

SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF SOME
UNIQUE 4-SUBSTITUTED AMIDO-1, 2-DIHYDRO-2-
SUBSTITUTED PHENYL-1,5-DIMETHYL-3H-PYRAZOL-3-
ONE

THESIS SUBMITTED
UNIVERSITY OF MUMBAI

FOR THE DEGREE OF
MASTER OF SCIENCE
IN
CHEMISTRY

SUBMITTED BY
AMITA VIJAY MAYEKAR

UNDER THE GUIDANCE OF
DR. ANIL BOBADE

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

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SOME UNIQUE 4-SUBSTITUTED AMIDO-1,2-
DIHYDRO-2-SUBSTITUTED PHENYL-1,5-
DIMETHYL-3H-PYRAZOL-3-ONE**

A THESIS SUBMITTED
TO THE
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**M.Sc. (BY RESEARCH) DEGREE
IN CHEMISTRY**

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MISS. AMITA VIJAY URMILA MAYEKAR

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BOB

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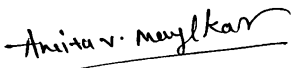
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STATEMENT BY THE CANDIDATE

As required by the University Regulation No. R.2190, I Wish to state that the work embodied in this thesis titled **"Synthesis and antimicrobial activity of some unique 4-substituted amido-1,2-dihydro-2-substituted phenyl-1,5-dimethyl-3H-pyrazol-3-one"** forms my own contribution to the research work carried out under the guidance of **Dr. Anil S. Bobade** at the **Haffkine Institute for Training, Research and Testing, Mumbai-12**. This work has not been submitted for any degree of this or any other University. Whenever references have been made to previous works of others, it has been clearly indicated as such and included in the Bibliography.

Signature of Candidate

 .

Miss. Amita Vijay Mayekar

Certified by



Dr. Anil S. Bobade,
Department of Chemotherapy,
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INTRODUCTION

INTRODUCTION

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

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

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

Basic research

Basic research (also called fundamental or pure research) has as its primary objective the advancement of knowledge and the theoretical understanding of the relations among variables. It is exploratory and often driven by the researcher's curiosity, interest, and intuition. Therefore, it is sometimes conducted without any practical end in mind, although it may have unexpected results pointing to practical applications. The terms "basic" or "fundamental" indicate that, through theory generation, basic research provides the foundation for further, sometimes applied research.

Research processes

Scientific research

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

Formation of the topic:

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

Research methods

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

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

Research can also fall into two distinct types:

- Primary research
- Secondary research

Primary research (also called **field research**) involves the collection of data that does not already exist. This can be through numerous forms, including questionnaires and telephone interviews

amongst others. This information may be used in such things as questionnaires, magazines, and Interviews.

The term is widely used in market research and competitive intelligence.

- May be very expensive because many people need to be confronted.
- By the time the research is complete it may be out of date.
- People may have to be employed or avoid their primary duties for the duration of the research.
- People may not reply if emails or letters are used.

Secondary research (also known as desk research) involves the summary, collection and/or synthesis of existing research rather than primary research, where data is collected from, for example, research subjects or experiments.

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

Secondary research can come from either internal or external sources^[1-3].

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

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

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

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

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

Process of drug discovery

The process from identification of a target to entry of the drug into the market has the following steps^[5]:

a) Discovery

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

More often, the hits can come from synthetic sources, such as historical compound collections and combinatorial chemistry.

b) Optimization

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

c) Development

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

d) Biological testing

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

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

The field of medicinal chemistry has evolved from an emphasis on the synthesis, isolation and characterization of drugs, to an increases

awareness of the biochemistry of diseased states and the design of drugs for the prevention of diseases. An important aspect of the medicinal chemistry has been to establish a relationship between chemical structure and biological activity.

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

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

Antimicrobial activity:

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

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

In the last decades, the high use of antimicrobial agents has been recognized as an important public health challenge. Their widespread

use has been linked to the development of bacterial resistance responsible for increases in morbidity, mortality, hospital stay duration and healthcare costs associated with untreatable infections ^[6] .

The history of antimicrobials begins with the observations of Pasteur and Joubert ^[7], who discovered that one type of bacteria could prevent the growth of another. They did not know at that time that the reason one bacteria failed to grow was that the other bacteria were producing an antibiotic. Technically, antibiotics are only those substances that are produced by one microorganism that kill, or prevent the growth, of another microorganism. Of course, in today's common usage, the term antibiotic is used to refer to almost any drug that cures a bacterial infection. Antimicrobials include not just antibiotics, but synthetically formed compounds as well.

Main classes

Antibiotics

Antibiotics are generally used to treat bacterial infections. The toxicity to humans and other animals from antibiotics is generally considered to be low. However, prolonged use of certain antibiotics can decrease the number of gut flora, which can have a negative impact on health. Some recommend that during or after prolonged antibiotic use, that one should consume probiotics and eat reasonably to replace destroyed gut flora.

The term antibiotic originally described only those formulations derived from living organisms but is now applied also to synthetic antimicrobials, such as the sulfonamides.

The discovery, development, and clinical use of antibiotics during the 20th century have substantially decreased mortality from bacterial infections. The antibiotic era began with the pneumatic application of nitroglycerine drugs, followed by a “golden” period of discovery from approximately 1945 to 1970, when a number of structurally diverse, highly effective agents were discovered and

developed. However, since 1980 the introduction of new antimicrobial agents for clinical use has declined. Paralleled to this there has been an alarming increase in bacterial resistance to existing agents.

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

Antiviral drugs

Antiviral drugs are a class of medication used specifically for treating viral infections. Like antibiotics, specific antivirals are used for specific viruses. They are relatively harmless to the host, and therefore can be used to treat infections. They should be distinguished from vermicides, which actively deactivate virus particles outside the body.

Most of the antivirals now available are designed to help deal with HIV; herpes viruses, best known for causing cold sores and genital herpes, but actually causing a wide range of diseases; the hepatitis B and C viruses, which can cause liver cancer; and influenza A and B viruses. Researchers are now working to extend the range of antivirals to other families of pathogens.

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

Antifungal drugs

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

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

The discovery of antimicrobials like penicillin and tetracycline paved the way for better health for millions around the world. Before 1941, the year penicillin was discovered; no true cure for gonorrhea, strep throat, or pneumonia existed. Patients with infected wounds often had to have a wounded limb removed, or face death from infection. Now, most of these infections can be easily cured with a short course of antimicrobials.

However, the future effectiveness of antimicrobial therapy is somewhat in doubt. Microorganisms, especially bacteria, are becoming resistant to more and more antimicrobial agents. Bacteria found in hospitals appear to be especially resilient, and are causing increasing difficulty for the sickest patients—those in the hospital. Currently, bacterial resistance is combated by the discovery of new drugs. However, microorganisms are becoming resistant more quickly than new drugs are being found, thus, future research in antimicrobial therapy may focus on finding how to overcome resistance to antimicrobials, or how to treat infections with alternative means.

Types of Infections for study purposes:

Bacterial Infections:

The development of resistance to current antibacterial therapy continues to drive the search for more effective agents. Bacteria are commonly responsible for many diseases which were considered until recently to be resistant to chemotherapy. The bacteria were thought to be primitive in structure and so could be influenced by chemicals which are not highly toxic to the host. Many attempts have been made to combat bacterial infections in animals by the use of drugs, but most of them have met with little or no success, with the exceptions of Morgenroth and Levy's partial success in year 1911. In combating bacterial infections of the blood stream, they found that ethyl dihydrocuprein (ophthocin), a quinine derivative, would cure pneumococcal infection in mice. Heidelberger and Jacobs coupled diazotized p-amino benzene sulphonyl amide with dihydro cuprien and found that the resultant compound had only mild activity and was not further investigated ^[8, 9].

However, in 1935, Domagk^[10], Mietzsch and Klarer^[11] first dispelled the failure of earlier scientists by discovering Prontosil and reported that this compound cures Streptococcal infections in mice and Staphylococcal infection in rabbit. This period was considered to be a brilliant era in the chemotherapy of microbial infections.

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

Fungal Infections:

There has been a recent increase in the worldwide prevalence of invasive fungal infections ^[12].

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

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

Considerable progress has been made in the chemotherapy of microbial disease. Yet, the development of resistance by the microbes to the drugs is an important problem faced by the chemotherapist. The rapidity of development of resistance varies widely under different circumstances. Besides the potent drugs used in the clinical therapy over years are being reported to show newer side effects. Thus there is always a need for search for newer compounds with potent activity, good tolerances, lesser side effects and increased efficacy against resistant microbial strains ^[13].

The development of new, rapid and clean synthetic routes toward focused libraries of nitrogen-containing heterocycles is of great importance to both medicinal and synthetic chemists^[14-16]. Consequently, the design and development of procedures for the generation of new heterocycles by means of multi step reaction is a matter of growing interest^[17].

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

Literature survey revealed that pyrazole and pyrazolone ring system represent an important class of compound not only for their theoretical interest but also for their antipyretic, antidepressant, anti-inflammatory, antiviral, antibacterial, antifungal activities^[18-23] to a lesser extent. They are also used as insecticides and herbicides. Recently, some aryl Pyrazoles are reported to have non-nucleoside HIV-1 reverse transcriptase inhibitory activity^[24].

HISTORY OF PYRAZOLE DERIVATIVES:

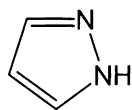
The first Pyrazole {I} compound was prepared by Knorr^[25], in 1883, from phenylhydrazine and a β -keto ester.

Pyrazole {I} refers both to the class of simple aromatic ring organic compounds of the heterocyclic series characterized by a 5-membered ring structure composed of three carbon atoms and two nitrogen atoms in adjacent positions and to the unsubstituted parent compound being so composed and having pharmacological effects on humans.

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

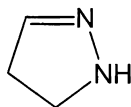
In medicine, Pyrazoles are used for their analgesic, anti-inflammatory, antipyretic, antiarrhythmic, tranquilizing, muscle relaxing, psychoanaleptic, anticonvulsant, monoamineoxidase inhibiting, antidiabetic and antibacterial activities ^[26-30].

Structurally related compounds are **pyrazoline** {II} and **pyrazolidine** {III}.



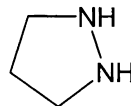
Pyrazole

{I}



Pyrazoline

{II}

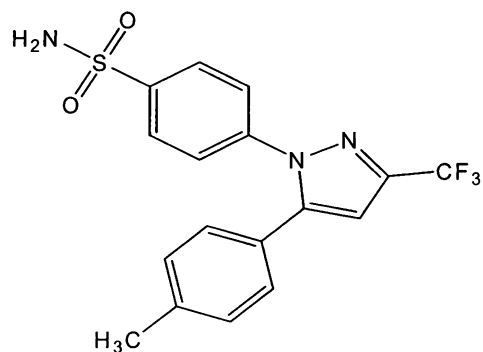


Pyrazolidine

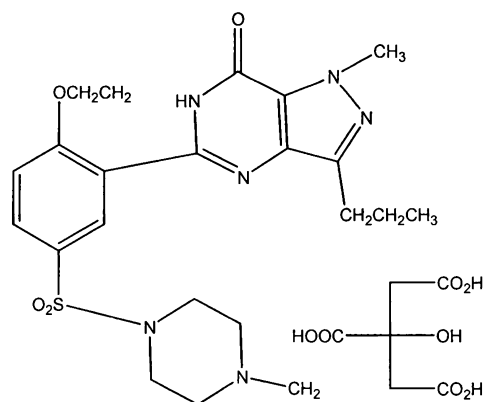
{III}

Substituted Pyrazoles are an important class of compounds in the pharmaceutical industry. Particularly interesting to medicinal chemists are the 1,3,5-trisubstituted and 1,3,4,5-tetrasubstituted Pyrazoles, which constitute the core structures of commercial drugs such as **Celebrex** {IV} and **Viagra** {V}.

The only member of this class currently available is celecoxib (CELEBREX). It is one of the selective COX-2 inhibitors and was approved for marketing in the United States in 1998.



Celebrex {IV}

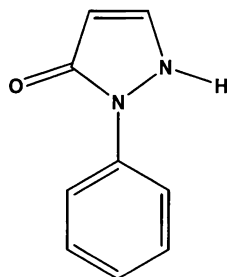


Viagra {V}

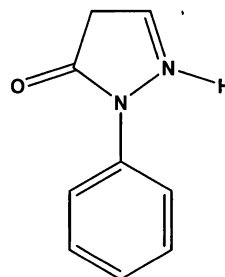
Pyrazolone {VI}, a five-membered ring lactam, is a derivative of pyrazole that has an additional keto ($=\text{O}$) group.

It has a molecular formula of $\text{C}_3\text{H}_4\text{N}_2\text{O}$.

It has the following structure:



3-PYRAZOLONE



5-PYRAZOLONE

{VI}

The Pyrazolone ring is a prominent structural motif found in numerous pharmaceutical active compounds. This is mainly due to the ease preparation and the important biological activity. Pyrazolone framework plays an essential role in biologically active compounds and therefore represents an interesting template for combinatorial as well as medicinal chemistry^[31-33].

PYRAZOLONE DERIVATIVES:

This group of drugs includes phenylbutazone, oxyphebutazone, antipyrine, aminopyrine, and dipyrone. These drugs have been in clinical use for many years.

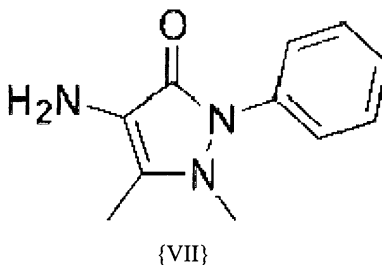
Other Examples Of Derivatives Include:

- Apazone
- Nifenazone
- Propyphenazone
- Kebuzone
- Suxibuzone
- Sulfamazone

For the research project the important drugs are as follows:

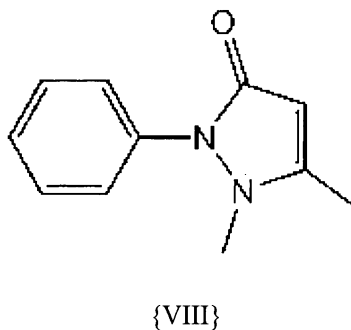
AMPYRONE (4-Aminoantipyrine):

Ampyrone {VII} is a metabolite of aminopyrine with analgesic, anti-inflammatory and antipyretic properties. Due to the risk of agranulocytosis its use as a drug is discouraged. Instead it is used as a reagent for biochemical reactions producing peroxides or phenols. Ampyrone stimulates liver microsomes and is also used to measure extracellular water.



PHENAZONE (Antipyrine):

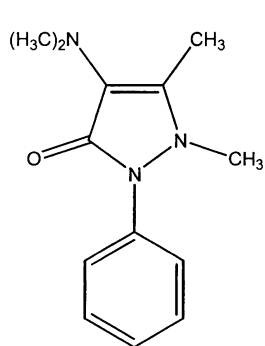
Phenazone or phenazon {VIII} is an analgesic. It was first synthesized by Ludwig Knorr in 1883. It is formed by reducing diortho-dinitrodiphenyl with sodium amalgam and methyl alcohol or by heating diphenylene-ortho-dihydrazine with hydrochloric acid to 150°C. It crystallizes in needles which melt at 156°C.



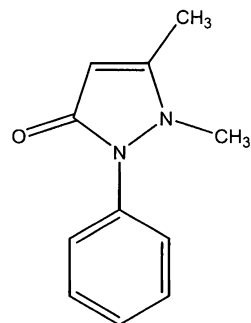
Hundred of compounds in the pyrazolone series have been synthesized since the discovery of the basic compound. However, only Antipyrine and Aminopyrine have been successful as mild analgesics and antipyretics.

Antipyrine (phenazone) {VIII} was introduced into medicine in 1884. Methylation of 1-phenyl-3-methyl-5-pyrazolone with methyl iodide and alkali in alcohol, methylsulfate and alkali, methyl benzensulfinate and alkali in benzene, or by methyl alcohol and hydrochloric acid gives the N-methyl derivative known as antipyrine. A few years later, a closely related compound, aminopyrine (puramidon) {IX} was synthesized and used clinically. Both antipyrine and aminopyrine were originally employed for antipyresis, but are now more often prescribed for analgesia. Both antipyrine and aminopyrine are phenyl-pyrazolon derivatives, aminopyrine being the 4-dimethylamino derivative of antipyrine.

Their structural formulas are as follows:-



AMINOPYRINE
{IX}



ANTIPYRINE
{VIII}

Plan Of Work

PLAN OF WORK:

Recent research work reported that pyrazolone derivative incorporating Antipyrine or phenazone (1-phenyl-2,3-dimethyl-3-pyrazoline-5-one) and 4-Aminoantipyrine moiety has attracted a great deal of interest due to its wide applications in the field of pharmaceuticals^[34-37]. Antipyrine was the first pyrazolone derivative used in the management of pain and inflammation and their derivatives have attracted the attention of several research groups due to their potential activities. In this context, broad spectra of bioactive antipyrine and 4-aminoantipyrine derivatives have been investigated and diversities of bioactive such as analgesic, anti-inflammatory, antimicrobial and anticancer activity have been reported^[38-44].

The antibacterial and antifungal activity sought our attention because antimicrobial resistance developed by important pathogens has increased in the last decade^[45, 46]. Besides, emerging and re-emerging bacterial infectious diseases still cause death and disability worldwide^[46].

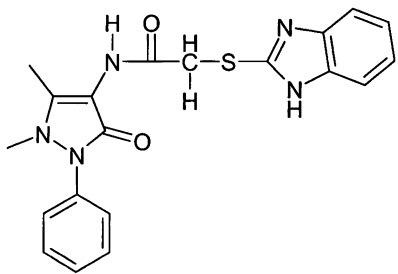
In view of the above-mentioned findings and as continuation of our effort to identify new candidates that may be of value in designing new, potent, selective and less toxic antimicrobial agents, we reported herein the synthesis of some new heterocycles incorporating antipyrine moiety.

Part 2:

Synthesis of 4-substituted amido-2-substituted phenyl-1,5-dimethyl-3-pyrazolone:

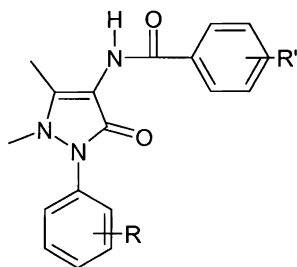
The survey of literature revealed that amido linkage is well known for its antimicrobial, antifungal and antitubercular activities^[47-50], A. N. Evstropov et al^[51] showed some amide derivatives having antiviral activity incorporating antipyrine moiety.

N. Karali et al^[52] showed some antimicrobial benzazole derivatives incorporating antipyrine moiety with amido linkage {X}.



{X}

So, we introduce the amide linkage at 4th position of antipyrine to get the novel substituted amido compounds which have the following general structure {XI}.



{XI}

Where, R = H, 4-Cl, 3,4-Dichloro etc.; R' = 2,6-Difluoro, H, 4-Cl etc.

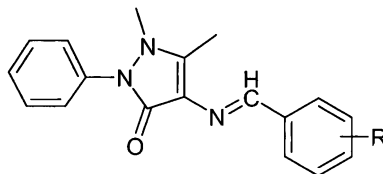
Part 3:

Synthesis of 4-substituted benzylidene-2-substituted phenyl-1,5-dimethyl-3-pyrazolone:

The presence of azomethine linkage in compounds has been known to decrease their toxicity considerably^[53, 54].

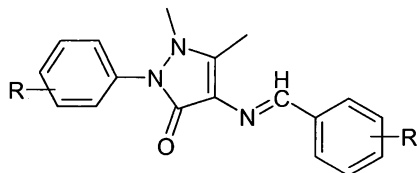
Schiff bases from biological active benzaldehyde have shown encouraging antimicrobial activity *in vitro*^[55, 56].

Recently, Yogesh Kumar Vaghasiya et al^[2] reported the synthesis of Schiff bases derived from vanillin and 4-aminoantipyrine with antibacterial activity {XII}.



{XII}

Taking in to account the pharmacophoric importance of antipyrine derivatives and the importance of azomethine linkage that imparts antimicrobial activity, Schiff bases of 4-substituted aminoantipyrine and different substituted benzaldehyde were synthesized which have the following general structure {XIII}.



{XIII}

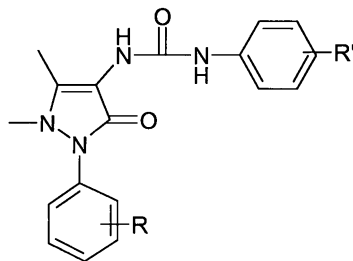
Where, R = H, 4-Cl, 3,4-Dichloro etc. ; R' = 4-Cl, 2,4-Dichloro etc.

Part 4:

Synthesis of 4-substituted phenyl urea-2-substituted phenyl-1,5-dimethyl-3-pyrazolone:

Pharmacologically, urea derivatives have been reported to possess versatile uses^[57, 58], so we decided to condense the 4th position of

aminoantipyrine with different substituted phenyl isocyanates to get the biological active urea linkage at 4th position of antipyrine which have the following general structure {XIV}.



{XIV}

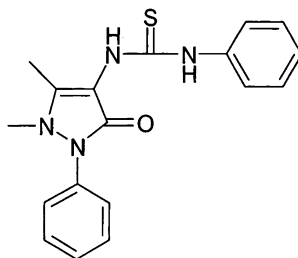
Where, R = H, 4-Cl, 3,4-Dichloro etc.; R' = 4-Ethoxy, H, 2,4-Dichloro etc.

Part 5:

Synthesis of 4-substituted phenyl thiourea-2-substituted phenyl-1, 5-dimethyl-3-pyrazolone:

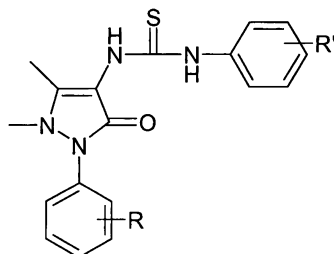
The compound contain —N—C(=S)— group which acts as the pharmacophore. Thiourea group has lots of pharmaceutical activities. Also, 1,3-disubstituted thiourea are well documented in the literature for their biological activity.

Recently, Silvio Cunha et al^[59] reported the antimicrobial properties of thiourea derivative with antipyrine moiety {XV}.



{XV}

These references prompted us to include the substituted thiourea group at 4th position of substituted aminoantipyrine which have following general structure {XVI}.



{XVI}

Where, R = H, 4-Cl, 3,4-Dichloro etc.; R' = 4-Methoxy, H, 3-Cl, 4-Fluoro etc.

All the synthesized compounds have been characterized by TLC, FT-IR, NMR and elemental analysis.

All the synthesized compounds were subjected for their antibacterial screening against *Staphylococcus aureus* and *Salmonella typhi* as a bacterial strains and antifungal activity against *Candida albicans* as fungal strain *in vitro*.

CHEMISTRY

Chemistry of pyrazolone derivatives:

3-Methyl-1-phenyl-5-pyrazolone (PMP) has different biological effects as antipyrine metabolite and is a good biological active molecule [51, 52, 60-64]. In recent years, several other pyrazolones were synthesized such as acyl pyrazolone derivatives^[65], Schiff base of pyrazolone^[66, 67] and thiourea derivatives of pyrazolone incorporating antipyrine moiety [63, 64, 68].

Antipyrine was first prepared in 1884 by heating "Oxymethylchinizin" with methyl iodide and methanol in a sealed tube at 100°C. That same year, antipyrine was obtained by heating acetoacetic ester with phenylhydrazine at 100-150°C to form phenylmethylpyrazolone and then alkylating by heating with a methyl halide. In 1887, antipyrine was prepared by heating phenylmethylhydrazine with acetoacetic ester at 130-160°C.

In 1891, an alkylation process was introduced in which phenylmethylpyrazolone was methylated by means of sodium methyl sulfate and 50% hydriodic acid and which gave an 80% yield of antipyrine. In 1932, a methylation procedure which did not require pressure equipment was described; 1-phenyl-3-methylpyrazolone was heated in anisole to 135°C, and methyl bromide was bubbled through the mass for 10 hr. After removal of the anisole by steam distillation, a 95% yield of antipyrine was obtained^[69].

For research project, the basic pyrazolone intermediate, substituted 4-aminoantipyrine was chosen, prepared from phenyl hydrazine, which involved steps like diazotization, methylation, nitrosation and reduction.

Chemistry of Diazonium compounds:

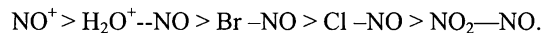
Diazonium compounds or diazonium salts are a group of organic compounds sharing a common functional group with the characteristic structure of $R-N^+X^-$ where, R can be any organic residue such as alkyl or aryl and X is an inorganic or organic anion such as a halogen. Historically, diazonium salts have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

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

The most important method for the preparation of diazonium salts is treatment of aromatic amines such as aniline with sodium nitrite in the presence of a mineral acid. In aqueous solution these salts are unstable at temperature higher than $+5^\circ\text{C}$; the $-\text{N}^+\equiv\text{N}$ group tends to be lost as N_2 , i.e. nitrogen gas. Typically diazonium compounds are not isolated and once prepared, used immediately in further reactions.

Since most of the diazotizations are performed in aqueous solutions, protonated nitrous acid (from sodium nitrite and hydrochloric acid)^[70] is usually used. Free NO^+ should only appear in concentrated acid and in the course of conversion with complex NO^+ salts.

The sequence of reactivity is shown as below-



I] Aqueous Solution:

Normal diazotization of aromatic amines with nitrous acid carried out in dilute aqueous mineral acid and usually yields the diazonium salt in solution. The direct method starts from solution or suspension of the amines in dilute hydrochloric acid (approximately 2.5 mole acid / mole

amine), which is treated with the molar quantity of concentrated aqueous sodium nitrite solution at 0°– 10°C. The conversion proceeds rapidly and almost quantitatively^[71]. The end point can be determined by numerous methods and an excess of nitrous acid can easily be removed by adding urea which react with nitrous acid to form nitrogen, carbon dioxide and water or sulphamic acid^[72].

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

II] Concentrated acids^[73]:

Weakly basic amines bearing strongly electron-attracting substituents on the aromatic or heteroaromatic nucleus are diazotized preferentially in concentrated acids, since hydrolysis of the generated diazonium compound occurs in aqueous solution with increasing dilution. Good results have been obtained with concentrated sulphuric acid.

III] Organic solvents^[74, 75]:

In order to prepare a solid diazonium salt diazotization of aromatic amines is carried out in organic solvent (glacial acetic acid, methanol, ethanol, formamide, DMF, acetone) by means of nitrous acid esters (preferably pentyl nitrile).

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

Victor Meyer reported the coupling of diazonium salt with an activated aliphatic carbon atom.

It is generally assumed that the hydrazone is a stable form when coupling occurs at a methyl or methylene carbon and azo structure is

formed when coupling occurs at a methinyl carbon without elimination of the group.

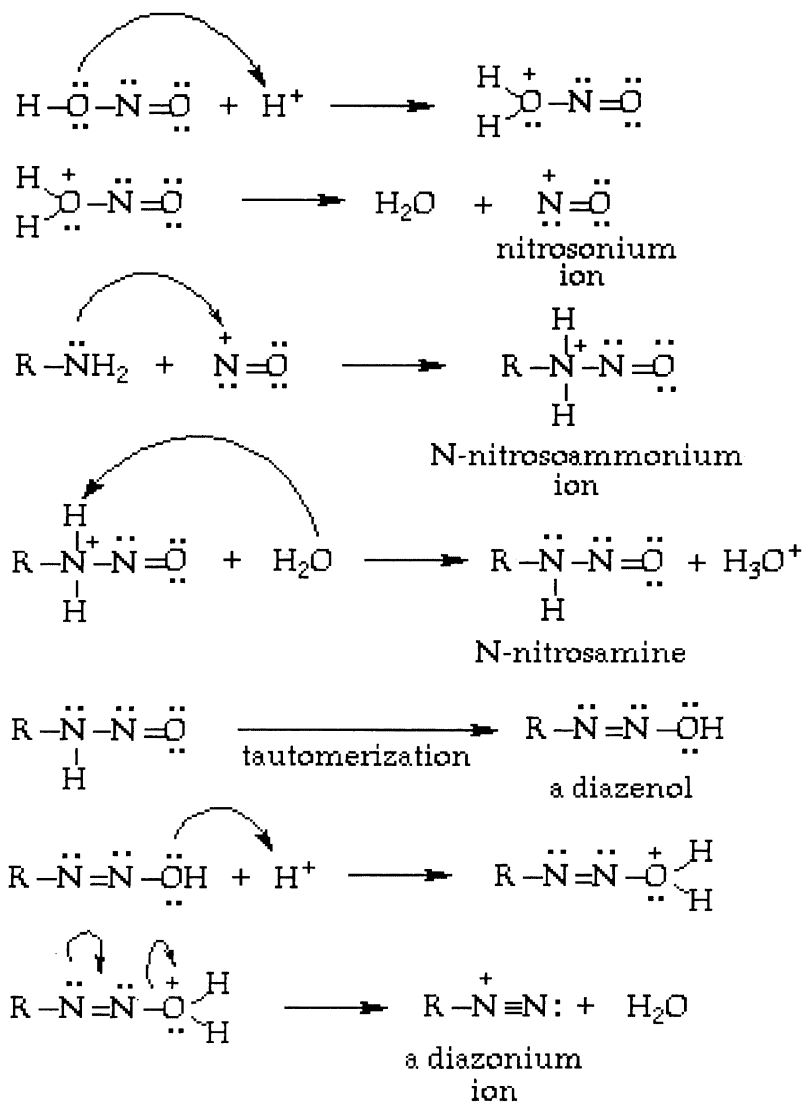
Reactant ratio:

Equivalent amount of diazonium salt and reactant are commonly employed. Excess of diazonium salt should be avoided as it can couple again with the product such as hydrazone to form a formazan derivative. This reaction is favored by strongly acidic solution. Reactions are usually rapid and the product can soon be isolated after the addition of diazonium salt.

Diazotization of Amine involved following steps:-

1. The amine is dissolved in dilute hydrochloric or sulfuric acid and the Solution is cooled to 0-5°C.
2. A solution of sodium nitrite in water is gradually added to the solution of the amine salt during the course of about 5 minutes and then the combined solution (1 + 2) are allowed to stand for an additional 5 to 10 minutes at 0°C.
3. At the end of this time diazotization is usually complete.
4. Throughout the entire process the temperature of the solution must be below 10°C preferably below 5°C.
5. The liberation of nitrous acid from sodium nitrite & the formation of the diazonium chloride require two equivalents of acids.
6. Nitrosation a process for the introduction of a nitroso group or its equivalent into an organic structure by a replacement of an activated α -hydrogen by a nitroso groups was discovered by victor Meyer in 1873. The nitroso group can be introduced into an organic structure through the addition of a nitrating mixture in the diazonium salt.

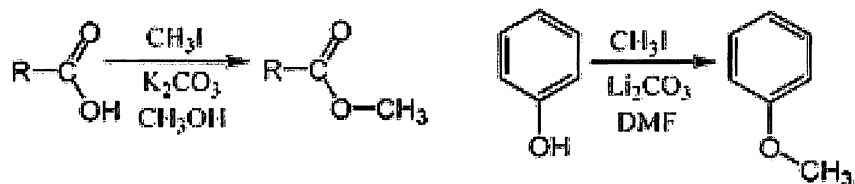
Mechanism of Diazotization ^[76] :



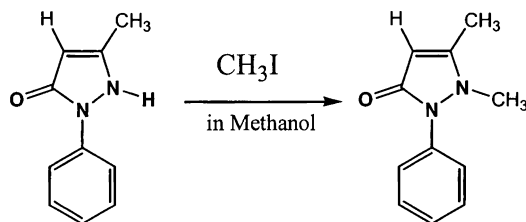
Chemistry of Methylation:

Methylation in the chemical sciences denotes the attachment or substitution of a methyl group on various substrates. This term is commonly used in chemistry, biochemistry, soil science and the biological sciences^[77].

The term methylation in organic chemistry refers to the alkylation process used to describe the delivery of a CH₃ group. This is commonly performed using electrophilic methyl sources - iodomethane, dimethyl sulfate, dimethyl carbonate, or less commonly with the more powerful (and more dangerous) methylating reagents of methyl triflate or methyl fluorosulfonate (magic methyl), which all react via S_N² nucleophilic substitution^[78]. For example a carboxylate may be methylated on oxygen to give a methyl ester, an alkoxide salt RO⁻ may be likewise methylated to give ether, ROCH₃, or a ketone enolate may be methylated on carbon to produce a new ketone.



In the present work Pyrazolone derivative is methylated on Nitrogen to give antipyrine.



Chemistry of Nitrosation:

Nitrosation, a process for the introduction of a nitroso group or its equivalent into an organic structure by a replacement of an activated α -hydrogen by a nitroso group, was discovered by Victor Meyer in 1873 [79, 80].

The nitroso derivatives containing the R-NO functionality.

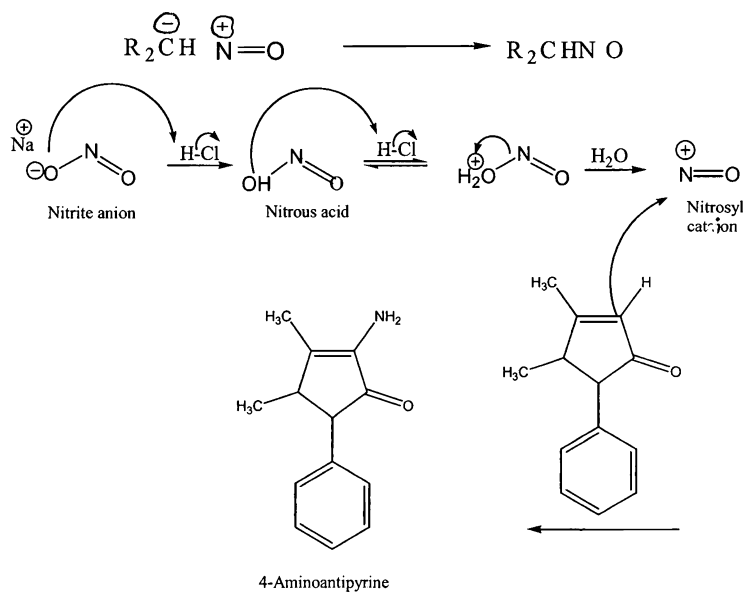
Their structure must be R-N=O.

Types of nitroso compounds:

1. C-nitroso compounds:

It is a characteristic of the c-nitroso compounds, which are blue if aliphatic or green if aromatic. An aliphatic hydrocarbon containing a tertiary hydrogen atom ($>\text{CH}-$) is readily nitrated. In the reduction of a nitro compound the nitroso compound is an intermediate, by this is meant the direct replacement of hydrogen from tertiary carbon atom of hydrocarbon by the nitroso group is called C-Nitrosation [81].

Mechanism of C-Nitrosation:

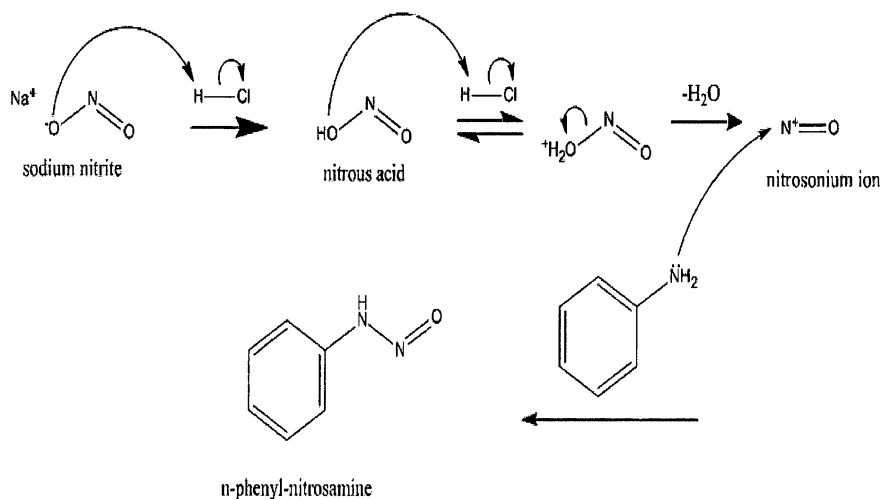


2. N-nitroso compounds:

When secondary amines are treated with nitrous acid, N-nitroso compounds are formed^[82]. Tertiary amines have also been N-nitrosated but in these cases one cleaves so that the product is the nitroso derivative of the secondary amine^[83].

Nitrosation proceeds rapidly at 0°C, usually with sodium nitrite and aqueous HCl or H₂SO₄ (this mixture yield nitrous acid HNO₂).

Mechanism of N-Nitrosation:



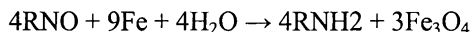
Generally, nitrosation requires an active substrate because NO^+ is much less reactive than NO_2^+ . Tertiary aromatic amines substituted in the ortho position generally do not react with HONO, probably because the ortho substituent prevents planarity of the dialkylamino group, without which the ring is no longer activated. This is an example of steric inhibition of resonance. Addition of Lewis acid increases the rate of nitrosation, suggesting that the nitrosating species is polarized No^+No^- ^[84].

Chemistry of reduction of nitroso compounds:

It has long been known that the nature of the reduced product actually formed depends critically upon the reduction conditions. Thus, although nitroso 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^[85, 86].

The reduction of nitroso compounds involves the progressive replacement of oxygen in the –NO group by hydrogen. Under suitable conditions aromatic nitroso compounds are readily reduced to amino compounds by means of acidic, reducing systems such as metal like iron and tin. In the solution of the metal in acetic acid, the nitroso compound is reduced and amine may be recovered from the reaction mixture by making it alkaline, by steam distillation or by filtration.

If catalytic method is eliminated, iron and zinc are the metal 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.

Zinc is a powerful reducing agent but utilization of its reducing action 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.

Aminopyrine was first prepared in 1893 by Stolz^[87]. He nitrosated antipyrine by treating it with sodium nitrite in acid solution. The nitrosoantipyrine was reduced to the amine with zinc dust and acetic acid, and was methylated by heating with methyl iodide in alcohol under pressure.

Reduction under pressure:

The reduction of the nitroso compound to the corresponding amino compound may be carried out at ordinary temperature and pressure merely by reducing the nitroso compound in a mixture containing the catalyst suspended in an inert medium such as water or alcohol^[88]. This suspension should be agitated sufficiently vigorously to give a very fine dispersion of hydrogen in the inert medium. Alternatively other methods may be adopted which provide a fine dispersion of the gaseous particles of the hydrogen throughout the reaction mixture. The reduction is conveniently carried out using water as a suspension medium but it is possible to have present other inert substances or even acids or bases such as formic or acetic acid or ammonia. If nickel is used as a catalyst the amount and concentration of acid should not be such as to dissolve the metal. Higher temperature may be used if desired, although since this is not necessary, It is preferred that the reaction be carried out at approximately room temperature should not be permitted to rise substantially above 85°C. The hydrogen may be diluted with inert gases, such as nitrogen without interfering with the reaction^[89].

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. In general, the process according to the

invention can be carried out by dissolving the 4-nitroso compound in the suitable solvent, adding the selected quantity of Raney nickel as catalyst and hydrogenating the solution at the pressure selected. The reaction time required for this purpose is governed by the quality and concentration of the solution and catalyst, by the reaction temperature and by the hydrogen pressure selected. Accordingly, it is not possible to quote a reaction time applicable to every case, and the reaction time can actually vary between a few minutes and a few hours. On completion of the reaction, the catalyst is separated off in known manner, for example by filtration or centrifugation. The product is subsequently isolated from the catalyst-free solution in known manner, for example by distillation or by crystallization^[90].

Catalytic methods:

This method^[91, 92] 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 reduction, the inability to dissipate adequately the heat of hydrogenation^[93].

The most common catalyst for the hydrogenation of nitroso compounds are platinum (low pressure) and Raney nickel (high pressure). Supporting catalysts such as platinum on carbon^[94, 95], palladium on carbon^[95-97] 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 vapors 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 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.

Solvent 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 safely added to ethyl alcohol in portions and mixed until it is wetted with the solvent, before another addition is made.

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

Equipments for reduction under pressure:

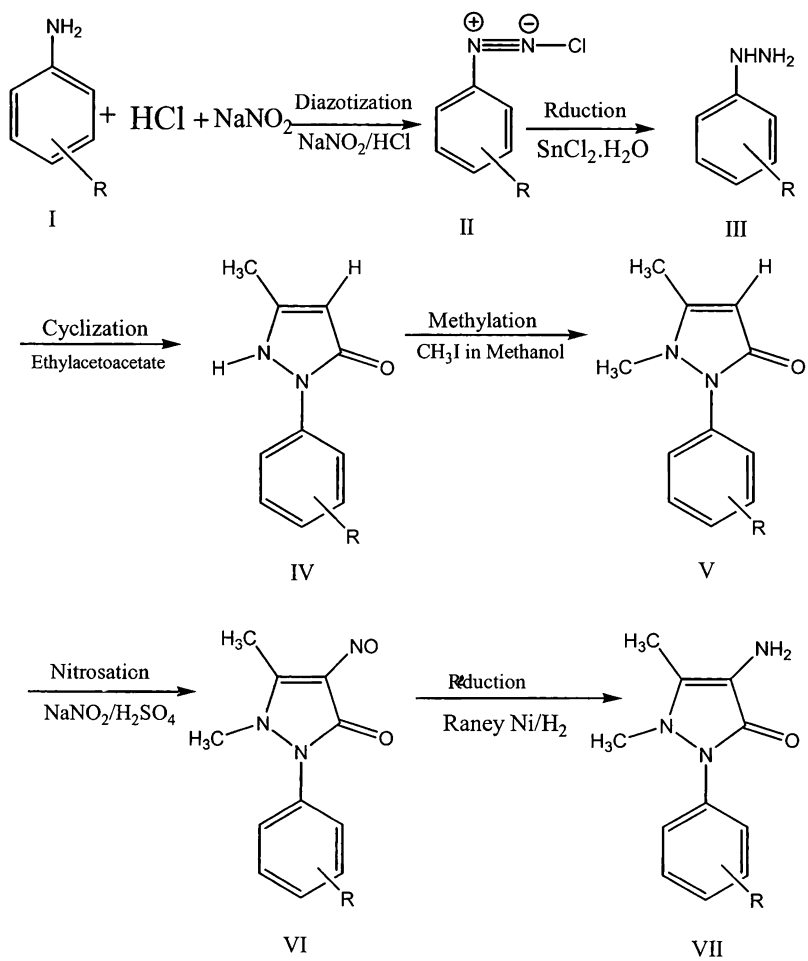
For operation, the vessel containing solvents, reactants and the catalyst is placed in the reactor. Hydrogen is added up to 150 Psi and the stirrer is started with heating.

The progress of the reaction is followed by observation of the drop in pressure. Although, catalytic hydrogenation 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 condition but can be hazardous in high pressure reduction.

SCHEMATIC
REPRESENTATION

Scheme 1

Synthesis of substituted 4-aminoantipyrine



Where R = H, 4-Cl, 4-OCH₃, etc

EXPERIMENTAL

Experimental:

Substituted 4-aminoantipyrine is used as a starting drug intermediate for synthesis of all 4-series which is prepared by following steps:

Synthesis of these intermediates involves the following steps:

1. Preparation of substituted phenyl hydrazine.
2. Preparation of substituted pyrazolone.
3. Methylation of pyrazolone to get Antipyrine.
4. Nitrosation and reduction of Antipyrine.

1. Preparation of substituted phenyl hydrazine:

General method for preparing arylhydrazines ^[98, 99]:

Stirred slurry of the aniline (20ml, 0.20 mol) in 80 ml of conc. HCl and was treated drop wise at 0°C or below, with (17 gm, 0.20 mol) of sodium nitrite in 70 ml of cold water. The mixture was stirred for 15 minutes at about 0°C, filtered and if necessary added to 100gm of chopped ice before proceeding with the reduction. Drop wise addition with stirring at about 0°C of stannous chloride dihydrate (140 gm, 0.6 mol) in 140 ml of cold concentrated HCl, then produced slurry of tin double salt of hydrazine hydrochloride. This slurry was refrigerated overnight, filtered and the precipitate was washed with saturated sodium chloride followed by 2:1 petroleum ether – ether. The filtered solid was treated with excess concentrated NaOH and the hydrazine was extracted into ether.

All phenyl hydrazines on exposure to air or light decompose, so it is safe to prepare it in to its salt form.

For salt formation the ether extract was washed, dried, decolorized and saturated with dry HCl at ice bath temperature. If necessary, the hydrochloride was recrystallised from methanol, ethanol, water and some mixture of these solvents.

Using the similar procedure, Simple phenyl hydrazine and 4-Chloro phenyl hydrazine was prepared.

1. Simple Phenyl Hydrazine

B.P.: 240°C

Lit.^[100] B.P.: 242°C

Yield: 58%

It can be crystallized on cooling in an ice bath

M.P.: 22°C

Lit.^[100] M.P.: 23 °C

Yield: 58%

Simple Phenyl Hydrazine Hydrochloride:

M.P.: 240°C

Lit.^[100]M.P:240-250 °C

Yield: 60%

2. 4-Chloro Phenyl hydrazine Hydrochloride

M.P.: 221°C

Lit.^[99]M.P: 221.5-223 °C

Yield: 57 %

Preparation of Dichloro phenyl hydrazine^[101]:

500 ml of conc. HCl was stirred into a solution of (25 gm, 0.15 mol) of 3,4-dichloro aniline in 100 ml of glacial acetic acid at room temperature and the resulting solution was cooled at 0°C or below. The mixture was stirred with the solution of (11 gm, 0.15mol) of sodium nitrite in 70 ml of water and the cold solution of the diazonium salt was stirred rapidly and treated drop wise with cold solution of (100 gm, 0.45 mol) of stannous chloride dihydrate in 100 ml of concentrated HCl. The insoluble complex salt was collected by filtration. Washed with a little saturated aqueous NaCl and the 3,4-dichloro phenyl hydrazine is liberated from the salt by treatment of the latter with the ether. The ether solution was concentrated until crystallization of the 3,4-dichloro phenyl hydrazine occurred. Purification could be effected by re-crystallization from ether.

3. 3',4'-Dichloro Phenyl hydrazine Hydrochloride

M.P.: 215°C

Lit.^[98] M.P: 212-217°C

Yield: 50 %

Para-Methoxy phenyl hydrazine ^[102]:

The most satisfactory method of preparing this substance appears to be the following:

Procedure:

P-anisidine (38 gm, 0.3 mol) is mixed with concentrated hydrochloric acid (300 ml), when a thick crystalline paste of the hydrochloride separates, this is well stirred and diazotized at 0°C with a solution of sodium nitrite (20.7 gm, 0.3 mol in 90 ml of water). A well cooled solution of stannous chloride (210 gm, 0.9 mol in concentrated hydrochloric acid 180 ml) is added to the purple diazonium solution and this causes the separation of a yellow crystalline solid which after standing for two or three hours becomes colorless. After being collected and thoroughly pressed, the mass is decomposed by shaking with caustic potash solution (400 ml of 25%), care being taken to keep the mixture well cooled. The free base separates as a light brown crystalline solid which is collected, washed with a very little water and dried over sulphuric acid in vacuum desiccators. The hydrazine is purified by dissolving it in the minimum of hot toluene, then filtering it and then adding about 3 volumes of light petroleum, which precipitates the base into shining plates.

4. P-methoxy Phenyl hydrazine

M. P.: 63°C

Lit.^[103] M.P.: 65°C

Yield: 44%

P-methoxy Phenyl Hydrazine is very unstable. So, it is immediately converted in to its salt form.

M. P.: 160°C

Lit.^[98] M.P.: 160-162°C

Yield: 44%

2. Preparation of substituted pyrazolone (1-Substituted Phenyl-3-Methyl-5-Pyrazolone) using phenyl hydrazine ^[104]:

Took 250 ml round bottom flask in ice bath, a mixture of (13 gm, 0.1 mol) of redistilled ethyl acetoacetate and (10.8 gm, 0.1 mol) phenyl hydrazine was placed in it with constant stirring. The reaction is exothermic so keep the flask was kept in ice bath, after complete addition, slowly product was separated. This mixture was refluxed on water bath for 2 hours. The mixture was stirred from time to time. The heavy reddish syrup was allowed to cool down and about 10 ml of ether was added to it to remove colored impurities, with constant stirring.

For recrystallization, ether was removed completely, the product was dried, then recrystallised with hot water. Colourless crystals were formed.

Chemical Test for Conformation of the Pyrazolone Derivatives (C.T.):

1. When boiled with acetone, produced red color.
2. With aqueous FeCl_3 gives red color in alcohol, which on boiling turns to purple precipitate due to formation of pyrazole blue.

Purity was checked by T.L.C. using ethyl acetate: Chloroform (1:4) system.

Using the same procedure following Pyrazolone derivatives were prepared.

1. General Name: 1-Phenyl-3-Methyl-5-Pyrazolone

IUPAC Name: 1-Methyl-2-phenyl-1,2-dihydro-pyrazol-3-one

M.P.: 128°C

M.P. (Lit.)^[104]: 127°C

Yield: 67 %

2. General Name: 1-(4'-Chloro Phenyl)-3-Methyl-5-Pyrazolone**IUPAC Name: 2-(4-Chloro-phenyl)-1-methyl-1,2-dihydro-****Pyrazol-3-one**

M.P.: 124°C

Yield: 60 %

Elemental analysis of C₁₀H₉ClN₂O

	C %	H %	N %
Calculated	57.57	4.35	13.43
Found	57.05	4.20	13.09

3. General Name: 1-(3',4'-Dichloro Phenyl)-3-Methyl-5-Pyrazolone**IUPAC Name: 2-(3,4-Dichloro-phenyl)- 1-methyl-1,2-dihydro-****Pyrazol-3-one**

M.P.: 129°C

Yield: 62 %

Elemental analysis of C₁₀H₈Cl₂N₂O

	C %	H %	N %
Calculated	49.41	3.32	11.52
Found	49.06	3.28	11.24

Using phenyl hydrazine hydrochloride ^[105]:

In a 50 ml round bottom flask provided with a reflux condenser, placed (7.3 gm, 0.05 mol) of phenyl hydrazine hydrochloride, (9.3 gm, 0.1 mol) of crystallized sodium acetate, (6.5 gm, 6.4 ml, 0.05 mol) of redistilled ethylacetoacetate and 10 ml of ethanol. The mixture was refluxed for 1 hour on a water bath and then cooled in ice bath. About 5% sodium carbonate solution was added to it; it was filtered and washed with two 5 ml portions of ether. The crude product was recrystallised from 15 ml of toluene, then it was again washed with ether.

Using the same procedure the P-Methoxy pyrazolone derivative was prepared.

4. General Name: 1-(4'-Methoxy Phenyl)-3-Methyl-5-Pyrazolone

IUPAC Name: 2-(4-Methoxy-phenyl)- 1-methyl-1,2-dihydro-pyrazol-3-one

M.P.: 132°C

Yield: 48 %

Elemental analysis of $C_{11}H_{12}N_2O_2$

	C %	H %	N %
Calculated	64.69	5.92	13.72
Found	64.34	5.79	13.48

3. Methylation of pyrazolone to get antipyrine Using methyl iodide ^[106] :

In a pressure vessel, (1.75 gm, 0.01 mol) of 1-phenyl-3-methyl-5-pyrazolone in 10 ml of methanol was taken. The reaction mixture was first completely dissolved in to solvent before placing it in to pressure vessel. To the reaction mixture, Methyl iodide (5 gm, 2.2 ml, 0.03 mol) in 10 ml of methanol was added. The pressure vessel was heated to develop vapour pressure up to 110-115°C, with stirring for 2 hrs. The reaction mixture was allowed to cool down to room temperature. The excess of methanol was removed by distillation and the product obtained was decolorized by sulphuric acid. The residue was treated with a warm concentrated solution of NaOH, and the oil which separates was removed by shaking with toluene. The toluene layer on evaporation deposited the antipyrine as a Colourless crystalline solid.

Purity was checked by T.L.C. using Ethyl acetate: Chloroform (1:4) system.

Conformation Test:

1. Aqueous solution turns red litmus blue due to its basic nature.
2. With NaNO_2 and dilute HCl yield a green colored solution
3. Produces an orange color with FeCl_3

1. General Name: Antipyrine

IUPAC Name: 1,5-Dimethyl-2-phenyl-1,2-dihydro-pyrazol-3-one

M.P.: 115°C

M.P. (Lit.)^[106] : 113°C

Yield: 72%

2. IUPAC Name: 2-(4-Chloro-phenyl)-1,5dimethyl -1,2-dihydro-pyrazol-3-one

M.P.: 115°C

Yield: 72%

Elemental analysis of $\text{C}_{11}\text{H}_{11}\text{ClN}_2\text{O}$

	C %	H %	N %
Calculated	59.33	4.98	12.58
Found	59.12	4.88	12.64

3. IUPAC Name: 2-(3,4-Dichloro-phenyl)-1,5dimethyl -1,2-dihydro-pyrazol-3-one

M.P.: 115°C

Yield: 72%

Elemental analysis of $C_{11}H_{10}Cl_2N_2O$

	C %	H %	N %
Calculated	51.39	3.92	10.90
Found	51.43	3.87	11.00

4. IUPAC Name: 2-(4-Methoxy-phenyl)-1,5dimethyl -1,2-dihydro-pyrazol-3-one

M.P.: 115°C

Yield: 72%

Elemental analysis of $C_{12}H_{14}N_2O_2$

	C %	H %	N %
Calculated	66.04	6.47	12.84
Found	66.10	6.48	12.80

Using Dimethyl sulphate^[107]:

1-phenyl-3-methyl-5-pyrazolone (1.75 gm, 0.01 mol) and dimethyl sulphate (1.30 gm, 0.01mol) were mixed in cool condition to prevent sudden reaction. This mixture was then heated very cautiously on oil bath at 100°C. The temperature was automatically raised to about 130°C. When there was increase in temperature the mass was heated to about 155-160°C and this temperature was maintained for about 6 hrs. Then about 10 ml of water was added Subsequently, NaOH solution (35%) was added and stirred till reaction mixture was neutralized. The charge was then heated to about 100°C for 2hrs. The reaction mixture was cooled at room temperature and the base was extracted with toluene.

Charcoal treatment was given to this and it was concentrated under reduced pressure to get corresponding antipyrine derivative.

Purity was checked by T.L.C. using Ethyl acetate: Chloroform (1:4) system.

1. IUPAC Name: 1,5-Dimethyl-2-phenyl-1,2-dihydro-pyrazol-3-one

M.P.: 115°C

M.P. (Lit.)^[106] : 113°C

Yield: 72%

4. Nitrosation and Reduction of Antipyrine^[108].

Took a three-necked round bottom flask equipped with mechanical stirrer, dropping funnel, thermometer and dry ice-acetone bath was taken. To this was added (0.56 gm, 0.057 ml, 0.05 mol) of 66% sulfuric acid and was cooled rapidly to 0°C and was added (1.8 gm, 0.01 mol) of 1-phenyl-2,3-dimethyl-5-pyrazolone dissolved in 5 ml of water. This solution was stirred for 2 hrs and the temperature was maintained at -10° to 0°C. While a solution of sodium nitrite 40% by volume strength was added through a dropping funnel, the tip of which extended nearly to the bottom of the flask. The complete addition of sodium nitrite solution and diazotization required 20 to 25 minutes. The end of the Nitrosation was indicated by a slight blue colour on starch iodide paper.

This nitroso mixture was filtered off to remove impurities dissolved in the mother liquor. The filtered nitroso compound was pasted up with water and ice to a smooth mixture with a temperature of 2-5°C. In a pressure vessel this nitroso mixture was taken with 20 ml of ethanol in to it. To the reaction mixture Raney Ni catalyst (86%) was added. The pressure vessel was first flushed with nitrogen and then hydrogen gas was passed in the vessel up to 70 psi. It was heated up to 50-60°C, with stirring for 2 hrs. The reaction mixture was allowed to

cool down to room temperature and Raney Ni 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 corresponding 4-aminoantipyrine.

Purity was checked by T.L.C. using Toluene: Methanol (1:4) system.

1. General Name: 4-Aminoantipyrine

IUPAC Name: 4-Amino-1,5-dimethyl-2-phenyl-1,2-dihydro-pyrazol-3-one

M.P.: 115°C

M.P. (Lit.)^[109]: 109°C

Yield: 72%

Elemental analysis of C₁₁H₁₃N₃O

	C %	H %	N %
Calculated	65.01	6.45	20.67
Found	65.08	6.54	20.57

2. IUPAC Name: 4-Amino-2-(4-chloro-phenyl)-1,5-dimethyl-1,2-dihydro-pyrazol-3-one

M.P.: 112°C

Yield: 62%

Elemental analysis of C₁₁H₁₂ClN₃O

	C %	H %	N %
Calculated	55.59	5.09	17.68
Found	55.47	5.02	17.58

**3. IUPAC Name: 4-Amino- 2-(3,4-dichloro-phenyl)-1,5dimethyl
-1,2-dihydro-pyrazol-3-one**

M.P.: 110°C

Yield: 58%

Elemental analysis of $C_{11}H_{11}Cl_2N_3O$

	C %	H %	N %
Calculated	48.55	4.07	15.44
Found	48.52	4.10	15.48

**4. IUPAC Name: 4-Amino-2-(4-methoxy-phenyl)-1,5dimethyl -
1,2-dihydro-pyrazol-3-one**

M.P.: 107°C

Yield: 52%

Elemental analysis of $C_{12}H_{15}N_3O_2$

	C %	H %	N %
Calculated	61.79	6.48	18.01
Found	61.87	6.34	18.06

Condensed these 4-aminoantipyrines with different substituted benzoyl chloride to get the corresponding amide derivatives, substituted benzaldehydes to get the bezylidine derivatives, substituted phenyl isocyanates to get the phenyl urea derivatives and substituted phenyl isothiocyanates to get the phenyl thiourea derivatives.

Modifications in the preparation of intermediates (Scheme 1):

1. Preparation of substituted phenyl hydrazine.

The classical method for preparation of phenyl hydrazine which involved diazotization of anilines and immediate reduction with sodium sulfite is deleted due to poor yield of the product, and modified by using stannous chloride dihydrated in which the slurry of the intermediate compound is refrigerated over night and after that it is neutralized with aqueous alkali and extracted with toluene to get good yield of the corresponding phenyl hydrazines.

2. Preparation of substituted pyrazolone.

Cyclization of Ethylacetoacetate with corresponding phenyl hydrazine is first carried out at ice-bath temperature and the reflux the reaction mixture to get good yield of the product.

3. Methylation of pyrazolone to get antipyrine.

Methylation under Pressure is very faster, simple, less time consuming method. So, instead of using Dimethyl sulfate for methylation, it is preferred that Methyl iodide for methylation procedure.

4. Nitrosation and reduction of antipyrine.

Reduction of Nitroso mixture using sodium sulfide is very hazardous and time consuming. So, modified this method by using Raney nickel and Hydrogen gas under pressure. This will also improved the quality and quantity of the final drug intermediate.

Taking all these factors in to consideration, the modified method allows the reaction to occur at a faster rate and it was also found that, the final product used as a drug intermediate obtained was relatively free of impurities.

PART 2

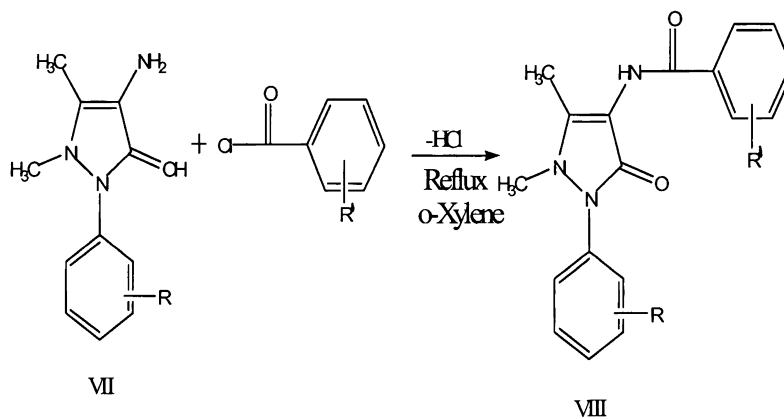
***Synthesis of 4-substituted
amido-2 substituted phenyl-
1, 5-dimethyl-3-pyrazolone***

SCHEMATIC
REPRESENTATION

Scheme 2:

Synthesis of 4-substituted amido-2-substituted phenyl-1,5-dimethyl-3-pyrazolone.

In this series biologically active pyrazolone derivative (VII) condensed with different substituted biologically active benzoyl chlorides in o-xylene to get the corresponding amide derivatives (VIII).



Where, R = H, 4-Cl, etc.; R' = 2, 6-Fluoro, H, 4-Chloro etc.

CHEMISTRY

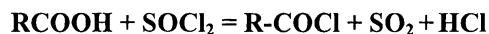
Chemistry of acid chlorides:

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

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



The use of thionyl chloride^[110] for the preparation of acid chlorides presents many advantages in a large number of cases. The reaction results in the formation of sulphur dioxide and hydrogen chloride in addition to the acid chloride and the isolation of the chloride are for this reason a simple matter.



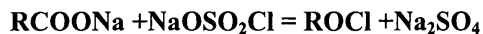
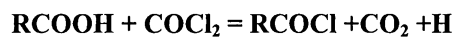
The thionyl chloride must be pure as impure product has been known to cause a somewhat explosive decomposition in some instances. The procedure consists of mixing the chlorinating agent with acid and heating the mixture, if necessary. The product may be isolated by fractional distillation or if solid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

Phosphorous oxy-chloride reacts with the sodium salt of carboxylic acid to give acid chloride. This method is of importance with

acids which undergo decomposition with other chlorides of phosphorous.



The reaction of phosgene^[111] with carboxylic acids and p-toluene sulphonylchloride or of sodium chlorosulphonate with salts of carboxylic acids is also used for the preparation of acid chlorides.



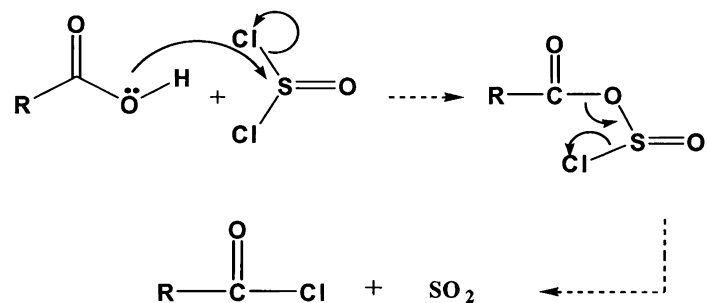
The acid chlorides are obtained readily through the reaction of sulphuryl chlorides with anhydrous salts of organic acids. The reagents are well mixed and subjected to distillation.

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

Reaction mechanism:

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

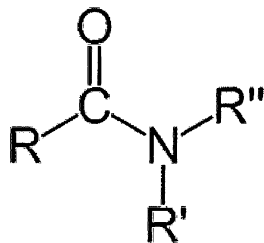
JAIN
JAIN KIN institute
BANGALORE



Chemistry of amides:

Amides are derived from carboxylic acids. A carboxylic acid contains the $-\text{COOH}$ group, and in an amide the $-\text{OH}$ part of that group is replaced by an $-\text{NH}_2$ group.

So amides contain the $-\text{CONH}_2$ group.



The amide linkage is one of the most fundamental and widespread chemical bonds in nature^[112]. Amides are common moieties in naturally occurring substances like proteins and peptides and are also found in various synthetic materials. This makes the amide functionality an important building block in synthetic chemistry and many methods for its synthesis were reported in the literature^[113-117].

Amide compounds have attracted much attention because of their extensive applications in the pharmaceutical industry; they have been

generally used as a protective group in synthetic routes, an amide functional group is contained in many bioactive compounds or drugs, and amide modification is an important optimization methodology for new chemical entities in medicinal chemistry^[114, 118]. Furthermore, peptide bond, a special kind of amide bond in life science, constitutes the backbone of proteins.

The stable and polar amide functionality is an important unit among the organic molecules present in natural occurring materials. It is also found in many synthetic substances, an intermediate or as active pharmaceutical product or prodrugs^[119]. Due to its interest in organic synthesis the preparation of amides from the corresponding amines is an important and well known transformation. It has been noted recently that around 66% of all preliminary screening reactions in industrial medicinal chemistry laboratories involve amide formation^[120-122].

Since, amide bond linkage is ubiquitous and important core structure in pharmaceuticals, chemicals, as well as many natural products^[123-125] and hence it was thought to synthesize new amide linkage incorporating antipyrene moiety by condensation of 4-substituted aminoantipyrene and different substituted acid chlorides.

EXPERIMENTAL

Experimental:

Synthesis of benzoyl chloride:

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

Yield: 66%

B.P.: 196°C

B.P. Lit. ^[104] : 198°C

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

Synthesis of 4-substituted amido-2-substituted phenyl-1, 5-dimethyl-3-pyrazolone:

General Name: Synthesis of 4-(2,6-difluoro benzamido)-2-phenyl-1,5-dimethyl-3-pyrazolone ^[126].

IUPAC Name: N-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-2,6-difluoro-benzamide

Took a 100 ml round bottom flask equipped with 'Y' bent which carried dropping funnel with guard tube and double walled water condenser with guard tube. A solution of 4-aminoantipyrine (0.22 gm, 0.001 mol) in 30 ml of o-xylene was placed in the flask. Pyridine (0.02 g) was added in it. The reaction mixture was refluxed on the heating mantle. The solution of 2, 6-Difluoro benzoyl chloride (0.2 gm, 0.14 ml, 0.001 mol) in 20 ml o-xylene was dropwise added in it, while reaction mixture was in refluxing condition. After complete addition

also the refluxion was continued for 2.5 hrs. The solid compound began to separate during the reflux. Reaction mixture was cooled and filtered, and compound was recrystallised from tetrahydrofuran.

M.P.: 205°C

Yield: 72%

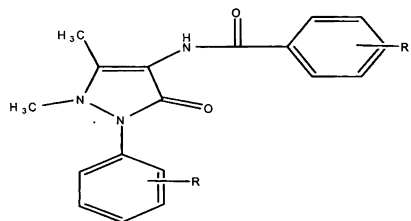
Elemental analysis of $C_{18}H_{15}F_2N_3O_2$

	C %	H %	N %
Calculated	62.97	4.40	11.07
Found	61.14	4.70	11.67

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

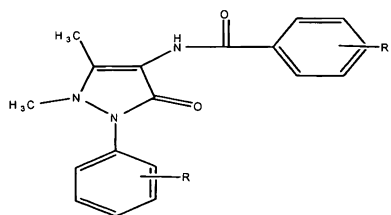
1.

Table NO. 1: 4-substituted amido-2-substituted phenyl-1, 5-dimethyl-3-pyrazolone:



Sr. No	R	R'	Molecular Formula	M.P. (°C) (uncor rected)	Yield (%)	Elemental Analysis (%)					
						Calculated			Found		
						C	H	N	C	H	N
1	H	H	C ₁₈ H ₁₇ N ₃ O ₂	175	89	70.34	5.58	13.67	70.50	5.02	14.10
2	H	2,6-diF	C ₁₈ H ₁₅ F ₂ N ₃ O ₂	205	87	62.97	4.40	11.07	61.14	4.70	11.67
3	H	3,4-Cl ₂	C ₁₈ H ₁₅ Cl ₂ N ₃ O ₂	110	89	57.46	4.02	11.17	56.99	4.48	11.60
4	H	2-F	C ₁₈ H ₁₆ FN ₃ O ₂	112	86	66.45	4.96	12.92	66.92	4.56	13.32
5	H	4-Cl	C ₁₈ H ₁₆ ClN ₃ O ₂	115	88	63.25	4.72	12.29	62.78	4.32	12.15

Table NO. 1: 4-substituted amido-2-substituted phenyl-1, 5-dimethyl-3-pyrazolone:



6	4-Cl	4-Cl	C ₁₈ H ₁₅ Cl ₂ N ₃ O ₂	130	70	57.46	4.02	11.17	57.90	3.60	11.10
7	4-Cl	4-OCH ₃	C ₁₉ H ₁₈ ClN ₃ O ₃	180	64	61.38	4.88	11.30	61.20	4.46	10.97
8	3,4-Cl ₂	4-OCH ₃	C ₁₉ H ₁₇ Cl ₂ N ₃ O ₃	210	58	56.17	4.22	10.34	56.08	4.16	10.54
9	3,4-Cl ₂	4-Cl	C ₁₈ H ₁₄ Cl ₃ N ₃ O ₂	165	55	52.64	3.44	10.23	52.82	3.28	10.56
10	3,4-Cl ₂	4-F	C ₁₈ H ₁₄ Cl ₂ FN ₃ O ₂	122	52	54.84	3.58	10.66	54.48	3.62	10.58

SPECTRAL ANALYSIS:

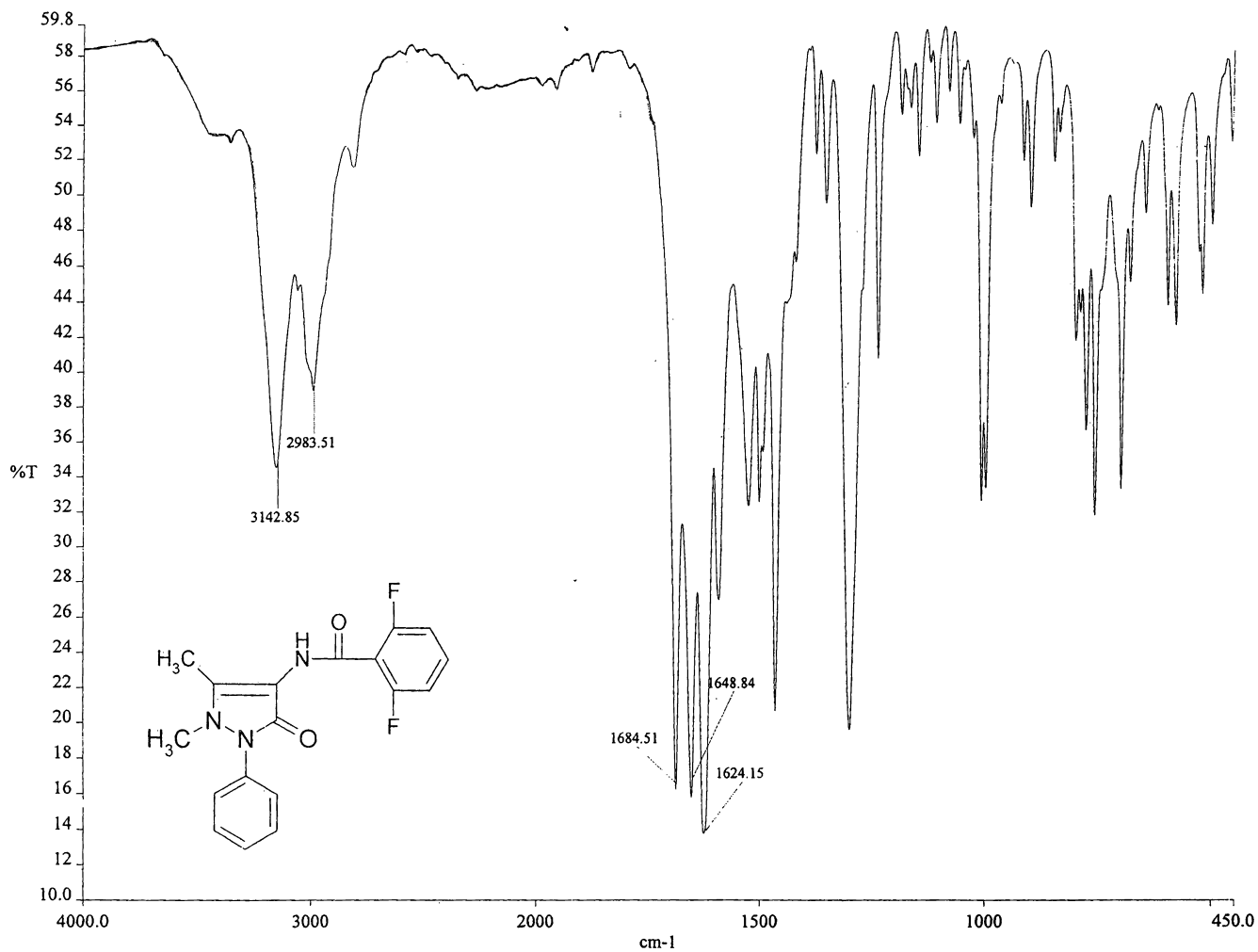
Spectral studies of 4-(2, 6-difluoro benzamido)-2-phenyl -1, 5-dimethyl-3-pyrazolone (Table No. 1 compound No.2)

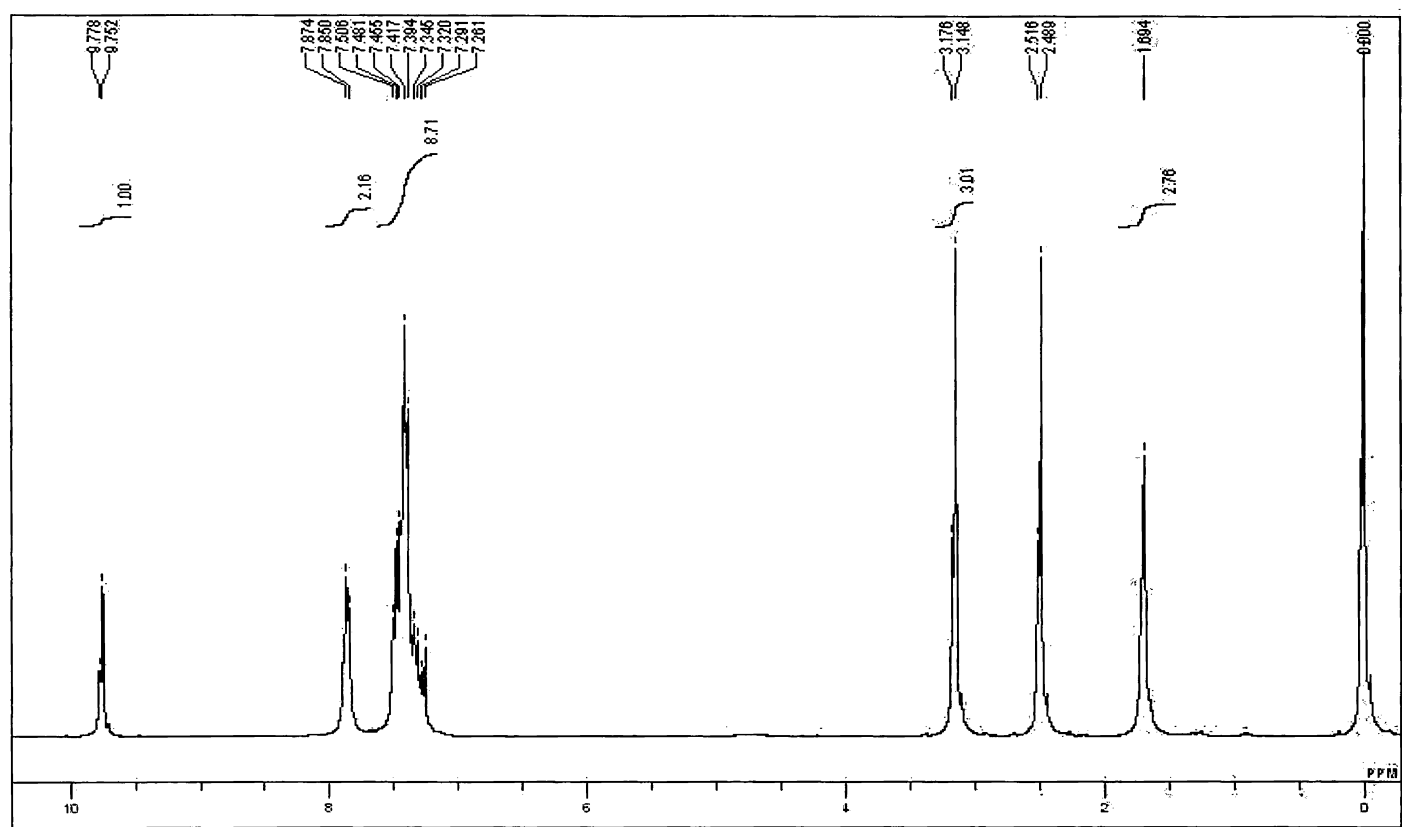
IR (KBr) cm^{-1}

Wave number	Assignment
3148.35	N-H Str (sec.amide)
1681.76	Aromatic ketone (C=O)
1648.84	Amide C=O Str.
1624.15	C=C Str.
2983.51	Ar. H

PMR (DMSO -d₆) δ :

1.69	(s, 3H, C-CH ₃)
3.148-3.176	(d, 3H, N-CH ₃)
7.261-7.874	(m, 11H, Ar-H)
9.752-9.778	(d, 1H, -NH)





PART 3

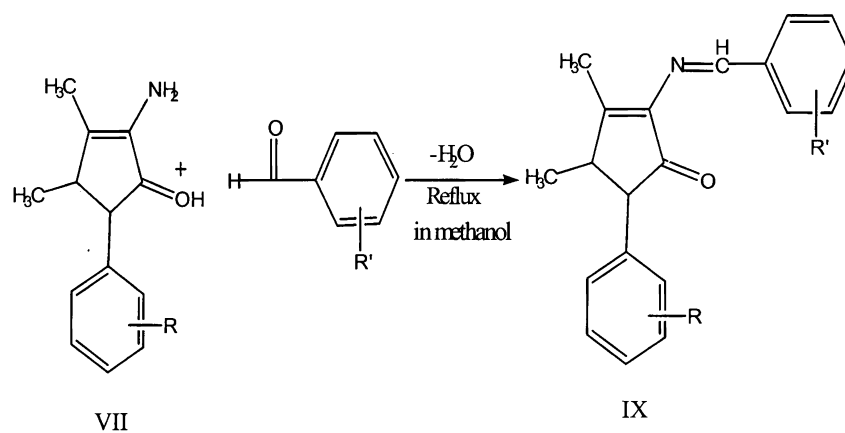
***Synthesis of 4-substituted
benzylidene-2-substituted
phenyl-1, 5-dimethyl-3-
pyrazolone***

SCHEMATIC
REPRESENTATION

Scheme 3:

Synthesis of 4-substituted benzylidene-2-substituted phenyl-1,5-dimethyl-3-pyrazolone.

In this series different substituted benzaldehydes condensed with different substituted 4-aminoantipyrine (VII) to get corresponding biologically active Schiff bases (IX).



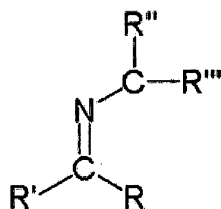
Where, R = H, 4-Cl etc.; R' = 2,6-Dichloro, H etc.

CHEMISTRY

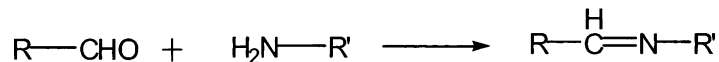
Chemistry of Schiff bases:

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

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



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



Schiff^[127] for the first time reported condensation of this kind and hence the condensation products are referred to as “Schiff bases”.

Schiff bases are characterized by the $-N=CH-$ (imine) group which is important in elucidating the mechanism of transamination and racemisation reaction in biological systems^[128, 129]. Due to the great flexibility and diverse structural aspects, a wide range of Schiff bases have been synthesized and their complexation behavior studied^[130].

Pelton et al^[131] had shown the antifungal and antibacterial activity of some substituted salicylaldehydes. Schiff bases from different aldehydes have shown encouraging anti-tuberculosis activity *in vitro* in our laboratory^[132]. Also the presence of an azomethine linkage in

compounds has been shown to decrease their toxicity considerably ^[54-56, 133].

Many procedures have been described in the literature for the synthesis of Schiff bases ^[134-136].

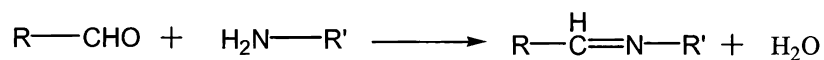
The important methods are listed below:

1. The simplest method appears to be the one in which equimolar quantities of an amine and aldehydes are condensed by boiling in methanol or ethanol^[2].
2. In 1946, Tipson and clapp^[137] obtained Schiff bases by refluxing the amine and the aldehydes in toluene with a few drops of piperidine. The water formed during the course of the reaction was removed by employing a Dean-stark trap.
3. In 1950, Hayes et al^[138] prepared Schiff bases by heating a mixture of an aldehydes and an amine in benzene and removing the water formed during the reaction using Dean-Stark apparatus.
4. In 1953, Giovambettista and Rabassa ^[139] suggested the use of acetic acid as a catalyst for the synthesis of Schiff bases in good yield.
5. In 1971, the condensation of hydrazine and an aldehydes using glacial acetic acid as a solvent was described.

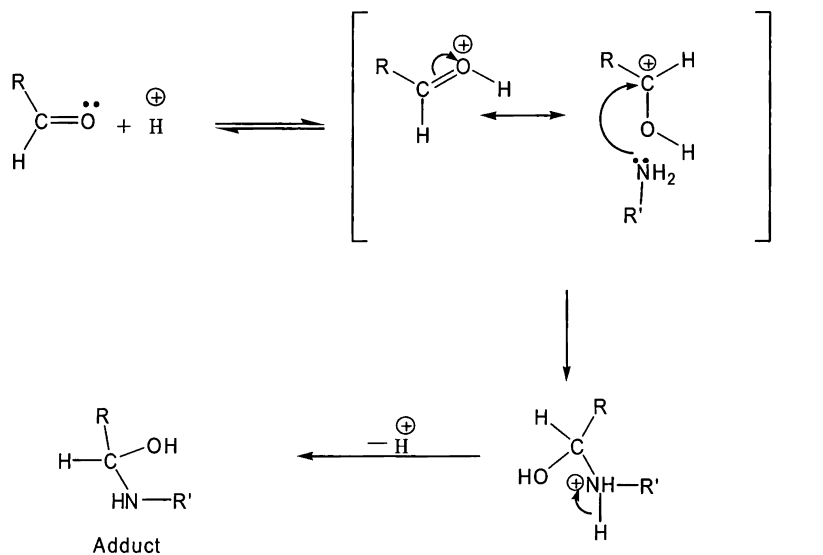
As the first method was found to give good yields it was followed initially. The addition of few drops of acetic acid was found to improve the yield. Later on, during the course of investigation the last method using acetic acid as a solvent was found to be the best for the following reasons.

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

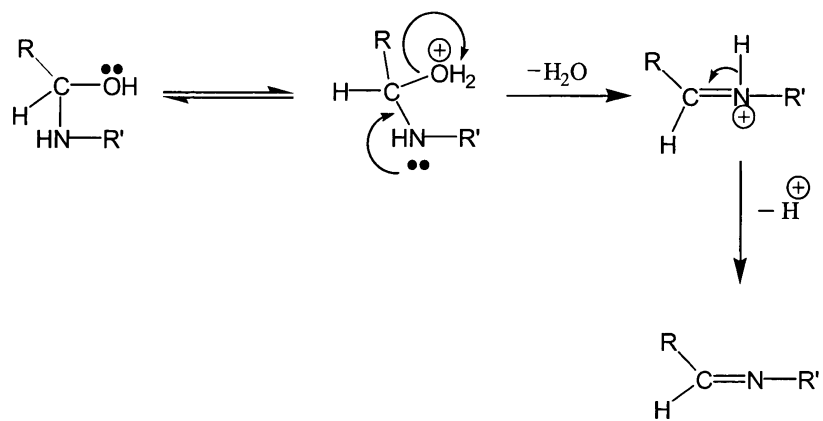
Reaction mechanism:



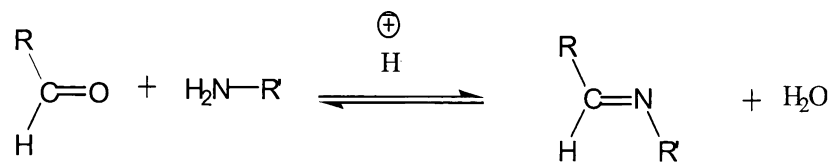
The amine and the aldehydes are heated in the presence of an acid. The acid protonated the carbonyl oxygen and promotes the attack of nucleophilic amine.



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



The overall reaction:



EXPERIMENTAL

Experimental:

Synthesis of 4-substituted bezylidine-2-substituted phenyl-1, 5-dimethyl-3-pyrazolone:

For above synthesis all the commercially available benzaldehydes were used.

General Name: Synthesis of 4-(2'',6''-Dichloro bezylidine)-2-(4'-Chloro) phenyl-1,5-dimethyl-3-pyrazolone .

IUPAC Name: 2-(4-Chloro-phenyl)-4- {[1-(2,6-dichloro phenyl) meth-(E)-ylidene]-amino}-1,5-dimethyl-pyrazolidin - 3-one

In a 50 ml round bottom flask equipped with reflux condenser, 2,6-Dichloro benzaldehyde (0,18 gm, 0.01 mol) dissolved in 25 ml of methanol was added into 4- (4'-chloro) aminoantipyrine (0.25 gm, 0.001 mol) and few drops of glacial acetic acid which act as catalyst was added in to it. The mixture was refluxed for 5-6 hrs at 80-90°C in a water bath. The resulting solution was cooled to room temperature, and then poured onto crushed ice with constant stirring. The precipitate was filtered off and washed with sodium bisulfite solution to remove the excess aldehydes. The product was crystallized from hot methanol and dried.

M.P.: 170°C

Yield: 78%

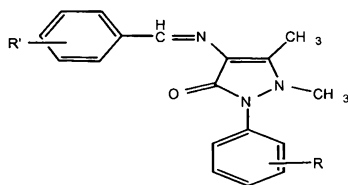
Elemental analysis of $C_{18}H_{16}Cl_3N_3O$

	C %	H %	N %
Calculated	54.50	4.07	10.59
Found	54.32	3.69	10.46

Similarly other substituted bezylidine derivatives were prepared and reported along with their melting points and analytical data in Table No. 2.

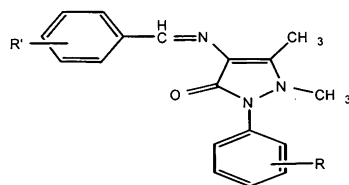
The benzaldehydes used for this synthesis from commercially available Sigma-Aldrich Chemicals.

Table NO. 2: 4-substituted benzylidene-2-substituted phenyl-1, 5-dimethyl-3-pyrazolone:



Sr. No.	R	R'	Molecular Formula	M.P. (°C)	Yield (%)	Elemental Analysis (%)					
						Calculated			Found		
						C	H	N	C	H	N
1	H	2,5(OCH ₃) ₂	C ₂₀ H ₂₁ Cl ₂ N ₃ O	240	78	68.36	6.02	11.96	68.40	6.10	12.06
2	H	2,4-(Cl) ₂	C ₁₈ H ₁₅ Cl ₂ N ₃ O	240	74	60.01	4.20	11.66	60.15	4.12	11.76
3	H	2-F	C ₁₈ H ₁₆ FN ₃ O	180	70	69.89	5.21	13.58	69.76	5.36	13.68
4	H	4-OC ₂ H ₅	C ₂₀ H ₂₁ N ₃ O ₂	70	76	71.62	6.31	12.53	71.58	6.38	12.42
5	4-Cl	4-F	C ₁₈ H ₁₅ ClFN ₃ O	235	65	62.89	4.40	12.22	62.32	4.08	12.57
6	4-Cl	2,4-(Cl) ₂	C ₁₈ H ₁₄ Cl ₃ N ₃ O	230	63	54.78	3.58	10.65	54.32	3.69	10.46

Table NO. 2: 4-substituted benzylidene-2-substituted phenyl-1, 5-dimethyl-3-pyrazolone:



7	4-Cl	4-OC ₂ H ₅	C ₂₀ H ₂₀ ClN ₃ O ₂	90	60	64.95	5.45	11.36	64.70	5.25	11.67
8	4-Cl	2,5(OCH ₃) ₂	C ₂₀ H ₂₀ ClN ₃ O ₃	140	62	62.26	5.22	10.89	62.09	5.18	10.95
9	3,4-diCl	2,4-(Cl) ₂	C ₁₈ H ₁₃ Cl ₄ N ₃ O	220	65	50.38	3.05	9.79	50.58	3.22	9.64
10	3,4-diCl	4-OC ₂ H ₅	C ₂₀ H ₁₉ Cl ₂ N ₃ O	265	59	59.42	4.74	10.39	59.32	4.64	10.30
11	3,4-diCl	4-F	C ₁₈ H ₁₄ Cl ₂ FN ₃ O	205	60	57.16	3.73	11.11	57.31	3.54	11.00
12	4-OCH ₃	4-Cl	C ₁₉ H ₁₈ ClN ₃ O ₂	122	52	64.14	5.10	11.81	64.39	5.01	11.83

SPECTRAL ANALYSIS:

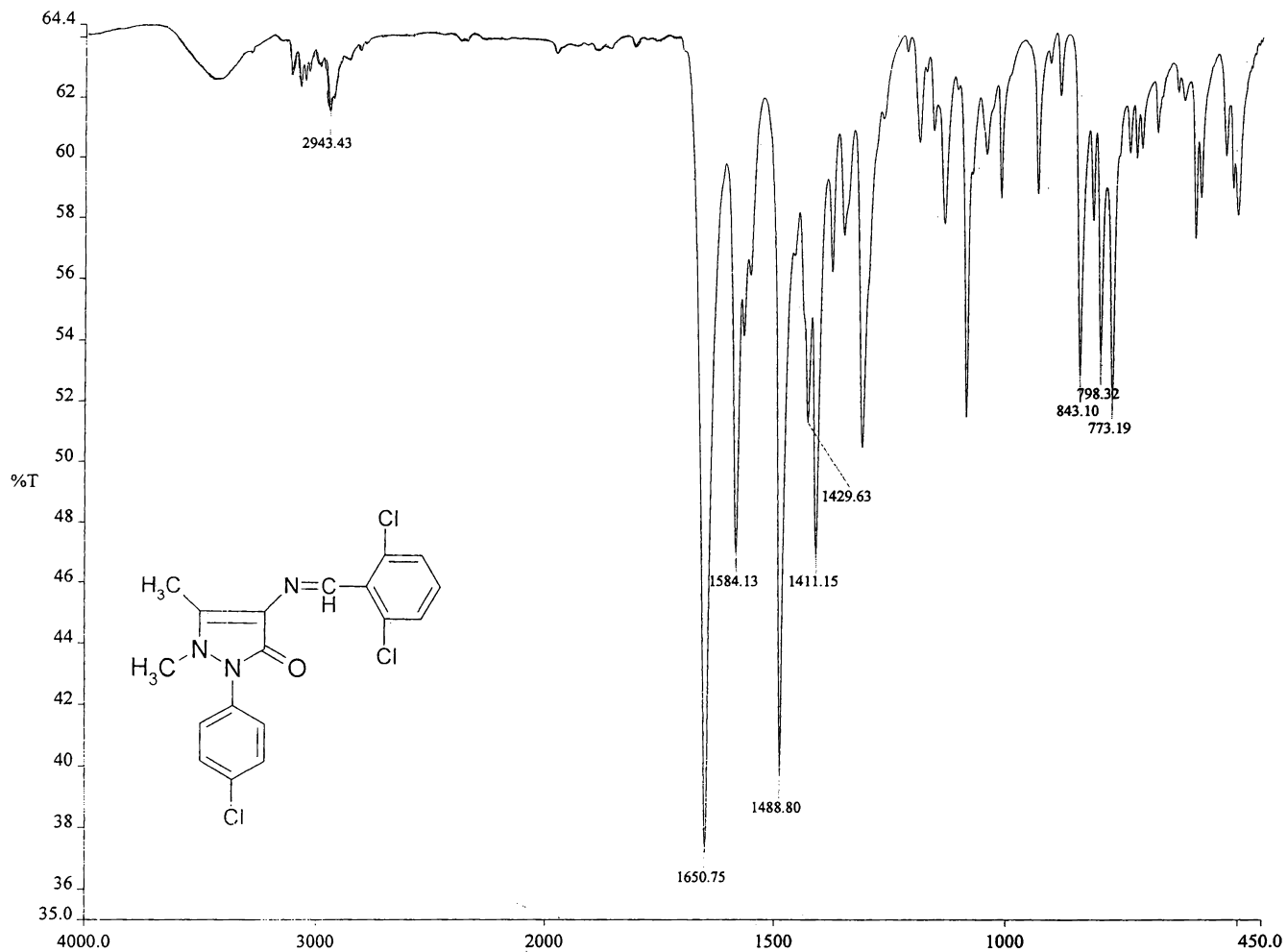
Spectral studies of 4-(2, 6-dichloro bezylidine)-2-(4-chloro phenyl) -1, 5-dimethyl-3-pyrazolone (Table No. 2 compound No.5)

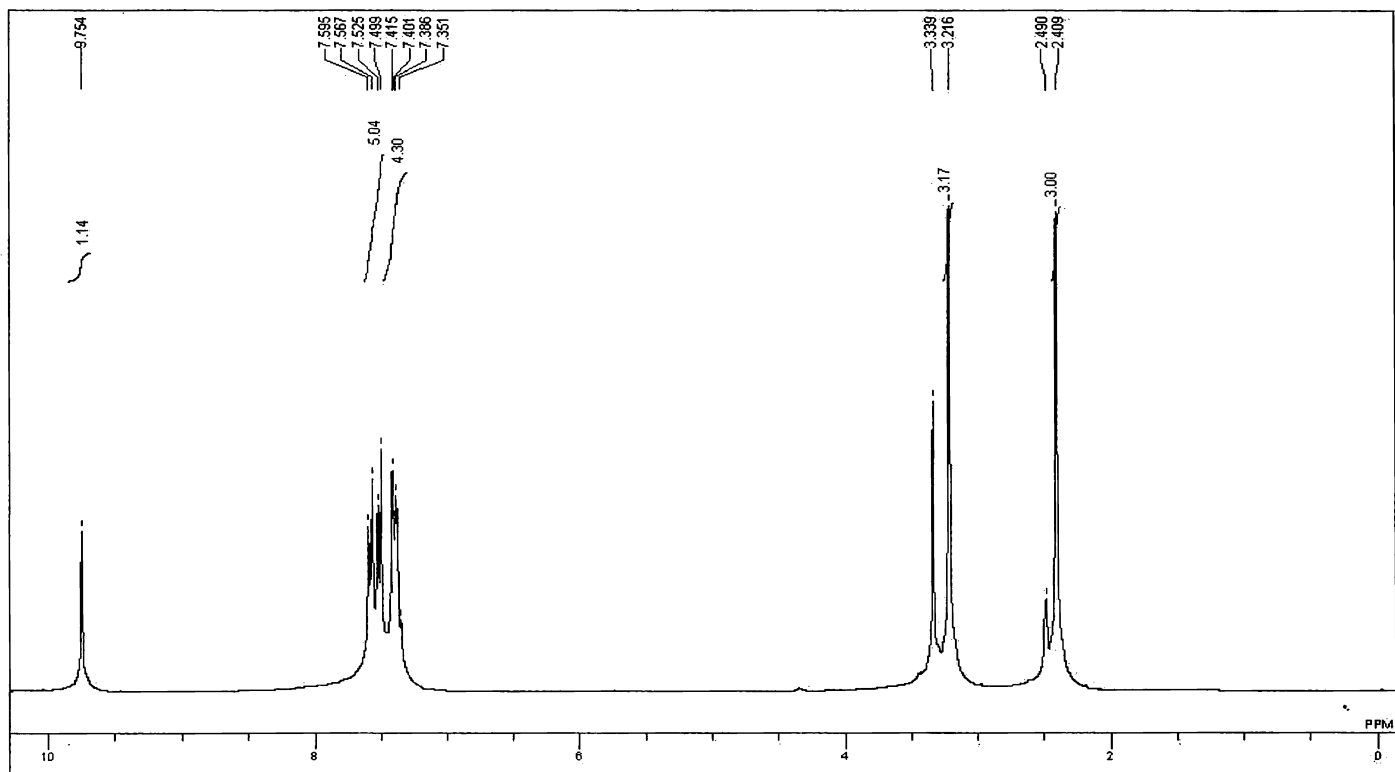
IR (KBr) cm⁻¹

Wave number	Assignment
1486.99	Schiff base (C=N)
1648.84	C=O Str.
1583.00	C=C Str.
770-850	C-Cl Str.
2939.56	Ar. H

PMR (DMSO -d₆) δ:

2.409	(s, 3H, C-CH ₃)
3.216	(s, 3H, N-CH ₃)
7.351-7.595	(m, 9H, Ar-H)
9.754	(s, 1H, N=CH)





Part 4

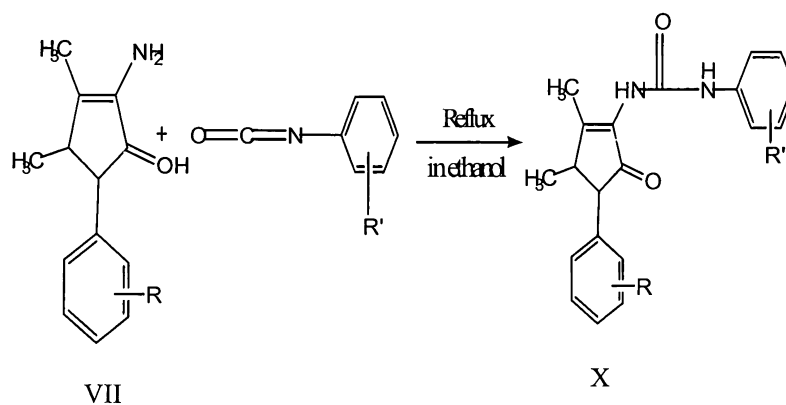
***Synthesis of 4-substituted
phenyl urea-2-substituted
phenyl-1, 5-dimethyl-3-
pyrazolone***

SCHEMATIC
REPRESENTATION

Scheme 4

Synthesis of 4-substituted phenyl urea-2-substituted phenyl-1,5-dimethyl-3-pyrazolone.

In this series substituted 4-aminoantipyrine (VII) condensed with substituted phenyl isocyanates to get biologically active phenyl urea derivatives (X).



R = H, 4-Cl, etc. ; R' = 2,4-Dichloro, H etc.

CHEMISTRY

Chemistry of Urea:

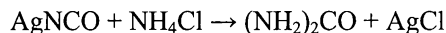
Urea is an organic compound with the chemical formula $(\text{NH}_2)_2\text{CO}$.

Urea is also known by the International Nonproprietary Name (INN) **carbamide**, as established by the World Health Organization. For example, the medicinal compound *hydroxyurea* (old British Approved Name) is now *hydroxycarbamide*. Other names include carbamide resin, isourea, carbonyl diamide, and carbonyldiamine.

Discovery

Urea was first discovered in urine in 1773 by the French chemist Hilaire Rouelle.

It was the first organic compound to be artificially synthesized from inorganic starting materials, in 1828 by Friedrich Wöhler, who prepared it from silver isocyanate through a reaction with ammonium chloride:



Although Wöhler was attempting to prepare ammonium cyanate, by forming urea, he inadvertently discredited vitalism, the theory that the chemicals of living organisms are fundamentally different from inanimate matter, thus starting the discipline of organic chemistry.

This discovery prompted Wöhler to write triumphantly to Berzelius:

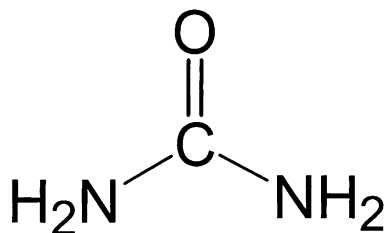
"I must tell you that I can make urea without the use of kidneys, either man or dog. Ammonium cyanate is urea."

Wöhler's historic preparation of "artificial" urea demonstrated to the scientific world that an organic compound could be synthesized, not only by a living organism, but by the working chemist. For many, then, Wöhler is considered the true father of organic chemistry.

It is found in mammalian and amphibian urine as well as in some fish, Birds and reptiles excrete only uric acid, comprising a different form of nitrogen metabolism that requires less water.

Structure

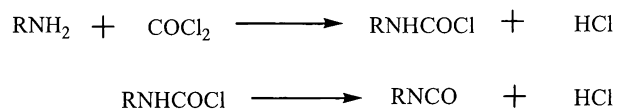
Urea is highly soluble in water and has a pK_a close to zero. Therefore it is an efficient way for the human body to expel excess nitrogen. Its high solubility is due to extensive hydrogen bonding with water, up to eight hydrogen bonds may form - two from the oxygen atom, one from each hydrogen atom and one from each nitrogen atom.



Method of preparation:

A great number of methods of preparation of isocyanates are reported in the literature ^[140-145]. These can conveniently be classed according to the reaction involved. The most common method involves the reaction between an amine or its salts and phosgene ^[143].

Reaction:



Medical use:

Urea is used in topical dermatological products to promote rehydration of the skin. If covered by an occlusive dressing, 40% urea preparations may also be used for nonsurgical debridement of nails. This drug is also used as an earwax removal aid. Like saline, urea injection is used to perform abortions. It is also the main component of an alternative medicinal treatment referred to as urine therapy.

They are also used in the numerous pharmaceutical and biological activities such as antibacterial, antithyroid, hypnotic and anaesthetic, hypoglycemic, anthelmintic, diuretic and biocidal ^[146, 147].

EXPERIMENTAL

Experimental:**Synthesis of 4-fluoro phenyl isocyanate ^[143]:**

A solution of 4-fluoroaniline (22.22 gm, 0.2 mole) in 500 ml of dry toluene was added dropwise with stirring to a solution of phosgene (20% in 100 ml of dry toluene, 0.2 mole) maintained at -10 to 0°C. The reaction mixture was stirred at 25°C for 2 hours. After the addition was completed, it was then heated slowly to reflux temperature. Toluene was removed by distillation at atmospheric pressure. Vacuum distillation of residue yielded 4-fluorophenyl isocyanate.

Yield: 60%

B.P.: 65°C

B.P. (Lit.) ^[148, 149]: 68°C

Similarly 2,4-dichloro phenyl isocyanate was prepared by using the same method at room temperature, and the remaining phenyl isocyanates were used from commercially available Sigma-Aldrich chemicals.

Synthesis of 4-substituted phenyl urea-2-substituted phenyl-1, 5-dimethyl-3-pyrazolone:

General Name: Synthesis of 4-(4'-ethoxy phenyl urea)-2-(4'-chloro phenyl)-1, 5-dimethyl-3-pyrazolone

IUPAC Name: 1-[2-(4-Chloro-phenyl)-1,5-dimethyl-3-oxo-2,3-dihydro-1H-pyrazol-4-yl]-3-(4-ethoxy-phenyl)-urea

Took a 50 ml round bottom flask equipped with reflux condenser. In this was added (0.18 gm, 0.2 ml, 0.001 mol) of 4-Ethoxy phenyl isocyanate and (0.25 gm, 0.001 mol) of 4-(4'-chloro) aminoantipyrine in 25 ml of ethanol. The reaction mixture was heated under reflux for 5 hrs. Then evaporated under vacuum and the residue was allowed to cool. The precipitate was filtered off, washed with ethanol and crystallized from 80% ethanol.

M.P.: 235°C

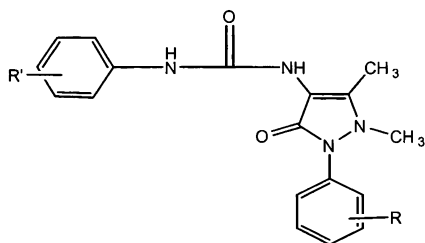
Yield: 65%

Elemental analysis of $C_{20}H_{21}Cl_3N_4O_3$

	C %	H %	N %
Calculated	59.93	5.28	13.98
Found	59.41	5.36	14.06

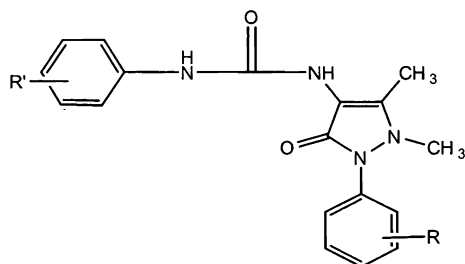
Similarly other substituted Phenyl urea derivatives were prepared and reported along with their melting points and analytical data in Table No. 3.

Table NO.3: 4-substituted phenyl urea-2-substituted phenyl-1, 5-dimethyl-3-pyrazolone:



Sr. N o.	R	R'	Molecular Formula	M.P. (°C)	Yield (%)	Elemental Analysis (%)					
						Calculated			Found		
						C	H	N	C	H	N
1	H	2,5-OCH ₃	C ₂₀ H ₂₂ N ₄ O ₄	240	70	62.82	5.80	14.65	62.28	5.40	14.35
2	H	2,4-diCl	C ₁₈ H ₁₇ N ₄ O ₂ Cl ₂	240	70	55.26	4.12	14.32	55.32	3.98	14.04
3	H	4- OC ₂ H ₅	C ₂₀ H ₂₂ N ₄ O ₃	212	68	65.56	6.05	15.29	65.75	5.80	15.45
4	H	4-F	C ₁₈ H ₁₇ N ₄ O ₂ F	235	60	63.52	5.03	16.46	63.45	5.21	16.09
5	4-Cl	4-OC ₂ H ₅	C ₂₀ H ₂₁ N ₄ O ₃ Cl	90	55	59.93	5.28	13.98	59.41	5.36	14.06

Table NO.3: 4-substituted phenyl urea-2-substituted phenyl-1, 5-dimethyl-3-pyrazolone:



6	4-Cl	2-F	C ₁₈ H ₁₆ N ₄ O ₂ ClF	230	66	57.68	4.30	14.95	57.27	4.43	14.20
7	4-Cl	2,4-diCl	C ₁₈ H ₁₅ N ₄ O ₂ Cl ₃	140	57	50.79	3.55	13.16	50.70	3.21	15.89
9	4-Cl	2,5-(OCH ₃) ₂	C ₂₀ H ₂₂ N ₄ O ₄ Cl	70	55	57.63	5.08	13.44	57.48	4.87	13.47
8	3,4-diCl	2,4-diCl	C ₁₈ H ₁₄ N ₄ O ₂ Cl ₄	180	65	46.98	3.07	12.18	46.68	3.10	12.28
10	3,4-diCl	4-OC ₂ H ₅	C ₂₀ H ₂₀ N ₄ O ₃ Cl ₂	220	63	55.18	4.63	12.87	55.34	4.12	12.57
11	3,4-diCl	2-F	C ₁₈ H ₁₅ N ₄ O ₂ Cl ₂ F	265	52	52.83	3.69	13.69	52.62	3.65	13.48

SPECTRAL ANALYSIS:

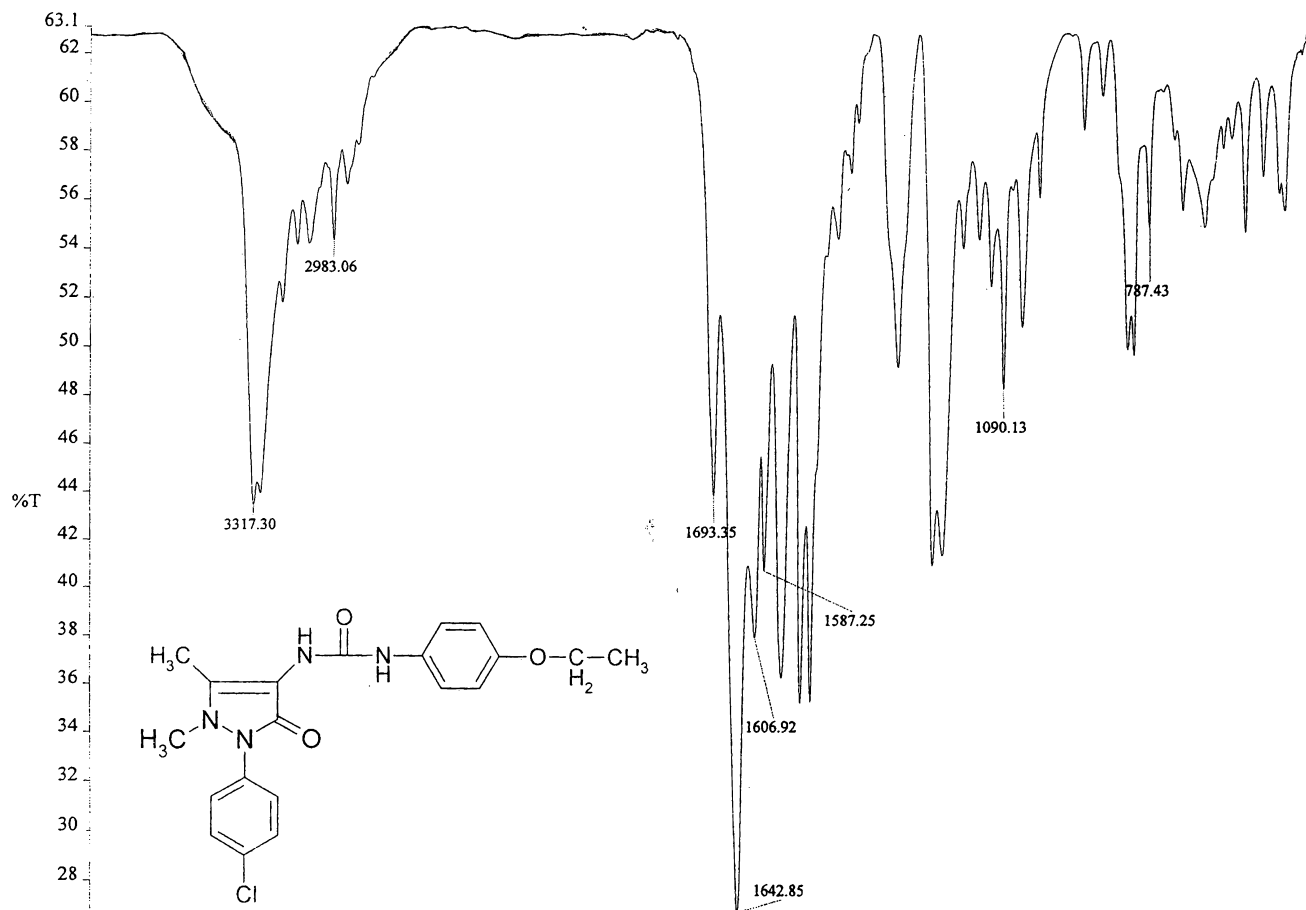
Spectral studies of 4-(4'-ethoxy phenyl urea)-2-(4'-chloro phenyl) -1, 5-dimethyl-3-pyrazolone (Table No. 3 compound No.5)

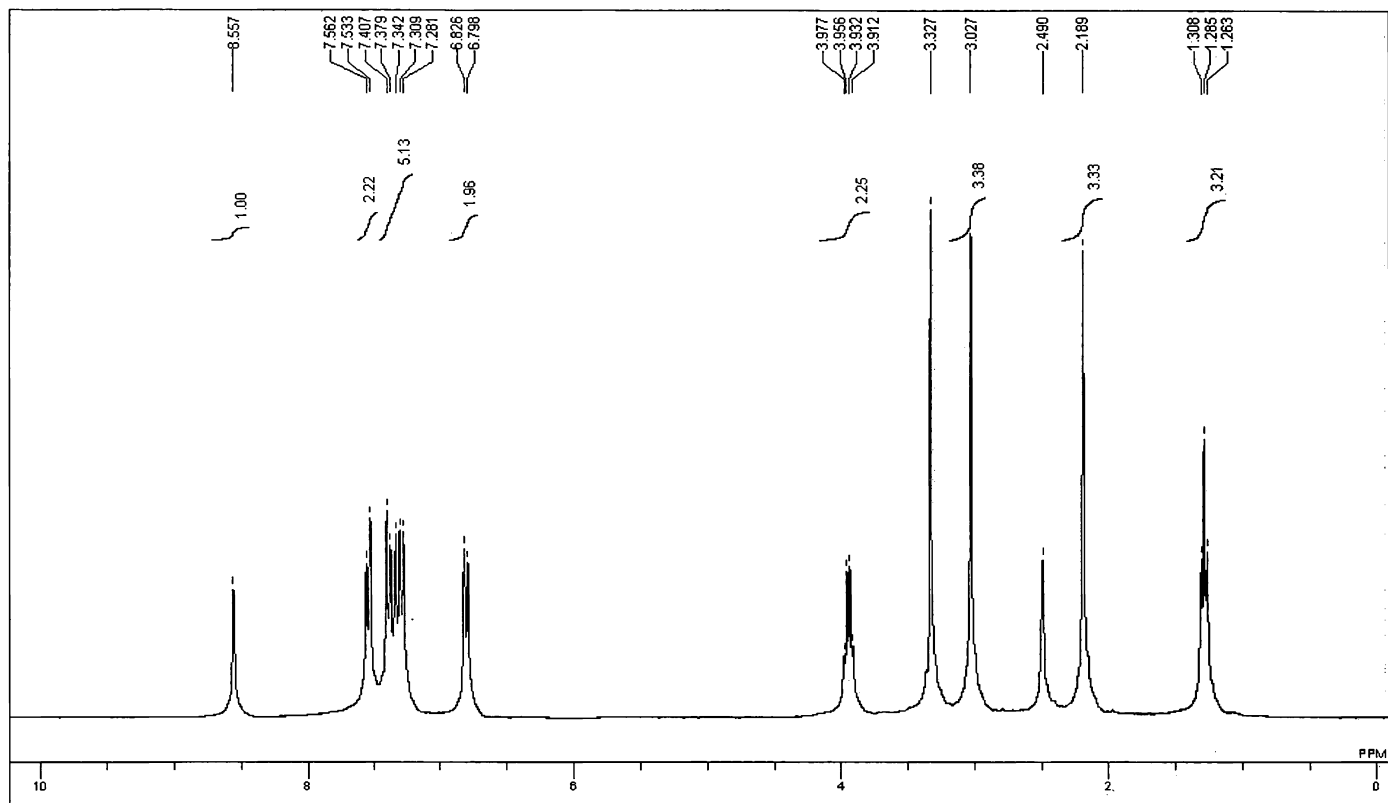
IR (KBr) cm^{-1}

Wave number	Assignment
1693.35, 1642.85	C=O Str.
1606.92	C=C Str.
3317.30	NH Str.
2983.06	Ar. H
1587.25	N-CH ₃
1090.13	C-O Str.
787.43	C-Cl

PMR (DMSO -d₆) δ :

1.263-1.308	(t, 3H, C-CH ₃)
2.189	(s, 3H, N-CH ₃)
3.027	(s, 3H, OCH ₃ CH ₂)
3.912-3.977	(q, 3H, OCH ₃ CH ₂)
6.798-7.562	(m, 8H, Ar-H, 1H, -NH)
8.557	(s, 1H, -NH)





Part 5

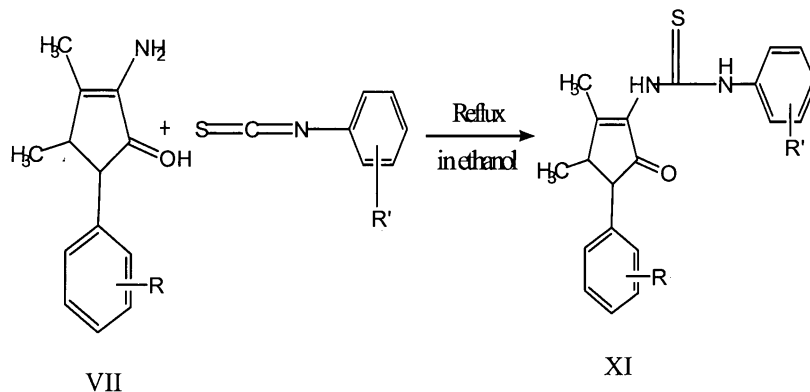
***Synthesis of 4-substituted
phenyl thiourea-2-
substituted phenyl-1, 5-
dimethyl-3-pyrazolone***

SCHEMATIC
REPRESENTATION

Scheme 5

Synthesis of 4-substituted phenyl thiourea-2-substituted phenyl-1,5-dimethyl-3-pyrazolone.

In this series substituted phenyl isothiocyanates condensed with different substituted 4-aminoantipyrine (VII) to get corresponding biologically active phenyl thiourea derivatives (XI).



Where, R = H, 4-Cl, 4-OCH₃ etc.; R' = 4-Methoxy, 3-Chloro etc.

CHEMISTRY

Chemistry of thiourea:

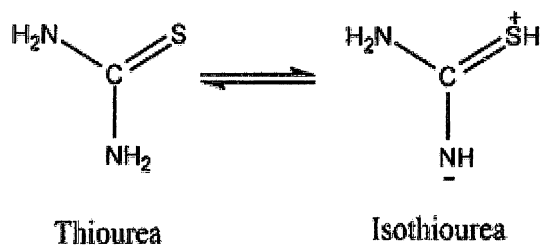
Thiourea is an organic compound of carbon, nitrogen, sulfur and hydrogen, with the formula CSN_2H_4 or $(\text{NH}_2)_2\text{CS}$. It is similar to urea, except that the oxygen atom is replaced by a sulfur atom. The properties of urea and Thiourea differ significantly because of the relative electro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

Moreover, thioureas are important building blocks in synthesis of hetrocycles.

Structure and bonding

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

Thiourea occurs in two tautomeric forms:



Synthesis of thiourea

Thioureas, in general, are well defined crystalline compounds of high stability.

The main part of chemistry of thioureas consists of reactions, due to their nucleophilic reactivity. The centre of reaction is located either at nitrogen atom or at sulfur atom. Substitution at single nitrogen gives 1-

monosubstituted, whereas, substitution at both nitrogen atom gives 1,3-disubstituted derivatives of thioureas. The s-substituted thioureas are referred as pseudothioureas.

There are several synthetic methods known for preparation of thiourea derivatives. Many variations have been applied to each of these when circumstances demanded it.

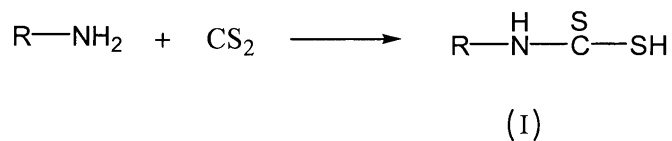
The global annual production of thiourea is around 10,000 tons. About 40% is produced in Germany, another 40% in China, and 20% in Japan. Thiourea can be prepared from ammonium thiocyanate but more commonly is synthesized by the reaction of hydrogen sulfide with calcium cyanamide in the presence of carbon dioxide.

In this chapter synthesis of monosubstituted thioureas is discussed.

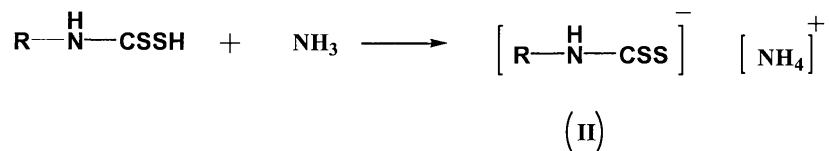
General method for the preparation of 1-monosubstituted thioureas are:

(A) Reaction between carbon disulphide amines:

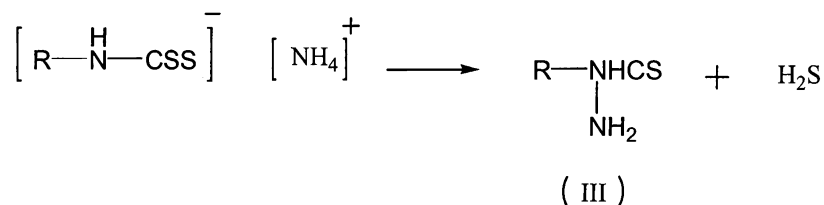
The reaction between carbon disulfide and primary / secondary / aliphatic or aromatic amine gives dithiocarbamic acid (I) at room temperature.



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

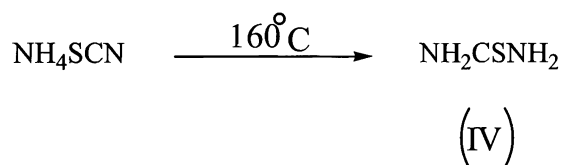


This salt of monosubstituted dithiocarbamate on heating gives 1-monosubstituted-thiourea (III).

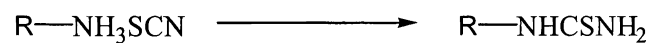


(B) Action of alkali thiocyanate on amine hydrochloride:

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



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

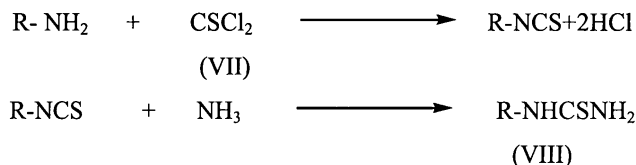


The reaction can be carried out in either-

- (a) An inert organic solvent
- (b) Aqueous medium.

(D) Organic isothiocyanates and ammonia:

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



For the preparation of 1-amino-substituted thioureas, not a single method of general application is known.

The available method that is, isomeric change of amine isothiocyanate into corresponding 1-monosubstituted thioureas is applicable only to aromatic primary amines and on limited basis to aromatic and mixed secondary amines ^[150-153]. The reaction of ammonia with isothiocyanates is restricted in measure, because the isothiocyanates can not be prepared very easily ^[154]. The use of thiophosgene is very much restricted by its being expensive and toxic in nature. Also isothiocyanate formed during the reaction reacts with excess of amine forming 1,3-disubstituted thiourea. The reaction of isothiocyanate with primary amine gives 1,3-unsymmetrical disubstituted thiourea^[155]. This method is advantageous for the synthesis of 1-monosubstituted thioureas, if an unwanted group is removed easily by hydrolysis.

Applications of thiourea derivatives:

Thioureas derivatives have attracted much attention due to their lots of biological, pharmacological and industrial applications. A variety of thiourea derivative exhibit analgesic, anti-inflammatory, antitubercular, antimicrobial, anticancer, herbicidal, anti-HIV, antiviral, HDL-elevating, analgesic, antibacterial and antifungal activities ^[22, 57, 58, 156-159].

EXPERIMENTAL

Experimental:

Synthesis of Phenyl Isothiocyanate:

In a 250 ml three necked round bottom flask equipped with mercury sealed mechanical stirrer, water condenser, and a dropping funnel, was taken thiophosgene (1.3 gm, 0.88 ml, 0.011 mol) in 50 ml glacial acetic acid. To it was added a solution of aniline (1 gm, 1 ml, 0.01 mol) in chloroform (100 ml), dropwise, maintaining the temperature at 0°C. The reaction mixture was stirred further at 0°C for 4 hours. The chloroform layer was separated and washed with water, till it became neutral. The chloroform layer was dried over anhydrous Na_2SO_4 , the solvent was removed under reduced pressure.

Yield: 69%

B.P: 118-121°C

B. P. (Lit.) ^[160]: 120-121°C

Similarly some phenyl isothiocyanates were prepared by the similar method and others were used from commercially available Sigma-Aldrich Chemicals.

Synthesis of 4-substituted phenylthiourea-2-substituted phenyl-1, 5-dimethyl-3-pyrazolone.

General Name: Synthesis of 4-(4''-Methoxy phenyl thiourea)-2-phenyl-1,5-dimethyl-3-pyrazone:

IUPAC Name: 1-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-3-(4-methoxy-phenyl)-thiourea

To a solution of 4-aminoantipyrine (0.25 gm, 0.001 mol) in benzene (10 ml), 4-methoxy phenyl isothiocyanate (0.2 gm, 0.17 ml, 0.001 mol) was added dropwise with stirring, in ice-bath. The reaction mixture was left for 3 hours at room temperature, after which, the solvent was evaporated and crude solid was treated with petroleum ether. Recrystallization of product was carried out with Chloroform and petroleum ether (4:1).

M.P.: 202°C

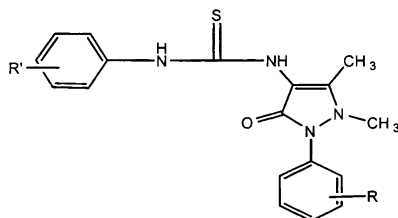
Yield: 78%

Elemental analysis of $C_{19}H_{20}N_4O_3S$

	C %	H %	N %	S %
Calculated	61.94	5.47	15.21	8.70
Found	61.50	5.67	13.20	9.70

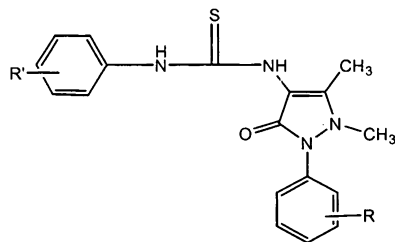
Similarly other substituted Phenyl thiourea derivatives were prepared and reported along with their melting points and analytical data in Table No. 4.

Table NO.4: 4-substituted phenyl thiourea-2-substituted phenyl-1, 5-dimethyl-3-pyrazolone:



Sr. No	R	R'	Molecular Formula	M.P (°C)	Yield (%)	Elemental Analysis (%)					
						Calculated			Found		
						C	H	N	C	H	N
1	H	H	C ₂₀ H ₂₂ N ₄ O ₄	240	70	63.88	4.17	11.67	60.12	4.18	11.50
2	H	3-Cl	C ₁₈ H ₁₇ N ₄ O ₂ Cl ₂	240	70	57.98	4.60	15.03	56.20	4.23	11.32
3	H	4-OCH ₃	C ₂₀ H ₂₂ N ₄ O ₃	212	68	61.94	5.47	15.21	61.50	5.67	13.20
4	H	4-F	C ₁₈ H ₁₇ N ₄ O ₂ F	235	60	59.64	4.42	16.36	59.36	4.03	16.22
5	4-Cl	4-F	C ₂₀ H ₂₁ N ₄ O ₃ Cl	90	55	54.7	3.5	10.6	54.3	3.6	10.4

Table NO.4: 4-substituted phenyl thiourea-2-substituted phenyl-1, 5-dimethyl-3-pyrazolone:



6	4-Cl	4-OCH ₃	C ₁₈ H ₁₆ N ₄ O ₂ ClF	230	66	56.64	4.75	13.91	56.52	4.65	13.76
7	4-Cl	3-Cl	C ₁₈ H ₁₅ N ₄ O ₂ Cl ₃	140	57	53.08	3.96	13.75	53.14	3.58	13.42
8	3,4-diCl	4-F	C ₁₈ H ₁₄ N ₄ O ₂ Cl ₄	180	65	50.83	3.55	13.17	50.36	3.70	13.02
9	3,4-diCl	4-OCH ₃	C ₂₀ H ₂₀ N ₄ O ₃ Cl ₂	220	63	52.18	4.15	12.81	51.98	4.69	12.43
10	3,4-diCl	3-Cl	C ₁₈ H ₁₅ N ₄ O ₂ Cl ₂ F	265	52	48.94	3.42	12.68	48.68	3.76	12.85

SPECTRAL ANALYSIS:

**Spectral studies of 4-(4'-Methoxy phenyl thiourea)-2-phenyl -1,
5-dimethyl-3-pyrazolone (Table No. 4, compound No.3)**

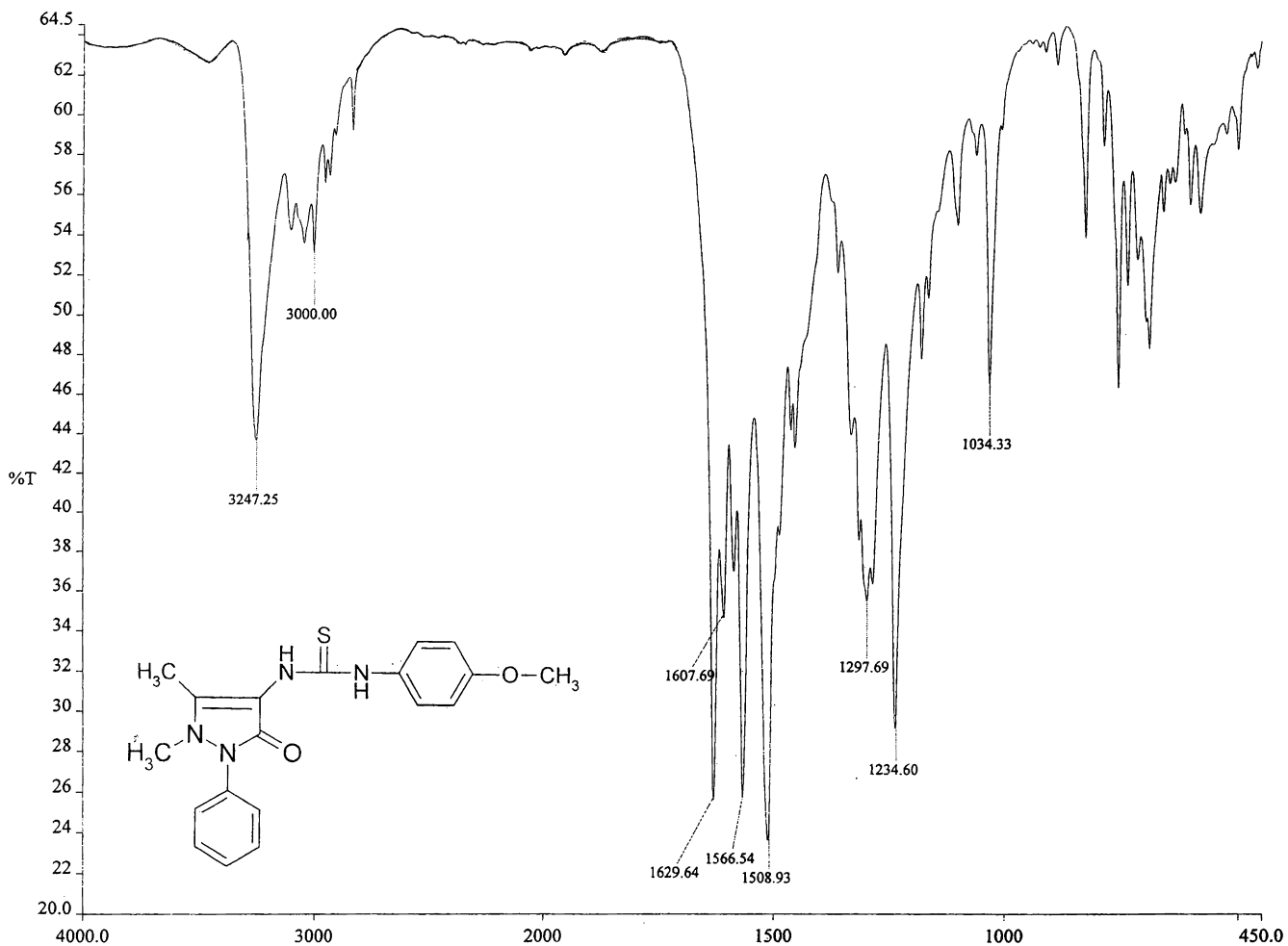
IR (KBr) cm^{-1}

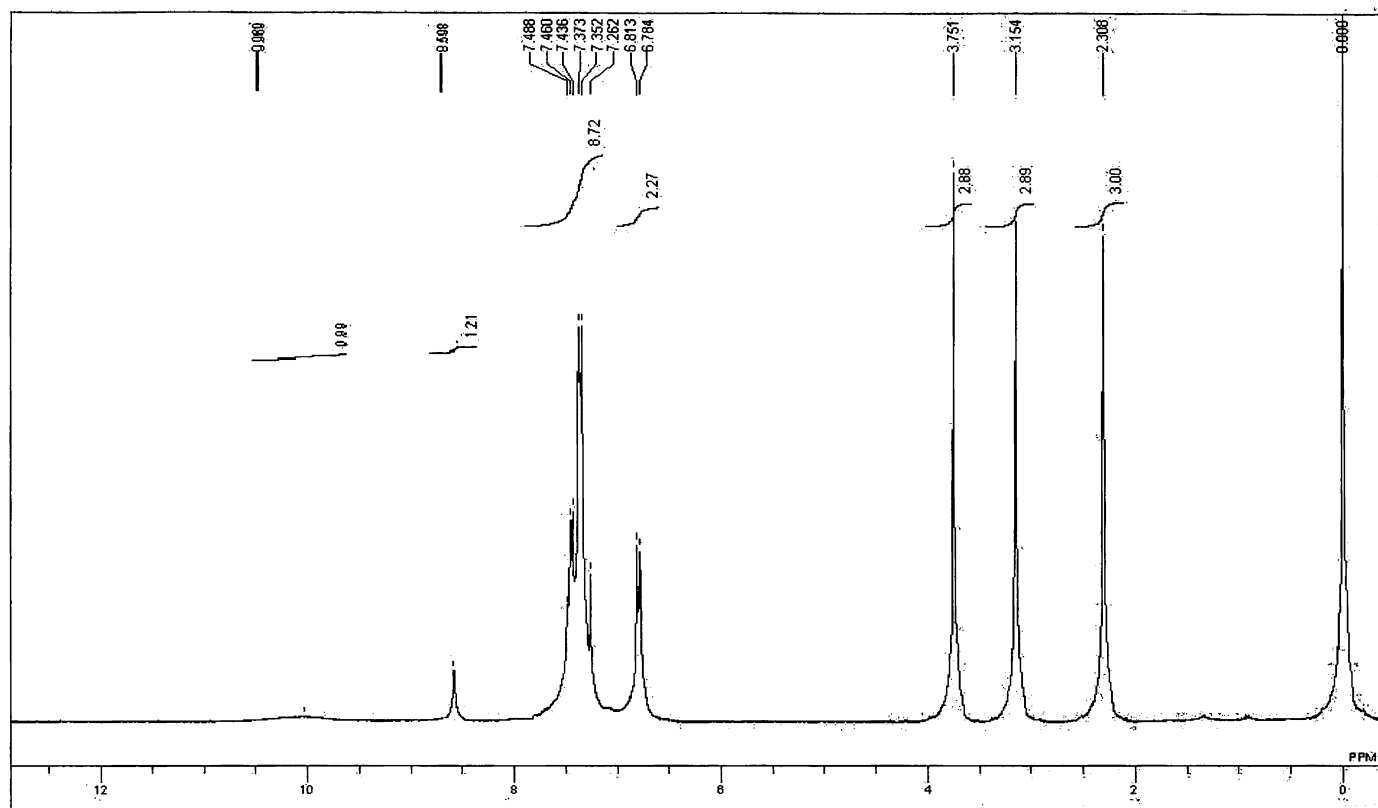
Wave number	Assignment
1629.64	C=O Str.
1607.69	C=C Str.
3247.25	NH Str.
3000.00	Ar. H
1234.60	C=S Str.

PMR (DMSO -d₆) δ :

2.308	(s, 3H, C-CH ₃)
3.154	(s, 3H, N-CH ₃)
3.751	(s, 3H, O <u>CH₃</u>)
6.784-7.488	(m, 8H, Ar-H)
8.598	(s, 1H, Ar-NH)
9.969	(s, 1H, -NH)

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PART 6

***Biological Screening,
Results and Discussion***

Part 6:

Biological Screening, Results and Discussion

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

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

Drug Stock Solution

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

Controls:

Media Control

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

Culture Control

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

Solvent Control

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

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

Turbidity Control

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

Standard Control

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

Antibacterial Screening:

The test compounds have been subjected to *in vitro* screening against Gram-positive *S. aureus* and Gram-negative *S. typhi* using tube dilution 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, 50, 20 and 10 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).

Antifungal Screening:

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

Test Medium:

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

Preparation of the Innoculum:

Candida albicans

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

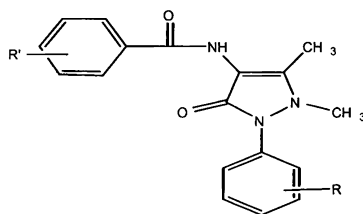
Screening procedure

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

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

The results of the biological screening are recorded in Table 5, 6, 7, 8.

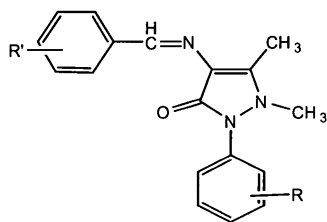
Table No. 5: Antimicrobial activity of Synthesis of 4-substituted benzamido-2-substituted phenyl-1, 5-dimethyl-3-pyrazolone.



Sr.No.	R	R'	Antibacterial Activity in $\mu\text{g/ml}$		Antifungal Activity in $\mu\text{g/ml}$
			<i>S. aureus</i> ATCC 3750	<i>S. typhi</i> NCTC 786	<i>C. albicans</i>
1	H	H	200	200	200
2	H	2,6-Difluoro	10	10	100
3	H	3,4-Dichloro	200	100	200
4	H	2-F	100	50	100
5	H	4-Cl	N.A.	100	N.A.
6	4-Cl	4-Cl	50	50	50
7	4-Cl	4-OCH ₃	50	50	200
8	3,4-Dichloro	4-OCH ₃	50	100	N.A.
9	3,4-Dichloro	4-Cl	100	200	100
10	3,4-Dichloro	4-F	100	50	100

N.A. indicates inactive at 200 $\mu\text{g/ml}$

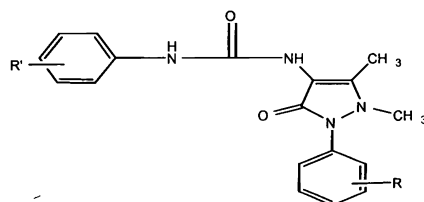
Table No. 6: Antimicrobial activity of Synthesis of 4-substituted benzilidene-2-substituted phenyl-1, 5-dimethyl-3-pyrazolone.



Sr. No.	Substitution		Antibacterial Activity in $\mu\text{g/ml}$		Antifungal Activity in $\mu\text{g/ml}$
	R	R'	<i>S. aureus</i> ATCC 3750	<i>S. typhi</i> NCTC 786	<i>C. albicans</i>
1	H	2,5-OCH ₃	100	100	200
2	H	2,4-Dichloro	200	100	200
3	H	4-OC ₂ H ₅	100	100	N.A.
4	H	2-F	50	100	100
5	4-Cl	4-OC ₂ H ₅	10	50	200
6	4-Cl	2,4-Dichloro	50	50	100
7	4-Cl	2,5-Dimethoxy	200	10	100
8	4-Cl	4-F	50	200	50
9	3,4-Dichloro	2,4-Dichloro	100	100	200
10	3,4- Dichloro	4-OC ₂ H ₅	200	100	200
11	3,4- Dichloro	4-F	50	200	50

N.A. indicates inactive at 200 $\mu\text{g/ml}$

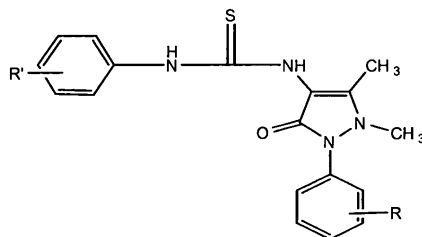
Table No.7: Antimicrobial activity of Synthesis of 4-substituted phenyl urea-2-substituted/unsubstituted phenyl-1, 5-dimethyl-3-pyrazolone.



Com- pound	R	R'	Antibacterial Activity in		Antifungal
			<i>S. aureus</i> ATCC 3750	<i>S. typhi</i> NCTC 786	Activity in $\mu\text{g/ml}$
1	H	2,5-Dimethoxy	100	100	200
2	H	2,4-Dichloro	200	100	N.A.
3	H	4- Ethoxy	200	200	200
4	H	4-F	200	50	50
5	4-Cl	4- Ethoxy	50	200	50
6	4-Cl	4-F	10	50	200
7	4-Cl	2,4- Dichloro	100	50	100
8	4-Cl	2,5-Dimethoxy	200	10	100
9	3,4-Dichloro	2,4-Dichloro	100	100	N.A.
10	3,4-Dichloro	4- Ethoxy	50	100	100
11	3,4-Dichloro	4-F	200	200	N.A.

N.A. indicates inactive at 200 $\mu\text{g/ml}$

Table no. 8: Antimicrobial activity of Synthesis of 4-substituted phenyl thiourea-2-substituted phenyl-1, 5-dimethyl-3-pyrazolone.



Com- pound	R	R'	Antibacterial Activity in µg/ml		Antifungal Activity in µg/ml
			<i>S. aureus</i> ATCC 3750	<i>S. typhi</i> NCTC 786	<i>C. albicans</i>
1	H	H	N.A.	200	N.A.
2	H	3-Cl	200	N.A.	N.A.
3	H	4-OCH ₃	100	200	N.A.
4	H	4-F	200	N.A.	N.A.
5	4-Cl	4-F	100	100	N.A.
6	4-Cl	4-OCH ₃	N.A.	N.A.	N.A.
7	4-Cl	3-Cl	100	100	100
8	3,4DiChloro	4-F	100	N.A.	100
9	3,4-Dichloro	4-OCH ₃	100	100	N.A.
10	3,4-Dichloro	3-Cl	N.A.	100	N.A.

N.A. indicates inactive at 200µg/ml

RESULTS

&

DISCUSSION

RESULT AND DISCUSSION:

The present study is based on the synthesis of novel drug intermediates with possible biological activity. Though several methods are available for the synthesis of 4-aminoantipyrine, but they have their own limitations. In the present research work, various experimental trials were made to standardize the synthesis of substituted 4-aminoantipyrine.

It involves following steps:

1. Preparation of Phenyl Hydrazines

The classical method for the preparation of phenyl hydrazine which involved diazotization of aniline and immediate reduction with sodium sulphite^[104, 161, 162]. If the sodium sulphite solution contains excess of alkali a black tar is formed when the solution is warmed and very little phenyl hydrazine was obtained.

It is best to use stannous chloride dihydrated solution in cool concentrated HCl for reduction of diazonium salt of aniline to give corresponding phenyl hydrazine^[98, 99].

For extraction, the use of toluene as a solvent instead of benzene is good for quality and quantity of the product.

The direct use of phenyl hydrazine for next intermediate is good but due to its toxicity it is preferred to use its hydrochloride form. For the formation of phenyl hydrazine hydrochloride salt, the toluene extract is dried over solid potassium hydroxide flasks, and then add slowly to cool dry hydrochloric acid at ice bath temperature to get the good yield of the product^[98].

2. The cyclization procedure is an addition of ethyl acetoacetate to phenyl hydrazine solution. It is exothermic reaction^[105]. So before addition it is necessary to cool the solutions. Addition is done at ice bath

temperature (5-10°C). One drop addition of glacial acetic acid as a catalyst will improve the yield of the product.

3. Methylation of the pyrazolone is done by known classical method i.e. by using dimethyl sulfate in non aqueous medium^[104, 107]. Here the practical yield of the product is very poor. This was modified by using PTC (phase transfer catalyst)^[163, 164]. Reaction is done in aqueous and organic medium to improve the yield of the product. But this workup was found to be costly, time consuming and hazardous. This was deleted and replaced with methyl iodide treatment of the reaction mass under vapour pressure followed by neutralization with cold sodium hydroxide solution; this modification gives good yield of the product and reaction is clean and smooth.

4. During workup for nitrosation and reduction of antipyrine, according to literature procedure the nitroso compound is reduced by sodium sulfide^[165]. It is very hazardous procedure due to liberation of H₂S gas for long period. Also it is a very time consuming and pollution-prone procedure. The addition of sodium sulfide to nitroso mixture, stirring and product formation will take nearly 10-12 hrs. This was deleted and modified by the reduction of nitroso compound by using Raney Nickel and H₂ gas under pressure with the literature support^[166-168]. Thus the major drawbacks were eliminated in the procedure. This makes the process less hazardous, simple and economical. Within the 4-5 hrs the product is collected which saves 10 cumbersome hours.

In all the above steps, wherever extraction is required toluene is found to be the best solvent for organic compounds instead of benzene. This change in the solvent system made the reaction non-hazardous. It was also found that the final product which was obtained was relatively free of impurities with a sharp melting point.

The method utilized for synthesis has a number of advantages.

- Minimum numbers of reaction steps are involved in the preparation.
- The reactions are simple, non-hazardous, economical, non-polluting and less time consuming.
- The yield of the intermediates and the final product are satisfactory.

The 4-aminoantipyrine is used as a potent drug intermediate for the synthesis of novel heterocyclic compounds and condensed this moiety with different substituted acid chlorides, benzaldehyde, phenyl isocyanates, phenyl isothiocyanates to get biological active amide linkage, bezylidine derivatives, phenyl urea and phenyl thiourea derivatives respectively.

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

To evaluate their efficacy they were screened for their antibacterial and antifungal activity *in vitro*.

PART 2:

In this series there are total 10 compounds which have been screened for their antibacterial and antifungal activity. (Table no. 5, pg. no. 99)

The antimicrobial activity of this series revealed the following observations.

1. In this series, compound no. 2, Table no.5, Pg no. 99, shows excellent antibacterial activity at 10µg/ml against *S. aureus* and

S. typhi, thus difluoro substitution was found to contribute to the antibacterial activity.

2. The substitutions like chloro, dichloro, methoxy also contribute to the antibacterial activity. These compounds show antibacterial activity at 50 to 200µg/ml against *S. aureus* and *S. typhi* (compound no. 3, 4, 5, 6, 7, 8, 9, 10, Table no .5, pg. no. 99).
3. In this series (compound no.1, Table no. 5, Pg. no. 99) without any substitution in both the phenyl rings do not contribute significantly towards the antibacterial and antifungal activities.
4. In case of antifungal screening, the (compound no. 6, Table no. 5, pg.no.99) show good antifungal activity at 50µg/ml against *Candida albicans*.

PART 3:

In this series there are total 11 compounds which have been screened for their antibacterial and antifungal activity. (Table no. 6, pg. no. 100)

The antimicrobial activity of this series revealed the following observations.

1. In this series (Compound No.4, 5, 6, 7, 8 and 11, Table No.6, Pg no.100) inhibits antibacterial activity against *S. aureus* and *S. typhi* at 10 to 50µg/ml.
2. All the synthesized compounds showed moderate to good antifungal activity against *Candida albicans* (Table No.6, Pg. No.100, Compound No.1 to 11).

PART 4:

In this series there are total 11 compounds which have been screened for their antibacterial and antifungal activity. (Table no. 7, pg. no. 101)

The antimicrobial activity of this series revealed the following observations.

1. Of all the 11 substitutions made, Compounds of phenyl urea derivatives (Table No.7, Pg. no. 101, Compound No. 4, 5, 6, 7, 8 and 10) were found to be the most active against *S. aureus* and *S. typhi* with MIC of 10µg/ml and 50µg/ml.
2. Also other five substitutions (Table No.7, Pg. no. 101, Compound No.1, 2, 3, 9 and 11) have shown moderate activity against *S. aureus* and *S. typhi* at 100-200µg/ml for both organisms.
3. Moderate antifungal activity upto 50µg/ml was observed for Flouro, Chloro and Ethoxy substitutions (Table No.7, pg. no. 101, Compound No.4 and 5) and other substitutions like Dichloro , Dimethoxy (Table No.7, pg. no. 101, Compound No.1, 3, 6, 7, 8 and 10) have given compounds with activity upto 200µg/ml against *Candida albicans*. The remaining substitutions were found to be inactive against *Candida albicans*.

PART 5:

In this series there are total 10 compounds which have been screened for their antibacterial and antifungal activity. (Table no. 8, pg. no. 102)

The antimicrobial activity of this series revealed the following observations.

1. The compounds synthesized in this series have not shown any encouraging antibacterial activity. Most compounds have exhibited activity in the range of 100 - 200µg/ml against both *S. aureus* and *S. typhi*.
2. In antifungal screening only chloro and fluoro substitutions (Table No.8, pg. no. 102, Compound No.7 and 8) have shown moderate activity against *Candida albicans* at 100µg/ml.

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APPENDIX

APPENDIX

1. The Melting points have been determined in open capillaries in a Campbell melting point apparatus, and are uncorrected.
2. The Thin Layer Chromatography of the compounds have been run on silica gel glass plates.
3. The Infrared Spectra were recorded on Perkin Elmer FTIR Spectrometer at Department Of Chemistry, IIT, Powai.
4. The PMR Spectra were recorded on JEOL-300 MHz spectrometer using TMs as the internal reference, at Institute of Science, Fort, Mumbai.
5. Carbon, Nitrogen and Hydrogen analysis were carried out on Thermo-Finnigan Flash EA 1112, at Regional Sophisticated Analytical Instrument Facilities, IIT, Powai.

SYNOPSIS

SYNOPSIS

**OF THE THESIS TO BE SUBMITTED
TO THE UNIVERSITY OF MUMBAI
FOR THE DEGREE OF**

MASTER OF SCIENCE IN ORGANIC CHEMISTRY

TITLE OF THE PROPOSAL: **SYNTHESIS AND ANTIMICROBIAL
ACTIVITY OF SOME UNIQUE 4-
SUBSTITUTED AMIDO-1,2-DIHYDRO-2-
SUBSTITUTED PHENYL-1,5-DIMETHYL-3H-
PYRAZOL-3-ONE.**

NAME OF THE **:** **AMITA VIJAY MAYEKAR.**
CANDIDATE

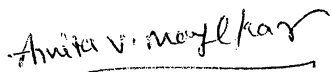
BASIC QUALIFICATION **:** **B.Sc (MARCH 2004)**
OF THE CANDIDATE

NAME AND **DR.ANIL SHRIDHAR BOBADE**
DESIGNATION **:** **(SCIENTIFIC OFFICER.)**
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(MS. A.V.MAYEKAR)


SIGNATURE OF THE GUIDE
(DR. A.S.BOBADE)

INTRODUCTION

Organic chemistry can be termed as living physical science because it encompasses in itself the boundary less ability of the limitless.

In this constant endeavor, the organic chemist is always in search of new synthetic routes or improving the existing ones to synthesize the well known or an entirely new compounds.

One field, which attracts the organic chemist the most, is that of heterocyclic compound chemistry. Heterocyclic compounds are such compounds, which have a cyclic structure with two or more atoms in the ring, one of which is essentially a carbon atom, the other being considered as heteroatom, they can be of any size varying from three members onwards. The heteroatom can be any atom; yet the most common are N, O and S. The number of possible heterocyclic compounds is limitless and enormous number of heterocyclic compounds known and it is increasing rapidly day by day. The synthesis of heterocycles gained importance because these compounds are very widely distributed in nature and are essential for life. They play a vital role in the metabolism of all living cells.

For example, pyrazole as well as pyrazole containing compounds have been reported to show a broad spectrum of biological activity such as antimicrobial¹, antibacterial and antifungal², antipyretic³, antiinflammatory⁴, antitumor⁵ and antihypertensive. Keeping in view the biological importance and medicinal utility of these compounds; four series of new pyrazolone derivatives having pyrazole moiety have been synthesized. Due to bioactivity associated with pyrazole and pyrazole containing compounds, researchers and chemists are very much interested in pyrazole chemistry. Pyrazole ring is a prominent structural motif found in numerous pharmaceutically active compounds. This is mainly due to the ease of preparation and the important biological activity.

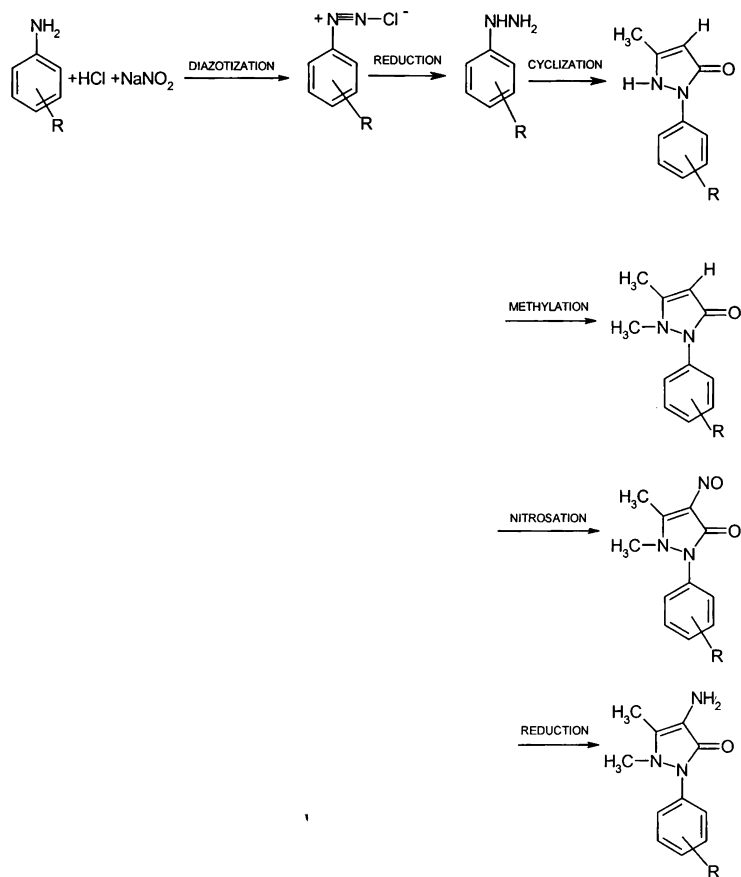
Pyrazole framework plays an essential role in biologically active compounds and therefore for combinatorial (one of the new methods logically developed by researchers in the pharmaceutical industries to reduce the time and cost associated with effective and competitive new drugs which involves the rapid synthesis or the computer simulation of a large number of different but structurally related molecules) as well as medicinal chemistry.

Based on these aspects efforts have been made to synthesize heterocyclic molecules having pyrazolone moiety and will be documented in the thesis. An interesting addition in the thesis is the synthesis of drug intermediates having azomethine linkage, amido linkage, urea and thiourea linkages prepared from the parent pyrazolone molecule. For synthesis of all these, drug **4-aminoantipyrine** (substituted) is used as the starting material.

Synthesis of these intermediates involves the following steps:

1. Preparation of substituted phenylhydrazine.
2. Preparation of substituted pyrazolone.
3. Methylation of pyrazolone to get antipyrine.
4. Nitrosation and reduction of antipyrine.

SCHEMATIC REPRESENTATION:



$\text{R} = 4\text{-Cl}, 4\text{-OCH}_3, \text{H}$ etc.

The thesis will consist of the following parts:

PART 1: Introduction

PART 2: Synthesis of 4-substituted amido-2-substituted phenyl-1, 5-dimethyl-3-pyrazolone.

PART 3: Synthesis of 4-substituted benzylidene-2-substituted phenyl-1, 5-dimethyl-3-pyrazolone.

PART 4: Synthesis of 4-substituted phenyl urea-2-substituted phenyl-1, 5-dimethyl-3-pyrazolone.

PART 5: Synthesis of 4-substituted phenylthiourea-2-substituted phenyl-1, 5-dimethyl-3-pyrazolone.

PART 1:

Introduction

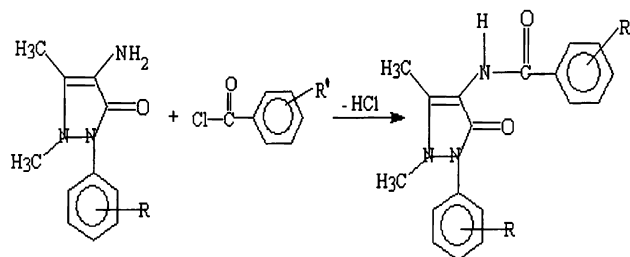
This part of the thesis will be devoted to the general introduction which will highlight the background of current research project. This will also cover a modest review of literature with reference to the selected heterocycles.

PART 2:

Synthesis of 4-substituted amido-2-substituted phenyl-1, 5-dimethyl-3-pyrazolone.

The most fruitful basis for the discovery of new drug is to start with an old drug. Since amido linkage is well known for its antimicrobial⁶, antifungal and antitubercular activities. Hence, it was thought that 4th position of the pyrazolone molecule coupled with different substituted benzoyl chlorides to get the amido linkage. Hence, series no. 1 having pyrazolone moiety as well as amido linkage were synthesized, with the hope of achieving biological activity. In this series we have prepared 10-12

compounds and have subjected them to antifungal and antibacterial screening.



R = H, 4-Cl, 4-OCH₃ etc.

R' = 2, 6-Dichloro, 4-Chloro etc.

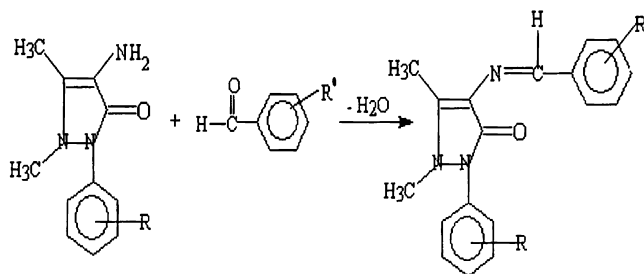
PART 3:

Synthesis of 4-substituted benzylidene-2-substituted phenyl-1, 5-dimethyl-3-pyrazolone.

Azomethine linkage itself is well known for its antitubercular activity, this type of linkage may reduce the toxicity of the compound. Since, the Schiff bases are characterized by –N=CH– (Imine) group which is important in elucidating the mechanism of transamination and racemisation reaction in biological system.

Literature survey shows that Schiff bases show bacteriostatic and bactericidal activity. Antitumor, anticancer, antibacterial, antifungal activity has been reported and they are also active against wide range organisms. eg. *Candida albicans*, *Staphylococcus aureus* etc⁷. Many Schiff bases are known to be medicinally important and are used to design medicinal compounds. So, we synthesized some new substituted Schiff bases obtained from substituted pyrazolone molecule condensing with various biologically active substituted benzaldehydes. In this

series we have prepared 18 compounds and have screened them for their antifungal and antibacterial activities.

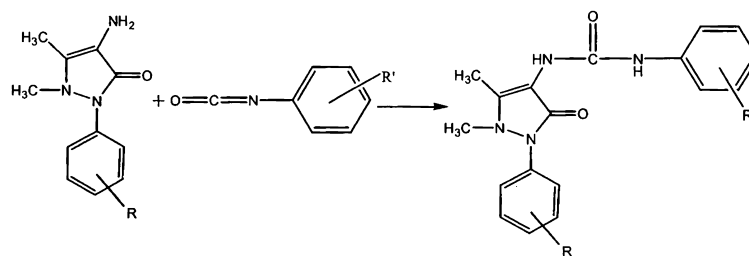


R = H, 4-Cl, 4-OCH₃ etc.; R' = 2, 6-Dichloro, 2,4-dimethoxy etc.

PART 4:

Synthesis of 4-substituted phenyl urea-2-substituted phenyl-1, 5-dimethyl-3-pyrazolone.

Urea derivatives possess wide therapeutic activity such as antibacterial⁸, antithyroid, hypnotic and anaesthetic, hypoglycemic, anthelmintic, diuretic and biocidal. Pyrazolone molecules are also biologically very important in heterocyclic system. Hence, it was thought that 4th position of pyrazolone molecules coupled with isocyanates. So the resulting 12 new novel urea derivatives might possess enhanced biological activity.

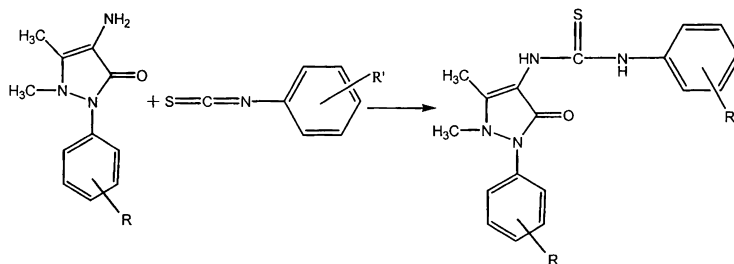


R = H, 4-Cl, 4-OCH₃ etc. R' = 2,4-Dichloro ; 2,5-Dimethoxy etc.

PART 5:

Synthesis of 4-substituted phenylthiourea-2-substituted phenyl-1,5 -dimethyl-3-pyrazolone.

Thiourea group has lots of pharmaceutical and biological activities⁹⁻¹⁴. The compound contains –NH-C=S group which acts as the pharmacophore. Substituted thiourea derivatives are known for antitubercular, antileprosy and antimalarial activities, antibacterial activities. So, we thought to include the thiourea group at 4th position of pyrazolone molecule with substituted isothiocyanates. We have prepared 12-15 new thiourea derivatives and studied for their anti-bacterial and anti-fungal activities.



R=H,4-Cl,4-OCH₃ etc.

R'= 4-Fluoro,3-Chloro etc.

All synthesized compounds have been characterized by TLC, FT-IR, NMR and Elemental Analysis.

PART 6:

Biological Screening

All the synthesized compounds were subjected to antibacterial and antifungal 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 micro gm/ml) against *S. typhi* and **Ampicillin** (MIC 1 micro gm/ml) against *S. aureus*.

For antifungal screening, the organisms used were *Candida albicans*. The medium used for growth was Sabouraud's Glucose Broth. The standard drugs used were Miconazole against *Candida albicans*.

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

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