

PREPARATION, SPECTROSCOPIC
CHARACTERISATION AND EVALUATION OF
POSSIBLE BIOLOGICALLY ACTIVE COMPOUNDS

THESIS SUBMITTED TO
UNIVERSITY OF BOMBAY

FOR THE DEGREE OF
MASTER OF PHARMACY
IN
PHARMACOLOGY

SUBMITTED BY
TUSHAR N LOKHANDE

UNDER THE GUIDANCE OF
DR. B.G. KHADSE

DEPARTMENT OF PHARMACOLOGY
HAFFKINE INSTITUTE FOR TRAINING, RESEARCH & TESTING
BOMBAY

Disclaimer

This research work belongs to

Mr. Lokhande Tushar N.

No content of this work can be
circulated or used for any commercial
purpose without the consent of
respective researcher

**PREPARATION, SPECTROSCOPIC
CHARACTERISATION AND EVALUATION OF
POSSIBLE BIOLOGICALLY ACTIVE COMPOUNDS**

**A
Thesis Submitted
To The
University Of Mumbai
For The Degree of
Master of Pharmaceutical Sciences
In
The Faculty Of Technology
(Pharmaceutical Chemistry)**

**By
Mr. Lokhande Tushar N.
B.Pharm.**

HAFFKINE INSTITUTE LIBRARY

Call No.

Acc No.

Price

Source

Donated

**DEPARTMENT OF CHEMOTHERAPY
HAFFKINE INSTITUTE FOR TRAINING, RESEARCH AND TESTING
A.D. MARG, PAREL, MUMBAI – 400 012**

2001

Acknowledgements

It is my pleasure to acknowledge the help and support of my research guide Dr. B.G. Khadse, M.Sc., Ph.D., Ex-Director, Haffkine Institute. He has been a constant source of encouragement during the entire period of my project. His able guidance coupled with valuable and timely suggestions during the entire course of this study, have been largely responsible for structuring this work and steering it clear through thick and thin. To him, I would remain truly indebted for life.

I take this opportunity to sincerely thank Dr. (Ms.) M.S. Phadke, Director, Haffkine Institute and Director of Medical Education and Research, Maharashtra, for smooth progression of my research activities.

I am thankful to Dr. S. S. Mahajani, Officer-In-Charge Training and Dr. M.G. Phatak, Head, Department of Chemotherapy for providing timely help.

It is with great pleasure, that I take this opportunity to thank Dr. Anil Bobade, Department of Chemotherapy, who has always extended moral support and timely insights during the tenure of this work.

My very special thanks to Dr. A.Y. Ambaye and Ms. Bharati Nadkarni for reviewing the manuscript and for valuable discussions, which helped me to present the work in this form.

I am thankful to Dr. S. V. Gadre, Ex-Asst. Director, Department of Bacteriology for progression of many difficult works and for suggestions in carrying out antimicrobial screening.

My very special thanks to Ms. P.A. Kirtikar, Mr. Pramod Andhere for carrying out the elemental analysis, Mr. K.R. Pawar and Ms. Meera Ramaya for carrying out the microbiological screening and Dr. Ravi Rayanade and Mr. S.P. Gagbhiye for suggestions regarding antimicrobial screening.

I am thankful to my labmates Mr. Sandeep Paranjape, Mr. Hemant Narkhede, Mr. Ravi Chambhare, Mr. Sagar Agnihotri, Mr. Sunil Narkhede, Ms. Sharvani Vingkar, Ms. Mukta Bohra for providing timely help and suggestions.

I am thankful to my colleagues and friends Mr. Chandrakant Bonde, Mr. Yogesh Kulkarni, Mr. Vinod Rane and Ms. Jaya John for providing soothing and pleasant environment. I will always cherish the time spent with them.

Special thanks are also due to Dr. Vilas Lokhande, Dr. Arvind Manian, Dr. T. Arulmoli, Dr. Suryakant Deshmukhi, Dr. Ashwini Chaudhari and Dr. Rajesh Oswal.

I take this opportunity to express my thankfulness towards several colleagues from other departments of the institute like Sameer, Rajendra, Mahendra, Viraj, Vandana, Rucha, Meghna, Severena, Jayshree, Suprabha, Sarita, Priti, Smita, Sunita, Yogini Leena, Vikrant and Suchita.

I am very much thankful to Dr. Arvind Manian, Mr. Umesh Mali for the great help in interpreting NMR spectra. I thank the RSIC Centre, IIT, Powai, for prompt assistance in providing NMR spectra.

I extend my appreciation to Mrs. M.V. Joshi and Mr. V.D. Mhapsekar and entire staff of the library for their everhelping nature.

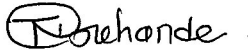
I must thank my relatives Mr. C.P. Mahajan, Prof. S.P. Chaudhari, Mr. P.E. Narkhede, Mr. Bindeshi Patil, Mr. Narendra Patil, Dr. Kishor Patil, Mr. Pramod Patil, Mr. Pravin Patil, Mr. Prabhakar Lokhande, Mr. Prashant Dhande and Mr. Yurvaraj Dhande for fruitful guidance and timely help

I gratefully acknowledge Mr. Manish Sheth for typing and formatting my thesis.

I am indebted to my parents, grandmother and late grandfather whose inspiration made me carry out this work. Love and affection of my brothers Rajendra and Chetan carries a special meaning for me, I cannot express in words the faith and trust put in me by my family whose encouragement and support have made this thesis see the light of day.

Last but not the least, I must thank God, the supreme power of the Universe for providing help in my most difficult times.

*Haffkine Institute
Mumbai 400012*


Tushar N. Lokhande

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.

Statement submitted under rule 0.413

Statement I :

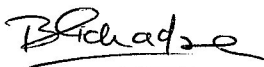
The work embodied in the Thesis to be submitted is original, based on the discovery of new facts, viz. the synthesis and characterization of new and unknown compounds of possible chemotherapeutic value. Also included in the Thesis, are new relations of facts, observed on the basis of in vitro testing.

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
Research Guide



Lokhande Tushar N.
Research Student

CONTENTS

	Page No
Part IA	
Introduction and Review of Literature	1-32
Part IB	
Plan of Work	33-42
Part IC	
Chemistry of	
a) Maleimides	43-50
b) Succinimides	51-57
c) Reduction of Nitro compounds	58-62
d) N-Nitrosation of amines	63-64
e) Acetylation of amines	65-67
Part ID	
Schematic Representation of series of compounds synthesized and drug intermediate.	68-72
Part II	
Synthesis of 4[4'-(4"-chlorophenoxy)-3'-chloroanilino]-N-(alkyl/aryl/substituted aryl and heteroaryl) succinimides	73-81
Part III	
Synthesis of 4-(pyrrolidino anilino)-N-((aryl/substituted aryl and heteroaryl) succinimides	82-84
Part IV	
A. Drug intermediate	85-86
Preparation of 1-acetyl-4-nitroso piperazinyI as a drug intermediate for N-(4-acetyl-1-piperazinyI)-4-fluorobenzamide monohydrate (FK-960).	
B. Spectroscopic characterization	87-91
Part V	
Biological screening, Results and Discussions.	92-101
Conclusion	102
References	103-111
Appendix	112
Synopsis	

Part IA

***Introduction
and
Review of Literature***

Part IA

INTRODUCTION

The use of organic transformations for the preparation of new compounds with more or less complex structures has had a profound influence on both organic chemistry and on modern civilization.

Medicinal chemistry covers three critical steps¹

- A discovery step, consisting of identification and production of new active substances, usually called lead compounds. The 'lead' compounds can originate from synthetic organic chemistry, from natural sources or from biotechnological processes.
- An optimization step, dealing mainly with the synthetic modification of the lead structure in order to improve potency, selectivity and to lessen toxicity.
- A development stage, consisting of optimization of the synthetic route for bulk products, and the modification of the pharmacokinetic and pharmaceutical properties of the active substance, to render it suitable for clinical use. This process can involve the preparation of chemical formulations that are better absorbed, or are more water soluble, or have sustained release properties.

Most of today's marketed medicines were found by chance or by systemic screening of large collections of compounds, either from natural sources or were man-made.

It is true that drug design is becoming more rational, but it has not yet eliminated the role of chance for the hard-working, mentally alert bench chemists. The vast majority of drugs still on the market were developed by a mixture of rational design, trial and error, hard graft and pure luck².

Frequently, the development of drugs has been based on 'ringing the changes' watching the literature, to see what works on related compounds and what doesn't and then trying out similar alterations to one's own work².

Even when drug design is followed on rational lines, good fortune often has a role to play. The development of the beta blocker, propranolol, was aided by such a slice of good fortune².

Current emphasis on high thorough screening and combinatorial chemistry approaches suggests that for the discovery of tomorrow's drugs "educates" chance will remain pivotal. In the pharmaceutical arena, the primary role for computational efforts has been in the discovery phase of drug development³, usually focused on *in vitro* testing. Reduction of screening costs or of the time required to identify a candidate are important goals of computer aided molecular design⁴.

Computer assisted molecular modeling (CMM) is relatively a new and rapidly developing tool in drug design. Computer graphics techniques allow the transformation of complex data sets obtained, (e.g. from theoretical or chemical calculations or X-ray diffraction patterns), into a picture on a computer screen. Chemical structures, whether small ligands or macromolecule, and their properties may thus be visualised, manipulated and matched or combined with other relevant molecules. Thus, the perspective of conventional molecular models, such as Dreiding or creatinine phosphokinase (CPK), has been dramatically expanded. In addition, the ever-increasing rate at which the atomic coordinates or soluble biomacromolecules such as enzymes are being elucidated and subsequently deposited in a publicly accessible form has indeed fueled structure-based drug design. The availability of the three-dimensional architecture of HIV protease, thymidylate synthase, thrombin, carbonic anhydrase and many more has led to at least an equal number of new chemical entities being evaluated and used in the clinic now. In such cases, CMM has certainly led to a more rational approach towards drug design, in particular when the binding domains for inhibitors and substrates are known from cocrystallization experiments. Thus, knowing the "Lock" definitely helps in designing the "key", although the interaction between newly synthesized ligands and target macromolecules may appear unexpected⁵.

There are several basic types of searching and discovery methods. Database-searching methods, such as DOCK, a program developed at the University of

California, San Francisco, identify molecules that are complementary in shape and chemical interactions to a user-supplied receptor structure⁶.

Three dimensional substructure searches (pharmacophore searches) find molecules that include selected functional groups or types of groups in specified geometric relations⁷.

Similarly searches rank database molecules by the number of features they have in common with a query⁸. In addition to these searching procedures, interaction site-based modeling^{9,10} and de novo compound design are becoming increasingly popular¹¹. Aligned with these searching and discovery methods are the more statistically derived methods, such as two-dimensional(2D) and three dimensional quantitative structure activity relationships (3D-QSAR), which attempt to correlate observed biological response with some calculable molecular properties. However, random synthesis might turn out to succeed where molecular modeling has to win its spurs.

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

- a) Identification of the lead compound
- b) Optimization of the lead compound
- c) Evaluation of delivery and metabolic issues
- d) Optimization of synthesis, and
- e) Animal and clinical testing

This research venture aims to hit upon some potent molecules effective against tuberculosis, bacterial infections and/or fungal infections. Hence, a literature survey of the infections and the existing compounds of these categories is included.

Review of Literature

Tuberculosis

“Cholera, bubonic plague, malaria and other diseases take with them hundreds and even thousands. But tuberculosis claims its victims in all civilized countries, in tens of thousands,” said Robert Koch, Father of bacteriology. True to this conviction tuberculosis continues unabated.

India harbours one-third of all tuberculosis(TB) cases in the World and surveys have shown that one Indian dies of TB every minute. Almost 600,000 patients die every year in the country, although TB today is wholly curable. Lack of awareness is one of the main reasons for the death¹².

According to Sudipto Roy of the Indian Medical Association “Nearly half the adult population in India today are victims of tuberculosis”. At the moment, there are around 14 million TB patients in the country, with three million new cases being added each year. “Half of all tuberculosis cases in India occur in the age group of 15-44 years, which are economically productive years. The prevention of TB deaths and effective control of this disease can lead to an estimated economic benefit of Rs. 2400 crores, which can have a significant impact on our economic progress” says an Indian Medical Association Survey. The TB epidemic is growing larger and more dangerous each year. The main cause for worry is the multidrug resistant form of tuberculosis (MDR TB), which shows very high mortality¹².

Preliminary reports of WHO from New Delhi have shown the incidence of MDR TB to be as high as 13 % of all TB cases. Since the MDR TB microbe is infectious and virulent, the proportion of MDR TB may increase substantially in the next few years. It is estimated that a single case of infectious TB spreads infection to 15-20 people in a year, according to Indian Medical Association sources¹².

Another problem with new strains of TB is the close nexus with HIV infection. The presence of HIV infection increases the chances of developing active TB from 5 percent to 50% during a lifetime, in a person who has got primary tuberculosis infection. Last year, about 10 percent of all TB cases worldwide were HIV associated, and this is expected to increase to increase to 15 percent by next year¹².

The National Tuberculosis Programme (NTP) was introduced in India for the first time in the year 1962. The principal objectives of the NTP are applicable throughout the country and they are largely to try and reduce the disease of tuberculosis. Among numerous diseases covered in the National Health Programme, Tuberculosis is the most prevalent, but it receives the lowest allocation¹³.

The WHO suggests that at least 5.6 million persons worldwide, mostly in the developing countries, carry dual infection, and more than 1.4 million cases of HIV related tuberculosis will occur by the year 2000¹⁴.

In India, as per reports nearly 50,000 persons die of tuberculosis, annually. The number of patients at any given time has been estimated to be atleast 9-10 million, of which one fourth (2-2.5 million) are likely to bear the infectious type¹⁵.

Information as well as education pertaining to the tuberculosis is extremely necessary, so that people are able to realize its seriousness and combat the disease in its initial stage.

Apart from *M. tuberculosis*, which is the principle mycobacteria responsible for causing tuberculosis, other causative mycobacteria include *M. bovis* and *M. africanum*. While *M. tuberculosis* is generally transmitted through airborne droplets dispersed by patients with infectious pulmonary or laryngeal disease, *M. bovis* is transmitted through milk from diseased cows.

Mycobacterial and other common bacterial infections differ in their host-parasite relationship. The common bacteria multiply rapidly and induce a quick, defensive response from the host. If the defence mechanism of the host is adequate to deal with the invasion, then the bacteria are eliminated, but if not, then the bacteria emerge victorious by overwhelming the host. In either case, the process is usually of short duration and the outcome of the contest is decided in a short time.

Mycobacterial infections such as tuberculosis, are chronic diseases which do not elicit a rapid response from the host. After initial infection and multiplication, the

bacilli may lie dormant for a long time without giving rise to any clinical symptoms. Often, however, the bacilli may be reactivated due to malnutrition, immunosuppressive drugs and diseases, including HIV infection. As the human system is endowed with a relatively high resistance through years of natural selection, particularly in our urban communities, the effect of this invasion by a slow growing but persistent organism, is to prolong the struggle, thus making the disease chronic.

Tuberculosis is characterized by the formation of nodular bodies or tubercles in the tissues, from which the disease derives its name. In most cases, the primary area of infection is the lungs, but other vulnerable areas include lymph nodes, kidneys, bones, the genital tract, the brain and the meninges. In case of infections of the lungs, the lesions often liquify and drain into the bronchial tree, giving rise to a persistent cough accompanied by sputum.

Tuberculosis causes extensive tissue destruction and virulent bacilli reside in the cavities and debris of the tissue, thus being relatively immune to any drug action. The tubercle formation, although serving a useful purpose of walling off the infection and preventing it from spreading, also protects walled off colonies from chemotherapeutic attack.

Also, tubercle bacilli are vulnerable to bactericidal drugs only when they are metabolically active and replicating. Here, the problem lies in the fact that a small subpopulation of the bacilli can remain dormant indefinitely, except for short periods, when they become transiently active, thus evading chemotherapeutic agents. Another difficulty is in the existence of drug resistant mutants in populations of bacilli hitherto unexposed to antibiotics.

A truly effective drug should, therefore, be capable of penetrating the tubercle and the debris, enter the phagocytes within which the bacilli are growing and finally destroy them. It should possess potent bactericidal/sterilizing activity against metabolically active and semidormant bacilli, while also preventing the emergence of resistant strains throughout the normally prolonged periods of treatment.

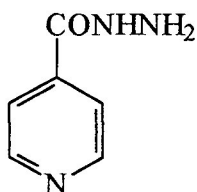
There is no known drug individually self-sufficient, possessing all the listed properties in its entirety. Hence, a combination of two or more drugs is used for the treatment, which may stretch to several months before a reasonable cure is effected.

Five drugs are currently considered essential in the control of tuberculosis- Isoniazid, Rifampicin, Streptomycin, Ethambutol and Pyrazinamide. Thiacetazone too is used widely to supplement Isoniazid in many developing countries due to its low cost. Other drugs of value in treating patients with multiple-drug-resistant disease include p-amino salicylic acid (PAS), Kanamycin, Cycloserine, Capreomycin, Viomycin and Ethionamide, but generally they are more toxic and expensive.

A review of all the known antitubercular agents would be voluminous and are in any case well recorded in other sources of information. Hence, a brief description of the essential drugs used for the control of the disease would be within the scope of this work.

Isoniazid (INH)

Isoniazid (I), the hydrazide of isonicotinic acid, was first described in 1912 but its action against the tubercle bacilli was only discovered in 1952. It is unrivalled in its bactericidal action and ensures the destruction of the bacilli within a few days of starting treatment. It is highly active against replicating tubercle bacilli but does not affect the semidormant species¹⁶ which resume multiplication after removal of contact with the drug.



(I)

INH is rapidly absorbed when administered either orally or parenterally and diffuses readily into all fluids and tissues. INH is a member of all antitubercular chemotherapeutic combinations currently recommended by the World Health Organisation (W.H.O.)

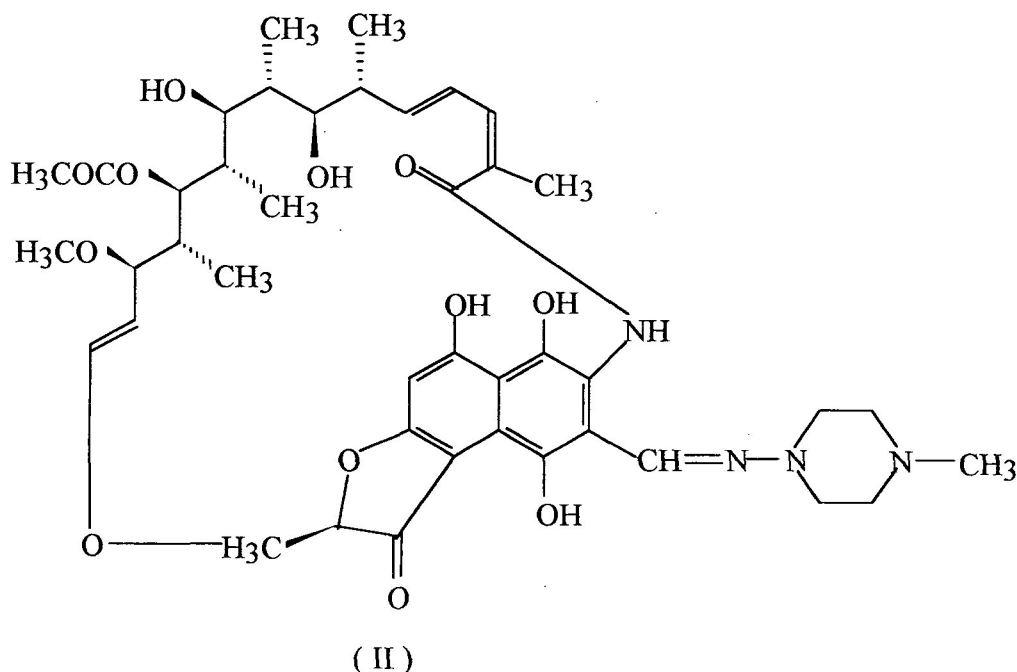
INH is generally well tolerated at the recommended dose, which is approximately 5 mg/kg (maximum 300 mg) daily, for adults and children. The most important side

effect of the drug is related to its toxicity, especially to the peripheral and central nervous system. Peripheral neuritis is the most common side reaction and is said to occur in 17% of the patients receiving 6mg/kg of Isoniazid daily. This can be prevented by administering 100mg of Pyridoxine, a naturally occurring form of Vitamin B₆.

Hepatitis is a not so common, but nevertheless a serious side effect associated with INH therapy. This is mostly prevented by a prompt withdrawal of treatment. A sharp rise in the serum concentrations of hepatic transaminases at the onset of treatment is usually not clinically significant. If the enzyme levels normalise on suspension of dosage then there is less chance of them rising once again when treatment is resumed.

Rifampicin

It is a semi-synthetic derivative of Rifamycin, a complex antibiotic which inhibits the synthesis of ribonucleic acid (RNA) in a broad range of microbial pathogens. It exhibits potent bactericidal and sterilizing action against tubercle bacilli, in both cellular and extra-cellular locations.



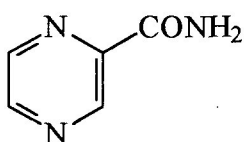
The effectiveness of Rifampicin(II) in the treatment of patients with drug-resistant tuberculosis was considered 'extraordinary' when it was first introduced¹⁷.

Rifampicin is believed to inhibit the synthesis of RNA by inactivating the deoxyribonucleic acid (DNA) dependent RNA polymerase, thus terminating the process of bacterial gene expression. Since resistance to Rifampicin develops rapidly, it is always administered in combination with other effective antimycobacterial agents.

As absorption of the drug is reduced when taken with food, it is preferably given about 30 minutes before meals at a dosage of 10 mg/kg (max. 600 mg) daily in both adults and children. It is usually well tolerated at recommended doses, although there are reports of severe gastrointestinal disturbances. Other adverse effects include skin rashes, fever and influenza-like syndrome.

Pyrazinamide

Pyrazinamide (III) is a synthetic pyrazine analogue of nicotinamide and was successfully used against human tuberculosis in 1952¹⁸. It is only weakly bactericidal against *M. tuberculosis* but has a potent sterilizing activity on organisms that exhibit slow growth owing to an acidic pH of the environment, such as inside macrophages. Thus, it is able to destroy tubercle bacilli which is otherwise left relatively untouched by other drugs. It is highly effective during the first two months of treatment and is administered at a dosage of 30 mg/kg daily, in both adults and children.



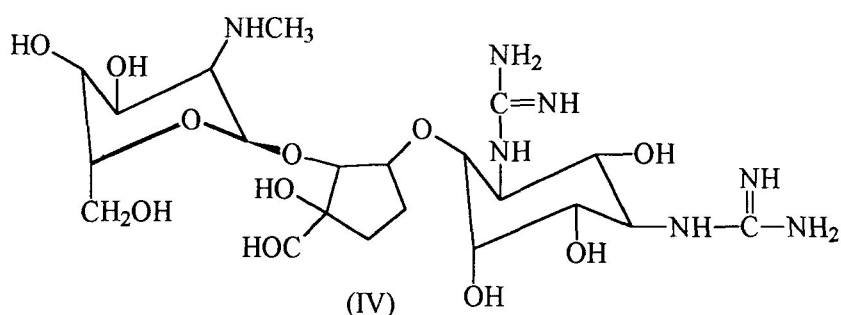
(III)

It is usually well tolerated but there have been complaints of slight flushing of the skin. An excessive exposure of the skin to sunshine may occasionally cause a reddish brown colouration of the exposed areas resembling sunburn. Other adverse effects are pain in the joints and sometimes attacks of gout, necessitating treatment.

Streptomycin

The antitubercular action of Streptomycin (IV), an antibiotic isolated from a culture of *Streptomyces griseus*, was first demonstrated by Schatz and Waksman in 1944. It is not absorbed from the intestinal tract, but after intramuscular administration it

diffuses readily into most body tissues and attains bactericidal concentrations in tuberculous cavities. This drug is of particular use when primary resistance to other drugs is suspected.

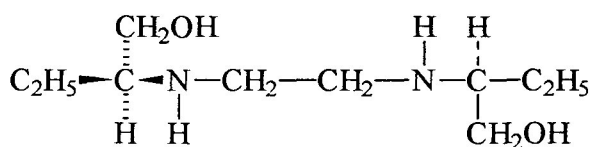


The normal dosage for adults and children is 15 mg/kg daily, while in patients over 60 years of age it should be used with caution. In case of children too, it is preferably avoided since the injections are painful and may also lead to irreversible auditory nerve damage. It should also not be used in pregnancy, as it crosses the placenta thus causing the same effect in the foetus.

Hypersensitivity reactions due to the use of Streptomycin are common and include severe vestibular damage. This was a high risk factor earlier but is uncommon with the currently recommended dosages. If there are complaints of headache, vomiting or vertigo, the dosage is reduced.

Ethambutol

Ethambutol (V) has been classified as a bacteriostatic drug¹⁹. It has been developed as a synthetic congener of related ethanediamines and is bactericidal against *M. tuberculosis*, *M. bovis* and some non-specific mycobacteria. It is used in combination with other antitubercular drugs to prevent or delay the emergence of resistant strains. Approximately 75 % of the strains of the human type are sensitive to Ethambutol at a concentration of 1 µg/ml. Bacterial resistance to the drug develops *in vivo* when it is given alone.



(V)

It is administered orally and is readily absorbed from the gastrointestinal tract. The normal dosage for adults and children ranges from 15-25 mg/kg daily to 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.

Ethambutol can bring about dose-dependent optic neuritis, readily resulting in impairment of visual acuity and color vision. If detected early, this effect is usually reversible, but blindness can occur if treatment is not discontinued promptly. Patients who are too young or who are otherwise unable to comprehend early should not receive this drug.

OTHER BACTERIAL INFECTIONS

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

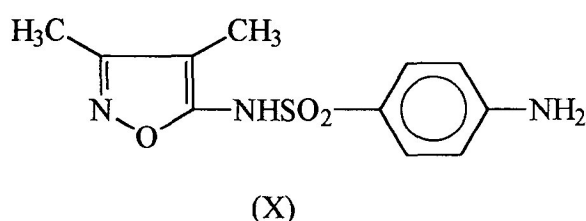
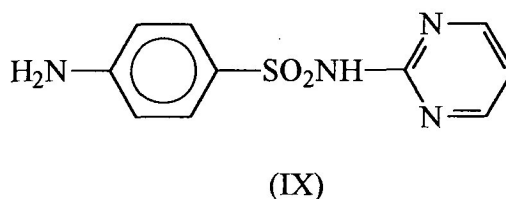
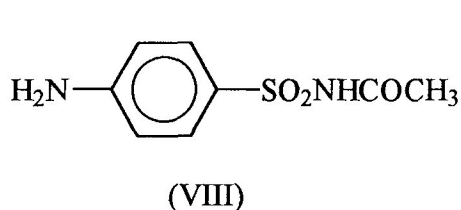
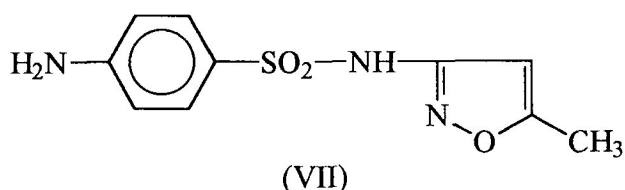
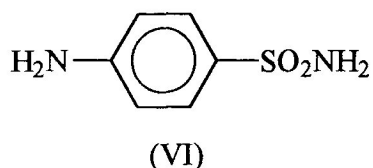
However, in 1935, Domagk²⁰, Mietzsch and Klarer²¹ first dispelled the failure of earlier scientists by discovering Prontosil, and reported that this compound cures *Streptococcal* infections in mice and *Staphylococcal* infections in rabbits. This period was considered to be a brilliant era in the chemotherapy of microbial infections.

The history of antibacterials has thus been dynamic, characterized 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. These drugs were the first synthetic chemotherapeutic agents employed to prevent and cure bacterial infections in man, and were quite effective in bringing about a sharp decline in the morbidity and mortality figures for the treatable infectious diseases. The important members of this class include Sulfanilamide (VI), Sulfamethoxazole (VII), Sulfacetamide (VIII), Sulfadiazine (IX) and Sulfisoxazole (X).



The sulfonamides have a wide range of antibacterial activity against both gram-positive and gram-negative bacteria. They are usually rapidly absorbed from the gastrointestinal tract, except in the bowel. Approximately about 70-100% of an oral dose is absorbed and finally eliminated from the body, partly as metabolic products; the largest fraction being excreted in the urine.

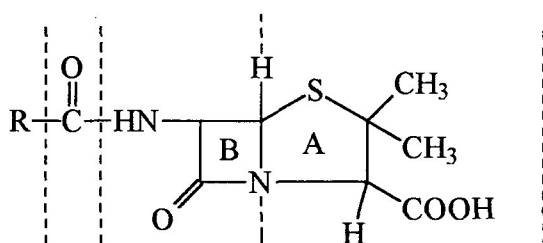
Strains of bacteria resistant to the sulfonamides have become common during recent years. Also, the advent of Penicillins and other broad spectrum antibiotics has

diminished the role of sulfonamides as antibacterial agents. However, the introduction of the combination of Trimethoprim and Sulfamethoxazole, known popularly as Cotrimoxazole, has resulted in the increased use of sulfonamides for the treatment of specific bacterial infections.

Penicillins and Cephalosporins :

Since Alexander Fleming's serendipitous discovery of Penicillin in 1928, Penicillins have remained one of the most important group of antibiotics. Despite the discovery of several other anti-bacterial 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 the treatment of a large number of infectious diseases.

The basic structure of Penicillin (XI) consists of a thiazolidine ring (A) connected to a β -lactam ring (B), which is attached to a side chain(R) through an amide carbonyl. The Penicillin nucleus is an important requirement for biological activity, alteration of which causes loss of all significant activity. Many of the bacterial and pharmacological characteristics of Penicillins are determined by its side chain, which can also alter the susceptibility of the resultant compounds to inactivating enzymes (β -lactamases).



(XI)

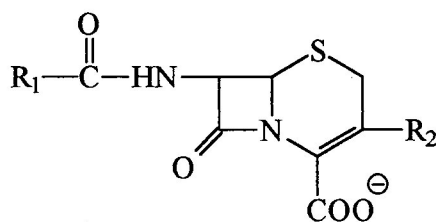
The Penicillins are classified according to their spectrum of antibacterial activity. While the pharmacological properties of the individual drug in the various classes would constitute a lengthy discussion, certain generalisations can, however, be made. Following absorption, Penicillins are widely distributed throughout the body. The therapeutic concentrations of these agents are quite rapidly achieved in tissues, but comparatively much lower levels are found in brain tissues and in intra-ocular fluids.

The Penicillins do not penetrate the living phagocytic cells to a great extent. This class of compounds is quite rapidly eliminated, particularly by glomerular filtration and renal tubular filtration, as a result of which high concentrations of the drug are found in the urine. Therefore, they exhibit short half-lives of approximately 30-60 minutes.

No sooner was the fact established that Penicillins are capable of eradicating microorganisms, than it was practically administered in every situation where there was a risk of bacterial infection. But after the investigations of prophylaxis under controlled conditions, it is now understood that Penicillin is highly efficient in some situations, quite the contrary in others and of questionable value in still others.

The commonly cited adverse effects in the usage of Penicillins, which are probably the most common causes of drug allergy, have been the hypersensitivity reactions. Such reactions could occur with any dosage form of Penicillin, but as the drug is rapidly eliminated, the allergic reactions usually subside. In some cases the symptoms may persist for one to two weeks after withdrawal of treatment, while in other cases, the reaction is mild and disappears during therapy with the drug. At the worst of circumstances, an extreme allergic reaction may even result in death of the patient.

The Cephalosporins (XII) 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.



(XII)

The first generation Cephalosporins exhibit good activity against gram-positive organisms 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 β -lactamase producing strains.

The Cephalosporins are widely used as antibiotics, and perhaps overused for conditions where much less expensive antibiotic could not provide relief. Nevertheless they are indeed useful as alternatives to Penicillins for a variety of infections in patients who suffer allergic reactions to Penicillins.

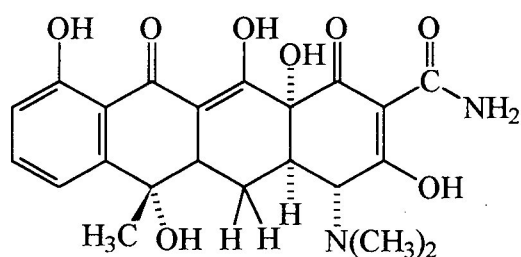
The Cephalosporins also find wide use in prophylaxis during and after surgery, wherein the first generation agents have been shown to be safe and effective. A few of the third generation Cephalosporins are the drugs of choice for meningitis, and in some cases are superior to the combination of Ampicillin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

The β -lactam antibiotics (Penicillins and Cephalosporins) suffer bacterial resistance due to a variety of factors. It may be caused by the inability of the agents to enter the site of action²². Also, it is possible for a sensitive strain to acquire resistance by mutation and has been described for gram-positive cocci²³.

Bacteria can also destroy the β -lactam antibiotics enzymatically. β -lactamases are capable of inactivating certain of these antibiotics and is the major term of this enzyme although different microorganisms synthesise a number of distinct β -lactamases. Depending on substrate specifications, these are often described as either Penicillinases or Cephalosporinases. Individual Penicillins and Cephalosporins vary in their susceptibility to these enzymes.

Tetracyclines :

Tetracyclines (XIII) 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.



(XIII)

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

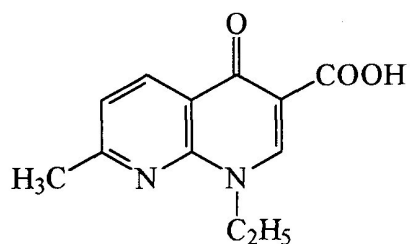
All Tetracyclines produce gastrointestinal irritation to varying degrees in some individuals, and this is more common after oral administration. Diarrhoea may also be a side effect which, though frequent and fluid, does not contain blood or leukocytes. Doxycycline, and to a lesser extent other derivatives, may produce mild to severe reactions in the skin of treated individuals exposed to sunlight.

Other important antibacterial agents include Chloramphenicol and furan derivatives such as Nitrofurantoin, Nitrofurazones, Furazolidone etc. Several of these drugs are active against various species of gram-positive and gram-negative microorganisms besides mycobacteria.

Quinolones :

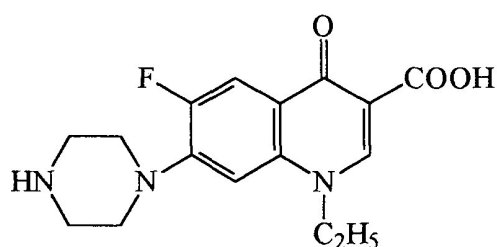
The quinolone carboxylic acids, or 4-quinolones, are a group of synthetic antibacterial agents structurally related to Nalidixic acid (XIV). Nalidixic acid is active against gram-negative bacteria but has little activity against *Pseudomonas* and gram-positive organisms, because of which their bactericidal use has generally been limited to the treatment of urinary tract infections.

Modifications in the structure of Nalidixic acid have 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.

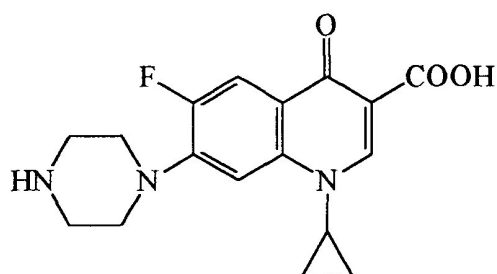


(XIV)

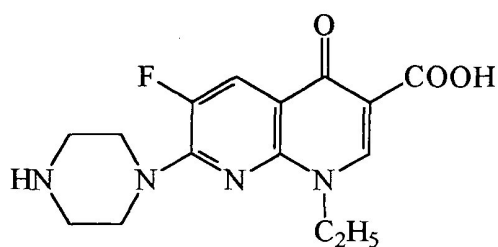
Addition of the 7-piperazinyl group and a fluorine atom at 6-position of the quinolone has produced a group of fluoroquinolones with a spectrum of activity broader than that of nalidixic acid, and they are found to be more useful for the treatment of systemic infections. They include Norfloxacin (XV), Ciprofloxacin (XVI), Enoxacin (XVII), Ofloxacin (XVIII) and Lomefloxacin (XIX). One disadvantage of all the quinolone antibacterial agents is that they are generally not recommended for use in children, adolescents, pregnant and breast feeding women, because of their propensity to cause joint erosions in immature animals.



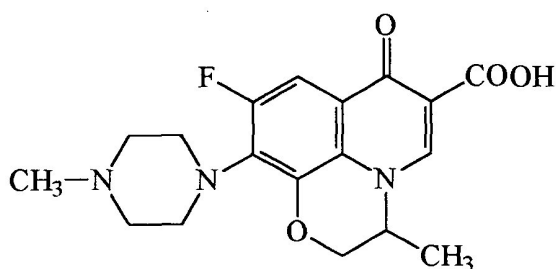
(XV)



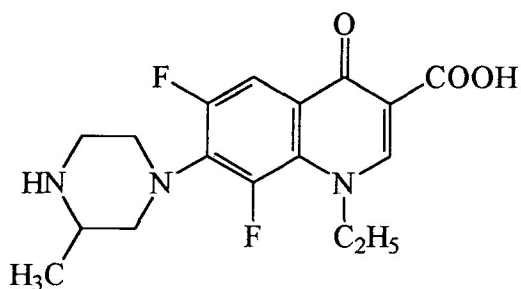
(XVI)



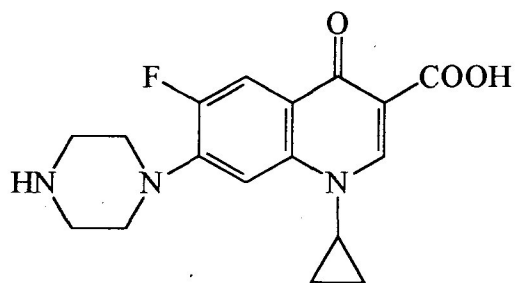
(XVII)



(XVIII)



(XIX)

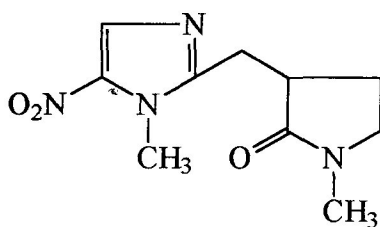


(XX)

Tefloxacin (XX) was demonstrated by J. L. Chen *et al*²⁴ to possess activity against Penicillin resistant *Staphylococcus*, *Streptococcus* and *Haemophilus* species. It was itself a β -lactamase inhibitor and displayed synergy with Ampicillin.

Miscellaneous :

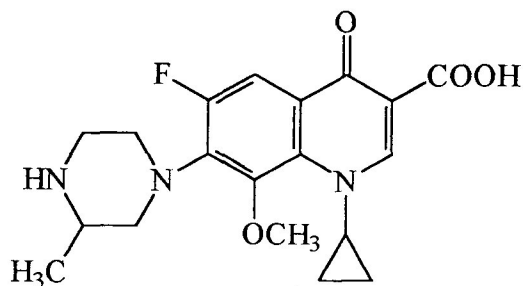
A series of 2-substituted-5-nitroimidazoles was reported to be active against anaerobic organisms. Compound (XXI) was unique in this series, and was found to be more potent both *in vitro* and *in vivo* as compared to Metronidazole.



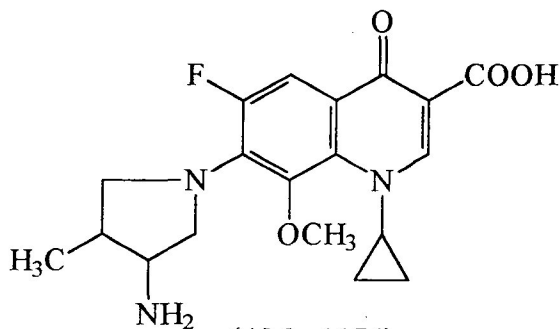
(XXI)

Quinolones :

The use of N-cyclopropyl and C-8 hetero atom substituents on quinolone nucleus continues to provide potent antibacterial agents. AM - 1155 and AM - 1174, new quinolones bearing 8-methoxy group, outperformed Ciprofloxacin against anaerobes and a range of gram-positive bacterial with potency upto 16 to 62 – fold greater than Ciprofloxacin²⁵.

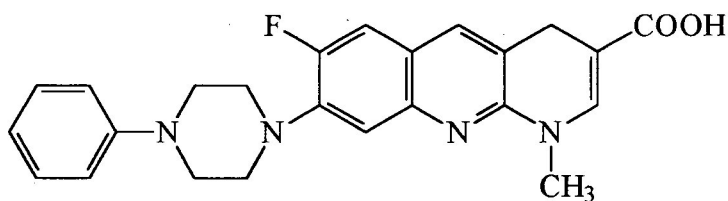


(AM - 1155)



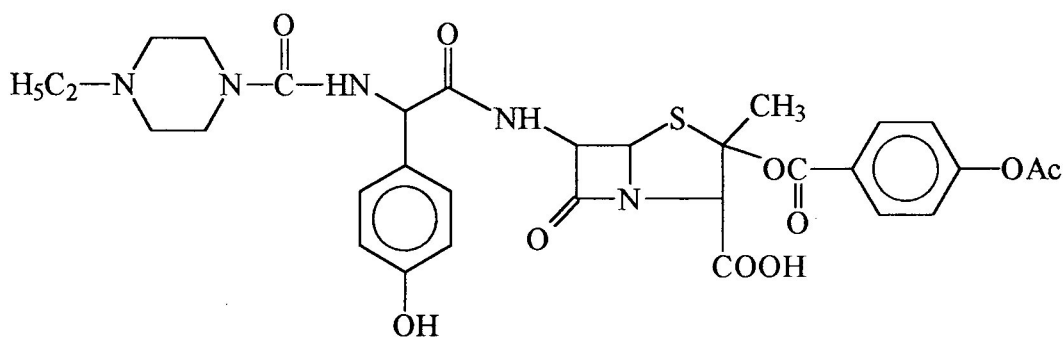
(AM - 1174)

RU -67829 (a compound in benzonaphthyridine series) was found to be more active against gram-positive organisms than Ciprofloxacin²⁶.



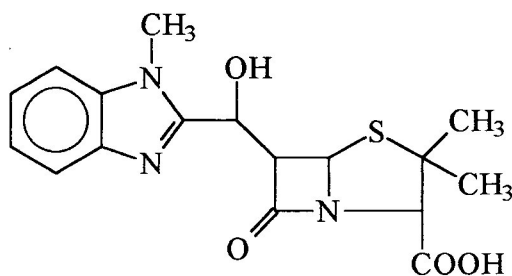
(RU - 67829)

In Penicillins, the catechol containing compounds (XXII) had outstanding activity against *Pseudomonas*, and stability to gram-negative β -lactamases, but not to the lactamases of *S. aureus*²⁷.



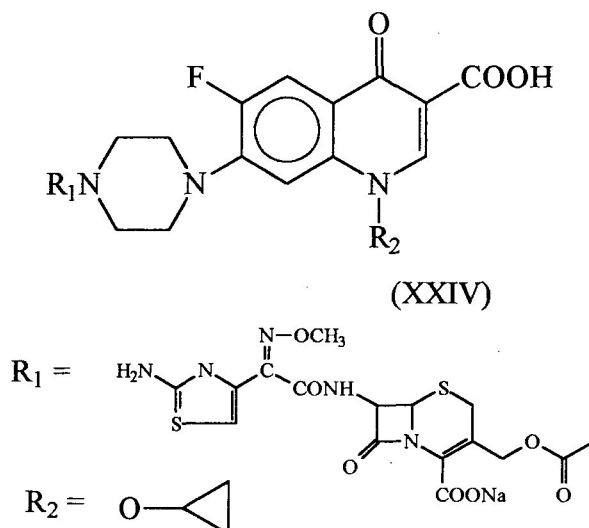
(XXII)

The stability to gram-positive β -lactamases could be achieved through the use of a (benzimidazole-2-yl)-hydroxymethyl group (XXIII).



(XXIII)

Recently, new β -lactam quinolone hybrids have been reported. The carbamate linked R0-24-4383 (XXIV) showed a broad spectrum of activity and exhibited aqueous solubility much greater than that of the quinolone component alone^{28,29}.



FUNGAL INFECTIONS

Fungi, like the human host, are eukaryotic organisms, and thus the number of suitable targets for therapeutic attack 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 defense mechanism, including patients with neoplastic diseases, organ-transplant recipients, diabetics and patients with AIDS. These patients are susceptible to an ever growing list of opportunistic fungi. From the data collected by Centre for Disease Control, it is known that among the 1 and 2 million healthy Americans who have already been infected with AIDS, a significant number of these cases are associated with various fungal infections. One yeast-like fungus, *Cryptococcus neoformans*, is reported to be the common reason for debilitating and life-threatening infections in AIDS patients¹⁷.

The agents which are currently in use as antifungals are classified and listed below:

Allylamines

Naftifine
Terbinafine

Triazoles

Fluconazole
Itraconazole
Terconazole

Imidazoles

Bifconazole
Butaconazole
Chlorimidazole
Clotrimazole
Econazole
Enilconazole
Fenticonazole
Isoconazole
Ketoconazole
Niconazole
Oxiconazole
Sulconazole
Tioconazole

Polyene macrolides

Amphotericin
Candididin
Hachimycin
Kanamycin
Nystatin
Pentamycin

Most of the antifungals given above are applied in the treatment of infections of hair, mucous membranes, nails and skin caused by *Candida dermatophytes*, fungi including *Epidermophyton*, *Microsporum*, *Trichophyton* and *Malassezia furfur*. The main agents, which are used in treating these infections, are listed below.

Superficial Fungal Infection

Candida infections
Tinea
Pityriasis versicolor

Main topical antifungal agents

Azoles, Polyene antifungals
Olamine, Haloprogin, Tolnaftate, Azoles, Ciclopirox,
Azoles, Ciclopirox, Olamine, Haloprogin, Tolnaftate

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

Systemic fungal infections

Aspergillosis
Blastomycosis

Main antifungal agents

Amphotericin
Amphotericin, Ketoconazole

Candidal infections:

Oesophageal
Disseminated
Coccidioidomycosis
Cryptococcosis
Histoplasmosis
Paracoccidioidomycosis

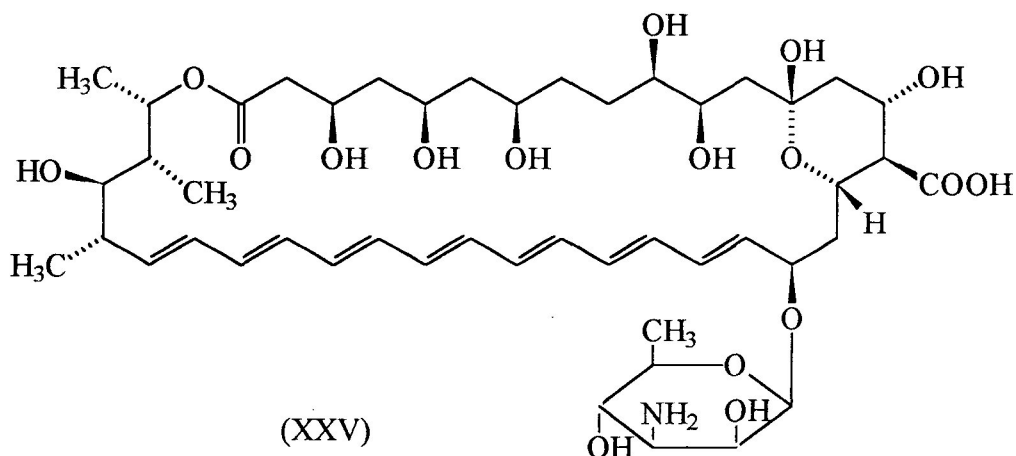
Fluconazole, Ketoconazole
Amphotericin, with or without Flucytosine
Amphotericin, Ketoconazole
Amphotericin, with or without Flucytosine, Fluconazole
Amphotericin, Ketoconazole
Amphotericin, Ketoconazole

A brief description about the major drugs used in treating fungal infections is presented in the following pages.

Amphotericin B

It was the first commercially available systemic antifungal drug, which 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 (XXV). It is amphoteric in nature, forming soluble salts in both basic and acidic environments.

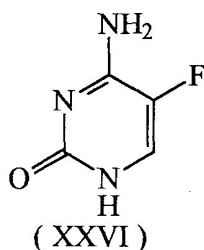
The primary mechanism of action of the polyenes in general, and Amphotericin B in particular, is binding to ergosterol, the principal sterol present in the cell membrane of sensitive fungi³².



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

Flucytosine

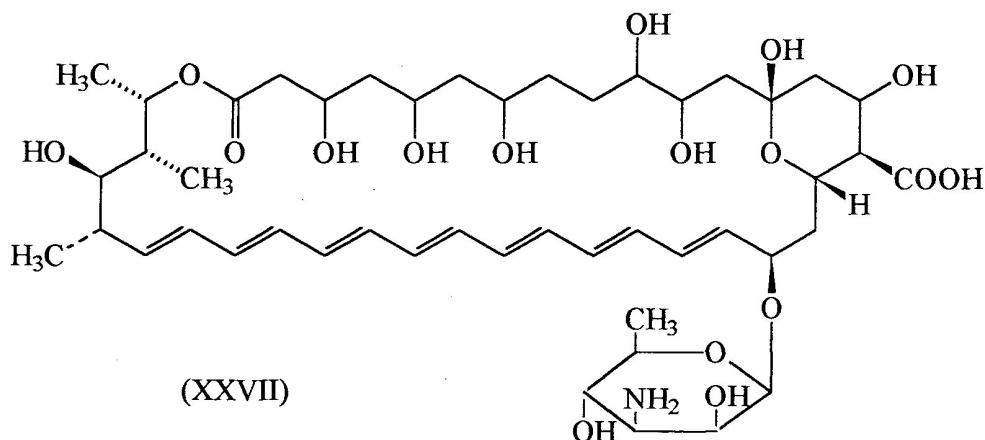
It is a fluorine analogue of Cytosine (XXVI). It was found to have *in vitro* and *in vivo* antifungal activity¹⁹. It is increasingly being used as an adjunct to Amphotericin B therapy.



The mechanism of action of Flucytosine is the disruption of protein synthesis by inhibition of DNA synthesis, and alteration of the amino acid pool by inhibition of RNA synthesis. It is used for treating infections caused by *Candida albicans*, *Cryptococcus neoformans* and *Torulopsis glabrata*.

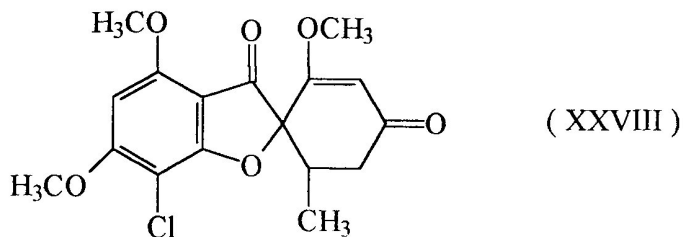
Nystatin

Nystatin(XXVII) is produced by *Streptomyces noursei*. It was discovered by Hazen and Brown²⁰ and possesses food antifungal activity. The toxicity after parenteral administration to animals has led to its use only topically or orally. The MIC of this drug for *Candida* species and *Cryptococcus neoformans* is in the range of 1.56-12.6 µg/ml.



Griseofulvin

Griseofulvin (XXVIII) was discovered by Oxford *et al* in 1939 as a metabolic product of *Penicillium griseofulvum*. Grove predicted its chemical structure in 1950²¹.



As a "cell-wall antibiotic" particularly active on *Dermatophytes*, Griseofulvin interferes with the biosynthesis of the chitinous elements of the hyphal wall. No resistant strains are observed among *Dermatophytes* during the treatment with this drug.

The synthetic antifungal agents

Metal containing molecules

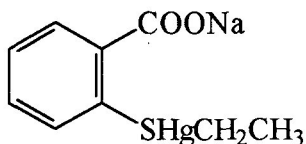
Heavy metal ions such as Ag^+ , Hg^{2+} , Cu^{2+} and Zn^{2+} have long been used as fungitoxicants in agriculture and also in topical ointments for dermatophytoses. A combination of a heavy metal atom with an organic molecule increases the activity, but a more important aspect is the selective toxicity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

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

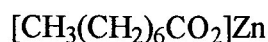
Zinc dimethyldithiocarbamate (XXIX), metal complex of 5-chloro-7-iodo-8-hydroxyquinoline(XXX), sodium ethyl mercurithio salicylate(XXXI), Zinc caprylate (XXXII) and pyrithione (XXXIII).



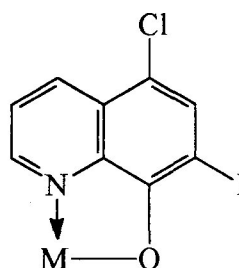
(XXIX)



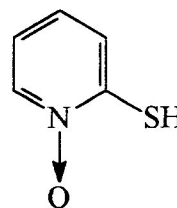
(XXXI)



(XXXII)



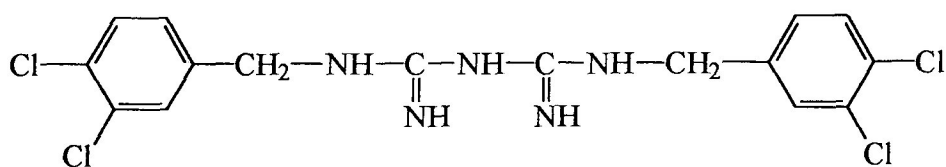
(XXX)



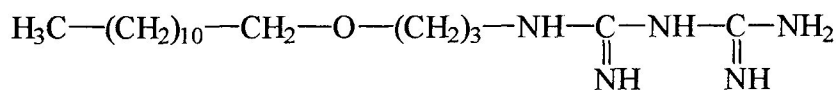
(XXXIII)

Chelating agents

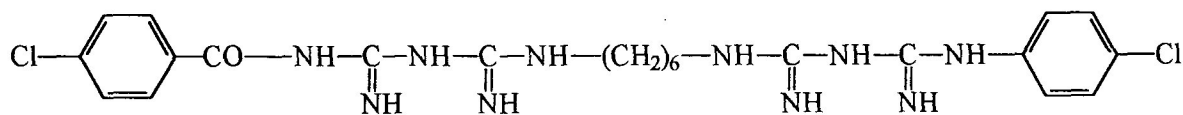
Among chelating agents with considerable *in vitro* antifungal potency are substituted biguanides like N^1 , N^5 -bis-(3,4-dichlorobenzyl) biguanide(XXXIV), N^1 -Y lauroxypropyl biguanide(XXXV), 1,6-bis-(5-chlorophenyl biguanidine) hexane(XXXVI)³⁶ etc.



(XXXIV)



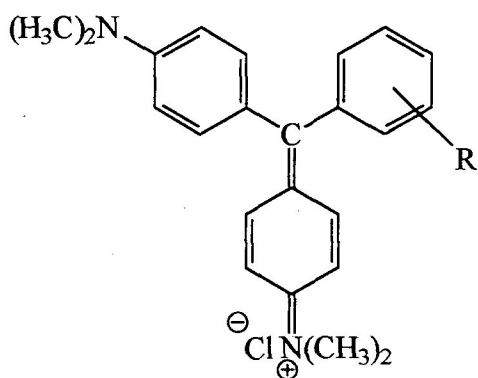
(XXXV)



(XXXVI)

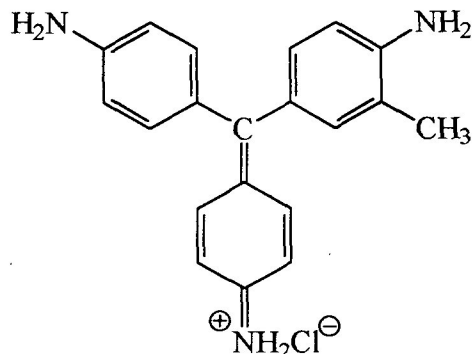
Aniline Dyes

Aniline dyes, such as malachite green(XXXVII), gentian violet(XXXVIII), methyl rosaniline chloride (XXXIX) have been employed in topical therapy of dermatophytoses as well as Candidiasis of skin and mucous membranes.



(XXXVII) R = H

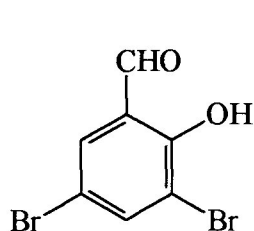
(XXXVIII) R = N(CH₃)₂



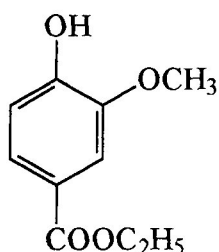
(XXXIX)

Phenolics and Diamides

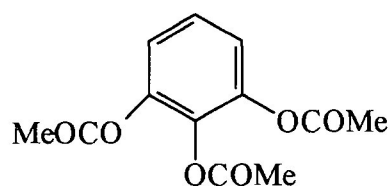
Phenolic compounds are known for their antifungal potency. Among the numerous phenolic compounds that have been used externally for mycotic infections are phenol, phenyl mercuric nitrate, cresol, m-cresyl acetate, benzoic acid, 4-hydroxybenzoic acid, salicylic acid, salicylanilide, 3,5-dibromo salicylaldehyde (XXXX), ethyl vanillate(XXXXI), pyrogallol triacetate(XXXXII), 3-iodo-2-propynyl-2',4',5'-trichlorophenyl ether(XXXXIII), Haloprogin 1,8-hydroxyanthranol (XXXXIV) and tetrachlorobenzoquinone(XXXXV).



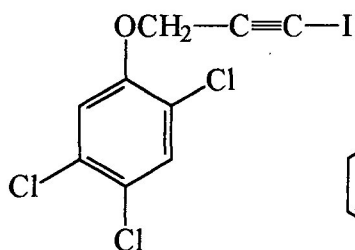
(XXXX)



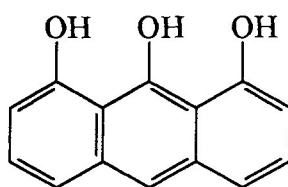
(XXXXXI)



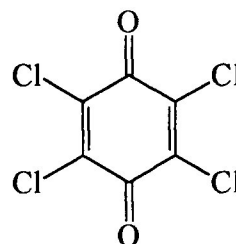
(XXXXXII)



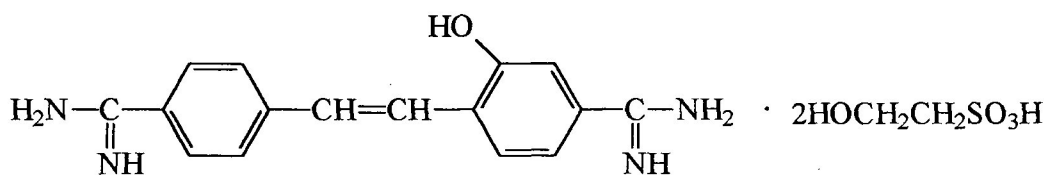
(XXXXXIII)



(XXXXXIV)



(XXXXXV)

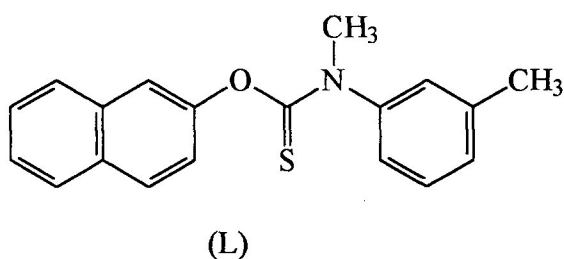
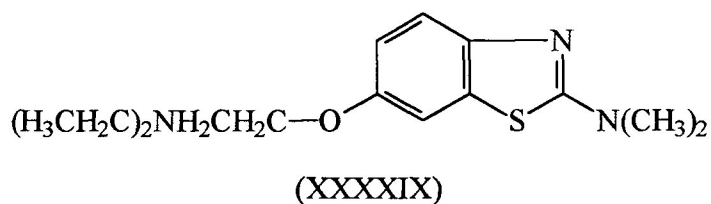
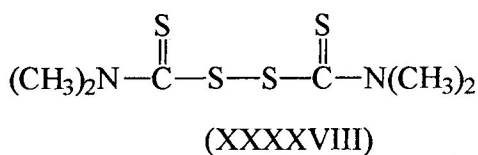
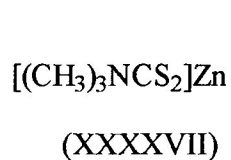


(XXXXXVI)

Several biamidines (e.g. stibamidine, propamidine, pentamidine) are antifungal *in vitro*. 2-hydroxystilbiamidine diisothionate (XXXXXVI) has been successfully employed in patients with blastomycosis. It is less toxic than Amphotericin B³⁷.

Sulfur-containing molecules as antifungal agents

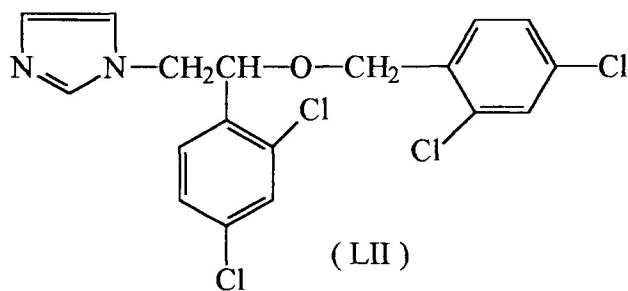
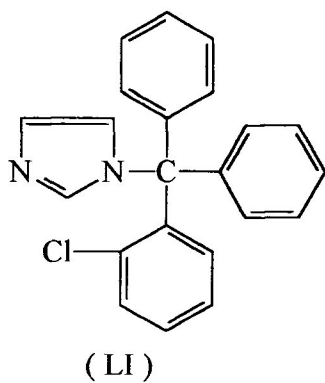
A variety of organic compounds which contain divalent sulfur attached to carbon atom have *in vitro* fungistatic action, and many of these have been employed in the topical therapy of dermatophytoses. Among these are dithiocarbamates (XXXXXVII), tetraethylthiuram disulfide (XXXXXVIII) and benzothiazoles such as 6-(diethylaminoethoxy)-2-dimethyl amino derivatives (XXXXXIX)^{38,39}.



A widely used compound with unusually high *in vitro* and *in vivo* activity against the dermatophytes is naphthiocarbamate of N-methyl-p-toluidine (Tolnaftate), (L). It is also effective against candidal lesions⁴⁰.

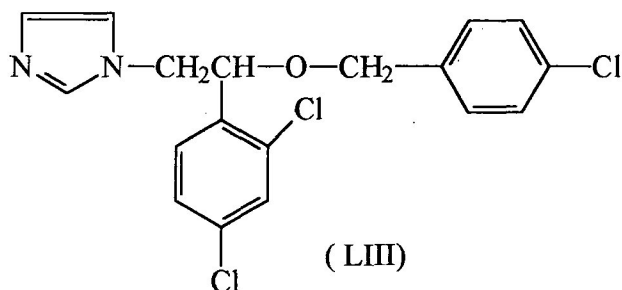
Imidazole antifungals

Among all the imidazole compounds, three distinct compounds are actually therapeutic antifungals, with very wide spectra against yeast and filamentous fungi. They are Clotrimazole (LI), Miconazole (LII) and Econazole (LIII).



Clotrimazole was developed by investigators at the Bayer Laboratories in 1969, and is utilized topically in superficial mycoses, or orally in clinical trials in deep mycoses⁴¹.

Miconazole⁴²(LII) is a well known antifungal drug developed by the investigators at Janssen Pharmaceutica in 1969. It is utilized in superficial mycoses and by oral / intravenous routes in deep mycoses, in clinical experimental trials.

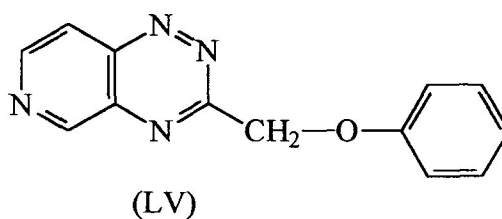
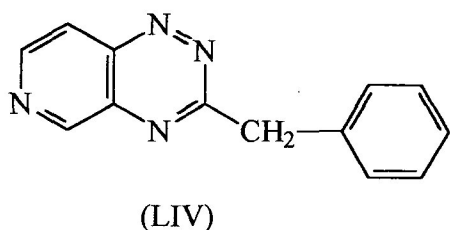


Econazole(LIII) is a phenethyl imidazole derivative. Like Miconazole, it is utilized as a topical agent in superficial mycoses and as a systemic antifungal, by oral or intravenous routes, in deep mycoses⁴³.

The mode of action of imidazoles studied by Preusser⁴⁴ on *Candida albicans* and *Trichophyton rubrum* shows that at a concentration 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 (LIV) and oxyfungin (LV) have been described as antifungal agents by Wright^{45,46}.



Triazoles

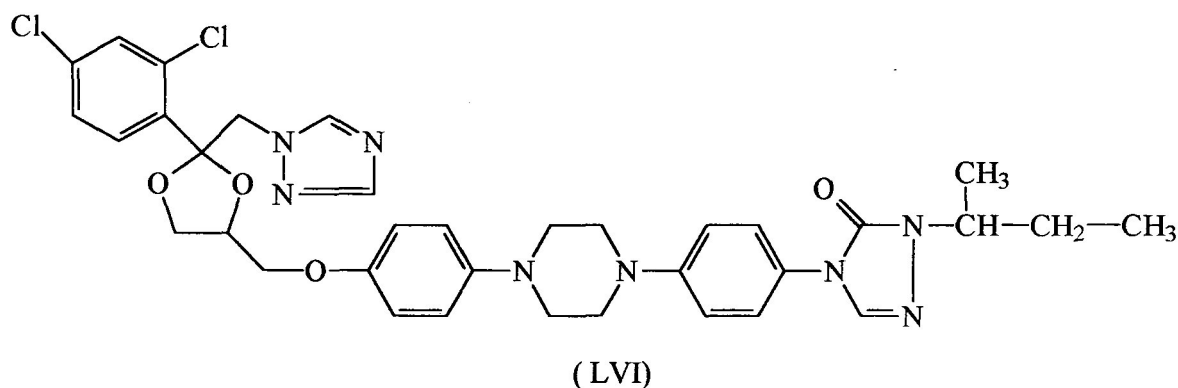
The replacement of imidazole ring with the triazole offers many advantages to these newer azoles⁴⁷.

- 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.

There are a number of such compounds that have recently been licensed, or are currently being developed and tested.

Itraconazole

Itraconazole (LVI) is a water-soluble, lipophilic triazole analogue that exhibits excellent activity against most fungal pathogens.

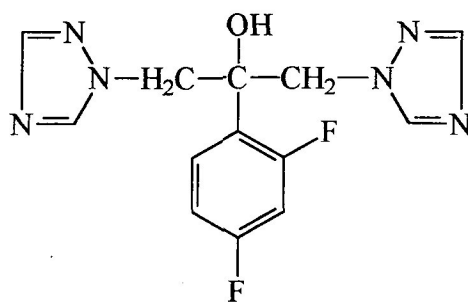


When compared with Ketoconazole, its potential advantages include a broader spectrum of activity (including *Aspergillus* spp.), a more desirable pharmacokinetic profile and less toxicity⁴⁸.

Most of the adverse reactions reported are transient and include gastrointestinal disturbance, dizziness, headache, depressed libido (with normal testosterone levels) and leucopenia⁴⁹.

Fluconazole

Fluconazole (LVII) 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 and the mechanism of action is inhibition of fungal C-14 demethylase.



(LVII)

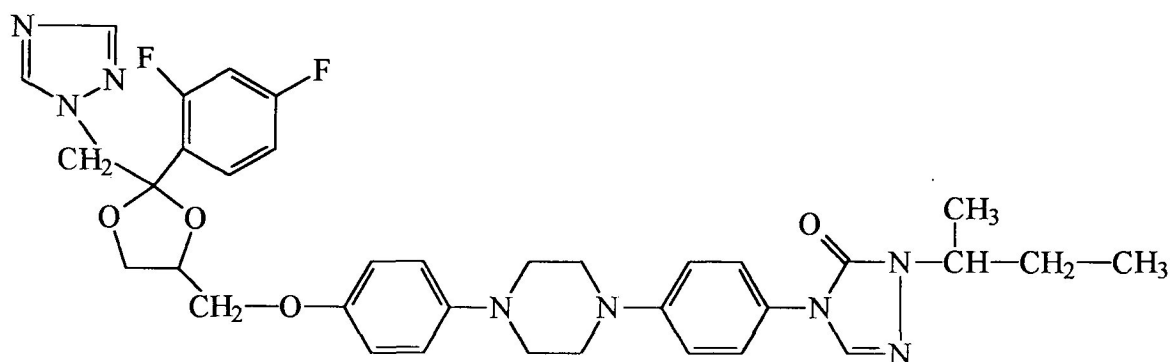
A major point of confusion during the development of this drug, however, has been its relatively low activity *in vitro* compared to its high *in vivo* activity.

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 the patients receiving the drug. Fluconazole is also a potential candidate for prevention and early treatment of disseminated candidiasis in granulocytopenic patients⁵¹.

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

Saperconazole

Saperconazole (LVIII) is a water-soluble, lipophilic fluorinated triazole with a chemical structure similar to Itraconazole, except that the two chlorine atoms are replaced by fluorine.



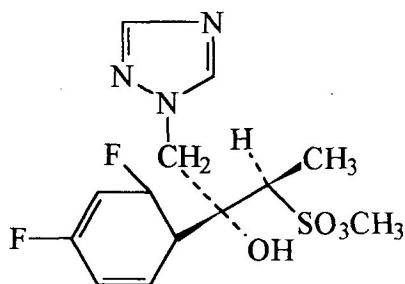
(LVIII)

It has a broad spectrum of antifungal activity against, *Cryptococcus* spp and *Aspergillus* spp³⁵.

OTHER AZOLES

SCH 39304

SCH 39304 (LIX) is a N-substituted triazole with a broad spectrum of antifungal activity. It is active orally, parenterally and topically.

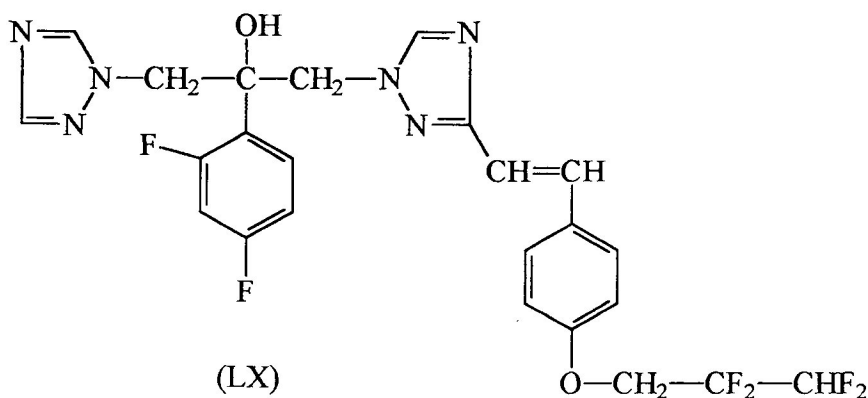


(LIX)

The potent broad spectrum *in vivo* activity of SCH 39304 against *Candida*, *Aspergillus*, *Cryptococcus*, *Blastomyces*, *Coccidioides* and *dematiaceous* moulds holds exceptional promise as a valuable agent. SCH 39304 has been shown to cause hepatocellular carcinoma in animals, and probably became an orphan drug only available to patients who fail to respond to all other antifungal drugs.

ICI 195739

ICI 195739 (LX) is an orally active bis-triazole with a broad spectrum of antifungal activity⁵³.



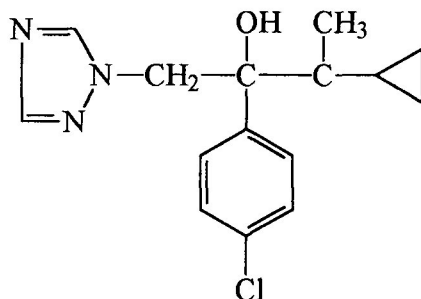
(LX)

Like other antifungal azoles, it is a potent inhibitor of fungal C-14 sterol demethylation. However, ICI 195739 is more freely permeable than Ketoconazole

and Fluconazole, and therefore, organisms that are resistant due to reduced azole uptake are more sensitive to this drug⁵⁴.

SDZ 89-845

SDZ 89-845 (LXI) is a triazole antifungal agent that has shown activity in a number of animal models against superficial and systemic fungal infections⁵⁵.



(LXI)

Part IB

Plan of Work

Part IB

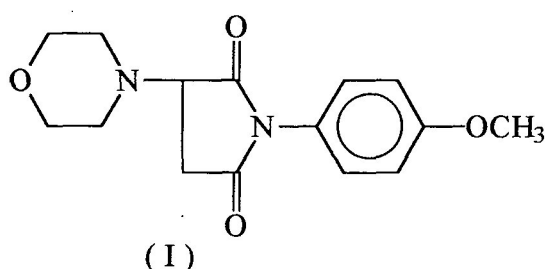
PLAN OF WORK

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

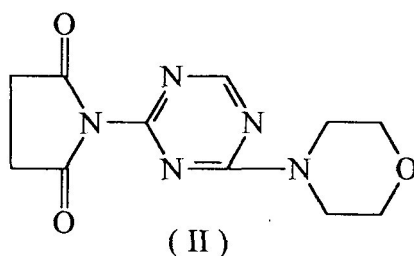
The present work has been planned keeping in mind the various chemical structures of biologically active moieties.

Literature survey revealed that succinimide derivatives possess anticonvulsant⁵⁶, fungicidal⁵⁷, antibacterial⁵⁸, antitubercular⁵⁹ and antiarthritis⁶⁰ activity. Hence, it was decided to select succinimide as the 'lead' moiety. This was supported by the following reports.

Earlier studies have shown that 4-morpholino-N-(4'-methoxy phenyl) succinimide(I) inhibits *in vitro* growth of *M. tuberculosis* at a MIC of 0.39 µg/ml⁶¹.



Also, the succinimides having heteroaryl substituents at 1-position are found to exhibit anti-microbial activity, for instance 2-succinimido-4-morpholino-1,3,5- triazine (II) is reported to be an antiviral agent⁶².



Part II

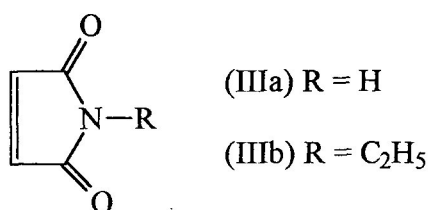
Synthesis of 4[4'(4''-chlorophenoxy)-3'-chloroanilino]-N-(alkyl/ aryl/ substituted aryl and heteroaryl) succinimides

It involves the following steps

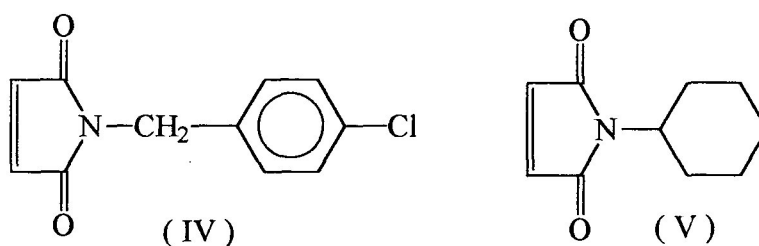
A) Synthesis of N- alkyl / aryl / substituted aryl and heteroaryl maleimides.

N-substituted maleimide are reported to possess antimitotic⁶³, antimicrobial⁶⁴ and insecticidal⁶⁵ activity.

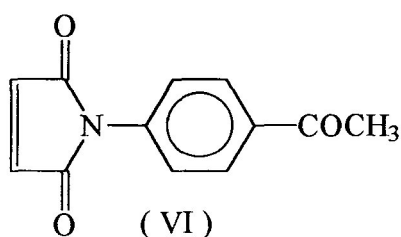
Unsubstituted maleimide (IIIa) and N-ethyl maleimide (IIIb) exhibit antibacterial activity⁶⁴.



N-(p-chlorobenzyl) maleimide (IV) and N-cyclohexyl maleimide (V) show a marked antifungal⁶⁶ activity against *Candida albicans* and *Trichophyton mentagrophytes*.

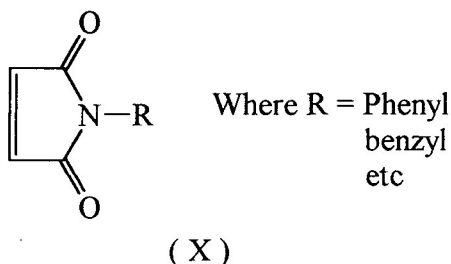


4-(p-acetyl phenyl) maleimide (VI) inhibits the growth of *Aspergillus foetidus*, *Aspergillus niger* and *Aspergillus oryzae*⁶⁷.



Also, quinoline derivatives are well known for their antimalarial, antifungal and antibacterial effects. Derivatives like 5-amino-6-chloro-8 thiocyanato quinoline (VII) and 2-[(5-nitro-2-furyl) vinyl] quinoline (VIII) were found to be active against *Candida albicans* and *Streptococcal*⁶⁸ infections, while 8-amino-6-methyl quinoline⁶⁹ (IX) was

Considering above facts different N-substituted maleimides (X) were synthesized as the intermediate for the synthesis of various succinimides (Scheme 1, pg. 68).

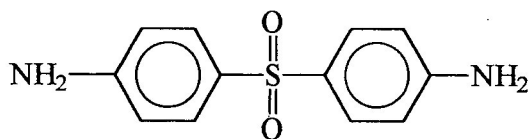


This moiety was used on the basis of a study carried out to relate antitubercular activity to chemical structure among diphenyl ethers, which highlights the following facts⁷².

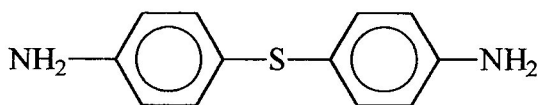
The introduction of 1 or 2 chloro substituents increases the antitubercular activity. Further halogenation has little additional effect. The greatest activity is shown by

compounds in which the halogens are shared by both the benzene nuclei. This prompted us to use the diphenyl ether substituent.

Also, 4, 4'- diaminodiphenyl sulfone (Dapsone) (XI) has been successfully used in the treatment of leprosy, which is a mycobacterial infection.



(XI)



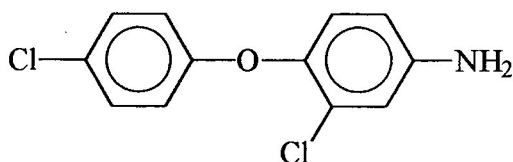
(XII)

4,4'-diphenyl sulfide (XII) has shown *in vivo* antitubercular activity in experimental mice, which was attributed to the *in vivo* oxidation of sulfide to sulfone⁷³.

This example brings forth the significance of the sulfide linkage.

Therefore, an attempt has been made to replace -S- by -O- to check the effect of such an isosteric replacement, by using a diphenyl ether substitution.

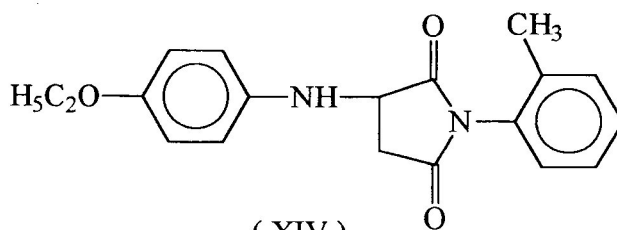
Thus considering the above facts the following type of diphenyl ether linkage (XIII) was incorporated on substituted maleimides to obtain title compounds i.e. succinimides.



(XIII)

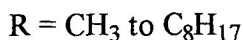
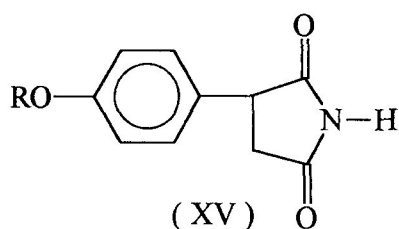
C) Synthesis of 4[4'(4''-chlorophenoxy)-3'-chloroanilino]-N-(alkyl / aryl / substituted aryl and heteroaryl) succinimides.

4(4'-ethoxyanilino)-N-(2-methyl phenyl) succinimide (XIV) is reported to be an antitubercular agent⁷⁴.



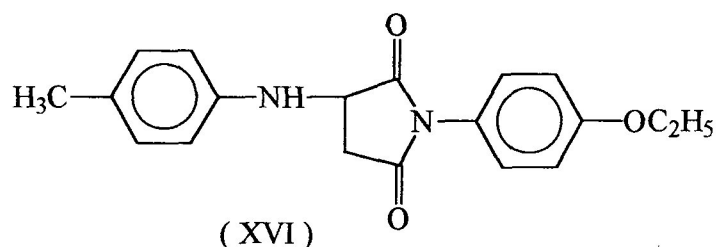
(XIV)

Earlier, Akopyan *et al*⁷⁵ noted antispasmodic activity in the compounds of type (XV).



The activity is influenced by the length of the C-chain at the alkoxy group. It increases from C_1 to C_4 , but subsequent increase in the number of C atoms reduce the activity of molecule.

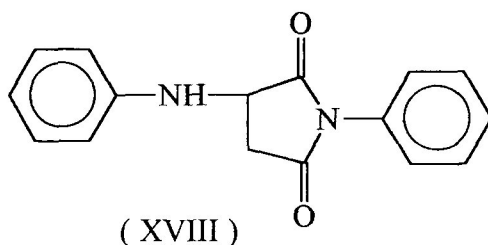
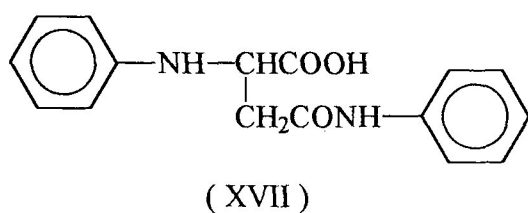
Earlier studies carried out at our laboratory have shown that 4(4'-methylanilino)-N-(4-ethoxyphenyl) succinimide⁷⁶ (XVI) inhibits *in vitro* growth of *M. tuberculosis* at a MIC of 0.78 $\mu\text{g/ml}$.



2-Benzyl succinimides are reported to be anticonvulsants⁷⁷.

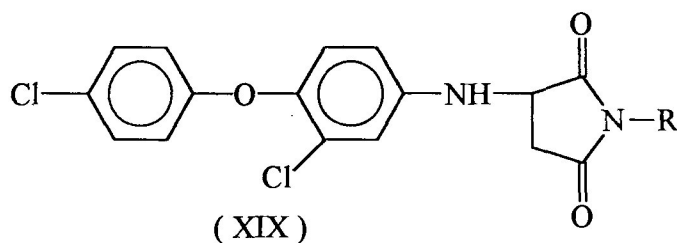
Succinimide derivative are also known to possess antifungal activity⁷⁸.

The derivatives of succinimides are used as intermediates⁷⁷ for the synthesis of some active antitubercular compounds, like for e.g. succinomonanilides (XVII), which has MIC of 0.04 $\mu\text{g/ml}$. For the synthesis of (XVII), (XVIII) is used as a starting material.



One important point taken into account here was that succinimide derivatives (e.g. like XVIII) may metabolise after their administration in the body and may form open chain compounds (like XVII) which have good antitubercular activity.

Thus, considering the importance of substituted maleimides and the diphenyl ether linkage in which halogens are shared by both benzene nuclei, various succinimides(XIX) were synthesized by condensing substituted maleimides with 4(4'-chlorophenoxy)-3-chloroaniline (as per scheme 2, pg. 69).



Where R = Phenyl, Benzyl, p-n-butoxy phenyl, 3-quinolinyl etc.

Part III

Synthesis of 4-(pyrrolidino anilino)-N- (aryl / substituted aryl and heteroaryl) Succinimides

It consist of the following parts

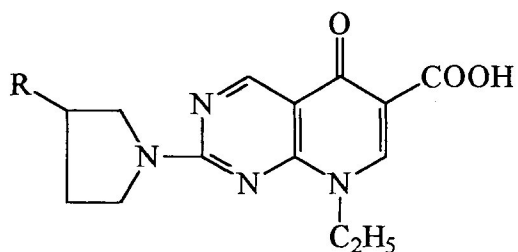
A) Synthesis of N-aryl /substituted aryl and heteroaryl maleimides

The importance of maleimides, used in the synthesis of the title compounds, has already been discussed in part IIA (pg. 34).

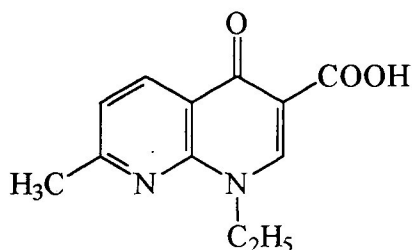
The same maleimides were used in this series also.

B) Synthesis of 1-(4-Aminophenyl) pyrrolidine

Piromidic acid (XX) and its metabolite, hydroxy piromidic acid (XXI), containing N-pyrrolidino substituents posses antibacterial activity⁷⁹ comparable to that of nalidixic acid(XXII).

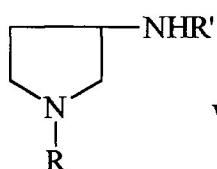


(XX) : R = H
(XXI) : R = OH



(XXII)

1-aryl-3-(lower alkyl) aminopyrrolidines (XXIII) are useful as intermediates and as bronchodilators and antifibrillatory agents⁸⁰.



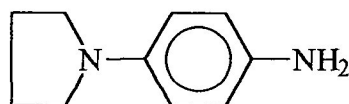
where, R = Aryl
R' = Alkyl

(XXIII)

Deguchi *et al* reported N-alkyl-pyrrolidine derivatives as antihistamines⁸¹.

Also, compounds like N-[1-(cyclopropyl-methyl)-3-ethyl-3-pyrrolidiny] phenol exhibited analgesic and antimorphine effects⁸².

Thus, considering the above facts 1-(4-Aminophenyl) pyrrolidine (XXIV) was introduced at 4 position of substituted maleimide to obtain title compounds i.e. succinimides

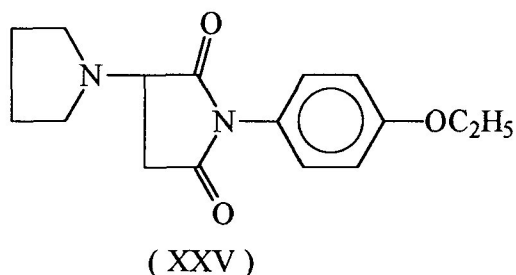


(XXIV)

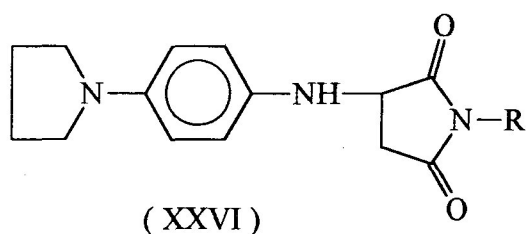
C) Synthesis of 4-(pyrrolidino anilino)-N-(aryl//substituted aryl and heteroaryl) succinimides

Succinimide derivatives possess the anticonvulsant⁵⁶, fungicidal⁵⁷, antibacterial⁵⁸, antitubercular⁵⁹ and antiarthritis⁶⁰ activity already discussed.

Earlier studies carried out in our laboratory revealed that 4-(N-pyrrolidino)-N-(4-ethoxyphenyl) succinimide (XXV) was active *in vitro* at a concentration of 1.56 µg/ml against *M. tuberculosis*.



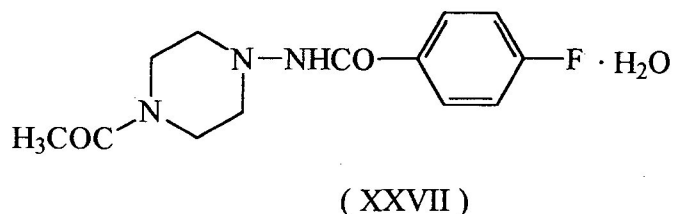
Thus considering the importance of pyrrolidino substituents at 4-position, it was decided to use long-chain substituents, which is also one of the strategies of drug development. The following type of compounds were synthesized by condensing different substituted maleimides with 1-(4-Aminophenyl) pyrrolidine to obtain the title compounds (XXVI) as per scheme 3.



where R= phenyl, benzyl, p-n-butoxy phenyl, 3-quinolinyl etc.

Part IV

Preparation of 1-acetyl-4-nitroso piperazine as a drug intermediate for N-(4-acetyl-1-piperazinyl)-4-fluorobenzamide monohydrate(FK-960)⁸³



FK-960 (XXVII) is a putative antidementia agent. It was discovered at Fujisawa through a rational drug-screening program⁸³.

The unique pharmacological profile of FK-960 makes this compound an interesting alternative to cholinergic agonists or cholinesterase inhibitors in the treatment of Alzheimer's disease.

FK-960 is presently in phase II clinical trials.

Alzheimer's disease (AD) is a progressive, irreversible, neurological disorder. The clinical presentation is characterized by core deficits in cognition-memory, praxis, and language⁸⁴, and is commonly associated with a variety of psychiatric disturbances and marked deterioration in behavior^{83,85}.

It is the most common form of dementing illness associated with both severe disability in carrying out the activities of everyday life and reduced life expectancy after disease onset, compared to age matched controls^{86,87}.

By virtue of its protracted, unremitting course, AD can produce a significant impact upon the emotional health of caregivers⁸⁶ and the economic integrity of the health care system.

Reliable estimates for the prevalence of dementia among persons under the age of 65 are not available due to the frequency of this condition in the younger population, the absence of uniform spontaneous reporting procedures and the difficulty of screening a sufficiently large sample for evidence of cognitive loss and associated features through survey.

The prevalence of Alzheimer's disease is strongly associated with advancing age⁸⁹; ranging from 3% among persons aged 65 to 74 years, up to 47% of those over 85 years. In the context of these epidemiological data and an aging population, the pharmaceutical industry has made substantial investments in central nervous system research, into the etiology, patho-physiology and potential therapeutic applications for Alzheimer's Disease.

Clinical development efforts for cognitively impaired elderly and AD occur in an increasingly competitive environment, characterized by mechanistically diverse test

compounds, large research budgets, fewer attempted provital studies in patients with Alzheimer's disease and the use of multiple measures within these studies to facilitate hypothesis generation based upon results.

In literature, FK-960 was obtained by 2 procedures, which are covered in the experimental section (Scheme 4, pg. 71).

In our laboratory, 1-acetyl 4-nitroso piperazine, the intermediate for FK-960, was obtained by the following modified procedure (scheme-5, pg 72).

1. First, nitrosation⁹⁰ of piperazine was done with NaNO_2/HCl in water to obtain N-nitroso piperazine.
2. Then, N-nitroso piperazine was acetylated⁹¹ with acetic anhydride in a small amount of glacial acetic acid to obtain 1-acetyl-4-nitroso piperazine.

Modifications :

1. In our laboratory, N-nitroso piperazine was first obtained from piperazine, since piperazine is easily available and is relatively cheaper.
2. N-nitroso piperazine was successfully obtained without using reagents, such as benzyloxy carbonyl chloride as a blocking agent. This avoids one step, and ultimately time and money.
3. Acetylation of N-nitroso piperazine with acetic anhydride in glacial acetic acid is cost effective as compared to acetylation in acetyl chloride and pyridine in dioxane, as per literature scheme 3 B.
4. Also, according to the literature scheme 3A, acetylation in acetic anhydride in the presence of sodium hydroxide give rise to inorganic impurities, which are difficult to be isolated.

Taking all these factors into consideration, the method of synthesis was suitably modified, thereby making the process commercially viable.

Part 1C

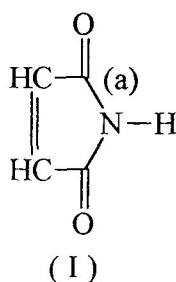
Chemistry

Part I C

a) CHEMISTRY OF MALEIMIDES

Maleimides are important intermediates in the preparation of various substituted succinimides of biological value.

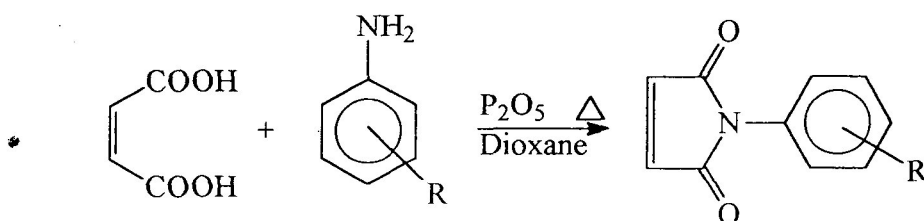
Maleimide (I) is a five membered ring having two carbonyl groups on the carbon adjacent to the double bond. N-substituted aryl, alkyl and heteroaryl maleimides can be synthesized by the cyclization of a variety of bifunctional derivatives, such as the carboxylic acids and derivatives, diamides or amidic acids. Almost all procedures involve the formation of carbonyl carbon-nitrogen bond (a) (shown in I).

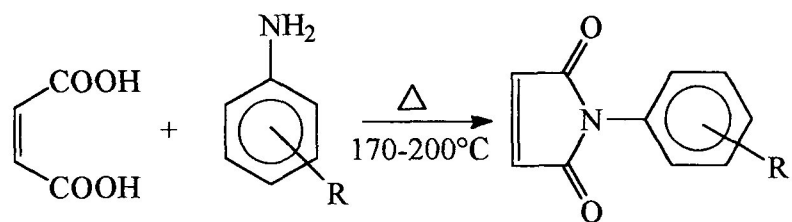


The different methods of preparation of N-substituted aryl, alkyl and heteroaryl maleimides are as follows:

i) From carboxylic acid and derivatives:

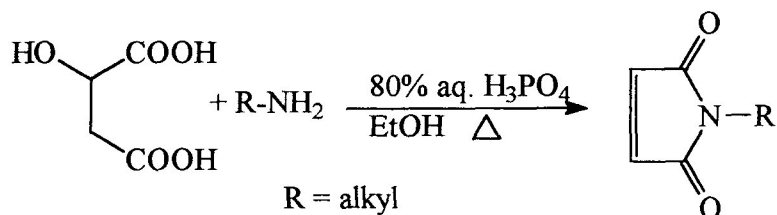
Five membered cyclic imides are readily formed from dicarboxylic acids and substituted amines with or without the presence of catalyst. N-aryl maleimides are obtained in 55% yields when a mixture of maleic acid and substituted aniline is heated with phosphorus pentoxide (dehydrating agent) in dioxane, or by the direct fusion of maleic acid and substituted aniline^{92,93}.





R = alkyl/aryl

Lukes and Pergal⁹⁴ obtained N-alkyl maleimides in about 70% yields by dehydrating a mixture of malic acid and primary alkyl amine with 80% aqueous phosphoric acid.

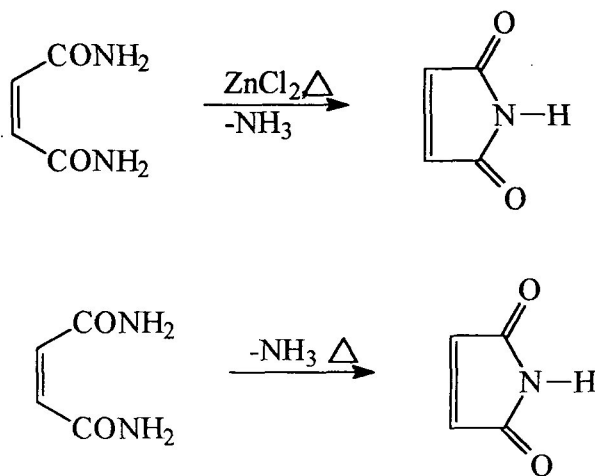


R = alkyl

Although, malic acid forms maleimide, in relatively better yields than maleic acid, the reaction is not feasible in the laboratory as the chemicals required are not readily available and the mode of isolation of the final product is not satisfactory.

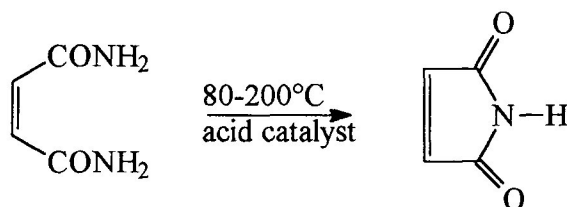
ii) From diamides and amidic acids:

Diamides are converted to cyclic imides on heating alone or in the presence of a catalyst^{95,96}. Maleic acid diamide that is maleamide forms maleimide on heating with ZnCl_2 , or even in the absence of it.

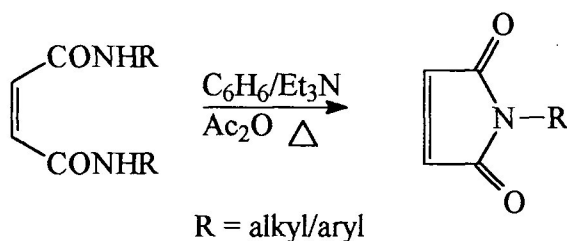


α,β -unsaturated carboxylic acid amides can be cyclized to α,β -dicarboxylic acid imide at 80-200°C in a solvent containing 0.01-20% of an acid catalyst like sulfur

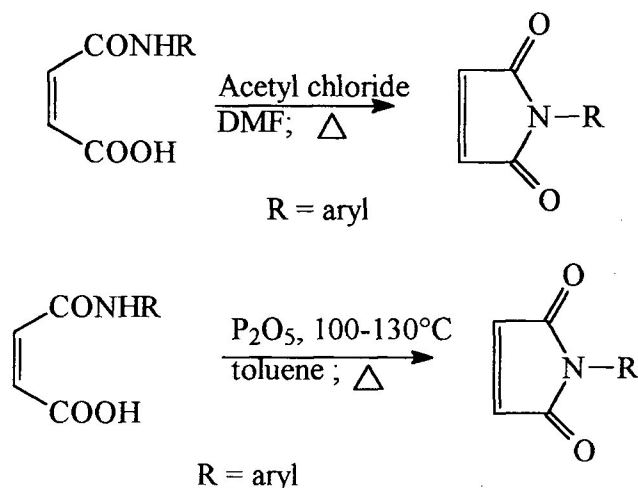
trioxide, chlorosulfonic acid, a phosphoric acid and organic sulfonic acid, or an organophosphoric acid⁹⁷.

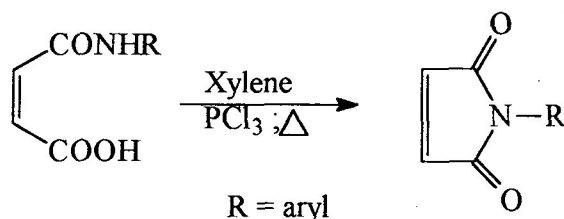


According to Sauers and Cotter⁹⁸, N-alkyl and N-aryl maleimides can be prepared in about 60% yield by cyclo-dehydration of the corresponding N-alkyl and N-aryl maleamic acids in the presence of trialkylamine, acetic anhydride or ketene in an inert organic diluent. Ketene and acetic anhydride form acetic acid after the abstraction of water from the maleamic acid. Triethylamine neutralizes the acetic acid *in situ* and the reaction proceeds in the forward direction.

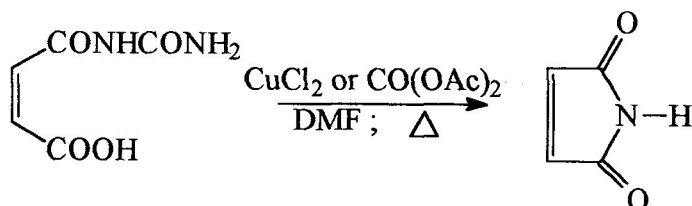


Maleamic acids form maleimides in about 65% yield when treated in dialkyl formamide or HCONEt_2 at -10°C to 90°C , with a dehydrating agent like acetyl chloride, benzyl chloride, phosphorus pentoxide, phosphorus trichloride, antimony chloride (SbCl_5), ClCO_2Et or sulfonyl chloride^{99,100}.





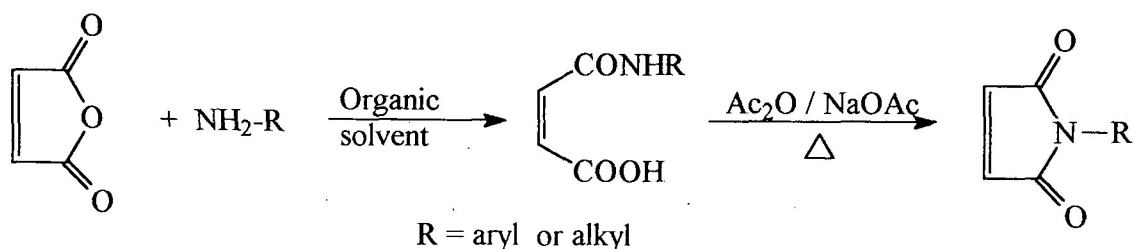
Takaya and co-workers^{101,102} have synthesized maleimide by heating N-carbamoyl maleamic acid with dehydrating agents like, cupric chloride, zinc chloride, nickel chloride or cobalt acetate, in an inert solvent at 50-180°C.



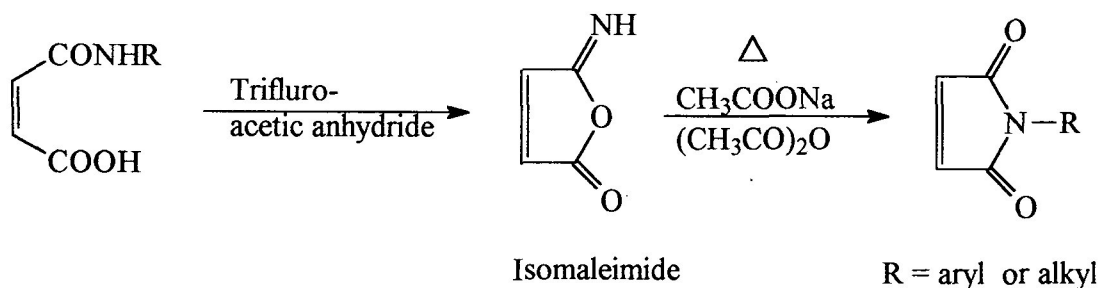
N-Substituted maleamic acids bearing electron withdrawing substituents such as N-p-carboxyphenyl or N-ethoxyphenyl fail to cyclize sometimes¹⁰³. For the preparation of N-substituted maleimides from the corresponding amidic acid, the method of Sauers *et al*⁹⁸ appears to be the most suitable. The reaction is obtained in good yields without any side products.

iii) From anhydrides :

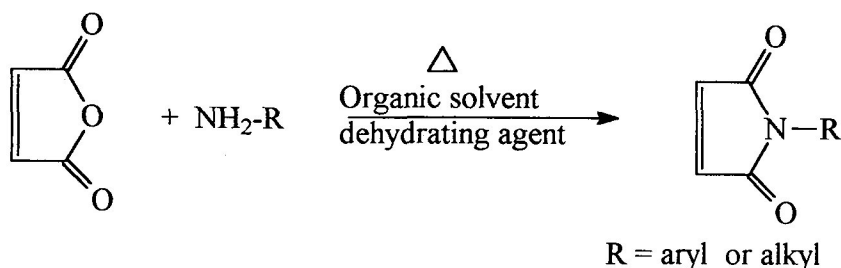
Burley and Haylett¹⁰⁴ reacted different primary amines with maleic anhydride in acetic acid to produce maleamic acid, which on heating in the presence of acetic anhydride formed the corresponding maleimides in about 70% yields. Acetic acid can be replaced by benzene, acetone or ether, and better yields (80%) are obtained when the maleamic acid is heated with acetic anhydride plus anhydrous sodium or potassium acetate¹⁰⁵⁻¹⁰⁷. Acetic anhydride extracts a molecule of water from the maleamic acid to form acetic acid, while anhydrous sodium or potassium acetate acts as an additional dehydrating agent.



When trifluoroacetic anhydride is used as the dehydrating agent instead of acetic anhydride, isomaleimide is formed, which can be rearranged to the more thermodynamically stable maleimide by heating it strongly with sodium acetate and acetic anhydride¹⁰⁸.

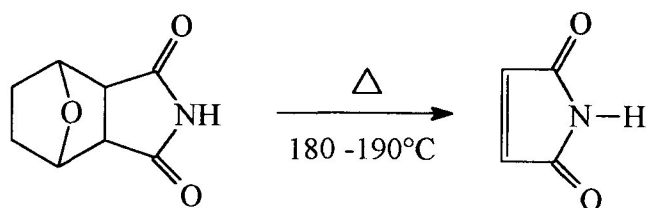


When Maleic anhydride and substituted amines are heated in an organic solvent in the presence of a dehydrating agent like phosphorus pentoxide, p-toluene sulfonic acid sulfur trioxide, sulfuric acid, or phosphoric acid, substituted maleimide is obtained in 80-90% yields^{97,109,110}. Maleic anhydride forms 99-100% pure maleimide in higher yields than maleic acid; the reaction temperature in the case of maleic anhydride is lower and hence, there is a low probability of the formation of resinous by-products.

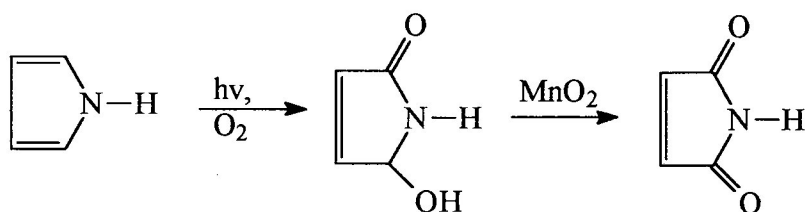


iv) Miscellaneous methods:

For the synthesis of maleimide, Prill¹¹¹ employed a dienophile exchange reaction, in which bicyclic imide adducts were heated in a gas flow system with excess of maleic anhydride, while Twaney¹¹² effected similar decomposition without the use of an added diene acceptor.



Kwart and Burchuk¹¹³ oxidised pyrrole with potassium dichromate and sulfuric acid to afford maleimide in low yields. The photolytic oxidation of pyrrole followed by oxidation with manganese dioxide gives better yields of maleimide¹¹⁴.



From the various methods discussed above for the preparation of maleimides, the methods reported by Istratoin *et al*¹⁰⁶ was employed. This method was employed as the chemicals required were easily available, the time taken was less and the work-up of the reaction was easy and clean.

The N-substituted aryl, alkyl and heteroaryl maleimides were synthesized as shown in Scheme 1 (pg. 68). The different amines (0.05 mole) were added to a solution of maleic anhydride (0.05 mole) in acetone at room temperature to obtain different maleamic acids; which when heated at 90-100°C for one hour in acetic anhydride (3 moles of that of maleamic acid) containing anhydrous sodium acetate (half mole of that of maleamic acid) formed the corresponding maleimides in 65-85 % yield.

The different amines used in the synthesis of maleimides were available in our laboratory. Only, p-n-propoxyaniline^{115,116} and p-n-butoxy aniline^{115,116,117} were prepared by the known literature procedure.

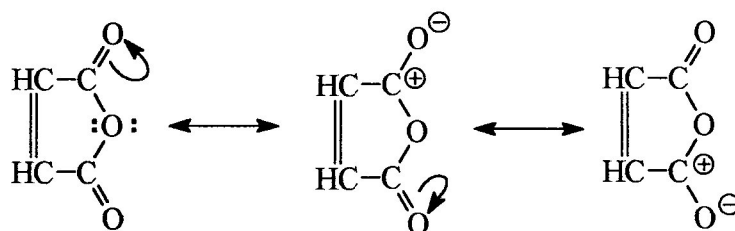
Reaction Mechanism:

Maleic anhydride is a very potent dienophile as it has two carbonyl groups on the carbon adjacent to the double bond. The carbon-oxygen bond of a carbonyl group is a reactive double bond. Its bond energy (179 Kcal) is more than two carbon-oxygen single bonds (2 x 8.5 Kcal), in contrast to the carbon-carbon double bond (145.8 Kcal), which is weaker than two carbon-carbon single bonds (2 x 82.6 Kcal). The greater strength of carbon-oxygen double bond as compared to the carbon-oxygen single bond is because repulsion between the unshared electrons is greater for single bonded oxygen than double bonded oxygen. The reactivity of carbonyl group is primarily due to the difference in electronegativity between carbon and oxygen,

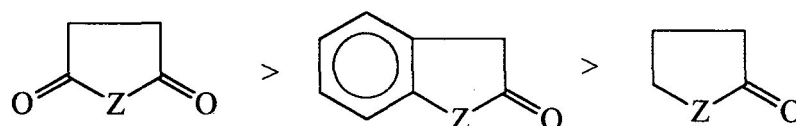
which leads to a considerable contribution of dipole response form with oxygen negative and carbon positive.



As the carbonyl groups are electron withdrawing due to the electronegativity of their oxygen atom, maleic anhydride has the following resonance structures.

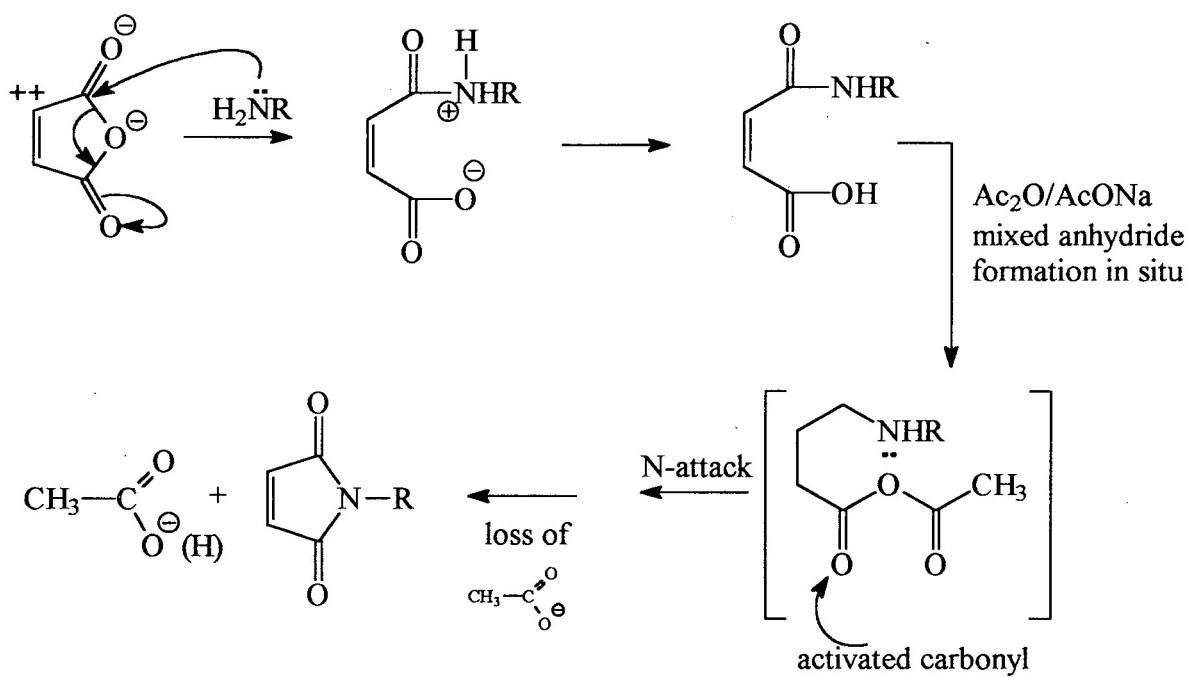


The unshared electrons on the nitrogen atom of amines play a key role in the reaction of amines with cyclic anhydrides. Nucleophilic reagents (amines) attack the carbonyl carbon atom of the cyclic anhydride. If the carbonyl group is adjacent to the hetero atom, the ring is opened, but if it is not adjacent, a carbonyl addition compound results. The ease of ring opening depends on the hetero atom ($S > O \gg NH$) and on the ring type.



Z = heteroatom

Ring opening by nucleophilic reagents necessitates the hetero atom in gaining a negative charge; if the nucleophile is uncharged a deprotonation step occurs during or following the nucleophilic substitution reaction. The probable reaction mechanism for formation of N-substituted aryl/alkyl and heteroaryl maleimide is shown below.



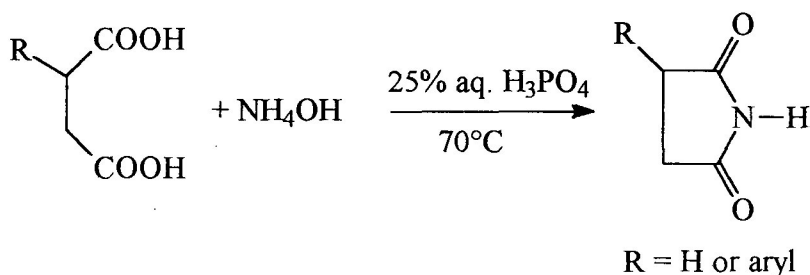
R = alkyl/aryl, etc

b) CHEMISTRY OF SUCCINIMIDES

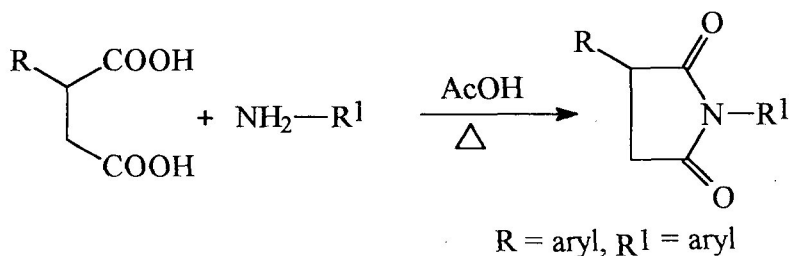
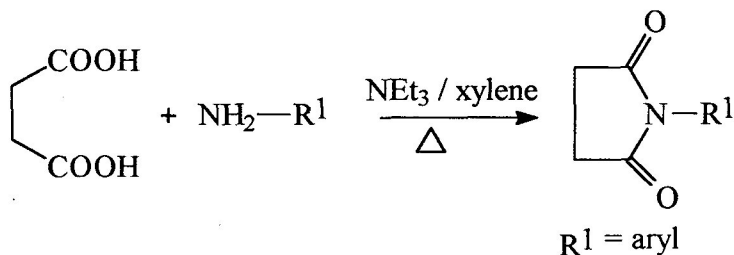
Succinimides were prepared as early as 1835¹¹⁸, not long after the first synthesis of an organic compound from inorganic material by Wohler in 1829. Succinimides can be synthesized by the cyclization of a variety of bifunctional derivatives, such as succinic acid, succinamides, succinic anhydride and maleamic acids, or by addition reaction with maleimides. The different methods of preparation of substituted succinimides are discussed below:

i) From succinic acid and succinic anhydride:

Lange *et al*¹¹⁹ synthesized substituted succinimides by heating substituted succinic acid at 175°C with ammonium hydroxide. Later, Fuerst *et al*¹²⁰ modified this method to obtain better yields of succinimides; the succinic acid was heated at 70°C with ammonium hydroxide in the presence of 25% aqueous phosphoric acid.

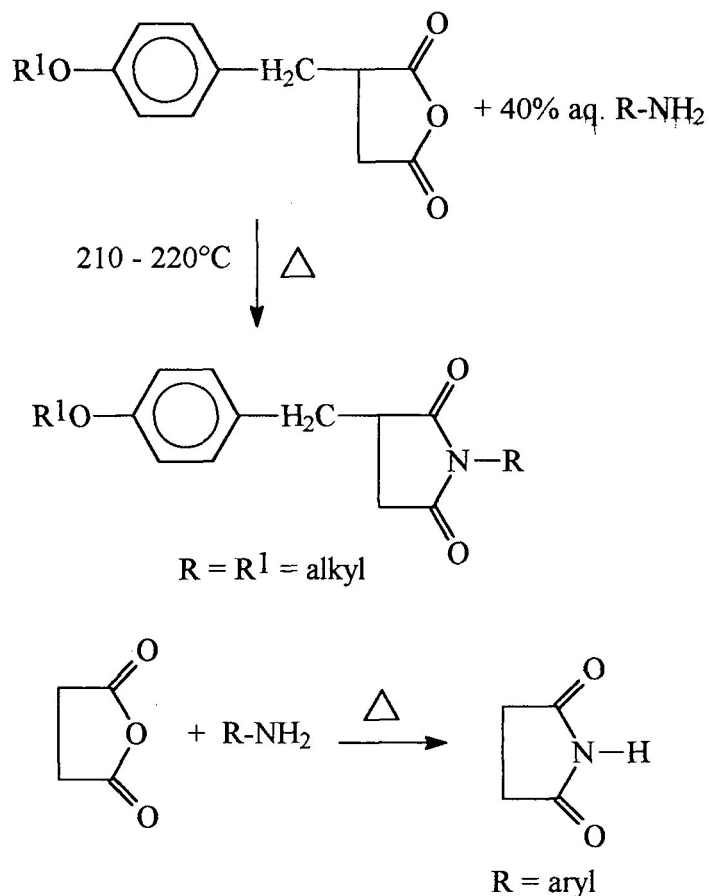


Heating succinic acid with an aryl or alkyl amine in an organic solvent like xylene, acetic acid, gives corresponding succinimide in good yields^{121,122}.



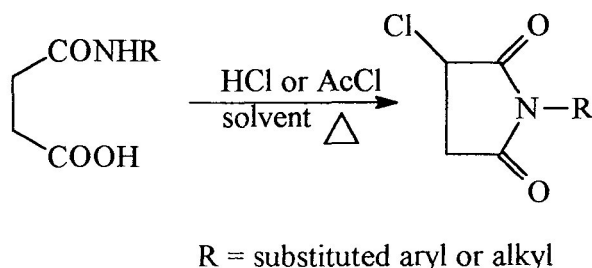
In 1951, Hoey and Lester¹²³ compared the yields of N-substituted succinimides formed by heating succinic acid with primary amines, either at 230-260°C in a sealed tube, or at 124-175°C followed by distillation of water. The latter method was found to give better yields of N-substituted succinimides.

Succinic anhydride when treated with an amine at 200-220°C in a solvent or even in the absence of it, also formed substituted succinimide¹²¹.

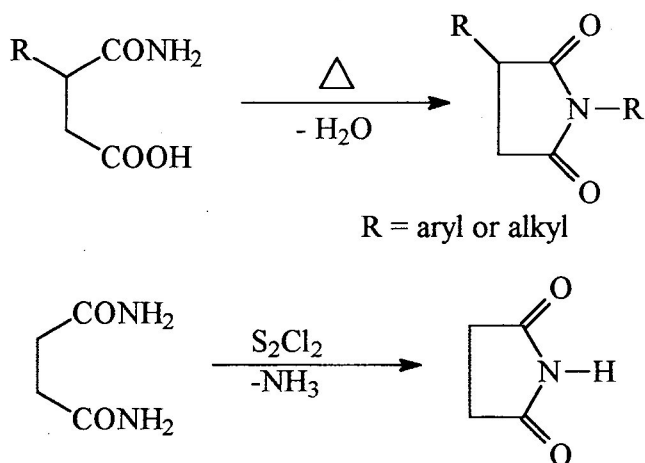


ii) From maleamic acid, succinamic acid and succinamide

Roderick¹²⁴ and Jakob¹²⁵ have reported that maleamic acids form succinimides when heated with hydrochloric acid or acetyl chloride in an organic solvent.

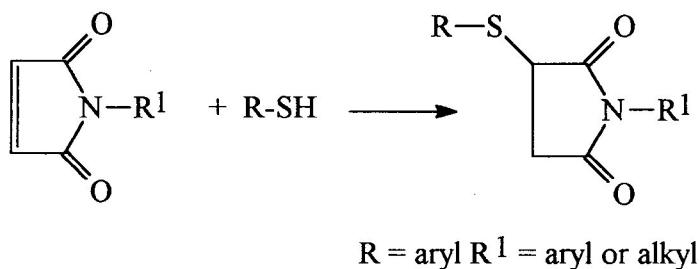


Succinamic acid and succinamide are both readily converted to succinimide when heated. Hope and Wiles¹²⁶ used sulfur monochloride to promote intramolecular elimination of ammonia from succinamide to give succinimide.

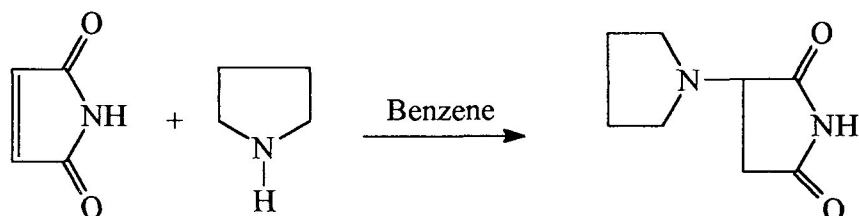
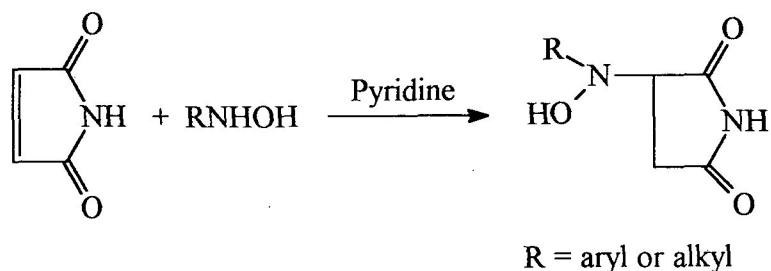


iii) From maleimides

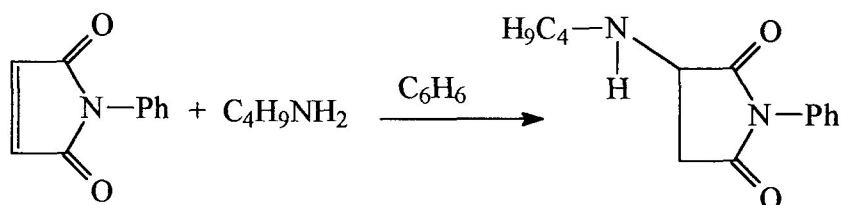
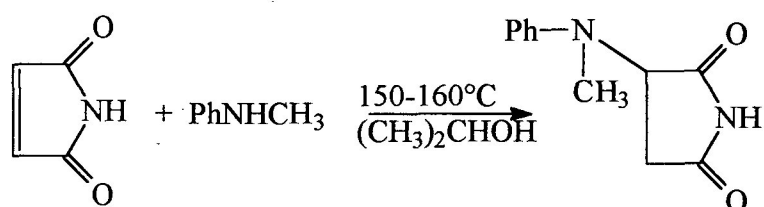
N-aryl, alkyl and heteroaryl maleimides add a number of reagents to give substituted derivatives of N-aryl, alkyl and heteroaryl succinimides. The reaction of mercaptoacetic acid, o-mercaptobenzoic acid and other arylthiols with N-ethyl or N-phenyl maleimides yields the product from addition of the S-H group to the carbon-carbon double bond^{127,128}.



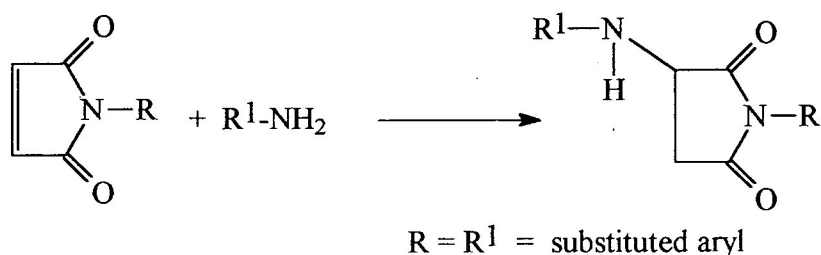
Primary and secondary amines add to the double bond in maleimide at room temperature in the cold or even at higher temperature to give succinimides; for example, phenyl hydroxylamine and hydroxylamine in dry pyridine add to the double bond to give the 4-(hydroxyamino) derivative¹²⁹ while the reaction between pyrrolidine and maleimide gives 4-(N-pyrrolidino) succinimide¹³⁰.



Dawson and co-workers^{105,131,132} prepared 4-(N-substituted)-N-aryl or alkyl succinimides by treating the appropriate maleimides with an amine. Thus, 4-(N-methylanilino) succinimide was obtained in about 50% yield by heating maleimide and N-methylaniline at 150-160°C, followed by the addition of isopropyl alcohol; due to steric hindrance high temperature is necessary for the completion of the reaction. 4-(N-butyl)-N-phenyl succinimide was obtained in about 62% yield by refluxing butylamine and N-phenyl maleimide in benzene.

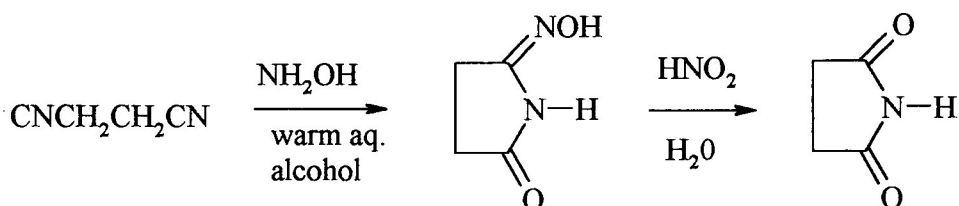


However, Kul'chitskaya *et al*¹³³ prepared 4-(substituted anilino)-N-aryl succinimides in 70-90% yields by arylation of the appropriate N-aryl maleimide with substituted aniline.

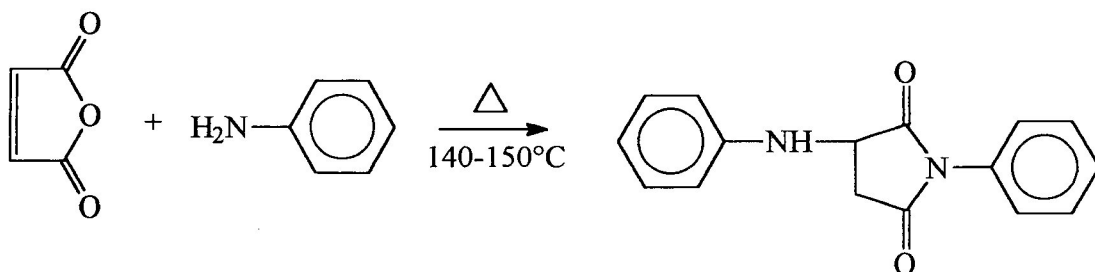


iv) Miscellaneous methods

The heating of an appropriate acid halide with an ammonium halide under anhydrous conditions can bring about the formation of cyclic imides such as succinimide¹³⁴. Succinonitriles may be treated in a solution of dry methanol with liquid ammonia to yield 2,5-diaminopyrrolidine, which may then be hydrolyzed in two steps to give succinimide. Succinonitrile reacts with hydroxylamine in warm aqueous alcohol solution to give 5-hydroxyimino-2-pyrrolidone, which can be treated with nitrous acid in aqueous acid so that succinimide is obtained¹³⁵.



Recently a new method was introduced by Padsalgikar¹³⁶ for the preparation of symmetrically substituted aryl amino succinimides. The aryl amino succinimides, with the same substituent on both the N-atoms, were prepared by heating a mixture of maleic anhydride and an aryl amine at 140-150°C.



Succinimides can also be prepared by treating a solution of acrylonitrile in dilute sulfuric acid with sodium cyanide¹³⁷. Scarpati¹³⁸ oxidised 2-formyl pyrrole with hydrogen peroxide in the presence of pyridine to obtain succinimide.

The reaction of acrylamide with carbon monoxide at 160-180°C and 100-300 atm. using Raney cobalt salts or cobalt carbonyls as catalyst, gave succinimide in 65-80% yields¹³⁹.

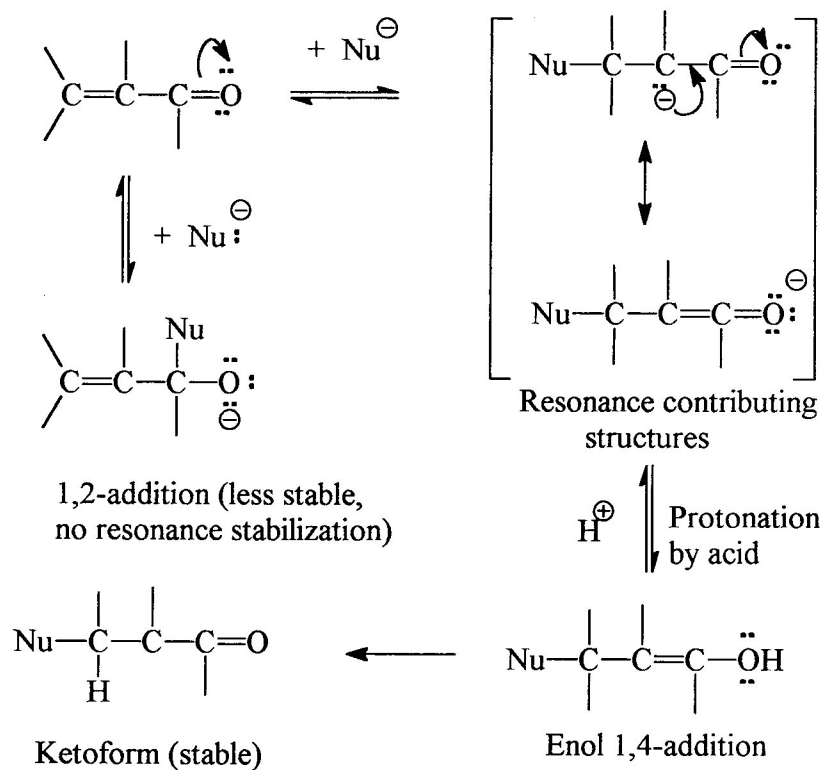
The 4 - [4'(4''- chlorophenoxy) - 3' - chloroanilino] - N - (alkyl / aryl / substituted aryl / heteroaryl) succinimides and 4-pyrrolidine anilino-N-(aryl / substituted aryl/ heteroaryl) succinimides were synthesized in 2 steps.

- i. The preparation of N-alkyl / aryl / substituted aryl and heteroaryl maleimides;
- ii. Reaction of 4(4'-chlorophenoxy)-3-chloroaniline and 1-(4-aminophenyl) pyrrolidino with substituted maleimides to obtain the corresponding succinimides.

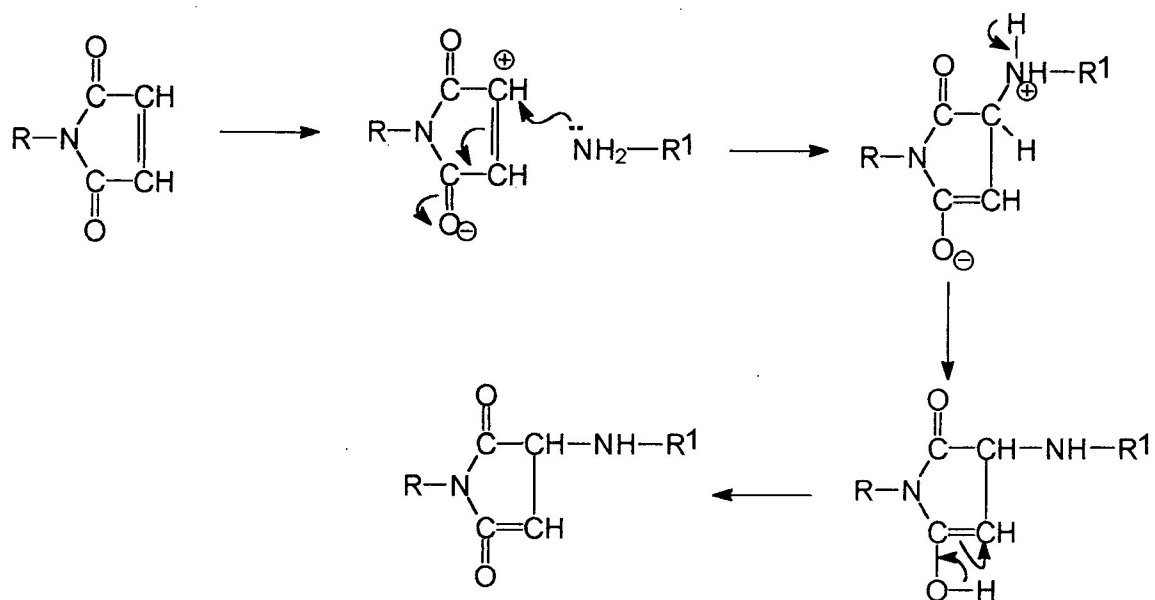
The N-substituted maleimides (0.001 mole) and 4(4'-chlorophenoxy)-3-chloroaniline (0.001 mole) or 1-(4-aminophenyl) pyrrolidine (0.001 mole) were stirred in an organic solvent for 6 hours to obtain the corresponding succinimides.

Reaction mechanism:

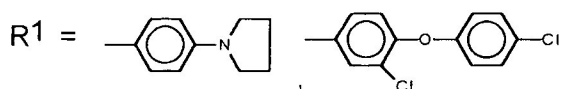
Maleimide and its N-substituted derivatives possess an electron deficient double bond. Primary and secondary amines undergo simple addition reaction across the double bond. The addition occurs in analogy to additions of amino compounds to α,β -unsaturated ketones²³¹. The carbon-carbon double bond is activated towards nucleophilic addition by the carbonyl group. Nucleophilic attack occurs at the carbon-carbon double bond to give the 1,4-addition product. Ammonia, primary and secondary amines add to α,β -unsaturated ketones to exclusively give the 1,4-addition product. Although the nucleophilic addition can also occur at the carbonyl carbon atom, the product formed is relatively unstable under the reaction conditions and is converted to the more stable 1,4- addition product¹⁴⁰.



The probable mechanism of addition of aryl amines across the double bond in the N-substituted maleimide is as follows: -



R = substituted aryl, alkyl or heteroaryl etc

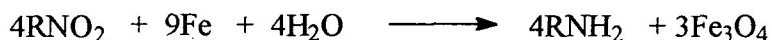


c) CHEMISTRY OF REDUCTION OF NITRO COMPOUNDS

It has long been known that the nature of the reduced product actually formed depends critically upon the reduction conditions. Thus, although nitro compounds were able to be reduced by using metals such as iron, tin, zinc (in acidic condition), sodium sulphide, ammonium polysulphide (in alkaline medium), ferric chloride, platinum dioxide and Raney Nickel etc., the preferred reduction reaction was performed by using activated Raney Nickel^{141,142}.

The reduction of nitro compounds involves the progressive replacement of oxygen in the $-\text{NO}_2$ group by hydrogen. Under suitable conditions aromatic nitro compounds are readily reduced to amino compounds by means of acidic, reducing systems such as metals like 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 by 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. Moreover it cannot be used for reduction of single nitro group on a polynitro compound, nor can it be used on substrates harmed by acid media (e.g. some ethers and thioethers). The 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 the retention of configuration¹⁴³. 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 aluminum 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. Successful efforts are to be

found out where an abundance of zinc is supplied during the entire reaction period. If the experimenter is fortunate, the hydrochloride of amine will separate; but if unfortunate, he must make the entire mixture strongly alkaline and extract with a suitable solvent. In this, very often a problem of emulsion formation takes place.

The present authors are impressed with the smooth generation of hydrogen from mossy tin and acid, but prejudiced the difficulty of work-up if the amine hydrochloride does not precipitate from the acid solution. The entire mixture should be added slowly to a strong alkaline solution. If tin hydroxide precipitates and remains for any length of time, no reasonable amount of sodium hydroxide dissolves the flocculant solid. Certainly, this method does not lend itself well to large scale reductions. On the other hand, stannous chloride is a useful and selective reducing agent for nitro groups when carbonyl groups are present, such as in reduction of m-nitrobenzaldehyde.

Amalgamated aluminium is another reducing agent best adopted to neutral or basic reducing conditions. For example, 2-nitroacenaphthene suspended in boiling ethanol, when treated with amalgamated aluminium and then portionwise with boiling water is reduced in 85% yield to the corresponding amino compound.

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 the catalyst.

Catalytic methods:

This method^{144,145} is clean and work-up is so easy that it should be considered the method of choice in the research laboratory. The only pitfalls are the possibility of poisoning of the catalyst or, for large-scale reductions, the inability to dissipate adequately the heat of hydrogenation¹⁴⁶.

The most common catalysts for the hydrogenation of nitro compounds are platinum (low pressure) and Raney nickel (high pressure). Supporting catalysts such as platinum on carbon^{147,148} palladium on carbon^{149,150,151} are also used.

Solvents and media used for catalytic reductions

Any solvent that is pure and does not react with the substrate or the 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 alcohols, particularly, methyl and ethyl alcohols. The vapours of methyl alcohol, however, may ignite in the presence of palladium or platinum catalyst, 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 a decrease in the reaction rate.

Solvents that form peroxide should be avoided. For example, dioxane should be used after thorough 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 the 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 a 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 the catalyst is placed in the reactor. Hydrogen is added upto 150 psi and the stirrer is started with heating.

The progress of the 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 other chemical reaction. After hydrogenation, most catalysts (except for copper chromate) are more pyrophoric because of the presence of adsorbed

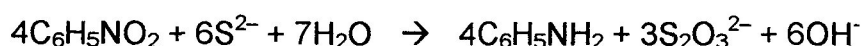
hydrogen on their surface. Therefore, care must be taken during filtration. The filter paper must be covered with liquid at all times and should not be allowed to become dry. Any spent catalyst can be destroyed by digestion in hydrochloric acid. Do not heat the reaction mixture to the desired working temperature before starting agitation. This may be safe under low pressure conditions but can be hazardous in high-pressure reductions.

Zinin reduction

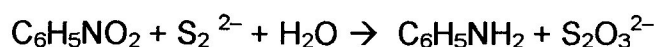
The zinin reduction is a method for the reduction of nitro compounds by negative divalent Sulfur (Sulfide, sulfhydrylate, and polysulfides). This versatile reaction has been used for plant-scale manufacture of aromatic amines when other reduction media are destructive to sensitive compounds, or result in undesired side reactions. It is used with more sensitive compounds that would not be compatible with acid media. A closer look at the technology may lead to the development of processes, which offer advantages over many current catalytic and iron reduction methods. For instance, a recent article describes a continuous process for reducing 1-nitronaphthalene with aqueous sodium disulfide¹⁵².

Mechanism:

The stoichiometry of the zinin reduction is illustrated by the original zinin reduction of nitrobenzene with aqueous ammonium sulfide.



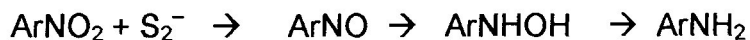
If reductant is disulfide, the equation is analogous to



The medium generally used was aqueous methanol and the rate increased rapidly with increasing concentration of water. It was shown that when sufficient alkali present to keep equilibrium for on the side of disulfide ion, the rate of is first order. Electron-withdraw in substituents speed up the reaction considerably.



Azoxybenzene reduces very slowly as compared to nitrobenzene. These observations suggest that the rate determining step is the attack of disulfide ion on the nitro group. Probably the first product is nitroso, compound, which is rapidly reduced to a hydroxyl amine and then to amine.



The kinetic studies suggest that the disulfide ion be used rather than the sulfide ion. As high a concentration of water should be used as is consistent with the complete or partial solution of the nitro compound in the reaction mixture. Enough excess alkali should be used to have almost all the reductant present as sulfide ion or disulfide ion and excess reductant should be used to ensure a rapid reduction that does not stop at an intermediate stage¹⁵².

Application:

Generally, zinin reaction is applied to the reduction of mono-and poly-nitro arenes to the corresponding amines and nitro amines, but has found application in the conversion of some nitroso and azo compounds to amines. It can also be used for the preparation of some hydroxylamines and benzotriazoles, starting with the nitro compounds.

Generally, the reagents used are ammonium, sodium or potassium sulfides, hydrosulfides, or polysulfides. Manganous sulfide and ferrous sulfide are also used. An improvement in reductions with alkali sulfides by the addition of selenium has been suggested¹⁵³.

The positions of the substituents play an important role in the progress of the reaction. e.g. in dinitro and trinitrophenols and their ethers, a nitro group ortho to the hydroxy or alkoxy is group preferentially reduced.

Generally, catalytic hydrogenation requires more expensive equipment and the techniques are considerably more difficult if one takes into account catalyst preparation, catalyst poisoning hazards, and risk of reducing other groups.

d) CHEMISTRY OF N-NITROSATION OR N-NITROSO-DE-HYDROGENATION OF AMINES

When secondary amines are treated with nitrous acid, N-nitroso compounds are formed¹⁵⁴.

The reaction can be accomplished by using dialkyl, diaryl, or alkyl arylamines, and even with mono-N-substituted amides.

Tertiary amines have also been N-nitrosated, but in these cases one group cleaves, so that the product is the nitroso derivative of the secondary amine¹⁵⁵. The group that cleaves appears as an aldehyde or ketone.

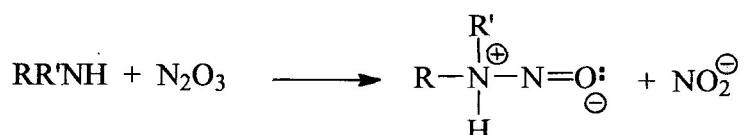
Other reagents have also been used, for example NOCl, which is useful for amines or amides that are not soluble in acidic aqueous solutions, or when the N-nitroso compounds are highly reactive. N-nitroso compounds are also formed by treatment of amines with gaseous N₂O₃ or N₂O₄¹⁵⁶.

The mechanism of nitrosation is as shown below-

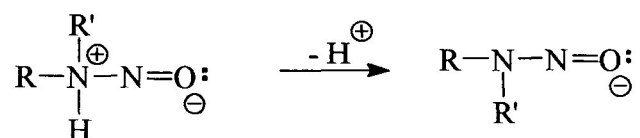
Step I



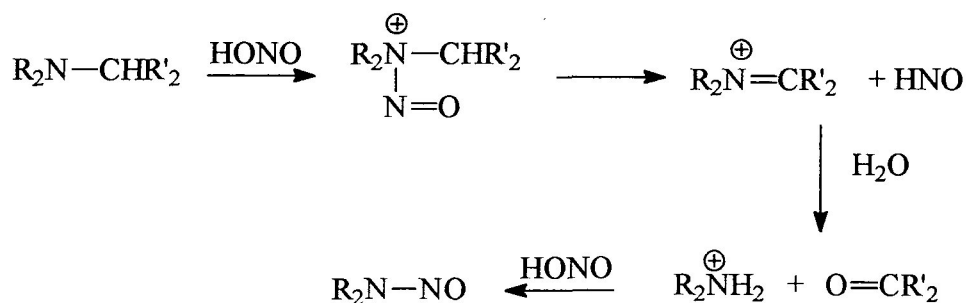
Step II



Step III



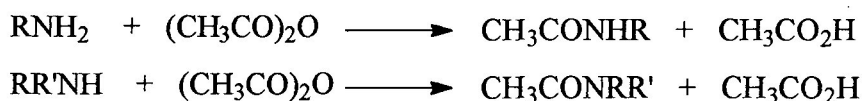
Since this species cannot lose a proton, it is stable and the reaction ends there. The following has been the mechanism for the reaction with tertiary amines¹⁵⁷.



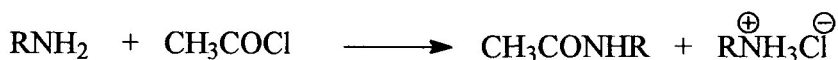
Generally, nitrosation requires an active substrate because NO^+ is much less reactive than NO^{2+} . Kinetic studies have shown that NO^+ is at least 10^{14} times less reactive than NO^{2+} . Tertiary aromatic amines substituted in the ortho position generally do not react with HONO, probably because the ortho substituent prevents planarity of the dialkylamino group, without which the ring is no longer activated. This is an example of steric inhibition of resonance. Addition of Lewis acid increases the rate of nitrosation, suggesting that nitrosating species is polarized NO^+NO^- .¹⁵⁸

CHEMISTRY OF ACETYLATION OF AMINES

Primary and secondary amines are best acetylated with acetic anhydride :



Acetyl chloride is not so satisfactory since an equivalent quantity of the amine hydrochloride is simultaneously produced.



Methods

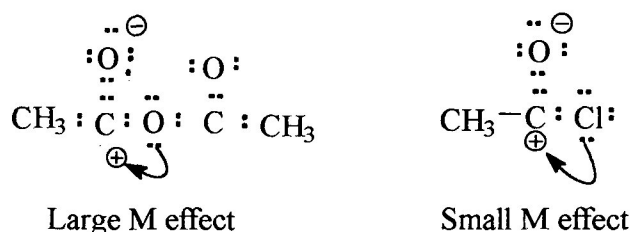
1) Reflux gently in a test tube under a short air condenser 1 g of the base with 2.5 moles [or 3.0 g (3.0 ml) if molecular weight is unknown] of acetic anhydride for 10-15 minutes. Cool the reaction mixture and pour it into 20 ml of cold water. Boil to decompose the excess of acetic anhydride. When cold, filter the residual insoluble acetyl derivative and wash it with a little cold water. Recrystallise from water or from dilute ethanol.

Certain ortho substituted derivatives of aromatic amines are difficult to acetylate under the above conditions owing to steric hindrance. The process is facilitated by the addition of a few drops of concentrated sulphuric acid, which acts as a catalyst, and the use of a large excess of acetic anhydride.

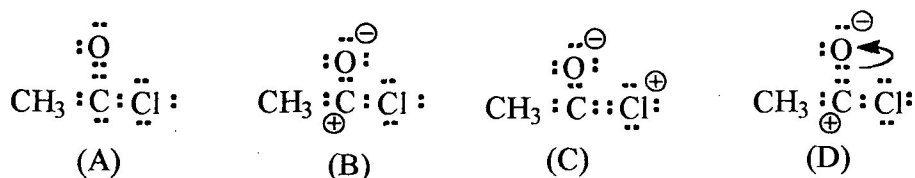
2) Excellent results may be obtained by conducting the acetylation in aqueous solution. Dissolve 0.5 g of the amine in 2 M hydrochloric acid, and add a little crushed ice. Introduce a solution of 5 gm of hydrated sodium acetate in 25 ml. of water, followed by 5 ml. of acetic anhydride. Shake the mixture in the cold until the smell of acetic anhydride disappears. Collect the solid acetyl derivative, and recrystallise it from water or dilute alcohol¹⁵⁹.

The acid anhydride resembles the acid chlorides as acylating agents. The chief difference between these two types of compounds in their acylating activity is that the anhydrides are less vigorous in their reaction and, therefore, often more convenient than the acid chlorides, which sometimes are quite violent. Acetic anhydride is used as an acetylating agent in the production of cellulose acetate, aspirin, acetanilide, as well as a large number of industrial and medicinal products.

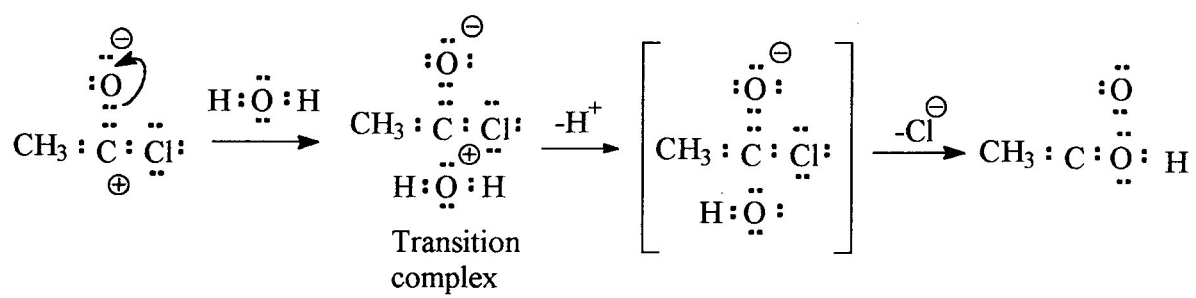
The fact that acetic anhydride reacts more slowly than acetyl chloride is a logical consequence of its electronic structure. In acetic anhydride, the acetate group has a greater tendency to exhibit an M effect than does the chlorine atom in acetyl chloride. Consequently, the rate of which the acid anhydride can react with an electron donor (water, alcohol, ammonia and so forth) is much slower than the rate of the corresponding reaction of an acid chloride¹⁶⁰.



The mechanism of the reaction between electron donors, such as the hydroxyl and amino groups, and an acid chloride (acetyl chloride, for example) is easily understood by considering the electronic formulas of an acid chloride.



In formula A, the carbon atom of the carbonyl group has its full complement (Octet) of electrons and is comparatively unreactive. In formula B, however, the carbonyl carbon has but six electrons and may act as an acceptor of electrons from a group (or molecule), which is an electron donor. Since the chlorine atom is a poor electron donor, the highly polarized form of the carbonyl group (formula B) is only slightly decreased by a shift of electrons from the chlorine atom (formula C). Over-all the carbonyl carbon atom is somewhat deficient in electrons (formula D) and acts as an electron acceptor from an atom which has unshared electrons. For example, the reaction of acetyl chloride and water is initiated by the formation of a transition complex between the carbonyl carbon atom (electron poor) and the oxygen atom (electron rich)¹⁶⁰.



Part ID

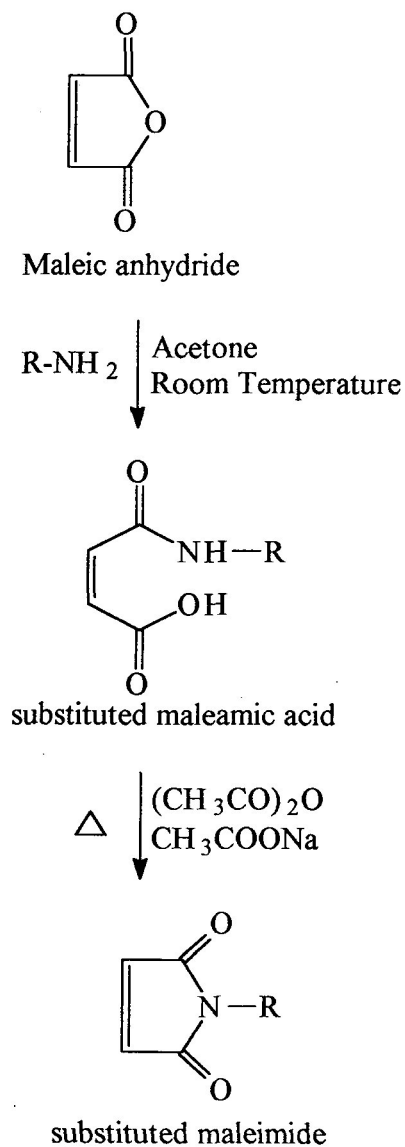
***Schematic Representation
of series of compounds
synthesized and drug
intermediate***

Part ID

**SCHEMATIC REPRESENTATION OF THE SERIES OF COMPOUNDS
SYNTHESIZED AND DRUG INTERMEDIATES**

Scheme 1

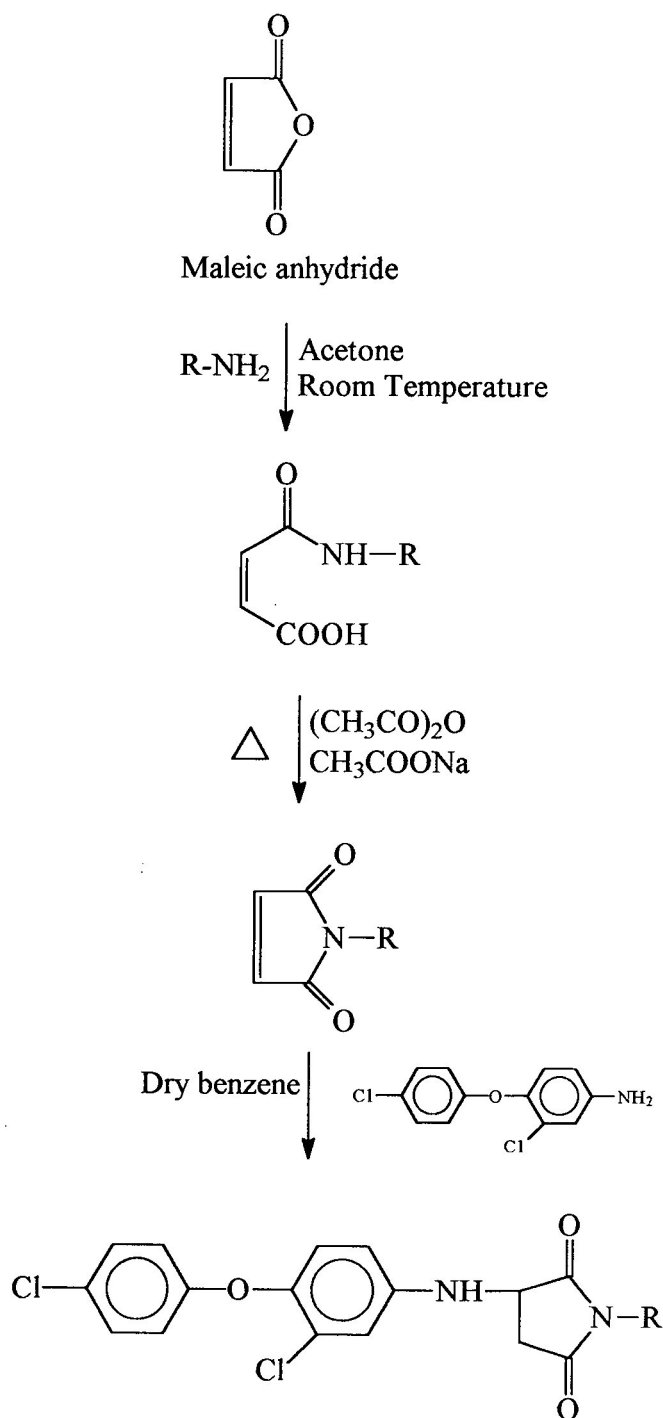
Synthesis of N-alkyl/aryl/substituted aryl and heteroaryl maleimides



where R = alkyl / aryl / substituted
aryl and heteroaryl

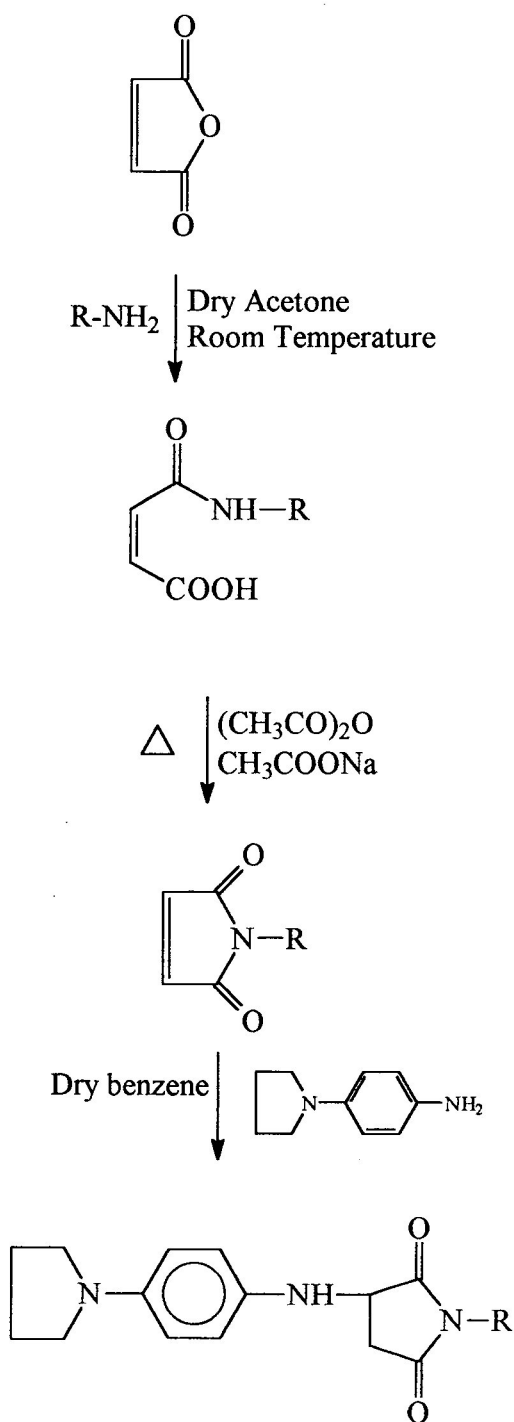
Scheme 2

Synthesis of 4 [4'(4''-chlorophenoxy)-3'-chloroanilino]-N-(alkyl / aryl / substituted aryl and heteroaryl) succinimides



where, R = C_6H_5 , $CH_2C_6H_5$,
Ph- $OC_4Hg(n)$ etc

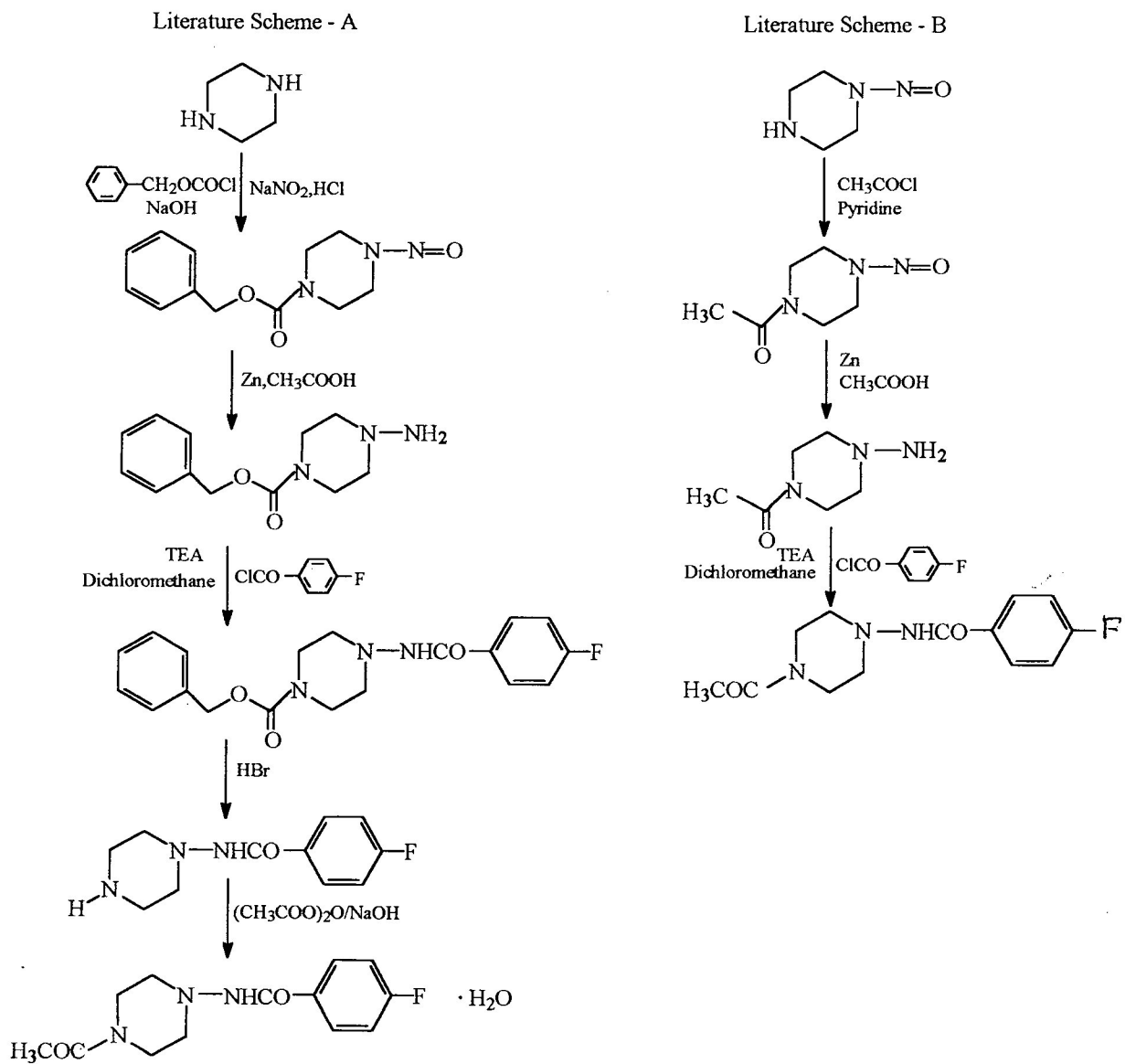
Scheme 3
Synthesis of 4-(pyrrolidino anilino)-N-(aryl / substituted aryl and heteroaryl)
succinimides



where, $R = Ph, CH_2-C_6H_5,$
 $Ph-OC_3H_7(n),$ etc

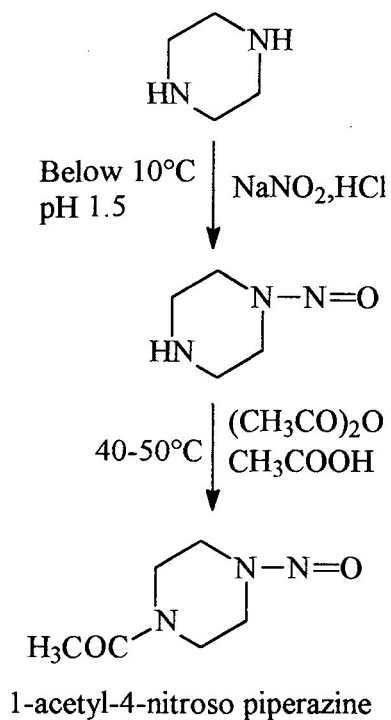
Scheme 4

Synthesis of N-(4-acetyl-1-piperazinyl)-4-fluorobenzamide monohydrate



Scheme 5 (modified scheme)

Synthesis of 1-acetyl-4-nitroso piperazine as a drug intermediate for N-(4-acetyl-1-piperazinyl)-4-fluorobenzamide monohydrate (FK-960)



Experimental

Part II

***Synthesis of 4[4'(4"-
chlorophenoxy)-3'-
chloroanilino]-N-
(alkyl/aryl/substituted
aryl and heteroaryl)
succinimides***



Part II

Synthesis of 4[4'(4''-chlorophenoxy)-3'-chloroanilino]-N-(alkyl/aryl / substituted aryl and heteroaryl) succinimides.

This part deals with the synthesis of 4[4'(4''-chlorophenoxy)-3'-chloroanilino]-N-(alkyl / aryl / substituted aryl and heteroaryl) succinimides which involves the following steps.

- A. Synthesis of N -alkyl / aryl / substituted aryl and heteroaryl maleimides
- B. Synthesis of 4(4'-chlorophenoxy)-3-chloroaniline
- C. Synthesis of 4[4'(4''-chlorophenoxy)-3'-chloroanilino]-N-(alkyl / aryl / substituted aryl and heteroaryl) succinimides.

A. Synthesis of N- alkyl / aryl / substituted aryl and heteroaryl maleimides

a) Synthesis of N-phenyl maleimide

To a solution of maleic anhydride (8 gm., 0.081 M) in dry acetone, taken in a three necked flask equipped with a mercury seal mechanical stirrer, dropping funnel and reflux condenser carrying guard tube, aniline (7.59 gm, 7.44 ml., 0.081 M) dissolved in dry acetone was added at room temperature, with vigorous stirring. The N-phenyl maleamic acid which separated out within one hour of stirring was filtered and washed with dry acetone, and dried.

Yield: 11.72 gm. (75.2%)

M.P. 208-209°C

This N-phenyl maleamic acid (11.72 gm. 0.061 M) was heated in a 100 ml round bottomed flask fitted with a reflux condenser and a guard tube, along with anhydrous sodium acetate (2.41 gm, 0.029 M) and acetic anhydride (20.4 gm., 0.2 M, 18.88 ml) at 100°C for 2-3 hours. The reaction mixture was then poured on crushed ice. The solid that separated out was filtered, washed with water and dried. The crude product was crystallised from ethanol to obtain pure N-phenyl maleimide. Purity was checked by T.L.C. using ethyl acetate: Hexane (1:4) system.

Yield : 7.4 gm (70.4%)

M.P. 89-90°C

(Lit¹⁰⁷ 89-89.8°C)

b) Preparation of N-(p-n-propoxy phenyl) maleimide

It involves the following steps

Synthesis of N-Propyl bromide

To a 48% hydrobromic acid (25 gm., 16.8 ml; 0.31 M) solution contained in 250 ml three necked flask fitted with a mechanical stirrer, dropping funnel and a distillation unit, concentrated sulfuric acid (7.5 gm; 4.1 ml; 0.076 M) was added in portions, with constant stirring. The reaction mixture was cooled to room temperature, and n-propyl alcohol (7.2 g; 855 ml; 0.12 M) was added to it in portions. Then, concentrated sulfuric acid (6 gm, 3.28ml, 0.062M) was then gradually introduced and the reaction mixture was simultaneously slowly distilled until no more oily drops of n-propyl bromide passed over. The crude product was washed successively with equal volumes of water, concentrated hydrochloric acid, water, 5% sodium bicarbonate solution and water. The oily layer was separated, dried over calcium chloride and distilled to obtain pure n-propyl bromide

Yield : 12.05 gm (81.6%),

B.P. 69-72°C

(Lit¹¹⁵. B.P. 70-72°C)

Synthesis of 4-n-propoxy acetanilide

A solution of paracetamol (9.6 g, 0.06 M), potassium hydroxide (3.36 g, 0.06 M) and n-propyl bromide (7.38g; 0.06 M) in ethanol (50 ml) were taken in a round bottomed flask and refluxed on a steam bath for 4 hours, in the absence of atmospheric humidity. The reaction mixture was filtered and the filtrate was concentrated under reduced pressure to obtain crude 4-n-propoxy acetanilide

Yield : 9.84 gm. (84.98%)

M. P. 119-120°C

(Lit¹¹⁶ M.P. 118-120°C)

Synthesis of 4-n-propoxy aniline

4-n-propoxy acetanilide (7.8 gm, 0.04 M) and 18% hydrochloric acid (30 ml) was taken in a round bottomed flask equipped with reflux condenser and heated on a

burner for 2 hours. The mixture was then cooled to 0°C and made alkaline with 10% sodium hydroxide solution. The oil which separated after neutralization was extracted with ether. The ether extract was washed with water, dried over sodium sulfate and concentrated. The residual oil was distilled under reduced pressure to obtain pure 4-n-propoxy aniline.

Yield : 5.1 gm. (83.6%)

B.P. 136-138°C / 11 mm

(Lit¹¹⁶ B.P. 137°C / 11 mm)

Synthesis of *N*-(4-n-propoxy phenyl) maleimide

To a solution of maleic anhydride (3.30 g; 0.033 M) in dry acetone (50 ml) taken in a three necked flask equipped with a mercury seal mechanical stirrer, dropping funnel and reflux condenser carrying a guard tube, p-n-propoxy aniline (5.1 gm, 0.033 M) was added at room temperature with vigorous stirring. The 4-n-propoxy phenyl maleamic acid which separated out within one hour of stirring was filtered, washed with dry acetone, and dried.

Yield : 4.30 g (84.64 %)

M.P. 192°C

This 4-n-propoxy phenyl maleamic acid (4.30 gm, 0.017 M) was heated in a 100 ml round bottomed flask, fitted with reflux condenser and a guard tube, along with anhydrous sodium acetate (0.69 g, 0.0085 M) and acetic anhydride (5.2 gm, 0.051 M, 4.81 ml) at 100°C for 2-3 hours the reaction mixture was then poured on crushed ice and the separated solid was filtered, washed with water and dried. The crude compound was crystallized from ethanol to obtain pure *N*-(4-n-propoxy phenyl) maleimide. Purity was checked by T.L.C. using ethyl acetate: Hexane (1:4) system.

Yield : 2.85 g (72.3%)

M. P. 96°C

c) Synthesis of *N*-(4-benzene carboxylic acid) maleimide

To a solution of maleic anhydride (3.26; 0.033 M) in dry acetone (30 ml), taken in a three necked flask equipped with a mercury seal mechanical stirrer, dropping funnel

and reflux condenser carrying a guard tube, 4-aminobenzoic acid (4.56 g, 0.033 M) dissolved in dry acetone was added at room temperature, with vigorous stirring. The 4-benzene carboxylic maleamic acid which separated out within one hour of stirring, was filtered washed with dry acetone and dried.

Yield 6.65 g (85.0 %)

M. P. 230°C

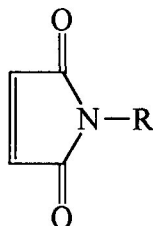
This 4-benzene carboxylic maleamic acid (6.65g, 0.033 M) was heated in a 100 ml round bottomed flask fitted with a reflux condenser and guard tube along with anhydrous sodium acetate (1.35 g, 0.0165 M) and acetic anhydride (10.09 g., 0.099 M, 9.35 ml)) at 100°C for 2-3 hrs. The reaction mixture was poured on crushed ice and the solid that separated out was filtered, washed with water and dried. The crude compound was crystallized from ethanol to obtain pure N-(4-benzene carboxylic acid) maleimide. Purity was checked by T.L.C. using ethyl acetate hexane (1:4) system.

Yield 4.56 gm (74.3%)

M.P. 260°C

Similarly, all other N-substituted maleimides were prepared by the above procedure. The physical data of these synthesized maleimides is recorded in Table 1, pg 77.

Table I
N- alkyl / aryl / substituted aryl and heteroaryl maleimides



Sr No	R	Mol. Formula	Mol. weight	M.P. (°C)	Yield (%)	Elemental Analysis(%)					
						Calculated			Found		
						C	H	N	C	H	N
1	Methyl	C ₅ H ₅ NO ₂	111	94-96	84.7	54.05	4.50	12.61	54.35	4.71	12.85
2	Phenyl	C ₁₀ H ₇ NO ₂	173	89-90	83.5	69.36	4.06	8.09	69.56	4.25	8.3
3	Benzyl	C ₁₁ H ₉ NO ₂	187	92	71.27	70.58	4.81	7.40	70.19	5.02	7.09
4	4-chlorophenyl	C ₁₀ H ₆ NO ₂ Cl	207.5	119-120	75.4	57.83	2.89	6.74	57.75	3.00	6.85
5	4-carboxylic phenyl	C ₁₁ H ₇ NO ₄	217	260	78.7	60.82	3.22	6.45	60.61	3.00	6.74
6	4-methoxy phenyl	C ₁₁ H ₉ NO ₃	203	147	76	65.02	4.43	6.89	65.35	4.53	7.16
7	4-ethoxy phenyl	C ₁₂ H ₁₁ NO ₃	217	132	74.2	66.19	5.07	6.45	66.35	5.28	6.84
8	4-n-propoxy phenyl	C ₁₃ H ₁₃ NO ₃	231	96	81.2	67.53	5.63	6.06	67.89	5.45	5.91
9	4-n-butoxy phenyl	C ₁₄ H ₁₅ NO ₃	245	81	80.6	68.51	6.12	5.72	68.40	5.96	5.93
10	4-nitro diphenyl sulfide	C ₁₆ H ₁₀ N ₂ O ₄ S	326	182	67.9	58.89	3.06	8.58	59.10	3.35	8.88
11	3- quinoliny	C ₁₃ H ₈ N ₂ O ₂	224.	142	69.2	69.64	3.57	12.50	69.31	3.82	12.79
12	8-quinoliny	C ₁₃ H ₈ N ₂ O ₂	224	218	66.8	69.64	3.57	12.50	70.02	3.34	12.75

B) Synthesis of 4(4'-chlorophenoxy)-3-chloroaniline

It involves 2 steps

i) Synthesis of 4(4'-chlorophenoxy)-3-chloronitro benzene

To a solution of 4-chloro phenol (18 gm, 0.13 M) in dry DMF taken in a three necked flask equipped with a mercury seal mechanical stirrer, dropping funnel and reflux condenser carrying a guard tube, Potassium carbonate (19.20 g, 0.13 M) was added. This reaction was allowed to continue at 40-50°C for 1-1.5 hours, and then 3,4-dichloro nitro benzene (26.68 g, 0.13 M) dissolved in dry DMF was added to the reaction mixture through the dropping funnel over a period of one hour. The reaction was then allowed to continue at 110-130°C for 7-8 hours in an oil bath. The reaction was monitored on T.L.C. using Ethyl acetate: Hexane (1:4) system.

After completion of reaction the whole reaction mixture was dumped on crushed ice and a yellow coloured solid that separated out was filtered, washed with water and dried. The crude compound was crystallized from methanol to obtain pure 4(4'-chlorophenoxy)-3-chloro nitrobenzene. The purity was checked by T.L.C. using ethyl acetate : Hexane (1:4) system.

Yield : 29.96 gm (75.4%)

M.P. : 108-109°C

(Lit ¹⁶¹ M.P. 108-110°C)

ii) Reduction of 4 (4'-chlorophenoxy)-3-chloro nitrobenzene to obtain 4(4'-chlorophenoxy)-3-chloroaniline.

The title compound was obtained by 2 reductive procedures

a) In a pressure vessel, 4(4'-chlorophenoxy)-3-chloronitrobenzene (4 gm, 0.014 M) in methanol was taken. The reaction mixture was heated on water bath before placing it in the pressure vessel, to dissolve the compound. To the reaction mixture Raney Ni catalyst (8.5%) was added. The pressure flask was first flushed with nitrogen and then hydrogen gas was passed in the flask upto 70 psi. It was heated upto 50-60°C, with stirring for 2 hrs. The reaction mixture was allowed to cool down to room temperature and Raney nickel was filtered out. The Raney Ni was

decomposed with 10% HCl. To the filtrate charcoal treatment was given and it was concentrated under reduced pressure to get 4(4'-chlorophenoxy)-3-chloroaniline.

Yield : 2.94 gm (82.3%)

M.P. : 80°C

Lit¹⁶² M. P. 80-81°C

b) To a solution of 4(4'-chlorophenoxy)-3-chloronitro benzene (4 gm, 0.014 M) in methanol taken in a 3 necked flask equipped with a mercury seal mechanical stirrer, and reflux condenser hydrated sodium polysulphide (16gm) was added in portions over a period of 1 hour. Then the reaction mixture was refluxed for 6 hours and was monitored on T.L.C. using Ethyl acetate : Hexane (1:4) system.

After completion of the reaction, methanol was removed under reduced pressure. The residue obtained was dumped in water. The creamish coloured product obtained was crystallized from methanol to obtain pure 4(4'-chlorophenoxy)-3-chloroaniline. The purity was checked by T.L.C. using Ethyl acetate : Hexane (1:4) system.

Yield : 2.59 gm (72.6%)

M.P. : 81°C

Lit¹⁶² M.P. 80-81°C

The yield of sodium polysulphide reduction method is less, but it is easy and less hazardous and does not involve use of complicated systems for reduction. Therefore Sodium sulphide reduction method was successfully used in this project.

C) Synthesis of 4[4'(4''-chlorophenoxy)-3'-chloroanilino]-N-(alkyl / aryl / substituted aryl and heteroaryl) succinimides

a) Preparation of 4[4'(4''-chlorophenoxy)-3'-chloroanilino] -N- phenyl succinimide
(Compound No. 2 Table II, pg 81)

In a 50 ml. round bottomed flask fitted with a guard tube, N-phenyl maleimide (0.33 gm; 0.00196 M) and dry benzene (25 ml) were placed. To this, 4(4'-chlorophenoxy)-3-chloroaniline (0.50 gm, 0.00196 M) was added at room temperature and the reaction mixture was stirred at room temperature for 6 hours. After discarding a small

amount of sticky mass which formed on chilling the solution, the mother liquor was concentrated under reduced pressure. Addition of petroleum ether to the solution gave the crude product, which was then purified from methanol to obtain the pure product. The purity was checked by T.L.C. using Toluene : Methanol (10 : 1) system.

Yield : 0.5 gm. (59.88%)

Elemental analysis

	C%	H%	N%
Calculated	61.82	3.47	6.55
Found	61.55	3.43	6.54

b) Preparation of 4[4'(4''-chlorophenoxy)-3'-chloroanilino]-N-(4-n-propoxyphenyl)succinimide (Compound No. 8, Table II, pg. 81)

In a 50 ml. round bottomed flask fitted with a guard tube, N-4-n-propoxy phenyl maleimide (0.45 gm, 0.00196 M) and dry benzene (25ml) were placed. To this 4(4'-chlorophenoxy)-3-chloroaniline (0.50 gm, 0.00196 M) was added at room temperature and the reaction mixture was stirred at room temperature for 6 hours. After discarding a small amount of sticky mass which formed on chilling the solution, the mother liquor was concentrated under reduced pressure. Addition of petroleum ether to the solution gave the crude product, which was then purified from methanol to obtain the pure product. The purity was checked by T.L.C. using Toluene: Methanol (10 : 1) system.

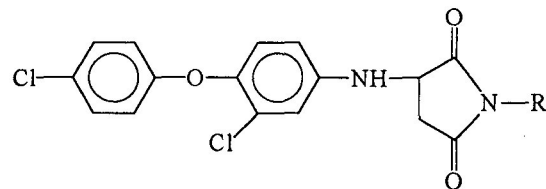
Yield : 0.58 gm (61.06%)

Elemental analysis

	C%	H%	N%
Calculated	61.85	4.53	5.77
Found	61.50	4.32	5.71

Similarly, the other 4[4'(4''-chlorophenoxy)-3'-chloroanilino]-N- alkyl / aryl / substituted aryl and heteroaryl succinimides were prepared and are tabulated with their physical data in table II, pg 81. The spectral data of the representative compounds has been included in the section – 'Spectroscopic characterization'

Table II
4 [4'(4''-chlorophenoxy)-3'- chloroanilino]-N-(alkyl /aryl / substituted aryl and heteroaryl) succinimides



Sr No	R	Mol. Formula	Mol. weight	M.P. (°C)	Yield (%)	Elemental Analysis (%)					
						Calculated			Found		
						C	H	N	C	H	N
1	methyl	C ₁₇ H ₁₄ N ₂ O ₃ Cl ₂	365	Oil	62.31	55.89	3.83	7.67	56.01	3.84	8.06
2	phenyl	C ₂₂ H ₁₆ N ₂ O ₃ Cl ₂	427	Oil	59.88	61.82	3.47	6.55	61.55	3.43	6.54
3	benzyl	C ₂₃ H ₁₈ N ₂ O ₃ Cl ₂	441	Oil	56.60	62.58	4.08	6.34	62.33	4.40	6.46
4	4-chlorophenyl	C ₂₂ H ₁₅ N ₂ O ₃ Cl ₃	461.5	Oil	60.00	57.20	3.25	6.06	57.59	3.44	5.83
5	4-carboxylic phenyl	C ₂₃ H ₁₆ N ₂ O ₅ Cl ₂	471	110-112	58.2	58.59	3.39	5.94	58.59	3.40	6.11
6	4-methoxy phenyl	C ₂₃ H ₁₈ N ₂ O ₄ Cl ₂	455	Oil	59.77	60.65	3.95	6.15	60.29	3.75	5.75
7	4-ethoxy phenyl	C ₂₄ H ₂₀ N ₂ O ₄ Cl ₂	471	180-182	60.5	61.14	4.24	5.94	60.83	4.10	6.24
8	4-n-propoxy phenyl	C ₂₅ H ₂₂ N ₂ O ₄ Cl ₂	485	Oil	61.06	61.85	4.53	5.77	61.50	4.32	5.41
9	4-n- Butoxy phenyl	C ₂₆ H ₂₄ N ₂ O ₄ Cl ₂	499	Oil	61.85	62.52	4.80	5.61	62.82	4.64	5.37
10	4-Nitro diphenyl sulfide	C ₂₈ H ₁₉ N ₃ O ₅ SCl ₂	580	126-128	52.12	57.93	3.27	7.24	57.58	3.12	7.34
11	3- Quinoliny	C ₂₅ H ₁₇ N ₃ O ₃ Cl ₂	478	Oil	60.4	62.76	3.55	8.78	62.74	3.47	8.74
12	8-quinoliny	C ₂₅ H ₁₇ N ₃ O ₃ Cl ₂	478	Oil	60.43	62.76	3.55	8.78	62.83	3.28	8.71

Part III

***Synthesis of 4-(pyrrolidino
anilino)-N-((aryl /
substituted aryl and
heteroaryl) succinimides***

Part III

Synthesis of 4-(pyrrolidino anilino)-N-(aryl / substituted aryl and heteroaryl) succinimides

This part deals with the synthesis of pyrrolidine anilino-N-aryl / substituted aryl and heteroaryl succinimides which involves the following steps

- A. Synthesis of N-aryl / substituted aryl and heteroaryl maleimides
- B. Synthesis of 1-(4-aminophenyl) pyrrolidine
- C. Synthesis of 4-(pyrrolidino anilino)-N-(aryl / substituted aryl and heteroaryl) succinimides.

A) Synthesis of N-aryl / substituted aryl and heteroaryl maleimides

The synthetic procedure for maleimides which will be used in the synthesis of title compounds, has been described in part II A, pg 73-76 and their physical data is recorded in table I, pg 77.

B) Synthesis of 1-(4-aminophenyl) pyrrolidine

It involves the following 2 steps

i) Synthesis of 1-(4-nitrophenyl) pyrrolidine

A mixture of 4-chloronitro benzene (26.8 g; 0.16 mole) and pyrrolidine (24.2 g; 0.34 mole) were heated at 95-100°C in a sealed tube for 6 hours. The reaction mixture was stirred with 100 ml. of water. The product separated was isolated by filtration, washed with water, recrystallised twice from acetic acid and dried at 110°C.

Yield : 20.0 gm (61.34%)

M.P. 167-167.5°C

(Lit.¹⁶³ M.P. 167-168°C)

ii) Reduction of 1-(4-nitrophenyl) pyrrolidine to obtain 1-(4-aminophenyl) pyrrolidine

Tin foil (10.0 g) was gradually added to a mixture of 1-(4-nitrophenyl) pyrrolidine (3.84 g; 0.02 mole) in concentrated hydrochloric acid (40 ml.) The mixture was heated for 5 hours and left overnight. Then the solution was filtered. Crude crystalline hydrochloride was collected and washed with water. Charcoal treatment was then given to the filtrate. It was evaporated to half its volume on water bath in a China dish. The concentrate was chilled in ice-salt mixture to obtain crystals of

hydrochloride salt of 4-aminophenyl pyrrolidine. This hydrochloride was basified with liquor ammonia under ice-cold condition. Then the solution was filtered and the filtrate was extracted thrice with ether, dried over anhydrous Na₂SO₄ and the ether was removed under reduced pressure. The oil separated was distilled under vacuum. Purity was checked by T.L.C. using Toluene : Methanol (1:1) system.

Yield: 2.26 g (70.0%);

B.p.: 180-185°C / 16 mm

(Lit ¹⁶⁴ B.P. 180-185°C / 16 mm)

C) Synthesis of 4-(pyrrolidino anilino)-N-(aryl / substituted aryl and heteroaryl) succinimides

a) Preparation of 4-(Pyrrolidino anilino)-N-phenyl succinimide (Compound No. 1, Table III, pg 84)

In a 50 ml. round bottomed flask equipped with a guard tube, N-phenyl maleimide (0.83 gm, 0.0048 mole) and dry benzene (25 ml) were placed. To this, 1-(4-aminophenyl) pyrrolidine (0.78 gm, 0.0048 mole) was added at room temperature and the reaction mixture was stirred at room temperature for 6 hours. After discarding a small amount of sticky mass which formed on chilling, the solution was concentrated under reduced pressure. Addition of petroleum ether to the solution gave the crude product, which was then purified from methanol to obtain the pure product. The purity was checked by T.L.C. using Toluene : Methanol (10 : 1) system.

Yield: 0.89gm (58.2%)

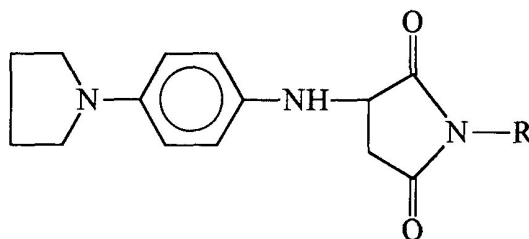
M.P: 178-179°C

Elemental analysis

	C%	H%	N%
Calculated	74.76	6.54	8.72
Found	74.67	6.30	9.08

Similarly, the other pyrrolidino anilino-N- aryl / substituted aryl and heteroaryl succinimides were prepared and are tabulated with their physical data in table no. III. The spectral data of the representative compounds has been included in the section - 'Spectroscopic Characterisation'.

Table III
4-(Pyrrolidino anilino)-N-(aryl / substituted aryl and heteroaryl) succinimides



Sr No	R	Mol. Formula	Mol. weight	M.P. (°C)	Yield (%)	Elemental Analysis (%)					
						Calculated			Found		
						C	H	N	C	H	N
1	phenyl	C ₂₀ H ₂₁ N ₂ O ₂	321	178-179	58.2	74.76	6.54	8.72	74.67	6.40	9.08
2	benzyl	C ₂₁ H ₂₃ N ₃ O ₂	349	Oil	54.2	72.20	6.59	12.03	72.00	6.20	11.85
3	4-chlorophenyl	C ₂₃ H ₂₀ N ₃ O ₂ Cl	369.5	278-280	55	64.95	5.41	11.36	64.60	5.05	11.00
4	4-carboxyl phenyl	C ₂₂ H ₁₅ N ₂ O ₃ Cl ₃	379	> 290	58.5	66.49	5.54	11.08	66.53	5.34	11.14
5	4-ethoxy phenyl	C ₂₂ H ₂₅ N ₃ O ₃	379	Oil	55.2	69.95	6.59	12.20	70.00	6.30	12.30
6	4-n-propoxy phenyl	C ₂₃ H ₂₇ N ₂ O ₃	379	165-167	57.3	72.82	7.12	7.38	72.44	7.23	7.53
7	4-n-butoxy phenyl	C ₂₄ H ₂₉ N ₃ O ₃	407	218-220	54.6	70.76	7.12	10.31	70.40	7.00	10.11
8	4-nitro diphenyl sulfide	C ₂₆ H ₂₄ N ₄ O ₄ S	488	132-134	54.7	63.93	4.91	11.47	63.80	4.80	11.20
9	3- quinoliny	C ₂₃ H ₂₂ N ₄ O ₂	386	145	59.05	71.5	5.69	14.50	71.2	5.72	14.49
10	8- quinoliny	C ₂₃ H ₂₂ N ₄ O ₂	386	Oil	60.02	71.5	5.69	14.50	71.3	5.68	14.53

Part IVA

Drug intermediate

Preparation of 1-acetyl-4-nitroso piperazinyl as a drug intermediate for N-(4-acetyl-1-piperazinyl)-4-fluorobenzamide monohydrate (FK-960)

Part IV A

Preparation of 1-acetyl-4-nitroso piperazine as a drug intermediate for N-(4-acetyl-1-piperazinyl)-4-fluorobenzamide monohydrate (FK-960).

This part involves preparation of 1-acetyl-4-nitroso piperazine as an intermediate for N-(4-acetyl-1-piperazinyl)-4-fluorobenzamide monohydrate (FK-960)

FK-960 was obtained by 2 procedures as mentioned in the literature⁸³.

A) The acetylation of 1-nitroso piperazine with acetyl chloride and pyridine in dioxane gives 1-acetyl-4-nitroso-piperazine, which is reduced with Zn and acetic acid/water to 1-acetyl-4-amino-piperazine. Finally, this compound is acylated with 4-fluorobenzoyl chloride in the presence of triethylamine using dichloromethane as the solvent.

B) The nitrosation of piperazine with NaNO_2 / HCl in water followed by condensation with benzyloxy carboxyl chloride by means of NaOH yields 1- (benzyloxycarbonyl)-4-nitrosopiperazine, which is reduced with Zn and acetic acid / water, as before, to corresponding amino derivative. The acylation of this amino derivative with 4-fluorobenzyl chloride and triethylamine in dichloromethane affords N-[4-(benzyloxycarbonyl) piperazin-1-yl]-4-fluorobenzamide, which is deprotected with HBr in acetic acid to yield N-(1-piperazinyl)-4-fluorobenzamide. Finally, this compound is acetylated with acetic anhydride / NaOH in water.

In our laboratory 1-acetyl-4-nitroso piperazine, the intermediate for FK-960, was prepared by the following modified procedure.

It involves 2 steps -

1) Preparation of N-nitroso piperazine

Sodium nitrite (6.9 gm, 0.1 M) was mixed with water (approx. 30 ml) and stirred till it dissolved completely, and cooled in ice bath below 10°C , piperazine 8.6 gm. (0.1 mole) was dissolved in 30 ml. of water and added in portions to the sodium nitrite solution mixture, maintaining the temperature below 10°C . The mixture was stirred for 30 min. Then 36% HCl solution was added to the reaction mixture till pH became 1.5 while maintaining the temperature below 10°C . The reaction mixture was again stirred for 30 min. Later, the reaction mixture was shaken with 30 ml dichloromethane and the dichloro methane layer was separated. Two more washes

with dichloromethane were given. Then the reaction mixture was basified and extracted with 3 portions of dichloromethane, 30 ml each. This dichloromethane layer was evaporated to get mononitrosopiperazine. The purity is checked by T.L.C. using chloroform: methanol (10 : 1) system.

Yield: 6.93 gm. (60.3%)

B.P. = 125-126°C

Lit B.P.⁹⁰ = 123-125°C

2) Preparation of 1-acetyl-4-nitroso piperazine

4-nitroso piperazine 3.3 gm (0.0286 M) was dissolved in glacial acetic acid (5 ml.) and stirred on a water bath, maintaining temperature in between 40-50°C. To this solution, acetic anhydride 2.91 gm. (2.70 ml, 0.0286 M) was added dropwise, with the help of a dropping funnel, and mixture was kept on stirring for 2 hrs. maintaining the temperature in between 40-50°C.

Later, the solvent was removed by vacuum distillation. The remaining solution was dumped on crushed ice. As no separation of products was seen, the solution was extracted with dichloromethane and the excess dichloromethane was removed under pressure. The viscous, oily liquid obtained was refrigerated to obtain 1-acetyl-4-nitroso piperazine as a cream coloured solid. The product obtained was recrystallised from methanol⁹¹. The purity of product was checked by T.L.C. using Toluene: Methanol (1:1) system.

Yield: 3.21 gm (71.4%)

M.P.: 74-76°C

Elemental analysis

	C%	H%	N%
Calculated	45.85	7.006	26.75
Found	45.60	7.00	27.15

The intermediate synthesized was confirmed by elemental analysis and spectral characterization, which is included in the corresponding section of 'Spectroscopic Characterisation.

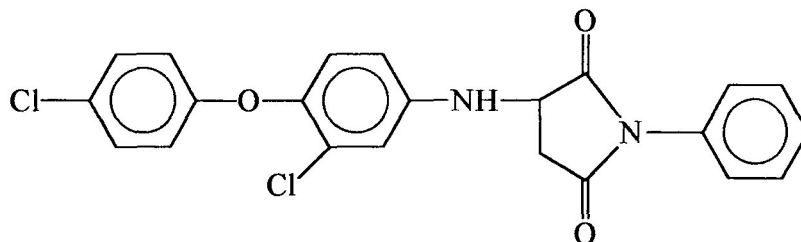
Part IVB

Spectroscopic Characterisation

Part IV B

Spectroscopic characterisation :

4[4'(4''-chlorophenoxy)-3'-chloroanilino]-N-phenyl succinimide



IR (CHCl_3) cm^{-1}

Wavenumber (cm^{-1})	Assignment
2927.8	Aromatic C-H stretching vibration
3040.5	Aromatic symmetric ether
3366.1	-NH stretching of aromatic amine
1700.7	Carbonyl stretching vibration of 5-membered cyclic ketone
752.8	C-Cl stretching
1231.09, 1074.5	C-O stretching of diaryl ether
1406.4, 1494.02, 1587.94	C=C stretching vibration
1287.34	C-N stretching

PMR (CDCl_3) δ :

6.76-7.79	(m, 12 H, Ar-H)
3.67.3.86	[m, 4H, (3H, CH-CH ₂ of succinimide), (1H, -NH)]

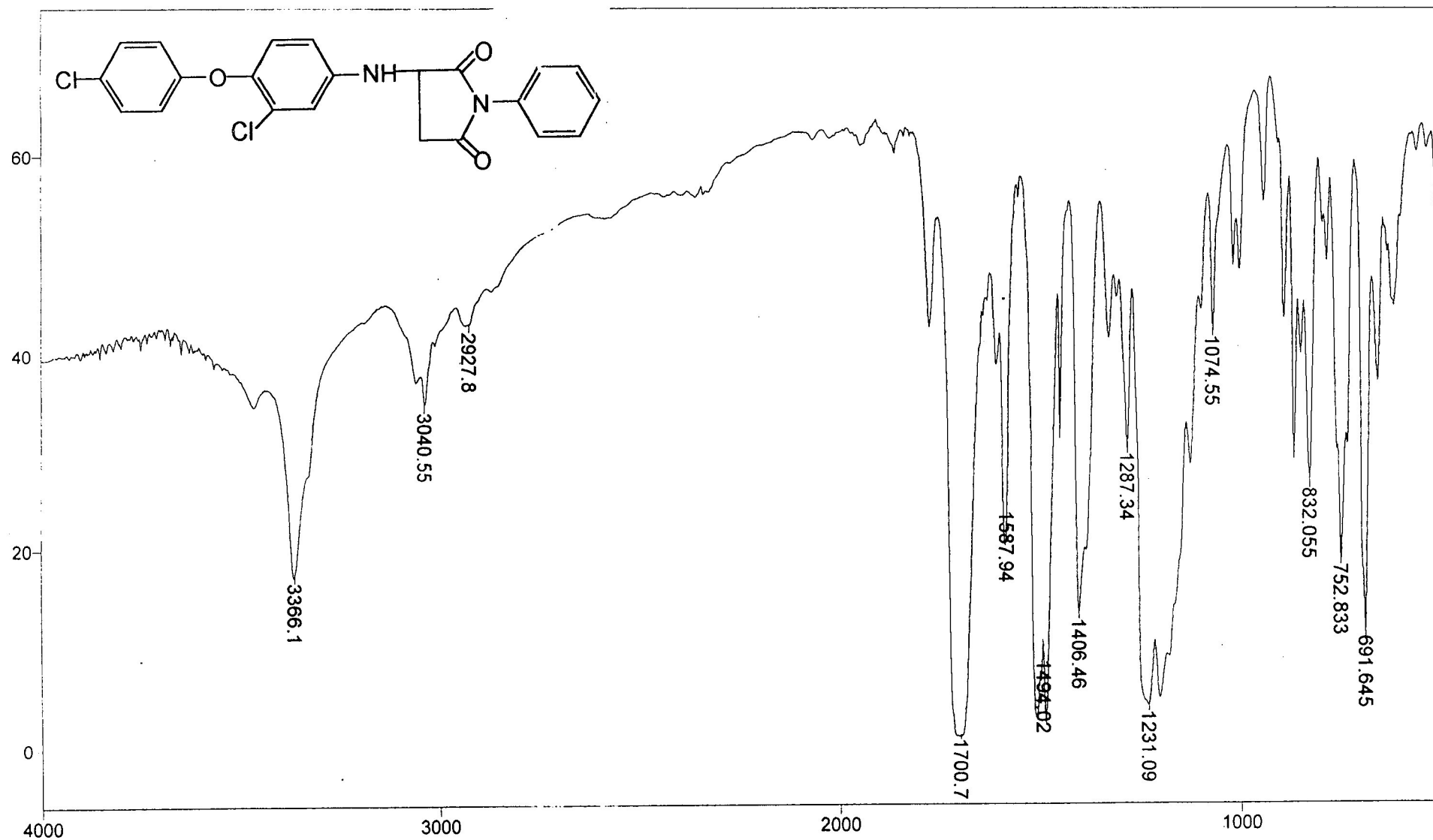
Sample Description: 4(4'-chlorophenoxy)-3-chloroanilino-N-phenyl succinimide.

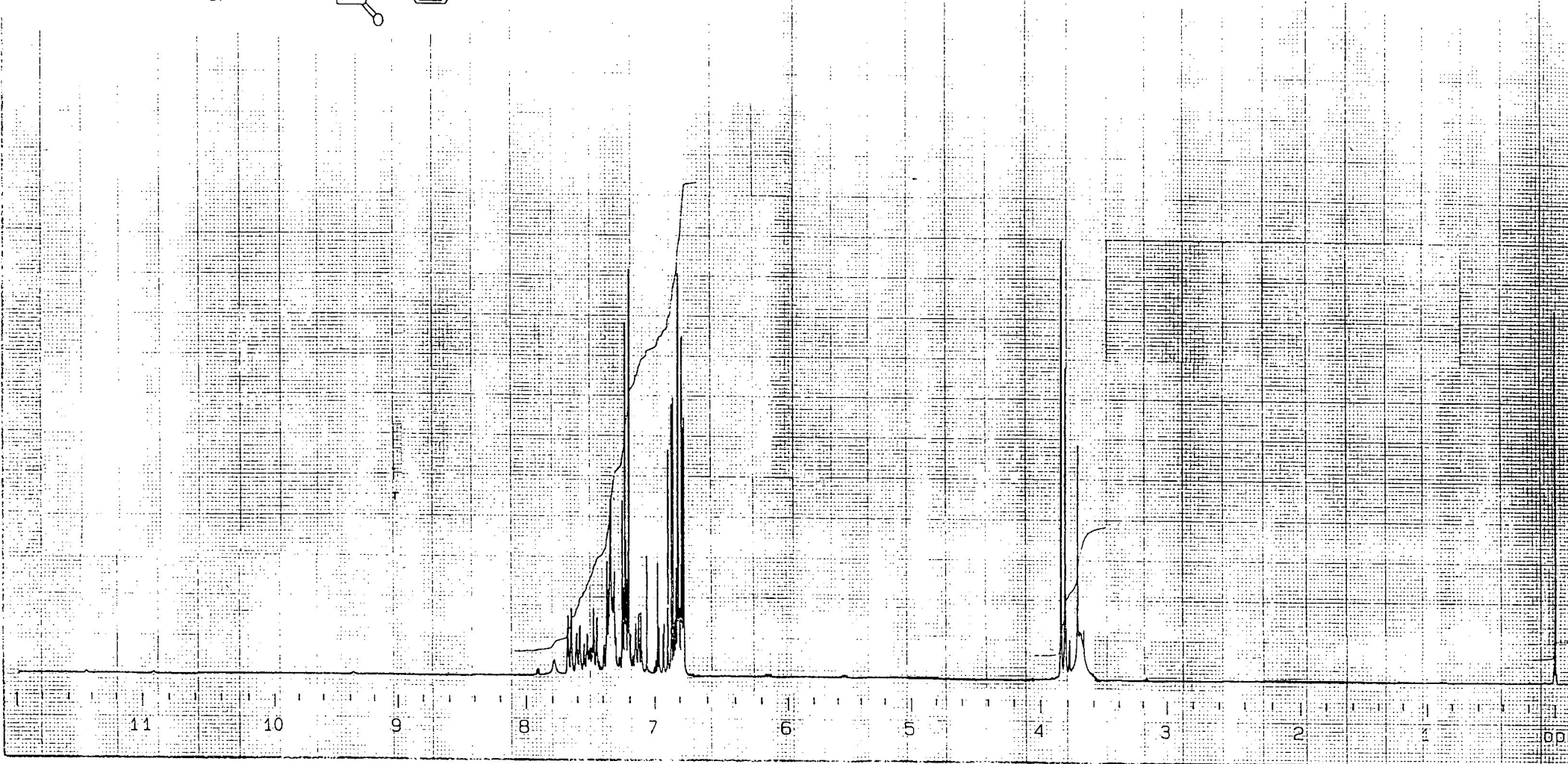
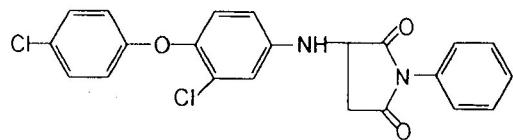
Scans= 10 Slow

Res=4 cm⁻¹

Apod= Cosine

Zero Filling= 1 x

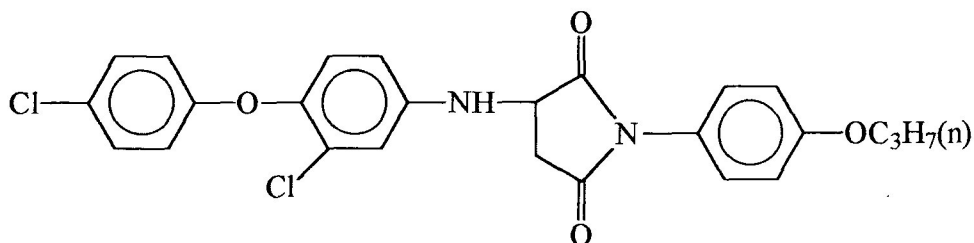




OBSERVE	Nucleus	H1	Freq	299,9486	Offset	-1769.6	EN	not used	Pulse Sequence	s2pul	SAMPLE	Number	
	Spec. Wdt	5998.8	Offset	1200.0	Phase	20	LR	0.100, not used	Tube O.D.		S-1	File	BS-1.fid
	Acq. Tm	4.001	Delay	0	Mode	C	Wdt	3693.7	Temp	not used		Date	Mar 17 99
	Pulse Wdt	10.0	Transients	800	Pr. Wdt	17.5	Power Mark	not used	Solvent	CDCl3		WHO	300

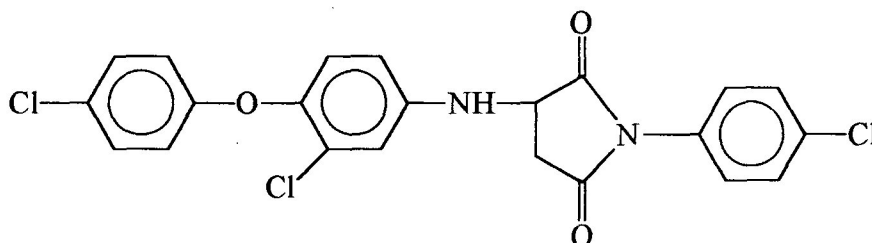
4[4'(4''-chlorophenoxy)-3'-chloroanilino]-N-(4-n-propoxy phenyl) succinimide.

IR (CHCl₃) cm⁻¹



Wavenumber (cm ⁻¹)	Assignment
2877.05, 2933.67, 2971.82	Aromatic C-H stretching vibration
3328.8	-NH stretching of aromatic amines
1700.07	Carbonyl stretching vibration of 5-membered cyclic ketone
742.6	C-Cl stretching
1249.9	C-O stretching of diaryl ether
1406.4, 1512.8, 1587.94	C = C stretching vibration
1299.82	C-N stretching

4[4'(4''-chlorophenoxy)-3'-chloroanilino]-N-(4-chlorophenyl) succinimide



IR (CHCl₃) cm⁻¹

Wavenumber (cm ⁻¹)	Assignment
2852.71, 2921.68	Aromatic C-H stretching vibration
3364.71	-NH stretching of aromatic amines
1704.64	Carbonyl stretching vibration of 5-membered cyclic ketone
690.1	C-Cl stretching
1247.19, 1017.92	C-O stretching of diaryl ether
1408.07, 1511.32	C = C stretching vibration
1202.78	C-N stretching

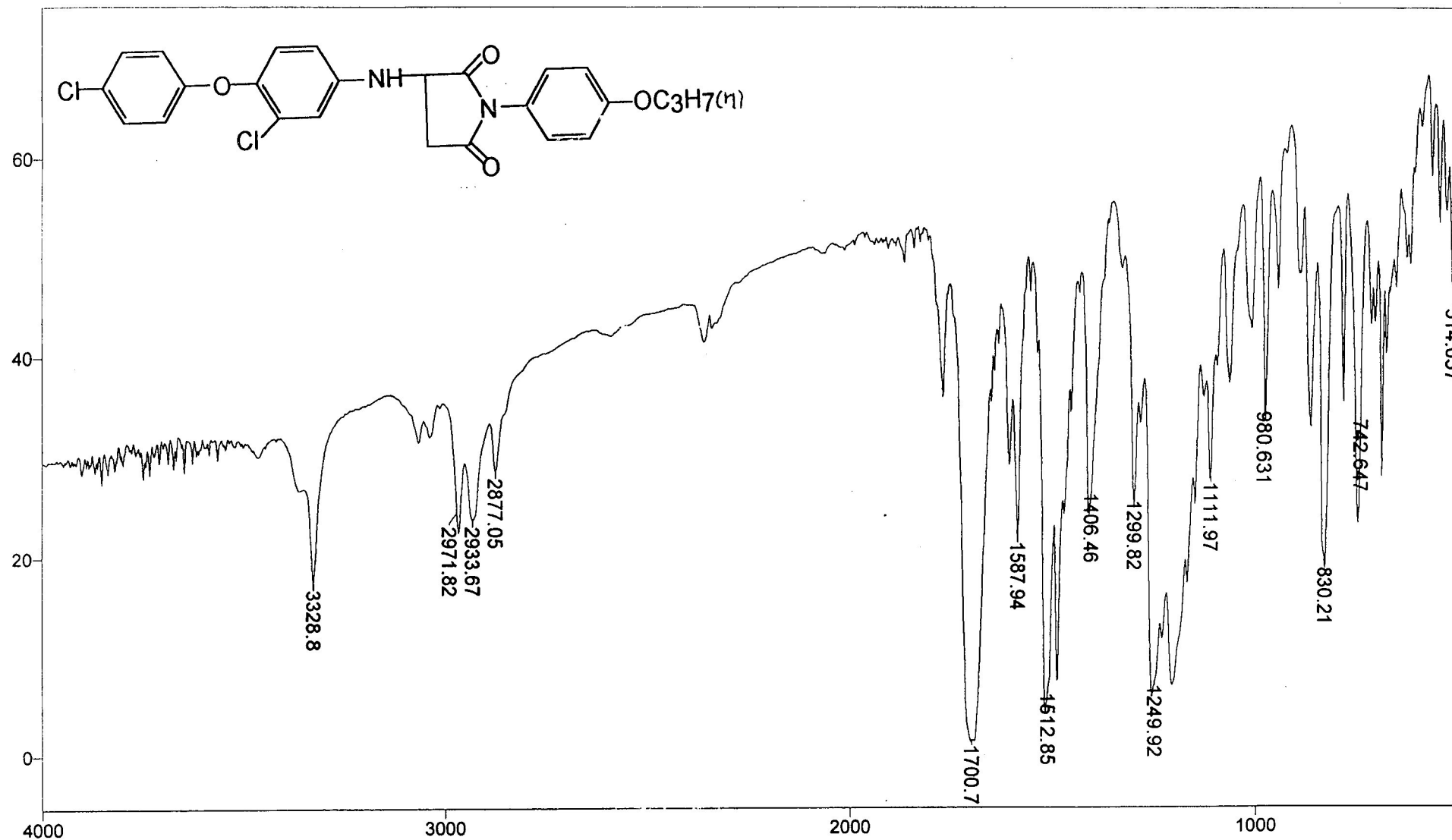
Sample Description: 4(4'-chlorophenoxy)-3-chloroanilino-N-P-propoxyphenyl succinimide.

Scans= 10 Slow

Res=4 cm-1

Apod= Cosine

Zero Filling= 1 x



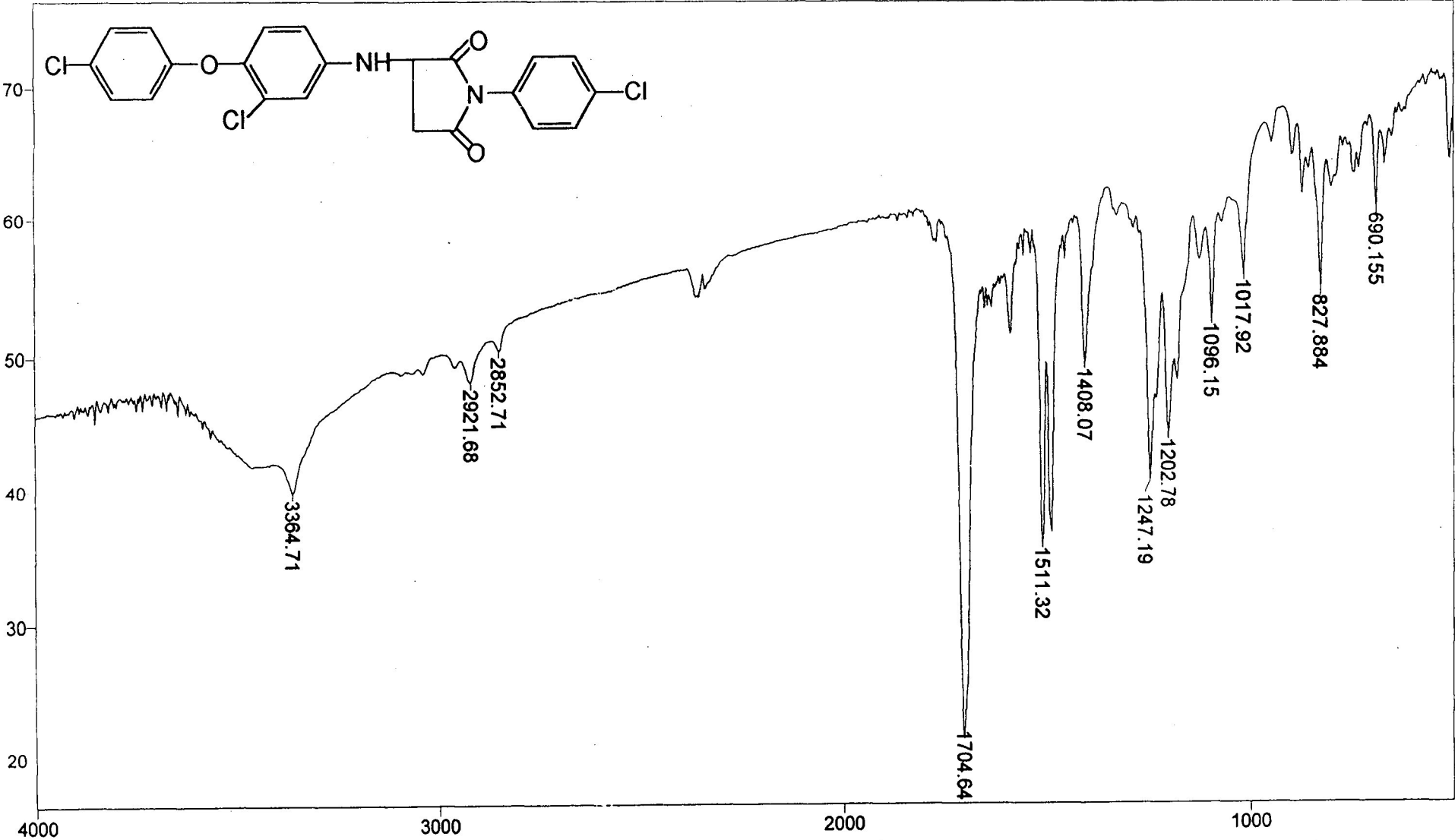
Sample Description:

Scans= 10 Slow

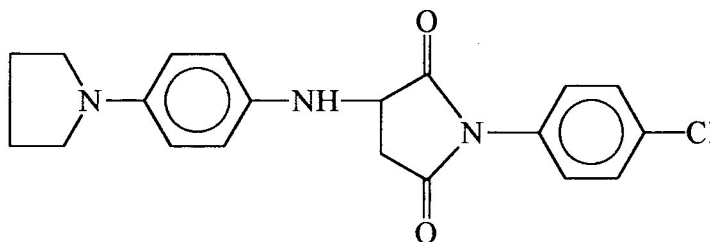
Res=4 cm-1

Apod= Cosine

Zero Filling= 1 x



4-(pyrrolidino anilino)-N-(4-chlorophenyl) succinimide



IR (KBr) cm^{-1}

Wavenumber (cm^{-1})	Assignment
2965.04, 3109.52	Aromatic C-H stretching vibration
3309.9	-NH stretching of aromatic amines
1700.7	Carbonyl stretching vibration of 5-membered cyclic ketone
1375.15	C-N stretching
725.91	C-Cl stretching
1512.85, 1644.2	C = C stretching vibration

PMR (CDCl_3) δ :

6.5-7.7	(m, 8 H, Ar-H)
3.2-3.4	(t, 4H, pyrrolidino)
2.1-2.2	[m, 4H, (3H, CH-CH ₂ of succinimide), (1H, -NH)]
2.0-2.1	(Pentet, 4H, pyrrolidino)

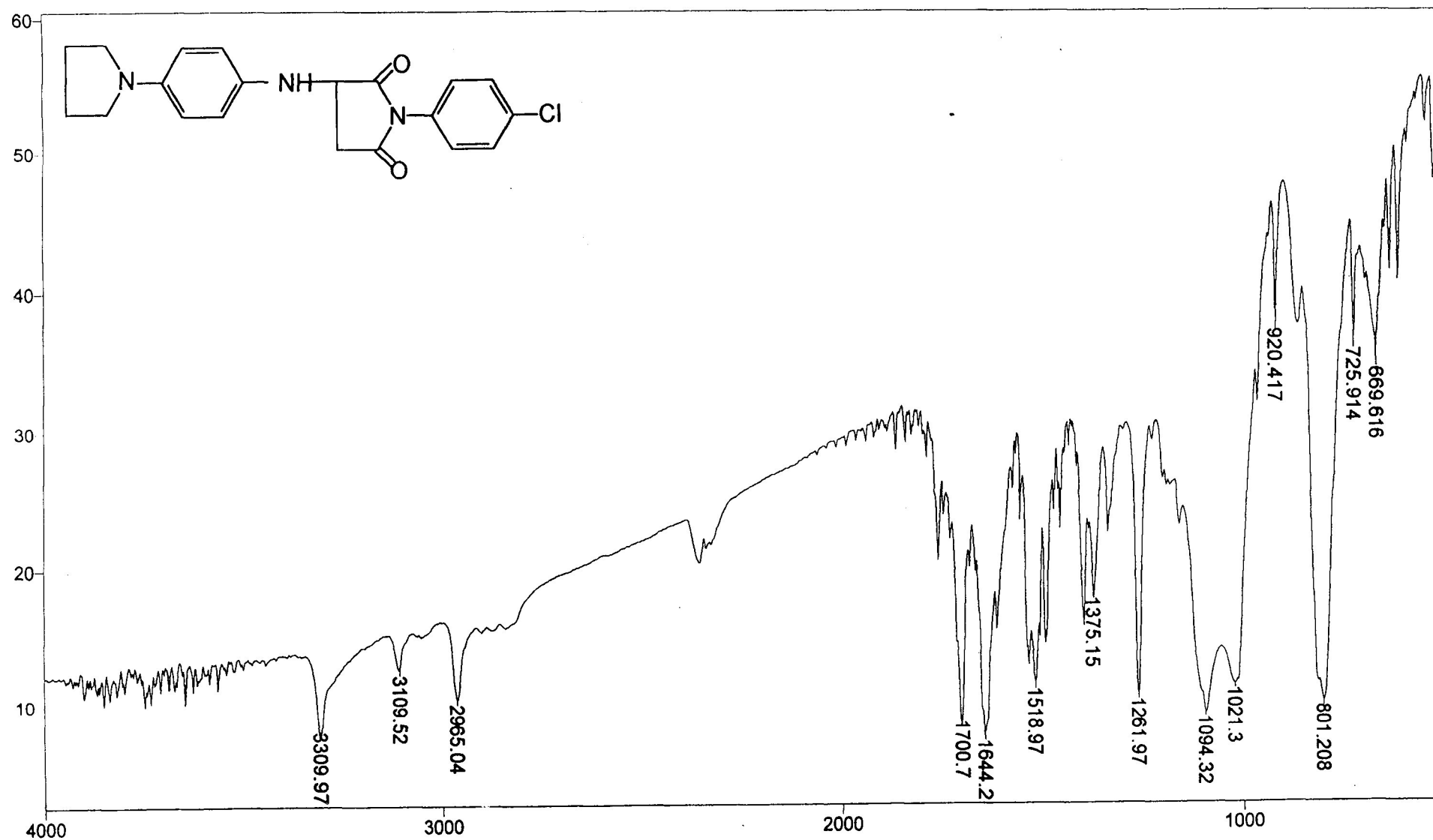
Sample Description: Pyrrolidineanilino-N-P-chlorophenyl succinimide.

Scans= 10 Slow

Res=4 cm⁻¹

Apod= Cosine

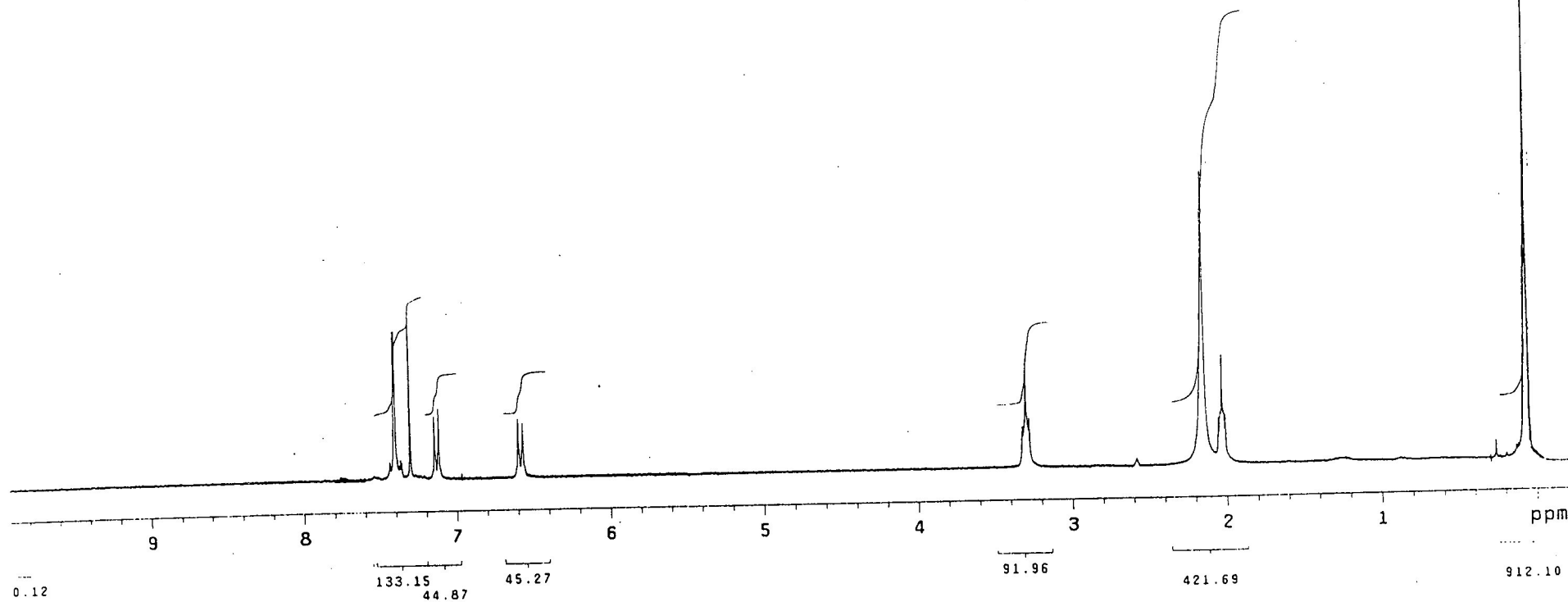
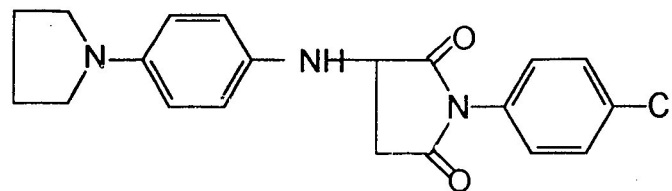
Zero Filling= 1 x



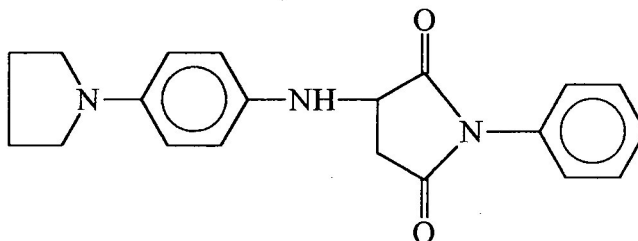
PSU-IV

exp2 s2pul

SAMPLE		DEC. & VT	
date	Jul 18 2000	dn	H1
solvent	cdcl3	dof	0
file	exp	dm	n
ACQUISITION		dmm	c
sfrq	299.949	dmf	200
tn	H1	dip	20
at	4.033	temp	not used
np	48384	PROCESSING	
sw	5998.8	lb	0.10
fb	3300	fn	not used
bs	8	math	1
pw	10.0		
pw	10.0	werr	
tpwr	not used	wexp	
d1	0	wbs	
tof	1000.0	wnt	
nt	800	DISPLAY	
ct	40	sp	-71.2
alock	n	wp	3051.7
gain	0	vs	619
FLAGS		sc	0
il	n	wc	250
in	y	hzmm	12.21
dp	y	is	66333.69
hs	nn	rfl	909.3
		rtp	0
		th	2
		ins	20.000
		nm	ph



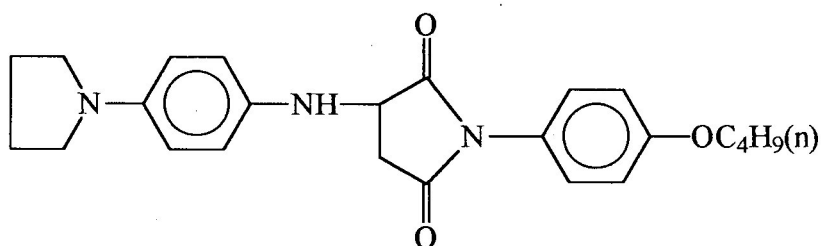
4-(Pyrrolidino anilino)-N-phenyl succinimide



IR (KBr) cm^{-1}

Wavenumber (cm^{-1})	Assignment
2965.4, 3109.5	Aromatic C-H stretching vibration
3316.2	-NH stretching of aromatic amines
1706.81	Carbonyl stretching vibration of 5-membered cyclic ketone
1373.9	C-N stretching
1525.33, 1638.08	C = C stretching vibration

4-(pyrrolidino anilino)-N-(4-n-butoxyphenyl) succinimide



IR (KBr) cm^{-1}

Wavenumber (cm^{-1})	Assignment
2865.18, 2956.73, 3109.52	Aromatic C-H stretching vibration
3307.84	-NH stretching of aromatic amines
1700.7	Carbonyl stretching vibration of 5-membered cyclic ketone
1368.7	C-N stretching
1093.3, 1118.3	N – butoxy
1512.8, 1631.7	C = C stretching vibration

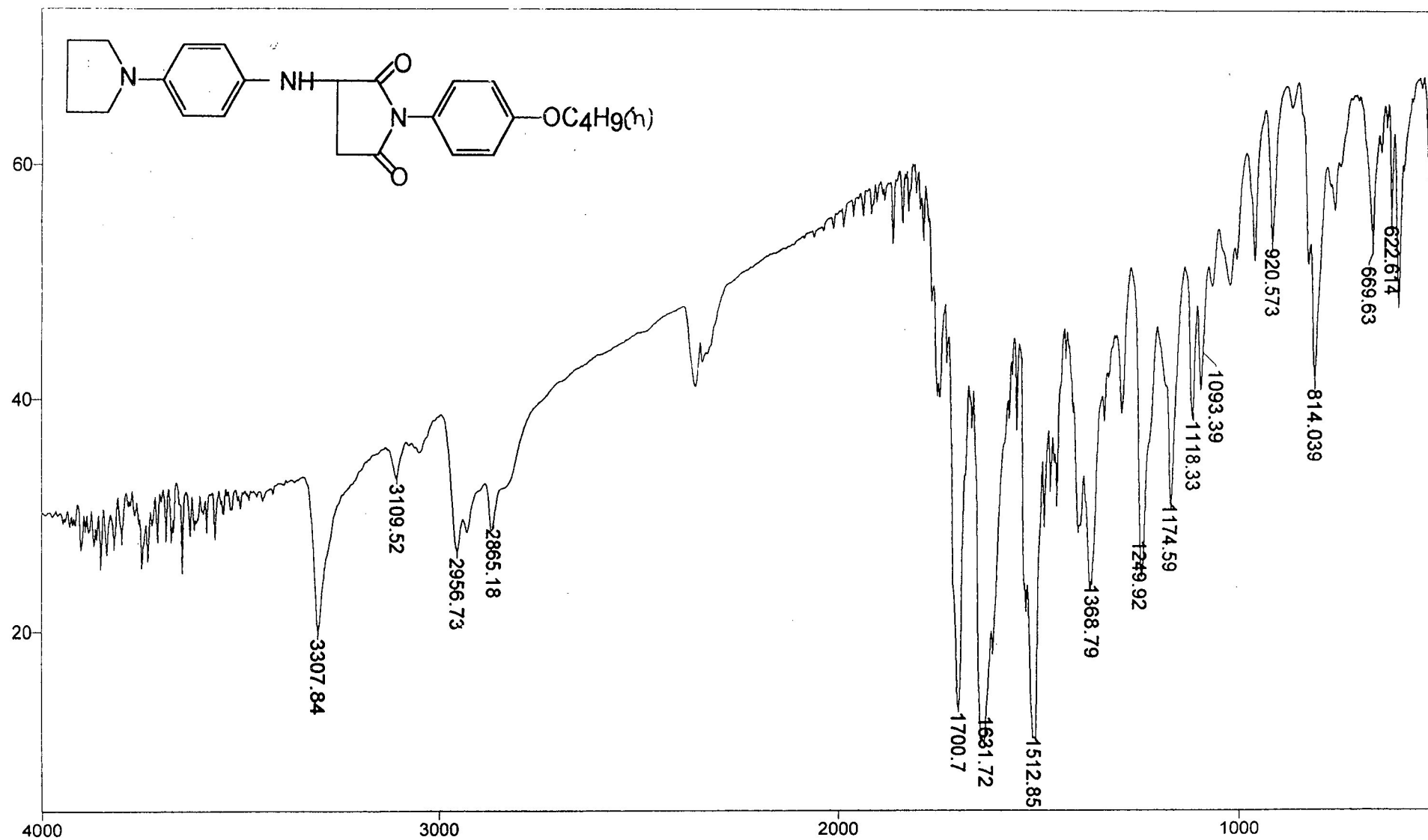
Sample Description: Pyrrolidineanilino-N-P-butoxyphenyl succinimide.

Scans= 10 Slow

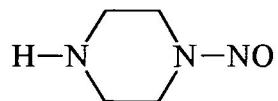
Res=4 cm-1

Apod= Cosine

Zero Filling= 1 x



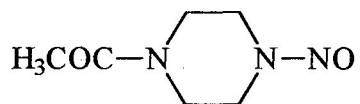
N-nitroso piperazine



IR (CHCl₃) cm⁻¹

Wavenumber (cm ⁻¹)	Assignment
2940.51	-CH stretch
1362.43	-CH bend
1418.9	-H-NO vibrations
3372.4	-NH bend

1-acetyl-4-nitroso piperazine



IR (KBR) cm⁻¹ spectrum

Wavenumber (cm ⁻¹)	Assignment
2940.51	-CH stretch
3026.47, 1356.32	- CH ₃ CO stretch
1437.77	-N-NO vibrations
1644.2	C = O stretch of acetyl
3262.22	C =O overtone

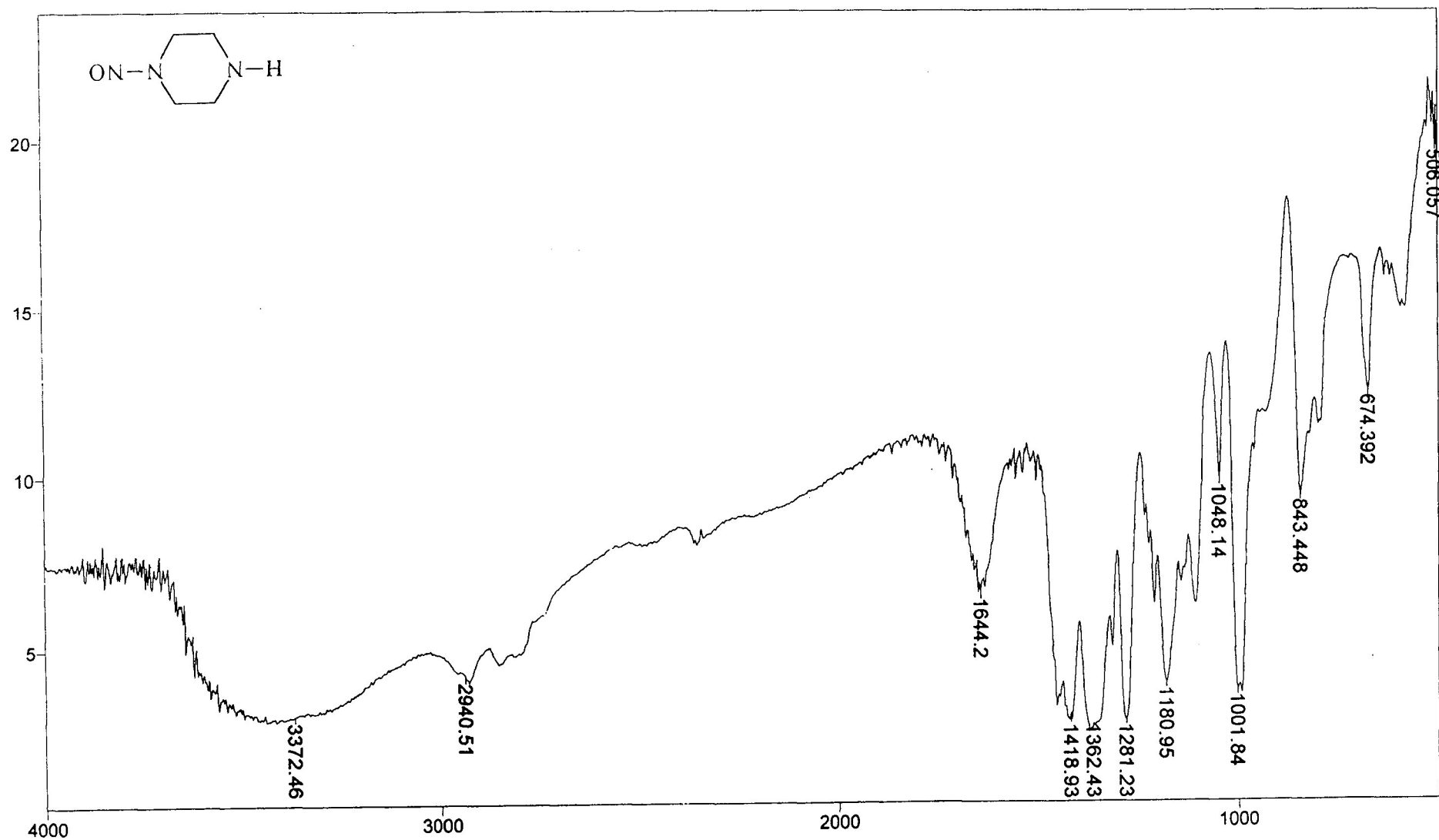
Sample Description: 4-nitroso piperazine

Scans= 10 Slow

Res=4 cm⁻¹

Apod= Cosine

Zero Filling= 1 x



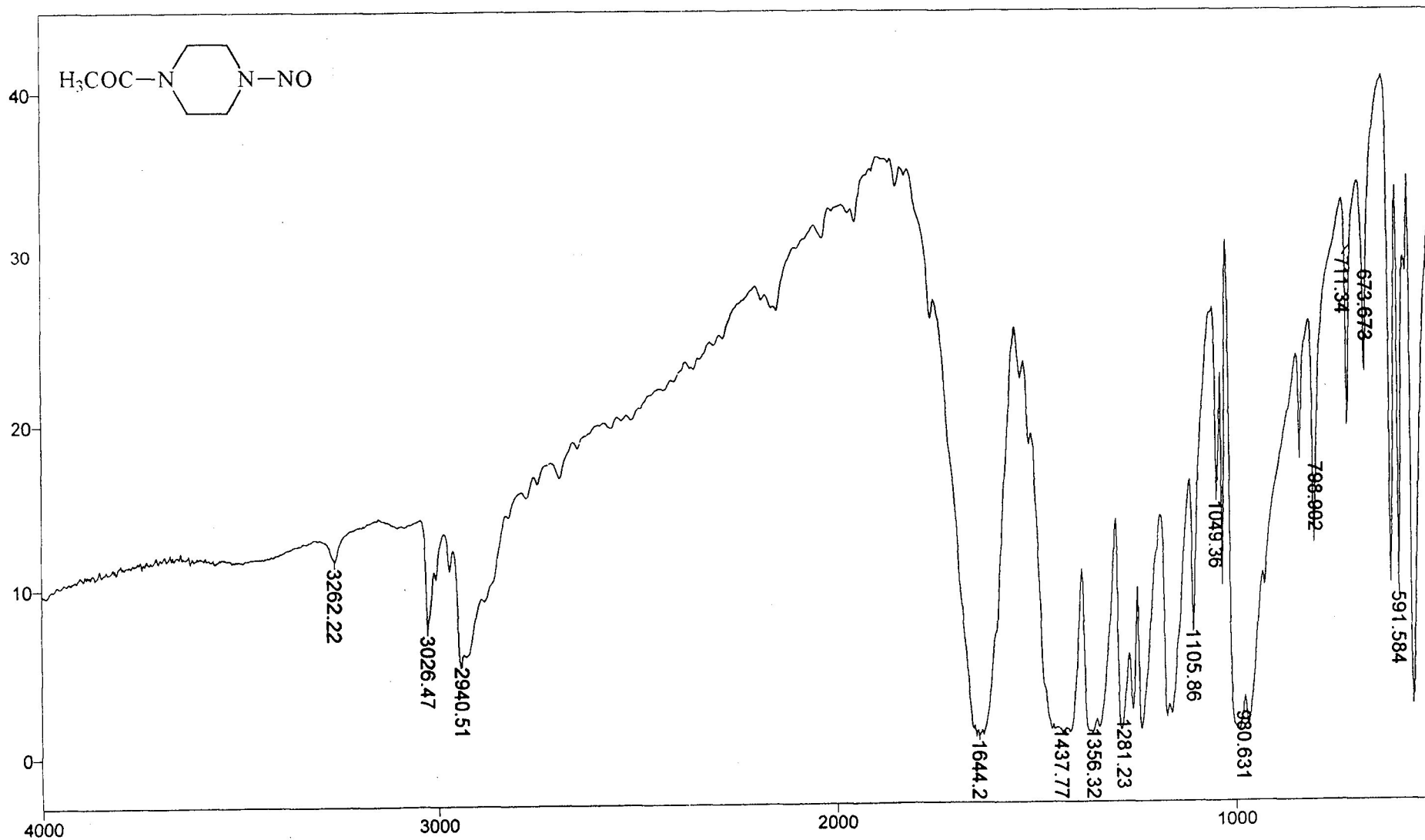
Sample Description: 1-acetyl 4-nitroso piperazine

Scans= 10 Slow

Res=4 cm-1

Apod= Cosine

Zero Filling= 1 x



Part V

Biological Screening, Results and Discussions

Part V

Biological screening, results and discussion

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

The various screenings carried out include the *in vitro* study against Gram-positive *Staphylococcus aureus* ATCC 3750 and Gram-negative *Salmonella typhi* NCTC 786 and *in vitro* study of activity against fungal strains, such as *Candida albicans*, *Trichophyton mentagrophytes* and *Trichophyton rubrum* was carried out.

Drug stock solution

The test compound (10 mg.) was dissolved in a suitable solvent (10 ml.) such as DMF, ethanol, dil. HCl as the case may be, so as to prepare a stock solution of 1000 µg/mg. From this solution, the drug concentrations viz. 200, 100, 40 and 20 µg/ml were made. For lower dilutions the stock solution was diluted to prepare concentrations such as 10, 4 and 2 µg/ml.

Controls:

Media control

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

Culture control

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

Solvent control

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

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

Turbidity control

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

Standard control

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

Antibacterial screening

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

Muller Hinton broth was used as the culture medium. Sterilized medium was dispensed in each borosilicate glass test tube (150 + 20 mm). The drug solution was added in order to attain final drug concentrations of 200, 100, 40, 20, 10, 4, 2 and 1 µg/ml.

Innoculum of standard suspension (0.1 ml of test organism strain which contains 10^6 bacilli/ml) was added. The tubes were incubated at 37°C for 48 hours and then examined for the presence or absence of growth of the organism. The lowest concentration which showed no visible growth was taken as an end point and is known as the minimum inhibitory concentration (MIC).

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

Antitubercular screening

The test compounds were subjected to *in vitro* screening by tube dilution method employing the human virulent H₃₇R_V strain of *Mycobacterium tuberculosis*.

The medium used was Kirchner's and Middlebrook 7H9 broth base with ADC growth supplement (0.480 gm. per 100 ml. of medium).

The sterile medium was dispensed in each borosilicate test tube to attain final dilutions of 200, 100, 40, 20, 10, 4, 2 and 1 µg/ml. Inoculum of standard suspension, i.e. 0.1 ml of H₃₇R_v strain of *M. tuberculosis* containing 10⁶ bacilli/ml, was added. The tubes were incubated at 37°C for 10 days and then examined for the presence or absence of growth of the test organism. The lowest concentration which showed no visible growth was taken as the end point. Isoniazid (MIC = 0.04 µg/ml) was used as a standard.

Antifungal screening

The organisms employed for the test were *Candida albicans*, *Trichophyton mentagrophytes* and *Trichophyton rubrum*. These organisms were maintained on Sabouraud's agar slants by regular subculturing. *C. albicans* was cultured every week and the rest of the organisms were cultured at the interval of 12 days.

Test medium

Serafataller's medium was employed for growth and pH of the medium was adjusted to 5.2. The sterile medium was dispensed in each borosilicate test tube. The drug solution was added in order to get the final concentrations of 200, 100, 40, 20, 10, 4 and 2 µg/ml.

Preparation of the inoculum

Candida albicans

A 48 hours old growth on Sabouraud's agar slant was suspended in 4-5 ml of the test medium with the help of a platinum loop. The cell count of the suspension (yeast phase) was carried out using Neubauer chamber and then diluted with requisite volume of the medium to give a final concentration of 5 x 10⁶ cells per ml.

T. rubrum and *T. mentagrophytes*

To a 12 to 14 hours old growth on Sabouraud's agar slant, 5 ml. of sterile test medium and 3-4 sterile glass beads (3mm diameter) were added and gently shaken

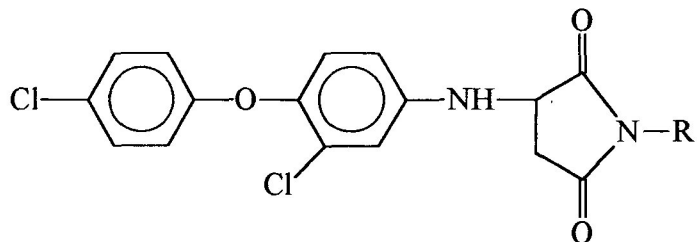
for 10 minutes. The supernatant was decanted into another sterile tube and the count of spores in the suspension was carried out using Neubauer chamber. The final strength of the inoculum was adjusted to 5×10^6 spores per ml., using the test medium.

Screening Procedure

To each test tube was added 0.1 ml. of the standardized inoculum of the test organism. The tubes were then incubated at room temperature in a dark place. The presence or absence of growth was noted visually after 7 days in case of *Candida albicans* and after 14 days in case of *T. rubrum* and *T. mentagrophytes*

Miconazole was used as the standard drug for control, which has a MIC of 6.25 µg/ml against *C. albicans* and 1.56 µg/ml against *T. mentagrophytes* and *T. rubrum*. The results of the Biological Screening are recorded in Table IV, V, VI, VII (pg. 96-99)..

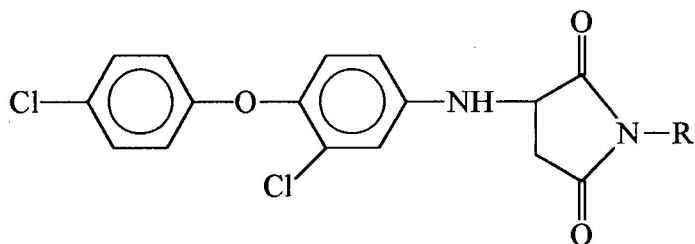
Table IV
Antimicrobial activity of 4[4'(4''-chlorophenyl)-3'-chloroanilino]- N-(alkyl / aryl / substituted aryl and heteroaryl(succinimides



Sr. No.	Substitution	Antibacterial activity in µg/ml		
		H ₃₇ R _v strain of <i>M. tuberculosis</i>	<i>S. aureus</i> ATCC 3750	<i>S. typhi</i> NCTC 786
1	Methyl	100	40	100
2	phenyl	NA	10	10
3	benzyl	100	10	10
4	4-chlorophenyl	NA	4	4
5	4-carboxylic phenyl	NA	100	100
6	4-methoxy phenyl	100	NA	100
7	4-ethoxy phenyl	100	100	100
8	4-n- propoxy phenyl	100	10	10
9	4-n-butoxy phenyl	100	10	10
10	4-nitro diphenyl sulfide	100	100	NA
11	3-quinoliny	100	100	100
12	8-quinoliny	100	40	100

NA indicates inactive at 200 µg/ml

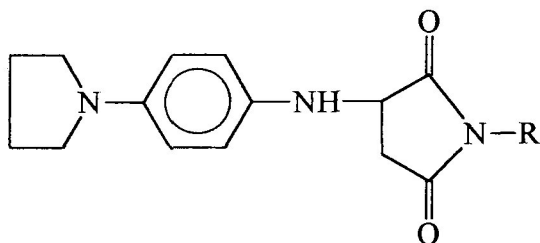
Table V
Antifungal activity of 4[4'(4''-chlorophenoxy)-3'-chloroanilino]- N-(alkyl / aryl / substituted aryl and heteroaryl) succinimides



Sr. No.	Substitution	Antifungal activity in µg/ml		
		<i>C. albicans</i>	<i>T. mentagrophytes</i>	<i>T. rubrum</i>
1	Methyl	NA	NA	NA
2	phenyl	40	40	40
3	benzyl	200	40	40
4	4-chlorophenyl	40	40	40
5	4-carboxylic phenyl	NA	100	100
6	4-methoxy phenyl	NA	NA	NA
7	4-ethoxy phenyl	20	20	10
8	4-n- propoxy phenyl	40	40	40
9	4-n-butoxy phenyl	40	40	40
10	4-nitro diphenyl sulfide	NA	100	NA
11	3-quinolinyl	NA	NA	NA
12	8-quinolinyl	NA	NA	100

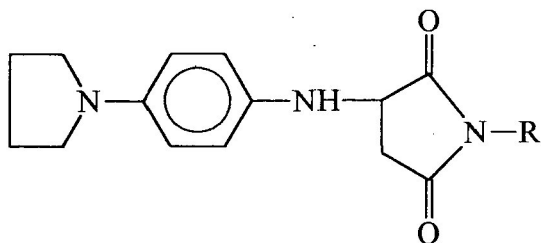
NA indicates inactive at 200 µg/ml

Table VI
Antibacterial activity of 4-(pyrrolidino anilino)-N-(aryl / substituted aryl and heteroaryl) succinimides



Sr. No.	Substitution	Antibacterial activity in µg/ml		
		H ₃₇ R _v strain of <i>M. tuberculosis</i>	<i>S. aureus</i> ATCC 3750	<i>S. typhi</i> NCTC 786
1	phenyl	40	100	100
2	benzyl	100	100	100
3	4-chlorophenyl	100	100	100
4	4-carboxylic phenyl	100	100	100
5	4-ethoxy phenyl	100	100	100
6	4-n- propoxy phenyl	100	100	100
7	4-n-butoxy phenyl	100	100	100
8	4-nitro diphenyl sulfide	100	100	100
9	3-quinolinyl	100	100	100
10	8-quinolinyl	100	100	100

Table VII
Antifungal activity of 4-(pyrrolidino anilino)-N-(aryl / substituted aryl and heteroaryl) succinimides



Sr. No.	Substitution	Antifungal activity in µg/ml		
		<i>C. albicans</i>	<i>T. mentagrophytes</i>	<i>T. rubrum</i>
1	Phenyl	NA	NA	NA
2	Benzyl	NA	NA	40
3	4-chlorophenyl	NA	NA	NA
4	4-carboxylic phenyl	10	100	20
5	4-ethoxy phenyl	NA	NA	NA
6	4-n- propoxy phenyl	NA	NA	NA
7	4-n-butoxy phenyl	NA	NA	NA
8	4-nitro diphenyl sulfide	NA	NA	NA
9	3-quinolinyl	NA	100	NA
10	8-quinolinyl	NA	NA	100

NA indicates inactive at 200 µg/ml

DISCUSSION

The present work was focused on the synthesis of novel heterocyclic compounds with possible biological activity. To evaluate their efficacy, they were screened for their antibacterial, antitubercular and antifungal activity, *in vitro*.

In part II synthesis of 4[4'(4''-chlorophenoxy) -3' -chloroanilino]- N-(alkyl / aryl / substituted aryl and heteroaryl) succinimides has been described. The antimicrobial and antifungal activity of this series revealed the following observations.

1. In this series, 4[4'(4'' - chlorophenoxy) - 3' - chloroanilino]- N-(4-n - chlorophenyl) succinimide (compound no. 4, Table No. IV, pg. 96) shows excellent antibacterial activity at 4 µg/ml against *S. aureus* and *S. typhi*. Thus, 4-chlorophenyl substituent was found to contribute to the antibacterial activity.
2. Also, the other N-substitution like phenyl, benzyl, 4-n-propoxy phenyl, 4-n-butoxyphenyl, also contribute to the antibacterial activity. These compounds show antibacterial activity at 10 µg/ml, against both the organisms (Compound No. 2,3,8 and 9 respectively, Table No. IV, pg. 96).
3. Other substitutions like methyl, 4-carboxylic phenyl, 4-methoxyphenyl, 4-ethoxyphenyl, 4-nitrodiphenyl sulfide, 3-quinolinyl, 8-quinolinyl do not show any significant contribution towards antibacterial activity. (Compound No. 1, 5, 6, 7, 10, 11 and 12 respectively, Table No. IV, pg. 96).
4. The compound with 4-chlorophenyl substituent (Compound No. 4, Table No. IV, pg. 96) show excellent antibacterial activity, but at the same time this substituent does not have any significant antimycobacterial activity. Also, the substitutions like phenyl, 4-carboxylic phenyl do not show any antimycobacterial activity. (Compound No. 2 and 5, Table No. IV, pg. 96).
5. In this series of compounds N-methyl / phenyl / substituted aryl and heteroaryl substitutions do not contribute significantly towards the antimycobacterial activity.
6. In case of antifungal screening, the N-substitutions like phenyl, 4-chlorophenyl, 4-n-propoxyphenyl, 4-n-butoxyphenyl show antifungal activity at 40 µg/ml against *C. albicans*, *T. mentagrophytes*, and *T. rubrum* (Compound No. 2,4,8 and 9 respectively, Table No. V, pg 97). Benzyl substitution showed activity at 200 µg/ml against *C. albicans* and at 40 µg/ml against *T. rubrum* and *T. mentagrophytes* (Compound No. 3, Table No. V, pg. 97).

7. Excellent antifungal activity was obtained in case of 4[4'(4"- chlorophenoxy) - 3-chloroanilino]- N-(4-ethoxyphenyl) succinimide (Compound No. 7, Table No. V, pg. 97). This compound shows activity at 20 µg/ml against *C. albicans* and *T. mentagrophytes*, and at 10 µg/ml against *T. rubrum*. However, this compound does not show any significant antibacterial activity.

In part III, synthesis of 4-(pyrrolidino anilino)-N-(aryl / substituted aryl and heteroaryl) succinimides was carried out. The antimicrobial and antifungal screening of this series of compounds reveals the following points:

1. The 4-(pyrrolidino anilino)- N - (aryl / substituted aryl and heteroaryl) succinimides inhibits *S. aureus* and *S. typhi* at a concentration of 100 µg/ml *in vitro*. 4-(pyrrolidino anilino)- N- phenyl succinimide (Compound No. 1 and Table No. VI, pg. 98) inhibits *M. tuberculosis* at a concentration of 40 µg/ml, while other substituents show activity at 100 µg/ml only. Hence, various substitutions on the nitrogen were not found to have any significant effect on the antibacterial or antitubercular activity.
2. Antifungal screening of this series of compounds reveals that only 4-(pyrrolidino anilino)-N-(4-carboxylic phenyl) succinimide (Compound No. 4 and Table No. VII, pg. 99) is active at 10 µg/ml against *C. albicans*, at 100 µg/ml against *T. mentagrophytes* and at 20 µg/ml against *T. rubrum*. The other analogs like 4-(pyrrolidino anilino)-N-benzyl succinimide (Compound No. 2, Table No. VII, pg. 99) showed activity at 40 µg/ml against *T. rubrum*, while 4-(pyrrolidino anilino)-3-N-quinolinyl succinimide(compound No. 9, Table No. VII, pg. 99)was found to inhibit *T. mentagrophytes* at 100 µg/ml. Also 4-(pyrrolidino anilino)-8-N- quinolinyl succinimide(Compound No. 10, Table No.VII, pg. 99) exhibited activity at 100 µg/ml against *T. rubrum*. In general, this series showed poor antifungal activity, with a majority of compounds showing no activity at all.

Conclusion

CONCLUSION

The conclusion from the biological screening of these 2 series of compounds is

- 4(4'-chlorophenoxy)-3-chloroaniline and 1-(4-aminophenyl) pyrrolidine substitutions at 4-position of the substituted maleimide does not significantly contribute to antimycobacterial activity.
- Antibacterial screening of both the series reveals that 4(4'-chlorophenoxy)-3-chloroaniline substituted at 4-position of substituted maleimides contributed significantly towards the antibacterial activity as compared to 1-(4-aminophenyl) pyrrolidine substitution at 4-position of the substituted maleimides. Also, in case of 4[4'(4''-chlorophenoxy)-3'-chloroanilino]-N-(methyl / phenyl / substituted aryl and heteroaryl) succinimides, activity is also influenced by the substitution at 1-N of the maleimide. Thus, in this series of compounds activity is influenced by the combined effect of substitution of 4(4'-chlorophenoxy)-3-chloroaniline at 4-position of maleimides, and 1-N-substitution on the maleimides.
- Also, it can be seen that 4(4'-chlorophenoxy)-3-chloroaniline present at 4-position of the substituted maleimides contributes significantly towards antifungal activity. In case of 4[4'(4''-chlorophenoxy)-3'-chloroanilino]-N-(methyl / phenyl / substituted aryl and heteroaryl) succinimides activity is greatly influenced by 1-N substitution on the maleimide. However, in case of 4-(pyrrolidino anilino)-N-(phenyl/ substituted aryl and heteroaryl) succinimides the effect of substitution at 1-N positions of the maleimides is not significant. 1-(4-aminophenyl) pyrrolidine at 4-position of the substituted maleimides also does not significantly contribute towards the antifungal activity. In this series of compounds, only 4-(pyrrolidino anilino)-N-(4-carboxylic phenyl) succinimides show some promising antifungal activity. In spite of the fact that not many promising compounds have been obtained in this research venture, it has brought to light the significance of the succinimides moiety, which needs to be explored further as regards antibacterial, antimycobacterial and antifungal activity. The introduction of the 'right kind' of substitution on this molecule will definitely yield potent compounds.

References

REFERENCES

1. Camille G. Wermuth, **The Practice of Medical Chemistry**, Academic press, London, P : 4 (1996).
2. Graham L. Patrick, **An Introduction to Medicinal Chemistry**, Oxford University Press, P:101-102 (1995).
3. J. A. Dimasi, N. R. Bryant and L. Lasagna, New Drug Development in the United States from 1963 to 1990, **Clin. Pharmacol. Ther.**, **50** : 471-486 (1991).
4. I. D. Kuntz, structure-based strategies for drug design and discovery, **Science** **257** : 1078-1082 (1992).
5. Drugs and the Pharmaceutical Sciences, **Receptor Based Drug Design**, edited by Poyl Leff, Marcel Dekker, Inc. **89** : 323-325.
6. R. DesJarlais, R. P. Sheridan et al., **J. Med. Chem.**, **31** : 722-729 (1998).
7. Y. C. Martin, 3D database searching in drug design, **J. Med. Chem.**, **3** : 2145-2154 (1992).
8. P. J. Artymiuk, P. A. Bath et al., **J. Chem. Inf. Compute. Sci.**, **32** : 617-630 (1992).
9. P. J. Goodford, **J. Med. Chem.**, **28** : 849-857 (1985).
10. A. Miranker and Karplus, **Proteins Struct. Funct. Genet.**, **11**:29-34 (1991).
11. R. S. Bohacek and C. McMartin, **J. Am. Chem. Soc.** **116** : 5560-5571 (1994).
12. Somshanka Bondyopadhyway, New threat from multi-drug resistant tuberculosis, **The Times of India** (April 27, 2000).
13. Balarka Batabyal, Tuberculosis rampant in the city, **The Times of India**, (June 5, 2000).
14. Laal S, Samanich K. M. and Sonnenberg M. G. **J. Infect. Dis.**, **76** : 133 (1997).
15. Ranadive S. N. and Banerjee K., **ICMR Bulletin**, **19**:11, (1989).
16. Mackness G. B., Smith N., **Amer Rev. Tubercul.**, **67**:322 (1953).
17. Davidson P. t., Goble M, Lester W., **Chest**, **61**:574-578 (1972) cited from **Drugs** **43** : 5, 1992 Drug treatment of Tuberculosis. (1992).
18. Kushner, s, Dalalian H, Sanjuro J. L., Bach F L, Safir S R, **J. Am. Chem. Soc.** **74**: 3617 (1952).
19. Kucks M A, Pects E A, Forbes M, **Am. Rev. Resp. Dis.**, **87** : 805-806 (1963).
20. Dogmagk G, G, **Deut. Med. Wochensehr.**, **61** : 250 (1931).

21. Mietzsch F and Klarer J., **Ger Pat.** 607537 (1935) Cited from **Chem. Abstr.** **29** : 4135 (1935).
22. Jaffe A, Chabbert Y A, Semonin O, **Antimicrobial Agents and Chemotherapy** **22** : 942 (1982).
23. Malouin F, Bryan L E, **Antimicrobial Agents and Chemotherapy**, **30** : 1 (1986).
24. Chen Y L; **J. Antibiot**, **44** : 970 (1991).
25. Mark J. Suto John M. Domagata and Paul F. Miller, **Annual Reports in Medicinal Chemistry**, Academic Press WC, California, **27** : 119, (1992).
26. Barrear M., Antoine M, Beni Chon J L, Deseonclosis J F, Girand P, Robin M, Wentzler S, Picault G, Desnotter J F, Rotin O, and Bouanchaud D H, **31st Interscience Conference on Antimicrobial Agents and Chemotherapy**, 369 (1991).
27. Barlkovitz D, Corraz A, Dietoner K, Georjopapadakoa N, Keith D Mook, R. PnuessD and Wei CC, **31st Interscience Conference on Antimicrobial Agents and Chemotherapy**, 824 (1991).
28. Hirai K, Hissaka M, Niwat Y, Yasui T, Fukuda H, Ishizaki t, Suzur S, and Nishino K, **30th International Conference on Antimicrobial Agents and Chemotherapy**, 385 (1990).
29. Biskid G, Dikuch H A, Fallat V, keith D D, Lipschitz ER, McGarry D H, Rossman P and Siebelist J, **30th International Conference on Antimicrobial Agents and Chemotherapy**, 403 (1990).
30. Lyman C A, Walsh T J, **Drugs** **44** : 9 (1992).
31. Kovacs J A, Koracs AA, Blis M, Wright W C, Gill VJ, Tuazon CV, **Ann. International Medical**, **103** : 533 (1985).
32. Kerridge D., **Advances in Microbiological Physiology**, **27** : 1 (1986).
33. Grunberg E., Titsworth E., Bennet M.,; **Antimicrobial Agents and Chemotherapy**, **3** : 566 (1964).
34. Hazen E L, Brown R, **Science**, 423 (1950).
35. Grove J F, Ismay D., Macmillan J., Mulhottan TPC, Rogers Mat, **Chem Ind.**, **11** : 219 (1951).
36. Weinberg ED, **Annals of New York Academy of Sciences**, **148** : 587 (1968).

37. Leavell UW Jr., **The Diagnosis and Treatment of Fungal Infections**, Robinson HM Ed, Thomas Springfield, **111** : 89 (1974).
38. Campbell Ah, **Experiemtnal Chemotherapy** Vol. 3 Schnitzer RJ, Hawking F. Eds, Academic Press New York (1964). P. 56
39. Brown R., **Experimental Chemotherapy**, Vol. 3 Schnitzer RJ, Hawking F. Eds, Academic Press New York (1964). P. 60
40. Rippon J W, **Medical Mycology**, Suanders, Philadelphia, p. 531 (1974).
41. Plempel M, Bartmann K, Buchel K H, Rogel E, **Post Grade Med. J.**, **50** : 11 (1974).
42. Thienpont D, van Cj, Nveten JM, Niemegur CJE, Marsboom R, **Arzneimittel Forschung**, **25** : 224 (1975).
43. Godefroi EF, Heeres J, Van CJ, **J. Med. Chem**, **12** : 784 (1969).
44. Preusser Hj, von Dw, **Trichophyton ruburm Mykosen**, **58** : 453 (1975).
45. Wright GC, Bayless AV, Gray JE, **Germen Offen** 2,427,382 cited from **Chem. Abstr. 82** : 171087 (1975).
46. Wright GC, Bayless Av, Gray JE, **German Offen** 2,427,377 cited from **Chem Abstr. 82** : 171088 (1975).
47. Richardson K, Cooper K, Marriott MS, Tarbit MH, Troke PF, **Review of Infection Diseases**, **12** : S 267 (1990).
48. Warnick DW, **J. Antimicrobial and Chemotherapy**, **24** : 275 (1989).
49. Grabill Jr, **Infections Disease Clinics of North America**, **2** : 805 (1988).
50. Saag MS, Dismukes WF, **Emphasis in New Triazoles Antimicrobial Agents and Chemotherapy.**, **32** :1 (1988).
51. Welch TJ, McCully C, Rinaldi MG, Walance JC, Babs FM, **Antimicrobial Agents and Chemotherapy**, **34** : 1281(1990).
52. Van CJ, Van GF, Janssen PAJ, **Revista Iberica de Micologia** 5(1988).
53. Ryley JF, Mcgregor S, Wilson RG, **Annals of New York Academy of Sciences** **544** : 310 (1988).
54. Formtling R A, **Clinical Microbiology Reviews**, **1** : 187 (1988).
55. Schaude M, Petramyi J. Nungassnar J. G., et al, **Revisa Iberica de Micologia**, 5 (1988).
56. **Diss. Abstr. Int. B**; 45 (11), 3511; cited from **Chem. Abstr.**, **103** : 104821e (1985).

57. Haerberle, Norman *et al*; **Eur. Pat. Appl.** EP 173, 284; cited from **Chem. Abstr.**, **105** : 37470 r (1986).
58. Wilmheim sandermann *et al*. **Ger. Offen**, **2**, 1977, 641, 935, Cited from **Chem. Abstr**, **88**, 37604 u (1978).
59. Korhalokar A., Khadse B. G., **Indian Drugs**, **29(7)** : 306-307 (1992).
60. Buchanan, O. H. and Freyberg, R. H., **J. Pharmacol Exp. Ther.**, **82** : 391 (1944).
61. V.M. Ragnekar and B.G. Khadse, **Ind. Jour. Pharm. Sci.**, **46 (6)** : 196-8 (1984).
62. N. Giraldi, Danenico Artini and willy Longeman, **Gen. Pat**; **1**, 159, 459 (Dec. 19, 1963); cited from **Chem. Abstr.**; **61** : 8325 d (1964).
63. Friedmann, E., Marrian, D. H. and Simon-Reves, L. **Brit. J. Pharmacol.**, **4** : 105 (1949).
64. a) Torgenson, D. C., Hensley, W. H. and Lambrich, J. A. **Contrib. Boyce Thompson Inst.**, **22** : 67 (1963); cited from **Chem. Rev.**; **70** : 466 (1970).
 b) Marrian, D. H., Friendmann, E. and ward, Jean, L. **Biochem. J.**, **54** : 65 (1953).
65. Dury, Franz Reichenederkari, Adolphi, Heinrich, and Stumeeyer, Herbert, **Ger. Offen. Pat.**; **1**, 146, 694 (1963); cited from **Chem. Abstr.**; **59** : 3277 f (1963).
66. Vigier, J., Boucherie, A. (Lab. Chim..Toxicol. Fac. Med. Pharm. Geenoble, Fr. **Bull. Trav. Soc. Pharm. Lyon.**, **15(3)** : 83-90 (1971).
67. Ferenczy, L., Markovics, B. and 250lt., **J. Acta Univ. Szeged; Acta Biol.**; **7 (34)** : 75 (1961); cited from **Chem. Abstr.**, **60**: 4503 e (1964).
68. Koji Miura; **Antimicro. Agents Chemother.**; 275, 1962; Cited from **Chem. Abstr.**, **59** : 13233b (1963).
69. P. Juhasz, I. Bekesi **J. Uri. Biochem. Parmacol**; **13 (2)** : 235-239 (1964).
70. Chaturvedi, R. V. et al. **Natl. Acod. Sci. Letl. (India)**, **10 (12)** : 417-18 (1987); cited from **Chem. Abstr.**, **109** : 107584 g (1988).
71. Moyer, R. L. **Rev. Med. France**, 31 (1947).
72. Vincent C. Bany, J. G. Belton, ML. Conalty, *et al.*, **Proc. Roy. Irish Acad.** **53 B** : 61-83 (1950); cited from **Chem. Abstr.**, **45** : 9501e, (1951).

73. M. C. Khosla, J. D. Kohli and N. Anand; **J. Sci. Ind. Res. (India)**, **18C** : 51 (1959).
74. Wilhelm Sandermann et al. **Ger. Offen** 2, 641, 935, 1977; cited from **Chem. Abstr.**, **88** : 37604 (1978).
75. Akopryan, N. E. and Gerasimyan, N. D. **Bio. Zh. Arm.**, **24(2)** : 84 (1971); Cited from **Chem. Abstr.**, **75** : 61869 K (1971).
76. V. M. Ragnekar and B. G. Khadse, **Ind. Jour. Chem.** **25B(3)** : 342-4 (1986).
77. Nargund, K. S. *et al.* **Indian Drugs**, **22 (3)** : 121-2 (1984).
78. **Schizuoka Coll. Pharm. Sci.**, Shizuoka, Japan 422104 (11) : 1198-206, 1984; cited from **Chem. Abstr.**, **102** : 125085 n (1985).
79. Shimizu, M., Sekine, y; Higuchi, H. Suzuki, H; H. Nakamura, S. and Makamura, K. **Antimicrob. Agents Chemother**, 123 (1970). Cited from **Burger's Medicinal Chemistry**, Part II, P : 68 (1979).
80. Dawson, Norman D. (Robins, A. H., co; Inc.) V. S. 3,433, 801, 1969; Cited From **Chem. Abstr.** **70** : 115002q (1969).
81. Deguchi, Yoshio; Koto, *et al*, (Fujisawa Pharmaceutical Co. Ltd.) Japan 6905, 221, (1969) ; cited from **Chem. Abstr.** **70** : 115003 r (1969).
82. Lockhart, Ian M. (Parke, Davis and Co.) **Ger. Offen.** 1914, 925, 1969; cited from **Chem. Abstr.**, **72** : 43433x (1970).
83. Fujisawa Pharmaceutical Co. Ltd. (JP); **Drug Fut**, **22(8)** : 830-832 (1997).
84. C. L. Grady, J. V. Haxby, N. L. Schala Geter, G. Berg, S. I. Rapoport, **Neurology**, **36** : 1390-1392 (1986).
85. C.D. Nicholson, **Psychopharm.** (Berl), **101 (2)** : 147-159 (1990).
86. R. S. Frackowiak, C. Pozzilli, N. Legg et al, **Brain**, **104** : 649-654 (1981).
87. D. S. Olton, G. L. Wenk, **Psychopharmacology: The Third Generation of Progress**, Raven Press, New York (1987).
88. C. Flicker, M. Serby, and S. H. Ferris, **Psychopharmacology** (Berl), **100(2)** : 243-250 (1990).
89. C. H. Vanderwolf, **Brain Res.** **504(2)** : 192-198 (1989).
90. Gavande Aparna, Kulkarni V. M. Thesis titled as "Synthesis of Drug Intermediate' , University of Mumbai (1998).
91. Richard Baltzly, Johannes and S. Buck *et al*, **J. Am. Chem. Soc.** **66** : 263-6 (1944).

92. Barakat, M. Z., Shehab, S. K. and El-sadr, M. M. **J. Chem. Soc.**, p. 4133 (1957).
93. Toshiaki, Ozaki, Fukashi, Horiuchi and Akira, Fujinami. **Ger. Offen**, **1**, 812, 206 (1969); cited from **Chem. Abstr.**, **71** : 124013 s (1969).
94. Luckes, R. and Pergal, M. **Collection Czech. Chem. Commu.**, **27** : 1387 (1962).
95. Rinkes, I. J. **Rec. Trav. Chim.**, **48** : 960 (1929); cited from 'Chemistry of Amides', John Wiley and Sons Ltd., p. 335 (1970).
96. Anschütz, r. and Wirtz, Q., **Ann. Chem.**, **239** : 154 (1887); cited from 'Chemistry of Amides,' John Wiley and Sons Ltd. P. 335 (1970).
97. Imperial Chemical Industries Ltd. **Neth. Appl.**, 6,412,344 (1965); cited from **Chem. Abstr.**, **63** : 8260a (1965).
98. Sauers, C. K. and Cotter, R. J., **U. S. Pat.**, 3,018,290 (1962); cited from **Chem. Abstr.**, **56** : 99761 (1962).
99. Sato, Hisao, **Japan Kokai**, 75, 126, 6599 (1975); Cited from **Chem. Abstr.**, **85** : 62687t (1976).
100. Kretov, a. E. and Kul'chitskaya, N. E., **J. Gern. Chem. USSR.**, **26** : 221 (1956); cited from **Chem. Abstr.**, **50** : 13771 g (1956).
101. Takaya, Tsuguo and Okuda, Yukio. **Japan. Kokai.**, 75,36,453 (1975); Cited from **Chem. Abstr.** **83** : 78646y (1975).
102. Takaya, Tsuguo and Koike, Hiroshi., **Japan Kokai.**, 7688, 959 (1976); Cited from **Chem. Abstr.**, **86** : 105974z (1977).
103. "Comprehensive Organic Chemistry", Ed., Sir Derek Barton and W. David Ollis., Pergamon Press Ltd., **2** : 959, (1979).
104. Burley, R. W. and Haylett, T. **Chem. and Ind.**, 1285 (1959).
105. Dawson, N. D. and Cale, a. D. Jr. **U. S. Pat.**, **3** : 433, 802 (1969); Cited from **Chem. Abstr.** **70** : 96612w (1969).
106. Istratou, Rodica, Farcasin, Malvina and Nicolan, Cd. **Rev. Roum. Chim.**, **12**, 1429 (1967).
107. 'Organic Synthesis', Ed., Baumgarten, H. E., John Wiley and Sons, Inc., **5** : 944 (1973).
108. Cotter, R. J., Sauers, C. K. and Whelan, J. M., **J. Org. Chem.**, **26** : 10 (1961).
109. Abblard, Jean and Baudoin, Michel, **Ger. Offen.**, 2,751,901 (1978); Cited from **Chem. Abstr.**, **89** : 109072p (1978).

110. Oba, Masayuki, Kawamata, Motoo, Tsuboi, Hikotada and Koga, Shinjii, **Japan Kokai**, Tokyokoho, 79,66,671 (1979); Cited from **Chem. Abstr.**, **91** : 193168w (1979).
111. Prill, E. J., **U. S. Pat.**, 2,524,145 (1950); Cited from **J. Am. Chem. Soc.**, **76** : 2835 (1954).
112. Tawney, P. O., **U. S. Pat.**, 2,524,145 (1950); cited from **J. Am. Chem. Soc.**, **76** : 2835 (1954).
113. Kwart, H. and Burchuk, I. **J. Am. Chem. Soc.**, **74** : 3094 (1952).
114. DeMayo, P. and Reid, S. T. **Chem. and Ind.**, 1576 (1962).
115. Vogel, A. I. '**A Text Book of Practical Organic Chemistry**' 3rd Ed., p. 277 (1956).
116. Profft, E., Runge, F. and Jumar, A. **J. Prakt. Chem.**, **1(4)** : 57 (1954).
117. Sekera, A., Boroansky, A., Jakubee, I., Palat, K. and Vrba, C., **Ceskoslov. Farm.**, **5** : 388 (1956); Cited from **Chem. Abstr.**, **51** : 8669a (1957).
118. Darcet, E. **Ann. Chim, Phys.**, **58** : 294 (1835); Cited from **Chem. Revs.**, **70** : 439 (1970).
119. Lange, Jerzy., Urbanski, Tadensz and Venulet, Jan., **Roczuli Chem.**, **36** : 1631 (1962); Cited from **Chem. Abstr.**, **59** : 9868d (1963).
120. Fuerst, Ernst. Winderl, Siegfried., Wittever, Arnold and Hoffmann, Herwig. **Ger. Offen.**, 2,313,386 (1974); Cited from **Chem. Abstr.**, **82** : 3825b (1975).
121. Katsuji, Nodera and Akira, Fujinami., **Ger. Offen.**, **2** : 100, 800 (1971); Cited from **Chem. Abstr.**, **75** : 118127y (1971).
122. Bruszewski, Zdzislaw, **Pol. Patent**, 103,705 (1979); Cited from **Chem. Abstr.**, **93**, 8013x (1980).
123. Hoey, G. B. and Lester, C. T., **J. Am. Chem. Soc.**, **73** : 4473 (1951).
124. Roderick, W. R., **J. Am. Chem. Soc.**, **79** : 1710 (1957).
125. Jakob, Wolfgang and Meyer, Karl Heinrich, **Ger. Offen.**, 2,720,311 (1978); Cited from **Chem. Abstr.**, **90** : 71777c (1979).
126. Hope, P. and Wiles, L. A., **J. Chem. Soc.**, p. 4583 (1965).
127. Marrian, D. H. **J. Chem. Soc.**, Part II, p. 1515 (1949).
128. Mustafa, A., Asker, W., Khattab, S. and Zayed, S.M.A.D. **J. Org. Chem.**, **26** : 787 (1961).
129. Jolles, E. **Gazz. Chim. Ital.**, 65,1221 (1935); Cited from **Chem. Revs.**, **70** : 451 (1970).

130. Sharpless, N. E. and Flavin, M., **Biochem.**, **5(9)** : 2963 (1966).
131. Dawson, N. D. **U. S. Patent**, 3,433,801 (1969); Cited from **Chem. Abstr.**, **70** : 115002 g (1969).
132. Dawson, N. D., Cale, A. D., Jr. and Lusfond, D. D. **Fr. Patent**, 1,551,862 (1969); Cited from **Chem. Abstr.**, **72** : 43432w (1970).
133. Kul'chitskaya, N. E., Kremlev, M. M., Egorova, N. L. and Romanenko, V. D. **Vop. Khim. Khim. Tekhnol.**, **26** : 9 (1972); Cited from **Chem. Abstr.**, **78** : 84217x (1973).
134. Hargreaves, M. K., Pritchard, J. G. and Dave, H. R. **Chem. Revs.**, **70** : 439 (1970).
135. Elvidge, J. A. and Linstead, R. P. **J. Chem. Soc.**, Part I : 442 (1954).
136. Padsalgikar, K. N. **M. Sc. Thesis**, University of Bombay (1971).
137. Ciba Ltd., **Swiss Patent**, 275,798 (1951); Cited from **Chem. Abstr.**, **47** : 1732 (1953).
138. Scarpati, R. and Santacroce, C. **Rend. Accad. Sci. Fis. Mat. Napples**, **28** : 27 (1961); Cited from **Chem. Revs.**, **70** : 442 (1970).
139. Falbe, J. and Korte, F. **Ber.**, **95** : 2680 (1960).
140. 'Organic Chemistry', Ed., Neckers, D. C. and Doyle, M. P. John Wiley and Sons, Inc., p. 908 (1977).
141. Vystrcil and Prokes, **Chem. Listy.**, **46** : 670 (1952), Cited from **Chem. Abstr.**, **48** : 165 (1954).
142. Grammaticakis, P., **Bull. Soc. Chi. France**, p. 207, (1953).
143. Butler, K. and Patridge, M. W., **J. Chem. Soc.**, p. 2398, (1959).
144. Smith, H. E., **J. Org. Chem.**, **31** : 684 (1966).
145. Augustin, R. L., **Catalytic Hydrogenation**, Marcell, Dekker, New York, p. 91-92 (1965).
146. Rylander, P. N., **Catalytic Hydrogenation over platinum metals**, Academic Press, New York, P : 168-203 (1967)
147. Allen, C. F. H. and Allan, J. Van, Organisation, **Syn. Coll.**, **3** : 63 (1965).
148. Augustin, R. L., **Catalytic Hydrogenation**; Marcell Dekker, New York, P : 152 (1965).
149. Brown, H. C. and Sivarankarn, **J. Am. Chem. Soc.**, **84** : 2828 (1962).
150. Augustin, R. L., **Catalytic Hydrogenation**, Marcell Dekker, New York P : 36 (1965).

151. Berson, J. A. and Cohen, T; **J. Org. Chem.**, **20** : 1461 (1951).
152. G. M. Tomokkin and B. I. Kissin, **Khim Prom**; **3** : 79 (1960), Cited from **Chem. Abstr.** **55** : 469 (1961).
153. F. Feigl and P. W. West, **Anal. Chem.**, **19** : 351 (1947).
154. Sandler and Karo, "**Organic functional group preparations**", **2** : 424-450, Academic Press; New York, (1971).
155. Hein, **J. Chem. Educ.** **40** : 181 (1963).
156. Challis and Kryptopoulos, **J. Chem. Soc.**, Perkin Trans **7** : 299 (1979).
157. a) Smith and Loeppky, **J. Am. Chem. Soc.**, **89** : 1147 (1967).
b) Gowenlock, Hutchison, Little, **J. Chem. Soc.**, Perkin Trans **2** : 1110 (1979).
158. T. G. Bonner, R. A. Hancock, G. Yousif and F. R. Rolle, **J. Chem. Soc.**, (B); 1237 (1969).
159. B. S. Furniss, A. J. Hannaford, V. Rogers, P. W. G. Smith, A. R. Totchell, **Vogel's Textbook of Practical Organic Chemistry**, 4th Edition, ELBS P : 1129, (1978).
160. Ray Q. Brewster, **Organic Chemistry**, 2nd edition, Prentice Hall, Inc. P : 202-204, 210.
161. Mason, G. W., Brooker, E. G. **Proc. N. Z. Weed Pest Contr. Conf.**, No. 21 163-72 (Eng) (1968) Cited from **Chem. Abstr**, **70** : 77494s (1969).
162. Sjogren, E. B., Rider, M. A., Nelson P. H. *et al*, **J. Med. Chem.**, **34** : 3295-3301 (1991).
163. Luvalle, J. E., Glass, D. b. and Weissberger, A. **J. Am. Chem. Soc.**, **70** : 2227 (1948).
164. Badische Anilin and Soda-Fabrik (I.G. Farbenindustrie Akt. – Ges. "In Auflosung") (August Weickemann, Inventor) – **Ger.** 803,903, Apr. 12, 1951, cited from **Chem. Abstr.**, **45** : 8047 a (1951).

Appendix

APPENDIX

1. The Melting points have been determined in open capillaries in a Veego VMP-1 melting point apparatus, and are uncorrected.
2. The TLC of the compounds have been run on Eastman Kodak precoated Silica gel plates of type K301 R and E. Merck precoated silica gel plates of type 60 F 254.
3. The Infrared Spectra were recorded on BOMEM MB-104 Fourier Transform Infrared Spectrometer.
4. The PMR Spectras were recorded on Varian 300 MHz spectrometer using TMS as the internal reference, at RSIC, IIT, Powai.
5. Carbon, nitrogen and hydrogen analyses were carried out on Heraeus C, H, N, Elemental Analyzer at the Haffkine Institute.

Synopsis

SYNOPSIS
OF THE THESIS TO BE SUBMITTED TO
THE UNIVERSITY OF MUMBAI
FOR THE DEGREE OF
MASTER OF PHARMACEUTICAL SCIENCES
IN THE FACULTY OF TECHNOLOGY.

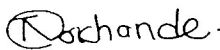
TITLE OF THE THESIS : Preparation, Spectroscopic characterisation and evaluation of possible biologically active compounds.

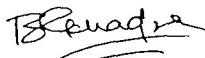
NAME OF THE CANDIDATE : Mr. Lokhande Tushar N.

NAME AND DESIGNATION OF THE GUIDE : Dr. B.G. Khadse
Ex-Director and head of Dept. of Chemotherapy
Haffkine Institute, Parel, Mumbai-12.

PLACE OF RESEARCH : Department of Chemotherapy,
Haffkine Institute,
Parel, Mumbai-12.

NUMBER AND DATE OF REGISTRATION : 41, Feb.01, 1999.

SIGNATURE OF THE STUDENT : 

SIGNATURE OF THE GUIDE : 

SYNOPSIS

Modern drug design is a multidisciplinary activity involving the combined expertise of chemists, biochemists, biologists, pharmacologists, theoretical and computational chemists, together with many others, who take part in the drug development program.

The principle goal of medicinal chemistry is the discovery of lead compounds, their molecular modifications, the preparation of drug, and the explanation of their molecular mode of action. Lead compounds are discovered by screening, but if a biochemical aberration can be established as an etiological factor in a given disease, metabolites involved in the biochemical lesions can become lead molecules. In a few cases, the reactions of drug with active receptor sites, especially enzymatic cofactors, have been analyzed and these serve as the guides for designing lead compounds. The rationale for molecular modification is based on bioisosterism¹.

However, there is a constant need for the discovery of new drugs due to the resistance being exhibited by different micro-organisms against various existing drugs. The emergence of multidrug resistant *Mycobacterium tuberculosis* and other such resistant strains of bacteria are a major threat to medicinal chemistry.

An attempt has been made in this research project to try and find out an answer to this problem. All compounds have been synthesized with the hope of achieving novel anti-tubercular, anti-bacterial and antifungal activity.

All synthesized compounds have been characterized by TLC, M.P. (recorded in open capillaries and are uncorrected), FT-IR (recorded on Hartmann - Braun, MB-104 series), NMR (recorded at RSIC, IIT, Mumbai) and carbon, hydrogen and nitrogen analysis (recorded on Heraeus CHN elemental analyzer) Haffkine Institute.

The thesis will consist of the following parts :

PART- I : Introduction, Review of Literature and Plan of work.

PART-II : Synthesis of 4[4'(4''-chlorophenoxy)-3'-chloroanilino] -N- (alkyl / aryl / substituted aryl / heteroaryl) succinimides.

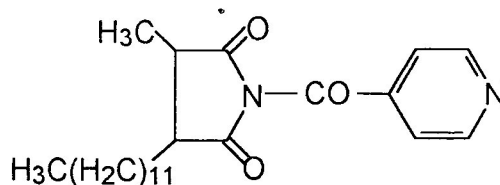
PART-III : Synthesis of 4-(pyrrolidine anilino)-N-(aryl / substituted aryl / heteroaryl) succinimides.

PART-IV : Synthesis of 1-acetyl, 4-nitroso piperazine as drug intermediate for N- (4-acetyl -1- piperaziny) -4 - fluoro benzamide monohydrate(FK-960).

PART-V : Biological Screening, results and discussion.

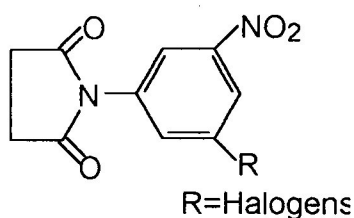
PART-II : Synthesis of 4 [4' (4''-chlorophenoxy)-3'-chloroanilino]-N-(alkyl / aryl / substituted aryl / heteroaryl) succinimides.

Literature survey revealed that succinimide derivatives possess anticonvulsant², fungicidal³, antibacterial⁴ and antitubercular⁵ activity. Furthermore, N-isonicotinamido- 3-dodecyl-4-methyl succinimide (I) was reported to exhibit excellent antitubercular activity⁶.



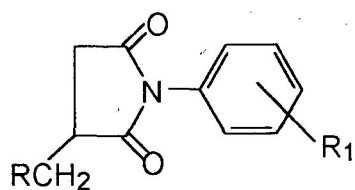
(I)

Also, 1-(3-nitro-5-halogenophenyl) pyrrolidine-2,5-dione (II) was found to possess a good fungicidal⁷ activity.



(II)

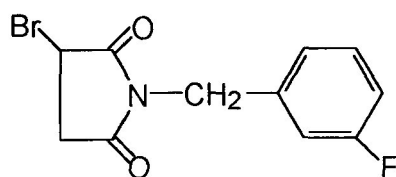
Substituted 1-phenyl-3-methyl pyrrolidinediones (III) were also reported to possess fungicidal activity³.



R=Halo, CN, SCN etc.
R₁=Halo, CN, NO₂ etc.

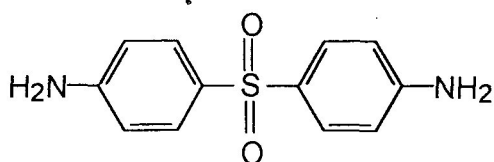
(III)

The following compound (IV) also shows fungicidal activity⁸.

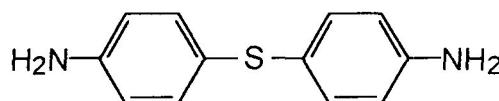


(IV)

4,4'-diaminodiphenyl sulfone (V) has been successfully used in the treatment of leprosy which is also a mycobacterial infection. 4,4'-diphenyl sulfide (VI) has shown *in vivo* antitubercular activity in experimental mice, which was attributed to the *in vivo* oxidation of sulfide to sulfone⁹. This example brings forth the significance of the sulfide linkage.

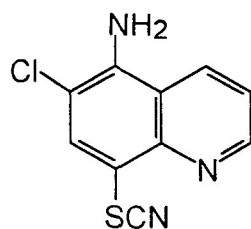


(V)

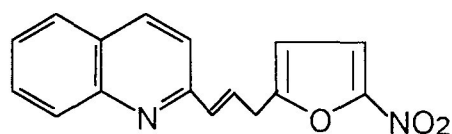


(VI)

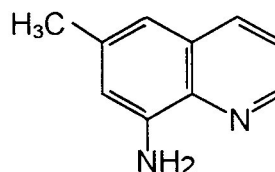
However, we decided to replace -S- by -O- to check the effect of such an isosteric replacement. Another parameter used for assessment was the introduction of electron withdrawing groups on the parent moiety. Also, quinoline derivatives are well known for their antimalarial, antifungal and antibacterial effects. Derivatives like 5-amino-6-chloro-8-thiocyanato quinoline (VII) and 2-[(5-Nitro-2-furyl)vinyl]quinoline(VIII) were found to be active against *Candida albicans*¹⁰ and *Streptococcal*¹¹ infections, while 8-amino-6-methyl quinoline¹² (IX) was claimed to possess antitubercular activity. Hence, quinoline substitution was also introduced on the parent moiety.



(VII)

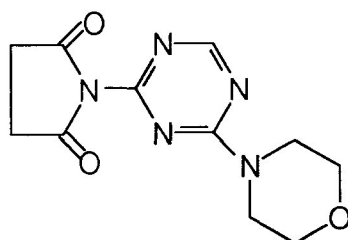


(VIII)



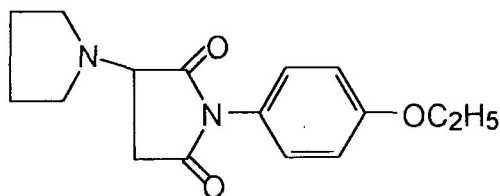
(IX)

Succinimides having heteroaryl substituents at 1-position are found to exhibit antimicrobial activity, for instance, 2-succinimido-4-morpholino-1,3,5-triazine (X) is reported to be an antiviral agent¹³.



(X)

Earlier studies carried out in our laboratory revealed that 4-(N-pyrrolidino)-N-(4-ethoxyphenyl) succinimide (XI) was active *in vitro* at the concentration 1.56 µg/ml against *M. tuberculosis*.

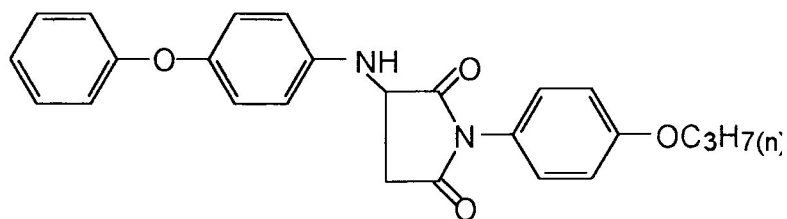


(XI)

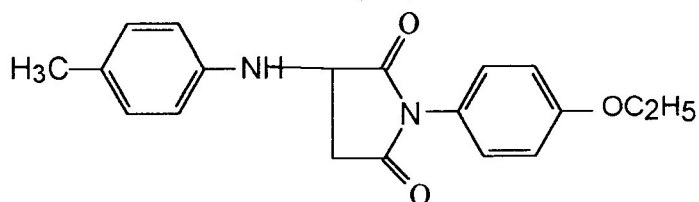
Antimicrobial studies carried out in our laboratory revealed that 4-(4'-phenoxyanilino)-N-(4-n-propoxyphenyl) succinimide (XII) inhibited the growth of *M. tuberculosis in vitro* at the MIC of 0.5 µg/ml. This activity is comparable to that of the standard drug, Isonicotinic acid hydrazide (INH).

Earlier studies have revealed that 4-(4'-methylanilino)-N-(4-ethoxyphenyl) succinimide¹⁴ (XIII) inhibits the *in vitro* growth of *Mycobacterium tuberculosis* at a MIC

of 0.78 $\mu\text{g/ml}$. These two examples indicate that alkoxy groups play an important role as regards antitubercular activity.

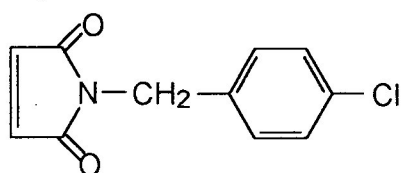


(XII)



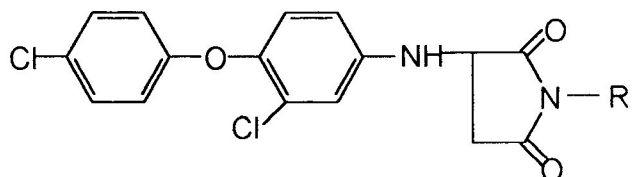
(XIII)

Also, various substituted maleimides are used as intermediates¹⁵ for anti-microbial drugs and are reported to possess fungicidal and bactericidal¹⁶ activity. eg - N-(p-chloro benzyl) maleimide (XIV) showed a marked antifungal¹⁷ activity against *C. albicans* and *T. mentagrophytes*.



(XIV)

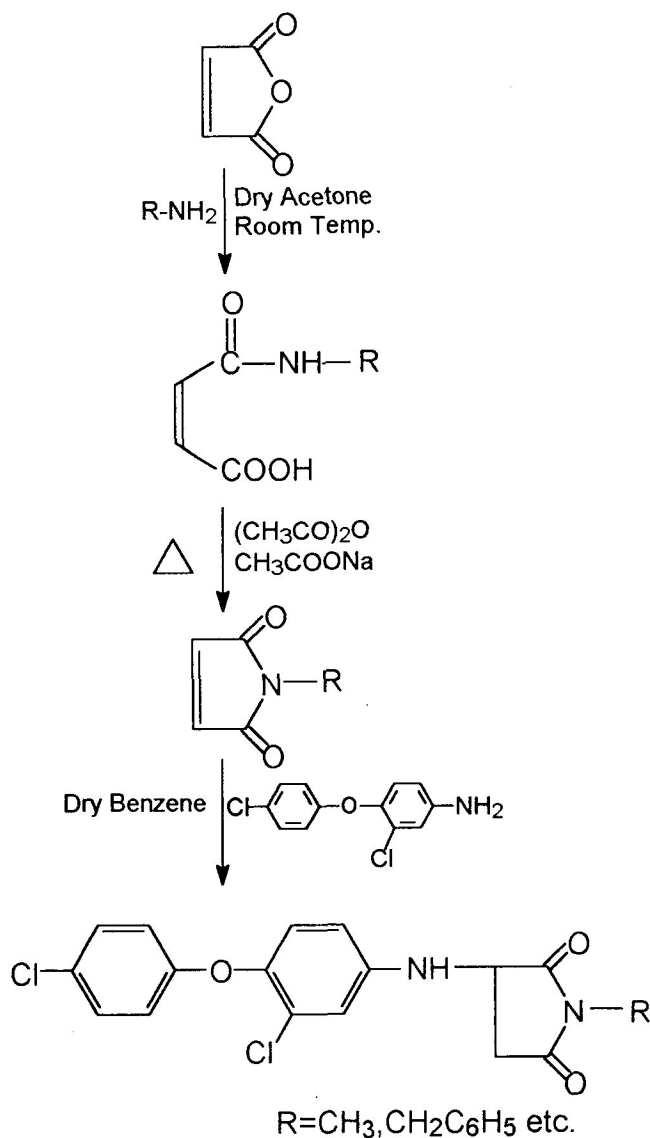
Thus, taking the importance of succinimide molecule into account, following compounds (XV) were synthesized as per scheme-1.



R=Ph-OC₃H₇(n), Ph-OC₄H₉(n), Ph-Cl etc.

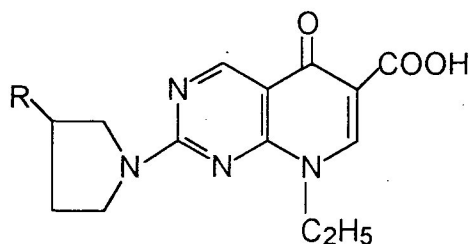
(XV)

SCHEME-1



Part-III : Synthesis of 4-(Pyrrolidino anilino)-N-(aryl/substituted aryl and heteroaryl) succinimides.

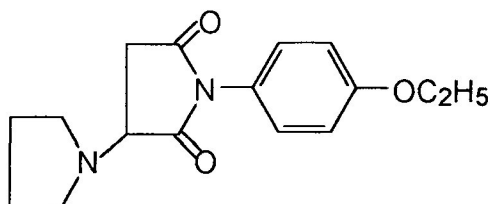
Piromidic acid (XVI) and its metabolite, hydroxy piromidic acid (XVII), containing N-pyrrolidino substituent possess antibacterial activity¹⁸ comparable to that of Nalidixic acid.



XVI: R=H;

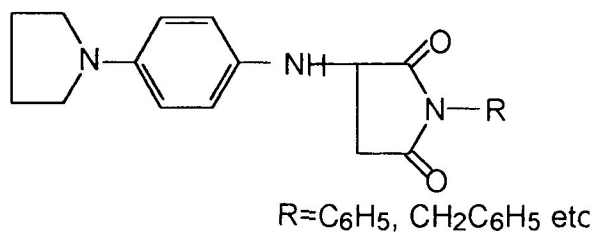
XVII: R=OH.

Earlier studies carried out in our laboratory revealed that 4-(N-pyrrolidino)-N-(4-ethoxy phenyl) succinimide (XVIII) was active *in vitro* at the concentration of 1.56 $\mu\text{g/ml}$ against *M. tuberculosis*. This proves the importance of pyrrolidine group.



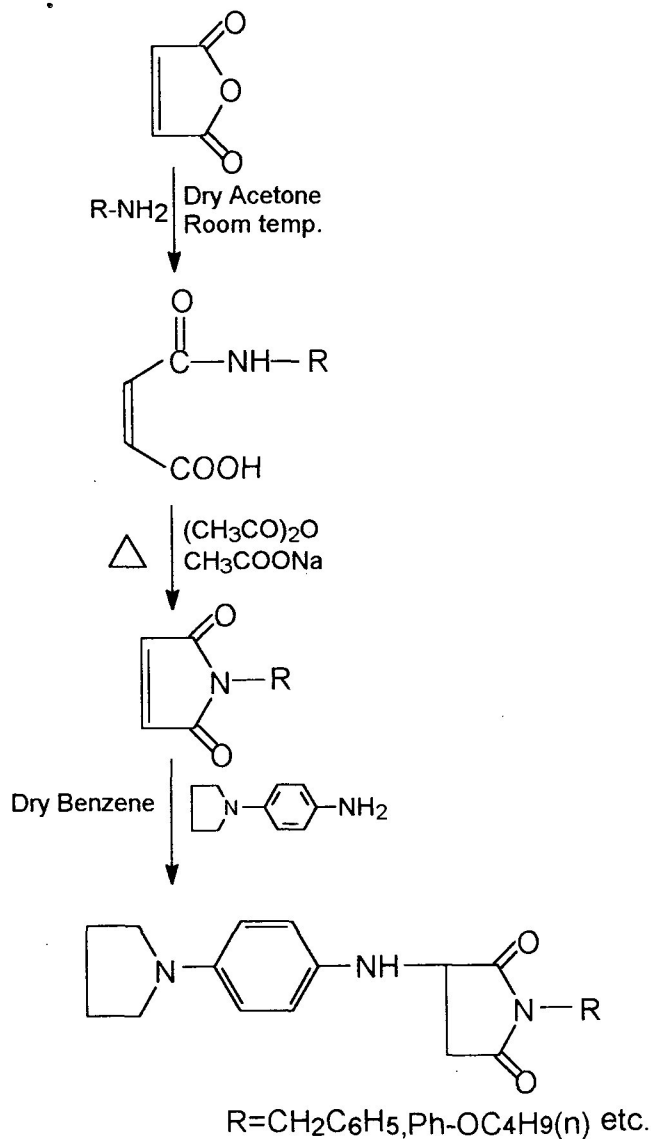
(XVIII)

The importance of succinimide moiety is already discussed in part -II. Therefore, it was decided to incorporate long chain substituents which is also one of strategies of drug development. Thus the following type of compounds were synthesized by condensing different substituted maleimide with 4-aminophenyl pyrrolidine to obtain the title compounds (XIX) as shown in scheme-2.



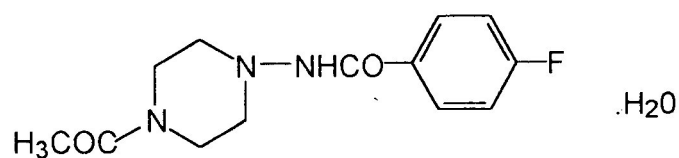
(XIX)

SCHEME-2



PART-IV : Preparation of 1-acetyl, 4-nitroso piperazine as drug intermediate for N-(4-acetyl-1-piperazinyl)-4-fluorobenzamide monohydrate (FK-960)¹⁹

FK-960 (XX) is a putative antidementia agent. It was discovered at Fujisawa, through a rational drug screening program. The unique pharmacological profile of FK-960 makes this compound an interesting alternative to cholinergic agonists or cholinesterase inhibitors in the treatment of Alzheimer's disease. It was shown to improve memory deficits in rats in passive avoidance.

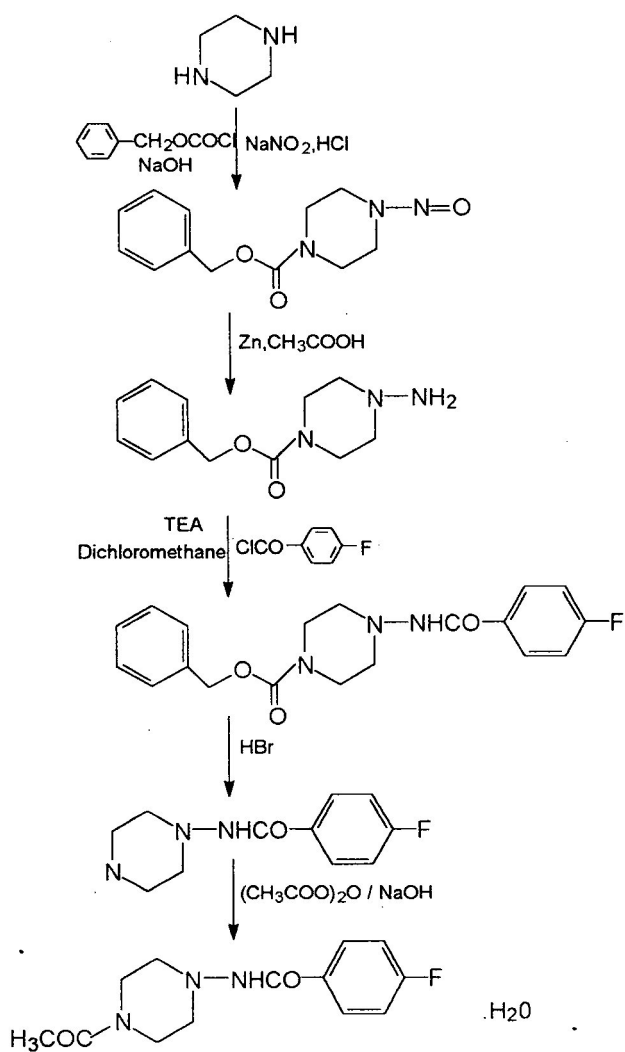


(XX)

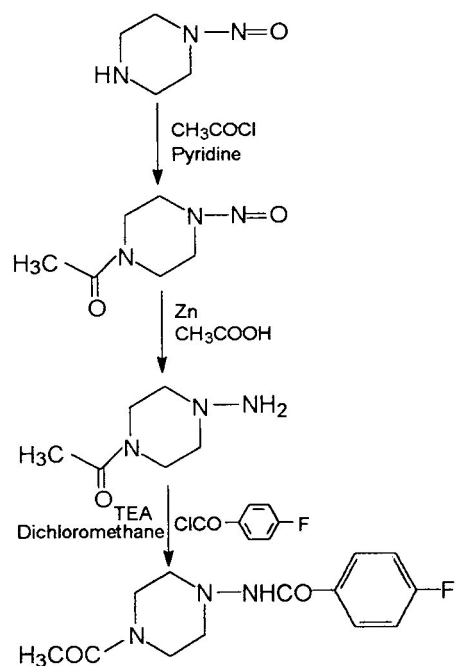
FK-960 is currently in phase II clinical trials.

SCHEME-3

Literature scheme-A



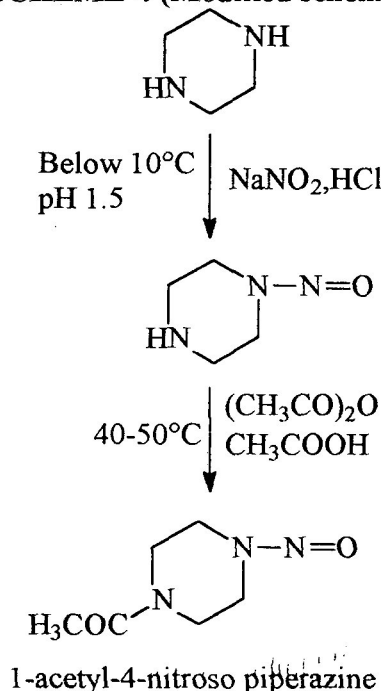
Literature scheme - B



Modifications :

1. In our laboratory N-nitroso piperazine was first obtained from piperazine, since it is easily available and is relatively cheaper.
2. N-nitroso piperazine was successfully obtained without using reagent such as benzyloxy carbonyl chloride as a blocking agent. This avoids one step, and ultimately time and money.
3. Acetylation of N-nitroso piperazine with acetic anhydride in glacial acetic acid is cost effective as compared to acetylation in acetyl chloride and pyridine in dioxane, as per literature scheme 3B.
4. Also, according to the literature scheme 3A, acetylation in acetic anhydride in the presence of sodium hydroxide gives rise to inorganic impurities which are difficult to be isolated.

Taking all these factors into consideration, the method of synthesis was suitably modified, thereby making the process commercially viable.

SCHEME-4 (Modified scheme)

PART-V: Biological screening.

All the synthesized compounds were subjected to antibacterial, antifungal and antitubercular screening.

For antibacterial screening, the organisms used were *Staphylococcus aureus* ATCC 3750 and *Salmonella typhi* NCTC 786. The medium used was Muller Hinton Broth. The standard drugs used were Trimethoprim (MIC 1 µg/ml) against *S. typhi* and Ampicillin (MIC 0.01 µg/ml) against *S. aureus*.

For antitubercular screening, H₃₇R_V strain of *M. tuberculosis* was used. The medium used for this screening was Kirchner's and Middlebrook 7 H9 broth base with ADC grown supplement. The standard drug used was Isoniazid (MIC 0.04 µg/ml).

For antifungal screening, the organisms used were *Candida albicans*, *Trichophyton mentagrophytes* and *Trichophyton rubrum*. The medium used for growth was Sraufataller medium. The MIC of Miconazole is 6.25 µg /ml against *C.albicans* and 1.56 µg /ml against *T.mentagrophytes* and *T. rubrum*.

The literature survey, experimental and biological details will be dealt within the thesis.

REFERENCES:

1. F.G. De Las Heras and S Vega; "Medicinal Chemistry Advances", Pergamon Press, 1 (1980).
2. Diss. Abstr. Int. B; 45(11),3511; Cited from Chem. Abstr.,103, 104821e (1985).
3. Haeberle, Norman *et al*; Eur. Pat. Appl. EP 173, 284; Cited from Chem. Abstr.,105, 37470r (1986).
4. Wilmheim Sandermann *et al* . Ger. offen, 2, 1977, 641, 935, Cited from Chem Abstr., 88, 37604u (1978).
5. Korhalokar A., Khadse B.G.; Indian Drugs, 29(7) pp:306-307 (1992).
6. Proct. Roy. Irish Acad.,57(B), 37-45 Cited from Chem. Abstr. ,50, 340g (1956)
7. Kato, Toshiro *et al*. Japan Kokai 7688, 631; Cited from Chem .Abstr., 85, 172785f, (1976).

8. Haeberle, Norman *et al*; **Ger. Offen** DE 3635, 282; **Cited from Chem. Abstr.**, **109**, 144606u (1998).
9. M.C. Khosla, J.D. Kohli and N. Anand; **J. Sci. Ind. Res.** (India), **18c**, 51 (1959).
10. Yashiki Hamada and hisayashi Sugihora Yakugu Zasshi.; **83**,138-142(1963), **Cited from Chem. Abstr.**, **59**, 5134d (1963).
11. Koji Miura; **Antimicro. Agents Chemother.**, 1962, 275; **Cited from Chem. Abstr.**, **59**, 13233b (1963).
12. P. Juhasz , I. Bekesi, **J Uri. Biochem. Pharmacol**; **13**(2), 235-239g, (1964)
13. N. Giraldi, Domenico Artini and Willy Longeman, **Gen. Pat**; **1**, 159, 459 (Dec. 19, (1963); **Cited from Chem. Abstr.**, **61**, 8325d (1964).
14. V.M. Rangnekar and B.G. Khadse **Ind. Jour. Chem.** **25B**(3), 342-4 (1986)
15. Nakazawa, Mikiro; Miki, Shigio (New Jpn. Chem. Co Ltd.) Jpn. Kokai Tokkyo Koho Jp., 62,138,468(87,138,468); **Cited from Chem. Abstr.**, **108**(9), 664 (1988)
16. Umio, Suminori; Kariyone, Kazuo; Nakamura, Hitoshi (Fujisawa Pharm. co. ltd.) **Japan** 6830, 309; **Cited from Chem. Abstr.**, **70**, 77625k (1969).
17. Vigier, J., Boucherie, A. (Lab. Chim. toxicol. Fac. **Bull. Trav. Soc. Pharm. Lyon**, **15**(3), 83-90(1971).
18. Shimizu, M., Sekine, Y., Higuchi, H. Suzuki, H., Nakamura, S. and Makamura, k. **Antimicrob. Agents Chemother.**, **123** (1970).
19. Fujisawa Pharmaceutical Co. Ltd. (JP).; **Drug fut.** **22**(8), 830-832, (1997).

ERRATA

[illegible]

