

SYNTHESIS AND STUDY OF SUBSTITUTED
BENZOTHAZOLE, TRIAZOLE AND PYRAZOLE AS
POSSIBLE ANTIMICROBIAL AGENTS

THESIS SUBMITTED
UNIVERSITY OF MUMBAI

FOR THE DEGREE OF
MASTER OF SCIENCE
IN
ORGANIC CHEMISTRY

SUBMITTED BY
VRUSHALI NANDKISHOR PATIL

UNDER THE GUIDANCE OF
DR. ANIL BOBADE

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

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Ms. Vrushali Nandkishor Namrata Patil

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ACHARYA DONDE MARG, PAREL, MUMBAI - 400012.

FEBRUARY 2010

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*Dedicated to my Family
&
my brother Hemant Dada*

Acknowledgement

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Vrushali Nandkishor Patil

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 “ Synthesis and study of substituted Benzothiazoles, Triazoles & Pyrazoles as possible antimicrobial 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, Parel, 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

Vrushali Nandkishor Patil

Certified by



Signature of Guide

Dr. Anil S. Bobade

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*Introduction &
Review of Literature*

Introduction

'No scientific discipline is currently as hotly pursued as chemistry; if one not compelled, therefore, to apply its astounding results to man?.....chemists concentrate the fragrance of cinnamon into a few drops and distill from treacherous poison and innocent plants-----the amazing medications. Yet there is more to come. If chemistry keeps its promises..... Whatever is alive , will be perfect; and in the end man will live for centuries, free from pain and suffering, in indestructible health, until he dissolves serenely, in peace.'

---JOHANNES GAUB (1734)

Research is defined as human activity based on intellectual application in the investigation of matter. The primary purpose for applied research is discovering, interpreting, and the development of methods and systems for the advancement of human knowledge on a wide variety of scientific matters of our world and the universe. Research can use the scientific method, but need not do so.

Scientific research relies on the application of the scientific method, a harnessing of curiosity. This research provides scientific information and theories for the explanation of the nature and the properties of the world around us. It makes practical applications possible. Scientific research is funded by public authorities, by charitable organizations and by private groups, including many companies. Scientific research can be subdivided into different classifications according to their academic and application disciplines.

The term research is also used to describe an entire collection of information about a particular subject.

Basic research

Basic research (also called fundamental or pure research) has as its primary objective the advancement of knowledge and the theoretical understanding of the relations among variables. It is exploratory and often driven by the researcher's curiosity, interest, and intuition. Therefore, it is sometimes conducted without any

practical end in mind, although it may have unexpected results pointing to practical applications. The terms “basic” or “fundamental” indicate that, through theory generation, basic research provides the foundation for further, sometimes applied research.

Research processes

Scientific research

Generally, research is understood to follow a certain structural process. Though step order may vary depending on the subject matter and researcher, the following steps are usually part of most formal research, both basic and applied:

Formation of the topic:

- Hypothesis
- Conceptual definitions
- Operational definitions
- Gathering of data
- Analysis of data
- Test, Revising of Hypothesis
- Conclusion

Research methods

The goal of the research process is to produce new knowledge, which takes three main forms:

- Exploratory research, which structures and identifies new Problems
- Constructive research, which develops solutions to a Problem
- Empirical research, which tests the feasibility of a solution Using empirical evidence

Research can also fall into two distinct types:

- ❖ Primary research
- ❖ Secondary research

➤ **Primary research** (also called **field research**) involves

- The collection of data that does not already exist. This can be through numerous forms, including questionnaires and telephone interviews amongst others. This information may be used in such things as questionnaires, magazines, and Interviews. The term is widely used in market research and competitive intelligence.
- May be very expensive because many people need to be confronted.
- By the time the research is complete it may be out of date.
- People may have to be employed or avoid their primary duties for the duration of the research.
- People may not reply if emails or letters are used.

➤ **Secondary research** (also known as desk research) involves

The summary, collection and/or synthesis of existing research rather than primary research, where data is collected from, for example, research subjects or experiments.

The term is widely used in market research and in medical research. The principal methodology in medical secondary research is the systematic review, commonly using meta-analytic statistical techniques, although other methods of synthesis, like realist reviews and meta-narrative reviews, have been developed in recent years.

Secondary research can come from either internal or external sources ¹⁻³.

Medical research is the basic research, applied research, or translational research conducted to aid the body of knowledge in the field of medicine. Medical research can be divided into two general categories: the evaluation of new treatments for both safety and efficacy in what are termed clinical trials, and all other research that contributes to the development of new treatments. The latter is termed preclinical research if its goal is specifically to elaborate knowledge for the development of new therapeutic strategies ⁴.

Preclinical research is research in basic science, which precedes the clinical trials, and is almost purely based on theory and animal experiments.

New treatments come about as a result of other, earlier discoveries often unconnected to each other, and in various fields. Sometimes the research is done for non-medical purposes, and only by accident contributes to the field of medicine (for example, the discovery of penicillin). Clinicians use these discoveries to create a treatment regimen, which is then tested in clinical trials.

Medicinal or Pharmaceutical chemistry is a discipline at the intersection of chemistry and pharmacology involved with designing, synthesizing and developing pharmaceutical drugs. Medicinal chemistry involves the identification, synthesis and development of new chemical entities suitable for therapeutic use. It also includes the study of existing drugs, their biological properties, and their quantitative structure-activity relationships. **Quantitative structure-activity relationship (QSAR)** (sometimes **QSPR**: quantitative structure-property relationship) is the process by which chemical structure is quantitatively correlated with a well defined process, such as biological activity or chemical reactivity.

Pharmaceutical chemistry is focused on quality aspects of medicines and aims to assure fitness for the purpose of medicinal products.

➤ **Process of drug discovery**

The process from identification of a target to entry of the drug into the market has the following steps⁵:

a) Discovery

The first step of drug discovery involves the identification of new active compounds, often called "hits", which are typically found by screening many compounds for the desired biological properties. These hits can come from natural sources, such as plants, animals, or fungi. More often, the hits can come from synthetic sources, such as historical compound collections and combinatorial chemistry.

b) Optimization

Another step in drug discovery involves further chemical modifications in order to improve the biological and physiochemical properties of a given lead compound library. Chemical modifications can improve the recognition and binding geometries (pharmacophores) of the lead compounds, their affinities and pharmacokinetics, or indeed their reactivity and stability during their metabolic degradation.

c) Development

The final step involves the rendering the lead compounds suitable for use in clinical trials. This involves the optimization of the synthetic route for bulk production, and the preparation of a suitable drug formulation.

d) Biological testing

Biological activity or testing is an expression describing the beneficial or adverse effects of a drug on living matter. When the drug is a complex chemical mixture, this activity is exerted by the substance's active ingredient or pharmacophore but can be modified by the other constituents. The main kind of biological activity is a substance's toxicity. Activity is generally dosage-dependent and it is not uncommon to have effects ranging from beneficial to adverse for one substance when going from low to high doses. Whereas a material is considered bioactive if it has interaction with or effect on any cell tissue in the human body, pharmacological activity is usually taken to describe beneficial effects, i.e. the effects of drug candidates.

For centuries, man has observed that natural substances can be used for their nutritional value and for the treatment of diseases, while being aware of their possible toxic and lethal effects.

The field of medicinal chemistry has evolved from an emphasis on the synthesis, isolation and characterization of drugs, to an increases awareness of the biochemistry of diseased states and the design of drugs for the prevention of diseases. An important aspect of the medicinal chemistry has been to establish a relationship between chemical structure and biological activity.

In developing drugs with specific activities, several approaches are used. The effect of natural products or synthetic drugs is determined on various biological systems or screens for “leads” to agents specific against various diseases. Once the effect of the drug is known, the medicinal chemist and the pharmacologist work together to improve the activity of the known active molecule or the “lead molecule”. This process normally goes through a synthesis - biological test – synthesis – biological test cycle until a drug with a desired activity is obtained.

The most frequent approach to obtaining drugs to be used for treating a particular disease is to synthesize analogs of drugs that are known to be effective in the treatment of the disease. The pharmacophore is a chemical segment of a molecule that is responsible for its biological actions. Normally, it is found that a specific type of biological activity of the molecule is dependent upon more than just one functional group. Hence, it becomes important to find such pharmacophores and join them together to obtain a moiety that acts as “lead”. Various substitutions are then carried out on this moiety to obtain active molecules.



➤ **Antimicrobial activity**

An antimicrobial is a substance that kills or inhibits the growth of microbes such as bacteria, fungi, or viruses. Antimicrobial drugs either kill microbes (microbicidal) or prevent the growth of microbes (microbistatic).

Antimicrobial therapy has only emerged over the last 60 years as a practical proposition and has become one of the pillars of modern medicine.

In the last decades, the high use of antimicrobial agents has been recognized as an important public health challenge. Their widespread use has been linked to the development of bacterial resistance responsible for increases in morbidity, mortality, hospital stay duration and healthcare costs associated with untreatable infections ⁶.

The history of antimicrobials begins with the observations of Pasteur and Joubert⁷, who discovered that one type of bacteria could prevent the growth of another. They did not know at that time that the reason one bacteria failed to grow was that the other bacteria were producing an antibiotic. Technically, antibiotics are only those substances that are produced by one microorganism that kill, or prevent the growth, of another microorganism. Of course, in today's common usage, the term antibiotic is used to refer to almost any drug that cures a bacterial infection. Antimicrobials include not just antibiotics, but synthetically formed compounds as well.

➤ **Antibacterial agents**

The essence of antimicrobial chemotherapy is selective toxicity to kill or inhibit the microbes without harming the patient. The early 1940's saw the appearance of potent, non-toxic chemotherapeutic agents selectively active against bacteria, and thus revolutionized the treatment of bacterial infections. The discovery of "miracle drugs"- Sulfonamide, Penicillin and Streptomycin provided respite from many infections, thus reducing the morbidity and mortality among humans and their domestic hands. But the advantage was short-lived because of the quite emergence of multidrug resistant bacteria. Hence, intense research in this field has become all the more important.

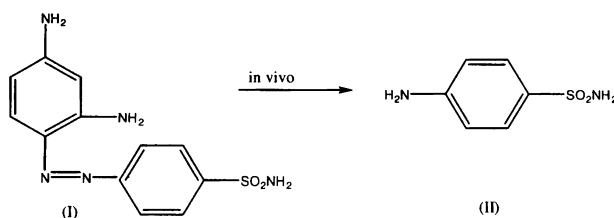
Review of literature gives the major developments in the field of antimicrobial chemotherapy.

Systematic antibacterials include a number of classes, which are enlisted below-

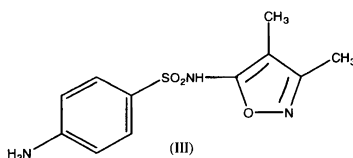
Sulfur Containing drugs

Sulfonamides:

Sulfonamides were discovered in the mid 1930's. Protonsil rubrum [I], a red dye was observed by Domagk⁸ to be taken up selectively by bacterial cells in case of *Streptococcal* infections in mice and *Staphylococcal* infections in rabbits. The dye killed the bacteria. Fractionation led to identification of an active substance known as amino benzene sulfonamide (sulfanilamide) [II]⁹, a colorless cleavage product formed by liver metabolism of the drug, which is responsible for the activity.



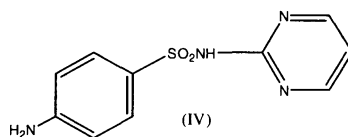
Sulfonamides are predominantly bacteriostatic in nature. They inhibit the enzyme dihydropteroate synthetase, an important biocatalyst needed for the biosynthesis of folic acid derivatives and ultimately DNA. They do this by competitive inhibition of p-amino benzoic acid (PABA) at the active site, due to structural resemblance. Sulfonamide is incorporated into tetrahydrofolic acid molecule by condensation with a dihydropteroate diphosphate precursor under the influence of dihydropteroate synthetase. Sulfonamides also act as antimetabolites by preventing the synthesis of purine nucleotide and thymidine. In human beings, folic acid is directly supplied in diet and is synthesized. Thus, sulfonamides may be considered safe for human cells. Bacteria, which are capable of taking up preformed folic acid, are intrinsically resistant to sulfonamides.



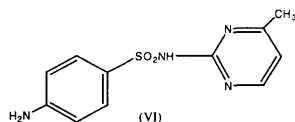
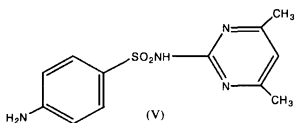
Sulfisoxazole [III]¹⁰ is one of the most popular sulfonamides in present use, which has a pKa of 5.0. It has a broad antimicrobial spectrum and is clinically used for urinary tract infection and as a backup for other more preferred agents.

It is well absorbed after oral administration, widely distributed and excreted by the kidneys. It maintains adequate blood levels for prolonged period.

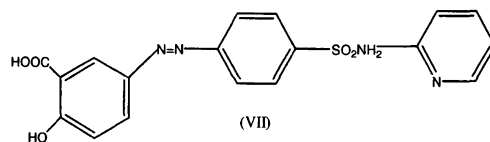
Plasmid-mediated resistance is commonly developed, particularly among Gram-negative organisms, due to decreased sensitivity to dihydropteroate synthetase. Super infections, allergic rashes, photosensitivity and drug fever are some of the common side effects of the drug.



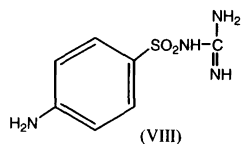
Sulphadiazine [IV] has retained pre-eminence because of its broad spectrum of activity, low toxicity and high tissue penetration. It can be used for meningococcal meningitis provided the strains of *Neisseria meningitides* involved are sulfonamide sensitive¹¹.



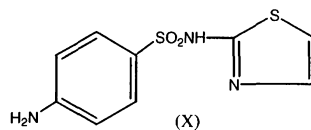
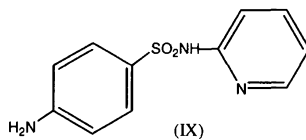
Sulphamethazine [V] and Sulphamerazine [VI]¹²⁻¹⁶ have greater tolerance and high solubility. Multiple (triple) sulfas, a 1:1:1 combination of three sulphapyrimidines, namely, sulphadiazine, sulphamethazine and sulphamerazine, is a very potent combination.



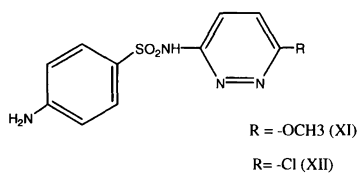
Sulphasalazine [VII] is absorbed in the duodenum and is converted to Para amino salicylic acid (PAS) which is used for ulcerative colitis and Crohn's disease because of its anti-inflammatory effect.



Sulphaguanidine [VIII] was extensively used for intestinal infections. Now, it has been withdrawn in many countries¹⁷ due to poor absorption and toxicity.

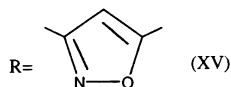
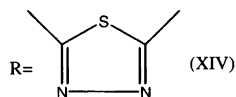
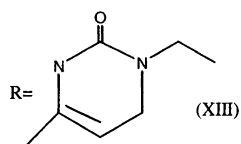
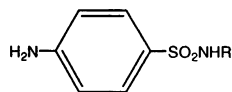


Sulphapyridine [IX]¹⁸, reported in 1938, was in use for the treatment of pneumonia and remained as a drug of choice until it was replaced by sulphathiazole [X]¹⁹.



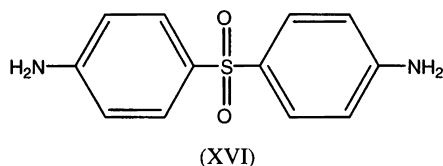
Long-acting sulfonamides include Sulphamethoxypyridazine [XI]¹⁹, which has a half life of about 37 hours. Thus, it needs to be administered only once a day. Sulphachloropyridazine [XII], a related sulphonamide, has a half-life of 7 hours and is useful for urinary tract infections (UTI).

Other sulfonamides include Sulphacytine [XIII], Sulphametiazole [XIV], Sulphamethoxazole [XV], etc.



Sulphones:

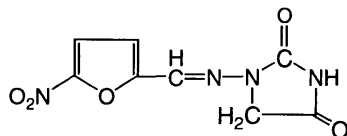
The parent sulphone, Dapsone (4,4'-sulphonyl dianiline) [XVI], is a prototype for a variety of analogues that have been studied widely. Dapsone²⁰ is effective against *Mycobacterium leprae* at low concentrations. Its mode of action is similar to that of sulfonamides. p,p-diamino diphenyl sulphone (DDS) has unique beneficial effects on some dermal inflammatory conditions, especially dermatologic, and it is likely that this activity may contribute to its activity against *M. leprae*.



NON-SULFUR DRUGS

Nitrofurantoin:

Nitrofurantoin [XVII] was first synthesized by Hayes²¹ by the condensation of 1-aminohydantoin with 5-nitro-2-furfuraldehyde. The drug is active against many strains of the common urinary tract pathogens.



[XVII]

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.

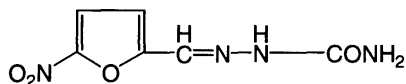
The compound is rapidly and completely absorbed from the gastrointestinal tract. Antibacterial levels in the blood are not attained but approximately 40% of the drug is excreted in the urine²². Concentrations that are bactericidal to most gram-positive & gram negative organisms are achieved in urine. Intravenous administration of the drug has received limited trial in the treatment of acute severe urinary tract infections²³. 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 Scott²⁴ have synthesized this compound [XVIII] 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

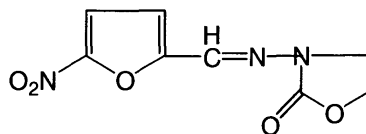
of some value in the therapy of sleeping sickness in man²⁵ caused by *Trypanosome gambiense*.



[XVIII]

Furazolidone:

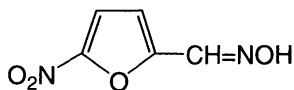
Furazolidone [XIX] was synthesized by Drake and Hayes²⁶. The drug has been reported to be of value in the treatment of intestinal infections due to *Shigella* and *Salmonella* organisms²⁷. It has an outstanding activity against these pathogenic bacterial infections. Excellent anticoccidial & antitrichomoral activity is also evident.



[XIX]

Nifuroxime:

Nifuroxime [XX] is prepared by treating 5-nitrofurfural with hydroxylamine in alcohol. It is patented²⁸ as a urinary antibacterial agent.



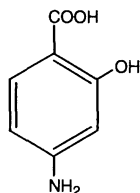
[XX]

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:

Bernheim²⁹ 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 Lehmann³⁰ to test a large series of related compounds for antituberculosis activity.

The most effective was PAS [XXI]



[XXI]

PAS was found to be highly bacteriostatic *in vitro* and also active orally against experimental infections in animals³¹⁻³³. PAS is specifically active against mycobacteria and against only certain species in this genus³⁴. It is equally effective against streptomycin-resistant strains³³. It is of interest to find that PAS has little effect on the respiration of mycobacteria nor does it inhibit the stimulating effect salicylates³⁵. *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.

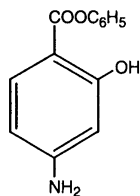
The action of PAS in combination therapy is two-fold. Firstly, it produces an antibacterial effect by competing with P-Aminobenzoic acid [PABA] in biochemical synthesis. Secondly, it enhances the serum concentration of INH 2-4 fold in man by undergoing competitive acetylation.

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.

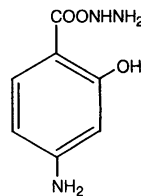
PAS is associated with 2% to 4% incidence of untoward effects. The most important being irritation of the gastrointestinal tract, peptic ulceration, renal irritation, hypersensitivity, dysfunction of the liver and thyroid.

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 [XXII] was claimed to be as active as streptomycin subcutaneously and equivalent to PAS orally^{36,37}.



[XXII]

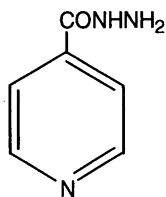


[XXIII]

PAS hydrazide [XXIII] was active *in vitro*. It's effect was inhibited by PABA and there was cross resistance with PAS, but not with INH³⁸.

Iso-nicotinic acid Hydrazide [INH]:

In 1945, Chorn³⁹ 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 [XXIV] 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's⁴⁰⁻⁴³.



[XXIV]

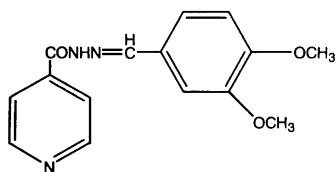
INH is both tuberculostatic and tuberculocidal *in vitro*; The minimal tuberculostatic concentration is 0.025 to 0.05 µg/ml. It is not active against the 'atypical' group. It is bactericidal even at its MIC.

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.

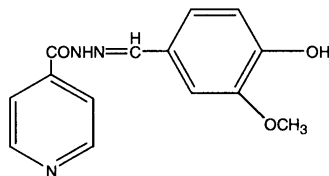
INH Hydrazones:

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



[XXV]



[XXVI]

Clinically verazide [XXV] 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-hydroxybenzylidine derivative was about half as active as INH and less than 1/10th as toxic⁶⁰. The 3-methoxy-4-hydroxybenzylidine derivative [Phthivazid, XXVI] has been extensively used clinically in Soviet Union⁴⁴.

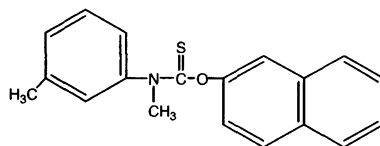
➤ *Antifungal agents*

Most of the fungal infections (mycoses) involve superficial invasion of the skin or the mucous membranes of body orifices. These diseases, which can usually be controlled by local application of an antifungal agent, are conveniently divided into two etiological groups.

- 1) Dermatophytosis (Tinea infections), which is contagious superficial epidermal infections, caused by various *Epidermophyton*, *Microsporum* and *Trichophyton* species.
- 2) Mycosis caused by pathogenic saprophytic yeasts, which are contagious and include *Aspergillosis*, *Blastomycosis*, *Candidiasis*, *Cryptococcosis*, *Histoplasmosis* etc. Under certain conditions, some of these species are capable of invading deep body cavities and causing systemic mycosis, which are less contagious. Such infections may become serious and occasionally life threatening and are difficult to treat.

During the earlier decades of the 20th century, the synthetic agents employed topically included fatty acids such as zinc undecylenate, salicylates such as N-phenyl salicyl amide, aniline dyes such as malachite green, gentian violet, quinolones such as 5-chloro-7-iodo-8-quinolinol, phenols like cresol, phenyl mercuric nitrate, chelating agents like biguanides etc. these were later replaced by Amphotericin B, Flucytosin and then more recently by two substituted imidazoles, Miconazole and Ketoconazole.

Tolnaftate:



[XXVII]

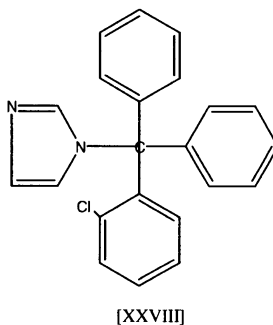
In 1960, the o-2-naphthyl ester of N-dimethyl thiocarbanilic acid (Tolnaftate) was found to have good activity against dermatophytes when applied topically. Poor permeability of Tolnaftate [XXVII] limits its use in therapy. It is available in a number of dosage forms for the treatment of ringworm, athletes foot etc. In susceptible fungi, Tolnaftate suppresses squalene epoxidase, which converts squalene to ergosterol and thereby affects cell wall formation.

➤ Substituted azoles

The substituted imidazoles represent a versatile group of antifungal agents with a unique mechanism of action. The earlier members reported were Clotrimazole [XXVIII]⁴⁵ and Miconazole [XXIX]⁴⁶, in 1969. These agents are active against dermatophytes such as *Trichophyton*, *Epidermophyton*, *Microsporum* and yeasts like *Candida albicans*. They are also active systemically in infections caused by *Cryptococci*, *Blastomyces*, *Histoplasma*, *Paracoccidioides* etc.

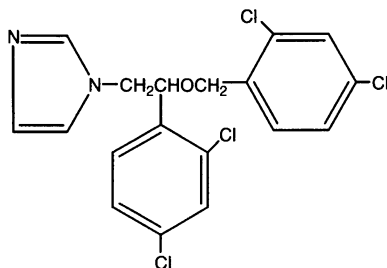
The mechanism of action of the drug is elucidated⁴⁷ extensively. At high concentrations (micromolar), they are fungicidal, while at low concentrations (nanomolar), they are fungistatic. The fungicidal effect is associated with damage to the cell wall with the loss of essential cell components, such as potassium ions and amino acids. The imidazole inhibits the cytochrome P-450 oxidase enzyme that catalyses the 14- α -demethylation of lanosterol to ergosterol.

Clotrimazole:



Clotrimazole [XXVIII] is a broad-spectrum antifungal agent use topically for the treatment of tinea infection and Candidiasis. As it causes severe gastric disturbances, it is not suitable for systemic use.

Miconazole:

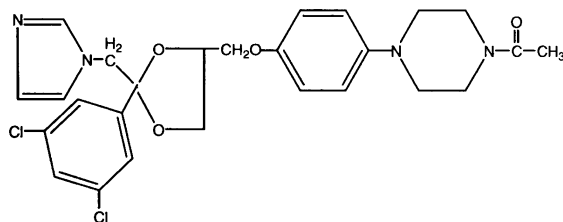


[XXIX]

Miconazole [XXIX] is intended for the treatment of serious systemic fungal infections like Candidiasis, Cryptococcosis, Petriellidiasis and Paracoccididomycosis. The adverse reactions include thrombophlebitis, pruritis, fever and G.I. disturbances.

Miconazole is solubilised with polyethylene glycol and castor oil to facilitate adequate intestinal absorption.

Ketoconazole:



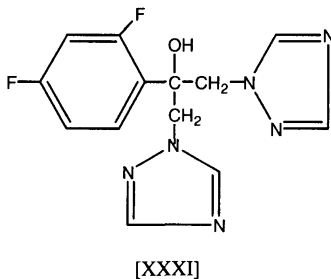
[xxx]

Ketoconazole [XXX] is administered orally for the treatment of systemic mycosis. It requires gastric acidity for absorption. Antacids and drugs like H₂-histamine antagonists and anticholinergics that inhibit gastric secretion, interfere with

its absorption. It is metabolized to the inactive form and is excreted by the enterohepatic route.

Ketoconazole is recommended for systemic mycosis such as Coccidiomycosis, Blastomycosis, Histoplasmosis, Chromomycosis, Paracoccidiomycosis and severe cutaneous infections. Hepatotoxicity is a major adverse effect. Also, levels of testosterone and corticosterone are lowered at high doses of Ketoconazole.

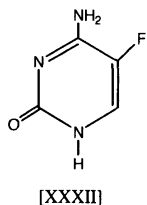
Fluconazole:



The absorption of Fluconazole [XXXI] does not depend upon gastric acidity; more over, the drug can be administered intravenously. It readily crosses the blood brain barrier, and thus, is useful for fungal meningitis.

Other azoles include Econazole, Butaconazole, Fenticonazole, Oxiconazole, Sulconazole, Ticonazole, Itraconazole and Terconazole.

Flucytosine:



This fluorinated pyrimidine was first synthesized in the 1950s as an antilukemic drug. Flucytosine [XXXII] has limited antifungal activity and sensitive strains of *Aspergillus*, *Candida*, *Cladosporium* and *Cryptococcus* are inhibited⁴⁸.

The incorporation of fluorinated pyrimidine into RNA, following selective deamination to 5-fluorouracil in the fungus, appears to be required for activity. 5-fluorouracil (5-Fu) gets metabolized to 5-fluoro-2'-deoxyuridylic acid, which suppresses DNA synthesis by inhibiting thymidylate synthetase. 5-Fu is extensively used in the treatment of various human cancers.

Due to chance of development of resistance, it is used in combination with Amphotericin B (polyene antibiotic), which lowers the risk.

Other active compounds include alkyl amines like Naftifine and Terbinafine, Triacetin, Acrisorcin, Haloprogin etc.

Types of Infections for study purposes:

➤ Bacterial Infections:

The development of resistance to current antibacterial therapy continues to drive the search for more effective agents. Bacteria are commonly responsible for many diseases which were considered until recently to be resistant to chemotherapy. The bacteria were thought to be primitive in structure and so could 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 exceptions of Morgenroth and Levy's partial success in year 1911. In combating bacterial infections of the blood stream, they found that ethyl dihydrocuprein (ophthocin), a quinine derivative, would cure pneumococcal infection in mice. Heidelberger and Jacobs coupled diazotized p-amino benzene sulphonyl amide with dihydro cuprien and found that the resultant compound had only mild activity and was not further investigated^{49,50}.

However, in 1935, Domagk⁵¹, Mietzsch and Klarer⁵² first dispelled the failure of earlier scientists by discovering Prontosil and reported that this compound cures Streptococcal infections in mice and Staphylococcal infection in rabbit. This period was considered to be a brilliant era in the chemotherapy of microbial infections.

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

➤ Fungal Infections:

There has been a recent increase in the worldwide prevalence of invasive fungal infections⁵³.

Fungi, like the human host, are eukaryotic organisms, and thus the number of suitable targets for therapeutic attacks is limited. Nonetheless, considerable progress in treating fungal infections has been achieved through better application of established antifungal agents and development of promising new investigational agents. This ever expanding battery of antifungal agents exhibits a wide variety of pharmacokinetic profiles, mechanism of actions and toxicity.

The incidents of systemic mycoses continue to rise steadily. This increase is due in part to improved recognition and diagnosis of fungal infections, but also to the prolonged survival of patients with global defects in their host defence mechanism, including patients with neoplastic diseases, organ transplant recipients, diabetic and patients with AIDS. These patients are susceptible to an ever growing list of opportunistic fungi. From the data collected by center for disease control it is known that among one and two million healthy Americans that have already been infected with AIDS, a significant number of these cases are associated with various fungal infections. One yeast-like fungus, *Cryptococcus Neoformans*, is reported to be the common reason for debilitating and life threatening infections in AIDS patients.

Considerable progress has been made in the chemotherapy of microbial disease. Yet, the development of resistance by the microbes to the drugs is an important problem faced by the chemotherapist. The rapidity of development of resistance varies widely under different circumstances. Besides the potent drugs used in the clinical therapy over years are being reported to show newer side effects. Thus there is always a need for search for newer compounds with potent activity, good tolerances, lesser side effects and increased efficacy against resistant microbial strains⁵⁴.

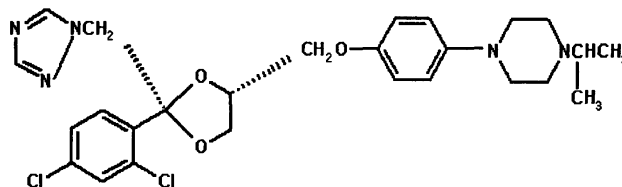
The development of new, rapid and clean synthetic routes toward focused libraries of nitrogen-containing heterocycles is of great importance to both medicinal and synthetic chemists⁵⁵⁻⁵⁷. Consequently, the design and development of procedures for the generation of new heterocycles by mean of multi step reaction is a matter of growing interest⁵⁸.

The present work has been planned keeping in mind the various chemical structures of biologically active moieties.

MARKETED FORMULATIONS WHICH CONTAIN 1,2,4-TRIAZOLE RING

1. Terconazole, U.S.P.

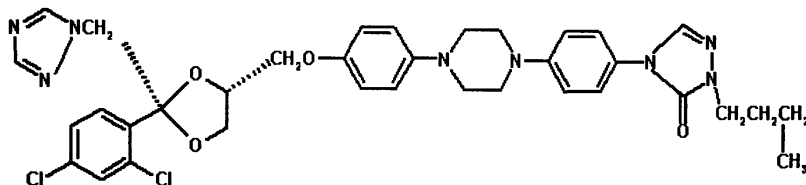
It is a triazole derivative that is used exclusively for the control of valvo-vaginal moniliasis caused by candida albicans and other candida species.



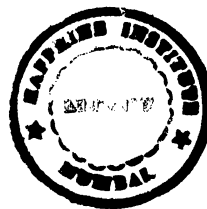
[XXXIII]

2. Itraconazole, U.S.P.

Itraconazole is an orally active, broad-spectrum antifungal agent that has become an important alternative to ketoconazole. The primary indications for itraconazole are for the treatment of systemic fungal infection including blastomycosis, histoplasmosis (including patients infected with HIV virus) and sporotrichosis.

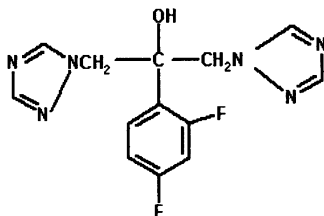


[XXXIV]



3. **Fluconazole**, U.S.P.

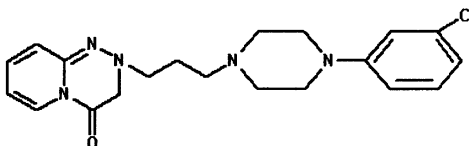
It is a water-soluble bis-triazole with broad-spectrum antifungal properties that is suitable for both oral and intravenous administration as the free base⁵⁹.



[XXXV]

4. **Trazodone**, U.S.P.

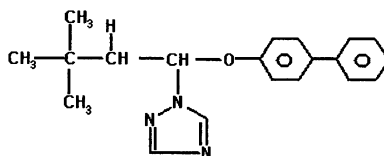
It is a triazolopyridine antidepressant, which selectively inhibits central serotonin uptake.



[XXXVI]

5. **Bittertanol**, USP

It is used as broad-spectrum fungicide⁶⁰.



[XXXVII]

➤ **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.

Progress in the field of chemotherapy is invariable 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.

Benzothiazole and Triazole moieties have played an important role in medicinal chemistry in the past few decades. Pyrazole and Schiff's base 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 and triazole attracted our attention for synthesizing new sulfur derivative compounds having potential antimicrobial activity.s

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.

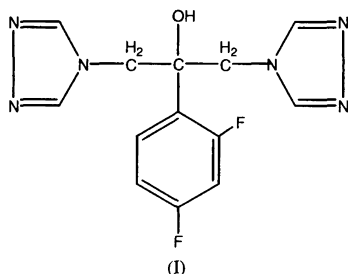
The present work in this thesis has been planned keeping in mind the various chemical structures of existing antibacterial agents and antifungal agents.

PART 2:- Synthesis of -substituted-thiobenzoic acid-s-{5-(acetyl-benzothiazol-2-yl-amino)-methyl}-[1,3,4]oxadiazole-2-yl

Among the various types of known heterocyclic ring system, benzothiazole derivatives plays vital role in biological field such as antitubercular, antiallergic, anti-inflammatory and fungicidal activity⁶¹⁻⁶⁴.

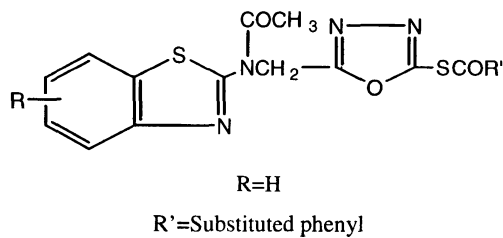
2-Amino-6-substituted benzothiazole are known to exhibit excellent antibacterial activity. According to Mayer's hypothesis⁶⁵, "A morphological relationship between bacteria and fungi may be parallel by a bio-chemical relationship". i.e. substance act as antifungal agents are likely to exhibit antitubercular activity also.

The importance of 1,2,4-triazole moiety as a major pharmacophoric requirement to exhibit enhance antifungal activity is well manifested by its inevitable presence in most recent antifungal drugs, viz. Flucanazole⁶⁶ (I).



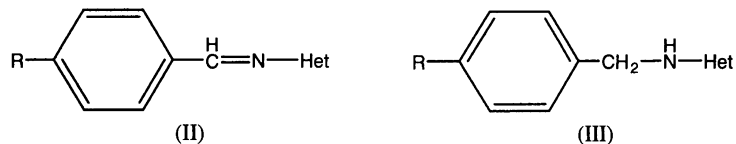
The presence of Oxygen and nitrogen in the heterocyclic system has attracted the attention of medicinal chemist because the diverse biological activities and profound efficacy. The recent review of literature has highlighted the significance of medicinal importance of oxadiazole and their derivatives⁶⁷.

2,5-Disubstituted 1,3,4-Oxadiazole constitute a unique class of Nitrogen and Oxygen containing five membered heterocycles. Oxadiazole⁶⁸ derivatives have been found to exhibit diverse biological activities such as antibacterial, fungicidal, insecticidal, anti-inflammatory and anti-HIV. Thus taking into consideration the above all observations, the synthesis of the derivatives of benzothiazole with 1,3,4-oxadiazole having acid chloride moiety were synthesized.



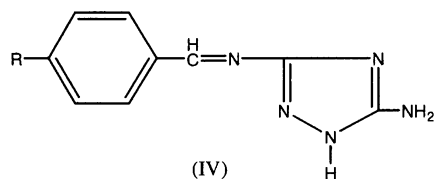
PART 3:- Synthesis of N-(substituted benzylidinoamino)-5-mercapto-4H-(1,2,4-triazol-3-yl- methyl)-N-benzothiazol-2-yl-acetamido

R. Fioravanti *et al*⁶⁹ have reported the antimicrobial activity of some new heteroaryl benzylamine (II) and their Schiff base (III). The amino triazoles are treated with various aldehydes to get Schiff base.



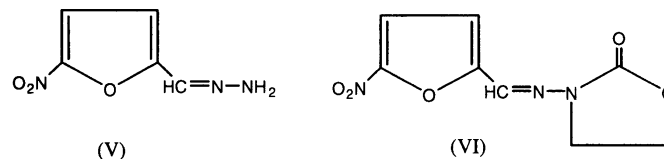
R = H, CH₃, Br, F etc.

The azomethine linkage is known to impart biological activity to various heterocyclic moieties e.g. Straukas J. *et al*⁷⁰ have reported the anti-inflammatory activity of triazole Schiff base (IV).



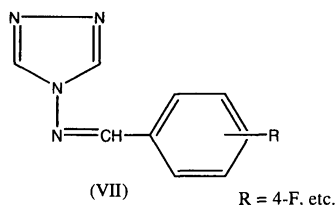
R = 2-Cl, 4-F, 4-OCH₃ etc.

Many of the biologically active compounds such as Nitrofurantoin (V), Nitrifurazone (VI)⁷¹ are used as antibacterial and anti-infective agents. These compounds also contain the azomethine linkage, which enhances the antimicrobial activity.

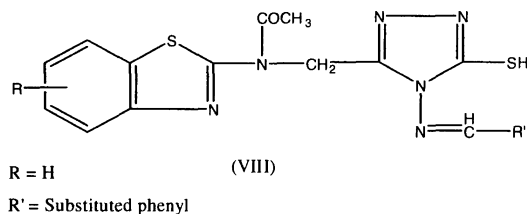


Taking into account the pharmacophoric importance of triazole in various newly introduced antifungal drugs like Flucanazole, Itraconazole, Saperconazole etc and importance of azomethine linkage that imparts antimicrobial activity, Schiff bases of 3-substituted-4-amino-5-mercapto-1,2,4-triazole were synthesized which have the

following general structure (VII). They were tested for their antifungal, antibacterial activity.



Fluorine containing drug such as Fluconazole (I) have been proved to be very specific drug for the treatment of microbial disease and they owe their activity due to the presence of fluorine which increases the lipophilicity of the molecule⁷². Considering this spectral property of fluorinated compound, Schiff bases were synthesized by condensation with various fluorinated and other aromatic aldehyde(VIII).



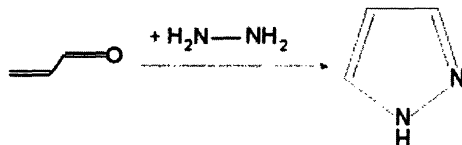
PART 4:- Synthesis of [1-[1-benzothiazol-2-yl-3-(substituted phenyl)-1H-pyrazol-4-yl]-meth-(E)-ylidene]-4-(substituted phenyl)-amine

Pyrazole as well as pyrazole containing compounds have been reported to show a broad spectrum of biological activity such as antimicrobial⁷³, antibacterial and antifungal⁷⁴. Literature survey revealed that pyrazole and pyrazolone ring system represent an important class of compound not only for their theoretical interest but also for their antipyretic, antidepressant, anti-inflammatory, antiviral, antibacterial, antifungal activities⁷⁵⁻⁸⁰ to a lesser extent, They are also used as insecticides and herbicides. Recently, some aryl Pyrazoles are reported to have non-nucleoside HIV-1 reverse transcriptase inhibitory activity⁸¹.

The first Pyrazole (IX) compound was prepared by Knorr⁸², in 1883, from phenylhydrazine and a β -keto ester. Pyrazole refers both to the class of simple aromatic ring organic compounds of the heterocyclic series characterized by a 5-

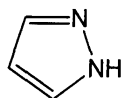
membered ring structure composed of three carbon atoms and two nitrogen atoms in adjacent positions and to the unsubstituted parent compound being so composed and having pharmacological effects on humans.

Pyrazoles are produced synthetically through the reaction of α , β -unsaturated aldehydes with hydrazine and subsequent dehydrogenation.



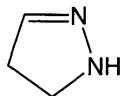
(IX)

In medicine, Pyrazoles are used for their analgesic, anti-inflammatory, antipyretic, antiarrhythmic, tranquilizing, muscle relaxing, psychoanaleptic, anticonvulsant, monoamineoxidase inhibiting, antidiabetic and antibacterial activities⁸³⁻⁸⁷. Structurally related compounds are **pyrazoline** (X) and **pyrazolidine** (XI)



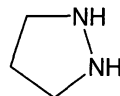
Pyrazole

(IX)



Pyrazoline

(X)

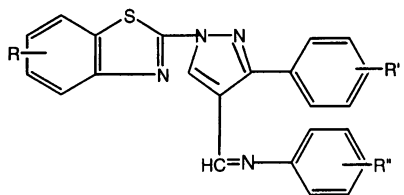


Pyrazolidine

(XI)

Substituted Pyrazoles are an important class of compounds in the pharmaceutical industry. Keeping in view the biological importance and medicinal utility of these compounds, two series of new pyrazole derivatives have been synthesized.

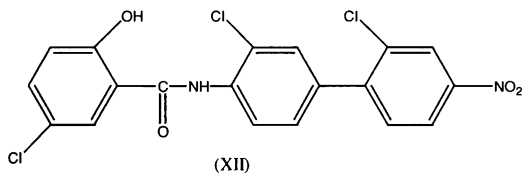
Benzothiazole derivatives as mentioned earlier in Part II for their wide range of activities. Therefore, it was considered worthwhile to explore the synthesis of compounds built upon benzothiazole skeleton incorporating pyrazolyl moiety with the hope of potentiating the activity of two such units in the same compound were prepared to obtain title compound (XII).



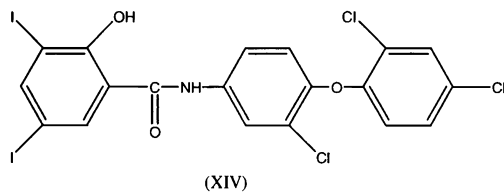
(XII)

PART 5 :- Synthesis of substituted-benzoic acid [1-[1-benzothiazol-2-yl-3-(substituted phenyl)-1H-pyrazol-4-yl]-meth-(E)-ylidene] hydrazide

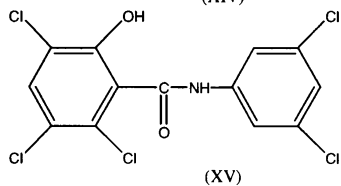
The acetamido group in the molecule was responsible for its activity. Aromatic amides have been proved to be useful in the field of medicinal chemistry. Niclosamide (XIII), Rafoxanide (XIV), Oxyclozanide (XV) etc are the drugs which owe their activity to the amide linkage.



(XII)

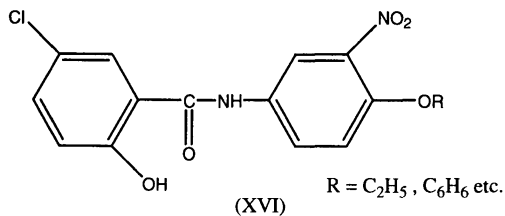


(XIV)

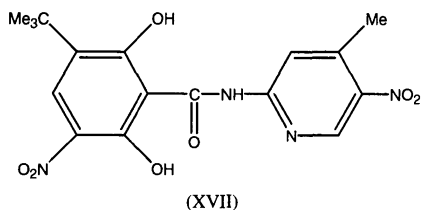


(XV)

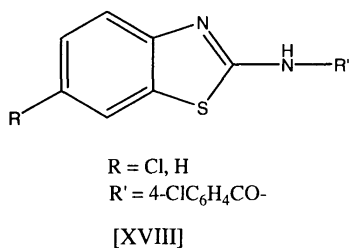
Further, the presence of -CONH linkage also plays an important role in imparting antifungal and antitubercular⁸⁸ activity to the molecule, which is reiterated by following compounds (XVI).



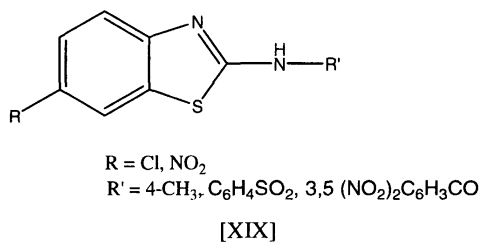
The interesting example of substituted pyridylamide (XVII) shows activity against *F. hepatica* and the contribution of -CONH linkage to the activity are good.



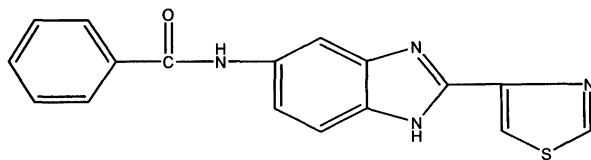
Benzothiazole is a well known established antifungal as well as bactericidal moiety in fat, paraffin and rubber industries⁸⁹. The amido linkage is much important for retaining the activity in the lead molecule (XVIII)



The 6-substituted-2-substituted benzothiazolyl amido derivative [XIX] showed good activity against *Streptococcus* S-86 at 25µg/ml concentration⁹⁰.

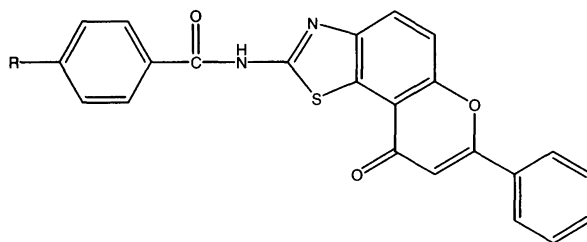


D.A Denham *et. al.*⁹¹ reported excellent antifilarial activity of 5-benzamido-2-(4-thiazolyl) benzimidazole [XX].



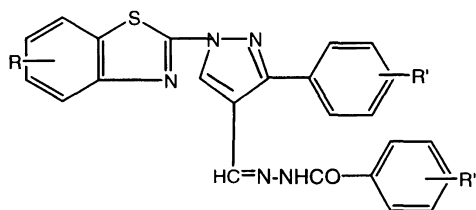
[XX]

Recently, Mantripragada Narayenu Sambaiah Thote⁹² has reported a lead molecule [XXI] containing the benzothiazole moiety and having benzamido substitution in position-2.



[XXI]

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 [XXII] 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.



[XXII]



Chemistry

CHEMISTRY

- ❖ Chemistry of 2-Amino Benzothiazole
- ❖ Chemistry of 1,2,4-Triazole
- ❖ Chemistry of Hydrazide
- ❖ Chemistry of Dithiocarbamate salt
- ❖ Chemistry of acid chloride
- ❖ Chemistry of Schiff Base
- ❖ Chemistry of Amide

CHEMISTRY OF 2-AMINO BENZOTHAZOLE

The benzothiazole ring contains a phenyl ring fused to a thiazole ring. 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.

SYNTHESIS OF 2-AMINO 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 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 dioxide⁹³ 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 chlorides⁹⁴, sulfuryl chlorides³⁷ or certain N-chloroamides⁹⁵⁻⁹⁷ can also be used but do not appear to possess any advantages over bromine or chlorine.

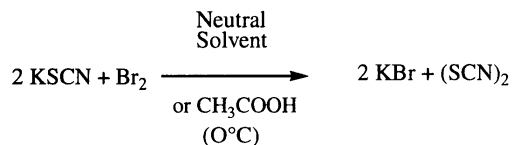
Solvents that have been used with thiocyanogen include benzene, bromobenzene, carbon tetrachloride, chloroform, ether, ethylene bromide, carbon disulfide, petroleum ether, methyl acetate, nitromethane, anhydrous formic acid and acetic acid. At low temperature such solvents as saturated solutions of alkali thiocyanates in methanol^{98,99} or acetone¹⁰⁰ 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^{101,102}.

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^{101,103,104}. 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

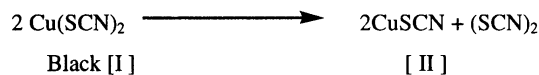
low concentration of the reagent, maintained in this way minimises polymerisation. The reagent is generated from the thiocyanate salts by chemical reactions.

➤ **THIOCYANOGEN GENERATED FROM SALTS BY CHEMICAL REACTIONS:**

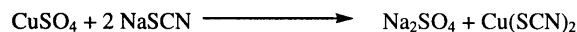
In this method the alkali metal thiocyanate is dissolved in acetic acid or formic acid^{104,105}, or better in neutral solvent like methyl acetate, acetone or methanol,^{106,107} and then the compound to be thiocyanated is introduced to the above solution. Bromine or chlorine is added to the cooled solution 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 thiocyanate¹⁰⁸, 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 (I) to cuprous salt (II).



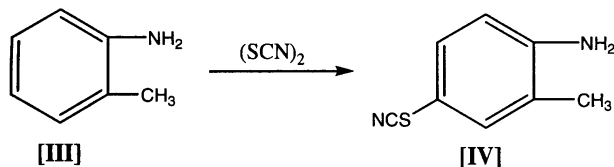
Cupric thiocyanate (II), prepared in advance, or a paste of cupric sulphate and sodium thiocyanate in equivalent proportions is added to a solution of the compound in methanol or acetic acid and the mixture is warmed to 35°-80°C, until the black cupric thiocyanate has changed completely to the white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



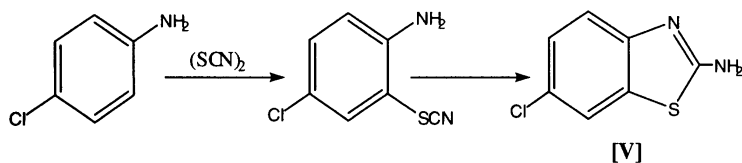
This procedure has the advantage over the others of permitting higher temperatures for thiocyanation.

➤ 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 [III] is converted to 4-thiocyano-o-toluidine [IV] (80% yield)¹⁰⁹.



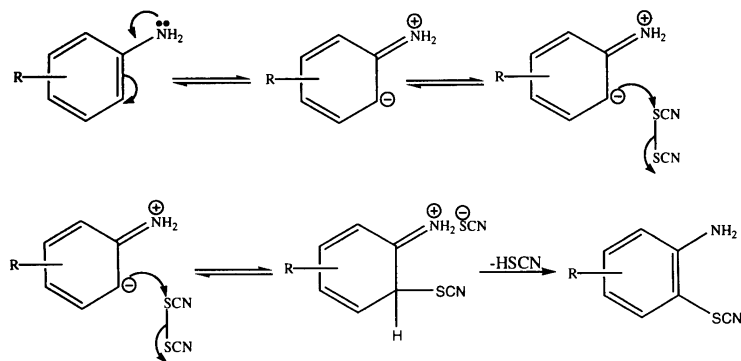
The thiocyanation takes place in the position ortho to the primary amine group. The final product in such cases is often an amino benzothiazole formed by the secondary reaction between the amino group and the adjacent thiocyno group. In this secondary reaction, since the amino group is having lone pair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



Mechanism:

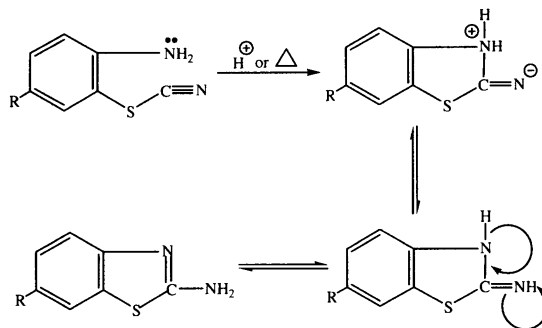
The mechanism of thiocyanogen reaction probably consists of the initial attack of thiocyanogen to the activated site of 4-substituted aniline where due to the mesomeric effect of amino group, the ortho position is having surplus of electrons

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.

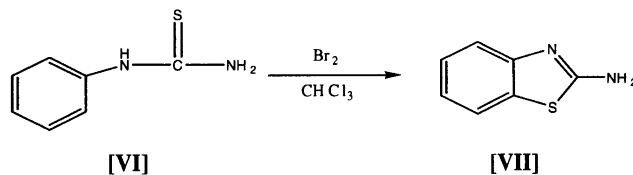
The intermediate, under the influence of acid and heating adds an amino function to the adjacent thiocyno group as:



➤ HUGERSCHOFF SYNTHESSES:

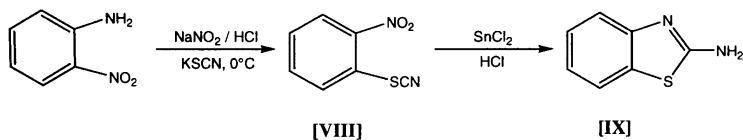
This synthesis is restricted to the preparation of 2-amino derivatives of the benzothiazoles [VII] and employs the action of oxidising agent such as bromine, upon aryl thioureas [VI] in an inert solvent such as chloroform, ethylene dichloride,

chlorobenzene or acetic acid. Sulfuryl chloride can be substituted for bromine¹¹⁰⁻¹¹². However, potassium ferrocyanide in alkaline media, the reagent for the Jacobson ring closure, gives no thiazole from the arylthiourea^{113,114}.



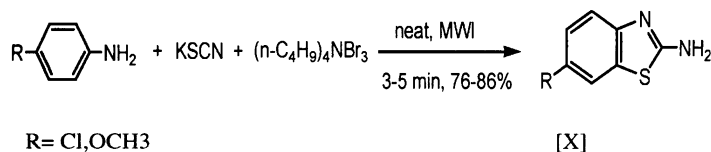
Generally, the yields in the Hegerschoff's reaction are excellent and frequently nearly quantitative.

2-Amino benzothiazoles [IX] have been prepared from o-thiocyano-nitro compounds of the type [VIII] by reduction using stannous chloride in HCl and FeSO₄ in ammonia^{115,116}.



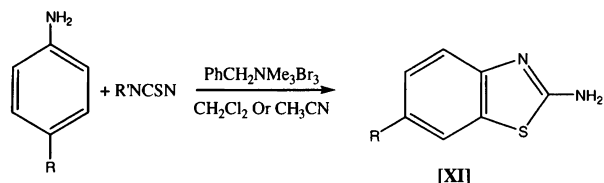
➤ NEWER METHODS:

1) 2-Aminobenzothiazoles [X] were efficiently synthesized by Zheng *et al.*¹¹⁷ using stable, crystalline tetrabutylammonium tribromide instead of toxic, corrosive liquid bromine under solvent-free and microwave irradiation condition.

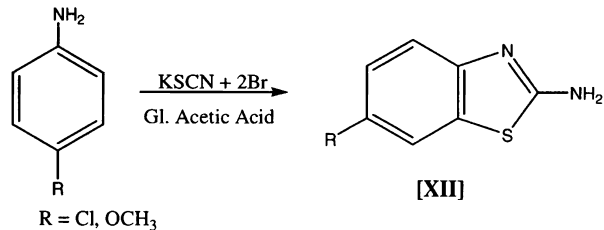


2) Jordan *et al.*¹¹⁸ prepared 2-aminobenzothiazole [XI] derivatives using benzyltrimethyl ammonium tribromide (PhCH₂NMe₃Br₃), which is a stable,

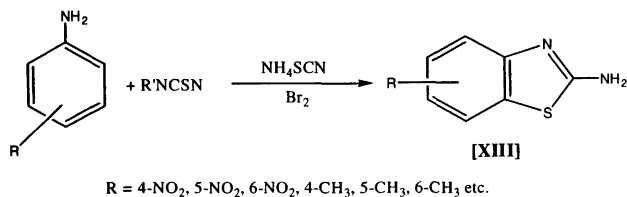
electrophilic bromine source for the conversion of substituted arylthioureas to 2-aminobenzothiazoles under mild conditions in a variety of solvents with good yields. One of the key benefits for this reagent when compared with molecular bromine in ease of addition and handling, which minimizes the risk 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 $\text{Ph-CH}_2\text{NMe}_3\text{Br}_3$.



3) Matsui *et. al.*¹¹⁹ prepared 6-substituted-2-aminobenzothiazoles [XII] by the reaction of 4-substituted anilines with potassium thiocyanate in presence of bromine.

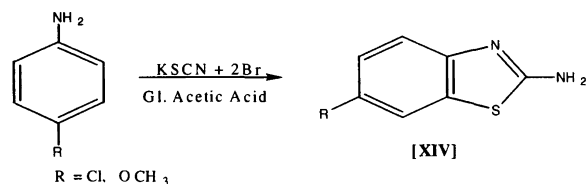


3) Patel and Agravat¹²⁰ synthesized various 4(5 or 6)-substituted-2-aminobenzothiazoles [XIII] with help of 4(5 or 6)-substituted anilines which was reacted with ammonium thiocyanate and bromine.

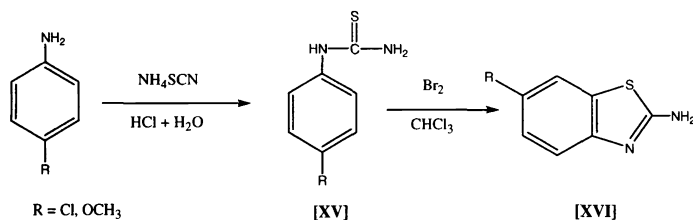


5) Jimonet *et. al.*¹²¹ synthesized various substituted-2-benzothiazolamines derivatives by different methods:

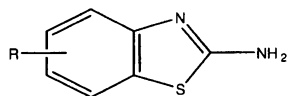
Method A:- One-pot reaction of appropriate anilines with thiocyanogen generated from bromine and alkaline thiocyanate in acetic acid medium led to formation of the desired product [XIV] in moderate yields.



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



In the present study, the raw materials viz. 2-amino substituted benzothiazoles [XVII] are synthesized by thiocyanating substituted anilines with KSCN and bromine in acetic acid at cold conditions, according to the literature methods¹²¹.



R = 5,6-DiCl, 6-CH₃, etc

[XVII]

Chemistry of Hydrazide

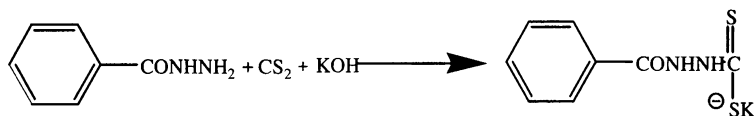
Acid hydrazides are obtained by the reaction of methyl or ethyl esters with hydrazine hydrate.



The general procedures followed for the synthesis of acid hydrazides in the present work is analogous to that reported by Vogel¹²².

Chemistry of Dithiocarbamate salts

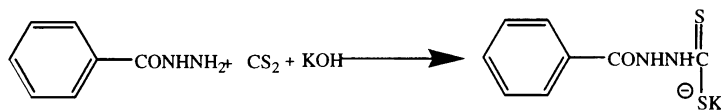
- i. Busch and starke¹²³ obtained crystalline potassium salt of 2-benzylidithio carbazinic acid (XVIII) by stirring together benzhydrazide (XIX), carbon disulphide and a solution of KOH in absolute alcohol for 2-3 hours at 35-40°C and then for 12-18 hrs at room temperature. Later Hoggarth synthesized the potassium salts of aryl dithiocarbamate using the same procedures¹²⁴.



[XVIII]

[XIX]

- ii. Reid¹²⁵ et al prepared the potassium salt [XXI] by stirring together KOH, CS₂ and aryl hydrazide in absolute alcohol for 12-16 hours and the product was washed with dry ether.



[XX]

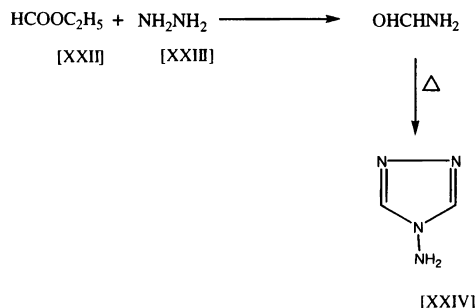
[XXI]

In present investigation, the method employed for the synthesis of potassium salts of substituted aryl dithiocarbamate is that of Ried et al.; the advantage of this method is the better yield obtained. However the reaction mixture was stirred for 4-5 hours only, as it was seen that the yields obtained after stirring for 4-5 hours or more were the same.

CHEMISTRY OF TRIAZOLE

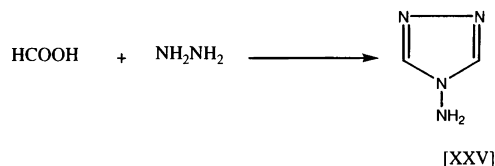
Methods of preparation of 4-Amino triazole and its derivatives:

1. 4-Amino triazole (XXII) has been prepared¹²⁶ by the reaction of ethyl formate (XXIII) with hydrazine (XXIV) to give formhydrazide, which was heated at 150 to 200° C to effect self condensation to give (XXIV).



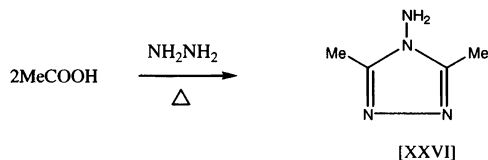
[XXIV]

Similar interaction of formic acid and hydrazine gave good yield of 4-Amino triazole (XXV)^{127,128}

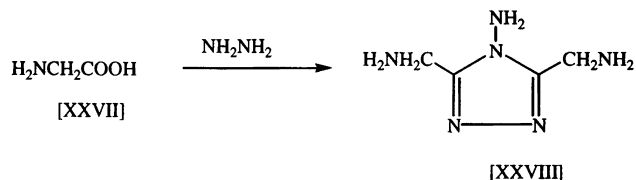


[XXV]

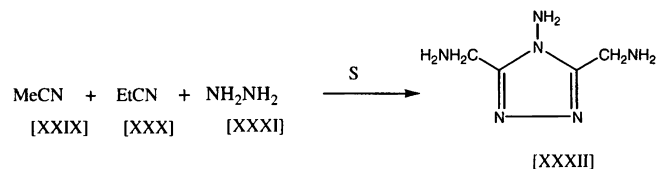
2. Other carboxylic acids and their functional derivatives have also been used to prepared alkyl and substituted alkyl -4-Amino triazoles, e.g. acetic acid and hydrazine hydrate at high temperature give 3,5-dimethyl-4-Amino triazole (XXVI)¹²⁹



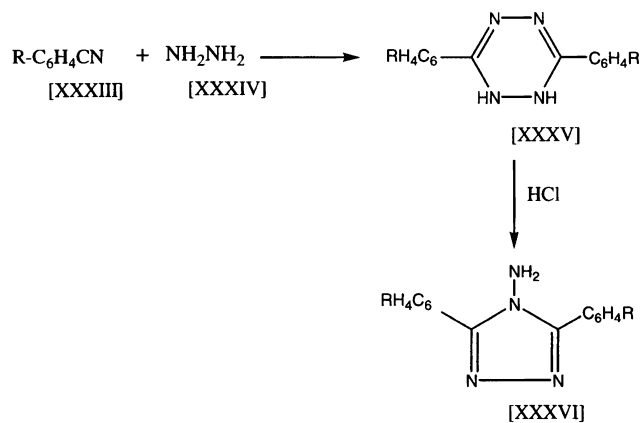
3. Similar reaction have been reported for the preparation of 4-Amino-3,5-bis (amino alkyl) triazole (XXVII)¹³⁰⁻¹³¹, which is prepared by the reaction of glycine (XXVII) with hydrazine to give (XXVII).



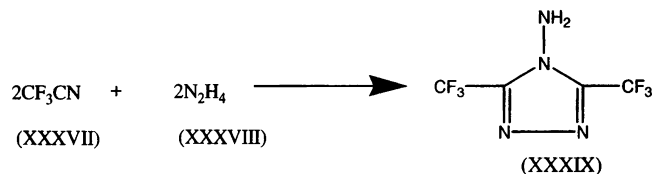
4. Sulfur and sulfur containing compounds catalyze the formation of 4-Amino triazoles e.g. 4-Amino triazole (XXXII) substituted at 3,5-position with different substituents was prepared by the reaction of equimolar amount of acetonitrile (XXIX) and propionitrile (XXX) with a mixture of hydrazine (XXXI) and sulfur¹³².



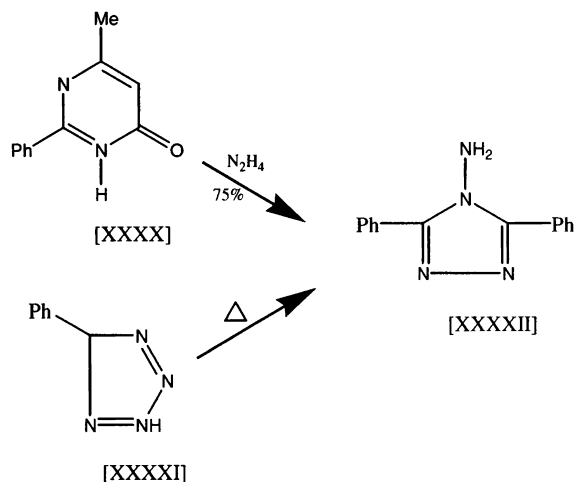
5. The preparation of 4-Amino-3,5-diaryl triazoles (XXXVI) are usually effected by the acidic rearrangement of 1,2-dihydro tetrazines (XXXV), which are obtained by the reaction of equimolar amount of arynitriles (XXXIII) and hydrazine (XXXIV) in refluxing benzene or toluene¹³³.



6. Perfluoro alkyl triazole (XXXIX) are prepared by the various methods e.g. perfluoro acetonitrile (XXXVII) with hydrazine (XXXVIII) in a sealed tube at room temperature gave (XXXIX)¹³⁴



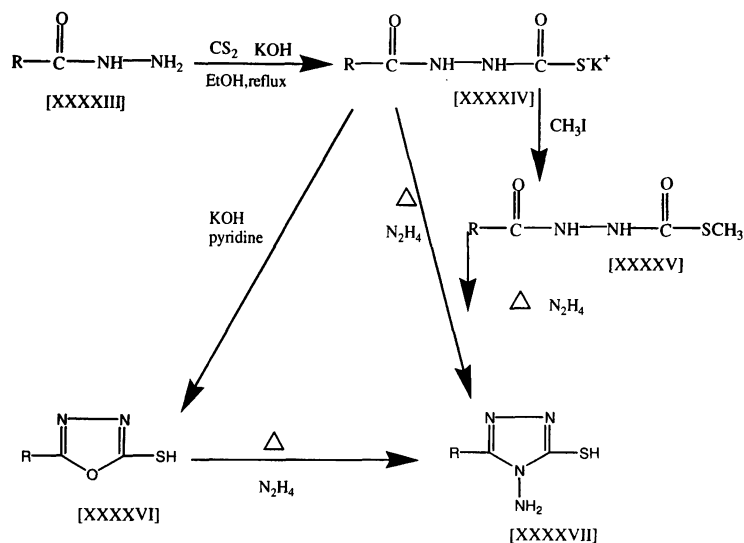
7. 4-amino -3,5-diphenyl triazoles (XXXXII) are also formed by the decomposition of other hetero aromatic ring systems e.g. reaction of pyrimidine (XXXX) with hydrazine¹³⁵. Also the thermolysis of 5-phenyl tetrazole (XXXXI) in various solvents¹³⁶ gives (XXXXII).



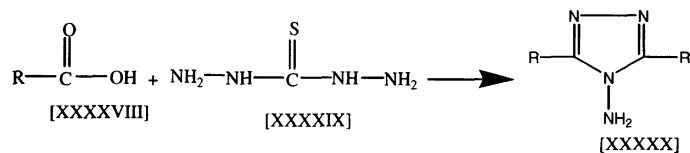
Methods of preparation of 3-(substituted phenyl)-4-amino-5-mercapto-1,2,4-triazole:

1. One of the methods for synthesis of triazole is Hoggarth synthesis¹³⁷. This methodology involves the condensation of carboxylic acid hydrazides with carbon disulphide and potassium hydroxide to yield the potassium dithiocarbazate (XXXXIV) which after s-alkylation with methyl iodide,

undergo ring closure with an excess of hydrazine, to produce the amino thiols (XXXXVII) in good yields.



2. More recently, Heindel and Reid have reported the direct conversion of the carbazate salt by the reaction with excess of hydrazine, this avoids 5-methylation, resulting in the higher yield and a shorter reaction time¹²⁵.
3. Alternatively, oxadiazoles (XXXXVI) can be converted to triazoles (XXXXVII) by the reaction with hydrazine.
4. It has also been reported that the same carboxylic acid react with thiocarbonylhydrazide to afford directly the 3-substituted-4-amino-5-mercapto-1,2,4-triazoles (XXXXX). It has also been found that the reaction of thiocarbonylhydrazide with carboxylic acids can be conveniently applied to both aliphatic and aromatic acid derivatives^{138,139}.



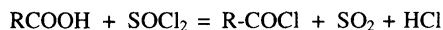
Chemistry of acid chlorides

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

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

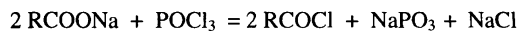


The use of thionyl chloride¹⁴⁰ for the preparation of acid chlorides presents many advantages in a large number of cases. The reaction results in the formation of sulphur dioxide and hydrogen chloride in addition to the acid chloride and the isolation of the chloride are for this reason a simple matter.

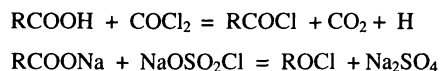


The thionyl chloride must be pure as impure product has been known to cause a somewhat explosive decomposition in some instances. The procedure consists of mixing the chlorinating agent with acid and heating the mixture, if necessary. The product may be isolated by fractional distillation or if solid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

Phosphorous oxy-chloride reacts with the sodium salt of carboxylic acid to give acid chloride. This method is of importance with acids which undergo decomposition with other chlorides of phosphorous.



The reaction of phosgene¹⁴¹ with carboxylic acids and p-toluene sulphonylchloride or of sodium chlorosulphonate with salts of carboxylic acids is also used for the preparation of acid chlorides.

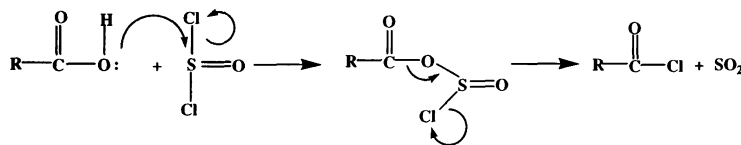


The acid chlorides are obtained readily through the reaction of sulphuryl chlorides with anhydrous salts of organic acids. The reagents are well mixed and subjected to distillation.

In the present work some acid chlorides were prepared by known methods using corresponding benzoic acids and the some commercially available acid chlorides were used from Sigma-Aldrich Chemicals.

➤ **Reaction mechanism:**

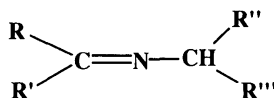
Acids and their salts are the only common starting materials of acid chlorides and several reagents are utilized as a source of nucleophilic chloride. All the reagents from reactive intermediate so, that the leaving group in the substitution is not hydroxyl group.



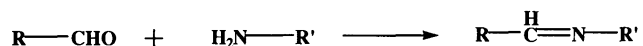
Chemistry of Schiff bases

A Schiff base (or azomethine), named after Hugo Schiff, is a functional group that contains a carbon-nitrogen double bond with the nitrogen atom connected to an aryl or alkyl group—but not hydrogen.

Schiff bases are of the general formula $R_1R_2C=N-R_3$, where R_3 is an aryl or alkyl group that makes the Schiff base a stable imine. A Schiff base derived from aniline, where R_3 is a phenyl or substituted phenyl, can be called an *anil*.



Schiff bases are formed by the condensation of an amine and the carboxyl group of aldehydes.



Schiff ¹⁴² for the first time reported condensation of this kind and hence the condensation products are referred to as “Schiff bases”.

Schiff bases are characterized by the $-\text{N}=\text{CH}-$ (imine) group which is important in elucidating the mechanism of transamination and racemisation reaction in biological systems^{143,144}. Due to the great flexibility and diverse structural aspects, a wide range of Schiff bases have been synthesized and their complexation behavior studied ¹⁴⁵.

Pelton et al¹⁴⁶ had shown the antifungal and antibacterial activity of some substituted salicylaldehydes. Schiff bases from different aldehydes have shown encouraging anti-tuberculosis activity *in vitro* in our laboratory ¹⁴⁷. Also the presence of an azomethine linkage in compounds has been shown to decrease their toxicity considerably ¹⁴⁸.

Many procedures have been described in the literature for the synthesis of Schiff bases ¹⁴⁹⁻¹⁵¹.

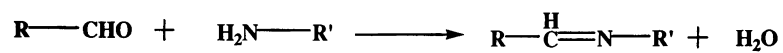
The important methods are listed below:

1. The simplest method appears to be the one in which equimolar quantities of an amine and aldehydes are condensed by boiling in methanol or ethanol.
2. In 1946, Tipson and clapp¹⁵² obtained Schiff bases by refluxing the amine and the aldehydes in toluene with a few drops of piperidine. The water formed during the course of the reaction was removed by employing a Dean-stark trap.
3. In 1950, Hayes et al¹⁵³ prepared Schiff bases by heating a mixture of an aldehydes and an amine in benzene and removing the water formed during the reaction using Dean-Stark apparatus.
4. In 1953, Giovambettista and Rabassa¹⁵⁴ suggested the use of acetic acid as a catalyst for the synthesis of Schiff bases in good yield.
5. In 1971, the condensation of hydrazine and an aldehydes using glacial acetic acid as a solvent was described.

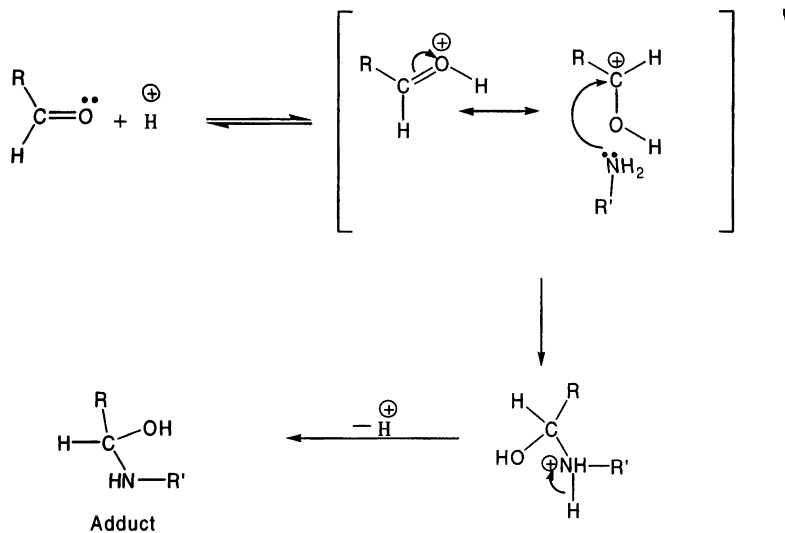
As the first method was found to give good yields it was followed initially. The addition of few drops of acetic acid was found to improve the yield. Later on, during the course of investigation the last method using acetic acid as a solvent was found to be the best for the following reasons.

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

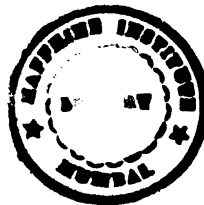
Reaction mechanism:



The amine and the aldehydes are heated in the presence of an acid. The acid protonated the carbonyl oxygen and promotes the attack of nucleophilic amine.



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



Chemistry of amides

Amides are derived from carboxylic acids. A carboxylic acid contains the –COOH group, and in an amide the –OH part of that group is replaced by an –NH₂ group. So amides contain the –CONH₂ group. (R-CO-NR'R'')

The amide linkage is one of the most fundamental and widespread chemical bonds in nature¹⁵⁵. Amides are common moieties in naturally occurring substances like proteins and peptides and are also found in various synthetic materials. This makes the amide functionality an important building block in synthetic chemistry and many methods for its synthesis were reported in the literature¹⁵⁶⁻¹⁶⁰

Amide compounds have attracted much attention because of their extensive applications in the pharmaceutical industry; they have been generally used as a protective group in synthetic routes, an amide functional group is contained in many bioactive compounds or drugs, and amide modification is an important optimization methodology for new chemical entities in medicinal chemistry¹⁶¹⁻¹⁶⁵. Furthermore, peptide bond, a special kind of amide bond in life science, constitutes the backbone of proteins.

The stable and polar amide functionality is an important unit among the organic molecules present in natural occurring materials. It is also found in many synthetic substances, an intermediate or as active pharmaceutical product or prodrugs¹⁶⁶. Due to its interest in organic synthesis the preparation of amides from the corresponding amines is an important and well known transformation. It has been noted recently that around 66% of all preliminary screening reactions in industrial medicinal chemistry laboratories involve amide formation¹⁶⁷⁻¹⁶⁹

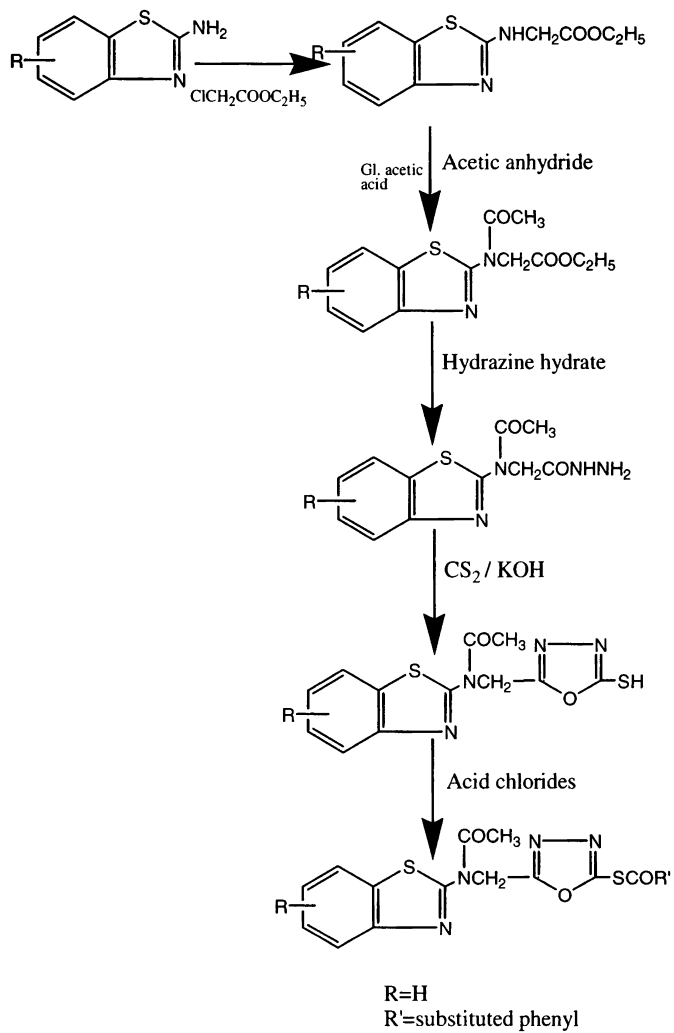
Since, amide bond linkage is ubiquitous and important core structure in pharmaceuticals, chemicals, as well as many natural products¹⁷⁰⁻¹⁷² and hence it was thought to synthesize new amide linkage by condensation of formylated pyrazolyl benzothiazole and different substituted benzhydrazide.

*Schematic
Representation*

SCHEMATIC REPRESENTATION

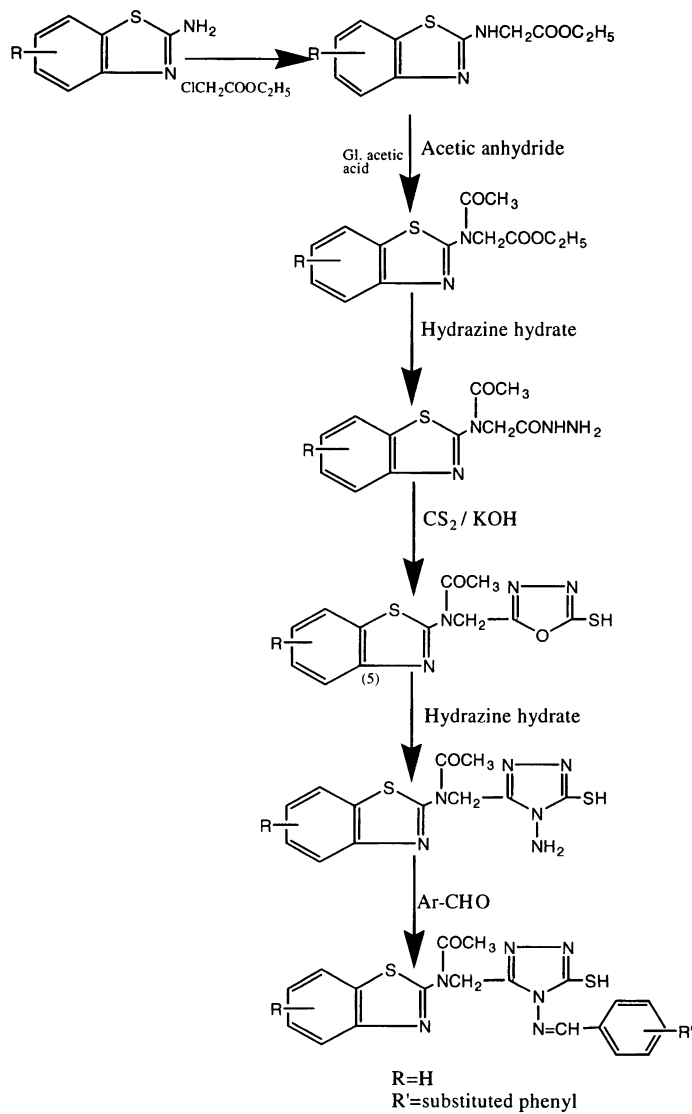
Synthesis of -substituted-thiobenzoic acid-s-{5-[(acetyl-benzothiazol-2-yl-amino)-methyl]-[1,3,4]oxadiazole-2-yl}

Scheme I



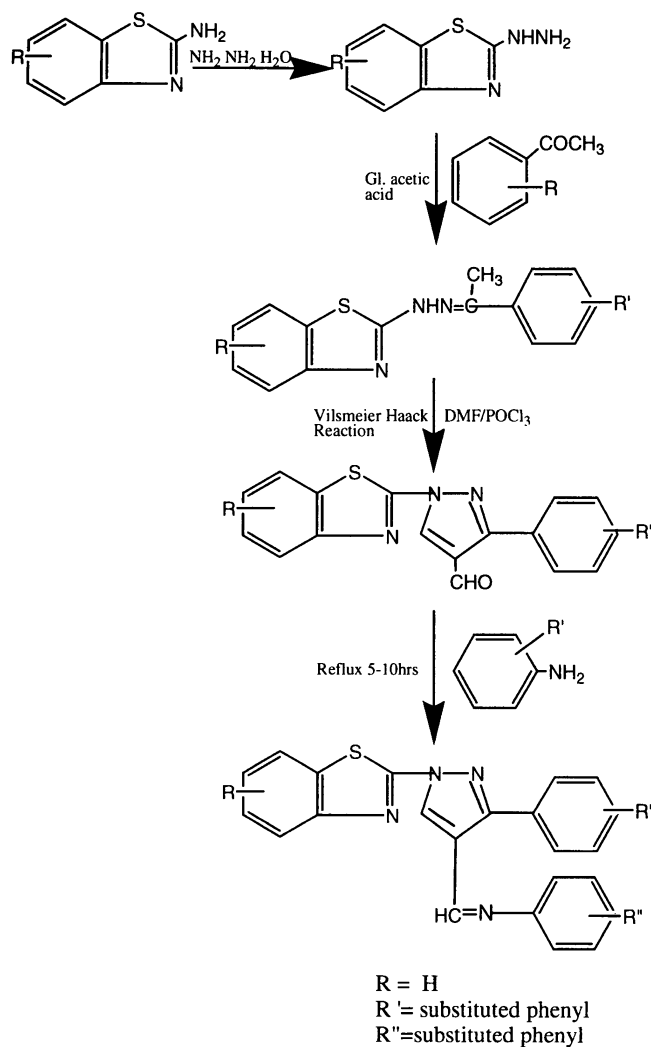
Synthesis of N-(*o*-(substituted benzylideneamino)-5-mercapto-4H-(1,2,4-triazol-3-yl-methyl)-N-benzothiazol-2-yl-acetamido

Scheme II



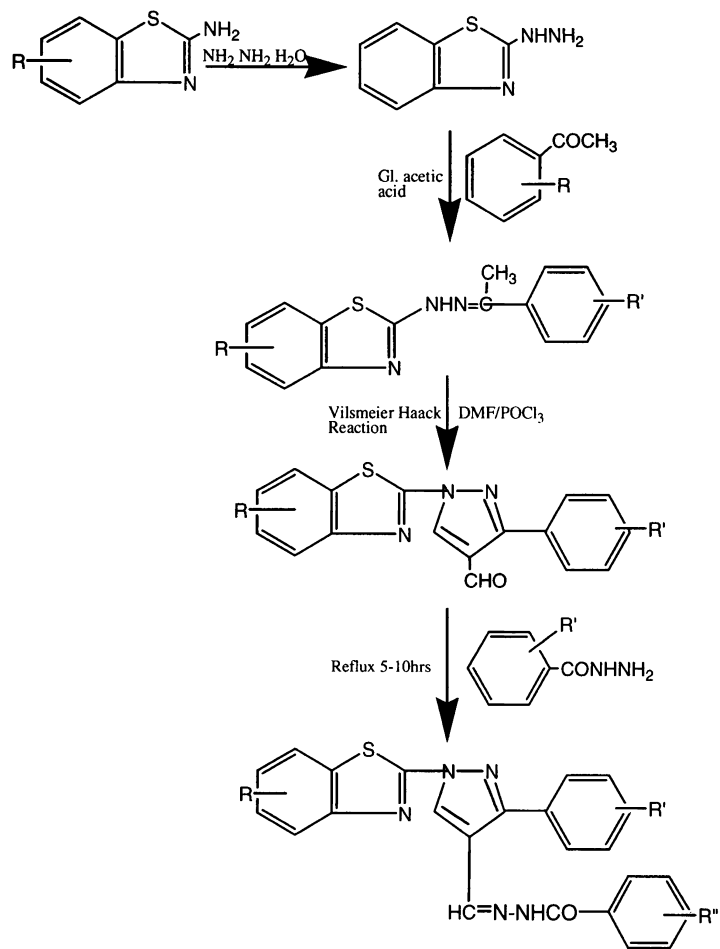
Synthesis of [1-[1-benzothiazol-2-yl-3-(substituted phenyl)-1H-pyrazol-4-yl]-meth-(E)-ylidene]-4-(substituted phenyl)-amine

Scheme III



Synthesis of substituted-benzoic acid [1-[1-benzothiazol-2-yl-3-(substituted phenyl)-1H-pyrazol-4-yl]-meth-(E)-ylidene] hydrazide

SCHEME IV



R = H
 R' = substituted phenyl
 R'' = substituted phenyl

Experimental

EXPERIMENTAL

This section of the thesis deals with the synthetic details of the compounds synthesized. The purity of the raw materials used in various synthetic reactions was ascertained by melting point (Confirmed by the literature M.P.) and TLC. The completion of the reaction was monitored by TLC and reaction continued until both the reactants were consumed or largely consumed. Product isolated, purified and recrystallised using suitable solvent system. The product obtained was then characterized by elemental and spectral analysis.

Part II: Synthesis of -substituted-thiobenzoic acid-s-{5-(acetyl-benzothiazol-2-yl-amino)-methyl}-[1,3,4]oxadiazole-2-yl

➤ Step-1 : Synthesis of 2-N-(ethyl aceto acetate) benzothiazole

In a 100 ml round bottom flask, 1.5 gm (0.01 M) of 2-amino benzothiazole connected to reflux condenser with guard tube to maintain dry condition to the reaction. To this 2.07 gm (0.15 M) of anhydrous potassium carbonate was dissolved in 10 ml of anhydrous acetone by warming on water bath. when mixture is almost dissolved, Chloro ethyl acetate 1.225 ml (0.01 M) was added and reflux for 20 hrs.on water bath.

After completion of the reaction, the solvent distilled off and residue was poured into crushed ice. The solid which is obtained wash with cold water and recrystallized with ethanol.

M.P = 110°C.

Yield = 51 %.

Colour & nature = Pale yellow amorphous powder

Elemental analysis: C₁₁H₁₂N₂O₂ S

	C%	H%	N%
Calculated	55.93	5.08	11.38
Found	55.60	4.99	11.05

➤ **Step-2 : Synthesis of 2-N-(acetyl ethyl aceto acetate) amino benzothiazole**

Take 2.36 gm (0.01 M) of compound to this add 0.3 ml (0.03 M) of acetic unhydride and 5 ml of glacial acetic acid. Reflux the reaction mixture for about 15 min. on water bath with occasional shaking. The reaction mixture was cooled, pour the content slowly in cold water. Filter the solid and washed with excess of water and recrystallized with ethanol.

M.P = 120-125°C.

Yield = 64 %.

Colour & nature = Pale yellow amorphous powder

Elemental analysis: $C_{13}H_{14}N_2O_3S$

	C%	H%	N%
Calculated	56.11	5.03	10.07
Found	56.34	4.83	10.36

➤ **Step-3 : Synthesis of 2-N-acetyl-(acethydrazide) benzothiazole**

The 2.78 gm (0.01 M) of compound dissolved in 10 ml of ethanol. To this solution add 2.5 ml of (0.05 M) of hydrazine hydrate and 3-4 drops of conc. HCl dropwise with intermittent shaking. After addition was completed, the reaction mixture was refluxed for 3-4 hrs. The reaction mixture was then poured into crushed ice thus the product separated as solid was filter and recrystallized with ethanol.

M.P.= 160°C

Yield = 56%

Colour & nature = White amorphous powder

Elemental Analysis: $C_{11}H_{12}N_4O_2S$

	C%	H%	N%
Calculated	50.00	4.54	21.21
Found	50.28	4.19	21.68

➤ **Step-4 : Synthesis of 2-N-acetyl –[(2'-methyl-5'-mercapto)-1',3',4'-oxadiazolyl] benzothiazole**

In two necked 250 ml round bottom flask equipped with a dropping funnel and reflux condenser, a solution of compound 2.72 gm (0.01 M) in absolute alcohol and KOH 0.5 gm (0.01 M) in water was refluxed on water bath for 1 hr. The solution of Carbon disulfide 0.76 ml (0.01 M) in absolute alcohol was added dropwise. The reaction mixture was refluxed till evolution of H₂S gas ceases. Thereafter it was cooled, diluted with cold water and acidified with glacial acetic acid. The resulting solid was washed with water and crystallized from ethanol.

M.P.= 147°C

Yield = 41%

Colour & nature = Yellow amorphous powder

Elemental Analysis: C₁₉H₁₄N₄O₃S₂

	C %	H %	N %
Calculated	55.60	3.41	13.65
Found	55.89	3.08	13.55

➤ **Step-5 : Synthesis of benzoyl chloride:**

A mixture of benzoic acid (24.4 gm, 0.02 mol) and freshly distilled thionyl chloride (32.57 gm, 0.3 mol) in 50 ml dry benzene was taken in 250 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 atmospheric pressure¹⁷³.

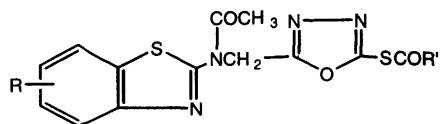
Sr. No	Substituent	Observed B.P. (°C)	Reported B.P.(°C)	Yield (%)
1	2-Fluoro	73	66-67	68
2	4-Fluoro	72	69-70	74
3	3-Chloro	75	71-76	65
4	4-Chloro	113	111	79

Similarly, some benzoyl chlorides were prepared in the laboratory by using the same method and others were used from Sigma-Aldrich Chemicals.

➤ **Step 6 :- Synthesis of substituted-thiobenzoic acid-s-{5-(acetyl-benzothiazol-2-yl-amino)-methyl}-[1,3,4]oxadiazole-2-yl.**

In 100 ml three necked round bottom flask equipped with dropping funnel, reflux condenser and guard tube was placed a mixture of 2-N-acetyl –[(2'-methyl-5'-mercapto)-1',3',4'-oxadiazolyl] benzothiazole, 0.1gm (0.02 M) stirred at 50-60°C for about 30 min .To this add dropwise a solution of substituted benzoyl chloride 0.177gm (0.001 M)in 10 ml dry 1,4-Dioxane. After addition , the reaction mass was stirred for 2 hrs at the same temp. The solution was poured into crushed ice. The solid separates out was filtered , washed with water and dried. It was then recrystallized from ethanol.

Table 1:- Synthesis of -substituted-thiobenzoic acid-s-{5-(acetyl- benzothiazol-2-yl-amino)-methyl}-[1,3,4]oxadiazole-2-yl



Sr. No.	R	R'	Molecular Formula	M.P. (°C)	Yield (%)	Nature	Colour	Elemental Analysis (%)					
								Calculated			Found		
								C	H	N	C	H	N
1	H	4-F	C ₁₉ H ₁₃ FN ₄ O ₃ S ₂	152	69	Amorphous	White	53.27	3.03	13.08	53.30	3.00	13.04
2	H	2-F	C ₁₉ H ₁₃ FN ₄ O ₃ S ₂	171	45	Amorphous	Yellowish White	53.27	3.03	13.08	53.24	3.01	13.17
3	H	3-Cl	C ₁₉ H ₁₃ Cl N ₄ O ₃ S ₂	180	51	Amorphous	Pale Yellow	51.30	2.92	12.60	51.52	2.87	12.87
4	H	2-Cl	C ₁₉ H ₁₃ Cl N ₄ O ₃ S ₂	195	58	Amorphous	Pale Yellow	51.30	2.92	12.60	51.26	2.86	12.48
5	H	2,5-DiCH ₃	C ₁₈ H ₁₃ N ₂ Cl ₃ O ₃ S	163	48	Amorphous	Yellow	57.53	4.10	12.78	57.29	4.22	12.89

SPECTRAL ANALYSIS:

➤ Spectral studies of 4-Fluorophenylthiobenzoic acid-s-{5-(acetyl-benzothiazol-2-yl-amino)-methyl}-[1,3,4]oxadiazole-2-yl.

PMR (CDCl₃)δ: -

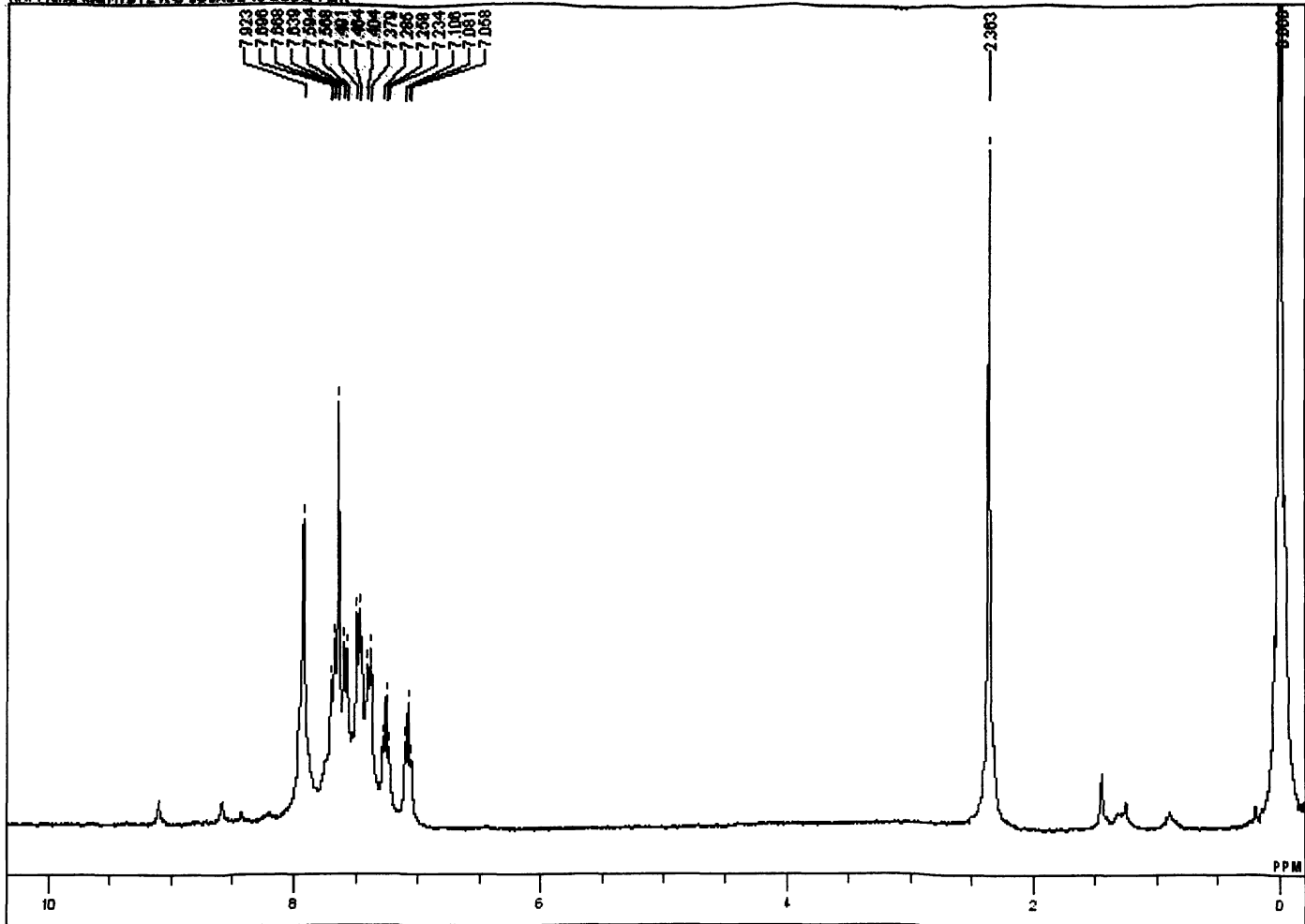
7.0-7.9	(m, 8H ,Ar-H)
2.3	(s,3H, -COCH ₃)

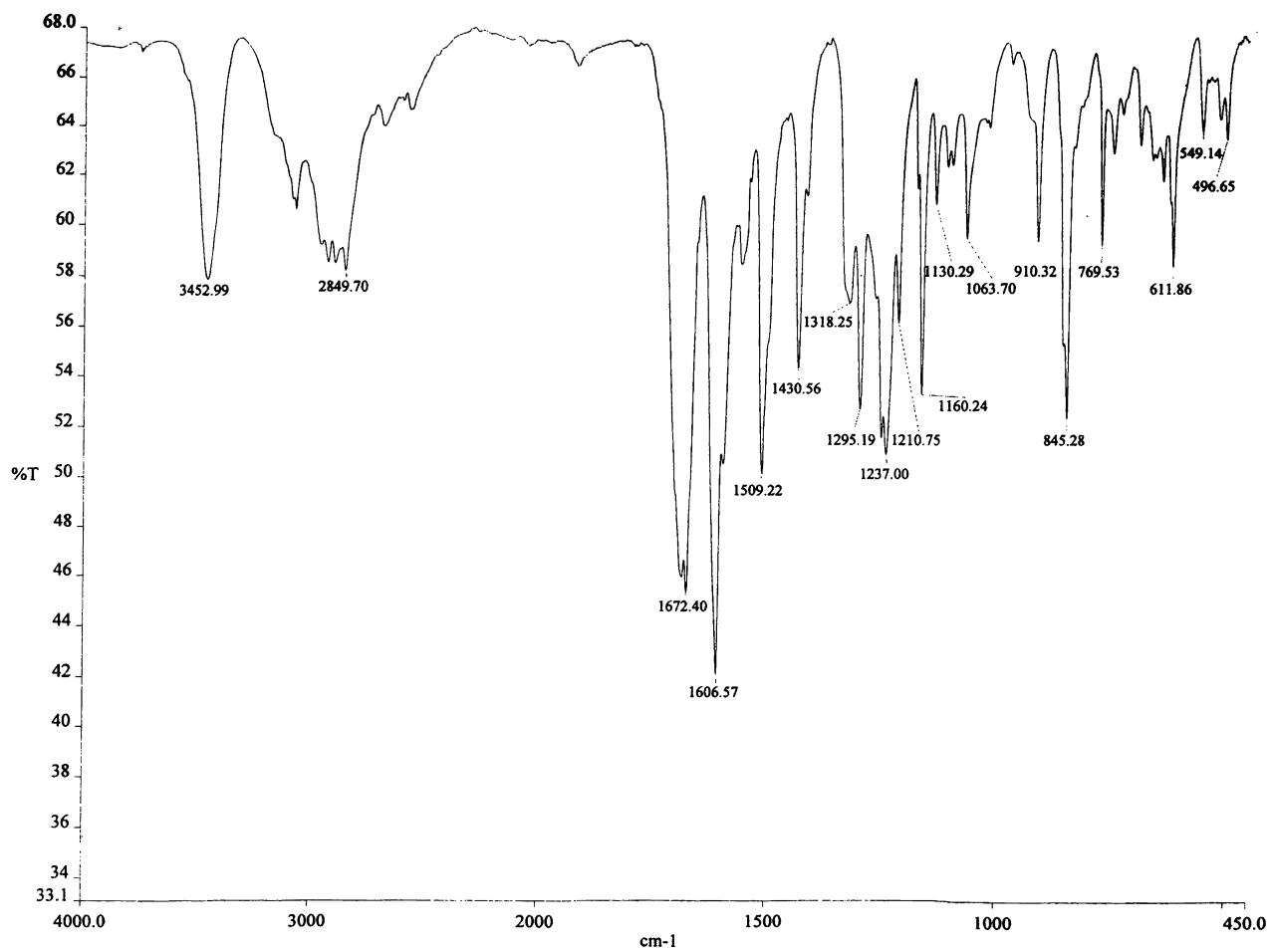
IR (KBR) cm⁻¹

Wave Number	Assignment
2849-3452	(Ar-H)
1672	(C=O)
1295-1318	(R-O-R)
746	(-C-Cl)

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HAFKINS-INSTITUTE-A-S-80\AD5-16-CDCE-PMR





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PART III :- Synthesis of N-(substituted benzyldinamino)-5-mercapto-4H-(1,2,4-triazol-3-yl- methyl)-N-benzothiazol-2 yl-acetamido

2-N-(acetyl acetohydrazide) amino benzothiazole which is synthesized as mentioned in **Part-II**.

The above mentioned compound can be synthesized by two methods:-

1. The reaction of 2-N-acetyl-[(2'-methyl-5'-mercapto)-1',3',4'-oxadiazolyl]-benzothiazole (Scheme I) with Hydrazine hydrate in ethanol which was refluxed for 2 hrs.
2. The second method involved the formation of potassium salt of 2-N-acetyl-(acethydrazide) benzothiazole (Scheme I) and then it was further treated with hydrazine hydrate to get the desired compound.

In the present research work, method (2) was used because of high yield and the potassium salt was more stable than 2-N-acetyl –[(2'-methyl-5'-mercapto)-1',3',4'-oxadiazolyl] benzothiazole.

N-(substituted benzyldinamino)-5-mercapto-4H-(1,2,4-triazol-3-yl- methyl)-N-benzothiazol-2-yl-acetamido was synthesized by cyclization of potassium salt of 2-N-acetyl-(acethydrazide) benzothiazole with hydrazine hydrate. The potassium salt of 2-N-acetyl-(acethydrazide) benzothiazole de was in turned prepared by the reaction of substituted aryl hydrazide with carbon disulfide and potassium hydroxide as detailed in procedure below,

➤ **Synthesis of potassium salt of 2-N-acetyl-(acethydrazide) benzothiazole**

In a 500ml conical flask, KOH (0.84gm;0.015) was placed in 200 ml ethanol. The solution was stirred on a magnetic stirrer at a room temperature. To the stirred solution was added (2.695gm;0.01mole) of aryl hydrazide in small portions ensuring the earlier portion was dissolved completely. A separating funnel was clamped over the conical flask, containing 11.4 gm of carbon disulfide (9ml;0.15mole)¹⁷⁴.

After all the aryl hydrazide was dissolved, CS₂ was added drop wise ensuring no rise in the temperature of the reaction mixture. As the addition of the CS₂ completion, a yellowish white solid separated from the solution and the whole

solution thickened up. 150ml of ethanol was further added & the mixture was further stirred for next 4-5 hrs. It was then diluted with 200 ml ether and solid was filtered. Further washing of solvent ether was given to solid filtered & solid which was obtained was dried in a vacuum at 65°-75°C for 4hrs.

A mixture of potassium salt of 2-N-acetyl-(acethydrazide) benzothiazole (2.03gm;0.05mole), 80% excess of Hydrazine hydrate and water 10 ml was heated under reflux for 4 hrs. The colour of the solution became green during heating. The reaction mixture was cooled, diluted with water and neutralized with conc. Hydrochloric acid. The white solid obtained was filtered, washed thoroughly with water and finally crystallized from ethanol¹⁷⁵.

Yield :- 72%

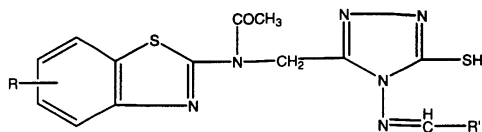
M.P:- 107°-108°C

➤ **Synthesis of N-(-substituted benzylidinamino)-5-mercapto-4H-(1,2,4-triazol-3-yl- methyl)-N-benzothiazol-2-yl-acetamido**

In a 100ml round bottom flask, a solution of 4-amino-5-mercapto-4H-(1,2,4-triazol-3-yl- methyl)-N-benzothiazol-2-yl-acetamido (0.001M) in a glacial acetic acid (20ml) and benzaldehyde (0.001 M) was refluxed for 20 mins. The reaction mixture was cooled, diluted with water and the resulting solid was washed with water and crystallized from ethanol.



Table 2:- Synthesis of N-(-substituted benzylidinamino)-5-mercapto-4H-(1,2,4-triazol-3-yl- methyl)-N-benzothiazol-2-yl-acetamido



Sr. No.	R	R'	Molecular Formula	M.P. (%)	Yield ($^{\circ}\text{C}$)	Nature	Colour	Elemental Analysis (%)					
								Calculated			Found		
								C	H	N	C	H	N
1	H	4-Cl	$\text{C}_{19}\text{H}_{15}\text{N}_6\text{ClOS}_2$	152	70	Amorphous	Yellow	51.52	3.38	18.98	51.48	3.06	18.55
2	H	3,4,5-TriOCH ₃	$\text{C}_{21}\text{H}_{22}\text{N}_6\text{O}_4\text{S}_2$	171	45	Amorphous	Yellow	51.85	4.52	17.28	51.93	4.89	17.36
3	H	2,4-DiCl	$\text{C}_{19}\text{H}_{14}\text{N}_6\text{Cl}_2\text{OS}_2$	180	53	Amorphous	White	47.79	2.93	17.60	47.93	2.99	17.35
4	H	2,3-DiOH	$\text{C}_{19}\text{H}_{16}\text{N}_6\text{O}_3\text{S}_2$	195	57	Amorphous	White	60.63	4.25	22.34	60.31	4.89	22.02
5	H	4-COOH	$\text{C}_{20}\text{H}_{16}\text{N}_6\text{O}_3\text{S}_2$	163	49	Amorphous	Yellow	53.09	3.53	18.58	53.11	3.02	18.56
6	H	2-OH-5-OCH ₃	$\text{C}_{21}\text{H}_{18}\text{N}_6\text{O}_3\text{S}_2$	202-205	61	Amorphous	White	54.07	3.86	18.02	54.29	3.23	18.46

SPECTRAL ANALYSIS:

Spectral studies of N-(4-Chlorobenzylidnamino)-5-mercapto-4H-(1,2,4-triazol-3-yl- methyl)-N-benzothiazol-2-yl-acetamido

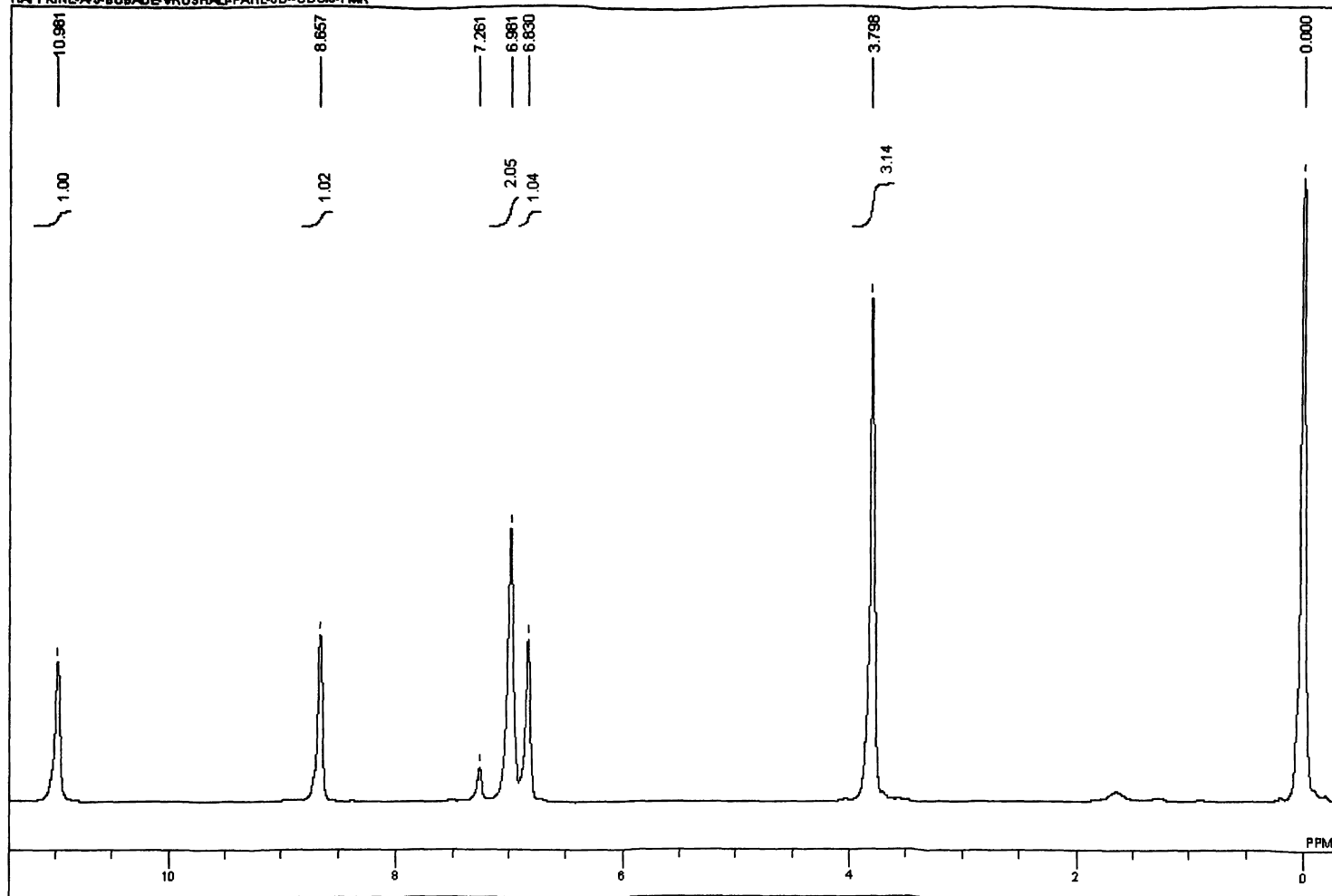
PMR (CDCl₃) δ : -

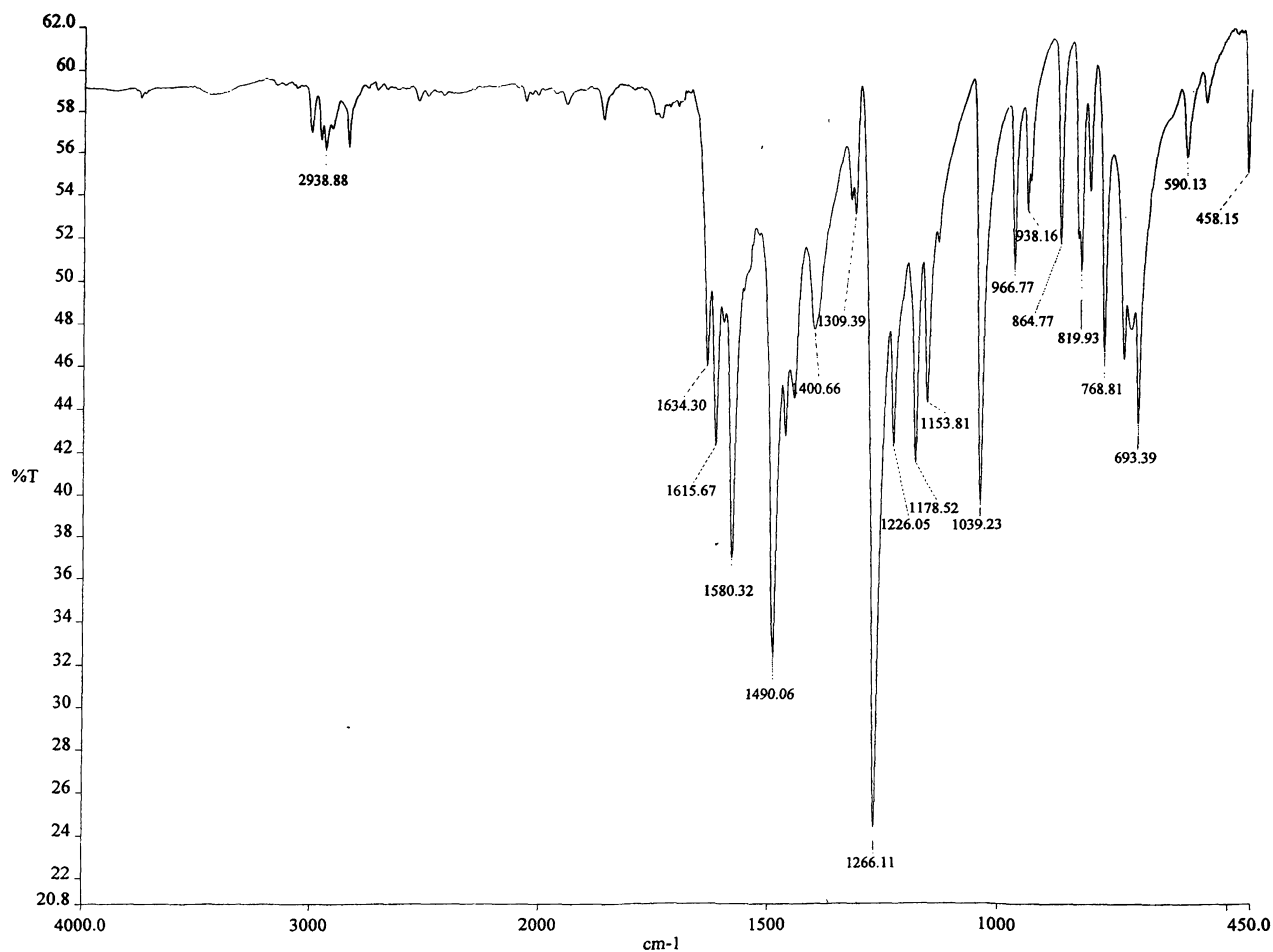
3.7	(s,6H,-OCH ₃ , -COCH ₃)
6.8-8.6	(m, 8H, ArH),
10.98	(s,2H,-SH,-OH)

IR (KBR) cm⁻¹

Wave Number	Assignment
2800-3100	(Ar-H)
1615-1634	(C=O)
1039	(-OC H ₃)
1580	(C=N)

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HAFFKINE A S-80\BADEV RUSHALI-PATIL-0D--CDCl3-PMR





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PART IV:- Synthesis of [1-[1-benzothiazol-2-yl-3-(substituted phenyl)-1H-pyrazol-4-yl]-meth-(E)-ylidene]-4-(substituted phenyl)-amine

Step 1: Synthesis of 2-hydrazino benzothiazole

Method : 1] From 2-Amino Benzothiazole¹⁷⁶

Conc. HCl (6 ml) was added drop wise with stirring to hydrazine hydrate (6 ml) at 5-10°C temp. To it ethylene glycol (24 ml) and 2-Amino Benzothiazole 4.5 gm, (0.03 M) .The mixture refluxed for 3 hrs. till the solid crystals appears. Cool, washed with water and recrystallized from ethanol.

M.P.= 195 °C

Yield = 81%

Colour & nature = Pale yellow amorphous powder

Method : 2] From 2-Mercapto Benzothiazole¹⁷⁷

Mixture of 2-Mercapto Benzothiazole 2.5 gm (0.01 M) dissolved in ethanol and hydrazine hydrate 10 ml (0.2 M) was heated about 7 hrs. A crystallized solid separates during refluxing was cooled, filtered and washed with 5% aq. Alkali, then with water. Recrystallized from ethenol

M.P.= 195 °C

Yield = 53%

Elemental Analysis: C₁₉H₁₄N₄O₃S₂

	C%	H%	N%
Calculated	55.60	3.41	13.65
Found	55.11	3.89	13.71

Step 2 : Synthesis of Benzothiazol-2-yl hydrazone

2-hydrazino benzothiazole 1.65 gm (1.5 mmol), appropriate substituted acetophenone (2.2 m mol) and glacial acetic acid (2-3 drops) were taken in absolute ethanol (20 ml). This mixture was refluxed on water bath for 5-10 hrs (monitored on TLC).

On cooling solid separates out which was filtered, washed with water and recrystallized with ethanol.

Sr.No.	R	M.P.(°C)	Yield %
1	3,4-Dichloro	216	59
2	2,4-dihydroxy	240-245 (dec.)	73
3	4-Fluoro	145	68

The Elemental analysis of **Compound No.1** is given below,

Elemental Analysis: $C_{15}H_{11}Cl_2N_3S$

	C%	H%	N%
Calculated	53.57	3.27	12.50
Found	53.23	3.10	12.11

Step 3 : Synthesis of Formylated Pyrazolyl Benzothiazole

Method : 1] Conventional method¹⁷⁸

Substituted Benzothiazol-2-yl hydrazone 1.68 ml, (0.005 M) was dissolved in 6 ml of DMF and this kept in ice cold condition.

To this Vilsmeier Haack reagent [(1.5 ml) of POCl₃ was added drop by drop with stirring in (6 ml) of DMF] is added with stirring at room temp. for 4 hrs. then content was poured into crushed ice (previously neutralized with NaHCO₃ or liq.NH₃) solid separates out which was filter washed with water, dried and recrystallized from ethanol.

Method : 2] Microwave method

The Substituted Benzothiazol-2-yl hydrazone 1.68 ml, (0.005 M) was added in mixture of Vilsmeier-Haack reagent in open Erlenmeyer flask and irradiated in microwave oven for 5 sec. After completion of the reaction, product was collected was describe in Method-1.

Sr. No.	R	M.P. (°C)	Time (hrs.) Conventional method	Time (sec.) Microwave method	Yield % Conventional method	Yield % Microwave method
1	3,4-DiCl	200-204	4	5	48	95
2	2,4-DiOH	Above300 (dec.)	5	6	68	93
3	4-F	150	4	5	88	95

The Elemental analysis of **Compound No.1** is given below,

Elemental Analysis: C₁₅H₁₁Cl₂N₃S

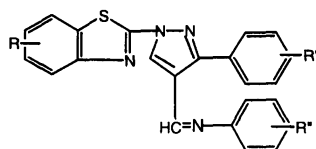
	C%	H%	N%
Calculated	53.57	3.27	12.50
Found	53.69	3.45	12.89

Step 4:- Synthesis of [1-[1-benzothiazol-2-yl-3-(substituted phenyl)-1H-pyrazol-4-yl]-meth-(E)-ylidene]-4-(substituted phenyl)-amine

Formylated pyrazolyl benzothiazole 1.87 gm, (0.005 M) in round bottom flask add 0.5 ml (0.005 M) of 4-Fluoro aniline in acetic acid .Reflux for 8 hrs. Cool ,Pour the content in ice cold water. Filter the solid and recrystallized from methanol.

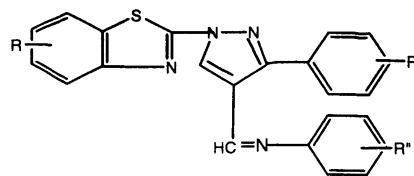
Similarly other substituted Benzothiazolyl derivatives were prepared and reported along with their melting points and analytical data in Table No.3

Table No. 3: Synthesis of [1-[1-benzothiazol-2-yl-3-(substituted phenyl)-1H-pyrazol-4-yl]-meth-(E)-ylidene]-4-(substituted phenyl)-amine



Sr No		R'	R''	Molecular formula	M.P (°C)	Yield %	Nature	Colour	Elemental Analysis					
									Calculated			Found		
									C	H	N	C	H	N
1.	H	3,4-DiCl	4-Cl	C ₂₃ H ₁₃ Cl ₃ N ₄ S	143	71	Crystlline	Yellow	59.76	2.19	11.15	59.77	2.49	11.39
2.	H	3,4-DiCl	3,4-DiCl	C ₂₃ H ₁₂ Cl ₄ N ₄ S	205	45	Crystlline	Brown	59.10	2.78	11.19	59.26	2.41	11.43
3.	H	3,4-DiCl	4-F	C ₂₃ H ₁₃ Cl ₂ FN ₄ S	145	51	Amorphus	White	53.28	2.31	10.81	53.62	2.61	11.03
4.	H	3,4 DiCl	2,5-DiOCH ₃	C ₂₅ H ₁₁ Cl ₂ N ₄ O ₂ S	196	58	Amorphus	Yellow	57.08	2.68	11.58	57.36	2.85	11.04
5.	H	2,4-DiOH	2,5-DiOCH ₃	C ₂₅ H ₁₉ N ₄ O ₂ S	263	48	Amorphus	Red	55.06	2.67	13.84	55.12	2.89	13.94
6.	H	2,4-DiOH	2,4-DiF	C ₂₃ H ₁₄ F ₂ N ₄ O ₂ S	269	53	Amorphus	Yellow	49.11	1.77	12.45	49.34	2.01	12.86

Table No. 3: Synthesis of [1-[1-benzothiazol-2-yl-3-(substituted phenyl)-1H-pyrazol-4-yl]-meth-(E)-ylidene]-4-(substituted phenyl)-amine



Sr no.	R	R'	R''	Molecular formula	M.P (⁰ C)	Yield (%)	Nature	Colour	Elemental Analysis					
									Calculated			Found		
									C	H	N	C	H	N
7.	H	2,4-DiOH	4-F	C ₂₃ H ₁₅ FN ₄ O ₂ S	150	64	Amorphus	Red	63.82	2.34	11.91	63.46	2.16	11.72
8.	H	4-F	4-F	C ₂₃ H ₁₄ F ₂ N ₄ S	99	65	Amorphus	Pale Yellow	68.33	4.32	12.75	68.13	4.19	12.79
9.	H	4-F	2,4-DiF	C ₂₃ H ₁₃ F ₃ N ₄ S	120	71	Crystlline	Pale Yellow	63.59	2.99	12.90	63.22	3.15	13.06
10.	H	4-F	3-Cl-4-F	C ₂₃ H ₁₃ ClF ₂ N ₄ S	155	75	Crystlline	Brown	61.33	2.88	12.43	61.01	3.09	12.11
11.	H	4-F	3,4-DiCl	C ₂₃ H ₁₃ Cl ₂ FN ₄ S	274	68	Amorphus	Yellow	61.01	2.06	12.06	61.35	2.01	12.46
12.	H	4-F	2,5-DiOCH ₃	C ₂₅ H ₁₉ FN ₄ O ₂ S	189	62	Crystlline	White	65.23	2.88	12.65	65.21	2.89	12.53

SPECTRAL ANALYSIS:

Spectral studies of [1-[1-benzothiazol-2-yl-3-(3,4-dichlorophenyl)-1H-pyrazol-4-yl]-meth-(E)-ylidene]-4-(4-chlorophenyl)-amine

PMR (CDCl₃) δ : -

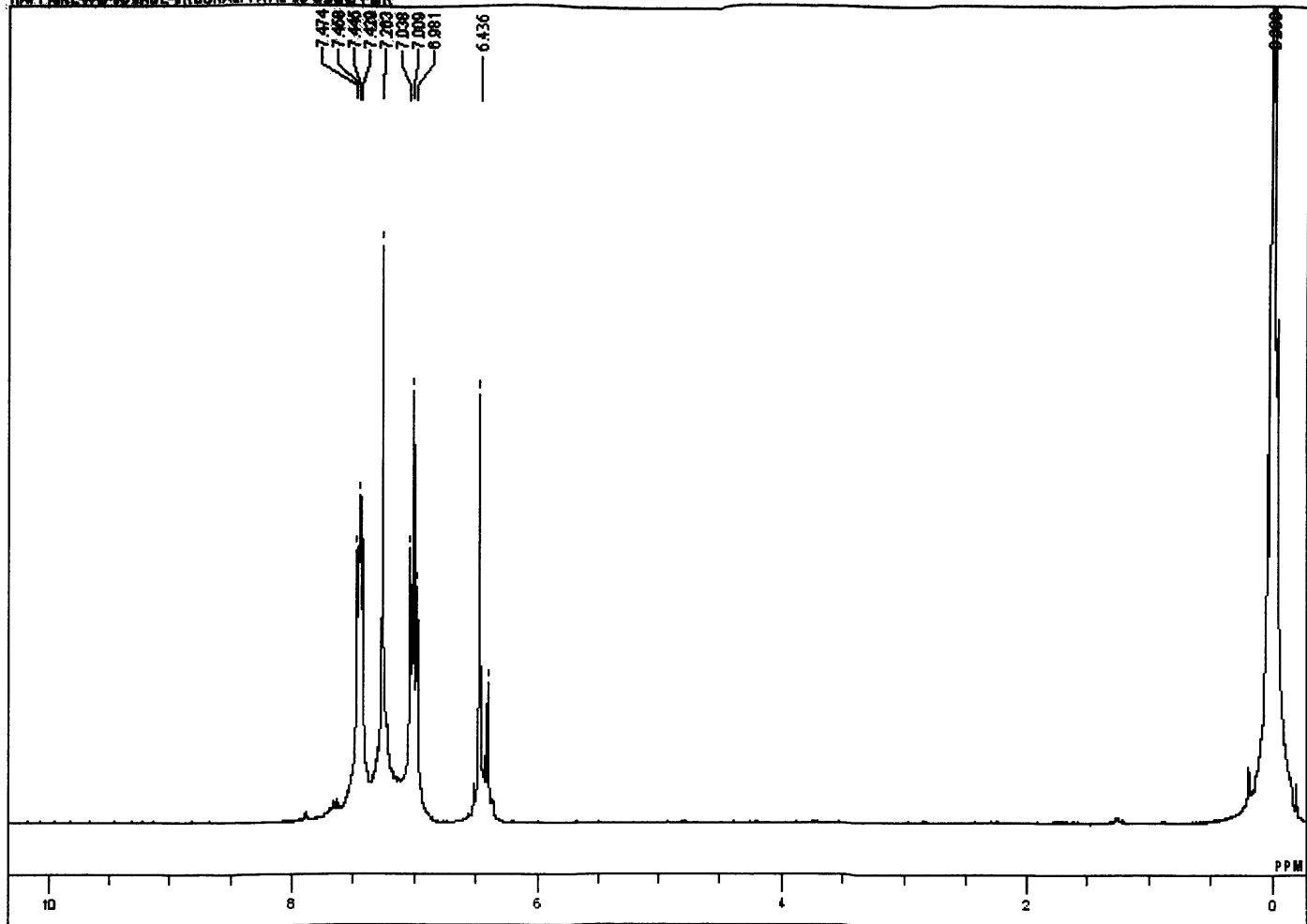
6.9-7.4 [m,(11H, ArH),
(1H, 5-CH),
(1H, N=CH)]

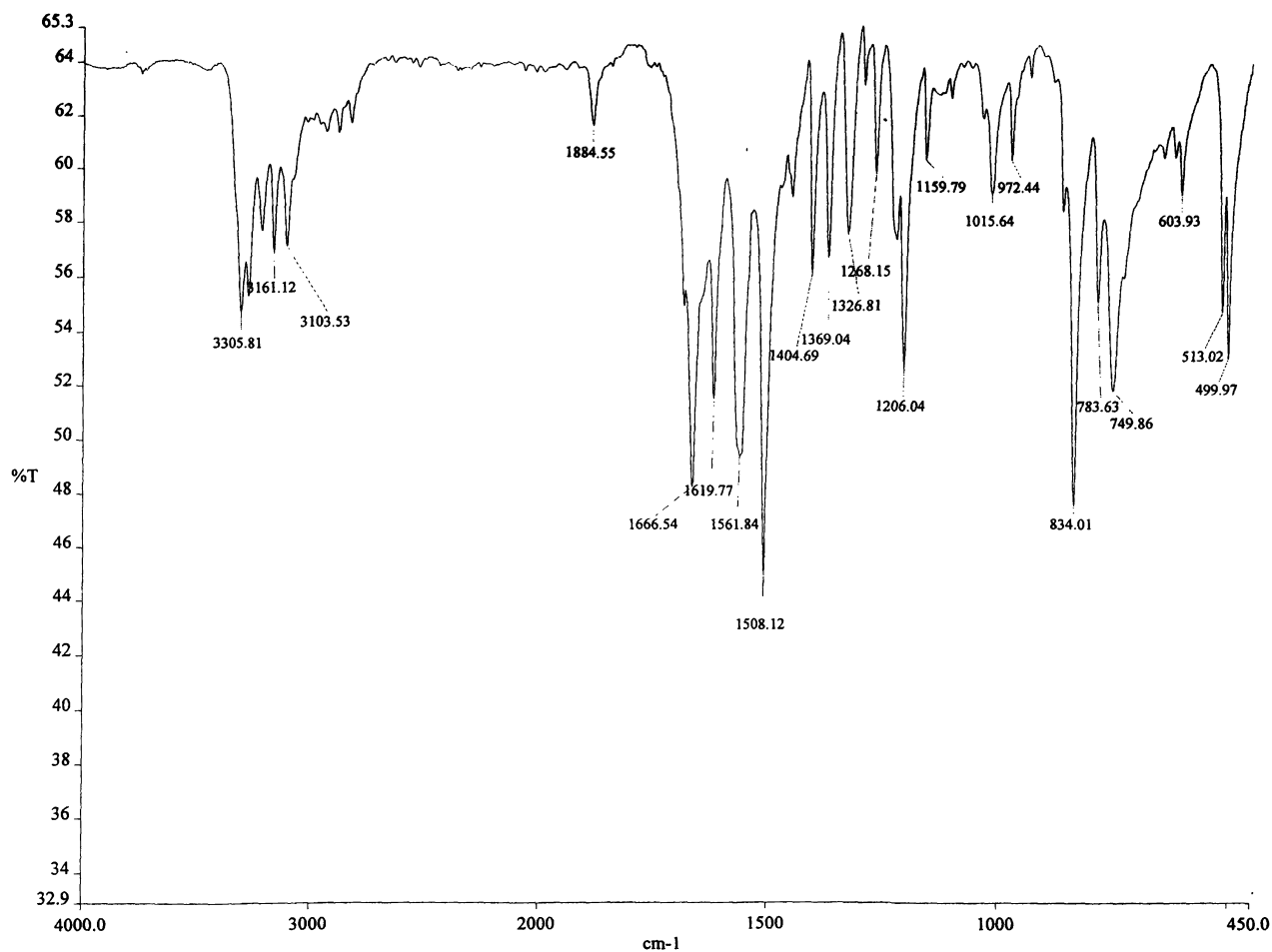
IR (KBR) cm⁻¹

Wave Number	Assignment
3103-3305	(Ar-H)
1508	(C=C)
1561	(C=N)
749	(C-Cl)
1015	(C-F)



0.2.1.DIFFRACTION 4-8-10 EADE-VRUSHALI-PATIL-10-CDCl3-PMR.2k
HAFTKNE-4-10 EADE-VRUSHALI-PATIL-10-CDCl3-PMR





3a.003

PART V :- Synthesis of substituted-benzoic acid [1-[1-benzothiazol-2-yl]-3-(substitutedphenyl)-1H-pyrazol-4-yl]-meth-(E)-ylidene] hydrazide

Formylated pyrazolyl benzothiazole was the starting material which was synthesized as mentioned in Part-4 (Scheme-III)

Step 4 :- Preparation of substituted benzhydrazide

Preparation of substituted benzhydrazide involves two steges-

- a) Synthesis of substituted benzoate
- b) Preparation of substituted benzhydrazide

a) Synthesis of substituted benzoate

In 500 ml round bottom flask a mixture of 1.565 gm (0.01M) of substituted benzoic acid 3.2 ml (0.1M) of absolute methanol and 4-5 drops of conc.H₂SO₄ was placed. A few small porcelain pieces were added and mixture was reflux gently on water bath for 4 hrs. The formation of ester was confirmed by TLC and quantitative test for ester¹⁷⁹

The excess of methanol was removed under reduced pressure and the reaction mixture was cooled. The residue was poured into about 5 ml of water contained in a separating funnel and the flask was rinsed with the some more distilled water.

The lower layer of the ester was carefully separated. A strong solution of sodium bicarbonate was added to the aqueous layer in the separating funnel until all free acid is removed ,which is evidenced by no further evolution of CO₂.

Carbon tetrachloride was added to the solution and shaken vigorously releasing the pressure occasionally. After separation of the two layer was dried by passing it over anhydrous magnesium sulphate. The CCl₄ was boiled off and the ester passed over anhydrous CaCl₂

Yield =1.3 gm (76%)

b) Preparation of substituted benzhydrazide

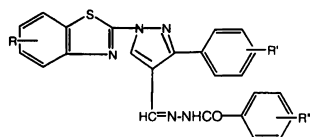
5 ml of hydrazine hydrate was placed in a 100 ml round bottom flask connected to a reflux condenser. The flask was warmed on a water bath and 0.01 M of substituted benzoate was added dropwise with occasional shaking. After the addition was completed, the reaction mixture was refluxed for 1 hrs. period. The reaction mixture was then poured into 250 ml beaker, solid product was separated. It was filtered under reduced pressure and recrystallized from rectified spirit¹⁸⁰

Sr.No.	substituted benzhydrazide	M.P..(°C)	Reported M.P.
1.	p-toluic benzhydrazide	116	118
2.	p-chloro benzhydrazide	155-158	163
3.	p--fluoro benzhydrazide	160	162-163
4.	p-hydroxy benzhydrazide	264	265
5.	p-methoxy benzhydrazide	136	136-140

Step 5 :- Synthesis of substituted-benzoic acid [1-[1-benzothiazol-2-yl-3-(substituted phenyl)-1H-pyrazol-4-yl]-meth-(E)-ylidene] hydrazide

Formylated pyrazolyl benzothiazole 1.87 gm, (0.005 M) in round bottom flask add 0.5 ml of substituted benzhydrazide in methanol. Reflux for 8 hrs. Cool Pour the content in ice cold water. Filter the solid and recrystallized from methanol. Analytical data given below in Table No.4

Table 4: Synthesis of substituted-benzoic acid [1-[1-benzothiazol-2-yl-3-(substituted phenyl)-1H-zpyrazol-4-yl]-meth-(E)-ylidene] hydrazide



Sr no	R	R'	R''	Molecular formula	M.P (^o C)	Yield (%)	Nature	Colour	Elemental Analysis					
									Calculated			Found		
									C	H	N	C	H	N
1	H	3,4-DiCl	4-Cl	C ₂₃ H ₁₃ Cl ₃ N ₄ S	143	69	Amorphus	Yellow	54.71	2.65	13.29	54.51	2.43	13.08
2	H	3,4-DiCl	4-OH	C ₂₃ H ₁₂ Cl ₄ N ₄ S	205	45	Crystalline	Pale Yellow	56.69	2.95	13.77	56.36	3.09	13.81
3	H	2,4-DiOH	4-Cl	C ₂₃ H ₁₃ Cl ₂ FN ₄ S	145	51	Amorphus	Red	58.83	3.26	14.30	58.70	3.01	14.69
4	H	4-F	4-OH	C ₂₅ H ₁₉ N ₄ O ₂ S	263	74	Crystalline	Reddish Brown	63.01	3.50	15.31	63.32	3.90	15.78
5	H	4-F	4-Cl	C ₂₃ H ₁₄ F ₂ N ₄ O ₂ S	269	53	Amorphus	Pale Yellow	60.56	3.15	14.72	60.19	3.55	14.32
6	H	4-F	4-CH ₃	C ₂₃ H ₁₅ FN ₄ O ₂ S	150	64	Crystalline	White	65.93	3.95	15.38	65.54	4.68	15.12
7	H	2,4-DiOH	4- CH ₃	C ₂₃ H ₁₄ F ₂ N ₄ S	99	63	Amorphus	Yellow	68.64	4.34	16.01	68.90	4.03	16.49

SPECTRAL ANALYSIS:

Spectral studies Synthesis of 4-Chlorobenzoic acid [1-[1-benzothiazol-2-yl-3-(3,4-dichlorophenyl)-1H-pyrazol-4-yl]-meth-(E)-ylidene] hydrazide

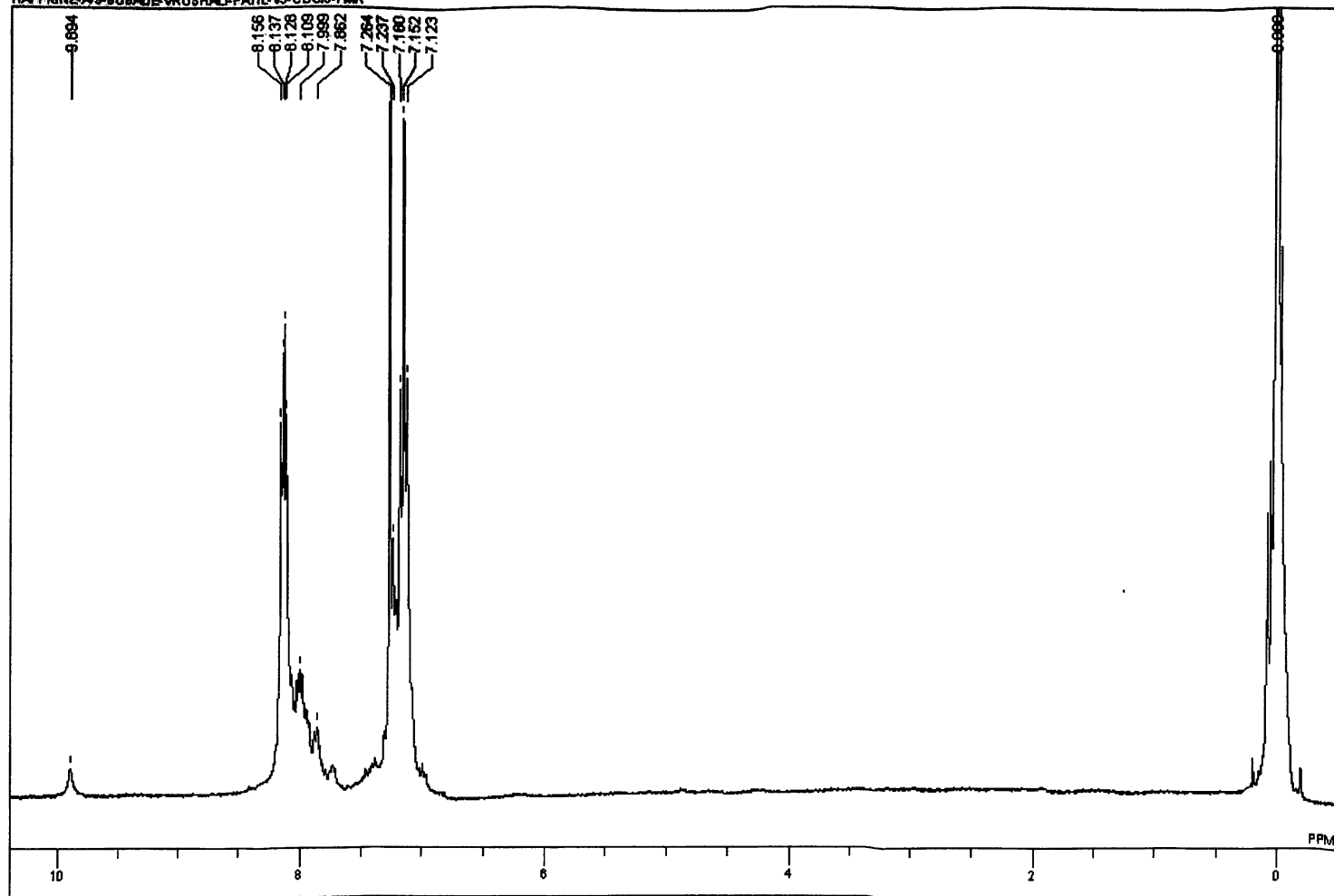
PMR (CDCl₃)δ: -

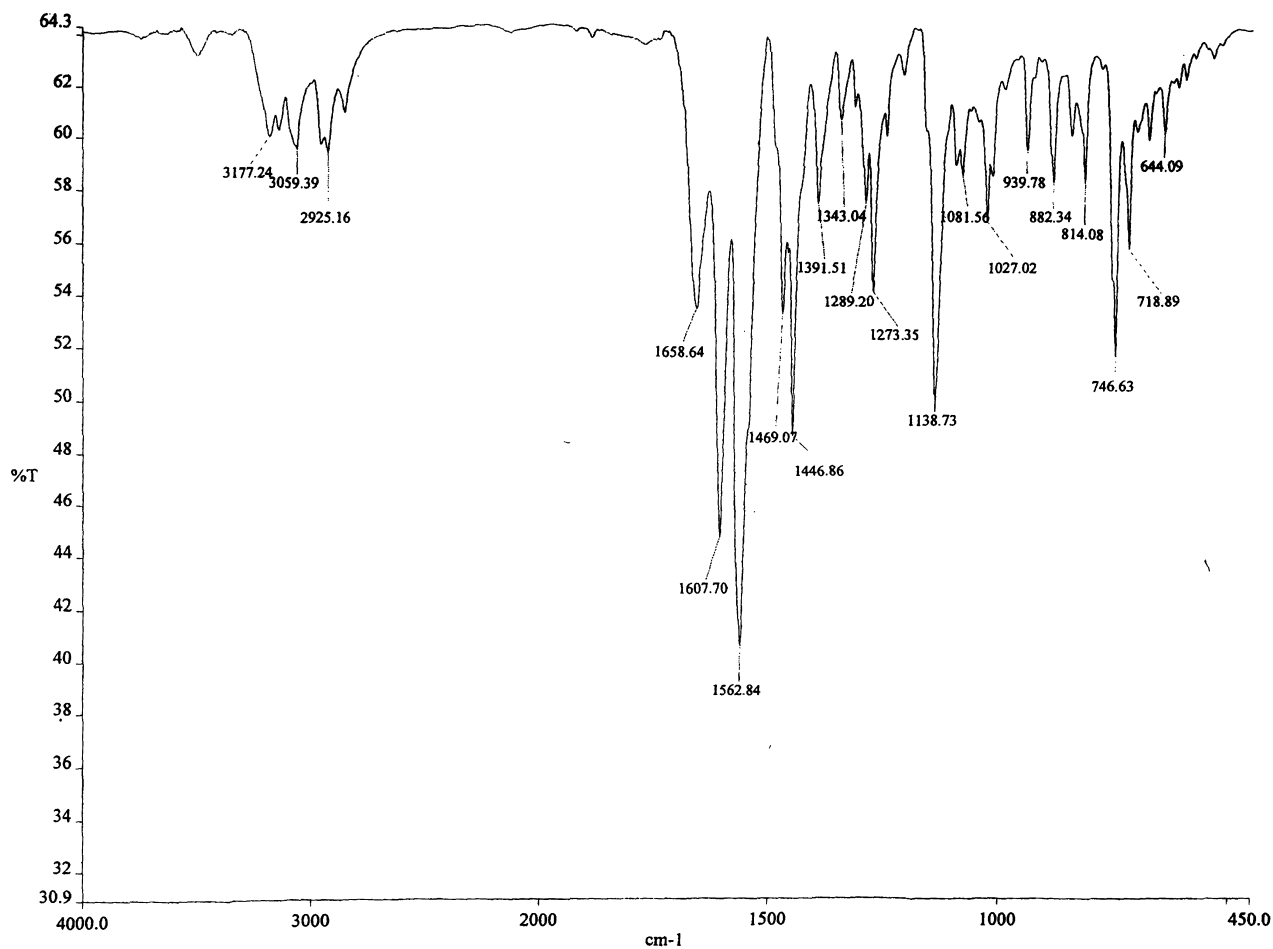
7.1-7.9 [m,(13H), (11H, ArH)
(1H, 5-CH),
(1H, N=CH)]

IR (KBR) cm⁻¹

Wave Number	Assignment
2925-3177	(Ar-H)
1607	(-NH) amide
1562	(C=N)
746	(C-Cl)
1658	(C=O)

D:\6.2.09\HAFFKINS-A-S-BORADEVRUSHAL-PATIL-V3-CDCl3-PMR.xls
HAFFKINS-A-S-BORADEVRUSHAL-PATIL-V3-CDCl3-PMR





1b.003

*Biological Screening, Results
&
Discussion*

PART VI :- Biological screening, Results and Discussion.

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*.

Details involved in the screening program is elaborated in the following section:

➤ Drug Stock Solution:

The test compound (10mg) was dissolved in a suitable solvent (10ml) such as DMF, ethanol, dil. HCl, DMSO as the case may be, so as to prepare a stock solution of 1000µg/ml. From this solution, the drug concentration viz. 200, 100, 50 and 20µg/ml were made. For lower dilutions the stock solution was diluted to prepare concentrations such as 20, 10, 5, 2µg/ml.

➤ **Controls:**

1. **Media Control:** Sterilized medium was kept as a control so as to assure the sterility of the medium. If this control shows growth of any type then results are considered invalid.
2. **Culture Control:** It contains sterilized medium and culture of the organism. 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 solvent used for preparation of the drug stock solution. If the growth is not observed, it can be concluded that solvent inhibits the growth of the organism and hence in that case results are considered invalid.
4. **Turbidity Control:** It contains medium and the drug stock solution. The turbidity is observed against the growth in different drug dilutions. It assures reliability against misinterpretation between growth and turbidity due to the drug.
5. **Standard Control:** It contains medium, organism culture and dilution of the standard drug stock solution corresponding to that of the drugs under test. The inhibition of growth was observed against that in standard.



➤ **Antibacterial Screening:**

The test compounds have been subjected to *in vitro* screening against Gram-positive *S. aureus* and Gram-negative *S. typhi* using tube dilution technique¹⁸¹.

Muller Hinton broth was used as a culture medium. DMSO was used as a solvent for the preparation of drug stock solution. Sterilized medium was dispensed in each borosilicate glass test tube (150+20mm). The drug solution was added in order to attain final drug concentrations as 200,100,50,20,10,5,2,1µg/ml etc.

Innoculum of standard suspension (0.1ml of the test organism strain which contains 10⁶ bacilli/ml) was added. The tubes were incubated at 37°C for 48 hours and then examined for the presence or absence of the growth of the organism. The lowest concentration, which showed no visible growth, was taken as the Minimum Inhibitory Coefficient (MIC). Ampicillin (MIC= 0.01µg/ml against Gram-positive *S.aureus*) and Trimethoprim (MIC= 1.0µg/ml against Gram-negative *S.typhi*) were used as the standard drugs.

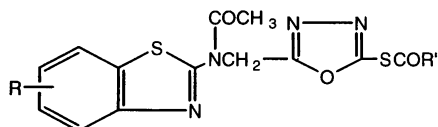
➤ **Antifungal Screening:**

The organisms used for the test were *Candida albicans* and *Aspergillus niger*. Again the method employed was the tube dilution method.

Sabraoud's medium was used as the growth media (pH=5.6). The sterile media was dispensed in a sterile borosilicate test tube. The drug solution was added in order to get the final drug concentrations of 200, 100, 50, 20, 10, 5, 2 µg/ml etc. The tubes were incubated at room temperature (28-30°C) in a dark place. *C. albicans* and *A. niger* were incubated for 7 days. The presence or absence of the growth was observed visually. The lowest concentration at which no growth was observed was taken as the end point (MIC).

Miconazole (MIC= 6.25 µg/ml against *C. albicans* and *A. niger*) was used as a standard drug.

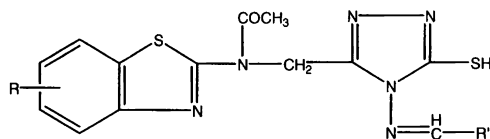
Table No. 1: Antimicrobial activity of Synthesis of substituted- thiobenzoic acid-s-{5-(acetyl-benzothiazol-2-yl-amino)-methyl}-[1,3,4]oxadiazole-2-yl



Compound	Substituents		Anti-microbial Activity($\mu\text{g /ml}$) [MIC]			
	R	R'	S.aureus	S.typhi	C.albicans	A.niger
1.	H	4-Fluoro	50	100	100	50
2.	H	4-Chloro	200	100	200	100
3.	H	2-Chloro	200	200	100	200
4.	H	2,5-Dimethoxy	100	50	100	100
5.	H	2-Fluoro	50	50	100	100

- Ampicillin (MIC-0.04 $\mu\text{g/ml}$) used as standard against *S. aureus*.
- Trimethoprim (MIC 0.01 $\mu\text{g/ml}$) used as standard against *S. typhi*.
- Miconazole (MIC 6.25 $\mu\text{g/ml}$) as standard against *C. albicans* and *A. niger*.
- N.A.- Inactive at 200 $\mu\text{g/ml}$.

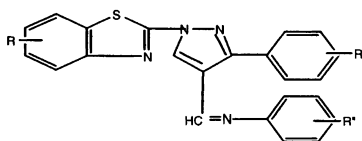
Table No. 2: Antimicrobial activity of Synthesis of N-(*-*substituted benzylidinamino)-5-mercapto-4H-(1,2,4-triazol-3-yl- methyl)-N-benzothiazol-2-yl-acetamido



Compound	Substituents		Anti-microbial Activity($\mu\text{g/ml}$) [MIC]			
	R	R'	S.aureus	S.typhi	C.albicans	A.niger
1.	H	4-Chloro	100	N.A.	100	100
2.	H	3,4,5,- Trimethoxy	100	200.	200	100
3.	H	2,4-Dichloro	N.A.	N.A.	100	200
4.	H	2,3- Dihydroxy	N.A.	200	100	50
5.	H	2-hydroxy-5- methoxy	100	100	100	50

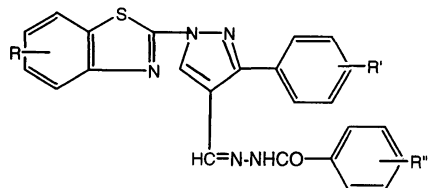
- Ampicillin (MIC-0.04 $\mu\text{g/ml}$) used as standard against *S. aureus*.
- Trimethoprim (MIC 0.01 $\mu\text{g/ml}$) used as standard against *S. typhi*.
- Miconazole (MIC 6.25 $\mu\text{g/ml}$) as standard against *C. albicans* and *A. niger*.
- N.A.- Inactive at 200 $\mu\text{g/ml}$.

Table No. 3: Antimicrobial activity of Synthesis of [1-[1-benzothiazol-2-yl-3-(substituted phenyl)-1H-pyrazol-4-yl]-meth-(E)-ylidene]-4-(substituted phenyl)-amine



comp	Substituents			Anti-microbial Activity($\mu\text{g/ml}$)			
	R	R'	R''	S. aureus	S. typhi	C. albican	A. niger
1.	H	3,4-Dichloro	4-Chloro	50	100	200	100
2.	H	3,4-Dichloro	3,4-Dichloro	100	100	100	100
3.	H	3,4-Dichloro	4-Fluro	50	100	100	200
4.	H	3,4-Dichloro	2,5-Dimethoxy	100	50	100	100
5.	H	2,4-Dihydroxy	2,5-Dimethoxy	100	100	200	200
6.	H	2,4-Dihydroxy	2,4-Difluoro	50	100	100	100
7.	H	2,4-Dihydroxy	4-Fluro	100	50	100	100
8.	H	4-Fluro	4-Fluro	50	20	50	50
9.	H	4-Fluro	2,4-Difluoro	50	20	50	100
10.	H	4-Fluro	3-Chloro-4-Fluro	50	200	100	50
11.	H	4-Fluro	3,4-Dichloro	100	100	100	100
12.	H	4-Fluro	2,5-Dimethoxy	50	50	50	50

Table No. 4: Antimicrobial activity of Synthesis of substituted-benzoic acid [1-[1-benzothiazol-2-yl-3-(substituted phenyl)-1H-pyrazol-4-yl]-meth-(E)-ylidene] hydrazide



Comp	Substituents			Anti-microbial Activity (µg /ml)[MIC]			
	R	R'	R''	S.aureus	S.typhi	C.albicans	A.niger
1.	H	3,4-Dichloro	4-Chloro	100	200	200	200
2.	H	3,4-Dichloro	4-Hydroxy	100	200	200	N.A
3.	H	2,4-Dihydroxy	4-Chloro	100	100	200	100
4.	H	4-Fluro	4-Hydroxy	N.A.	100	200	100
5.	H	4-Fluro	4-Chloro	20	100	200	100
6.	H	4-Fluro	4-Methyl	200	100	N.A	100
7.	H	2,4-Dihydroxy	4-Methyl	100	100	N.A	N.A

- Ampicillin (MIC-0.04 µg/ml) used as standard against *S. aureus*.
- Trimethoprim (MIC 0.01 µg/ml) used as standard against *S. typhi*.
- Miconazole (MIC 6.25 µg/ml) as standard against *C. albicans* and *A. niger*.
- N.A.- Inactive at 200 µg/ml.

RESULT AND DISSCUSION

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.

Discussion For Sereis I & II:

1. During workup for the synthesis of 2-N-(ethyl aceto acetate) Benzothiazole, K_2CO_3 was added in excess amount to maintain the alkaline condition which is responsible for the formation of potassium salt.
Futher addition of Ethyl chloro acetate was done in andhydrous condition which is necessary for the reaction. Small amount of moisture can alter the yield in Series I and II.
2. Schiff bases gives better yield when methanol is used as a solvent and acetic acid is use as catalyst in Series I this procedure did not required Dean-Stark apparatus.
3. Triazole moiety can be synthesized by two methods, one which includes Oxadiazole as an intermediate and another through the formation of potassium salt of hydrazide. Hydrazide intermediate was found to be highly hygroscopic while salt was more stable & could be easily prepared and stored.

Discussion For Sereis II & III

4. In the synthesis of 2-Hydrazino benzothiazole, from 2-Amino- benzothiazole, better yield was obtained when Ethylene glycol was used as a solvent in place of methanol or ethanol.
5. In the synthesis of 2-Hydrazino Benzothiazole, preparation from 2-Amino benzothiazole gives better yield than preparation from 2-Mercapto benzothiazole.
6. Vilsmeier-Haack reaction was carried out by both ways i.e.by Conventional and Microwave method.
Microwave method has following advantage over Conventional method :
 1. Less time consuming.
 2. More yield.
 3. Less impurities.

Discussion related to Biological screening:

The present research project deals with the synthesis of several novel heterocyclic moieties and assessment of their possible therapeutic value. In order to evaluate their efficacy and relate structural aspects with biological activity, these compounds were screened for *in vitro* antibacterial and antifungal activities.

In Series I, the synthesis of substituted- thiobenzoic acid-s-{5- (acetylbenzothiazol-2-yl-amino)-methyl}-[1,3,4]oxadiazole-2-yl have been described. The following points are to be noted related to Biological screening:

- 1) Presence of Fluorine moiety also showed promising activity against both bacterial strains as well as the fungal strain.
- 2) The substitutions like chloro, methoxy also contribute to the antibacterial activity. These compounds show antibacterial activity at 50 to 200µg/ml against *S. aureus* and *S. typhi*
- 3) Presence of electron withdrawing groups showed moderate antibacterial and antifungal activity

In Series II, the synthesis of N-(-substituted benzylidinamino)-5-mercapto-4H-(1,2,4-triazol-3-yl- methyl)-N-benzothiazol-2-yl-acetamido has been explained. Following observations have been made:

1. The substitutions like chloro, dichloro, methoxy, hydroxy also contribute to the antibacterial activity. These compounds show antibacterial activity at 50 to 200µg/ml against *S. aureus* and *S. typhi*
2. Chloro gr. not showed remarkable Anti-microbial activity hence Halogen substitution is not active against *S. aureus* and *S. typhi*.

In Series III, the synthesis [1-[1-benzothiazol-2-yl-3-(substituted phenyl)-1H-pyrazol-4-yl]-meth-(E)-ylidene]-4-(substituted phenyl)-amine has been explained. It was noted that:-

1. All the compounds screened showed moderate to good activity against all the four pathogenic strains.
2. Azomethine linkage on the Benzothiazolyl pyrazole has definitely improved its Anti-bacterial action.
3. Mono halo substituents have exhibited better action [comp.no.9, Table no.3]
4. Schiff's base in which halo substituents is present at para position of phenyl ring are more active than those in which halogens are at meta and ortho position.
5. Presence of Fluorine moiety also showed promising activity against both bacterial strains as well as the fungal strain.

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

1. The presence of amide linkage and Halogen substituents such as chloro, fluoro has shown excellent increase in Anti-bacterial activity but amide linkage is not beneficial as far as Antifungal activity is concerned.
2. In case of Anti-bacterial screening Fluoro and Chloro derivatives has shown best activity against *S.aureus* upto 20 µg/ml.

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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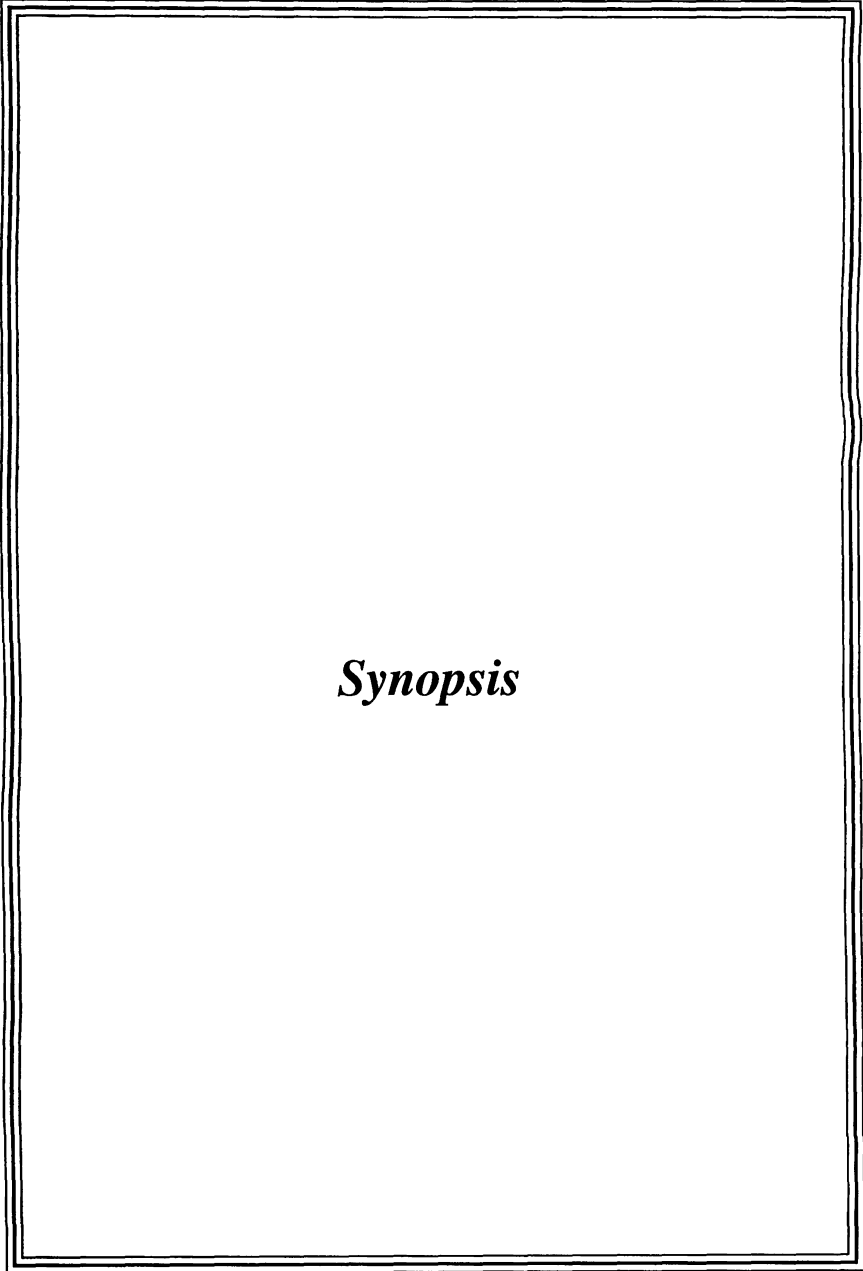
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Appendix

APPENDIX

1. The melting points have been determined in open capillaries on a Thermonik Melting Point Apparatus (Campbell Electronics, Mumbai, India) and are uncorrected.
2. Thin Layer Chromatography of the compounds have been run on E. Merck per coated silica gel plates of type 60 F 254.
3. Infrared spectra were recorded on a BOMEM MB-104 Fourier Transform Infrared Spectrometer.
4. PMR was recorded on JEOL 300 MHz spectrometer using TMS as internal reference at Institute of Science, Mumbai.



Synopsis

SYNOPSIS
OF THE THESIS TO BE SUBMITTED TO
THE UNIVERSITY OF MUMBAI
FOR THE DEGREE OF
MASTERS OF SCIENCE
IN THE FACULTY OF ORGANIC CHEMISTRY.

TITLE OF THE THESIS	: Synthesis and study of substituted Benzothiazoles, Triazoles & Pyrazoles As possible Antimicrobial agents.
NAME OF THE CANDIDATE	: Ms. Vrushali Nandkishor Patil.
QUALIFICATION	: B.Sc. Chemistry (March 2006).
NAME AND DESIGNATION OF THE RESEARCH SUPERVISOR	: Dr. Anil S. Bobade. (Senior Scientific Officer Class-I)
PLACE OF RESEARCH	: Department of Chemotherapy Haffkine Institute for Training, Research and Testing. Parel, Mumbai-12.
NUMBER AND DATE OF REGISTRATION	: 24, Aug. 13, 2007.
DATE OF SUBMISSION	: 31 st Aug 2009

**SIGNATURE OF
STUDENT**

**SIGNATURE OF THE
RESEARCH SUPERVISOR**

SYNOPSIS

“Man’s search for health is unending- A quest for longer, happier and healthier life. Yet, diseases and sufferings remain a point of his struggle for existence”. The fight against disease has entered its Third revolution- The molecular biological revolution and the fourth or ‘Genomic Revolution’ already on the horizon. The first Revolution has followed an increased understanding of chemistry during 1940’s – the Chemotherapeutic revolution. For several thousand years man has used herbs and potions as medicines but it is only since the mid nineteenth century that serious efforts were made to isolate and purify the active principles of these remedies. Since then a large variety of biologically active compounds have been obtained and their structures were determined. These natural compounds became the lead compounds for a major synthetic effort where chemist made literally thousands of analogues in an attempt to improve on what nature has provided. The vast majority of these drugs was carried out with no real design or reason, but out of the results came an appreciation of certain tactics which generally worked.

“Chemotherapy is an aid to nature”. Late Dr. Frank Hawking (1963) defined Chemotherapy as “The search for chemical compounds which will destroy infective parasites or organisms without destroying their animal host and the employment of such compounds to cure the corresponding diseases”.

All successful chemotherapeutic agents have certain properties in common. They must exert an antimicrobial action whether inhibitory or lethal, in high dilution and in the complex chemical environment which they encounter in the body.

Secondly since they are brought into contact with every tissue in the body, they must as far as possible be without harmful effect on the function of any organ.

The essence of chemotherapy is selective toxicity- to kill or inhibit the microbe without harming the patient. The properties of certain chemicals to destroy one

form of life without harming another is the corner stone of modern antimicrobial chemotherapy.

The practice of medicinal chemistry is devoted to the discovery and development of new agents for treating diseases.

Most of the activity in this discipline is directed to new natural or synthetic organic compounds.

Although the search for new entities is being increasingly governed by rational and high throughput techniques, serendipitous discoveries do still occur. The modern era is characterized by the fact that new drugs are being developed within a much stricter regulatory framework, with greater emphasis being placed on the quality of life. The process of establishing a new drug is exceedingly complex and involves the talents of people from a variety of disciplines, including chemistry, biochemistry physiology, pharmacology, pharmaceuticals and medicine.

Medicinal chemistry is concerned mainly with the organic, biological and analytical chemical aspects of this process. Thus it occupies a strategic position at the interface of chemistry and biology. Based on this aspect efforts have been made to synthesize molecules with incorporation of halogenated substituent's on various active moieties such as triazoles, pyrazoles etc and will be documented in the thesis.

The thesis will consist of the following parts:-

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

PART 2 :-Synthesis of -substituted-thiobenzoic acid-s-{5-{(acetyl-benzothiazol-2-yl-amino)-methyl}-[1,3,4]oxadiazole-2-yl}

PART 3 :- Synthesis of N-(-substituted benzylidinamino)-5-mercapto-4H-(1,2,4-triazol-3-yl- methyl)-N-benzothiazol-2-yl-acetamido

PART 4 :- Synthesis of [1-[1-benzothiazol-2-yl-3-(substituted phenyl)-1H-pyrazol-4-yl]-meth-(E)-ylidene]-4-(substituted phenyl)-amine

PART 5 :- Synthesis of substituted-benzoic acid [1-[1-benzothiazol-2-yl-3-(substituted phenyl)-1H-pyrazol-4-yl]-meth-(E)-ylidene] hydrazide

PART 6 :- Biological screening, Results and Discussion.

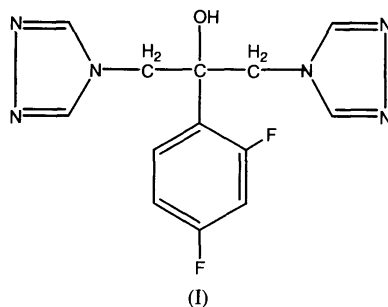
PART 1:- This part will give general introduction covering salient aspects of the research project and will also include **Review of Literature, Plan of Work** and will highlight the objectives involved in the synthetic work.

PART 2 :- Synthesis of -substituted-thiobenzoic acid-s-{5-[(acetyl-benzothiazol-2-yl-amino)-methyl]-[1,3,4]oxadiazole-2-yl}

Among the various types of known heterocyclic ring system, benzothiazole derivatives plays vital role in biological field such as antitubercular, antiallergic, anti-inflammatory and fungicidal activity¹⁻⁴.

2-Amino-6-substituted benzothiazole are known to exhibit excellent antibacterial activity. According to Mayer's hypothesis⁵, "A morphological relationship between bacteria and fungi may be parallel by a bio-chemical relationship". i.e. substance act as antifungal agents are likely to exhibit antitubercular activity also.

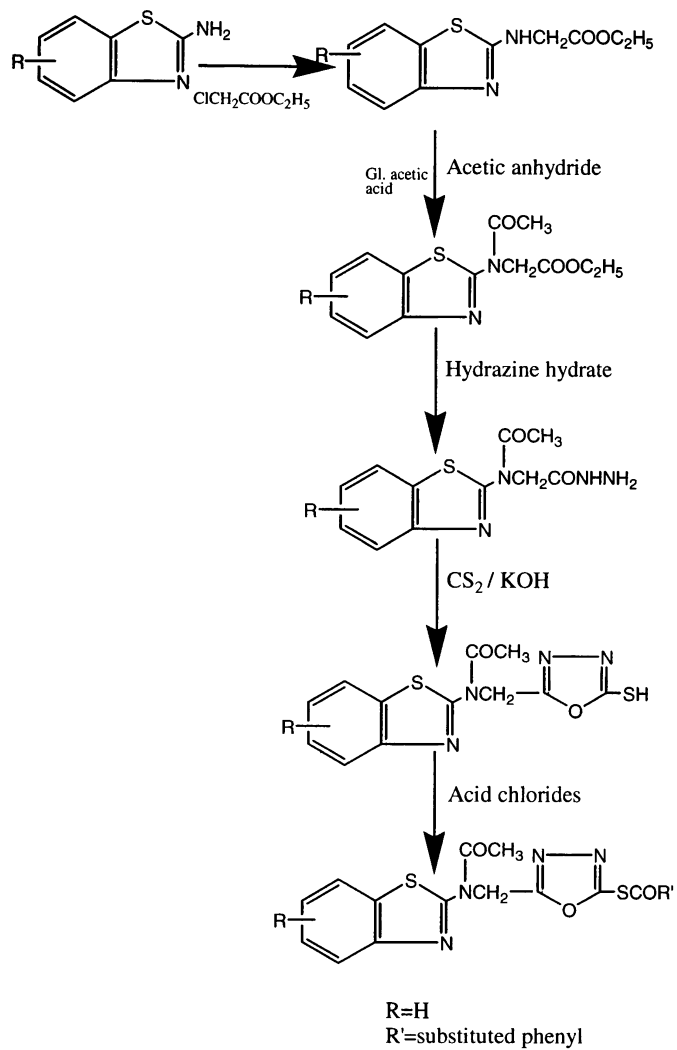
The importance of 1,2,4-triazole moiety as a major pharmacophoric requirement to exhibit enhance antifungal activity is well manifested by its inevitable presence in most recent antifungal drugs, viz. Flucanazole⁶ (I).



The presence of Oxygen and nitrogen in the heterocyclic system has attracted the attention of medicinal chemist because the diverse biological activities and profound efficacy. The recent review of literature has highlighted the significance of medicinal importance of oxadiazole and their derivatives⁷.

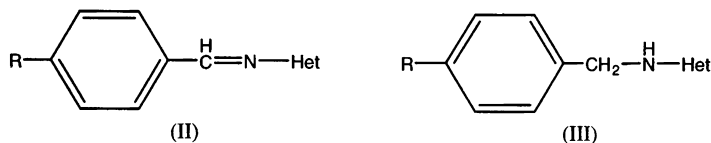
2,5-Disubstituted 1,3,4-Oxadiazole constitute a unique class of Nitrogen and Oxygen containing five membered heterocycles. Oxadiazole⁸ derivatives have been found to exhibit diverse biological activities such as antibacterial, fungicidal, insecticidal, anti-inflammatory and anti-HIV. Thus taking into consideration the above all observations, the synthesis of the derivatives of benzothiazole with 1,3,4-oxadiazole having acid chloride moiety were synthesized.

Scheme I



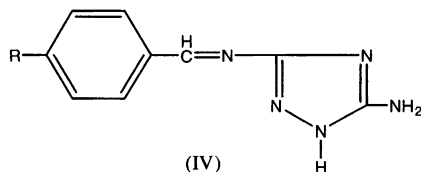
PART 3:- Synthesis of N-(substituted benzylidnamino)-5-mercapto-4H-(1,2,4-triazol-3-yl- methyl)-N-benzothiazol-2-yl-acetamido

R. Fioravanti *et al*⁹ have reported the antimicrobial activity of some new heteroaryl benzylamine (II) and their Schiff base (III). The amino triazoles are treated with various aldehydes to get Schiff base.



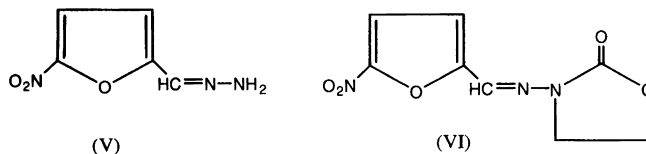
R = H, CH₃, Br, F etc.

The azomethine linkage is known to impart biological activity to various heterocyclic moieties e.g. Straukas J. *et al*¹⁰ have reported the anti-inflammatory activity of triazole Schiff base (IV).



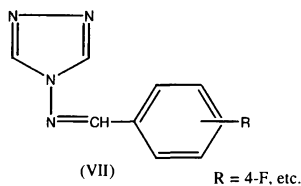
R = 2-Cl, 4-F, 4-OCH₃ etc.

Many of the biologically active compounds such as Nitrofurantoin (V), Nitrofurazone (VI)¹¹ are used as antibacterial and anti-infective agents. These compounds also contain the azomethine linkage, which enhances the antimicrobial activity.

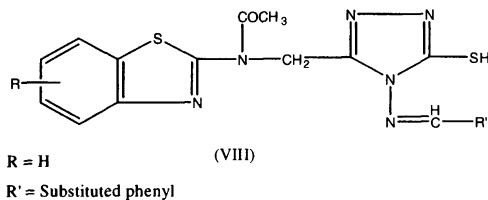


Taking into account the pharmacophoric importance of triazole in various newly introduced antifungal drugs like Fluconazole, Itraconazole, Saperconazole etc and importance of azomethine linkage that imparts antimicrobial activity, Schiff bases

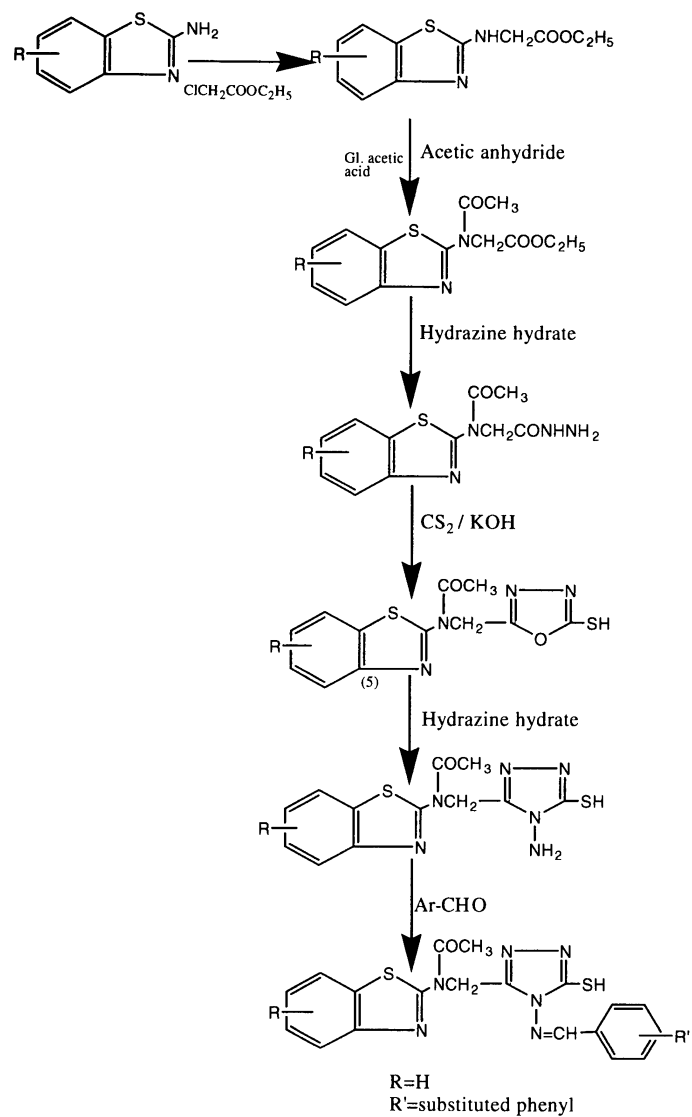
of 3-substituted-4-amino-5-mercapto-1,2,4-triazole were synthesized which have the following general structure (VII). They were tested for their antifungal, antibacterial activity.



Fluorine containing drug such as Fluconazole (I) have been proved to be very specific drug for the treatment of microbial disease and they owe their activity due to the presence of fluorine which increases the lipophilicity of the molecule¹². Considering this spectral property of fluorinated compound, Schiff bases were synthesized by condensation with various fluorinated and other aromatic aldehyde(VIII).



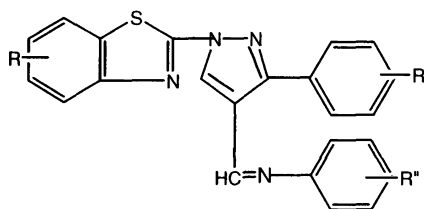
Scheme II



PART 4:- Synthesis of [1-[1-benzothiazol-2-yl-3-(substituted phenyl)-1H-pyrazol-4-yl]-meth-(E)-ylidene]-4-(substituted phenyl)-amine

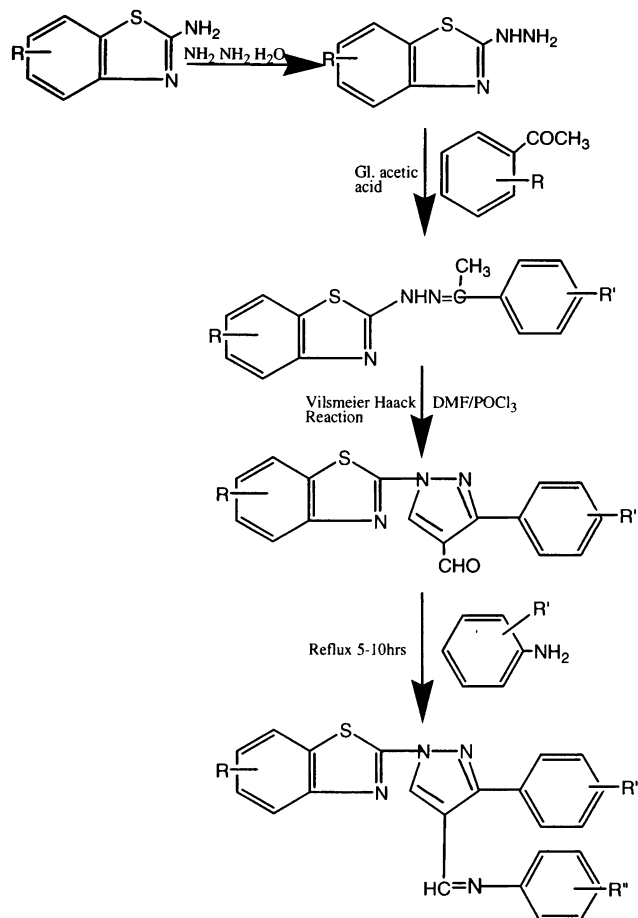
Pyrazole as well as pyrazole containing compounds have been reported to show a broad spectrum of biological activity such as antimicrobial¹³, antibacterial and antifungal¹⁴. Keeping in view the biological importance and medicinal utility of these compounds, two series of new pyrazole derivatives have been synthesized.

Benzothiazole derivatives as mentioned earlier in Part II for their wide range of activities. Therefore, it was considered worthwhile to explore the synthesis of compounds built upon benzothiazole skeleton incorporating pyrazolyl moiety with the hope of potentiating the activity of two such units in the same compound were prepared to obtain title compound (IX). The schematic representation shown on next page.



(IX)

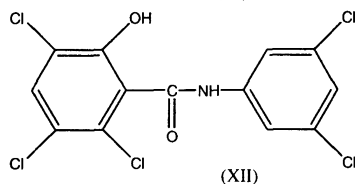
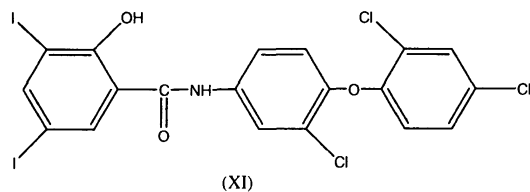
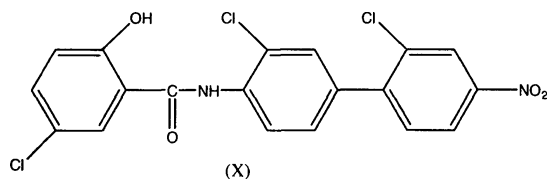
Scheme III



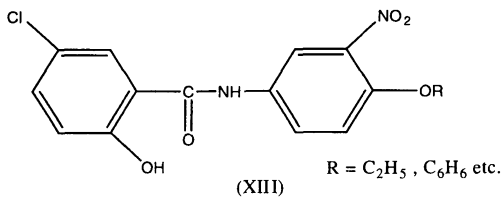
R = H
 R' = substituted phenyl
 R'' = substituted phenyl

PART 5 :- Synthesis of substituted-benzoic acid [1-[1-benzothiazol-2-yl-3-(substituted phenyl)-1H-pyrazol-4-yl]-meth-(E)-ylidene] hydrazide

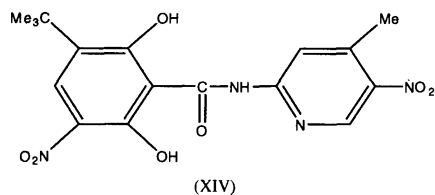
The acetamido group in the molecule was responsible for its activity. Aromatic amides have been proved to be useful in the field of medicinal chemistry. Niclosamide (X), Rafoxanide (XI), Oxyclozanide (XII) etc are the drugs which owe their activity to the amide linkage.



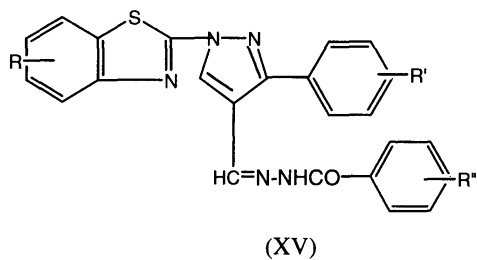
Further, the presence of –CONH linkage also plays an important role in imparting antifungal and antitubercular¹⁵ activity to the molecule, which is reiterated by following compounds (XIII).



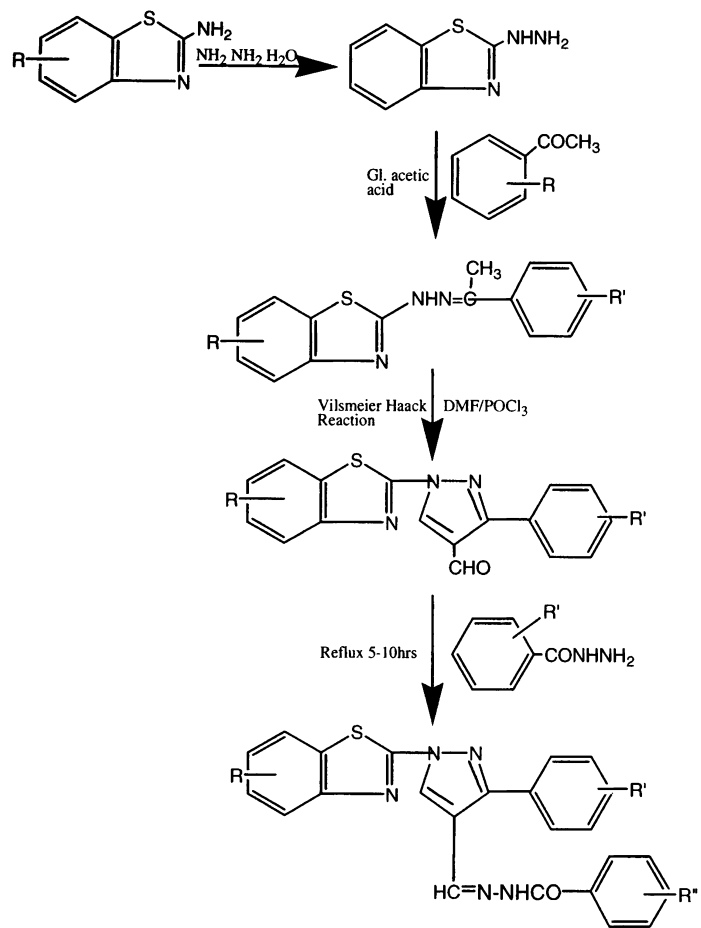
The interesting example of substituted pyridylamide (XIV) shows activity against *F. hepatica* and the contribution of –CONH linkage to the activity are good.



Hence, taking into consideration the importance of benzothiazole nucleus as mentioned earlier in PART-2 the pharmacophoric importance of pyrazole ring with acetamido linkage were prepared to obtain the title compound (XV)



SCHEME IV



R =H
 R'= substituted phenyl
 R''=substituted phenyl

PART 6:- Biological screening, Results and Discussion.

All the compounds from series I, II, III and IV were synthesized and were confirmed by Thin Layer Chromatography (TLC), Carbon Hydrogen Nitrogen (CHN) analysis, Nuclear Magnetic Resonance (NMR) Spectroscopy, Infra-Red (IR) Spectroscopy and same compounds were subjected to microbiological screening, which involves anti-bacterial and anti-fungal screening.

Anti-bacterial screening:

The compounds synthesized were subjected to *in vitro* test against *Staphylococcus aureus* ATCC 3750 and *Salmonella typhi* NCTC 786. The method used was that of tube dilution and the medium employed in the screening was Mueller- Hinton Broth¹⁶. The standard drugs used were Ampicillin (MIC 0.1 µg/ml) against *S. aureus* and Trimethoprim (MIC 0.1 µg/ml) against *S. typhi*.

Anti-fungal screening:

Compounds reported in series I, II, III and IV were also screened for their anti-fungal activity by tube dilution technique¹⁷ 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.

An attempt has been made to obtain some promising leads for anti-microbial compounds. The synthetic and microbiological evaluation of the compounds will be dealt in the thesis.

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