

SYNTHESIS OF SOME SUBSTITUTED OXADIAZOLES,
TRIAZOLES AND THIAZOLIDINONES AS POSSIBLE
ANTIMICROBIAL AGENTS

THESIS SUBMITTED
UNIVERSITY OF MUMBAI

FOR THE DEGREE OF
MASTER OF SCIENCE
IN
ORGANIC CHEMISTRY

SUBMITTED BY
KUSHAL HARESHWAR DEVYANI DAWANE

UNDER THE GUIDANCE OF
DR. ANIL BOBADE

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

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Mr. Kushal Hareshwar Devyani Dawane

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DAW

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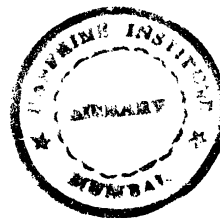
Ph. D

**DEPARTMENT OF CHEMOTHERAPY
HAFFKINE INSTITUTE FOR TRAINING, RESEARCH AND
TESTING.**

ACHARYA DONDE MARG, PAREL, MUMBAI - 400012.

FEBRUARY 2010

Dedicated to my Family
&
Friends



Acknowledgement

I express with all sincerity my deep sense of gratitude, indebtedness & thankfulness to my respected guide Dr. Anil S. Bobade ,Ph.D, Head of the Dept., Chemotherapy, Haffkine Institute, whose deep dedication, constant encouragement & constructive thinking during the entire course of my study has greatly helped me to present this work. It is with pleasure that I recall my years of association with him

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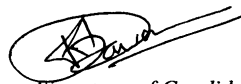
I shall never be able to express my deep indebtedness to my family for their massive sacrifice and support without which the thesis could never have been produced in reality.

Last, but not the least I would like to thank GOD Almighty who gave me the strength and determination in every walk of life, and helped me conquer the mountains of difficulties instead of removing them from my path.

Kushal Dawane

STATEMENT BY THE CANDIDATE

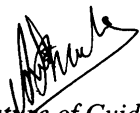
*As required by the University Regulation No. R. 2190, I wish to state that the work embodied in this thesis titled “**Synthesis and study of some substituted Oxadiazoles, Triazoles & Thiazolidinones as possible Antimicrobial Agents**” forms my own contribution to the research work carried out under 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

Kushal Hareshwar Dawane

Certified by



Signature of Guide

Dr. Anil S. Bobade

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Introduction

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)

Medicinal chemistry is multifaceted discipline that encompasses synthetic organic chemistry, natural chemistry products, enzymology, chemical biology, structural biology and computational methods, all of which are aimed at the discovery and development of new therapeutic agents. Medicinal chemistry is by nature an interdisciplinary science and practitioners have a strong background in organic chemistry, coupled with a broad understanding of biological concepts related to cellular drug targets.

The field of medicinal chemistry has evolved from an emphasis on the synthesis, isolation and characterization of drugs, to an increased 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 relation between a chemical structure and biological activity. An increased considerations in recent years has been to correlate the chemical structure with the chemical reactivity or physical properties and these correlations can be in turn be related to their therapeutic actions. Although a great deal of success in understanding the relationship between chemical structure and biological activity has been observed in numerous areas where there are still many human sufferings which require new and improve drugs.



Process of drug discovery

Drug discovery has its roots in the beginning of mankind. Even our polygenetic cousins, the chimpanzees have been observed to chew certain leaves to alleviate gastrointestinal distress. Likewise, in the evolution of early hunter gatherer communities, it was only a short step from gathering vegetation to be eaten to assuage the hunger pangs, to gathering material to be ingested to diminish the pain and discomfort from illness and injury. The process from identification of a target to entry of the drug into the market has the following steps¹:

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.

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.

Review of Literature

Review of Literature

Chemotherapy is the practice of administering chemical preparations to a diseased or an infected person with the aim of relieving the disease symptoms or eliminating the infection. This particular section, review of literature specifically deals with various chemotherapeutic agents used in the treatment of infectious diseases. They are listed as below.

Bacterial Infections:

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

However, Domagk², Mietzsch and Klarer³ in 1935, first dispelled the failure of earlier scientists by discovery of Prontosil, and reported that this compound cured streptococcal infections in mice and staphylococcal infection in rabbits. This era was considered to be a brilliant era in chemotherapy of microbial infections.

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

Synthetic Antibacterial Agents:

Sulfur containing compounds:

➤ Sulfonamides:

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

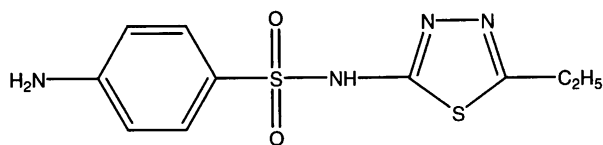
In 1936, Colebrook and Kenny⁴, as well as in 1936, Buttle et al.⁵ report their favourable clinical results with prontosil and sulfanilamide in puerperal sepsis and meningococcal infections. These two reports awakened the medical profession to the new field of bacterial chemotherapy, and experimental and clinical articles in great profusion soon appeared.

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

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

Numerous derivatives of para-aminobenzene sulphonamides (sulfanilamide) were synthesized and studied. Among these, sulfadimethoxine, sulfameter, sulfaethadione, sulfamethozypyridazine and sulfamethozypyridazine acetyl are to be marked as long acting sulfonamides.

➤ **Sulfaethidole:**

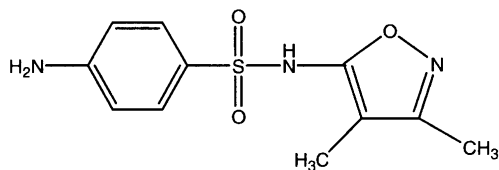


[I]

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

➤ **Mixed Sulfonamides:**

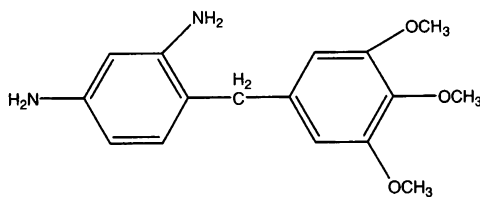
The danger of crystal formation in the kidneys on administration of sulfonamides has been reduced greatly through the use of more soluble sulfonamides such as sulfisoxazole [II].



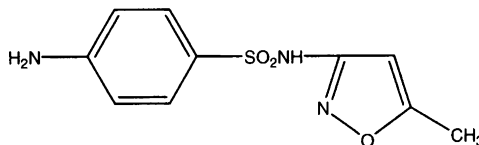
[II]

This danger may be diminished still further by administering mixtures of sulfonamides. Further, the introduction of trimethoprim [III] in combinations with sulfamethoxazole [IV] constitutes an important advancement in the development of clinically effective antimicrobial agents and represents the practical application of a theoretical consideration; that is, if two drugs act on sequential steps in the pathway of

an obligate enzymatic reaction in bacteria, the result of their combination will be supra-additive. Extensive biochemical studies on this combination have proved it.



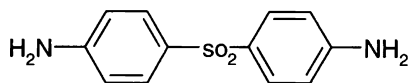
[III]



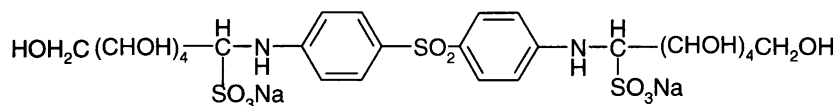
[IV]

➤ **Sulfones:**

The sulfones first attracted interest because of their chemical relationship to the sulfonamides. Of the six possible diaminophenyl sulfones having one amino group in each ring, only the 4,4'-isomer [Dapsone, V] was active⁶. Dapsone was found in 1937 to be 30 times more active and only 15 times as toxic as sulfanilamides when used in streptococcal infections in mice. Against pathogenic strains of Tubercle Bacillus, it was active at 10 µg/ml. Dapsone and its glucose bisulphite derivatives [VI] were the first compounds to exhibit a good life-extending effect on tuberculosis in animals^{7,8} and stimulated extensive structural modifications. At that time, the drug was considered too toxic for administration to men, and attempts were initiated to find a compound with a better therapeutic index.



[V]

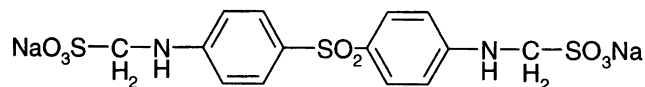


[VI]

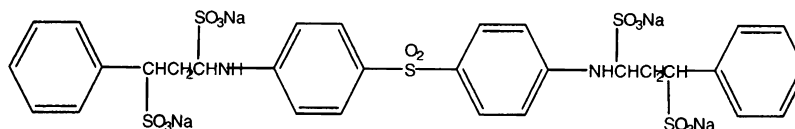
Shepard⁹ has done extensive work on dapsone in leprosy. This bacteriostatic drug has been shown to possess sensitivity of 0.02 µg/ml on microorganisms of untreated leprosy patients¹⁰.

The mechanism of action of the sulfones is probably similar to that of the sulfonamides since both possess approximately the same range of antibacterial activity and both antagonise PADA. The most common untoward effect is haemolysis of varying degree. Anorexia, nausea and vomiting may follow the administration of sulfones. Isolated instances of headache, nervousness, drug fever and variety of skin rashes have been reported by Rapoport and Guss¹¹.

Activity was exhibited by several mono¹²⁻¹⁴ and bis¹⁴ Schiff bases of aromatic aldehydes and aldehyde bisulphite complexes such as glucosulfone [VIII]^{8,15}, aldesulfone [Diazone, VII]¹⁶⁻²⁰.



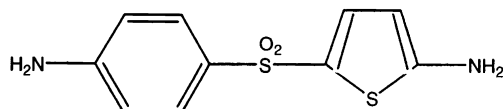
[VII]



[VIII]

These compounds had weak in vitro activity under conditions that prevented hydrolysis and the parent sulfone was almost always detected in vivo^{21,22}.

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

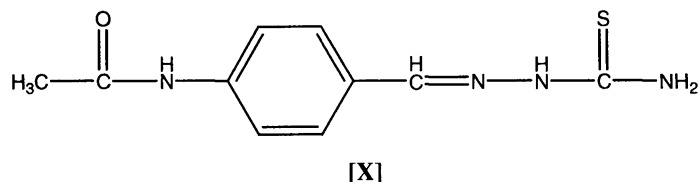


[IX]

Thiosemicarbazone:

The tuberculostatic activity of thiosemicarbazones and related drugs was first observed by Dogmak et al.,²³. The weak antituberculosis activity of sulfathiazole led to the preparation of series of analogues^{24,25}. The thiadiazoles and their precursor thiosemicarbazones were tested for activity. These compounds included the active benzal thiosemicarbazones along with numerous inactive aliphatic analogues.

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



Amithiozone was active orally against experimental infections in animals, the activity being comparable to streptomycin²⁶⁻²⁸. It also suppressed the emergence of resistance in these experimental infections. Its high tuberculostatic activity was not inhibited by PABA as would be expected from structure-activity relationship.

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

NON-SULFUR DRUGS:

Furan Dervatives:

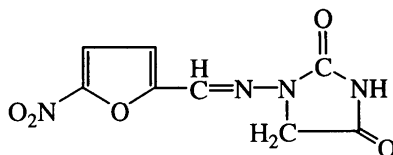
Derivatives of furan possess antimicrobial activity. The presence of nitro group in the 5-position of the furan ring confers antibacterial activity on mainly 2-substituted furans. Dodd and Stillman²⁹ prepared a large number of nitro furans and their non-nitrated analogs and found that the nitro group conferred antibacterial activity on the various furan derivatives. Although, hundreds of broadly active derivatives have been prepared, only a few have enjoyed clinical success. The nitrofurans are active against certain gram-positive and a variety of gram-negative bacteria, some fungi and the protozoan *Trichomonas vaginalis*.

It has been generally concluded³⁰⁻³² that the antibacterial activity is the greatest in those compounds whose nitro group is the most readily reduced. A bacteriostatic action is exerted upon most bacteria in the concentration range of 1:100,000 to 1:200,000. Bactericidal concentrations are approximately twice as great. The mechanism of the antibacterial action of the furan derivatives is unknown, but it is presumed that the compounds probably interfere with enzymatic processes essential to bacterial growth.

On an individual basis, the various agents are useful topically and orally under certain conditions. When used in combination with antibiotics like chloramphenicol and erythromycin, the nitrofurans were found to be synergistic³³. Little or no development of cross-resistance is so far evident³⁴⁻³⁶.

➤ **Nitrofurantoin:**

Nitrofurantoin [XI] was first synthesized by Hayes³⁷ by the condensation of 1-aminohydantoin with 5-nitro-2-furfuraldehyde. The drug is active against many strains of the common urinary tract pathogens.



[XI]

Nitrofurantoin is bacteriostatic at concentrations of 5 to 10 $\mu\text{g/ml}$ and bactericidal at 100 $\mu\text{g/ml}$ but it is not known whether a bactericidal occurs in vivo. The antibacterial activity is higher in acidic urine.

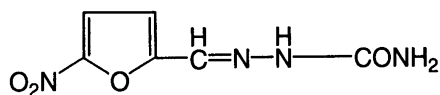
The compound is rapidly and completely absorbed from the gastrointestinal tract. Antibacterial levels in the blood are not attained but approximately 40% of the drug is excreted in the urine³⁸. Concentrations that are bactericidal to most gram-positive & gram negative organisms are achieved in urine. Intravenous administration of the drug has received limited trial in the treatment of acute severe urinary tract infections³⁹. The average dose of Nitrofurantoin results in a urinary concentration of approximately 20 $\mu\text{g}/100\text{ml}$.

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 Scott⁴⁰ have synthesized this compound [XII] 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 man⁴¹ caused by Trypanosome gambiense.

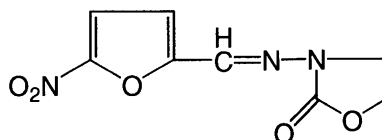




[XII]

➤ **Furazolidone:**

Furazolidone [XIII] was synthesized by Drake and Hayes⁴². The drug has been reported to be of value in the treatment of intestinal infections due to *Shigella* and *Salmonella* organisms⁴³. It has an outstanding activity against these pathogenic bacterial infections. Excellent anticoccidial & antitrichomonal activity is also evident.

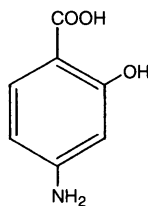


[XIII]

PAS (*p*-aminosalicylic acid) and its derivatives:

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

The most effective was PAS [XIV]



[XIV]

PAS was found to be highly bacteriostatic in vitro and also active orally against experimental infections in animals⁴⁶⁻⁴⁸ PAS is specifically active against mycobacteria and against only certain species in this genus⁴⁹. It is equally effective against streptomycin-resistant strains⁴⁶. It is of interest to find that PAS has little effect on the respiration of mycobacteria nor does it inhibit the stimulating effect salicylates⁵⁰. In vitro, most strains of Mycobacterium Tuberculosis are sensitive to a concentration of 1 µg/ml of PAS

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

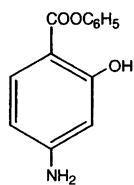
The action of PAS in combination therapy is two-fold. Firstly, it produces an antibacterial effect by competing with P-Aminobenzoic acid [PABA] in biochemical synthesis. Secondly, it enhances the serum concentration of INH 2-4 fold in man by undergoing competitive acetylation.

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

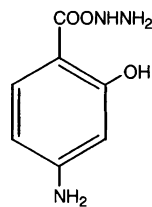
PAS is associated with 2% to 4% incidence of untoward effects. The most important being irritation of the gastrointestinal tract, peptic ulceration, renal irritation, hypersensitivity, dysfunction of the liver and thyroid.

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

Attention was turned to prepare derivatives that would be absorbed and eliminated more slowly. The phenyl ester [XV] was claimed to be as active as streptomycin subcutaneously and equivalent to PAS orally^{51,52}.



[XV]

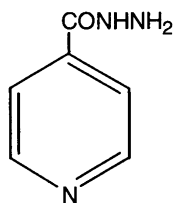


[XVI]

PAS hydrazide [XVI] was active in vitro. It's effect was inhibited by PABA and there was cross resistance with PAS, but not with INH⁵³.

➤ **Iso-nicotinic acid Hydrazide [INH]:**

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



[XVII]

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

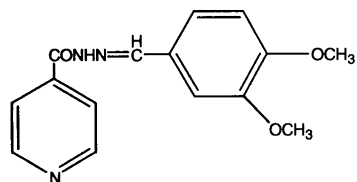
INH is strikingly superior to streptomycin. Unlike streptomycin, INH penetrates cells and also cerebrospinal fluid and is just as effective against intracellularly located bacilli as it is against those growing in culture media.

➤ **INH Hydrazones:**

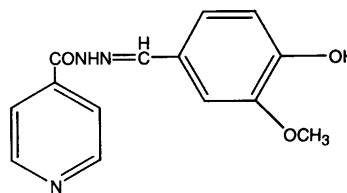
The very high activity and relatively low toxicity of INH promoted the synthesis of an extremely large number of structural analogues by many groups of workers. A small number of compounds had high activity, some because of

conversion in vivo INH. Despite claims to the contrary, none of the derivatives appears to be superior to INH.

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



[XVIII]

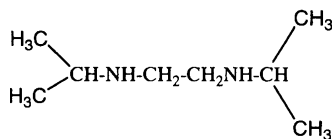


[XIX]

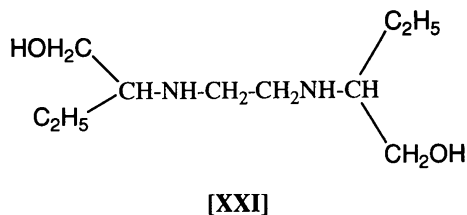
Clinically Verazide [XVIII] was originally claimed to exert a more marked and prolonged antituberculous effect than INH and 3-4 times less toxic. Later the same workers concluded that clinically the compound showed no advantage over INH and caused toxic side-effects attributable to veratraldehyde formed by hydrolysis⁵⁹. The 2-hydroxybenzylidene derivative was about half as active as INH and less than 1/10th as toxic⁶⁰. The 3-methoxy-4-hydroxybenzylidene derivative [Phthivazid, XIX] has been extensively used clinically in Soviet Union⁶¹.

➤ **Ethambutol:**

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



[XX]



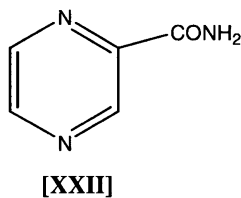
About 75% of the strains of the human type of *M. tuberculosis* are sensitive to 1 µg/ml of ethambutol.

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

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

➤ **Pyrazinamide:**

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



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

The Antibiotics

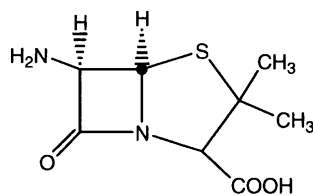
➤ *β-lactam antibiotics:*

The 1990 worldwide commerce in antibiotics was measured in tonnages and its value is in excess of seven billion. About half of this is associated with β-lactam antibiotics alone.

β-lactam antibiotics are believed to act by selectively inhibiting the cell wall synthesis⁶⁴, specifically, by inhibiting the biosynthesis of the dipeptidoglycan that is needed to provide strength and rigidity to the cell wall. Penicillin and Cephalosporin acylate specific bacterial D-alanine transpeptidase⁶⁵, thereby rendering it inactive for cross-linking of peptides.

➤ *Penicillins:*

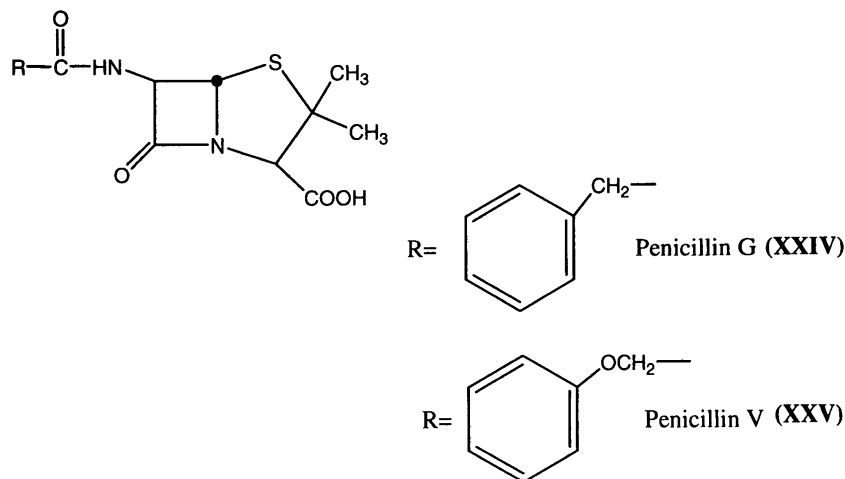
In 1929, Alexander Fleming for the first time discovered *Penicillium notatum* causing partial lysis of *Staphylococcal* colonies. Later on, Penicillin was isolated and gradually large-scale production also took place. The penicillin field was then expanded to include orally active penicillins, broad spectrum and enzymatically stable penicillins.



(XXIII)

6-amino penicillanic acid (XXIII), i.e. the fundamental nucleus, is prepared by using naturally occurring fungal enzyme that causes selective side chain cleavage of penicillins without breaking the β-lactam bond. More recently, selective chemical processes have been devised for accomplishing biosynthetic analogues.

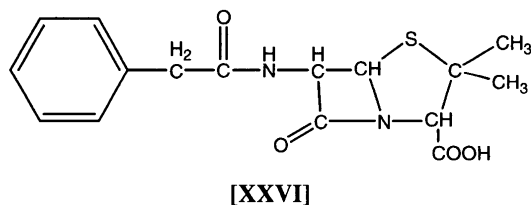
In the presence of phenyl acetic acid, benzyl penicillin (penicillin G) (XXIV) and with phenoxy acetic acid, phenoxy methyl penicillin (penicillin V) (XXV) was synthesized by fermentation.



➤ **Benzyl penicillin:**

Resistance develops in the bacteria by the deactivation of the penicillin by the enzymes namely β -lactamase catalyses hydrolysis of β -lactam ring whereas acylase is responsible for hydrolysis of acylamino side chain. In Gram-negative organisms, the resistance develops due to poor penetrability.

Among the four different penicillins i.e F, G, X & K, the G or Benzyl penicillin [XXVI] has exhibited the most prominent properties. It inhibits the growth of the more sensitive organisms, which include almost all Gram positive pathogens and some Gram negative pathogens. Mandelstam and Rogers⁶⁶ have shown that, at least with Staphylococci, benzyl penicillin acts by inhibiting the biosynthesis of cell wall mucopeptide, without interfering with protein synthesis.



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

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

➤ **Ampicillin:**

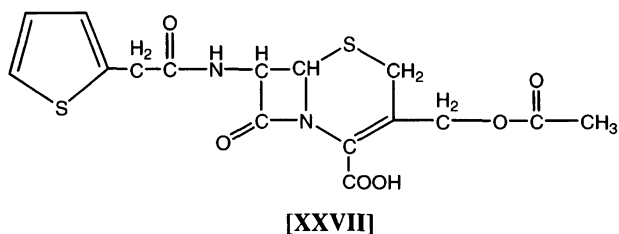
In 1961, Holinson and Stevens⁶⁷, have reported this semi synthetic α -aminobenzyl penicillin, which was administered orally as the free acid, which unlike most other penicillins is soluble only to the extent of about 10% in water.

Ampicillin is slightly less active than benzyl against most Gram positive bacteria. Its outstanding property is an activity four to eight times greater than that of benzyl penicillin against various Gram negative bacilli^{68,69}. It is bactericidal and appears to be as free from toxicity as benzyl penicillin. Gastric intolerance and rashes are the only side effects.

A few antibiotics containing thiophene ring:

i) **Cephalothin:**

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



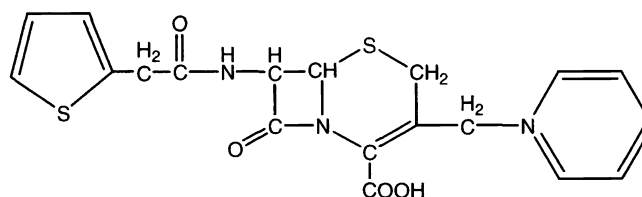
Cephalothin is active against both Gram positive and Gram negative micro-organisms. Gram positive bacteria are highly sensitive to this agent, many being

suppressed by concentrations of 0.004 µg/ml. Gram negative bacteria are inhibited by 1 to 6 µg/ml. Almost all strains of *Klebsiella* are sensitive at 4 to 16 µg/ml. Cephalothin is not active against viruses, yeasts and fungi. Bacteria susceptible to Cephalothin are sensitive over approximately the same range of concentrations of Cefazolin and Cephapirin. Cephalothin and its congeners inhibit bacterial cell-wall synthesis in a manner similar to that of penicillin. Cephalothin produces renal damage only rarely.

ii) **Cephaloridine:**

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

Mycobacterium fortuitum is sensitive to cephaloridine but not to cephalothin.



[XXVIII]

Cephalodrin C and its semi-synthetic congeners induce the synthesis of penicillinase by *B. cereus* and *Staph. aureus*. Cephalothin is highly resistant to this enzyme, but cephaloridine appears to be more susceptible to its effect⁷⁰.

Cephaloridine is nephrotoxic^{71,72}. Renal injury due to this drug occurs most often with administration of 6g or more per day. Large doses of cephaloridine result in a high incidence of granular casts in the urine.

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

➤ **Aminoglycoside antibiotics:**

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

i] **Streptomycin:**

The first public announcement of the discovery of Streptomycin from *Streptomyces griseus* was made by Schatz, Bugie and Waksman⁷³ early in 1944, and it was soon shown to inhibit the growth of the tubercle bacillus and a number of Gram positive and Gram negative micro-organisms in vitro and in vivo. The activity of streptomycin in vivo is essentially suppressive.

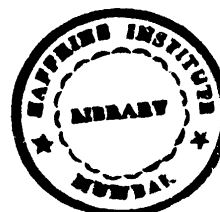
It was the first clinically effective drug to become available for the treatment of tuberculosis. At first, it was given in large doses, but problems related to toxicity and the development of resistant micro-organisms seriously limited its usefulness. This led to administration of the antibiotic in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

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

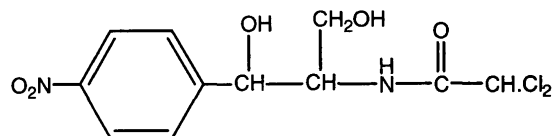
Since other effective tuberculostatic agents have become available, the use of streptomycin for the treatment of pulmonary tuberculosis has been greatly reduced.

ii] **Dihydrostreptomycin:**

Dihydrostreptomycin is a synthetic modification of streptomycin which reduces the toxicity while maintaining the activity. It is also produced by *Streptomyces*. It has essentially the same activity as that of streptomycin⁷⁴⁻⁷⁶ and is less toxic to the vestibular apparatus but more toxic to the auditory branch and could cause delayed severe deafness without warning symptoms⁷⁷.



➤ **Chloramphenicol:**



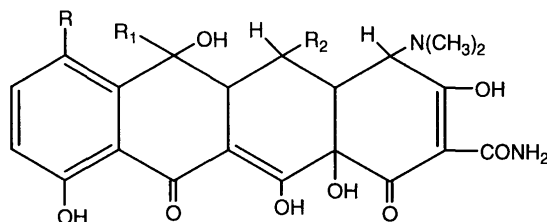
[XXIX]

Ehrlich et al.⁷⁸ and Carter et al.⁷⁹ had discovered the first broad spectrum antibiotic, chloramphenicol [XXIX]. In vitro it is active against a wide range of Gram positive and Gram negative bacteria. Chloramphenicol is strictly bacteriostatic against almost all bacteria species. The same bacteriostatic effect is seen in vivo.

It is a choice drug for infection of *Salmonella typhi*, although this organism remains viable for long periods and will regrow on removal from exposure to the drug⁸⁰.

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

➤ **Tetracyclines:**



[XXX]

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

The tetracyclines possess a wide range of antimicrobial activity against Gram positive and Gram negative bacteria, which overlaps that of many other antimicrobial drugs. With establishment of their in vitro antimicrobial activity, effectiveness in experimental infections, and pharmacological properties, the tetracyclines rapidly became widely used in therapy⁸¹⁻⁸³. Tetracyclines are also effective against some micro-organisms innately insensitive to many chemotherapeutic agents, such as rickettsiae, Mycoplasma and amoebae.

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

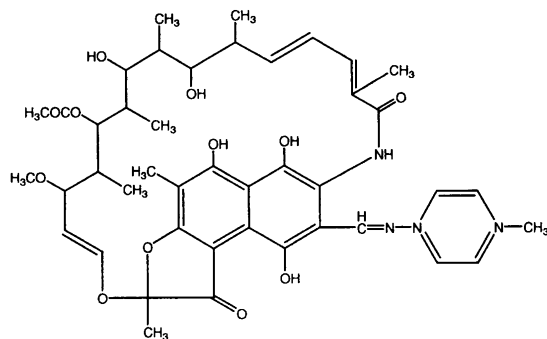
There are still a large number of antibiotics that needs to be mentioned. Prominent ones are macrolides, for example, erythromycin, oleandomycin, peptide antibiotics like polymyxins, bacitracin, antifungal antibiotics like nystatin, amphotericin, griseofulvin which are used in clinical practice. The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

➤ **Rifampicin:**

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

The drug is zwitterion and is soluble in organic solvents and in water at acidic pH⁸⁴.

Rifampicin [XXXI] inhibits the growth of most Gram positive bacteria, as well as Gram negative micro-organisms such as *Escherichia Coli*, *Pseudomonas*, *Indole* positive and negative proteus and *Klebsiella*^{85,86}. Also this drug is highly active against *Neisseria meningitides*, minimal inhibitory concentration range from 0.1 to 0.8 $\mu\text{g/ml}$ ⁸⁷.



[XXXI]

Rifampicin in concentrations of 0.005 to 0.2 $\mu\text{g/ml}$ is inhibitory to *M. tuberculosis* in vitro^{88,89} and is also active against atypical mycobacteria.

Micro-organisms, including mycobacteria develop resistance to rifampicin rapidly in vitro as a one-step process. This also appears to be the case in vivo, and this antibiotic must therefore not be used alone. The drug finds its greatest usefulness in the initial treatment of pulmonary tuberculosis in combination with INH in an outpatient therapeutic regimen⁹⁰. Rifampicin is a drug of choice for chemoprophylaxis of meningococcal disease in household contacts of patients.

Rifampicin does not cause untoward effects with great frequency. The most notable problem is the development of jaundice. It also produces gastrointestinal disturbances such as nausea, vomiting, abdominal cramps, diarrhoea and sometimes mild allergic reaction. Rifampicin causes oculotoxicity, which disappears when it is withdrawn from the treatment⁹¹.

Fungal Infections:

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

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

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

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

The discovery that specific fungi are the etiologic agents of specific infectious diseases preceded the work of Pasteur and Koch on pathogenic bacteria by three decades. Nevertheless, medical mycology has not received as much attention as have other areas of medical microbiology and at present there exist neither clinically available vaccines nor useful antisera for mycotic diseases. The incidence of systemic mycoses continues to rise steadily. This increase is due in part to the prolonged survival of patients with defects in their host defence mechanism, including patients with neoplastic diseases, organ transplant recipients, diabetics and patients with AIDS. These patients are susceptible to an ever-growing list of opportunistic fungi. From the data collected by the Center for Disease control, it is known that among the 1 and 3 million healthy Americans who have already been infected with AIDS, a significant number of these cases are associated with various fungal infections⁹². One

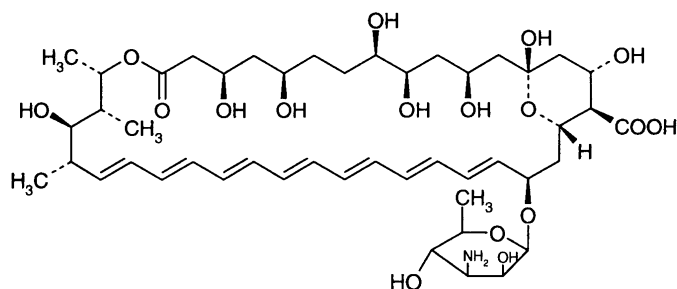
yeast-like fungus, *Cryptococcus neoformans*, is reported to be the most common debilitating and life-threatening infections in AIDS patients⁹³.

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

➤ **Amphotericin B:**

This antibiotic produced by *Streptomyces nodusus*, was discovered in 1955. It became the first systemic antifungal drug. Amphotericin B [XXXII] is a polyene macrolide with seven conjugated double bond (a heptene), an internal ester, a free carboxyl group and a glycoside side chain with a primary amino group. The drug is amphoteric, insoluble in water and is not absorbed via oral or intramuscular routes and is very active against pathogenic yeasts⁹⁴.

The primary mechanism of action of the polyenes in general, and amphotericin B in particular, is binding to ergosterol, the principal sterol present in the cell membrane of sensitive fungi⁹⁵.



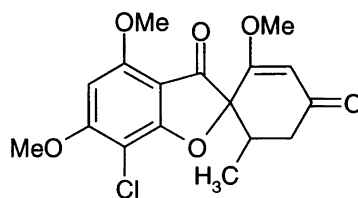
[XXXII]

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

➤ **Griseofulvin:**

Although Oxford A.E et al discovered this antibiotic, a product of *Penicillium griseofulvum* [XXXIII], in 1939; its antifungal activity was not utilized until 1951.

Groove predicted its chemical structure in 1950⁹⁶. However, in 1958, the antibiotic was found to be effective against dermatophytes when administered orally. The fluoro analogues retain the potency of the original drug but the bromo and dichloro analogues are ineffective. The replacement of the methoxy substituent on the cyclohexane ring either with propoxy or butoxy, increased invert activity, whereas substitution of an amino acid group at this site eliminates activity.

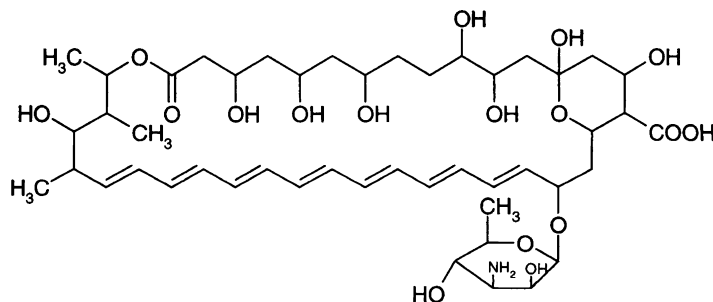


[XXXIII]

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

➤ **Nystatin:**

Nystatin [XXXIV] is produced by *Streptomyces noursei*. It was discovered by Hazen and Brown⁹⁷ and possesses good antifungal activity. Toxicity after parenteral administration to animals has led to its topical or oral use.

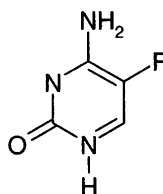


[XXXIV]

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

➤ **5-Fluorocytosine:**

This Fluorinated pyrimidine [XXXV] was first synthesized in the 1950s as a potential antileukemic drug. It was found to have in vitro and in vivo antifungal activity⁹⁸. It is being increasingly used as an adjunct to Amphotericin B therapy. Accordingly, in *Aspergillus*, *Candida* and *Cryptococcus* infections, the drug is combined with Amphotericin B.

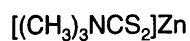


[XXXV]

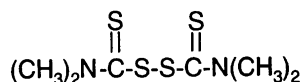
The susceptible fungal cells deaminate 4-fluorocytosine to 5-fluorouracil. The latter is metabolized to 5-fluoro-2'-deoxyuridylic acid, which suppresses DNA synthesis, by inhibition of thymidylate synthetase. The drug can also be incorporated into RNA to cause inhibition of protein synthesis. 5-fluorocytosine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

Sulfur containing molecules as antifungal agents:

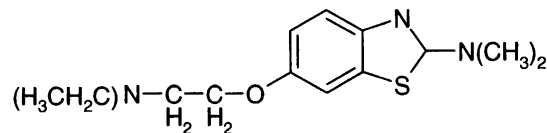
A variety of organic compounds that contain divalent sulfur attached to the carbon atom having in vitro fungistatic action, and many of these have been employed in topical therapy of dermatophytoses. Among these are dithiocarbamates [XXXVI], tetraethylthiuram disulfide [XXXVII] and benzothiazoles [XXXVIII] such as 6-diethylaminoethoxy-2-dimethyl amino derivatives [XXXIX]^{99,100}.



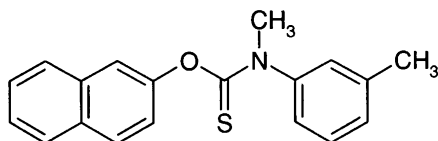
[XXXVI]



[XXXVII]



(XXXVIII)

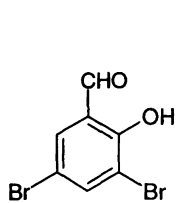


(XXXIX)

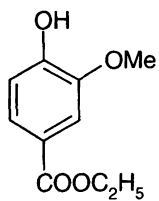
A widely used compound with unusually high in vitro and in vivo activity against the dermatophytes is o-2-naphthyl ester of m-N-dimethylthiocarbanilic acid (Tolnaftate). It is also effective against Candidal lesions¹⁰¹.

Phenolics and Diamides:

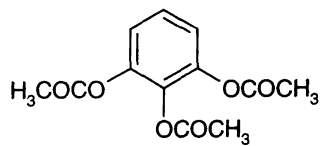
Phenolic compounds were known for their antifungal potency. Among the numerous phenolic compounds that have been used externally for mycotic infections are phenol, phenyl mercuric nitrate, cresol, m-cresyl acetate, benzoic acid, 4-hydroxy benzoic acid, salicylic acid, salicylanilide, 3,5-dibromo salicylaldehyde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



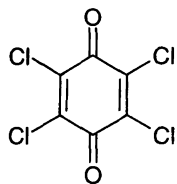
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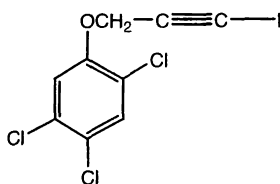
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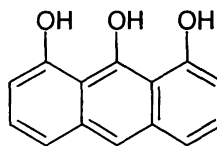
[XLII]



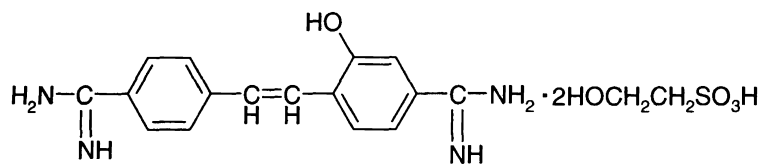
[XLIII]



[XLIV]



[XLV]

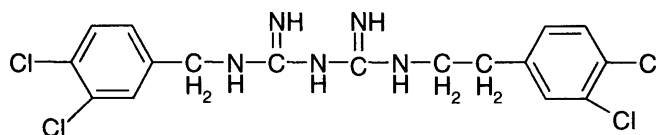


[XLVI]

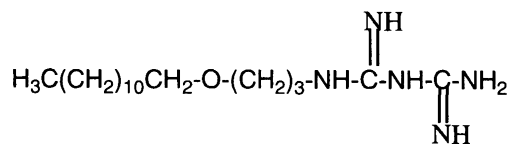
Several biamidines (e.g. stibamide, propamide, pentamide) are antifungal in vitro. 2-hydroxy stilbamidine diisothionate [XLVI] has been successfully employed in patients with blastomycosis. It is less toxic than Amphotericin B¹⁰².

➤ **Chelating Agents:**

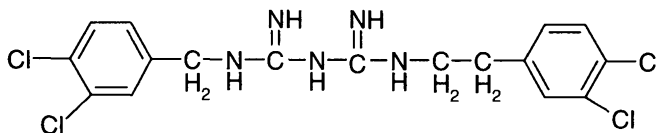
Among the chelating agents with considerable in vitro antifungal potency are substituted biguanides like N',N⁵-bis-(3,4-dichlorobenzyl) biguanide [XLVII], N'-Y-laurooxypropyl biguanide [XLVIII], 1,6-bis-(5-p-chlorophenyl biguanidine) hexane [XLIX]¹⁰³.



[XLVII]



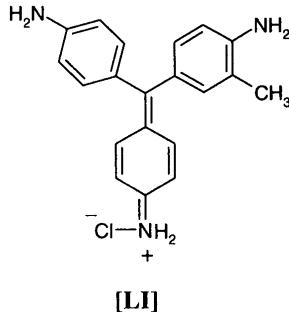
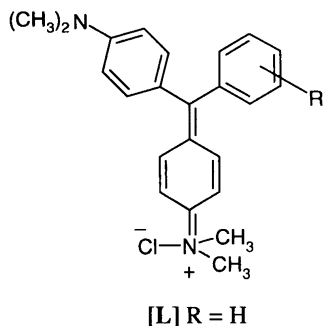
[XLVIII]



[XLIX]

➤ **Aniline Dyes:**

Aniline dyes such as malachite green [L], gentian violet [LI], methyl rosaniline chloride [LII] etc. have been employed in topical therapy of dermatophytoses as well as Candidiasis of skin mucous membranes.



➤ **Azoles:**

During the past quarter century, a series of imidazoles and triazoles have been synthesized and examined for antifungal activity. Various members of this series inhibit a broad spectrum of fungal pathogens. Some of the drugs are employed topically, for cutaneous yeasts as well as dermatophytic lesions. Imidazoles (two nitrogen atoms in a 5-membered ring) include Butaconazole, Cloconazole, Clotrimazole, Econazole, Fentiazole, Ketoconazole, Miconazole, Oxiconazole, Sulconazole and Tioconazole. Triazoles (three nitrogen atoms in a 5-membered ring) include Fluconazole, Itraconazole and Terconazole.

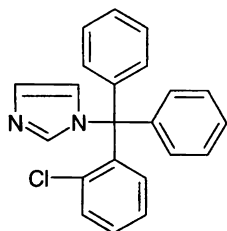
Clotrimazole [LII] was developed by investigators of the Bayer Laboratories in 1969, and is utilized topically in superficial mycoses or orally in clinical trials for deep mycoses¹⁰⁴.

Miconazole [LIII] is a well-known antifungal drug developed by the investigators of Janssen Pharmaceutica in 1969. It is utilized topically in superficial mycoses and by oral and intravenous routes in deep mycoses in clinical experimental trials¹⁰⁵.

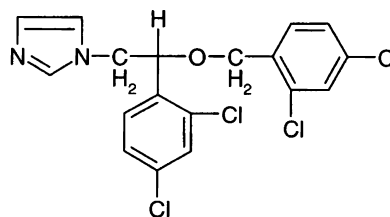
Econazole [LIV] is a phenethyl imidazole derivative. Like Miconazole, it is utilized as a topical agent in superficial mycoses and as a systemic antifungal by oral and intravenous routes in deep mycoses¹⁰⁶.

Fluconazole [LV] is a water-soluble fluorine substituted bis-triazole. It has comparable activity to that of Ketoconazole in inhibition of fungal C-14 demethylase. It is also potential candidate for prevention and early treatment of disseminated candidates in granulocytopenic patients¹⁰⁷.

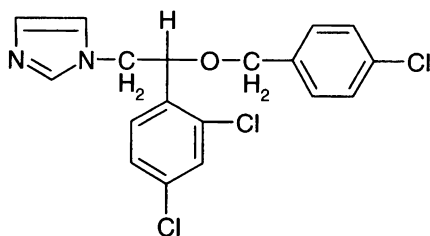
Itraconazole [XLVI] is a water-soluble, lipophilic triazole analogue that exhibits excellent activity against most fungal pathogens. When compared with Ketoconazole, its potential advantages include a broader spectrum of activity (including *Aspergillus* sp and *Sporothrix*), a more desirable pharmacokinetic profile and less toxicity¹⁰⁸.



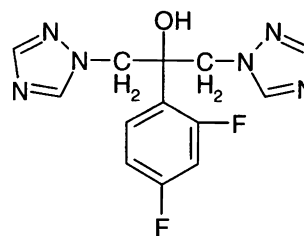
[LII]



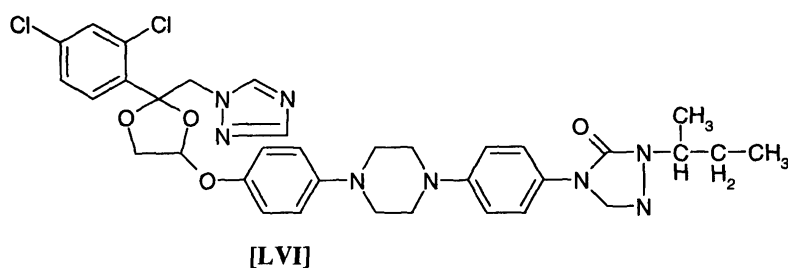
[LIII]



[LIV]



[LV]



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

IMPORTANT FACTS

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

Classification and Mechanism of action:

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

The antibacterial agents especially the antibiotics presently available maybe classified into several groups on the basis of their mechanisms of action^{109,110,111}.

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

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

Adverse reactions to chemotherapeutic agents:

The adverse reactions produced by anti-infective agents are of three general types. The concept of toxic effects and hypersensitivity reactions caused by drugs is same. More distinctive, however, are biological and metabolic alterations in normal microbial flora and supra-infections and interference with nutrition. These effects maybe induced, in varying degree by the antimicrobials¹¹².

Host determinants of response to antimicrobial agents:

Although, the nature of the infection determines the type of therapy, the innate host factors, unrelated to the disorder, are often the prime determinants of not only the type of drug selected but also of its dose, route of administration, risk and nature of untoward effects, and therapeutic effectiveness. Among such factors are age, genetic background, pregnancy, concurrent diseases, allergy, nervous system abnormalities, indigenous microbial flora, hepatic function, renal function and electrolyte balance and host's defense mechanisms¹¹³.

Resistance of micro-organisms to antimicrobial agents:

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

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

Properties of ideal chemotherapeutic agents:

The ideal chemotherapeutic should have the following properties. The compound should exhibit selective and effective antimicrobial activity, and it should be bactericidal rather than bacteriostatic. While it might be desirable that an agent

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

Search for new agents:

Literature reports show very closely that biological activity is very specific with regard to structure. Any change in the structure will significantly alter the activity and quite often may completely remove it. It also happens that if the important portion is retained, other changes do not materially affect the activity.

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

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

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

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

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

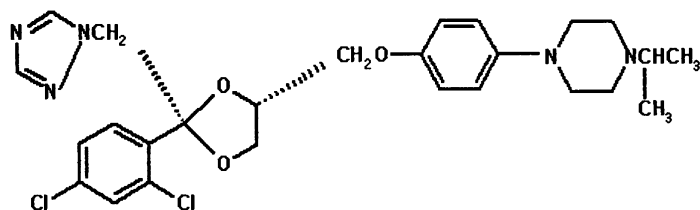
Benzothiazole, Thiazolidinone & Triazole moieties have played an important role in medicinal chemistry in the past few decades. Sulfur containing derivatives especially sulfonamides have been the pinnacle of success for almost over a century in the field of progressive pharmacy. Acid chlorides moieties have shown an increase in antimicrobial activity when used in combination with other medicinally important molecule.

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

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

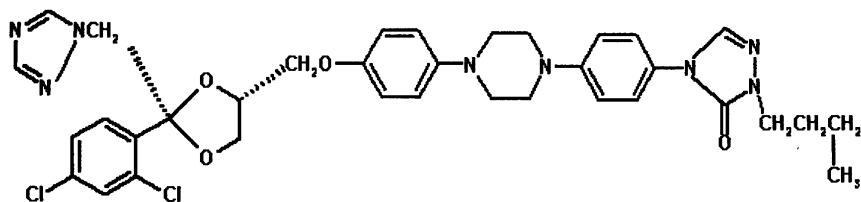
1. Terconazole, USP

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



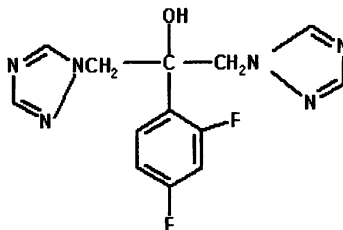
2. Itraconazole, USP

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.



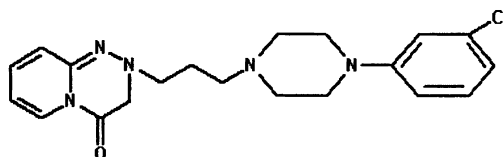
3. Fluconazole, USP

It is a water-soluble bis-triazole with broad-spectrum antifungal properties that is suitable for both oral and intravenous administration as the free base¹¹⁴.



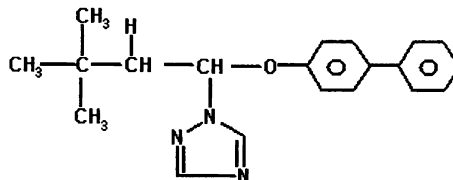
4. Trazodone, USP

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



5. Bittertanol

It is used as broad-spectrum fungicide¹¹⁵.



Plan of Work

Plan of work

Pharmaceutical research, particular in the field of medicinal chemistry, provides a basis for the development of new approaches to combat human diseases. The biomedical research can be basic (seeking an understanding of biological phenomenon which is unknown) or applied (using principles which are known to produce desired new product or effect). In either case research has the effect of obviating the established therapies in favor of new modalities which would be safer and more effective.

As the abilities of the chemist to synthesize isolate and characterize new compounds grew and as biologists learnt more about the actions of the drugs and about the biological mechanisms in health and diseases, the search of new drugs gradually began on a more rational basis. Today, useful drugs arise from natural products, chemical synthesis or combination of both sources by the means of approaches that vary in a complete spectrum between serendipitous discovery and rational synthesis.

Organic chemists in pharma industry synthesize hundreds of new compounds every week. In most cases, the chemist has a specific reason for synthesizing a particular compound; usually based on the theoretical considerations, medicinal chemistry, biological mechanism or a combination of all these three. In the past three decades, the biological sciences have undergone a major redirection towards molecular understanding of biological systems.

The search for chemical structures that which exhibit pharmacological activity is a difficult goal for the organic chemist. Compounds are submitted for screening different types of biological & pharmacological action. Observations on interesting and repeatable biological activity opens new pathway for additional chemical research efforts in the expansion of series and often leads significant new medicinal products.

Organic chemist has succeeded in the deliberate chemical alteration of the natural drugs and produces derivatives which are either more potent or superior in other respect. Such modifications of known pharmacodynamics compounds both synthesize and natural, constitutes one of the main kind of research efforts in the field of Chemotherapy.

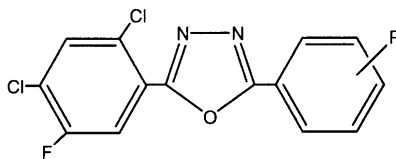
An important aspect of medicinal chemistry has been to establish a relationship between chemical structures and biological action. An increased consideration in research years has been to correlate between chemical structure with reactivity or physical properties and these correlations in turn, be related to therapeutic action.

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

Part 2:- Synthesis of 5-[2', 4', 6'-Trichlorophenoxy methyl]-2-(substituted Thiocarbamoyl)-1, 3, 4-Oxadiazole.

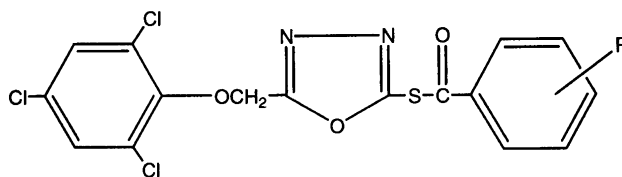
2,5-Disubstituted 1,3,4-Oxadiazole constitute a unique class of Nitrogen & Oxygen containing five membered heterocycles. Oxadiazole¹¹⁶ derivatives have been found to be exhibit diverse biological activities such as anti bacterial, fungicidal, anti inflammatory & anti HIV.

Recently Hosam Saad et,al¹¹⁷; have reported 2-aryl-5-substituted-1,3,4-Oxadiazoles show anti bacterial activity. Xiuman Zheng et,al¹¹⁸; also reported 2,5-Disubstituted Oxadiazole (I) shows significant insecticidal activity.



(I)

Hence taking into considerations the above facts it was planned to synthesize the series of compound having combination of 2,5-Disubstituted Oxadiazole with different biologically active acid chlorides so as to study their anti bacterial & anti fungal properties. In this series about 5-6 compounds prepared which will be confirmed by spectral analysis & will be tested for their anti microbial activities. The title compound has following general structure (II).

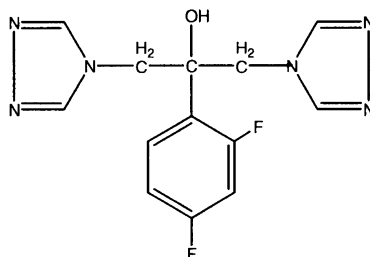


(II)

R= 4-Cl, 4-F

Part 3:- Synthesis of 3-[2', 4', 6'-Trichlorophenoxy methyl]-4-(substituted aniline)-5-aryl-1,2,4-Triazole.

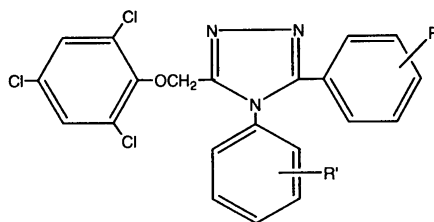
The importance of 1,2,4-Triazole moiety as a major pharmacophoric requirement to exhibit enhanced anti fungal activity is well manifested by its inevitable presence in the most recent anti fungal drug viz Flucanazole¹¹⁹(III).



(III)

4 -Fluoro aniline is used as a starting material for the synthesis of various anti bacterial¹²⁰ & insecticides¹²¹. 3-chloro-4-fluoro aniline is used in the synthesis of various pesticides¹²². A derivative of benzoic acid shows fungicidal activity¹²³.

Thus taking into consideration the above facts, the series of compounds having benzoic acid, aniline & Triazole moiety has been synthesized. In this series about 10-11 compounds will be prepared which will be confirmed by spectral analysis & will be tested for their anti microbial activities. The title compound has following general structure (IV).



(IV)

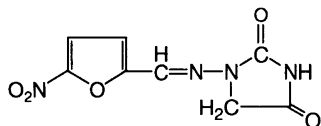
R= 4-Cl, 4-OC₂H₅

R'= 4-Cl, 4-OC₂H₅

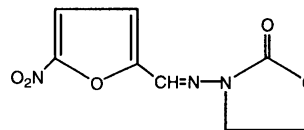
Part 4:- Synthesis of 3-[2',4',6'- Trichlorophenoxy methyl]- 5-(substituted Thiocarbonyl)-4-(substituted-N-benzylidenyl-amino)-1,2,4-Triazole.

Schiff's bases from 5-chlorosalicylaldehyde, 4-bromosalicylaldehyde & 4-N,N-dimethyl amino benzaldehyde have been reported to have encouraging tuberculostatic action¹²⁴. The presence of Azomethine linkage in compounds has also been known to decrease the toxicity considerably¹²⁵.

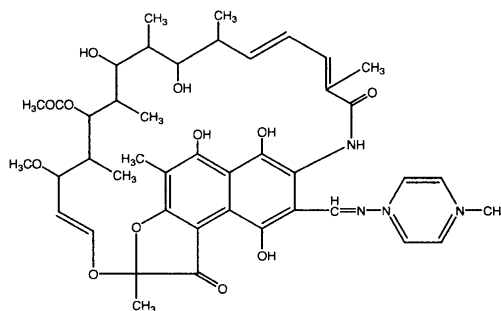
The presence of Azomethine linkage in variety of biologically active compounds such as Nitrofurantoin(V), ~~Nitrofurazone~~ (VI) and also in the fields of antibiotics such as Rifampicin(VII) has been proved to be very essential.



(V)

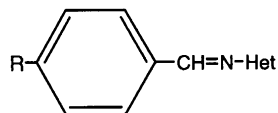


(VI)



(VII)

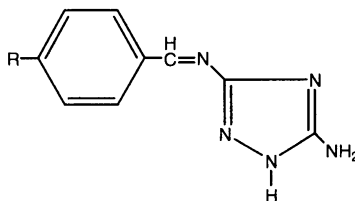
R.Fioranvati, et,al; have reported anti microbial activity of some new hetero aryl benzyl amines & their Schiff bases¹²⁶(VIII).



(VIII)

R= C₆H₅, 4-CH₃C₆H₄, 4-BrC₆H₄, 4-F C₆H₄,etc

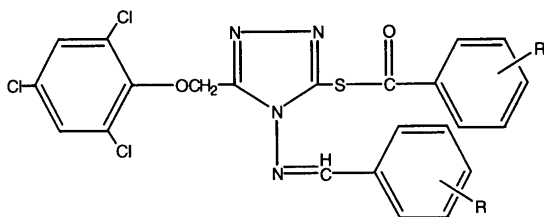
Straukas J.et al¹²⁷; have reported the anti-inflammatory activity of triazole Schiff base (IX).



[IX]

1,2,4-Triazole derivatives as mentioned earlier in the Part 2 are very pharmacological important. Compounds containing 1,2,4-Triazole nucleus & n-benzylidenyl derivatives also posses various biological activity. The thiol grouping at 5th position enhances the anti tubercular property of Triazole¹²⁸.A Mercapto group [-SH] is an important toxophore responsible for pesticidal activity¹²⁹.

Hence taking into consideration of all above mentioned facts, a series of compounds of Mercapto Triazole with different acid chlorides has been synthesized so as to study their anti bacterial & anti fungal properties. In this series about 10-12 compounds will be synthesized which will be confirmed by spectral analysis & will be subjected to their anti bacterial & anti fungal activites. The title compound has following general structure (X).



[X]

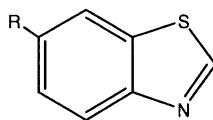
R= 4-Cl, 4-F, etc

R'=4-Cl, 4-COOH, etc

Part 5:- Synthesis of 3-(6-Chloro-benzathiazol-2-ylamino)-2-(substituted-phenyl)-thiazolidin-4-one.

Heterocycles bearing Thiazole, Sulphur & Nitrogen constitute the core structure of number of pharmacologically & biologically active compounds. Among these various types of known heterocyclic ring system, benzothiazole nucleus has found to be present in various medicinal compound having anti tubercular, anti allergic, anti inflammatory & anti fungal activity¹³⁰.

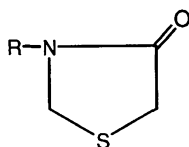
In benzothiazole derivatives, the substitution at 6th position by chloro, methoxy, etc can cause an increase in anti parasitic activity¹³¹(XI).



(XI)

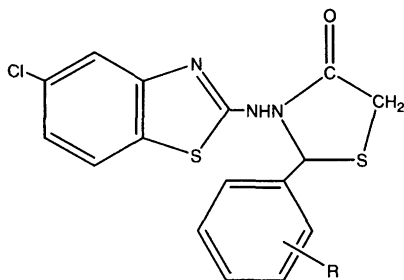
R=Cl,OCH₃

4-Thiazolidinones (VII) derivatives play a vital role in pharmaceutical sciences owing to their wide biological & industrial applications¹³².The derivatives of 4-Thiazolidinones have also occupied a unique place in the field of Medicinal chemistry due to a wide range of pharmacological activities^{133,134}(XII).



(XII)

Based on the above facts, a series of compound have been synthesized by condensing various substituted Benzaldehyde with Hydrazino benzothiazole to get substituted Schiff's bases which was then cyclised with Mercapto acetic acid to get the final compound which has both Benzothiazole & 4-Thiazolidinone moiety. In this series about 10 compounds will be synthesized which will be confirmed by spectral analysis & will be tested for their anti microbial activites. The title compound has following general structure (XIII).



[XIII]

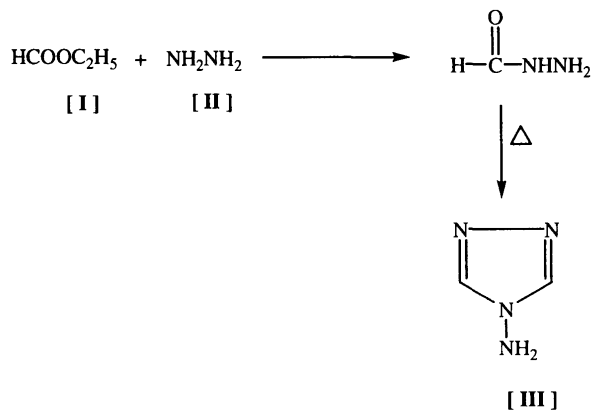
R= 4-Cl, 4-COOH, etc

Chemistry

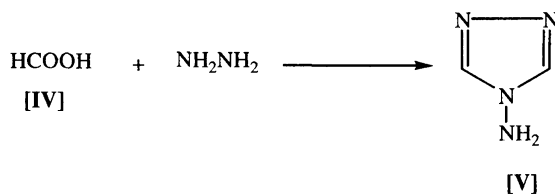
CHEMISTRY OF TRIAZOLE

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

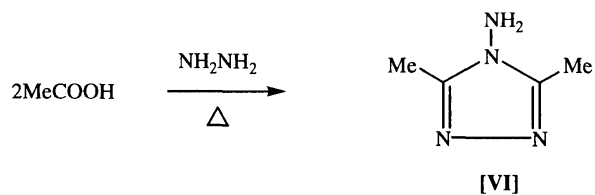
1. 4-Amino triazole (III) has been prepared¹³⁵ by the reaction of ethyl formate (I) with hydrazine (II) to give formhydrazide, which was heated at 150 to 200° C to effect self condensation to give (III).



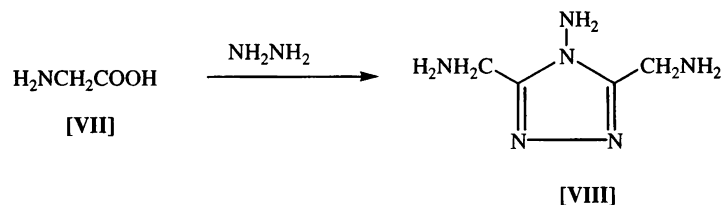
Similar interaction of formic acid (IV) and hydrazine gave good yield of 4-Amino triazole (V)^{136,137}.



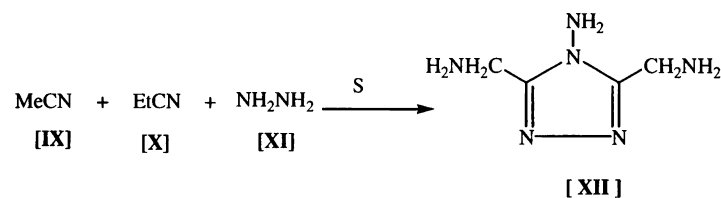
2. Other carboxylic acids and their functional derivatives have also been used to prepared alkyl and substituted alkyl -4-Amino triazoles, e.g. acetic acid and hydrazine hydrate at high temperature give 3,5-dimethyl-4-Amino triazole (VI)¹³⁸.



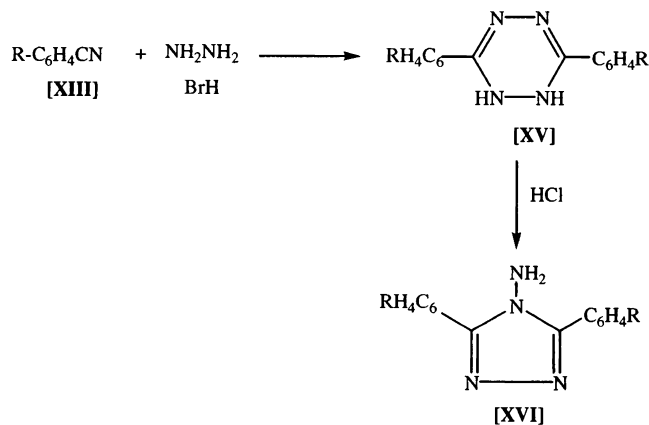
3. Similar reaction have been reported for the preparation of 4-Amino-3,5-bis (amino alkyl) triazole (VIII)^{139,140}, which is prepared by the reaction of glycine (VII) with hydrazine to give (VIII).



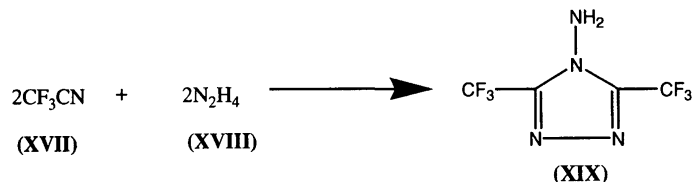
4. Sulfur and sulfur containing compounds catalyze the formation of 4-Amino triazoles e.g. 4-Amino triazole (XII) substituted at 3,5-position with different substituents was prepared by the reaction of equimolar amount of acetonitrile (IX) and propionitrile (X) with a mixture of hydrazine (XI) and sulfur¹⁴¹.



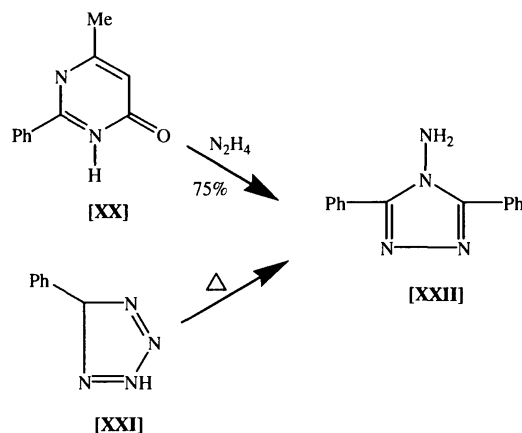
5. The preparation of 4-Amino-3,5-diaryl triazoles (XVI) are usually effected by the acidic rearrangement of 1,2-dihydro tetrazines (XV), which are obtained by the reaction of equimolar amount of aryl nitriles (XIII) and hydrazine (XIV) in refluxing benzene or toluene¹⁴².



6. Perfluoro alkyl triazole (XIX) are prepared by the various methods e.g. perfluoro acetonitrile (XVII) with hydrazine (XVIII) in a sealed tube at room temperature gave (XIX)¹⁴³.

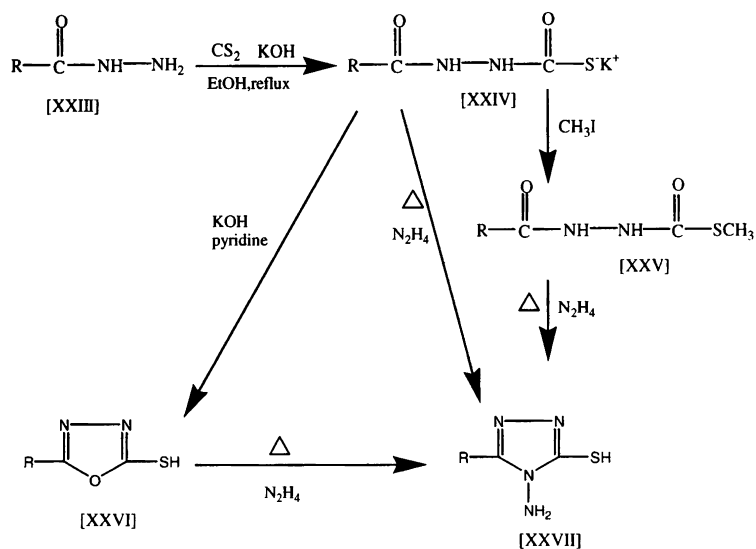


7. 4-amino -3,5-diphenyl triazoles (XXII) are also formed by the decomposition of other hetero aromatic ring systems e.g. reaction of pyrimidine (XX) with hydrazine¹⁴⁴. Also the thermolysis of 5-phenyl tetrazole (XXI) in various solvents¹⁴⁵ gives (XXII).

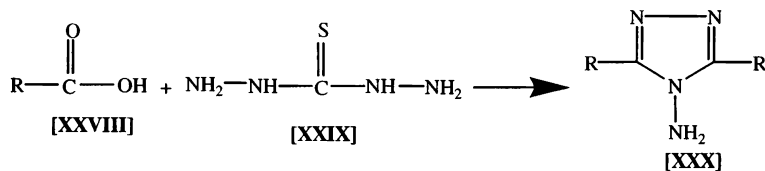


Methods of preparation of 3-(substituted phenyl)-4-amino-5-mercapto-1,2,4-triazole:

1. One of the methods for synthesis of triazole is Hoggarth synthesis¹⁴⁶. This methodology involves the condensation of carboxylic acid hydrazides with carbon disulphide and potassium hydroxide to yield the potassium dithiocarbazate (XXIV) which after s-alkylation with methyl iodide, undergo ring closure with an excess of hydrazine, to produce the amino thiols (XXVII) in good yield.



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 time¹⁴⁷.
3. Alternatively, oxadiazoles (XXVI) can be converted to triazoles (XXVII) by the reaction with hydrazine.
4. It has also been reported that the same carboxylic acid (XXVIII) react with thiocarbo-hydrazide (XXIX) to afford directly the 3-substituted-4-amino-5-mercapto-1,2,4-triazoles (XXX). It has also been found that the reaction of thiocarbohydrazide with carboxylic acids can be conveniently applied to both aliphatic and aromatic acid derivatives^{148,149}.



Chemistry of Hydrazide

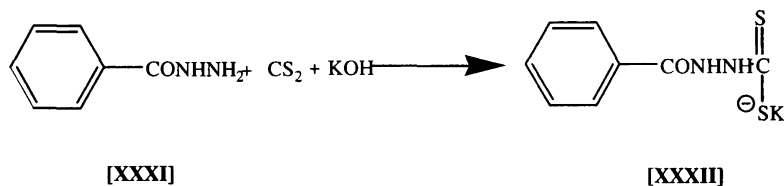
Acid hydrazides are obtained by the reaction of methyl or ethyl esters with hydrazine hydrate.



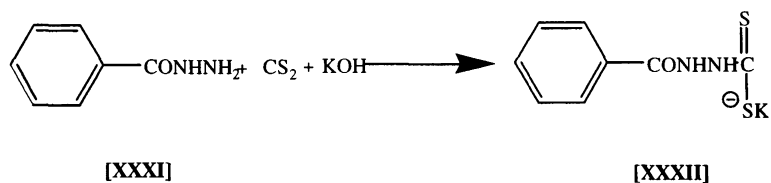
The general procedures followed for the synthesis of acid hydrazides in the present work is analogous to that reported by Vogel¹⁵⁰.

Chemistry of Dithiocarbamate salts

1. Busch and starke¹⁵¹ obtained crystalline potassium salt of 2-benzylidithio carbazinic acid (XXXII) by stirring together benzhydrazide (XXXI), 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 procedures¹⁵².



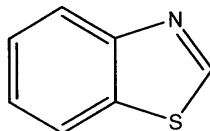
2. Reid¹⁴⁷ et al prepared the potassium salt [XXXIII] by stirring together KOH, CS₂ and aryl hydrazide in absolute alcohol for 12-16 hours and the product was washed with dry ether.



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.

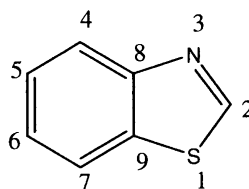
CHEMISTRY OF 2-AMINO BENZOTHAZOLE

The benzothiazole ring contains a phenyl ring fused to a thiazole ring as indicated in the structure (I)



[I]

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



[II]

SYNTHESIS OF 2-AMINO-6-SUBSTITUTED BENZOTHAZOLES

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 dioxide¹⁵³, 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 chlorides¹⁵⁴, sulfuryl chlorides¹⁵⁵ or certain N-chloroamides^{156, 157, 158} 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^{159, 160} or acetone¹⁶¹ can be used. The yield in the

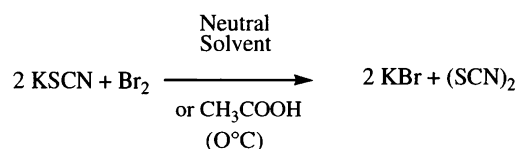
thiocyanation of amine is 20%-30% higher when the reaction is carried out in a neutral medium like methanol rather than in acetic acid. The formation of a thiazole is also inhibited in a neutral solvent. Ether is usually not satisfactory because the solvent is attacked and because some of the amine is precipitated as the thiocyanate^{162,163}.

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^{162,164,165}. 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 minimizes polymerisation. The reagent is generated from the thiocyanate salts by chemical reactions.

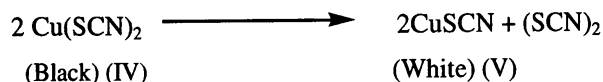
THIOCYANOGEN GENERATED FROM SALTS BY CHEMICAL REACTIONS:

In this method the alkali metal thiocyanate (III) is dissolved in acetic acid or formic acid^{165,166}, or better in neutral solvent like methyl acetate, acetone or methanol^{159,167} 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.

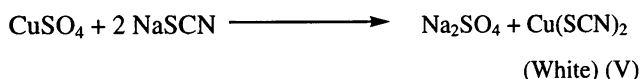
The chemical transformation can be written as below:-



Cupric thiocyanate¹⁶⁸, the use of which may be considered as still another modification of this general procedure, shows promise of being effective. It releases thiocyanogen merely by the dissociation of the cupric (IV) to cuprous salt (V).



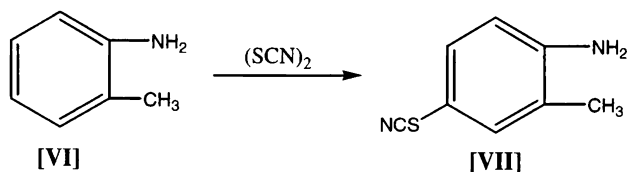
Cupric thiocyanate (IV), prepared in advance, or a paste of cupric sulphate and sodium thiocyanate in equivalent proportions is added to a solution of the compound in methanol or acetic acid and the mixture is warmed to 35°-80°C, until the black cupric thiocyanate has changed completely to the white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



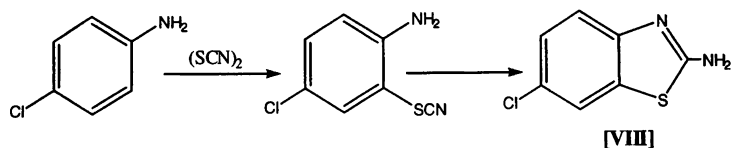
This procedure has the advantage over the others of permitting higher temperatures for thiocyanation.

THIOCYANATION OF AROMATIC AMINES:

The thiocyanation group (-SCN) is introduced into aromatic amines with rapidity. It enters preferentially at a free para position if available, otherwise at ortho position. For example, o-toluidine (VI) is converted to 4-thiocyano-o-toluidine (VII) (80% yield) ¹⁶⁹.

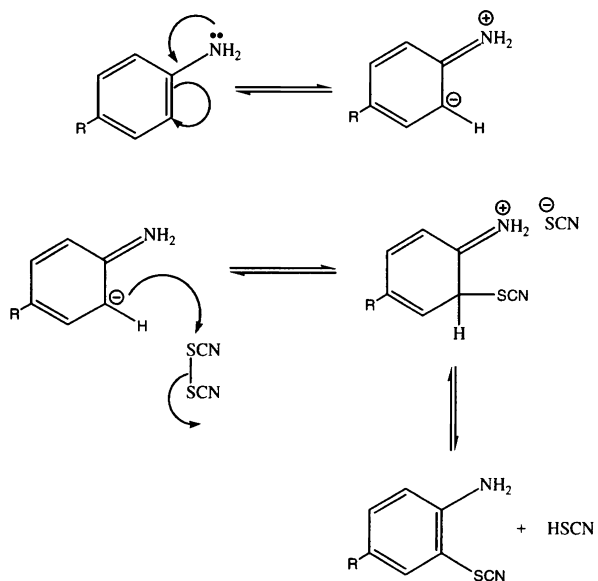


The thiocyanation takes place in the position ortho to the primary amine group. The final product in such cases is often an amino benzothiazole formed by the secondary reaction between the amino group and the adjacent thiocyano group. In this secondary reaction, since the amino group is having lone pair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



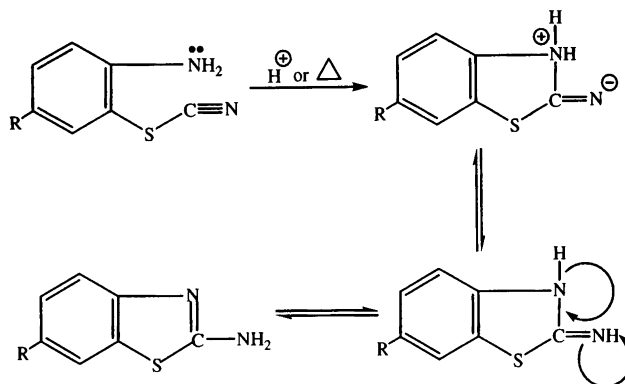
Mechanism:

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



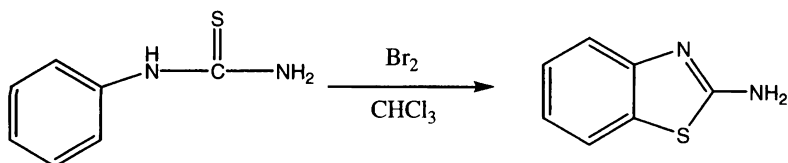
The unstable intermediate species immediately loses a proton to retain the aromatic character of the molecule which gives higher stability.

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

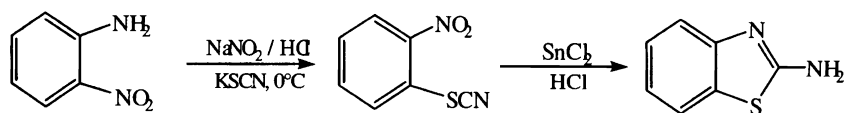


HUGERSCHOFF SYNTHESIS:

This synthesis is restricted to the preparation of 2-amino derivatives of the benzothiazoles (X) and employs the action of oxidising agent such as bromine, upon aryl thioureas (IX) in an inert solvent such as chloroform, ethylene dichloride, chlorobenzene or acetic acid. Sulfuryl chloride can be substituted for bromine¹⁷⁰⁻¹⁷². However, potassium ferrocyanide in alkaline media, the reagent for the Jacobson ring closure, gives no thiazole from the arylthiourea^{173,174}.

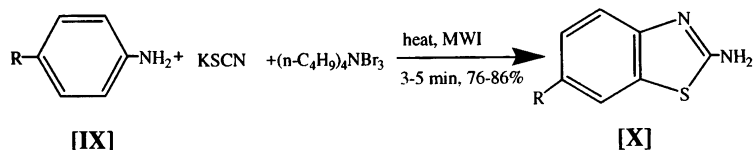


Generally, the yields in the Hugerschoff's reaction are excellent and frequently nearly quantitative. 2-Amino benzothiazoles (XIII) have been prepared from o-thiocyanonitro compounds of the type (XI) by reduction using stannous chloride in HCl and FeSO₄ in ammonia^{175,176}.



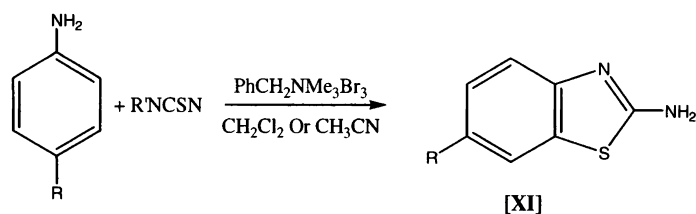
NEWER METHODS:

- 1) 2-Aminobenzothiazoles [X] were efficiently synthesized by Zheng et. al.¹⁷⁷ using stable, crystalline tetrabutylammonium tribromide instead of toxic, corrosive liquid bromine under solvent-free and microwave irradiation condition.

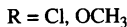


R = Cl, OCH₃

- 2) Jordan et. al.¹⁷⁸ 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 is ease of addition and handling, which minimizes the risk of forming brominated side products. They have extended the use of this reagent to a direct, one-pot synthesis of 2-aminobenzothiazoles from either aryl isothiocyanate and anilines or tetrabutylammonium thiocyanate and anilines in the presence of a stoichiometric amount of Ph-CH₂NMe₃Br₃.

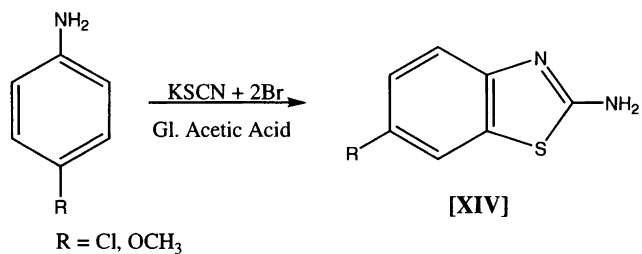


- 3) Matsui et. al.¹⁷⁹ prepared 6-substituted-2-aminobenzothiazoles [XII] by the reaction of 4-substituted anilines with potassium thiocyanate in presence of bromine.

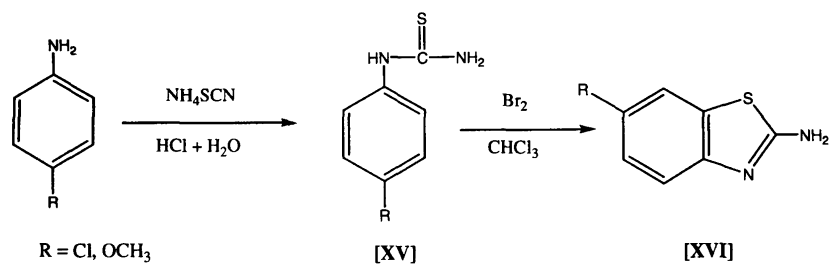


R = 4-NO₂, 5-NO₂, 6-NO₂, 4-CH₃, 5-CH₃, 6-CH₃ etc.

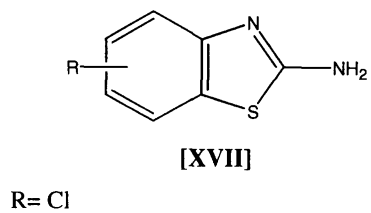
Method A: One-pot reaction of appropriate anilines with thiocyanogen generated from bromine and alkaline thiocyanate in acetic acid medium led to formation of the desired product [XIV] in good to moderate yields.



60

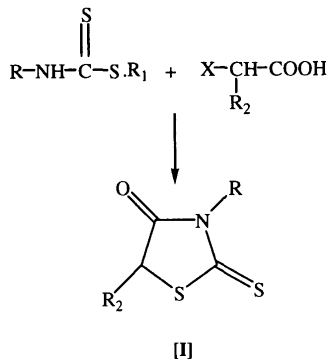


In the present study, the raw material viz. 2-amino 6-chloro-benzothiazoles [XVII] are synthesized by thiocyanating substituted anilines with KSCN and bromine in acetic acid at cold conditions, according to the literature methods¹⁸¹.

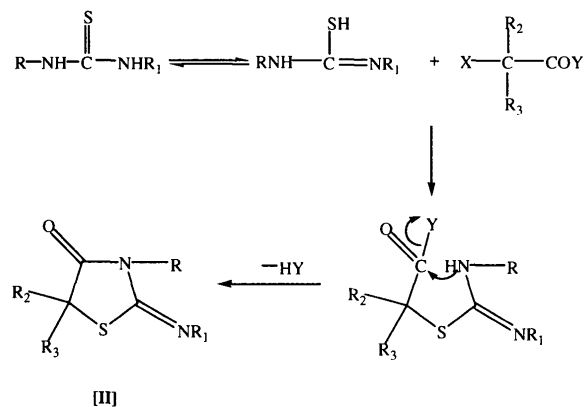


CHEMISTRY OF 4-THIAZOLIDINONES

The dithiocarbamates, formed by the reaction of primary amine with carbon disulfide in the presence of a base, react with α -haloalkanoic acids in the presence of NaHCO_3 to give substituted 2-thiono-4-thiazolidinones (I)^{182,183}.

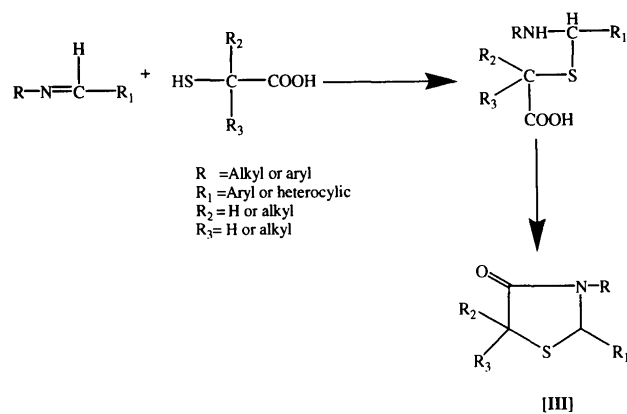


Substituted 2-imino-4-thiazolidinones (II) are obtained in good yields by the reaction of symmetrical and unsymmetrical thioureas with various substituted and unsubstituted α -haloalkanoic acids, their esters, acid chlorides, amides or carbamates^{184,185}. The reaction proceeds via intermediate isothiurea which cyclizes in refluxing acetic acid, ethanol or benzene in the presence of sodium acetate or pyridine.

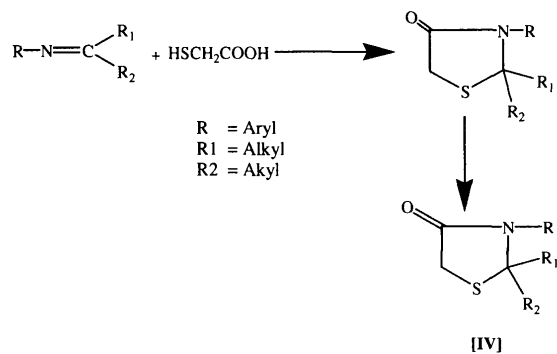


α -Mercaptoalkanoic acids have been extensively used for the synthesis of 4-thiazolidinones. The substituted and unsubstituted α -Mercaptoalkanoic acids reacts conveniently with Schiff bases of aromatic and heterocyclic aldehydes and aliphatic or

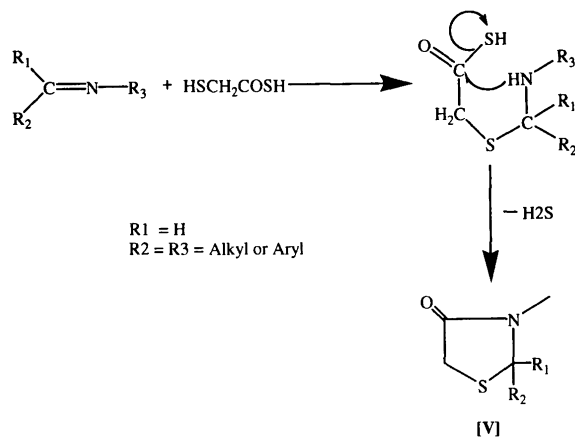
aromatic amines in different solvents to give a variety of 2-substituted-4-thiazolidinones (III)^{186,187}.



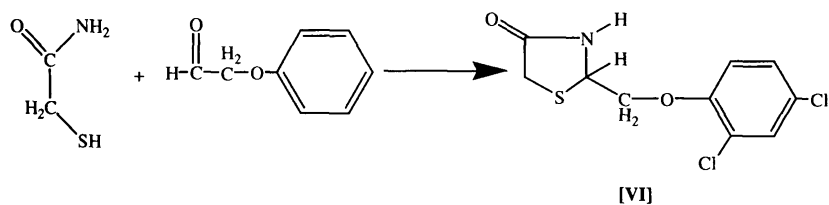
Schiff bases obtained by the condensation of ketones and amines also reacts with α -Mercaptoacetic acid to give 2,2-disubstituted -4-Thiazolidinones(IV)¹⁸⁸.



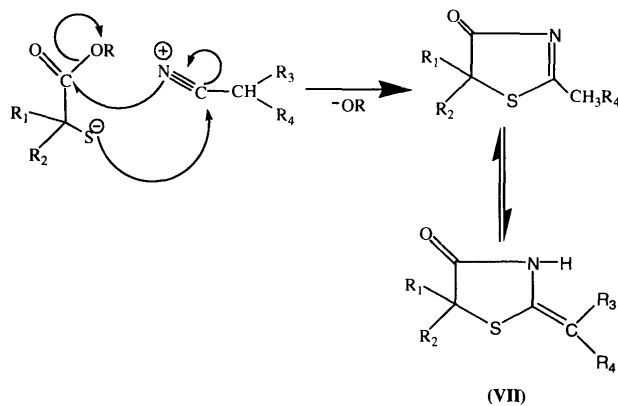
α -Mercaptothiocarboxylic acid can also be used for the synthesis of 4-thiazolidinones by the reaction with different Schiff bases¹⁸⁹ by refluxing in ether.



α -Mercaptoacetamide reacts with the carbonyl function of aldehydes in the presence of catalytic amounts p-toluenesulfonic acid or boron trifluoride etherate in inert solvents to give 2-substituted-4-thiazolidinones (VI)¹⁹⁰.



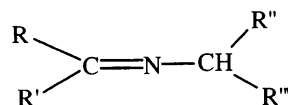
Substituted and unsubstituted α -Mercaptoacetic acid esters reacts smoothly with the compounds containing activated nitrile groups in the presence of an equivalent amount of alcoholate to give 4-thiazolidinones(VII)¹⁹¹.



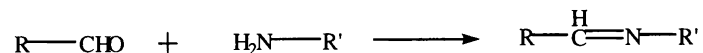
Chemistry of Schiff bases

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 ¹⁹² 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^{193,194} Due to the great flexibility and diverse structural aspects, a wide range of Schiff bases have been synthesized and their complexation behavior studied¹⁹⁵.

Pelton et al¹⁹⁶ 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 laboratory¹⁹⁷. Also the presence of an azomethine linkage in compounds has been shown to decrease their toxicity considerably¹⁹⁸

Many procedures have been described in the literature for the synthesis of Schiff bases¹⁹⁹⁻²⁰¹.

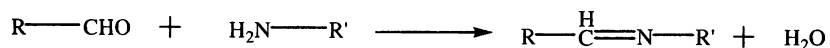
The important methods are listed below:

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 clapp²⁰² 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 al²⁰³ 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 Rabassa ²⁰⁴ 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 carboxyl oxygen and promotes the attack of nucleophilic amine.

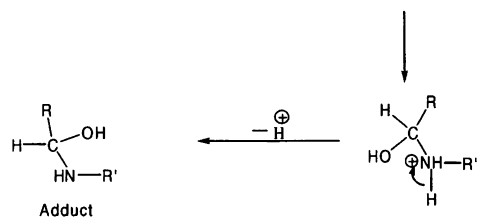
Step5:- 5- [2', 4', 6'-Trichlorophenoxy methyl]-2 -(substituted Thiocarbamoyl)-
1, 3, 4 – Oxadiazole.

In 100 ml three necked round bottom flask, with dropping funnel fitted with the guard tube and a double walled reflux condenser also with a guard tube, was placed a solution of compound 2-(2',4',6'-trichlorophenoxymethyl)-5-mercapto-1,3,4-Oxadiazole (3.11g; 0.01 mol) in dry pyridine, on an oil bath. To this add dropwise a solution of p-Chloro benzoyl chloride (1.2ml; 0.001 mol) in 10 ml dry pyridine. The solution was poured into crushed ice. The solid separates out was filtered, washed with water and dried. It was then recrystallized from methanol²¹¹.

Yield :- 51%

M.P:- 180°C

Similarly, other derivatives were synthesized and reported along with their melting points and other important data in Table-2.



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

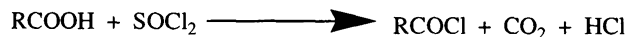
CHEMISTRY OF ACID CHLORIDES

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

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



The use of thionyl chloride²⁰⁵ for the preparation of acid chlorides presents many advantages in a large number of cases. The reaction result in the formation of Sulphur dioxide and hydrogen chloride in addition to the acid chloride, and the isolation of the chloride is for this reason, a simple matter.

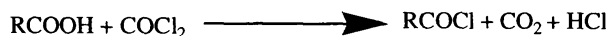


The thionyl chloride must be pure, as the impure product has been known to cause somewhat explosive decomposition in some instances. The procedure consists of mixing the chlorinating agent with acid and heating the mixture, if necessary. The product may be isolated by fractional distillation or if solid,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

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

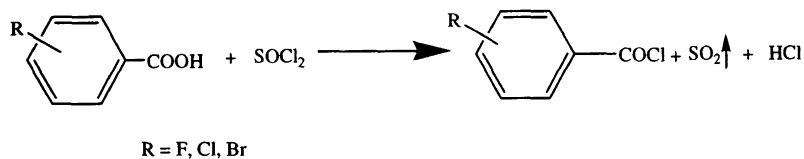


The reaction of phosgene²⁰⁶ with carboxyl acids, and p -toluene sulphochloride or of sodium chlorosulphonate with salts of carboxylic acids are also used for the preparation of acid chlorides.



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

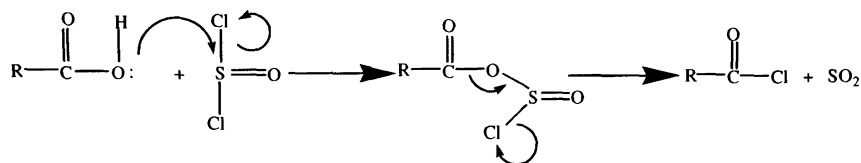
Thus, in the present work all the acid chlorides were prepared by reacting thionyl chloride with corresponding acids.



Reaction mechanism

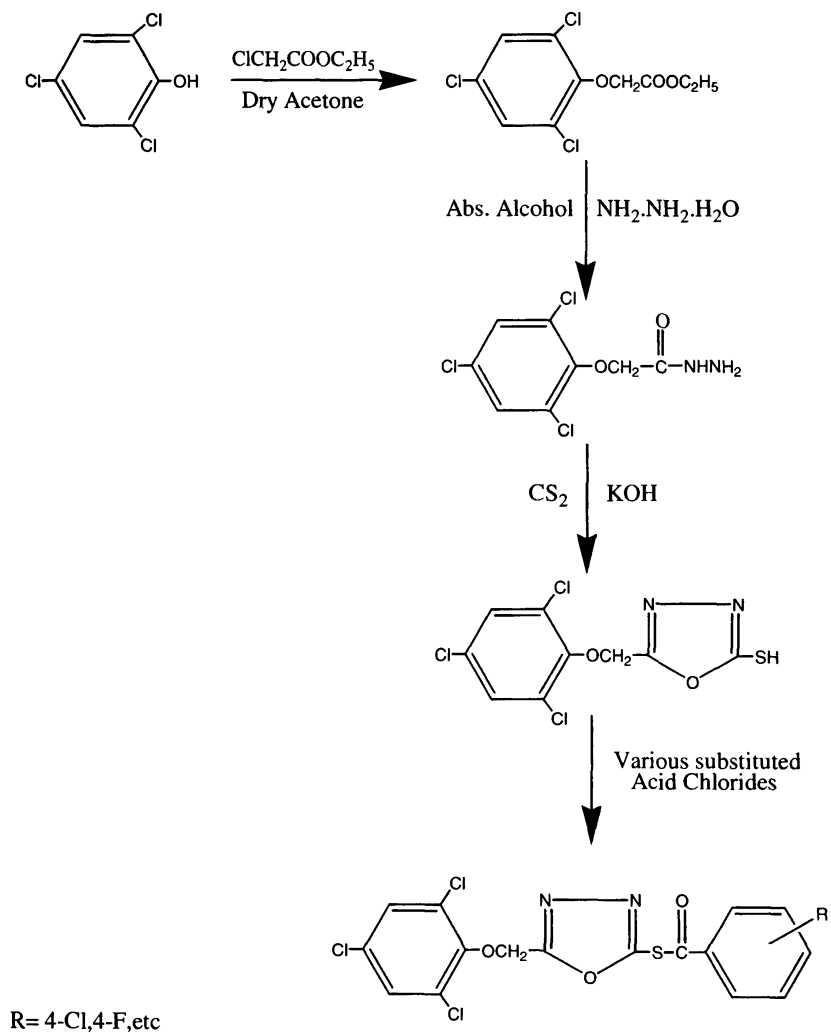


Acids and their salts are the only common starting materials for the synthesis of acid chlorides and several reagents are utilized as a source of nucleophilic chloride. All the reagents form reactive intermediate (I), so that the leaving group in the substitution is not hydroxy group.

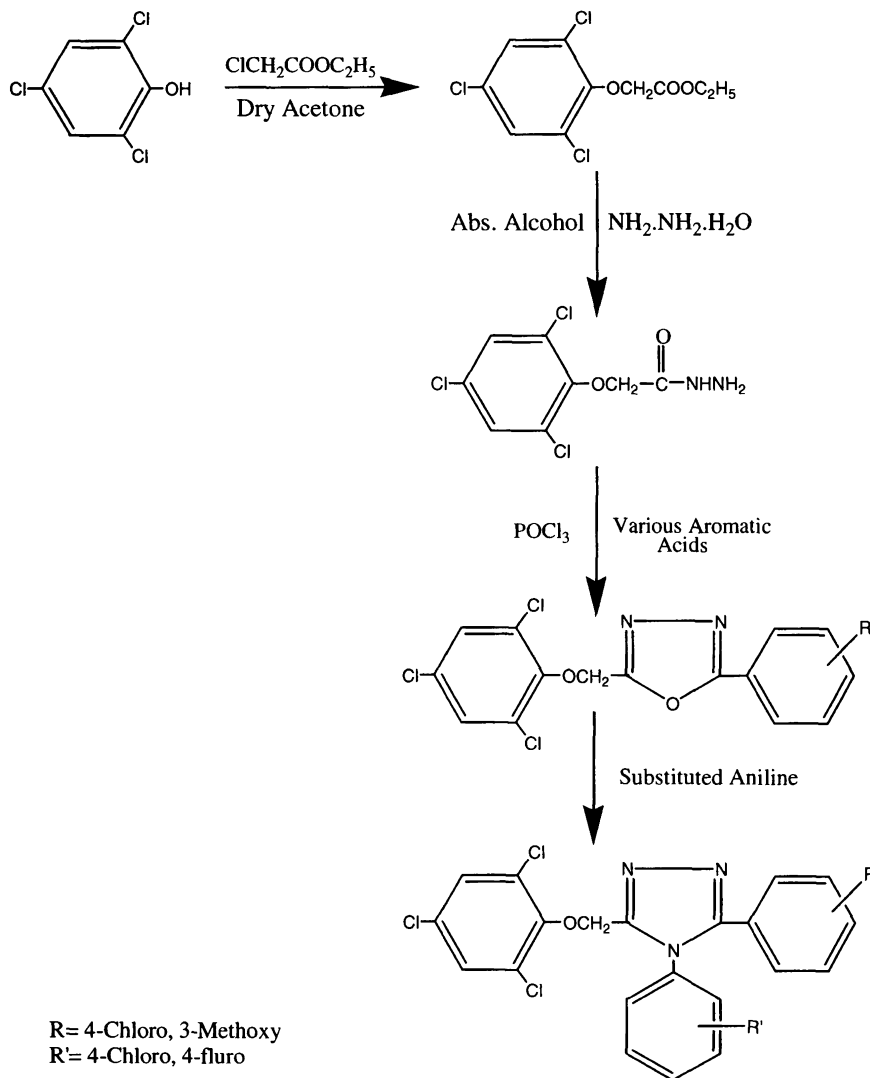


*Schematic
Representation*

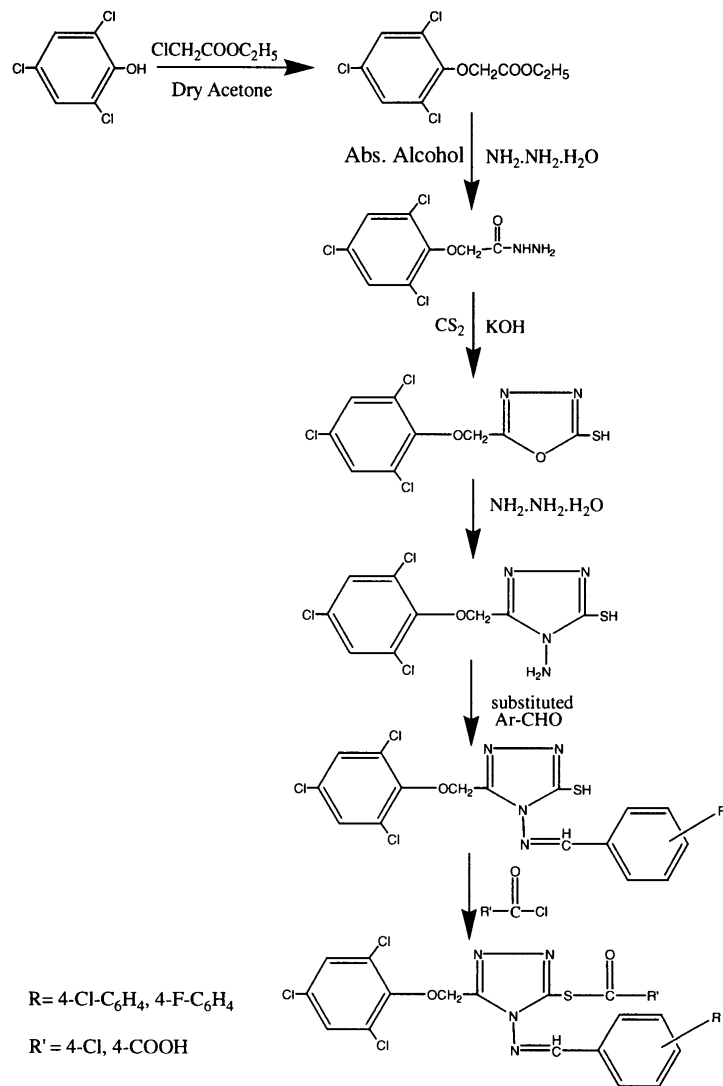
**SCHEME I :- Synthesis of 5 – [2', 4', 6'-Trichlorophenoxy methyl]-2–
(substituted Thiocarbamoyl) – 1, 3, 4 – Oxadiazole.**



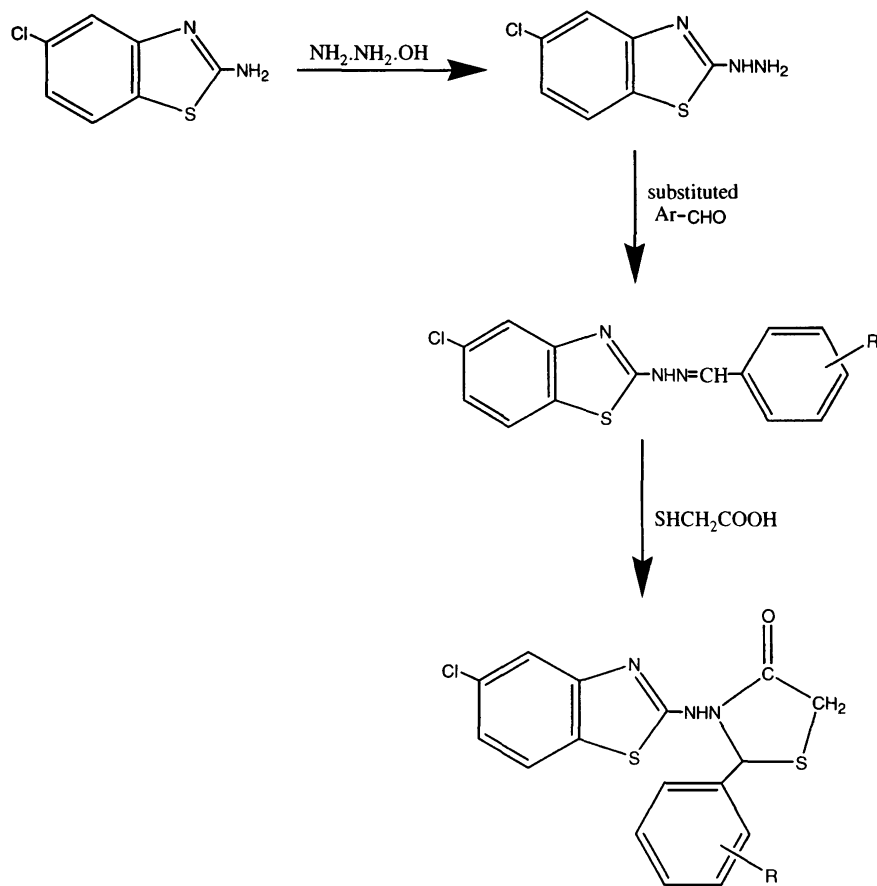
SCHEME II:- Synthesis of 3 -[2', 4', 6'- Trichlorophenoxy methyl]-4-(substituted aniline) - 5 - aryl - 1, 2, 4 - Triazole.



SCHEME III :- Synthesis of 3-[2',4',6'- Trichlorophenoxy methyl]- 5-(substituted Thiocarbonyl)-4-(substituted-N-benzylidenyl amino)-1,2,4-Triazole.



SCHEME IV :- Synthesis of 3-(6-Chloro-benzathiazol-2-ylamino)-2-(substituted phenyl)-thiazolidin-4-one.



$\text{R} = 4\text{-Cl}, 4\text{-COOH}, \text{etc.}$

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 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 5 – [2', 4', 6'-Trichlorophenoxy methyl]-2– **(substituted Thiocarbamoyl) – 1, 3, 4 – Oxadiazole.**

IUPAC Name: - Synthesis of substituted thiobenzoic acid S-[5-(2,4,6- **trichlorophenoxy methyl)-[1,3,4]oxadiazol-2-yl].**

Step1:- Synthesis of 2,4,6-trichlorophenoxyacetate

In two neck 500ml flat bottomed flask equipped with a reflux condenser, dropping funnel and a guard tube, 2,4,6-trichlorophenol (19.75g; 0.1 mol) and potassium carbonate (11.2g; 0.2 mol) was refluxed on a magnetic stirrer for 2 hrs to form a potassium salt in dry acetone (50 ml). Then ethylchloro acetate (10.8ml; 0.1 mol) was added drop wise and the mixture was further stirred for 6 hrs. The excess of acetone was removed under pressure and the residue was cooled and poured onto crushed ice. Liquid product was obtained which was isolated by ether extraction.

Yield:- 60%

B.P:- 190-195°C

Density :- 1.313 gm/ml

Step2:- Synthesis of 2,4,6-trichlorophenoxyacetyl hydrazide

A mixture of 2,4,6-trichlorophenoxyacetate(2.83g; 0.01mol) and hydrazine hydrate (1.94ml; 0.04 mol) in absolute alcohol was refluxed in a 100ml round bottom flask on a water bath for 5 hrs. The reaction mixture was then cooled and poured onto crushed ice. The separated crystals was then filtered and recrystallized from ethanol.

Yield:-74%

M.P:- 154°C

Elemental Analysis of $C_8H_7Cl_3N_2O_2$

	C%	H%	N%
Calculated	35.62	2.46	9.87
Found	36.79	2.83	9.44

Step3:- Synthesis of 2-(2',4',6'-trichlorophenoxymethyl)-5-mercapto-1,3,4-Oxadiazole

In a two neck 500ml round bottom flask equipped with a dropping funnel and reflux condenser, a solution of 2,4,6-trichlorophenoxyacetyl hydrazide (2.7g; 0.01 mole) in absolute alcohol and potassium hydroxide (0.5g) in water was refluxed on a water bath for 1 hr. The solution of carbon disulfide (1.8ml; 0.03mol) in absolute alcohol was added drop wise. The reaction mixture was refluxed till evolution of H_2S gas. 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.

Yield:-70%

M.P:- 168°C

Elemental Analysis of $C_9H_5Cl_3N_2O_2S$

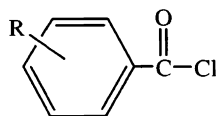
	C%	H%	N%
Calculated	34.67	1.60	8.98
Found	35.81	1.75	8.89

Step4:- Synthesis of substituted benzoyl chlorides

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

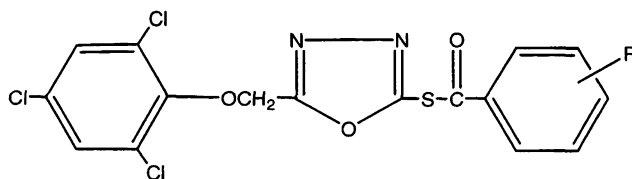
Similarly, other substituted benzoyl chlorides were synthesized and reported along with their melting points and other important data in Table-1

Table:-1:- Substituted Benzoyl Chlorides



Sr. No	Substituent (benzoyl-chloride)	Observed B.P.	Reported B.P.	Yield (%)	References
1	H	198	197	62	207
2	2-Fluoro	73	66-67	68	208
3	3-Chloro	224	222	73	209
4	4-Chloro	113	111	79	209
5	2,6-Difluoro	68	68-69	61	210

Table 2:- 5- [2', 4', 6'-Trichlorophenoxy methyl]-2 -(substituted Thiocarbamoyl)- 1, 3, 4 – Oxadiazole



Sr no.	R (benzoyl chloride)	Molecular formula	M.P (⁰ C)	Yield (%)	Elemental Analysis					
					Calculated			Found		
					C	H	N	C	H	N
1	H	C ₂₁ H ₁₂ Cl ₅ N ₃ O	152	69	46.26	2.16	6.73	46.55	2.34	6.91
2	3-Chloro	C ₂₁ H ₁₂ Cl ₄ FN ₃ O	171	45	42.66	1.77	6.22	42.83	1.41	6.55
3	4-Chloro	C ₂₁ H ₁₁ Cl ₆ N ₃ O	180	51	42.66	1.77	6.22	42.21	1.35	6.59
4	4-Fluro	C ₂₃ H ₁₇ Cl ₄ N ₃ O ₃	195	58	44.29	1.84	6.45	44.48	1.99	6.55
5	2,5-Dimethyl	C ₂₃ H ₁₇ Cl ₄ N ₃ O ₂	163	48	48.70	2.93	6.31	48.53	2.73	6.73
6	3-Methoxy	C ₂₂ H ₁₅ Cl ₄ N ₃ O ₂	202-205	62	45.79	2.46	6.28	45.92	2.88	6.56

SPECTRAL ANALYSIS

Spectral Studies of 5- [2', 4', 6'-Trichlorophenoxy methyl]-2 -(4-Chloro phenyl-Thiocarbamoyl)-1, 3, 4 – Oxadiazole [Table No.2; Compound No. 3]

PMR (DMSO-d₆) δ : -

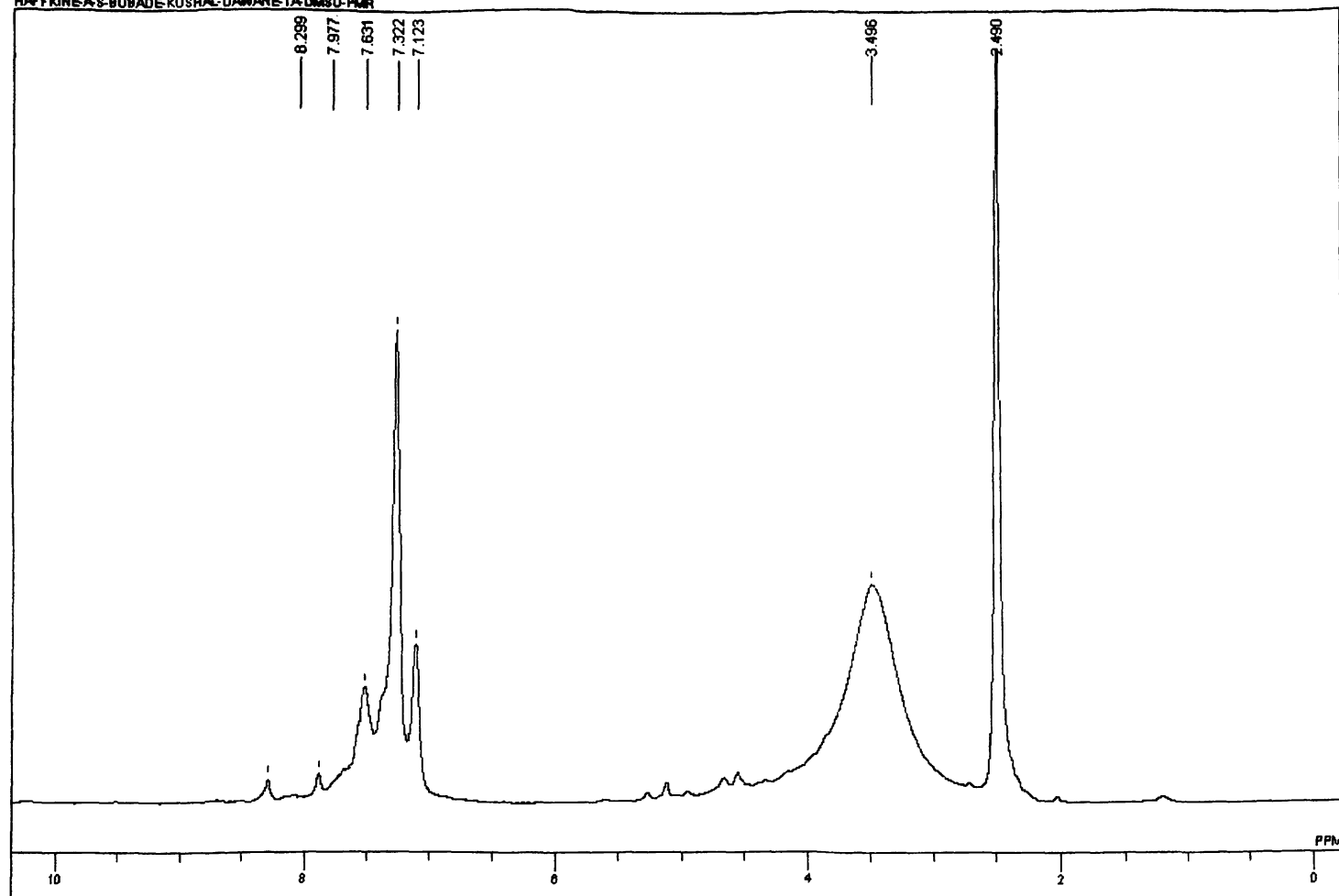
3.496 : [s, 2H (-OCH₂)]
7.123-8.299 : [m, 6H (Ar-H)]

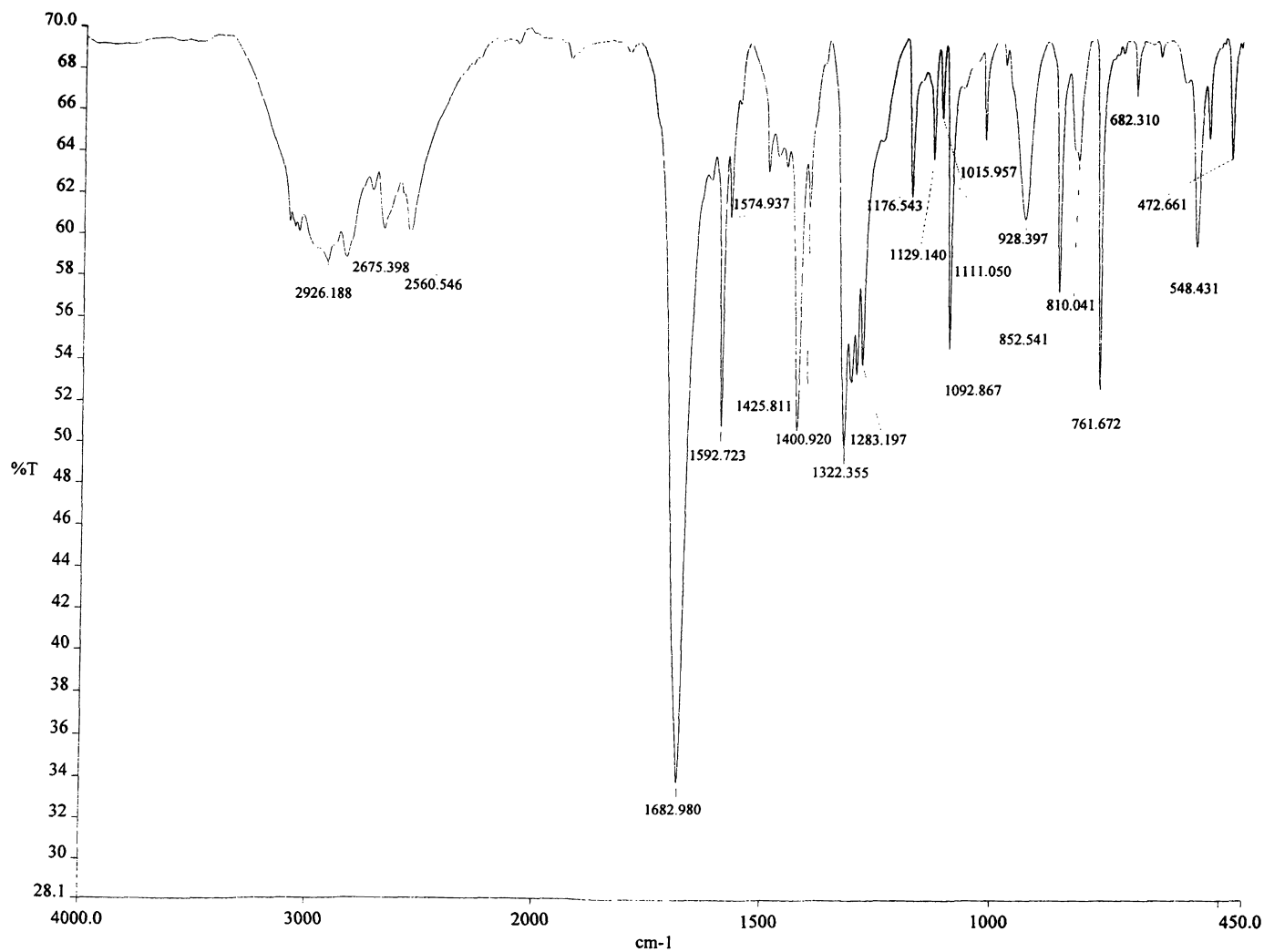
IR (KBR)

Wavenumber

1) 2500-3100 cm⁻¹ : Ar-H str
2) 1322 cm⁻¹ -C-O-C-
3) 1682 cm⁻¹ C=C str.
4) 761 cm⁻¹ (C-Cl)
5) 850-930 cm⁻¹ tetra sub. benzene

D:\14.12.09\HAFFKINE-A-S-80\BADE KUSHAL-DAWANE-1A\DMSO-PMR.xls
HAFFKINE-A-S-80\BADE KUSHAL-DAWANE-1A\DMSO-PMR





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Part II: - Synthesis of 3 –[2', 4', 6'- Trichlorophenoxy methyl]–4–(substituted-aniline) – 5 – aryl – 1, 2, 4 – Triazole.

IUPAC Name:- Synthesis of 3,4 –Bis-(substituted phenyl)-5-(2,4,6-Trichlorophenoxymethyl)-4H-[1,2,4]triazole.

2,4,6-trichlorophenoxyacetyl hydrazide was the starting material which was synthesized as mentioned earlier in Part I (Step 1 & 2).

Step3:- Synthesis of 2-(2',4',6'-trichlorophenoxymethyl)-5-aryl-1,3,4-Oxadiazole

The 2,4,6-trichlorophenoxyacetyl hydrazide (2.69g; 0.01mol) was placed in a 50ml round bottom flask equipped with a reflux condenser on a water bath. The solution of an p-Chloro benzoic acid (1.57g; 0.01 mol) in POCl₃ (5ml) was then added with constant stirring and the resultant mixture was refluxed for 5-6 hrs. The contents was then cooled and poured onto crushed ice and neutralized with NaHCO₃. The resultant solid was washed with water and crystallized with ethanol.

Yield:-55%

M.P:- 130°-132°C

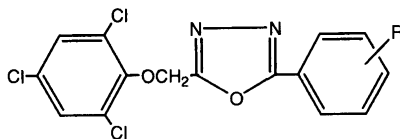
The Elemental Analysis for the above is given below,

Elemental Analysis of C₁₅H₈N₂C₁₄O₂

	C%	H%	N%
Calculated	46.15	2.05	7.17
Found	46.47	2.45	7.41

Similarly, other methoxy derivative was prepared by condensing 3-methoxy benzoic acid with 2,4,6-trichlorophenoxyacetyl hydrazide during the course of this work and the details have been mentioned in Table-3.

Table 3 :- 2-(2',4',6'-trichlorophenoxy methyl)-5-aryl-1,3,4-Oxadiazole



Sr. No.	R (Benzoic acid)	Yield (%)	M.P. (°C)
1	4-Chloro	55	130-132
2	3-Methoxy	63	175

**Step 4:- Synthesis of 3-[2', 4', 6'- Trichlorophenoxy methyl]-4-(substituted aniline)
- 5 - aryl - 1, 2, 4 - Triazole.**

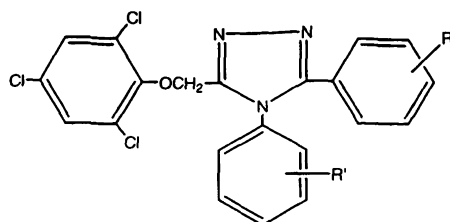
In a 50ml round bottom flask was taken a mixture of 2-(2',4',6'-trichlorophenoxy methyl)-5-(4-chloro phenyl)-1,3,4-Oxadiazole (0.78g; 0.002mol) and P-Chloro aniline (0.260g; 0.002mol) in a dry pyridine was refluxed on a water bath for 4-5hrs. The resultant mixture was cooled and poured on to crushed ice. The resultant solid was washed with cold water, dried and crystallized with ethanol.

Yield:- 58%

M.P:- 148 °C

Similarly, other derivatives were synthesized and reported along with their melting points and other important data in Table-4.

Table 4:- 3 - [2', 4', 6'- Trichlorophenoxy methyl]-4-(substituted aniline) - 5 - aryl - 1, 2, 4 - Triazole



Sr no.	R (Benzoic acid)	R' (Aniline)	Molecular formula	M.P (^o C)	Yield (%)	Elemental Analysis					
						Calculated			Found		
						C	H	N	C	H	N
1	4-Chloro	4-Chloro	C ₂₁ H ₁₂ Cl ₅ N ₃ O	148	58	50.45	2.40	10.51	50.64	2.68	10.86
2	4-Chloro	4-Fluro	C ₂₁ H ₁₂ Cl ₄ FN ₃ O	158	55	52.17	2.84	8.69	52.32	2.69	8.31
3	4-Chloro	2,4-dichloro	C ₂₁ H ₁₁ Cl ₆ N ₃ O	167	45	47.19	2.05	9.67	47.45	2.37	9.79
4	4-Chloro	2,5-dimethoxy	C ₂₃ H ₁₇ Cl ₄ N ₃ O ₃	182	52	52.57	3.23	8.00	52.75	3.39	8.48
5	4-Chloro	4-Ethoxy	C ₂₃ H ₁₇ Cl ₄ N ₃ O ₂	174	38	54.22	3.33	8.25	55.57	3.66	8.49
6	3-Methoxy	4-Chloro	C ₂₂ H ₁₅ Cl ₄ N ₃ O ₂	163	60	53.33	3.03	8.48	54.51	3.38	8.25
7	3-Methoxy	4-Fluro	C ₂₂ H ₁₅ Cl ₃ FN ₃ O ₂	155	59	55.17	3.13	8.77	55.69	3.49	8.93
8	3-Methoxy	2,4-dichloro	C ₂₂ H ₁₄ Cl ₆ N ₃ O ₂	185	57	46.72	2.47	7.43	46.87	2.78	7.88
9	3-Methoxy	2,5-dimethoxy	C ₂₄ H ₂₀ Cl ₃ N ₃ O ₄	207	48	55.33	3.84	8.06	56.11	3.98	8.46
10	3-Methoxy	4-Ethoxy	C ₂₃ H ₂₀ Cl ₃ N ₃ O ₃	192	43	56.04	4.06	8.52	56.21	4.70	8.95

SPECTRAL ANALYSIS

Spectral Studies of 3 – [2', 4', 6'- Trichlorophenoxy methyl]–4–(4-Chloro aniline)–
5– (4-chloro phenyl) – 1, 2, 4 – Triazole

PMR (CDCl₃) δ : -

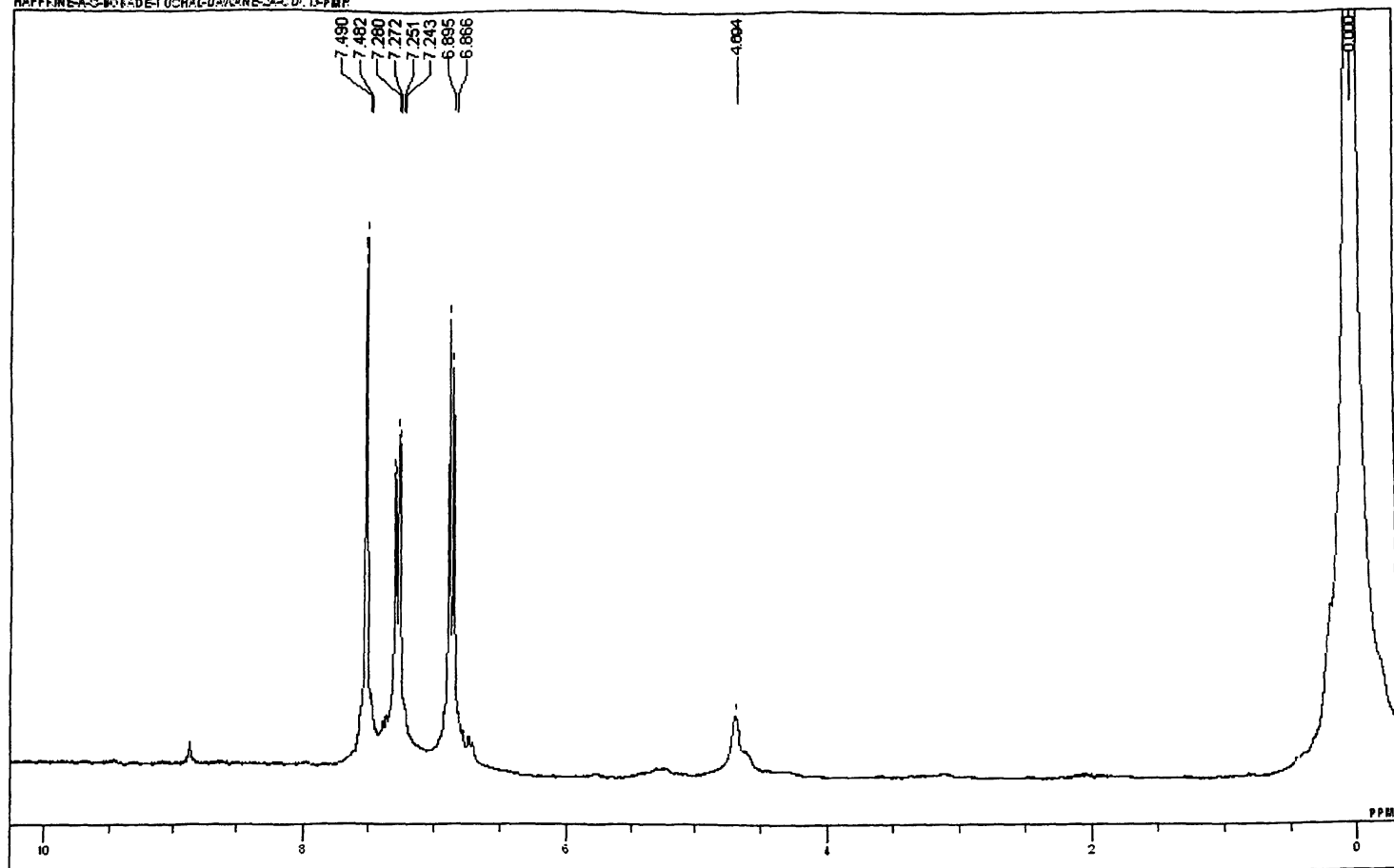
4.694 : [s, 2H (-OCH₂)]
6.866-7.490 : [m, 10H (Ar-H)]

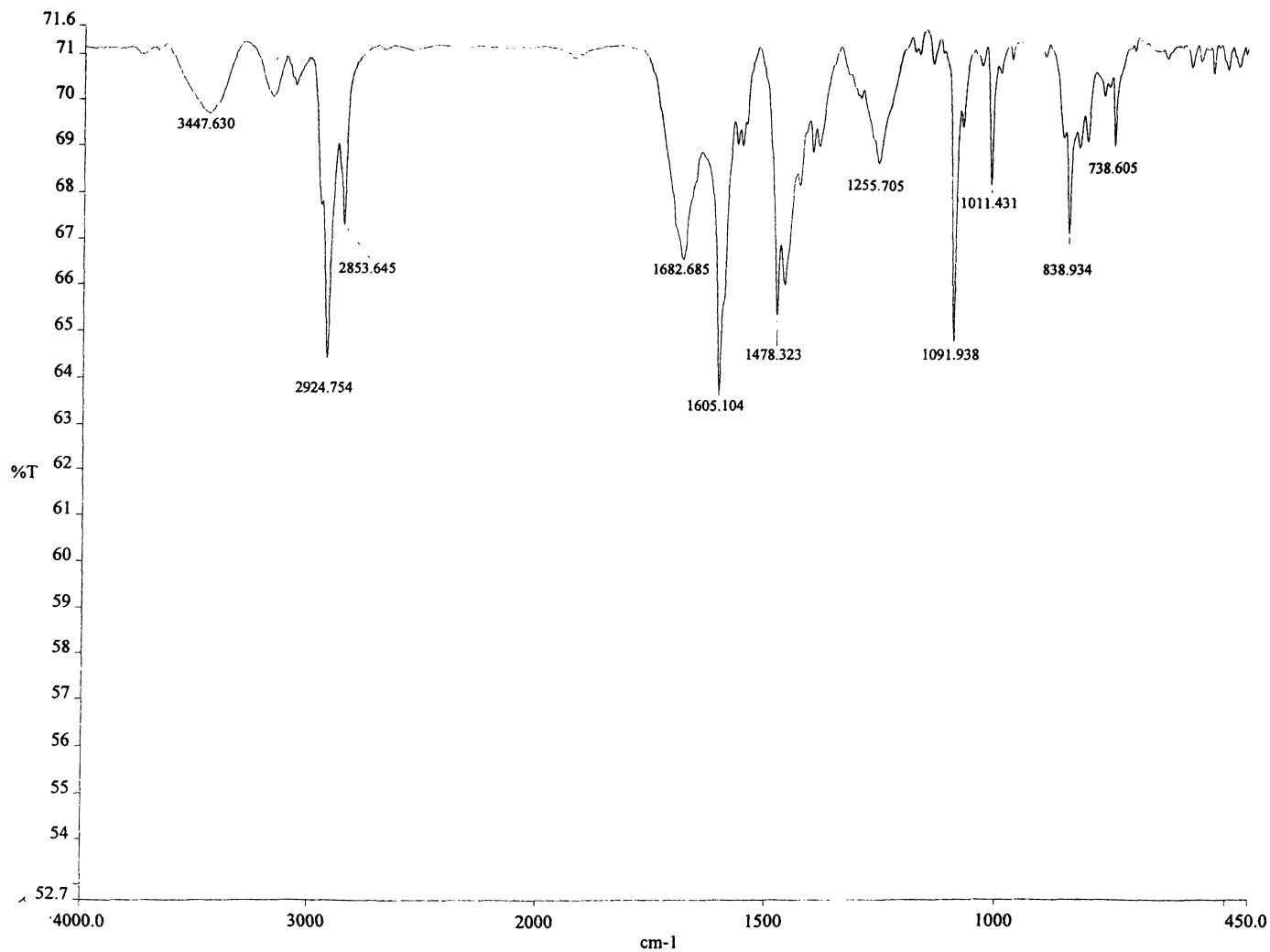
IR (KBR)

Wavenumber

1) 2853-1478 cm⁻¹ : Ar-H str
2) 1255-1478 cm⁻¹ –C–O–C–
3) 1605 cm⁻¹ C=N str.
4) 738 cm⁻¹ (C-Cl)
5) 1478 cm⁻¹ : (CH def.CH₂)

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HAFFINE-A-S-BOBADE-I-UCHAL-DAVANE-CA-CD-13-PMR





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Part IV:- Synthesis of 3-[2',4',6'- Trichlorophenoxy methyl]- 5-(substituted-Thiocarbonyl)-4-(substituted-N-benzylidenyl amino)-1,2,4-Triazole.

IUPAC Name:- Synthesis of 4-substituted-thiobenzoic acid 4-[[1-(substituted phenyl)-meth-(E)-ylidene]amino]-5-(2,4,6-Trichlorophenoxy methyl)-4H-[1,2,4]-triazol-3-yl.

Synthesis of 3-(2',4',6'-Trichlorophenoxy)methyl)-4-amino-5-mercapto-1,2,4-triazole

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

1. The reaction of 2-(2',4',6'-trichlorophenoxy)methyl)-5-mercapto-1,3,4-Oxadiazole (Scheme I) with Hydrazine hydrate in dry pyridine which was refluxed for 2 hrs.
2. The second method involved the formation of dithiocarbamate salt of 2,4,6-trichlorophenoxy acetyl hydrazide 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 dithiocarbamate salt was more stable, more easier & less time consuming than 2-(2',4',6'-trichlorophenoxy)methyl)-5-mercapto-1,3,4-Oxadiazole. 2,4,6-trichlorophenoxy-acetyl hydrazide was the starting material which was synthesized as mentioned earlier in Part I (Step 1 & 2).

3-(2',4',6'-Trichlorophenoxy)methyl)-4-amino-5-mercapto-1,2,4-triazole was synthesized by cyclization of dithiocarbamate salt of 2,4,6-trichlorophenoxy acetyl hydrazide. The dithiocarbamate salt of 2,4,6-trichlorophenoxy acetyl hydrazide was in turned prepared by the reaction of substituted aryl hydrazide with carbon disulfide and potassium hydroxide as detailed in procedure below,

Step3:- Synthesis of potassium salt of 2,4,6-trichlorophenoxyacetyl hydrazide²¹².

In a 500ml conical flask, KOH (0.84g; 0.015mol) was placed in 200ml absolute alcohol. The solution was stirred on a magnetic stirrer at a room temperature. To the stirred solution was added (2.69g; 0.01mol) 2,4,6-trichlorophenoxyacetyl 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; 0.15mol). 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 absolute alcohol was further added & the mixture was further stirred for next 4-5 hrs. It was then diluted with 200ml ether 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.

Yield:-80%

Step4:- Synthesis of 3-(2',4',6'-Trichlorophenoxyethyl)-4-amino-5-mercapto-1,2,4-triazole.

A mixture of potassium salt of 2,4,6-trichlorophenoxyacetyl hydrazide (2.03g; 0.05mol), 80% Hydrazine hydrate (4.9ml; 0.1mol) and water 10ml 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 ethanol²¹³.

Yield :-72%

M.P:- 107°-108°C

Elemental Analysis of C₉H₇Cl₃N₄OS

	C%	H%	N%
Calculated	33.17	2.15	17.20
Found	33.95	2.78	17.55

Step5:- Synthesis of 3-(2',4',6'-Trichlorophenoxy)methyl)-4-(N-benzylidiny amino)-5-mercapto-1,2,4-triazole

In a 100ml round bottom flask, a solution of 3-(2',4',6'-Trichlorophenoxy-methyl)-4-amino-5-mercapto-1,2,4-triazole(3.25 g; 0.01 moles) in a glacial acetic acid (20ml) and p-Chloro benzaldehyde (1.41g; 0.01 mol) 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.

Yield:- 55%

M.P:- 149°C

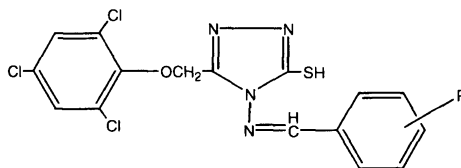
The Elemental Analysis for the above is given below,

Elemental Analysis of $C_{16}H_{10}N_4Cl_4OS$

	C%	H%	N%
Calculated	42.85	2.23	12.50
Found	43.05	2.59	12.85

Similarly other substituted triazole derivatives were prepared by condensing different substituted benzaldehydes and reported along with their melting points and yield in Table No. 5

Table No. 5 :- 3-(2',4',6'-Trichlorophenoxy)methyl)-4-(N-benzylidiny amino)-5-mercapto -1,2,4-triazole



Sr. No.	R (benzaldehyde)	Yield (%)	M.P.(°C)
1.	H	55	149
2.	4-Chloro	58	142
3.	4-Methoxy	59	172

Step6:- Synthesis of 3-[2',4',6'- Trichlorophenoxy methyl]- 5-(substituted-Thiocarbonyl)-4-(substituted-N-benzylidenyl amino)-1,2,4-Triazole.

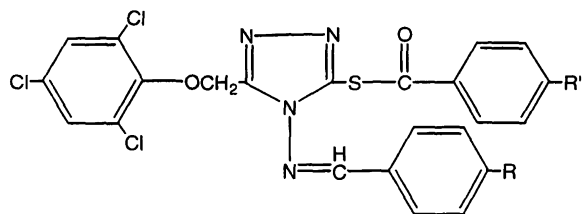
In 100 ml three necked round bottom flask, with dropping funnel fitted with the guard tube and a double walled reflux condenser also with a guard tube, was placed a solution of compound 3-(2',4',6'-Trichlorophenoxymethyl)-4-(N-benzylidenyl amino)-5-mercapto-1,2,4-triazole (3.11g; 0.01moles) in dry pyridine, on an oil bath. To this add dropwise a solution of p-Chloro benzoyl chloride (1.2ml; 0.001 mol) in 10 ml dry pyridine. The solution was poured into crushed ice. The solid separates out was filtered, washed with water and dried. It was then recrystallized from methanol²¹¹.

Yield:- 51%

M.P:- 220 °C

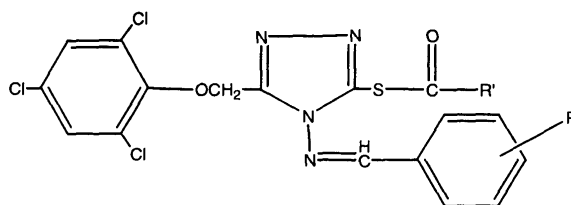
Similarly other substituted Triazole derivatives were prepared and reported along with their melting points and analytical data in Table No.6

Table 6:- 3-[2',4',6'- Trichlorophenoxy methyl]- 5-(substituted Thiocarbonyl)-4-(substituted-N-benzylidenyl-amino)-1,2,4-Triazole



Sr no.	R (benzaldehyde)	R' (Benzoyl Chloride)	Molecular formula	M.P (^o C)	Yield (%)	Elemental Analysis					
						Calculated			Found		
						C	H	N	C	H	N
1.	H	4-Chloro	C ₂₃ H ₁₄ N ₄ Cl ₄ O ₂ S	190	45	50.00	2.53	10.14	50.35	2.69	10.56
2.	H	4-fluro	C ₂₃ H ₁₄ N ₄ Cl ₃ FO ₂ S	175	49	51.54	2.61	10.54	51.95	2.79	10.93
3.	H	2,5-dimethoxy	C ₂₅ H ₁₉ N ₄ Cl ₃ O ₂ S	205	53	54.99	3.48	10.26	54.50	3.96	10.69
4.	H	3-Methoxy	C ₂₄ H ₁₇ N ₄ Cl ₃ O ₃ S	165	59	52.60	3.10	10.22	52.98	3.56	10.58
5.	4-Chloro	4-Chloro	C ₂₃ H ₁₃ N ₄ Cl ₅ O ₂ S	220	51	47.50	2.21	9.54	47.88	2.48	9.81
6.	4-Chloro	4-Fluro	C ₂₃ H ₁₃ N ₄ Cl ₄ FO ₂ S	238	48	48.42	2.28	9.82	48.73	2.41	9.98

Table 6:- 3-[2',4',6'- Trichlorophenoxy methyl]- 5-(substituted Thiocarbonyl)-4-(substituted-N-benzylidenyl-amino)-1,2,4-Triazole



7.	4-Chloro	2,5-dimethoxy	$C_{25}H_{18}N_4Cl_4O_2S$	253	43	51.72	3.10	9.65	51.96	3.39	9.86
8.	4-Chloro	3-Methoxy	$C_{24}H_{16}N_4Cl_3O_3S$	210	51	50.88	2.82	9.89	51.05	2.99	9.98
9.	4-Ethoxy	4-Chloro	$C_{24}H_{16}N_4Cl_4O_3S$	172	40	49.48	2.74	9.62	49.81	2.93	9.95
10.	4-Ethoxy	4-fluro	$C_{24}H_{16}N_4Cl_3FO_3S$	185	56	50.92	2.82	9.90	51.08	2.96	10.08
11.	4-Ethoxy	2,5-dimethoxy	$C_{25}H_{21}N_4Cl_3O_2S$	168	58	55.76	3.75	10.00	55.93	3.89	10.31
12.	4-Ethoxy	3-Methoxy	$C_{25}H_{19}N_4Cl_3O_4S$	218	47	51.94	3.29	9.69	52.23	3.56	9.85

SPECTRAL ANALYSIS

Spectral Studies of 3-[2',4',6'-Trichlorophenoxy methyl]-5-(4-Chloro phenyl

Thiocarbamoyl)-4-(4-Chloro-N-benzylidenylamino)-1,2,4-Triazole

[Table No. 5; Compound No. 6]

PMR (DMSO-d₆) δ: -

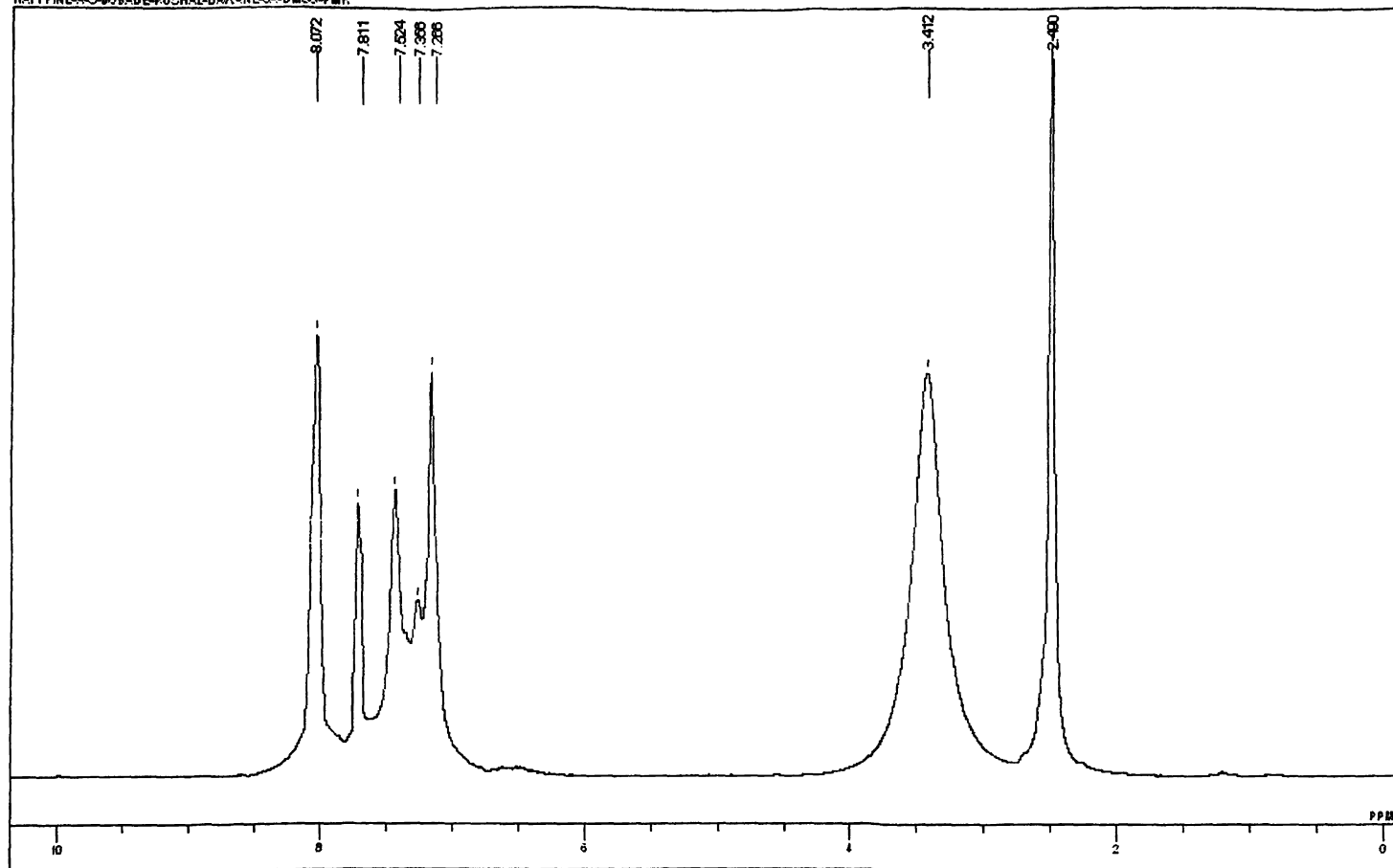
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7.266-8.072	[m, 11H (10H Ar-H), 1H (N=CH)]

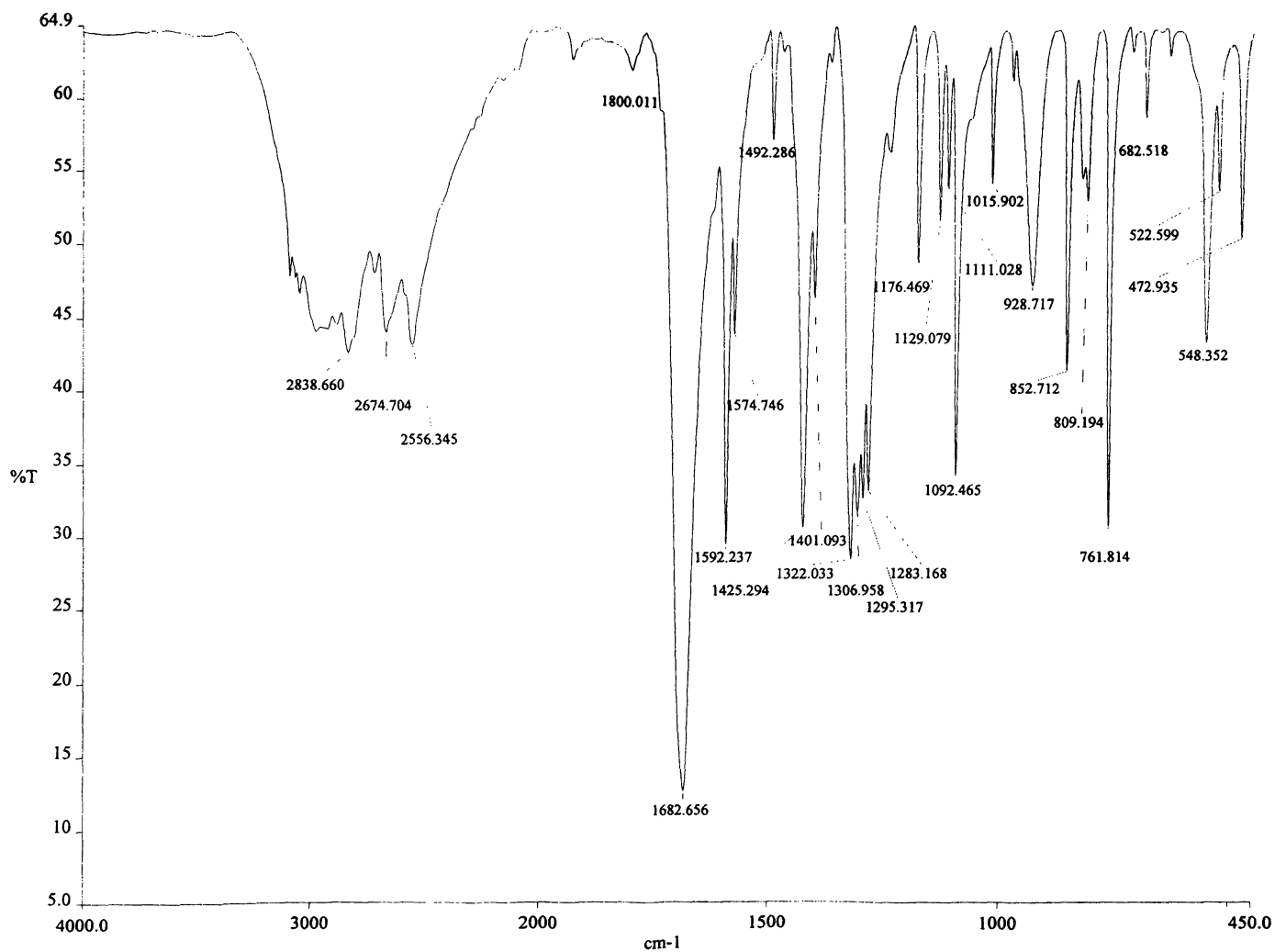
IR (KBR)

Wavenumber

1) 2556-2838 cm ⁻¹	Ar-H str
2) 1574-1592 cm ⁻¹	C=N
3) 1682 cm ⁻¹	: C=O
4) 761 cm ⁻¹	(C-Cl)
5) 852-928 cm ⁻¹	tetra substituted benzene

014.12.09HAFFINE-A-O-BOBADE-LUCHAL-DAYANE-3A-DMSO-PMR 26
HAFFINE-A-O-BOBADE-LUCHAL-DAYANE-3A-DMSO-PMR





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Part V:- Synthesis of 3-(6-Chloro-benzathiazol-2-ylamino)-2-(substituted phenyl)-thiazolidin-4-one.

Step 1:- Synthesis of 6-Chloro- 2-amino benzothiazole

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

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

Yield:- 68%

M.P.:- 197°C

Lit. M.P¹⁸¹:- 198°C

Elemental Analysis of C₇H₅ClN₂S

	C%	H%	N%
Calculated	45.52	2.71	15.17
Found	45.81	2.95	15.37

Step 2:- Synthesis of 6-Chloro-2-hydrazino benzothiazole

In a 100ml flat bottom flask, conc. HCl (4 ml) was added dropwise with stirring to Hydrazine hydrate (4 ml) at 5–10°C. To this, a mixture of Ethylene glycol (16 ml) and 6-Chloro-2-amino benzothiazole was added & refluxed for 3 hours on a water bath. On cooling, solid separates out, which was filtered, washed with water and recrystallized with ethanol.

M.P.: - 216°C

Yield: - 75%

Lit. M.P.²¹⁴: - 216°C

Elemental Analysis of C₇H₆ClN₃S

	C%	H%	N%
Calculated	42.10	3.00	21.05
Found	42.35	3.25	21.22

Step 3:- Synthesis of 6-Chloro benzothiazol-2-yl hydrazones

In a round bottom flask of 100 ml, 6-Chloro-2-hydrazino benzothiazole (1.99 g; 0.01 mol) and p-chloro benzaldehyde (1.085 g; 0.01 mol) were dissolved in 50 ml of abs. ethanol. To this a few drops of gl. Acetic acid was added. The reaction mixture was refluxed on a water bath for 5-6 hrs. It was cooled and poured over crushed ice; the solid thus obtained was filtered, washed with water and recrystallized from ethanol.

Yield:- 69%

M.P:- 273 °C

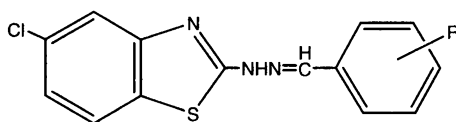
The Elemental Analysis for the above is given below,

Elemental Analysis of C₁₄H₉Cl₂N₃S

	C%	H%	N%
Calculated	60.86	2.79	13.04
Found	60.58	2.91	13.32

Similarly, other 6-Chloro benzothiazol-2-yl hydrazones were prepared by condensing various substituted aromatic benzaldehyde with 6-Chloro-2-hydrazino benzothiazole during the course of this work and the details have been mentioned in Table 7.

Table 7:- 6-Chloro benzothiazol-2-yl hydrazones



Sr. No.	R (benzaldehyde)	Yield (%)	M.P.(°C)
1.	H	72	232
2.	4-chloro	69	273
3.	4-carboxy	60	300-303
4.	4-hydroxy	58	258
5.	4-fluro	67	282
6.	2-nitro	71	265
7.	2,3-dihydroxy	55	275
8.	2,6-dichloro	63	260
9.	2,4-dimethoxy	52	248
10.	3,4,5-trimethoxy	56	240

Step 4:- Synthesis of 3-(6-Chloro benzothiazol-2-ylamino)-2-(substituted-phenyl)-thiazolidin-4-one.

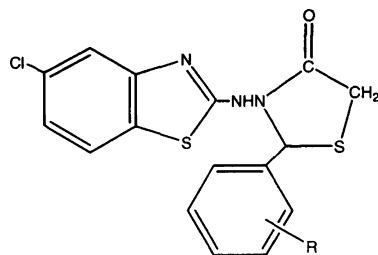
In a round bottom flask of 100 ml, 6-Chloro benzothiazol-2-yl-4-chloro-hydrazones (2.93g; 0.01mol) and Thioglycollic acid (0.6ml; 0.01 mol) were dissolved in DMF (15ml). To this a pinch of anhyd. ZnCl_2 was added. The reaction mixture was refluxed on a water bath for 15–18 hrs. It was cooled and poured over crushed ice, the solid thus obtained was filtered, washed with water and recrystallized from ethanol.

Yield:- 59%

M.P:- 245 °C

Similarly other substituted 4-Thiazollidinones derivatives were prepared and reported along with their melting points and analytical data in Table No. 8.

Table 8:- 3-(6-Chloro-benzathiazol-2-ylamino)-2-(substituted-phenyl)-thiazolidin-4-one.



Sr no.	R	Molecular formula	M.P (°C)	Yield (%)	Elemental Analysis (%)					
					Calculated			Found		
					C	H	N	C	H	N
1.	H	C ₁₆ H ₁₂ ClN ₃ OS ₂	172	61	53.11	3.31	11.61	53.23	3.58	11.87
2.	4-chloro	C ₁₆ H ₁₁ Cl ₂ N ₃ OS ₂	245	59	48.48	2.77	10.6	48.56	2.93	10.84
3.	4-carboxy	C ₁₇ H ₁₂ ClN ₃ O ₃ S ₂	260	48	50.31	2.95	10.35	50.65	2.73	10.61
4.	4-hydroxy	C ₁₆ H ₁₂ ClN ₃ O ₂ S ₂	204	45	50.86	3.71	11.12	50.23	3.98	11.25
5.	4-fluro	C ₁₆ H ₁₁ ClFN ₃ OS ₂	228	57	50.59	2.89	11.06	50.76	2.53	11.46
6.	2-nitro	C ₁₆ H ₁₁ ClN ₄ O ₃ S ₂	212	56	47.23	2.70	13.77	47.57	2.81	13.52
7.	2,3-dihydroxy	C ₁₆ H ₁₂ ClN ₃ O ₃ S ₂	218	43	48.79	3.04	10.67	48.99	3.39	10.23
8.	2,6-dichloro	C ₁₆ H ₁₀ Cl ₃ N ₃ OS ₂	230	51	44.59	2.32	9.75	44.91	2.76	9.93
9.	2,4-dimethoxy	C ₁₈ H ₁₆ ClN ₃ O ₃ S ₂	196	59	56.91	4.21	11.06	56.65	4.63	11.46
10.	3,4,5-trimethoxy	C ₁₉ H ₁₈ ClN ₃ O ₄ S ₂	188	50	50.49	3.98	9.30	50.78	3.69	9.41

SPECTRAL ANALYSIS

Spectral Studies of 3-(6-Chloro-benzathiazol-2-ylamino)-2-(4-Chlorophenyl)-thiazolidin-4-one.

[Table No. 8; Compound No. 2]

PMR (DMSO-d₆) δ : -

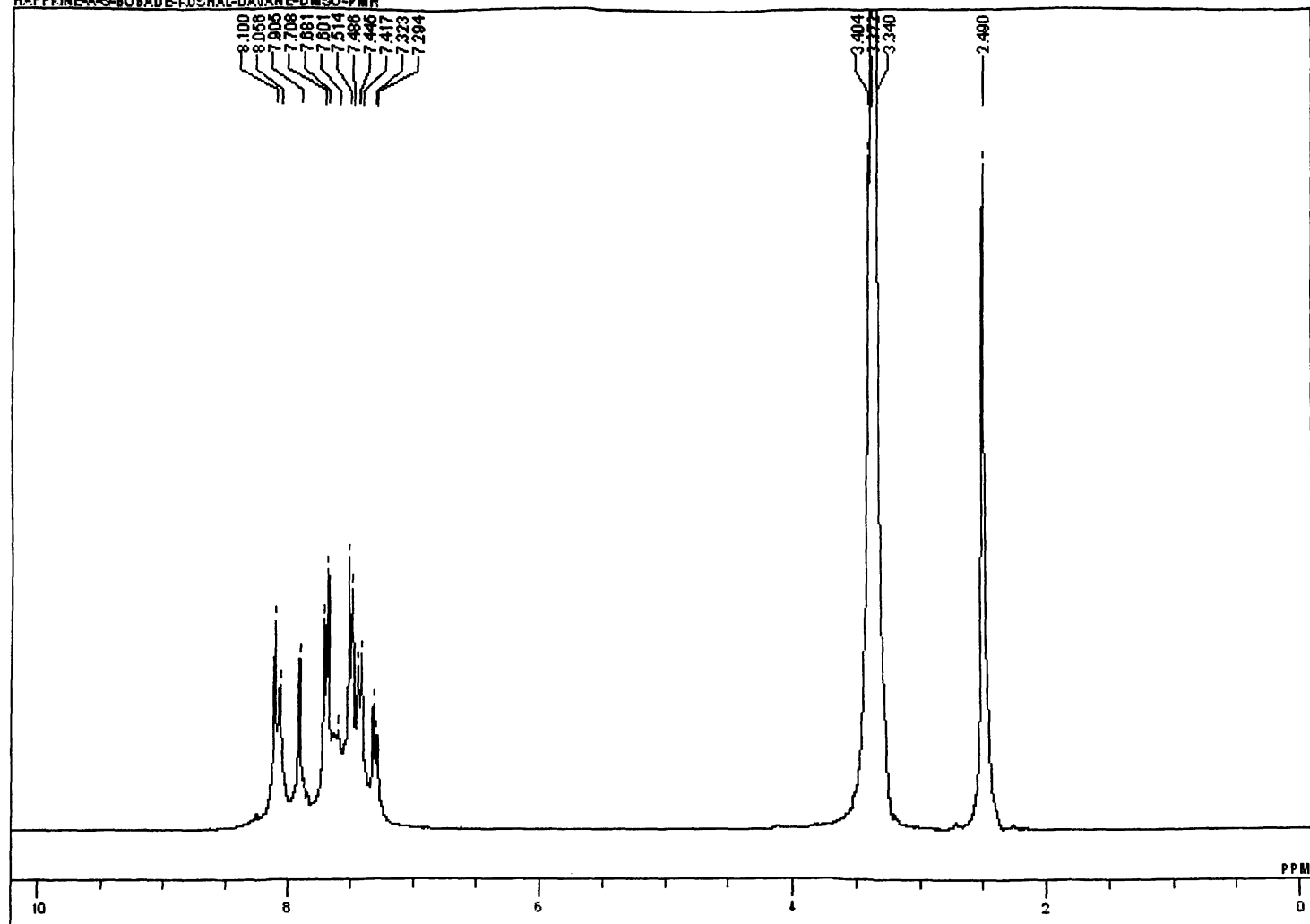
7.294-8.100		[m, 8H; 7H(Ar-H), 1H NH]
3.404	:	[s, 1H, HC-S]
3.340	:	[s, 2H (-SCH ₂)]

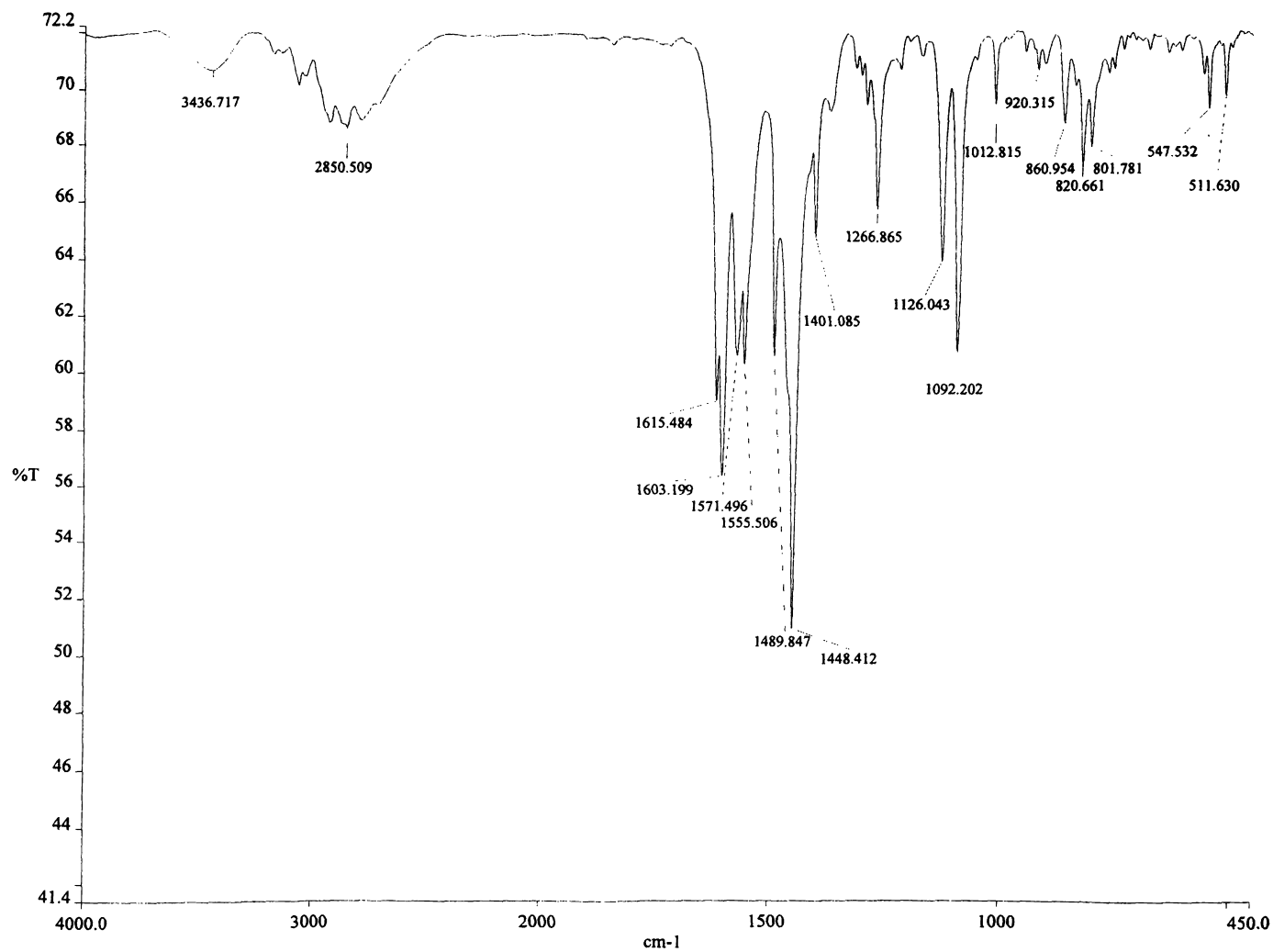
IR (KBR)

Wavenumber

1) 2800-2900 cm ⁻¹		Ar-H str
2) 1555-1571 cm ⁻¹	:	C=C str.
3) 1630-1615 cm ⁻¹		C=O
4) 820 cm ⁻¹		(C-Cl)
5) 3436 cm ⁻¹		N-H
6) 1126 cm ⁻¹		C=N

D:\7.1.09\HAFFKINE-A-G-BOSADE-KUSHAL-DAVANE-141-DM50-PMR.2k
HAFFKINE-A-G-BOSADE-KUSHAL-DAVANE-DM50-PMR





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Biological Screening

Biological Screening

All the synthesized compounds in the present work were subjected to antibacterial and antifungal screening.

The various screenings carried out include the in vitro study against Gram-positive *Staphylococcus aureus* ATCC 3750 and Gram-negative *Salmonella typhi* NCTC 786 and in vitro study of activity against fungal strain such as *Candida albicans* & *Aspergillus Niger* was carried out.

Drug Stock Solution

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

Controls:

Media Control

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

Culture Control

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

Solvent Control

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

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

Turbidity Control

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

Standard Control

It contains the medium, culture organism and dilution of similar order of standard drug corresponding to that of the drug under test. The inhibition of growth was observed against the 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 technique²¹⁶.

Muller Hinton broth was used as the culture medium. Sterilized medium was dispensed in each borosilicate glass test tube (150 +20 mm). The drug solution was added in order to attain final drug concentrations of 200, 100, 50, 20 and 10 µg/ml.

Innoculum of standard suspension (0.1 ml of test organism strain which contains 10⁶ bacilli/ml) was added. The tubes were incubated at 37⁰C for 48 hours and then examined for the presence or absence of growth of the organism. The lowest concentration which showed no visible growth was taken as an end point and is known as the minimum inhibitory concentration (MIC).

Ampicillin (MIC = 0.01 µg/ml against Gram-positive *S. aureus*) and Trimethoprim (MIC = 1 µg/ml against Gram-negative *S. typhi* were used as the standard drugs).

Antifungal Screening:

The organisms employed for the test were *Candida albicans*. This organism was maintained on Sabouraud's agar slants by regular sub culturing. *C. albicans* was cultured every week.

Test Medium:

The sterile medium was dispensed in each borosilicate test tube. The drug solution was added in order to get the final concentrations of 200, 100, 50, 20, and 10 µg/ml.

Preparation of the Inoculum:***Candida albicans***

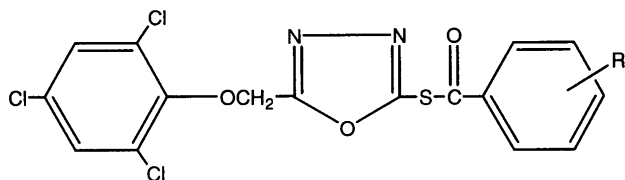
A 48 hours old growth on Sabouraud's agar slants was suspended in 4-5 ml of the test medium with the help of a platinum loop. The cell count of the suspension was carried out using Neubauer Chamber and then diluted with requisite volume of the medium to give a final concentration of 5×10^6 cells per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

Screening procedure

To each test tube was added 0.1 ml of the standardized inoculum of the test organism. The tubes were then incubated at 36°C. The presence or absence of growth was noted visually after days in case of *Candida albicans*.

Miconazole was used as the standard drug for control, which has a MIC of 6.25 µg/ml against *Candida albicans*.

Table No. 1:- Antimicrobial activity of Substituted [5 – {2', 4', 6'-Trichlorophenoxy methyl}-2-(substituted Thiocarbamoyl) – 1, 3, 4 – Oxadiazole].



Comp.	Substituents R	Anti Microbial Activity (μ/ml) [MIC]			
		Anti bacterial		Anti Fungal	
		S. aureus	S. typhi	C. albicans	A. Niger
1.	H	200	100	100	NA
2.	3-Chloro	200	NA	100	200
3.	4-Chloro	100	200	200	200
4.	4-Fluro	100	100	200	100
5.	2,5-Dimethyl	50	100	50	100
6.	3-Methoxy	40	50	100	100

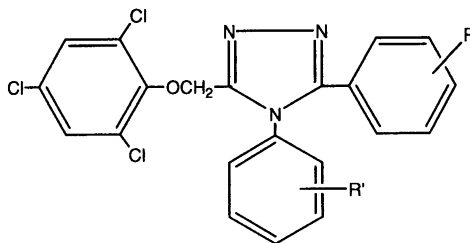
Ampicillin (MIC-0.04 μg/ml) used as standard against *S. aureus*.

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.

**Table No. 2:- Antimicrobial activity of Substituted [3 – [2', 4', and 6'-Trichloro-
phenoxy- methyl]-4-(substituted aniline) – 5 – aryl – 1, 2, 4 – Triazole].**



Comp.	Substituents		Anti-microbial Activity($\mu\text{g/ml}$) [MIC]			
			Anti bacterial		Anti Fungal	
	R (Benzoic acid)	R' (Aniline)	S. aureus	S. typhi	C. Albicans	A. Niger
1.	4-Chloro	4-Chloro	50	100	200	100
2.	4-Chloro	4-Fluro	100	40	50	100
3.	4-Chloro	2,4-Dichloro	100	100	40	100
4.	4-Chloro	4-Methoxy	200	200	100	200
5.	4-Chloro	4-Ethoxy	200	200	200	N.A
6.	3-Methoxy	4-Chloro	50	100	200	100
7.	3-Methoxy	4-Fluro	100	50	100	200
8.	3-Methoxy	2,4-Dichloro	50	200	50	100
9.	3-Methoxy	4-Methoxy	200	200	100	100
10.	3-Methoxy	4-Ethoxy	100	200	200	200

Ampicillin (MIC-0.04 $\mu\text{g/ml}$) used as standard against S. aureus.

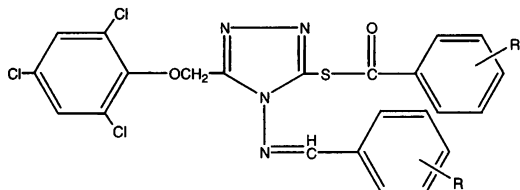
Trimethoprim (MIC 0.01 $\mu\text{g/ml}$) used as standard against S. typhi.

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 Substituted 3-[2',4',6'- Trichlorophenoxy methyl]-5-(substituted Thiocarbonyl)-4- (substituted-N-benzylidenyl amino)-1,2,4-Triazole].



Comp.	Substituents		Anti-microbial Activity($\mu\text{g/ml}$) [MIC]			
			Anti bacterial		Anti Fungal	
	R (Benzaldehyde)	R' (Benzoic acid)	S. aureus	S. Typhi	C. albicans	A. Niger
1.	H	4-Chloro	200	100	100	200
2.	H	4-Fluoro	100	50	100	100
3.	H	2,5-dimethyl	200	200	100	200
4.	H	3-methoxy	200	200	200	200
5.	4-Chloro	4-Chloro	100	100	100	50
6.	4-Chloro	4-Fluoro	100	50	100	100
7.	4-Chloro	2,5-dimethyl	200	100	200	100
8.	4-Chloro	3-Methoxy	200	200	200	200
9.	4-Ethoxy	4-Chloro	200	N.A.	200	200
10.	4-Ethoxy	4-Fluoro	200	200	100	100
11.	4-Ethoxy	2,5-dimethyl	200	200	100	N.A.
12.	4-Ethoxy	3-methoxy	N.A.	200	200	N.A.

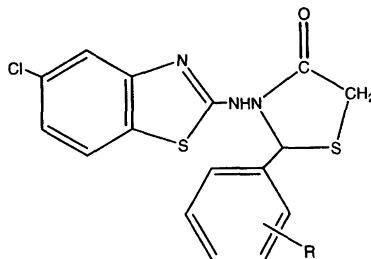
Ampicillin (MIC-0.04 $\mu\text{g/ml}$) used as standard against *S. aureus*.

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. 4:- Antimicrobial activity of 3-(6-Chloro-benzathiazol-2-ylamino)-2-(substituted phenyl)-thiazolidin-4-one.



Comp.	Substituents		Anti-microbial Activity ($\mu\text{g/ml}$) [MIC]			
			Anti bacterial		Anti Fungal	
	R	R'	S. aureus	S. Typhi	C. albicans	A. Niger
1.	H	4-chloro	100	200	100	200
2.	4-chloro	4-fluoro	50	100	200	200
3.	4-Carboxy	4-chloro	100	200	100	200
4.	4-hydroxy	3-methoxy	50	200	100	100
5.	4-fluro	4-chloro	100	200	100	100
6.	2-nitro	4-fluoro	200	200	50	100
7.	2,3-dihydroxy	2,5-dimethyl	100	100	50	100
8.	2,6-dichloro	3-methoxy	50	100	100	200
9.	2,4-dimethoxy	4-chloro	200	100	50	100
10.	3,4,5-trimethoxy	4-fluoro	50	100	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 $\mu\text{g/ml}$.

*Results
&
Discussion*

RESULT AND DISCUSSION

The present investigation was focused on the synthesis of novel organic compounds i.e derivatives of Oxadiazole, Triazole & Thiazolidinone as a possible therapeutic value. To evaluate their efficacy, they were screened for their in vitro antibacterial and antifungal activity.

In **Series I**, the synthesis of substituted **5 – {2', 4', 6'-Trichlorophenoxy - methyl}-2-(substituted Thiocarbamoyl)-1, 3, 4 – Oxadiazole** have been described. The following points are to be noted:

1. Presence of the electron donating substituents such as methyl, methoxy, attached to the phenyl ring showed remarkable anti bacterial & anti fungal activity.
2. Presence of the electron withdrawing substituents, attached to the phenyl ring showed moderate anti bacterial & anti fungal activity.
3. All the compounds of this series showed good to moderate potency against all four pathogenic strains as shown in the **Table 1**.

In **Series II**, the synthesis of **3 – {2', 4', 6'-Trichlorophenoxy methyl]-4-(substituted aniline)- 5 – aryl – 1, 2, 4 – Triazole** has been explained. Following observations have been made:

1. All the compounds of this series showed good to moderate potency against all four pathogenic strains shown in **Table 2**.
2. Dichloro substituted aniline substituents showed good to moderate potency against all three pathogenic strains.
3. Compound with Fluro substituents at para position gave a remarkable activity at 50 µg/ ml against S-typhi & at 40 µg/ ml against C-albicans.

In **Series III**, the Synthesis of **3 – [2', 4', 6'- Trichlorophenoxy methyl]– 4 – (substituted aniline) – 5 – aryl – 1, 2, 4 – Triazole** has been explained. Following observations have been made:

1. The compound with 4-Fluoro substituents has shown a remarkable activity than the other halogens substituents.
2. Electronegative groups like methoxy ,2,5-Dimethyl were found to be active at 200 µg/ml for anti-bacterial screening.
3. Presence of electron withdrawing group i.e. chloro has shown good to moderate activity against all the pathogenic strains.

In **Series IV**, the Synthesis of **3-(6-Chloro-benzathiazol-2-ylamino)-2-(substituted phenyl)-thiazolidin-4-one** has been explained. Following observations have been made:

1. All the compounds in this series showed moderate to good potency against the pathogenic strains.
2. Electronegative groups like hydroxy, methoxy, nitro showed a remarkable anti-bacterial activity at 50 µg/ml against *S. aureus* pathogenic strain.
3. Presence of electron withdrawing group showed moderate anti-bacterial and anti-fungal activity.

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

Discussion

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.

1. During workup for the synthesis of (2',4',6'-Trichlorophenoxy acetate) (**Scheme I, step 1**), K_2CO_3 was added in excess amount to maintain the alkaline condition which is responsible for the formation of potassium salt. Further addition of Ethyl chloro acetate was done in anhydrous condition which is necessary for the reaction. Small amount of moisture can alter the yield.
2. Triazole moiety can be synthesized by two methods (**Scheme III, step 3**), 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.
3. For the synthesis of 2-Hydrazino benzothiazole from 2-Amino-6-chloro-benzothiazole (**Scheme IV, step 2**), better yield was obtained when Ethylene glycol was used as a solvent in place of methanol or ethanol.
4. For the reaction of Acid chlorides (**Scheme I, step 5**), the reaction took place smoothly in presence of dry Pyridine as a solvent in place of dry 1,4-dioxane.
5. Mercapto acetic acid was found to be the best solvent for the cyclization of Schiff bases than 1,4-Dioxane or Dimethyl Formamide (**Scheme IV, step 4**).

Though it may be concluded that the present investigation is not successful in rendering any new organic compound more active than the existing drugs, in the class of infections for which these compounds were screened. It may be worth while to pursue further investigations with related compounds bearing in mind certain trends observed in the present investigation as a part of the efforts to discover novel and potent drugs.

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APPENDIX

1. The melting points have been determined in open capillaries on a Thermo-nik 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.

<u>TITLE OF THE THESIS</u>	: Synthesis and study of some substituted Oxadiazoles, Triazoles & Thiazolidinones As possible Antimicrobial agents.
<u>NAME OF THE CANDIDATE</u>	: Dawane Kushal Hareshwar Devyani
<u>QUALIFICATION</u>	: B.Sc. Chemistry (March 2006).
<u>NAME AND DESIGNATION</u>	: Dr. Anil S. Bobade.
<u>OF THE RESEARCH</u>	(Senior Scientific Officer Class-I)
<u>SUPERVISOR</u>	
<u>PLACE OF RESEARCH</u>	: Department of Chemotherapy Haffkine Institute for Training, Research and Testing. Parel, Mumbai-12.
<u>NUMBER AND DATE OF</u>	: 25, Aug. 13, 2007.
<u>REGISTRATION</u>	
<u>DATE OF SUBMISSION</u>	: 31 st Aug 2009



SIGNATURE OF
STUDENT



SIGNATURE OF THE
RESEARCH SUPERVISOR

SYNOPSIS

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

However, the true era of Medicinal Chemistry really began in 1930's with the preparation and use of antibacterial sulfonamide drugs. The history of medicine was from that time on, to be dominated by medicinal therapeutics. Today a large number of diseases are cured or at least controlled by drugs therapy. Thus, the fight against bacterial and fungal infections has been largely won and significant progress has been made in treating mental, pulmonary, inflammatory and cardiovascular conditions. In addition, some cancers and certain forms of leukemia are cured by chemotherapy.

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

Medicinal Chemistry is the study of those chemicals which are expected to have beneficial effects on the infected living systems. At its heart is synthetic organic chemistry. But no medicinal chemist is merely interested in the chemical properties of the molecule under investigation. The entire logic and all efforts of the synthesis of new compound are for an anticipated biological activity. But it happens most frequently that the joint efforts result in compound with lack of activity.

Amidst the frequent, but much needed failures and seldom occurring breakthroughs, the strive in medicinal chemistry continues for various reasons; more silently for-

- 1) Obtaining compound with higher efficiency and lower toxicity.
- 2) To hit upon moieties that tackle drug resistance of various microorganism.

One aspect of Medicinal Chemistry deals with the incorporation of newly emerged pharmacophores on already existing biologically active moieties to produce newer molecules with higher efficacy. One such genuine effort has substituted been made to synthesize molecules with incorporation of halogenated substitutes on various active moieties such as Oxadiazole, Triazole and Thiazolidinones 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-(2,4,6-trichlorophenoxy methyl)-[1,3,4]oxadiazol-2-yl]ester.
[Synthesis of 5 – [2', 4', 6'-Trichlorophenoxy methyl]-
2 – (substituted Thiocarbonyl) – 1, 3, 4 – Oxadiazole.]

Part 3 :- Synthesis of 3,4 –Bis-(substituted phenyl)-5-(2,4,6- Trichlorophenoxy-methyl)-4H-[1,2,4]triazole.
[Synthesis of 3 – [2', 4', 6'- Trichlorophenoxy methyl]-
4 – (substituted aniline) – 5 – aryl – 1, 2, 4 – Triazole.]

Part 4 :- Synthesis of 4-substituted-thiobenzoic acid 4-{[1-(substituted phenyl)-meth-(E)-ylidene]-amino}-5-(2,4,6-Trichlorophenoxy methyl)-4H-[1,2,4]- triazol-3-yl ester.
[Synthesis of 3-[2',4',6'- Trichlorophenoxy methyl]-
5-(substituted Thiocarbonyl)-4-(substituted-N-benzylidenyl amino)-
1,2,4-Triazole.]

Part 5 :- Synthesis of 3-(6-Chloro-benzothiazol-2-ylamino)-2-(substituted-phenyl)-thiazolidin-4-one.

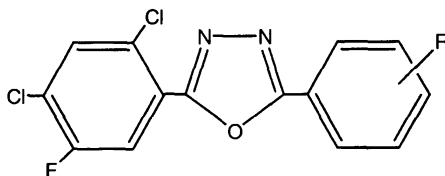
Part 6 :- Biological screening, Result, & Discussion

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

Part 2:- Synthesis of substituted thiobenzoic acid S-[5-(2,4,6-trichlorophenoxy methyl)-[1,3,4]oxadiazol-2-yl].

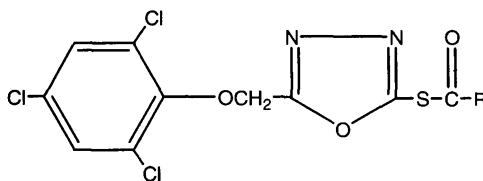
2,5-Disubstituted 1,3,4-Oxadiazole constitute a unique class of Nitrogen & Oxygen containing five membered heterocycles. Oxadiazole¹ derivatives have been found to be exhibit diverse biological activity such as anti bacterial, fungicidal, anti inflammatory & anti HIV.

Recently Hosam Saad et,al²; have reported 2-aryl-5-substituted-1,3,4-Oxadiazoles show anti bacterial activity. Xiuman Zheng et,al³; also reported 2,5-Disubstituted Oxadiazole (I) shows significant insecticidal activity.



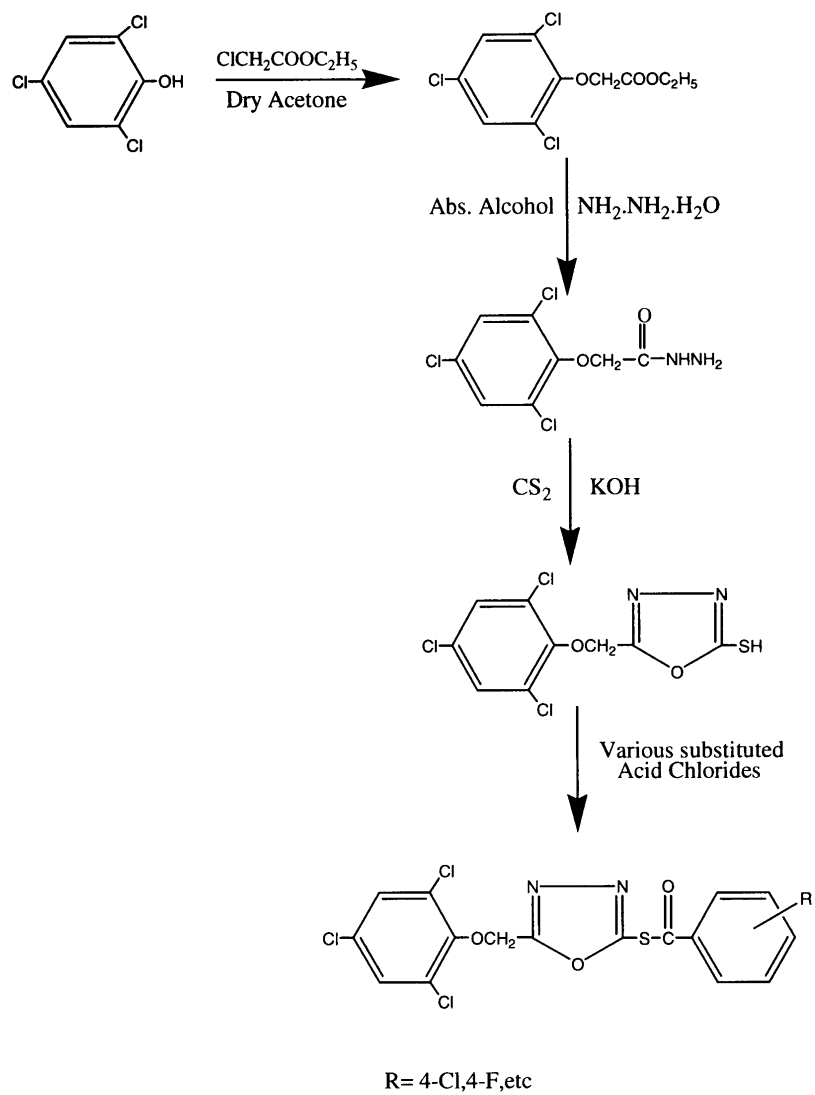
(I)

Hence taking into considerations the above facts it was planned to synthesize the series of compound having combination of 2,5-Disubstituted Oxadiazole with different acid chlorides so as to study their anti bacterial & anti fungal properties. The title compound has following structure.



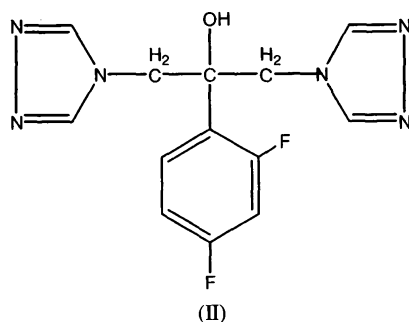
R= 4-ClC₆H₄, 4-FC₆H₄,etc

SCHEME:- I



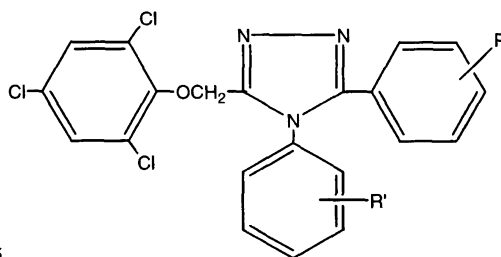
Part 3:- Synthesis of 3,4 –Bis-(substituted phenyl)-5-(2,4,6-Trichlorophenoxy methyl)-4H-[1,2,4]triazole.

The importance of 1,2,4-Triazole moiety as a major pharmacophoric requirement to exhibit enhanced anti fungal activity is well manifested by its inevitable presence in the most recent anti fungal drug viz Flucanazole⁴(II).



4 –Fluro aniline is used as a starting material for the synthesis of various anti bacterial⁵ & insecticides⁶. 3-chloro-4-fluro aniline is used in the synthesis of various pesticides⁷. A derivative of benzoic acid shows fungicidal activity⁸.

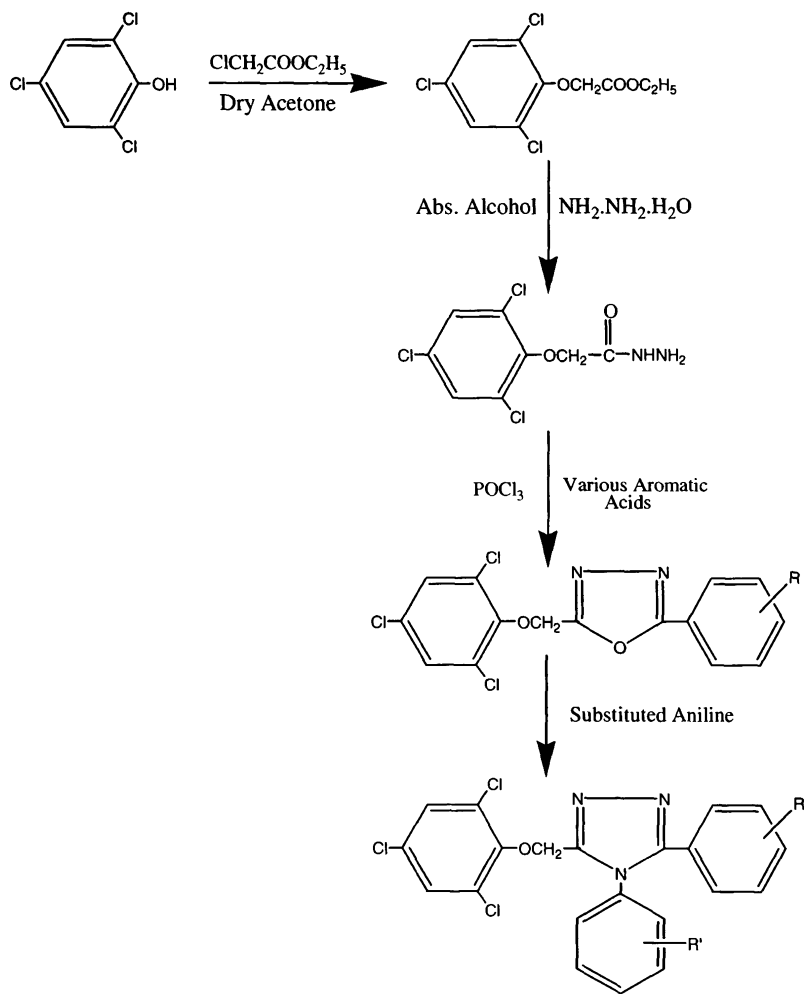
Thus taking into consideration the above facts, the series of compounds having benzoic acid, aniline & Triazole moiety has been synthesized.



R= 4-Cl, 3-OCH₃

R'= 4-Cl, 4-OC₂H₅

SCHEME:- II



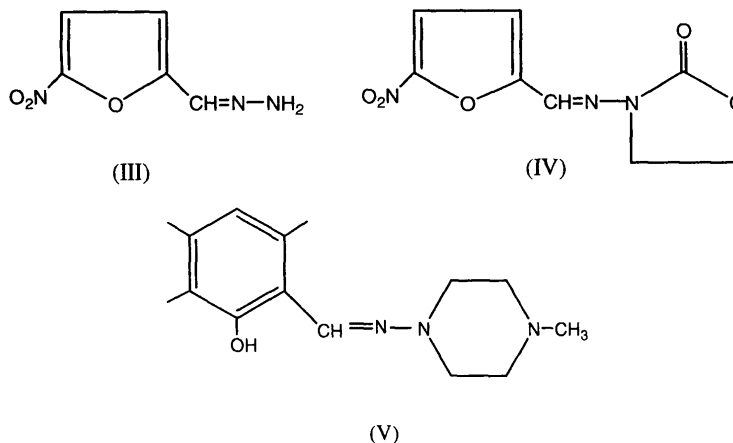
$\text{R} = 4\text{-Cl}, 3\text{-OCH}_3$

$\text{R}' = 4\text{-Cl}, 4\text{-OC}_2\text{H}_5$

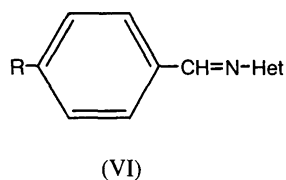
Part 4:- Synthesis of 4-substituted-thiobenzoic acid 4-[[1-(substituted phenyl)meth-(E)-ylidene]-amino]-5-(2,4,6-Trichlorophenoxy)methyl)-4H [1,2,4]- triazol-3-yl.

Schiff's bases from 5-chlorosalicylaldehyde, 4-bromosalicylaldehyde & 4-N,N-dimethyl amino benzaldehyde have been reported to have encouraging tuberculostatic action⁹. The presence of Azomethine linkage in compounds has also been known to decrease the toxicity considerably¹⁰.

The presence of Azomethine linkage in variety of biologically active compounds such as Nitrofurantoin(III), Nitrofurazone(IV) and also in the fields of antibiotics such as Rifampicin(V) has been proved to be very essential.



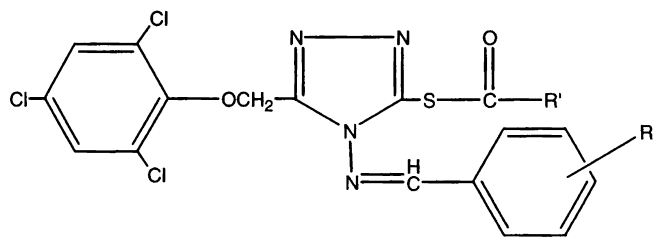
R.Fioranvati, et,al; have reported anti microbial activity of some new hetero aryl benzyl amines & their Schiff bases¹¹(VI).



R=C₆H₅, 4-CH₃C₆H₄, etc.

1,2,4-Triazole derivatives as mentioned earlier in the Part 2 are very pharmacological important. Compounds containing 1,2,4-Triazole nucleus & n-benzylidenyl derivatives also possess various biological activity. The thiol grouping at 5th position enhances the anti tubercular property of Triazole¹². A Mercapto group [-SH] is an important toxophore responsible for pesticidal activity¹³.

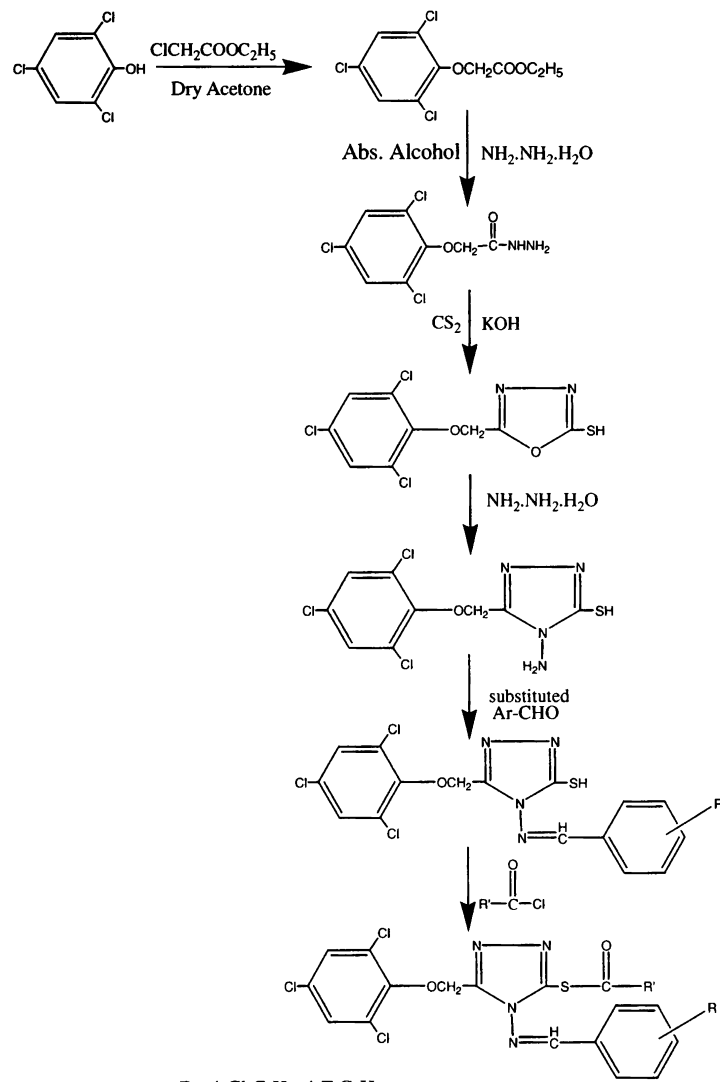
Hence taking into consideration of all above mentioned facts, a series of compounds of mercapto Triazole with different acid chlorides has been synthesized so as to study their anti bacterial & anti fungal properties. The title compound will have following structure.



R = 4-Cl-C₆H₄, 4-F-C₆H₄

R' = 4-Cl, 4-COOH

SCHEME:- III



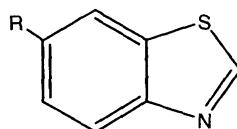
$\text{R} = 4\text{-Cl-C}_6\text{H}_4, 4\text{-F-C}_6\text{H}_4$

$\text{R}' = 4\text{-Cl}, 4\text{-COOH}$

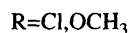
Part 5:- Synthesis of 3-(6-Chloro-benzathiazol-2-ylamino)-2-(substituted-phenyl)-thiazolidin-4-one.

Heterocycles bearing Thiazole, Sulphur & Nitrogen constitute the core structure of number of pharmacologically & biologically active compounds. Among these various types of known heterocyclic ring system, benzothiazole nucleus has found to be present in various medicinal compound having anti tubercular, anti allergic, anti inflammatory & anti fungal activity¹⁴.

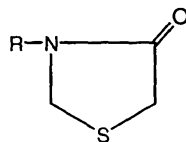
In benzothiazole derivatives, the substitution at 6th position by chloro, methoxy, etc can cause an increase in anti parasitic activity¹⁵(VII).



(VII)



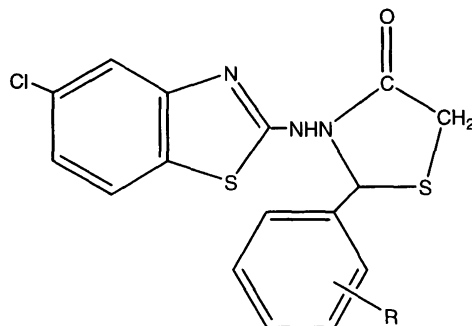
4-Thiazolidinones (VII) derivatives play a vital role in pharmaceutical sciences owing to their wide biological & industrial applications¹⁶.The derivatives of 4-Thiazolidinones have also occupied a unique place in the field of Medicinal chemistry due to a wide range of pharmacological activities^{17,18}(VIII).



(VIII)

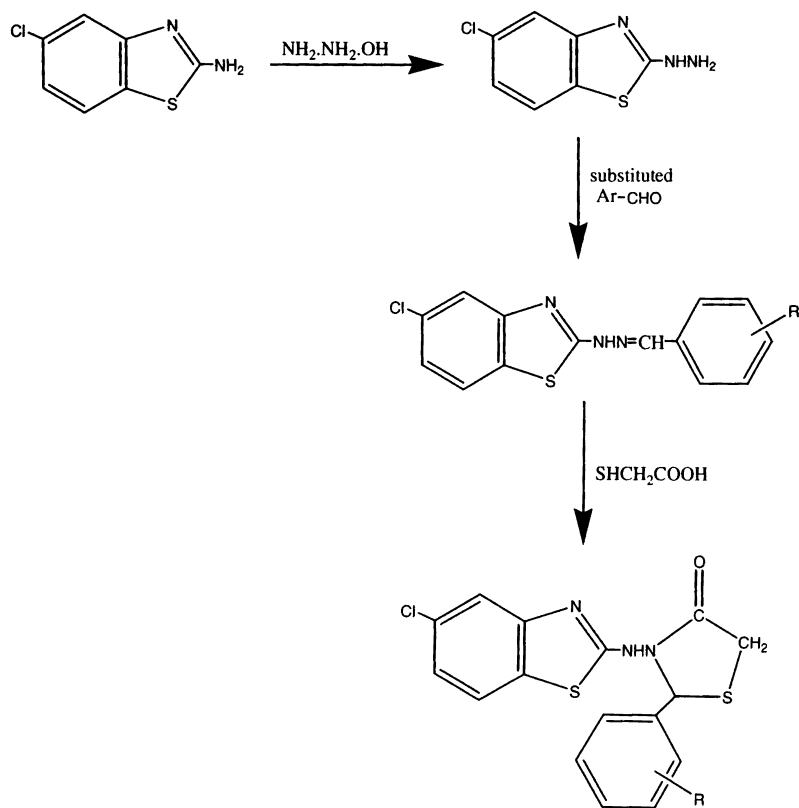
Based on the above facts, a series of compound have been synthesized by condensing various substituted Benzaldehyde with Hydrazino benzothiazole to get substituted Schiff's bases which was then cyclised with Mercapto acetic acid to get the final compound which has both Benzothiazole & 4-Thiazolidinone moiety. In this series 10

compounds have been synthesized which will be studied for their Anti bacterial & Anti fungal properties. The Final Compound will have the following structure



R= 4-Cl, 4-COOH

Scheme:- IV



R= 4-Cl, 4-COOH

Biological Screening:

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

Anti Bacterial Screening:-

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

The **in vitro** antimicrobial screening was carried out by tube dilution method against various species of Gram Positive and Gram Negative bacteria viz. **Staphylococcus Aureus. ATCC 3750** and **Salmonella Typhi. NCTC786**. The culture medium used was Mueller-Hinton Broth¹⁹.

Anti Fungal Screening:-

Some compounds reported in series in I, II,III and IV were also screened for their antifungal activity by the tube dilution technique²⁰ against fungal strains viz.

Candida Albicans.

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

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

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