

SYNTHESIS OF ORGANIC COMPOUNDS AS POSSIBLE ANTIBACTERIAL AND ANTIFUNGAL AGENTS

THESIS SUBMITTED
UNIVERSITY OF MUMBAI

FOR THE DEGREE OF
MASTER OF PHARMACY

SUBMITTED BY
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UNDER THE GUIDANCE OF
DR. B.G. KHADSE

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

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Synthesis Of Organic Compounds As Possible Antibacterial And Antifungal Agents

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A
Thesis
Submitted To
The University Of Mumbai
For The Degree Of Master Of Pharmacy
In
The Faculty Of Technology
By

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B. Pharm.

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STATEMENT

Statement submitted under rule 0.412

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

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Statement I :

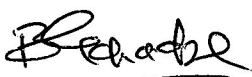
The work embodied in the Thesis to be submitted is original, based on the discovery of new facts, viz. the synthesis and characterization of new and unknown compounds of possible chemotherapeutic value. Also included in the Thesis, are new relation of facts, observed on the basis of in vitro testing.

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

Statement II

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

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



Dr. B.G. Khadse
Signature of Research Guide



Sonali D. Wayangankar
Signature of the Research Student

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*INTRODUCTION
AND
REVIEW OF LITERATURE*



“Now that the liability to, and danger of, disease are to a great extent circumscribed..... the efforts of chemotherapeutics are directed as far as possible to fill up the gaps left in this ring.....”.

Paul Ehrlich

Diseases are an increasing concern for mankind. Management of these diseases is exceptionally frustrating because of the difficulties in establishing the diagnosis. Furthermore, existing therapeutic agents are suboptimal for the immunocompromised patients. The developing countries have been experiencing an epidemiological transition, with rapid aging of the population together with an increasing incidence of noncommunicable diseases linked to changes in life-style.

The growing prevalence of cancer, cardio vascular disease, diabetes and other chronic conditions in addition to the long-standing problems of communicable diseases such as cholera, malaria and tuberculosis impose a double burden on healthier systems in these countries. In addition, there is the pandemic of Human Immuno Deficiency Virus (HIV) infection and Acquired Immuno Deficiency Syndrome (AIDS) which impose a particularly heavy burden on developing countries.

The recent World Health Organisation report¹ reveals that diseases caused the death of 50 million people throughout the world, of these 50 million, about 6.8 million deaths are registered in the developed market economies, 4.2 million in Eastern Europe, 7 million in the group of least developed countries and 32 million elsewhere in the developing world. Another reliable data² on causes of death show that, the infectious and parasitic diseases continue to pose serious risk to adult health in developing countries. This category of diseases accounts for about 17 million deaths or almost half of all deaths occurring in developing countries. Tuberculosis claims around 3 million lives each year, 90% of which are among the adult population. Pneumonia is the cause of about 2 million adult deaths per year in developing countries. Other major health risks for human include malaria, schistosomiasis, hepatitis B each of which account for several hundred thousand deaths each year¹.

In order to evolve and develop new approaches, new mechanisms, new resources that can expedite the process of finding ways for medicinal chemist's realisation of overcoming all these obstacles, it is essential to do research on new molecules.

To-day, research in pharmaceuticals is becoming ever more expensive with the result that end-products are priced out of the reach of developing countries. Yet, pressures from manufacturers and donors to adopt these costly new technologies, and even from the general public, are intense. The trend seems to be towards more and more expensive treatments for diseases affecting fewer and fewer people. In general, it is hoped that research will generate new and improved preventive, therapeutic and enabling technologies, giving attention to simplicity and adaptability, to meet the needs of developing countries like India and others.

The present investigation is the attempts in this direction for obtaining possible solutions to some of the persistent health problems that affect human life, especially bacterial and fungal infections.

BACTERIAL INFECTIONS

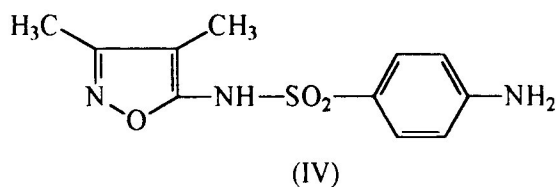
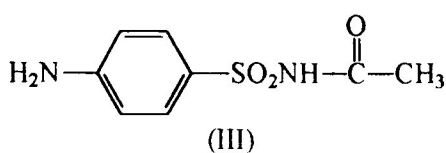
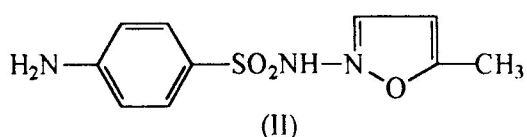
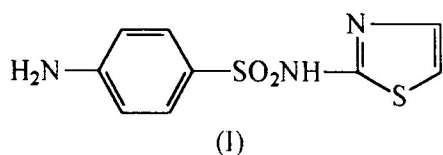
The development of resistance to current antibacterial therapy continues to drive the search for more effective agents. Bacteria are commonly responsible for many diseases, which were considered until recently to be resistant to chemotherapy. The bacteria were thought to be primitive in structure to be influenced by chemicals which are not highly toxic to the host. Many attempts have been made to combat bacterial infections in animals by the use of drugs, but most of them have met with little or no success with the exception of morgenroth and Lavy's partial success in the year 1911, in combating bacterial infections of the blood stream. They found that ethyldihydrocuprein (Optochin), a quinine derivative, would cure pneumococcal infection in mice. The discovery of Prontosil was considered to be a brilliant era in chemotherapy of microbial infections.

It is administered orally and it is readily absorbed from the gastrointestinal tract. The normal dosage for adults and children ranges from 15-25 mg/kg or 40 mg/kg three times weekly. The dosage is carefully calculated on a weight basis to avoid toxicity and is reduced in patients with impaired renal function.

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

Sulfonamides :

The term 'sulfonamide' is usually employed as a generic name for derivatives of p-aminobenzenesulfonamide (sulfanilamide). These drugs were the first, systemic chemotherapeutic agents employed to prevent and cure bacterial infections in man and were quite effective in bringing about a sharp decline in the morbidity. Important members of this class include sulfathiazole (I), sulfamethoxazole (II), sulfacetamide (III), sulfisoxazole (IV).



The sulfonamides have a wide range of antibacterial activity against Gram-positive and Gram-negative bacteria. They are usually rapidly absorbed from the gastrointestinal tract except those specially designed for their local effects in the bowel. Approximately about 70-100% of an oral dose is absorbed and are finally eliminated from the body partly as the unchanged drugs and partly as metabolic products, the largest fractions being excreted in the urine.

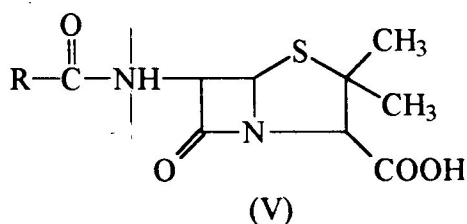
Strains of bacteria resistant to the sulfonamides have become common during recent years. Also the advent of penicillins and other antibacterials has diminished the role of the sulfonamides as antibacterial agents. However, the introduction of the combination of

trimethoprim and sulfamethoxazole, known popularly as co-trimoxazole, has resulted in increased use of sulfonamides for the treatment of specific bacterial infections.

Penicillins and Cephalosporins :

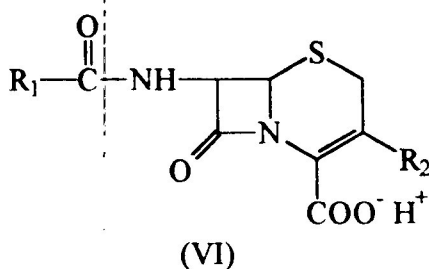
Since Alexander Fleming's serendipitous discovery of penicillin in 1928 it has remained one of the most important group of antibiotics. Despite the discovery of several other antibacterial agents, the penicillins are widely used with new derivatives being produced every year. Due to their unique advantages,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

The basic structure of penicillin (V) consists of a thiazolidine ring connected to a beta-lactam ring to which is attached a side chain (R). The penicillin nucleus is an important requirement for biological activity, alteration of which causes loss of all significant activity. Many of the antibacterial and pharmacological characteristics of penicillins are determined by its side chain which can also alter the susceptibility of the resultant compounds to inactivating enzymes (beta-lactamases).



No sooner was the fact established that penicillins are capable of eradicating microorganisms, it was practically administered in every situation where there was a risk of bacterial infection.

The cephalosporins (VI) have shown tremendous growth over the past decade and are usually classified by the 'generations' system which is based on the general features of antibacterial activity.



The first generation cephalosporins exhibit good activity against Gram-positive bacteria and relatively lower activity against Gram-negative bacteria. The second-generation cephalosporins exhibit better activity against Gram-negative microorganisms but are inferior to the third-generation cephalosporins. The third-generation cephalosporins are generally less active against Gram-positive bacteria as compared to first-generation agents, but are much more active against beta-lactamase producing strains.

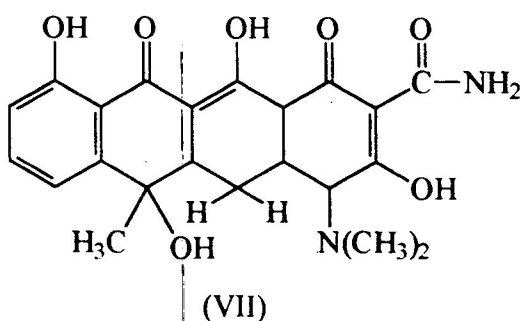
The cephalosporins are widely used antibiotics, perhaps overused for conditions where much less expensive antibiotics could provide relief.

The cephalosporins also find wide use in prophylaxis during and after surgery, wherein the first-generation agents have been shown to be safe and effective. A few of the third-generation cephalosporins are the drugs of choice for meningitis and in some cases superior to a combination of ampicillin and chloramphenicol.

The beta-lactam antibiotics (penicillins and cephalosporins) suffer bacterial resistance due to a variety of factors. It may be caused by the inability of the agent to enter the site of action³. Also it is possible for a sensitive strain to acquire resistance by mutation and has been described for Gram-positive cocci⁴.

Tetracyclines :

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



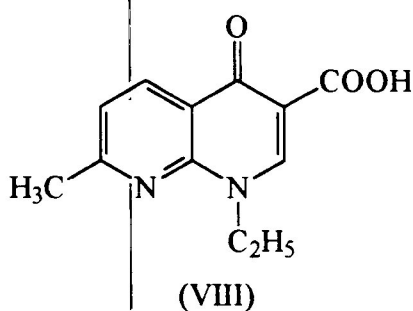
Microorganisms that have become resistant to one tetracycline frequently exhibit resistance to the other members of the family. The bacterial species possibly exhibit resistance only after exposure to the drug. The tetracyclines were initially useful for treatment of infections with aerobic Gram-negative bacteria but most species are now relatively resistant. Nevertheless, more than 90% of strains of Haemophilus influenzae may still be sensitive to either tetracycline or doxycycline.

Other important antibacterial agents include chloramphenicol and the furan derivatives such as nitro-furantoin, nitrofurazone, furazolidone, etc. Several of the drugs active against M. tuberculosis discussed earlier are also active against various species of Gram-positive and Gram-negative microorganisms besides mycobacteria.

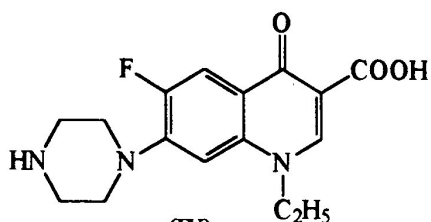
Quinolones :

The quinolone carboxylic acids, (or) 4-quinolones are a group of synthetic antibacterial agents structurally related to nalidixic acid (VIII). Nalidixic acid is active against Gram-negative bacteria. Bactericidal use has generally been limited to the treatment of urinary tract infections.

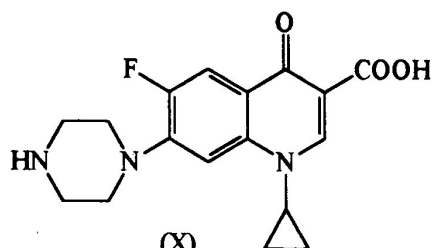
Modification of the structure of nalidixic acid has produced related antibacterial agents such as oxolinic acid, piromidic acid, cinoxacin, and acrosoxacin. None has been considered to represent a significant clinical advance over nalidixic acid.



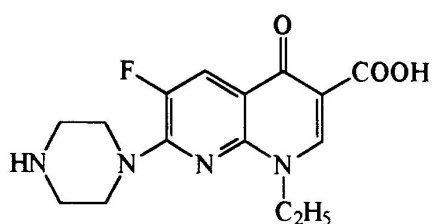
Addition of the 7-piperazinyl group and a fluorine atom at position 6 has produced a group of fluoroquinolones with a broad spectrum useful for the treatment of systemic infections. They include norfloxacin (IX), ciprofloxacin (X), enoxacin (XI), ofloxacin (XII).



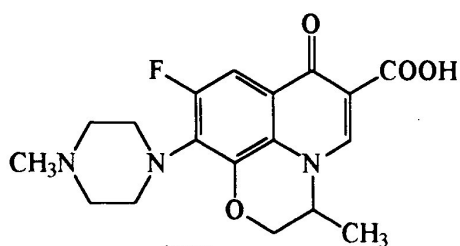
(IX)
Norfloxacin



(X)
Ciprofloxacin



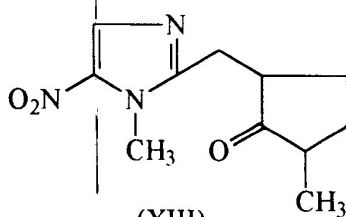
(XI)
Enoxacin



(XII)
Ofloxacin

Miscellaneous :

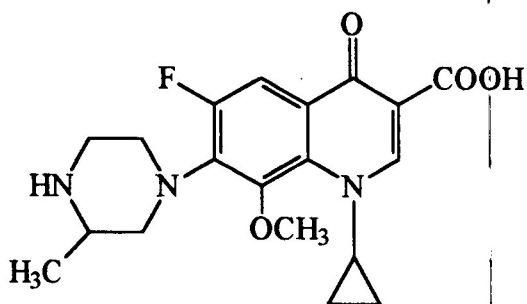
Activity against anaerobic organisms was reported for a series of 2-substituted-5-nitroimidazoles. Compound (XIII) was unique in this series, and was found to be more potent both *in vitro* and *in vivo* than metronidazole.



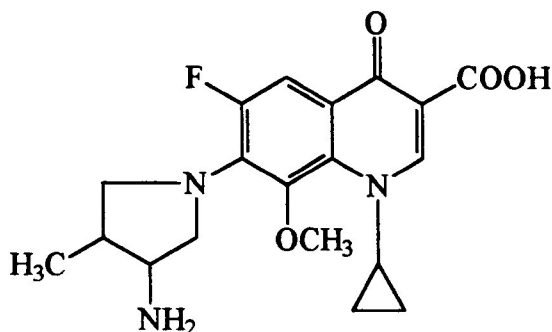
(XIII)

Fluoro Quinolones :

Use of N-cyclopropyl and C-8 heteroatom substituents continued to provide potent antibacterial agents. AM-1155 and AM-1174, new quinolones bearing an 8-methoxy group, outperformed ciprofloxacin against anaerobes and a range of Gram-positive bacteria with potency upto 16 to 62-fold greater than ciprofloxacin⁵.



AM - 1155



AM - 1174

FUNGAL INFECTIONS :

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

The incidence of systemic mycoses continues to rise steadily. This increase is due in part to improved recognition and diagnosis of fungal infections, but also to the prolonged survival of patients with global defects in their host defence mechanisms including patients with neoplastic diseases, organ transplant recipients, diabetics and patients with AIDS. These patients are susceptible to an ever growing list of opportunistic fungi. One yeast-like fungus, Cryptococcus neoformans, is reported to be the common cause of debilitating and life-threatening infections in AIDS patients⁶.

The agents which are currently in use as anti-fungals are classified and listed below :

Allylamines	Triazoles
Naftisine	Fluconazole
Terbinafine	Itraconazole
	Tereonazole
Imidazoles	Polyene macrolides
Bifonazole	Amphotericin
Butoeonazole	Candicidin
Chlormidazole	Hachimycin
Chlotrimazole	Hamycin
Econazole	Klatamycin
Enileonazole	Nystatin
Fenticonazole	Pentamycin
Isoconazole	
Ketoconazole	
Miconazole	
Oxiconazole	
Sulconazole	
Tioconazole	

Most of the antifungal agents in the Table are applied topically in the treatment of infections of hair, mucous membranes, nails, or skin by candida (candidiasis), dermatophyte fungi including Epidermophyton, Microsporum, Trichophyton (tinea, ringworm) and Malassezia furfur. The main agents which are used in treating these infections are listed below :

Superficial fungal Infection	Main Topical Antifungal Agents
Candida infection	azoles, polyene antifungals
Tinea	azoles, ciclopirox, olamine haloprogin, tolnaftate
Pityriasisi versicolor	azoles, ciclopirox, olamine haloprogin, tolnaftate

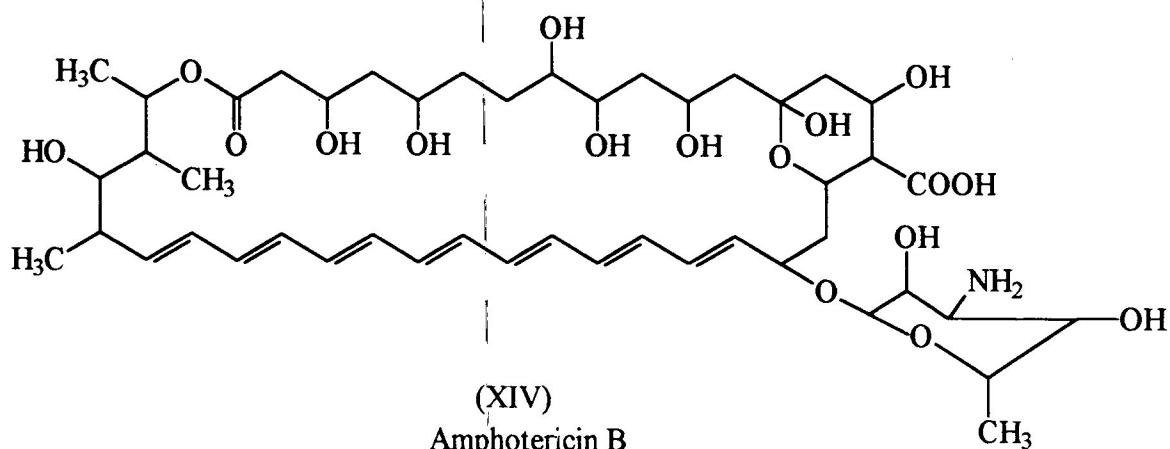
Systemic fungal infections tend to occur in immunocompromised patients and include infections due to *Aspergillus*, *Blastomyces*, *Candida*, *Coccidioides*, *Cryptococcus*, *Histoplasma*. The principal drugs which are used in such infections are listed below :-

Systemic fungal infections	Main antifungal agents
Aspergillosis	Amphotericin
Blastomycosis	Amphotericin, Ketoconazole
<u>Candidal Infection</u>	
Oesophageal	Fluconazole, Ketoconazole
disseminated	Amphotericin with or without flucytosine
Coccidioidomycosis	Amphotericin, Ketoconazole
Cryptococcosis	Amphotericin with or without flucytosine, fluconazole
Histoplasmosis	Amphotericin, Ketoconazole
Paracoccidioidomycosis	Amphotericin, Ketoconazole

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

Amphotericin-B :

It is the first commercially available systemic antifungal drug, and remains the cornerstone for therapy in critically ill patients with systemic fungal infections. It was first isolated in the 1950s from *Streptomyces nodosus*. It is a polyene macrolide that consists of seven conjugated double bonds, an internal ester, a free carboxylic group and a glycoside chain with a primary amino group (XIV). It is amphoteric, forming soluble salts in both basic and acidic environments.

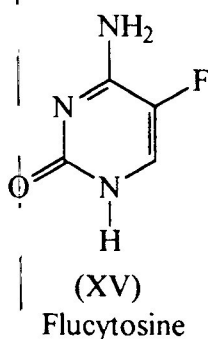


The primary mechanism of action of the polyenes in general and amphotericin B in particular, is due to binding to ergosterol, the principal sterol present in the cell membrane of sensitive fungi⁷.

Flucytosine :

It is a fluorine analogue of cytosine (XV), synthesised in the 1950s as a potential antineoplastic agent. It was not effective against tumours but was found to have in vitro and in vivo antifungal activity⁸.

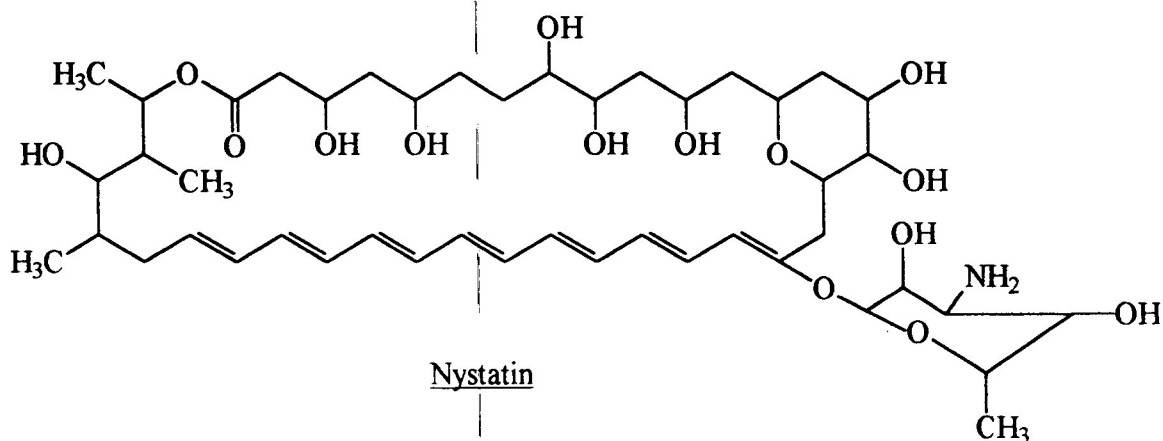
It has been increasing in usage as an adjunct to amphotericin B therapy.



The mechanisms of action of flucytosine are the disruption of protein synthesis by inhibition of DNA synthesis, and alteration of the amino acid pool by inhibition of RNA synthesis. It combines with amphotericin B to exhibit synergistic action in both in vitro and in vivo systems. It has few side effects. However, it is still in use for treating the infections caused by Candida albicans, Cryptococcus neoformans and Torulopsis glabrata.

Nystatin :

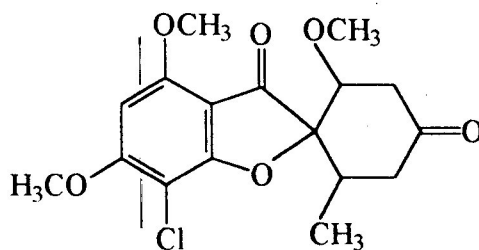
It is produced by Streptomyces noursei. It was discovered by Hazen and Brown⁹ and possesses good antifungal activity. Toxicity after parenteral administration to animals has led to its use only topically or orally.



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

Griseofulvin (XVI) :

It was discovered by Oxford et al. in 1939 as a metabolic product of Penicillium griseofulvum. Grove¹⁰ predicted its chemical structure in 1950.



(XVI)

As a 'Cell-wall antibiotic' particularly active on dermatophytes, griseofulvin interferes with biosynthesis of the chitinous elements of the hyphal wall.

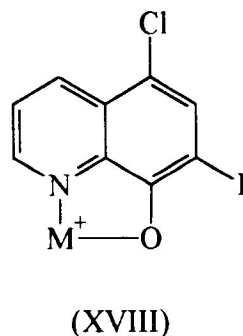
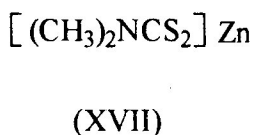
THE SYNTHETIC ANTIFUNGAL AGENTS

Metal containing molecules :

Heavy metal ions such as Ag^+ , Hg^+ , Cu^{2+} and Zn^{2+} have long been used as fungitoxicants in agriculture and also in topical ointments for dermatophytoses. A combination of heavy metal atom with an organic molecule increases the activity and more important is the selective toxicity of metal.

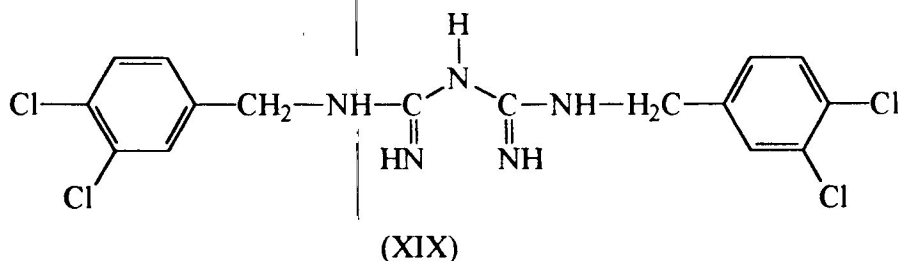
Examples of antifungal compounds that are metal complexes, are given below :

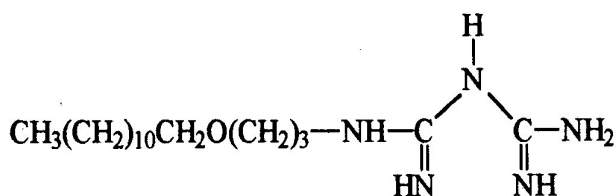
Zinc dimethyldithio carbamate (XVII), metal complex of 5-chloro-7-iodo-8-hydroxyquinoline (XVIII)



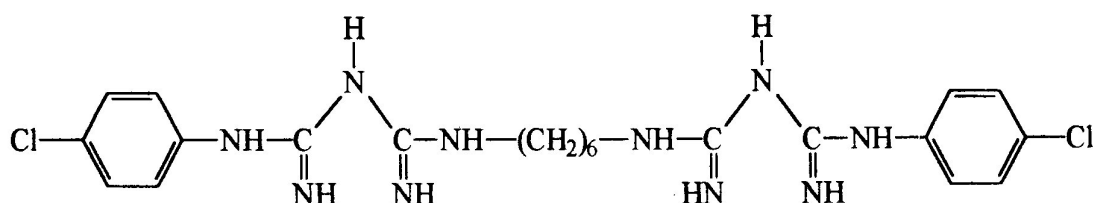
Chelating Agents :

Among chelating agents with considerable *in vitro* antifungal potency are substituted biguanides as N^1 , N^5 -bis-(3,4-dichlorobenzyl) biguanide¹¹ (XIX), N^1 -r-Lauroxy propyl biguanide¹². (XX), 1,6-bis-(5-p-chlorophenyl biguanidine) hexane (XXI).





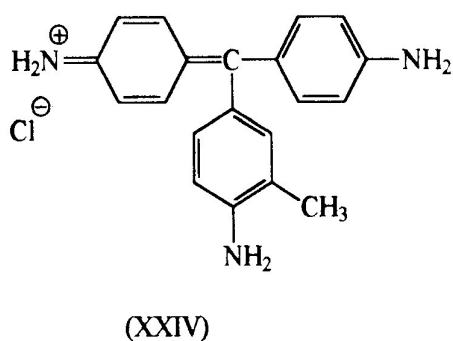
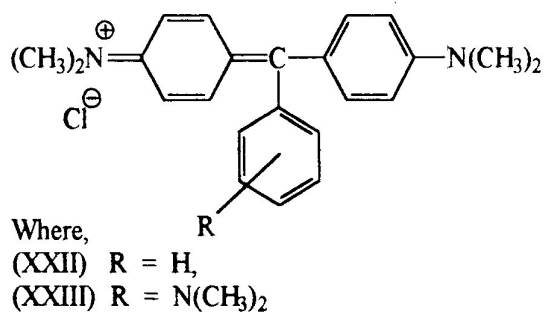
(XX)



(XXI)

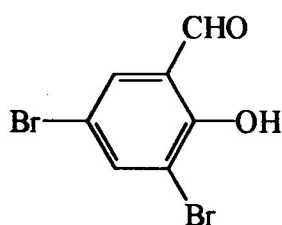
Aniline Dyes :

Aniline Dyes, such as malachite green (XXII), gentian violet (XXIII), methyl rosaniline chloride (XXIV) have been employed in topical therapy of dermatophytes as well as for candidiasis of the skin and mucous membranes.

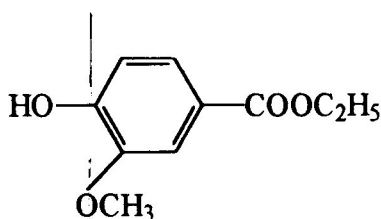


Phenolics and Diamides :

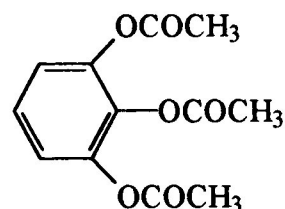
Phenolic compounds were known for their antifungal potency. Among the numerous phenolic compounds that have been used externally for mycotic infections are phenol, phenylmercuric nitrate, cresol, m-cresyl acetate, benzoic acid, p-hydroxybenzoic acid, salicylic acid, salicylanilide, 3,5-dibromosalicylaldehyde (XXV), ethyl vanillate (XXVI), pyrogallol triacetate (XXVII).



(XXV)

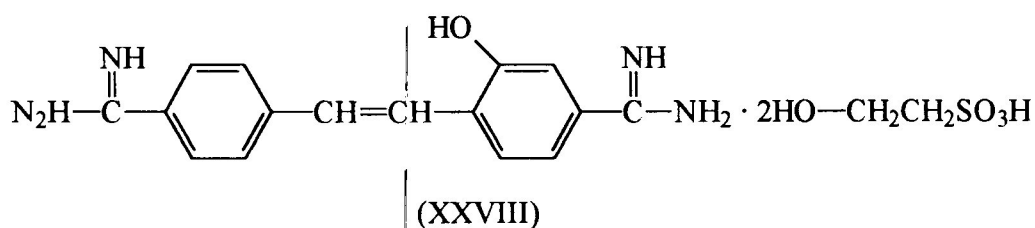


(XXVI)



(XXVII)

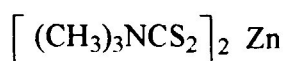
Several biamidines (e.g., stibamidine, propamidine, pentamidine) are antifungal in vitro. 2-Hydroxystilbimidine diisothionate (XXVIII) has been successfully employed in patients with blastomycosis¹³. It is less toxic than amphotericin B.



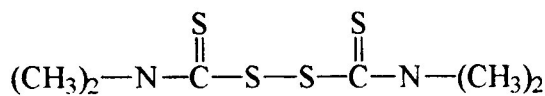
(XXVIII)

Sulfur Containing Molecules as Antifungal Agents :

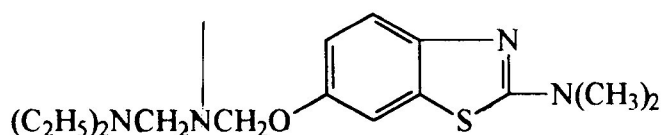
A variety of organic compounds that contain divalent sulfur attached to carbon atom have in vitro fungistatic action, and many of these have been employed in topical therapy of dermatophytoses. Among these, are dithiocarbamates (XXIX), tetraethylthiuram disulfide (XXX), and benzothiazoles such as 6-(β -diethylaminoethoxy)-2-dimethylamino derivatives(14,15). (XXXI).



(XXIX)

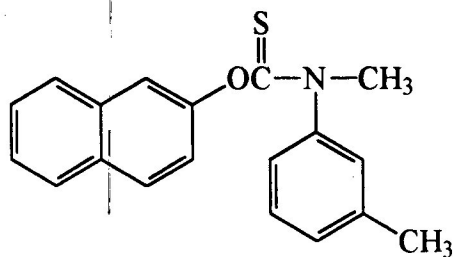


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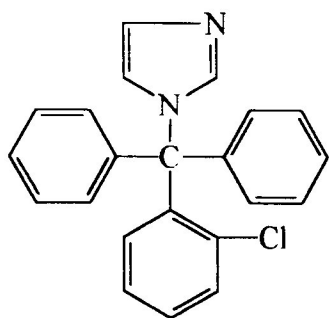
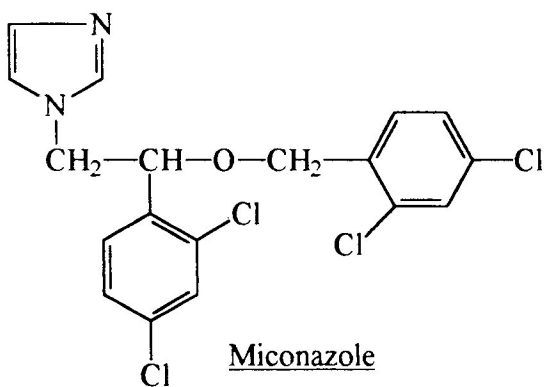
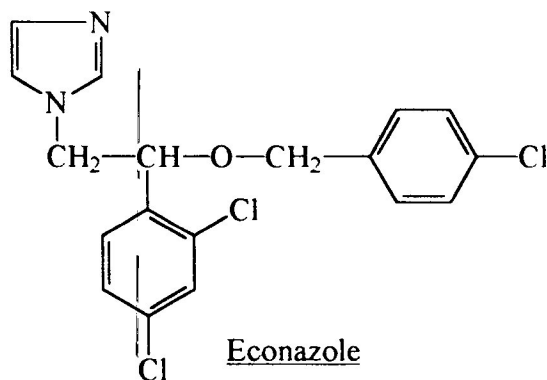
A widely used compound with unusually high in vitro and in vivo activity against the dermatophytes is O-2-naphthyl ester of m-N-dimethylthiocarbanilic acid (XLIII) (Tolnaftate). It is also effective against candidal lesions¹⁶.



(XXXII)

Imidazole Antifungals :

Among all the imidazole compounds, three distinct compounds are actually therapeutic antifungals with very wide spectra against yeast and filamentous fungi. They are clotrimazole, miconazole and econazole.

ClotrimazoleMiconazoleEconazole

Clotrimazole [1-(o-chlorotriptyl)-imidazole] was developed by investigators of the Bayer Laboratories¹⁷ in 1969, and is utilized topically in superficial mycoses or orally in clinical trials in deep mycoses.

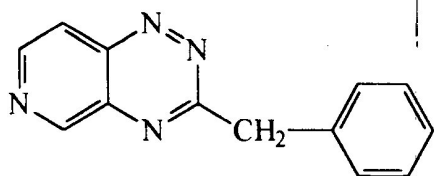
Miconazole is a well known antifungal drug developed by the investigators of Janssen Pharmaceuticals¹⁸ in 1969. It is utilized topically in superficial mycoses and by oral or intravenous routes in deep mycoses in clinical experimental trials.

Econazole is a phenethyl imidazole derivative, 1-[2,4-dichloro-(p-chlorobenzyloxy) phenethyl]-imidazole and belongs to the series described by Godefroi et al¹⁹. Like miconazole, it is utilized as topical agent in superficial mycoses and as a systemic antifungal by oral intravenous routes in deep mycoses.

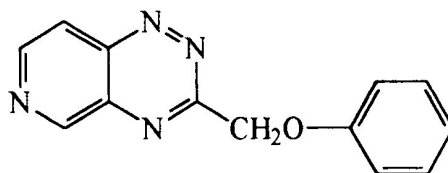
The mode of action studied by Preusser²⁰ on Candida albicans and Trichophyton rubrum shows that at concentrations as low as 1.1×10^{-7} M alterations on the cellular membranes and lysis of the intracytoplasmic organelles, occurred with decomposition of ribosomes.

Miscellaneous :

Some pyridotriazines, Triafungin and Oxyfungin have been described as antifungal agents by Wright et al²¹.



Triafungin



Oxyfungin

TRIAZOLES :

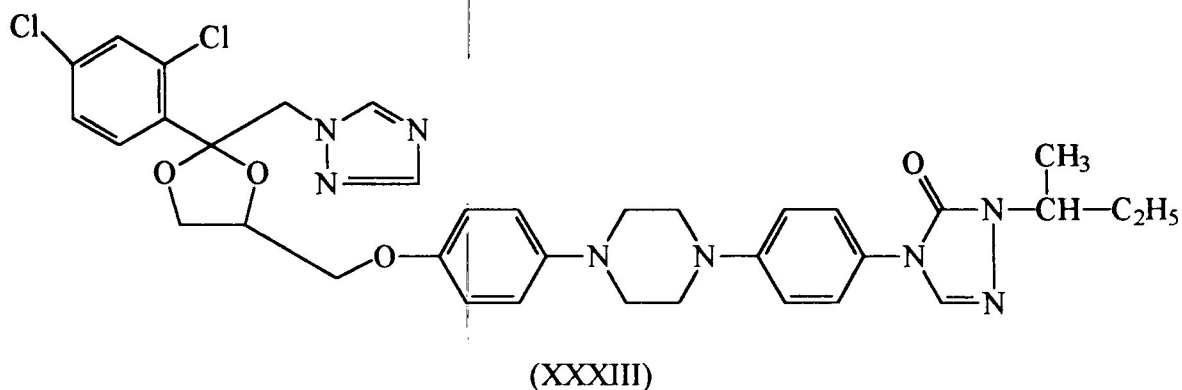
The substitution of the triazole ring for the imidazole ring offers many advantages to these newer azoles²².

- 1) It allows for greater polarity, which may improve solubility and reduce protein binding.

2) The triazole ring is less nucleophilic and more resistant to metabolic attack.

Itraconazole (XXXIII) :

It is water soluble, lipophilic triazole analogue that exhibits excellent activity against most fungal pathogens.

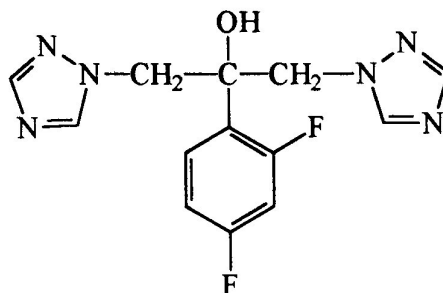


When compared with ketoconazole, its potential advantages include a broader spectrum of activity (including *Aspergillus* spp. and *Sporothrix schenckii*), a more desirable pharmacokinetic profile and less toxicity²³.

Most of the adverse reactions reported²⁴ are transient and include gastrointestinal disturbances, dizziness, headache, depressed libido (with normal testosterone levels) and leucopenia.

Fluconazole (XXXIV) :

It is a water-soluble fluorine substituted bis-triazole that has been shown to be effective against a variety of fungal infections in immunocompetent and immunocompromised hosts. Fluconazole has comparable activity to that of ketoconazole in inhibition of fungal C-14 demethylase. A major point of confusion during the development of this drug, however, has been its relatively low activity *in vitro* compared with its high *in vivo* activity.



(XXXIV)

It possesses pharmacokinetic properties that are distinct from the previously discussed azole derivatives.

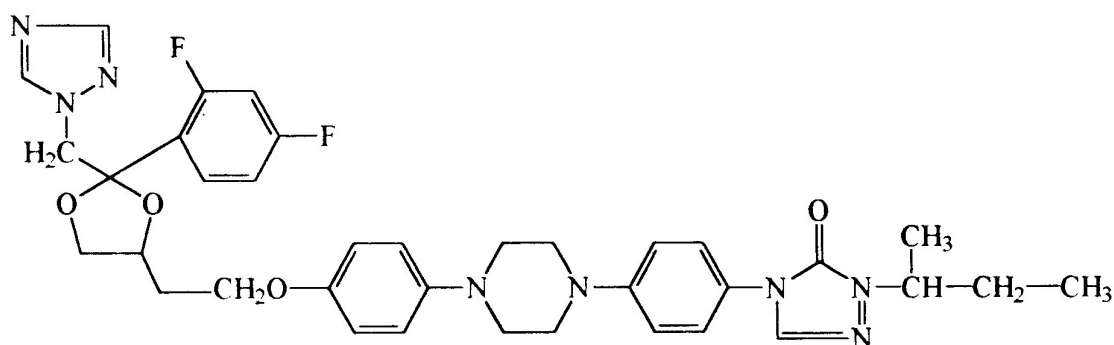
It has been well tolerated with very few dose limiting side effects. Nausea, other GI symptoms and elevation in hepatic transaminases have been reported in 5% of patients receiving the drug²⁵.

Fluconazole is also a potential candidate for prevention and early treatment of disseminated candidiasis in granulocytopenic patients²⁶.

Finally, fluconazole is also currently being studied for primary treatment of histoplasmosis and sporotrichosis.

Saperconazole (XXXV) :

It is water soluble, lipophilic fluorinated triazole with similar chemical structures to itraconazole, with two chlorine atoms replaced by fluorine.

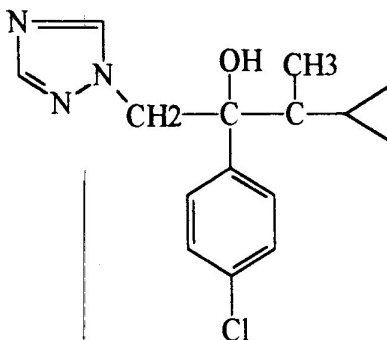


(XXXV)

therefore, organisms that are resistant due to reduced azole uptake are more sensitive to this drug²⁹.

SDZ 89-845 :

It is a triazole antifungal agent that has shown³⁰ activity in a number of animal models of superficial and systemic fungal infections.



SDZ 89 - 845

Non-Steroidal Anti-inflammatory Drugs :

These drugs may be defined as “Classic” nonsteroidal anti-inflammatory agents because they are active in the carrageenan induced edema, adjuvant arthritis, and ultraviolet-erythema assays, and are antipyretic.

Inflammation, however is a normal, essential, protective response to any noxious stimulus that may threaten the host and may vary from a localized reaction to a complex responses involving the whole organism.

Anti-inflammatory drugs modify inflammatory responses to diseases, but are not curative and do not remove the underlying cause of the disease. An ideal anti-inflammatory drug should affect only aberrant, uncontrolled inflammation and not interfere with the normal inflammatory response, which is a part of the body's vital defense mechanism to invading microorganisms and other environmental insults.

In the past 25 years, intensive research has been aimed at discovering nonsteroidal anti-inflammatory drugs with the therapeutic effectiveness of the glucocorticoids but without their

undesirable side effects. About 30 nonsteroidal anti-inflammatory drugs (NSAIDs) have been marketed in the United States and England. The drugs that have been introduced seem to have substantial sales. Data suggest that introduction of a new NSAID is not associated with a corresponding reduction in the prescribing of the other NSAIDs³¹. Nearly every drug firm in the United States is trying to capture a share of the large and expanding market for drugs to treat rheumatoid arthritis and related disorders.

Although improved therapy is still desperately needed for the rheumatic diseases, especially rheumatoid arthritis and osteoarthritis, beneficial drugs are available. These products relieve pain, swelling, and inflammation and enable many patients with mild to moderate rheumatic disease to lead essentially normal lives. They include drugs such as aspirin and the salicylates, indomethacin, sulindac, ibuprofen, fenoprofen, naproxen, tolmetin, penicillamine, meclofenamic acid, and the gold compounds.

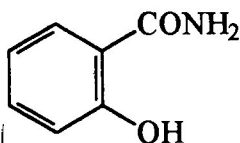
The sequence of events of carrageenan-induced edema of the rat paw has been delineated. The action of the initial edema mediators, histamine and serotonin, is succeeded by a second phase in which increased vascular permeability is maintained by kinin release. This is followed by a third phase, prostaglandin release, which is associated with the migration of leukocytes into the site of inflammation. Nonsteroidal anti-inflammatory agents suppress this migration. Activation and release of all the foregoing mediators depend on an intact complement system. For comparison, indomethacin and phenylbutazone are 148 and 3.3 times, respectively, as potent as aspirin in producing 50% inhibition of carrageenan-induced rat paw edema³².

Because the clinically useful nonsteroidal anti-inflammatory drugs alter widely in chemical and physical properties, attempts have been made to compare the biochemical properties of these compounds to determine possible modes of action. These drugs are polyvalent: they are able to modulate more than one molecular or cellular event thought to be concerned in the inflammatory event.

Nonsteroidal anti-inflammatory drugs have many biochemical activities³³. They are said to inhibit formation of the inflammatory mediators (Histamine, Serotonin, Prostaglandins, and Kinins) and to moderate the activity of the inflammatory process. They are also believed to

Salicylamide :

This drug, o-hydroxybenzamide, has been suggested as an effective substitute for aspirin in rheumatic disease.



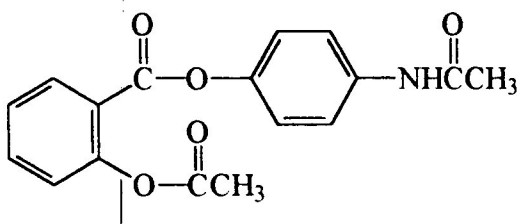
It is stable in liquid preparations, does not cure gastrointestinal ulceration, and can be taken by persons allergic to aspirin.

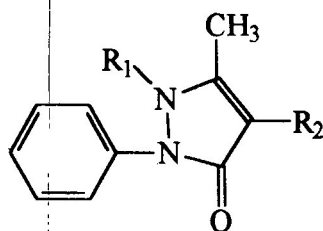
Salicylamide has been reported to be an effective analgesic for pain of rheumatic disease when administered in doses of at least 6 g. Other investigators, however, have been unable to find any anti rheumatic activity clinically under double-blind conditions, and have concluded that salicylamide has no useful antiinflammatory or antirheumatic activity³⁵. Because salicylamide is not hydrolyzed to salicylate, its analgesic activity depends on the entire molecule,

Derivatives of aniline and p-aminophenol, as exemplified by acetaminophen, have analgesic activity similar to that of aspirin but lack anti inflammatory and anti rheumatic activity. Preparations containing combinations of salicylamide and acetaminophen therefore seem to have little use in the treatment of rheumatic disease.

Benorylate :

Benorylate (Benoral), marketed in Europe as an anti-inflammatory drug, is an ester of acetylsalicylic acid with acetaminophen. The *in vivo* hydrolysis gives two compounds with complementary activity in different drug classes. Benorylate is reported to cause less gastrointestinal bleeding than aspirin³⁶. Acetaminophen nephrotoxicity, however, is reported to be enhanced by concurrent aspirin administration³⁷. Studies of benorylate in the U.S.A were halted when test animals developed eye damage.



5- Pyrazolone Derivatives :

Antipyrine (Phenazone) $R_1 = \text{CH}_3$, $R_2 = \text{H}$

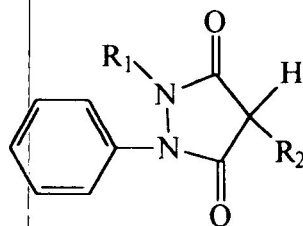
Aminopyrine (amidopyrine) $R_1 = \text{CH}_3$, $R_2 = \text{N}(\text{CH}_3)_2$

Dipyrone (methampyrone), Narone, and pyrilgin $R_1 = \text{CH}_3$, $R_2 = \text{H}_3\text{CNCH}_2\text{SO}_3\text{Na}$

Antipyrine and aminopyrine have analgesic, antipyretic, and antirheumatic activities similar to those of aspirin and sodium salicylate, and were formerly widely used for the same purposes. Fatal agranulocytosis caused by their application (especially associated with dipyrone and aminopyrine) led to curtailment of their use.

3,5-Pyrazolidinedione Derivatives :

The 5- pyrazolones are important because they led to the potent phenylbutazone class of anti-inflammatory agents.



Phenylbutazone (Butazolidin) $R_1 = \text{C}_6\text{H}_5$; $R_2 = n\text{-C}_4\text{H}_9$

Oxphenbutazone (Tandearil) $R_1 = p\text{-HOC}_6\text{H}_4$; $R_2 = n\text{-C}_4\text{H}_9$

Sulfinpyrazone (Anturane) $R_1 = \text{C}_6\text{H}_5$; $R_2 = \text{CH}_2\text{CH}_2\text{SOC}_6\text{H}_5$

Phenylbutazone had analgesic and antipyretic properties but showed no advantages over antipyrine and aminopyrine. The sodium salt of phenylbutazone, however, was found to solubilize several sparingly soluble drugs, including aminopyrine.

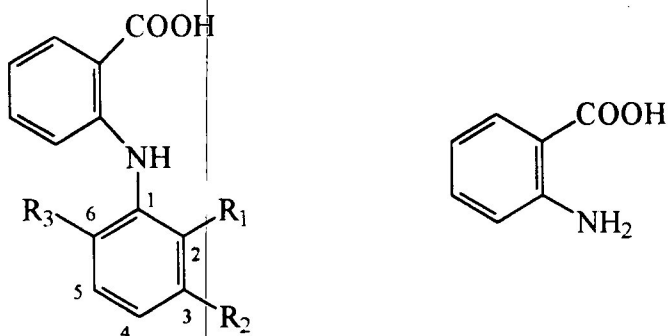
Phenylbutazone competes for the same binding sites on human plasma albumin as coumarin anticoagulants, sulfonamides, tolbutamide, indomethacin, and glucocorticoids. This competition and displacement of drug from binding sites may result in increased pharmacologic and toxic activity.

Mode of Action :

Phenylbutazone displays the diverse biochemical reactions listed earlier for the nonsteroidal anti inflammatory agents . Some of the biochemical modes of action proposed for aspirin may also apply to phenylbutazone. These include uncoupling of oxidative phosphorylation, inhibition of prostaglandin synthesis, inhibition of the histamine-forming enzyme histidine decarboxylase, stabilization of lysosomal membranes, and inhibition of mucopolysaccharide biosynthesis.

N-Arylanthranilic Acids (Fenamates) :

Derivatives of anthranilic acid, such as mefenamic acid, meclofenamic acid, and flufenamic acid, are potent analgesic and anti-inflammatory agents. These fenamates are nitrogen analogs of salicylic acid; anthranilic acid is also an o-substituted benzoic acid.



Mefenamic acid, introduced in the United States in 1967 has been approved by the Food and Drug Administration only for use in the management of pain and the treatment of primary dysmenorrhoea. Meclofenamic acid, as the sodium salt (generic name, meclofenamate sodium), was released in the United States in 1980 for treatment of rheumatoid arthritis and osteoarthritis. Flufenamic acid is marketed in Great Britain for the treatment of rheumatic disease.

In clinical studies, phenylbutazone was approximately twice as potent as flufenamic acid and five times as active as mefenamic acid in arthritic patients.

Mefenamic acid is absorbed relatively slowly, mainly from the small intestine, and reaches maximal blood levels in 2 to 4 hours.

The mechanisms of anti-inflammatory and analgesic activity are probably similar to those of the other acidic nonsteroidal anti-inflammatory agents.

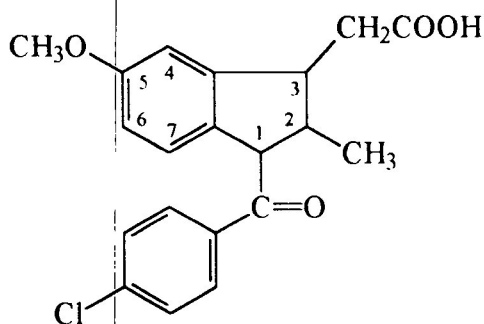
Mefenamic acid is indicated for the relief of mild to moderate pain, especially that following dental extraction.

Aryl- and Heteroarylacetic Acid Derivatives :

These are important nonsteroidal anti-inflammatory agents making up a large part of the nonsteroidal anti-inflammatory prescription drug market. These drugs may be classified according to the aryl or heteroaryl moiety.

Indomethacin was introduced in the United States in 1965 and is one of the most potent nonsteroidal anti-inflammatory agents.

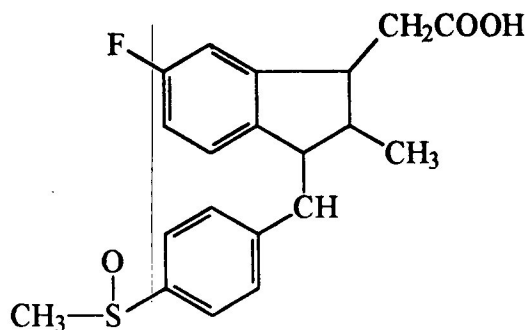
Indomethacin is rapidly absorbed and excreted, with peak blood levels occurring within 2 hours after oral administration.



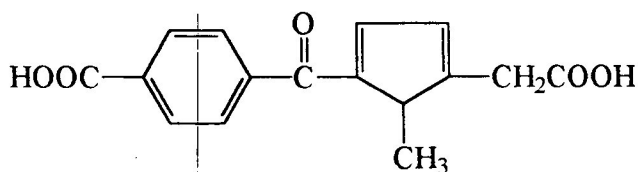
The mode of action may be polyvalent and similar to that of the other acidic anti-inflammatory agents, such as aspirin, phenylbutazone, and mefenamic acid. The anti-inflammatory effects of indomethacin are not a result of stimulation of the production or release of corticosteroids.

Sulindac :

An anti-inflammatory agent marketed in the United States starting in 1978.



The heterocyclic nitrogen atom was removed from indomethacin in an effort to lessen the central nervous system and gastric irritancy side effects of the parent drug. The resultant indene isoster, cis-1-(p- chlorobenzylidenyl) indene 3-acetic acid,(sulindac) proved to be an active compound lacking central nervous system symptoms and showing less irritation to the gastrointestinal tract.



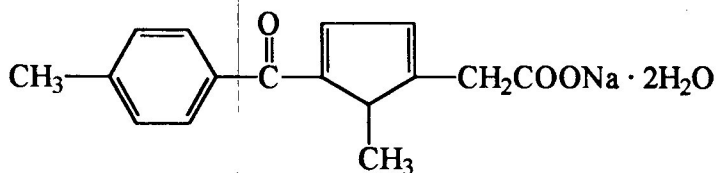
Sulindac is a “pro-drug” its active form is the sulfide metabolite, which has a relatively long half-life, making possible a clinical schedule of twice-daily administration.

Pyrroleacetic Acid Derivatives :**Tolmetin, U.S.P**

Tolmetin (as the sodium salt) was introduced in the United States in 1976 as a new drug for the relief of inflammation and pain of rheumatoid arthritis.

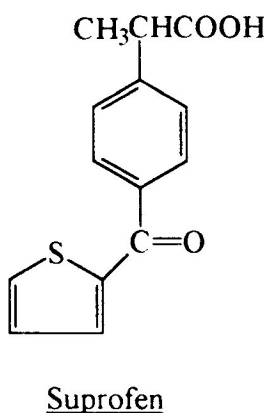
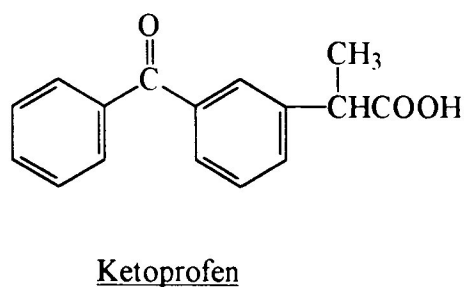
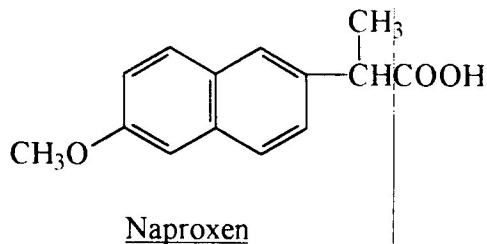
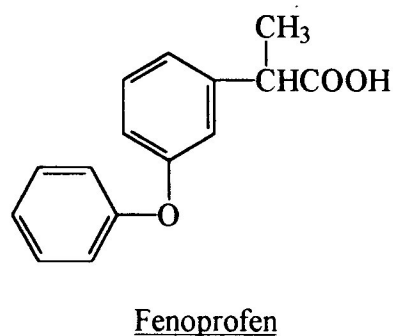
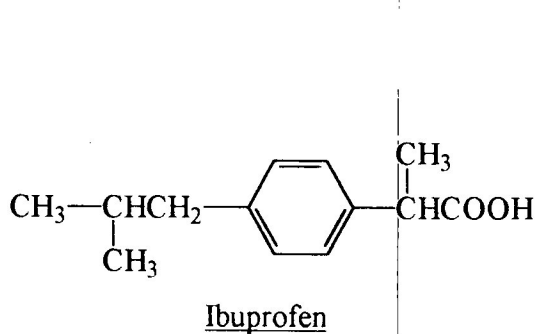
Tolmetin sodium is rapidly absorbed and readily eliminated after oral administration, reaching peak plasma levels within 30 to 60 minutes, with half life of 60 minutes.

Tolmetin sodium inhibits prostaglandin formation by inhibiting prostaglandin synthetase. This may be its primary mechanism of anti-inflammatory activity. Tolmetin sodium also inhibits polymorph migration, has antipyretic activity, and decreases capillary permeability.



Phenyl- α -Methylacetic Acid Derivatives :

Many phenyl- α -methylacetic acid derivatives have been synthesized and found to possess anti inflammatory activity; four such agents presently commercially available in the United States are ibuprofen, fenoprofen, ketoprofen, suprofen and Naproxen.



Ibuprofen U.S.P. :

Ibuprofen is rapidly absorbed when administered orally, reaching peak blood levels in 1 hour, and decreasing to about one-half of peak levels in 4 hours.

The mode of action of ibuprofen is similar to that of the other acidic nonsteroidal anti-inflammatory agents in being related to inhibition of prostaglandin formation.

Fenoprofen is a typical lipid-soluble carboxylic acid and is rapidly absorbed on oral administration, achieving peak plasma levels within 2 hours, with a plasma half life of approximately 3 hours. Fenoprofen's inhibits the rate of collagen-induced platelet aggregation, shows in vitro activation of the fibrinolytic system, stabilizes lysosomal membranes, and inhibits phagocytosis and complement functions.

It is rapidly and completely absorbed from the gastro-intestinal tract, giving in the plasma the unchanged compound, hydroxylated metabolites and their respective glucuronides.

Suprofen :

Suprofen is rapidly and completely absorbed from the gastrointestinal tract with peak serum levels occurring within 1 hour of oral administration.

Suprofen is approximately six times as potent as Retoprofen.

Naphthaleneacetic Acid Derivatives :

Naproxen is an acidic, highly albumin-bound drug. After oral administration, it is promptly and fully absorbed. The mean half-life of the drug is 13 hours, close to ideal for twice-daily administration.

Naproxen like most other acidic nonsteroidal anti-inflammatory agents, inhibits prostaglandin synthesis, this is the most probable mechanism of action.

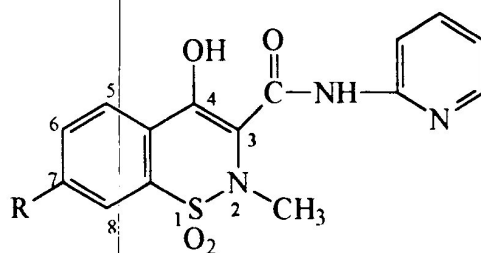
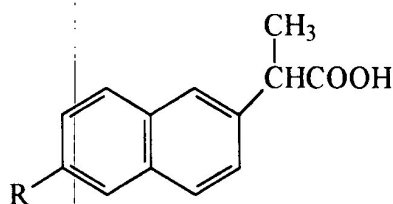
A new class of nonsteroidal anti-inflammatory compounds that are not carboxylic acids is exemplified by the potent agent piroxicam.

Piroxicam is well absorbed after oral administration with peak drug plasma concentrations reached within 3 to 5 hours after administration.

Piroxicam is potent inhibitor of prostaglandin biosynthesis through inhibition of the cyclooxygenase step of arachidonic acid metabolism, and thus may have a mechanism of activity similar to that of the other acidic nonsteroidal anti-inflammatory drugs.

Topical Anti-Inflammatory Agents :

The use of these drugs may be extended to that of topical, nonsteroidal anti-inflammatory agents. The butyl ester of flufenamic acid and the 2 pyridinemethanol ester of ibuprofen have been marketed in Japan for the topical treatment of acute and chronic eczema and of contact and atopic dermatitis, Piroxicam, an amide derivative of ketoprofen, is available in Spain in aerosol form for topical treatment of inflammatory and painful musculoskeletal disorders.



Eicosanoids-Prostaglandins, Thromboxanes and Leukotrienes :

Considerable evidence has supported the role of prostaglandins as primary contributors to conditions of inflammation, pain and fever and has provided the concept that nonsteroidal anti-inflammatory drugs act by inhibiting the biosynthesis of prostaglandins³⁸. Prostaglandins are a group of cyclopentane derivatives formed from 20 carbon polyunsaturated fatty acids by most mammalian tissues, and they exhibit a variety of physiologic activities. The prostaglandins, by

influencing biologic processes near their point of release and by possessing mechanisms for their inactivation at or near the locus of release, have therefore been referred to as “local hormones”.

Sulphonanilides :

Nimesulide is the leading molecule of a new chemical class, the sulphonanilides, which has shown a significant inhibitory selectivity towards cyclooxygenase-2 without affecting cyclooxygenase-1. It is a new non-steroidal anti-inflammatory agent given orally or rectally on a twice a daily basis in a number of inflammatory and pain states.

1. Inflammation :

Several clinical trials have demonstrated the efficacy and tolerability of Nimesulide in the treatment of acute inflammatory conditions of soft tissues and Osteo-articular system.

The action of Nimesulide appears to differ from that of the most conventional NSAIDs in this respect that Nimesulide selectively inhibits the formation of pro-inflammatory prostaglandins with less effect on these prostaglandins that have a gastroprotective action. As a consequence Nimesulide is better tolerated by the G.I. tract than most other NSAIDs while exerting equivalent efficacy. It is worthy of note that Nimesulide may be used in the treatment of inflammation associated with asthma with a reduced risk of inducing obstructive pulmonary reactions as occurs with other NSAIDs.

2. Fever :

In preclinical investigations, Nimesulide shows greater potency and a longer duration of action than paracetamol. Indeed the (E.D. 50) for Nimesulide was 200 fold less than that of paracetamol.

3. Pain status :

Nimesulide has proven to be a potent and fast acting analgesic in the treatment of a variety of painful conditions, including those associated with sports injuries, oncology and post operative trauma.

4. Platelet Anti-Aggregation Activity

Pharmacodynamic properties :

The potency of anti-inflammatory response of Nimesulide were compared with Indomethacin, Phenylbutazone, Deflumidone and Aspirin. It was found 41, 4, 4 and 1 for Indomethacin, Phenylbutazone, Deflumidone and Aspirin respectively where as for Nimesulide it was 120.

Pharmacokinetics :

Nimesulide has been reported to be almost completely absorbed from gastrointestinal tract after an oral dose.

Mechanism of Action :

Nimesulide produces irreversible inhibition of prostaglandins synthesis equally inhibiting formation of PGE_2 and PGF_2 . At doses that inhibit acute inflammation. Nimesulide has no effect on the arachidonic acid metabolism in organs such as stomach, kidneys and lungs normally effected by other NSAIDs. It acts by free radical scavenging, effect on histamine release and activity, effect on neutrophil peroxide, cartilage degradation and metalloprotease synthesis, phosphodiesterase type, inhibition of the release of tumor necrosis factors and thereby decreasing cytokinin release.

Side effects :

At present there are no large long term studies to adequately assess the overall incidence of side effects with Nimesulide. Heart burn, excessive perspiration, flushing, hyperexcitability and skin rash are the occassional side effects reported. The incidence of adverse events usually ranging between 0 to 10% increase with a longer duration of therapy.

Place of Nimesulide in therapy :

Studies to date suggest that Nimesulide is an effective anti-inflammatory and analgesic agent in the treatment of osteoarthritis, some chronic respiratory tract inflammatory conditions, otorhinolaryngological inflammation, soft tissue and oral cavity inflammation, chronic genitourinary tract inflammation as well as in several other conditions such as dysmenorrhoea

and phlebitis / thrombosis. Nimesulide has also generally been associated with a lower incidence of side effects than most comparative drugs.

The efficacy of Nimesulide in the treatment of various conditions associated with the inflammatory process reflects its novel spectrum of action and hence this drug was chosen for synthesis. Clinical trials with Nimesulide and reference NSAID's have demonstrated Nimesulide to be better tolerated than diclofenac, ketoprofen, naproxen, piroxicam and other widely used agents for the relief of the pain and inflammation.

*PLAN
OF
WORK*



The search for new effective and safe drugs has become increasingly sophisticated and costly. Because of a growing awareness of the potential dangers endangered by foreign agents in the body, it is necessary to demonstrate that a drug does not have short and long term deleterious effects on health. It is also necessary to demonstrate that a drug will perform the pharmacological role for which it is recommended.

The main approach 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 groups in the drug molecule known as molecular modification.

The second concerns the physical properties determining 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 and analogues are synthesised.

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

For the treatment of bacterial and fungal infections a number of drugs may be available but they have limited usefulness on account of their toxicity and their ability to combat the emergence of resistance by the invading organism. Thus, there is a definite bridge to be built up by the newer chemotherapeutic agents which can exhibit broad spectrum activity as far as eradication of the infections, toxicity and inexpensiveness of the drug is concerned. Therefore, absolute relentless effort is the necessity of the present state of chemotherapy of bacterial and fungal infections.

Part II

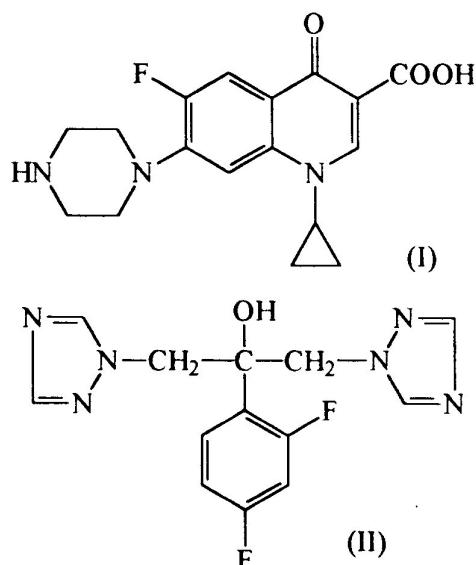
Synthesis of 2-(2,4-difluoro phenyl)-3- (1H-1,2,4-triazole-5-yl) propan-2-ol, 1(substituted phenyl) tetrazoles.

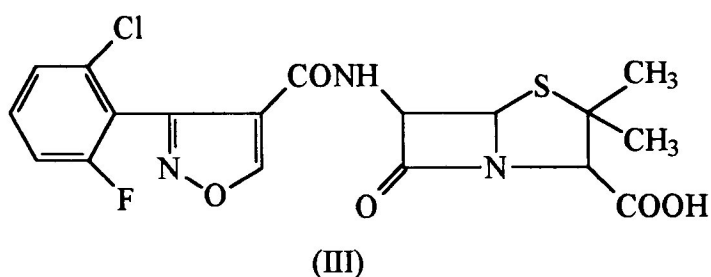
Extensive biological investigations of organic fluorine compounds during the past few decades have been rapidly translated into useful applications in the field of medicinal chemistry. The interest in the fluorine containing compounds which hopes to alter the pharmacological properties of drugs for the better is based primarily in the following considerations :

1. Fluorine, the second smallest atom as measured by atomic radius and internuclear distance to carbon would most closely resemble bioactive hydrogen analogues with respect to steric requirement at enzyme receptore sites.
2. Fluorine imparts increased oxidative and thermal stability because the binding energy of the carbon-fluorine bond exceeds that of the carbon-hydrogen bond by over 17 Kcal/mol.
3. Fluorine leads to increased lipid solubility in membranes that would enhance the rate of absorption and in vivo transport.

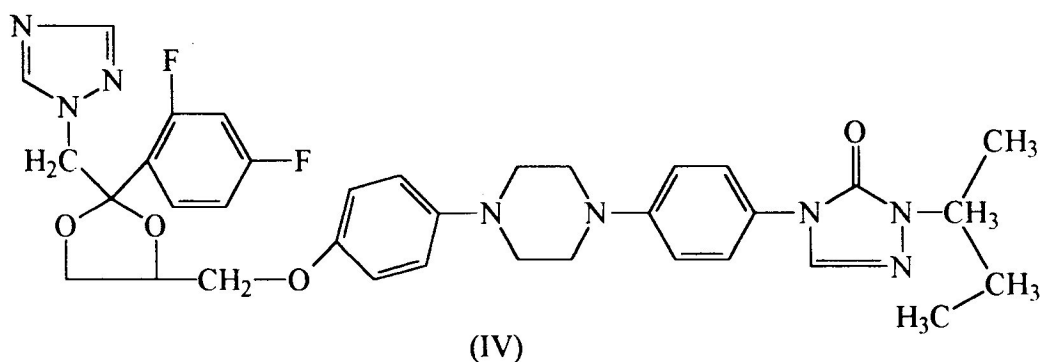
In this regard the tri fluoro methyl group is amongst the most lipophilic of all substituents.

Numerous fluorinated compounds are presently in therapeutic use, viz., Ciprofloxacin (I) (antibacterial), Fluconazole (II) (antifungal), Flucloxacillin (III) (antibacterial).

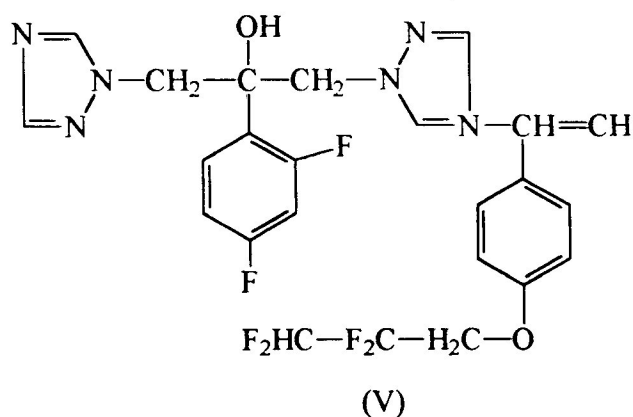




Literature study revealed that the triazole group was less susceptible to metabolic inactivation than an imidazole moiety, but yet it retained the ability to interact with the cytochrome P-450 unit in the fungal C-14 demethylase enzyme. Replacement of imidazole by a 1,2,4-triazole-1-yl unit gave a drug which was twice as potent in the standard murine systemic candidosis model as the corresponding imidazole analogue.

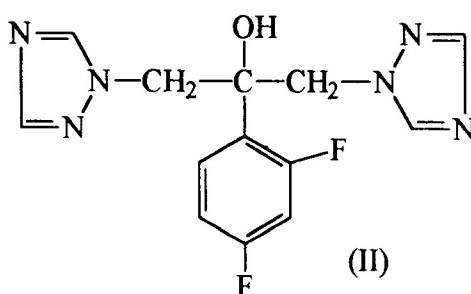


Saperconazole (IV) is water soluble, lipophilic fluorinated triazole with similar chemical structures to itraconazole with the two chlorine atoms replaced by fluorine. It has a broad spectrum of antifungal activity including the dimorphic fungi. *Aspergillus* species, phacohycomycetes and *Cryptococcus* species.



It is an orally active bis-triazole with broad spectrum antifungal activity²⁸.

Like other antifungal azoles, it is a potent inhibitor of fungal C-14 sterol demethylase. However ICI 195739 is more freely permeable than ketococazole and therefore, organisms that are resistant due to reduced azole uptake are more sensitive to this drug²⁹.



Fluconazole is a water-soluble fluorine substituted bis-triazole that has been shown to be effective against a variety of fungal infections in immunocompetent and immunocompromised hosts. Fluconazole has comparable activity to that of ketoconazole in inhibition of fungal C-14 demethylase. A major point of confusion during the development of this drug. However, has been it's relatively low acitvity in vitro compared with its high in vivo activity.

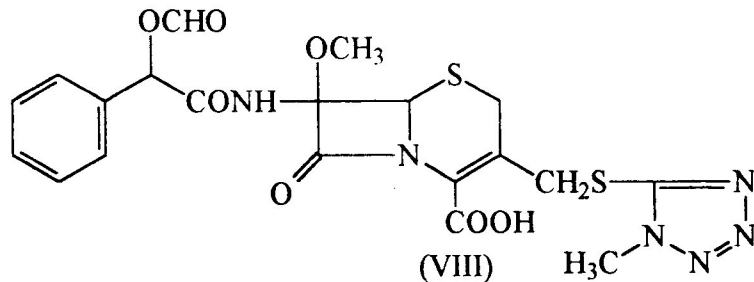
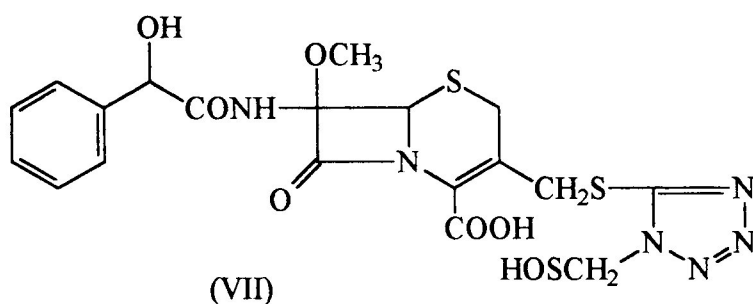
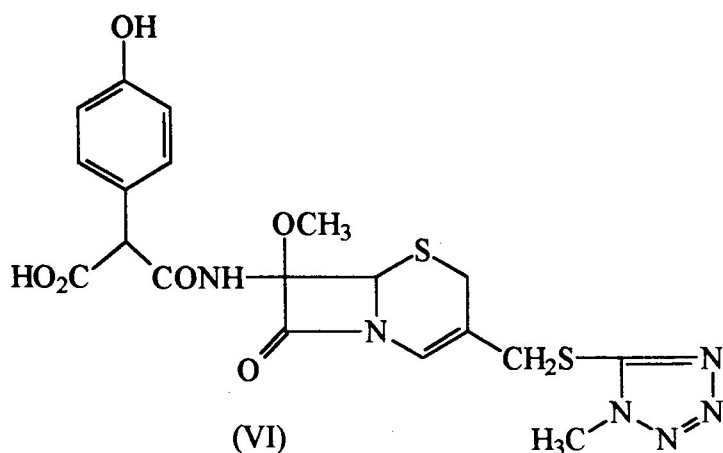
It possesses pharmacokinetic properties that are distinct from the previously discussed azole derivatives.

It has been well tolerated with very few dose limiting side effects. Nausea, other GI symptoms and elevation in hepatic transaminases have been reported in 5% of patients receiving the drug²⁵.

Fluconazole is also a potential candidate for prevention and early treatment of disseminated candidiasis in granulocytopenic patients²⁶.

Finally, fluconazole is also currently being studied for primary treatment of histoplasmosis and sporotrichosis. It exhibits 2,4-difluoro phenyl group as the basic moiety which is responsible for the drugs activity.

Tetrazoles also belong to a category of azoles and compounds containing tetrazole nucleus are known to possess antibacterial and antifungal activity⁴².

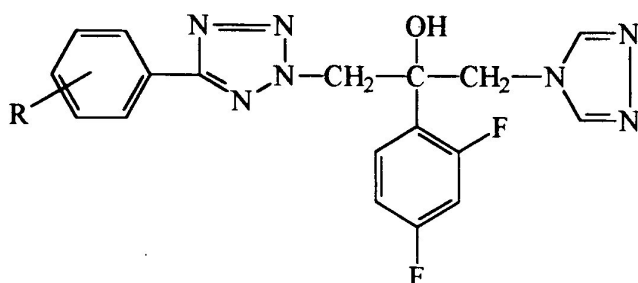


Above mentioned are some of the cephalosporins antibiotics presently used in the United States⁴². These drugs possess the triazole nucleus responsible for activity against gram-negative bacilli. e.g. Moxalactum(VI) and Cephmandole (VIII), anaerobes e.g. Cefonicid (VII).

As seen above triazoles and tetrazoles are the two main bioactive moieties responsible for broad spectrum antibacterial and antifungal activity. Considering the biological importance of fluoro compounds and the above mentioned two bioactive moieties an attempt was made to synthesize a combination of 1,2,4 triazole nucleus and a tetrazole nucleus which gave birth to a series of nine new compounds having halogenated substituents such as fluoro, trifluoro and chloro on the phenyl ring of the tetrazole moiety. These compounds were synthesized and

screened for possible activity against gram-positive and gram-negative bacterial and other fungal infections with an aim to obtain enhanced biological activity.

These nine compounds were as follows :



R = ortho Cl, meta Cl, para Cl

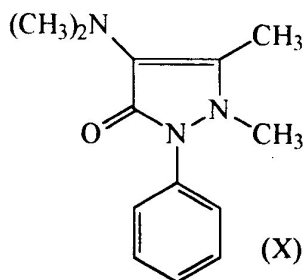
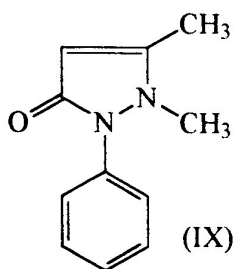
R = ortho F, meta F, para F

R = ortho CF₃, meta CF₃, para CF₃

Part III : Synthesis of 1-thiocarbamoyl-3-methyl-4-(aryl-hydrazono)-2-pyrazolin-5-one

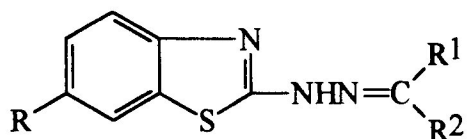
The chemistry and wide range of pharmaceutical properties of 2-pyrazoline-5-one derivatives have been cited in literature^{44,47}. In view of potential biological activities of aryl pyrazoles and pyrazoline-5-one it was considered worthwhile to synthesize some of these compounds as possible antibacterial agents^{45,46}.

Both antipyrine (IX) and aminopyrine (X) have antirheumatic potencies against rheumatic diseases associated with known infections caused by bacterial and fungal agents. Both of them possess pyrazolinone nucleus⁴³.



Many N,N-disubstituted hydrazones of 3-formyl rifamycin SV had high activity both *in vitro* and *in vivo*, against gram positive bacteria and moderate activity against gram negative bacteria⁴². The hydrazone of 3-formyl rifamycin SV with N-amino-N'-methyl piperazine (rifampicin) was the most active *in vivo* and was selected for clinical use. It is active against

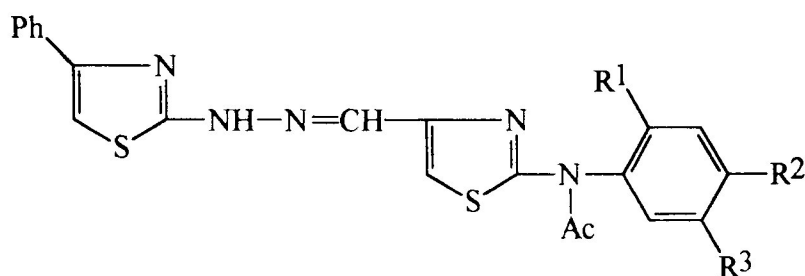
gram positive bacteria at lower concentrations and against gram negative bacteria at concentrations of 1 - 20 µg/ml. In 1993 Zhou *et al*⁴⁸ have reported compounds of type (XI) which are active against *S. aureus*.



R¹ = aryl
R² = halo, H

(XI)

In the same year Likhate *et al*⁴⁹ have reported 2-substituted thiazolyl hydrazones (XII) which are active against *Salmonella typhosa*, gram positive and gram negative bacteria.

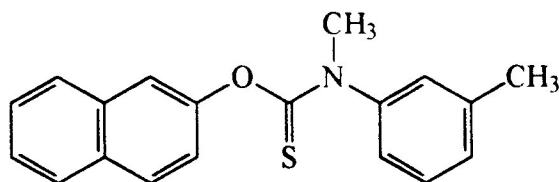


R¹, R³ = H, Cl; R² = aryl

(XII)

Sulfur containing molecules as antifungal agents :

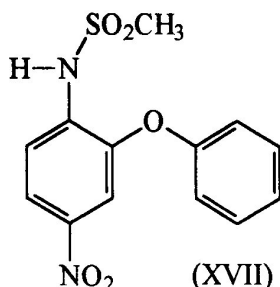
A variety of organic compounds that contain divalent sulfur attached to carbon atom gave *in vitro* fungistatic action and many of these have been employed in topical therapy of dermatophytoses.



(XIII)

Tolnaftate(XIII) is effective against candidal lesion.

Part IV : Synthesis of Nimesulide [A potent non steroidal anti inflammatory drug (NSAID)] by different routes



Nimesulide(XVII) is the leading molecule of a new class, the sulphonanilides, which has shown a significant inhibitory selectivity towards cyclooxygenase 2 without affecting cyclooxygenase 1, cyclooxygenase 2 is the inducible form of the enzyme found almost exclusively in inflammatory tissues but largely devoid of physiological activity in healthy tissue, in which cyclooxygenase 1 plays important regulating roles (particularly gastric and renal physiological control). This significant inhibitory selectivity results in equivalent efficacy against pain and inflammation but with a better safety profile particularly at the gastro intestinal level.

The drug 'Nimesulide' was chosen for synthesis due to the favourability of following reasons :

1. It is remarkably safe NSAID, very effective as treatment for painful and inflammatory processes and a useful alternative for patients at risk of adverse reactions with classical NSAIDS i.e. elderly subjects and patients sensitive to acetyl salicylic acid and other NSAIDs who need antiinflammatory treatment.
2. On the basis of an index score of severity, Nimesulide (score 21) showed a less ulcerogenic trend than diclofenac (score 28).
3. The hepatic toxicity profile of Nimesulide was shown to be safer than that of other commonly used NSAIDs.
4. The incidence of adverse reactions with 8.9% with Nimesulide with 16.7% with reference to drugs such as Diclofenac, Ibuprofen, Indomethacin, Piroxicam, etc.

5. Comparative clinical trials with Nimesulide and reference NSAIDs have demonstrated Nimesulide to be better tolerated especially with regard to gastrointestinal toxicity, than Diclofenac, Ketoprofen, Naproxen, Piroxicam and other widely used agents for the relief of the pain and inflammation.

Several clinical trials have demonstrated the efficacy and tolerability of Nimesulide in the treatment of acute inflammatory conditions of soft tissues and osteo articular system. NSAIDs inhibit the synthesis of prostaglandins, however the action of Nimesulide appears to differ from that of most conventional NSAIDs in this respect that it selectively inhibits the formation of pro-inflammatory prostaglandins with less effect on those prostaglandins that have a gastroprotective action. As a consequence, Nimesulide is better tolerated by the GI tract than most other NSAIDs while exerting equivalent efficacy.

Nimesulide may be used in the treatment of inflammation associated with asthma with a reduced risk of inducing obstructive pulmonary reactions, as occurs with other NSAIDs.

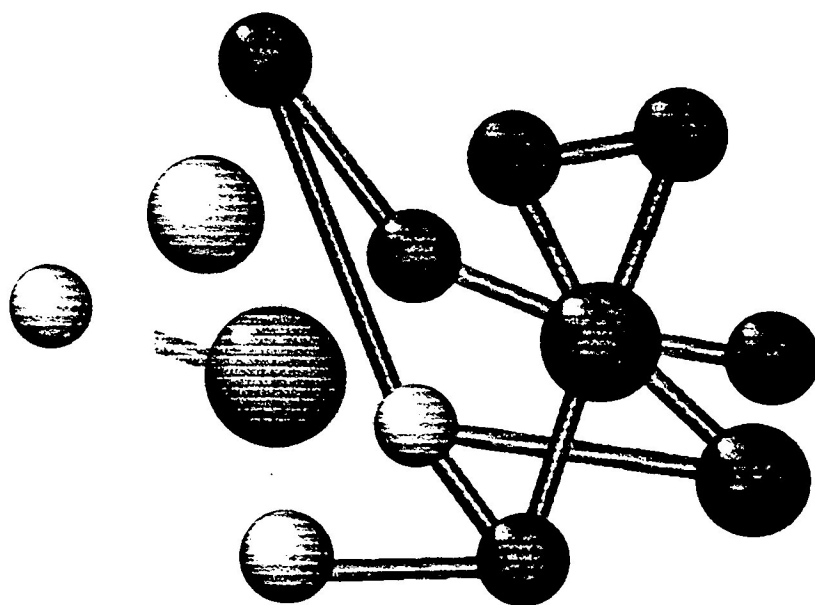
Nimesulide shows greater potency and a longer duration of action than Paracetamol.

Since the efficacy of Nimesulide in the treatment of various conditions associated with the inflammatory process reflects its novel spectrum of action, this drug was chosen for synthesis.

Nimesulide was prepared as follows :

Condensation of ortho chloro nitro benzene with phenol gave nitro diphenyl ether which was reduced to amino diphenyl ether which on further condensation with methane sulfonyl chloride gave 2-phenoxy methane sulfonamide which on nitration gave the desired drug.

CHEMISTRY



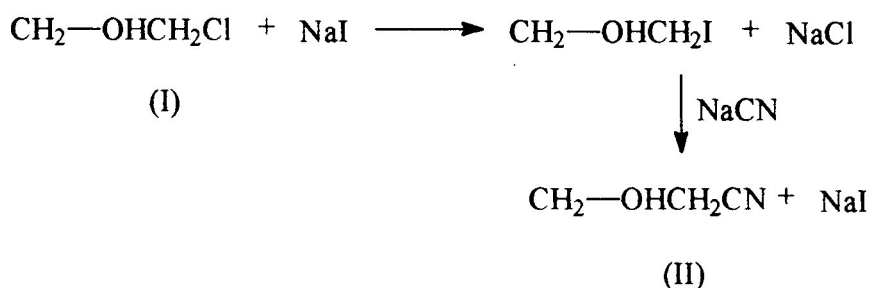
CHEMISTRY
OF
NITRILES

Nitriles are prepared by the classical methods described in the literature such as :

1) From Halides :

a) From aliphatic halides :

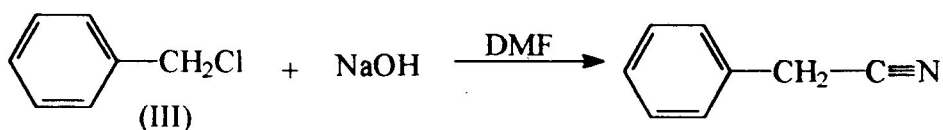
The metal cyanides react with alkyl (I) or aryl halides to provide mainly aliphatic nitriles (II). The reactivity of the halides in decreasing order is $-I > -Br > -Cl$. Therefore, the yields of nitriles obtained from primary halides are increased by the introduction of a promoter such as sodium iodide, which by exchange converts the chloride first into the iodide, which in turn, reacts with the metallic cyanide to form the nitrile.



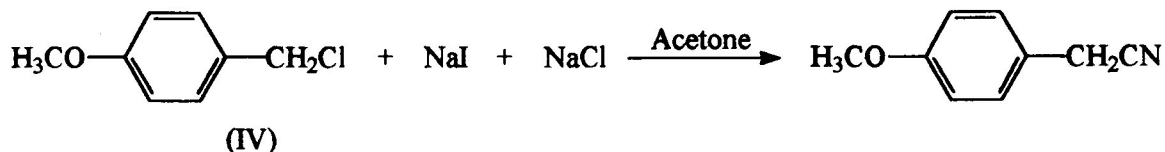
Other metallic cyanides used to form nitriles are potassium cyanide or cuprous cyanide.



The yields in such cases vary from 75-90%. The solvent - alcohol, accelerates the reaction very slowly and higher solvents like ethylene glycol⁵⁰, methyl cellosolve⁵¹, tetrahydrofurfuryl alcohol⁹² and DMF improve the yields; but with secondary halides, their yields are low and tertiary halides give little or no nitriles. The conversion of alkyl halides (III) into nitriles is accomplished best with sodium or cuprous cyanide with pyridine, quinoline, dimethyl formamide⁵³ and N-methyl pyrrolidine⁵⁴ as solvents. The reaction time can be shortened by the addition of small amount of a nitrile and traces of copper sulphate as catalyst.



While, halide like anisyl chloride (IV) gives its nitrile in 75% yield when it is treated with sodium iodide and sodium cyanide in dry acetone⁵⁵.

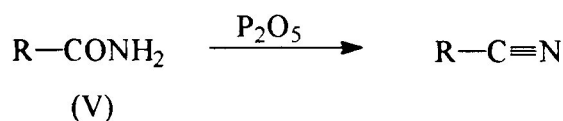


b) From heterocyclic halides :

The halides of pyridine⁵⁶, quinoline⁵⁷, yield nitrile satisfactorily with cuprous cyanide alone⁵⁴.

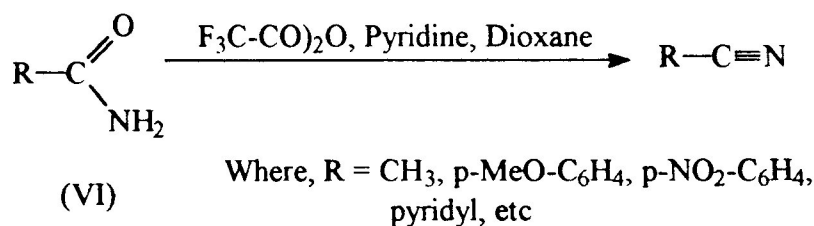
2) From amides :

The preparation of nitriles by the removal of water from amide (V), can be performed in 60-70% yields by using usual dehydrating agents like phosphorus pentoxide⁵⁸ or phosphorous pentachloride⁵⁹.



Sometimes, dehydrating agents like carbonyl chloride⁶⁰, benzene and p-toluene sulphonyl chloride⁵⁸⁻⁶¹, phosphorus oxychloride are used in the nucleophilic solvents such as pyridine or tertiary amines. Even addition of salts like sodium chloride⁶² or sodium metabisulphite⁶³ to phosphorus oxychloride, enhance the effectiveness of the reagents.

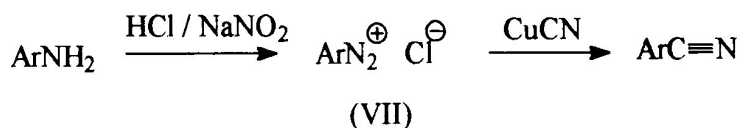
The recent convenient synthesis of nitriles⁶⁴ under mild conditions, makes use of the trifluoroacetic anhydride / pyridine system, which leads to a rapid amide(VI) to nitrile transformation with 85-95 % yields.



The excellent yields and mild reaction conditions of the new method, are especially useful for thermally labile nitriles.

3) From diazonium salts (Sandmeyer's reaction) :

The replacement of aromatic amino groups by cyanide, is obtained in 65-85% yields by the action of cuprous cyanide with diazonium compounds (VII).



Double or complex salts of cuprous cyanide such as $\text{Na}_3\text{Cu}(\text{CN})_5\text{NH}_3$ ⁶⁵ has also been employed to a great extent. While, carrying out the reaction, it is important to neutralize the the diazonium salt before addition to the cyanide solution, in order to avoid the escape of hydrogen cyanide. o-Tolunitrile⁶⁶ can be obtained by this procedure in 64-70% yield.

4) From aldehydes :

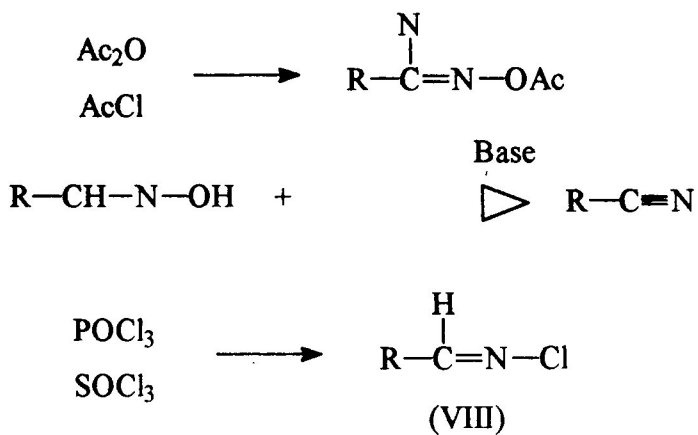
Many methods have been reported for the conversion of aldehydes to nitriles e.g., (a) dehydration of aldoximes using dicyclohexyl carbodiimide⁶⁷, carbonyl biimidazole⁶⁸, or phosgene immonium chloride⁶⁹, (b) 1,2-elimination of o-substituted aldoxime^{70, 71} and (c) 1,2-elimination of $\text{R}-\text{CH}=\text{N}-\text{N}-(\text{CH}_3)_3$ and $\text{R}-\text{CH}=\text{N}-\text{tosyl}$ by base⁷².

Method "A" is discussed under the title "dehydration of aldoximes" and Methods "B" and "C" are discussed under the headings "1,2-elimination of o-substituted aldoximes.

Method - A (Dehydration of aldoximes) :

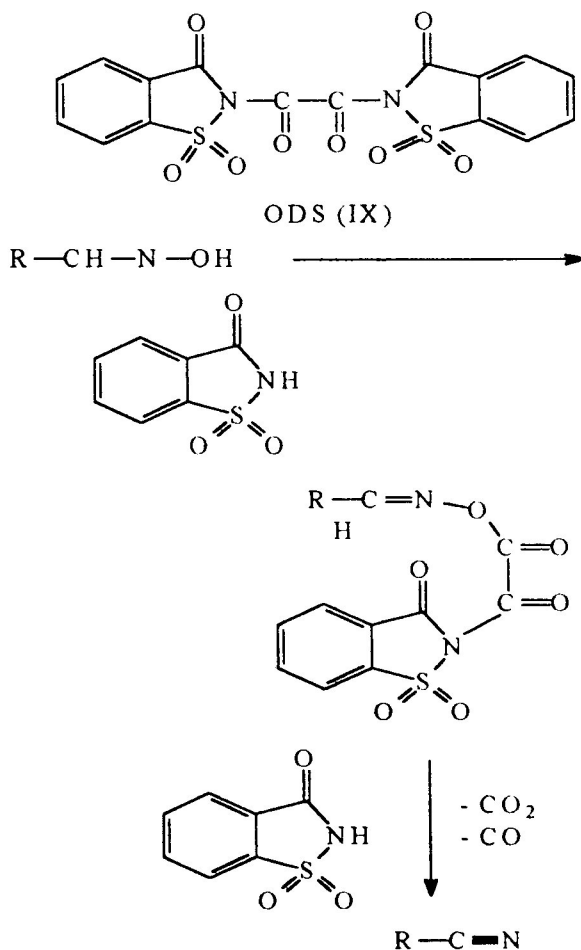


The dehydration of aldoximes represents one of the preparative method for nitriles from corresponding aldehydes. Large number of dehydration agents are employed to accomplish the dehydration of aldoximes⁷³. e.g. acetic anhydride, phosphorus oxides, thionyl chloride, acetyl chloride, etc. In all these cases, the dehydrating agent forms a reactive complex (VIII) which renders the aldoximes to lose a water molecule easily.



Recently, new dehydrating agents have been introduced e.g., dicyclohexyl carbodiimide⁶⁷, carbonyl biimidazole⁶⁸, and phosgene immonium chlorides⁶⁹.

Very recently Kitagawa, T. et al.⁷⁴ described new dehydrating agent, 2-2'-oxalyldi (o-sulfobenzimide) (ODS) (IX) for convenient one-pot synthesis of nitriles from aldoximes. The possible mechanism proposed was as follows :



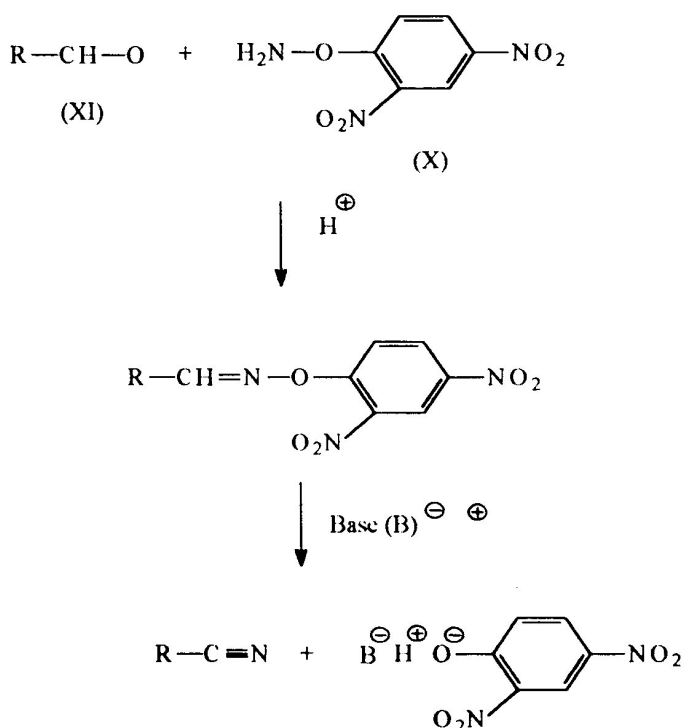
These reagents are highly reactive and involves very clean reaction at room temperature or cold temperature.

Methods - B & C : (1,2-Elimination of o-substituted aldoximes) :

Method B

This is the method recently developed by Miller et al.⁷¹ for accomplishing the conversion of aldehydes to nitriles. This method differs from other routine methods in the fact that the experimentalist need not isolate and purify the intermediate oximes or related aldehyde derivatives, although he may do so, if he wishes. This method, therefore, saves one step in the aldehyde to nitrile conversion.

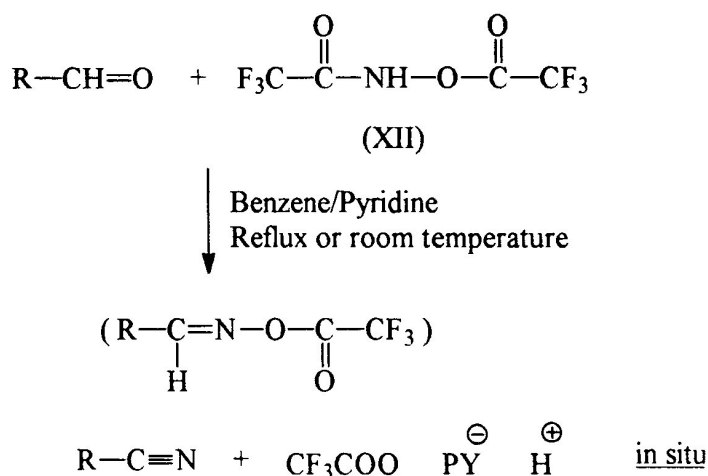
The development of efficient amino protecting groups has facilitated the synthesis of a variety of sensitive compounds, including o-substituted hydroxyl amines⁷⁵. Although a number of these are highly unstable⁷⁶, other have significant synthetic utility. Their electrophilic character has been exploited in their increasing use as aminating agents⁷⁷⁻⁷⁹. Their nucleophilicity has been demonstrated by their reaction with ketones to form substituted oximes. Thus, 2,4-dinitrophenyl hydroxyl amine(X) can be reacted with aromatic or alkyl aldehydes (XI) to form corresponding o-substituted aldoximes.



Nitriles can be prepared from these o-substituted aldoximes by 1,2-elimination reaction using excess base [KOH or $N(C_2H_5)_3$], as shown above. The development of a deep yellow to red colour of the 2,4-dinitrophenolate ion indicates the extent of completion of the reaction.

Method C :

This method is a one-step reaction involving the use of O-N-bis(trifluoroacetyl) hydroxyl amine (XII) as one of the reagent as follows :



The reaction conditions are very mild and directly affords nitrile, eliminating the intermediate step⁸⁰. The yields are in the range of 70-90 %.

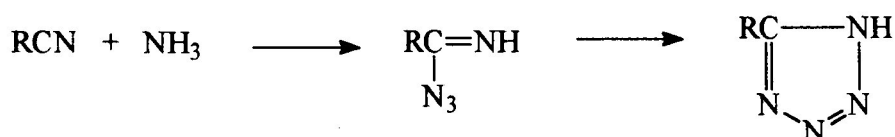
The reagent could be obtained by reacting trifluoroacetic anhydride with hydroxyl amine hydrochloride in suitable solvents. The intermediate aldoxime is so unstable that it could not be isolated and instantly decomposed to furnish nitrile; thus, eliminating the intermediate step.

Among the nitrogen containing derivatives of aldehydes which have been utilised in preparing nitriles, the oxime derivatives were the most successfully used in our present work to prepare substituted phenyl nitriles which are obtained from their corresponding substituted aldehydes by first converting them to aldoximes, followed by dehydration to get nitriles.

The oximes were achieved by condensing corresponding aldehydes (0.05 mole) at reflux temperature with a solution of hydroxylamine hydrochloride (0.06 mole) in ethanol, in presence of aqueous solution of sodium carbonate (0.075 mole). In some cases, where oximes do not precipitate out, the reaction mixture has to be saturated with gaseous carbon dioxide and could to get the oximes in very good yields. These oximes give respective nitriles in the range of 55-90% yields after dehydration with 94-96% acetic anhydride.

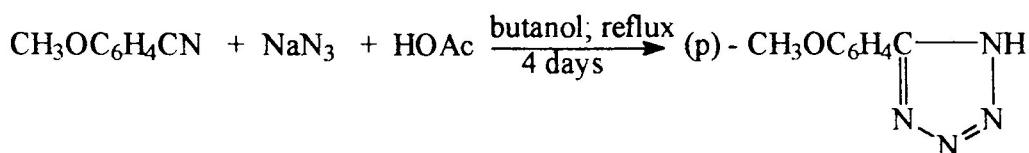
CHEMISTRY
OF
TETRAZOLES

Tetrazoles are formed by addition of hydrazoic acid to compounds with carbon-nitrogen unsaturation such as nitriles, cyanates, thiocyanates, isocyanides, cyanamides, carbodimides, isocyanates and isothiocyanates. Nitriles combined with hydrazoic acid to form 5-mono substituted tetrazoles. In this, as well as in other synthesis of 1-, 5-, and 1,5 substituted tetrazoles, the presumed intermediates are imide azides which cyclize to the isomeric tetrazoles. As a rule, it is impossible to isolate the intermediate imide azide.

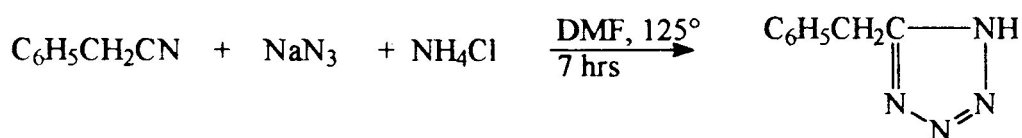


Tetrazoles, itself is formed by the interaction of hydrogen cyanide and hydrogen azide in alcoholic solution⁸¹ yields of 5- alkyl or aryl-tetrazoles are generally high⁸² but the reaction usually requires several days for completion.

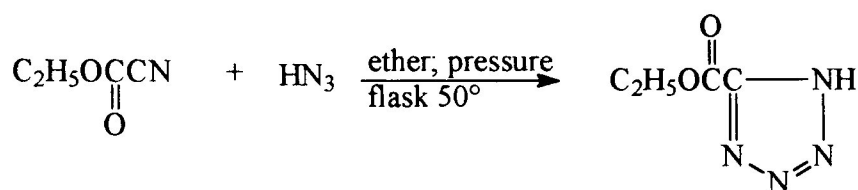
Several methods are available that avoid the separate preparation of hydrazoic acid solutions. In one procedure sodium azide and glacial acetic acid are heated with nitrile in 2-propanol or butanol^{82,83}.



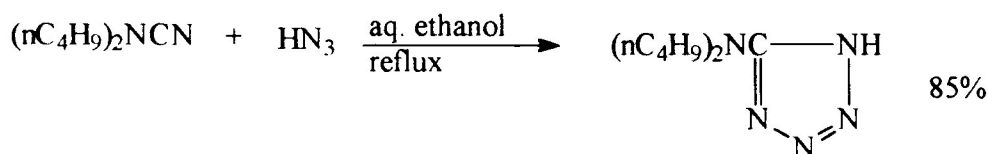
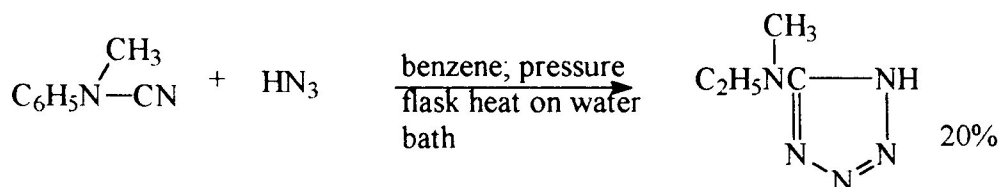
Ammonium azides, prepared in situ from sodium azide under ammonium or substituted ammonium chlorides⁸⁴⁻⁸⁵ combined readily with nitriles in relatively short reaction times. Dimethyl formamide is a good reaction medium because of its solvent power for inorganic azides.



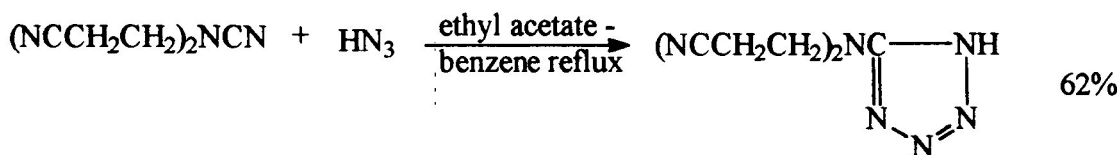
Increasing the positive charge on the carbon of a nitrile group facilitates the attack of azide ion. This may be achieved by the addition of small excess of either ammonium azides or Lewis acids which exert their influence by co-ordinating with the nitrile nitrogen. If strongly electro negative groups are directly attached to cyanide, the addition of hydrogen azide often proceeds under milder conditions than with alkyl or aryl substituents. Ethyl cyanoformates, cyanogen bromide, and cyanogen, e.g. combined with hydrazoic acid in ether solution⁸⁶ to form the corresponding tetrazoles.



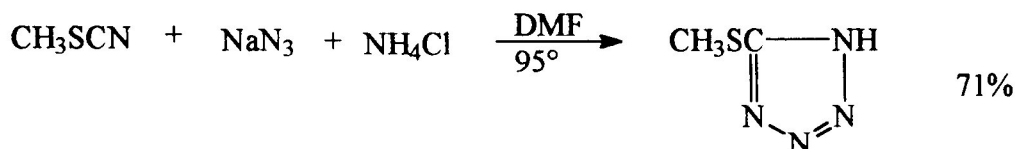
Disubstituted cyanamides also may be converted to tetrazoles, as illustrated by the reactions of methyl phenyl cyanamide⁸⁷ and dibutylcyanamide⁸⁸ chemical reaction.



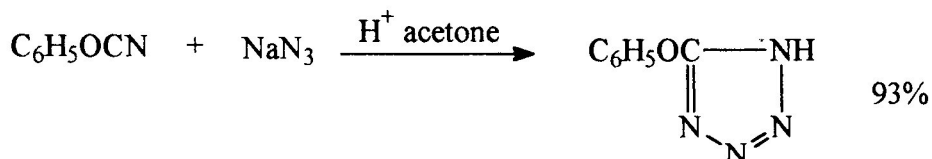
Reaction of bis (2-cyanoethyl) cyanamide with hydrazoic acid takes place only at the more polarised cyanamide nitrile and not at the nitrile groups of the cyanoethyl radicals⁸⁹. Similarly, more electro negative aryl nitriles react faster with ammonium azides after three hrs. at 100°C⁸⁴ to give 5 substituted phenyl tetrazoles.



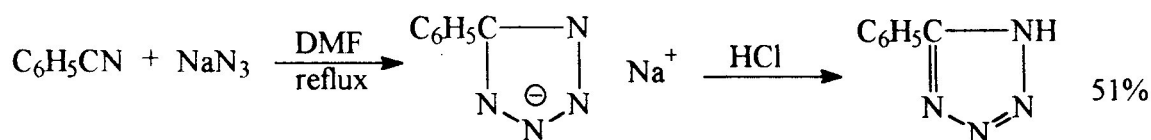
Thiocyanates also react as nitriles and are converted by the sodium azide-ammonium chloride method to 5-alkyl or 5-aryl-thio tetrazoles^{105,84}.



Cyanates add hydrazoic acid under mild conditions to form 5-substituted tetrazoles ethers¹⁰⁶.

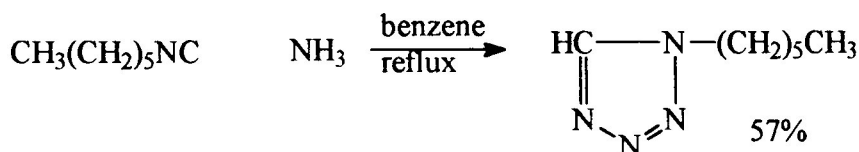
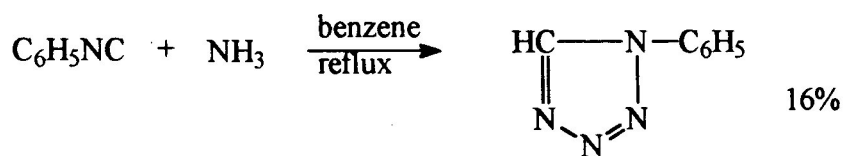


Since the azide - cyanides reaction proceeds by combination of the azide ion with the cyanide carbon, preformed hydrazoic acid is not essential to the reaction. Indeed, sodium azide, with no added acid during the reaction, combines with benzo nitrile in dimethyl formamide to form sodium 5-phenyl tetrazole, acidification liberates the free tetrazole⁸⁴.

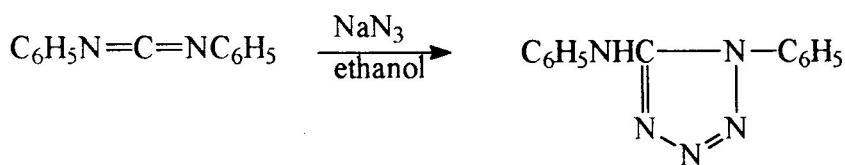


Trifluoro aceto nitrile reacts exothermally with sodium azide in aceto nitrile at room temperature to give sodium 5-trifluoro methyl tetrazole in 75% yield⁹⁰. Aluminium azide in tetrahydrofuran also reacts with nitriles^{91,92}, a quantitative yield of 5-phenyl tetrazole results from benzonitrile after heating for 24 hrs. with this reagent, but only one third of the available azide groups are utilised.

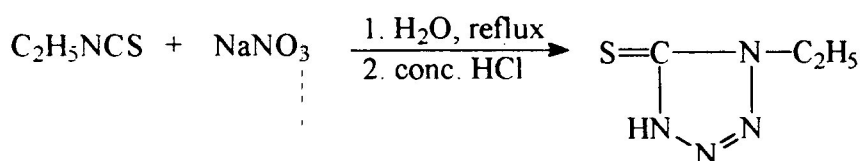
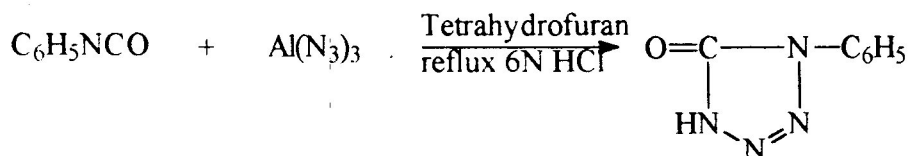
Inter-action of hydrazoic acid with isocyanides in ether or benzene solution produces 1-substituted tetrazoles^{93,94,95,96,97}.



Carbodiimides react with hydrogen azide to form 1,5-disubstituted tetrazoles with a nitrogen substituent in the 5 position. The method has been applied to both alkyl⁹⁸ and aryl carbodiimides^{99,100}

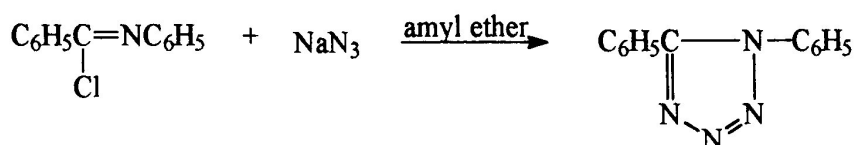


Separate preparation of carbodiimides for this synthesis may be avoided by starting with thiourea derivatives. Isocyanates¹⁰¹ and isothiocyanates^{102,103} led respectively to 1 substituted tetrazolinones and tetrazolinethiones.



The reaction between imide chlorides and azide salts constitute a general synthesis of 1-mono substituted and 1,5 disubstituted tetrazoles.

Thus reaction of the imidechloride of benzanilide with sodium azide produces 1,5-diphenyl tetrazole¹⁰⁴ by displacement of chlorine.



The nature of the product appears to be affected by many factors such as nature of aromatic compounds, the reaction temperature and duration of the reaction i.e. larger period of time favours the yield of tetrazole.

The method (2) is comparatively favourable with other methods of the preparation of tetrazoles because of its simplicity and satisfactory yields^{84,85}.

In the present synthetic work, the 5 (substituted phenyl) tetrazoles have been synthesized as per method (2) essentially due to the following reasons:

- 1) Easy availability of raw materials;
- 2) Cost effective operation;
- 3) Non-hazardous operation due to the use of azide salt instead of hydrazoic acid;
- 4) High reactivity of nitriles in relatively short reaction time with good reaction medium (DMF).
- 5) Good yields.

CHEMISTRY
OF
TRIAZOLES

Methods of Preparation :

Intra molecular condensation: Ring closures in Alkaline Media.

Ring closure of acyl derivatives of semicarbazides, thiosemicarbazides, or amino-guanidines in alkaline solutions is a method widely applied for the preparation of s-triazoles. Starting materials are obtained on acylation of semicarbazide, thiosemicarbazide, aminoguanidine, or their derivatives. The required acyl semi-carbazides or acylthiosemicarbazides are also available from the combination of acylhydrazines or thiocarbohydrazide and potassium cyanide or thiocyanate^{107,108}.

With acylthiosemicarbazides as starting materials for S-triazoles, competitive thiadiazole formation may be diminished or eliminated by operating in strong alkali¹⁰⁹.

Ring closures in Acidic Media :

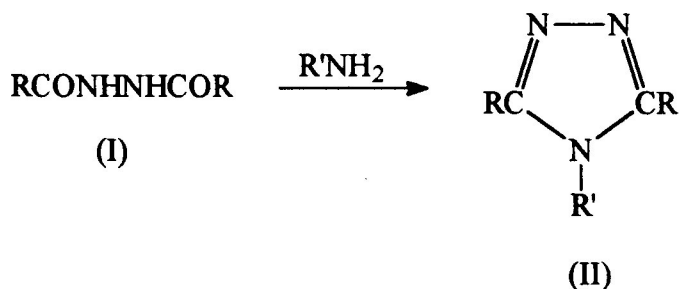
The tendency of acyl thiosemicarbazides to be transformed in the presence of acids into thiadiazoles rather than triazoles has limited the number of S-triazole derivatives obtained from this combination of starting materials. Certain triazole ring closures require dehydrogenation.

Oxidative ring closures in the presence of peroxide oxidation of s-alkyl thiosemicarbazones with hydrogen peroxide is reported to give better yields of triazoles, atleast in certain instances, than are obtained from oxidation with ferric chloride¹¹⁰.

Thermally induced Ring Closure :

Several acyl derivatives of semicarbazides, thiosemicarbazides, and aminoguanidines change into 2-triazoles when heated.

Intermolecular condensation of Diacylhydrazines and amines. On treating diformylhydrazine (I, R = H) with an excess of ammonia in an autoclave at 200°C for 24 hours, 1, 2, 4 - triazole (II, R = R = H) is obtained in good yield¹¹¹.

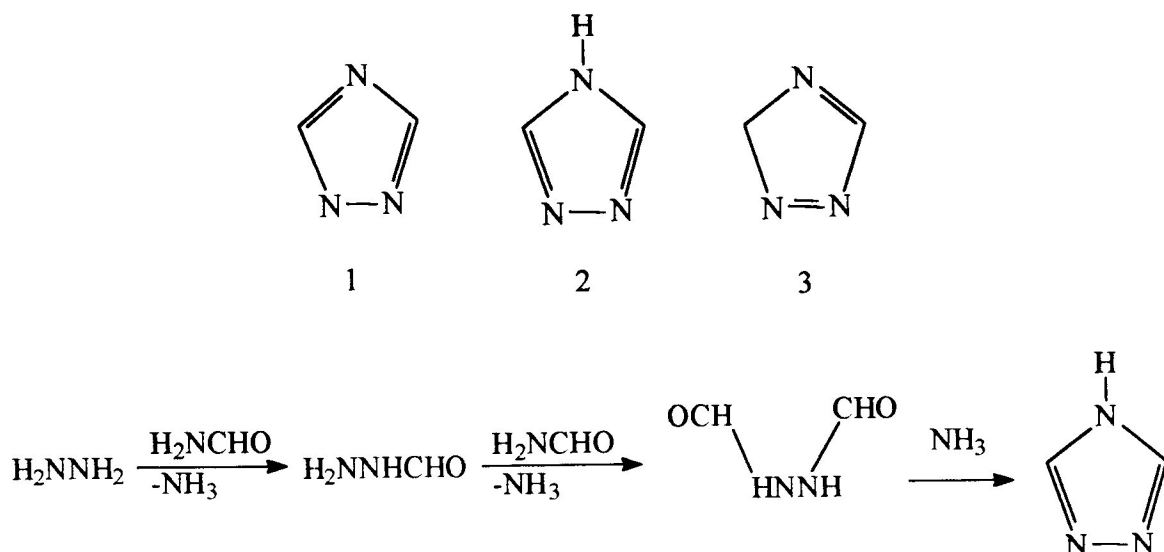


Under the same conditions a mixture of hydrazine, ammonia, and formamide gives comparable yields of 1, 2, 4-triazole. Triazoles have been obtained from N-acylamido-dithiocarbamic acids and amines, diacyl Amines and Hydrazines (Einhorn-Brunner reaction).

The formation of triazoles from diacylamines and hydrazines has been designated the Einhorn-Brunner reaction¹¹². Hydrazine or its monosubstituted derivatives are combined with symmetrical or unsymmetrical diauflamines in an organic acid, in an acid-buffered solution, or in the presence of pyridine hydrochloride. Stronger acids give lower yields of triazoles and enhance the formation of hydrazides, ammonium salts and compounds of unknown structure. Isomeric triazoles are not produced from unsymmetrical diacyl amines^{113,114}.

Heating hydrazides and amides at high temperatures in the absence of a solvent produces S-triazoles¹¹⁵ by a process known as the Pellizzari reaction¹¹⁶. Acyl hydrazidines may be intermediates. Characteristic low yields are attributed to loss of amides through dehydration into the corresponding nitriles which do not participate in the Pellizzari reaction leading to triazoles. Unsubstituted 1,2,4- triazole has been prepared from formamide and formyl hydrazine.

The heteroaromatic triazole ring system is composed of five atoms, two carbons, and the three nitrogens, which can be arranged in two combinations to give either 1,2,3- triazole or 1,2,4- triazole. Although two NH (1 and 2) and one CH₂ (3) tautomeric forms are possible for 1,2,4- triazole, this structure is best represented as a positively charged hydrogen associated with the resonance stabilized triazole anion.



The 1,2,4-1 H- triazole notation is used to describe a 1 N - substituted triazole. Pellizzari obtained the parent ring system from the reaction of formyl hydrazine with formamide.

Condensation of hydrazine sulfate with formamide was reported to give a 53 % yield of s-triazole (1)¹¹⁷. Ainsworth and Jones observed that a large quantity of ammonia was evolved in the reaction of hydrazine with Formamide, and to prevent the loss of ammonia the intermediate N,N - di- Formyl hydrazine was reacted with excess ammonia in a pressure vessel to give a 70 to 80 % yield of s-triazole (1)¹¹⁸. A Further improvement in the yield of s-triazole (1) resulted from the work of Grundmann and Ratz, who obtained a 95 % yield of s-triazole(1). From the interaction of S-triazine (4) with hydrazine hydrochloride¹¹⁹. Apparently, the intermediate amidrazone (5) was initially formed, which was postulated to react with another molecule of s- triazine (4) to give s-triazole (1). However, the acid- catalysed self condensation of amidrazones is well documented¹²⁰⁻¹²², and s-triazole (1) might be formed via intermediate 6. With hydrazine rather than it's hydrochloride, s- triazine (4) reacted to give 1,2 - diformyl hydrazine dihydrazone.

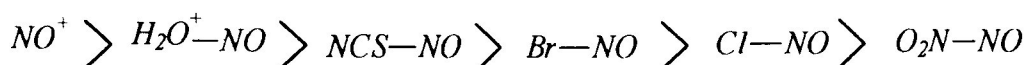
CHEMISTRY
OF
DIAZONIUM SALTS



Recently the intermediate step of a primary nitroso amine could be isolated in the course¹²³ of the diazotization of an appropriate amine .

Since most diazotizations are performed in aqueous medium, protonated nitrous acid (from sodium nitrite and acid)¹²⁴ is usually used, free NO^+ should only appear in concentrated acid and in the course of conversions with complex NO^+ salts.

Reactivity decreases under normal conditions in the following sequence:-



1) In aqueous medium :

Normal diazotization of aromatic amines with nitrous acid is carried out in dilute aqueous mineral acids and usually yields the diazonium salts in solution. The direct method starts from solutions or suspensions of the amines in dilute hydrochloric acid (approximately 2.5 mol acid per mol amine) which are treated with the molar amount of concentrated aqueous sodium nitrite solution at 0-10°C. The conversions proceed rapidly and almost quantitatively¹²⁵, the end point can be determined by numerous methods, and an excess of nitrous acid can easily be removed by adding urea which reacts with nitrous acid to form nitrogen, carbon dioxide and water or sulphamic acid¹²⁶.

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

2) In concentrated acids¹²⁷ :

Weakly basic amines bearing strongly electron attracting substituents on the aromatic (or hetero aromatic) nucleus are diazotized preferentially in concentrated acids, since hydrolysis¹²⁸ of the generated diazonium compounds occurs in aqueous solutions with rising dilution. Good results have been described with concentrated sulphuric acid¹²⁹.

3) In organic solvents¹³⁰ :

Usually, in order to prepare solid diazonium salts, diazotizations of aromatic amines are carried out in organic solvents (glacial acetic acid¹³¹, methanol, ethanol, formamide, DMF, acetone) by means of nitrous acid esters (preferentially pentyl nitrile)

A diazonium salt will couple with an aliphatic compound containing an activated carbon-hydrogen bond. This discussion is limited to those reactions in which both nitrogen atoms of the diazonium salt are retained in the resulting molecule.

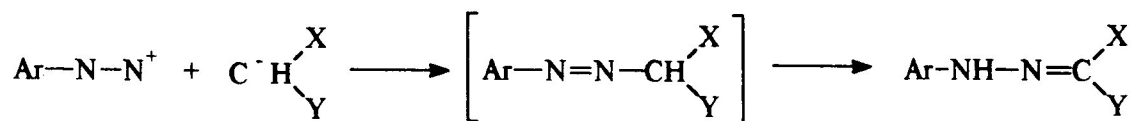
Victor Meyer was the first to report the coupling of a diazonium salt with an activated aliphatic carbon atom 1.

Much of the early work with the coupling reaction was prompted by the desire to determine whether the products were of the azo or hydrazone structure. However it is generally assumed that the hydrazone is the stable form whenever coupling occurs at a methyl or methylene carbon and where coupling occurs on a methinyl carbon without the elimination of group only the azo structure is possible.

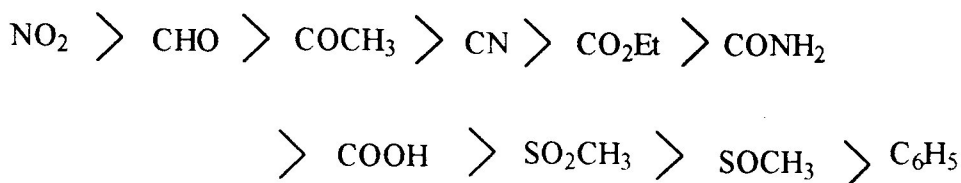
The reactions of β - Keto esters with diazonium salts have been studied extensively. Products from ethyl acetoacetate and over fifty different diazonium salts have been reported. Good yields of the hydrazones of α , β - diketo esters are obtained if 1 mole of the diazonium salt is employed.

CHEMISTRY
OF
HYDRAZONES

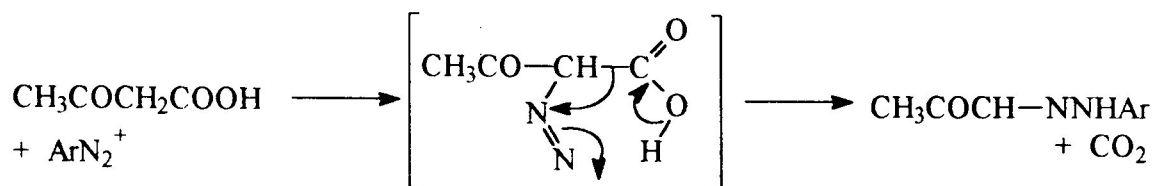
Aliphatic compounds containing an activated methylene group couple with diazonium salts to form aryl hydrazones^{140,141}. The reaction is regarded as an electrophilic attack of the diazonium ion on the carbanion derived from the methylene compound, forming an unstable azo compound which spontaneously tautomerizes to a hydrazone:



The reaction is usually carried out in cold aqueous solutions buffered with sodium acetate, but the PH of the medium can be lowered for strongly activated methylene compounds. The activating effect of the substituents (X and Y) for the coupling can be arranged according to Hunig and Boes¹⁴² in the decreasing order.

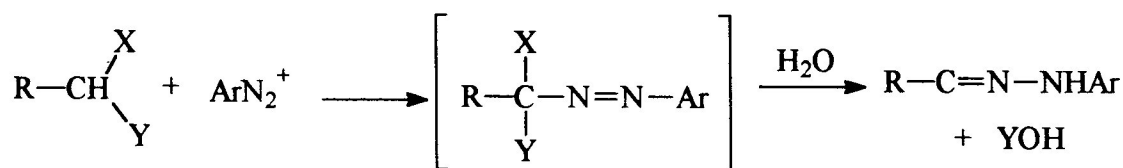


Other activating groups are the tertiary sulphonium and quaternary pyridinium ions, and heterocyclic residues such as 2- pyridyl and 2- quinolyl,. When one of the activating groups is a carboxyl, coupling is accompanied by decarboxylation, resembling the nitrosative decarboxylation :

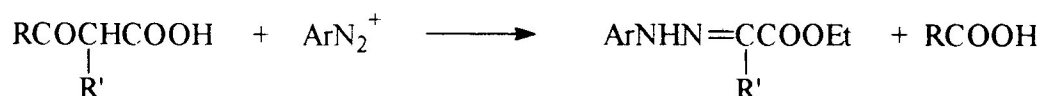


Active methyl groups situated at the 2 or 4 position to the ring nitrogen of heterocyclic compounds such as 2- picoline¹⁴³ and 9 methyl acridine¹⁴⁴ can also couple with a diazonium salt to yield a tetra hydrazone. The activity of the methyl group is increased if the heteroatom is first quaternarized to the onium salts.

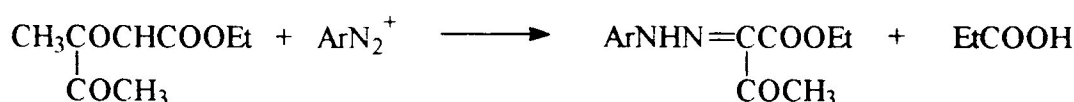
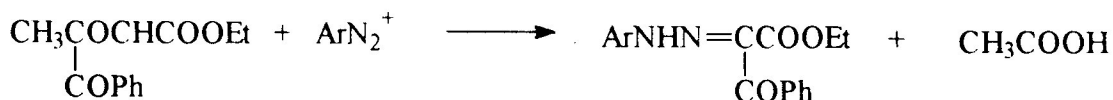
A methinyl carbon carrying at least two activating groups reacts with diazonium salts to form an unstable azo compound, which is subsequently transformed to aryl hydrazone by hydrolytic cleavage of one of the electron attracting groups.



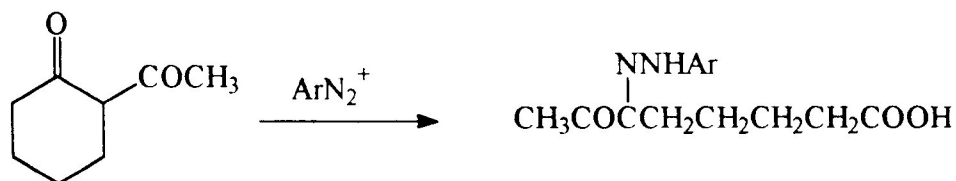
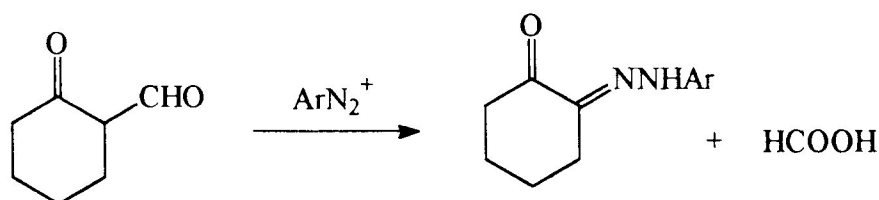
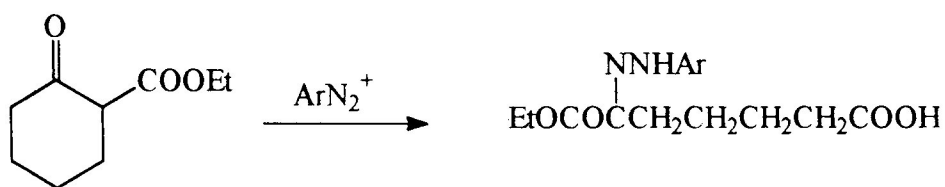
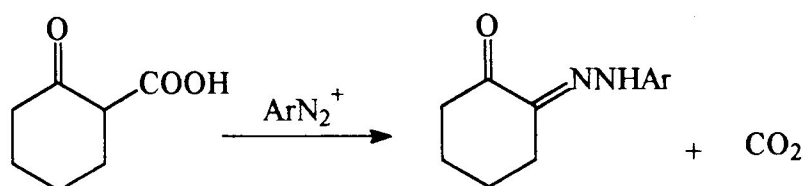
The reaction is known as the Japp-Klingemann reaction, and has been reviewed^{141,145}. Groups that usually undergo splitting are carboxyl, acyl, and alkoxy carbonyl. The scission of the latter probably follows a prior saponification. Examples.



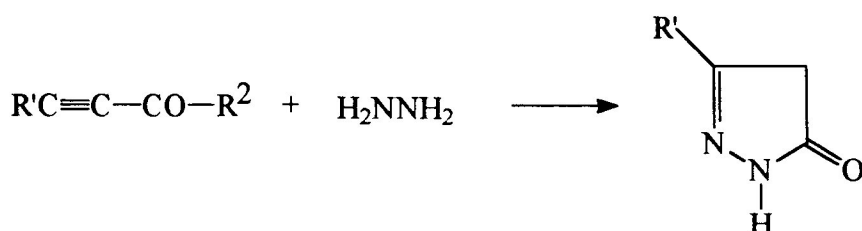
In diacylacetic esters, the acyl group corresponding to the weaker acid is more readily cleaved. For example¹⁴⁶.



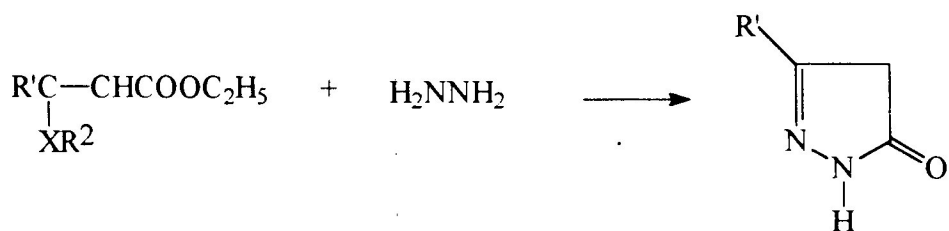
The behaviour of various 2 - substituted cyclohexanones illustrates the tendencies of various activating groups to undergo splitting relative to that of the ring carbonyl. The reaction can follow two courses, either side- chain splitting or a ring opening. With 2- carboxyl and 2- formyl groups, the side-chain is preferentially cleaved, whereas the corresponding ethoxycarbonyl and acetyl residues are more strongly bound and consequently the cyclohexanone ring is opened^{145,147}.



A number of syntheses of 2-pyrazolin-5-ones depend on the reaction of compounds, other than β -ketoesters, substituted in the β -position or of α , β unsaturated acids, esters and amides. The most frequently used unsaturated compounds are those having a triple bond in the α , β -position^{148,166}.



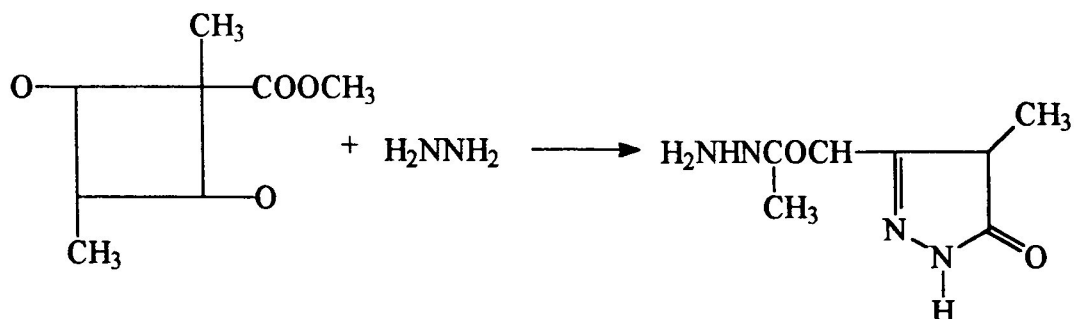
The reaction of ketene with aryl hydrazines forms 2 - pyrazolin - 5 ones^{151,152}.



The first product formed is the hydrazone of the corresponding acetoacethydrazide. This then cyclizes to the desired product at elevated temperatures.

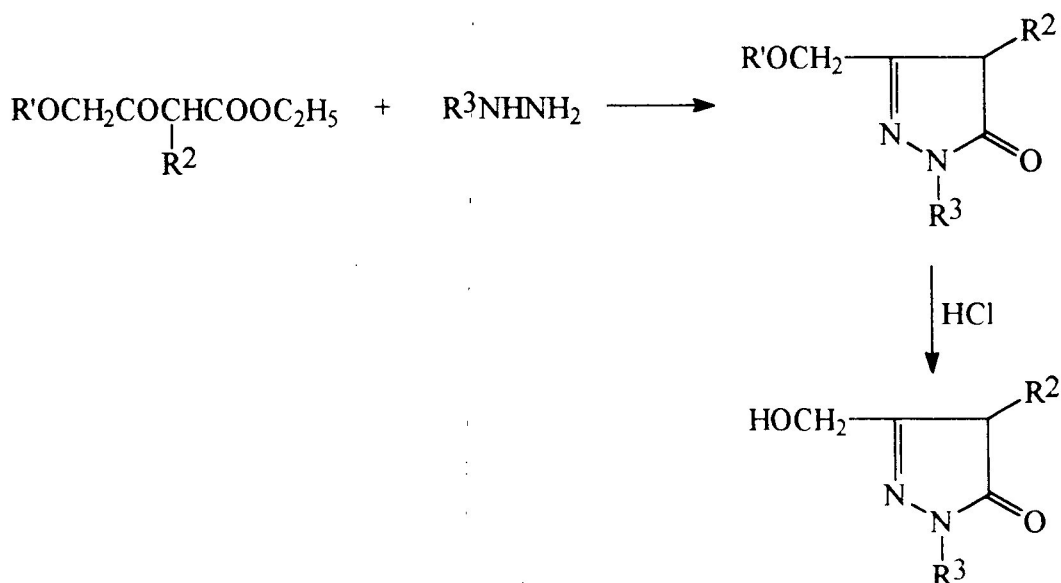


A rather unusual synthesis of pyrazolinones from a cyclobutane derivative has been reported. This probably involves cleavage of the 1,3 - dione to a β -ketohydrazide which cyclizes.

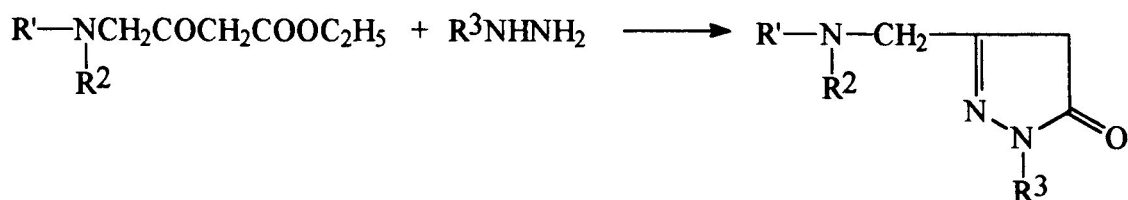


The most outstanding chemical property of 2-pyrazolin-5-ones is the activity of the hydrogen atoms at C-4. This position is very reactive, undergoing the characteristic condensations and substitutions of the active methylene group. Aldehydes and ketones react readily at this position giving bispyrazolinones. The formation of the bispyrazolinone can be suppressed by use of acetic acid as the condensing agent^{149,150}.

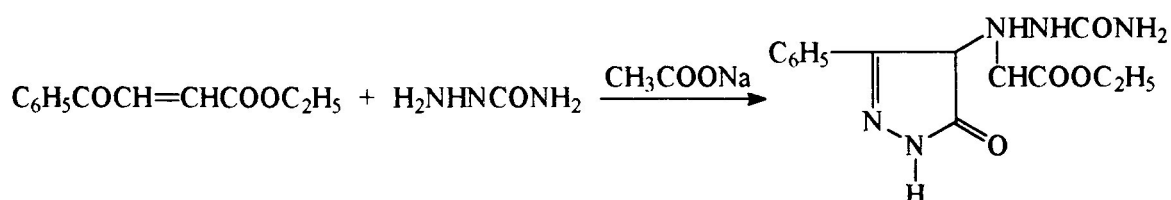
Reaction of the appropriate alkoxy or phenoxyacetic esters with hydrazine^{151,152,153} gives 2 - pyrazolin - 5- ones.



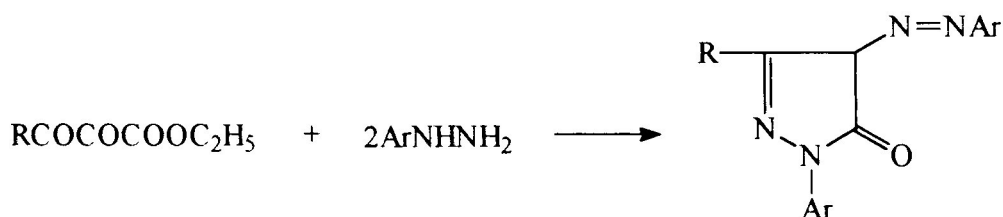
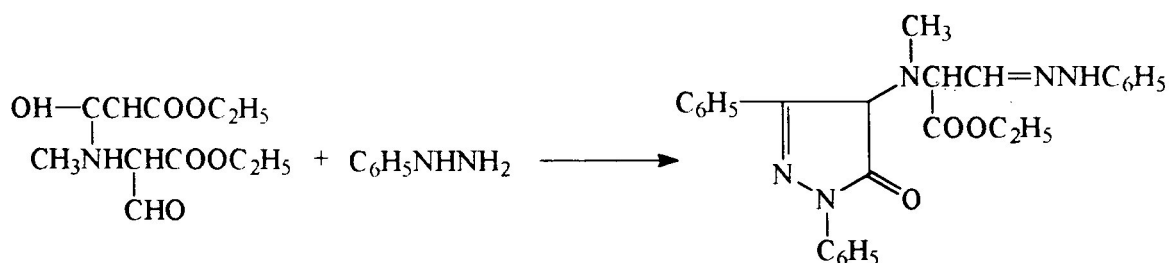
Synthesis from an appropriate β -ketoester or derivative and a hydrazine^{154,155,156}



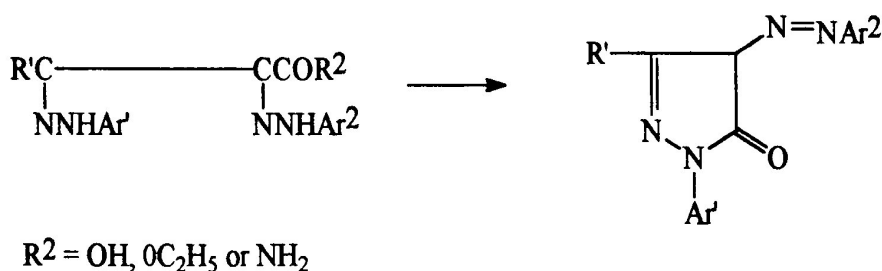
Condensation of one molecule of semicarbazide with the ketone carbonyl is followed by cyclization to the C-3 of the ester with elimination of ammonia after addition of a second molecule of semicarbazide



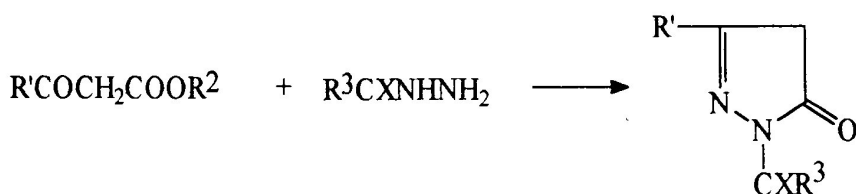
The cyclisation of tertiary amino β - aldehydoester to a pyrazolinone has been reported¹⁶⁰.



α , β diketoesters with hydrazides^{157,158,159}. Cyclization of a variety of bishydrazones of α , β - diketo acids^{160,167}, α , β - diketoesters(161,162,163) and α,β -diketoamides^{164,169} using either acid, base or heat, to 4 aryl-2-pyrazolin-5-ones have been reported^{165,168}.



Functional group substituents on N-1 of 2-pyrazolin-5-ones are acyl, sulfonyl and various carboxyl derivatives, such as carbalkoxy, amides, thioamides, hydrazides, thiohydrazides and amidines. The usual synthesis of these compounds is by the classical 2-pyrazolin-5-one synthesis reaction of a β -ketoester with a hydrazine. In these cases the hydrazines are of special types such as hydrazide, semicarbazide, carbazide or their imino or thio analogs. Sometimes the hydrazone intermediates are isolated and cyclized by heating.



$\text{R}_3 = \text{alkyl, aralkyl, NH}_2, \text{NH}_2\text{NH}, \text{C}_6\text{H}_5\text{NHNH}$

$\text{X} = \text{O, S, NH}$

SYNTHESIS OF 1-THIOCARBAMOYL-3-METHYL-4 (ARYLHYDRAZONO) 2-PYRAZOLIN-5-ONE.

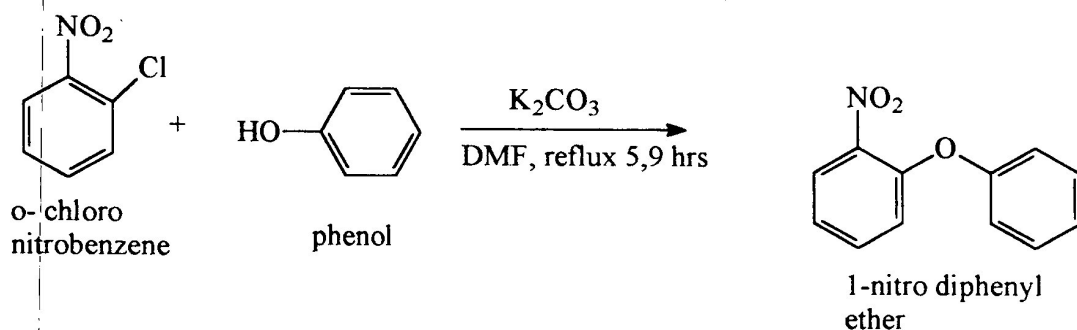
To an arylamine (0.05 mole) dissolved in a mixture of conc. HCl (40 ml) and water (30 ml) and cooled to 0°C in an ice bath, a cold aqueous solution of sodium nitrite (0.07 mole) was added. The diazonium salt solution was filtered into a cooled solution of ethyl acetoacetate (0.05 mole) and sodium acetate (0.6 mole) in ethanol (100 ml). The resulting solid was washed with water and crystallised from ethanol (20 ml). To this solid (0.02 mole) dissolved in glacial acetic acid (20 ml) in a single necked 100 ml round bottom flask, a solution of thiosemicarbazide (0.02 mole) in glacial acetic acid (25 ml) was added and the mixture was refluxed for 4 hours. It was then cooled and allowed to stand overnight to give a crystalline solid.

This procedure was adopted as it required reasonable time to isolate the final product. The formation of the diazonium salt and the hydrazono compound was easier. The isolation of the hydrazono product was easier. The reaction conditions and availability of reactants was much simpler. The yields were good within the range of 60-80%.

CHEMISTRY
OF
REDUCTION

Nimesulide is a new non-steroidal anti-inflammatory agent given orally or rectally twice a day. It produces irreversible inhibition of prostaglandins synthesis equally inhibiting formation of PGE1 and PGE2. It is used in the treatment of osteoarthritis, rheumatoid arthritis, acute and chronic respiratory tract inflammation, otorhinolaryngological inflammation, dysmenorrhea and thrombophlebitis. Heart burn, excessive perspiration and skin rash are the occasional side effects.

N-(4-Nitro-2-phenoxy phenyl) methane sulfonamide (Nimesulide) is commonly synthesized by the following route.

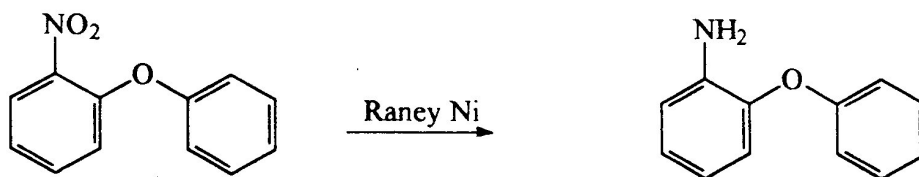


Nitro Benzene

The solvent used was DMF with K_2CO_3 (basic condition) due to its favourability in the process of condensation. The Cl atom at o-position readily combines with the hydrogen atom of phenol to liberate HCl gas forming an ether linkage in these conditions.

Reduction of 2-nitro diphenyl ether to 2-amino diphenyl ether

It has long been known that the nature of the product, actually formed depends critically upon the reduction conditions. Thus although 2-amino diphenyl ether is reduced by using sodium sulphide, ferric chloride, platinum dioxide and Raney Nickel the preferred reduction reaction was performed by using Raney Nickel^{170,171}, by sodium sulphide Flakes or by ferric chloride.



Reduction of 2-nitro diphenyl ether

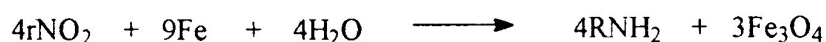
In the pressure vessel was taken 2-nitro diphenyl ether (2.15 g; 0.01 mole) in methanol (30 ml). The reaction mixture was heated on water-bath before placing in pressure vessel to dissolve the compound. Raney Nickel 5 % was added to reaction mixture. This reaction mixture was hydrogenated in a pressure reactor. After complete reduction, the contents of the vessel were filtered through fluted filter paper. The Raney Nickel was decomposed with dilute HCl (10 %). The filtrate was concentrated under reduced pressure. The residue was recrystallised from hot methanol.

Yield (1.48g; 80.0 %)

Reduction of nitro compounds involves the progressive replacement of oxygen in the - NO₂ group by hydrogen. Under suitable conditions aromatic nitro compounds are readily reduced to amino compounds by means of acidic reducing systems using such metals as iron and tin.

In the solution of the metal in acetic acid, the nitro compound is reduced and the amine may be recovered from the reaction mixture by making it alkaline, by steam distillation or filtration.

If catalytic methods are eliminated, iron and zinc are the metals of choice for reductive procedures, in the research laboratory. The advantage of iron is that only a catalytic amount of acid is needed to be added and the iron oxide precipitates out to permit a relatively simple separation.



The disadvantage is that the quality of iron powder from various commercial sources is variable. Some powders give spontaneous, exothermic reduction. Reduction with this reagent may be carried out in benzene or aqueous alcohol. Furthermore, iron powder in acetic acid is capable of reducing aliphatic optically active nitro compounds to the amines with retention of configuration¹⁷². Indeed, iron powder and acetic acid is a Favourable combination for

reduction of aromatic nitro compounds. On the other hand, basic reducing agents such as lithium aluminium hydride lead to racemization.

Zinc is a powerful reducing agent, but utilization of its reducing action is wasteful because of rapid reaction with an acid such as HCl. Stannous chloride is a useful and selective reducing agent for nitro groups when carbonyl groups are present.

Amalgamated aluminium is another reducing agent best adopted to neutral or basic reducing conditions.

Reduction using ferric chloride

2-nitro diphenyl ether (4.3 g 0.02 mole) was dissolved in toluene (15 ml) till clear solution was obtained. Then the mixture was heated to 80-85°C. At 80°C FeCl₃ solution (0.5 g in 5 ml water) was added dropwise and stirred for 1 hour at 90°C. After 1 hour the mixture was filtered and washed with portions of toluene. Toluene was distilled under vacuum to give 1-amino diphenyl ether.

(Yield 3.03 g 82.0 %)

Reduction using Raney Nickel

Since all the amine derivatives are highly susceptible to oxidation, the reduction was done by catalytic hydrogenation using Raney nickel as catalyst.

Catalytic methods :

This method^{173,174}, is clean and work-up is so easy that it should be considered the method of choice in the research laboratory. The only pitfall are the possibility of poisoning of the catalyst or, for large scale reductions, the inability to dissipate adequately the heat of hydrogenation¹⁷⁵.

The most common catalyst for the hydrogenation of nitro compounds are platinum (low pressure) and Raney nickel (high pressure). Supporting catalyst such as platinum on carbon^{176,177}, palladium on carbon^{178,179,180} are used.

Solvents and media used for catalytic reductions

Any solvent that is pure and does not react with substrate or products of reduction can be used. Water is the safest solvent but many organic compounds are insoluble in it. The most commonly used ones are the alcohols, particularly, methyl and ethyl alcohols. The vapours of methyl alcohol, however, may ignite in presence of palladium, platinum catalysts and some times with Raney Nickel (depending on its activity). Nevertheless, it is safe if the catalyst is first added to some water or methyl cellosolve provided that neither of these affects the reaction. Once the catalyst is completely covered, the alcohol can be safely added.

Benzene may be used (only if thiophene-free), although it attacks the rubber stoppers in the parr apparatus and is itself strongly bound to the surface of most catalysts, resulting in decrease in reaction rate.

Solvents that form peroxide should be avoided. For example, dioxane should be used after thoroughly purification and distillation.

Addition of catalyst :

In general, catalysts active in low pressure reductions, can be safely added to ethyl alcohol. If a large amount of catalyst is to be used, it should be added in portions and mixed until it is wetted with the solvent before another addition is made.

One should not attempt to carry out reduction in pressure reactor, filled beyond about 60-65% capacity, because a free space that is too small results in poor mixing and prolonged reaction time.

Equipment :

For operation, the vessel containing solvent, reactant and catalyst is placed in the reactor, hydrogen is added upto 150 psi and the stirrer is then started with heating. The progress of reaction is followed by observation of the drop in pressure. Although, catalytic hydrogenation is potentially hazardous, with proper care it can be as safe as any chemical reaction. After hydrogenation most catalysts (except for copper chromate) are more pyrophoric because of hydrogen absorbed on their surface during the reduction. Therefore, care must be taken during

filtration. The filter paper must be covered with liquid at all times and not allowed to become dry. Any spent catalyst can be destroyed by digestion in hydrochloric acid. Do not heat the reaction mixture to the desired working temperature before starting agitation. This may be safe under low pressure conditions but can be hazardous is higher pressure reductions.

The overall yields of catalytic hydrogenation are 60-80% and the product obtained in this case is in fairly pure state.

Reduction with sodium sulphide :

In a 100 ml round bottom flask sodium sulphide (2.64 g, 0.03 mole) finely powdered was placed. 2-nitro diphenyl ether (6.15 g 0.03 mole) dissolved in 40 ml methanol was added to it dropwise and the mixture was refluxed for 7-8 hour. After cooling the mixture was dumped in ice with stirring. The ice cold solution was then extracted with ethyl acetate to isolate the product.

(Yield: 4.33 g, 82.0 %)

Preparation of 2-amino diphenyl ether hydrochloride :

Conc. HCl (11.7 N, 7 ml) was added to 2-amino diphenyl ether at 10-20°C till pH 3-4 to get 2-amino diphenyl ether hydrochloride.

M.P. 170 -172°C

Yield (3.92 g, 82.0 %)

Preparation of N-(2 Phenoxy Phenyl) methane Sulfonamide :

2-amino diphenyl ether hydrochloride (2.21 g ; 0.01 mole) was dissolved in water (5 ml). Caustic KOH was added till pH =7. The free base was extracted in dichloromethane. 3ml of pyridine was added and the mixture was cooled to 5°C. Methane sulfonyl chloride (1.14g 0.01 mole) was added at 10-20°C for period of 5 hours. The mixture was digested at 10-20°C for 2 hours. Then conc. HCl (11.7 N, 15 ml) was added. After separating the dichloro methane layer it was distilled and water was added with stirring till precipitate was obtained m.p 123-125°C

(Yield = 2.35 g, 90.0 %)

CHEMISTRY
OF
NITRATION

Preparation N(4- Nitro -2- Phenoxy phenyl) methane sulfonamide :

Nitration of organic substances, a reaction discovered in 1834 (Mitcherlich, synthesis of nitrobenzene) is one of the most common and important reaction in organic chemistry. The aim of nitration is to substitute one or several hydrogen atoms in the aromatic or hetero-aromatic nucleus by one or several nitro groups.

The number of nitro groups entering the organic molecule depends on the concentration of the acid used, on temperature and the duration of the process, on the nature of the substance being nitrated and on other factors. In the course of nitration, the main reaction is accompanied by side reactions which diminish the yield of main product i.e. the nitro compound. The oxidising effect of nitric acid is responsible for one of these undesirable side reactions. This oxidising effect is enhanced by rise in temperature due to water content of reaction mixture and in the amount of nitrogen oxides in the nitric acid. The introduction of a nitro group into an organic molecule is accompanied by the formation of a molecule of water. The accumulation of water in the reaction mixture lowers the concentration of nitric acid. If nitric acid alone is used as a nitrating agent, the lowering of its concentration below a certain limit leads to practical ceasing of the reaction and complete utilization of the nitric acid becomes impossible. Hence in practice a mixture of nitric acid and sulphuric acid is used for nitration. This is the so called nitrating mixture.

The temperature at which nitration is carried out is another very important factor affecting the course of the process. Nitration is an exothermic reaction and when a mixture of nitric acid and sulphuric acid is used in the nitration of aromatic compounds a large amount of additional heat is given off by the dilution of sulphuric acid with water formed in the reaction. Hence it is usually necessary to use external cooling and to mix the reagents gradually. Variation in temperature not only affects the number of nitro groups entering a molecule but also the position which the nitro group enters. It is self evident therefore, that for each particular nitration there is an optimal temperature. The introduction of the nitro group into aromatic or heterocyclic compounds exhibiting aromatic properties is always affected by means of nitric acid or nitrating mixture. It has also been possible to obtain nitro compounds by the action of nitric acid alone, although better results are commonly obtained by using the nitrating mixture and other nitrating agents. Even when anhydrous nitric acid is used for nitrating aromatic and

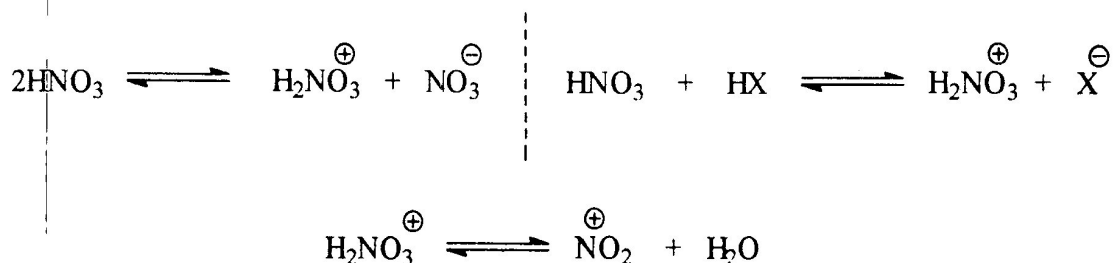
heterocyclic compounds its dilution by the water formed during the reaction rises its oxidising properties at the expense of its nitrating properties.

Nitration with a mixture of nitric acid and sulphuric acid

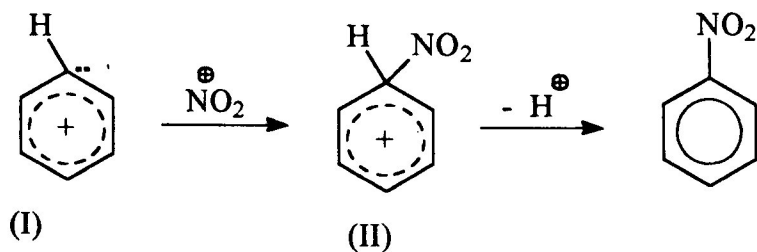
The most widespread method for nitrating aromatic compound, uses a mixture of nitric acid with concentrated sulphuric acid (nitrating mixture). This mixture is a more powerful nitrating agent than nitric acid alone. The presence of sulphuric acid not only contributes its dehydrating properties but also enhances the nitrating action of nitric acid, whilst diminishing its oxidising properties. However sulphuric acid serves as a good solvent for many compounds which is another favourable factor in nitration processes.

Mechanism :

It is now accepted that nitration is due to the action of a positively charged ion (i.e. a cation) NO_2^+ . Therefore, a nitration by ionic substitution (or displacement) is an electrophilic substitution, according to the well known nomenclature introduced by Ingold. There was overwhelming evidence that in all of the nitrating systems (i.e. HNO_3 and nitric acid in mineral acids) the nitronium ion (NO_2^+) was the effective electrophile. The process involved could be written as follows, in the first case heterolysis of nitric acid being dependent upon auto protonation and the second being dependent upon the action of a stronger acid (HX)



In electrophilic substitution, the two electrons which form the new covalent bond (i.e. the bond between the aromatic compound and the electrophilic reagent) are both supplied by the aromatic compound (I).



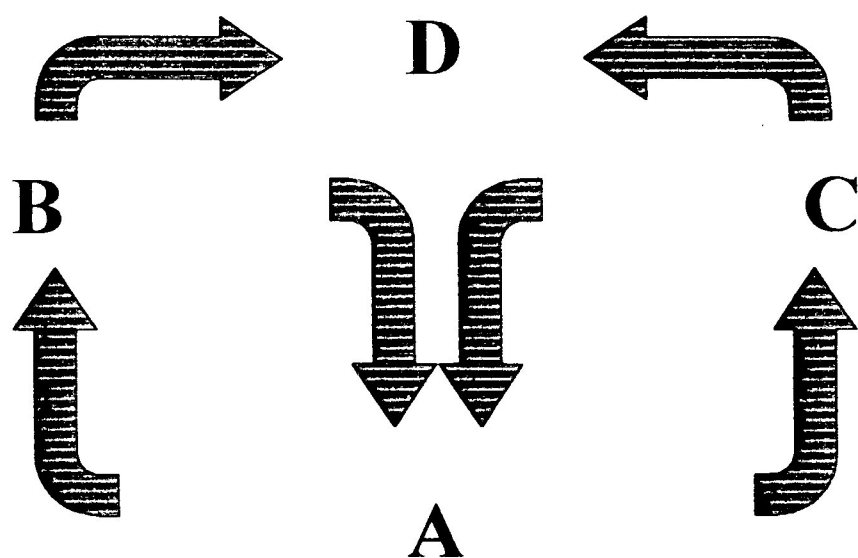
At first, two free electrons are supplied by the aromatic system (Electron sextet). The addition of the electrophilic agent NO_2^+ results in the formation of the intermediate complex, (II) (Wheland intermediate)¹⁸².

According to Melander^{183,184}, the formation of II constitutes the rate determining step. The overall reaction is of second order and follows the Sp^2 mechanism i.e. bimolecular electrophilic substitution.

N-(2-phenoxy phenyl) methane sulfonamide (2.0 g, 0.007 mole) was dissolved in glacial acetic acid (8 ml) at 45°C. Conc. Nitric acid (d : 1.5, 4 ml) was added in portions maintaining the temperature at 45-50°C. The mixture was digested at 30-35°C for 1 hour and then cooled to 0°C. The solid was filtered and crystallised from 10 ml ethanol.

M.P. 145°C Yield 2.23 g (95.0 %)

*SCHEMATIC
REPRESENTATION
OF THE SERIES OF
COMPOUNDS SYNTHESISED.*

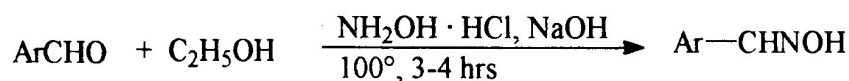


SCHEMATIC REPRESENTATION OF THE SERIES OF THE COMPOUNDS SYNTHESISED

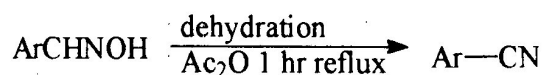
Following are the the schematic representations of the various series of compounds reported in the present investigation :

1. Synthesis of 2-(2,4-difluoro phenyl)-3-(1H-1,2,4- triazol-1-yl) propan-2-ol, 1 (substituted phenyl) tetrazole (III)

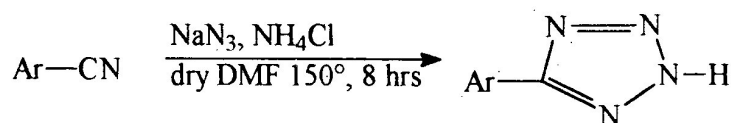
Step I :



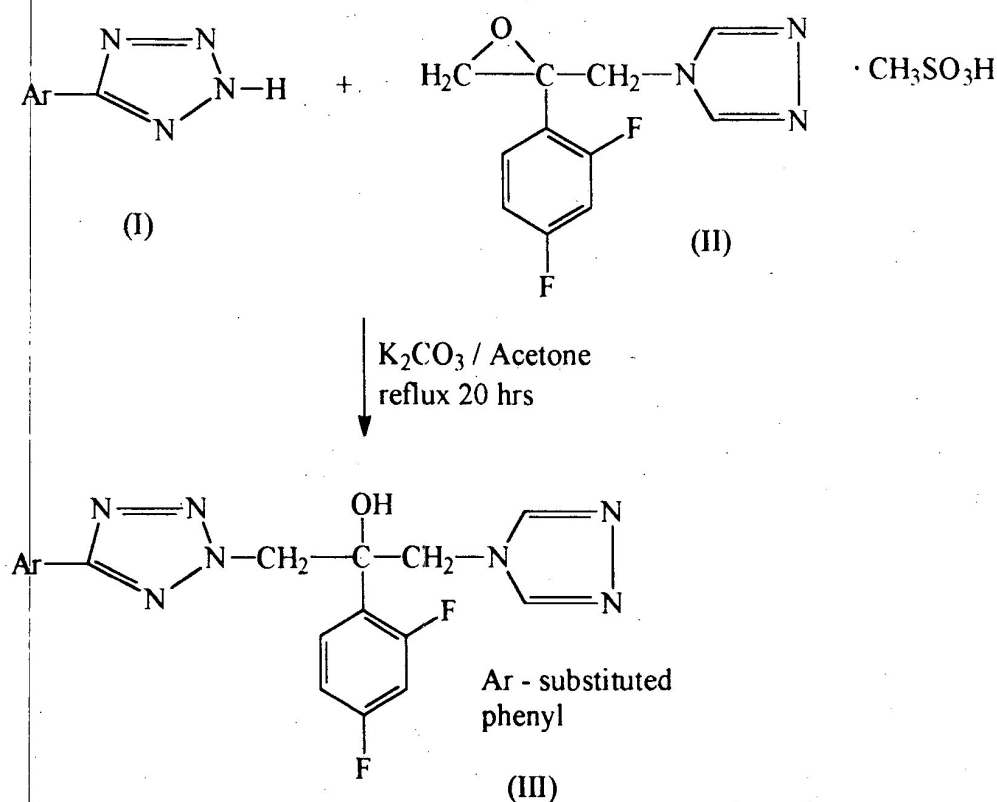
Step II :



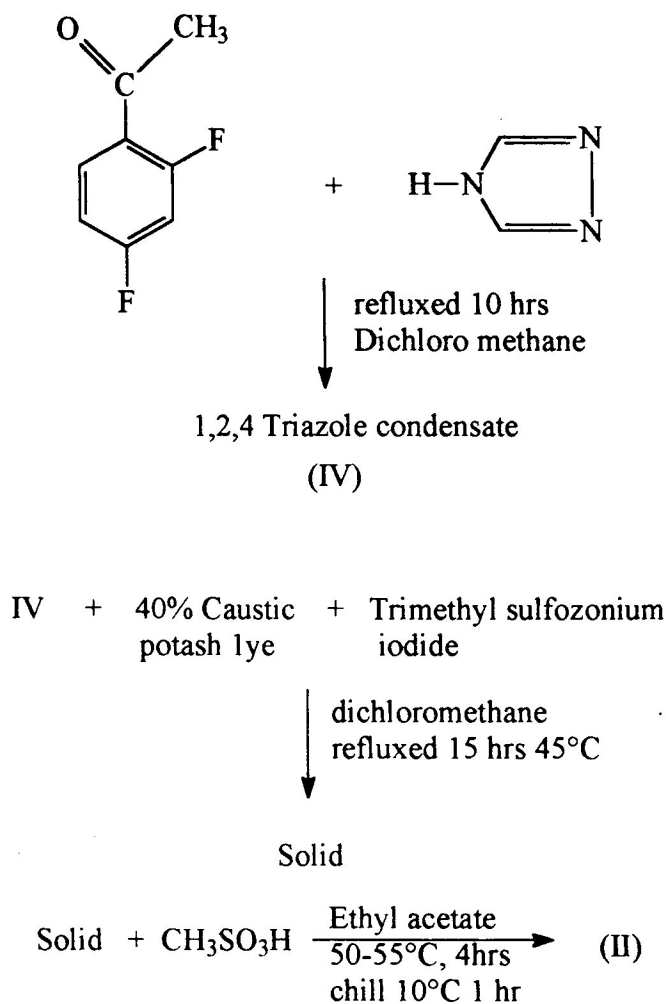
Step III :



Step IV :

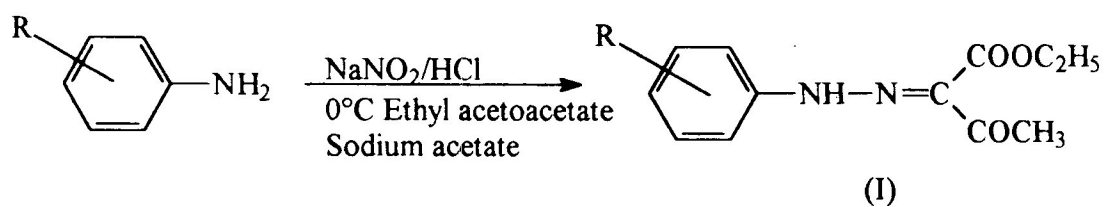


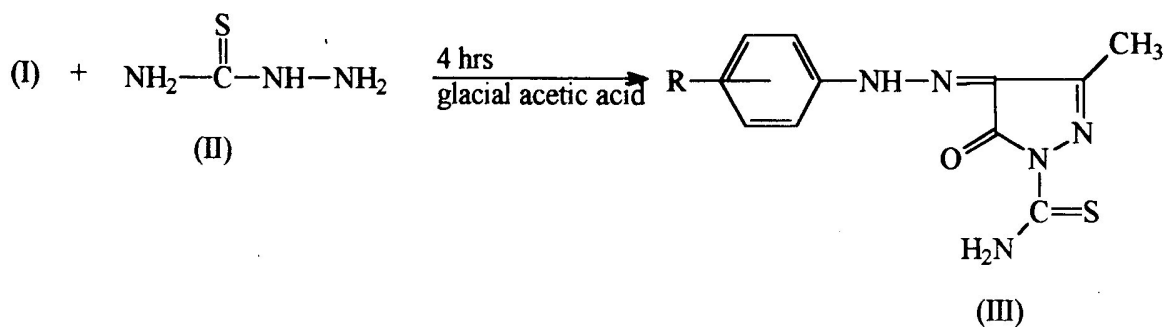
SYNTHESIS OF II



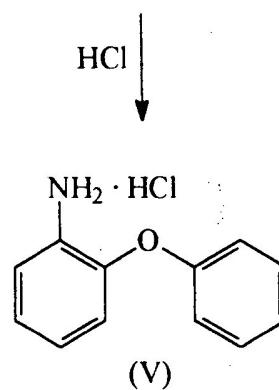
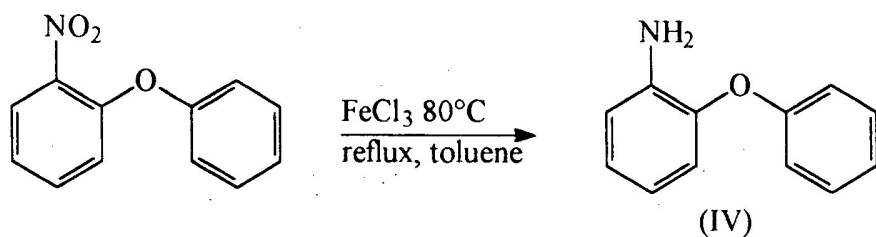
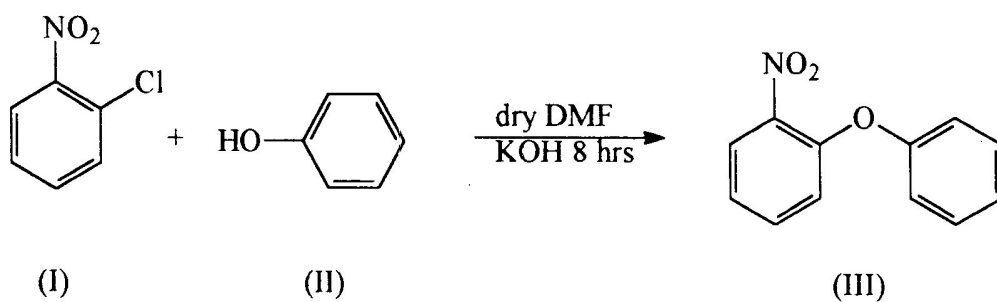
2. Synthesis of 1-thiocarbamoyl-3-methyl-4(aryl hydrazono)-2- pyrazolin-5-ones

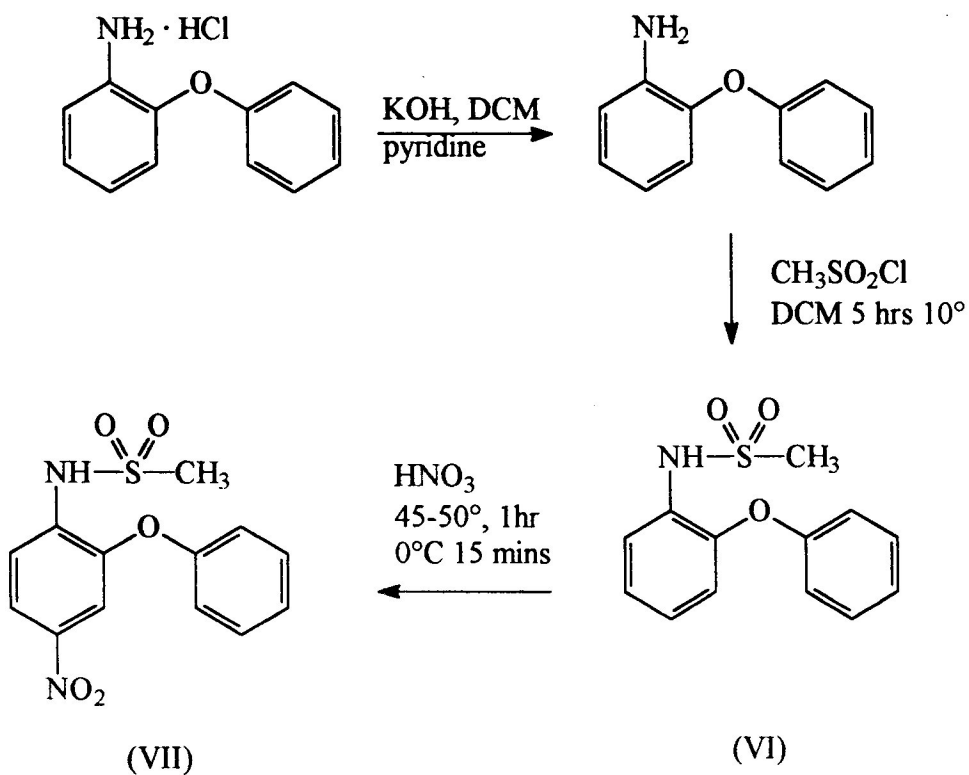
Step I :



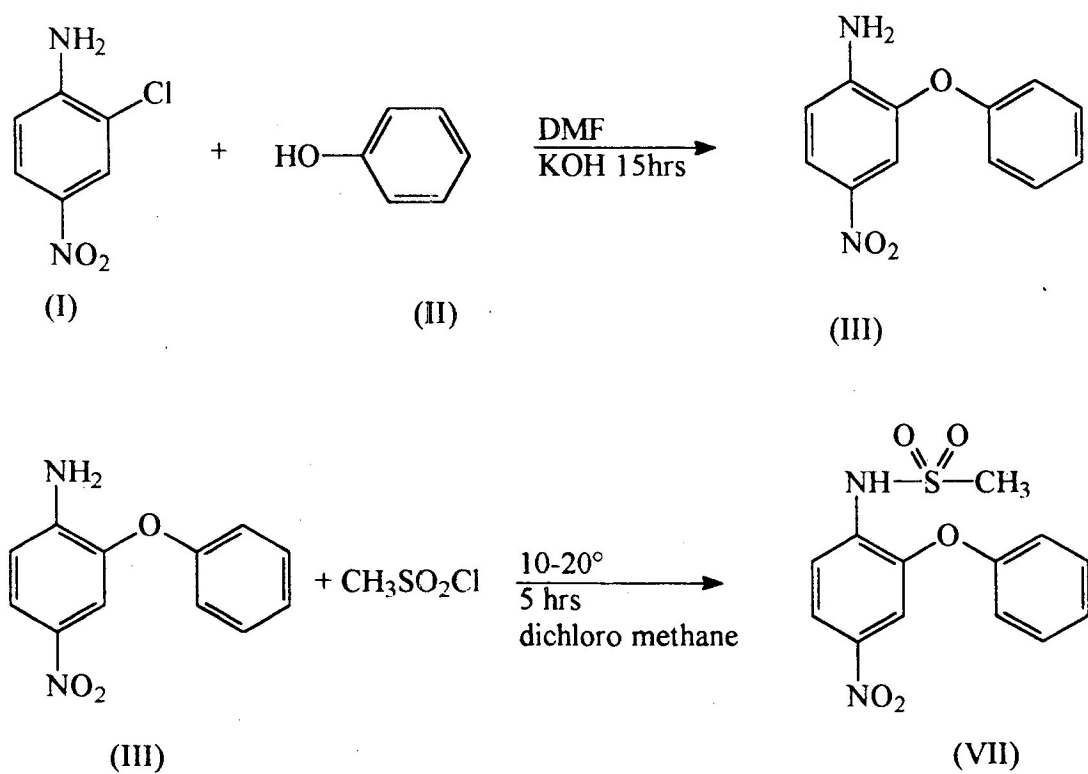
Step II :

R = Cl, F, OCH₃, etc.

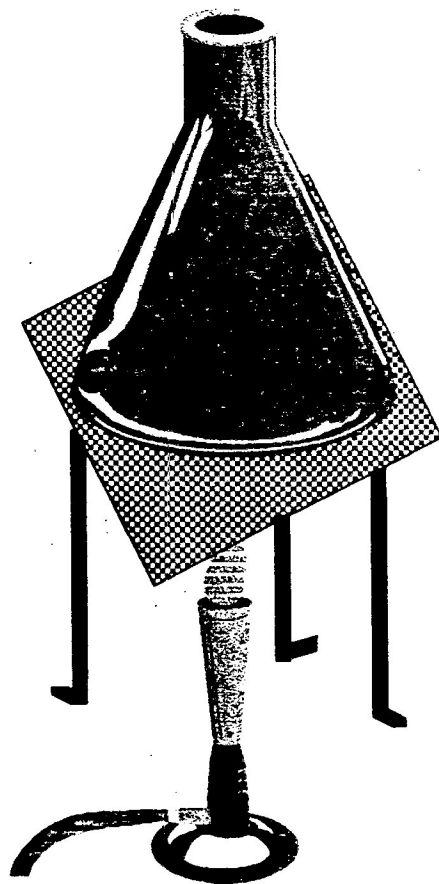
3. Synthesis of N-(4-nitro-2-phenoxy phenyl) methane sulfonamide (Nimesulide)



Route 2 :



EXPERIMENTAL



SYNTHESIS OF
2-(2,4-DIFLUORO PHENYL)-
3(1 H- 1,2,4-TRIAZOL-1-YL)
PROPANE-2-OL.
1 (SUBSTITUTED) PHENYL
TETRAZOLES

PART II

EXPERIMENTAL :

Synthesis of 2-(2,4 difluoro phenyl)-3-(1H-1, 2, 4 - triazol-1-yl) propan-2-ol, 1(substituted phenyl) tetrazoles which involves the following steps:

- i) preparation of 1-(substituted phenyl) aldoximes.
- ii) preparation of various 1-(substituted phenyl) nitriles.
- iii) preparation of various 1-(substituted phenyl) tetrazoles.
- iv) preparation of 2-(2,4-difluoro phenyl)-3-(1H-1,2,4-triazol-1-yl) propan-2-epoxide derivative of methane sulfonic acid.
- v) preparation of the title compounds by condensation of 2-(2,4-difluoro phenyl)-3-(1H'-1,2,4-triazol-1-yl) propan -2-epoxide with appropriate substituted phenyl tetrazoles

i) Preparation of 1-substituted phenyl aldoximes.

The preparation of the title compound involves various intermediates whose preparations are mentioned here:-

1-(2 fluoro phenyl) aldoxime :

A 500 ml three necked round bottom flask, provided with a mercury sealed stirrer, a dropping funnel and a condenser, was mounted on a steam-bath. In this was placed 2 - Fluoro benzaldehyde (6.2 g; 0.05 mole) dissolved in 95 % warm ethanol (30 ml) and to it was added a warm solution of hydroxyl amine hydrochloride (4.2. g; 0.06 mole) in water (5 ml). After stirring for five minutes, a solution of sodium hydroxide (3.0 g; 0.075 mole) in water (40ml) was added. The mixture was stirred for additional ten minutes and kept at room temperature for three hours. Then crushed ice (25 g) was added in it. The separated aldoxime was filtered, washed with cold water and dried.

Yield: 6.72 g (88.0 %)

1-(3 Fluoro phenyl) aldoxime :

A 500 ml three necked round bottomed Flask, provided with a mercury sealed stirrer, a dropping Funnel and a condensor, was mounted on a steambath. In this was placed 3 Fluoro benzaldehyde (6.2 g; 0.05 mole) dissolved in 95 % warm ethanol (30 ml) and to it was added a warm solution of hydroxyl amine hydrochloride (4.2 g ; 0.06 mole) in water (5ml). After

stirring for five minutes, a solution of sodium hydroxide (3.0g; 0.075 mole) in water (40 ml) was added. The mixture was stirred for additional ten minutes and kept at room temperature for three hours. Then crushed ice (25g) was added in it. The separated aldoxime was filtered, washed with cold water and dried .

Yield: 6.87 g (90.0 %)

1-(4 Fluoro phenyl) aldoxime :

A 500 ml three necked round bottomed Flask, provided with a mercury sealed stirrer, a dropping Funnel and a condenser, was mounted on a steam bath. In this was placed 4 Fluoro benzaldehyde (6.2g; 0.05 mole) dissolved in 95 % warm ethanol (30 ml) and to it was added a warm solution of hydroxyl amine hydrochloride (4.2 g; 0.06 mole) in water (5ml). After stirring for five minutes, a solution of sodium hydroxide (3.0g; 0.075mole) in water (40ml) was added. The mixture was stirred for additional ten minutes and kept at room temperature for three hours. Then crushed ice (25g) was added in it. The separated aldoxime was filtered, washed with cold water and dried.

Yield: 7.17 g (94.0 %)

1-($\alpha\alpha\alpha$ -Trifluoro -2- tolualdoxime) :

A 500ml three necked round bottomed Flask, provided with a mercury sealed stirrer, a dropping funnel and a condenser, was mounted on a steam-bath. In this was placed ($\alpha\alpha\alpha$ -Trifluoro -2- tolualdehyde (8.7 g, 0.05 mole) dissolved in 95 % warm ethanol (30ml) and to it was added a warm solution of hydroxyl amine hydrochloride (4.20; 0.06 mole) in water (5 ml). After stirring for five minutes, a solution of sodium hydroxide (3.0g; 0.075 mole) in water (40 ml) was added. The mixture was stirred for additional ten minutes and kept at room temperature for three hours. Then crushed ice (25 g) was added in it. The separated aldoxime was filtered, washed with cold water and dried.

Yield 7.93 g (84.0 %)

1- ($\alpha\alpha\alpha$ - Trifluoro -3 - tolualdoxime) :

A 500 ml three necked round bottomed flask, provided with a mercury sealed stirrer, a dropping funnel and a condenser, was mounted on a steam bath. In this was placed $\alpha\alpha\alpha$ -trifluoro-3-tolualdehyde (8.7 g; 0.05 mole) dissolved in 95% warm ethanol (30 ml) and to it

was added a warm solution of hydroxyl amine hydrochloride (4.2 g; 0.06 mole) in water (5 ml). After stirring for 5 minutes, a solution of sodium hydroxide (3.0 g; 0.075 mole) in water (40 ml) was added. The mixture was stirred for additional 10 minutes and kept at room temperature for 3 hours. Then crushed ice (25 g) was added in it. The separated aldoxime was filtered, washed with cold water and dried.

Yield 6.10 g (80.0 %).

1-($\alpha\alpha\alpha$ - Trifluoro - 4 - tolualdoxime) :

A 500 ml three necked round bottomed flask, provided with a mercury sealed stirrer, a dropping funnel and a condenser was mounted on a steam bath. In this was placed $\alpha\alpha\alpha$ -Trifluoro-4-tolualdehyde (8.7 g; 0.05 mole) dissolved in 95% warm ethanol (30 ml) and to it was added a warm solution of hydroxyl amine hydrochloride (4.2 g; 0.06 mole) in water (5 ml). After stirring for 5 minutes, a solution of sodium hydroxide (3.0 g; 0.075 mole) in water (40 ml) was added. The mixture was stirred for additional ten minutes and kept at room temperature for three hours. Then crushed ice (25 g) was added in it. The separated aldoxime was filtered, washed with cold water and dried.

Yield : 6.63 g (87.0%).

1-(2 chloro phenyl) aldoxime

A 500 ml three necked round bottomed flask, provided with a mercury sealed stirrer, a dropping funnel and a condenser was mounted on a steam bath. In this was placed 2 chloro benzaldehyde (7.02 g; 0.05 mole) dissolved in 95% warm ethanol (30 ml) and to it was added a warm solution of hydroxyl amine hydrochloride (4.2 g; 0.06 mole) in water (5 ml). After stirring for 5 minutes a solution of sodium hydroxide (3.0 g; 0.07 mole) in water (40 ml) was added. The mixture was stirred for additional ten minutes and kept at room temperature for three hours. Then crushed ice (25 g) was added in it. The separated aldoxime was filtered, washed with cold water and dried.

Yield : 7.14 g (92.0%).

1-(3 chloro phenyl) aldoxime :

A 500 ml three necked round bottomed flask, provided with a mercury sealed stirrer, a dropping funnel and a condenser was mounted on a steam bath. In this was placed 3 chloro benzaldehyde (7.02 g; 0.05 mole) dissolved in 95% warm ethanol (35 ml) and to it was added a warm solution of hydroxyl amine hydrochloride (4.2 g; 0.06 mole) in water (5 ml). After stirring for 5 minutes a solution of sodium hydroxide (3.0 g; 0.75 mole) in water (40 ml) was added. The mixture was stirred for additional ten minutes and kept at room temperature for three hours. Then crushed ice (25 g) was added in it. The separated aldoxime was filtered, washed with cold water and dried.

Yield: 7.45 g (96.0%).

1-(4 Chloro phenyl) aldoxime:

A 500 ml three necked round bottomed flask, provided with a mercury sealed stirrer, a dropping funnel and condenser, was mounted on a steam bath. In this was placed 4 chloro benzaldehyde (7.02g; 0.05 mole) dissolved in 95 % warm ethanol (30 ml) and to it was added a warm solution of hydroxyl amine hydrochloride (4.2 g; 0.06 mole) in water (5 ml). After stirring for five minutes, a solution of sodium hydroxide (3.0g; 0.075 mole) in water (40 ml) was added. The mixture was stirred for additional ten minutes and kept at room temperature for three hours. Then crushed ice (25g) was added in it. The separated aldoxime was filtered, washed with cold water and dried .

Yield: 6.28 g (81.0 %)

(ii) Preparation of various 1 - (substituted phenyl) nitriles

The (2 Fluoro phenyl) aldoxime (4.96 g; 0.04 mole) was refluxed with acetic anhydride (10.0 g ; 94 %) in 250 ml round bottom flask equipped with a condenser. The mixture was refluxed for 1 hr, cooled to room temperature, it was poured on crushed ice with stirring. The separated compound was filtered, washed with cold water and recrystallised from ethanol.

Yield: 4.11 g (85.0 %)

b.p. 88°C/21 mm

Lit b.p.¹⁸⁵ 90°C/21 mm

The (3 Fluoro phenyl) aldoxime (4.96 g; 0.04 mole) was refluxed with acetic anhydride (10.0 g ; 94 %) in 250 ml round bottom flask equipped with a condenser. The mixture was refluxed

for 1 hr, cooled to room temperature, it was poured on crushed ice with stirring. The separated compound was filtered, washed with cold water and crystallised from ethanol.

Yield: 3.81 g (79.0%)

b.p. 179-180°C/753mm

Lit. b.p.¹⁸⁵ 182-183°C/753 mm

$\alpha\alpha$ -Trifluoro-3 tolualdoxime (6.96 g; 0.04 mole) was refluxed with acetic anhydride (10.0 g; 94 %) in 250ml round bottom flask equipped with a condenser. The mixture was refluxed for 1 hr, cooled to room temperature, it was poured on crushed ice with stirring. The separated compound was filtered, washed with cold water and recrystallised from ethanol.

Yield 4.29 g (89 %)

b.p. 187° C

Lit b.p.¹⁸⁶ 189°C

$\alpha\alpha$ - Trifluoro-2-tolualdoxime (6.96 g, 0.04 mole) was refluxed with acetic anhydride (10.0 g; 94 %) in 250 ml round bottom flask equipped with a condenser. The mixture was refluxed for 1 hr, cooled to room temperature, it was poured on crushed ice with stirring. The separated compound was filtered, washed with cold water and recrystallised from ethanol.

Yield 3.61 g (75 %)

b.p. 149 - 150°C

Lit b.p.¹⁸⁶ 153°C

The (2 chloro phenyl) aldoxime (6.22 g ; 0.04 mole) was refluxed with acetic anhydride (10.0 g; 94%) in 250 ml round bottom flask equipped with a condenser. The mixture was refluxed for 1 hr, cooled to room temperature. It was poured on crushed ice with stirring. The separated compound was filtered, washed with cold water and recrystallised from ethanol.

Yield 5.0 g (91 %)

M.P. 90-92°C

Lit M.P.¹⁸⁶ 94°C

The (3 chloro phenyl) aldoxime (6.22 g; 0.04 mole) was refluxed with acetic anhydride (10.0g; 94 %) in 250 ml round bottom flask equipped with a condensor. The mixture was refluxed for

1 hr, cooled to room temperature. It was poured on crushed ice with stirring. The separated compound was filtered, washed with cold water and recrystallised from ethanol.

Yield: 4.5 g (82%)

M.P. 60-62°C

Lit M.P.¹⁸⁶ 64°C

4 Fluoro benzonitrile, $\alpha\alpha\alpha$ -trifluoro 4-tolunitrile, 4 chloro benzonitrile prepared from the corresponding aldoxime, following the procedure adopted above for the preparation of 2 Fluoro benzonitrile from 2 - Fluoro benzaldehyde.

Substituted nitriles



Sr. No.	R	Yield (%)	M.P/B.P. (°C)		Reference
			Obtained	Reported	
1	2- Fluoro Phenyl	85.0	88°/21mm	90°/21mm	186
2	3- Fluoro phenyl	79.0	179°/753mm	182°/753mm	186
3	4-Fluoro phenyl	87.0	163°/10mm	165°/10mm	186
4	$\alpha\alpha\alpha$ -trifluoro 2-toluyyl	75.0	149	153	187
5	$\alpha\alpha\alpha$ -trifluoro 3 -toluyyl	89.0	187	189	187
6	$\alpha\alpha\alpha$ -trifluoro 4-toluyyl	81.0	3%	41	187
7	2-chloro phenyl	91.0	92	94	186
8	3-chloro phenyl	82.0	62	64	186
9	4-chloro phenyl	94.0	50	52	186

iii) Preparation of various 1 -(substituted phenyl) tetrazoles.

2 Fluoro benzonitrile (6 g; 0.05 mole) was refluxed with dimethyl formamide (20 ml) in 100 ml round bottom flask equipped with a condenser. The nitrile was refluxed with Sodium azide (6.5 g; 0.1 mole), Ammonium chloride (3.5 g; 0.066 mole) for 8 to 10 hours. After 10 hours the solution was filtered. The solvent DMF was evaporated under reduced pressure. Then 4-5 ml conc. HCl was added to the residue. The Flocculant precipitate was Filtered and recrystallised from alcohol.

Yield: 6.83 g (84.0 %)

M.P. 130°C

Lit M.P.¹⁸⁷ 132°C

3 Fluoro phenyl 2-H Tetrazole and 4 Fluoro phenyl 2-H tetrazole were prepared from the corresponding benzonitrile, following the procedure adopted above for the preparation of 2 Fluoro phenyl 2-H Tetrazole from 2 Fluoro benzonitrile.

$\alpha\alpha\alpha$ -tri fluoro 2-tolunitrile (8.5g; 0.05 mole) was refluxed with dimethyl Formamide (20 ml) in 100 ml round bottom flask equipped with a condenser. The nitrile was refluxed with Sodium azide (6.5g; 0.1 mole), Ammonium chloride (3.5g ; 0.066mole) for 8-10 hours. After 10 hours the solution was filtered. The solvent DMF was evaporated under reduced pressure. Then 4-5 ml conc. HCl was added to the residue. The Flocculant precipitate was filtered and recrystallised from alcohol.

Yield: 8.5 g (80.0 %)

M.P. 135°C

Lit M.P.¹⁸⁸ 137°C

$\alpha\alpha\alpha$ tri fluoro-3- toluy 2-H tetrazole and $\alpha\alpha\alpha$ tri fluoro 4-toluy 2-H tetrazole were prepared from the corresponding tolunitrile, following the procedure adopted above for the preparation of $\alpha\alpha\alpha$ tri fluoro-2-toluy 2-H tetrazole from $\alpha\alpha\alpha$ -trifluoro 2 -tolunitrile.

2 chloro benzonitrile (6.87 g ; 0.05 mole) was refluxed with dimethyl Formamide (20 ml) in 100 ml round bottom flask equipped with a condenser, The nitrile was refluxed with Sodium azide (6.5g ,0.1 mole), Ammonium chloride (3.5 g, 0.066 mole) for 8-1- hours. After 10 hours the solution was filtered. The solvent DMF was evaporated under reduced pressure. Then 4-5 ml conc. HCl was added to the residue. The flocculant precipitate was filtered and recrystallised from alcohol.

Yield 7.91 g (88%)

M.P. 210°C

Lit M.P.¹⁸⁷ 212°C

3 chloro phenyl 2 - H tetrazole and 4 chloro phenyl 2-H tetrazole were prepared from the corresponding benzonitrile, following the procedure adopted above for the preparation of 2-chloro phenyl 2 H tetrazole from 2 chloro benzonitrile.

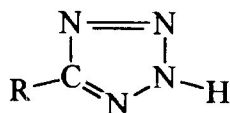
A Preparation of 1,2,4 - triazole

1,3,5 - Triazine (6.48 g , 0.07 M) was refluxed with hydrazine hydrochloride (5.47 g, 0.07 M) in ethanol (30 ml) in 100 ml round bottom flask equipped with a condensor for 4-5 hours. The intermediate solid formed was further condensed with Triazine (5.67 g; 0.07M) in ethanol (20 ml) for 3-4 hours to give 1,2,4 - triazole.

Yield: 5.24 g 95.0 %

M.P. 123°C,

Lit M.P. 119 -121°C.



Sr. No.	R	Yield (%)	M.P. (°C)		Reference
			Obtained	Reported	
1	2- Fluoro Phenyl	84.0	130	132	188
2	3- Fluoro phenyl	86.0	120	122	188
3	4-Fluoro phenyl	81.0	196	199	188
4	ααα-trifluoro 2-toluyI	80.0	135	137	189
5	ααα-trifluoro 3 -toluyI	91.0	153	155	189
6	ααα-trifluoro 4-toluyI	94.0	217	220	189
7	2-chloro phenyl	88.0	210	212	188
8	3-chloro phenyl	78.0	105	108	188
9	4-chloro phenyl	95.0	259	262	188

iv) Preparation of 2 - (2,4-difluoro phenyl) -3-(1 H - 1,2,4- triazol -l-yl) propan-2-epoxide derivative of methane sulfonic acid.

2,4 difluoro acetophenone (10.92 g, 0.07 M) and 1,2,4 triazole (4.76 g ,0.07 M) were stirred and refluxed for 10 hours in 90 ml dichloro methane at 60°C to give 12.5g of 1,2-4 triazole condensate. This condensate, 57 ml 40 % caustic potash lye, Trimethyl sulfoxonium iodide (11.2 g, 0.07 M) was stirred and refluxed in 110 ml dichloro methane for 15 hrs at 45°C. Layer of dichloro methane was seperated. After extracting remaining aqueous layer with 30 ml of dichloro methane for 3 times (3 x 30 ml). The organic layer of dichloro methane was combined and washed with 25 ml of water for 2 times (2 x 25 ml) and dried over sodium sulphate. The solvent dichloro methane was recovered completely under reduced pressure and 60 ml ethyl acetate was added in residue. The mixture was heated to 50-55°C. Methane sulfonic acid (4.9g in 15 ml ethyl acetate) was added at 50-55°C and stirred for half hour. Then it was chilled at 10-15°C for 1 hour. The solid which separated out was washed with chilled ethyl acetate and dried on air.

Yield: 13 g

M.P 132-135°C

Lit. M.P.¹⁹⁰ 128-129°C

v) Preparation of the title compounds by condensation of 2-(2,4- difluoro phenyl) -3-(1 H-1,2,4 - triazol-l-yl) propan- 2- epoxide with appropriate substituted phenyl tetrazoles.

2 Fluoro phenyl 2 H - tetrazole (8.2 g, 0.05 mole) was dissolved in 30 ml acetone. Methane sulfonic acid derivative (16.65 g, 0.05 mole) was dissolved in 30 ml acetone. The above two reactants were combined and refluxed in acetone for 15-20 hours with anhydrous K₂CO₃ (16.6g, 0.01 mole). The acetone was evaporated under reduced pressure. To the residue 20 ml water was added and it was extracted with ethyl acetate (20 ml). Then ethyl acetate was evaporated under reduced pressure to get the solid.

Yield: 17.64 g (88.0 %)

M.P. 215°C

v) Preparation of the title compounds by condensation of 2-(2,4- difluoro phenyl) -3-(1 H-1,2,4 - triazol-1-yl) propan- 2- epoxide with appropriate substituted phenyl tetrazoles. 2-(2,4 - Difluoro phenyl) -3- (1 H-1,2,4- triazol -1-yl) Propan -2-ol, 1(3-Fluoro phenyl) tetrazole and 2-(2,4 difluoro phenyl) -3- (1 H-1,2,4-triazol -1-yl)propan- 2-ol, 1(4 fluoro phenyl) tetrazole were prepared from the corresponding tetrazoles, following the procedure adopted above for the preparation of 2- (2,4-difluoro phenyl)-3-(1 H-1,2,4-triazol-1-yl) propan - 2- ol, 1(2 Fluoro phenyl) tetrazole from 2 fluoro phenyl 2-H tetrazole.

$\alpha\alpha\alpha$ trifluoro 2-toluy 2 H tetrazole (10.7g ; 0.05 mole) was dissolved in 30 ml acetone. Methane sulfonic acid derivative (16.65g; 0.05 mole) was dissolved in 30 ml acetone. The above two reactants were combined and refluxed in acetone for 15-20 hours with anhydrous K_2CO_3 , (16.6g; 0.1 mole). The acetone was evaporated under reduced pressure. To the residue 20 ml water was added and it was extracted with ethyl acetate (20ml). Then ethyl acetate was evaporated under reduced pressure to get the solid.

Yield 20.7 g (85.0%)

M.P 250°C

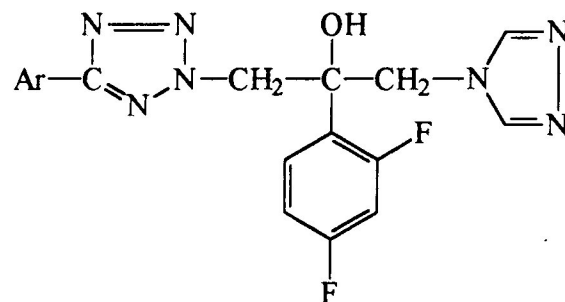
v) Preparation of the title compounds by condensation of 2-(2,4- difluoro phenyl) -3-(1 H-1,2,4 - triazol-1-yl) propan- 2- epoxide with appropriate substituted phenyl tetrazoles. 2- (2,4 - difluoro phenyl) -3- (1 H -1,2,4 - triazol -1-yl) propan -2-ol, 1($\alpha\alpha\alpha$ trifluoro 3 toluy 2 H tetrazole and 2- (2,4 - difluoro phenyl) -3-(1H--1,2,4- triazol-1-yl) propan -2-ol, 1 ($\alpha\alpha\alpha$ trifluoro 4 toluy 2H tetrazole were prepared from the corresponding tetrazoles, following the procedure adopted above for the preparation of 2 -(2,4 - difluoro phenyl) -3-(1 H -1,2,4 - triazol -1-yl) propan-2-ol, 1($\alpha\alpha\alpha$ trifluoro 2 toluy 2 H tetrazole from $\alpha\alpha\alpha$ trifluoro 2- toluy -2- H tetrazole.

2-chloro phenyl tetrazole (9.02g; 0.05 mole) was dissolved in 30 ml acetone. Methane sulfonic acid derivative (16.65g ; 0.05 mole) was dissolved in 30 ml acetone. The above two reactants were combined and refluxed in acetone for 15-20 hours with anhydrous K_2CO_3 (6.6g ; 0.1 mole). The acetone was evaporated under reduced pressure. To the residue 20 ml water was added and it was extracted with ethyl acetate (20 ml). Then ethyl acetate was evaporated under reduced pressure to get the solid.

Yield 17.36 g (83.0 %)

M.P 270°C

2-(2,4- difluoro phenyl) -3- (1H-1,2,4 - Triazol-1-yl) propan-2-ol, 1 (3 chloro phenyl) 2 H tetrazole and 2-(2,4 - difluoro phenyl) -3- (1H- 1,2,4- triazol 1-yl) propan-2-ol, 1(4 chloro phenyl) 2 H tetrazole were prepared from the corresponding tetrazoles, following the procedure adopted above for the preparation of 2- (2,4 - difluoro phenyl) -3- (1 H-1,2,4- triazol -1 yl) propan- 2-ol, 1(2 chloro phenyl) 2 H tetrazole from 2 chloro phenyl 2 H tetrazole.



Sr	Ar	Mol. Formula	Yield	M.P. °C	Elemental Analysis					
					Calculated			Found		
					C%	H%	N%	C%	H%	N%
1	2-fluoro Phenyl	C ₁₈ H ₁₄ N ₇ OF ₃	88%	215	53.86	3.49	24.43	53.82	3.46	24.0
2	3- fluoro Phenyl	C ₁₈ H ₁₄ N ₇ OF ₃	93%	247	53.86	3.49	24.43	53.84	3.45	24.45
3	4- fluoro Phenyl	C ₁₈ H ₁₄ N ₇ OF ₃	91%	260	53.86	3.49	24.43	53.88	3.52	24.47
4	2-Tri fluoro methyl Phenyl	C ₁₉ H ₁₄ N ₇ OF ₅	85%	250	50.55	3.10	21.72	50.58	3.12	21.75
5	3-Tri fluoro methyl Phenyl	C ₁₉ H ₁₄ N ₇ OF ₅	94%	180	50.55	3.10	21.72	50.53	3.08	21.70
6	4-Tri fluoro methyl Phenyl	C ₁₉ H ₁₄ N ₇ OF ₅	87%	230	50.55	3.10	21.72	50.51	3.07	21.71
7	2-chloro Phenyl	C ₁₈ H ₁₄ N ₇ OF ₂ Cl	83%	270	51.73	3.35	23.47	51.71	3.32	23.45
8	3-chloro Phenyl	C ₁₈ H ₁₄ N ₇ OF ₂ Cl	95%	240	51.73	3.35	23.47	51.76	3.38	23.49
9	4-chloro Phenyl	C ₁₈ H ₁₄ N ₇ OF ₂ Cl	80%	290	51.73	3.35	23.47	51.74	3.37	23.46

Spectral studies of 2-(2,4 - difluoro phenyl) - 3 -(1 H- 1,2,4- triazol-1-yl) propan -2 -ol, 1(4- fluoro phenyl) tetrazole.(Compound No.1)

IR (KBr) cm^{-1} : 3436(OH); 838,3199 (disubstituted aromatic); 2924(CH_2),1621 (C=N); 1275(N=N);1141(tetrazole);1094 (fluorination);966(1,2,4 triazole).

Spectral studies of 2-(2,4 - difluoro phenyl) - 3 -(1 H- 1,2,4- triazol-1-yl) propan -2 -ol, 1(4 trifluoromethyl phenyl) tetrazole.(Compound No.4)

IR (KBr) cm^{-1} : 3430 (OH); 850,3199 (disubstituted aromatic); 2924(CH_2),1619 (C=N); 1280 (N=N);1121 (tetrazole);1071 (difluorination);967 (1,2,4 triazole), 1021(CF_3).

Spectral studies of 2-(2,4 - difluoro phenyl) - 3 -(1 H- 1,2,4- triazol-1-yl) propan -2 -ol, 1(2 chloro phenyl) tetrazole.(Compound No.7)

IR (KBr) cm^{-1} : 3442 (OH); 832,3199 (disubstituted aromatic); 2918 (CH_2),1613 (C=N); 1276 (N=N);1095 (tetrazole);1045 (difluorination);970 (1,2,4 triazole), 633 (mono chlorination).

PMR Spectral Analysis (Compound No. 7)

PMR (DMSO) δ 2.510 (4 H , s , CH_2)

δ 3.457 (1 H , s , OH)

δ 6.601 (2 H , d , N-CH=N)

δ 7.0 - 8.04 (7 H , m , aromatics)

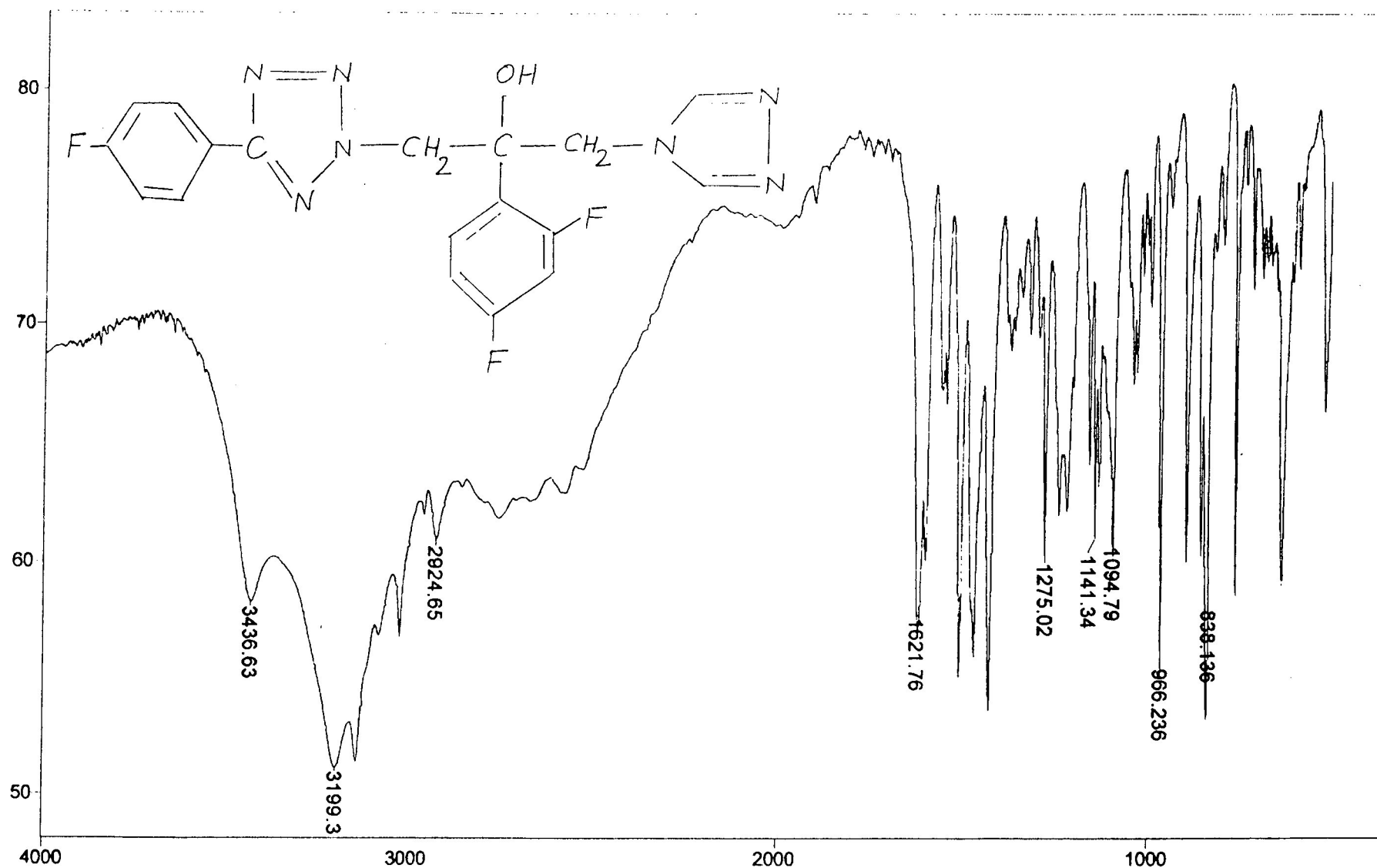
Sample Description: fluconazole derivative

Scans= 10 Slow

Res=4 cm-1

Apod= Cosine

Zero Filling= 1 x



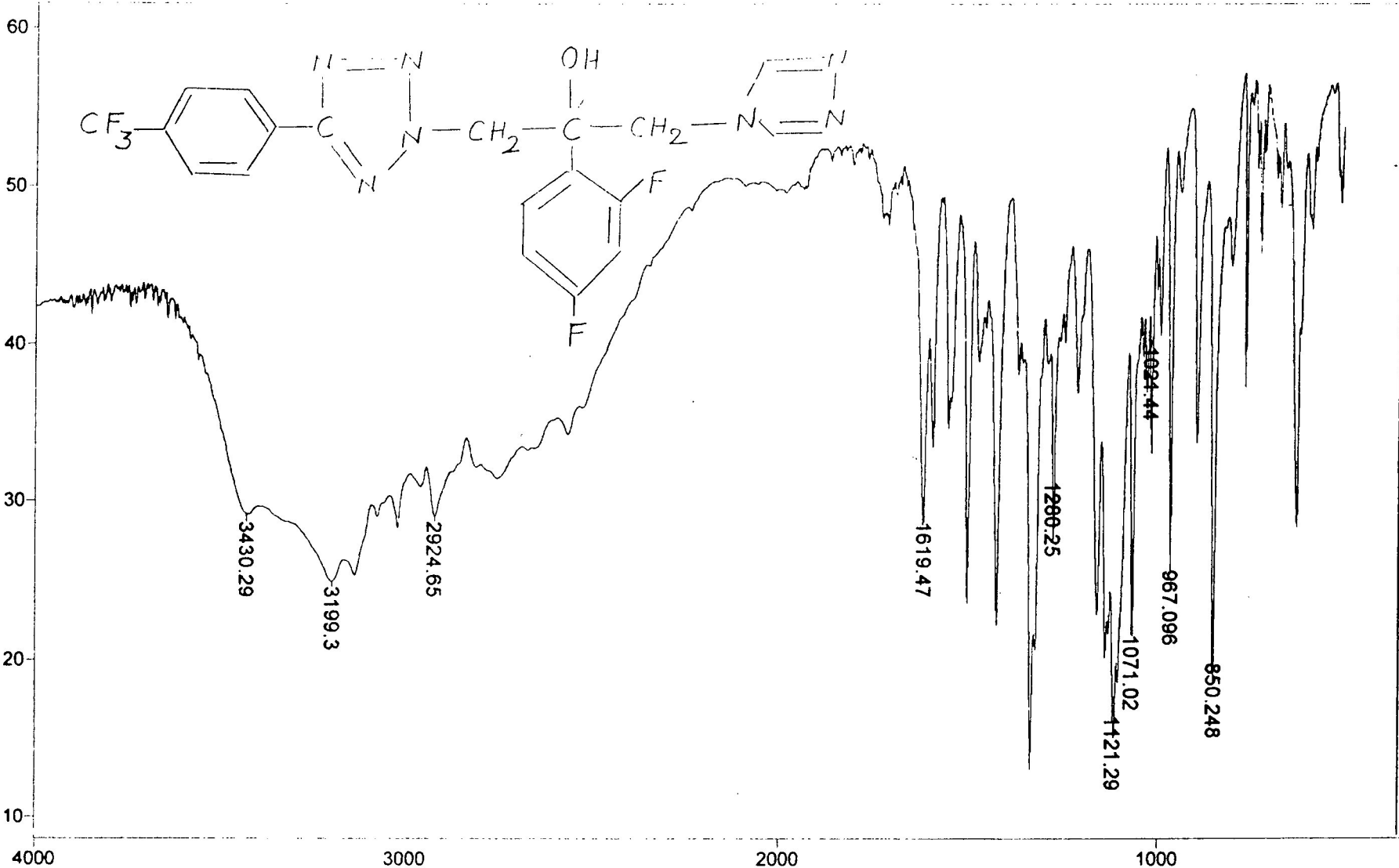
Sample Description: fluconazole derivative

Scans= 10 Slow

Res=4 cm-1

Apod= Cosine

Zero Filling= 1 x



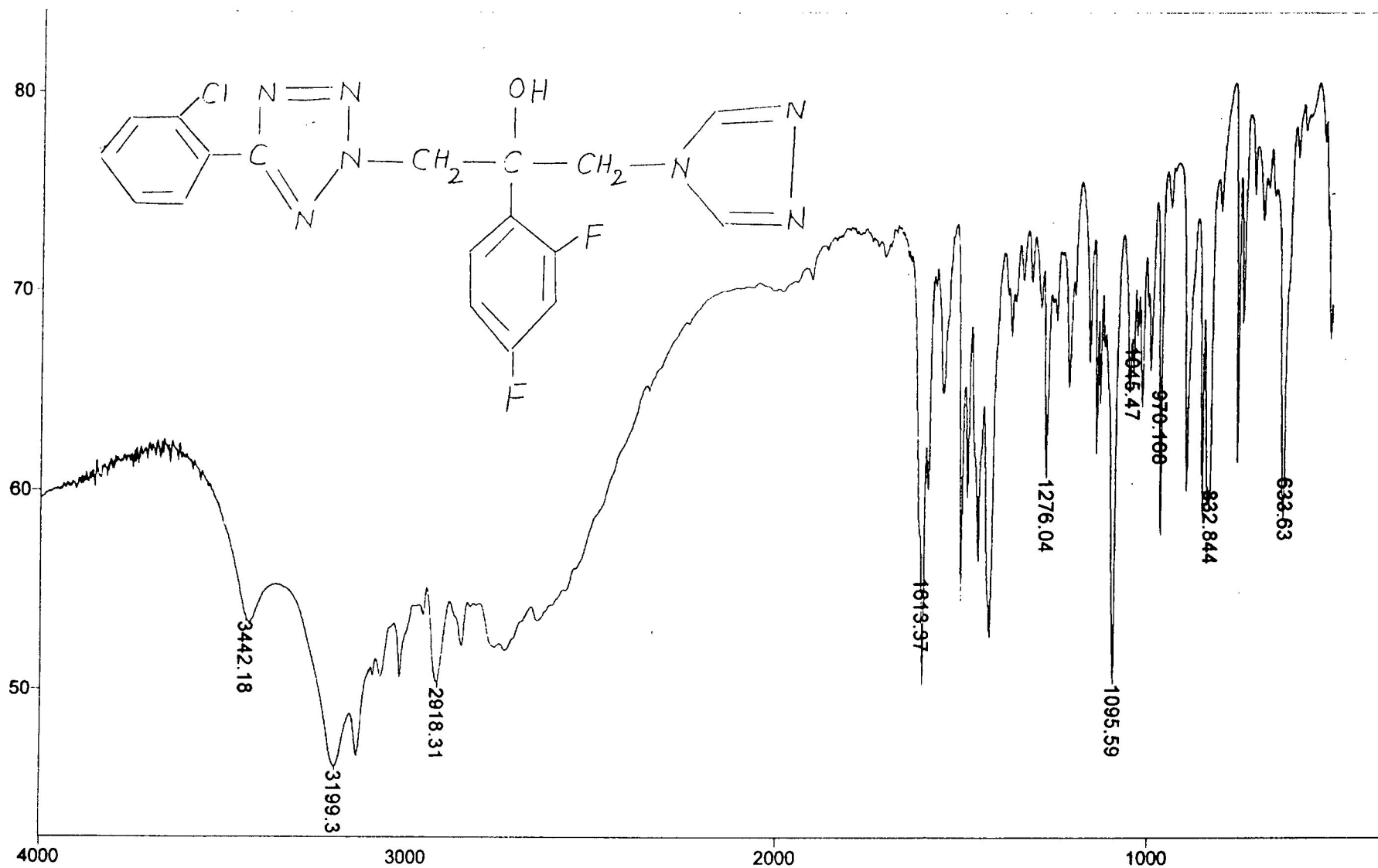
Sample Description: fluconazole derivative

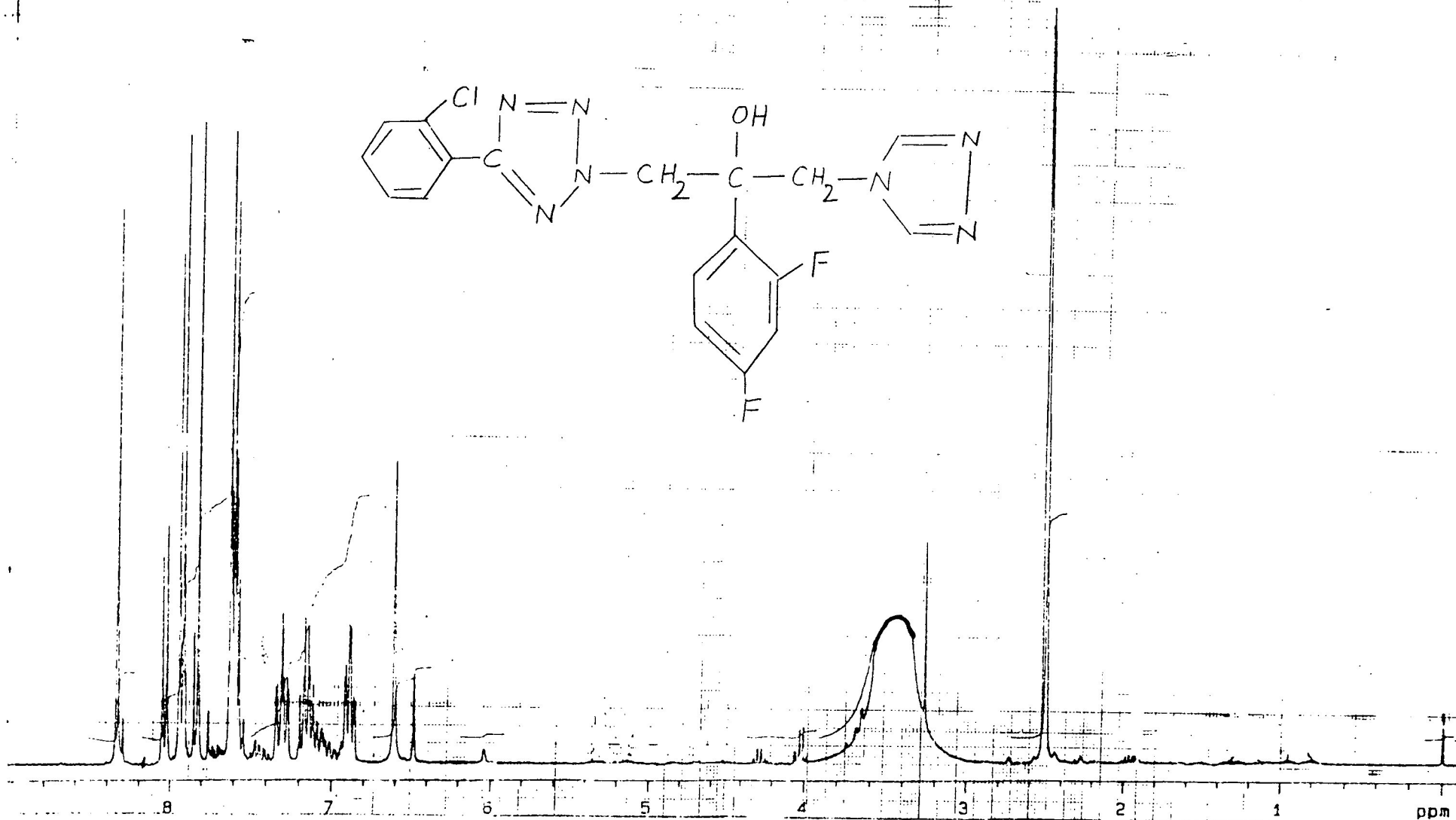
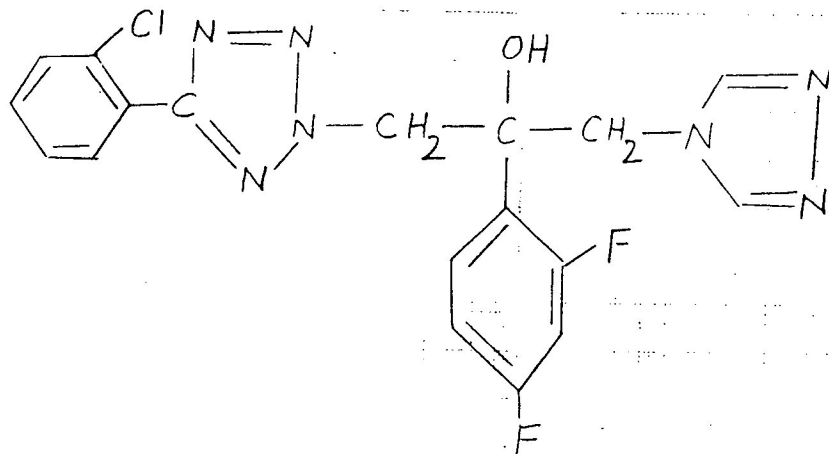
Scans= 10 Slow

Res=4 cm-1

Apod= Cosine

Zero Filling= 1 x





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10.0
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900.0
0
264

H1
1370.8
20
200
not used

not used
0.100
27.6.8

not used
-31.1

not used
a2pul
N7
26.0
0.00

N7.11d

Dec 17 97

300

*SYNTHESIS OF
1-THIOCARBAMOYL-3-
METHYL-4 (ARYL
HYDRAZONO) 2-
PYRAZOLIN-5- ONE*

PART III

SYNTHESIS OF 1-THIOCARBAMOYL-3-METHYL-4 (ARYL HYDRAZONO) 2-PYRAZOLIN-5- ONE

This part includes synthesis of 1-Thiocarbamoyl-3-methyl-4(aryl hydrazono)F-2- pyrazolin-5-one which involves the following steps.

- i) Preparation of substituted aryl diazonium salt of corresponding substituted aryl amine.
- ii) Preparation of ethyl 2-substituted aryl hydrazono-3-oxo butyrates.
- iii) Preparation of the title compounds by condensation of (2) with thiosemicarbazide in the presence of glacial acetic acid.

2) Preparation of ethyl 2-(3 chloro 4 Fluoro phenyl hydrazono) -3- oxobutyrate

To 3 chloro 4 fluoro aniline (7.25g, 0.05 mole) dissolved in a mixture of conc. HCl (40ml) and water (30 ml) and cooled to 0°C in an ice bath, a cold aqueous solution of sodium nitrite (4.83g, 0.07 mole) was added. The diazonium salt solution was filtered into a cooled solution of ethyl acetoacetate (6.5g ; 0.05 mole) and sodium acetate. (49.2 g; 0.6 mole) in ethanol (100ml). The resulting solid was washed with water and recrystallised from ethanol (20 ml).

M.P 117 -120°C

Lit m.p 119°C

Yield (12.55 g, 88.0 %)

Ethyl 2-(2 chloro 6 fluoro phenyl hydrazono) -3- oxobutyrate, Ethyl 2-(4 chloro -3- fluoro phenyl hydrazono) -3- oxobutyrate and Ethyl 2- (6 chloro 2 Fluoro phenyl hydrazono) -3- oxobutyrate were prepared from the corresponding substituted aryl amine, following the procedure adopted above for the preparation of ethyl 2-(3 chloro 4 fluoro phenyl hydrazono) -3- oxobutyrate from 3 chloro 4 fluoro aniline.

Preparation of ethyl 2 - (3,5 dichloro 4 methoxy phenyl hydrazono) -3- oxobutyrate.

To 3,5 dichloro 4 methoxy aniline (9.6 g; 0.05 mole) dissolved in a mixture of conc HCl (40ml) and water (30 ml) and cooled to 0°C in an ice bath, a cold aqueous solution of sodium nitrite (4.83g; 0.07 mole) was added . The diazonium salt solution was filtered into a cooled solution of ethyl acetoacetate (6.5 g; 0.05 mole) and sodium acetate (49.2 g; 0.6 mole) in

ethanol (100 ml). The resulting solid was washed with water and crystallised from ethanol (20ml).

M.P 95°C

Lit M.P. 97°C

Yield 12.82 g (77.0 %)

Ethyl 2-(2,4 dichloro 6 methoxy phenyl hydrazono)-3-oxobutyrate, Ethyl 2-(2,6 dichloro 4 methoxy phenyl hydrazono)-3-oxobutyrate were prepared from the corresponding substituted aryl amine, following the procedure adopted above for the preparation of Ethyl 2-(3,5 dichloro 4 methoxy phenyl hydrazono)-3-oxobutyrate from 3,5 dichloro 4 methoxy aniline.

Preparation of ethyl 2- (4 Fluoro phenyl hydrazono) 3 -oxobutyrate

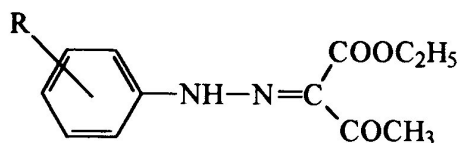
To 4 fluoro aniline (5.55 g; 0.05 mole) dissolved in a mixture of conc HCl (40 ml) and water (30 ml) and cooled to 0°C in an ice bath, a cold aqueous solution of sodium nitrite (4.83 g; 0.07 mole) was added. The diazonium salt solution was filtered into a cooled solution of ethyl acetoacetate (6.5g; 0.05 mole) and sodium acetate (49.2 g; 0.6 mole) in ethanol (20ml)

M.P. 170°C

Lit M.P 172°C

(Yield : 10.32 g, 82.0 %)

Ethyl -2- (2 Fluoro phenyl hydrazono) -3- oxo butyrate and Ethyl -2- (3 Fluoro phenyl hydrazono) -3- oxobutyrate were prepared from the corresponding substituted aryl amine, following the procedure adopted above for the preparation of ethyl -2- (4 fluoro phenyl hydrazono) -3- oxobutyrate from 4 fluoro aniline.



Sr. No.	R	M.P. (°C)		Yield	Solvent
		Found	Reported		
1	3 chloro 4 fluoro	117	119	88.0 %	Ethanol
2	2 chloro 6 fluoro	140	142	78.0 %	Ethanol
3	3,5 dichloro 4 methoxy	95	97	77.0 %	Ethanol
4	2,4 dichloro 6 methoxy	135	138	75.0 %	Ethanol
5	2,6 dichloro 4 methoxy	101	103	87.0 %	Ethanol
6	4 fluoro	170	172	82.0 %	Ethanol
7	2 fluoro	140	144	91.0 %	Ethanol
8	3 fluoro	155	157	81.0 %	Ethanol
9	4 chloro 3 fluoro	125	127	84.0 %	Ethanol
10	6 chloro 2 fluoro	87	89	93.0 %	Ethanol

3) Preparation of 1- Thio carbamoyl -3-methyl -4- (3 chloro 4 fluoro phenyl hydrazono) -2- pyrazolin-5-one .

To ethyl -2- (3 chloro 4 fluoro hydrazono) -3-oxobutyrate (5.93g, 0.02 mole) dissolved in glacial acetic acid (20ml) in a 100 ml round bottom flask, a solution of thiosemicarbazide (1.8 g, 0.02 mol) in glacial acetic acid (25 ml) was added and the mixture was refluxed for 4 hours. It was then cooled and allowed to stand overnight. The resulting solid was dried and recrystallised from ethanol (15 ml)

M.P. 230-233° C

Yield (4.13 g, 66.0 %)

1- Thio carbamoyl -3- methyl -4- (2 chloro 6 Fluoro phenyl hydrazono) -2- pyrazolin -5-one, 1-Thio carbamoyl -3-methyl -4-(4 chloro 3 fluoro phenyl hydrazono) -2-pyrazolin -5-one, and 1-Thiocarbamoyl-3 methyl-4-(4 chloro 3Fluoro phenyl hydrazono) 2-pyrazolin - 5 one,and 1-Thiocarbamoyl -3-methyl-4-(6 chloro 2 Fluoro Phenyl hydrazono)2-pyrazolin-5-one were prepared from the corresponding ethyl 2-(aryl hydrazono)-3-oxobutyrate, following the

procedure adopted above for the preparation of 1-Thio carbamoyl-3-methyl -4- (3-chloro 4-fluoro phenyl hydrazono) -2- pyrazolin -5- one from ethyl 2- (3- chloro -4- fluoro phenyl hydrazono) -3- oxo-butyrate

Preparation of 1 - Thiocarbamoyl -3-methyl-4 (3,5 dichloro 4 methoxy hydrazono) -2-pyrazolin- 5-one

To ethyl 2- (3,5 dichloro 4 methoxy phenyl hydrazono) -3- oxobutyrate (6.66 g; 0.02 mole) dissolved in glacial acetic acid (20 ml) in a single necked 100 ml round bottom flask, a solution of thiosemicarbazide (1.8 g 0.02 mole) in glacial acetic acid (25 ml) was added and the mixture was refluxed for 4 hours. It was then cooled and allowed to stand overnight. The resulting solid was dried and recrystallised from ethanol (15 ml)

M.P. 210 - 213°C

Yield: 5.18 g; 72.0 %

1- Thiocarbamoyl-3-methyl-4-(2,4 dichloro 6 methoxy phenyl hydrazono) -2- pyrazolin-5-one; 1- Thiocarbamoyl-3-methyl-4-(2,6 dichloro 4 methoxy phenyl hydrazono) -2- pyrazolin-5-one were prepared from the corresponding ethyl 2- aryl hydrazono -3 oxobutyrate, following the procedure adopted above for the preparation of 1- Thiocarbamoyl-3-methyl-4 (3,5 dichloro 4 methoxy hydrazono) -2-pyrazolin-5-one from ethyl 2-(3,5, dichloro 4 methoxy phenyl hydrazono) -3- oxobutyrate.

Preparation of 1 - Thiocarbamoyl -3-methyl -4 (fluoro phenyl hydrazono) -2- pyrazolin- 5-one

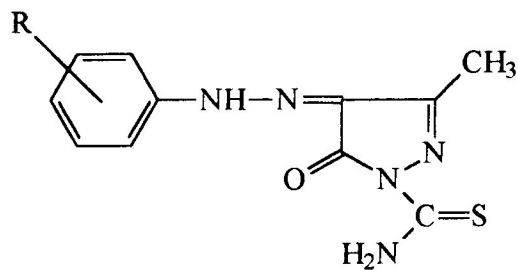
To ethyl 2-(4 fluoro phenyl hydrazono) -3- oxobutyrate (5.04 g; 0.02 mole) dissolved in glacial acetic acid (20 ml) in a single necked 100ml round bottom flask, a solution of thiosemicarbazide (1.8 g; 0.02 mole) in glacial acetic acid (25 ml) was added and the mixture was refluxed for 4 hours . It was then cooled and allowed to stand overnight. The resulting solid was dried and crystallised from ethanol (15 ml)

M.P. 235°C -237°C

Yield 4.46 g 80.0 %

1-Thiocarbamoyl 3-methyl-4-(2 fluoro phenyl hydrazono) -2- pyrazolin-5-one and 1-Thiocarbamoyl-3-methyl-4-(3 Fluoro phenyl hydrazono)-2 pyrazolin-5-one were prepared

from the corresponding ethyl 2- aryl hydrazono-3-oxobutyrate following the procedure adopted above for the preparation of 1 Thiocarbamoyl-3-methyl-4 (4 fluoro phenyl hydrazono) -2-pyrazolin-5-one from ethyl 2-(4fluoro phenyl hydrazono) -3- oxobutyrate.



Sr	R	Mol. Formula	Yield	M.P. °C	Elemental Analysis					
					Calculated			Found		
					C%	H%	N%	C%	H%	N%
1	3 Chloro 4 fluoro	C ₁₁ H ₉ ClFN ₅ OS	66	230	42.10	2.87	22.32	42.12	2.89	22.34
2	2 Chloro 6 fluoro	C ₁₁ H ₉ ClFN ₅ OS	71	215	42.10	2.87	22.32	42.08	2.85	22.30
3	3,5 dichloro 4 methoxy	C ₁₁ H ₁₁ Cl ₂ N ₅ O ₂ S	72	210	40.0	3.05	19.44	39.8	3.03	19.46
4	2,4 dichloro 6 methoxy	C ₁₁ H ₁₁ Cl ₂ N ₅ O ₂ S	64	240	40.0	3.05	19.44	40.1	3.04	19.47
5	2,6 dichloro 4 methoxy	C ₁₁ H ₁₁ Cl ₂ N ₅ O ₂ S	67	230	40.0	3.05	19.44	40.3	3.07	19.45
6	4 fluoro	C ₁₁ H ₁₀ FN ₅ OS	80	235	47.31	3.58	25.08	47.29	3.56	25.07
7	2 fluoro	C ₁₁ H ₁₀ FN ₅ OS	79	205	47.31	3.58	25.08	47.33	3.60	25.09
8	3 fluoro	C ₁₁ H ₁₀ FN ₅ OS	76	195	47.31	3.58	25.08	47.3	3.57	25.05
9	4 chloro 3 fluoro	C ₁₁ H ₉ ClFN ₅ OS	73	225	42.10	2.87	22.32	42.07	2.86	22.29
10	6 chloro 2 fluoro	C ₁₁ H ₉ ClFN ₅ OS	81	205	42.10	2.87	22.32	42.11	2.88	22.33

1-Thiocarbamoyl-3-methyl- 4(3 chloro 4 fluoro hydrazono)- 2- pyrazolin-5-one
(Compd No.1)

IR (KBr) cm^{-1} : 3266,3384 (NH_2); 1693 ($\text{C}=\text{N}$); 818 ($\text{N}-\text{C}=\text{S}$);1049 ($\text{C}=\text{S}$);
1103 (monofluorination);791(monochlorination);1480($\text{C}-\text{CH}_3$);1220($\text{C}=\text{O}$)
1253 (NH stretching).

1-Thiocarbamoyl-3-methyl- 4(3,5dichloro 4 methoxy hydrazono)- 2- pyrazolin-5-one
(Compd No.3)

IR (KBr) cm^{-1} : 3324,3455 (NH_2); 1697 ($\text{C}=\text{N}$); 809 ($\text{N}-\text{C}=\text{S}$);1102 ($\text{C}=\text{S}$);
670,770(dichlorination) ;2930 ($\text{C}-\text{OCH}_3$); 1476($\text{C}-\text{CH}_3$);1216($\text{C}=\text{O}$)
1257 (NH stretching).

PMR Spectral Analysis of Compound No. 1

PMR (DMSO) : δ 2.269 (3 H, s, CH_3)
 δ 7.25 - 7.92 (3 H, m, aromatic)
 δ 8.9 (2 H, s, $\text{NH}_2-\text{C}=\text{S}$)
 δ 9.46 (1 H, s, $\text{NHN}=\text{C}$)

PMR Spectral Analysis of Compound No. 3

PMR (DMSO) : δ 2.2723 (3H, s, CH_3)
 δ 3.896 (3H, s, Ar- OCH_3)
 δ 7.25 -7.82 (2H, m, aromatic)
 δ 8.892 (2H, s, $\text{CS}-\text{NH}_2$)
 δ 9.46 (1H, s, $\text{NH}-\text{N}=\text{C}$)

1-Thiocarbamoyl-3-methyl- 4(4 fluoro hydrazono)- 2- pyrazolin-5-one
(Compd No.6)

IR (Kbr) cm^{-1} : 3266,3382(NH_2); 1689 ($\text{C}=\text{N}$); 832 ($\text{N}-\text{C}=\text{S}$);1048 ($\text{C}=\text{S}$);
1110(mono fluorination); 1492($\text{C}-\text{CH}_3$);1219($\text{C}=\text{O}$)
1243 (NH stretching).

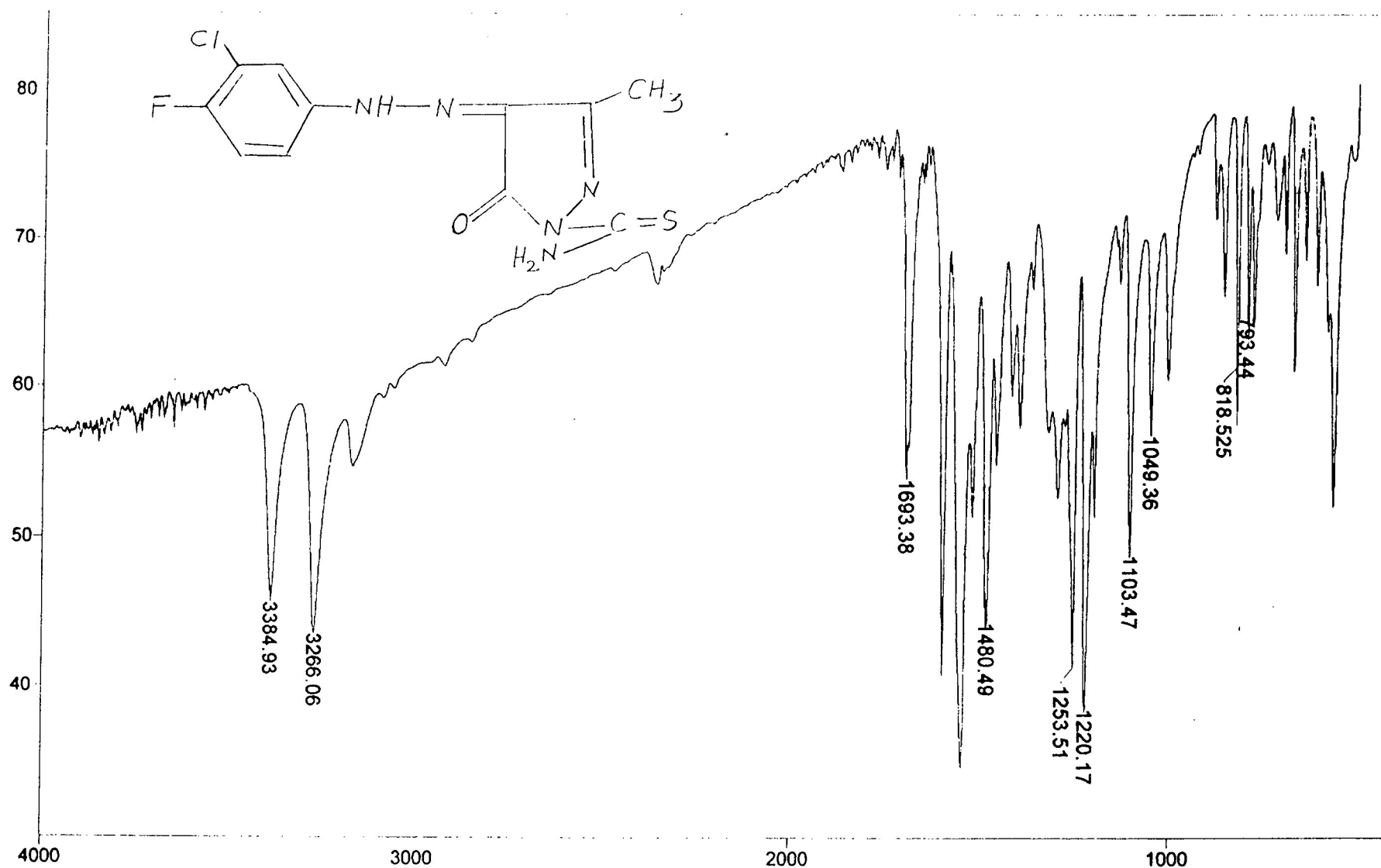
Sample Description: pyrazolinone derivative of 3 chloro 4 fluoro aniline

Scans= 10 Slow

Res=4 cm-1

Apod= Cosine

Zero Filling= 1 x



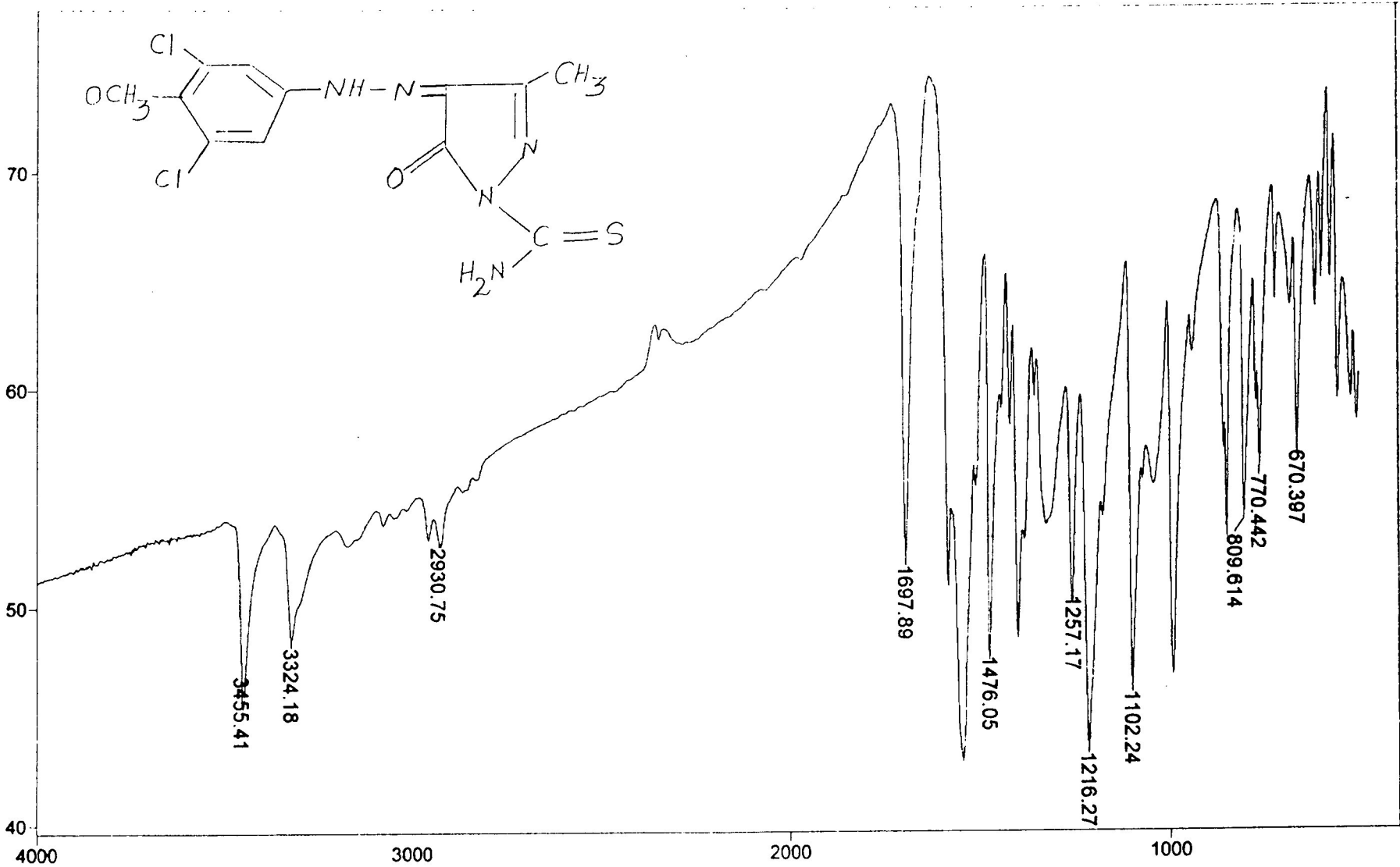
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Scans= 10 Slow

Res=4 cm-1

Apod= Cosine

Zero Filling= 1 x



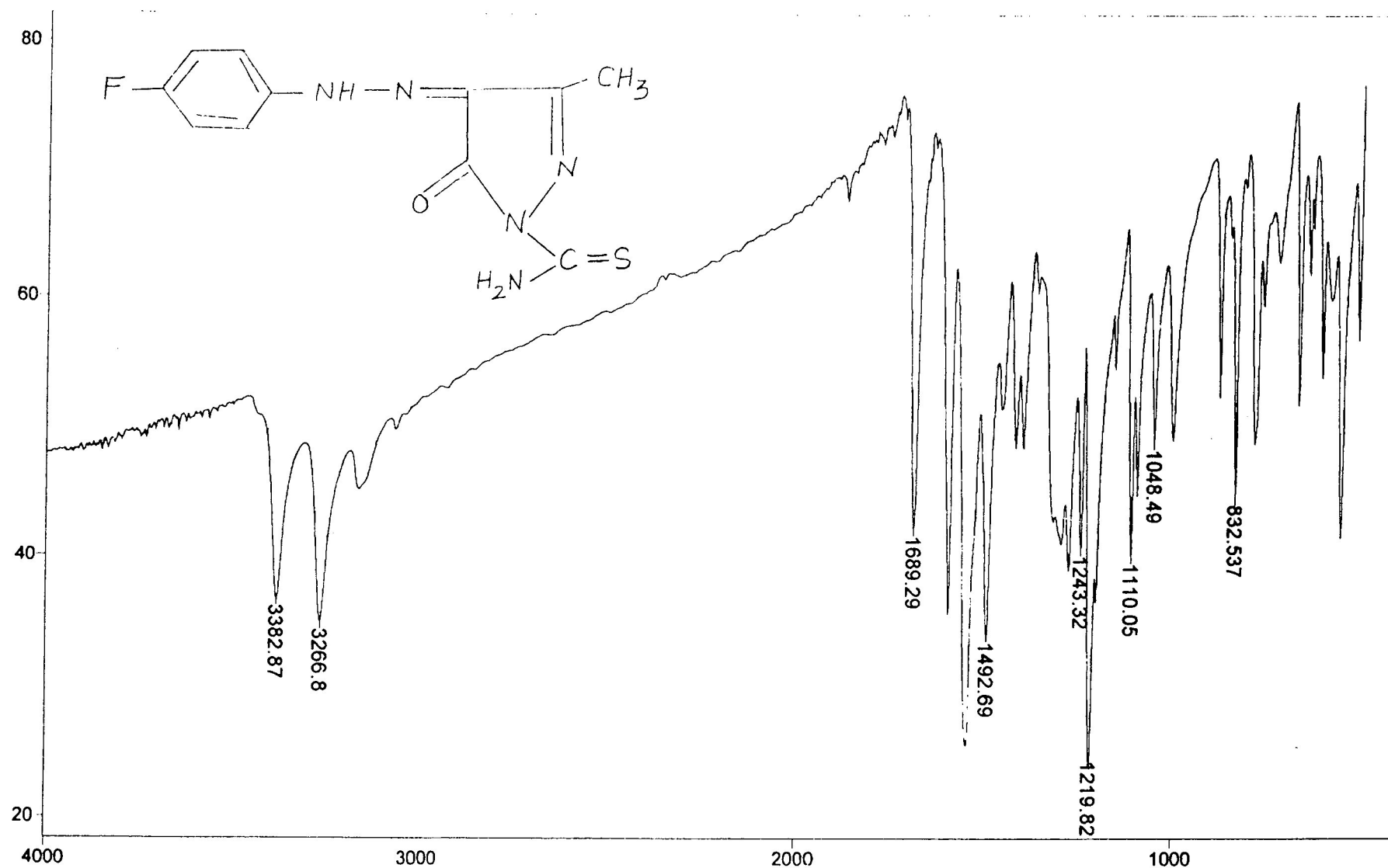
Sample Description: pyrazolinone derivative of 4 fluoro aniline

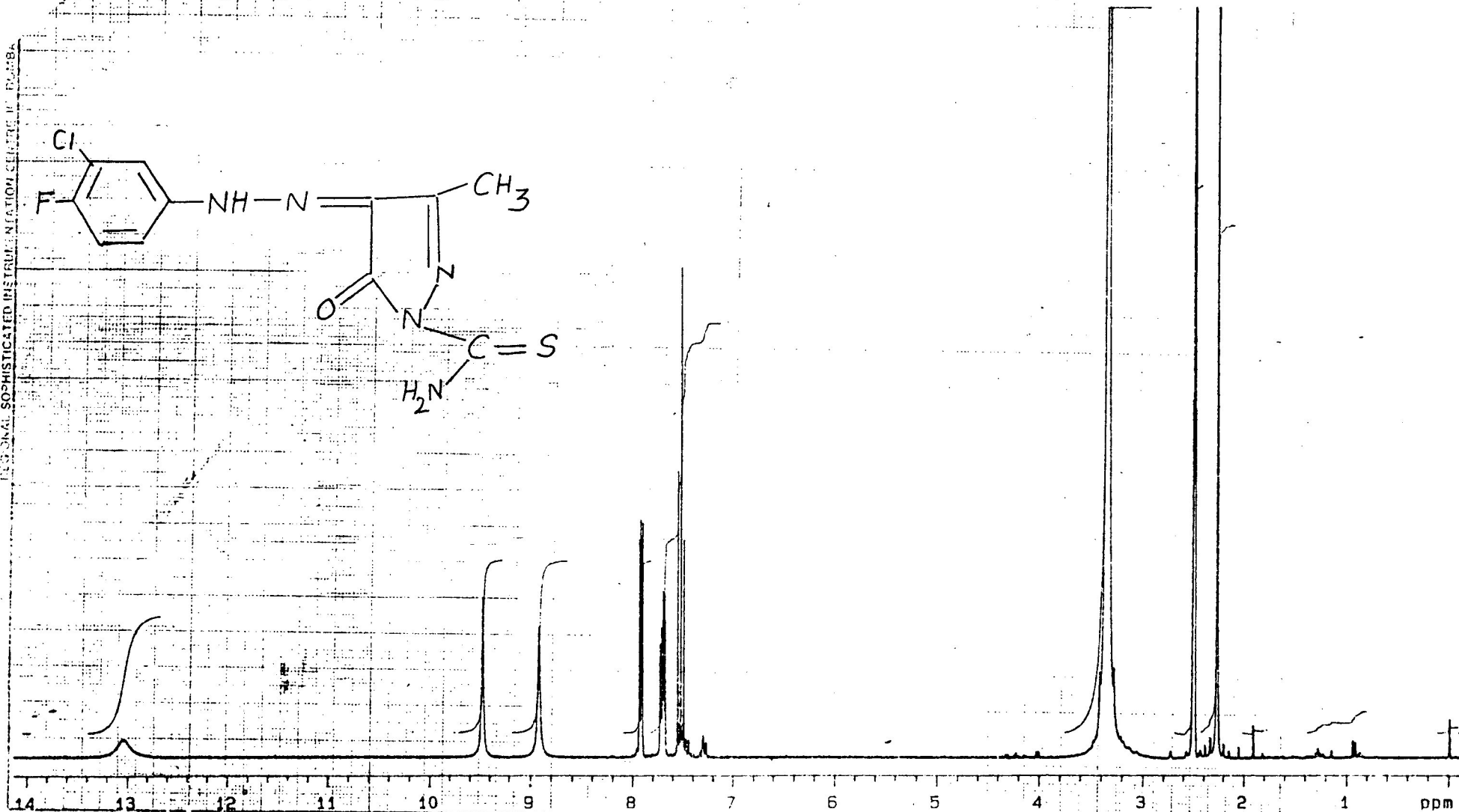
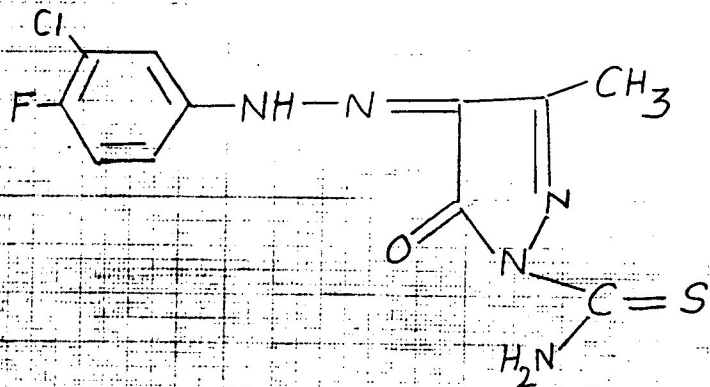
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Res=4 cm-1

Apod= Cosine

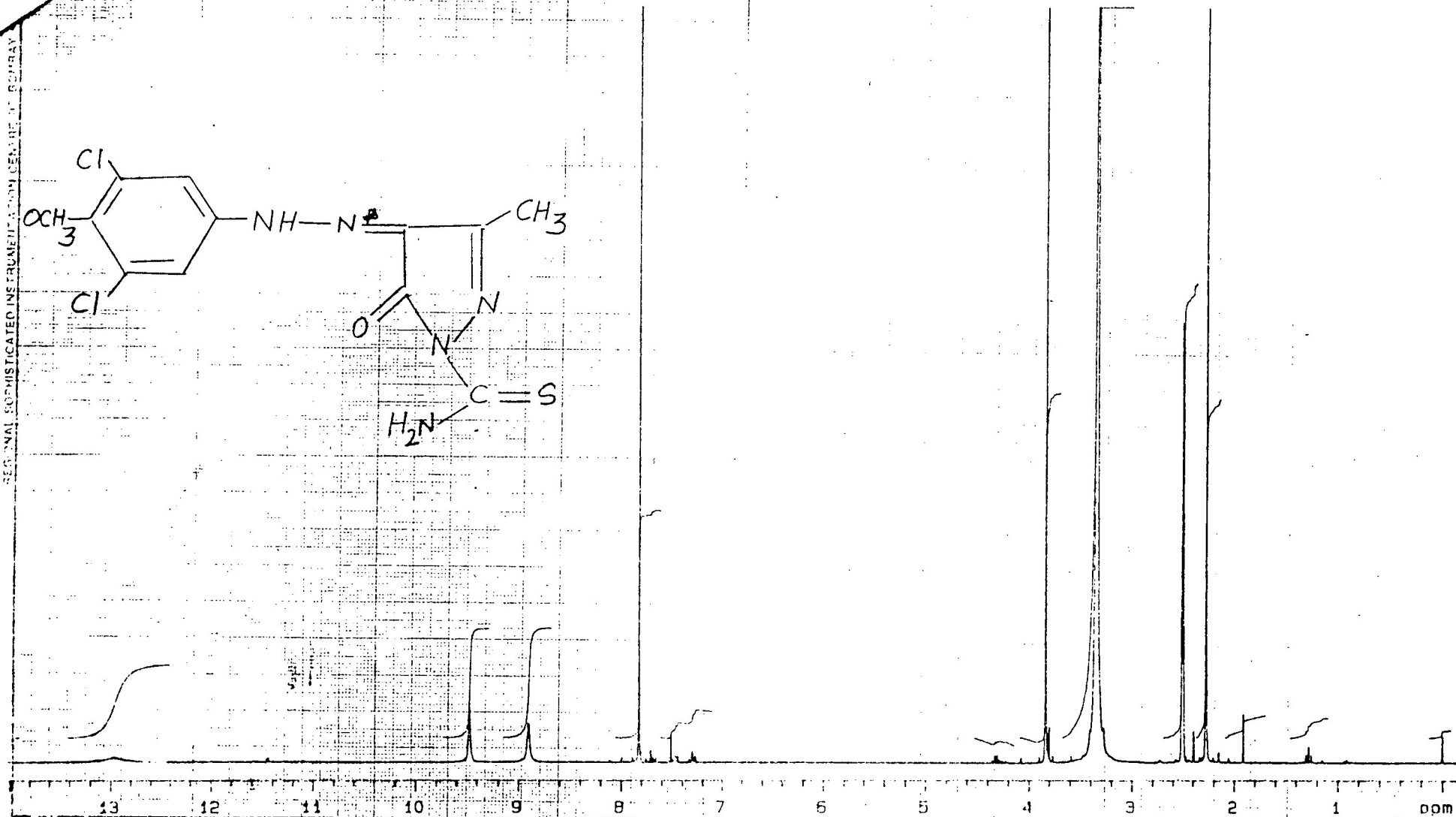
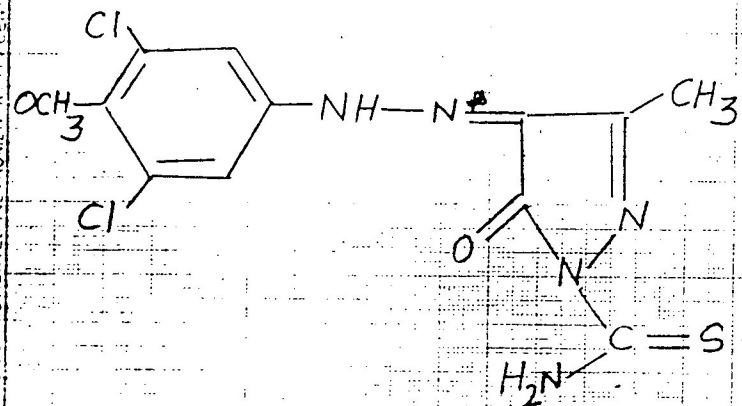
Zero Filling= 1 x





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							-34.8
							not used
							25.0
							DMSO
							300

TS1.fid
Dec 17 97
300



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*SYNTHESIS OF
DRUG
INTERMEDIATE OF
NIMESULIDE
AND NIMESULIDE ITSELF*

Part IV

Synthesis of Nimesulide (a potent non steroidal antiinflammatory drug (NSAID) by two different routes).

Preparation of Nimesulide involves following different steps.

1. Preparation of 2-nitro diphenyl ether
2. Preparation of 2-amino diphenyl ether
3. Preparation of 2-amino diphenyl ether hydrochloride
4. Condensation of 2-amino diphenyl ether hydrochloride with methane sulphonyl chloride
5. Preparation of the title compound by nitration of N-(2-phenoxy phenyl) methane sulfonamide

1) Synthesis of 2-nitro diphenyl ether

Ortho chloro nitro benzene (4.7 g; 0.03 mole) was placed in a 100ml three necked round bottom flask. Phenol (3.76 g; 0.04 mole) was added dropwise to the solution of ortho chloro nitro benzene in dry dimethyl formamide (20 ml). KOH (2.24 g, 0.04 mole) was added and the mixture was refluxed for 8-9 hours. Then the mixture was dumped in ice cold water (30ml) to get yellow coloured precipitate of 2-nitro diphenyl ether.

M.P. 38-40°C

Lit M.P. 35°C

Yield 5.64 g (88.0 %)

2) Preparation of 2-amino diphenyl ether

2- nitro diphenyl ether (4.3 g, 0.02 mole) was dissolved in toluene (15ml) till clear solution was obtained. Then the mixture was heated to 80-85°C At 80°C FeCl_3 solution (0.5 g in 5 ml water) was added dropwise and stirred at 1 hour at 90°C. After 1 hour the mixture was filtered and washed with portions of toluene. Toluene was distilled under vacuum to give 2-amino diphenyl ether.

3) Preparation of 2-amino diphenyl ether hydrochloride

Conc HCl (11.7 N, 7 ml) was added at 10-20°C till pH 3-4 to get 2-amino diphenyl ether hydrochloride.

M.P. 170-172°C

Yield (3.63 g, 82.0 %)

4) Preparation of N-(2- phenoxy phenyl) methane sulfonamide.

2-amino diphenyl ether hydrochloride (2.21 g 0.01 mole) was dissolved in water (5 ml) caustic KOH was added till pH =7. The free base was extracted in dichloro methane 3ml of pyridine was added and the mixture was cooled to 5°C Methane sulfonyl chloride(1.14 g 0.01 mole) was added at 10-20°C for period of 5 hours. The mixture was digested at 10-20°C for 2 hours. Then conc. HCl (11.7N, 15 ml) was added. After separating the dichloro methane layer it was distilled and water was added with stirring till precipitate was obtained.

M.P.123-125°C

Yield : 2.35 g; (90.0%)

5. Preparation of N-(4-Nitro-2-phenoxy phenyl) methane sulfonamide.

N-(2-phenoxy phenyl) methane sulfonamide,(2.0 g, 0.007 mole) was dissolved in glacial acetic acid (8 ml) at 45°C. Conc nitric acid (4 ml) was added in portions maintaining the temperature at 45-50°C. The mixture was digested at 30-35°C for one hour and then cooled to 0°C. The solid was filtered and recrystallised from 10 ml ethanol.

M.P. 145°C

Lit.M.P 143°C.

Yield: 2.23 g(95.0%).

	Cal	Found	Molecular Formula
C	50.64%	50.62%	C ₃ H ₂ N ₂ O ₅ S
H	3.92%	3.95%	
N	9.09%	9.06%	

TLC system - benzene: methanol (9:1)

The second route for the preparation of Nimesulide involves following steps:

- i) Condensation of ortho chloro 4 nitro aniline with phenol
- ii) Preparation of the title compound by condensation of 2-amino 4 nitro diphenyl ether with methane sulfonyl chloride.

iii) Condensation of ortho chloro 4 nitro aniline with phenol.

Ortho chloro 4 nitro aniline (5.16 g; 0.03 mole) was placed in a 100 ml three necked round bottom flask. Phenol (3.76 g; 0.04 mole) was added drop wise to the solution of ortho chloro-4- nitro aniline in dry dimethyl formamide (20 ml) KOH (2.24 g; 0.04 mole) was added and the mixture was refluxed for 15-17 hours. Then the mixture was dumped in ice cold water (30 ml) to get yellow precipitate of 2-amino 5 nitro diphenyl ether.

M.P. 39-41°C

Yield: 6.08 g; (89.0%).

(iv) 2-amino 5 nitro diphenyl ether (4.56 g; 0.02 mole) was cooled to 5°C. Then methane sulfonyl chloride (2.28 g; 0.02 mole) was added at 10-20°C for period of 5 hours. The mixture was digested at 10-20°C for 2 hours in dichloro methane (30 ml). After separating the dichloro methane layer it was distilled and water was added with stirring till precipitate of final compound was obtained.

M.P. 145°C

Lit. M.P. 143°C

Yield: 1.8 g; (76.68%)

	Cal	Found	Molecular Formula
C	50.64%	50.61%	C ₁₃ H ₉ N ₂ O ₅ S
H	3.92%	3.94%	
N	9.09%	9.05%	

TLC system - hexane : ethyl acetate (9:1)

Spectral studies of N-(2-phenoxy phenyl) methane sulfonamide.

IR (KBr) cm^{-1} : 3242 (NH- stretching); 1257 (ether linkage); 1151,1322 (R-SO₂ -NH)

Spectral studies of N-(4 - nitro -2-phenoxy phenyl) methane sulfonamide.

IR (KBr) cm^{-1} : 3286 (NH- stretching);1519 (NO₂); 1244 (ether linkage);
1151,1344 (R-SO₂ -NH)

PMR Spectral Analysis of N-(2-phenoxy phenyl) methane sulfonamide.

PMR (CDCl₃) : δ 3.1 (3 H, s, -SCH₃)

δ 1.6 (1 H, s, -NH-) (D₂O exchangeable)

δ 7.06 - 7.78 (9 H, m, aromatics)

PMR Spectral Analysis of N-(4 - nitro -2-phenoxy phenyl) methane sulfonamide.

PMR (CDCl₃) : δ 3.1 (3 H, s, -SCH₃)

δ 1.6 (1 H, s, -NH-) (D₂O exchangeable)

δ 7.06 - 7.78 (8 H, m, aromatics)

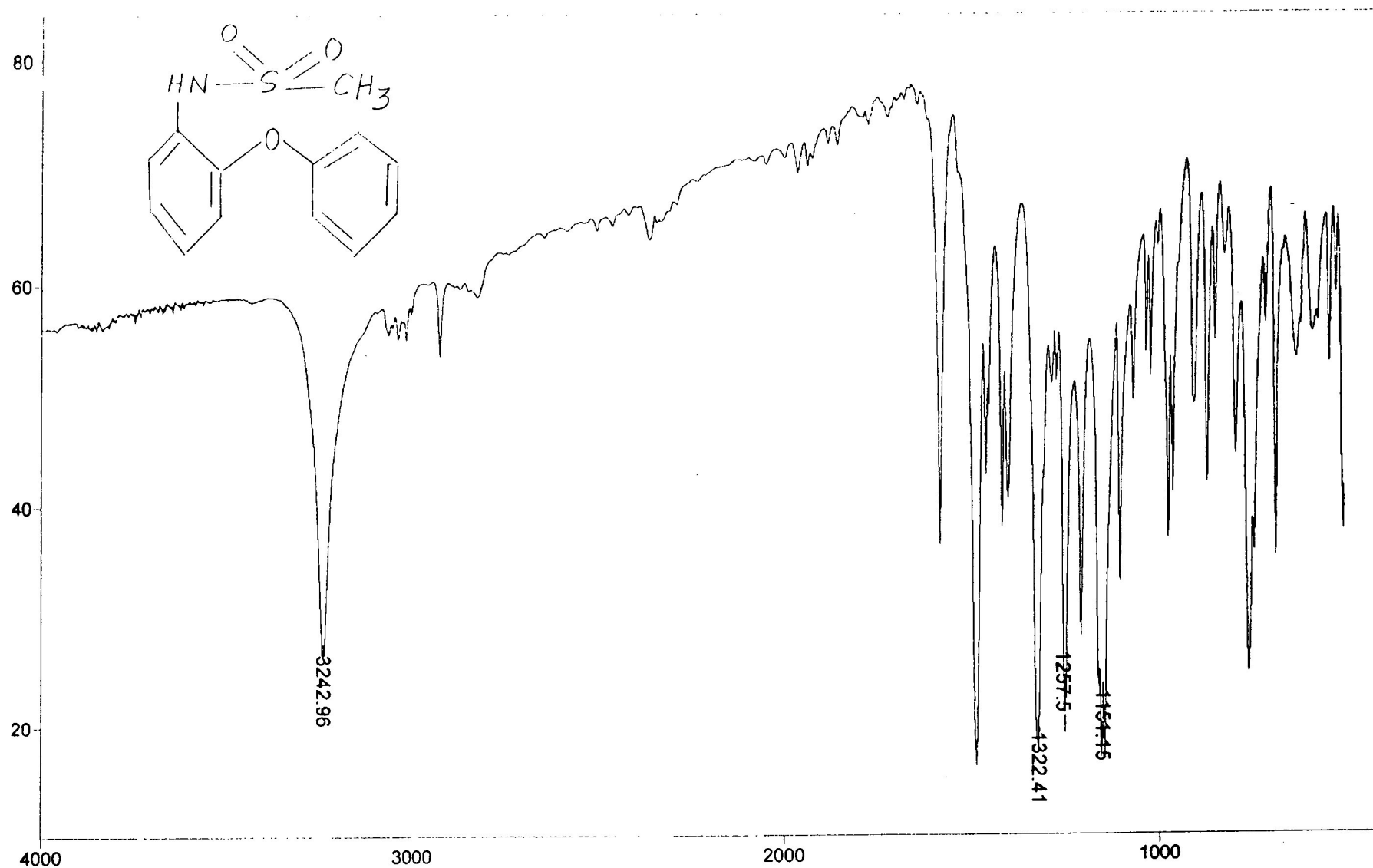
Sample Description: INTERMEDIATE OF NIMESULIDE

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Res=4 cm-1

Apod= Cosine

Zero Filling= 1 x



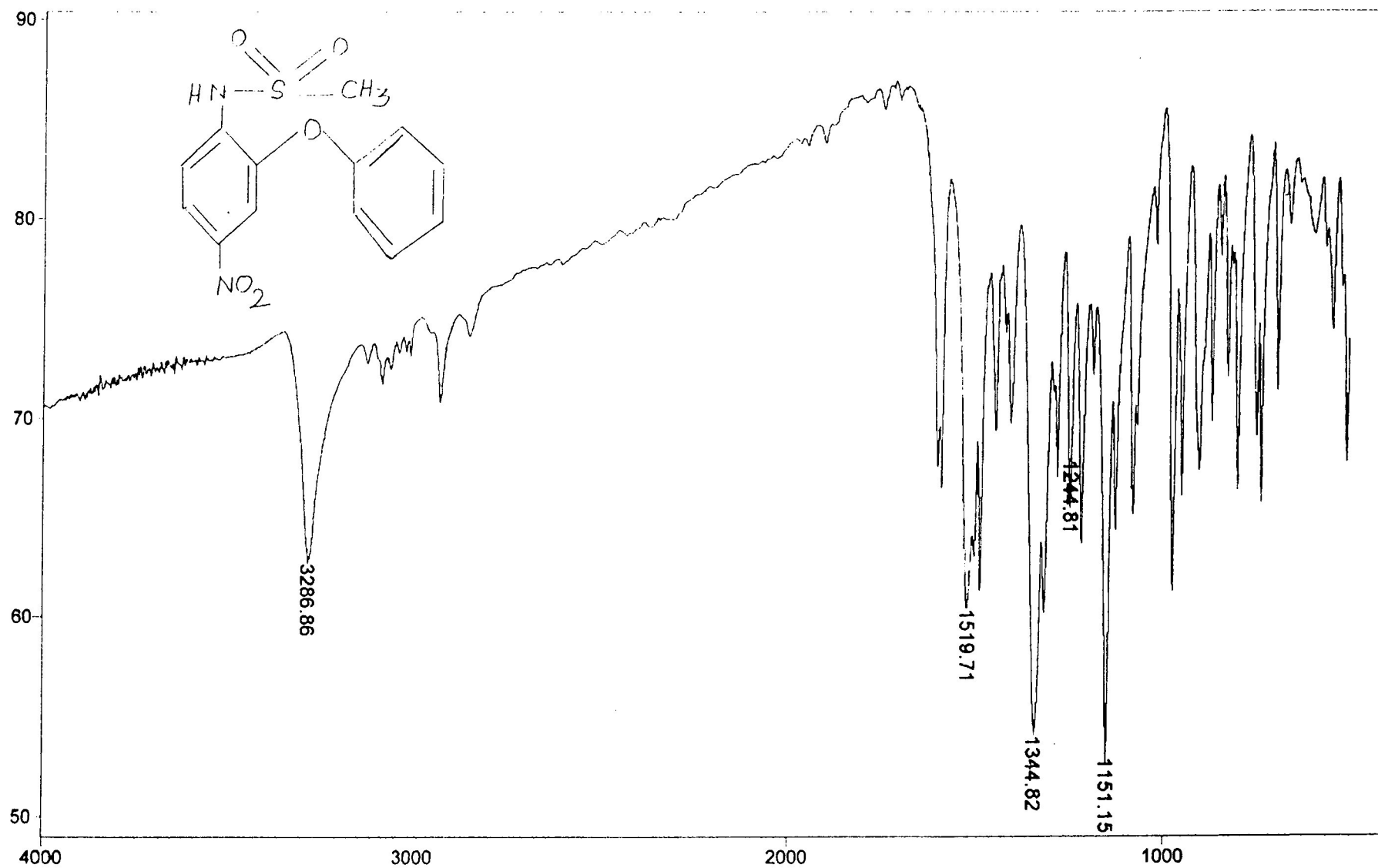
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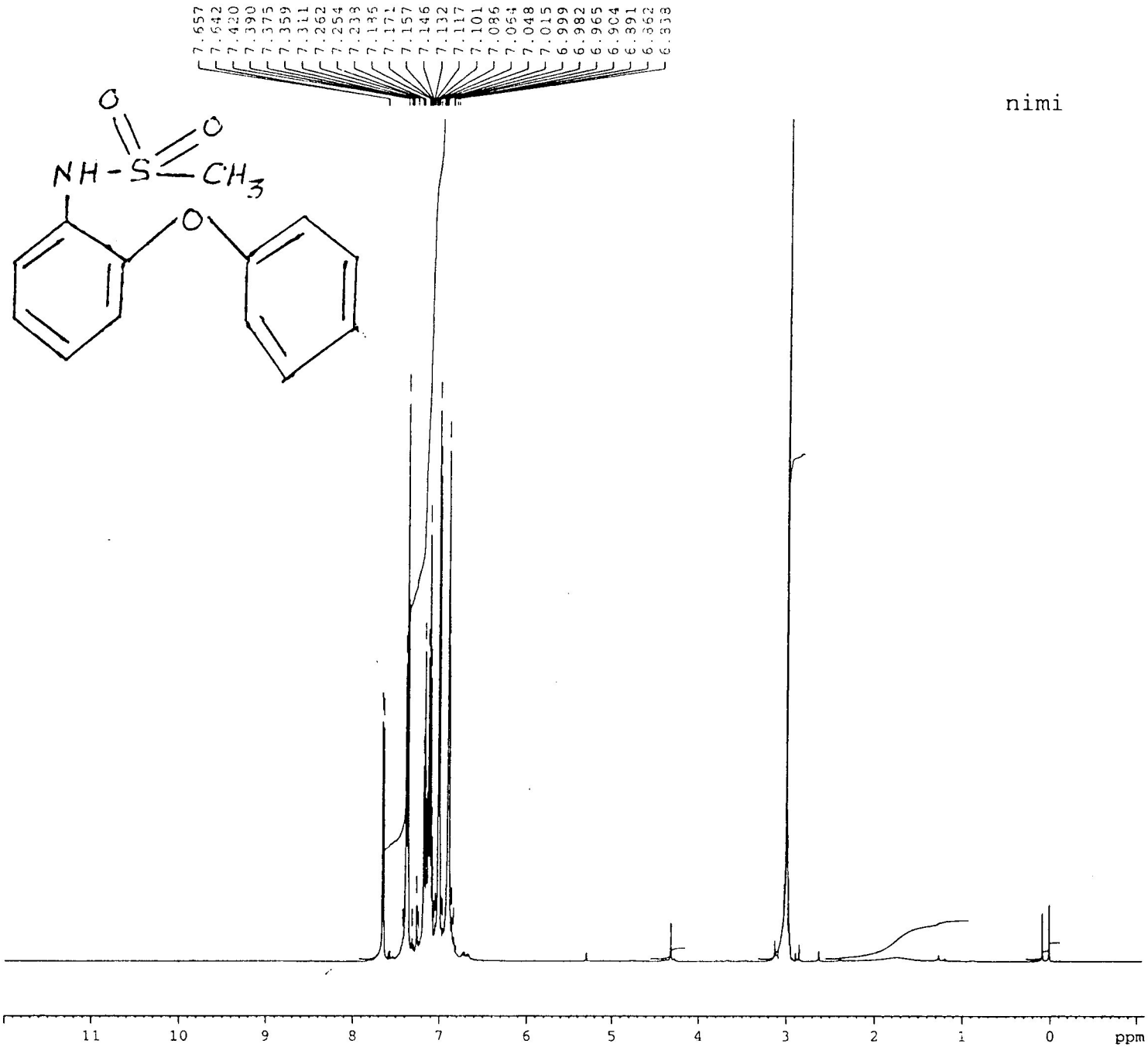
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Res=4 cm-1

Apod= Cosine

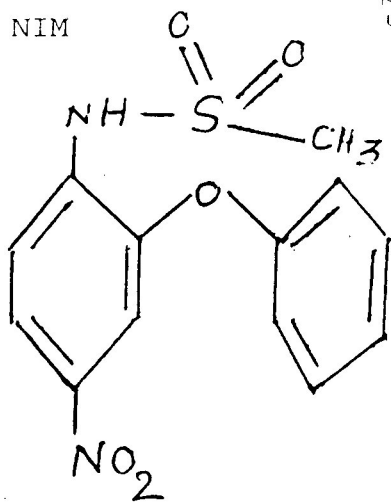
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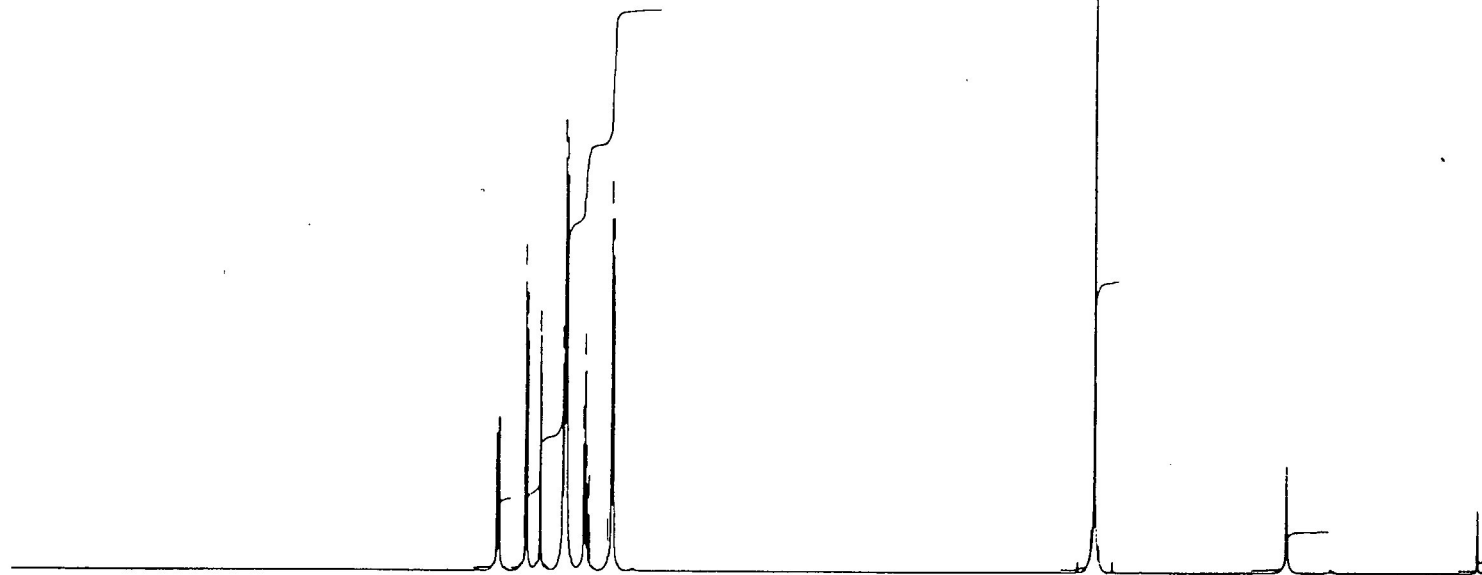


nimi

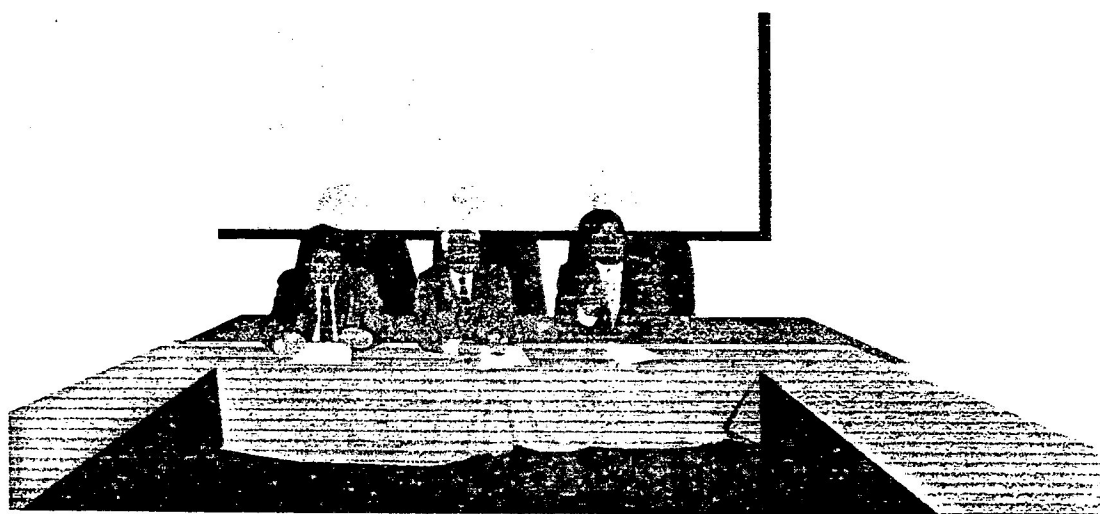
NIM



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7.775
7.766
7.757
7.753
7.649
7.474
7.459
7.444
7.310
7.295
7.260
7.263
7.112
7.353
7.167



*BIOLOGICAL RESULTS
AND
DISCUSSION*



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

The various screening programmes carried out have been in the in vitro study against various species of Gram-positive and Gram-negative bacteria viz. Staphylococcus aureus ATCC 3750 and (S. aureus) Salmonella typhi NCTC 786 (S. typhi) for estimating antibacterial activity.

Similarly, various screening programmes conducted have been in the invitro study against various strains of fungi, viz., Candida albicans, Trichophyton mentagrophytes and Trichophyton rubrum.

The methodology adopted for the various biological screening programmes undertaken in this study has been summarised below :

MATERIALS AND METHODS

Test solution:

Ten milligrams of the test substance was dissolved in 1 ml of the suitable solvent (DMF, acetic acid, methanol) and then diluted to 10 ml with distilled water for antifungal screening. For antibacterial screening 10 ml of DMF was used. The solution was added to the sterile medium by sterile medium to give a final concentration of 200, 100, 40, 20 10, 5, 2, 1 μ g/ml of the medium. The technique employed in this process was tube dilution technique¹⁹¹.

Antibacterial screening

The test solution was added in graded concentrations aseptically to the tubes containing sterile test medium (Mueller Hinton nutrient broth)¹⁹² pH was 7.2 -7.4. To each test tube was then added 0.1 ml of standard inoculum (48 hours old culture of the test organism) (5×10^{-6} spores of the organism).

After mixing the contents, the tubes were incubated at 37°C and examined after 48 hours for the presence or absence of the growth of the test organism. The lowest concentration at which no growth was observed was taken as the minimum inhibitory concentration .

Ampicillin sodium was used as standard drug for control (MIC of Ampicillin Sodium is 0.01 µg/ml for *S. aureus*) and Trimethoprim was also used as standard drug for control (MIC of Trimethoprim is 1 µg/ml for *S. typhi*). Tubes without any drug were kept for comparison of turbidity.

Antifungal screening

The organisms were maintained on Sabouraud's agar slants by regular subculturing. *C. albicans* was sub-cultured every week and *T. mentagrophytes* and *T. rubrum* at the interval of 12 days.

Test Medium:-

The liquid medium described by Schraufstaller *et al*, was used for screening the compounds for their antifungal activity. The final pH of the medium was adjusted to 5.2. This test medium was dispensed in requisite amounts varying from 3.7 to 4.8 ml in sterile tubes. To these were added the requisite test solutions in graded concentrations varying from 200 to 1 µg/ml.

PART II

Synthesis of 2-(2,4-difluoro phenyl) 3-(1 H-1,2,4, triazol -1-yl) propan-2-ol, 1 (Substituted phenyl) tetrazoles.

The screening results exhibit the activity of the test compounds against the Gram positive and Gram-negative bacteria taken for the test viz, S. aureus, S. typhi. Only 2 Fluoro, 3 Fluoro, 4 Fluoro and 2 trifluoro methyl substitutions in the phenyl ring have exhibited excellent activity ranging between 1- 2 µg/ml against S. aureus and good activity at 20µg/ml against S. typhi. 3-trifluoro methyl, 4 trifluoro methyl, 2 chloro, 3 chloro and 4 chloro substituteions in the phenyl ring exhibited good activity at 10 µg/ml against S. aureus and 20 µg/ml against S. typhi.

The antifungal screening results show an interesting trend based on the different substitutions carried out in the phenyl ring. The chloro substituted compounds (No 7, No 8, No 9) have exhibited the highest activity at 1µg/ml against C. albicans, excellent activity ranging between 5-10 µg/ml against T. rubrum, 1-5 µg/ml against T. mentagrophytes. The fluoro substituted compounds (No. 1, No. 2, No. 3) have exhibited excellent activity ranging between 1- 5 µg/ml for T. rubrum and T. mentagrophytes. The tri fluoro methyl substituted compounds (No. 4, No. 5, No. 6) showed highest activity at 1µg/ml against T. mentagrophytes, good activity ranging between 1-5 µg/ml against C. albicans and excellent activity between 1-10 µg/ml against T. rubrum.

Synthesis of (2,4 - Difluoro phenyl)-3-(1H-1,2,4-triazol-1-yl) propan-2-ol substituted phenyl) Tetrazoles

Table No. I

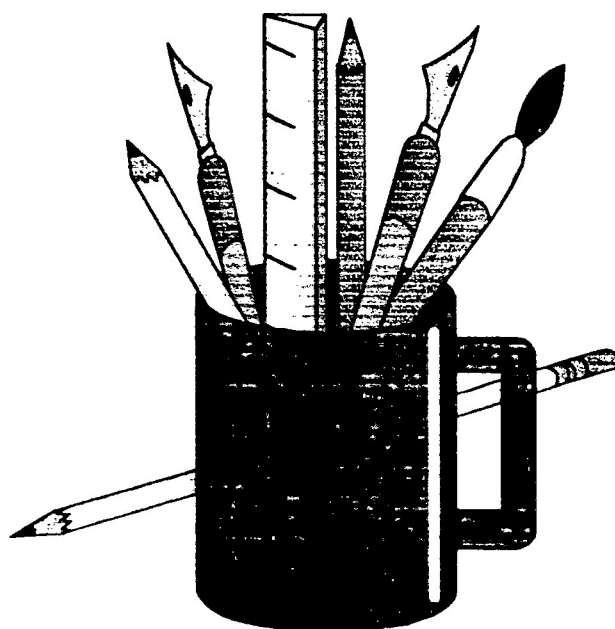
Sr No		Antibacterial activity (µg/ml)		Antifungal activity (µg/ml)		
		<u>S. aureus</u>	<u>S. typhi</u>	<u>C. albicans</u>	<u>T. rubrum</u>	<u>T. mentagrophytes</u>
1	2F	1	20	2	10	10
2	3F	1	20	1	10	10
3	4F	2	20	5	1	1
4	2CF ₃	1	20	2	1	1
5	3CF ₃	10	20	1	10	1
6	4CF ₃	10	20	2	1	1
7	2Cl	10	20	1	10	2
8	3Cl	10	20	1	5	1
9	4Cl	10	20	1	5	5

The present study has seen the synthesis of novel organic molecules which have been screened for their possible therapeutic value against bacterial and fungal infections.

In the Part II, the synthesis of 2- (2,4- difluoro phenyl) -3 - (1H -1,2,4 -triazol -l-yl) propan -2-ol, 1 (substituted phenyl) tetrazoles, have been described which have been screened for their antibacterial activity against S. aureus, S. typhi. These compounds have also been screened for their antifungal activity against T. rubrum, T. mentagrophytes and C. albicans. All the compounds in this series were found active when compared to the standard drugs.

In Table I the antibacterial / antifungal activity results of 2-(2,4- difluoro phenyl) -3 (1 H-1,2,4 triazol-1-yl) propan-2-ol, 1 (substituted phenyl) tetrazoles have been described. It is observed that the antifungal activity is favoured by the presence of Cl, F, CF₃ group, while the antibacterial activity is less favoured by the presence of electron-withdrawing groups such as Cl, F, CF₃.

CONCLUSION



Thus it may be concluded that in the tetrazole series, the presence of an electron withdrawing group is favoured for the antifungal activity.

The biological activity results against bacterial and fungal infections reveal the following trends.

- 1) All the compounds exhibit good activity against various Gram-positive and Gram - negative bacteria viz, S. aureus and S. typhi.
- 2) All the compounds exhibit excellent activity against various fungi viz, C. albicans, T. rubrum and T. mentagrophytes
- 3) All the compounds exhibit good, antibacterial activity i.e. 20 µg/ml against S. Typhi
- 4) The presence of the Fluoro substituent at the 2 and 3- position of the phenyl ring contributed to the enhancement of the antibacterial activity of the compounds against S. aureus in comparison to the Fluoro substituent at the 4- position of the phenyl ring.
- 5) The compounds with trifluoro methyl substituents at the 3 and 4-position of the phenyl ring were found to be less active against S. aureus than the compound with trifluoro methyl substituent at the 2- position of the phenyl ring.
- 6) The presence of the chloro substituent at the 2,3, and 4 position of the phenyl ring showed same activity against S. aureus.
- 7) The presence of the Fluoro substituent at the 2 and 3 position of the phenyl ring showed same activity against S. aureus.
- 8) The presence of the trifluoro methyl substituent at the 3 and 4 position of the phenyl ring exhibited similar activity against S. aureus.
- 9) The 2 Fluoro, 3 Fluoro and 2 trifluoro methyl analogues were found to be most active against S. aureus in comparison with other synthesized analogues in this series.
- 10) All the chloro derivatives showed same activity against C. albicans.

- 11) 2 trifluoro methyl and 4 trifluoro methyl analogues exhibited similar activity against C. albicans.
- 12) 3 Fluoro, 2 Fluoro and 4 Fluoro derivatives showed variation in the activity in the descending order against C. albicans. 3 fluoro was most active compared to 2 and 4 fluoro analogues.
- 13) 3 fluoro, 3- trifluoro methyl and 2,3,4, chloro analogues were found to be most active compared to other compounds in the series against C. albicans.
- 14) 2 fluoro and 3 fluoro analogues showed similar activity against T. rubrum and T. mentagrophytes. In the same way 4 fluoro, 2 trifluoro methyl group and 4 trifluoro methyl group exhibited same activity against T. rubrum and T. mentagrophytes.
- 15) 2Cl, 3 Cl, and 3CF₃ groups were more active against T. mentagrophytes than T. rubrum.
- 16) 4 Cl derivatives showed same degree of activity against both T. mentagrophytes and T. rubrum.
- 17) 4F, 3Cl (2,3,4) CF₃ analogues were found to be most active against T. mentagrophytes.
- 18) 4 F, 2 CF₃ and 4CF₃, analogues were highly active against T. rubrum amongst all the compounds in the series.

It may be concluded that the present investigation has resulted in the discovery of new heterocyclic molecules which would selectively act against various Gram-positive, Gram-negative bacteria and fungi that are infecting human beings.

Part III

Synthesis of 1- thiocarbamoyl -3- methyl - 4 (aryl hydrazono) -2- pyrazolin -5-one

The screening results exhibit the activity of the test compounds against the Gram positive and Gram-negative bacteria taken for the test viz, S. aureus and S. typhi. 2-chloro- 6 fluoro(Compound No. 2), 3,5 dichloro 4 methoxy(Compound No. 3), and 2,6 dichloro 4 methoxy(Compound No. 5) analogues have exhibited excellent activity upto 1.56 µg/ml against both S. aureus and S. typhi respectively. 2 fluoro(Compound No. 7) and 4 fluoro(Compound No. 6) derivatives showed good activity upto 20 µg/ml against both S. aureus and S. typhi. 4 chloro- 3 fluoro(Compound No. 9); 6 chloro-2 fluoro(Compound No. 10) analogues also showed good activity upto 20 µg/ml against both S. aureus and S. typhi. 3 fluoro and 2,4, dichloro 6 methoxy(Compound No. 4) analogues showed low activity upto 200 µg/ml against S. aureus and S. typhi.

The screening results also exhibited activity of the test compounds against C. albicans, T. rubrum and T. mentagrophytes.

2,6 dichloro 4 methoxy(Compound No. 5) and 6 chloro- 2 fluoro(Compound No. 10) analogues were found to be highly active. They showed activity upto 1µg/ml for all the three organisms like C. albicans, T. mentagrophytes and T. rubrum. 4 chloro 3 fluoro(Compound No. 9) analogue showed good activity upto 20 µg/ml against all the above three organisms used in antifungal screening 3 chloro 4 fluoro(Compound No. 1) and 2,4 dichloro 6 methoxy analogue exhibited very good activity upto 2 µg/ml against C. albicans, T. rubrum and T. mentagrophytes respectively.

2 chloro 6 fluoro(Compound No. 2), 3,5 dichloro 4 methoxy(Compound No. 3) and 4 fluoro(Compound No. 6) analogues showed same pattern of activity upto 2 µg/ml for C. albicans. All the three fluoro derivatives exhibited same activity upto 1µg/ml for T. mentagrophytes. 4 fluoro(Compound No. 6) and 2 -fluoro(Compound No. 7) derivatives showed same activity for T. rubrum also with an exception of 3 fluoro(Compound No. 8) derivative which showed good activity upto 20 µg/ml for C. albicans and comparatively low activity upto 40 µg/ml for T. rubrum. 4 fluoro(Compound No. 6) derivative was found to be

highly active amongst all the three fluoro derivatives against C. albicans with an activity upto 2 µg/ ml which was followed by comparatively less active 2 fluoro derivative with an activity upto 5µg/ml.

3,5 dichloro 4 methoxy(Compound No. 3) analogue showed same amount of activity upto 5 µg/ml., for both T. mentagrophytes and T. rubrum respectively 2 chloro - 6 fluoro(Compound No. 2) derivative was found to be more active upto 5 µg/ml for T. rubrum compared to 10 µg/ml for T. mentagrophytes.

Conclusions :

In table II the antibacterial/antifungal activities of 1 - Thiocarbamoyl -3- methyl- 4 (aryl hydrazono) -2-2 pyrazolin- 5- one have been described

- 1) This screening was performed with a main aim in mind to see whether there was any change in the activity upon interchanging the positions of the substituents on the phenyl ring.
- 2) While comparing the 3 chloro 4 fluoro and 4 chloro 3 fluoro analogue a remarkable observation was made regarding the difference in activity of the two compounds against all the three organisms used for antifungal screening. The 3 chloro 4 fluoro derivative showed same activity upto 2 µg/ml for all the three organisms viz C. albicans, T. mentagrophytes and T. rubrum. Similarly 4 chloro 3 fluoro analogue showed same activity upto 20 µg/ml for the same three organisms.

Thus chloro group at 3 position is more favourable for activity than chloro group at 4 position in the phenyl ring. Whereas in contrast to that fluoro group at 4 position is more favourable for activity than at 3 position in the phenyl ring. It has been proved that interchanging the positions of the substituents in the above two compounds lead to distinct diversification in the antifungal activity with a wide range of 2 µg/ml - 20 µg/ml

- 3) 2 chloro 6 fluoro derivative showed descending order of activity for C. albicans, T. rubrum and T. mentagrophytes respectively i.e. 2 $\mu\text{g/ml}$ for C. albicans 5 $\mu\text{g/ml}$ for T. rubrum and 10 mg/ml for T. mentagrophytes. On mutually interchanging the positions of the substituents the activity was enhanced upto 1 $\mu\text{g/ml}$ for the same three organisms respectively. Thus fluoro group at position 2 was preferable than at 6 position while chloro group at 6 position was preferable than at 2 position.
- 4) 4 fluoro analogue was found to be most active upto 2 $\mu\text{g/ml}$ for C. albicans, followed by 2 fluoro analogue with an activity of 5 $\mu\text{g/ml}$ 3 fluoro analogue was found to be less active in comparison with the other two. It showed activity upto 20 $\mu\text{g/ml}$ for C. albicans. All the three fluoro derivatives were found to be equally active upto 1 $\mu\text{g/ml}$ respectively for T. mentagrophytes 4 fluoro and 2 fluoro analogue showed same activity upto 1 $\mu\text{g/ml}$ resp. for T. rubrum. But fluoro group when placed at 3 position showed remarkable decrease in activity upto 40 $\mu\text{g/ml}$ for same species i.e. T. rubrum.
- 5) 2,6 dichloro 4 methoxy and 3,5 dichloro 4 methoxy analogues when compared 2,6 dichloro 4 methoxy was found to be more active than the other analogue. It showed same activity upto 1 $\mu\text{g/ml}$ for all the three organisms resp. 3,5 dichloro 4 methoxy derivative was more active against C. albicans upto 2 $\mu\text{g/ml}$ than against T. mentagrophytes and T. rubrum. This derivative showed same activity response upto 5 $\mu\text{g/ml}$ for both T. mentagrophytes and T. rubrum respectively. Thus without changing the position of methoxy group it was proved that substitution of chloro group at both 2 and 6 position was favourable for activity than at 3 and 5 position in the phenyl ring.
- 6) 2,4, dichloro 6 methoxy derivative was less active than the 2,6 dichloro 4 methoxy derivative. Thus shifting of methoxy group from 4 position of phenyl ring to 6 position lead to decrease in activity. Similarly chloro group at 2 and 6 position was preferred over substitution at 2 and 4 position and also 3 and 5 position in the phenyl ring. 2,4 dichloro 6 methoxy derivative showed same degree of activity upto 2 $\mu\text{g/ml}$ for all the three organisms respectively.

In conclusion reports for antibacterial screening against S. aureus and S. typhi the following points are highlighted.

- 1) Except for the 3 chloro 4 fluoro analog all the remaining nine analogues proved to exhibit same degree of activity individually for both S. aureus and S. typhi.
- 2) Compound No. 1 and No. 9 when compared with each other showed that compound No. 1 was more active against both organisms than compound No. 9. Thus shifting of chloro group from 3 position to 4 position of phenyl ring reduces the activity against both the organisms while shifting of fluoro group from 3 to 4 position of phenyl ring enhances the activity against both the organisms.
- 3) Mutually interchanging the positions of the chloro and fluoro substituents in the phenyl ring of compound No 2 and compound No. 10 showed large variation in the activity. 6 position is favourable for fluoro group and 2 position is favourable for chloro group was the ultimate conclusion with wide range of activity between 1.56 µg/ml - 25 µg/ml for both the organisms.
- 4) 3 fluoro derivative was one of the least active derivative compared to 2 and 4 fluoro derivative. The activity showed range between 20-200 µg/ml for both the organisms.
- 5) Compound No. 3 and 5 showed maximum activity upto 1µg/ml against S. aureus and S. typhi. There was no change in activity on interchanging the positions of chloro groups in the phenyl ring.
- 6) Interchanging the position of methoxy group showed change in activity of compound No 3, 4, 5 and 6, methoxy derivative was less active than the 4 methoxy derivative which was highly active. Similarly 2 and 4 substitutions by chloro group was not favourable over 2 and 6 positions in compound no. 5 and 3 and 5 positions in compound no. 3. Thus among dichloro methoxy combinations 2,4, dichloro 6 methoxy derivative was least active against both S. aureus and S. typhi.

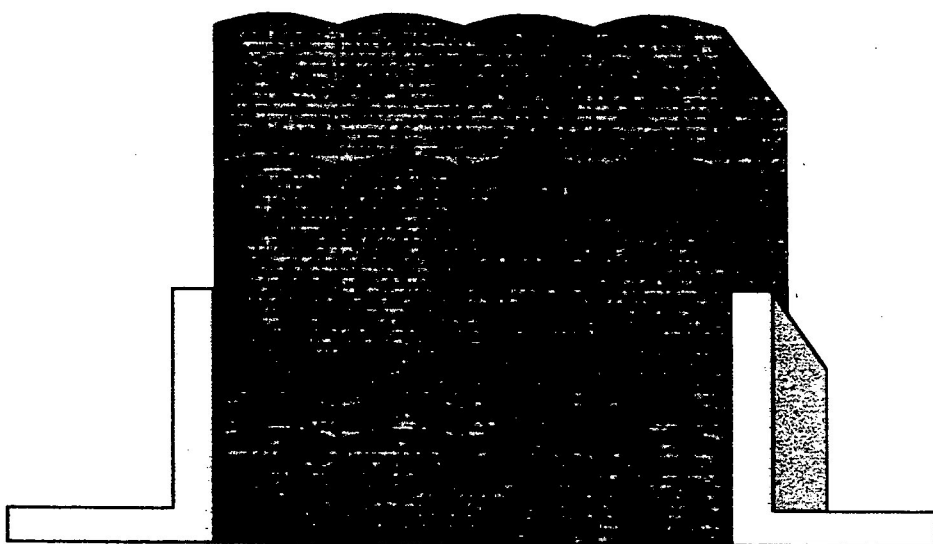
This by mutually interchanging the positions of the substituents in the phenyl ring the antibacterial and antifungal activity of the analogues can be enhanced or decreased.

Synthesis of 1- thiocarbamoyl -3-methyl -4 (aryl-hydrazono) -2-pyrazolin -5-one*

Table II

Sr No		Antibacterial activity ($\mu\text{g/ml}$)		Antifungal activity ($\mu\text{g/ml}$)		
		<u>S. aureus</u>	<u>S. typhi</u>	<u>C. albicans</u>	<u>T. rubrum</u>	<u>T. mentagrophytes</u>
1	3Cl, 4F	10	1	2	2	2
2	2Cl, 6F	1	1	2	10	5
3	3,5 diCl, 4OCH ₃	1	1	2	5	5
4	2,4 diCl, 6OCH ₃	200	200	2	2	2
5	2,6 diCl, 4OCH ₃	1	1	1	1	1
6	4F	20	20	2	1	1
7	2F	20	20	5	1	1
8	3F	200	200	20	40	1
9	3F, 4Cl	20	20	20	20	20
10	2F, 6Cl	20	20	1	1	1

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APPENDIX

APPENDIX

1. The melting points have been determined in Open capillaries in a veego VMP - 1 melting point apparatus.
2. The TLC of the compounds have been run on Eastman Kodak precoated silica gel plates of type K 301 R and E. Merck pre -coated silica gel plates of type 60 F 254.
3. The Infrared spectra was recorded on a BOMEM MB - 104 Fourier Transform Infrared Spectrometer.
4. The PMR spectra was recorded on Brucker AC 80 MHz and 500 MHz Spectrometer and Varian 300 MHz and 600 MHz spectrometer using TMS as internal reference.

SYNOPSIS

**A SYNOPSIS
OF THE THESIS TO BE SUBMITTED TO
THE UNIVERSITY OF MUMBAI
FOR THE DEGREE OF MASTER OF PHARMACY
IN THE FACULTY OF TECHNOLOGY**

TITLE OF THE THESIS :

SYNTHESIS OF ORGANIC COMPOUNDS AS POSSIBLE
ANTIBACTERIAL AND ANTIFUNGAL AGENTS.

NAME OF THE CANDIDATE : Miss SONALI D. WAYANGANKAR

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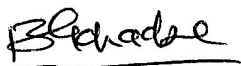
PLACE OF RESEARCH : DEPARTMENT OF CHEMOTHERAPY
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**NUMBER AND DATE OF
REGISTRATION :** 16 / 4th DEC. 1995.

SIGNATURE OF THE STUDENT :



SIGNATURE OF THE GUIDE :



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

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

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

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

The essence of chemotherapy is selective toxicity- to kill or inhibit the microbe without harming the patient. The properties of certain chemicals to destroy one form of life without harming another is the corner stone of modern antimicrobial chemotherapy.

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

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

For many centuries the search for therapeutic substances centered on the investigation of herbs grown in the gardens of religious establishments. In the nineteenth century the advent of analytical and synthetic chemistry signalled an important change in direction. However the true era of medicinal chemistry really began in the 1930's with the preparation and use of the antibacterial sulfonamide drugs. The history of medicine was from that time on to be dominated by medicinal therapeutics. Today, a large number of diseases are cured or at least controlled by drug therapy.

Thus the fight against bacterial and fungal infections has been largely won and significant progress has been made in treating mental, pulmonary, gastrointestinal, inflammatory and cardiovascular conditions.

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 characterised 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, pharmaceutics and medicine.

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

The thesis will consist of the following parts.....

PART-1

Introduction, Review of literature and Plan of Work

PART-2

SYNTHESIS of 2-(2,4- difluoro phenyl)-3-(1 H-1,2,4-triazol-1-yl) propan-2-ol, 1(substituted phenyl) tetrazoles.

PART-3

SYNTHESIS of 1 - Thiocarbamoyl - 3 - methyl - 4 - (aryl hydrazono) - 2 - pyrazolin - 5 - one.

PART-4

SYNTHESIS of Nimesulide (A potent NSAID) by different routes.

PART-5

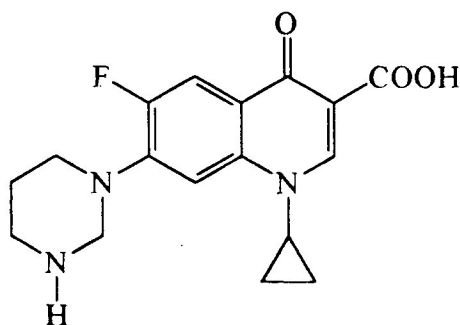
Biological screening.

PART - 2**SYNTHESIS of 2 - (2,4- DIFLUORO PHENYL) - 3 - (1 H-1, 2, 4 - TRIAZOL - 1 - YL) PROPAN - 2 -OL, 1(SUBSTITUTED PHENYL) TETRAZOLES.**

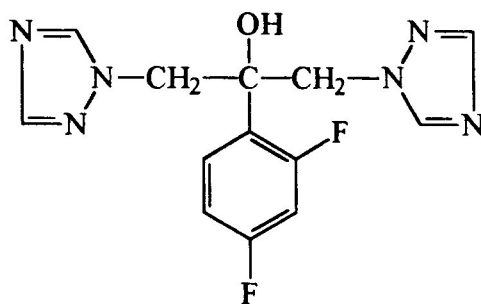
Extensive biological investigations of organic fluorine compounds during the past few decades have been rapidly translated into useful applications in the field of medicinal chemistry¹⁶ The interest in the fluorine containing compounds which hopes to alter the pharmacological properties of drugs for the better is based primarily in the following considerations:-

- 1) Fluorine, the second smallest atom as measured by atomic radius and internuclear distance to carbon would most closely resemble bioactive hydrogen analogues with respect to steric requirements at enzyme receptor sites.
- 2) Fluorine imparts increased oxidative and thermal stability because the binding energy of the carbon-fluorine bond exceeds that of the carbon-hydrogen bond by over 17Kcal/mol.
- 3) Fluorine leads to increased lipid solubility in membranes that would enhance the rate of absorption and in vivo transport. In this regard the trifluoro methyl group is amongst the most lipophilic of all substituents.

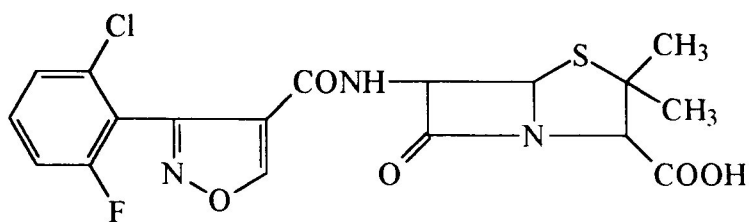
Numerous fluorinated compounds are presently in therapeutic use. viz Ciprofloxacin (antibacterial), Fluconazole (antifungal), Flucloxacillin (antibacterial).



(i)

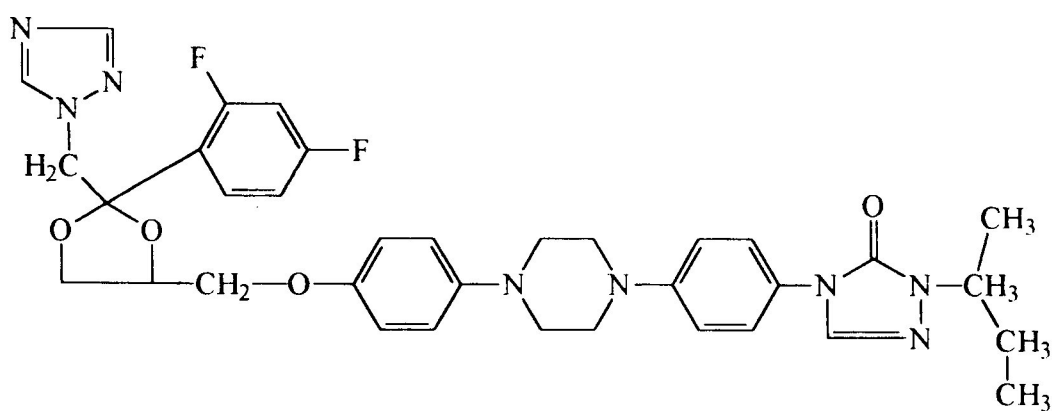


(ii)



(iii)

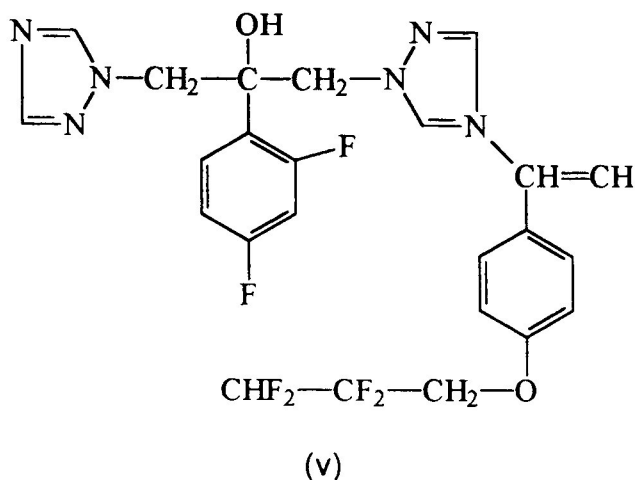
Literature study revealed that the triazole group was less susceptible to metabolic inactivation than an imidazole moiety, but yet it retained the ability to interact with the cytochrome P-450 unit in the fungal C-14 demethylase enzyme. Replacement of imidazole by a 1,2,4-triazol-1-yl unit gave a drug which was twice as potent in the standard murine systemic candidosis model as the corresponding imidazole analogue.



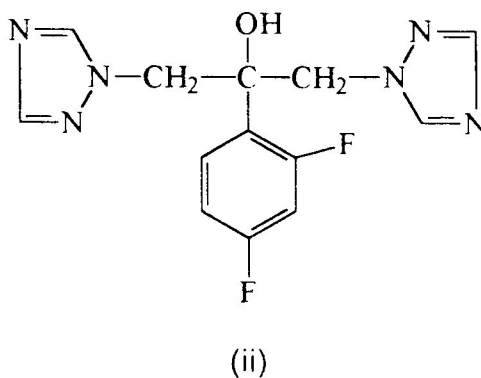
(iv)

Saperconazole is water soluble, lipophilic fluorinated triazole with similar chemical structures to itraconazole, with two chlorine atoms replaced by fluorine. It has a broad spectrum of

antifungal activity including the dimorphic fungi *Aspergillus* species, *Phacohyphomycetes* and *Cryptococcus* species.

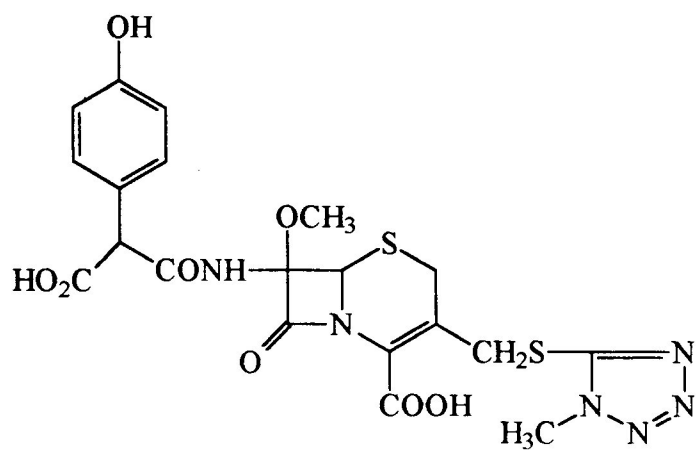


ICI 195739 is an orally active bis-triazole with broad spectrum antifungal activity.

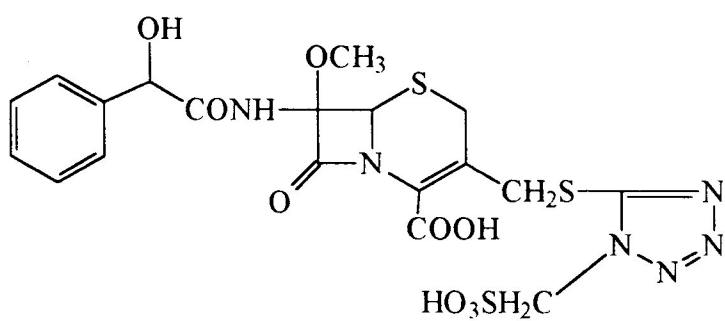


FLUCONAZOLE

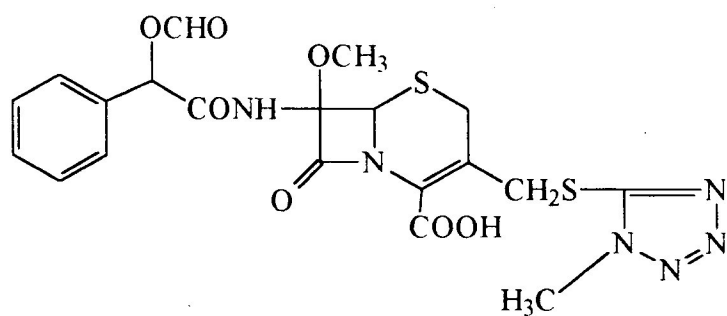
Fluconazole is a safe effective broad spectrum antifungal agent that may be given by both the oral and intravenous routes, in a once daily dosing schedule⁷. The safety appears to be due to fluconazole's remarkable selectivity for the fungal C-14 demethylase, rather than related enzymes present in man.



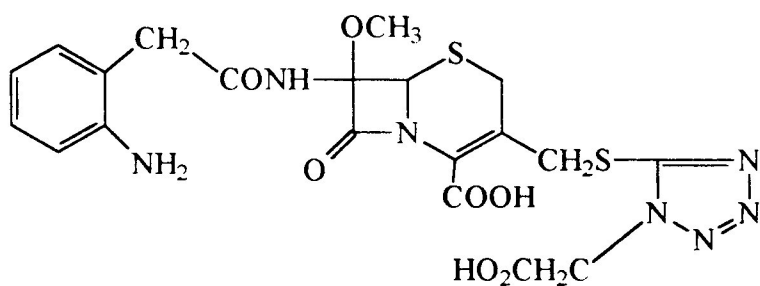
(vi)



(vii)



(viii)



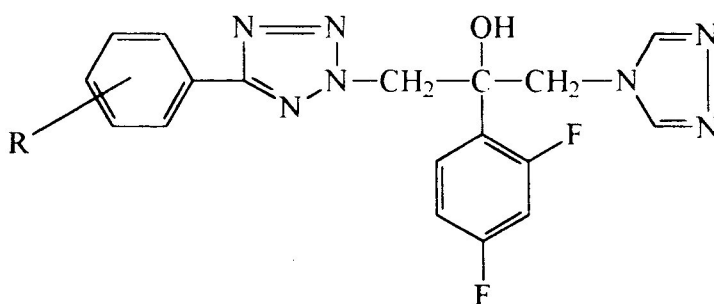
(ix)

Considering the biological importance of fluoro compounds and various bioactive moieties two series of compounds having halogenated substituents such as fluoro, trifluoro and chloro was prepared. Tetrazoles also belong to a category of azoles and are known to possess antibacterial and antifungal activity⁶.

Above mentioned are some of the cephalosporin antibiotics presently used in the United States⁶. These drugs possess the tetrazole nucleus responsible for activity against gram-negative bacilli for e.g Cephmandole, anaerobes for e.g Cefonicid and *Pseudomonas* species for e.g Ceforanide.

Hence an attempt was made to synthesize a combination of 1,2,4 triazole nucleus and a tetrazole nucleus which gave birth to a series of nine new compounds having halogenated substituents on the phenyl ring of the tetrazole moiety. These compounds were synthesized with an aim to obtain enhanced biological activity.

These nine compounds were as follows:



(xix)

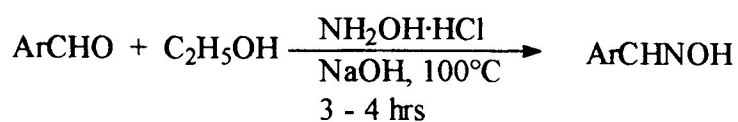
R ; ortho Cl, meta Cl, para Cl

R ; ortho F, meta F, para F

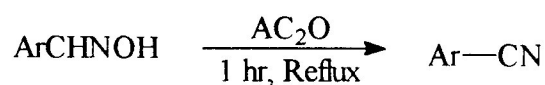
R ; ortho CF₃, meta CF₃, para CF₃

GENERAL SCHEME OF SYNTHESIS OF PART II

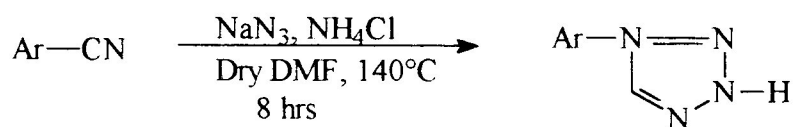
Step I



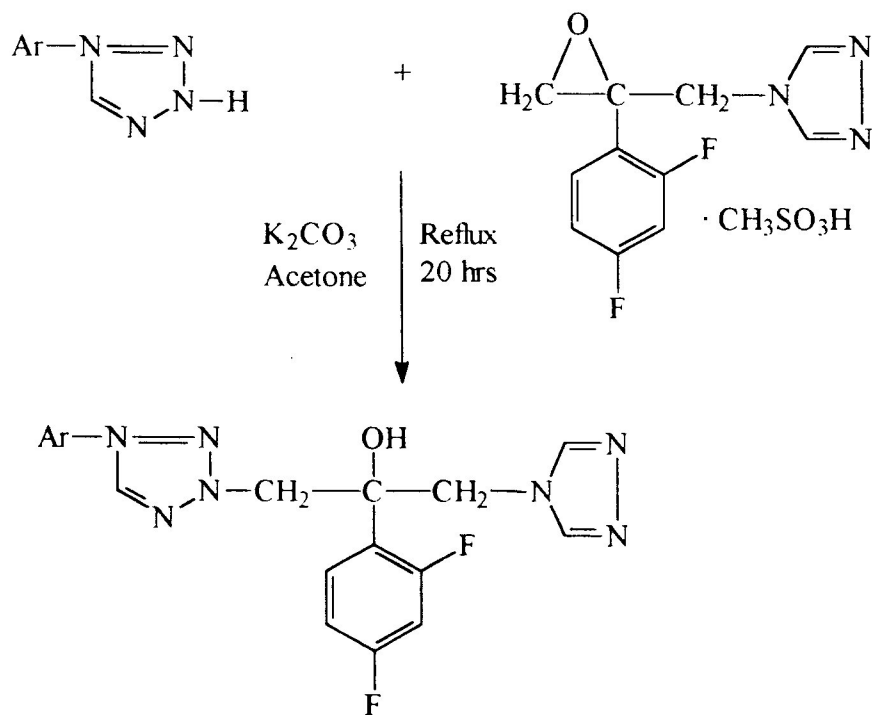
Step II



Step III



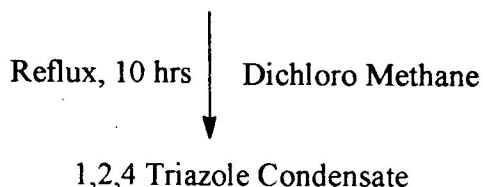
Step IV



SYNTHESIS OF 2nd RAW MATERIAL IN STEP 4

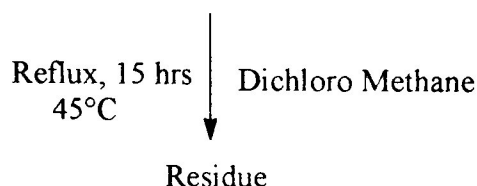
Step I

2, 4 Difluoro Acetophenone + 1,2,4 Triazole

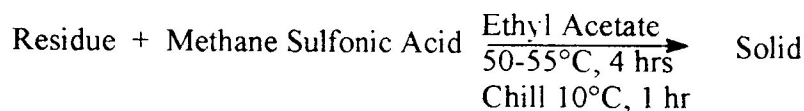


Step II

1,2,4 Triazole Condensate + 40% Caustic Potash Lye
+ Trimethyl Sulfozonium Iodide



Step III



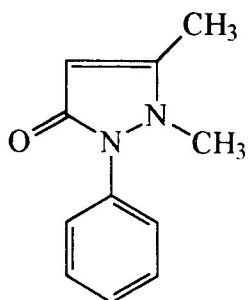
GENERAL STRUCTURE AND PHYSICAL CONSTANTS OF THE DERIVATIVES OF PART 2

	Substituent	m.p ($^{\circ}\text{C}$)	Yield (%)
1	2 fluoro phenyl	215	88
2	3 fluoro phenyl	247	93
3	4 fluoro phenyl	260	91
4	2 trifluoro phenyl	225	88
5	3 trifluoro phenyl	180	94
6	4 trifluoro phenyl	230	87
7	2 chloro phenyl	270	83
8	3 chloro phenyl	240	95
9	4 chloro phenyl	290	80

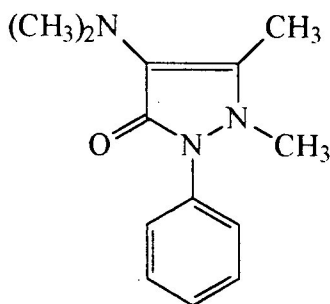
PART 3 :**SYNTHESIS OF 1-THIOCARBAMOYL-3-METHYL-4-(ARYL-HYDRAZONO)-2-PYRAZOLIN-5-ONE.**

The chemistry and wide range of pharmaceutical properties of 2-pyrazoline-5-one derivatives have been cited in literature^{12,15}. In view of potential biological activities of aryl pyrazoles and pyrazolin-5-one it was considered worthwhile to synthesize some of these compounds as possible antibacterial agents^{13,14}.

Both antipyrine and aminopyrine have antirheumatic potencies against rheumatic diseases associated with known infectious bacterial and fungal agents. Both of them possess pyrazolinone nucleus⁶.

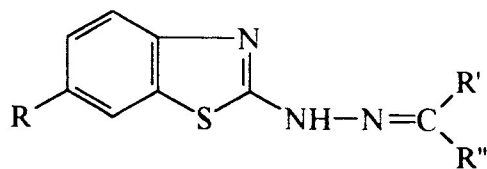


(x)



(xi)

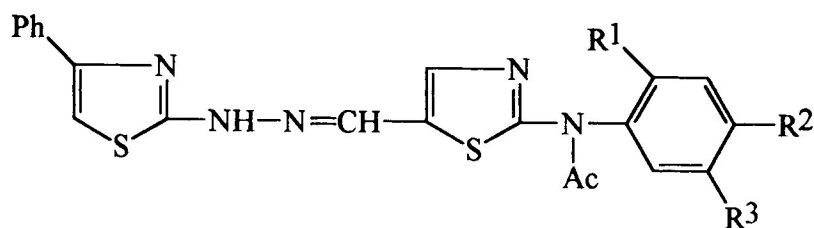
Many of N,N-disubstituted hydrazones of 3-formyl rifamycin SV had high activity both in vitro and in vivo, against gram positive bacteria and moderate activity against gram negative bacteria⁶. The hydrazone of 3-formyl rifamycin SV with N-amino-N'-methyl piperazine (rifampicin) was the most active in vivo and was selected for clinical use. It is active against gram positive bacteria at lower concentrations and against gram negative bacteria at concentrations of 1-20 µg/mL. In 1993 Zhou et al¹⁷ have reported compounds of type which are active against *S. aureus*.



R' = aryl, alkyl
R'' = halo, H

(xvi)

In the same year Likhate et al¹⁸ have reported 2-substituted thiazolyl hydrazones which are active against *Salmonella typhosa*, Gram positive and Gram negative bacteria.

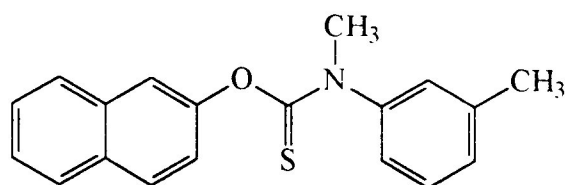


$R', R_3 = H, Cl, R_2 = \text{aryl}$

(xvii)

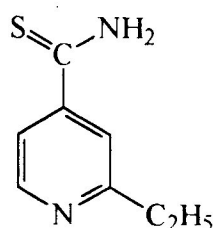
Sulfur containing molecules as antifungal agents:

A variety of organic compounds that contain divalent sulfur attached to carbon atom gave invitro fungistatic action and many of these have been employed in topical therapy of dermatophytoses.



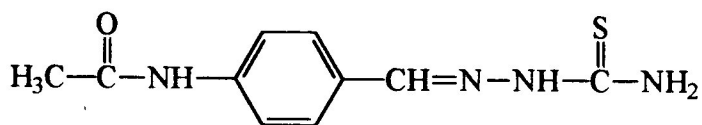
(xii)

Tolnaftate is effective against candidal lesions. The presence of thioamide linkage in ethionamide is responsible for antimycobacterial activity.



(xiii)

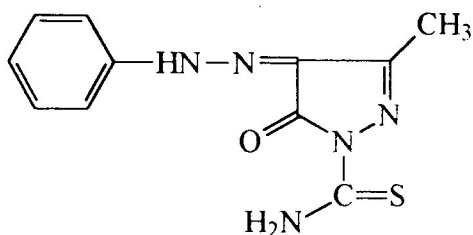
Amithiozone(xiv) is also one compound which possesses thioamide linkage responsible for antimycobacterial activity.



(xiv)

Hence, a series of ten compounds possessing the pyrazolinone ring, the hydrazono linkage as well as the thioamide linkage were prepared and tested for their antibacterial and antifungal activities. Compounds were tested to see whether any remarkable difference was observed in activity on interchanging the positions of the substituents on the phenyl ring and whether the thioamide linkage contributed to some bacteriostatic activity.

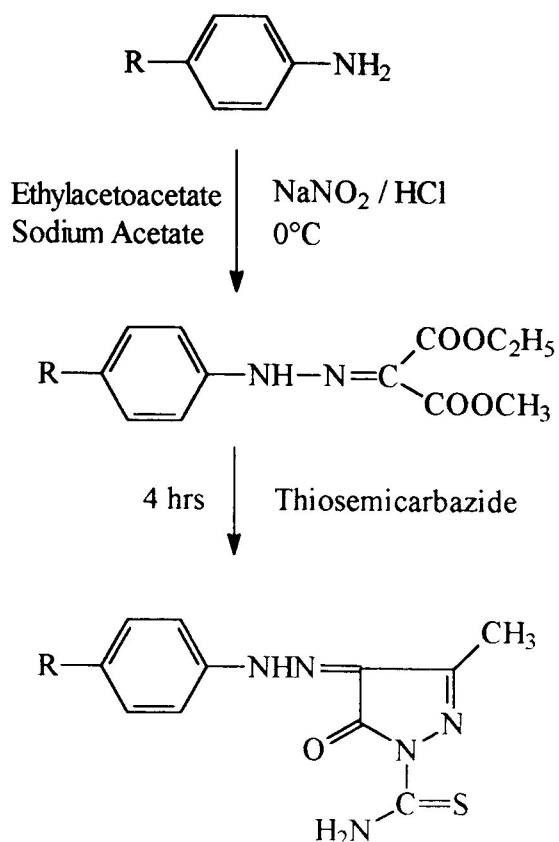
The following series (xviii) of compounds were prepared:



(xviii)

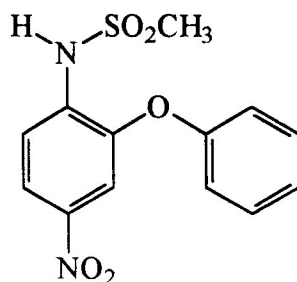
- 1) R : 3Cl ; 4F
- 2) R : 2Cl ; 6F
- 3) R : 3Cl ; 4OCH₃ ; 5Cl
- 4) R : 2Cl ; 6OCH₃ ; 4Cl
- 5) R : 2Cl ; 4OCH₃ ; 6Cl
- 6) R : 2F
- 7) R : 3F
- 8) R : 4F
- 9) R : 4Cl ; 3F
- 10) R : 6Cl ; 2F

SCHEME OF SYNTHESIS OF PART 3



PHYSICAL CONSTANTS OF THE DERIVATIVES IN PART 3

SUBSTITUENT(R)	m.p.($^\circ C$)	YIELD(%)
3 chloro,4 fluoro	230	66
2 chloro, 6 fluoro	215	71
3,5 dichloro 4 methoxy	210	72
2,4 dichloro 6 methoxy	240	64
2,6 dichloro 4 methoxy	230	67
4 fluoro	235	80
2 fluoro	205	79
3 fluoro	195	76
4chloro, 3 fluoro	225	73
6 chloro, 2 fluoro	205	81

PART 4 :**SYNTHESIS OF NIMESULIDE [A POTENT NON STEROIDAL ANTIINFLAMMATORY DRUG (NSAID)] BY DIFFERENT ROUTES.**

(xv)

Nimesulide is a non steroidal antiinflammatory drug (NSAID) that selectively inhibits cyclooxygenase², the inducible form of the enzyme found almost exclusively in inflammatory tissues but largely devoid of activity in healthy tissues, in which cyclooxygenase¹ plays important regulating roles^{10,11}.

Since Nimesulide is the leading molecule of a new class, the sulphonanilides, this drug was chosen for synthesis^{8,9} due to the favourability of following reasons :

- 1) It is remarkable safe NSAID, very effective as treatment for painful and inflammatory processes.
- 2) On the basis of an index score of severity, nimesulide showed a less ulcerogenic trend than diclofenac.
- 3) The hepatic toxicity profile of nimesulide was shown to be safer than that of other commonly used NSAIDs.
- 4) The incidence of adverse reactions was 8.9% with nimesulide and 16.7% with reference drugs such as diclofenac, Ibuprofen etc.
- 5) Nimesulide shows greater potency and a longer duration of action than paracetamol.

Since the efficacy of Nimesulide in the treatment of various conditions associated with the inflammatory process reflects its novel spectrum of action this drug was chosen for synthesis.

Nimesulide was prepared by the following two routes:

Route 1:

Condensation of ortho chloro nitrobenzene with phenol gave nitro diphenyl ether, which was reduced to amino diphenyl ether which on further condensation with methane sulfonyl chloride gave 2 phenoxy methane sulfonanilide, which on nitration gave the desired drug.

Route 2 :

Condensation of ortho chloro 4 nitro aniline with phenol gave amino 4 nitro diphenyl ether which on further condensation with methane sulfonyl chloride gave the final drug.

Though Route 2 involves less number of steps in the synthesis as compared to Route 1, Route 1 was preferred because it gave better yields in less duration .

The synthesis via Route 2 in comparison with route 1 was much more time consuming with lower yields. The formation of amino 4 nitro diphenyl ether took 35 to 40 hours due to the steric hindrance of amino group at position 1.

Raw materials required for route 1 were readily and easily available as compared to those required for route 2.

As all the factors favoured route 1, the same route was employed for the synthesis of Nimesulide.

PART 5 BIOLOGICAL SCREENING

The compounds reported herein have been subjected to elemental and spectroscopic analysis, and the results were found to be satisfactory.

The compounds reported in parts 2&3 have been screened for their in vitro antibacterial and antifungal activities.

ANTIBACTERIAL SCREENING

The compounds described in parts 2 & 3 were also subjected to in vitro tests against *S. aureus* and *S. typhi*. The culture medium employed in the screening was Mueller-Hinton broth. Six compounds have exhibited good activity i.e between 12.5 µg/ml - 25µg/ml for *S. aureus* and 25µg/ml - 50µg/ml for *S. typhi*.

The standard drugs used for antibacterial screening were AMPICILLIN against *S. aureus* and TRIMETHOPRIM against *S. typhi*.

The standard dose of AMPICILLIN is 0.01µg/ml and that of TRIMETHOPRIM is 1µg/ml.

ANTIFUNGAL SCREENING

The compounds have also been screened for their antifungal activity in vitro by the Tube Dilution technique against fungal strains viz *Candida albicans*, *T. rubrum* and *T. mentagrophytes*. Nine compounds showed very good activity i.e between 12.5 µg/ml - 25µg/ml against *T. mentagrophytes* and *T. rubrum*. These compounds showed excellent activity i.e between 1.56µg/ml - 3.12µg/ml against *C. albicans*. The standard drug used was MICONAZOLE for antifungal screening. The standard dose of MICONAZOLE is 6.25µg/ml for *C. albicans*, 1.56µg/ml for *T. mentagrophytes* and 1.56µg/ml for *T. rubrum*.

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