SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL STUDIES OF SOME NEW N-BRIDGED HETEROCYCLES

Thesis

Submitted in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

by

SHRIDHAR ASHOK MALLADI

DEPARTMENT OF CHEMISTRY

NATIONAL INSTITUTE OF TECHNOLOGY KARNATAKA,

SURATHKAL, MANGALORE – 575025.

October, 2012

DECLARATION

I hereby ***declare*** that the Research Thesis entitled **"Synthesis, Characterization and Biological Studies of Some New N-Bridged Heterocycles"** which is being submitted to the **National Institute of Technology Karnataka, Surathkal** in partial fulfillment of the requirements for the award of the Degree of **Doctor of Philosophy** in **Chemistry** is a bonafide report of the research work carried out by me. The material contained in this Research Thesis has not been submitted to any University or Institution for the award of any degree.

Shridhar Ashok Malladi
Reg. No. 082007CY08F07
Department of Chemistry

Place: NITK-Surathkal
Date:

CERTIFICATE

This is to ***certify*** that the Research Thesis entitled **"Synthesis, Characterization and Biological Studies of Some New N-Bridged Heterocycles"** submitted by **Shridhar Ashok Malladi** (Register Number: 082007CY08F07) as the record of the research work carried out by him is accepted as the Research Thesis submission in partial fulfillment of the requirements for the award of degree of Doctor of Philosophy.

Dr. Arun M. Isloor
Research Guide
Date:

Chairman-DRPC
Date:

Dedicated
To my
Beloved Parents
Family Members
and
Friends

ACKNOWLEDGEMENT

From the depth of my heart I express my deep sincere gratitude to the almighty for the blessings he had bestowed upon me to do this work.

For his faith in me, his encourage, his motivation & inspiration made me to reach these heights, he is one and only Dr. Arun M. Isloor, Associate Professor, Department of Chemistry, National Institute of Technology Karnataka, Surathkal. My mentor, my guide reflects with his incredible personality and lightened up my life with indomitable determination. I deem it as my privilege to work under his able guidance. I ever remain grateful to him.

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At this juncture I think of my family members whose selfless sacrificial life and their great efforts with pain and tears and unceasing prayers have enabled me to reach the present position in life. I am forever indebted to my father who planted the seed of idea to do research and his encouragement in attaining my goal.

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Shridhar Malladi

ABSTRACT

Antibiotics, first introduced in the 1940s, dramatically reduced illnesses and deaths caused by bacterial infections. Before the introduction of antibiotics, infectious diseases claimed countless victims. But a hallmark of antibiotics is that they lose their effectiveness over time as bacteria naturally evolve and mutate and so become resistant to the medicine's effects. The rate of growth of antimicrobial resistance has accelerated due to the widespread global use of antibiotics. It is important to find out newer, safer and more effective antibiotics with broad-spectrum of activity. Heterocyclic compounds by virtue of their specific activity could be employed in the treatment of infectious diseases. A systematic investigation of this class of heterocyclic lead revealed that pyrazole containing pharmacoactive agents play important role in medicinal chemistry. The prevalence of pyrazole cores in biologically active molecules has stimulated the need for elegant and efficient ways to make these heterocyclic lead. Owing to the pharmacological importance of pyrazole and its derivatives, in the present work, it has been contemplated to couple various biologically active heterocyclic moieties with pyrazole through active functional systems to form a new molecular framework. Accordingly, different series, viz. triazolothiadiazole (**P_{1-10}**), oxadiazole (**P_{11-24}**), thiazole (**P_{25-38}**), Schiff base (**P_{39-48}**), Cyanopyridone (**P_{49-63}**) and pyrazoline derivatives (**P_{64-74}**) carrying pyrazole ring as core structure have been designed and synthesized. Structures of the newly synthesized compounds were confirmed by FT-IR, ^{1}H NMR, ^{13}C NMR, mass spectral studies followed by elemental analyses. The newly synthesized compounds were tested for their antimicrobial activity. Selected compounds were also screened for anti-inflammatory and antioxidant activity. Some of the synthesized compounds were found to exhibit potent activity. The acute oral toxicity study for few of the biologically active compounds was also performed. Molecular docking studies of selected compounds were carried out for better understanding of the drug-receptor interaction.

Key words: Pyrazole, Triazolothiadiazole, Oxadiazole, Schiff base, Cyanopyridone, Pyrazoline, Biological and Molecular docking studies.

CONTENTS

LIST OF SCHEMES, FIGURES AND TABLES

FIGURES

SCHEMES

TABLES

ABBREVIATIONS

DMSO	:	Dimethyl sulfoxide
IR	:	Infrared
NMR	:	Nuclear Magnetic Resonance
LC-MS	:	Liquid Chromatography-Mass Spectrometry
TLC	:	Thin Layer Chromatography
$POCl_3$	:	Phosphorus oxychloride
PDB	:	Protein Data Bank
LGA	:	Lamarckian Genetic Algorithm
COX	:	Cylcooxygenase
MIC	:	Minimum Inhibitory Concentration
ATCC	:	American Type Culture Collection
MTCC	:	Microbial Type Culture Collection
OECD	:	Organization for Economic Co-operation and Development
DPPH	:	2,2-diphenyl-1-picrylhydrazyl
BHT	:	Butylated hydroxytoluene
EDTA	:	Ethylenediaminetetraacetic acid
TCA	:	Trichloroacetic acid
TBA	:	Thio-barbituric acid
PMS	:	Phenazine methosulphate
NBT	:	Nitroblue tetrazolium
H_2O_2	:	Hydrogen peroxide

CHAPTER - 1

GENERAL INTRODUCTION

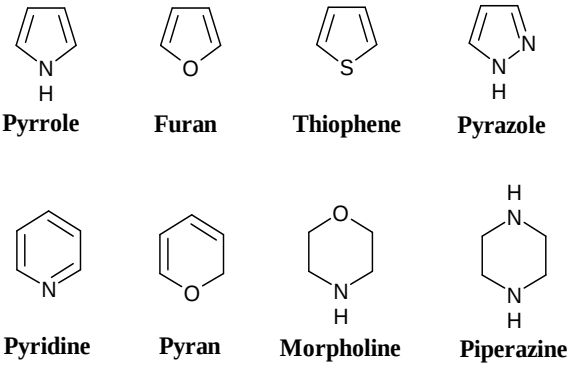
Abstract

This chapter includes general introduction to heterocyclic chemistry and chemistry of pyrazoles. It also covers a detailed literature survey on pyrazole based entities carrying various pharmacophoric heterocycles. A brief account on biological importance of various pyrazole derivatives has also been discussed in this chapter. Further, it covers the scope and objectives of the present research work.

1.1 INTRODUCTION TO HETEROCYCLIC CHEMISTRY

Medicinal chemistry is the application of chemical research techniques to the synthesis of pharmaceutical compounds. During the early stages of medicinal chemistry development, scientists were primarily concerned with the isolation of medicinal agents found in plants. Today, scientists in this field are also equally concerned with the creation of new synthetic drug compounds. Medicinal chemistry is almost always geared toward drug discovery and development.

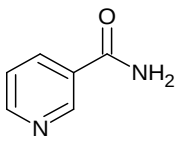
Heterocyclic compounds are a class of organic compounds whose molecules contain one or more ring of atoms with at least one heteroatom being an element other then carbon, most frequently oxygen, nitrogen or sulphur. Heterocyclic compounds probably constitute the largest and most varied family of organic compounds. The most common heterocycles are those having five- or six-membered rings and containing heteroatoms of nitrogen (N), oxygen (O), or sulfur (S). Some of the common heterocycles are mentioned below,



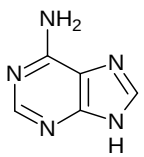
In the medicinal world, the chemistry of heterocycles has played a vital role in combating many deadly diseases. The role of heterocyclic compounds has become increasingly important not only in the medicinal field but also provided an important platform for the rapid exchange of research in the areas of agriculture, photography, biocide formulation and polymer science. Heterocyclic systems occur in a wide variety of natural and synthetic compounds and are essential to life in various ways. Certain derivatives are produced in nature by various animals and plants. Frequently, the naturally occurring heterocycles are structurally complex. Most of the sugars and their derivatives including vitamin-C, for instance, exist largely in the form of five membered (furan) or six membered (pyran) rings containing one oxygen atom.

The delicious and appetizing flavors of many everyday foods are heterocycles. The odour of fried potatoes is principally due to 2,5-dimethyl pyrazine. The aroma of coffee is due to furfuryl mercaptan. The aroma of new baked bread is due to 3-hydroxyfuran. Heterocyclic rings constitute a large number of synthetic dyes and drugs. A few of the useful condensed heterocyclic compounds are quinoline, isoquinoline, acridine, phenanthridine, caffeine, allopurinol etc.

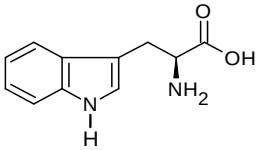
Heterocycles are of great importance in the metabolic activities. Among these few compounds are thiamin, riboflavin, nicotinic acid, pyridoxine folic acid, biotin, adenine the B_{12} family, vitamin-E, chlorophyll, haemoglobin, purine and pyrimidine components of nucleic acids, harmones etc. Most members of the vitamin-B group possess heterocyclic rings containing nitrogen. One example is vitamin-B_6 [pyridoxine] which is a derivative of pyridine essential in amino acid metabolism (Ullah 2004). Some of the structures of common naturally occurring heterocycles are given below,



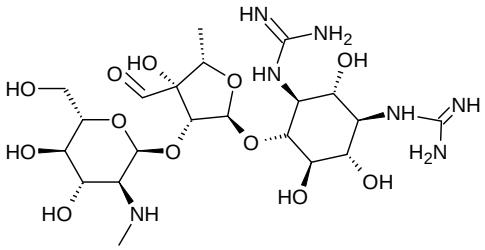
Vitamin B_3



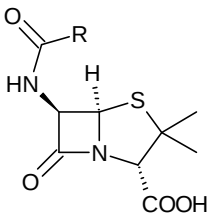
Vitamin B_4



Tryptophan



Streptomycin



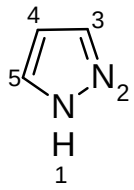
Penicillin

In view of the general observation that the biological activities are invariably associated with a large variety of heterocyclic systems such as pyrazole, triazole, thiazole, oxadiazole, thiadiazole, pyridine, pyrazoline etc. A large number of their new derivatives have been synthesized and extensively studied for various pharmacological properties. The fast growing literature on heterocycles in recent years demonstrates their increasing significance in the pharmaceutical field.

Pyrazole, which forms the infrastructure of the present research work, is a unique class of heterocycle possessing diverse biological properties. Owing to the pharmacological importance of pyrazole, the present research work has been planned to synthesize new pyrazole derivatives, which combine two or more bio-labile components. A brief account on pyrazole chemistry and biological importance of various pyrazole derivatives have been highlighted in the following sections.

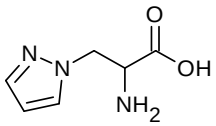
1.2 CHEMISTRY OF PYRAZOLES

Pyrazole (**S-1.1**) is a class of simple aromatic compound of the heterocyclic series characterized by a five-membered ring structure composed of the three carbon atoms and two nitrogen atoms in adjacent positions.



S-1.1

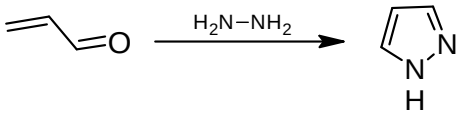
In 1959, the first natural pyrazole, 1-pyrazolyl-alanine (**S-1.2**), was isolated from seeds of watermelons.



1-pyrazolyl-alanine

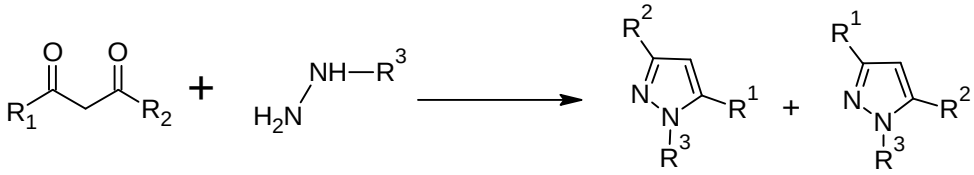
S-1.2

Pyrazoles are produced synthetically through the reaction of α, β-unsaturated aldehydes with hydrazine and subsequent dehydrogenation (**S-1.3**).



S-1.3

Pyrazoles can also be synthesized by the condensation of 1,3-diketones with arylhydrazines.



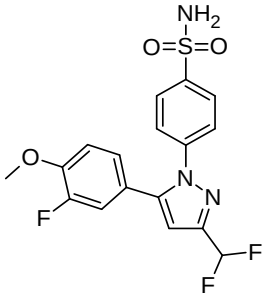
S-1.4 **S-1.5**

1.3 BIOLOGICAL IMPORTANCE OF PYRAZOLES

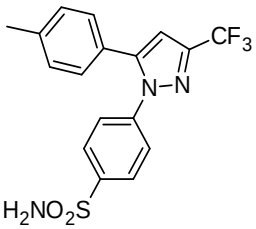
Pyrazoles are class of compounds which play an important role in medicinal chemistry. The synthesis of pyrazoles remains of great interest due to the wide applications of such heterocycles in the pharmaceutical and agrochemical industry. The prevalence of pyrazole cores in biologically active molecules has stimulated the need for elegant and efficient ways to make these heterocyclic lead.

Pyrazoles are heterocyclic compound having a diverse biological activity when compared to other compounds. Pyrazoles are known for their anti-arthritic (Rangari et al. 1990), fungicidal (Delany et al. 1991), antipyretic, antibacterial (Holla et al. 2000), analgesic (Isloor et al. 2000), antitubercular (Chovatia et al. 2007), anti-

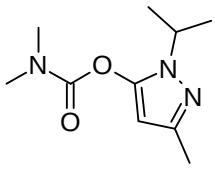
inflammatory (Youssef et al. 2010), anticancer (Balbi et al. 2011), anti-HIV and antimicrobial (Siddiqui et al. 2011) activities.



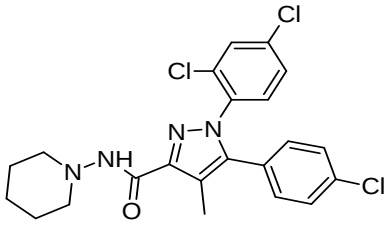
S-1.6
Deracoxib
Anti-inflammatory



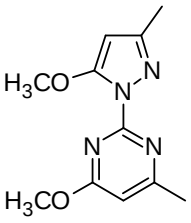
S-1.7
Celecoxib
Anti-inflammatory



S-1.8
Isolan
Insecticide

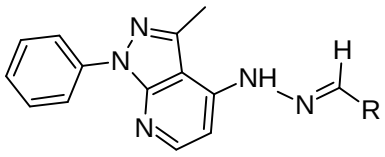


S-1.9
Rimonabant
Anti-obesity drug



S-1.10
Epirizole
Analgesic, Anti-inflammatory

Gaston et al. (1996) reported the synthesis and analgesic activity of the new functionalized arylcarbaldehyde 4-(1-phenyl-3-methylpyrazolo[3,4-b]pyridine) hydrazone derivatives (**S-1.11**).

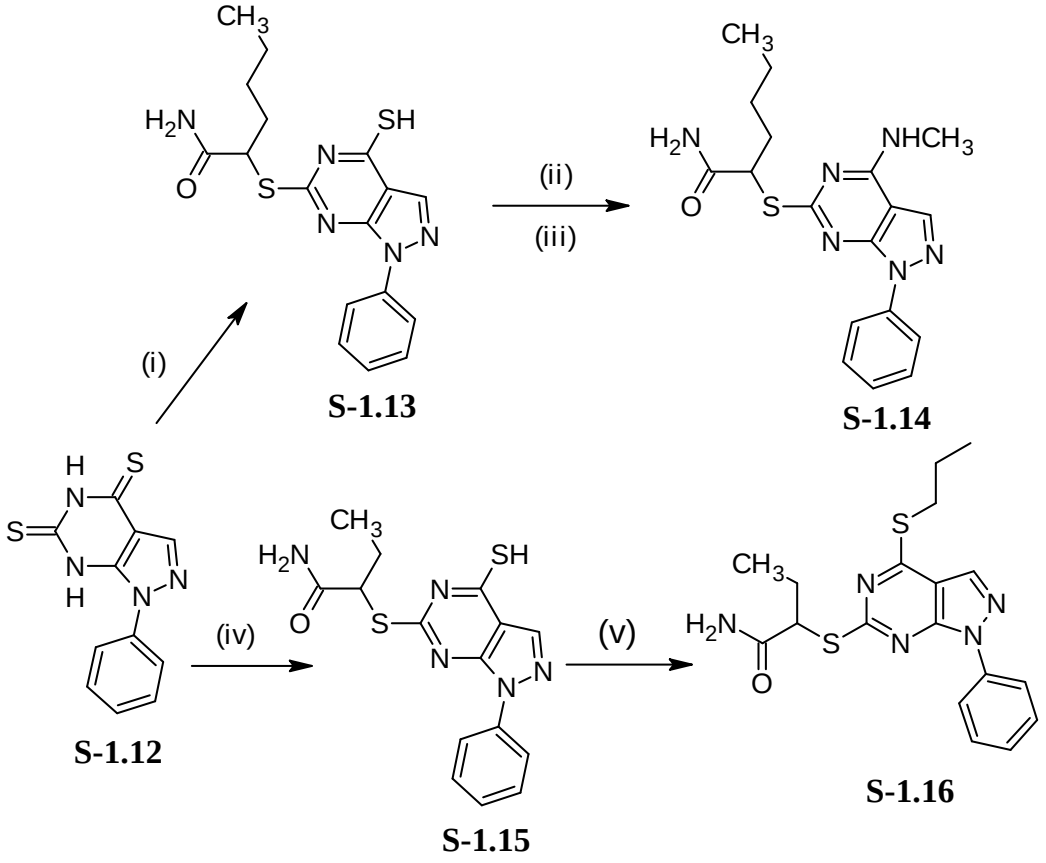


S-1.11

Where R = 2-NO_2-C_6H_4, 4-NO_2-C_6H_4, 2-CF_3-C_6H_4, 4-OCH_3-C_6H_4, 4-F-C_6H_4, 4-CN-C_6H_4, 2-furyl

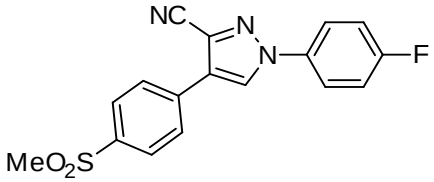
Poulsen and Quin (1996) reported the synthesis and receptor binding at A_1 and A_{2a} receptors of 1-phenylpyrazolo[3,4-*d*]pyrimidines (**S-1.12** to **S-1.16**) substituted at

C_6 with thioethers containing distal amide substituents and alkyl branching and substituted at C-4 with alkylamine or alkylthiol. These compounds were shown to be an A_1 antagonist. They lack the sugar moiety which is a requisite for agonist activity.



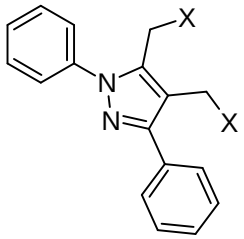
i) $CH_3(CH_2)_3CH(Br)CONH_2$, Pyridine, RT ii) CH_3I, NaOH (aq), RT
iii) CH_3NH_2 (g), EtOH, 110 ˚C iv) $CH_3CH_2CH(Br)CONH_2$, Pyridine, RT
v) C_3H_7I, NaOH (aq), 60 ˚C

1,4-Diaryl pyrazole derivative (**S-1.17**) was synthesized by Tsuji et al. (1997). The compound was tested for anti-inflammatory activity to develop anti-inflammatory agents with fewer side effects than existing non-steroidal anti-inflammatory drugs. The structure activity relationship was extensively studied.



S-1.17

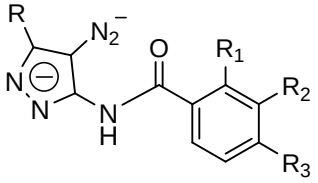
A series of pyrazole derivatives with two and three alkylating sites (**S-1.18**) were prepared by Katayama and Oshiyama (1997). Synthesized compounds were investigated for their anticancer activity.



S-1.18

Where X= Cl, Br, OH, OCOOPh, $OCONH_2$

Daidone and his group (1998) reported the antimicrobial and antineoplastic activities of new 4-diazopyrazole derivatives (**S-1.19**).

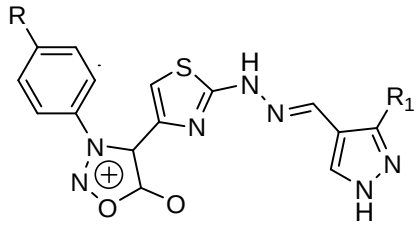


S-1.19

Where R = CH_3, C_6H_5 R_1 = H, NO_2, Cl

R_2 = H, Cl R_3 = H, Cl, CH_3, OCH_3, CF_3, NO_2, I, Br, F

Isloor et al. (2000) reported the synthesis of 3-aryl-4-(substituted pyrazolidine hydrazine-4-thiazolyl) sydnones (**S-1.20**) and its analgesic, anticonvulsant activities.

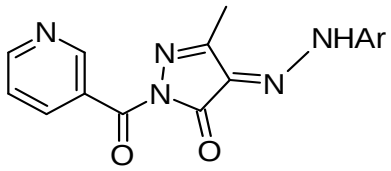


S-1.20

Where R = H, CH_3, OCH_3

R_1 = C_6H_5, 4-Cl-C_6H_4, 4-OCH_3-C_6H_4, 4-CH_3-C_6H_4

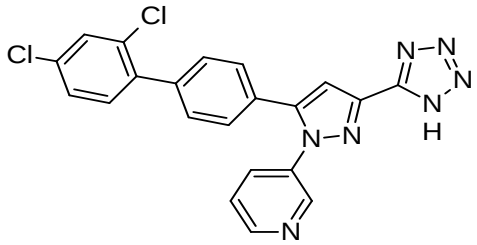
Kalluraya and Chimbalkar (2001) reported a series of pyrazolines of the type (**S-1.21**). These compounds were tested for antibacterial and antifungal activity.



S-1.21

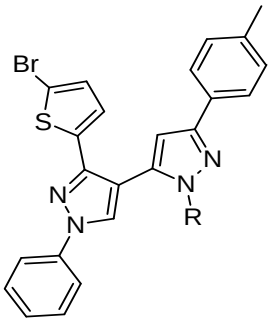
Ar= 4-$NO_2C_6H_4$, 2-NO_2-4-$CH_3C_6H_3$, 3,5-$Cl_2C_6H_3$, C_6H_5, 2-CH_3-4-ClC_6H_3, 2,5-$(CH_3)_2C_6H_3$, 4-ClC_6H_4, 2,5-$Cl_2C_6H_3$, 2-ClC_6H_4, 2-COC_6H_4

Pyrazole (**S-1.22**) was synthesized by Finn et al. (2003) with significantly improved potency on bacterial methionyl-t-RNA synthetase and selectivity over human methionyl-t-RNA synthetase.



S-1.22

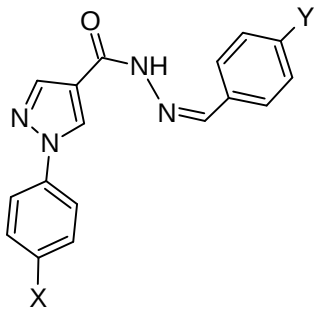
The synthesis of novel series of structurally related 1*H*-pyrazolyl derivatives (**S-1.23**) described by the Bekhit and Abdel-Aziem (2004). All the newly synthesized compounds were tested for their anti-inflammatory and antimicrobial activities. COX-1 and COX-2 inhibitory activities, ulcerogenic effects and acute toxicity were also determined.



Where R= H, C_6H_5CO, C_6H_5NHCS, CH_3CO, CHO, CH_3SO_2, 4-$CH_3C_6H_4SO_2$

S-1.23

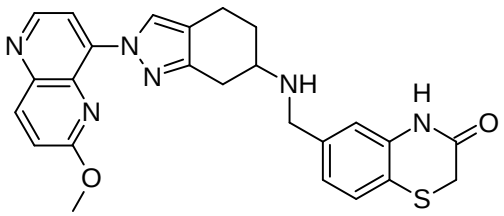
Bernardino et al. (2006) reported the synthesis and leishmanicidal activities of 1*H*-pyrazole-4-carbohydrazides (**S-1.24**) and found that among all the 1*H*-pyrazole-4-carbohydrazides derivatives examined, the most active compounds were those with X = Br, Y = NO_2 and X = NO_2, Y = Cl derivatives which showed to be most effective on promastigotes forms of *Leishmania amazonensis* than on *Leishmania chagasi* and *Leishmania braziliensis* species.



S-1.24

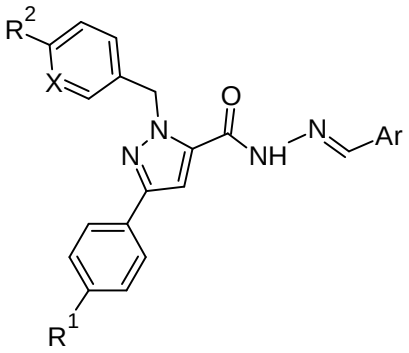
Where X = OCH_3, NO_2, Br
Y = H, OH, CN, OCH_3, Br, Cl, F, SCH_3, NO_2, CH_3

Gomez and his group (2007) described the synthesis and preliminary SAR analysis of novel pyrazole derivatives as potent inhibitors of type II topoisomerases. Their investigation report revealed that compound **S-1.25**, a tetrahydroindazole core structure, was one of the most active compounds, with potent antibacterial activity against both Gram-positive and Gram-negative organisms.



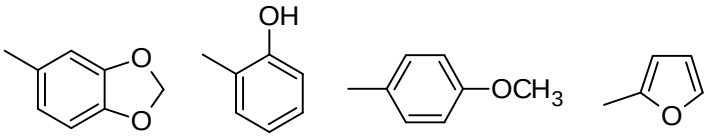
S-1.25

Xia et al. (2008) reported a series of novel 1-arylmethyl-3-aryl-1*H*-pyrazole-5-carbohydrazide hydrazone derivatives (**S-1.26**) and the effects of all the compounds on A549 cell growth were investigated. The results revealed that all compounds had almost inhibitory effects on the growth of A549 cells.

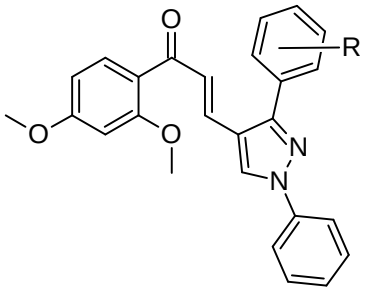


S-1.26

Where R^1 = H, Cl, OMe; R^2 = H, Cl, *t*-Bu; X = C, N

Ar = 

A research group led by Bandgar and his co-workers (2009) synthesized a novel series of pyrazole chalcones (**S-1.27**) and investigated for their anti-inflammatory, antioxidant and antimicrobial activity. Their investigation report revealed that compounds with R = 4-H, 4-OCH_3 and 3-Cl on phenyl ring of pyrazole moiety exhibited promising IL-6 inhibitory, free radical scavenging and antimicrobial activities.

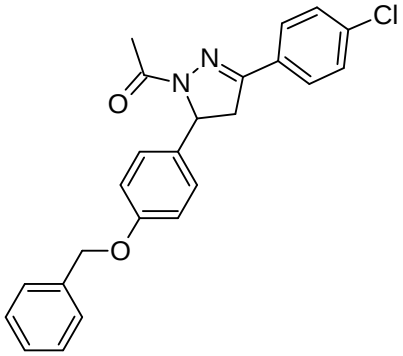


S-1.27

Where R = H, CH_3, OCH_3, Cl, Br, F, 3-Cl, 2-Cl, 2,4-Cl_2, 2,4-OCH_3

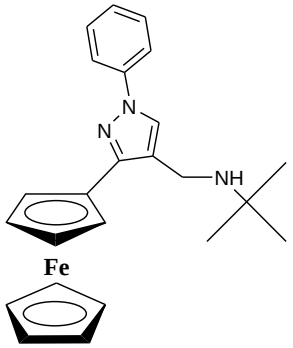
El-Sabbagh and his researchers (2009) synthesized some new pyrazole and thiazole derivatives and evaluated for their antiviral activity. The antiviral activity for such novel compounds against a broad panel of viruses in different cell cultures

revealed that N-acetyl 4,5-dihydropyrazole (**S-1.28**) was the only active one at subtoxic concentrations against vaccinia virus (Lederle strain) in HEL cell cultures with a 50% effective concentration (EC_{50}) value of 7 mg/mL.

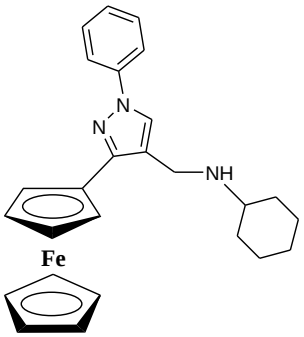


S-1.28

Damljanovic et al. (2009) reported synthesis and antimicrobial activity of some new pyrazole derivatives containing a ferrocene unit. The most active compounds were the amines **S-1.29** and **S-1.30**, showing reduction of bacterial and fungal growth comparable or higher than that exhibited by the standards.

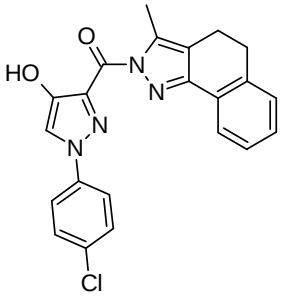


S-1.29

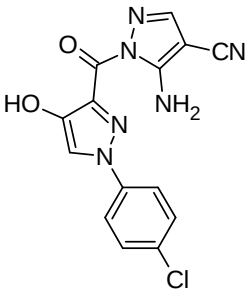


S-1.30

Rostom (2010) reported the synthesis of some 1-(4-chlorophenyl)-4-hydroxy-1*H*-pyrazol-3-carbonyl derivatives linked to nitrogenous heterocyclic ring systems as potential antitumor agents. The tricyclic naphthopyrazole (**S-1.31**) and the aminocyanopyrazole (**S-1.32**) could be classified as the most active antitumor members identified in this study.

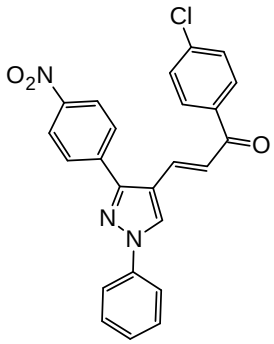


S-1.31

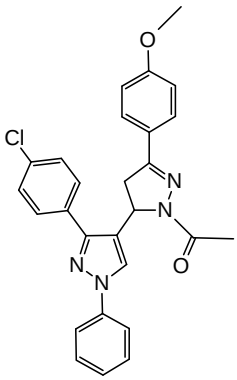


S-1.32

Insuasty et al. (2010) reported the synthesis of novel pyrazolic analogues of chalcones and their 3-aryl-4-(3-aryl-4,5-dihydro-1H-pyrazol-5-yl)-1-phenyl-1*H*-pyrazole derivatives as potential antitumor agents. The anticancer evaluation data revealed that among the compounds studied, derivatives **S-1.33** and **S-1.34** exhibited high activity patterns against different cancer cell lines.

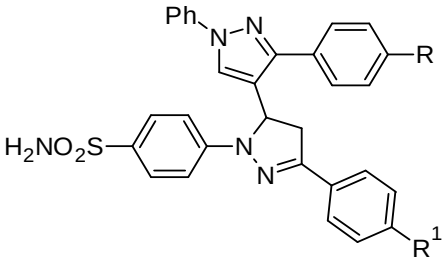


S-1.33



S-1.34

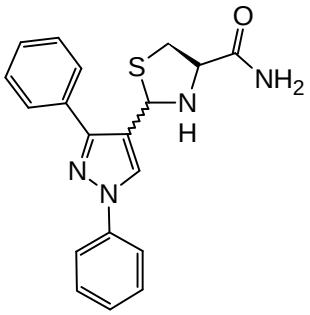
Sharma et al. (2010) reported the synthesis and biological evaluation of some pyrazolylpyrazolines (**S-1.35**) as anti-inflammatory-antimicrobial agents. The compound with substituent R= Br and R^1= H have been identified as the most biologically active member within this study with an interesting dual anti-inflammatory and antibacterial profile.



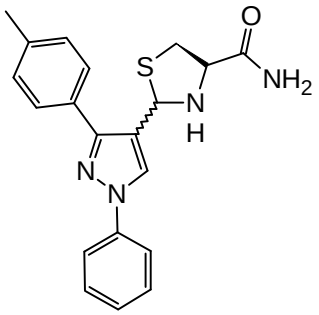
S-1.35

Where R=H, CH_3, F, Br R^1=H, CH_3, F, Br

Bekhit and his co-workers (2010) reported the synthesis and biological evaluation of some thiazolylpyrazole derivatives as dual anti-inflammatory-antimicrobial agents. The results revealed that compounds **S-1.36** and **S-1.37** represented a fruitful matrix for the development of a new class of dual anti-inflammatory-antimicrobial agents.

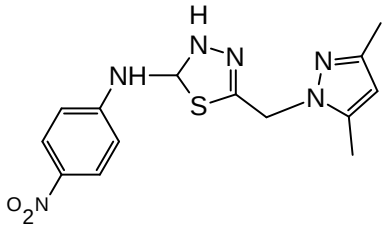


S-1.36



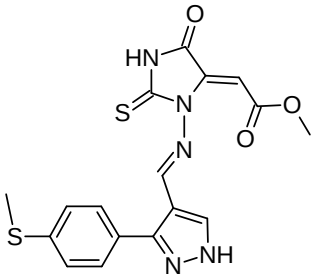
S-1.37

El-Moghazy and his associates (2011) synthesized some new pyrazole derivatives and investigated for their anti-inflammatory activity. The results revealed that the compound **S-1.38** emerged as most effective anti-inflammatory agent among the synthesized molecules.



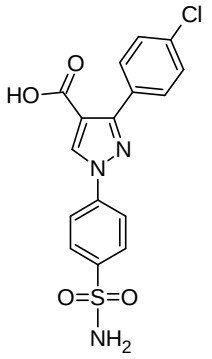
S-1.38

Vijesh et al. (2011) synthesized some new pyrazole incorporated imidazole derivatives and subjected for their antimicrobial activity. Results revealed that the compound **S-1.39** was found to be potent antimicrobial agent in the series.

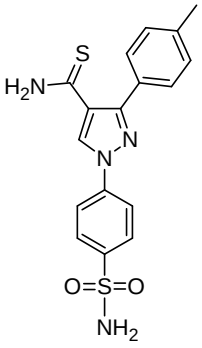


S-1.39

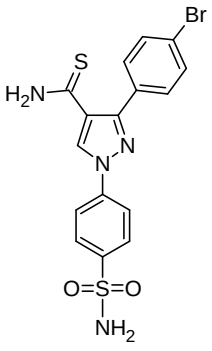
Sharma et al. (2011) reported the synthesis and biological evaluation of some 4-functionalized-pyrazoles as antimicrobial agents. Three tested compounds, **S-1.40**, **S-1.41** and **S-1.42** exhibited moderate antibacterial activity against gram-positive bacteria.



S-1.40

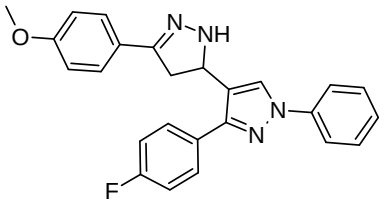


S-1.41



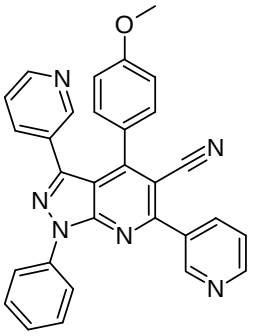
S-1.42

Khunt and his researchers (2012) synthesized a series of N-phenyl-3-(4-fluorophenyl)-4-substituted pyrazoles and tested for antimycobacterial activity *in vitro* against *Mycobacterium tuberculosis H37Rv* strain using the BACTEC 460 radiometric system. The results revealed that among the synthesized compounds, **S-1.43** was identified as the most active molecule with an IC_{50} of 0.47 µM.

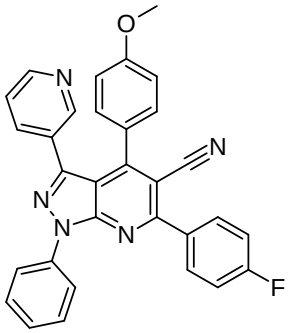


S-1.43

El-borai et al. (2012) synthesized the pyrazolo[3,4-b]pyridines under microwave irradiation through multicomponent reaction and evaluated their antitumor and antimicrobial activities. The results revealed that most of the tested compounds showed excellent activity against the gram-negative rather than the gram-positive bacteria. Compounds **S-1.44** and **S-1.45** showed significant antitumor activity (IC_{50} = 3.43-3.75 µg/mL).

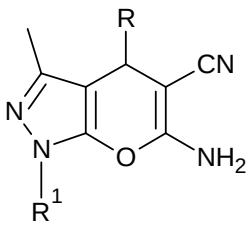


S-1.44



S-1.45

Mandha and his co-workers (2012) reported an ecofriendly green approach for synthesis of substituted pyrano[2,3-*c*]pyrazoles (**S-1.46)** via a multicomponent one pot approach in aqueous ethanol medium under totally non-catalytic conditions. The synthesized compounds were evaluated for their antibacterial, anti-inflammatory and cytotoxic activities and found that most of the synthesized compounds showed promising activity.



S-1.46

Where,
R = 3-OH-Ph, 4-Br-Ph, 4-CH_3-Ph, C_6F_5, 3-pyridinyl, 2-thienyl, 4-$C_{11}H_5Cl_3NO$, 3-OC_6H_5-Ph, 3-$C_{11}H_5Cl_3NO$
R^1 = H, Ph

1.4 SCOPE AND OBJECTIVES

Antimicrobial resistance-also known as drug resistance-occurs when microorganisms such as bacteria, viruses, fungi and parasites change in ways that render the medications used to cure the infections they cause ineffective. When the microorganisms become resistant to most antimicrobials they are often referred to as "*superbugs*". This is a major concern because a resistant infection may kill, can spread to others, and imposes huge costs to individuals and society.

Serious infections caused by bacteria that have become resistant to commonly used antibiotics have become a major global healthcare problem in the 21st century. Currently, only a limited number of antibiotics are available to treat multidrug-resistant strains of infectious bacteria, and resistance to even the newest antimicrobial agents is appearing.

The discovery and development of antimicrobials, also called "*miracle drugs*", has been one of the most important advances in the history of modern medicine. In the present scenario, there is an urgent and continuous need of exploration and development of cheaper, effective drugs with better bioactive potential and least side effects.

Heterocyclic compounds by virtue of their specific activity could be employed in the treatment of infectious diseases. Review of literature indicated that pyrazole and its derivatives play a significant role in the development of pharmacologically important molecules. Keeping this in view, some new N-bridged heterocycles particularly pyrazole containing heterocycles have been synthesized starting from simple molecules and investigated for their preliminary microbiological evaluation.

The main objectives of the present research work are as follows:

- Synthesis of new pyrazole derivatives carrying interesting pharmacophores like triazole, thiazole, oxadiazole, thiadiazole, pyridine, pyrazoline.
- Characterization of new compounds by IR, ^{1}H NMR, ^{13}C-NMR and Mass spectrometry, followed by elemental analysis.
- X-ray crystallographic studies of selected compounds for elucidation of final three-dimensional structure.

- Evaluation of *in vitro* antimicrobial studies for all the series whereas, antioxidant and anti-inflammatory activities for selected series of newly synthesized compounds.
- Study of structure activity relationship with reference to biological activity.

The thesis has been divided into eight chapters.

Chapter 1: This is an introductory chapter, which deals with a brief account of synthesis, reactions and biological activities of some pyrazole derivatives based on the publications appearing in the chemical literature up to August 2012. The main objectives of the present research work were also explained here.

Chapter 2: This chapter describes the synthesis, characterization and biological evaluation of some new 3,6-disubstituted-1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazoles bearing pyrazole moiety. The final product, thiadiazoles were obtained in reasonable yields by condensing 3-substituted-4-amino-5-mercapto-1,2,4-triazoles with various 3-substituted pyrazole-4-carboxylic acids in the presence of phosphorous oxychloride. The synthesized compounds have been characterized by recording their IR, NMR and Mass spectra followed by elemental analysis. Preliminary antimicrobial and anti-inflammatory activity has been carried out for all the target compounds. A molecular docking study of selected compounds which has exhibited good anti-inflammatory has carried out for better understanding of drug receptor interaction.

Chapter 3: This chapter emphasis on the synthesis of new 2,5-disubstituted-1,3,4-oxadiazoles and their antimicrobial studies. 1,3,4-oxadiazole derivatives bearing pyrazole moiety were synthesized by reacting various substituted pyrazole-4-carboxylic acids with different acid hydrazides in phosphorous oxychloride. All the synthesized compounds were characterized by IR, NMR, Mass spectra and elemental analyses. Synthesized compounds were screened for their *in vitro* antibacterial activity and antifungal activity. The result of such studies has been described in this chapter.

Chapter 4: This chapter deals with synthesis, characterization and biological studies of 2,4-disubstituted thiazole derivatives. The target compounds were synthesized by the reaction of various 3-aryl-1H-pyrazole-4-carbaldehyde thiosemicarbazones with phenacyl bromides. The synthesized compounds were characterized by spectral

studies. Furhter antimicrobial antioxidant activities of target compounds were carried out.

Chapter 5: In this chapter, synthesis of some new Schiff bases bearing triazole and a pyrazole skeleton has been discussed. The Schiff bases were synthesized by the condensation of 4-amino-5-substituted-4H-1,2,4-triazole-3-thiol with various 3-substituted-pyrazoles-4-carboxaldehydes in presence of concentrated sulphuric acid in ethanol-dioxane mixture. Structures of the synthesized compounds were confirmed by spectral studies. Newly synthesized compounds were evaluated for their *in vitro* antimicrobial activity.

Chapter 6: This chapter highlights the synthesis of a new series of 4,6-disubstituted-3-cyano-2-pyridone derivatives. The final compounds were obtained by the reaction of 3-substituted-1*H*-pyrazole-4-carbaldehydes, various acetyl compounds, ethyl cyanoacetate and ammonium acetate in ethanolic medium via one-pot multicomponent reaction. The structures of the desired compounds were characterized by spectral studies and were screened for their *in vitro* antimicrobial activity. The result of such studies has been highlighted in this chapter.

Chapter 7: This chapter deals with the synthesis and biological studies of new 3-aryl-4-(3-aryl-4,5-dihydro-1*H*-pyrazol-5-yl)-1-phenyl-1*H*-pyrazole derivatives. First step involves the synthesis of pyrazolic chalcones which were synthesized from a Claisen-Schmidt reaction of 3-aryl-1-phenylpyrazol-4-carboxaldehydes with several acetophenone derivatives. Subsequently, the cyclocondensation reaction of chalcones with phenylhydrazine in acetic acid medium afforded the target compounds. The synthesized compounds were characterized by spectral studies and were screened for their *in vitro* antimicrobial activity.

Chapter 8: The summary and conclusions of present research work has been discussed in this chapter.

CHAPTER - 2

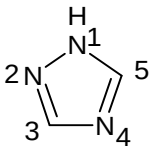
SYNTHESIS AND BIOLOGICAL ACTIVITIES OF SOME NEW 3,6-DISUBSTITUTED-1,2,4-TRIAZOLO-[3,4-b]-1,3,4-THIADIAZOLES BEARING PYRAZOLE MOIETY

Abstract

This chapter describes a detailed literature survey on 1,2,4-triazole, 1,3,4-thiadiazole and their condensed nucleus systems (triazolothiadiazoles). It also includes the synthesis and characterization of newly designed pyrazole containing triazolothiadiazole derivatives. Further, the antimicrobial and anti-inflammatory activities of the synthesized compounds have been discussed in this chapter.

2.1 INTRODUCTION

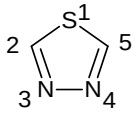
1,2,4-Triazoles (**S-2.1**) represent an overwhelming and rapid developing field in modern heterocyclic chemistry. From literature it is predictable that, 1,2,4-triazoles represent important pharmacophores, and play a vital role as medicinal agents.



S-2.1

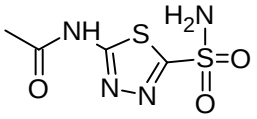
A degree of respectability has been bestowed for 1,2,4-triazole derivatives due to their wide range of biological activities such as analgesic (Turan-Zitouni et al. 1999), anti-inflammatory (Tozkoparan et al. 2000), antitubercular (Kucukguzel et al. 2001), antifungal (Hirpara et al. 2003), antibacterial (Holla et al. 2001; Prakash et al. 2004; Sunil et al. 2011), antiviral (Somorai et al. 2006), antimicrobial (Isloor et al. 2009), anticancer (He et al. 2010) and antihypertensive (Siddiqui et al. 2011) properties. 4-amino-3-mercapto-1,2,4-triazole derivative is an ideal heterocycle by virtue of its vicinal nucleophiles amino and mercapto groups constitutes a ready-made building block for construction of various substituted organic heterocycles (Collin et al. 2003).

Thiadiazole is a five-membered ring system containing two nitrogen and one sulphur atom. They occur in nature in four isomeric forms viz. 1,2,3-thiadiazole; 1,2,5-thiadiazole; 1,2,4-thiadiazole and 1,3,4-thiadiazole. The 1,3,4-thiadiazole isomer (**S-2.2)** of thiadiazole series provide a bulk of literature on thiadiazole.

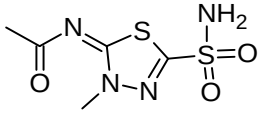


S-2.2

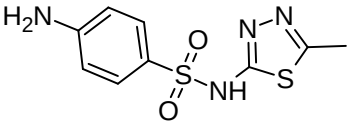
Thiadiazole can act as the bio-isosteric replacement of the thiazole moiety. So it acts like third and fourth generation Cephalosporins, hence can be used in antibiotic preparations. Many drugs containing thiadiazole nucleus are available in the market such as Acetazolamide (**S-2.3)**, Methazolamide (**S-2.4)**, Sulfamethazole (**S-2.5)**, etc.



S-2.3



S-2.4

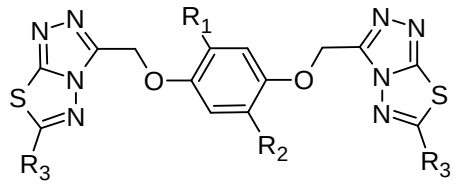


S-2.5

In recent years, the chemistry of 1,2,4-triazoles and their fused heterocyclic derivatives has received considerable attention owing to their synthetic and effective biological importance. Heterocycles bearing a symmetrical triazoles or 1,3,4-thiadiazole moiety exhibit broad spectrum of pharmacological properties such as antimicrobial, anticancer, antitubercular, antiinflammatory, analgesic and anticonvulsant activities (Dogan et al. 2002; Walczak et al. 2004; Schenone et al. 2006).

Derivatives of 1,2,4-triazole and 1,3,4-thiadiazole condensed nucleus systems (triazolothiadiazoles) found to have diverse pharmacological activities including unique anti-inflammatory, anti-edema, and analgesic properties (Mathew et al. 2006; Karthikeyan et al. 2007; Prasad et al. 2009). A triazolo thiadiazole system may be viewed as a cyclic analogue of two very important components, thiosemicarbazide and biguanide which often display diverse biological activities.

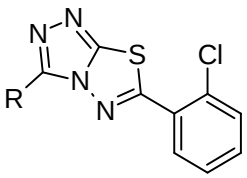
Holla et al. (2002) reported the synthesis and anticancer studies of bis-triazolothiadiazoles (**S-2.6**). The newly synthesized compounds were screened for their anticancer activity against a panel of 60 cell lines derived from seven cancer types namely, lung, colon, melanoma, renal, ovarian, CNS and leukemia. Results revealed that some of the tested compounds showed promising anticancer properties.



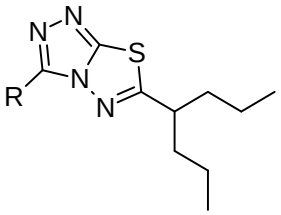
S-2.6

R_1=H, Cl, *t*Bu; R_2=H, Cl; R_3=C_6H_5, 4-$NO_2C_6H_4$, 2-ClC_6H_4, 4-ClC_6H_4, $C_6H_5CH_2$.

Swamy et al. (2006) reported the synthesis of pharmaceutically important condensed heterocyclic 3,6-disubstituted-1,2,4-triazolo-1,3,4-thiadiazole derivatives (**S-2.7** and **S-2.8)** and tested for their antimicrobial activity. Antimicrobial results revealed that the compounds bearing -C_2H_5, C_6H_5 and p-CH_3-C_6H_4 groups on triazole nucleus showed significant inhibition against all the strains tested, when compared to standard drugs.



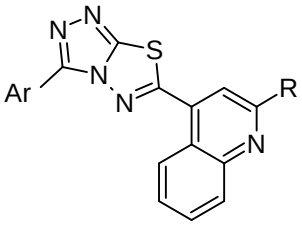
S-2.7



S-2.8

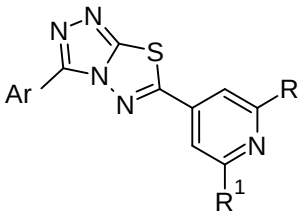
Where R = -CH_3, -C_2H_5, C_6H_5, p-CH_3-C_6H_4, p-Cl-C_6H_4

Mathew and his co-workers (2007) reported the synthesis and pharmacological activities of 3,6-disubstituted-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazoles (**S-2.9, S-2.10, S-2.11**). Synthesized compounds were studied for their antibacterial, antifungal, anti-inflammatory and analgesic activities and found that some of the tested compounds showed significant pharmacological activities.



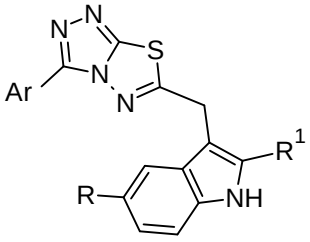
S-2.9

R = C_6H_5, CH_3, H



S-2.10

R = OH, Cl, F
R^1= OH, H

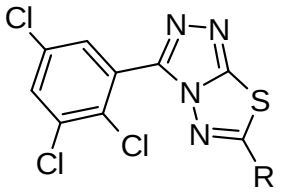


S-2.11

R = OCH_3, H
R^1= CH_3, H

Where Ar = 2-Cl-5-OCH_3-C_6H_3, 3,4-OCH_3- C_6H_3-CH_2, 2-CH_3-furyl

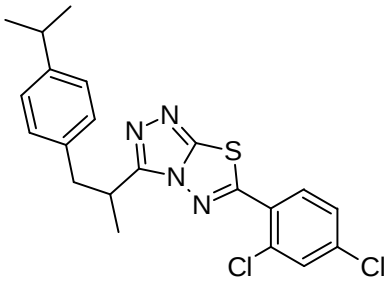
Karegoudar et al. (2008) reported the synthesis of 1,2,4-triazolo-1,3,4-thiadiazoles bearing trichlorophenyl moiety (**S-2.12**). Newly synthesized compounds were screened for their antimicrobial and anti-inflammatory activities. Some of the compounds exhibited promising antimicrobial and anti-inflammatory activities.



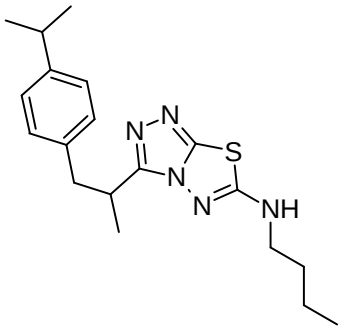
S-2.12

Where R = 4-CH_3-C_6H_4, 4-OCH_3-C_6H_4, 3,4-Cl_2- C_6H_3, 3,5-CH_3- C_6H_3, -OCH_2- C_6H_5, 5-quinolinyl, C_6H_5, Pyridyl, 5-isoquinolyl, 2-bromopyridyl.

Synthesis and evaluation of anti-inflammatory activity of 1,2,4-triazolo [3,4-b][1,3,4]thiadiazole derivatives of Ibuprofen and biphenyl-4-yloxy acetic acid was reported by Amir et al. (2008). The results showed that compounds (**S-2.13** and **S-2.14**) having 2,4-dichlorophenyl and n-butyl amino groups, respectively, was found to be the highest, being slightly less than Ibuprofen, but equivalent to Flurbiprofen. In general the presence of 2,4-dichlorophenyl, 4-chloroprene, n-butyl amino and 4-aminophenyl groups at C-6 of triazolo-thiadiazole ring resulted in high anti-inflammatory activity.



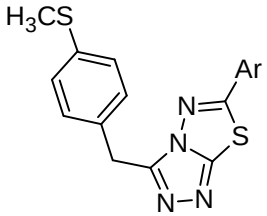
S-2.13



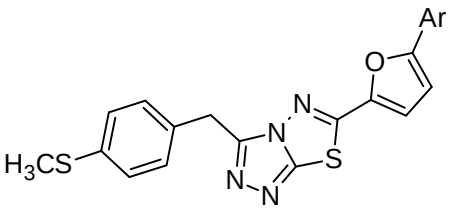
S-2.14

The synthesis and antimicrobial activities of some new triazolothiadiazoles containing 4-methylthiobenzyl moiety **(S-2.15 and S-2.16)** was reported by Prasad et

al. (2009). The preliminary results revealed that some of the compounds exhibited promising antimicrobial activities.



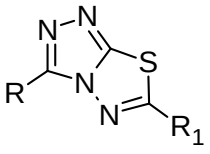
S-2.15



S-2.16

Where Ar = 2,3,4-Cl_3-C_6H_2, 4-SCH_3-C_6H_4-CH_2, 2,4-Cl_2-5-F-C_6H_2, C_6H_4-CH_2-, 4-NO_2-C_6H_4, 4-OH-C_6H_4, 4-Br-C_6H_4, 4-F-C_6H_4, 2,5-Cl_2-C_6H_3, 2-Cl-4-NO_2-C_6H_3

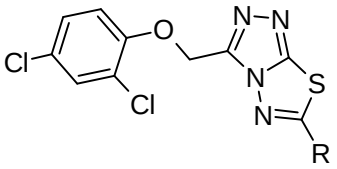
Husain et al. (2009) described the synthesis and biological evaluation of 3,6-disubstituted-1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazoles (**S-2.17**). The compounds were evaluated for their anti-inflammatory, analgesic, ulcerogenic, and lipid peroxidation actions. Some of the newly synthesized compounds showed very good anti-inflammatory activity with low GI toxicity and reduced lipid peroxidation. These compounds also showed interesting profile of analgesic activity in acetic acid-induced writhing test. The findings of the study indicated that the synthesized compounds have superior GI safety profile along with reduction in lipid peroxidation as compared to that of the standard.



S-2.17

Where R = $C_6H_5CH_2$-, $C_6H_5OCH_2$-, 2-OHC_6H_4-
R_1= $C_6H_5CONHCH_2$-, 2-BrC_6H_4-, 3-BrC_6H_4-, 4-BrC_6H_4-,
2-C_6H_5CO-C_6H_4-, $C_{10}H_7CH_2$-, $C_8H_6NCH_2$-, $C_6H_5COCH_2CH_2$-

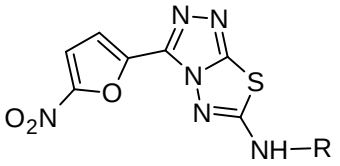
El Shehry and his researchers (2010) described the synthesis, anti-inflammatory and molluscicidal activities of 3-((2,4-dichlorophenoxy)methyl)-1,2,4-triazolo-thiadiazoles (**S-2.18)**. The compounds bearing p-methoxyphenyl and 2,4-dichlorophenoxy groups on thiadiazole ring showed potent anti-inflammatory activity.



S-2.18

Where R = 2,4-$NO_2C_6H_3$, 4-$OCH_3C_6H_4$, 2-furyl, 2,4-dichlorophenoxy

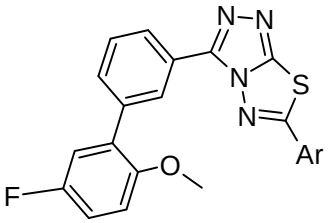
Badr and Barwa (2011) reported the synthesis of [1,2,4]triazolo[3,4-b][1,3,4] thiadiazoles (**S-2.19**) starting from 5-nitro-2-furoic acid and evaluation of their antimicrobial activity. The results revealed that most of the tested compounds showed interesting antibacterial activity against *Staphylococcus aureus*.



S-2.19

Where R = C_2H_5, n-C_4H_9, C_6H_5, 4-F-C_6H_4, 4-CH_3-C_6H_4-CH_2

Ramaprasad et al. (2012) reported the microwave assisted synthesis of triazolothiadiazole analogues as anticancer and antibacterial agents. The antibacterial and anticancer screening results showed that compounds bearing 4-F, 3-CF_3 and 2-F, 5-NO_2 groups in the phenyl ring of thiadiazole exhibited the highest activity.



S-2.20

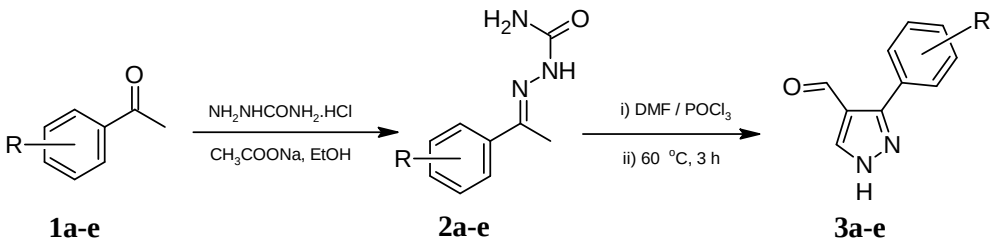
The literature survey highlights that 1,2,4-triazole and 1,3,4-thiadiazole condensed nucleus system found to have diverse pharmacological activities. Therefore it was planned to investigate a composite system, which combine these two biolabile components in a ring together to give a compact and planar structure and screened for their antimicrobial and anti-inflammatory activities.

2.2 MATERIALS AND METHODS

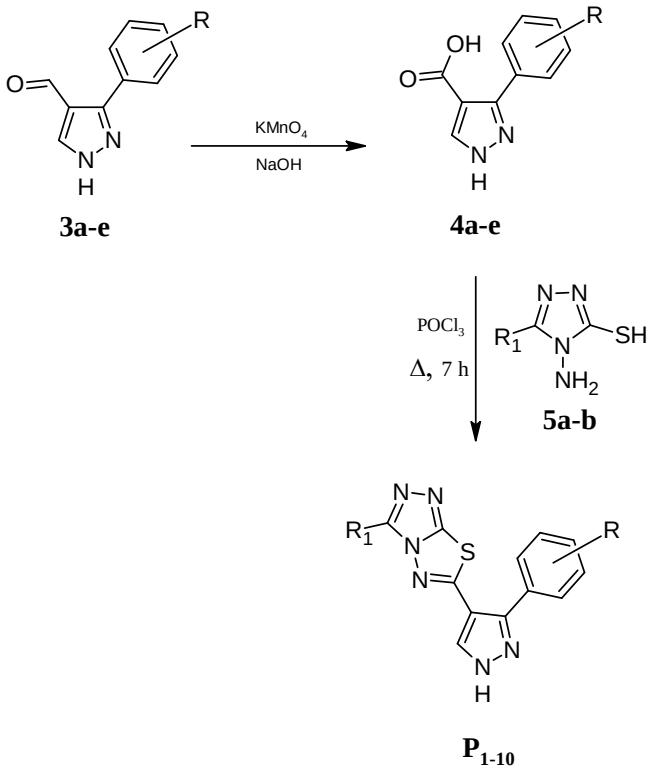
The starting material 3-substituted-1*H*-pyrazole-4-carbaldehydes **(3a-e)** were synthesized from corresponding acetophenones through multi-step reactions (**Scheme 2.1**). First step involves the reaction of various 4-substituted acetophenones (**1a-e**) with semicarbazide hydrochloride in presence of sodium acetate to form respective semicarbazones (**2a-e)**. Further these semicarbazones were subjected to Vilsmayer-Haack reaction to yield 3-substituted-1*H*-pyrazole-4-carbaldehydes **(3a-e)** (Baraldi et al. 1997; Lebedev et al. 2005).

Further, oxidation of 3-substituted-pyrazole-4-carbaldehydes using potassium permanganate in the presence of base (Lebedev et al. 2005) yielded corresponding acid (**4a-e**). 3-Substituted-4-amino-5-mercapto-1,2,4-triazoles (**5a-b**) were synthesized as reported in the literature (Dhaka et al. 1974, Reid and Heindel, 1976). Subsequently, condensation of 3-substituted-4-amino-5-mercapto-1,2,4-triazoles (**5a-b**) with various 3-substituted-pyrazole-4-carboxylic acids (**4a-e**) in the presence of phosphorous oxychloride afforded a series of 3,6-disubstituted-1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazoles (**P_{1-10}**) (**Scheme 2.2**)

Melting points were determined by open capillary method. The IR spectra were recorded on a Thermo Nicolet avatar 330-FT-IR spectrophotometer. ^{1}H NMR spectra were recorded (DMSO-d_6) on a Bruker and Varian (400 MHz) spectrometer and ^{13}C NMR were recorded (DMSO-d_6) on a Bruker (100 MHz). Chemical shift values are given in δ scales.



Scheme 2.1 Synthetic route for 3-substituted-1*H*-pyrazole-4-carbaldehydes (**3a-e**)



3a-e **4a-e** **5a-b** **P_{1-10}**

R= H, 4-OCH_3, 4-F, 4-Cl, 2,4-Cl
R_1= C_2H_5, C_3H_7

Scheme 2.2 Synthetic route for 3,6-disubstituted-1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazoles **P_{1-10}**

The mass spectra were recorded on LC-MS-Agilent 1100 series and API 2000 LC/MS/MS system. Single crystal X-ray analysis was performed using Bruker APEXII CCD diffractometer. Elemental analyses were performed on a Flash EA 1112 series CHNS-O Analyzer. The completion of the reaction was checked by thin layer chromatography (TLC) on silica gel coated aluminium sheets (silica gel 60 F_{254}). Commercial grade solvents and reagents were used without further purification.

2.3 EXPERIMENTAL

The experimental protocols followed for the synthesis of compounds **P_{1-10}** is given in the following section.

General procedure for the synthesis of 3-(4-substituted phenyl)-1*H*-pyrazole-4-carboxylic acid (4a–e)

3-substituted-pyrazol-4-carbaldehyde (**3a-e**) (0.01 mol) was dissolved with stirring in a solution of 2 g of NaOH in 40 mL of water. The mixture was cooled to

15 ˚C, and a solution of $KMnO_4$ (0.0088 mol) in 40 mL of water was quickly added. The mixture was stirred for 30 min at 20 ˚C and then heated to 95-100˚C until the solution becomes completely decolorized. The solution was cooled and filtered to remove MnO_2 precipitate. Then, the filtrate was acidified with Conc. HCl to pH 3. The resulting solid was filtered off, washed with water and dried (Lebedev et al., 2005).

General procedure for the synthesis of 3-Substituted-4-amino-5-mercapto-1,2,4-triazoles (**5a-b**)

A mixture of propionic or butyric acid (0.1 mol) and thiocarbohydrazide (0.08 mol) are stirred and heated at reflux until solution is achieved. The resultant solution was cooled to room temperature, diluted with 30 mL of diethyl ether and refrigerated for 1 hour. The crude white solid was obtained which was then filtered and recrystallized from water to gives 3-Substituted-4-amino-5-mercapto-1,2,4-triazoles (Dhaka et al. 1974, Reid and Heindel, 1976).

4-amino-5-ethyl-4H-1,2,4-triazole-3-thiol (m.p. 146-148 ˚C.)

4-amino-5-propyl-4H-1,2,4-triazole-3-thiol (m.p. 103-105 ˚C.)

General procedure for the synthesis of 3,6- disubstituted-1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazoles ($P_{1\text{-}10}$)

An equimolar mixture of respective triazole (**5**) (0.001 mol) and 3-(4-substitutedphenyl)-1H-pyrazole-4-carboxylic acid (**4**) (0.001 mol) was dissolved in 5 mL of dry phosphorous oxychloride. The resulted solution was further refluxed for 7 h. Excess phosphorous oxychloride was then distilled off and the mixture was gradually poured into crushed ice with stirring. The mixture was allowed to stand overnight and the solid was separated. The separated solid was filtered, washed thoroughly with cold water, followed by 20% $NaHCO_3$ solution and recrystallized from a mixture of dioxane and ethanol.

3-ethyl-6-(3-phenyl-1 *H* -pyrazol-4-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (P_1)

FT-IR (cm^{-1}): 3390 (N-H-str), 3066 (aromatic C-H-str), 2909 (aliphatic C-H-str), 1604 (C=N), 1481 (C=C) (**Figure 2.1**); ^{1}H NMR (DMSO-d_6): δ 1.30(t, 3H, *J*=7.6 Hz, CH_3), 2.98 (q, 2H, *J*=7.6 Hz, CH_2), 7.71-7.49 (m, 5H, Ar-H), 8.43 (s, 1H, pyrazole-5H), 13.70 (s, 1H, Pyrazole N-H) (**Figure 2.2**). ^{13}C NMR: 159.8, 152.4, 148.2, 146.2, 136.2, 129.7, 129.3, 129.1, 128.4, 108.7, 18.0, 10.6 (**Figure 2.3**). MS: m/z = 297

(M+1) (**Figure 2.4**). Anal. calcd. for $C_{14}H_{12}N_6S$: C, 56.74; H, 4.08; N, 28.36. Found: C, 56.69; H, 4.04; N, 28.39 %.

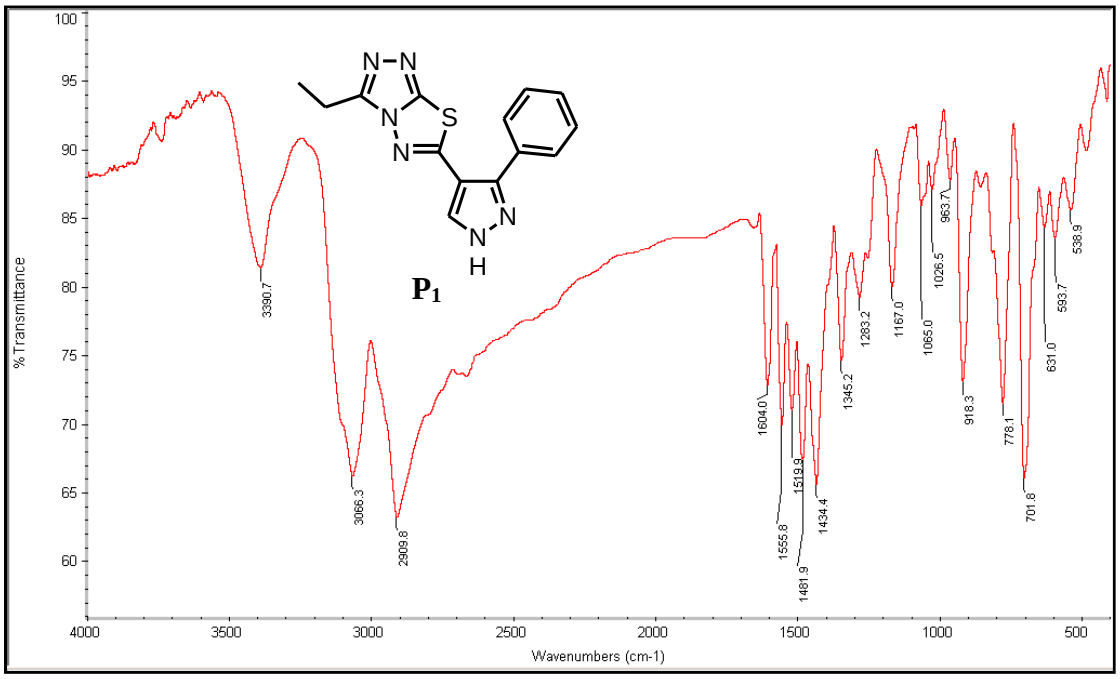


Figure 2.1 IR spectrum of compound **P_1**

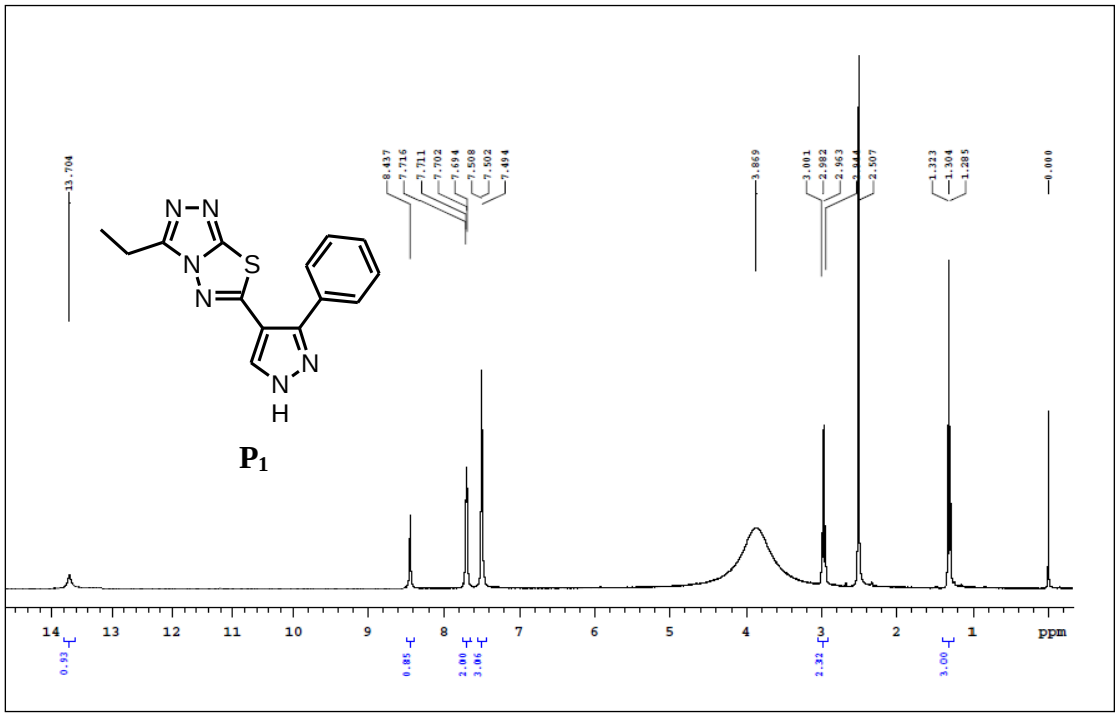
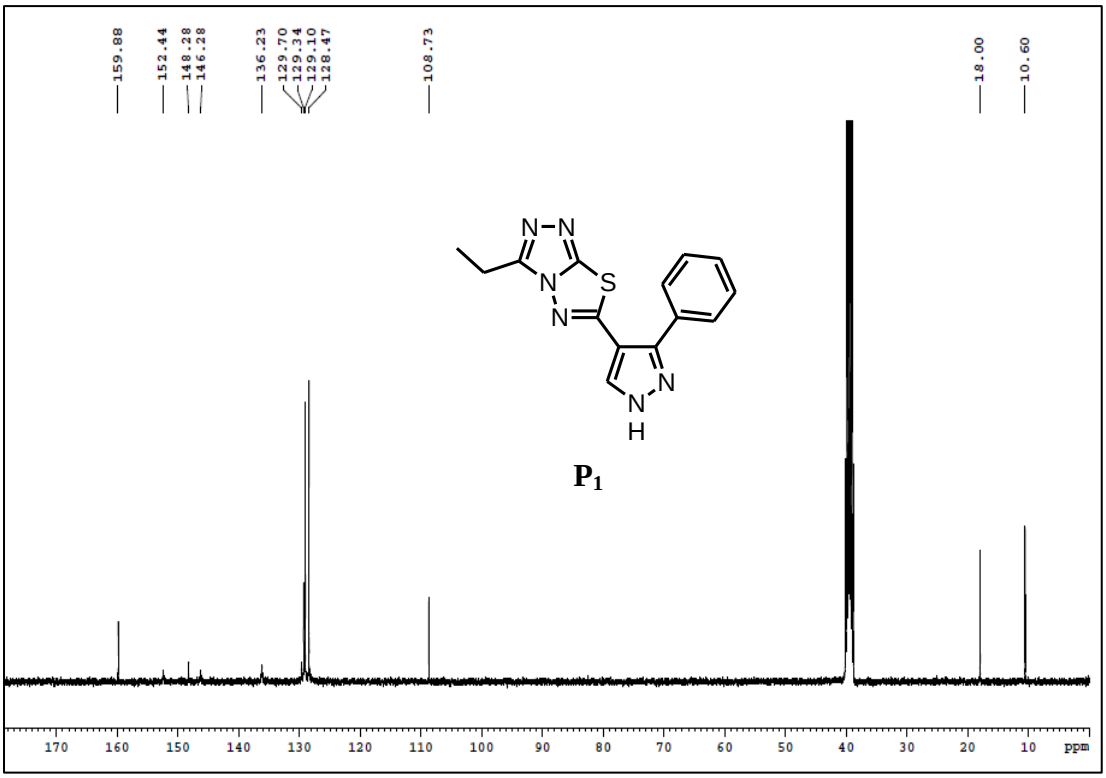


Figure 2.2 ^{1}H NMR spectrum of compound **P_1**



P₁

Figure 2.3 ^{13}C NMR spectrum of compound **P₁**

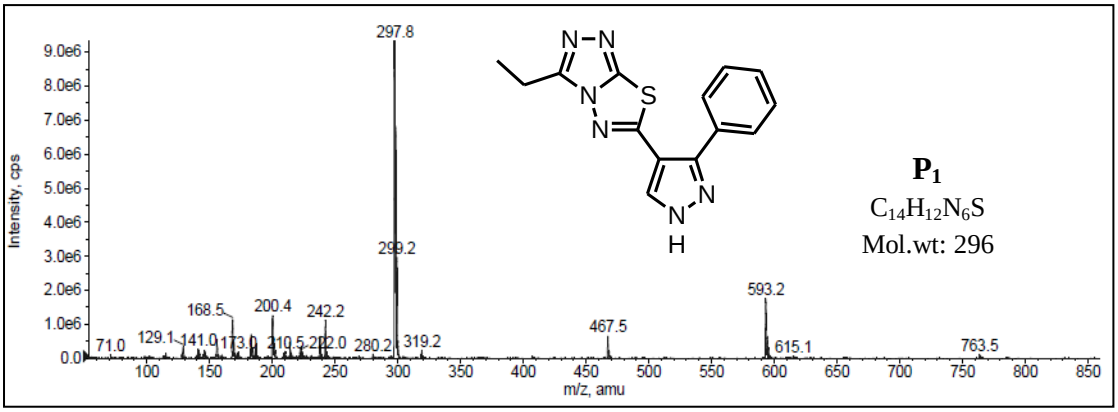


Figure 2.4 Mass spectrum of compound **P₁**

3-ethyl-6-(3-(4-fluorophenyl)-1*H*-pyrazol-4-yl)-[1,2,4]triazolo[3,4-b][1,3,4] thiadiazole (P₂)

FT-IR (cm^{-1}): 3397(N-H-str), 3091 (aromatic C-H-str), 2929 (aliphatic C-H-str), 1605 (C=N), 1479 (C=C); ^{1}H NMR (DMSO-d$_6$): δ 1.30 (t, 3H, *J*=7.6 Hz, CH$_3$), 2.98 (q, 2H, *J*=7.6 Hz, CH$_2$), 7.75-7.79 (m, 2H, Ar-H), 7.30-7.35 (t, 2H, J_{ortho}= 9 Hz, Ar-H), 8.46 (s, 1H, pyrazole-5H), 13.70 (s, 1H, Pyrazole N-H). ^{13}C NMR: 159.6, 148.7,

131.8, 131.8, 115.9, 115.7, 109.4, 18.5, 11.2. MS: m/z = 315 (M+1) (**Figure 2.5**). Anal. calcd. for $C_{14}H_{11}FN_6S$: C, 53.49; H, 3.53; N, 26.74. Found: C, 53.45; H, 3.58; N, 26.77 %. The crystal structure of compound **P_2** has been given in **Figure 2.6**.

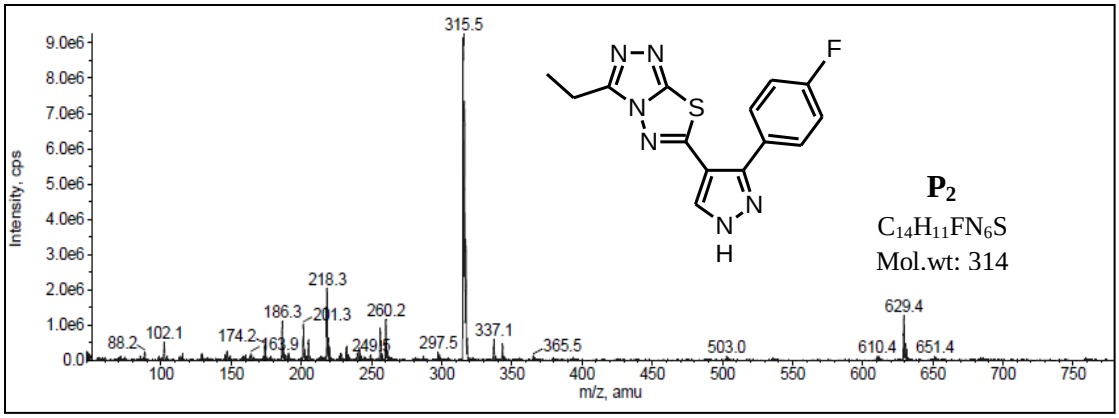


Figure 2.5 Mass spectrum of compound **P_2**

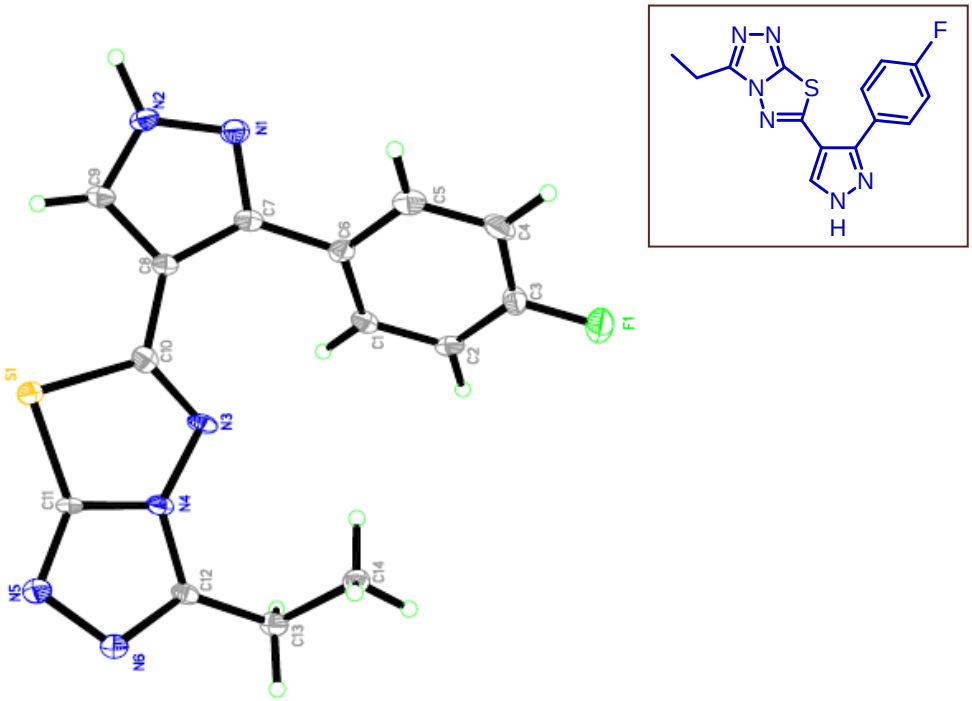


Figure 2.6 The single crystal X-ray structure of compound **P_2**

3-ethyl-6-(3-(4-methoxyphenyl)-1*H*-pyrazol-4-yl)-[1,2,4]triazolo[3,4-b][1,3,4] thiadiazole (P_3)

FT-IR (cm^{-1}): 3350 (N-H-str), 3111 (aromatic C-H-str), 2931 (aliphatic C-H-str), 1613 (C=N), 1472 (C=C); ^{1}H NMR (DMSO-d_6): δ 1.33 (t, 3H, *J*=7.6 Hz, CH_3), 2.99 (q, 2H, *J*=7.6 Hz, CH_2), 3.83 (s, 3H, -OCH_3), 7.05-7.07 (d, 2H, *J*= 8.0 Hz, Ar-H), 7.62-7.65 (d, 2H, *J* =8.8 Hz, Ar-H), 8.34 (s, 1H, pyrazole-5H), 13.70 (s, 1H, Pyrazole N-H). ^{13}C NMR: 159.7, 131.0, 114.4, 109.1, 55.7, 18.5, 11.3. MS: m/z = 327 (M+1). Anal. calcd. for $C_{15}H_{14}N_6OS$: C, 55.20; H, 4.32; N, 25.75. Found: C, 55.17; H, 4.36; N, 25.78 %.

3-ethyl-6-(3-(4-chlorophenyl)-1*H*-pyrazol-4-yl)-[1,2,4]triazolo[3,4-b][1,3,4] thiadiazole (P_4)

FT-IR (cm^{-1}): 3360 (N-H-str), 3173 (aromatic C-H-str), 2940 (aliphatic C-H-str), 1579 (C=N), 1498 (C=C); ^{1}H NMR (DMSO-d_6): δ 1.16 (t, 3H, *J*=7.6 Hz, CH_3), 2.50 (q, 2H, *J*=7.2 Hz, CH_2), 7.46-7.48 (d, 2H, *J*=8.8 Hz, Ar-H), 7.78-7.80 (d, 2H, *J*=8.4 Hz, Ar-H), 8.37 (s, 1H, pyrazole-5H), 13.70 (s, 1H, Pyrazole N-H) (**Figure 2.7**). MS: m/z = 331 (M+1). Anal. calcd. for $C_{14}H_{11}ClN_6S$: C, 50.83; H, 3.35; N, 25.41. Found: C, 50.86; H, 3.31; N, 25.45 %.

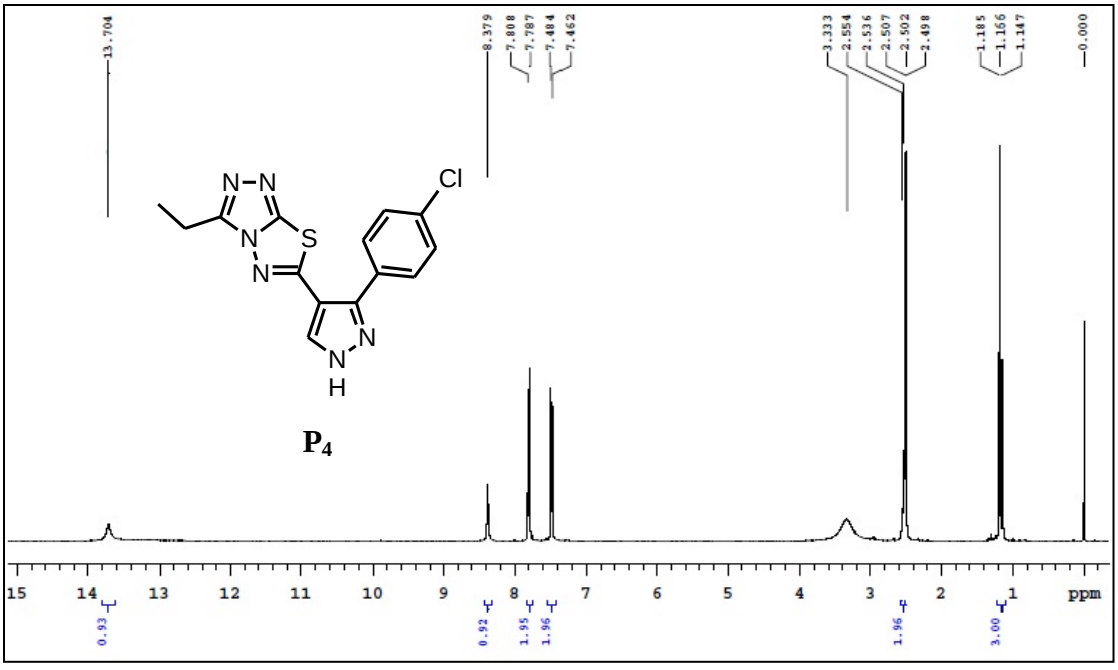


Figure 2.7 ^{1}H NMR spectrum of compound **P_4**

3-propyl-6-(3-phenyl-1*H*-pyrazol-4-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (P_5)

FT-IR (cm^{-1}): 3389 (N-H-str), 3091 (aromatic C-H-str), 2918 (aliphatic C-H-str), 1613 (C=N), 1479 (C=C); ^{1}H NMR (DMSO-d_6): δ 0.93 (t, 3H, *J*=7.2 Hz, CH_3), 1.77 (m, 2H, CH_2), 2.93 (t, 2H, *J*=7.4 Hz, CH_2), 7.47-7.71 (m, 5H, Ar-H), 8.43 (s, 1H, pyrazole-5H), 13.70 (s, 1H, Pyrazole N-H). MS: m/z = 311 (M+1). Anal. calcd. for $C_{15}H_{14}N_6S$: C, 58.05; H, 4.55; N, 27.08. Found: C, 57.96; H, 4.49; N, 26.98 %.

3-propyl-6-(3-(4-fluorophenyl)-1*H*-pyrazol-4-yl)-[1,2,4]triazolo[3,4-b][1,3,4] thiadiazole (P_6)

FT-IR (cm^{-1}): 3403 (N-H-str), 3102 (aromatic C-H-str), 2964 (aliphatic C-H-str), 1603 (C=N), 1483 (C=C);^{1}H NMR (DMSO-d_6): δ 0.92 (t, 3H, *J*=7.6 Hz, CH_3), 1.75 (m, 2H, CH_2), 2.91 (t, 2H, *J*=7.4 Hz, CH_2), 7.74-7.77 (m, 2H, Ar-H), 7.29-7.33 (t, 2H, J_{ortho}= 8.6 Hz, Ar-H), 8.46 (s, 1H, pyrazole-5H), 13.71 (s, 1H, Pyrazole N-H). MS: m/z = 329 (M+1). Anal. calcd. for $C_{15}H_{13}FN_6S$: C, 54.87; H, 3.99; N, 25.59. Found: C, 54.82; H, 3.94; N, 25.56 %.

3- propyl-6-(3-(4-methoxyphenyl)-1*H*-pyrazol-4-yl)-[1,2,4]triazolo[3,4-b][1,3,4] thiadiazole (P_7)

FT-IR (cm^{-1}): 3360 (N-H-str), 3081 (aromatic C-H-str), 2951 (aliphatic C-H-str), 1611 (C=N), 1463 (C=C); ^{1}H NMR (DMSO-d_6): δ 0.94 (t, 3H, *J*=7.6 Hz, CH_3), 1.78 (m, 2H, CH_2), 2.93 (t, 2H, *J*=7.4 Hz, CH_2), 3.82 (s, 3H, -OCH_3), 7.03-7.06 (d, 2H, *J*= 8.8 Hz, Ar-H), 7.61-7.63 (d, 2H, *J*=8.8 Hz, Ar-H), 8.32 (s, 1H, pyrazole-5H), 13.68 (s, 1H, Pyrazole N-H). MS: m/z = 341 (M+1). Anal. calcd. for $C_{16}H_{16}N_6OS$: C, 56.45; H, 4.74; N, 24.69. Found: C, 56.41; H, 4.78; N, 24.72 %.

3- propyl-6-(3-(4-chlorophenyl)-1*H*-pyrazol-4-yl)-[1,2,4]triazolo[3,4-b][1,3,4] thiadiazole (P_8)

FT-IR (cm^{-1}): 3391 (N-H-str), 3088 (aromatic C-H-str), 2928 (aliphatic C-H-str), 1566 (C=N), 1476 (C=C); ^{1}H NMR (DMSO-d_6): δ 0.92 (t, 3H, *J*=7.4 Hz, CH_3), 1.74 (m, 2H, CH_2), 2.90 (t, 2H, *J*=7.4 Hz, CH_2), 7.52-7.53 (d, 2H, *J*=7.2 Hz, Ar-H), 7.73-7.75 (d, 2H, *J*=8.4 Hz, Ar-H), 8.57 (s, 1H, pyrazole-5H), 13.80 (s, 1H, Pyrazole N-H). MS: m/z = 345 (M+1), 347 (M+2). Anal. calcd. for $C_{15}H_{13}ClN_6S$: C, 52.25; H, 3.80; N, 24.37. Found: C, 52.29; H, 3.77; N, 24.33 %.

3-ethyl-6-(3-(2,4-dichlorophenyl)-1*H*-pyrazol-4-yl)-[1,2,4]triazolo[3,4-b] [1,3,4] thiadiazole (P_9)

FT-IR (cm^{-1}): 3320 (N-H-str), 3097 (aromatic C-H-str), 2926 (aliphatic C-H-str), 1582 (C=N), 1479 (C=C); ^{1}H NMR (DMSO-d_6): δ 1.20 (t, 3H, *J*=7.6 Hz,CH_3), 2.89 (q, 2H, *J*=7.6 Hz, CH_2), 7.59-7.81 (m, 3H, Ar-H), 8.62 (s, 1H, pyrazole-5H). ^{13}C NMR: 158.8, 152.5, 148.6, 135.5, 134.9, 134.1, 129.6, 128.1, 111.2, 18.5, 11.01. MS: m/z = 365 (M^+), 367 (M+2), 369 (M+4). Anal. calcd. for $C_{14}H_{10}Cl_2N_6S$: C, 46.04; H, 2.76; N, 23.01. Found: C, 46.07; H, 2.71; N, 23.08 %.

3- propyl-6-(3-(2,4-dichlorophenyl)-1*H*-pyrazol-4-yl)-[1,2,4]triazolo[3,4-b] [1,3,4] thiadiazole (P_{10})

FT-IR (cm^{-1}): 3325 (N-H-str), 3099 (aromatic C-H-str), 2875 (aliphatic C-H-str), 1583 (C=N), 1472 (C=C); ^{1}H NMR (DMSO-d_6): δ 0.86 (t, 3H, *J*=7.2 Hz, CH_3), 1.62 (m, 2H, CH_2), 2.82 (t, 2H, *J*=7.6 Hz, CH_2), 7.59-7.81 (m, 3H, Ar-H), 8.64 (s, 1H, pyrazole-5H), 13.79 (s, 1H, Pyrazole N-H). ^{13}C NMR: 158.8, 152.4, 147.6, 134.9, 134.0, 129.5, 128.0, 111.1, 26.7, 19.9, 13.8. MS: m/z = 379 (M^+), 381 (M+2), 383 (M+4). Anal. calcd. for $C_{15}H_{12}Cl_2N_6S$: C, 47.50; H, 3.19; N, 22.16. Found: C, 47.47; H, 3.14; N, 22.19 %.

2.4 PHARMACOLOGY

2.4.1 Antimicrobial activity

Antimicrobial studies have been carried out at Department of Biochemistry, Kuvempu University, Shimoga, India. The following bacteria and fungi were used for the experiment. Bacteria: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853. All bacterial strains were maintained on nutrient agar medium at ± 37 ˚C. Fungi: *Aspergillus flavus* ATCC 15547, *Chrysosporium keratinophilum* ATCC 14803 and *Candida albicans* MTCC 227 are used in this study. All fungi strains were maintained on potato dextrose agar (PDA) at ± 25 ˚C. These cultures were obtained from the Department of Microbiology, Kuvempu University, Shimoga, India.

Antibacterial activity

The antibacterial activity of newly synthesized compounds **(P_{1-10})** was determined by "well-plate" method in Mueller-Hinton Agar (Rocha et al. 1995;

Arthington-Skaggs et al. 2000). The compounds were tested against a panel of pathogenic microorganisms, including *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Microorganism strains were maintained on nutrient agar medium at 37 ˚C. The cultures were inoculated in fresh 10 mL nutrient broth to yield an initial suspension of approximately 10-100 cfu/mL. All broths were then incubated statically at the aforementioned temperatures for microorganisms, for 18-24 h so that all cells were in the stationary phase. Susceptibility of the test organism to the compounds was determined by employing in the "well-plate" technique. The bacterial suspensions were diluted tenfold in distilled water, and 0.1 mL from the appropriate dilution was spread plated on nutrient agar in order to give a population of approximately 10^6 cfu/plate. Twenty milliliters of agar media was poured into each petri dish. Excess of suspension was decanted and plates were dried by placing in incubator at 37 ˚C for 1 h. Six millimeter diameter well were then punched carefully using a sterile cork borer and 30 µL of test solutions of different concentrations (1 mg/mL and 0.5 mg/mL) were added into each labeled well. The same procedure was repeated for different micro-organisms. Each experiment was carried out in triplicate. After the inoculation of organism and compound, the Petri plates were incubated for 24 h at 37 ˚C. After the incubation, the inhibition zone was measured and the values for Dimethylsulphoxide (DMSO) were subtracted to get the actual values. Streptomycin was used as standard drug.

Antifungal activity

Antifungal studies of newly synthesized compounds **(P_{1-10})** were carried out against *Aspergillus flavus*, *Chrysosporium keratinophilum* and *Candida albicans*. Sabourands agar media was prepared by dissolving peptone (10 g), D-glucose (40 g) and agar (20 g) in distilled water (1000 mL) and adjusting the pH to 5.7. Normal saline was used to make a suspension of spore of fungal strains for lawning. A loopful of particular fungal strain was transferred to 3 mL saline to get a suspension of corresponding species. Twenty milliliters of agar media was poured into each petri dish. Excess of suspension was decanted and plates were dried by placing in incubator at 37 ˚C for 1 h. Six millimeter diameter well were then punched carefully using a sterile cork borer and 30 µL of test solutions of different concentrations (1 mg/mL and 0.5 mg/mL) were added into each labeled well. A control was also

prepared for the plates in the same way using DMSO solvent. The Petri dishes were prepared in triplicate and maintained at 25 ˚C for 72 h. Antifungal activity was determined by measuring the diameter of inhibition zone. Activity of each compound was compared with Fluconazole as standard (MacLowry et al. 1970 and Portillo et al. 2001).

2.4.2 Anti-inflammatory activity

Screening of anti-inflammatory drugs by Carrageenan induced paw edema method (Hu et al. 2008 and Maria et al. 2008)

The anti-inflammatory activity of the test compounds was carried out using Carrageenan-induced rat paw edema model according to Winter et al. (1962) by employing 1% Carrageenan solution as the phlogistic agent. Edema was induced in the left hind paw of Wistar rats (200-250 g) by the sub-plantar injection of 0.1 mL of 1% Carrageenan in distilled water. Both sexes were used. Each group composed of six animals. The animals which were bred in the laboratory were housed under standard conditions and received a diet of commercial food pellets and water *ad libitum* during the maintenance but they were entirely fasted during the experiment period. The study was carried out at Department of Pharmacology, Nitte Gulabi Shetty Memorial College of Pharmacology, Deralakatte, Mangalore, India. The study was approved by the Institutional Ethics Committee for animal experimentation KSHEMA, Deralakatte, Mangalore, India. The studies were conducted in accordance with recognized guidelines on animal experimentation.

The test compounds were given intraperitoneally 30 min after Carrageenan injection. The paw volume was measured plethysmometrically at 0 and 3 h after the carrageenan injection. The difference in the paw volume of the injected and the control were compared for each animal. The percentage inhibition of edema was calculated using the formula,

$$\% \text{ Edema inhibition} = 100 - (V_{test} / V_{control}) \times 100$$

Statistical analysis

All experimental groups were composed of 6 animals. Data obtained from animal experiments were expressed as mean ± standard error (S.E.M.). The statistical significance of difference between groups were assessed by means of analysis of variance (ANOVA) followed by Dunnet's test.

2.4.3 Molecular docking studies

The ligands were drawn in ChemDraw Ultra 6.0 (Chem Office package) assigned with proper 2D orientation and the structure of each compound was analyzed for connection error in bond order. OSIRIS, an ADMET based Java library layer that provides reusable cheminformatics functionality and is an entirely in-house developed drug discovery informatics system was used to predict the total drug score via *in silico* (Sander et al. 2009). Energy of the molecules was minimized using Dundee PRODRG2 server (Schuttelkopf et al. 2004). The energy minimized compounds were then read as input for AutoDock 4.2, in order to carry out the docking simulation (Morris et al. 2009). All the heteroatoms were removed from the 1PXX.pdb, to make complex less receptor free of any ligand before docking. The Graphical User Interface program "AutoDock Tools" was used to prepare, run, and analyze the docking simulations. Kollman united atom charges, solvation parameters and polar hydrogen's were added to the receptor for the preparation of protein in docking simulation. Since ligands are not peptides, Gasteiger charge was assigned and then non-polar hydrogens were merged. AutoDock requires pre-calculated grid maps, one for each atom type, present in the ligand being docked and its stores the potential energy arising from the interaction with macromolecule. This grid must surround the region of interest (active site) in the macromolecule. In the present study, the binding site was selected based on the amino acid residues, which are involved in binding with Diclofenac of COX-2 as obtained from PDB with ID 1PXX which would be considered as the probable best accurate region as it is solved by experimental crystallographic data (Rowlinson et al. 2003). Therefore, the grid was centered at the region including all the ten amino acid residues (Val349, Ser530, Leu352, Tyr385, Tyr348, Trp387, Gly526, Ala527, Met522 and Leu384) that surround active site. The grid box size was set at 56, 44, and 54 A˚ for x, y and z respectively, and the grid center was set to 26.472, 25.806 and 12.52 for

x, y and z respectively, which covered all the ten amino acid residues in the considered active pocket. AutoGrid 4.0 Program, supplied with AutoDock 4.0 was used to produce grid maps. The spacing between grid points was 0.375 angstroms. The Lamarckian Genetic Algorithm (LGA) was chosen to search for the best conformers. During the docking process, a maximum of ten conformers was considered for each compound. All the AutoDock docking runs were performed in Intel CentrinoCore2Duo CPU @ 2.20 GHz of IBM system origin, with 2 GB DDR RAM. AutoDock 4.0 was compiled and run under Windows XP Service Pack 3 operating system.

2.5 RESULTS AND DISCUSSION

2.5.1 Chemistry

The structures of the synthesized compounds (**P_{1-10}**) were characterized by IR, NMR, mass spectral and elemental analyses. The reaction between **4a-e** and **5a-b** resulted in the formation of cyclized products **P_{1-10}**. The absence of NH_2 (δ 5.6) and SH (δ 13.4) peaks in ^{1}H-NMR spectra of **P_{1-10}** which were present in **5a-b** can be attributed for the involvement of these functional groups in the formation of cyclized products and also cyclisation was further confirmed by the absence of NH_2 (3260 cm^{-1}) absorption band in the IR spectrum of **P_{1-10}**. IR spectrum of **P_1** exhibited bands at 3390, 3066, 2909, 1604 and 1481 cm^{-1} which corresponds to N-H, aromatic C-H, aliphatic C-H, C=N and C=C respectively. The ^{1}H-NMR of **P_1** showed a singlet at δ 13.7 and 8.43 which were due to NH and 5H proton of pyrazole ring. A multiplet was observed between δ 7.71-7.49 which corresponds to Ar-H. Aliphatic protons which resonated as quartet and triplet at δ 2.98 and 1.30 can be assigned to -CH_2-CH_3 respectively. The mass spectrum of **P_1** showed molecular ion peak at m/z = 297 (M+1), which is in agreement with the molecular formula $C_{14}H_{12}N_6S$. Similarly, the spectral values for all the compounds and C, H, N analyses are given in the experimental part. Among the synthesized compounds the structure of **P_2** was confirmed by single crystal X-ray analysis as well (Fun et al. 2010). The compound **P_2** (**Figure 2.6**) consists of a fluorophenyl ring (F1/C1-C6), a pyrazole ring (N1/N2/C7/C8/C9) and a 3-ethyl-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole moiety (S1/N3-N6/C10-C14). The [1,2,4] triazolo[3,4-b][1,3,4] thiadiazole ring system is essentially planar (maximum

deviation = 0.022 (3) Å for atom N4) and is inclined at angles of 15.00 (18) and 52.82 (16)˚ with respect to the pyrazole and phenyl rings. Bond lengths and angles are within normal ranges. The characterization data of compounds $\mathbf{P_{1\text{-}10}}$ and crystallographic data of $\mathbf{P_2}$ have been provided in **Table 2.1** and **Table 2.2** respectively.

Table 2.1 Characterization data of the compounds $\mathbf{P_{1\text{-}10}}$

Compounds	R^1	R	Molecular formula (Mol.Wt)	Yield (%)	M.p. (˚C)
$\mathbf{P_1}$	C_2H_5	H	$C_{14}H_{12}N_6S$ (296)	68	217-219
$\mathbf{P_2}$	C_2H_5	4-F	$C_{14}H_{11}FN_6S$ (314)	74	206-207
$\mathbf{P_3}$	C_2H_5	4-OCH_3	$C_{15}H_{14}N_6OS$ (326)	65	222-224
$\mathbf{P_4}$	C_2H_5	4-Cl	$C_{14}H_{11}ClN_6S$ (330)	72	290-293
$\mathbf{P_5}$	C_3H_7	H	$C_{15}H_{14}N_6S$ (310)	56	219-221
$\mathbf{P_6}$	C_3H_7	4-F	$C_{15}H_{13}FN_6S$ (328)	61	187-189
$\mathbf{P_7}$	C_3H_7	4-OCH_3	$C_{16}H_{16}N_6OS$ (340)	67	213-215
$\mathbf{P_8}$	C_3H_7	4-Cl	$C_{15}H_{13}ClN_6S$ (344)	69	220-222
$\mathbf{P_9}$	C_2H_5	2,4-Cl	$C_{14}H_{10}Cl_2N_6S$ (365)	71	200-202
$\mathbf{P_{10}}$	C_3H_7	2,4-Cl	$C_{15}H_{12}Cl_2N_6S$ (379)	77	203-205

2.5.2 Biological activity

The newly synthesized compounds $\mathbf{P_{1\text{-}10}}$ were tested for their antibacterial activity (*in vitro)* against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* and their activity was compared to a well-known commercial antibiotic, Streptomycin. Antibacterial activity was carried out by "well-plate" method by measuring its zone of inhibition. Antibacterial results revealed that the compounds $\mathbf{P_2}$, $\mathbf{P_3}$, $\mathbf{P_4}$, $\mathbf{P_6}$, $\mathbf{P_7}$, $\mathbf{P_8}$ exhibited good antibacterial activity, which might be possibly due to the presence of electron releasing ability of alkyl chain to condensed nucleus. Apart from alkyl chain, these compounds contain substituents such as 4-fluorophenyl, 4-methoxyphenyl and 4-chlorophenyl which may be another reason for their enhanced

activity. The absence of such pharmocophore in compounds **P_1** and **P_5** failed to exhibit antibacterial activity.

Table 2.2 Crystallographic data for compound **P_2**

Crystal Data	**P_2**
Emperical formula	$C_{14}H_{11}FN_6S$
Formula weight	314.35
Temperature (K)	100
Wavelength (Å)	0.71073
Crystal system	Orthorhombic
Space group	*Pca*2_1
Cell dimensions	
a (Å)	35.053 (2)
b (Å)	3.8463 (2)
c (Å)	9.9482 (6)
α (˚)	90
β (˚)	90
γ (˚)	90
Volume (Å^3)	1341.26
Z	4
Density (Mg m^{-3})	1.557
F (000)	648
Θ range for data collection (˚)	2.3-30.0

All the synthesized compounds were also tested for its antifungal activity (*in vitro)* against *Aspergillus flavus*, *Chrysosporium keratinophilum* and *Candida albicans* by measuring its average zone of inhibition. Antifungal activity result reveals that among the tested compounds, **P_2** and **P_6** showed good antifungal activity against *Aspergillus flavus* and *Chrysosporium kerantinophilum*. Remaining compounds showed poor activity against all the tested fungal strains. The activity exhibited by the compounds **P_2** and **P_6** may be attributed to the presence of 4-fluorophenyl substituent on pyrazole ring. Further, the introduction of substitutions such as chloro and 2,4-dichloro on phenyl ring of pyrazole produced a decrease in antifungal activity. In addition, the

presence of electron donating group i.e. 4-methoxy substituent failed to exhibit antifungal activity in compounds **P_3** and **P_7**. The results of antibacterial and antifungal studies are shown in **Table 2.3** and **Table 2.4** respectively.

Table 2.3 Antibacterial activity of compounds **P_{1-10}**

Compound code	**Zone of inhibition (mm)**					
	Escherichia coli		***Staphylococcus aureus***		***Pseudomonas aeruginosa***	
Conc. in µg/mL	1000	500	1000	500	1000	500
P_1	00	00	00	00	00	00
P_2	14±0.1	09±0.2	12±0.2	08±0.1	13±0.2	09±0.1
P_3	13±0.1	08±0.1	11±0.1	07±0.2	14±0.2	10±0.2
P_4	13±0.1	09±0.2	13±0.1	08±0.2	13±0.1	07±0.1
P_5	00	00	00	00	00	00
P_6	13±0.2	07±0.2	12±0.1	07±0.2	12±0.2	08±0.2
P_7	12±0.1	06±0.2	12±0.1	08±0.2	11±0.2	07±0.1
P_8	12±0.1	07±0.2	11±0.1	08±0.1	12±0.2	08±0.2
P_9	04±0.1	02±0.1	05±0.2	03±0.1	04±0.2	02±0.1
P_{10}	03±0.1	01±0.1	04±0.2	02±0.2	04±0.1	02±0.1
Streptomycin (Std.)	16±0.2	10±0.1	15±0.2	10±0.2	16±0.2	13±0.2

The synthesized compounds were also screened for anti-inflammatory activity by Carrageenan induced paw edema method. Among the screened compounds, **P_8** showed significant anti-inflammatory activity at percentage inhibition of 64.7, compared to the standard drug Diclofenac sodium which showed the percentage inhibition at 80.4. Similarly compounds **P_4** and **P_5** have showed percentage inhibition of 56.9. However remaining compounds showed poor anti-inflammatory activity. Results of anti-inflammatory activity have been presented in **Table 2.5**.

The significant contribution of compound **P_8** might be possibly due to the presence of p-chlorophenyl substituent on third position of pyrazole ring and propyl chain on triazole moiety. Similarly, presence of ethyl chain with p-chlorophenyl group in compound **P_4** and propyl chain with phenyl group as substituent in compound **P_5** can be attributed for its moderate activity.

Table 2.4 Antifungal activity of compounds $\mathbf{P_{1\text{-}10}}$

Compound code	Zone of inhibition (mm)					
	Aspergillus flavus		***Chrysosporium keratinophilum***		***Candida albicans***	
Conc. in µg/mL	1000	500	1000	500	1000	500
$\mathbf{P_1}$	02±0.1	01±0.2	04±0.1	02±0.1	05±0.1	04±0.1
$\mathbf{P_2}$	10±0.2	07±0.1	12±0.1	09±0.2	06±0.1	03±0.1
$\mathbf{P_3}$	00	00	00	00	00	00
$\mathbf{P_4}$	03±0.1	02±0.2	04±0.1	03±0.1	05±0.1	03±0.1
$\mathbf{P_5}$	02±0.1	01±0.1	02±0.2	00	03±0.1	00
$\mathbf{P_6}$	09±0.2	06±0.1	13±0.1	08±0.1	03±0.1	01±0.1
$\mathbf{P_7}$	00	00	00	00	00	00
$\mathbf{P_8}$	03±0.1	01±0.2	03±0.1	02±0.1	04±0.2	01±0.1
$\mathbf{P_9}$	05±0.1	03±0.1	04±0.1	02±0.1	06±0.1	02±0.1
$\mathbf{P_{10}}$	04±0.1	02±0.1	05±0.1	02±0.1	04±0.1	02±0.1
Fluconazole (Std.)	13±0.2	10±0.1	17±0.2	15±0.2	22±0.2	20±0.2

2.5.3 Molecular docking studies

Considering the well obtained *in vitro* results, it was thought worthy to perform molecular docking studies, screening for supportive coordination between *in silico* studies with *in vitro* results. Considering Cyclooxygenase-2 (COX-2) as the target receptor, comparative and automated docking studies with newly synthesized candidate lead compounds was performed to determine the best *in silico* conformation. The Lamarckian genetic algorithm, inculcated in the docking program AutoDock 4.2, was employed for the purpose. **Figure 2.8** shows the native crystal structure of Diclofenac bound to the Cyclooxygenase active site of COX-2 obtained from Protein Data Bank (http://www.pdb.org/pdb/home/home.do) with the PDB ID 1PXX (Rowlinson et al. 2003). The docking of receptor COX-2 with newly synthesized candidate ligands exhibited well established bonds with one or more amino acids in the receptor active pocket. Docking studies were performed for compounds $\mathbf{P_4}$, $\mathbf{P_5}$, $\mathbf{P_8}$ & Diclofenac. The active pocket was considered to be the site where Diclofenac was complexed in COX-2 of 1PXX. The active pocket consisted of

10 amino acid residues as Val349, Ser530, Leu352, Tyr385, Tyr348, Trp387, Gly526, Ala527, Met522 and Leu384 as shown in **Figure 2.9**. The synthesized ligand molecules having 2D structure were converted to energy minimized 3D structures and were further used for *in silico* protein-ligand docking. **Figure 2.10** shows the images of ligands docked separately to COX-2 including the considered standard Diclofenac. **Table 2.6** shows the Binding energy and Inhibition constant of all the four compounds including standard Diclofenac.

Table 2.5 Anti-inflammatory activity of compounds $\mathbf{P_{1\text{-}10}}$

Treatment	Dose (mg/kg)	Initial paw volume (mL) (b)	Paw volume after 3 h (mL) (a)	Edema volume (a-b)	Percentage inhibition
Control	vehicle	0.93±0.01	2.20±0.04	1.27±0.02	
Diclofenac	10	0.80±0.04	1.05±0.02	0.25±0.04	80.4**
$\mathbf{P_1}$	20	0.8±0.08	1.78±0.01	0.90±0.09	29.4*
$\mathbf{P_2}$	20	0.85±0.04	1.83±0.06	0.98±0.02	23.5*
$\mathbf{P_3}$	20	0.85±0.01	1.90±0.08	1.05±0.01	17.6
$\mathbf{P_4}$	20	0.83±0.09	1.38±0.02	0.55±0.04	56.9**
$\mathbf{P_5}$	20	0.85±0.02	1.40±0.04	0.55±0.08	56.9**
$\mathbf{P_6}$	20	0.93±0.09	2.00±0.09	1.05±0.04	12.0
$\mathbf{P_7}$	20	0.95±0.04	2.38±0.01	0.93±0.02	13.5
$\mathbf{P_8}$	20	0.85±0.01	1.28±0.04	0.45±0.08	64.7**
$\mathbf{P_9}$	20	0.98±0.09	1.93±0.02	0.95±0.01	25.5*
$\mathbf{P_{10}}$	20	0.83±0.08	1.80±0.02	0.98±0.09	23.5*

*= $p<0.05$ compared to control ** = $p<0.01$ compared to control

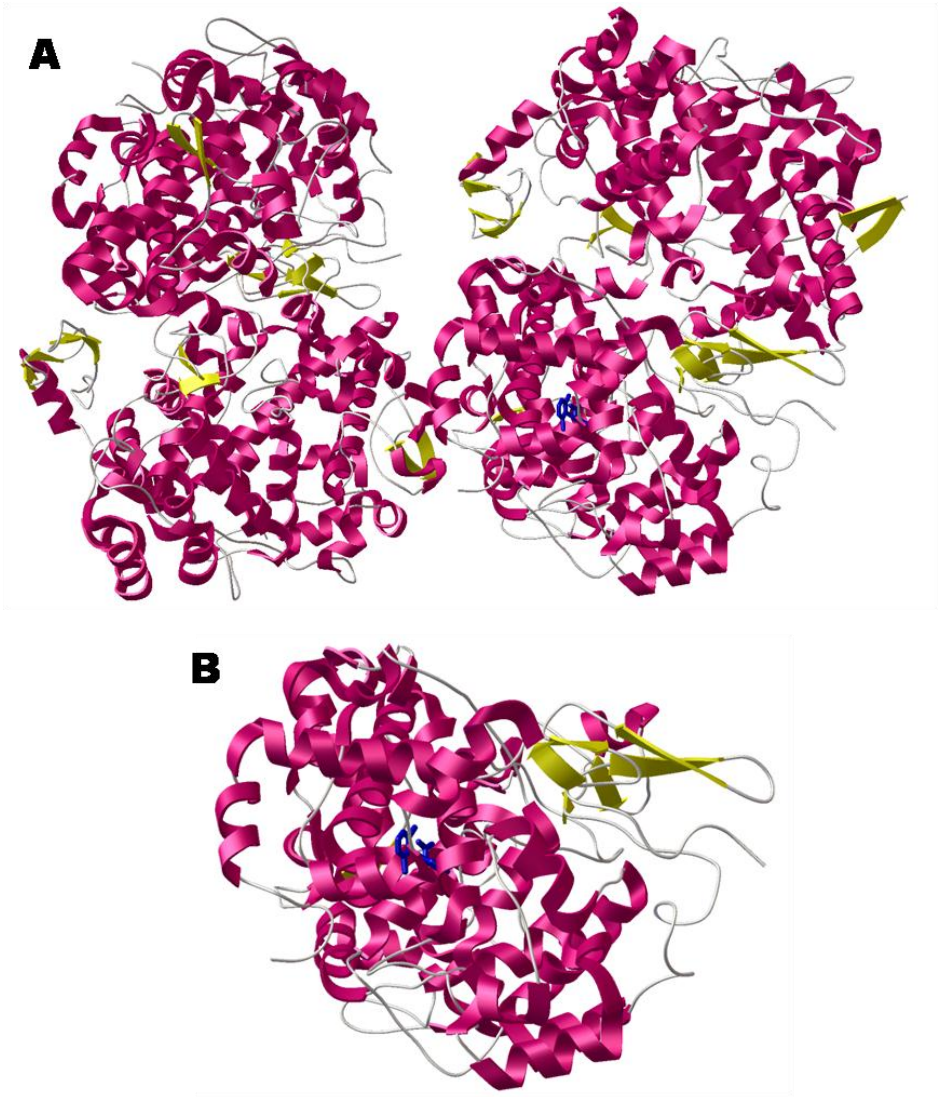


Figure 2.8 A) Secondary structure of COX-2 (PDB ID 1PXX) complexed with Diclofenac (complete protein), showing its dimeric nature with two identical subunits in each monomer. B) Chain A (part of complete protein) complexed with Diclofenac.

Molecular docking studies revealed that all three synthesized molecules showed good binding energy towards the target protein ranging from -9.1 kJ $mol^{-1}$to-8.76 kJ mol^{-1}. Finally considering both *in vitro* and *in silico* molecular docking results, among the synthesized molecules **P_8** showed the best result and can be considered as the best inhibitor of COX-2.

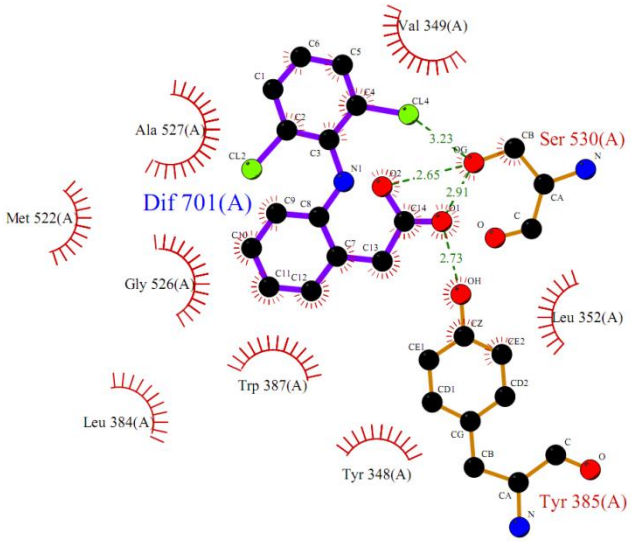


Figure 2.9 PDB sum's ligplot results for 1PXX, showing all 10 amino acid residues of active pocket.

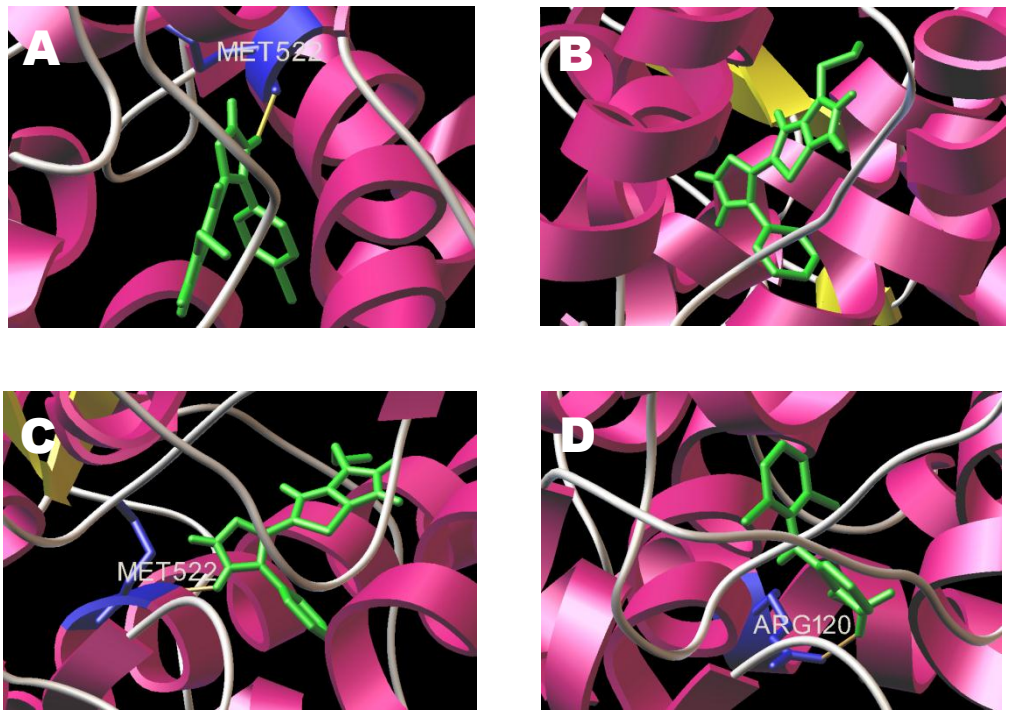


Figure 2.10 Showing all ligands docked in best of its conformation. A) $\mathbf{P_4}$ forming 1H bond with Met522. B) $\mathbf{P_5}$ forming no H bonds. C) $\mathbf{P_8}$ forming 1H bond with Met522. D) Diclofenac forming 1H bonds with Arg120.

Table 2.6 Binding Energy (kJ mol^{-1}) and Inhibition Constant of **P_4**, **P_5**, **P_8** including standard Diclofenac.

Compound	Binding Energy (kJ mol^{-1})	Inhibition Constant
P_4	-8.76	406.41 nM
P_5	-8.92	287.8 nM
P_8	-9.1	212.03 nM
Diclofenac	-7.49	3.22 µM

2.6 CONCLUSIONS

In the present study, a series of 3,6-disubstituted 1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazoles bearing pyrazole moiety were synthesized, characterized by spectral studies and screened for their preliminary antimicrobial and anti-inflammatory activities. Compound **P_2** was analyzed for its molecular structure by single crystal X-ray crystallography. Antimicrobial investigation revealed that the compounds **P_2**, **P_3**, **P_4**, **P_6**, **P_7**, **P_8** showed good antibacterial activity whereas compounds **P_2** and **P_6** showed good antifungal activity among the synthesized compounds when tested against different microbial strains. Further, anti-inflammatory studies revealed that compound **P_8** exhibited significant activity whereas **P_4** and **P_5** were found to be moderately active among the synthesized compounds. Docking studies were performed for compounds **P_4**, **P_5**, **P_8** & Diclofenac among which **P_8** showed the best result and can be considered as the best inhibitor of COX-2.

CHAPTER - 3

SYNTHESIS AND BIOLOGICAL EVALUATION OF NEWER ANALOGUES OF 2,5-DISUBSTITUTED 1,3,4-OXADIAZOLE CONTAINING PYRAZOLE MOIETY AS ANTIMICROBIAL AGENTS

Abstract

This chapter describes a detailed literature survey on 1,3,4-oxadiazole and its derivatives. It also includes the synthesis and characterization of newly designed pyrazole containing 2,5-disubstitued 1,3,4-oxadiazole derivatives. Further, the antimicrobial activities of the synthesized compounds have been discussed.

3.1 INTRODUCTION

Oxadiazole is a five membered heterocyclic aromatic compound having two carbons, two nitrogen and one oxygen atoms with general formula $C_2H_2ON_2$. The four possible isomers of oxadiazole are 1,2,3-oxadiazole (**S-3.1)**, 1,2,4-oxadiazole **(S-3.2)**, 1,2,5-oxadiazole (**S-3.3)** and 1,3,4-oxadiazole **(S-3.4)**. Out of these 1,3,4-oxadiazoles are found to be biologically most potent.

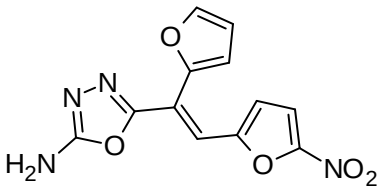
S-3.1

S-3.2

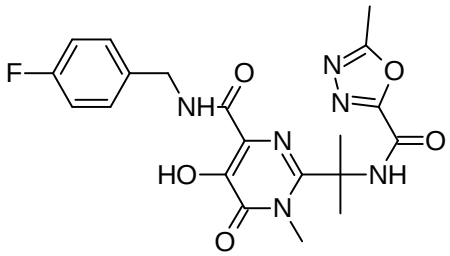
S-3.3

S-3.4

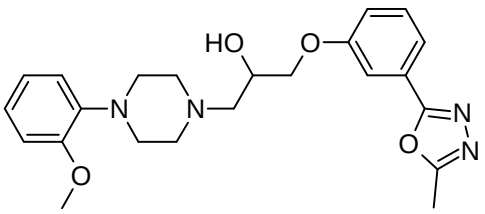
The capacity of 1,3,4-oxadiazole nucleus to undergo variety of chemical reaction have made it medicinal backbone on which number of potential molecules can be constructed. A few therapeutic agents available in the market such as Fumarizole (**S-3.5)**, Raltegravir (**S-3.6)**, Nesapidil (**S-3.7)**, Tiodazosin (**S-3.8)** etc posses 1,3,4-oxadiazole nucleus.



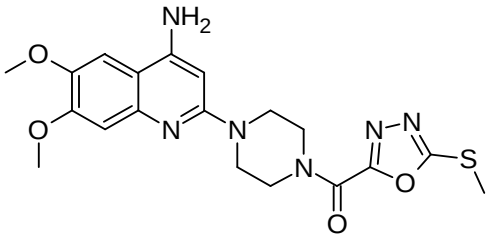
S-3.5



S-3.6



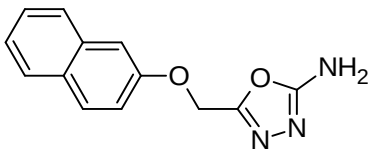
S-3.7



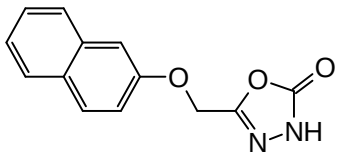
S-3.8

2,5-Disubstituted 1,3,4-oxadiazoles display a wide spectrum of activities such as anti-inflammatory (Silvestrini and Pagatti 1961), antimalarial (Hutt et al. 1970), antifungal (Sharma and Bahel 1982) anticonvulsant (Omar et al. 1984) and antibacterial (Jain et al. 2009; Chandrakantha et al. 2010). Substituted 1,3,4-oxadiazoles are of considerable pharmaceutical and material interest, which is documented by a steadily increasing number of publications and patents. For instance, 2-amino-1,3,4-oxadiazoles act as muscle relaxant (Yale and Losee 1966) and exhibit antimitotic activity. Analgesic, anti-inflammatory, anticonvulsive, diuretic and antiemetic properties are exhibited by 5-aryl-2-hydroxymethyl-1,3,4-oxadiazole derivatives (Adelstein et al. 1976).

Sahin et al. (2002) reported the synthesis of two series of 1,3,4-oxadiazole derivatives. The antimicrobial properties of the compounds were investigated against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* bacterial strains and against fungal strains such as *Candida albicans*, *Candida krusei* and *Candida parapsilosis.* Among the synthesized compounds, **S-3.9** and **S-3.10** exhibited significant activity against *Candida krusei.*

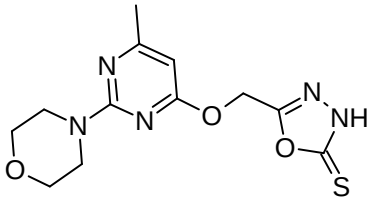


S-3.9

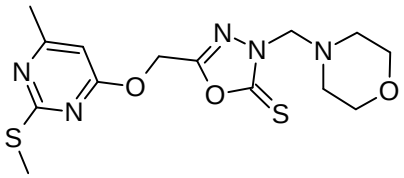


S-3.10

The synthesis of 5-(6-methyl-2-substituted 4-pyrimidinyloxymethyl)-2,3-dihydro-1,3,4-oxadiazole-2-thiones and their 3-morpholinomethyl derivatives were described by Jakubkiene and his co-workers (2003). Anti-inflammatory activity was carried out for the synthesized compounds and the results indicated that compounds **S-3.11, S-3.12** were the most active of all the tested ones.

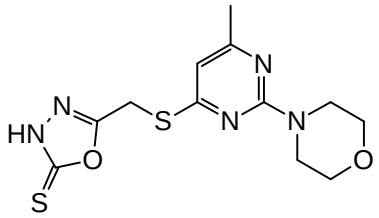


S-3.11



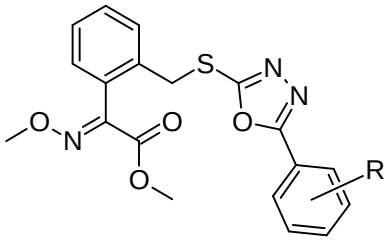
S-3.12

Burbuliene et al. (2004) described the synthesis and anti-inflammatory activity of derivatives of 5-[(2-disubstitutedamino-6-methyl-pyrimidin-4-yl)-sulfanylmethyl]-3H-1,3,4-oxadiazole-2-thiones. The results showed that compound **S-3.13**, containing morpholinyl substituent in the second position of pyrimidine ring, was found to be most potent among the series.



S-3.13

Li et al. (2006) described the stereoselective synthesis and fungicidal activities of (*E*)-α-(methoxyimino)-benzeneacetate derivatives containing 1,3,4-oxadiazole ring (**S-3.14)**. The results revealed that all the tested compounds exhibited potent fungicidal activity against *Rhizoctonia solani*, *Botrytis cinereapers*, *Gibberella zeae*, *Physalospora piricola* and *Bipolaris mayclis*.

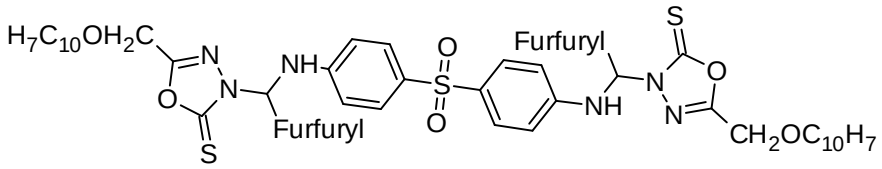


S-3.14

Where R = H, 4-MeO, 4-C_6H_5-CH_2O, 3-Cl, 2,3-Cl_2, 2,4-Cl_2, 2,5-Cl_2, 2,4-Cl_2-5-F, 2-F, 4-F, 2-F-4-Br, 2,3-F_2, 4-CF_3, 2-I, 4-NO_2

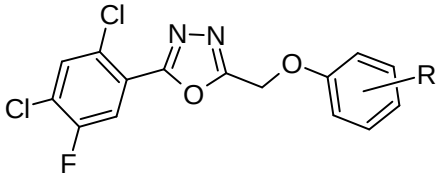
Ali and Shaharyar (2007) synthesized a series of oxadiazole Mannich bases by reacting oxadiazole derivatives, dapsone and appropriate aldehyde in the presence of methanol. Compound **S-3.15** was found to be the most promising compound active

against *Mycobacterium tuberculosis* H37Rv and isoniazid (INH) resistant *Mycobacterium tuberculosis.*

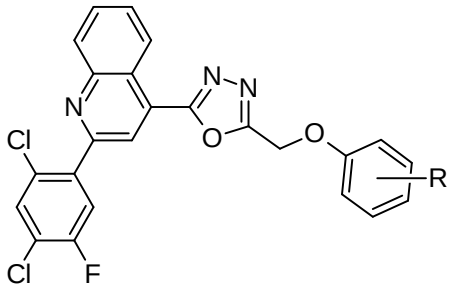


S-3.15

Karthikeyan et al. (2008) synthesized a series of 2,4-dichloro-5-fluorophenyl bearing 1,3,4-oxadiazoles (**S-3.16 and S-3.17)** and studied their antimicrobial properties. The results showed that among the synthesized oxadiazoles, compounds with methyl, chloromethyl and dichloro substituents in the phenyl ring at fifth position of oxadiazoles were found to increase the antimicrobial activity.



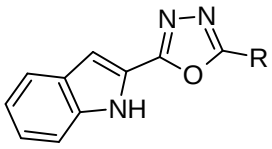
S-3.16



S-3.17

Where R = 4-CH_3, 2-CH_3, 2-Cl, 4-Cl-2-CH_3, 4-Cl-3-CH_3, 4-Cl, 2,4-Cl_2

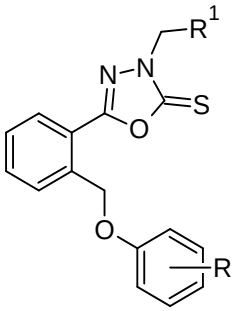
A series of diverse 5-(3-indolyl)-2-(substituted)-1,3,4-oxadiazoles (**S-3.18)** have been synthesized by Kumar et al. (2009) which represent a novel class of potent and selective anticancer agents. The SAR studies revealed that compounds either with 4-pyridyl or 3-pyridyl substitution were found to be potent and selective.



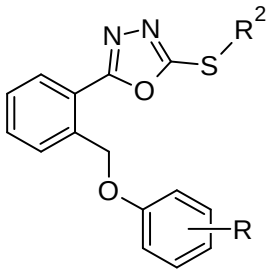
S-3.18

Where R = C_6H_5, CH_2-C_6H_5, 4-pyridyl, 3-pyridyl, 4-OCH_3-C_6H_4, 4-Cl-C_6H_4, 3,4-di-OCH_3-C_6H_3, 2,3,4-tri-OCH_3-C_6H_2, 4-BnO-3-CH_3O-C_6H_3, 4-BnO-3-CH_3-OC_6H_3, 4-HO-3-$CH_3OC_6H_3$

Naveena et al. (2010) synthesized some disubstituted 1,3,4-oxadiazoles carrying 2-(aryloxymethyl)phenyl moiety (**S-3.19 and S-3.20**) and carried out their antimicrobial activity. Some of the compounds showed good activity against tested microbial strains.



S-3.19



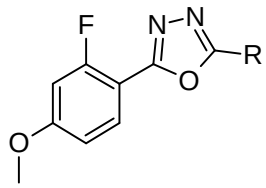
S-3.20

Where R = 2-CH_3, 3-CH_3, 4-CH_3, 4-Cl

R^1 = morpholine, -NH-C_6H_3-2-Cl-5-CH_3, -NH-C_6H_4-3-CF_3, -NH-C_6H_3-2-$COCF_3$-4-Cl

R^2 = ethyl, benzyl, 2,4-dichlorobenzyl, 4-Cl-benzyl, 3-CH_3-benzyl

New series of 1,3,4-oxadiazole derivatives containing 2-fluoro-4-methoxy moiety (**S-3.21**) were synthesized by Chandrakantha et al. (2010). The synthesized compounds were subjected for antimicrobial studies. Among the screened samples, compounds having 3-bromo-2-methyl phenyl group and 2,3,4-trifluoro phenyl group as substituents exhibited excellent antibacterial activity.

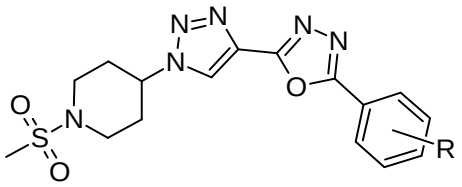


S-3.21

Where R = 2-F-5-OCH_3-C_6H_3, 2-CF_3-C_6H_4, 3-Br-2-CH_3-C_6H_3, 2,3,4-F_3-C_6H_2, 3-CN-C_6H_4,
2,3-di-CH_3-C_6H_3, 2-Cl-pyridyl, 2,3-F_2-6-NO_2-C_6H_2, 4-F-C_6H_4

Sangshetti et al. (2011) demonstrated the use of sodium bisulfite for the synthesis of 2,5-disubstituted 1,3,4-oxadiazole (**S-3.22**) from hydrazides and aromatic aldehydes using conventional as well as microwave synthesis in good yield. All the synthesized compounds were tested for *in vitro* antifungal activity. From the antifungal activity data, it was observed that compound with chloro and hydroxyl

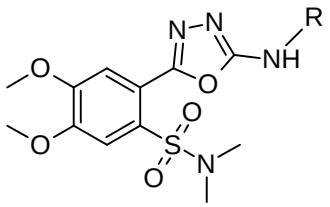
substituents are the most active among all tested compounds against most of the tested organisms.



S-3.22

Where R = H, OH, Cl

Zoumpoulakis et al. (2012) reported the synthesis of novel sulfonamide-1,2,4-triazoles, 1,3,4-thiadiazoles and 1,3,4-oxadiazoles (**S-3.23)**, as potential antibacterial and antifungal agents. The results indicated that increase of the length of the aliphatic chain, increases lipophilicity which is mandatory for antibacterial activity. More specifically, oxadiazole analogues with propyl and butyl chains exhibited the best MIC activity over most of the studied bacteria.



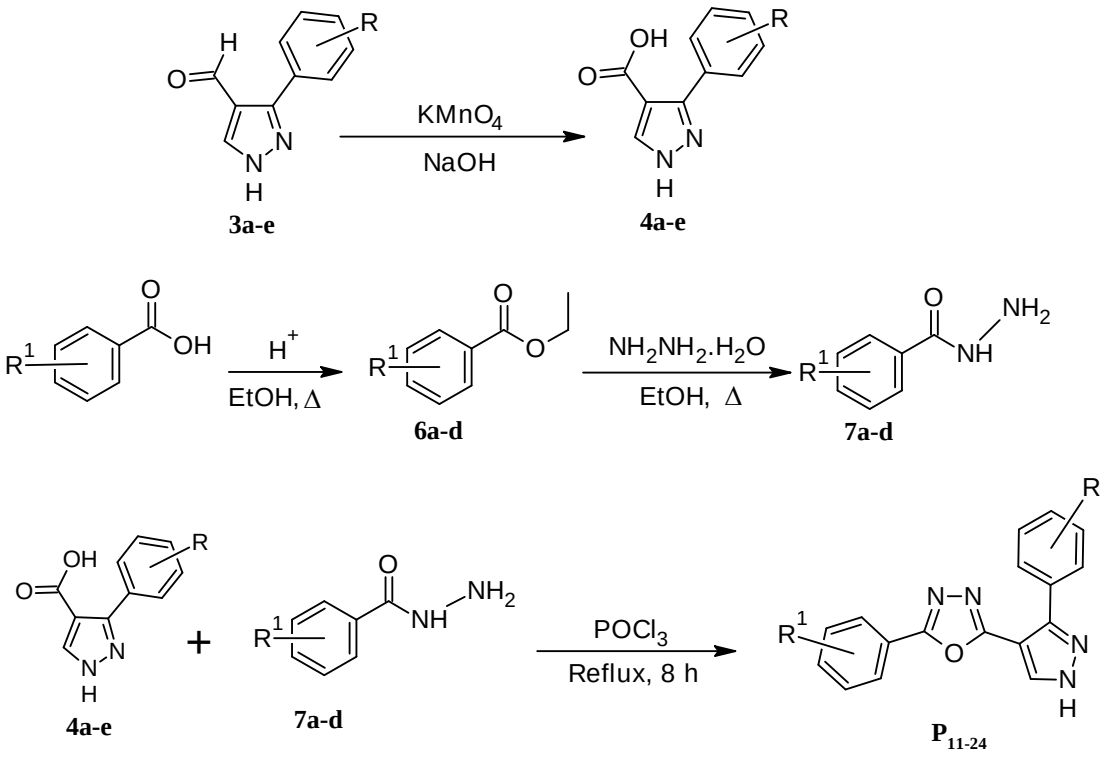
S-3.23

Where R = -$CH_2CH_2CH_3$, -$CH_2CH_2CH_2CH_3$

Literature review reveals that chemical modification of bioactive components is one of the most common approaches in drug discovery with improved therapeutic effect and the wide occurrence of nitrogen containing heterocycles in bioactive natural products and pharmaceuticals have made them important synthetic targets. In view of the potential biological activity of members of the 1,3,4-oxadiazole and pyrazole ring systems, it was planned to synthesize a series of new 2,5-disubstituted-1,3,4-oxadiazoles emphasizing, in particular, on the strategy of combining two chemically different but pharmacologically compatible heterocycles in a single frame work.

3.2 MATERIALS AND METHODS

In the current work, aromatic esters (**6a-d**) were synthesized from the appropriate aromatic acids by treating with ethanol in the presence of catalytic amount of Conc. sulphuric acid. Reaction of compound (**6a-d)** with hydrazine hydrate yielded corresponding acid hydrazides (**7a-d**) (Husain and Ajmal, 2009). Similarly, 3-substituted-pyrazole-4-carboxylic acids were synthesized as per the reported procedure (Lebedev et al., 2005) as discussed in **Chapter 2**. Subsequently condensation of substituted acid hydrazides (**7a-d**) with various 3-substituted-pyrazole-4-carboxylic acids **(4a-e)** in presence of phosphorous oxychloride afforded a series of 2,5-disubstituted-1,3,4-oxadiazoles $\mathbf{P_{11\text{-}24}}$. The synthetic route has been outlined in **Scheme 3.1**.



R = H, 4-OCH_3, 4-F, 4-Cl, 2,4-Cl
R^1= 4-Cl, 4-OCH_3, 2-Cl, 4-NO_2

Scheme 3.1 Synthetic route for 2,5-disubstituted-1,3,4-oxadiazole derivatives $\mathbf{P_{11\text{-}24}}$

All the laboratory grade reagents were obtained commercially. The reaction was monitored by thin layer chromatography, which was performed on Merck precoated plates (silica gel. 60 F_{254}, 0.25 mm) and was visualized by fluorescence quenching under UV light (254 nm). Melting points were determined by open capillary method. The IR spectra were recorded on a Thermo Nicolet avatar 330-FT-IR spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded (DMSO-d_6) on a Bruker (400 MHz & 100 MHz). Chemical shift values are given in δ scales. The mass spectra were recorded on LC-MS-Agilent 1100 series. Elemental analyses were performed on a Flash EA 1112 series CHNS-O Analyzer.

3.3 EXPERIMENTAL

The experimental protocols followed for the synthesis of compounds **$P_{11\text{-}24}$** is given in the following section.

The synthetic procedure for the synthesis of intermediate compounds **3a-e** has been discussed in **Chapter 2**.

General procedure for the synthesis of 3-(4-substitutedphenyl)-1*H*-pyrazole-4-carboxylic acid (4a-e)

3-Substituted- pyrazole-4-carbaldehydes (**3a-e**) (0.01 mol) was dissolved with stirring in a solution of 2g of NaOH in 40 mL of water. The mixture was cooled to 15˚C, and a solution of $KMnO_4$ (1.39 g, 0.0088 mol) in 40 mL of water was quickly added. The mixture was stirred for 30 min at 20 ˚C and then heated to 100 ˚C until the solution becomes completely decolorized. The solution was cooled and filtered to remove MnO_2 precipitate. Then the filtrate was acidified with Conc. HCl to pH 3. The resulting solid was filtered off, washed with water and dried (Lebedev et al. 2005).

General procedure for the synthesis of 2,5-disubstituted-1,3,4-oxadiazoles ($P_{11\text{-}24}$)

An equimolar mixture of respective substituted acid hydrazides **(7a-d)** (0.001 mol) and 3-(4-substituted phenyl-1H-pyrazole-4-carboxylic acids **(4a-e)** (0.001 mol) was dissolved in 5 mL of dry phosphorous oxychloride. The resulted solution was further refluxed for 8 h. Excess of phosphorous oxychloride was then distilled off and the mixture was gradually poured into crushed ice with stirring. The separated solid was filtered, washed thoroughly with cold water followed by 20 % $NaHCO_3$ solution and recrystallized from a mixture of DMF and water.

2-(4-chlorophenyl)-5-(3-phenyl-1*H*-pyrazol-4-yl)-1,3,4-oxadiazole (P_{11})

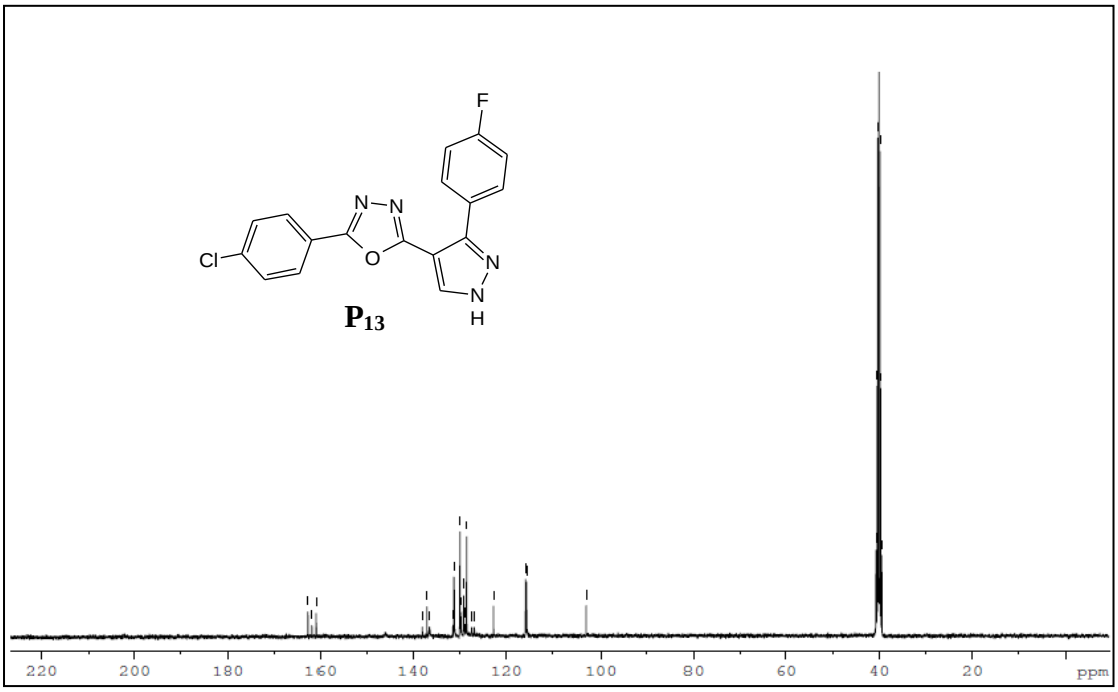
FT-IR (cm^{-1}): 3143 (N-H-str), 3053 (C-H-str), 1593 (C=N), 1531 (C=C), 1087 (C-O-C); ^1H NMR (DMSO-d_6): δ 13.79 (s,1H, pyrazole-NH), 8.7 (s, 1H,pyrazole-5H), 7.55-8.0 (m, 9H, Ar-H). MS: m/z = 321 (M-1). Anal. calcd. for $C_{17}H_{11}ClN_4O$: C, 63.26; H, 3.44; N, 17.36. Found: C, 63.23; H, 3.49; N, 17.30 %.

2-(4-chlorophenyl)-5-(3-(4-chlorophenyl)-1*H*-pyrazol-4-yl)-1,3,4-oxadiazole (P_{12})

FT-IR (cm^{-1}): 3160 (N-H-str), 3021 (C-H-str), 1603 (C=N), 1554 (C=C), 1090 (C-O-C); ^1H NMR (DMSO-d_6): δ 13.71 (s,1H, pyrazole-NH), 8.67 (s, 1H,pyrazole-5H), 7.52-8.23 (m, 8H, Ar-H). MS: m/z = 357 (M^+), 359 (M+2), 361 (M+4). Anal. calcd. for $C_{17}H_{10}Cl_2N_4O$: C, 57.16; H, 2.82; N, 15.69. Found: C, 57.11; H, 2.86; N, 15.65 %.

2-(4-chlorophenyl)-5-(3-(4-fluorophenyl)-1*H*-pyrazol-4-yl)-1,3,4-oxadiazole (P_{13})

FT-IR (cm^{-1}): 3182 (N-H-str), 3077 (C-H-str), 1609 (C=N), 1542 (C=C), 1089 (C-O-C); ^1H NMR (DMSO-d_6): δ 13.73 (s,1H, pyrazole-NH), 8.5 (s, 1H,pyrazole-5H), 7.32-7.98 (m, 8H, Ar-H). ^{13}C NMR: 162.59, 160.78, 136.97, 131.38, 131.30, 130.04, 129.87, 128.60, 122.78, 115.77, 115.55, 103.14 (**Figure 3.1**). MS: m/z = 341(M+1), 343 (M+2) (**Figure 3.2**). Anal. calcd. for $C_{17}H_{10}ClFN_4O$: C, 59.92; H, 2.96; N, 16.44. Found: C, 59.96; H, 2.90; N, 16.49 %.



P_{13}

Figure 3.1 ^{13}C NMR spectrum of compound **P_{13}**

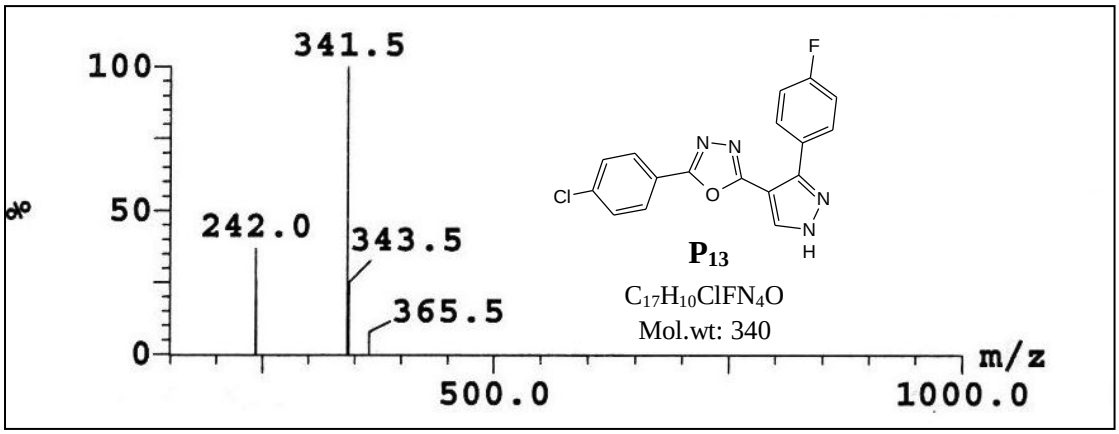


Figure 3.2 Mass spectrum of compound **P_{13}**

2-(4-chlorophenyl)-5-(3-(4-methoxyphenyl)-1*H*-pyrazol-4-yl)-1,3,4-oxadiazole (P_{14})

FT-IR (cm^{-1}): 3143 (N-H-str), 3014 (C-H-str), 1605 (C=N), 1516 (C=C), 1088 (C-O-C); ^{1}H NMR (DMSO-d_6): δ 13.76 (s,1H, pyrazole-NH), 8.39 (s, 1H,pyrazole-5H), 7.05-7.97 (m, 8H, Ar-H), 3.82 (s, 3H, -OCH_3). ^{13}C NMR: 162.5, 160.34, 136.92, 130.50, 130.12, 130.01, 129.87, 129.28, 128.56, 122.81, 122.63, 114.21, 102.63, 55.74. MS: m/z = 353 (M+1), 355 (M+2). Anal. calcd. for $C_{18}H_{13}ClN_4O_2$: C, 61.28; H, 3.71; N, 15.88. Found: C, 61.25; H, 3.75; N, 15.84 %.

2-(4-chlorophenyl)-5-(3-(2,4-dichlorophenyl)-1*H*-pyrazol-4-yl)-1,3,4-oxadiazole (P_{15})

FT-IR (cm^{-1}): 3155 (N-H-str), 3078 (C-H-str), 1599 (C=N), 1543 (C=C), 1093 (C-O-C); ^{1}H NMR (DMSO-d_6): δ 13.81 (s,1H, pyrazole-NH), 8.64 (s, 1H,pyrazole-5H), 7.56-7.85 (m, 7H, Ar-H). MS: m/z = 391 (M^+). Anal. calcd. for $C_{17}H_9Cl_3N_4O$: C, 52.14; H, 2.32; N, 14.31. Found: C, 52.18; H, 2.38; N, 14.36 %.

2-(4-methoxyphenyl)-5-(3-phenyl-1*H*-pyrazol-4-yl)-1,3,4-oxadiazole (P_{16})

FT-IR (cm^{-1}): 3143 (N-H-str), 3063 (C-H-str), 1604 (C=N), 1490 (C=C), 1077 (C-O-C); ^{1}H NMR (DMSO-d_6): δ 13.71 (s,1H, pyrazole-NH), 8.66 (s, 1H,pyrazole-5H), 7.12-8.21 (m, 9H, Ar-H), 3.83 (s, 3H, -OCH_3). MS: m/z = 319(M+1). Anal. calcd. for $C_{18}H_{14}N_4O_2$: C, 67.91; H, 4.43; N, 17.60. Found: C, 67.95; H, 4.47; N, 17.64 %.

2-(3-(4-chlorophenyl)-1*H*-pyrazol-4-yl)-5-(4-methoxyphenyl)-1,3,4-oxadiazole (P_{17})

FT-IR (cm^{-1}): 3143 (N-H-str), 3064 (C-H-str), 1608 (C=N), 1543 (C=C), 1086 (C-O-C); ^1H NMR (DMSO-d_6): δ 13.82 (s,1H, pyrazole-NH), 8.71 (s, 1H,pyrazole-5H), 7.12-8.24 (m, 8H, Ar-H), 3.84 (s, 3H, -OCH_3). MS: m/z = 353 (M+1), 354 (M+2). Anal. calcd. for $C_{18}H_{13}ClN_4O_2$: C, 61.28; H, 3.71; N, 15.88. Found: C, 61.24; H, 3.75; N, 15.83 %.

2-(3-(4-fluorophenyl)-1*H*-pyrazol-4-yl)-5-(4-methoxyphenyl)-1,3,4-oxadiazole (P_{18})

FT-IR (cm^{-1}): 3144 (N-H-str), 3073 (C-H-str), 1610 (C=N), 1546 (C=C), 1087 (C-O-C); ^1H NMR (DMSO-d_6): δ 13.79 (s,1H, pyrazole-NH), 8.67 (s, 1H,pyrazole-5H), 7.15-8.04 (m, 8H, Ar-H), 3.84 (s, 3H, -OCH_3). MS: m/z = 337 (M+1). Anal. calcd. for $C_{18}H_{13}FN_4O_2$: C, 64.28; H, 3.90; N, 16.66. Found: C, 64.24; H, 3.93; N, 16.69 %.

2-(2-chlorophenyl)-5-(3-phenyl-1*H*-pyrazol-4-yl)-1,3,4-oxadiazole (P_{19})

FT-IR (cm^{-1}): 3163 (N-H-str), 3102 (C-H-str), 1611 (C=N), 1536 (C=C), 1020 (C-O-C); ^1H NMR (DMSO-d_6): δ 13.73 (s,1H, pyrazole-NH), 8.64 (s, 1H,pyrazole-5H), 7.52-7.96 (m, 9H, Ar-H). MS: m/z = 323 (M+1). Anal. calcd. for $C_{17}H_{11}ClN_4O$: C, 63.26; H, 3.44; N, 17.36. Found: C, 63.23; H, 3.47; N, 17.39 %.

2-(2-chlorophenyl)-5-(3-(4-chlorophenyl)-1*H*-pyrazol-4-yl)-1,3,4-oxadiazole (P_{20})

FT-IR (cm^{-1}): 3247 (N-H-str), 3138 (C-H-str), 1602 (C=N), 1559 (C=C), 1087 (C-O-C) (**Figure 3.3**); ^1H NMR (DMSO-d_6): δ 13.84 (s,1H, pyrazole-NH), 8.65 (s, 1H,pyrazole-5H), 7.54-7.99 (m, 8H, Ar-H) (**Figure 3.4**). MS: m/z = 357 (M^+) (**Figure 3.5**). Anal. calcd. for $C_{17}H_{10}Cl_2N_4O$: C, 57.16; H, 2.82; N, 15.69. Found: C, 57.13; H, 2.86; N, 15.64 %.

2-(2-chlorophenyl)-5-(3-(4-fluorophenyl)-1*H*-pyrazol-4-yl)-1,3,4-oxadiazole (P_{21})

FT-IR (cm^{-1}): 3248 (N-H-str), 3137 (C-H-str), 1601 (C=N), 1557 (C=C), 1086 (C-O-C); ^1H NMR (DMSO-d_6): δ 13.79 (s,1H, pyrazole-NH), 8.61 (s, 1H,pyrazole-5H), 7.33-7.98 (m, 8H, Ar-H) (**Figure 3.6**). MS: m/z = 341 (M+1). Anal. calcd. for $C_{17}H_{10}ClFN_4O$: C, 59.92; H, 2.96; N, 16.44. Found: C, 59.95; H, 2.93; N, 16.41 %.

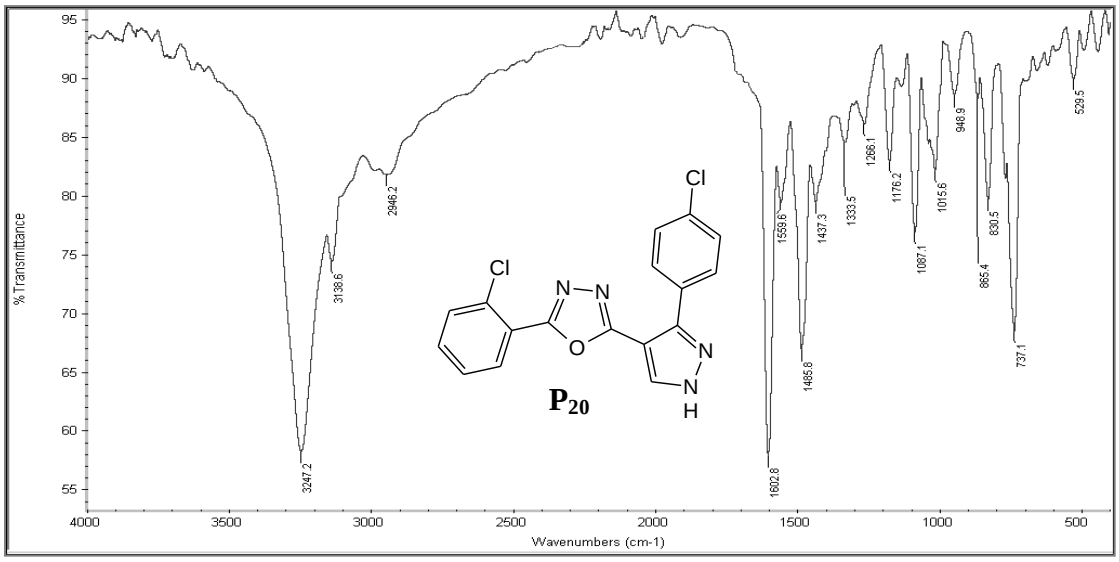


Figure 3.3 IR spectrum of compound **P_{20}**

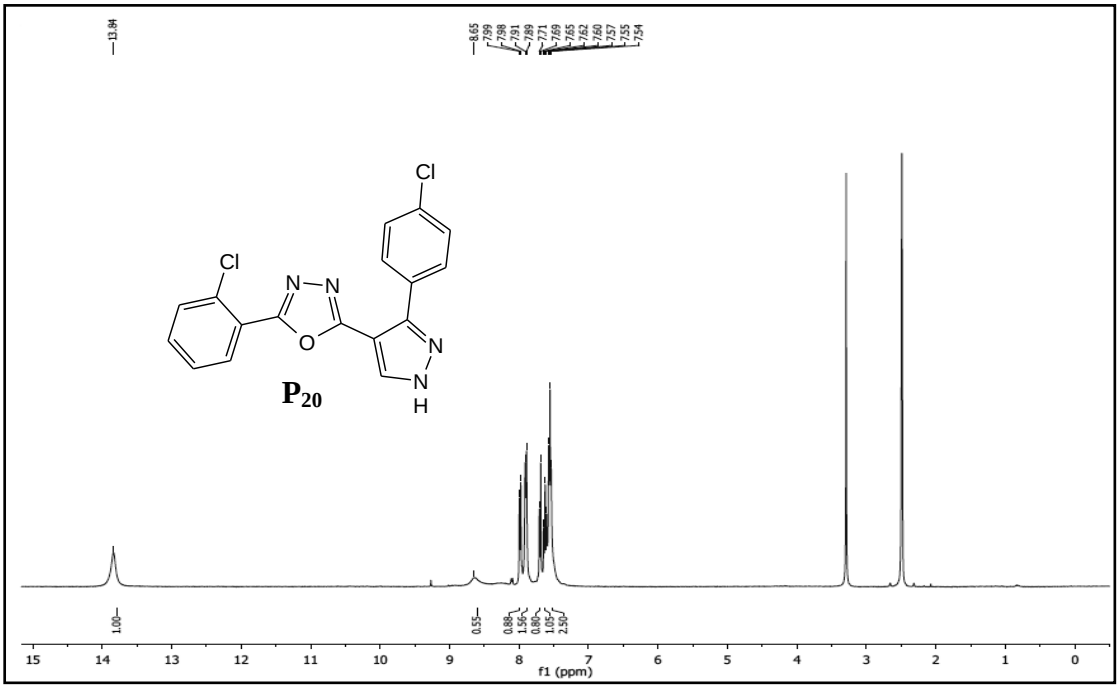


Figure 3.4 ^{1}H NMR spectrum of compound **P_{20}**

2-(2-chlorophenyl)-5-(3-(4-methoxyphenyl)-1*H*-pyrazol-4-yl)-1,3,4-oxadiazole (P_{22})

FT-IR (cm^{-1}): 3272 (N-H-str), 3129 (C-H-str), 1602 (C=N), 1543 (C=C), 1100 (C-O-C); ^{1}H NMR (DMSO-d_6): δ 13.71 (s,1H, pyrazole-NH), 8.59 (s, 1H,pyrazole-5H), 7.05-7.98 (m, 8H, Ar-H), 3.82)s, 3H, -OCH_3). MS: m/z = 353 (M+1). Anal. calcd. for $C_{18}H_{13}ClN_4O_2$: C, 61.28; H, 3.71; N, 15.88. Found: C, 61.24; H, 3.74; N, 15.83 %.

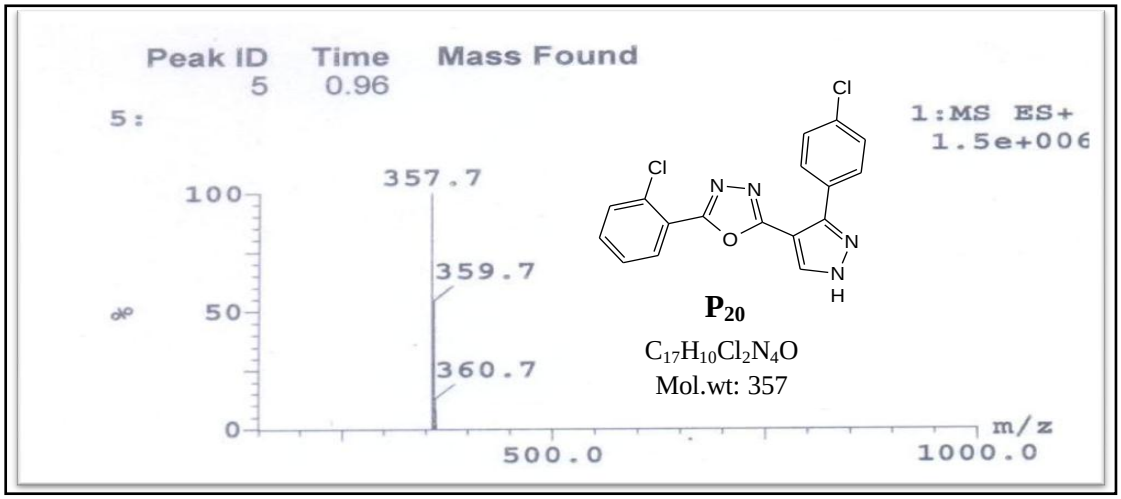


Figure 3.5 Mass spectrum of compound **P_{20}**

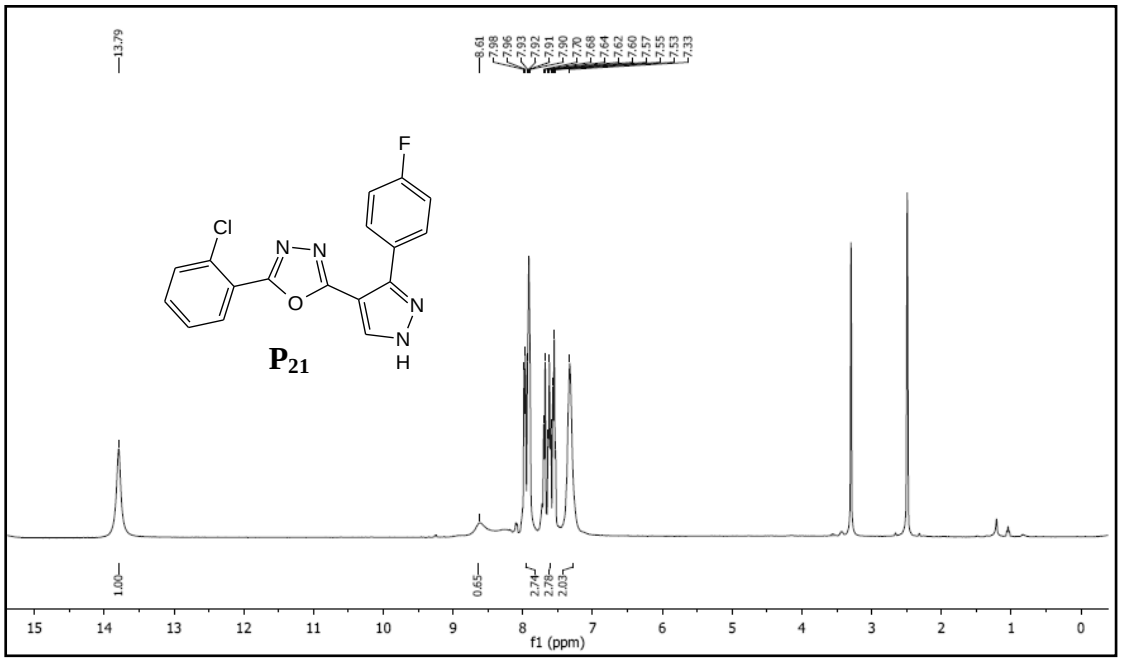


Figure 3.6 ^{1}H NMR spectrum of compound **P_{21}**

2-(4-nitrophenyl)-5-(3-phenyl-1*H*-pyrazol-4-yl)-1,3,4-oxadiazole (P_{23})

FT-IR (cm^{-1}): 3120 (N-H-str), 3070 (C-H-str), 1596 (C=N), 1519 (C=C), 1075 (C-O-C); ^{1}H NMR (DMSO-d_6): δ 13.76 (s,1H, pyrazole-NH), 8.72 (s, 1H,pyrazole-5H), 7.52-8.43 (m, 8H, Ar-H). MS: m/z = 334 (M+1). Anal. calcd. for $C_{17}H_{11}N_5O_3$: C, 61.26; H, 3.33; N, 21.01. Found: C, 61.22; H, 3.37; N, 21.06 %.

2-(3-(4-chlorophenyl)-1*H*-pyrazol-4-yl)-5-(4-nitrophenyl)-1,3,4-oxadiazole (P_{24})

FT-IR (cm^{-1}): 3157 (N-H-str), 3111 (C-H-str), 1603 (C=N), 1558 (C=C), 1093 (C-O-C); ^{1}H NMR (DMSO-d_6): δ 13.76 (s,1H, pyrazole-NH), 8.58 (s, 1H,pyrazole-5H),

7.57-8.44 (m, 8H, Ar-H). MS: m/z = 368 (M+1). Anal. calcd. for $C_{17}H_{10}ClN_5O_3$: C, 55.52; H, 2.74; N, 19.04. Found: C, 55.56; H, 2.71; N, 19.01 %.

3.4 PHARMACOLOGY

3.4.1 Antimicrobial activity

The *in vitro* antimicrobial activity of newly synthesized compounds **P_{11-24}** were determined by "well- plate" method in Mueller-Hinton Agar as explained in **Chapter 2** by measuring the diameter of inhibition zone (mm). In this work *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 were used to investigate the antibacterial activity whereas *Aspergillus flavus* ATCC 15547, *Chrysosporium keratinophilum* ATCC 14803 and *Candida albicans* MTCC 227 were used to study antifungal activity. These cultures were obtained from the Department of Microbiology, Kuvempu University, Shimoga, India. All fungi strains were maintained on potato dextrose agar (PDA) at ± 25 ˚C.

3.5 RESULTS AND DISCUSSION

3.5.1 Chemistry

The structures of the synthesized compounds (**P_{11-24}**) were characterized by IR, NMR, mass spectral and elemental analyses. Analytical and spectral data of all synthesized compounds were in full agreement with the proposed structures. IR spectrum of compound **P_{20}** showed absorption bands at 3247, 3138, 1602, 1559, 1087 cm^{-1} which were due to the N-H, C-H, C=N, C=C and C-O-C groups respectively . In ^{1}H-NMR spectra, all protons were seen according to the expected chemical shift and integral values. The ^{1}H-NMR spectrum of **P_{20}** showed a singlet at δ 13.84 corresponds to pyrazole NH proton. A singlet at δ 8.65 was due to pyrazole 5H proton. Also, at δ 7.99-7.54 a multiplet was observed which was due to aromatic protons. The mass spectrum of **P_{20}** showed molecular ion peak at m/z = 357 (M^+), which is in agreement with the molecular formula $C_{17}H_{10}Cl_2N_4O$. Similarly the spectral values for all the compounds and C, H, N analyses are given in the experimental part and the characterization data has been provided in **Table 3.1.**

Table 3.1 Characterization data of the compounds $\mathbf{P_{11\text{-}24}}$

Compounds	R^1	R	Molecular formula (Mol.wt.)	Yield (%)	M.p. (^oC)
$\mathbf{P_{11}}$	4-Cl	H	$C_{17}H_{11}ClN_4O$ (322)	66	223-225
$\mathbf{P_{12}}$	4-Cl	4-Cl	$C_{17}H_{10}Cl_2N_4O$ (357)	68	126-128
$\mathbf{P_{13}}$	4-Cl	4-F	$C_{17}H_{10}ClFN_4O$(340)	75	273-275
$\mathbf{P_{14}}$	4-Cl	4-OCH_3	$C_{18}H_{13}ClN_4O_2$ (352)	73	176-178
$\mathbf{P_{15}}$	4-Cl	2,4-Cl	$C_{17}H_9Cl_3N_4O$ (391)	67	234-236
$\mathbf{P_{16}}$	4-OCH_3	H	$C_{18}H_{14}N_4O_2$ (318)	64	133-135
$\mathbf{P_{17}}$	4-OCH_3	4-Cl	$C_{18}H_{13}ClN_4O_2$ (352)	71	238-240
$\mathbf{P_{18}}$	4-OCH_3	4-F	$C_{18}H_{13}FN_4O_2$ (336)	62	240-242
$\mathbf{P_{19}}$	2-Cl	H	$C_{17}H_{11}ClN_4O$ (322)	69	139-141
$\mathbf{P_{20}}$	2-Cl	4-Cl	$C_{17}H_{10}Cl_2N_4O$ (357)	65	241-243
$\mathbf{P_{21}}$	2-Cl	4-F	$C_{17}H_{10}ClFN_4O$(340)	61	214-216
$\mathbf{P_{22}}$	2-Cl	4-OCH_3	$C_{18}H_{13}ClN_4O_2$ (352)	67	185-187
$\mathbf{P_{23}}$	4-NO_2	H	$C_{17}H_{11}N_5O_3$ (333)	65	131-133
$\mathbf{P_{24}}$	4-NO_2	4-Cl	$C_{17}H_{10}ClN_5O_3$ (367)	63	146-148

3.5.2 Biological activity

The newly synthesized compounds $\mathbf{P_{11\text{-}24}}$ were tested for their antibacterial activity (*in vitro)* against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* and their activity was compared to a well-known commercial antibiotic, Streptomycin. Antibacterial activity was carried out by "well-plate" method by measuring its zone of inhibition. The compounds $\mathbf{P_{11\text{-}24}}$ were screened for their antibacterial activity in triplicate against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* at two different concentrations of 1000, 500 µg/mL as shown in **Table 3.2**.

Table 3.2 Antibacterial activity of compounds $\mathbf{P_{11\text{-}24}}$

Compound code	Zone of inhibition (mm)					
	Escherichia coli		***Staphylococcus aureus***		***Pseudomonas aeruginosa***	
Conc. in µg/mL	1000	500	1000	500	1000	500
$\mathbf{P_{11}}$	04±0.2	01±0.1	02±0.1	00	03±0.2	01±0.2
$\mathbf{P_{12}}$	07±0.1	05±0.2	08±0.2	06±0.2	04±0.2	03±0.1
$\mathbf{P_{13}}$	00	00	00	00	00	00
$\mathbf{P_{14}}$	00	00	00	00	00	00
$\mathbf{P_{15}}$	09±0.1	07±0.2	09±0.2	06±0.1	10±0.2	08±0.1
$\mathbf{P_{16}}$	07±0.1	06±0.1	09±0.1	07±0.2	08±0.2	06±0.1
$\mathbf{P_{17}}$	04±0.2	02±0.1	04±0.1	02±0.2	05±0.1	02±0.1
$\mathbf{P_{18}}$	00	00	00	00	00	00
$\mathbf{P_{19}}$	12±0.2	10±0.1	11±0.1	10±0.1	12±0.2	09±0.1
$\mathbf{P_{20}}$	13±0.1	10±0.2	12±0.1	09±0.2	11±0.1	09±0.2
$\mathbf{P_{21}}$	11±0.1	08±0.1	10±0.1	08±0.2	12±0.1	07±0.1
$\mathbf{P_{22}}$	11±0.2	09±0.1	12±0.1	10±0.2	11±0.1	08±0.2
$\mathbf{P_{23}}$	03±0.2	02±0.1	04±0.1	03±0.1	04±0.2	01±0.1
$\mathbf{P_{24}}$	00	00	00	00	00	00
Streptomycin (Std.)	16±0.2	10±0.1	15±0.2	10±0.2	16±0.2	13±0.2

The investigation of antibacterial screening data revealed that most of the tested compounds showed moderate to good bacterial inhibition. Compound $\mathbf{P_{19}}$ exhibited equipotent activity as that of standard against *Escherichia coli* and *Staphylococcus aureus* at 500 µg/mL. Compound $\mathbf{P_{20}}$ exhibited equipotent activity as that of standard against *Escherichia coli* whereas slightly less than that of standard against *Staphylococcus aureus* and *Pseudomonas aeruginosa* at 500 µg/mL. $\mathbf{P_{22}}$ also inhibited the growth of *Staphylococcus aureus* similarly as that of standard whereas slightly less than that of standard against *Escherichia coli* and *Pseudomonas aeruginosa at* 500 µg/mL. Compounds $\mathbf{P_{15}}$ and $\mathbf{P_{16}}$ were found to be active against all the tested bacterial strains. Compound $\mathbf{P_{12}}$ showed moderate activity against

Escherichia coli and *Staphylococcus aureus*. Remaining compounds showed fair or poor activity against tested bacterial strains.

Table 3.3 Antifungal activity of compounds $\mathbf{P_{11\text{-}24}}$

Compound code	Zone of inhibition (mm)					
	Aspergillus flavus		***Chrysosporium keratinophilum***		***Candida albicans***	
Conc. in µg/mL	1000	500	1000	500	1000	500
$\mathbf{P_{11}}$	00	00	00	00	00	00
$\mathbf{P_{12}}$	03±0.2	01±0.1	04±0.1	03±0.1	04±0.1	03±0.1
$\mathbf{P_{13}}$	00	00	00	00	00	00
$\mathbf{P_{14}}$	00	00	00	00	00	00
$\mathbf{P_{15}}$	08±0.2	06±0.1	06±0.1	04±0.1	06±0.2	03±0.2
$\mathbf{P_{16}}$	06±0.1	04±0.1	05±0.1	03±0.2	04±0.2	02±0.2
$\mathbf{P_{17}}$	04±0.1	03±0.2	06±0.1	05±0.2	04±0.1	02±0.1
$\mathbf{P_{18}}$	00	00	00	00	00	00
$\mathbf{P_{19}}$	10±0.2	08±0.1	09±0.1	07±0.1	09±0.2	06±0.1
$\mathbf{P_{20}}$	09±0.1	08±0.2	07±0.2	06±0.1	04±0.2	02±0.1
$\mathbf{P_{21}}$	05±0.1	03±0.1	04±0.2	03±0.1	05±0.1	02±0.1
$\mathbf{P_{22}}$	06±0.1	05±0.2	04±0.1	03±0.1	05±0.1	04±0.1
$\mathbf{P_{23}}$	04±0.2	01±0.1	04±0.1	02±0.1	03±0.2	01±0.2
$\mathbf{P_{24}}$	00	00	00	00	00	00
Fluconazole (Std.)	13±0.2	10±0.1	17±0.2	15±0.2	22±0.2	20±0.2

All the synthesized compounds were also tested for its antifungal activity (*in vitro)* against *Aspergillus flavus*, *Chrysosporium keratinophilum* and *Candida albicans* by measuring its average zone of inhibition (**Table 3.3**). Fluconazole was used as standard for antifungal activity. Among the tested compounds, $\mathbf{P_{19}}$ and $\mathbf{P_{20}}$ showed good antifungal profile against *Aspergillus flavus*, and *Chrysosporium keratinophilum* at concentration of 500 µg/mL when compared with standard. $\mathbf{P_{15}}$, $\mathbf{P_{16}}$ and $\mathbf{P_{22}}$ showed moderate activity against *Aspergillus flavus* but showed poor activity

against rest of two microorganisms. Remaining compounds showed poor activity against the tested microorganisms.

The enhanced activity of **P_{19}** and **P_{20}** can be attributed due to presence of 2-chlorophenyl group attached to fifth position of oxadiazole and presence of phenyl and p-chlorophenyl substituents on pyrazole ring which is attached to second position of oxadiazole. The presence of 2-chlorophenyl substituent (fifth position of oxadiazole) along with 4-fluorophenyl, 4-methoxyphenyl substituent on pyrazole ring in **P_{21}** and **P_{22}** respectively may be the reason for its enhanced activity. Compounds **P_{15}** and **P_{16}** contain 4-chlorophenyl and 4-methoxyphenyl substituent attached to oxadiazole nucleus along with 2,4-dichlorophenyl and phenyl substituents on pyrazole ring may be the reason for its activity.

It can be concluded that the compounds **P_{19}**, **P_{20}, P_{21}** and **P_{22}** which contain 2-chlorophenyl substituent on fifth position of oxadiazole ring may increase the antimicrobial profile of the compound.

3.6 CONCLUSIONS

In summary, a new series of 2,5-disubstituted-1,3,4-oxadiazoles were synthesized and screened for their antimicrobial activity against various microorganisms. Among the synthesized compounds, **P_{19}** and **P_{20}** showed excellent antimicrobial activity against various tested microorganisms. Hence it can be concluded that the compounds **P_{19}** and **P_{20}** are identified as the most potent antimicrobial agents in the present series and deserve further investigation in order to clarify the mode of action at molecular level responsible for the activity observed.

CHAPTER - 4

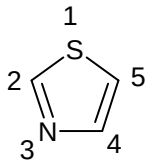
SYNTHESIS AND EVALUATION OF ANTIMICROBIAL ANTIOXIDANT, ACTIVITIES OF NEW 2,4-DISUBSTITUTED THIAZOLES

Abstract

This chapter describes a detailed literature survey on 1,3-thiazole and its derivatives. It also includes the synthesis and characterization of newly designed pyrazole containing 1,3-thiazole derivatives. Antimicrobial and antioxidant studies of such compounds have been discussed in this chapter.

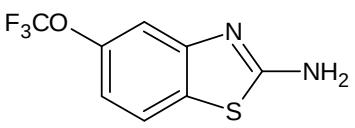
4.1 INTRODUCTION

Thiazole (**S-4.1**) is a heterocyclic organic compound that has a five-member ring molecular structure (C_3H_3NS) containing three carbon atoms, one sulfur atom, and one nitrogen atom. Thiazole itself is a clear to pale yellow flammable liquid with a pyridine-like odor. The thiazole moiety is a crucial part of vitamin-B1 (thiamine) and epothilone.

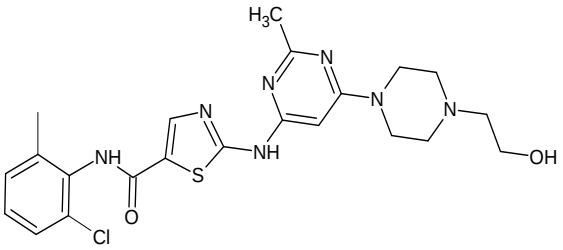


S-4.1

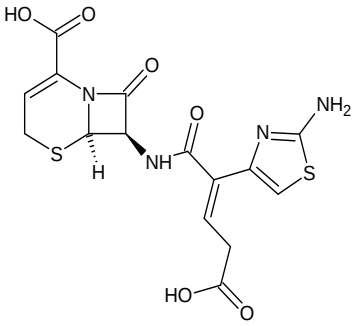
Thiazoles have been frequently discovered as a vital component of novel and structurally diverse natural products that exhibit a wide variety of biological activities. Their presence in peptides, their ability to bind to proteins, DNA, and RNA, as well as the exceptional range of antitumor, antiviral, and antibiotic activities of thiazole containing compounds have directed numerous synthetic studies and new applications. Thiazole ring system has found in many natural and synthetic products with a wide range of pharmacological activities, such as antiviral, anticancer, antibacterial, antifungal, anticonvulsant, antiparkinsonian and anti-inflammatory activities that can be well illustrated by the large number of drugs in the market containing this functional group. Some of the commercial drugs which have the thiazole moiety are as follows,



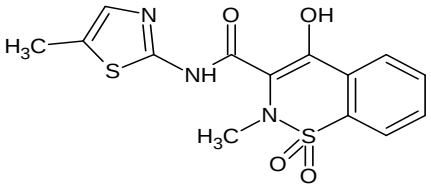
S-4.2
Riluzole (Anticonvulsant)



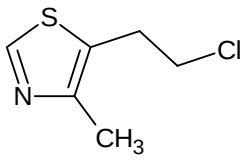
S-4.3
Dasatinib
(Tyrosine Kinase inhibitor)



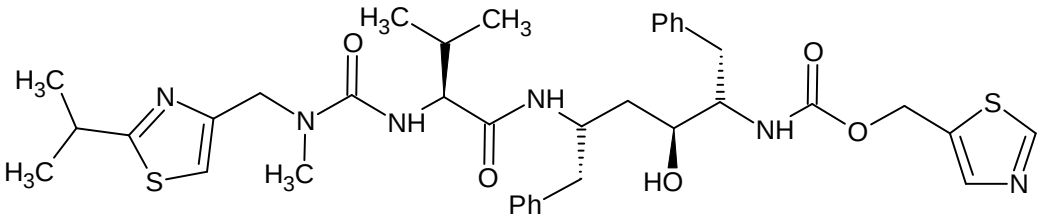
S-4.4
Ceftibuten
(Cephalosporin antibiotic)



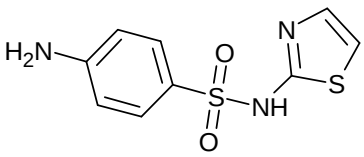
S-4.5
Meloxicam (Anti-inflammatory)



S-4.6
Clomethiazole (sedative)

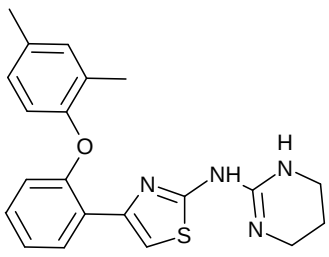


S-4.7
Ritonavir (Antiviral)



S-4.8

Sulfathiazole
(Antimicrobial)

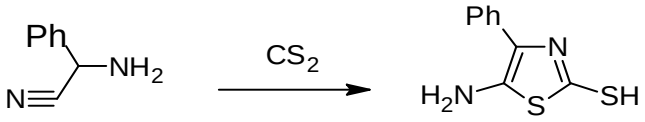


S-4.9

Abafungin
(Antifungal)

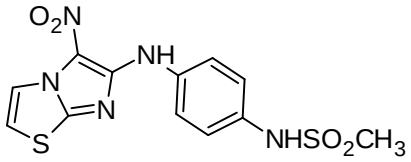
Despite their importance from pharmacological and synthetic point of view, comparatively few methods for their preparation have been reported in the literature. Because of the importance of thiazoles and their ease of synthesis, the literature and patents are rich in the synthesis and applications of thiazoles.

The Cook-Heilbron reaction involves the reaction of alpha aminonitriles with salts and esters of dithioacids, carbon disulfide, carbon oxysulfide and isothiocyanates under extremely mild conditions to form 5-amino-2-mercapto thiazole (**S-4.10**) (Cook et al. 1949).



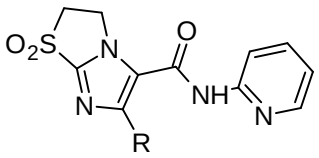
S-4.10

Andreani et al. (1993) synthesized 6-anilinoimidazo[2,1-b]thiazoles and evaluated the cytotoxic activity against HeLa cell lines. The results revealed that among the synthesized only compound **S-4.11** was found to be active.



S-4.11

Andreani et al. (1995) reported the synthesis and fungicide activity of 2,3-dihydroimidazo[2,1-b]thiazole-5-carboxamides (**S-4.12**). The results from a fungicide test showed that the two most active amides are those arising from 2-aminopyridine which contain methyl and chloro substituent on imidazole ring.

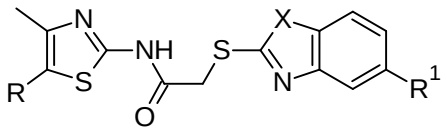


S-4.12

Where R = CH_3, Cl

Turan-Zitouni et al. (2003) synthesized some 2-[(benzazole-2-yl)thioacetyl amino]thiazole derivatives (**S-4.13**) and tested them for antimicrobial activity and

toxicity. The results revealed that all the synthesized compounds were found to be active against the tested microbial strains.



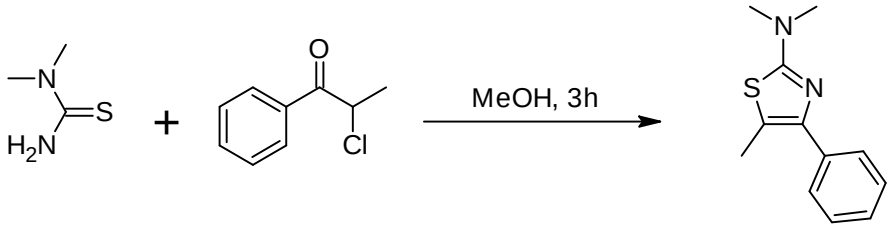
S-4.13

Where R = H, CH_3, $COOC_2H_5$

R^1 = H, Cl, NO_2, CH_3

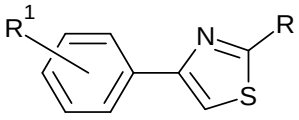
X = NH, O, S

Hantzsch thiazole synthesis is one of the oldest methods for the synthesis of thiazoles moiety (**S-4.14**) where a reaction between haloketones and thioamides takes place (Sheldrake et al. 2006). The reaction takes place due to the strong nucleophilicity of the sulfur atom in thioamides or thioureas, and gives fantabulous yields for simple thiazoles but low yields for some substituted thiazoles, as of dehalogenation.



S-4.14

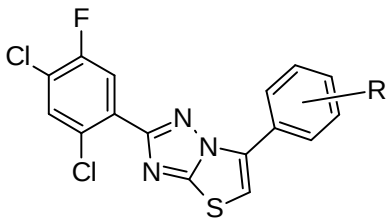
Potewar et al. (2007) reported the synthesis of 2,4-disubstituted thiazoles (**S-4.15**) using ionic liquid under ambient conditions. Authors observed some of the important features such as enhanced reaction rate, mild reaction conditions, high yields and green aspects such as avoiding hazardous organic solvents, toxic catalysts and waste, ease of recovery and reuse of this novel reaction medium. The above methodology was successfully applied for a practical synthesis of an anti-inflammatory agent, Fanetizole.



S-4.15

Where,
R^1= H, p-CH_3, p-OCH_3, p-Cl
R = NH_2, CH_3, $NHCH_2Ph$

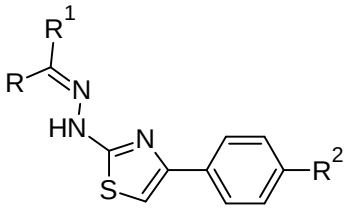
Karthikeyan (2009) reported the synthesis, analgesic, anti-inflammatory and antimicrobial studies of 2,4-dichloro-5-fluorophenyl containing thiazolotriazoles (**S-4.16**). The compounds with R= 4-fluoro, 4-chloro and 2,4-dichloro-5-fluorophenyl at sixth position of the thiazolotriazole ring system showed excellent anti-inflammatory activity. Analgesic activity studies revealed that compounds with R= 4-chloro, 4-bromo and 2,4-dichloro-5-fluorophenyl exhibited good analgesic activity. Compounds bearing R= 4-fluoro, 4-chloro and 2,4-dichloro-5-fluorophenyl at sixth position of the thiazolotriazole ring system displayed excellent antibacterial activity. Antifungal screening studies revealed that compounds with R= 4-chloro and 2,4-dichloro-5-fluorophenyl showed excellent antifungal activity.



Where,
R = 4-OCH_3, 4-CH_3, 4-F, 4-Cl, 4- Br, 4-NO_2, 2,4-Cl and 2,4-Cl-5-F

S-4.16

Bharti and her coworkers (2010) synthesized some new Schiff bases (**S-4.17**) containing 2,4-disubstituted thiazole ring and studied their antibacterial and antifungal activities. Most of the synthesized compounds exhibited good antibacterial as well as antifungal activities.



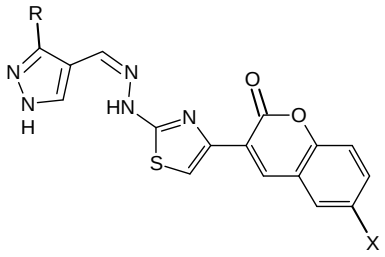
S-4.17

Where R = H, C_6H_5

R^1 = C_6H_5, 2-F-C_6H_4, 2-OCH_3-C_6H_4, 2,4-OH-C_6H_3, 3,4,5-OCH_3-C_6H_2, 4-NO_2-C_6H_4, 4-OH-3-OCH_3-C_6H_3, 4-OCH_3-3-OH-C_6H_3, 3,4-OCH_3-C_6H_3, 3-indolyl

R^2 = OCH_3, Br

Vijesh et al. (2010) synthesized some 2,4-disubstituted thiazoles (**S-4.18**) and evaluated their antimicrobial properties. The results revealed that compounds having 2,5-dichlorothiophene substituent and 2,4-dichlorophenyl substituent showed significant antibacterial activity against all tested microorganisms.

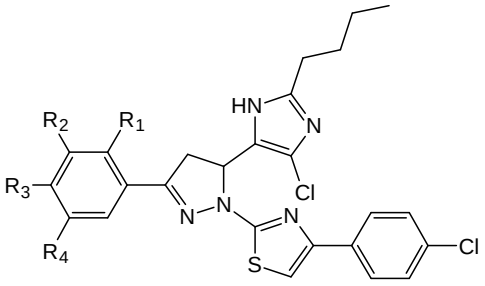


Where R = 4-SCH_3-C_6H_4, 2,4-Cl_2-C_6H_3, biphenyl, 2,4-Cl_2-thiophene

X = H, Br

S-4.18

Dawane and his coworkers (2010) developed a novel, efficient and environmentally benign methodology towards the synthesis of 1-(4-(4-chlorophenyl)-2-thiazolyl)-3-aryl-5-(2-butyl-4-chloro-1*H*-imidazol-5yl)-2-pyrazolines (**S-4.19**) by the reaction of chalcones with 2-hydrazino-thiazole in PEG-400 as an alternative reaction solvent. Most of the compounds were found to be active in the current study.



Where,

R_1= OH, H

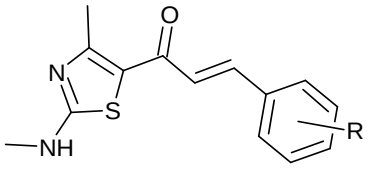
R_2= H, I, Br

R_3= H, Cl, Me, NH_2, OCH_3

R_4= H, Cl, Me, I, Br

S-4.19

Liaras and his researchers (2011) reported the synthesis and biological evaluation of thiazole based chalcones (**S-4.20)** as potent antimicrobial agents. The investigation of antimicrobial screening data revealed that almost all the compounds exhibited greater activity than reference drugs.

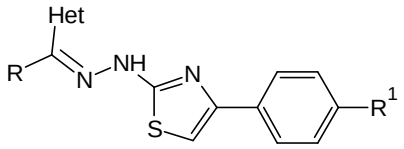


S-4.20

Where,

R = H, 4-NO_2, 3-NO_2, 4-Cl, 3-Cl, 2-Cl, 4-OMe, 2-OMe, 2,6-Cl_2, 2,4-Cl_2

Chimenti et al. (2011) reported the synthesis and biological evaluation of novel 2,4-disubstituted-1,3-thiazoles **(S-4.21)** as anti-*Candida* spp. agents. The results of such study showed that the presence of heterocyclic or bicyclic rings on hydrazone moiety revealed a promising selective inhibitory activity especially against *Candida albicans* and *Candida glabrata*.

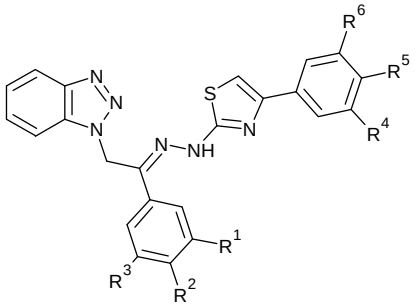


S-4.21

Where,

R= H, CH_3 ; R^1= CH_3, OCH_3 ; Het= 2-furyl, 2-thienyl, 2-pyridyl, 1-napthyl, Benzodioxol-5-yl, 3-indolyl, 3-coumarinyl

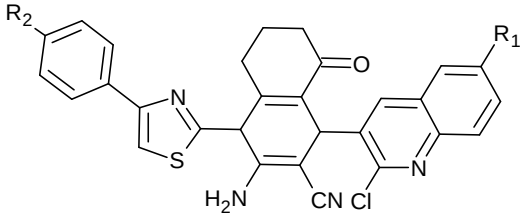
Gaikwad et al. (2012) with the aim of investigating their antimicrobial activity reported the synthesis of new benzotriazole derivatives clubbed with thiazole moiety (**S-4.22**). The investigation of antimicrobial screening data revealed that most of the tested compounds showed moderate to good microbial inhibitions.



Where,
R^1, R^3, R^4, R^6 = H, CF_3
R^2, R^5 = F, Cl, Br, NO_2, H

S-4.22

Shah et al. (2012) reported the synthesis of biquinoline derivatives (**S-4.23**) containing a thiazole moiety by a one-pot, base-catalyzed cyclocondensation reaction of 2-chloro-3-formyl quinoline, malononitrile and enaminone and studied their antimicrobial properties.



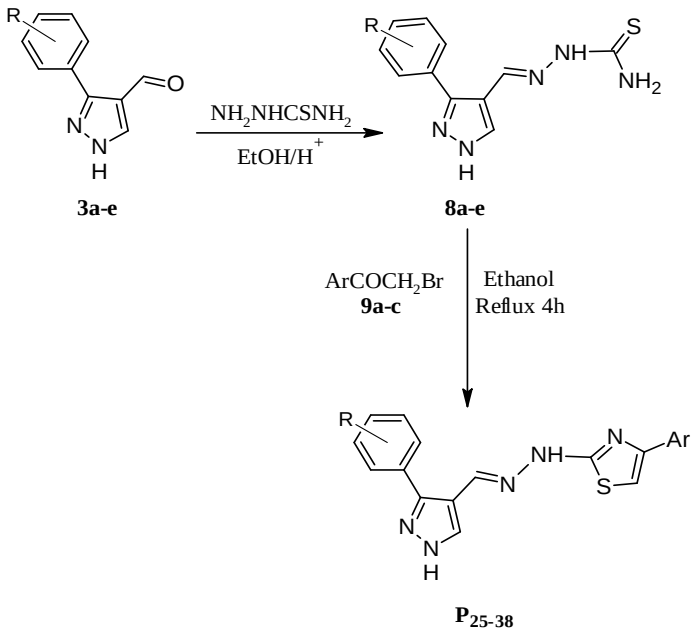
Where,
R_1 = H, CH_3, OCH_3, Cl
R_2 = H, Cl, OH

S-4.23

Looking at the medicinal importance of thiazole and pyrazole moieties, it was contemplated to synthesize some new class of heterocyclic molecules in which both the moieties (thiazole and pyrazole) are present with an attempt to develop potential bioactive molecules.

4.2 MATERIALS AND METHODS

3-aryl-1*H*-pyrazole-4-carbaldehydes (**3a-e**) were synthesized by the Vilsmayer Haack reaction of semicarbazones (Lebedev et al. 2005). The starting materials, 3-aryl-1*H*-pyrazole-4-carbaldehyde thiosemicarbazones (**8a-e)** were synthesized by refluxing equimolar amount of 3-aryl-1-*H*-pyrazole-4-carbaldehydes with thiosemicarbazide in absolute ethanol. Substituted phenacyl bromides (**9a-c**) were prepared according to the procedure reported in literature (Furniss et al. 1996). The target molecules $\mathbf{P_{25\text{-}38}}$ were obtained in reasonably good yield by refluxing 3-aryl-1*H*-pyrazole-4-carbaldehyde thiosemicarbazones (**8a-e**) with various phenacyl bromides (**9a-c**) in ethanol. The reaction pathway has been summarized in **Scheme 4.1**.



Where R = H, 4-OCH_3, 4-F, 4-Cl, 2,4-Cl
Ar = C_6H_5, 4-OCH_3-C_6H_4, 4-F-C_6H_4

Scheme 4.1 Synthetic route for 2,4-disubstituted thiazole derivatives $\mathbf{P_{25\text{-}38}}$

Melting points were determined by open capillary method. The IR spectra were recorded on Thermo Nicolet avatar 330-FT-IR spectrophotometer. ^{1}H-NMR spectra were recorded (DMSO-d_6) on a Bruker and Varian (400 MHz) spectrometer whereas ^{13}C-NMR spectra were recorded on Bruker (100 MHz) spectrometer using TMS as internal standard. Chemical shift values are given in δ (ppm) scales. The mass spectra were recorded on a LC-MS-Agilent 1100 series. Elemental analyses were performed on a Flash EA 1112 series CHNS-O Analyzer. The completion of the reaction was checked by thin layer chromatography (TLC) on silica gel coated aluminium sheets (silica gel 60 F254). Commercial grade solvents and reagents were used without further purification.

4.3 EXPERIMENTAL

The experimental protocols followed for the synthesis of compounds **8a-e** and **P_{25-38}** is given in the following section.

The synthetic procedure for the synthesis of intermediate compounds **3a-e** has been discussed in **Chapter 2**.

General procedure for the synthesis of 3-aryl-1*H*-pyrazole-4-carbaldehyde thiosemicarbazone (8a-e)

An equimolar mixture of the appropriate 3-aryl-1*H*-pyrazole-4-carbaldehyde **(3a-e)** (0.005 mol) and thiosemicarbazide (0.005 mol) in absolute ethanol (10 mL) was refluxed on a water bath in presence of catalytic amount of concentrated sulphuric acid for 2 h. The solid mass that separated on cooling was collected by filtration, washed with ethanol, dried and recrystallized from ethanol.

3-(phenyl)-1*H*-pyrazole-4-carbaldehyde thiosemicarbazone (8a)

Yield 88%, m.p. 249-250 ˚C. FT-IR (cm^{-1}): 3436, 3239 (N-H-str), 1582 (C=N), 1470 (C=C), 1096 (C=S) (**Figure 4.1**). MS: m/z = 246 (M+1) (**Figure 4.2**).

3-(4-methoxyphenyl)-1*H*-pyrazole-4-carbaldehyde thiosemicarbazone (8b)

Yield 88%, m.p. 241-242 ˚C. FT-IR (cm^{-1}): 3435, 3268 (N-H-str), 1609 (C=N), 1483 (C=C), 1105 (C=S). MS: m/z =276 (M+1).

3-(4-fluorophenyl)-1*H*-pyrazole-4-carbaldehyde thiosemicarbazone (8c)

Yield 83%, m.p. 251-253 ˚C. FT-IR (cm^{-1}): 3444, 3300 (N-H-str), 1557 (C=N), 1480 (C=C), 1101 (C=S). MS: m/z =264 (M+1).

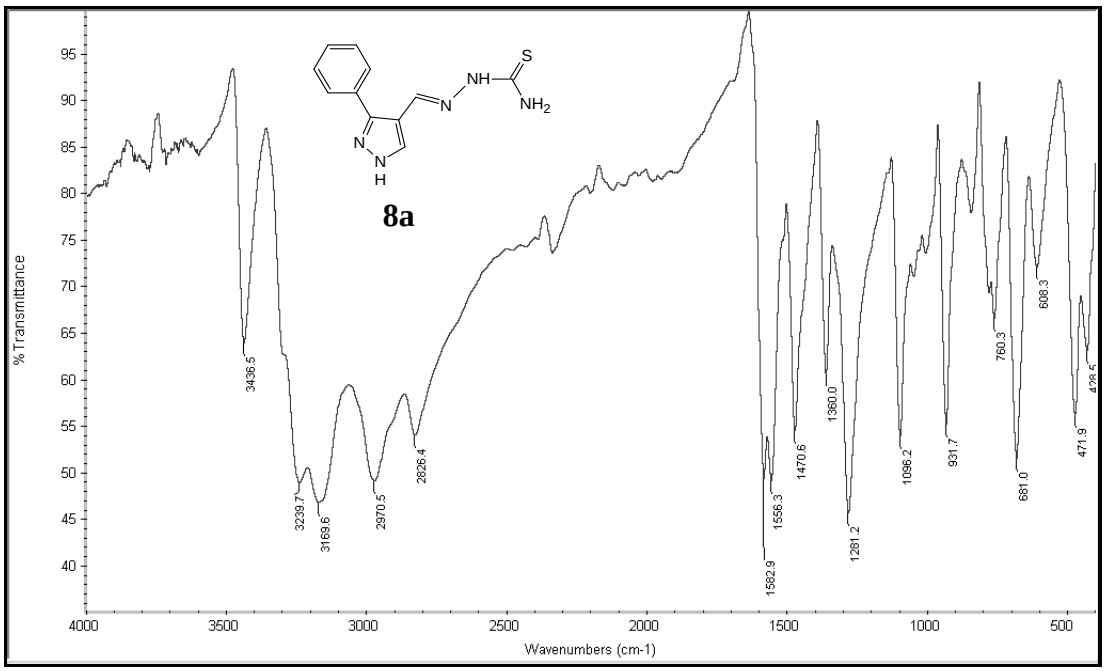


Figure 4.1 IR spectrum of compound **8a**

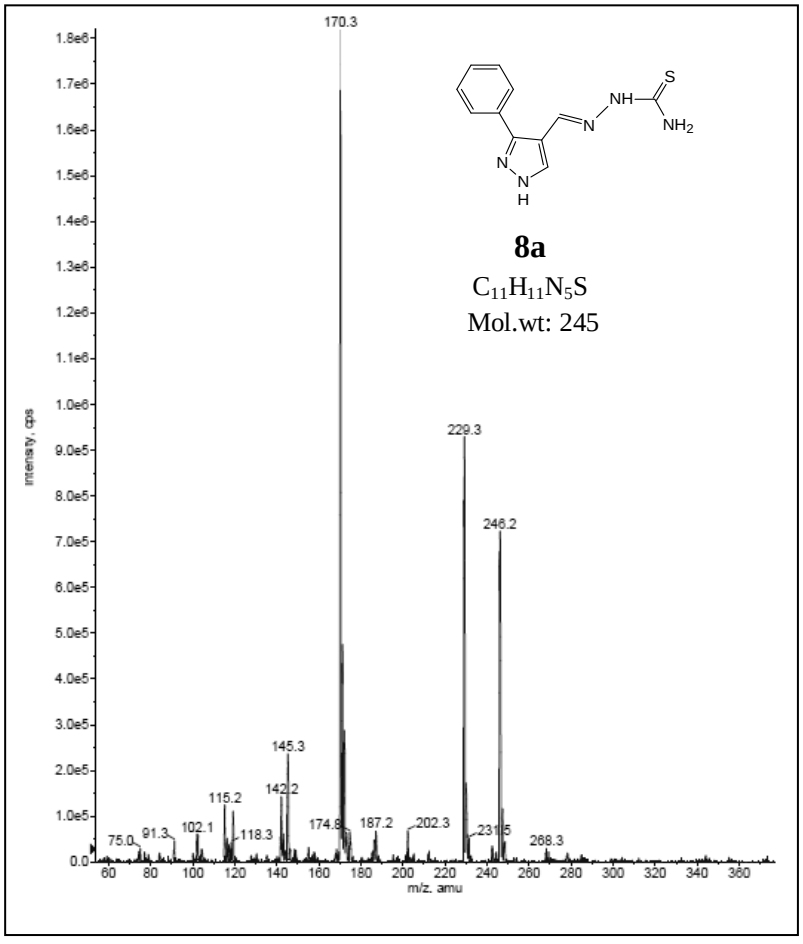


Figure 4.2 Mass spectrum of compound **8a**

3-(4-chlorophenyl)-1*H*-pyrazole-4-carbaldehyde thiosemicarbazone (8d)

Yield 89%, m.p. 267-269 ˚C. FT-IR (cm^{-1}): 3446, 3381 (N-H-str), 1605 (C=N), 1476 (C=C), 1098 (C=S), 835 (C-Cl). MS: m/z =280 (M+1), 282 (M+2).

3-(2,4-dichlorophenyl)-1*H*-pyrazole-4-carbaldehyde thiosemicarbazone (8e)

Yield 81%, m.p. 213-215 ˚C. FT-IR (cm^{-1}): 3402, 3255 (N-H-str), 1608 (C=N), 1462 (C=C), 1068 (C=S), 936, 817 (C-Cl) . MS: m/z =314 (M+), 316 (M+2), 318 (M+4).

General procedure for the synthesis of 2-bromo-1-(4-substituedphenyl)ethanone 9a-c

A solution of substituted acetophenone (0.04 mol) in 30 mL of dry ether was placed in a dry three-necked flask fitted with addition funnel. The solution was cooled in an ice bath, 0.1 g of anhydrous $AlCl_3$ was introduced and 6.4 g (0.04 mol) of bromine was added gradually from a addition funnel with stirring. After all the bromine has been added, the ether and dissolved HBr were removed under reduced pressure. The solid mass obtained was washed several times with a 1:1 mixture of water and petroleum ether. The crystals of substituted phenacyl bromides were filtered and recrystallised from ethanol and dried over vacuum for several hours and their purity was confirmed by recording thier melting points (Paul et al. 2003).

2-bromo-1-phenylethanone- M.p. 48-50 ˚C

2-bromo-1-(4-methoxyphenyl)ethanone- M.p. 67-69 ˚C

2-bromo-1-(4-fluorophenyl)ethanone- M.p. 47-49 ˚C.

General procedure for the synthesis of 4-aryl-2-(2-((3-aryl-1*H*-pyrazol-4-yl) methylene)hydrazinyl)thiazole P_{25-38}

An equimolar mixture of 3-aryl-1*H*-pyrazole-4-carbaldehyde thiosemi-carbazone, **8a-e** (0.001 mol) and substituted phenacyl bromides **9a-c** (0.001 mol) in absolute ethanol was refluxed for 4 h. After completion of the reaction, the reaction mixture was allowed to cool. The solid thus separated was collected by filtration and recrystallized using ethanol-dioxane mixture.

3-[phenyl]-1*H*-pyrazole-4-carbaldehyde[4-phenyl-1,3-thiazol-2-yl]hydrazone (P_{25})

FT-IR (cm^{-1}): 3320 (N-H-str), 3013 (C-H-str), 1610 (C=N), 1485 (C=C) (**Figure 4.3**); ^{1}H-NMR (DMSO-d_6): δ 11.8 (s,1H, pyrazole-NH), 8.19 (s,1H, N=CH), 8.04 (s, 1H, pyrazole-5H), 7.3-7.84 (m, 10H, Ar-H), 7.29 (s, 1H, thiazole-5H) (**Figure 4.4**); ^{13}C-

NMR: 168.6, 149.5, 137.22, 134.4, 131.1, 129.2, 129.0, 128.9, 128.6, 128.2, 126.1, 114.2, 103.8 (**Figure 4.5**). MS: m/z = 344 (M-1) (**Figure 4.6**). Anal. calcd. for $C_{19}H_{15}N_5S$: C, 66.07; H, 4.38; N, 20.27. Found: C, 66.10; H, 4.34; N, 20.23 %.

3-[4-methoxyphenyl]-1*H*-pyrazole-4-carbaldehyde[4-phenyl-1,3-thiazol-2-yl]hydrazone (P_{26})

FT-IR (cm^{-1}): 3406 (N-H-str), 3043 (C-H-str), 1615 (C=N), 1438 (C=C); ^{1}H-NMR (DMSO-d_6): δ 11.9 (s,1H, pyrazole-NH), 8.17 (s,1H, N=CH), 7.99 (s, 1H, pyrazole-5H), 7.08-7.84 (m, 9H, Ar-H), 7.29 (s, 1H, thiazole-5H), 3.83 (s, 3H, -OCH_3); ^{13}C-NMR: 168.6, 160.0, 149.2, 144.7, 137.6, 134.3, 130.0, 129.1, 128.7, 128.2, 126.1, 123.1, 114.7, 113.7, 103.8, 55.78. MS: m/z = 376 (M+1). Anal. calcd. for $C_{20}H_{17}N_5OS$: C, 63.98; H, 4.56; N, 18.65. Found: C, 63.94; H, 4.53; N, 18.60 %.

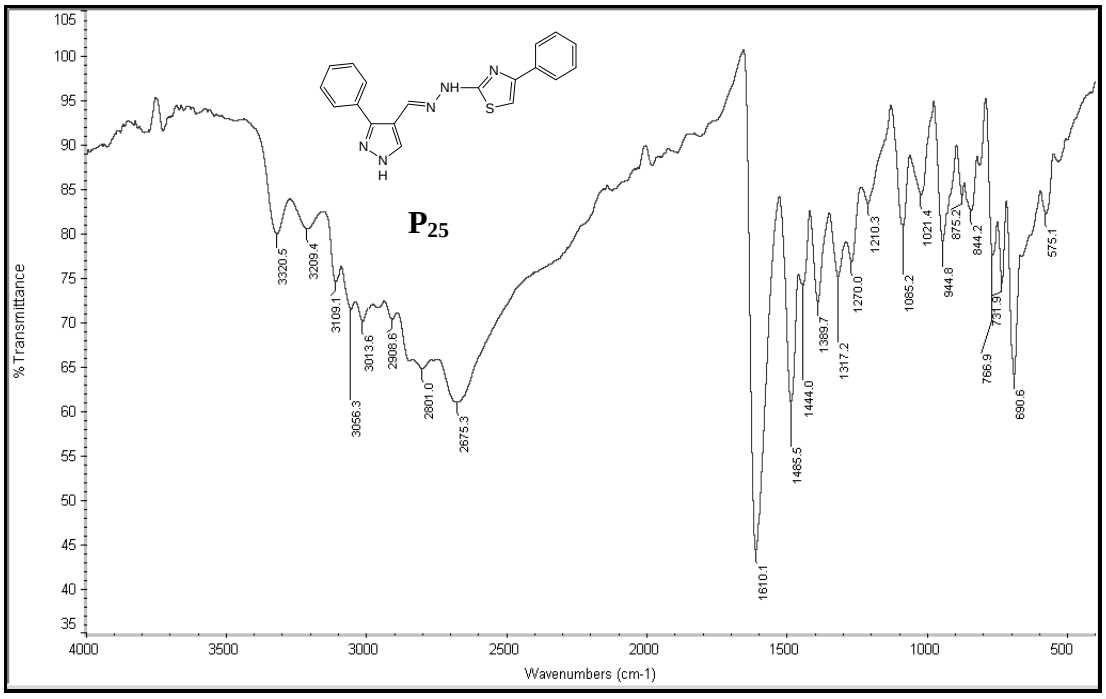


Figure 4.3 IR spectrum of compound **P_{25}**

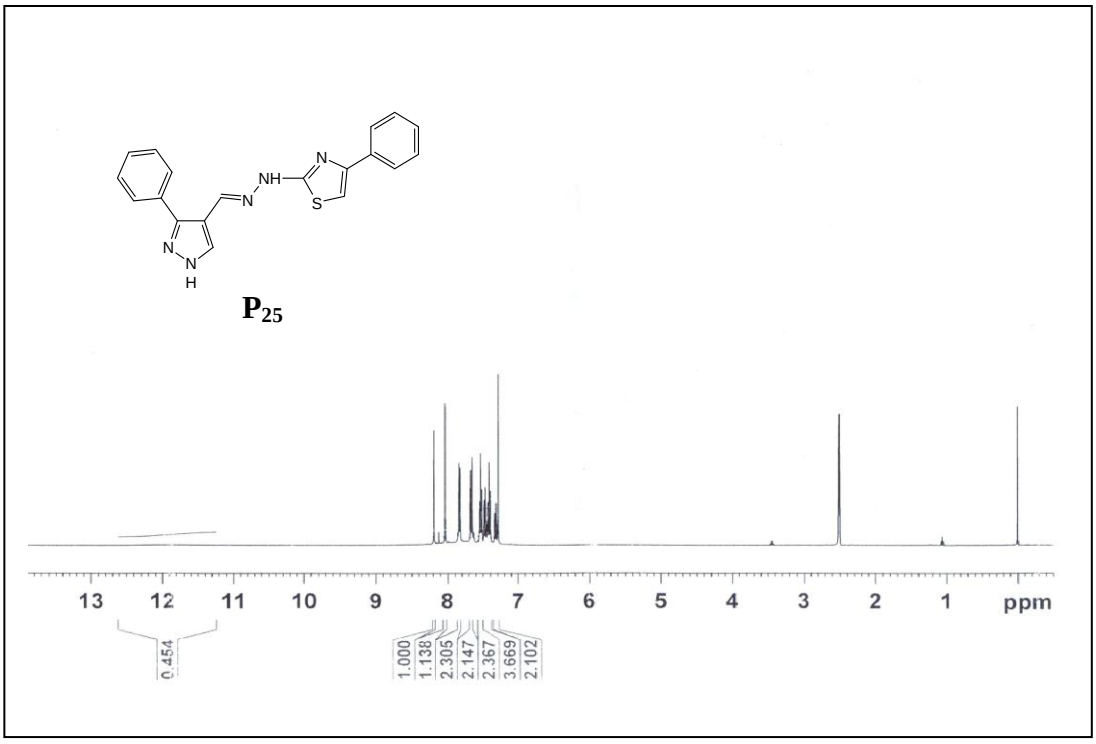


Figure 4.4 ^{1}H NMR spectrum of compound **P_{25}**

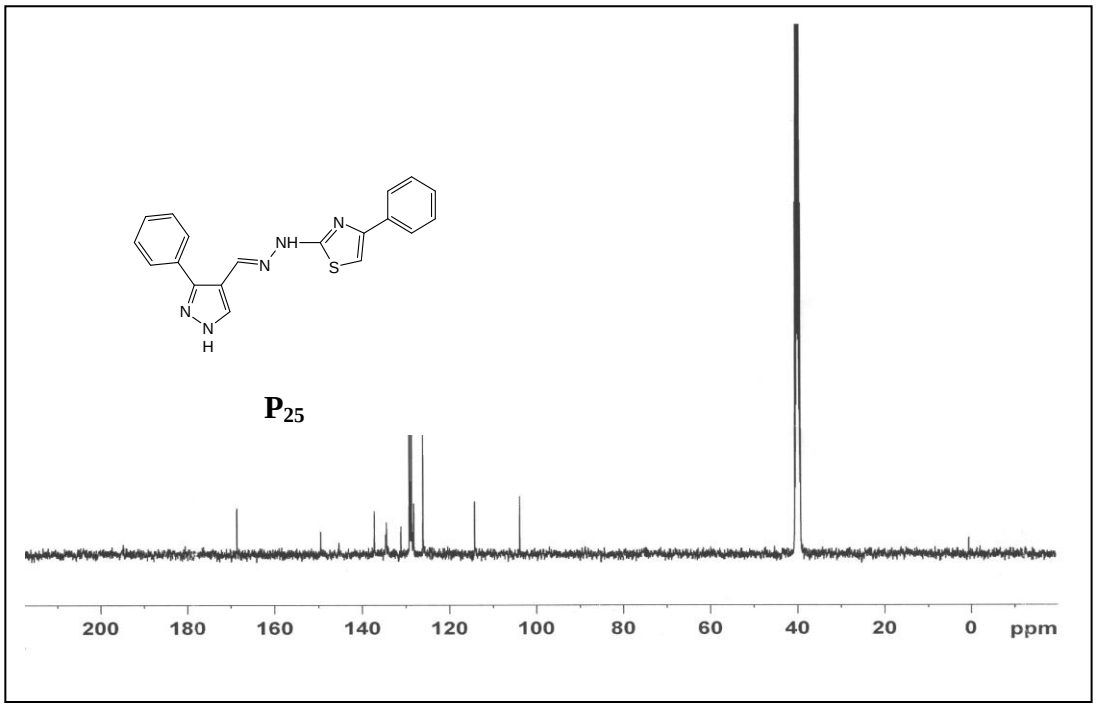


Figure 4.5 ^{13}C NMR spectrum of compound **P_{25}**

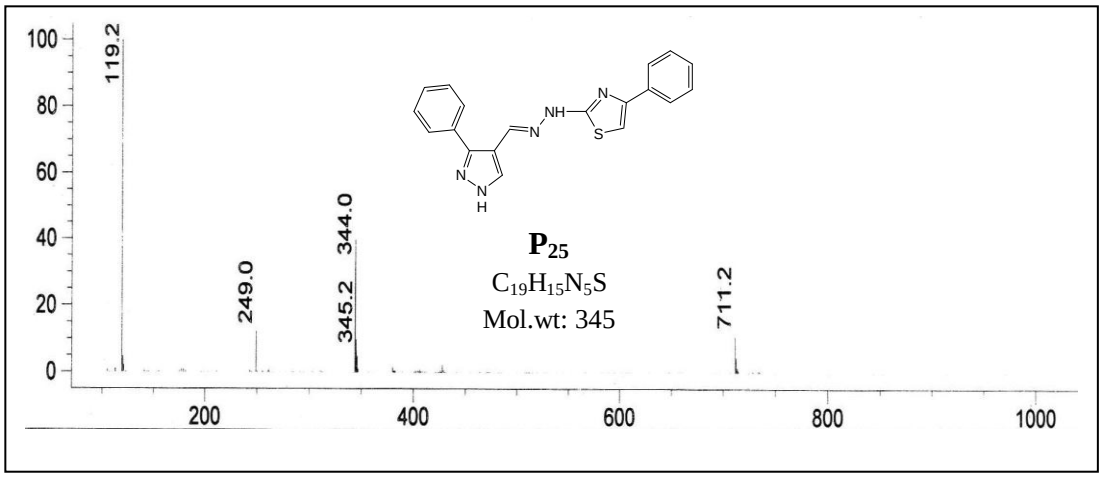


Figure 4.6 Mass spectrum of compound **P_{25}**

3-[4-fluorophenyl]-1*H*-pyrazole-4-carbaldehyde[4-phenyl-1,3-thiazol-2-yl] hydrazone (P_{27})

FT-IR (cm^{-1}): 3352 (N-H-str), 3040 (C-H-str), 1634 (C=N), 1501 (C=C), 1095 (C-F); ^{1}H-NMR (DMSO-d_6): δ 11.9 (s,1H, pyrazole-NH), 8.11 (s,1H, N=CH), 8.04 (s, 1H, pyrazole-5H), 7.28-7.84 (m, 9H, Ar-H), 7.27 (s, 1H, thiazole-5H); ^{13}C-NMR: 168.6, 150.1, 136.6, 134.8, 130.9, 130.8, 129.1, 128.1, 126.1, 116.1, 115.9, 114.3, 103.8. MS: m/z = 364 (M+1). Anal. calcd. for $C_{19}H_{14}FN_5S$: C, 62.79; H, 3.88; N, 19.27. Found: C, 62.75; H, 3.84; N, 19.23 %

3-[4-chlorophenyl]-1*H*-pyrazole-4-carbaldehyde[4-phenyl-1,3-thiazol-2-yl] hydrazone (P_{28})

FT-IR (cm^{-1}): 3397 (N-H-str), 3187 (C-H-str), 1622 (C=N), 1486 (C=C), 821 (C-Cl); ^{1}H-NMR (DMSO-d_6): δ 11.8 (s,1H, pyrazole-NH), 8.14 (s,1H, N=CH), 8.07 (s, 1H, pyrazole-5H), 7.29-7.85 (m, 9H, Ar-H), 7.28 (s, 1H, thiazole-5H); MS: m/z = 380 (M+1), 382 (M+2). Anal. calcd. for $C_{19}H_{14}ClN_5S$: C, 60.07; H, 3.71; N, 18.44. Found: C, 60.10; H, 3.68; N, 18.40 %.

3-[2,4-dichlorophenyl]-1*H*-pyrazole-4-carbaldehyde[4-phenyl-1,3-thiazol-2-yl] hydrazone (P_{29})

FT-IR (cm^{-1}): 3332 (N-H-str), 3085 (C-H-str), 1619 (C=N), 1485 (C=C), 943, 796 (C-Cl); ^{1}H NMR (DMSO-d_6): δ 11.9 (s,1H, pyrazole-NH), 8.09 (s,1H, N=CH), 7.89 (s, 1H, pyrazole-5H), 7.28-7.82 (m, 8H, Ar-H), 7.22 (s, 1H, thiazole-5H); ^{13}C NMR: 168.7, 150.1, 135.8, 134.7, 134.6, 134.3, 133.7, 132.3, 131.4, 129.5, 129.1, 128.0,

127.7, 126.0, 115.9, 103.7. MS: m/z = 414 (M^+), 416 (M+2), 418 (M+4). Anal. calcd. for $C_{19}H_{13}Cl_2N_5S$: C, 55.08; H, 3.16; N, 16.90. Found: C, 55.10; H, 3.11; N, 16.94%.

3-[phenyl]-1*H*-pyrazole-4-carbaldehyde[4-methoxyphenyl-1,3-thiazol-2-yl] hydrazone (P_{30})

FT-IR (cm^{-1}): 3372 (N-H-str), 3019 (C-H-str), 1605 (C=N), 1497 (C=C); ^{1}H NMR (DMSO-d_6): δ 11.9 (s,1H, pyrazole-NH), 8.20 (s,1H, N=CH), 8.04 (s, 1H, pyrazole-5H), 7.44-7.77 (m, 9H, Ar-H), 7.12 (s, 1H, thiazole-5H), 3.79 (s, 3H, -OCH_3); MS: m/z = 376 (M+1). Anal. calcd. for $C_{20}H_{17}N_5OS$: C, 63.98; H, 4.56; N, 18.65. Found: C, 63.95; H, 4.52; N, 18.61%.

3-[4-methoxyphenyl]-1*H*-pyrazole-4-carbaldehyde[4-methoxyphenyl-1,3-thiazol-2-yl]hydrazone (P_{31})

FT-IR (cm^{-1}): 3381 (N-H-str), 3013 (C-H-str), 1604 (C=N), 1497 (C=C); ^{1}H NMR (DMSO-d_6): δ 11.8 (s,1H, pyrazole-NH), 8.10 (s,1H, N=CH), 7.95 (s, 1H, pyrazole-5H), 7.75-7.77 (d, 2H, *J*=7.6 Hz, Ar-H), 7.58-7.60 (d, 2H, *J*=7.6 Hz, Ar-H), 7.08-7.10 (d, 2H, *J*=8.0 Hz, Ar-H), 6.96-6.98 (d, 2H, *J*=8.4 Hz, Ar-H), 7.10 (s, 1H, thiazole-5H), 3.79 and 3.83 (s, 6H, -OCH_3); ^{13}C NMR: 168.5, 160.0, 159.5, 130.0, 129.3, 127.5, 123.1, 114.7, 114.4, 114.2, 113.7, 101.8, 55.7, 55.6. MS: m/z = 406 (M+1). Anal. calcd. for $C_{21}H_{19}N_5O_2S$: C, 62.21; H, 4.72; N, 17.27. Found: C, 62.23; H, 4.69; N, 17.24%.

3-[4-fluorophenyl]-1*H*-pyrazole-4-carbaldehyde[4-methoxyphenyl-1,3-thiazol-2-yl] hydrazone (P_{32})

FT-IR (cm^{-1}): 3373 (N-H-str), 3013 (C-H-str), 1605 (C=N), 1498 (C=C), 1095 (C-F); ^{1}H NMR (DMSO-d_6): δ 11.77 (s,1H, pyrazole-NH), 8.08 (s,1H, N=CH), 8.0 (s, 1H, pyrazole-5H), 6.93-7.75 (m, 8H, Ar-H), 7.06 (s, 1H, thiazole-5H), 3.76 (s, 3H, -OCH_3); MS: m/z = 394 (M+1). Anal. calcd. for $C_{20}H_{16}FN_5OS$: C, 61.06; H, 4.10; N, 17.80. Found: C, 61.09; H, 4.13; N, 17.85%.

3-[4-chlorophenyl]-1*H*-pyrazole-4-carbaldehyde[4-methoxyphenyl-1,3-thiazol-2-yl] hydrazone (P_{33})

FT-IR (cm^{-1}): 3369 (N-H-str), 3010 (C-H-str), 1606 (C=N), 1497 (C=C), 828 (C-Cl); ^{1}H NMR (DMSO-d_6): δ 11.9 (s,1H, pyrazole-NH), 8.13 (s,1H, N=CH), 8.05 (s, 1H, pyrazole-5H), 6.91-7.75 (m, 8H, Ar-H), 7.09 (s, 1H, thiazole-5H), 3.77 (s, 3H, -

OCH_3); MS: m/z = 410 (M+1), 412 (M+2). Anal. calcd. for $C_{20}H_{16}ClN_5OS$: C, 58.60; H, 3.93; N, 17.09. Found: C, 58.57; H, 3.95; N, 17.12%.

3-[phenyl]-1*H*-pyrazole-4-carbaldehyde[4-fluorophenyl-1,3-thiazol-2-yl]hydrazone (P_{34})

FT-IR (cm^{-1}): 3348 (N-H-str), 3020 (C-H-str), 1613 (C=N), 1497 (C=C), 1094 (C-F); ^1H NMR (DMSO-d_6): δ 11.9 (s,1H, pyrazole-NH), 8.12 (s,1H, N=CH), 8.0 (s, 1H, pyrazole-5H), 7.19-8.0 (m, 8H, Ar-H), 7.23 (s, 1H, thiazole-5H); MS: m/z = 364 (M+1). Anal. calcd. for $C_{19}H_{14}FN_5S$: C, 62.79; H, 3.88; N, 19.27. Found: C, 62.76; H, 3.84; N, 19.23%.

3-[4-methoxyphenyl]-1*H*-pyrazole-4-carbaldehyde[4-fluorophenyl-1,3-thiazol-2-yl] hydrazone (P_{35})

FT-IR (cm^{-1}): 3342 (N-H-str), 3027 (C-H-str), 1613 (C=N), 1493 (C=C), 1091 (C-F); ^1H NMR (DMSO-d_6): δ 11.73 (s,1H, pyrazole-NH), 8.08 (s,1H, N=CH), 7.93 (s, 1H, pyrazole-5H), 7.05-7.87 (m, 8H, Ar-H), 7.22 (s, 1H, thiazole-5H), 3.81 (s, 3H, -OCH_3); MS: m/z = 394 (M+1). Anal. calcd. for $C_{20}H_{16}FN_5OS$: C, 61.06; H, 4.10; N, 17.80. Found: C, 61.02; H, 4.15; N, 17.83%.

3-[4-fluorophenyl]-1*H*-pyrazole-4-carbaldehyde[4-fluorophenyl-1,3-thiazol-2-yl] hydrazone (P_{36})

FT-IR (cm^{-1}): 3331 (N-H-str), 3028 (C-H-str), 1602 (C=N), 1493 (C=C), 1090 (C-F); ^1H NMR (DMSO-d_6): δ 11.8 (s,1H, pyrazole-NH), 8.09 (s,1H, N=CH), 8.01 (s, 1H, pyrazole-5H), 7.19-7.87 (m, 8H, Ar-H), 7.22 (s, 1H, thiazole-5H); MS: m/z = 382 (M+1). Anal. calcd. for $C_{19}H_{13}F_2N_5S$: C, 59.83; H, 3.44; N, 18.36. Found: C, 59.86; H, 3.41; N, 18.38%.

3-[4-chlorophenyl]-1*H*-pyrazole-4-carbaldehyde[4-fluorophenyl-1,3-thiazol-2-yl] hydrazone (P_{37})

FT-IR (cm^{-1}): 3336 (N-H-str), 3014 (C-H-str), 1602 (C=N), 1490 (C=C), 1089 (C-F), 832 (C-Cl); ^1H NMR (DMSO-d_6): δ 11.8 (s,1H, pyrazole-NH), 8.09 (s,1H, N=CH), 8.04 (s, 1H, pyrazole-5H), 7.19-7.87 (m, 8H, Ar-H), 7.23 (s, 1H, thiazole-5H); MS: m/z = 398 (M+1), 400 (M+2). Anal. calcd. for $C_{19}H_{13}ClFN_5S$: C, 57.36; H, 3.29; N, 17.60. Found: C, 57.33; H, 3.25; N, 17.63%.

3-[2,4-dichlorophenyl]-1*H*-pyrazole-4-carbaldehyde[4-fluorophenyl-1,3-thiazol-2-yl] hydrazone (P_{38})

FT-IR (cm^{-1}): 3407 (N-H-str), 3020 (C-H-str), 1606 (C=N), 1496 (C=C), 1093 (C-F), 948, 833 (C-Cl); ^1H NMR (DMSO-d_6): δ 11.75 (s,1H, pyrazole-NH), 8.06 (s,1H, N=CH), 7.87 (s, 1H, pyrazole-5H), 7.19-7.83 (m, 7H, Ar-H), 7.17 (s, 1H, thiazole-5H); MS: m/z = 432 (M^+), 434 (M+2), 436 (M+4). Anal. calcd. for $C_{19}H_{12}Cl_2FN_5S$: C, 52.79; H, 2.80; N, 16.20. Found: C, 52.82; H, 2.84; N, 16.25%.

4.4 PHARMACOLOGY

4.4.1 Antimicrobial activity

The *in vitro* antimicrobial activity of newly synthesized compounds **P_{25-38}** were determined by "well-plate" method in Mueller-Hinton Agar as explained in **Chapter 2** by measuring the diameter of inhibition zone (mm). In this work *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 were used to investigate the antibacterial activity whereas *Aspergillus flavus* ATCC 15547, *Chrysosporium keratinophilum* ATCC 14803 and *Candida albicans* MTCC 227 were used to study antifungal activity. These cultures are obtained from the Department of Microbiology, Kuvempu University, Shimoga, India. All fungi strains were maintained on potato dextrose agar (PDA) at ± 25 ˚C.

4.4.2 Acute toxicity and behavioral studies

Acute toxicity studies have been carried out at Department of Biochemistry, Kuvempu University, Shimoga, India. The animals used in the present study such as Swiss albino mice weighing 20-25 g were procured from Veterinary College, Bangalore, Karnataka, India. Animals were maintained in colony cages at 25±2 ˚C, relative humidity of 45-55 %, maintained under 12 h light and dark cycle and were fed with standard animal feed and water. Animals were maintained under standard conditions in an animal house. The entire animals were acclimatized for a week before use.

The acute toxicity test was carried out according to the Organization for Economic Co-operation and Development (OECD) guidelines to establish the effective dose of test compounds after obtaining ethical clearance from Animal Ethics Committee of S.J.M. College of Pharmacy, Chitradurga-577501, Karnataka, India.

Albino mice of either sex weighing between 20-25 g were grouped starved for 24 h with water prior to test. On the day of the experiment, animals were administered with different compounds to different groups in an increasing dose of 250, 500, 1000, 1500 2000, 3000 and 4000 mg/kg body weight orally. The acute toxic symptoms and the behavioral changes produced by the test compounds were observed continuously for 4 h and at 8 h, 12 h and 24 h onset of toxic symptoms and gross behavioral changes were also recorded (Segovia et al. 2002).

4.4.3 Antioxidant Studies (*in vitro*)

Antioxidant studies was carried out at Department of Biochemistry, Kuvempu University, Shimoga, India.

DPPH radical scavenging assay

The free radical scavenging activity of test samples $\mathbf{P_{25\text{-}38}}$ was measured by DPPH (2,2-diphenyl-1-picrylhydrazyl) according to literature (Brand-Williams et al. 1995). 100 µg/mL of each test sample and standard BHT (Butylated hydroxytoluene) was taken in different test tubes and the volume was adjusted to 1 mL using methanol. Freshly prepared 2 mL of 0.1 mM DPPH solution was mixed and vortexed thoroughly and left in dark for 30 min. The absorbance of stable DPPH• was measured at 517 nm. The DPPH control (containing no sample) was prepared using the same procedure. Radical scavenging activity was expressed as the inhibition percentage and was calculated using the equation of DPPH radical scavenging activity.

DPPH radical scavenging activity (%) = $(A_{Control} - A_{Sample}/A_{Control}) \times 100$.

Where $A_{Control}$ is the absorbance of control, A_{Sample} is the absorbance of sample /standard BHT (Butylated hydroxytoluene).

Nitric oxide scavenging activity

Sodium nitroprusside in aqueous solution at physiological pH spontaneously produce nitric oxide, which reacts with oxygen to produce nitrite ions, which can be determined by using the Griess Illosvoy reaction of Garrat (1964). Griess Illosvoy reagent was slightly modified using naphthylethylenediamine dihydrochloride (0.1 %, w/v) instead of 1-naphthylamine (5 %). The reaction mixture (3 mL) containing 2 mL of 10 mM sodium nitroprusside, 0.5 mL of phosphate buffer saline (pH 7.4, 0.01 M) and 0.5 mL (100 µg/mL) of different test samples $\mathbf{P_{25\text{-}38}}$ was incubated for 150 min at

25 ˚C. The reaction mixture (0.5 mL) was mixed with 1 mL of sulphanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 min for the completion of diazotization reaction. The resultant mixture was then added with 1 mL of naphthylethylenediamine dihydrochloride (0.1%) and allowed to stand for 30 min in diffused light. The absorbance of the pink coloured chromophore was measured at 540 nm against the corresponding blank solution. Scavenging capacity of test samples was compared with standard drug BHT.

Nitric Oxide scavenging activity (%) = $(A_{Control} - A_{Sample}/A_{Control}) \times 100$

Where $A_{Control}$ is the absorbance of control, A_{Sample} is the absorbance of sample/ standard BHT (Butylated hydroxytoluene).

Hydroxyl radical (OH•) scavenging activity

The hydroxyl radical scavenging activity was determined according to the modified method of Chung (1997). The Fenton reaction mixture containing 200 µL of 10mM $FeSO_4.7H_2O$, 200 µL of 10 mM EDTA (Ethylene diaminetetraacetic acid) and 200 µL of 10 mM 2-deoxyribose was mixed with 1.2 mL of 0.1 M phosphate buffer (pH 7.4) containing 100µg/mL of different concentration of test samples **P_{25-38}**. Freshly prepared 200 µL of 10 mM H_2O_2 was added to the mixture and incubated for 4 h at 37 ˚C. Later, 1 mL of 2.8% TCA (Trichloroacetic acid) and 1 mL of 1% TBA (Thio-barbituric acid) were added and placed in boiling water bath for 10 min. The mixture was brought to room temperature and centrifuged at 2000 rpm for 5 min and absorbance was measured at 532 nm. The percentage of hydroxyl radical scavenging activity was calculated by employing the following formula and compared with the standard BHT.

Hydroxyl radical scavenging activity (%) = $(A_{sample}/ A_{blank}) \times 100$

Where A_{Sample} is the absorbance of sample, A_{blank} is the absorbance of blank.

Superoxide anion scavenging activity

Using the method of Nishikimi (1972) the superoxide anion scavenging activity was determined, wherein a mixture of 1 mL of NBT (Nitroblue tetrazolium) (156_MNBT in 100 mM phosphate buffer, pH 7.4) 1mL NADH (468_M in 100 mM phosphate buffer, pH 7.4) and 0.1 mL (100 µg/mL) of test samples **P_{25-38}** was prepared in water. To this mixture 100 µL of PMS (Phenazine methosulphate)

solution (60_M PMS in 100 mM phosphate buffer, pH 7.4) was added to start the reaction. The reaction mixture was incubated at 25 ˚C for 5 min, and the absorbance was measured at 560 nm against blank. Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging activity. BHT was used as standard.

Superoxide scavenging activity (%) = $(A_{Control} - A_{Sample} / A_{Control}) \times 100$

Where $A_{Control}$ is the absorbance of control, A_{Sample} is the absorbance of sample/ standard BHT (Butylated hydroxytoluene).

4.5 RESULTS AND DISCUSSION

4.5.1 Chemistry

The structures of the synthesized compounds **P_{25-38}** were characterized by IR, NMR, mass spectral and elemental analyses. Analytical and spectral data of all synthesized compounds were in full agreement with the proposed structures. IR spectrum of compound **P_{25}** showed absorption bands at 3320, 3013, 1610, 1485 and 1085 cm^{-1} which is due to the N-H, C-H, C=N, C=C and C-S groups respectively. The ^{1}H-NMR spectrum of **P_{25}** showed a singlet at δ 11.8 corresponds to pyrazole N-H proton. An N=CH proton resonated as singlet at δ 8.1. Thiazole-5H proton and pyrazole-5H proton resonated as singlet at δ 7.29 and 8.0 respectively. The mass spectrum of **P_{25}** showed molecular ion peak at m/z = 344 (M-1), which is in agreement with the molecular formula $C_{19}H_{15}N_5S$. Similarly the spectral values for all the compounds and C, H, N analyses are given in the experimental part and the characterization data has been provided in **Table 4.1**.

4.5.2 Biological activity

The *in vitro* antibacterial activity of newly synthesized compounds (**P_{25-38}**) were determined by "well-plate" method (Arthington-Skaggs et al. 2000; Rocha et al. 1995). In this work, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* were used to investigate the activity. The test compounds were dissolved in dimethyl sulfoxide (DMSO) at concentrations of 1000 and 500 μg/mL. The antibacterial screening revealed that some of the tested compounds showed good inhibition against various tested microbial strains (**Table 4.2**). The results indicated that among the tested compounds, **P_{29}** and **P_{38}** showed excellent activity against all the

bacterial strains at concentrations of 1 and 0.5 mg/mL compared to standard drug Streptomycin.

Table 4.1 Characterization data of the compounds $\mathbf{P_{25\text{-}38}}$

Compounds	R	Ar	Molecular formula (Mol.wt.)	Yield (%)	M.p. (^oC)
$\mathbf{P_{25}}$	H	C_6H_5	$C_{19}H_{15}N_5S$ (345)	86	209-210
$\mathbf{P_{26}}$	4-OCH_3	C_6H_5	$C_{20}H_{17}N_5OS$ (375)	72	220-221
$\mathbf{P_{27}}$	4-F	C_6H_5	$C_{19}H_{14}FN_5S$ (363)	88	125-127
$\mathbf{P_{28}}$	4-Cl	C_6H_5	$C_{19}H_{14}ClN_5S$ (379)	74	150-152
$\mathbf{P_{29}}$	2,4-Cl	C_6H_5	$C_{19}H_{13}Cl_2N_5S$ (414)	87	180-183
$\mathbf{P_{30}}$	H	4-OCH_3-C_6H_4	$C_{20}H_{17}N_5OS$ (375)	89	229-231
$\mathbf{P_{31}}$	4-OCH_3	4-OCH_3-C_6H_4	$C_{21}H_{19}N_5O_2S$ (405)	85	239-241
$\mathbf{P_{32}}$	4-F	4-OCH_3-C_6H_4	$C_{20}H_{16}FN_5OS$ (393)	87	240-242
$\mathbf{P_{33}}$	4-Cl	4-OCH_3-C_6H_4	$C_{20}H_{16}ClN_5OS$ (409)	82	243-245
$\mathbf{P_{34}}$	H	4-F-C_6H_4	$C_{19}H_{14}FN_5S$ (363)	84	229-231
$\mathbf{P_{35}}$	4-OCH_3	4-F-C_6H_4	$C_{20}H_{16}FN_5OS$ (393)	88	241-243
$\mathbf{P_{36}}$	4-F	4-F-C_6H_4	$C_{19}H_{13}F_2N_5S$ (381)	85	258-260
$\mathbf{P_{37}}$	4-Cl	4-F-C_6H_4	$C_{19}H_{13}ClFN_5S$ (397)	88	252-254
$\mathbf{P_{38}}$	2,4-Cl	4-F-C_6H_4	$C_{19}H_{12}Cl_2FN_5S$ (432)	72	232-233

The compounds $\mathbf{P_{30}}$, $\mathbf{P_{31}}$, $\mathbf{P_{33}}$, $\mathbf{P_{36}}$ & $\mathbf{P_{37}}$ showed good activity against *Escherichia coli* and *Staphylococcus aureus* whereas found to be moderately active against *Pseudomonas aeruginosa*. The inhibitory effect of $\mathbf{P_{34}}$ was more against *Escherichia coli* than compared to *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. $\mathbf{P_{26}}$ was found to be moderately active only against *Staphylococcus aureus* than compared to rest of tested strains. Compounds $\mathbf{P_{32}}$ and $\mathbf{P_{35}}$ failed to exhibit antibacterial activity against the tested microorganisms.

Newly synthesized compounds (**P_{25-38}**) were also screened for their antifungal activity against *Aspergillus flavus*, *Chrysosporium keratinophilum* and *Candida albicans*. Fluconazole was used as standard drug. The compounds were dissolved in DMSO and antimicrobial activity was determined by "well-plate" method (MacLowry et al. 1970; Portillo et al. 2001) at concentration of 1000 and 500 μg/mL. Results of antifungal studies have been presented in **Table 4.3**.

Table 4.2 Antibacterial activity of compounds **P_{25-38}**

Compound code	Zone of inhibition(mm)					
	Escherichia coli		***Staphylococcus aureus***		***Pseudomonas aeruginosa***	
Conc. in μg/mL	1000	500	1000	500	1000	500
P_{25}	07±0.2	05±0.2	08±0.2	06±0.2	05±0.1	03±0.2
P_{26}	06±0.1	02±0.2	09±0.2	07±0.2	08±0.2	06±0.1
P_{27}	09±0.2	06±0.2	06±0.1	04±0.2	07±0.2	05±0.2
P_{28}	06±0.2	04±0.1	08±0.1	05±0.1	08±0.2	04±0.2
P_{29}	13±0.2	10±0.2	14±0.1	12±0.1	16±0.1	13±0.1
P_{30}	10±0.1	07±0.1	11±0.2	08±0.2	10±0.1	08±0.2
P_{31}	11±0.2	09±0.2	09±0.2	07±0.1	10±0.2	07±0.2
P_{32}	00	00	00	00	00	00
P_{33}	10±0.2	07±0.2	10±0.1	08±0.1	07±0.2	03±0.2
P_{34}	13±0.2	10±0.1	09±0.2	07±0.1	10±0.2	08±0.1
P_{35}	00	00	00	00	00	00
P_{36}	10±0.2	08±0.2	08±0.1	06±0.2	09±0.2	07±0.1
P_{37}	09±0.2	07±0.1	08±0.2	07±0.2	10±0.1	07±0.2
P_{38}	15±0.2	12±0.2	12±0.1	11±0.2	16±0.2	13±0.1
Streptomycin (Std.)	16±0.2	10±0.1	15±0.2	10±0.2	16±0.2	13±0.2

The results indicated that among the tested compounds, **P_{29}** and **P_{38}** showed good activity against *Aspergillus flavus*, *Chrysosporium keratinophilum* at concentrations of 1000 and 500 μg/mL compared to standard drug Fluconazole. **P_{27}** and **P_{36}** were found to be active only against *Aspergillus flavus* whereas slightly active against rest of two fungal strains. None of the synthesized compounds were superior

to the standard drug Fluconazole against various tested microbial strains, however the antifungal activities of some of the compounds are comparable to those of standard.

Table 4.3 Antifungal activity of compounds $\mathbf{P_{25\text{-}38}}$

Compound code	**Zone of inhibition(mm)**					
	Aspergillus flavus		***Chrysosporium keratinophilum***		***Candida Albicans***	
Con in µg/mL	1000	500	1000	500	1000	500
$\mathbf{P_{25}}$	04±0.2	02±0.2	06±0.2	05±0.2	05±0.1	03±0.2
$\mathbf{P_{26}}$	05±0.1	03±0.1	04±0.2	03±0.1	03±0.1	01
$\mathbf{P_{27}}$	06±0.1	05±0.2	04±0.1	03±0.1	05±0.1	04±0.1
$\mathbf{P_{28}}$	06±0.2	04±0.1	05±0.1	03±0.1	04±0.1	03±0.1
$\mathbf{P_{29}}$	11±0.1	09±0.1	09±0.1	07±0.1	10±0.2	08±0.2
$\mathbf{P_{30}}$	00	00	00	00	00	00
$\mathbf{P_{31}}$	03±0.1	01	02±0.1	00	03±0.1	00
$\mathbf{P_{32}}$	00	00	00	00	00	00
$\mathbf{P_{33}}$	00	00	00	00	00	00
$\mathbf{P_{34}}$	04±0.1	01	03±0.1	01	04±0.1	02±0.1
$\mathbf{P_{35}}$	00	00	00	00	00	00
$\mathbf{P_{36}}$	06±0.2	05±0.1	04±0.2	03±0.1	05±0.1	03±0.1
$\mathbf{P_{37}}$	04±0.2	02±0.1	03±0.2	01	05±0.2	02
$\mathbf{P_{38}}$	10±0.1	08±0.1	09±0.1	07±0.1	11±0.1	08±0.1
Fluconazole (Std.)	13±0.2	10±0.1	17±0.2	15±0.2	22±0.2	20±0.2

Antioxidant activity of compounds **($\mathbf{P_{25\text{-}38}}$)** were examined by performing DPPH radical, nitric oxide, hydroxyl radical and superoxide anion scavenging activity (**Table 4.4**). Antioxidant studies reveals that all the compounds showed significant DPPH scavenging activity (>80 %) except for $\mathbf{P_{27}}$, $\mathbf{P_{28}}$, $\mathbf{P_{29}}$, $\mathbf{P_{30}}$. Nitric oxide activity, OH radical scavenging activity and superoxide scavenging activity was significantly high in case of $\mathbf{P_{26}}$, $\mathbf{P_{34}}$ and $\mathbf{P_{38}}$. Although $\mathbf{P_{30}}$ compound showed significant nitric oxide and OH radical scavenging activity, it fails to give significant value for super

oxide scavenging activity. The graphical representation of scavenging activity (%) of compounds has been presented in **Figure 4.7**.

Table 4.4 Scavenging activity (%) of compounds $\mathbf{P_{25\text{-}38}}$

Compounds	DPPH Assay	Nitric oxide Assay	OH Radical Assay	Superoxide anion scavenging activity
$\mathbf{P_{25}}$	80.88	60.46	60.46	66.46
$\mathbf{P_{26}}$	86.69	77.49	77.49	82.49
$\mathbf{P_{27}}$	68.22	58.22	58.22	62.22
$\mathbf{P_{28}}$	70.00	60.24	60.24	64.24
$\mathbf{P_{29}}$	74.55	64.56	64.56	62.32
$\mathbf{P_{30}}$	76.62	76.62	76.62	68.56
$\mathbf{P_{31}}$	83.06	61.46	61.46	63.24
$\mathbf{P_{32}}$	82.56	62.16	62.16	65.16
$\mathbf{P_{33}}$	84.47	64.42	64.42	60.64
$\mathbf{P_{34}}$	86.08	72.24	72.24	76.24
$\mathbf{P_{35}}$	84.10	64.10	64.10	68.46
$\mathbf{P_{36}}$	85.11	66.11	66.11	66.87
$\mathbf{P_{37}}$	84.74	62.56	62.56	64.26
$\mathbf{P_{38}}$	86.18	74.34	74.34	78.56
BHT	92.42	88.56	89.56	85.86

As regards the relationships between the structure of the heterocyclic scaffold and the detected antimicrobial properties, clearly, introduction of 2,4-dichloro substitution on pyrazole ring in $\mathbf{P_{29}}$ and $\mathbf{P_{38}}$ enhanced the antimicrobial properties. It was observed that the introduction of chloro, methoxy and fluoro groups on pyrazole as well as in thiazole ring is favored too for antibacterial activity (**$\mathbf{P_{26}}$, $\mathbf{P_{30}}$, $\mathbf{P_{31}}$, $\mathbf{P_{33}}$, $\mathbf{P_{34}}$, $\mathbf{P_{36}}$ & $\mathbf{P_{37}}$)** except in case of $\mathbf{P_{32}}$ and $\mathbf{P_{35}}$.

The acute oral toxicity study for compounds $\mathbf{P_{29}}$ and $\mathbf{P_{38}}$ was also carried out by following the OECD guidelines No. 420. The experimental studies revealed that the compounds have revealed good safety profile till the uppermost dose (2000

mg/kg). Further, no significant gross behavioral changes were observed in experimental animals except in the 3000 and 4000 mg/kg of all organic compounds, which showed depression on the first day and dead on second day.

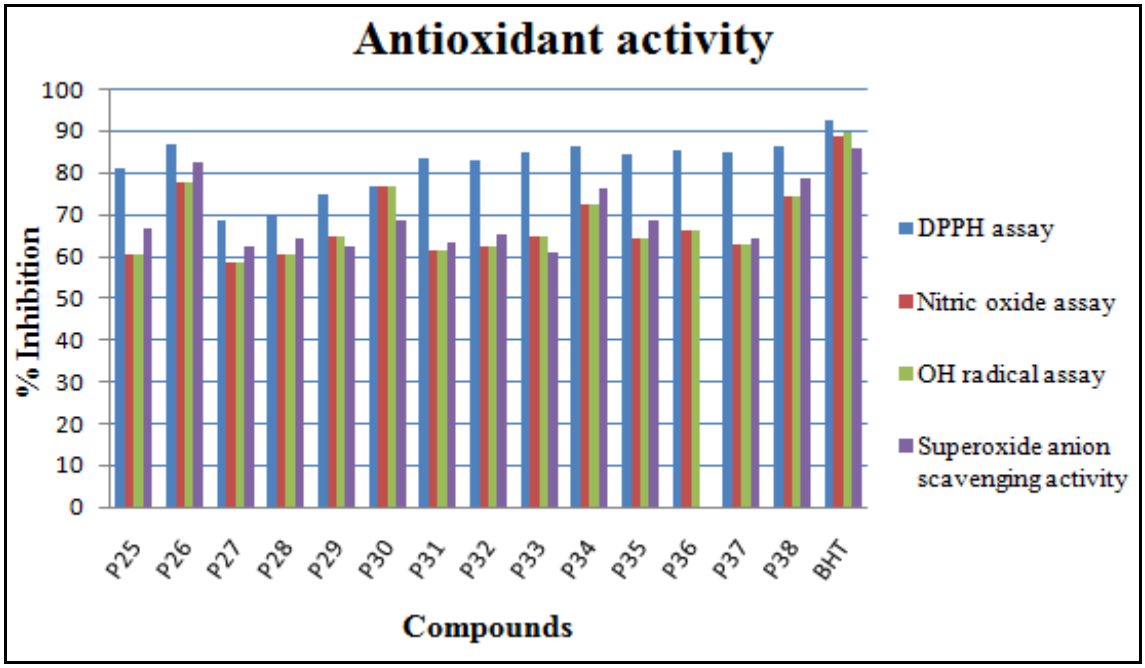


Figure 4.7 Graphical representation of scavenging activity (%) of $\mathbf{P_{25\text{-}38}}$

4.6 CONCLUSIONS

A series of new thiazoles derivatives containing pyrazole ring were synthesized and subjected for their antimicrobial and antioxidant activities. The antibacterial result revealed that among the synthesized compounds, $\mathbf{P_{29}}$ and $\mathbf{P_{38}}$ showed excellent activity against all the bacterial strains at concentrations of 1000 and 500 µg/mL compared to standard drug Streptomycin. The antifungal results also indicated that the compounds $\mathbf{P_{29}}$ and $\mathbf{P_{38}}$ exhibited good activity against all the fungal strains at concentrations of 1000 and 500 µg/mL compared to standard drug Fluconazole, while other compounds were found to be moderately active.

Antioxidant studies reveals that all the compounds showed significant amount of DPPH activity (>80 %) except for $\mathbf{P_{27}}$, $\mathbf{P_{28}}$, $\mathbf{P_{29}}$, $\mathbf{P_{30}}$. Nitric oxide activity, OH radical scavenging activity and superoxide scavenging activity was significantly high in case of $\mathbf{P_{26}}$, $\mathbf{P_{34}}$ and $\mathbf{P_{38}}$.

CHAPTER - 5

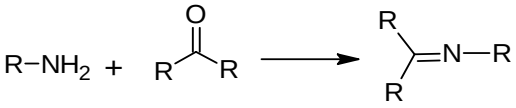
SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF SOME NEW PYRAZOLE BASED SCHIFF-BASES

Abstract

This chapter describes a detailed literature survey on Schiff bases derived from 1,2,4-triazole analogues. It also includes the synthesis and characterization of Schiff bases carrying triazole and pyrazole entities. Antimicrobial studies of the synthesized compounds have also been discussed in this chapter.

5.1 INTRODUCTION

Compounds with the structure of –C=N– (azomethine group) (**S-5.1**) are known as Schiff bases, which are usually synthesized by the condensation of primary amines and active carbonyl groups according to the following schemes.



S-5.1

where 'R' may be an alkyl or aryl group. Schiff bases that contain aryl substituent are substantially more stable and more readily synthesized, while those which contain alkyl substituents are relatively unstable. Schiff bases of aliphatic aldehydes are relatively unstable and readily polymerizable, while those of aromatic aldehydes having effective conjugation are more stable.

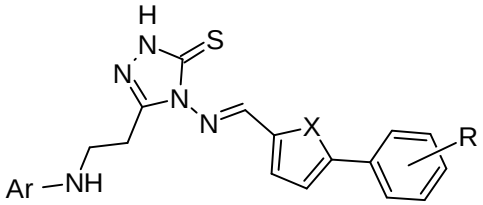
Schiff bases gain importance in medicinal and pharmaceutical field due to the most versatile organic synthetic intermediates for the synthesis of many heterocyclic ring systems like azetidinones (Kalsi et al. 1990), thiazolidinones (Kucukguzel et al. 2006) etc.

The chemistry of 1,2,4-triazole and their fused heterocyclic derivatives have received considerable attention owing to their synthetic and effective biological importance. 1,2,4-triazole moieties have incorporated into variety of therapeutically interesting drug candidates including antiviral (Ribavarin), anti migraine **(**Rizatriptan), antifungal (Fluconazole), antianxiety compounds (Alprazolam). Moreover sulphur containing heterocyclic compounds represent an important group of compounds that are promising on practical application (Holla et al. 2003; Wu et al. 2007 and Isloor et al. 2010). The pharmacological importance of heterocycles derived from 1,2,4-triazole paved the way towards active research in triazole chemistry.

The chemistry of Schiff bases derived from 1,2,4-triazole analogues has been an interesting field of study for a long time. It is well known from the literature that,

Schiff bases derived from 1,2,4-triazoles displayed excellent biological properties. In particular, they show antitubercular (Patole et al. 2006), antimalarials, anticonvulsant, anti-inflammatory (Bhandari et al. 2008), antibacterial, antifungal (Isloor et al. 2009) and anticancer (Sunil et al. 2011) properties. They are important structures in the medicinal and pharmaceutical fields and it has been suggested that the azomethine linkage might be responsible for the biological activities displayed by Schiff bases.

Kalluraya et al. (1996) reported the synthesis and biological activities of 3-substituted-anilinomethyl-4-(5-substituted-2-furfurylidene)amino-1,2,4-triazole-5-thiones (**S-5.2**). The newly synthesized compounds were screened for their antibacterial and antifungal activities.

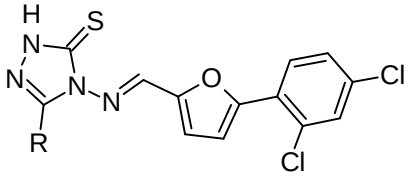


S-5.2

Where Ar = 4-CH_3-C_6H_4, 2-OCH_3-C_6H_4

R = 4-Cl, 4-NO_2; X = O, S

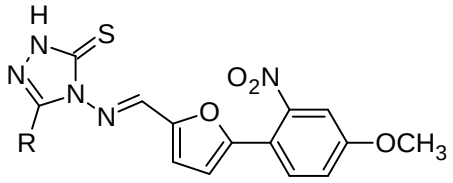
Holla et al. (2000) synthesized some 3-substituted-4-[5-(2,4-dichlorophenyl)-2-furfurylidine]amino-5-mercapto-1,2,4-triazoles (**S-5.3**) and tested for their antimicrobial activity.



S-5.3

Where R = H, CH_3, C_2H_5, C_3H_7, Ph, 4-chlorophenoxymethyl, 2-chlorophenoxymethyl, 4-chloro-3-methyl-phenoxymethyl, 2,4-dichlorophenoxymethyl, 3,4-dimethylphenoxy methyl.

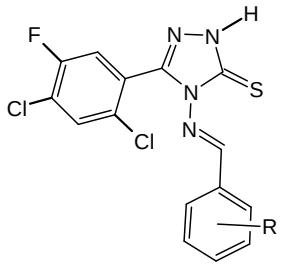
Holla et al. (2003) synthesized some 3-substituted 4-[5-(4-methoxy-2-nitro phenyl)-2-furfurylidene]amino-5-mercapto-1,2,4-triazoles (**S-5.4**) and investigated their anticancer properties. Some of the compounds were found to be biologically potent.



S-5.4

Where R = H, CH_3, C_2H_5, C_3H_7, 2-Cl-$C_6H_4OCH_2$, 4-Cl-$C_6H_4OCH_2$, 2,4-Cl2-$C_6H_3OCH_2$, 4-Cl-3-Me-$C_6H_3OCH_2$

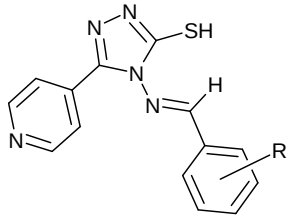
Karthikeyan et al. (2006) reported the synthesis and biological activity of Schiff bases (**S-5.5**) bearing 2,4-dichloro-5-fluorophenyl moiety. The antimicrobial results revealed that most of the Schiff bases showed good antibacterial and antifungal activity.



S-5.5

Where R = 4-$N(CH_3)_2$, 4-Cl , 3,4-O-CH_2-O-

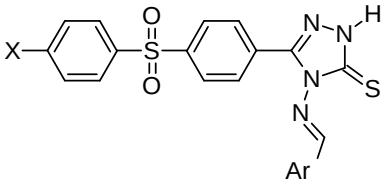
Khanmohammadi et al. (2008) synthesized a series of new Schiff base hydrazones (**S-5.6**) by condensation reaction of 4-amino-3-(4-pyridine)-5-mercapto-1,2,4-triazole with various aldehydes and/or dialdehydes and investigated their antimicrobial properties. The results of such studies revealed that Schiff bases with 4-chloro, 4-methyl, 4-methoxy, 2,4-dichloro and 2-hydroxy substituents showed good inhibition against *Staphylococcus aureus.*



S-5.6

Where R = 4-NO_2, 4-Cl, 4-Me, 4-OMe, 2,3-di-Cl, 2,4-di-Cl, 2,6-di-Cl, 2-OH, 3-OMe-2-OH, 4- OMe-2-OH

Almajan et al. (2009) reported the synthesis of some new 4-substituted 5-[4-(4-X-phenylsulfonyl)phenyl]-2,4-dihydro-3*H*-1,2,4-triazole-3-thiones (**S-5.7**). The potential antibacterial effects of the synthesized compounds were investigated using the *Acinetobacter baumanii* ATCC 19606; *Citrobacter freundii* ATCC 8090; *Pseudomonas aeruginosa* ATCC 9027; *Enterococcus faecalis* ATCC 19433; *Staphylococcus aureus* ATCC 12600; *Staphylococcus epidermidis* ATCC 14990; *Bacillus subtilis* ATCC 6633 strains. Some of them exhibited promising activities against *Acinetobacter baumanii* and *Bacillus subtilis.*

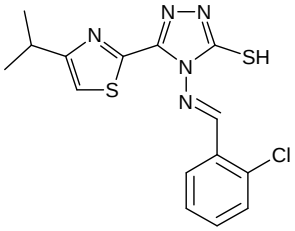


S-5.7

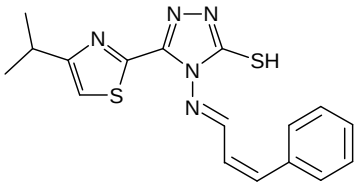
Where X = H, Cl, Br

Ar = C_6H_5, 2-OMe-C_6H_4, 3-NO_2-C_6H_4, 4-$N(CH_3)_2$-C_6H_4, Furyl

Suresh Kumar and his co-workers (2010) described the synthesis of some novel 2-substituted-5-[isopropylthiazole] clubbed 1,2,4-triazole and 1,3,4-oxadiazoles as potential antimicrobial and antitubercular agents. The antimicrobial activity of the Schiff base series revealed that all the tested compounds possessed moderate to good inhibition, compounds (**S-5.8**) and (**S-5.9**) showed comparatively good activity against all tested microbial strains and excellent inhibition towards *Micobacterium tuberculosis* H_{37}Rv at MIC 4 mg/mL.

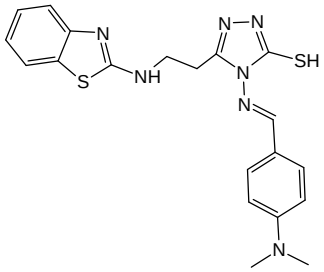


S-5.8

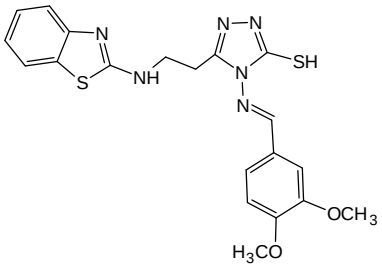


S-5.9

Soni et al. (2010) reported the synthesis and antimicrobial properties of Schiff bases of benzothiazole derivatives. From the activity studies, it was concluded that among all benzothiazole derivatives, (**S-5.10**) showed maximum antibacterial whereas (**S-5.11**) showed maximum antifungal activity.

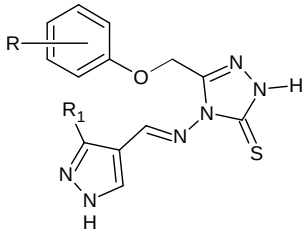


S-5.10



S-5.11

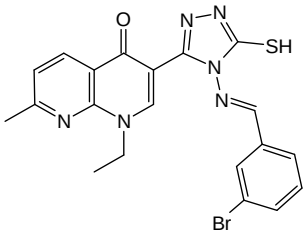
Sunil et al. (2011) synthesized some new Schiff bases (**S-5.12**) and evaluated for *in vitro* cytotoxic properties against Hep G2 cell lines. The results revealed that the compounds bearing halogen atoms showed good cytotoxic activity.



S-5.12

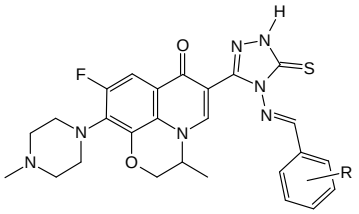
Where R = H, 2-Cl, 2-CH_3, 4-CH_3
R_1 = 4-Cl-C_6H_4, 4-F-C_6H_4

Aggarwal et al. (2011) reported the synthesis of nalidixic acid based 1,2,4-triazole derivatives and evaluated for *in vitro* antimicrobial activity. The antimicrobial results revealed that, among the azomethine derivatives of 1,2,4-triazole, compound (**S-5.13**) was the most potent against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*.



S-5.13

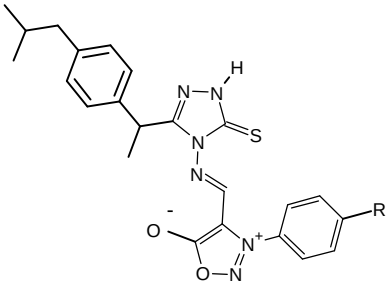
Hu et al. (2012) reported the synthesis and antitumor activity of s-triazole Schiff bases derived from Ofloxacin (**S-5.14**). The results revealed that the Schiff bases possessing a free phenol group exhibit more potent activity than the other tested compounds.



S-5.14

Where R = H, 4-OCH_3, 2-OH, 2-OCH_3, 3,4-OCH_2O, 3,4,5-$(OCH_3)_3$, 3-OH-4-OCH_3, 4-OH-3-OCH_3, 4-Cl, 4-F, 4-NO_2

Nithinchandra et al. (2012) reported the synthesis, characterization and pharmacological activity of some new Schiff bases (**S-5.15**) containing sydnone. The synthesized compounds were tested for their anti-inflammatory and analgesic properties and found that the Schiff bases possessing electron donating groups on sydnone will enhance the biological activity.



S-5.15

Where R = H, CH_3, OCH_3 X = O, CH_2, NCH_3, NC_2H_5, NC_6H_5

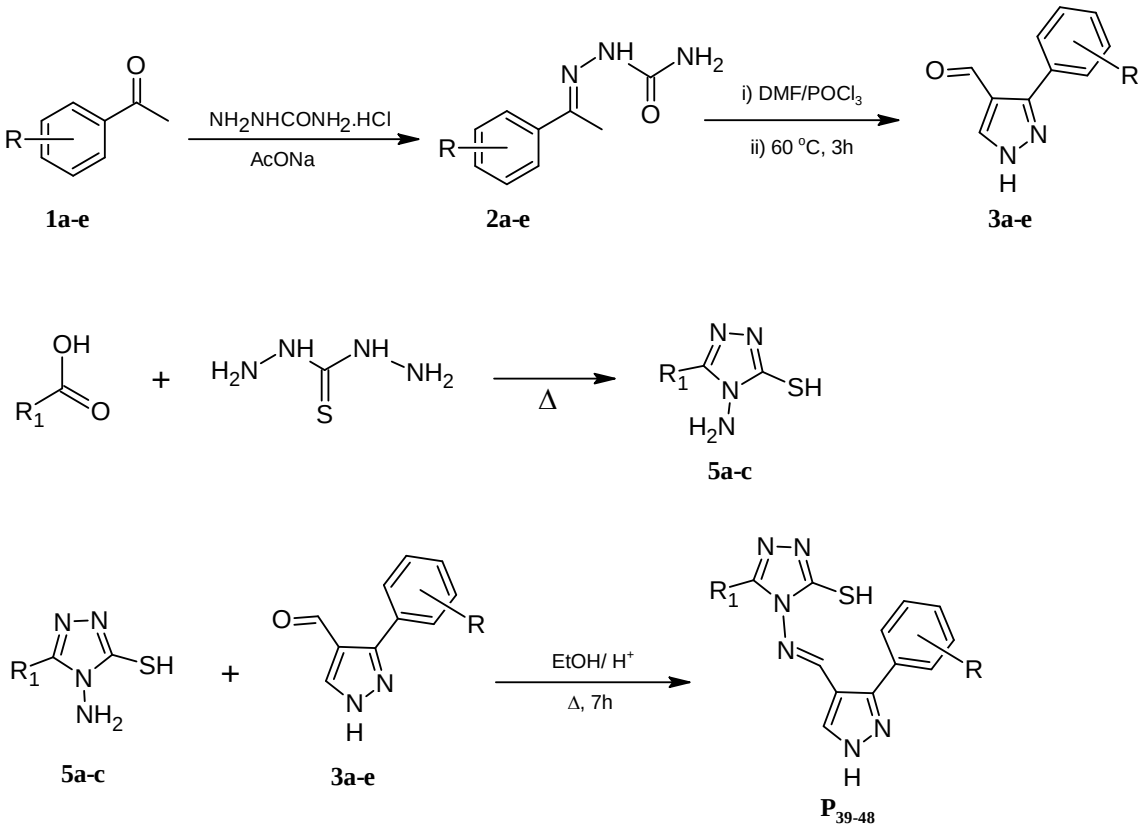
Literature survey revealed triazole derivatives which belong to an important group of heterocyclic compounds, which have been the subject of extensive study in the recent past. Based on the biological applications of 1,2,4-triazole, azomethine and pyrazole entities, it was planned to synthesize some new Schiff base derivatives bearing triazole and pyrazole skeletons and investigate their antimicrobial properties.

5.2 MATERIALS AND METHODS

3-Substituted-1*H*-pyrazole-4-carbaldehydes (**3a-e**) were synthesized by the Vilsmayer Haack reaction of semicarbazones (Baraldi et al. 1997; Lebedev et al. 2005). 3-Substituted-4-amino-5-mercapto-1,2,4-triazoles (**5a-b**) were synthesized as reported in the literature (Dhaka et al. 1974; Reid and Heindel, 1976). The Schiff bases (**P_{39-48}**) were synthesized by the condensation of 3-substituted-4-amino-5-mercapto-1,2,4-triazoles (**5a-b**) with various 3-substituted-1*H*-pyrazole-4-

carbaldehydes (**3a-e**) in presence of concentrated sulphuric acid in ethanol-dioxane mixture. The synthetic route has been outlined in **Scheme 5.1**.

Melting points were determined by open capillary method. The IR spectra were recorded on a Thermo Nicolet avatar 330-FT-IR spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded (DMSO-d_6) on a Bruker (400 MHz) spectrometer using TMS as internal standard.



Where R_1 = H, C_2H_5, C_3H_7

R = H, 4-OCH_3, 4-F, 4-Cl, 2,4-Cl

Scheme 5.1 Synthetic route for Schiff base derivatives $\mathbf{P_{39\text{-}48}}$

Chemical shift values are given in δ scales. The mass spectra were recorded on LC-MS-Agilent 1100 series and API 2000 LC/MS system. Elemental analyses were performed on a Flash EA 1112 series CHNS-O analyzer. The completion of the reaction was checked by thin layer chromatography (TLC) on silica gel coated aluminium sheets (silica gel 60 F254). Commercial grade solvents and reagents were used without further purification.

5.3 EXPERIMENTAL

The experimental protocols followed for the synthesis of compounds **P_{39-48}** are given in the following section.

Synthesis of 4-amino-5-substituted-4*H*-1,2,4-triazole-3-thiol (5a-c)

The compounds were synthesized according to previously reported procedure (Isloor et al. 2009).

Synthesis of 3-substituted-1*H*-pyrazole-4-carbaldehydes (**3a-e**)

The compounds were synthesized according to previously reported procedure (Lebedev et al. 2005) as discussed in **Chapter 2**.

General procedure for the synthesis of 4-[(3-Substituted-1*H*-pyrazol-3-yl)methylene amino]-5-substituted-4*H*-1,2,4-triazole-3-thiols (P_{39-48})

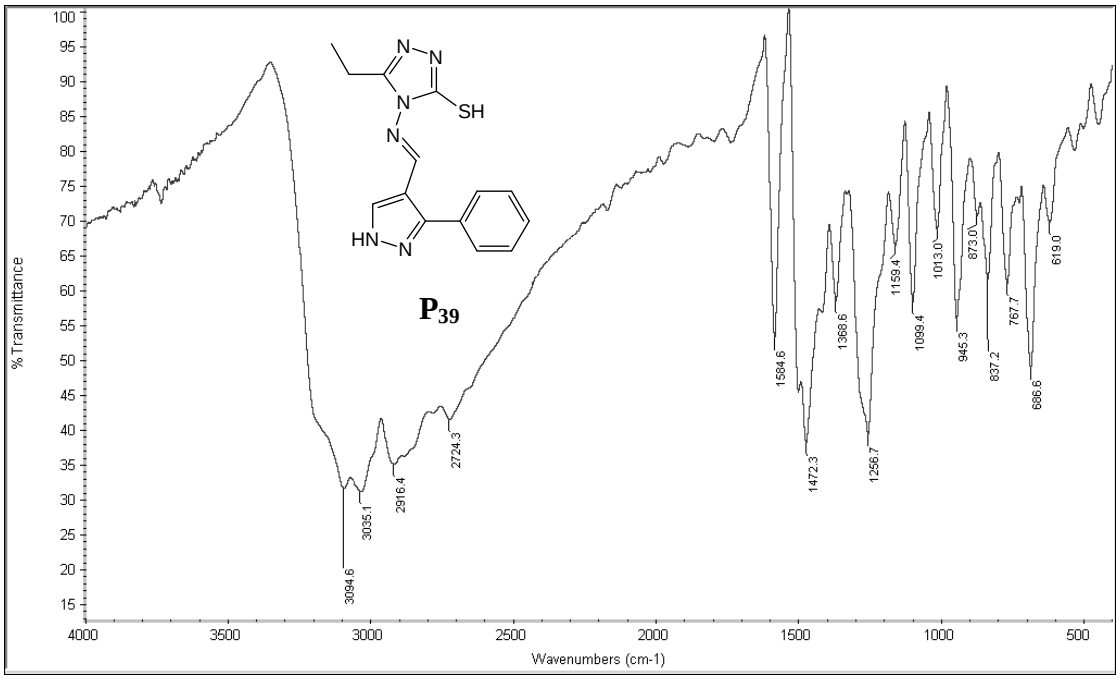
Equimolar mixture of 4-amino-5-substituted-4*H*-1,2,4-triazole-3-thiol **1a-c** (0.001 mol) and *3*-(4-substitutedphenyl)-1*H*-pyrazole-4-carbaldehyde **2a-e** (0.001 mol) was refluxed in ethanol-dioxane mixture (8 mL) for 7 h in presence of catalytic amount of concentrated sulphuric acid. The resulting solution was cooled to room temperature and the precipitated solid was filtered under suction, washed with ethanol and recrystallized from ethanol-dioxane mixture.

5-Ethyl-4-{[(E)-(3-phenyl-1*H*-pyrazol-4-yl)methylidene]amino}-4*H*-1,2,4-triazole -3-thiol (P_{39})

FT-IR (cm^{-1}): 3094 (N-H), 3035 (Ar-H), 2916 (C-H), 1584 (C=N) (**Figure 5.1**). ^{1}H NMR (DMSO-d_6): δ= 13.67 (bs, 2H, SH & NH), 9.76 (s, 1H, N=CH), 8.23 (s, 1H, pyrazole-5H), 7.5-7.75 (m, 5H, Ar-H), 2.69 (q, 2H, -CH_2), 1.21 (t, 3H, -CH_3) (**Figure 5.2**). MS: m/z = 299 (M+1) (**Figure 5.3**). Anal. calcd. for $C_{14}H_{14}N_6S$: C, 56.36; H, 4.73; N, 28.17; Found C, 56.29; H, 4.68; N, 28.11 %.

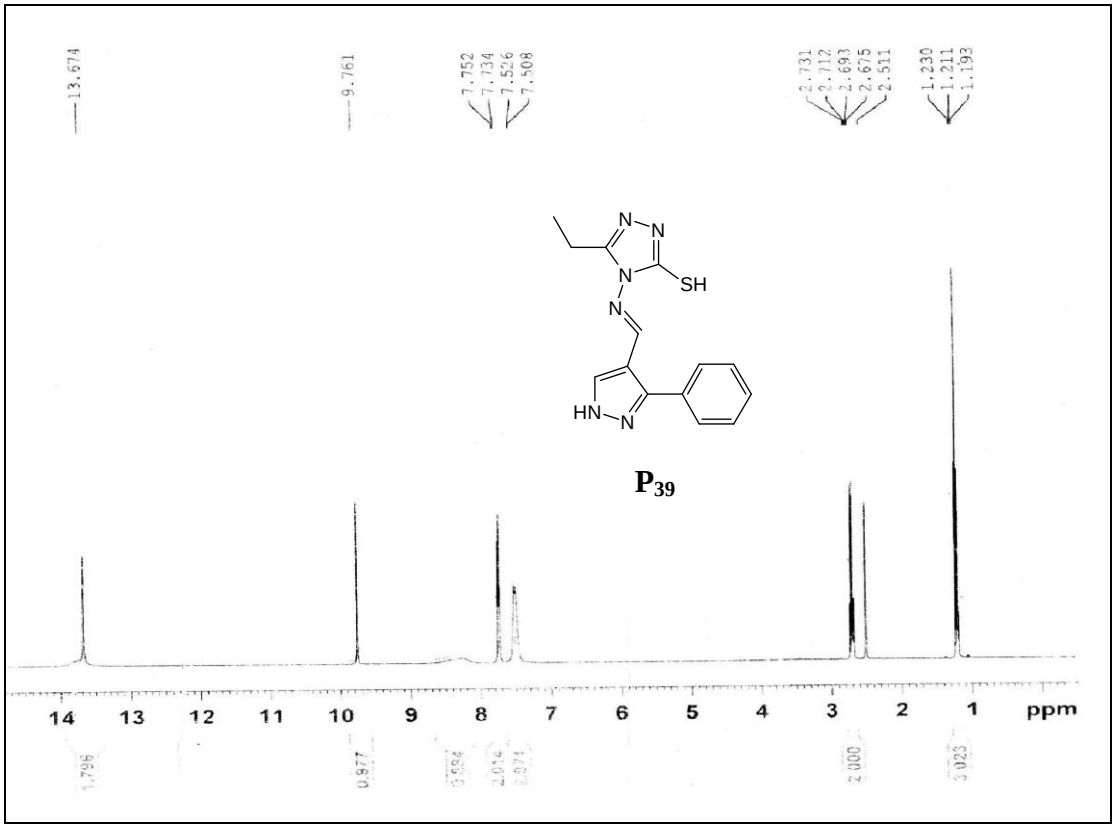
5-Ethyl-4-({(E)-[3-(4-methoxyphenyl)-1*H*-pyrazol-4-yl]methylidene}amino)-4*H*-1,2,4-triazole-3-thiol (P_{40})

FT-IR (cm^{-1}): 3103 (N-H), 3013 (Ar-H), 2929 (C-H), 1603 (C=N), 1169 (C-O). ^{1}H NMR (DMSO-d_6): δ 13.66 (bs, 2H, SH & NH), 9.68 (s, 1H, N=CH), 8.26 (s, 1H, pyrazole-5H), 7.67 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.0 (d, 2H, *J* = 8.4 Hz, Ar-H), 3.82 (s, 3H, -OCH_3), 2.67 (q, 2H, -CH_2), 1.21 (t, 3H, -CH3). ^{13}C NMR: 161.5, 160.4, 158.9, 152.4, 136.6, 130.1, 114.9, 111.9, 55.7, 18.7, 10.5 (**Figure 5.4**). MS: m/z = 329 (M+1). Anal. calcd. for $C_{15}H_{16}N_6OS$: C, 54.86; H, 4.91; N, 25.59; Found C, 54.81; H, 4.88; N, 25.53 %.



P39

Figure 5.1 IR spectrum of compound **P39**



P39

Figure 5.2 ^{1}H NMR spectrum of compound **P39**

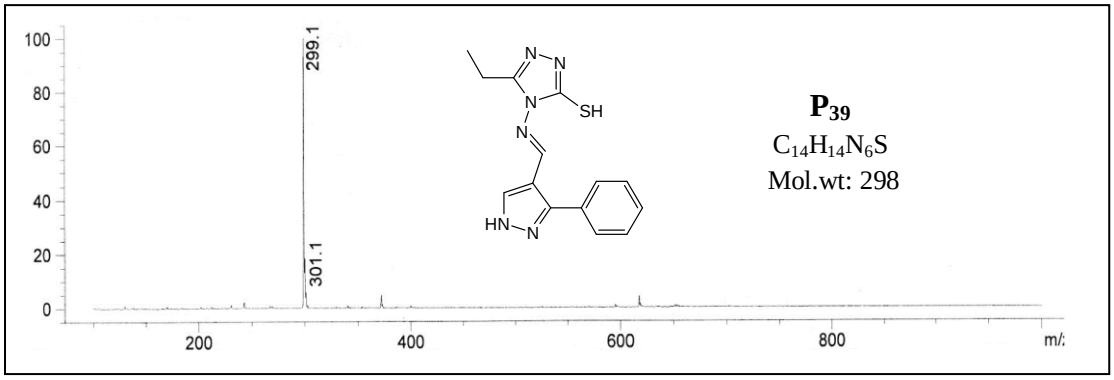


Figure 5.3 Mass spectrum of compound **P_{39}**

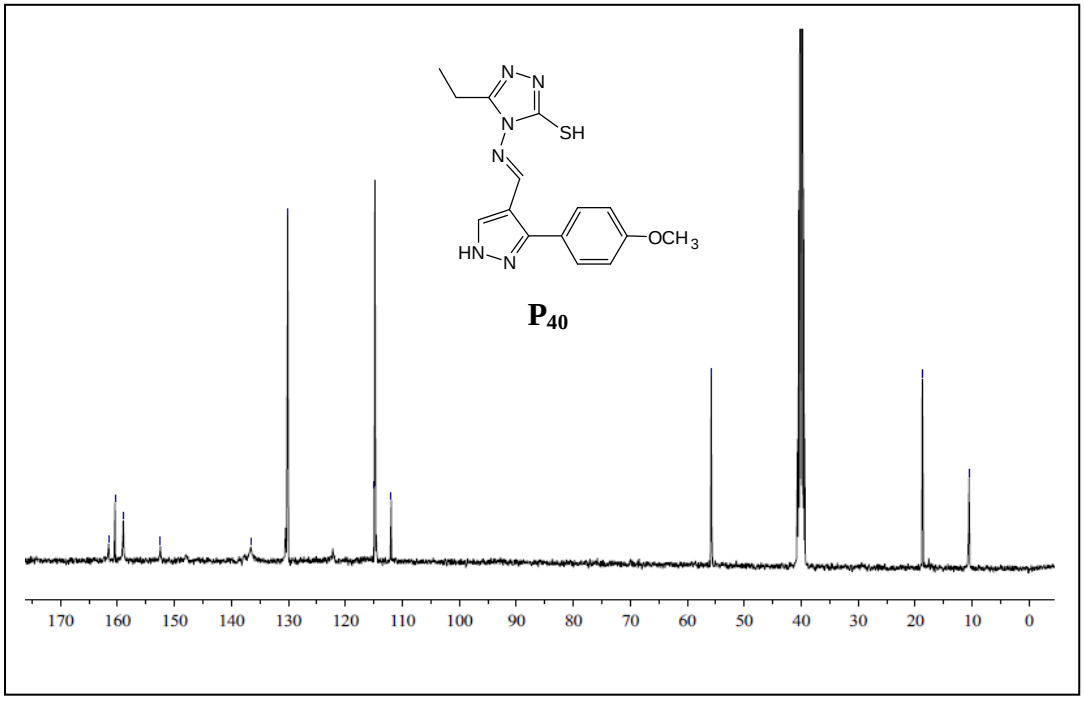
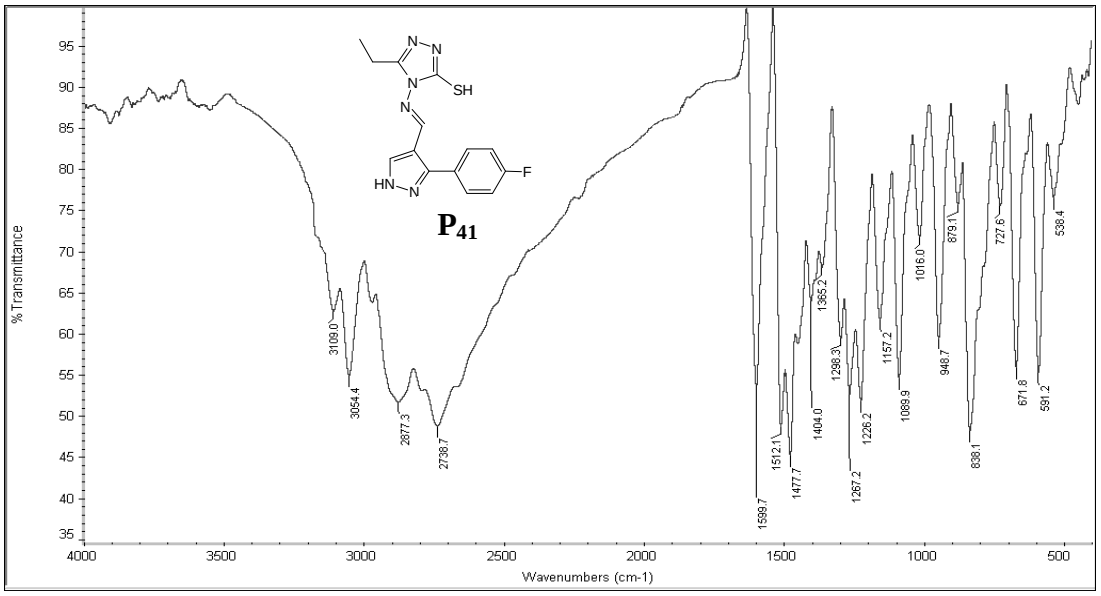


Figure 5.4 ^{13}C NMR spectrum of compound **P_{40}**

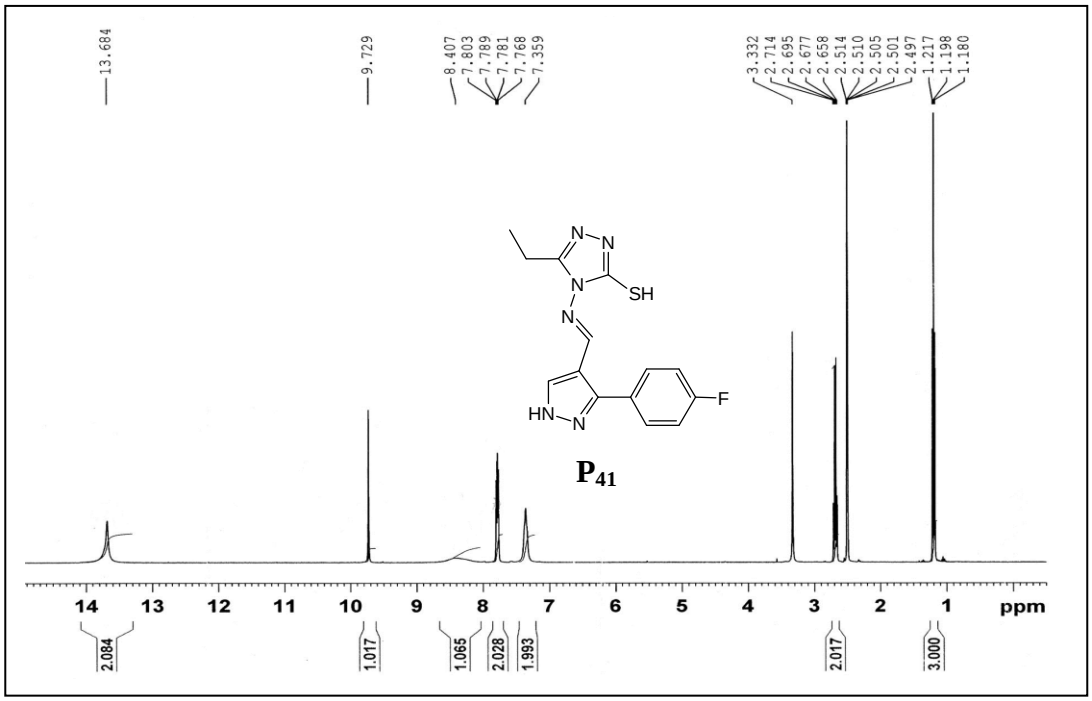
5-Ethyl-4-({(E)-[3-(4-fluorophenyl)-1*H*-pyrazol-4-yl]methylidene}amino)-4*H*-1,2,4-triazole-3-thiol (P_{41})

FT-IR (cm^{-1}): 3109 (N-H), 3054 (Ar-H), 2877 (C-H), 1599 (C=N), 1157 (C-F) (**Figure 5.5**). ^{1}H NMR (DMSO-d_6): δ 13.68 (bs, 2H, SH & NH), 9.72 (s, 1H, N=CH), 8.4 (s, 1H, pyrazole-5H), 7.35-7.80 (m, 4H, Ar-H), 2.65 (q, 2H, -CH_2), 1.19 (t, 3H, -CH_3) (**Figure 5.6**). MS: m/z = 317 (M+1). Anal. calcd. for $C_{14}H_{13}FN_6S$: C, 53.15; H, 4.14; N, 26.57; Found C, 53.11; H, 4.09; N, 26.52 %.



P41

Figure 5.5 IR spectrum of compound **P_{41}**



P41

Figure 5.6 ^{1}H NMR spectrum of compound **P_{41}**

4-({(E)-[3-(4-chlorophenyl)-1*H*-pyrazol-4-yl]methylidene}amino)-5-ethyl-4*H*-1,2,4-triazole-3-thiol (P_{42})

FT-IR (cm^{-1}): 3129 (N-H), 3078 (Ar-H), 2911 (C-H), 1597 (C=N), 831 (C-Cl). ^1H NMR (DMSO-d_6): δ 13.69 (bs, 2H, SH & NH), 9.76 (s, 1H, N=CH), 8.57 (s, 1H, pyrazole-5H), 7.75 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.57 (d, 2H, *J* = 8.4 Hz, Ar-H), 2.65 (q, 2H, $-CH_2$), 1.19 (t, 3H, $-CH_3$). MS: m/z = 333 (M+1). Anal. calcd. for $C_{14}H_{13}ClN_6S$: C, 50.52; H, 3.94; N, 25.25; Found C, 50.47; H, 3.88; N, 25.19 %.

4-({(E)-[3-(2,4-dichlorophenyl)-1*H*-pyrazol-4-yl]methylidene}amino)-5-ethyl-4*H*-1,2,4-triazole-3-thiol (P_{43})

FT-IR (cm^{-1}): 3125(N-H), 3051 (Ar-H), 2974 (C-H), 1596 (C=N), 853 (C-Cl). ^1H NMR (DMSO-d_6): δ 13.67 (bs, 2H, SH & NH), 9.74 (s, 1H, N=CH), 8.54 (s, 1H, pyrazole-5H), 8.0 (d, 1H, *J* = 7.8 Hz, Ar-H), 7.56 (d, 1H, *J* = 2.8 Hz, Ar-H), 7.45 (m, 1H, Ar-H). MS: m/z = 366 (M^+). Anal. calcd. for $C_{14}H_{12}Cl_2N_6S$: C, 45.79; H, 3.29; N, 22.88; Found C, 45.73; H, 3.22; N, 22.83 %.

4-{[(E)-(3-phenyl-1*H*-pyrazol-4-yl)methylidene]amino}-4*H*-1,2,4-triazole-3-thiol (P_{44})

FT-IR (cm^{-1}): 3138 (N-H), 3014 (Ar-H), 2971 (C-H), 1569 (C=N). ^1H NMR (DMSO-d_6): δ 13.8 (s, 1H, SH), 13.75 (bs, 1H, NH), 9.54 (s, 1H, N=CH), 8.77 (s, 1H, triazole-5H), 8.13 (s, 1H, pyrazole-5H), 7.52-7.75 (m, 5H, Ar-H), 3.82 (s, 3H, $-OCH_3$). MS: m/z = 271 (M+1). Anal. calcd. for $C_{12}H_{10}N_6S$: C, 53.32; H, 3.73; N, 31.09; Found C, 53.27; H, 3.68; N, 31.14 %. Crystal structure of **P_{44}** has also been solved **(Figure 5.7)**.

4-({(E)-[3-(4-methoxyphenyl)-1*H*-pyrazol-4-yl]methylidene}amino)-4*H*-1,2,4-triazole-3-thiol (P_{45})

FT-IR (cm^{-1}): 3137 (N-H), 3067 (Ar-H), 2935(C-H), 1598 (C=N), 1167 (C-O). ^1H NMR (DMSO-d_6): δ 13.89 (s, 1H, SH), 13.68 (bs, 1H, NH), 9.47 (s, 1H, N=CH), 8.78 (s, 1H, triazole-5H), 8.13 (s, 1H, pyrazole-5H), 7.66 (d, 2H, *J* = 6.8 Hz, Ar-H), 7.09 (d, 2H, *J* = 8.4 Hz, Ar-H), 3.82 (s, 3H, $-OCH_3$). MS: m/z = 301 (M+1). Anal. calcd. for $C_{13}H_{12}N_6OS$: C, 51.99; H, 4.03; N, 27.98; Found 51.93; H, 4.06; N, 27.91 %.

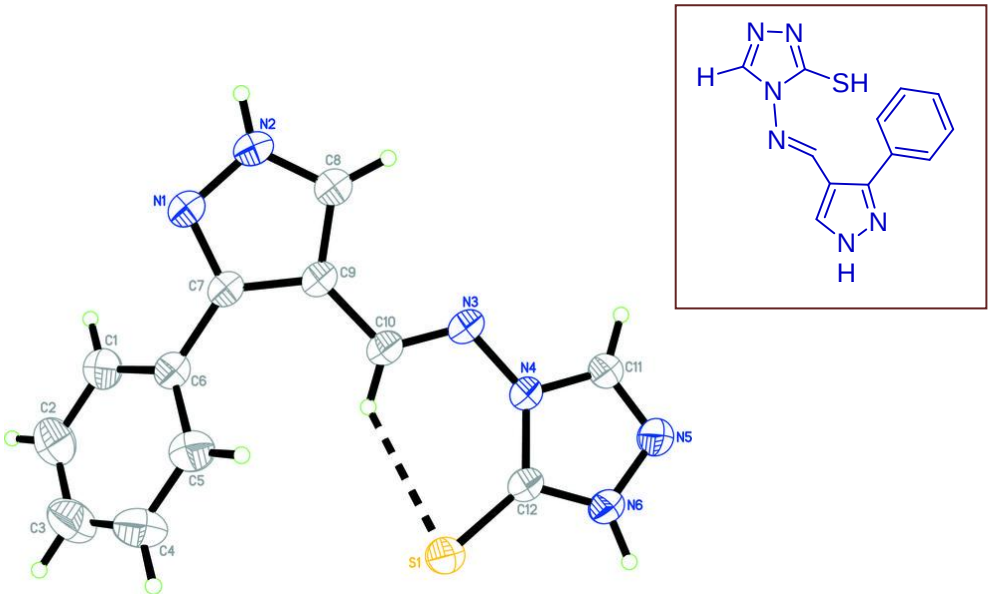


Figure 5.7 The Single crystal X-ray structure of compound **P_{44}**

4-({(E)-[3-(4-fluorophenyl)-1*H*-pyrazol-4-yl]methylidene}amino)-4*H*-1,2,4-triazole-3-thiol (P_{46})

FT-IR (cm^{-1}): 3139 (N-H), 3072 (Ar-H), 2969 (C-H), 1600 (C=N), 1148 (C-F). ^1H NMR (DMSO-d_6): δ 13.90 (s, 1H, SH), 13.76 (bs, 1H, NH), 9.48 (s, 1H, N=CH), 8.79 (s, 1H, triazole-5H), 8.20 (s, 1H, pyrazole-5H), 7.35-7.83 (m, 4H, Ar-H). MS: m/z = 289 (M+1). Anal. calcd. for $C_{12}H_9FN_6S$: C, 49.99; H, 3.15; N, 29.15; Found C, 49.94; H, 3.11; N, 29.12 %.

4-({(E)-[3-(4-chlorophenyl)-1*H*-pyrazol-4-yl]methylidene}amino)-4*H*-1,2,4-triazole-3-thiol (P_{47})

FT-IR (cm^{-1}): 3131 (N-H), 3021 (Ar-H), 2964 (C-H), 1599 (C=N), 822 (C-Cl). ^1H NMR (DMSO-d_6): δ 13.91 (s, 1H, SH), 13.76 (bs, 1H, NH), 9.48 (s, 1H, N=CH), 8.52 (s, 1H, triazole-5H), 8.12 (s, 1H, pyrazole-5H), 7.53-7.81 (m, 4H, Ar-H). MS: m/z = 305 (M+1). Anal. calcd. for $C_{12}H_9ClN_6S$: C, 47.29; H, 2.98; N, 27.58; Found C, 47.23; H, 2.94; N, 27.52 %.

4-({(E)-[3-(4-fluorophenyl)-1*H*-pyrazol-4-yl]methylidene}amino)-5-propyl-4*H*-1,2,4-triazole-3-thiol (P_{48})

FT-IR (cm^{-1}): 3154 (N-H), 3048 (Ar-H), 2963 (C-H), 1610 (C=N), 1244 (C=S), 1154 (C-F). ^1H NMR (DMSO-d_6): δ 13.71 (bs, 1H, SH & NH), 9.72 (s, 1H, N=CH), 8.37 (s, 1H, pyrazole-5H), 7.33-7.79 (m, 4H, Ar-H), 2.64 (t, 2H, -CH_2), 1.64 (m, 2H, -

CH_2), 0.92 (t, 3H, -CH_3). MS: m/z = 331 (M+1). Anal. calcd. for $C_{15}H_{15}FN_6S$: C, 54.53; H, 4.58; N, 25.44; Found C, 54.48; H, 4.51; N, 25.41 %.

5.4 PHARMACOLOGY

Antimicrobial studies has been carried out at Department of Microbiology, Veterinary College, Hebbal, KVAFSU, Bangalore, India.

5.4.1 Antimicrobial activity

All the newly synthesized compounds (**P_{39-48}**) were screened for their antimicrobial activity. For this, *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* (ATCC 6633), *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 microorganisms were employed. New compounds were also screened for their antifungal activity against *Candida albicans* MTCC 227. Antimicrobial study was assessed by Minimum Inhibitory concentration (MIC) by serial dilution method (Mackie and Mc. Cartney 1989). Ceftriaxone was used as standard for antibacterial studies and antifungal activity was compared with the standard drug Fluconazole. Several colonies of above mentioned bacteria's were picked off afresh on isolation plate and inoculated in corresponding tubes containing 5 mL of trypticase soya broth. The broth was incubated for 6 h at 37 ˚C until there was visible growth. Mc Farland No.5 standard was prepared by adding 0.05 mL of 1% w/v $BaCl_2.2H_2O$ in Phosphate Buffered Saline (PBS) to 9.95 mL of 1% v/v H_2SO_4 in PBS. The growth of all the four cultures was adjusted to Mc Farland No.5 turbidity standard using sterile PBS. This gives a 10^8 cfu/mL suspension. The working inoculums of aforementioned four different microorganisms containing 10^5 cfu/mL suspension was prepared by diluting the 10^8 cfu/mL suspension, 10^3 times in trypticase soya broth.

Preparation of antimicrobial suspension (1 mg/mL)

Dissolved 10 mg of each compound in 10 mL of Dimethyl formamide to get 1 mg/mL concentration.

Preparation of dilutions

In all, for each of the 10 anti-microbial compounds and standard antimicrobials i.e. Ceftriaxone for antibacterial and Fluconazole for antifungal, 30 tubes of 5 mL capacity were arranged in 5 rows with each row containing 6 tubes. Then 1.9 mL of trypticase soya broth was added in the first tube in each row and then 1 mL in the remaining tubes. Now, 100 µL of anti-microbial suspension dissolved in

Dimethylformamide was added to the first tube in each row and then after mixing the content, 1 mL was serially transferred from these tubes to the second tube in each of the rows. Then the contents in the second tube of each of the rows were mixed and transferred to the third tube in each of the rows.

This serial dilution was repeated till the sixth tube in each of the rows. This provided anti-microbial concentrations of 50, 25, 12.5, 6.25, 3.125, 1.6125 µg/mL in the first to sixth tube respectively in each row. Finally, 1 mL of 10^5 cfu/mL of *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans* suspension were added to the first, second, third, fourth and fifth rows of tubes respectively. Along with the test samples and Ceftriaxone/Fluconazole (standards), the inoculums control (without antimicrobial compound) and broth control (without anti-microbial compound and inoculum) were maintained. All the test sample and control tubes were then incubated for 16 h at 37 ˚C.

Interpretation

After incubation, the tubes showing no visible growth were considered to be representing the MIC. Inoculums control showed visible growth, where as the broth control showed no growth.

5.5 RESULTS AND DISCUSSION

5.5.1 Chemistry

The structures of the synthesized compounds **$P_{39\text{-}48}$** were characterized by IR, NMR, mass spectral and elemental analyses. Analytical and spectral data of all synthesized compounds were in full agreement with the proposed structures. The IR spectrum of compound **P_{39}** showed absorption bands at 3094, 3035, 2916, 1584 which were due to N-H, Ar-H, C-H and C=N groups respectively. The ^{1}H-NMR spectrum of **P_{39}** showed broad singlet at δ 13.67 which was due to presence of S-H and N-H protons. The Schiff base proton i.e. -N=CH and pyrazole-5H proton resonated as singlet at δ 9.76 and 8.23 respectively. A quartet at δ 2.69 and a triplet at δ 1.21 confirm the presence of alkyl side chain protons. The mass spectrum of **P_{39}** showed molecular ion peak at m/z = 299 (M+1), which is in agreement with the molecular formula $C_{14}H_{14}N_6S$. Furthermore, the structure of compound **P_{44}** was confirmed by single crystal X-ray analysis (Fun et al. 2011). In the compound **P_{44}**, $C_{12}H_{10}N_6S$, a

weak intramolecular C-H···S hydrogen bond stabilizes the molecular conformation. The pyrazole and triazole rings form a dihedral angle of 17.82 (8)˚. The molecule adopts an *E* configuration with respect to the central C=N double bond. The spectral values for all the compounds and C, H, N analyses are given in the experimental part. The characterization and crystallographic data have been provided in **Table 5.1** and **Table 5.2** respectively.

Table 5.1 Characterization data of the compounds $\mathbf{P_{39\text{-}48}}$

Compounds	R_1	R	Molecular formula (Mol.wt.)	Yield (%)	M.p. (˚C)
$\mathbf{P_{39}}$	C_2H_5	H	$C_{14}H_{14}N_6S$ (298)	79	217-219
$\mathbf{P_{40}}$	C_2H_5	4-OCH_3	$C_{15}H_{16}N_6OS$ (328)	65	227-229
$\mathbf{P_{41}}$	C_2H_5	4-F	$C_{14}H_{13}FN_6S$ (316)	63	242-244
$\mathbf{P_{42}}$	C_2H_5	4-Cl	$C_{14}H_{13}ClN_6S$ (332)	67	248-250
$\mathbf{P_{43}}$	C_2H_5	2,4-Cl	$C_{14}H_{12}Cl_2N_6S$ (366)	64	238-240
$\mathbf{P_{44}}$	H	H	$C_{12}H_{10}N_6S$ (270)	69	253-255
$\mathbf{P_{45}}$	H	4-OCH_3	$C_{13}H_{12}N_6OS$ (300)	66	244-246
$\mathbf{P_{46}}$	H	4-F	$C_{12}H_9FN_6S$ (288)	63	276-278
$\mathbf{P_{47}}$	H	4-Cl	$C_{12}H_9ClN_6S$ (304)	76	283-285
$\mathbf{P_{48}}$	C_3H_7	4-F	$C_{15}H_{16}FN_6S$ (330)	66	225-227

5.5.2 Biological activity

All the newly synthesized compounds were screened for their antimicrobial activity. For this, *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans* microorganisms were employed. Anti-microbial study was assessed by Minimum Inhibitory concentration (MIC) by serial dilution method. Antibacterial investigation revealed that most of the synthesized compounds have shown good to moderate activity against tested

microorganisms. In particular, compound **P_{44}** has exhibited excellent activity against *Staphylococcus aureus* with an MIC value of 1.6125 µg/mL whereas it has shown activity equal to that of standard drug Ceftriaxone (1.6125 µg/mL) against *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* microorganisms. **P_{41}** exhibited equipotent activity as that of standard drug Ceftriaxone for all four tested microorganisms. Compound **P_{39}** showed similar activity as that of standard against *Staphylococcus aureus* and *Pseudomonas aeruginosa* with an MIC values of 3.125 µg/mL and 1.6125 µg/mL respectively. Similarly, **P_{40}** also exhibited equipotent activity as that of standard against *Staphylococcus aureus* and *Escherichia coli* with MIC values of 3.125 µg/mL and 1.6125 µg/mL respectively. Compounds **P_{42}** and **P_{46}** also showed similar activity against *Staphylococcus aureus* as that of standard (3.125 µg/mL).

Table 5.2 Crystallographic data for compound **P_{44}**

Crystal Data	**P_{44}**
Emperical formula	$C_{12}H_{10}N_6S$
Formula weight	270.32
Temperature (K)	296
Wavelength (Å)	0.71073
Crystal system	Monoclinic
Space group	$P2_1/c$
Cell dimensions	
a (Å)	4.1180 (4)
b (Å)	17.9237 (16)
c (Å)	17.0787 (15)
α (˚)	90
β (˚)	97.352 (3)
γ (˚)	90
Volume ($Å^3$)	1250.2
Z	4
Density (Mg m^{-3})	1.436
F (000)	560
Θ range for data collection (˚)	2.7-32.1

Antifungal activity of the synthesized compounds was tested against *Candida albicans*. The antifungal results revealed that among the tested compounds, **P_{39}** and **P_{44}** showed excellent results with an MIC value of 3.125 µg/mL. Compounds **P_{41}**, **P_{42}**, **P_{46}** and **P_{47}** exhibited equipotent activity as that of standard drug Fluconazole which showed MIC value of 6.25 µg/mL. The activity exhibited by compounds **P_{40}** and **P_{45}** was comparatively less than compared to standard drug.

The structure activity relationship of synthesized compounds highlights that the excellent antibacterial activity of **P_{44}** can attributed due to presence of unsubstituted phenyl ring on pyrazole moiety. Also it was observed that the substituents like methoxy and fluoro on fourth position of phenyl ring of pyrazole showed good antibacterial activity against the tested microorganisms. However, in case of **P_{48}** the decrease in activity may be observed which may be due to increase in length of the alkyl chain on triazole ring.

Table 5.3 Antimicrobial activity of compounds **$P_{39\text{-}48}$**

Compound code	Antibacterial activity				Antifungal activity
	Staphylococcus aureus	*Bacillus subtilis*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Candida albicans*
P_{39}	3.125	3.125	6.25	1.6125	3.125
P_{40}	3.125	3.125	1.6125	3.125	12.5
P_{41}	3.125	1.6125	1.6125	1.6125	6.25
P_{42}	3.125	3.125	6.25	6.25	6.25
P_{43}	12.5	3.125	3.125	6.25	12.5
P_{44}	1.6125	1.6125	1.6125	1.6125	3.125
P_{45}	12.5	12.5	12.5	***	12.5
P_{46}	3.125	3.125	12.5	6.25	6.25
P_{47}	6.25	12.5	12.5	6.25	6.25
P_{48}	12.5	***	***	6.25	***
Standard	3.125	1.6125	1.6125	1.6125	6.25
Inoculum control	Growth observed	Growth observed	Growth observed	Growth observed	Growth observed
Broth control	No growth	No growth	No growth	No growth	No growth

*** Indicates growth in all concentrations

The antifungal activity exhibited by compounds **P_{39}** and **P_{44}** is may be due to the presence of unsubstituted phenyl ring on pyrazole moiety. However, substituting the phenyl group with chloro and fluoro (**P_{41}**, **P_{42}**, **P_{46}** and **P_{47}**) also favored too for its antifungal activity. The replacement of halogen substituent by methoxy group in compound **P_{40}** and **P_{45}** led to decrease in its activity. Compound **P_{43}** which has 2,4-dichloro substitution pattern did not favor much for its activity. Even though the compound **P_{48}** which has fluoro substituent, it failed to inhibit the growth of *Candida albicans* and reason may be the same i.e., increased alkyl chain length on triazole ring.

5.6 CONCLUSIONS

In the present work, a series of new pyrazole based Schiff bases were synthesized and evaluated for their antimicrobial activities against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa* and *Candida albicans* microorganisms by serial dilution method. The compound **P_{44}** has exhibited excellent activity against *Staphylococcus aureus* than compared to standard drug Ceftriaxone whereas similar activity as that of standard against remaining three microorganisms. Compound **P_{41}** was active at same concentration as that of standard in case of all four microorganisms. The antifungal results revealed that among the tested compounds, **P_{39}** and **P_{44}** showed excellent antifungal activity.

CHAPTER - 6

SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF SOME NEW PYRAZOLE CONTAINING CYANOPYRIDONE DERIVATIVES

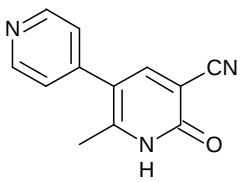
Abstract

This chapter describes a detailed literature survey on 3-cyanopyridine and its derivatives. It also includes the synthesis and characterization of newly synthesized pyrazole containing 4,6-disubstituted-3-cyano-2-pyridone derivatives. Antimicrobial studies of such compounds have been discussed in this chapter.

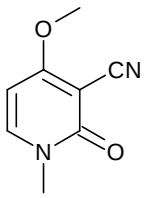
6.1 INTRODUCTION

Functionalized nitrogen and oxygen containing heterocycles play a predominant role in medicinal chemistry and they have been intensively used as scaffolds for drug development. The rapid assembly of molecular diversity is an important goal of synthetic organic chemistry and is one of the key paradigms of modern drug discovery. The synthesis of the pyridine containing heterocyclic systems occupies an important place in the realm of synthetic organic chemistry, due to their therapeutic and pharmacological properties (Gilchrist et al. 2001; Henry et al. 2004; Bagley et al. 2005; Vijesh et al. 2011). They have emerged as integral backbones of over 7000 existing drugs (Li et al. 1999; Vacher et al. 1999). The pyridine nucleus is an integral part of anticancer and anti-inflammatory agents (Amr et al. 2006; Son et al. 2008) too.

On the other hand, cyanopyridone and cyanopyridine derivatives have shown to posses promising antimicrobial and anticancer activities (Hammam et al. 2000). The interest in 3-cyano-2(1*H*)-pyridone and their derivatives is due to their wide range of practical uses as medicinal compounds (Dorigo et al. 1993). In addition, the pharmacological and physiological activity of 3-cyanopyridines has attracted much attention in recent years with the synthesis and the study of the nonglycosidic cardiotonic agent Milrinone (**S-6.1**). The 3-cyanopyridin-2-(1*H*)-one nucleus is also the structural basis of the alkaloid Ricinine (**S-6.2**) the first known alkaloid containing a cyano-group.

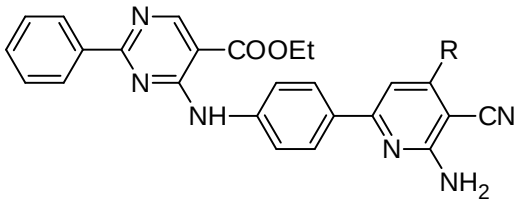


S-6.1



S-6.2

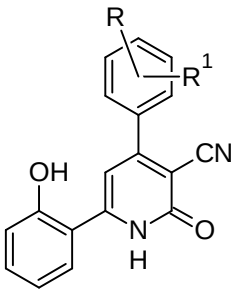
EI-Kerdawy et al. (1990) synthesized some 2-amino-3-cyanopyridine derivatives (**S-6.3**) and tested them for *in vitro* antimicrobial activity. Some of them were proved to be active.



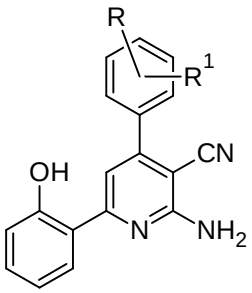
S-6.3

Where R = 2-Cl-C_6H_4, 4-Cl-C_6H_4, 4-Br-C_6H_4, 4-NO_2-C_6H_4, 4-pyridyl, 2-thienyl

Anti-inflammatory, analgesic and antipyretic activities of 4,6-disubstituted 3-cyanopyridine-2-ones (**S-6.4**) and 3-cyano-2-aminopyridines (**S-6.5**) was reported by Manna et al. (1992). Most of the pyridone and pyridine derivatives showed remarkable activity due to presence of electron withdrawing power of the substituents and by their position on the 4-aryl group.



S-6.4

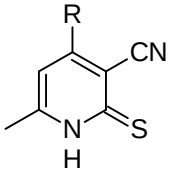


S-6.5

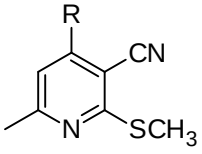
Where R = H, 2-Cl, 2-OCH_3

R^1 = 2-Cl, 3-Cl, 4-Cl, 2-OCH_3, 4-OCH_3, 5-OCH_3, 2-Br, 3-Br, 4-Br, 4-CH_3, 3-NO_2, 4-$N(CH_3)_2$

Attabbi et al. (1999) synthesized some cyanopyridine derivatives (**S-6.6 and S-6.7**) and evaluated their antimicrobial properties. The antimicrobial activity revealed that most of the compounds showed excellent activity.



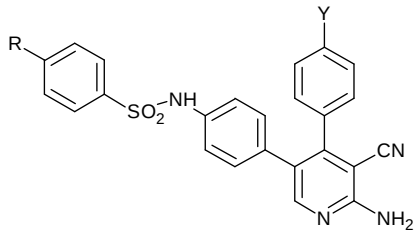
S-6.6



S-6.7

Where R = 4-Cl-C_6H_4, 2-furyl

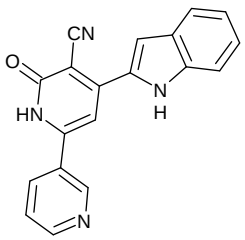
Moustafa and Ahmad (2003) reported synthesis and antimicrobial activity of some new cyanopyridines (**S-6.8**), isoxazoles, pyrazoles, and pyrimidines bearing sulfonamide moiety. Antimicrobial investigation revealed that some of the compounds showed good inhibition against gram positive bacteria.



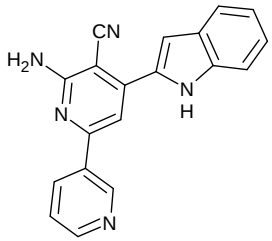
S-6.8

Where R = H, CH_3
Y = H, NO_2, OCH_3

Abd El-Latif et al. (2007) reported the synthesis, reactions, and pharmacological screening of heterocyclic derivatives using nicotinic acid as a natural synthon (**S-6.9** & **S-6.10**). The pharmacological screening showed that many of the synthesized compounds have good analgesic and anticonvulsant activities comparable to Valdecoxib and Carbamazepine as reference drugs.

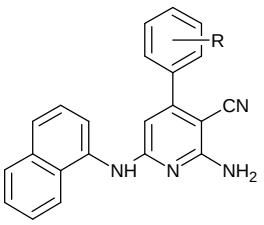


S-6.9



S-6.10

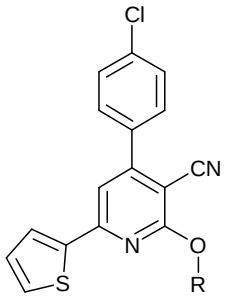
Konda and his group (2010) synthesized some new 2-amino-3-cyano-4-aryl-6-(1-napthyl amino)-pyridines (**S-6.11**) and evaluated for their antibacterial properties. The investigation of antimicrobial screening data revealed that most of the compounds showed good zone of inhibition.



Where R = 2-OH, 2-OH-5-Cl, 2-OH-3-Br-5-Cl, 4-OH, 4-Cl, 4-OCH_3, 3-OCH_3-4-OH

S-6.11

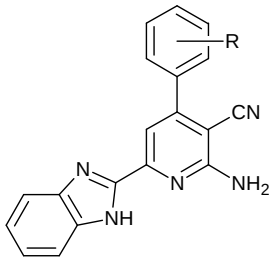
El-Sayed et al. (2011) reported the synthesis, antitumor and antimicrobial activities of 4-(4-chlorophenyl)-3-cyano-2-(*β*-O-glycosyloxy)-6-(thien-2-yl)-nicotinonitrile (**S-6.12**). Selected members of these compounds were screened for antitumor and antibacterial activity. Some of the compounds showed good antibacterial and antitumor activities.



S-6.12

Where R = glycosyl, galactosyl, xylosyl and lactosyl

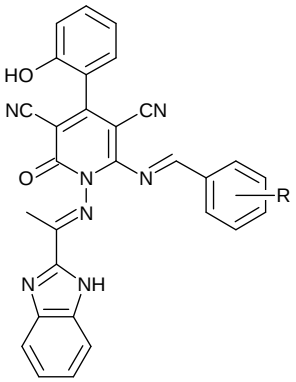
Yadav et al. (2011) reported the synthesis and antimicrobial activity of some new 2-subsituted benzimidazole derivatives carrying pyridine (**S-6.14**). The investigation of antimicrobial activity revealed that some of the compounds showed significant activity.



S-6.13

Where R = H, 4-OCH_3, 3,4-OCH_3, 3,4,5-OCH_3, 4-Cl, 4-$N(CH_3)_2$

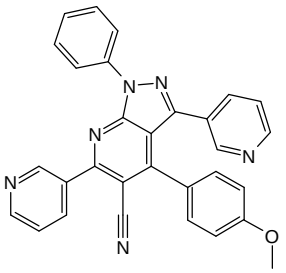
Desai et al. (2012) synthesized a series of 1-(1-(1H-benzo[d] imidazol-2-yl)ethylideneamino)-6-(arylideneamino)-4-(2-hydroxyphenyl)-2-oxo-1,2-dihydro pyridine-3,5-dicarbonitriles (**S-6.13**) and tested for their antimicrobial activity. The compounds bearing chloro and hydroxy groups exhibited very good to excellent antimicrobial activity.



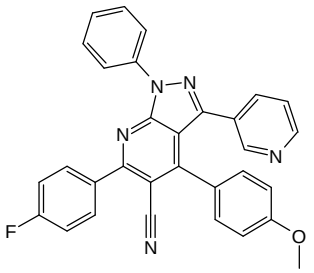
S-6.14

Where R = H, 4-OH, 4-Cl, 2-Cl, 4-N,N-$(CH_3)_2$, 3-OCH_3, 3-NO_2, 4-Cl, 3,4,5-(OCH_3)

El-borai et al. (2012) synthesized pyrazolo[3,4-b]pyridines under microwave irradiation in multicomponent reactions and evaluated for their antitumor and antimicrobial properties. Antimicrobial results revealed that most of compounds exhibited good activity. Antitumor results showed that among the synthesized compounds, **S-6.15** and **S-6.16** were found to be most effective ones.

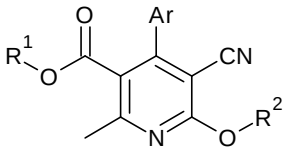


S-6.15



S-6.16

Reddi and his researchers (2012) described the montmorillonite K-10 mediated green synthesis of cyano pyridines **(S-6.17)** and their evaluation as potential inhibitors of PDE4. The results revealed that some of the synthesized cyano pyridines showed PDE4B inhibitory properties in vitro and good interactions with PDE4B protein *in silico*.



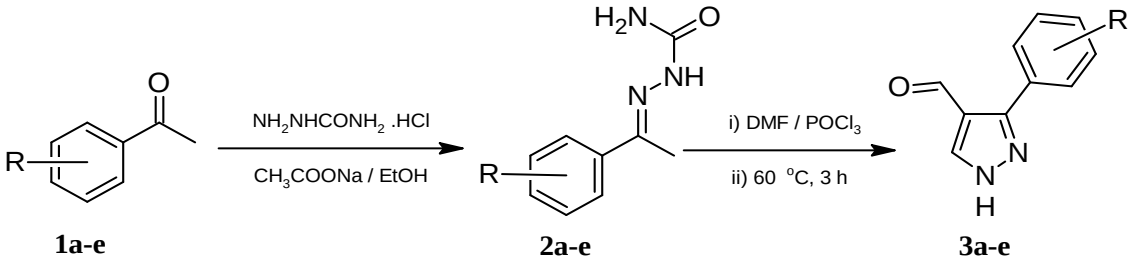
S-6.17

Where Ar = 4-Br-C_6H_4, 4-OMe-C_6H_4, 4-F-C_6H_4, 2,4-di-F-C_6H_3, 4-CF_3-C_6H_4, C_6H_5

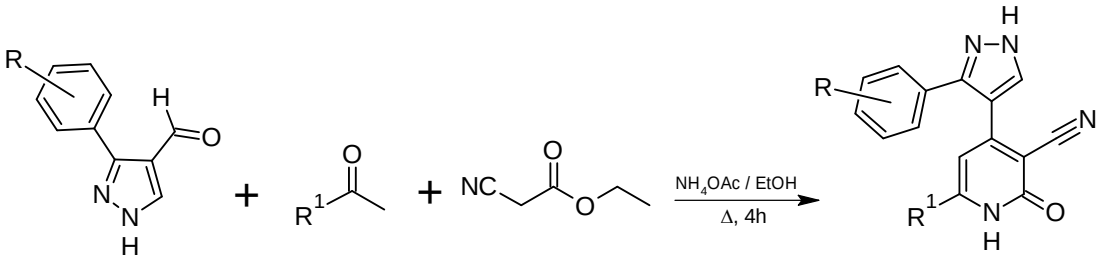
R^1/R^2 = Et, Me

In view of these observations and with a view to further explore the pharmacological profile of this class of compounds, it was contemplated to synthesize some new 4,6-disubstituted-3-cyano-2-pyridone derivatives bearing pyrazole skeleton and evaluate their antimicrobial properties.

6.2 MATERIALS AND METHODS



1a-e **2a-e** **3a-e**



3a-e **10a-c** **11** **P_{49-63}**

Where R^1 = Thienyl, 1-napthyl, 5-Cl-thienyl

R = H, 4-F, 4-Cl, 2,4-Cl, 4-CH_3, 4-OCH_3

Scheme 6.1 Synthetic route for 4,6-disubstituted-3-cyanopyridones **P_{49-63}**

The starting material 3-substituted-1*H*-pyrazole-4-carbaldehydes **(3a-e)** were synthesized from corresponding acetophenones through multi-step reactions as discussed in **Chapter 2**. Further**,** 4,6-disubstituted-3-cyano-2-pyridone derivatives were synthesized via one-pot multicomponent reaction of 3-substituted-1*H*-pyrazole-4-carbaldehydes **(3a-e)**, various acetyl compounds **(10a-c),** ethyl cyanoacetate **(11)** and ammonium acetate in ethanolic medium (**Scheme 6.1**).

Melting points were determined by open capillary. The IR spectra were recorded on a Thermo Nicolet avatar 330-FT-IR spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded (DMSO-d_6) on a Varian (400 MHz), Bruker (400 MHz) spectrometer using TMS as internal standard. Chemical shift values are given in δ scales. The mass spectra were recorded on API 2000 LC/MS/MS system. Elemental

analyses were performed on a Flash EA 1112 series CHNS-O Analyzer. The completion of the reaction was checked by thin layer chromatography (TLC) on silica gel coated aluminium sheets (silica gel 60 F254). Commercial grade solvents and reagents were used without further purification.

6.3 EXPERIMENTAL

The experimental protocols followed for the synthesis of compounds $\mathbf{P_{49\text{-}63}}$ are given in the following section.

Synthesis of 3-substituted-1*H*-pyrazole-4-carbaldehydes (**3a-e**)

The compounds were synthesized according to previously reported procedure (Lebedev et al. 2005) as discussed in **Chapter 2**.

General procedure for the synthesis of 4,6-disubstituted-3-cyano-2-pyridones ($\mathbf{P_{49\text{-}63}}$)

A mixture of 3-substituted-1*H*-pyrazole-4-carbaldehydes **3a-e** (0.001 mol) corresponding acetyl compounds **10a-c** (0.001 mol), ethyl cyanoacetate **11** (0.0012 mol) and ammonium acetate (0.008 mol) were refluxed in ethanol (5 mL) for 4 h. The reaction mixture was cooled to obtain precipitate which was filtered, washed with ethanol and recrystallized from ethanol:dioxane mixture to get pure product.

2-oxo-4-(3-phenyl-1*H*-pyrazol-4-yl)-6-(thiophen-2-yl)-1,2-dihydropyridine-3-carbonitrile ($\mathbf{P_{49}}$)

FT-IR (cm^{-1}): 3229, 3090 (N-H), 2211 (C≡N), 1635 (C=O), 1594 (C=N) (**Figure 6.1**); ^{1}H NMR (DMSO-d_6): δ 13.53 (bs,1H, pyrazole-NH), 12.66 (s, 1H, pyridone ring NH), 8.32 (s, 1H, pyrazole-5H), 7.18-7.89 (m, 8H, Ar-H), 6.23 (s, 1H, pyridone-5H) (**Figure 6.2**); MS: m/z = 345 (M+1) (**Figure 6.3**). Anal. calcd. for $C_{19}H_{12}N_4OS$: C, 66.26; H, 3.51; N, 16.27. Found: C, 66.20; H, 3.55; N, 16.24%.

4-[3-(4-fluorophenyl)-1*H*-pyrazol-4-yl]-2-oxo-6-(thiophen-2-yl)-1,2-dihydro pyridine-3-carbonitrile ($\mathbf{P_{50}}$)

FT-IR (cm^{-1}): 3245, 3088 (N-H), 2205 (C≡N), 1637 (C=O), 1594 (C=N) **(Figure 6.4)**; ^{1}H NMR (DMSO-d_6): δ 13.54 (bs,1H, pyrazole-NH), 12.63 (s, 1H, pyridone ring NH), 8.34 (s, 1H, pyrazole-5H), 7.15-7.93 (m, 7H, Ar-H), 6.14 (s, 1H, pyridone-5H): ^{13}C NMR: 163.7, 163.4, 161.3, 152.0, 131.5, 130.48, 130.4, 129.3, 117.1, 116.2,

116.0, 114.3, 106.6 **(Figure 6.5)**; MS: m/z = 363 (M+1) **(Figure 6.6)**. Anal. calcd. for $C_{19}H_{11}FN_4OS$: C, 62.97; H, 3.06; N, 15.46. Found: C, 62.91; H, 3.02; N, 15.41 %.

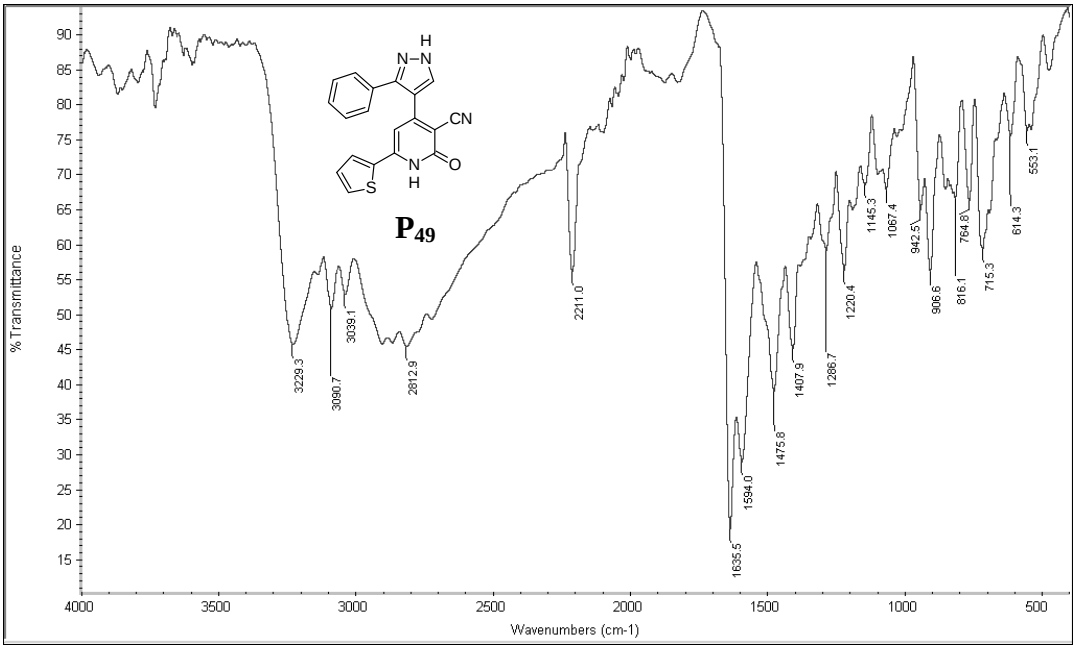


Figure 6.1 IR spectrum of compound **P_{49}**

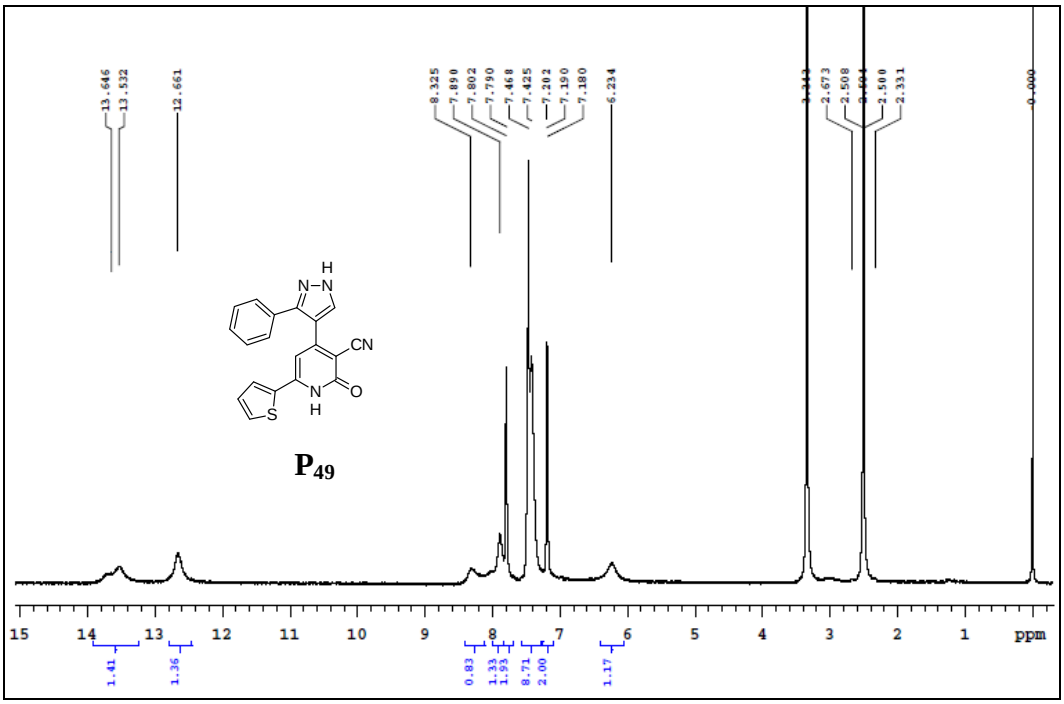


Figure 6.2 ^{1}H NMR spectrum of compound **P_{49}**

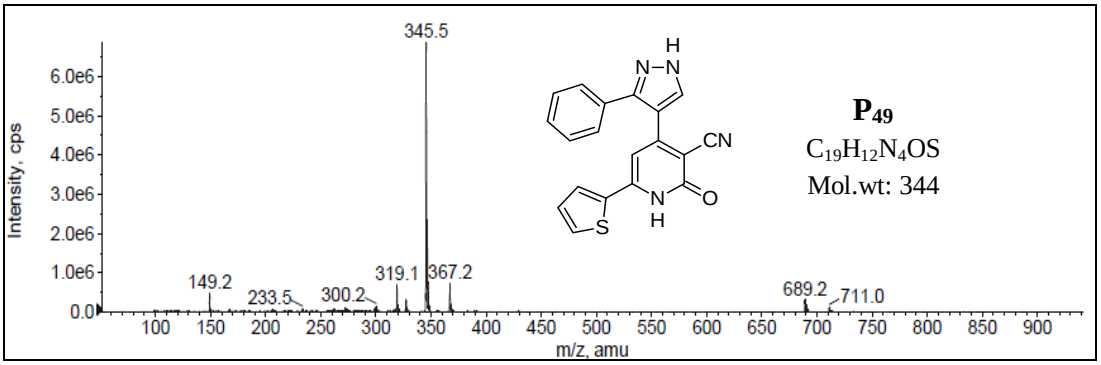


Figure 6.3 Mass spectrum of compound **P_{49}**

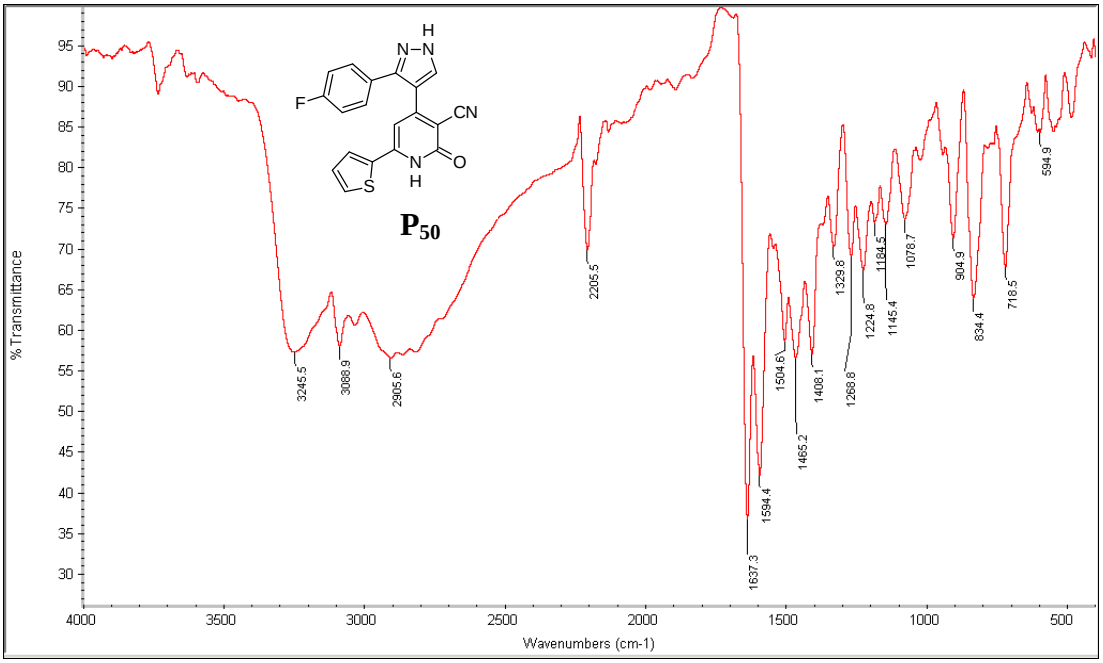


Figure 6.4 IR spectrum of compound **P_{50}**

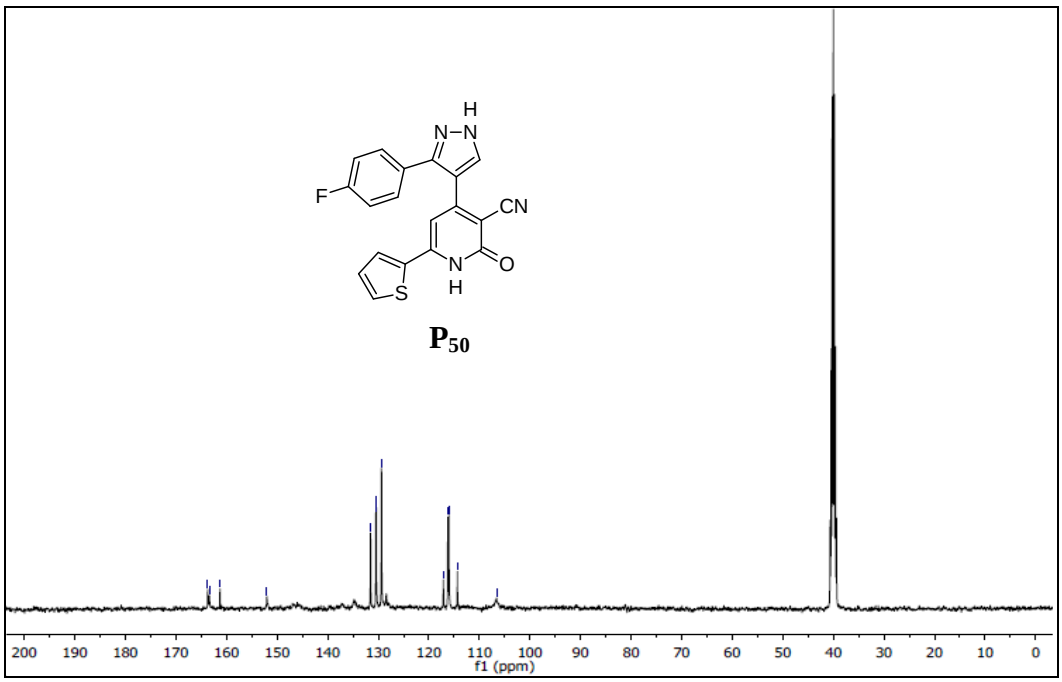


Figure 6.5 ^{13}C NMR spectrum of compound **P_{50}**

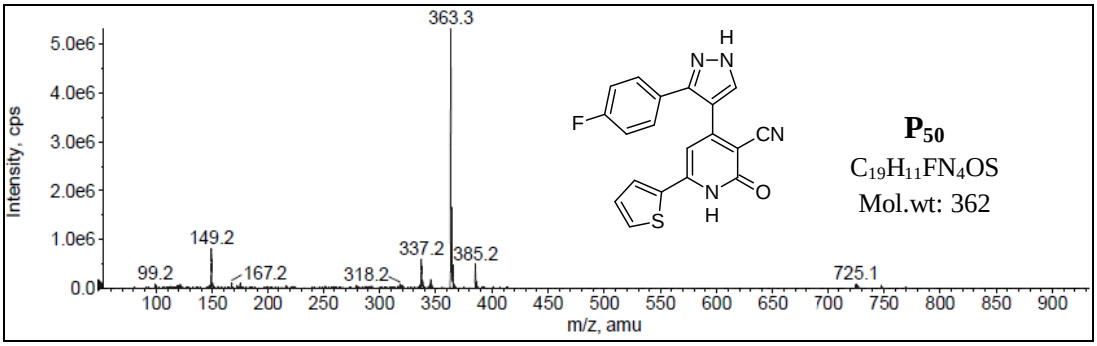


Figure 6.6 Mass spectrum of compound **P_{50}**

4-[3-(4-chlorophenyl)-1*H*-pyrazol-4-yl]-2-oxo-6-(thiophen-2-yl)-1,2-dihydro pyridine-3-carbonitrile (P_{51})

FT-IR (cm^{-1}): 3224, 3080 (N-H), 2216 (C≡N), 1638 (C=O), 1594 (C=N); ^{1}H NMR (DMSO-d_6): δ 13.56 (bs,1H, pyrazole-NH), 12.67 (s, 1H, pyridone ring NH), 8.24 (s, 1H, pyrazole-5H), 7.20-7.92 (m, 7H, Ar-H), 6.30 (s, 1H, pyridone-5H) **(Figure 6.7)**; MS: m/z = 379 (M+1). Anal. calcd. for $C_{19}H_{11}ClN_4OS$: C, 60.24; H, 2.93; N, 14.79. Found: C, 60.19; H, 2.89; N, 14.75 %.

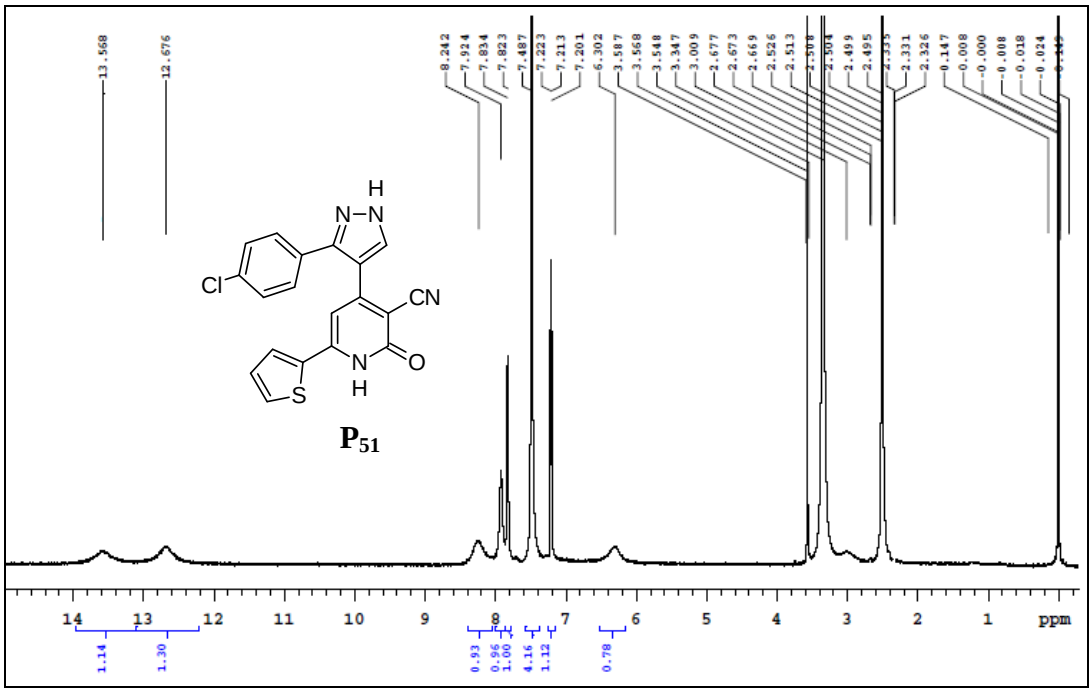


Figure 6.7 ^{1}H NMR spectrum of compound **P_{51}**

4-[3-(2,4-dichlorophenyl)-1*H*-pyrazol-4-yl]-2-oxo-6-(thiophen-2-yl)-1,2-dihydro pyridine-3-carbonitrile (P_{52})

FT-IR (cm^{-1}): 3199, 3088 (N-H), 2214 (C≡N), 1649 (C=O), 1606 (C=N); ^{1}H NMR (DMSO-d_6): δ 13.54 (bs,1H, pyrazole-NH), 12.65 (s, 1H, pyridone ring NH), 8.38 (s, 1H, pyrazole-5H), 7.17-7.82 (m, 6H, Ar-H), 6.15 (s, 1H, pyridone-5H); MS: m/z = 412 (M^{+}). Anal. calcd. for $C_{19}H_{10}Cl_2N_4OS$: C, 55.22; H, 2.44; N, 13.56. Found: C, 55.18; H, 2.39; N, 13.51 %.

4-[3-(4-methylphenyl)-1*H*-pyrazol-4-yl]-2-oxo-6-(thiophen-2-yl)-1,2-dihydro pyridine-3-carbonitrile (P_{53})

FT-IR (cm^{-1}): 3321, 3088 (N-H), 2807 (Aliphatic C-H), 2213 (C≡N), 1632 (C=O), 1596 (C=N); ^{1}H NMR (DMSO-d_6): δ 13.56 (bs,1H, pyrazole-NH), 12.67 (s, 1H, pyridone ring NH), 8.11 (s, 1H, pyrazole-5H), 7.17-7.85 (m, 7H, Ar-H), 6.42 (s, 1H, pyridone-5H), 2.32 (s, 3H, -CH_3); ^{13}C NMR: 163.2, 152.4, 145.9, 138.2, 136.9, 135.7, 131.5, 129.7, 129.3, 128.1, 117.1, 114.0, 106.7, 96.7, 21.0; MS: m/z = 359 (M+1). Anal. calcd. for $C_{20}H_{14}N_4OS$: C, 67.02; H, 3.94; N, 15.63. Found: C, 67.06; H, 3.91; N, 15.59 %.

6-(naphthalen-1-yl)-2-oxo-4-(3-phenyl-1*H*-pyrazol-4-yl)-1,2-dihydropyridine-3-carbonitrile (P_{54})

FT-IR (cm^{-1}): 3121, 3088 (N-H), 2221 (C≡N), 1634 (C=O), 1594 (C=N); ^{1}H NMR (DMSO-d_6): δ 13.54 (bs,1H, pyrazole-NH), 12.66 (s, 1H, pyridone ring NH), 8.32 (s, 1H, pyrazole-5H), 7.20-7.88 (m, 13H, Ar-H), 6.24 (s, 1H, pyridone-5H). ^{13}C NMR: 162.1, 153.2, 150.3, 133.4, 131.1, 130.9, 130.2, 129.3, 128.8, 128.6, 128.0, 127.7, 126.9, 117.7, 114.2, 109.7, 100.05; MS: m/z = 389 (M+1). Anal. calcd. for $C_{25}H_{16}N_4O$: C, 77.30; H, 4.15; N, 14.42. Found: C, 77.26; H, 4.12; N, 14.38 %.

4-[3-(4-methoxyphenyl)-1*H*-pyrazol-4-yl]-6-(naphthalen-1-yl)-2-oxo-1,2-dihydro pyridine-3-carbonitrile (P_{55})

FT-IR (cm^{-1}): 3217, 3088 (N-H), 2220 (C≡N), 1638 (C=O), 1586 (C=N); ^{1}H NMR (DMSO-d_6): δ 13.55 (bs,1H, pyrazole-NH), 12.65 (s, 1H, pyridone ring NH), 8.15 (s, 1H, pyrazole-5H), 7.02-8.05 (m, 11H, Ar-H), 6.0 (s, 1H, pyridone-5H) 3.79 (s, 3H, -OCH_3). MS: m/z = 419 (M+1). Anal. calcd. for $C_{26}H_{18}N_4O_2$: C, 74.63; H, 4.34; N, 13.39. Found: C, 74.59; H, 4.31; N, 13.34 %.

4-[3-(4-fluorophenyl)-1*H*-pyrazol-4-yl]-6-(naphthalen-1-yl)-2-oxo-1,2-dihydro pyridine-3-carbonitrile (P_{56})

FT-IR (cm^{-1}): 3291, 3066 (N-H), 2211 (C≡N), 1657 (C=O), 1599 (C=N); ^{1}H NMR (DMSO-d_6): δ 13.56 (bs,1H, pyrazole-NH), 12.78 (s, 1H, pyridone ring NH), 8.36 (s, 1H, pyrazole-5H), 7.31-8.06 (m, 11H, Ar-H), 6.02 (s, 1H, pyridone-5H). ^{13}C NMR: 163.8, 162.0, 153.1, 150.5, 133.4, 130.8, 130.3, 128.9, 128.0, 127.5, 126.9, 125.5, 125.0, 117.1, 116.3, 116.1, 114.3, 109.6; MS: m/z = 407 (M+1). Anal. calcd. for $C_{25}H_{15}FN_4O$: C, 73.88; H, 3.72; N, 13.79. Found: C, 73.81; H, 3.65; N, 13.74 %.

4-[3-(4-chlorophenyl)-1*H*-pyrazol-4-yl]-6-(naphthalen-1-yl)-2-oxo-1,2-dihydro pyridine-3-carbonitrile (P_{57})

FT-IR (cm^{-1}): 3217, 3058 (N-H), 2207 (C≡N), 1655 (C=O), 1600 (C=N); ^{1}H NMR (DMSO-d_6): δ 13.53 (bs,1H, pyrazole-NH), 12.76 (s, 1H, pyridone ring NH), 8.26 (s, 1H, pyrazole-5H), 7.47-8.06 (m, 11H, Ar-H), 6.03 (s, 1H, pyridone-5H). ^{13}C NMR: 162.1, 152.9, 150.6, 133.4, 130.9, 130.3, 129.3, 128.9, 128.0, 127.5, 126.9, 125.6, 125.0, 117.1, 114.5, 109.7, 100.1; MS: m/z = 423 (M+1). Anal. calcd. for $C_{25}H_{15}ClN_4O$: C, 71.01; H, 3.58; N, 13.25. Found: C, 71.05; H, 3.53; N, 13.20 %.

4-[3-(2,4-dichlorophenyl)-1*H*-pyrazol-4-yl]-6-(naphthalen-1-yl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (P_{58})

FT-IR (cm^{-1}): 3145, 3058 (N-H), 2215 (C≡N), 1647 (C=O), 1592 (C=N); ^{1}H NMR (DMSO-d_6): δ 13.55 (bs,1H, pyrazole-NH), 12.66 (s, 1H, pyridone ring NH), 8.40 (s, 1H, pyrazole-5H), 7.44-8.06 (m, 11H, Ar-H), 5.87 (s, 1H, pyridone-5H). MS: m/z = 456 (M^+). Anal. calcd. for $C_{25}H_{14}Cl_2N_4O$: C, 65.66; H, 3.09; N, 12.25. Found: C, 65.61; H, 3.01; N, 12.19 %.

4-[3-(4-methylphenyl)-1*H*-pyrazol-4-yl]-6-(naphthalen-1-yl)-2-oxo-1,2-dihydro pyridine-3-carbonitrile (P_{59})

FT-IR (cm^{-1}): 3215, 3069 (N-H), 2221 (C≡N), 1653 (C=O), 1612 (C=N); ^{1}H NMR (DMSO-d_6): δ 13.53 (bs,1H, pyrazole-NH), 12.68 (s, 1H, pyridone ring NH), 8.36 (s, 1H, pyrazole-5H), 7.40-8.02 (m, 12H, Ar-H), 5.83 (s, 1H, pyridone-5H) 2.34 (s, 3H, -CH_3). MS: m/z = 403 (M+1). Anal. calcd. for $C_{26}H_{18}N_4O$: C, 77.59; H, 4.5; N, 13.92. Found: C, 77.53; H, 4.45; N, 13.88 %.

6-(5-chlorothiophen-2-yl)-2-oxo-4-(3-phenyl-1*H*-pyrazol-4-yl)-1,2-dihydro pyridine-3-carbonitrile (P_{60})

FT-IR (cm^{-1}): 3217, 3088 (N-H), 2210 (C≡N), 1646 (C=O), 1602 (C=N); ^{1}H NMR (DMSO-d_6): ^{1}H NMR (DMSO-d_6): δ 13.52 (bs,1H, pyrazole-NH), 12.72 (s, 1H, pyridone ring NH), 8.26 (s, 1H, pyrazole-5H), 7.23-7.98 (m, 7H, Ar-H), 6.24 (s, 1H, pyridone-5H). MS: m/z = 379 (M+1). Anal. calcd. for $C_{19}H_{11}ClN_4OS$: C, 60.24; H, 2.93; N, 14.79. Found: C, 60.21; H, 2.97; N, 14.74 %.

6-(5-chlorothiophen-2-yl)-4-[3-(4-fluorophenyl)-1*H*-pyrazol-4-yl]-2-oxo-1,2-dihydropyridine-3-carbonitrile (P_{61})

FT-IR (KBr, υ_{max} cm^{-1}): 3224, 3089 (N-H), 2213 (C≡N), 1646 (C=O), 1602 (C=N); ^{1}H NMR (DMSO-d_6): ^{1}H NMR (DMSO-d_6): δ 13.55 (bs,1H, pyrazole-NH), 12.72 (s, 1H, pyridone ring NH), 8.18 (s, 1H, pyrazole-5H), 7.18-7.87 (m, 6H, Ar-H), 6.47 (s, 1H, pyridone-5H). MS: m/z = 397 (M+1). Anal. calcd. for $C_{19}H_{10}ClFN_4OS$: C, 57.51; H, 2.54; N, 14.12. Found: C, 57.47; H, 2.50; N, 14.08 %.

4-[3-(4-chlorophenyl)-1*H*-pyrazol-4-yl]-6-(5-chlorothiophen-2-yl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (P_{62})

FT-IR (cm^{-1}): 3254, 3085 (N-H), 2212 (C≡N), 1630 (C=O), 1583 (C=N); ^{1}H NMR (DMSO-d_6): ^{1}H NMR (DMSO-d_6): δ 13.53 (bs,1H, pyrazole-NH), 12.72 (s, 1H,

pyridone ring NH), 8.18 (s, 1H, pyrazole-5H), 7.09-7.75 (m, 6H, Ar-H), 6.24 (s, 1H, pyridone-5H). MS: m/z = 412 (M^+). Anal. calcd. for $C_{19}H_{10}Cl_2N_4OS$: C, 55.22; H, 2.44; N, 13.56. Found: C, 55.17; H, 2.39; N, 13.50 %.

6-(5-chlorothiophen-2-yl)-4-[3-(4-methylphenyl)-1*H*-pyrazol-4-yl]-2-oxo-1,2-dihydropyridine-3-carbonitrile (P_{63})

FT-IR (cm^{-1}): 3257, 3083 (N-H), 2213 (C≡N), 1627 (C=O), 1585 (C=N); ^{1}H NMR (DMSO-d_6): ^{1}H NMR (DMSO-d_6): δ 13.55 (bs,1H, pyrazole-NH), 12.66 (s, 1H, pyridone ring NH), 8.07 (s, 1H, pyrazole-5H), 7.21-7.66 (m, 6H, Ar-H), 6.72 (s, 1H, pyridone-5H) 2.32 (s, 3H, -CH_3). MS: m/z = 393 (M+1). Anal. calcd. for $C_{20}H_{13}ClN_4OS$: C, 61.14; H, 3.34; N, 14.26. Found: C, 61.11; H, 3.29; N, 14.20 %.

6.4 PHARMACOLOGY

6.4.1 Antimicrobial activity

The *in vitro* antimicrobial activity of newly synthesized compounds **P_{49-63}** were determined by "well-plate" method in Mueller-Hinton Agar as explained in **Chapter 2** by measuring the diameter of inhibition zone (mm). In this work *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 were used to investigate the antibacterial activity whereas *Aspergillus flavus* ATCC 15547, *Chrysosporium keratinophilum* ATCC 14803 and *Candida albicans* MTCC 227 were used to study antifungal activity. These cultures are obtained from the Department of Microbiology, Kuvempu University, Shimoga, India. All fungi strains were maintained on potato dextrose agar (PDA) at ± 25 ˚C.

6.4.2. Acute toxicity and behavioral studies

Acute toxicity studies have been carried out at Department of Biochemistry, Kuvempu University, Shimoga, India. The animals used in the present study such as Swiss albino mice weighing 20-25 g were procured from Veterinary College, Bangalore, Karnataka, India. Animals were maintained in colony cages at 25±2 ˚C, relative humidity of 45-55 %, maintained under 12 h light and dark cycle and were fed with standard animal feed and water. Animals were maintained under standard conditions in an animal house. The entire animals were acclimatized for a week before use.

The acute toxicity test was carried out for the compounds **P_{50}**, **P_{51}**, **P_{52}**, **P_{55}**, **P_{61}**, **P_{62}** and **P_{63}** according to the Organization for Economic Co-operation and Development (OECD) guidelines to establish the effective dose of test compounds after obtaining ethical clearance from Animal Ethics Committee of S.J.M. College of Pharmacy, Chitradurga-577501, Karnataka, India. Albino mice of either sex weighing between 20-25 g were grouped starved for 24 h with water prior to test. On the day of the experiment, animals were administered with different compounds to different groups in an increasing dose of 250, 500, 1000, 1500 2000, 3000 and 4000 mg/kg body weight orally. The acute toxic symptoms and the behavioral changes produced by the test compounds were observed continuously for 4 h and at 8 h, 12 h and 24 h onset of toxic symptoms and gross behavioral changes were also recorded (Segovia et al. 2002).

6.5 RESULTS AND DISCUSSION

6.5.1 Chemistry

The structures of the synthesized compounds (**$P_{49\text{-}63}$**) were characterized by IR, NMR, mass spectral and elemental analyses. Analytical and spectral data of all synthesized compounds were in full agreement with the proposed structures. The IR spectrum of compound **P_{49}** showed absorption bands at 3229 & 3090, 2211, 1635, 1594 cm^{-1} which corresponds to N-H, C≡N, C=O and C=N stretching respectively. Similarly, its ^{1}H NMR spectrum showed two singlets at δ 13.53 and 12.66 which were due to presence of pyrazole N-H and N-H of pyridone ring respectively. Further, the pyrazole-5H and aromatic proton of pyridone ring resonated at δ 8.32 and 6.23 which confirm the structure. The mass spectrum of **P_{49}** showed molecular ion peak at m/z = 345 (M+1), which is in agreement with the molecular formula $C_{19}H_{12}N_4OS$. Similarly the spectral values for all the compounds and C, H, N analyses are given in the experimental part and the characterization data has been provided in **Table 6.1.**

6.5.2 Biological activity

The new compounds **$P_{49\text{-}63}$** were tested for their antibacterial activity (*in vitro)* at a concentration of 1000 and 500 µg/mL against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* and their activity was compared to a well-known commercial antibiotic, Streptomycin. The results were recorded for each tested

compound as the average diameter of inhibition zones of bacterial growth surrounding the well in millimetres. The newly synthesized compounds exhibited variable antibacterial activity against the above tested bacterial strains. The results indicated that among the tested compounds, **P_{50}**, **P_{51}**, **P_{52}**, **P_{55}**, **P_{61}**, **P_{62}** and **P_{63}** showed good antibacterial activity towards all bacterial strains at concentrations of 1000 and 500 µg/mL when compared with standard drug. Rest of compounds showed fair or poor activity. Results of antibacterial studies have been presented in **Table 6.2**.

Table 6.1 Characterization data of the compounds **$P_{49\text{-}63}$**.

Compounds	R^1	R	Molecular formula (Mol.wt.)	Yield (%)	M.p. (°C)
P_{49}	Thienyl	H	$C_{19}H_{12}N_4OS$ (344)	53	>300
P_{50}	Thienyl	4-F	$C_{19}H_{11}FN_4OS$ (362)	56	>300
P_{51}	Thienyl	4-Cl	$C_{19}H_{11}ClN_4OS$ (378)	51	>300
P_{52}	Thienyl	2,4-Cl	$C_{19}H_{10}Cl_2N_4OS$ (412)	49	>300
P_{53}	Thienyl	4-CH_3	$C_{20}H_{14}N_4OS$ (358)	56	>300
P_{54}	1-napthyl	H	$C_{25}H_{16}N_4O$ (388)	58	>300
P_{55}	1-napthyl	4-OCH_3	$C_{26}H_{18}N_4O_2$ (418)	60	>300
P_{56}	1-napthyl	4-F	$C_{25}H_{15}FN_4O$ (406)	57	>300
P_{57}	1-napthyl	4-Cl	$C_{25}H_{15}ClN_4O$ (422)	50	>300
P_{58}	1-napthyl	2,4-Cl	$C_{25}H_{14}Cl_2N_4O$ (456)	48	>300
P_{59}	1-napthyl	4-CH_3	$C_{26}H_{18}N_4O$ (402)	56	>300
P_{60}	5-Cl-thienyl	H	$C_{19}H_{11}ClN_4OS$ (378)	45	>300
P_{61}	5-Cl-thienyl	4-F	$C_{19}H_{10}ClFN_4OS$ (396)	48	>300
P_{62}	5-Cl-thienyl	4-Cl	$C_{19}H_{10}Cl_2N_4OS$ (412)	51	>300
P_{63}	5-Cl-thienyl	4-CH_3	$C_{20}H_{13}ClN_4OS$(392)	53	>300

Table 6.2 Antibacterial activity of compounds $\mathbf{P_{49\text{-}63}}$.

Compound code	Zone of inhibition (mm)					
	Escherichia coli		***Staphylococcus aureus***		***Pseudomonas aeruginosa***	
Conc. in µg/mL	1000	500	1000	500	1000	500
$\mathbf{P_{49}}$	00	00	00	00	00	00
$\mathbf{P_{50}}$	07±0.1	05±0.2	08±0.2	06±0.2	06±0.2	04±0.1
$\mathbf{P_{51}}$	09±0.2	06±0.2	06±0.1	04±0.2	07±0.2	05±0.2
$\mathbf{P_{52}}$	08±0.2	06±0.2	09±0.2	07±0.1	07±0.2	05±0.2
$\mathbf{P_{53}}$	00	00	00	00	00	00
$\mathbf{P_{54}}$	00	00	00	00	00	00
$\mathbf{P_{55}}$	06±0.2	04±0.1	07±0.1	05±0.1	08±0.2	04±0.2
$\mathbf{P_{56}}$	00	00	00	00	00	00
$\mathbf{P_{57}}$	03±0.2	01±0.1	05±0.1	03±0.1	04±0.2	02±0.2
$\mathbf{P_{58}}$	04±0.2	01±0.1	02±0.1	00	03±0.2	01±0.2
$\mathbf{P_{59}}$	00	00	00	00	00	00
$\mathbf{P_{60}}$	00	00	00	00	00	00
$\mathbf{P_{61}}$	07±0.1	05±0.2	08±0.2	06±0.2	06±0.2	04±0.1
$\mathbf{P_{62}}$	06±0.2	04±0.1	07±0.1	05±0.1	08±0.2	04±0.2
$\mathbf{P_{63}}$	06±0.1	04±0.2	07±0.3	04±0.2	09±0.2	07±0.1
Streptomycin (Std.)	16±0.2	10±0.1	15±0.2	10±0.2	16±0.2	13±0.2

All the synthesized compounds were also tested for its antifungal activity (*in vitro)* against *Aspergillus flavus*, *Chrysosporium keratinophilum* and *Candida albicans* by measuring its average zone of inhibition. Fluconazole was used as standard for antifungal activity. Among the synthesized, compound $\mathbf{P_{51}}$ showed moderate activity against *Aspergillus flavus* at concentrations of 1000 and 500 µg/mL. Remaining compounds showed poor antifungal activity against all fungal strains compared to standard drug Fluconazole **(Table 6.3)**.

The acute oral toxicity study for compounds $\mathbf{P_{50}}$, $\mathbf{P_{51}}$, $\mathbf{P_{52}}$, $\mathbf{P_{55}}$, $\mathbf{P_{61}}$, $\mathbf{P_{62}}$ and $\mathbf{P_{63}}$ was also carried out by following the OECD guidelines No. 420. The experimental

studies revealed that the compounds were quite safe up to 2000 mg/kg and no deaths of animals were recorded. Further, no significant gross behavioral changes were observed in experimental animals except in the 3000 and 4000 mg/kg of all organic compounds, which showed depression on the first day and dead on second day.

Table 6.3 Antifungal activity of compounds $\mathbf{P_{49\text{-}63}}$.

Compound code	**Zone of inhibition (mm)**					
	Aspergillus flavus		***Chrysosporium keratinophilum***		***Candida albicans***	
Conc in µg/mL	1000	500	1000	500	1000	500
$\mathbf{P_{49}}$	00	00	00	00	00	00
$\mathbf{P_{50}}$	05±0.1	03±0.1	04±0.2	03±0.1	05±0.1	02±0.1
$\mathbf{P_{51}}$	06±0.1	05±0.2	04±0.1	03±0.1	05±0.1	04±0.1
$\mathbf{P_{52}}$	03±0.2	01±0.1	04±0.1	03±0.1	04±0.1	03±0.1
$\mathbf{P_{53}}$	00	00	00	00	00	00
$\mathbf{P_{54}}$	00	00	00	00	00	00
$\mathbf{P_{55}}$	04±0.1	03±0.2	06±0.1	05±0.2	04±0.1	02±0.1
$\mathbf{P_{56}}$	00	00	00	00	00	00
$\mathbf{P_{57}}$	03±0.1	02±0.1	03±0.1	01±0.1	03±0.1	01±0.1
$\mathbf{P_{58}}$	04±0.1	02±0.1	05±0.1	02±0.1	04±0.1	02±0.1
$\mathbf{P_{59}}$	00	00	00	00	00	00
$\mathbf{P_{60}}$	00	00	00	00	00	00
$\mathbf{P_{61}}$	04±0.2	01±0.1	04±0.1	02±0.1	03±0.2	01±0.2
$\mathbf{P_{62}}$	00	00	00	00	00	00
$\mathbf{P_{63}}$	00	00	00	00	00	00
Fluconazole (Std.)	13±0.2	10±0.1	17±0.2	15±0.2	22±0.2	20±0.2

The results of antimicrobial study reveals that presence of substituent's on the phenyl ring attached to the fourth position of pyrazole ring plays important role. The activity exhibited by $\mathbf{P_{50}}$, $\mathbf{P_{51}}$, $\mathbf{P_{52}}$, $\mathbf{P_{55}}$, $\mathbf{P_{61}}$, $\mathbf{P_{62}}$ and $\mathbf{P_{63}}$ is may be due to the presence of groups like, chloro, fluoro, methyl and methoxy attached to fourth position of

phenyl rings of pyrazole ring. This is also supported by the previous reports (Bandgar et al. 2009). However, in general, compounds containing a halogen substituents showed better antibacterial activity than the compounds with other substituent's (Sharma et al. 2011). The absence of such pharmacophore on phenyl ring fails to exhibit both antibacterial as well as antifungal activity. From the antimicrobial results we can conclude that, synthesized compounds are specific antibacterial agents.

6.6 CONCLUSIONS

In the present work, a series of new 4,6-disubstituted-3-cyano-2-pyridone derivatives were synthesized and characterized by IR, ^{1}H NMR, ^{13}C NMR, mass and elemental analyses. All the compounds were screened for its antimicrobial activity. Antibacterial results indicated that the compounds **P_{50}**, **P_{51}**, **P_{52}**, **P_{55}**, **P_{61}**, **P_{62}** and **P_{63}** showed good antibacterial activity towards all bacterial strains when compared to standard drug Streptomycin. **P_{51}** showed moderate antifungal activity whereas remaining compounds showed poor antifungal activity compared to other synthesized compounds. The acute oral toxicity study for compounds **P_{50}**, **P_{51}**, **P_{52}**, **P_{55}**, **P_{61}**, **P_{62}** and **P_{63}** was also carried out. The experimental studies revealed that the compounds were quite safe up to 2000 mg/kg and no deaths of animals were recorded.

CHAPTER - 7

SYNTHESIS OF NEW 3-ARYL-4-(3-ARYL-4,5-DIHYDRO-1*H*-PYRAZOL-5-YL)-1-PHENYL-1*H*-PYRAZOLE DERIVATIVES AS POTENTIAL ANTIMICROBIAL AGENTS

Abstract

This chapter describes a detailed literature survey on pyrazolines and its derivatives. The synthesis and characterization of newly designed 3-*aryl-4-(3-aryl-4,5-dihydro-1H-pyrazol-5-yl)-1-phenyl-1H-pyrazole derivatives has been included in this chapter. Antimicrobial studies of such compounds have also been discussed.*

7.1 INTRODUCTION

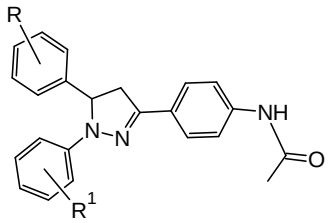
Pyrazolines are well known and important nitrogen containing five membered heterocyclic compounds. Several pyrazoline derivatives have been found to possess considerable biological activities which stimulated research activities in this field. After the pioneer work of Fischer and Knoevenagel in the late nineteenth century, the reaction of α,β-unsaturated aldehyde and ketones with hydrazines became one of the most popular method for the preparation of 2-pyrazolines.

The study of pyrazolines has attracted medicinal chemists due to their diverse biological properties such as antitumor, immunosuppressive, antibacterial, anti-inflammatory, anticancer, antidiabetic, and antidepressant properties (Holla et al. 2000, Bansal et al. 2001, Ahn et al. 2004 and Prasad et al. 2005, Manna et al. 2005, Karthikeyan et al. 2007, Ratkovic et al. 2010). The recent success of pyrazole COX-2 inhibitor (Reddy et al. 2008) has further highlighted the importance of these heterocycles in medicinal chemistry. A systematic investigation of this class of compounds revealed that pyrazole containing pharmacoactive agents play important role in medicinal chemistry.

1,2-Diaryl substituted heterocyclic system occurs in so many diverse classes of biologically interesting low molecular-weight compounds that it would be an understatement to link it to the ease of synthesis of the vicinal diaryl system (Kubinyi et al. 2004). Drugs based on a pyrazole ring bearing two adjacent aryl groups in a vicinal relation have often been occupying a position in the list of best selling pharmaceutical products since the beginning of this decade (Kubinyi et al. 2004). Recently, there has been considerable amount of progress in 1,3-diarylpyrazole chemistry because of the recognition of importance of the pyrazole structure in biological processes as antiparasitic (Rathelot et al. 2002), antiangiogensis (Abadi et al. 2003), antitubercular (Chovatia et al. 2007), antiviral (Hashem et al. 2007), analegesic and anxiolytic activity (Shetty and Bhagat 2008), antitumor (Joksovic et al.

2009), antimicrobial (Thumar and Patel 2011) and anti-inflammatory (Kumar et al. 2012) etc.

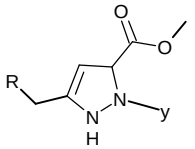
Singh et al. (1974) reported the anticonvulsant activity and selective inhibition of nicotinamide adenine dinucleotide-dependent oxidations by 1,3,5-trisubstituted pyrazolines (**S-7.1**). In general, pyrazolines possessing a 2,4-dinitrophenyl substituent at fifth position of the pyrazoline moiety possessed greater anticonvulsant activity.



S-7.1

Where R = H, Cl, OCH_3; R^1 = H, 2-NO_2, 4-NO_2, 2,4-$(NO_2)_2$

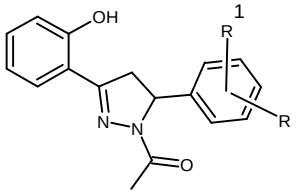
Agarwal et al. (1989) reported the preparation of pyrazolines (**S-7.2**) from methyl 4-oxooctadec-2(*E*)-enoate and their antimicrobial activity. The antimicrobial results revealed that all the compounds showed good antibacterial and antifungal activity.



S-7.2

Where R = $CH_3(CH_2)_{12}$; y = H, C_6H_5, 4-CH_3-C_6H_4

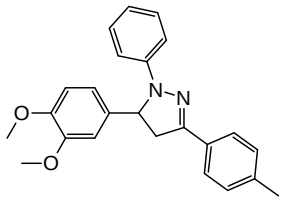
The synthesis, anti-inflammatory, analgesic and antipyretic activities of N-acetyl-Δ^2-pyrazolines (**S-7.3**) were reported by Manna et al. (1992). The synthesized compounds showed good anti-inflammatory and analgesic activity. The presence of substituents on the aryl group of the N-acetyl-Δ^2-pyrazoline derivatives was responsible for their anti-inflammatory and analgesic activity.



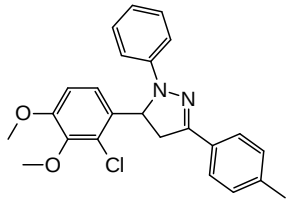
S-7.3

Where R = H, 2-Cl, 2-OCH_3
R_1 = H, 2-Cl, 3-Cl, 4-Cl, 2-Br, 3-Br, 4-Br, 2-OCH_3, 4-CH_3, 4-$N(CH_3)_2$, 4-OCH_3, 5-OCH_3

Palaska et al. (1996) synthesized some 1,3,5-triphenyl-2-pyrazolines and their antidepressant activity was investigated by Porsolt behavioral despair test. 1-Phenyl- 3-(4-methylphenyl)-5-(3,4-dimethoxyphenyl)-2-pyrazoline (**S-7.4**) and 1-phenyl-3-(4-methylphenyl)-5-(2-chloro-3,4-dimethoxyphenyl)-2-pyrazoline (**S-7.5**) showed significant antidepressant activity compared with Clomipramine and Tranylcypromine.

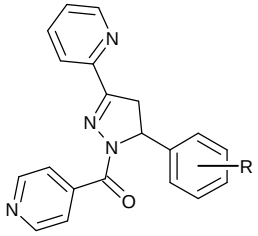


S-7.4



S-7.5

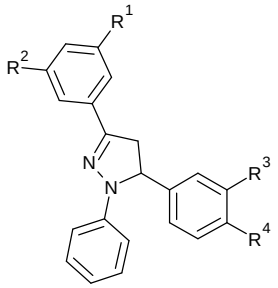
Mamolo et al. (2001) reported the synthesis and antimycobacterial activity of 5-aryl-1-isonicotinoyl-3-(pyridin-2-yl)-4,5-dihydro-1H-pyrazole derivatives (**S-7.6**). The results of such studies revealed that all the synthesized compounds exhibited an interesting *in vitro* antimycobacterial activity against the tested strains of *Mycobacterium tuberculosis*.



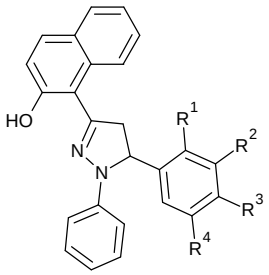
S-7.6

Where R = H, 2-Cl, 3-Cl, 4-Cl, 2-Br, 3-Br, 4-Br, 2-F, 3-F, 4-F, 2-CH_3, 3-CH_3, 4-CH_3

Prasad et al. (2005) synthesized some 1,3,5-triphenyl-2-pyrazolines (**S-7.7**) and 3-(2″-hydroxynaphthalen-1″-yl)-1,5-diphenyl-2-pyrazolines (**S-7.8**). The antidepressant activity of these compounds was evaluated and the results revealed that the compounds possessing electron-releasing groups such as dimethylamino, methoxy, and hydroxyl substituents, on both the aromatic rings at third and fifth positions of pyrazolines, considerably enhanced the antidepressant activity when compared to the pyrazolines having no substituents on the phenyl rings.



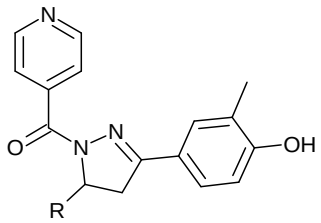
S-7.7



S-7.8

Where R^1 = H, OH; R^2 = H, CH_3, Br, OCH_3; R^3 = H, Br, OCH_3, Cl; R^4 = -$N(CH_3)_2$, H, OCH_3

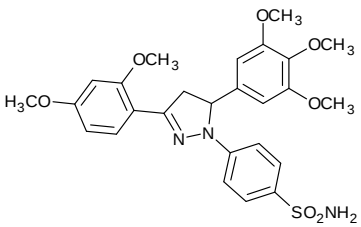
Shaharyar and his researchers (2006) synthesized a series of N′-nicotinoyl-3-(4'-hydroxy-3'-methyl phenyl)-5-(substitutedphenyl)-2-pyrazolines (**S-7.9**) by the reaction of isoniazid (INH) with chalcones and were tested for their antimycobacterial activity *in vitro* against *Mycobacterium tuberculosis* H37Rv (MTB) and INH-resistant *Mycobacterium tuberculosis* (INHR-MTB) using the agar dilution method.



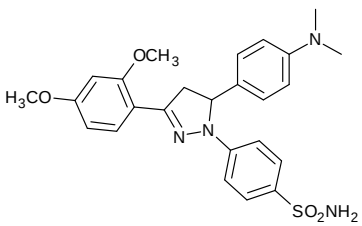
S-7.9

Where,
R = 4-OCH_3-C_6H_4, 4-Cl-C_6H_4, 4-$N(CH_3)$-C_6H_4, C_6H_5, Furyl, 3,4-$(OCH_3)_2$-C_6H_3, 4-F-C_6H_4, 2-Cl-C_6H_4, 2,6-Cl_2-C_6H_3, 3-NO_2-C_6H_4

Rathish et al. (2009) reported the synthesis and anti-inflammatory activity of some new 1,3,5-trisubstituted pyrazolines bearing benzene sulfonamide. The results revealed that compounds **S-7.10** and **S-7.11** were found to be more active than Celecoxib throughout the study.



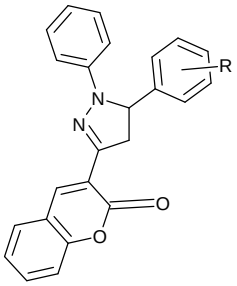
S-7.10



S-7.11

Synthesis and pharmacological evaluation of a novel series of 5-(substituted) aryl-3-(3-coumarinyl)-1-phenyl-2-pyrazolines (**S-7.12)** as novel anti-inflammatory

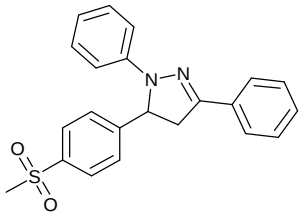
and analgesic agents was described by Khode and his coworkers (2009). Among the prepared compounds, compounds with substituents like 4-Chloro, 2,4-dichloro, 3-methoxy, 4-fluoro exhibited significant anti-inflammatory activity.



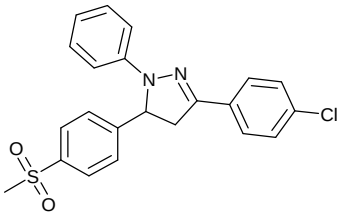
S-7.12

Where R = H, 4-OCH_3, 4-NMe_2, 4-Cl, 2,4-Cl, 3-OCH_3, 4-F
3-NO_2, 4-CH_3, 2-NO_2, 4-OH

Sivakumar et al. (2010) synthesized 1,3,5-triphenyl-2-pyrazolines and tested them for their anti-infective activity. The *in vitro* anti-infective activities (antimycobacterial, antibacterial and antifungal) revealed that, compared to the thiomethyl substitution, methylsulfonyl substitution in the A-ring leads to higher activity, namely compound **S-7.13** (high antimycobacterial activity) and **S-7.14** (high activity against bacterial and fungal strains) are found to be more active than the rest.

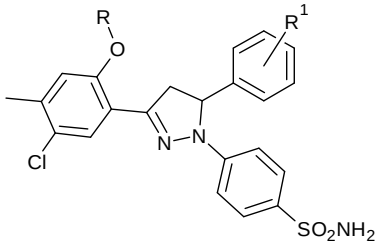


S-7.13



S-7.14

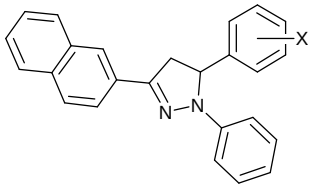
Bashir et al. (2011) reported the synthesis of some new 1,3,5-trisubstituted pyrazolines (**S-7.15)** bearing benzene sulfonamide as anticancer and anti-inflammatory agents. Few of the compounds were found to be active in the synthesized series.



Where
R = H, CH_3
R^1 = H, 2-OH, 2-Cl, 4-Cl,
3,4-OCH_3, 3,4,5-OCH_3

S-7.15

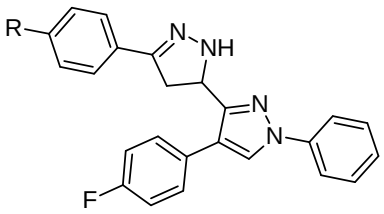
Sakthinathan et al. (2012) reported the synthesis, spectral studies and antimicrobial activities of some 2-naphthyl pyrazoline derivatives **(S-7.16)**. Antimicrobial results revealed that most of the synthesized compounds were found to be active against tested microorganisms.



S-7.16

Where X= H, 3-Br, 4-Br, 2-Cl, 3-Cl, 4-Cl, 4-F, 2-OCH_3, 4-CH_3

Shelke et al. (2012) synthesized fluorinated pyrazolines (**S-7.17)** using green technique and investigated their anti-infective properties. Antimicrobial results showed that most of the compounds showed good MIC (Minimum Inhibitory Concentration) values against tested microbial strains.



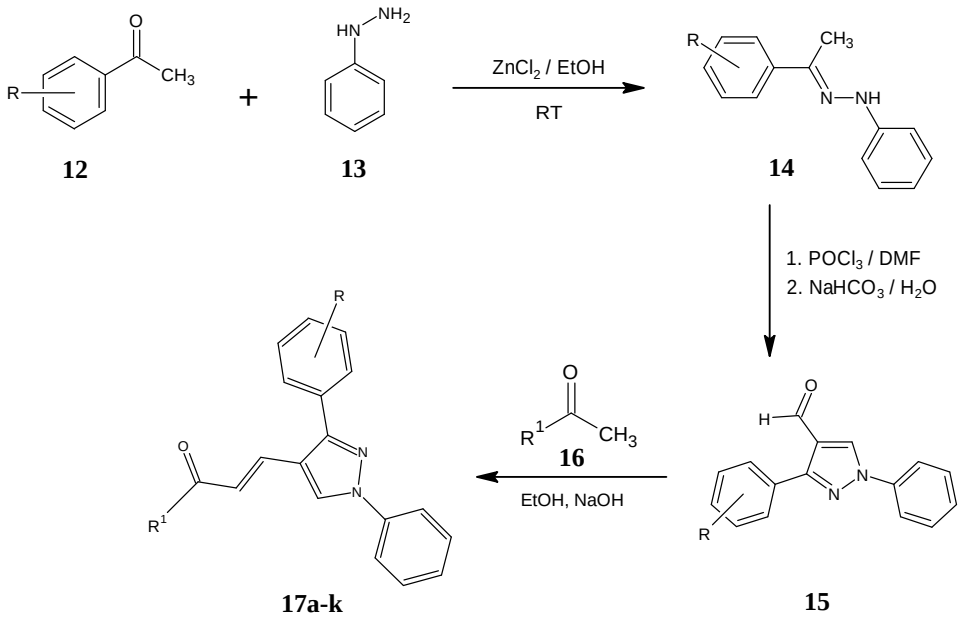
S-7.17

Where R = H, CH_3, F, Cl, Br

Owing to the significant contribution made by the researchers in synthesizing various pyrazoline derivatives and investigating them for their bioactivity, it was thought worthwhile to synthesize some new 3-aryl-4-(3-aryl-4,5-dihydro-1*H*-pyrazol-5-yl)-1-phenyl-1*H*-pyrazole derivatives and evaluate their antimicrobial properties.

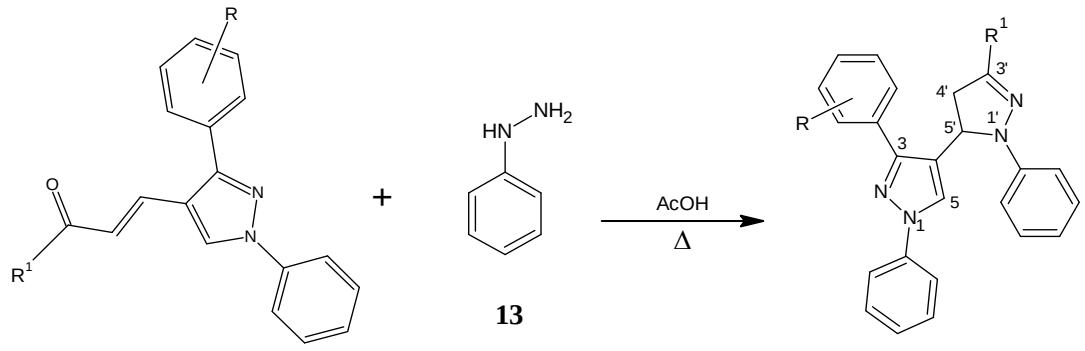
7.2 MATERIALS AND METHODS

In order to obtain the pyrazolic chalcones, the corresponding 4-formylpyrazolic precursors **15** were initially synthesized from the phenylhydrazones **14** (prepared by condensation of the respective acetophenones **12** and phenylhydrazine **13**); following a similar procedure described by Kira and his group (Kira et al. 1969). In this sense, the Vilsmeier–Haack reagent ($POCl_3$/DMF) was employed affording compounds **15**. Subsequently, the Claisen–Schmidt condensation of the obtained aldehydes **15** with the acetophenones **16** afforded the corresponding pyrazolic chalcones **17** (**Scheme 7.1**).



Scheme 7.1 Synthesis of new pyrazolic chalcones **17a-k**.

Continuing with the synthetic approach, the cyclocondensation of the chalcones **17a-k** with phenylhydrazine **13** in the presence of acetic acid as solvent, afforded the desired products $\mathbf{P_{64\text{-}74}}$ (**Scheme 7.2**).



17a-k **13** **P_{64-74}**

Where R = H, Cl, Br, OCH_3

R^1 = Biphenyl, 4-SCH_3-C_6H_4, 2,4-Cl-C_6H_3

Scheme 7.2 Synthesis of new pyrazoline derivatives **P_{64-74}**

Melting points were determined by open capillary method. The IR spectra were recorded on a Thermo Nicolet avatar 330-FT-IR spectrophotometer. ^{1}H-NMR spectra were recorded (DMSO-d_6) on a Bruker (400 MHz) spectrometer. Chemical shift values are given in δ scales. The mass spectra were recorded on LC-MS-Agilent 1100 series. Elemental analyses were performed on Elementar Vario Micro CHNS analyzer. The completion of the reaction was checked by thin layer chromatography (TLC) on silica gel coated aluminium sheets (silica gel 60 F254). Single crystal X-ray analysis was performed using Bruker APEXII CCD diffractometer. Commercial grade solvents and reagents were used without further purification.

7.3 EXPERIMENTAL

The experimental protocols followed for the synthesis of compounds **P_{64-74}** is given in the following section.

7.3.1 Synthesis and Characterization

General procedure for the preparation of chalcones (17a-k)

To a cold, stirred mixture of methanol (20 mL) and sodium hydroxide (12.09 mmol) was added appropriate acetophenone **16** (4.03 mmol). The reaction mixture was stirred for 10 min. To this was added appropriate formyl pyrazole **15** (4.03 mmol) followed by tetrahydrofuran (30 mL). The solution was further stirred for 2 h at 0 ˚C and then at room temperature for 5 h. It was then poured into ice cold water. The

resulting solution was neutralized with dil. HCl. The solid so separated was filtered, washed with water, dried and crystallized from ethanol to afford product **17a-k**.

(2E)-3-[3-(4-chlorophenyl)-1-phenyl-1*H*-pyrazol-4-yl]-1-[4-biphenyl]prop-2-en-1-one (17a)

FT-IR (cm^{-1}): 3122-3055 (C-H), 1659 (C=O), 1593 (C=N) (**Figure 7.1**); MS: m/z = 461 (M+1) (**Figure 7.2**). Anal. calcd. for $C_{30}H_{21}ClN_2O$: C, 78.17; H, 4.59; N, 6.08. Found: C, 78.11; H, 4.54; N, 6.01 %.

(2E)-3-[3-(4-bromophenyl)-1-phenyl-1*H*-pyrazol-4-yl]-1-[4-biphenyl]prop-2-en-1-one (17b)

FT-IR (cm^{-1}): 3116-3052 (C-H), 1656 (C=O), 1596 (C=N); MS: m/z = 507 (M+2). Anal. calcd. for $C_{30}H_{21}BrN_2O$: C, 71.29; H, 4.19; N, 5.54. Found: C, 71.23; H, 4.14; N, 5.48 %.

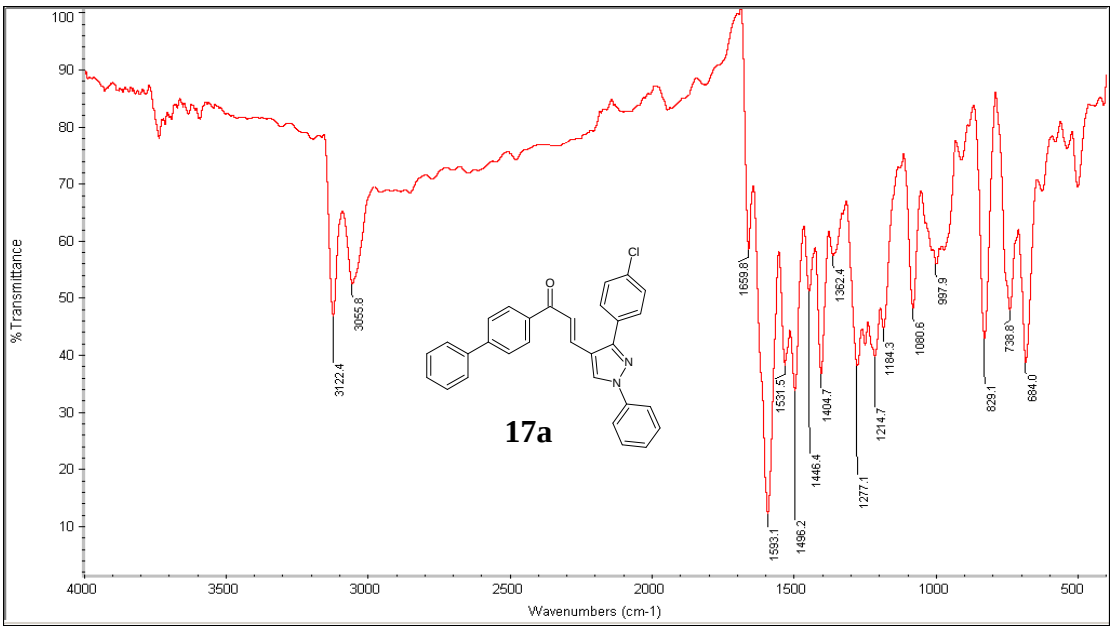


Figure 7.1 IR spectrum of compound **17a**

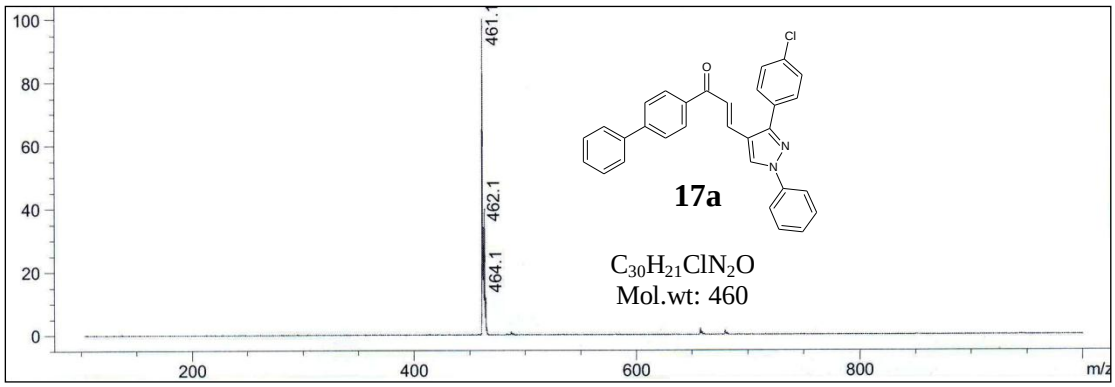


Figure7.2 Mass spectrum of compound **17a**

(2E)-3-[3-(4-methoxyphenyl)-1-phenyl-1*H*-pyrazol-4-yl]-1-[4-biphenyl]prop-2-en-1-one (17c)

FT-IR (cm^{-1}): 3118-3047 (C-H), 1654 (C=O), 1580 (C=N); MS: m/z = 457 (M+1). Anal. calcd. for $C_{31}H_{24}N_2O_2$: C, 81.56; H, 5.30; N, 6.14. Found: C, 81.49; H, 5.26; N, 6.11%.

(2E)-3-(1,3-diphenyl-1*H*-pyrazol-4-yl)-1-[4-(methylsulfanyl)phenyl]prop-2-en-1-one (17d)

FT-IR (cm^{-1}): 3122-3051 (C-H), 1662 (C=O), 1586 (C=N); MS: m/z = 397 (M+1). Anal. calcd. for $C_{25}H_{20}N_2OS$: C, 75.73; H, 5.08; N, 7.07. Found: C, 75.68; H, 5.01; N, 7.02 %.

(2E)-3-[3-(4-chlorophenyl)-1-phenyl-1*H*-pyrazol-4-yl]-1-[4-(methylsulfanyl) phenyl]prop-2-en-1-one (17e)

FT-IR (cm^{-1}): 3118-3057 (C-H), 1647 (C=O), 1583 (C=N); MS: m/z = 431 (M+1). Anal. calcd. for $C_{25}H_{19}ClN_2OS$: C, 69.68; H, 4.44; N, 6.50. Found: C, 69.62; H, 4.38; N, 6.46 %.

(2E)-3-[3-(4-bromophenyl)-1-phenyl-1*H*-pyrazol-4-yl]-1-[4-(methylsulfanyl) phenyl] prop-2-en-1-one (17f)

FT-IR (cm^{-1}): 3116-3060 (C-H), 1648 (C=O), 1583 (C=N); MS: m/z = 477 (M+2). Anal. calcd. for $C_{25}H_{19}BrN_2OS$: C, 63.16; H, 4.03; N, 5.89. Found: C, 63.11; H, 3.98; N, 5.80 %.

(2E)-3-[3-(4-methoxyphenyl)-1-phenyl-1*H*-pyrazol-4-yl]-1-[4-(methylsulfanyl) phenyl] prop-2-en-1-one (17g)

FT-IR (cm^{-1}): 3114-3053 (C-H), 1657 (C=O), 1592 (C=N); MS: m/z = 427 (M+1). Anal. calcd. for $C_{26}H_{22}N_2O_2S$: C, 73.21; H, 5.20; N, 6.57. Found: C, 73.17; H, 5.15; N, 6.51 %.

(2E)-1-(2,4-dichlorophenyl)-3-(1,3-diphenyl-1*H*-pyrazol-4-yl)prop-2-en-1-one (17h)

FT-IR (cm^{-1}): 3121-3066 (C-H), 1687 (C=O), 1586 (C=N); MS: m/z = 419 (M+). Anal. calcd. for $C_{24}H_{16}Cl_2N_2O$: C, 68.75; H, 3.85; N, 6.68. Found: C, 68.71; H, 3.79; N, 6.60 %. Crystal structure of **17h** has been presented in **Figure 7.3**.

(2E)-3-[3-(4-chlorophenyl)-1-phenyl-1*H*-pyrazol-4-yl]-1-(2,4-dichlorophenyl) prop-2-en-1-one (17i)

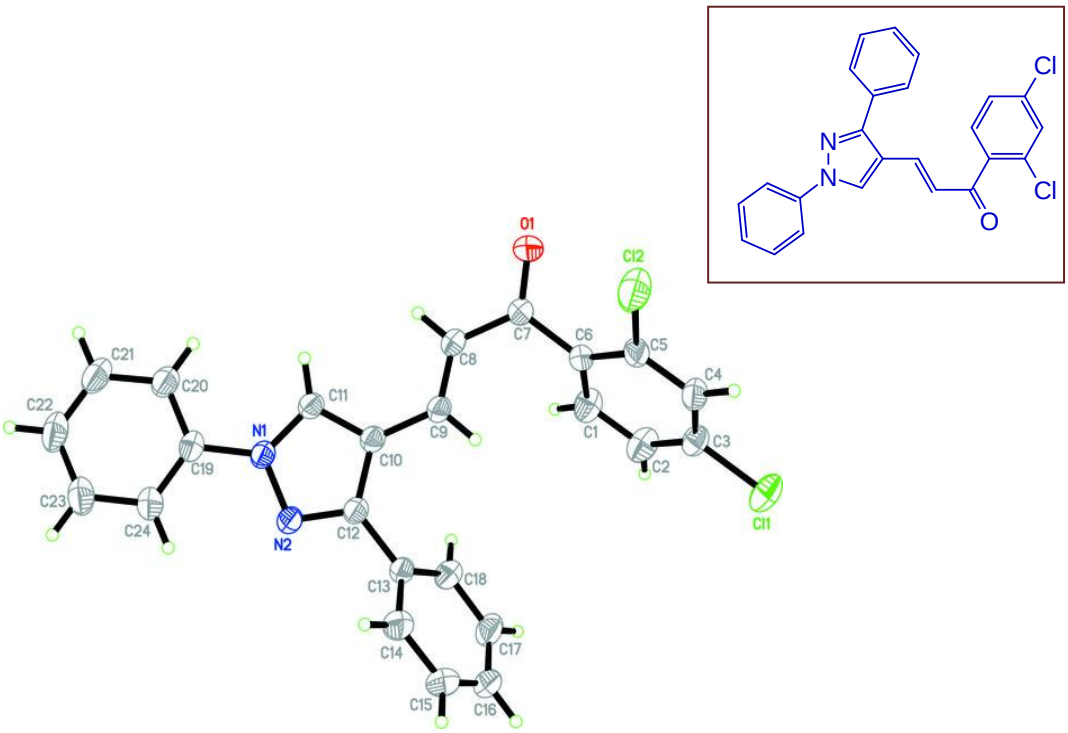
FT-IR (cm^{-1}): 3118-3088 (C-H), 1658 (C=O), 1585 (C=N). MS: m/z = 454 (M+1). Anal. calcd. for $C_{24}H_{15}Cl_3N_2O$: C, 63.53; H, 3.33; N, 6.17. Found: C, 63.47; H, 3.29; N, 6.11%.

(2E)-3-[3-(4-bromophenyl)-1-phenyl-1*H*-pyrazol-4-yl]-1-(2,4-dichlorophenyl) prop-2-en-1-one (17j)

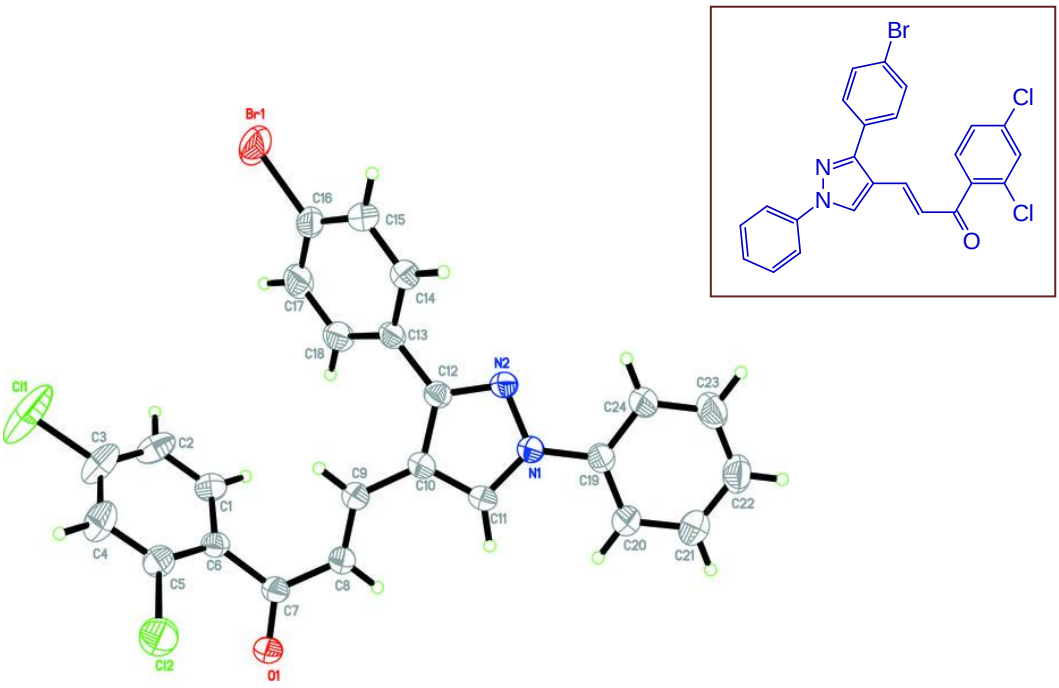
FT-IR (cm^{-1}): 3122-3060 (C-H), 1683 (C=O), 1584 (C=N). MS: m/z = 499 (M+1). Anal. calcd. for $C_{24}H_{15}BrCl_2N_2O$: C, 57.86; H, 3.03; N, 5.62. Found: C, 57.81; H, 2.97; N, 5.58 %. Crystal structure of **17j** has been presented in **Figure 7.3**.

(2E)-1-(2,4-dichlorophenyl)-3-[3-(4-methoxyphenyl)-1-phenyl-1*H*-pyrazol-4-yl]prop-2-en-1-one (17k)

FT-IR (cm^{-1}): 3118-3071 (C-H), 1687 (C=O), 1610 (C=N); MS: m/z = 449 (M+). Anal. calcd. for $C_{25}H_{18}Cl_2N_2O$: C, 66.83; H, 4.04; N, 6.23. Found: C, 66.78; H, 3.97; N, 6.18 %.



17h



17j

Figure 7.3 The Single crystal X-ray structures of compounds **17h** & **17j**

General procedure for the synthesis of pyrazolines P_{64-74}

A mixture of chalcone **17a-k** (1.0 mmol) and phenylhydrazine **13** (1.5 mmol) was refluxed in glacial acetic acid (8 mL) for 4-5 h. Reaction progress was monitored by TLC and the precipitate formed after cooling was filtered off and recrystallized from ethanol, affording compounds **P_{64-74}.**

3-(4-chlorophenyl)-1-phenyl-4-(1-phenyl-3-biphenyl-4,5-dihydro-1*H*-pyrazol-5-yl)-1*H*-pyrazole (P_{64})

FT-IR (cm^{-1}): 3042 (Ar-C-H), 2923 (C-H), 1590 (C=N), 1492 (C=C) (**Figure 7.4)**; ^{1}H-NMR (DMSO-d_6): δ 8.36 (s, 1H, pyrazole-5H), 7.83-7.69 (m, 10H, Ar-H), 7.55 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.48-7.41 (m, 5H, Ar-H), 7.27 (dd, 1H, *J* = 7.2 Hz, Ar-H), 7.15 (dd, 2H, *J* = 8 Hz, Ar-H), 7.03 (d, 2H, *J* = 7.6 Hz, Ar-H), 6.73 (dd, 1H, *J* = 7.2 Hz, Ar-H), 5.58-5.53 (dd, 1H, *J* = 7.6 Hz, 12.2 Hz, 5′-H of pyrazoline), 4.08-4.01 (dd, 1H, *J* = 12 Hz, 17.2 Hz, 4′-H of pyrazoline), 3.31-3.25 (dd, 1H, *J* = 7.6 Hz, 17.6 Hz, 4′-H of pyrazoline) (**Figure 7.5**). ^{13}C-NMR (DMSO-d_6): δ 148.9, 147.8, 145.0, 140.6, 139.9, 139.5, 130.1, 129.9, 129.4, 129.3, 129.2, 127.0, 126.8, 119.5, 118.7, 113.8, 56.4, 42.8 (**Figure 7.6**). MS: m/z = 551 (M+1) (**Figure 7.7**). Anal. calcd. for $C_{36}H_{27}ClN_4$: C, 78.46; H, 4.94; N, 10.17. Found: C, 78.39; H, 4.88; N, 10.13 %.

3-(4-bromophenyl)-1-phenyl-4-(1-phenyl-3-biphenyl-4,5-dihydro-1*H*-pyrazol-5-yl)-1*H*-pyrazole (P_{65})

FT-IR (cm^{-1}): 3041 (Ar-C-H), 2920 (C-H), 1590 (C=N), 1492 (C=C); ^{1}H-NMR (DMSO-d_6): δ 8.38 (s, 1H, pyrazole-5H), 7.85-7.69 (m, 12H, Ar-H), 7.51-7.37 (m, 5H, Ar-H), 7.29 (dd, 1H, *J* = 7.4 Hz, Ar-H), 7.17 (dd, 2H, *J* = 8 Hz, Ar-H), 7.05 (d, 2H, *J* = 7.6 Hz), 6.76 (dd, 1H, *J* = 7.2 Hz, Ar-H), 5.61-5.56 (dd, 1H, *J* = 7.6 Hz, 12.2 Hz, 5′-H of pyrazoline), 4.11-4.03 (dd, 1H, *J* = 12 Hz, 17.2 Hz, 4′-H of pyrazoline), 3.33-3.27 (m, 1H, 4′-H of pyrazoline merged with peak of HOD). ^{13}C-NMR (DMSO-d_6): δ 148.9, 147.8, 145.0, 140.6, 139.9, 139.5, 132.1, 130.4, 129.9, 129.4, 129.3, 128.1, 127.8, 127.2, 127.0, 126.8, 119.5, 118.7, 113.8, 56.4, 42.8. MS: m/z = 595 (M+1). Anal. calcd. for $C_{36}H_{27}BrN_4$: C, 72.61; H, 4.57; N, 9.41. Found: C, 72.57; H, 4.52; N, 9.37 %.

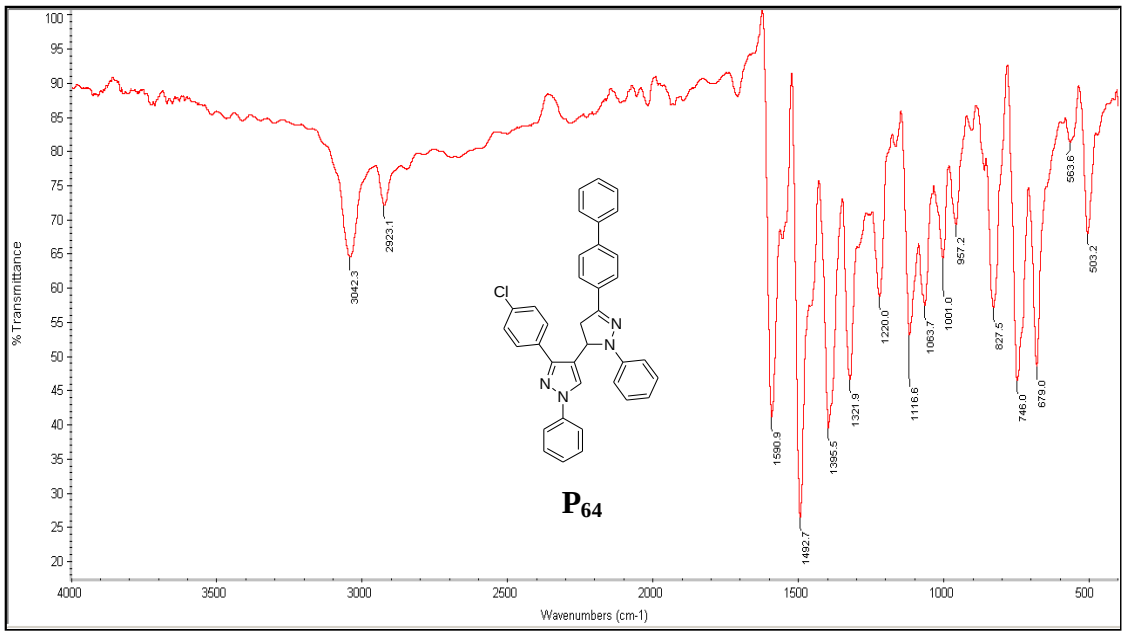


Figure 7.4 IR spectrum of compound **P_{64}**

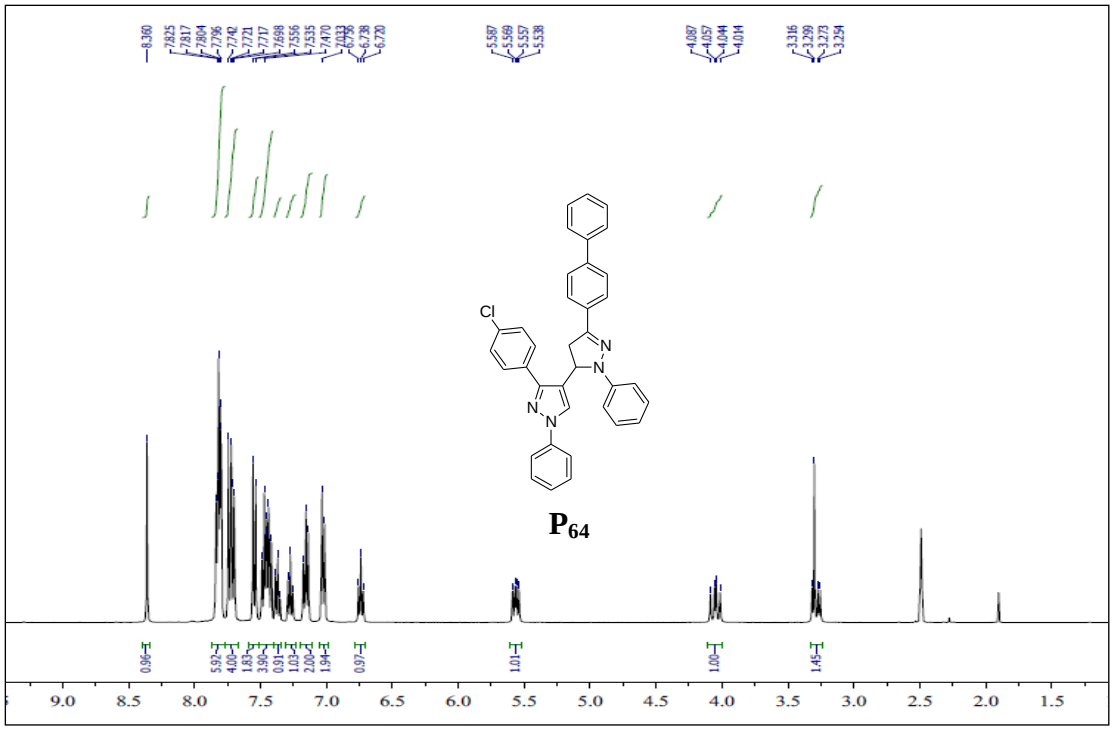


Figure 7.5 ^{1}H NMR spectrum of compound **P_{64}**

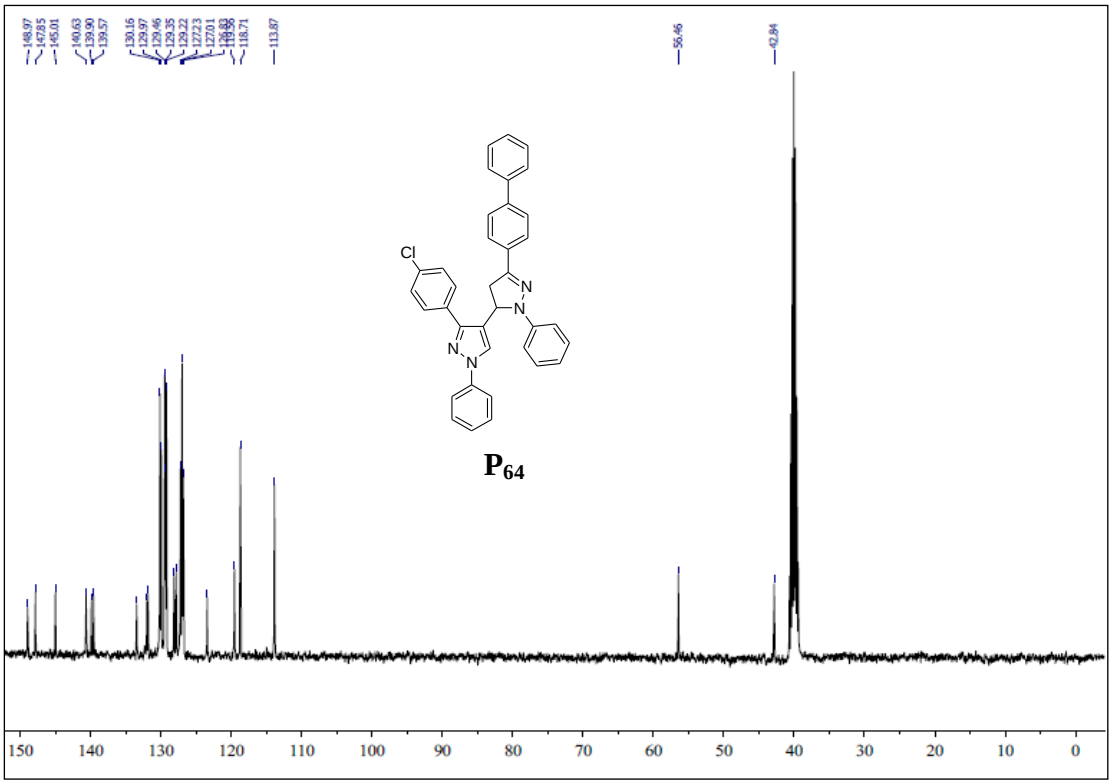


Figure 7.6 ^{13}C NMR spectrum of compound **P_{64}**

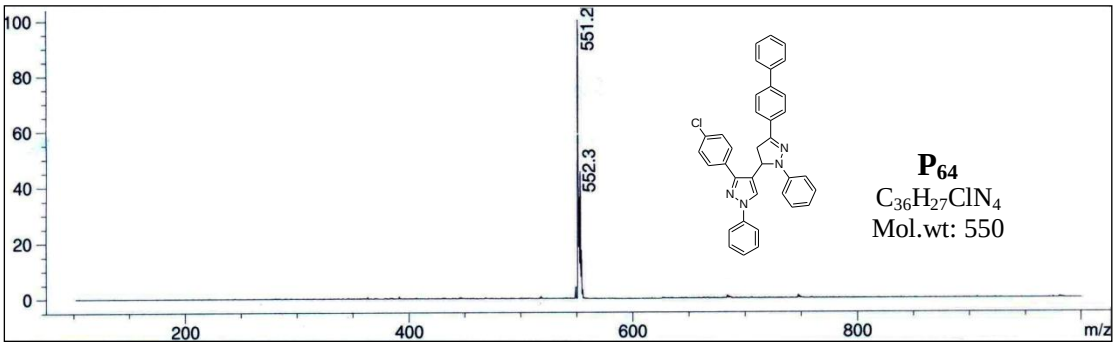


Figure 7.7 Mass spectrum of compound **P_{64}**

3-(4-methoxyphenyl)-1-phenyl-4-(1-phenyl-3-biphenyl-4,5-dihydro-1*H*-pyrazol-5-yl)-1*H*-pyrazole (P_{66})

FT-IR (cm^{-1}): 3031 (Ar-C-H), 2939 (C-H), 1593 (C=N), 1491 (C=C); ^{1}H-NMR (DMSO-d_6): δ 8.31 (s, 1H, pyrazole-5H), 7.83-7.80 (m, 4H, Ar-H), 7.74-7.70 (m, 6H, Ar-H), 7.49-7.35 (m, 5H, Ar-H), 7.25 (dd, 1H, *J* = 7.4 Hz, Ar-H), 7.14 (dd, 2H, *J* = 8 Hz, Ar-H), 7.07-7.0 (m, 4H, Ar-H), 6.73 (dd, 1H, *J* = 7.2 Hz, Ar-H), 5.54-5.49

(dd, 1H, *J* = 7.6 Hz, 12.4 Hz, 5′-H of pyrazoline), 4.08-4.01 (dd, 1H, *J* = 12.4 Hz, 17.2 Hz, 4′-H of pyrazoline), 3.80 (s, 3H, -OCH_3), 3.32-3.26 (m, 1H, 4′-H of pyrazoline merged with peak of HOD). ^{13}C-NMR (DMSO-d_6): δ 159.7, 150.0, 147.7, 145.0, 140.5, 139.9, 139.7, 131.9, 129.9, 129.7, 129.4, 129.3, 128.1, 127.4, 127.2, 127.0, 126.8, 126.6, 125.5, 122.9, 119.4, 118.5, 114.6, 113.8, 56.5, 55.6, 42.8. MS: m/z = 547 (M+1). Anal. calcd. for $C_{37}H_{30}N_4O$: C, 81.29; H, 5.53; N, 10.25. Found: C, 81.24; H, 5.49; N, 10.20 %. Crystal structure of **P_{66}** has been given in **Figure 7.8**.

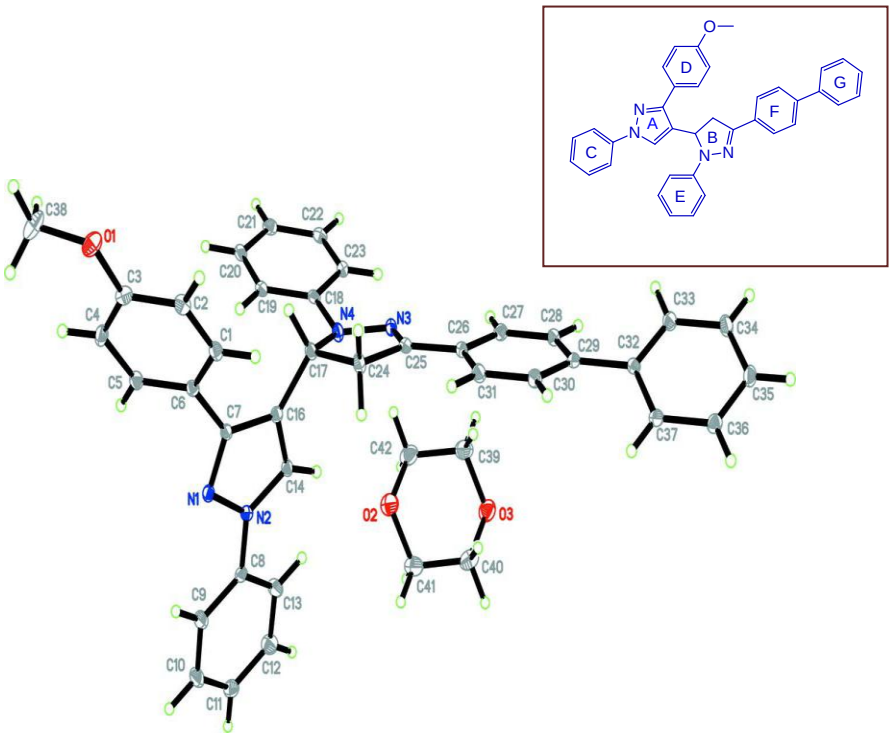


Figure 7.8 The Single crystal X-ray structure of compound **P_{66}**

4-(3-(4-(methylthio)phenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazol-5-yl)-1,3-diphenyl-1*H*-pyrazole (P_{67})

FT-IR (cm^{-1}): 3042 (Ar-C-H), 2917 (C-H), 1590 (C=N), 1492 (C=C); ^{1}H-NMR (DMSO-d_6): δ 8.33 (s, 1H, pyrazole-5H), 7.83-7.77 (2d, 4H, *J* = 8 Hz, *J* = 7.2 Hz, Ar-H), 7.67 (d, 2H, *J* = 8.4, Ar-H), 7.52-7.41 (m, 5H, Ar-H), 7.30-7.24 (m, 3H, Ar-H), 7.12 (dd, 2H, *J* = 7.8 Hz, Ar-H), 6.98 (d, 2H, *J* = 7.6 Hz, Ar-H), 6.71 (dd, 1H, *J* = 7.2 Hz, Ar-H), 5.51-5.46 (dd, 1H, *J* = 7.6 Hz, 12.2 Hz, 5′-H of pyrazoline), 4.05-3.97 (dd, 1H, *J* = 12.4 Hz, 17.0 Hz, 4′-H of pyrazoline), 3.27-3.21 (dd, 1H, *J* = 8.0 Hz, 17.0 Hz, 4′-H of pyrazoline), 2.49 (s, 3H, -SCH_3, merged with DMSO-d_6 peak). ^{13}C-NMR

(DMSO-d_6): δ 150.1, 147.8, 145.1, 139.6, 139.5, 129.9, 129.3, 129.2, 128.6, 128.4, 126.8, 126.6, 126.1, 119.3, 118.6, 113.7, 56.49, 42.9, 14.9. MS: m/z = 487 (M+1). Anal. calcd. for $C_{31}H_{26}N_4S$: C, 76.51; H, 5.39; N, 11.51. Found: C, 76.47; H, 5.34; N, 11.47 %.

3-(4-chlorophenyl)-4-(3-(4-(methylthio)phenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazol-5-yl)-1-phenyl-1*H*-pyrazole (P_{68})

FT-IR (cm^{-1}): 3038 (Ar-C-H), 2916 (C-H), 1586 (C=N), 1488 (C=C); ^{1}H-NMR (DMSO-d_6): δ 8.35 (s, 1H, pyrazole-5H), 7.83-7.78 (m, 4H, Ar-H), 7.67 (d, 2H, *J* = 8.8, Ar-H), 7.54 (d, 2H, *J* = 8.8 Hz, Ar-H), 7.43 (dd, 2H, *J* = 8.0 Hz, Ar-H), 7.30-7.27 (m, 3H, Ar-H), 7.13 (dd, 2H, *J* = 8.0 Hz, Ar-H), 6.99 (d, 2H, *J* = 7.6 Hz, Ar-H), 6.72 (d, 1H, *J* = 7.4 Hz, Ar-H), 5.53-5.48 (dd, 1H, *J* = 7.6 Hz, 12.4 Hz, 5′-H of pyrazoline), 4.03-3.96 (dd, 1H, *J* = 12.0 Hz, 17.2 Hz, 4′-H of pyrazoline), 3.25-3.19 (dd, 1H, *J* = 7.6 Hz, 17.2 Hz, 4′-H of pyrazoline), 2.49 (s, 3H, -SCH_3, merged with DMSO-d_6 peak). ^{13}C-NMR (DMSO-d_6): δ 148.9, 147.8, 145.1, 139.5, 130.1, 129.9, 129.3, 129.2, 127.8, 126.9, 126.6, 126.1, 119.4, 118.7, 113.8, 56.4, 42.8, 14.9. MS: m/z = 521 (M+1). Anal. calcd. for $C_{31}H_{25}ClN_4S$: C, 71.45; H, 4.84; N, 10.75. Found: C, 71.39; H, 4.80; N, 10.71 %.

3-(4-bromophenyl)-4-(3-(4-(methylthio)phenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazol-5-yl)-1-phenyl-1*H*-pyrazole (P_{69})

FT-IR (cm^{-1}): 3034 (Ar-C-H), 2916 (C-H), 1586 (C=N), 1490 (C=C); ^{1}H-NMR (DMSO-d_6): δ 8.34 (s, 1H, pyrazole-5H), 8.03 (d, 1H, *J* = 8.4 Hz, Ar-H), 7.94 (d, 1H, *J* = 7.6 Hz, Ar-H), 7.87-7.56 (m, 5H, Ar-H), 7.45-7.41 (m, 3H, Ar-H), 7.30-7.25 (m, 3H, Ar-H), 7.13 (dd, 2H, *J* = 8.0 Hz, Ar-H), 6.99 (d, 2H, *J* = 7.6 Hz, Ar-H), 6.72 (dd, 1H, *J* = 7.2 Hz, Ar-H), 5.53-5.48 (dd, 1H, *J* = 7.6 Hz, 12.2 Hz, 5′-H of pyrazoline), 4.03-3.96 (dd, 1H, *J* = 12.0 Hz, 17.2 Hz, 4′-H of pyrazoline), 3.25-3.19 (dd, 1H, *J* = 7.6 Hz, 17.2 Hz, 4′-H of pyrazoline), 2.49 (s, 3H, -SCH_3). MS: m/z = 565 (M^+). Anal. calcd. for $C_{31}H_{25}BrN_4S$: C, 65.84; H, 4.46; N, 9.91. Found: C, 65.79; H, 4.41; N, 9.88 %.

3-(4-methoxyphenyl)-4-(3-(4-(methylthio)phenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazol-5-yl)-1-phenyl-1*H*-pyrazole (P_{70})

FT-IR (cm^{-1}): 3011 (Ar-C-H), 2917 (C-H), 1591 (C=N), 1491 (C=C); ^{1}H-NMR (DMSO-d_6): δ 8.30 (s, 1H, pyrazole-5H), 7.81 (d, 2H, *J* = 7.6 Hz, Ar-H), 7.71 (d, 2H,

J = 8.8 Hz, Ar-H), 7.65 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.42 (dd, 2H, *J* = 8.0 Hz, Ar-H), 7.30-7.23 (m, 4H, Ar-H), 7.12 (dd, 2H, *J* = 8.0 Hz, Ar-H), 7.06 (d, 2H, *J* = 8.8 Hz, Ar-H), 6.98 (d, 2H, *J* = 7.6 Hz, Ar-H), 6.71 (dd, 1H, *J* = 7.2 Hz, Ar-H), 5.48-5.43 (dd, 1H, *J* = 7.6 Hz, 12.2 Hz, 5′-H of pyrazoline), 4.03-3.95 (dd, 1H, *J* = 12.0 Hz, 17.2 Hz, 4′-H of pyrazoline), 3.8 (s, 3H, -OCH_3), 3.25-3.19 (dd, 1H, *J* = 7.6 Hz, 17.2 Hz, 4′-H of pyrazoline), 2.49 (s, 3H, -SCH_3). MS: m/z = 517 (M+1). Anal. calcd. for $C_{32}H_{28}N_4OS$: C, 74.39; H, 5.46; N, 10.84. Found: C, 74.34; H, 5.41; N, 10.80 %.

4-(3-(2,4-dichlorophenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazol-5-yl)-1,3-diphenyl-1*H*-pyrazole (P_{71})

FT-IR (cm^{-1}): 3058 (Ar-C-H), 2871 (C-H), 1590 (C=N), 1494 (C=C); ^{1}H-NMR (DMSO-d_6): δ 8.39 (s, 1H, pyrazole-5H), 7.83 (d, 2H, *J* = 7.6 Hz, Ar-H), 7.78-7.72 (m, 3H, Ar-H), 7.67 (d, 1H, *J* = 2.4 Hz, Ar-H), 7.51-7.4 (m, 6H, Ar-H), 7.28 (dd, 1H, *J* = 7.4 Hz, Ar-H), 7.14 (dd, 2H, *J* = 8.0 Hz, Ar-H), 7.0 (d, 2H, *J* = 8.0 Hz, Ar-H), 6.75 (dd, 1H, *J* = 7.4 Hz, Ar-H), 5.59-5.54 (dd, 1H, *J* = 7.6 Hz, 12.2 Hz, 5′-H of pyrazoline), 4.15-4.08 (dd, 1H, *J* = 12.0 Hz, 17.4 Hz, 4′-H of pyrazoline), 3.42-3.36 (dd, 1H, *J* = 7.6 Hz, 17.2 Hz, 4′-H of pyrazoline).MS: m/z = 508 (M^+). Anal. calcd. for $C_{30}H_{22}Cl_2N_4$: C, 70.73; H, 4.35; N, 11.00. Found: C, 70.69; H, 4.31; N, 10.97 %.

3-(4-chlorophenyl)-4-(3-(2,4-dichlorophenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazol-5-yl)-1-phenyl-1*H*-pyrazole (P_{72})

FT-IR (cm^{-1}): 3047 (Ar-C-H), 2904 (C-H), 1590 (C=N), 1496 (C=C); ^{1}H-NMR (DMSO-d_6): δ 8.5 (s, 1H, pyrazole-5H), 7.83 (d, 2H, *J* = 7.6 Hz, Ar-H), 7.77-7.72 (m, 3H, Ar-H), 7.67 (d, 1H, *J* = 2.4 Hz, Ar-H), 7.53-7.43 (m, 5H, Ar-H), 7.28 (dd, 1H, *J* = 7.4 Hz, Ar-H), 7.16 (dd, 2H, *J* = 8.0 Hz, Ar-H), 7.02 (d, 2H, *J* = 8.0 Hz, Ar-H), 6.76 (dd, 1H, *J* = 7.4 Hz, Ar-H), 5.61-5.56 (dd, 1H, *J* = 7.6 Hz, 12.0 Hz, 5′-H of pyrazoline), 4.13-4.06 (dd, 1H, *J* = 12.4 Hz, 17.2 Hz, 4′-H of pyrazoline), 3.40-3.33 (dd, 1H, *J* = 7.6 Hz, 17.6 Hz, 4′-H of pyrazoline). MS: m/z = 543 (M+1). Anal. calcd. for $C_{30}H_{21}Cl_3N_4$: C, 66.25; H, 3.89; N, 10.30. Found: C, 66.20; H, 3.83; N, 10.25 %.

3-(4-bromophenyl)-4-(3-(2,4-dichlorophenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazol-5-yl)-1-phenyl-1*H*-pyrazole (P_{73})

FT-IR (cm^{-1}): 3035 (Ar-C-H), 2922 (C-H), 1589 (C=N), 1495 (C=C); ^{1}H-NMR (DMSO-d_6): δ 8.4 (s, 1H, pyrazole-5H), 7.83 (d, 2H, *J* = 7.6 Hz, Ar-H), 7.77 (d, 1H, *J* = 8.4 Hz, Ar-H), 7.69-7.64 (m, 4H, Ar-H), 7.48-7.43 (m, 4H, Ar-H), 7.28 (dd, 1H,

J = 7.4 Hz, Ar-H), 7.16 (dd, 2H, *J* = 8.0 Hz, Ar-H), 7.02 (d, 2H, *J* = 8.0 Hz, Ar-H), 6.76 (dd, 1H, *J* = 7.2 Hz, Ar-H), 5.61-5.56 (dd, 1H, *J* = 7.6 Hz, 12.2 Hz, 5′-H of pyrazoline), 4.13-4.05 (dd, 1H, *J* = 12.4 Hz, 17.4 Hz, 4′-H of pyrazoline), 3.39-3.33 (dd, 1H, *J* = 7.6 Hz, 17.2 Hz, 4′-H of pyrazoline). MS: m/z = 587 (M+1). Anal. calcd. for $C_{30}H_{21}BrCl_2N_4$: C, 61.25; H, 3.60; N, 9.52. Found: C, 61.21; H, 3.57; N, 9.48 %.

4-(3-(2,4-dichlorophenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazol-5-yl)-3-(4-methoxy phenyl)-1-phenyl-1*H*-pyrazole (P_{74})

FT-IR (cm^{-1}): 3064 (Ar-C-H), 2926 (C-H), 1594 (C=N), 1489 (C=C); ^{1}H-NMR (DMSO-d_6): δ 8.36 (s, 1H, pyrazole-5H), 7.82, 7.80, 7.78 (d, 3H, *J* = 8 Hz, Ar-H), 7.67-7.64 (d, 3H, *J* = 12 Hz, Ar-H), 7.48-7.42 (m, 3H, Ar-H), 7.26 (dd, 1H, *J* = 6 Hz, Ar-H), 7.15 (t, 2H, *J* = 6 Hz, Ar-H), 7.05-7.0 (m, 4H, Ar-H), 6.76 (dd, 1H, *J* = 8 Hz, Ar-H), 5.57-5.52 (dd, 1H, *J* = 8 Hz, 12 Hz, 5′-H of pyrazoline), 4.13-4.06 (dd, 1H, *J* = 12 Hz, 16 Hz, 4′-H of pyrazoline), 3.80 (s, 3H, -OCH_3), 3.40-3.34 (dd, 1H, *J* = 8 Hz, 16 Hz, 4′-H of pyrazoline). Anal. calcd. for $C_{31}H_{24}Cl_2N_4O$: C, 69.02; H, 4.48; N, 10.39. Found: C, 68.97; H, 4.41; N, 10.34 %.

7.4 PHARMACOLOGY

7.4.1 Antimicrobial activity

The *in vitro* antimicrobial activity of newly synthesized compounds **P_{64-74}** were determined by "well-plate" method in Mueller-Hinton Agar as explained in **Chapter 2** by measuring the diameter of inhibition zone (mm). In this work *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 were used to investigate the antibacterial activity whereas *Aspergillus flavus* ATCC 15547, *Chrysosporium keratinophilum* ATCC 14803 and *Candida albicans* MTCC 227 were used to study antifungal activity. These cultures are obtained from the Department of Microbiology, Kuvempu University, Shimoga, India. All fungi strains were maintained on potato dextrose agar (PDA) at ± 25 ˚C.

7.5 RESULTS AND DISCUSSION

7.5.1 Chemistry

The structural elucidation of compounds **17a-k** was based on the spectral data (IR, mass spectrometry and Elemental analysis). The IR spectrum of compound **17a** showed absorption bands at 3122-3055, 1659 & 1593 cm^{-1} which were due to

presence of C-H, C=O and C=N respectively. The mass spectrum of **17a** showed molecular ion peak at m/z = 461 (M+1), which is in agreement with the molecular formula $C_{30}H_{21}ClN_2O$ (**Figure 7.2**). The characterization data of compounds **17a-k** has been provided in **Table 7.1.**

Table 7.1 Characterization data of the compounds **17a-k**.

Compounds	**R**	**R^1**	**Molecular formula (Mol.wt.)**	**Yield (%)**	**M.p. (˚C)**
17a	Cl	biphenyl	$C_{30}H_{21}ClN_2O$ (460)	79	158-160
17b	Br	biphenyl	$C_{30}H_{21}BrN_2O$ (505)	78	165-167
17c	OCH_3	biphenyl	$C_{31}H_{24}N_2O_2$ (456)	81	183-185
17d	H	4-SCH_3-C_6H_4	$C_{25}H_{20}N_2OS$ (396)	75	136-138
17e	Cl	4-SCH_3-C_6H_4	$C_{25}H_{19}ClN_2OS$ (430)	73	160-162
17f	Br	4-SCH_3-C_6H_4	$C_{25}H_{19}BrN_2OS$ (475)	74	156-158
17g	OCH_3	4-SCH_3-C_6H_4	$C_{26}H_{22}N_2O_2S$ (426)	74	140-142
17h	H	2,4-Cl-C_6H_3	$C_{24}H_{16}Cl_2N_2O$ (419)	76	133-135
17i	Cl	2,4-Cl-C_6H_3	$C_{24}H_{15}Cl_3N_2O$ (453)	71	165-167
17j	Br	2,4-Cl-C_6H_3	$C_{24}H_{15}BrCl_2N_2O$ (498)	80	184-186
17k	OCH_3	2,4-Cl-C_6H_3	$C_{24}H_{18}Cl_2N_2O$ (449)	80	174-176

Furthermore, the intermediate compounds **17h** and **17j** was confirmed by single crystal X-ray analysis (**Figure 7.3**) (Fun et al. 2011a, 2011b). In compound **17h**, the the phenyl (C1-C6) ring and the two benzene (C13-C18 and C19-C24) rings form dihedral angles of 64.29 (9), 53.15 (10) and 7.06 (10)˚, respectively, with the pyrazole ring (N1/N2/C10-C12). The phenyl ring also forms dihedral angles of 65.06 (10) and 67.80 (10)˚ with the two benzene rings (C13-C18 and C19-C24), respectively. The benzene rings form a dihedral angle of 52.32 (10)˚. The molecule

17h exists in trans conformation with respect to the acyclic C8=C9 bond [bond length = 1.330 (2) Å]. Bond lengths and angles are within normal ranges. Similarly, in compound **17j**, the benzene (C19-C24) ring and the two phenyl (C1-C6 and C13-C18) rings form dihedral angles of 10.34 (16), 50.23 (16) and 40.95 (15)˚, respectively, with the pyrazole ring (N1/N2/C10-C12). The benzene ring also forms dihedral angles of 56.89 (17) and 38.81 (16)˚ with dichloro-bound phenyl (C1-C6) and bromo-bound phenyl (C13-C18) rings, respectively. The phenyl rings form a dihedral angle of 89.57 (17)˚. The molecule **17j** exists in *trans* configuration with respect to the acyclic C8=C9 bond [bond length = 1.336 (4) Å]. Bond lengths and angles are within normal ranges.

Similarly, the IR spectrum of compound $\mathbf{P_{64}}$ showed absorption bands at 3042-2923, 1590 &1492 which were due to C-H, C=N & C=C respectively (**Figure 7.4**). In the ^{1}H NMR spectrum of compounds $\mathbf{P_{64}}$ (**Figure 7.5**) the two methylenic 4′-H protons and the stereogenic 5′-H proton of the pyrazolinic moiety displayed a typical ABX type pattern of doublet of doublet. Thus, the two methylene protons (4′-H) displayed two signals; doublet of doublet at δ 4.08-4.01 ppm with coupling constants of 12 Hz and 17.2 Hz and a doublet of doublet at δ 3.31-3.25 ppm with coupling constants of 7.6 Hz and 17.6 Hz. Methine proton (5′-H) resonated as doublet of doublet at δ 5.54-5.49 ppm with coupling constants of 7.6 Hz and 12.2 Hz. The pyrazole 5-H resonated as singlet at δ 8.36. In some cases, the doublet of doublet of methylene proton around δ 3.31 has not been observed clearly because of merging of this particular proton with the water signal from DMSO-d_6. The main signals in the ^{13}C NMR for $\mathbf{P_{64}}$ corresponds to C-4′ at δ 42.8, C-5′ δ 56.4 ppm and the quaternary C-3′ at δ 148.9 ppm. The mass spectrum of $\mathbf{P_{64}}$ showed molecular ion peak at m/z = 551(M+1), which is in agreement with the molecular formula $C_{36}H_{27}ClN_4$. The characterization data of compounds $\mathbf{P_{64\text{-}74}}$ has been provided in **Table 7.2.** Furthermore, the structure of compound $\mathbf{P_{66}}$ was confirmed by single crystal X-ray analysis (Fun et al. 2012). 1,4-dioxane was used as crystallization solvent for $\mathbf{P_{66}}$, which is in association with the crystal. In the crystal structure of $\mathbf{P_{66}}$, the ring A (N1/N2/C7/C14/C16), B (N3/N4/C17/C24/C25), C (C1–C6), D (C8–C13), E (C18-C23), F (C26-C31) and G (C32-C37) are essentially planar. The dihedral angle between the least-square planes of the rings are A/B = 74.09 (10)˚, A/C = 42.50 (10)˚,

A/D = 8.04 (11)˚, A/E = 86.29 (9)˚, A/F = 77.25 (9)˚, A/G = 83.37 (9)˚, B/C = 55.81 (8)˚, B/D = 74.18 (10)˚, B/E = 19.64 (8)˚, B/F = 3.18 (8)˚, B/G = 30.67 (8)˚, C/D = 49.32 (9)˚, C/E = 71.40 (8)˚, C/F = 57.94 (8)˚, C/G = 86.47 (8)˚, D/E = 86.48 (9)˚, D/F = 77.36 (9)˚, D/G = 86.35 (9)˚, E/F = 16.50 (7)˚, E/F = 20.45 (7)˚ and F/G = 28.72 (7)˚.The crystallographic data of compound **16h**, **16j** and **P_{66}** has been summarized in **Table 7.3**.

Table 7.2 Characterization data of the compounds **$P_{64\text{-}74}$**.

Compounds	R	R^1	Molecular formula (Mol.wt.)	Yield (%)	M.p. (˚C)
P_{64}	Cl	biphenyl	$C_{36}H_{27}ClN_4$ (550)	76	211-213
P_{65}	Br	biphenyl	$C_{36}H_{27}BrN_4$ (594)	85	224-226
P_{66}	OCH_3	biphenyl	$C_{37}H_{30}N_4O$ (546)	87	163-165
P_{67}	H	4-SCH_3-C_6H_4	$C_{31}H_{26}N_4S$ (486)	81	208-210
P_{68}	Cl	4-SCH_3-C_6H_4	$C_{31}H_{25}ClN_4S$ (520)	78	219-221
P_{69}	Br	4-SCH_3-C_6H_4	$C_{31}H_{25}BrN_4S$ (565)	76	206-208
P_{70}	OCH_3	4-SCH_3-C_6H_4	$C_{32}H_{28}N_4OS$ (516)	72	210-212
P_{71}	H	2,4-Cl-C_6H_3	$C_{30}H_{22}Cl_2N_4$ (508)	71	196-198
P_{72}	Cl	2,4-Cl-C_6H_3	$C_{30}H_{21}Cl_3N_4$ (542)	69	185-187
P_{73}	Br	2,4-Cl-C_6H_3	$C_{30}H_{21}BrCl_2N_4$ (586)	70	163-165
P_{74}	OCH_3	2,4-Cl-C_6H_3	$C_{31}H_{24}Cl_2N_4O$ (539)	68	160-162

7.5.2 Biological activity

The newly synthesized compounds **$P_{64\text{-}74}$** were tested for their antibacterial activity (*in vitro)* against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* and their activity was compared to a well-known commercial antibiotic, Streptomycin. Antibacterial activity was carried out by "well-plate" method by measuring its zone of inhibition.

Table 7.3 Crystallographic data for compounds **17h**, **17j** & $\mathbf{P_{66}}$.

Crystal Data	**17h**	**17j**	$\mathbf{P_{66}}$
Emperical formula	$C_{24}H_{16}Cl_2N_2O$	$C_{24}H_{15}BrCl_2N_2O$	$C_{37}H_{30}N_4O.C_4H_8O_2$
Formula weight	419.29	498.19	634.75
Temperature (K)	296	296	100
Wavelength (Å)	0.71073	0.71073	0.71073
Crystal system	Triclinic	Monoclinic	Triclinic
Space group	$P\bar{1}$	$P2_1/c$	$P\bar{1}$
Cell dimensions			
a (Å)	9.6185 (8)	11.4203 (14)	11.1189 (2)
b (Å)	10.6596 (9)	9.9357 (13)	13.0541 (2)
c (Å)	11.8537 (10)	19.656 (3)	13.0852 (2)
α (°)	67.377 (2)	90	117.309 (1)
β (°)	75.777 (1)	94.653 (3)	90.468 (1)
γ (°)	69.934 (2)	90	98.558 (1)
Volume ($Å^3$)	1044.64	2222.9	1662.48
Z	2	4	2
Density (Mg m^{-3})	1.333	1.489	1.268
F (000)	432	1000	672
Θ range for data collection (°)	2.4-28.8	2.9-22.2	2.3-29.9

The compounds $\mathbf{P_{64\text{-}74}}$ were screened for their antibacterial activity in triplicate against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* at two different concentrations of 1000, 500 µg/mL as shown in (**Table 7.4**). Results revealed that in general, most of the tested compounds showed good activity against all the three bacterial strains. Six compounds $\mathbf{P_{64}}$, $\mathbf{P_{65}}$, $\mathbf{P_{68}}$, $\mathbf{P_{69}}$, $\mathbf{P_{72}}$ and $\mathbf{P_{73}}$ showed broad spectrum activity against all the tested bacterial strains. Compounds $\mathbf{P_{66}}$, $\mathbf{P_{70}}$ and $\mathbf{P_{74}}$ were also slightly active against tested bacterial strains. All the synthesized compounds were also tested for its antifungal activity (*in vitro)* against *Aspergillus flavus*, *Chrysosporium keratinophilum* and *Candida albicans* by measuring its

average zone of inhibition (**Table 7.5**). Fluconazole was used as standard for antifungal activity. Among the tested compounds, **P_{64}**, **P_{65}**, **P_{68}**, **P_{69}**, **P_{72}** and **P_{73}** were found to be moderately active against *Aspergillus flavus* and *Chrysosporium keratinophilum.* These compounds are also found to be slightly active against *Candida albicans.*

Table 7.4 Antibacterial activity of compounds **$P_{64\text{-}74}$**.

Compound code	**Zone of inhibition (mm)**					
	Escherichia coli		***Staphylococcus aureus***		***Pseudomonas aeruginosa***	
Conc. in µg/mL	1000	500	1000	500	1000	500
P_{64}	07±0.1	05±0.2	08±0.2	06±0.2	04±0.2	06±0.1
P_{65}	09±0.2	06±0.2	06±0.1	04±0.2	07±0.2	05±0.2
P_{66}	04±0.2	02±0.1	04±0.1	02±0.2	05±0.1	02±0.1
P_{67}	03±0.1	01±0.2	02±0.2	00	03±0.1	02±0.1
P_{68}	05±0.1	04±0.2	08±0.2	05±0.1	07±0.1	04±0.1
P_{69}	07±0.1	06±0.1	09±0.1	07±0.2	08±0.2	06±0.1
P_{70}	02±0.2	01±0.1	04±0.2	03±0.2	04±0.1	02±0.2
P_{71}	00	00	00	00	00	00
P_{72}	06±0.2	04±0.2	07±0.2	04±0.1	08±0.1	06±0.1
P_{73}	08±0.1	05±0.1	08±0.1	06±0.1	05±0.1	05±0.1
P_{74}	03±0.2	02±0.1	04±0.1	03±0.1	04±0.2	01±0.1
Streptomycin (Std.)	16±0.2	10±0.1	15±0.2	10±0.2	16±0.2	13±0.2

Based on these preliminary results, it can be seen that all the six compounds **P_{64}**, **P_{65}**, **P_{68}**, **P_{69}**, **P_{72}** and **P_{73}** showing good antimicrobial activity have a halogen, chloro or bromo as one of the substituents. However, in general, compounds containing a halogen substituents showed better antibacterial activity than the compounds with other substituent's which is also supported by the previous report (Sharma et al. 2011). From the antimicrobial results we can conclude that, synthesized compounds are specific antibacterial agents. Hence they are ideally suited for further modifications to obtain more efficacious antibacterial compounds.

Table 7.5 Antifungal activity of compounds $\mathbf{P_{64\text{-}74}}$.

Compound code	Zone of inhibition (mm)					
	Aspergillus flavus		***Chrysosporium keratinophilum***		***Candida albicans***	
Conc. in µg/mL	1000	500	1000	500	1000	500
$\mathbf{P_{64}}$	05±0.1	03±0.1	04±0.2	03±0.1	05±0.1	02±0.1
$\mathbf{P_{65}}$	06±0.1	05±0.2	04±0.1	03±0.1	05±0.1	04±0.1
$\mathbf{P_{66}}$	00	00	00	00	00	00
$\mathbf{P_{67}}$	00	00	00	00	00	00
$\mathbf{P_{68}}$	03±0.1	02±0.1	03±0.1	01±0.1	03±0.1	01±0.1
$\mathbf{P_{69}}$	04±0.1	03±0.2	06±0.1	05±0.2	04±0.1	02±0.1
$\mathbf{P_{70}}$	00	00	00	00	00	00
$\mathbf{P_{71}}$	00	00	00	00	00	00
$\mathbf{P_{72}}$	04±0.1	02±0.1	05±0.1	02±0.1	04±0.1	02±0.1
$\mathbf{P_{73}}$	04±0.2	01±0.1	04±0.1	02±0.1	03±0.2	01±0.2
$\mathbf{P_{74}}$	00	00	00	00	00	00
Fluconazole (Std.)	13±0.2	10±0.1	17±0.2	15±0.2	22±0.2	20±0.2

7.6 CONCLUSIONS

The present study highlights the synthesis and investigation of antimicrobial properties of a new series of pyrazolylpyrazolines with the hope of discovering new structure leads serving as antimicrobial agents. The structure of intermediate compounds **17h, 17j** and the final compound $\mathbf{P_{66}}$ has been solved by single crystal X-ray analysis. Antimicrobial results showed that the compounds $\mathbf{P_{64}}$, $\mathbf{P_{65}}$, $\mathbf{P_{68}}$, $\mathbf{P_{69}}$, $\mathbf{P_{72}}$ and $\mathbf{P_{73}}$ showed broad spectrum activity against all the tested bacterial strains. Antifungal results showed that among the tested compounds, $\mathbf{P_{64}}$, $\mathbf{P_{65}}$, $\mathbf{P_{68}}$, $\mathbf{P_{69}}$, $\mathbf{P_{72}}$ and $\mathbf{P_{73}}$ were found to be moderately active against *Aspergillus flavus* and *Chrysosporium keratinophilum* whereas slightly active against *Candida albicans.*

CHAPTER - 8

SUMMARY AND CONCLUSIONS

SUMMARY AND CONCLUSIONS

8.1 SUMMARY

The discovery and development of antimicrobials, also called "*miracle drugs*", has been one of the most important advances in the history of modern medicine. In the present scenario, there is an urgent and continuous need of exploration and development of cheaper, effective drugs with better bioactive potential and least side effects.

Heterocyclic compounds by virtue of their specific activity could be employed in the treatment of infectious diseases. Review of literature indicated that pyrazole and its derivatives play a significant role in the development of pharmacologically important molecules. Keeping this in view, some new N-bridged heterocycles particularly pyrazole containing heterocycles have been synthesized starting from simple molecules and investigated for their preliminary microbiological evaluation.

Accordingly, different series, viz. triazolothiadiazole, 1,3,4-oxadiazole, thiazole, Schiff base, cyanopyridone and pyrazoline derivatives carrying pyrazole ring as core structure have been designed. Pharmacological studies of the new pyrazole derivatives were also performed.

Based on the literature survey the following biological activities have been studied.

- Triazolothiadiazole series: Antimicrobial and Anti-inflammatory activity
- 1,3,4-oxadiazole series: Antimicrobial activity
- Thiazole series: Antimicrobial and Antioxidant activity
- Schiff base series: Antimicrobial activity
- Cyanopyridone series: Antimicrobial activity
- Pyrazoline series: Antimicrobial activity

Antimicrobial studies were assessed by well plate method by measuring the zone of inhibition in millimeter (mm) and by Minimum Inhibitory Concentration (MIC) by serial dilution method. Antioxidant activity of compounds were examined by performing DPPH radical, nitric oxide, hydroxyl radical and superoxide anion scavenging activity. The acute oral toxicity study for selected biologically active compounds was performed as per OECD guidelines. Molecular docking studies of the

selected biologically potent compounds were carried out for better understanding of the drug-receptor interaction.

8.2 CONCLUSIONS

The following conclusions can be drawn from the present research work.

- Six different types of new molecules in combination with pyrazole have been successfully designed. While designing, important pharmacophoric structures such as triazolothiadiazole, 1,3,4-oxadiazole, thiazole, triazole Schiff base and pyrazoline moieties have been incorporated.
- Structures of new compounds have been established using FTIR, ^{1}H NMR, ^{13}C NMR and mass spectrometry, followed by elemental analysis.
- Structures of selected new compounds have been confirmed by X-ray crystallographic studies.
- Newly synthesized target molecules have been evaluated for their antibacterial, antifungal, anti-inflammatory and antioxidant activities.
- Some of the synthesized compounds were found to exhibit potent antimicrobial/ anti-inflammatory/antioxidant activity.
- Among the six series of synthesized compounds, three series of compounds viz. 1,3,4-oxadiazoles (**P_{11-24}**), Thiazoles (**P_{25-38}**) and Schiff bases (**P_{39-48}**) showed excellent antimicrobial activity. In 1,3,4-oxadiazoles series **P_{19}**, **P_{20}**, in thiazole series **P_{29}**, **P_{38}** and in Schiff bases series **P_{41}** and **P_{44}** exhibited excellent antimicrobial activity when compared to standard drug. In case of 1,3,4-oxadiazoles, 2-chlorophenyl substituent on oxadiazole plays important role as antimicrobial agent. Thiazole series which has 2,4-dichlorophenyl substituent on pyrazole ring favors antimicrobial activity. For any compounds to behave as antioxidants it should possess hydrogen donor atoms. Since thiazole series contain C=N-NH- linker they can be used as antioxidants. Schiff base series having phenyl and p-fluorophenyl substituent on pyrazole ring favors for its antimicrobial activity. The presence of propyl chain in triazole ring and p-chlorophenyl substituent in pyrazole ring among 1,3,4-thiadiazole series is suitable to exhibit anti-inflammatory activities. The reason why these family of compounds are

important because the mode of interaction of these compounds with the target protein is effective than compared to other compounds.

- A combination of pyrazole with certain other biologically important heterocyclic entities in a single frame work has enhanced the biological activity. Hence they are ideally suited for further modifications to obtain more efficient antimicrobial/ anti-inflammatory/antioxidant compounds.

8.3 SCOPE FOR FUTURE WORK

- Electron donating functional groups on the phenyl ring of pyrazole moiety can be replaced by electron withdrawing groups such as -NO_2, -CN, -SO_3H, -COR, -CO_2H, -$CONH_2$ and their antimicrobial properties can be further investigated.
- Antimicrobial activity of the compounds synthesized in the present work has been tested only against normal bacteria. Further the antimicrobial activity of these newly synthesized compounds can be extended to multidrug-resistant bacterial strains such as Vancomycin-resistant Staphylococcus aureus (VRSA), Methicillin-resistant Staphylococcus aureus (MRSA), Oxacillin-resistant Staphylococcus aureus (ORSA) etc.
- The newly synthesized compounds which showed excellent antimicrobial activity *in vitro* can be further subjected for *in vivo* biological activities.
- In recent years, antimicrobial properties of rhodanine analogues have been investigated extensively for multidrug-resistant bacterial strains. These rhodanine analogues can be coupled with various substituted pyrazole-4-carbaldehydes and can be screened for antimicrobial activity against various multidrug-resistant bacterial strains.

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1. **Shridhar Malladi**, Arun M Isloor, Shrikrishna Isloor, D.S. Akhila. (2011). "Synthesis, Characterization and Antibacterial activity of Some New Pyrazole based Schiff-bases." *Arabian J. Chem.*, DOI:10.1016/j.arabjc.2011.10.009. (*An Elsevier publication*, IF-1.367)
2. **Shridhar Malladi**, Arun M. Isloor, Prashanth Shetty, Hoong Kun Fun, Sandeep Telkar, Riaz Mahmood and Nishitha Isloor. (2012). "Synthesis and anti-inflammatory evaluation of some new 3,6-disubstituted-1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazoles bearing pyrazole moiety." *Med. Chem. Res.*, 21, 3273-3280. (IF-1.272)
3. **Shridhar Malladi**, Arun M Isloor, Peethambar S.K, Shivananda K.N. (2012). "Synthesis and evaluation of antioxidant, antimicrobial activities of new 2,4-disubstituted thiazoles." *Int. J. Pharm. Res*., 4, 81-88.
4. **Shridhar Malladi**, Arun M Isloor, Peethambar S.K, Ganesh B M and Palusa Sanathkumar Goud. (2012). "Synthesis and antimicrobial activity of some new pyrazole containing cyanopyridone derivatives." *Der Pharma Chemica*, 4, 43-52.
5. **Shridhar Malladi**, Arun M Isloor, Peethambar S.K and Hoong Kun Fun. (2012). "Synthesis of new 3-aryl-4-(3-aryl-4,5-dihydro-1*H*-pyrazol-5-yl)-1-phenyl-1*H*-pyrazole derivatives as potential antimicrobial agents." *Med. Chem. Res*., DOI 10.1007/s00044-012-0267-8. (IF-1.272)
6. **Shridhar Malladi**, Arun M Isloor, and Peethambar S.K, "Synthesis and biological evaluation of newer analogues of 2,5-disubstituted-1,3,4-oxadiazole containing pyrazole moiety as antimicrobial agents." (Communicated to *Eur. J. Med. Chem.*, January 2013).

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2. **Shridhar Malladi**, Arun M. Isloor, Prashanth Shetty, Hoong Kun Fun, "Synthesis and anti-inflammatory evaluation of some new 3,6-disubstituted-1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazoles bearing pyrazole moiety." ICC, 29^{th} Annual Conference, 19^{th}-21^{st} December 2010, Dept. of Chemistry, Panjab University, Chandigarh.
3. **Shridhar Malladi**, Arun M Isloor, Shrikrishna Isloor, Hoong Kun Fun, "Synthesis, Characterization and Antibacterial activity of Some New Pyrazole based Schiff-bases." International Conference on Vistas in Chemistry, 11^{th}-13^{th} October 2011, Indira Gandhi Centre for Atomic Research Kalpakkam, Tamilnadu.
4. **Shridhar Malladi**, Arun M Isloor, Garudachari B and Peethambar S.K, "Synthesis and antimicrobial evaluation of some pyrazole containing cyanopyridone derivatives through one-pot multicomponent reaction." International Conference on Synthetic and Structural Chemistry (ICSSC-2011), 8^{th}-10^{th} December, 2011, Mangalore University, Mangalore.

CURRICULUM VITAE

SHRIDHAR A MALLADI
Department of Chemistry
NITK-Surathkal
Mangalore-575025
India
Email: shridhar_chem@yahoo.co.in
Phone: +91-9739149479

Education

1. Ph.D. Thesis: "Synthetic, Characterization and Biological Studies of Some New N-bridged Heterocycles".

   Supervisor: Dr. Arun M Isloor
2. M.Sc. Chemistry, Kuvempu University, 77.98 %, 2007, Karnataka, India.
3. B.Sc. Chemistry, Karnataka University, 65.33 %, 2005, Karnataka, India.

Work Experience

- Worked as project trainee at *Bioorganics and Applied Materials*, Bangalore from August 2007 to Febraury 2008.
- Worked as scientist at *Syngene International Ltd*. (A Biocon Company) from March 2008 to December 2008.

Synthetic Expertise & Skills

- Handled 10 mg- 5 kg scale reactions
- Purification of bulk products by crystallization techniques
- Handled reagents like DIBAL-H, Diethylzinc, Palladium, Lithium Aluminum Hydride.
- Analytical interpretation of organic molecules by IR, ^{1}H & ^{13}C NMR & LCMS.

Instruments handled

- Thermo Nicolet avatar 330-FT-IR spectrophotometer
- UV-Visible spectrophotometer
- Fluorescence spectrophotometer
- Radley's Parallel Synthesizer
- Buchi Rotary Evaporator (1L & 20 L capacity)

Research publications

1. **Shridhar Malladi**, Arun M Isloor, Shrikrishna Isloor, D.S. Akhila "Synthesis, Characterization and Antibacterial activity of Some New Pyrazole based Schiff-bases." *Arabian Journal of Chemistry*, (2011), DOI:10.1016/j.arabjc.2011.10.009. (*An Elsevier publication*, IF-1.367)
2. **Shridhar Malladi**, Arun M. Isloor, Prashanth Shetty, Hoong Kun Fun, Sandeep Telkar, Riaz Mahmood and Nishitha Isloor, "Synthesis and anti-inflammatory evaluation of some new 3,6-disubstituted-1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazoles bearing pyrazole moiety." *Medicinal Chemistry Research*, 21 (2012) 3273-3280. (IF-1.272)
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9. B. Chandrakantha, Arun M. Isloor, Prakash Shetty, Shrikrishna Isloor, **Shridhar Malladi**, Hoong Kun Fun, "Synthesis, characterization and antimicrobial activity of novel ethyl 1-(N-substituted)-5-phenyl-1*H*-pyrazole-4-carboxylate derivatives". *Medicinal Chemical Research,* (2011), DOI 10.1007/s00044-011-9796-9.
10. Arun M. Isloor Dhanya Sunil, Prakash Shetty, **Shridhar Malladi**, K. S. R. Pai, Naseer Maliyakkl, "Synthesis, characterization, anticancer, and antioxidant activity of some new thiazolidin-4-ones in MCF-7 cells". *Medicinal Chemical Research,* (2012), DOI 10.1007/s00044-012-0071-5.
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Personal details

Address for correspondence:	#421/B/17(2), Mrutyunjaynagar, Near APMC Yard, Bailhongal, Belgaum, Karnataka State -561102 India.
E-mail:	shridhar_chem@yahoo.co.in shridhar.malladi@gmail.com
Telephone:	+91-9739149479
Date of birth:	07-12-1984
Sex:	Male
Marital status:	Single
Nationality:	Indian
Language known:	English, Kannada, Hindi and Telgu

References

1. Dr. Arun M Isloor
Assoc. Professor
Dept. of Chemistry
NITK-Surathkal
Mangalore-575025
Mob: +91-9448523990
Email: isloor@yahoo.com

2. Dr. A. Chittaranjan Hegde
Professor and Head
Dept. of Chemistry
NITK-Surathkal
Mangalore-575025
Mob: +91-9980360242
Email: hegdeac@rediffmail.com

3. Dr. Lokesh Babu
Research Investigator
BBRC-Syngene (A Biocon Company)
Biocon Park , Plot 2&3,
Bommasandra-Jigani Link Road,
Bangalore-560099
Mob: +91-9008700211
Email: b_lokesh01@rediffmail.com
lokesh.babu@syngeneintl.com