

5,6-DISUBSTITUTED BENZOTHAZOLE AND
BENZIMIDAZOLE SULFONAMIDES/SULFONYL SULFIDES AS
POSSIBLE THERAPEUTIC AGENTS

THESIS SUBMITTED
UNIVERSITY OF MUMBAI

FOR THE DEGREE OF
MASTER OF SCIENCE
IN
CHEMISTRY

SUBMITTED BY
RAJEEV RAMA ROHINI VARMA

UNDER THE GUIDANCE OF
DR. ANIL BOBADE

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

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M.Sc. (BY RESEARCH) DEGREE
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**UNDER THE GUIDANCE OF
Dr. ANIL S. BOBADE**

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**DEPARTMENT OF CHEMOTHERAPY,
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SEPTEMBER 2009**

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VAR

STATEMENT BY THE CANDIDATE

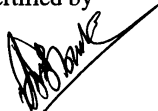
As required by the University Regulation No. R. 2190, I wish to state that the work embodied in this thesis titled “5,6-Disubstituted Benzothiazole and Benzimidazole sulfonamides/sulfonyl sulfides as possible therapeutic agents” forms my own contribution to the research work carried out under the guidance of **Dr. Anil S. Bobade** at the Department of Chemotherapy, **Haffkine Institute for Training, Research and Testing, Mumbai-12**. This work has not been submitted for any other degree of this or any other University. Whenever references have been made to previous works of others, it has been clearly indicated as such and included in the Bibliography.



Signature of Candidate

Rajeev R. Varma

Certified by



Signature of Guide

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Dedicated
to
My Family,
Friends &
Relatives

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Rajeev Varma

INDEX

Pg. No.

Acknowledgement

Introduction	1
Review of Literature	3
Plan of Work	44

Series I:

Synthesis of 2-(4'-amino-3'-methylphenyl) sulfonamido-5,6-disubstituted benzothiazoles

1. Chemistry of Benzothiazoles	51
2. Chlorosulfonation of Acetanilides	61
3. Schematic Representation	66
4. Experimental	67

Series II:

Synthesis of 2-(4'-Substituted benzamido-3'-methylphenyl)sulfonamido -5,6-disubstituted benzothiazoles.

1. Chemistry of Acid Chlorides & 2-Benzamido benzothiazoles	74
2. Schematic Representation	80
3. Experimental	81

Series III:

Synthesis of 2-(4'-acetamido-3'-methylphenyl)sulfonyl-2-yl mercapto-5,6-disubstituted benzimidazoles.

1. Chemistry of Benzimidazoles	84
2. Schematic Representation	90
3. Experimental	91

Series IV:

Synthesis of 2-(4'-phenyl thioureido-3'-methyl phenyl) sulfonamido-5,6-disubstituted benzothiazoles.

1. Chemistry of Thioureas	99
2. Schematic Representation	104
3. Experimental	105

Results & Discussions

1. Chemistry	107
2. Spectral Results	110

Biological Screening & Results

1. Screening Methods	114
2. Results of Organic Drugs tested against pathogenic strains	119
3. Conclusion of Research Work	123

References	126
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Appendix	141
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Published Paper

Synopsis	i-xii
-----------------------	--------------

INTRODUCTION

Chemotherapy is defined as the study and the use of agents, which are selectively more toxic to the invading organisms than to the host. Paul Ehrlich, the pioneer in chemotherapy, was more absolute in his concept and used the term to describe the cure of an infectious disease without injury to the host. This idea has been rather closely approached by the antibiotics. The scientific principles of chemotherapy were established chiefly during the period 1919-1935.

The first 30 years of the 20th century saw the development of useful chemotherapeutic agents, among which were organic compounds containing heavy metals such as arsenic, mercury and antimony, certain dyes and a few modifications of the quinine molecule. These agents represented extremely important advances, but even so, had many drawbacks. The next 30 years of the 20th century comprise the period of the greatest advance in the area of chemotherapy. During this time the sulfonamides, sulfones, many phenols and their derivatives, the antimalarial agents, the surfactants and of great importance, the antibiotics were studied and introduced into medicinal practice. The development of these newer drugs has relegated some of the older ones to positions of minor importance or of historical interest only.

The modern era of the chemotherapy of infections started with the clinical use of sulfanilamides in 1936. The golden age of antimicrobial therapy began with the production of penicillin in 1941, when this compound, first discovered in 1929, was mass produced and made available for limited clinical trial for the first time. Subsequent discoveries of antibiotics were the result of highly planned efforts.

Each year about 50 million people die throughout the world. Of these, about 6.8 million deaths are registered in the developing market economies, 4.2 million in eastern Europe, 7 million in the group of least developed countries and 32 million elsewhere in the developing world. This catalogue of diseases account for about 17 million deaths or almost half of all deaths occurring in developing countries

Reliable data on causes of death show that, the infectious and parasitic diseases continue to pose serious risk to adult health in developing countries.

Research in vaccines, pharmaceuticals and medical equipments are becoming even more expensive with the result that, end products are priced out of the reach of developing countries. Yet pressures from manufacturers and donors to adopt these costly new technologies and even from the general public are intense. The trend seems to be more and more expensive treatments for diseases affecting less number of people. In general, it is hoped that R&D will develop new and improved preventive therapeutic and enabling technologies, giving attention to simplicity and adaptability to meet the needs of developing countries.

Hence, we must develop new approaches, mechanisms, partnerships and resources that can expedite this process. The problems now being faced may not be susceptible to approaches that have been applied in the past. We cannot continue doing what we have always done. Tomorrow cannot be just more of yesterday. We need flexibility and pragmatism as much as innovation but the stress must invariably be on action.

The work presented in this thesis is yet another humble effort in the field of medicinal chemistry, though very minute even to be regarded as a small step in offering a practical solution to the innumerable problems, nevertheless, a devoted effort to sincerely contribute to a healthier and happier human life. The work deals with some of the commonest ailments viz. bacterial and fungal infections though not considered fatal by and large. But even today these irritate physicians at times, when coupled with other chronic conditions and also due to the resistance offered by infecting organisms to various forms of therapy.

REVIEW
OF
LITERATURE

Features of clinical importance are described below under individual drugs.

BACTERIAL INFECTIONS:

The development of resistance to current antibacterial therapy continues to drive the search for more effective agents. Bacteria are more 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 drugs, but most of them have met with little or no success with the exception of Morgenroth & Levy's partial success in the year 1911, in combating bacterial infections of the blood stream. They found that ethyl dihydrocuprein (optochin) a quinine derivative would cure pneumococcal infection in mice. Hidelberger and Jacobs, coupled diazotised p-aminobenzene sulfonamide with dihydrocuprein and found that the resultant compound has only mild activity, and was not further investigated.

However, Domagk¹, Mietzsch and Klarer² in 1935, first dispelled the failure of earlier scientists by discovery of Prontosil, and reported that this compound cured streptococcal infections in mice and staphylococcal infection in rabbits. This era was considered to be a brilliant era in chemotherapy of microbial infections.

The history of antibacterials has been dynamic, characterised by the constant emergence of new challenges followed by investigation, discovery and production of new drugs. A complete review of 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:

Sulfur containing compounds:

1) Sulfonamides:

The sulfonamide drugs were the first effective chemotherapeutic agents to be employed systemically for the prevention and cure of bacterial infections in man. Before penicillin became generally available, the sulfonamides were the mainstay of antibacterial chemotherapy.

In 1936, Colebrook and Kenny³, as well as in 1936, Buttle *et al.*⁴ report their favourable clinical results with prontosil and sulfanilamide in *puerperal sepsis* and meningococcal infections. These two reports awakened the medical profession to the new field of bacterial chemotherapy, and experimental and clinical articles in great profusion soon appeared.

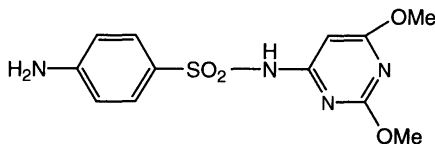
A vast number of derivatives of sulfanilamides were subsequently synthesized. This resulted in introduction of sulfathiazole, sulfapyridine, sulfamerazine, sulfadimidine, etc into clinical practice. Many of these have been tested for their clinical value in various bacterial, protozoal and viral infections.

Sulfonamides have a wide range of antimicrobial activity against both gram-positive and gram-negative organisms. With a few exceptions, there is a direct correlation between their efficacy *in vitro* and *in vivo*. In general, the sulfonamides exert only a bacteriostatic effect in the body and cellular and humoral defense mechanisms of the host are essential for the final eradication of the infection.

Numerous derivatives of para-aminobenzene sulphonamides (sulfanilamide) were synthesized and studied. Among these, sulfadimethoxine, sulfameter, sulfaethadione, sulfamethoxy pyridazine

and sulfamethoxy pyridazine acetyl are to be marked as long acting sulfonamides.

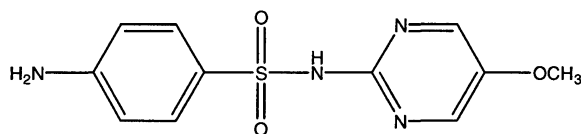
Sulfadimethoxine :



[I]

The actions and uses of Sulfadimethoxine [I] are those of the antimicrobial sulfonamides in general. However, it is characterised by slow renal excretion and hence has not only long duration of action but also a negligible incidence of crystalluria. It is absorbed rapidly and the incidence of kidney damage is reported to be low. A disadvantage to its long duration of action is that when toxicity occurs, the drug cannot be removed quickly from the body.

Sulfamer (Sulfamethoxydiazine):

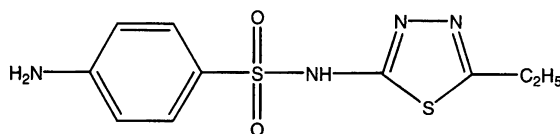


[II]

Sulfamer [II] is a long acting sulfonamide, which is readily absorbed from the gastrointestinal tract. Following oral administration, measurable levels of the drug reaches in approximately two hours and peak serum levels occur within 4 to 8 hours. Limited data suggest that measurable amounts of the drug are still present in the plasma 96 hours after the drug is administered; approximately, 90% of the sulfonamide in

plasma is non-acetylated. Because of the long lasting blood levels of the sulfameter, it is administered orally as a single daily dose, preferably after breakfast.

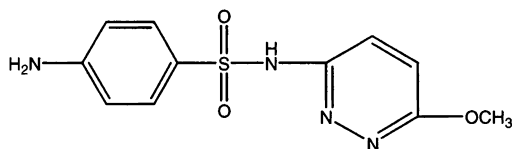
Sulfaethidole:



[III]

Sulfaethidole [III] shares the antimicrobial actions and uses of sulfadiazine, except that it poorly penetrates the blood brain barrier and hence cannot be employed to treat meningitis. This drug is useful in urologic therapy. It has biologic half-life of about 8 hours in man, but with sustained released preparations, a much longer duration of action is achieved. The degree of acetylation of the drug in the body is low; only 5% to 10% of the drug in the urine is in the conjugate form since the solubility of the unchanged drug is fairly high, the incidence of crystalluria is considerably lower than that of sulfadiazine.

Sulfamethoxypyridazine :

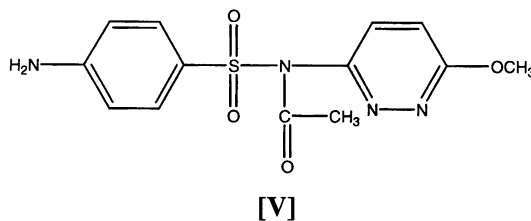


[IV]

Sulfamethoxypyridazine [IV] is absorbed rapidly from the gastrointestinal tract but has a very slow rate of excretion, a longer duration of action is obtained and less frequent administration is required than is the case with other sulfonamides. A diminished incidence of renal toxicity has been noted, but in general, the same side

effects and untoward reactions may be expected as with other sulfonamides.

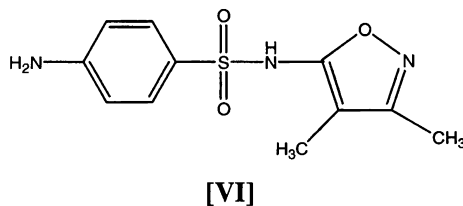
Sulfamethoxypyridazine Acetyl:



Sulfamethoxypyridazine acetyl [V] has the same actions and uses as the parent sulfonamides, sulfamethoxypyridazine, except that it is tasteless and therefore, better suited to paediatric use as a liquid medication.

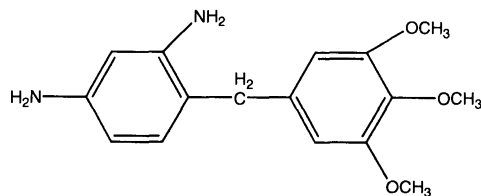
Mixed Sulfonamides:

The danger of crystal formation in the kidneys on administration of sulfonamides has been reduced greatly through the use of more soluble sulfonamides such as sulfisoxazole [VI].

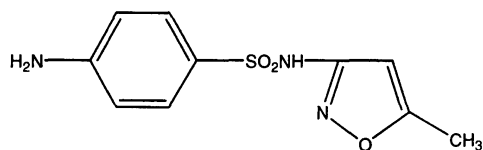


This danger may be diminished still further by administering mixtures of sulfonamides. Further, the introduction of trimethoprim [VII] in combinations with sulfamethoxazole [VIII] constitutes an important advancement in the development of clinically effective antimicrobial agents and represents the practical application of a

theoretical consideration; that is, if two drugs act on sequential steps in the pathway of an obligate enzymatic reaction in bacteria, the result of their combination will be supra-additive. Extensive biochemical studies on this combination have proved it.



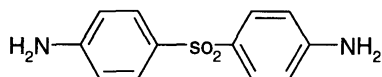
[VII]



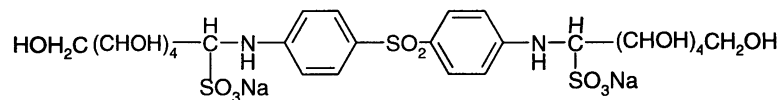
[VIII]

2) Sulfones:

The sulfones first attracted interest because of their chemical relationship to the sulfonamides. Of the six possible diaminophenyl sulfones having one amino group in each ring, only the 4,4'-isomer [Dapsone, IX] was active⁵. Dapsone was found in 1937 to be 30 times more active and only 15 times as toxic as sulfanilamides when used in streptococcal infections in mice. Against pathogenic strains of Tubercle Bacillus, it was active at 10 µg/ml. Dapsone and its glucose bisulphite derivatives [X] were the first compounds to exhibit a good life-extending effect on tuberculosis in animals^{6,7} and stimulated extensive structural modifications. At that time, the drug was considered too toxic for administration to men, and attempts were initiated to find a compound with a better therapeutic index.



[IX]

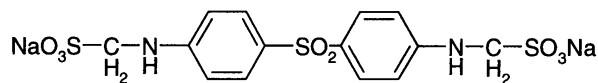


[X]

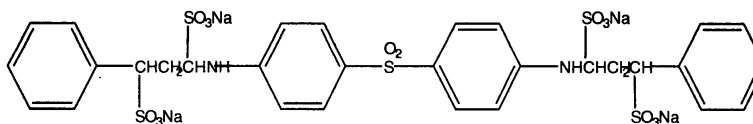
Shepard⁸ has done extensive work on dapsone in leprosy. This bacteriostatic drug has been shown to possess sensitivity of 0.02 µg/ml on microorganisms of untreated leprosy patients⁹.

The mechanism of action of the sulfones is probably similar to that of the sulfonamides since both possess approximately the same range of antibacterial activity and both antagonise PADA. The most common untoward effect is haemolysis of varying degree. Anorexia, nausea and vomiting may follow the administration of sulfones. Isolated instances of headache, nervousness, drug fever and variety of skin rashes have been reported by Rapoport and Guss¹⁰.

Activity was exhibited by several mono¹¹⁻¹³ and bis¹³ Schiff bases of aromatic aldehydes and aldehyde bisulphite complexes such as glucosulfone [X]^{7,14}, aldesulfone [Diazone, XI]^{15,16} and Solasulfone [XII]¹⁷⁻¹⁹.



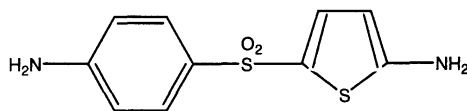
[XI]



[XII]

These compounds had weak *in vitro* activity under conditions that prevented hydrolysis and the parent sulfone was almost always detected *in vivo*^{20,21}.

Appreciable activity was retained when only one aminophenyl moiety was replaced by an amino heterocycle, the most active being promizole [XIII] wherein the amino and the sulfones group occupy the relatively same positions.

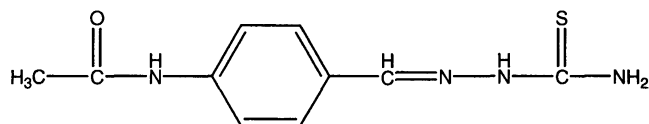


[XIII]

Thiosemicarbazone:

The tuberculostatic activity of thiosemicarbazones and related drugs was first observed by Dogmak *et al.*,²². The weak antituberculosis activity of sulfathiazole led to the preparation of series of analogues^{23,24}. The thiadiazoles and their precursor thiosemicarbazones were tested for activity. These compounds included the active benzal thiosemicarbazones along with numerous inactive aliphatic analogues.

Study of structure-activity relationship suggested that 4-acetylaminobenzaldehyde thiosemicarbazones (amithiozone) [XIV] was the most promising of these compounds. It is the one on which a great deal of work has been done.



[XIV]

Amithiozone was active orally against experimental infections in animals, the activity being comparable to streptomycin²⁵⁻²⁷. It also suppressed the emergence of resistance in these experimental infections.

Its high tuberculostatic activity was not inhibited by PABA as would be expected from structure-activity relationship.

In 1954, Lowe was the first to discover the effectiveness of aminothiozone in leprosy. It appears to exert a greater effect on the tuberculoid than on the leprometous form of the disease. It is not as well tolerated in man as in animals. The principal obstacle is its toxicity. The most common untoward effect of the thiosemicarbazones and derivatives are anorexia, nausea and vomiting. The drug may depress bone marrow function. Some degree of anaemia is observed in a large percentage of patients receiving amithiozone. The fairly high incidence of jaundice in patients receiving amithiozone suggests that it is hepatotoxic; the effect on the liver disappears when treatment is stopped.

NON-SULFUR DRUGS:

Furan Derivatives:

Derivatives of furan possess antimicrobial activity. The presence of nitro group in the 5-position of the furan ring confers antibacterial activity on mainly 2-substituted furans. Dodd and Stillman²⁸ prepared a large number of nitro furans and their non-nitrated analogs and found that the nitro group conferred antibacterial activity on the various furan derivatives. Although, hundreds of broadly active derivatives have been prepared, only a few have enjoyed clinical success. The nitrofurans are active against certain gram-positive and a variety of gram-negative bacteria, some fungi and the protozoan *Trichomonas vaginalis*.

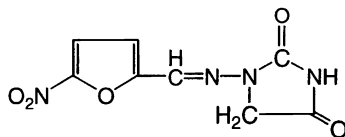
It has been generally concluded²⁹⁻³¹ that the antibacterial activity is the greatest in those compounds whose nitro group is the most readily reduced. A bacteriostatic action is exerted upon most bacteria in the concentration range of 1:100,000 to 1:200,000. Bactericidal concentrations are approximately twice as great. The mechanism of the

antibacterial action of the furan derivatives is unknown, but it is presumed that the compounds probably interfere with enzymatic processes essential to bacterial growth.

On an individual basis, the various agents are useful topically and orally under certain conditions. When used in combination with antibiotics like chloramphenicol and erythromycin, the nitrofurans were found to be synergistic³². Little or no development of cross-resistance is so far evident³³⁻³⁵.

Nitrofurantoin:

Nitrofurantoin [XV] was first synthesized by Hayes³⁶ by the condensation of 1-aminohydantoin with 5-nitro-2-furfuraldehyde. The drug is active against many strains of the common urinary tract pathogens.



[XV]

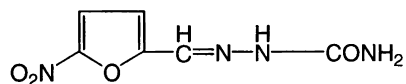
Nitrofurantoin is bacteriostatic at concentrations of 5 to 10 µg/ml and bactericidal at 100 µg/ml but it is not known whether a bactericidal occurs *in vivo*. The antibacterial activity is higher in acidic urine.

The compound is rapidly and completely absorbed from the gastrointestinal tract. Antibacterial levels in the blood are not attained but approximately 40% of the drug is excreted in the urine³⁷. Concentrations that are bactericidal to most gram-positive & gram negative organisms are achieved in urine. Intravenous administration of the drug has received limited trial in the treatment of acute severe urinary tract infections³⁸. The average dose of Nitrofurantoin results in a urinary concentration of approximately 20 µg/100ml.

The most common untoward effects are nausea, vomiting and diarrhoea. The incidence is less if the drug is administered with milk or other food or is used in a smaller dosage.

Nitrofurazone:

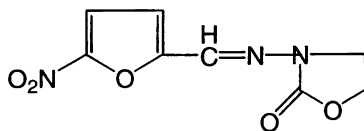
Stillman and Scott³⁹ have synthesized this compound [XVI] from 5-nitrofurfuraldehyde and semicarbazide hydrochloride in the presence of sodium acetate. The drug has broad spectrum antibacterial activity against most gram-positive and gram-negative surface pathogens. It has been used in the treatment of mixed infections of superficial wounds and diseases of the skin. The compound is effective in the systemic treatment of trypanosomal infection in animals, and it is reported to be of some value in the therapy of sleeping sickness in man⁴⁰ caused by *Trypanosome gambiense*.



[XVI]

Furazolidone:

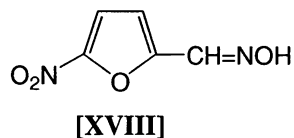
Furazolidone [XVII] was synthesized by Drake and Hayes⁴¹. The drug has been reported to be of value in the treatment of intestinal infections due to *Shigella* and *Salmonella* organisms⁴². It has an outstanding activity against these pathogenic bacterial infections. Excellent anticoccidial & antitrichomoral activity is also evident.



[XVII]

Nifuroxime:

Nifuroxime [XVIII] is prepared by treating 5-nitrofurfural with hydroxylamine in alcohol. It is patented⁴³ as a urinary antibacterial agent.

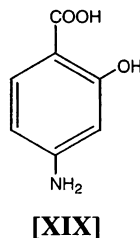


The compound has wide antibacterial and excellent antifungal activity. *Candida Albicans*, various microspores, and *Trichophyton* species are susceptible. The product is also effective against *Trichomonas vaginalis* and is often formulated in combinations with Furazolidone in the form of suppositories.

PAS (p-aminosalicylic acid) and its derivatives:

Bernheim⁴⁴ observed that benzoates and salicylates stimulated the respiration of virulent but not avirulent mycobacteria. It was suggested that such compounds might be involved in the oxidative metabolism of the bacilli, and that substituted benzoates and salicylates might interfere with these processes. This led Lehmann⁴⁵ to test a large series of related compounds for antituberculosis activity.

The most effective was PAS [XIX]



PAS was found to be highly bacteriostatic *in vitro* and also active orally against experimental infections in animals⁴⁶⁻⁴⁸. PAS is specifically active against mycobacteria and against only certain species in this genus⁴⁹. It is equally effective against streptomycin-resistant strains⁴⁸. It is of interest to find that PAS has little effect on the respiration of mycobacteria nor does it inhibit the stimulating effect salicylates⁵⁰. *In vitro*, most strains of *Mycobacterium Tuberculosis* are sensitive to a concentration of 1 µg/ml of PAS

PAS is mainly used as a secondary drug in combination with a primary drug like INH. PAS is readily absorbed from the gastrointestinal tract and maximal blood concentration is reached within 1.5 to 2 hours. Sodium PAS is absorbed even more rapidly. It appears to be distributed throughout the total body water.

The action of PAS in combination therapy is two-fold. Firstly, it produces an antibacterial effect by competing with P-Aminobenzoic acid [PABA] in biochemical synthesis. Secondly, it enhances the serum concentration of INH 2-4 fold in man by undergoing competitive acetylation.

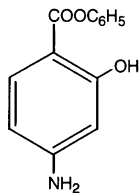
Over 80% of the drug is excreted in the urine in first 10 hours; more than 50% is in the form of the acetylated compound in man. The largest portion of the remainder is made up of the free acid.

PAS is associated with 2% to 4% incidence of untoward effects. The most important being irritation of the gastrointestinal tract, peptic ulceration, renal irritation, hypersensitivity, dysfunction of the liver and thyroid.

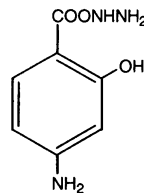
One drawback to the use of PAS is its rapid absorption and excretion which makes large, frequent doses necessary if adequate blood levels are to be maintained. Amongst the derivatives of PAS only those which generated PAS showed activity.

Attention was turned to prepare derivatives that would be absorbed and eliminated more slowly. The phenyl ester [XX] was

claimed to be as active as streptomycin subcutaneously and equivalent to PAS orally^{51,52}.



[XX]

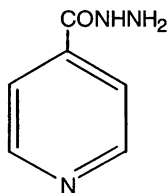


[XXI]

PAS hydrazide [XXI] was active *in vitro*. Its effect was inhibited by PABA and there was cross resistance with PAS, but not with INH⁵³.

Iso-nicotinic acid Hydrazide [INH]:

In 1945, Chorine⁵⁴ reported that nicotinamide possessed tuberculostatic action. Examination of the related compounds revealed that many pyridine derivatives, including several congeners of isonicotinic acid, possess tuberculostatic activity. Because the thiosemicarbazones were known to inhibit *M.tuberculosis*, the thiosemicarbazone of isonicotinaldehyde was synthesized and studied. INH [XXII] an intermediate for the synthesis of isonicotinaldehyde was also tested for its antitubercular activity. The exceptional antitubercular activity of INH was reported by three groups of workers independently and essentially simultaneously in the early 1950's⁵⁵⁻⁵⁸.



[XXII]

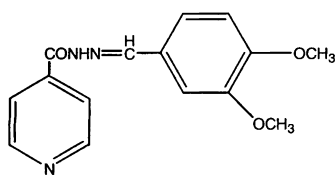
INH is both tuberculostatic and tuberculocidal *in vitro*; The minimal tuberculostatic concentration is 0.025 to 0.05 µg/ml. It is not active against the 'atypical' group. It is bactericidal even at its MIC.

INH is strikingly superior to streptomycin. Unlike streptomycin, INH penetrates cells and also cerebrospinal fluid and is just as effective against intracellularly located bacilli as it is against those growing in culture media.

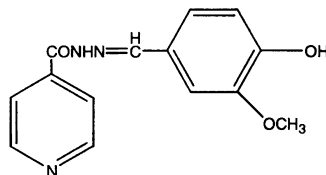
INH Hydrazones:

The very high activity and relatively low toxicity of INH promoted the synthesis of an extremely large number of structural analogues by many groups of workers. A small number of compounds had high activity, some because of conversion *in vivo* INH. Despite claims to the contrary, none of the derivatives appears to be superior to INH.

Substituted benzylidene derivatives have received particular attention and a number of them have been tested



[XXIII]



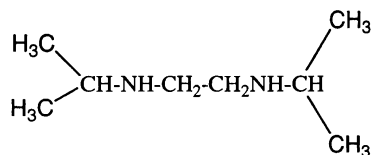
[XXIV]

Clinically verazide [XXIII] was originally claimed to exert a more marked and prolonged antituberculous effect than INH and 3-4 times less toxic. Later the same workers concluded that clinically the compound showed no advantage over INH and caused toxic side-effects attributable to veratraldehyde formed by hydrolysis⁵⁹. The 2-hydroxybenzylidene derivative was about half as active as INH and less than 1/10th as toxic⁶⁰. The 3-methoxy-4-hydroxybenzylidene derivative

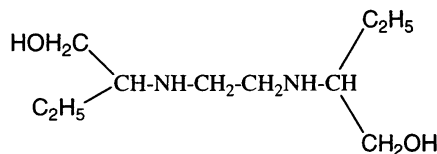
[Phthivazid, XXIV] has been extensively used clinically in Soviet Union⁶¹.

Ethambutol:

While screening selected compounds, Thomas and co-workers⁶² found that N,N'-Diisopropylethylenediamine [XXV] was effective in the treatment of experimental tuberculous infections in mice. A number of congeners of this compound were examined; the one that eventually proved to be the most tuberculostatic was ethylenediaminedi-1-butanol dihydrochloride. *In vitro* and *in vivo* studies revealed that the d-form of this substance [Ethambutol, XXVI] exhibited activity 200 times more than what l-isomer did.



[XXV]



[XXVI]

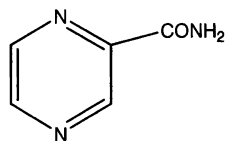
About 75% of the strains of the human type of *M. tuberculosis* are sensitive to 1 µg/ml of ethambutol.

The most important side effect is optic neuritis resulting in decrease of visual acuity and loss of ability to perceive the colour green. Recovery usually occurs when ethambutol is withdrawn; the time required is a function of the degree of visual impairment⁶³.

Ethambutol has been used with notable success in the therapy of human tuberculosis of various forms, in combination with INH. Because of greater tuberculostatic activity, lower incidence of toxic side effects and better patient acceptance, it has essentially replaced PAS in this effluent country.

Pyrazinamide:

Pyrazinamide [XXVIII] exhibits tuberculostatic activity *in vitro* only at slightly acidic pH. The growth of tubercle bacilli within monocytes *in vitro* is completely inhibited by the drug in a concentration of 12.5 µg/ml.



[XXVIII]

Pyrazinamide is more effective than PAS, cycloserine or viomycin in experimental infections with human strains of *M. Tuberculosis* in mice and in guinea pigs. When the drug is used alone *in vivo* the disease is initially controlled but soon relapses as resistance develops. When it is administered simultaneously with INH, the bacteria remain sensitive but the infection may not be eradicated.

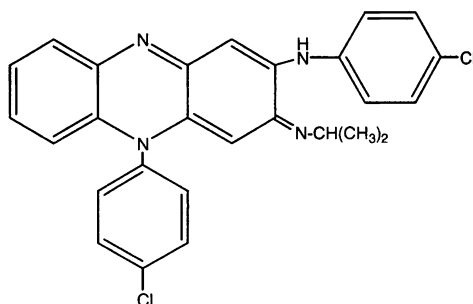
Pyrazinamide is a secondary drug. It is less effective and considerably toxic than several of the other drugs. All patients who are being treated with pyrazinamide should be hospitalized.

Basic Dyes:

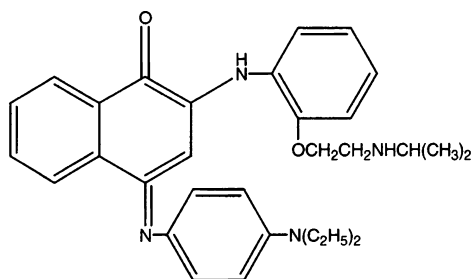
The basic dyes include the phenazines, phenoxazines and naphthoquinonimines, which have similarities in structure and properties.

The clofazimine [XXVIII] is one of the most potent antituberculotics known equal or superior to INH under very demanding test conditions⁶⁴. There is no *in vitro* or *in vivo* cross-resistance with INH, PAS or streptomycin and it prevents emergence to INH resistance using the chronic mouse infection⁶⁵. However, its clinical efficacy seems to be thwarted by inability to achieve the required blood levels⁶⁶.

The naphthoquinonimines [XXIX] is one of the most active of the series, being orally about 20% as active as INH and about equal to streptomycin when given subcutaneously.



[XXVIII]



[XXIX]

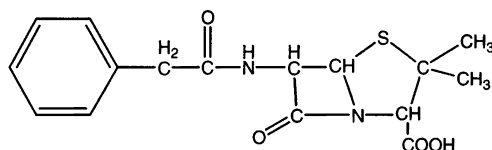
Antibiotics:

The development of antibiotics is unquestionably one of the major advances in chemotherapy in recent years. Wakaman who used the word “antibiotic” defined it as substances produced by micro-

organisms antagonistic to the growth or life of others in high dilution. A number of antibiotic substances are known to exert antimicrobial activity by virtue of their ability to inhibit the synthesis of bacterial cell wall material⁶⁷. It started with tyrothricin of Dubos (1934) and penicillin of Alexander Fleming, which revolutionised the treatment of bacterial infections. Other penicillins were obtained by appropriate precursors in a suitable liquid medium. Subsequent development gave long acting forms of benzyl penicillin and semi synthetic penicillins and cephalosporins.

Benzyl penicillin:

Among the four different penicillins i.e F, G, X & K, the G or Benzyl penicillin [XXX] has exhibited the most prominent properties. It inhibits the growth of the more sensitive organisms, which include almost all Gram positive pathogens and some Gram negative pathogens. Mandelstam and Rogers⁶⁸ have shown that, at least with Staphylococci, benzyl penicillin acts by inhibiting the biosynthesis of cell wall mucopeptide, without interfering with protein synthesis.



[XXX]

The salts of benzyl penicillin are very freely diffusible; after intramuscular injection absorption occurs within a few minutes to produce a high concentration in the blood.

Procaine penicillin, benethamine penicillin and benzethine penicillin have shown satisfactory results because of lower solubility. When these are administered as suspension of crystals they dissolve at the site of injection.

Ampicillin:

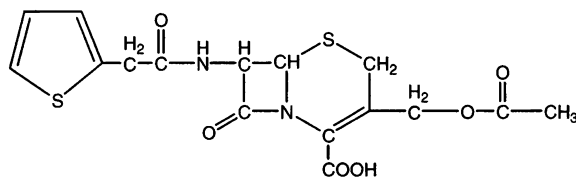
In 1961, Holinson and Stevens⁶⁹, have reported this semi synthetic α -aminobenzyl penicillin, which was administered orally as the free acid, which unlike most other penicillins is soluble only to the extent of about 10% in water.

Ampicillin is slightly less active than benzyl against most Gram positive bacteria. Its outstanding property is an activity four to eight times greater than that of benzyl penicillin against various Gram negative bacilli^{70,71}. It is bactericidal and appears to be as free from toxicity as benzyl penicillin. Gastric intolerance and rashes are the only side effects.

A few antibiotics containing thiophene ring:

i) Cephalothin:

Cephalothin [XXXI] is a semisynthetic derivative of cephalosporin C. Other semisynthetic congeners presently available for clinical use are Cefazolin, Cephapirin, Cephaloridine, Cephradine and Cephaloglycin.



[XXXI]

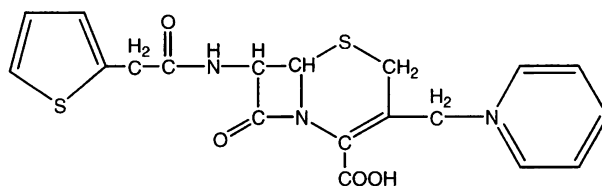
Cephalothin is active against both Gram positive and Gram negative micro-organisms. Gram positive bacteria are highly sensitive to this agent, many being suppressed by concentrations of 0.004 $\mu\text{g/ml}$. Gram negative bacteria are inhibited by 1 to 6 $\mu\text{g/ml}$. Almost all strains of *Klebsiella* are sensitive at 4 to 16 $\mu\text{g/ml}$. Cephalothin is not active

against viruses, yeasts and fungi. Bacteria susceptible to Cephalothin are sensitive over approximately the same range of concentrations of Cefazolin and Cephapirin. Cephalothin and its congeners inhibit bacterial cell-wall synthesis in a manner similar to that of penicillin. Cephalothin produces renal damage only rarely.

ii) Cephaloridine:

The general range of activity and antibacterial spectrum of cephaloridine [XXXII] closely approximates that of Cephalothin, although some strains of *E. Coli* may be somewhat more sensitive to the former. It also appears to be more active than Cephalothin against *Cl. Perfringens* (Welchii).

Mycobacterium fortuitm is sensitive to cephaloridine but not to cephalothin.



[XXXII]

Cephalodprin C and its semi-synthetic congeners induce the synthesis of penicillinase by *B. cereus* and *Staph. aureus*. Cephalothin is highly resistant to this enzyme, but cephaloridine appears to be more susceptible to its effect⁷².

Cephaloridine is nephrotoxic^{73,74}. Renal injury due to this drug occurs most often with administration of 6g or more per day. Large doses of cephaloridine result in a high incidence of granular casts in the urine.

Cephaloridine is effective in the therapy of bronchitis due to *H. influenzae*. This drug has also been found when employed as an aerosol in patients with purulent bronchitis.

Aminoglycoside antibiotics:

The aminoglycoside antibiotics – Streptomycin, Gentamycin and Kenamycin – contain amino sugars in glycosidic linkage. The aminoglycosides are used almost exclusively to treat infections caused by Gram negative bacteria. They act to inhibit protein synthesis in susceptible micro-organisms.

i) Streptomycin:

The first public announcement of the discovery of Streptomycin from *Streptomyces griseus* was made by Schatz, Bugie and Wakaman⁷⁵ early in 1944, and it was soon shown to inhibit the growth of the tubercle bacillus and a number of Gram positive and Gram negative micro-organisms *in vitro* and *in vivo*. The activity of streptomycin *in vivo* is essentially suppressive.

It was the first clinically effective drug to become available for the treatment of tuberculosis. At first, it was given in large doses, but problems related to toxicity and the development of resistant micro-organisms seriously limited its usefulness. This led to administration of the antibiotic in smaller quantities; however, when given alone over a period, the micro-organism became resistant.

Streptomycin may cause allergic reactions such as skin rashes, fever, eosinophilia, and anaphylactic shock and minor dysfunction of the eighth cranial nerve.

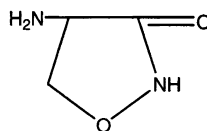
Since other effective tuberculostatic agents have become available, the use of streptomycin for the treatment of pulmonary tuberculosis has been greatly reduced.

ii) Dihydrostreptomycin:

Dihydrostreptomycin is a synthetic modification of streptomycin which reduces the toxicity while maintaining the activity. It is also produced by *Streptomyces*. It has essentially the same activity as that of streptomycin⁷⁶⁻⁷⁸ and is less toxic to the vestibular apparatus but more toxic to the auditory branch and could cause delayed severe deafness without warning symptoms⁷⁹.

Amino acid Antibiotics:

i) Cycloserine:

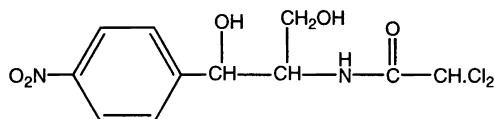


[XXXIII]

Cycloserine [XXXIII] is a D-4-amino-isoxazolidine-3-one. It is a broad spectrum antibiotic produced by *Streptomyces orchidaceus*. Cycloserine is inhibitory for *M. tuberculosis* in concentrations of 5 to 20 µg/ml *in vitro*. There exists no cross resistance between cycloserine and other tuberculostatic agents. *In vivo*, cycloserine is more effective in man than what the results in experimental animals would suggest.

Reactions to cycloserine most commonly involve the central nervous system. The untoward effects include headache, confusion, nervousness, irritability, and psychotic states with suicidal tendencies.

Chloramphenicol:



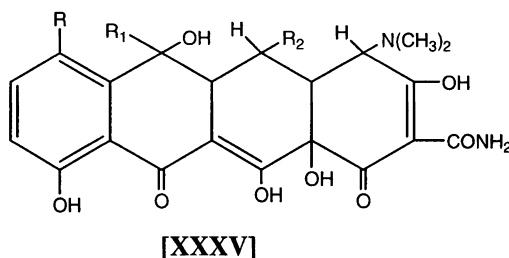
[XXXIV]

Ehrlich *et al.*⁸⁰ and Carter *et al.*⁸¹ had discovered the first broad spectrum antibiotic, chloramphenicol [XXXIV]. *In vitro* it is active against a wide range of Gram positive and Gram negative bacteria. Chloramphenicol is strictly bacteriostatic against almost all bacteria species. The same bacteriostatic effect is seen *in vivo*.

It is a choice drug for infection of *Salmonella typhi*, although this organism remains viable for long periods and will regrow on removal from exposure to the drug⁸².

The usual route of administration is oral and absorption is complete. The peak blood level, reached in two or four hours, is about 8-12 µg/ml. About 60% of the chloramphenicol in the blood is bound to protein. Excretion is mainly renal, 90% of the dose being detected in the urine. Nausea, vomiting, diarrhoea and bone marrow depression are the side effects noted.

Tetracyclines:



In 1948, when aureomycin, the first of the tetracyclines was discovered, the only other antibiotics in general use were penicillin and streptomycin. The tetracyclines [XXXV] are a family of closely related antibiotics of which chlorotetracycline, oxytetracycline and tetracycline have been in use for over 10 years.

The tetracyclines possess a wide range of antimicrobial activity against Gram positive and Gram negative bacteria, which overlaps that of many other antimicrobial drugs. With establishment of their *in vitro*

antimicrobial activity, effectiveness in experimental infections, and pharmacological properties, the tetracyclines rapidly became widely used in therapy⁸³⁻⁸⁵. Tetracyclines are also effective against some micro-organisms innately insensitive to many chemotherapeutic agents, such as rickettsiae, Mycoplasma and amoebae.

All the tetracyclines are adequately but incompletely absorbed from the gastrointestinal tract. They are excreted in the urine and faeces, the primary route for most being the kidney. Various skin reactions, burning of the eyes, brown and black casting of the tongue, fever of varying degree and eosinophilia may occur when these agents are administered.

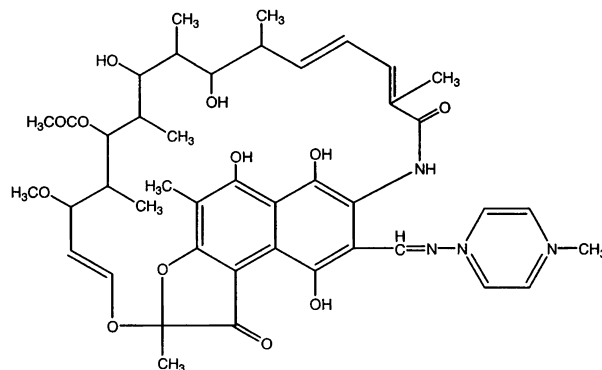
There are still a large number of antibiotics that needs to be mentioned. Prominent ones are macrolides, for example, erythromycin, oleandomycin, peptide antibiotics like polymyxins, bacitracin, antifungal antibiotics like nystatin, amphotericin, griseofulvin which are used in clinical practice. The most promising antibiotic of recent origin is Rifamycin

Rifampicin:

The rifamycins are a group of related 2-acylamino-1,4-naphthoquinones prepared by *Streptomyces mediterranei*. One of the five components separated is rifamycin B. Rifamycin B can be readily converted to rifamycin SV, a more active component, through a series of reactions. Resistance to rifamycin developed fairly rapidly, apparently as a one-step mutation process. In order to enhance the activity, rifamycin SV was structurally modified to 3-formyl-rifamycin SV which was highly active. The best among all the structural variations tried was rifampicin. The hydrazone formed from N-amino-N'-methyl piperazine and 3-formyl rifamycin SV.

The drug is zwitterion and is soluble in organic solvents and in water at acidic pH⁸⁶.

Rifampicin [XXXVI] inhibits the growth of most Gram positive bacteria, as well as Gram negative micro-organisms such as *Escherichia Coli*, *Pseudomonas*, Indole positive and negative proteus and *Klebsiella*^{87,88}. Also this drug is highly active against *Neisseria meningitides*, minimal inhibitory concentration range from 0.1 to 0.8 µg/ml⁸⁹.



[XXXVI]

Rifampicin in concentrations of 0.005 to 0.2 µg/ml is inhibitory to *M. tuberculosis in vitro*^{90,91} and is also active against atypical mycobacteria.

Micro-organisms, including mycobacteria develop resistance to rifampicin rapidly *in vitro* as a one-step process. This also appears to be the case *in vivo*, and this antibiotic must therefore not be used alone. The drug finds its greatest usefulness in the initial treatment of pulmonary tuberculosis in combination with INH in an outpatient therapeutic regimen⁹². Rifampicin is a drug of choice for chemoprophylaxis of meningococcal disease in household contacts of patients.

Rifampicin does not cause untoward effects with great frequency. The most notable problem is the development of jaundice. It also produces gastrointestinal disturbances such as nausea, vomiting, abdominal cramps, diarrhoea and sometimes mild allergic reaction. Rifampicin causes oculotoxicity, which disappears when it is withdrawn from the treatment⁹³.

FUNGAL INFECTIONS:

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

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

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

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

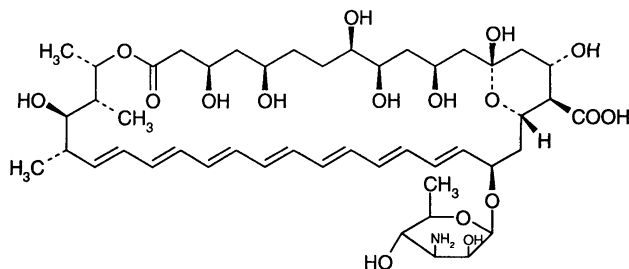
The discovery that specific fungi are the etiologic agents of specific infectious diseases preceded the work of Pasteur and Koch on pathogenic bacteria by three decades. Nevertheless, medical mycology has not received as much attention as have other areas of medical microbiology and at present there exist neither clinically available vaccines nor useful antisera for mycotic diseases. The incidence of systemic mycoses continues to rise steadily. This increase is due in part to the prolonged survival of patients with defects in their host defence mechanism, including patients with neoplastic diseases, organ transplant recipients, diabetics and patients with AIDS. These patients are susceptible to an ever-growing list of opportunistic fungi. From the data collected by the Center for Disease control, it is known that among the 1 and 3 million healthy Americans who have already been infected with AIDS, a significant number of these cases are associated with various fungal infections⁹⁴. One yeast-like fungus, *Cryptococcus neoformans*, is reported to be the most common debilitating and life-threatening infections in AIDS patients⁹⁵.

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

Amphotericin B:

This antibiotic produced by *Streptomyces nodusus*, was discovered in 1955. It became the first systemic antifungal drug. Amphotericin B [XXXVII] is a polyene macrolide with seven conjugated double bond (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 yeasts⁹⁶.

The primary mechanism of action of the polyenes in general, and amphotericin B in particular, is binding to ergosterol, the principal sterol present in the cell membrane of sensitive fungi⁹⁷.

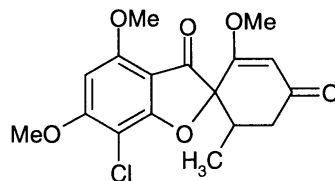


[XXXVII]

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

Griseofulvin:

Although Oxford A.E *et al* discovered this antibiotic, a product of *Penicillium griseofulvum* [XXXVIII], in 1939; its antifungal activity was not utilized until 1951. Groove predicted its chemical structure in 1950⁹⁸. However, in 1958, the antibiotic was found to be effective against dermatophytes when administered orally. The fluoro analogues retain the potency of the original drug but the bromo and dichloro analogues are ineffective. The replacement of the methoxy substituent on the cyclohexane ring either with propoxy or butoxy, increased invert activity, whereas substitution of an amino acid group at this site eliminates activity.

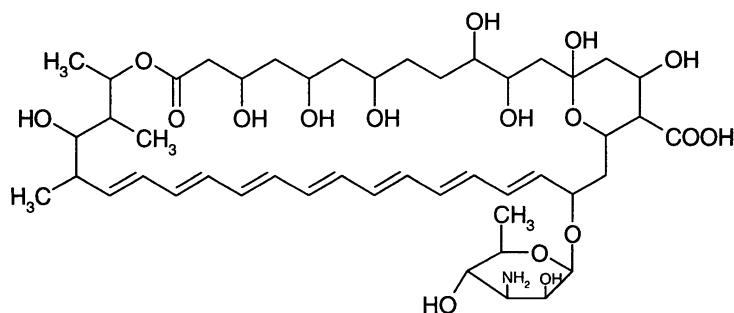


[XXXVIII]

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 [XXXIX] is produced by *Streptomyces noursei*. It was discovered by Hazen and Brown⁹⁹ and possesses good antifungal activity. Toxicity after parenteral administration to animals has led to its topical or oral use.

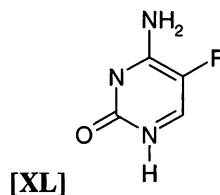


[XXXIX]

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

5-Fluorocytosine:

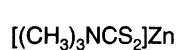
This Fluorinated pyrimidine [XL] was first synthesized in the 1950s as a potential antileukemic drug. It was found to have *in vitro* and *in vivo* antifungal activity¹⁰⁰. It is being increasingly used as an adjunct to Amphotericin B therapy. Accordingly, in *Aspergillus*, *Candida* and *Cryptococcus* infections, the drug is combined with Amphotericin B.



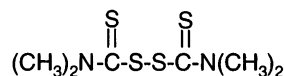
The susceptible fungal cells deaminate 4-fluorocytosine to 5-fluorouracil. The latter is metabolized to 5-fluoro-2'-deoxyuridylic acid, which suppresses DNA synthesis, by inhibition of thymidylate synthetase. The drug can also be incorporated into RNA to cause inhibition of protein synthesis. 5-fluorocytosine is extensively used in a number of human malignancies.

Sulfur containing molecules as antifungal agents:

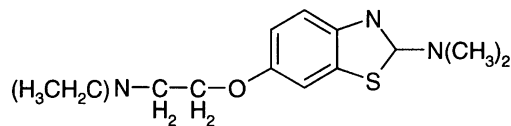
A variety of organic compounds that contain divalent sulfur attached to the carbon atom having *in vitro* fungistatic action, and many of these have been employed in topical therapy of dermatophytoses. Among these are dithiocarbamates [XLI], tetraethylthiuram disulfide [XLII] and benzothiazoles [XLIII] such as 6-diethylaminoethoxy-2-dimethyl amino derivatives [XLIV]^{101,102}.



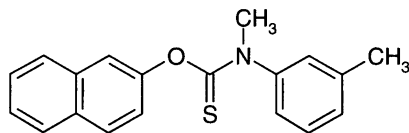
[XLI]



[XLII]



(XLIII)

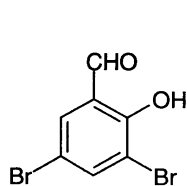


(XLIV)

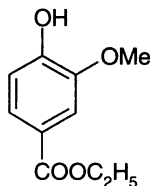
A widely used compound with unusually high *in vitro* and *in vivo* activity against the dermatophytes is o-2-naphthyl ester of m-N-dimethylthiocarbamic acid (Tolnaftate). It is also effective against *Candidal* lesions¹⁰³.

Phenolics and Diamides:

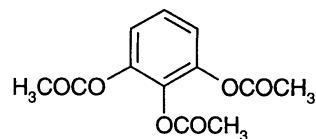
Phenolic compounds were known for their antifungal potency. Among the numerous phenolic compounds that have been used externally for mycotic infections are phenol, phenyl mercuric nitrate, cresol, m-cresyl acetate, benzoic acid, 4-hydroxy benzoic acid, salicylic acid, salicylanilide, 3,5-dibromo salicylaldehyde [XLV], ethyl vanillate [XLVI], pyrogallol triacetate [XLVII], 3-iodo-2-propyl-2',4',5'-trichlorophenyl ether [XLVIII], haloprogin 1,8-hydroxyanthranol [XLIX] and tetrachlorobenzoquinone [L].



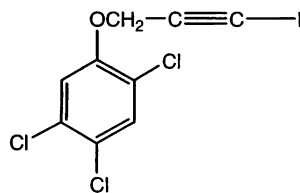
[XLV]



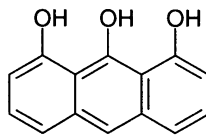
[XLVI]



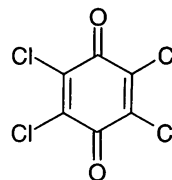
[XLVII]



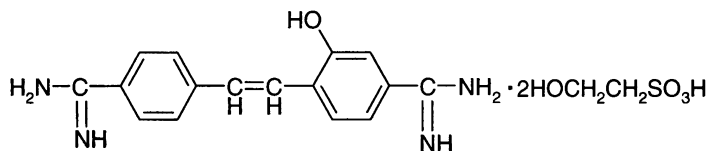
[XLVIII]



[XLIX]



[L]

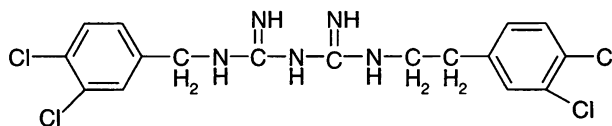


[LI]

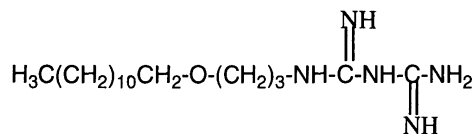
Several biamidines (e.g. stibamidine, propamidine, pentamidine) are antifungal *in vitro*. 2-hydroxy stilbamidine diisothionate [LI] has been successfully employed in patients with blastomycosis. It is less toxic than Amphotericin B¹⁰⁴.

Chelating Agents:

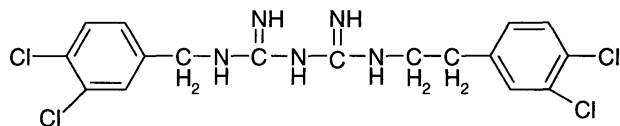
Among the chelating agents with considerable *in vitro* antifungal potency are substituted biguanides like N',N⁵-bis-(3,4-dichlorobenzyl) biguanide [LII], N'-Y-laurooxypropyl biguanide [LIII], 1,6-bis-(5-p-chlorophenyl biguanidine) hexane [LIV]¹⁰⁵.



[LII]



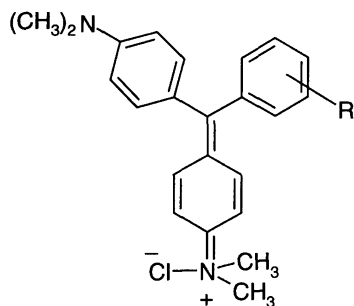
[LIII]



[LIV]

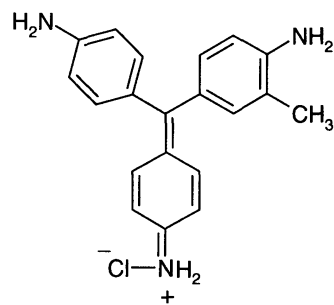
Aniline Dyes:

Aniline dyes such as malachite green [LV], gentian violet [LVI], methyl rosaniline chloride [LVII] etc have been employed in topical therapy of dermatophytoses as well as Candidasis of skin mucous membranes.



[LV] R = H

[LVI] R = N(CH₃)₂



[LVII]

Azoles:

During the past quarter century, a series of imidazoles and triazoles have been synthesized and examined for antifungal activity. Various members of this series inhibit a broad spectrum of fungal

pathogens. Some of the drugs are employed topically, for cutaneous yeasts as well as dermatophytic lesions. Imidazoles (two nitrogen atoms in a 5-membered ring) include Butaconazole, Cloconazole, Clotrimazole, Econazole, Fentiazole, Ketoconazole, Miconazole, Oxiconazole, Sulconazole and Tioconazole. Triazoles (three nitrogen atoms in a 5-membered ring) include Fluconazole, Itraconazole and Terconazole.

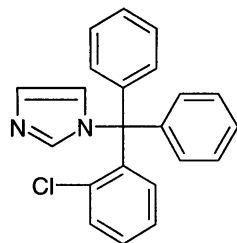
Clotrimazole [LVIII] was developed by investigators of the Bayer Laboratories in 1969, and is utilized topically in superficial mycoses or orally in clinical trials for deep mycoses¹⁰⁶.

Miconazole [LIX] is a well-known antifungal drug developed by the investigators of Janssen Pharmaceutica in 1969. It is utilized topically in superficial mycoses and by oral and intravenous routes in deep mycoses in clinical experimental trials¹⁰⁷.

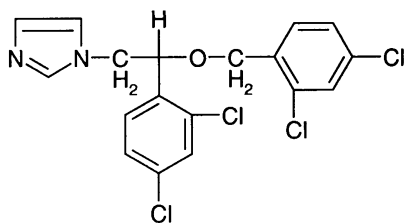
Econazole [LX] is a phenethyl imidazole derivative. Like Miconazole, it is utilized as a topical agent in superficial mycoses and as a systemic antifungal by oral and intravenous routes in deep mycoses¹⁰⁸.

Fluconazole [LXI] 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 potential candidate for prevention and early treatment of disseminated candidiasis in granulocytopenic patients¹⁰⁹.

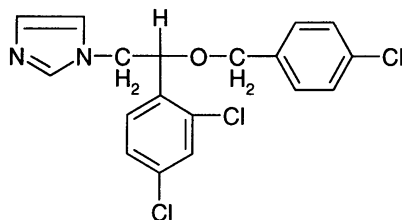
Itraconazole [LXII] is a water-soluble, lipophilic triazole analogue that exhibits excellent activity against most fungal pathogens. When compared with Ketoconazole, its potential advantages include a broader spectrum of activity (including *Aspergillus* sp and *Sporothrix*), a more desirable pharmacokinetic profile and less toxicity¹¹⁰.



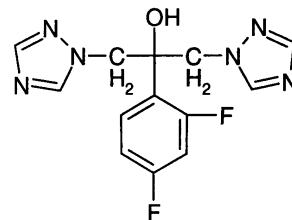
[LVIII]



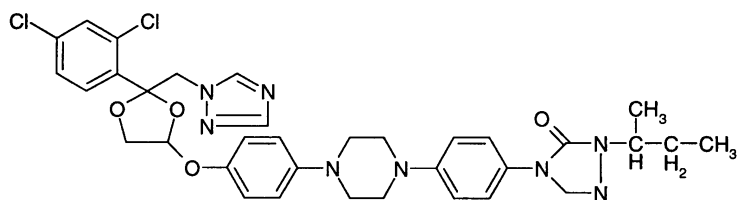
[LIX]



[LX]



[LXI]



[LXII]

Azoles exert antifungal activity by inhibiting cytochrome P-450 that catalyses the 14 α -demethylation of lanosterol to ergosterol. Although the azoles are considered to have a wide antifungal spectrum, individual differences in activity exist. 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

IMPORTANT FACTS

The knowledge acquired so far with the uses of drugs including antibiotics in a variety of conditions lead to certain important facts as noted below:

Classification and Mechanism of action:

Antibacterial drugs are not effective against all micro-organisms. The 'spectrum' of activity of each drug is probably the result of several factors, the chief of which relates to the primary mechanism of action.

The antibacterial agents especially the antibiotics presently available maybe classified into several groups on the basis of their mechanisms of action^{111,112,113}.

1. **Agents inhibiting the synthesis of the bacterial cell wall:** The penicillins, the cephalosporins, cycloserine, vancomycin, restocatin and bacitracin.
2. **Agents affecting the permeability of the cell membrane:** The polymyxins, colistimethate, the polyene antifungal agents aystatin and amphotericin.
3. **Agents primarily inhibiting protein synthesis by their effects on ribosomes:** Chloramphenicol, the tetracyclines, the aminoglycosides, the macrolide antibiotics, erythromycin and oleandomycin, and lincomycin and clindamycin.
4. **Agents affecting nucleic acid metabolism:** Rifampin and nalidixic acid
5. **The antimetabolites:** The sulfonamides, trimethoprim, amino salicylic acid and the sulfones.

Although this classification appears valid on the basis of the available evidence, it may require modification with more definitive information. Besides, the general mechanism of action of a few agents remains unknown.

Adverse reactions to chemotherapeutic agents:

The adverse reactions produced by anti-infective agents are of three general types. The concept of toxic effects and hypersensitivity reactions caused by drugs is same. More distinctive, however, are biological and metabolic alterations in normal microbial flora and supra-infections and interference with nutrition. These effects maybe induced, in varying degree by the antimicrobials¹¹⁴.

Host determinants of response to antimicrobial agents:

Although, the nature of the infection determines the type of therapy, the innate host factors, unrelated to the disorder, are often the prime determinants of not only the type of drug selected but also of its dose, route of administration, risk and nature of untoward effects, and therapeutic effectiveness. Among such factors are age, genetic background, pregnancy, concurrent diseases, allergy, nervous system abnormalities, indigenous microbial flora, hepatic function, renal function and electrolyte balance and host's defense mechanisms¹¹⁵.

Resistance of micro-organisms to antimicrobial agents:

The phenomenon of the development of drug resistance is not universal among either the micro-organisms or the drugs. The development of resistance to an antibiotic involves a stable genetic change, heritable from generation. Any of the mechanism that result in alteration of bacterial genetic composition can operate, and bacteria may thus become resistant to antimicrobial agents by mutation, transduction, transformation or conjugation. The first three mechanisms are particularly involved in the development of drug insensitivity in Gram-

positive cocci, while all four maybe responsible for the acquisition of resistance by Gram negative bacilli.

Concurrent therapy with two or more antimicrobial agents may greatly diminish the speed of developing bacterial resistance, since microorganisms resistant to one drug may still be killed by the other. The classical therapeutic example of this principle is in the drug treatment of tuberculosis. Resistance of the *tubercle bacillus* to a single tuberculostatic compound such as isoniazid, for example, is appreciably delayed when this drug is given together with another effective agent such as streptomycin or ethambutol.

Properties of an ideal chemotherapeutic agent:

The ideal chemotherapeutic should have the following properties. The compound should exhibit selective and effective antimicrobial activity, and it should be bactericidal rather than bacteriostatic. While it might be desirable that an agent kills a broad range of micro-organisms, problems of suprainfection often intervene. Bacteria should not develop resistance to the drug. Its antimicrobial efficacy must not be materially reduced by body fluids, exudates, plasma proteins or tissue enzymes. Characteristics of absorption, distribution, fate and excretion should be such that bactericidal levels in the blood, tissues and body fluids including the cerebrospinal fluid are rapidly reached and maintained for prolonged periods. Excretion of the drug in the urine in bactericidal concentrations is of great value in urinary tract infections, and renal injury must not result from such excretions. Finally, and obviously, the many general characteristics desirable in any pharmacological agent must also be present.

Search for new agents:

Literature reports show very closely that biological activity is very specific with regard to structure. Any change in the structure will significantly alter the activity and quite often may completely remove it.

It also happens that if the important portion is retained, other changes do not materially affect the activity.

Chemical and physical properties play a vital role in the type of activity exhibited. Cationic or anionic molecules show antibacterial properties. The active compounds may act by penetrating cells easily. They may cause lysis of the cell membrane and achieve rapid bactericidal action. Some drugs are capable of bringing about alteration in the membranes of the cell in order to penetrate the cell.

Important enzymes could be blocked by substances whose molecular structure resembled those of their substrates. Notable is the action of sulfonamides on dihydrofolate synthetase. Lysozyme is an enzyme hydrolysing a polysaccharide substrate present in the cell wall.

The chemical constitution as related to activity meant considerations of their pK values, density of electrons and the three dimensional geometry of the structure. The receptor theory, biological antagonism and bioisosterism are the main principles involved in structure-activity relationships. The study of hydrophobic, steric and electronic effects in quantitative terms has enormous potential. These help to understand the forces underlying intrinsic drug activity without knowing anything precisely about the structure of the particular receptor that is involved.

Progress in the field of chemotherapy is invariably made by following up a novel biological observation, which can be explained in terms of action of well defined chemical entities. Drugs with a given group could have two quite opposite actions. One may mimic a natural metabolite while the other may show exactly opposite action.

A new light on structure-activity relationships was provided by an investigation on the antimicrobial properties of 8-Hydroxyquinoline. Neither quinoline nor phenol is at all antibacterial at a dilution of 1:5000 where as 8-Hydroxyquinoline is active at 1:500,000. It is possible that the biological properties could be enhanced by combining the two individual moieties in a single molecule.

Benzothiazole and Benzimidazole moieties have played an important role in medicinal chemistry in the past few decades. Sulfur containing derivatives especially sulfonamides have been the pinnacle of success for almost over a century in the field of progressive pharmacy. Benzamide and thiourea moieties have shown an increase in antimicrobial activity when used in combination with other medicinally important molecules.

Hence it was because of these reasons, the two important molecules i.e benzothiazole (having benzamide and thiourea moiety) and benzimidazole attracted our attention for synthesizing new sulfur derivative compounds having potential antimicrobial activity.

PLAN

OF

WORK

Plan of Work

The discovery of a new drug is made by designing compounds that would effectively interfere with any of vital nutritional, metabolic, and respiratory or other life processes of the invading organisms so as to suppress their further growth, leaving the rest to the body's protective mechanisms, unaffected or intact.

The main approaches to drug design can be of two types:

- 1.) The group or moiety approach
- 2.) The physiochemical approach of the molecule as a whole.

The group of moiety approach implies elimination, introduction or substitution of certain moieties or group in the drug molecule known as molecular modification.

The second concerns the physical properties determining the biological transport of drugs. It deals with the formation of drug enzyme complex i.e., protein binding, especially, plasma protein binding, to induce easy transport for the drug action.

In drug design, the aim must be thorough investigation of well chosen or designed compounds taking into consideration the electronic effect, hydrophobic effect or steric effect. The most widely used mode of approach so far has been the use of some drug of known structure as a model from which other congeners or homologues and analogues are synthesized.

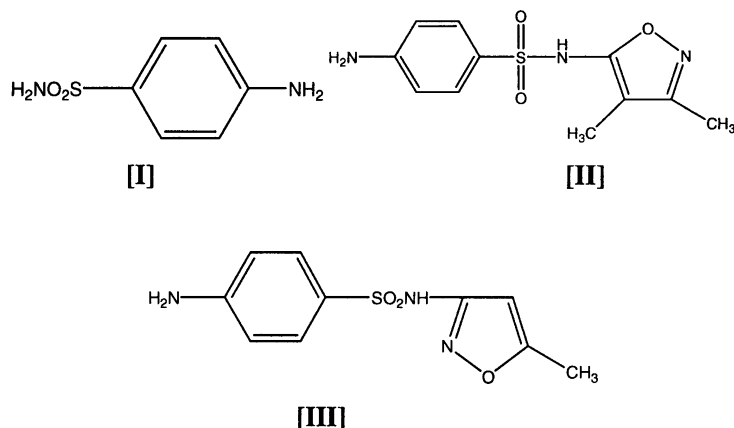
The present work in this thesis has been planned keeping in mind the various chemical structures of existing antibacterial agents and antifungal agents.

For the treatment of bacterial and fungal infections, a number of drugs may be available but they have limited usefulness on account of their toxicity and their ability to combat the emergence of resistance by the invading organism. Thus, there is a definite bridge to be built up by the newer chemotherapeutic agents, which can exhibit broad-spectrum

activity as far as eradication of the infections, toxicity and inexpensiveness of the drug is concerned. Therefore, absolute unrelenting effort is the necessity if the present state of chemotherapy of bacterial and fungal infections.

Series I: Preparation of 2-(4'-amino-3'-methylphenyl)sulfonamido-5,6-disubstituted benzothiazoles.

Sulfonamides were the first, systemic chemotherapeutic agents employed to prevent and cure bacterial infections. They have a wide range of antibacterial activity against both Gram-positive and Gram-negative bacteria. The important members of this class include sulfonilamide [I], sulfamethoxazole [II], sulfisoxazole [III].

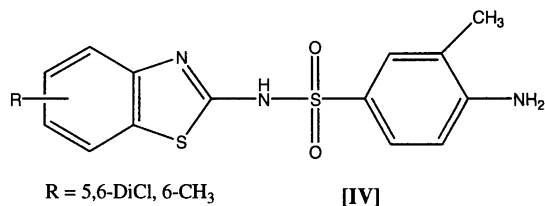


In the above mentioned drugs, the sulfonamide moiety is considered to be very essential for their activity against bacterial infections.

Among the various types of known heterocyclic ring systems, benzothiazole nucleus is found to be present in various medicinally

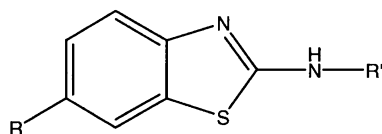
important compounds having antiparasitic^{116,117}, antibacterial^{118,119}, antiviral¹²⁰, antitubercular^{121,122} and antifungal¹²³ activity.

Taking a lead from these known facts, it was proposed to synthesize various biologically active compounds by incorporating sulfonamide moiety at the 2nd position of 5,6-disubstituted & 6-substituted benzothiazoles. These compounds [IV] were then checked for their activity against bacterial as well as fungal infections.



Series II: Synthesis of 2-(4'-Substituted benzamido-3'-methylphenyl)sulfonamido-5,6-disubstituted benzothiazoles

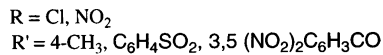
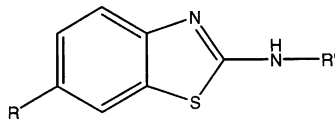
2-Substituted benzamido benzothiazoles are useful as bacteriocides and fungicides. Benzothiazole is a well known established antifungal as well as bactericidal moiety in fat, paraffin and rubber industries¹²⁴. The amido linkage is much important for retaining the activity in the lead molecule [V]



R = Cl, H
R' = 4-ClC₆H₄CO-

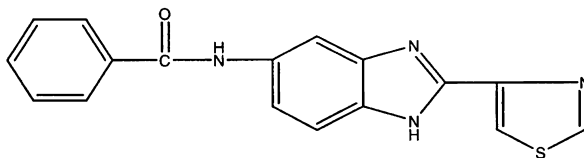
[V]

The 6-substituted-2-substituted benzothiazolyl amido derivative [VI] showed good activity against *Streptococcus* S-86 at 25µg/ml concentration¹²⁵.



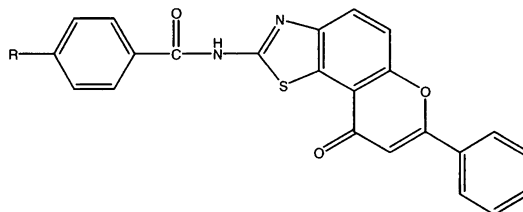
[VI]

D.A Denham *et. al.*¹²⁶ reported excellent antifilarial activity of 5-benzamido-2-(4-thiazolyl) benzimidazole [VII].



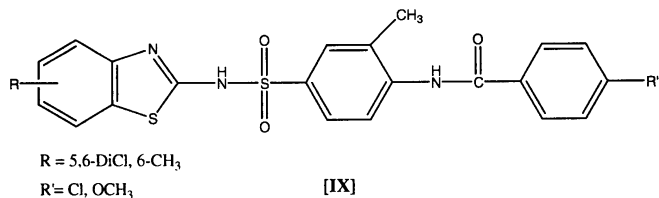
[VII]

Recently, Mantripragada Narayenu Sambaiah Thote¹²⁷ has reported a lead molecule [VIII] containing the benzothiazole moiety and having benzamido substitution in position-2.



[VIII]

All the above examples indicate the importance of the amido linkage in biologically active moieties. Taking into account the above mentioned facts, it was decided to synthesize new molecules [IX] where the active benzothiazole moiety is substituted in position-2 with substituted benzamido group, which will be further studied for their antibacterial and antifungal activity.

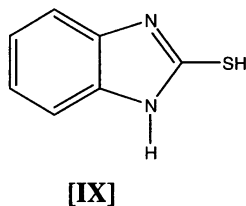


Series III : Synthesis of 2-(4'-acetamido-3'-methylphenyl)sulfonyl-2-ylmercapto-5,6-disubstituted benzimidazoles

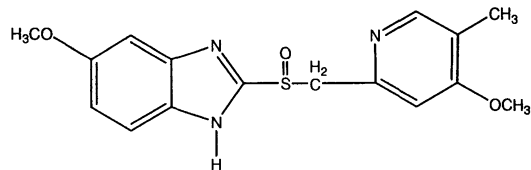
Benzimidazole is one of the oldest and chemically well-studied nitrogen heterocyclics. It has been quite lately recognised as the versatile nucleus for building molecules possessing broad spectrum of biological activity such as powerful anthelmintic, antimicrobial, pesticidal, antimalarial, anticancer,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

Benzimidazoles have also been reported to be an important group of systemic fungicides currently in use for controlling the plant diseases¹²⁸.

2-Mercapto benzimidazole [IX] and its derivatives have been reported in literature for their antibacterial and antifungal properties^{129,130}.

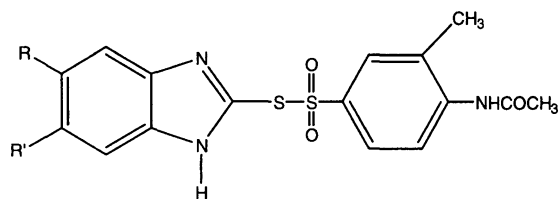


5-methoxy-2-mercapto benzimidazole has been found as an intermediate for *Omeprazole* [XI]¹³¹ which is a potent antiulcer agent and is used in the treatment of Zollinger Ellison syndrome



[XI]

From the above literature findings and taking into consideration the importance of benzimidazole nucleus, it was decided to synthesize compounds of the type [XII] and be tested for their potency against bacterial and fungal infection.

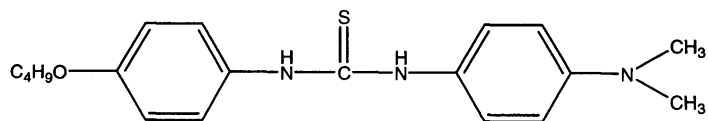


R = Naphthoxy, Methoxy
R' = Cl, H.

[XII]

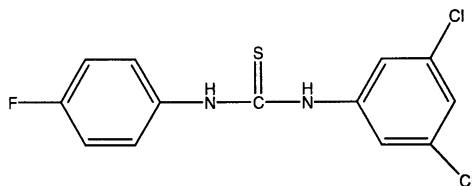
Series IV: Synthesis of 2-(4'-phenylthiourea-3'-methylphenyl) sulfonamido-5,6-disubstituted benzothiazoles

1,3-Disubstituted thioureas are well documented in literature for their biological activity eg: *Thiambutsine*¹³² [XIII]



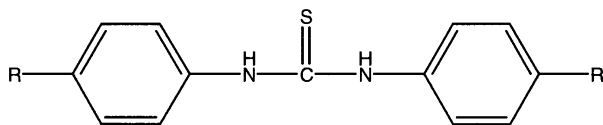
[XIII]

Thioureas not only possess antitubercular & antileprotic activity but they show significant antifungal activity as well. *Fluonilide*¹³³ [XIV] is a well-known antifungal agent, which is simple substituted thiourea.



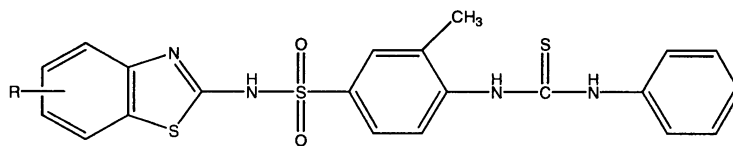
[XIV]

Mayer *et. al.*¹³⁴ have reported few diphenyl thiourea derivatives of general structure [XV], the activity of which exceeds the activity of streptomycin.



[XV]

Taking all the above facts into consideration and keeping in mind the biological importance of the benzothiazole and sulfonamide groups discussed previously, it was decided to synthesize thiourea derivatives [XVI] incorporated with sulfonamide group at the second position of the benzothiazole molecule, which will be then tested for its antibacterial as well as antifungal activity.



R = 5,6-DiCl, 6-CH₃

[XVI]

Series I:

Synthesis of 2-(4'-amino-3'-methylphenyl)sulfonamido-5,6-disubstituted benzothiazoles.

[4-Amino-N-(5,6-disubstituted-benzothiazol-2-yl)-3-methyl-benzenesulfonamide]

- 1) *Chemistry of Benzothiazoles*
- 2) *Chlorosulfonation of Acetanilides*
- 3) *Schematic Representation*
- 4) *Experimental*

thiocyano group (-SCN) through the use of thiocyanogen (SCN)₂ is commonly referred as thiocyanation.

Since the reagent is extremely unstable, certain precautions must be observed for its use in the reactions. Thiocyanogen is a liquid which on cooling forms a colourless crystalline solid melting between -3°C to -2°C. At room temperature it polymerises rapidly to a reddish orange, amorphous mass of indefinite composition known as pseudothiocyanogen. Although, relatively stable in inert dry solvents, thiocyanogen may polymerise in solution, especially, under the catalytic effect, influence of heat, light and moisture.

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

METHODS FOR THE USE OF THIOCYANOGEN IN SUBSTITUTION REACTIONS

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

- i) free thiocyanogen in organic solvents ;
- ii) thiocyanogen liberated gradually in organic solvents from a metal thiocyanate by various reagents.

FREE THIOCYANOGEN

This method was first to be employed and is still a useful procedure when the reaction involved is slow, as in addition reactions or when the product is difficult to purify. Usually the only contaminant of the product other than the starting material is polythiocyanogen, which is entirely insoluble in water and organic solvents.

The reagent is prepared by the action of an oxidising agent upon thiocyanic acid in an organic solvent and is accomplished by means of such reagents as lead tetra acetate, lead peroxide or manganese dioxide¹³⁵, but the yield is so low that the preparation of metal

thiocyanate is much to be preferred. Lead thiocyanate reacts rapidly and quantitatively with bromine to form thiocyanogen and lead bromine, which is removed rapidly by filtration. Halogen carriers such as phenyliodo chlorides¹³⁶, sulfuryl chlorides¹³⁷ or certain N-chloroamides^{138, 139, 140} can also be used but do not appear to possess any advantages over bromine or chlorine.

Solvents that have been used with thiocyanogen include benzene, bromobenzene, carbon tetrachloride, chloroform, ether, ethylene bromide, carbon disulfide, petroleum ether, methyl acetate, nitromethane, anhydrous formic acid and acetic acid. At low temperature such solvents as saturated solutions of alkali thiocyanates in methanol^{141, 142} or acetone¹⁴³ can be used. The yield in the thiocyanation of amine is 20%-30% higher when the reaction is carried out in a neutral medium like methanol rather than in acetic acid. The formation of a thiazole is also inhibited in a neutral solvent. Ether is usually not satisfactory because the solvent is attacked and because some of the amine is precipitated as the thiocyanate^{144, 145}.

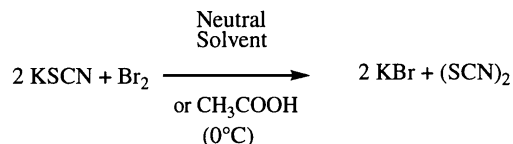
Moisture must be excluded from thiocyanation solution in order to prevent hydrolysis. Another troublesome side reaction, particularly in concentrated solutions is polymerisation, which is induced by light, heat and the presence of hydrolysis products. Polymerisation is reported to be dependent upon the dielectric constants of the solvents^{144, 146, 147}. Stirring is said to retard polymerisation. For the usual synthetic reaction the preferred procedure is to generate thiocyanogen in a solution of the substance to be thiocyanated at a rate equal to the rate of the removal by reaction. The low concentration of the reagent, maintained in this way minimises polymerisation. The reagent is generated from the thiocyanate salts by chemical reactions.

THIOCYANOGEN GENERATED FROM SALTS BY CHEMICAL

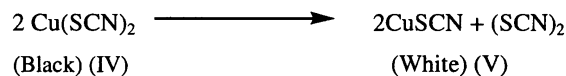
REACTIONS:

In this method the alkali metal thiocyanate (III) is dissolved in acetic acid or formic acid^{147,148}, or better in neutral solvent like methyl acetate, acetone or methanol^{141,149} and then the compound to be thiocyanated is introduced to the above solution. Bromine or chlorine is added to the cooled solution at such a rate that the thiocyanogen reacts as fast as it is liberated. The neutral solvent that is susceptible to attack by halogen is protected by saturating the solution with an appropriate alkali metal halide or by using a large excess of an alkali metal thiocyanate in the reaction mixture. Other reagent for producing thiocyanogen from potassium thiocyanate have been described.

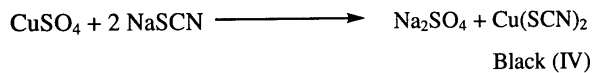
The chemical transformation can be written as below:-



Cupric thiocyanate¹⁵⁰, the use of which may be considered as still another modification of this general procedure, shows promise of being effective. It releases thiocyanogen merely by the dissociation of the cupric (IV) to cuprous salt (V).



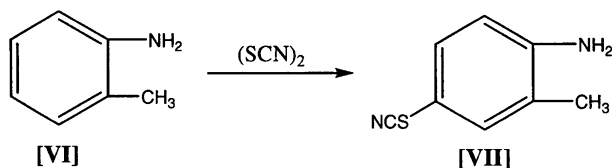
Cupric thiocyanate (IV), prepared in advance, or a paste of cupric sulphate and sodium thiocyanate in equivalent proportions is added to a solution of the compound in methanol or acetic acid and the mixture is warmed to 35°-80°C, until the black cupric thiocyanate has changed completely to the white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



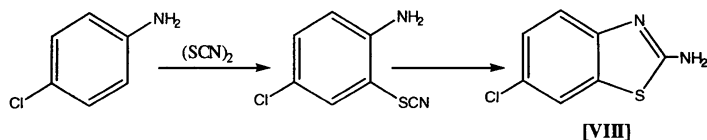
This procedure has the advantage over the others of permitting higher temperatures for thiocyanation.

THIOCYANATION OF AROMATIC AMINES:

The thiocyanation group (-SCN) is introduced into aromatic amines with rapidity. It enters preferentially at a free *para* position if available, otherwise at ortho position. For example, o-toluidine [VI] is converted to 4-thiocyano-o-toluidine [VII] (80% yield)¹⁵¹.

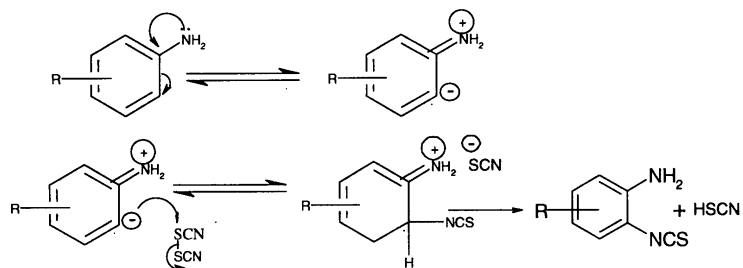


The thiocyanation takes place in the position ortho to the primary amine group. The final product in such cases is often an amino benzothiazole formed by the secondary reaction between the amino group and the adjacent thiocyano group. In this secondary reaction, since the amino group is having lone pair of electrons, it will be added to the C=N bonds of the thiocyano group to give resultant 2-amino-6-substituted benzothiazole [VIII]



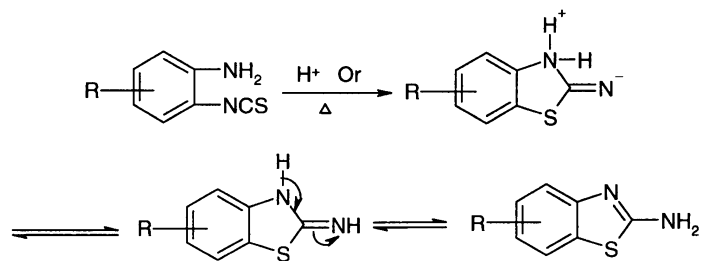
Mechanism:

The mechanism of thiocyanogen reaction probably consists of the initial attack of thiocyanogen to the activated site of 4-substituted aniline where due to the mesomeric effect of amino group, the ortho position is having surplus of electrons which rendered the site to act as a good electron rich centre, to give an intermediate species as:-



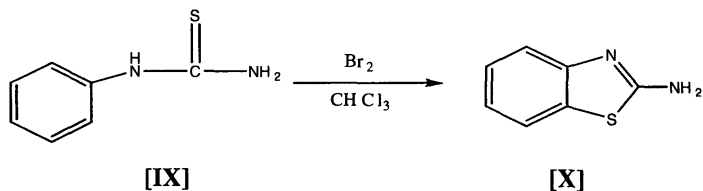
The unstable intermediate species immediately loses a proton to retain the aromatic character of the molecule which gives higher stability.

The intermediate, under the influence of acid and heating adds an amino function to the adjacent thiocyano group as:



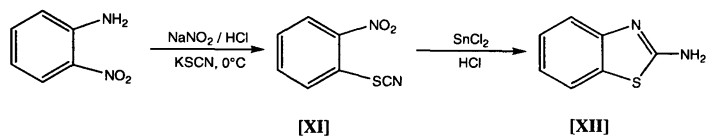
HUGERSCHOFF SYNTHESSES:

This synthesis is restricted to the preparation of 2-amino derivatives of the benzothiazoles [X] and employs the action of oxidising agent such as bromine, upon aryl thioureas [IX] in an inert solvent such as chloroform, ethylene dichloride, chlorobenzene or acetic acid. Sulfuryl chloride can be substituted for bromine¹⁵²⁻¹⁵⁴. However, potassium ferrocyanide in alkaline media, the reagent for the Jacobson ring closure, gives no thiazole from the arylthiourea^{155,156}.



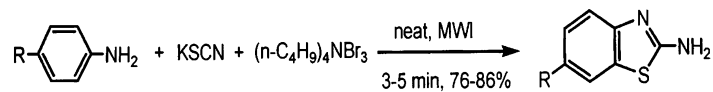
Generally, the yields in the Hegerschhoff's reaction are excellent and frequently nearly quantitative.

2-Amino benzothiazoles [XII] have been prepared from o-thiocyano-nitro compounds of the type [XI] by reduction using stannous chloride in HCl and FeSO₄ in ammonia^{157,158}.



NEWER METHODS:

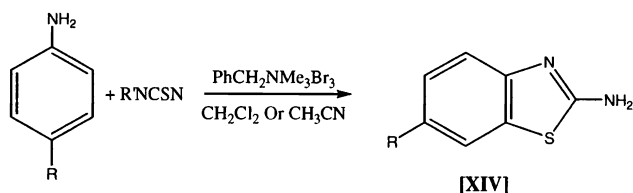
1) 2-Aminobenzothiazoles [XIII] were efficiently synthesized by Zheng *et. al.*¹⁵⁹ using stable, crystalline tetrabutylammonium tribromide instead of toxic, corrosive liquid bromine under solvent-free and microwave irradiation condition.



R = Cl, OCH₃

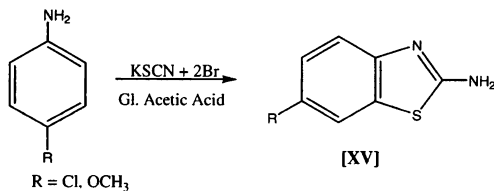
[XIII]

2) Jordan *et. al.*¹⁶⁰ prepared 2-aminobenzothiazole [XIV] derivatives using benzyltrimethylammonium tribromide (PhCH₂NMe₃Br₃), which is a stable, electrophilic bromine source for the conversion of substituted arylthioureas to 2-aminobenzothiazoles under mild conditions in a variety of solvents with good yields. One of the key benefits for this reagent when compared with molecular bromine in ease of addition and handling, which minimizes the risk of forming brominated side products. They have extended the use of this reagent to a direct, one-pot synthesis of 2-aminobenzothiazoles from either aryl isothiocyanate and anilines or tetrabutylammonium thiocyanate and anilines in the presence of a stoichiometric amount of Ph-CH₂NMe₃Br₃.



[XIV]

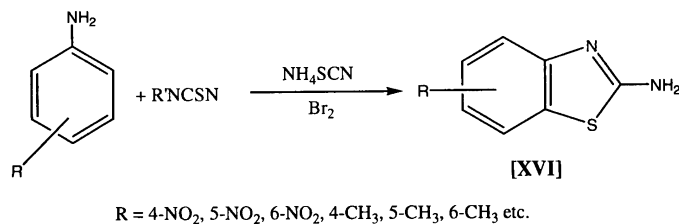
3) Matsui *et. al.*¹⁶¹ prepared 6-substituted-2-aminobenzothiazoles [XV] by the reaction of 4-substituted anilines with potassium thiocyanate in presence of bromine.



R = Cl, OCH₃

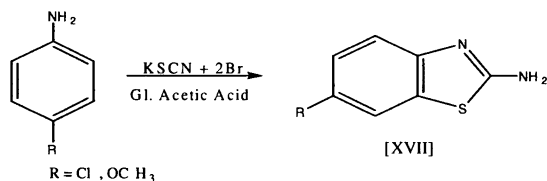
[XV]

4) Patel and Agraval¹⁶² synthesized various 4(5 or 6)-substituted-2-aminobenzothiazoles [XVI] with help of 4(5 or 6)-substituted anilines which was reacted with ammonium thiocyanate and bromine.

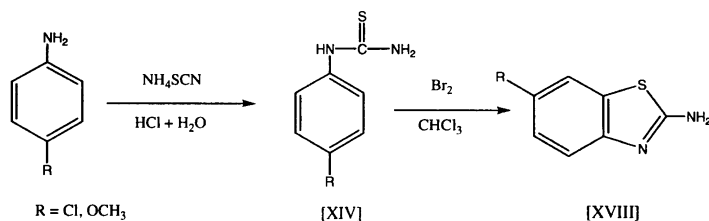


5) Jimonet *et. al.*¹⁶³ synthesized various substituted-2-benzothiazolamines derivatives by different methods:

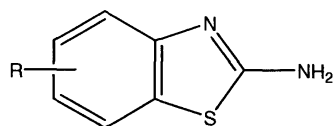
Method A: One-pot reaction of appropriate anilines with thiocyanogen generated from bromine and alkaline thiocyanate in acetic acid medium led to formation of the desired product [XVII] in good to moderate yields.



Method B: In this method cyclization of phenylthiourea [IX] with bromine in chloroform leads to the formation of 2-amino benzothiazole [XVIII].



In the present study, the raw materials *viz.* 2-amino substituted benzothiazoles [XIX] are synthesized by thiocyanating substituted anilines with KSCN and bromine in acetic acid at cold conditions, according to the literature methods¹⁶³.

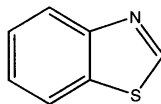


R = 5,6-DiCl, 6-CH₃, etc

[XIX]

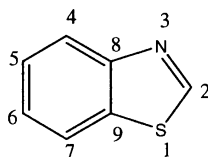
CHEMISTRY OF BENZOTHAZOLES

The benzothiazole ring contains a phenyl ring fused to a thiazole ring as indicated in the structure [I]



[I]

These compounds were formed only by compounds in which the nitrogen and sulfur atoms were ortho to each other. They were expressed as derivatives of o-amino thiophenols. The numbering system for benzothiazole [II] is as follows:



[II]

SYNTHESIS OF 2-AMINO-6-SUBSTITUTED BENZOTHAZOLES & 2-AMINO-5,6-DISUBSTITUTED BENZOTHAZOLES

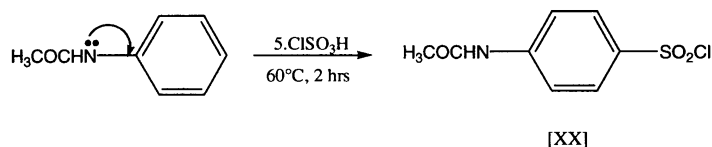
TYPE – A (THIOCYANATION REACTION):

Thiocyanation is one of the many organic reactions useful to synthetic organic chemist. It is a simple electrophilic reaction showing close similarity with halogenation. Therefore, the reaction is often classified as pseudohalogenation and the reagent as pseudohalogen. As far as chemical reactivity is concerned the thiocyanogen behaves exactly similar as halogen. The direct substitution of hydrogen atom by a

CHLOROSULFONATION OF ACETANILIDES

Aromatic amines are often protected by acetylation before chlorosulfonation because reaction of the resultant acetanilides reduces decomposition of the product. An important example is the chlorosulfonation of acetanilide to form 4-(acetamido)benzenesulfonyl chloride [XX]. Smiles and Stewart¹⁶⁴ reported that the synthesis went in excellent yield (80%) by treatment of acetanilide with the reagent (five equivalents) at 60 °C for 2 hours.

The reaction is of considerable commercial importance because 4-(acetamido) benzenesulfonyl chloride is the precursor of a range of sulfonamide antibacterial and other drugs¹⁶⁵.



In the above chlorosulfonation, the large steric size of the acetamido group effectively precludes o-sulfonation, so the p-sulfonyl chloride is essentially the sole product isolated. Studies of the chlorosulfonation of acetanilide with excess chlorosulfonic acid (five equivalents) in the temperature range 0 to 100°C indicated that the optimum temperature for the production of the monosulfonyl chloride was 55-60°C. Some disulfonation occurred > 40°C, polysulfonation > 70°C and even at 100°C (2 hours), the reaction gave no sulfone¹⁶⁶.

Improvements in the manufacture of acetylsulfanilyl chloride, involved the recovery of sulfuric acid¹⁶⁷, use of more reagent (six equivalents) at 40°C (3 hours) and subsequent treatment with dilute sulfuric acid afforded 84% yield¹⁶⁸.

The quantity of chlorosulfonic acid employed may be reduced to 2.3-3.1 equivalents of reagent to substrate reacted at 30-32°C for 40-60

minutes followed by addition of further reagent to give a reagent to substrate ratio of 4.87:1 followed by stirring the reaction mixture at 40-42°C¹⁶⁹. Reaction of acetanilide with chlorosulfonic acid in the presence of benzene < 95°C afforded a mixture of the desired sulfanilyl chloride and benzenesulfonic acid¹⁷⁰. The chlorosulfonation of acetanilide by excess chlorosulfonic acid occurs in two stages and the kinetics has been determined¹⁷¹. In the reaction, the product yield was found to be inversely proportional to the concentration of the byproducts i.e. hydrogen chloride and sulfuric acid. The use of a mixture of chlorosulfonic acid and excess sulfur trioxide enhanced the yield of product by formation of pyrosulfuryl chloride which reacted with the byproducts to form chlorosulfonic acid¹⁷².

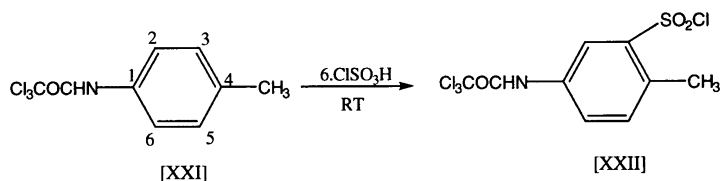
Recent studies¹⁷³ showed that in the chlorosulfonation of acetanilide, the molar excess of the reagent could be reduced to 3.48 without effecting the quality or yield (> 77.4%) of the product. Acetanilide has also been reacted with chlorosulfonic acid (4.8 equivalents) at 50 ± 2 °C, followed by hydrolysis, extraction and crystallization to yield dry N⁴-acetylsulfanilyl chloride.

Several substituted acetanilides have also been treated with chlorosulfonic acid, thus 2- and 3-chloro; 2,5- and 2,6-dichloroacetanilides reacted with excess reagent to give the corresponding 4-sulfonyl chlorides¹⁷⁴.

With 3-chloroacetanilide, the crude sulfonyl chloride could be characterized as the hydrazide¹⁷⁴, or purified by recrystallization from benzene giving 18% yield¹⁷⁵. The 2- and 3-chloroacetanilides reacted with reagent (four to five equivalents) at 60-80°C (4 hours) to give low yields of the sulfonyl chlorides, but chlorosulfonation of the 2,5-dichloro analogue demanded prolonged reaction at 80°C (8 hours), while the 2,6-derivative needed even longer reaction time (14 hours). Surprisingly, all attempts to chlorosulfonate 2,3-dichloroacetanilide failed, possibly due to a 'buttressing effect' of the bulky acetamido group on the 2-chlorine atom¹⁷⁴. Side chain substituted α -mono-, α,α ,di- and

α -trichloroacetanilides reacted smoothly with excess chlorosulfonic acid (five equivalents) at 60°C to give excellent yields of the corresponding p-sulfonyl chlorides¹⁷⁶.

α -Bromoacetanilide-p-sulfonyl chloride is used as an amide-linking agent¹⁷⁷. p-Methyl- α,α,α -trichloroacetanilide [XXI] reacted with excess reagent (six equivalents) at room temperature (8 hours) to give the sulfonyl chloride (XXII, 50%)¹⁷⁶.

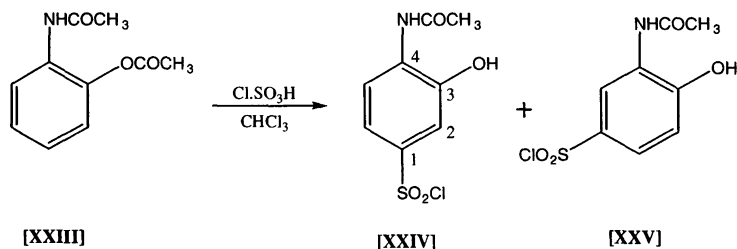


In this reaction, chlorosulfonation occurs *ortho* to the methyl group, since the relatively large size of the trichloroacetamido group precludes attack in the 2-position. The introduction of the powerful electron-donating methoxy group into acetanilide greatly facilitates chlorosulfonation. 2- and 4-Methoxyacetanilide both reacted easily with chlorosulfonic acid to give excellent yields of the corresponding 5- and 3-sulfonyl chlorides. Sulfonation has occurred respectively *p*- and *o*- to the methoxy group which clearly controls the orientation of sulfonation. In contrast, chlorosulfonation of 3-methoxyacetanilide afforded only a moderate yield of the 4-sulfonyl chloride¹⁷⁸.

2-Methyl-, 2-methoxy- and 4-methylacetanilide all reacted with chlorosulfonic acid in boiling chloroform to give good yields of the corresponding sulfonyl chlorides. Several of the derived sulfonyl hydrazides showed antibacterial activity¹⁷⁹.

2,5-Dimethoxyacetanilide reacted with chlorosulfonic acid (three equivalents) at 60°C (1 1/2 hours) to yield the 4-sulfonyl chloride (53%)¹⁶⁹. Later, other workers¹⁸⁰ discovered that a much improved yield

(93%) of the product (used in the dye industry) was obtained by prolonged treatment of 2,5-dimethoxyacetanilide with a mixture of chlorosulfonic acid and thionyl chloride. 2-Acetoxyacetanilide [XXIII], by warming with the reagent (five equivalents) in chloroform at 50-60°C (2 hours), suffered o-deacetylation yielding a mixture of the sulfonyl chlorides [XXIV] and [XXV] (79%)¹⁸¹. NMR spectroscopy showed that the major product was 4-acetamido-3-hydroxybenzenesulfonyl chloride, indicating that the acetamido moiety was the predominant directive influence on the orientation of sulfonation rather than the hydroxyl group. This result may be explained in terms of hydrogen bonding between the carbonyl and hydroxyl groups¹⁸¹. 4-Acetoxyacetanilide with chlorosulfonic acid (five equivalents) at 60-70°C (2 hours) afforded 5-acetamido-2-hydroxybenzenesulfonyl chloride (50%)¹⁸¹.



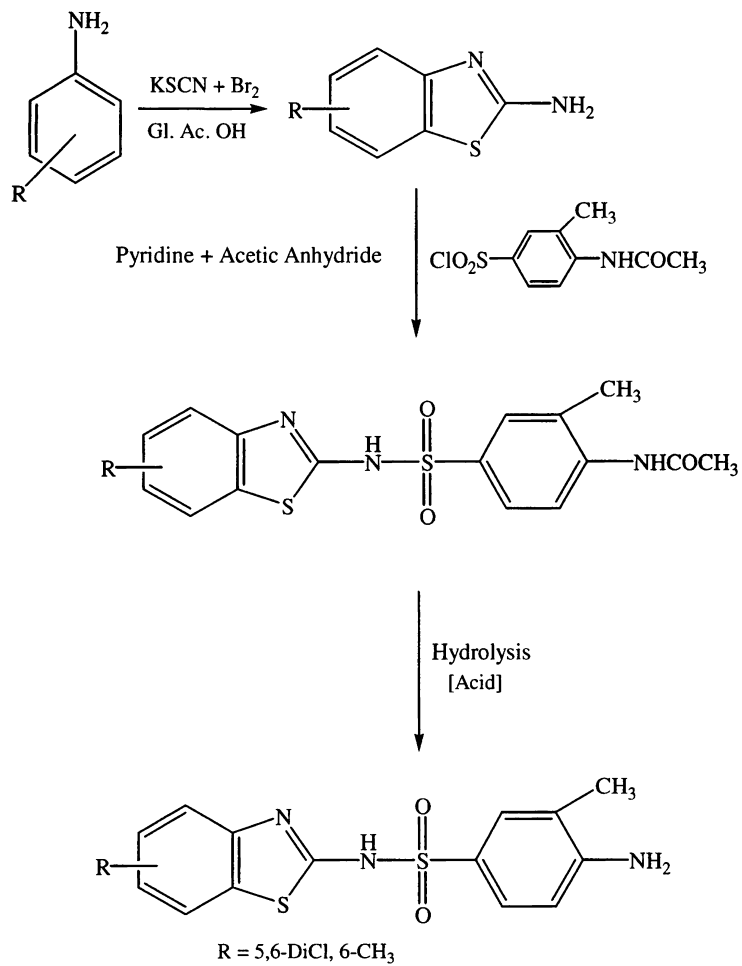
In this example, sulfonation occurs in the 3-position (*ortho* to the hydroxyl group) since the large acetamido group inhibits 2-sulfonation. Attempted chlorosulfonation of 3-acetoxyacetanilide failed; the reaction only gave black tars¹⁸¹. 2,4-Dimethylacetanilide reacted with chlorosulfonic acid to give 5-acetamido-2,4-dimethyl benzenesulfonyl chloride (69%)¹⁸².

Sulfonation occurred, as expected, o-/p- with respect to the methyl groups since the position *para* to the acetamido group is blocked. 3,5-Dimethylacetanilide reacted with excess reagent (four equivalents) at 90°C (3 hours) to give 4-acetamido-2,6-dimethylbenzenesulfonyl

chloride which is used in the preparation of aldose reductase inhibitors^{183,184}.

1-Acetamidonaphthalene reacts with chlorosulfonic acid (three equivalents) at 60-65°C for 2 hours to yield 4-acetamidonaphthalenesulfonyl chloride (45%)¹⁸⁵. Previous workers¹⁸⁶, by performing the chlorosulfonation at 80°C (1 hour), obtained a lower yield of the product (31%).

Schematic Representation of SERIES- I



Experimental:

- 1) Preparation of 2-aminosubstituted benzothiazoles.
- 2) Preparation of 2-methyl acetanilide.
- 3) Preparation of 4-acetamido-3-methylbenzenesulfonyl chloride.
- 4) Preparation of 2-(4'-acetamido-3'-methylphenyl) sulfonamido - substituted benzothiazoles.
- 5) Preparation of 2-(4'-amino-3'-methylphenyl) sulfonamido-substituted benzothiazoles.

1) Preparation of 2-amino substituted benzothiazoles:

In a 500 ml three necked flask equipped with a mechanical stirrer, short air condensor, and dropping funnel, was placed a solution of 3,4-dichloroaniline (17.8 gm; 0.11 mol) in 150 ml of glacial acetic acid. Then a solution of potassium thiocyanate (25.76 gm; 0.265 mol) in 100 ml glacial acetic acid was introduced in it, stirred this mixture well. The temperature of the reaction mixture was maintained between 10–15°C. using ice-bath.

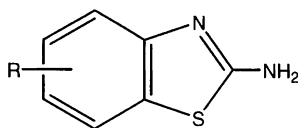
A mixture of bromine (20.0 gm, 6.41 ml; 0.25 mol) in 60 ml glacial acetic acid was introduced dropwise, with stirring over a period of 2–3 hours. The temperature of the reaction mixture was maintained between 5–10°C throughout the addition. Stirred for 18–22 hours after complete addition at room temperature. Then the reaction mixture was warmed on water-bath, diluted with 400 ml of hot water, and filtered. Cooled the filtrate and neutralized with saturated sodium carbonate solution. Crude 2-amino-5,6-dichloro benzothiazole was precipitated out. It was then recrystallized from ethanol.

Yield: 16.2 gm (67.2%);

M.P.: 186°C, Lit¹⁸⁹ M.P.: 185–190

Similarly, other 2-aminosubstituted benzothiazoles were prepared during the course of this work and the details have been mentioned in Table-I

Table-I



R	Yield (%)	M.P (°C)	Lit. M.P(°C)
6-CH ₃	64.1%	135	132.5–133.5 ¹⁸⁷
6-OCH ₃	66.3%	164	165-167 ¹⁸⁸
5,6-DiCl	67.2%	187	185–190 ¹⁸⁹
5-Cl, 6-F	55.6%	218	217–220 ¹⁹⁰
6-Cl	67.3%	198	199-200 ¹⁹¹
6-OC ₂ H ₅	65.5%	163	161 ¹⁹²
6-NO ₂	64.8%	249	247–248 ¹⁹³
6-Br	62.4%	210	212 ¹⁹⁴

2-amino-4,6-difluorobenzothiazole was available in the lab procured from Sigma-Aldrich.

2) Preparation of 2-methyl acetanilide:

O-toluidine (26.6 ml; 0.249 mol), Acetic anhydride (25.4 ml; 0.249 mol), glacial acetic acid (15 ml, 0.249 mol) and 0.1 gm of zinc dust were taken in a 500 ml round bottom flask equipped with a reflux condenser. The mixture was refluxed for 30 minutes and the hot liquid was poured in a thin stream into one litre beaker containing 500 ml of cold water, whilst the stirring was continued. The crude product was separated out. It was filtered at the suction pump and washed with a little cold water. It was recrystallized from ethanol and air-dried.

Yield: (29.8 g; 80.32%)

M.P.: 109°C (Lit.¹⁹⁵: 110°C)

3) Preparation of 4-acetamido-3-methylbenzenesulfonyl chloride:

In a 500 ml round bottom flask, fitted with a mechanical stirrer is placed 29 gm (16.5 ml; 0.25 mol) of chlorosulfonic acid. The flask is surrounded by a cooling bath and cooled with running water to *about* 12–15°C. To the chlorosulfonic acid, was added gradually 14.9 gm (0.1 mol) of 2-methyl acetanilide. This required about fifteen minutes, if the temperature is maintained at approximately 15°C. Since large volume of hydrogen chloride is evolved, the reaction was conducted inside the fuming hood. After all the 2-methyl acetanilide has been added, the mixture was heated to 60°C for two hours to complete the reaction.

The syrupy liquid was poured slowly, with stirring into 1 kg of ice to which just enough water has been added to make stirring easy. The decomposition of the excess chlorosulfonic acid was carried out in the hood. The solid sulfuryl chloride which separated was collected on a suction funnel and was washed with water.

The crude material was dried by pressing on a porous plate and then recrystallized from dry benzene. It formed into thick colourless prisms.

Yield: 19.7 gm (79.4%)

The above compound being highly unstable in nature was not characterized and was instantly used up for further reactions¹⁹⁶.

4) Preparation of 2-(4'-acetamido-3'-methylphenyl) sulfonamido -
5,6-dichloro benzothiazoles:

In a 100 ml round bottom flask equipped with double wall condenser with a guard tube, was placed a solution of 2-amino-5,6-dichloro benzothiazole (2.19 g; 0.01 mol) in pyridine (4 ml, dry) and acetic anhydride (20 ml). To this mixture was added 4-acetamido-3-methylbenzenesulfonyl chloride (2.48 gm; 0.01 mol). The reaction mixture was refluxed for 2 hours on water bath. The reaction mixture was then poured onto 20 ml of water; the solid obtained i.e 2-(4'-acetamido-3'-methylphenyl) sulfonamido-5,6-dichloro benzothiazole was filtered and recrystallized from dil. ethanol (80%).

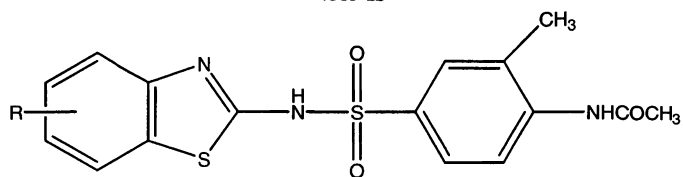
Yield: (2.68 g; 62.3 %)

M.P.: 257°C

Elemental Analysis:

	C %	H %	N %
Calculated	44.66	3.04	9.76
Percentage	44.92	3.26	9.37

Similarly, other derivatives were prepared by the above method and their physical data have been mentioned in Table-II



Sr. No.	R	M.P (°C)	Yield (%)	Mol. Wt.	Mol. Formula	Elemental Analysis (C, H, N)					
						Calculated			Found		
						C%	H%	N%	C%	H%	N%
1	6-OCH ₃	216	62	391	C ₁₇ H ₁₇ N ₃ O ₄ S ₂	52.16	4.38	10.73	51.91	4.10	10.44
2	6-CH ₃	212	58	375	C ₁₇ H ₁₇ N ₃ O ₃ S ₂	54.38	4.56	11.19	54.66	4.32	10.89
3	5,6-DiCl	257	62	430	C ₁₆ H ₁₃ Cl ₂ N ₃ O ₃ S ₂	44.66	3.04	9.76	44.92	3.26	9.37
4	5-Cl, 6-F	232	56	414	C ₁₆ H ₁₃ ClFN ₃ O ₃ S ₂	46.43	3.17	10.15	46.11	3.53	10.38
5	6-Cl	232	61	396	C ₁₆ H ₁₄ ClN ₃ O ₃ S ₂	48.54	3.56	10.61	48.18	3.25	10.22
6	6-Br	205	59	440	C ₁₆ H ₁₄ BrN ₃ O ₃ S ₂	43.64	3.20	9.54	43.31	3.51	9.71
7	6-NO ₂	292	57	406	C ₁₆ H ₁₄ N ₄ O ₅ S ₂	47.28	3.47	13.78	46.92	3.12	13.49
8	6-OC ₂ H ₅	210	59	405	C ₁₈ H ₁₉ N ₃ O ₄ S ₂	53.32	4.72	10.36	53.09	4.55	10.12
9.	4-F, 6-F	185	58	397	C ₁₆ H ₁₃ F ₂ N ₃ O ₃ S ₂	48.35	3.30	10.57	48.18	3.06	10.24

5) Preparation of 2-(4'-amino-3'-methylphenyl) sulfonamido -5,6-dichloro benzothiazole:

In a 50 ml round bottom flask fitted with a water condenser, was placed 2-(4'-acetamido-3'-methyl phenyl) sulfonamido-5,6-dichloro benzothiazole (0.86 g; 0.002 mol) and a solution of 50 ml glacial acetic acid (80%) was added to it. This reaction mixture was refluxed for 4-6 hours with occasional shaking and the resultant clear solution was filtered and was diluted with ice-cold water (50 ml) and was further neutralized with liq. ammonia. The compound was then made free from traces of glacial acetic acid (if any) by stirring it in a sodium carbonate solution (5-10%). The compound was then recrystallized from absolute alcohol.

Yield: (0.494 g; 63.6 %)

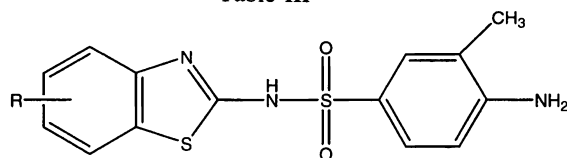
M.P.: 249°C

Elemental Analysis:

	C %	H %	N %
Calculated	43.30	2.86	10.82
Percentage	43.68	2.55	10.49

Similarly, other derivatives were prepared by the above method and their physical data have been mentioned in Table-III

Table-III



Sr. No.	R	M.P (°C)	Yield (%)	Mol. Wt.	Mol. Formula	Elemental Analysis (C, H, N)					
						Calculated			Found		
						C%	H%	N%	C%	H%	N%
1	6-OCH ₃	222	60	349	C ₁₅ H ₁₅ N ₃ O ₃ S ₂	51.56	4.33	12.03	51.22	4.04	11.79
2	6-CH ₃	203	62	333	C ₁₅ H ₁₅ N ₃ O ₂ S ₂	54.03	4.53	12.60	53.81	4.19	12.36
3	5,6-DiCl	249	63	388	C ₁₄ H ₁₁ Cl ₂ N ₃ O ₂ S ₂	43.30	2.86	10.82	43.68	2.55	10.49
4	5-Cl, 6-F	244	59	372	C ₁₄ H ₁₁ ClFN ₃ O ₂ S ₂	45.22	2.98	11.30	45.59	2.67	10.96
5	6-Cl	218	63	354	C ₁₄ H ₁₂ ClN ₃ O ₂ S ₂	47.52	3.42	11.82	47.16	3.09	11.48
6	6-Br	195	62	398	C ₁₄ H ₁₂ BrN ₃ O ₂ S ₂	42.22	3.04	10.55	42.57	2.78	10.20
7	6-NO ₂	287	58	364	C ₁₄ H ₁₂ N ₄ O ₄ S ₂	46.14	3.32	15.38	46.51	3.01	15.06
8	6-OC ₂ H ₅	230	65	363	C ₁₆ H ₁₇ N ₃ O ₃ S ₂	52.87	4.71	11.56	52.52	4.40	11.39
9.	4-F, 6-F	198	61	355	C ₁₄ H ₁₁ F ₂ N ₃ O ₂ S ₂	47.31	3.12	11.82	47.87	3.49	11.45

Series II:

*Synthesis of 2-(4'-Substituted
benzamido-3'-methylphenyl)
sulfonamido-5,6-disubstituted
benzothiazoles*

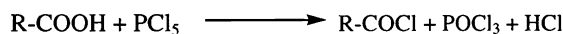
*{4-Chloro-N-[4-(5,6-disubstituted-benzothiazol-2-yl
sulfamoyl)-2-methyl-phenyl]-benzamide}*

- 1) Chemistry of Acid Chlorides &
2-Benzamido Benzothiazoles*
- 2) Schematic Representation*
- 3) Experimental*

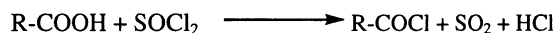
Chemistry of Acid Chlorides & 2-Benzamido Benzothiazoles

Acid chlorides are primarily used 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, sulfuryl 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.

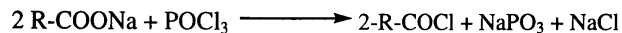


The use of thionyl chloride¹⁹⁷ for the preparation of acid chlorides presents many advantages in a large number of cases. The reaction results in the formation of sulfur dioxide and hydrogen chloride, in addition to the acid chloride, and the isolation of the chloride, is for this reason, a simple matter.

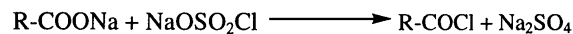
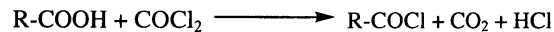


The thionyl chloride must be pure, as the impure product has been known to cause somewhat explosive decomposition in some instances. The procedure consists in mixing the chlorinating agent with acid and heating the mixture, if necessary. The product may be isolated by fractional distillation or if solid,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

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

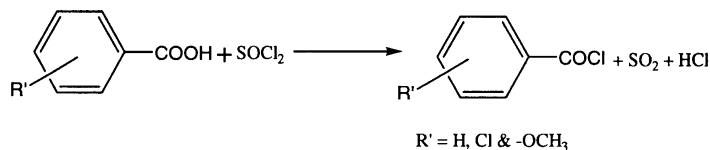


The reaction of phosgene¹⁹⁸ with carboxyl acids, p-toluene sulfochloride or of sodium chlorosulfonate with salts of carboxylic acids are also used for the preparation of acid chlorides.

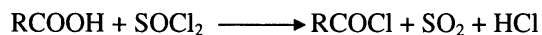


The acid chlorides are obtained readily through the reaction of sulfuryl chlorides with anhydrous salts of organic acids. The reagents are well mixed and subjected to distillation.

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



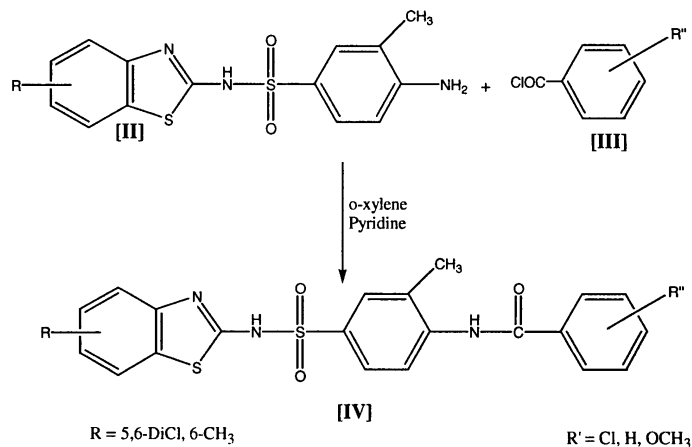
Reaction mechanism:



Acids and their salts are the only common starting materials for the synthesis of acid chlorides and several other reagents are utilized as sources of nucleophilic chloride. All the reagents form reactive intermediate [I], so that the leaving group in the substitution is not hydroxyl group.

Chemistry of Benzamido Benzothiazoles:

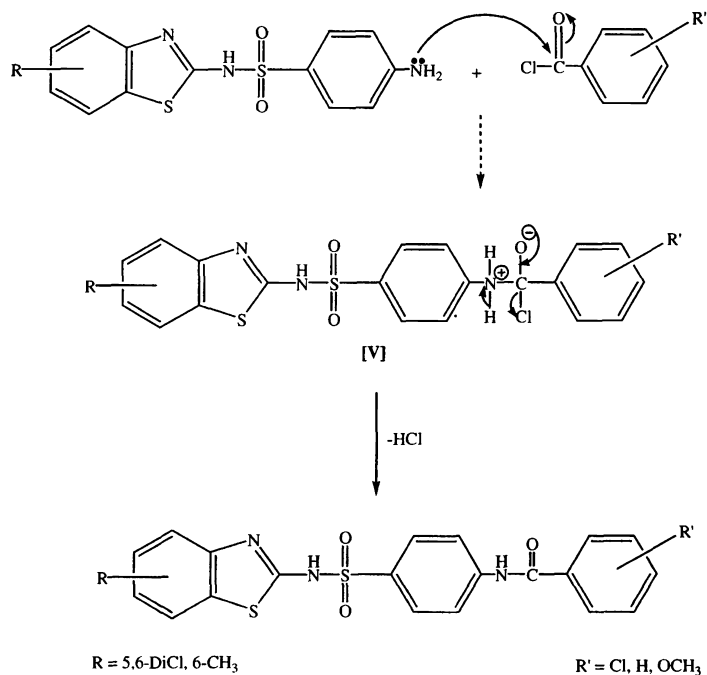
Simple substituted benzanilides are reported for various types of biological activities e.g. as antifungal¹⁹⁹, antihelminthic and antimicrobial agents²⁰⁰. Also, the amino derivatives of substituted benzothiazoles are reported to possess marginal bactericidal activity²⁰¹. In view of this, few 2-(4'-Substituted benzamido-3'-methyl phenyl) sulfonamido-5,6-disubstituted benzothiazoles (IV) were synthesized by condensing substituted benzoyl chlorides (III) with 2-[4'-amino-5'-methyl phenyl] sulfonamido -5,6-disubstituted benzothiazoles (II) in non-polar solvent at elevated temperature. The synthetic route can be represented as follows:-



2-amino-substituted benzothiazoles reacts with acid chlorides in benzene at reflux temperature²⁰². Similar reaction conditions were tried for the above reaction and it was found that o-xylene was the best solvent for reflux than benzene.

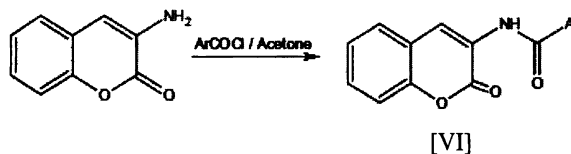
Possible mechanism of the reaction:

The acid chlorides are readily attacked by nucleophile at the carbonyl carbon. The resulting adduct [V] eliminates the chloride ion. So, although, each step of the reaction is theoretically reversible, the reaction by-product is hydrogen chloride gas, which was *usually* abstracted by the base from the reaction mixture; shifting the equilibrium to the side of the product.

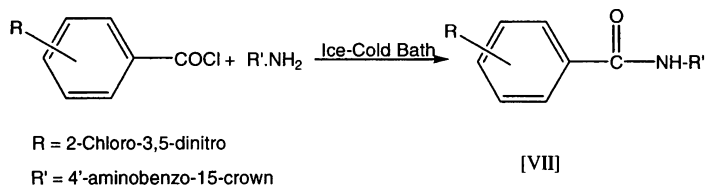


Different methods for preparation of Benzamide derivatives:

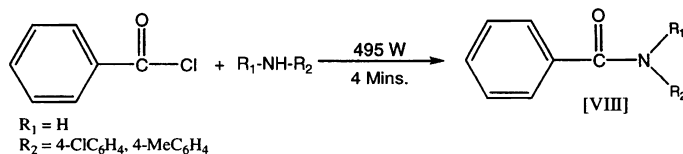
Maddi *et al*²⁰³ synthesized some benzamido derivatives (~ 65% yield) [VI] by adding a solution of substituted benzoyl chloride dissolved in dry acetone to a solution of an amine dissolved in dry pyridine at room temperature.



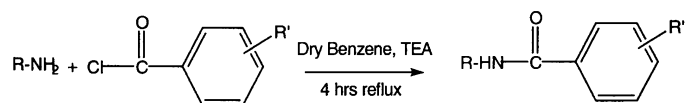
Baratoi *et al*²⁰⁴ obtained benzamido derivatives (55% yield) [VII] in ice cold conditions by adding ice-cold acid chloride in acetone solution to an amine dissolved in water/acetone mixture which was further cooled in ice-cold conditions.



Microwave method was also employed by Yanqiu Li *et al*²⁰⁵ by exposing the mixture of an amine and benzoyl chloride in a crucible at 495 W which resulted in benzamido derivatives giving 90% yield [VIII]



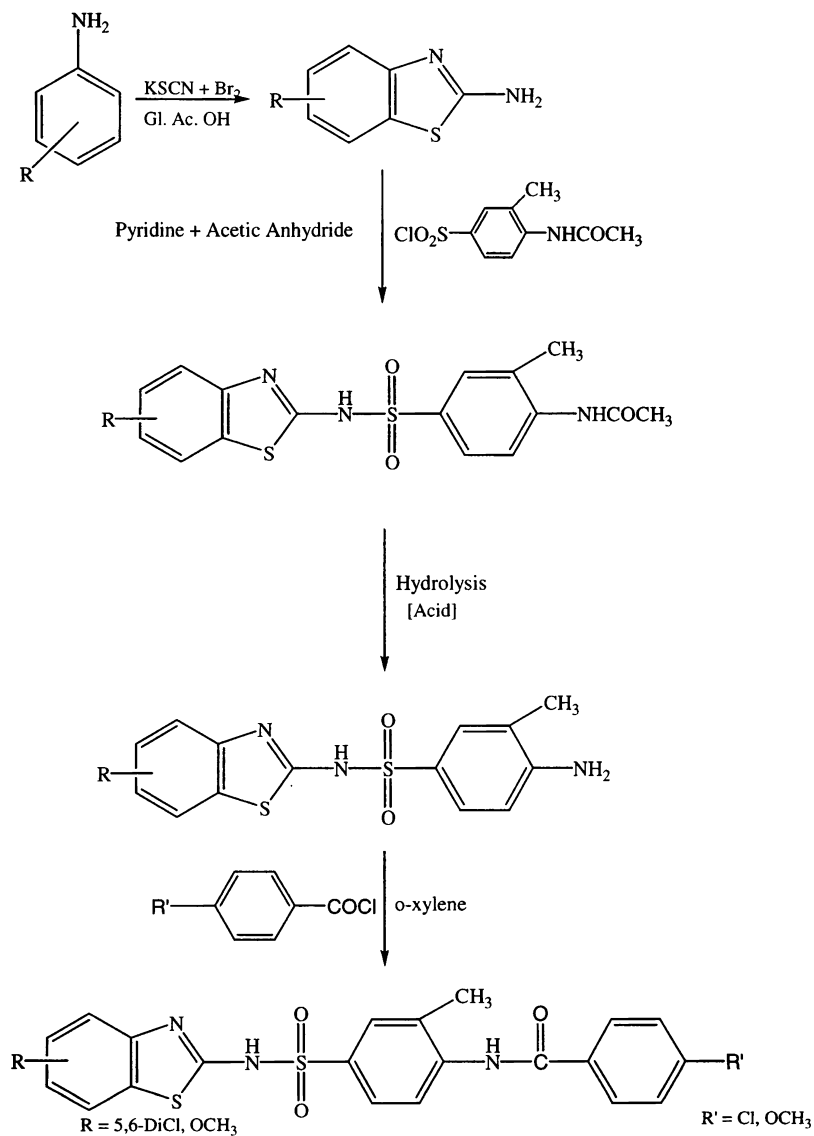
Agarwal *et al*²⁰⁶ synthesized benzamido derivatives [IX] by dissolving an amine, substituted benzoyl chloride and triethyl amine in a neutral solvent like dry benzene and continuing heating at reflux temperature.



R = 4-aryl-1-(p-amino benoyl) piperazine derivatives
R' = 4-OCH₃, 2-OCH₃, 3-OCH₃ [IX]

O-xylene was a better solvent to dissolve the reactants. When 2-(4'-amino-3'-methyl phenyl) sulfonamido-5,6-disubstituted benzothiazoles were reacted with acid chloride in o-xylene at reflux temperature in the presence of pyridine as catalyst, on work up directly gave the pure product.

Schematic Representation of Series II:



Experimental:

- 1) Preparation of 2-amino substituted benzothiazoles.
- 2) Preparation of 2-methyl acetanilide.
- 3) Preparation of 4-acetamido-3-methylbenzenesulfonyl chloride.
- 4) Preparation of 2-(4'-acetamido-3'-methylphenyl) sulfonamido-5,6-disubstituted benzothiazoles.
- 5) Preparation of 2-(4'-amino-3'-methylphenyl) sulfonamido-5,6-disubstituted benzothiazoles.
- 6) Preparation of substituted benzoyl chlorides.
- 7) Preparation of 2-(4'-Substituted benzamido-3'-methyl phenyl) sulfonamido-5,6-disubstituted benzothiazoles.

Step 1) to Step 5) have been explained in detail in Series –I, pg. nos. 68–73.

6) Preparation of substituted benzoyl chlorides.

Synthesis of Benzoyl chloride:

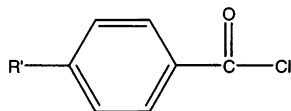
A mixture of benzoic acid (3.66 gm, 0.03 mol) and freshly distilled thionyl chloride (6.25 gm, 0.05 mol) in 25 ml dry benzene was taken in 100 ml round bottom flask equipped with reflux condenser and a guard tube. The reaction mixture was refluxed on steam bath with occasional shaking till evolution of HCl gas ceases. Excess of thionyl chloride was distilled out along with benzene. The residual oil was distilled under vacuum at 15 mm.

Yield: 2.41 ml (69%)

B.P = 197°C

Lit.²⁰⁷ B.P.:198°C

Similarly, p-methoxy benzoyl chloride and p-chloro benzoyl chloride have been prepared [X] and their physical constants have been given in the Table-IV



[X]

Table-IV:

R'	Yield (%)	B.P.	Lit. B.P.	Reference
-OCH ₃	71.5%	260°C	262°C	208
-Cl	73.3%	224°C	222°C	209

7) **Preparation of Synthesis of 2-(4'-Substituted benzamido-3'-methyl phenyl) sulfonamido-5,6-disubstituted benzothiazoles**

In a 100 ml round bottom flask equipped with a 'Y' bent tube, which holds a dropping funnel along with a guard tube and double wall water condenser also fitted with a guard tube was placed a solution of 2-(4'-amino-3'-methylphenyl) sulfonamido-5,6-dichloro benzothiazole (0.388 gm; 0.001 mol) in 30 ml o-xylene. Pyridine (0.02 g, 0.0001 mol) was added in it. The reaction mixture was refluxed on a heating mantle. A solution of p-chloro benzoyl chloride (0.175 gm, 0.13 ml; 0.001 mol) in 10 ml o-xylene was added dropwise in it using a dropping funnel, while reaction mixture was in refluxing condition. After complete addition, it was continued to reflux for 2.5 hours. The compound begins to separate towards the end of the reflux process. The reaction mixture was cooled and compound was separated.

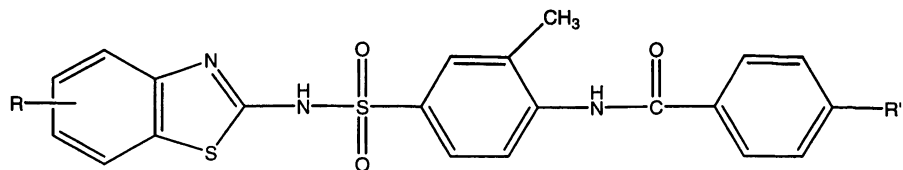
Yield: 0.35 gm (66.4 %)

M. P.: 272°C

Percentage	C %	H %	N %
Calculated	47.87	2.68	7.98
Found	48.11	2.93	7.74

Similarly, other substituted benzamido derivatives were synthesized and reported along with their melting points and other important data in Table-V

Table-V



Sr. No.	R	R'	M.P (°C)	Yield (%)	Mol Wt	Mol. Formula	Elemental Analysis (C, H, N)					
							Calculated			Found		
							C%	H%	N%	C%	H%	N%
1	6-OCH ₃	Cl	198	63.1	488	C ₂₂ H ₁₈ ClN ₃ O ₄ S ₂	54.15	3.72	8.61	53.89	3.33	8.28
2	6-CH ₃	H	192	65.5	437	C ₂₂ H ₁₉ N ₃ O ₃ S ₂	60.39	4.38	9.60	60.65	4.61	9.29
3	6-CH ₃	Cl	228	66.2	472	C ₂₂ H ₁₈ ClN ₃ O ₃ S ₂	55.98	3.82	8.90	56.15	3.32	8.41
4	6-CH ₃	OCH ₃	223	64.8	467	C ₂₃ H ₂₁ N ₃ O ₄ S ₂	59.08	4.53	8.99	58.77	4.27	8.65
5	5,6-DiCl	H	210	67.1	492	C ₂₁ H ₁₅ Cl ₂ N ₃ O ₃ S ₂	51.22	3.07	8.53	51.49	3.24	8.86
6	5,6-DiCl	Cl	272	66.4	527	C ₂₁ H ₁₄ Cl ₃ N ₃ O ₃ S ₂	47.87	2.68	7.98	48.11	2.93	7.74
7	5,6-DiCl	OCH ₃	200	65.6	522	C ₂₂ H ₁₇ Cl ₂ N ₃ O ₄ S ₂	50.58	3.28	8.04	50.29	3.51	8.28
8	5-Cl, 6-F	Cl	253	63.8	510	C ₂₁ H ₁₄ Cl ₂ FN ₃ O ₃ S ₂	49.42	2.76	8.23	49.13	2.46	7.97
9	5-Cl, 6-F	OCH ₃	238	62.5	506	C ₂₂ H ₁₇ ClFN ₃ O ₄ S ₂	52.22	3.39	8.30	52.48	3.05	8.56
10	6-NO ₂	Cl	305	64.4	503	C ₂₁ H ₁₅ ClN ₄ O ₅ S ₂	50.15	3.01	11.14	50.38	3.34	11.46
11	6-NO ₂	OCH ₃	267	63.9	498	C ₂₂ H ₁₈ N ₄ O ₆ S ₂	53.00	3.64	11.24	53.32	3.30	11.09

Series III:

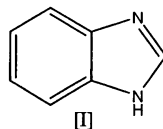
Synthesis of 2-(4'-acetamido-3'-methylphenyl)sulfonyl-2-yl mercapto-5,6-disubstituted benzimidazoles

{4-Acetylamino-3-methyl-benzenethio sulfonyl acid S-(5,6-disubstituted-1H-benzimidazol-2-yl) ester}

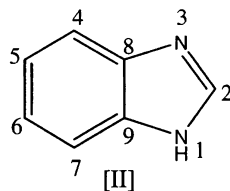
- 1) Chemistry of Benzimidazoles*
- 2) Schematic Representation*
- 3) Experimental*

CHEMISTRY OF BENZIMIDAZOLES

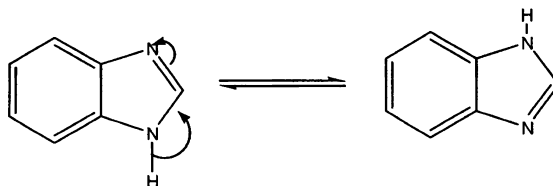
The benzimidazole contains a phenyl ring fused to an imidazole ring as indicated in the structure [I]



Since compounds of this type were formed by the loss of water, they are 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:



Benzimidazole which contains a hydrogen atom attached to nitrogen in the 1-position readily tautomerise²¹⁰.



Synthesis of benzimidazoles:

Some systematic reviews on benzimidazoles were published in 1951²¹¹ and 1953²¹², although subsequent surveys have appeared in articles^{213,214} covering the chemistry of both imidazoles and benzimidazoles. In the some reviews²¹⁵, a compilation of the synthesis, reactions and spectroscopic properties of benzimidazoles is presented.

General Methods:

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

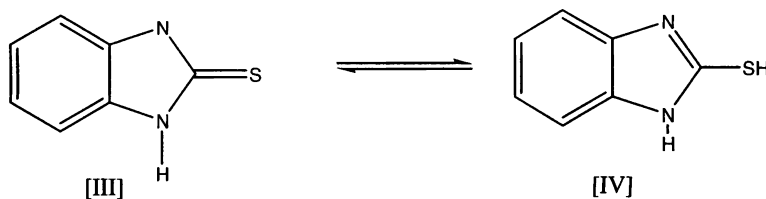
- a) By reacting o-phenylene diamine with carboxylic acids and various other derivatives^{216,217}.
- b) By reacting o-phenylene diamines with acid chlorides^{218,219}, ester²²⁰ or acid anhydride²²¹.
- c) By reacting o-phenylene diamine with amide²²² or nitrile²²³.
- d) By reacting o-phenylene diamine with aldehyde²²⁴ or ketone²²⁵.
- e) By reacting o-phenylene diamine with urea²²⁶ or thiourea²²⁷.
- f) By reacting o-phenylene diamine with isothiocyanate²²⁸.
- g) By reacting o-phenylene diamine with organic acids²²⁴.

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.

2-Mercapto benzimidazole:

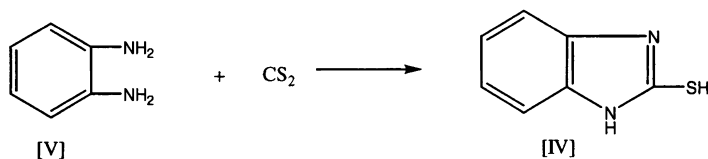
2-(3H) Benzimidazole thione (III) and 2-mercapto benzimidazole (IV) are tautomeric substances analogous to 2-(3H) benzimidazolone and 2-hydroxy benzimidazole.



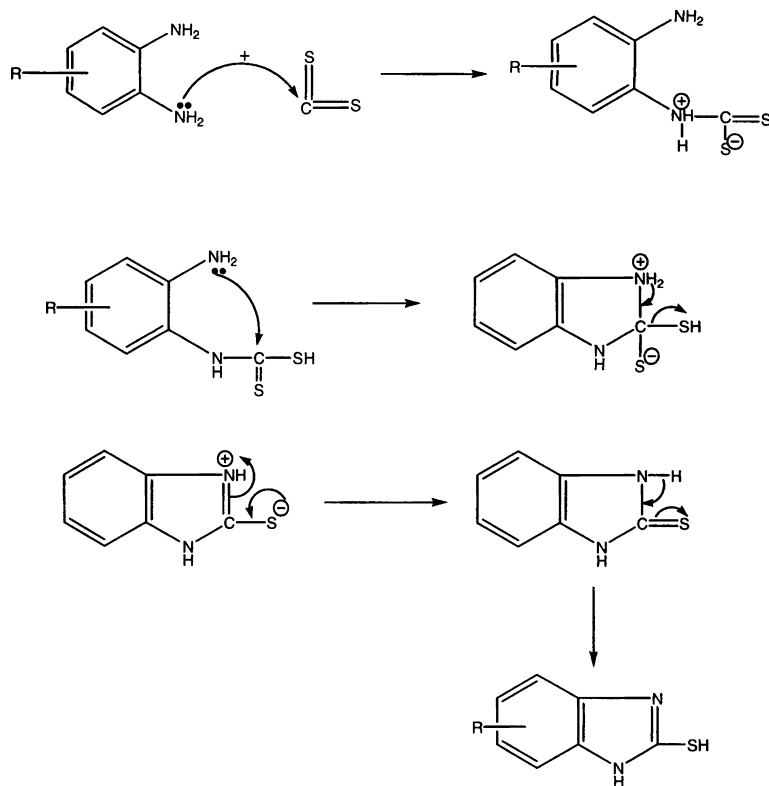
Different Methods of preparation of 2-Mercapto benzimidazole:

(a) Synthesis from o-phenylene diamine and carbon disulfide:

2-mercapto benzimidazole (IV) in general may be very conveniently prepared by this method. The reactants, o-phenylene diamine (V) and carbon disulfide are condensed by heating in the alcoholic solution with or without addition of alkali. 2-mercapto benzimidazole itself has been prepared by this method^{229,230}.



Probable Mechanism:

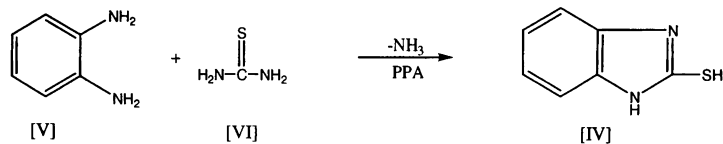


R = 5-Phenoxy-6-Chloro, 5-Naphthoxy-6-Chloro, 5-Methoxy

O-phenylene diamine containing substituents in the phenyl ring have been used, including those containing alkyl²³¹, halogen²³², phenyl²³³, aniline²³⁴ and sulfonamido²³⁵ groups.

(b) From o-phenylene diamine and thiourea:

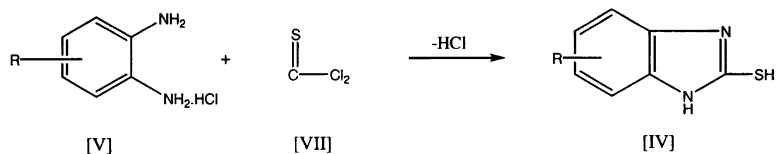
This reaction is analogous to the preparation of benzimidazole derivatives from o-phenylene diamine (V) and thiourea (VI) using Poly phosphoric acid as catalyst. Substituted o-phenylene diamine and thiourea are heated at 170°-180°C²²⁷.



The excellent yields of substituted 2-mercapto benzimidazoles (IV) can be obtained by using amyl alcohol at reflux temperature till evolution of ammonia gas ceases.

(c) From o-phenylene diamine and thiophosgene:

Billeter and Steiner obtained substituted 2-mercaptobenzimidazoles (IV) and 5-methyl-2-mercapto benzimidazole by the action of thiophosgene (VII) on o-phenylene diamine (V) and 3,4-diamino toluene, respectively^{236,237}.



R = H, 5- Methyl, 5-Arsonic Acid

(d) Miscellaneous methods:

2-mercapto benzimidazole can be prepared in 84-86% yield by the action of potassium ethyl xanthate on o-phenylene diamine²³⁸. 2-Mercapto benzimidazoles have also been prepared from o-phenylene diamine and ammonium thiocyanate in the presence of catalytic amount of ammonium chloride at 140°C for 1–3 hours. By this method, 2-mercapto benzimidazole and 5-chloro, and 5-methyl derivatives of 2-mercapto benzimidazoles have been prepared²²⁴.

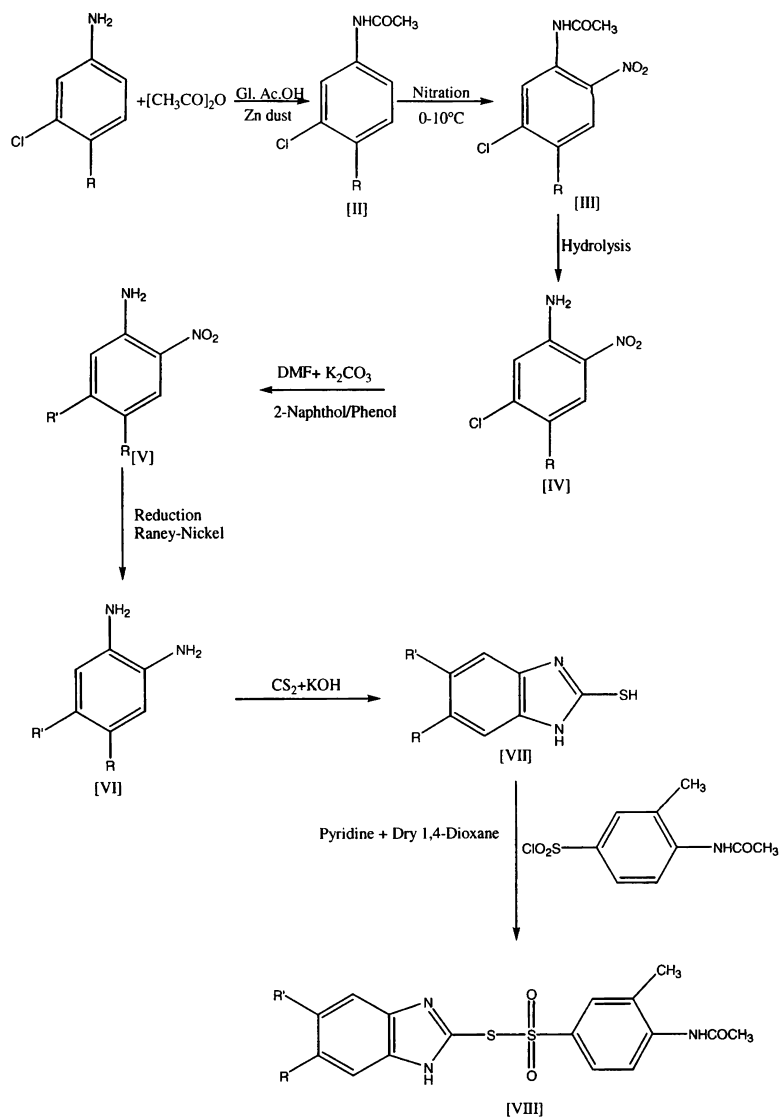
In the present investigation, 5,6-disubstituted-2-mercapto benzimidazoles were prepared by the following manner.

3-Chloro acetanilide/3,4-dichloro acetanilide [II] were nitrated at 0°-5°C to give 5-chloro-2-nitroacetanilide/4,5-dichloro-2-nitroacetanilide [III], which on further hydrolysis gives 5-chloro-2-nitroaniline/4,5-dichloro-2-nitro aniline [IV]. The resultant products were further reacted with Phenol etc and anhydrous potassium carbonate in presence of Dimethyl Formamide (D.M.F.) to 5-substituted-2-nitroaniline/5-substituted-4-chloro-2-nitroaniline [V]. Further, it was reduced with hydrazine hydrate and Raney-Nickel to give 5-substituted-ortho phenylene diamine/5-substituted-4-chloro ortho phenylene diamine [VI]. These ortho phenylene diamines on condensation with potassium ethyl xanthate under reflux conditions gave 5-substituted -2-mercapto benzimidazole/5-substituted-6-chloro-2-mercapto benzimidazole [VII].

These substituted mercapto benzimidazoles obtained as above were condensed with 4-acetamido-3-methyl benzene sulfonyl chloride in presence of pyridine and using dry 1,4-dioxane or Dichloromethane as a solvent to give different derivatives of title compound [VIII].

The schematic diagram leading to the formation of the different derivatives of the title compounds summarising the various reactions discussed so far is given in the scheme below.

Schematic Representation of Series III:



R' = 5-Phenoxy, 5-Naphthoxy, 5-Methoxy; R = Cl & H

EXPERIMENTAL

- 1) Synthesis of 3,4-dichloro acetanilide.
- 2) Synthesis of 4,5-dichloro-2-nitro acetanilide.
- 3) Synthesis of 4,5-dichloro-2-nitro aniline.
- 4) Synthesis of 5-Phenoxy-4-chloro-2-nitroaniline.
- 5) Synthesis of 5-Phenoxy -4-chloro ortho phenylene diamine.
- 6) Synthesis of 5-substituted -6-chloro-2-mercapto benzimidazole.
- 7) Synthesis of 2-(4'-acetamido-3'-methylphenyl)sulfonyl-2-yl
mercapto-5,6-disubstituted benzimidazoles

1) Synthesis of 3,4-dichloro acetanilide:

In a 500 ml round bottom flask equipped with a reflux condensor was placed 3,4-dichloro aniline (32.40 g; 0.2 mol), acetic anhydride (23 g; 21 ml, 0.2 mol), glacial acetic acid (14 ml; 0.2 mol) and 0.1 g of zinc dust. The reaction mixture was refluxed for 30 minutes and then was allowed to cool down at room temperature. It was then poured into 500 ml of ice-water mixture. The solid separated was filtered, washed with cold water and dried. It was then recrystallized from ethanol.

Yield: 1) 3-chloro acetanilide: 27.8 g (82 %)

M.P: 119°-120°C (Lit.²³⁹ M.P.:121°C)

2) 3,4-dichloro acetanilide : 33.2 g (81.3 %)

M.P: 124°C (Lit. M.P.²⁴⁰:125–127°C)

2) Synthesis of 4,5-dichloro-2-nitro acetanilide:

In a 500 ml round bottom flask equipped with a mechanical stirrer, dropping funnel and thermometer was placed a solution of 3,4-dichloro acetanilide (10.20 g; 0.05 mol) in 10 ml conc. sulfuric acid and it was stirred till all the reactant was thoroughly dissolved in conc. sulfuric acid. The temperature of the reaction mixture was brought down between 0°–10°C, following which at this temperature the nitrating mixture (conc. sulfuric acid 10 ml, d: 1.84 and conc. nitric acid 2.2 ml, d: 1.42) was added drop wise. The reaction mixture was further stirred for 2 hours at the same temperature. The reaction mixture i.e the nitrating product was precipitated by pouring the acid mixture on crushed ice with vigorous stirring. It was then neutralized by liquor ammonia. The crude product was filtered, washed with ice-cold water and recrystallized from ethanol.

Yield: 1) 5-chloro-2-nitro acetanilide: 7.7 g (71.8 %)

M.P : 116°C (Lit.²⁴¹ M.P.: 117°-118°C)

2) 4,5-dichloro-2-nitro acetanilide: 9.2 (73.8 %)

M.P : 122°C (Lit.²⁴² M.P. 123°-124°C)

3) Synthesis of 4,5-dichloro-2-nitro aniline:

A solution of 4,5-dichloro-2-nitro acetanilide (24.9 g; 0.1 mol) in 30 ml boiling ethanol was added to a solution of potassium hydroxide (5.6 g; 0.1 mol) in 10 ml water. The reaction mixture was refluxed for 40 minutes and cooled. The alcohol was distilled out under vacuum. The residue was poured into crushed ice and was made slightly acidic with dilute hydrochloric acid. The solid separated out was filtered, washed with cold water and dried. It was further recrystallized from ethanol.

Yield: 1) 5-chloro-2-nitro aniline: 12.1 g (70.2 %)

M.P: 122°C (Lit.²⁴¹ M.P.: 122°-124°C)

2) 4,5-dichloro-2-nitro aniline: 14.4 g (70 %)

M.P: 177°C (Lit.²⁴² M.P.: 178°-179°C)

4) Synthesis of 5-Phenoxy-4-chloro-2-nitro aniline.

A mixture of phenol (0.94 g, 0.01 mol) and potassium carbonate (1.38 g, 0.01 mol) were refluxed in 25 ml of dried dimethyl formamide. The reaction mixture was stirred at 140°-160°C for two hours. To this solution 4,5-dichloro-2-nitro aniline (2.07 g; 0.01 mol) dissolved in 15 ml dry dimethyl formamide (D.M.F.) was added at the same reflux temperature. Reaction mass was further stirred for 6-8 hours. It was then poured in crushed ice and the solid separated was filtered, dried and recrystallized from ethanol.

Yield:

1) 5-phenoxy-4-chloro-2-nitro aniline: 1.85 g (70 %)

M.P.:136°C

Elemental Analysis:

	C %	H %	N %
Calculated	54.46	3.43	10.58
Found	54.82	3.19	10.27

2) 5-naphthoxy-2-nitro aniline: 1.91 g (68.2 %)

M.P.: 119-120°C

Elemental Analysis:

	C %	H %	N %
Calculated	68.56	4.32	9.99
Found	68.23	4.08	9.63

3) 5-naphthoxy-4-chloro-2-nitro aniline: 2.24 gm (71.2 %)

M.P.:163°C

Elemental Analysis:

	C %	H %	N %
Calculated	61.06	3.52	8.90
Found	61.39	3.76	8.54

5) Synthesis of 5- Phenoxy-4-chloro ortho phenylene diamine.

In a 500 ml round bottom flask equipped with a mechanical stirrer, dropping funnel and a reflux condenser was placed 1 gm of Raney-Nickel and 80% hydrazine hydrate (10 ml, 0.16 mol) in 30 ml ethanol. The mixture was heated at 40°-50°C. To this 5-phenoxy-4-chloro-2-nitro aniline (2.64 gm, 0.01 mol) in 25 ml alcohol was added dropwise. The reaction mixture was refluxed for 6-8 hours till reduction

was completed. The solution was filtered and the ethanol was distilled out under vacuum. Raney-Nickel was decomposed in 10% hydrochloric acid. Filtrate was concentrated and poured into crushed ice, where the solid separated was filtered, dried and recrystallized from ethanol.

Yield:

1) 5-phenoxy-4-chloro-o-phenylene diamine: 1.21 gm (51.7%)

M.P.: 82°–84°C.

Elemental Analysis:

	C %	H %	N %
Calculated	61.41	4.72	11.94
Found	61.18	4.44	11.75

2) 5-napthoxy-o-phenylene diamine: 1.29 gm (51.6 %)

M.P.: 79°–81°C

Elemental Analysis:

	C %	H %	N %
Calculated	76.78	5.64	11.19
Found	76.56	5.39	10.92

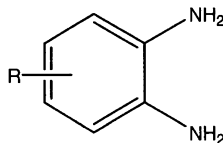
3) 5-napthoxy-4-chloro-o-phenylene diamine: 1.44 gm (50.6 %)

M.P.: 147°C

Elemental Analysis:

	C %	H %	N %
Calculated	67.49	4.60	9.84
Found	67.10	4.29	9.51

Other O-phenylene diamine (OPDA) derivatives prepared:



Sr. No.	R	Melting Point (°C)		Yield (%)	Ref.
		Observed	Reported		
1.	4,5-Dichloro	161°C	162-163°C	58.3	243
2.	5-Methoxy	47-48°C	45-46°C	66.2	244
3.	5-Chloro	70-72°C	69-70°C	60.6	245

6) Synthesis of 5-Phenoxy-6-chloro-2-mercapto benzimidazole.

In conical flask, containing potassium hydroxide was added 25 ml ethanol and 5 ml water. The mixture was warmed on waterbath till all the solid goes into the solution. The temperature of the solution was brought down to room temperature and to which was added carbon disulfide (3.375g, 2.75 ml, 0.04 mol) with stirring.

In a 500 ml three-neck round bottom flask equipped with a dropping funnel and a reflux condenser was placed 5-phenoxy-4-chloro-ortho phenylene diamine (0.586 gm, 0.0025 mol) in 200 ml ethanol. To this was added potassium ethylxanthate solution which was prepared as mentioned above. The mixture was further refluxed for 6-8 hours until fumes of hydrogen sulfide ceases. The reaction mixture was cooled and then concentrated under vacuum to remove most of the ethanol. The concentrated filtrate was poured into crushed ice and neutralised with dilute hydrochloric acid. The solid separated was filtered, washed with water and dried. It was then recrystallized from ethanol.

Yield:

1) 5-phenoxy-6-chloro-2-mercapto benzimidazole: 0.45 gm (65.1%)

M.P.: 226°C

Elemental Analysis:

	C %	H %	N %
Calculated	56.42	3.28	10.12
Found	56.21	3.51	9.91

2) 5-naphthoxy-2-mercapto benzimidazole: 0.47 gm (64.3 %)

M.P.: 261°–263°C

Elemental Analysis:

	C %	H %	N %
Calculated	69.84	4.14	9.58
Found	70.15	3.79	9.20

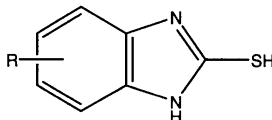
3) 5-naphthoxy-6-chloro-2-mercapto benzimidazole: 0.49 gm (60 %)

M.P.: 236°–238°C

Elemental Analysis:

	C %	H %	N %
Calculated	62.42	3.39	8.57
Found	62.75	3.08	8.22

Other 2-Mercapto Benzimidazole derivatives prepared:



Sr. No.	R	Melting Point (°C)		Yield (%)	Ref.
		Observed	Reported		
1.	5,6-Dichloro	341–343°C	344–346°C	64.2%	246
2.	5-Chloro	260°C	262°C	68.5%	247
3.	5-Methoxy	253–255°C	255-256 °C	73.1%	247

7) Synthesis of 2-(4'-acetamido-3'-methylphenyl)sulfonyl-2-yl mercapto-5-phenoxy-6-chloro benzimidazole.

In a three neck 500 ml round bottom flask was added 4-acetamido-3-methyl benzene sulfonyl chloride* (1.24 gm; 0.005 mol) along with pyridine (0.4 ml; 0.005 mol) in dichloromethane (40 ml). To this mixture, was added 2-mercapto-5-phenyloxy-6-chloro benzimidazole (0.69 gm; 0.0025 mol) dissolved in 160 ml of dichloromethane, very slowly for 2 hours maintaining the temperature at 4°C. The mixture was vigorously stirred for 24 hours.

The pyridinium chloride was filtered off and the solution concentrated. The precipitating product (i.e the title compound) was then isolated, dried and washed three times with ethyl alcohol. The residue was recrystallized from the same.

Yield: 1.55 gm, 63.5 %

M.P.: 245°C

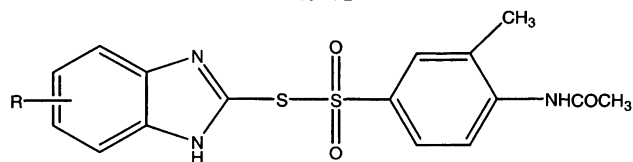
Elemental Analysis:

	C %	H %	N %
Calculated	54.15	3.72	8.61
Found	53.80	3.39	8.25

* Synthesized on pg. no. 71

Similarly other benzimidazole derivatives were prepared adopting the above procedure and their data have been mentioned in Table-VI

Table-VI



Sr. No	R	M.P (°C)	Yield (%)	Mol Wt	Mol. Formula	Elemental Analysis (C, H, N)					
						Calculated			Found		
						C %	H %	N %	C %	H %	N %
1	H	255°C	68.2%	361	C ₁₆ H ₁₅ N ₃ O ₃ S ₂	53.17	4.18	11.63	52.85	3.91	11.50
2	5-phenoxy-6-chloro	245°C	63.5%	488	C ₂₂ H ₁₈ ClN ₃ O ₄ S ₂	54.15	3.72	8.61	53.80	3.39	8.25
3	5-(2-napthyloxy)-6-chloro	259°C	61.2%	538	C ₂₆ H ₂₀ ClN ₃ O ₄ S ₂	58.04	3.75	7.81	57.71	3.46	7.63
4	5-(2-napthyloxy)	233°C	57.7%	504	C ₂₆ H ₂₁ N ₃ O ₄ S ₂	62.01	4.20	8.34	62.36	3.97	8.08
5	5-OCH ₃	268°C	64%	391	C ₁₇ H ₁₇ N ₃ O ₄ S ₂	52.16	4.38	10.73	51.88	4.16	10.40
6	5,6-DiCl	256°C	65.3%	430	C ₁₆ H ₁₃ Cl ₂ N ₃ O ₃ S ₂	44.66	3.04	9.76	44.91	2.79	9.58
7	5-Cl	277°C	63.7%	396	C ₁₆ H ₁₄ ClN ₃ O ₃ S ₂	48.54	3.56	10.61	48.15	3.74	10.32

Series IV:

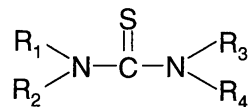
*Synthesis of 2-(4'-phenyl
thiourea-3'-methylphenyl)
sulfonamido-5,6-disubstituted
benzothiazoles*

*{N-(5,6-Disubstituted-benzothiazol-2-yl)-3-
methyl-4-(3-phenyl-thioureido)-benzene
sulfonamide}*

- 1) Chemistry of Thiourea*
- 2) Schematic Representation*
- 3) Experimental*

CHEMISTRY OF THIOUREAS

Thioureas are generally represented by the structure [I]



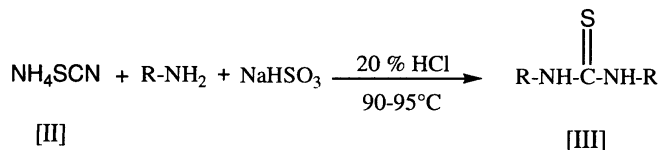
[I]

The chemistry essentially deals with the reaction promoted by the nucleophile reactivity of these compounds, the centre of the reaction being located either at S-atom or N-atom. Formation of the thiazole ring with a haloacetyl group is of pharmaceutical importance.

GENERAL METHODS OF SYNTHESIS:

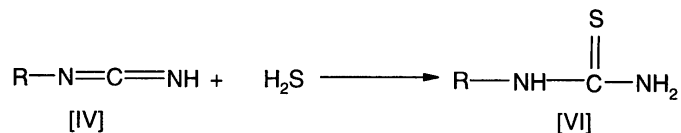
(a) Action of alkali thiocyanate on amines:

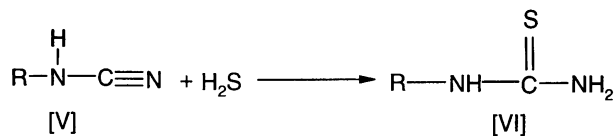
When ammonium thiocyanate [II] was heated together with 20% HCl, sodium bisulfite and an appropriate amine at 90-95°C for 10-12 hours it rearranges to give thiourea derivatives²⁴⁸ [III].



(b) Thiohydrolysis of carbo-imides and cyanamides:

On passing hydrogen sulphide gas to substituted carbo imides [IV] and cyanamides [V] corresponding thioureas [VI] are formed.

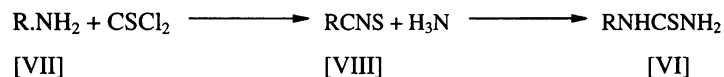




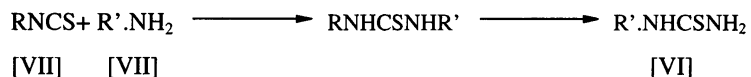
The reaction is mostly depended on the ability of carboimides and cyanamides to add to H₂S.

(c) Action of isothiocyanate on ammonia or primary amine:

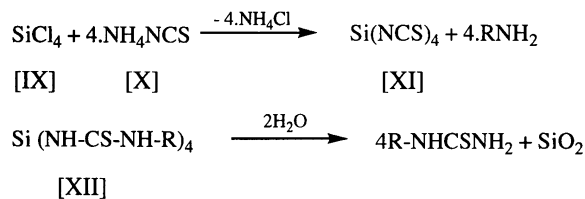
(i) Primary amines [VII] react with thiophosgene to give isothiocyanates [VIII] which further reacts with ammonia to give corresponding thioureas.



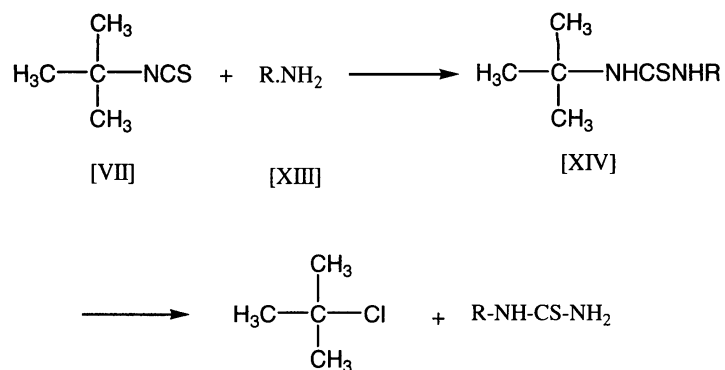
(ii) Isothiocyanates [VIII] reacts with primary amines [VII] to give unsymmetric 1,3 disubstituted thioureas. Substituted isothiocyanates are chosen such that after disubstituted product is formed the substituted group can be easily removed by hydrolysis or heterolysis to obtain monosubstituted thioureas of corresponding primary amine.



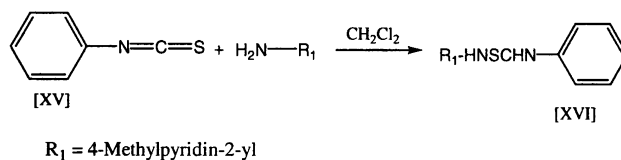
Neville *et al*²⁴⁹ have reported a method using silicon tetrathiocyanate [XI] as a thiocarbonylating agent. It is prepared by the action of ammonium thiocyanate on silicon tetrathiocyanate [XI] in benzene which on treatment with primary amine yields a silicon complex [XII] which on further hydrolysis gives monosubstituted thioureas.



Vijaykumar Nair²⁵⁰ has reacted tertbutylisothiocyanate [VII] with substituted aromatic, aliphatic and heterocyclic primary amines [XIII] to give disubstituted unsymmetric thioureas [XIV], which on hydrolysis gives respective monosubstituted thioureas.

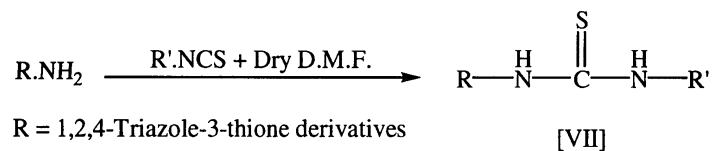


Souad Kasmi-Mir *et al*²⁵¹ have synthesized substituted phenyl thioureas [XVI] by the action of phenyl isothiocyanate [XV] on appropriate aromatic amines using dichloromethane as a solvent.



Rasmussen *et al*²⁵² have reported improved procedures for the preparation of cycloalkyl, arylalkyl and aryl thioureas by the action of benzoyl isothiocyanate on anilines in acetone and debenzoylation of the resultant N-aryl, N-benzoyl thioureas with 5% aqueous NaOH.

Thiourea derivatives [VII] were prepared by Eser Ilhan *et al*²⁵³ when amines were treated with appropriate isothiocyanates at equimolar amounts when dry Dimethyl formamide (D.M.F.) was used as a solvent.



R = 1,2,4-Triazole-3-thione derivatives

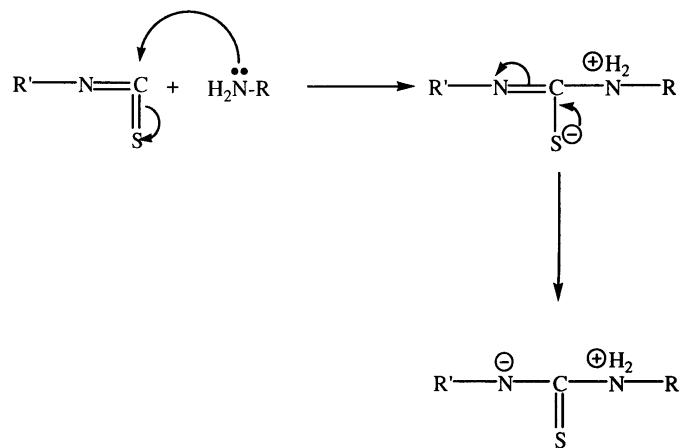
R' = 4-CH₃.C₆H₅, 3-Cl-C₆H₅ etc.

Reaction Mechanism:

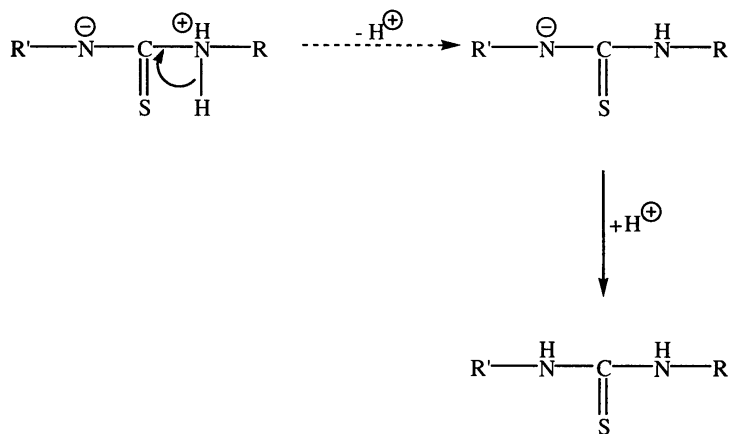


Simple addition of the nucleophile (R'-NH₂) to the carbon atom of the isothiocyanate group (R-N=C=S) give thiourea derivatives as the addition product as shown below:

i) **Attack of the nucleophile to the carbon atom of the isothiocyanate group:**

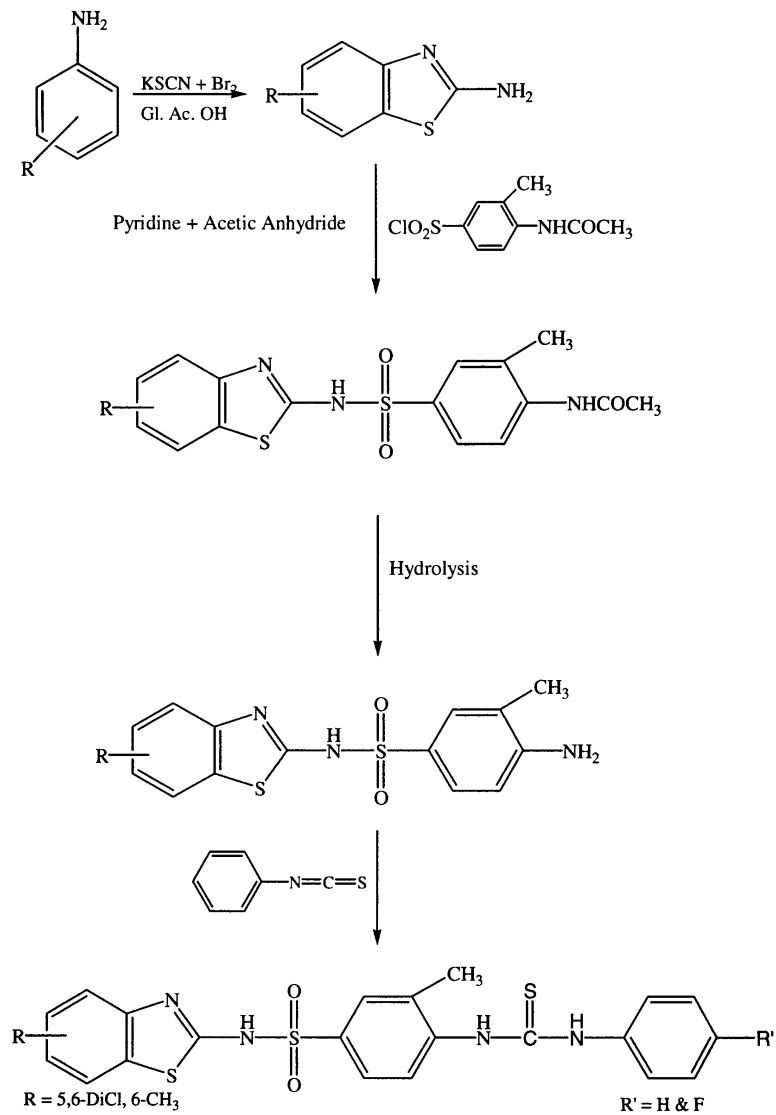


ii) **Transfer of hydrogen ion:**



The reaction products are very stable and formed very rapidly.

Schematic Representation of Series IV:



Experimental:

- 1) Preparation of 2-amino substituted benzothiazoles.
- 2) Preparation of 2-methyl acetanilide.
- 3) Preparation of 4-acetamido-3-methylbenzenesulfonyl chloride.
- 4) Preparation of 2-(4'-acetamido-3'-methylphenyl) sulfonamido-5,6-disubstituted benzothiazoles.
- 5) Preparation of 2-(4'-amino-3'-methylphenyl) sulfonamido-5,6-disubstituted benzothiazoles.
- 6) Preparation of Phenyl Isothiocyanate.
- 7) Preparation of 2-(4'-phenylthioureido-3'-methylphenyl) sulfonamido-5,6-disubstituted benzothiazoles.

Step 1) to Step 5) have been explained in detail in Series –I, pg. nos. 68–73.

6) Preparation of Phenyl Isothiocyanate:

In a 250 ml three necked round bottom flask equipped with mercury sealed mechanical stirrer, water condenser, and a dropping funnel, was taken thiophosgene (1.3 gm, 0.88 ml; 0.0115 mol) in 50 ml glacial acetic acid. To it was added a solution of aniline, (0.93 gm, 0.86 ml, 0.01 mol) in chloroform (100 ml), dropwise maintaining the temperature at 0°C. The reaction mixture was stirred further at 0°C for 4 hours. The chloroform layer was separated and washed with water, till it became neutral. The chloroform layer was dried over anhydrous Na₂SO₄, the solvent was removed under reduced pressure.

Yield : 0.98 gm; 0.86 ml (73 %)
B.P : 219°C
Lit B.P.²⁵⁴ : 221°C

4-Fluoro Phenyl Isothiocyanate was available in the lab, which was acquired from Sigma-Aldrich.

7) Preparation of 2-(4'-phenylthioureido-3'-methylphenyl) sulfonamido-5,6-disubstituted benzothiazoles.

A mixture of 2-(4'-amino-3'-methylphenyl) sulfonamido-5,6-dichloro benzothiazole (0.388 gm, 0.001 mol) and Phenyl Isothiocyanate (0.135 gm, 0.11 ml, 0.001 mol) in 20 ml ethanol was refluxed for 4-6 hours on water-bath. The solvent was removed under vacuum and the residue was taken in hexane, triturated well and filtered. The resultant product was then recrystallized from ethanol.

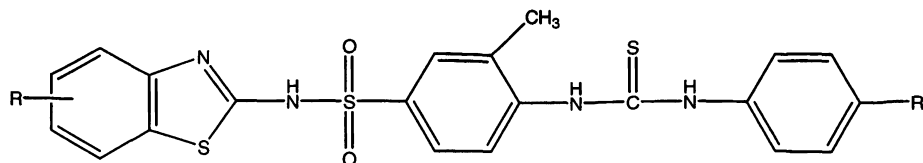
Yield : 0.36 gm (68.8 %)

M.P : 240°C

Percentage	C %	H %	N %
Calculated	48.18	3.08	10.70
Found	47.87	2.79	10.43

All the other thiourea derivatives were prepared by similar procedure during the course of this work. Their physical data has been given in Table-VII

Table-VII



Sr. No.	R	R'	M.P (°C)	Yield (%)	Mol. Wt.	Mol. Formula	Elemental Analysis (C, H, N)					
							Calculated			Found		
							C%	H%	N%	C%	H%	N%
1	6-CH ₃	H	227 °C	63.5	468	C ₂₂ H ₂₀ N ₄ O ₂ S ₃	56.39	4.30	11.96	56.73	4.11	11.59
2	6-OCH ₃	H	230 °C	66.4	484	C ₂₂ H ₂₀ N ₄ O ₃ S ₃	54.52	4.16	11.56	54.18	3.83	11.22
3	5,6- DiCl	H	240 °C	68.8	523	C ₂₁ H ₁₆ Cl ₂ N ₄ O ₂ S ₃	48.18	3.08	10.70	47.87	2.79	10.43
4	5- Cl, 6-F	H	256 °C	65.7	507	C ₂₁ H ₁₆ ClFN ₄ O ₂ S ₃	49.75	3.18	11.05	49.37	3.51	10.82
5	6-NO ₂	H	258 °C	66.8	499	C ₂₁ H ₁₇ N ₅ O ₄ S ₃	50.49	3.43	14.02	50.15	3.22	14.29
6	6-OC ₂ H ₅	H	227 °C	64.2	498	C ₂₃ H ₂₂ N ₄ O ₃ S ₃	55.40	4.45	11.24	55.28	4.17	11.58
7	6-Cl	H	208 °C	65.1	489	C ₂₁ H ₁₇ ClN ₄ O ₂ S ₃	51.58	3.50	11.46	51.24	3.31	11.10
8	6-Br	H	222 °C	66.3	533	C ₂₁ H ₁₇ BrN ₄ O ₂ S ₃	47.28	3.21	10.50	47.66	2.97	10.22
9	4-F, 6-F	H	217 °C	61.8	490	C ₂₁ H ₁₆ F ₂ N ₄ O ₂ S ₃	51.41	3.29	11.42	51.30	3.64	11.19
10	6-NO ₂	F	284 °C	65.9	517	C ₂₁ H ₁₆ FN ₅ O ₄ S ₃	48.73	3.12	13.53	48.45	2.82	13.21
11	6-CH ₃	F	235 °C	64.4	486	C ₂₂ H ₁₉ FN ₄ O ₂ S ₃	54.30	3.94	11.51	54.56	4.28	11.17
12	6-OC ₂ H ₅	F	242 °C	65.3	516	C ₂₃ H ₂₁ FN ₄ O ₃ S ₃	53.47	4.10	10.84	53.16	4.45	10.44

RESULTS
&
DISCUSSION

1) Chemistry

2) Spectral Results

CHEMISTRY

In the course of this research work, following points describes the observations, difficulties faced, failed attempts and alternative methods chosen in the synthesis of novel organic drugs.

Series I: Synthesis of 2-(4'-amino-3'-methylphenyl)sulfonamido-5,6-disubstituted benzothiazoles:

- 1) In the preparation of 4-acetamido-3-methyl benzene sulfonyl chloride, the product obtained were lumps of the solid material which were broken and the mixture was stirred for several minutes, the residue was filtered and washed with a solution of 10% Sodium Bicarbonate and then with cold water. The crude product was used in the next step, immediately.
- 2) Following the hydrolysis in glacial acetic acid, the product obtained was neutralized until it was alkaline (checked by pH paper), followed by stirring the product in 5-10% Sodium Carbonate solution to make it free from traces of glacial acetic acid.
- 3) The presence of aromatic primary amino group was affirmed by CT²⁵⁵ (confirmatory test) by taking 0.01g of compound in 1:1 HCl, cooled and was added few drops of NaNO₂ solution. This was then added to a cold solution of β -naphthol dissolved in NaOH, resulting in orange-red colour dye which confirmed the presence of aromatic primary amino group.

Series II: Synthesis of 2-(4'-Substituted benzamido-3'-methylphenyl)
sulfonamido-5,6-disubstituted benzothiazoles:

- 1) Most of the times, after reflux was completed, the compounds were obtained after the solution was kept overnight.
- 2) Pyridine was used as a catalyst.

Series III: Synthesis 2-(4'-acetamido-3'-methylphenyl)sulfonyl-2-
ylmercapto-5,6-disubstituted benzimidazoles:

- 1) Only 5-6 compounds were prepared in this series due to reduced yields obtained after reduction method.
- 2) Failed attempts of reducing substituted Ortho nitro anilines included reducing under pressure²⁵⁶ [pressure reactor, Raney-Nickel Catalyst], reducing using Zinc powder as catalyst²⁵⁷ and reducing using Iron Powder as catalyst²⁵⁸.
- 3) An alternate method²⁵⁹ was chosen for reduction of 4-methoxy-2-nitro aniline, where reduction was carried out within 15 minutes using Raney-Nickel as catalyst and formic acid as reducing agent. This method resulted in 65-70% yield but unfortunately didn't work for any other compounds.
- 4) Dry 1,4-Dioxane was mostly used as an aprotic solvent in the preparation of the title compound.

Series IV: Synthesis of 2-(4'-phenylthioureido-3'-methylphenyl)
sulfonamido-5,6-disubstituted benzothiazoles:

- 1) It was observed that sharp crystals were obtained for electron donating groups like $-\text{CH}_3$, $-\text{OCH}_3$, etc. after the reaction mixture was cooled.
- 2) Similarly, the reaction mixture was poured into crushed ice for compounds having electron-withdrawing groups like 5,6- DiCl , $-\text{Cl}$, etc to get the title compound.

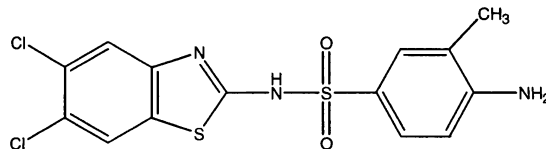
It was not possible to conduct the spectral analysis of all the synthesized compounds since the institute does not have adequate facilities, funding and instruments for the same. The researchers, therefore, have conducted spectral analysis of few compounds at IIT Powai, Department of Chemistry for IR and Institute of Science for NMR results.

SPECTROSCOPIC RESULTS

Series I

2-(4'-amino-3'-methylphenyl) sulfonamido-5,6-dichloro benzothiazole

[Synthesized on pg .no. 73]

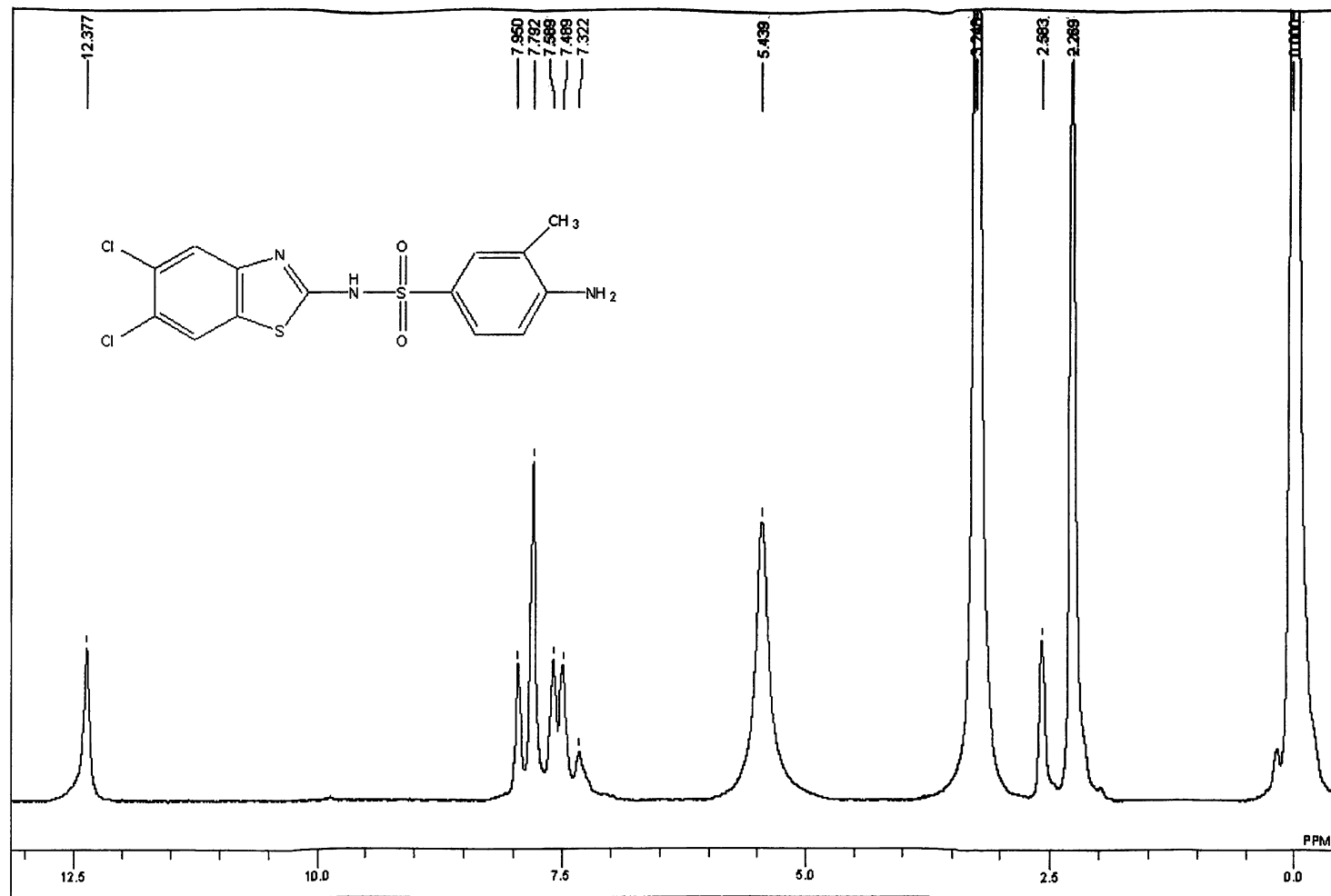


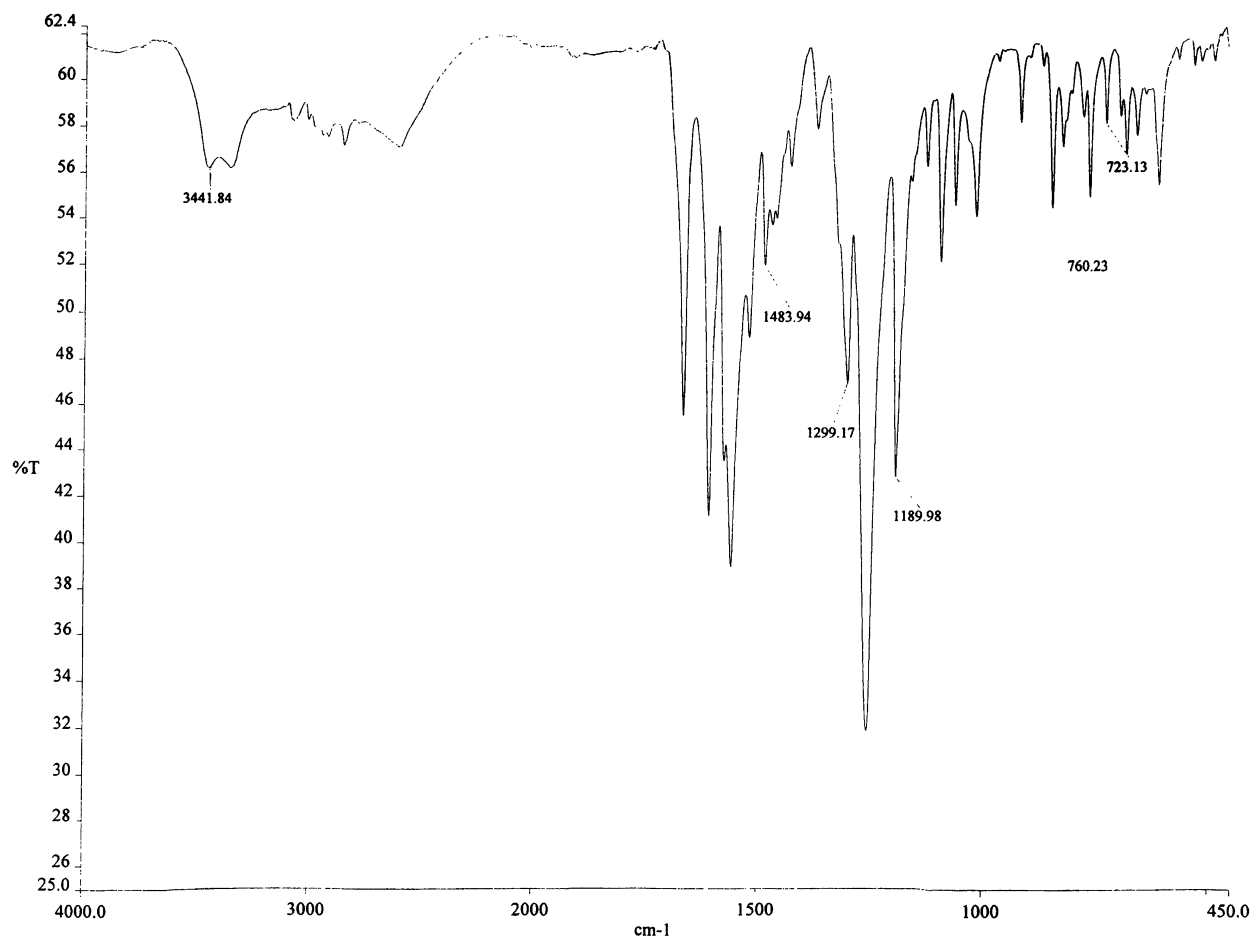
PMR (DMSO-d₆) δ : -

2.269	: [s, 3H (-CH ₃)]
5.439	: [bs, 2H (-NH ₂)]
7.322-7.950	: [m, 5H (Ar-H)]
12.377	: [s, 1H (-SO ₂ -NH-)]

IR (KBR)

1) -SO ₂ NH-	: 1189 cm ⁻¹ , 1299 cm ⁻¹
2) -NH ₂	: 3441 cm ⁻¹
3) Thiazole	: 1483 cm ⁻¹
4) (-C-Cl)	: 760 cm ⁻¹ , 723 cm ⁻¹

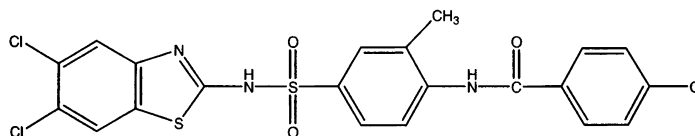




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Series II

2-(4'-{4''-chloro-} benzamido-3'-methylphenyl) sulfonamido -5,6-dichloro benzothiazole : [Synthesized on pg .no. 82-83]



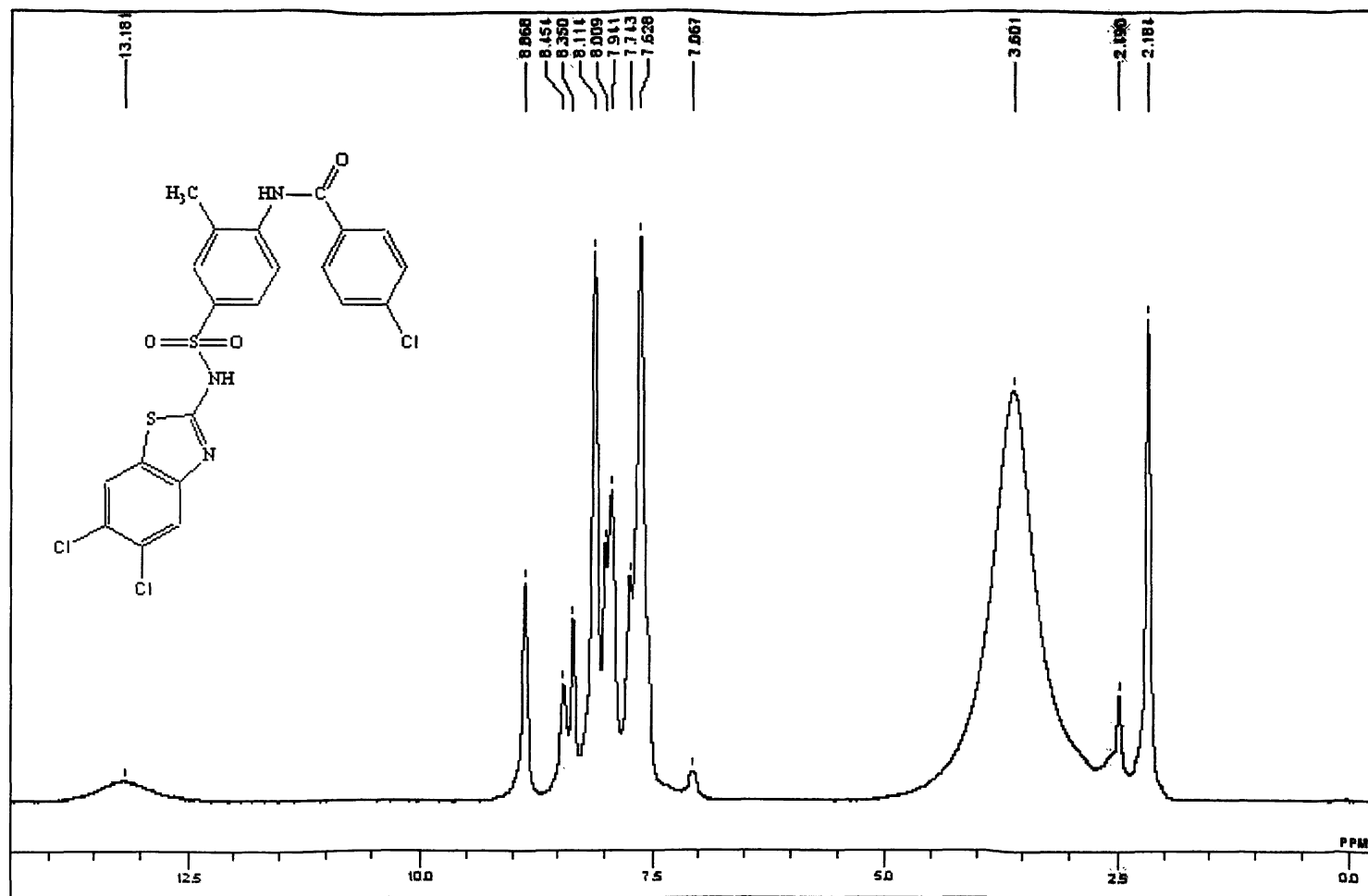
PMR (DMSO-d₆) δ : -

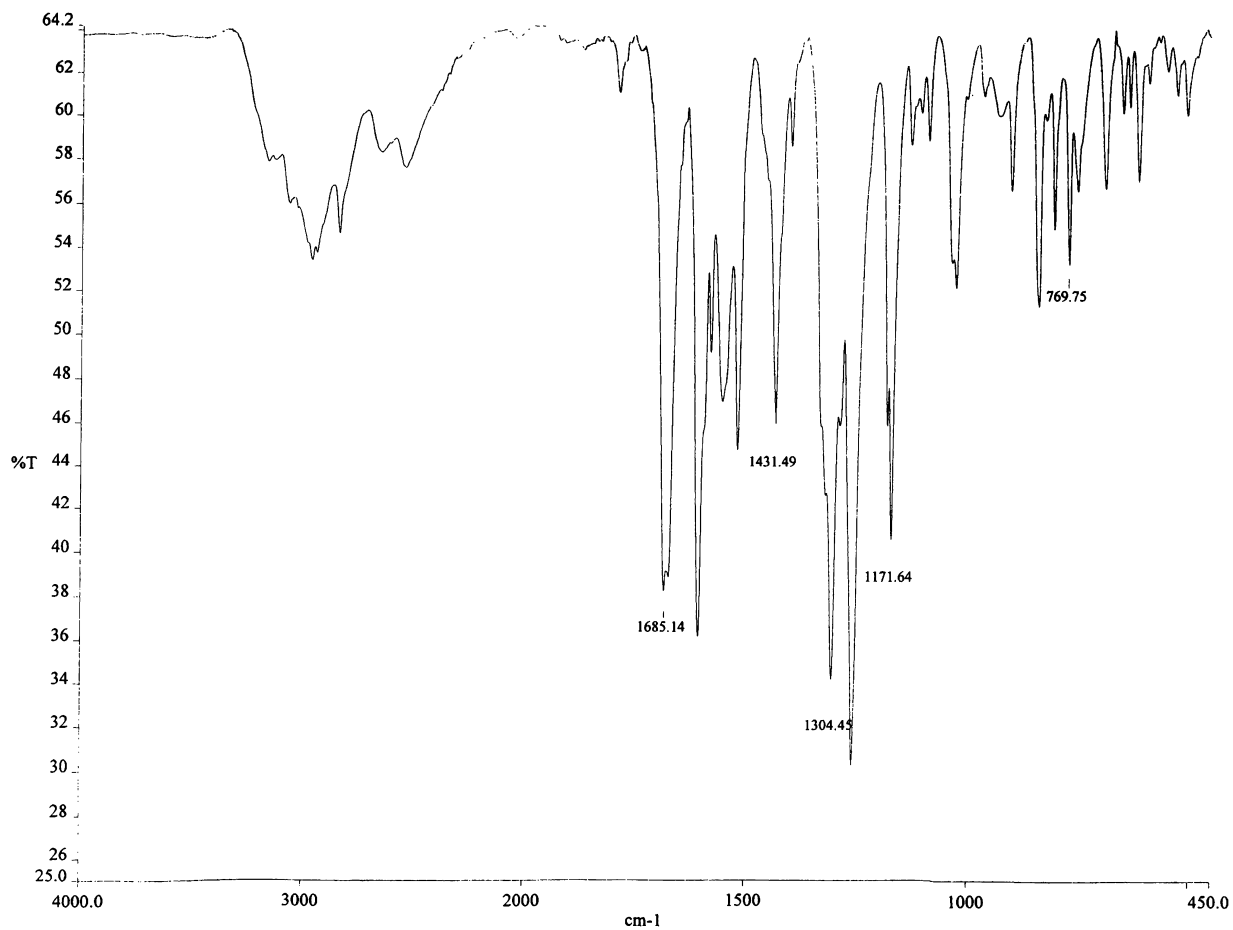
2.184	: [s, 3H(-CH ₃)]
7.628-8.454	: [m, 9H(Ar-H)]
8.868	: [s, 1H(-CO-NH-)]
13.181	: [s, 1H(-SO ₂ -NH-)]

IR (KBR)

1) -SO ₂ NH -	: 1171 cm ⁻¹ , 1304 cm ⁻¹
2) -HN-C=O	: 1685cm ⁻¹
3) Thiazole	: 1431 cm ⁻¹
4) -C-Cl	: 769 cm ⁻¹

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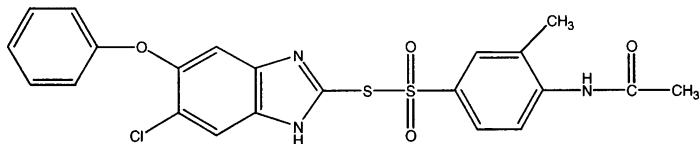




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Series III

2-(4'-acetamido-3'-methyl phenyl)sulfonyl-2-yl mercapto-5-phenoxy-6-chloro benzimidazole. [Synthesized on pg. no. 98]

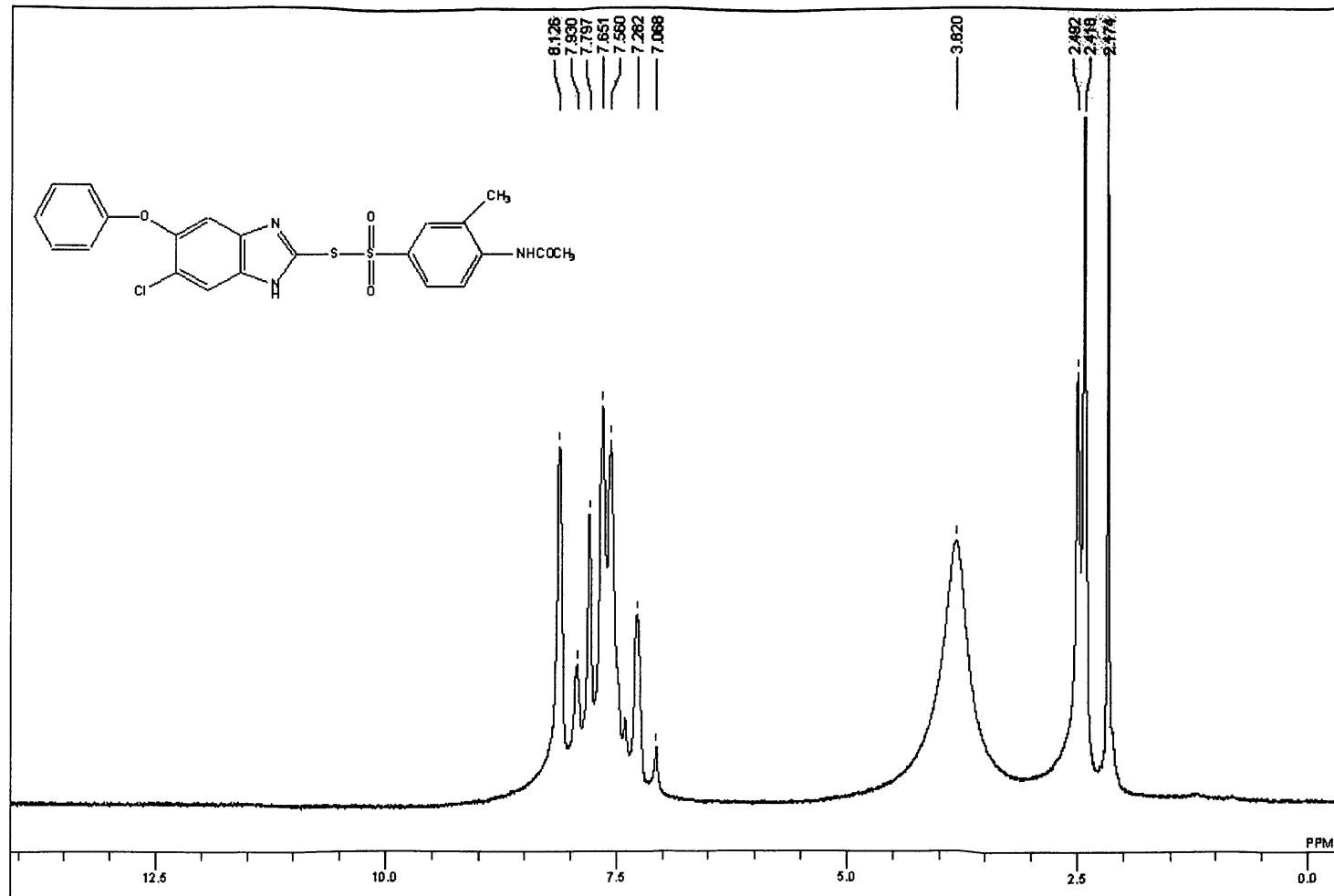
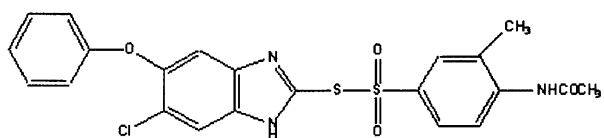


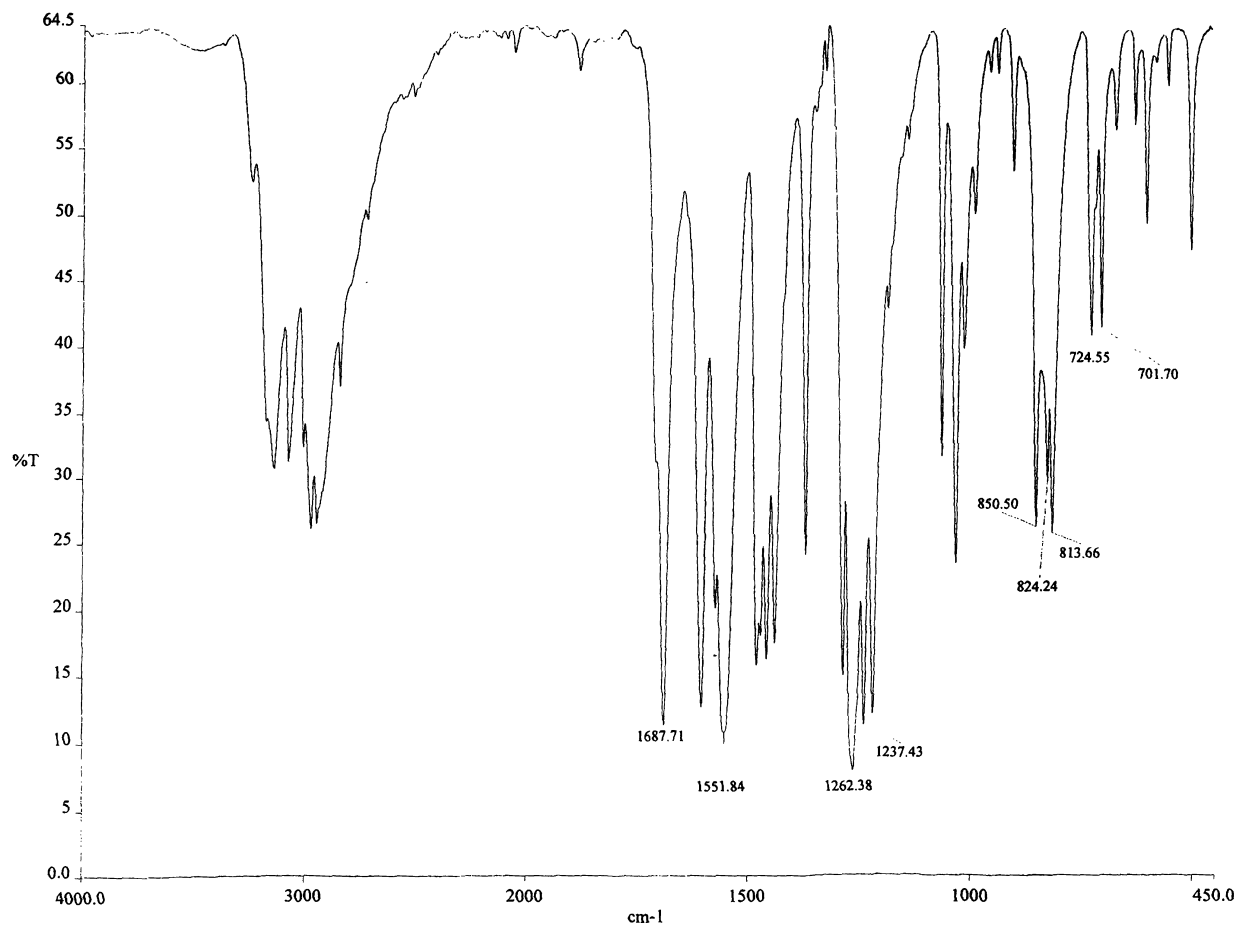
PMR (DMSO-d₆) δ: -

2.174	: [s, 3H(Ar-CH ₃)]
2.418	: [s, 3H(-CO-CH ₃)]
7.068-8.126	: [m, 12H(Ar-H, -NHCO- , -NH Imidazole)]

IR (KBR)

1) -C=N [Imidazole]	: 1551 cm ⁻¹
2) Aromatic ether [Ar-O-Ar]	: 1237 cm ⁻¹ , 1262 cm ⁻¹
3) -C=O [Acetamido]	: 1687 cm ⁻¹
4) 5-substituted benzimidazole	: 850 cm ⁻¹ , 824 cm ⁻¹ , 813 cm ⁻¹
5) -C-Cl-	: 724 cm ⁻¹ , 701 cm ⁻¹

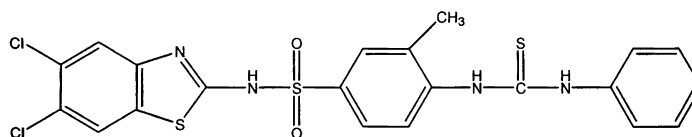




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Series IV

2-(4'-phenylthioureido-3'-methylphenyl) sulfonamido -5,6-dichloro
benzothiazole. [Synthesized on pg. no. 106]

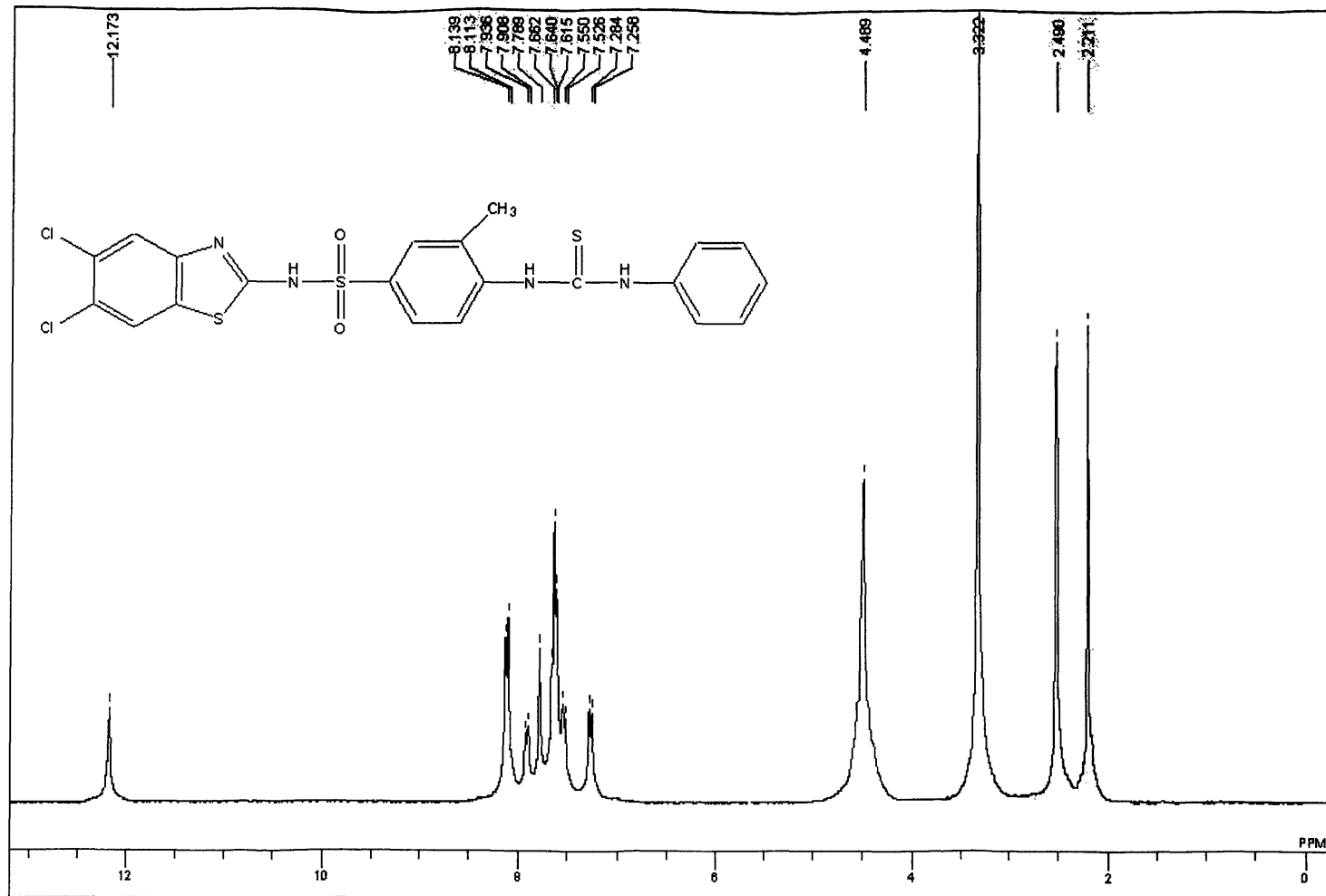


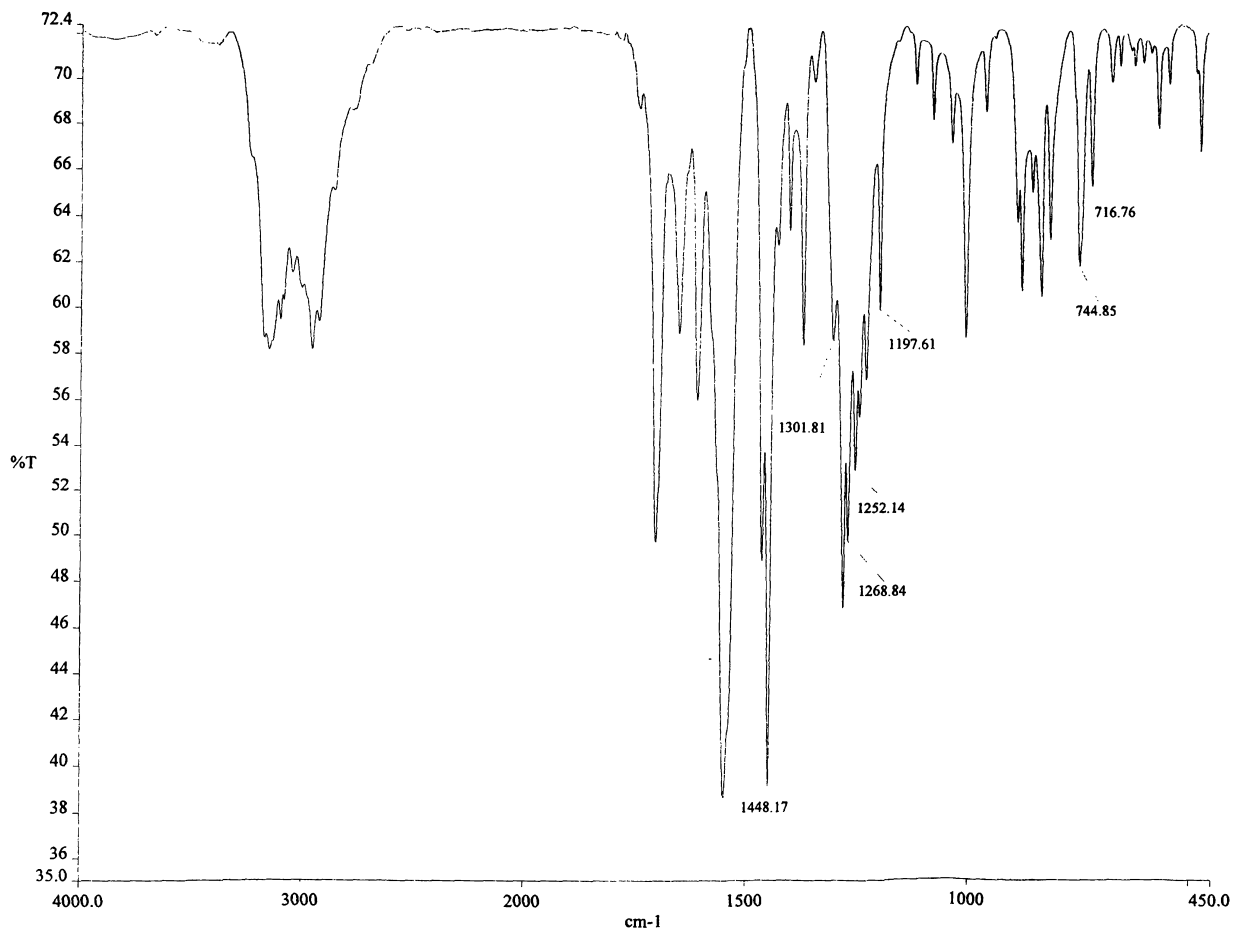
PMR (DMSO-d₆) δ : -

2.211	: [s, 3H(-CH ₃)]
4.489	: [bs, 2H(NH-CS-NH)]
7.258-8.139	: [m, 10H(Ar-H)]
12.173	: [s, 1H(-SO ₂ -NH-)]

IR (KBR):

1) -SO ₂ NH -	: 1197 cm ⁻¹ , 1301 cm ⁻¹
2) -C=S	: 1252 cm ⁻¹ , 1268cm ⁻¹
3) Thiazole	: 1448 cm ⁻¹
4) -C-Cl	: 744 cm ⁻¹ , 716 cm ⁻¹





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Biological Screening & *Results*

- 1) *Screening Methods.*
- 2) *Results of Organic Drugs
tested against pathogenic
strains.*
- 3) *Conclusion of Research
Work.*

Biological Screening & Results:

The development in chemotherapy of synthetic drugs totally depends upon the biological results of the chemotherapeutic agents, which ultimately give the guidelines to the research worker in the selection of agents for further clinical studies and set the pattern for future clinical studies and set the pattern of future drug evaluation.

Taking this into consideration the above fact, in our present work, different heterocyclic derivatives, derived from different biological important moieties, were prepared and were screened for their *in vitro* antibacterial activity against one gram positive bacteria viz. *Staphylococcus aureus* (ATCC 10148) and one gram negative bacteria viz. *Salmonella typhi* (NCTC 786) as well as their antifungal activity against the fungal strain *Candida Albicans*.

Biological Screening :-

The compounds synthesized in the present study have been subjected to antibacterial and antifungal screening based on their structural features.

The *in vitro* testing of these compounds was carried out against gram positive and gram negative bacteria viz. *Staphylococcus aureus* (ATCC 10148) *Salmonella typhi* (NCTC 786) and against fungal culture, *Candida albicans*.

SCREENING AGAINST BACTERIAL INFECTIONS :-

The test compounds have been subjected to *in vitro* screening by the tube dilution technique²⁶⁰ against gram positive and gram negative bacteria viz. *S. aureus* and *S. typhi*, using **Mueller-Hinton (M.H.)** broth as the culture medium.

SCREENING PROCEDURE:-

Preparation of Drug Solution :-

0.01 gm (10 μ g) of drug sample dissolved in 10 ml Dimethyl Sulfoxide (D.M.S.O.) to give stock solution of 100 μ g/ml.

Serial dilutions of drug samples can be prepared as follows:

2 ml drug stock solution + 8 ml M.H. broth	- 200 μ g
1 ml drug stock solution + 9 ml M.H. broth	- 100 μ g
0.5 ml drug stock solution + 9.5 ml M.H. broth	- 50 μ g
0.4 ml drug stock solution + 9.6 ml M.H. broth	- 40 μ g
0.2 ml drug stock solution + 9.8 ml M.H. broth	- 20 μ g
0.1 ml drug stock solution + 9.9 ml M.H. broth	- 10 μ g

Controls:

1. Media Control:

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

2. Culture Control:

It contains sterilized medium and culture of the organism under test. It should show growth in the medium which assures optimum pH, and sufficient nutrients to promote growth. If no growth is observed, then the medium is considered to be faulty and results are not reliable.

3. Solvent Control:

It contains sterilized medium, solvent used to dissolve the drug and the suspended culture in it. Organism should show growth in the quantity of solvent used to prepare the drug stock solution. If growth is

not observed, it can be concluded that the solvent inhibits the growth of the organism under the test and the results are not valid.

4. Turbidity Control:

It contains the medium and the drug stock solution. The turbidity is observed against the growth in different drug dilutions. It assures reliability against misinterpretations between growth and turbidity due to the growth.

5. Standard Control:

Order of standard drug corresponding to that of the drug under test. The inhibition of growth was observed against the standard

Sterile Mueller–Hinton broth was dispensed in each borosilicate test tube (150x20 mm) as per requirement.

Muellar-Hinton both was dispensed in each borosilicate test tube (150x20 mm) as per requirement, and was further autoclaved at 15 pound pressure at 120⁰C for half an hour. This sterile media was used for further procedure. The drug compounds 0.01 gm (10 µg) were dissolved in D.M.S.O (10 ml).

The serial dilutions were made in such a way as to give various final drug concentrations of 200, 100, 50, 40, 20, 10 µg/ml etc. The inoculum of standard suspension (0.1ml) of the desired bacterial strain (*S.aureus*, *S. typhi*) containing 10⁶ bacilli/ was added at the end. The tubes were incubated at 37⁰C for 24 hours and then examined for the presence or absence of the growth of the test organisms. The lowest concentration which showed no visible growth was taken as end point called as Minimum Inhibitory Concentration (M.I.C.)

Control tubes with Ampicillin and Trimethoprim were used as standard drugs and tubes without drugs were kept for comparison. Also, the solvent D.M.S.O. used for dissolution of test samples was

similarly tested to see that they did not affect the growth of the culture at the concentration used under these experimental conditions.

[Ampicillin (M.I.C = 0.01 µg/ml) against Gram positive bacteria *S. aureus* and Trimethoprim (M.I.C = 1 µg/ml) against gram negative bacteria *S. typhi* and Miconazole (M.I.C = 0.625 µg/ml) against *C. albicans*.]

SCREENING AGAINST FUNGAL INFECTIONS :-

The organism employed in the test was *Candida Albicans*; which was maintained on Sabouraud's agar slants by regular sub culturing *C. albicans* was subcultured every week.

SCREENING PROCEDURE :-

The test compounds have been subjected to *in vitro* screening by the tube dilution method serial dilutions of drug samples were prepared as explained earlier only the difference is that instead of Mueller Hinton broth, culture media employed was Sabouraud's broth. Serial dilutions of drug samples were prepared in graded concentrations varying from 200 µg/ml to 10 µg/ml.

Preparation of Innoculum :-

Candida albicans :-

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

Sabouraud's broth was dispensed in each borosilicate test tube (150x20 mm) as per requirement and autoclaved. The drug compounds 0.01 gm (10 µg) were dissolved in D.M.SO. (10 ml.). The serial dilutions were made in such a way that to give various final drug

concentrations of 200, 100, 50, 40, 20, 10 µg/ml etc. The inoculum of standard suspension (0.1 ml) of

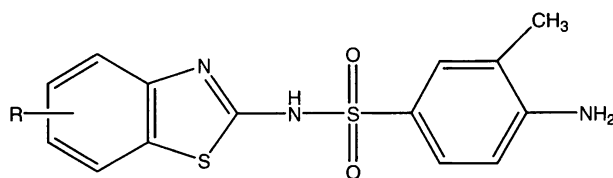
Candida albicans strain containing 5×10^6 cells per ml was added at the end. The tubes were incubated at 37°C for 48 hours and then examined for the presence or absence of the growth of the test organisms. The lowest concentration which showed no visible growth was taken as end point called as Minimum Inhibitory Concentration (MIC)

Control tubes with Miconazole as standard drug & tubes without any drug were kept for comparison. Also the solvent D.M.S.O. used for dissolution of test samples was similarly tested to see that they did not affect the growth of the culture at the concentration used under these experimental conditions.

Miconazole was used as a standard compounds for the antifungal screening program. It is active against *Candida albicans* at a concentration of 0.625 µg/ml.

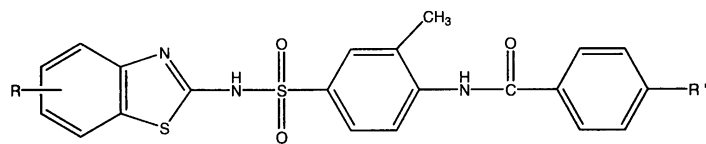
Results of Organic Drugs tested against pathogenic strains

Series I:



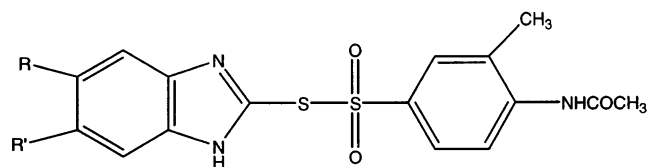
Sr. No.	R	S. Aureus ($\mu\text{g/ml}$)	S. Typhi ($\mu\text{g/ml}$)	C. Albicans ($\mu\text{g/ml}$)
1	6-OCH ₃	200	100	50
2	6-CH ₃	10	10	20
3	5,6-DiCl	200	100	100
4	5-Cl,6-F	100	100	100
5	6-NO ₂	100	200	100
6	6-Cl	200	200	100
7	6-Br	100	200	100
8	6-OC ₂ H ₅	100	100	100
9	4-F,6-F	100	50	50

Series II:



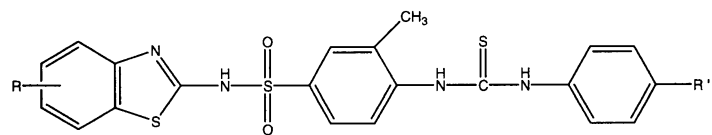
Sr. No.	R	R'	S. Aureus ($\mu\text{g/ml}$)	S. Typhi ($\mu\text{g/ml}$)	C. Albicans ($\mu\text{g/ml}$)
1	6-OCH ₃	Cl	100	200	100
2	6-CH ₃	H	100	200	50
3	6-CH ₃	Cl	100	100	100
4	6-CH ₃	-OCH ₃	100	100	20
5	5,6-DiCl	H	100	200	200
6	5,6-DiCl	Cl	200	100	100
7	5,6-DiCl	-OCH ₃	200	100	100
8	5-F,6-Cl	Cl	200	200	100
9	5-F,6-Cl	-OCH ₃	200	100	50
10	6-NO ₂	Cl	200	200	100
11	6-NO ₂	-OCH ₃	200	100	100

Series III:



Sr. No.	R	R'	S. Aureus (µg/ml)	S. Typhi (µg/ml)	C. Albicans (µg/ml)
1	H	H	200	200	200
2	Phenoxy	Cl	200	200	200
3	Naphthoxy	Cl	200	200	100
4	Naphthoxy	H	200	100	100
5	Methoxy	H	200	200	200
6	Cl	Cl	200	100	200
7	Cl	H	200	100	200

Series IV:



Sr. No.	R	R'	S. Aureus ($\mu\text{g/ml}$)	S. Typhi ($\mu\text{g/ml}$)	C. Albicans ($\mu\text{g/ml}$)
1.	6-CH ₃	H	100	100	50
2.	6-OCH ₃	H	100	100	100
3.	5,6-DiCl	H	200	100	100
4.	5-Cl,6-F	H	200	100	100
5.	6-NO ₂	H	200	200	100
6.	6-Cl	H	200	200	200
7.	6-Br	H	200	200	100
8.	6-OC ₂ H ₅	H	200	100	200
9.	4,6-DiF	H	100	50	50
10.	6-NO ₂	F	100	200	100
11.	6-OC ₂ H ₅	F	100	100	100
12.	6-CH ₃	F	100	100	50

Conclusion of Research Work.

The present investigation was focused on the synthesis of novel organic compounds i.e benzothiazole sulfonamides and benzimidazole sulfonyl sulfides of possible therapeutic value. To evaluate their efficacy, they were screened for their *in vitro* antibacterial and antifungal activity.

In Series I, the synthesis of 2-(4'-amino-3'-methylphenyl) sulfonamido-5,6-disubstituted benzothiazoles have been described. The following points are to be noted:

- 1) Presence of electron donating groups especially methyl group attached to the sixth position showed remarkable antibacterial as well as antifungal activity.
- 2) Presence of electron withdrawing groups showed moderate antibacterial and antifungal activity
- 3) Presence of Fluorine moiety also showed promising activity against both bacterial strains as well as the fungal strain.

In Series II, the synthesis of 2-(4'-Substituted benzamido-3'-methylphenyl) sulfonamido-5,6-disubstituted benzothiazoles has been explained. Following observations have been made:

- 1) All the compounds screened showed moderate to good activity against all the three pathogenic strains

- 2) Methyl substituted benzothiazole was the better of all the organic drugs in terms of antifungal activity synthesized in this series.
- 3) All the compounds irrespective of the group attached to the benzothiazole ring showed activity in the same range in terms of antibacterial activity.

In Series III, the synthesis 2-(4'-acetamido-3'-methylphenyl)sulfonyl-2-ylmercapto-5,6-disubstituted benzimidazoles has been explained. It was noted that:-

- 1) All the compounds showed moderate activity.
- 2) Benzimidazole derivatives having Naphthoxy moiety showed better fungal activity than the rest of the drugs synthesized in this series.
- 3) Among all the drugs prepared during this research, derivatives of this series showed least potency against both the bacterial strains.

In Series IV, synthesis of 2-(4'-phenylthioureido-3'-methylphenyl)sulfonamido-5,6-disubstituted benzothiazoles has been described. Following points were noted:-

- 1) All the compounds screened in this series showed moderate to good potency against the pathogenic strains.
- 2) Difluoro substituted benzothiazole derivative showed remarkable activity against both the bacterial strains and the fungal strain.

- 3) Electron donating groups attached to the benzothiazole group did not impart encouraging activity against the bacterial strains.

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. It may be worth while to pursue further investigations with related compounds bearing in mind certain trends observed in the present investigation as a part of the efforts to discover novel and potent drugs.

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APPENDIX

- 1) Melting points were determined in open glass capillaries using Theronik Precision melting point cum Boiling Point Apparatus [Model C-PMB-2] and were uncorrected.
- 2) Purity of the compounds was verified by precoated T.L.C. plates [E. Merck Kieselgel].
- 3) Elemental analysis was carried out on Thermo Finnegan Flash and EA-1112.
- 4) The IR Spectra were recorded using KBr Pellets on [Bomem FTIR MB-10452 M 5903L] spectrometer
- 5) The ^1H -NMR Spectra were recorded on [Joel-AMX-400 (300 MHz)] spectrometer using T.M.S. as an internal standard.
- 6) Solvents used in the synthesis were redistilled, and dried by using suitable drying agents, wherever required

SYNTHESIS AND BIOLOGICAL ACTIVITY OF SOME SUBSTITUTED BENZOTHAIAZOLES

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Various 6-substituted-2-(4-acetamido-5-methylphenylsulfonamido) benzothiazoles **III** and 6-substituted-2-(4-amino-5-methylphenylsulfonamido) benzothiazoles **IV** containing different substituents have been synthesised by condensation of 2-amino-6-substituted benzothiazoles with 4-acetamido-3-methylbenzenesulfonyl chloride in acetic anhydride and pyridine mixture. The structures of the compounds (**III**) and (**IV**) have been established on the basis of their elemental analysis and spectral data. The compounds thus prepared were screened for their antimicrobial activity.

Benzothiazoles exhibit antibacterial^{1,2} and antifungal^{3,4} activity.

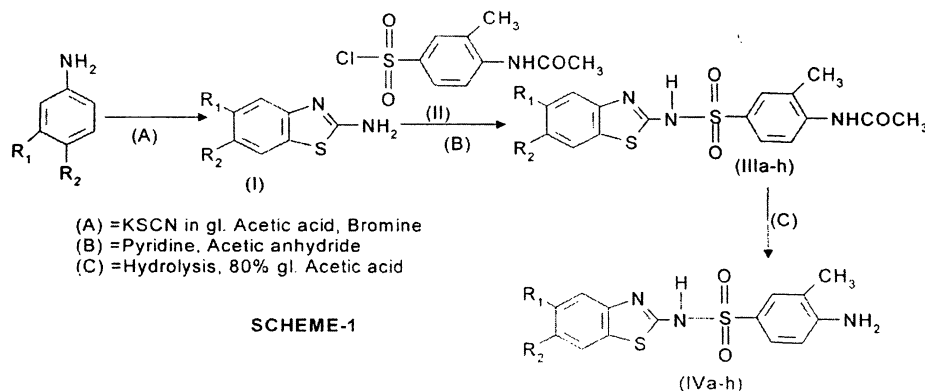
In view of these reports we have synthesized several substituted benzothiazoles (**III**, **IV**) having sulfonamido moiety with a view to study their antimicrobial activity.

III were prepared by the reaction of 2-amino-6-substituted benzothiazoles (**I**) with 4-acetamido-3-methyl benzenesulfonyl chloride using acetic anhydride and pyridine mixture giving acetamido derivatives (**IIIa-h**). Hydrolysis of the acetamido derivatives **III** with 80% gl acetic acid, resulted in new compounds [**IVa-h**].

4-Acetamido-3-methylbenzenesulfonyl chloride was prepared by chlorosulfonation of 2-methyl acetanilide using standard methods⁵.

Antimicrobial screening

The synthesized compounds were screened for their *in vitro* antibacterial and antifungal activities against *Staphylococcus aureus* (ATCC 3750), *Salmonella typhi* (NCTC 786) and *Candida albicans*. The min inhibitory conc (MIC) was determined using tube dilution method according to the standard procedure⁶. DMSO was used as a solvent with appropriate controls. Ampicillin (MIC=0.01 µg/ml against *S. aureus*) and trimethoprim (MIC=1 µg/ml against *S. typhi*) were used as standard drugs. Miconazole was used as antifungal standard drug. It was found that the compounds having electron donating groups (**IIIa**, **IIIb**, **IIIc**, **IVa**, **IVb**, **IVc**) were more potent than the compounds with electron withdrawing



SYNOPSIS

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**OF THE THESIS TO BE SUBMITTED
TO THE UNIVERSITY OF MUMBAI
FOR THE DEGREE OF**
MASTER OF SCIENCE IN ORGANIC CHEMISTRY

TITLE OF THE PROPOSAL : 5,6-DISUBSTITUTED BENZOTHAZOLE
AND BENZIMIDAZOLE
SULFONAMIDES/SULFONYL SULFIDES AS
POSSIBLE THERAPEUTIC AGENTS.

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CANDIDATE

BASIC QUALIFICATION : B.Sc. [MARCH 2005]
OF CANDIDATE

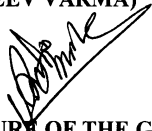
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INTRODUCTION

Only half a century ago, man relied almost exclusively on nature to produce the organic drugs he needed, and the contributions of pharmacy were confined largely to the preparation of extracts, tinctures or other dosage forms of the crude drugs and to the isolation of active principals, especially alkaloids and glycosides. Synthetic drugs began to appear at a noticeable rate in the 1920s, and this generally is attributed to the very large expansion of the **American chemical Industry fostered by World War I.**

However, the true era of medicinal chemistry really began in the 1930s with the preparation and use of anti-bacterial sulfonamide drugs. The history of medicine from that time onwards is dominated by medicinal therapeutics.

Today, a large number of diseases are cured or atleast controlled by drug therapy. Though the battle against bacterial and fungal infections has been largely won, infectious diseases are still a major health hazard for humankind.

Many Chemotherapeutic agents are available to combat these diseases, but among them only a few can be termed as broadly useful. Even the most potent anti-microbials do not generally cure infections solely by virtue of their action against invading organism; they also require help from host's defense mechanisms

Hence, majority of anti-microbial drugs play only subsidiary role either because of their weak activity or their small margin of safety or due to rapid emergence of resistance or due to poor defense mechanism of the host.

In search of new drugs three procedures are often followed:

I] The routine screen: where compounds from varying sources are tested for their effect against a predetermined disease or diseased state.

II] Structural Modification: where compounds whose biological effects that are known are used as models for design of new compounds for similar biological effects.

III] Biochemical Design: where a compound is patterned to act in lieu of a manner similar to a known biochemical substance.

In view of these, the search for an ideal chemotherapeutic substance is done either by taking a compound with known therapeutic action and then introducing substituents to arrive at a more powerful new compound, or it may also be done by examining a cross section of compounds belonging to diverse groups and detect in them the structural features associated with therapeutic activity.

In developing countries, the resources available for the control of any disease are limited. Therefore the development of inexpensive but effective, acceptable but non-toxic drug regimens for mass treatment of patients is of great importance.

Most common drugs that are used as drug intermediates are sulfur-containing compounds

Iago Goldston quoted in *Behind the Sulfa Drugs: A short history of Chemotherapy* (1943) that sulfa drugs are the 'crowning achievement' of curative medicine¹. Sulfonamide linkage is the necessary feature of all the sulfa drugs and proved its validity for biological activity. It has been known that sulfonamide group has been considered as an important pharmacophore for enhancing the biological activities of various commercially exploited drugs namely acetazoleamide & methazolamide².

Literature, study reveals that benzothiazole moiety is an important pharmacophore & exhibits outstanding biological properties³

and biological activity of the same has been further established by Asad & Falmy⁴.

Benzimidazoles are the most important group of fungicides currently in use for controlling plant diseases⁵. A development occurred in the field of benzimidazole with the emergence of broad-spectrum anti-microbial compound⁶. Bacteriostatic activity was reported for 2-Mercaptobenzimidazole against *Staphylococcus Aureus*⁷.

Considering, all the above factors it was decided that a series of Sulfur containing derivatives of both Benzothiazoles and Benzimidazoles would be synthesized and tested for their biological activity.

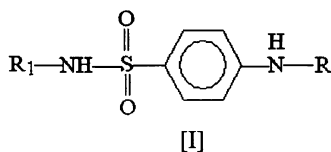
Thus the thesis of the planned work will be divided into following sections:

- 1] Introduction
- 2] Review of Literature
- 3] Plan of Work
- 4] Series I: Synthesis of 2-(4'-amino-3'-methylphenyl)sulfonamido-5,6-disubstituted benzothiazoles
- 5] Series II: Synthesis of 2-(4'-Substituted benzamido-3'-methylphenyl)sulfonamido-5,6-disubstituted benzothiazoles
- 6] Series III: Synthesis of 2-(4'-acetamido-3'-methylphenyl)sulfonyl-2-ylmercapto-5,6-disubstituted benzimidazoles
- 7] Series IV: Synthesis of 2-(4'-phenylthiourea-3'-methylphenyl)sulfonamido-5,6-disubstituted benzothiazoles
- 8] Results & Discussion
- 9] Biological Screening.

Series I: Synthesis of 2-(4'-amino-3'-methylphenyl)sulfonamido-5,6-disubstituted benzothiazoles.

Varieties of Benzothiazole derivatives are known in literature for their prodigious range of activities. Particularly they are more valuable as anti-parasitic⁸, anti-inflammatory⁹, fungicidal¹⁰, herbicidal¹¹ & anti-bacterial¹² agents

P-acetamido benzenesulfonyl chloride [I] is considered as the basic intermediate of all sulfonamides.¹³

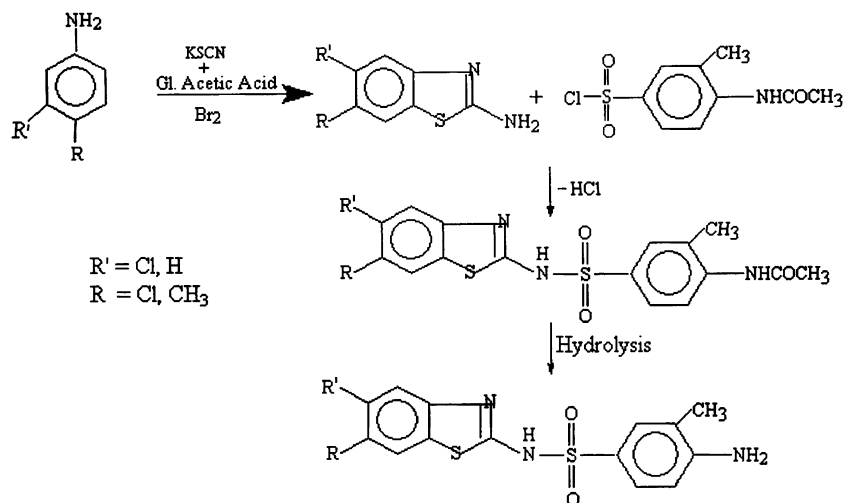


In benzothiazole derivatives, substitution at 6-position by methoxy, ethoxy, nitro, chloro, bromo can cause an increase in anti-parasitic activity¹⁴.

Benzothiazoles having a nitro moiety in the ring were reported in literature and are considerably active as anti-inflammatory agents and anti-analgesic drugs¹⁵.

Sulfonamides completely retarded the growth of fungi¹⁶. Considering all the above factors and biological activity of the mentioned moieties, it was decided to prepare a number of benzothiazole derivatives having sulfonamide linkage and be screened for anti-bacterial and anti-fungal tests.

Schematic Representation :



Series II: Synthesis of 2-(4'-Substituted benzamido-3'-methylphenyl)sulfonamido-5,6-disubstituted benzothiazoles.

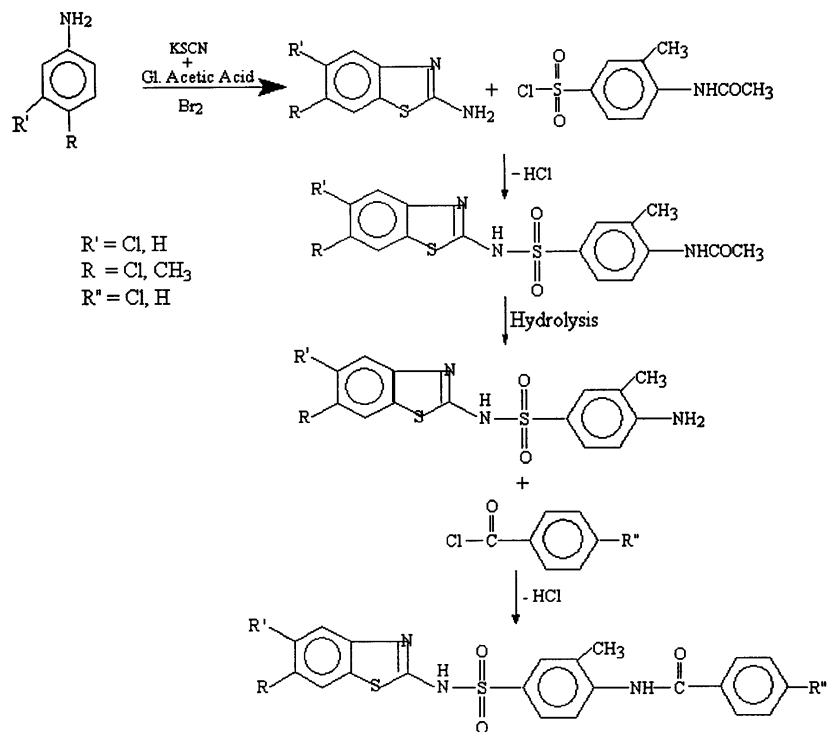
Remarkable advances have been achieved in bactericidal and fungicidal fields by introducing an amido linkage in benzothiazole derivatives.

Eg: 2-substituted benzamido benzothiazole are used as bactericides and fungicides¹⁷

The amido linkage is much important for retaining the activity in the lead molecule.

The above examples indicate the essentiality of the amido linkage into the molecule for activity. Taking this idea into mind the synthesis of 5,6-disubstituted and 6-substituted benzothiazoles having benzamido moiety in the 2nd position were synthesized and tested for their anti-fungal and anti-bacterial activity.

Schematic Representation :

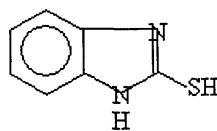


Series III: Synthesis of 2-(4'-acetamido-3'-methylphenyl)sulfonyl-2-ylmercapto-5,6-disubstituted benzimidazoles

According to the literature, Benzimidazole derivatives generally exhibit versatile pharmacological activities, such as antibacterial, antifungal, antihelmintic, anti-allergic, antineoplastic, local analgesic, antihistaminic, vasodilative, hypotensive and spasmolytic activities.¹⁸

Mercaptans show powerful antimycobacterial activity as demonstrated by Mayer in 1941¹⁹.

It is also known that thiosulfonate linkages are moderate antifungals having more potency than corresponding disulfides²⁰.

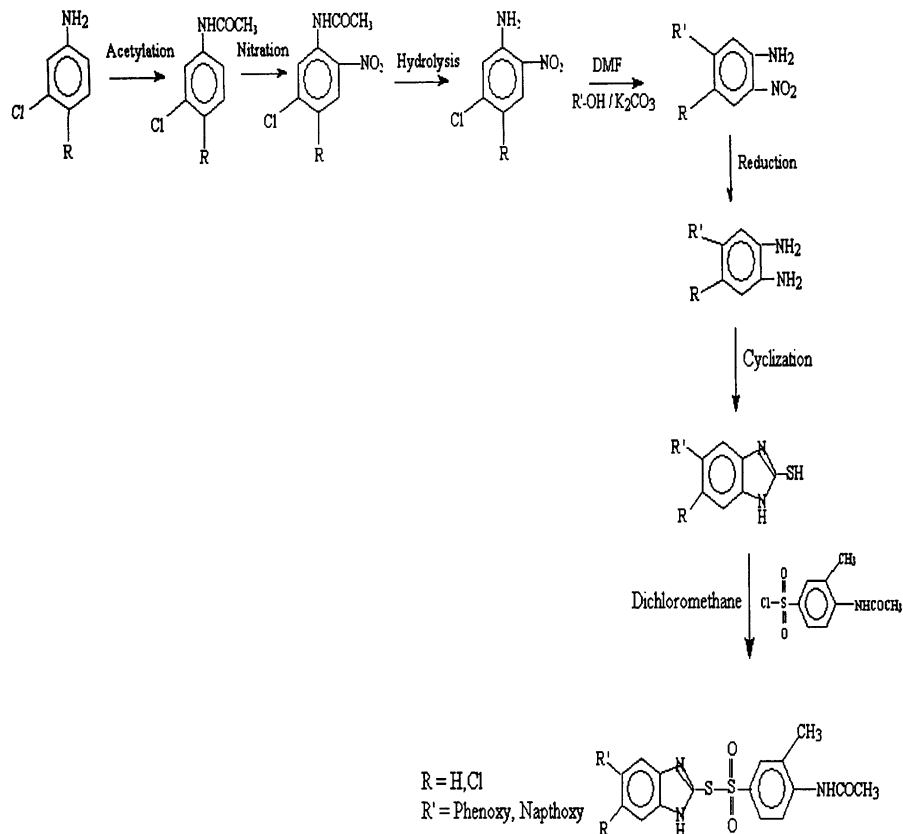


[II]

A number of 2-Thiobenzimidazoles [II] have demonstrated a broad spectrum of significant biological activity i.e. plant growth inhibition^{21,22}, bactericidal, fungicidal and virucidal activities²³.

Hence, it was planned, according to the gathered information from the above literature that new derivatives of 5,6-disubstituted / 5-substituted-2-mercapto benzimidazoles having sulfonyl sulfides a.k.a thiosulfonate linkage at 2nd position of the benzimidazole group which will be screened for antibacterial and antifungal tests.

Schematic Representation :



Series IV: Synthesis of 2-(4'-phenylthiourea-3'-methylphenyl) sulfonamido-5,6-disubstituted benzothiazoles

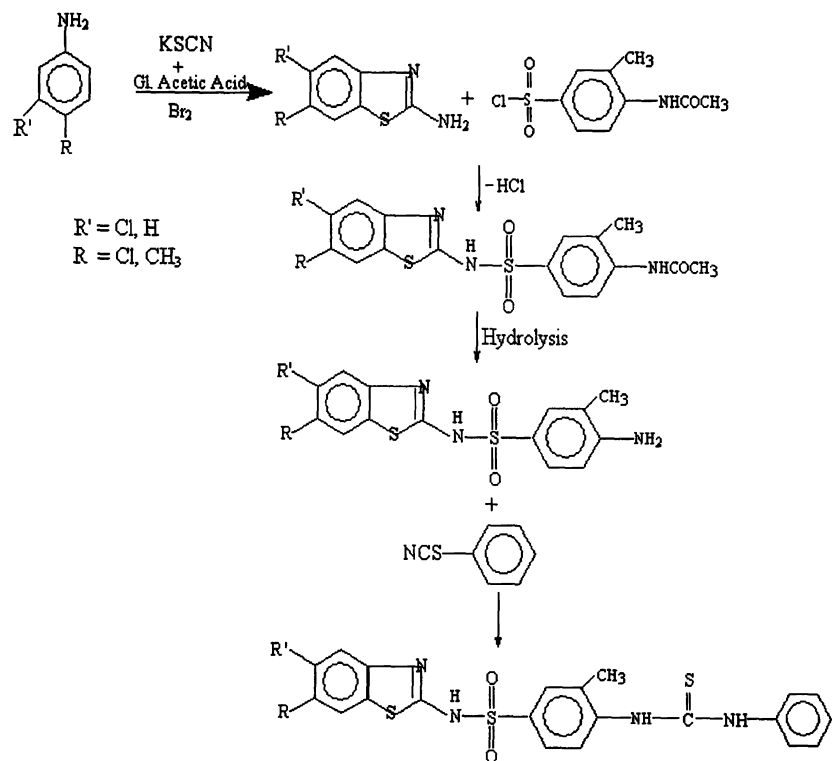
Thiourea derivatives have been found to possess wide therapeutic activities²⁴.

Substituted Thiourea Derivatives are known for their antitubercular²⁵, antileprotic²⁶ and antimalarial²⁷ activities.

Thiourea not only confers antibacterial, anti-tubercular or anti-leprotic activity but also has been reported to possess antifungal as well as antiviral action²⁸.

Keeping in mind the biological activities of substituted benzothiazoles and sulfonamides in previous series, we also decide to synthesize compounds having Thiourea moiety at the 2nd position of the benzothiazoles ring which also includes a sulfonamido linkage.

Schematic Representation :



Hence, from every series 6-10 compounds were synthesized and were subjected to Elemental analysis, spectroscopic analysis, anti-fungal and antibacterial screening.

Biological Screening:

As mentioned previously the compounds herein have been characterized by TLC, Elemental analysis and Spectroscopic analysis like IR and NMR.

The compounds reported in Series I, II III & IV have been subjected to antifungal and antibacterial screening *in vitro*.

The *in vitro* antimicrobial screening was carried out by tube dilution method against various species of Gram Positive and Gram Negative bacteria viz. Staphylococcus Aureus. ATCC 3750 and Salmoenlla Typhi 786. The culture medium used was Mueller-Hinton Broth²⁹.

Some compounds reported in series in I, II,III and IV were also screened for their antifungal activity by the tube dilution technique³⁰ against fungal strains viz. Candida Albicans.

In different screening methods, the standard drugs employed were trimethoprim as general anti-bacterial agent and miconazole as an anti-fungal agent.

The results of the above antibacterial and antifungal screening will be discussed in the thesis.

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