

SYNTHESIS OF ORGANIC COMPOUNDS OF POSSIBLE THERAPEUTIC VALUE

THESIS SUBMITTED TO
UNIVERSITY OF MUMBAI

FOR THE DEGREE OF
MASTER OF PHARMACEUTICAL SCIENCES
IN
PHARMACEUTICAL CHEMISTRY

SUBMITTED BY
CHANDAN P. JAGTAP

UNDER THE GUIDANCE OF
DR. B.G. KHADSE

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

Disclaimer

This research work belongs to

Mr Chandan P. Jagtap

No content of this work can be
circulated or used for any commercial
purpose without the consent of
respective researcher

**Synthesis of
Organic Compounds of Possible
Therapeutic value**

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

547.785.5:615

JAG

By

**Mr. Chandan P. Jagtap
B. Pharm.**

**HAFFKINE INSTITUTE LIBRARY,
MUMBAI.**

**Call No.
Price**

**Acc No. 423
Source Purchased.
Donated.**

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

1998

ACKNOWLEDGEMENTS

I express with all sincerity my deep sense of gratitude, indebtedness and thankfulness to my respected guide Dr. B. G. Khadse, M.Sc., Ph.D., Director, Haffkine Institute. Whose deep dedication, constant encouragement and constructive thinking during the entire course of study has greatly helped me to present this work. It is with pleasure that I recall my years of association with him.

I am grateful to Dr. V. L. Yemul, former Director, Haffkine Institute for providing excellent research facilities during the course of this work.

I am thankful to Dr. S. V. Gadre, Assistant Director, Department of Bacteriology for various suggestions in carrying out antimicrobial screening.

It is with great pleasure, that I take this opportunity to thank Dr. Anil Bobade, Dept. of Chemotherapy, who has always extended moral support and timely insights during my most difficult times.

My very special thanks to Mr. Hemant Narkhede and Ms. Bharati Kulkarni for reviewing the manuscript and for the valuable discussions which helped me to present the work in this form. It is great pleasure to thank Mr. Ravi Chambhare, Mr. Sandeep Paranjape, Mr. Sagar Agnihotri, Ms. Sharvani Vingkar, Ms. Mukta Bohra, Ms. Sonali Wayagankar for their kind co-operation during this work.

I am very much thankful to my senior colleagues Dr. Arvind Manian, Dr. Anup Barde and my colleague Ms. Pallavi Kharkar, for the great help they provided in interpreting NMR spectra. I thank the Regional Sophisticated Instrumentation Center, IIT Powai, for prompt assistance in providing NMR spectra.

I take this opportunity to express my thankfulness towards several colleagues from the other departments of the institute Masoud, Viraj, Arun, Vandana and Smita.

I thank Mrs. P. A. Kirtikar, Mr. L. G. Mistry for carrying out the elemental analysis, Mr. K. R. Pawar for carrying out the microbiological screening and Dr. Mrs. V. R. Kamath for carrying out the antimalarial screening. I also thank the entire staff of Dept. of Chemotherapy for their help.

I extend my appreciation to Ms. M. V. Joshi and the entire staff of the library for their everhelping nature and for maintaining a beautiful library.

I gratefully acknowledge Mr. Manish Sheth for formatting my thesis.

I am indebted to my parents and my sisters, Shraddha, Megha and Swati, whose patience and inspiration made me carry out this work. My most special thanks to my brother-in-law Ganesh, for helping me during the most crucial part of the work which I may never be able to reciprocate.

I thank my dearest friends Surendra, Mandar, Rahul, and Sammit for supporting and encouraging me during the course of my study.

I am indebted to God for providing me help in my most difficult times.



STATEMENT

Statement submitted under rule 0.412

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

Statement submitted under rule 0.413

Statement I :

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

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

Statement II :

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

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

Dr. B. G. Khadse
Signature of Research Guide

Chandan P. Jagtap
Signature of the Research Student

CONTENTS

	Page
PART I A - INTRODUCTION, REVIEW OF LITERATURE PLAN OF WORK	1
PART I B - CHEMISTRY OF	
♦ BENZIMIDAZOLE	47
♦ 2-METHYL THIOPSEUDOUREA-1,3-DICARBOXYLIC ACID DIMETHYL ESTER	49
♦ METHYL 5 - (SUBSTITUTED THIO) - 2 - BENZIMIDAZOLE CARBAMATE	50
♦ 2 - MERCAPTO BENZIMIDAZOLE	51
♦ ACID CHLORIDES	54
♦ 5 - METHOXY - 2 - (THIOCARBAMOYL SUBSTITUTED) BENZIMIDAZOLE	56
♦ DIAZONIUM SALTS	58
♦ REDUCTION	61
PART I C - SCHEMATIC REPRESENTATION OF THE SERIES OF COMPOUNDS SYNTHESISED	65
PART II - SYNTHESIS OF METHYL 5 - (SUBSTITUTED THIO) - 2 - BENZIMIDAZOLE CARBAMATE	67
PART III - SYNTHESIS OF 5 - METHOXY - 2 - (THIOCARBAMOYL SUBSTITUTED) BENZIMIDAZOLE	82
PART IV - SYNTHESIS OF DRUG INTERMEDIATE OF TRICLOSAN AND TRICLOSAN ITSELF	96
PART V - BIOLOGICAL RESULTS AND DISCUSSIONS	105
CONCLUSION	115
REFERENCES	117
APPENDIX	125
SYNOPSIS	



PART IA

**INTRODUCTION, REVIEW OF LITERATURE AND PLAN OF
WORK**



INTRODUCTION

Humankind has been subject to ubiquitous problem of disease since before the dawn of recorded history¹. One presumes that mankind has been searching for suitable therapy for nearly as long. This was a desperately difficult enterprise given the acute nature of most infections and the nearly total lack of understanding of their origins, which was prevalent until the last century. Man has waged a continuous battle against the various diseases that afflict him. Today, a large number of diseases are cured, or at least controlled by a drug therapy. Thus, the fight against bacterial and fungal infections has been largely won, and significant progress has been made in treating mental, pulmonary, gastrointestinal, inflammatory and cardiovascular conditions. In addition, some cancers and certain forms of leukemia are also cured by chemotherapy.

Until relatively a few years ago, there was a sense of optimism that the long struggle for control over infectious diseases was almost over. Smallpox was eradicated. Poliomyelitis, guinea worm disease, leprosy, chagas disease and neonatal tetanus were targeted for eradication or elimination. Antimicrobial drugs were effectively suppressing countless infections.

But cautious optimism has turned into a fatal complacency that is costing millions of lives every year. The struggle for control, far from being over, has reached a critical stage. Infectious diseases remain the world's leading cause of death, accounting for at least 17 million (about 33%) of the 32 million people who die each year.

Diseases that used to be restricted geographically, such as Cholera, are now striking in regions once thought safe. While some diseases have been almost subdued, others such as Malaria and Tuberculosis that have always been among our greatest enemies are fighting back with renewed ferocity. The role of infectious agents in the development of many types of Cancer is becoming more evident.

New and emerging diseases, combined with the rapid spread of pathogens resistant to antibiotics and of disease carrying insects resistant to insecticides, are daunting

challenges to human health. The new diseases range from AIDS to little known but equally lethal viral infections. In many cases, their source is unknown, as is the reason for their emergence. More often than not, no specific treatment is available for them. Thus, to combat these problems, rigorous and intensified research in an attempt to discover newer and more effective drugs is essential. The Medicinal Chemist, who forms the base of the pyramid constituting drug research, is constantly searching for novel moieties which can be exploited for chemotherapeutic purposes. The development of any drug is a lengthy, costly exercise, which requires the combined activities of chemist, biologist, pharmacist and clinicians. It is in the discovery phase, that Medicinal Chemist plays this major role.

The present investigations are the attempts of such a Medicinal Chemist for possible solutions to some of the persistent health problems that affect human life, namely, bacterial, fungal and malarial infections.

BACTERIAL INFECTIONS :

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

However, Domagk², Mietzsch and Klarer³ in 1935, first dispelled the failure of earlier scientists by discovering Prontosil, and reported that this compound cures *Streptococcal* infection in mice and *Staphylococcal* infection in rabbit. This period

was considered to be a brilliant era in chemotherapy of microbial infections.

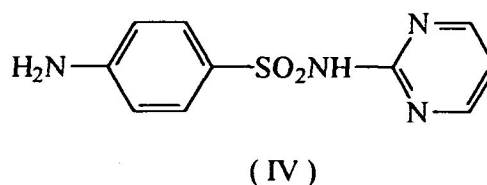
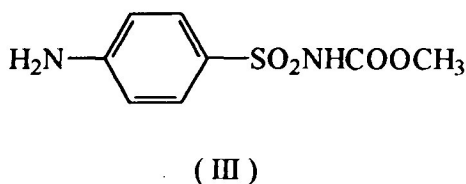
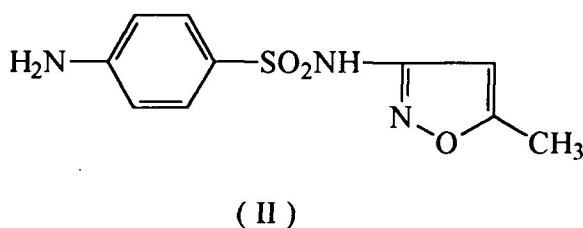
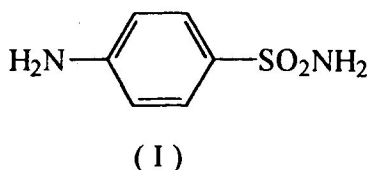
The history of antibacterials has been dynamic, characterized by the constant emergence of new challenges followed by investigation, discovery and the production of new drugs. A complete review of the various agents would be beyond the scope of this work and hence is not attempted. The following pages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

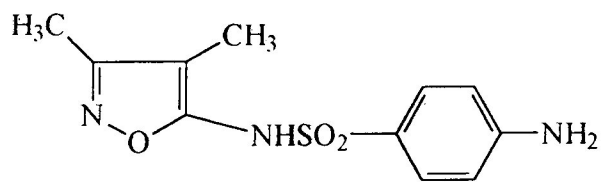
Synthetic Antibacterial agents :

Sulphonamides

Sulphonamides were the first, systemic chemotherapeutic agents employed to prevent and cure bacterial infections in man and were quite effective in bringing about a sharp decline in the morbidity and mortality figures for the treatable infectious diseases. The Sulfonamides are bacteriostatics, when used in achievable doses and, inhibit the enzyme dihydropteroate synthetase, an important biocatalyst needed for the biosynthesis of folic acid derivatives, and ultimately DNA. They do it by competing at the active site with p-aminobenzoic acid (PABA), a normal structural component of folic acid derivatives.

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





(V)

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

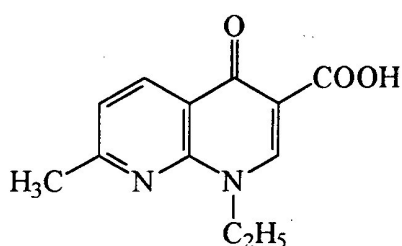
Strains of bacteria resistant to the sulphonamides have become common during recent years. Also, the advent of penicillins and other antibiotics has diminished the role of sulphonamides as antibacterial agents. However, the introduction of the combination of trimethoprim and sulphamethoxazole, known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

Quinolones

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

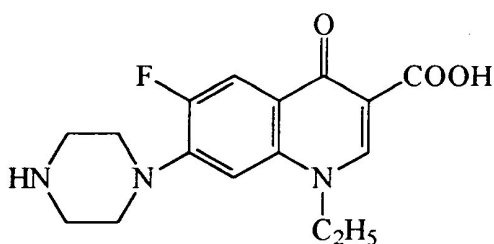
The Quinolone carboxylic acids or 4-Quinolones are the group of synthetic antibacterial agents structurally related to nalidixic acid (VI). Nalidixic acid is active against gram-negative bacteria but has little activity against *Pseudomonas* and gram-positive organisms because of which its bactericidal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

Modification of the structure of nalidixic acid has produced related antibacterial agents such as oxolinic acid, pyromidic acid, cinoxacin and acrosoxacin. None has been considered to represent a significant clinical advantages over nalidixic acid.

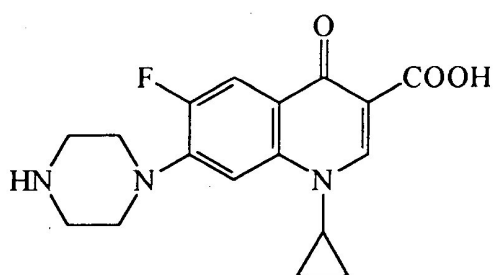


(VI)

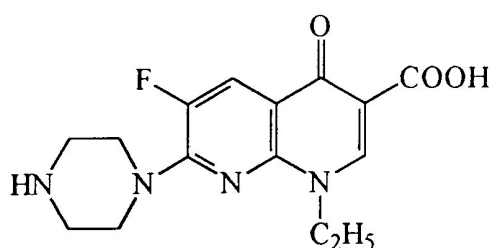
Addition of the 7-piperazinyl group and a fluorine atom at position 6 has produced the group of fluoroquinolones with a broad spectrum of activity than nalidixic acid and are most useful for the treatment of systemic infections. They include norfloxacin (VII), ciprofloxacin (VIII), enoxacin (IX), ofloxacin (X) and lomefloxacin (XI). One disadvantage of all quinolone antibacterial agents is that they are generally not recommended for use in children, adolescent, pregnant and breast feeding women, because of there propensity to cause joint erosion in immature animals.



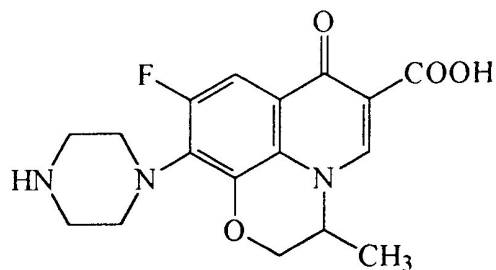
(VII)



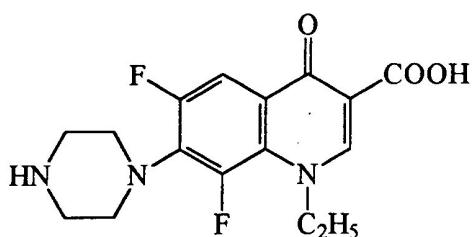
(VIII)



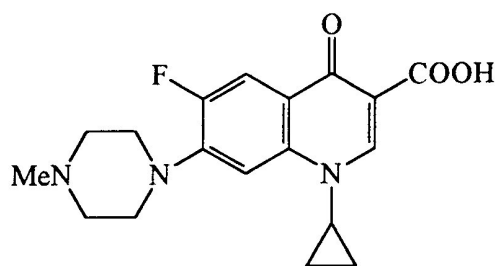
(IX)



(X)



(XI)

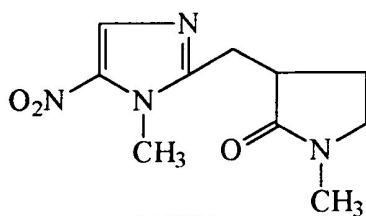


(XII)

Tefloxacin (XII) was demonstrated by Y. L. Chen ⁴ for activity against penicillin resistant *Staphylococcus*, *Streptococcus* and *Haemophilus*. It was itself a β -lactamase inhibitor and displayed synergism with ampicillin.

Miscellaneous

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



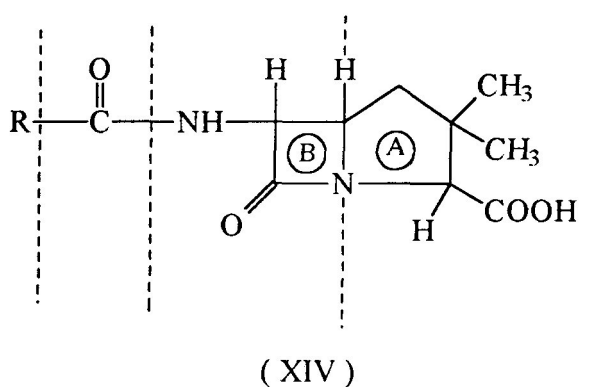
(XIII)

The minimum pharmacophore required for significant antibacterial activity consists of the 4-pyridine ring with a 3-carboxylic acid group. The mechanism of action of Quinolones is prevention of the detachment of gyrase from DNA.

Penicillins and Cephalosporins

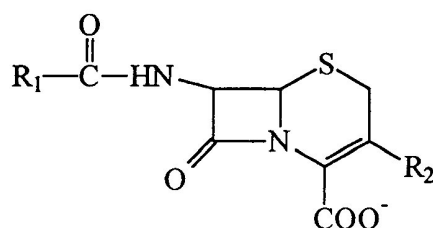
Since Alexander Fleming's serendipitous discovery of Penicillin in 1928 it has remained one of the most important groups of antibiotics. Despite the discovery of several other antibacterial agents, the penicillins are widely used with new derivatives being produced every year. Due to their unique advantages,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

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



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

No sooner was the fact established that penicillins are capable of eradicating microorganisms, it was practically administered in every situation where there was a risk of bacterial infection. But after the investigation 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 others. The commonly sited adverse effect in the usage of penicillins, which was probably the most common cause of drug allergy, has been the hypersensitivity reactions. Such reactions could occur with any dosage form of penicillin, but as the drug is rapidly eliminated the allergic reactions usually subside. In some cases, the symptoms may persist for one to two weeks after withdrawal of treatment, while in other cases the reaction is mild and disappears during therapy with the drug. At the worst of circumstances, an extreme allergic reaction may even result in death of the patient.



(XV)

The Cephalosporins (XV) have shown tremendous growth over the past decade and are usually classified by the "generations" system, which is based on the general features of antibacterial activity. The first generation Cephalosporins exhibit good activity against gram-positive bacteria and relatively low activity against gram-negative bacteria. The second generation Cephalosporins exhibit better activity against gram-negative microorganisms but are inferior to third generation Cephalosporins. The third generation Cephalosporins are generally less active against gram-positive bacteria as compared to first generation agents, but are much more active against beta lactamase producing strains.

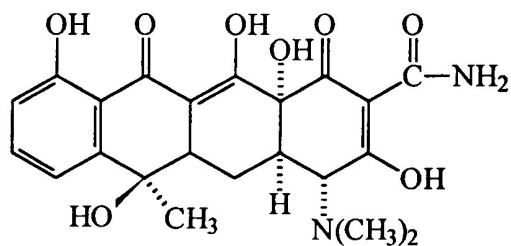
The Cephalosporins are widely used antibiotics, perhaps overused for conditions where much less expensive antibiotics could provide relief. Nevertheless they are indeed useful as alternatives to penicillins for a variety of infections inpatients who

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

The beta-lactam antibiotics (Penicillins and Cephalosporins) suffer bacterial resistance due to a variety of factors. It may be caused by the inability of the agent to enter the site of action⁵. Also, it is possible for a sensitive strain to acquire resistance by mutation and has been described for gram-positive cocci⁶. Bacteria can also destroy the beta-lactam antibiotic enzymatically. Beta lactamases are capable of inactivating certain of these antibiotics and is the major form of this enzyme, although different microorganisms synthesize a number of distinct beta-lactamases. Depending on substrate specificities these are often described as either penicillinases or cephalosporinases. Individual Penicillins and Cephalosporins vary in their susceptibility to these enzymes.

Tetracycline

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



(XVI)

Microorganisms that have become resistant to one type of tetracycline frequently exhibit resistance to the other members of the family. The bacterial species possibly

exhibit resistance only after exposure to the drug. The tetracyclines are initially useful for treatment of infections with aerobic gram-negative bacteria but most species are now relatively resistant. Nevertheless, more than 90% of strains of *Haemophilus influenza* may still be sensitive to either tetracycline or doxycycline.

All the tetracyclines produce gastrointestinal irritation to varying degrees in some individuals and is more common after oral administration. Diarrhoea may also be a side effect, which though frequent and fluid, does not contain blood or leukocytes. Doxycycline, and to a lesser extent other derivatives, may produce mild to severe reaction in the skin of treated individuals exposed to sunlight. Other important antibacterial agents include Chloramphenicol and the furan derivatives such as nitrofurantoin, furazolidone etc. Several of the drugs active against *M. tuberculosis* discussed earlier are also active against various species of gram-positive and gram-negative microorganisms besides mycobacteria.

FUNGAL INFECTIONS

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

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

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

Severe toxic and haemorrhagic diseases can occur in animals and humans after ingestion of harvested grains and nuts in which saprophytic fungi have grown and

produced toxins. Likewise, hemolytic, neurotoxic, and gastrointestinal diseases are associated with ingestion of certain types of saprophytic mushrooms. Antifungal drugs are powerless in the treatment of mycotoxic diseases.

The discovery that specific fungi are the etiologic agents of specific infectious diseases preceded by three decades the work of Pasteur and Koch on pathogenic bacteria. Nevertheless, medical mycology has not received as much attention as have other areas of medical microbiology and at present there exist neither clinically available vaccines nor useful antiserum for mycotic diseases. The incidence of systemic mycoses continues to rise steadily. This increase is due in part to improved recognition and diagnosis of fungi infections, but also to the prolonged survival of patients with defects in their host defense mechanism including patients with neoplastic diseases, organ transplant recipients, diabetics and patients with AIDS. These patients are susceptible to an ever-growing list of opportunistic fungi. From the data collected by the Center for Disease Control, it is known that among the 1 and 2 million healthy Americans who have already been infected with AIDS, a significant number of these cases are associated with various fungal infections⁷. One yeast-like fungus, *Cryptococcus neoformans*, is reported to be the common of debilitating and life-threatening infections in AIDS patients⁸.

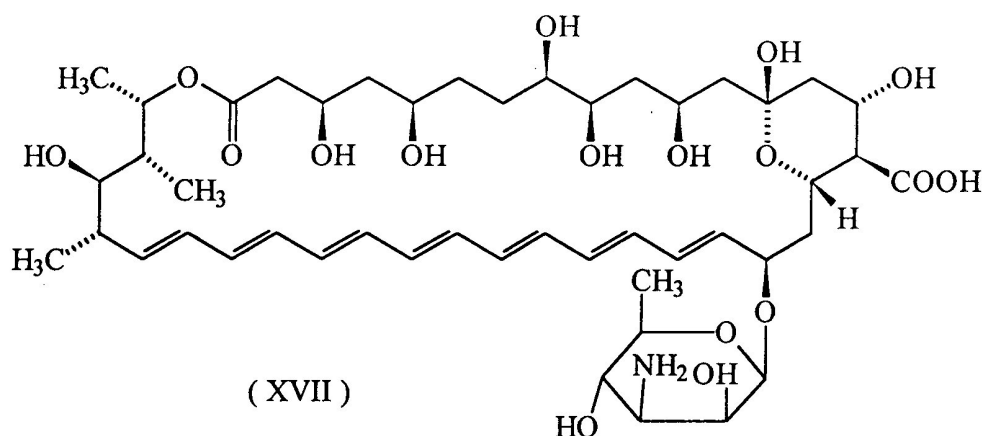
The following pages represent a brief description about major principal drugs that are used in treating fungal infections.

Amphotericin B

This antibiotic produced by *Streptomyces nodusus*, was discovered in 1955. It became the first systemic antifungal drug. Amphotericin B (XVII) is a polyene macrolide with seven conjugated double bonds (a heptene), an internal ester, a free carboxyl group and a glycoside side chain with a primary amino group. The drug is amphoteric, insoluble in water and is not absorbed via oral or intramuscular routes and is very active against pathogenic yeasts⁹.

The primary mechanism of action of the polyenes in general and amphotericin B in

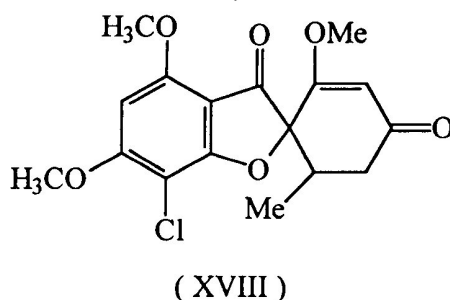
particular is due to binding to ergosterol, the principal sterol present in the cell membrane of sensitive fungi¹⁰.



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

Griseofulvin

Although Oxford discovered this antibiotic, a product of *Penicillium griseofulvum* (XVIII), in 1939, its antifungal activity was not utilized until 1951. Groove predicted its chemical structure in 1950¹¹. However in 1958, the antibiotic was found to be effective against dermatophytes when administered orally. The fluoro analogues retain the potency of the original drug but the bromo and dichloro analogues are ineffective. Replacement of the methoxy substituent on the cyclohexane ring either with propoxy or butoxy increased invert activity whereas substitution of an amino acid group at this site eliminates activity.

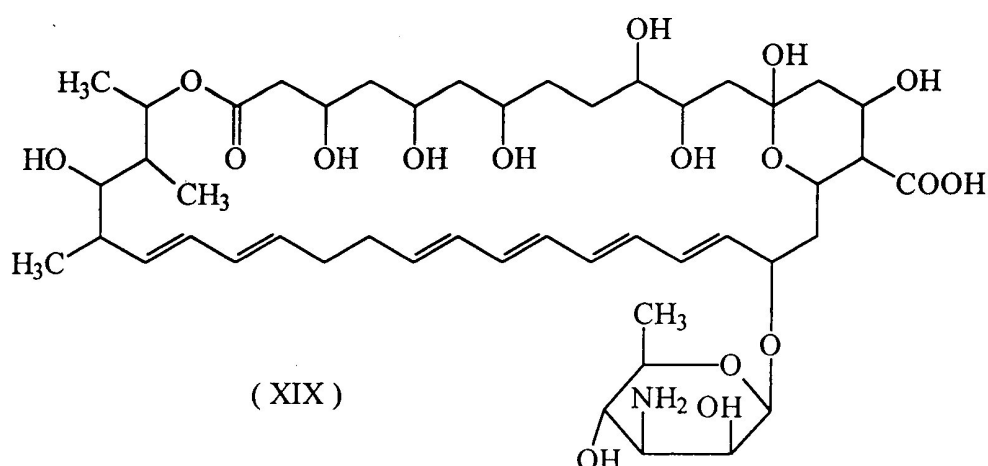


In susceptible fungi, griseofulvin binds to RNA and to microtubule associated

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

Nystatin

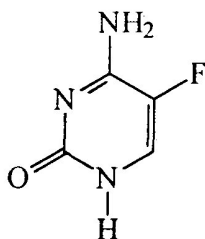
Nystatin (XIX) is produced by *Streptomyces noursei*. It was discovered by Hazen and Brown¹² and possesses good antifungal activity. Toxicity after parenteral administration to animals has led to its use only topically or orally.



The minimum inhibitory concentration of this drug for *Candida* species, *Cryptococcus neoformans* and the dimorphic fungi causing systemic mycoses is in the range of 1.56-12.6 $\mu\text{g/ml}$.

5-Fluorocytosine

This fluorinated pyrimidine (XX) was first synthesized in 1950s as a potential antileukemic drug. It was found to have *invitro* and *invivo* antifungal activity¹³. It has been increasingly in usage as an adjunct to amphotericin B therapy. Accordingly, in *Aspergillus*, *Candida* and *Cryptococcus* infections, the drug is combined with Amphotericin B.

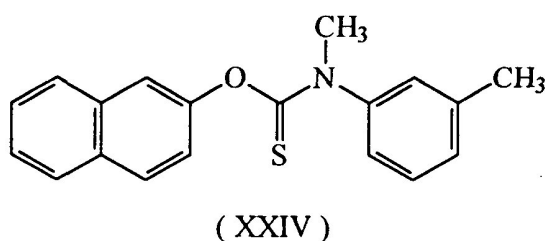
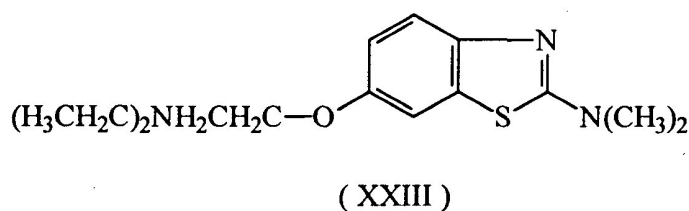
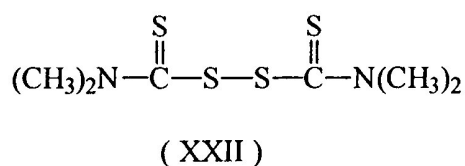
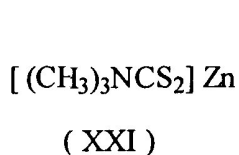


(XX)

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

Sulphur containing molecules as antifungal agents

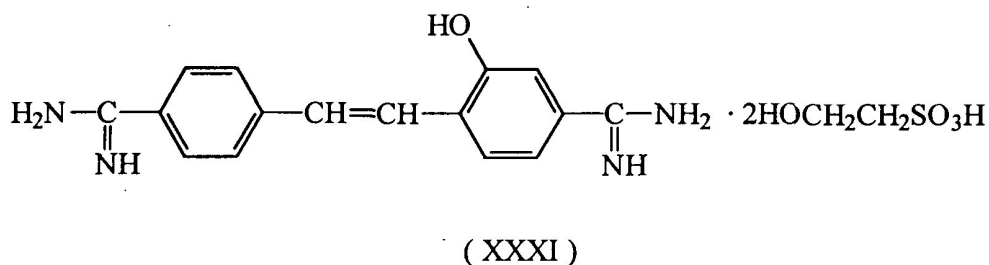
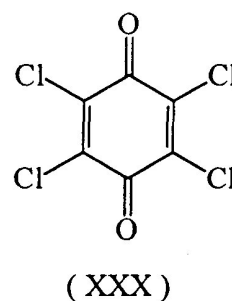
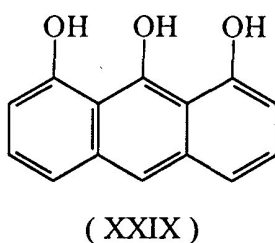
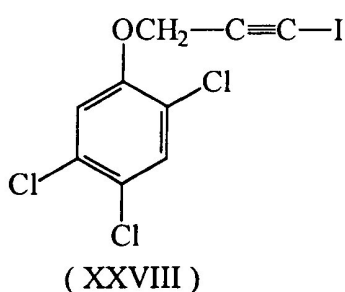
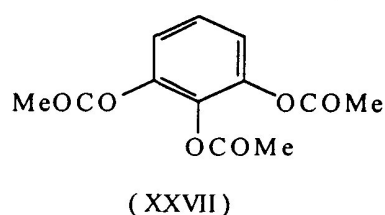
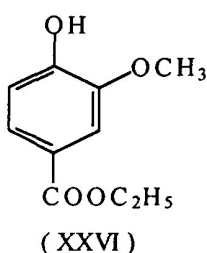
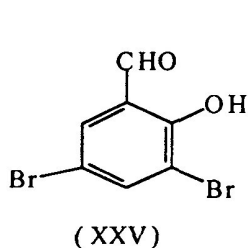
A variety of organic compounds that contain divalent sulphur attached to carbon atom have *invitro* fungistatic action, and many of these have been employed in topical therapy of dermatophytoses. Among these are dithiocarbamates (XXI), tetraethylthiuram disulphide (XXII) and benzothiazoles such as 6-(b-diethylaminoethoxy)-2-dimethyl amino derivatives (XXIII)^{14,15}.



A widely used compound with unusually high *in vitro* and *in vivo* activity against the dermatophytes is o-2-naphthyl ester of *m*-N-dimethylthiocarbanilic acid (XXIV) (Tolnaftate). It is also effective against Candidal lesions¹⁶.

Phenolics and Diamides

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

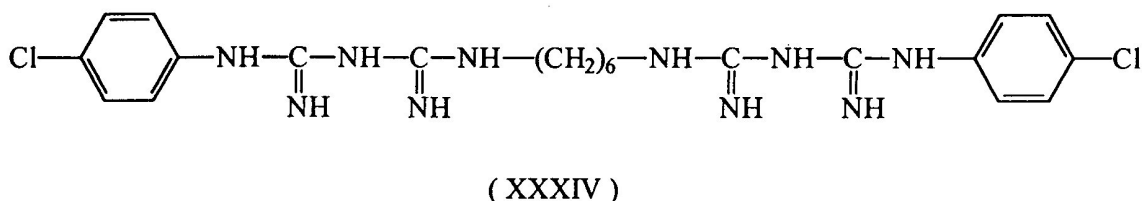
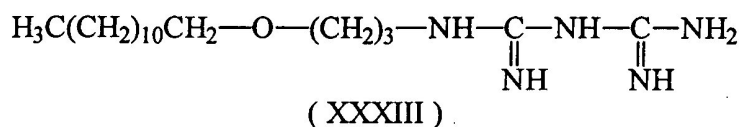
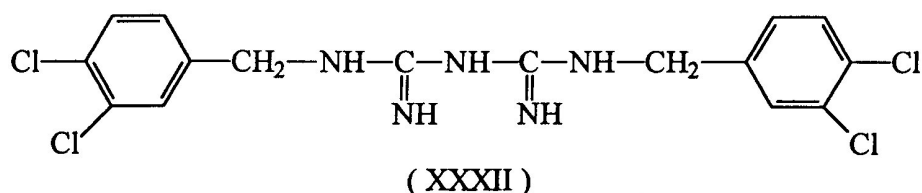


Several biamidines (e.g. stibamidine, propamidine, pentamidine) are antifungal *in*

vitro. 2-hydroxy stilbamidine diisothionate (XXXI) has been successfully employed in patients with blastomycosis. It is less toxic than Amphotericin B¹⁷.

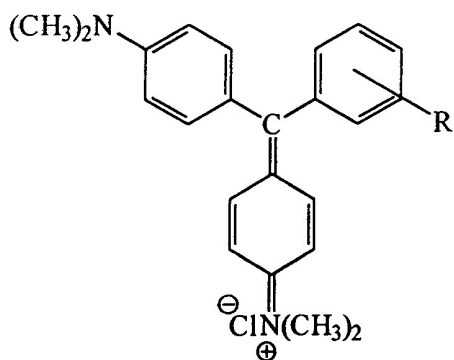
Chelating Agents

Among chelating agents with considerable *in vitro* antifungal potency are substituted biguanides as N', N⁵-bis- (3,4-dichlorobenzyl) biguanide (XXXII), N'-Y-laurooxypropyl biguanide (XXXIII), 1,6-bis- (5-p-chlorophenyl biguanidine) hexane (XXXIV)¹⁸.

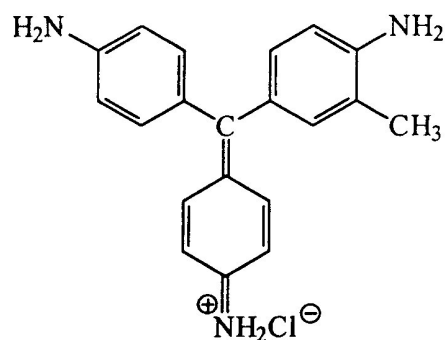


Aniline Dyes

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



(XXXV) R = H
(XXXVI) R = N(CH₃)₂



(XXXVII)

Azoles

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

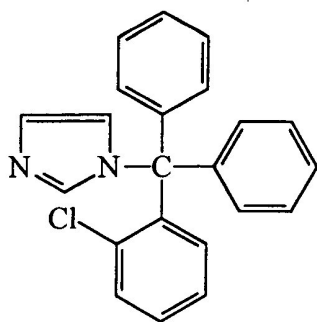
Clotrimazole (XXXVIII) was developed by investigators of the Bayer Laboratories in 1969, and is utilized topically in superficial mycoses or orally in clinical trials in deep mycoses¹⁹.

Miconazole (XXXIX) is a well-known antifungal drug developed by the investigators of Janssen Pharmaceuticals in 1969. It is utilized topically in superficial mycoses and by oral, intravenous routes in deep mycoses in clinical experimental trials²⁰.

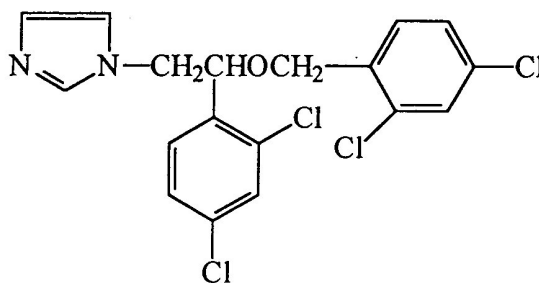
Econazole (XXXX) is a phenethyl imidazole derivative. Like Miconazole, it is utilized as a topical agent in superficial mycoses and as a systemic antifungal by oral and intravenous routes in deep mycoses²¹.

Fluconazole (XXXXI) is a water-soluble fluorine substituted bis-triazole. It has comparable activity to that of Ketoconazole in inhibition of fungal C-14 demethylase. It is also a potential candidate for prevention and early treatment of disseminated candidiasis in granulocytopenic patients²².

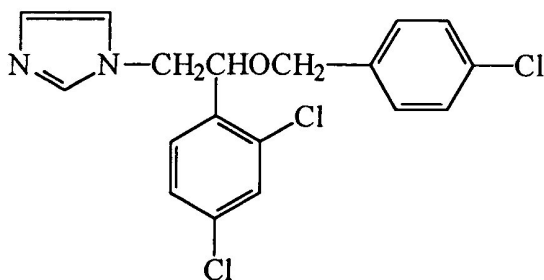
Itraconazole (XXXXII) is a water soluble, lipophilic triazole analogue that exhibits excellent activity against most fungal pathogens. When compared with Ketoconazole, its potential advantages include a broader spectrum of activity (including *Aspergillus* sp. and *Sporothrix schenckii*), a more desirable pharmacokinetic profile and less toxicity²³.



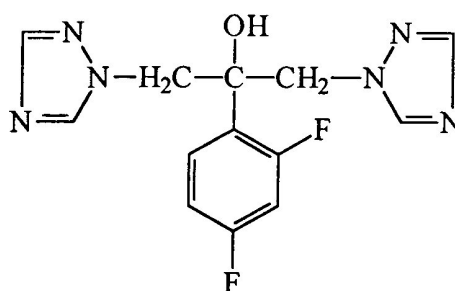
(XXXVIII)



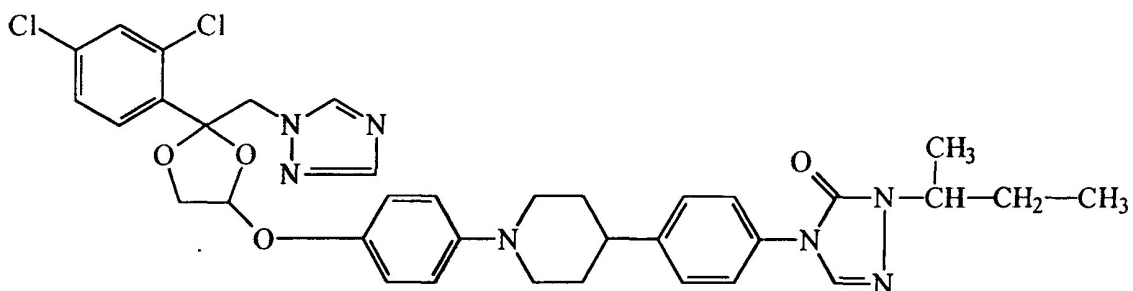
(XXXIX)



(XXXX)



(XXXXI)



(XXXXII)

Azoles exert antifungal activity by inhibiting cytochrome P-450 that catalyses the 14α - demethylation of lanosterol to ergosterol. Although the azoles are considered to have a wide antifungal spectrum, individual differences in activity occur. For instance, itraconazole is much more active than ketoconazole against the agents of aspergillosis and sporotrichosis. Similarly, fluconazole is active against *Candida tropicalis*, whereas ketoconazole is active against *Candida krusei*, an agent resistant to fluconazole.

ANTIMALARIAL DRUGS

Malaria is one of the most serious, complex and refractory health problems facing humanity this century. The disease, presenting over 120 million clinical cases annually infects some 300-500 million of the world's people. It is estimated that between 1.5 and 2.7 million people die from malaria every year, either directly or in association with acute respiratory infections and anemia, and upto 1 million of those deaths are among children younger than five years old. More persons die from malaria each year than have died from AIDS in the last seventeen years.

Human malaria is caused by four species of protozoan parasites of the *Plasmodium* genus. These are *Plasmodium falciparum*, *P. vivax*, *P. ovale* and *P. malariae*, each of which presents slightly different clinical symptoms. *P. falciparum* is the most widespread of the four geographically and the most pernicious, causing a majority of malaria-related morbidity and mortality.

An insect vector, the female anophelous mosquito, transmits parasites from one person to another. In most malaria-prone areas, several species are able to transmit

the parasite and the exact species responsible vary from region to region. Male mosquito does not transmit the disease. These mosquitoes are present in almost all countries in the tropics and subtropics and they bite during nighttime hours, from dusk to dawn. The parasites develop in the gut of the mosquito and are passed on in the saliva when an infected mosquito bites a person. The blood carries the parasites to the victim's liver where they invade the cells and multiply. After 9-16 days they return to the blood and penetrate the red cells, where they multiply and begin destroying the red cells.

The signs and symptoms of malaria illness are variable, but most patients experience fever. Other symptoms often include headache, back pain, chills, muscle ache, increased sweating, malaise, nausea, and sometimes vomiting, diarrhoea, and cough. Early in an infection, the cyclic patterns of fever may not be noticeable but later, a clear cyclic trend with symptoms recurring at regular intervals occurs. Of the four species of parasite, only *Falciparum* malaria can progress rapidly to the cerebral stage, where infected red cells obstruct the blood vessels in the brain. Untreated cases can progress to coma, renal failure, liver failure, pulmonary edema, convulsions, and death. Although infections with *P. vivax* and *P. ovale* often cause less serious illness, parasites may remain dormant in the liver for many months or even years later. Malaria is diagnosed by the clinical symptoms and by microscopic examination of the blood. Stained thick and thin blood smears are used to diagnose malaria and to quantify the level of parasitemia. Giemsa-stained thin smears are used to differentiate between the species of parasite. Antimalarial drugs can normally cure malaria. The symptoms quickly disappear once the parasites are killed. The standard measures of clinical antimalarial drug efficiency are fever clearance time and parasite clearance time. In certain geographic regions, however, the parasites have developed resistance to antimalarial agents, particularly chloroquine.

In the last several years, it has been noted that microscopic examination of blood is an inadequate method for detecting low levels of parasitemia. The lack of sensitivity seldom affects treatment and diagnosis in acute cases, but it does not limit

understanding of the degree to which malaria is chronic. In an endemic area, polymerase chain reaction studies revealed that more than 90% of the exposed population at any one time was chronically infected with *P. falciparum*²⁴.

The choice of antimalarial agent for treatment in each particular case is determined by a multiplicity of factors such as the parasite species causing the infection, the acquired immune status of the patient, the susceptibility of the parasite strain to antimalarial agents, the facilities and resources available for health care, and the genetic make-up of the patient, to list a few. During pregnancy, women are at high risk of death from falciparum malaria. Also at risk are children who are prone to severe attacks until they develop immunity.

The Parasite

The four species of human malaria parasites are evolutionarily, morphologically and clinically distinct. *P. Vivax*, *P. malariae* and *P. ovale* are closely related on an evolutionary basis to a number of simian malarias. *P. falciparum* appears more closely related to avian malarias and is of more recent origin perhaps within the last 10,000 years²⁵. Recently, a new species, dubbed *P. vivax* like parasite, has been described that infects humans²⁶. Its morphological characteristics show it to be similar to *P. vivax*, but analysis of its DNA indicates that the sequence for the circumsporozoite protein (CS) gene is quite different from that of *P. vivax*. The CS protein is the major surface protein of the sporozoite stage of the parasite and has been studied as a source of malaria vaccine antigens.

The life cycle of the parasite in both mosquitoes and humans is complex, as is the terminology of the various parasite development stages. When an infected mosquito bites, sporozoites are injected into the blood stream of the human victim and then travel to liver tissue where they invade parenchyma cells. During development and multiplication in the liver, known as the preerythrocytic stage, the host is asymptomatic. After a variable period of time (6-8 days for vivax, 9 days ovale, 12-16 days malariae, 5-7 days *falciparum*), merozoites (5,000 to 40,000 per sporozoite) are released from the liver and the parasites take up residence in the red blood cells

(erythrocytic stage). In nonrelapsing malarias (*falciparum* and *malariae*), no parasites are left in the liver; the infection moves entirely into the blood stage. In relapsing malarias, some of the merozoites (or sporozoites) differentiate into a dormant nondividing stage (hypnozoites), providing a reservoir of parasites in the liver that can be activated for up to five years following the initial infection. Invasion of the red cell by a merozoite results in the development of the trophozoite stage. The parasite feeds upon the protein portion of hemoglobin and hemozoin, a waste product, accumulates in the host cell cytoplasm. After the parasite undergoes nuclear divisions, the red blood cells burst and merozoites, parasite waste, and cell debris are released. The presence of the debris is the cause of the episodes of fever and chills associated with malaria. The merozoites released by the red cell rupture go on to infect more erythrocytes. Time intervals between cell rupture (fever), infection of other erythrocytes, and then their rupture (new bouts of fever) are characteristic of the parasite species. A few merozoites become differentiated into male and female gametocytes, forms that are dormant in humans. When a mosquito takes a blood meal from the infected human, the gametocytes begin sexual reproduction in the digestive tract of the mosquito. Ultimately, sporozoites form and then reside in the mosquito saliva, ready for a new round of infection.

Humans are not the only creatures that are parasitised by *Plasmodia* species. Several other parasites and their hosts have been used extensively for research in malaria. *Plasmodium berghei*, *P. vinckei*, and *P. yoelii* are useful models for malaria in mice and rats while *P. cynomolgi* and *P. knowlesi* are studied in owl monkey (aotus) and in splenectomized chimpanzee.

The most common method for evaluating the antimalarial activity of the drugs and experimental compounds is the microdilution technique introduced by Desjardins and coworkers in 1979²⁷ and modified by Milhous²⁸. Cultured intraerythrocytic asexual forms of *P. falciparum* are treated with serial dilutions of compounds to be tested. Inhibition of uptake of [$G-^3H$] hypoxanthine by the parasites serves as the indicator of antimalarial activity.

Resistance

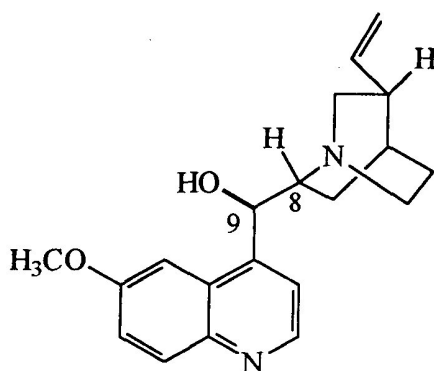
Drug resistance in malaria has been defined as the “ability of a parasite strain to survive and / or multiply despite the administration and absorption of a drug given in doses equal to, or higher than, those usually recommended, but within the limits of tolerance of the subject”²⁹. The phenomenon of drug resistance has become common in many infectious diseases; tuberculosis, staphylococci, streptococci, HIV, syphilis. Drug resistance was perhaps first recognised in the treatment of malaria. Early in this century, it was noted that some cases of malaria responded more poorly to quinine, the only drug available at the time. At present, the increasing occurrence of chloroquine-resistant strains of *P. falciparum* has inspired global drug-design and development efforts in an attempt to identify new agents for the treatment of resistant strains of *Plasmodia*. The sensitivity of a parasite strain to a drug can be classified into four general groups; sensitive (S), in which clearance of asexual parasitemia occurs within 7 days of the initiation of treatment without subsequent recrudescence; slightly resistant (RI), in which asexual parasitemia is cleared as in sensitive cases but recrudescence follows; moderately resistant (RII), in which the level of asexual parasitemia is reduced markedly but not cleared fully; and highly resistant (RIII), in which little or no reduction in asexual parasitemia occurs.

Drug pressure is considered to be the main causative factor of selecting and propagating resistant *P. falciparum* in a particular locality or area. Current efforts at analysing the molecular mechanisms of resistance are offering new approaches for drug resistance. Mutations in key molecular targets had been identified for antifolate resistant strains. Rapid efflux of chloroquine from resistant parasites can be reversed by co-administration of certain agents. A clearer understanding of the molecular basis of resistance may provide improved therapeutic strategies for use in resistant organisms.

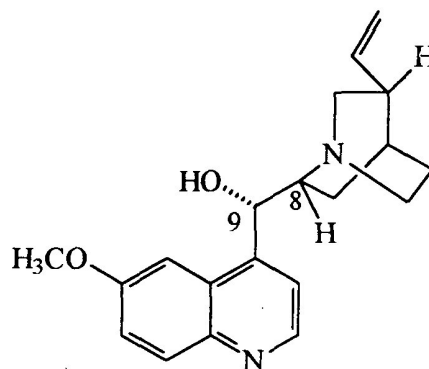
Drugs used in the treatment and prophylaxis of Malaria

Quinine and Quinidine

Quinine may be claimed without exaggeration as the drug to have relieved more human sufferings than any other in history. In the 19th century, the active principles, the cinchona alkaloids, quinine (L), quinidine (LI), cinchonine, and cinchonidine, were isolated and purified. The total synthesis of quinine by Woodward and Doering in 1944-1945 was a landmark in modern synthetic chemistry^{30,31}. The stereochemical differences among the cinchona alkaloids results in differences in potency. Quinidine is two- to three-fold more active than quinine in both chloroquine-sensitive and chloroquine-resistant strains of *P. falciparum*.



(L)
(-) - 8S, 9R



(LI)
(+) - 8R, 9S

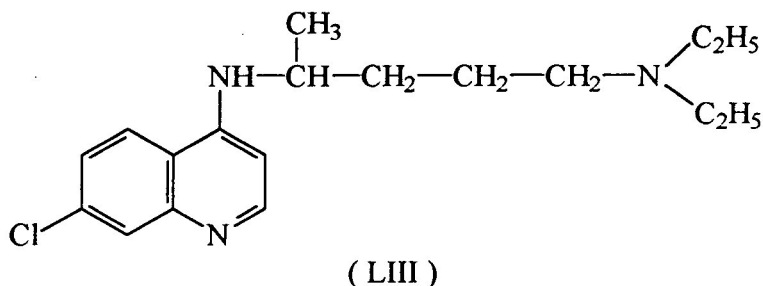
Since the development of potent synthetic agents, quinine had fallen into disuse as a first-line antimalarial. But with the advent of multidrug resistant strains, quinine has returned as an important agent in severe cases involving drug resistant parasites. It is almost always used in combination with tetracycline. Effective treatment usually involves a seven days course of the combination. The mechanism of action of quinine may involve inhibition of heme polymerization. Quinidine inhibits heme polymerase with an IC₅₀ value of 90μM; quinine of 300μM. Quinine binds to both serum albumin and α1-acid glycoprotein (AAG) in human plasma.

Quinine and quinidine have a low therapeutic index and concerns about toxicity intrude. The symptom complex called cinchonism is associated with the use of the cinchona alkaloids. Excess quinine or quinidine can induce the symptoms consisting

of hearing loss, tinnitus, visual disturbances, rashes, vertigo, nausea, vomiting, and central nervous system changes including headache, confusion, and loss of consciousness. Quinine may cause myocardial depression, prolongation of QT interval, and peripheral vasodilatation. Quinidine has more potent intrinsic antimalarial properties than quinine. Quinidine has more pronounced cardiac effects than quinine and has greater tendency to induce hypotension. Hypoglycemia is another important complication associated with the cinchona use.

Chloroquine

Chloroquine (LIII) is one of the most successful antimicrobial agents and was synthesised in 1934. Chloroquine remains the favoured agent for susceptible malaria. Commercially available chloroquine is racemic. The main metabolite is desethylchloroquine, which is equally active as chloroquine in sensitive strains of *P. falciparum*. Chloroquine acts upon the intra-erythrocytic stages of parasite life cycle; at therapeutic concentrations, it has no effect upon the sporogonic, exoerythrocytic and mature sexual stages.

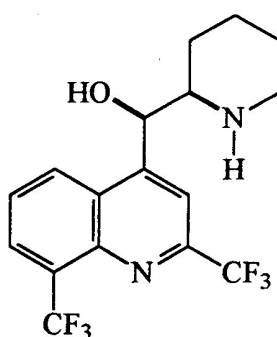


In chemoprophylaxis, lower doses of chloroquine taken daily were shown to provide adequate drug concentrations, as good as higher doses weekly or twice weekly. Compliance may be better with a daily tablet either as a single agent or in a combined formulation with proguanil. Chloroquine can intercalate into DNA but only at concentrations much higher than are effective *in vivo*. It has been shown that chloroquine concentrations as low as 20 μ M inhibit the transition to Z-DNA.

Mefloquine

Mefloquine (LIV) was selected from a group of nearly 300 quinoline methanol agents due to its high activity in animal models. The synthesis of racemic mefloquine was

reported in 1971 along with isomers that differed in the position of the trifluoromethyl groups around the quinoline system. The 2,8-bis- (trifluoromethyl) arrangement proved to be the most active of the series. Although the compounds that possessed an aryl group at the 2-position of the quinoline ring were found to have augmented antimalarial activity, this structural feature also conferred unacceptable levels of phototoxicity.



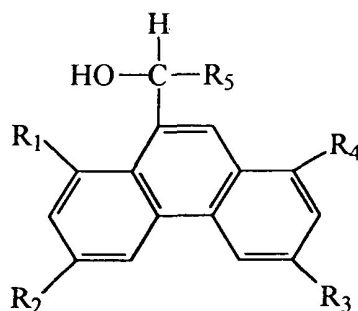
(LIV)

Mefloquine, a 4-quinolinemethanol structurally related to quinine, is an orally active agent effective against the intraerythrocytic stages of the plasmodium life cycle. It has no effect against mature gametocytes. Three hypotheses have been put forward to explain the action of mefloquine. (1) DNA intercalation may be important in the action of the fast acting blood schizonticidal agents. Even so, there is no evidence that mefloquine does intercalate with DNA. (2) Mefloquine and other quinoline antimalarials have been shown to inhibit parasite growth at concentrations that also increase pH in the vacuoles. (3) The parasite food vacuole is the primary target of mefloquine action and the affinity of mefloquine for free heme is high.

Halofantrine

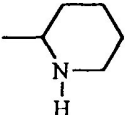
Phenanthrenemethanols were first discovered to possess antimalarial activity during the drug discovery efforts of World War II. In SAR studies, it was determined that the dibutylamino side chain was optimal for the substituted 9-phenanthrene system. Initial human trials confirmed that halofantrine (LV) was a promising new agent for the treatment of malaria. The first two members of the series that were examined in depth were WR33,063 (LVI) and WR122,455 (LVII). Both the compounds displayed

good activity in chloroquine-resistant strains but high cure rates required multiple doses over several days.



(LV) : $R_5 = \text{CH}_2\text{CH}_2\text{N}[(\text{CH}_2)_3\text{CH}_3]_2$; $R_2 = \text{CF}_3$
 $R_3 = R_4 = \text{Cl}$

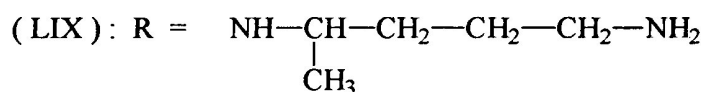
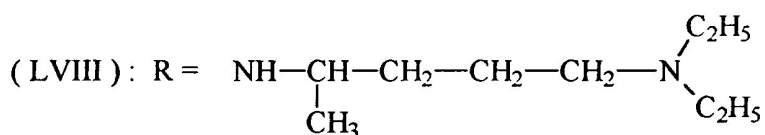
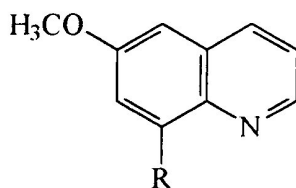
(LVI) : $R_5 = \text{CH}_2\text{N}[(\text{CH}_2)_6\text{CH}_3]_2$; $R_2 = \text{Br}$

(LVII) : $R_5 =$  ; $R_2 = \text{CF}_3$; $R_3 = \text{CF}_3$

Halofantrine is schizonticidal with selective activity against the erythrocytic stages of plasmodia, especially the more mature parasitic stages. The drug accumulates in the parasitised red blood cells with concentration upto 60% higher than in uninfected cells; erythrocytes with matured parasites showed the highest accumulations³². There is no observed action on gametocytes or hypnozoites. The drug is a potent and selective inhibitor of the catalytic subunit of cyclic AMP-dependent protein kinase.

Primaquine

The prototype 8-aminoquinoline was pamaquine (LVIII), introduced in 1925. Due to human toxicity, the use of pamaquine declined, and other 8-aminoquinolines were synthesized. Of this, primaquine has been used extensively as the antirelapse drug of choice. Primaquine (LIX) is essentially identical in curative properties against *P.cyanomologi*³³.



Quinocide an isomer of primaquine, has been used extensively in Eastern Europe and the former Soviet Union and has very similar therapeutic properties and side effects, though its chemotherapeutic index is somewhat lower. Primaquine is rapidly absorbed from the gastrointestinal tract reaching maximal concentration 1-3hrs after administration. The most serious adverse effect associated with primaquine is intravascular hemolysis. The usual 14-day curative regimen of primaquine has been reported to lead to a number of other symptoms including anorexia, nausea, cyanosis, epigastric distress, abdominal pain, cramps, malaise, dark urine, vomiting and vague chest pains. Primaquine clears hypnozoites from the liver and effects a radical cure of relapsing malarias.

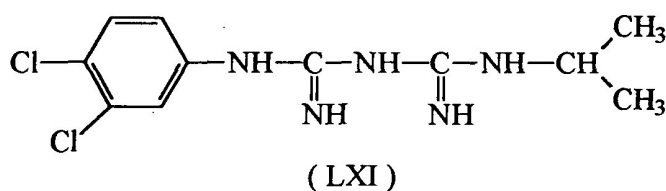
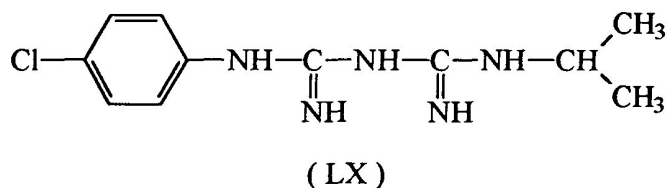
Antifolates

A number of agents have been discovered that effect antimalarial activity through the inhibition of the parasite dihydrofolate pathways. These agents are divided into two types: inhibitors of dihydrofolate reductase (DHFR) and those that inhibit dihydropteroate synthetase (DHPS).

a) Proguanil and Chloroproguanil

Proguanil (LX) was the most active of a series of biguanides screened in 1940s. It proved to be an excellent casual prophylactic in falciparum malaria as well as satisfactory suppressive of vivax malaria, although the drug is not active against hypnozoites. A related compound, chloroproguanil (LXI) has been used occasionally and studies suggest that it may be a good alternative to proguanil in areas of drug

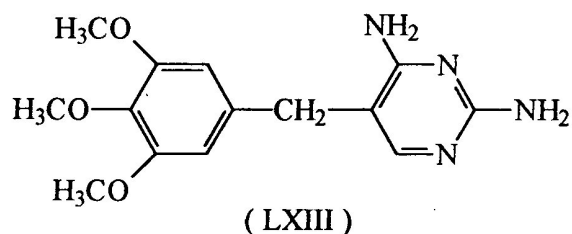
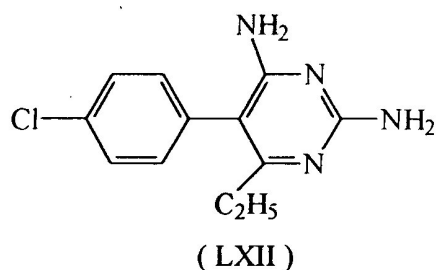
resistance. Proguanil is a tissue schizonticide, active against pre-erythrocytic liver stages. Because of their slow action and because resistance seemed to develop quickly, it was used in combination with chloroquine and was found to be an effective prophylactic regimen in an endemic area with high levels of chloroquine resistant strains.



The antimalarial activity of proguanil resides in a cyclised metabolite, cycloguanil; proguanil is then a prodrug form of cycloguanil. Proguanil acts as a mimic of dihydrofolate. The drug competitively inhibits the parasite enzyme dihydrofolate reductase (DHFR, tetrahydrofolate dehydrogenase). Plasmodia require a tetrahydrofolate cofactor in order to synthesise pyrimidine *de novo* since they have no salvage pathways for pyrimidines.

b) Pyrimethamine

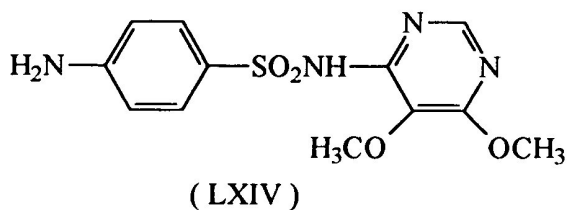
Pyrimethamine (LXII) was developed by Burroughs Wellcome in 1950. Despite its wide margin of safety, pyrimethamine is no longer used as a single agent due to the ease with which resistance develops to the drug when given alone. A related agent, both structurally and in action, is Trimethoprim (LXIII). It is much less used in the treatment of malaria and always in combination. Pyrimethamine acts by inhibition of the parasite DHFR.

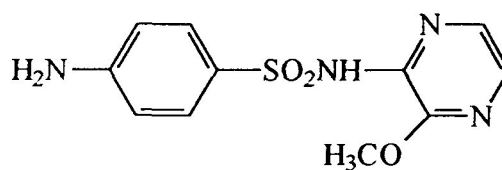


The basis for the selective action of pyrimethamine is differential binding to dihydrofolate reductase in different species of plasmodia and in the mammalian host and the extraordinary sensitivity to the action of nuclear division of the malaria parasite at the time of development of the schizonts in the erythrocytes and in the liver tissue.

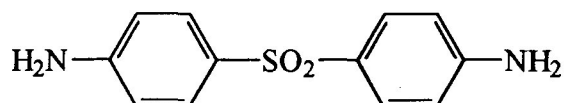
c) Sulphonamides and Sulphones

Upon the discovery that sulphanilamide had appreciable antimalarial activity, a number of related sulphonamides were tested for activity. Two longer acting agents, sulphadoxine (LXIV) and sulphalene (LXV), were identified as the most promising members of the group. These studies led to the assessment of dapsone (LXVI), a sulphone drug, used for leprosy. Sulphamethoxazole and sulphisoxazole are other examples of this class of agents.





(LXV)



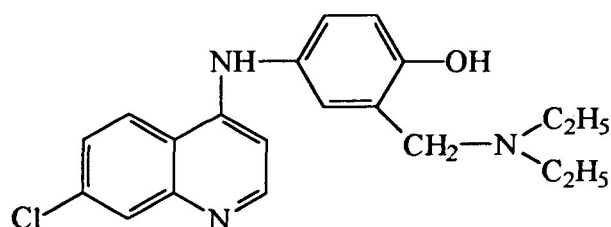
(LXVI)

Sulphonamides and sulphones are selectively toxic to malaria parasites because they inhibit a plasmodial enzyme, dihydropteroate synthetase (DHPS) that is not present in mammals. DHPS catalyses the combination of p-aminobenzoic acid (PABA) with a substituted pterin. The product, dihydropteroate, is ultimately converted into dihydrofolate. The folate co-factors are necessary in the biosynthesis of pyrimidines. Sulpha drugs compete with PABA and are converted into nonmetabolizable adducts; the result is a depletion of the folate co-factor pool.

Other agents

Amodiaquine

Amodiaquine (LXVII) is a 4-aminoquinoline, structurally related to chloroquine. As a fast acting blood schizonticide, it has been shown to be effective against chloroquine resistant strains of *P. falciparum*, despite the similarity in chemical structures.

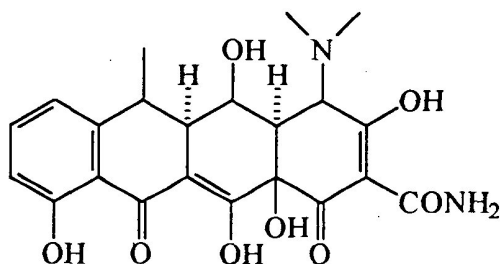


(LXVII)

Amodiaquine was thought to be well tolerated until it began to be used widely for prophylaxis. At that point, evidence of its long-term toxicity emerged. Severe adverse effects associated with amodiaquine are agranulocytosis and liver toxicity.

Antibiotics

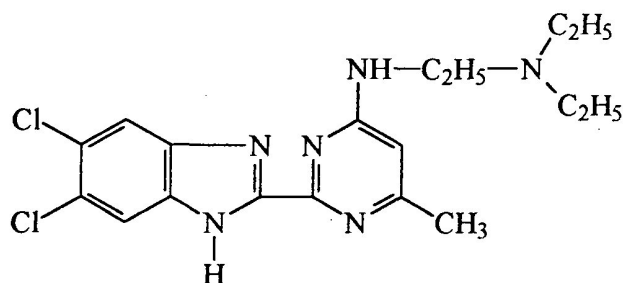
(LXVIII)



(LXIX)

Benzimidazoles

A number of 2-amino benzimidazole derivatives were prepared and tested for their antimalarial activity against *P. berghei* and *P. gallanicum*. L. N. Werbel³⁴ synthesized almost 138 novel benzimidazoles, many of which showed curative antimalarial activity in animal models.



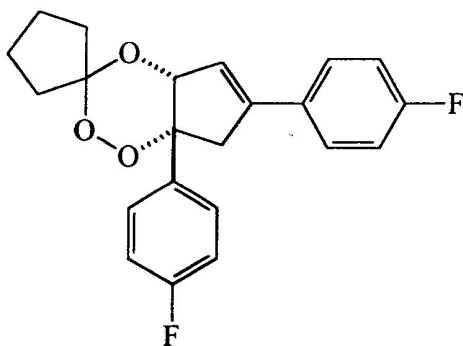
(LXX)

Most potent compound which was undertaken for clinical trial was 5,6-dichloro-2-[4-(2-diethylamino)-ethyl amino,6-methyl,pyrimidinyl] Benzimidazole (LXX). It showed marked activity against cycloguanil resistant line of *P.berghei*.

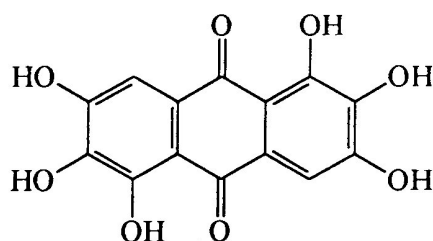
EXPERIMENTAL AGENTS

Synthetic Experimental Antimalarial agents

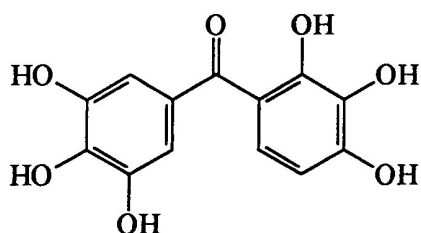
A number of new structural lead compounds have been discovered or prepared in the quest for new antimalarial agents. Although many trioxane compounds have been prepared and studied, Fenozan-50F (LXXI) is the first trioxane, which was identified as the most active agent. The compound is active against blood stages of *P. berghei*, *P. yoelii*, and even against a wide spectrum of drug-resistant parasite strains. Rufigallol (LXXII), easily prepared by dehydration of gallic acid, displayed an IC₅₀ of 35nM against chloroquine resistant *P. falciparum in vitro* and was the most active compound in the group. When combined with exifone (LXXIII), a strong potentiation of antimalarial effect was noted. Also, one of the earliest known antimalarial agents after quinine was the synthetic thiadiazine dye, methylene blue (LXXIV).



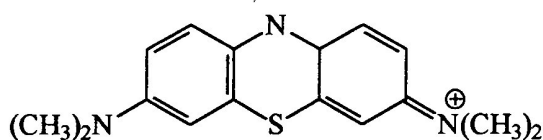
(LXXI)



(LXXII)



(LXXIII)

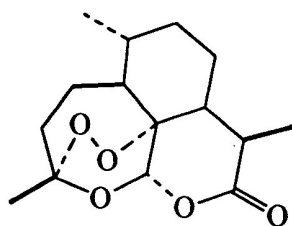


(LXXIV)

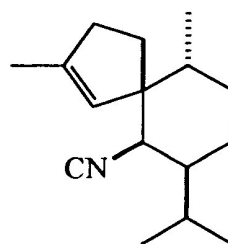
Antimalarial Natural Products

Artemisinin

Artemisinin (Quing hao su) (LXXV) is the active antimalarial component of the chinese herb Quing hao (*Artemisia annua*). Although the herb has been used for malaria therapy in China for over 1000years, the active component of the herb was not isolated and characterized until 1972, when Chinese scientist showed it to be a novel antimalarial compound with structure of sesquiterpene lactone. Subsequently, several of its derivatives have been studied which represents a new series of antimalarial compounds with a high level of blood schizonticidal activity against chloroquine-resistant malaria parasites.

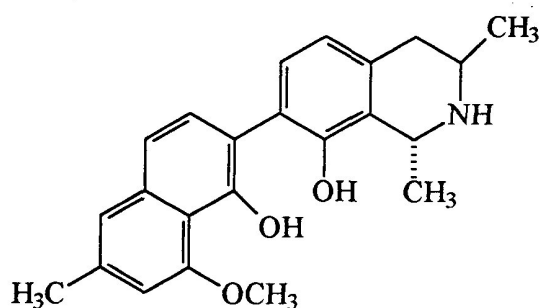


(LXXV)



(LXXVI)

Many natural products have been shown to have antimalarial activity in a variety of screenings. The initial reports of isonitrile compounds, isolated from marine organisms, that had significant antimalarial activity was that of axisonitrile-3 (LXXVI) from the sponge *Acanthella klethra*. Naphthylisoquinoline alkaloids have been recovered from tropical plant species. One of the compounds, dioncophylline B (LXXVII), possesses activity against a chloroquine-resistant strain of *P. falciparum* with an IC_{50} of 63ng/mL.



(LXXVII)



PLAN OF WORK

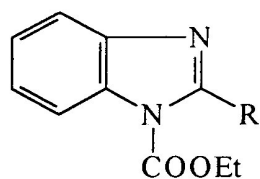
The earlier pages have seen a discussion on three of the major illnesses viz. Bacterial, Fungal and Malarial infections, that have afflicted man from ancient times to the present day. The progress of the chemotherapy of these infections during this past decades is also characterised by an impressive, though not unexpected increase in the number of observations on fastness. Hence, the development of resistance to the drugs by microorganisms and parasites is an important problem facing the chemotherapist. Drug resistance can be defined as the temporary or permanent loss of the initial sensitivity of the microorganism or parasite to the effect of the active substance. The rapidity of development of resistance varies widely under different circumstances. Besides, the potent drug used in the clinical therapy over years is being reported to show newer side effects. Hence, continuous efforts at discovery of newer and better molecules as chemotherapeutic agents are mandatory and the present work is a further step in this direction. Novel heterocyclic molecules have been synthesised as detailed in the following parts and evaluated for their chemotherapeutic properties and 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 adaptation in light of the recent trends in drug research in incorporating newly emerged pharmacophores on existing moieties.

PART – II

Synthesis of methyl 5-(substituted thio)-2-benzimidazolecarbamate.

Among the various types of known heterocyclic ring system different thio substituted benzimidazole derivatives have demonstrated significant biological activities^{35,36}. So in this part it was proposed to undertake the synthesis and biological study of benzimidazole derivatives.

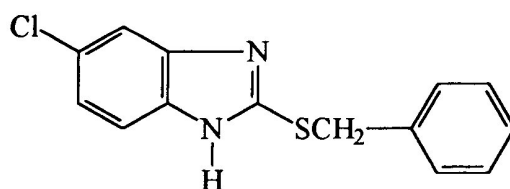
Also, literature survey had revealed that Benzimidazole carbamates³⁷ (I) had found possess antifungal, anthelmintic and antibacterial properties.



R = 2-Thiazolyl methyl

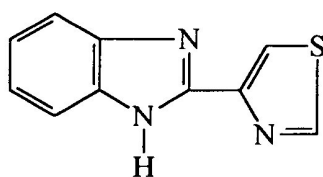
(I)

During the literature survey, it was found that 2-mercaptobenzimidazole derivatives³⁸, namely 5 (6)-chloro-2- (benzylthio) benzimidazole (II) possess antifungal activity against *Trichophyton interdigitale*, at a dilution 1:290,900 or 3.4 µg/ml. Similarly, bacteriostatic activity was also reported for 2-mercaptobenzimidazole against *S. aureus*³⁹.



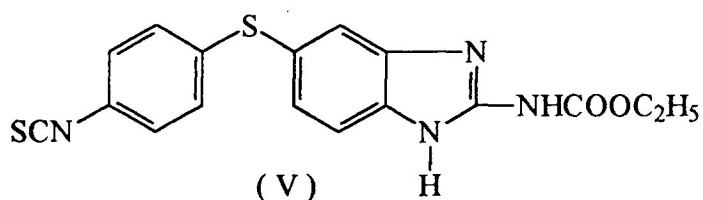
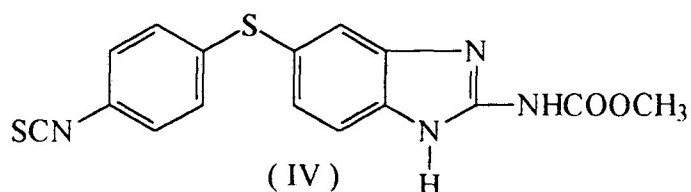
(II)

Ever since the discovery of 2-(4'-thiazolyl) benzimidazole⁴⁰ (III) (thiabendazole) in 1961 as the first broad-spectrum anthelmintic, several thousand benzimidazoles have been prepared but less than twenty have been commercially exploited.

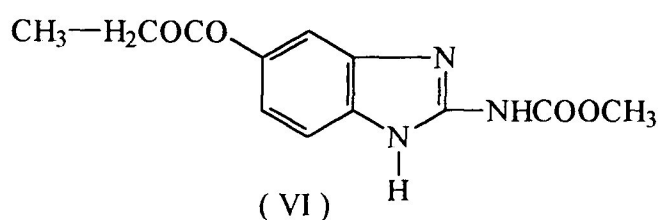


(III)

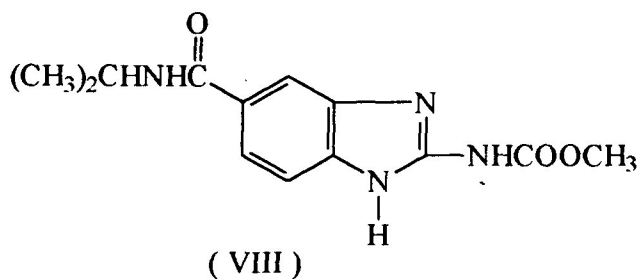
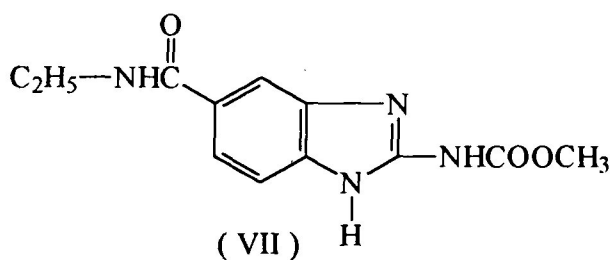
Syed Abuzar and Satyavan Sharma⁴¹ had synthesised and studied anthelmintic activity of 2-substituted 5(6) Aryl thio-benzimidazoles. Compounds (IV) and (V) given to the rats infected with *H. nana* at the oral doses of 250mg/kg x 3 and 250mg/kg x 1 caused 100% elimination of the tapeworms along with their scolices.



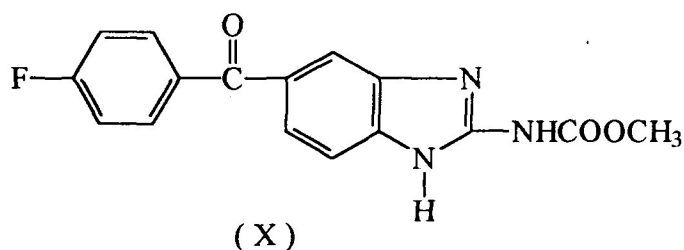
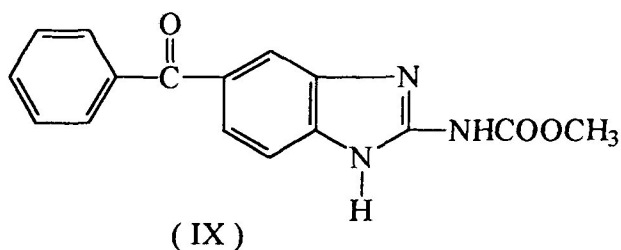
Recently, Ram et al synthesised 5-carboxy 1H benzimidazole-2-carbamates (VI) which were found to be curative against adult *B.pahangi* microfilariae in jirds at the dose of 12.5mg/kg when given for 5 days and 82% effective at a dosage of 6.25 mg/kg when given for 5 days and also *A. vitae* in jirds at 25mg/kg when given for 5 days⁴².



Also (VII) and (VIII) were found to show activity against *B. pahangi* in jirds at 25mg/kg x 5 days and 6.25mg/kg x 5 days⁴².

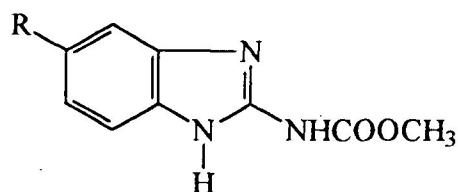


Alkyl benzimidazole-2-carbamate like Mebendazole⁴³ (IX) and Flubendazole⁴⁴ (X) are broad spectrum anthelmintics containing methoxy carbonyl amino group at the 2-position and the benzoyl group at the 5-position. Both are major pharmacophoric requirements for anthelmintic activity.

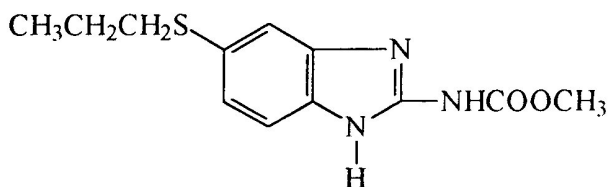


Taking into account importance of this carbamate linkage (-NHCOOCH₃) present in the above compounds, this linkage was introduced in our compounds.

Smith, Kline and French laboratories have prepared a series of 5(6)-alkyl, aryl and alkoxybenzimidazole-2-carbamates with marked anthelmintic activity like Oxibendazole⁴⁵ (XI) and Parbendazole⁴⁶ (XII).



- (XI) R = OPr
 (XII) R = n- Bu



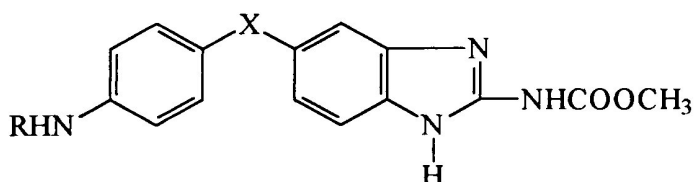
(XIII)

Albendazole (XIII) is a benzimidazole anthelmintic possessing broad spectrum activity against gastrointestinal, roundworms, tapeworms, lungworms and liver flukes⁴⁷. It has been demonstrated to possess high activity against *A. caninum* in dogs at a dose of 50mg/kg given for three days. The sheep carrying moniezia infection are cured to 100% by albendazole at a dose of 10mg/kg.

Recently, Albendazole has been reported to possess antimalarial activity⁴⁸. It inhibits polymerisation to microtubules and it is active against larval stages of cestodes of the genus *Echinococcus*.

Also, the antimalarial activity of three imidazoles viz; Ketoconazole, Diacylketoconazole and Miconazole has been disclosed against chloroquine resistant *Plasmodium falciparum in vitro*⁴⁹.

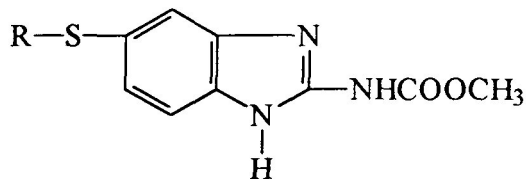
Subhash Chandra and Satyavan Sharma⁵⁰ had carried out antimalarial activity of Alkyl 5(6)(4-aminophenyl) sulphono benzimidazole-2-carbamate (XIV).



(XIV) X = S, R = COCH₃, R = CH₃
X = SO₂, R = COCH₃, R = CH₃, etc.,

The overall promise of this literature sparked a rebirth of our interest in the 2,5(6)-disubstituted benzimidazoles as an ideal parasitic agent and led to the preparation of an array of new congeners.

Taking lead from these known facts, it was proposed to synthesize and evaluate the antibacterial, antifungal and antimalarial efficacy of methyl 5-(substituted thio)-2-benzimidazole carbamate (XV).



(XV)

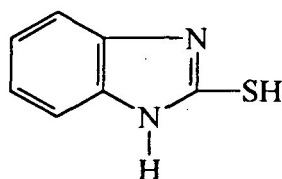
R = 5- methyl thiadiazole
 = benzimidazole
 = 5-methoxy benzimidazole, etc.

PART - III

Synthesis of 5-methoxy-2-(thiocarbamoyl substituted) benzimidazole.

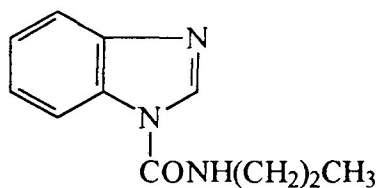
The pharmacological history of several thousands of imidazole derivatives has been established over 30 years, after the discovery of the antibacterial and antifungal activity of benzimidazole by Wolley⁵¹ (1944) which later led to the synthesis of benzimidazole derivatives with strong antiprotozoal and anthelmintic properties. The discovery of a new antibacterial (Azomycin), a trichomonicide (Metronidazole) and anthelmintic agents (Thiabendazole and Carbendazole) which contain prominent imidazole nucleus, has added impetus to investigations in these areas with particular emphasis on the synthesis of newer benzimidazole derivatives for pharmacological screening.

A number of 2-thiobenzimidazole derivatives (XVI) have demonstrated a broad spectrum of significant biological activity i.e., plant growth inhibition^{52,53}, bactericidal, fungicidal and virucidal activities³⁵.



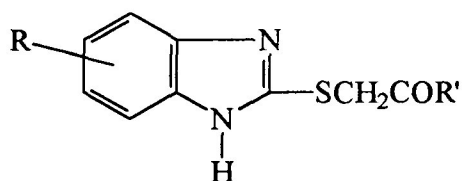
(XVI)

Benomyl⁵⁴ (XVII) is another antifungal compound having benzimidazole moiety with a carbamido linkage.



(XVII)

The following type of Benzimidazoles (XVIII) are known for their diverse biological properties⁵⁵.

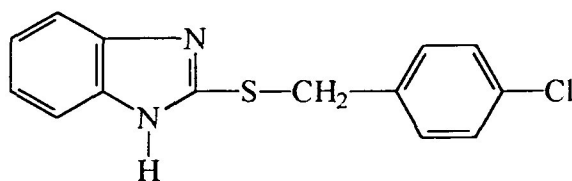


(XVIII)

R = H, CH₃, Cl, NO₂

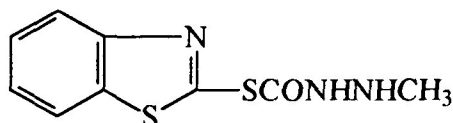
R' = Me, Ph

A development occurred in the field of benzimidazole with the emergence of broad-spectrum antimicrobial compound⁵⁶ (XIX).



(XIX)

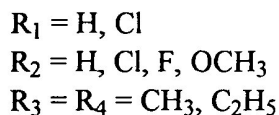
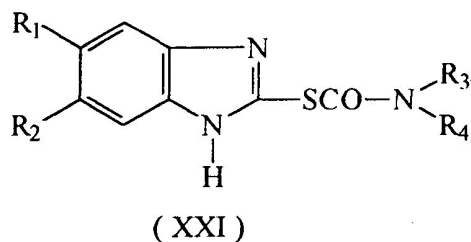
Also, Assad and Falmy⁵⁷ synthesized benzothiazoles (XX) having thiocarbamoyl linkage at 2-position and were found to show inhibition zone of 9-18mm on various bacteria at a concentration of 0.01g/100ml.



(XX)

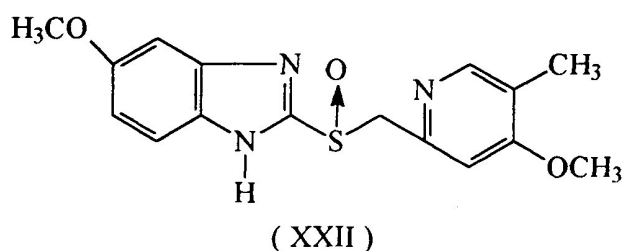
Very recently, Sanjay Palav and B. G. Khadse⁵⁸ (XXI) have reported a series of 5,6-disubstituted-2-(N, N-dialkylthiocarbamido) benzothiazoles as potential

chemotherapeutic agents having antibacterial, antifungal and larvicidal activity.

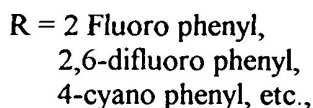
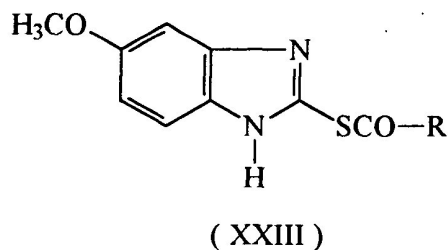


All the above examples indicate the importance of the benzimidazole nucleus and thiocarbamoyl linkage at the two position of the benzimidazole nucleus.

Also, 5-methoxy-2-mercaptobenzimidazole has been found as an intermediate for Omeprazole⁵⁹ (XXII) which is a potent antiulcer agent and is used in the treatment of Zollinger Ellison syndrome.



Taking into account the above facts and biological importance, a series of compounds (XXIII) containing the combination of 5-methoxy-2-mercaptobenzimidazole with substituted acid chlorides have been effected so as to study their antibacterial and antifungal properties.



PART - IV

Synthesis of Triclosan

This invention relates to the synthesis of 2,4,4'-trichloro-2'-hydroxydiphenylether, a bacteriostatic useful in the formulation of cosmetics and soaps. Triclosan has broad-spectrum antibacterial activity against vegetative organisms. It is used in the disinfection of the skin, in surgical scrubs and in deodorants, in concentrations of 0.1 to 2%⁶⁰.

Triclosan⁶¹ is a chlorinated bisphenol antiseptic, effective against gram-positive and gram-negative bacteria and fungi with variable or poor activity against *Pseudomonas sp.* It is also used in soaps, creams and oral hygiene products. However, there have been isolated reports of contact dermatitis. Methicillin resistant *S.aureus*(MRSA) infection on a surgical unit was successively controlled by handwashing and bathing using triclosan. A further report of successful control of endemic MRSA in a surgical unit has been made.

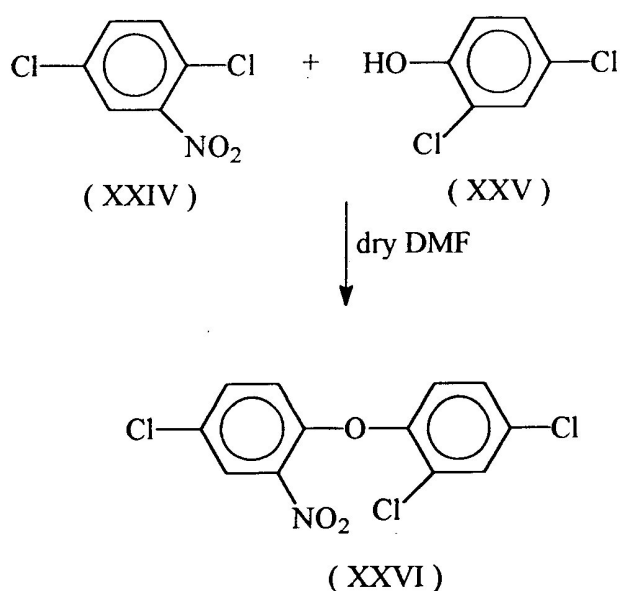
Thus, Triclosan has been proved to be an important bacteriostatic agent found in popular deodorants and antiperspirants⁶².

The bacteriostatic 2,4,4'-trichloro-2'-hydroxydiphenylether covered by U.S. Pat. No. 3,506,720 is annually marketed in million-pound quantities under the trademark IRGASAN DP-300 for use in soaps and cosmetics.

This being an important drug, the synthesis of this compound was indigenously carried out in our laboratory. Triclosan has been prepared in three steps.

Step 1 Synthesis of 2,4,4'-trichloro-2'-nitrodiphenylether (TNDE)

This TNDE⁶³ (XXVI) compound was obtained by condensation of equimolar quantities of 2,5-dichloronitrobenzene (XXIV) and 2,4-dichlorophenol (XXV) in dry dimethylformamide as the solvent.

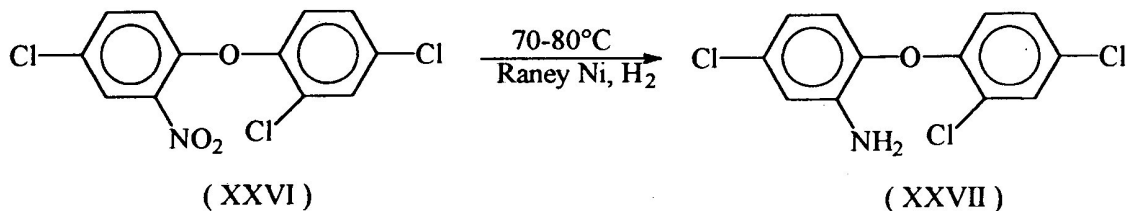


Step 2 Synthesis of 2,4,4'-trichloro-2'-aminodiphenylether (TADE)

This TADE (XXVII) was obtained by the reduction of TNDE that has been carried out by different routes.

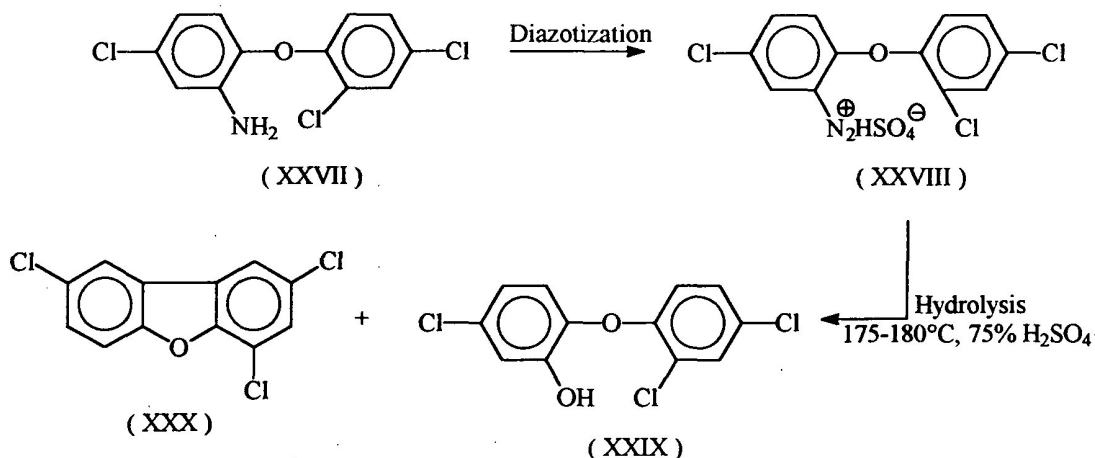
- Reduction by sodium sulphide flakes gave very less yield (40%) and it was difficult to remove inorganic impurities.
- Also, reduction by activated iron powder and 10% hydrochloric acid gave fewer yields (40%) and removal of inorganic impurities was difficult.
- Reduction by activated Raney nickel in presence of hydrogen gas in pressure reactor with methanol as solvent gave a good yield (85-90%) and was more feasible.

So, it was preferred to carry out the reduction of TNDE by activated Raney nickel and hydrogen gas.



Step 3 Synthesis and purification of 2,4,4'-trichloro-2'-hydroxydiphenylether

Triclosan (XXIX) was produced from 2,4,4'-trichloro-2'-aminodiphenylether by diazotization and hydrolysis according to the equation below.



The present work comprises the steps of diazotizing the sulphate salt of TADE, instead of hydrochloride salt and hydrolyzing the diazo compound (XXVIII) by 75% sulphuric acid at $175-180^\circ\text{C}$, distilling the oily layer by chlorinated solvent. Then solvent was removed under reduced pressure and it was treated with 10% sodium hydroxide solution. The resulting solution was extracted with organic solvent to remove impurities. The aqueous layer was acidified by 10% hydrochloric acid and extracted with chlorinated solvent and it was removed under reduced pressure to get resultant Triclosan (XXIX). Products of this synthesis, in addition to the desired phenol triclosan obtained in 32% yield includes 2,4,8-trichloro benzofuran (XXX) as the major impurity and traces of more than ten other compounds including phenolics and non-phenolics related to or derived from the furan compound.

PART IB

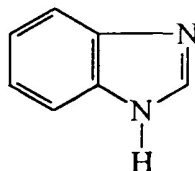
CHEMISTRY

THIS PART DEALS WITH THE CHEMISTRY OF ALL THE COMPOUNDS / INTERMEDIATES INVOLVED IN THE WORK.

- ♦ BENZIMIDAZOLE
- ♦ METHYL 5 – (SUBSTITUTED THIO) – 2 – BENZIMIDAZOLE CARBAMATE
- ♦ 2- METHYL THIOPSEUDO UREA - 1,3 - DICARBOXYLIC ACID DIMETHYL ESTER
- ♦ 2 – MERCAPTOBENZIMIDAZOLE
- ♦ ACID CHLORIDES
- ♦ 5 – METHOXY – 2 – (THIOCARBAMOYL SUBSTITUTED) BENZIMIDAZOLE
- ♦ DIAZONIUM SALTS
- ♦ REDUCTION

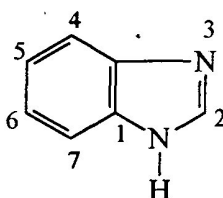
CHEMISTRY OF BENZIMIDAZOLE

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



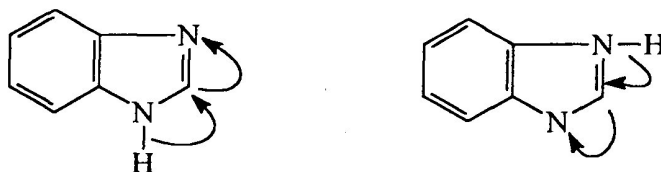
(I)

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 *o*-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 tautomerise⁶⁴.



Synthesis of benzimidazole

The last systematic reviews on benzimidazoles were published in 1951⁶⁵ and 1953⁶⁶ although subsequent surveys have appeared in articles⁶⁷⁻⁶⁹ covering the chemistry of both imidazoles and benzimidazoles. In the latest reviews⁷⁰, 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 acids^{71,72}
- b) by reacting O – phenylene diamines with acid chlorides^{73, 74}, ester⁷⁵ or acid anhydride⁷⁶.
- c) by reacting O – phenylene diamines with amide⁷⁷ or nitrile⁷⁸.
- d) by reacting O – phenylene diamines with aldehyde⁷⁹ or ketone⁸⁰.
- e) by reacting O – phenylene diamines with urea⁸¹ or thiourea⁸².
- f) by reacting O – phenylene diamines with isothiocyanate⁸³.

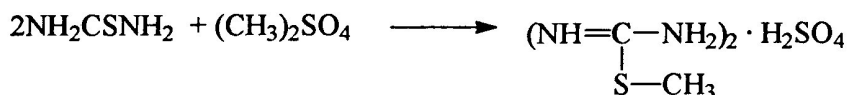
Properties of benzimidazoles

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) :

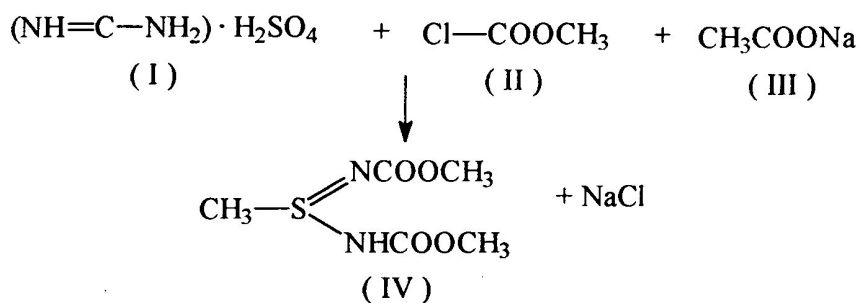
Two moles of thiourea were treated with 2 moles of dimethyl sulphate and refluxed for one hour. The compound was recrystallized from 95% ethanol. The compound was isolated in 79% yield.



2 – Methyl thiopseudo urea – 1, 3 – dicarboxylic acid dimethyl ester : (Carbamic acid [(methylthio) methylidyne] – 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. Then it was treated with sodium acetate (III) (0.028 mole). Reaction mixture was kept overnight.

Next day, acetic acid and methyl chloroformate was distilled out under reduced pressure. The compound (IV) was precipitated on cooling, the yield is 70%.



CHEMISTRY OF METHYL 5 – (SUBSTITUTED THIO) –2- BENZIMIDAZOLE CARBAMATE

In the present investigation, methyl 5 - (substituted thio) benzimidazole carbamates were prepared by the following manner.

The 4 – (substituted thio) – 1, 2- phenylene diamine is the key intermediate for the synthesis of 5 - (substituted thio) benzimidazole carbamate. The most convenient route adapted is that from *m* – chloro aniline (I). It was initially acetylated and the acetyl derivative (II) was then nitrated with concentrated HNO_3 and concentrated H_2SO_4 at 0 – 5°C, to give 5 – chloro – 2 – nitro acetanilide (III). It was then hydrolysed with 10 % KOH to give 5 – chloro – 2 – nitro aniline (IV). The purified 5 – chloro – 2 – nitro aniline was further reacted in the presence of anhydrous K_2CO_3 with substituted thio compounds by using DMF as solvent to obtain the corresponding 5 – (substituted thio) – 2 – nitro aniline (V) derivatives.

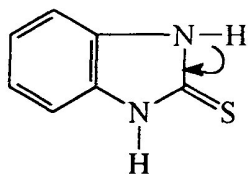
The above 5 – (substituted thio) – 2 – nitro aniline (V) derivatives were further converted to the corresponding 4 – (substituted thio) – 1, 2- phenylene diamine (VI) by reduction with activated Raney nickel in presence of hydrogen gas or with anhydrous sodium sulphide.

The 4 – (substituted thio) – 1, 2- phenylene diamines obtained then were condensed with one mole of the 2 – methyl thiopseudo urea – 1, 3 – dicarboxylic acid dimethyl ester (X). In the first step of the reaction, due to lone pair of electrons of the NH_2 group of heterocyclic attached to the carbon atom of $\underline{\text{S}}$ – methyl guanidine an intermediate species is formed. This species immediately loses CH_3SH and retain the aromatic character of the molecule which gives higher stability. After condensation of (VI) and (X) compounds we get the desired methyl 5 – (substituted thio) – 2 – benzimidazole carbamate. (XI)

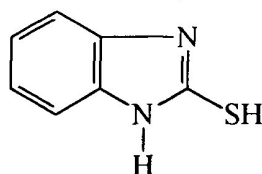
The schematic diagram leading to the formation of the title compounds summarizing the various reactions discussed so far is given in the scheme I.

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.



(I)

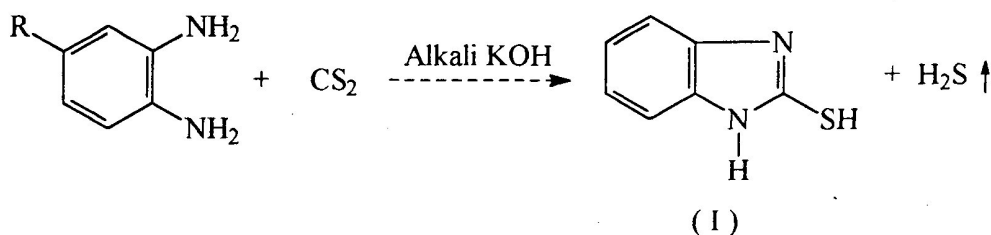


(II)

Different methods of 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 – mercapto benzimidazole itself has been prepared by this method^{84,85}.

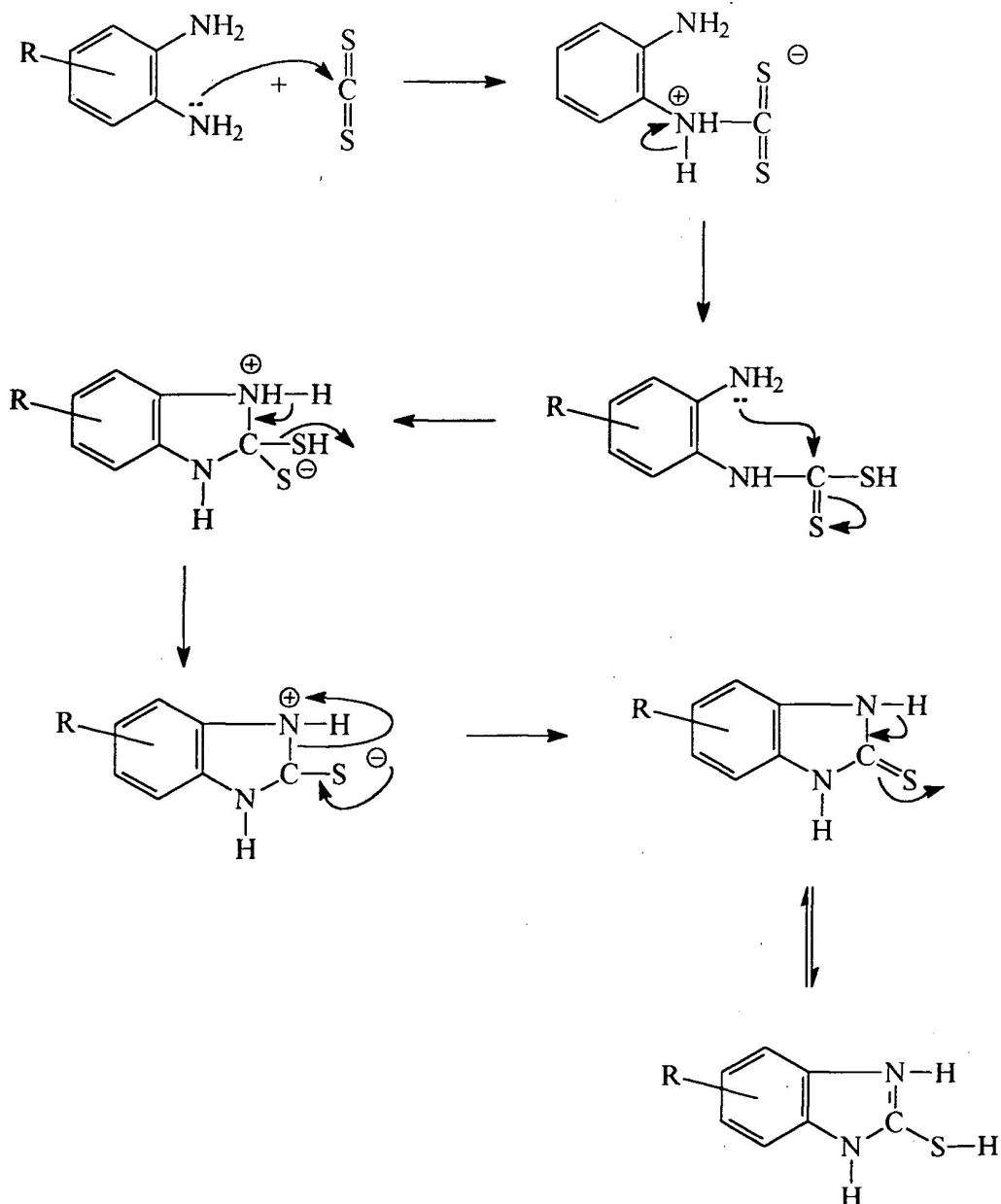


O – phenylene diamines containing substituents on the phenyl ring have been used, including those containing alkyl⁸⁶, halogen⁸⁷, phenyl⁸⁸, aniline⁸⁹ and sulphonamido⁹⁰ groups.

LIBRARY
HAFFKINE INSTITUTE
MUMBAI.

423

Probable reaction mechanism



b) Synthesis from *O* – phenylene diamine and thiourea

This reaction is analogous to the preparation of 2 – hydroxy benzimidazole from *O* – phenylene diamines and urea. Substituted – 2 – mercapto benzimidazoles (V) may be prepared by this method by heating substituted *O* – phenylene diamine dihydrochloride (III) and thiourea (IV) to 170 – 180°C⁹¹. Excellent yields have been obtained by carrying out the reaction in amyl alcohol and heating under reflux until the evolution of ammonia becomes negligible.

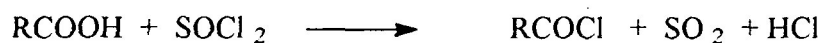
CHEMISTRY OF ACID CHLORIDES

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

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



The use of thionyl chloride⁹⁸ 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.

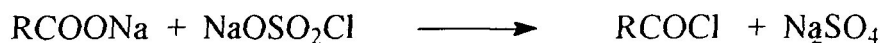


The thionyl chloride must be pure, as the impure product has been known to cause somewhat explosive decomposition in some instances. The procedure consists of mixing the chlorinating agent with acid and heating the mixture, if necessary. The product may be isolated by fractional distillation or if solid,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

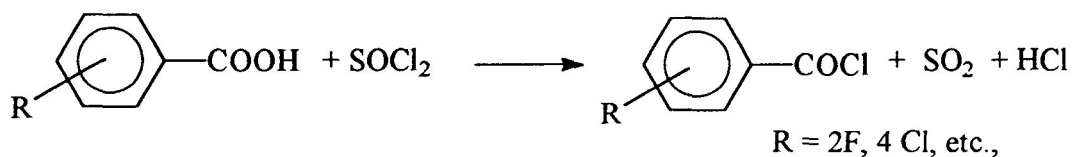


The reaction of phosgene⁹⁹ with carboxyl acids, and *p* – toluene sulphonyl chloride or of sodium chlorosulphonate with salts of carboxylic acids are also used for the preparation of acid chlorides.



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

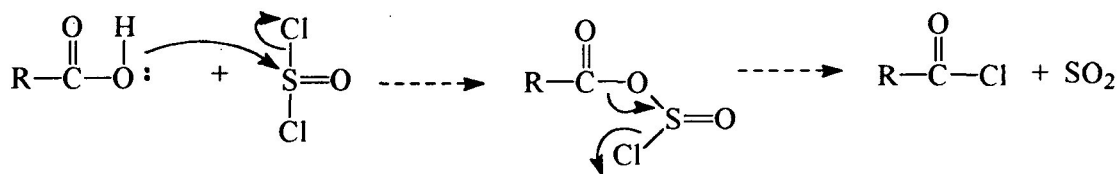
Thus, in the present work all the acid chlorides were prepared by reacting thionyl chloride with corresponding acids.



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.



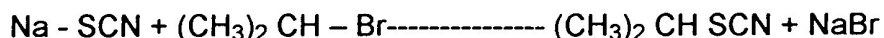
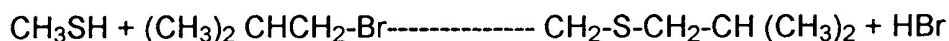
CHEMISTRY OF 5 – METHOXY – 2 – (THIOCARBAMOYL SUBSTITUTED) BENZIMIDAZOLE

In the present investigation, 5 – methoxy – 2 – (thiocarbamoyl substituted) benzimidazole were prepared by the following manner.

4 – methoxy – 1, 2 – phenylene diamine is the major intermediate required for the synthesis of 5 – methoxy - 2 - mercapto benzimidazole. The most convenient route adapted is that from 4 – methoxy acetanilide (I). It was nitrated with concentrated HNO_3 and concentrated H_2SO_4 at $0 - 5^\circ\text{C}$, to give 2 – nitro – *p* - acetanisidide (II). It was then hydrolysed with 10 % HCl . The above nitro derivative was further reduced by activated Raney nickel in presence of hydrogen gas to give the desired intermediate 4 – methoxy – 1, 2 – phenylene diamine (III). This (III) compound on condensation with Potassium methyl xanthate under reflux conditions gives 5 – methoxy - 2 - mercapto benzimidazole (IV).

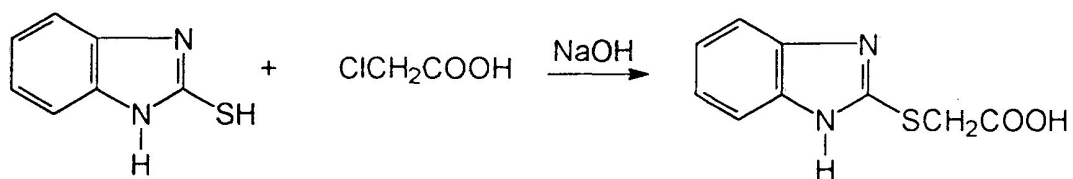
Reaction of 2 – mercapto benzimidazole

In general, sulphur compounds show greater nucleophilic reactivity than their oxygen analogues and substitutions by sulphur containing nucleophiles often occur under very mild conditions, giving high yields. The common leaving groups include halide, sulphonate ions, as usual and water (from conjugate acids of alcohols).

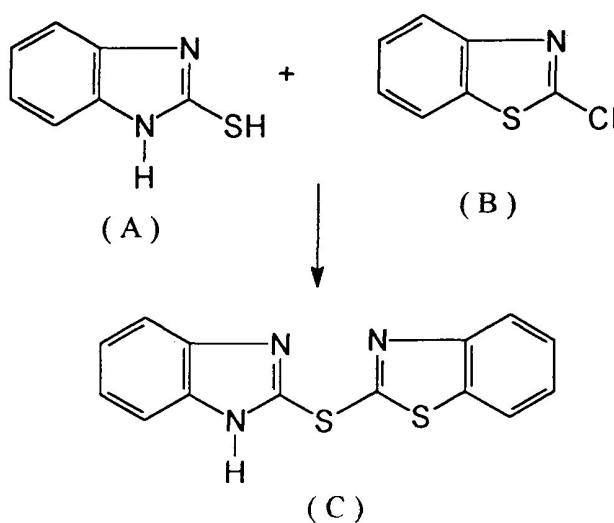


2 – mercapto benzimidazoles are generally stable substances and soluble in dilute alkali.

Alkylation occurs readily with replacement of the 2 – mercapto hydrogen to yield s – alkylated derivatives¹⁰⁰. 2 – mercapto benzimidazole may be treated with chloroacetic acid by refluxing in 2N sodium hydroxide solution¹⁰¹.



Also, 2 – mercapto benzimidazole (A) reacted with 2 – chloro benzothiazole (B) yields 2 – (benzothiazole – 2' – mercapto) – benzimidazole hydrochloride (C) in 86 % yield ¹⁰².



Similarly, 5 – methoxy – 2 – mercapto benzimidazole (IV) obtained as mentioned above was condensed with substituted acid chlorides (VII) in presence of triethylamine using 1, 4 – dioxane as the solvent to give the desired compounds (VIII).

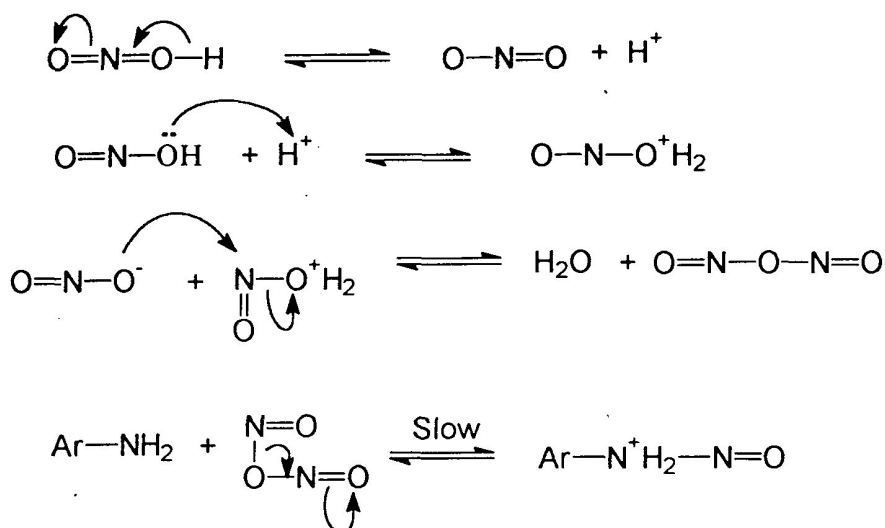
The schematic diagram leading to the formation of the title compounds summarizing the various reactions discussed so far is given in the scheme II.

CHEMISTRY OF DIAZONIUM SALTS

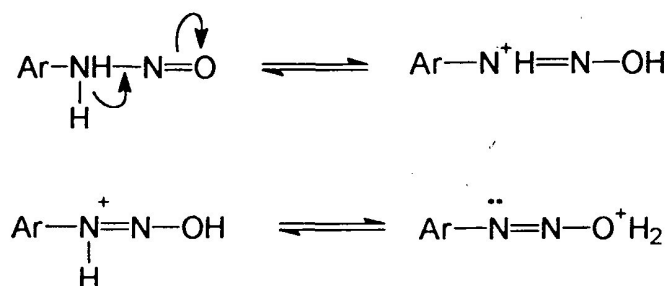
Formation of aromatic diazonium salts from the corresponding amines by diazotization :

The reaction of amino salt with nitrous acid is known as diazotization. Diazonium salts were prepared first by Petercriess in 1858 by the action of nitrous acid on the salt of an aromatic amine.

A detail study of kinetics of diazotization has shown that it involves nucleophilic attack by amine on dinitrotrioxide, which is in equilibrium with nitrous acid.



Normally, the free amine is the reactive species even though its concentration may be very small in the acidic solutions used. The conjugate acid of the primary nitroso amine by a series of proton transfer and loss of water, gives this diazonium ion.

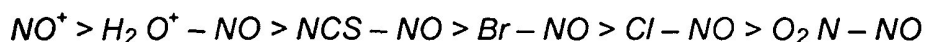




Recently, the intermediate step of a primary nitroso amine could be isolated in the course¹⁰³ of the diazotization of an appropriate amine.

Since most diazotizations are performed in aqueous medium, protonated nitrous acid (from sodium nitrite and acid)¹⁰⁴ is usually used free NO⁺ should only appear in concentrated acid and in the course of conversions with complex NO⁺ salts.

Reactivity decreases under normal conditions in the following sequence :-



1) In aqueous medium

Normal diazotization of aromatic amines with nitrous acid is carried out in dilute aqueous mineral acids and usually yields the diazonium salts in solution. The direct method starts from solutions or suspensions of the amines in dilute hydrochloric acid (approximately 2.5 mol acid per mol amine) which are treated with the molar amount of concentrated aqueous sodium nitrite solution at 0-10°C. The conversion proceeds rapidly and almost quantitatively¹⁰⁵, the end point can be determined by numerous methods, and an excess of nitrous acid can easily be removed by adding urea which reacts with nitrous acid to form nitrogen, carbon dioxide and water or sulphamic acid¹⁰⁶.

Since nitrous acid is an unstable compound, it is generated in the presence of the amine by reaction between sodium nitrite and a mineral acid, usually hydrochloric acid or sulphuric acid. Excess acid serves to keep the mixture strongly acidic, which is necessary to prevent undesirable side reactions.

2) In concentrated acids¹⁰⁷ :

Weakly basic amines bearing strongly electron attracting substituents on the

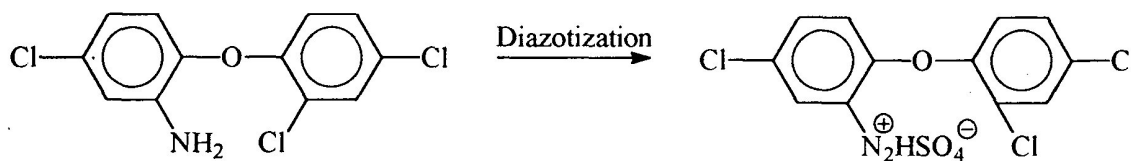
aromatic (or hetero aromatic) nucleus are diazotized preferentially in concentrated acids, since hydrolysis¹⁰⁸ of the generated diazonium compounds occurs in aqueous solutions with rising dilution. Good results have been described with concentrated sulphuric acid¹⁰⁹

3) In organic solvents¹¹⁰:

Usually, in order to prepare solid diazonium salts, diazotization of aromatic amines is carried out in organic solvents (glacial acetic acid¹¹¹, methanol, ethanol, formamide, DMF, acetone) by means of nitrous acid esters (preferentially pentyl nitrile).

DIAZOTIZATION OF 2, 4, 4' – TRICHLORO – 2' – AMINO DIPHENYL ETHER

To the 2, 4, 4' – trichloro – 2' – amino diphenyl ether (0.034 mole) dissolved in a mixture of concentrated H₂SO₄ (15ml) and water (20ml) was cooled to 0°C in an ice bath, a cold aqueous solution of sodium nitrite (0.038 mole) was added dropwise within 15–20 min. After completion of the diazotization, the diazonium salt solution gives a positive starch iodide paper test.



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 able to be 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^{112,113}.

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 configuration¹¹⁴. 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^{115,116} 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 hydrogenation¹¹⁷.

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^{118,119}, palladium on carbon^{120 121 122} 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 about 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 higher 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.

Reduction of 2, 4, 4' – trichloro - 2-nitro diphenyl ether (TNDE)

In a pressure vessel was taken TNDE (0.02 mole) in methanol (50ml). The reaction mixture was heated on waterbath before placing in a pressure vessel to dissolve the compound. The reaction mixture was hydrogenated in a pressure reactor. After complete reduction the contents of the vessel were filtered through fluted filter paper. The Raney nickel was decomposed with dil.HCl (10%). The filtrate was concentrated under reduced pressure to get the product 2, 4, 4' –trichloro –2- nitro diphenyl ether.

Reduction of 5 – (2 – imidazolidine thione) – 2 – nitro aniline

In a similar manner given above, reduction of 5 – (2 – imidazolidine thione) – 2 – nitro aniline was carried out.

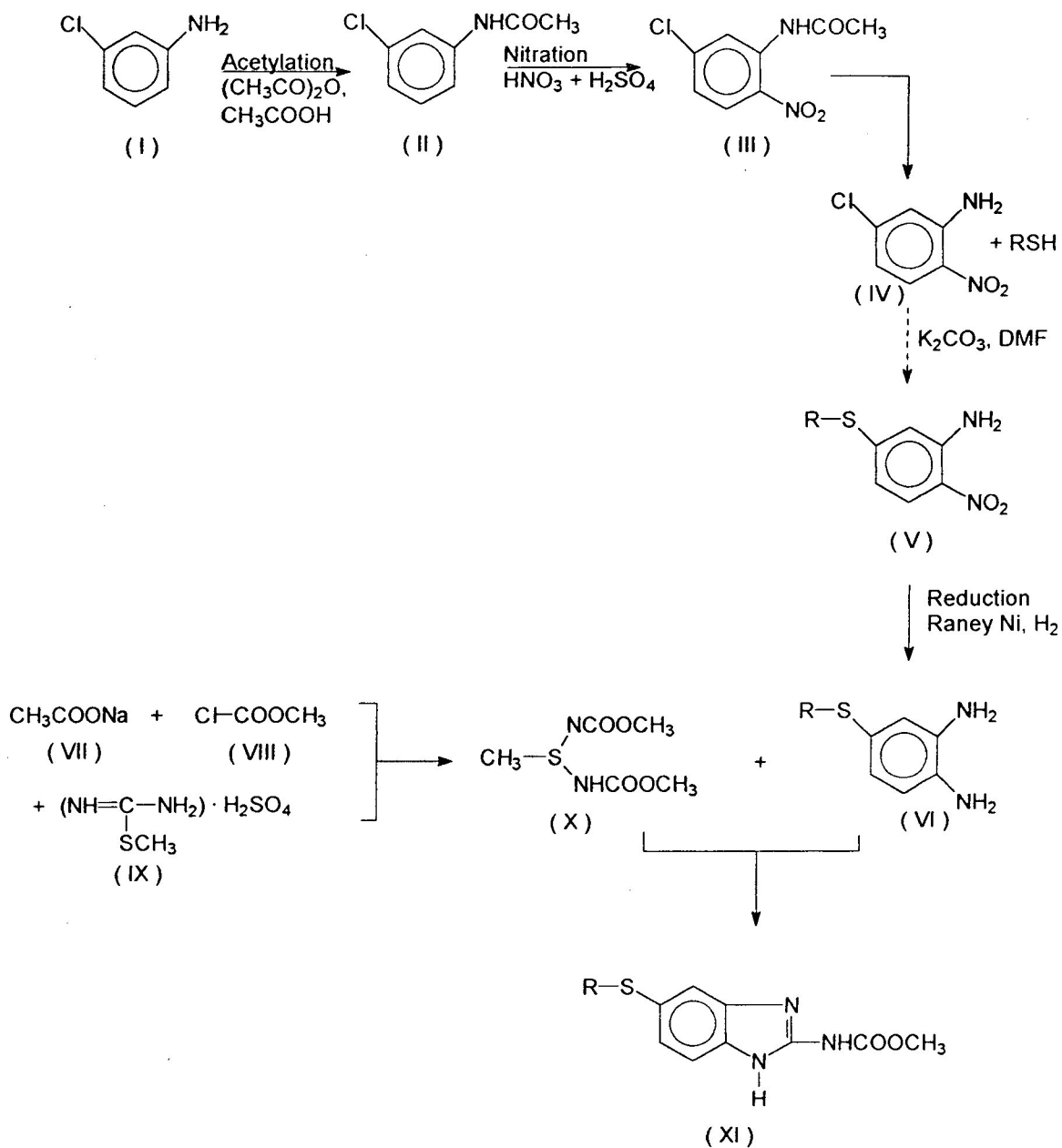
Reduction of 3 – nitro – 4 – amino anisidine

In a similar manner given above, reduction of 3 – nitro – 4 – amino anisidine was carried out.

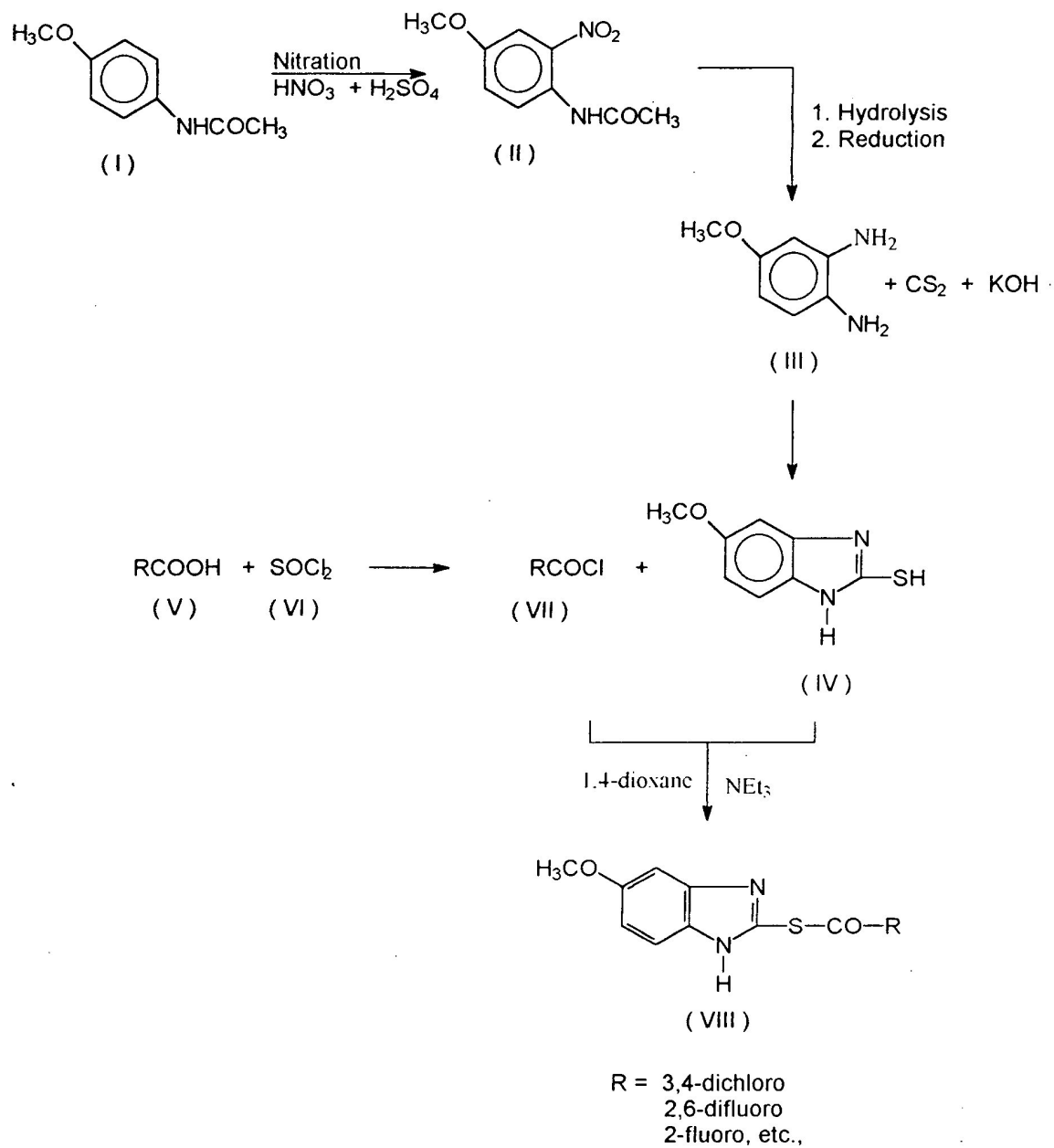
PART IC

SCHEMATIC REPRESENTATION OF THE SERIES OF
COMPOUNDS SYNTHESIZED

SCHEME I
Synthesis of methyl-5-(substituted thio)-2-benzimidazole carbamate



SCHEME II
Synthesis of 5-methoxy (thiocarbamoyl substituted) benzimidazole



PART II

SYNTHESIS OF
METHYL 5 – (SUBSTITUTED THIO) – 2 –BENZIMIDAZOLE
CARBAMATE

This part deals with the synthesis of Methyl 5 - (substituted thio) -2- benzimidazole.

carbamate which involves the following steps.

- 1) Synthesis of 5 – chloro -2- nitro aniline.
- 2) Synthesis of 5 – (substituted thio) –2- nitro aniline.
- 3) Synthesis of 4 – (substituted thio) – 1,2 phenylene diamine.
- 4) Synthesis of 2 – methyl thiopseudo urea – 1,3 – dicarboxylic acid dimethyl ester.
- 5) Synthesis of title compounds by cyclization of 4 – (substituted thio) – 1,2 phenylene diamine with 2 – methyl thiopseudo urea – 1,3 – dicarboxylic acid dimethyl ester.

1) Synthesis of 5-chloro-2-nitro aniline :

It involves three steps :

- a) Synthesis of 3 – chloro acetanilide.
- b) Synthesis of 5 – chloro – 2 – nitro acetanilide.
- c) Synthesis of 5 – chloro – 2 – nitro aniline.

a) Synthesis of 3 – chloro acetanilide :

In a 500ml round bottomed flask, equipped with a reflux condenser, was placed 28.05 g (0.22mole) of *m* – chloro aniline, 22.45g (20.78ml ; 0.22mole) of acetic anhydride, 13.21g (12.7ml ; 0.22mole) of glacial acetic acid and 0.1g of zinc dust. The mixture was boiled gently for 30min and then the hot liquid was poured in a thin stream into a one liter beaker containing 500ml of cold water whilst stirring was continued. It was cooled by using ice and the crude product was filtered at the pump and washed with a little cold water. It was dried on filter paper in the air. It was recrystallised and air dried.

Yield : 32.64gm (80.0%)

M.P.— 78°C.

Lit.¹²³ M.P.-- 77 – 79°C.

b) Synthesis of 5 – chloro – 2 – nitro acetanilide :

A solution of 3 – chloro acetanilide 9.275g (0.05mole) in 10ml concentrated sulphuric acid was taken into 50ml three necked round bottomed flask, equipped with a mechanical stirrer, a thermometer and a dropping funnel. It was stirred at 0 to 10°C. Nitrating mixture (Conc. H₂SO₄ 10ml, d –1.84 and nitric acid 2.2ml, d –1.42) was added dropwise at 0 to 10°C. The reaction mixture was stirred for further 2 hours at this temperature. Then reaction mixture i.e., nitrating product was precipitated by pouring the acid mixture on crushed ice with vigorous stirring, the temperature being maintained at 0°C. It was neutralized by liquor ammonia, the crude 5 – chloro – 2 – nitro acetanilide was filtered, washed with ice cold water, until neutral pH. The product was recrystallised from ethanol.

Yield : 8.98gm (76.5%)

M.P.— 116°C.

Lit.¹²⁴ M.P.— 116 – 118°C.

c) Synthesis of 5 – chloro – 2 – nitro aniline :

To a solution of 18.5 g (0.1mole) of 5 – chloro – 2 - nitro acetanilide in 30ml of boiling ethyl alcohol added a solution of 5.6 g (0.1mole) of KOH in 10ml of water. It was refluxed for 40 minutes. It was diluted with 120ml of water and distilled until 15ml of distillate (alcohol and water) are collected. The residue was poured into 150ml of cold water. The 5 – chloro – 2 – nitro aniline was separated out. It was filtered at the pump and washed with cold water. The crude material was purified from ethanol.

Yield : 13.04gm (75.6%)

M.P.--- 121°C.

Lit.¹²⁵ M.P.-- 122 – 124°C.

2) Synthesis of 5 – (substituted thio) – 2 – nitro aniline

Synthesis of 5 – (2 – imidazole thio) – 2 – nitro aniline

In a dry 100ml three necked round bottomed flask equipped with a dropping funnel, reflux condenser with a calcium chloride guard tube, mechanical stirrer was placed a mixture of 2 – mercapto imidazole (5.108g, 0.05mole) in a 25ml of dried dimethyl formamide and freshly oven dried potassium carbonate (6.910g, 0.05mole). The reaction mixture stirred at 50 – 60°C for 30min. To this is added dropwise a solution of 5 – chloro – 2 – nitro aniline (8.625g, 0.05mole) in a 15ml of dried dimethyl formamide at the same temperature. After addition the reaction mass was stirred at 115 – 120°C for 6 – 8 hrs. It was then poured into crushed ice. The solid separated out was filtered and washed with cold water. It was then recrystallised from ethanol.

Yield : 7.21g (64%)

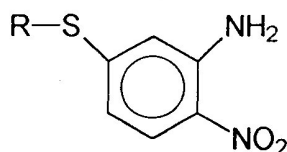
M.P.-- 103 - 108°C.

Elemental analysis of C₉H₈N₄O₂S

	C%	H%	N%
Calculated	45.76	3.38	23.72
Found	45.51	3.26	23.58

In a similar manner all the others 5 – (substituted thio) – 2 – nitro aniline have been synthesised and whose physical data has been represented in Table No.- 1.

Table 1
5- (Substituted thio) – 2 - nitro aniline



SR	R	YIELD %	M. P.° C
1	5 – methyl thiadiazole	62	248
2	Benzimidazole	57	231
3	5 – methoxy benzimidazole	59	246
4	1, 3, 4, - triazole	64	261
5	Benzothiazole	61	255
6	Imidazole	64	265
7	Benzoxazole	57.5	244
8	Isoquinazolinone	58	284
9	Quinoxaline	62	264
10	5 – thio (2' – methyl carbamate benzimidazolyl) thiadiazole	56	242

3) Synthesis of 4 – (substituted thio) – 1, 2 phenylene diamine

In a pressure reactor equipped with a water condenser and stirrer was placed 5 –(2 – imidazole thio) – 2 – nitro aniline (4.72g, 0.02mole) in 40ml ethanol and activated Raney nickel (5%). The pressure flask was first flushed with nitrogen and then hydrogen gas was passed in the flask upto 60 PSI. The reaction mixture was heated upto 50 – 60°C with stirring for 2 hrs. The reaction mixture was allowed to cool down to room temperature and activated raney nickel was filtered out. Raney nickel was decomposed with dil. HCl (10%). The filtrate was concentrated under reduced pressure to get the residue. The residue was recrystallised from ethanol.

Yield : 3.7 g (89%)

M.P. : 265°C

Similarly other 4 – (substituted thio) – 1, 2 phenylene diamine have been prepared.

4) Synthesis of 2 – methyl thiopseudourea -1, 3 – dicarboxylic acid dimethyl ester

In a 100ml three necked round bottomed flask equipped with a stirrer, water condenser and dropping funnel placed a solution of S – methyl iso – thiourea sulphate (7.8g, 0.028mole) in 7ml water. Into this mixture methyl chloroformate (4.4ml, 0.057mole) was added dropwise. After complete addition of methyl chloroformate the reaction mixture was stirred well for 30 – 45min. at this temperature then added acetic acid till the pH shows 5. After this addition, reaction mixture was stirred for 1 – 2 hrs vigorously and maintained the P^H 5. Sodium acetate (2.3g, 0.028mole) was added within 15 – 20 min. Reaction mixture was kept for overnight at room temperature with stirring.

Next day, methyl chloroformate (Excess) and acetic acid was distilled out. The compound was precipitated on cooling at room temperature. The compound was recrystallised from chloroform.

Yield : 4.04 g

M.P.-- 100-101°C.

Lit.¹²⁶ M.P.-- 100-102°C.

5) Synthesis of title compounds by cyclization of 4 – (substituted thio) – 1,2 phenylene diamine with 2 – methyl thiopseudo urea – 1,3 – dicarboxylic acid dimethyl ester.

Synthesis of methyl 5 – (imidazole thio) – 2 – benzimidazole carbamate by cyclization of 4 – (imidazole thio) – phenylene diamine with 2 – methyl thiopseudo urea – 1,3 – dicarboxylic acid dimethyl ester.

In a 100 ml round bottomed flask equipped with a reflux condenser was placed a mixture of 4 – (imidazole thio) – phenylene diamine (2.5g, 0.012mole) in 15 – 20ml ethanol and 2 – methyl thiopseudo urea – 1,3 – dicarboxylic acid dimethyl ester (2.67g, 0.013mole). The reaction mixture was kept under reflux for 10 – 12 hrs. on water bath. The progress of the reaction was indicated by the evolution of methyl mercaptan detected by its strong odour and darkening of lead acetate paper when placed at the opening of the condenser. The completion of reaction was determined by slow and gradual darkening of the lead acetate paper. The ethanol was distilled out under reduced pressure. The product was recrystallised from dimethyl formamide.

Yield : 2.1g (60%)

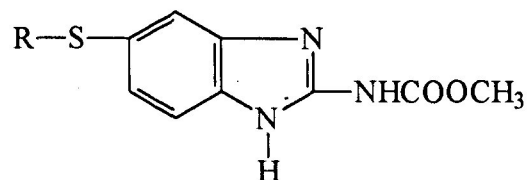
M. P. --- > 200°C.

Elemental analysis of $C_{12}H_{11}N_5O_2S$

	C%	H%	N%
Calculated	49.82	3.80	24.22
Found	49.91	3.45	24.12

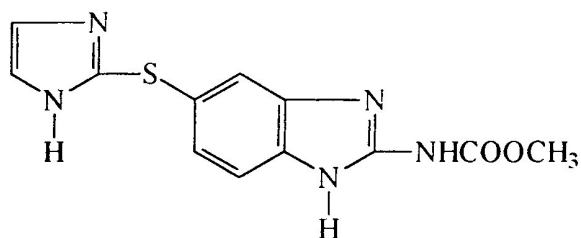
Similarly other methyl 5 – (substituted thio) – 2 – benzimidazole carbamate have been synthesised and their physical constants have been given in Table 2.

Table : 2
methyl-5-(Substituted thio) benzimidazole carbamate



Sr. No.	R	Molecular Formula	Mol. Wt.	M.P. °C	Yield %	Elemental calculated			Analysis found		
						C	H	N	C	H	N
1	5-methyl thiadiazole	C ₁₂ H ₁₁ N ₅ O ₂ S ₂	321	>250 °	78.80	44.85	3.42	21.80	44.60	3.22	21.58
2	Benzamidazole	C ₁₆ H ₁₃ N ₅ O ₂ S	339	>250 °	74.86	56.64	3.83	20.64	56.44	3.52	20.43
3	5-methoxy benzimidazole	C ₁₇ H ₁₅ N ₅ O ₃ S	351	>250 °	75.75	58.11	4.27	19.94	58.36	4.51	19.57
4	1,3,4,-thiazole	C ₁₁ H ₁₀ N ₆ O ₂ S	290	>250 °	59.00	45.51	3.44	28.96	45.13	3.73	28.60
5	Benzothiazole	C ₁₆ H ₁₂ N ₄ O ₂ S ₂	356	>250 °	73.50	53.93	3.37	15.73	53.62	3.33	15.49
6	Imidazolidine	C ₁₂ H ₁₁ N ₅ O ₂ S	289	>250 °	60.00	49.82	3.8	24.22	49.91	3.45	24.12
7	Benzoxazole	C ₁₅ H ₁₂ N ₄ O ₃ S	328	>250 °	68.00	54.87	3.65	17.07	54.47	3.53	17.25
8	Isoquinazolinone	C ₁₇ H ₁₃ N ₅ O ₃ S	367	>250 °	61.00	55.58	3.54	19.07	55.65	3.25	19.32
9	Quinoxaline	C ₁₇ H ₁₃ N ₅ O ₂ S	351	>250 °	65.00	58.11	3.70	19.94	58.36	3.43	19.52
10	5-thio(2'-methyl carbamate benzimidazolyl) thiadiazole	C ₂₀ H ₁₆ N ₈ O ₄ S ₃	528	>250 °	68.80	45.45	3.03	21.21	45.19	3.13	21.53

Spectral Studies of 5-(substituted thio) – 2 – benzimidazole carbamate [Table No.2]
 methyl 5 – (imidazole thio) – 2 – benzimidazole carbamate (comp. no. 6)



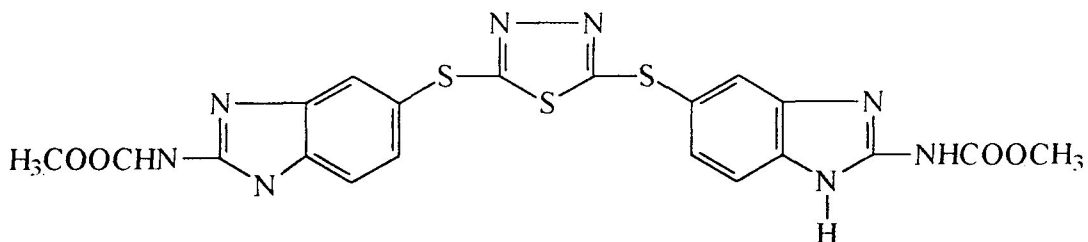
IR (KBr) cm^{-1}

3364	(N-H Stretching, NHCOOCH_3)
2949	(C-H Stretching, alkyl group)
1694	(C=O Stretching, NHCOOCH_3)
1588, 1519, 1457	(imidazole group)
1297	(C-O Stretching of ester)
770, 857, 914	(5-substituted benzimidazole group)
651	(C-S Stretching)

PMR (DMSO - d_6) δ :

3.76	(S, 3H, CH_3)
7.1 - 7.45	[m, 7H, (Ar - H, imidazole and benzimidazole - NH)]
11.6	(S, 1H, $-\text{NHCOOCH}_3$)

Methyl 5 – [5 – thio (2' – methyl carbamate benzimidazolyl) thiadiazole thio] – 2 – benzimidazole carbamate (comp. no. 10)



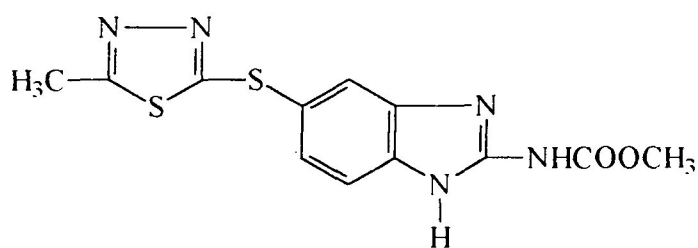
IR (KBr) cm^{-1}

3361	(N-H Stretching, NHCOOCH_3)
2949	(C-H Stretching, alkyl group)
1707	(C=O Stretching, NHCOOCH_3)
1638, 1588	(C=N Stretching, heterocyclic ring)
1526	(ring stretching vibration of 1,3,4-thiadiazole)
1268	(C-O Stretching of ester)
1088	(1,3,4- thiadiazole group)
770, 851, 914	(5-substituted benzimidazole group)
651	(C-S Stretching)

PMR (DMSO - d_6) δ :

3.76	(S, 6H, 2- CH_3)
7.06 - 7.95	[m, 8H, (6H, Ar - H)(2H, benzimidazole - NH)]
11.6	(S, 2H, 2- NHCOOCH_3)

Methyl – 5 – (5-methyl thiadiazole thio) – 2 – benzimidazole carbamate



IR (KBr) cm^{-1} (comp. no.1)

3336	(N-H Stretching, NHCOOCH_3)
2924	(C-H Stretching, alkyl group)
1700	(C=O Stretching, NHCOOCH_3)
1644, 1588	(C=N Stretching, heterocyclic ring)
1457	(ring stretching vibration of 1,3,4-thiadiazole)
1271	(C-O Stretching of ester)
1096	(1,3,4- thiadiazole group)
770, 857, 914	(5-substituted benzimidazole group)
657	(C-S Stretching)

File # 1: BMETP

Mode= 2 (Mid-IR)

4/3/97 8:52 AM

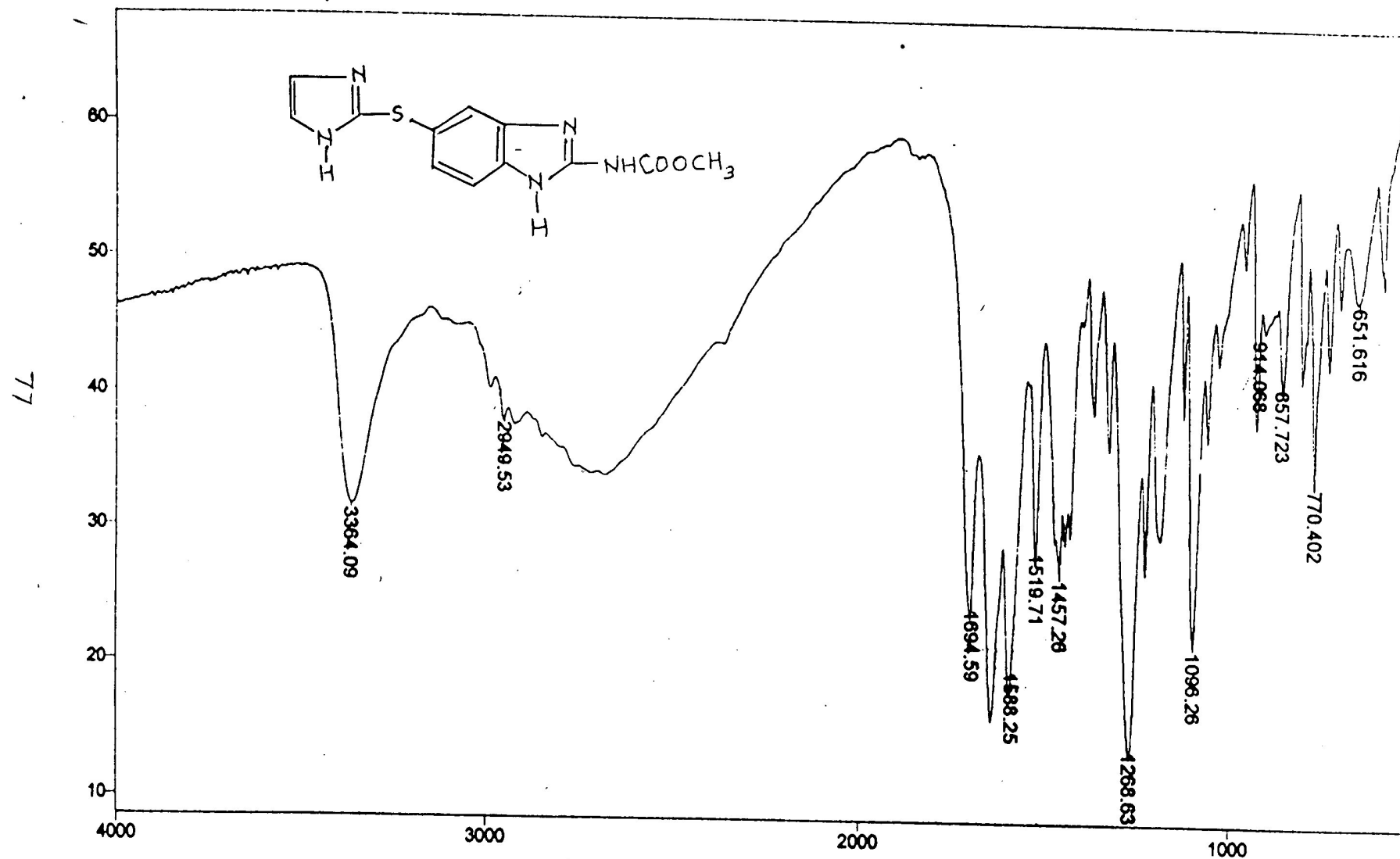
Sample Description: benzimidazole analogue.

Scans= 10 Slow

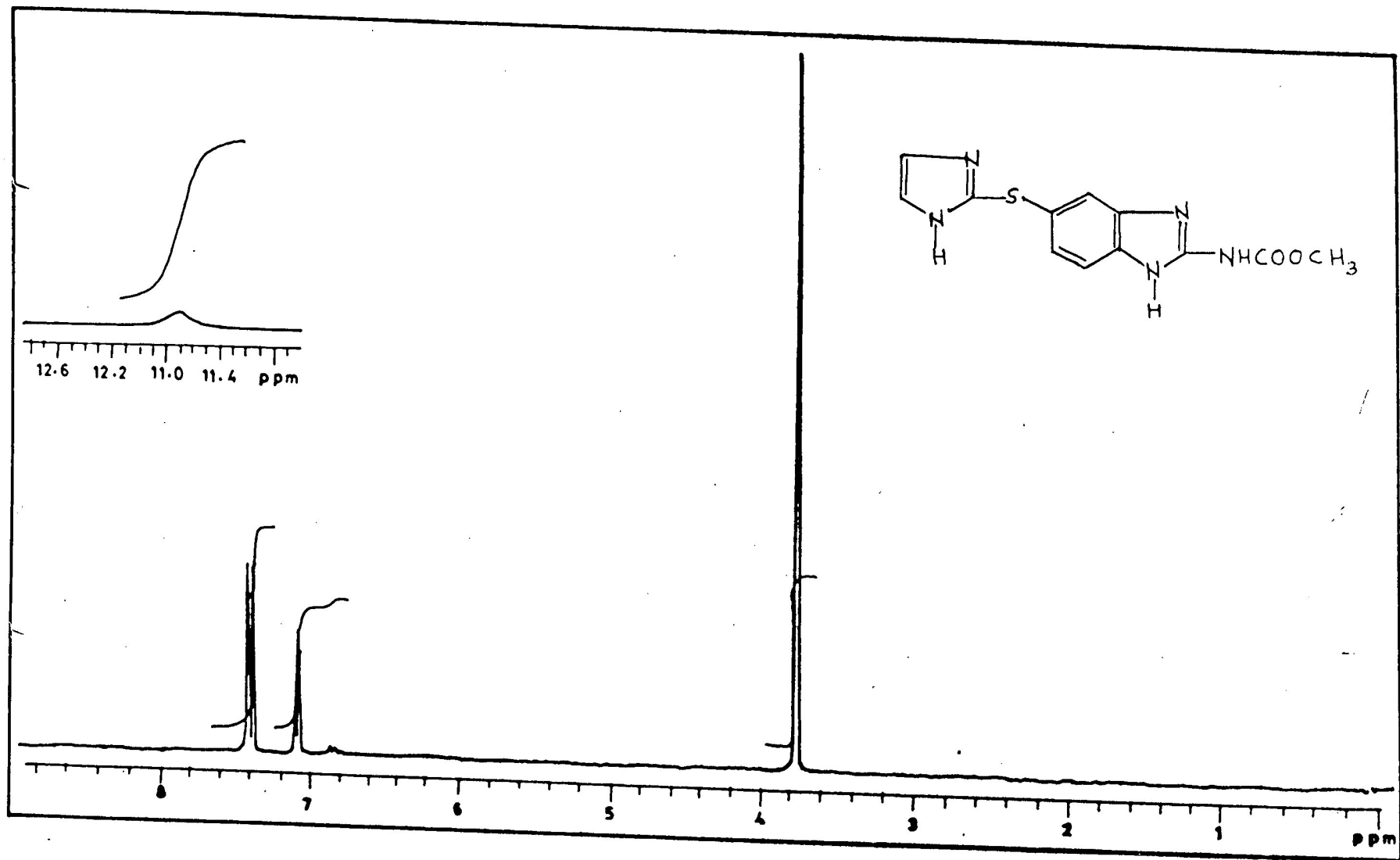
Res=4 cm-1

Apod= Cosine

Zero Filling= 1 x



Transmittance / Wavenumber (cm-1)



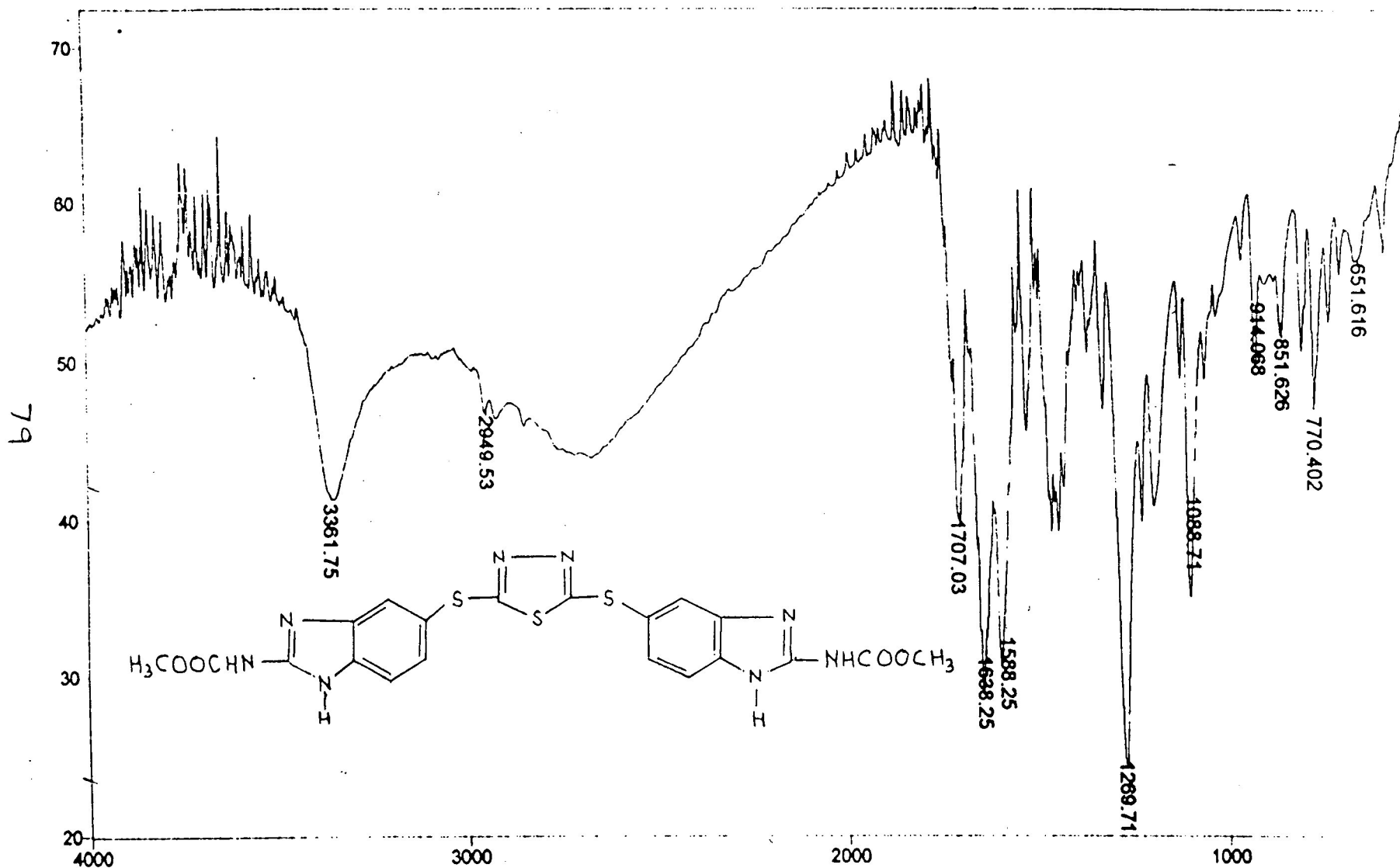
Sample Description: benzimidazole

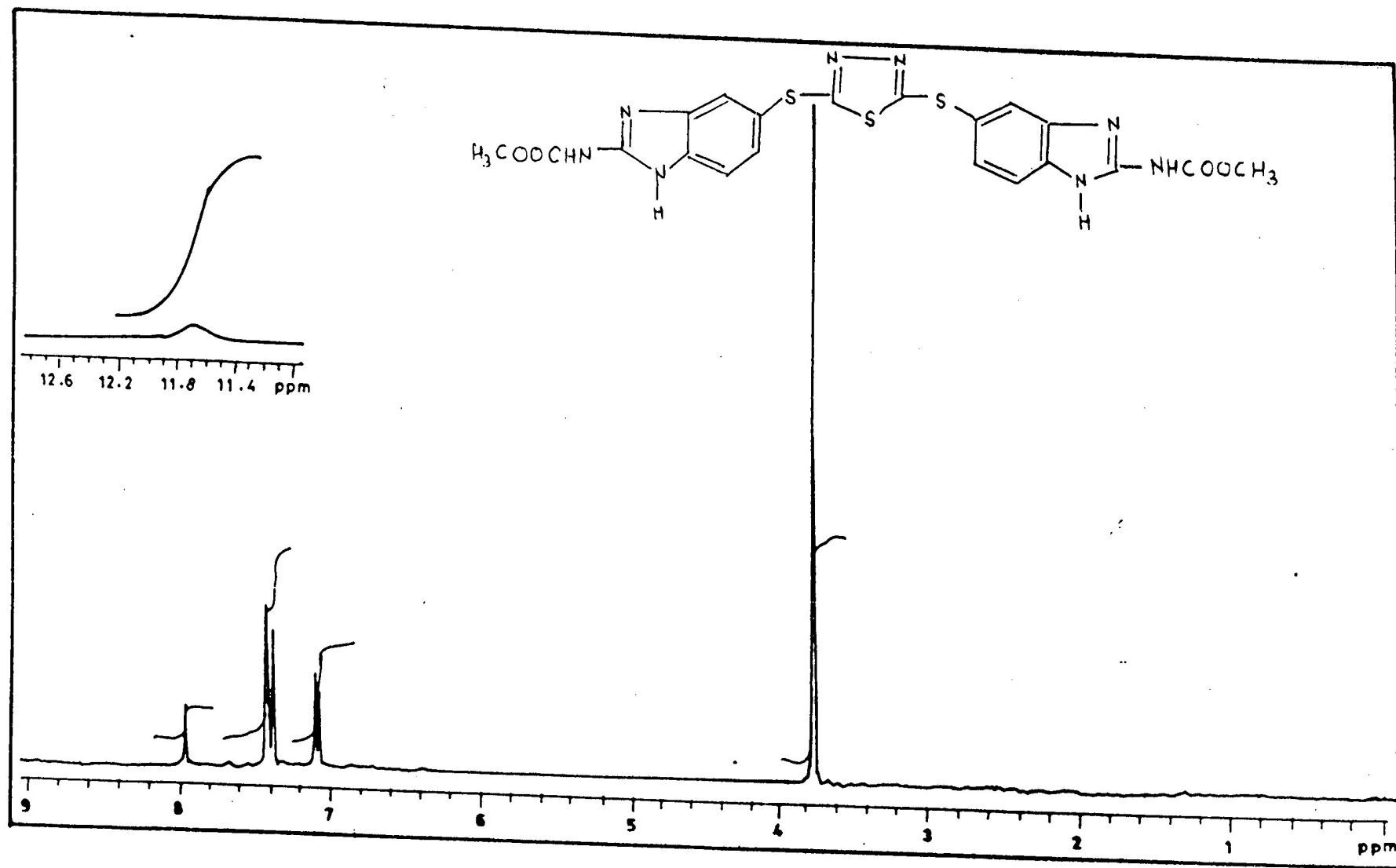
Scan= 10 Slow

Res=4 cm-1

Apod= Cosine

Zero Filling= 1 x





File #1: BMTDZ

Mode= 2 (Mid-IR)

11/26/96 9:59 AM

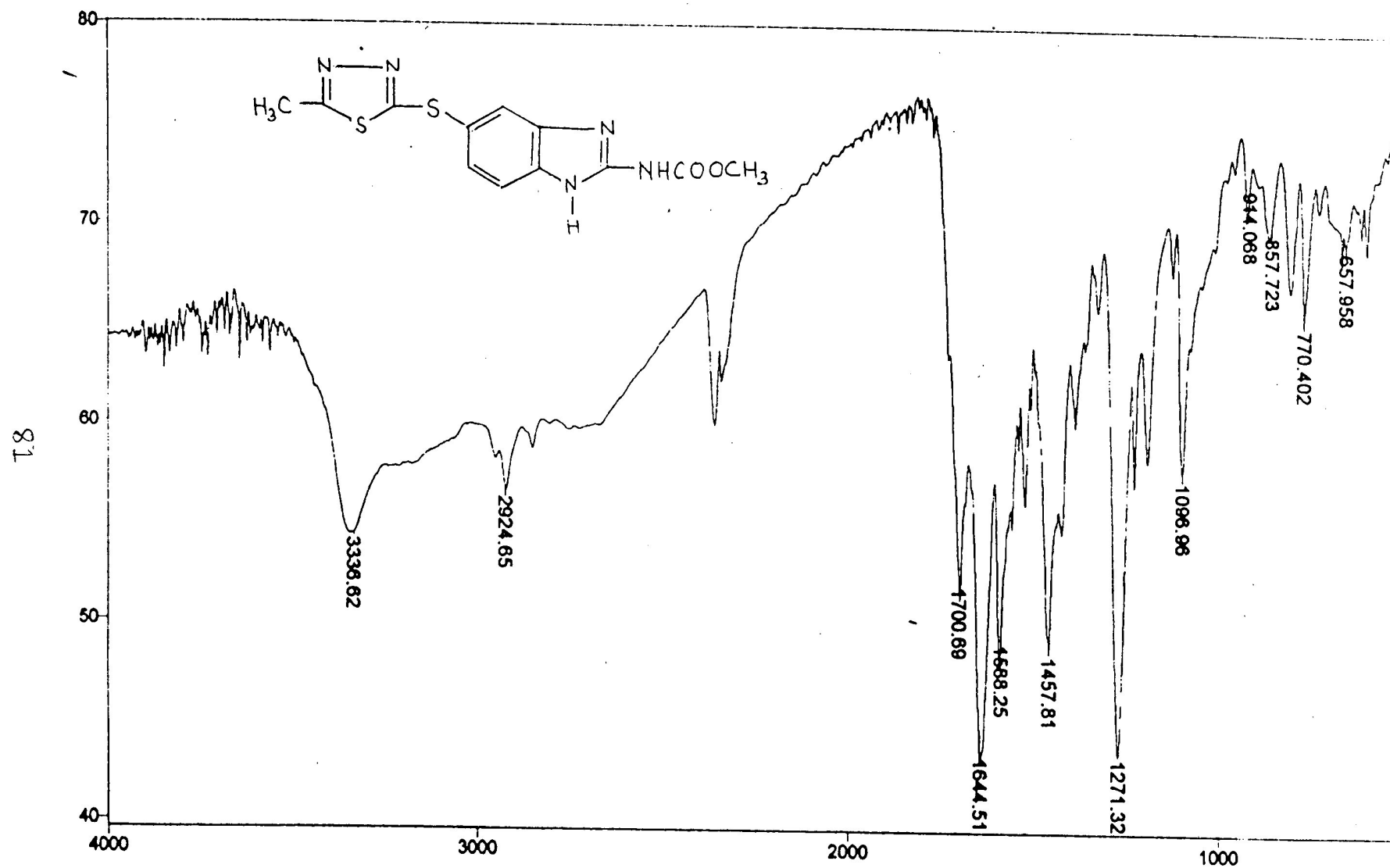
Sample Description: benzimidazole

Scans= 10 Slow

Res=4 cm-1

Apod= Cosine

Zero Filling= 1 x



Transmittance / Wavenumber (cm-1)

PART III

SYNTHESIS OF

5 – METHOXY – 2 – (THIOCARBAMOYL SUBSTITUTED)

BENZIMIDAZOLE



This part deals with the synthesis of 5 – methoxy – 2 - (thiocarbamoyl substituted) benzimidazole which involves the following steps

- 1) Synthesis of 4 – methoxy – 1,2 – phenylene diamine.
- 2) Synthesis of 5 – methoxy – 2 – mercapto benzimidazole.
- 3) Synthesis of substituted acid chloride.
- 4) Synthesis of title compounds by condensation of 5 – methoxy – 2 – mercapto benzimidazole with substituted acid chlorides.

1) Synthesis of 4 – methoxy – 1, 2 – phenylene diamine

It involves three steps :

- a) Synthesis of 2 – nitro – *p*- acetanisidide.
- b) Synthesis of 5 – methoxy – 2 – nitro aniline.
- c) Synthesis of 4 – methoxy – 1, 2 – phenylene diamine.

a) Synthesis of 2 – nitro – *p*- acetanisidide.

A solution of 4 – methoxy acetanilide 9.45g (0.05mole) in 10ml concentrated sulphuric acid was taken into 50 ml three – necked round bottomed flask, equipped with a mechanical stirrer, a thermometer and a dropping funnel. It was stirred at 0 to 10°C. Nitrating mixture (Conc. H₂SO₄ 10ml, d –1.84 and nitric acid 2.2ml, d –1.42) was added dropwise at 0 to 10°C. The reaction mixture was stirred for further 2 hrs. at this temperature. Then reaction mixture i.e., nitrating product was precipitated by pouring the acid mixture on crushed ice with vigorous stirring, the temperature being maintained at 0 ° C. It was neutralised by liquor ammonia, the crude 2 – nitro – *p* – acetanisidide was filtered, washed with ice cold water, until neutral pH. The product was recrystallised from ethanol.

Yield : 9.0 g (77%)

M. P. --- 116°C.

Lit.¹²⁷ M. P. --- 117°C.

b) Synthesis of 5 – methoxy – 2 – nitro aniline

To a solution of 23.4g (0.1mole) of 2 – nitro – *p* – acetanisidide in 30 ml of boiling ethyl alcohol added a solution of 5.6g (0.1mole) in 10 ml of water. It was refluxed for 40 min. It was then diluted with 120 ml of water and distilled until 75 ml of distillate (alcohol and water) was collected. The residue was poured into 150 ml of cold water. The 5 – methoxy – 2 – nitro aniline was separated out. It was filtered at the pump and washed with cold water. The crude material was purified from ethanol.

Yield : 12.432g (74%)

M. P. --- 127°C.

Lit.¹²⁷ M. P. – 129°C.

c) Synthesis of 4 – methoxy – 1, 2 – phenylene diamine.

In a pressure reactor equipped with a water condenser, mechanical stirrer was placed 4 – methoxy – 2 – nitro aniline (3.36g, 0.02mole) in 40 ml ethanol and activated Raney nickel (5%). The pressure vessel was first flushed with nitrogen and then hydrogen was passed in the vessel upto 60PSI. The reaction mixture was heated upto 50 – 60°C with stirring for 2 hrs. The reaction mixture was allowed to cool down to room temperature and Raney nickel was filtered out. The Raney nickel was decomposed with dil. HCl (10%). The filtrate was concentrated under reduced pressure to get the product.

Yield : 2.45g (89%)

M. P. --- 51°C.

Lit.¹²⁸ M. P. --- 50-52°C.

2. Synthesis of 5 – methoxy – 2 – mercapto benzimidazole

To potassium hydroxide (5.6g, 0.01mole) was added 150 ml ethanol and 30 ml water. The mixture was warmed on waterbath till all the solid goes into the solution. To this cold solution, was added carbon disulphide (13.5g, 11ml, 0.17mole) with stirring.

In a 250 ml three necked round bottomed flask equipped with a dropping funnel and a reflux condenser was placed 4 – methoxy – 1, 2 – phenylene diamine (13.8g, 0.1mole) in 200 ml ethanol. To this above reaction mixture potassium ethyl xanthate solution was added dropwise. The mixture was further refluxed for 6 hrs. until fumes of hydrogen sulphide gas ceases. The reaction mixture was cooled and then concentrated under vacuum to remove most of the ethanol. The oil obtained was diluted with water and then neutralised with dilute hydrochloric acid. The solid separated was filtered, washed with water and dried. It was then recrystallised from ethanol.

Yield : 13.5g (75%)

M. P. --- 155°C

Lit.¹²⁹ M. P. --- 156-158°C

3. Synthesis of substituted acid chlorides

Synthesis of 2, 6 – difluoro benzoyl chloride

A mixture of 2, 6 – difluoro benzoic acid (3.16g, 0.03 mole) and freshly distilled thionyl chloride (3.75g, 0.03 mole) in 25 ml dry benzene was taken in 100 ml round bottomed flask equipped with a reflux condenser and a guard tube. The reaction mixture was refluxed on steam bath with occasional shaking till evolution of HCl gas ceases. Excess of thionyl chloride was distilled out along with benzene. The residual oil was distilled under vacuum at 15mm.

Yield : 2.75g (78%)

B. P._{15mm} = 77°C

Lit.¹³⁰ B. P._{15mm} = 76-79°C.

Similarly other substituted acid chlorides have been prepared and their physical constants have been given in Table 3.

Table 3
SUBSTITUTED ACID CHLORIDES



Sr. No	R	Yield %	B.P.		References
			Observed	Reported	
1	2, 6 – difluorophenyl	78	77(15 mm)	76-79(15 mm)	130
2	4 – fluorophenyl	82.5	72 (20 mm)	69.70 (15 mm)	131
3	4 – chlorophenyl	79.5	113 (20 mm)	111 (18 mm)	132
4	2 - fluorophenyl	82	74(20 mm)	66.67 (15 mm)	131

4. **Synthesis of title compounds by condensation of 5 – methoxy – 2 – mercapto benzimidazole with substituted acid chlorides.**

Synthesis of 5 – methoxy – 2 – (2, 6 – difluoro phenyl thiocarbamoyl) benzimidazole :

In a 100 ml three necked dry round bottomed flask with a dropping funnel, reflux condenser with a guard tube, was placed a mixture of triethylamine (0.003 mole, 0.10g) and a solution of 5 – methoxy – 2 – mercapto benzimidazole (0.36g, 0.002 mole) in 10 ml dry 1, 4 – dioxane. The reaction mixture was stirred on a mechanical stirrer at 50 – 60°C for about 30 min. To this is added dropwise a solution 2, 6 – difluoro benzoyl chloride (0.177g, 0.001mole) in 10 ml dry 1, 4 – dioxane at the same temperature. After the addition, the reaction mass was stirred for 2 hrs. It was kept overnight at room temperature. The solution was poured onto crushed ice. The solid separated out was filtered and washed with water and dried. It was then recrystallised from ethanol.

Yield : 0.40g (63%)

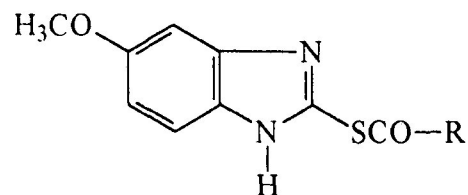
M. P. --- > 250°C.

Elemental analysis of $C_{15}H_{10}F_2N_2O_2S$

	C%	H%	N%
Calculated	56.25	3.125	8.75
Found	56.47	3.146	8.49

Similarly other 5 – methoxy – 2 – (thiocarbamoyl substituted) benzimidazoles have been synthesised and their physical constants have been given in Table 4.

Table : 4
5-methoxy-2-(thiocarbamoyl substituted) benzimidazole

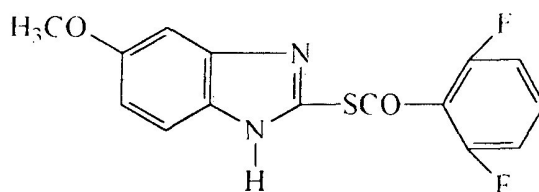


Sr. No	R	Molecular Formula	Mol. Wt.	M.P. °C	Yield %	Elemental calculated			Analysis found		
						C	H	N	C	H	N
1	2,6-difluorophenyl	C ₁₅ H ₁₀ F ₂ N ₂ O ₂ S	320	> 200°	63.00	56.25	3.125	8.75	56.47	3.146	8.49
2	4-fluorophenyl	C ₁₅ H ₁₁ N ₂ O ₂ FS	302	> 200°	68.50	59.60	3.43	9.27	59.43	3.52	9.48
3	4-cyanophenyl	C ₁₆ H ₁₁ N ₃ O ₂ S	309	> 200°	63.40	62.10	3.55	13.59	62.40	3.51	13.42
4	4-chlorophenyl	C ₁₅ H ₁₁ N ₂ O ₂ ClS	318.5	> 200°	72.00	56.51	3.45	8.87	56.58	3.56	8.93
5	2-fluorophenyl	C ₁₅ H ₁₁ N ₂ O ₂ FS	302	> 200°	73.25	59.60	3.43	9.27	59.25	3.62	9.81
6	4-methoxyphenyl	C ₁₆ H ₁₄ N ₂ O ₃ S	314	> 200°	65.50	61.14	4.45	8.91	61.45	4.56	8.68
7	cyclopropyl	C ₁₂ H ₁₂ N ₂ O ₂ S	248	> 200°	63.02	58.06	4.83	11.29	58.04	4.56	11.52
8	3,4-dichlorophenyl	C ₁₅ H ₁₀ N ₂ O ₂ Cl ₂ S	353	> 200°	68.80	50.99	2.83	7.93	50.62	2.46	7.58
9	Morpholinyl	C ₁₃ H ₁₅ N ₃ O ₃ S	293	> 200°	73.00	53.24	5.11	14.33	53.40	5.18	14.45
10	3,4-dimethoxyphenyl	C ₁₆ H ₁₄ N ₂ O ₃ S	344	> 200°	63.20	59.30	4.65	8.13	59.42	4.72	8.42

Spectral Studies of 5 – methoxy – 2 – (thiocarbamoyl substituted) benzimidazoles

[Table no. 4.]

5-methoxy – 2 – (2, 6 – difluoro phenyl thiocarbamoyl) benzimidazole (comp. no. 1)



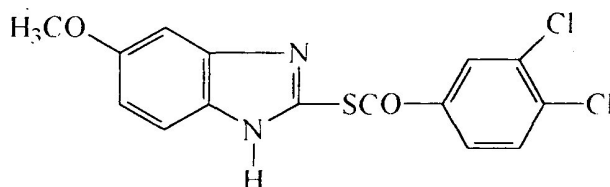
IR (KBr) cm^{-1}

3086	(N-H Stretching of benzimidazole)
2930	(C-H Stretching, CH_3)
1701	(C=O Stretching)
1632, 1588	(C=N Stretching, heterocyclic ring)
1463	(C=C Skeletal vibration of benzene)
1282, 1157	(C-O Stretching of OCH_3)
1088	(C-F Stretching)
789, 826, 914	(5- substituted benzimidazole)
692	(C-S Stretching)

PMR (DMSO - d_6) δ :

2.49 - 2.50	[S, 3H, Ar- OCH_3]
7.01-8.34	[m, 7H,(6H, Ar - H, imidazole- NH)]

5 – methoxy - 2 – (3,4 – dichloro phenyl thiocarbamoyl) benzimidazole (Compound no. 8)



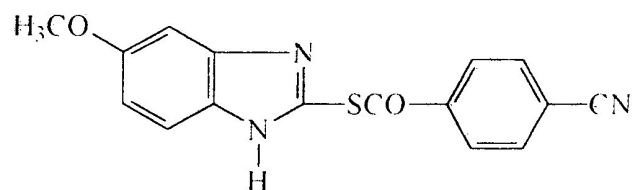
IR (KBr) cm^{-1}

3086	(N-H Stretching of benzimidazole)
2930	(C-H Stretching, CH_3)
1701	(C=O Stretching)
1585	(C=N Stretching, heterocyclic ring)
1031	(C-O Streching of OCH_3)
930, 855, 824	(5- substituted benzimidazole)
739	(C-Cl streching)
657	(C-S streching)

PMR (DMSO - d_6) δ :

2.49 - 2.50	[S, 3H, Ar- OCH_3]
6.67 - 8.12	[m, 7H (6H, Ar-H) (1H, benzimidazole -NH)]

5 - methoxy - 2 - (4 - cyano phenyl thiocarbamoyl) benzimidazole (comp. no. 3)



IR (KBr) cm^{-1}

3065	(N-H Stretching of benzimidazole)
2833	(C-H Stretching, CH_3)
2226	($\text{C}\equiv\text{N}$ Streching)
1701	(C=O Stretching)
1631, 1556	(C=N Stretching, heterocyclic ring)
1180, 1156	(C-O Stretching of OCH_3)
930, 855, 824	(5- substituted benzimidazole)
692	(C-S Streching)



#1: BZBCL4R

Mode= 2 (Mid-IR)

10/28/97 11:56 AM

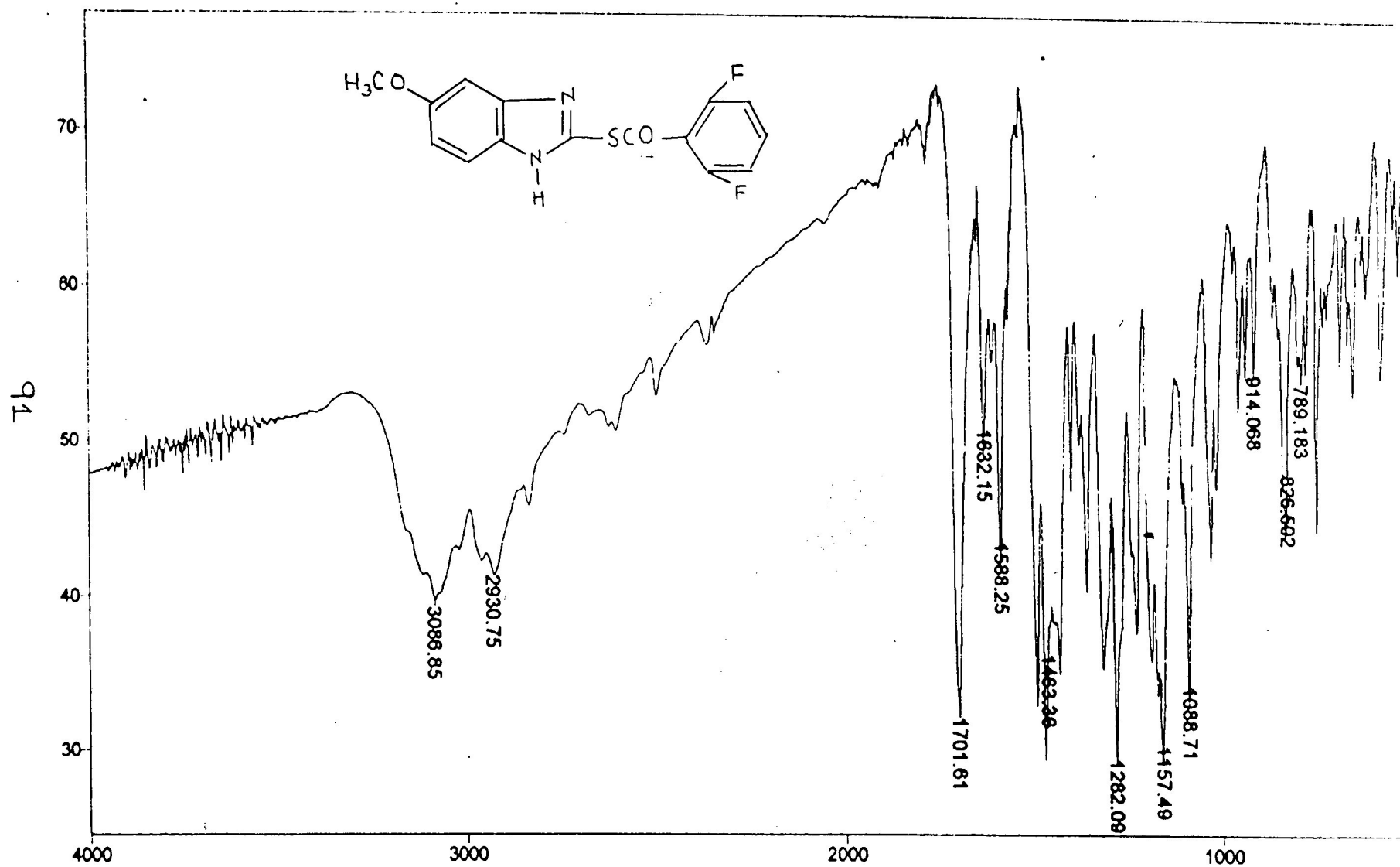
Sample Description: 4-fluorobenzocarbonylthiobenz

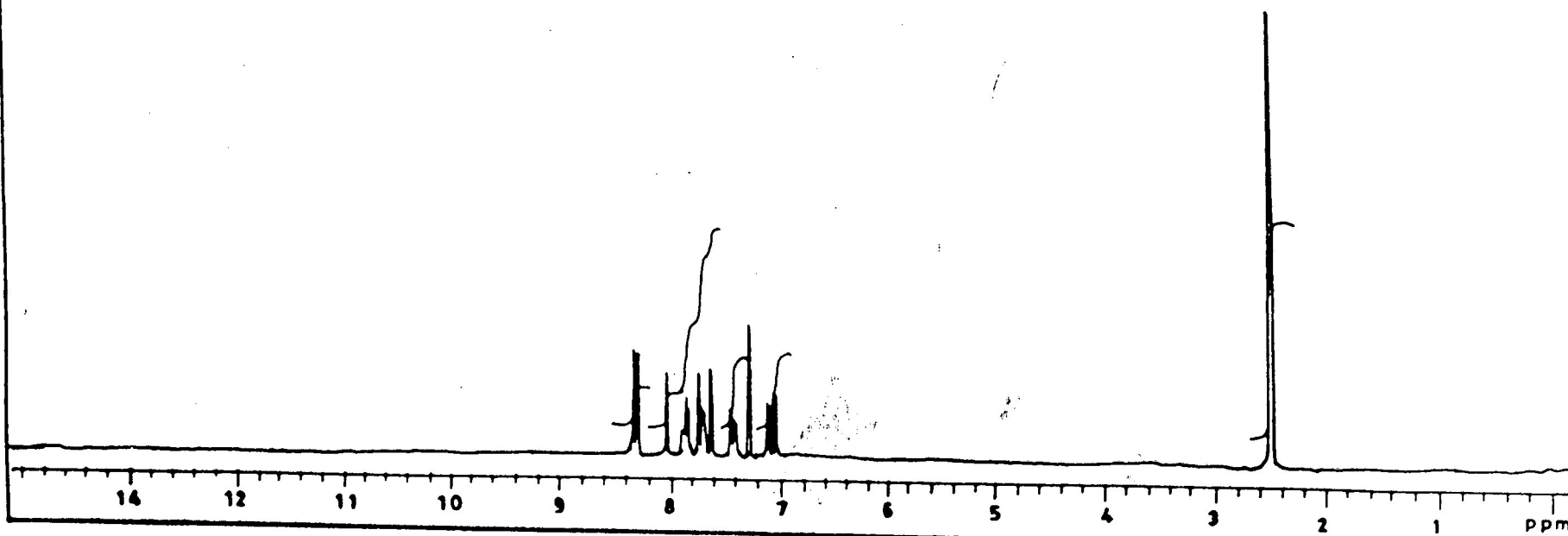
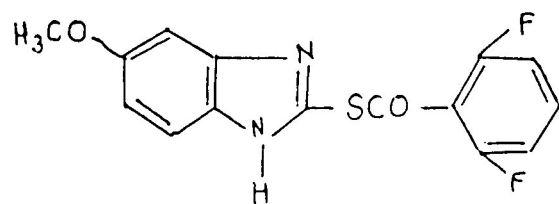
Scan= 10 Slow

Res=4 cm⁻¹

Apod= Cosine

Zero Filling= 1 x





1 = BZBCL5

Mode= 2 (Mid-IR)

10/28/97 12:18 PM

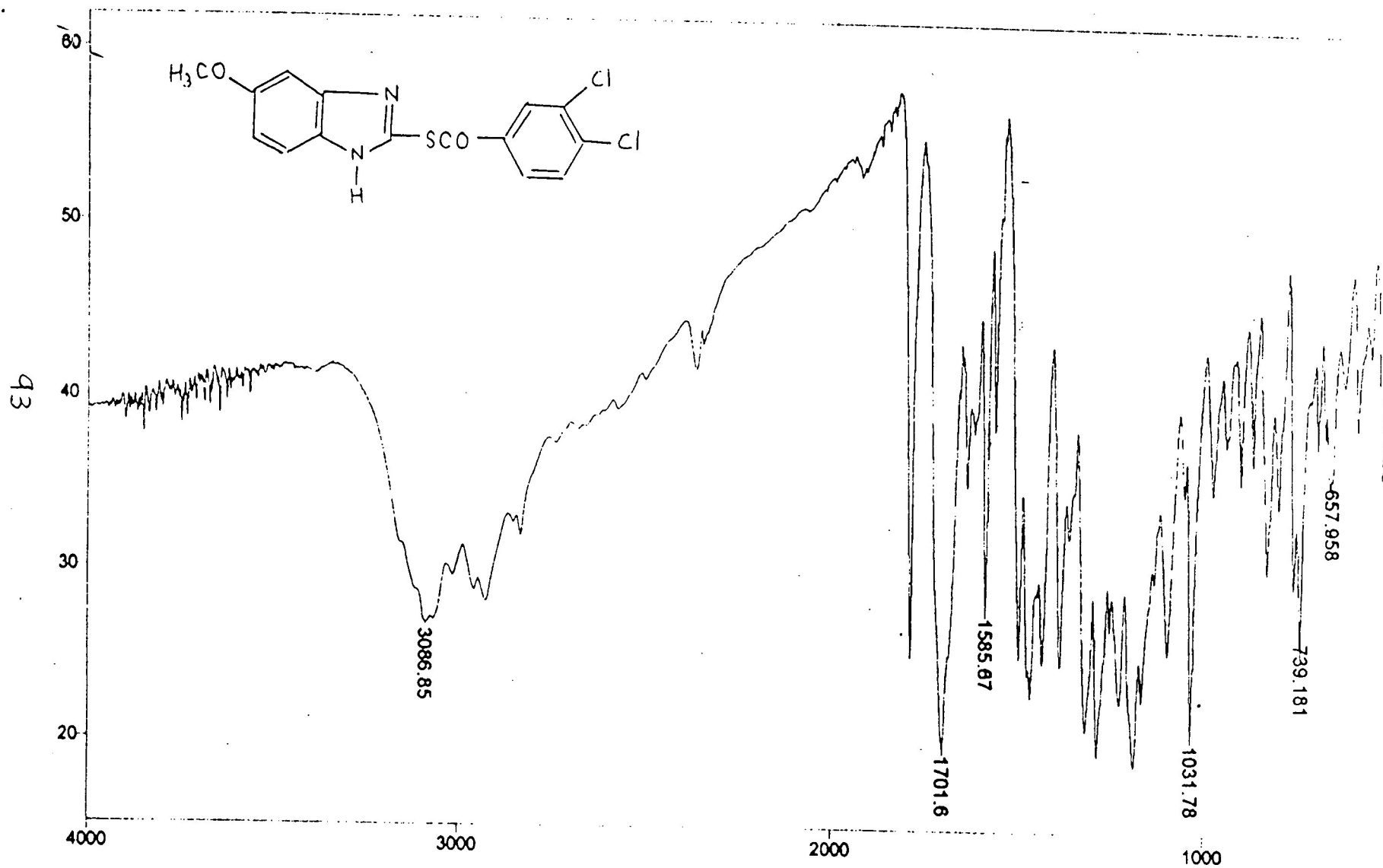
Sample Description: 3,4-dichlorobenzocarbonylthioindole

Scan= 10 Slow

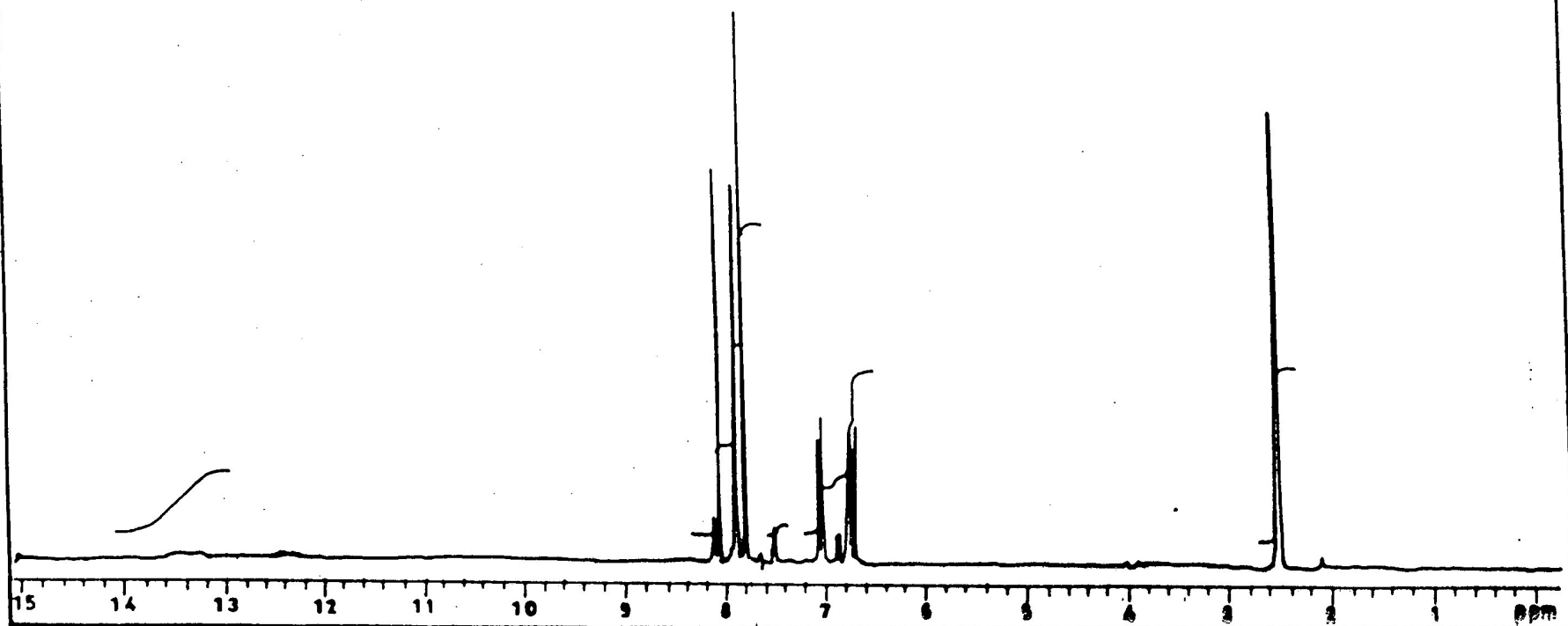
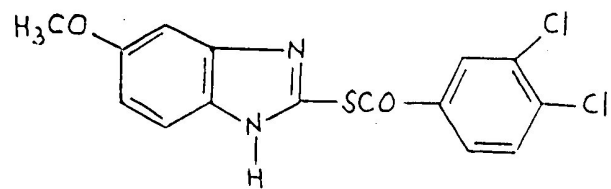
Res=4 cm-1

Apod= Cosine

Zero Filling= 1 x



94



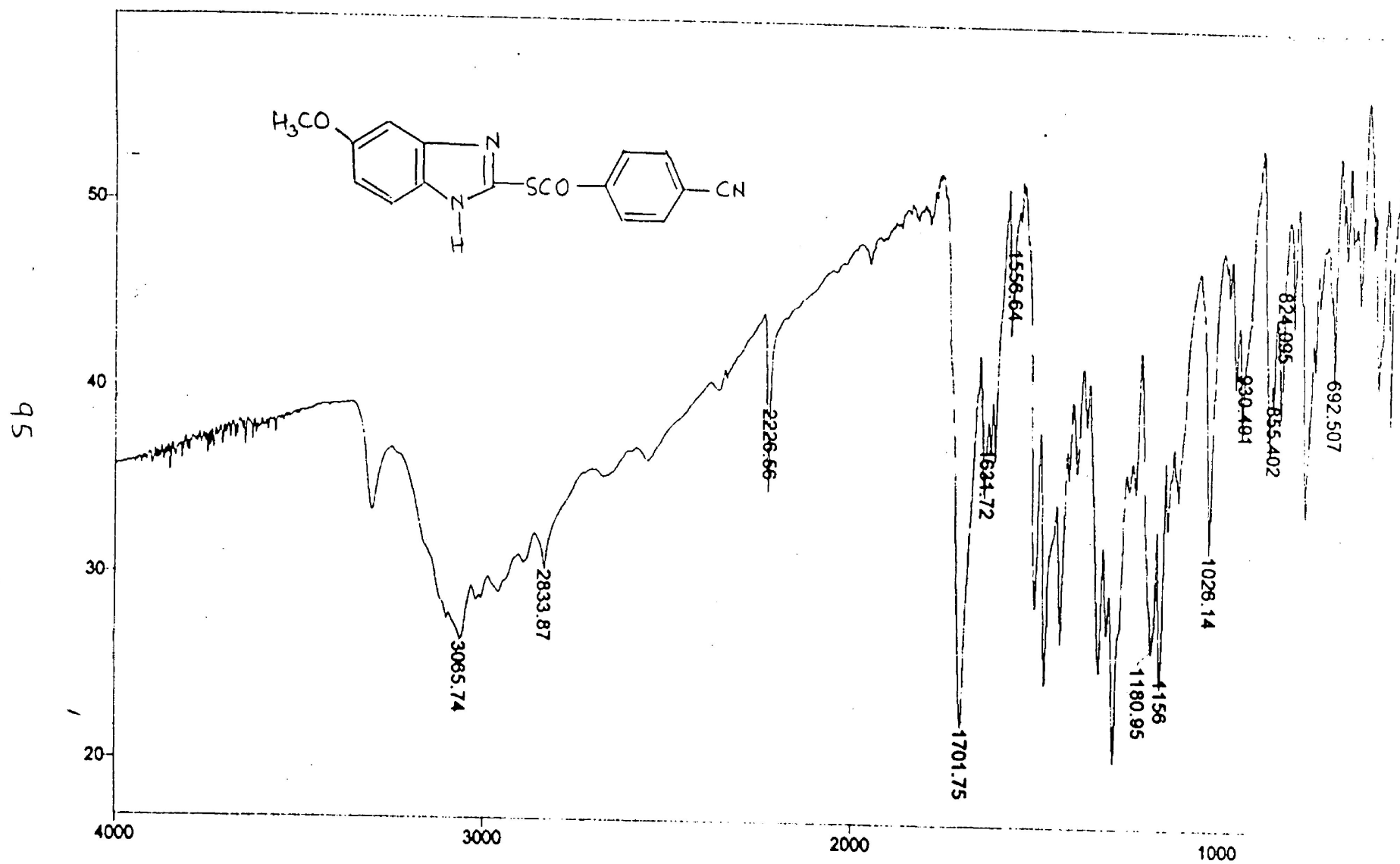
Sample Description: 4cyanobenzothiocarbamylbenz

Saves= 10 Slow

Res=4 cm-1

Apod= Cosine

Zero Filling= 1 x



PART IV

SYNTHESIS OF
DRUG INTERMEDIATE OF TRICLOSAN AND TRICLOSAN
ITSELF

Synthesis of 2, 4, 4' – trichloro – 2' – hydroxy diphenyl ether (Triclosan)

Preparation of Triclosan involves following different steps,

- 1) Preparation of 2, 4, 4' – trichloro – 2' – nitrodiphenyl ether (TNDE).
- 2) Preparation of 2, 4, 4' – trichloro – 2' – aminodiphenyl ether (TADE).
- 3) Preparation and purification of 2, 4, 4' – trichloro – 2' – hydroxydiphenyl ether.

1) Preparation of 2, 4, 4' – trichloro – 2' – nitrodiphenyl ether (TNDE).

In a 250 ml three necked round bottomed flask equipped with water condenser, mechanical stirrer and dropping funnel was placed (8.49 g, 0.0521mole) 2, 4 – dichloro phenol dissolved in 10 ml dry dimethyl formamide. To this was added (8.64g, 0.0625mole) of anhydrous potassium carbonate. The reaction mixture was then stirred and heated to 70°C in oil bath for 30min. To this reaction mixture was added 2, 5 – dichloronitrobenzene (10.0g, 0.0521mole) dissolved in 10 ml of dry dimethyl formamide at the same temperature (70°C). After addition the reaction mixture was refluxed for 6 – 8hrs. Excess of dimethyl formamide was removed under reduced pressure. The residue was poured into 100g of crushed ice. Resultant solid was filtered, washed with water. It was then recrystallised from methanol.

Yield : 13.40g (81.7%)

M. P. --- 84°C.

Lit.⁶³ M. P. --- 85°C.

2. Preparation of 2, 4, 4' – trichloro – 2' – aminodiphenyl ether (TADE).

In a pressure reactor vessel equipped with a water condenser, mechanical stirrer was placed 2, 4, 4' – trichloro – 2' – nitrodiphenyl ether (10g, 0.034mole) in 50 ml methanol and activated Raney nickel (8.5%). The pressure flask was first flushed with nitrogen and then hydrogen gas was passed in the flask upto 60 PSI. The reaction mixture was heated upto 50 – 60°C with stirring for 2 hrs. The reaction mixture was allowed to cool down to room temperature and Raney nickel was filtered out. The Raney nickel was decomposed with 10% dil. HCl. The filtrate was concentrated under reduced pressure to get the product TADE.

Yield : 7.97g (88%).

3. Preparation and purification of 2, 4, 4' – trichloro – 2' – hydroxydiphenyl ether.

In a 100 ml beaker was placed 2, 4, 4' – trichloro – 2' – aminodiphenyl ether (10g, 0.034mole) dissolved in a mixture of conc. H_2SO_4 (15ml) and water (20ml). Then the reaction mass was cooled to 0°C in an ice bath and a cold aqueous solution of sodium nitrite (2.66g, 0.038mole) was added dropwise within 15 – 20 min. Upon completion of the diazotization the diazonium salt solution gives positive starch iodide paper test.

In a 250 ml three necked round bottomed flask equipped with a water condenser, dropping funnel was placed with 20 ml water and 22.5 ml conc. H_2SO_4 . The mixture is heated to $175 - 180^\circ\text{C}$. Water is permitted to distil through the condenser so that the temperature can be maintained. The diazotization mass was charged from the dropping funnel into the flask slowly around one hour, while the mixture was maintained at $175 - 180^\circ\text{C}$. Upon completion of the addition the mixture is held until hydrolysis is complete by the negative diazo spot test. The completely hydrolysed mixture was cooled to $100 - 110^\circ\text{C}$ and the resultant layers are permitted to settle out. The top oily layer consisting of desired material was separated from the bottom spent acid layer.

This oily layer was neutralised with 10% NaOH solution and 5 – 8 ml of tetrachloroethylene (TCE) was added and is stirred for 15 min, until the phases separate. The bottom TCE/ furan layer split from (top) aqueous product layer. The aqueous product layer was extracted with washings of TCE to remove non-phenolic organic impurities. The aqueous layer was neutralised to pH 6-7 by 10% hydrochloric acid and extracted with dichloromethane which was removed under reduced pressure to get triclosan.

Yield : 3.15g (32%)

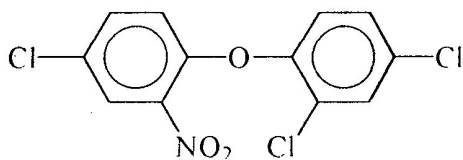
M. P. --- 54°C .

Lit.¹³³ M. P. --- 54 to 56°C .

Elemental analysis of $\text{C}_{12}\text{H}_7\text{O}_2\text{Cl}_3$

	C%	H%
Calculated	49.74	2.41
Found	49.46	2.63

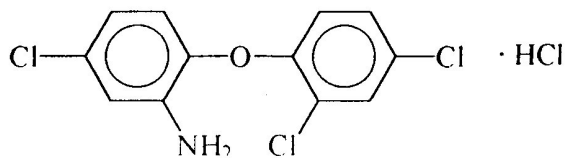
Spectral Studies of 2,4,4'-trichloro-2'-nitro diphenyl ether.



IR (KBr) cm^{-1}

3086	(C-H stretching of benzene ring)***
1600, 1469	(C=C, skeletal vibration)
1525	(C-NO ₂ , aromatic, asym)
1344	(C-NO ₂ , aromatic, sym)
1259	(C-O stretching, diarylether)
1051, 1095	(C-H in plane deformation)
807, 870	(C-H out of plane deformation of benzene ring; two adjacent H atoms, one H atom)

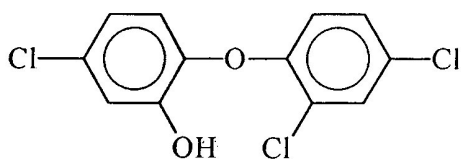
Spectral studies of 2,4,4'-trichloro-2'-amino hydrochloride diphenyl ether.



IR (KBr) cm^{-1}

3424	(N-H stretching, aromatic amine)
2843, 2574	(NH ₃ ⁺ Cl ⁻ amine hydrochloride)
1625	(NH deformation, primary amine)
1469	(C=C, skeletal vibration)
1319	(C-N stretching of amine)
1264	(C-O stretching of diarylether)
1095, 1051	(C-H in plane deformation)
843, 870	(C-H out of plane deformation of benzene; two adjacent H atoms, one H atom)
747	(C-Cl stretching)

Spectral Studies of 2,4,4'-trichloro-2'-hydroxy diphenyl ether



IR (KBr) cm^{-1}

3311

(O-H stretching)

1600, 1469

(C=C, skeletal vibration)

1347

(O-H, deformation of phenol)

1232

(C-O stretching of diaryl ether)

1182

(C-O stretching of phenol)

1051

(C-H in plane deformation)

822, 909

(C-H out of plane deformation of benzene;
two adjacent H atoms, one H atom)

745

(C-Cl stretching)

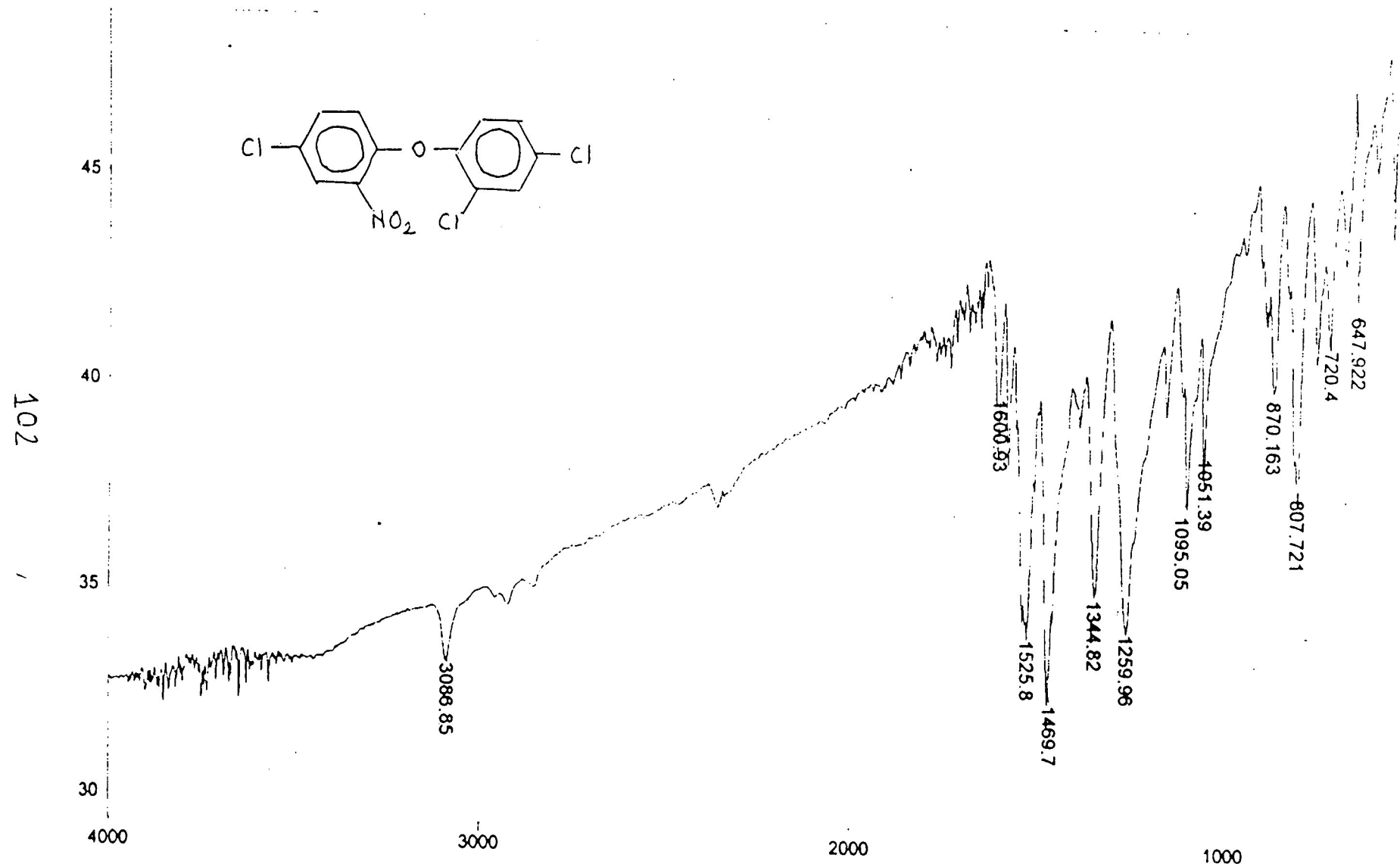
Sample Description: diphenylether

Scans= 10 Slow

Res=4 cm-1

Apod= Cosine

Zero Filling= 1 x



Transmittance / Wavenumber (cm-1)

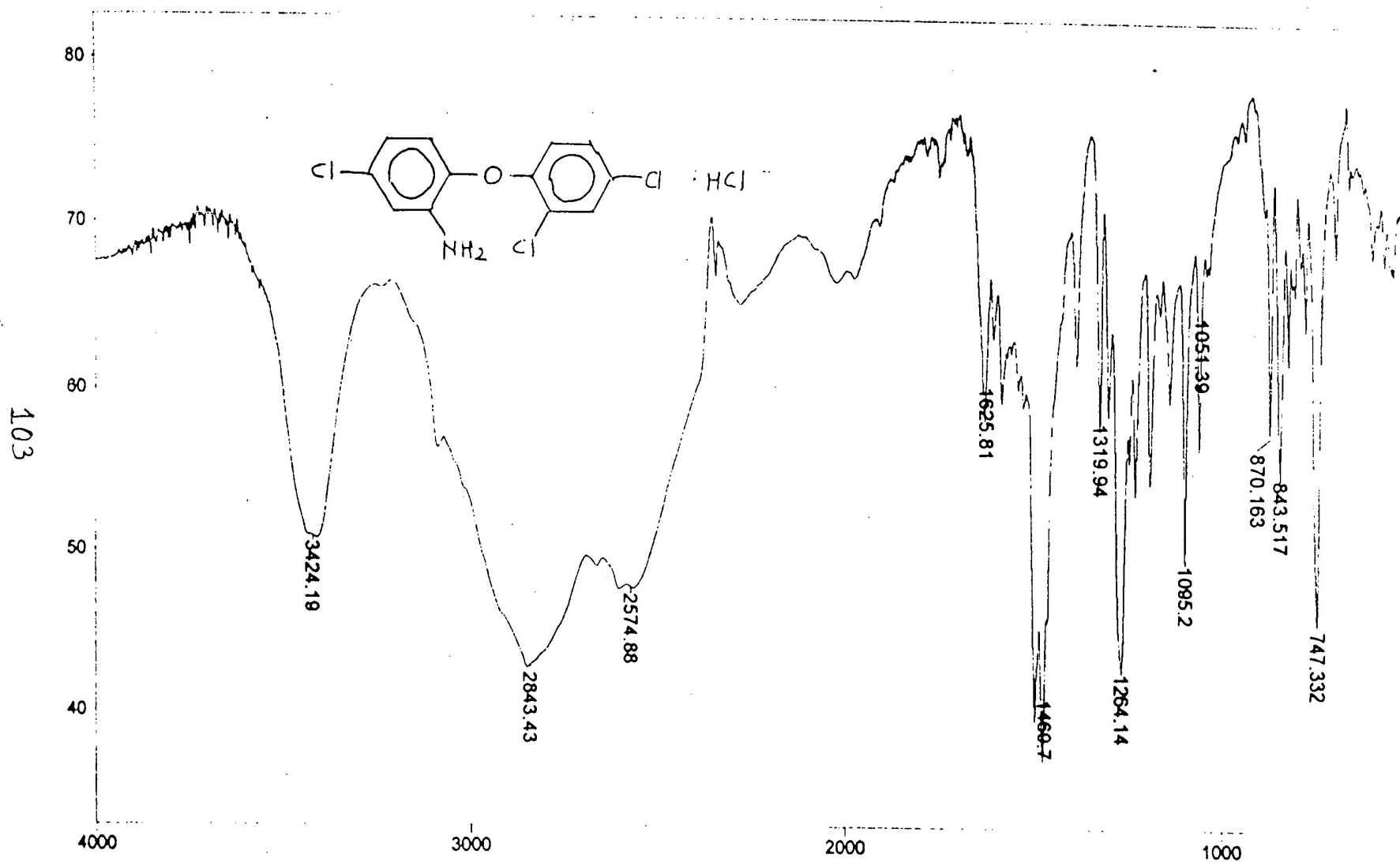
Sample Description: diphenyletheraminohydrochloride

Scans= 10 Slow

Res=4 cm-1

Apod= Cosine

Zero Filling= 1 x



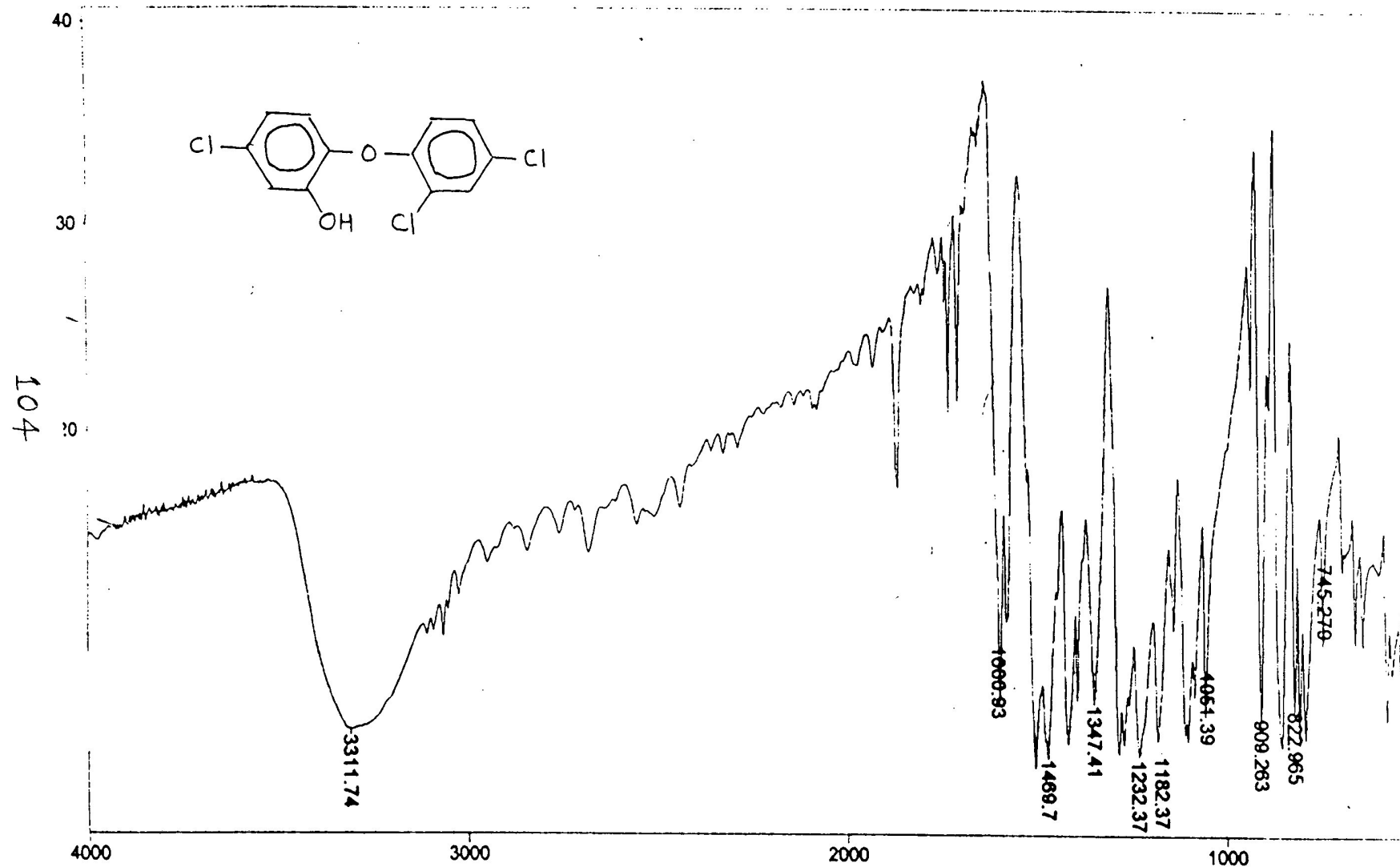
* Description: triclosan

10 Slow

Res=4 cm⁻¹

Apod= Cosine

Zero Filling= 1 x



PART V

BIOLOGICAL RESULTS AND
DISCUSSIONS

BIOLOGICAL RESULTS AND DISCUSSIONS

The compounds synthesized in the present investigation have been subjected to various biological screening programme based on their structural features so as to ascertain their suitability as potential chemotherapeutic agents.

The various screening program carried out have been in the *in vitro* study against various species of Gram-positive and Gram-negative bacteria. viz. *Staphylococcus aureus* ATCC 3750 (*S. aureus*) and *Salmonella typhi* NCTC 786 (*S. typhi*) for estimating antimicrobial activity. Literature studies have revealed that compounds from Part I may have antimalarial activity. In view of this finding we have evaluated our compounds for their antimalarial activity using rodent malaria model with rodent malarial strain of *P. berghei* in mice *in vivo*.

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

Antimalarial Screening

Chemotherapeutic research is closely associated with the development of adequate screening methods which guide the workers for future investigations.

The first isolation of the rodent malaria was reported by Vincke and Lips¹³⁴. Thereafter many species and subspecies of rodent malaria have been isolated and divided into two groups, the berghie group and the Vinckei group. The group used for the majority of chemotherapy research is the berghei group.

Therefore, ten compounds were screened for their antimalarial activity employing rodent malarial strain of *Plasmodium berghei* in mice *in vivo* by Rane's Preliminary Rapid Screening Method¹³⁵. Pyrimethamine was used as the standard drug for comparison.

Advantages of the Rodent Model :

The rodent plasmodium is considered to be the most important tool in the primary phase of the search for better antimalarial drugs. Its unparalleled simplicity and convenience helps in the screening of a large number of compounds. *P. berghei* infections in the mouse can suggest ways of administering new agents most effectively at relatively low costs and can determine the value of a new agent in the face of established resistance to existing drugs.

To test the blood schizonticidal activity in the rodent malaria system, two tests were mentioned in a report by a W.H.O. Scientific group¹³⁶ (W.H.O., 1973). The tests were :-

- a) Evaluation of activity by assessment of survival time : Rane test for blood schizonticidal screening².
- b) Evaluation of activity by means of parasite counts : The 4-Day Suppressive test using *P. berghei* (in swiss mice) modified from Thurston¹³⁷.

Susceptibility of *P. berghei* to different hosts :

Satyaprakash et al.¹³⁸ investigated the susceptibility of various animals to blood induced infection with *P. berghei*. The animals like monkeys, rabbits, guinea, pigs, cats, etc. were investigated. The authors demonstrated that none of the animals inoculated with *P. berghei* developed the infection. The infectivity of *P. berghei* to several other animals was studied by various workers during 1950's and the animals found susceptible were golden hamster, gerbil, bat and squirrel. The infectivity of *P. berghei* to the rodents like mice and rats were found susceptible was a breakthrough for antimalarial screening.

The *P. berghei* infection in laboratory mouse is the most economically and feasible approach since it performs as a primary and secondary screen before monkey malaria screens. Hence the *P. berghei* strain maintained in Haffkine Institute was used for this study.

Rane's Preliminary Rapid Screening Method

This system is based on the use of mice inoculated intraperitoneally with a asexual blood parasites, and treated three days latter with one subcutaneous injection of the test compound. Antimalarial activity is assessed by comparing survival times of treated mice to infected, nontreated controls. All mice are albino type, breed in Haffkine laboratory. Test male weigh from 18-20g and are all approximately of same age. Each mouse recieves about 1×10^6 parasitized erythrocytes drawn from donor mice infected four days earlier with *P. berghei*. Test compounds are dissolved or suspended in Tween-80 and distilled water. Deaths that occur before the sixth day, when infected, untreated controls begin to die, are regarded as toxic deaths. Each compound is administered initially in four graded doses diluted fourfolds into groups of five mice per dose level. A control group of 5 mice which was not administered any drug was kept as untreated control. In each test pyrimethamine was used as the standard for comparison. The dose at which double the survival time was attained was considered the Minimum Effective Dose (M.E.D.). Dose range selected was 160, 80, 40 and 20 mg/kg. Mice alive after 40days after infection are considered cured.

Observations and Results :

1. Untreated infected animals died within 6 to 7 days average. The survival time of these controls varied with every batch of experiment.
2. The animals administered with the drug and did not recover from the infection were observed till death.
3. The drug which enhanced the survival time (twice the period of untreated control mice) was considered to be active.
4. The smallest dose at which the survival time was doubled was considered Minimum Effective Dose (MED).
5. Deaths within 48 hrs after administration of compounds were considered toxic (T).

Antibacterial Screening

The test compounds have been subjected to *in vitro* screening by the tube dilution technique¹³⁹ against various species of Gram-positive and Gram-negative bacteria viz. *S. aureus*, and *S. typhi* using Mueller-Hinton broth as the culture medium.

Screening Procedure

Sterile Mueller-Hinton broth (4.35 ml) was dispersed in each borosilicate test tube (150 X 20 mm). The test compounds (20 µg) were dissolved in a suitable solvent (10ml) such as dimethyl formamide (DMF), dilute HCl, ethanol as the case may be.

The serial drug dilution were made in such a way as to give various final drug concentrations of 200, 100, 50, 25, 12.5, 6.25 µg/ml. The inoculum of standard suspension (0.1 ml) of the desired bacterial strain (*S. aureus* or *S. typhi*) containing 10⁶ bacilli / ml was added at the end. The tubes were incubated at 37°C for 24 hrs. 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).

Control tubes with ampicillin as standard drug and tubes without any drug were kept for comparison. Also the solvents used for dissolving the substances under study were similarly tested to see that they did not affect the growth of the culture at the concentrations used under these experimental conditions. Ampicillin inhibited the growth of *S. aureus* and *S. typhi* at concentrations of 0.5 and 0.4 µg/ml respectively.

Antifungal Screening

The organisms employed in the test were 1) *Candida albicans* 2) *Trichophyton rubrum* 3) *Trichophyton mentagrophytes*. These organisms were maintained on Sabouraud's agar slants by regular subculturing. *C. albicans* was cultured every week and the rest of the organisms at the interval of 12 days.

Test Medium

The liquid medium described by E. Joan Stokes et.al¹⁴⁰ was used for screening the compounds for their antifungal activity. The final pH of the medium was adjusted to 5.2. This test medium was dispensed in requisite varying from 4.4 to 4.85 ml in sterile tubes. To these were added the requisite test solution in graded concentrations varying from 200 to 6.25 mg / ml.

Preparation of innoculum

Candida albicans

A 48 hours old growth on Sabouraud's agar slant was suspended in 4-5 ml of the test medium with the help of platinum loop. The cell count of suspension (yeast phase) was carried out using Neubaur chamber and then diluted with requisite volume of the medium to give a final concentration of 5×10^6 cells per ml.

T.rubrum and T. mentagrophytes

To a 12 to 14 days old growth on Sabouraud's agar slant, 5 ml of sterile test medium and 3-4 sterile glass beads (3 mm diameter) were added and gently shaken for 10 minutes. The supernatant was decanted into another sterile tube and the count of spores in the suspension was carried out using Neubauer chamber. Final strength of the innoculum was adjusted to 5×10^6 spores per ml, using test medium.

Screening Procedure

To each test was then added 0.1 ml of standardized innoculum of the test organism. The tubes were then incubated at room temperature (28-30°C) in a dark place. The presence or absence of growth was noted visually after 7 days in case of *Candida albicans* and after 14 days in case of *T. rubrum* and *T. mentagrophytes*.

Miconazole was used as standard drug for control which have MIC of 6.25µg/ml for *C. albicans* and 1.56µg/ml for *T. mentagrophytes* and *T.rubrum*. Tubes without any drug were also kept for comparison.

RESULTS AND DISCUSSION

PART I :

METHYL 5 – (SUBSTITUED THIO) – 2 – BENLIMIDAZOLE CARBAMATE

Antibacterial and Antifungal screening

In this series 10 compounds have been synthesized. Most of the compounds in these series were found to have moderate activity. All the compounds were found to be moderately active at the concentration of 100 µg / ml against *S. aureus* and *S. typhi*. (Table 5).

The antifungal testing results shows good pattern as compared to the antibacterial screening. Most of the compounds i.e. compounds no. 1,2,3,4,9,10 (Table 5) in these series were found to be inactive against *C. albicans* and compound no. 5,6,7,8 showed moderate activity at the concentration of 200 µg / ml against *C. albicans*.

Also, compounds no. 1,3,4,6,7,8,9,10 showed moderate activity at the concentration of 100 µg / ml whereas compound no. 2 and 5 were found to be active at the concentration of 50 µg/ml against *T. rubrum* respectively.

Also, compound no. 1, 4, 5, 6, 7, 8, 9 showed moderate activity at the concentration of 100 µg/ml against *T. mentagrophytes* whereas compound no. 2,3,10 shows activity at the concentration of 50 µg/ml.

Antimalarial Screening

About 10 compounds were screened against *Plasmodium berghei* in mice for antimalarial activity (Table 6). The dose is mentioned in mg/kg. These compounds have been screened by Rane's test, out of which two compounds were inactive and other compounds showed activity in the range of 40 – 160 mg/kg. The test compound no. 7 methyl 5 – (1, 3, 4 – triazole –2' – thio) – 2 – benzimidazole carbamate had shown good antimalarial activity at the concentration of 40 mg/kg. Also, compound no. 3, 6, 9, 10, showed moderate activity at the concentration of 80 mg/kg against *Plasmodium berghei*. Compound no. 4 and 5 showed activity at the concentration of 160 mg/kg against *Plasmodium berghei*.

PART II :

5 – METHOXY – 2 – (SUBSTITUTED THIOCARBAMOYL) BENZIMIDAZOLE

Antibacterial and Antifungal Screening

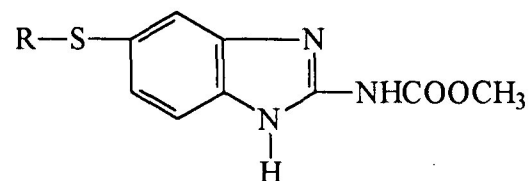
In this series totally ten compounds were screened against the bacterial species of *S. aureus* and *S. typhi*. The results once again were very discouraging. All the compounds have shown moderate activity at the concentration of 100 µg/ml against both *S. aureus* and *S. typhi* (Table 7) Only compound no. 4 and 6 has shown activity at 50 µg / ml for *S. typhi*.

All these compounds were subjected for antifungal activity and the results were somewhat encouraging as compared to the bacterial screening (Table 7) Two compounds (compound no.1 & 2) had shown no activity against any fungal species. Compound no. 3, 10 had shown moderate activity at the concentration of 100 µg / ml against all the fungal species i.e. *C. albicans*, *T. rubrum*, *T. mentagrophytes*. Two compounds no.5 and 9 had shown activity against all three fungal species at 50 µg / ml. Rest of compounds have shown activity at 200 µg / ml.

Above results shows that electron withdrawing group enhances the activity of the parent moiety against fungal species.

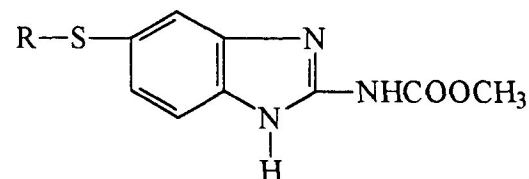
**LIBRARY
HAFFKINE INSTITUTE
MUMBAI.**

Table : 5
Antimicrobial Screening of methyl-5-(Substituted thio) benzimidazole carbamate



Sr. No.	R	Antibacterial		Antifungal		
		S. aureus	S. typhi	C. albicans	T. mentagrophytes	T. rubrum
1	2-mercapto-5-methyl thiadiazole	100	100	-	100	100
2	2-mercaptobenzimidazole	100	100	-	50	50
3	2-mercapto-imidazole	100	100	-	100	50
4	2--mercapto-5-thio-(2'methylcarbamate benzimidazolyl) thiadiazole	100	100	-	100	100
5	2-mercapto-benzoxazole	100	100	200	50	100
6	2-mercaptoisoquinazolinone	100	100	200	100	100
7	2-mercapto-1,3,4,-triazole	100	100	200	100	100
8	2-mercapto-quinoxaline	100	100	200	100	100
9	2-mercapto-benzothiazole	100	100	-	100	100
10	5-methoxy-2-mercaptobenzimidazole	100	100	-	100	50

Table : 6
Antimalarial Screening of methyl-5-(Substituted thio) benzimidazole carbamate



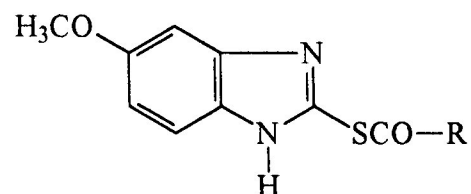
Sr. No.	R	Mean survival time (in days)				Remark
		20	40	80	160	
1	2-mercapto-5-methyl thiadiazole	5.4	7.8	8.6	10.2	Inactive
2	2-mercaptobenzimidazole	4.8	4.8	6.4	8.2	Inactive
3	2-mercapto-imidazole	8.4	10.2	12.4	12.8	Active at 80 and 160
4	2--mercapto-5-thio-(2'methylcarbamate benzimidazolyl) thiadiazole	4.4	6.4	8.8	11.10	Active at 160
5	2-mercapto-benzoxazole	7.0	8.4	9.0	12.0	Active at 160
6	2-mercaptoisoquinazolinone	6.2	8.8	11.4	12.2	Active at 80 and 160
7	2-mercapto-1,3,4,-triazole	8.6	11.2	12.4	13.8	Active at 40, 80 and 160
8	2-mercapto-quinoxaline	7.4	8.2	10.0	11.4	Active at 160
9	2-mercapto-benzothiazole	8.8	10.0	10.6	11.4	Active at 80 and 160
10	5-methoxy-2-mercaptobenzimidazole	8.2	9.8	11.8	12.6	Active at 80 and 160

The standard drug used was Pyrimethamine, having activity at 20 mg/kg.

Sex - Male, Wt, of mice 18-20 g; No. of mice per group - 5

Mean survival time of control- 5.4 days.

Table : 7
Antimicrobial Screening of 5-methoxy-2-substituted thio carbamoyl benzimidazole



Sr. No.	R	Antibacterial		Antifungal		
		S. aureus	S. typhi	C. albicans	T. mentagrophytes	T. rubrum
1	2-fluorophenyl	100	100	-	-	-
2	2,6-difluorophenyl	100	100	-	-	-
3	4-methoxyphenyl	100	100	100	100	100
4	4-chlorophenyl	100	50	200	200	200
5	3,4-dichlorophenyl	100	100	50	50	50
6	4-fluorophenyl	100	50	100	200	200
7	Morpholine	100	100	-	100	-
8	4-cyanophenyl	100	100	200	200	-
9	Cyclopropane	100	100	50	50	50
10	3,4-dimethoxyphenyl	100	100	100	100	100

‘ - ’ = Inactive at 200 µg/ml

CONCLUSION

CONCLUSION

In the present investigation, novel heterocyclic molecules have been synthesized which have been screened for their possible therapeutic value. To evaluate their efficacy, they were screened for their *in vitro* antibacterial and antifungal activity.

One of the series i.e. Part II has also been subjected to *in vivo* antimalarial activity. In Part II the synthesis of methyl 5 – (substituted thio) benzimidazole carbamate has been described and to evaluate their efficacy, they were screened for their *in vitro* antibacterial and antifungal activity and also *in vivo* antimalarial activity, the results of which are given in Tables 5 and 6. The antimicrobial activity of this series revealed that :

- 1) All compounds having carbamate linkage -NHCOOCH_3 at two position of benzimidazole with 5 substituted thio compounds had shown moderate activity against the bacterial strains i.e. both *S. aureus* and *S. typhi*.
- 2) Also all compounds in this series had shown moderate activity against the fungal strains i.e. *C. albicans*, *T. rubrum*, *T. mentagrophytes*. The antifungal activity is imparted to some compounds as an effect of substitution of heterocyclic moieties like benzimidazole, benzoxazole at the 5 – position of benzimidazole carbamates.

Hence, Methyl carbamate linkage have not shown any significant increase in antibacterial and antifungal activity.

- 3) Carbamate linkage at benzimidazoles have produced biologically active anthelmintics such as albendazole, mebendazole, flubendazole, oxibendazole, etc. Recently, Albendazole having carbamate linkage -NHCOOCH_3 at the 2- position had reported antimalarial activity. Also, the incorporation of carbamate linkage -NHCOOCH_3 at the two position of our basic moiety i.e. benzimidazole moiety imparts good antimalarial activity. It was also found that substitution at position 5 of the benzimidazole moiety by a 2- mercapto triazole nucleus imparts good antimalarial activity.

In Part III the synthesis of 5 – methoxy – 2 – (thiocarbamoyl substituted) benzimidazole has been described and to evaluate their efficacy, they were screened for their *in vitro* antibacterial and antifungal activity. The results of which are given in Table 7. From various antimicrobial screening following observations are made :

- 1) All compounds have moderate activity against the bacterial strains.
- 2) The antifungal activity is favored by the presence of electron withdrawing group in the phenyl ring.
- 3) The thiocarbamoyl linkage at the 2 – position of benzimidazole moiety imparts better antifungal activity rather than antibacterial activity.

Though it may be concluded that the present investigation is not successful in rendering any new organic compound more active than the existing drugs in the class of infections for which these compounds were screened, we conclude that benzimidazole carbamates can be considered as a ideal lead compound for antimalarial screening and needs extensive and vigorous study for the development of more active analogs.

Hence, it may be worth while to pursue 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.

REFERENCES

REFERENCES

1. Foye, William O., 'Principles of Medicinal Chemistry', 4th Ed., p 1.
2. Dogmagk G, G, **Deut. Med. Wochensehr**, **61**, (1931) 250.
3. Mietzsch F and Klarer J., **Ger Pat. 607537** (1935) Cited from **Chem.Abstr.** **29** : 4135 (1935).
4. Chen, Y. L., **J. Antibiot.**, **44**, (1991) 970.
5. Jaffe, A., Chabbert, Y.A., Semonin, O., **Antimicrobial agents and chemotherapy**, **22**, (1982) 942.
6. Malouin, F., Bryan, L.E., **Antimicrobial Agents and chemotherapy**, **30**, (1986) 1
7. Lyman, C. A., Walsh, T. J., **Drugs**, **44**, (1992) 9.
8. Kovacs, J. A., Koracs, A. A., Blis, M., Wright, W. C., Gill, V. J., Tuazon, C. V., **Ann. Intern. Med.**, **103**, (1964) 533.
9. Asaad, Falmy, M., **Egypt J. Chem.**, **32**, (3) 1989, (Pub.1991), 367 –74 (Eng)
10. Kerridge, D., **Advances in Microbiological physiology**, **27**, (1986) 1.
11. Grove, J. F., Ismay, D., Macmillan, J., Mulhottan, TPC., Rogers, Mat., **Chem. Ind.**, **11**, (1951) 219.
12. Hazen, E. L., Brown, R., **Science**, (1950) 423.
13. Grunberg, E., Titsworth, E., Bennet, M., **Antimicrobial agents and chemotherapy**, **3**, (1964) 566.
14. Campbell Ah, **Experimental Chemotherapy**, Vol. 3, Schnitzer RJ, Hawking F. Eds. Academic press, New York (1964).
15. Brown R, **Experimental Chemotherapy**, Vol. 3, Schnitzer RJ, Hawking F. Eds. Academic press, New York (1964).
16. Rippon J W, **Medical Mycology Suanders**, Philadelphia (1974) 531.
17. Leavell UW Jr. **The Diagnosis and treatment of Fungal infections**, Robinson HM Ed, Thomas Springfield, 111, (1974) 89.
18. Weinberg ED, **Annals of New York Academy of Sciences**, **148**, (1968) 587.
19. Plempel M, Bartmann K, Buchel K H, Rogel E, **Post Grad Med. J.** **50**, (1974) 11.

20. Thienpont D, Van Cj, Nveten JM, Niemegur CJE, Marsboom R, **Arzneimittel Forschung**, **25**, (1975) 224.
21. Godefroi EF, Heeres J, Van Cj, **J. med. Chem.**, **12**, (1969) 784.
22. Welch TJ, McCully C, Rinaldi MG, Wallace JC, Babs FM, **Antimicrobial agents and chemotherapy**, **34**, (1990) 1281.
23. Warnick DW, **J. Antimicrobial and chemotherapy**, **24**, (1989) 275.
24. E. Bottius, A. Guanzirolli, J.-F. Trape, C. Rogier, L. Konate, and P. Druilhe, **Trans. R. Soc. Trop. Med. Hyg.**, **90**, (1996) 15-19. Cited from **Burger's Medicinal Chemistry and Drug Discovery**, Vol. 5, 5th Ed. p 5 edited by Manfred E. Wolff.
25. White N J, **Lancet**, **341**, (1993) 793.
26. S. H. Quari, Y.-P. Shi, I. F. Goldman, V. Udhaykumar, M. P. Alpers, **Lancet**, **341**, 7(1993) 780-783..
27. R. E. Desjardins, C. J. Canfield, D. E. Haynes, and J. D. Chulay, **Antimicrobial Agents and Chemotherapy**, **16**, (1979) 710-718.
28. W. K. Milhous, N. F. Weatherley, J. F. Bowdre, and R. E. Desjardins, **Antimicrobial Agents and Chemotherapy**, **27**, (1985) 525-530.
29. W. H. Wernsdorfer and D. Payne, **Pharmacology and Therapeutics**, **50**, (1991) 95-121.
30. R. B. Woodward and W. E. Doering, **J. Am. Chem. Soc.**, **67**, (1945) 860-874.
31. R. B. Woodward and W. E. Doering, **J. Am. Chem. Soc.**, **66**, (1944) 849.
32. B. Cenni and B. Betschart, **Chemotherapy (Basel)**, **41**, (1995) 153-158.
33. L. H. Schmidt, S. Alexander, L. Allen and J. Rasco, **Antimicrobial Agents and Chemotherapy**, **12**, (1977) 51-60.
34. Werbel LN, Curry A, Elslager EF, and Hess C., **J. Het. Chem.**, **10**, (1973) 363-82.
35. Vida J A., **Tetrahedron Letters**, (1972) 3921.
36. Rovnyah, G., Narayanan, V. H., Naugwitz, R. D., and Cimarusti, C. M., Cited from **Chem. Abstr.** **84** : (1976) 105596m.
37. **Chimetron**, Fr. Patent, 1,450,670, Aug26, 1966, Cited from **Chem. Abstr.** **67** : (1967) 2192x.

38. Shotaro, Nakajima, Ichiro Tanaka, Teniya Seki, and Toshio Anmo, **Yakugaku Zasshi.**, **78**, (1958) 1378-82, Cited from **Chem. Abstr.** **53** : (1959) 8124b.
39. Balio, Alessandro, **Ricerca Sci.**, **20**, (1950) 1268.
40. H. D. Brown, A. R. Matzuk, I. R. Ilves, L. H. Peterson, S. A. Harris, L. H. Sarett, J. R. Egerton, J. J. Yakstis, W. C. Campbell and A. C. Cuckler, **J. Am. Chem. Soc.** **83**, (1961) 1764.
41. Syed Abuzar, K V B Rao, Satyavan Sharma, Suman Gupta and J. C. Katyar, **Indian Journal of Chemistry Vol 24B**, February 1983, p 178 – 181.
42. Ram S, Wise D C, Wotring L L, Mc Call J W, Townsend L B, **J. Med. Chem.**, **35** (1992) p 539.
43. D Walker and D Knight, **Vet. Rec.** **90**, (1972) 58.
44. D. Duwel, **Pestic. Sci.** **8**, (1977) 550.
45. Smith Kline and French Laboratories : **Brit. Pat.** **1, 123, 317** (1968), Cited from **Chem. Abstr.** **69** : (1968) 96722k.
46. P. Actor, E. L. Anderson, C. J. Dicuollo, R. J. Feralauto, J. R. E. Hoover, J. F. Pagano, L. R. Ravin, S. F. Scheidy, R. J. Stedman and V. J. Theodorides : **Nature (London)** **215**, (1967) 321.
47. V. J. Theodorides, R. J. Gyurik, W. D. Kingsbury and R. C. Parish, **Experientia**, **32**, (1976) 702.
48. Dieckmann – Schuppert – A ; Franklin – RM, **Cell – Biol – Int – Rep.** **1989 May ; 13 (5)** : 411 – 8.
49. Pfaller, N. A. and Krogstad, D. J., **Antimicrobial Agents and Chemotherapy**, **92**, (1982) 917-919.
50. Subhash Chandra, P. K. Das, G. P. Dutta, G.D.P. Dutta, Syed Abuzar and Satyavan Sharma, **Indian Journal of Malariology**, **Vol. 24**, June 1987, p 17–19.
51. Wolley. **J. Biol. Chem.**, **152** (1944).
52. Bossche, H. V., Den, Rochette, F., and Horig, C., **Adv. Pharmacol. Chemotherp.**, **19**, 67 (1982).
53. Van Allan, Deacon, **Org. Synth. Coll.**, **IV**, 569 (1963).

54. Adams, Charles Dewitt, Schlatter Rudolph., **Ger. Offen., 1,956,157** (1970),
Cited from **Chem. Abstr. 73** : (1970) p 3536.
55. Lozanova K H, Vasiler G, Kalcheva V, (Inst. Plant Physiol., 113, Sofia Bulg.)
Doki Bulg. Akaad, Nauk, 44 (9), (1991) 115-8 (Engl.), Cited from **Chem. Abstr., 116** : (1992) 235516q.
56. Aries Robert **Fr. 1,509,192**, Cited from **Chem. Abstr., 70** : (1969) 57842q.
57. Asaad, Falmy M, **Egypt J. Chem., 32, (3)** 1989, 367-74 (Eng.).
58. Sanjay D. Palav, Barsu G. Khadse and Ms. V. R. Kamath, **Indian Drugs, 34, (7)**, July 1997. P 407-409.
59. 'The Merck Index', **12th Ed.**, Edited by Budhavari S, Mary Adele, J. O. Neil, Smith A, Heckelman P E, Goanne F, Kennary, p 6977.
60. 'Remingtons Pharmaceutical Sciences' **18th Ed.**, p 1173, Mack publishing company Easton, Pennsylvania 18042.
61. 'Martindale', The Extra Pharmacopoeia, **12th Ed.**, Edited by E. F. James, Reynolds., p 805.3.
62. William Foye, Thomas, L. Lemke., 'Principles of Medicinal Chemistry', **4th Ed.**, p 81
63. W. V. Farvar., **J. Appl. Chem. London, 10, Pt. 5**, 207-13 (1960).., Cited from **Chem. Abstr. 55** : 4420e (1961)..
64. Day, A. R., **Electronic Mechanisms of Organic Reactions**, American Book Co., Newyork (1950), p 242 – 3.
65. Wright, J. B., **Chem. Rev. 48** (1951) p 377 – 541.
66. Hofmann, K., **The Chem. Of Heterocyclic compounds Part I**, Weissbeger, A. Ed., Interscience, N. Y. (1953) p 247.
67. Schipper, E. S. and Day, A. R., **Heterocyclic compounds, Vol. 5**, Elderfield, R. C. Ed., Wiley, N. Y. (1957), p 267, 281 – 84.
68. Pozhanskii, A. F., Garnovskii, A. D. and Simmonov, A. M., **Russ. Chem. Rev., 35** (1966) p 122.
69. Grimmett, M. R., (Med. Tech. Pub. Co.), **Int. Rev. Sci. Org. Chem.**, Seroney (1973)
70. Preston, P. N., **Chem. Rev., 74 (3)** (1974) p 279 – 316.

71. Fischer, O. and Veiel, O, **Ber.**, **38** (1905).
72. Phillips, M. A., **J. Chem. Soc.**, **28** (1929) p 2820.
73. Green, H. and Day, A. R., **J. Am. Chem. Soc.**, **64** (1947) p 1167.
74. McKee, R. L. and Bost, R. W., **J. Am. Chem. Soc.**, **65** (1947) p 471.
75. Sexton, W. A., **J. Chem. Soc.**, (1942), p 303
76. Sinsminov, A. M., Ismail Skill, V. A., **Chem. Abstr.**, **35** (1941) p 2870.
77. Lopeland, R. A. B. and Day, A. R., **J. Am. Chem. Soc.**, **65** (1943) p 1872.
78. Hollegas, E. L. and Wagner, E. C., **J. Org. Chem.**, **9** (1944) p 31 – 49.
79. Wright, J. B., **J. Am. Chem. Soc.**, **71** (1949) p 2035.
80. Widerfield, R. C. and Kreysa, F. J., **J. Am. Chem. Soc.**, **70** (1948) p 44.
81. Kymi, O., **J. Prakt. Chem.**, **75** (2) (1907) p 323.
82. Ministry, S. M. and Guha, P. C., **J. Ind. Chem. Soc.**, **7** (1930) p 793.
83. Omar, A., Hohsen, M. E., Habib, H. S. and Aboulwata, Omaimam, **Synthesis**, (1977), p 864 – 5.
84. Bywater, W. G., McGiverty, D. A. and Jensch, M. D., **J. Pharmacol.**, **85** (1945) 14.
85. Kawaoka, Y., **J. Soc. Chem. Ind. Japan**, **43** (suppl.), (1940) 275 ; cited through : **Chem. Abstr.**, **35** (1941) p 2368
86. Jacobson, P., **Ann.**, **287** (1895) p 150, 190.
87. Fischer, O. and Limner, F., **J. Prakt. Chem.**, **74** (1906) p 60.
88. Dziurzynski, M., **Chem. Zentr.**, **79** (1908) p 948.
89. James, A. J. and Turner, E. E., **J. Chem. Soc.**, (1950) p 1515.
90. Allen, C. F. H., Bell, A. and Wilson, C. V., **J. Am. Chem. Soc.**, **66** (1944) p 835.
91. Kym, O., **J. Prakt. Chem.**, **75** (2) (1907) p 324.
92. Billeter, O. and Steiner, A., **Ber.**, **20** (1887) p 229, 231.
93. Everett, J. G., **J. Chem. Soc.**, (1929) p 677.
94. Cope, A. C., **Org. Synth.**, **30**, John Wiley and Sons, p 56.
95. Fischer, O. and Limmer, F. J., **J. Prakt. Chem.**, **74** (2) (1906) p 60.
96. Lellmann, E., **Ann.**, **228** (1885) p 244.
97. Everett, J. G., **J. Chem. Soc.**, (1930) 2402 – 8.

98. Auwers and Risse., **Ber.** **64**, (1931) p 2220.
99. Hentschel, **Ber.** **17**, (1884) p 1285.
100. Morgen, G. T. and Challenor, W. A., **J. Chem. Soc.**, **119** (1921) p 1537 – 46.
101. Everett, J. G., **J. Chem. Soc.**, (1932) p 3042.
102. Scott, W. and Watt, G. W., **J. Org. Chem.**, **2** (1937) p 152.
103. Dorofienko, G. N. Andreichikov and Trukhan, G eE Khim, Geterotki, Soedin, **1344** (1974), quoted from : Squil and Patai, **The Chemistry of Diazonium and diazogroup, Part II**, p 655.
104. Wilcox, T. H., **J. Am. Dyest. Rept.**, **55**, 891 (1966).
105. Putter, R., George. Thieme Verlag Stuttgart, Hauber- Weyl- Muller, **Methodender Organischem Chemie, Vol X, Part III, 4th ed.**, p 16 (1965).
106. Schank, K., **Methodicum Chemicum**, Vol. 6 (Ed Zymalkowski, F), Academic Press, New York, San Francisco, London p 164 (1975), Georg Thieme Publishers, Stuttgart (1975).
107. Putter, R., George. Thieme Verlag Stuttgart, Hauber- Weyl- Muller, **Methodender Organischem Chemie, Vol X, Part III, 4th ed.**, p 22 (1965).
108.
 - i. Matrka, Z. M, Sagner, Z., Chmatal, V. Sterba V and Vesley, **M Coll. Czech., Commum** **32**, 1462(1967).
 - ii. Putter, R., George. Thieme Verlag Stuttgart, Hauber- Weyl- Muller, **Methodender Organischem Chemie, Vol X, Part III, 4th ed.**, p 12 (1965)
109.
 - i. Fierz- David, H. E. and Blangley, L., **Grundlegende Operationen der Farbenchemie**, **8th ed.** Springer Verlag Wien, p 244 (1952).
 - ii. Putter, R., George. Thieme Verlag Stuttgart, Hauber- Weyl- Muller, **Methodender Organischem Chemie, Vol X, Part III, 4th ed.**, p 22 (1965).
110. **Brit. Pat. 741, 228 [C.A., 50, 9913 (1956)] ; Brit. Pat. 736, 568 [C.A., 50, 13097 (1956)].**
111. Putter, R., George. Thieme Verlag Stuttgart, Hauber- Weyl- Muller,

Methodender Organischem Chemie, Vol X, Part III, 4th ed., p 28 (1965).

112. Vystroil and Prokes, **Chem. Listy.**, **46**, 670 (1952), cited from **C. A.** :**48**, 165 (1954).
113. Grammaticakis, P., **Bull. Soc. Chim. France**, 207 (1953).
114. Butler, K. and Patridge, M. W., **J. Chem. Soc.**, 2398, (1959).
115. Smith, H. E., **J. Org. Chem.**, **31**, 684 (1966).
116. Augustin, R. L., **Catalytic Hydrogeneration**, Marcell Dekker, New York (1965), p 91 – 92.
117. Rylander, P. N., **Catalytic Hydrogenation over platinum metals**, Academic press, New York, p 168 – 203 (1967).
118. Allen, C. F. H. and Allan, J. Van, Organisations, **Syn. Coll.**, **Vol. 3**, 63 (1965).
119. Augustin, R. L., **Catalytic Hydrogeneration**, Marcell Dekker, New York (1965), p 152.
120. Brown, H. C. and Sivarankarn. **J. Am. Chem. Soc.**, **84**, 2828 (1962).
121. Augustin, R. L., **Catalytic Hydrogeneration**, Marcell Dekker, New York (1965), p 36.
122. Berson, J. A. and Cohen, T., J Organisations., **Chem.**, **20**, 1461 (1955).
123. Gyurik, R. J., Theodorides, V. J., U S 3,052,103, Cited from **Chem. Abstr.** **84** : 31074r (1976).
124. Haugwitz, Rudiger, D., Cruthers, Larry R., U S 4, 145, 431, Cited from Chem. Abstr. 91: 27301q (1979).
125. Archer, G. A., Ning R. J., Kalish R. J., **J. M. C.** **20**, 1312 (1977).
126. Lednicer, D., **The Org. Chem. Of Drug Synthesis**, **Vol. 2**, p 352.
127. I. Heilbron, and H. M. Bunbury, 'Dictionary of Organic Compounds', **IV**, 2107.
128. A. McCoubrey and W. Webster., **J. Chem. Soc.**, **1948**, p 1719-20.
129. Nakagawa, Satoshi; Fukanaga, Toshihiro, oda, Ryoza, **Jpn. Kokai, Tokkyo Koho J P**, 06, 279, 416, 4th October, 1994, 6 pp.
130. 'Dictionary of Organic Compounds', Chapman & Hall. 6th Edn. **Vol. 4 D-O-05135**.

131. Kennichi, F., Hisao, K., Taichiro, O., Yoshiaki, I., Hiroshi, S., **Nippon Kagaku Zasshi.**, **79**, 1120-4 (1958), Cited from **Chem. Abstr.** **54** : 5379a (1960).
132. I. Heilbron, and H. M. Bunbury, '**Dict. Org. Compds.**,' **I**, 472 (1953).
133. '**The Merck Index**', **12th Ed.**, Edited by Budhavari S, Mary Adele, J. O. Neil, Smith A, Heckelman P E, Goanne F, Kennary, p .
134. Vincke, I. and Lips, M. **Ann. Soc. Belge. Med. Trop.**, **28**, 97 – 104 (1948).
Quoted from : "**Antimalarial Drugs**", Vol. I, p 225 (1986).
135. Peters, W., '**Chemotherapy and Drug Resistance in Malaria Academic**', Newyork, p 64 – 136 (1970).
136. Crowin, H., Bruce, A. H., Zong-ru-guo, Cynthia, D. et al. **J. Med. Chem.**, **27**, p 129 – 143 (1984).
137. Thurston, J. P., **Trans. Roy. Soc. Trop. Med. Hyg.**, **44**, p 703 – 706 (1950).
138. Satyaprakash, Krishnaswami, A. K. and Ramkrishnan, S. P., **Ind. J. Malaria.**, **6**, p 175 – 182 (1952).
139. Koneman, E. V., Janda, W. M., Allen, S. D., Sommers, H. M., Dowell, V. R., Winn, W. C., "**Color Atlas And Textbook Of Diagnostic Microbiology**", J. B. Lippincott Company, **3rd Edn.**, (1988), 487.
140. E. Joan Stokes, **Clinical Bacteriology**, **4th Edn.**, (1975), p 226.

APPENDIX

APPENDIX

1. The melting points have been determined in Open capillaries in a veego VMP – 1 melting point apparatus.
2. The TLC of the compounds have been run on Eastman Kodak precoated silica gel plates of type K 301 R and E. Merck pre – coated silica gel plates of type 60 F 254.
3. The Infrared spectra was recorded on a BOMEM MB – 104 Fourier Transform Infrared Spectrometer.
4. The PMR Spectra were recorded on Brucker AC 80 MHz. Spectrometer and Varian 300 MHz. Spectrometer using TMS as internal reference.

SYNOPSIS

SYNOPSIS

For many centuries the search for therapeutic substances centered on the investigation of herbs grown in the gardens of religious establishment where the sick found the shelter. In the nineteenth century, the advent of analytical and synthetic chemistry signaled an important change in direction in that alkaloids were purified, and simple molecules such as acetylsalicylic acid were synthesised.

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 was, from that time on, to be dominated by medicinal therapeutics. Today, a large number of diseases are cured, or atleast controlled by drug therapy. Thus, the fight against bacterial and fungal infections, has been largely won, and significant progress has been made in treating mental, pulmonary, inflammatory and cardiovascular conditions. In addition, some cancers and certain forms of leukaemia are cured by chemotherapy.

Taken in the prospective sense Medicinal Chemistry relates to the design and production of compounds that can be used in medicine for prevention, treatment or cure of human and animal diseases. Taken in the retrospective sense, Medicinal Chemistry covers three critical steps :

- # A discovery test, consisting of the identification and production of new active substances usually called lead compounds. The lead compounds can originate from synthetic organic chemistry, from natural sources, or from biotechnological processes.
- # An optimization step, that deals mainly with the synthetic modification of the lead structure in order to improve potency, selectivity and lessen toxicity. Its characteristics are establishment and analysis of structure activity relationships.
- # A development step, consisting of the optimization of the synthetic route for bulk production and the modification of the pharmacokinetic and the pharmaceutical properties of the active substance to render it suitable for clinical use.

Although the search for new entities is being increasingly governed by rational and high throughput techniques, serendipitous discoveries do still occur, as in the old days of the medicinal herbs.

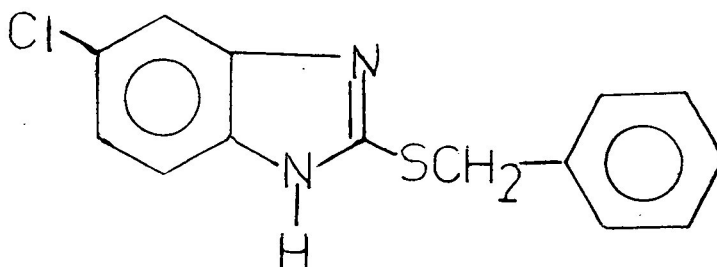
The thesis would consist of 'Introduction', 'Review of Literature' and 'Plan of Work' in PART-1. Further it would be subdivided into different parts as briefly summarised here.

PART- 2

Synthesis of methyl 5- (substituted thio) - 2- benzimidazolecarbamate.

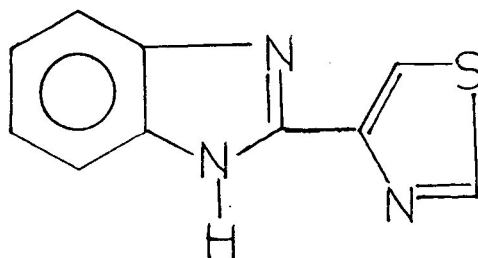
Benzimidazole is one of the oldest and chemically well studied Nitrogen heterocyclics, it has been quite lately recognised as the versatile nucleus for building molecules possessing broad spectrum of biological activity such as powerful anthelmintic, antimicrobial, pesticidal, antimalarial, anticancer and pharmacological activities.

During the literature survey, it was found that 2-mercapto benzimidazole derivatives¹, namely, 5(6)-chloro-2-(benzylthio) benzimidazole (I) possesses antifungal activity against *Trichophyton interdigitale*, at a dilution 1:290,900 or 3.4ug/ml. Similarly, bacteriostatic activity was also reported for 2-mercapto benzimidazole² against *S.aureus*.



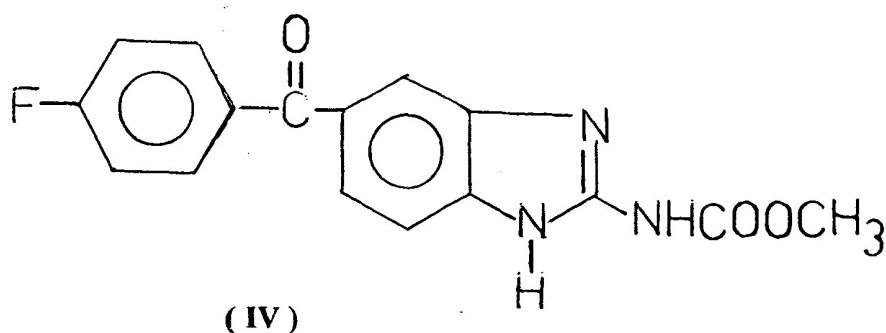
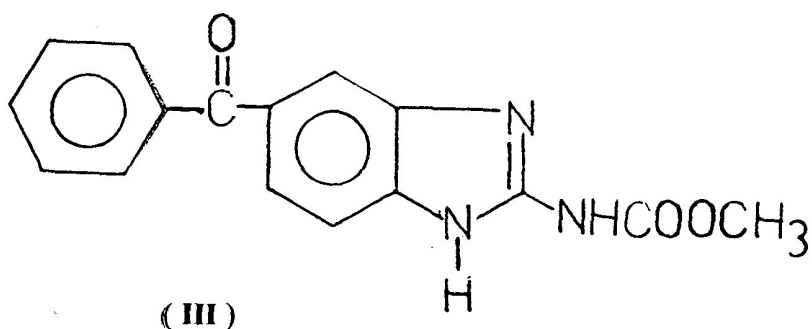
(I)

Ever since the discovery of 2-(4'-thiazolyl) benzimidazole³ (thiabendazole) (II) in 1961 as the first broad spectrum anthelmintic, several thousand benzimidazoles has been prepared but less than twenty have been commercially exploited.



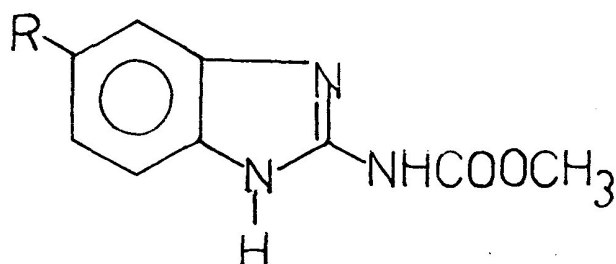
(II)

Alkyl benzimidazole-2-carbamate like Mebendazole⁴ (III) and Flubendazole⁵ (IV) which are broad spectrum anthelmintic containing methoxy carbonyl amino group at the 2-position and the benzoyl group at the 5-position, both are major pharmacophoric requirements for anthelmintic activity.



Taking into account this carbamate linkage, -NHCOOCH_3 , present in the above compounds this linkage was introduced in our compounds.

Smith, Kline and French laboratories have prepared a series of 5(6)-alkyl, aryl and alkoxybenzimidazole-2-carbamates with marked anthelmintic activity like Oxibendazole⁶ (V) and Parbendazole⁷ (VII).

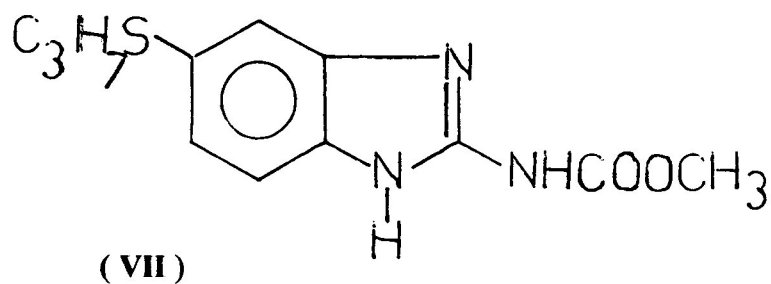


(V) $\text{R} = \text{OPr}$

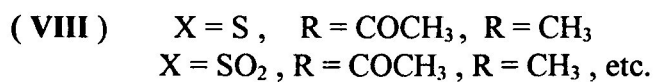
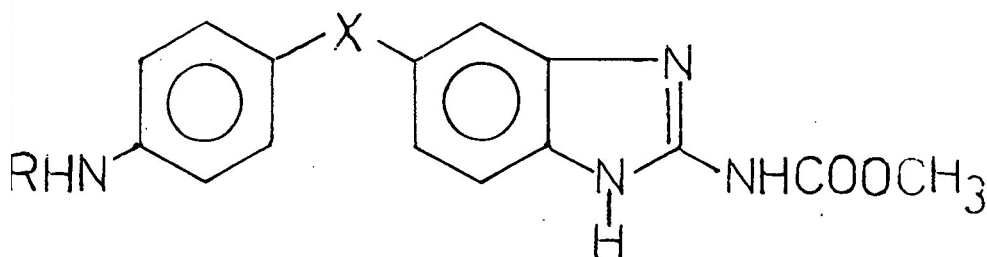
(VI) $\text{R} = \text{n-Bu}$

Albendazole (VII) is a benzimidazole anthelmintic possessing broad spectrum activity against gastrointestinal, round worms, tape worms, lung worms and liver flukes⁸. It has been demonstrated to possess high activity against *A. caninum* in dogs at a dose of 50mg/kg given for three days⁹. The sheep carrying moniezia infection are cured to 100% by Albendazole at a dose of 10mg/kg.

Recently, Albendazole has been reported to possess antimalarial activity¹⁰.

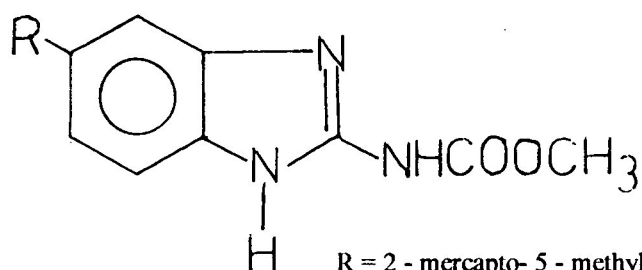


Also, Subhash Chandra and Satyavan Sharma¹¹ had carried out antimalarial activity of Alkyl 5(6)(4-aminophenyl) sulphonyl benzimidazole-2-carbamate (VIII).



Thus, considering the importance of 2,5(6)disubstituted benzimidazoles as a ideal antiparasitic agent, Synthesis of such compounds was made. Also some of the compounds showed structural resemblance with 4,4-diamino diphenyl sulphone, a potent antimalarial.

This prompted us to evaluate the,antibacterial,antifungal and antimalarial efficacy of 5-(substituted thio)- 2- benzimidazolecarbamate (IX).



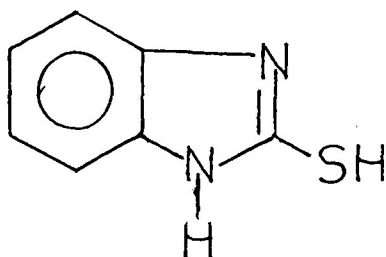
(IX)

R = 2 - mercapto- 5 - methyl thiadiazole,
 2 - mercapto- benzimidazole,
 2 - mercapto-5 - methoxy benzimidazole,
 2 - mercapto- 1, 3, 4 - triazole,
 2 - mercapto- benzothiazole, etc.

PART-3

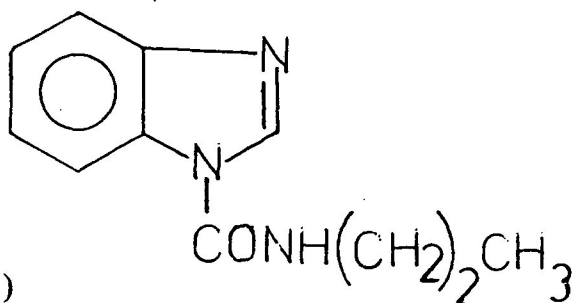
Synthesis of 5-methoxy-2-(thio substituted benzoyl) benzimidazole.

The literature study reveals that 2-mercapto benzimidazole¹² (X) itself is a bacteriostatic and antifungal agent.



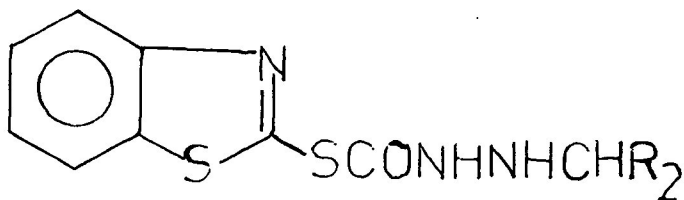
(X)

Benomyl¹³ (XI) is another antifungal compound having benzimidazole moiety with a carbamido linkage.



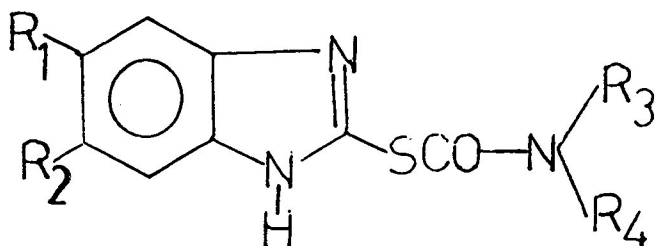
(XI)

Also, Asaad and Falmy¹⁴ synthesised benzothiazoles (XII) having thiocarbamoyl linkage at 2-position and were found to show inhibition zone of 9-18mm on various bacteria at a concentration of 0.01g \ 100 ml.



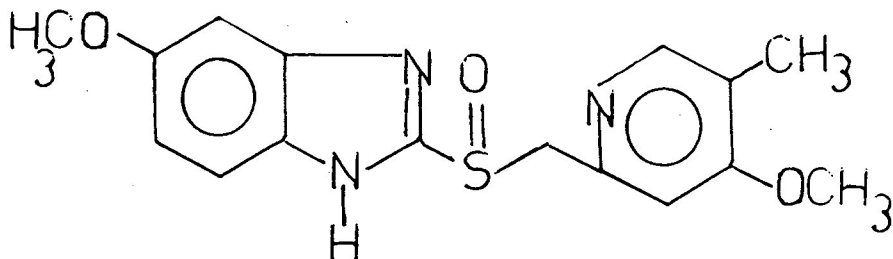
(XII)

Very recently, Sanjay Palav and B. G. Khadse¹⁵ (XIII) had reported a series of 5,6-disubstituted-2-(N,N-dialkylthiocarbamido) benzothiazoles, as a potential chemotherapeutic activity such as antibacterial, antifungal and larvicidal activity.



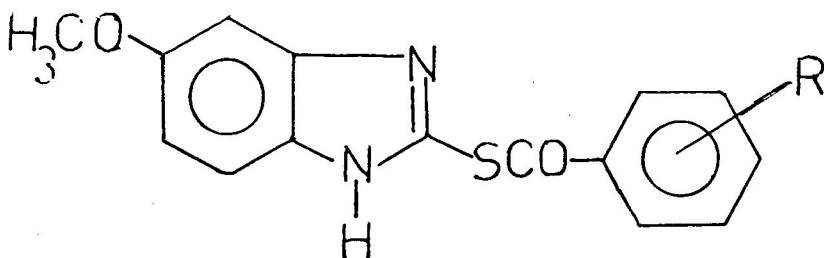
(XIII) $R_1 = H, Cl$
 $R_2 = H, Cl, F, OCH_3$
 $R_3 = R_4 = CH_3, C_2H_5$

Also, 5-methoxy-2-mercaptobenzimidazole has been found as an intermediate for Omeprazole¹⁶ (XIV) which is a potent antiulcer agent and used in treatment of Zollinger Ellison syndrome.



(XIV)

In view of the above findings a series of compounds, thus containing the combination of 5-methoxy-2-mercapto benzimidazole with different acid chlorides (XV) have been effected so as to study their antibacterial and antifungal properties.



(XV) $R = 2\text{-fluoro}, 2,4\text{-difluoro}, 3,4\text{-difluoro}, 4\text{-cyano}, \text{etc.}$

PART 4

Synthesis of Triclosan.

Triclosan¹⁷ is a chlorinated bisphenol antiseptic, effective against gram positive and most gram negative bacteria. It is also active against fungi. It is used in soaps, creams and

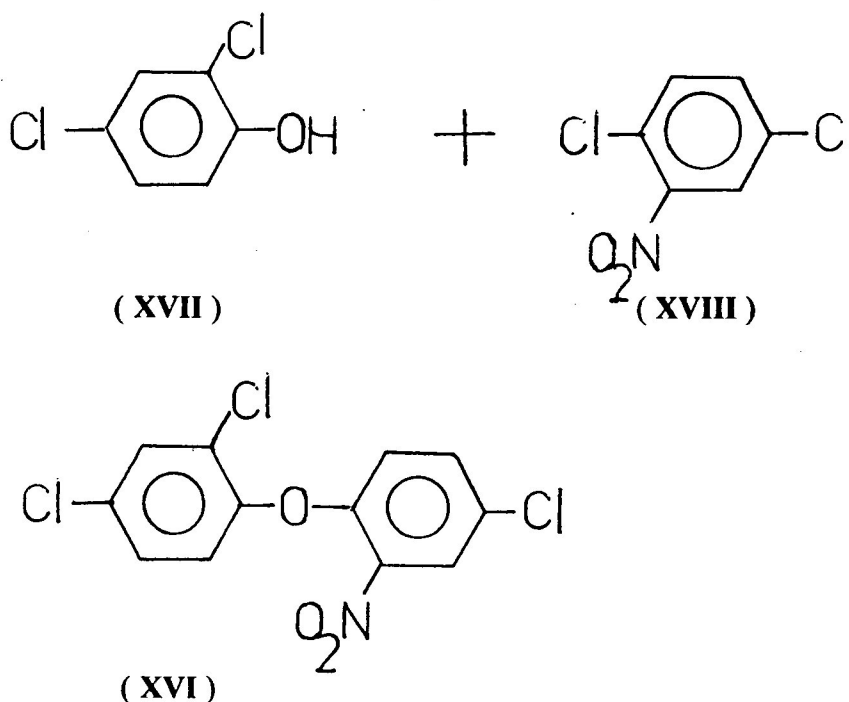
solutions in concentrations of upto 2% for disinfection of the skin prior to surgery, infections or veinpuncture. It is also used in oral hygiene products. There have been isolated reports of contact dermatitis.

Methicillin resistant staphylococcus aureus infection on a surgical unit was successfully controlled by hand washing and breathing using Triclosan.

Thus, Triclosan has been proved to be a important bacteriostatic agent found in popular deodorants and antiperspirants¹⁸.

This being an important drug, the synthesis of this compound was indigenously carried out in our laboratory. Triclosan has been prepared in three steps.

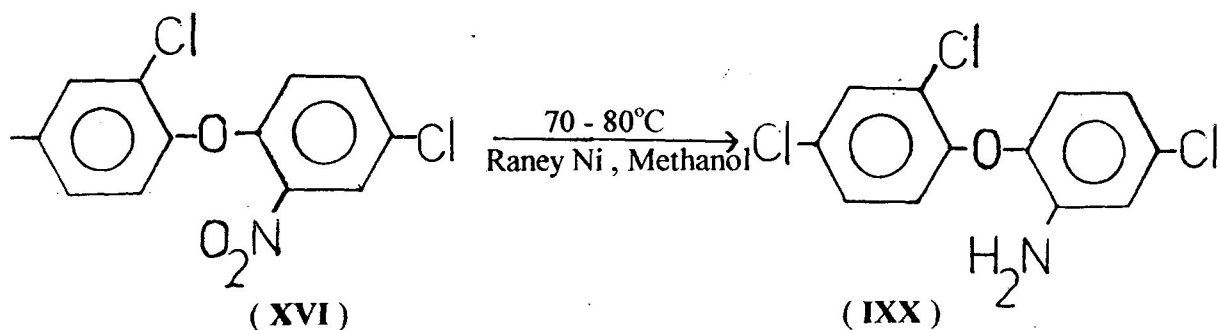
Step 1 : Synthesis of 2,4,4'-trichloro-2'-nitrodiphenylether (TNDE) (XVI) was obtained by condensation of equimolar quantities of 2,4-dichlorophenol (XVII) and 2,5-dichloronitrobenzene (XVIII) in dry dimethylformamide.



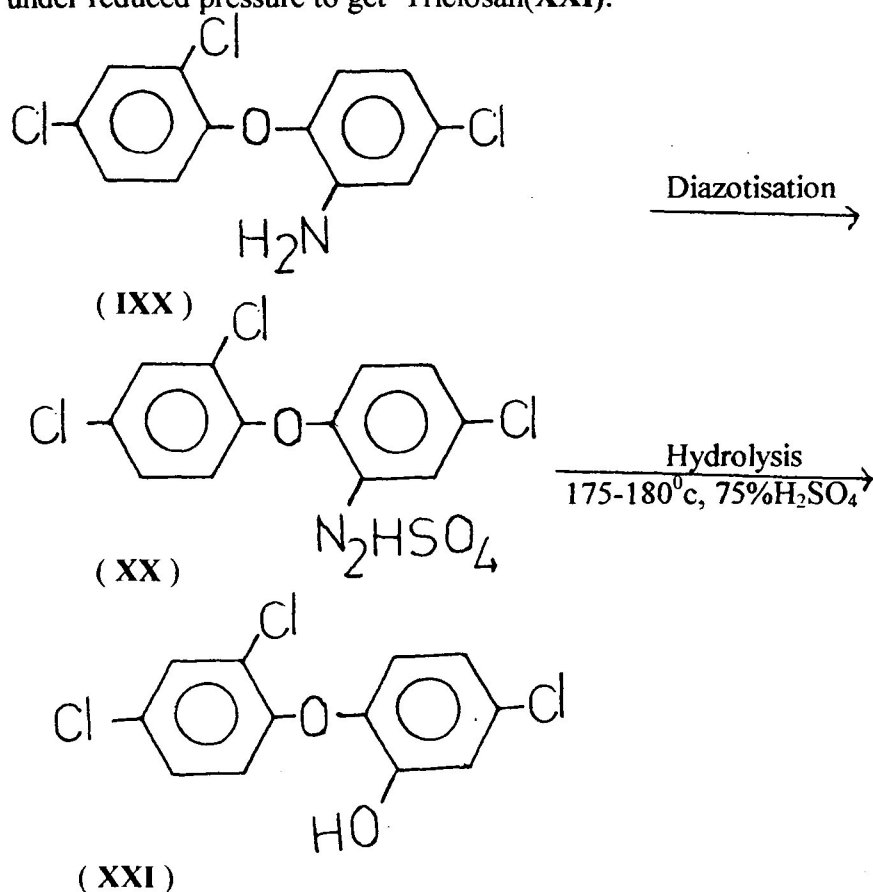
Step 2 : Synthesis of 2, 4, 4' - trichloro - 2' - aminodiphenylether (TADE) (XVIII) was obtained by reduction of TNDE which has been carried out in three different routes.

- Reduction by sodium sulphide flakes gave very less yield 40% and it was difficult to remove inorganic impurities.
- Also, reduction by activated iron powder and 10% hydrochloric acid gave less yield 40% and removal of inorganic impurities was difficult.
- Reduction by activated Raney nickel and hydrogen gas in pressure reactor in methanol as solvent had given good yield 85 - 90% and was more feasible.

So, it was preferred to carry out the reduction of TNDE by activated Raney nickel and hydrogen gas.



Step 3 : Synthesis and purification of 2, 4, 4' - trichloro - 2' - hydroxydiphenylether (Triclosan) which comprises the steps of diazotizing the sulphate salt of TADE, instead of hydrochloride salt, and hydrolysing the diazo compound (XX) by 75% sulphuric acid at 175 - 180°C, distilling the oily layer by chlorinated solvent. Then solvent was removed under reduced pressure and it was treated with 10% sodium hydroxide solution. The resulting solution was extracted with organic solvent to remove impurities. The aqueous layer was acidified by 10% hydrochloric acid and extracted with chlorinated solvent and it was removed under reduced pressure to get Triclosan (XXI).



PART-5

Biological Screening :

The compounds reported herein have been characterised by TLC, elemental analysis and spectroscopic analysis like IR, NMR and results have found to be quite satisfactory.

The compounds reported in part 2 and part 3 have been subjected for its antibacterial and antifungal screenings in vitro.

About twenty compounds have been subjected for antibacterial screening in vitro by the tube dilution technique using Gram positive bacteria and Gram negative bacteria viz., S. aureus, S. typhi. The culture medium used was Mueller-Hinton broth¹⁹. This compounds have shown moderate activity in the range of 100-200µg/ml.

Some compounds have been screened for its antifungal activity in vitro by the tube dilution technique²⁰ against fungal strains viz., C. albicans, T. rubrum and T. mentagrophytes. Most of this compounds had not shown any significant activity.

The standard drugs used for antibacterial screening were **AMPICILLIN** against S. aureus and **TRIMETHOPRIM** against S. typhi, **MICONAZOLE** for antifungal screening. The Standard dose of Ampicillin is 0.01µg/ml and that of Trimethoprim is 1µg/ml. The Standard dose of Miconazole is 6.25µg/ml for C. albicans, 1.56µg/ml for T. rubrum, T. mentagrophytes and 1.56µg/ml for T. rubrum.

Literature studies have revealed that compounds from part 1 may have antimalarial activity. In view of this finding we have evaluated our compounds for their antimalarial activity using rodent malaria model with rodent malarial strain of P. berghei in mice in vivo.

Single-Dose Regimen :

Rane's Preliminary Rapid Screening Method²¹.

This system is based on the use of mice inoculated intraperitoneally with a asexual blood parasites, and treated three days latter with one subcutaneous injection of the test compound. Antimalarial activity is assessed by comparing survival times of treated mice to infected, nontreated-controls. All mice are albino type, breed in Haffkine laboratory. Test male weigh from 18 to 20g and are all approximately of same age. Each mouse receives about 1×10^6 parasitized erythrocytes drawn from donor mice infected four days earlier with P. berghei. Test compounds are dissolved or suspended in Tween-80 and distilled water. Deaths that occur before the sixth day, when infected, untreated controls begin to die, are regarded as toxic deaths. Each compound is administered initially in four graded doses diluted fourfolds into groups of five mice per dose level.

In each test Pyrimethamine and Cycloguanil Hydrochloride were included for comparison. The dose at which double the survival time was attained was considered the Minimum Effective Dose (M.E.D). Mice alive after 40 days after infection are considered are cured.

About ten compounds have been screened by Rane's test, out of which two compounds were inactive and other compounds showed activity in the range of 40-160mg/kg. The test compound 5-(1,3,4-triazole-2'-thio)-2-benzimidazole carbamate had shown good antimalarial activity 40mg/kg. Also, 5-(benzothiazole-2'-thio)-2-benzimidazole carbamate and 5-(5'-methoxy-benzimidazole-2'-thio)-2-benzimidazole carbamate showed activity about 80mg/kg.

REFERENCES

1. Nakajima, Shotaro., Tonak, Ichivo.; Seki, Teniya.; Anmo, Toxhlo.; Yakugaka zasshi 78, 1378-82 (1958). Cited from Chem. Abstr.: 53: 8124b (1959).
2. Balio; Alessandro; Ricerca. Sci. ; 20: 1268 (1950).
3. Brown, H.D.; Matzuk, A.R.; Ilves, I.R.; Peterson, L.H.; Harris, S.A.; Egerton, J.R.; Yakstis, J.J.; Campbell, W.C.; Cuckler, A.C.; J. Am. Chem. Soc.; 83,1764 (1961).
4. Walker, D. & Knight, D.; Vet. Rec. 90,58 (1972).
5. Duwel, D.; Pestic. Sci.; 8,550 (1977).
6. Smith, Kline & French Lab.; Briti. Pat. ; 1,123,317 (1968); Cited from Chem. Abstr.; 69: 96722k (1968).
7. Actor, P.; Anderson, E.L.; Ravin, L.R.; Dicuollo, C.J.; Ferlauto, R.J.; Hoover, J.R.E.; Pangono, J.F.; Scheidy, S.F.; Stedman, R.F.J. & Theodorides, V.J.; Nature (London); 215,321 (1967)
8. Van Schalkwyk, P.C.; Geyser, T.L.; Recio, M. & Erasmus, F.P.G.; J.S. Afr. Vet. Ass.; 50,31 (1979).
9. Theodorides, V.J.; Gyurik, R.J.; Kingsbury, W.D. & Parish, R.C.; Experientia ; 32,702 (1976).
10. Dieckmann-Schuppert-A; Franklun, R.M.; Cell-Bio-Int-Rep.; May 1989;13 (5); 411-8.
11. Chandra, S.; Das, P.K.; Dutta, G.P.; Dutta, G.D.P.; Abuzar, S. & Sharma, S.; Indian Journal of Maliriology. Vol. 24, June 1987, pp. 17-19.
12. Allan, V.; Deacon,.; Org. Syn.; Vol. 9; 569 (1963).
13. Adams; Dewitt, C.; Schlatter; Rudolph; Ger. Offen.; 1,956,157 (1970); Cited from Chem. Abstr: 73, 3536m (1970).
14. Assad; Falmy, M.; Egypt J. Chem.; 1989 (Pub. 1991), 32(3), 367-74 (Eng).
15. Palav, S.D.; Khadse, B.G. & Kamath, V.R.; Indian Drugs, 34 (7), July 1997.
16. ' The Merck Index'; Twelfth Edition; Edited by Budhavari, S.; Mary Adele J.O.Neil; Smith, A.; Heckelman, P.E.; Goanne, F.; Kennary; pp. 6977.

17. 'Martindale'; The Extra Pharmacopoeia; Thirteenth edition; Edited by James, E.F.; Reynolds; pp.805.3.
18. Foye, William.; Thomas; Lemke, L.; 'Principles of Medicinal Chemistry'; Fourth edition; pp. 814.
19. Koneman, E.V.; Janda, W.M.; Allen, S.D.; Sommers, H.M.; Dowell, V.R.; Winn, W.C.; 'Color Atlas and Textbook of Diagnostic Microbiology'.J. P. Lippincott company, Third edition.; (1988) 487.
20. E.Joan Stokes, 'Clinical Bacteriology', Fourth edition (1975), 226.
21. Peters, W.; 'Chemotherapy and Drug Resistance in Malaria Academic', Newyork, pp. 64-136 (1970).

