

SYNTHESIS AND EVALUATION OF NOVEL HETEROCYCLIC COMPOUNDS OF POSSIBLE THERAPEUTIC VALUE

THESIS SUBMITTED
UNIVERSITY OF MUMBAI

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

SUBMITTED BY
NARKHEDE, HEMANT B.

UNDER THE GUIDANCE OF
DR. B. G. KHADSE

DEPARTMENT OF CHEMOTHERAPY
HAFFKINE INSTITUTE FOR TRAINING, RESEARCH & TESTING
BOMBAY

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Mr. Hemant B. Narkhede

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**Synthesis and Evaluation
of Novel Heterocyclic Compounds
of possible Therapeutic Value**

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

**By
Mr. Hemant B. Narkhede
B.Pharm.**

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

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Haffkine Institute

..... Hemant B. Narkhede

Mumbai-400 012.

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 I:

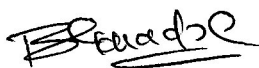
The work embodied in the Thesis to be submitted is original, based on the discovery of new facts viz. The synthesis and characterization of new and unknown compounds of possible chemotherapeutic value. Also included in this Thesis, are new relation of facts, observed on the basis of *in vitro* testing.

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

Statement II:

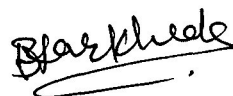
During the study, all available information of related nature published in scientific journals by previous workers in allied fields, has been used as a source of information, as indicated in the "References".

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



Dr. B.G. Khadse

Signature of Research Guide



Mr. Hemant B. Narkhede

Signature of Research Student.

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Synopsis

Introduction and Review of Literature

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

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

To add to this, there is emergence of bacterial resistance to a number of antimicrobial agents such as macrolides, quinolones, vancomycin and β -lactam antibiotics. Many of these strains are also resistant to macrolides, tetracyclines, aminoglycosides and fluoroquinolones. Multi drug resistant strains of *Mycobacterium tuberculosis* have emerged in several countries including United States³. Resistance of *Bacterioides fragilis* to β -lactam ranges from 3 to 30%; nearly all of them are

resistant to tetracyclines. This rapid and extensive emergence of resistant bacteria has resulted in a clear cut need to discover new chemotherapeutic agents.

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

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

The wide prevalence of parasitic diseases are essentially due to distinctive climatic conditions, poor environmental sanitation, low socio-economic status, lack of health education and other related factors.

TYPE OF INFECTIONS

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

Filariasis is a group of infections that cause disability and severe disfigurement of legs. It is transmitted by the bite of a mosquito.

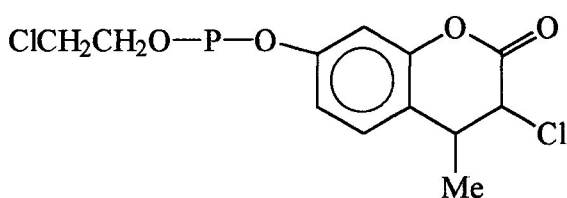
Strongyloides stercoralis has a direct effect in causing intractable diarrhoea. Schistosomiasis is another serious malady, which causes liver, gut and bladder fibrosis from schistosome eggs, deposited by worms.

Anthelmintics are drugs that are used to rid the body of parasite worms, i.e. helminths. The term anthelmintic should not be restricted to drugs acting locally to expel worms from the gastrointestinal tract. There are several types of worms that penetrate tissues, and drugs used to combat systemic infestations should also be included under the general term anthelmintic. Therefore, the anthelmintics may be defined broadly as drugs used to combat any type of helminthiasis.

Anthelmintic drugs :

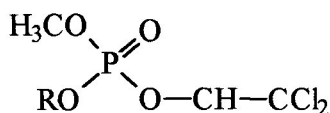
1) Organophosphorous compounds

Many organophosphorous compounds do possess efficacy *in vivo* against intestinal nematodes, but most cannot be used for therapy, either because the spectrum of activity is too narrow or because the therapeutic index is too low⁴. Although haloxon (I) is unsuitable for administration to humans because a certain degree of delayed neurotoxicity has been associated with its use, nevertheless, in animals, it is an extremely useful, inexpensive drug. It is especially suitable for the treatment of ascariasis in young puppies, and in cattle, for instance, it is certainly one of the safest and most effective of the currently available anthelmintic agents⁵.



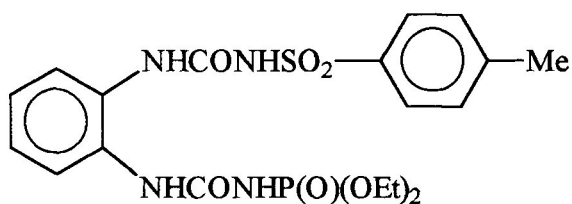
(I) Haloxon

The other organophosphorous compounds with nematocidal properties include the *dichlovos* (II) *cogner vincofos* (III)⁶ and *diuredosan* (IV)⁷ which is a recently announced broad spectrum anthelmintic agent that is active against a variety of intestinal nematodes and cestodes in dogs and cats.



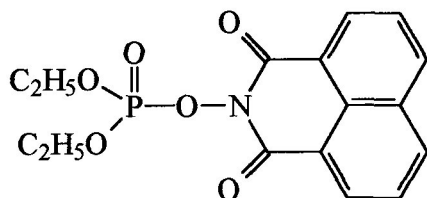
(II) Diclofos R = Me

(III) Vincofos R = n-C₈H₁₇

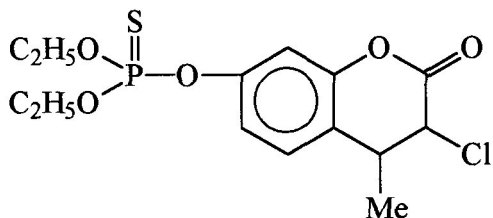


(IV) Diuredosan

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



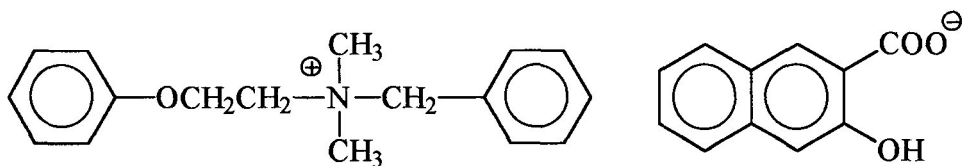
(V) Naphthalophos



(VI) Coumaphos

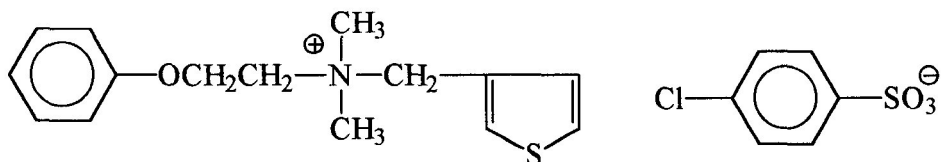
2) Bephenium and Thenium :

Bephenium hydroxynaphthoate (VII), introduced 20 years ago to human clinical medicine is still the drug of choice for treatment of hookworm infection. It has the added advantage that it is effective against the roundworm *Ascaris lumbricoides*, and against *Trichostrongylus* species when administered as a single oral dose⁸.



(VII) Bephenium Hydroxynaphthoate

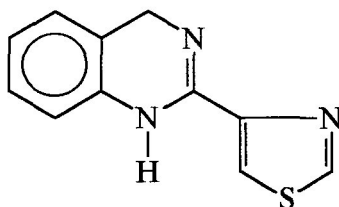
Another drug that emerged from the structure activity relationship studies of this series of compounds was veterinary anthelmintic agent Thenium closylate(VIII), for use against hookworms in dogs.



(VIII) Thenium closylate

3) Benzimidazoles :

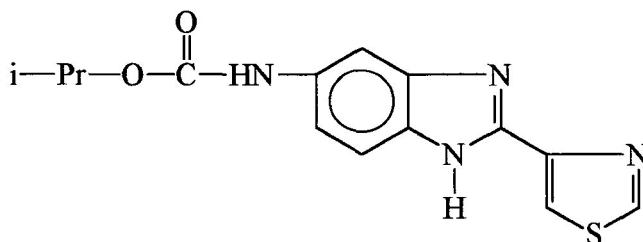
Introduction of 2-(4-thiazolyl)-benzimidazole (Thiabendazole)⁹ (IX) in 1961, by workers from the Merck laboratories was the first of the modern, truly broad spectrum gastrointestinal nematodes, useful for the eradication of most intestinal nematodes. This was quickly followed by a generation of various benzimidazoles, several of which are currently used in the treatment of human helminth infections.



(IX) Thiabendazole

Thiabendazole is a very good agent for the treatment of hookworm infections and is most effective in multiple dose therapy achieving cure rates to the order of 75-83%. It also shows anti-inflammatory activity, which may be advantageous in the treatment of human hookworm infections. However, it suffers a disadvantage of being readily deactivated in sheep due to hydroxylation at its 5-position¹⁰.

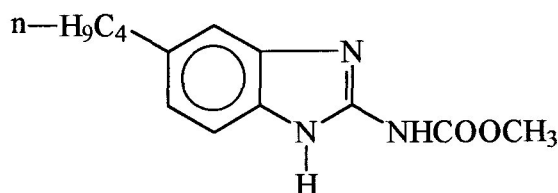
As part of an extensive programme investigating structural modification of thiabendazole, workers at Merck examined the effect of introducing various substituents into the 5-position of the molecule. The most active compound to emerge from the study was 5-i-propoxycarbonylamino derivative known as Cambendazole (X).



(X) Cambendazole

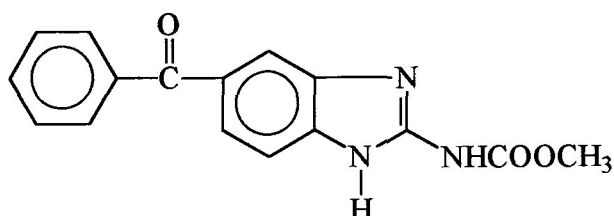
Cambendazole is of low toxicity, has a similar spectrum of activity as that of thiabendazole and is several times more potent than thiabendazole.

Parbendazole(XI)¹¹ was introduced by Smith-Kline and French Laboratories and it exhibited significant activity against different helminth parasites, although its action is rather limited against hookworms. It shows about 66-88% clearance of *A. caninum* infections in dogs at an oral dose of 20 mg/kg. It represents the first of the benzimidazole anthelmintic agents to contain the methyl carbamate moiety in the 2-position.



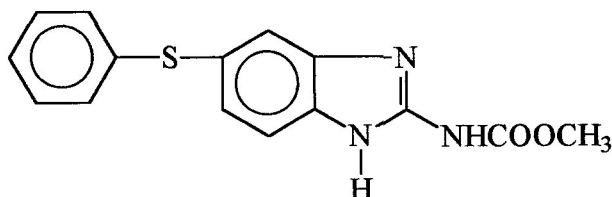
(XI) Parbendazole

Parbendazole was soon followed by Mebendazole (XII), marketed by Janssen Pharmaceutica, and it has been confirmed as a remarkably safe, broad spectrum anthelmintic that is especially useful in the treatment of human whipworm (*Trichuris trichiura*) and pinworm (*Enterobius vermicularis*) infections¹².



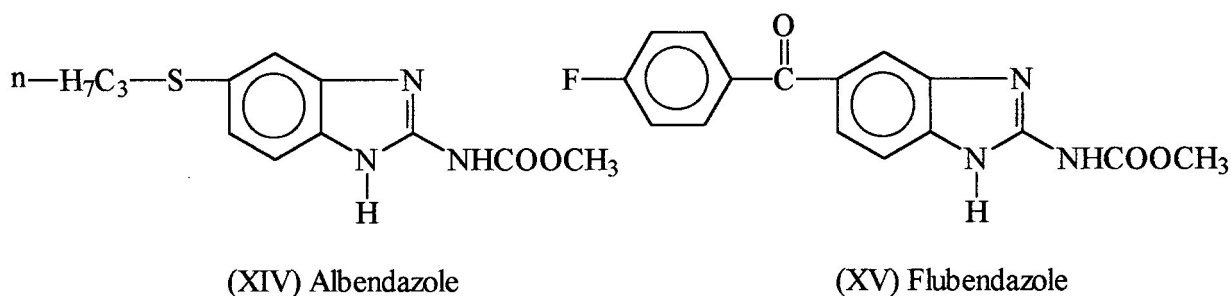
(XII) Mebendazole

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



(XIII) Fenbendazole

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



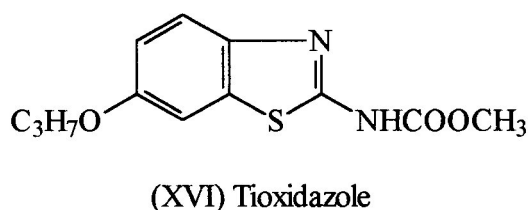
Albendazole exhibits high efficacy against *A. caninum* in dogs at a dose of 50mg/kg given for three days while it shows relatively weak activity if a single dose of 250 mg/kg is administered.

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

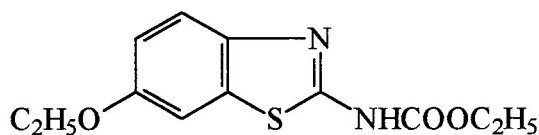
Thus it can be seen that benzimidazoles represent the only class of truly broad spectrum anthelmintics. However, poor drug absorption and lack of water solubility are problems characteristic of these compounds, which limit their usage.

Benzothiazoles and related compounds

Attempts to extend the functional characteristics of the benzimidazole class of compounds to the benzothiazoles with a view to develop possible anthelmintics have met with some success. Several substituted benzothiazoles have been synthesised of which methyl-6-propoxybenzothiazole-2-carbamate (XVI) (Tioxidazole) has emerged as a potent anthelmintic compound for intestinal worms¹³. At a dose of 50mg/kg for five days it shows excellent activity against *N. dubius* in mice.

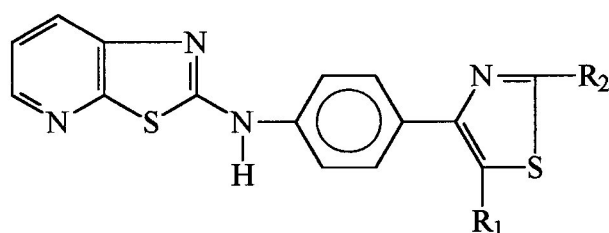


Another benzothiazole of similar structure ethyl-6-ethoxybenzothiazole-2-carbamate (XVII) exhibits over 90% clearance of *H. contortus* and *B. trigonocephalum* in lambs at a dose of 200mg/kg¹³.

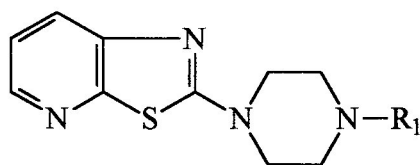


(XVII)

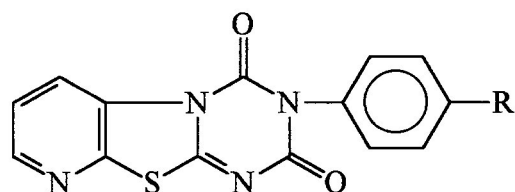
A few pyridothiazoles (XVIII-XX) have been reported to possess anthelmintic activity¹⁴⁻¹⁶ of which (XVIII) caused 90% mortality of adult *N. dubius* worms at a dose of 1mg/kg. The other two compounds (XIX) and (XX), exhibited moderate activity against *N. dubius* in mice.



(XVIII)



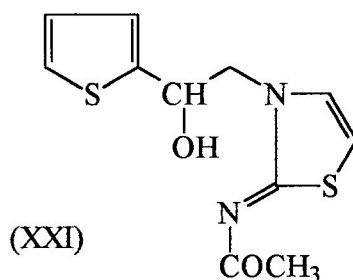
(XIX)



(XX)

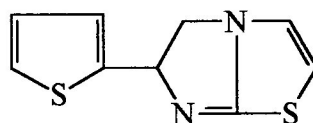
Imidazothiazoles :

In the 1960's, Janssen Pharmaceutica, while engaged in the search for a broad spectrum anthelmintic, discovered that thiazothienol (XXI) showed promising activity in chickens¹⁷.



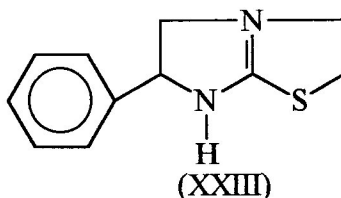
(XXI)

While (XXI) also exhibited activity against sheep nematodes, it was ineffective in rats or mice suggesting that the active principle was probably a metabolite. Thereafter, chicken were dosed with (XXI) and thiazothieliet(XXII), isolated from the faeces(XXII) was found to be far more potent than its precursor and is also active in both mice and rats¹⁷.



(XXII)

With the Imidazothiazole as the lead compound, other such molecules were synthesised of which Tetramisole (XXIII) represents a broad spectrum anthelmintic. The anthelmintic activity is only due to its S(-) isomer, termed Levamisole.



(XXIII)

Tetramisole is also used for veterinary purpose and exhibits high activity against various gastrointestinal nematodes of sheep, dogs, fowl, cattle and horses. In humans, it gives a 27-75% cure rate against hookworm infections at a dose of 2.5 - 5.0 mg/kg given for a period of three days.

Tuberculosis :

Tuberculosis is one of the most widespread infections known to man. This disease is largely caused by *Mycobacterium tuberculosis*, an 'acid fast' bacilli. It has been estimated that 1.7 billion people or a third of the world's population, harbor the pathogen *Mycobacterium tuberculosis*, and every year 8 million individuals develop new clinical disease causing 3 million deaths¹⁸.

It attacks both humans and animals, affects all ages and every organ in the body, and ranges from latent to hyperacute. Infection with human immunodeficiency virus

(HIV) is the most potent risk factor for development of tuberculosis (TB) and by 1995, 3.1 million people were expected to be co-infected with HIV and TB according to World Health Organisation.

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

1. Caseation necrosis, a unique feature of tuberculosis, allows the bacilli to remain in a dormant condition by sealing off the communication of necrotic area with the communicating channel like bronchus and ureter. When the communication opens, the solid caseous material is autolysed and the liquid contents are expelled, resulting in development of cavity with a caseous lining. When the dormant bacilli gets an access to the atmospheric oxygen, they multiply rapidly, producing a spread of the infection and an increase in the size of cavity. Bronchial stenosis or ureteric constriction, which may occur as sequelae, prevent adequate drainage of the caseous materials¹⁹.
2. Mycobacterial infections such as tuberculosis are chronic diseases, which do not elicit a rapid response from the host. After initial infection and multiplication, the bacilli may lie dormant for a long time without giving rise to any of the clinical symptoms. Often, however, the bacilli may be reactivated due to malnutrition, immunosuppressive drugs and diseases including HIV infection.
3. The cavity formation tends to block the blood vessels supplying the necrotic area making penetration by antitubercular drugs difficult. The caseated material itself is also impenetrable to majority of the chemotherapeutic agents.
4. Tubercle bacilli remain viable and multiply even when ingested by macrophages. The chemotherapeutic agents, with few exceptions, penetrate poorly into the macrophages.

5. Also Tubercle bacilli are vulnerable to bactericidal drugs only when they are metabolically active and replicating. Here, the problem lies in the fact that small subpopulation of bacilli can remain dormant indefinitely except for short periods when they become transiently active, thus evading chemotherapeutic agents. Another difficulty is in the existence of drug resistant mutants in populations of bacilli hitherto unexposed to antibiotics.

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

No effective chemotherapeutic agent was available for treating till as late as 1940. The treatment then mainly consisted of rest, fresh air and good food. In addition, surgical methods like phrenic nerve avulsion, artificial pneumothorax and pneumoperitoneum were widely employed¹⁹. The discovery of effective para-aminosalicylic acid by Bernheim in 1941 was the first step in the development of effective therapeutic agents. The other two commonly used agents, Streptomycin and Isoniazid were discovered in 1944 and 1952, respectively.

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

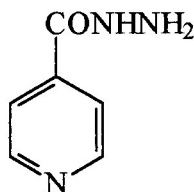
Following are the currently used drugs in treatment of tuberculosis.

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

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

Isoniazid (INH)

Isoniazid (XXIV), the hydrazide of isonicotinic acid was first described in 1912 but its action against the tubercle bacilli was only discovered in 1952. It is unrivalled in its bactericidal action and ensures the destruction of the bacilli within a few days of starting treatment. It is highly active against replicating tubercle bacilli but does not affect the semidormant species²⁰ which resume multiplication after removal of contact with the drug.



(XXIV) Isoniazid

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

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

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

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

Rifampicin :

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

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

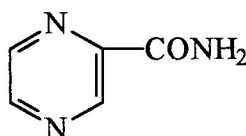
Streptomycin :

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

It is more actively bactericidal in proliferative than in the resting phase of bacterial growth. The normal dosage for adults and children is 15 mg/kg daily, while in patients over 60 years of age it should be used with caution. It should be avoided during pregnancy as it crosses the placenta thus causing the same effect in the foetus. The injections are very painful and must be administered by deep intramuscular route; 1 injection with approximate dosage per day is sufficient but barely tolerable. Duration of treatment is 1-2 months²¹.

Pyrazinamide :

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



(XXV) Pyrazinamide

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

Ethambutol :

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



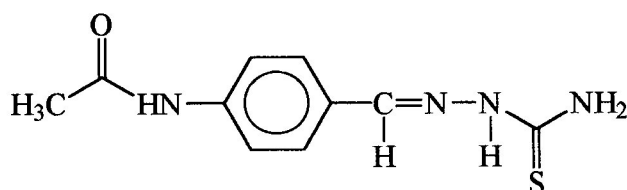
(XXVI) Ethambutol

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

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

Thiacetazone :

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



(XXVII) Thiacetazone

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

Other bacterial infections :

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

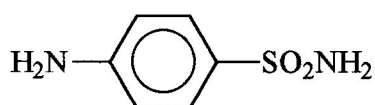
Not surprisingly, therefore, mankind's earliest therapeutic successes against infecting organisms are in the form of plant extracts. Observations that natural substances controlled the spectacular symptoms of certain diseases led to the initial recognition of two other ancient remedies : Quinine, obtained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

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

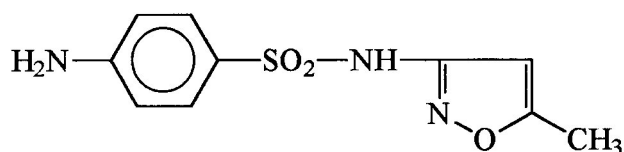
The following pages present a brief summary of the important classes of compounds used for this purpose.

Sulfonamides :

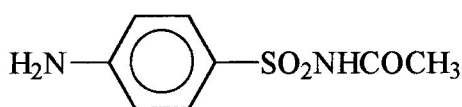
The term sulfonamide is usually employed as a generic name for derivatives of p-aminobenzenesulfonamide. Domagk²², Mietzsch and Klarer²³ in 1935 discovered Prontosil, and reported that this compound cures *Streptococcal* infections in mice and *Staphylococcus* infections in rabbits. After this success, sulfonamides were the first synthetic chemotherapeutic agents employed to prevent and cure bacterial infections in man and were quite effective in bringing about a sharp decline in the morbidity and mortality figures for the treatable infectious diseases. Important members of this class include Sulfanilamide (XXVIII), Sulfamethoxazole (XXIX), Sulfacetamide (XXX), Sulfadiazine (XXXI) and Sulfisoxazole (XXXII).



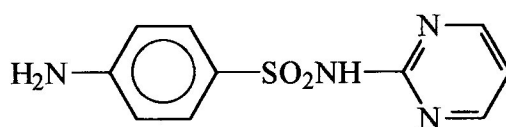
(XXVIII) Sulfanilamide



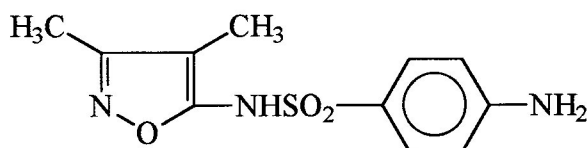
(XXIX) Sulfamethoxazole



(XXX) Sulfacetamide



(XXXI) Sulfadiazine



(XXXII) Sulfisoxazole

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

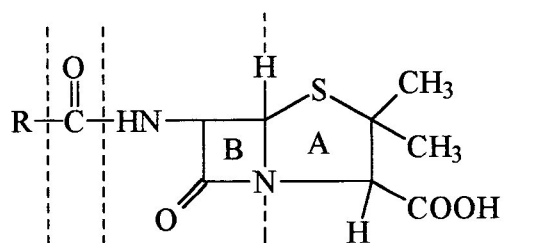
Strains of bacteria resistant to the sulfonamides have become common during recent years. Also, the advent of Penicillins and other antibiotics has diminished the role of sulfonamides as antibacterial agents. However, the introduction of the combination of Trimethoprim and Sulfamethoxazole, known popularly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

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

Penicillins and Cephalosporins :

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

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



(XXXIII)

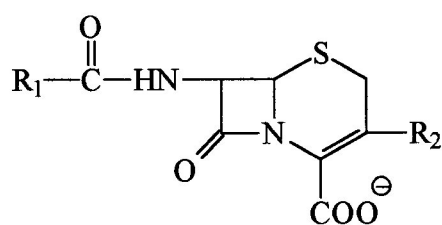
The penicillins are classified according to their spectrum of antibacterial activity. While the pharmacological properties of the individual drug in the various classes would constitute a lengthy discussion, certain generalisations can, however, be made. Following absorption, penicillins are widely distributed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

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

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

The commonly cited adverse effects in the usage of penicillin, which are probably the most common causes of drug allergy, have been the hypersensitivity reactions. Such reactions could occur with any dosage form of penicillin, but as the drug is rapidly eliminated the allergic reactions usually subside. In some cases the symptoms may persist for one to two weeks after withdrawal of treatment while in other cases the reaction is mild and disappears during therapy with the drug. At the worst of circumstances an extreme allergic reaction may even result in death of the patient.

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



(XXXIV)

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

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

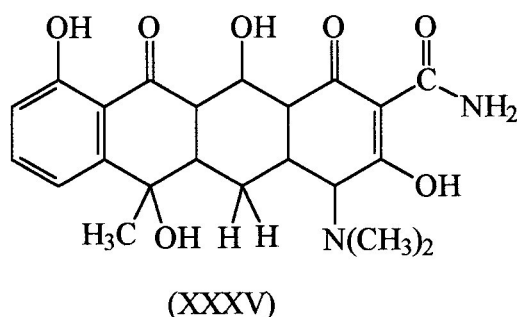
The cephalosporins also find wide use in prophylaxis during and after surgery, wherein the first generation agents have been shown to be safe and effective. A few of the third generation cephalosporins are the drugs of choice for meningitis and in some cases superior to combination of Ampicillin and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

The β -lactum antibiotics (penicillins and cephalosporins) suffer bacterial resistance due to a variety of factors. It may be caused by the inability of the agents to enter the site of action²⁴. Also, it is possible for a sensitive strain to acquire resistance by mutation and has been described for gram-positive cocci²⁵.

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

Tetracyclines :

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



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

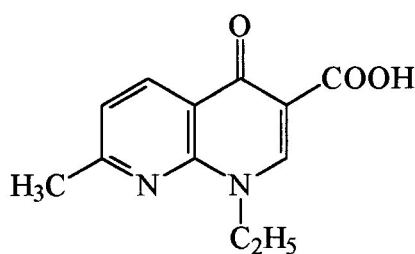
All Tetracyclines produce gastrointestinal irritation to varying degrees in some individuals and this is more common after oral administration. Diarrhoea may also be a side effect, which though frequent and fluid, does not contain blood or leukocytes. Doxycycline and to a lesser extent other derivatives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

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

Quinolones :

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

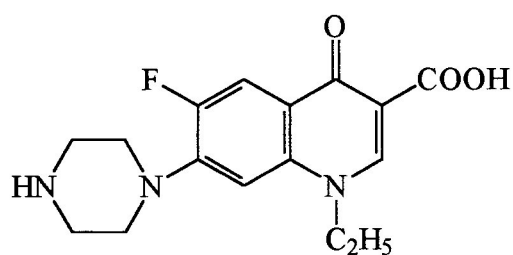
Modifications in the structure of Nalidixic acid has produced related antibacterial agents such as oxolinic acid, piromidic acid, cinoxacin and acrosoxacin. None has been considered to represent a significant clinical advance over nalidixic acid.



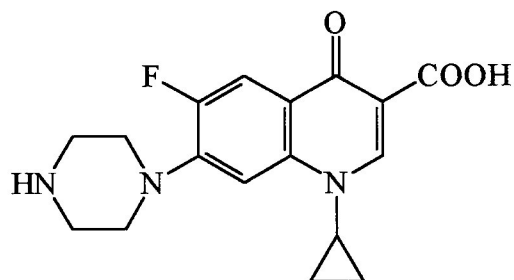
(XXXVI) Nalidixic acid

Addition of the 7-piperazinyl group and a fluorine atom at position 6 has produced a group of fluoroquinolones with a broad spectrum of activity than nalidixic acid, and they are more useful for the treatment of systemic infections. They include Norfloxacin (XXXVII), Ciprofloxacin (XXXVIII), Enoxacin (XXXIX), Ofloxacin (XL) and Lomefloxacin (XLI). One disadvantage of all the quinolone antibacterial agents is that they are generally not recommended for use in children, adolescent, pregnant and

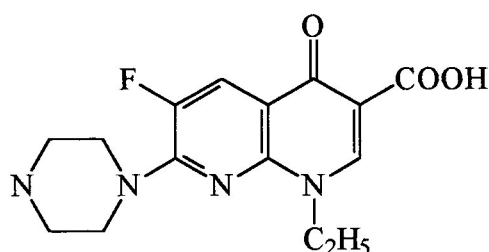
breast feeding women, because of their propensity to cause joint erosions in immature animals.



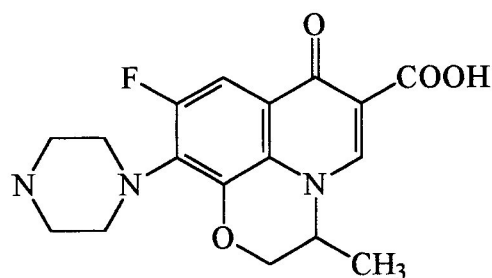
(XXXVII) Norfloxacin



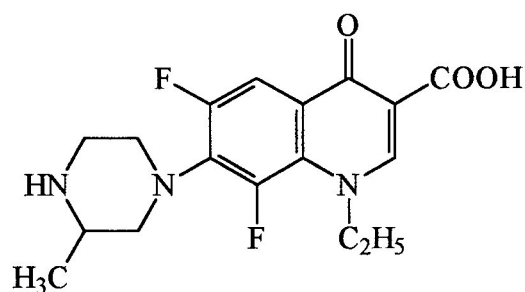
(XXXVIII) Ciprofloxacin



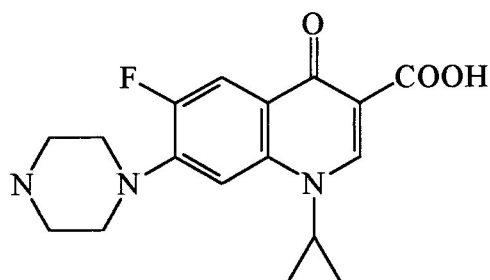
(XXXIX) Enoxacin



(XL) Ofloxacin



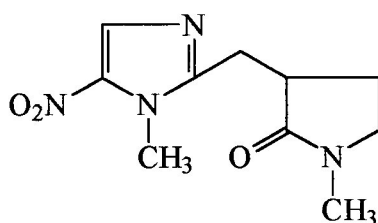
(XLI) Lomefloxacin



(XLII) Tefloxacin

Tefloxacin (XLII) was demonstrated by J. L. Chen et al²⁶ to possess activity against penicillin resistant *Staphylococcus*, *Streptococcus* and *Haemophilus*. It was itself a β -lactamase inhibitor and displayed synergy with Ampicillin.

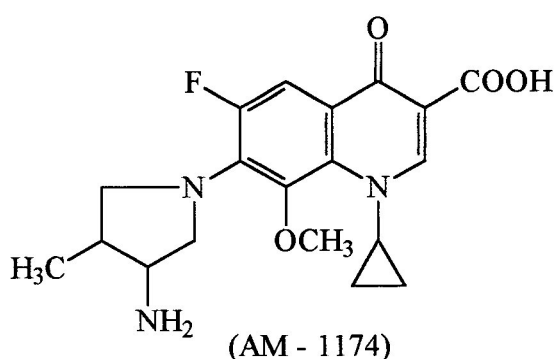
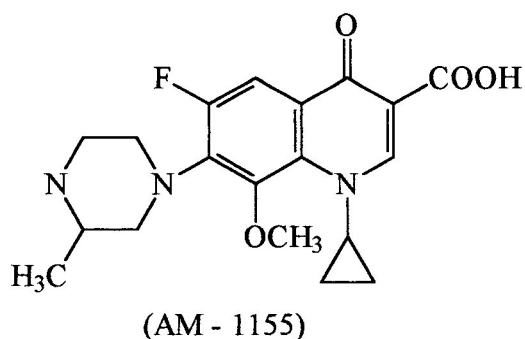
Miscellaneous : Activity against anaerobic organisms was reported for a series of 2-substituted-5-nitroimidazoles. Compound (XLIII) was unique in this series, and was found to be more potent both *in vitro* and *in vivo* than Metronidazole.



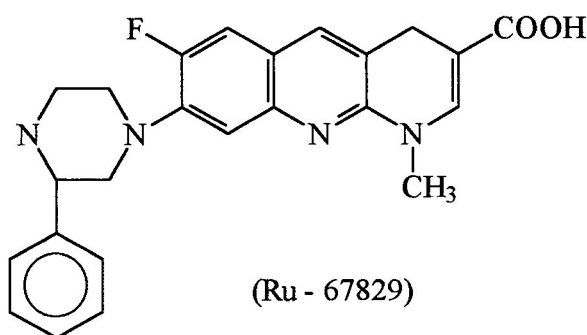
(XLIII)

Quinolones :

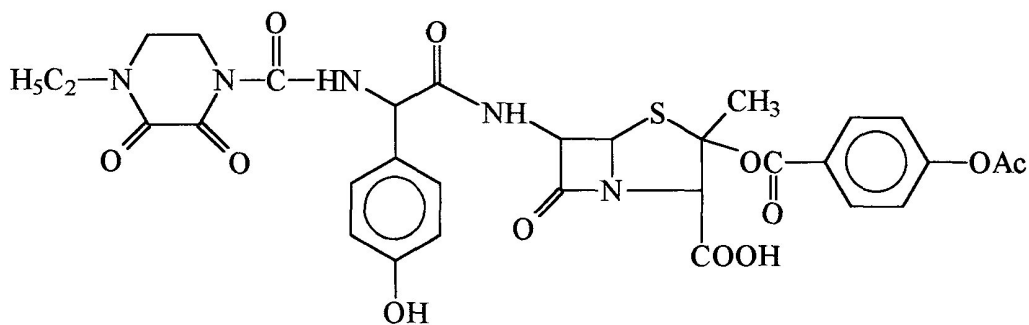
Use of N-cyclopropyl and C-8 hetero atom substituents continued to provide potent antibacterial agents. AM – 1155 and AM – 1174, new quinolones bearing 8-methoxy group, outperformed Ciprofloxacin against anaerobes and a range of gram-positive bacterial with potency upto 16 to 62 – fold greater than Ciprofloxacin²⁷.



RU –67829 (a compound in benzonaphthyridine series) was found to be more active against gram-positive organisms than Ciprofloxacin²⁸.

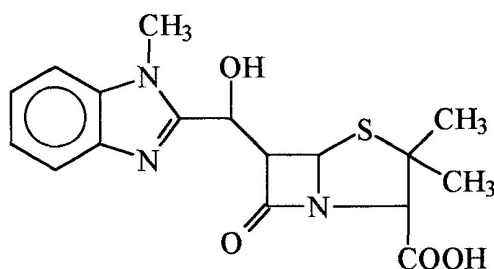


In penicillins, the catechol containing compounds (LXIV) had outstanding activity against *Pseudomonas*, and stability to gram-negative β -lactamases, but not to the lactamases of *S. aureus*²⁹.



(XLIV)

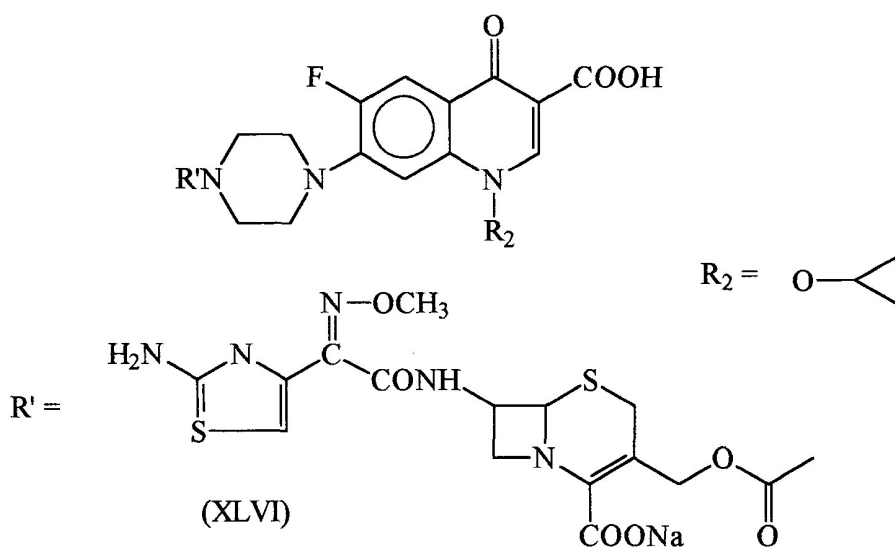
The stability to gram-positive β -lactamases could be achieved through the use of a (benzimidazole-2-yl) hydroxymethyl group at R-position (XLV).



(XLV)

AM – 1174 exhibited activity 128-fold that of Ciprofloxacin against Ciprofloxacin-resistant strains of *S. aureus*³⁰.

New β -lactum quinolone hybrids have been reported. The carbamate linked R0-24-4383 (XLVI) showed broad spectrum activity and exhibited aqueous solubility much greater than that of the quinolone component alone³¹.



Viral infections :

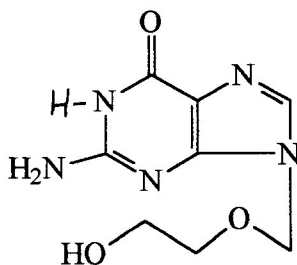
The development of compounds useful for the prophylaxis and therapy of viral disease has presented an even more difficult problem than those encountered in search for drugs effective in disorders produced by other microorganisms. The reason for this is that virus are obligate intracellular parasites that require the active participation of metabolic processes of the invaded cell. Thus, agents that may inhibit or cause the death of viruses are also very likely to injure the host cells that harbour them. Another problem is that certain viruses multiply in the cytoplasm while others do so in the nucleus of the infected cell. A drug which acts on a virus in the cytoplasm may not necessarily act on virus that multiplies in the nucleus and vice-versa.

Although the search for substances that might be of use in the management of viral infections has been long and intensive, very few agents have been found to have clinical applicability. However, even these have exhibited very narrow activity, limited to only few specific viruses.

Eight drugs are currently approved for treating virus infections in United States. They include : Purine nucleoside derivatives, Vidarbine, Acyclovir, Ganciclovir, Famciclovir and Valacyclovir; Pyrimidine nucleoside analogs, Idoxuridine and Trifluridine, and the pyrophosphate analogs, Foscarnet.

Purine nucleoside analogs :

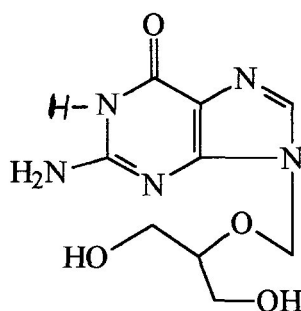
Researchers discovered Acyclovir (ACV) (XLVII), a synthetic guanosine analog bearing an acyclic side chain, over a two decades ago. It was found to be a potent inhibitor of Herpes virus replication. It is practically nontoxic to uninfected cells and is normally inactive against virus in other families^{32,33}.



(XLVII) Acyclovir

Acyclovir acts by inhibiting primer template function in replicating DNA molecules after being incorporated³⁴.

After the success of Acyclovir, large number of analogs of Acyclovir were prepared. Gancyclovir (XLVIII), one such analog, was found to be very potent inhibitor of Herpes virus replication with HCMV being more susceptible to HSV³⁵.

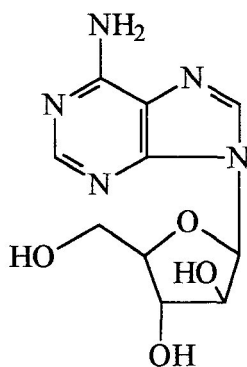


(XLVIII) Gancyclovir

Gancyclovir has an additional hydroxymethylene moiety in the acyclic side chain. This minor structural modification markedly affects the spectrum and safety profile of Gancyclovir. Unlike Acyclovir, the antiviral spectrum of Gancyclovir extends beyond herpesviridae. Gancyclovir substantially reduces HBV and DHBV replicating *in vitro* and *in vivo*, but it is currently indicated for HCMV-related diseases, like retinitis and pneumonia, that occur in immunocompromised individuals. The drug is not as safe as Acyclovir as it can damage uninfected cells.

Vidarbine :

Vidarbine (XLIX) is an adenosine analog that has D-arabinose as a sugar moiety.

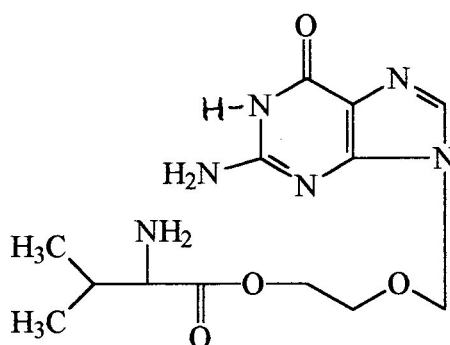


(XLIX) Vidarbine

Vidarbine displays broad spectrum antiviral activity *in vitro* but its mechanism of action and selectivity is still unresolved, Vidarbine is currently used to manage various types of herpes infections but it has been replaced by Acyclovir for most indications.

Valacyclovir :

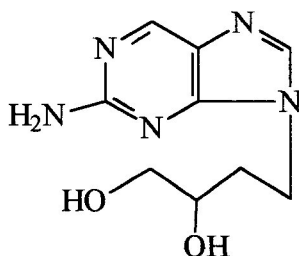
Further development in acyclovir resulted in many new analogs that are active against viral infections. One such approach was the esterification of Acyclovir with amino acids which resulted in discovery of a new analog Valacyclovir (L), the L-valyl ester.



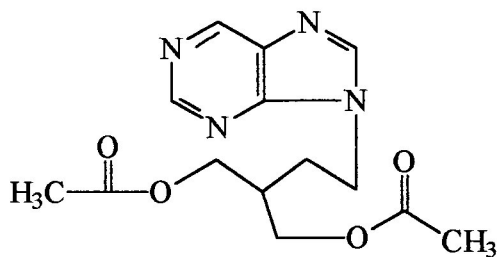
(L) Valacyclovir

Valacyclovir is a prodrug for Acyclovir. It has no intrinsic antiviral activity. The prodrug is rapidly converted to Acyclovir in humans by a hydrolase present in the liver and gut wall. Drug displays a mean absolute bioavailability of 54% and a three fold increase in oral bioavailability over Acyclovir.

An ester prodrug strategy also led to the development of famciclovir (LII), the diacetyl, 6-deoxy derivative of pencyclovir (LI).



(LI) Pencyclovir

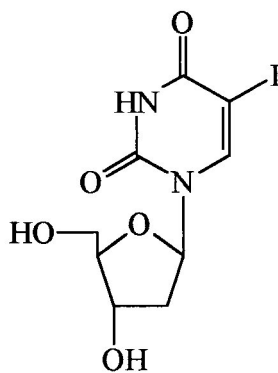


(LII) Famciclovir

Famicyclovir is the oral drug form currently approved for clinical use against acute herpes zoster and genital herpes. The prodrug is metabolised primarily by loss of acetyl groups prior to oxidation and it delivers high blood levels of Pencyclovir, about seventy per cent, after oral dosing.

Pyrimidine Nucleoside Derivatives.

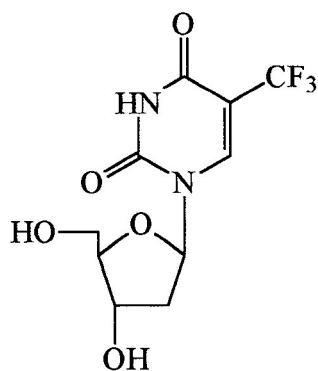
Idoxuridine (LIII), is the first nucleoside based antiviral agent that was licensed in U.S. for clinical use.



(LIII) Idoxuridine

The drug is indicated only for topical treatment of HSV keratitis because its action is mutagenic and metabolically unstable. Phosphorylated drug metabolites interfere with various biochemical processes including cell and virus DNA replication.

Trifluridine (LIV), another pyrimidine nucleoside derivative is the drug of choice for herpes keratitis. The drug is more efficacious than Idoxuridine and can be administered at lower dose frequency.



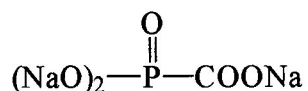
(LIV) Trifluridine

Metabolic activation of Trifluridine is not strictly dependent on herpes nucleoside kinase, as in the case of Idoxuridine. The monophosphorylated metabolite of

Trifluridine inhibits denovo biosynthesis of thymidylate but the drug's precise mechanism of action of the drug is not completely defined.

Pyrophosphate analogs :

The only pyrophosphate analog available as an antiviral drug is Foscarnet (LV).



(LV) Foscarnet

Foscarnet is a nonnucleoside drug used to manage DNA virus infection. The drug was discovered as a selective antiviral agent almost three decades ago^{35,36}. It is active against herpes virus, VV and certain RNA viruses. Foscarnet inhibits DNA synthetic reactions noncompetitively with respect to deoxynucleoside triphosphates. The drug acts preferentially on virus polymerase³⁷. Intravenous administration of Foscarnet is currently approved for treating various HCMV- associated diseases as well as Acyclovir resistant HSV infections.

Plan of Work

Plan of work

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

Voltaire³⁸ (1694-1774) stated "Therapeutics is the pouring of a drug of which one knows nothing, into a patient of whom one knows less."

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

All successful chemotherapeutic agents should have certain properties in common :

1. They must exert antimicrobial action whether inhibitory or lethal in high dilutions and in the complex chemical environment, which they encounter in the body.
2. Also, since they are brought into contact with every tissue in the body, they must possibly, act without any disrupting or harmful effects on the normal functions of any organ.

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

Subsequently, there have been immense advances in the knowledge of the anatomy, chemical composition and metabolism of various microbial cells. This new knowledge has been helpful in understanding what drugs do to the infecting organism.

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

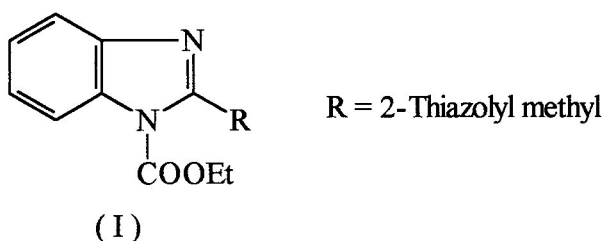
The logic supporting the work presented in this thesis was formulated, bearing in mind the biological activities of known moieties and attempting certain structural modifications or adaptations in the light of the recent trends in drug research, in incorporating newly emerged pharmacophores on existing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

Part II : Synthesis of 2,5-disubstituted-1-[N-(1-ethyl methyl) sulphonyl] benzimidazoles.

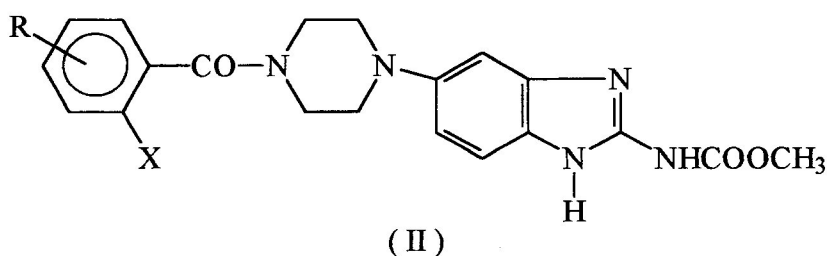
Among the various types of known heterocyclic ring systems, different thio substituted benzimidazole derivatives have demonstrated significant biological activities^{39,40}.

So, in this part it, was proposed to undertake the synthesis and biological study of benzimidazole derivatives.

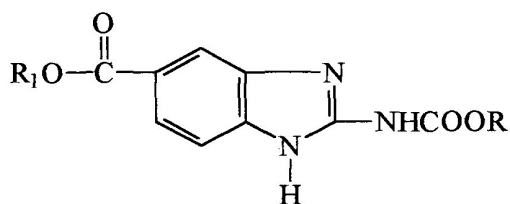
Also, literature survey had revealed that benzimidazole carbamates⁴¹ (I) had been found to possess antifungal, anthelmintic and antibacterial properties.



Some of the methyl-5-(4-salicyloyl-piperazin-1-yl)-benzimidazole-2-carbamates (II) had been found to possess potential anthelmintic activity⁴² in experimental mice.



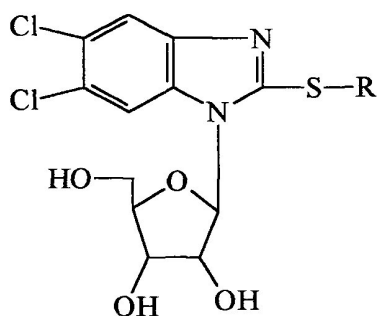
The 2,5-disubstituted benzimidazole derivatives have been studied extensively for their biological activities. During literature survey it was found that certain alkyl-5-(alkoxy carbonyl)1H-benzimidazole-2-carbamates (III) and related derivatives were active as antineoplastic and antifilarial compounds at concentrations less than 1 μ g/ml⁴³.



(III)

R = Alkyl, R₁ = Alkyl, Alkylaryl

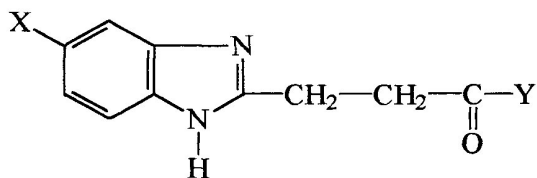
Several 2-alkylthio and 2-benzylthio derivatives of 5,6-dichloro-1-(β -D-ribofuranosyl) benzimidazole (IV) have been found to possess antiviral activity⁴⁴ against Herpes Simplex Virus – 1 (HSV-1).



(IV)

R = alkyl, substituted benzyl

Propionic acid derivatives of benzimidazole were also found to possess potent antibacterial and antifungal activities⁴⁵. 5-chloro-1H-benzimidazole-2-propionic acid derivatives (V) were found to have potent antibacterial and antifungal activities against *Staphylococcus aureus* and *Candida albicans*, respectively.

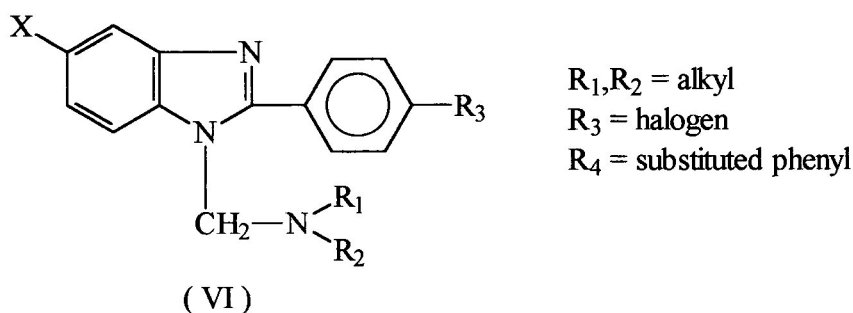


(V)

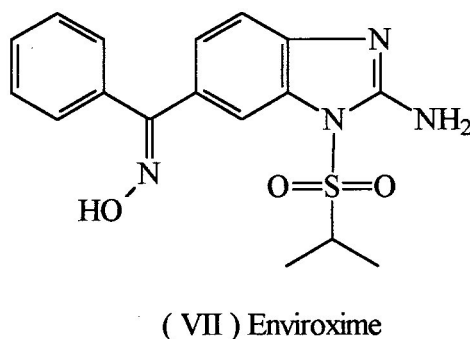
X = H, Cl

Y = OH, OCH₃, OC₂H₅, NH₂,NHNH₂, NHOH

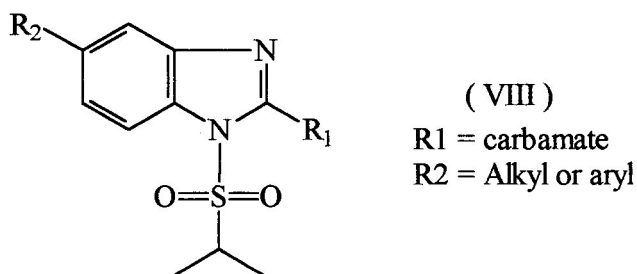
Recently, 1-dialkylaminomethyl - 2 - (4 - substituted phenyl) - 5 - substituted benzimidazole derivatives (VI) were found to be highly active against bacteria and fungi. Some of them were found to be more active than Streptomycin against *Enterococcus faecalis* and *Pseudomonas aeruginosa*⁴⁶.



Also, 6-[[[(Hydroxylimino) phenyl] methyl]-1-[1-methylethyl]-sulphonyl]-1-H-benzimidazole-2-amine, (Enviroxime) (VII) and its analogs were found to be highly active against Rhinovirus multiplications^{47,48}.

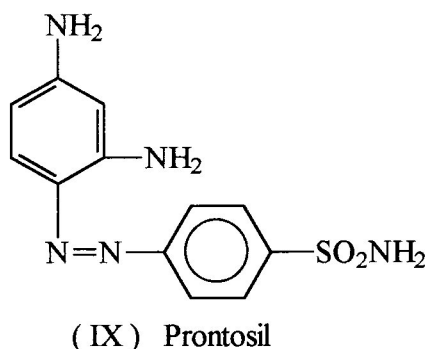


All these promising references prompted us to prepare a series of compounds containing substitutions at 1,2 and 5 positions of benzimidazole. Ten 2,5-disubstituted -1-[N-(1-ethyl methyl)sulphonyl] benzimidazoles (VIII) were prepared in this series and were tested for anthelmintic, antibacterial and antitubercular activities.



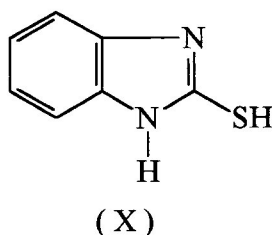
Part III : Synthesis of 5-chloro-2-(1-H-benzimidazole) sulfonamides and 5-fluoro-2-(1-H-benzimidazole) sulfonamides.

The discovery of antibacterial activity of Prontosil (IX), a sulfonamide, in early 1930's was the beginning of the era of chemotherapy⁴⁹. There after sulfonamides have been studied extensively and large number of sulfonamides were prepared and tested for their antibacterial activities. Presently there are about two dozen sulfonamides in clinical use as antibacterials.

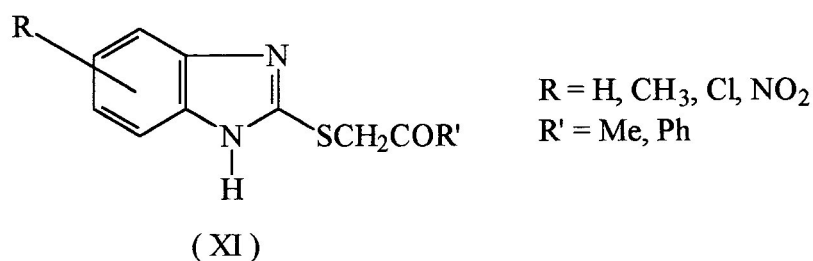


The pharmacological history of several thousands of imidazole derivatives has been established over 30 years, after the discovery of antibacterial and antifungal activity of benzimidazole by Wolley⁵⁰ (1944) which later led to the synthesis of benzimidazole derivatives with strong antiprotozoal and anthelmintic properties.

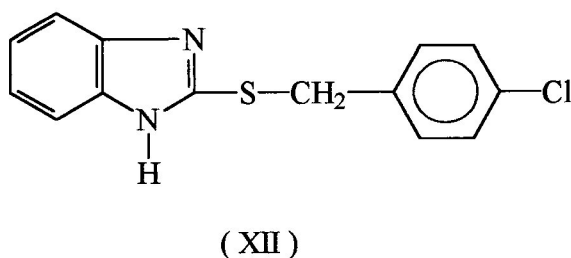
A number of 2-thiobenzimidazole derivatives (X) have demonstrated a broad spectrum of significant biological activity, i.e. plant growth inhibitors^{51,52}, bactericidal⁵³, fungicidal and virucidal³⁹ activities.



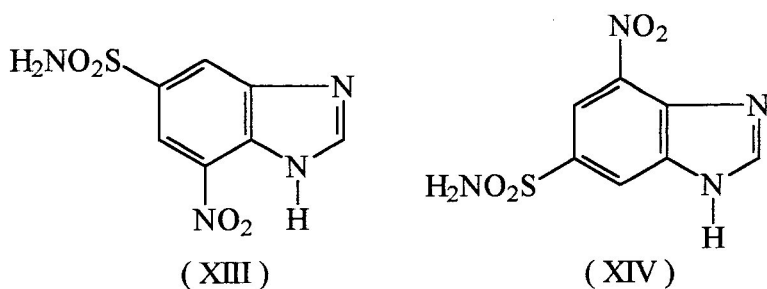
The following type of Benzimidazoles (XI) are known for their diverse biological properties⁵⁴.



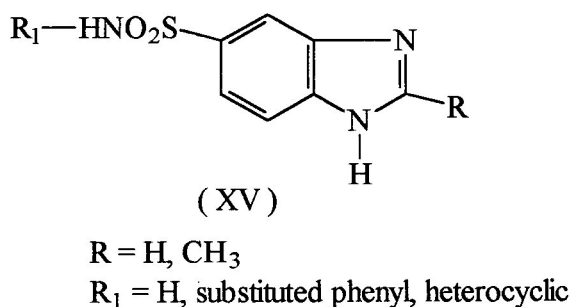
The S-benzyl derivatives of 2-mercaptobenzimidazole were found to be broad spectrum antimicrobials. 4-chlorobenzyl analog (XII) was found to be most active broad spectrum antimicrobial⁵⁵.



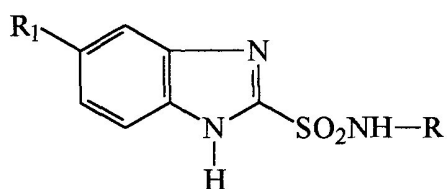
7-nitro-5-benzimidazole sulfonamides (XIII) and 4-nitro-6-benzimidazole sulfonamide (XIV) were found to be excellent antibacterials⁵⁶.



Similarly, 5(or 6)-benzimidazole sulfonamides (XV) and its 2-methyl derivatives were found to inhibit the growth of Polio and Influenza B virus⁵⁷.



Taking into account the above facts and biological importance, a series of 5-halo-2-benzimidazole sulfonamides (XVI) was prepared so as to study their antibacterial and anti-tubercular properties.



(XVI)

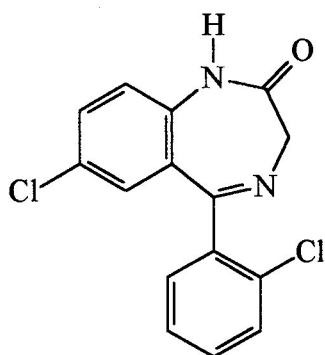
R = heterocyclic

R₁ = Cl, F

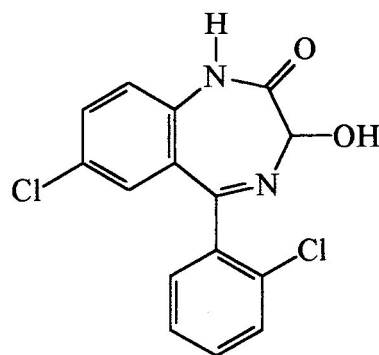
Part IV : Synthesis of 2-amino-2',5-dichloro benzophenone.

Due to advances in healthcare, the life expectancy has been dramatically increased. This has ultimately resulted in many diseases associated with aging such as autoimmune, cardiac and those directly affecting central and peripheral nervous system. Diseases of elderly are generally associated with anxiety and depression. Indeed, statistical data in U.S.A. showed that one in four people may anticipate progressive impairment of cognitive functions⁵⁸.

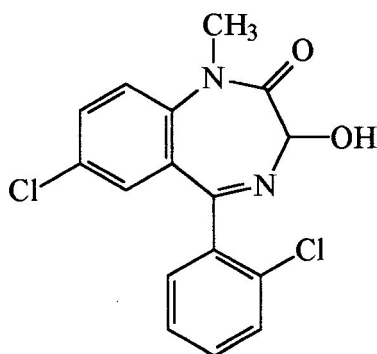
Benzodiazepines are widely used as centrally acting therapeutic agents. They display potent tranquilizing, muscle relaxant, sedative, hypnotic and convulsant activities⁵⁹. Now a days there are about two dozen of benzodiazepines in clinical use. Delorazepam (XVII), Lorazepam (XVIII), Lormetazepam (XIX), Cloxazolam (XX) and Triazolam (XXI) are amongst the most important benzodiazepines in clinical use as sedatives and hypnotics.



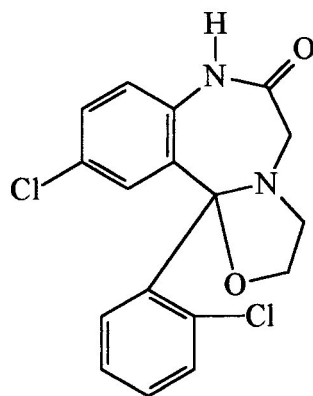
(XVII) Delorazepam



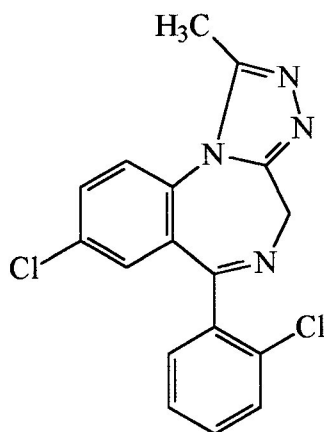
(XVIII) Lorazepam



(XIX) Lormetazepam



(XX) Cloxazolam



(XXI) Triazolam

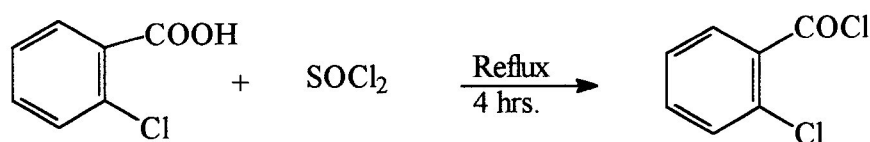
All these drugs are highly potent and safe.

All these drugs are synthesised from a common raw material, i.e. 2-amino-2',5-dichloro benzophenone. As it is totally imported at present, there is a great need to develop a simplified preparative process from readily available raw materials. Taking into account the importance of this specific fine chemical, synthesis of this chemical was included in the present work.

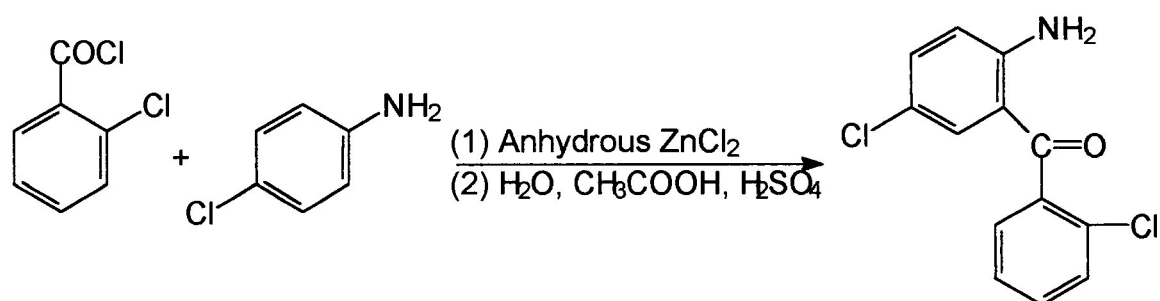
Synthesis of 2-amino-2',5-dichloro benzophenone is carried out by Friedel-Crafts acylation. The reaction involves condensation of 4-chloroaniline with 2-chlorobenzoyl chloride in presence of Lewis acid to form a ketone⁵⁹.

The synthesis involves two steps

1. Preparation of 2-chlorobenzoyl chloride
 2. Condensation of 2-chlorobenzoyl chloride with 4-chloroaniline
1. Acid chloride is prepared by refluxing 2-chlorobenzoic acid in thionyl chloride with DMF (in catalytic amount) for 4 hours.



2. Preparation of 2-amino-2',5-dichlorobenzophenone

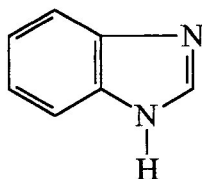


4-chloroaniline is added to 2-chloro-benzoyl chloride at 140°C to which is added (XXII)
anhydrous Zinc chloride at $180\text{--}200^\circ\text{C}$. The reaction mixture was stirred at 230°C for 2 hrs and poured in cold water. The resulting solid was refluxed in a hydrolysing mixture of water, acetic acid and conc. H_2SO_4 at 140°C for 4 hours. The mixture was allowed to cool and poured in crushed ice. 2-amino-2',5-dichlorobenzophenone separates as a greenish yellow solid. The raw materials required for the synthesis are indigenously available and cheap.

Chemistry

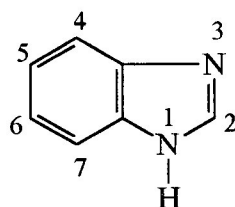
CHEMISTRY OF BENZIMIDAZOLE

The benzimidazole contains a phenyl ring fused to an imidazole ring as indicated in the structure (I)



(I)

Since compounds of this type are formed by the loss of water, they were called “anhydro - bases” in the very early literature. These compounds were formed only by compounds in which the nitrogen containing groups were ortho to each other. They were expressed as derivatives of Q -phenylene diamines. The numbering systems for benzimidazole (II) is as follows :



(II)

Benzimidazole, which contains a hydrogen atom attached to nitrogen in the 1 – position, readily tautomerises⁶⁰.



Synthesis of benzimidazole

The last systematic reviews on benzimidazoles were published in 1951⁶¹ and 1953⁶² although subsequent surveys have appeared in articles⁶³⁻⁶⁵ covering the chemistry of both imidazoles and benzimidazoles. In the latest reviews⁶⁶, a compilation of the synthesis, reactions and spectroscopic properties of benzimidazoles is presented.

General methods

Synthesis of benzimidazoles starts practically with o - phenylene diamine. In the literature survey, we have come across the following methods used for the synthesis of benzimidazoles :

- a) by reacting o - phenylene diamines with carboxylic acids⁶⁷⁻⁶⁸.
- b) by reacting o - phenylene diamines with acid chlorides^{69,70} ester⁷¹ or acid anhydride⁷².
- c) by reacting o - phenylene diamines with amide⁷³ or nitrile⁷⁴.
- d) by reacting o - phenylene diamines with aldehyde⁷⁵ or ketone⁷⁶.
- e) by reacting o - phenylene diamines with urea⁷⁷ or thiourea⁷⁸.
- f) by reacting o - phenylene diamines with isothiocyanate⁷⁹.

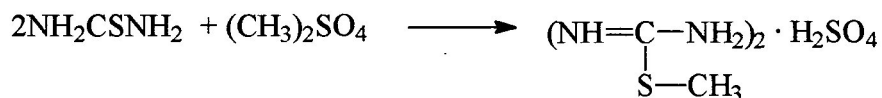
Properties of benzimidazoles

The benzimidazole nucleus is very stable in character. It does not get easily oxidized by reduction. Benzimidazoles are weakly basic, but somewhat less basic than imidazoles. Accordingly, they are, in general, soluble in dilute acids. Benzimidazoles are also sufficiently acidic to be soluble in aqueous alkali and form N- metallic compounds.

CHEMISTRY OF 2-METHYL THIOPSEUDO UREA –1,3 - DICARBOXYLIC ACID DIMETHYL ESTER

S – Methyl isothiurea sulphate : (Pseudourea – 2 – methyl – 2 – thiosulphate) :

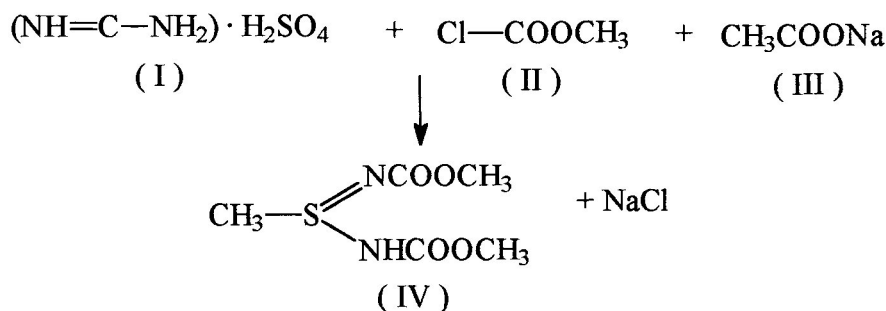
Two moles of thiourea were treated with 2 moles of dimethyl sulphate and refluxed for one hour. The compound was recrystallized from 95% ethanol. The compound was isolated in 79% yield.



2 – Methyl thiopseudo urea – 1, 3 – dicarboxylic acid dimethyl ester : (Carbamic acid [(methylthio) methylidene] – di – dimethyl ester) :-

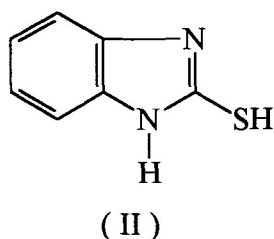
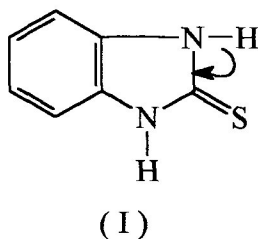
A solution of S – methyl isothiurea sulphate (I) (0.028 mole) in water was treated with methyl chloroformate (0.057 mole) (II) with stirring. Then the reaction mixture was treated with 10 % NaOH solution at low temperature and adjusted to pH 8.0. After 2hrs, it was treated with acetic acid and the pH was adjusted to 5.0. It was then treated with sodium acetate (III) (0.028 mole). Reaction mixture was allowed to rest overnight.

Next day, acetic acid and methyl chloroformate were distilled out under reduced pressure. The compound (IV) was precipitated on cooling, to obtain a yield of 70%.



CHEMISTRY OF 2 – MERCAPTOBENZIMIDAZOLE

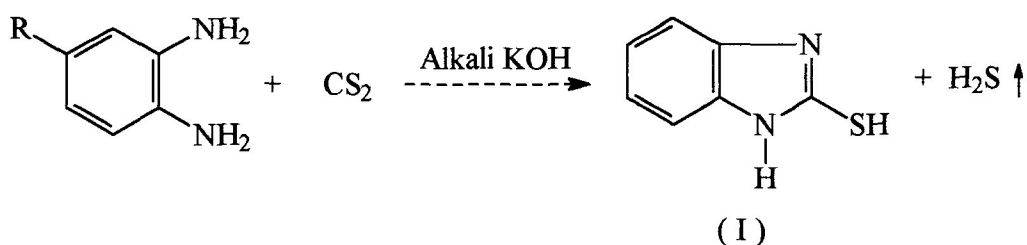
2 -(3H) benzimidazole thione (I) and 2 – mercapto benzimidazole (II) are tautomeric substances analogous to 2 – (3H) benzimidazolone and 2 – hydroxy benzimidazole.



Different methods for preparation of 2 – mercapto benzimidazole

a) Synthesis from O – phenylene diamine and carbondisulphide

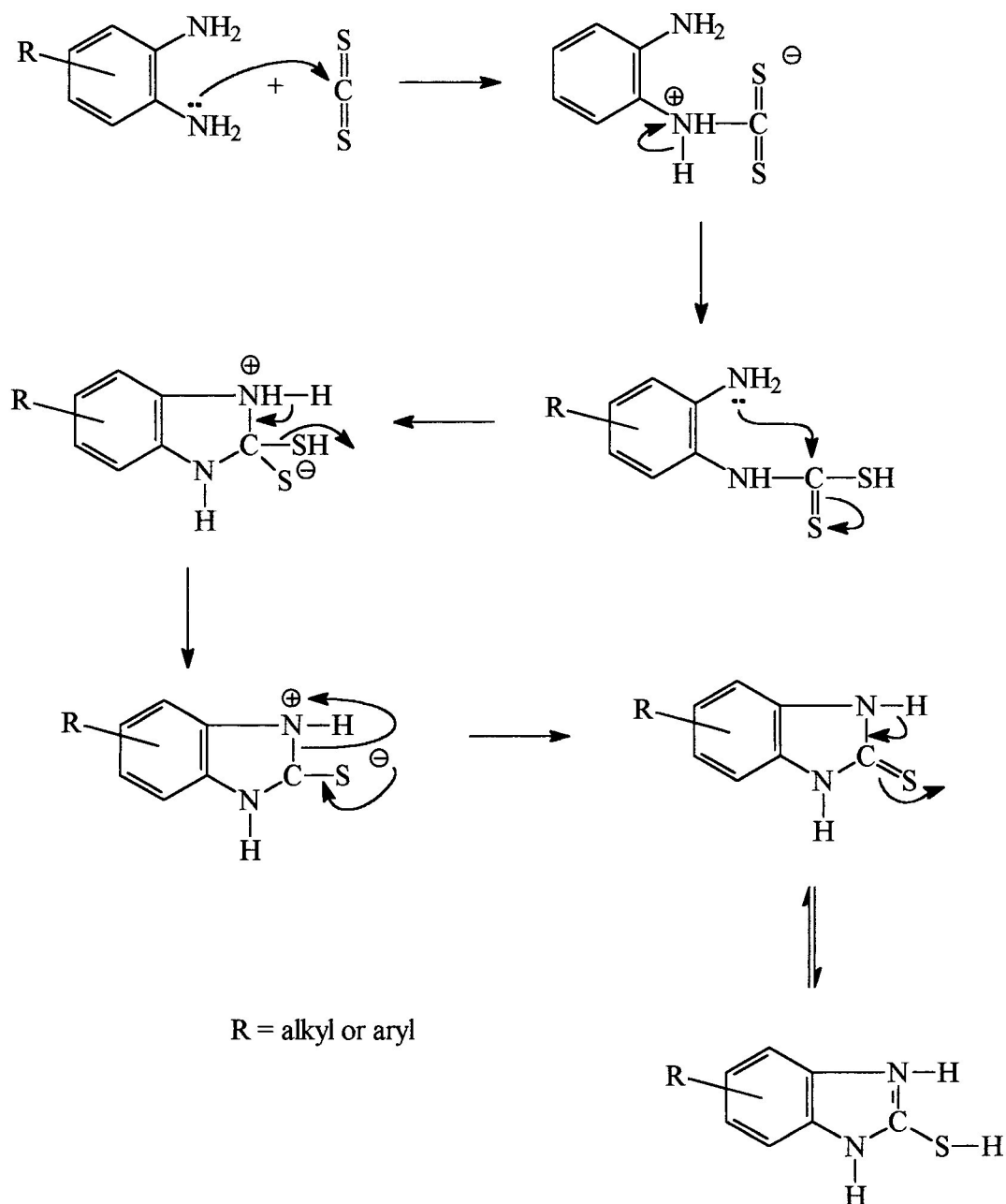
2 – mercapto benzimidazole, in general, may be prepared very conveniently by this method. The reaction is carried out usually by heating the reactants in alcoholic solution with or without the addition of alkali to the reaction mixture. 2 – mercaptobenzimidazole itself has been prepared by this method^{80,81}.



R = Alkyl of aryl group

O – phenylene diamines containing substituents on the phenyl ring have been used, which include those containing alkyl⁸², halogen⁸³, phenyl⁸⁴, aniline⁸⁵ and sulphonamido⁸⁶ groups.

Probable reaction mechanism



b) Synthesis from O – phenylenediamine and thiourea

This reaction is analogous to the preparation of 2 – hydroxy benzimidazole from O – phenylene diamines and urea. Substituted – 2 – mercaptobenzimidazoles (V) may be prepared by this method by heating substituted O – phenylene diamine dihydrochloride (III) and thiourea (IV) to 170 – 180°C⁸⁷. Excellent yields have been obtained by carrying out the reaction in amyl alcohol and heating under reflux until the evolution of ammonia becomes negligible.


$$\text{R-C}_6\text{H}_3(\text{NH}_2)_2 + \text{CSCl}_2 \xrightarrow{-2\text{HCl}} \text{R-C}_6\text{H}_3\text{N}_2\text{SH}$$

R = Alkyl or aryl

2 – mercaptobenzimidazole can be prepared in 84 – 86.5 % yield by the action of potassium ethyl xanthate on O – phenylene diamine⁹⁰. 2 – mercaptobenzimidazoles have also been prepared from O – phenylene diamine dihydrochloride and ammonium thiocyanate. A solution of the two substances is usually evaporated to dryness on a steam- bath and then heated for short time. By this method, 2 – mercaptobenzimidazole⁸³ and 5 – chloro⁹¹, 4 – methyl⁹² and 5 – methyl derivatives of 2 – mercaptobenzimidazole have been prepared.

CHEMISTRY OF ACID CHLORIDES

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

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



The use of thionyl chloride⁹³ for the preparation of acid chlorides presents many advantages in a large number of cases. The reaction results in the formation of sulphur dioxide and hydrogen chloride in addition to the acid chloride, and the isolation of the chloride, is for this reason, a simple matter.

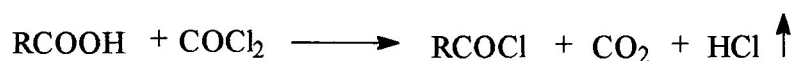


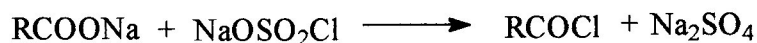
The thionyl chloride must be pure, as the impure product has been known to cause somewhat explosive decomposition in some instances. The procedure consists of mixing the chlorinating agent with acid and heating the mixture, if necessary. The product may be isolated by fractional distillation or if solid,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

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



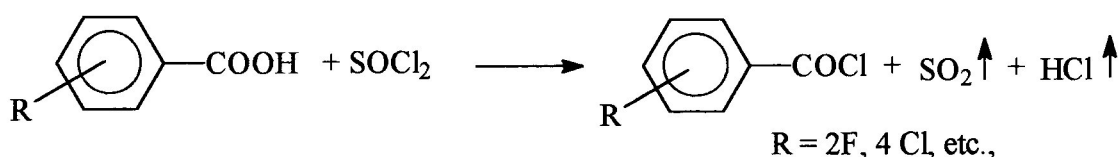
The reaction of phosgene⁹⁴ with carboxylic acids, and *p* – toluene sulphochloride or of sodium chlorosulphonate with salts of carboxylic acids are also used for the preparation of acid chlorides.





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

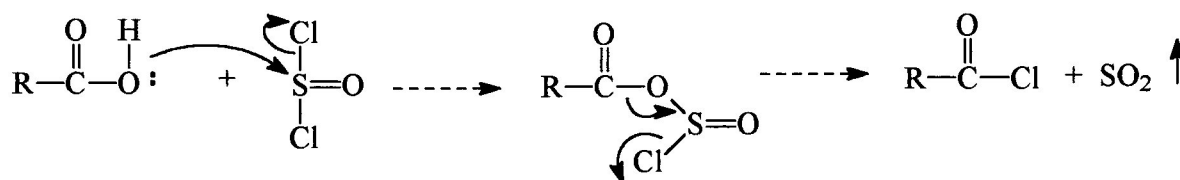
In the present work all the acid chlorides were prepared by reacting thionyl chloride with corresponding acids.



Reaction mechanism :



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

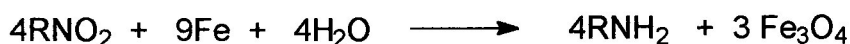


CHEMISTRY OF REDUCTION OF NITRO COMPOUNDS

It has long been known that the nature of the product actually formed depends critically upon the reduction conditions. Thus, although nitro compounds were reduced by using metals such as iron, tin, zinc (in acidic condition), sodium sulphide, ammonium polysulphide (in alkaline medium), ferric chloride, platinum dioxide and Raney Nickel etc., the preferred reduction reaction was performed by using activated Raney Nickel^{95,96}.

Reduction of nitro compounds involves the progressive replacement of oxygen in the - NO₂ group by hydrogen. Under suitable conditions, aromatic nitro compounds are readily reduced to amino compounds by means of acidic reducing systems such as metals as iron and tin. In the solution of the metal in acetic acid, the nitro compound is reduced and the amine may be recovered from the reaction mixture by making it alkaline, by steam distillation or filtration.

If catalytic methods are eliminated, iron and zinc are the metals of choice for reductive procedures, in the research laboratory. The advantage of iron is that only a catalytic amount of acid is needed to be added and the iron oxide precipitates out to permit a relatively simple separation.



The disadvantage is that the quality of iron powder from various commercial sources is variable. Some powders give spontaneous, exothermic reduction. Reduction with this reagent may be carried out in benzene or aqueous alcohol. Furthermore, iron powder in acetic acid is capable of reducing aliphatic, optically active nitro compounds to the amines with the retention of configuration⁹⁷. Indeed, iron powder and acetic acid is a favorable combination for reduction of aromatic nitro compounds. On the other hand, basic reducing agents such as lithium aluminium hydride lead to racemization.

Zinc is a powerful reducing agent, but utilization of its reducing action is wasteful because of rapid reaction with an acid such as HCl. Successful efforts are to be found out when an abundance of zinc is supplied during the entire reaction period. If

the experimenter is fortunate, the hydrochloride of amine will separate but if unfortunate, he must make the entire mixture strongly alkaline and extract with suitable solvent. In this, very often a problem of emulsion formation comes across.

The present authors are impressed with the smooth generation of hydrogen from mossy tin and acid, but prejudiced the difficulty of work-up if the amine hydrochloride does not precipitate from the acid solution. The entire mixture should be added slowly to a strong alkaline solution. If tin hydroxide precipitates and remains for any length of time, no reasonable amount of sodium hydroxide dissolves the flocculent solid. Certainly, this method does not lend itself well to large scale reductions. On the other hand, stannous chloride is a useful and selective reducing agent for nitro groups when carbonyl groups are present such as in reduction of *m* – nitrobenzaldehyde.

Amalgamated Aluminium is another reducing agent best adopted to neutral or basic reducing conditions. For example, 2-nitroacenaphthene suspended in boiling ethanol, when treated with amalgamated Aluminium, and then portionwise with boiling water, is reduced in 85% yield to amino compound.

Reduction using Raney Nickel

Since all the amine derivatives are highly susceptible to oxidation, the reduction was done by catalytic hydrogenation using Raney nickel as catalyst.

Catalytic methods :

This method^{98,99} is clean and work-up is so easy that it should be considered the method of choice in the research laboratory. The only pitfalls are the possibility of poisoning of the catalyst or, for large scale reductions, the inability to dissipate adequately the heat of hydrogenation¹⁰⁰.

The most common catalysts for the hydrogenation of nitro compounds are platinum (low pressure) and Raney Nickel (high pressure). Supporting catalyst such as platinum on carbon^{101,102}, palladium on carbon^{103,104,105} are used.

Solvents and media used for catalytic reductions

Any solvent that is pure and does not react with substrate or products of reduction can be used. Water is the safest solvent but many organic compounds are insoluble in it. The most commonly used ones are alcohol, particularly, methyl and ethyl alcohols. The vapours of methyl alcohol, however, may ignite in presence of palladium, platinum catalyst and some times with Raney Nickel (depending on its activity). Nevertheless, it is safe if the catalyst is first added to some water or methyl cellosolve, provided that neither of these affects the reaction. Once the catalyst is completely covered, the alcohol can be safely added.

Benzene may be used (only if thiophene-free), although it attacks the rubber stoppers in the parr apparatus and is itself strongly bound to the surface of most catalysts, resulting in decrease in reaction rate.

Solvents that form peroxide should be avoided. For example, dioxane should be used after thorough purification and distillation.

Addition of catalyst :

In general, catalysts active in low pressure reductions, can be safely added to ethyl alcohol. If a large amount of catalyst is to be used, it should be added in portions and mixed until it is wetted with the solvent before another addition is made.

One should not attempt to carry out reduction in pressure reactor, filled beyond 60-65% capacity, because a free space that is too small results in poor mixing and prolonged reaction time.

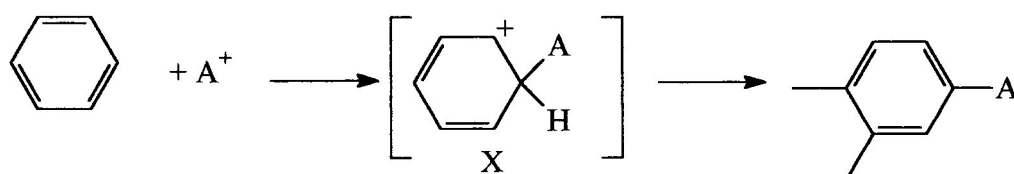
Equipment :

For operation, the vessel containing solvent, reactant and catalyst is placed in the reactor, hydrogen is added upto 150 psi and the stirrer is started with heating. The progress of reaction is followed by observation of the drop in pressure. Although, catalytic hydrogenation is potentially hazardous, with proper care it can be as safe as any chemical reaction. After hydrogenation most catalysts (except for copper chromate) are more pyrophoric because of hydrogen absorbed on their surface during the reduction. Therefore, care must be taken during filtration. The filter paper

must be covered with liquid at all times and not allowed to become dry. Any spent catalyst can be destroyed by digestion in hydrochloric acid. Do not heat the reaction mixture to the desired working temperature before starting agitation. This may be safe under low pressure conditions but can be hazardous in high pressure reductions.

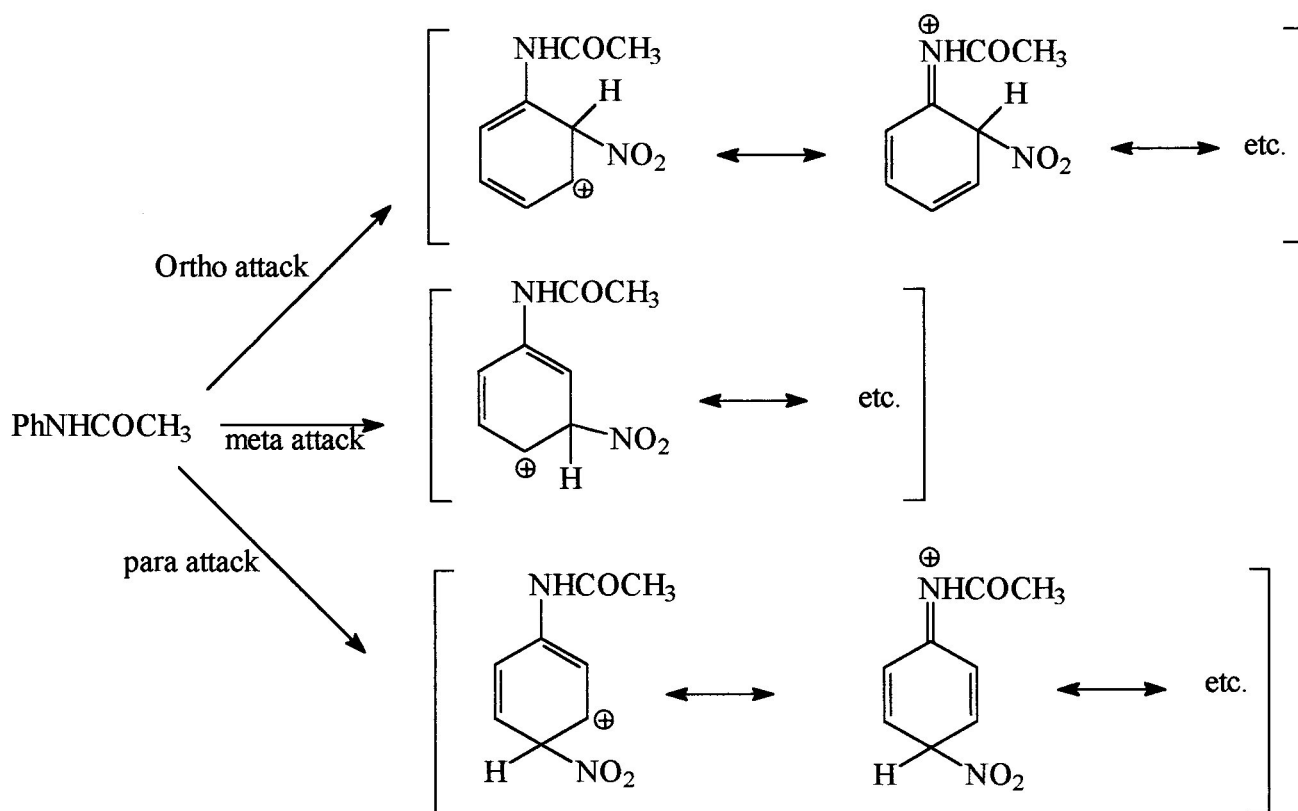
The overall yields of catalytic hydrogenation in our experiments are 60-80% and the product obtained in this case is in fairly pure state.

Nitration : Of the various types of electrophilic-substitution reactions, nitration is most widely studied. The attacking electrophile in these reactions is often, though not always, the nitronium ion NO_2^+ . In great majority of such reactions, the leaving group is the proton, and the reaction then requires the breakage of a rather strong C-H bond. With aromatic substrates, however, there is one important circumstance that favors electrophilic substitution. The attacking electrophile may form a new bond before the old bond breaks, and the energy that becomes available when the new bond forms may be used to help bring about the breakage of the old bond. It might then be anticipated that the attack on an aromatic system by an electrophile A^+ will pass through an intermediate of the type X, in which both the attacking species and leaving group are bound to the substrate.



Nitration of acetanilide :

Representative structures for each of the possible cation intermediates are shown as follows¹⁰⁶ :



In the intermediate for *meta* attack, the positive charge carried in by the NO_2^+ ion must remain on the benzene ring until the proton departs, completing the substitution reaction. On the other hand, with *ortho* or *para* attack, π electron density drifts from the electron rich nitrogen atom into the ring, dispersing the positive charge, and lowering the energy both of the intermediate and the transition state leading to it. It is thus concluded that in the nitration of acetanilide, *ortho* and *para* positions are readily attacked than *meta* positions, although it can not be said which of the two positions is more reactive.

In case of acetanilide, nitrogen bound to ring has one unshared and available pair of electron, and in each case electron density from this pair drifts into the ring to help disperse the positive charge carried in by the electrophile attacking either at the

ortho or the *para* position. If the *para* position of the acetanilide is blocked, *ortho* substituted isomer is obtained.

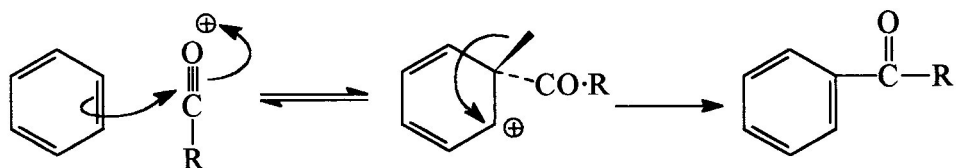
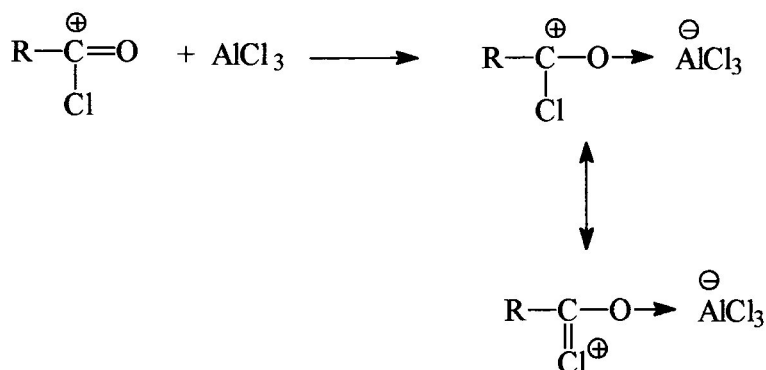
Friedel-Crafts acylation :

The acylation reaction is formulated as an attack of the ring by an electrophilic agent, which is often represented as the acylium ion ($R-C^+=O$). The acylating agent usually is obtained from an acid chloride or anhydride by treatment with a Lewis acid. The resulting Lewis salt is the source of the acylating agent.

The oxygen atom of the carbonyl group co-ordinates with Lewis acid to give a complex in which the electron deficit of the carbon atom of the carbonyl group is enhanced.

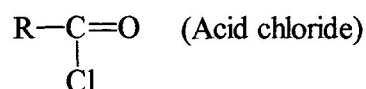
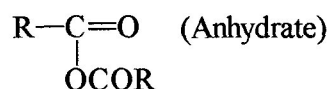
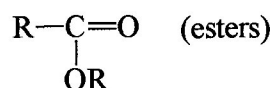
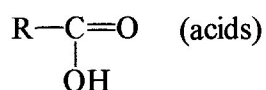
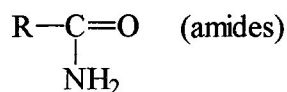


Acylium ion formed from acid chlorides are more stable than ordinary carbanium ions because the electron shortage is delocalised to the oxygen and halogen atom.



Although acid chlorides are the most common acylating agents, other acid derivatives and even the acids themselves can be used. On the basis of the known

order of increasing electron releasing power of atoms and groups, these acylating agents fall in the following order of increasing effectiveness¹⁰⁷.



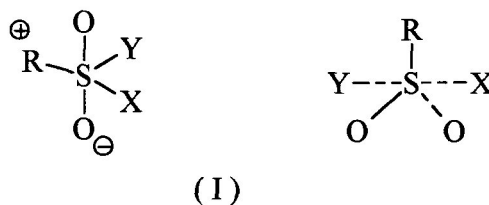
The acylation reaction is affected by many factors other than the character of the reactants, like temperature, time, pressure, degree of mixing and manner of introduction of the reagents.

Lewis acids react with ketones to form stable complexes that are removed as the reaction proceeds; for this reason, it is necessary to use at least one mole of the catalyst.

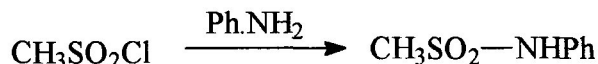
In case of Friedel-Craft acylation reactions, when *para* is unsubstituted, the *p*-isomer is the chief product. When *p*-position is blocked, *o*-derivatives are formed.

Chemistry of sulfonyl chloride.

Sulfonamides are highly crystalline, particularly the derivatives from sulfonyl chloride. The preparation of sulfonamide from sulfonyl chloride is a nucleophilic substitution at RSO_2Cl in a manner similar to the attack of R-COCl , but the mechanism is not the same because a 'tetrahedral' intermediate in this case (I) would have five groups on the central atom¹⁰⁸.



This mechanism more closely resembles the $\text{S}_{\text{N}}2$ 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 :



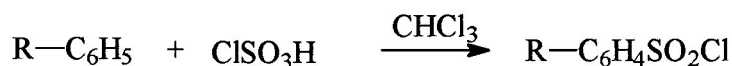
Preparation of sulfonyl chloride :

The sulfonyl chlorides are prepared by various methods.

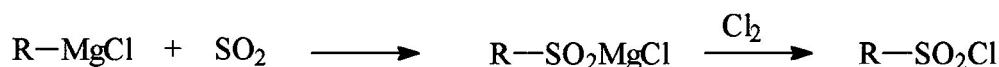
1. Sulfonyl chloride is made by reacting sodium sulfonate with PCl_5 or POCl_3 at 180°C ¹⁰⁵. In this reaction, benzene sulfonate reacts with the PCl_5 or POCl_3 to yield benzene sulfonyl chloride.



Chlorosulfonic acid is utilized for the conversion of aromatic hydrocarbons to sulfonyl derivatives and can be used in the characterization of unknown hydrocarbons¹⁰⁹. In this, chlorosulfonyl chloride is used to provide the sulfonyl chloride ($-\text{SO}_2\text{Cl}$) 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 chloroform¹¹⁰.

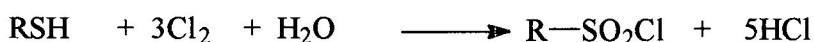


Sulfonyl chlorides have also been prepared by the reaction of Grignard reagent first with SO_2 , and then with chlorine gas¹¹¹.



Aliphatic hydrocarbons can also be chlorosulfonated with sulfonyl chloride derivatives¹¹², e.g. 2,3-dimethyl butane yields 20% of 2,3-dimethyl butane-1-sulfonyl chloride. More recently, photolytic chlorosulfonation of alkane has been reported to yield alkanesulfonyl halide^{113, 114}. In this mixture C_{10} - C_{20} alkane sulfonyl chlorides were prepared by photolytic chlorosulfonation of a paraffin mixture with chlorine and a large excess of SO_2 . 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 chloride¹¹⁵. 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 joined directly to a carbon of heterocyclic ring. In such a case, sulfonyl chlorides (sulfonamide) are usually prepared by chlorosulfonation or conversion to sulfonic acid and acid chloride, followed by the amidation to get sulfonamide. The oxidative

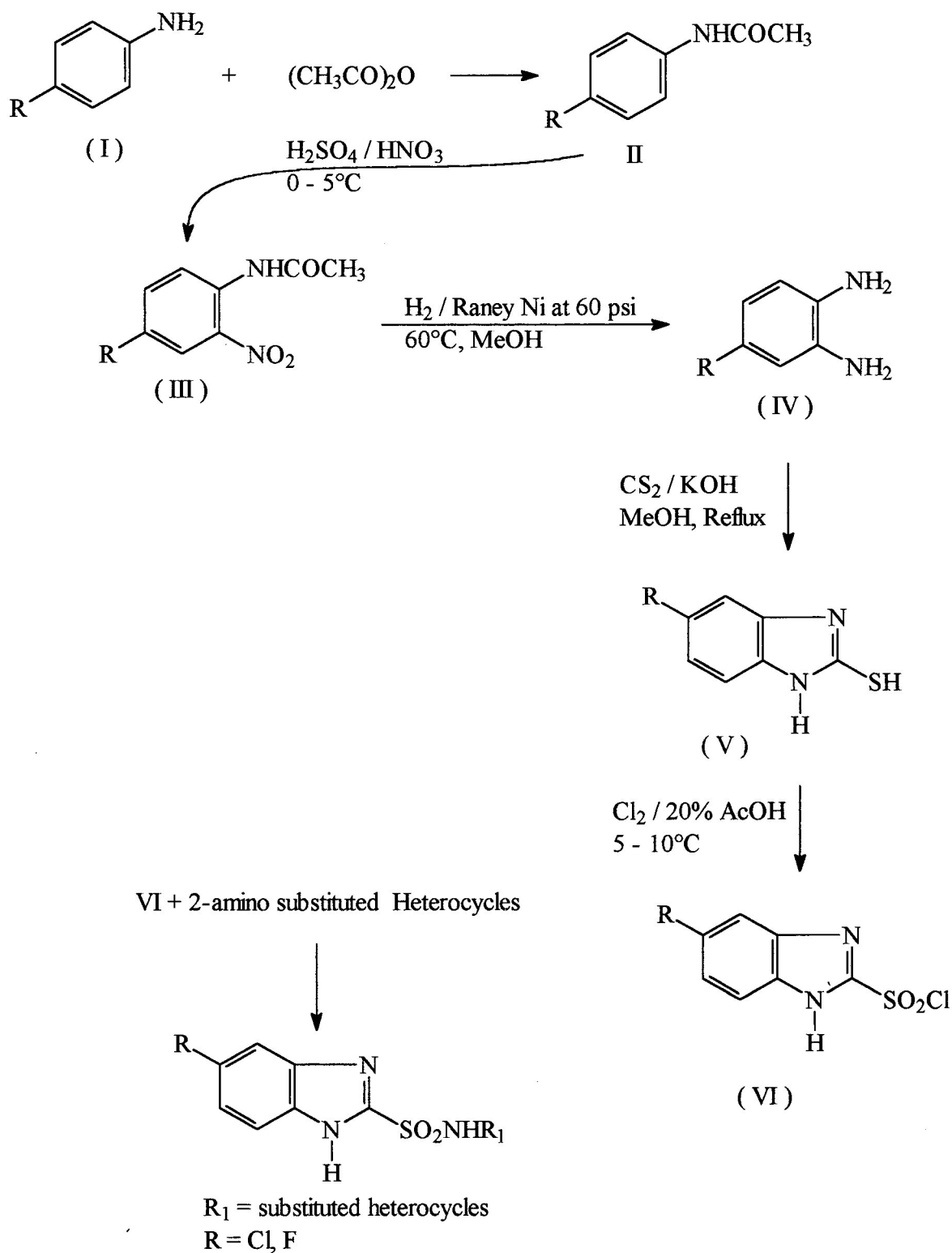
chlorination at low temperature of such heterocycles has been found to be quite widely applicable to the preparation of these compounds.

Same 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 give the corresponding chloro derivative, e.g. 2-mercaptobenzimidazole converted to 2-chlorobenzimidazole when the reaction is carried out for a long period at room temperature¹¹⁶.

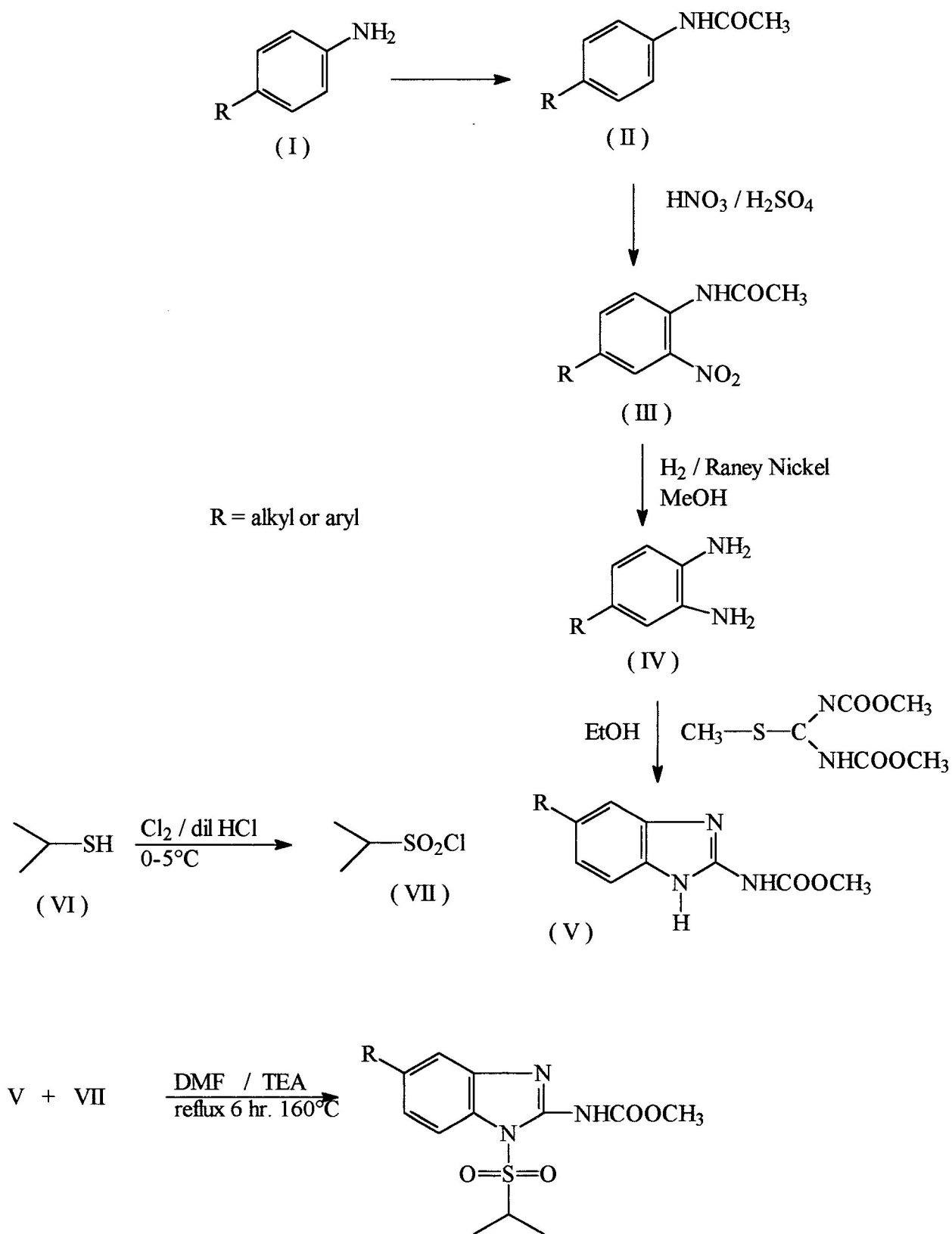
SCHEMATIC REPRESENTATION OF THE SERIES OF COMPOUNDS SYNTHESISED

Scheme II

Synthesis of 5-chloro(or fluoro)-benzimidazole-2-sulfonamide



Synthesis of 1-isopropylsulfonyl-2-methyl carbamate-5-(alkyl/aryl-substituted) benzimidazoles



Synthetic Part

Synthesis of 5-chloro (or fluoro)-2-benzimidazole sulfonamides

Synthesis involves following steps :

1. preparation of 4-chloroacetanilide
2. preparation of 2-nitro-4-chloroacetanilide
3. preparation of 4-chloro-1,2-phenylenediamine
4. preparation of 4-chloro-2-nitro aniline
5. preparation of 5-chloro-2-mercaptobenzimidazole
6. preparation of 5-chloro-2-benzimidazole
7. condensation of 5-chloro-2-benzimidazole sulfonyl chloride with various 2-amino heterocycles.

1. Preparation of 4-chloroacetanilide

In a dry conical flask placed in ice-bath, 12.75 g (0.1M) 4-chloroaniline was dissolved in 20 ml of acetic acid. To this was added 10.2 g (9.5 ml, 0.1M) of acetic anhydride, dropwise with vigorous stirring. Stirring was continued till all the acetic anhydride was added. The reaction mixture was allowed to stand for half an hour in ice-bath and solid separated was filtered. It was washed thoroughly with ice-cold water and dried. It was recrystallised from methanol.

Yield - 12.9 gm, 76%

M.P. 170°C

Reported¹¹⁷ M.P. 169.5°C

2. Preparation of 4-chloro-2-nitroacetanilide

In a conical flask placed in an ice-salt bath was taken 8.45 g (0.05M) of 4-chloro acetanilide. To this was added 10 ml of conc. H_2SO_4 and it was stirred vigorously to form fine suspension. Temperature of the mixture was kept below 0°C. To this was added a mixture of 3.15 g (2.2 ml, 0.05M) of HNO_3 ($d = 1.42$) and 4.9 g (2.66 ml, 0.05M) of conc. H_2SO_4 dropwise with vigorous stirring so that temperature does not cross 0°C. When all the mixture was added, the reaction mixture was allowed to stand below 0°C for 15-20 minutes and dumped in crushed ice. The solid was filtered and washed with cold water till the pH of water was neutral. Solid was dried in hot air oven at 60°C for 4 hours. It was recrystallised from methanol.

Yield = 7.2 g, 67.35%

M.P. = 104°C

Reported¹¹⁸ M.P. = 104°C

3. Preparation of 4-chloro-2-nitroaniline

In a round bottom flask equipped with water condenser was placed 10.7 g (0.05M) of 4-chloro-2-nitroacetanilide. It was dissolved in 40 ml of methanol. To this was added 2 g (0.05M) of NaOH and the reaction mixture was refluxed for 2 hours on water bath. Any solid separated was filtered off and methanol was removed under reduced pressure. The residue was dumped in cold water and solid separated was filtered. It was recrystallised from methanol.

Yield = 6.2 g (72%)

M.P. = 115°C

Reported¹¹⁸ M.P. = 116-117°C

4. Preparation of 4-chloro-1,2-phenylenediamine

5 g (0.028M) of 4-chloro-2-nitroaniline was dissolved in 50 ml of methanol in a pressure vessel. To this was added 0.5g of activated Raney Nickel (Nitro to amino) and pressure vessel was closed. 60 psi of Hydrogen gas was passed in the pressure vessel and the reaction mixture was stirred at 60°C. Reaction was monitored on TLC. When reaction was completed mixture was filtered to separate Raney Nickel. It was destroyed in dil. HCl. The filtrate was given charcoal treatment and methanol was removed under reduced pressure to obtain solid.

Yield = 3.2 g, 77.66%

M.P. = 75°C

Reported¹¹⁹ M.P. = 76°C

5. Preparation of 5-chloro-2-mercaptobenzimidazole

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

and acidified with dil. HCl till pH of the solution becomes acidic. The solid separated was filtered and dried.

Yield = 6.2 g, 67.2 %

M.P. = 289-290°C

Reported¹²⁰ M.P. = 290-292°C

6. Preparation of 5-chloro-2-benzimidazole sulfonyl chloride

In a three necked round bottomed flask equipped with an air condenser, gas inlet tube and a stopper, 6.15 g (0.33M) 5-chloro-2-mercaptobenzimidazole was suspended in 150 ml of 20% acetic acid. Flask was placed in ice-bath so that the temperature of the reaction mixture does not exceed 5-8°C. Chlorine gas was bubbled through the mixture while stirring it magnetically. Chlorine was passed for 1 hour and the mixture was filtered on suction and air-dried. It was used immediately after drying.

Yield = 5 g , 59.8%

7. Preparation of final compounds by condensation of 5-chloro-2-(1-H) benzimidazole sulfonyl chloride and 2-amino heterocycles.

Preparation of 2-[(2'-pyridinyl)-sulfonamido]-5-chloro (1-H) benzimidazole.

In a dry three necked flask equipped with a water condenser, and guard tube, dropping funnel, stopper and magnetic needle, 1 g (0.0039M) of 5-chloro-2-benzimidazole sulfonyl chloride was dissolved in 10 ml of dry acetone. To this was added 0.39 g (0.55 ml, 0.004M) of triethylamine. The mixture was stirred so as to reach temperature of 60°C. At this temperature, 0.41 g (0.039M) of 2-aminopyridine dissolved in 5 ml of dry acetone was added dropwise through the dropping funnel over a period of 15 minutes. The reaction mixture was then stirred at 60°C for 3 hours. The white solid separated was removed by filtration. Filtrate was concentrated to get the desired product, 5-chloro-2-[2'pyridinyl] sulfonamido benzimidazole. It was crystallised from methanol.

Yield = 0.6 g, 48%

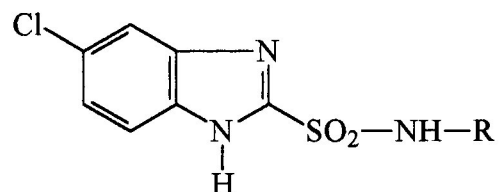
M.P. = 145°C

	C	H	N
Calculated	46.67	2.91	18.1
Found	46.42	2.84	17.96

Similarly, other compounds in this series were prepared and are listed in Table No.

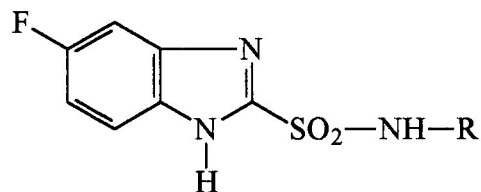
1. Similarly sulfonamides of 5-fluoro-2-mercapto benzimidazole were prepared and are listed in Table No. 2.

TABLE 1 : 5-CHLORO-2-BENZIMIDAZOLE SULFONAMIDES

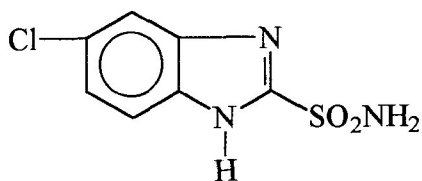


Sr. No.	R	Molecular Formula	Mol. Wt.	M .P. °C	Yield %	Elemental calculated			Elemental found		
						C	H	N	C	H	N
1	Hydrogen	C ₇ H ₆ ClN ₃ O ₂ S	231.5	265	52	36.28	2.59	18.01	36.1	2.49	17.9
2	Pyridine	C ₁₂ H ₉ ClN ₄ O ₂ S	308.5	145	58	46.67	2.91	18.1	46.42	2.84	17.96
3	Thiazole	C ₁₀ H ₇ ClN ₄ O ₂ S ₂	314.5	181-182	59	38.15	2.22	17.8	38.44	2.18	17.62
4	Pyrimidine	C ₁₁ H ₈ ClN ₅ O ₂ S	309.5	202-204	55	42.64	2.58	22.61	42.95	2.55	22.34
5	4,6-dimethyl pyrimidine	C ₁₃ H ₁₂ ClN ₅ O ₂ S	337.5	230	68	46.22	3.55	20.74	46.1	3.52	20.44
6	4-methyl pyridine	C ₁₃ H ₁₁ ClN ₄ O ₂ S	322.5	182	65	48.37	3.41	17.36	47.9	3.45	17.18
7	5-nitro thiazole	C ₁₀ H ₆ ClN ₅ O ₄ S ₂	359.5	270	69	33.37	1.66	19.47	33.25	1.72	19.28
8	Thiadiazole	C ₉ H ₆ ClN ₅ O ₂ S ₂	315.5	168	64	34.23	1.9	22.18	33.95	1.55	21.97

TABLE 2 : 5-FLUORO -2-BENZIMIDAZOLE SULFONAMIDES

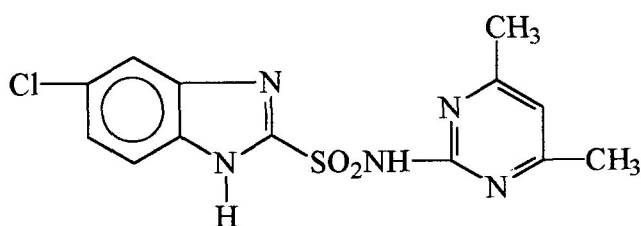


Sr. No.	R	Molecular Formula	Mol. Wt.	M .P. °C	Yield %	Elemental calculated			Elemental found		
						C	H	N	C	H	N
1	Hydrogen	C ₇ H ₆ FN ₃ O ₂ S	215	232	62	39.06	2.79	19.53	38.79	2.33	19.41
2	Pyridine	C ₁₂ H ₉ FN ₄ O ₂ S	292	187	68	49.31	3.08	19.17	48.91	2.94	18.87
3	Thiazole	C ₁₀ H ₇ FN ₄ O ₂ S ₂	298	144	58	40.26	2.34	18.79	39.94	1.98	18.43
4	Pyrimidine	C ₁₁ H ₈ FN ₅ O ₂ S	293	207	65	45.05	2.73	19.11	44.86	2.54	18.84
5	4,6-dimethyl pyrimidine	C ₁₃ H ₁₂ FN ₅ O ₂ S	321	212	69	48.59	3.73	21.8	48.61	3.66	21.7
6	4-methyl pyridine	C ₁₃ H ₁₁ FN ₄ O ₂ S	306	152	56	50.98	3.59	18.3	50.67	3.29	18.0



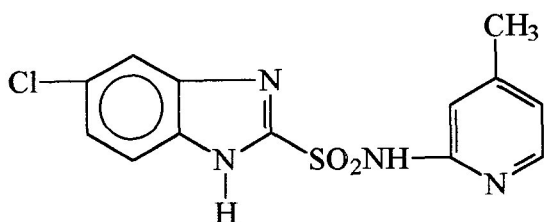
1180.95, 1343.84
3316.2
704.98
817.736, 980.631
1431.41, 1469.07

sulfonamide
free amine group
chlorine
5-substituted benzimidazole
C-C skeletal model of C₆H₆



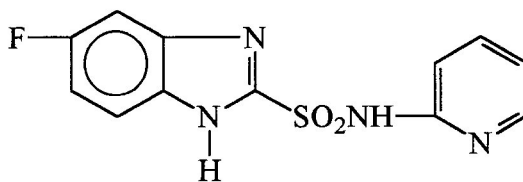
3303.48
2921.68, 2990.41
1187.31, 1343.84
742.647
1231.09
999.464
930.49, 924.095

NH of sulfonamide
methyl group of pyrimidine
sulfonamide
chlorine
CH in plane deformation vibrations of pyrimidine
pyrimidine
5-substituted BI

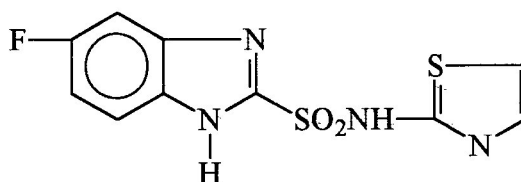


3309.84
2977.94
742.647
1343.84
1481.55
805.262, 986.746
1218.61

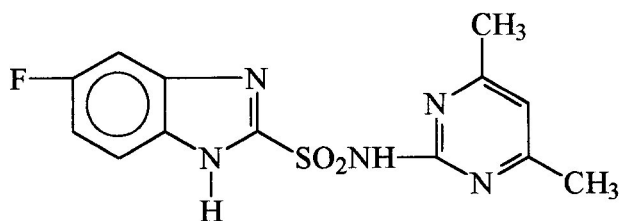
NH stretching
methyl group
chlorine
sulfonamide
C-C skeletal model of C₆H₆
5-substituted benzimidazole
pyridine



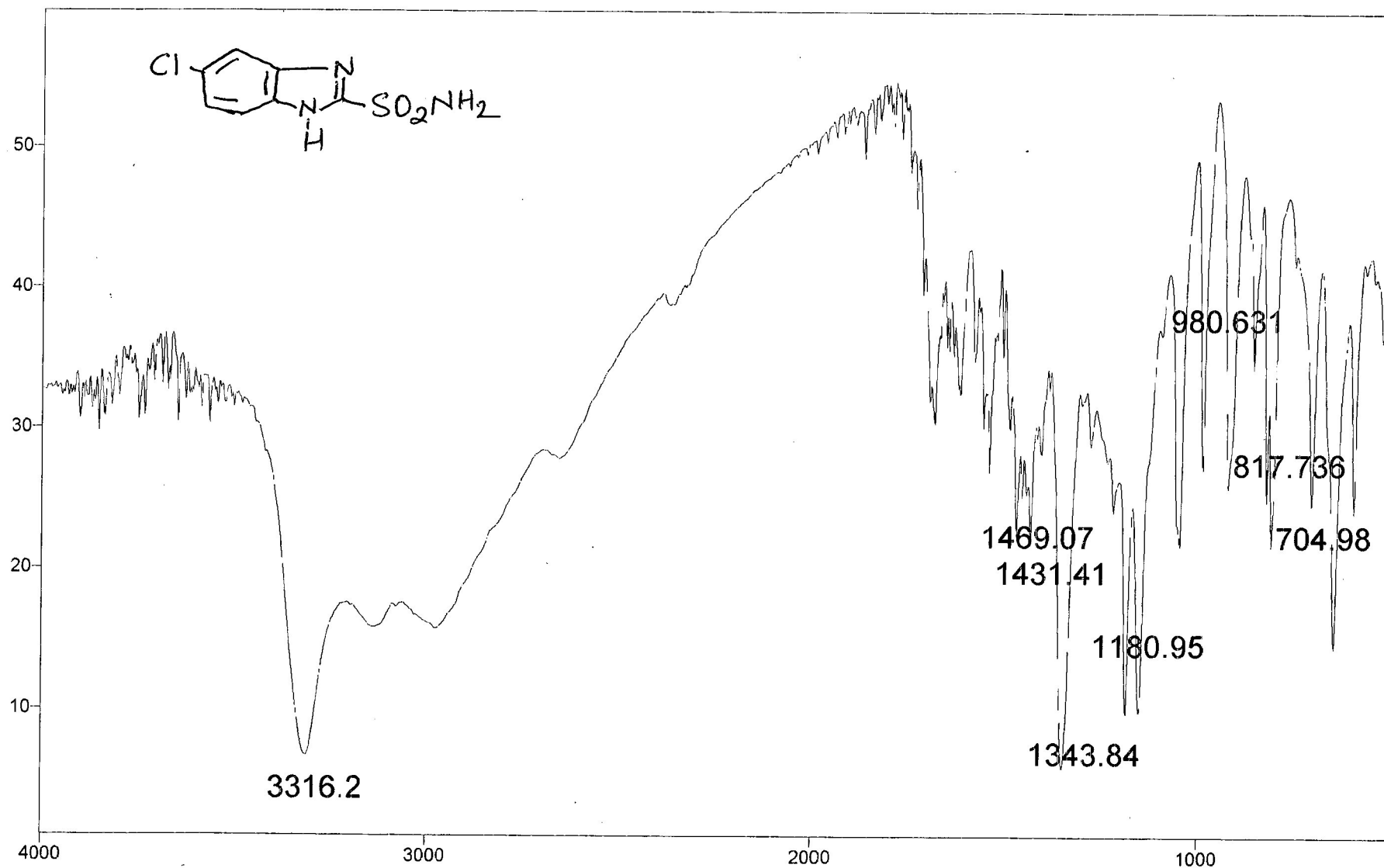
1337.48	sulfonamide
3309.84	NH stretching
1055.72	fluorine
980.631, 836.569	5-substituted benzimidazole
1462.71	C-C skeletal model of C ₆ H ₆
1105.86	pyridine

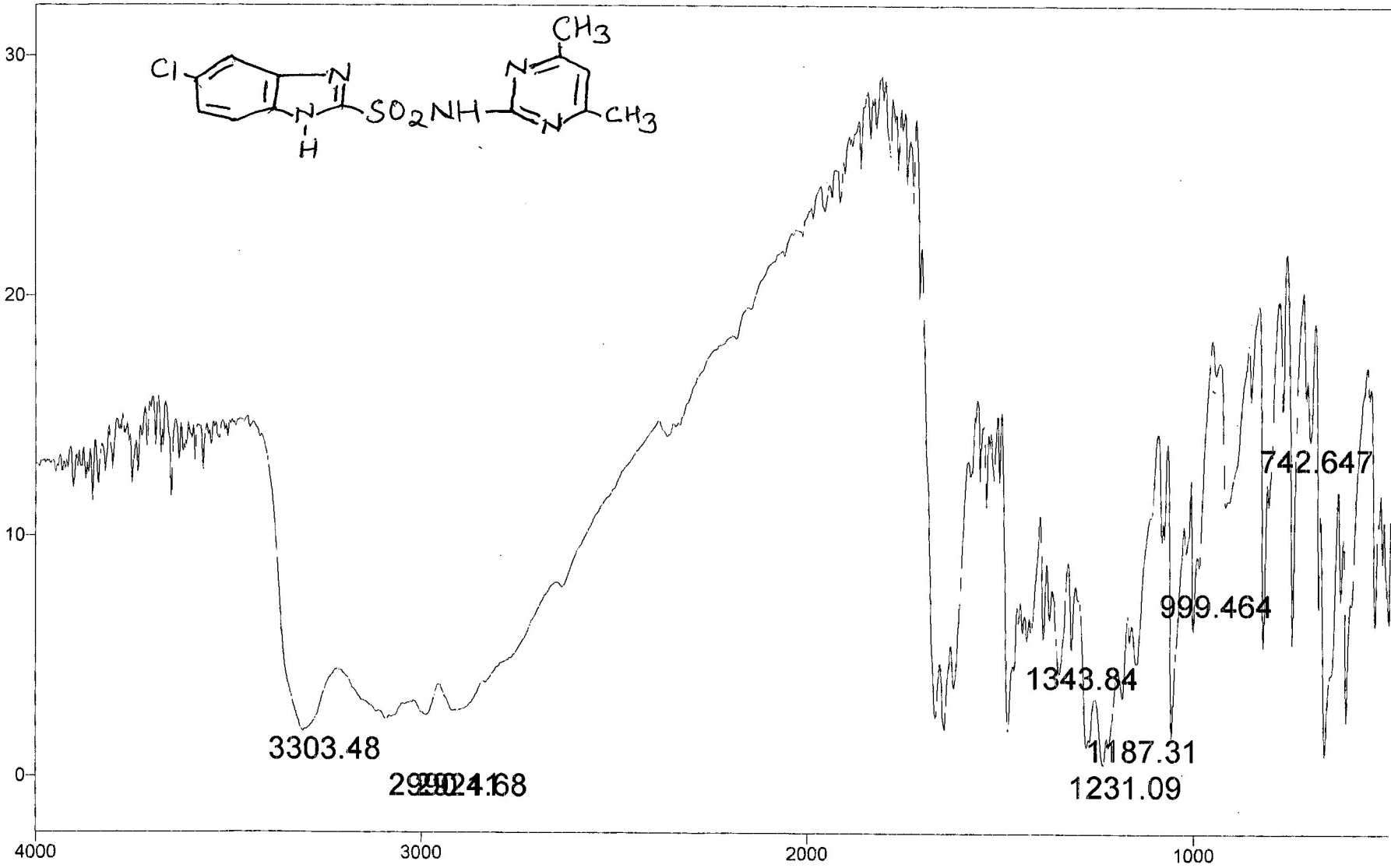


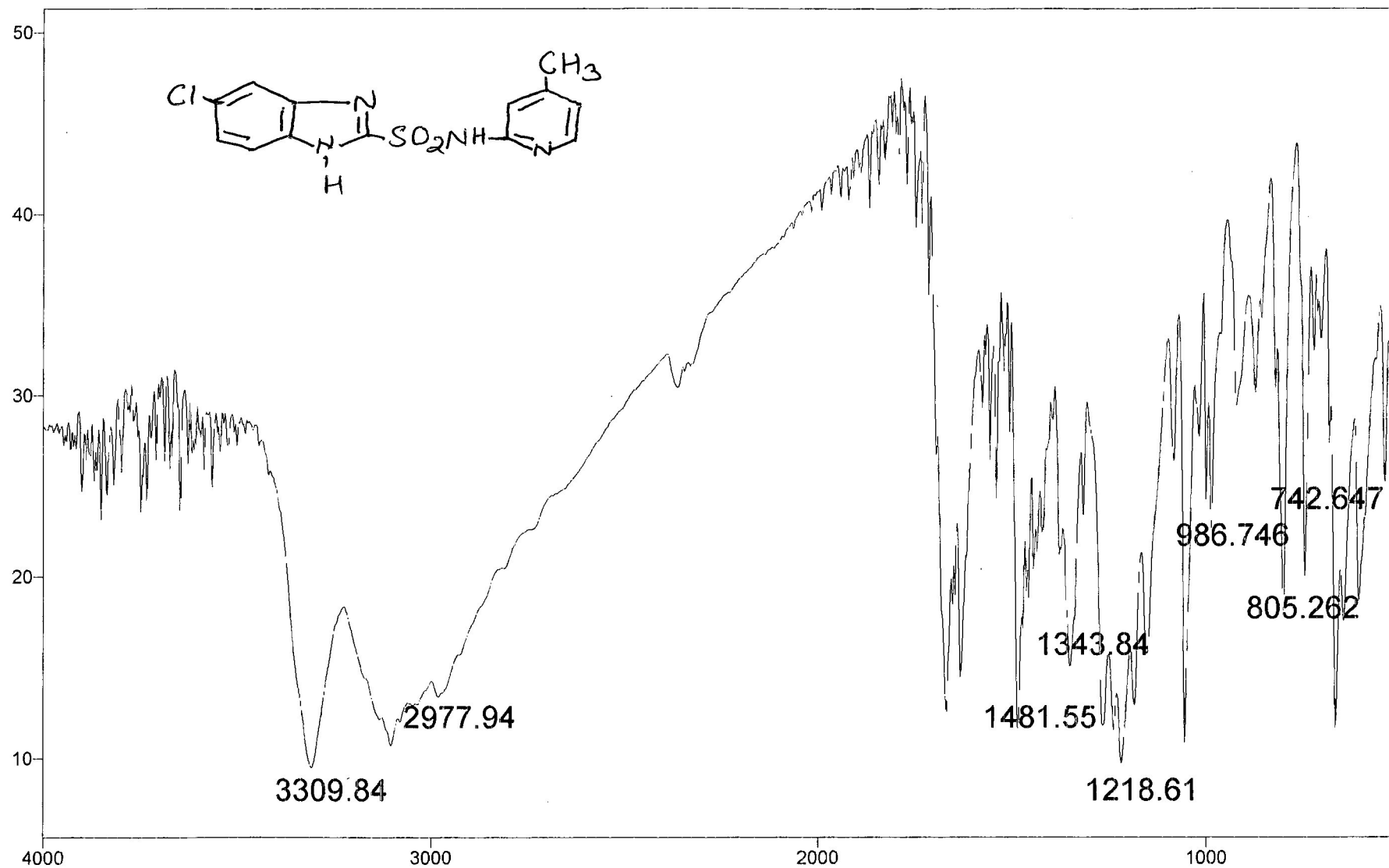
3460.02	NH stretching
1306.18	sulfonamide
1099.5	fluorine
980.631, 830.21	5-substituted benzimidazole
1437.77	C-C skeletal model of C ₆ H ₆
1055.72	thiazole

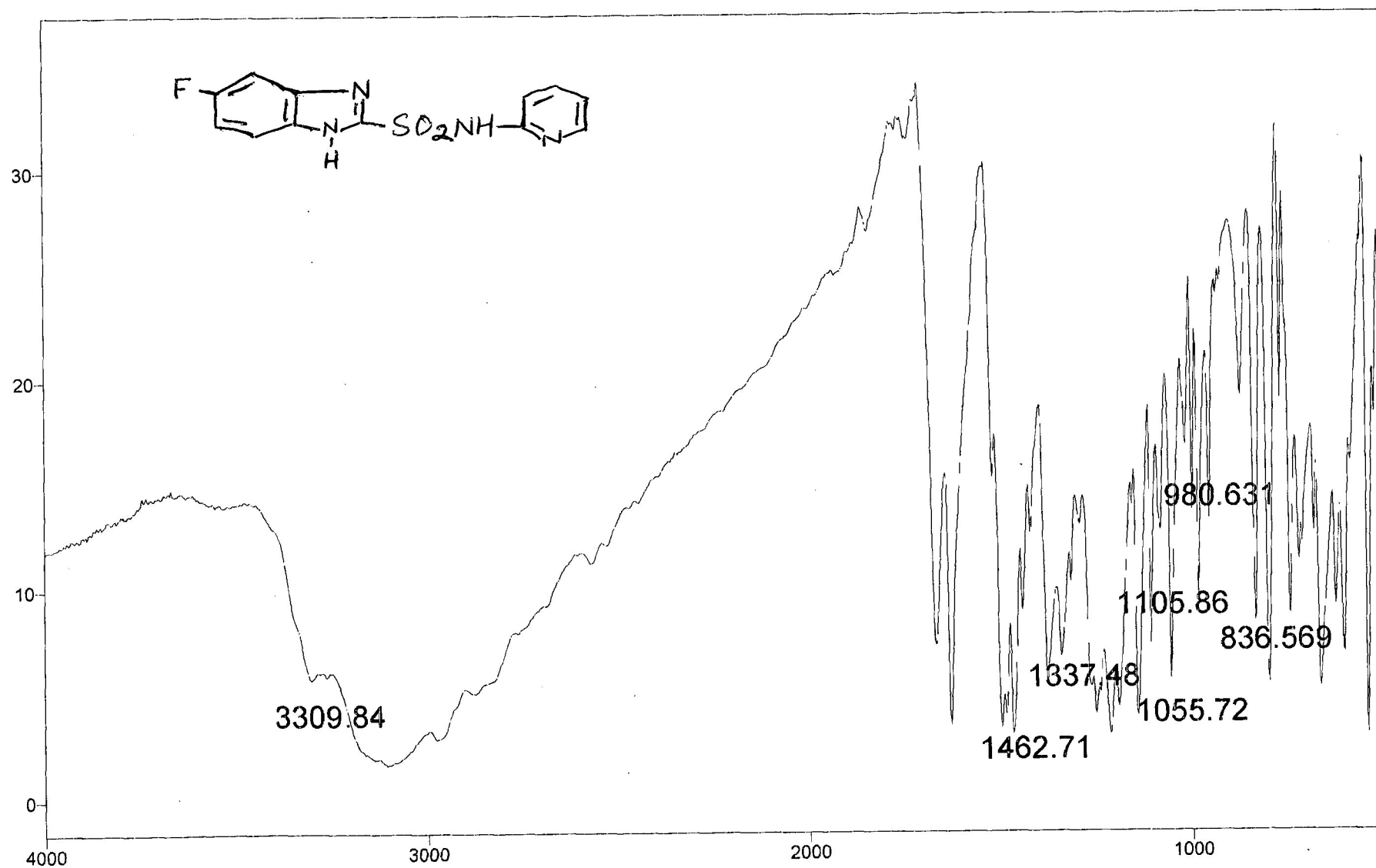


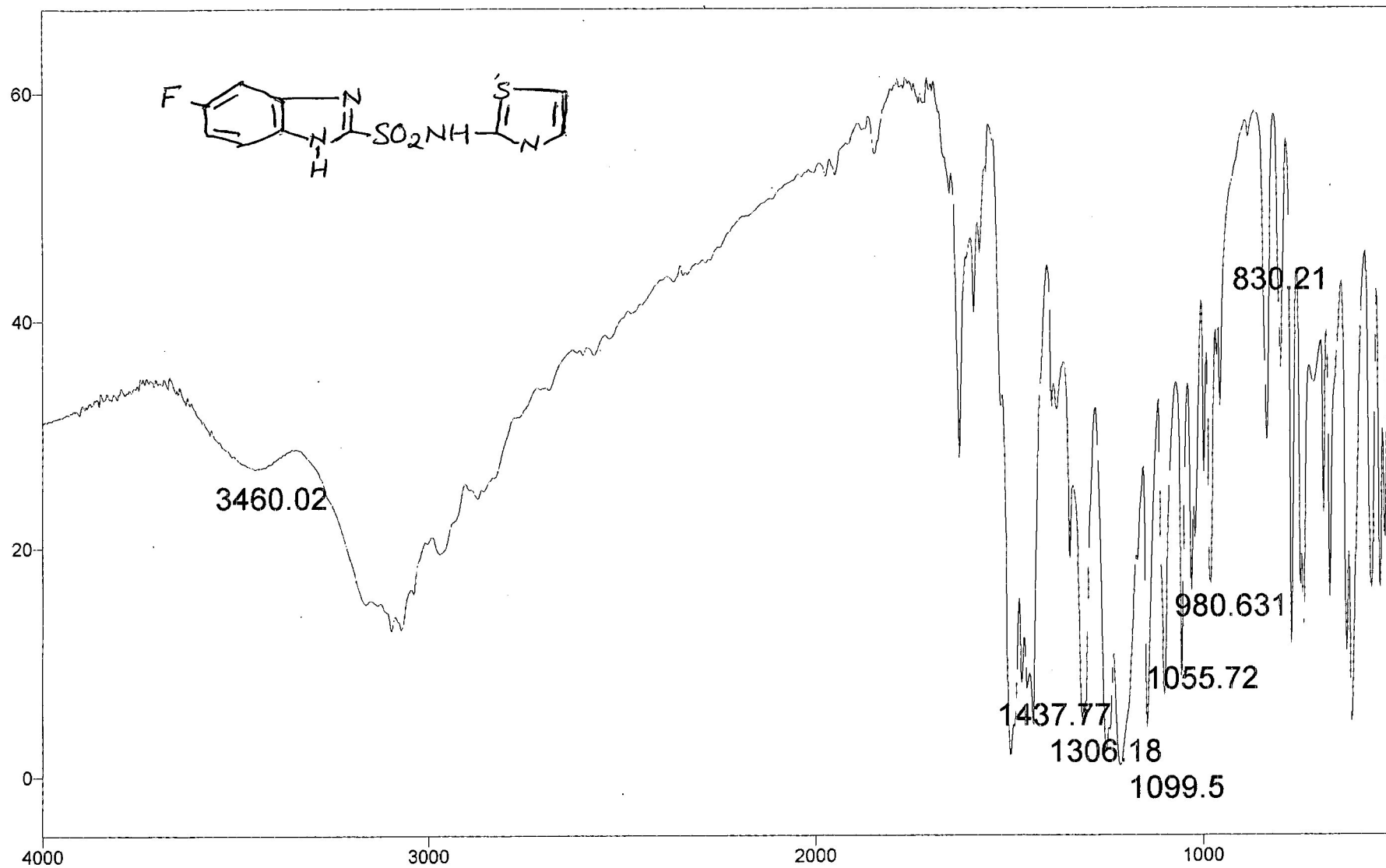
3291.01	NH stretching
2984.3	methyl groups
1343.84	sulfonamide
1055.72	fluorine
836.569, 980.631	5-substituted benzimidazole
1469.07	C-C skeletal model of C ₆ H ₆
999.464	pyrimidine
1237.2	CH- in plane deformation vibration of pyrimidine

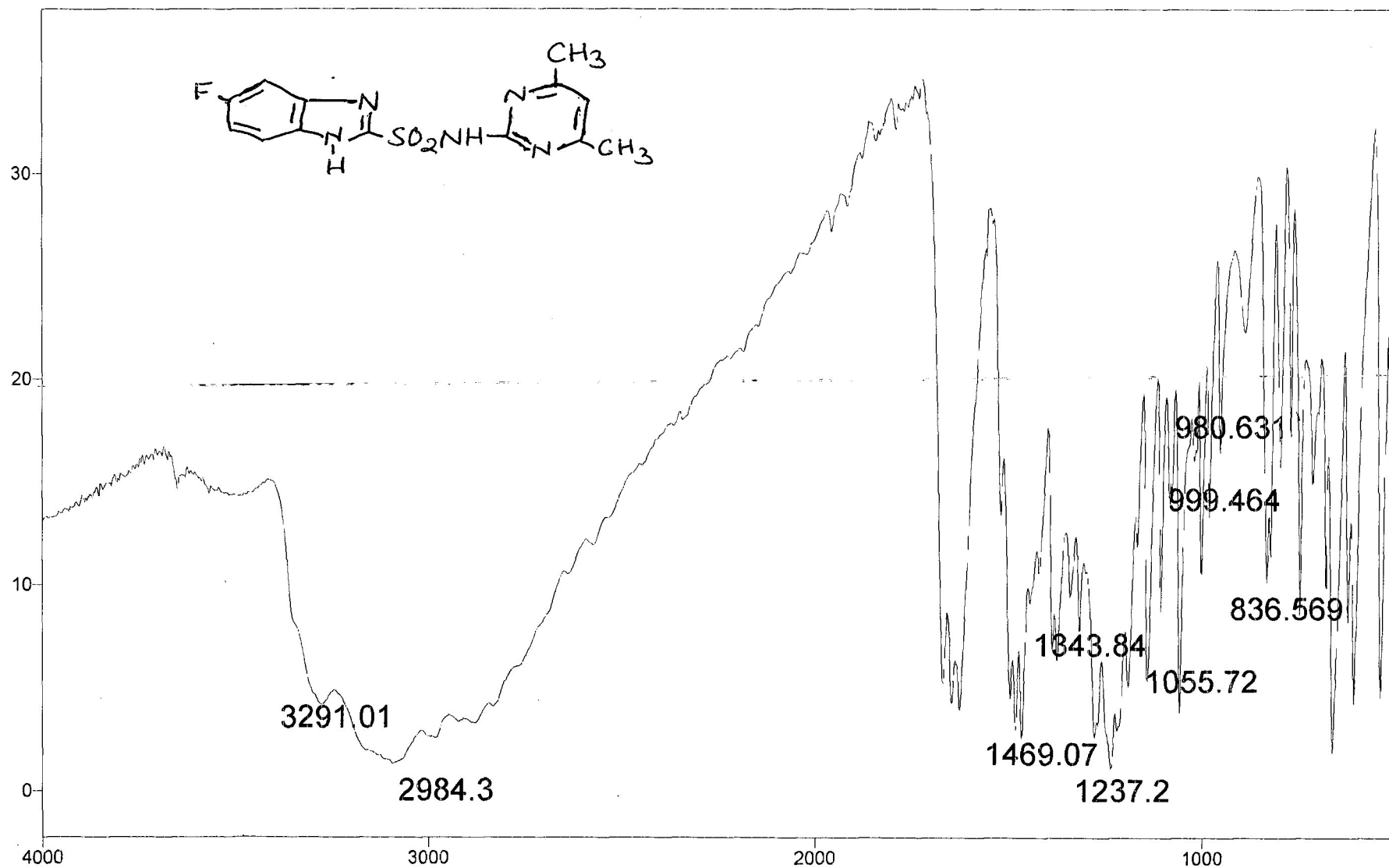












Nucleus	MHz	41
Spec. Width	Hz	6888.8
Acq. Time	sec	2.000
Pulse Width	μ sec	10.0
Transmits		400
Delay	sec	0
Offset	Hz	1800.0
Freq.	MHz	289.9486

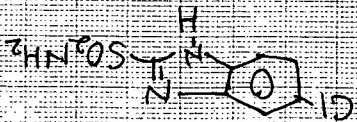
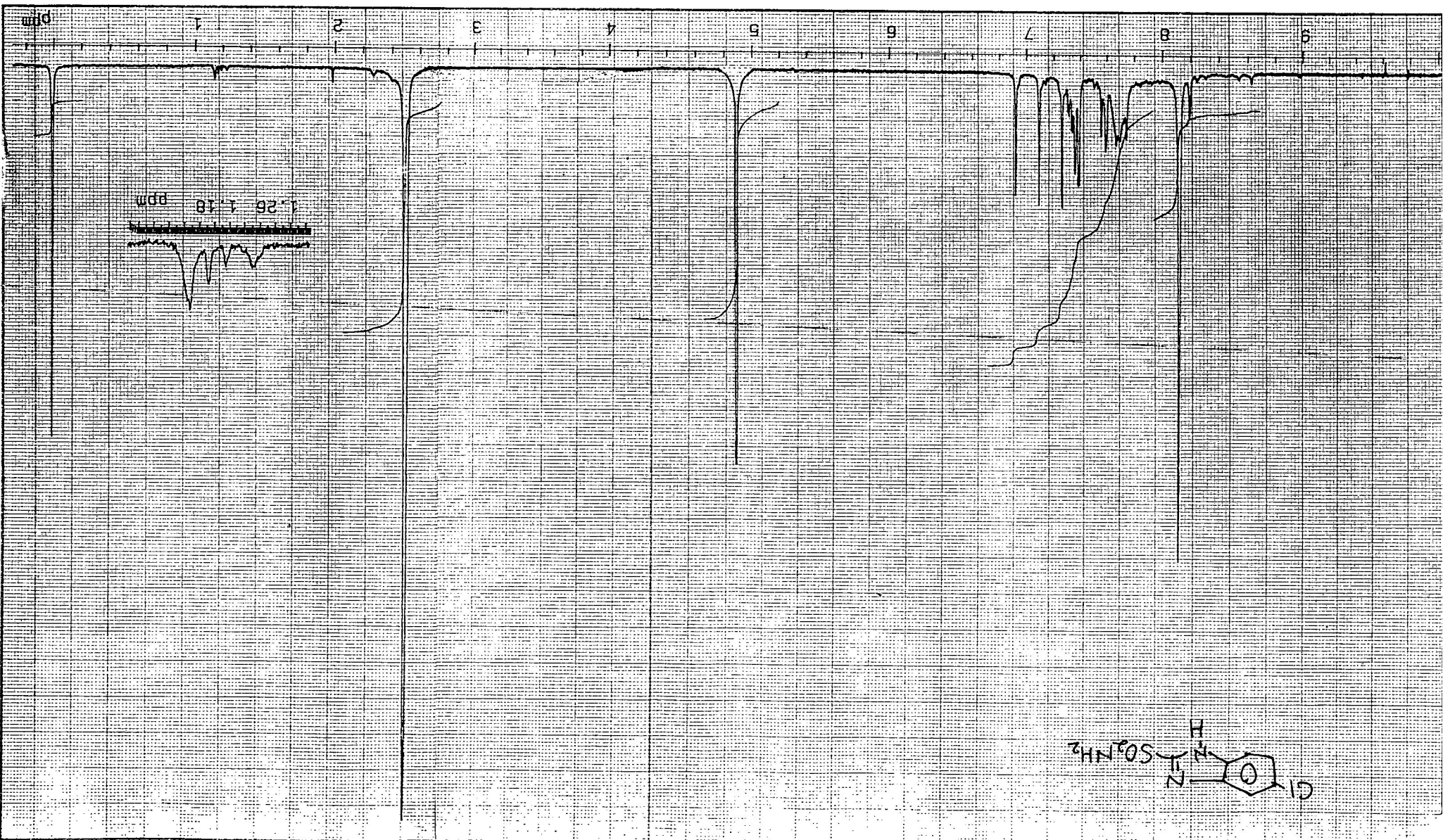
Nucleus	H1	Offset	-778.0	Hz
Mode	M	Power	10	dB
Modulation Mode	C	Freq.	200	Hz
Pulse Width	_____	Power Mode	MAN 0888	

Reference

Width	3102.9	Hz/pwm	Start	-87.9	Hz/pwm
LB	0.200	Hz	used		sec
FM	327.88	K	RE		sec

Run Sequence _____
 Tube (C) _____
 Temp. _____ °C
 Solvent: _____
 DMSO _____
 SAMPLE _____

Number _____
File _____
Date _____
VXPRO _____
300



Synthesis of 1-N-isopropyl-2,5-disubstituted benzimidazole

Synthesis of 1-N-2,5-disubstituted benzimidazoles involves following steps

1. Synthesis of 2,5-disubstituted-1-H-benzimidazole.
2. Condensation of 2,5-disubstituted –1-H-benzimidazoles with isopropylsulfonyl-chloride.

1. *Synthesis of 2-methylthiopseudourea-1,3-dicarboxylic acid dimethyl ester.*

In a 100 ml three necked round bottom flask equipped with stirrer, water condenser and dropping funnel were placed a solution of 5-methyl iso-thiourea sulphate (7.8g, 0.028 M) in 7 ml water. Into this mixture, methyl chloroformate (4.4 ml, 0.057M) was added dropwise at 5-10°C. The whole mixture was stirred for 1 hour at the same temperature. To this was added 25% NaOH solution dropwise, maintaining the temperature between 10-15°C till the pH of the solution was 8. At this pH, white solid was separated out which was collected by filtration.

Yield = 4.04 g (70.13%)

M.P. = 100-101°C

Reported¹²¹ M.P. = 100-102°C

2. *Synthesis of 2,5-disubstituted-1-H-benzimidazoles*

Different 4-substituted-1,2-phenylene diamines were cyclised by 2-methyl thiopseudourea-1,3-dicarboxylic acid dimethyl / diethyl ester using ethyl alcohol as solvent to obtain ethyl / methyl-5-substituted-2-(1)-H-benzimidazole carbamates.

Procedure for preparation of methyl-5-(propylthio)-1-H-benzimidazole-2-carbamate.

In a dry round bottom flask equipped with double surface water condenser 3.62 g (0.02M) of 4-propylthio-1,2-phenylenediamine was dissolved in 30 ml of ethyl alcohol. To this was added 4.12g (0.02M) of 2-methyl thiopseudourea-1,3-dicarboxylic acid dimethyl ester, and the reaction mixture was refluxed on water bath for 7-8 hours. The expected product precipitated as solid from the reaction mixture. The reaction mixture was allowed to cool to room temperature and the solid was separated by filtration. It was recrystallised from alcohol.

Yield = 3.7 g (75.5%)

M.P. 207 °C

Reported¹²² M.P. 208-210°C

Reported data of these compounds is given in Table No. 3.

All other methyl / ethyl-5-substituted -1,2 phenylenediamines were obtained in our laboratory.

3. Preparation of isopropyl sulfonyl chloride

In a 100 ml three necked round bottom flask equipped with air condenser, gas inlet tube and stopper was taken 60ml of 4N HCl. The flask was placed in an ice-bath. 10 ml of 2-propanethiol was added to the flask and chlorine was bubbled through the mixture while stirring for 3 to 4 hours at 5-8°C. Isopropyl sulfonyl chloride is accumulated at the bottom of the flask. The whole mixture was transferred to a separating funnel and heavy layer of isopropyl sulfonyl chloride was separated out. It was subjected to distillation and pure isopropyl sulfonyl chloride was collected at 74-75°C at 19mm pressure

Yield = 11.8 g , 9.3 ml, 62.93%

Reported¹²³ B.P. = 74-75°C/19mm of Hg.

4. Preparation of methyl-1-isopropyl sulfonyl-5-propylthio-2-benzimidazole carbamate

In a 100 ml three necked round bottom flask equipped with water condenser, dropper, stopper and magnetic needle was taken 1.325 g (0.005M) of methyl 5-propylthio-1-H-benzimidazole carbamate. It was dissolved in 4 ml of dry DMF. To this was added 0.6 g (0.85 ml, 0.006M) of triethyl amine and the mixture was heated to 60°C. To this added was 0.85 g (0.67 ml, 0.006M) of isopropyl sulfonyl chloride in 2 ml of dry DMF, dropwise. The reaction mixture was heated at 155-160°C with stirring for 6-8 hours. DMF was removed under reduced pressure and residue was dumped in ice-cold water. The solid separated was filtered and recrystallised from DMF.

Yield = 1.2 g , 64.86%

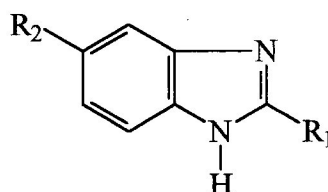
M.P. = 222°C

	C	H	N
Calculated	61.78	5.66	11.32
Found	61.48	5.54	11.28

Cambendazole was obtained as a standard sample from our laboratory.

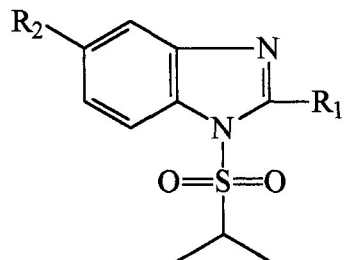
Physical data and analysis reports of these compounds are given in Table No. 4

TABLE 3 : 2,5-DISUBSTITUTED BENZIMIDAZOLES

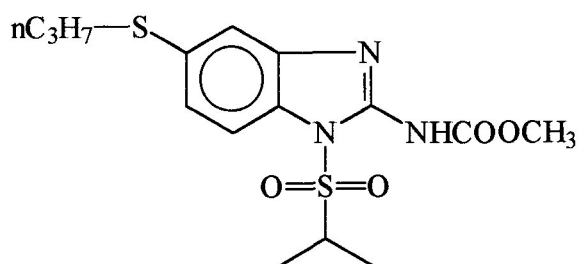


Sr. No.	R ₁	R ₂	M.P. found °C	M.P. reported °C
1	Methyl carbamate	Benzoyl	288-289	288.5
2	Methyl Carbamate	n-butyl	226	225-227 (dec.)
3	Methyl Carbamate	n-propylthio	207	208-210
4	Methyl Carbamate	Hydrogen	>280	300-302
5	Methyl Carbamate	Propoxy	229-230	230-230.5
6	Methyl Carbamate	Thio phenyl	232-233 (dec.)	233 (dec.)

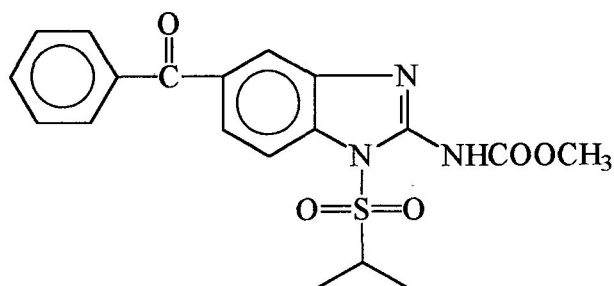
TABLE 4 : 2,5-DISUBSTITUTED 1-(N-ISOPROPYL SULFONYL) BENZIMIDAZOLES



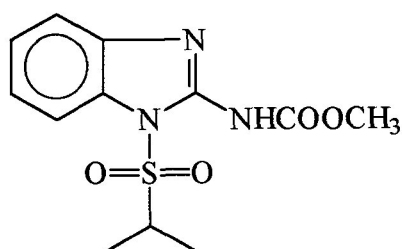
Sr. No	R ₁	R ₂	Molecular Formula	Molecular Weight	MP (°C)	Yield %	Elemental Analysis %					
							Calculated			found		
							C	H	N	C	H	N
1	Methyl carbamate	Benzoyl	C ₁₉ H ₁₉ N ₃ O ₅ S	401	160	64	56.85	4.73	10.47	56.55	4.56	10.58
2	Methyl carbamate	n-butyl	C ₁₆ H ₂₃ N ₃ O ₄ S	353	192	62	54.39	11.89	13.72	54.31	11.72	13.52
3	Methyl carbamate	n-propyl thio	C ₁₅ H ₂₁ N ₃ O ₄ S ₂	371	222	68	61.78	5.66	11.32	61.48	5.54	11.28
4	Thiazole	Isopropyl amido	C ₁₇ H ₂₀ N ₄ O ₃ S ₂	392	200-3	57	60.58	4.92	14.28	60.69	4.63	14.14
5	Ethyl carbamate	Hydrogen	C ₁₃ H ₁₇ N ₃ O ₄ S	311	199	71	50.16	5.12	13.50	50.48	4.94	13.29
6	Methyl carbamate	Hydrogen	C ₁₂ H ₁₅ N ₃ O ₄ S	297	229	65	48.01	3.27	14.14	48.14	3.51	14.02
7	Methyl carbamate	Chlorine	C ₁₂ H ₁₄ ClN ₃ O ₄ S	331.5	255	74	46.17	4.12	12.66	46.11	4.10	12.49
8	Methyl carbamate	5-n-propyl-thio-6-chloro	C ₁₅ H ₂₀ ClN ₃ O ₄ S ₂	405.5	205	68	44.38	4.93	10.35	44.67	4.79	10.22
9	Methyl carbamate	n-propoxy	C ₁₅ H ₂₁ N ₃ O ₅ S ₂	387	267-8	61	42.11	3.03	10.85	41.98	2.96	10.68
10	Methyl carbamate	Phenyl thio	C ₁₈ H ₁₉ N ₃ O ₄ S ₂	405	227	60	62.62	3.96	10.37	62.48	4.02	10.28



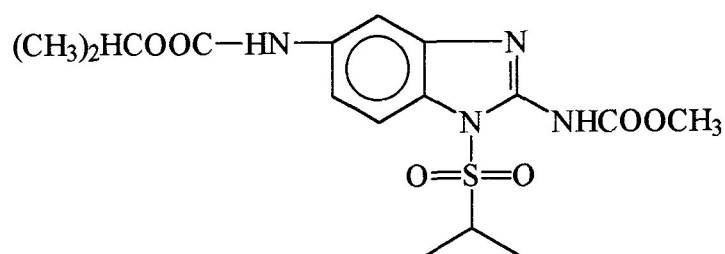
1368.79	SO ₂ -N linkage
1706.81	carbamate
2965.46, 2927.8, 2802.5	alkyl groups
961.798, 824.09	5-substituted benzimidazole
692.5	C-S-stretching
3366.1	NH stretching
1287.34	C-O stretching of NHCOOCH ₃
1462.71	C-C skeletal model of benzene



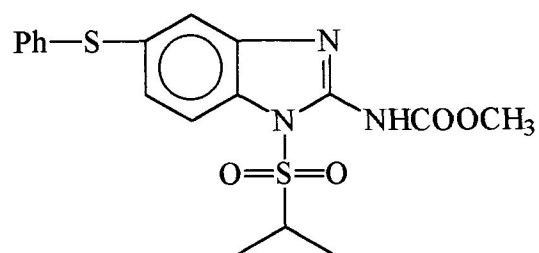
830.21, 974.272	5-substituted benzimidazole
1318.65	SO ₂ -N linkage
1713.17	carbamate
1443.85	C-C skeletal model of benzene
1650.56	C=O
3334.79	NH stretching NHCOOCH ₃
1287.34	C-O stretching of ester
2965.46	alkyl groups
2815.29	wide peak of isopropyl group



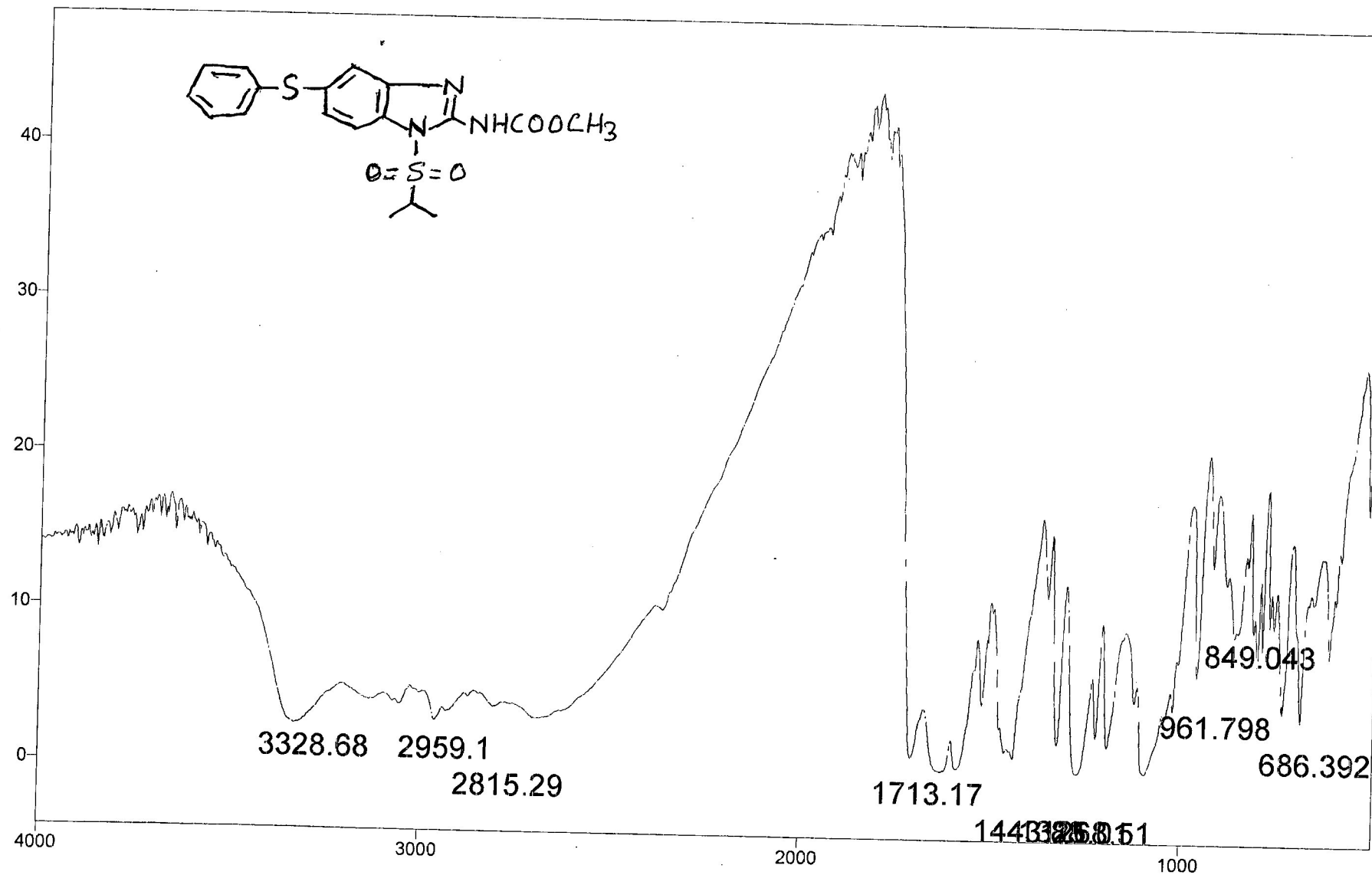
1713.17	carbamate
3327.32	NH stretching (NHCOOCH ₃)
13.25.01	SO ₂ -N stretching
2959.1	alkyl group
2802.57	isopropyl group
961.789, 836.56	5-substituted benzimidazole
1450.24	C-C skeletal model of benzene
1287.34	C-O stretching of NHCOOCH ₃

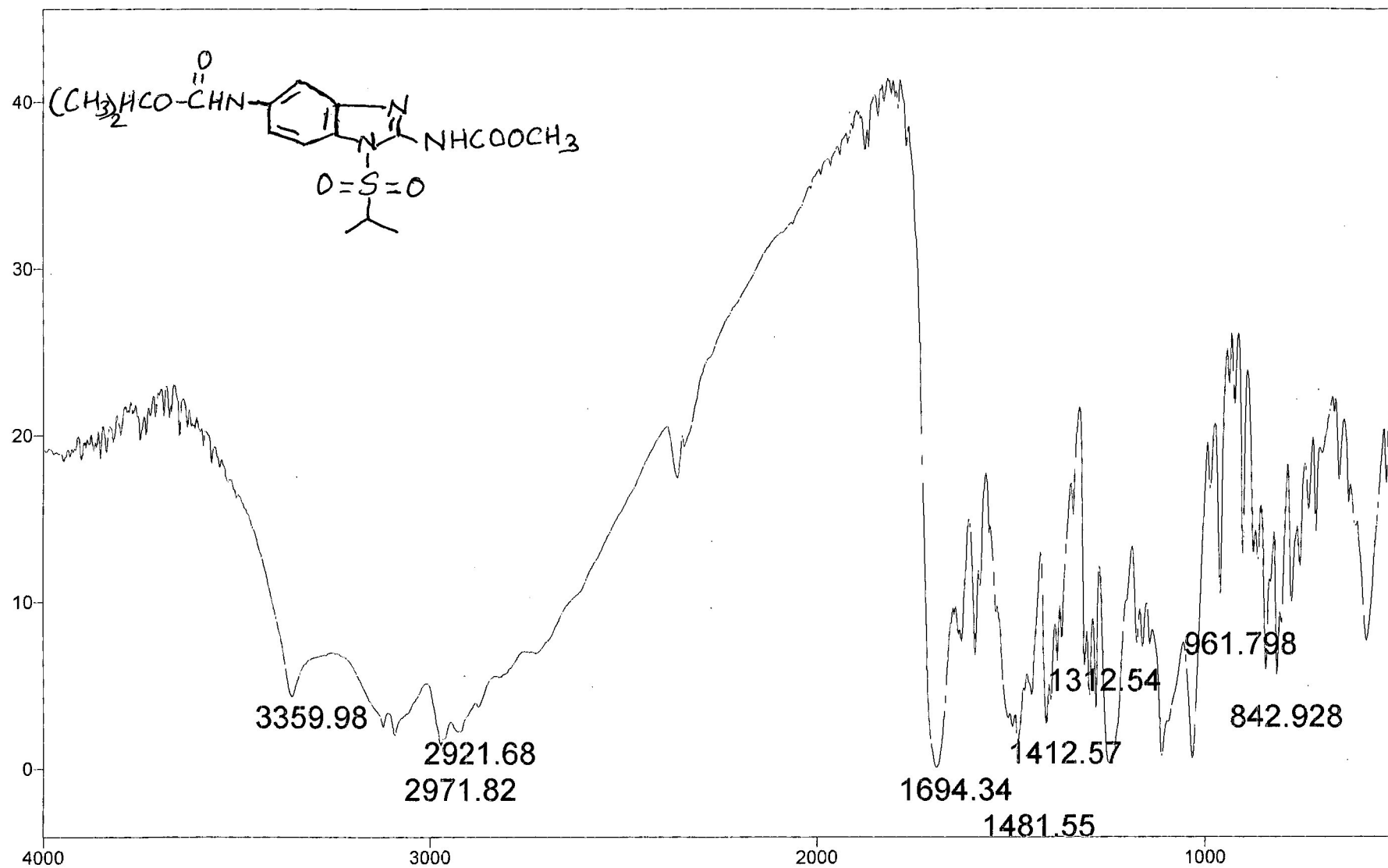


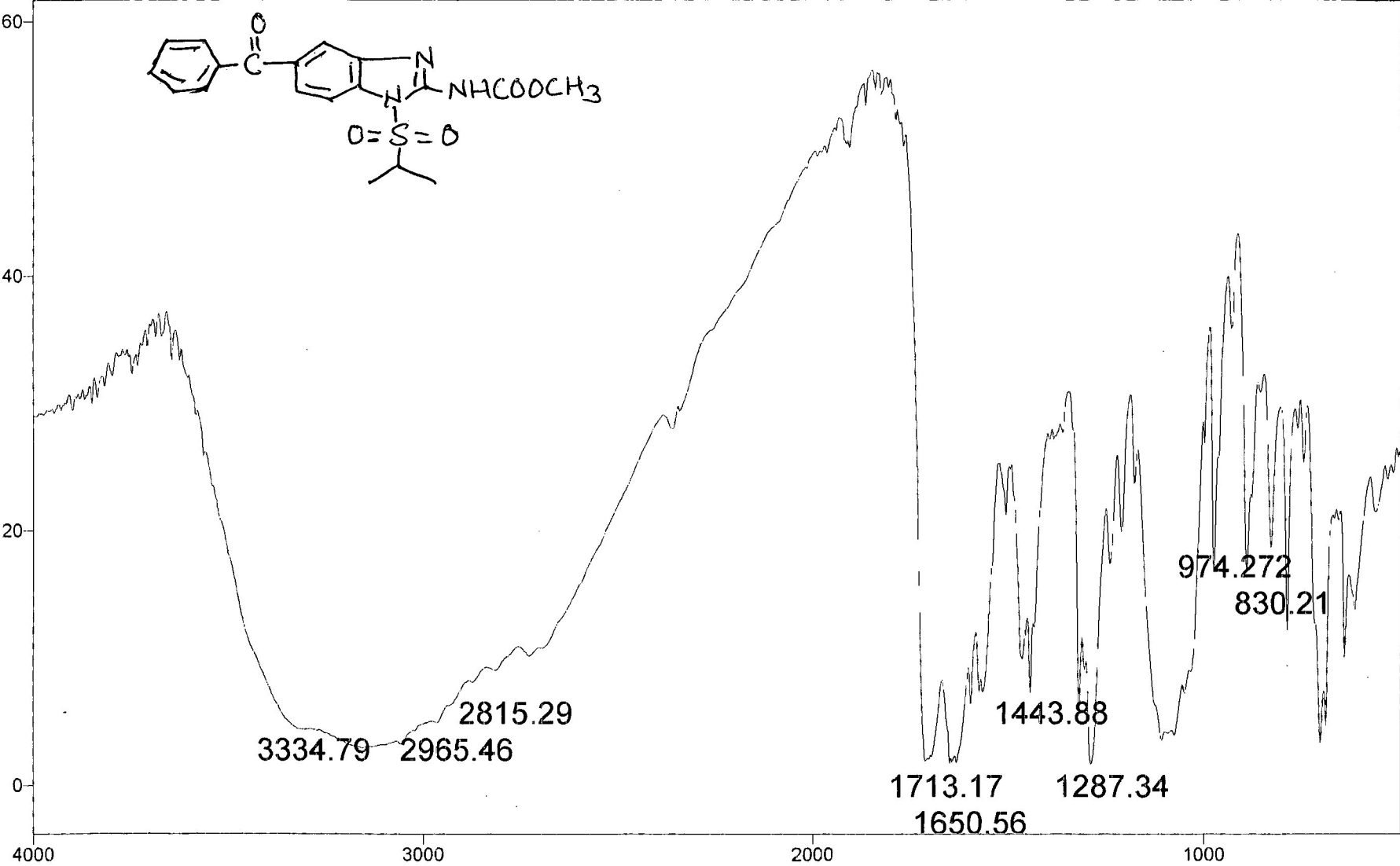
1694.34	carbamate
2971.82	alkyl groups
2921.68	alkyl groups
3359.98	NH of carbamate
1481.55, 1425.57	C-C skeletal model of C ₆ H ₆
1312.54	SO ₂ -N linkage
842.928, 961.798	5-substituted benzimidazole

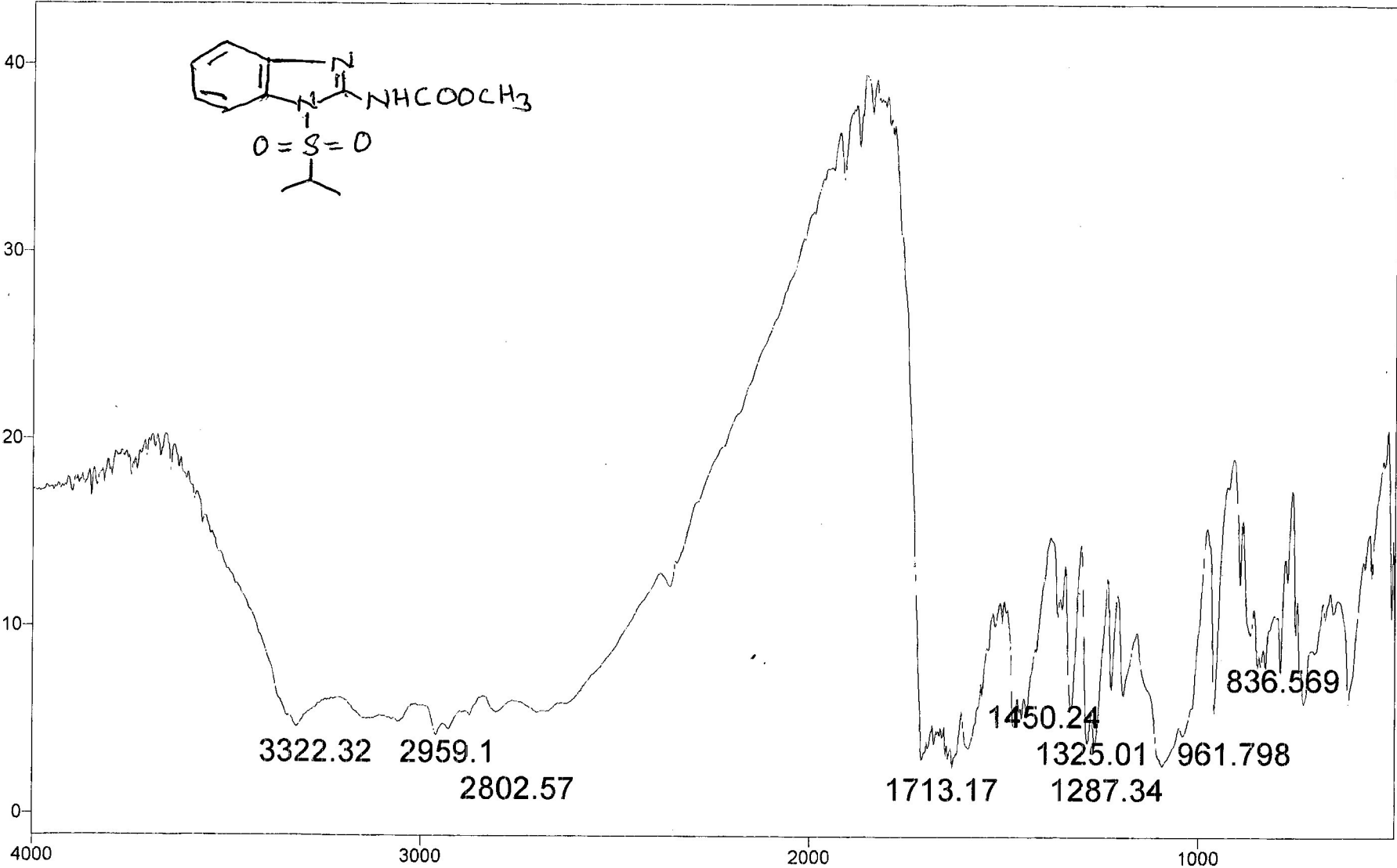


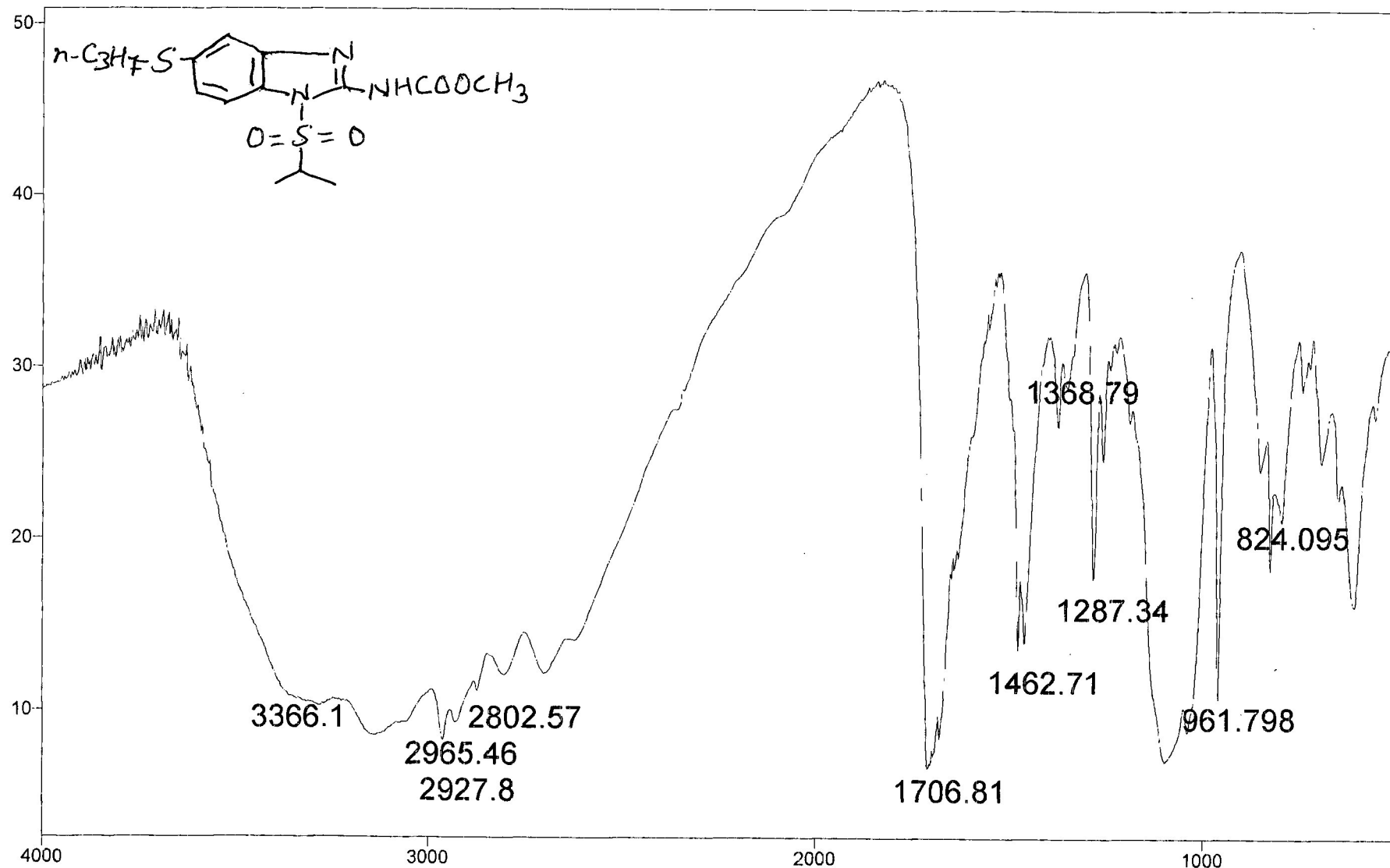
1713.17	carbamate
2815.29, 2959.1	alkyl groups
3328.68	NH of carbamate
1443.88	C-C skeletal model of C ₆ H ₆
1325.01	SO ₂ -N linkage
1268.51	C-O stretching of ester
849.043, 961.798	5-substituted benzimidazole
686.392	C-S stretching

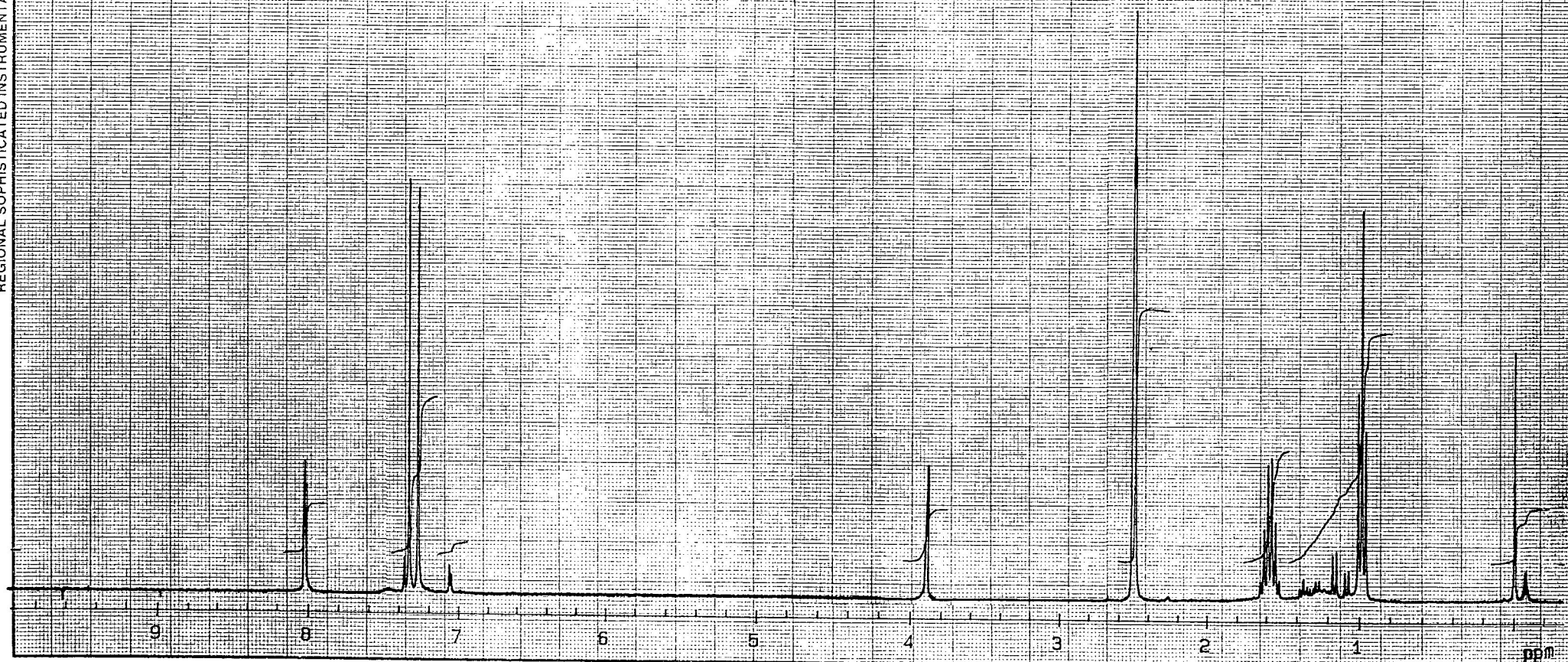
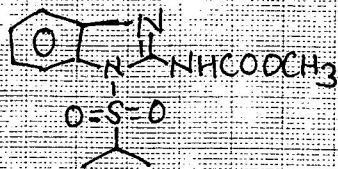












OBSERVE

Nucleus H1 Freq. 289.8488 MHz
 Spec. Width 6998.8 Hz Offset 1800.0 Hz
 Acq. Time 4.001 sec Delay 0 sec
 Pulse Width 10.0 μ sec Transients 108

DECOUPLE

Nucleus H1 Offset -776.0 Hz
 Mode n Power 10 dB
 Modulation Mode c Freq. 200 Hz
 Pulse Width μ sec Power Map not used

PLOT/PROCESSING

FN 32788 K RE sec CD sec
 LB 0.100 Hz AF not used sec QCD
 Width 3083.0 Hz/ppm Start -97.0 Hz/ppm
 Reference

EXPERIMENT

Pulse Sequence s2pu1 SAMPLE
 Tube O.D. mm
 Temp. 25.0 °C
 Solvent DMSO IPSC1

Number
 File
 Date Jan 15 89
 VXP0 300

Synthesis of 2-amino-2'5-dichlorobenzophenone

Antipsychotics are the drugs used to treat psychotropic disorders. Benzodiazepines are very important class of antipsychotics. Delorzepam, Lorazepam, Cloxazolam and Triazolam are amongst the currently used benzodiazepines.

Synthesis of all these drugs starts from a common raw material, i.e. 2-amino-2',5-dichloro benzophenone. This section deals with the synthesis of this very important fine chemical, which is currently totally imported in India.

Synthesis involves two steps

1. Preparation of 2-chlorobenzoyl chloride
2. Condensation of 2-chlorobenzoyl chloride with 4-chloroaniline.

1. Preparation of 2-chlorobenzoyl chloride

In a 100 ml dry round bottom flask, equipped with water condenser and a guard tube was placed 5g (0.032M) of 2-chlorobenzoic acid. To this was added 7.25 g (4.45 ml) of freshly distilled thionyl chloride and two drops of DMF as a catalyst. The whole mixture was refluxed in an oil bath at 85°C for 4-5 hours. The excess of thionyl chloride was removed under reduced pressure along with benzene. The remaining liquid was subjected to distillation and pure 2-chlorobenzoyl chloride was collected at 137-139°C / 60mm of Hg.

Yield = 4.2 g, (3 ml), 75.13%

Reported¹²⁴ B.P. = 137-139°C / 60 mm of Hg.

2. Condensation of 2-chlorobenzoic acid with 4-chloroaniline

In a 100 ml three necked round bottom flask equipped with mechanical stirrer, air condenser with guard tube and a stopper was placed 17.13 g (12.4 ml, 0.0979 M) of 2-chlorobenzoyl chloride. It was heated upto 130-140°C. At this temperature, 5 g (0.39M) of 4-chloroaniline was added in portions with vigorous stirring, over a period of 30 minutes. The temperature was increased upto 180-200°C. At this temperature anhydrous Zinc chloride 6.25 g (0.045M) was added in portions, over a period of 30 minutes. The temperature was increased upto 230°C and maintained for 3 hours. After 3 hours, it was cooled to 120°C. To this was added 50 ml of boiling water and

the whole mixture was stirred for 15-20 minutes. The contents were poured into a beaker. The supernatant liquid was removed and the residue was again placed in three necked flask. To this was added 50 ml of boiling water. Air condenser was replaced with water condenser. The reaction mixture was again refluxed for 15-20 minutes at 120°C. Again water was removed and residue was treated with boiling water. After three such treatments with boiling water, residue was treated with 5% NaOH solution. It was washed with cold water and again placed in three necked flask. To this was added a mixture of 8.6 ml water, 12.4 ml acetic acid and 16 ml conc. Sulphuric acid. The mixture was refluxed at 130-140°C for 14 hours. It was allowed to cool to room temperature and poured on crushed ice. Slightly sticky solid separated out. The water was decanted and solid was again treated with crushed ice. The solid becomes granular. Again water was decanted and solid was treated with 5%NaOH solution. It was decanted and washed with cold water. The solid was separated by filtration and recrystallised from methanol.

Yield = 7.3 g, 70%

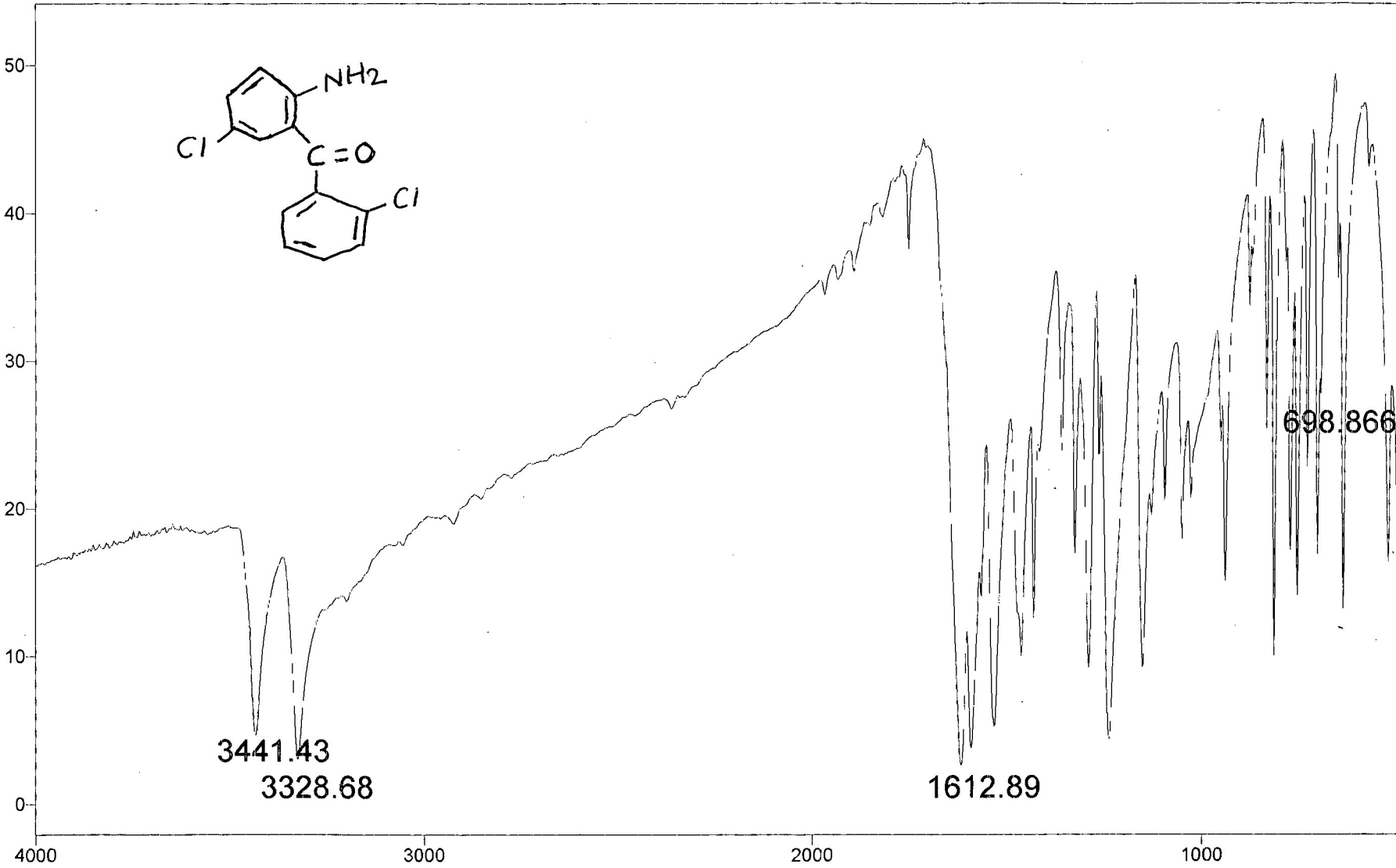
M.P. = 87-88°C

Reported¹²⁵ M.P. = 88-89°C

There are only few methods reported for the preparation of this fine chemical. In our laboratory, we tried to synthesise this compound by various methods¹²⁶⁻¹²⁹ but we could not succeed. We tried to hydrolyse the complex by conc. HCl, acetic acid and many other combinations but we found the mixture of water, acetic acid and conc. Sulphuric acid as the best combination for hydrolysis. We have also made the modifications in the original synthetic method and made it more economical and less hazardous. The modifications which we have made are as follows :

1. In the literature procedure, the starting material used was 4-chlorobenzanilide. This was replaced by 4-chloroaniline in our laboratory, which is a lot cheaper than 4-chlorobenzanilide.
2. In literature method three hot water washings, each of two hours were given which were replaced in our laboratory by giving two fifteen minute washings of hot water followed by treatment with 5% sodium hydroxide solution. This estimates the quantity of unreacted 2-chlorobenzoyl chloride. All this work can be accomplished in one hour, which saves five cumbersome hours.

3. During workup, according to literature, the reaction mixture is to be dumped into the water, then it is extracted with organic solvents like benzene or ethyl acetate. The organic layer is treated with sodium bicarbonate and the organic layer is evaporated to get the crude product. In our laboratory, we dumped the reaction mixture in ice-cold water. Water was decanted and solid was treated with 5% sodium hydroxide solution. After five minutes, the solid was collected by filtration. Thus, we avoided the extraction with organic solvents which is a costly and hazardous method in chemical industry. We made the procedure less hazardous and more economical than the literature procedure. The yield, we obtained, was same as that of literature method. The quality and purity of the product are comparable with the commercial sample.



Biological Results and Discussion

Biological Results and Discussion

The compounds synthesised in the present work have been subjected to various biological screening programmes based on their chemical and structural features so as to ascertain their suitability as potential chemotherapeutic agents.

The various screening programmes carried out have been the *in vitro* study against various species of gram-positive and gram-negative bacteria. viz. *Staphylococcus aureus* ATCC 3750 (*S. aureus*) and *Salmonella typhi* ATCC 786 (*S. typhi*) for estimating antimicrobial activity. Literature studies have revealed that compounds from Part II may have anthelmintic activity. In the view of this finding we have evaluated our compounds for their anthelmintic activity using *H. nana*. The methodology adapted for the various biological screening programmes undertaken in this study has been described in the following section.

Antibacterial screening :

The test compounds have been subjected to *in vitro* screening by the tube dilution technique¹³⁰ against various species of gram-positive and gram-negative bacteria viz. *S. aureus* and *S. typhi* using Muller Hinton broth as culture medium.

Screening procedure :

Sterile Muller-Hinton broth was dispensed in each borosilicate test tube (150 x 20 mm). The drug solution was added in order to attain final drug concentration as 200, 100, 40, 20, 4 and 2 µg/ml.

At the end, inoculation of standard suspension, (0.1ml of the desired bacterial strain (*S. aureus* or *S. typhi*) containing 10^6 bacilli/ml) was added. The tubes were incubated at 37°C for 48 hours and then examined for the presence or absence of growth of the test organism.

The lowest concentration, which showed no visible growth was taken as 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 programmes. MIC of Ampicillin is 0.01 µg/ml against *S. aureus* and that of Trimethoprim is 1 µg/ml against *S. typhi*.

Antitubercular Screening :

The test compounds have been subjected to *in vitro* screening by tube dilution technique employing the human virulent H₃₇Rv strain of *Mycobacterium tuberculosis*. In this method, Kirchner's medium containing Tween-80, as described by Rake et al¹³¹, was used.

Kirchner's medium (sterilised) was dispensed in each borosilicate tube and to this 0.5ml of fresh, sterile horse serum was added. The drug solution was added in each tube so as to attain final drug concentrations as 200, 100, 40, 20, 10, 4, 2 µg/ml. Finally, the inoculum of standard suspension, i.e. 0.1ml of *M. tuberculosis* (H₃₇Rv strain) 10⁶ bacilli per ml, was added. The tubes were incubated at 37°C for ten days and then examined for presence or absence of growth of the test organism. The lowest concentration, which showed no viable 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.

Anthelmintic screening :

Compounds synthesised in Part II were screened for their *in vivo* anthelmintic activity against *Hymenolepis nana* in albino mice. The method used is the one described by Agarwal, Alka et al¹³².

Screening procedure :

Each male mouse of albino strain (18-20 gms., 4 weeks old), of the total 105 mice was given an oral inoculum of 100 mature eggs collected from mature worm of *H. nana*. The infected mice were divided into 21 groups of 5 mice each. 2 groups of mice were used for each of the 10 test compounds. The two groups were treated on 17th or 18th day post infection with either single dose of 250 mg/kg or repeated dose

of 100 mg/kg of each compound. One group of mice was kept as control. The mice were sacrificed on the 5th post treatment day.

At autopsy the entire small intestine was removed and laid on a background of black glass and contents were removed by expression. The number of heads of *H. nana* worm was counted.

The drug action is expressed in terms of percentage efficacy (% efficacy) on the basis of reduction of worms following treatment. The drug response was calculated on the mice cleared of the infection as compared to the untreated control group. The anthelmintic activity of the compounds was grouped as per the following chart, which expresses degrees of activity according to percentage efficacy¹³³.

75 – 99%	→	very active
50 – 74 %	→	active
34 – 49 %	→	slightly active
25-33 %	→	possibly active

Results and Discussion

Part II : 1-isopropyl sulfonyl-2,5-disubstituted 1-H-benzimidazole

All the 10 compounds in this series were screened for their antibacterial and antitubercle activity (Table No. 5)

Of the 10 compounds screened for antibacterial activity, six compounds (i.e. compound nos. 2, 4, 5, 7, 8, 9) exhibited good activity of 10µg/ml against gram-positive bacteria.

Also, four compounds (i.e. compound nos. 2, 7, 9, 10) exhibited a good activity of 10µg/ml against gram-negative bacteria. Six compounds (i.e. compound nos. 1, 3, 4, 5, 6, 8) exhibited an activity of 100 µg/ml against gram-negative bacteria.

Antitubercular screening showed more encouraging results. Six compounds (i.e. compound nos. 1, 2, 3, 4, 6, 7) showed antitubercular activity at 10 µg/ml. Three compounds showed activity at 40µg/ml (compound nos. 8, 9, 10) and only one compound (compound no. 5) showed moderate activity at 100 µg/ml against tubercular bacilli.

Anthelmintic screening (Table no. 6) showed variable results. Three compounds (i.e. 1, 8, and 9) seemed to be very active, i.e. percent efficacy is more than 75%. Five compounds (i.e. compound nos. 3, 4, 6, 7 and 10) showed clearance from 50-74%, which indicates that these are active. Two compounds (i.e. Compound nos. 2 and 5) exhibited low activity in the range of 34-49% indicating that these compounds are slightly active.

Part III : 5-chloro-2-benzimidazole sulfonamides and 5-fluoro-2-benzimidazole sulfonamides.

Total 14 sulfonamides were synthesised during the present course and all of them were subjected to antibacterial and antitubercular screening programme (Table no. 7 and 8. Compounds (i.e. compound nos. 3, 4, and 5) of chloro substituted sulfonamides exhibited good antibacterial activity at 10 µg/ml against *S. aureus*. Compound no. 1, 2, 6, and 8) exhibited moderate activity at 100µg/ml against *S. aureus*. Activity of these compounds against *S. typhi* was discouraging. Only two

compounds (compound nos. 7 and 8) exhibited activity at 40µg/ml and the rest of the compounds exhibited moderate activity at 100µg/ml against *S. typhi*.

In case of antitubercular activity, seven compounds out of eight chloro substituted sulfonamides exhibited good activity at 10 µg/ml and only one compound (i.e. compound no. 8) exhibited antitubercular activity at 20µg/ml.

Three compounds (i.e. compound nos. 3, 4 and 5) in fluoro substituted sulfonamides exhibited good activity at 10µg/ml against *S. aureus*. Two compounds (i.e. compound nos. 1 and 6) exhibited activity at 40 µg/ml and only one compound (i.e. compound no. 2) exhibited moderate activity at 100µg/ml against *S. aureus*. Activity of fluoro substituted sulfonamides against *S. typhi* was discouraging. Only three compounds (i.e. compound nos. 4, 5 and 6) exhibited activity at 40 µg/ml against *S. typhi* and remaining three compounds exhibited activity at 100 µg/ml.

5-fluoro substituted sulfonamides also exhibited good antitubercular activity. Five compounds (i.e. compound nos. 1, 2, 3, 4 and 6) exhibited activity at 10 µg/ml and only one compound exhibited activity at 20 µg/ml.

CONCLUSION

The present investigation was focussed on the synthesis of novel heterocyclic compounds of possible therapeutic value. To evaluate their efficacy, they were screened for their *in vitro* antitubercular activity against H₃₇Rv strain of *Mycobacterium tuberculosis* and antibacterial activity against *Staphylococcus aureus* ATCC 3750 and *Salmonella typhi* NCTC 786 and also *in vivo* anthelmintic activity against *Hymenolepis nana*.

In part II, synthesis of 2,5-disubstituted 1-N-isopropyl sulfonylbenzimidazoles has been described. In this series standard anthelmintic drugs such as Mebendazole, Albendazole, Cambendazole etc., were used and hydrogen at 1-N-position of benzimidazole was substituted by isopropyl sulfonyl group as it was shown in literature¹³⁴ that this group imparts antiviral activity and lipophilicity.

The antimicrobial activity of the series (Table no. 4) reveals the following points.

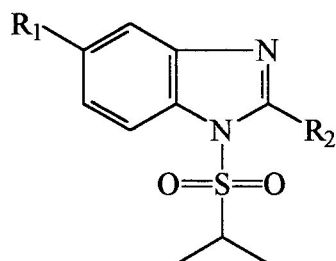
1. Although it was thought that isopropyl sulfonyl group at position 1 would impart activity, encouraging results were not obtained. Six out of ten compounds exhibited activity at 10 µg/ml against *S. aureus* whereas only four exhibited this activity against *S. typhi*. Remaining compounds showed activity at 100µg/ml. None of the compound was active below 10 µg/ml against bacteria.
2. Similarly in case of antitubercular screening, the activity ranges from 10 µg/ml to 100 µg/ml. Therefore, our assumptions that isopropyl sulfonyl group may impart activity to standard anthelmintic drugs was not upto mark as we found discouraging results.
3. As the compounds used were standard anthelmintic drugs, we screened these ten compounds for their *in vivo* anthelmintic activity (Table no. 5). It was found that activity of all these compounds was substantially reduced and only three compounds out of ten exhibited good anthelmintic activity.

Part III described synthesis of 5-chloro/fluoro-2-benzimidazole sulfonamides. The results of antibacterial and antitubercular screening are summarised in table nos. 6 and 7. It was found that when R is hydrogen, the antibacterial activity was very low. When hydrogen was replaced by sulfur or nitrogen containing heterocycles, the activity against *S. aureus* increases upto 10 µg/ml, but these compounds are ineffective against *S. typhi*.

In case of antitubercular activity no variation was observed whether R is hydrogen or heterocycle. All the compounds exhibited similar activity of 10 µg/ml with few exceptions.

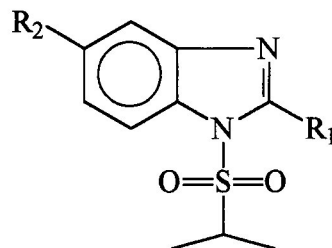
Though it may be concluded that the present investigation is not successful in rendering any new organic compound more active than existing drugs, it may be worthwhile to pursue further investigations with related compounds bearing in mind certain trends observed in the present investigation as part of the efforts to discover novel and potent drugs.

**TABLE 5 : 2,5-DISUBSTITUTED-1-(N-ISOPROPYL SULFONYL)
BENZIMIDAZOLES**



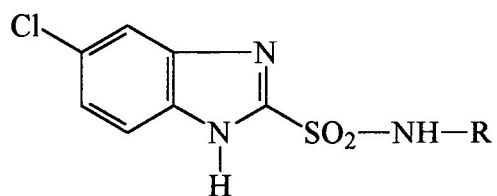
Sr. No.	R ₁	R ₂	Antibacterial		Anti T.B. (µg/ml)
			<i>S. aureus</i> (µg/ml)	<i>S. typhi</i> (µg/ml)	
1	Methyl carbamate	Benzoyl	100	100	10
2	Methyl carbamate	n-butyl	10	10	10
3	Methyl carbamate	n-propyl-thio	100	100	10
4	Thiazole	Isopropyl amido	10	100	10
5	Ethyl carbamate	Hydrogen	10	100	100
6	Methyl carbamate	Hydrogen	100	100	10
7	Methyl carbamate	Chlorine	10	10	10
8	Methyl carbamate	5-n-propyl-thio-6-chloro	10	100	40
9	Methyl carbamate	n-propoxy	10	10	40
10	Methyl carbamate	Phenyl-thio	100	10	40

Table 6 : Disubstituted 1-(N-isopropyl sulfonyl) benzimidazole



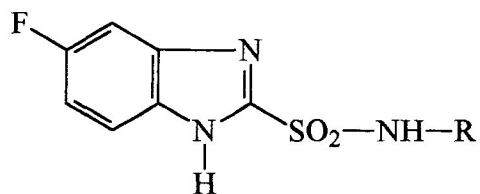
Sr. No	R1	R2	Single dose 250 mg/kg		Single dose 100 mg/kg	
			% efficacy	Drug response	% efficacy	Drug response
1	Methyl carbamate	Benzoyl	87.09	60	75.26	20
2	Methyl carbamate	n-butyl	48.38	-	34.4	-
3	Methyl carbamate	n-propyl-thio	54.30	-	50.53	-
4	Thiazole	Isopropyl amido	62.90	40	54.83	20
5	Ethyl carbamate	Hydrogen	50.00	-	44.08	-
6	Methyl carbamate	Hydrogen	60.75	20	53.22	-
7	Methyl carbamate	Chlorine	64.51	40	60.21	20
8	Methyl carbamate	5-n-propyl-thio-6-chloro	88.17	80	78.49	40
9	Methyl carbamate	n-propoxy	77.95	40	66.66	20
10	Methyl carbamate	Phenyl thio	54.30	20	45.16	-

Table 7 : 5-chloro-2-(1-H)-benzimidazole sulfonamides



Sr. No.	R	Antibacterial		Anti T.B. (µg/ml)
		<i>S. aureus</i> (µg/ml)	<i>S. typhi</i> (µg/ml)	
1	Hydrogen	100	100	10
2	Pyridine	100	100	10
3	Thiazole	10	100	10
4	Pyrimidine	10	100	10
5	4,6-dimethyl pyridine	10	100	10
6	4-methyl pyridine	100	100	10
7	5-nitro thiazole	20	40	10
8	Thiadiazole	100	40	20

TABLE 8 : 5-FLUORO-2-(1-H)-BENZIMIDAZOLE SULFONAMIDES



Sr. No.	R	Antibacterial		Anti T.B. (µg/ml)
		<i>S. aureus</i> (µg/ml)	<i>S. typhi</i> (µg/ml)	
1	Hydrogen	40	100	10
2	Pyridine	100	100	10
3	Thiazole	10	100	10
4	Pyrimidine	10	40	10
5	4,6-dimethyl pyridine	10	40	20
6	4-methyl pyridine	40	40	10

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SYNOPSIS

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

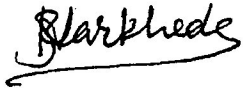
TITLE OF THE THESIS : Synthesis and evaluation of novel heterocyclic compounds of possible therapeutic value.

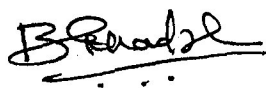
NAME OF THE CANDIDATE : Mr. Hemant B. Narkhede.

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

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

NUMBER AND DATE OF REGISTRATION : 27/ Feb. 18, 1997

SIGNATURE OF THE STUDENT : 

SIGNATURE OF THE GUIDE : 

Medicinal chemistry, according to Burger, "tries to be based on the ever increasing hope that biochemical rationales for drug discovery may be found". In contrast, he described pharmaceutical chemistry as being concerned with modifications of structures having known physiologic or pharmacologic effects and with analysis of drugs.

The practice of medicinal chemistry is devoted to the discovery and development of new agents for treating diseases. Most of the activity in this discipline is directed to new natural or synthetic organic compounds. Inorganic compounds continue to have their place in therapy, but organic compounds with increasingly specific pharmacologic activities are clearly the dominant force.

From ancient days, the search for therapeutic substances centered on the investigation of herbs grown in gardens of religious establishments where the sick found shelter. However, the true era of medicinal chemistry really began in the 1930s with the preparation and use of the antibacterial sulfonamide drugs. The history of medicine, from that time on is dominated by medicinal therapeutics. Today, a large number of diseases are cured, or at least controlled by drug therapy. Though the battle against bacterial and fungal infections has been largely won, infectious diseases are still a major health hazard for humankind. Heterocyclic derivatives play an important role in the control of various microbial diseases. Hence, in the present study, a new series of heterocyclic compounds has been synthesised and studied for their properties as antibacterial and antifungal agents. Also 2-amino 2',5-dichloro benzophenone, which is a vital intermediate for several psychotropic drugs, has been synthesised during this course.

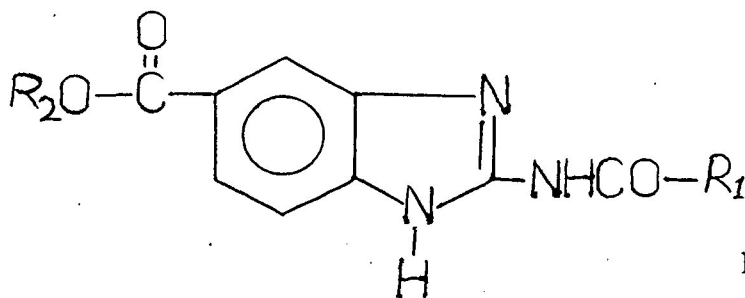
The thesis would consist of the following parts,

1. Introduction and review of literature.
2. Synthesis of 2,5-disubstituted-1-[N-(1-ethyl methyl) sulphonyl]benzimidazoles.
3. Synthesis of 5-chloro-2-(1-H-benzimidazole)sulfonamides and 5-fluoro-2-(1-H-benzimidazole) sulfonamides.
4. Synthesis of 2-amino-2',5-dichloro benzophenone : An important intermediate for psychotropic drugs.
5. Biological screening.

Part 2.

Synthesis of 2,5-disubstituted-1-[N-(1-ethyl methyl)sulphonyl]benzimidazoles.

The 2,5-disubstituted benzimidazole derivatives have been studied extensively for their biological activities. Certain alkyl 5-(alkoxy carbonyl)1-H-benzimidazole-2-carbamates (I) and related derivatives are found to be highly active (at concentrations less than 1µg/ml) as antineoplastic and antifilarial agents¹.

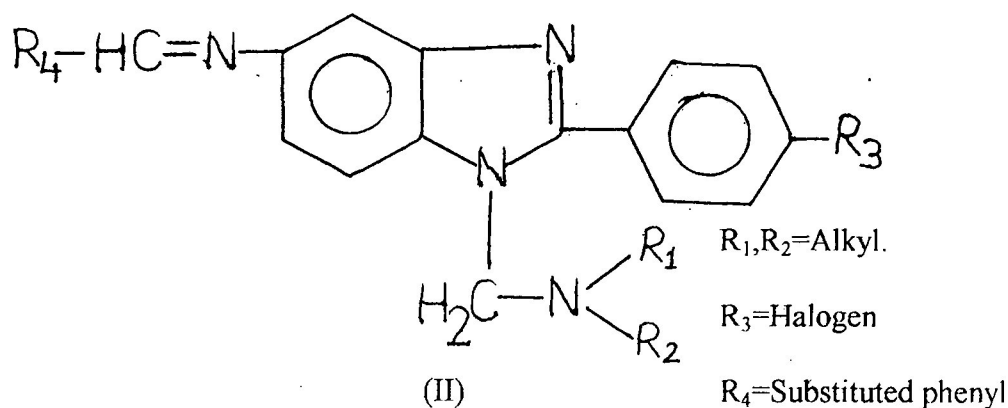


(I)

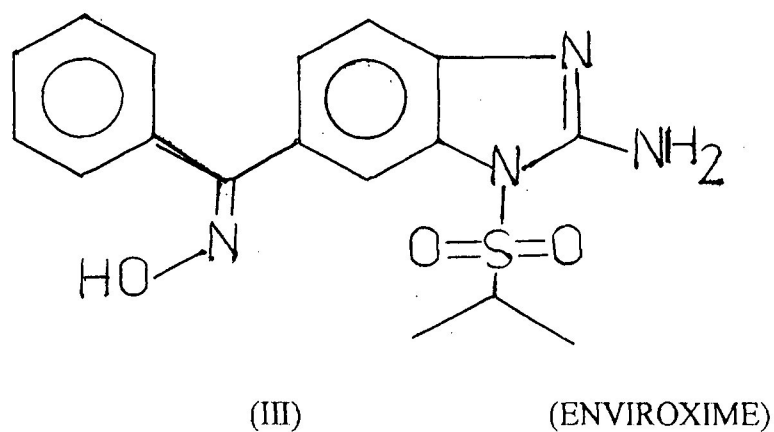
R₁= Alkyl, Alkylaryl.

R₂= Alkyl.

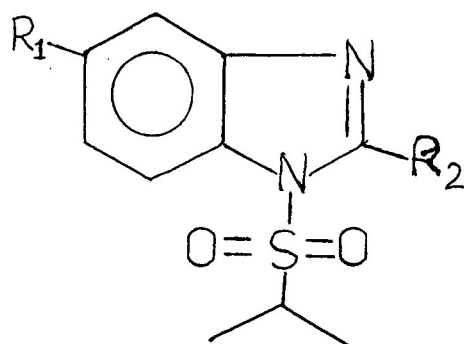
Some 1-dialkylaminomethyl-2-(4-substituted phenyl)-5-substituted benzimidazole derivatives (II) were found to be highly active against bacteria and fungi. Some of them were found to be more active than Streptomycin against *Enterococcus faecalis* and *Pseudomonas aeruginosa*².



Also, 6-[[[(Hydroxylimino)phenyl]methyl]-1-[(1-methylethyl)sulphonyl]-1-H-benzimidazole-2-amine, (Enviroxime) (III) and its analogs are found to be highly active against Rhinovirus multiplication^{3,4}.



Considering the above different references a series of compounds containing 1-[N-(1-methylethyl)sulphonyl]benzimidazole (IV) as basic nucleus is prepared with an aim to get compounds with improved activities. All these compounds are tested for their biological activities such as antibacterial, anti-tubercular, anthelmintic and anti-HIV activities.



(IV)

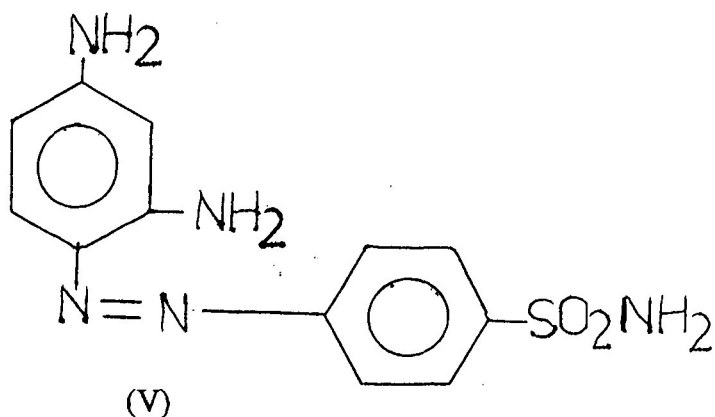
R_1 =Alkyl or aryl

R_2 =Carbamate

Part 3.

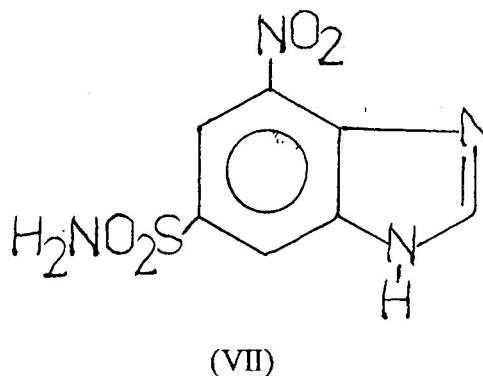
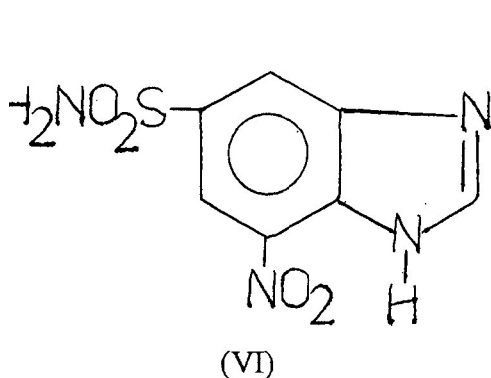
Synthesis of 5-chloro-2-(1-H-benzimidazole)sulphonamides and 5-fluoro-2-(1-H-benzimidazole)sulphonamides.

The discovery of the antibacterial activity of Prontosil (V), a sulfonamide, in early 1930s was the beginning of the era of chemotherapy⁵. Thereafter sulphonamides have been studied extensively and large number of sulphonamides were prepared and tested for their antibacterial activities. Presently there are about two dozen sulphonamides in clinical use as antibacterials.

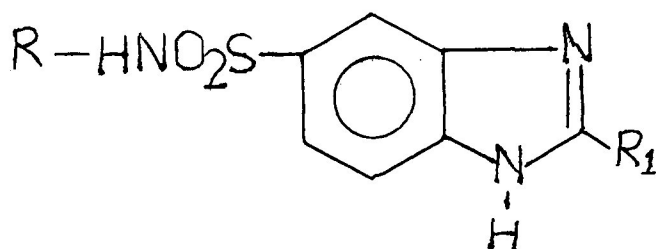


2-mercaptobenzimidazole is well known for its bacteriostatic⁶ and bactericidal⁷ activities.

7-nitro-5-benzimidazolesulfonamides(VI) and 4-nitro-6-benzimidazolesulfonamides(VII) , have been found to be excellent antibacterials⁸.



Similarly, 5(or 6)-benzimidazolesulfonamides(VIII) and its 2-methyl derivatives are found to inhibit the growth of polio and influenza B virus⁹.



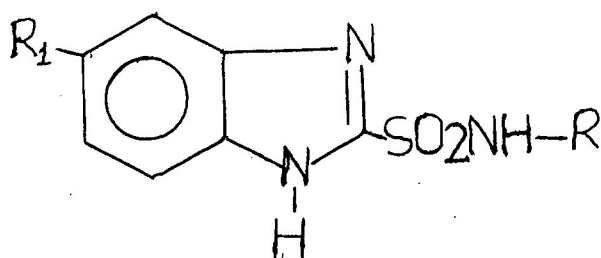
R= H, substituted phenyl,

Heterocyclic.

(VIII)

R₁= H, CH₃.

The well known antibacterial activity of sulphonamides and above facts prompted us to synthesise 5-chloro and 5-fluoro-2-benzimidazole sulphonamides(IX) in order to get biologically active compounds.



R= Heterocyclic.

(IX)

R₁= Cl, F.

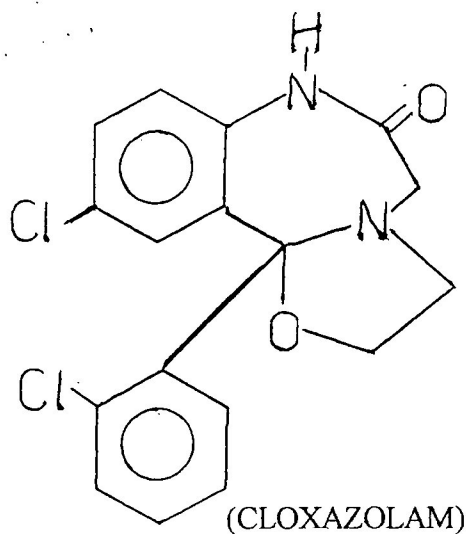
Part 4.

Synthesis of 2-amino-2',5-dichloro benzophenone.

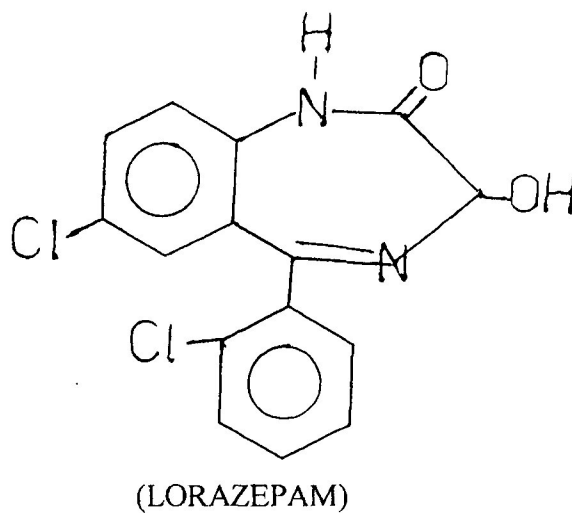
Advances in healthcare have dramatically increased life expectancy. Consequently, the incidence of many diseases associated with aging, including autoimmune, cardiac and metabolic diseases, as well as those directly affecting central and peripheral nervous systems, has increased. Diseases of elderly are associated with anxiety and depression. Improved agents for treatment of these disorders and various dementias as well as therapeutic agents to improve, limit or reverse the neurodegenerative process represents major challenges in CNS-related drug research. Indeed, statistical data in America showed that one in four people may anticipate progressive impairment of cognitive functions¹⁰.

Anxiety is treated by benzodiazepines. While effective, benzodiazepines are sedative and muscle relaxants. Now a days there are about two dozen benzodiazepines in clinical use. Cloxazolam(X), Lorazepam(XI) and Triazolam(XII) are amongst these benzodiazepines. All these drugs are highly potent and safe¹¹.

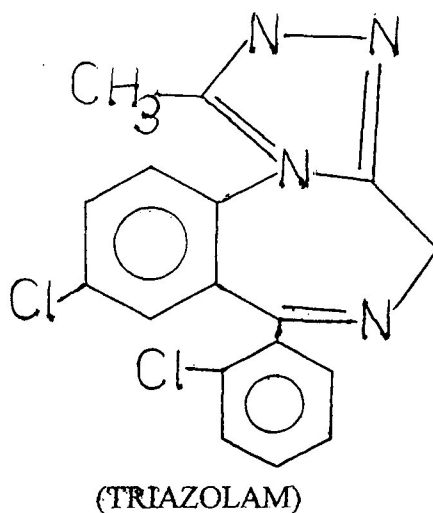
DRUG	DURATION	DOSE
Cloxazolam	Long acting	3 to 12 mg daily
Lorazepam	Short acting	1 to 6 mg daily
Triazolam	Very short acting	125 to 250µg at night for management of insomnia



(X)



(XI)



(XII)

Synthesis of all these useful drugs starts from 2-amino-2',5-dichloro benzophenone.

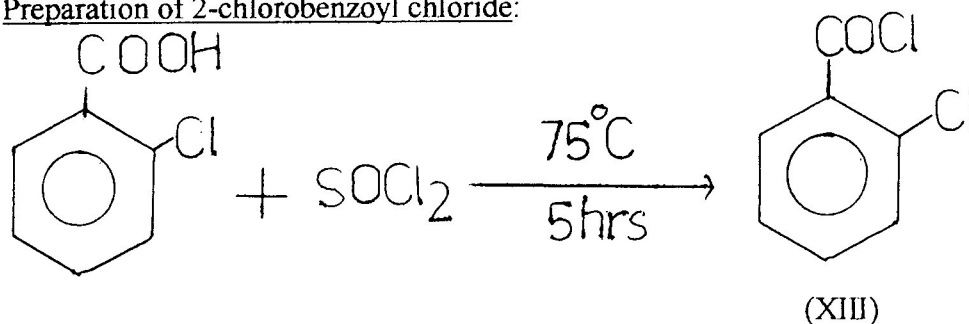
However, at present it is totally imported and there is a great need to develop a simplified preparative process for this specialised fine chemical from readily available raw materials. In literature, no much details are reported. Hence, synthesis of this important compound was carried out in our laboratory by various methods¹²⁻¹⁵ in order to get improved yield. Of these, following method was found to be the most suitable method for synthesis of 2-amino-2',5-dichloro benzophenone¹⁶.

The synthesis involves two steps:

1. Preparation of 2-chlorobenzoylchloride.
2. Condensation of 2-chlorobenzoylchloride and 4-chloroaniline.

The raw materials required for the synthesis of this compound, viz. 2-chlorobenzoic acid and 4-chloroaniline are indigenously available and are cheap.

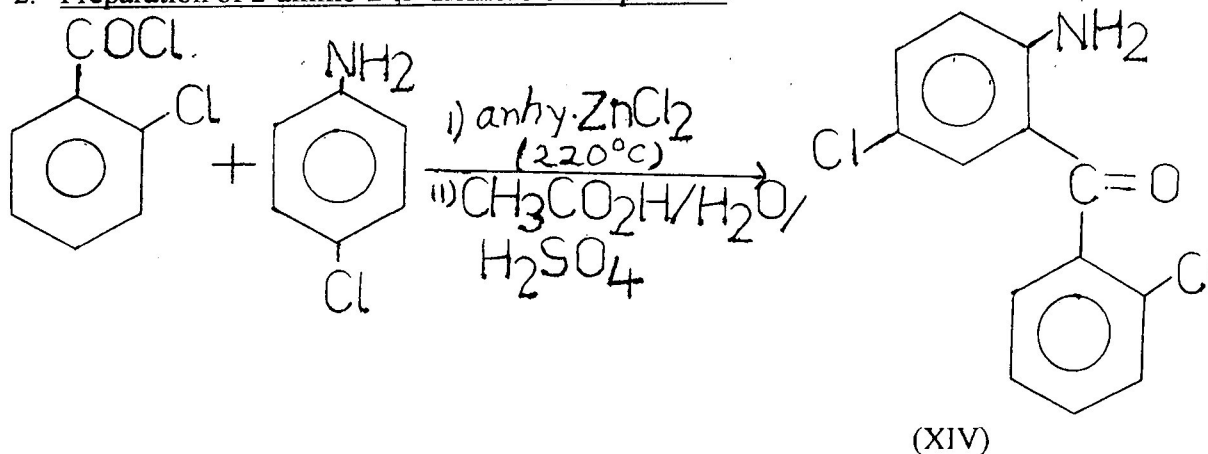
1. Preparation of 2-chlorobenzoyl chloride:



2-chlorobenzoic acid was refluxed in excess of thionyl chloride for 5 hours.

Excess of thionyl chloride was removed under reduced pressure and acid chloride was distilled off to obtain 2-chlorobenzoyl chloride (XIII).

2. Preparation of 2-amino-2',5-dichloro benzophenone.



4-chloroaniline (5g, 0.39M) was added to 2-chlorobenzoyl chloride (17.13g, 12.4ml, 0.0979M) at 140°C. To this was added anhydrous zinc chloride (6.25g, 0.045M) at 220°C.

It was stirred for two hours at this temperature and poured in water. The residue was refluxed in the mixture of water(8.6ml), acetic acid(12.4ml) and conc. sulphuric acid(16ml) at 130⁰C for 14 hours. It was then poured in cold water and the solid obtained was collected by filtration and purified to get 2-amino-2',5-dichlorobenzophenone (XIV). Yield obtained was 71%. m.p.= 87-88⁰C.

Modifications:

1. In the procedure reported in the literature, the starting material used was 4-chloro benzanilide. This was replaced by 4-chloroaniline in our laboratory, which is a lot cheaper than 4-chlorobenzanilide.
2. In literature method, three hot water washings, each of two hours, have been given. In our laboratory we gave two fifteen-minute washings of hot water followed by treatment with 5% sodium hydroxide solution. This eliminates unreacted 2-chlorobenzoic acid chloride. All this work can be accomplished in one hour which saves five cumbersome hours.
3. During workup, according to literature procedure, reaction mixture is to be dumped in water, then it is extracted with organic solvents like benzene or ethyl acetate. The organic layer is treated with sodium bicarbonate solution and then the organic solvent is evaporated to get the crude product. In our laboratory, we dumped the reaction mixture in ice-cold water. Water was decanted and solid was suspended in 5% sodium hydroxide solution. However, solvent extraction which is a costly and hazardous method in chemical industry is deleted and replaced with cold water treatment of the reaction mass followed by washing with NaOH solution. This makes the process much less hazardous,

simpler and economical. After five minutes, the solid was collected by filtration. The quality and purity of the product remained comparable with the commercial sample.

V. **Biological screening:**

The compounds reported in parts 2&3 have been subjected to *in vitro* antitubercular and antibacterial screening.

The test compounds (10 mg) were dissolved in suitable solvent (10 ml) such as dimethylformamide, ethanol, dil. HCl as the case may be, so as to prepare drug stock solution 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 concentration, the drug stock solution was diluted to prepare various stock solutions such as 10, 5, 2.5 µg/ml etc.

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 al*¹⁷ was used.

The tubes were incubated at 37°C for ten days and then examined for the presence or absence of growth of the test organism. The lowest concentration which showed no visible growth was taken as the end point, i.e. Minimum Inhibitory Concentration. Isoniazid was used as a standard drug and was also subjected to same screening pattern. Minimum Inhibitory concentration of Isoniazid is 0.04 µg/ml.

The test compounds have also 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 medium¹⁸. 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*.

The compounds in part 2 were also subjected to in vivo anthelmintic screening¹⁹. These compounds were tested for their activity against *H.nana* in albino mice. The standard drug used for anthelmintic screening was Mebendazole.

Seven compounds in part 2 exhibited anti-bacterial activity upto 10 µg/ml against *S. typhi* and *S. aureus*. Five compounds from part 3 exhibited anti-bacterial activity upto 10 µg/ml. Seven compounds in part 2 exhibited anti-tubercular activity upto 10 µg/ml. Results of anthelmintic activity are awaited.

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