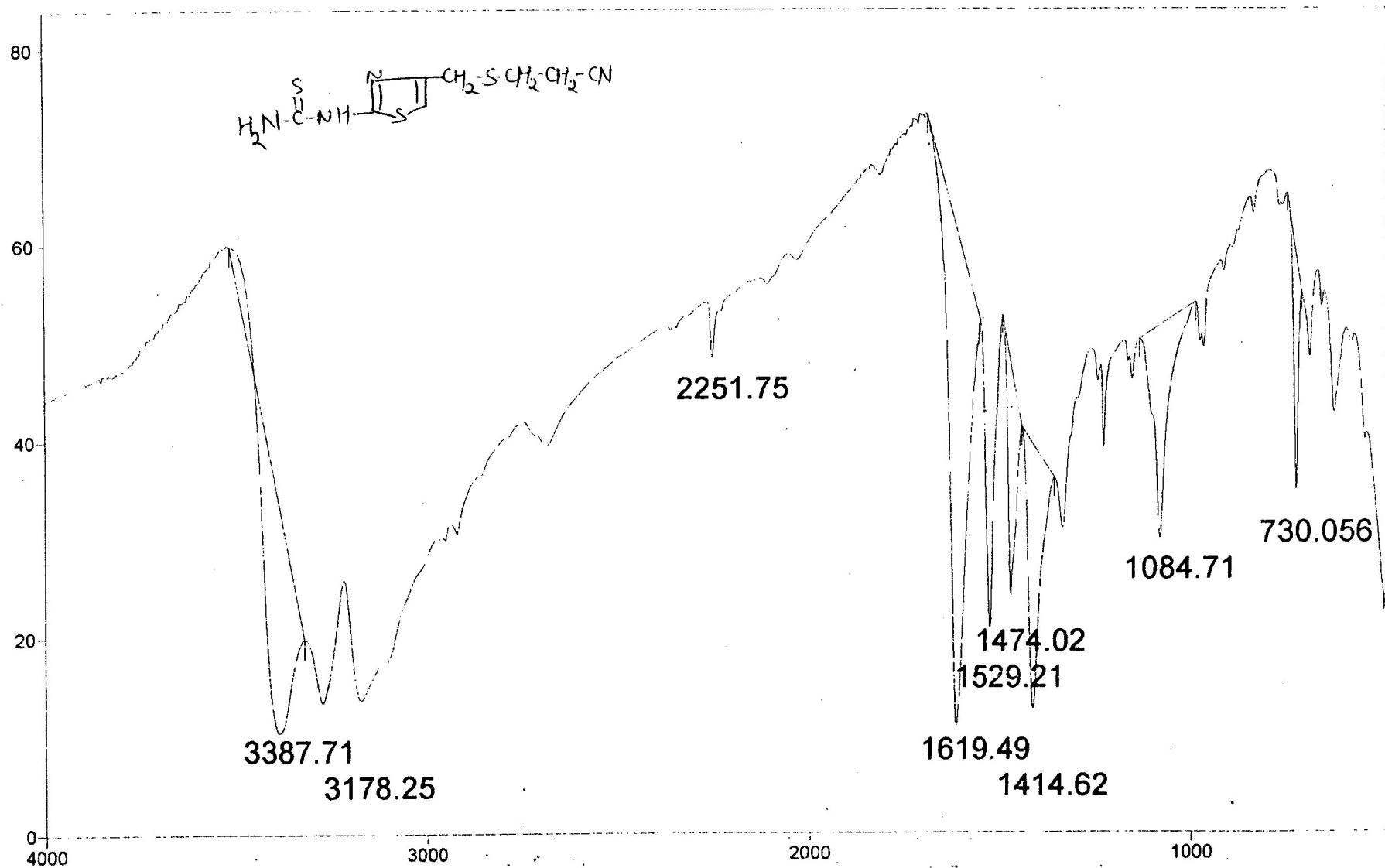
3387	(N-H Stretching)
2250	(C≡N nitrile)
1619	(NH ₂ deformation)
1529	(C=N thiazole)
1414	(C-N thiourea)
1084	(C=S thiourea)

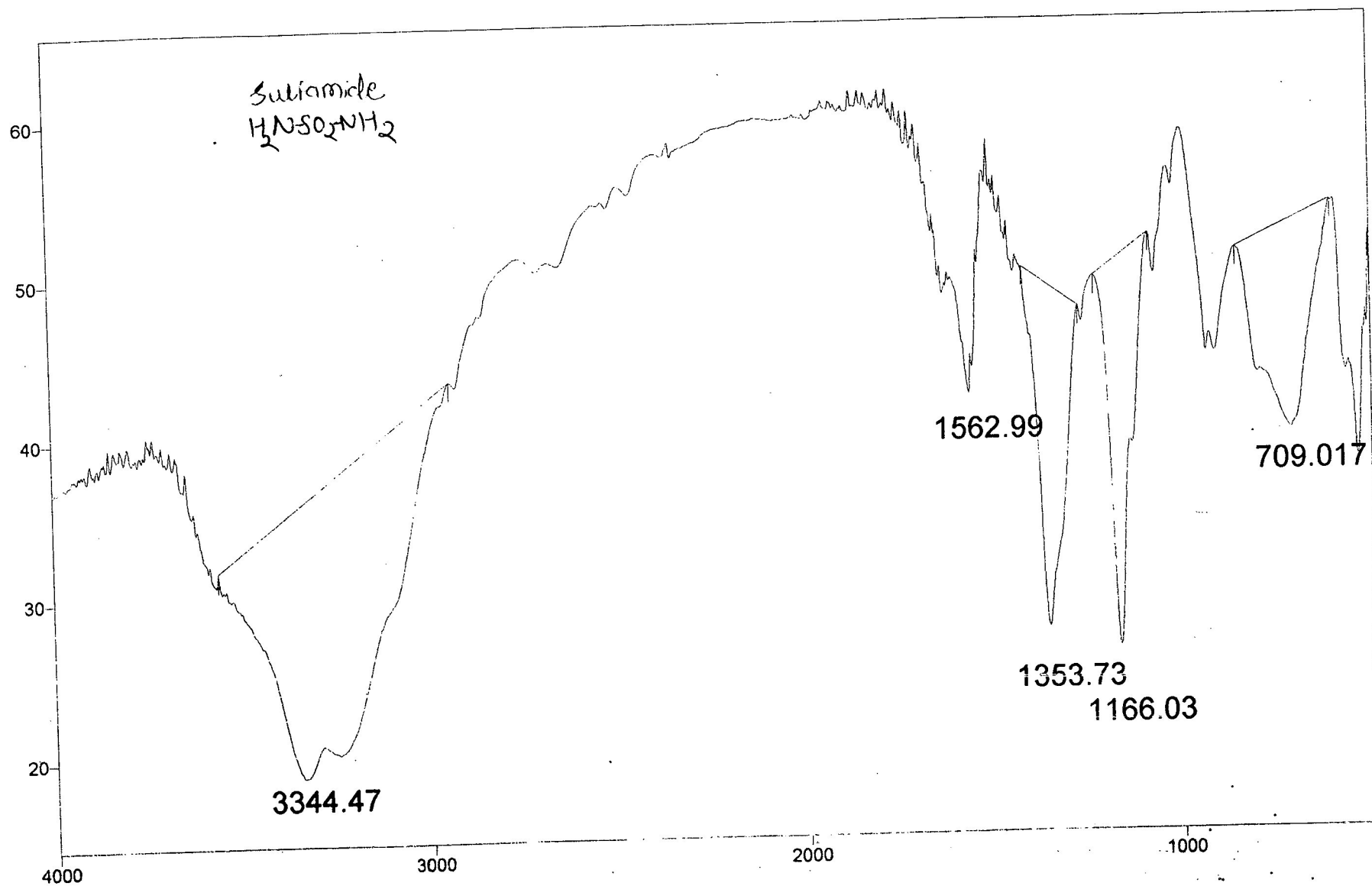
sulfamide

IR (KBr) cm^{-1}

3238	(N-H Stretching)
1564	(NH ₂ deformation)
1360	(sulfonamide S=O)
1168	(sulfonamide N-H)



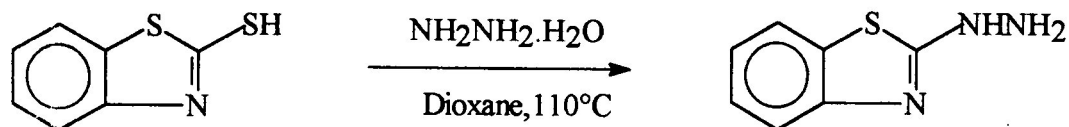




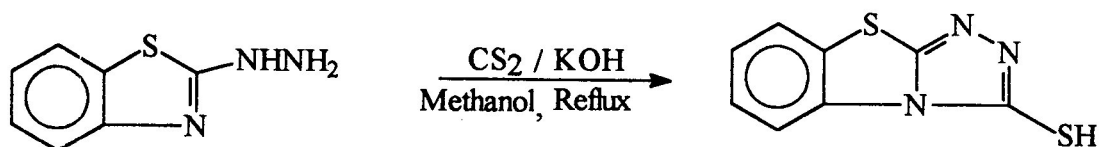
Scheme I

Synthesis of 3-N{[substituted heterocyclic/phenyl]-s-triazolo-(3,4-b)-benzothiazole} -sulfonamide.

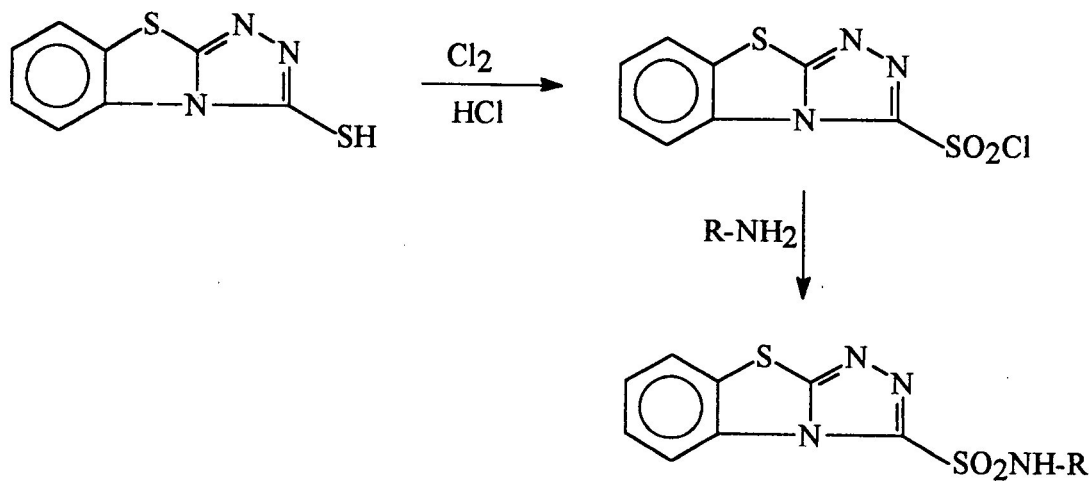
STEP 1



STEP 2



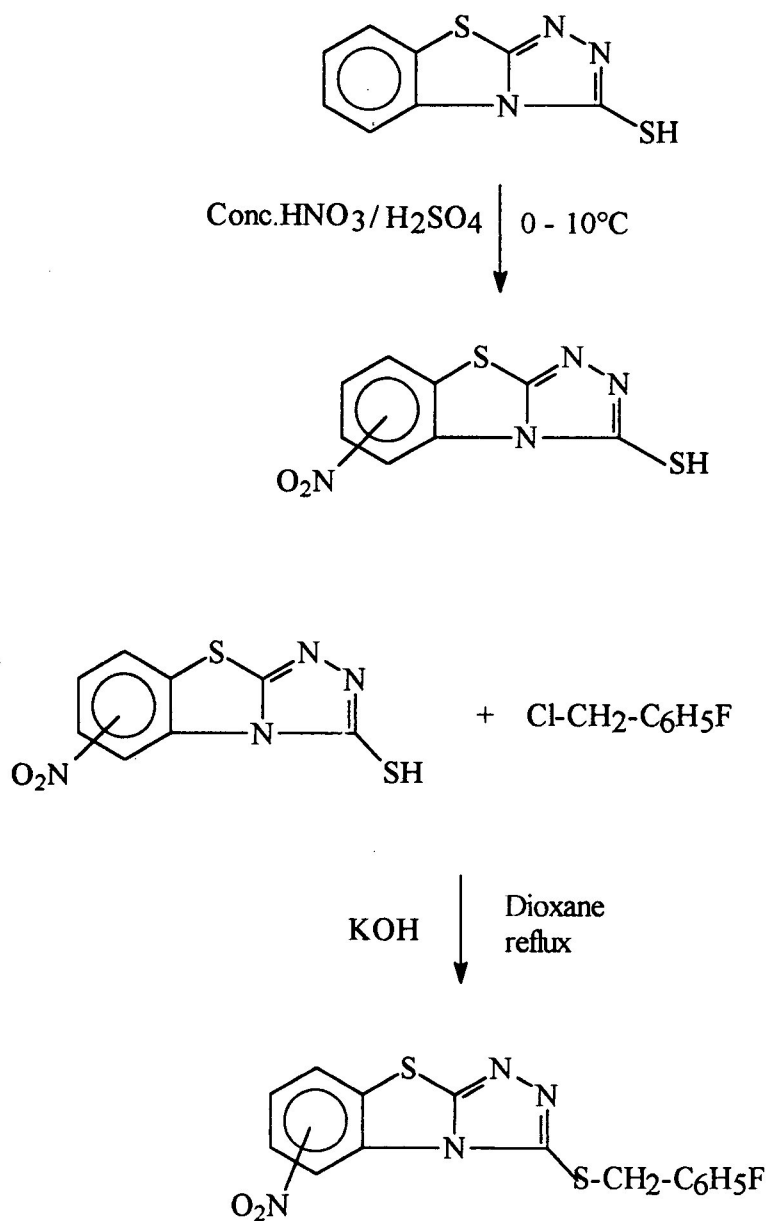
STEP 3



R : Heterocyclic/Phenyl

Scheme II

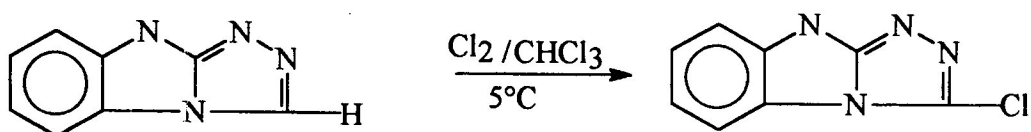
Synthesis of 5/6 - Nitro – 3 - {[substituted phenyl] - s - triazolo-(3,4-b)-benzothiazole} thione.



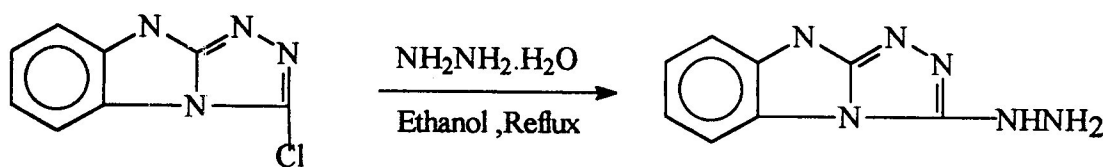
Scheme III

Synthesis of 3-Hydrazino-N (substituted/ heterocyclic) methylene -s-triazolo- (3,4-b)- benzothiazole

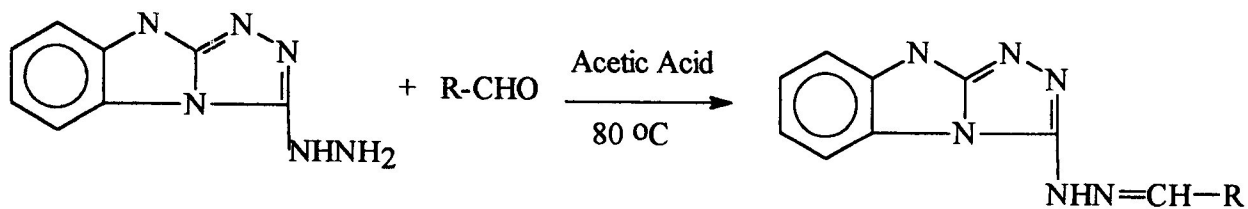
STEP 1



STEP 2



STEP 3



R :Substituted Phenyl

Biological Evaluation

BIOLOGICAL EVALUATION OF COMPOUNDS

The compounds synthesized in the present work have been subjected to various biological screening programmes. These compounds were screened for

- in vitro activity against H₃₇RV strain of *Mycobacterium tuberculosis*,
- in vitro study against Gram positive and Gram-negative bacteria viz. *Staphalococcus aureus* ATCC 3750 and *Salmonella typhi* NCTC 786, and
- in vitro study against various fungal strains, viz. *Candida albicans*, *Tricophyton mentagrophytes* and *Tricophyton rubrum*.

The methodology adopted for the various biological screening programs undertaken in this study has been described in the following section .

The test compounds (10 mg) were dissolved in a suitable solvent (10 ml) such as dimethylformamide, ethanol, dil. HCl as the case may be, so as to prepare a drug stock solution of concentration 1000µg/ml. From this stock solution following final drug concentrations were made - 200, 100, 40, 20 µg/ml. For lower final drug concentrations, the drug stock solution was diluted to prepare various stock solutions such as 10, 5, 2.5µg/ml etc.

CONTROLS

1. Media control : Sterilized medium was kept as media control so as to assure sterility of medium.
2. Organism control : It contains sterilized medium and organism. Organism should grow in the medium, which means that the medium is useable to support the growth.
3. Solvent control : This includes sterilized medium, solvent and culture. This acts as a control for the solvent used in testing.

ANTITUBERCULAR SCREENING :

The test compounds have been subjected to in vitro screening by tube dilution technique employing the human virulent H₃₇RV strain of *M. tuberculosis*. In this method, Kirchner's medium containing tween-80 as described by Rake et al¹³¹ 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 of 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* (H₃₇RV strain) 10⁶ bacilli/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 (MIC). Isoniazid was used as a standard drug and was also subjected to the 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 µg/ml. etc. At the end, inoculum of standard suspension, 0.1ml of the desired bacterial strain

(*S. aureus* or *S. typhi*) containing 10⁶ 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 µg/ml against *S. aureus* and that of Trimethoprim is 1µg/ml against *S. typhi*.

Results and discussion

Part II

Section I

In this section 12 novel compounds were synthesized . All these compounds along with starting material 3-mercapto-s-triazolo-(3,4-b) benzothiazole were subjected to anti microbial screening programs like antibacterial and anti-tubercular screening. This series consists of sulfonamido linkage.

Antibacterial activity

Antibacterial screening of all 12 compounds was carried out against Gram-positive and Gram-negative bacteria viz. *Staphylococcus aureus* ATCC 3750 and *Salmonella typhi* NCTC 786. All compounds showed moderate activity of 100µg/ml against *Staphylococcus aureus* and *Salmonella typhi*.

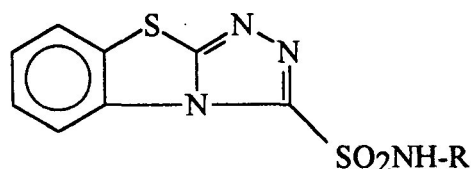
Antitubercular activity

In case of antitubercular screening, sulfonamide linkage showed increase in activity. In antitubercular screening following four compounds showed good activity.

1. 3-N-[(2-pyridinyl)-s-triazolo-(3,4-b)benzothiazole]-Sulfonamide
2. 3-N-[(4-methyl-2-pyridinyl)-s-triazolo-(3,4-b)benzothiazole]-Sulfonamide
3. 3-N-[(2-pyrimidinyl)-s-triazolo-(3,4-b)benzothiazole]-Sulfonamide
4. 3-N-[(4,6-dimethyl-2-pyrimidinyl)-s-triazolo-(3,4-b)benzothiazole] Sulfonamide

Compound no. 11 was the only compound found inactive at 200µg/ml against *M. tuberculosis*

Table no 4 : Biological activity of 3-N-[(substituted heterocyclic/phenyl)-s-triazolo-(3,4-b)benzothiazole]-Sulfonamide



Sr. No.	R	<i>Mycobacterium tuberculosis</i> (µg / ml)	<i>Staphylococcus aureus</i> (µg / ml)	<i>Salmonella typhi</i> (µg / ml)
1	H	10	100	100
2	2-pyridine	2	100	100
3	4-methyl-2-pyridine	1	100	100
4	5-chloro-2-pyridine	10	100	100
5	2,6 dimethyl-2-pyrimidine	2	100	200
6	5-nitro-2-thiazole	100	200	100
7	2-thiazole	100	100	100
8	4,6 dihydroxy-2-pyrimidine	50	200	100
9	2-pyrimidine	2	100	100
10	4-fluorophenyl	50	100	100
11	2,5-dimethyl phenyl	N.A.	100	100
12	3-chloro-4-fluorophenyl	50	100	100

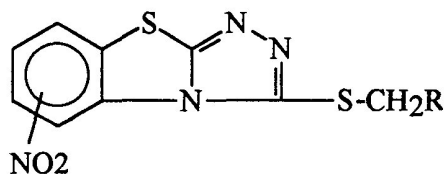
Section II

In this section 4 compounds were prepared , which contain s-benzyl linkage and nitro group attached to the aromatic ring. The anti bacterial activity and antimycobacterial activity of these compounds was determined.

In case of antibacterial activity, all four compounds showed moderate activity, i.e. 100µg/ml activity against *Staphylococcus aureus* and *Salmonella typhi*.

In case of antitubercular activity all four compounds showed good activity in the range of 1-20µg/ml. The most active compound was 3[(4-fluorobenzyl)thio]-s-triazolo-(3,4-b) benzothiazole, which inhibited *M. tuberculosis* at 1.0µg/ml.

Table no 5 : Biological activity of 3[(substituted benzyl)thio]-s-triazolo-(3,4-b) benzothiazole



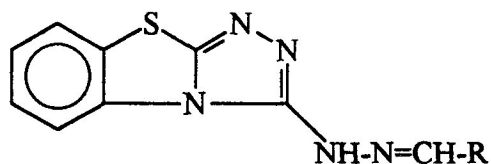
Sr. No.	R	<i>Mycobacterium tuberculosis</i> (µg / ml)	<i>Staphylococcus aureus</i> (µg / ml)	<i>Salmonella typhi</i> (µg / ml)
1	4-fluorophenyl	1	100	100
2	2-fluorophenyl	10	100	100
3	3,4-dichlorophenyl	10	100	100
4	4-trifluoromethylphenyl	20	100	100

PART III

In this part 6 novel compounds were synthesized along with the starting material 3-hydrazino-s-triazolo-(3,4-b) benzothiazole, and their antibacterial and anti tubercular activity testing was carried out. All six compounds showed moderate activity of 100µg/ml against *S. aureus*, *S. typhi* and *M. tuberculosis*.

The activity of individual compounds with their substitutions are given in the table below.

Table no 6 : Biological activity of 3- hydrazino-N-(substituted/Heterocyclic)methylene-s-triazolo(3,4-b)-benzothiazole.



Sr. No.	R	<i>Mycobacterium tuberculosis</i> (μg / ml)	<i>Staphylococcus aureus</i> (μg / ml)	<i>Salmonella typhi</i> (μg / ml)
1	4-chlorophenyl	100	100	100
2	Phenyl	100	100	100
3	2,4-dimethoxyphenyl	100	100	100
4	3,4,5-trimethoxyphenyl	200	100	100
5	4-trifluoromethylphenyl	100	100	100
6	N-methylpyrrole	100	100	100

CONCLUSION

The present work was undertaken for the synthesis of novel heterocyclic compounds of possible anti-tubercular and anti-bacterial activity.

Part II deals with the synthesis of sulfonamide and S-benzyl linkage of S-triazolobenzothiazole-3-thione. The anti-microbial screening reveals the following points :

- The presence of sulfonamide linkage on the present moiety has shown excellent increase in anti-tubercular activity of the parent compound, 3-triazolobenzothiazole-3-thione.
- The different sulfonamides synthesized in section - (I) showed deviation in activity. Close observation of the final compounds showed that the sulfonamide with aminopyridine, aminopyrimidine and its relative compounds has considerable impact on anti-tubercular activity. At the same time, sulfonamides with aromatic amines showed decreased activity than the parent moiety, S-triazolobenzothiazole. In case of sulfonamide of thiazole and substituted thiazole, there is an unexpected decrease in activity against *M. tuberculosis*.
- In case of compounds with S-benzyl linkage reported in section - (II), the activity is retained, and a few compounds show an increase in the anti-tubercular activity.

This may be due to the S-benzyl linkage on S-triazolobenzothiazole-3-thione and also due to the nitro group attached to the benzene nucleus on the principal moiety.

4- fluoro substituents seem to be more favorable for anti-tubercular activity.

- In case of anti-bacterial activity, there is a higher increase or decrease in the activity against *S. aureus* and *S. typhi*. All the compounds showed moderate activity of 100 µg/ml in both sulfonamide and S-benzyl linkage of S-triazolobenzothiazole.
- In part III, the synthesis of 3-hydrazino-(N-substituted phenyl/N-methyl/ pyrrole) methylene- S-triazolobenzothiazole has been described. From various screening programs following observations are made.

The presence of hydrazinomethyl linkage in 3-hydrazino-S-triazolo(3,4-b) benzothiazole has no considerable impact on either anti-bacterial or anti-mycobacterial activity. All the 6 compounds in this series showed moderate activity of 100 µg/ml and hence, it is concluded that the hydrazino methane linkage does not have any special impact on anti-bacterial and anti-tubercular activity.

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

TITLE OF THE THESIS : Synthesis of organic compounds
of possible biological activity.

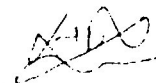
NAME OF THE CANDIDATE : Mr. Sandeep D. Paranjape.

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

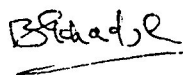
PLACE OF RESEARCH : Department of Chemotherapy,
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**NUMBER AND DATE
OF REGISTRATION** : 26/ Feb. 18, 1997

SIGNATURE OF THE STUDENT:



SIGNATURE OF THE GUIDE



The increasing clinical importance of drug resistant bacterial pathogens has lent additional urgency to microbiological and antibacterial research . As pointed out in a number of reviews, there have been relatively rapid and dramatic changes in antibiotic resistance patterns in both, community and institutional setting, which is likely to have considerable impact on the therapeutic strategies for treatment of bacterial infections . Most worrisome as a public health threat, is the recent increase in the incidence of tuberculosis caused by multidrug resistant strains of *Mycobacterium tuberculosis*¹.

The WHO has estimated that within the next ten years, 30 million people worldwide will die of tuberculosis². At present INH, Streptomycin, Rifampin etc. are the drugs of choice in different combinations in the treatment of tuberculosis. Now a days because of multidrug resistant strains of *M. tuberculosis* and also due to AIDS, tuberculosis has once again become a major health problem in India as well as world wide. Therefore, there is an equally urgent need for new research approaches to help combat the spread of tuberculosis and other microbial infections.

Thus, keeping in mind the above facts, efforts have been made to synthesise heterocyclic compounds and will be documented in the thesis. The thesis will also contain the synthesis of intermediate of Famotidine. Famotidine is a H₂ antagonist and has the potential to substitute Cimetidine, Ranitidine.

The thesis will consist of the following parts -

Part 1 - Introduction, Review of literature and plan of work.

Part 2 - Synthesis of sulfonamide and S-benzyl derivative of substituted/unsubstituted s-triazolo-(3,4-b)-benzothiazole.

Part 3 - Synthesis of 3-hydrazino-N-(substituted/heterocyclic) methylene-s-triazolo-(3,4-b)-benzothiazole.

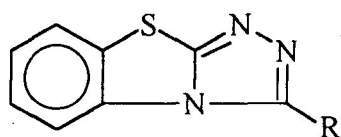
Part 4 - Synthesis of 3-[[[2-thioureido-4-thiazolyl)methyl]thio}propionitrile as intermediate of Famotidine.

Part 5 - Biological screening.

Part 2

Synthesis of sulfonamide and S-benzyl derivative of substituted/unsubstituted-s-triazolobenzothiazole.

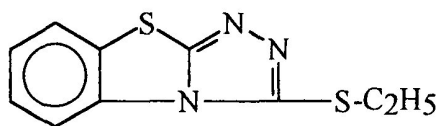
In the literature, triazolobenzothiazole and its derivatives exhibited potent anti-tubercular³ and anti-bacterial activity⁴. 3-substituted-s-triazolo-(3,4b)benzothiazole³(I) (R=H,OH, NH₂, SH, COOH, COOEt, etc) prepared by Gvozdijakova, A. *et al*⁵ showed anti-mycobacterial activity. Of these compounds, the derivative with R= SH was found to be the most active analog in vitro.



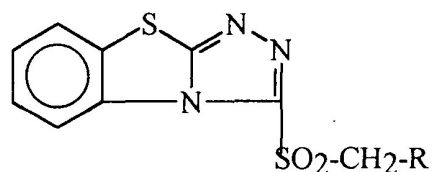
(I)

R= SH, H, NH₂, etc

s-alkyl (II) and sulphone (III) derivatives of triazolobenzothiazole⁶ were found to be more active anti-tubercular agents as compared to the derivatives synthesised by Gvozdiakova, A. *et al.* This is thought to be due to the s-alkyl linkage introduced into the molecule.

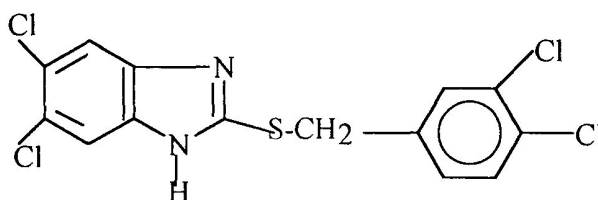


(II)



(III) R=phenyl

In our laboratory a number of 2-thiobenzimidazole derivatives were prepared and tested for their antitubercular activity, of which 5,6-dichloro-2-(3,4 dichlorobenzyl) thiobenzimidazole (IV) showed antitubercular activity at the concentration of 3.12 μ g/ml⁷.



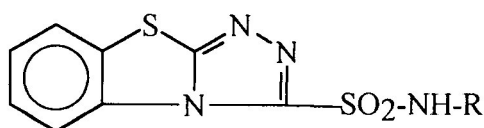
(IV)

Thus, considering the importance of s-benzyl linkage, the work in this line was continued.

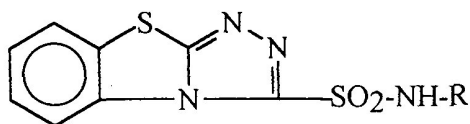
In 1932 Domagk⁸ observed that Prontosil protected mice against *Streptococcal* infection and rabbits against *Staphylococcal* infection. The effect of Prontosil was most probably due to its in vivo conversion to the active metabolite, Sulfanilamide. Hence, sulphonamides were introduced in the treatment of bacterial infections. They still continue to be an important class of anti-microbial agents. These drugs are commonly used in preventing *Streptococcal* infections and rheumatic fever. Sulfonamides are broad spectrum, predominantly bacteriostatic and relatively slow acting analogs of p-aminobenzoic acid (PABA).

A combination of Sulphamethoxazole and Trimethoprim has been found to be useful, both for prophylaxis and treatment of *Pneumocystis carinii* pneumonia in patients with AIDS.

Hence, considering the importance of the s-benzyl, sulphone and sulphonamido linkages, we decided to synthesise heterocyclic sulfonamides (V) and s-benzyl derivatives of s-triazolo(3,4-b) benzothiazole-3-thione (VI).



(V) R- Heterocyclic, substituted phenyl



(VI) R=2-fluorophenyl, 4-fluorophenyl,
3,4-dichlorophenyl, 4-trifluoromethylphenyl,
R' = NO₂

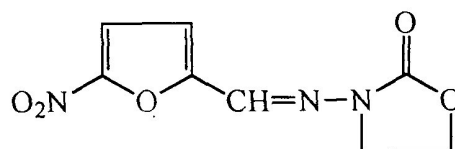
The synthesis of all sulphonamides and s-benzyl derivatives of 3-mercapto triazolobenzothiazole was confirmed by IR spectroscopy and NMR spectroscopy. Elemental analysis of the compounds was also carried out. They were then subjected to biological screening.

Part 3

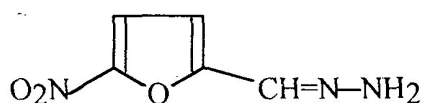
Synthesis of 3-hydrazino-N-(substituted phenyl/heterocyclic)methylene-s-triazolo (3,4-b) benzothiazole.

Schiff bases have proved their advantages in a number established drugs as well as in a number of anti-microbial compounds. Schiff bases from 5-chlorosalicylaldehyde, 5-bromosalicylaldehyde have been reported to have encouraging tuberculostatic activity⁹. The presence of azomethine linkage in the compound has also been known to decrease toxicity considerably¹⁰.

Many of the biologically active compounds, such as synthetic anti-bacterials like Nitrofurantoin(VII), Nitrofurazone(VIII) were used as antibacterial and anti-infective agents, respectively. These compounds contain azomethine linkage which enhanced antimicrobial activity.

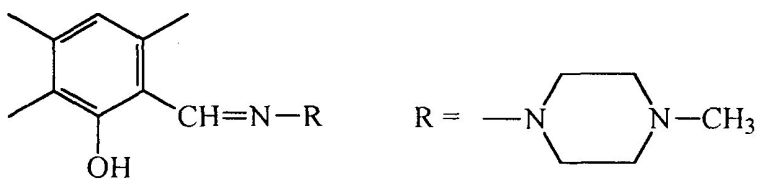


(VII) Nitrofurantoin



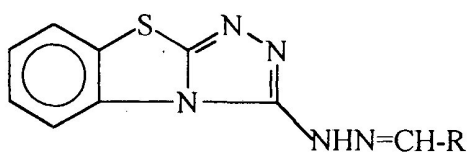
(VIII) Nitrofurazone

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



(IX) Rifampin

Considering this activity and the importance of hydrazino-methine linkage as in Nitrofurazone, it was proposed to synthesise hydrazino-methine linkage of 3 hydrazino s-triazolobenzothiazole, by condensing it with different halogenated benzaldehydes (X). Structures of these compounds were confirmed by elemental analysis, IR and NMR spectra and finally, they were subjected to biological screening.

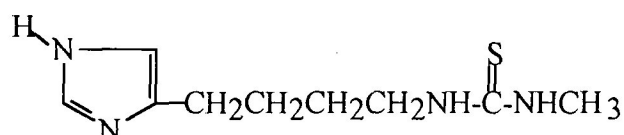


(X) R= chlorophenyl, fluorophenyl,
trifluoromethylphenyl, N-methyl pyrrole etc.

Part 4

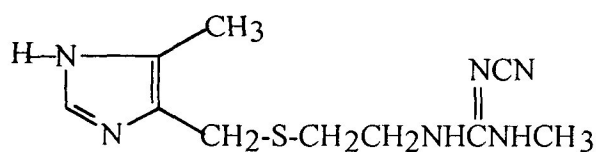
Synthesis of 3{[(2-thioureido-4-thiazolyl)methyl]thio}propionitrile as intermediate of Famotidine.

Peptic ulcer conditions involve ulceration in the oesophagus, stomach, duodenum or jejunum. Treatment of this involves a simple principle - "No acid, no ulcer". Hence, drugs which inhibit gastric acid secretion were developed. A series of compounds useful in preventing gastric acid secretion was synthesised, with Burimamide (XI) being the first drug to demonstrate H₂-antagonism.



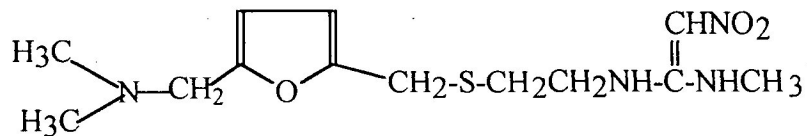
(XI) Burimamide

A structural modification of this prototype led to the evolution of more potent H₂-antagonists like Cimetidine(XII).



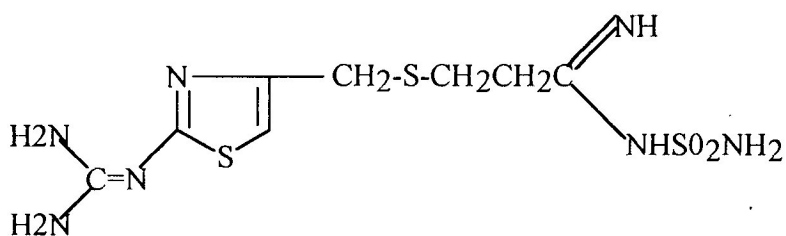
(XII) Cimetidine

Further studies have shown that structurally unique non-imidazole derivatives, such as Ranitidine (XIII), are more active antagonists as compared to Cimetidine.



(XIII) Ranitidine

In 1986, Famotidine (XIV), which is 2-guanidinothiazole isoster H_2 -antagonist was introduced. This drug contains N-amino sulphonylimidamide, which represents polar moiety on side chain and seems to be responsible for the higher potency of the drug. The drug showed a high potency in animals (dogs) and so the studies were extended to human beings. The clinical trials proved conclusively that a single dose of 40mg of Famotidine was sufficient to prevent gastric acid secretion.



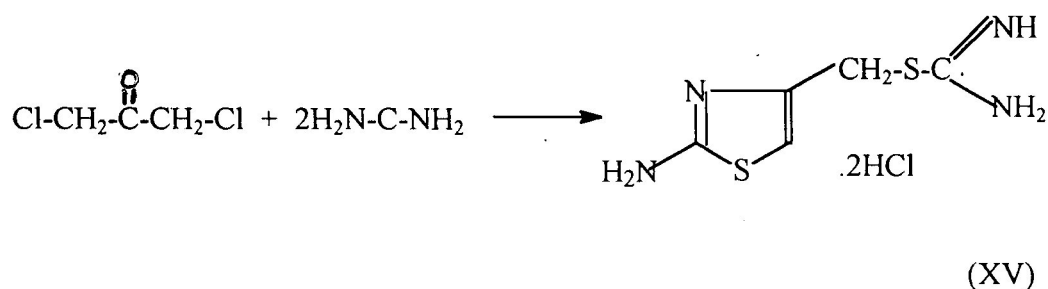
(XIV) Famotidine

Famotidine is 39 times more potent than Cimetidine in histamine-stimulated Heidenhain pouch dog¹². It also proved to be a well absorbed drug. When administered

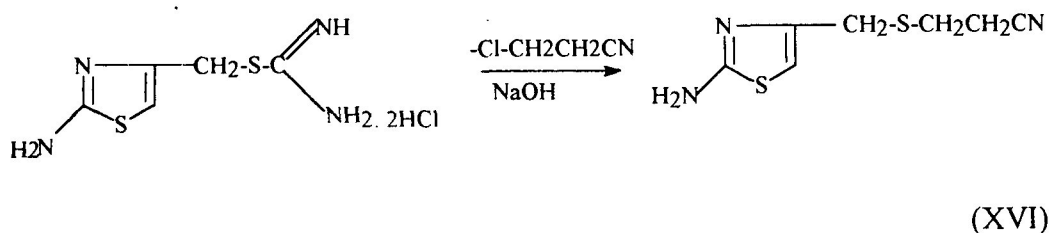
intraduodenally to the pylorus-ligated rat, Famotidine was found to be 50 times more active than Cimetidine in inhibiting acid output. Thus, Famotidine is a potent histamine H₂-antagonist and a powerful inhibitor of gastric acid secretion. Extensive clinical studies showed its efficacy in the treatment of peptic ulcer and related conditions¹². Yanagisawa, I. *et al* carried out the synthesis of Famotidine^{11,12} with a yield of 54.7%.

Hence, it was decided to synthesize an intermediate of Famotidine with improved yield. A modification was carried out at different steps to improve yield at every step, to make the process cost-effective and to avoid the use of costly fine chemicals like benzoyl isothiocyanate.

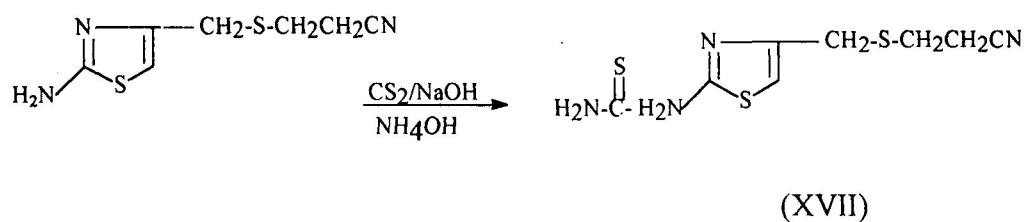
The basic intermediate, Thiuronium salt of thiazole (XV), was prepared by reaction of 1,3-dichloroacetone with thiourea as given below.



3-[[[(2-aminothiazolyl) methyl]thio} propionitrile (XVI) was prepared from thiuronium salt and chloropropionitrile. At this stage, the process was modified by using water : 1,4-dioxane mixture (50:50) in presence of KOH, which improved the yield by about 15% over the reported method, which uses water : ethanol as the solvent.



(Thioureido)thiazole (XVII) was prepared from (XVI) by reaction with carbon disulphide and ammonia.



Because of this modification, one complete step from the established procedure is avoided. Also, the process becomes economical as the use of benzoyl isothiocyanate, a hazardous and costly fine chemical, is completely avoided.

Biological results and discussion.

About 22 new compounds were synthesised during the course of this study . All these compounds were screened in vitro against H₃₇RV strain of *Mycobacterium tuberculosis* and also against *Salmonella typhi* and *Staphylococcus aureus*.

The testing was carried out by the method of Rake *et al*¹³. 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 the drug stock solutions of concentration 1000 µg/ml . From this stock solution, following final drug concentrations were made - 200,100,40,20 µg/ml. For lower final drug concentrations, the drug stock solution was diluted to prepare various stock solutions such as 10, 5, 2.5 µg/ml etc.

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 the same screening pattern. Minimum Inhibitory concentration of Isoniazid is 0.04 µg/ml. Among the 22 compounds tested, 8 compounds were moderately active at 100µg/ml, 9 compounds were found to exhibit good activity in the range of 12.5 - 50µg/ml and three compounds exhibited better activity at 3.56µg/ml. One compound exhibited an activity of 1.56µg/ml and the most active compound was 3-(4 fluoro benzyl)thio-triazolobenzothiazole which exhibited activity below 1 µg/ml.

The anti-bacterial screening was carried out using Mueller-Hinton broth as the growth medium. 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 µg/ml against *S. aureus* and that of Trimethoprim is 1 µg/ml against *S. typhi*. All the compounds were found to exhibit moderate activity, i.e. 100 µg/ml against both the organisms.

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