

SYNTHESIS AND STUDY OF NEW HETEROCYCLIC
ORGANIC COMPOUNDS AS POSSIBLE THERAPEUTIC
AGENTS

THESIS SUBMITTED
UNIVERSITY OF MUMBAI

FOR THE DEGREE OF
MASTER OF SCIENCE
IN
ORGANIC CHEMISTRY

SUBMITTED BY
YOGESH PRABHAKAR PRAVINA JADHAV

UNDER THE GUIDANCE OF
DR. ANIL BOBADE

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

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Mr Yogesh Prabhakar Pravina Jadhav

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A

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JAD

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Ph. D

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**DEPARTMENT OF CHEMOTHERAPY
HAFFKINE INSTITUTE FOR TRAINING, RESEARCH AND
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MARCH 2010

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 New Heterocyclic Organic Compounds As Possible Therapeutic Agents*" forms my own contribution to the research work carried out under the guidance of Dr. Anil S. Bobade at the Department Of Chemotherapy, Haffkine Institute, 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

YOGESH PRABHAKAR JADHAV

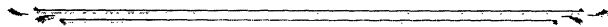
Certified by



Signature of Guide

Dr. Anil S. Bobade

DEDICATED TO MY FAMILY



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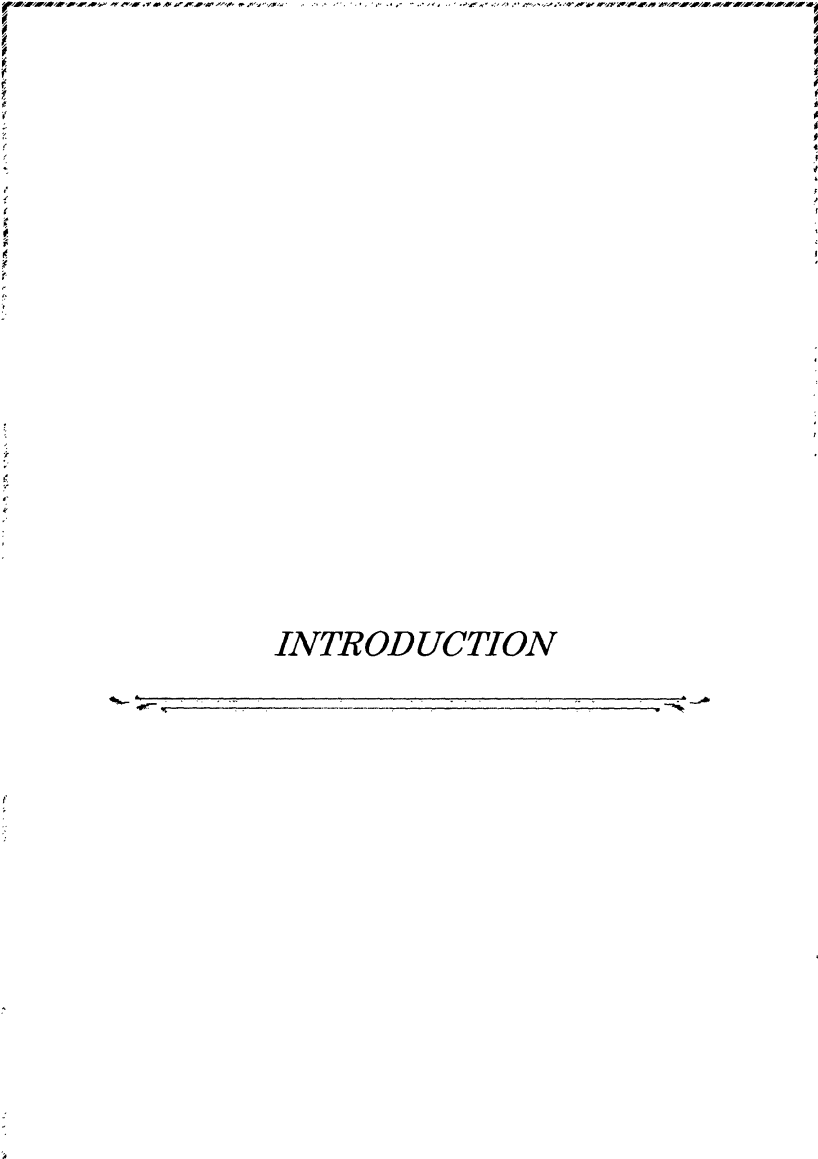
I wish though shall never be able to express my deep indebtedness to my family for their massive sacrifice and support without which the thesis could never have been produced in reality. More important than anything else has been the support of my family. My parents Mr. Prabhakar Jadhav and Pravina Jadhav, my sister Harshala and grandma Sumitra who stood by me through thick and thin, and patiently backed me all throughout my career. I shall immense debt of gratitude that I bear to Mr. and Mrs. Pratap Jadhav my uncle, the blaze of inspiration and moral support they have provided to me. I have shared rather special relation with my best friends Amol and Prashant Sawant the blaze of inspiration and moral support they have provided to me.

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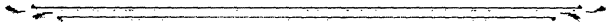
YOGESH P. JADHAV

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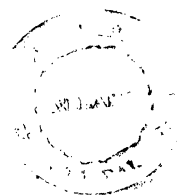
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INTRODUCTION



INTRODUCTION



"Those who fail to read history are destined to suffer the repetition of its mistakes"

From time immemorial man has been interested in trying to control disease. The medicine man, the priest, the herbalist and the magician, all undertook in various ways to cure man's disease and/or to bring relief to the sick. In an almost complete absence of scientific medical knowledge, it would not be fair to say that the early practitioner of medicine contributed nothing to the alleviation of man's suffering to disease. Medical knowledge in fact has been derived, to a very great degree, from the intuitive and observational propositions and cumulative experiences gleaned from others. A history of medicine thus contributes a review of accomplishment and errors, false theories and misinformation and mistaken interpretations. It is also a study of the evolution of man and of human knowledge down the ages; of the biographies of eminent individuals who developed medicine, of the discoveries and inventions in different historical periods; and of the ever-concepts, goal and objectives of medicine. In the course of its evolution, which proceed by stages, with advances and halts, medicine has richly from the traditional cultures of which it is a part, and later from biological and natural sciences and more recently from social and behavioral sciences. Medicine is thus built on the best of the past.

For all human history until the 1880s, medicines had been derived almost exclusively from natural products, plant, animal, or mineral. Among these sources, plant extracts were by far the most important. The first great medical achievement of analytical organic chemistry in the nineteenth century was the isolation of active principles of plants, including morphine from opium and quinine from cinchona bark, and their characterization as members of a new chemical class, the alkaloids. With rapid development of analytical, synthetic, and structural organic chemistry in the middle decades of the nineteenth century, chemists moved beyond isolation of natural products to their synthesis. More important, they began to recognize the potential medical usefulness of organic compounds prepared in the laboratory. A crucial turning point

came in the 1880s, with the realization by German chemists that certain coal tar derivatives have powerful fever- and pain reducing properties. One of these compounds, phenacetin, introduced in 1887, was manufactured by the German fine chemicals company Bayer and found widespread use in medicine. Recognition of the pharmaceutical potential of coal tar derivatives, joined with increasing industrial connections to academic organic chemistry and legal and economic incentives for diversification, propelled companies such as Bayer, which were already in possession of extensive knowledge of coal tar chemistry through work on synthetic dyes, into pharmaceutical research. One of the important early products of the Bayer pharmaceutical laboratories, established in the 1890s, was acetylsalicylic acid, marketed in 1899 under the trade name Aspirin.

We live in an age of powerful medicinal drugs. Antibiotics cure or prevent bacterial infections, diuretics and other medicines help to control hypertension, antiretrovirals add years to the lives of those infected with HIV. The list of substances that cure or palliate our ills is very long and getting longer every year. The “explosion” of knowledge during 20th century has made medicine more complex, and treatment more costly, but the benefits of modern medicine have not yet penetrated the social periphery in many countries. The glaring contrasts in the state of health between the developed and developing countries, between the rural and urban areas, and between the rich and poor have attracted worldwide criticism as “social injustices”. The commitment of all countries, under the banner of the World Health Organization, is to wipe out the inequalities in the distribution of health resources and services, and attain the goal of health care for all. The goal of modern medicine is no longer merely treatment of sickness. The other and more important goals which have emerged are prevention of disease, promotion of health and improvement of the quality of life of individual and communities. In other words, the scope of medicine has considerably broadened during recent years.



REVIEW OF LITERATURE

REVIEW OF LITERATURE

On Christmas Day 1932, the German chemical combine I.G. Farbenindustrie filed a patent application on behalf of a red dye that its medical researchers and chemists had found to have a remarkable ability to cure certain kinds of bacterial infections in mice. By the time the patent was awarded just over two years later, the dye, now called by the trade name Prontosil, had been found to have the same curative powers in humans. Announced in a brief publication in a German medical journal in February 1935 by Gerhard Domagk, the medical researcher responsible for the work, Prontosil went on the market and became available to doctors in April 1935. In retrospect, it is clear that the introduction of Prontosil marked a turning point in the history of medicine. As the first of the compounds called sulfonamides or, more familiarly, sulfa drugs, Prontosil initiated a revolution in the therapeutics and management of bacterial infections. Within a few years, feared diseases such as streptococcal infections (including childbed fever and septicemia), pneumonia, meningitis, dysentery, gonorrhea, and urinary tract infections, were brought under a substantial measure of control by chemotherapy. The medical success of the sulfa drugs gave a powerful impetus to the expansion of the international pharmaceutical industry and of the research and development enterprise within it. The same success contributed heavily to the opening of what was widely referred to as an era of miracle drugs, with its attendant optimism and raised expectations of medicine, accelerated appearance of new medicines as a result of increased levels of research, regulatory dilemmas, and unanticipated therapeutic problems. Finally, the sulfa drugs proved extraordinarily fruitful as starting points for molecular modifications that led to new drugs or classes of drugs not only for bacterial infections (eventually including tuberculosis) but also for leprosy (Hansen's disease), diabetes, hyperthyroidism, gout, heart disease, and hypertension. The reach of research based on the sulfa drugs extended beyond human into veterinary medicine and even to the discovery of new herbicides.

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. Various Chemotherapeutic agents depending on their uses can broadly classified as follows

1. Antibiotics

Antibiotics are used to treat bacterial infections. The toxicity to humans and other animals from antibiotics is generally considered to be low. However, prolonged use of certain antibiotics can decrease the number of gut flora, which can have a negative impact on health. Some recommend that during or after prolonged antibiotic use, that one should consume probiotics and eat reasonably to replace destroyed gut flora. The term antibiotic originally described only those formulations derived from living organisms but is now applied also to synthetic antibacterial drugs. The discovery, development, and clinical use of antibiotics during the 20th century have substantially decreased mortality from bacterial infections. The antibiotic era began with the pneumatic application of nitroglycerine drugs, followed by a “golden” period of discovery from approximately 1945 to 1970, when a number of structurally diverse, highly effective agents were discovered and developed. However, since 1980 the introduction of new antimicrobial agents for clinical use has declined. Paralleled to this there has been an alarming increase in bacterial resistance to existing agents.

Antibiotics are among the most commonly used drugs. For example, 30% or more hospitalized patients are treated with one or more courses of antibiotic therapy. However, antibiotics are also among the drugs commonly misused by physicians, e.g. usage of antibiotic agents in viral respiratory tract infection. The inevitable consequence of widespread and injudicious use of antibiotics has been the emergence of antibiotic-resistant pathogens, resulting in the emergence of a serious threat to global public health. The resistance problem demands that a renewed effort be made to seek antibacterial agents effective against pathogenic bacteria resistant to current antibiotics. One of the possible strategies towards this objective is the rational localization of bioactive phytochemicals.

2. Antiviral drugs

Antiviral drugs are a class of medication used specifically for treating viral infections. Like antibiotics, specific antivirals are used for specific viruses. They are relatively harmless to the host, and therefore can be used safely to treat viral infections. They should be distinguished from vermicides, which actively deactivate virus particles outside the body. Most of the antivirals now available are designed to help deal with HIV; herpes viruses, best known for causing cold sores and genital herpes, but actually causing a wide range of diseases; the hepatitis B and C viruses, which can cause liver cancer; and influenza A and B viruses. Researchers are now working to extend the range of antivirals to other families of pathogens.

Antiviral drugs work by inhibiting the virus either before it enters the cell, stopping it from reproducing, or in some cases preventing it from exiting the cell. However, like antibiotics, viruses may evolve to resist the antiviral drug.

3. Antifungal drugs

An antifungal drug is medication used to treat fungal infections such as athlete's foot, ringworm, candidiasis (thrush), serious systemic infections such as cryptococcal meningitis, and others.

Antifungals work by exploiting differences between mammalian and fungal cells to kill off the fungal organism without dangerous effects on the host. Unlike bacteria, both fungi and humans are eukaryotes. Thus fungal and human cells are similar at the molecular level. This means it is more difficult to find a target for an antifungal drug to attack that does not also exist in the infected organism. Consequently, there are often side-effects to some of these drugs. Some of these side-effects can be life-threatening if the drug is not used properly.

❖ Some Important Synthetic Antimicrobial Agents

A. Sulfur Containing Compounds (Sulfa Drugs):

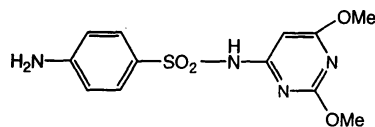
1. Sulfonamides:

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

In 1936, Colebrook and Kenny¹, as well as in 1936, Buttle *et al.*² report their favourable clinical results with prontosil and sulfanilamide in *puerperal sepsis* and meningococcal infections. These two reports awakened the medical profession to the new field of bacterial chemotherapy, and experimental and clinical articles in great profusion soon appeared. A vast number of derivatives of sulfanilamides were subsequently synthesized. This resulted in introduction of sulfathiazole, sulfapyridine, sulfamerazine, sulfadimidine, etc into clinical practice. Many of these have been tested for their clinical value in various bacterial, protozoal and viral infections. Sulfonamides have a wide range of antimicrobial activity against both gram-positive and gram-negative organisms. With a few exceptions, there is a direct correlation between their efficacy *in vitro* and *in vivo*. In general, the sulfonamides exert only a bacteriostatic effect in the body and cellular and humoral defense mechanisms of the host are essential for the final eradication of the infection.

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

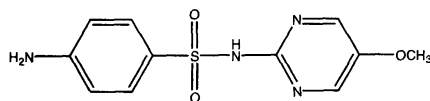
i) Sulfadimethoxine :



[I]

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

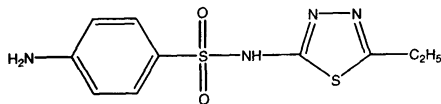
ii) Sulfameter (Sulfamethoxydiazine):



[II]

Sulfameter [II] is a long acting sulfonamide, which is readily absorbed from the gastrointestinal tract. Following oral administration, measurable levels of the drug reaches in approximately two hours and peak serum levels occur within 4 to 8 hours. Limited data suggest that measurable amounts of the drug are still present in the plasma 96 hours after the drug is administered; approximately, 90% of the sulfonamide in plasma is non-acetylated. Because of the long lasting blood levels of the sulfameter, it is administered orally as a single daily dose, preferably after breakfast.

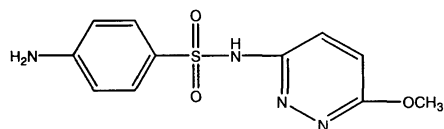
iii) Sulfaethidole :



[III]

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

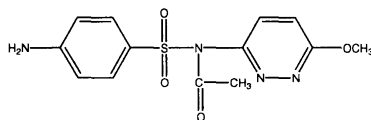
iv) Sulfamethoxypyridazine :



[IV]

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

v) Sulfamethoxypyridazine Acetyl :



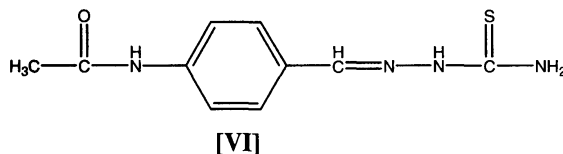
[V]

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

2. Thiosemicarbazone:

The tuberculostatic activity of thiosemicarbazones and related drugs was first observed by Domagk *et al.*³ The weak antituberculosis activity of sulfathiazole led to the preparation of series of analogues.^{4,5} 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) [VI] 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 streptomycin.^{6,7} 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 anemia 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.

B. Furan Derivatives :

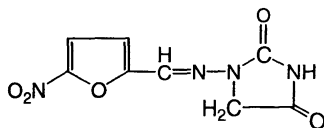
Derivatives of furan possess antimicrobial activity. The presence of nitro group in the 5-position of the furan ring confers antibacterial activity on mainly 2-substituted furans. Dodd and Stillman⁸ prepared a large number of nitro furans and their non-nitrated analogs and found that the nitro group 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 concluded⁹⁻¹¹ 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 synergistic.¹² Little or no development of cross-resistance is so far evident.¹³⁻¹⁵

1. Nitrofurantoin :

Nitrofurantoin [VII] was first synthesized by Hayes¹⁶ by the condensation of 1-aminohydantoin with 5-nitro-2-furfuraldehyde. The drug is active against many strains of the common urinary tract pathogens.



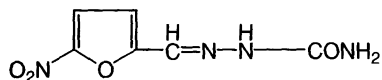
[VII]

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 urine.¹⁷ 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 infections.¹⁸ The average dose of Nitrofurantoin results in a urinary concentration of approximately 20 $\mu\text{g}/100\text{ml}$. The most common effects are nausea, vomiting and diarrhea. The incidence is less if the drug is administered with milk or other food or is used in a smaller dosage.

2. Nitrofurazone :

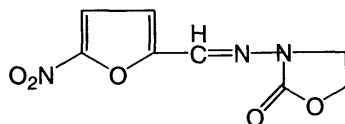
Stillman¹⁹ have synthesized this compound [VIII] from 5-nitrofurfuraldehyde and semicarbazide hydrochloride in the presence of sodium acetate. The drug has broad spectrum antibacterial activity against most gram-positive and gram-negative surface pathogens. It has been used in the treatment of mixed 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 man²⁰ caused by *Trypanosome gambiense*.



[VIII]

3. Furazolidone :

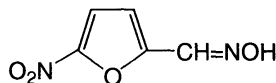
Furazolidone [IX] was synthesized by Drake and Hayes.²¹ The drug has been reported to be of value in the treatment of intestinal infections due to *Shigella* and *Salmonella* organisms.²² It has an outstanding activity against these pathogenic bacterial infections. Excellent anticoccidial & antitrichomoral activity is also evident.



[IX]

4. Nifuroxime :

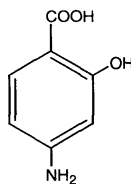
Nifuroxime [X] is prepared by treating 5-nitrofurfural with hydroxylamine in alcohol. It is patented²³ as a urinary antibacterial agent. The compound has wide antibacterial and excellent antifungal activity. *Candida Albicans*, various microspores, and *Trichophyton* species are susceptible. The product is also effective against *Trichomonas vaginalis* and is often formulated in combinations with Furazolidone in the form of suppositories.



[X]

C. p-Aminosalicylic acid (PAS) and its derivatives:

Bernheim²⁴ 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 Lehmann²⁵ to test a large series of related compounds for antituberculosis activity. The most effective was PAS [XI]



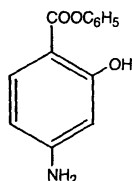
[XI]

PAS was found to be highly bacteriostatic *in vitro* and also active orally against experimental infections in animals.²⁶⁻²⁸ PAS is specifically active against mycobacteria and against only certain species in this genus.²⁹ It is equally effective against

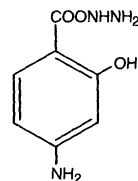
streptomycin-resistant strains.³⁰ It is of interest to find that PAS has little effect on the respiration of mycobacteria nor does it inhibit the stimulating effect salicylates.³¹ *In vitro*, most strains of *Mycobacterium Tuberculosis* are sensitive to a concentration of 1 µg/ml of PAS

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.

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 [XII] was claimed to be as active as streptomycin subcutaneously and equivalent to PAS orally.^{32,33}



[XII]



[XIII]

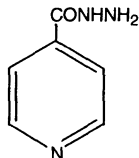
PAS hydrazide [XIII] was active *in vitro*. It's effect was inhibited by PABA and there was cross resistance with PAS, but not with INH.

D. Iso-nicotinic acid derivatives

1. Iso-nicotinic acid Hydrazide [INH] :

In 1945, Chorin³⁴ 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 [XIV] 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's.³⁵⁻³⁸

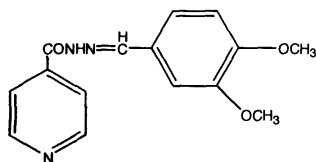


[XIV]

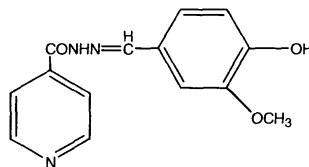
INH is both tuberculostatic and tuberculocidal *in vitro*; The minimal tuberculostatic concentration is 0.025 to 0.05 µg/ml. It is not active against the 'atypical' group. It is bactericidal even at its MIC. INH is strikingly superior to streptomycin. Unlike streptomycin, INH penetrates cells and also cerebrospinal fluid and is just as effective against intracellularly located bacilli as it is against those growing in culture media.

2. 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



[XV]



[XVI]

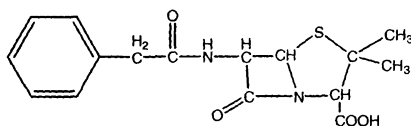
Clinically verazide [XV] was originally claimed to exert a more marked and prolonged antituberculous effect than INH and 3-4 times less toxic. Later the same workers

concluded that clinically the compound showed no advantage over INH and caused toxic side-effects attributable to veratraldehyde formed by hydrolysis.³⁹

The 2-hydroxybenzylidene derivative [XVI] was about half as active as INH and less than 1/10th as toxic.⁴⁰ The 3-methoxy-4-hydroxybenzylidene derivative [Phthivazid, XII] has been extensively used clinically in Soviet Union.⁴¹

E. Benzyl Penicillin:

Among the four different penicillins i.e F, G, X & K, the G or Benzyl penicillin [XVII] 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 Rogers⁴² have shown that, at least with *Staphylococci*, benzyl penicillin acts by inhibiting the biosynthesis of cell wall mucopeptide, without interfering with protein synthesis.



[XVII]

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.

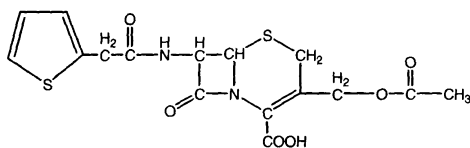
1. Ampicillin:

In 1961, Rolinson and Stevens,⁴³ 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.^{44,45} It is bactericidal and

appears to be as free from toxicity as benzyl penicillin. Gastric intolerance and rashes are the only side effects. Few antibiotics containing thiophene ring are :

2. Cephalothin:

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

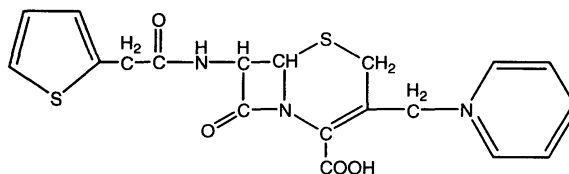


[XVIII]

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.

3. Cephaloridine:

The general range of activity and antibacterial spectrum of cephaloridine [XIX] 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).



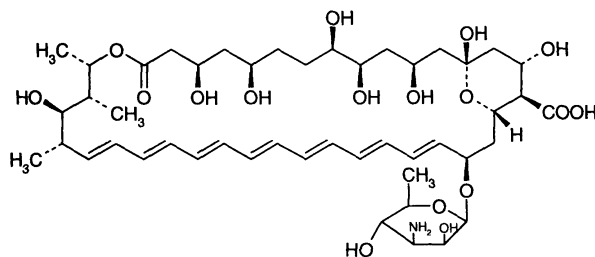
[XIX]

Mycobacterium fortuitum is sensitive to cephaloridine but not to cephalothin. Cephalodprin C and its semi-synthetic congeners induce the synthesis of penicillinase by *B. cereus* and *Staph. aureus*. Cephalothin is highly resistant to this enzyme, but cephaloridine appears to be more susceptible to its effect.⁴⁶

Cephaloridine is nephrotoxic.^{47,48} 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.

F. Amphotericin B:

This antibiotic produced by *Streptomyces nodusus*, was discovered in 1955. It became the first systemic antifungal drug. Amphotericin B [XX] 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 yeasts.⁴⁹ 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 fungi.⁵⁰

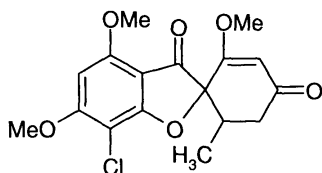


[XX]

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.

G. Griseofulvin:

Although Oxford A.E et al discovered this antibiotic, a product of *Penicillium griseofulvum* [XXI], in 1939; its antifungal activity was not utilized until 1951. Groove predicted its chemical structure in 1950.⁵¹ 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.

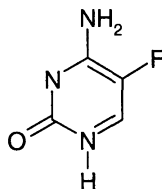


[XXI]

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.

H. 5-Fluorocytosine:

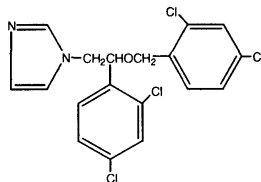
This Fluorinated pyrimidine [XXII] was first synthesized in the 1950s as a potential antileukemic drug. It was found to have *in vitro* and *in vivo* antifungal activity.⁵² It is being increasingly used as an adjunct to Amphotericin B therapy. Accordingly, in *Aspergillus*, *Candida* and *Cryptococcus* infections, the drug is combined with Amphotericin B.



[XXII]

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.

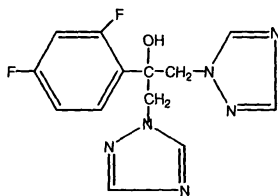
I. Miconazole:



[XXIII]

Miconazole [XXIII] is intended for the treatment of serious systemic fungal infections like Candidiasis, Cryptococcosis, Petriellidiasis and Paracoccididomycosis. The adverse reactions include thrombophlebitis, pruritis, fever and G.I. disturbances. Miconazole is solubilised with polyethylene glycol and castor oil to facilitate adequate intestinal absorption.

J. Fluconazole:



[XXIV]

The absorption of Fluconazole [XXIV] does not depend upon gastric acidity; more over, the drug can be administered intravenously. It readily crosses the blood brain barrier, and thus, is useful for fungal meningitis. Other azoles include Econazole, Butaconazole, Fenticonazole, Oxiconazole, Sulconazole, Ticonazole, Itraconazole and Terconazole.

❖ Factors Producing Limitations For Use of Chemotherapeutic Agents

A. 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 antimicrobials.⁵³

B. 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 mechanisms.⁵⁴

C. Resistance of micro-organisms to antimicrobial agents:

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

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

❖ Properties of an Ideal Chemotherapeutic Agent:

The ideal chemotherapeutic should have the following properties. The compound should exhibit selective and effective antimicrobial activity, and it should be bactericidal rather than bacteriostatic. While it might be desirable that an agent kills a broad range of micro-organisms, problems of supra-infection 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.

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

PLAN OF WORK

PLAN OF WORK

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

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

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

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

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

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

The present work in this thesis has been planned keeping in mind the various chemical structures of existing antibacterial agents and antifungal agents. This thesis includes synthesis and study of biological activity of heterocyclic molecules containing more than one biological active heterocyclic moiety. Biological importance of parent heterocyclic moiety and method used to synthesize target molecule is shortly noted in following four sections.

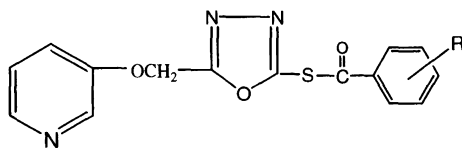
Scheme I :- 2-(Substituted thiocarbamoyl)-5-(3-pyridoxymethylene)-1,3,4-oxadiazole

Large number of pyridine derivatives exhibit interesting biological activities. Especially 3-Pyridine derivatives such as Isoniazides and Nicotinamide are commonly used as drug for the treatment of tuberculosis.⁵⁵

The presence of Oxygen and nitrogen in the heterocyclic system has attracted the attention of medicinal chemist because the diverse biological activities and profound efficacy. Oxadiazole nucleus, constitute a unique class of nitrogen and oxygen containing five membered heterocycles associated with various biological activities such as anti-bacterial, anti-inflammatory, pesticidal, anti-hypertensive.⁵⁶⁻⁵⁹

The recent review of literature has highlighted the significance of medicinal importance of oxadiazole and their derivatives.⁶⁰ Also it has been reported that 2,5-disubstituted-1,3,4-oxadiazoles shows good anti-bacterial and significant pesticidal activity.^{61,62}

Thus taking into consideration the above all observations, the derivatives of pyridoxo compound containing 1,3,4-oxadiazole and thiocarbamoyl moieties were planned to synthesize. Method can be summarized as, 3-Pyridoxylacetaldehyde on reaction with CS₂ in mild alkaline condition gives 1,3,4-oxadiazole ring with thiol group at 2nd position which when condensed with various acid chlorides yield the respective 2-(Substituted thiocarbamoyl)-5-(3-pyridoxymethylene)-1,3,4-oxadiazole. Structure [I] represent the title compound. We planned to synthesize 5-6 derivatives of title compound. Structure will be confirmed by elemental analysis and spectral study.

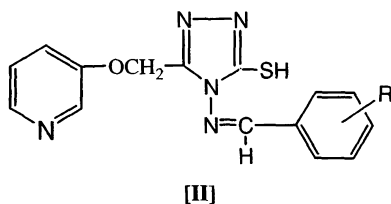


[I]

**Scheme II :- 3-(3-Pyridoxymethylene)-4- (N-substituted benzylidinylamino)-
5 -mercapto-1,2,4-triazole**

Many heterocyclic compounds containing 1, 2, 4-triazole ring are well known for their diverse pharmacological properties like anti-inflammatory, anti-microbial, anti-convulsant and anti-depressant activity.⁶³⁻⁶⁶ Schiff bases, which contains a carbon-nitrogen double bond with nitrogen atom connected to an aryl or alkyl group i.e. azomethine linkage, are well documented to show encouraging anti-microbial activity.⁶⁷ Also presence of azomethine linkage in compound has also been known to decrease the toxicity considerably.⁶⁸ Anti-microbial activity of some hetero aryl benzyl amines and their Schiff bases (IV) is reported by Fioranvati, R. et al.⁶⁹ Also Manna P. et al⁷⁰ and Trivedi V. et al.⁷¹ make considerable efforts to show biological activity related to azomethine linkage.

These observations lead us to include triazole ring and azomethine linkage along with pyridine entity in present study. Synthetic method for title compound can be summarized as, 3-Pyridoxy-2-mercapto-1,3,4-oxadiazole on subsequent reaction with excess of hydrazine hydrate and various substituted benzaldehydes generate title compound 3-(3-pyridoxymethylene)-4-(N-substitutedbenzylidinylamino)-5-mercapto-1,2,4-triazoles. Structure [II] represents the title compound. We planned to synthesize 5-6 analogs of title compound. Structure will be confirmed by CHN analysis, IR and NMR study.



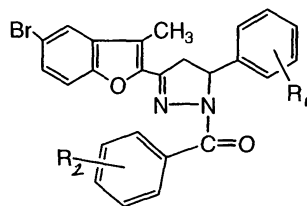
**Scheme III :- 5-Aryl-3-(5-bromo-3-methylbenzofuran-2-yl)-1-substituted
benzoylpyrazol-2-enes**

Furans are five membered heterocycles containing oxygen as hetero atom. Furans and benzofurans are associated with wide spectrum of biological activity.^{72,73}

Also pyrazoles as well as pyrazole containing compounds have been reported to show a broad spectrum of biological activity such as anti-microbial, anti-pyretic, anti-inflammatory and anti-tumor.⁷⁴⁻⁷⁷

A series of benzofurylethanolamine analogs of propafenone have been prepared and evaluated for multidrug resistance-reversing activity by Gerhard et al.⁷⁸ An excellent correlation of biological data with calculated lipophilicity values was found for benzofurans. Electrophysiological effects of dronedarone (a noniodinated benzofuran derivative) in the rabbit heart were observed by Wei Sun et al.⁷⁹ in 1999.

These facts encouraged us to synthesize new heterocyclic molecule bearing benzofuran and pyrazole moieties into a single molecule. Method planned to synthesize title compound can be summarized as 2-Cinnamoyl-5-bromo-3-methylbenzofurans after reacting with hydrazine hydrate yield respective 1H-pyrazoline which on reaction with acid chlorides yields 1-substituted benzoyl pyrazolines. Structure [III] represents the title compound. We planned to synthesize 5-6 derivatives of title compound. Structure will be confirmed by elemental analysis and spectral study.



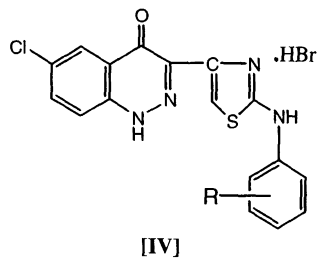
[III]

Scheme IV :- 2-(Substituted anilino)-4-(6-chloro-1H-cinnoline-4-one) thiazole .HBr

Thiazoles constitute the core structure of number of pharmacological and biological active compounds. Variety of thiazole derivatives are well known for their anti-microbial, anti-allergic, anti-tubercular and anti-inflammatory activities.⁸⁰⁻⁸³

Similarly cinnoline ring system shows wide spectrum of biological activities. 1-Alkyl-3-carboxycinnoline-4-ones exhibit good anti-microbial activity. Considering this biological activity of cinnoline moiety, it is incorporated with thiazole ring to get title compound.⁸⁴⁻⁸⁵

Thus taking into consideration the above all observations, the derivatives of 1H-Cinnoline incorporated with thiazole were planned to synthesize. Synthetic method can summarize as, α -brominated 3-Acetyl-1H-cinnoline-4-one when condensed with substituted thiourea gives 2-(Substituted anilino)-4-(6-chloro-1H-cinnoline-4-one) thiazole .HBr. Structure [IV] represents the title compound. We planned to synthesize 8-10 derivatives of title compound. Structure confirmation will be carried out using CHN analysis and spectral study.



Synthetic approach towards title molecules of above four sections is represented in “**Schematic Representation**” (page no. 53-56). All compounds synthesized in present work, subjected for antibacterial and antifungal screening using tube dilution method.

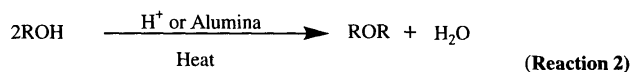
CHEMISTRY OF INTERMEDIATES

- ❖ *Chemistry of Ether Linkage*
- ❖ *Chemistry of Triazole*
- ❖ *Chemistry of Benzofurans*
- ❖ *Chemistry of Diazonium Compounds*
- ❖ *Chemistry of Cinnoline*
- ❖ *Chemistry of Bromination*
- ❖ *Chemistry of Thiourea*
- ❖ *Chemistry of Schiff Bases*
- ❖ *Chemistry of Acid Chlorides*

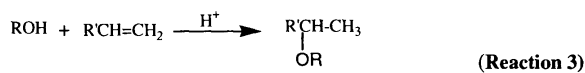
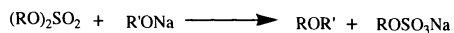
CHEMISTRY OF INTERMEDIATES

❖ Chemistry of Ether Linkage

Ethers are the organic compounds having oxygen atom bonded to two carbon atoms i.e. [C-O-C] linkage. The common methods used to produce ethers in laboratory are the *Williamson Synthesis* (Reaction 1) and the dehydration of alcohols using acids or inorganic oxides such as alumina (Reaction 2).

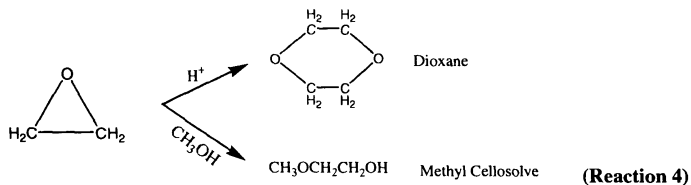


Other methods involve the reaction of dialkyl sulfates with sodium phenolates and the addition of olefins to alcohols or phenols using acid catalysts (Reaction 3).



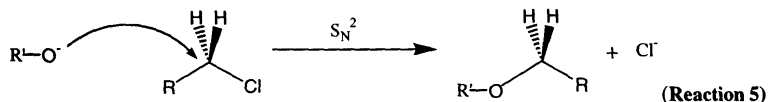
The laboratory reaction with dialkyl sulfate is not preferred since it has been reported that these reagents may cause carcinogenic reactions.

Ethers are also formed by the base or acid catalyzed condensation of oxides with themselves or alcohols or phenols. (Reaction No. 4)



Williamson Synthesis⁸⁶

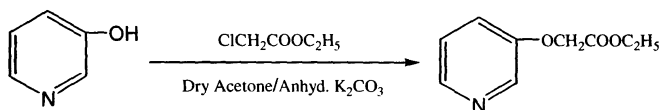
Williamson synthesis is applicable to the aromatic as well as the aliphatic series and it the basis of large scale production of commercial epoxy resins and crown ethers.



Primary halides gives best yields since secondary and tertiary halides dehydrohalogenate to give olefins. Alkyl phenyl ethers are prepared from aqueous or alcoholic solution of alkali phenolates and alkyl halides.^{87,88} Benzyl halides are easily replaced by alkoxy groups in high yields.⁸⁹

Ethers tend to absorb and react with oxygen from air to form peroxide. Some time thus peroxides cause an explosive detonation on distillation or concentration. In many cases heat, shock or friction can also cause decomposition.

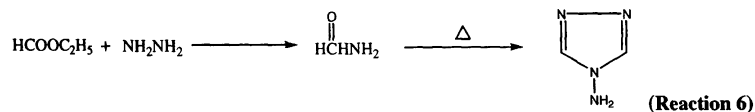
In present study we use Williamson synthesis as a synthetic tool to obtain desirable ether linkage in target molecule. Synthesis of 3-Pyridoxyacetate can schematically represent as



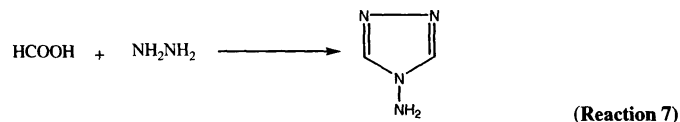
❖ *Chemistry Of Triazole*

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

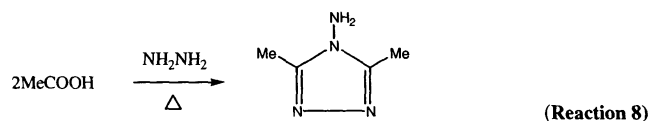
1. 4-Amino triazole has been prepared⁹⁰ by the reaction of ethyl formate with hydrazine to give form hydrazide, which was heated at 150 to 200° C to effect self condensation.



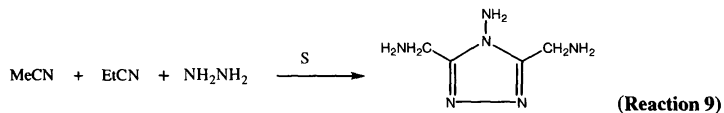
Similar interaction of formic acid and hydrazine gave good yield of 4-Amino triazole.^{91,92} (Reaction 7)



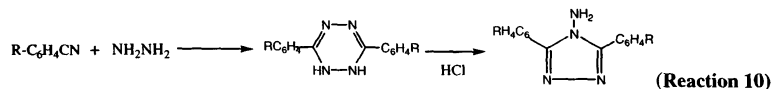
2. Other carboxylic acids and their functional derivatives have also been used to prepared alkyl and substituted alkyl-4-Amino triazoles, e.g. acetic acid and hydrazine hydrate at high temperature give 3,5-dimethyl-4-Amino triazole⁹³



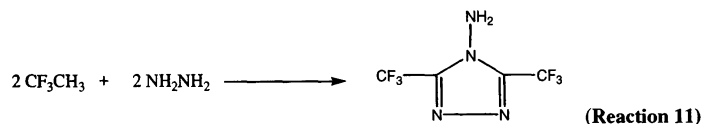
3. Sulfur and sulfur containing compounds catalyze the formation of 4-Amino triazoles e.g. 4-Amino triazole substituted at 3,5-position was prepared by the reaction of equimolar amount of acetonitrile and propionitrile with a mixture of hydrazine and sulfur.⁹⁴



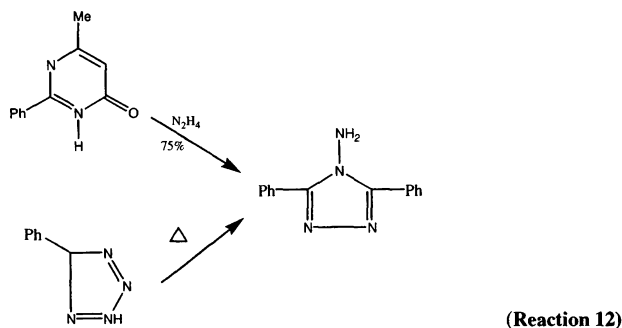
4. The preparation of 4-Amino-3,5-diaryl triazoles are usually effected by the acidic rearrangement of 1,2-dihydro tetrazines, which are obtained by the reaction of equimolar amount of arynitriles and hydrazine in refluxing benzene or toluene.⁹⁵



5. Perfluoro alkyl triazole are prepared by the various methods e.g. perfluoro acetonitrile with hydrazine in a sealed tube at room temperature gave 3,5 Substituted-4-aminotriazole.⁹⁶



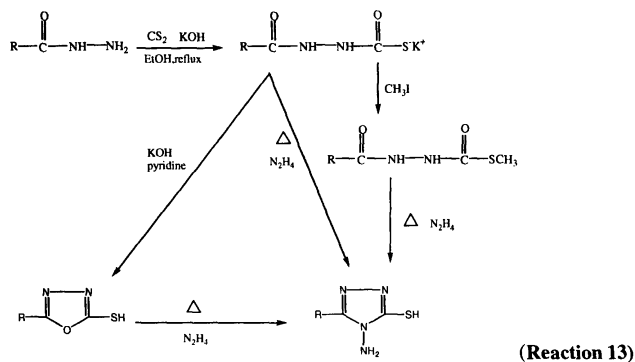
6. 4-amino-3,5-diphenyl triazoles are also formed by the decomposition of other hetero aromatic ring systems e.g. reaction of pyrimidine with hydrazine⁹⁷ and the thermolysis of 5-phenyl tetrazole in various solvents.⁹⁸



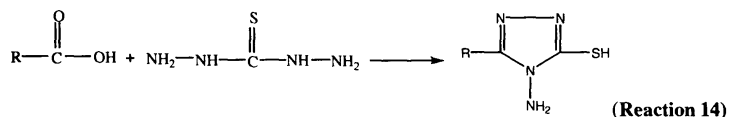
Methods of preparation of 3-(substituted phenyl)-4-amino-5-mercapto-1,2,4-triazole:

1. One of the methods for synthesis of triazole is *Hoggarth synthesis*.⁹⁹ This methodology involves the condensation of carboxylic acid hydrazides with carbon disulphide and potassium hydroxide to yield the potassium dithiocarbazate which after s-alkylation with methyl iodide, undergo ring closure with an excess of hydrazine, to produce the amino thiols in good yields. (Reaction 13)
2. More recently, Heindel and Reid¹⁰⁰ have reported the direct conversion of the carbazate salt by the reaction with excess of hydrazine, this avoids 5-methylation,

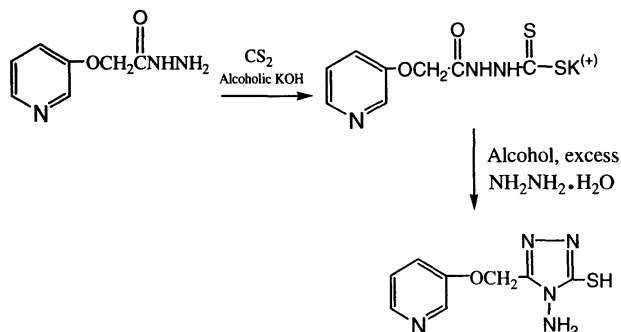
resulting in the higher yield and a shorter reaction time. Alternatively, oxadiazoles can be converted to triazoles by the reaction with hydrazine. (Reaction 13)



- It has also been reported that the carboxylic acids react with thiocarbo-hydrazide to afford directly the 3-substituted-4-amino-5-mercapto-1,2,4-triazoles. It has also been found that the reaction of thiocarbohydrazide with carboxylic acids can be conveniently applied to both aliphatic and aromatic acid derivatives.^{101,102}

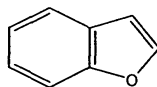


In present study, the method employed for the synthesis of amino triazole ring is that of Ried et al.,¹⁰⁰ the advantage of this method is the better yield obtained however the reaction mixture was stirred for 4-5 hours only, as it was seen that the yields obtained after stirring for 4-5 hours or more were the same. Synthesis of 3-(3-Pyridoxymethyl)-4-amino-5-mercapto-1,2,4-triazole can schematically represent as

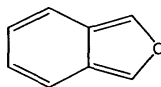


❖ Chemistry of Benzofuran

Two isomeric benzofurans exist. Benzo[b]furan [Structure I], which is the most common isomer was synthesized for first time by Fittig and Ebert¹⁰³ in 1883 and is the subject of several reviews.^{104,105} Benzo[c]furan or isobenzofuran [Structure II], is an essentially stable molecule that is, nevertheless, quite reactive toward dienophiles and oxygen.



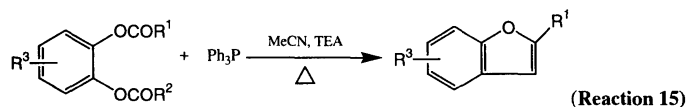
[Structure I]



[Structure II]

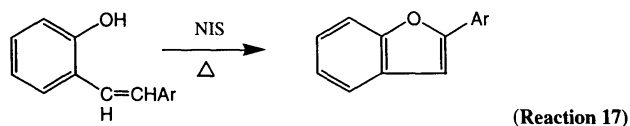
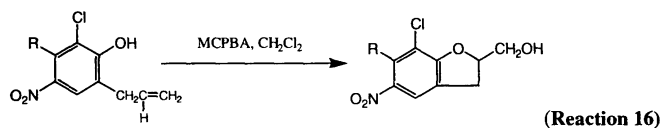
Several methods reported for the synthesis of Benzofuran

1. 2-Acyloxybenzoyl alcohol or bromide reacts with triphenyl phosphine hydrobromide to give the benzylphosphonium salt which is converted by triethylamine into benzofuran.¹⁰⁶

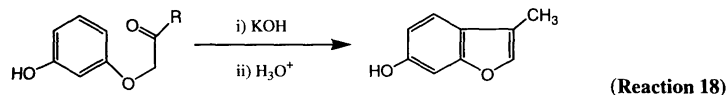


2. Many methods have been described of converting an alkenyl or alkynyl phenol into a benzofuran; whereas alkyne gives furan directly, the alkene usually needs either an additional dehydrogenation (or oxidation) step or a reagent which combines both

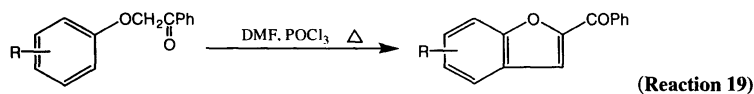
cyclizing and oxidative steps.¹⁰⁷ Osmium tetra-oxideperoxate (OsO_4 , NaIO_4) or 3-chloroperbenzoic acid (MCPBA) are example of such reagents which generate 2,3-dihydrofuran-2-methanol¹⁰⁸ while N-iodosuccinimide yields the furan ring in one step and usually high yield.¹⁰⁹



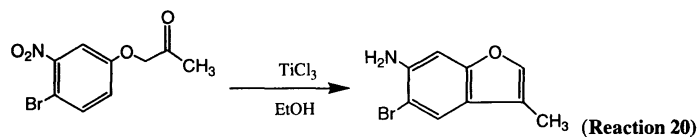
3. Cyclodehydration reactions provide a common route to benzofurans. The cyclodehydration occurs either thermally or chemically and can result in rearrangement products. When aryloxyacetone is treated with standard dehydrating reagents (H_2SO_4 , ZnCl_2 , POCl_3 , KOH etc) the corresponding alkylbenzofuran is isolated.¹¹⁰



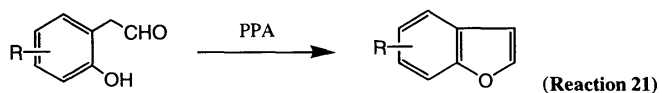
4. Benzofuran also obtained using Vilsmeier reagent, which supply a carbon atom and converts a reactive methyleneoxy group into a furan under mild conditions.^{111,112}



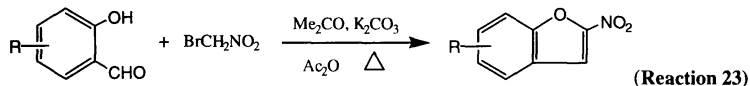
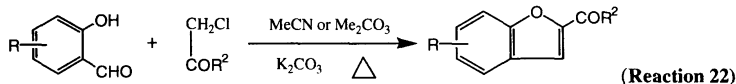
5. The key step in the total synthesis of secofuranoeremophilane utilizes titanium trichloride in ethanol to cyclize ether to benzofuran.¹¹³



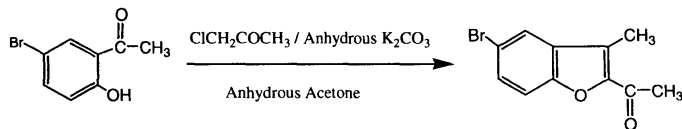
6. Thermal ring closure with subsequent cyclodehydrogenation of ortho-substituted phenols is known as the *Hansch Reaction* and can be demonstrated by the acid-catalyzed cyclization of 2-hydroxybenzaldehyde.¹¹⁴



7. Salicylaldehydes reacts with reactive methylene compounds such as bromonitromethane¹¹⁵ or α -halogenoketones^{116,117} to form good yields of benzofurans.



In present study we synthesized benzofuran moiety using 5-Bromo-2-hydroxyacetophenone and reactive methylene compound i.e. Chloroacetone. Choice of this method is regarded to the desired product, ease of procedure, yield and availability of chemicals, equipments in our laboratory. The synthesis of 5-Bromo-2-hydroxyacetophenone can be represented as



❖ *Chemistry of Diazonium compounds:*

Diazonium compounds or diazonium salts are a group of organic compounds sharing a common functional group with the characteristic structure of $R-N^+X^-$ where, R can be any organic residue such as alkyl or aryl and X is an inorganic or organic anion such as a halogen. Historically, diazonium salts have been developed as important intermediates in the organic synthesis of dyes.

The process of forming diazonium compound is called diazotation, diazoniating, or diazotization. The reaction was discovered by Peter Griess in 1858, which subsequently discovered several reactions of the new compound.

Kinetics of diazotization are very complicated as the nitrosating agents may be nitrous acid, dinitrogen trioxide (N_2O_3) or $NOCl$. N_2O_3 is the more powerful diazotizing agent than nitrous acid.¹¹⁸

Since most of the diazotizations are performed in aqueous solutions, protonated nitrous acid (from sodium nitrite and hydrochloric acid)¹¹⁹ is usually used. The most important method for the preparation of diazonium salts is treatment of aromatic amines such as aniline with sodium nitrite in the presence of a mineral acid. In aqueous solution these salts are unstable at temperature higher than $+5^\circ C$; the $[—\overset{+}{N}\equiv N]$ group tends to be lost as N_2 , i.e. nitrogen gas. Typically diazonium compounds are not isolated and once prepared, used immediately in further reactions.

The liberation of nitrous acid from sodium nitrite and the formation of the diazonium chloride requires two equivalents of acid. In actual practice the amount of acid must be 2.5 to 3 equivalents in order to prevent the combination of benzenediazonium chloride with undiazotized aniline to form a yellow precipitate of diazoamino benzene. Free NO^+ should only appear in concentrated acid and in the course of conversion with complex NO^+ salts.

The other important method for preparing diazonium salts involves the reaction of nitrous acid with an aromatic nitroso compound. Reactive aromatic nuclei, such as

aromatic ring containing powerful donor groups (phenyls and ter. amines), react with nitrous acid to give C-nitroso compound. In the presence of an excess of nitrous acid these compounds react further to give good yield of diazonium salt. Less reactive aromatic compounds can also be converted into diazonium salts through the nitroso compounds, but the reaction sequence under these circumstances is more complicated.¹¹⁸

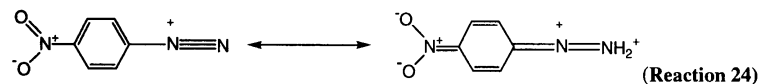
The sequence of reactivity of diazotizing agents is shown as below-



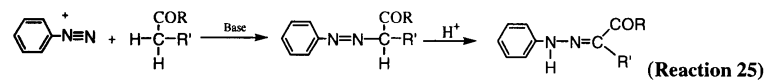
Diazo Coupling

The variety and complexity of behavior shown by diazonium ion is very great indeed and interpretation of their chemistry has been subject of renowned controversies. This has arisen since allowance was not always made for competing equilibria in solution and since several parallel reactions (e.g. ionic, free radical), whose relative impression can be changed by small variations in solvent or reaction condition, can occur.¹²⁰

Arenediazonium ions $[\text{ArN}_2^{(+)}]$ are relatively weak electrophile and hence react only with electron rich aromatic substrate like aryl amines phenols. Aromatic compounds like anisol, mesitylene, acylated anilines or phenolic esters are ordinarily not reactive enough to be suitable substrate. However they may be coupled to activate $[\text{ArN}_2^{(+)}]$. An electron withdrawing substituent in para position of $[\text{ArN}_2^{(+)}]$ increases the electrophilicity of diazo group by concentrating the positive charge at terminal nitrogen.

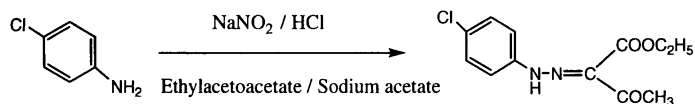


Reaction of an $[\text{ArN}_2^{(+)}]$ species with an aliphatic substrate is possible if a sufficiently acidic C-H bond is present e.g. with β -keto esters and malonic esters.¹²¹ The reaction mechanism is likely to be of (S_{E}^{-1}) type, an electrophilic substitution at aliphatic carbon.



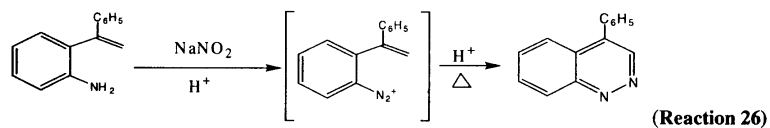
The diazo coupling with C-H acidic aliphatic substrate is a feature of the *Japp-Klingemann reaction*, specifically coupling of $[\text{ArN}_2^{(+)}]$ with 1,3-dicarbonyl substrate ($\text{R}' = \text{COR}$).

In present study we diazotized 4-Chloroaniline hydrochloride using sodium nitrite solution. Thus aryl diazonium salt obtained is coupled with ethyl acetoacetate (C-H acidic aliphatic substrate) to give Ethyl-2-(4-chlorophenyl hydrazono)-3-oxybutarate.

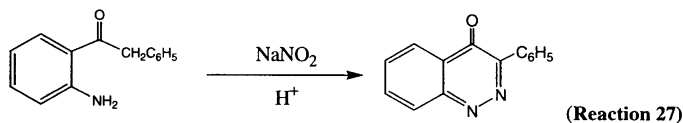


❖ Chemistry of Cinnoline

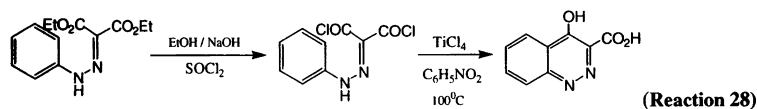
The most widely used method to prepare cinnolines which possess alkyl, aryl or heteroaryl groups in either the 3- and/or 4-position is called the *Widman-Stoermer synthesis*.¹²² Generally, a diazotized o-aminoarylethylene cyclizes upon standing to generate the cinnoline. Diazotization of the amine generates an intermediate diazonium ion, which undergoes slow decomposition and concomitant cyclization to give cinnoline ring.



Similarly, the *Borsche synthesis*¹²³ utilizes the thermal decomposition of a diazonium salt; the corresponding 4-hydroxycinnoline is prepared from the substituted 2-aminoacetophenone.¹²⁴



Hydrazone is convenient starting materials in cinnoline syntheses as shown by the *Friedel-Crafts* cyclization of mesoxalyl chloride phenylhydrazones. Hydrazone is hydrolysed carefully to the diacid, transformed to the corresponding acid chloride, then cyclized in the presence of a Lewis acid to give cinnoline ring system.¹²⁵



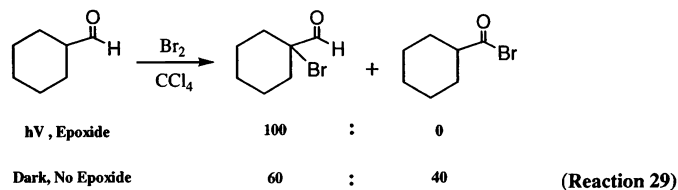
❖ Chemistry of Bromination

Use of various brominating agents for different substrates is well known. Some of the widely used brominating agents are molecular bromine (Br_2), potassium bromate, sodium hypobromate, pyridine hydrobromide perbromide, Quaternary ammonium perhalogenides, N-bromo succinamide etc.

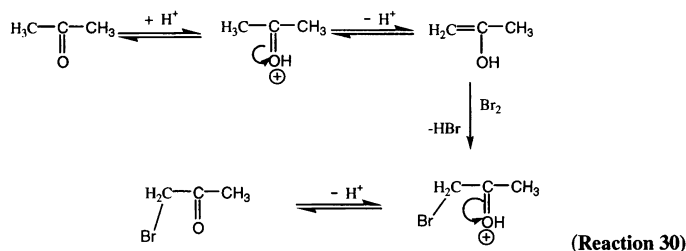
1. Bromine (Br_2)

Molecular bromine is a powerful brominating and oxidizing agent hence it can initiate or participate in ring cleavage and rearrangement. Electrophilic aromatic substitution occurs in presence of Lewis acids to provide brominated aromatics. Monobromination usually occurs due to the deactivating nature of bromine. However, highly reactive aromatics such as phenols, anilines and poly-alkylbenzenes are frequently polybrominated even in absence of catalyst.

Less reactive carboxylic esters are frequently converted to acid halide, α -brominated and subsequently re-esterified in one pot. The bromination of ketones is believed to occur via acid-catalyzed enolization, followed by electrophilic attack on the enol form.¹²⁶ Unsymmetrical ketones can give rise to mixture of bromoketones due to mixture of enols. Radical bromination in presence of epoxides (as acid scavengers) allows for substitution at more highly substituted position. (Reaction No. 29)



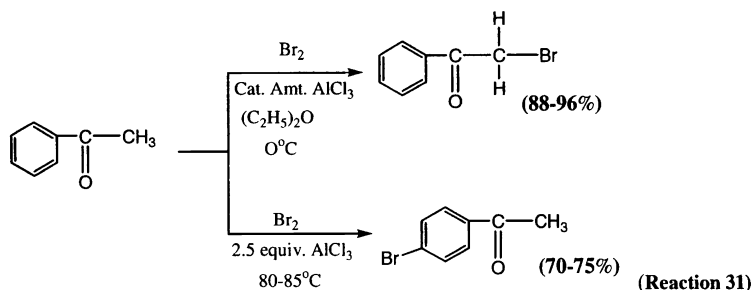
The reaction of ketone with bromine proceeds by acid-catalysed enolation of ketone and subsequent electrophilic attack on enol by bromine; loss of proton from intermediate oxonium ion leads to the bromoketone.



For majority of ketones, which exist largely in keto rather than enol form, enolization is the rate determining step and overall rate of bromination is independent of nature or concentration of bromine except at very low concentration. It should note that formation of the enol requires presence of both, an acid and a base in an inert solvent such as CCl_4 or chloroform. In such a system base present are unprotonated ketones and halide ion. An excess of Lewis acid such as AlCl_3 serves to convert these bases to their conjugate acid and to prevent enolization. The preparation of phenacyl bromide from acetophenone and bromine in presence of catalytic amount of aluminum chloride is well known procedure.¹²⁷ Pearson and Pope¹²⁸ had found that, if sufficient catalyst is used to complex completely the carbonyl compound without solvent and to activate the halogen, side-chain halogenation is suppressed completely and nuclear halogenation takes place. The result brought about by the large excess of catalyst used, of necessity one equivalent to complex the carbonyl compound and of desirability an extra equivalent to complex the halogen and accelerate the rate of reaction, has been called the *swamping catalyst effect*. The acid produces a greater increase in the rate of ring bromination than it does in

the rate of keto-enol interconversion, the rate-determining step in side-chain bromination.

The following equation illustrate use of this technique to prevent bromination α - to a carbonyl group during bromination of an aromatic nucleus.¹²⁹



2. N-Bromosuccinimide (NBS)

N-Bromosuccinimide (NBS) is a reagent which is used in radical substitution and electrophilic addition reactions in organic chemistry. NBS can be considered a one convenient source of bromine.

NBS is commercially available. It can also be synthesized in laboratory by adding sodium hydroxide and bromine to an ice-water solution of succinimide. The NBS product precipitates out and can be collected by filtration. Although NBS is easier and safer to handle than bromine, precautions should be taken to avoid inhalation. NBS will decompose over time giving off bromine. Pure NBS is white, but it is often found to be off-white or brown colored by bromine. In general, reactions involving NBS are exothermic.

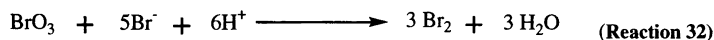
NBS will react with alkenes in an aqueous solvent to give bromohydrins. The preferred conditions are the portionwise addition of NBS to a solution of the alkenes in 50% aqueous DMSO, DMF, THF or tert-butanol at 0°C. Formation of a bromonium ion and immediate attack by water gives strong *Markovnikov addition*.^{130,131} Side reactions includes the formation of α -bromo-ketones and dibromo compounds. These can be minimized by the use of freshly recrystallized NBS.

Standard conditions for using NBS in allylic and/or benzylic bromination involves refluxing a solution of NBS in anhydrous CCl_4 with a radical initiator, usually benzoyl peroxide, irradiation or both to effect radical initiation.¹³² The allylic and benzylic radical intermediates formed during this reaction are more stable than other carbon radicals and the major products are allylic and benzylic bromides. This is also called the *Wohl-Ziegler reaction*. The carbon tetrachloride must be maintained anhydrous throughout the reaction, as the presence of water may likely hydrolyze the desired product.¹³³ Barium carbonate is often added to maintain anhydrous and acid-free conditions.

Electron-rich aromatic compounds, such as phenols, anilines, and various aromatic heterocycles can be brominated using NBS. Using DMF as the solvent gives high levels of para-selectivity.^{134,135}

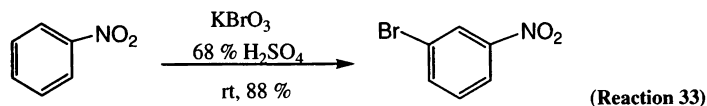
3. Potassium Bromate (KBrO_3)

Potassium bromate is generally used for deactivated aromatics. It is also a convenient source of molecular bromine. Potassium bromate and Sodium bromate react with bromide ion in dilute acid solution to produce bromine.



As such, it is a convenient reagent for the bromination of alkenes, phenols, salicylic acid, aniline and other activated compounds. Synthetically useful brominations of methyl ketones, 3-Substituted cyclohexenones, thiophene, organoboranes and sulfonylhydrazides have been reported.¹³⁶⁻¹⁴⁰ Potassium bromate in absence of added bromide is an effective brominating agent. An efficient procedure for the bromination of deactivated aromatic compound is developed with the use of potassium bromate in sulfuric acid, in the absence of added bromide. For example, nitrobenzene has been brominated (Reaction No. 33) in 88% yield in this fashion. This method is arguably superior to other methods for nitrobenzene bromination.¹⁴¹ Other deactivated aromatics that can be brominated by this method include benzoic acid, halobenzoic acid, phthalic acid, hydrobenzoic acid, acetophenone and benzophenone etc. In the case of

acetophenone, the bromination occurred at aromatic ring and not on the methyl group, which is normal position for attack using molecular bromine.¹⁴²



4. Phenyltrimethylammonium tribromide [PTT] ($\text{PhN}^+\text{Me}_3\text{Br}_3^-$)

It is a quaternary ammonium perhalogenides, being solid compounds, constitute convenient halogen sources. Among this kind of compounds *Pyridine hydrobromide perbromide* is the most popular. *Phenyltrimethylammonium tribromide* (PTT) has the advantages of high stability and ease of preparation.

When dissolved in tetrahydrofuran (THF), PTT is a source of (Br_3^-) ions, the properties of which are different from these of molecular bromine. Particularly, it is much less electrophile and less reactive towards aromatic ring and double bonds, and is thus selective brominating reagent for ketones and ketals when the molecule has double bonds or activated aromatic nuclei which would be attacked by bromine.

Anhydrous THF contributes to the selectivity of the reagents because of the stability of (Br_3^-) ion in this solvent. Moreover, THF acts as buffer by reaction with the liberated hydrobromic acid, which is why PTT in tetrahydrofuran can also be very useful if the substrate bears acid-sensitive functions. The importance of the solvent appears in the bromination of 1,5-cyclooctanedione; in THF, the pure cis-trans-cis isomer is isolated,¹⁴³ whereas in dichloromethane, a mixture of two tetrabromo ketones is obtained.¹⁴⁴ It must be emphasized that anhydrous tetrahydrofuran must be used because small amount of water can greatly retard the rate of bromination of ketones, with resulting decreased selectivity.

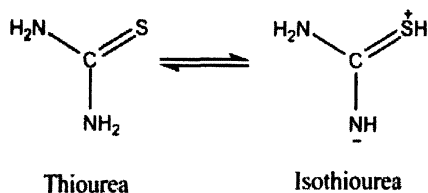
Other uses of this reagent have also been described. Tosylhydrazones undergo oxidation to tosylazoalkenes, using PTT followed by treatment with base; this reaction fails with molecular bromine, dioxane dibromide or N-bromosuccinimide in a range of solvents.¹⁴⁵ PTT has also been recommended for the aromatization of certain 6H-Pyrrolo-benzothiazoles.¹⁴⁶

❖ *Chemistry of Thiourea:*

Thiourea is an organic compound of carbon, nitrogen, sulfur and hydrogen, with the formula CSN_2H_4 or $(\text{NH}_2)_2\text{CS}$. It is similar to urea, except that the oxygen atom is replaced by a sulfur atom. The properties of urea and Thiourea differ significantly because of the relative electro negativities of sulfur and oxygen. Thiourea is a versatile reagent in organic synthesis. "Thioureas" refers to a broad class of compounds with the general structure $(\text{R}^1\text{R}^2\text{N})(\text{R}^3\text{R}^4\text{N})\text{C}=\text{S}$. Thioureas are related to thioamides, e.g. $\text{RC}(\text{S})\text{NR}_2$, where R is ethyl, methyl etc. Moreover, thioureas are important building blocks in synthesis of hetrocycles.

Structure and bonding

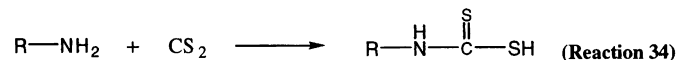
Thiourea is a planar molecule. The $\text{C}=\text{S}$ bond distance is $1.60 \pm 0.1 \text{ \AA}$ for a wide range of derivatives. This indicates that the $\text{C}=\text{S}$ bond is insensitive to the nature of the substituent. Thiourea occurs in two tautomeric forms:



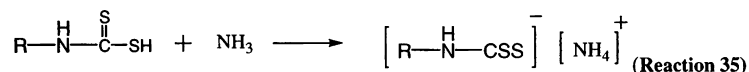
Thioureas, in general, are well defined crystalline compounds of high stability. The main part of chemistry of thioureas consists of reactions, due to their nucleophilic reactivity. The centre of reaction is located either at nitrogen atom or at sulfur atom. Substitution at *single nitrogen* gives 1-monosubstituted, whereas, substitution at both nitrogen atom gives 1,3-disubstituted derivatives of thioureas. The s-substituted thioureas are referred as pseudothiureas. There are several synthetic methods known for preparation of thiourea derivatives. Many variations have been applied to each of these when circumstances demanded it. Thiourea can be prepared from ammonium thiocyanate but more commonly is synthesized by the reaction of hydrogen sulfide with calcium cyanamide in the presence of carbon dioxide. General methods for the preparation of 1-monosubstituted thioureas are:

1. Reaction between carbon disulphide amines:

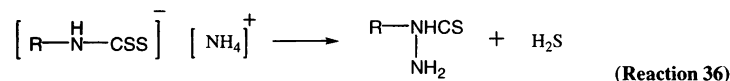
The reaction between carbon disulfide and primary / secondary / aliphatic or aromatic amine gives dithiocarbamic acid at room temperature.



Dithiocarbamic acid when reacted with molar proportion of ammonia, gives ammonium dithiocarbamate.

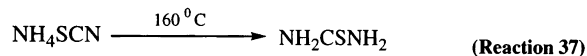


This salt of monosubstituted dithiocarbamate on heating gives 1-monosubstituted-thiourea.

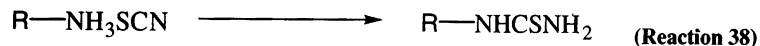


2. Action of alkali thiocyanate on amine hydrochloride:

It has been known that heating ammonium thiocyanate at 160°C for several hours causes it to rearrange to thiourea.



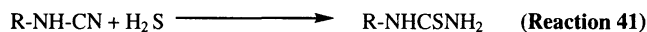
The same rearrangement occurs when the ammonium ion is mono or disubstituted but not when it is tri- or tetra-substituted. Use of this rearrangement is made, in preparing 1-monosubstituted thioureas.



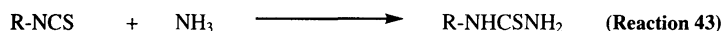
The reaction can be carried out in either in an inert organic solvent or aqueous medium. Chlorobenzene is commonly used as a solvent. It is saturated with hydrogen chloride and the amine and thiocyanate are added. The mixture is then heated at 110-120°C. One mole of the amine is dissolved in water containing 1.3 moles of hydrogen chloride. Ammonium thiocyanate is added and after a reflux period the solution is evaporated to dryness on a steam bath. The residue is heated for several additional hours, taken up in

$$\begin{array}{c}
 \text{R-NH}_2 + \text{NH}_4\text{SCN} + \text{HCl} \longrightarrow \text{NH}_4\text{Cl} + \text{HSCN} \\
 \text{H-S} \equiv \text{C} \equiv \text{N} \\
 \updownarrow \\
 \text{S}=\text{C}=\text{NH} \\
 \xleftarrow{\text{R-NH}_2} \text{R}-\overset{\text{H}}{\underset{|}{\text{N}}}-\overset{\text{S}}{\underset{||}{\text{C}}}-\text{NH}_2
 \end{array}
 \quad (\text{Reaction 39})$$

This method is based on the ability of carbodimides and cyanamides to add H_2S



Primary amines react with thiophosgene to give an isothiocyanate, which reacts with ammonia to give corresponding thioureas.



The reaction of ammonia with isothiocyanates is restricted in measure, because the isothiocyanates can not be prepared very easily. The use of thiophosgene is very much restricted by its being expensive and toxic in nature. Also isothiocyanate formed during the reaction reacts with excess of amine forming 1,3-disubstituted thiourea. The reaction of isothiocyanate with primary amine gives 1,3-unsymmetrical disubstituted thiourea.¹⁵⁰

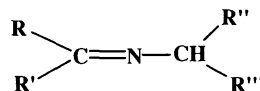
This method is advantageous for the synthesis of 1-monosubstituted thioureas, if an unwanted group is removed easily by hydrolysis.

Thioureas derivatives have attracted much attention due to their lots of biological, pharmacological and industrial applications. A variety of thiourea derivative exhibit analgesic, anti-inflammatory, antitubercular, antimicrobial, anticancer, herbicidal, anti-HIV, antiviral, HDL-elevating, analgesic, antibacterial and antifungal activities.¹⁵¹⁻¹⁵⁴

❖ *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.



Schiff¹⁵⁵ 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.^{156,157} Due to the great flexibility and diverse structural aspects, a wide range of Schiff bases have been synthesized and their complexation behavior studied.¹⁵⁸

Pelton et al¹⁵⁹ had shown the antifungal and antibacterial activity of some substituted salicylaldehydes. Schiff bases from different aldehydes have shown encouraging anti-tuberculosis activity *in vitro* in our laboratory.¹⁶⁰ Also the presence of an azomethine linkage in compounds has been shown to decrease their toxicity considerably.¹⁶¹ Many procedures have been described in the literature for the synthesis of Schiff bases.¹⁶²⁻¹⁶⁴

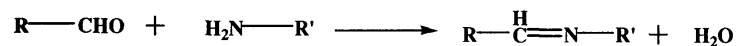
Some important methods are listed below:

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 Clapp¹⁶⁵ 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 al¹⁶⁶ 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 1955, Giovambettista and Rabassa¹⁶⁷ 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.

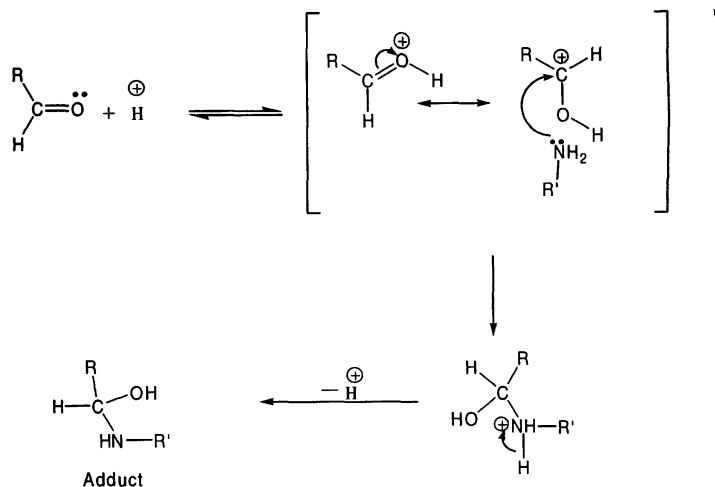
As the first method was found to give good yields it was followed initially. The addition of few drops of acetic acid was found to improve the yield. Later on, during the course of investigation the last method using acetic acid as a solvent was found to be the best for the following reasons.

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.



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

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

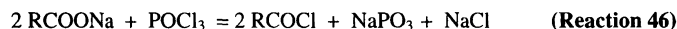


The use of thionyl chloride¹⁶⁸ for the preparation of acid chlorides presents many advantages in a large number of cases. The reaction results in the formation of sulphur dioxide and hydrogen chloride in addition to the acid chloride and the isolation of the chloride are for this reason a simple matter.

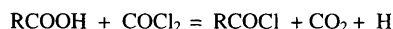


The thionyl chloride must be pure as impure product has been known to cause a somewhat explosive decomposition in some instances. The procedure consists of mixing the chlorinating agent with acid and heating the mixture, if necessary. The product may be isolated by fractional distillation or if solid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

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



The reaction of phosgene¹⁶⁹ with carboxylic acids and p-toluene sulphonylchloride or of sodium chlorosulphonate with salts of carboxylic acids is also used for the preparation of acid chlorides.

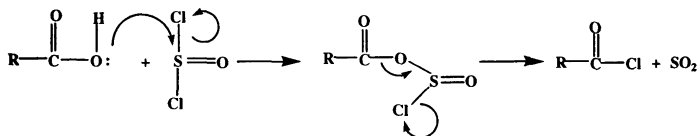


The acid chlorides are obtained readily through the reaction of sulphuryl chlorides with anhydrous salts of organic acids. The reagents are well mixed and subjected to distillation.

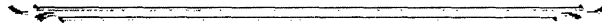
In the present work some acid chlorides were prepared by known methods using corresponding benzoic acids and the some commercially available acid chlorides were used from Sigma-Aldrich Chemicals.

➤ **Reaction mechanism:**

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

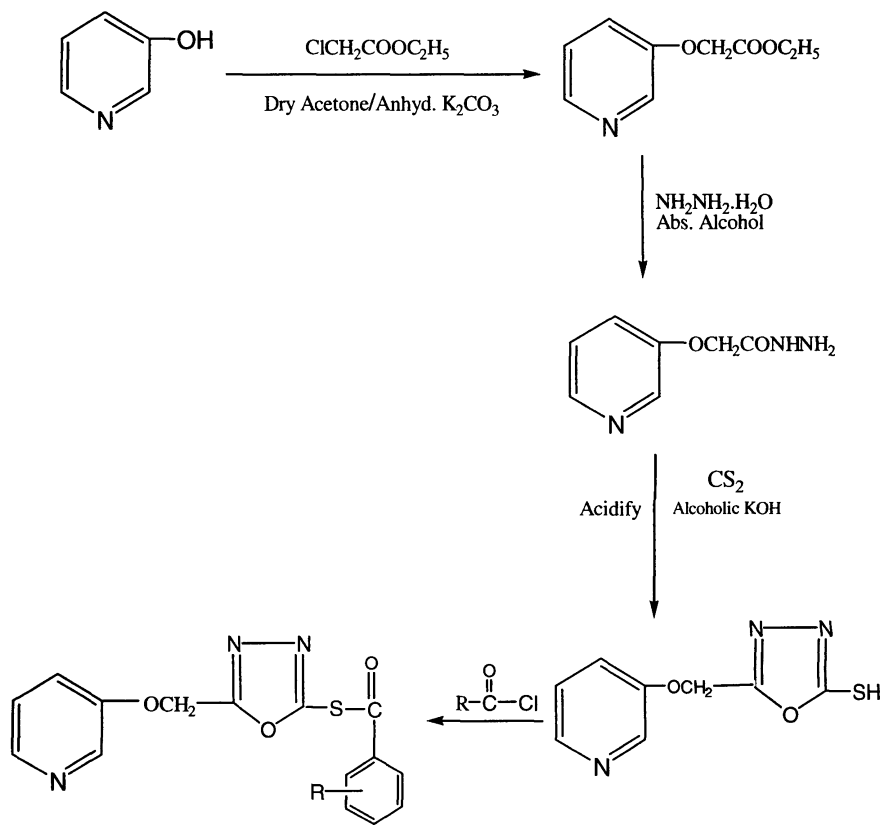


SCHEMATIC REPRESENTATION

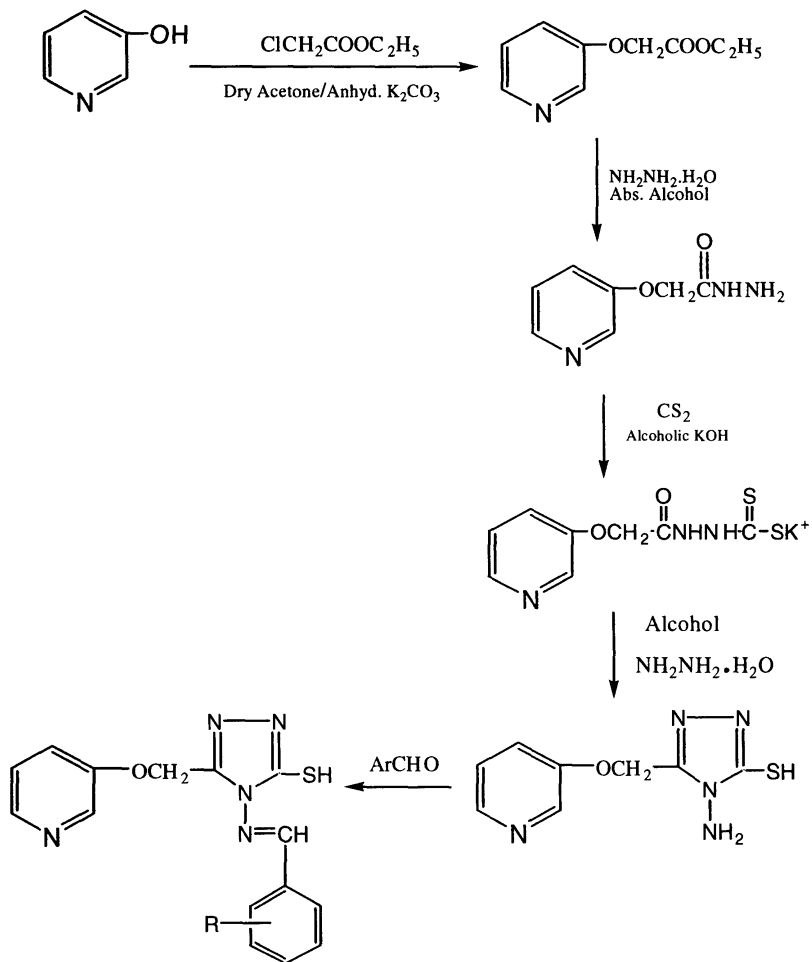


SCHEMATIC REPRESENTATION

Scheme I:- Synthesis of 2-(Substituted thiocarbamoyl)-5-(3-pyridoxymethylene)-1,3,4-oxadiazole

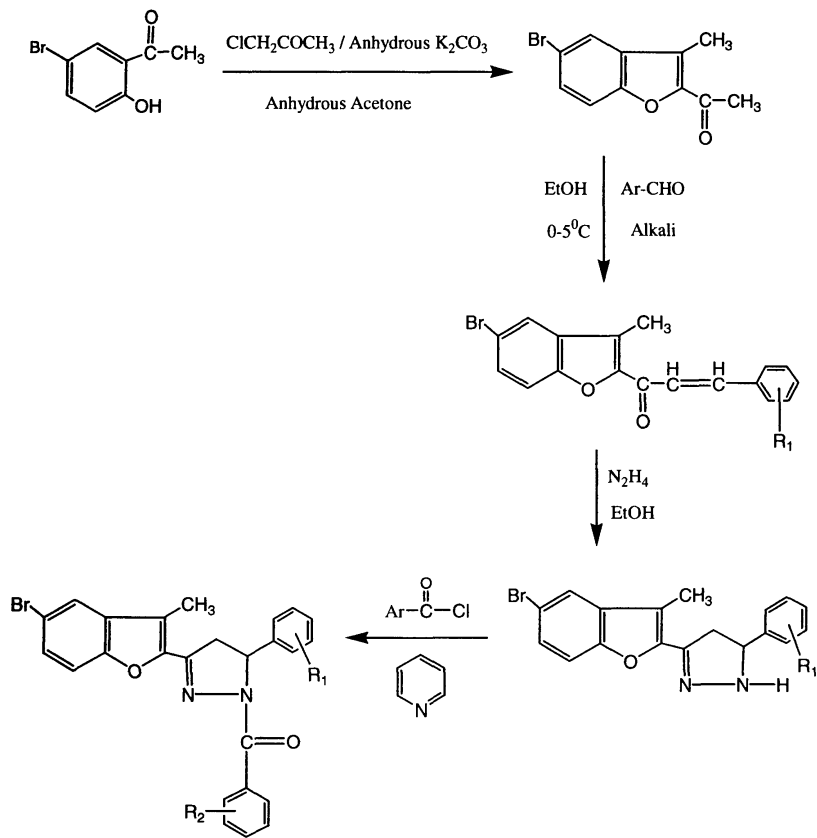


Scheme II :- Synthesis of 3-(3-Pyridoxymethylene)-4-(N-substituted benzylidinylamino)-5-mercapto-1,2,4-triazole



R = -Cl, -F, -OH etc.

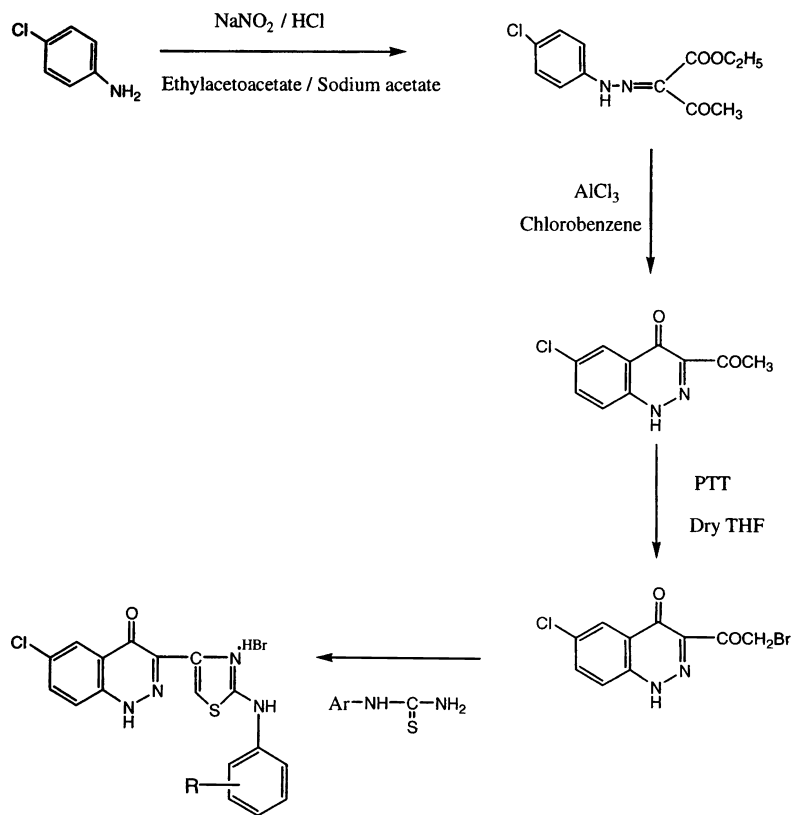
Scheme III :- 5-Aryl-3-(5-bromo-3-methylbenzofuran-2-yl)-1-(substituted benzoyl)-pyrazol-2-enes.



$\text{R}_1 = 3\text{-Cl}, 4\text{-Cl}$ etc.

$\text{R}_2 = 4\text{-F}, 4\text{-Cl}$ etc.

**Scheme IV :- 2-(Substituted anilino)-4-(6-chloro-1H-cinnoline-4-one) thiazole
.HBr**



R = -Cl, -F, -OCH₃ etc.

EXPERIMENTAL



EXPERIMENTAL

Scheme I:- *Synthesis of 2-(Substituted thiocarbamoyl)-5-(3-pyridoxymethylene)-1,3,4-oxadiazole*

▪ Synthesis of 3-pyridoxy acetyl hydrazide (Ia)

A mixture of 3-hydroxy pyridine 9.5g (0.1mole) and anhydrous potassium carbonate 34.5g (0.25mole) was stirred and refluxed in anhydrous acetone for 6 hours. Cooled to room temperature and 11.1ml (0.1mole) of ethyl chloroacetate was added to it dropwise with stirring. After complete addition stirred for one hours and whole reaction extracted with ether.

Excess of solvent was evaporated in vacuum. Residual liquid was added dropwise to a stirred solution of 9.7ml (0.2mole) of hydrazine hydrate in absolute alcohol at room temperature. Mixed well and refluxed for 3 hours.

Excess of solvent was distilled off. After cooling white amorphous solid was obtained. Crystallized from ethanol.

Yield = 48% , M.P.=148⁰C

Elemental Analysis (C ₇ H ₉ N ₃ O ₂)	C %	H %	N %
Calculated	50.29	5.43	25.14
Found	50.40	5.19	24.86

▪ **Synthesis of 2-(3-pyridoxymethyl)-5-mercapto-1,3,4-oxadiazole (Ib)**

To a stirred ethanolic solution of 4g of KOH and 1.67g (0.01mole) of 3-pyridoxy acetyl hydrazide (Ia), 1.8ml (0.03mole) of CS₂ was added at room temperature. Then the reaction mixture was refluxed till evolution of H₂S ceased. Thereafter it was cooled, diluted with cold water and acidified with glacial acetic acid. The white cotton wooly solid separated was washed with water and crystallized from ethanol.

Yield = 66%, M. P. = 125⁰C

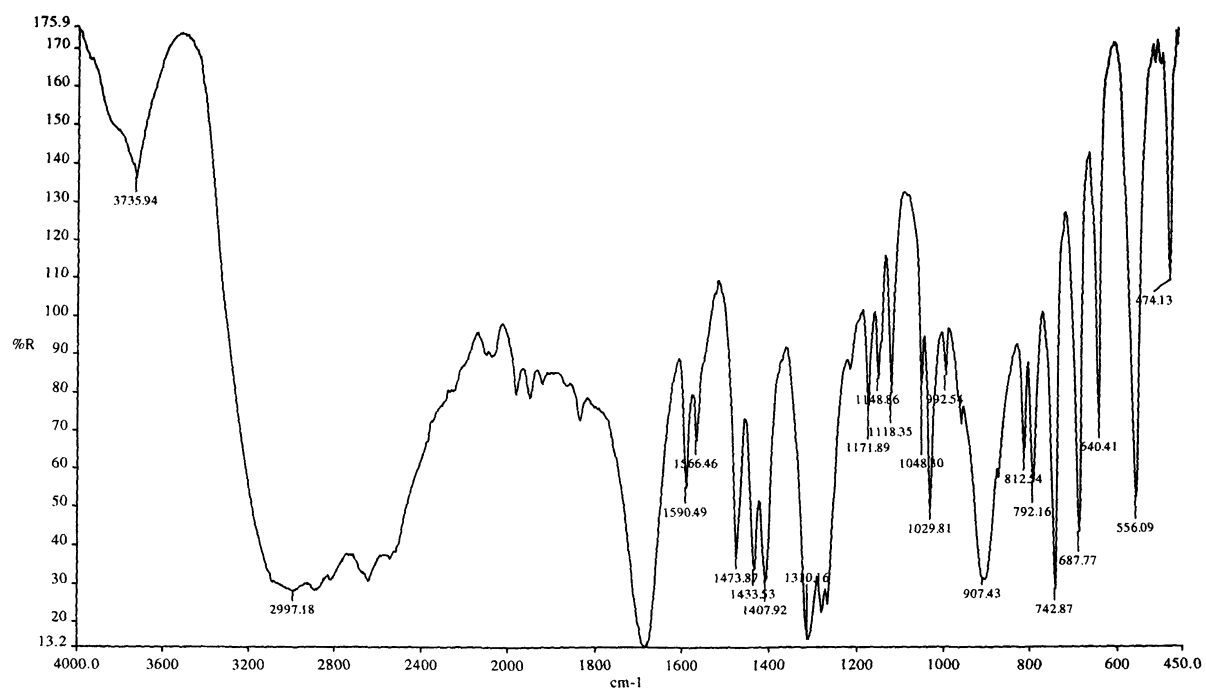
Elemental Analysis (C ₉ H ₁₁ N ₃ O ₂ S)	C %	H %	N %
Calculated	47.99	4.92	18.65
Found	49.53	4.99	18.48

➤ **Synthesis of benzoyl chloride**

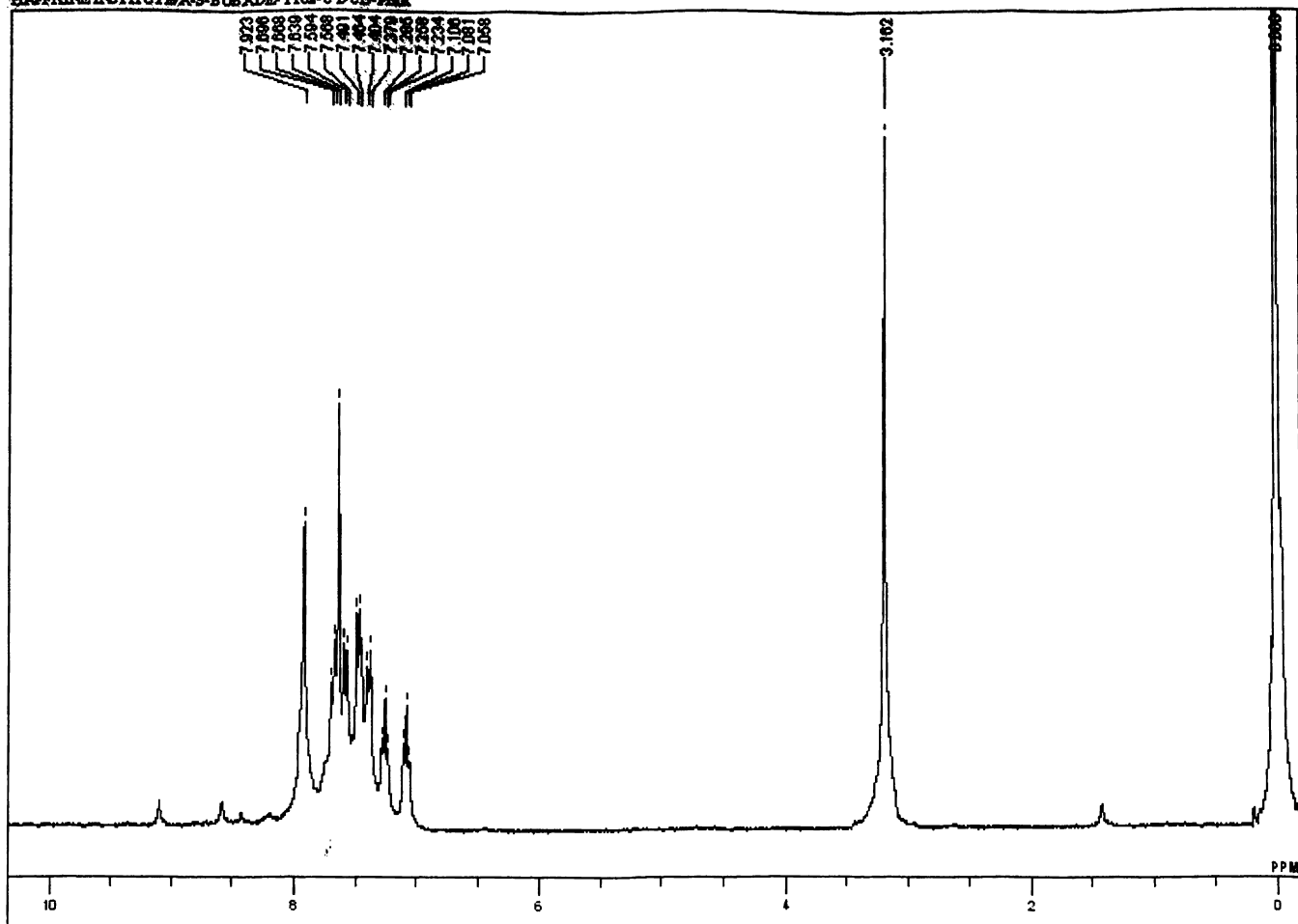
A mixture of 4-Fluorobenzoic acid (24.4 gm, 0.02 mol) and freshly distilled thionyl chloride (32.57 gm, 0.3 mol) in 50 ml dry benzene was taken in 250 ml round bottom flask equipped with reflux condenser and a guard tube. The reaction mixture was refluxed on steam bath with occasional shaking till evolution of HCl gas ceases. Excess of thionyl chloride was distilled out along with benzene. The residual oil was distilled under vacuum.

Yield = 68%, B.P. Observed = 72°C Lit¹⁷⁰ = 69-70°C

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



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HAFKINE INSTITUTE A-S-BORADE V102-C DCD-PMRA



▪ **Synthesis of 2-(3-pyridoxymethyl)-5-(4-bromophenyl)-1,3,4-triazole**

(Compound No. 102, Table No. 1, page no. 59)

Solution of 2.09g (0.01mole) of 2-(3-pyridoxymethyl)-5-mercapto-1,3,4-oxadiazole (**Ib**) and 1.4ml (0.001mole) of 4-bromobenzoyl chloride in dry pyridine was refluxed for 2 hours. Cooled and poured into crushed ice. Solid thus separated was filtered, washed with 20ml of water and crystallized from ethanol.

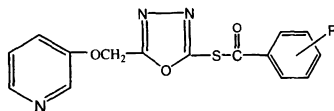
Yield = 58%, M. P. = 160⁰C

IR (KBr) cm⁻¹ -: 2997-3300 (C-H str. Aromatic), 1690 (C=O Str.), 1590 (C=C str. Aromatic), 1310 (C-O-C str Ether linkage), 907 (C-H def Aromatic), 556 (C-Br str).

¹H NMR (CDCl₃) δ ppm -: 3.16 (s, 2H, -OCH₂), 7.0-7.92 (m, 8H, Ar-H).

All other analogs were prepared using similar method, physical properties and elemental analysis results of which are recorded in Table No. 1.

Table No. 1 :- Physical properties of 2-(Substituted thiocarbamoyl)-5-(3-pyridoxymethylene)-1,3,4-oxadiazole derivatives.



Sr. No	R	Yield (%)	M.P. (°C)	Colour & Nature	Elemental Analysis			
					%	C	H	N
101	4-Chloro	55	156	Brown Amorphous Solid	Cal.	51.79	2.87	12.08
					Found	51.63	2.65	11.82
102	4-Bromo	58	160	Brown Amorphous Solid	Cal.	45.91	2.55	10.71
					Found	46.12	2.73	10.56
103	4-Fluro	61	152	Yellow Amorphous Solid	Cal.	54.38	3.02	12.68
					Found	54.11	3.34	12.91
104	2-Bromo	52	168	Gray Amorphous Solid	Cal.	45.91	2.55	10.71
					Found	45.68	2.36	10.88
105	4-Methoxy	47	173	Off White Amorphous Solid	Cal.	55.97	3.79	12.24
					Found	55.61	3.58	12.42

**Scheme II :- Synthesis of 3-(3-Pyridoxymethylene)-4-(N-substituted
benzylidinylamino)-5-mercapto-1,2,4-triazole**

▪ **Synthesis of 3-(3-pyridoxymethyl)-4-amino-5-mercapto-1,2,4-triazole. (IIa)**

To a stirred solution of 4g of KOH in 40ml absolute alcohol 1.67g (0.01mol) of 3-pyridoxyacetyl hydrazide (**Ia**) was added in small portions ensuring the earlier portion was dissolved completely. After that 9.0ml carbon disulfide (0.15mole) was added dropwise ensuring no rise in the temperature of the reaction mixture. During addition yellowish solid separated from the solution and the whole solution thickened up. 150ml of absolute alcohol was added & the mixture was further stirred for next 4-5 hrs. It was *then diluted* with 20ml ether & solid was filtered. Further washing of ether was given to solid and dried in a vacuum at 65°-75°C for 4hrs. M. P. = 168⁰C

A mixture of above obtained, excess of 80% Hydrazine hydrate 9.0ml (0.2mol) and 10ml water 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 ethanol.

Yield = 75%, M.P. = 220⁰C.

Elemental Analysis (C ₁₀ H ₁₆ N ₅ OS)	C %	H %	N %
Calculated	47.22	6.34	27.54
Found	47.55	5.98	27.15

▪ **Synthesis of 3-(3-pyridoxymethyl)-4-(N-3-chlorobenzylidinylamino)-5-mercapto-1,2,4-triazole.** (Compound No. 202, Table No. 2, page no. 62)

In a 100ml round bottom flask, a solution of 3.25g (0.01mole) of 3-(3-pyridoxymethyl)-4-amino-5-mercapto-1,2,4-triazole (**IIa**) in a glacial acetic acid (20ml) and 3-chlorobenzaldehyde (1.40g; 0.01 mol) was refluxed for 20 minutes. The reaction mixture was cooled, diluted with water and the resulting solid was washed with water and crystallized from ethanol.

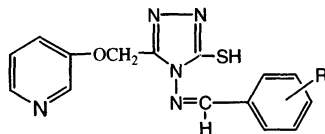
Yield = 52%, M.P. = 218⁰C.

IR(KBr) cm⁻¹ :- 3066-2935 (C-H str. Aromatic), 1505 (N=C str.), 1323 (C-O-C str. Ether linkage), 1128 (C-F str.), 928 (C-H def. Aromatic).

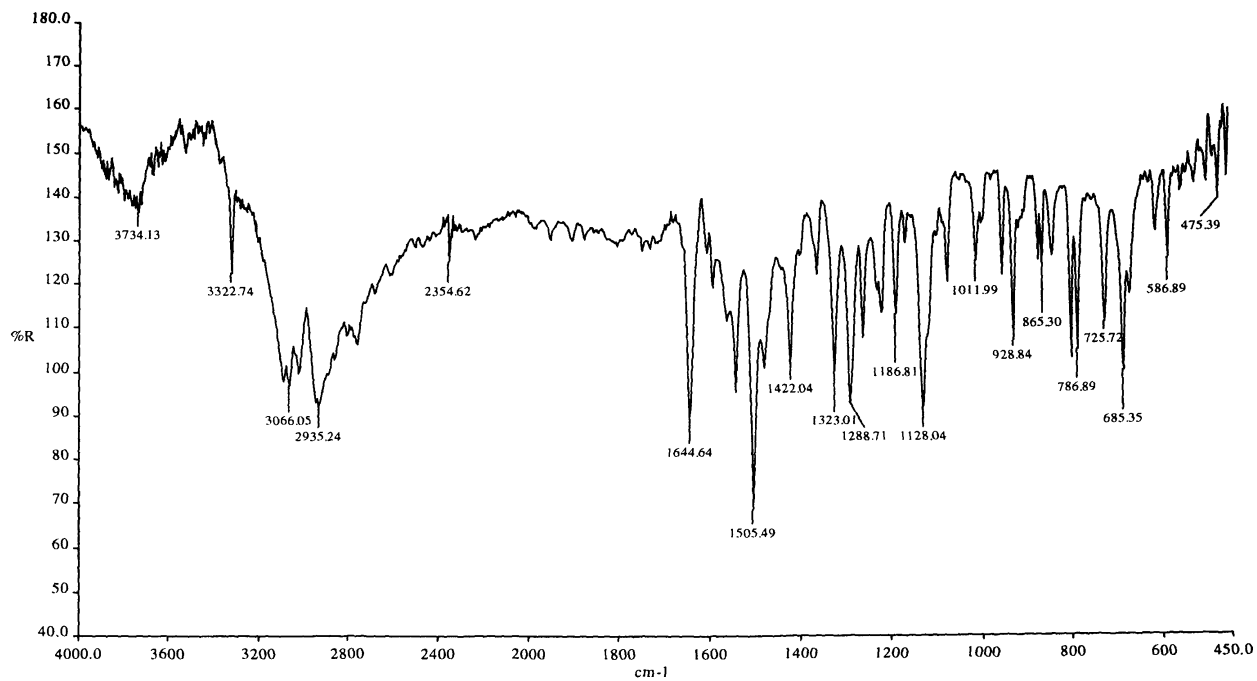
¹H NMR (CDCl₃) δ ppm :- 3.16 (s, 2H, -OCH₂-), 6.9-8.2 (m, 9H, [8H Ar., 1H N=CH-Ar]), 9.87 (s, 1H, S-H).

Similarly other substituted triazole derivatives were prepared by using different substituted benzaldehydes. Particulars of these synthesized compounds are noted in Table No. 2.

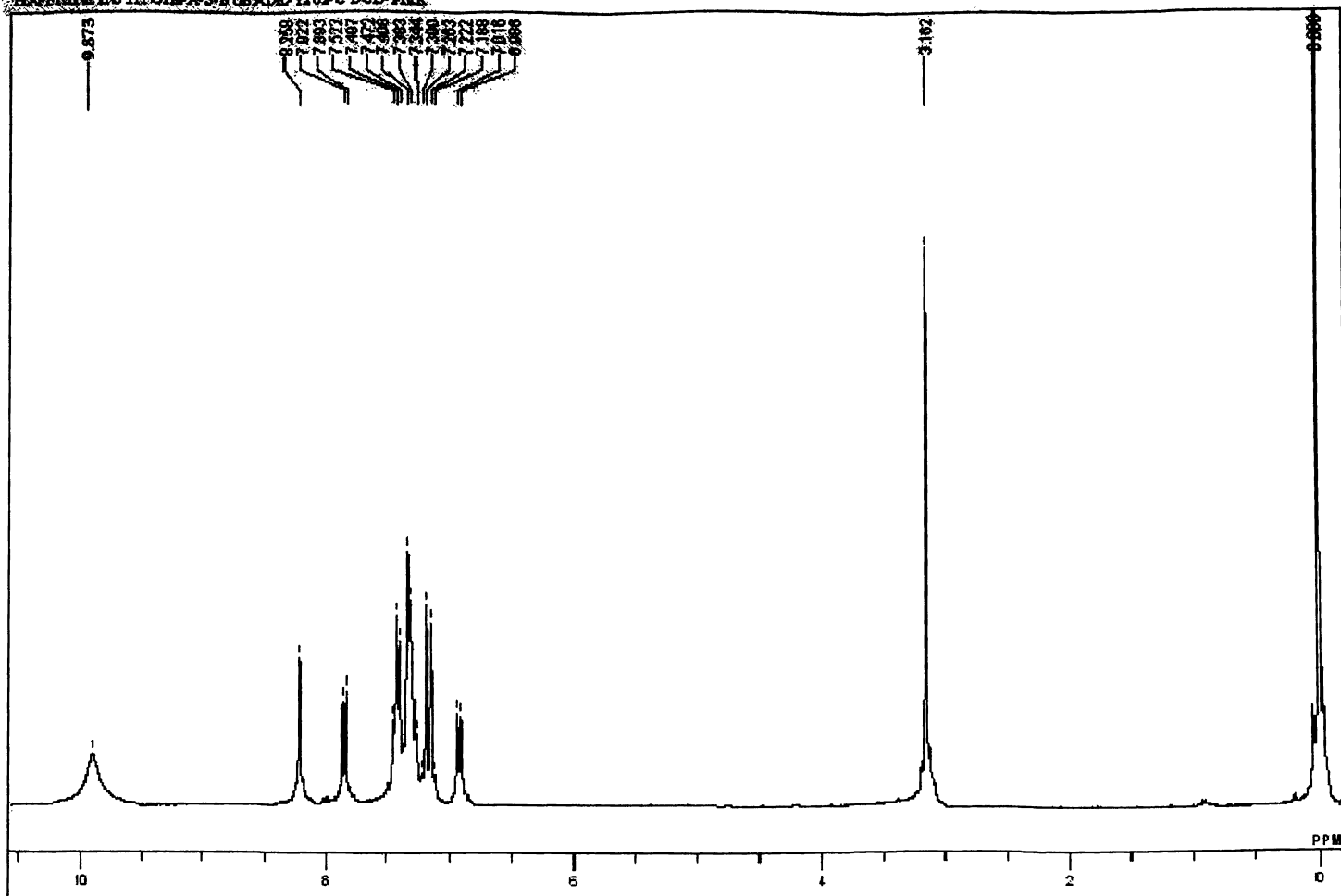
Table No. 2 :- Physical properties of 3-(3-Pyridoxymethylene)-4-(N-substituted benzylidinylamino)-5-mercapto-1,2,4-triazole derivatives.



Sr. No	R	Yield (%)	M.P. (°C)	Colour & Nature	Elemental Analysis			
					%	C	H	N
201	4-Chloro	55	205	Brown Amorphous Solid	Cal.	52.09	3.47	20.26
					Found	52.36	3.30	19.03
202	3-Chloro	52	218	Yellow Amorphous Solid	Cal.	52.09	3.47	20.26
					Found	52.19	3.41	20.17
203	4-Fluro	67	189	Yellow Amorphous Solid	Cal.	54.71	3.64	21.27
					Found	54.85	3.51	21.49
204	4-Hydroxy	56	193	Yellow Amorphous Solid	Cal.	55.04	3.97	21.40
					Found	55.36	3.66	21.23
205	2,4-Dimethoxy	47	198	Yellow Amorphous Solid	Cal.	53.48	4.73	19.49
					Found	53.65	4.93	19.16



DMF- d_6 HAFFKINE DISTILLATE A-S-B OBADE Y202-C DCB-PMRA1
HAFFKINE DISTILLATE A-S-B OBADE Y202-C DCB-PMR



Scheme III :- 5-Aryl-3-(5-bromo-3-methylbenzofuran-2-yl)-1-(substituted benzoyl)-pyrazol-2-enes.

▪ **Synthesis of 5-Bromo-3-methyl-2-acetylbenzofuran (IIIa)**

To stirred mixture of 2.15g (0.01mole) of 5-Bromo-2-hydroxy acetophenone and 3.45g (0.025mole) of anhydrous K_2CO_3 was added, 1.0ml (0.01mole) of chloroacetone dropwise over a period of 10 minutes. Reaction mixture was stirred for 3 hours. Allowed to cool and poured into crushed ice. Solid thus separated was filtered washed with 20ml cold water, crystallized with ethanol.

Yield = 60%, M. P. = $183^{\circ}C$

Elemental Analysis ($C_{11}H_9BrO_2$)	C %	H %	N %
Calculated	52.20	3.58	-
Found	52.45	3.73	-

▪ **Synthesis of 5-Bromo-2-cinnamoyl-3-methylbenzofurans (IIIb)**

To a stirred solution of 2.52g (0.01mole) of 5-Bromo-3-methyl-2-acetylbenzofuran (**IIIa**) in 10ml of anhydrous ethanol was added 1.40g (0.01mole) of 3-chlorobenzaldehyde at room temperature. The reaction mixture was cooled to 0-5°C and aqueous solution of sodium hydroxide (70%, 5ml) was added in portion while stirring. After complete addition stirring was continued for 3 hours and contents allowed to stand overnight. The solution was diluted with water and neutralized with dilute HCl. The off-white colored solid thus separated was collected, dried and crystallized from ethanol.

Yield = 55%

M. P. = 135°C

Elemental Analysis (C ₁₈ H ₁₂ BrClO ₂)	C %	H %	N %
Calculated	57.55	3.22	-
Found	57.68	2.94	-

▪ **Synthesis of 5-(3-Chlorophenyl)-3-(5-bromo-3-methylbenzofuran-2-yl)-1H-pyrazolines (IIIc)**

A mixture of 3.91g (0.01mole) of 5-Chloro-2-cinnamoyl-3-methyl benzofuran and 2.5g (0.15mole) of hydrazine hydrate in 50ml anhydrous ethanol was refluxed for 3 hours. Excess of solvent was distilled off. Solid collected and crystallized from methanol.

Yield = 75%, M. P. = 146⁰C

Elemental Analysis (C ₁₈ H ₁₄ BrClN ₂ O ₂)	C %	H %	N %
Calculated	55.48	3.62	7.19
Found	55.68	3.95	7.43

▪ **Synthesis of 5-(3-Chlorophenyl)-3-(5-bromo-3-methyl benzofuran-2-yl)-1-(4-fluorobenzoyl)-pyrazolines.** (Compound No. 303, Table No. 3, page no. 66)

A mixture of 1.94g (0.005mole) of 1H-pyrazoline (IIIc) and 0.6ml (0.005mole) of 3-Fluorobenzoyl chloride in anhydrous pyridine (10ml) was refluxed for one hour. Then neutralized with cold dilute HCl. The solid separated was filtered and crystallized from ethanol.

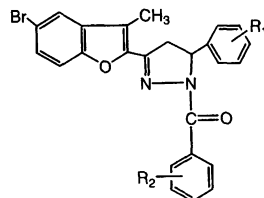
Yield = 67%, M. P. = 189⁰C

IR(KBr) cm⁻¹ -: 3177-3071 (C-H str. Aromatic), 1689 (C=O str.), 1555 (N=C str. Pyrazoline), 1263 (C-O-C str. Benzofuran), 1065 (C-F str.), 825 (C-H def. Aromatic), 722 (C-Cl str.)

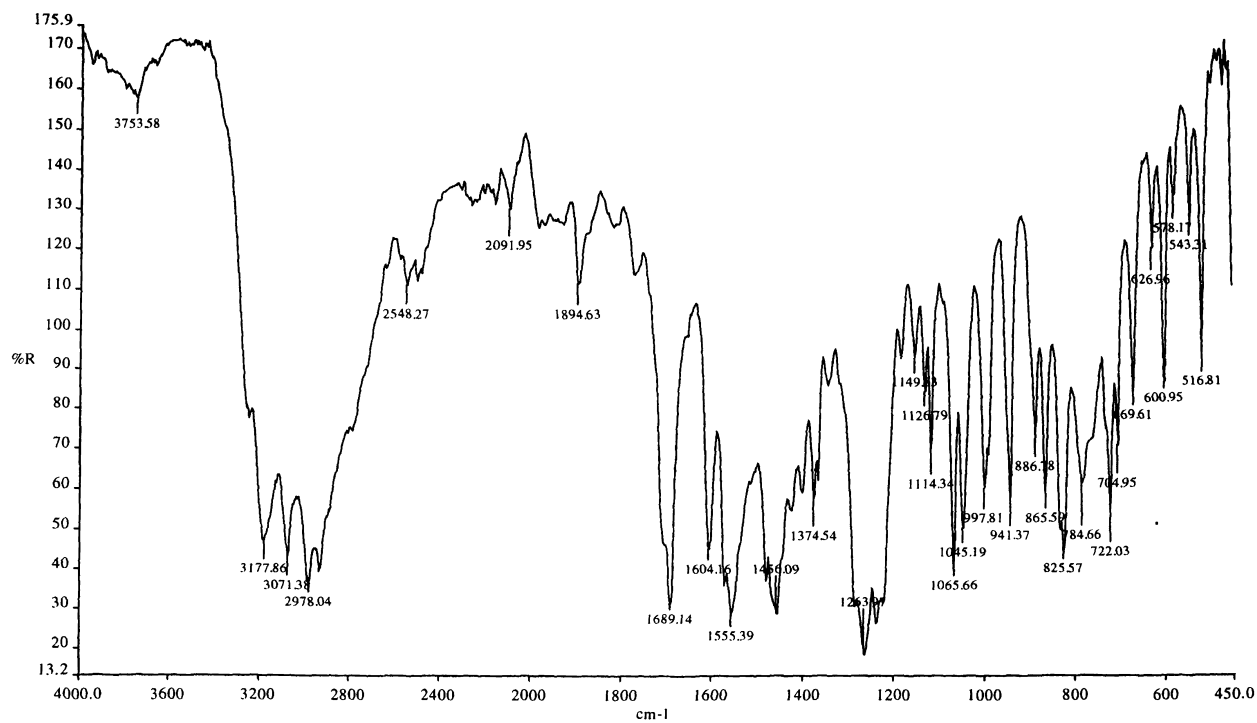
¹H NMR (CDCl₃) δ ppm -: 1.67 (s, 3H, -CH₃), 2.29-2.30 (d, 2H, -CH₂, Pyrazoline), 3.67-3.82 (t, 1H, -CH, Pyrazoline), 6.78-7.48 (m, 11H, Aromatic).

All other substituted derivatives were prepared in similar manner and physical data is recorded in Table No. 3.

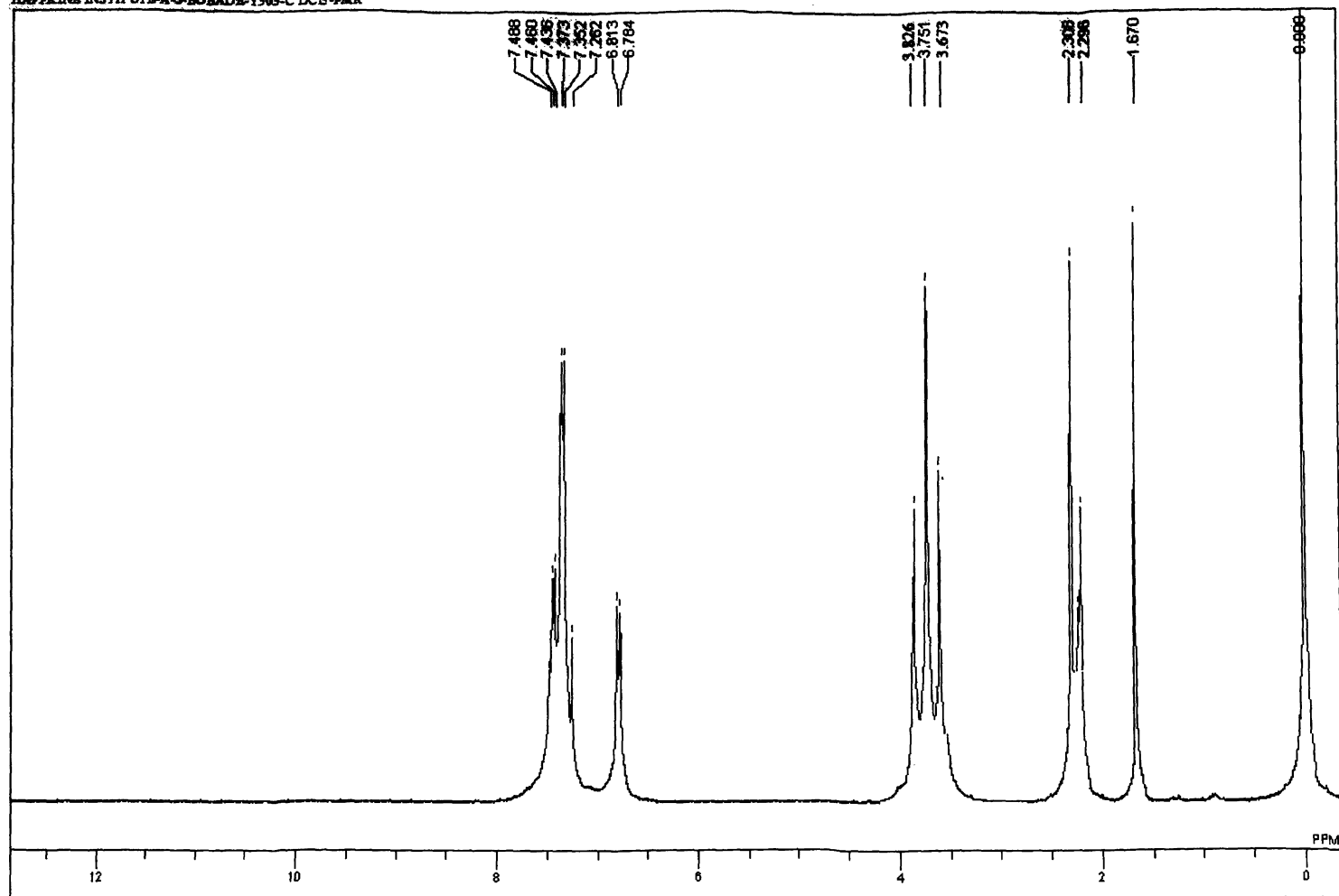
Table 3:- Physical properties of 5-Aryl-3-(5-bromo-3-methylbenzofuran-2-yl)-1-(substituted benzoyl)-pyrazol-2-enes



Sr. No	Substituent	Yield (%)	M.P. (°C)	Colour & Nature	Elemental Analysis			
					%	C	H	N
301	R ₁ = 3-Chloro	55	205	Off White Amorphous Solid	Cal.	56.92	3.22	5.31
	R ₂ = 4-Chloro				Found	56.67	3.36	5.48
302	R ₁ = 4-Chloro	52	218	Brown Amorphous Solid	Cal.	56.92	3.22	5.31
	R ₂ = 4-Chloro				Found	56.59	3.06	5.56
303	R ₁ = 3-Chloro	67	189	Brown Amorphous Solid	Cal.	58.65	3.32	5.47
	R ₂ = 3-Fluro				Found	58.25	3.54	5.63
304	R ₁ = 4-Chloro	56	193	Off White Amorphous Solid	Cal.	58.88	3.53	5.49
	R ₂ = 4-Hyroxy				Found	58.92	3.37	5.61
305	R ₁ = 4-Chloro	47	198	White Amorphous Solid	Cal.	58.64	3.80	5.06
	R ₂ = 2,4-Dimethoxy				Found	58.75	3.99	5.41



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HAFKINE INSTITUTE-A-S-BORADE-Y303-C DCE-PMR



**Scheme IV :- 2-(Substituted anilino)-4-(6-chloro-1H-cinnoline-4-one) thiazole
.HBr**

▪ **Synthesis of Ethyl-2-(4-chlorophenyl hydrazono)-3-oxybutarate (IVa)**

To the magnetically stirred 50ml 50% HCl , 4g (0.03mole) of finely powdered 4-Chloroaniline was slowly added at room temperature. Solution allowed to stirred for 30 min to form hydrochloride salt of 4-chloroaniline. That suspension was cooled to 0-5⁰C using salt-ice bath. To this cooled suspension, saturated solution of 4.14g (0.06mole) of NaNO₂ was added into the solution through the funnel, taking care that reaction temperature should not raised above 5⁰C.

After 10 minutes of complete addition of NaNO₂ solution, the presence of excess of nitrous acid in reaction mixture is checked by using starch-iodine paper test.

Cooled diazonium salt solution thus obtained was slowly added (through cotton filter if required) into the stirred solution of 4.0ml of Ethylacetoacetate, 40ml ethanol, 49.20g of Sodium acetate and 40ml water, at 5-10⁰C.

Yellow coloured product obtained is filtered and crystallized through 80% methanol to obtained fine yellow crystals.

Yield = 70 %

M. P. Lit¹⁷¹ = 94⁰C, Obtained = 94⁰C.

Elemental Analysis (C ₁₃ H ₁₅ ClN ₂ O ₃)	C %	H %	N %
Calculated	53.64	4.88	10.43
Found	53.48	4.72	10.11

▪ **Synthesis of 3-Acetyl-6-chloro cinnoline-4-one (IVb)**

Solution of 2.68g (0.01mole) of Ethyl-2-(4-chlorophenyl hydrazono)-3-oxybutarate **[IVa]** in 15ml of anhydrous chlorobenzene was heated upto 50°C on oil bath in a round bottom flask equipped with water condenser and guard tube. 2.6g (0.02mole) of AlCl₃ was added to it carefully in portions. After complete addition reaction mixture was refluxed for 4-5 hours and allowed to cool. Then 20ml of dilute HCl was added to it and heated on water bath to decompose the complex formed.

Cooled to room temperature and filtered the greenish yellow solid product obtained. Crystallized with methanol.

Yield = 65%

M. P. Lit¹⁷¹ = 207°C, Obtained = 205°C

Elemental Analysis (C ₁₀ H ₇ ClN ₂ O ₂)	C %	H %	N %
Calculated	53.95	3.17	12.58
Found	53.48	3.32	12.21

▪ **Synthesis of selective brominating agent Phenyl trimethyl ammonium tribromide (PTT)¹⁷²**

Step I :- Preparation of Phenyltrimethyl ammonium sulfomethylate.

Solution of 12.1ml (0.01mole) of freshly distilled N,N – Dimethyl aniline in 50ml of anhydrous toluene was prepared in 250ml flat bottom flask equipped with thermometer, magnetic stirrer and water condenser. Solution was stirred and heated at 40⁰C for one hour. Heating was stopped and 9.0ml (0.095mole) of dimethyl sulfate was added dropwise within 20 minutes. Colourless sulfomethylate starts to crystallize and temperature rise was observed upto 60⁰C. Reaction stirred at room temperature for 1.5 hour then heated on water bath for one hour.

Allowed to cool and excess of solvent was decanted, as sulfomethylate formed was highly hygroscopic, difficult to filter and melting point determination. Product obtained was dried in vacuum at 50-60⁰C.

Yield = 45%

Step II :- Preparation of Phenyltrimethyl ammonium tribromide (PTT)

Solution of 15.0g (0.06mole) of sulfomethylate in 15ml of 48% hydrobromic acid diluted with 15ml of water was magnetically stirred in 100ml round bottom flask. 3.28ml (0.064mole) of bromine was added slowly to above stirred solution over 20 minutes. An orange-yellow precipitate starts to separates out. Reaction mixture stirred for 5-6 hour at room temperature.

The product obtained was filtered, washed with 10ml water, crystallized from acetic acid and air dried under efficient hood.

Yield = 80%

M.P. Lit¹⁷² = 113-115⁰C, Obtained = 115⁰C

▪ **Synthesis of 3-Bromoacetyl-6-chloro-1H-cinnoline-4-one (IVc)**

Suspension of 1.11g (0.005mole) 3-Acetyl-6-chloro cinnoline-4-one **[IVb]** in 10ml of anhydrous (sodium dried) tetrahydrofuran (THF) was stirred in 50ml flat bottom flask. 1.88g (0.005mole) of phenyltrimethyl ammonium tribromide (PTT) was added to stirred suspension at room temperature. Milky yellowish precipitates starts to generate. Stirred 30 minutes after complete addition, product obtained was filtered and dried in vacuum. Product obtained was pure enough and no further purification required.

Yield = 85%, M. P. = 185⁰C

Elemental Analysis (C ₁₀ H ₆ BrClN ₂ O ₂)	C %	H %	N %
Calculated	39.83	2.01	9.29
Found	39.66	2.32	9.55

▪ **Synthesis of 4-Fluorophenyl thiourea**

Solution of 1.64g (0.01mole) of dry 4-Fluoroaniline hydrochloride, 0.97g (0.01mole) of potassium thiocyanate in anhydrous chlorobenzene was refluxed for 6 hours. Solid thus obtained was crushed, filtered, washed with water and crystallized with 80% methanol.

Yield = 70%, M. P. = 173⁰C

Elemental Analysis (C ₁₀ H ₆ BrClN ₂ O ₂)	C %	H %	N %
Calculated	52.45	2.59	14.39
Found	52.19	2.32	14.65

All other substituted thioureas used in this work were prepared in similar manner.

▪ **Synthesis of 2-(4-Fluorophenylanilino)-4-(6-chloro-1H-cinnoline-4-one)thiazole .HBr** (Compound No. 405, Table No. 4, page no. 72)

Solution of 1.5g (0.005mole) of 1H-cinnolinone (**IVc**) and 4-Chloro phenyl thiourea 0.93g (0.005mole) in 10ml of ethanol were prepared separately, mixed in round bottom flask and refluxed for 5 hours. Solid thus obtained was filtered, washed with 10ml of water and crystallized from methanol.

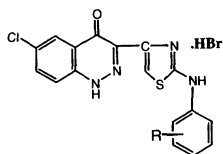
Yield = 60%, M. P. = 190⁰C

IR (KBr) cm⁻¹ -: 3736 (N-H str.), 3200–3000 (C-H str. Aromatic), 1690 (C=O str.), 1400–1600 (Thiazole), 1087 (C-F str.)

¹H NMR (CDCl₃) δ ppm -: 6.51 (s, 1H, –CH, Thiazole), 7.26–7.87 (m, 7H, Aromatic), 9.77 (s, 2H, N-H, Cinnilnone & Anilino)

All other substituted analogs were prepared in similar manner physical properties and elemental analysis of which are recorded in Table No. 4.

Table No. 4 :-
Physical properties and Elemental analysis of 2-(Substituted anilino)-4-(6-chloro-1H-cinnoline-4-one) thiazole .HBr

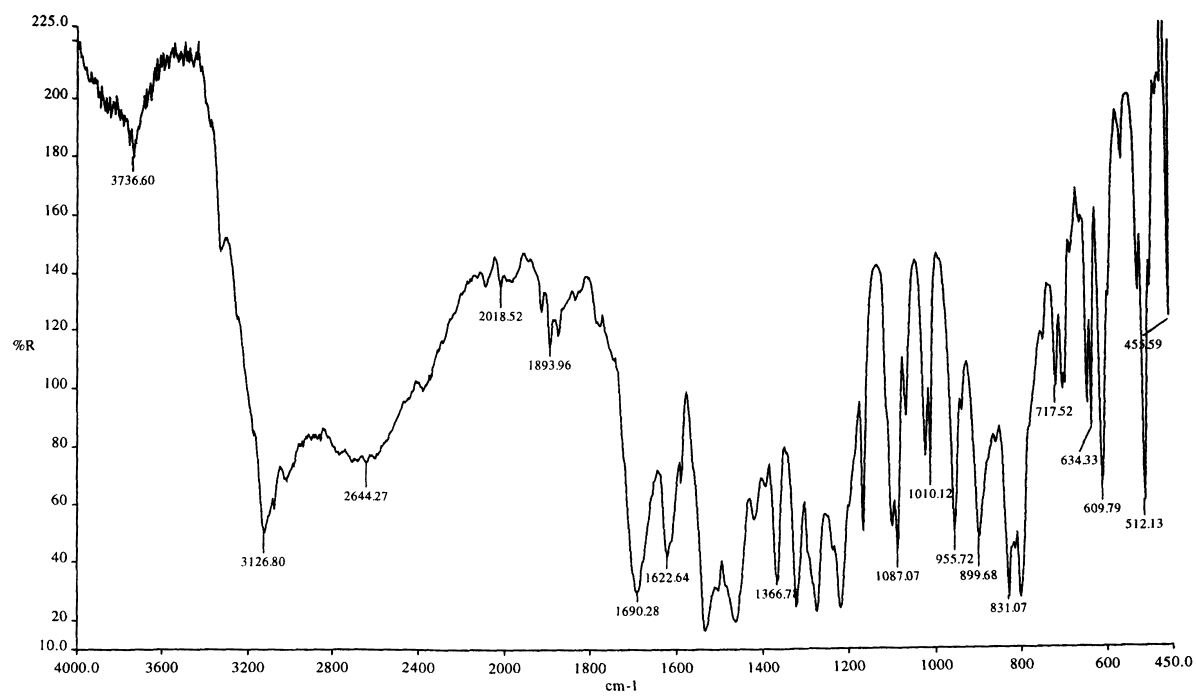


Sr. No	R	Yield (%)	M.P. (°C)	Colour & Nature	Elemental Analysis			
					%	C	H	N
401	4-Methyl	55	205	Yellow crystalline solid	Cal.	48.07	3.14	12.46
					Found	48.29	3.25	12.06
402	3-Methoxy	52	218	Yellow crystalline solid	Cal.	46.42	3.03	12.03
					Found	46.16	3.23	12.42
403	4-Chloro	67	189	Yellow crystalline solid	Cal.	43.43	2.36	11.92
					Found	43.52	2.56	11.63
404	2-Chloro	56	193	Yellow Amorphous solid	Cal.	43.43	2.36	11.92
					Found	43.18	2.58	11.79
405	4-Fluro	47	198	Yellow crystalline solid	Cal.	45.00	2.44	12.35
					Found	45.35	2.19	12.63

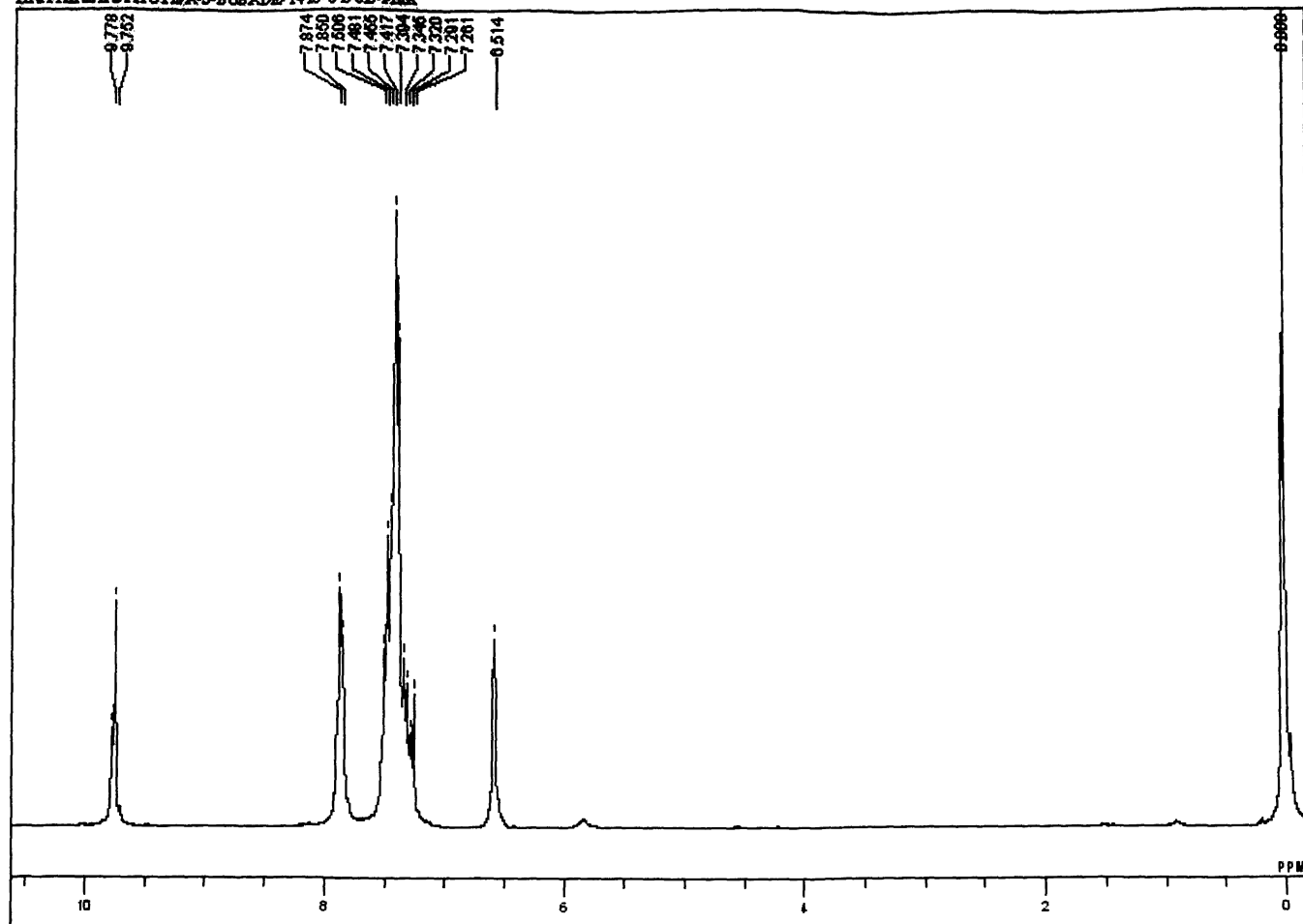
Table Continued.....

Table No. 4 Continued.....

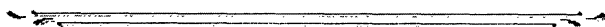
Sr. No	R	Yield (%)	M.P. (°C)	Colour & Nature	Elemental Analysis			
					%	C	H	N
406	4-Bromo	55	205	Yellow crystalline solid	Cal.	39.68	2.15	10.89
					Found	39.86	2.43	10.68
407	3-Chloro-4-Fluro	52	218	Yellow Amorphous solid	Cal.	41.83	2.06	11.48
					Found	41.62	2.28	11.25
408	3,5-Dichloro	67	189	Yellow Amorphous solid	Cal.	40.46	2.00	11.10
					Found	40.29	2.34	11.39
409	2,3-Dichloro	56	193	Brown crystalline solid	Cal.	40.46	2.00	11.10
					Found	40.19	2.16	11.24
410	3,4-Dichloro	47	198	Yellow crystalline solid	Cal.	40.46	2.00	11.10
					Found	40.68	1.84	11.49



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HAFKINE INSTITUTE A-3-B0BADE Y4 10-C DCB-PMRA



*BIOLOGICAL SCREENING, RESULTS AND
DISCUSSION*



BIOLOGICAL SCREENING, RESULTS AND DISCUSSION

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

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

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

➤ Drug Stock Solution:

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

➤ Controls:

1. **Media Control:** Sterilized medium was kept as a control so as to assure the sterility of the medium. If this control shows growth of any type then results are considered invalid.
2. **Culture Control:** It contains sterilized medium and culture of the organism. It should show growth in the medium, which assures optimum pH and sufficient nutrients to promote growth. If no growth is observed, then the medium is considered to be faulty and results are not reliable.

3. **Solvent Control:** It contains sterilized medium, solvent used to dissolve the drug and the suspended culture in it. Organism should show growth in the solvent used for preparation of the drug stock solution. If the growth is not observed, it can be concluded that solvent inhibits the growth of the organism and hence in that case results are considered invalid.
4. **Turbidity Control:** It contains medium and the drug stock solution. The turbidity is observed against the growth in different drug dilutions. It assures reliability against misinterpretation between growth and turbidity due to the drug.
5. **Standard Control:** It contains medium, organism culture and dilution of the standard drug stock solution corresponding to that of the drugs under test. The inhibition of growth was observed against that in standard.

➤ **Antibacterial Screening:**

The test compounds have been subjected to *in vitro* screening against Gram-positive *S. aureus* and Gram-negative *S. typhi* using tube dilution technique.¹⁷³

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

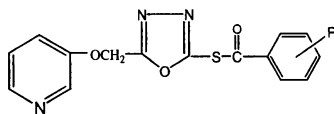
Innoculum of standard suspension (0.1ml of the test organism strain which contains 10^6 bacilli/ml) was added. The tubes were incubated at 37°C for 48 hours and then examined for the presence or absence of the growth of the organism. The lowest concentration, which showed no visible growth, was taken as the Minimum Inhibitory Coefficient (MIC). Ampicillin (MIC= 0.01µg/ml against Gram-positive *S.aureus*) and Trimethoprim (MIC= 1.0µg/ml against Gram-negative *S.typhi*) were used as the standard drugs.

➤ **Antifungal Screening:**

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

Sabraoud's medium was used as the growth media (pH=5.6). The sterile media was dispensed in a sterile borosilicate test tube. The drug solution was added in order to get the final drug concentrations of 200, 100, 50, 20, 10, 5, 2 µg/ml etc. The tubes were incubated at room temperature (28-30°C) in a dark place. *C. albicans* and *A. niger* were incubated for 7 days. The presence or absence of the growth was observed visually. The lowest concentration at which no growth was observed was taken as the end point (MIC). Miconazole (MIC= 6.25 µg/ml against *C. albicans* and *A. niger*) was used as a standard drug.

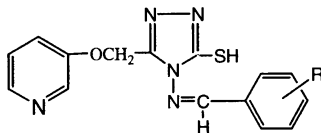
Table No. 1 :- Biological screening of 2-(Substituted thiocarbamoyl)-5-(3-pyridoxymethylene)-1,3,4-oxadiazole derivatives.



Comp. No.	Substituents R	Anti Microbial Activity (μ /ml) [MIC]			
		Anti bacterial		Anti Fungal	
		S. aureus	S. typhi	C. albicans	A. niger
101.	4-Chloro	50	100	50	100
102.	4-Bromo	200	200	200	200
103.	4-Fluro	50	50	100	50
104.	2-Bromo	N.A.	200	N.A.	N.A.
105.	4-Methoxy	50	100	100	100

- Ampicillin (MIC-0.04 μ g/ml) used as standard against *S. aureus*.
- Trimethoprim (MIC 0.01 μ g/ml) used as standard against *S. typhi*.
- Miconazole (MIC 6.25 μ g/ml) as standard against *C. albicans* and *A. niger*.
- N.A. -: Not active at 200 μ g/ml.

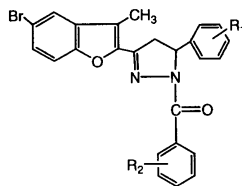
Table No. 2 :- Biological screening of 3-(3-Pyridoxymethylene)-4- (N-substituted benzylidinylamino)-5-mercapto-1,2,4-triazole derivatives.



Comp. No.	Substituents R	Anti Microbial Activity (μ /ml) [MIC]			
		Anti bacterial		Anti Fungal	
		S. aureus	S. typhi	C. albicans	A. Niger
201.	4-Chloro	50	50	50	50
202.	3Chloro	100	200	100	100
203.	4-Fluro	50	100	50	50
204.	4-Hydroxy	200	N.A.	100	N.A.
205.	2,4-Dimethoxy	N.A.	100	100	100

- Ampicillin (MIC-0.04 μ g/ml) used as standard against *S. aureus*.
- Trimethoprim (MIC 0.01 μ g/ml) used as standard against *S. typhi*.
- Miconazole (MIC 6.25 μ g/ml) as standard against *C. albicans* and *A. niger*.
- N.A. -: Not active at 200 μ g/ml.

Table 3:- Biological screening of 5-Aryl-3-(5-bromo-3-methylbenzofuran-2-yl)-1-(substituted benzoyl)-pyrazol-2-enes

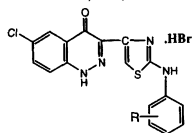


Comp. No.	Substituents		Anti-microbial Activity($\mu\text{g/ml}$) [MIC]			
			Anti bacterial		Anti Fungal	
	R	R'	S. aureus	S. typhi	C. Albicans	A. Niger
301.	3-Chloro	4-Chloro	100	200	100	200
302.	4-Chloro	4-Chloro	200	200	50	200
303.	4-Chloro	3-Fluro	100	100	100	100
304.	4-Chloro	4-Hydroxy	50	200	100	200
305.	4-Chloro	2,4-Dimethoxy	50	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. -: Not active at 200 $\mu\text{g/ml}$.

Table No. 4 :-

Biological screening of 2-(Substituted anilino)-4-(6-chloro-1H-cinnoline-4-one) thiazole .HBr



Comp. No.	Substituents R	Anti Microbial Activity (μ/ml) [MIC]			
		Anti bacterial		Anti Fungal	
		S. aureus	S. typhi	C. albicans	A. Niger
401.	4-Methyl	200	200	50	100
402.	3-Methoxy	200	200	200	200
403.	4-Chloro	100	100	100	100
404.	2-Chloro	100	100	100	100
405.	4-Fluro	50	50	50	100
406.	4-Bromo	N.A.	N.A.	200	100
407.	3-Chloro-4-Fluro	50	50	50	20
408.	3,5-Dichloro	50	50	200	100
409.	2,3-Dichloro	50	50	100	100
410.	3,4-Dichloro	50	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. -: Not active at 200 μg/ml.

DISCUSSION

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

In Series I, the synthesis of 2-(Substituted thiocarbamoyl)-5-(3-pyridoxymethylene)-1,3,4-oxadiazole derivatives have been described. The following points are to be noted related to Synthesis and Biological screening:

1. The key step in the synthesis of 3-pyridoxyacetyl hydrazide (**Ia**) is the formation of potassium salt of 3-hydroxy pyridine. For this anhydrous K_2CO_3 stirred with 3-hydroxy pyridine for about three to five hours at $50^\circ C$ to provide complete salt formation.
2. It was found that 3- pyridoxyacetate, as containing ester functional group tends to de-esterify in presence of water (humidity) and acid (liberated HCl). To overcome this, excess of K_2CO_3 (2.5 equivalent) in anhydrous condition was used. Also separation of 3- pyridoxyacetate from reaction mixture, found very difficult in our laboratory conditions. Hence we use ethereal extract of reaction mixture to prepare 3-pyridoxyacetyl hydrazide (**Ia**).
3. 2-(3-pyridoxymethyl)-5-mercapto-1,3,4-oxadiazole (**Ib**) was found highly hygroscopic hence must be used immediately when prepared.
4. Among all the five synthesized titled compounds, (Refer Table No. 1, page No. 78) 2-bromo substituent (Compound No. 104) is found not active, 4-bromo substituent (Compound No. 102) exhibited activity upto $200\mu g/ml$ and rest of the compounds shows good activity against *S.aureus*. The compounds with 2-bromo substituent (Compound No. 104) & 4-bromo substituent (Compound No. 102) exhibited activity upto $200\mu g/ml$ whereas rest of the compounds exhibited moderate to good activity upto $100\mu g/ml$ against *S.typhi*. 4-Fluro substitution (Compound No. 103) shows encouraging activity at $50\mu g/ml$ against *S.typhi*.
5. 2-Bromo substituent (Compound No. 104) is found not active against both fungal strains while 4-bromo substituent (Compound No. 102) exhibited activity upto

200µg/ml. All other substitutions shows moderate to good activity against both fungal strains.

In Series II, the synthesis of 3-(3-Pyridoxymethylene)-4-(N-substituted benzylidinylamino)-5-mercapto-1,2,4-triazole has been explained. Following observations have been made

1. In present investigation, the method employed for the synthesis of amino triazole ring is that of Ried et al.¹⁰⁰ the advantage of this method is the better yield obtained however the reaction mixture was stirred for 4-5 hours. Also potassium salt formed was found more stable than oxadiazole (**Ib**), hence can be stored under atmospheric conditions.
2. All the five synthesized compounds (Refer Table No. 2, page No. 79) exhibited activity upto 200µg/ml against *S.aureus* except 2,4-Dimethoxy substituent(Compound 205) agaist *S.aureus* and 4-hydroxy substituent (Compound No. 204) against *S.typhi*.
3. All the five synthesized compounds exhibited moderate activity upto 100µg/ml against *C.albicans*. 4-Chloro and 4-fluro substituent (Compound No. 201 & 203) shows encouraging activity against both fungal strains. Rest all the compounds except 4-hydroxy substituent (Compound No. 204), showed activity upto 100µg/ml against *A.niger*.
4. Schiff's base in which halo substituents is present at para position of phenyl ring are more active than those in which halogens are at meta and ortho position.

In Series III, the synthesis 5-Aryl-3-(5-bromo-3-methylbenzofuran-2-yl)-1-(substituted benzoyl)-pyrazol-2-enes has been explained. It was noted that:-

1. All the five synthesized compounds (Refer Table No. 3, page No. 80) showed moderate activity upto 200µg/ml among which 4-Hydroxy and 2,4-dimethoxy (Compound No. 304 & 305) showed activity upto 50µg/ml against *S.aureus*. The 4-fluoro derivative (Compound No. 303) exhibited activity at 100µg/ml while the other remaining compounds exhibited activity upto 200µg/ml against *S.typhi*.

2. All the compounds showed moderate activity against fungal species of *C.albicans* upto 100µg/ml exceptional of 2,4-Dimethoxy substitutions (Compound No. 305) with activity at 200µg/ml. The compounds 4-fluoro substitution (Compound No. 303) showed activity at 100µg/ml while remaining others at 200µg/ml against *A.niger* species except 2,4-Dimethoxy substitutions (Compound No. 305) which is found not active upto 200µg/ml.

In Series IV, synthesis of Biological screening of 2-(Substituted anilino)-4-(6-chloro-1H-cinnoline-4-one) thiazole .HBr has been described. Following points were noted related to Biological screening:-

1. Bromination of 3-Acetyl-6-chloro cinnoline-4-one (**IVb**) using molecular bromine (Br_2) gave low yield with mixture of by products (dibromination, ring bromination etc.), while bromination using Phenyltrimethylammonium tribromide [**PTT**] gave quantitative product (85%) and clean reaction. Also being solid and stable, handling and storage of **PTT** found convenient.
2. Chloro, Dichloro, Fluro substitutions showed activity upto 100µg/ml against *S.aureus* and *S.typhi*. (Refer Table No. 4, page No. 81) All other substitutions shows moderate activity with exception of 4-Bromo phenyl substitution which was found to be inactive at 200µg/ml against *S.aureus* and *S.typhi*.
3. All the compounds synthesized exhibited moderate antifungal activity against *C. albicans* and moderate activity against *A. niger* except for 3-chloro-4-fluro phenyl substitution, which exhibited good activity upto 20µg/ml. against *A. niger*.
4. Presence of Fluorine moiety also showed promising activity against both bacterial strains as well as the fungal strain.

Considering above findings it can be noted that with respect to the combined heterocyclic moieties in present study, Fluro and Chloro substituted analogs contributes noticeable to the biological activity while Bromo analogs does not show encouraging activity. Also Methyl, Methoxy, Dimethoxy and Hydroxy derivatives does not show promoting biological activity.

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APPENDIX

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**

MASTER OF SCIENCE IN ORGANIC CHEMISTRY

**TITLE OF THE THESIS : SYNTHESIS AND STUDY OF NEW HETEROCYCLIC
ORGANIC COMPOUNDS AS POSSIBLE THERAPEUTIC
AGENTS.**

**NAME OF THE : Mr. YOGESH PRABHAKAR PRAVINA JADHAV
CANDIDATE**

NAME OF THE GUIDE : DR. ANIL SHRIDHAR BOBADE

**PLACE OF RESEARCH : HAFFKINE INSTITUTE OF TESTING, RESEARCH AND
TRAINING, PAREL, MUMBAI-12.**

**NUMBER AND DATE : 23, 13TH Aug 2007
OF REGISTRATION**

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OF SYNOPSIS**

SIGNATURE OF THE GUIDE



(DR. A. S. BOBADE)

SIGNATURE OF THE CANDIDATE



(MR. YOGESH P. JADHAV)

SYNOPSIS

In the history of medicine few developments have been profound effect upon human life and society, due to the power to control infections caused by micro-organisms. Despite of these developments, bacterial diseases still present a serious problem owing to various reasons.

Many chemotherapeutic agents are available to combat these diseases, among them only a few can be termed as broadly useful. Even the most potent anti-microbials do not generally cure infection solely by virtue of their action against invading organism. They required help from the host's defense mechanism. Hence the majority of anti-microbial drugs play only subsidiary role, either because of their weak activity, their small margin of safety (toxicity), due to rapid emergence of resistance or due to poor defense mechanism of the host.

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

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

Several heterocyclic derivatives find important applications in medicinal chemistry. The benzimidazoles are synonymous with anthelmintic agents; thiazoles are useful for several purposes ranging from anti-bacterials to immuno-suppressive agents, pyridine derivatives are useful as anti-ulcer and anti-tubercular compounds; while iso-oxazoles find applications in penicillins and semi synthetic antibiotics. Such a number of heterocyclic derivatives play an important role in the control of various microbial diseases and hence, in this present study a new series of heterocyclic compounds have been synthesized and studied for their properties as anti-bacterial and anti-fungal agents.

The thesis would consist of following seven parts

Part I -: Introduction, Plan of work

Part II -: Chemistry of intermediates

Part III-: Synthesis of 2-(Substituted thiocarbamoyl)-5-(3-pyridoxymethylene)-1,3,4-oxadiazole

Part IV-: Synthesis of 3-(3-Pyridoxymethylene)-4-(N-substituted benzylidinylamino)-5-mercapto-1,2,4-triazole

Part V-: 5-Aryl-3-(5-bromo-3-methylbenzofuran-2-yl)-1-substituted benzoylpyrazol-2-enes

Part VI -: 2-(Substituted anilino)-4-(6-chloro-1H-cinnoline-4-one) thiazole .HBr

Part VII -: Biological screening, Results and Discussion

Part I -: Introduction, Plan of work

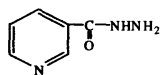
This part will give general introduction covering salient features of the research projects and also include plan of work which will highlight the objectives involved in the synthetic work.

Part II -: Chemistry of intermediates

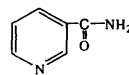
This part will consist of chemistry of intermediates involved in this thesis and approach toward their synthesis

Part III -: Synthesis of 2-(Substituted thiocarbamoyl)-5-(3-pyridoxymethylene)-1,3,4-oxadiazole

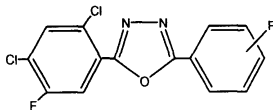
Large number of pyridine derivatives exhibit interesting biological activities. Especially 3-Pyridine derivatives such as Isoniazides (I) and Nicotinamide (II) are commonly used as drug for the treatment of tuberculosis¹. 1, 3, 4-Oxadiazole nucleus, constitute a unique class of nitrogen and oxygen containing five membered heterocycles associated with various biological activities such as anti-bacterial, anti-inflammatory, pesticidal, anti-hypertensive.²⁻⁵ Recently, it has been reported that 2,5-disubstituted-1,3,4-oxadiazoles(III) shows good anti-bacterial and significant pesticidal activity.⁶⁻⁷



I

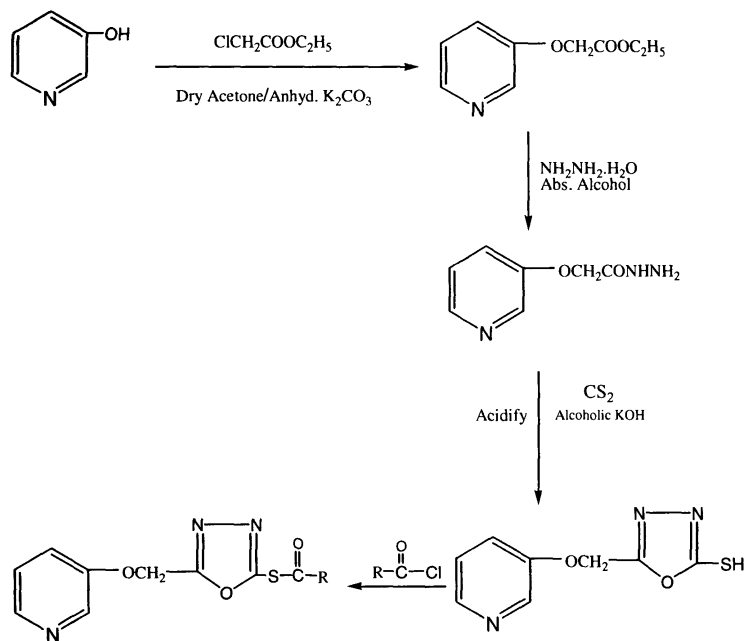


II



III

After reviewing these facts we synthesize a new series of molecules consisting of two biological active moieties viz. 3-substituted pyridine and 2, 5-disubstituted-1,3,4-oxadiazoles. Synthetic method for preparation of title compound is represented schematically as follows



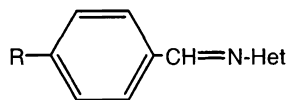
R = C₆H₄Cl, C₆H₄F etc.

Six derivatives of title compound were synthesized and after structure confirmation by spectral study they were subjected for *in vitro* antimicrobial screening.

Part IV-: Synthesis of 3-(3-Pyridoxymethylene)-4-(N-substituted benzylidinylamino)-5-mercapto-1,2,4-triazole

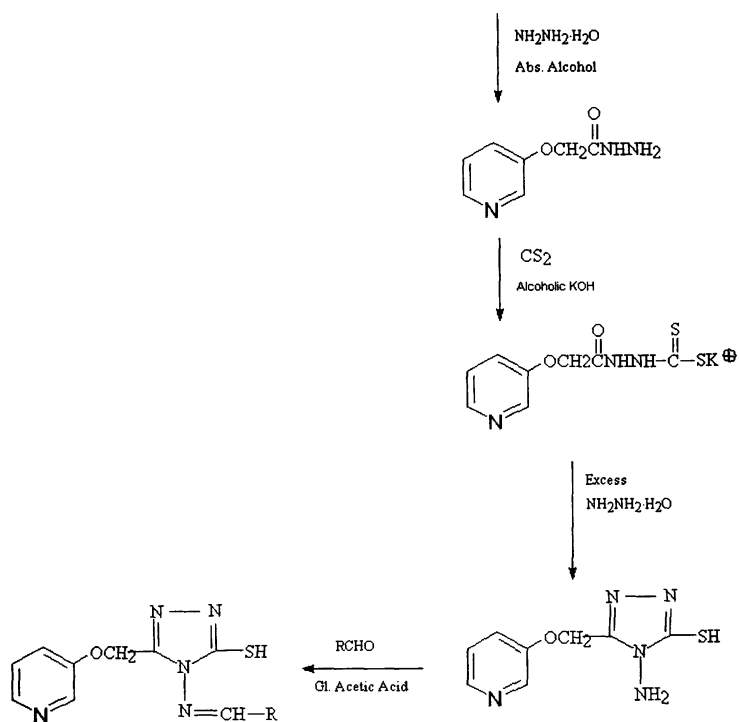
Many heterocyclic compounds containing 1, 2, 4-triazole ring are well known for their diverse pharmacological properties like anti-inflammatory, anti-microbial, anti-convulsant and anti-depressant activity.⁸⁻¹¹

Schiff bases, which contains a carbon-nitrogen double bond with nitrogen atom connected to an aryl or alkyl group i.e. azomethine linkage, are well documented to show encouraging anti-microbial activity.¹² Also presence of azomethine linkage in compound has also been known to decrease the toxicity considerably.¹³ Anti-microbial activity of some hetero aryl benzyl amines and their Schiff bases (IV) is reported by Fioranvati, R. et al.^{14a} Also Manna P. et al^{14b} and Trivedi V. et al^{14c} make considerable efforts to show biological activity related to azomethine linkage.



IV

After studying these references we tried to develop following type of compound in which pyridine molecule was incorporated at 3rd position while an azomethine linkage at 4th position of 1,2,4-triazole. Synthesis of title compound schematically represented on next page.

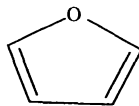


$\text{R} = \text{C}_6\text{H}_4\text{Cl}, \text{C}_6\text{H}_4\text{F}$ etc.

Five derivatives of title compound were synthesized and after structure confirmation by spectral study they were subjected for *in vitro* antibacterial and antifungal screening.

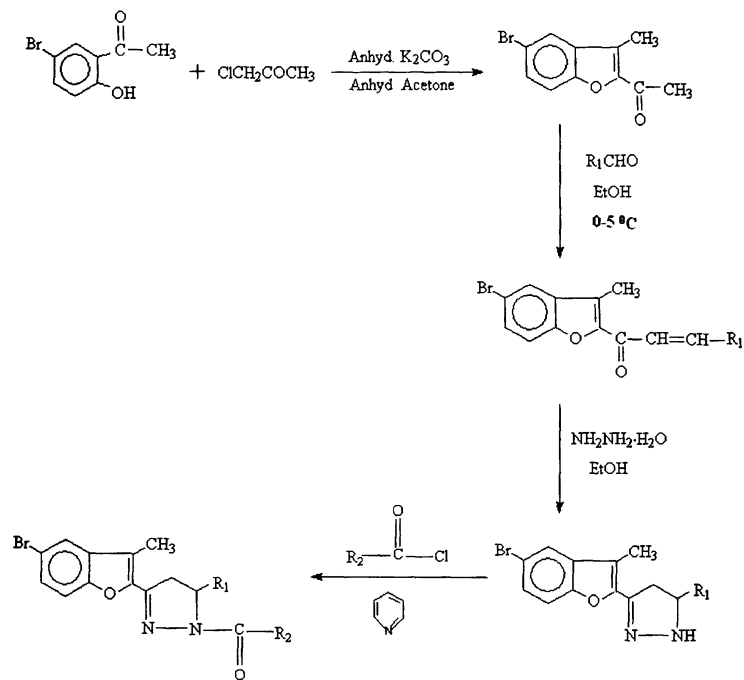
Part V:- 5-Aryl-3-(5-bromo-3-methylbenzofuran-2-yl)-1-substituted benzoylpyrazol-2-enes

Furans (V) are five membered heterocycles containing oxygen as hetero atom. Furans and benzofurans are associated with wide spectrum of biological activity.¹⁵⁻¹⁶



V

Also pyrazoles as well as pyrazole containing compounds have been reported to show a broad spectrum of biological activity such as anti-microbial, anti-pyretic, anti-inflammatory and anti-tumor.¹⁷⁻²⁰ These facts encouraged us to synthesize new heterocyclic molecule bearing benzofuran and pyrazole moieties into a single molecule. Schematic representation of synthesis of title compound is given below.



$R_1 = C_6H_5, C_6H_4Cl$ etc.

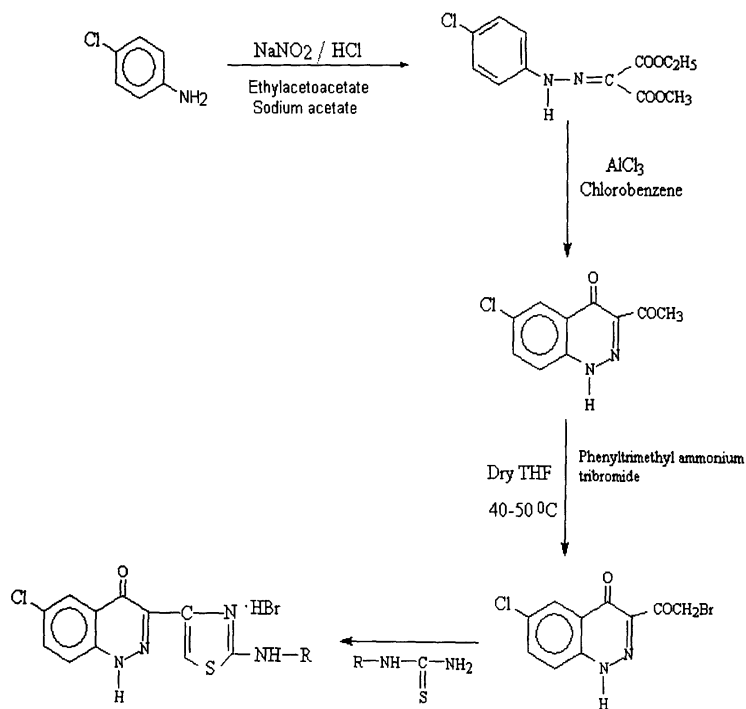
$R_2 = C_6H_4Cl, C_6H_4F$ etc.

Six derivatives of title compound were synthesized and after structure conformation by spectral study they were subjected for *in vitro* antimicrobial screening.

Part VI -: 2-(Substituted anilino)-4-(6-chloro-1H-cinnoline-4-one) thiazole .HBr

Thiazoles constitute the core structure of number of pharmacological and biological active compounds. Variety of thiazole derivatives are well known for their anti-microbial, anti-allergic, anti-tubercular and anti-inflammatory activities.²¹⁻²⁴

Similarly cinnoline ring system shows wide spectrum of biological activities.²⁵ 1-Alkyl-3-carboxycinnoline-4-ones exhibit good anti-microbial activity.²⁶ Considering this biological activity of cinnoline moiety, it is incorporated with thiazole ring to get title compound. Synthetic scheme of title compound is represented as follows.



$\text{R} = \text{C}_6\text{H}_4\text{Cl}, \text{C}_6\text{H}_4\text{F}$ etc.

Ten derivatives of title compound were synthesized and after structure conformation by spectral study they were subjected for *in vitro* antifungal and antibacterial screening.

Part VII --: Biological screening, Results and Discussion

In this project we synthesized totally 25-30 compounds and course of reaction were confirmed by Thin Layer Chromatography (TLC). Synthesized compound were subjected to Carbon Hydrogen Nitrogen (CHN) analysis. Few compounds were subjected for spectral study namely Nuclear Magnetic Resonance (NMR) Spectroscopy and Infra-Red (IR) Spectroscopy for structure conformation. After structure confirmation compounds were subjected to in vitro antimicrobial screening, which involves anti-bacterial and anti-fungal screening.

Anti--bacterial screening:

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

Anti-fungal screening:

Compounds synthesized were also screened for *in vitro* anti-fungal activity by tube dilution technique against fungal strains viz. *Candida albicans* using the standard drug Miconazole.

The synthetic and microbiological evaluation of the compounds will be dealt in the thesis.

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