SYNTHESIS, CHARACTERIZATION AND ANTITUBERCULAR STUDIES OF SOME PYRAZOLE BASED MOLECULES

Thesis

Submitted in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

by

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June, 2016

DECLARATION

I hereby *declare* that the research thesis entitled **"Synthesis, characterization and antitubercular studies of some pyrazole based molecules"** 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*** is a *bonafide* report of the research work carried out by me. The material contained in this thesis has not been submitted to any University or Institution for the award of any degree.

Nagabhushana
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Department of Chemistry

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Date:

CERTIFICATE

This is to *certify* that the thesis entitled **"Synthesis, characterization and antitubercular studies of some pyrazole based molecules"** submitted by **Nagabhushana (Register Number: CY12F03)** 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. Udaya Kumar D.
Research Guide

Chairman-DRPC

Dedicated

to my family

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Mr. Nagabhushana

ABSTRACT

Tuberculosis, caused by bacillus called *Mycobacterium tuberculosis*, is one of the major diseases and is second largest killer disease after HIV/AIDS. In the last few decades, extensive research is going on globally targeting effective heterocyclic candidates to develop newer entities to combat against these infectious agents. Pyrazole is such a member of the heterocyclic family that constantly draws interest for the development of newer drug moiety due to its wide spectrum of pharmacological applications. In particular, literatures on pyrazole derivatives have shown quite good response in terms of antitubercular and antibacterial property. Owing to this therapeutic nuance of pyrazole and its derivatives, in the current work, it has been contemplated to integrate various potent heterocyclic motifs with the pyrazole skeleton to form a new molecular framework. Accordingly, five different libraries of pyrazole based compounds comprising of 1,2,3-triazole (**P1**-**P24**), 1,3,4-oxadiazole (**P25**-**P48**), two series of quinoline (**P49 -P89**) and isoniazid analogs (**P90**-**P107**) have been successfully synthesized through multistep organic synthetic protocols. Chemical structures of the prepared molecules were established by various spectroscopic techniques viz. ^{1}H NMR, ^{13}C NMR, ESI-MS and elemental analyses. Additionally, 3-dimensional structures of few target molecules were confirmed by single crystal X-ray diffraction studies. Further, the synthesized title compounds were subjected to preliminary *in vitro* antitubercular studies against *Mycobacterium tuberculosis H37Rv* strain and antibacterial screening against three common infectious bacterial strains of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*. The active antitubercular molecules in each series were identified and tested for their toxicity on the benign non-cancerous cells. The *in silico* molecular modeling studies of these active derivatives were also carried out.

Key words: Pyrazole derivatives, SC-XRD, antitubercular studies, antibacterial screening, non-cancerous cells, *in silico* molecular modeling

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NOMENCLATURE

ABBREVIATIONS

^{13}C NMR	Carbon-13 nuclear magnetic resonance
^{1}H NMR	Proton nuclear magnetic resonance
Ac_2O	Acetic anhydride
AcOH	Acetic acid
AIDS	Acquired immune deficiency syndrome
ATCC	American type culture collection
CADD	Computer aided drug design
CCDC	Cambridge crystallographic data centre
DCM	Dichloromethane
DIPEA	*N*,*N*-Diisopropylethylamine
DMF	Dimethyl formamide
DMSO	Dimethyl sulfoxide
E. coli	*Escherichia coli*
EAA	Ethyl acetoacetate
EDCI	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
ESI-MS	Electrospray ionization mass spectrometry
EtOAc	Ethyl acetate
EtOH	Ethanol
H_2SO_4	Sulfuric Acid
HATU	1-[Bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*] pyridinium 3-oxid hexafluorophosphate
HCl	Hydrochloric acid
HIV	Human immunodeficiency virus
HOBt	Hydroxybenzotriazole
INH	Isoniazid
$LiAlH_4$	Lithium aluminium hydride
LiOH	Lithium hydroxide
MDR-TB	Multidrug-resistant tuberculosis

MeOH	Methanol
MIC	Minimum inhibitory concentration
mp	Melting point
MTB	*Mycobacterium tuberculosis*
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NaH	Sodium hydride
NaN_3	Sodium azide
NaOEt	Sodium ethoxide
NCEs	New chemical entities
ORTEP	Oak ridge thermal ellipsoid plot program
P. aeruginosa	*Pseudomonas aeruginosa*
PDB	Protein data bank
PEG	Polyethylene glycol
$POCl_3$	Phosphorus oxychloride
PPA	Polyphosphoric acid
QSAR	Quantitative structure–activity relationship
RT	Room temperature
S. aureus	*Staphylococcus aureus*
SC-XRD	Single crystal X-Ray diffraction
SI	Selectivity index
t-BuOH	Tertiary butanol
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TMS	Tetramethylsilane
UV	Ultraviolet
WHO	World health organization
XDR-TB	Extensively drug-resistant tuberculosis

SYMBOLS AND UNIT

α	Alpha
β	Beta
γ	Gamma
δ	Delta
g	Gram
µg	Microgram
µL	Microlitre
mL	Millilitre
mmol	Millimole
ppm	Parts per million
min	Minutes
%	Percent
ºC	Degree Celsius
h	Hour
Hz	Hertz
>	Greater than
<	Less than
Å	Angstrom
±	Plus or minus
m/z	Mass to charge ratio

CHAPTER 1

INTRODUCTION

Abstract

This chapter encompasses a detailed introduction on pyrazole, its chemistry and importance of pyrazole and its derivatives in drug discovery and design. It also covers a thorough literature survey on pyrazole based molecules which exhibit potent biological activity. Further, scope and objectives of the present work are highlighted.

1.1 MEDICINAL CHEMISTRY AND HETEROCYCLES

Research in medicinal chemistry involves design and development of new compounds that are useful for drug discovery. The drug discovery process involves a team of researchers from a wide range of disciplines such as chemistry in particular organic chemistry, biology, biochemistry, pharmacology, medicine, mathematics, computational chemistry and others.

Cyclic organic compounds which contain at least one atom other than carbon atom in the ring system are known as heterocyclic compounds. Nitrogen, oxygen and sulfur are the most common hetero atoms present in these systems. However, hetero cyclic rings containing other hetero atoms such as phosphorus and silicon are also widely known. The majority of pharmaceuticals and agrochemicals are heterocyclic compounds. Also cosmetics, reprography, information storage devices and plastic materials are prepared using heterocyclic compounds. Synthesis and development of variety of heterocycles has become a major division in synthetic organic chemistry research over the last one century, particularly because of the wide range of pharmaceutical activity exhibited by these classes of molecules. Further, the role of heterocyclic compounds has become increasingly important in other fields of scientific research as well which provided an important platform for the rapid development of research in the areas such as agriculture, photography, dyes, polymer science etc.

Heterocyclic compounds play a vital role in biological system and are widely spread in nature particularly in plant alkaloids, nucleic acids, anthocyanins and flavones as well as in hemoglobin and chlorophyll. Also some of the proteins, vitamins and hormones contain heterocyclic rings. Many of the natural drugs such as theobromine, quinine, papaverine, emetine, atropine, theophylline, procaine, reserpine, codeine, morphine are heterocycles. Almost all the compounds we know as synthetic drugs such as isoniazid, metronidazole, diazepam, azidothymidine,

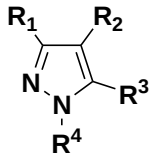
barbiturates, chlorpromazine, antipyrine, methotrxate and catopril also are heterocycles. Because of their increasing significance in the medicinal field, a lot of research activities, particularly on design and development of new heterocycles of pharmacological interest, have been put forward during the recent years, all over the world. Some of the interesting properties about heterocyclic compounds make it more enthusiastic for the medicinal chemist to develop variety of new molecules having pharmacological importance.

1) Availability of the low cost raw materials
2) Synthetic feasibility
3) Chemical stability
4) Capacity to incorporate functional groups either as constituents or as part of the ring system.

The pyrazole ring system possesses all the above mentioned properties and hence it is being considered as one of the key members in the field of medicinal chemistry, protein chemistry, natural product chemistry and so on.

1.2 CHEMISTRY OF PYRAZOLE AND ITS DERIVATIVES

Pyrazole is a five membered heterocyclic aromatic organic compound with a molecular formula $C_3H_4N_2$, consisting of three carbon atoms and two nitrogen atoms in adjacent positions. There are four different substitution positions available on the ring and hence variety of pyrazole derivatives could be synthesized as depicted below.

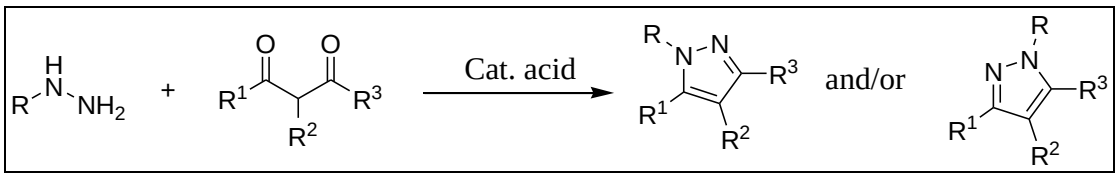


R^1, $R^{2,}$ R^3 and R^4 = H, X (halide), aromatic, heterocyclic or any other group

A number of synthetic protocols have been reported in the literature to prepare pyrazole derivatives. Some of the important methods which give variety of pyrazole derivatives are as follows.

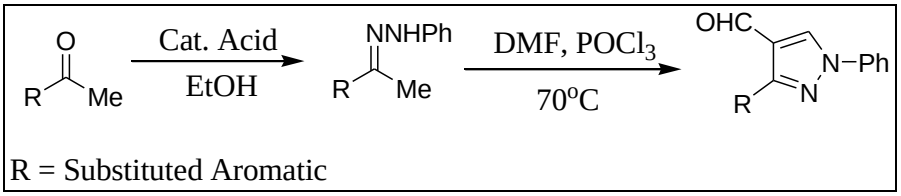
The Knorr pyrazole synthesis is a well-known reaction for the synthesis of pyrazole derivatives which involves reaction of hydrazine or its derivatives and a 1,3-

dicarbonyl compound using an acid catalyst. Drawback of this method is that, with unsymmetrical dicarbonyl compounds it ends up with a mixture of two isomers, separation of which is difficult (Knorr, 1883) (**Scheme 1.1**).



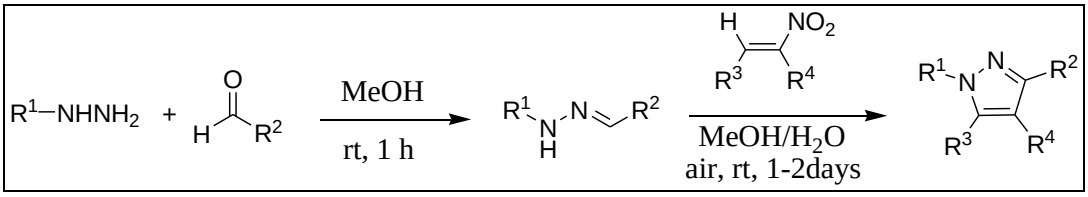
Scheme 1.1

Kira et al. (1969) reported the synthesis of pyrazole derivatives by Vilsmeier-Haack reaction. The synthesis involves the cyclization of acetophenone phenylhydrazone by dimethyl formamide (DMF) and phosphorous oxychloride ($POCl_3$) adduct at 70-80 ^{0}C (**Scheme 1.2**).



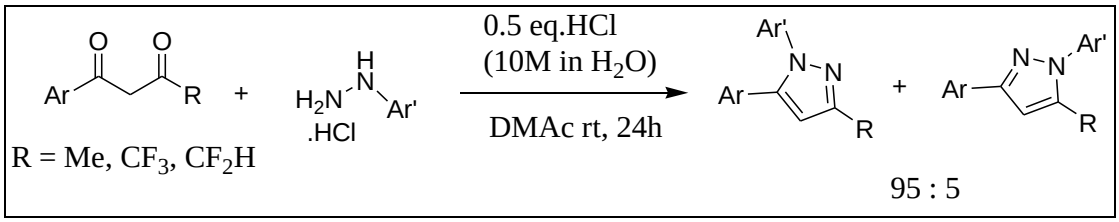
Scheme 1.2

A novel one-pot regioselective synthesis of substituted pyrazoles was described by Deng and Mani (2006) by the reaction between *N*-monosubstituted hydrazones and nitro-olefins at room temperature (**Scheme 1.3**).



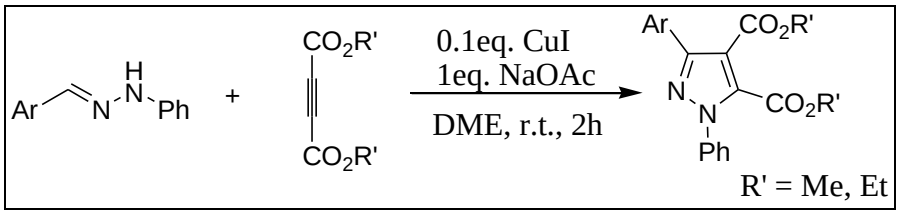
Scheme 1.3

Gosselin et al. (2006) investigated a novel route for the synthesis of highly regeoselective 1-aryl-3,4,5-substituted pyrazole derivatives based on the condensation of 1,3-diketones with arylhydrazines using dilute hydrochloric acid (HCl) and *N*,*N*-dimethylacetamide as the solvent (**Scheme 1.4**).



Scheme 1.4

Ma et al. (2011) demonstrated easy and efficient method for the synthesis of polysubstituted pyrazoles from phenylhydrazones and dialkyl acetylenedicarboxylates using copper based catalyst (**Scheme 1.5**).

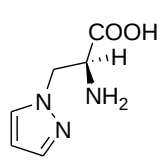


Scheme 1.5

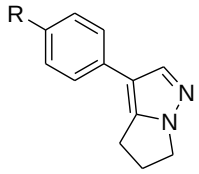
1.2.1 Pyrazole: A versatile moiety

Pyrazole derivatives are rarely found in nature probably due to difficulty in the formation of N-N bond by the living organism (Suri et al. 2012). However, a few pyrazole derivatives have been successfully isolated. These naturally occurring pyrazole derivatives found to exhibit a wide range of pharmacological activities and hence a lot of effort has been devoted to identify and isolate pyrazole derivatives from various natural sources. The first pyrazole derivative isolated from natural product is an isomer of histidine, (S)-2-amino-3-(1*H*-pyrazol-1-yl)propanoic acid from *Citrullus vulgaris* in juice of watermelon in 1957 (Eicher and Hauptmann, 2003). It is a naturally occurring amino acid which possesses excellent antidiabetic activity (Noe and Fowden, 1959).

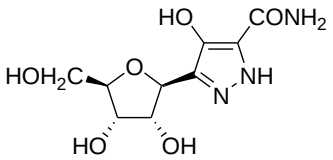
A pyrazole alkaloid, Withsomnine has been originally isolated from the roots of Indian medicinal plants Solanaceae in 1966 which acts as an anti-depressant both to central nerves system and circulatory system (Wube et al. 2008). Withsomnine also acts as mild analgesic and inhibitor of COX-1, COX-2 and TBL_4 enzymes. 4-Hdroxywithsomnine and 4-methoxywithsomnine which is isolated from root bark of *Newbouldia leavis* is used in the treatment of various disorders like worm infections, conjunctivitis, migraine, and different forms of orchitis (Pangerl et al. 2010).



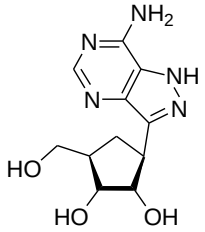
(*S*)-2-amino-3-(1*H*-pyrazol-1-yl)propanoic acid



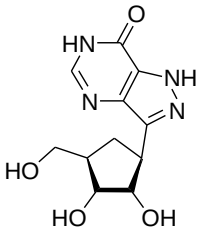
R = H ; Withasomnine
R = OH ; 4'-Hydroxywithasomnine
R = OMe ; 4'-Methoxywithasomnine



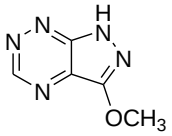
Pyrazofurin



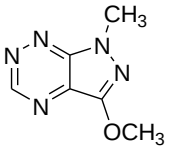
Formycin A



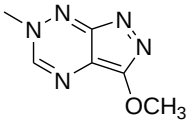
Formycin B



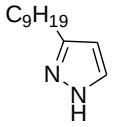
Fluviol A



Fluviol C



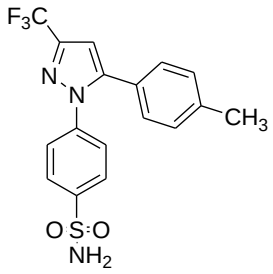
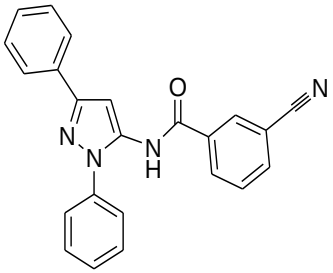
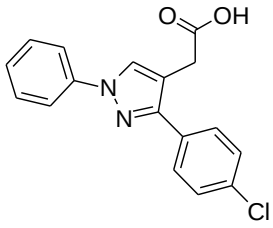
Fluviol E

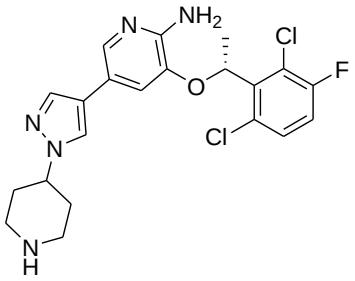
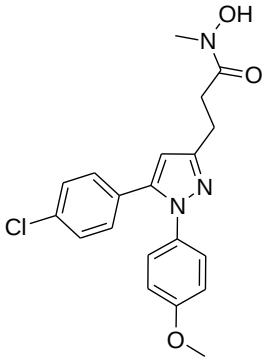
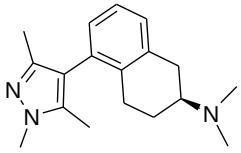
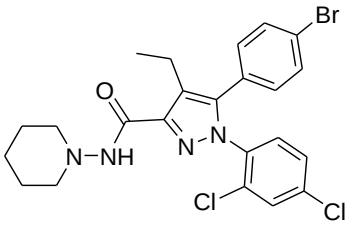
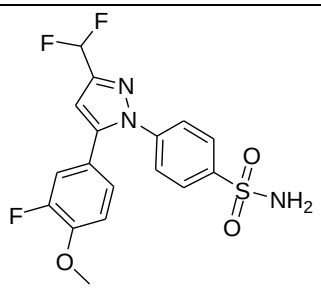
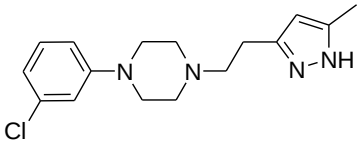


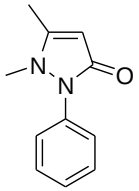
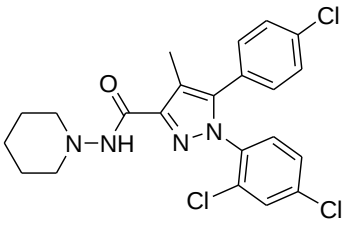
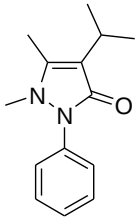
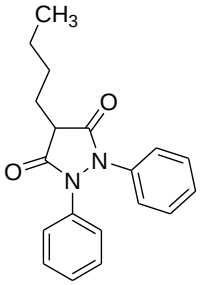
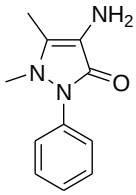
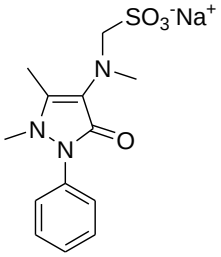
3-nonyl-1*H*-pyrazole

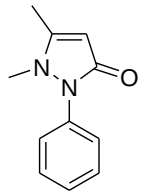
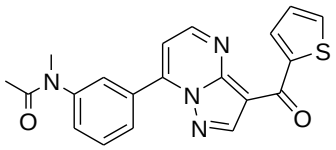
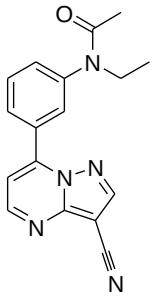
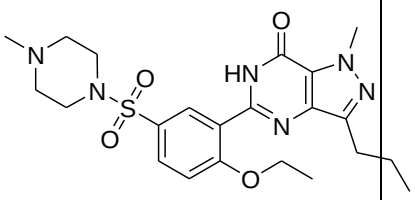
Pyrazofurin is another pyrazole containing natural product isolated from fermentation of strain of a *Streptomyces candidus* (Crain et al. 1973). It showed promising antitumor as well as antiviral activity (Petrie et al. 1986). Formycin A and Formycin B are the two C-nucleoside antibiotics produced by the culture filtrates of the rice mold *Nocardiea interforma* and *Streptomyces lavendulae*, both are found to exhibit good antitumor as well as antibiotic properties (Buchanan et al. 1980). Fluviols are the bicyclic nitrogen rich antibiotics isolated from *Pseudomonas fluorescenes*. They are classified as Fluviol A, Fluviol B, Fluviol C, Fluviol D and Fluviol E. Among Fluviols, Fluviol E found to inhibit the growth of both gram positive and gram negative bacteria there by exhibiting broad spectrum of antimicrobial activity. Fluviol C showed moderate antitumor activity, weak antimicrobial activity and is less toxic compare to Fluviol E whereas Fluviol A, the dimethyl derivative of fluviol E is found to be very less toxic and exhibited a strong antitumor effect (Smirnov et al. 1997). Another pyrazole containing natural product

known as 3-nonyl-1*H*-pyrazole, isolated from *Houttuynia cordata*, a plant of the piperacae family from tropical Asia has been found to be very good antimicrobial agent. Natural pyrazole derivatives exhibit wide spectrum of biological activities and there is a wide scope for the structural modification of these derivatives to get more promising drug like molecules. There are many synthetic pyrazole containing drugs available in the market. Some of the important drugs among them are listed below.

Sl. No.	**Name of the drug**	**Structure**	**Application**
1	Celecoxib		It is a nonsteroidal anti-inflammatory drug (NSAID) and COX-2 inhibitor, used for the treatment of Osteoarthritis, rheumatoid arthritis, acute pain, painful menstruation etc.
2	CDPPB		Used for Schizophrenia a mental disorder, acts as antipsychotic in animals.
3	Lonazolac		It is a nonsteroidal anti-inflammatory drug.

4	Crizotinib		It is an anticancer drug used for the treatment of some non-small cell lung carcinoma.
5	Tepoxalin		It is also NSAID used for the treatment of disorders such as hip dysplacia and arthritis.
6	AS-19		Improves long term memory acquisition
7	Surinabant		Investigated as a potential treatment for nicotine addiction and smoking cessation.
8	Deracoxib		It is also NSAID used in veterinary medicine to treat osteoarthritis in dogs.
9	Mepiprazole		Aiding for anxiety neuroses.

10	Phenazone		Is an analgesic and antipyretic.
11	Rimonabant		It is an antiobesity drug.
12	Propyphenazone		It is also an analgesic, antipyretic and anti-inflammatory agent.
13	Phenylbutazone		It is a NSAID for the short term treatment of pain and fever in animals.
14	Ampyrone		Is an analgesic, antipyretic and anti-inflammatory drug.
15	Metamizole sodium		Is an analgesic and antipyretic drug.

16	Edaravone		It is a neuro protective agent used for treating neurological recovery following acute brain ischemia and subsequent cerebral infraction.
17	Indiplon		Used as a hypnotic sedative.
18	Zaleplon		Is a sedative hypnotic used in the management of insomnia.
19	Sildenafil (Viagra)		To treat erectile dysfunction and pulmonary arterial hypertension.

1.3 ANTIMICROBIALS AND THEIR IMPORTANCE

Microbes are very small living organisms. The majority of them can only be seen with a microscope, which magnifies their image. Microbes are so tiny and widely spread in nature, just a tea spoon of soil contains over a million of microbes. Same way in human body inside and out, for example the skin, mouth and the intestines are covered in millions of individual micro-organisms. More than 95% of the microbes are not harmful, but at the same time there are some micro-organisms that cause diseases in humans and animals which are termed as pathogens. Microbes occur in an amazing variety of shapes and sizes and they are divided into six major groups. They are Bacteria, Viruses, Fungi, Protozoa, Algae and Archaea. Some of the important diseases caused by microorganisms are as follows.

Infectious disease	Microbe that causes the disease	Type of microbe
Cold	*Rhinovirus*	Virus
Chickenpox	*Varicella zoster*	Virus
German measles	*Rubella*	Virus
AIDS	*Human immune deficiency virus*	Virus
Whooping cough	*Bordatella pertussis*	Bacterium
Bubonic plague	*Yersinia pestis*	Bacterium
Tuberculosis	*Mycobacterium tuberculosis*	Bacterium
Malaria	*Plasmodium falciparum*	Protozoan
Ringworm	*Trichophyton rubrum*	Fungus
Athletes' foot	*Trichophyton mentagrophytes*	Fungus

An antimicrobial is a substance that kills or inhibits the growth of microorganisms. Today, more than 15 different classes of antimicrobials are known, they differ in chemical structure and mechanism of action. Specific antimicrobials are necessary for the treatment of specific pathogens. After the discovery of antimicrobials there is a substantial reduction in threat posed by infectious diseases. The use of these antimicrobial drugs combined with improvement in sanitation, nutrition, housing and the advent of widespread immunization programs, has led to a dramatic drop in deaths from diseases that were previously widespread, untreatable and frequently fatal. Also, these drugs have contributed to the major gains in life expectancy by helping to control many serious infectious diseases. Because of this reason they are also commonly known as "**wonder drugs**".

1.3.1 History and mode of action of antimicrobials

Paul Ehrlich and Sahachiro Hata in 1910 discovered the first antimicrobial agents; they synthesized a series of organic arsenical compounds. One of the compounds, Arsphenamine with a trade name Salvarasan showed a very good antisyphilitic activity.

In 1928 the Scottish scientist and Nobel laureate, Alexander Fleming discovered that *Penicillium rubens* grown in the appropriate substrate found to inhibit the growth of *Staphylococcus aureus* (*S. aureus*) bacterium in a zone surrounding it.

This serendipitous observation is the base for the modern era antibiotic discovery. Penicillin came into clinical use in the 1940s, it is found to be outstanding agent in terms of safety and efficiency, led in the era of antimicrobial chemotherapy by saving the lives of many wounded soldier during World War II.

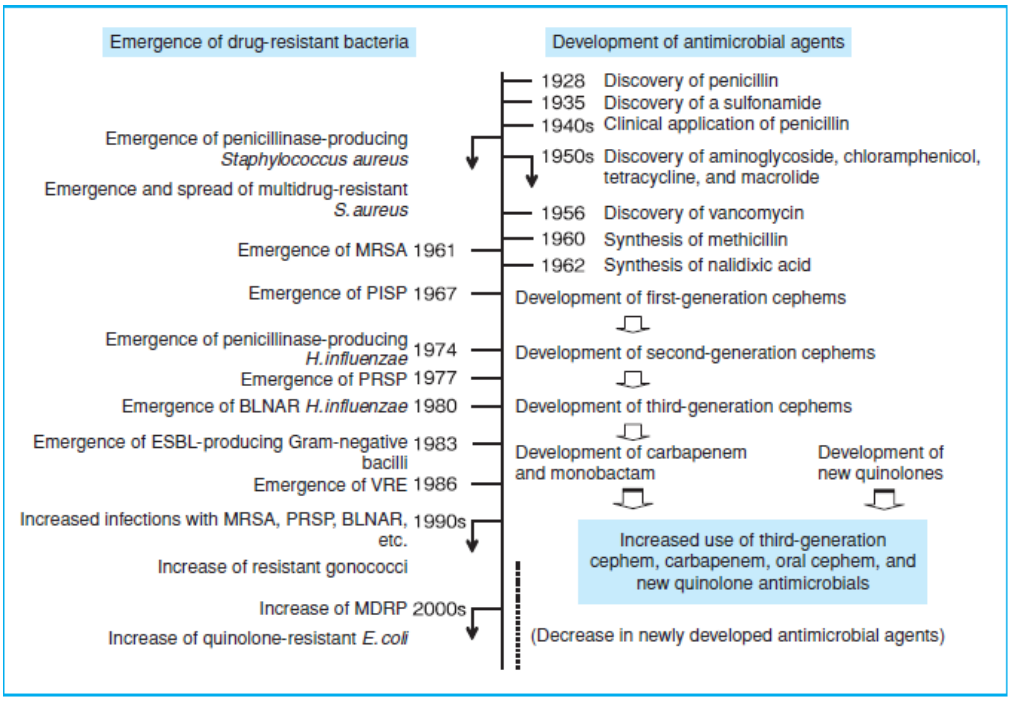


Figure 1.1 History of antimicrobials

(Source: Research and reviews, "History of antimicrobial agents and resistant bacteria." by Tomo Saga and Keizo Yamaguchi, JMAJ 52(2): 103–108, 2009)

Domagk and other researchers in 1935 discovered first sulfonamide drugs; and cemented the way for antibiotic revolution in medicine. During the subsequent decades many new class of antimicrobial drugs were discovered, leading to a golden age for antimicrobial chemotherapy. Aminoglycoside antibiotic, streptomycin, was developed from the soil bacterium *Streptomyces griseus*. Thereafter, chloramphenicol, macrolide, tetracycline and glycopeptides (e.g., vancomycin) were discovered from soil bacteria. In 1962, the synthetic antimicrobial agent nalidixic acid, a quinolone antimicrobial drug, was developed. A schematic representation of the history of antimicrobials is given in **Figure 1.1**.

Antibacterials can be divided into two types based on their effects on target cells. Substances that actually kill microorganisms are termed 'bactericidal'.

Examples of bactericidal drugs include penicillins, cephalosporins, aminoglycosides, and quinolones. Compounds that only inhibit the growth of microorganisms are termed 'bacteriostatic'. The decision to use a bactericidal or bacteriostatic drug to treat infection depends entirely upon the type of infection. Some examples of bacteriostatic drugs are tetracyclines, sulfonamides, macrolides etc.

Also, based on their range of activity, antimicrobial drugs can be classified as (i) narrow spectrum drugs, which are only active against a relatively small number of gram-positive organisms, (ii) moderate spectrum drugs, which are effective against gram-positive and the most systemic, enteric and urinary tract gram-negative pathogens, (iii) narrow and moderate spectrum drugs like beta-lactam antibiotics, (iv) broad spectrum drugs which are active against all prokaryotes with two exceptions, *Mycobacteria* and *Pseudomonas* and (v) drugs which are effective against *Mycobacteria* only.

1.3.2 Major diseases caused by bacteria

From the ancient days, bacteria have been responsible for some of the most deadly diseases and widespread epidemic of human civilization. Some of the deadly diseases caused by bacteria, such as tuberculosis, typhus, diphtheria, plague, typhoid, cholera, dysentery and pneumonia have taken a large toll of human being. Pneumonia, tuberculosis and diarrhea were the three leading causes of death at the beginning of the twentieth century. Antibiotic treatment, water purification and immunization (vaccination) have reduced the mortality rate in the twenty-first century to a large extent in the developed countries, also to the reasonable numbers in the under developed countries. Most of the bacterial diseases have been controlled at present, but many new bacterial pathogens have been identified in the past thirty years, and many bacterial pathogens, such as *S. aureus* and *Mycobacterium tuberculosis* (MTB) have emerged with new forms resistance to antimicrobial agents. Great attention is necessary; research and study are needed to control both old and new bacterial pathogens.

Hans Christian Gram found a method of differentiating bacterial species into two large groups known as gram-positive bacteria and gram-negative bacteria. The cell wall of gram-positive bacteria is a thick mesh-like made of peptidoglycan, and as a result are stained purple by crystal violet. The gram-negative bacteria have a thinner

layer of cell wall made of paptidoglycan, which show counter stain pink by the safranin. Some of the important bacterial pathogens and the diseases caused by them are listed in **Figure 1.2**.

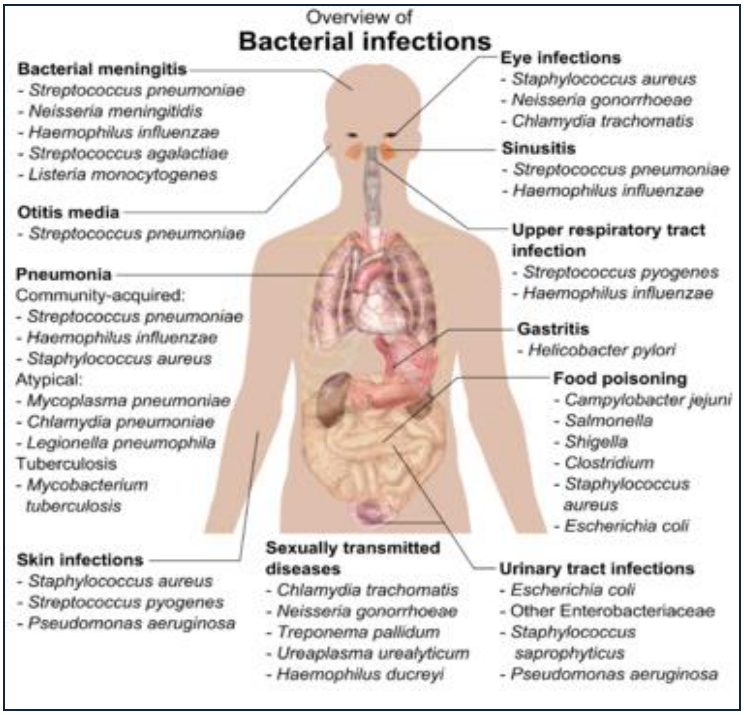


Figure 1.2 Overview of major bacterial infections and main species involved. (Source: Fisher, B., Harvey, R.P. and Champe, P.C. (2007). Lippincott's illustrated reviews: microbiology. Hagerstown, M.D.: Lippincott Williams & Wilkins. pp. Chapter 33, pages 367–392. ISBN 0-7817-8215-5)

1.3.2.1 *Mycobacterium tuberculosis*

Among all microbial diseases, Tuberculosis generally termed as TB is one of the most dangerous and deadly diseases. TB is a very old disease having an evidence of an approximately 17,000 years ago. Researchers also found the tubercular decay in spines of Egyptian mummies dating from 400BC. It is the second greatest killer worldwide due to a single infectious agent after HIV/AIDS. According to the 2015 report released from world health organization (WHO), 9.6 million people fell ill with TB and 1.5 million died in 2014 (**Figure 1.3**). Over 95% of the TB cases and deaths occur in low- and middle income countries of Asia and Africa. Out of 9.6 million cases of TB incidences, in India alone 2.2 million cases reported (**Figure 1.4**) which is a very large number compared to other global countries.

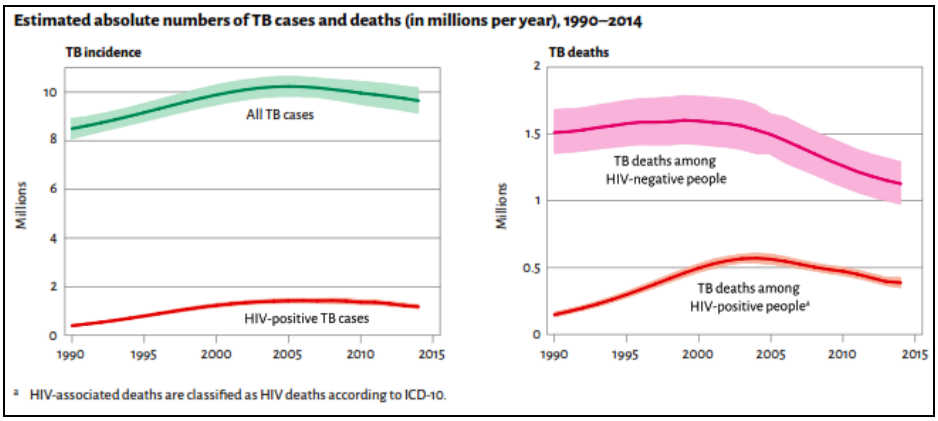


Figure 1.3 Global trends in estimated rates of TB incidence and mortality.

(Source: WHO global tuberculosis report 2015)

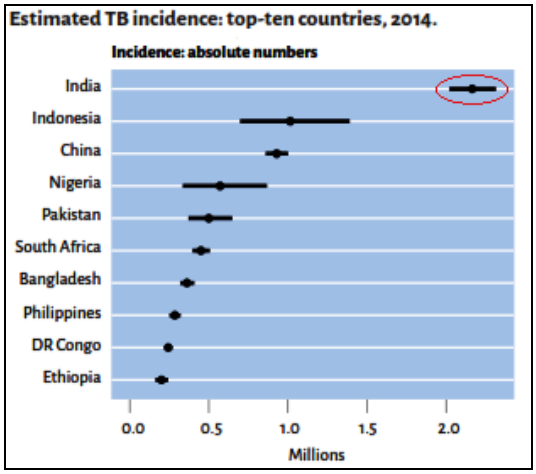


Figure 1.4 Estimated TB incidences in top ten countries, 2014

(Source: WHO global tuberculosis report 2015)

TB is an infectious disease caused by a type of bacterium called MTB (**Figure1.5**). TB primarily affects the lungs, but it can also affects the other parts of the body such as organs in the central nervous system, lymphatic system, circulatory system etc. When tuberculosis affects a person, the bacteria in the lungs multiply and cause pneumonia along with chest pain, blood in the cough and a prolonged cough. Also lungs and lymph nodes near the heart become enlarged. Once TB spreads to other parts of the body, it often interrupts the body immune system; later disease turns into an active state with pneumonia and damage to bones, kidneys and the meninges that lie in the spinal cord and brain. The classic symptoms of the tuberculosis disease are a chronic cough with blood tinged sputum, fever, weight loss and night sweats.

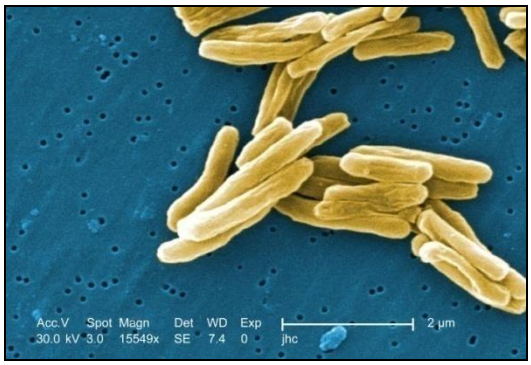


Figure 1.5 Scanning electron micrograph of *Mycobacterium tuberculosis*

The standard drugs for the short course treatment of tuberculosis include a mixture of four drugs: isoniazid (INH), rifampicin (RMP), pyrazinamide (PZA) and ethambutol (EMB) for two months then isoniazid and rifampicin alone for a further four months. These drugs are known as the first line TB drugs. There are also present cases like tuberculosis show resistance to first line therapy, known as extensively drug-resistant tuberculosis (XDR-TB) or multidrug-resistant tuberculosis (MDR-TB), in such cases the second-line drugs are implemented for the treatment of TB. Generally these drugs are less preferred due to one of the possible three reasons: it may be less effective than the first line drugs (e.g., *p*-aminosalicylic acid); it may have toxic side-effects (e.g., cycloserine); or it may be expensive and unavailable in many developing countries (e.g., fluoroquinolones).

Even first line TB drugs also show some or other side effects, isoniazid adverse effects include rashes, abnormal liver functions, hepatitis, mild central nervous system effects etc. Joint pain (arthralgia) is the most common side effect of pyrazinamide. TB treatment using rifampicin for long time leads to most serious problem called hepatotoxicity, and the patients receiving it often undergo baseline and frequent liver function tests to detect liver damage. Side effects of EMB are optic neuritis, red-green color blindness, arthralgia, hyperuricaemia, milk skin reactions etc. Due to many serious adverse effects most of the second and third line TB drugs are not recommended by WHO.

Despite the availability of strong and powerful antitubercular (antiTB) drugs, TB is still a leading threat for humanity. Recent emergence of new dominant forms of TB such as MDR-TB and XDR-TB are alarming the serious problem in TB control. Also there are few latest cases on a more dangerous and completely fatal form of

tuberculosis, totally drug resistance tuberculosis (TDR-TB) has been identified in three countries; India, Iran and Italy (Ballell et al, 2005; Velayati et al. 2009; Loewenberg 2012). It is also clear from the statistical data that the mortality rate and spread of the disease by TB infection in a Human Immunodeficiency Virus (HIV) infected human is very high when compared to a normal human (Corbett et al. 2003).

No new first line TB drug has been discovered in last fifty years after rifampicin. However, several new entities are currently in different stages of clinical trials (Ahirrao, 2008; Villemagne et al. 2012; Ginsberg, 2010; Cole and Riccardi 2011) and recently a new drug bedaquiline has been approved by Food and drug administration (FDA) for its use in drug resistant TB (Diacon et al. 2012; Mahajan, 2013; Butler et al. 2013). Currently there is an apparent need for fast acting TB drugs with fewer side effects, which are capable to eliminate the infection in a short period.

1.3.2.2 *Staphylococcus aureus*

Staphylococcus aureus (*S. aureus*) is a gram-positive bacterium found in human respiratory tract and on the skin. The common diseases of *S. aureus* are skin infections such as pimples and boils; it enters the blood and can cause number of problems in the body like bacteremia, toxic shock syndrome, pneumonia, meningitis etc. Some of these bacteria are also responsible for food poisoning. Penicillin is the most common choice of antibiotic for the treatment for *S. aureus* infections.

1.3.2.3 *Escherichia coli*

Escherichia coli (*E. coli*) are gram-negative bacteria usually found in the lower intestine of warm blooded organisms. Most of the *E.coli* strains are harmless, but some of them can cause serious food poisoning in humans, severe stomach cramps, urinary tract infections, diarrhoea (often bloody), vomiting and perhaps slight fever.

1.3.2.4 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa (*P. aeruginosa*) is a gram-negative, aerobic, rod shaped bacterium which leads commonly pulmonary tract infections, urinary tract infections, burns, wounds and other blood infections. Gram-negative pathogenic diseases are not easily controlled by antibiotics, because it has a fairly impermeable outer layer and it can also turn on pumps that remove antibiotics from the cell.

1.3.2.5 *Klebsiella pneumonia*

These are rod shaped gram-negative, anaerobic bacteria that colonizes the respiratory system. It can cause bronchopneumonia which results in the acute inflammation of the walls of the bronchioles and consequent congestion with pus. Treatment for this disease is difficult especially those with a weakened immune system or on long courses of certain antibiotics.

1.3.2.6 *Streptococcus pneumonia*

Streptococcus pneumonia is a gram-positive, anaerobic significant human pathogenic bacterium which causes lobar pneumonia, especially in young adults. Pneumococcal is the main vaccine for this infection. *Streptococcus pneumoniae* has a natural transformation system as a mechanism for genetic exchange (horizontal gene transfer) with related and unrelated species. It has been shown that in the last twenty years genes coding for alterations in penicillin binding proteins have been passed in this way so that the bacterium has got resistance to beta lactam antibiotics. As a result there is a decrease in impact on such diseases by older antibiotics.

1.4 MODE OF ACTION OF ANTIMICROBIALS

The antimicrobial agents function by attacking various cellular targets which include cell wall, plasma membrane, nucleic acids and protein synthesis of the microbe. The schematic representation of the mechanisms of action of antimicrobial agents is given in **Figure 1.6**. The precise mechanisms of action of antimicrobial drugs are still not clear, but the following possible views were proposed for their mode of action

- **Inhibition of cell wall synthesis**: Certain antimicrobials work by inhibiting the cell wall synthesis. Therefore, they have little effect on host cells, which do not contain peptidoglycan. Penicillin, bacitracin, cephalosporine and vancomycin act in this way.
- **Inhibition of protein synthesis**: Several antimicrobial agents like chloramphenicol, erythromycin, streptomycin, tetracyclines etc. act by inhibiting protein synthesis. As ribosomes of prokaryotic cells are slightly different from those of eukaryotes, they can be used as a target.

- **Injury to the plasma membrane**: This is a mode of action for certain antibacterials and antifungals. Antifungals are able to work mostly against fungus cell membranes because they contain ergosterol instead of cholesterol. However, these antimicrobials are potentially quite toxic to the host. The examples include polymixins (antibacterial), amphotericin B, miconazole and ketoconazole (antifungals).
- **Inhibition of nucleic acid synthesis**: These drugs interfere with DNA replication and transcription, but their selective toxicity varies. RMP and certain quinolone derivatives are the examples under this mode of action.
- **Inhibition of the synthesis of essential metabolites**: Generally sulfonamides and trimethoprim functions by this way. They interfere with the pathway on which bacteria synthesize folic acid. Since humans produce folic acid by a different pathway, these drugs have less effect on human cells.

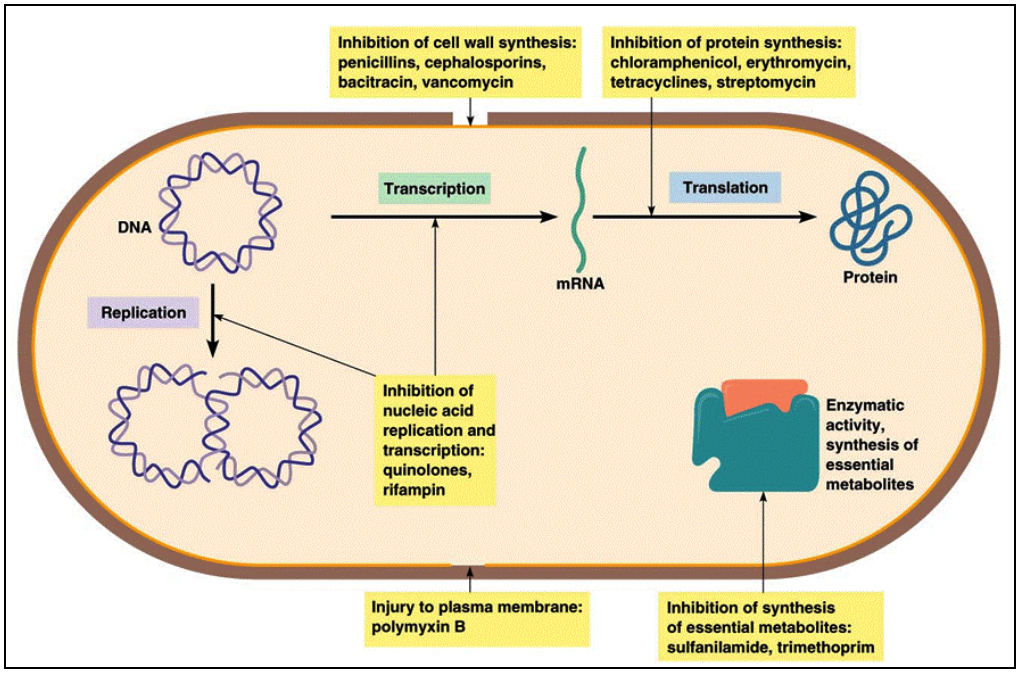


Figure 1.6 Schematic representation of mechanism of action on bacterial cell. (Source: http://classes.midlandstech.edu/carterp/Courses/bio225/chap20/lecture2.htm)

1.5 ANTIMICROBIAL SCREENING

In general, antimicrobial activity of any substance can be investigated by various screening methods (WHO/CDS/CSR/RMD, 2003). Both *in vitro* and *in vivo* methods are widely used for screening of compounds for antimicrobial activity.

Amongst them, *in vitro* methods are extensively used for the preliminary evaluation of antifungal, antibacterial and antiTB activities. Further, *in vivo* studies are employed on animal models of the human condition necessary to elucidate the mechanisms of antimicrobial action and to develop drugs that can control infection caused by pathogenic microbes. Furthermore, the toxicity (IC_{50}) studies of these molecules are performed on non-cancerous cells in order to pass them into phase trials.

During the last decades, several experimental procedures were developed for Antimicrobial Susceptibility Testing (AST) by Clinical and Laboratory Standards Institute (CLSI) that created standards to perform ASTs. These methods are extensively being used to determine the molecular potency against microbes. Generally, *in vitro* antimicrobial susceptibility testing methods are divided mainly into three types, viz. (i) diffusion, (ii) dilution and (iii) diffusion and dilution methods. Some of the important antimicrobial testing methods have been discussed in the following sections.

1.5.1 Diffusion methods

Diffusion method involves two important techniques, viz. Stokes method and Kirby-Bauer method. These methods are typically used for antimicrobial susceptibility testing, which are being well recommended by the National Committee for Clinical Laboratory Standards (NCCLS).

1.5.1.1 Stokes method: In this method a known quantity of bacteria is grown on agar plates in the presence of thin wafers containing relevant standard antibiotics. If the bacteria are susceptible to a particular antimicrobial, an area of clearing surrounds the wafer where bacteria are not capable of growing (called a zone of inhibition). Also, the rates of antimicrobial diffusion are determined and these values are used to estimate the bacteria's sensitivity to that particular antimicrobial agent. In general, larger zones correlate with smaller concentration of test compounds for a specific microorganism. This information can be used to choose appropriate antimicrobials to combat a particular infection.

1.5.1.2 Kirby method: The Kirby-Bauer test, known as the disk-diffusion method, is the most widely used antibacterial susceptibility test in determining the precise antibiotics used to treat the exact infection. This method relies on the inhibition of

bacterial growth measured under standard conditions. For this test, a culture medium, specifically the Mueller-Hinton agar, is uniformly and aseptically inoculated with the test organism and then filter paper discs, which are impregnated with a specific concentration of a particular antimicrobial, is placed on the medium. The organism will grow on the agar plate while the antimicrobial 'works' to inhibit the growth. If the organism is susceptible to a specific antimicrobial drug, there will be no growth around the disc containing the antibiotic. Thus, a 'zone of inhibition' can be observed and measured to determine the susceptibility to an antimicrobial for that particular organism.

1.5.2 Dilution methods

Dilution methods mainly include minimum inhibition concentration (MIC) method, which can be further classified as broth dilution and agar dilution methods.

1.5.2.1 Minimum inhibitory concentration (MIC) method: MIC method is generally used to determine the minimal concentration of antimicrobial to inhibit or kill the microorganisms completely. This can be achieved by dilution of antimicrobial solution in either agar or broth media. The dilutions are normally expressed in log2 serial dilutions (i.e. two fold). In this method, a pure culture of a single microorganism is grown in appropriate broth. The culture is standardized using standard microbiological techniques (nearly 1 million cells per milliliter). The compound under screening is diluted a number of times, 1:1, using a sterile diluents. After dilution, a volume of the standardized inoculums equal to the volume of the diluted compound is added to each dilution vessel bringing the microbial concentration to approximately 500,000 cells per milliliter. The inoculated, serially diluted antimicrobial agent is incubated. After incubation, the dilution vessels are observed for microbial growth, the results of which are usually indicated by turbidity or color change. The last tube in the dilution series that does not demonstrate growth corresponds to the minimum inhibitory concentration (MIC) of the antimicrobial agent.

1.5.2.2 Broth dilution method: The Broth dilution method is a simple technique for testing a small number of isolates, even single isolate. It involves serial dilution of the antimicrobial agent in a liquid medium, which is then inoculated with a standardized

number of organisms and incubated for a prescribed time. The lowest concentration of antibiotic preventing appearance of turbidity is considered to be the minimal inhibitory concentration. It has the added advantage that the same tubes can be taken for Minimum Bactericidal Concentrations (MBC) tests also.

1.5.2.3 Agar dilution method: In this method, the compounds under screening are diluted on log2 dilution intervals where each petri dish contains 50 per cent of the concentration of the given compound in the previous dilution. The diluted solution is incorporated into the agar medium and mixed by gentle rotation and poured into petri dish. A control plate without any antimicrobial agent incorporated into the medium is also used along with each compound tested, to check for growth of the test and control strains. Readings are recorded after the petri dishes have been incubated. The main advantage of the method is that it is possible to test several organisms on the same plate.

1.5.3 Dilution and diffusion method

Dilution and diffusion method is a convenient method to screen the antimicrobial susceptibility of any substance. It is also known as epsilometer test (E test). This 'exponential gradient' testing methodology is generally used for the quantitative antimicrobial screening wherein both dilution of antimicrobial and diffusion of antimicrobial into the medium involve. In this method, a thin inert carrier strip containing a predefined stable antimicrobial gradient is used. It is then applied onto an inoculated agar plate. Then, there is an immediate release of the drug. On incubation for 24 hours, a symmetrical inhibition ellipse is produced. The intersection of the inhibitory zone edge and the calibrated carrier strip indicates the MIC value over a wide concentration range (>10 dilutions) with inherent precision and accuracy. E test is simple, easy to perform and is a reliable method for determination of MIC. Also, it has been shown to be a good alternative to the agar and broth dilution tests, particularly for the strains such as *H. influenza*. However its cost and limited availability is a concern.

The latest 'genotypic' technique for detection of antimicrobial resistance genes has also been promoted as a way to increase the speed and accuracy of susceptibility testing. Numerous DNA based assays are being developed to detect

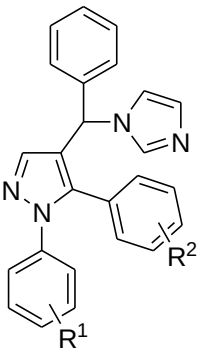
bacterial antibiotic resistance at the genetic level. These methods, when used in conjunction with phenotypic analysis, offer the promise of increased sensitivity, specificity, and speed in the detection of specific known resistance genes and can be used in tandem with traditional laboratory AST methods.

Although a variety of methods exist, the goal of *in vitro* antimicrobial susceptibility testing is to provide a reliable predictor of how an organism is likely to respond to antimicrobial therapy in the infected host. This type of information aids the clinician in selecting the appropriate antimicrobial agent, aids in developing antimicrobial use policy, and provides data for epidemiological surveillance. Such epidemiological surveillance data provide a base to choose the appropriate empirical treatment (first-line therapy) and to detect the emergence and/or the dissemination of resistant bacterial strains or resistance determinants in different bacterial species. The selection of a particular AST method is based on many factors such as validation data, practicality, flexibility, automation, cost, reproducibility, accuracy and individual preference.

1.3 LITERATURE SURVEY

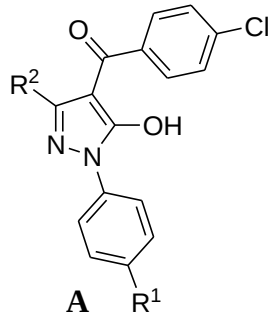
From the literatures it is observed that pyrazole derivatives also have exhibited promising antiTB properties. Some of the important literatures are listed below.

Menozzi et al. (2004) synthesized new halogenated 4-[*1H*-imidazol-1-yl(phenyl)methyl]-1,5-diphenyl-1*H*-pyrazoles and their *in vitro* assays for antimicrobial and antimycobacterial activities were carried out. Among the fifteen derivatives three compounds with a chloro substitution showed very good activity compared to the reference drugs. The replacement of chlorine with fluorine atom in these molecules led to inactive derivatives.

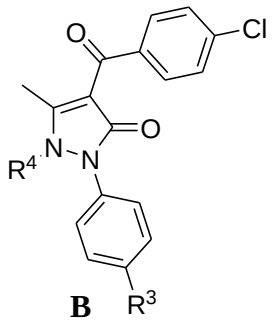


R^1 = H, 2-Cl, 2,4-Cl_2, 4-Cl, 2-F, 4-F, 2,4-F_2,
R^2 = H, 2-Cl, 4-Cl, 2-F, 4-F,

Castagnolo et al. (2008) reported the synthesis and antiTB activity and structural activity relationship (SAR) study of 21 novel pyrazole derivatives. Among these, compound with a 4-bromo substitution at R^1 and -CH_3 group at R^2 showed very good activity (MIC = 4μg/ml) against MTB-H_{37}Rv strain. Also, most of the compounds of series **A** were found to be better in terms of activity as compare to those of **B.**



A

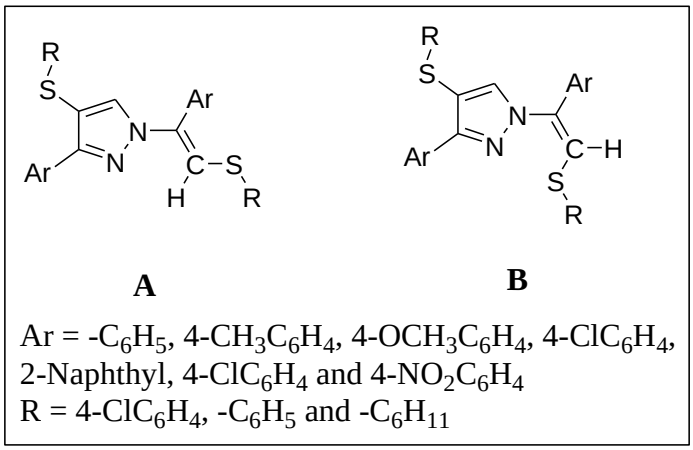


B

R^1 = H, Cl, F, Br, CH_3, Isopropyl
R^2 = CH_3, CF_3, Isopropyl, Ph

R3 = H, Cl
R^4 = CH_3, Bn, 4-F-Bn, 4-NO_2-Bn

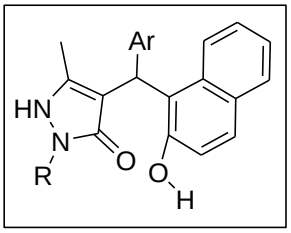
Manikannan et al. (2010) synthesized new isomeric pyrazole derivatives and the compounds were tested for antimycobacterial activity against MTB. It was found that isomeric compounds of **A** are more active than those of **B**. The 4-$NO_2C_6H_4$ derivative of isomer **A** was found to be the most potent molecule of the series with a MIC of 0.78 μg/ml.



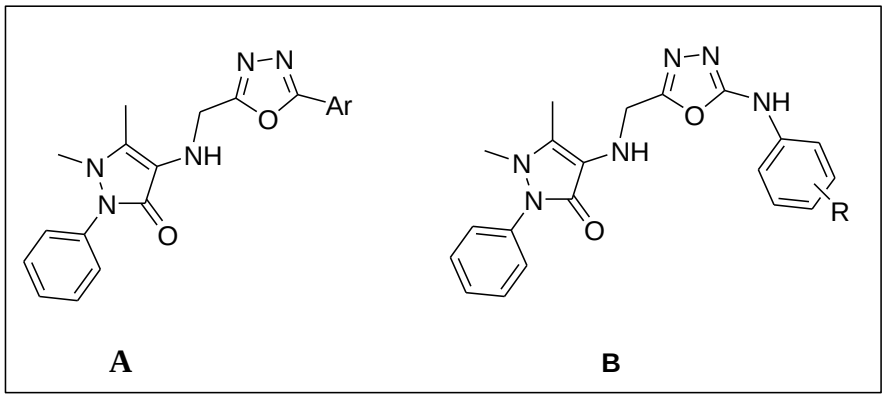
A **B**

Ar = -C_6H_5, 4-$CH_3C_6H_4$, 4-$OCH_3C_6H_4$, 4-ClC_6H_4, 2-Naphthyl, 4-ClC_6H_4 and 4-$NO_2C_6H_4$
R = 4-ClC_6H_4, -C_6H_5 and -C_6H_{11}

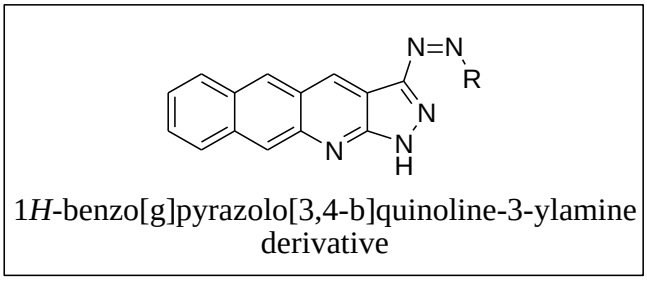
Gunasekaran et al. (2011) synthesized a series of 2-aryl-5-methyl-2,3-dihydro-1*H*-3-pyrazolones by one-pot, four component sequential reaction and the compounds were assayed for their inhibitory activity towards MTB using agar dilution method. The dervative 4-[(2,4-dichlorophenyl)(2-hydroxy-1-naphthyl)methyl]-2-(4-fluoro phenyl)-5-methyl-2,3-dihydro-1*H*-3-pyrazolone showed maximum potency with a MIC of 1.6 µM, which is 2.94 and 4.75 times more active than ciprofloxacin and EMB respectively.



Ahsan and coworkers in 2011 and 2012 reported the antimycobacterial investigation of two novel series of pyrazole-1,3,4-oxadiazole analogs. One of the derivatives of series **A** with a substitution Ar = 4-pyridinyl showed activity comparable to that of the standard drug isoniazide (MIC = 0.28 µg/ml). Also the compound was found to be nontoxic up to 62.5 µg/ml. In series **B** the derivative with substitution R = 4-Flouro was found to be the most active against MTB with MIC of 0.78 µg/ml and was nontoxic up to 62.5 µg/ml.

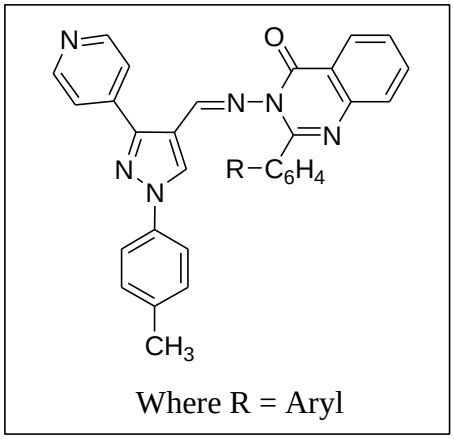


Parekh and Maheria in 2012 demonstrated antiTB and antibacterial evaluations of some novel phenyl pyrazolone-substituted 1*H*-benzo[*g*]pyrazolo[3,4-*b*]quinoline-3-yl-amine derivatives. All 10 new compounds were evaluated for their *in vitro* antibacterial activity against *S. aureus, Bacillus subtilis, E. coli*, and *P. aeruginosa*. Also the compounds were tested for *in vitro* antiTB activity against MTB. The microbial analysis revealed that benzoquinoline analogs combining with different substituted pyrazole are good antibacterial and antituberculosis agents with less toxic effects.



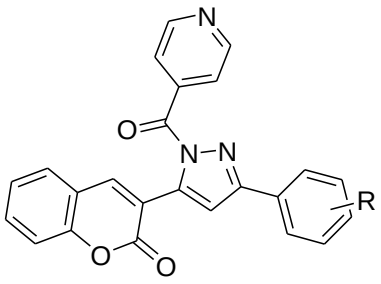
1*H*-benzo[g]pyrazolo[3,4-b]quinoline-3-ylamine derivative

Pandit and Dodiya (2013) synthesized twelve new derivatives of 2-(aryl)-3-(((3-(pyridin-4-yl)-1-(p-tolyl)-1*H*-pyrazol-4-yl)methylene) amino)-quinazolin-4(3*H*)-ones and carried out *in vitro* studies to inhibit the growth of MTB. From the screening studies they found that five compounds exhibited excellent antiTB activity with a MIC of <3.125 µg/mL.



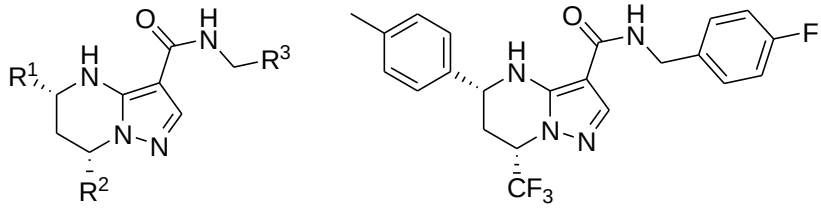
Where R = Aryl

Aragade et al. (2013) studied the antibacterial activity of various pyrazole derivatives derived from the isoniazid pharmacophore along with coumarin scaffold. The synthesized 11 compounds were investigated for their *in vitro* antimycobacterial activity against MTB using Resazurin MIC assay. The synthesized compounds exhibited MIC ranging from 0.625 to 2.50 µg/ml. Among the compounds tested, 3-[3-(4-fluorophenyl)-1-isonicotinoyl-1*H*-pyrazol-5-yl]-2*H*-chromen-2-one was found to be the most active with MIC of 0.625 µg/ml.



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

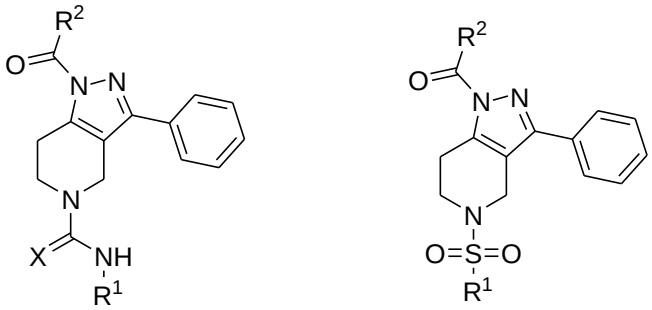
Yokokawa et al. (2013) synthesized tetrahydropyrazolo[1,5-*a*]pyrimidine derivatives and demonstrated the *in-vitro* screening against MTB. All the compounds were found to be good antiTB agents. They carried out *in vivo* studies using one of the active compounds in a mouse efficacy model, achieving the reduction of 3.5 log CFU of MTB after oral administration to infected mice once a day at 100 mg/kg for 28 days. Thus the compound could be a promising candidate for inclusion in combination therapies for both drug-sensitive and drug-resistant tuberculosis.



A **B**

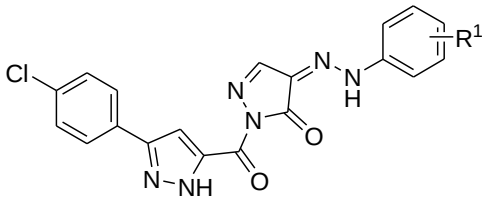
A - Tetrahydropyrazolo[1,5-*a*]pyrimidine dervivative
B - Potent candidate of tetrahydropyrazolo[1,5-*a*]pyrimidine dervivative

Samala et al. (2013) synthesized 3-phenyl-4,5,6,7-tetrahydro-1*H*-pyrazolo[4,3-*c*]pyridine derivatives and evaluated for MTB pantothenate synthetase (PS) inhibition study, *in vitro* activities against MTB and cytotoxicity against RAW 264.7 cell line. Among the compounds, 1-benzoyl-N-(4-nitrophenyl)-3-phenyl-6,7-dihydro-1*H*-pyrazolo[4,3-*c*]pyridine-5(4*H*)carboxamide was found to be the most active compound with IC_{50} of 5.87 ± 0.12 μM against MTB PS and inhibited MTB with MIC of 9.28 μM. Also the compound was found to be non-cytotoxic even at 50 μM concentration.

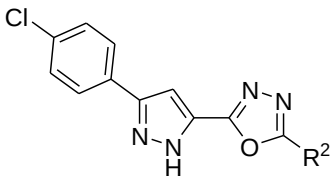


X = O or S; R_1 = Aryl, R_2 = Phenyl, Cyclohexyl

Horrocks et al. (2013) synthesized 20 novel 3-(4-chlorophenyl)-4-substituted pyrazole derivatives and tested *in vitro* antiTB activity of the compounds against MTB strain. The best activity was observed for the 4-fluoro substituted pyrazolinone derivative with MIC value 0.35 μg/ml which is only slightly less potent than the standard drug rifampicin. All the compounds were evaluated for *in vitro* antifungal activity against four pathogenic strains of fungi. The MIC values were compared with fluconazole and 5-fluorocytosine as standard agents.

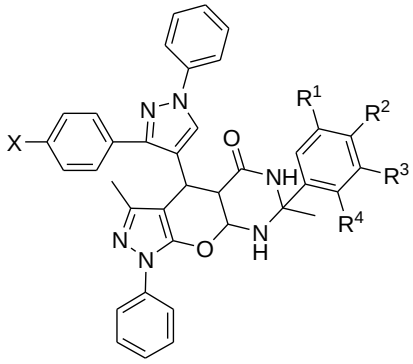


R^1 = H, 4-OCH_3, 2-Cl, 4-Cl, 4-NO_2, 3-Cl, 4-F, 3,4-$(Cl)_2$, 4-F, 2-CH_3, 4-COOH



R^2 = C_6H_5, 4-OCH_3-C_6H_4, 2-OH-C_6H_4, 2,4-$(OH)_2$-C_6H_3, 2-Cl-C_6H_4, 4-NO_2-C_6H_4, 4-NH_2-C_6H_4, -CH=CHC_6H_5, 2-C_5H_4N

Chobe et al. (2013) synthesized a new series of pyrano[2,3-*c*]pyrazole derivatives. The *in vitro* antimycobacterial activities of newly synthesized compounds were investigated against *Mycobacterium smegmatis*, *Mycobacterium pheli* and *Mycobacterium tuberculosis* species and most of the compounds were found to be good antiTB agents.

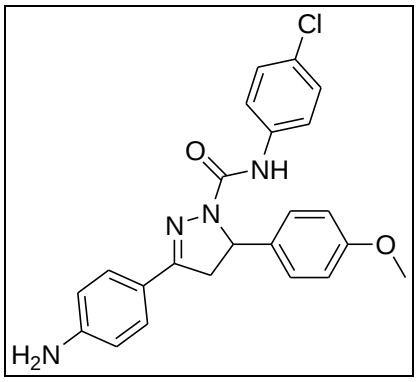


Pyrano[2,3-*c*]pyrazole derivative

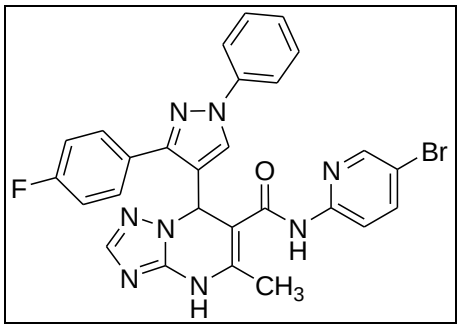
	a	b	c	d	e	f	g	h	i	j
R^1	-OH	-OH	-OH	-OH	-OH	-OH	-OH	-OH	-OH	-OH
R^2	-H	-H	-Cl	-I	-H	-H	-Cl	-I	-H	-I
R^3	-H	-OH	-OH	-H	-H	-OH	-OH	-H	-H	-H
R^4	-Cl	-H	-H	-Cl	-Cl	-H	-H	-Cl	-Cl	-Cl

Ahsan and Saini (2014) described the synthesis of 3-(4-aminophenyl)-5-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazole-1carboxamide/carbothioamide analogs and studied their antiTB activity against MTB. The derivative with 4-chlorophenyl

substitution on the carboxamide linkage was the most active agent in the series with MIC of 7.41 µM.



Bhatt et al. (2015) synthesized series of novel pyrazole linked triazolo-pyrimidine hybrids and evaluated for their antiTB activity against MTB. They also carried out molecular docking study of the active molecules against InhA enzyme of MTB. Furthermore, the enzyme inhibition assay, Absorption, distribution, metabolism and excretion (ADME) prediction assay and cytotoxic study revealed that one of the derivatives is emerged as the potent antiTB lead.



The above literature studies on activity of pyrazole derivatives against MTB undoubtedly suggests that derivatives of pyrazole are good pharmacophoric leads for antiTB agents and there is enough scope for further derivatization and structural modification to develop molecules with better activity.

1.4 SCOPE AND OBJECTIVES OF THE PRESENT WORK

At present, only a limited number of antiTB drugs are available to treat multidrug resistant strains of infectious TB bacteria, and resistance to even the latest antibiotic is appearing. Increasing antibiotic resistance in microbial populations has

necessitated the search for new antiTB agents. The current approaches to cure these diseases are very complex as they take several months of chemotherapy to eliminate completely the persistent bacteria. Moreover, the treatment of TB with the frontline drugs is associated with severe side effects. Thus, there is an urgent need for the development of newer antiTB drugs which are more selective and effective than the existing drugs, with safe ADME profile.

It is well established that small modifications in the structure of the target molecules alter their biological character as well as their physiochemical properties. Both steric and electronic factors are claimed to be prime determinants in the variation of biological activity. Generally, factors such as presence of hydrophobic and hydrophilic groups, binding site and solubility of the molecules are desirable features to exhibit medicinal activity to a great extent. Stereochemistry of the molecule also plays an important function in their biological activity. These parameters have major role in the discovery of new drugs in the modern pharmaceutical research.

Generally, a rational drug design process for a new antimicrobial agent could be achieved in many ways. One of the strategies is the identification of new targets through better understanding of molecular mechanisms of infections. Another way is to modify the structure of already existing drugs by improving the binding affinity to the receptor.

Against this background, in the present research study, it has been planned to design new pyrazole based compounds carrying biologically important pharmacophores on its active positions and synthesize them using standard procedures. Also, it has been thought of investigating their preliminary antiTB properties to understand the structure activity relationship. It has been hoped that the study would lead to develop new active antiTB agents, which may enter as potential candidates for second phase clinical studies. The expected novel dream-drug would be a suppressor for tuberculosis infection with shorter duration of action and less toxicity. Also, the correlation between structure activities of the new compounds would impart valuable information to assist development of new types of drugs in new millennium. Furthermore, the results of the research may be useful in understanding the mechanism of drug action.

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

- To design new pyrazole based molecular structures with possible antiTB activity.
- To synthesize new chemical entities (NCEs) related to pyrazole containing derivatives and optimization of their synthetic methods.
- To develop suitable purification methods for the NCEs like column chromatography and recrystallization techniques.
- To characterize the synthesized new compounds by using spectral methods like ^{1}H NMR, ^{13}C NMR and Mass spectrometry, followed by elemental analysis.
- Also to carry out single crystal X-ray diffraction studies (SCXRD) of selected compounds for elucidation of final three-dimensional structure.
- To evaluate the *in vitro* antiTB activity of the final compounds.
- To study the *in vitro* cytotoxicity for the active molecules against a non-cancerous cell line.
- To perform *in silico* molecular modeling studies for the active molecules in order to investigate their binding affinity with the target enzymes.
- To evaluate the *in vitro* antibacterial activity of the final compounds.

Keeping the above objectives in view and based on various literature supports, five new series of pyrazole based derivatives have been designed in the present work. In the first series, new pyrazole-1,2,3-triazole hybrid derivatives have been designed as antiTB agents. The synthetic and antiTB screening results of these derivatives are discussed in Chapter 2. Chapter 3 includes discussion on synthesis of series of compounds based on pyrazole-oxadiazole derivatives and their biological studies. Two new series of pyrazole-quinoline hybrid derivatives containing methylene hydrazine and carboxamide groups were synthesized, the detailed discussion of which along with the biological screening of these molecules are explained in Chapters 4 and 5. Chapter 6 includes the synthesis and biological evaluation of isoniazid-pyrazole analogs. In order to further rationalize our antiTB results we have taken active molecules of the each series and tested for their cytotoxicity against a normal cell line. The *in silico* molecular modeling studies of the active compounds were also

carried out against target enzymes. Additionally, all the molecules were tested against three common pathogenic bacterial strains by zone of inhibition method. Finally, outcomes of the present research work are summarized in chapter 7, followed by references and list of publications.

CHAPTER 2

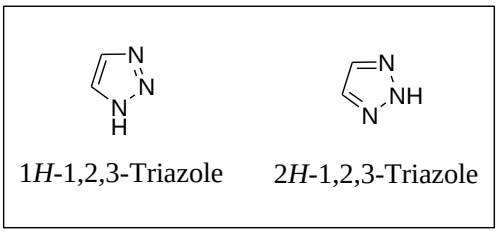
SYNTHESIS AND BIOLOGICAL ACTIVITY OF NEW 1-(PHENYL-1*H*-(PYRAZOL-3-YL)METHOXY)METHYL-1*H*-1,2,3-TRIAZOLE DERIVATIVES

Abstract

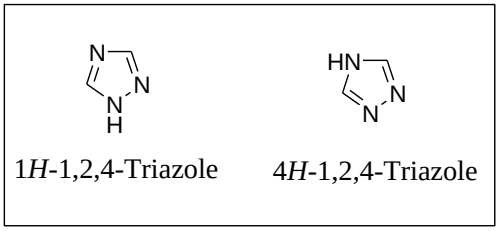
This chapter describes design and synthesis of new pyrazole and 1,2,3-triazole hybrid analogs. It also discusses their characterization by various spectral techniques followed by their antiTB and antibacterial screening studies.

2.1 INTRODUCTION

Triazole based hecterocyclic derivatives have gained great significance in medicinal chemistry research due to their wide spectrum of biological activities. It consists of a five membered ring which contain two carbons and three nitrogen atoms. There are two sets of isomers for triazole, 1,2,3-triazole and 1,2,4-triazole, that differ in the relative positions of the three nitrogen atoms. Each of these isomers has two tautomers that differ by, which nitrogen has a hydrogen bonded to it; 1,2,3-triazole (**S-2.1**) and 1,2,4-triazole (**S-2.2**).



S-2.1 1,2,3-triazole



S-2.2 1,2,4-triazole

Amongst two isomers, 1,2,3-triazoles have attained a unique place in synthetic field as they are found to be non-toxic, benign and stable. It is a well-known fact that 1,2,3-triazoles have not been isolated in any of the naturally occurring compounds (Mayot et al. 2005) and hence needs to be synthesized by facile and efficient synthetic routes. Also, with the invention of click chemistry of cycloaddition reactions, several 1,2,3-triazole derivatives have been synthesized and are found to possess a broad spectrum of pharmacological properties (Tron et al. 2008) such as antimicrobial (Holla et al. 2005), anticancer (Duan et al. 2013), antifungal (Aher et wl. 2009), antiviral (Zhou et al, 2005) and anti-inflammatory (Cunha et al. 2003) activities. Literatures demonstrated the promising antiTB properties of a number of *N*-substituted 1,2,3-triazole derivatives (Shanmugavelan et al. 2011; Gill et al. 2008; Addla et al. 2014; Addla et al. 2014; Nagesh et al. 2013; Kamal et al. 2013; Patpi et al. 2012; Surineni et al. 2015; Yempala et al. 2014), among which derivative **I-A09**

(Tan et al. 2009) is emerged to be a lead candidate and presently in preclinical trials. Additionally, the derivatives of 1,2,3-triazole possess remarkable metabolic stability and are proven to be amide surrogates in various bioactive compounds (Brik et al. 2005). Further, recent studies on azoles demonstrated their ability of forming hydrogen bonding and dipole interactions to biomolecular targets which results in improving their solubility in biological systems (Horne et al. 2004; Kushwaha et al. 2014). In recent years, the molecular hybridization concept has received significant importance in drug design and development, which involves combination of pharmacophoric moieties of two bioactive elements to produce a new hybrid lead compound with improved efficiency and efficacy, when compared to the parent compounds. It is also found that this strategy resulted in developing compounds with modified selectivity profile, different and/or dual modes of action and reduced undesired side effects (Viegas-Júnior et al. 2007; Ramprasad et al. 2015). With this background and pertaining to the literature reports on promising antiTB and antimicrobial activity of a number of pyrazole and 1,2,3-triazole derivatives (**Figure 2.1**), we have planned to amalgamate these two structural units in a single molecular framework and synthesized a library of pyrazole-1,2,3-traizole hybrid derivatives (**P1-P24**). All these synthesized final compounds were subsequently screened for antiTB activity against MTB strain by agar dilution method. The *in vitro* cytotoxicity studies against a normal Vero cell line of the active antiTB compounds were carried out by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Moreover, *in silico* molecular modeling studies on active antiTB derivatives were carried out against selected target enzymes. Further, antimicrobial studies on three bacterial strains were carried out using disc diffusion method.

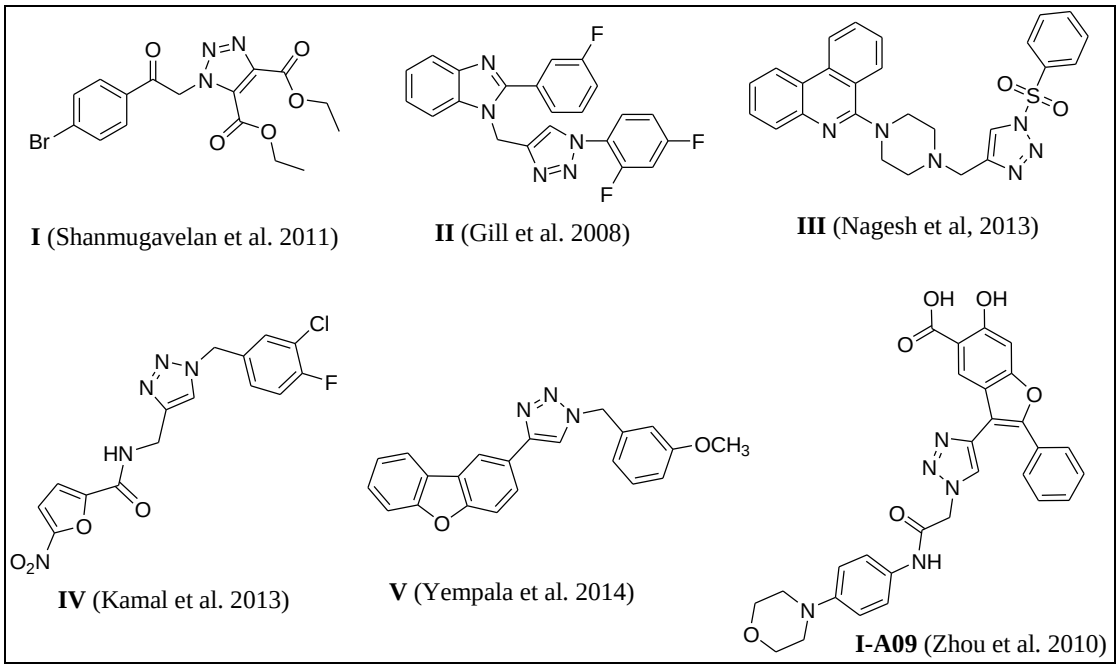
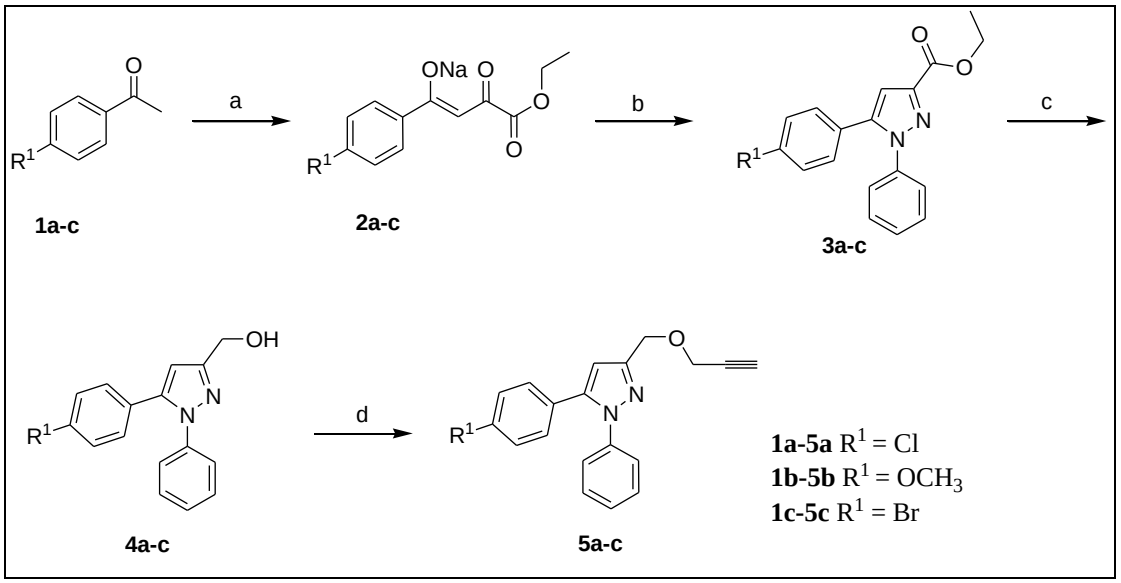


Figure 2.1 Representative 1,2,3-triazole based antiTB agents (**I-V** and **I-A09**).

2.2 CHEMISTRY

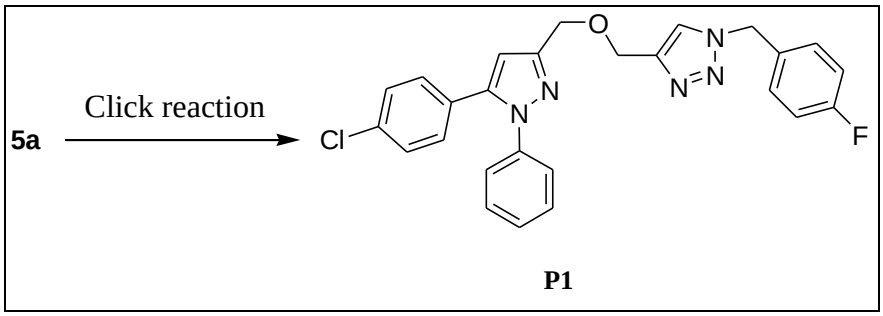
The synthetic route of intermediates **5a-c** is shown in **Scheme 2.1**. The protocol involves the Claisen condensation of 4-substituted acetophenones (**1a-c**) with diethyl oxalate in the presence of sodium ethoxide (NaOEt) in ethanol at room temperature (RT) to yield corresponding sodium salt of α,γ-diketoesters (**2a-c**). Esters of 5-aryl-1-phenyl-1*H*-pyrazole-3-carboxylic acids (**3a-c**) were synthesized in quantitatively good yield by the conventional cyclization reaction between **2a-c** and arylhydrazines in presence of acetic acid (AcOH). The ester derivatives were then reduced to corresponding alcohols (**4a-c**) in good yields by using lithium aluminium hydride ($LiAlH_4$) as the reducing agent. The propargylated scaffolds, 5-(4-aryl)-1-phenyl-3-((prop-2-ynyloxy)methyl)-1*H*-pyrazoles (**5a-c**) were obtained by the alkylation of alcohols **4a-c** with propargyl bromide in the presence of sodium hydride (NaH) (60%) in tetrahydrofuran (THF). The targeted regioselective 1,4-substituted 1,2,3-triazole derivatives (**P1-P24**) were synthesized using a multicomponent one pot click chemistry approach by treating the propargylated scaffolds (**5a-c)** with alkyl bromides or benzyl bromides in presence of catalytic amount of copper sulfate pentahydrate and sodium ascorbate in 2:1 mixture of polyethylene glycol-400 (PEG-400) and water at 50 °C (**Scheme 2.2**).



Scheme 2.1 Synthesis of the pyrazole-triazole based scaffolds. Reagents and conditions: a) Diethyl oxalate, NaOEt, ethanol, 0 °C to RT, 24h; b) Phenyl hydrazine, AcOH, 105 °C, 3h; c) $LiAlH_4$, diethyl ether, 0 °C to RT, 1h; d) NaH (60%), THF, propargyl bromide (80% in toluene), 0 °C to RT, 1h.

We tried using different solvent systems for the click reaction and the optimization was carried out with the reaction between **5a** and 4-fluorobenzyl bromide (**Table 2.1**). Among the solvents used, 2:1 mixture of PEG-400 and water was the most effective system which yielded a product (**P1**) yield of 84 %. The reaction using a mixture of t-butanol and water as the solvent also resulted in comparable product yield. PEG-400 is an interesting solvent in synthetic chemistry as it is non-toxic and inexpensive. Also it is soluble in water but show poor solubility in number of organic solvents. The greener and easy removable nature of PEG-400 prompted us to choose this solvent in the click reaction protocol.

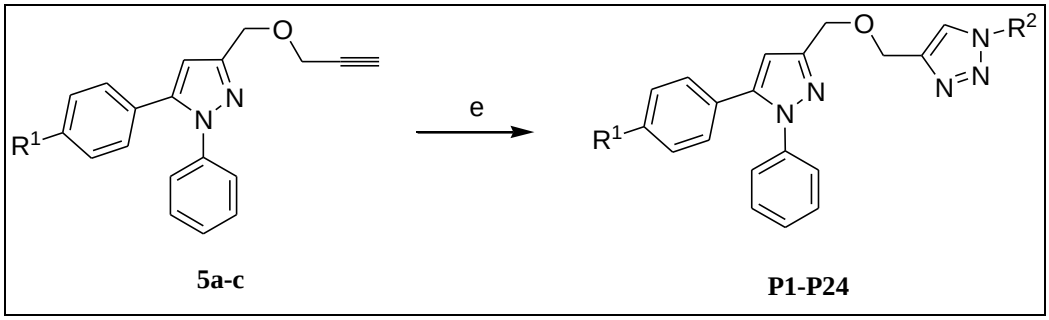
Table 2.1 Optimization of solvent for the click reaction of **5a**. Reagent and conditions: 4-Fluorobenzyl bromide, $CuSO_4.5H_2O$, NaN_3, sodium ascorbate, organic solvent/H_2O (2:1), 50 °C, 12h.



P1

Entry	Organic solvent	Time (h)	Yield[a] (%)
1	1,4-Dioxane	24	-
2	Ethanol	24	54
3	t-Butanol	12	80
4	PEG-400	12	84
5	Acetonitrile	24	-
6	DMF	24	48

[a] Isolated yield after column purification. ¯Required product not formed.



5a-c **P1-P24**

Scheme 2.2 Synthesis of target molecules. Reagent and conditions: e) Alkyl and benzyl bromides, $CuSO_4.5H_2O$, NaN_3, sodium ascorbate, PEG-400/H_2O (2:1), 50 °C, 12h.

2.3 EXPERIMENTAL

2.3.1 Materials and methods

All the reagents were purchased from lead reagent suppliers like Sigma Aldrich (Germany), Merck (India), Loba Chemie Pvt. Ltd. and Spectrochem Chemicals Pvt. Ltd. All the solvents used were laboratory reagent (LR) grade and were extra dried by distillation method. Thin layer chromatography (TLC) was carried out on coated aluminum sheets with 60 F_{254} silica gel (Merck KGaA). The intermediate and the final compounds were purified by column chromatography technique using silica gel 230-400 or 60-120 mesh size (Merck). A Stuart SMP3 (BIBBY STERLIN Ltd. UK) melting point apparatus was used for the determination of the melting points of the newly synthesized compounds and are uncorrected. Proton nuclear magnetic resonance (^{1}H NMR) spectra of all the compounds were recorded on Varian/Bruker 400 MHz spectrometers using tetra methyl silane (TMS) as internal standard and deuterated dimethyl sulfoxide (DMSO-d_6) as the solvent. Carbon 13 nuclear magnetic resonance (^{13}C NMR) spectra of the compounds were recorded using a Bruker 100 MHz NMR spectrometer in DMSO-d_6. A Thermo electron corporation EA-112 series C,H,N,S analyzer was used for the elemental analysis. Molecular mass of the compounds was determined using a Waters Q-Tof micro mass spectrometer with an electrospray ionization (ESI) source. The X-ray crystallographic analysis of the compound **P1** was carried out by fine-focus sealed tube graphite using Mo Kα source. The crystal structure solution was worked out by Bruker SMART APEXII DUO CCD diffractometer. All the atoms were located in different Fourier maps and refined isotropically, using a riding model and all the projections were generated using Oak Ridge Thermal Ellipsoid Plot (ORTEP) program.

2.3.2 Synthesis

Synthesis of sodium (*E*)-1-aryl-4-ethoxy-3,4-dioxobut-1-en-1-olate (2a-c).

Sodium (*E*)-1-(4-chlorophenyl)-4-ethoxy-3,4-dioxobut-1-en-1-olate (2a): To a stirred solution of 4-chloro acetophenone **1a** (6.18 g, 40 mmol) in anhydrous ethanol (12 mL), 1M NaOEt solution (5.45 g, 80 mmol) in ethanol was added at 0 °C. The reaction mass was allowed to warm to RT and stirred for 1 h. A solution of diethyl oxalate (11.69 g, 80 mmol) in anhydrous ethanol (20 mL) was added drop wise and

the reaction mixture was stirred at room temperature (RT) for 24 h. The progress of the reaction was monitored by TLC. Upon completion of the reaction, the precipitate obtained was filtered and washed with cold ethanol followed by diethyl ether and dried in vacuum to obtain the sodium salt (**2a**) as pale yellow solid. Yield 6.64 g (60%); pale yellow solid; mp 65-66 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.45 (d, 2H, J = 7.8 Hz), 7.16 (d, 2H, J = 7.8 Hz), 6.48 (s, 1H, CH), 4.31 (q, 2H, CH_2, J = 7.2 Hz), 1.32 (t, 3H, CH_3, J = 7.2 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 189.46, 169.41, 161.97, 139.53, 133.66, 130.30, 129.72, 98.57, 62.55, 14.26; Anal. calculated for $C_{12}H_{10}ClNaO_4$; C, 52.10; H, 3.64. Found: C, 52.05; H, 3.62.

Sodium (*E*)-4-ethoxy-1-(4-methoxyphenyl)-3,4-dioxobut-1-en-1-olate (2b): Compound **2b** was synthesized by following the above mentioned procedure by treating 4-methoxy acetophenone **1b** (6.0 g, 40 mmol) with diethyl oxalate (11.69 g, 80 mmol) in the presence of NaOEt (5.45, 80 mmol). Yield 6.20 g (57%); pale yellow solid; mp 89-90 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.26 (d, 2H, J = 8.0 Hz), 6.94 (d, 2H, J = 8.0 Hz), 6.41 (s, 1H, CH), 4.30 (q, 2H, CH_2, J = 7.2 Hz), 3.73 (s, 3H, OCH_3), 1.31 (t, 3H, CH_3, J = 7.2 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 189.50, 170.46, 160.88, 139.59, 132.69, 130.32, 129.55, 98.59, 62.52, 55.88, 14.25; Anal. calculated for $C_{13}H_{13}NaO_5$; C, 57.36; H, 4.81. Found: C, 57.33; H, 4.79.

Sodium (*E*)-1-(4-bromophenyl)-4-ethoxy-3,4-dioxobut-1-en-1-olate (2c): The above procedure was followed for the synthesis of compound **2c** by reacting 4-bromo acetophenone **1c** (7.96 g, 40 mmol) with diethyl oxalate (11.69 g, 80 mmol) in presence of NaOEt (5.45 g, 80 mmol). Yield 8.15 g (64%); yellow solid; mp 76-77 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.34 (d, 2H, J = 7.8 Hz), 7.19 (d, 2H, J = 7.8 Hz), 6.46 (s, 1H, CH), 4.30 (q, 2H, CH_2, J = 7.2 Hz), 1.30 (t, 3H, CH_3, J = 7.2 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 189.33, 169.49, 161.95, 132.53, 130.96, 130.37, 129.66, 98.51, 62.56, 14.25; Anal. calculated for $C_{12}H_{10}BrNaO_4$; C, 44.89; H, 3.14. Found: C, 44.88; H, 3.15.

Synthesis of ethyl 5-aryl-1-phenyl-1*H*-pyrazole-3-carboxylate (3a-c).

Ethyl 5-(4-chlorophenyl)-1-phenyl-1*H*-pyrazole-3-carboxylate (3a): To a stirred solution of compound **2a** (5.52 g, 20 mmol) in glacial AcOH (60 mL), phenyl

hydrazine (3.24 g, 30 mmol) was added under nitrogen atmosphere and heated at 105 °C for 3 h. The completion of the reaction was confirmed by TLC. Then the solvent was removed by evaporation under vacuum. The residue thus obtained was diluted with cold water (50 mL) and extracted with ethyl acetate (3 × 100 mL). The combined extract was washed with 10 % sodium bicarbonate solution, then with water followed by brine and dried over anhydrous sodium sulphate. The solvent was removed under reduced pressure and the crude product was purified by flash column chromatography (230-400 size mesh) using 20 % ethyl acetate in pet ether to afford the pure product **3a**. Yield 5.22 g (80%); yellow solid; mp 86-87 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.26-7.47 (m, 9H, ArH), 7.17 (s, 1H, pyrazole-CH), 4.34 (q, 2H, CH_2, J = 7.2 Hz), 1.32 (t, 3H, CH_3, J = 7.2 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 161.97, 144.02, 143.61, 139.46, 134.17, 131.18, 130.87, 129.82, 129.49, 129.33, 129.18, 129.11, 128.24, 126.15, 110.30, 61.03, 14.68; ESI-MS (*m/z*) 327.3 $(M+H)^+$; Anal. calculated for $C_{18}H_{15}ClN_2O_2$; C, 66.16; H, 4.63; N, 8.57. Found: C, 66.20; H, 4.64; N, 8.59.

Ethyl 5-(4-methoxyphenyl)-1-phenyl-1*H*-pyrazole-3-carboxylate (3b): The above procedure was followed for the synthesis of compound **3b** by reacting compound **2b** (6.0 g, 22 mmol) with phenyl hydrazine (3.58 g, 33 mmol) in glacial AcOH (65 mL). Yield 6.25 g (88%); yellow solid; mp 153-154 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.17-7.42 (m, 8H, ArH), 6.90 (d, 2H, ArH, J = 8.8 Hz), 4.36 (q, 2H, CH_2, J = 7.2 Hz), 3.73 (s, 3H, OCH_3), 1.32 (t, 3H, CH_3, J = 7.2 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 161.97, 160.12, 144.68, 141.49, 135.27, 132.71, 131.03, 129.98, 129.49, 129.41, 129.16, 129.10, 128.48, 126.15, 110.29, 61.06, 55.61, 14.69; ESI-MS (*m/z*) 323.1 $(M+H)^+$; Anal. calculated for $C_{19}H_{18}N_2O_3$; C, 70.79; H, 5.63; N, 8.69. Found: C, 70.71; H, 5.61; N, 8.66.

Ethyl 5-(4-bromophenyl)-1-phenyl-1*H*-pyrazole-3-carboxylate (3c): The above procedure was followed for the synthesis of compound **3c** by reacting compound **2c** (7.7 g, 24 mmol) with phenyl hydrazine (3.89 g, 36 mmol) in glacial AcOH (85 mL). Yield 7.82 g (89%); yellow solid; mp 98-99 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.25-7.47 (m, 9H, ArH), 7.17 (s, 1H, pyrazole-CH), 4.34 (q, 2H, CH_2, J = 7.2 Hz), 1.32 (t, 3H, CH_3, J = 7.2 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 161.91,

141.60, 140.76, 133.98, 132.53, 131.12, 130.45, 129.99, 129.66, 129.51, 129.11, 128.64, 126.32, 124.10, 110.28, 60.99, 14.67; ESI-MS (*m/z*) 372.2 $(M+H)^+$; Anal. calculated for $C_{18}H_{15}BrN_2O_2$; C, 61.64; H, 4.36; N, 3.78. Found: C, 61.73; H, 4.37; N, 3.79.

Synthesis of 5-aryl-1-phenyl-1*H*-pyrazol-3-yl)methanol (4a-c).

(5-(4-Chlorophenyl)-1-phenyl-1*H*-pyrazol-3-yl)methanol (4a): A solution of compound **3a** (5.00 g, 15 mmol) in diethyl ether (50 mL) was stirred in an ice-bath under nitrogen atmosphere at 0 °C. To this solution, $LiAlH_4$ (1.16 g, 30 mmol) was slowly added in portions. Then, the mixture was stirred at the same temperature for 1 h. After the completion of the reaction (as monitored by TLC), the reaction mass was quenched slowly with saturated ammonium chloride solution at 0 °C. The reaction mixture was then extracted with diethyl ether (3 × 100 mL), washed with water, then with brine solution and dried over anhydrous sodium sulphate. Then the solvent was removed under reduced pressure and the crude product **4a** obtained was taken as such for next step without further purification. Yield 4.05 g (93%); off white solid; mp 158-159 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.37-7.44 (m, 5H, ArH), 7.24 (s, 4H, ArH), 6.64 (s, 1H, pyrazole-CH), 5.25 (s, 1H, OH), 4.51(d, 2H, CH_2, J = 4.4 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 154.44, 142.40, 140.04, 133.53, 130.57, 129.62, 129.51, 129.16, 128.12, 125.61, 107.37, 57.68; ESI-MS (*m/z*) 285.3 $(M+H)^+$; Anal. calculated for $C_{16}H_{13}ClN_2O$; C, 67.49; H, 4.60; N, 9.84. Found: C, 67.54; H, 4.62; N, 9.87.

(5-(4-Methoxyphenyl)-1-phenyl-1*H*-pyrazol-3-yl)methanol (4b): The above procedure was followed for the synthesis of compound **4b** by reacting compound **3b** (7.0 g, 22 mmol) with $LiAlH_4$ (1.67 g, 44 mmol) in diethyl ether (70 mL). Yield 4.99 g (82%); off white solid; mp 102-103 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.23-7.40 (m, 7H, ArH), 6.89 (d, 2H, ArH, J = 8.8 Hz), 6.59 (s, 1H, pyrazole-CH), 5.24 (s, 1H, OH), 4.50 (d, 2H, CH_2, J = 4.4 Hz), 3.73 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 160.78, 154.40, 141.98, 140.88, 132.83, 131.02, 129.64, 129.16, 128.32, 125.51, 107.33, 67.68, 55.60; ESI-MS (*m/z*) 281.2 $(M+H)^+$; Anal. calculated for $C_{17}H_{16}N_2O_2$; C, 72.84; H, 5.75; N, 9.99. Found: C, 72.78; H, 5.78; N, 9.96.

(5-(4-Bromophenyl)-1-phenyl-1*H*-pyrazol-3-yl)methanol (4c): The above procedure was followed for the synthesis of compound **4c** by reacting compound **3c** (7.5 g, 20 mmol) with $LiAlH_4$ (1.52 g, 40 mmol) in diethyl ether (70 mL). Yield 6.25 g (94%); off white solid; mp 116-117 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.25-7.42 (m, 9H, ArH), 6.63 (s, 1H, pyrazole-CH), 5.25 (s, 1H, OH), 4.51(d, 2H, CH_2, J = 4.4 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 154.41, 141.01, 140.12, 133.46, 130.22, 130.15, 129.99, 128.19, 128.13, 125.58, 107.36, 67.69; ESI-MS (*m/z*) 330.1 $(M+H)^+$; Anal. calculated for $C_{16}H_{13}BrN_2O$; C, 58.38; H, 3.98; N, 8.51. Found: C, 58.43; H, 3.99; N, 8.54.

Synthesis of 5-aryl-1-phenyl-3-((prop-2-ynyloxy)methyl)-1*H*-pyrazole (5a-c).

5-(4-Chlorophenyl)-1-phenyl-3-((prop-2-ynyloxy)methyl)-1*H*-pyrazole (5a): In an atmosphere of nitrogen, NaH (60%) (0.06 g, 15 mmol) was taken in dry THF (40 ml) at 0 °C under stirring. To the above mixture compound **4a** (3.8 g, 13 mmol) was added in portions and the resulting solution was stirred for 15 min. Propargyl bromide (80 % in toluene) (2.23 g, 15 mmol) was added drop wise and the reaction mixture was allowed to warm to room temperature and stirred for additional 1 h. After the completion of the reaction, the reaction mixture was quenched by adding saturated ammonium chloride solution drop wise at 0 °C. The reaction mixture was then extracted with diethyl ether (3 × 100 mL), washed with water, then with brine solution and dried over anhydrous sodium sulphate. The solvent was removed under reduced pressure and the crude product was purified by column chromatography (230-400 size mesh) using 15 % ethyl acetate in pet ether to afford the pure compound **5a**. Yield 3.40 g (79%); yellow syrup; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.37-7.45 (m, 5H, ArH), 7.24-7.28 (m, 4H, ArH), 6.70 (s, 1H, pyrazole-CH), 4.58 (s, 2H, CH_2), 4.25 (d, 2H, CH_2, J = 1.6 Hz), 3.51 (s, 1H, CH); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 149.90, 142.73, 139.87, 133.68, 130.65, 130.40, 129.65, 129.21, 129.14, 128.37, 125.74, 108.20, 80.62, 77.91, 64.91, 57.39; ESI-MS (*m/z*) 323.3 $(M+H)^+$; Anal. calculated for $C_{19}H_{15}ClN_2O$; C, 70.70; H, 4.68; N, 8.68. Found: C, 70.67; H, 4.69; N, 8.71.

5-(4-Methoxyphenyl)-1-phenyl-3-((prop-2-ynyloxy)methyl)-1*H*-pyrazole (5b): The above procedure was followed for the synthesis of compound **5b** by reacting

compound **4b** (4.8 g, 17 mmol) with propargyl bromide (3.06 g, 20 mmol) using NaH (0.82 g, 20 mmol) in dry THF (50 mL). Yield 4.25 g (78%); Yellow syrup; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.22-7.44 (m, 7H, ArH), 6.90 (d, 2H, ArH, J = 8.8 Hz), 6.59 (s, 1H, pyrazole-CH), 4.57 (s, 2H, CH_2), 4.24 (d, 2H, CH_2, J = 1.6 Hz), 3.73 (s, 3H, OCH_3), 3.50 (s, 1H, CH); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 148.92, 143.83, 136.77, 131.68, 130.44, 130.39, 129.25, 129.19, 129.14, 128.17, 124.94, 108.17, 80.64, 77.90, 64.92, 57.38, 55.8; ESI-MS (*m/z*) 319.1 $(M+H)^+$; Anal. calculated for $C_{20}H_{18}N_2O_2$; C, 75.45; H, 5.70; N, 8.80. Found: C, 75.49; H, 5.72; N, 8.83.

5-(4-Bromophenyl)-1-phenyl-3-((prop-2-ynyloxy)methyl)-1*H*-pyrazole (5c): The above procedure was followed for the synthesis of compound **5b** by reacting the compound **4b** (6.0 g, 18 mmol) with propargyl bromide (3.25 g, 22 mmol) using NaH (0.88 g, 22 mmol) in dry THF (60 mL). Yield 5.35 g (80%); off white solid; mp 86-87 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.22-7.42 (m, 9H, ArH), 6.69 (s, 1H, pyrazole-CH), 4.57 (s, 2H, CH_2), 4.25 (d, 2H, CH_2, J = 1.6 Hz), 3.51 (s, 1H, CH); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 149.80, 141.65, 139.84, 133.58, 130.64, 130.38, 129.55, 129.20, 129.14, 128.36, 125.72, 108.16, 80.61, 77.91, 64.90, 57.39; ESI-MS (*m/z*) 368.1 $(M+H)^+$; Anal. calculated for $C_{20}H_{16}BrNO$; C, 62.14; H, 4.12; N, 7.63. Found: C, 62.20; H, 4.14; N, 7.66.

General procedure for the synthesis of final compounds (P1-P24).

A mixture of appropriate scaffold (**5a-c**) (0.1 g), bromo compound (1.1 eq), sodium azide (NaN_3) (1.1 eq), copper sulfate pentahydrate (0.2 eq) and sodium ascorbate (0.3 eq) was taken in 2:1 mixture of PEG-400 and water (3 mL). The mixture was stirred at 50 °C for 12 h. Completion of the reaction was confirmed by TLC. The reaction mixture was then filtered through celite, the residue was washed with 25 mL of ethyl acetate. The filtrate was extracted with ethyl acetate (3 × 25 mL). The organic layer was separated and washed twice with ammonia solution, then with water followed with brine solution and dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure and the crude product was purified by flash column chromatography (230-400 size mesh) using 60 % ethyl acetate in pet ether to afford the pure final products **P1-P24 (Table 2.2)**.

Table 2.2 Substitution pattern of final compounds **P1-P24**.

Compound	R^1	R^2	LogP/CLogP[a]
P1	-Cl	4-F-C_6H_4-CH_2-	6.52/5.42
P2	-Cl	4-CN-C_6H_4-CH_2-	6.40/4.71
P3	-Cl	4-OCH_3-C_6H_4-CH_2-	6.24/5.19
P4	-Cl	4-NO_2-C_6H_4-CH_2-	--/5.02
P5	-Cl	C_6H_5-CH_2-	6.36/5.28
P6	-Cl	2-F-C_6H_4-CH_2-	6.52/5.42
P7	-Cl	Cyclohexyl-	6.61/6.16
P8	-Cl	Cyclopentyl-	6.19/5.60
P9	-OCH_3	4-F-C_6H_4-CH_2-	5.84/4.66
P10	-OCH_3	4-CN-C_6H_4-CH_2-	5.71/3.95
P11	-OCH_3	4-OCH_3-C_6H_4-CH_2-	5.55/4.44
P12	-OCH_3	4-NO_2-C_6H_4-CH_2-	--/4.26
P13	-OCH_3	C_6H_5-CH_2-	5.68/4.52
P14	-OCH_3	2-F-C_6H_4-CH_2-	5.84/4.66
P15	-OCH_3	Cyclohexyl-	5.92/5.40
P16	-OCH_3	Cyclopentyl-	5.51/4.84
P17	-Br	4-F-C_6H_4-CH_2-	6.79/5.57
P18	-Br	4-CN-C_6H_4-CH_2-	6.67/4.86
P19	-Br	4-OCH_3-C_6H_4-CH_2-	6.51/5.35
P20	-Br	4-NO_2-C_6H_4-CH_2-	--/5.17
P21	-Br	C_6H_5-CH_2-	6.64/5.43
P22	-Br	2-F-C_6H_4-CH_2-	6.79/5.57
P23	-Br	Cyclohexyl-	6.88/6.30
P24	-Br	Cyclopentyl-	6.46/5.75

[a] Obtained from ChemDraw Ultra software

Spectral data of final derivatives (P1-P24)

1-(4-Fluorobenzyl)-4-(((5-(4-chlorophenyl)-1-phenyl-1*H*-pyrazol-3-yl)methoxy) methyl)-1*H*-1,2,3-triazole (P1): Yield 0.12 g (84%); off white solid; mp 114-115 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.22 (s, 1H, triazole-CH), 7.38-7.44 (m, 7H,

ArH), 7.18-7.29 (m, 6H, ArH), 6.69 (s, 1H, pyrazole-CH), 5.59 (s, 2H, CH_2), 4.64 (s, 2H, CH_2), 4.57 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 163.55, 161.12, 150.37, 144.66, 142.65, 139.89, 133.65, 132.83, 132.80, 130.76, 130.67, 130.64, 129.65, 129.26, 129.14, 128.33, 125.70, 124.58, 116.17, 115.95, 108.18, 65.62, 63.48, 52.45; ESI-MS (*m/z*) 474.3 $(M+H)^+$; Anal. calculated for $C_{26}H_{21}ClFN_5O$; C, 65.89; H, 4.47; N, 14.78. Found: C, 65.86; H, 4.45; N, 14.81.

4-((4-(((5-(4-Chlorophenyl)-1-phenyl-1*H*-pyrazol-3-yl)methoxy)methyl)-1*H*-1,2,3-triazol-1-yl)methyl)benzonitrile (P2): Yield 0.13 g (86%); off white solid; mp 80-81 °C; ^{1}H NMR (400MHz, DMSO-d_6) δ ppm 8.28 (s, 1H, triazole-CH), 7.85 (d, 2H, ArH, *J* = 8.4 Hz), 7.36-7.46 (m, 7H, ArH), 7.22-7.27 (m, 4H, ArH), 6.69 (s, 1H, pyrazole-CH), 5.73 (s, 2H, CH_2), 4.66 (s, 2H, CH_2), 4.58 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 150.36, 144.78, 142.65, 142.02, 139.89, 133.66, 130.64, 129.65, 129.25, 129.14, 129.11, 128.34, 125.71, 125.06, 119.00, 111.38, 108.19, 65.63, 63.45, 52.62; ESI-MS (*m/z*) 481.3 $(M+H)^+$; Anal. calculated for $C_{27}H_{21}ClN_6O$; C, 67.43; H, 4.40; N, 17.47. Found: C, 67.39; H, 4.41; N, 17.50.

1-(4-Methoxybenzyl)-4-(((5-(4-chlorophenyl)-1-phenyl-1*H*-pyrazol-3-yl)methoxy)methyl)-1*H*-1,2,3-triazole (P3): Yield 0.12 g (81%); Pale yellow solid; mp 92-93 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.16 (s, 1H, triazole-CH), 7.38-7.22 (m, 11H, ArH), 6.91 (d, 2H, ArH, *J* = 8.8 Hz), 6.69 (s, 1H, pyrazole-CH), 5.51 (s, 2H, CH_2), 4.63 (s, 2H, CH_2), 4.56 (s, 2H, CH_2), 3.72 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 159.59, 150.39, 144.58, 142.64, 139.90, 139.79, 133.65, 130.63, 130.04, 129.92, 129.64, 129.56, 129.26, 129.14, 128.47, 128.32, 125.70, 124.32, 114.58, 114.48, 108.18, 65.61, 63.51, 55.58, 52.81; ESI-MS (*m/z*) 486.3 $(M+H)^+$; Anal. calculated for $C_{27}H_{24}ClN_2O_2$; C, 66.73; H, 4.98; N, 14.41. Found: C, 66.67; H, 5.00; N, 14.43.

1-(4-Nitrobenzyl)-4-(((5-(4-chlorophenyl)-1-phenyl-1*H*-pyrazol-3-yl)methoxy)methyl)-1*H*-1,2,3-triazole (P4): Yield 0.12 g (78%); Pale yellow solid; mp 112-113 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.29 (s, 1H, triazole-CH), 8.23 (d, 2H, ArH, *J* = 8.8 Hz), 7.52 (d, 2H, ArH, *J* = 8.0 Hz), 7.38-7.44 (m, 5H, ArH), 7.22-7.27 (m, 4H, ArH), 6.69 (s, 1H, pyrazole-CH), 5.79 (s, 2H, CH_2), 4.66 (s, 2H, CH_2), 4.57

(s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 150.36, 147.69, 144.81, 143.97, 142.65, 139.88, 133.66, 130.66, 129.65, 129.45, 129.14, 128.34, 125.70, 125.11, 124.39, 108.19, 65.64, 63.46, 52.35; ESI-MS (*m/z*) 501.3 $(M+H)^+$; Anal. calculated for $C_{26}H_{21}ClN_6O_3$; C, 62.34; H, 4.23; N, 16.78. Found: C, 62.39; H, 4.26; N, 16.81.

1-Benzyl-4-(((5-(4-chlorophenyl)-1-phenyl-1*H*-pyrazol-3-yl)methoxy)methyl)-1*H*-1,2,3-triazole (P5): Yield 0.11 g (79%); Pale yellow syrup; ^1H NMR (400 MHz, DMSO-d_6) δ ppm 8.20 (s, 1H, triazole-CH), 6.89-7.38 (m, 14H, ArH,), 6.69 (s, 1H, pyrazole-CH), 5.63 (s, 2H, CH_2), 4.66 (s, 2H, CH_2), 4.57 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 161.12, 144.77, 144.64, 143.45 141.5, 139.89, 132.95, 132.83, 132.79, 130.76, 130.64, 130.61, 129.65, 129.26, 129.21, 128.33, 125.69, 124.57, 116.11, 115.90, 108.16, 65.62, 63.50, 52.42; ESI-MS (*m/z*) 456.4 $(M+H)^+$; Anal. calculated for $C_{26}H_{22}ClN_5O$; C, 68.49; H, 4.86; N, 15.36. Found: C, 68.55; H, 4.87; N, 15.40.

1-(2-Fluorobenzyl)-4-(((5-(4-chlorophenyl)-1-phenyl-1*H*-pyrazol-3-yl)methoxy) methyl)-1*H*-1,2,3-triazole (P6): Yield 0.12 g (82%); off white solid; mp 83-84 °C; ^1H NMR (400 MHz, DMSO-d_6) δ ppm 8.22 (s, 1H, triazole-CH), 7.18-7.40 (m, 13H, ArH), 6.69 (s, 1H, pyrazole-CH), 5.60 (s, 2H, CH_2), 4.64 (s, 2H, CH_2), 4.58 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 162.58, 162.12, 150.40, 143.66, 142.95, 139.91, 133.55, 132.93, 132.78, 130.66, 130.62, 130.60, 129.88, 129.64, 129.24, 128.25, 125.70, 124.68, 116.16, 115.90, 108.17, 65.63, 63.49, 52.44; ESI-MS (*m/z*) 474.3 $(M+H)^+$; Anal. calculated for $C_{26}H_{21}ClFN_5O$; C, 65.89; H, 4.47; N, 14.78. Found: C, 65.93; H, 4.48; N, 14.80.

4-(((5-(4-Chlorophenyl)-1-phenyl-1*H*-pyrazol-3-yl)methoxy)methyl)-1-cyclohexyl -1*H*-1,2,3-triazole (P7): Yield 0.97 g (68%); Pale yellow syrup; ^1H NMR (400 MHz, DMSO-d_6) δ ppm 8.20 (s, 1H, triazole-CH), 7.23-7.44 (m, 9H, ArH), 6.70 (s, 1H, pyrazole-CH), 4.63 (s, 2H, CH_2), 4.58 (s, 2H, CH_2); 3.47 (t, 1H, CH, J = 11.2 Hz), 2.04 (d, 2H, CH_2, J = 11.2 Hz), 1.69-1.83 (m, 8H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 150.51, 144.01, 142.88, 139.94, 132.17, 130.88, 129.67, 129.60, 128.36, 125.21, 122.76, 122.32, 108.11, 65.66, 63.66, 59.42, 33.32, 25.17, 25.11; ESI-

MS (*m/z*) 447.1 $(M+H)^+$; ESI-MS (*m/z*) 448.1 $(M+H)^+$; Anal. calculated for $C_{25}H_{26}ClN_5O$; C, 67.03; H, 5.85; N, 15.63. Found: C, 67.09; H, 5.87; N, 15.65.

4-(((5-(4-Chlorophenyl)-1-phenyl-1*H*-pyrazol-3-yl)methoxy)methyl)-1-cyclo pentyl-1*H*-1,2,3-triazole (P8): Yield 0.11 g (76%); Pale yellow syrup; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.20 (s, 1H, triazole-CH), 7.17-7.55 (m, 9H, ArH), 6.70 (s, 1H, pyrazole-CH), 4.63 (s, 2H, CH_2), 4.58 (s, 2H, CH_2), 3.95 (p, 1H, CH, J = 6.8 Hz), 1.68-2.19 (m, 8H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 151.01, 143.98, 142.36, 139.83, 132.16, 130.01, 130.51, 129.98, 128.36, 124.67, 123.98, 122.31, 108.15, 65.69, 63.58, 59.40, 33.33, 25.17, 25.08; ESI-MS (*m/z*) 434.1 $(M+H)^+$; Anal. calculated for $C_{24}H_{24}ClN_5O$; C, 66.43; H, 5.57; N, 16.14. Found: C, 66.39; H, 5.59; N, 16.17.

1-(4-Fluorobenzyl)-4-(((5-(4-methoxyphenyl)-1-phenyl-1*H*-pyrazol-3-yl)methoxy) methyl)-1*H*-1,2,3-triazole (P9): Yield 0.12 g (80%); Off white solid; mp 96-97 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.22 (s, 1H, triazole-CH), 7.35-7.42 (m, 5H, ArH), 7.18-7.35 (m, 6H, ArH), 6.90 (d, 2H, ArH, J = 8.8 Hz), 6.56 (s, 1H, pyrazole-CH), 5.70 (s, 2H, CH_2), 4.63 (s, 2H, CH_2), 4.54 (s, 2H, CH_2), 3.74 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm; 163.55, 161.12, 159.68, 150.14, 144.70, 143.75, 140.25, 132.82, 132.79, 130.76, 130.67, 130.23, 129.50, 128.04, 125.62, 125.52, 124.57, 122.70, 116.16, 115.95, 114.51, 107.36, 65.73, 63.45, 55.62, 52.46; ESI-MS (*m/z*) 470.3 $(M+H)^+$; Anal. calculated for $C_{27}H_{24}FN_5O_2$; C, 69.07; H, 5.15; N, 14.92. Found: C, 69.02; H, 5.17; N, 14.94.

4-((4-(((5-(4-Methoxyphenyl)-1-phenyl-1*H*-pyrazol-3-yl)methoxy)methyl)-1*H*-1,2,3-triazol-1-yl)methyl)benzo nitrile (P10): Yield 0.13 g (85%); Off white solid; mp 70-71 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.27 (s, 1H, triazole-CH), 7.84 (d, 2H, ArH, J = 7.6 Hz), 7.37-7.46 (m, 5H, ArH), 7.24 (d, 2H, ArH, J = 7.6 Hz), 7.14 (d, 2H, ArH, J = 8.4 Hz), 6.90 (d, 2H, ArH, J = 8.8 Hz), 6.57 (s, 1H, pyrazole-CH), 5.73 (s, 2H, CH_2), 4.64 (s, 2H, CH_2), 4.55 (s, 2H, CH_2); 3.74 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 159.68, 150.13, 144.83, 143.75, 142.03, 140.25, 133.20, 130.23, 129.51, 129.11, 128.04, 125.63, 125.05, 122.70, 119.00, 114.51, 111.37,

107.38, 65.74, 63.43, 55.63, 52.63; ESI-MS (*m/z*) 477.3 $(M+H)^+$; Anal. calculated for $C_{28}H_{24}N_6O_2$; C, 70.57; H, 5.08; N, 17.64. Found: C, 70.51; H, 5.10; N, 17.68.

1-(4-Methoxybenzyl)-4-(((5-(4-methoxyphenyl)-1-phenyl-1*H*-pyrazol-3-yl) methoxy)methyl)-1*H*-1,2,3-triazole (P11): Yield 0.12 g (81%); Yellow syrup; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.16 (s, 1H, triazole-CH), 6.90-7.43 (m, 13H, ArH,), 6.57 (s, 1H, pyrazole-CH), 5.51 (s, 2H, CH_2), 4.62 (s, 2H, CH_2), 4.54 (s, 2H, CH_2); 3.75 (s, 3H, OCH_3), 3.73 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 159.67, 159.58, 150.15, 144.61, 143.74, 140.24, 130.23, 130.04, 129.50, 128.47, 128.04, 125.63, 124.31, 122.70, 114.58, 114.50, 107.36, 65.70, 63.46, 55.62, 55.59, 52.80; ESI-MS (*m/z*) 482.1 $(M+H)^+$; Anal. calculated for $C_{28}H_{27}N_5O_3$; C, 79.84; H, 5.65; N, 14.54. Found: C, 79.86; H, 5.66; N, 14.56.

1-(4-Nitrobenzyl)-4-(((5-(4-methoxyphenyl)-1-phenyl-1*H*-pyrazol-3-yl)methoxy) methyl)-1*H*-1,2,3-triazole (P12): Yield 0.12 g (80%); Pale yellow solid; mp 109-110 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.29 (s, 1H, triazole-CH), 8.23(d, 2H, ArH, *J* = 8.8 Hz), 7.53(d, 2H, ArH, *J* = 8.0 Hz), 7.35-7.42 (m, 5H, ArH,), 7.24 (d, 2H, ArH, *J* = 6.9 Hz), 6.90 (d, 2H, ArH, *J* = 8.8 Hz), 6.57 (s, 1H, pyrazole-CH), 5.79 (s, 2H, CH_2), 4.65 (s, 2H, CH_2), 4.56 (s, 2H, CH_2); 3.74 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 159.68, 150.13, 147.69, 144.85, 143.98, 143.75, 140.25, 130.22, 129.50, 129.45, 128.04, 125.62, 125.09, 124.39, 122.69, 114.51, 107.38, 65.75, 63.44, 55.62, 52.35; ESI-MS (*m/z*) 497.3 $(M+H)^+$; Anal. calculated for $C_{27}H_{24}N_6O_4$; C, 65.31; H, 4.87; N, 16.93. Found: C, 65.37; H, 4.89; N, 16.90.

1-Benzyl-4-(((5-(4-methoxyphenyl)-1-phenyl-1*H*-pyrazol-3-yl)methoxy)methyl)-1*H*-1,2,3-triazole (P13): Yield 0.10 g (73%); Pale yellow solid; mp 85-86 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.20 (s, 1H, triazole-CH), 7.17-7.42 (m, 12H, ArH), 6.90 (d, 2H, ArH, *J* = 8.8 Hz), 6.56 (s, 1H, pyrazole-CH), 5.70 (s, 2H, CH_2), 4.62 (s, 2H, CH_2), 4.52 (s, 2H, CH_2), 3.73 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 159.67, 150.18, 144.95, 144.61, 143.71, 141.26, 130.25, 130.14, 129.78, 128.77, 128.34, 125.53, 124.32, 122.72, 114.58, 114.53, 107.35, 65.69, 63.46, 55.64, 52.81; ESI-MS (*m/z*) 452.3 $(M+H)^+$; Anal. calculated for $C_{27}H_{25}N_5O_2$; C, 71.82; H, 5.58; N, 15.51. Found: C, 71.80; H, 5.50; N, 15.53.

1-(2-Fluorobenzyl)-4-(((5-(4-methoxyphenyl)-1-phenyl-1*H*-pyrazol-3-yl)methoxy) methyl)-1*H*-1,2,3-triazole (P14): Yield 0.11 g (75%); Off white solid; mp 78-79 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.20 (s, 1H, triazole-CH), 6.69-7.42 (m, 13H, ArH), 6.57 (s, 1H, pyrazole-CH), 5.71 (s, 2H, CH_2), 4.62 (s, 2H, CH_2), 4.53 (s, 2H, CH_2), 3.73 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 163.58, 161.61, 159.72, 150.09, 144.68, 143.76, 140.25, 132.81, 132.83, 130.73, 130.70, 130.23, 129.48, 128.14, 125.63, 125.54, 124.57, 122.70, 116.19, 115.96, 114.51, 107.36, 65.72, 63.46, 55.63, 52.45; ESI-MS (*m/z*) 470.3 $(M+H)^+$; Anal. calculated for $C_{27}H_{24}FN_5O_2$; C, 69.07; H, 5.15; N, 14.92. Found: C, 69.10; H, 5.18; N, 14.91.

1-Cyclohexyl-4-(((5-(4-methoxyphenyl)-1-phenyl-1*H*-pyrazol-3-yl)methoxy) methyl)-1*H*-1,2,3-triazole (P15): Yield 0.10 g (70%); Pale yellow syrup; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.19 (s, 1H, triazole-CH), 6.98-7.47 (m, 9H, ArH), 6.69 (s, 1H, pyrazole-CH), 4.64 (s, 2H, CH_2), 4.53 (s, 2H, CH_2), 3.73 (s, 3H, OCH_3), 3.47 (t, 1H, CH, J = 11.2 Hz), 2.04 (d, 2H, CH_2, J = 11.2 Hz), 1.69-1.83 (m, 8H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 159.70, 150.92, 141.97, 140.86, 132.47, 131.79, 129.97, 129.83, 128.72, 125.60, 122.94, 122.33, 108.20, 65.69, 63.72, 59.42, 55.88, 33.33, 25.16, 25.09; ESI-MS (*m/z*) 444.1 $(M+H)^+$; Anal. calculated for $C_{26}H_{29}N_5O_2$; C, 70.41; H, 6.59; N, 15.79. Found: C, 70.36; H, 6.57; N, 15.83.

1-Cyclopentyl-4-(((5-(4-methoxyphenyl)-1-phenyl-1*H*-pyrazol-3-yl)methoxy) methyl)-1*H*-1,2,3-triazole (P16): Yield 0.10 g (76%); Pale yellow syrup; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.20 (s, 1H, triazole-CH), 7.14-7.43 (m, 7H, ArH,), 6.91 (d, 2H, ArH, J = 8.0 Hz) 6.58 (s, 1H, pyrazole-CH), 4.63 (s, 2H, CH_2), 4.56 (s, 2H, CH_2); 3.90 (p, 1H, CH, J = 7.2 Hz), 3.74 (s, 3H, OCH_3), 1.67-2.21 (m, 8H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 159.67, 150.21, 144.21, 143.72, 140.25, 130.22, 129.51, 128.04, 125.61, 123.14, 122.71, 114.51, 107.38, 65.73, 63.59, 61.38, 55.63, 33.30, 24.07; ESI-MS (*m/z*) 430.1 $(M+H)^+$; Anal. calculated for $C_{25}H_{27}N_5O_2$; C, 69.91; H, 6.34; N, 16.31. Found: C, 70.00; H, 6.36; N, 16.33.

1-(4-Fluorobenzyl)-4-(((5-(4-bromophenyl)-1-phenyl-1*H*-pyrazol-3-yl)methoxy) methyl)-1*H*-1,2,3-triazole (P17): Yield 0.11 g (80%); Off white solid; mp 94-95 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.20 (s, 1H, triazole-CH), 7.18-7.44 (m, 13H,

ArH), 6.70 (s, 1H, pyrazole-CH), 5.59 (s, 2H, CH_2), 4.63 (s, 2H, CH_2), 4.57 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 163.54, 161.10, 149.57, 144.68, 142.73, 141.99, 133.73, 132.91, 132.78, 130.78, 130.71, 130.54, 129.63, 129.11, 129.20, 128.30, 124.99, 124.62, 116.19, 116.06, 107.02, 65.63, 63.47, 52.45; ESI-MS (*m/z*) 519.1 $(M+H)^+$; Anal. calculated for $C_{26}H_{21}BrFN_5O$; C, 60.24; H, 4.08; N, 13.51. Found: C, 60.21; H, 4.10; N, 13.53.

4-((4-(((5-(4-Bromophenyl)-1-phenyl-1*H*-pyrazol-3-yl)methoxy)methyl)-1*H*-1,2,3-triazol-1yl)methyl)benzonitrile (P18): Yield 0.13g (88%); Yellow syrup; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.27 (s, 1H, triazole-CH), 7.83 (d, 2H, ArH, J = 8.4 Hz), 7.38-7.44 (m, 7H, ArH), 7.21-7.25 (m, 4H, ArH), 6.69 (s, 1H, pyrazole-CH), 5.73 (s, 2H, CH_2), 4.65 (s, 2H, CH_2), 4.58 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 150.37, 144.77, 142.58, 141.89, 139.96, 137.88, 130.59, 129.65, 129.45, 129.04, 129.13, 128.31, 127.32, 125.03, 118.99, 111.37, 108.17, 65.64, 63.46, 52.64; ESI-MS (*m/z*) 526.1 $(M+H)^+$; Anal. calculated for $C_{27}H_{21}BrN_6O$; C, 61.72; H, 4.03; N, 16.00. Found: C, 61.72; H, 4.01; N, 16.03.

1-(4-Methoxybenzyl)-4-(((5-(4-bromophenyl)-1-phenyl-1*H*-pyrazol-3-yl)methoxy) methyl)-1*H*-1,2,3-triazole (P19): Yield 0.13 g (87%); Off white solid; mp 58-59 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.18 (s, 1H, triazole-CH), 6.92-7.38 (m, 13H, ArH), 6.69 (s, 1H, pyrazole-CH), 5.50 (s, 2H, CH_2), 4.63 (s, 2H, CH_2), 4.57 (s, 2H, CH_2), 3.73 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 159.57, 150.38, 144.88, 142.64, 140.02, 139.89, 133.63, 130.62, 130.14, 129.96, 129.64, 129.55, 129.24, 129.14, 128.57, 128.30, 125.69, 124.30, 114.55, 114.38, 108.22, 65.60, 63.52, 55.57, 52.80; ESI-MS (*m/z*) 531.1 $(M+H)^+$; Anal. calculated for $C_{27}H_{24}BrN_5O2$; C, 61.44; H, 4.56; N, 13.20. Found: C, 61.49; H, 4.58; N, 13.23.

1-(4-Nitrobenzyl)-4-(((5-(4-bromophenyl)-1-phenyl-1*H*-pyrazol-3-yl)methoxy) methyl)-1*H*-1,2,3-triazole (P20): Yield 0.12 g (79%); Off white solid; mp 102-103 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.29 (s, 1H, triazole-CH), 8.23 (d, 2H, ArH, J = 8.8 Hz), 7.52 (d, 2H, ArH, J = 8.0 Hz), 7.38-7.44 (m, 5H, ArH), 7.22-7.27 (m, 4H, ArH), 6.69 (s, 1H, pyrazole-CH), 5.79 (s, 2H, CH_2), 4.66 (s, 2H, CH_2), 4.57 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 150.33, 147.30, 143.98, 143.90,

142.85, 140.86, 133.88, 130.76, 128.85, 129.65, 129.12, 128.44, 125.75, 125.19, 124.45, 108.19, 65.62, 63.45, 52.34; ESI-MS (*m/z*) 546.1 $(M+H)^+$; Anal. calculated for $C_{26}H_{21}BrN_6O_3$; C, 57.26; H, 3.88; N, 15.41. Found: C, 57.21; H, 3.90; N, 14.37.

1-Benzyl-4-(((5-(4-bromophenyl)-1-phenyl-1*H*-pyrazol-3-yl)methoxy)methyl)-1*H*-1,2,3-triazole (P21): Yield .09 g (69%); Off white solid; mp 91-92 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.20 (s, 1H, triazole-CH), 7.18-7.44 (m, 12H, ArH), 6.89 (d, 2H, ArH, J = 8.8 Hz), 6.57 (s, 1H, pyrazole-CH), 5.69 (s, 2H, CH_2), 4.62 (s, 1H, CH_2), 4.52 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 161.14, 144.73, 144.61, 143.39, 141.51, 140.39, 132.64, 132.22, 132.09, 130.89, 130.74, 130.52, 129.53, 129.26, 129.20, 128.30, 125.71, 124.54, 116.14, 115.91, 108.20, 65.63, 63.48, 52.41; ESI-MS (*m/z*) 501.1 $(M+H)^+$; Anal. calculated for $C_{27}H_{21}BrN_6O$; C, 62.41; H, 4.43; N, 14.00. Found: C, 62.42; H, 4.43; N, 14.03.

1-(2-Fluorobenzyl)-4-(((5-(4-bromophenyl)-1-phenyl-1*H*-pyrazol-3-yl)methoxy)methyl)-1*H*-1,2,3-triazole (P22): Yield 0.11 g (78%); Pale yellow solid; mp 95-96 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.20 (s, 1H, triazole-CH), 7.16-7.56 (m, 13H, ArH), 6.69 (s, 1H, pyrazole-CH), 5.67 (s, 2H, CH_2), 4.64 (s, 2H, CH_2), 4.57 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 161.88, 160.90, 150.44, 143.59, 142.84, 140.01, 133.56, 132.92, 132.79, 130.68, 130.64, 130.61, 129.77, 129.69, 129.36, 128.29, 125.79, 124.67, 116.15, 115.94, 108.18, 65.62, 63.52, 52.43; ESI-MS (*m/z*) 519.1 $(M+H)^+$; Anal. calculated for $C_{26}H_{21}BrFN_5O$; C, 60.24; H, 4.08; N, 13.51. Found: C, 60.29; H, 4.11; N, 13.53.

4-(((5-(4-Bromophenyl)-1-phenyl-1*H*-pyrazol-3-yl)methoxy)methyl)-1-cyclohexyl-1*H*-1,2,3-triazole (P23): Yield 0.09 g (67%); Yellow syrup; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.19 (s, 1H, triazole-CH), 7.16-7.57 (m, 9H, ArH), 6.70 (s, 1H, pyrazole-CH), 4.62 (s, 2H, CH_2), 4.57 (s, 2H, CH_2), 3.45 (m, 1H, CH), 2.04 (d, 2H, CH_2, J = 12 Hz), 1.23-1.82 (m, 8H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 150.47, 143.95, 142.67, 139.90, 132.07, 130.88, 129.67, 129.63, 128.35, 125.70, 122.51, 122.31, 108.18, 65.65, 63.68, 59.43, 33.32, 25.15, 25.08; ESI-MS (*m/z*) 493.1 $(M+H)^+$; Anal. calculated for $C_{25}H_{26}BrN_5O$; C, 60.98; H, 5.32; N, 14.22. Found: C, 60.94; H, 5.32; N, 14.23.

4-(((5-(4-Bromophenyl)-1-phenyl-1*H*-pyrazol-3-yl)methoxy)methyl)-1-cyclo pentyl-1*H*-1,2,3-triazole (P24): Yield 0.09 g (70%); Yellow syrup; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.19 (s, 1H, triazole-CH), 7.16-7.57 (m, 9H, ArH), 6.70 (s, 1H, pyrazole-CH), 4.63 (s, 2H, CH_2), 4.57 (s, 2H, CH_2), 3.96 (p, 1H, CH, J = 6.8Hz), 1.67-2.19 (m, 8H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 150.47, 150.20, 144.17, 142.67, 139.88, 132.06, 130.87, 129.66, 129.61, 129.54, 129.06, 128.86, 128.34, 127.98, 125.69, 123.16, 122.31, 108.18, 107.89, 65.61, 63.61, 63.03, 61.96, 60.28, 33.30, 33.01, 24.53, 24.40, 24.07; ESI-MS (*m/z*) 479.3 $(M+H)^+$; Anal. calculated for $C_{24}H_{24}BrN_5O$; C, 60.26; H, 5.06; N, 14.64. Found: C, 60.24; H, 5.04; N, 14.63.

2.4 PHARMACOLOGY

2.4.1 Antitubercular studies

Two-fold serial dilutions of each test compound/drug were prepared and incorporated into Middlebrook 7H11 agar medium with oleic acid, albumin, dextrose, and catalase (OADC) growth supplement to get final concentrations of 50, 25, 12.5, 6.25, 3.13, 1.56 and 0.78 µg/mL. Inoculum of MTB H37Rv ATCC 27294/XDR-TB was prepared from fresh Middlebrook 7H11 agar slants with OADC (Difco) growth supplement adjusted to 1 mg/mL (wet weight) in Tween 80 (0.05%) saline diluted to 10^{-2} to give a concentration of ~10^7 cfu/mL. Five microliters of this bacterial suspension was spotted onto 7H11 agar tubes containing different concentrations of the drug as discussed above. The tubes were incubated at 37 °C, and final readings (as MIC in µg/mL) were determined after 28 days. This method is similar to that recommended by the NCCLS for the determination of MIC in triplicate.

2.4.2 Cytotoxicity studies

Vero (African green monkey kidney) cell line was procured from National Centre for Cell Sciences (NCCS), Pune, India. Stock cells were cultured in MEM supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 µg/mL) and amphotericin B (5 µg/mL) in an humidified atmosphere of 5% CO_2 at 37 ºC until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 cm^2 culture flasks and all experiments were carried out in

96 microtitre plates (Tarsons India Pvt. Ltd., Kolkata, India). For cytotoxicity studies, each weighed test drugs were separately dissolved in distilled DMSO and volume was made up with MEM supplemented with 2% inactivated FBS to obtain a stock solution of 1 mg/mL concentration and sterilized by filtration. Serial two fold dilutions were prepared from this for carrying out MTT assay (Gundersen et al. 2011). The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 10^5 cells/mL using MEM containing 10% FBS. To each well of the 96 well microtitre plate, 0.1 mL of the diluted cell suspension (approximately 10,000 cells) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100 µL of different test concentrations of test drugs were added on to the partial monolayer in microtitre plates. The plates were then incubated at 37 °C for 3 days in 5% CO_2 atmosphere, and microscopic examination was carried out and observations were noted every 24 h interval. After 72 h, the drug solutions in the wells were discarded and 50 µL of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 h at 37 °C in 5% CO_2 atmosphere. The supernatant was removed and 100 µL of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% (IC_{50}) values is generated from the dose-response curves for each cell line. Selectivity index (SI) = IC_{50} against Vero cells/MIC against *M. tuberculosis*) values were also determined. The compound that exhibited selectivity index (SI) values greater than 10 against Vero cell line could be considered as nontoxic (Desai et al. 2015).

$$\% \text{ Growth Inhibition} = 100 - \left(\frac{\text{Mean OD of individual test group}}{\text{Mean OD of control group}} \text{ X } 100 \right)$$

2.4.3 Molecular docking studies

The crystal structure of InhA (PDB ID: 1P44), CYP121 (PDB ID: 4KTF) and TMPK (PDB ID: 4UNR) from species of MTB was taken from Protein Data Bank was utilized as template for docking explorations. The molecules were sketched using

Maestro panel of Schrodinger 2015-1 and were subjected for all possible conformation generation using LigPrep module. These molecules were docked to the active site of protein using extra precision (XP) mode of Glide.

2.4.4 Antibacterial studies

Newly synthesized organic compounds **P1-P24** were tested against a panel of pathogenic microorganisms which were maintained on nutrient agar medium at 37 °C. The cultures were inoculated into 10 mL fresh nutrient broth to yield an initial suspension of approximately 10–100 cfu/mL. All broths were incubated statically at the aforementioned temperature for 24 h. Susceptibility of the test organism to the organic compound was determined by well plate technique (Arthington-Skaggs et al. 2000; Rocha et al. 1995). The bacterial suspensions were serially diluted in saline and 0.1 mL from the appropriate dilution was spread on nutrient agar. The wells were dug using a sterile cork borer and the organic compounds dissolved in DMSO were added (1.0 and 0.5 mg/mL). Same procedure was repeated for all microorganisms and the petri plates were incubated for 18 h at 37 ^{o}C. After the incubation, the inhibition zone was measured and the values for DMSO solvent were subtracted to get the actual values.

2.5 RESULTS AND DISCUSSION

2.5.1 Chemistry

The structures of all the synthesized compounds were confirmed by ^{1}H NMR, ^{13}C NMR and mass spectral analysis as well as by elemental analysis. Cyclization of sodium (*E*)-1-(4-substituted)-4-ethoxy-3,4-dioxobut-1-en-1-olate (**2a-c**) to ethyl 5-(aryl)-1-phenyl-1*H*-pyrazole-3-carboxylate (**3a-c**) was evident by the presence of a singlet at δ 7.17 ppm due to the pyrazole ring proton, in the ^{1}H NMR spectrum (**Figure 2.2**). Also, the spectrum displayed additional five aromatic protons confirming the presence of N-phenyl ring. The reduction of the ester group in **3a-c** to give (5-(4-substituted)-1-phenyl-1*H*-pyrazol-3-yl)methanol (**4a-c**) was confirmed by the appearance of a signal corresponding to the -OH group at δ 5.25 ppm in the ^{1}H NMR spectrum (**Figure 2.3**). The structure of the compound was also supported by its ^{13}C NMR spectrum (**Figure 2.4**)**.** The absence of ethyl carboxylate peaks in the spectrum further confirms the reduction of the ester group. The alkylation of **4a-c**

using propargyl bromide to yield scaffolds **5a-c** was evidenced by the disappearance of -OH signal in the ^{1}H NMR spectrum of **5a-c**. Further, appearance of two new peaks at around δ 4.58 ppm for -OCH_2 and δ 4.25 ppm for alkyne -CH protons clearly confirmed the formation of the required products (**Figure 2.5**). The structure of the compound was also supported by its ^{13}C NMR spectrum (**Figure 2.6**)**.** The formation of triazole ring in **P1-P24** was supported by the characteristic 1,2,3-triazole ring (-CH) signal in the range δ 8.16 - 8.29 ppm in the ^{1}H NMR spectra. For instance, in compound **P1**, the triazole ring (-CH) signal appears as a sharp singlet at δ 8.22 ppm, all other aromatic -CH signals are in the range δ 7.18 - 7.44 (**Figure 2.7, Figure 2.9**). The sharp singlet at δ 6.69 ppm is due to pyrazole ring -CH and the benzyl -CH_2 appears at δ 5.59 ppm. The -CH_2 signals of the methylenedioxy linkage appear at δ 4.64 and 4.57 ppm, the higher chemical shift value is for -CH_2 group attached to the pyrazole ring (**Figure 2.8**). ESI-MS of **P1** showed a molecular ion peak at *m/z* 474.3, which tallies with its molecular formula $C_{26}H_{21}ClFN_5O$ (**Figure 2.11**). Further ^{13}C NMR spectrum and elemental analysis results clearly confirms the formation of **P1** (**Figure 2.10**)**.** The structural parameters, yield and melting point data of the target compounds (**P1-P24**) are presented in **Table 2.2.**

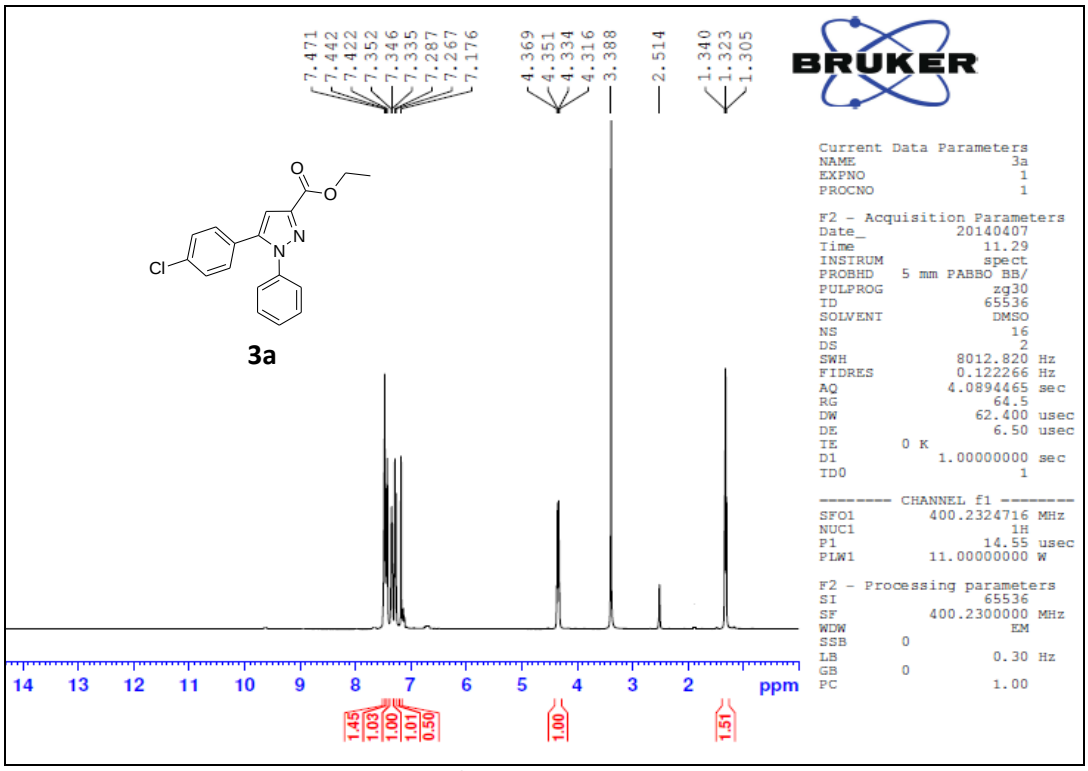


Figure 2.2 ^{1}H NMR spectrum of **3a.**

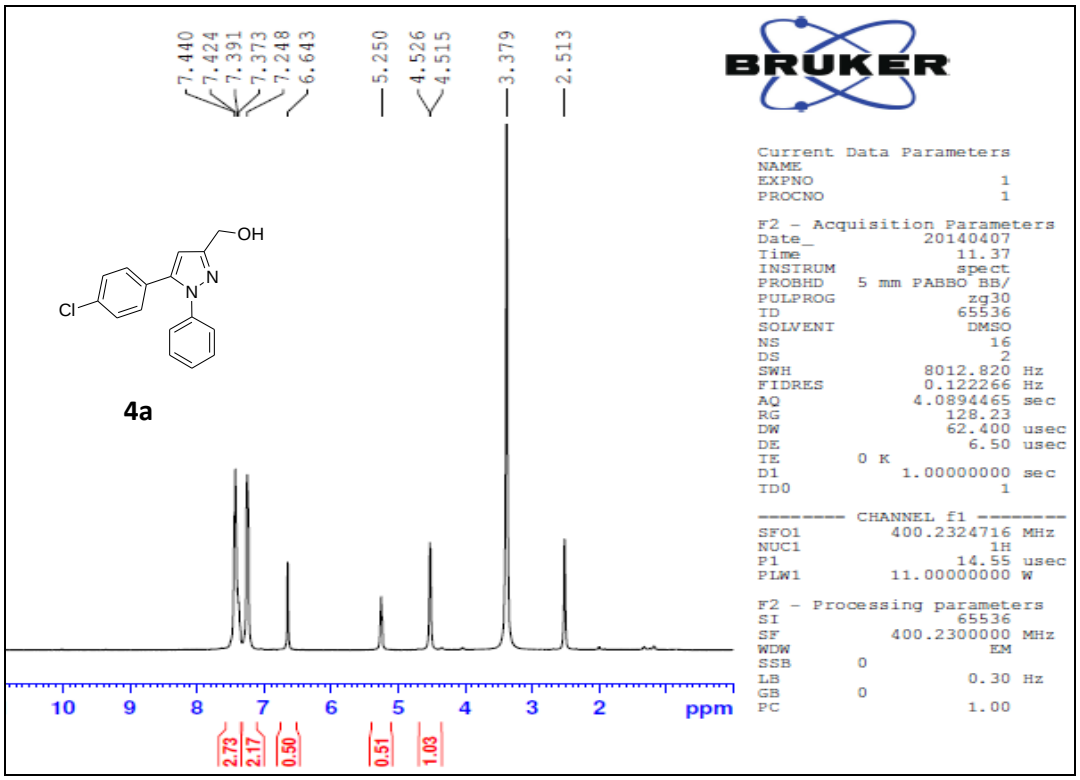


Figure 2.3 ^{1}H NMR spectrum of **4a.**

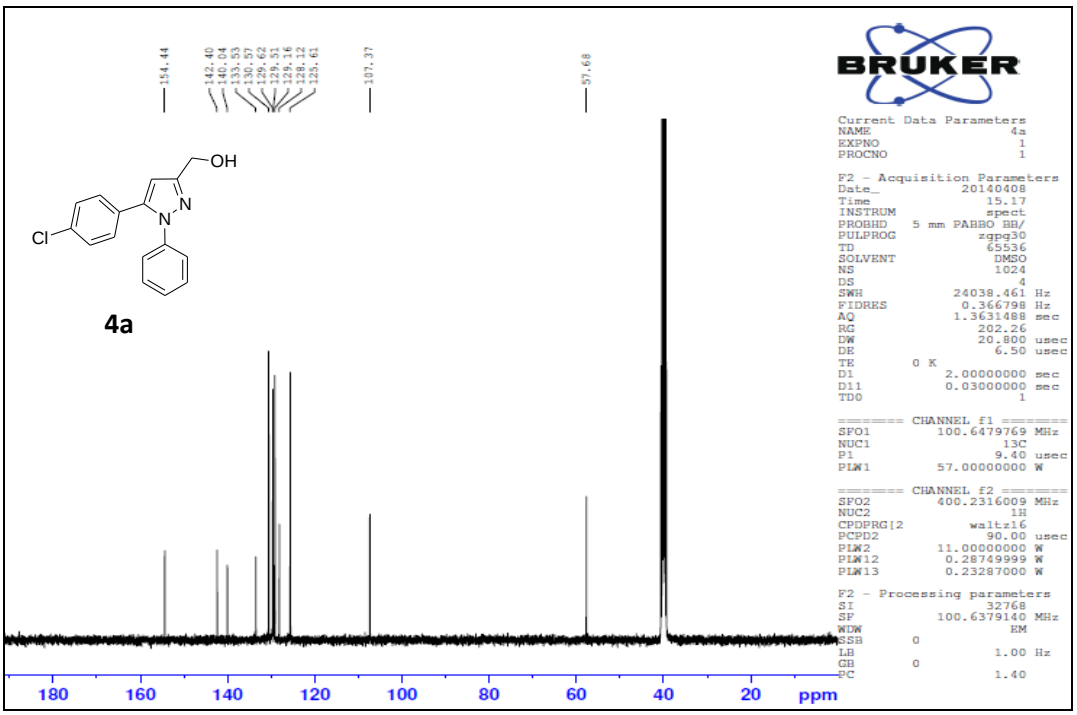


Figure 2.4 ^{13}C NMR spectrum of **4a.**

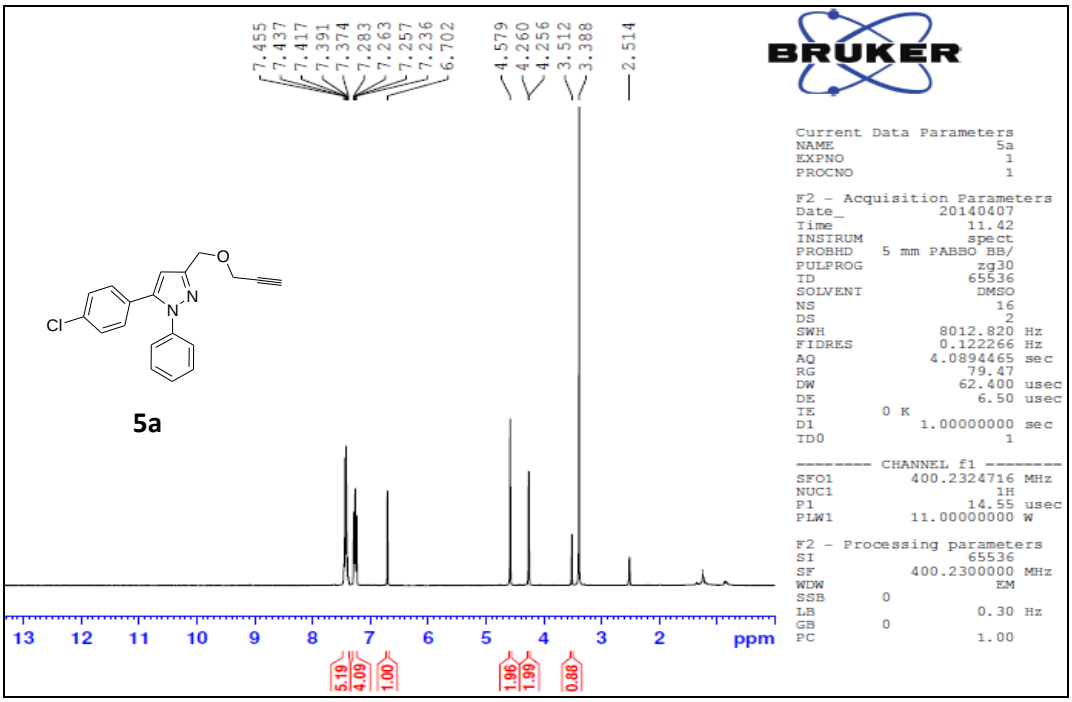


Figure 2.5 ^{1}H NMR spectrum of **5a.**

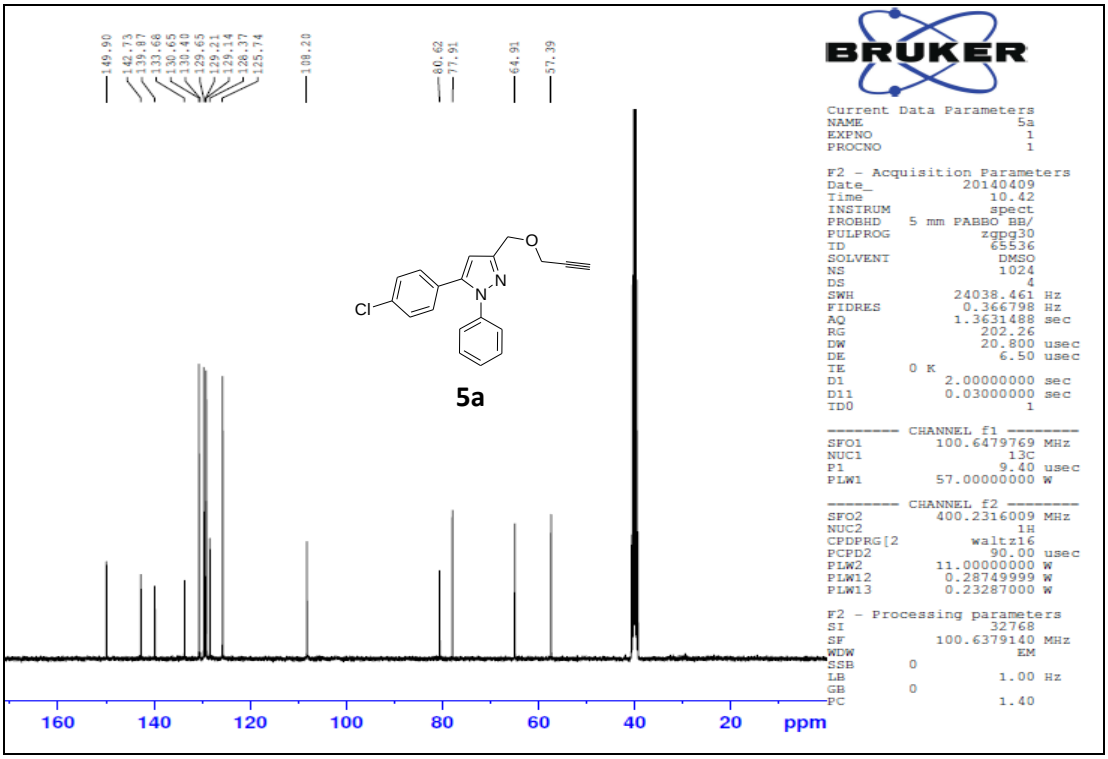


Figure 2.6 ^{13}C NMR spectrum of **5a**.

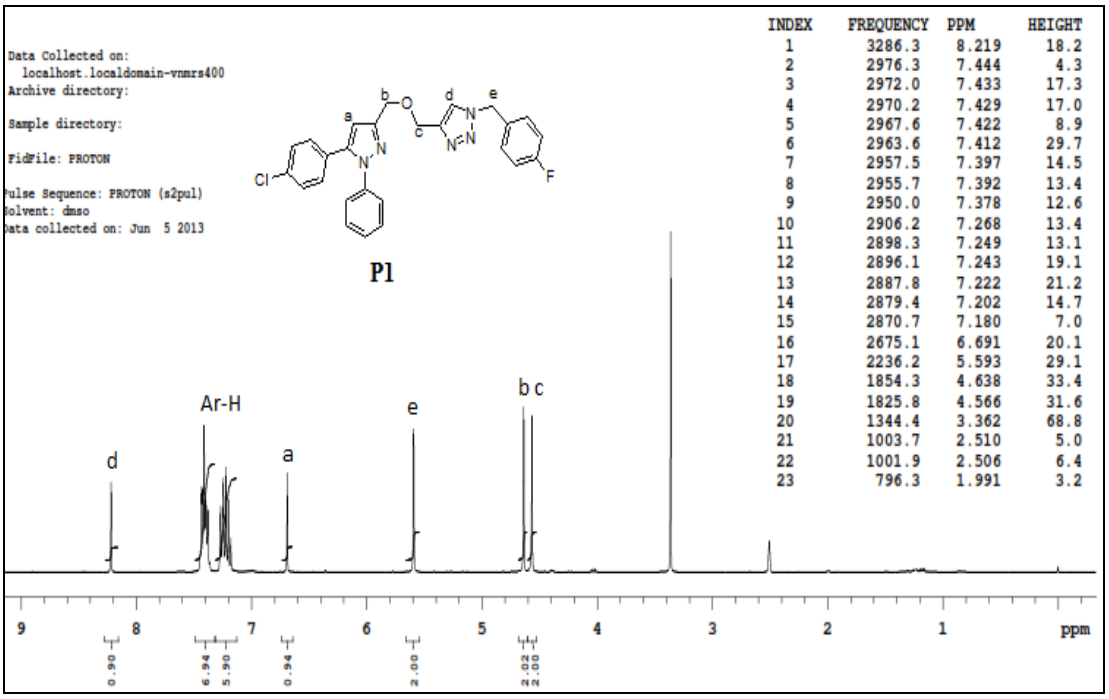


Figure 2.7 ^{1}H NMR spectrum of **P1**.

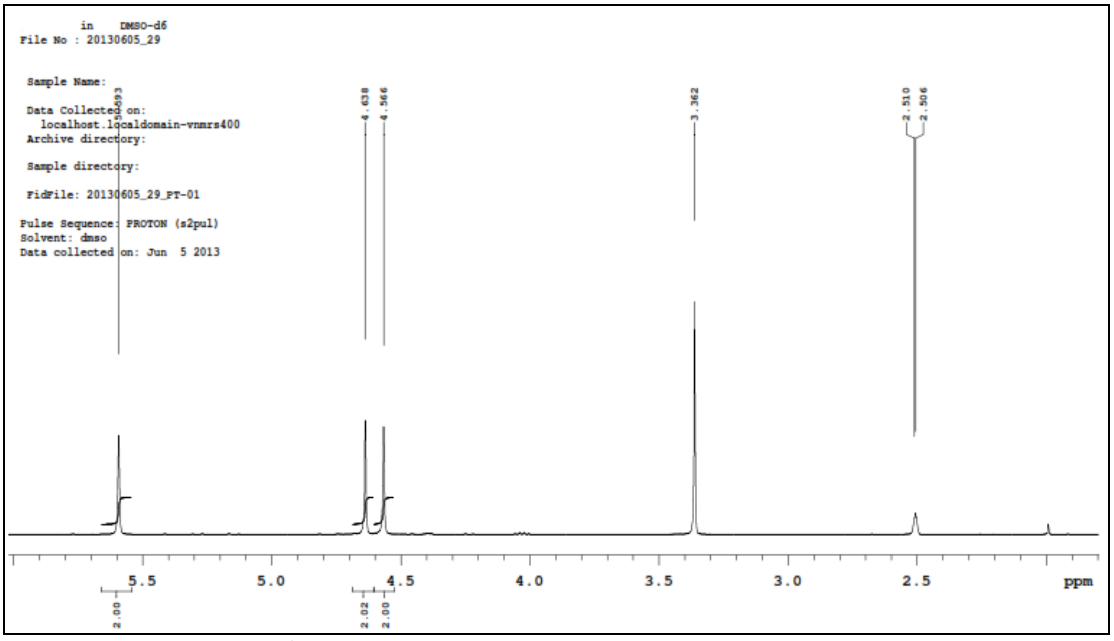


Figure 2.8 ^{1}H NMR spectrum of **P1** (expanded aliphatic region).

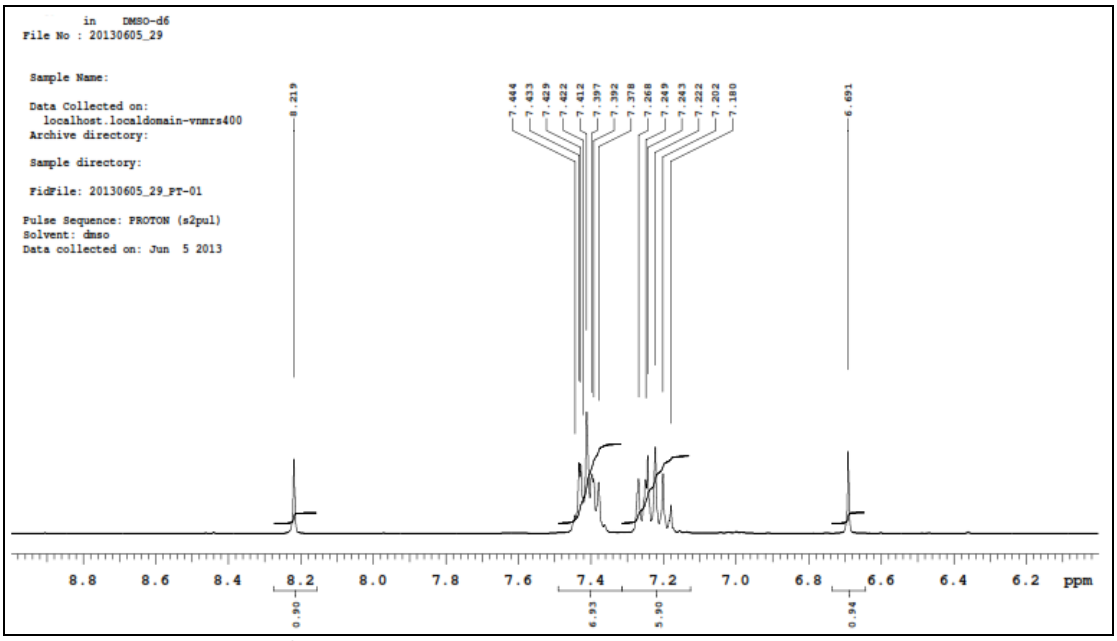


Figure 2.9 ^{1}H NMR spectrum of **P1** (expanded aromatic region).

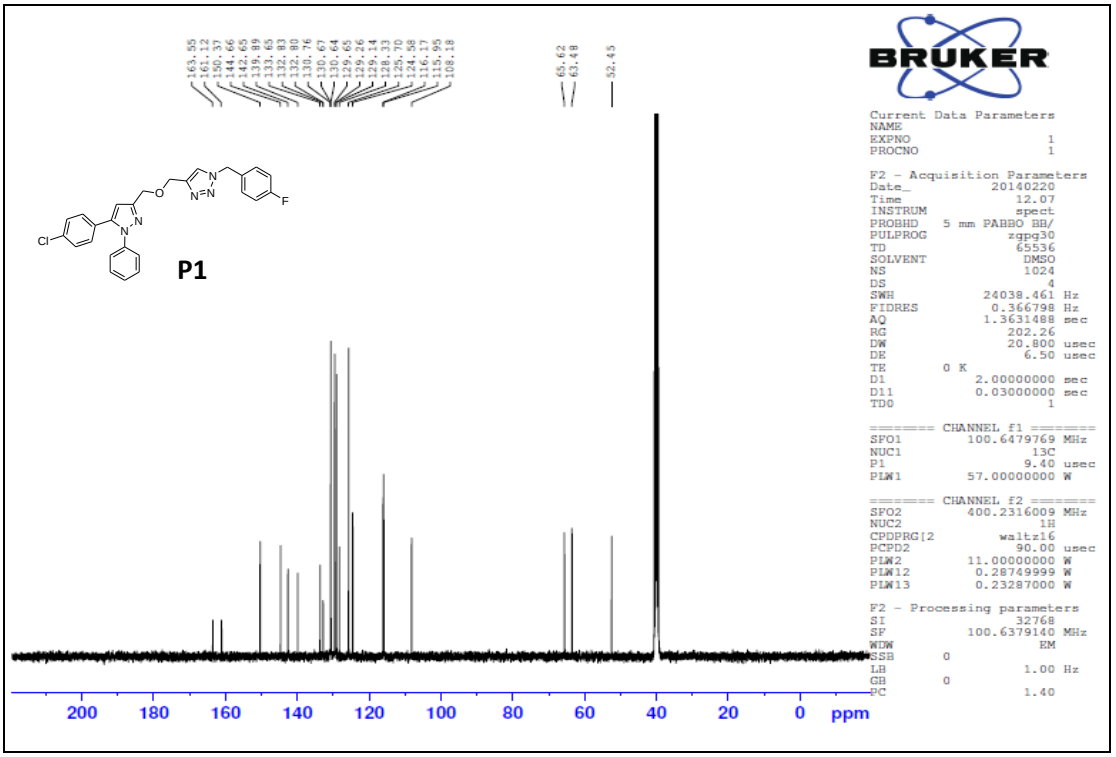


Figure 2.10 ^{13}C NMR spectrum of **P1.**

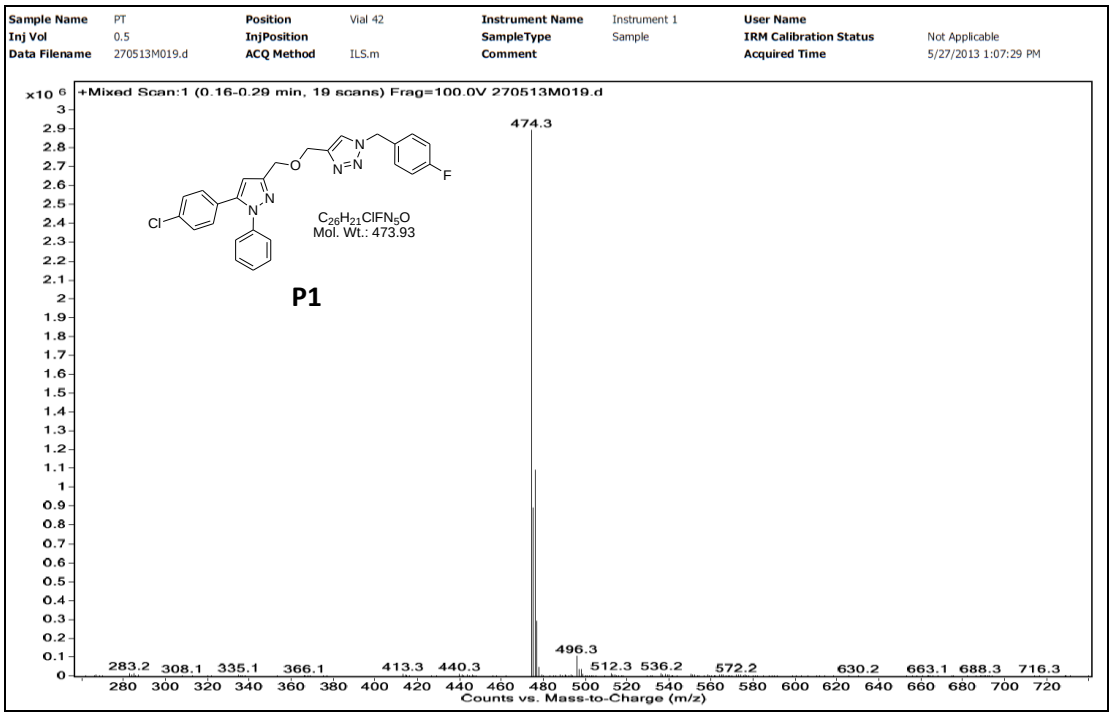


Figure 2.11 Mass spectrum of **P1.**

2.5.2 X-ray crystallographic analysis of P1.

The single crystal of final compound **P1** was grown by the slow evaporation of 1:1 mixture of methanol-chloroform solution of the compound at Rt. The suitable crystal required for the analysis was carefully isolated by using microscope and kept on goniometer tip for X-ray exposure. The structure of the crystal was solved by full matrix least-squares method using SHELXL-2013 package. All the atoms were positioned in different Fourier maps and refined isotropically, using a riding model and all the projections were generated using ORTEP. The ORTEP diagram (50% probability) of **P1** is depicted in **Figure 2.12** and the detailed crystal data is presented in **Table 2.3**.

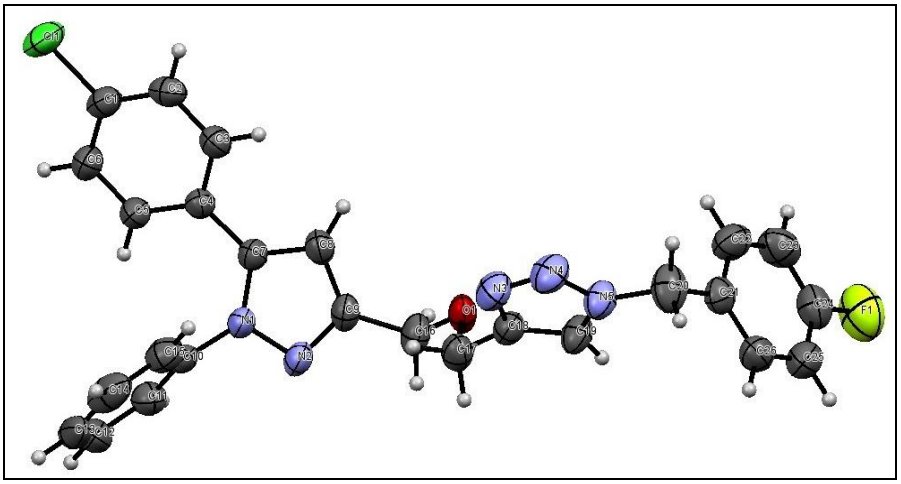


Figure 2.12 ORTEP diagram showing the X-ray crystal structure of **P1.**

Table 2.3 Crystal and structure refinement data for compound **P1.**

Parameters	**Crystal data**
Chemical formula	$C_{26}H_{21}ClFN_5O$
Formula weight	473.93
CCDC No.	1032622
Crystal system	Triclinic
Space group	P-1
a (Å)	5.6750(3)
b (Å)	10.7105(5)
c (Å)	19.9112(9)
Volume ($Å^3$)	1163.87(10)
Angle α, β, γ	79.5270(19), 85.8390(19), 78.1330(19)
Z	2
F_{000}	492.0
μ (mm^{-1})	0.201
Temperature (T)	296 K
Radiation wavelength (Å)	0.71073
Radiation type	Mo Kα
R-Factor (%)	4.3

2.5.3 Antitubercular studies

The antimycobacterial activity of the pyrazole-1,2,3-triazole hybrids (**P1-P24**) against MTB $H_{37}Rv$ (ATCC27294) was evaluated by agar dilution method and the MIC values are given in **Figure 2.13.** INH, EMB and PZA were used as positive drug standards. MIC is defined as the minimum concentration of compound required to completely inhibit the bacterial growth. Among twenty four compounds screened against MTB, 13 compounds (**P1-P7**, **P11-P13**, **P16** and **P22-P23**) showed MIC in the range 3.13 - 12.5 µg/mL. Out of eight 4-chlorophenyl substituted pyrazole derivatives (**P1-P8**), seven compounds showed significant activity against MTB, which suggests that 4-chlorophenyl substitution at position-5 of the pyrazole (**R^1**) ring is a decisive factor for the activity. Compound **P7** which contain a cyclohexyl substitution on the 1,2,3-triazole ring (**R^2**) was found to be the most active compound of the series with a MIC of 3.13 µg/mL. The inhibition activity of **P7** is comparable (in terms of MIC value) with that of the standard drug ethambutol. All the 4-chlorophenyl substituted target compounds (**P1-P8**), except **P8**, are either equipotent or more potent when compared with their respective 4-methoxyphenyl substituted (**P9-P16**) and 4-bromophenyl substituted (**P17-P24**) analogs. This structure-activity relationship further confirms the significant contribution of the 4-chlorophenyl substituent towards the inhibition activity of the molecules. The nature of the substituent on the 1,2,3-triazole ring (**R^2**) also affected the inhibition activity. However, we did not observe a general trend in the variation of the activity with respect to electron donating or withdrawing nature of these substituents. But, in case of 4-chlorophenyl substituted derivatives (**P1-P8**), those compounds which carry an electron-withdrawing functional group at **R^2** showed better inhibition activity than those compounds containing an electron releasing functionality. That is, compounds **P1**, **P2**, **P4** and **P6** which contain 4-F, 4-CN, 4-NO_2 and 2-F groups (at **R^2**) respectively exhibited MIC of 6.25 µg/mL, whereas compounds **P3** (which contain an electron-donating 4-OCH_3 group) and **P5** (which has no substituent on the phenyl ring) showed a higher MIC (12.5 µg/mL) value.

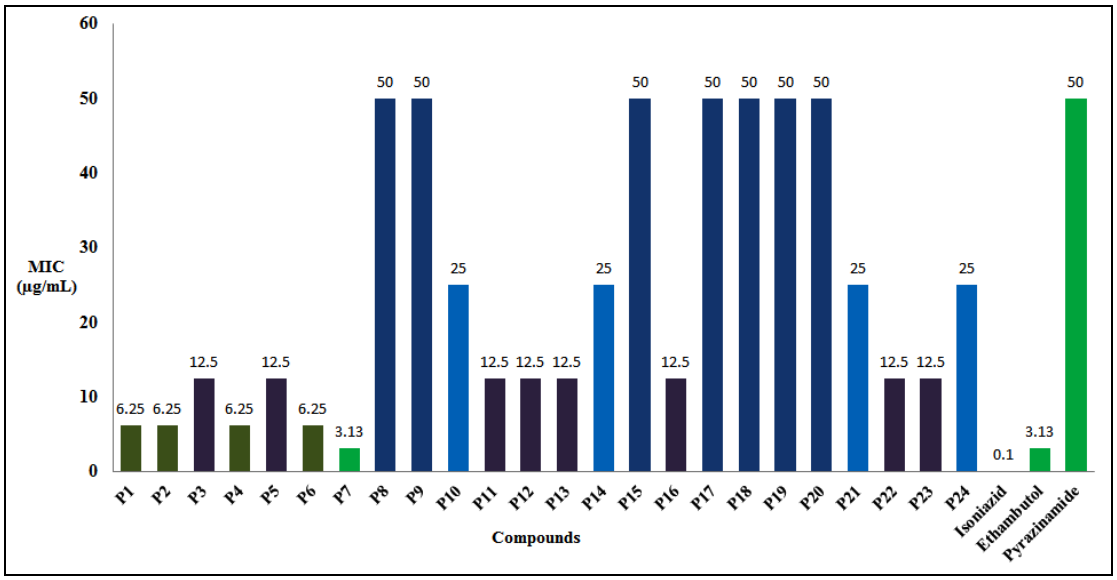


Figure 2.13 AntiTB activity of pyrazole 1,2,3-triazole hybrid derivatives **P1-P24**.

2.5.4 Cytotoxicity studies

The *in vitro* cytotoxicity studies of the active antiTB compounds with MTB MIC ≤ 6.25 µg/mL were carried out by MTT assay against a normal Vero cell line. The results of the cytotoxicity study are presented in the **Table 2.4**. All the active antiTB compounds **P1, P2, P4, P6** and **P7** exhibited low toxicity and the inhibition values were between 13.22 to 29.91 % at 65 µg/mL. Further, the high selectivity index (SI) of these derivatives (>37) clearly implies the suitability of the compounds for drug development as new antiTB agents.

Table 2.4 Cytotoxicity of active antiTB derivatives.

Compound	Percentage Inhibition[a]	IC_{50} (µg/mL)	SI[b]
P1	29.20±2.9	>1000	>160
P2	29.91±3.9	231.67	37.1
P4	20.30±1.3	986.67	157.9
P6	13.22±6.0	656.67	105.1
P7	17.62±5.1	646.67	206.6

[a] Percentage inhibition is at 62.5 µg/mL.

[b] Selectivity index (*in vitro*) = IC_{50} against Vero cells/MIC against *M. tuberculosis*.

2.5.5 Molecular docking studies

In order to understand the probable binding mode of the compounds, molecular modeling studies of the active antiTB compounds (**P1, P2, P4, P6** and **P7**) were performed against selected enzymes viz. InhA (PDB ID: 1P44), CYP121 (PDB ID: 4KTF) and TMPK (PDB ID: 4UNR) of MTB. Here, InhA is one of the enoyl acyl carrier protein reductase (ENR) from MTB. Also, the key first line drug isoniazid acts as an inhibitor of InhA. The drug binds tightly to the enoyl-acyl carrier protein reductase known as InhA, thereby blocking the natural enoyl-AcpM substrate and the action of fatty acid synthase. Similarly, *Mycobacterium tuberculosis* cytochrome P450 gene CYP121 is another enzyme found to be essential for viability of the bacterium. Many literatures demonstrated the strong binding of the enzyme with a number of azole based drugs. Recently, another enzyme thymidine monophosphate kinase (TMPK) has emerged as an attractive target for developing inhibitors of MTB growth. TMPK belongs to the family of transferases, specifically those transferring phosphorus containing groups. The protein structures of all the three enzymes were obtained from the protein data bank (PDB) were initially subjected to various processes such as removal of water molecules and removal of heteroatoms etc. using the Protein Preparation Wizard of Schrödinger 2015. The reference ligands were further re-docked with the active site residues of the corresponding proteins to validate the active site cavity. Re-docking results showed that all crystal compounds exhibited similar interactions as that of the original crystal structures and showed RMSD values between of 0.61 to 1.14 Å (**Figure 2.14**). The docking results were drawn based on docking score, hydrogen bonding, π-H and π-π interaction of the ligand with the protein (**Table 2.5**). The docking score is approximate mathematically calculated value used to predict the strength of the non-covalent interaction (also referred to as binding affinity) between two molecules after they have been docked. Scoring functions have also been developed to predict the strength of other types of intermolecular interactions, for example between two proteins or between protein and ligands etc. The docking score is expressed in negative value and the unit is KJ/mol. Higher the negative value represents batter fitting of the ligand with in the pockets of protein frame. The best docking pose of the representative compounds in the active

site of enzymes, InhA, CYP121 and TMPK are shown in **Figure 2.15**, **Figure 2.16** and **Figure 2.17** respectively.

Firstly, InhA of MTB which is one of the key enzymes validated as an effective antibacterial target was selected for docking studies. All five ligands (**P1, P2, P4, P6** and **P7**) have shown good docking score ranging from -8.74 to -10.72 (Table 4). The highest score was observed for ligand **P1** (-10.72). All ligands showed two common π-π interactions with amino acid residues Tyr 158 and Phe 149 (**Figure 2.15**), which is the common interaction of the 1P44 lagand as well. While the most active compound of the series **P8** showed an additional hydrogen bonding interaction with Gly 104, which may be one reason for enhanced activity of the compound. Additionally, docking studies were carried out with target enzymes of MTB, CYP121 and TMPK as well. It was observed that all ligands showed good interactions with the amino acid residues of CYP121 (**Figure 2.16**) with a good docking score (9.02 - 10.38) which is comparable with the score of the standard ligand. Similarly, docking studies with TMPK revealed the favorable interactions of the ligands with the amino acid residues (**Figure 2.17**). Overall, the docked ligands showed good docking score as well as multiple interactions with amino acid residues of all the three enzymes of MTB. Thus the antiTB profile of these active compounds (**P1, P2, P4, P6** and **P7**) could be due to their inhibition activity against the MTB enzymes.

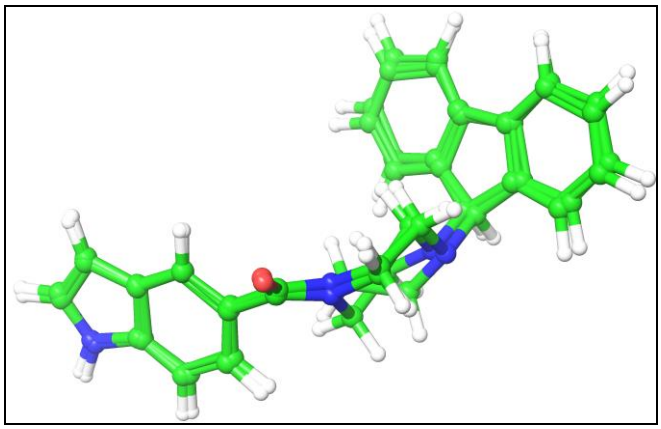


Figure 2.14 Superimposition of InhA ligand of PDB ID: 1P44 with re-docked ligand.

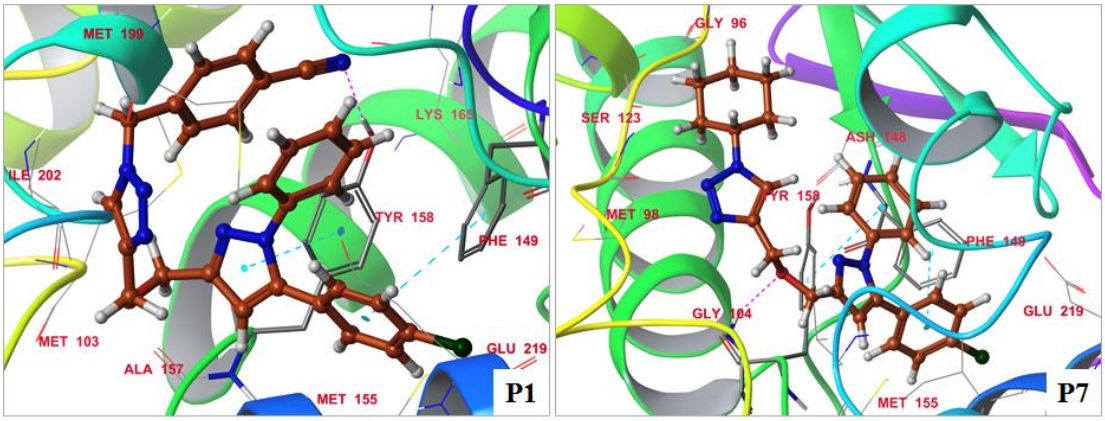


Figure 2.15 The docking pose of the compounds **P1** and **P7** in the active pocket of InhA.

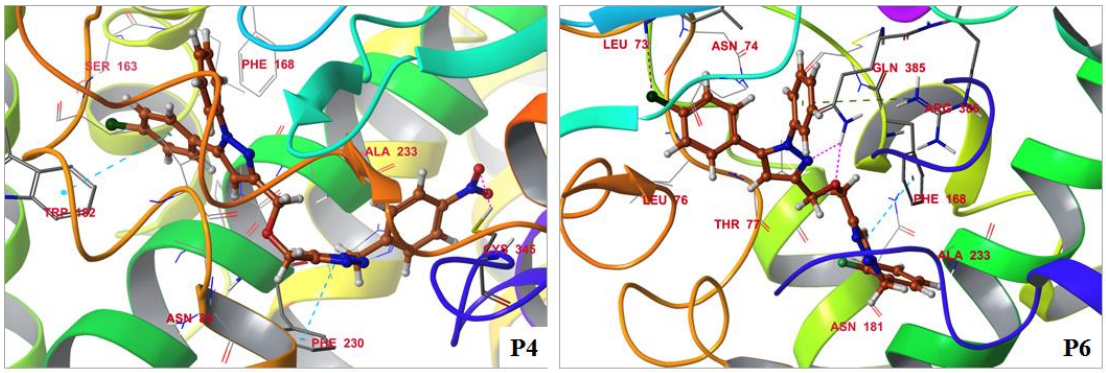


Figure 2.16 The docking pose of the compounds **P4** and **P6** in the active pocket of CYP121.

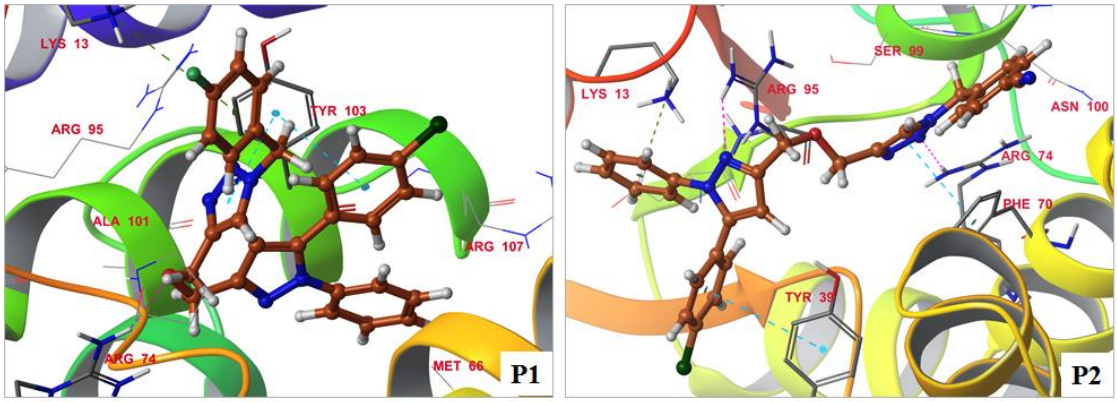


Figure 2.17 The docking pose of the compounds **P1** and **P2** in the active pocket of TMPK.

Table 2.5 Docking results of the active antiTB compounds

Compound ID	InhA		CYP121		TMPK	
	Docking score	**Amino acids that interacted with ligands**	**Docking score**	**Amino acids that interacted with ligands**	**Docking score**	**Amino acids that interacted with ligands**
Standard Ligand	-12.51	Tyr 158, Phe 149	-10.38	Gln 385, Val 228, Asn 85	-13.12	Asn 100, Tyr 103, Arg 74, Arg 95,
P1	-10.72	Tyr 158, Phe 149	-10.38	Trp 182, Phe 230	-7.34	Tyr 103, Arg 74, Lys 13
P2	-9.93	Tyr 158, Phe 149	-10.20	Phe 230	-4.74	Tyr 39, Arg 74, Arg 95, Phe 70, Lys 13
P4	-8.74	Tyr 158, Phe 149	-10.26	Trp 182, Phe 230, Cys 345	-7.45	Tyr 103, Arg 74, Phe 70,
P6	-9.38	Tyr 158, Phe 149	-9.21	Gln 385, Phe 168, Arg 386, Leu 73	-7.14	Tyr 103, Arg 74, Arg 95
P7	-8.82	Tyr 158, Phe 149, Gly104	-9.02	Gln 385, Phe 168	-7.28	Phe 70, Arg 107

2.5.6 Antibacterial studies

E. coli, *S. aureus* and *P. aeruginosa* are the common pathogens which cause harmful effects on human health. Some pyrazole (Ramesh et al. 1995) and 1,2,3-triazole (Kamal et al. 2013) derivatives showed promising activity against these pathogens. Hence we thought it is worthwhile to evaluate the antimicrobial activity of the new pyrazole-1,2,3-triazole derivatives (**P1-P24**) against these microorganisms. The antibacterial activity of the compounds was studied following the well plate method (zone of inhibition) by using streptomycin, the first class of antibiotic drug, as the standard. Compounds **P1**, **P3**, **P4**, **P5**, **P12** and **P23** showed promising activity against tested bacterial strains (**Table 2.6**). It is interesting to note that four of these compounds (**P1**, **P3**, **P4** and **P5**) contain a 4-chlorophenyl substitution at position-5 of the pyrazole ring. The antibacterial activity of derivatives **P4** and **P12** against *P. aeruginosa* is very close to that of streptomycin, the presence of nitro group in these molecules could be responsible for the observed activity. It is evident from **Table 2.6** that the target molecules show a better activity against *P. aeruginosa* strain as compared to other two bacterial strains.

Table 2.6 Antibacterial activity of compounds **P1-P24.**

Compound	**Zone of inhibition (mm)**					
	Staphylococcus Aureus		***Escherichia coli***		***Pseudomonas aeruginosa***	
Conc. in µg/ml	1	0.5	1	0.5	1	0.5
Control	00	00	00	00	00	00
Streptomycin	16.8±0.2	12.5±0.2	18.6±0.2	14.3±0.1	16.7±0.2	13.6±0.2
P1	**13±0.7**	**8±0.4**	**14±0.3**	**10±0.6**	**16±0.6**	**10±0.7**
P2	10±0.9	7±0.3	9±0.2	6±0.4	9±0.7	7±0.5
P3	**12±0.4**	**6±0.1**	**13±0.9**	**10±0.3**	**14±0.6**	**11±0.3**
P4	**13±0.2**	**8±0.2**	**14±0.1**	**9±0.7**	**16±0.3**	**12±0.8**
P5	**14±0.4**	**9±0.4**	**13±0.4**	**10±0.8**	**14±0.9**	**11±0.1**
P6	9±0.3	6±0.6	9±0.6	6±0.1	10±0.2	9±0.2
P7	12±0.7	7±0.3	9±0.7	5±0.6	11±0.6	6±0.4
P8	10±0.5	8±0.4	8±0.8	6±0.7	10±0.7	7±0.6
P9	9±0.2	7±0.6	9±0.2	7±0.2	9±0.3	5±0.8
P10	--	--	--	--	--	--
P11	--	--	--	--	--	--
P12	**14±0.7**	**10±0.5**	**13±0.3**	**8±0.5**	**16±0.8**	**12±0.3**

P13	7±0.3	4±0.9	10±0.4	9±0.8	10±0.4	7±0.4
P14	10±0.2	8±0.3	9±0.8	7±0.4	11±0.6	9±0.6
P15	9±0.5	7±0.2	6±0.7	2±0.8	11±0.7	7±0.7
P16	8±0.1	4±0.3	10±0.6	6±0.3	8±0.3	5±0.6
P17	--	--	--	--	--	--
P18	8±0.3	5±0.5	7±0.4	4±0.4	5±0.1	2±0.2
P19	--	--	--	--	--	--
P20	9±0.7	7±0.7	10±0.4	6±0.7	8±0.9	5±0.8
P21	--	--	--	--	--	--
P22	--	--	--	--	--	--
P23	**12±0.7**	**9±0.3**	**15±0.3**	**12±0.6**	**15±0.4**	**11±0.4**
P24	--	--	--	--	--	--

- : Bacteria resistant; Control: dimethylsulfoxide.

2.6 CONCLUSIONS

- We described the synthesis of a new series of pyrazole-1,2,3-triazole hybrids (**P1-P24**) using a multistep synthetic route in which substituted 1,2,3-triazole ring was constructed in the final step through click reaction protocol.
- The solvent system for the click reaction was optimized in terms of the product yield and the green solvent system, 2:1 mixture of PEG-400 and water, was the most effective among the solvent systems used.
- All the target compounds were characterized by ^{1}H NMR, ^{13}C NMR, mass spectral and elemental analyses and the structure of the final derivative **P1** was established by SC-XRD analysis.
- The *in vitro* antiTB screening of the molecules against MTB $H_{37}Rv$ strain revealed the better antiTB activity of 4-chlorophenyl substituted derivatives as compared to their 4-methoxyphenyl and 4-bromophenyl substituted analogs.
- The derivative (**P7**) with a 4-chlorophenyl substitution on the pyrazole ring and a cyclohexyl moiety on the 1,2,3-triazole ring is the best antiTB agent of the series with MIC of 3.13 µg/mL.
- Among 4-chlorophenyl substituted derivatives, an electron-withdrawing substituent on the 1,2,3-triazole ring enhanced the antiTB activity.
- The cytotoxicity study on active antiTB compounds also suggested that the compounds are nontoxic and have high selectivity index (>37).

- The structure and antiTB activity relationship was further supported by *in silico* molecular docking study of the active compounds against three enzymes of *M. tuberculosis*.
- Further, derivatives **P1**, **P3**, **P4**, **P5**, **P12** and **P23** exhibited significant antibacterial activity against the tested bacterial strains.
- With regard to the relationship between the structure of pyrazole-1,2,3-triazole hybrids and the detected antiTB and antimicrobial properties, it can be concluded that the chlorophenyl substituted pyrazole scaffolds are ideally suited for further structural modifications to obtain efficient antibacterial leads.

Appendix 2.1

Representative ^{1}H NMR, ^{13}C NMR and ESI-MS spectra of final compounds

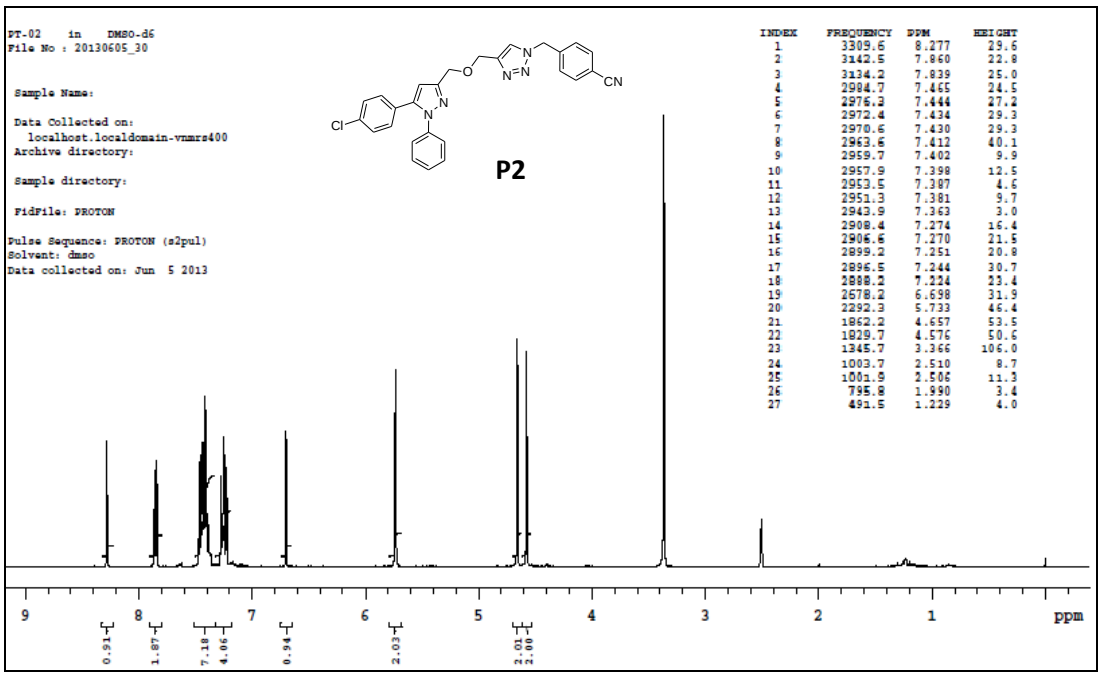


Figure 2.18 ^{1}H NMR spectrum of **P2.**

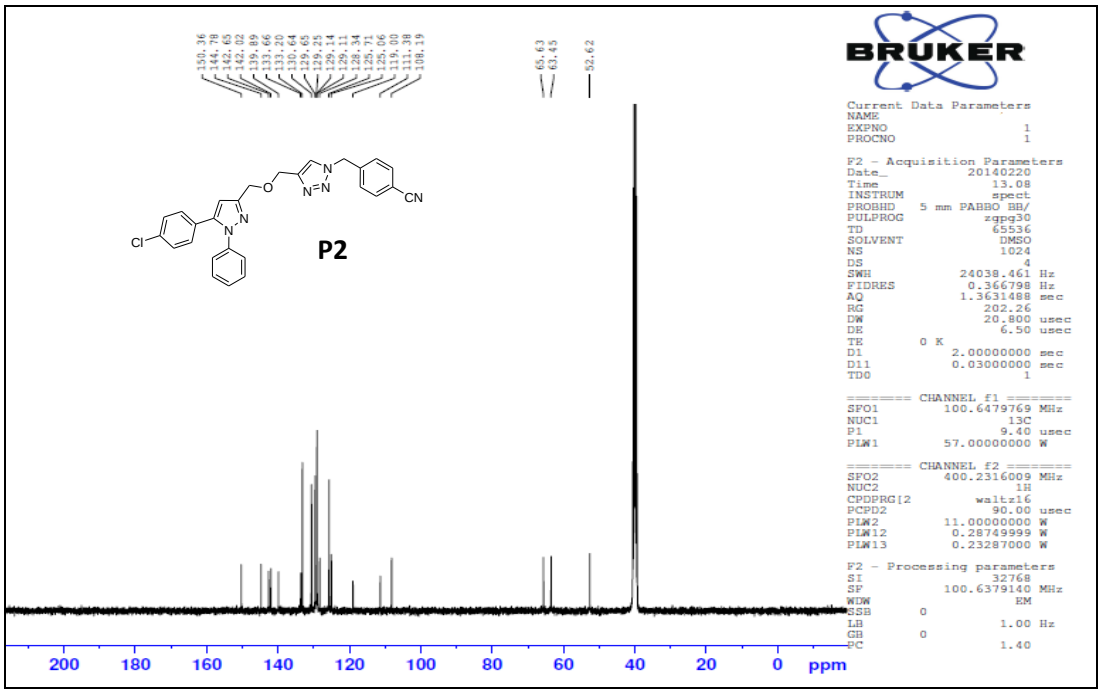


Figure 2.19 ^{13}C NMR spectrum of **P2.**

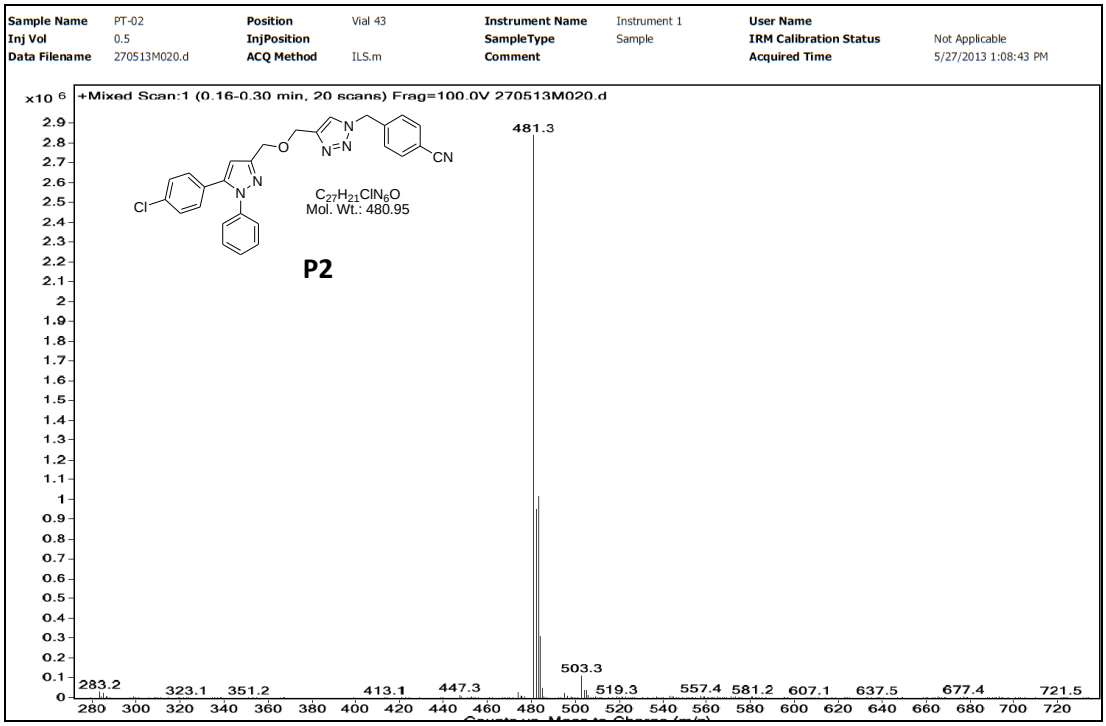


Figure 2.20 Mass spectrum of **P2.**

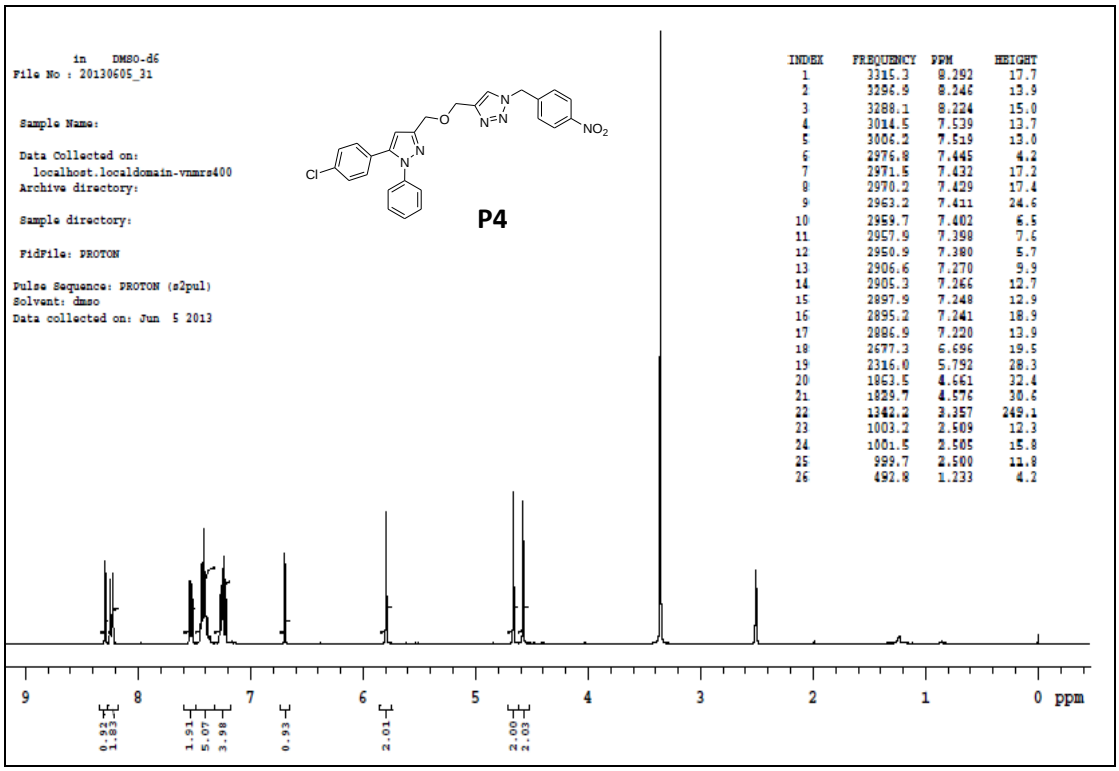


Figure 2.21 ^{1}H NMR spectrum of **P4.**

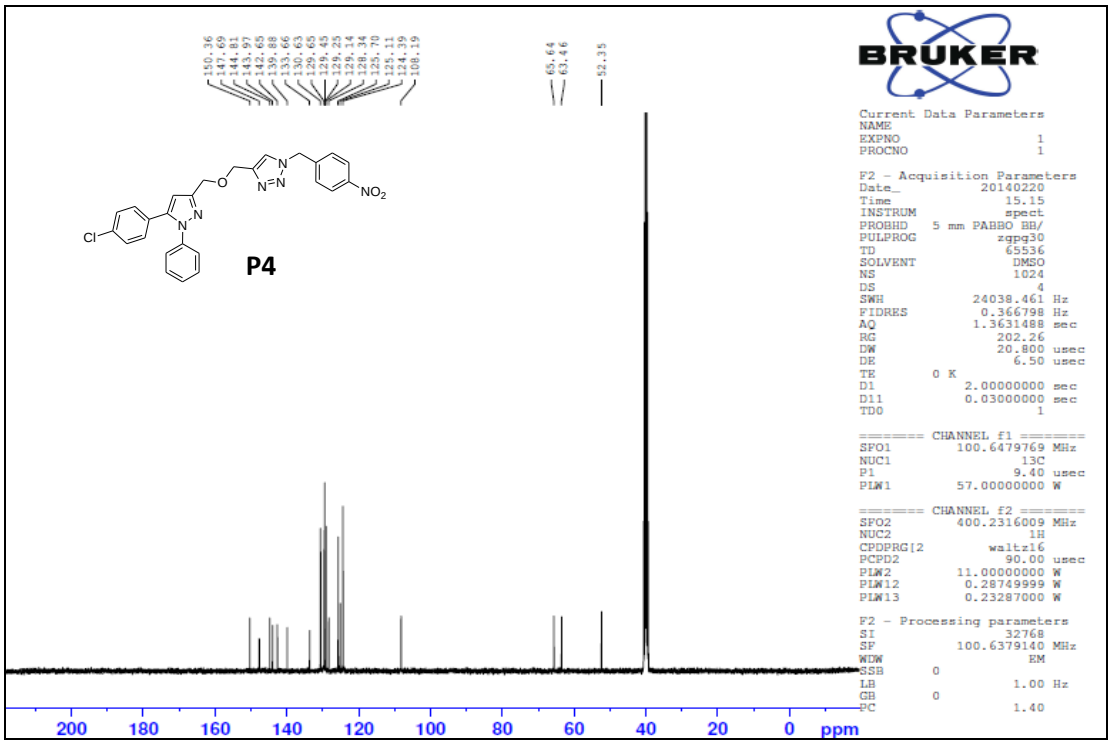


Figure 2.22 ^{13}C NMR spectrum of **P4.**

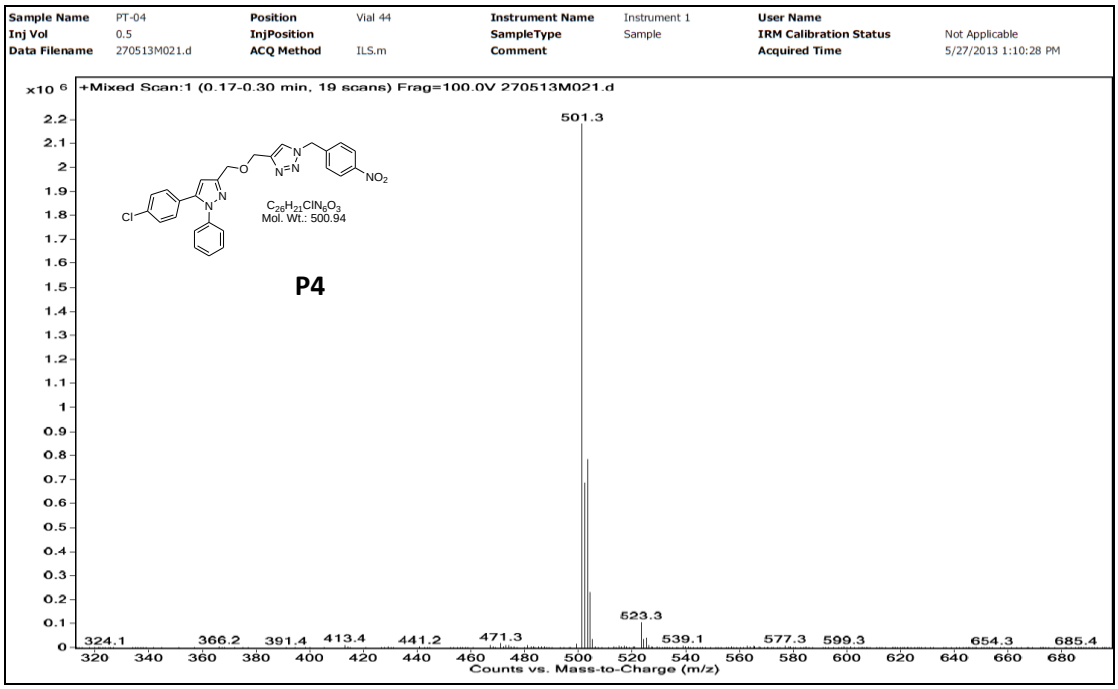


Figure 2.23 Mass spectrum of **P4.**

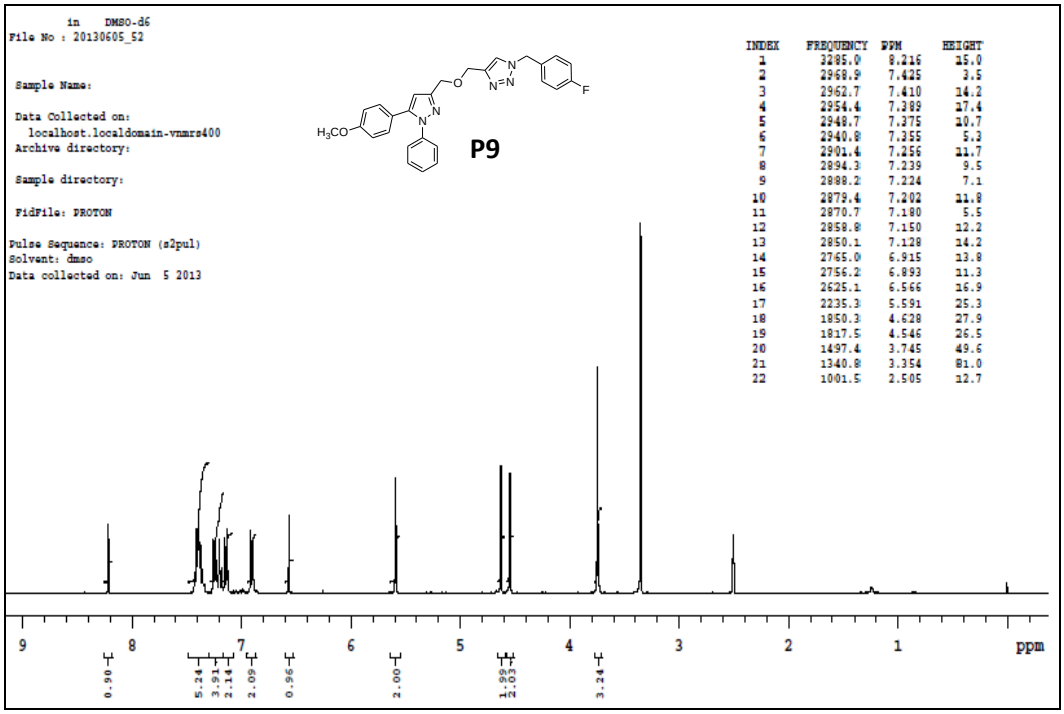


Figure 2.24 ^{1}H NMR spectrum of **P9.**

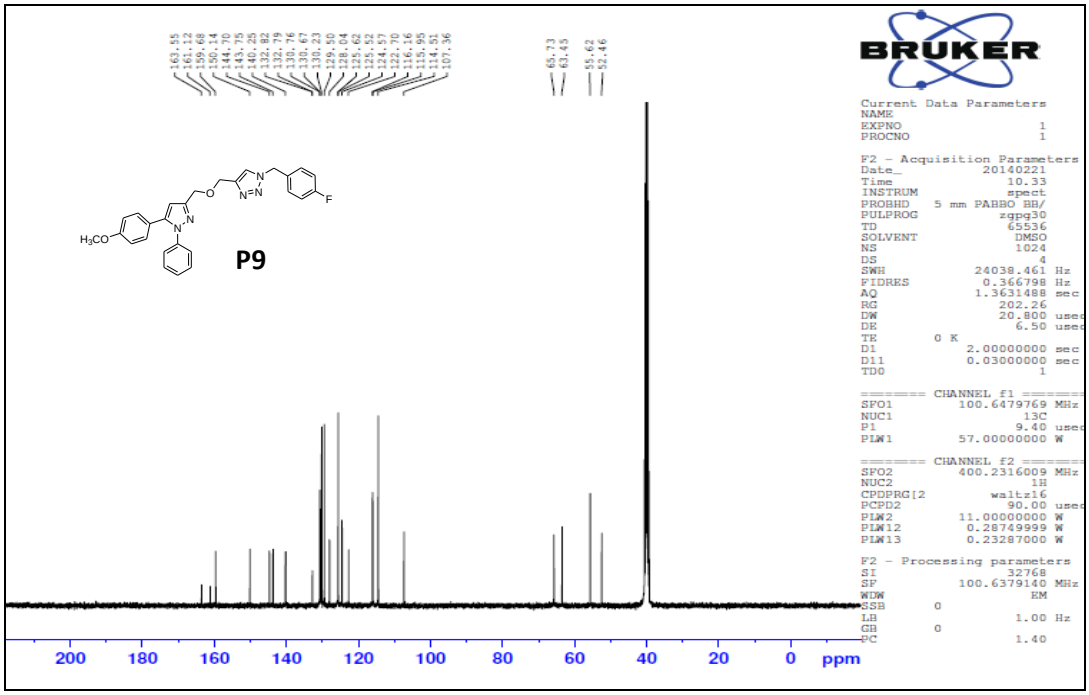


Figure 2.25 ^{13}C NMR spectrum of **P9.**

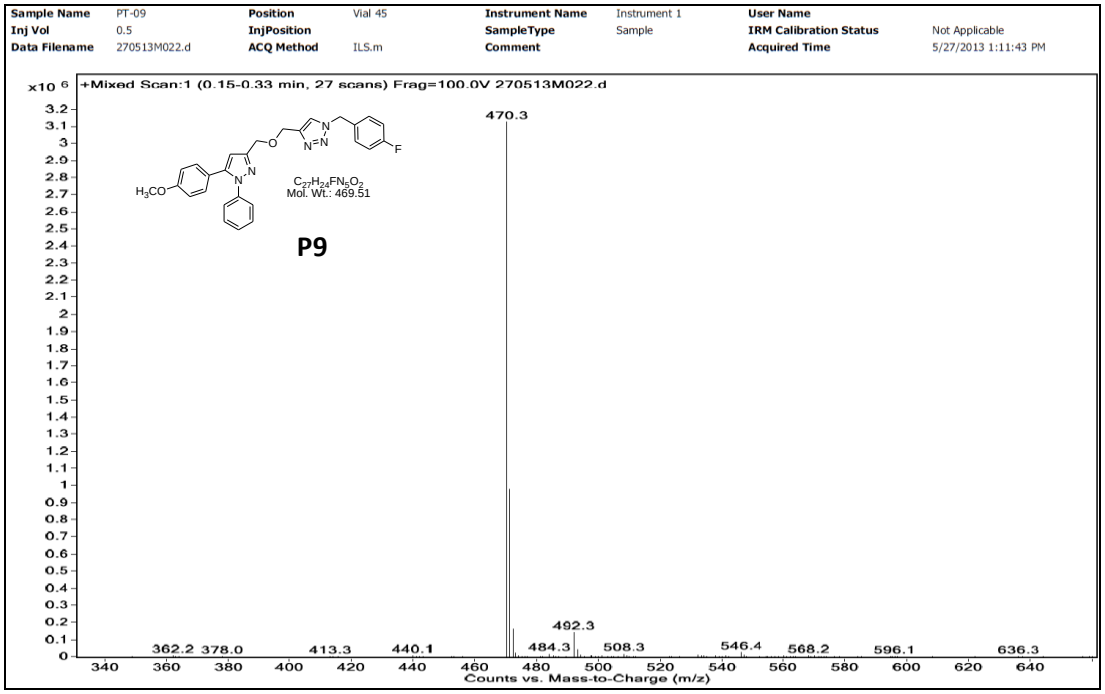


Figure 2.26 Mass spectrum of **P9.**

CHAPTER 3

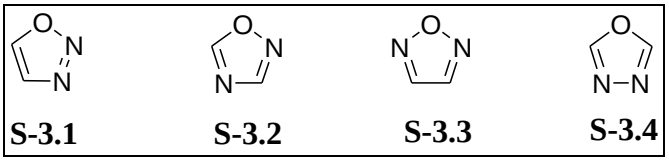
SYNTHESIS AND BIOLOGICAL ACTIVITY OF NEW 5-METHYL-1,3,4-OXADIAZOL-2-YL-1*H*-PYRAZOL-1-YL BENZAMIDE DERIVATIVES

Abstract

This chapter deals with synthesis of pyrazole derivatives carrying oxadiazole group and their characterization by spectral techniques. Further, it also describes the antiTB and antibacterial studies of these new compounds.

3.1 INTRODUCTION

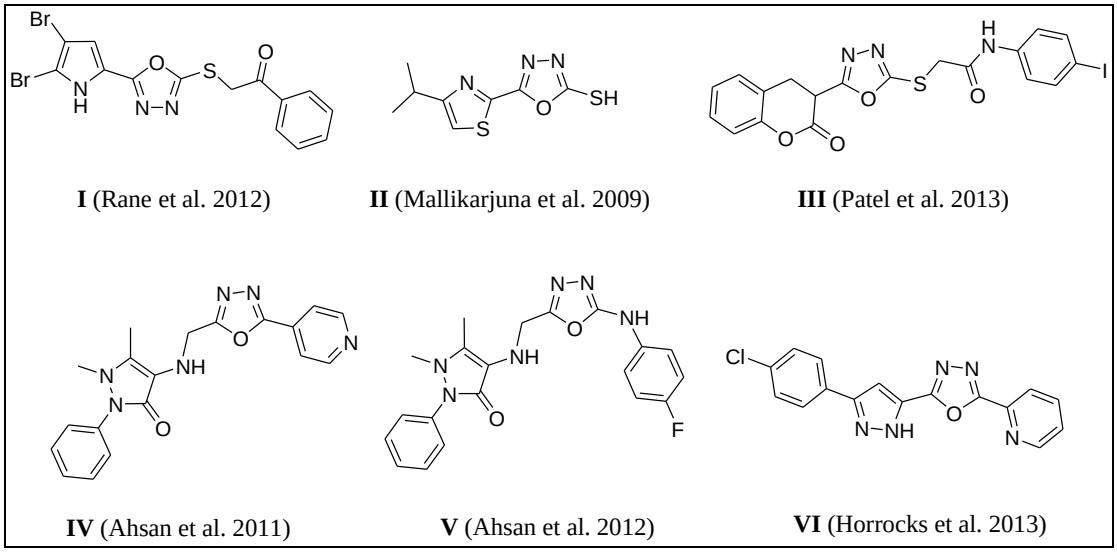
Oxadiazoles are five-membered heterocyclic compounds comprising of two carbons, two nitrogens and an oxygen atom with a general formula $C_2H_2N_2O$. They are considered to be derived from furan by the replacement of two carbon atoms (CH=) by two nitrogen (-N=) atoms, thereby resulting in the formation of isomers. Depending on the position of heteroatoms, there are four possible isomers of oxadiazole and are named as 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**).



Among the azole heterocycles, oxadiazole forms an important class of compounds in medicinal chemistry. They also act as bioisosteres of amides and esters as they enhance the biological activity by participating in hydrogen bonding interactions with different receptors. As a whole, they have acquired great prominence due to their broad spectrum of metabolic profile (Jiang et al. 2010). Further, the pharmacological activity of this heterocycle can also be attributed to the presence of toxophoric -N-C=O- linkage (Rigo and Couturier, 1985). Out of its four possible isomers, 1,3,4-oxadiazole nucleus provides a fertile source of bioactivity in the area of drug discovery and are widely exploited due to its varied pharmacological activities, such as antimicrobial (Chen et al. 2000; Şahin et al. 2002), anticonvulsant (Zarghi et al. 2005), anticancer (Valente et al. 2014, Miralinaghi et al. 2013) and in particular, antituberculosis (Rane et al. 2012; Mallikarjuna et al. 2009; Patel et al. 2013; Ahsan et al. 2011; Ahsan et al. 2012; Rane et al. 2013; Ali and Shaharyar, 2007). Hence, compounds containing 1,3,4-oxadiazole ring occupy an important place in medicinal chemistry. Some of the drug moieties which contain the 1,3,4-oxadiazole unit include,

zibotentan (an anti-cancer agent) (James and Growcott, 2009), furamizole (an anti-bacterial agent), raltegravir (HIV-integrase inhibitor), etc.

So, based on the promising antiTB activity of pyrazole and 1,3,4-oxadiazoles, we envisaged the molecular hybridization of these two pharmacophores. A recent report (Horrocks et al. 2013) on the significant antiTB activity of a series of pyrazole-oxadiazole hybrids (**VI**) further encouraged us in this direction (**Figure 3.1**). Hence, we have synthesized a new series of pyrazole-oxadiazole hybrids (**P25-P48**) and investigated their antiTB activity against MTB strains. In these molecules, the 1,3,4-oxadiazole ring is attached at position-3 of the pyrazole ring, the N1 of which carries a phenyl ring. Another important structural feature of these molecules is the presence of amide functionality. The amide group is introduced based on the fact that the two first line TB drugs, INH and PZA contain an amide (-CONH) linkage (**Figure 3.2**) as the major functional group. The benefit of this bond is that it easily forms hydrogen bond which increases the lipophilic character of the molecule. All these target derivatives were screened for their antiTB and antibacterial studies.



I (Rane et al. 2012) **II** (Mallikarjuna et al. 2009) **III** (Patel et al. 2013)

IV (Ahsan et al. 2011) **V** (Ahsan et al. 2012) **VI** (Horrocks et al. 2013)

Figure 3.1 Some of the active pyrazole and 1,3,4-oxadiazole based antiTB derivatives.

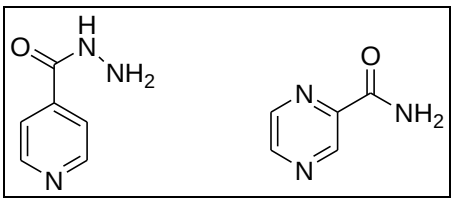
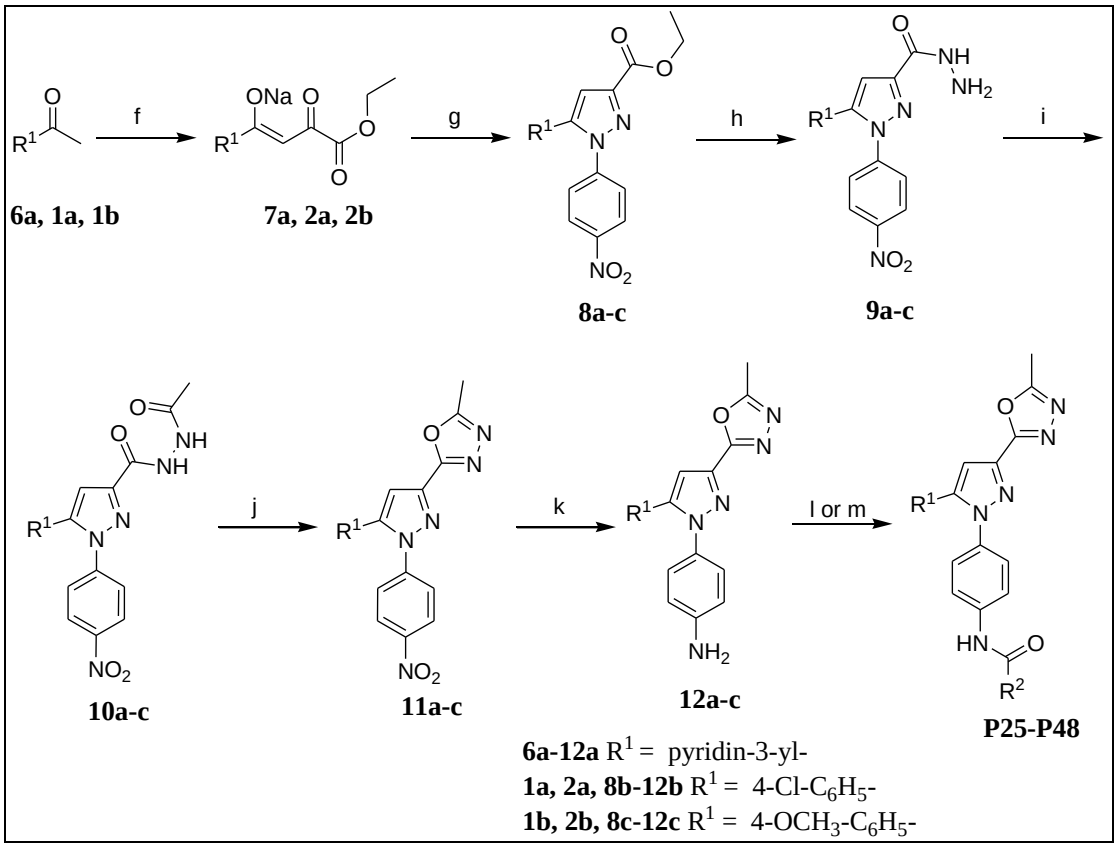


Figure 3.2 Structures of INH and PZA

3.2 CHEMISTRY

The synthetic route for the synthesis of final compounds **P25-P48** is illustrated in **Scheme 3.1.** In the first step, methyl aryl ketones (**6a, 1a, 1b**) were condensed with diethyl oxalate to give the corresponding sodium salt of α,γ-diketoesters (**7a, 2a, 2b**) in good yields. These intermediates upon cyclization with 4-nitro phenyl hydrazine in AcOH yielded corresponding derivatives of ethyl 1-(4-nitrophenyl)-5-aryl-1*H*-pyrazole-3-carboxylates (**8a-c**). The ester derivatives were then converted to corresponding hydrazides (**9a-c**) by treating them with hydrazine hydrate in alcoholic media at 80 °C and further reaction of these hydrazides with acetic anhydride in AcOH gave acetylated derivatives **10a-c**. The cyclodehydration reaction of these intermediates at 80 °C using excess of phosphorous oxychloride ($POCl_3$) resulted in oxadiazole intermediates **11a-c**, which upon reduction reaction using iron powder and saturated ammonium chloride solution in 1:1 mixture of methanol and water yielded three primary amine scaffolds **12a-c**. The target compounds **P25-P48** were conveniently synthesized from the amine precursors **12a-c** by using acid-amine coupling reaction. The target derivatives (**P25-P31, P33-P39** and **P41-P47**) were synthesized by coupling corresponding amine scaffold with different aromatic acids using the coupling agent *N*-[(dimethylamino)-1*H*-1,2,3-triazolo-[4,5-b]pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (HATU) and *N,N*-diisopropylethylamine (DIPEA) as the base in dimethyl formamide (DMF) media. While the acetamide derivatives (**P32, P40** and **P48**) were synthesized by the reaction of corresponding amine scaffold with acetic anhydride (Ac_2O) in presence of AcOH. All the final compounds were purified by silica gel column chromatography using 1:1 mixture pet ether and ethyl acetate as the eluent.



Scheme 3.1 Synthesis of pyrazole-oxadiazole hybrids (**P25-P48**). Reagents and conditions: f) Diethyl oxalate, NaOEt, ethanol, 0 °C to RT, 24 h; g) 4-Nitro phenyl hydrazine, HOAc, 105 °C, 3 h; h) $NH_2NH_2.H_2O$, ethanol, 80 °C, 3 h; i) Ac_2O, HOAc, 0 °C to RT 1 h; j) $POCl_3$, 80 °C, 4 h; k) Iron powder, saturated ammonium chloride solution, methanol, 70 °C, 5 h; l) Carboxylic acid, HATU, DIPEA, DMF, RT, 16 h; m) Ac_2O, HOAc, 0 °C to RT, 1 h.

3.3 EXPERIMENTAL

3.3.1 Materials and methods

Refer section **2.3.1**

3.3.2 Synthesis

Synthesis of sodium(*E*)-1-aryl-4-ethoxy-3,4-dioxobut-1-en-1-olate (7a, 2a and 2b).

Sodium(*E*)-4-Ethoxy-3,4-dioxo-1-(pyridin-3-yl)but-1-en-1-olate (7a): To a stirred solution of 3-acetyl pyridine **6a** (6.0 g, 50 mmol) in anhydrous ethanol (12 mL), 1M NaOEt solution (6.8 g, 100 mmol) in ethanol was added at 0 °C. The reaction mass was allowed to warm to RT and stirred for 1 h. A solution of diethyl oxalate (14.6 g, 100 mmol) in anhydrous ethanol (20 mL) was added drop wise and allowed to stir at

RT for 24 h. The progress of the reaction was monitored by TLC. Upon completion of the reaction, the precipitate obtained was filtered and washed with cold ethanol followed by diethyl ether and dried in vacuum to obtain sodium salt of the pure product as pale yellow solid **7a**. Yield 7.71 g (64%). Off white solid. mp 126-127 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.48-8.60 (m, 2H, H-Ar), 7.68 (m, 1H, H-Ar), 7.43 (m, 1H, H-Ar), 7.34 (s, 1H, H-CH), 4.32 (q, 2H, J = 6.8 Hz, H-CH_2), 1.33 (t, 3H, J = 6.8 Hz, H-CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 189.42, 171.81, 161.46, 153.11, 151.66, 133.73, 130.21, 100.66, 62.53, 14.25. Anal. calculated for $C_{11}H_{10}NNaO_4$; C, 54.33; H, 4.14; N, 5.76. Found: C, 54.30; H, 4.11; N, 5.74.

Sodium(*E*)-1-(4-chlorophenyl)-4-ethoxy-3,4-dioxobut-1-en-1-olate (2a) and Sodium(*E*)-4-ethoxy-1-(4-methoxyphenyl)-3,4-dioxobut-1-en-1-olate (2b):
The synthetic procedures and characterization data of intermediates **2a** and **2b** has already been discussed in section **2.3.2.**

Synthesis of ethyl 1-(4-nitrophenyl)-5-aryl-1*H*-pyrazole-3-carboxylate (8a-c).
Ethyl-1-(4-nitrophenyl)-5-(pyridin-3-yl)-1*H*-pyrazole-3-carboxylate (8a): To a stirred solution of the compound **7a** (7.5 g, 30 mmol) in AcOH (75 mL), 4-nitrophenyl hydrazine (7.08 g, 45 mmol) was added under nitrogen atmosphere and heated at 105 °C for 3 h. The completion of the reaction was confirmed by TLC. Then the solvent was removed by evaporation under vacuum. The residue thus obtained was diluted with cold water (50 mL) and extracted with ethyl acetate (3 × 100 mL). The combined extract was washed with 10 % sodium bicarbonate solution, then with water followed by brine solution and dried over anhydrous sodium sulphate. The solvent was removed under reduced pressure and the crude product was purified by flash column chromatography (230-400 size mesh) using 20 % ethyl acetate in pet ether to afford pure product **8a**. Yield 8.55 g (82%). Yellow solid. mp 136-137 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.60 (m, 2H, ArH), 8.31 (m, 2H, H-Ar), 7.63-7.72 (m, 3H, H-Ar), 7.43 (m, 1H, H-Ar), 7.34 (s, 1H, H-Ar), 4.37 (q, 2H, J = 7.07 Hz, H-CH_2), 1.33 (t, 3H, J = 7.2 Hz, H-CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 161.67, 150.52, 149.61, 147.28, 145.20, 143.99, 142.39, 136.83, 127.38, 126.94, 125.30, 125.25, 124.54, 124.12, 111.81, 61.30, 14.67. ESI-MS (m/z) 339.1 $(M+H)^+$. Anal. calculated for $C_{17}H_{14}N_4O_4$; C, 60.35; H, 4.17. N, 16.56. Found: C, 60.31; H, 4.14; N, 16.52.

Ethyl-5-(4-chlorophenyl)-1-(4-nitrophenyl)-1*H*-pyrazole-3-carboxylate (8b): The above procedure was followed for the synthesis of compound **8b** by reacting the compound **2a** (5.52 g, 20 mmol) with 4-nitrophenyl hydrazine (4.59 g, 30 mmol) in AcOH (60 mL). Yield 6.23 g (84%). yellow solid. mp 104-105 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.33 (d, 2H, J = 8.8 Hz, H-Ar), 7.62 (d, 2H, J = 8.8 Hz, H-Ar), 7.31-7.42 (m, 4H, H-Ar), 7.18 (s, 1H, H-Ar), 4.36 (q, 2H, J = 6.8 Hz, H-CH_2), 1.32 (t, 3H, J = 6.8 Hz, H-CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 161.97, 145.44, 144.21, 143.89, 132.51, 131.56, 129.68, 129.79, 128.41, 127.96, 127.10, 124.80, 124.77, 111.20, 61.25, 14.68. ESI-MS (m/z) 372.6 $(M+H)^+$. Anal. calculated for $C_{18}H_{14}ClN_3O_4$; C, 58.15; H, 3.80; N, 11.30. Found: C, 58.08; H, 3.76; N, 11.28.

Ethyl-5-(4-methoxyphenyl)-1-(4-nitrophenyl)-1*H*-pyrazole-3-carboxylate (8c): The above procedure was followed for the synthesis of compound **8c** by reacting the compound **2b** (6.0 g, 22 mmol) with 4-nitrophenyl hydrazine (5.06 g, 33 mmol) in glacial acetic acid (65 mL). Yield 6.88 g (85%). yellow solid. mp 158-159 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.31 (d, 2H, J = 7.2 Hz, H-Ar), 7.60 (d, 2H, J = 7.2 Hz, H-Ar), 7.17 (s, 1H, H-Ar), 7.14-6.99 (m, 4H, H-Ar), 4.35 (q, 2H, J = 6.8 Hz, H-CH_2), 3.76 (s, 3H, H-OCH_3), 1.32 (t, 3H, J = 6.8 Hz, H-CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 161.88, 160.81, 148.32, 146.71, 144.11, 133.99, 130.69, 129.76, 128.79, 128.04, 127.10, 126.83, 125.71, 109.78, 61.26, 55.75, 14.69. ESI-MS (m/z) 368.2 $(M+H)^+$. Anal. calculated for $C_{19}H_{17}N_3O_5$; C, 62.12; H, 4.66; N, 11.44. Found: C, 62.07; H, 4.68; N, 11.48.

Synthesis of 1-(4-nitrophenyl)-5-aryl-1*H*-pyrazole-3-carbohydrazide (9a-c).

1-(4-Nitrophenyl)-5-(pyridin-3-yl)-1*H*-pyrazole-3-carbohydrazide (9a): Compound **8a** (8.1 g, 24 mmol) was suspended in ethanol (80 mL), to which excess hydrazine hydrate ($NH_2NH_2.H_2O$) (3.60 g, 72 mmol) was added portion wise. The reaction mass was then refluxed for about 3 h. After the completion of reaction, the reaction mass was cooled and the solid obtained was filtered, washed with cold ethanol and dried under vacuum to obtain the hydrazide intermediate **9a**. Yield 6.05 g (78%). pale yellow solid. mp 209-210 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.74 (s, 1H, H-NH), 8.57-8.62 (m, 2H, H-Ar), 8.30-8.32 (m, 2H, H-Ar), 7.62-7.72 (m, 3H, H-Ar), 7.44 (m, 1H, H-Ar), 7.20 (s, 1H, H-Ar), 4.53 (br-s, 2H, H-NH_2). ^{13}C NMR (100

MHz, DMSO-d_6): δ 161.67, 150.43, 149.52, 148.00, 147.24, 146.93, 146.60, 144.16, 141.85, 136.78, 126.48, 125.66, 125.24, 124.89, 124.53, 124.17, 109.94. ESI-MS (m/z) 325.1 $(M+H)^+$. Anal. calculated for $C_{15}H_{12}N_6O_3$; C, 55.55; H, 3.73; N, 25.91. Found: C, 55.51; H, 3.71; N, 25.88.

5-(4-Chlorophenyl)-1-(4-nitrophenyl)-1*H*-pyrazole-3-carbohydrazide (9b): The above procedure was followed for the synthesis of compound **9b** by reacting the compound **8b** (6.0 g, 16 mmol) with hydrazine hydrate (2.42 g, 48 mmol) in ethanol (60 mL). Yield 4.67 g (81%). pale yellow solid. mp 173-174 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.67 (s, 1H, H-NH), 8.32 (d, 2H, J = 7.6 Hz, H-Ar), 7.59 (d, 2H, J = 7.6 Hz, H-Ar), 7.21-7.39 (m, 4H, H-Ar), 6.96 (s, 1H, H-Ar), 4.53 (br-s, 2H, H-NH_2). ^{13}C NMR (100 MHz, DMSO-d_6): δ 160.88, 145.99, 144.01, 139.89, 136.24, 130.93, 126.89, 126.71, 125.46, 125.20, 124.98, 124.15, 110.29. ESI-MS (m/z) 358.6 $(M+H)^+$. Anal. calculated for $C_{16}H_{12}ClN_5O_3$; C, 53.72; H, 3.68; N, 19.58. Found: C, 53.66; H, 3.66; N, 19.56.

5-(4-Methoxyphenyl)-1-(4-nitrophenyl)-1*H*-pyrazole-3-carbohydrazide (9c): The above procedure was followed for the synthesis of compound **9c** by reacting the compound **8c** (5.4 g, 15 mmol) with hydrazine hydrate (2.21 g, 45 mmol) in ethanol (55 mL). Yield 3.87 g (74%). pale yellow solid. mp 161-162 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.67 (s, 1H, H-NH), 8.29-8.32 (m, 2H, H-Ar), 7.59-7.61 (m, 2H, H-Ar), 7.23-7.26 (m, 2H, H-Ar), 6.97-6.99 (m, 3H, H-Ar), 4.50 (br-s, 2H, H-NH_2), 3.73 (s, 3H, H-OCH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 161.55, 160.48, 146.88, 145.89, 145.57, 144.21, 131.03, 127.78, 126.69, 126.31, 125.56, 124.90, 124.48, 124.15, 109.21, 55.71. ESI-MS (m/z) 353.1 $(M+H)^+$. Anal. calculated for $C_{17}H_{15}N_5O_4$; C, 57.79; H, 4.29; N, 19.82. Found: C, 57.74; H, 4.24; N, 19.80.

Synthesis of *N′*-acetyl-1-(4-nitrophenyl)-5-aryl-1*H*-pyrazole-3-carbohydrazide (10a-c).

***N′*-Acetyl-1-(4-nitrophenyl)-5-(pyridin-3-yl)-1*H*-pyrazole-3-carbohydrazide** (**10a**) The above synthesized hydrazide derivative **9a** (6.0 g, 18.5 mmol) was dissolved in 18 mL of AcOH under nitrogen atmosphere. To the resulting reaction mixture, Ac_2O (7.5 g, 74 mmol) was added drop wise at 0 °C, after completion of the addition the reaction mixture was stirred at room temperature for the 1 h. Completion of the

reaction was monitored on TLC using petether/ethyl acetate (60 : 40) as eluent. After completion of the reaction, the reaction mixture was poured in to ice-water to afford yellow precipitate which was then filtered by pump and dried; the solid obtained was recrystallized from ethanol. Yield 6.50 g (96%). yellow solid. mp 232-233 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.18 (s, 1H, H-NH), 9.89 (s, 1H, H-NH), 8.60 (d, 2H, J = 11.6 Hz, H-Ar), 8.34 (d, 2H, J = 2.0 Hz, H-Ar), 7.31-7.73 (m, 4H, H-Ar), 7.27 (s, 1H, H-Ar), 1.91 (s, 3H, H-CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.82, 161.21, 150.43, 149.89, 148.22, 148.21, 146.74, 146.60, 145.56, 142.15, 126.56, 125.76, 125.34, 124.89, 123.99, 109.92, 20.04. ESI-MS (m/z) 367.2 $(M+H)^+$. Anal. calculated for $C_{17}H_{14}N_6O_4$; C, 55.74; H, 3.85; N, 22.94. Found: C, 55.70; H, 3.83; N, 22.97.

***N'*-Acetyl-5-(4-chlorophenyl)-1-(4-nitrophenyl)-1*H*-pyrazole-3-carbohydrazide (10b):** Compound **10b** was synthesized according to the above mentioned procedure by treating **9b** (4.6 g, 12.8 mmol) with Ac_2O (3.94 g, 38.6 mmol) in AcOH (15 mL). Yield 4.88g (95%). yellow solid. mp 123-124 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.18 (s, 1H, H-NH), 9.88 (s, 1H, H-NH), 8.33 (d, 2H, J = 8.8 Hz, H-Ar), 7.62 (d, 2H, J = 8.8 Hz, H-Ar), 7.22-7.41 (m, 4H, H-Ar), 6.97 (s, 1H, H-Ar), 1.91 (s, 3H, H-CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.80, 160.82, 146.02, 145.02, 140.88, 136.28, 130.83, 126.81, 126.56, 125.90, 124.88, 124.68, 124.37, 110.19, 20.10. ESI-MS (m/z) 400.5 $(M+H)^+$. Anal. calculated for $C_{18}H_{14}ClN_5O_4$; C, 54.08; H, 3.53; N, 17.52. Found: C, 54.13; H, 3.52; N, 17.50.

***N'*-Acetyl-5-(4-methoxyphenyl)-1-(4-nitrophenyl)-1*H*-pyrazole-3-carbohydrazide (10c):** This compound was synthesized according to the above mentioned procedure by treating **9c** (3.8 g, 10.7 mmol) with Ac_2O (3.29 g, 32.3 mmol) in of AcOH (13 mL). Yield 4.04 g (95%). yellow solid. mp 139-140 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.19 (s, 1H, H-NH), 9.89 (s, 1H, H-NH), 8.32 (d, 2H, J = 8.8 Hz, H-Ar), 7.62 (d, 2H, J = 8.8 Hz, H-Ar), 7.25 (d, 2H, J = 8.8 Hz, H-Ar), 7.04 (s, 1H, H-Ar), 6.98 (d, 2H, J = 8.8 Hz, H-Ar), 3.81 (s, 3H, H-OCH_3), 1.91 (s, 3H, H-CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.81, 160.47, 160.33, 146.95, 146.79, 145.33, 145.12, 144.60, 144.52, 130.73, 127.55, 126.64, 126.54, 126.37, 125.14, 124.80, 121.41, 114.88,

109.15, 55.73, 20.06. ESI-MS (m/z) 396.2 $(M+H)^+$. Anal. calculated for $C_{19}H_{17}N_5O_5$; C, 57.72; H, 4.33; N, 17.71. Found: C, 57.68; H, 4.32; N, 17.75.

Synthesis of 2-(5-aryl-1-(4-nitrophenyl)-1*H*-pyrazol-3-yl)-5-methyl-1,3,4-oxa diazole (11a-c).

3-(3-(5-Methyl-1,3,4-oxadiazol-2-yl)-1-(4-nitrophenyl)-1*H*-pyrazol-5-yl)pyridine (11a): A mixture of **10a** (6.4 g, 88.9 mmol) and freshly distilled $POCl_3$ (32 mL) was heated at 80 °C for 4 h. The reaction was monitored by TLC. After completion of the reaction, excess of $POCl_3$ was distilled off. The residue thus obtained was stirred with ice water for 15 min. After this, the solid phase was filtered and dried to get crude compound **11a** which was then recrysatallized from hot ethanol. Yield 4.76 g (82%). pale yellow solid. mp 216-217 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.56-8.60 (m, 2H, H-Ar), 8.29-8.30 (m, 2H, H-Ar), 7.60-7.71 (m, 3H, H-Ar), 7.44 (m, 1H, H-Ar), 7.17 (s, 1H, H-Ar), 2.61 (s, 3H, H-CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.67, 160.43, 149.96, 148.42, 146.84, 146.63, 146.30, 126.97, 126.48, 126.42, 126.01, 125.24, 124.89, 124.17, 109.94, 11.04. ESI-MS (m/z) 349.1 $(M+H)^+$. Anal. calculated for $C_{17}H_{12}N_6O_3$; C, 58.62; H, 3.47; N, 24.13. Found: C, 58.68; H, 3.42; N, 24.09.

2-(5-(4-Chlorophenyl)-1-(4-nitrophenyl)-1*H*-pyrazol-3-yl)-5-methyl-1,3,4-oxadiazole (11b): Above procedure was followed by reacting **10b** (4.9 g, 12.2 mmol) with of $POCl_3$ (25 mL). Yield 3.51 g (78%). yellow solid. mp 144-145 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.33 (d, 2H, J = 7.6 Hz, H-Ar), 7.62 (d, 2H, J = 7.6 Hz, H-Ar), 7.24-7.38 (m, 4H, H-Ar), 6.96 (s, 1H, H-Ar), 2.60 (s, 3H, H-CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.86, 160.64, 145.72, 144.59, 139.24, 127.14, 126.89, 126.71, 125.65, 125.20, 124.98, 124.33, 109.40, 11.04. ESI-MS (m/z) 382.5 $(M+H)^+$. Anal. calculated for $C_{18}H_{12}ClN_5O_3$; C, 56.63; H, 3.17; N, 18.34. Found: C, 56.68; H, 3.20; N, 18.35.

2-(5-(4-Methoxyphenyl)-1-(4-nitrophenyl)-1*H*-pyrazol-3-yl)-5-methyl-1,3,4-oxadiazole (11c): Above procedure was followed by reacting **10c** (4.0 g, 10.1 mmol) with of $POCl_3$ (20 mL). Yield 3.15g (86%). yellow solid. mp 174-175 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.32 (d, 2H, J = 9.2 Hz, H-Ar), 7.63 (d, 2H, J = 9.6 Hz, H-Ar), 7.27-7.31 (m, 3H, H-Ar), 6.99 (d, 2H, J = 8.8 Hz, H-Ar), 3.78 (s, 3H, H-OCH_3), 2.61 (s, 3H, H-CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.47, 160.49, 159.69,

146.89, 145.74, 144.39, 139.11, 130.83, 127.66, 126.65, 126.39, 125.22, 124.86, 120.92, 114.89, 109.33, 108.52, 55.75, 11.03. ESI-MS (m/z) 378.1 $(M+H)^+$. Anal. calculated for $C_{19}H_{15}N_5O_4$; C, 60.47; H, 4.01; N, 18.56. Found: C, 60.48; H, 4.00; N, 18.55.

Synthesis of 4-(5-aryl-3-(5-methyl-1,3,4-oxadiazol-2-yl)-1*H*-pyrazol-1-yl)benzene amine (12a-c).

4-(3-(5-Methyl-1,3,4-oxadiazol-2-yl)-5-(pyridin-3-yl)-1*H*-pyrazol-1-yl) benzenamine (12a): Iron powder (3.93 g, 70 mmol) and saturated solution of ammonium chloride (15 mL) was added to compound **11a** (4.9 g, 14 mmol) in methanol (50 mL), the mixture was then stirred at 70 °C for 5 h. After completion (monitored by TLC), the reaction mixture was filtered through celite bed and the residue was washed with methanol (50 mL). The filtrate obtained was concentrated and the crude mass was extracted with ethyl acetate (3 x 50 mL). The organic layer was separated, washed with brine solution (2 x 25 mL), dried over anhydrous sodium sulfate and concentrated under reduced pressure. The crude residue thus obtained was purified by silica gel column chromatography using EtOAC/hexane (1:2) solvent mixture to give pure **12a**. Yield 2.58 g (69%). pale yellow solid. mp 198-199 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.54 (m, 2H, H-Ar), 7.68 (m, 1H, H-Ar), 7.41 (m, 1H, H-Ar), 7.36 (s, 1H, H-Ar), 7.01 (d, 2H, J = 8.8 Hz, H-Ar), 6.57 (m, 2H, H-Ar), 5.50 (br-s, 2H, H-NH_2), 2.59 (s, 3H, H-CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.05, 160.78, 148.89, 144.68, 143.79, 136.52, 133.97, 132.01, 131.99, 128.68, 127.64, 127.52, 127.20, 114.23, 112.82, 107.08, 14.56. ESI-MS (m/z) 319.1 $(M+H)^+$. Anal. calculated for $C_{17}H_{14}N_6O$; C, 64.14; H, 4.43; N, 26.40. Found: C, 64.10; H, 4.40; N, 26.44.

4-(5-(4-Chlorophenyl)-3-(5-methyl-1,3,4-oxadiazol-2-yl)-1*H*-pyrazol-1-yl) benzenamine (12b): The above procedure was followed for the synthesis of compound **12b** by reducing compound **11b** (3.8 g, 10 mmol) with iron powder (2.8 g, 50 mmol) in saturated NH_4Cl solution (12 mL) and ethanol (40 mL). Yield 2.69 g (74%). off white solid. mp 217-218 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.46 (m, 2H, H-Ar), 7.33 (m, 2H, H-Ar), 7.24 (s, 1H, H-Ar), 6.98 (d, 2H, J = 8.4 Hz, H-Ar), 6.57 (m, 2H, H-Ar), 5.48 (br-s, 2H, H-NH_2), 2.59 (s, 3H, H-CH_3). ^{13}C NMR (100

MHz, DMSO-d_6): δ 164.05, 160.78, 160.08, 149.80, 143.65, 137.24, 137.11, 133.97, 130.79, 130.71, 129.11, 128.41, 127.88, 127.31, 127.09, 114.01, 112.69, 107.12, 14.55. ESI-MS (m/z) 352.6 $(M+H)^+$. Anal. calculated for $C_{18}H_{14}ClN_5O$; C, 61.46; H, 4.01; N, 19.91. Found: C, 61.41; H, 3.98; N, 19.87.

4-(5-(4-Methoxyphenyl)-3-(5-methyl-1,3,4-oxadiazol-2-yl)-1*H*-pyrazol-1-yl) benzenamine (12c): The above procedure was followed for the synthesis of compound **12c** by reducing the compound **11c** (3.2 g, 8.5 mmol) with iron powder (2.4 g, 42 mmol) in saturated NH_4Cl solution (10 mL) and ethanol (30 mL). Yield 2.14 g (70%). Off white solid. mp 203-204 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.27-7.32 (m, 3H, H-Ar), 6.94-6.94 (m, 4H, H-Ar), 6.58 (d, 2H, J = 6.8 Hz, H-Ar), 5.48 (br-s, 2H, H-NH_2) 3.77 (s, 3H, H-OCH_3), 2.60 (s, 3H, H-CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.08, 160.36, 160.04, 149.44, 144.66, 142.89, 140.11, 132.97, 131.79, 130.01, 129.43, 129.21, 127.96, 127.66, 127.21, 114.06, 112.57, 108.75, 55.76, 14.54. ESI-MS (m/z) 348.2 $(M+H)^+$. Anal. calculated for $C_{19}H_{17}N_5O_2$; C, 65.69; H, 4.93; N, 20.16. Found: C, 65.64; H, 4.91; N, 20.19.

General procedure for synthesis of *N*-(4-(5-aryl-3-(5-methyl-1,3,4-oxadiazol-2-yl)-1*H*-pyrazol-1-yl)phenyl)benzamide (P25-P31, P33-P39 and **P41-P47).**

The acid derivatives (0.1g) were dissolved in dry DMF (2 mL) with stirring. About 1.1 equivalent of HATU was added and the resulting clear solution was stirred for 10 min. at RT. To this solution amine derivatives **12a-c** (1.1 eq) in DMF (1 mL) was injected and the resulting yellow solution was stirred for 20 min. Then 3.6 equivalent of DIPEA was added to this solution through syringe and the mixture was stirred for 16 h at room temperature; completion of the reaction was confirmed by TLC. The reaction mass was quenched with cold water and extracted with EtOAc (3 x 25 mL). The organic layer was separated, washed with brine solution (2 x 25 mL), dried over anhydrous sodium sulfate and concentrated under reduced pressure. The crude residue thus obtained was purified over silica gel (60-120 mesh size) column chromatography eluted with EtOAc/hexane (1:1) to give pure final analogs.

Table 3.1 Substitution pattern of final compounds **P25-P48**.

Compound	R^1	R^2	LogP/CLogP[a]
P25	pyridin-3-yl-	4-F-C_6H_5-	3.67/2.86
P26	pyridin-3-yl-	4-Cl-C_6H_5-	4.07/3.43
P27	pyridin-3-yl-	4-CH_3-C_6H_5-	4.00/3.15
P28	pyridin-3-yl-	4-OCH_3-C_6H_5-	3.38/2.77
P29	pyridin-3-yl-	4-NO_2-C_6H_5-	--/2.58
P30	pyridin-3-yl-	2-Cl-C_6H_5-	4.07/2.60
P31	pyridin-3-yl-	-C_6H_5	3.51/2.65
P32	pyridin-3-yl-	-CH_3	1.61/1.16
P33	4-Cl-C_6H_5-	4-F-C_6H_5-	5.56/5.07
P34	4-Cl-C_6H_5-	4-Cl-C_6H_5-	5.96/5.64
P35	4-Cl-C_6H_5-	4-CH_3-C_6H_5-	5.89/5.36
P36	4-Cl-C_6H_5-	4-OCH_3-C_6H_5-	5.28/4.98
P37	4-Cl-C_6H_5-	4-NO_2-C_6H_5-	--/4.79
P38	4-Cl-C_6H_5-	2-Cl-C_6H_5-	5.96/4.81
P39	4-Cl-C_6H_5-	-C_6H_5	5.40/4.86
P40	4-Cl-C_6H_5-	-CH_3	3.51/3.37
P41	4-OCH_3-C_6H_5-	4-F-C_6H_5-	4.88/4.28
P42	4-OCH_3-C_6H_5-	4-Cl-C_6H_5-	5.28/4.85
P43	4-OCH_3-C_6H_5-	4-CH_3-C_6H_5-	5.21/4.57
P44	4-OCH_3-C_6H_5-	4-OCH_3-C_6H_5-	4.59/4.18
P45	4-OCH_3-C_6H_5-	4-NO_2-C_6H_5-	--/3.99
P46	4-OCH_3-C_6H_5-	2-Cl-C_6H_5-	5.28/4.08
P47	4-OCH_3-C_6H_5-	-C_6H_5	4.72/4.07
P48	4-OCH_3-C_6H_5-	-CH_3	2.82/2.58

[a] Obtained from ChemDraw Ultra software.

4-Fluoro-*N*-(4-(3-(5-methyl-1,3,4-oxadiazol-2-yl)-5-(pyridin-3-yl)-1*H*-pyrazol-1-yl)phenyl)benzamide (P25): Yield 0.09 g (68%). Off white solid. mp 114-115 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.51 (s, 1H, H-CONH), 8.58 (s, 2H, H-Ar), 8.04 (t, 2H, J = 6.4 Hz, H-Ar), 7.88 (d, 2H, J = 8.4 Hz, H-Ar), 7.71 (d, 1H, J = 8.4 Hz, H-

Ar), 7.37-7.45 (m, 6H, H-Ar), 2.61 (s, 3H, H-CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 165.90, 165.21, 164.30, 163.41, 159.88, 150.22, 149.49, 142.32, 140.05, 138.04, 136.58, 134.51, 131.62, 131.02, 130.93, 126.71, 125.49, 124.03, 121.09, 115.99, 115.78, 108.28, 11.03. ESI-MS (m/z) 441.2 $(M+H)^+$. Anal. calculated for $C_{24}H_{17}FN_6O_2$; C, 65.45; H, 3.89; N, 19.08. Found: C, 65.50; H, 3.91; N, 19.11.

4-Chloro-*N*-(4-(3-(5-methyl-1,3,4-oxadiazol-2-yl)-5-(pyridin-3-yl)-1*H*-pyrazol-1-yl)phenyl)benzamide (P26): Yield 0.10 g (71%). Off white solid. mp 202-203 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.56 (s, 1H, H-CONH), 8.58 (s, 2H, H-Ar), 7.99 (d, 2H, J = 7.6 Hz, H-Ar), 7.88 (d, 2H, J = 7.6 Hz, H-Ar), 7.62-7.72 (m, 3H, H-Ar), 7.40-7.45 (m, 4H, H-Ar), 2.61 (s, 3H, H-CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 165.91, 165.24, 164.26, 163.39, 159.79, 150.08, 149.53, 142.31, 140.07, 138.05, 136.59, 134.51, 131.60, 131.01, 130.94, 129.11, 128.32, 126.76, 125.55, 124.06, 121.06, 108.31, 11.04. ESI-MS (m/z) 457.5 $(M+H)^+$. Anal. calculated for $C_{24}H_{17}ClN_6O_2$; C, 63.09; H, 3.75; N, 18.39. Found: C, 63.13; H, 3.73; N, 18.36.

4-Methyl-*N*-(4-(3-(5-methyl-1,3,4-oxadiazol-2-yl)-5-(pyridin-3-yl)-1*H*-pyrazol-1-yl)phenyl)benzamide (P27): Yield 0.09 g (69%). Off white solid. mp 222-223 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.56 (s, 1H, H-CONH), 8.55 (s, 1H, H-Ar), 8.49 (m, 1H, H-Ar), 7.88-7.99 (m, 4H, H-Ar), 7.40-7.72 (m, 7H, H-Ar), 2.61 (s, 3H, H-CH_3), 2.40 (s, 3H, H-CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 165.88, 165.09, 165.01, 163.40, 159.74, 149.78, 149.51, 142.31, 142.66, 141.35, 137.17, 136.51, 132.91, 131.66, 131.23, 129.99, 129.10, 128.84, 127.55, 124.85, 124.06, 121.42, 108.36, 25.03, 11.03. ESI-MS (m/z) 437.3 $(M+H)^+$. Anal. calculated for $C_{25}H_{20}N_6O_2$; C, 68.80; H, 4.62; N, 19.25. Found: C, 68.86; H, 4.60; N, 19.21.

4-Methoxy-*N*-(4-(3-(5-methyl-1,3,4-oxadiazol-2-yl)-5-(pyridin-3-yl)-1*H*-pyrazol-1-yl)phenyl)benzamide (**P28**): Yield 0.11 g (80%). Off white solid. mp 119-120 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.47 (s, 1H, H-CONH), 8.54 (m, 1H, H-Ar), 8.48 (m, 1H, H-Ar), 7.31-7.61 (m, 9H, H-Ar), 7.01 (d, 2H, J = 8.4 Hz, H-Ar), 3.85 (s, 3H, H-OCH_3), 2.60 (s, 3H, H-CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 165.76, 165.21, 165.08, 163.21, 159.73, 150.13, 148.97, 142.92, 142.29, 140.47, 136.17, 136.51, 132.65, 131.66, 131.20, 129.52, 129.19, 129.04, 127.55, 125.45, 114.49, 114.42,

108.31, 25.03, 55.84, 11.03. ESI-MS (m/z) 453.2 $(M+H)^+$. Anal. calculated for $C_{25}H_{20}N_6O_3$; C, 66.36; H, 4.46; N, 18.57. Found: C, 66.41; H, 4.43; N, 18.60.

***N*-(4-(3-(5-Methyl-1,3,4-oxadiazol-2-yl)-5-(pyridin-3-yl)-1*H*-pyrazol-1-yl)phenyl)-4-nitrobenzamide (P29):** Yield 0.11 g (74%). Off white solid. mp 126-127 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.79 (s, 1H, H-CONH), 8.59 (m, 2H, H-Ar), 8.39 (d, 2H, J = 8.8 Hz, H-Ar), 8.19 (d, 2H, J = 8.8 Hz, H-Ar), 7.90 (d, 2H, J = 9.2 Hz, H-Ar), 7.72 (d, 1H, J = 8.4 Hz, H-Ar), 7.42-7.46 (m, 4H, H-Ar), 2.61 (s, 3H, H-CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.69, 164.31, 159.86, 150.23, 149.73, 149.49, 142.34, 140.81, 139.65, 138.10, 136.59, 134.87, 129.77, 126.78, 125.47, 124.06, 121.25, 108.33, 11.03. ESI-MS (m/z) 468.3 $(M+H)^+$. Anal. calculated for $C_{24}H_{17}N_7O_4$; C, 61.67; H, 3.67; N, 20.98. Found: C, 61.70; H, 3.66; N, 20.96.

2-Chloro-*N*-(4-(3-(5-methyl-1,3,4-oxadiazol-2-yl)-5-(pyridin-3-yl)-1*H*-pyrazol-1-yl)phenyl)benzamide (P30): Yield 0.1 g (69%). Off white solid. mp 98-99 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.60 (s, 1H, H-CONH), 8.54 (m, 2H, H-Ar), 8.01 (m, 1H, H-Ar), 7.32-7.66 (m, 10H, H-Ar), 2.61 (s, 3H, H-CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 165.91, 165.27, 164.14, 159.69, 150.67, 149.44, 142.39, 140.07, 134.71, 133.73, 133.26, 132.90, 131.41, 130.91, 129.86, 128.33, 125.32, 124.51, 122.06, 121.14, 108.22, 11.03. ESI-MS (m/z) 457.4 $(M+H)^+$. Anal. calculated for $C_{24}H_{17}ClN_6O_2$; C, 63.09; H, 3.75; N, 18.39. Found: C, 63.15; H, 3.74; N, 18.37.

***N*-(4-(3-(5-Methyl-1,3,4-oxadiazol-2-yl)-5-(pyridin-3-yl)-1*H*-pyrazol-1-yl)phenyl) benzamide (P31):** Yield 0.1 g (75%). Off white solid. mp 126-127 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.52 (s, 1H, H-CONH), 8.56 (m, 1H, H-Ar), 8.51 (m, 1H, H-Ar), 7.83-7.31 (m, 12H, H-Ar), 2.61 (s, 3H, H-CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 165.88, 165.09, 163.40, 159.73, 149.78, 149.51, 142.31, 142.66, 141.35, 137.17, 136.51, 132.91, 131.66, 131.23, 129.99, 129.10, 128.84, 127.55, 124.85, 124.06, 121.42, 108.36, 11.03. ESI-MS (m/z) 423.1 $(M+H)^+$. Anal. calculated for $C_{24}H_{18}N_6O_2$; C, 68.24; H, 4.29; N, 19.89. Found: C, 68.20; H, 4.27; N, 19.88.

***N*-(4-(5-(4-Chlorophenyl)-3-(5-methyl-1,3,4-oxadiazol-2-yl)-1*H*-pyrazol-1-yl) phenyl)-4-fluorobenzamide (P33):** Yield 0.11 g (81%). Off white solid. mp 221-222 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.74 (s, 1H, H-CONH), 7.84-7.90 (m, 4H, H-

Ar), 7.71 (d, 2H, *J* = 8.4 Hz, H-Ar) 7.36-7.44 (m, 7H, H-Ar), 2.60 (s, 3H, H-CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.12, 165.28, 165.11, 159.91, 144.32, 140.21, 134.66, 131.52, 131.32, 130.67, 130.18, 129.89, 129.71, 129.44, 128.57, 127.82, 126.34, 123.39, 107.36, 11.03. ESI-MS (m/z) 474.4 $(M+H)^+$. Anal. calculated for $C_{25}H_{17}ClFN_5O_2$; C, 63.36; H, 3.62; N, 14.78. Found: C, 63.30; H, 3.63; N, 14.76.

4-Chloro-*N*-(4-(5-(4-chlorophenyl)-3-(5-methyl-1,3,4-oxadiazol-2-yl)-1*H*-pyrazol-1-yl)phenyl)benzamide (P34): Yield 0.09 g (66%). Off white solid. mp 215-216 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.75 (s, 1H, H-CONH), 7.91 (d, 2H, *J* = 7.6 Hz, H-Ar), 7.28-7.46 (m, 11H, H-Ar), 2.61 (s, 3H, H-CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.03, 165.36, 159.91, 143.47, 141.88, 137.67, 134.56, 134.24, 132.79, 131.23, 129.83, 129.70, 129.33, 129.25, 128.74, 127.01, 126.34, 123.44, 108.02, 11.03. ESI-MS (m/z) 491.1 $(M+H)^+$. Anal. calculated for $C_{25}H_{17}Cl_2N_5O_2$; C, 61.24; H, 3.49; N, 14.28. Found: C, 61.20; H, 3.47; N, 14.25.

***N*-(4-(5-(4-Chlorophenyl)-3-(5-methyl-1,3,4-oxadiazol-2-yl)-1*H*-pyrazol-1-yl) phenyl)-4-methylbenzamide (P35):** Yield 0.1 g (73%). Off white solid. mp 246-247 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.40 (s, 1H, H-CONH), 7.87-7.90 (m, 4H, H-Ar), 7.49 (d, 2H, *J* = 8.4 Hz, H-Ar), 7.33-7.37 (m, 7H, H-Ar), 2.61 (s, 3H, H-CH_3), 2.40 (s, 3H, H-CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 65.89, 164.54, 159.90, 143.50, 142.91, 141.12, 135.66, 134.76, 134.33, 132.51, 131.34, 129.88, 129.62, 129.37, 129.25, 127.02, 124.34, 123.44, 107.92, 25.01, 11.03. ESI-MS (m/z) 470.6 $(M+H)^+$. Anal. calculated for $C_{26}H_{20}ClN_5O_2$; C, 66.45; H, 4.29; N, 14.90. Found: C, 66.40; H, 4.26; N, 14.88.

***N*-(4-(5-(4-Chlorophenyl)-3-(5-methyl-1,3,4-oxadiazol-2-yl)-1*H*-pyrazol-1-yl) phenyl)-4-methoxybenzamide (P36):** Yield 0.1 g (70%). Off white solid. mp 218-219 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.31 (s, 1H, H-CONH), 7.97 (d, 2H, *J* = 8.4 Hz, H-Ar), 7.88 (d, 2H, *J* = 8.4 Hz, H-Ar), 7.49 (d, 2H, *J* = 8.4 Hz, H-Ar), 7.32-7.37 (m, 5H, H-Ar), 7.08 (d, 2H, *J* = 8.0 Hz, H-Ar), 3.85 (s, 3H, H-OCH_3), 2.61 (s, 3H, H-CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 165.78, 164.59, 164.15, 159.88, 143.75, 142.66, 135.48, 134.86, 132.53, 131.31, 129.87, 129.90, 129.42, 129.24, 127.10, 123.56, 121.41, 114.14, 107.91, 55.45, 11.03. ESI-MS (m/z) 486.1 $(M+H)^+$.

Anal. calculated for $C_{25}H_{20}ClFN_5O_2$; C, 64.27; H, 4.15; N, 14.41. Found: C, 64.27; H, 4.17; N, 14.39.

***N*-(4-(5-(4-Chlorophenyl)-3-(5-methyl-1,3,4-oxadiazol-2-yl)-1*H*-pyrazol-1-yl) phenyl)-4-nitrobenzamide (P37):** Yield 0.11 g (76%). Off white solid. mp 213-214 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.78 (s, 1H, H-CONH), 8.39 (d, 2H, J = 8.8 Hz, H-Ar), 8.20 (d, 2H, J = 8.4 Hz, H-Ar), 7.89 (d, 2H, J = 9.2 Hz, H-Ar), 7.33-7.50 (m, 7H, H-Ar), 2.61 (s, 3H, H-CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 165.86, 164.63, 159.90, 152.26, 143.89, 142.47, 141.70, 135.51, 134.56, 132.89, 131.21, 129.98, 129.90, 129.24, 129.12, 127.14, 123.52, 122.78, 121.40, 114.10, 108.01, 11.03. ESI-MS (m/z) 501.6 $(M+H)^+$. Anal. calculated for $C_{25}H_{17}ClN_6O_4$; C, 59.95; H, 3.42; N, 16.78. Found: C, 59.90; H, 3.40; N, 16.75.

2-Chloro-*N*-(4-(5-(4-chlorophenyl)-3-(5-methyl-1,3,4-oxadiazol-2-yl)-1*H*-pyrazol-1-yl)phenyl)benzamide (P38): Yield 0.11 g (72%). Off white solid. mp 239-240 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.76 (s, 1H, H-CONH), 7.82 (d, 2H, J = 8.8 Hz, H-Ar), 7.32-7.63 (m, 11H, H-Ar), 2.61 (s, 3H, H-CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 165.62, 164.28, 159.90, 144.04, 139.66, 137.94, 137.12, 134.79, 134.34, 131.77, 130.99, 130.77, 130.18, 129.44, 129.31, 128.09, 127.80, 126.68, 123.34, 107.97, 11.03. ESI-MS (m/z) 490.1 $(M+H)^+$. Anal. calculated for $C_{25}H_{17}Cl_2N_5O_2$; C, 61.24; H, 3.49; N, 14.28. Found: C, 61.29; H, 3.47; N, 14.26.

***N*-(4-(5-(4-Chlorophenyl)-3-(5-methyl-1,3,4-oxadiazol-2-yl)-1*H*-pyrazol-1-yl) phenyl)benzamide (P39):** Yield 0.09 g (72%). Off white solid. mp 124-125 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.48 (s, 1H, H-CONH), 7.88-7.97 (m, 4H, H-Ar), 7.32-7.62 (m, 10H, H-Ar), 2.61 (s, 3H, H-CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.62, 164.28, 159.90, 143.98, 142.23, 135.66, 135.94, 134.12, 132.79, 131.34, 130.99, 130.77, 129.18, 129.44, 129.31, 128.09, 127.80, 126.68, 122.34, 107.97, 11.03. ESI-MS (m/z) 456.2 $(M+H)^+$. Anal. calculated for $C_{25}H_{18}ClN_5O_2$; C, 65.86; H, 3.98; N, 15.36. Found: C, 65.81; H, 3.96; N, 15.33.

4-Fluoro-*N*-(4-(5-(4-methoxyphenyl)-3-(5-methyl-1,3,4-oxadiazol-2-yl)-1*H*-pyrazol-1-yl)phenyl)benzamide (P41): Yield 0.12 g (88%). Off white solid. mp 230-231 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.49 (s, 1H, H-CONH), 8.05 (m, 2H, H-

Ar), 7.86 (d, 2H, *J* = 8.8 Hz, H-Ar), 7.20-7.41 (m, 7H, H-Ar), 6.96 (d, 2H, *J* = 8.4 Hz, H-Ar), 3.77 (s, 3H, H-OCH_3), 2.60 (s, 3H, H-CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.52, 164.63, 164.29, 161.23, 159.89, 143.89, 142.26, 135.86, 134.54, 132.79, 130.96, 130.68, 129.96, 129.41, 129.26, 128.14, 127.76, 116.55, 115.36, 107.97, 55.36, 11.04. ESI-MS (m/z) 470.2 $(M+H)^+$. Anal. calculated for $C_{26}H_{20}FN_5O_3$; C, 66.52; H, 4.29; N, 14.92. Found: C, 66.46; H, 4.32; N, 14.97.

4-Chloro-*N*-(4-(5-(4-methoxyphenyl)-3-(5-methyl-1,3,4-oxadiazol-2-yl)-1*H*-pyrazol-1-yl)phenyl)benzamide (P42): Yield 0.12 g (86%). Off white solid. mp 205-206 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.55 (s, 1H, H-CONH), 7.90 (d, 2H, *J* = 8.0 Hz, H-Ar), 7.19-7.60 (m, 11H, H-Ar), 3.76 (s, 3H, H-OCH_3), 2.60 (s, 3H, H-CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.52, 164.83, 160.88, 159.87, 143.79, 142.24, 138.70, 135.53, 132.88, 130.91, 130.78, 129.83, 129.40, 129.33, 128.14, 127.56, 126.44 121.61, 115.37, 108.07, 55.37, 11.03. ESI-MS (m/z) 486.6 $(M+H)^+$. Anal. calculated for $C_{26}H_{20}ClN_5O_3$; C, 64.27; H, 4.15; N, 14.41. Found: C, 64.33; H, 4.17; N, 14.44.

***N*-(4-(5-(4-Methoxyphenyl)-3-(5-methyl-1,3,4-oxadiazol-2-yl)-1*H*-pyrazol-1-yl)phenyl)-4-methylbenzamide (P43):** Yield 0.1 g (76%). Off white solid. mp 220-221 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.37 (s, 1H, H-CONH), 7.87-7.89 (m, 4H, H-Ar), 7.19-7.36 (m, 7H, H-Ar), 6.96 (d, 2H, *J* = 8.8 Hz, H-Ar), 3.81 (s, 3H, H-OCH_3), 2.60 (s, 3H, H-CH_3), 2.39 (s, 3H, H-CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 165.47, 164.72, 161.24, 159.78, 143.89, 142.57, 140.58, 135.28, 134.28, 132.75, 130.96, 130.57, 129.41, 129.17, 129.01, 128.97, 127.52, 116.43, 115.40, 107.88, 55.37, 25.03, 11.04. ESI-MS (m/z) 466.1 $(M+H)^+$. Anal. calculated for $C_{27}H_{23}N_5O_3$; C, 69.66; H, 4.98; N, 15.04. Found: C, 69.72; H, 5.00; N, 15.01.

4-Methoxy-*N*-(4-(5-(4-methoxyphenyl)-3-(5-methyl-1,3,4-oxadiazol-2-yl)-1*H*-pyrazol-1-yl)phenyl)benzamide (P44): Yield 0.9 g (67%). Off white solid. mp 109-110 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.46 (s, 1H, H-CONH), 7.86 (d, 2H, *J* = 8.4 Hz, H-Ar), 7.24-7.52 (m, 7H, H-Ar), 7.09-7.6.95 (m, 4H, H-Ar), 3.76 (s, 3H, H-OCH_3), 3.74 (s, 3H, H-OCH_3), 2.61 (s, 3H, H-CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 165.64, 164.52, 164.71, 161.25, 159.88, 152.26, 142.44, 141.99, 135.41, 134.89, 132.56, 131.48, 129.97, 129.84, 129.21, 129.12, 125.26, 121.78, 120.41, 114.11,

114.02, 107.81, 55.40, 55.37, 11.03. ESI-MS (m/z) 482.3 $(M+H)^+$. Anal. calculated for $C_{27}H_{23}N_5O_4$; C, 67.35; H, 4.81; N, 14.54. Found: C, 67.41; H, 4.83; N, 14.57.

***N*-(4-(5-(4-Methoxyphenyl)-3-(5-methyl-1,3,4-oxadiazol-2-yl)-1*H*-pyrazol-1-yl) phenyl)-4-nitrobenzamide (P45):** Yield 0.1 g (72%). Off white solid. mp 141-142 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.78 (s, 1H, H-CONH), 8.38 (d, 2H, J = 8.8 Hz, H-Ar), 8.19 (d, 2H, J = 8.8 Hz, H-Ar), 7.28-7.61 (m, 9H, H-Ar), 3.74 (s, 3H, H-OCH_3), 2.61 (s, 3H, H-CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 165.74, 164.55, 161.11, 159.88, 151.46, 143.78, 142.52, 135.53, 134.42, 129.79, 129.62, 129.16, 127.19, 123.82, 122.65, 121.41, 114.15, 108.03, 55.38, 11.03. ESI-MS (m/z) 497.3 $(M+H)^+$. Anal. calculated for $C_{26}H_{20}N_6O_5$; C, 62.90; H, 4.06; N, 16.93. Found: C, 62.96; H, 4.04; N, 16.95.

2-Chloro-*N*-(4-(5-(4-methoxyphenyl)-3-(5-methyl-1,3,4-oxadiazol-2-yl)-1*H*-pyrazol-1-yl)phenyl)benzamide (P46): Yield 0.11 g (76%). Off white solid. mp 196-197 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.75 (s, 1H, H-CONH), 7.83 (d, 1H, J = 8.4 Hz, H-Ar), 7.29-7.61 (m, 12H, H-Ar), 3.74 (s, 3H, H-OCH_3), 2.61 (s, 3H, H-CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 165.65, 164.36, 160.89, 159.91, 142.06, 140.59, 137.31, 135.19, 134.83, 134.56, 130.99, 129.78, 129.51, 129.10, 128.78, 127.64, 126.61, 122.34, 121.23, 114.46, 107.97, 55.37, 11.03. ESI-MS (m/z) 486.5 $(M+H)^+$. Anal. calculated for $C_{26}H_{20}ClN_5O_3$; C, 64.27; H, 4.15; N, 14.41. Found: C, 64.31; H, 4.18; N, 14.42.

***N*-(4-(5-(4-Methoxyphenyl)-3-(5-methyl-1,3,4-oxadiazol-2-yl)-1*H*-pyrazol-1-yl) phenyl)benzamide (P47):** Yield 0.10 g (79%). Off white solid. mp 191-192 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.38 (s, 1H, H-CONH), 7.91 (d, 2H, J = 8.8 Hz, H-Ar), 7.29-7.62 (m, 12H, H-Ar), 3.74 (s, 3H, H-OCH_3), 2.61 (s, 3H, H-CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 165.06, 164.29, 160.87, 159.90, 143.98, 142.31, 135.77, 135.54, 134.69, 132.79, 130.94, 129.18, 129.47, 129.31, 128.42, 127.66, 126.68, 122.34, 121.36, 114.54 107.99, 11.03. ESI-MS (m/z) 452.2 $(M+H)^+$. Anal. calculated for $C_{26}H_{21}N_5O_3$; C, 69.17; H, 4.69; N, 15.51. Found: C, 69.23; H, 4.72; N, 15.55.

General procedure for synthesis of *N*-(4-(5-aryl-3-(5-methyl-1,3,4-oxadiazol-2-yl)-1*H*-pyrazol-1-yl)phenyl)benzamide (P32, P40, P48).

The appropriate amine derivative **12a-c** (0.1 g) was dissolved in AcOH (2 mL) under nitrogen atmosphere. To the resulting reaction mixture, Ac_2O (5 eq) was added drop wise at 0 °C, after completion of the addition the reaction mixture was stirred at room temperature for the 1 h. Completion of the reaction was monitored on TLC using hexane/EtOAc as eluent. After completion of the reaction, the reaction mixture was poured into ice-water to afford yellow precipitates which were filtered at pump and dried; the solid obtained was purified by silica gel column chromatography using 1:1 mixture hexane and EtOAc as the eluent.

***N*-(4-(3-(5-Methyl-1,3,4-oxadiazol-2-yl)-5-(pyridin-3-yl)-1*H*-pyrazol-1-yl) phenyl)acetamide (P32):** Yield 0.11 g (96%). Off white solid. mp 212-213 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.15 (s, 1H, H-CONH), 8.61 (s, 1H, H-Ar), 8.09-7.98 (m, 2H, H-Ar), 7.26-7.56 (m, 6H, H-Ar), 2.59 (s, 3H, H-CH_3), 2.05 (s, 3H, H-CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.15, 164.28, 159.87, 150.18, 149.45, 142.28, 140.28, 137.95, 136.50, 133.88, 126.84, 125.46, 123.99, 119.65, 108.15 24.52, 11.01. ESI-MS (m/z) 361.1 $(M+H)^+$. Anal. calculated for $C_{19}H_{16}N_6O_2$; C, 63.32; H, 4.48; N, 23.32. Found: C, 63.38; H, 4.50; N, 23.35.

***N*-(4-(5-(4-Chlorophenyl)-3-(5-methyl-1,3,4-oxadiazol-2-yl)-1*H*-pyrazol-1-yl) phenyl)acetamide (P40):** Yield 0.1 g (93%). Off white solid. mp 242-243 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.15 (s, 1H, H-CONH), 7.66 (d, 2H, J = 8.4 Hz, H-Ar), 7.45 (d, 2H, J = 8.0 Hz, H-Ar), 7.22-7.38 (m, 5H, H-Ar), 2.59 (s, 3H, H-CH_3), 2.06 (s, 3H, H-CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.14, 164.26, 159.88, 142.31, 140.14, 136.95, 136.42, 135.31, 131.88, 129.84, 128.46, 123.65, 119.69, 108.16, 24.53, 11.02. ESI-MS (m/z) 394.5 $(M+H)^+$. Anal. calculated for $C_{20}H_{16}ClN_5O_2$; C, 60.99; H, 4.09; N, 17.78. Found: C, 61.01; H, 4.08; N, 17.76.

***N*-(4-(5-(4-Methoxyphenyl)-3-(5-methyl-1,3,4-oxadiazol-2-yl)-1*H*-pyrazol-1-yl) phenyl)acetamide (P48):** Yield 0.11 g (96%). Off white solid. mp 149-150 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.15 (s, 1H, H-CONH), 7.66 (d, 2H, J = 8.4 Hz, H-Ar), 7.17-7.30 (m, 5H, H-Ar), 6.94 (d, 2H, J = 8.4 Hz, H-Ar), 3.76 (s, 3H, H-OCH_3), 2.59 (s, 3H, H-CH_3), 2.07 (s, 3H, H-CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.15,

164.28, 161.25, 159.90, 143.01, 141.89, 136.76, 135.41, 129.83, 126.64, 122.32, 121.36, 115.69, 108.11, 55.41, 24.54, 11.02. ESI-MS (m/z) 390.1 $(M+H)^+$. Anal. calculated for $C_{21}H_{19}N_5O_3$; C, 64.77; H, 4.92; N, 17.98. Found: C, 64.82; H, 4.94; N, 17.96.

3.4 PHARMACOLOGY

AntiTB studies and molecular docking studies were performed as discussed in the **Section 2.4**

3.4.1 Cytotoxicity studies

Exponentially growing cells (10^5 cells/well) were plated in 96-well plates. After treatment of cells with the xenobiotics, the cell monolayers were gently washed with warm phosphate-buffered saline (PBS) using a multichannel pipette. The cells were then exposed to different concentrations of drug (10 - 50 µg/mL) and kept for incubation for 48 h. After the incubation, 100 mL of MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) solution (5 µg/mL) was added to each well and cells were incubated for additional 2 - 3 h. After careful removal of incubation medium from the incubator, 100 µL of dimethyl sulphoxide was added and the plates were gently shaken to re-suspend formed formazan and waited for few minutes to form a homogenized color. The suspension was placed on a micro vibrator for 5 minute, and absorbances of wells containing cells and blanks were recorded at 490 nm. The experiment was executed in triplicate. The mean of the absorbance of wells was calculated with the same treatment after subtracting of blank absorbance. The results were normalized by considering control wells as 100% (maximum absorbance obtained), expressing then the results as percentage of controls.

3.4.2 Antibacterial studies

The compounds were screened for antibacterial activity using the disc diffusion method (Isenberg, 1992) by measuring the zone of inhibition. Bacterial strains of *S. aureus*, *P. aeruginosa* and *E. coli* were cultivated in brain heart infusion agar medium for 24 h. The culture suspensions were prepared and adjusted by comparing against 0.5 McFarland turbidity standard tubes. The hollow tube of 5 mm diameter was taken and heated. Pressed it on above inoculated Agar plate and removed it immediately by making a well in the plate. All The compounds were

dissolved in DMSO and appropriate dilutions were made (75 and 50 µg/mL) and added to respective wells. DMSO was used as a solvent and as control. After the inoculation of organism and compound, the petri plates were incubated for 18-24 h at 37 $^{\circ}$C. Inhibition zones formed on the medium were evaluated in millimeter (mm). The negative solvent control (DMSO) did not show any antimicrobial activity. Studies were performed in triplicate and the average reading was taken. Inhibition zones were compared with those of the reference discs.

3.5 RESULTS AND DISCUSSION

3.5.1 Chemistry

The structure of the intermediates and target compounds were confirmed by ^{1}H NMR, ^{13}C NMR and mass spectral techniques followed by elemental analysis. The ^{1}H NMR spectrum of **7a** displayed a triplet signal at δ 4.32 ppm and a quartet at δ 1.33 ppm due to methylene (-CH_2-) and methyl (-CH_3) protons respectively, of the ester (-$COOC_2H_5$) group. Further, the presence of signals in the region δ 8.60 - 7.34 ppm due to four aromatic protons of pyridine ring confirms the formation condensed product **7a**. The ^{1}H NMR spectra of compounds **8a-c** show additional four protons in the aromatic region and a sharp singlet (at δ 7.34, 7.18 and 7.17 ppm for **8a**, **8b** and **8c** respectively) due to new aromatic ring and pyarazole ring proton which confirm the formation of 1,3,5-trisubstituted pyrazoles (**Figure 3.3**). The structure of the compound was further confirmed by ^{13}C NMR spectrum (**Figure 3.4**). The ^{1}H NMR spectrum of **9a** shows two new signals at around δ 9.74 and 4.53 ppm corresponding to -NH- and -NH_2 protons respectively, of the carbohydrazide group (**Figure 3.5**). The structure of the compound was further confirmed by ^{13}C NMR spectrum **(Figure 3.6)**. Also, disappearance of triplet and quartet signals of ethyl ester protons further confirms the formation of the hydrazide intermediates. The -NH_2 signal was completely absent in the ^{1}H NMR spectrum of N-acylated intermediate **10a**, whereas two new signals appeared at δ 10.18 and 9.89 ppm respectively due to -NH and -CH_3 groups (**Figure 3.7)**. The successful cyclization of **10a-c** to form oxadiazole derivatives **11a-c** was established by both ^{1}H and ^{13}C NMR data. For instance, absence of both -NH- signals and shift of -CH_3 signal from δ 1.91 to 2.61 ppm the ^{1}H NMR spectrum and also shift of -CH_3 signal from δ 20.06 to 11.03 ppm in the ^{13}C NMR spectrum of **11a** supports the successful cyclization of **10a**. The synthesis of

intermediate **12a-c** by the reduction of nitro group in **11a-c** to amine group was well supported by the appearance of a new broad singlet for -NH_2 group at around δ 5.5 ppm in their ^{1}H NMR spectra (**Figure 3.8)**. The structure of target compounds, *N*-(4-(5-aryl-3-(5-methyl-1,3,4-oxadiazol-2-yl)-1*H*-pyrazol-1-yl)phenyl)-4-amides, was well confirmed by spectral methods and the characterization data are given in the experimental part. For instance, successful acid-amine coupling to form an amide group in target compound **P25** is evident by the disappearance of -NH_2 signal and appearance of new amide -NH- signal at δ 10.51 ppm in its ^{1}H NMR spectrum (**Figure 3.9)**. Further, the spectrum showed the presence of four additional protons in the aromatic region due to the introduction of a new phenyl ring. The ^{13}C NMR spectrum of **P25** matches well with its structure (**Figure 3.10)**. The mass spectrum of **P25** showed a molecular ion peak at m/z 441.2, which corresponds to its molecular formula $C_{24}H_{17}FN_6O_2$ (Mw = 440.43) (**Figure 3.11)**. Similarly, the formation of acetamide derivatives (**P32, P40** and **P48**) was confirmed by both ^{1}H and ^{13}C NMR spectra. For instance, formation of N-(4-(3-(5-methyl-1,3,4-oxadiazol-2-yl)-5-(pyridin-3-yl)-1H-pyrazol-1-yl)phenyl) acetamide (**P32**) was supported by the appearance of additional -CH_3 signals at δ 2.05 ppm and δ 24.52 ppm in its ^{1}H NMR and ^{13}C NMR spectra respectively whereas the -NH_2 signal was completely absent in the ^{1}H NMR spectrum. Further, the structure one of the final derivatives (**P40**) was achieved by SC-XRD analysis and the ORTEP diagram for the compound is shown in **Figure 3.12**.

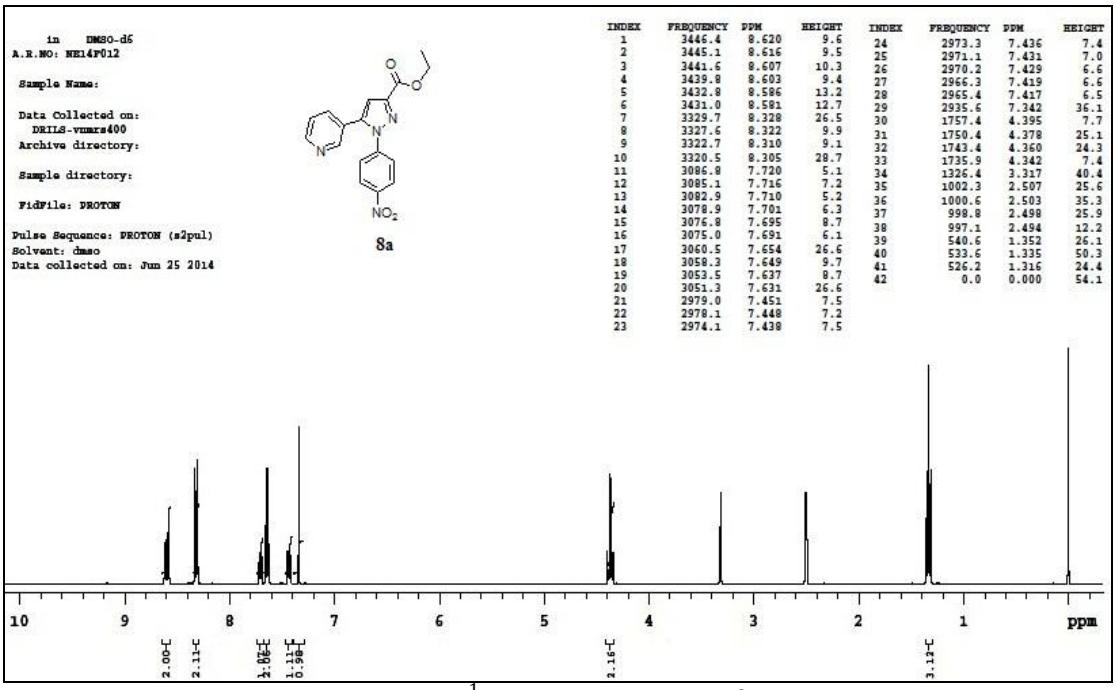


Figure 3.3 ^{1}H NMR spectrum of **8a.**

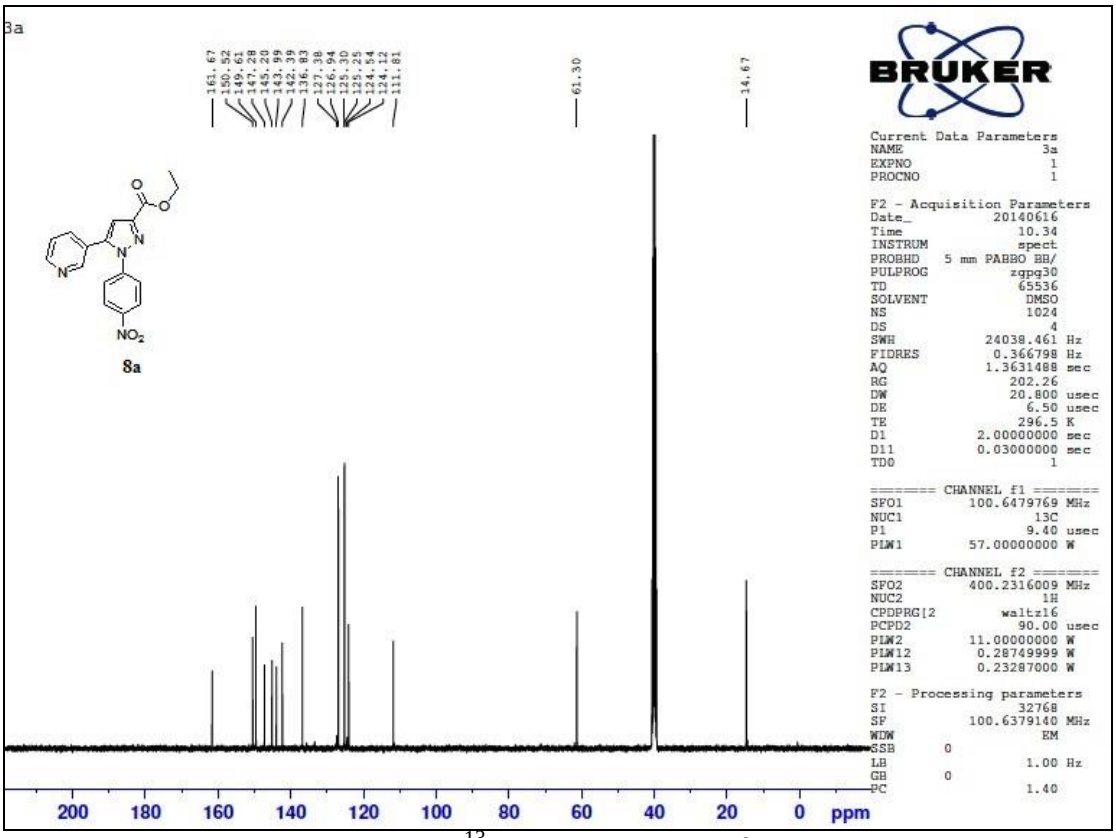


Figure 3.4 ^{13}C NMR spectrum of **8a.**

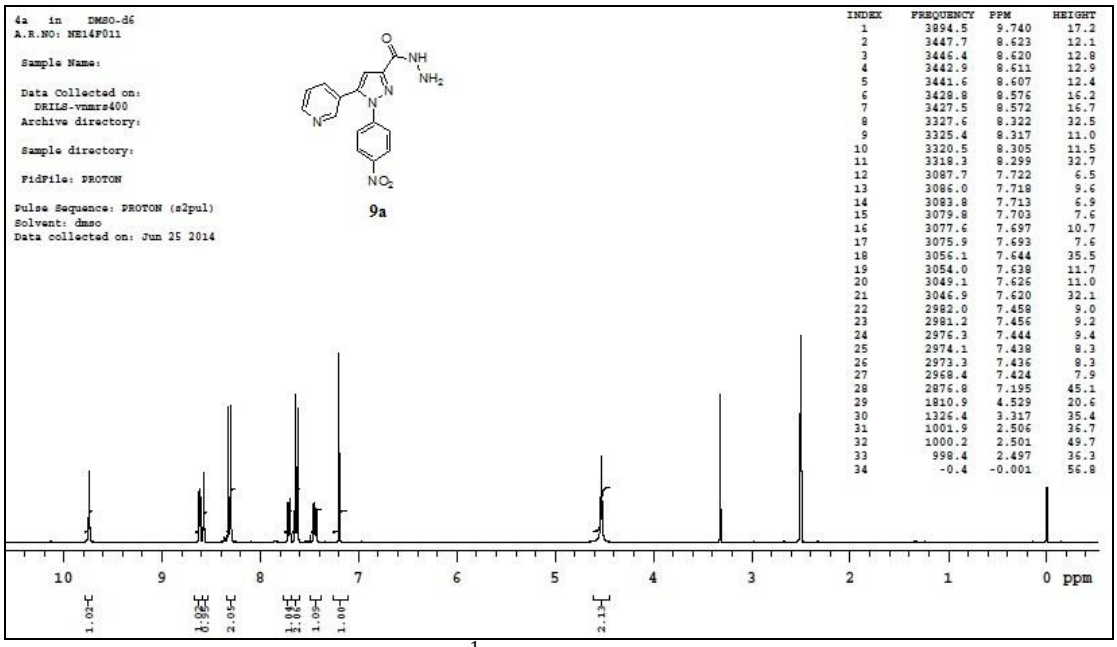


Figure 3.5 ^{1}H NMR spectrum of **9a.**

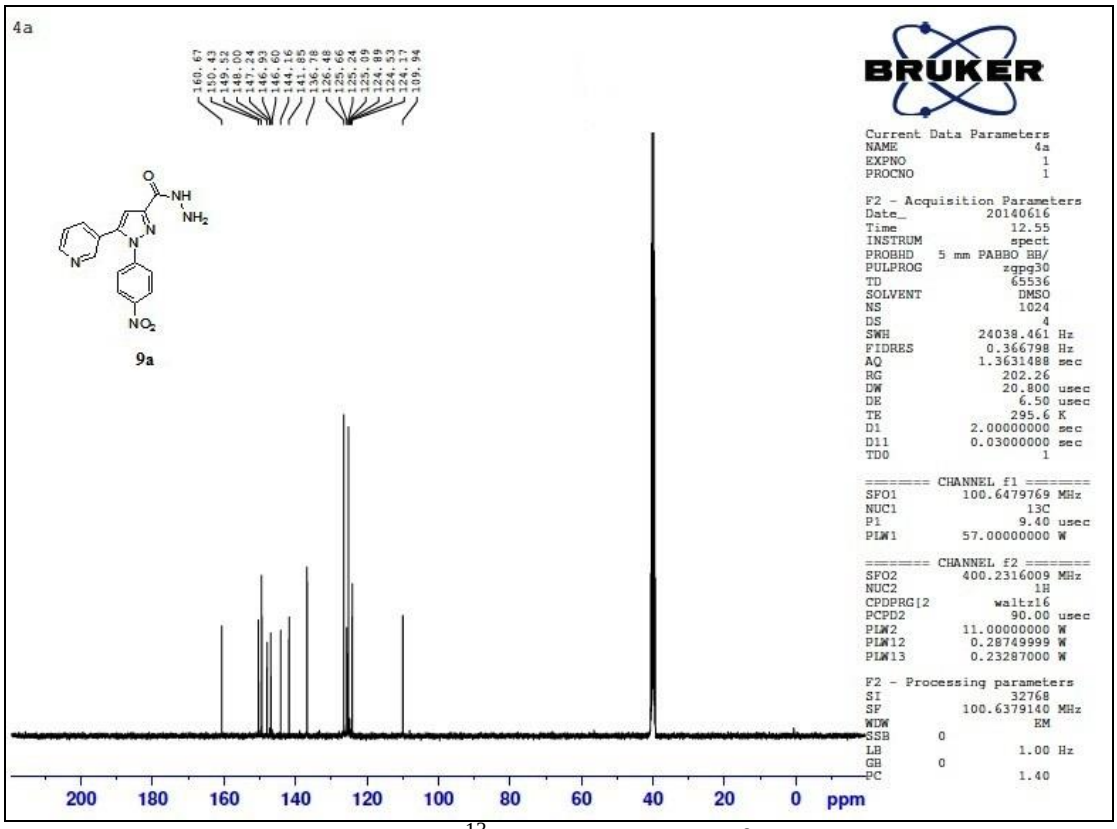


Figure 3.6 ^{13}C NMR spectrum of **9a.**

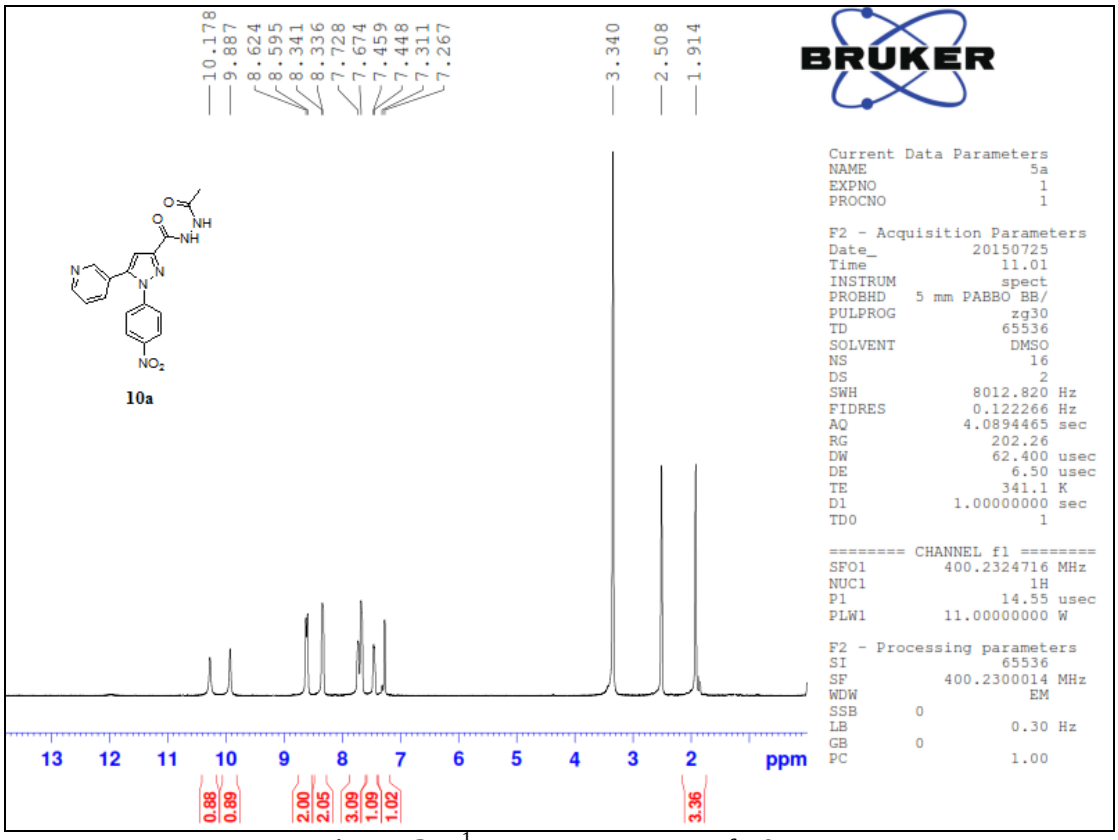


Figure 3.7 ^{1}H NMR spectrum of **10a.**

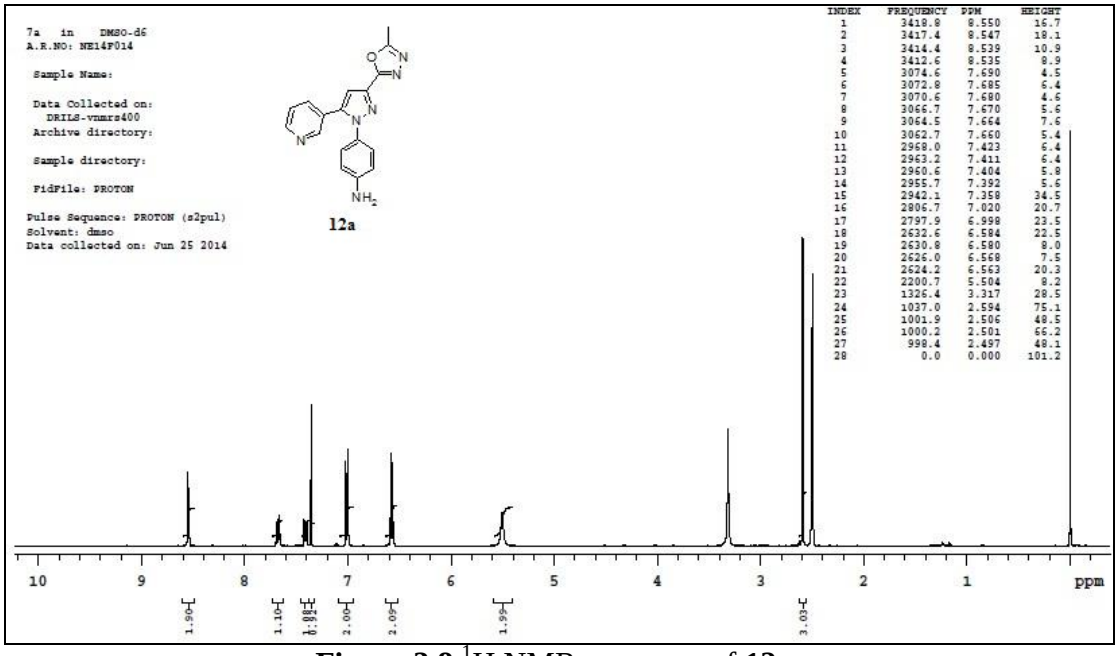


Figure 3.8 ^{1}H NMR spectrum of **12a.**

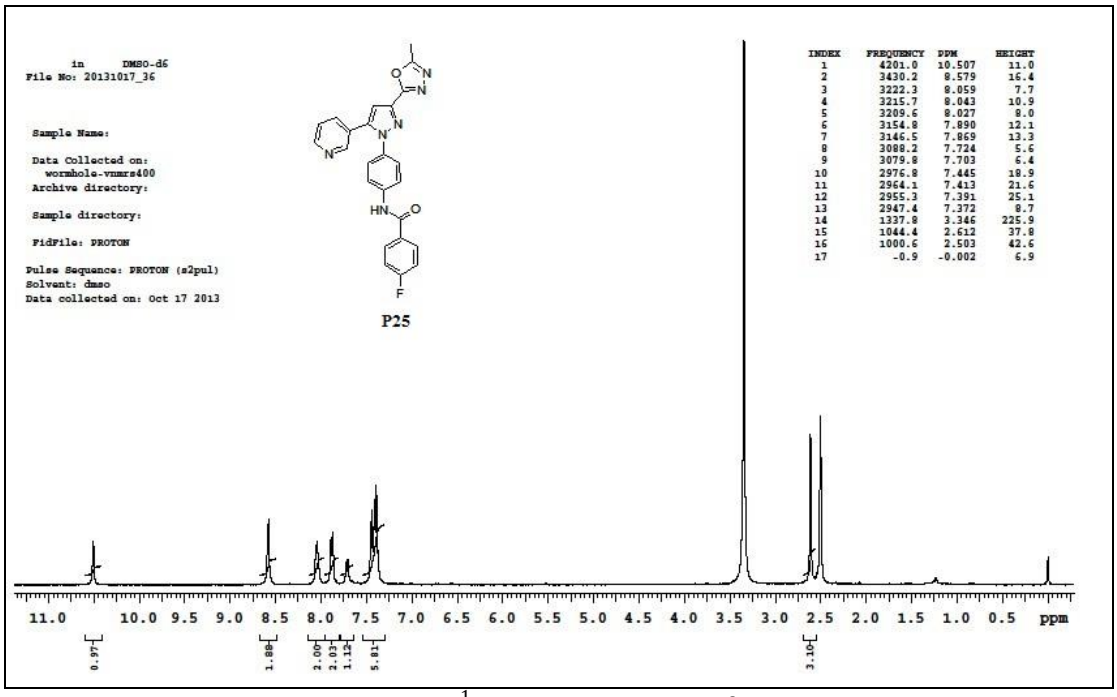


Figure 3.9 ^{1}H NMR spectrum of **P25.**

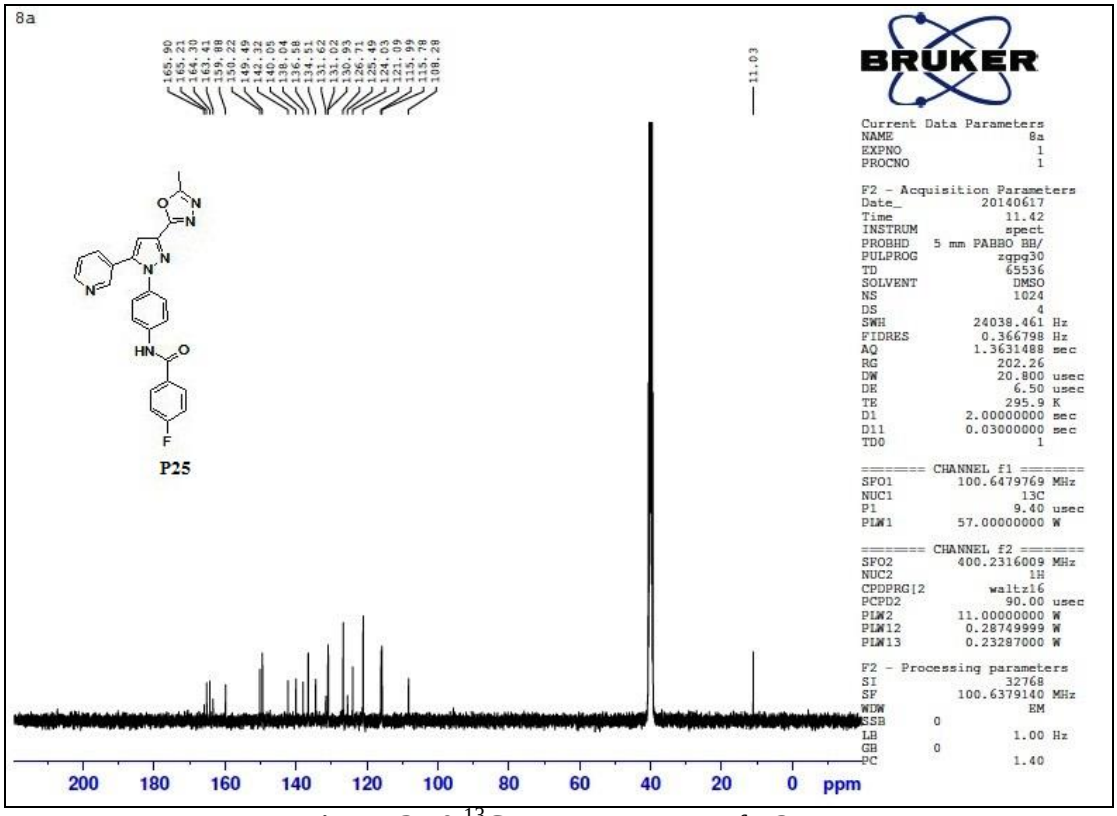


Figure 3.10 ^{13}C NMR spectrum of **P25.**

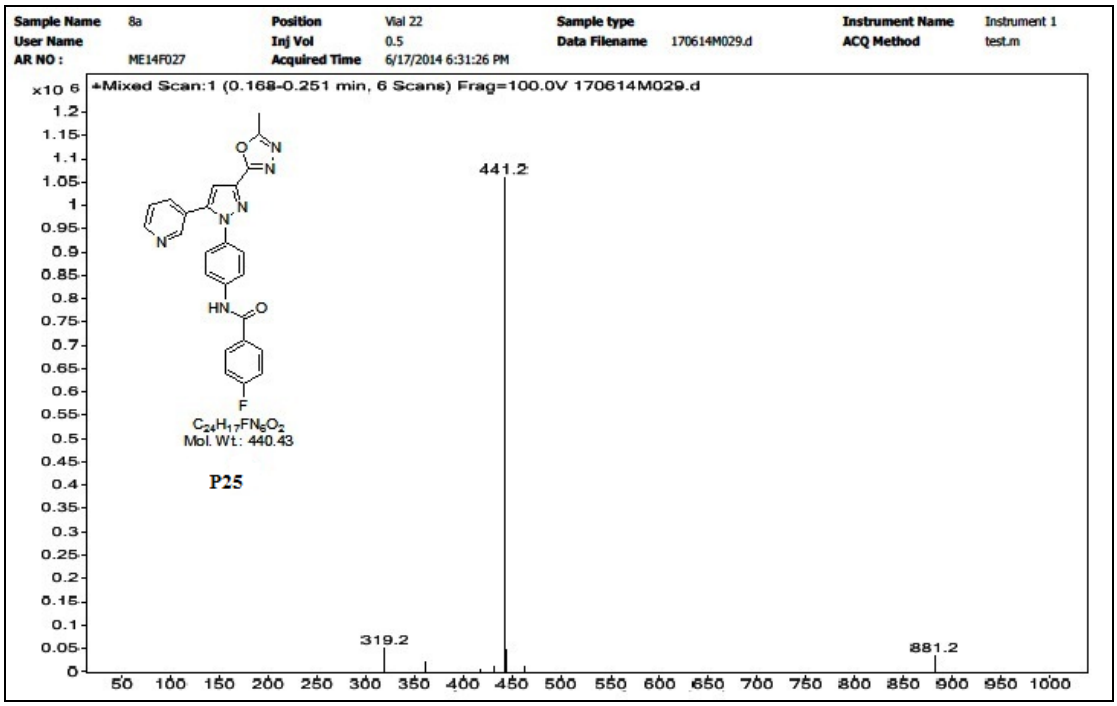


Figure 3.11 Mass spectrum of **P25.**

3.5.2 X-ray crystallographic analysis of P40.

The single crystal of one of the target compounds, *N*-(4-(5-(4-chlorophenyl)-3-(5-methyl-1,3,4-oxadiazol-2-yl)-1*H*-pyrazol-1-yl)phenyl)acetamide (**P40**) was grown by the slow evaporation of a 1:1 mixture of methanol-chloroform solution at RT through a parafilm plastic containing pinholes. The ORTEP diagram of **P40** and details of the crystal data are given in **Figure 3.12** and **Table 3.2** respectively.

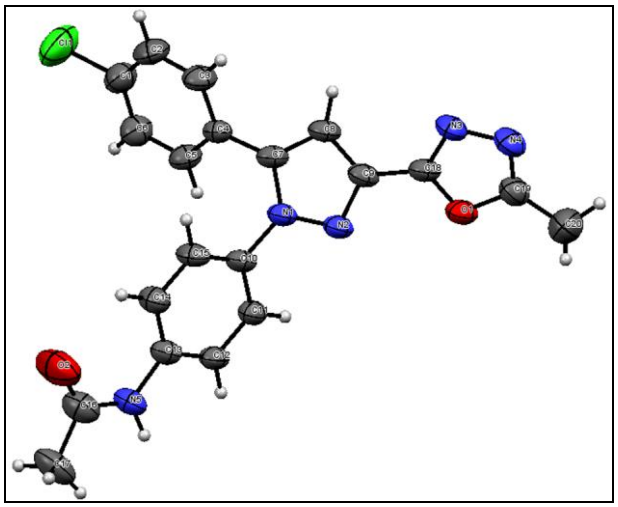


Figure 3.12 The ORTEP diagram showing the X-ray crystal structure of **P40.**

Table 3.2 Crystal and structure refinement data for compound **P40**.

Parameters	**Crystal data**
Chemical formula	$C_{20}H_{16}ClN_5O_2$
Formula weight	393.83
CCDC No.	1051636
Crystal system	Monoclinic
Space group	P 2_1
a (Å)	10.9334 (10)
b (Å)	7.3787 (8)
c (Å)	12.5319 (12)
Volume ($Å^3$)	1007.71 (17)
Angle α, β, γ	90, 94.623 (6), 90
Z	2
F_{000}	408.0
μ (mm^{-1})	0.215
Temperature (T)	296 K
Radiation wavelength (Å)	0.71073
Radiation type	Mo Kα
R-Factor (%)	5.37

3.5.3 Antitubercular studies

The *in vitro* antimycobacterial activity of the newly synthesized compounds **P25-P48** was evaluated by screening them against MTB. The comparison of the MIC values (in μg/mL) of **P25-P48** with the standard drugs is shown in **Figure 3.13.** The compound, 2-chloro-*N*-(4-(5-(4-chlorophenyl)-3-(5-methyl-1,3,4-oxadiazol-2-yl)-1*H*-pyrazol-1-yl)phenyl)benzamide (**P38**) is the lead molecule of the series with MIC of 1.56 μg/mL and is more potent than the standard EMB. Whereas, the non substituted benzamide derivative **P39** is the next most active molecule with MIC of 3.13 μg/mL, the activity of which is comparable with that of EMB. Derivatives **P29**, **P36** and **P48** showed promising antiTB activity with MIC of 6.25 μg/mL while moderate inhibition was observed with compounds **P37**, **P43**, **P44** and **P45** (MIC = 12.5 μg/mL). It is evident from the MIC values that the antiTB activity varies with different substitution

(R^1) at position-5 of the pyrazole ring. Further the activity also depends on nature and position of the substituent on the phenyl ring of the benzamide moiety (R^2). Among compounds derived from three different scaffolds (**12a-c**), most analogues of 4-chloro phenyl substituted pyrazole scaffold (**12b**) showed better antiTB activity when compared with the activity of those derived from pyridin-3-yl- (**12a**) and 4-methoxy phenyl substituted pyrazole (**12c**) scaffolds. Except the nitro derivative **P29**, no other pyridine derivatives showed promising activity. In the case of 4-chloro phenyl substituted pyrazoles, it is interesting to know that though 2-chloro benzamide derivative (**P38**) is significantly active (MIC = 1.56 µg/mL) corresponding 4-chloro derivative did not show a prominent activity (MIC = 50 µg/mL). This observation indicates that variation in the position (ortho or para) of the chloro group largely effects the activity of these molecules, the exact reason for this effect may be explored by further studies on structure-activity relationship or by computer aided modeling studies. The promising activity of 4-chloro phenyl substituted pyrazoles prompted us to investigate the activity of corresponding intermediates **9b**, **10b**, **11b** and **12b**. Among the four compounds, **10b** showed significant activity with MIC of 3.13 µg/mL (**Figure 3.13**) while the other three compounds were not so active (MIC = 50 µg/mL). It can be seen that the replacement of the N-acylcarbohydrazide (-$CONHNHCOCH_3$) functionality in **10b** with either a carbohydrazide (-$CONHNH_2$) group (**9b**) or an oxadiazole group (**11b**) reduced the activity of the molecules. This observation signifies the contribution of the acylcarbohydrazide functionality towards the antiTB activity. So, compound **10b** could be considered as a lead molecule for further structural modifications and biological studies in order to develop efficient antiTB drugs.

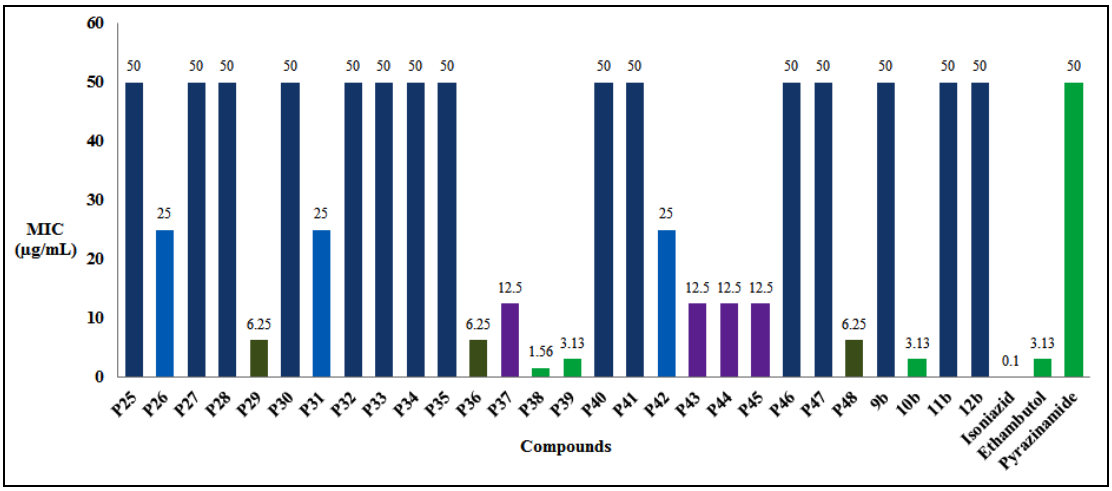


Figure 3.13 The antiTB activity of pyrazole-1,3,4-oxadiazole derivatives (**P25-P48**), and intermediates **9b**, **10b**, **11b** and **12b**.

3.5.4 Cytotoxicity studies

The *in vitro* cytotoxicity of the active molecules (MIC ≤ 6.25 µg/mL) was assessed by MTT assay against NIH 3T3 cells at 50 µg/mL concentration. The compounds are non-toxic at the tested concentration with cell growth inhibition values in the range 16.88 - 22.18% (**Figure 3.14**). Interestingly, intermediate **10b** showed the least toxicity (8.36% inhibition).

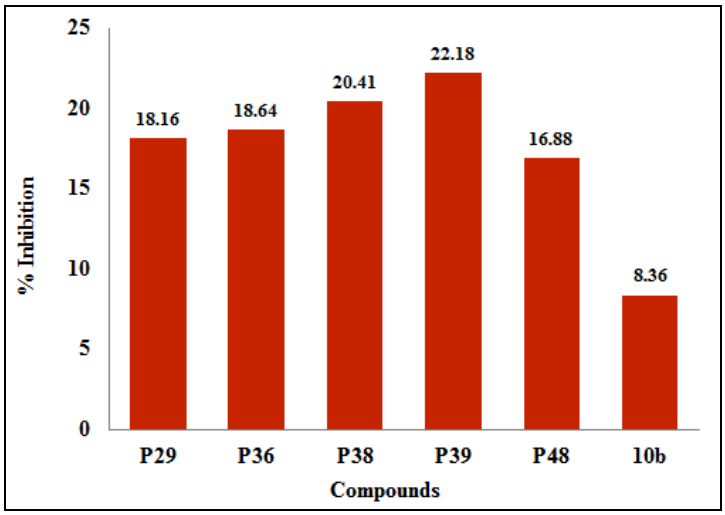


Figure 3.14 The cytotoxicity of the active derivatives against NIH 3T3 cells at 50 µg/mL concentration.

3.5.5 Molecular docking studies

The docking poses of the active antiTB molecules **P29, P36, P38, P39, P48** and **10b** against InhA showed moderated docking score (-6.48 to -8.49) and all the ligands showed at least one interaction with the amino acid residues. In the case of final derivatives (**P29, P36, P38, P39** and **P48)** interaction with Phe 149 (**Figure 3.15**) is found to be common which is also present in the standard ligand of 1P44 (**Table 3.3**). Intermediate **10b** showed interaction with Tyr 158. In case of CYP121 the docking score was found in the range of -7.43 to -8.69. It is interesting to note that the most active molecule of the series **P38** (MIC 1.56 µg/mL) showed high docking score of -8.60 and four significant interaction with the residues (**Figure 3.16**). In the case of protein TMPK all the ligands showed more than three interactions **(Figure 3.17)** but the docking score (-5.80 to -7.34) was found to be much lower than that of the standard ligand of the protein (-13.12). From the docking results it can be concluded that these molecules show stronger binding with the protein CYP121 than the other two proteins.

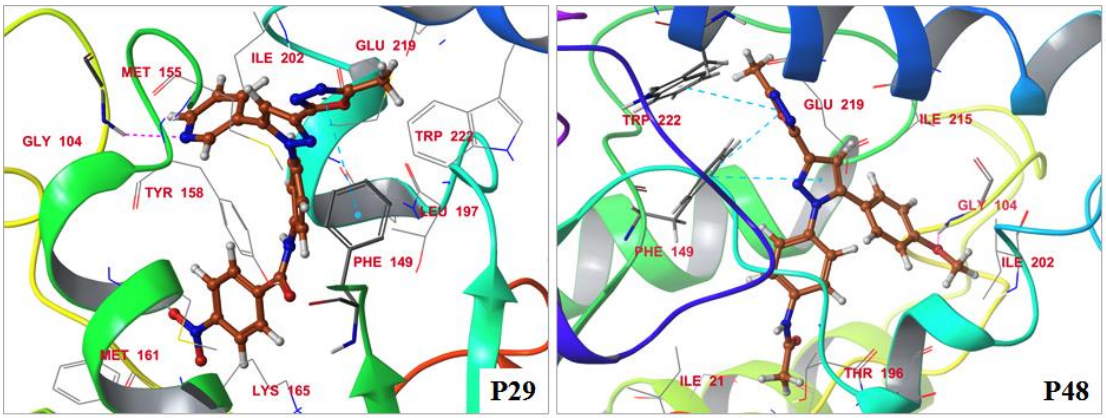


Figure 3.15 The docking pose of the compounds **P29** and **P48** in the active pocket of InhA.

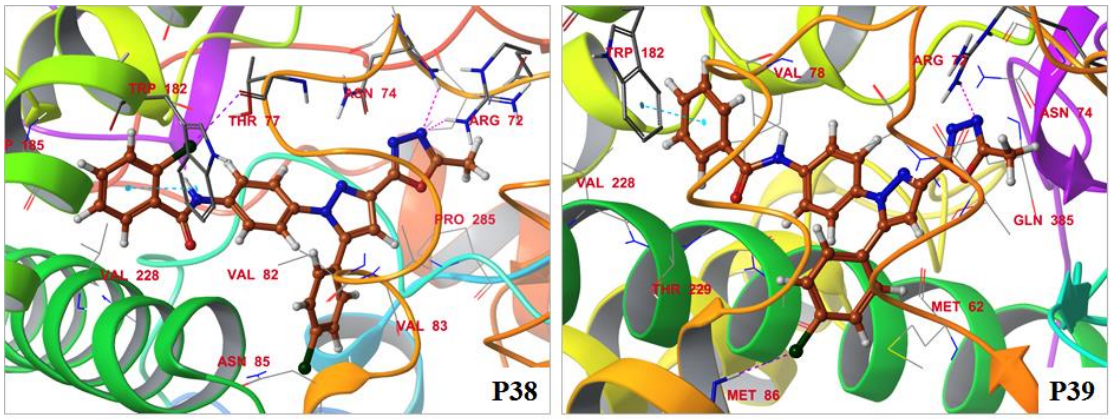


Figure 3.16 The docking pose of the compounds **P38** and **P39** in the active pocket of CYP121.

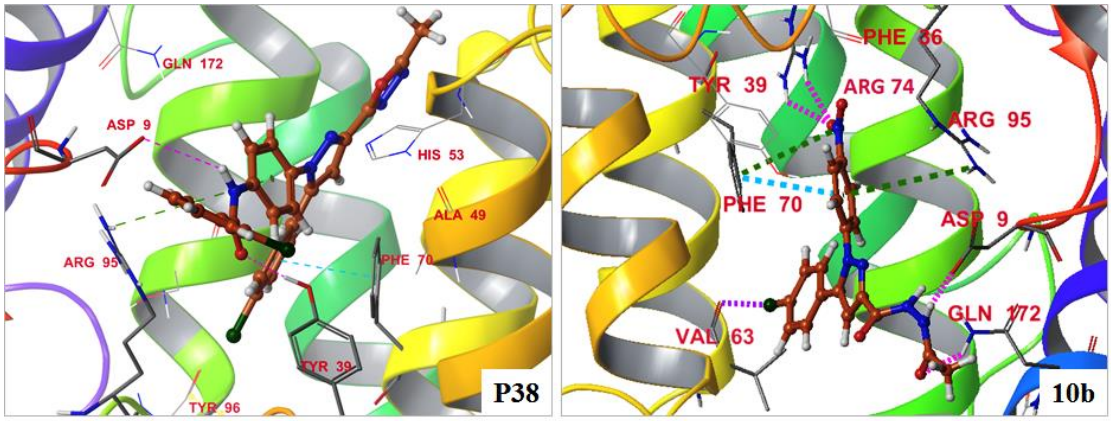


Figure 3.17 The docking pose of the compounds **P38** and **10b** in the active pocket of TMPK.

Table 3.3 Docking results of the active antiTB compounds

Compound ID	InhA		CYP121		TMPK	
	Docking score	**Amino acids that interacted with ligands**	**Docking score**	**Amino acids that interacted with ligands**	**Docking score**	**Amino acids that interacted with ligands**
Standard Ligand	-12.51	Tyr 158, Phe 149	-10.38	Gln 385, Val 228, Asn 85	-13.12	Asn 100, Tyr 103, Arg 74, Arg 95,
P29	-8.49	Phe 149,	-7.43	Asn 74	-5.80	Arg 95,

		Gly 104				Gly 10, Lys 13
P36	-7.57	Phe 149	-8.18	Asn 74, Trp 182	-6.09	Arg 95, Gly 10, Lys 13
P38	-8.23	Phe 149	-8.60	Trp 182, Thr 77, Asn 74, Arg 72	-6.45	Arg 95, Asp 9, Tyr 39, Phe 70
P39	-7.51	Phe 149	-8.35	Trp 182, Arg 72, Met 86	-6.19	Arg 95, Gly 10, Lys 13
P48	-7.81	Phe 149, Trp 222	-7.58	Asn 74, Asn 85	-7.34	Arg 95, Asp 9, Arg 74, Phe 70
10b	-6.48	Tyr 158	-6.70	Asn 74, Phe 168, Met 86	-6.74	Arg 95, Phe 70, Arg 74, Asp 9, Gln 172, Val 63,

3.5.6 Antibacterial studies

The *in vitro* antibacterial activities of the synthesized compounds **P25-P48** were determined via disc diffusion method by measuring zone of inhibition in millimeter. Ciprofloxacin was used as the standard drug. The compounds were screened against three common pathogenic bacterial strains *viz. S. aureus, E. coli* and *P. aeruginosa*. The test compounds were dissolved in dimethylsulfoxide (DMSO) and tested at two concentrations, 75 and 50 µg/mL. Each experiment was performed in triplicate and the average reading was taken considering its standard deviation. The zone of inhibition values of the tested compounds are presented in **Table 3.4.** On the basis of zone of inhibition values the compounds were classified as highly active (≥20 mm), moderately active (16-20 mm), and least active (≤15 mm). Accordingly, compounds **P25, P26** and **P31** have shown excellent activity compared with other compounds of the series. Notably, all these compounds are the analogs of pyridin-3-yl- substituted derivatives of pyrazole.

Table 3.4 Antibacterial activity of compounds **P25-P48**.

Compound	Zone of inhibition (mm)					
	Staphylococcus aureus		***Escherichia coli***		***Pseudomonas aeruginosa***	
Conc. in µg/ml	75	50	75	50	75	50
Control	00	00	00	00	00	00
Ciprofloxacin	26±0.1	21±0.2	32±0.2	27±0.2	21±0.2	18±0.1
P25	**24±0.1**	**20±0.3**	**28±0.2**	**25±0.2**	**19±0.3**	**16±0.1**
P26	**23±0.2**	**20±0.3**	**29±0.2**	**27±0.3**	**20±0.1**	**17±0.1**
P27	15±0.2	13±0.2	15±0.4	10±0.3	15±0.2	12±0.2
P28	**18±0.3**	**15±0.2**	**16±0.1**	**13±0.2**	**16±0.4**	**14±0.3**
P29	10±0.2	08±0.3	12±0.2	10±0.2	14±0.3	11±0.3
P30	08±0.1	06±0.1	12±0.2	10±0.2	14±0.2	12±0.4
P31	**22±0.4**	**20±0.3**	**26±0.3**	**20±0.3**	**20±0.4**	**17±0.1**
P32	12±0.2	09±0.2	10±0.1	08±0.2	12±0.4	10±0.2
P33	**18±0.1**	**16±0.2**	**22±0.2**	**20±0.3**	**18±0.1**	**15±0.2**
P34	**19±0.1**	**16±0.1**	**22±0.1**	**18±0.1**	**17±0.1**	**15±0.2**
P35	09±0.2	07±0.3	15±0.4	10±0.3	14±0.3	12±0.1
P36	09±0.1	06±0.2	15±0.3	10±0.2	10±0.2	07±0.4
P37	--	--	10±0.4	08±0.2	14±0.3	12±0.1
P38	--	--	10±0.4	08±0.2	15±0.2	14±0.2
P39	12±0.2	09±0.3	--	--	--	--
P40	13±0.3	11±0.3	12±0.1	09±0.1	12±0.1	10±0.1
P41	--	--	10±0.1	08±0.1	14±0.1	12±0.3
P42	12±0.2	09±0.4	14±0.2	10±0.2	14±0.3	10±0.1
P43	11±0.4	09±0.3	12±0.2	10±0.2	16±0.2	14±0.1
P44	10±0.1	08±0.3	12±0.2	10±0.2	--	--
P45	--	--	10±0.3	07±0.4	14±0.2	12±0.3
P46	**18±0.2**	**15±0.1**	**20±0.4**	**16±0.3**	**17±0.2**	**15±0.2**
P47	**19±0.2**	**14±0.2**	**18±0.4**	**16±0.3**	**18±0.4**	**14±0.1**
P48	12±0.4	07±0.3	10±0.3	07±0.2	14±0.1	12±0.3

--: Bacteria resistant; Control: dimethylsulfoxide

Thus the pyridine-3-yl substitution at position-5 of the pyrazole ring plays a pivotal role in enhancing the antibacterial activity of the molecule. Derivatives **P28, P33, P34, P46** and **P47** showed moderate antibacterial activity against all the three tested strains. Overall, five (**P25, P26, P33, P34** and **P46**) out of eight active compounds have electron-withdrawing halogen groups namely, fluoro (**P25** and **P33**) and chloro (**P26**, **P34** and **P46**), attached to the aryl ring of the carboxamide linkage ($\mathbf{R^2}$). This testifies that electron-withdrawing halogen group on the aryl ring of $\mathbf{R^2}$ plays a major role in enhancing the activity of the compounds.

3.6 CONCLUSIONS

- In this study, we demonstrated the synthesis, characterization and biological study of a new series of *N*-(4-(5-aryl-3-(5-methyl-1,3,4-oxadiazol-2-yl)-1*H*-pyrazol-1-yl)phenyl)-4-amide analogs.
- All the target compounds were characterized by ^{1}H NMR, ^{13}C NMR, mass spectral and elemental analyses and the structure of the final derivative **P40** was established by SC-XRD analysis.
- Nine compounds showed promising antiTB activity against MTB strain with MIC ≤ 12.5 µg/ml among which 2-chloro-*N*-(4-(5-(4-chlorophenyl)-3-(5-methyl-1,3,4-oxadiazol-2-yl)-1*H*-pyrazol-1-yl)phenyl)benzamide (**P38**) was the most active molecule with a MIC of 1.56 µg/mL.
- The structure and activity relationship revealed that the 4-chloro phenyl substituted pyrazole derivatives are better antiTB agents than the corresponding pyridine and 4-methoxy phenyl substituted derivatives.
- Among the 4-chloro phenyl substituted pyrazole intermediates (**9b**-**12b**), compound **10b** containing an N-acylcarbohydrazide group showed significant activity (MIC = 3.13 µg/mL).
- The cytotoxicity studies further establish that the active molecules of the series are non-toxic to a normal cell line and hence are potential lead molecules for the drug development process.
- *In silico* molecular modeling study revealed that the active compounds have a good binding pose in the active pockets of CYP121 protein.
- Compounds **P25, P26** and **P31** showed excellent inhibition activity against the tested bacterial strains.

Appendix 3.1

Representative ^{1}H NMR, ^{13}C NMR and ESI-MS spectra of final compounds

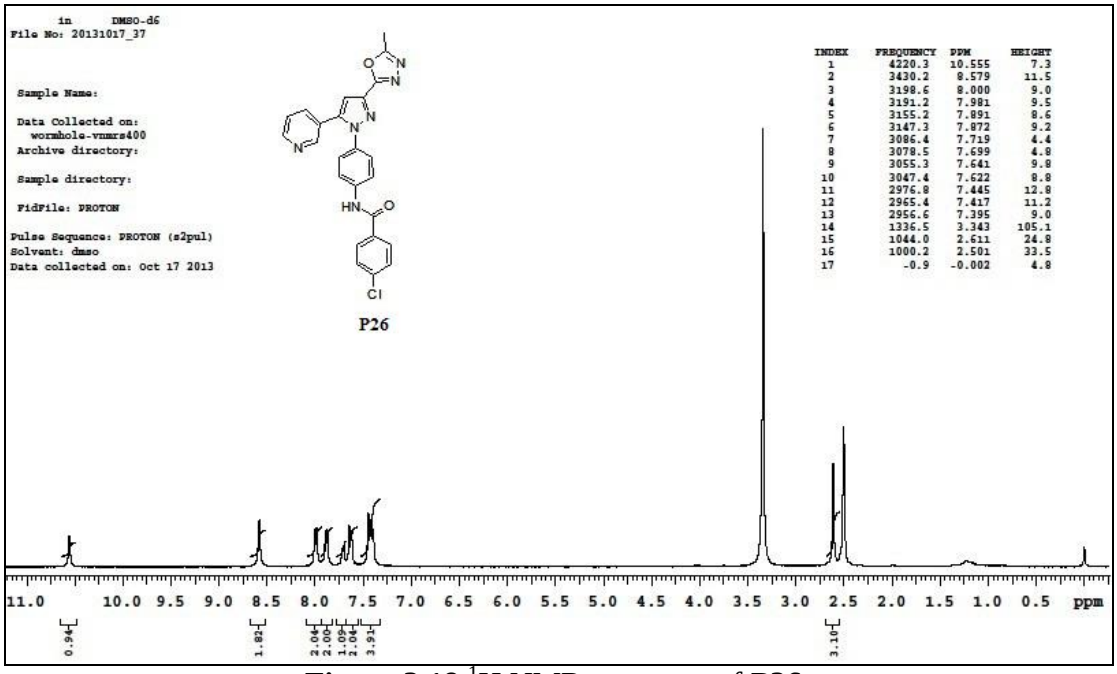


Figure 3.18 ^{1}H NMR spectrum of **P26.**

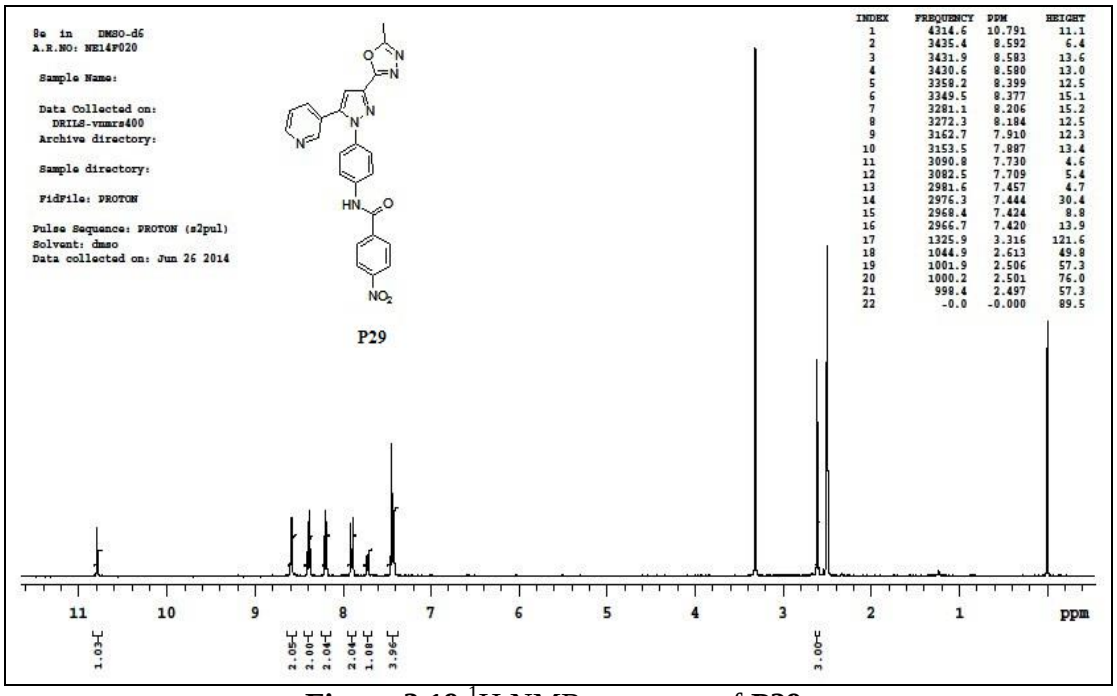


Figure 3.19 ^{1}H NMR spectrum of **P29.**

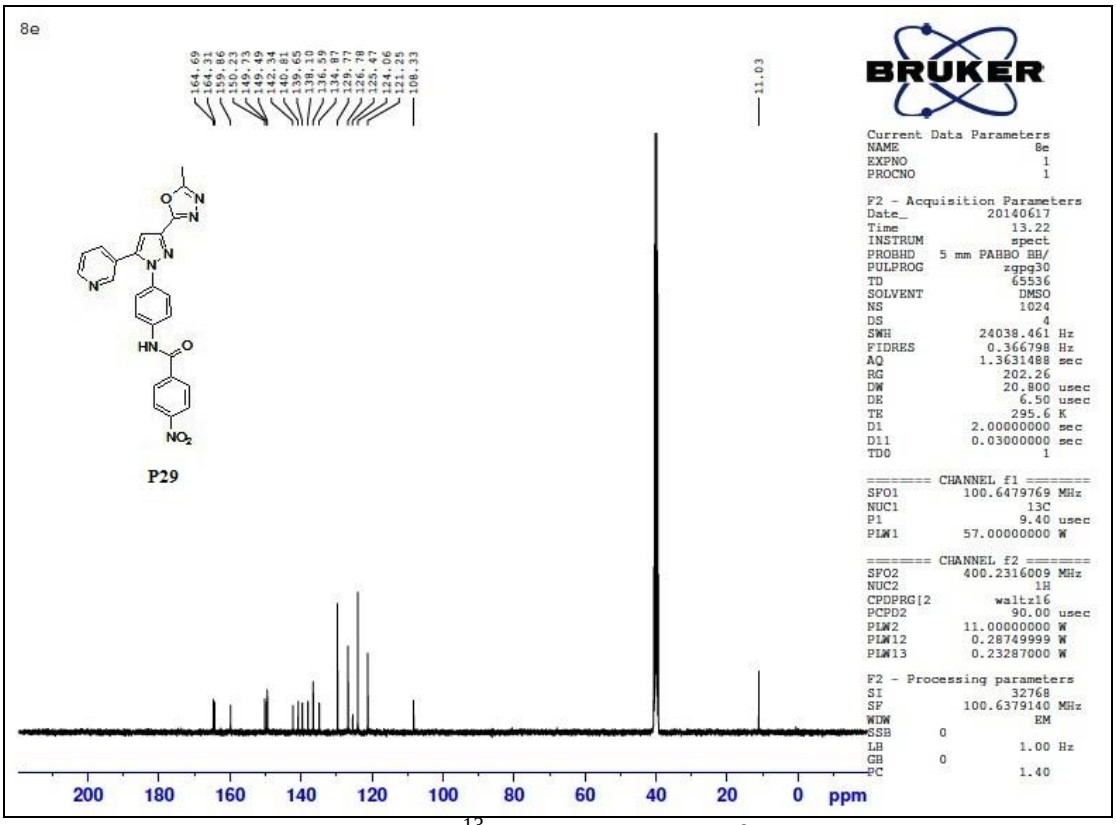


Figure 3.20 ^{13}C NMR spectrum of **P29.**

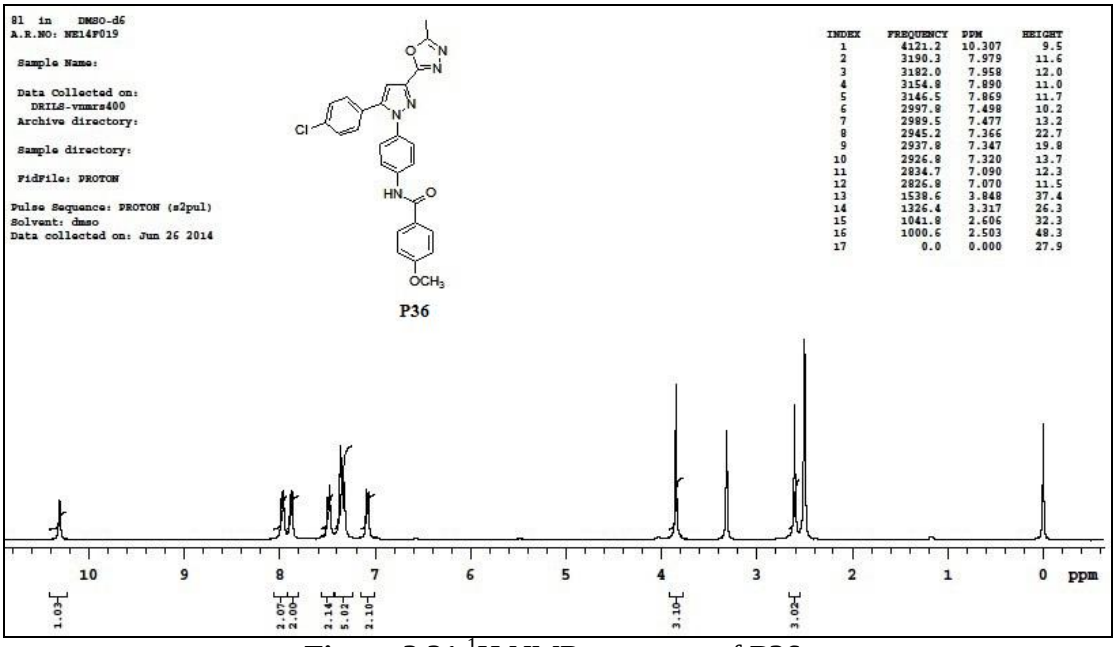


Figure 3.21 ^{1}H NMR spectrum of **P36.**

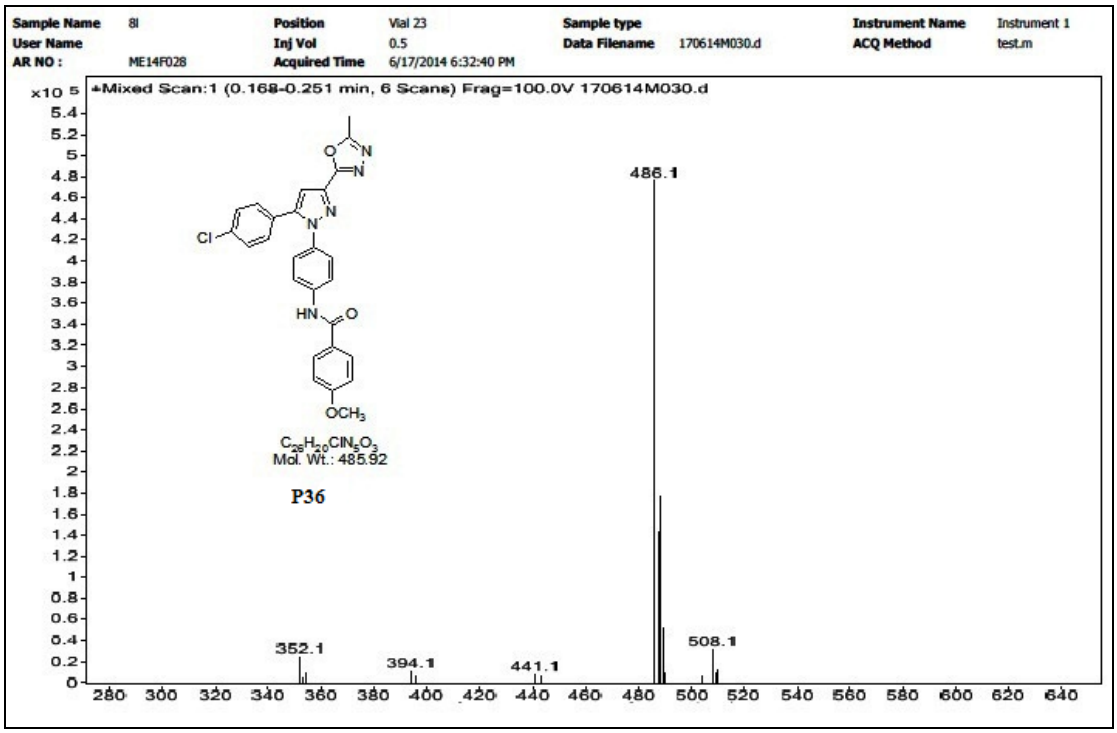


Figure 3.22 Mass spectrum of **P36.**

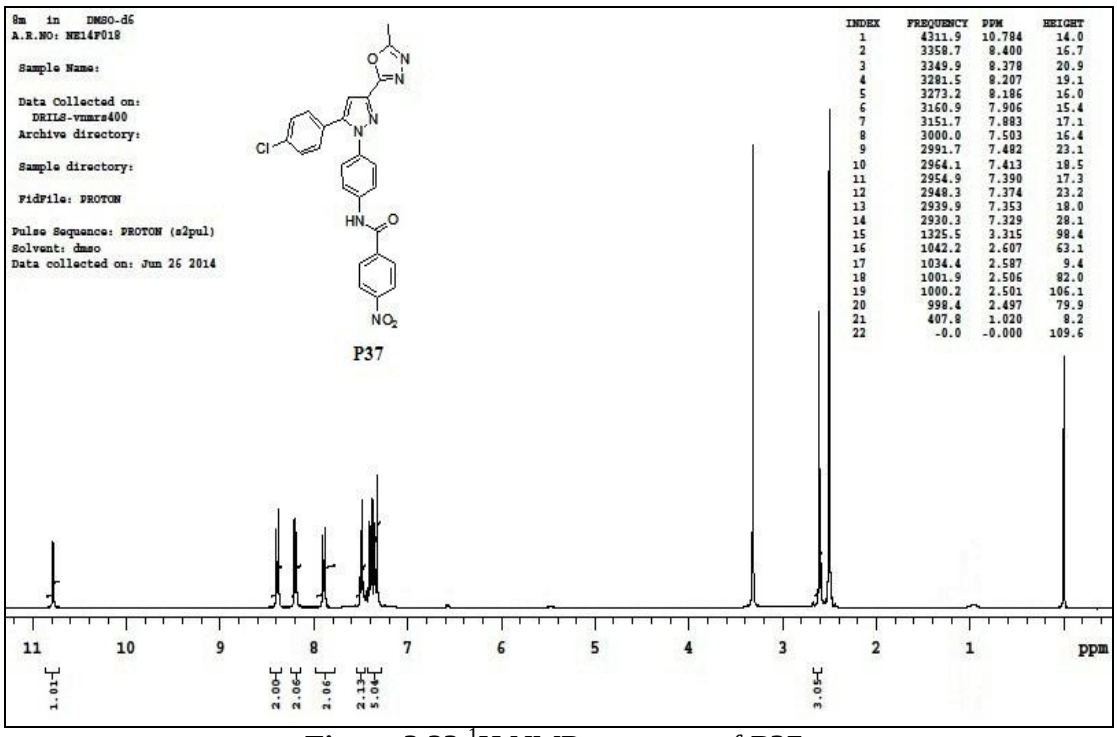


Figure 3.23 ^{1}H NMR spectrum of **P37.**

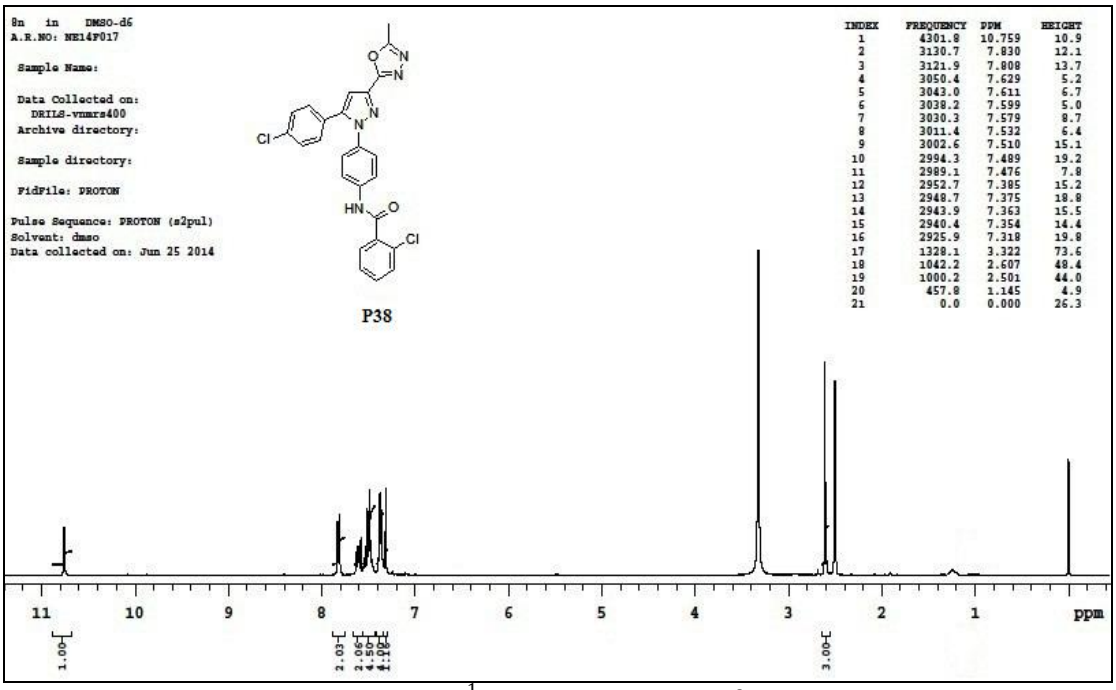


Figure 3.24 ^{1}H NMR spectrum of **P38.**

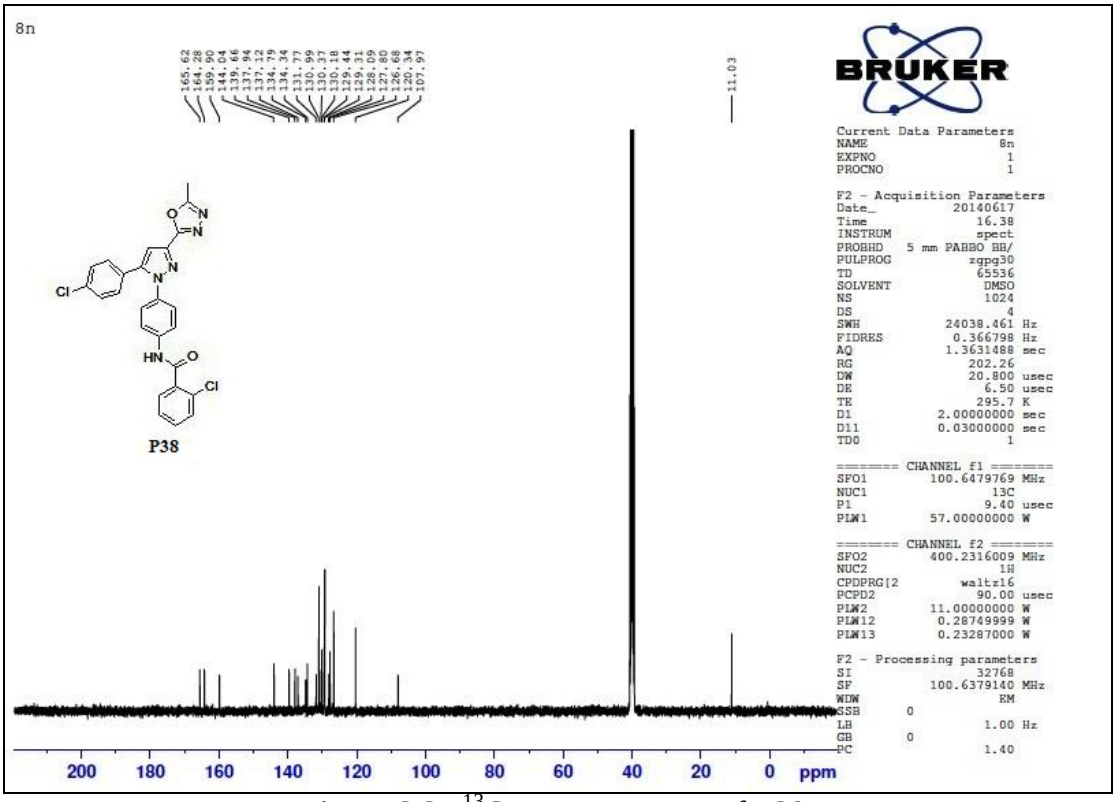


Figure 3.25 ^{13}C NMR spectrum of **P38.**

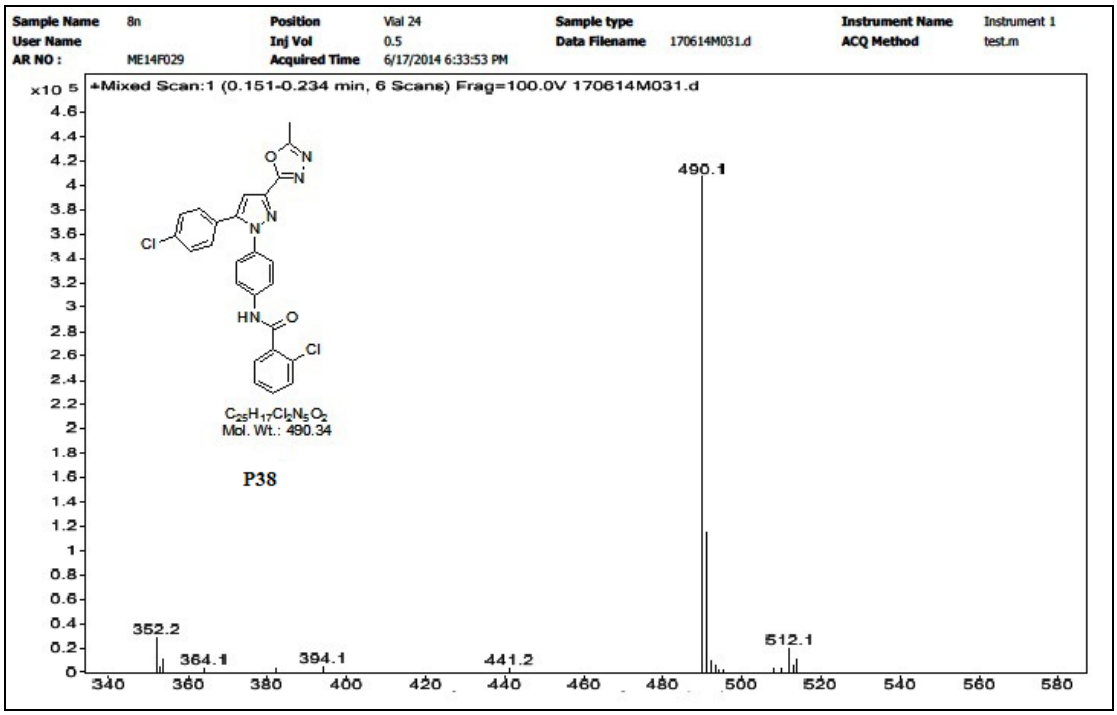


Figure 3.26 Mass spectrum of **P38.**

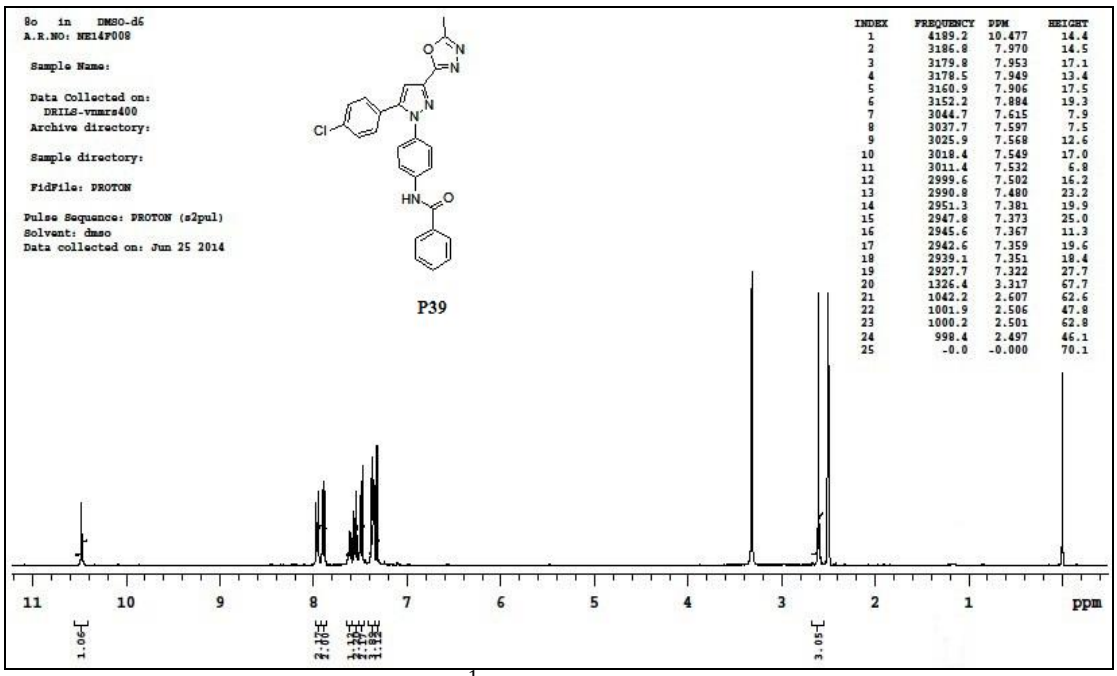


Figure 3.27 ^{1}H NMR spectrum of **P39.**

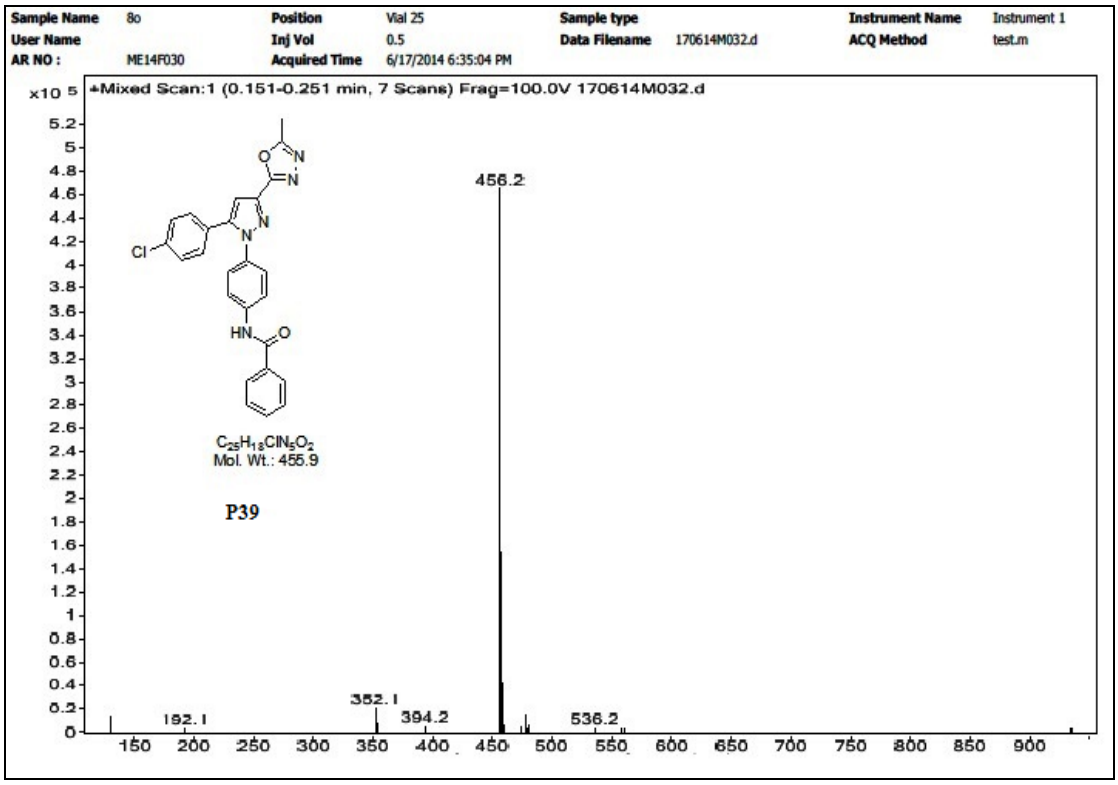


Figure 3.28 Mass spectrum of **P39.**

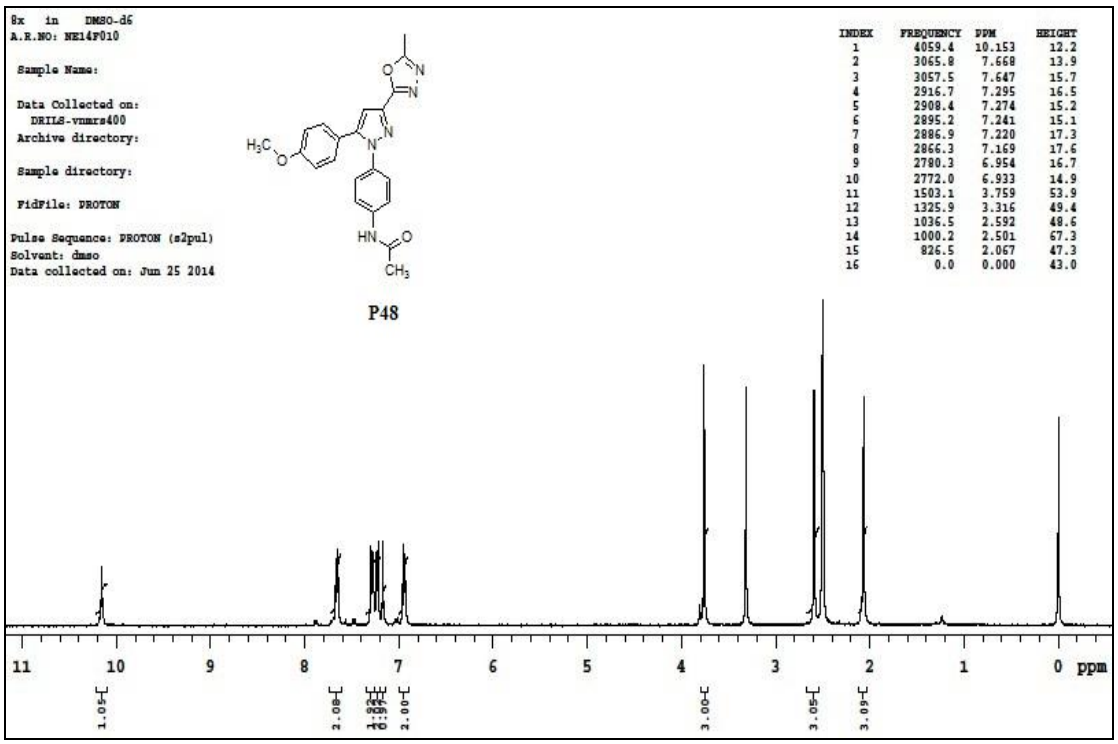


Figure 3.29 ^{1}H NMR spectrum of **P48.**

CHAPTER 4

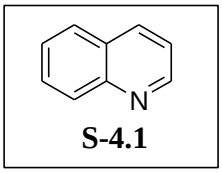
SYNTHESIS AND BIOLOGICAL ACTIVITY OF SOME NEW QUINOLINE-PYRAZOLE HYBRID DERIVATIVES

Abstract

The subsequent chapter elucidates the design and synthesis of new series of quinoline-pyrazole hybrid derivatives along with structural characterization of all the new intermediates and final molecules. In vitro antiTB and antibacterial investigation of all the title compounds is also included in the chapter.

4.1 INTRODUCTION

Quinoline (**S-4.1**) is a heterocyclic aromatic organic compound with the chemical formula C_9H_7N, also known as 1-azanaphthalene, wherein a benzene ring is fused to a pyridine at two adjacent carbon atoms. Quinoline has many analogies with naphthalene and pyridine in its molecular geometry, bond parameters, spectral and energy data.



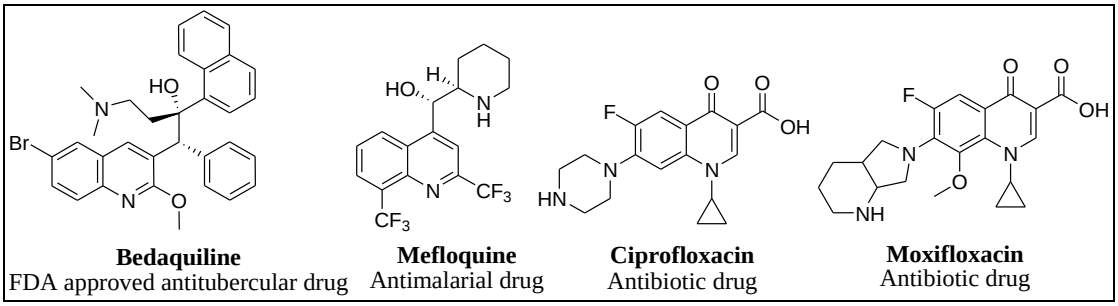
S-4.1

Quinoline moiety is of great importance to chemists as well as biologists since it is one of the key building elements for many naturally occurring compounds. It is interesting to note that the existing well-known antimalarial drugs like chloroquine, mefloquine, tafenoquine and primaquine contain quinoline as the core structural unit. Certain anticancer drugs such as camptothecin, topotecan, voreloxin and neratinib also contain quinoline as the active pharmacophores. Further, the pharmacological behavior of quinoline has been effectively utilized in antiretroviral and anti-inflammatory drugs, such as saquinavir and antrafenine. In addition, various antidepressant drugs such as viqualine, vabicaserin and talnetant containing quinoline as the core moiety still have a good pharmacological profile. Also, the quinoline based compounds such as montelukast, nedocromil and indacaterol are effective drugs for the treatment of asthma.

At present quinoline core has gathered an immense attention among the chemists as well as biologists as it is one of the key building block elements for antiTB and antibacterial drug derivatives. Recently, quinoline containing derivative, Bedaquiline was approved by USFDA as a new antiTB drug (Mahajan, 2013; Diacon

et al. 2012). Mefloquine, a quinoline derivative, is an orally administered drug used in the prevention and treatment of malaria. Interestingly, a number of its derivatives have been reported to possess very good antibacterial as well as antiTB activities (Mao et al. 2010; Mao et al. 2007; Dave and Rahatgaonkar, 2015). The quinoline based antibiotics, Ciprofloxacin and Moxifloxacin (which are used for a number of bacterial infections) show significant antitubercular activity as well and are recommended by WHO as second line antiTB drugs (**Figure 4.1**). In this perspective, we have chosen the quinoline skeleton for the design of new bioactive molecules.

Further, the chemistry of hydrazone derived from 3-aryl-1*H*-pyrazole-4-carbaldehyde has been an interesting field of study in medicinal chemistry for a long time (Pandit and Dodiya, 2013; Fan et al. 2010). In general, this synthetic protocol is widely employed to combine any two heterocycles through an azomethine (-C=N-) group. The protocol is simple and feasible which involves the condensation of hydrazine derivative and active carbonyl groups in the presence of a mineral acid. Pertaining to these observations and in search of new antiTB and antibacterial agents, we have designed a new series of hydrazones derived from quinoline and pyrazole units by employing the combinatorial chemistry approach, which is the present trend being practiced in the drug discovery process. The synthesized target compounds (**P49-P69)** were characterized, evaluated for their *in vitro* antiTB activity against MTB and antibacterial activity against three bacterial strains viz. *S. aureus*, *E. coli*, and *P. aeruginosa*.



Bedaquiline
FDA approved antitubercular drug

Mefloquine
Antimalarial drug

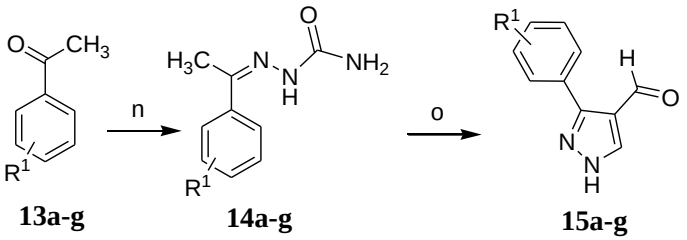
Ciprofloxacin
Antibiotic drug

Moxifloxacin
Antibiotic drug

Figure 4.1 Important quinoline containing drugs which possess promising antiTB activity.

4.2 CHEMISTRY

The synthetic route for quinoline-pyrazole hybrid derivatives (**P49-P69**) is represented in **Schemes 4.1-4.3**. Seven 3-aryl-1*H*-pyrazole-4-carbaldehydes (**15a-g**) were synthesized by the Vilsmeier-Haack formylation of respective semicarbazones (**14a-g**) (Lebedev et al. 2005).

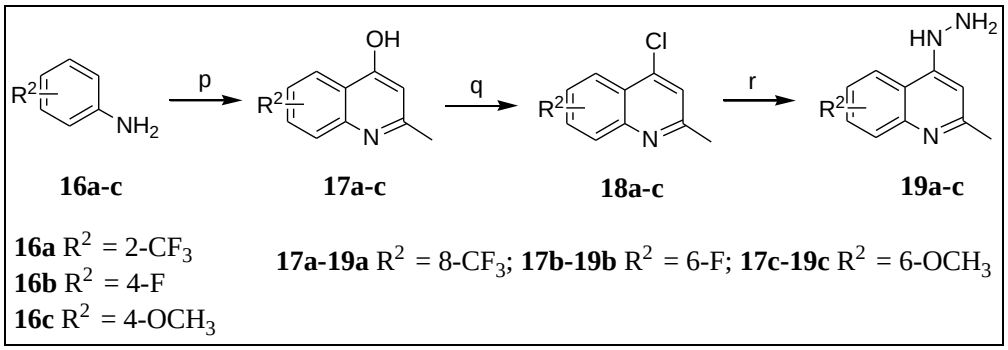


13a-g **14a-g** **15a-g**

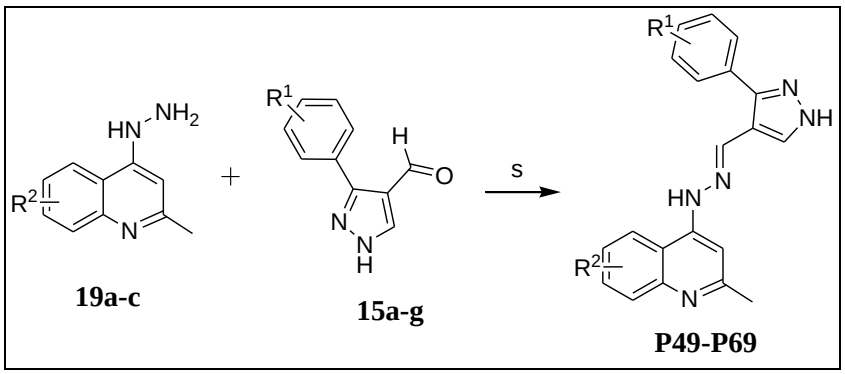
13a-15a R^1 = 4-F; **13b-15b** R^1 = 4-Cl; **13c-15c** R^1 = 3-Cl; **13d-15d** R^1 = 4-Br; **13e-15e** R^1 = 4-OCH_3; **13f-15f** R^1 = 4-CH_3; **13g-15g** R^1 = H

Scheme 4.1 Synthesis of the 3-aryl-1*H*-pyrazole-4-carbaldehyde (**15a-g**). Reagents and conditions: n) Semicarbazide hydrochloride, sodium acetate, ethanol, 80 °C, 8 h; o) $POCl_3$, DMF, 80 °C, 2 h.

Intermediates 1-(2-methylquinolin-4-yl)hydrazines (**19a-c)** were synthesized from the corresponding starting materials **16a-c**. The aniline derivatives (**16a-c**) were conventionally cyclized by heating with ethyl acetoacetate (EAA) in the presence of polyphosphoric acid (PPA) to yield substituted 2-methylquinolin-4-ol intermediates **(17a-c)**. The 4-hydroxy derivatives **17a-c** were then refluxed with freshly distilled $POCl_3$ to yield the corresponding 4-chloro derivatives (**18a-c)**, which were then treated with hydrazine hydrate in ethanol to give intermediates **19a-c**. The target compounds **P49-P69** were synthesized in good yield by refluxing an equimolar mixture of 3-aryl-1*H*-pyrazole-4-carbaldehyde (**15a-g**) and respective 1-(2-methylquinolin-4-yl)hydrazines (**19a-c**) in the presence of catalytic amount of sulphuric acid using ethanol as solvent.



Scheme 4.2 Synthesis of 1-(2-methyl-substituted)quinolin-4-yl)hydrazine derivatives (**19a-c**). Reagents and conditions: p) Ethyl acetoacetate, PPA, 150 °C, 2 h; q) $POCl_3$, 80 °C, 4 h; r) $NH_2NH_2.H_2O$, ethanol, 90 °C, 4 h.



Scheme 4.3 Synthesis of the pyrazole-quinoline based target compounds (**P49-P69**). Reagents and conditions: s) Ethanol, cat. sulfuric acid, 80 °C, 4 h.

4.3 EXPERIMENTAL

4.3.1 Materials and methods

Refer section **2.3.1**

4.3.2 Synthesis

Synthesis of 2-methyl-substituted-quinolin-4-ol derivatives (17a-c)

2-Methyl-8-(trifluoromethyl)quinolin-4-ol (**17a**): To an equimolar solution of 2-trifluoromethylaniline (10.0 g, 62.1 mmol) and EAA (8.01 g, 62.1 mmol), PPA (50 g, 5 w/w) was added and the reaction mixture was stirred at 150 ºC for 2 h. After the completion of the reaction (as monitored by TLC), the reaction mixture was poured into ice water (500 mL) slowly with vigorous stirring. The precipitated solid was filtered and completely dried in vacuum oven at 50 ºC to get the crude product as pale yellow solid. The crude product was taken as such for the next step without further

purification. Yield 11.0 g (78%); Off white solid; mp 181-182 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.37 (d, 1H, ArH, *J* = 8.4), 8.28 (d, 1H, ArH, *J* = 7.6 Hz), 7.68 (t, 1H, ArH, *J* = 7.8 Hz), 6.89 (s, 1H, ArH), 6.20 (brs, 1H, -OH), 2.70 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 163.84, 160.01, 158.23, 128.93, 127.64, 126.88, 125.55, 125.44, 124.16, 120.47, 25.82; ESI-MS (*m/z*) 228.0 $(M+H)^+$; Anal. calculated for $C_{11}H_8F_3NO$; C, 58.15; H, 3.55; N, 6.17. Found: C, 58.18; H, 3.55; N, 6.20.

6-Fluoro-2-methylquinolin-4-ol (17b): Compound **17b** was synthesized according to the above mentioned procedure by treating 4-fluoro aniline (10.0 g, 90.0 mmol) with EAA (11.71 g, 90.0 mmol) in PPA (50 g, 5 w/w). Yield 10.0 g (63%); Off white solid; mp 268-269 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.08 (d, 1H, ArH, *J* = 7.8), 7.77 (s, 1H, ArH), 7.38 (d, 1H, ArH, *J* = 7.8 Hz), 6.58 (s, 1H, ArH), 6.18 (brs, 1H, -OH), 2.71 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 176.56, 155.67, 149.02, 135.16, 125.94, 122.17, 119.91, 107.87, 104.74, 20.81; ESI-MS (*m/z*) 178.1 $(M+H)^+$; Anal. calculated for $C_{10}H_8FNO$; C, 67.79; H, 4.55; N, 7.91. Found: C, 67.82; H, 4.56; N, 7.90.

6-Methoxy-2-methylquinolin-4-ol (17c): Compound **17c** was synthesized according to the above mentioned procedure by treating 4-methoxy aniline (10.0 g, 81.0 mmol) with EAA (10.56 g, 81.0 mmol) in PPA (50 g, 5 w/w). Yield 10.7 g (69%); Off white solid; mp 303-304 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.88 (d, 1H, ArH, *J* = 7.4), 7.39 (s, 1H, ArH), 7.34 (d, 1H, ArH, *J* = 7.4 Hz), 6.49 (s, 1H, ArH), 6.19 (brs, 1H, -OH), 3.76 (s, 3H, OCH_3), 2.71 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 176.54, 158.21, 151.83, 138.31, 127.68, 121.89, 119.64, 106.17, 103.60, 55.74, 19.94; ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm ; ESI-MS (*m/z*) 208.3 $(M+H)^+$; Anal. calculated for $C_{11}H_{11}F_3NO_2$; C, 69.83; H, 5.86; N, 7.40. Found: C, 69.85; H, 5.88; N, 7.41.

Synthesis of substituted 4-chloro-2-methyl-quinoline derivatives (18a-c)

4-Chloro-2-methyl-8-(trifluoromethyl)quinoline (18a): A mixture of **17a** (10.0 g, 44.0 mmol) and freshly distilled $POCl_3$ (50 mL) was heated at 80 ºC for 4 h. The reaction was monitored by TLC. After completion of the reaction, excess $POCl_3$ was distilled off completely. The residue thus obtained was stirred with ice water for 15

min. After this, the solid phase was filtered and dried. Yield 8.74 g (81%); Off white solid; mp 66-67 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.40 (d, 1H, ArH, J = 8.4), 8.27 (d, 1H, ArH, J = 7.6 Hz), 7.71 (t, 1H, ArH, J = 7.8 Hz), 7.59 (s, 1H, ArH), 2.71 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 159.88, 158.24, 144.43, 131.03, 128.94, 125.21, 123.36, 121.66, 120.47, 119.53, 25.82; ESI-MS (*m/z*) 246.2 $(M+H)^+$; Anal. calculated for $C_{11}H_7ClF_3N$; C, 53.79; H, 2.87; N, 5.70. Found: C, 53.84; H, 2.88; N, 5.71.

4-Chloro-6-fluoro-2-methylquinoline (18b): This compound was synthesized according to the above mentioned procedure by treating **17b** (10.0 g, 56.4 mmol) with $POCl_3$ (50 mL). Yield 8.8 g (79.8%); Off white solid; mp 83-84 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.11 (d, 1H, ArH, J = 7.6), 7.84 (s, 1H, ArH), 7.45 (d, 1H, ArH, J = 7.6 Hz), 7.23 (s, 1H, ArH), 2.73 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 159.21, 158.38, 144.75, 130.42, 122.92, 121.23, 120.57, 119.35, 104.91, 25.44; ESI-MS (*m/z*) 196.3 $(M+H)^+$; Anal. calculated for $C_{10}H_7ClFN$; C, 61.40; H, 3.61; N, 7.16. Found: C, 61.44; H, 3.63; N, 7.18.

4-Chloro-6-methoxy-2-methylquinoline (18c): This compound was synthesized according to the above mentioned procedure by treating **17c** (10.0 g, 53.0 mmol) with $POCl_3$ (50 mL). Yield 8.0 g (73%); Off white solid; mp 98-99 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.97 (d, 1H, ArH, J = 7.4), 7.55 (s, 1H, ArH), 7.49 (d, 1H, ArH, J = 7.4 Hz), 7.18 (s, 1H, ArH), 3.75 (s, 3H, OCH_3), 2.71 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 156.59, 154.08, 142.65, 130.03, 125.72, 124.83, 121.62, 120.95, 102.68, 56.15, 24.81; ESI-MS (*m/z*) 208.0 $(M+H)^+$; Anal. calculated for $C_{11}H_{10}ClNO$; C, 63.62; H, 4.85; N, 6.75. Found: C, 63.64; H, 4.87; N, 6.72.

Synthesis of 1-(2-methyl-substituted)quinolin-4-yl)hydrazine derivatives (19a-c)

1-(2-Methyl-8-(trifluoromethyl)quinolin-4-yl)hydrazine (19a): Compound **18a** (8.0 g, 32.0 mmol) and hydrazine hydrate (20 mL) in ethanol (20 mL) was heated under reflux at 90 ºC for 4 h. Completion of the reaction was monitored by TLC. The reaction mixture was concentrated and allowed to cool. The solid product obtained was filtered, washed with water and dried. Yield 5.96 g (75%); Off white solid; mp 211-212 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.31 (s, 1H, -NH), 8.38 (d, 1H, ArH, J = 8.4), 8.13 (d, 1H, ArH, J = 7.6 Hz), 7.64 (t, 1H, ArH, J = 7.8 Hz), 6.95 (s,

1H, ArH), 4.36 (brs, 2H, -NH_2), 2.70 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 160.81, 158.23, 148.96, 129.32, 128.56, 126.17, 121.84, 120.66, 120.47, 119.36, 25.82; ESI-MS (*m/z*) 242.1 $(M+H)^+$; Anal. calculated for $C_{11}H_{10}F_3N_3$; C, 54.77; H, 4.18; N, 17.42. Found: C, 54.81; H, 4.19; N, 17.44.

1-(6-Fluoro-2-methylquinolin-4-yl)hydrazine (19b): This compound was synthesized according to the above mentioned procedure by treating **18b** (8.0 g, 41.0 mmol) with hydrazine hydrate (20 mL) in ethanol (20 mL). Yield 6.0 g (76%); Off white solid; mp 167-168 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.27 (s, 1H, -NH), 7.94 (d, 1H, ArH, *J* = 7.8), 7.51 (s, 1H, ArH), 7.28 (d, 1H, ArH, *J* = 7.8 Hz), 6.39 (s, 1H, ArH), 4.35 (brs, 2H, -NH_2), 2.68 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 159.65, 157.27, 145.12, 130.82, 118.85, 118.31, 116.90, 106.07, 98.91, 25.45; ESI-MS (*m/z*) 192.0 $(M+H)^+$; Anal. calculated for $C_{10}H_{10}FN_3$; C, 62.82; H, 5.27; N, 21.98. Found: C, 62.86; H, 5.25; N, 21.98.

1-(6-Methoxy-2-methylquinolin-4-yl)hydrazine (19c): This compound was synthesized according to the above mentioned procedure by treating **18c** (8.0 g, 38.6 mmol) with hydrazine hydrate (20 mL) in ethanol (20 mL). Yield 5.5 g (70%); Off white solid; mp 176-177 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.26 (s, 1H, -NH), 7.84 (d, 1H, ArH, *J* = 7.6), 7.29 (d, 1H, ArH, *J* = 7.6 Hz), 7.19 (s, 1H, ArH), 6.38 (s, 1H, ArH), 4.35 (brs, 2H, -NH_2), 3.74 (s, 3H, OCH_3), 2.67 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 156.07, 155.67, 144.35, 130.24, 122.05, 120.91, 116.65, 101.40, 100.92, 56.14, 24.82; ESI-MS (*m/z*) 204.0 $(M+H)^+$; Anal. calculated for $C_{11}H_{13}N_3O$; C, 65.01; H, 6.45; N, 20.68. Found: C, 64.97; H, 6.44; N, 20.70.

General procedure for the synthesis of the target compounds (P49-P69)

An equimolar mixture of 3-aryl-1*H*-pyrazole-4-carbaldehyde (**15a-g,** 0.1 g) and intermediate **19a-c** were dissolved in ethanol (2 mL). A catalytic amount of concentrated sulphuric acid was added and the reaction mixture was stirred at 80 ºC for 4 h, then kept at RT overnight. The resultant solid was filtered, washed with chilled ethanol, dried and recrystallized from ethanol-dioxane (1:2) mixture to afford the pure final compounds **P49-P69 (Table 4.1)**.

Table 4.1 Substitution pattern of final compounds (**P49**-**P69**).

Compound	R^1	R^2	Log P/Clog P[a]
P49	4-F	8-CF_3	5.48/5.72
P50	4-Cl	8-CF_3	5.88/6.29
P51	3-Cl	8-CF_3	5.88/6.29
P52	4-Br	8-CF_3	6.15/6.44
P53	4-OCH_3	8-CF_3	5.20/5.52
P54	4-CH_3	8-CF_3	5.81/6.06
P55	H	8-CF_3	5.33/5.56
P56	4-F	6-F	4.72/4.93
P57	4-Cl	6-F	5.12/5.50
P58	3-Cl	6-F	5.12/5.50
P59	4-Br	6-F	5.39/5.65
P60	4-OCH_3	6-F	4.44/4.73
P61	4-CH_3	6-F	5.05/5.27
P62	H	6-F	4.56/4.77
P63	4-F	6-OCH_3	4.44/5.02
P64	4-Cl	6-OCH_3	4.84/5.59
P65	3-Cl	6-OCH_3	4.84/5.59
P66	4-Br	6-OCH_3	5.11/5.74
P67	4-OCH_3	6-OCH_3	4.15/4.82
P68	4-CH_3	6-OCH_3	4.77/5.36
P69	H	6-OCH_3	4.28/4.86

[a] Obtained from ChemDraw Ultra 8.0 software.

(*E*)-1-((3-(4-fluorophenyl)-1*H*-pyrazol-4-yl)methylene)-2-(2-methyl-8-(trifluoromethyl)quinolin-4-yl)hydrazine (P49): Yield 0.16 g (93%); Off white solid; mp 264-265 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 13.44-13.37 (brs, 1H, NH), 10.93 (s, 1H, NH), 8.37-8.49 (m, 3H, ArH), 8.02 (d, 1H, ArH, J = 7.2 Hz), 7.72 (s, 2H, ArH), 7.17-7.55 (m, 4H, ArH), 2.55 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 161.86, 161.45, 147.52, 145.29, 137.76, 131.03, 130.95, 128.05, 126.80, 126.27, 125.67, 122.53, 116.62, 116.12, 114.80, 101.55, 26.23; ESI-MS (*m/z*)

414.0 $(M+H)^+$; Anal. calculated for $C_{21}H_{15}F_4N_5$; C, 61.02; H, 3.66; N, 16.94. Found: C, 60.97; H, 3.66; N, 16.96.

(*E*)-1-((3-(4-chlorophenyl)-1*H*-pyrazol-4-yl)methylene)-2-(2-methyl-8-(trifluoromethyl)quinolin-4-yl)hydrazine (P50): Yield 0.16 g (91%); Off white solid; mp 285-286 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 13.44 (s, 1H, NH), 10.95 (s, 1H, NH), 8.37-8.51 (m, 3H, ArH), 8.03 (d, 1H, ArH, J = 7.2 Hz), 7.51-7.72 (m, 5H, ArH), 7.18 (s, 1H, ArH), 2.55 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 160.44, 147.51, 145.27, 137.65, 134.81, 133.38, 132.59, 131.72, 130.62, 129.12, 128.01, 126.82, 122.55, 116.61, 114.99, 101.57, 26.22; ESI-MS (*m/z*) 430.0 $(M+H)^+$; Anal. calculated for $C_{21}H_{15}ClF_3N_5$; C, 58.68; H, 3.52; N, 16.29. Found: C, 58.64; H, 3.50; N, 16.33.

(*E*)-1-((3-(3-chlorophenyl)-1*H*-pyrazol-4-yl)methylene)-2-(2-methyl-8-(trifluoromethyl)quinolin-4-yl)hydrazine (P51): Yield 0.16 g (92%); Off white solid; mp 198-199 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 13.33-13.39 (brs, 1H, NH), 10.89 (s, 1H, NH), 8.38-8.51 (m, 3H, ArH), 8.01 (d, 1H, ArH, J = 7.02 Hz), 7.48-7.64 (m, 5H, ArH), 7.23 (s, 1H, ArH), 2.55 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 160.49, 148.03, 145.29, 139.31, 137.69, , 133.73, 132.68, 131.70, 130.63, 129.56, 129.27. 128.06, 127.41, 126.39, 125.74, 122.55, 116.59, 115.00, 101.56, 26.22; ESI-MS (*m/z*) 430.3 $(M+H)^+$; Anal. calculated for $C_{21}H_{15}ClF_3N_5$; C, 58.68; H, 3.52; N, 16.29. Found: C, 58.73; H, 3.51; N, 16.32.

(*E*)-1-((3-(4-bromophenyl)-1*H*-pyrazol-4-yl)methylene)-2-(2-methyl-8-(trifluoromethyl)quinolin-4-yl)hydrazine (P52): Yield 0.17 g (88%); Off white solid; mp 293-294 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 13.44 (s, 1H, NH), 10.95 (s, 1H, NH), 8.37-8.49 (m, 3H, ArH), 8.02 (d, 1H, ArH, J = 6.8 Hz), 7.47-7.80 (m, 5H, ArH), 7.18 (s, 1H, ArH), 2.55 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 160.45, 147.50, 145.31, 137.66, 133.79, 133.32, 132.58, 131.72, 129.13, 128.04, 125.57, 124.19, 122.58, 119.73, 116.58, 114.60, 101.55, 26.24; ESI-MS (*m/z*) 475.0 $(M+H)^+$; Anal. calculated for $C_{21}H_{15}BrF_3N_5$; C, 53.18; H, 3.19; N, 14.77. Found: C, 53.22; H, 3.18; N, 14.80.

(*E*)-1-((3-(4-methoxyphenyl)-1*H*-pyrazol-4-yl)methylene)-2-(2-methyl-8-(trifluoromethyl)quinolin-4-yl)hydrazine (P53): Yield 0.16 g (89%); Off white solid; mp 279-280 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 13.42 (s, 1H, NH), 10.89 (s, 1H, NH), 8.45-8.50 (m, 2H, ArH), 8.01-8.16 (m, 2H, ArH), 7.52-7.61 (m, 3H, ArH), 7.11-7.24 (m, 3H, ArH), 3.84 (s, 3H, OCH_3), 2.56 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 160.46, 159.96, 147.54, 145.29, 138.17, 138.01, 136.89, 134.44, 130.12, 127.99, 126.84, 122.49, 116.63, 114.68, 114.35, 101.51, 55.78, 26.24; ESI-MS (*m/z*) 426.0 $(M+H)^+$; Anal. calculated for $C_{22}H_{18}F_3N_5O$; C, 62.11; H, 4.26; N, 16.46. Found: C, 62.08; H, 4.28; N, 16.48.

(*E*)-1-(2-methyl-8-(trifluoromethyl)quinolin-4-yl)-2-((3-p-tolyl-1*H*-pyrazol-4-yl) methylene)hydrazine (P54): Yield 0.16 g (94%); Off white solid; mp 266-267 °C; ^{1}H NMR (400 MHz, DMSO-d_6) 13.29 (s, 1H, NH), 10.87 (s, 1H, NH), 8.02-8.48 (m, 3H, ArH), 8.00 (d, 1H, ArH, J = 7.2 Hz), 7.34-7.54 (m, 5H, ArH), 7.23 (s, 1H, ArH), 2.54 (s, 3H, CH_3), 2.48 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 160.47, 147.53, 145.29, 138.08, 137.74, 135.91, 133.31, 129.79, 128.69, 128.04, 126.82, 123.56, 122.50, 116.63, 114.61, 101.54, 26.24, 21.34; ESI-MS (*m/z*) 410.1 $(M+H)^+$; Anal. calculated for $C_{22}H_{18}F_3N_5$; C, 64.54; H, 4.33; N, 17.11. Found: C, 64.59; H, 4.31; N, 17.14.

(*E*)-1-(2-methyl-8-(trifluoromethyl)quinolin-4-yl)-2-((3-phenyl-1*H*-pyrazol-4-yl) methylene)hydrazine (P55): Yield 0.15 g (92%); Off white solid; mp 233-244 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 13.38 (s, 1H, NH), 10.89 (s, 1H, NH), 8.45-8.48 (m, 2H, ArH), 8.00 (d, 1H, ArH, J = 7.2 Hz), 7.48-7.64 (m, 7H, ArH), 7.23 (s, 1H, ArH), 2.48 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 160.48, 148.01, 145.28, 138.60, 137.69, 131.23, 130.47, 129.87, 129.62, 129.03, 128.38, 128.19, 127.74, 126.52, 125.71, 122.54, 119.88, 116.63, 114.59, 101.57, 26.22; ESI-MS (*m/z*) 396.1 $(M+H)^+$; Anal. calculated for $C_{21}H_{16}F_3N_5$; C, 63.79; H, 4.08; N, 17.71. Found: C, 63.83; H, 4.06; N, 17.68.

(*E*)-1-(6-fluoro-2-methylquinolin-4-yl)-2-((3-(4-fluorophenyl)-1*H*-pyrazol-4-yl) methylene)hydrazine (P56): Yield 0.14 g (94%); Off white solid; mp 235-236 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 13.28 (s, 1H, NH), 10.61 (s, 1H, NH), 8.39 (s, 1H, ArH), 7.26-8.13 (m, 8H, ArH), 7.11 (s, 1H, ArH), 2.39 (s, 3H, CH_3); ^{13}C NMR

(100 MHz, DMSO-d_6) δ ppm 161.98, 159.90, 157.56, 145.11, 141.26, 133.62, 130.99, 129.47, 128.19, 128.19, 127.87, 119.33, 118.81, 117.04, 115.35, 106.07, 100.83, 21.53; ESI-MS (*m/z*) 364.0 $(M+H)^+$; Anal. calculated for $C_{20}H_{15}F_2N_5$; C, 66.11; H, 4.16; N, 19.27. Found: C, 66.13; H, 4.18; N, 19.29.

(*E*)-1-((3-(4-chlorophenyl)-1*H*-pyrazol-4-yl)methylene)-2-(6-fluoro-2-methyl quinolin-4-yl)hydrazine (P57): Yield 0.18 g (94%); Off white solid; mp 271-272 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 13.36 (s, 1H, NH), 10.61 (s, 1H, NH), 8.39 (s, 1H, ArH), 7.39-8.18 (m, 8H, ArH), 7.07 (s, 1H, ArH), 2.38 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 159.91, 157.54, 145.13, 142.29, 140.44, 134.51, 131.08, 130.63, 129.79, 128.37, 127.26, 121.02, 119.11, 118.85, 115.29, 106.08, 100.85, 21.54; ESI-MS (*m/z*) 380.2 $(M+H)^+$; Anal. calculated for $C_{20}H_{15}ClFN_5$; C, 63.24; H, 3.98; N, 18.44. Found: C, 63.28; H, 3.99; N, 18.47.

(*E*)-1-((3-(3-chlorophenyl)-1*H*-pyrazol-4-yl)methylene)-2-(6-fluoro-2-methyl quinolin-4-yl)hydrazine (P58): Yield 0.19 g (93%); Off white solid; mp 209-210 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 13.36 (s, 1H, NH), 10.67 (s, 1H, NH), 8.40 (s, 1H, ArH), 7.47-8.22 (m, 8H, ArH), 7.05 (s, 1H, ArH), 2.38 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 159.91, 157.52, 145.11, 142.28, 140.36, 133.76, 131.28, 130.96, 129.59, 128.52, 128.38, 127.43, 120.23, 119.10, 118.85, 115.28, 106.08, 100.83, 21.53; ESI-MS (*m/z*) 380.1 $(M+H)^+$; Anal. calculated for $C_{20}H_{15}ClFN_5$; C, 63.24; H, 3.98; N, 18.44. Found: C, 63.29; H, 3.96; N, 18.41.

(*E*)-1-((3-(4-bromophenyl)-1*H*-pyrazol-4-yl)methylene)-2-(6-fluoro-2-methyl quinolin-4-yl)hydrazine (P59): Yield 0.2 g (90%); Off white solid; mp 218-219 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 13.31 (s, 1H, NH), 10.64 (s, 1H, NH), 8.39 (s, 1H, ArH), 7.31-8.10 (m, 8H, ArH), 7.07 (s, 1H, ArH), 2.38 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 159.91, 157.53, 145.10, 142.28, 140.31, 132.37, 131.76, 131.61, 128.29, 128.04, 127.38, 124.59, 120.21, 119.10, 115.28, 106.09, 100.84, 21.53; ESI-MS (*m/z*) 425.0 $(M+H)^+$; Anal. calculated for $C_{20}H_{15}BrFN_5$; C, 56.62; H, 3.56; N, 16.51. Found: C, 56.66; H, 3.59; N, 16.55.

(*E*)-1-(6-fluoro-2-methylquinolin-4-yl)-2-((3-(4-methoxyphenyl)-1*H*-pyrazol-4-yl) methylene)hydrazine (P60): Yield 0.17 g (88%); Off white solid; mp 211-212 °C;

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 13.28 (s, 1H, NH), 10.62 (s, 1H, NH), 8.39 (s, 1H, ArH), 7.34-8.12 (m, 6H, ArH), 7.03-7.11 (s, 3H, ArH), 3.75 (s, 3H, OCH_3), 2.39 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 160.44, 159.93, 157.51, 145.10, 142.29, 140.33, 131.29, 128.81, 127.82, 125.24, 120.23, 119.11, 118.85, 115.28, 115.02, 106.07, 100.83, 55.78, 21.53; ESI-MS (*m/z*) 376.1 $(M+H)^+$; Anal. calculated for $C_{21}H_{18}FN_5O$; C, 67.19; H, 4.83; N, 18.66. Found: C, 67.22; H, 4.85; N, 18.63.

(*E*)-1-(6-fluoro-2-methylquinolin-4-yl)-2-((3-p-tolyl-1*H*-pyrazol-4-yl)methylene) hydrazine (P61): Yield 0.17 g (89%); Off white solid; mp 202-203 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 13.28 (s, 1H, NH), 10.61 (s, 1H, NH), 8.39 (s, 1H, ArH), 7.33-8.13 (m, 8H, ArH), 7.11 (s, 1H, ArH), 2.54 (s, 3H, CH_3), 2.38 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 159.92, 157.51, 145.11, 142.24, 140.35, 137.73, 131.27, 130.71, 129.56, 127.88, 127.43, 120.24, 119.11, 118.88, 115.28, 106.07, 100.84, 26.24, 21.53; ESI-MS (*m/z*) 360.0 $(M+H)^+$; Anal. calculated for $C_{21}H_{18}FN_5$; C, 70.18; H, 5.05; N, 19.49. Found: C, 70.14; H, 5.04; N, 19.52.

(*E*)-1-(6-fluoro-2-methylquinolin-4-yl)-2-((3-phenyl-1*H*-pyrazol-4-yl)methylene) hydrazine (P62): Yield 0.17 g (92%); Off white solid; mp 201-202 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 13.31 (s, 1H, NH), 10.62 (s, 1H, NH), 8.40 (s, 1H, ArH), 7.26-8.11 (m, 9H, ArH), 7.10 (s, 1H, ArH), 2.38 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 159.91, 157.54, 145.09, 142.27, 140.36, 133.71, 131.21, 128.82, 128.47, 127.59, 127.43, 120.22, 119.10, 118.84, 115.27, 106.08, 100.86, 21.53; ESI-MS (*m/z*) 346.0 $(M+H)^+$; Anal. calculated for $C_{20}H_{16}FN_5$; C, 69.55; H, 4.67; N, 20.28. Found: C, 69.59; H, 4.68; N, 20.24.

(*E*)-1-((3-(4-fluorophenyl)-1*H*-pyrazol-4-yl)methylene)-2-(6-methoxy-2-methyl quinolin-4-yl)hydrazine (P63): Yield 0.16 g (84%); Off white solid; mp 215-216 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 13.26 (s, 1H, NH), 10.57 (s, 1H, NH), 8.37 (s, 1H, ArH), 7.28-7.73 (m, 8H, ArH), 7.02 (s, 1H, ArH), 3.87 (s, 3H, OCH_3), 2.48 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 161.96, 156.02, 155.68, 144.89, 142.73, 140.31, 131.06, 130.95, 128.84, 126.68, 120.84, 116.17, 115.95, 115.14, 105.08, 101.43, 100.87, 56.12, 21.54; ESI-MS (*m/z*) 376.1 $(M+H)^+$; Anal. calculated for $C_{21}H_{18}FN_5O$; C, 67.19; H, 4.83; N, 18.66. Found: C, 67.21; H, 4.82; N, 18.64.

(*E*)-1-((3-(4-chlorophenyl)-1*H*-pyrazol-4-yl)methylene)-2-(6-methoxy-2-methyl quinolin-4-yl)hydrazine (P64): Yield 0.18 g (94%); Off white solid; mp 222-223 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 13.27 (s, 1H, NH), 10.56 (s, 1H, NH), 8.37 (s, 1H, ArH), 7.32-7.73 (m, 8H, ArH), 7.04 (s, 1H, ArH), 3.86 (s, 3H, OCH_3), 2.48 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 156.03, 155.68, 144.86, 142.74, 140.33, 133.28, 131.22, 130.95, 128.47, 128.19, 127.73, 120.84, 116.17, 115.95, 105.07, 101.44, 100.88, 56.12, 21.53; ESI-MS (*m/z*) 392.1 $(M+H)^+$; Anal. calculated for $C_{21}H_{18}ClN_5O$; C, 64.37; H, 4.63; N, 17.87. Found: C, 64.39; H, 4.63; N, 17.89.

(*E*)-1-((3-(3-chlorophenyl)-1*H*-pyrazol-4-yl)methylene)-2-(6-methoxy-2-methyl quinolin-4-yl)hydrazine (P65): Yield 0.17 g (87%); Off white solid; mp 228-229 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 13.26 (s, 1H, NH), 10.57 (s, 1H, NH), 8.36 (s, 1H, ArH), 7.24-7.71 (m, 8H, ArH), 7.03 (s, 1H, ArH), 3.86 (s, 3H, OCH_3), 2.48 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 156.04, 155.67, 144.86, 142.72, 140.36, 134.18, 133.86, 130.54, 130.29, 126.63, 126.39, 126.02, 124.72, 121.14, 116.16, 115.95, 105.05, 101.45, 100.87, 56.13, 21.54; ESI-MS (*m/z*) 392.3 $(M+H)^+$; Anal. calculated for $C_{21}H_{18}ClN_5O$; C, 64.37; H, 4.63; N, 17.87. Found: C, 64.42; H, 4.64; N, 17.90.

(*E*)-1-((3-(4-bromophenyl)-1*H*-pyrazol-4-yl)methylene)-2-(6-methoxy-2-methyl quinolin-4-yl)hydrazine (P66): Yield 0.19 g (88%); Off white solid; mp 205-206 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 13.27 (s, 1H, NH), 10.58 (s, 1H, NH), 8.38 (s, 1H, ArH), 8.19 (s, 1H, ArH), 7.63-7.73 (m, 5H, ArH), 7.52 (d, 1H, ArH, J = 1.2 Hz), 7.25 (m, 1H, ArH), 6.99 (s, 1H, ArH), 3.87 (s, 3H, OCH_3), 2.48 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 156.05, 155.66, 144.87, 142.73, 140.35, 132.25, 131.92, 130.96, 128.88, 127.41, 122.38, 122.00, 120.91, 116.65, 115.31, 101.41, 100.90, 56.14, 21.57; ESI-MS (*m/z*) 437.1 $(M+H)^+$; Anal. calculated for $C_{21}H_{18}BrN_5O$; C, 57.81; H, 4.16; N, 16.05. Found: C, 57.86; H, 4.18; N, 16.02.

(*E*)-1-(6-methoxy-2-methylquinolin-4-yl)-2-((3-(4-methoxyphenyl)-1*H*-pyrazol-4-yl)methylene)hydrazine (P67): Yield 0.18 g (92%); Off white solid; mp 261-262 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 13.27 (s, 1H, NH), 10.54 (s, 1H, NH), 8.39 (s, 1H, ArH), 7.33-7.71 (m, 6H, ArH), 7.02-7.14 (m, 3H, ArH), 3.85 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 2.48 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 159.98,

156.02, 155.67, 144.88, 142.72, 140.30, 131.05, 129.89, 128.54, 126.71 126.47, 120.84, 116.16, 115.15, 114.60, 101.43, 100.89, 55.75, 56.10, 21.53; ESI-MS (*m/z*) 388.2 $(M+H)^+$; Anal. calculated for $C_{22}H_{21}N_5O_2$; C, 68.20; H, 5.46; N, 18.08. Found: C, 68.25; H, 5.49; N, 18.04.

(*E*)-1-(6-methoxy-2-methylquinolin-4-yl)-2-((3-p-tolyl-1*H*-pyrazol-4-yl) methylene)hydrazine (P68): Yield 0.16 g (90%); Off white solid; mp 188-189 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 13.26 (s, 1H, NH), 10.56 (s, 1H, NH), 8.36 (s, 1H, ArH), 7.19-7.71 (m, 8H, ArH), 7.04 (s, 1H, ArH), 3.86 (s, 3H, OCH_3), 2.49 (s, 3H, CH_3), 2.38 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 156.03, 155.67, 144.89, 142.74, 140.35, 131.06, 129.88, 128.50, 127.08, 126.72, 126.48, 120.83, 116.18, 115.14, 101.40, 100.93, 56.13, 24.82, 21.55; ESI-MS (*m/z*) 372.1 $(M+H)^+$; Anal. calculated for $C_{22}H_{21}N_5O$; C, 71.14; H, 5.70; N, 18.85. Found: C, 71.18; H, 5.68; N, 18.92.

(*E*)-1-(6-methoxy-2-methylquinolin-4-yl)-2-((3-phenyl-1*H*-pyrazol-4-yl) methylene)hydrazine (P69): Yield 0.16 g (89%); Off white solid; mp 183-184 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 13.27 (s, 1H, NH), 10.55 (s, 1H, NH), 8.37 (s, 1H, ArH), 7.22-7.72 (m, 9H, ArH), 7.03 (s, 1H, ArH), 3.85 (s, 3H, OCH_3), 2.49 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 156.03, 155.66, 144.91, 142.75, 140.34, 133.27, 131.01, 129.84, 128.43, 126.95, 126.72, 126.60, 120.82, 116.18, 115.92, 101.41, 100.90, 56.13, 21.54; ESI-MS (*m/z*) 358.0 $(M+H)^+$; Anal. calculated for $C_{21}H_{19}N_5$; C, 70.57; H, 5.36; N, 19.59. Found: C, 70.61; H, 5.33; N, 19.61.

4.4 PHARMACOLOGY

Cytotoxicity studies, molecular docking studies, antibacterial studies were performed as discussed in **Section 2.4.**

4.4.1 Antitubercular studies

Antimycobacterial activities of the final compounds were assessed against MTB using microplate alamar blue assay (MABA) (Lourenço et al. 2007; Franzblau et al. 1998). This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric method (Reis et al. 2004). Briefly, 200 µL of sterile deionized water was added to all outer perimeter

wells of sterile 96 wells plate to minimized evaporation of medium in the test wells during incubation. The 96 wells plate received 100 µL of the Middlebrook 7H9 broth and serial dilution of compounds were made directly on plate. The final drug concentrations tested were 100 to 0.2 µg/mL. Plates were covered and sealed with parafilm and incubated at 37 ºC for five days. After this time, 25 µL of freshly prepared 1:1 mixture of alamar blue reagent and 10% tween 80 was added to the plate and incubated for 24 h. A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth. The MIC was defined as lowest compound/drug concentration which prevented the color change from blue to pink.

4.5 RESULTS AND DISCUSSION

4.5.1 Chemistry

The compounds were purified by appropriate methods, recrystallization or column chromatography. All the synthesized compounds were characterized by recording their ^{1}H NMR, ^{13}C NMR and mass spectra followed by elemental analysis. For instance, formation of 2-methyl-8-(trifluoromethyl)quinolin-4-ol (**17a**) from 2-trifluoromethylaniline (**16a**) was evidenced by the appearance of new -OH signal as broad singlet at δ 6.20 ppm in the ^{1}H NMR spectrum of **17a**. The spectrum showed also a new singlet at δ 2.70 ppm due to -CH_3 protons thus confirming the completion of the cyclization reaction. Further, total proton count for compound **17a** matches very well with its structure. The ESI-MS of **17a** showed a molecular ion peak at *m/z* 228.0 $(M+H)^+$, which is in agreement with its molecular formula $C_{11}H_8F_3NO$. The complete disappearance of -OH signal in the ^{1}H NMR spectrum and a molecular ion peak at *m/z* 246.2 $(M+H)^+$ in the ESI-MS spectrum of **18a** confirms the chlorination at C-4 position of the quinoline ring. The ^{1}H NMR spectrum of intermediate **19a** displayed two new broad singlet signals at δ 4.36 and 8.31 ppm due to -NH_2 and -NH protons, respectively. The ESI-MS spectrum of **19a** displayed a molecular ion peak at *m/z* 242.1 $(M+H)^+$, which matches with its molecular formula, $C_{11}H_{10}F_3N_3$. Similarly, the structure of intermediates **17b-19b** and **17c-19c** were also confirmed by their spectral data. The ^{13}C NMR spectra and elemental analysis data of these intermediates further confirm their structure. Similarly, the formation of title compounds **P49-P69** was evidenced by spectral and elemental analysis. For instance, in the ^{1}H NMR spectrum of **P49 (Figure 4.2)**, the broad signal of -NH_2 at δ 4.36 ppm of quinoline

hydrazide (**19a**) was absent and the signal due to the -NH proton was shifted to δ 10.93 ppm thus confirming the formation of the hydrazone product. The ESI-MS of **P49** displayed a molecular ion peak at *m/z* 414.0 $(M+H)^+$, which matches with its molecular formula $C_{21}H_{15}F_4N_5$ (**Figure 4.4**). Further, the signals in the ^{13}C NMR spectrum are in well agreement with its structure **(Figure 4.3)**. In addition, the molecular structure of the compound **P49** was established by SC-XRD studies. The details of substitution patterns of the target compounds (**P49**-**P69**) are presented in **Table 4.1**.

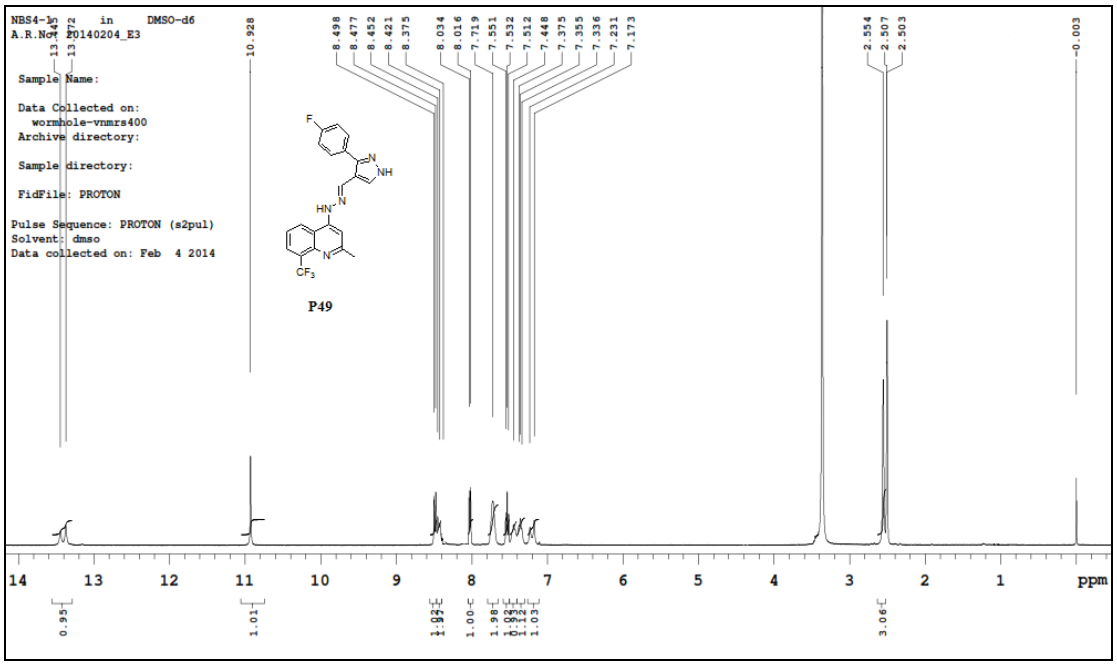


Figure 4.2 ^{1}H NMR spectrum of **P49.**

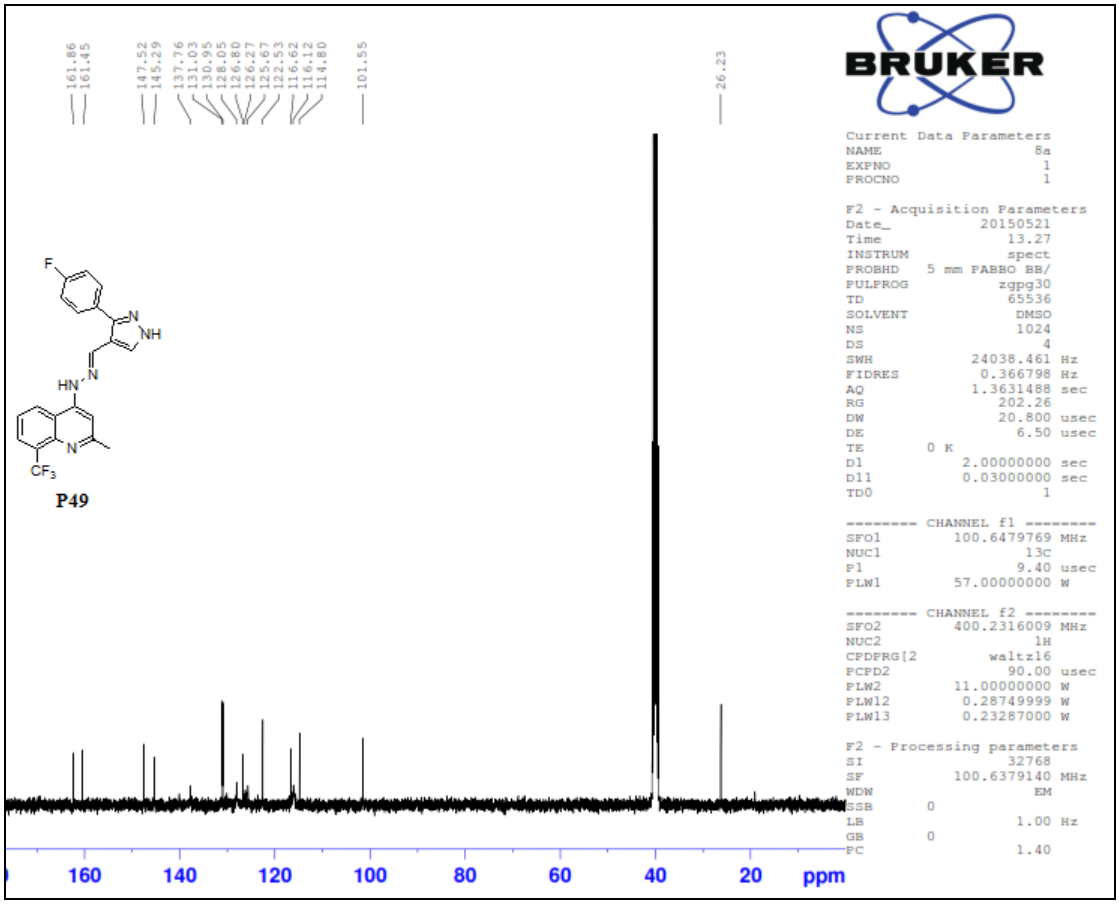


Figure 4.3 ^{13}C NMR spectrum of **P49.**

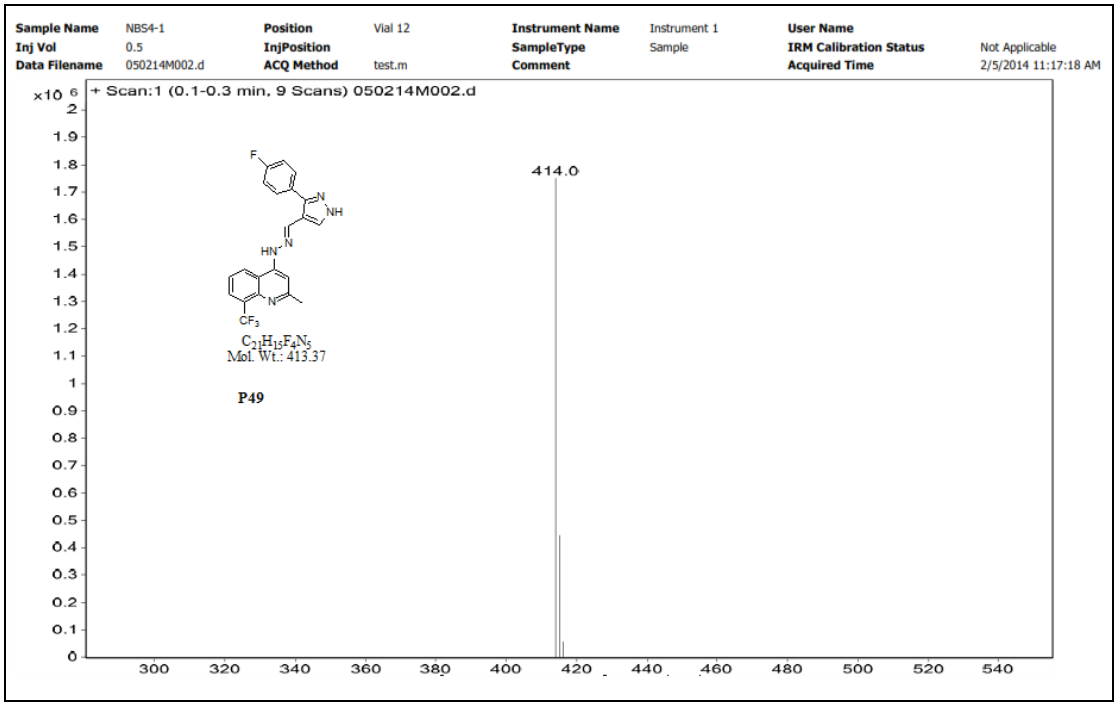


Figure 4.4 Mass spectrum of **P49.**

4.5.2 X-ray crystallographic analysis of P49.

The single crystal of one of the target compounds, (*E*)-1-((3-(4-fluorophenyl)-1*H*-pyrazol-4-yl)methylene)-2-(2-methyl-8-(trifluoromethyl)quinolin-4-yl)hydrazine (**P49**) was grown by the slow evaporation of a 1:1 mixture of methanol-dichloromethane solution at room temperature through a parafilm plastic containing pinholes. The ORTEP diagram for compound **P49** is shown in **Figure 4.5** and details of the crystal data are given in **Table 4.2**.

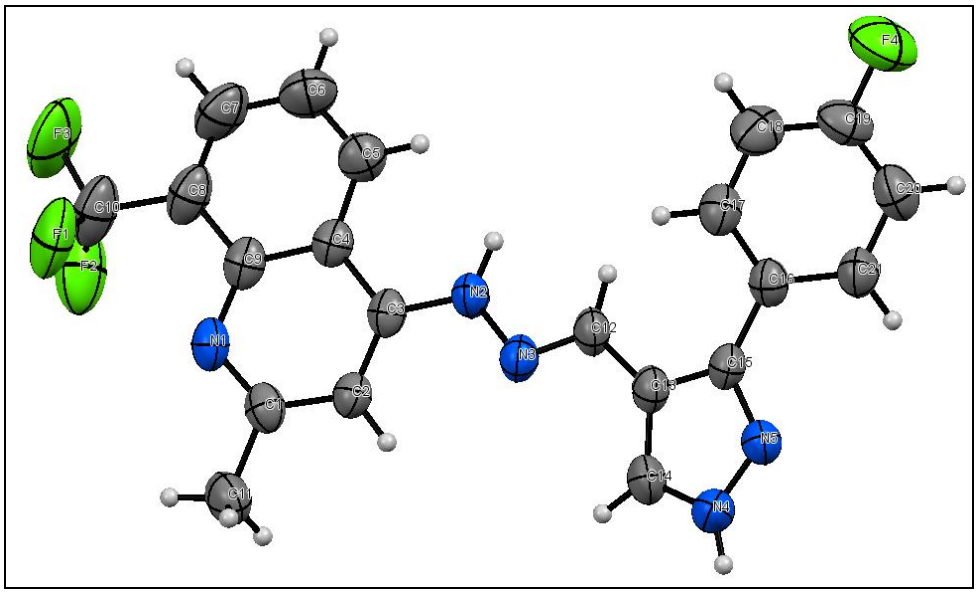


Figure 4.5 ORTEP diagram showing the X-ray crystal structure of **P49**.

Table 4.2 Crystal and structure refinement data for compound **P49.**

Parameters	**Crystal data**
Chemical formula	$C_{21}H_{15}F_4N_5$
Formula weight	413.38
CCDC No.	1418517
Crystal system	Monoclinic
Space group	P 2_1/c
a (Å)	9.1467(3)
b (Å)	19.1379(6)
c (Å)	11.2002(4)
Volume (Å ^{3})	1931.04(11)
Angle α, β, γ	90, 99.9580(17), 90

Z	4
F_{000}	848
μ (mm^{-1})	0.115
Temperature (T)	296 K
Radiation wavelength (Å)	0.71073
Radiation type	Mo Kα
R-Factor (%)	7.35

4.5.3 Antitubercular studies

The *in vitro* antimycobacterial activity in terms of MIC values, of the target compounds (**P49-P69)** and the standard drugs are represented in **Figure 4.6**. Four derivatives **P50**, **P51**, **P58** and **P63** showed significant inhibition activity with a MIC of 12.5 μg/mL and other nine compounds, **P49, P52, P53, P54, P57, P60, P61, P62** and **P69** displayed a moderate activity with MIC of 25 μg/mL. The compounds derived from 8-trifluoromethyl quinoline and 6-fluoro quinoline scaffolds exhibited superior inhibition activity than their 6-methoxy quinoline analogues. This observation clearly indicates that the trifluoromethyl and fluoro groups on the quinoline ring enhance the inhibition activity of the hybrid molecules. It is interesting to note that the structure of 8-trifluoromethyl quinoline derivatives (**P49-P55**) resemble with that of the drug, Mefloquine. Similarly, the 6-flouro quinoline derivatives (**P56-P62**) are structural analogues of Ciprofloxacin and Moxifloxacin. The presence of a 3/4-chlorophenyl group on the pyrazole ring enhanced the antiTB activity as seen in the case of compounds **P50, P51** and **P58**. These results suggests that the structural modification of the above mentioned drug molecules by a molecular hybridization approach could be a promising strategy to identify newer leads towards the development of potent antiTB agents.

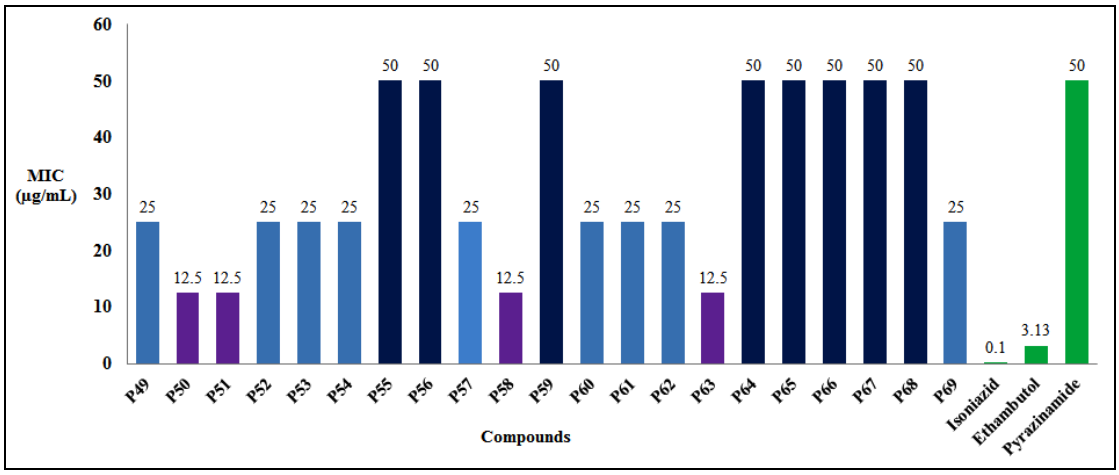


Figure 4.6 AntiTB activity of final derivatives (**P49-P69**).

4.5.4 Cytotoxicity studies

The active antiTB compounds with MIC of 12.5 µg/mL were further subjected to cytotoxicity studies against a Vero cell line. The percentage inhibition, IC_{50} and SI values are presented in **Table 4.3**. The low growth inhibition by the compounds indicates their nontoxic nature. Further, all four tested compounds showed SI value ≥ 10 which signifies their suitability for further development as antiTB agents.

Table 4.3. Cytotoxicity of active antiTB derivatives.

Compound	Percentage Inhibition[a]	IC_{50} (µg/mL)	SI[b]
P50	18.20	121.67	10
P51	6.73	240.00	19
P58	8.36	238.33	19
P63	6.34	>1000	>80

[a] Percentage Inhibition is at 62.5 µg/mL

[b] Selectivity index (*in vitro*) = IC_{50} against Vero cells/MIC against *M. tuberculosis*.

4.5.5 Molecular docking studies

The best docking pose of the representative compounds in the active site of enzymes, InhA, CYP121 and TMPK are shown in **Figures 4.7, 4.8** and **4.9** respectively. Firstly, InhA of MTB which is one of the key enzymes validated as an effective antibacterial target was selected for docking studies. All four ligands (**P50**, **P51**, **P58** and **P63**) have shown good docking score ranging from -7.64 to -10.88 (**Table 4.4**). The highest score was observed for ligand **P50** (-10.88). Except **P58**,

other three ligands showed two common π-π interactions with amino acid residues Phe 149 and Tyr 158 (**Figure 4.7**). Ligand **P63** showed an additional hydrogen bonding interaction with Ala 157. Additionally, docking studies were carried out with target enzymes CYP121 and TMPK as well. It was observed that all ligands showed three common interactions with the residues Trp 182, Gln 385 and Phe 168 (**Figure 4.8**) of CYP121with a good docking score (8.16 - 9.31). Ligand **P50** showed additional hydrogen bonding with Thr 229. Similarly, docking studies with TMPK revealed the favorable interactions of the ligands with the amino acid residues. Ligand **P58** displayed five interactions including two hydrogen bonds with Val 63 and Ser 99 (**Figure 4.9**); also it exhibited the highest docking score with both CYP121 and TMPK. Overall, the docked ligands showed good docking score as well as multiple interactions with amino acid residues of all the three enzymes of MTB. Thus the antiTB profile of these active compounds (**P50**, **P51**, **P58** and **P63**) could be due to their inhibition activity against the MTB enzymes.

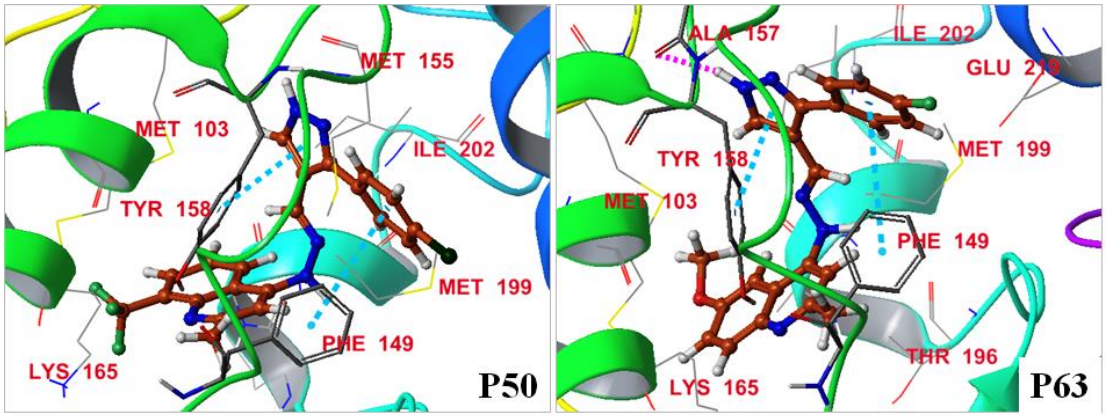


Figure 4.7 The docking pose of the compounds **P50** and **P63** in the active pocket of InhA.

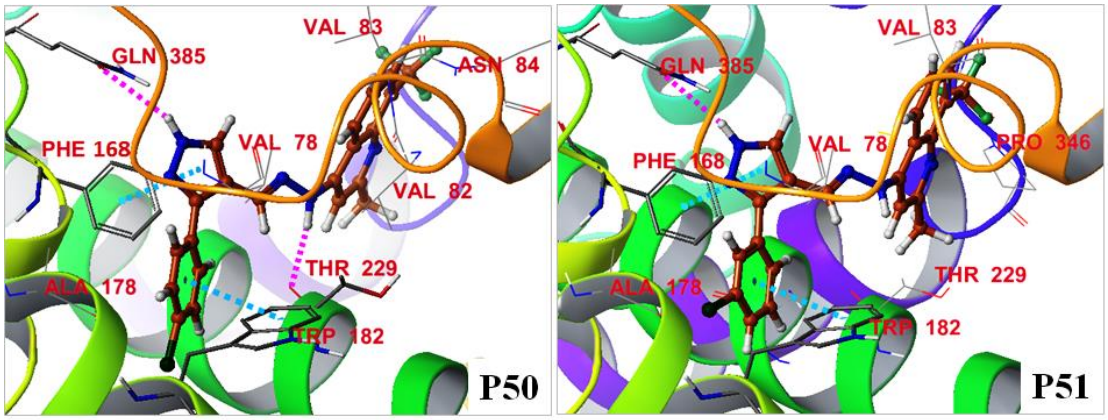


Figure 4.8 The docking pose of the compounds **P50** and **P51** in the active pocket of CYP121.

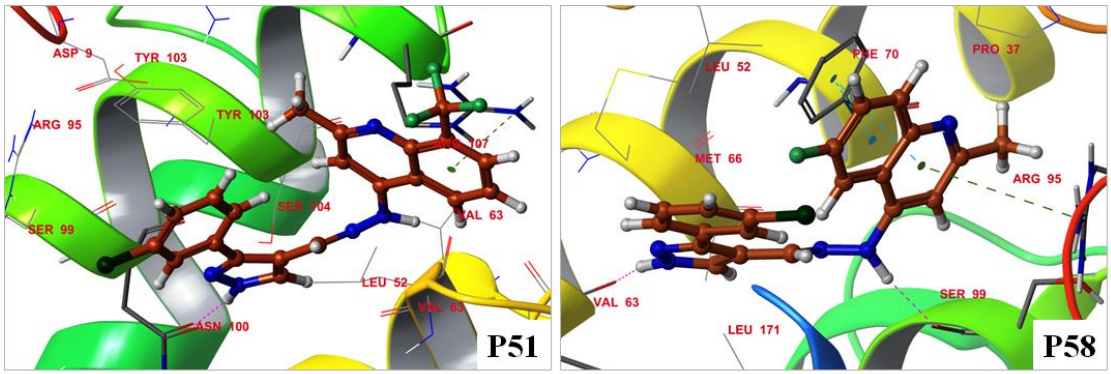


Figure 4.9 The docking pose of the compounds **P51** and **P58** in the active pocket of TMPK.

Table 4.4 Docking results of the active antiTB compounds

Compound ID	InhA		CYP121		TMPK	
	Docking score	**Amino acids that interacted with ligands**	**Docking score**	**Amino acids that interacted with ligands**	**Docking score**	**Amino acids that interacted with ligands**
Standard Ligand	-12.51	Tyr 158, Phe 149	-10.38	Gln 385, Val 228, Asn 85	-13.12	Asn 100, Tyr 103, Arg 74, Arg 95,
P50	-10.89	Phe 149,	-8.16	Trp 182, Gln 385,	-7.53	Ala 60,

		Tyr 158		Phe 168, Thr 229		Arg 107
P51	-7.64	Phe 149, Tyr 158	-8.63	Trp 182, Gln 385, Phe 168	-7.82	Asn 100, Arg 107
P58	-9.52	-	-9.31	Trp 182, Gln 385, Phe 168	-10.31	Val 63, Phe 70, Ser 99, Arg 95
P63	-9.39	Phe 149, Tyr 158, Ala 157	-8.23	Trp 182, Gln 385, Phe 168	-7.53	Arg 107, Arg 95, Phe 70

4.5.6 Antibacterial studies

The antibacterial activity results of the target compounds (**P49-P69)** are presented in **Table 4.5**. Four compounds **P50-P52** and **P55** showed significant inhibition activity against all three tested strains and rest of the compounds displayed moderate activity. Interestingly, all the active compounds are the derivatives of 8-triflouromethyl quinoline scaffold which indicates that the -CF_3 group plays a pivotal role in enhancing the inhibition activity of the molecules.

Table 4.5 Antibacterial activity of compounds **P49-P69**.

Compound	**Zone of inhibition (mm)**					
	Staphylococcus aureus		***Escherichia coli***		***Pseudomonas aeruginosa***	
Conc. in µg/ml	75	50	75	50	75	50
Control	00	00	00	00	00	00
Ciprofloxacin	26±0.1	21±0.2	32±0.2	27±0.2	21±0.2	18±0.1
P49	18±0.2	15±0.3	19±0.2	17±0.1	18±0.2	15±0.2
P50	**23±0.2**	**20±0.1**	**23±0.3**	**20±0.2**	**18±0.2**	**16±0.1**
P51	**20±0.3**	**18±0.1**	**24±0.2**	**21±0.1**	**18±0.1**	**15±0.3**
P52	**22±0.1**	**18±0.1**	**24±0.2**	**20±0.3**	**19±0.3**	**16±0.2**
P53	16±0.1	12±0.2	16±0.2	13±0.2	17±0.3	14±0.2
P54	14±0.1	11±0.1	17±0.1	14±0.2	19±0.2	15±0.2

P55	**21±0.2**	**18±0.1**	**24±0.1**	**20±0.1**	**20±0.2**	**18±0.3**
P56	09±0.3	06±0.3	12±0.2	10±0.2	11±0.3	09±0.1
P57	08±0.4	06±0.3	12±0.2	11±0.2	--	--
P58	10±0.1	07±0.3	16±0.4	14±0.1	12±0.1	10±0.4
P59	08±0.1	06±0.3	15±0.4	13±0.2	--	--
P60	09±0.2	06±0.2	16±0.3	14±0.3	12±0.4	10±0.2
P61	15±0.2	10±0.3	15±0.3	13±0.2	14±0.1	12±0.4
P62	17±0.2	15±0.4	11±0.4	10±0.2	15±0.1	12±0.2
P63	10±0.3	07±0.3	15±0.2	13±0.3	14±0.2	12±0.2
P64	16±0.1	13±0.2	16±0.1	14±0.1	15±0.2	12±0.1
P65	--	--	13±0.1	11±0.3	12±0.1	10±0.1
P66	11±0.1	09±0.2	15±0.1	13±0.3	16±0.3	12±0.2
P67	18±0.2	14±0.2	19±0.2	16±0.2	18±0.1	15±0.2
P68	11±0.3	08±0.3	13±0.3	10±0.2	12±0.2	10±0.3
P69	10±0.1	08±0.3	15±0.4	14±0.4	14±0.1	12±0.3

--: Bacterial resistant, Control: dimethylsulfoxide

4.6 CONCLUSIONS

- In conclusion, we have designed and synthesized a library of new quinoline-pyrazole hybrid derivatives and the molecules were characterized by spectral techniques. The structure of one of the derivatives (**P49**) was confirmed by single crystal XRD study.
- Derivatives **P50**, **P51**, **P58** and **P63** emerged as active antiTB leads which exhibited low toxicity profile and high selectivity index value.
- The molecular docking studies further revealed the strong interaction of the active compounds with the active site of the target enzymes.
- In the antibacterial studies, four compounds (**P50**-**P52** and **P55**) showed substantial inhibition activity against the tested bacterial strains.
- The compounds with 8-trifluoromethyl quinoline substitution exhibited remarkable antiTB as well as antibacterial activity and are promising lead molecules for further drug development.

- Overall, the results described here demonstrate the potential utility of the molecular hybridization strategy in designing new hybrid analogs of quinoline and pyrazole as potent antiTB and antibacterial agents for further lead optimization.

Appendix 4.1

Representative ^{1}H NMR, ^{13}C NMR and ESI-MS spectra of final compounds

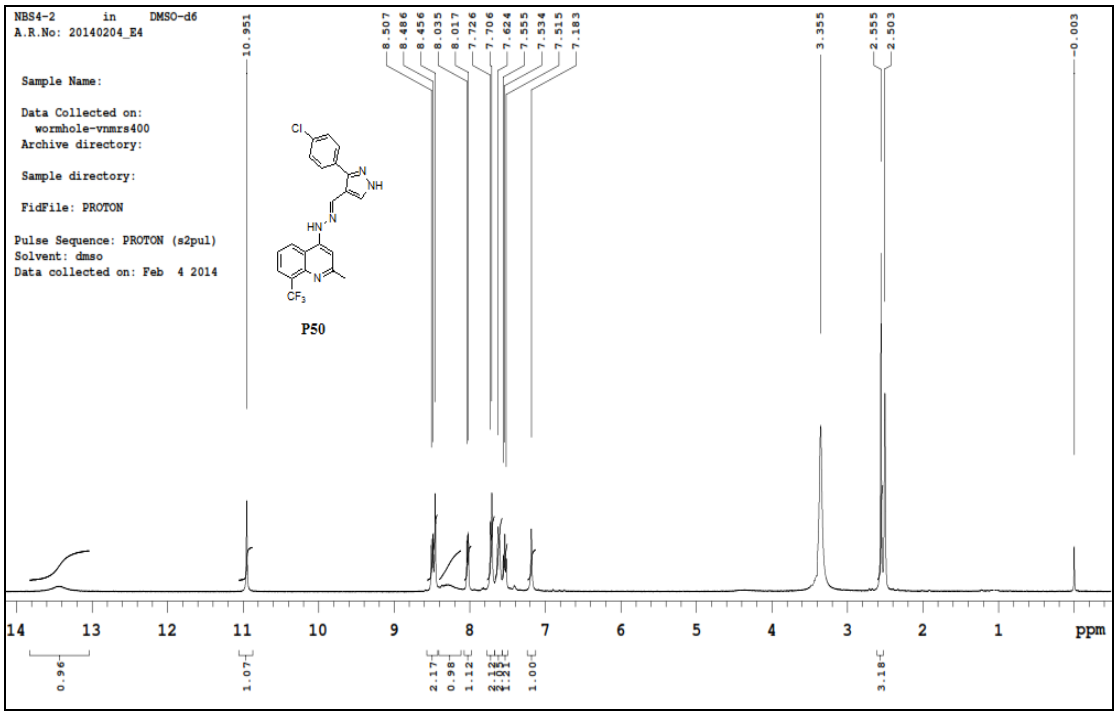


Figure 4.10 ^{1}H NMR spectrum of **P50.**

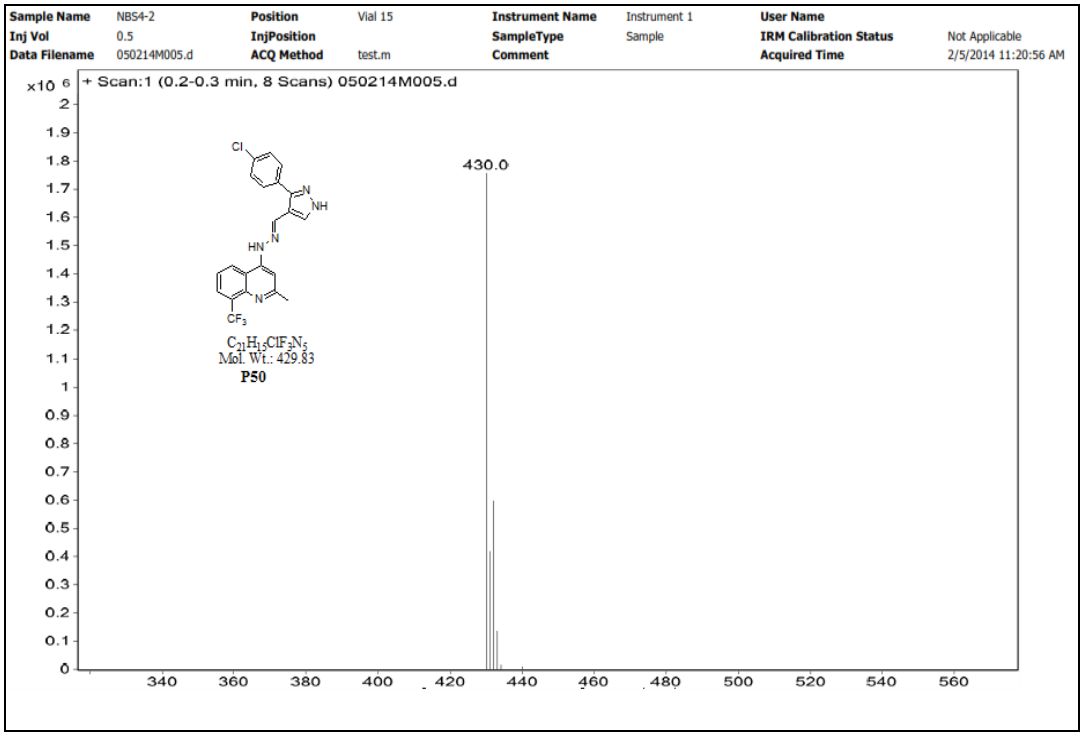


Figure 4.11 Mass spectrum of **P50.**

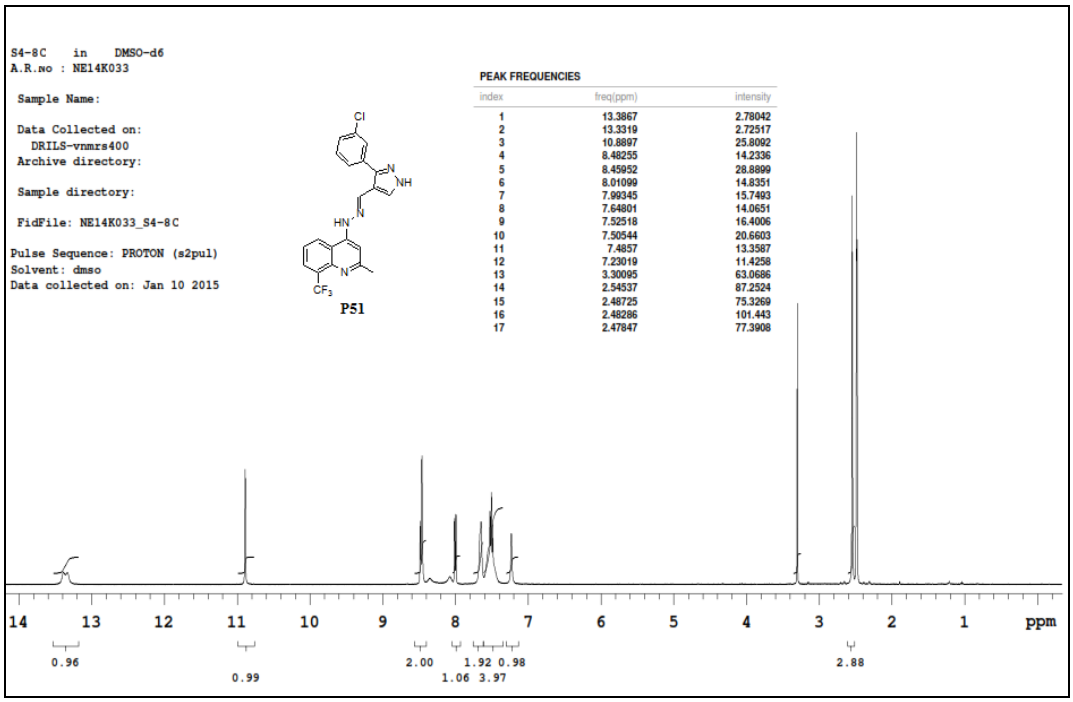


Figure 4.12 ^{1}H NMR spectrum of **P51.**

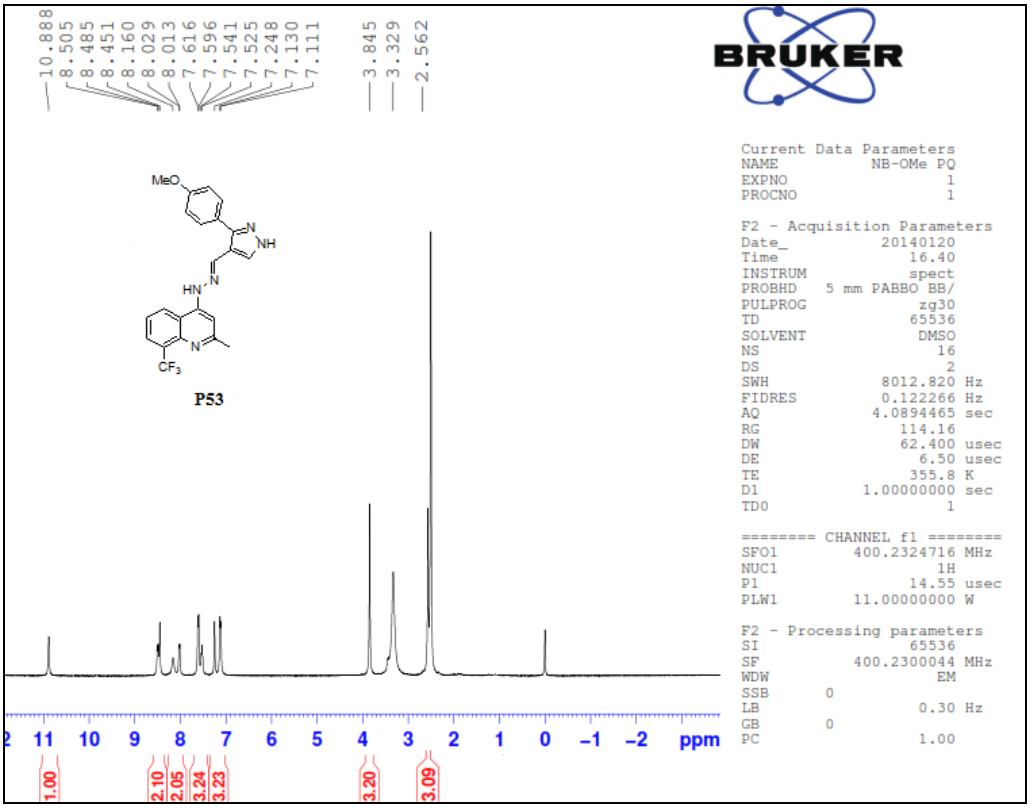


Figure 4.13 ^{1}H NMR spectrum of **P53.**

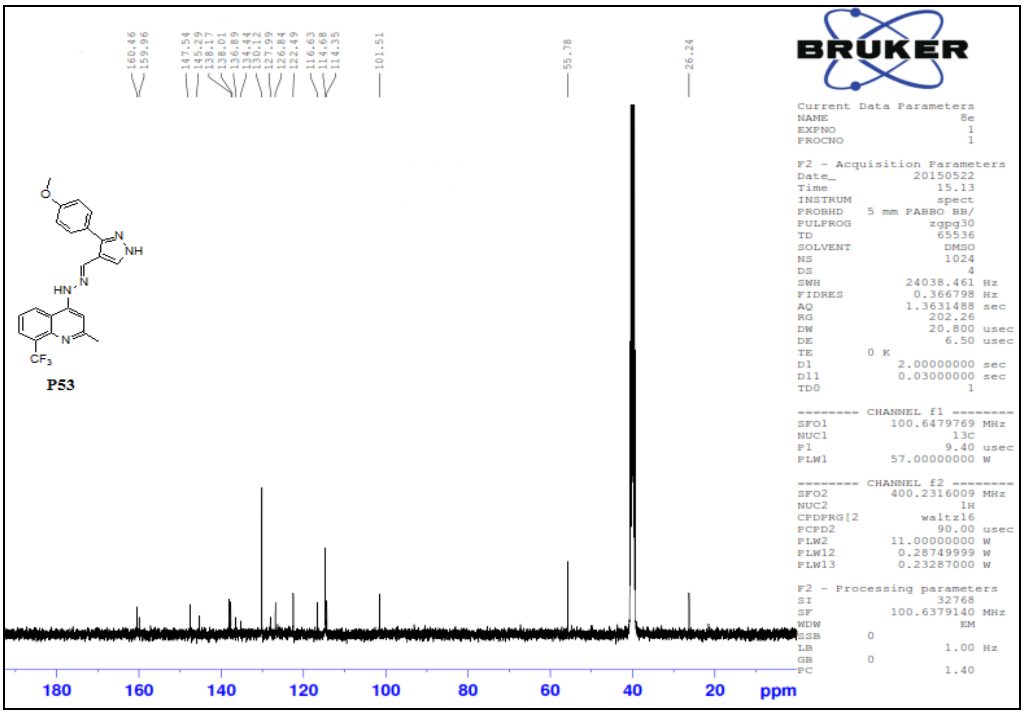


Figure 4.14 ^{13}C NMR spectrum of **P53.**

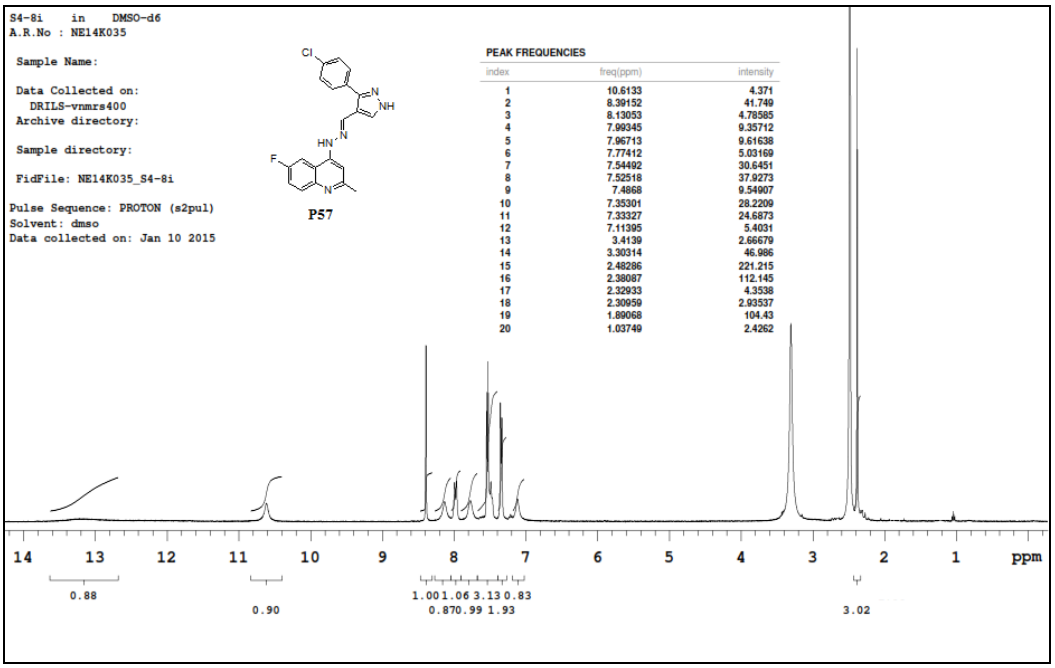


Figure 4.15 ^{1}H NMR spectrum of **P57.**

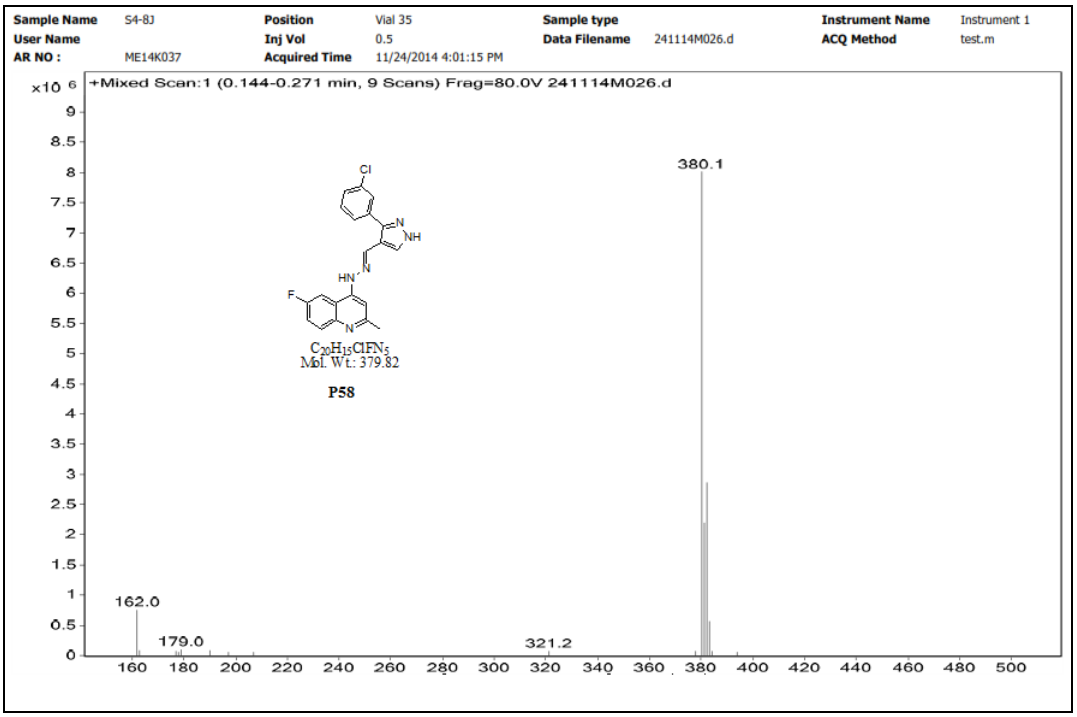


Figure 4.16 Mass spectrum of **P58.**

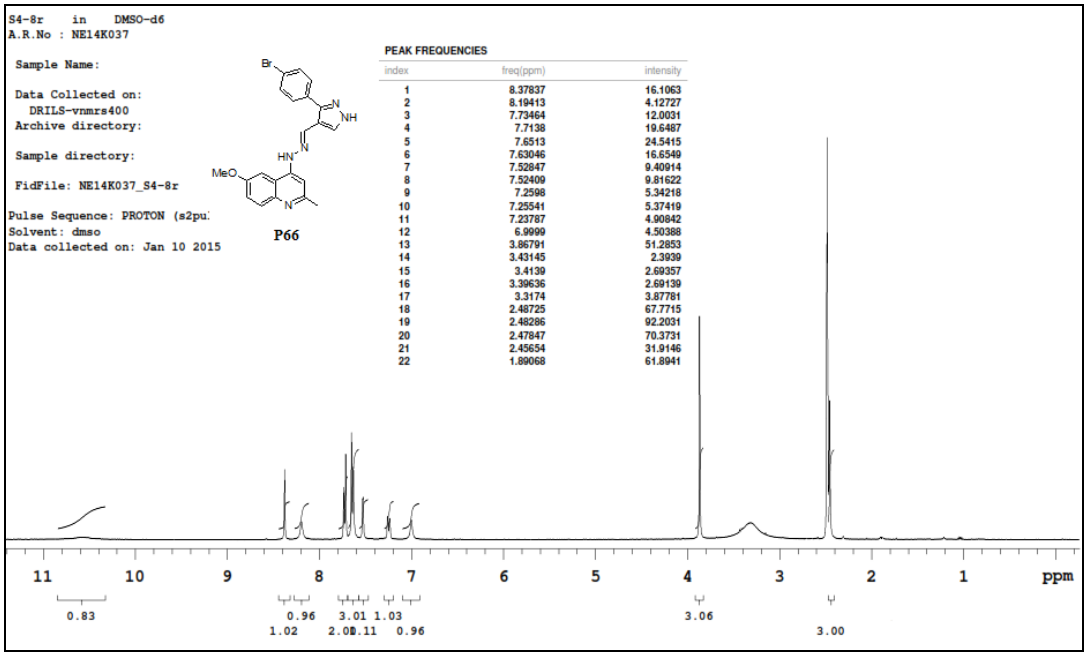


Figure 4.17 ^{1}H NMR spectrum of **P66.**

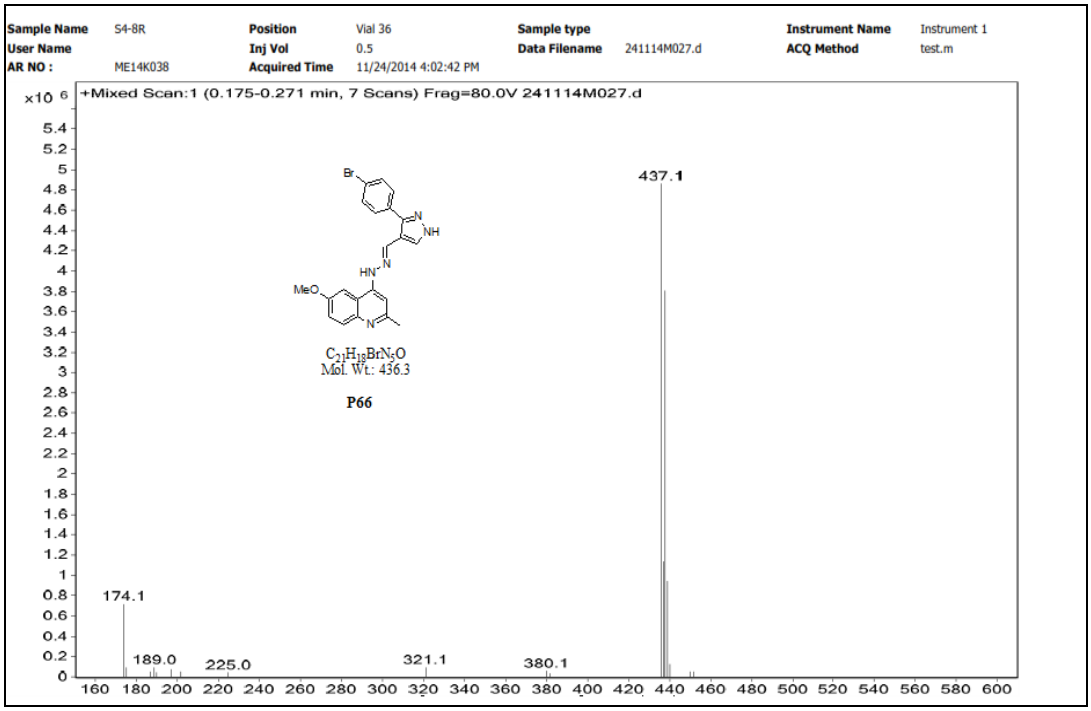


Figure 4.18 Mass spectrum of **P66.**

CHAPTER 5

SYNTHESIS AND BIOLOGICAL STUDIES OF NEW 1-(2-METHYL-8-(TRIFLUOROMETHYL)QUINOLIN-4 YL)-5-(PYRIDIN-3-YL)-1*H*-PYRAZOLE-3-CARBOXAMIDE DERIVATIVES

Abstract

This chapter explains the design and synthesis of new series of 8-trifluoromethylquinoline containing pyrazole-3-carboxamide derivatives along with structural characterization of all the newly formed intermediates and final molecules. Further in vitro antiTB and antibacterial study of all the target compounds is also included in the chapter.

5.1 INTRODUCTION

In the previous study (**Chapter 4**), it was observed that, variation of substitution on quinoline ring (-CF_3, -F and -OCH_3) has greatly altered the pharmacological action of the final compounds (**P49-P69**). It was further observed that the derivatives with 8-trifluoromethyl substitution on quinoline were found to be more active than 6-fluoro and 6-methoxy derivatives. Thus 8-trifluoromethyl substitution on quinoline ring plays major role in enhancing biological activity. In view of these observations, as a continuation of the previous work, it was decided to investigate new derivatives by retaining the 8-trifluoromethyl group on the quinoline pharmacophore in order to achieve more promising antitubercular and antibacterial leads.

As we know mefloquine, which contains a 8-trifluoromethyl substituted quinoline core, is a well-known antimalarial drug. Interestingly, it received a considerable attention recently as an antiTB agent because of its substantial activity against *Mycobacterium* species (Danelishvili et al. 2005; Sano et al. 2011). Further, the modified isoxazole (Mao et al. 2010), piperazine (Mao et al. 2007), pyrazolone (Eswaran et al. 2010) or oxazolidine (Gonçalves et al. 2010) derivatives of mefloquine (these heterocyclic rings were substituted at position-4 of the quinoline ring) showed enhanced antiTB activity (**Figure 5.1**). Considering the above points, we have planned to modify the structure of mefloquine by introducing a pyrazole-3-carboxamide pharmacophore at position-4 of the quinoline ring. In this regard we synthesized a novel series of 8-trifluoromethylquinoline containing pyrazole-3-carboxamide derivatives and screened them for their *in vitro* antiTB activity against MTB and antibacterial activity against three common pathogenic bacterial strains.

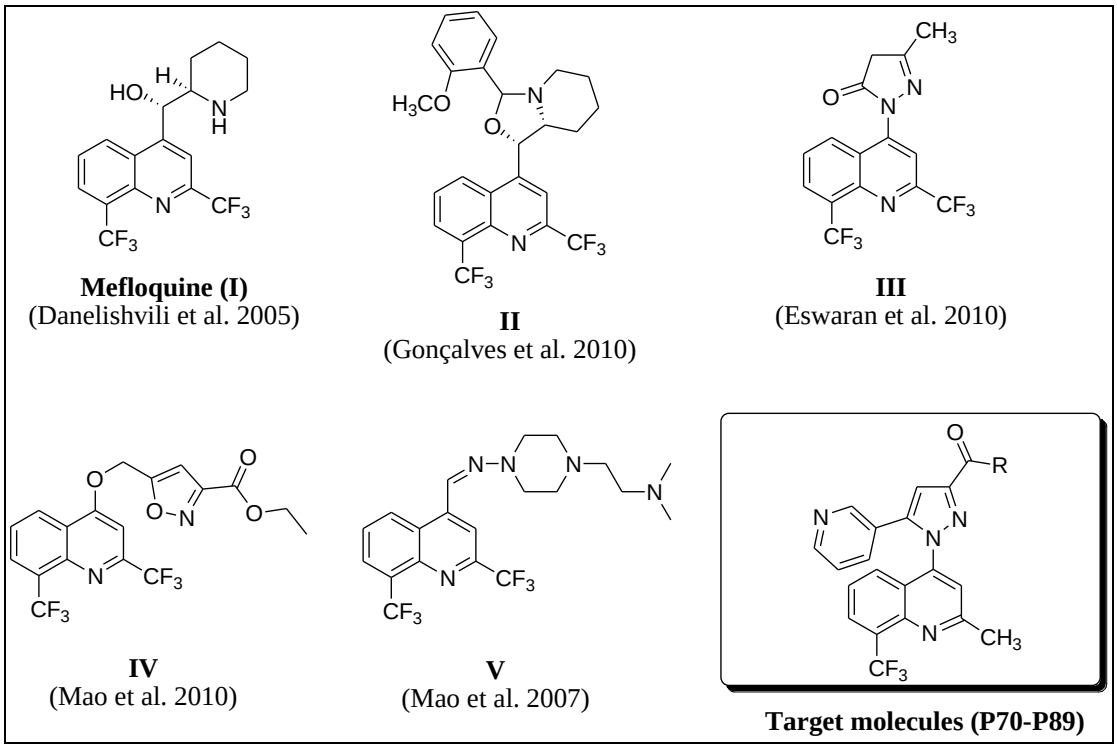
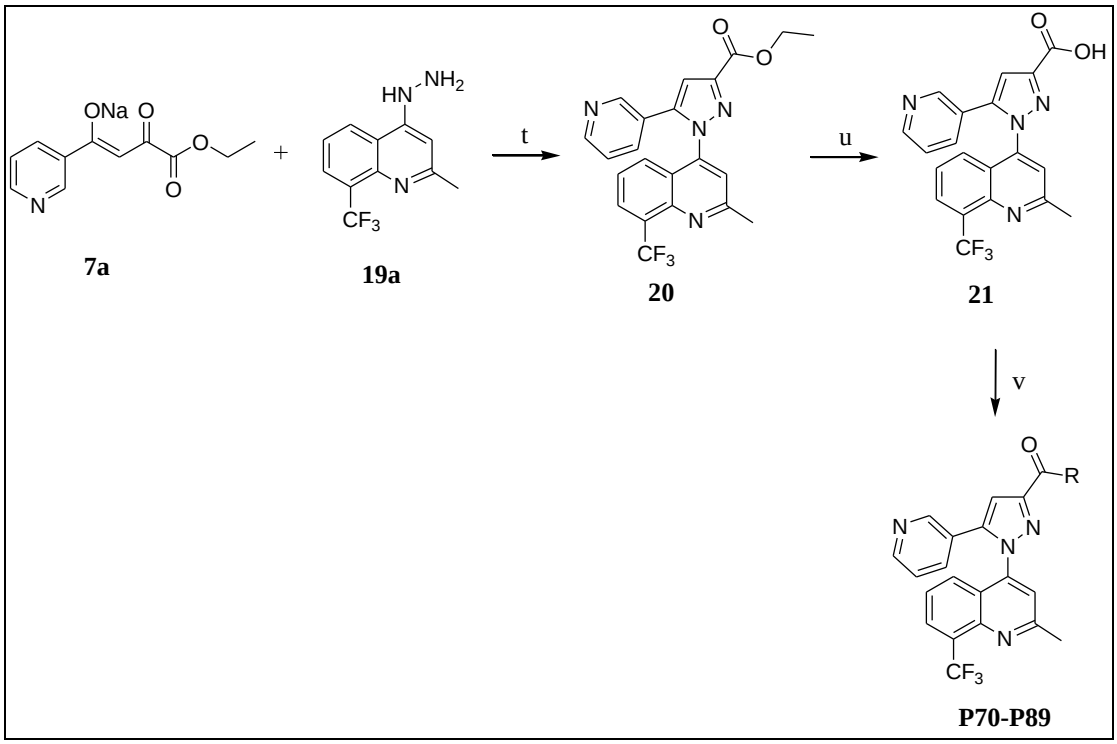


Figure 5.1 Examples of mefloquine based antiTB agents (I-V) and the general structure of target compounds **P70**-**P89**.

5.2 CHEMISTRY

The reaction sequence employed for the synthesis of target compounds (**P70**-**P89**) is shown in **Scheme 5.1**. The reaction between intermediates **7a** and **19a** in the presence of AcOH at 105 °C yielded ethyl 1-(2-methyl-8-(trifluoromethyl)quinolin-4-yl)-1*H*-pyrazole-3-carboxylate (**20**) in good yield. The carboxylic acid derivative **21**, which is the key intermediate for the synthesis of the target compounds, was synthesized by the lithium hydroxide (LiOH) mediated ester hydrolysis of intermediate **20** (Gokhale et al. 2015). The target derivatives (**P70**-**P89**) were synthesized by coupling the acid derivative (**21**) with different primary and secondary amines using HATU as the coupling agent and DIPEA as the base in DMF medium (Gokhale et al. 2015).



Scheme 5.2 Synthesis of the 8-trifluoromethyl quinoline-pyrazole based target compounds. Reagents and conditions: t) AcOH, 105 °C, 3 h; u) LiOH, THF/H_2O/MeOH (1:1:1), 8 h; v) Primary or secondary amine, HATU, DIPEA, DMF, 16 h.

5.3 EXPERIMENTAL

5.3.1 Materials and methods

Refer section **2.3.1**

5.3.2 Synthesis

The synthetic procedure followed for preparation of intermediates **7a** and **19a** has already been discussed in section **3.3.2** and **4.3.2** respectively.

Synthesis of ethyl 1-(2-methyl-8-(trifluoromethyl)quinolin-4-yl)-5-(pyridin-3-yl)-1*H*-pyrazole-3-carboxylate (20): To a stirred solution of compound **7a** (5.0 g, 20 mmol) in AcOH (50 mL), 1-(2-methyl-8-(trifluoromethyl)quinolin-4-yl)hydrazine **19a** (5.0 g, 20 mmol) was added under nitrogen atmosphere and heated at 105 °C for 3 h. The completion of the reaction was confirmed by TLC; upon completion of the reaction, the solvent was removed under vacuum. The residue obtained was diluted with water (50 mL) and extracted with EtOH (3 × 100 mL). The combined extract

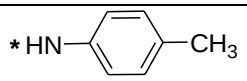
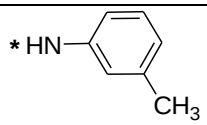
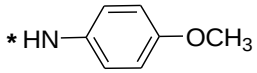
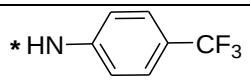
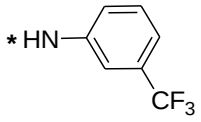
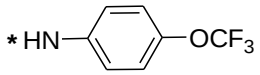
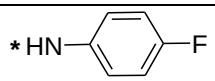
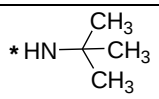
was washed with 10% sodium bicarbonate solution, then with water followed by brine solution and dried over anhydrous sodium sulphate. The solvent was removed under reduced pressure and the crude product was purified by flash column chromatography (60-120 size mesh) using 30% ethyl acetate in pet ether to afford the pure product (**20**) as yellow solid. Yield 7 g (80%); mp 155-156 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.50 (t, 1H, ArH, *J* = 2.0 Hz), 8.19 (d, 1H, ArH, *J* = 6.8 Hz), 7.76 (s, 1H, ArH), 7.55-7.70 (m, 4H, ArH), 7.43 (s, 1H, ArH), 7.27 (q, 1H, ArH, *J* = 7.8 Hz), 4.52 (q, CH_2, *J* = 7.2 Hz), 2.71 (s, 3H, CH_3), 1.31 (t, 3H, CH_3, *J* = 7.2 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 163.13, 161.50, 150.40, 148.96, 146.55, 145.02, 144.87, 143,67, 143.60, 135.91, 129.69, 128.25, 126.88, 126.80, 124,91, 124.03, 123.02, 122.44, 110.64, 61.34, 25.81, 14.64; ESI-MS (*m/z*) 427.1 $(M+H)^+$; Anal. calculated for $C_{22}H_{17}F_3N_4O_2$; C, 61.97; H, 4.02; N, 13.14. Found: C, 62.01; H, 4.02; N, 13.17.

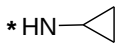
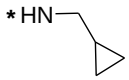
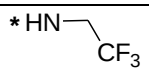
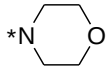
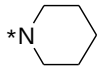
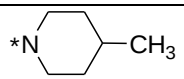
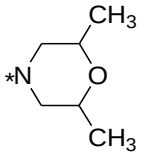
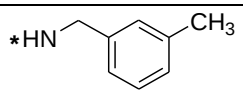
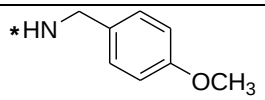
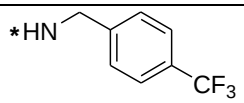
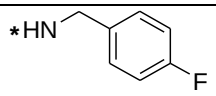
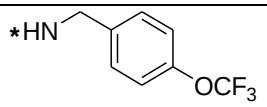
Synthesis of 1-(2-methyl-8-(trifluoromethyl)quinolin-4-yl)-5-(pyridin-3-yl)-1*H*-pyrazole-3-carboxylic acid (21): Compound **20** (6.5g, 15.2 mmol) was taken in 1:1 mixture of THF and MeOH (40 mL) and the solution was kept under stirring at 0 °C. To this solution, LiOH (1.89 g, 45 mmol) in water (20 mL) was added drop wise and the reaction mixture was stirred at RT for 8 h. The completion of the reaction was confirmed by TLC. Upon completion of the reaction, the solvent was removed under vacuum. The obtained residue was acidified with 1N HCl solution. The solid separated was filtered and recrystallized from diethyl ether to get pure product (**21**). Yield 4.74 g (78 %); pale yellow solid; mp 247-248 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 13.31 (br, 1H, COOH), 8.49 (d, 1H, ArH, *J* = 1.6 Hz), 8.40 (dd, 1H, ArH, *J* = 4.8, 2.0 Hz), 8.18 (d, 1H, ArH, *J* = 7.2 Hz), 7.75 (s, 1H, ArH), 7.55-7.71 (m, 3H, ArH), 7.41 (s, 1H, ArH), 7.26 (q, 1H, ArH, *J* = 7.2 Hz), 2.70 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 163.12, 160.89, 150.41, 148.96, 146.54, 145.01, 144.84, 143,66, 143.60, 135.71, 129.67, 128.26, 126.87, 126.81, 124,93, 123.94, 123.08, 122.47, 109.53, 25.81; ESI-MS (*m/z*) 399.0 $(M+H)^+$; Anal. calculated for $C_{20}H_{13}F_3N_4O_2$; C, 60.33; H, 3.29; N, 14.07. Found: C, 60.27; H, 2.28; N, 14.05.

General procedure for the synthesis of final compounds (P70-P89):

The acid derivative (**21**) (0.1g) was dissolved in dry DMF (2 mL) with stirring. To this, 1.1 equivalent of HATU was added and the resulting clear solution was stirred for 10 minutes at RT. To this solution, the corresponding amine derivative (1.1 eq) in DMF (1 mL) was injected and the resulting yellow solution was stirred for 20 min. Then, DIPEA (3.6 eq) was added through a syringe. The mixture was stirred for 16 h at RT; the completion of the reaction was confirmed by TLC. The reaction mass was quenched with cold water and extracted with EtOAc (3 x 25 mL). The organic layer was separated, washed with brine solution (2 x 25 mL), dried over anhydrous sodium sulfate and concentrated under reduced pressure. The crude residue thus obtained was purified over silica gel (60-120 mesh size) column chromatography using a solvent mixture of ethyl acetate and hexane (2:1) as the eluent to get the pure final compounds.

Table 5.1 Substitution pattern of final compounds **P70-P89**.

Compound	R	LogP/CLogP[a]
P70		5.85/5.75
P71		5.85/5.75
P72		5.24/5.18
P73		6.29/6.37
P74		6.29/6.37
P75		6.89/6.29
P76		5.52/5.42
P77		4.58/4.70

P78		4.00/3.82
P79		4.43/4.44
P80		4.68/4.26
P81		3.54/3.20
P82		4.67/4.23
P83		5.00/4.75
P84		4.18/4.24
P85		5.92/5.93
P86		5.31/5.35
P87		6.36/6.32
P88		5.59/5.58
P89		6.96/6.46

[a] Obtained from ChemDraw Ultra 8.0 software.

1-(2-Methyl-8-(trifluoromethyl)quinolin-4-yl)-5-(pyridin-3-yl)-*N*-p-tolyl-1*H*-pyrazole-3-carboxamide (P70): Yield 0.10 g (84 %); off white solid; mp 199-200 °C; ^{1}H NMR (400 MHz, DMSO-d$_6$) δ ppm: 10.18 (s, 1H, CONH), 8.54 (s, 1H, ArH), 8.46 (d, 1H, ArH, J = 4.0 Hz), 8.20 (d, 1H, ArH, J = 7.2 Hz), 7.85 (s, 1H, ArH), 7.60-7.76 (m, 5H, ArH), 7.50 (s, 1H, ArH), 7.28 (m, 1H, ArH), 7.12 (d, 2H, ArH, J = 8.0 Hz), 2.72 (s, 3H, CH_3), 2.25 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d$_6$) δ ppm:

161.50, 159.50, 150.44, 149.34, 148.93, 145.01, 144.06, 143.73, 136.42, 135.91, 133.31, 129.74, 129.47, 128.39, 126.89, 126.44, 126.15, 125.76, 125.01, 124.08, 123.27, 122.68, 121.00, 109.15, 25.83, 20.95; ESI-MS (*m/z*) 488.2 $(M+H)^+$; Anal. calculated for $C_{27}H_{20}F_3N_5O$; C, 65.52; H, 4.14; N, 14.37. Found: C, 65.49; H, 4.15; N, 14.39.

1-(2-Methyl-8-(trifluoromethyl)quinolin-4-yl)-5-(pyridin-3-yl)-*N*-m-tolyl-1*H*-pyrazole-3-carboxamide (P71): Yield 0.10 g (81 %); off white solid; mp 149-150 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 10.17 (s, 1H, CONH), 8.54 (s, 1H, ArH), 8.46 (d, 1H, ArH, *J* = 4.0 Hz), 8.19 (d, 1H, ArH, *J* = 7.2 Hz), 7.84 (s, 1H, ArH), 7.56-7.72 (m, 5H, ArH), 7.50 (s, 1H, ArH), 7.28 (m, 1H, ArH), 6.98-7.16 (m, 2H, ArH), 2.72 (s, 3H, CH_3), 2.27 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 161.49, 159.49, 150.44, 149.36, 149.00, 149.98, 144.10, 143.81, 136.38, 134.97, 134.88, 130.67, 128.66, 127.22, 126.62, 126.21, 125.48, 125.14, 124.01, 123.73, 123.31, 122.59, 121.04, 109.16, 25.84, 21.26; ESI-MS (*m/z*) 488.1 $(M+H)^+$; Anal. calculated for $C_{27}H_{20}F_3N_5O$; C, 65.52; H, 4.14; N, 14.37. Found: C, 65.56; H, 4.16; N, 14.40.

***N*-(4-Methoxyphenyl)-1-(2-methyl-8-(trifluoromethyl)quinolin-4-yl)-5-(pyridin-3-yl)-1*H*-pyrazole-3-carboxamide (P72):** Yield 0.11 g (85 %); off white solid; mp 161-162 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 10.17 (s, 1H, CONH), 8.53 (s, 1H, ArH), 8.46 (d, 1H, ArH, *J* = 4.4 Hz), 8.19 (d, 1H, ArH, *J* = 7.2 Hz), 7.87 (s, 1H, ArH), 7.62-7.77 (m, 5H, ArH), 7.50 (s, 1H, ArH), 7.29 (m, 1H, ArH), 7.08 (d, 2H, ArH, *J* = 8.4 Hz), 3.74 (s, 3H, OCH_3), 2.72 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 161.50, 159.49, 155.27, 150.45, 149.35, 148.99, 145.33, 144.08, 143.78, 135.66, 133.32, 130.22, 129.42, 126.77, 126.60, 126.17, 125.58, 125.11, 124.04, 123.31, 122.62, 121.11, 118.02, 108.88, 57.40, 25.83; ESI-MS (*m/z*) 504.3 $(M+H)^+$; Anal. calculated for $C_{27}H_{20}F_3N_5O_2$; C, 64.41; H, 4.00; N, 13.91. Found: C, 64.36; H, 4.02; N, 13.92.

1-(2-Methyl-8-(trifluoromethyl)quinolin-4-yl)-5-(pyridin-3-yl)-*N*-(4-(trifluoro methyl)phenyl)-1*H*-pyrazole-3-carboxamide (P73): Yield 0.10 g (77 %); off white solid; mp 158-159 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 10.66 (s, 1H, CONH), 8.55 (d, 1H, ArH, *J* = 2.0 Hz), 8.46 (dd, 1H, ArH, *J* = 4.8, 1.6 Hz), 8.19 (d, 1H, ArH,

J = 6.8 Hz), 8.06 (d, 2H, ArH, *J* = 8.4 Hz), 7.98 (s, 1H, ArH), 7.61-7.76 (m, 5H, ArH), 7.57 (s, 1H, ArH), 7.29 (m, 1H, ArH), 2.72 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 171.75, 162.77, 161.53, 160.25, 150.51, 148.96, 148.81, 145.00, 144.37, 143.66, 142.64, 135.94, 129.77, 128.32, 126.94, 126.38, 126.34, 126.17, 124.89, 124.09, 123.25, 122.73, 120.88, 109.40, 25.84; ESI-MS (*m/z*) 542.0 $(M+H)^+$; Anal. calculated for $C_{27}H_{17}F_6N_5O$; C, 59.89; H, 3.16; N, 12.93. Found: C, 59.94; H, 3.18; N, 12.95.

1-(2-Methyl-8-(trifluoromethyl)quinolin-4-yl)-5-(pyridin-3-yl)-*N*-(3-(trifluoro methyl)phenyl)-1*H*-pyrazole-3-carboxamide (P74): Yield 0.10 g (75 %); off white solid; mp 164-165 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 10.65 (s, 1H, CONH), 8.55 (d, 1H, ArH, *J* = 2.0 Hz), 8.46 (dd, 1H, ArH, *J* = 5.2, 2.0 Hz), 8.20 (d, 1H, ArH, *J* = 6.8 Hz), 8.08 (d, 2H, ArH, *J* = 8.0 Hz), 7.99 (s, 1H, ArH), 7.47-7.74 (m, 6H, ArH), 7.26 (m, 1H, ArH), 2.72 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 171.74, 162.21, 161.54, 160.26, 150.49, 149.03, 148.90, 144.92, 144.44, 143.57, 141.72, 136.74, 131.22, 129.30, 127.64, 126.76, 126.42, 126.12, 125.06, 124.26, 123.81, 121.43, 121.14, 111.02, 25.83; ESI-MS (*m/z*) 542.1 $(M+H)^+$; Anal. calculated for $C_{27}H_{17}F_6N_5O$; C, 59.8; H, 3.16; N, 12.93. Found: C, 59.92; H, 3.15; N, 12.96.

1-(2-Methyl-8-(trifluoromethyl)quinolin-4-yl)-5-(pyridin-3-yl)-*N*-(4-(trifluoro methoxy)phenyl)-1*H*-pyrazole-3-carboxamide (P75): Yield 0.10 g (73 %); pale yellow solid; mp 146-147 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 10.67 (s, 1H, CONH), 8.54 (d, 1H, ArH, *J* = 1.6 Hz), 8.46 (dd, 1H, ArH, *J* = 4.8, 2.0 Hz), 8.20 (d, 1H, ArH, *J* = 7.2 Hz), 7.99 (s, 1H, ArH), 7.44-7.72 (m, 6H, ArH), 7.22-7.34 (m, 3H, ArH), 2.72 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 171.72, 162.43. 161.66, 160.92, 160.24, 150.62, 148.96, 147.20, 146.86, 144.64, 143.52, 141.43, 133.75, 130.56, 129.64, 128.02, 126.95, 126.41, 125.30, 124.37, 123.84, 122.73, 120.88, 109.40, 25.84; ESI-MS (*m/z*) 558.0 $(M+H)^+$; Anal. calculated for $C_{27}H_{17}F_6N_5O_2$; C, 58.17; H, 3.07; N, 12.56. Found: C, 58.22; H, 3.06; N, 12.58.

***N*-(4-Fluorophenyl)-1-(2-methyl-8-(trifluoromethyl)quinolin-4-yl)-5-(pyridin-3-yl)-1*H*-pyrazole-3-carboxamide (P76):** Yield 0.09 g (76 %); off white solid; mp 161-162 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 10.22 (s, 1H, CONH), 8.54 (s,

1H, ArH), 8.46 (d, 1H, ArH, *J* = 4.4 Hz), 8.19 (d, 1H, ArH, *J* = 6.8 Hz), 7.85 (s, 1H, ArH), 7.61-7.76 (m, 5H, ArH), 7.49 (s, 1H, ArH), 7.27 (m, 1H, ArH), 7.06 (d, 2H, ArH, *J* = 7.6 Hz), 2.72 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 166.42, 161.49, 159.88, 159.50, 150.48, 149.56, 147.69, 145.12, 143.91, 136.83, 135.34, 133.74, 132.11, 130.56, 129.65, 126.83, 126.29, 126.11, 125.74, 125.01, 124.16, 122.68, 121.20, 109.21, 25.843; ESI-MS (*m/z*) 492.0 $(M+H)^+$; Anal. calculated for $C_{26}H_{17}F_4N_5O$; C, 63.54; H, 3.49; N, 14.25. Found: C, 63.59; H, 3.50; N, 14.26.

***N*-Tert-butyl-1-(2-methyl-8-(trifluoromethyl)quinolin-4-yl)-5-(pyridin-3-yl)-1*H*-pyrazole-3-carboxamide (P77):** Yield 0.10 g (86 %); off white solid; mp 166-167°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.49 (d, 1H, ArH, *J* = 2.0 Hz), 8.43 (dd, 1H, ArH, *J* = 4.8, 1.2 Hz), 8.17 (d, 1H, ArH, *J* = 6.8 Hz), 7.80 (s, 1H, ArH), 7.55-7.68 (m, 3H, ArH), 7.40 (s, 1H, CONH), 7.34 (s, 1H, ArH), 7.26 (m, 1H, ArH), 2.72 (s, 3H, CH_3), 1.37 (s, 9H, t-Bu); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 171.75, 161.55, 160.55, 150.34, 149.91, 148.83, 143.90, 143.78, 135.78, 129.63, 128.24, 126.42, 126.14, 125.75, 125.10, 124.06, 123.20, 122.70, 108.50, 28.99, 25.83; ESI-MS (*m/z*) 454.1 $(M+H)^+$; Anal. calculated for $C_{24}H_{22}F_3N_5O$; C, 63.57; H, 4.89; N, 15.44. Found: C, 63.59; H, 4.86; N, 15.42.

***N*-Cyclopropyl-1-(2-methyl-8-(trifluoromethyl)quinolin-4-yl)-5-(pyridin-3-yl)-1*H*-pyrazole-3-carboxamide (P78):** Yield 0.10 g (86 %); off white solid; mp 174-175 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.51 (d, 1H, ArH, *J* = 1.6 Hz), 8.46 (d, 1H, ArH, *J* = 4.8 Hz), 8.20 (d, 1H, ArH, *J* = 6.8 Hz), 7.81 (s, 1H, ArH), 7.57-7.69 (m, 4H, ArH and CONH), 7.38 (s, 1H, ArH), 7.27 (m, 1H, ArH), 2.87 (t, 1H, CH, *J* = 3.8 Hz), 2.72 (s, 3H, CH_3), 0.66 (t, 2H, CH_2, *J* = 3.6 Hz), 0.61 (t, 2H, CH_2, *J* = 3.8 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 162.25, 161.47, 150.34, 149.22, 148.86, 144.98, 143.81, 143.78, 135.81, 129.64, 128.35, 126.82, 126.41, 125.08, 124.06, 123.24, 123.03, 122.59, 108.61, 25.80, 23.11, 6.14; ESI-MS (*m/z*) 438.1 $(M+H)^+$; Anal. calculated for $C_{23}H_{18}F_3N_5O$; C, 63.15; H, 4.15; N, 16.01. Found: C, 63.20; H, 4.16; N, 15.98.

***N*-(Cyclopropylmethyl)-1-(2-methyl-8-(trifluoromethyl)quinolin-4-yl)-5-(pyridin-3-yl)-1*H*-pyrazole-3-carboxamide (P79):** Yield 0.10 g (82 %); pale yellow solid; mp

168-169 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.50 (d, 1H, ArH, *J* = 2.0 Hz), 8.46 (d, 1H, ArH, *J* = 4.8 Hz), 8.20 (d, 1H, ArH, *J* = 6.8 Hz), 7.80 (s, 1H, ArH), 7.55-7.69 (m, 4H, ArH and CONH), 7.37 (s, 1H, ArH), 7.27 (m, 1H, ArH), 2.98 (d, 2H, CH_2, *J* = 4.8 Hz), 2.72 (s, 3H, CH_3), 0.91 (t, 1H, CH, *J* = 3.6 Hz), 0.63-0.66 (m, 4H, CH_2, *J* = 3.8 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 162.69, 161.48, 150.35, 149.23, 148.85, 145.03, 143.88, 143.64, 135.66, 129.43, 128.36, 126.82, 126.39, 125.10, 123.98, 123.21, 122.87, 122.58, 108.62, 48.21, 25.81, 11.34, 3.52; ESI-MS (*m/z*) 452.0 $(M+H)^+$; Anal. calculated for $C_{24}H_{20}F_3N_5O$; C, 63.85; H, 4.47; N, 15.51. Found: C, 63.90; H, 4.49; N, 15.48.

1-(2-Methyl-8-(trifluoromethyl)quinolin-4-yl)-5-(pyridin-3-yl)-*N*-(2,2,2-trifluoro ethyl)-1*H*-pyrazole-3-carboxamide (P80): Yield 0.09 g (73 %); off white solid; mp 171-172 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 9.03 (t, 1H, CONH, *J* = 6.6 Hz), 8.51 (d, 1H, ArH, *J* = 2.0 Hz), 8.45 (dd, 1H, ArH, *J* = 4.8, 1.2 Hz), 8.19 (d, 1H, ArH, *J* = 6.8 Hz), 7.84 (s, 1H, ArH), 7.58-7.72 (m, 3H, ArH), 7.45 (s, 1H, ArH), 7.27 (m, 1H, ArH), 4.03 (q, 2H, CH_2, *J* = 6.6 Hz), 2.72 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 161.76, 161.45, 150.36, 149.22, 148.84, 144.93, 144.11, 143.81, 136.23, 129.41, 128.64, 128.30, 126.77, 126.49, 125.11, 123.99, 122.91, 122.86, 122.58, 108.70, 40.03, 25.80; ESI-MS (*m/z*) 480.1 $(M+H)^+$; ESI-MS (*m/z*) 480.1 $(M+H)^+$; Anal. calculated for $C_{22}H_{15}F_6N_5O$; C, 55.12; H, 3.15; N, 14.61. Found: C, 55.17; H, 3.16; N, 14.59.

(1-(2-Methyl-8-(trifluoromethyl)quinolin-4-yl)-5-(pyridin-3-yl)-1*H*-pyrazol-3-yl) (morpholino)methanone (P81): Yield 0.09 g (79 %); off white solid; mp 185-186 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.50 (d, 1H, ArH, *J* = 1.6 Hz), 8.45 (dd, 1H, ArH, *J* = 5.0, 1.8 Hz), 8.18 (d, 1H, ArH, *J* = 7.2 Hz), 7.76 (s, 1H, ArH), 7.74 (s, 1H, ArH), 7.55-7.65 (m, 2H, ArH), 7.31 (s, 1H, ArH), 7.27 (m, 1H, ArH), 3.91 (m, 2H, CH_2), 3.58-3.66 (m, 6H, CH_3), 2.72 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 171.74, 161.63, 161.42, 150.29, 149.52, 148.84, 144.96, 144.21, 142.12, 136.03, 129.65, 129.58, 128.38, 126.71, 124.95, 124.33, 123.18, 122.41, 109.52, 68.83, 48.00, 43.23, 25.80; ESI-MS (*m/z*) 468.1 $(M+H)^+$; Anal. calculated for $C_{24}H_{20}F_3N_5O_2$; C, 61.67, ; H, 4.31; N, 14.98. Found: C, 61.69; H, 4.33; N, 15.02.

(1-(2-Methyl-8-(trifluoromethyl)quinolin-4-yl)-5-(pyridin-3-yl)-1*H*-pyrazol-3-yl) (piperidin-1-yl)methanone (P82): Yield 0.10 g (84 %); off white solid; mp 175-176 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.52 (d, 1H, ArH, *J* = 1.6 Hz), 8.48 (dd, 1H, ArH, *J* = 4.8, 1.6 Hz), 8.21 (d, 1H, ArH, *J* = 6.8 Hz), 7.81 (d, 1H, ArH, *J* = 8.4 Hz), 7.72 (s, 1H, ArH), 7.57-7.69 (m, 2H, ArH), 7.27-7.31 (m, 2H, ArH), 3.80 (t, 2H, CH_2, *J* = 5.0 Hz), 3.65 (d, 2H, CH_3, *J* = 5.2 Hz), 2.72 (s, 3H, CH_3), 1.52-1,64 (m, 6H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 171.75, 161.65, 161.44, 150.31, 149.51, 148.95, 145.13, 143.66, 143.07, 135.92, 129.64, 129.59, 128.37, 126.68, 125.08, 124.04, 123.17, 122.30, 110.07, 72.86, 48.00, 43.23, 26.84, 25.90, 25.80, 24.52; ESI-MS (*m/z*) 465.2 $(M+H)^+$; Anal. calculated for $C_{25}H_{22}F_3N_5O$; C, 64.51; H, 4.76; N, 15.05. Found: C, 64.55; H, 4.77; N, 15.07.

(1-(2-Methyl-8-(trifluoromethyl)quinolin-4-yl)-5-(pyridin-3-yl)-1*H*-pyrazol-3-yl) (4-methyl piperidin-1-yl)methanone (P83): Yield 0.10 g (85 %); pale yellow solid; mp 183-184 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.52 (d, 1H, ArH, *J* = 1.6 Hz), 8.47 (dd, 1H, ArH, *J* = 5.0, 2.0 Hz), 8.20 (d, 1H, ArH, *J* = 6.8 Hz), 7.81 (d, 1H, ArH, *J* = 8.0 Hz), 7.71 (s, 1H, ArH), 7.57-7.69 (m, 2H, ArH), 7.26-7.31 (m, 2H, ArH), 3.64-3.68 (m, 4H, CH_2), 2.71 (s, 3H, CH_3), 1.36-1.62 (m, 5H, CH_3), 1.09 (d, 3H, CH_3, *J* = 4.8 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 172.45, 161.64, 160.89, 150.30, 149.51, 148.94, 145.18, 143.87, 143.23, 136.16, 129.64, 128.86, 128.36, 125.40, 125.13, 124.05, 122.69, 122.30, 110.19, 46.34, 46.11, 34.46, 31.72, 25.80, 21.07; ESI-MS (*m/z*) 480.1 $(M+H)^+$; Anal. calculated for $C_{26}H_{24}F_3N_5O$; C, 65.13; H, 5.04; N, 14.61. Found: C, 65.18; H, 5.00; N, 14.58.

(2,6-Dimethylmorpholino)(1-(2-methyl-8-(trifluoromethyl)quinolin-4-yl)-5-(pyridin-3-yl)-1*H*-pyrazol-3-yl)methanone (P84): Yield 0.10 g (78 %); pale yellow solid; mp 180-181 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.51 (d, 1H, ArH, *J* = 2.0 Hz), 8.46 (dd, 1H, ArH, *J* = 5.0, 2.0 Hz), 8.19 (d, 1H, ArH, *J* = 7.2 Hz), 7.77 (s, 1H, ArH), 7.74 (s, 1H, ArH), 7.56-7.65 (m, 2H, ArH), 7.30 (s, 1H, ArH), 7.27 (m, 1H, ArH), 3.85 (m, 2H, CH_2), 3.42-3.56 (m, 4H, CH_3), 2.72 (s, 3H, CH_3), 1.24-1.27 (m, 6H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 171.75, 161.69, 161.41, 150.31, 149.53, 148.85, 145.02, 144.21, 142.11, 136.03, 129.68, 129.60, 128.37, 126.71, 124.96, 124.32, 123.18, 122.41, 109.55, 70.15, 49.23, 49.21, 25.80, 21.58;

ESI-MS (*m/z*) 496.2 $(M+H)^+$; Anal. calculated for $C_{26}H_{24}F_3N_5O_2$; C, 63.02; H, 4.88; N, 14.13. Found: C, 62.97; H, 4.87; N, 14.11.

1-[2-Methyl-8-(trifluoromethyl)-4-quinolyl]-*N*-(m-tolylmethyl)-5-(3-pyridyl) pyrazole-3-carboxamide (P85): Yield 0.11 g (88 %); off white solid; mp 144-145 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 9.20 (t, 1H, CONH, *J* = 6.6 Hz), 8.49 (d, 1H, ArH, *J* = 2.0 Hz), 8.45 (dd, 1H, ArH, *J* = 5.6, 2.0 Hz), 8.18 (d, 1H, ArH, *J* = 6.8 Hz), 7.02-7.86 (m, 10H, ArH), 4.38 (d, 2H, CH_2, *J* = 6.0 Hz), 2.72 (s, 3H, CH_3), 2.28 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 171.74, 161.48, 161.11, 150.37, 149.05, 148.91, 147.62, 145.02, 144.06, 141.61, 138.34, 135.87, 129.94, 128.83, 127.85, 124.51, 124.41, 124.17, 122.65, 120.24, 110.06, 44.63, 25.80, 21.26; ESI-MS (*m/z*) 502.2 $(M+H)^+$; Anal. calculated for $C_{28}H_{22}F_3N_5O$; C, 67.06; H, 4.42; N, 13.96. Found: C, 67.11; H, 4.42; N, 13.97.

***N*-[(4-Methoxyphenyl)methyl]-1-[2-methyl-8-(trifluoromethyl)-4-quinolyl]-5-(3-pyridyl)pyrazole-3-carboxamide (P86):** Yield 0.11 g (84 %); off white solid; mp 116-117 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 9.18 (t, 1H, CONH, *J* = 6.6 Hz), 8.50 (d, 1H, ArH, *J* = 2.0 Hz), 8.46 (dd, 1H, ArH, *J* = 6.0, 2.0 Hz), 8.19 (d, 1H, ArH, *J* = 7.2 Hz), 6.98-7.72 (m, 10H, ArH), 4.37 (d, 2H, CH_2, *J* = 6.0 Hz), 3.74 (s, 3H, OCH_3), 2.72 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 171.74, 161.47, 161.10, 158.67, 150.41, 149.09, 148.92, 147.32, 144.07, 141.61, 135.34, 134.66, 133.64, 132.84, 129.93, 128.54, 127.85, 124.39, 124.31, 124.17, 122.66, 121.18, 110.74, 57.46, 44.63, 25.80; ESI-MS (*m/z*) 518.3 $(M+H)^+$; Anal. calculated for $C_{28}H_{22}F_3N_5O_2$; C, 64.99; H, 4.28; N, 13.53. Found: C, 64.96; H, 4.30; N, 13.55.

1-[2-Methyl-8-(trifluoromethyl)-4-quinolyl]-5-(3-pyridyl)-*N*-[[4(trifluoromethyl) phenyl]methyl]pyrazole-3-carboxamide (P87): Yield 0.11 g (79 %); off white solid; mp 139-140 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 9.12 (t, 1H, CONH, *J* = 6.2 Hz), 8.51 (d, 1H, ArH, *J* = 1.6 Hz), 8.44 (dd, 1H, ArH, *J* = 4.8, 1.2 Hz), 8.18 (d, 1H, ArH, *J* = 7.2 Hz), 7.81 (d, 1H, ArH, *J* = 8.4 Hz), 7.80 (s, 1H, ArH), 7.53-7.75 (m, 6H, ArH), 7.39 (s, 1H, ArH), 7.27 (m, 1H, ArH), 4.52 (d, 2H, CH_2, *J* = 6.0 Hz), 2.71 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 171.76, 161.47, 161.20, 150.39, 149.06, 148.90, 147.66, 145.09, 143.97, 139.56, 136.53, 135.44, 135.26, 134.76,

133.31, 129.84, 128.52, 127.37, 127.13, 125.57, 124.68, 122.34, 108.86, 43.26, 25.81; ESI-MS (*m/z*) 556.2 $(M+H)^+$; Anal. calculated for $C_{28}H_{19}F_6N_5O$; C, 60.54; H, 3.45; N, 12.61. Found: C, 60.58; H, 3.44; N, 12.64.

***N*-[(4-Fluorophenyl)methyl]-1-[2-methyl-8-(trifluoromethyl)-4-quinolyl]-5-(3-pyridyl)pyrazole-3-carboxamide (P88):** Yield 0.10 g (78 %); off white solid; mp 156-157 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 9.13 (t, 1H, CONH, *J* = 6.0 Hz), 8.50 (d, 1H, ArH, *J* = 1.6 Hz), 8.44 (dd, 1H, ArH, *J* = 6.0, 2.0 Hz), 8.18 (d, 1H, ArH, *J* = 7.2 Hz), 7.79 (s, 1H, ArH), 7.10-7.75 (m, 9H, ArH), 4.41 (d, 2H, CH_2, *J* = 6.4 Hz), 2.71 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 171.73, 161.46, 161.11, 160.53, 150.40, 148.97, 148.76, 147.31, 144.08, 137.49, 134.84, 134.65, 133.72, 132.88, 132.24, 129.92, 128.67, 127.43, 124.39, 124.34, 122.51, 121.25, 120.57, 109.76, 57.45, 44.03, 25.80; ESI-MS (*m/z*) 506.1 $(M+H)^+$; Anal. calculated for $C_{27}H_{19}F_4N_5O$; C, 64.16; H, 3.79; N, 13.86. Found: C, 64.20; H, 3.80; N, 13.85.

1-[2-Methyl-8-(trifluoromethyl)-4-quinolyl]-5-(3-pyridyl)-N-[[4-(trifluoro methoxy)phenyl]methyl]pyrazole-3-carboxamide (P89): Yield 0.11 g (82 %); pale yellow solid; mp 166-167 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 9.01 (t, 1H, CONH, *J* = 6.4 Hz), 8.52 (s, 1H, ArH), 8.46 (d, 1H, ArH, *J* = 3.6 Hz), 8.20 (d, 1H, ArH, *J* = 7.2 Hz), 7.81 (s, 1H, ArH), 7.29-7.76 (m, 9H, ArH), 4.47 (d, 2H, CH_2, *J* = 5.6 Hz), 2.70 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 171.75, 161.47, 161.19, 150.38, 149.04, 148.89, 147.64, 145.01, 144.01, 143.73, 139.60, 135.88, 129.72, 128.36, 126.85, 124.08, 123.16, 122.57, 121.75, 121.39, 108.79, 43.17, 25.80; ESI-MS (*m/z*) 572.1 $(M+H)^+$; Anal. calculated for $C_{28}H_{19}F_6N_5O_2$; C, 58.85; H, 3.35; N, 12.25. Found: C, 58.81; H, 3.36; N, 12.27.

5.4 PHARMACOLOGY

AntiTB, cytotoxicity studies studies and molecular docking studies were performed as discussed in the **Section 2.4** and antibacterial studies were performed as discussed in the **Section 3.4.**

5.5 RESULTS AND DISCUSSION

5.5.1 Chemistry

The structure of the synthesized intermediates and target compounds was confirmed by ^{1}H NMR, ^{13}C NMR and mass spectral techniques as well as by the elemental analysis. In the ^{1}H NMR spectrum of compound **20** (**Figure 5.2**), two new distinct signals at δ 4.52 (quartet) and 1.31 (triplet) ppm corresponds to -CH_2- and -CH_3 groups of the ethyl ester respectively. The proton of the C-4 pyrazole ring appeared as a sharp singlet at δ 7.43 ppm. In fact, the total proton count of the spectrum perfectly matched with its structure. Whereas, in the ^{13}C NMR spectrum, signals for -CH_2-, -CH_3 and C-4 pyrazole carbons appeared at δ 61.34 14.64 and 110.64 ppm respectively (**Figure 5.3**). Further, its three dimensional structure was proved by the SC-XRD analysis. The hydrolysis of ethyl ester (**20**) was clearly confirmed by the complete disappearance of the ethyl ester signals both in ^{1}H NMR and ^{13}C NMR spectra of the carboxylic acid derivative (**21**) (**Figure 5.4)**. In addition, the -COOH proton resonated at δ 13.31 ppm as a broad singlet. The formation of all the target compounds (**P70**-**P89**) was evidenced by ^{1}H NMR, ^{13}C NMR, mass spectral and elemental analysis data. For instance, in the ^{1}H NMR spectrum of **P70 (Figure 5.5)**, there is a new singlet signal at δ 10.18 ppm due to -CONH- proton (**Figure 5.6)**, whereas the signal due to -COOH proton disappeared completely. The spectrum shows a sharp singlet at δ 2.25 ppm which corresponds to the 4-methyl group on the aniline ring; this methyl group appeared at δ 20.95 ppm in the ^{13}C NMR spectrum (**Figure 5.7)**. The ESI mass spectrum of the molecule displayed the molecular ion peak at *m/z* 488.1 (M+1), this is in accordance with its molecular formula $C_{27}H_{20}F_3N_5O$ (Mol. Wt. = 487.48). Also, the structure of one of the final derivatives **P73** was confirmed by the SC-XRD analysis.

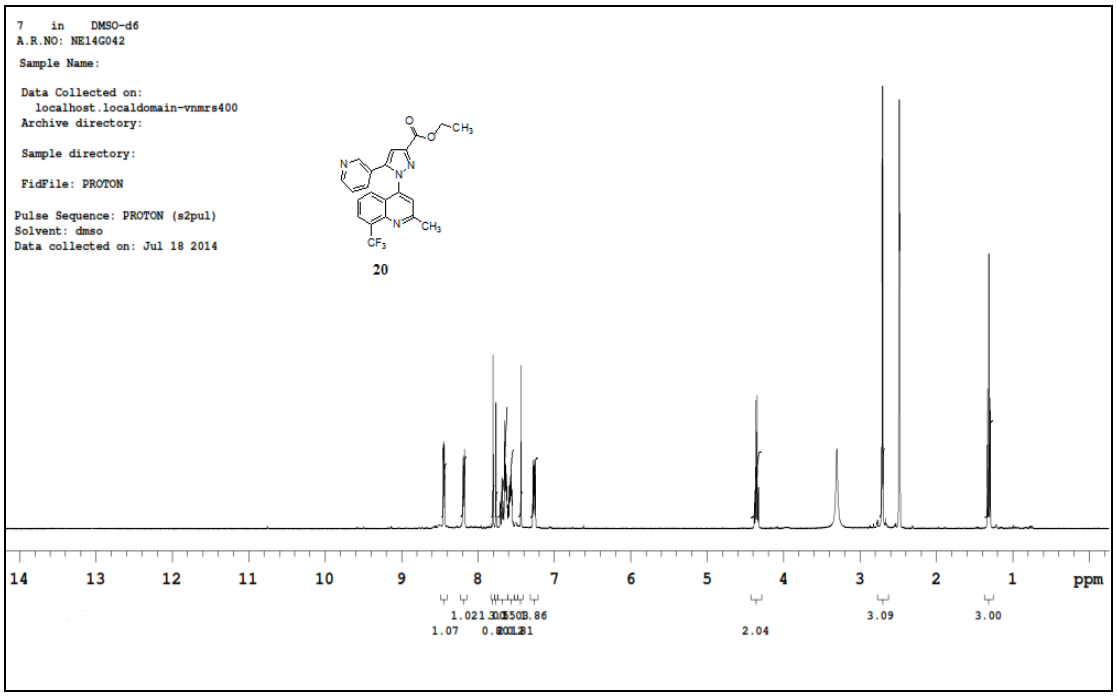


Figure 5.2 ^{1}H NMR spectrum of **20.**

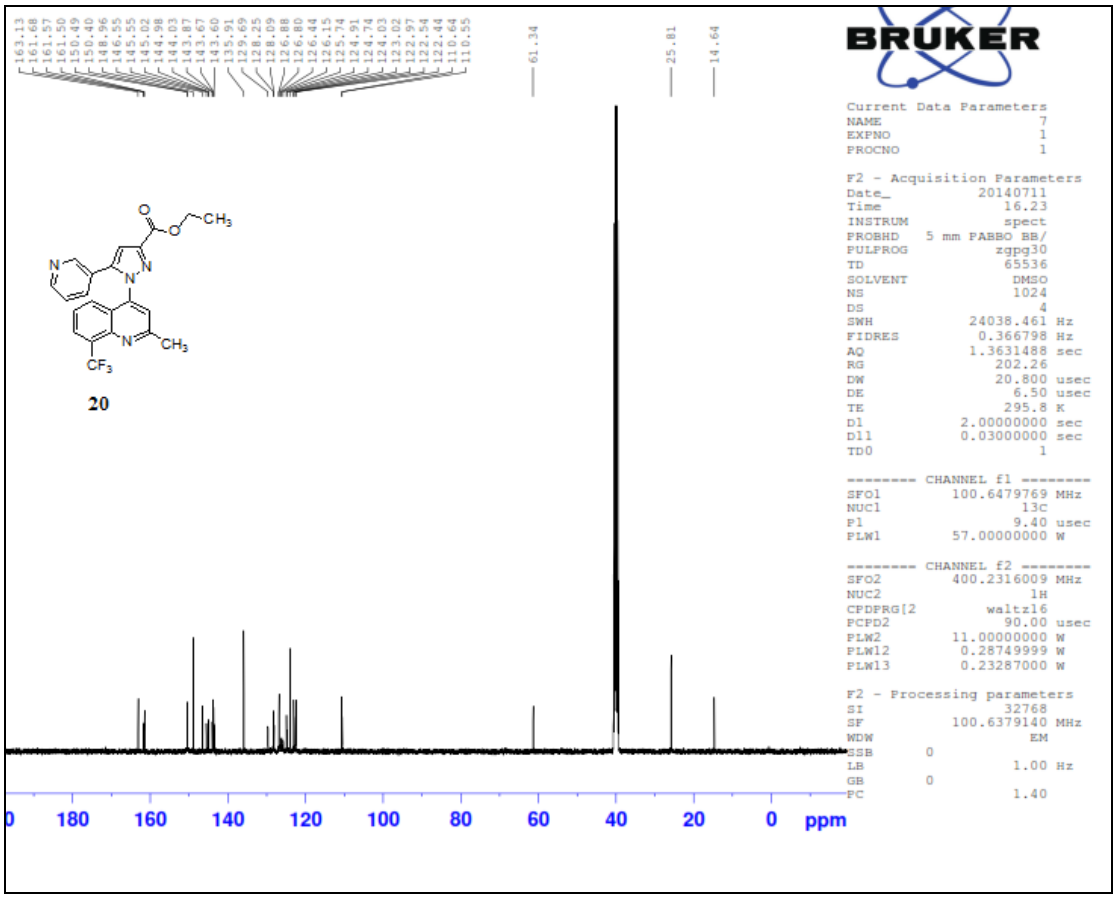


Figure 5.3 ^{13}C NMR spectrum of **20.**

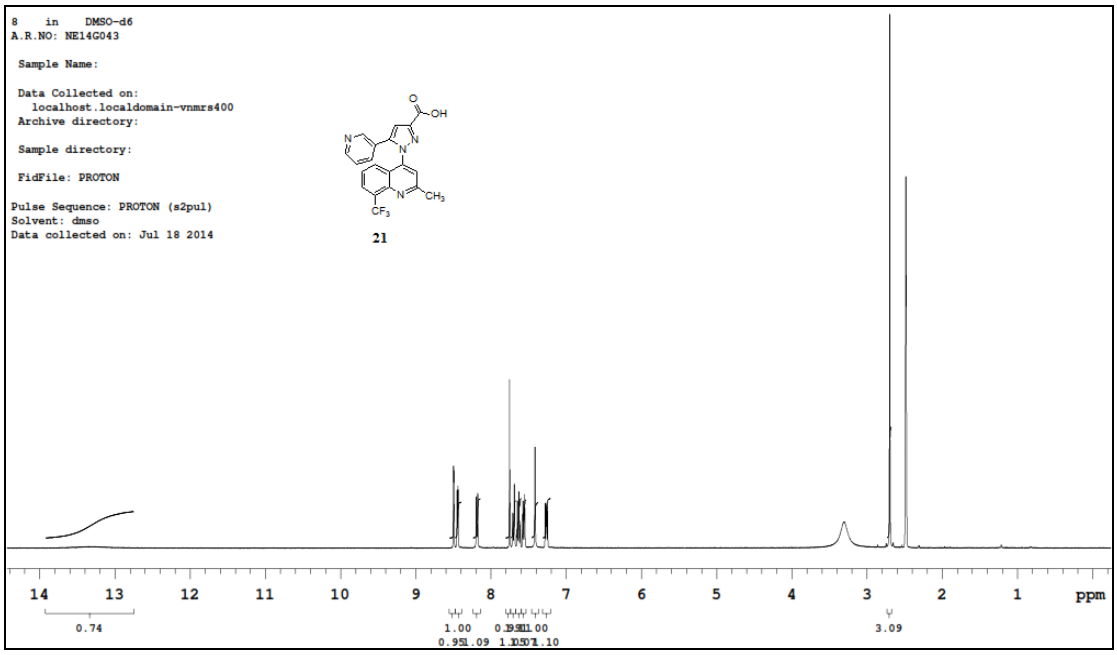


Figure 5.4 ^{1}H NMR spectrum of **21.**

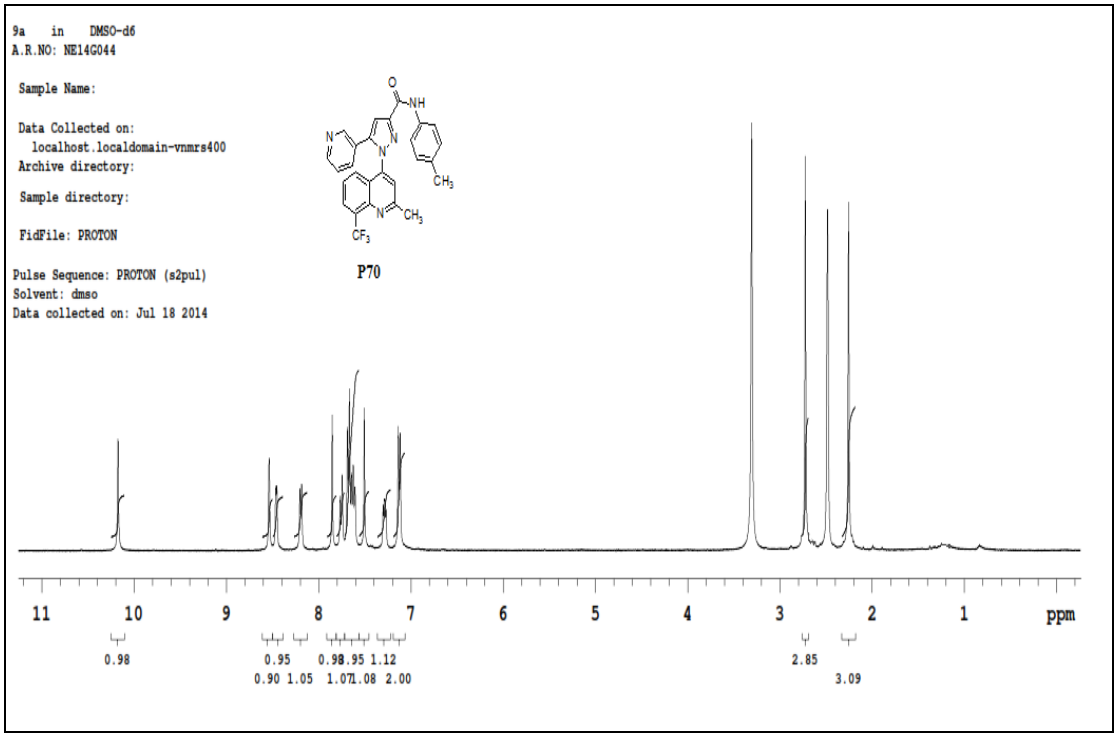


Figure 5.5 ^{1}H NMR spectrum of **P70.**

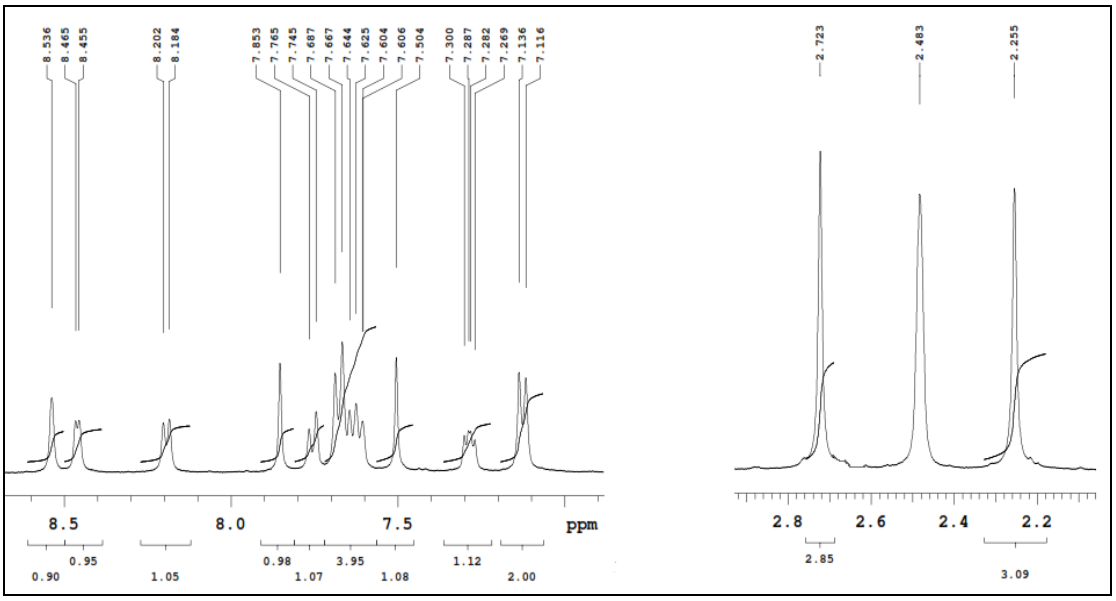


Figure 5.6 ^{1}H NMR of spectrum of **P70** (expanded)

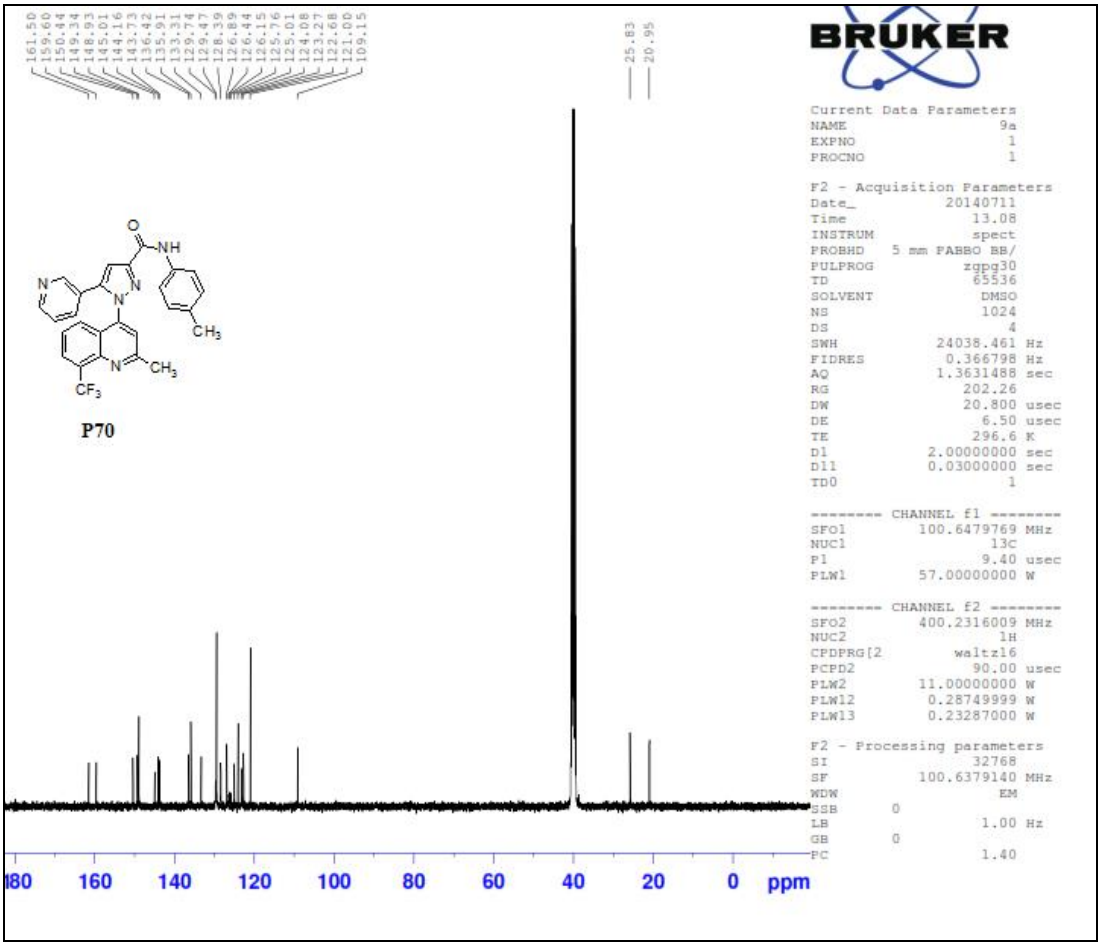


Figure 5.7 ^{13}C NMR spectrum of **P70.**

5.5.2 X-ray crystallographic analysis of 20 and P73

The single crystals of compounds **20** and **P73** were grown by the slow evaporation of their solution in 1:1 mixture of dichloromethane-methanol at RT. A crystal of an appropriate size was fixed on the X-ray diffractometer and the crystal data was collected at RT. The crystal structure was solved and refined by direct methods using SHELXL-2014/6 software. The ORTEP diagram showing the X-ray crystal structures of **20** and **P73** is shown in **Figure 5.8** and crystal structure refinement details are represented in **Table 5.2.**

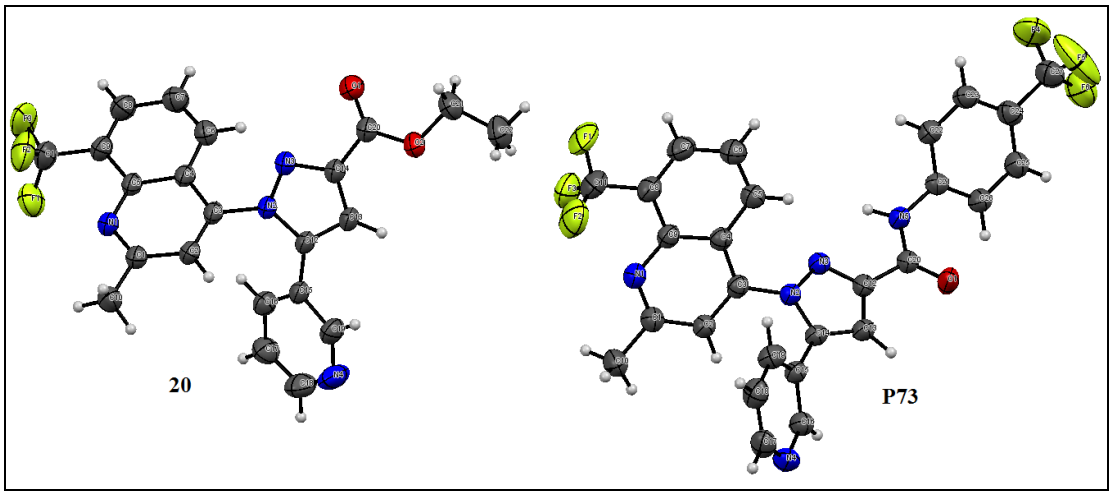


Figure 5.8 ORTEP diagram showing the X-ray crystal structures of **20** and **P73.**

Table 5.2 Crystal and structure refinement details of compounds **20** and **P73.**

Parameters	**Crystal data of 20**	**Crystal data of P73**
Chemical formula	$C_{22}H_{17}F_3N_4O_2$	$C_{27}H_{17}F_6N_5O$
Formula weight	426.39	541.45
CCDC No.	1051896	1051897
Crystal system	Monoclinic	Triclinic
Space group	P 21/n	P -1
a (Å)	11.2564(7)	9.225(5)
b (Å)	7.9005(4)	9.639(4)
c (Å)	22.1437(12)	27.465(14)
Volume ($Å^3$)	1968.67(19)	2344(2)
Angle α, β, γ	90, 91.41(3), 90	86.8(2),87.9(2), 74.1(2)

Z	4	4
F_{000}	880	1104
μ (mm^{-1})	0.114	0.130
Temperature (T)	296 K	296 K
Radiation wavelength (Å)	0.71073	0.71073
Radiation type	Mo Kα	Mo Kα
R-Factor (%)	5.58	5.08

5.5.3 Antitubercular studies

The antimycobacterial activity of the quinoline-pyrazole hybrids (**P70-P89**) and three intermediate compounds (**19a, 20** and **21**) against MTB was evaluated by agar dilution method and the MIC values are given in **Figure 5.9**. Among twenty three compounds screened against MTB, eight compounds *viz.* **P71, P72, P79, P80, P87-P89** and **21** showed significant activities with MIC values in the range 3.13 - 12.5 μg/mL. Compounds **P80** and **P89**, with a MIC of 3.13 μg/mL, are the most active compounds of the series. Their activity is comparable with that of the standard drug EMB. It was observed that the amides derived from benzylamine derivatives are more active than those derived from aniline analogues. For instance, compounds **P87-P89** showed superior activity than **P73**, **P76** and **P75** respectively. In benzylamine derivatives, the -CH_2- group attached to the amide -NH may help in reducing the steric crowd around the amide group, which intern helps the molecule to bind with the protein through hydrogen bonding. This effect is clearly evident while comparing the inhibition activity of **P78** and **P79**, just an additional -CH_2 group in **P79** doubles its activity. The presence of strong electron withdrawing -F, -CF_3 or -OCF_3 groups in the active compounds (**P80** and **P87-P89**) might also have contributed in enhancing their activity. The higher MIC values of secondary heterocyclic amine carrying targets **P81-P84** could be due to the increased steric hindrance on the amide linkage which prevents their interaction with the protein. The carboxylic acid intermediate **21** showed better activity than its ester analogue **20**. The higher polarity of the carboxylic group than the ester group may be responsible for the observed activity.

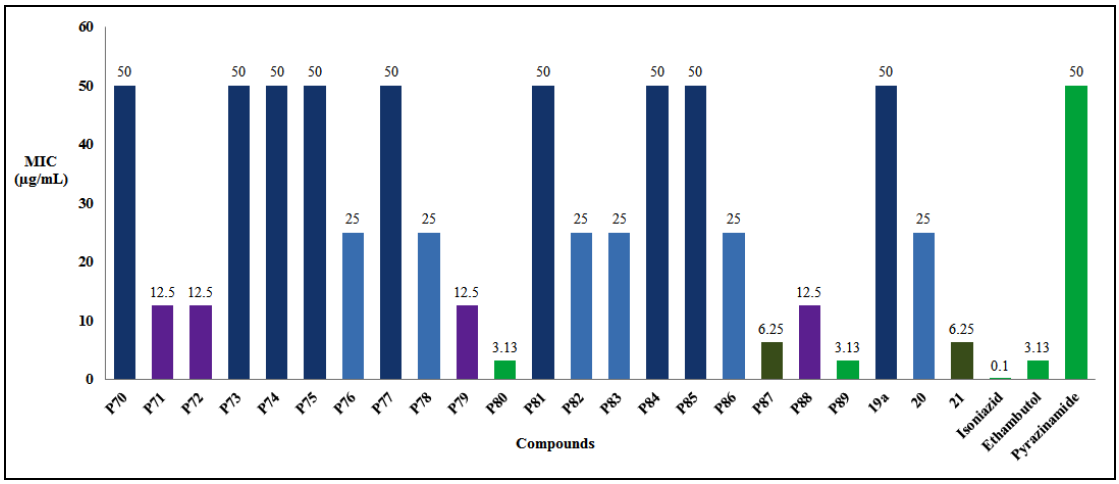


Figure 5.9 AntiTB activity of compounds **P70-P89, 19a, 20** and **21**.

5.5.4 Cytotoxicity studies

In order to determine the therapeutic potential of the newly synthesized molecules, the active derivatives with MTB inhibition ≤ 12.5 µg/mL were tested for their cytotoxicity against NIH 3T3 cell line using MTT assay and the results are tabulated in **Figure 5.10.** It can be seen that the compounds at 50 µg/mL do not inhibit the growth of the normal cell line much. The derivative **P71** was found to be the least toxic among the tested compounds with growth inhibition of only 8.36 %. The two potential derivatives **P80** and **P89** showed normal inhibition of 14.6 and 22.8 % respectively, which in turn proves the suitability of these molecules for further development as antiTB agents.

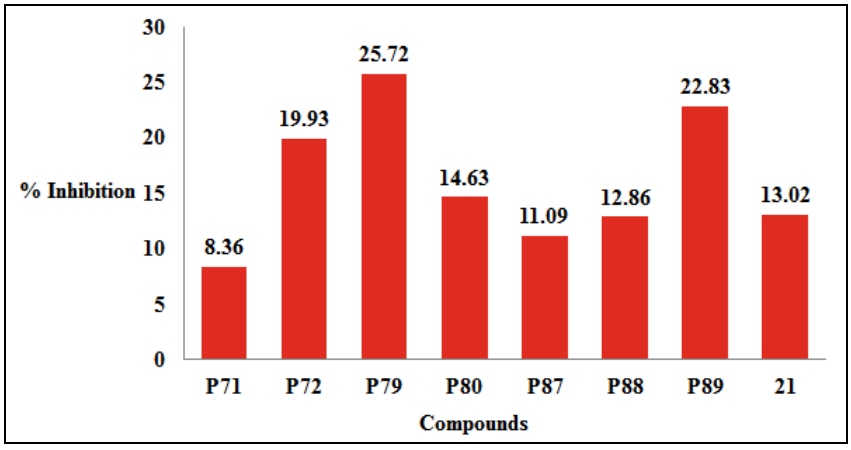


Figure 5.10 Cytotoxicity of the active quinoline-pyrazole derivatives (at a concentration of 50 µg/mL) against NIH 3T3 cells.

5.5.5 Molecular docking studies

The *in silico* molecular modeling results of the active antiTB molecules **P80, P87, P89** and **21** against three protein structures InhA, CYP121 and TMPK are tabulated in **Table 5.3**. In the case of InhA, all the four ligands showed one common interaction with Tyr 158 (**Figure 5.11**). The docking scores (-8.89 to -11.79) also found to be comparable with that of the standard ligand. When docked against CYP121, ligands **P87** (-9.10) and **P89** (-9.08) showed higher docking score than the other two ligands, but **P87** has only one interaction with the amino acid residue (**Figure 5.12**). Ligand **P89** has five interactions with good docking score (-9.08). The docking scores of these molecules against TMPK are much lower than that of the standard ligand. However, ligands **P80** and **P87** showed three and four interactions respectively (**Figure 5.13**), whereas other two ligands **P89** and **21** showed one interaction each. The observed antiTB activity and docking results reveal that the compounds possess good inhibition profile against the enzymes of MTB.

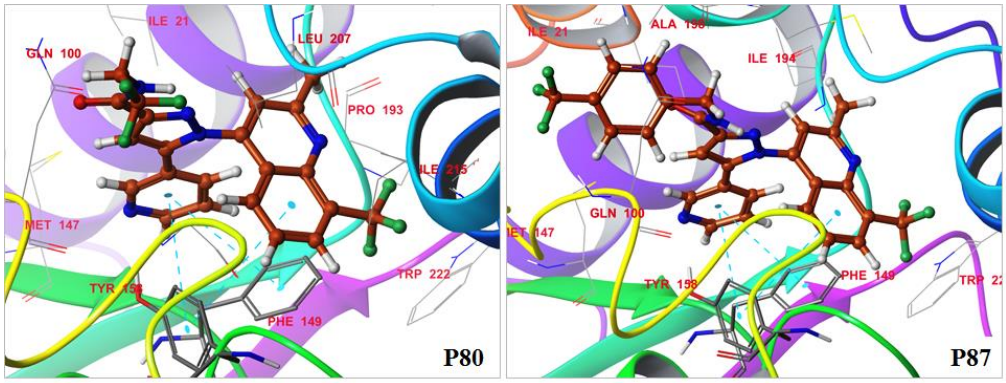


Figure 5.11 The docking pose of the compounds **P80** and **P87** in the active pocket of InhA.

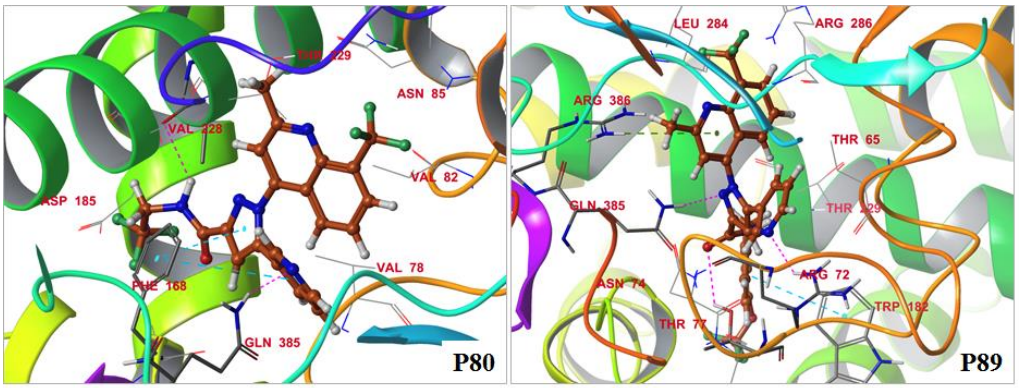


Figure 5.12 The docking pose of the compounds **P80** and **P89** in the active pocket of CYP121.

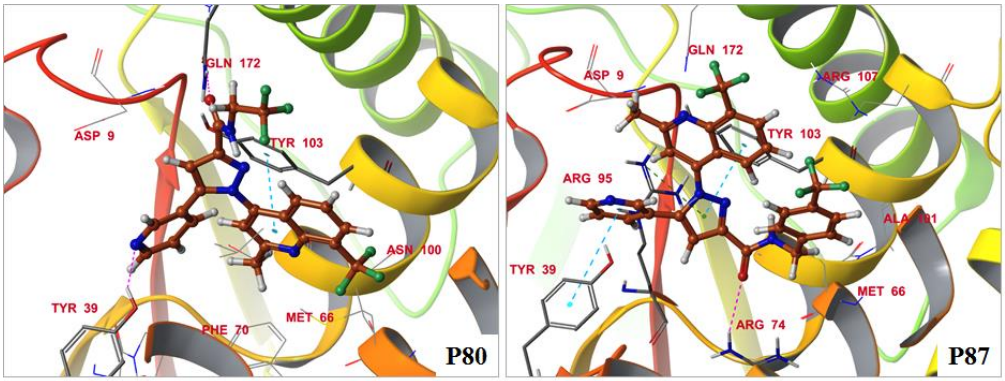


Figure 5.13 The docking pose of the compounds **P80** and **P87** in the active pocket of TMPK.

Table 5.3 Docking results of the active antiTB compounds

Compound ID	InhA		CYP121		TMPK	
	Docking score	**Amino acids that interacted with ligands**	**Docking score**	**Amino acids that interacted with ligands**	**Docking score**	**Amino acids that interacted with ligands**
Standard Ligand	-12.51	Tyr 158, Phe 149	-10.38	Gln 385, Val 228, Asn 85	-13.12	Asn 100, Tyr 103, Arg 74, Arg 95,
P80	-9.15	Tyr 158, Phe 149	-6.53	Val 228, Gln 385, Phe 168	-7.89	Tyr 39, Gln 172, Tyr 103
P87	-9.63	Tyr 158, Phe 149	-9.10	Phe 168	-8.28	Arg 74, Arg 95, Tyr 39, Tyr 103
P89	-11.79	Tyr 158	-9.08	Arg 386, Gln 385, Thr 77, Arg 72, Trp 182	-6.64	Arg 95
21	-8.89	Tyr 158, Gly 104	-5.99	Arg 72, Leu 73, Asn 74	-7.66	Arg 74

5.5.6 Antibacterial studies

The *in vitro* antibacterial activities of the newly synthesized compounds (P70-P89) are presented in **Table 5.4**. The screening results reveal that, most of the tested compounds show moderate inhibition against *S. aureus* and *P. aeruginosa* strains. Amongst the targets, intermediate **19a**, with significant inhibition against all the tested strains emerged as a promising lead molecule. The remarkable activity may be attributed to the presence of active hydrazine (-NH-NH_2) group in the molecule. Target compounds **P79** and **P80** with cyclopropylmethyl and trifluoroethyl substitution respectively on the amide nitrogen showed better inhibition activity than rest of the molecules, which may be due to lesser steric crowd around the amide group of these molecules. In general, substitution of amide nitrogen with less sterically hindered aliphatic groups containing an electron withdrawing moiety enhanced the inhibition activity of the molecules.

Table 5.4 Antibacterial activity of compounds **P70-P89**, **19a, 20** and **21**.

Compound	**Zone of inhibition (mm)**					
	Staphylococcus aureus		***Escherichia coli***		***Pseudomonas aeruginosa***	
Conc. in µg/ml	75	50	75	50	75	50
Control	00	00	00	00	00	00
Ciprofloxacin	26±0.1	21±0.2	32±0.2	27±0.2	21±0.2	18±0.1
P70	12±0.1	10±0.2	09±0.3	08±0.3	13±0.2	10±0.1
P71	11±0.2	10±0.2	10±0.1	07±0.2	14±0.1	12±0.2
P72	09±0.2	06±0.1	--	--	13±0.1	11±0.2
P73	17±0.3	14±0.3	--	--	11±0.3	10±0.3
P74	15±0.1	12±0.1	10±0.3	08±0.1	13±0.3	09±0.1
P75	14±0.1	11±0.2	09±0.1	07±0.3	14±0.2	11±0.3
P76	09±0.3	08±0.3	08±0.3	06±0.3	12±0.1	11±0.1
P77	11±0.1	09±0.3	09±0.1	07±0.1	11±0.2	09±0.3
P78	10±0.2	07±0.1	--	--	14±0.1	11±0.1
P79	**20±0.3**	**18±0.3**	**18±0.1**	**16±0.4**	**19±0.1**	**16±0.1**
P80	**22±0.1**	**18±0.1**	**19±0.2**	**17±0.1**	**21±0.1**	**17±0.1**
P81	11±0.3	09±0.2	10±0.2	08±0.3	14±0.2	10±0.1

P82	11±0.2	10±0.3	--	--	13±0.3	11±0.2
P83	10±0.2	09±0.2	--	--	13±0.3	09±0.3
P84	09±0.1	07±0.1	09±0.3	06±0.3	10±0.1	08±0.2
P85	11±0.1	10±0.1	14±0.1	11±0.1	12±0.1	09±0.2
P86	11±0.2	09±0.1	10±0.1	09±0.2	13±0.4	11±0.1
P87	14±0.2	12±0.2	13±0.3	10±0.2	10±0.1	08±0.1
P88	16±0.1	12±0.2	10±0.1	07±0.1	14±0.2	10±0.1
P89	15±0.3	12±0.1	10±0.1	08±0.1	13±0.1	11±0.2
19a	**24±0.3**	**20±0.1**	**31±0.2**	**27±0.3**	**20±0.3**	**17±0.2**
20	--	--	--	--	--	--
21	14±0.2	09±0.3	10±0.2	07±0.1	14±0.3	12±0.3

--: Bacteria resistant; Control: dimethylsulfoxide

5.6 CONCLUSIONS

- A new series of 8-trifluoromethyl quinoline containing pyrazole-3-carboxamide derivatives were synthesized, characterized and evaluated for their antiTB activity against MTB.
- The structures of the intermediate **20** and the final derivative **P73** were established by SC-XRD analysis.
- Derivatives, **P80** and **P89** emerged as the most active antiTB of the series with a MIC of 3.13 µg/mL.
- We attempted to establish a structural activity relationship (SAR) on the basis of hydrogen bonding interaction, electronic and steric factors.
- The carboxamides derived from benzylamine derivatives were more active than their aniline analogues.
- The hybrid amides with less sterically hindered substituents on the amide nitrogen exhibited enhanced antiTB activity.
- The cytotoxicity study of the active antiTB agents was also carried out which revealed the lack of general cellular toxicity of the molecules.
- These findings described here demonstrate the potential of these new quinoline-pyrazole derivatives for further optimization as active antiTB agents.

- The observed activity profile of the molecules was further supported by the molecular docking studies.
- Further, antibacterial screening of these compounds revealed that intermediate **19a** is nearly as potent as the standard drug, Ciprofloxacin.
- Two target compounds **P79** and **P80,** which have lesser steric crowd around the amide linkage, showed significant antibacterial activity whereas rest of the molecules displayed a moderate activity.

Appendix 5.1

Representative ^{1}H NMR, ^{13}C NMR and ESI-MS spectra of final compounds

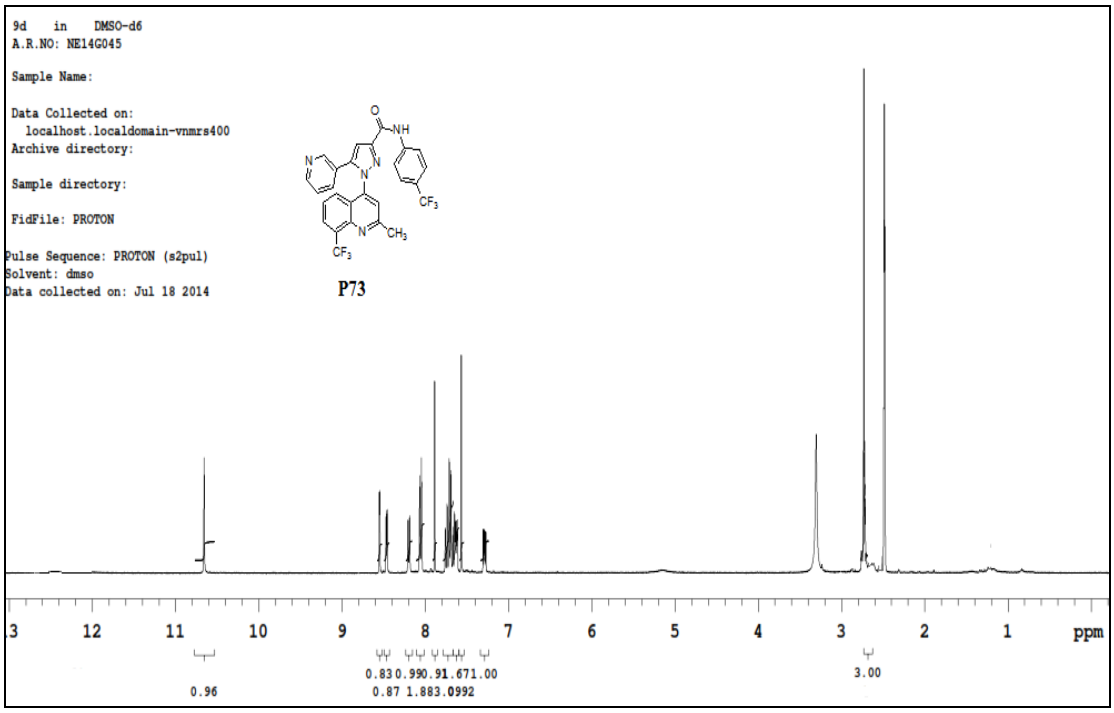


Figure 5.14 ^{1}H NMR spectrum of **P73.**

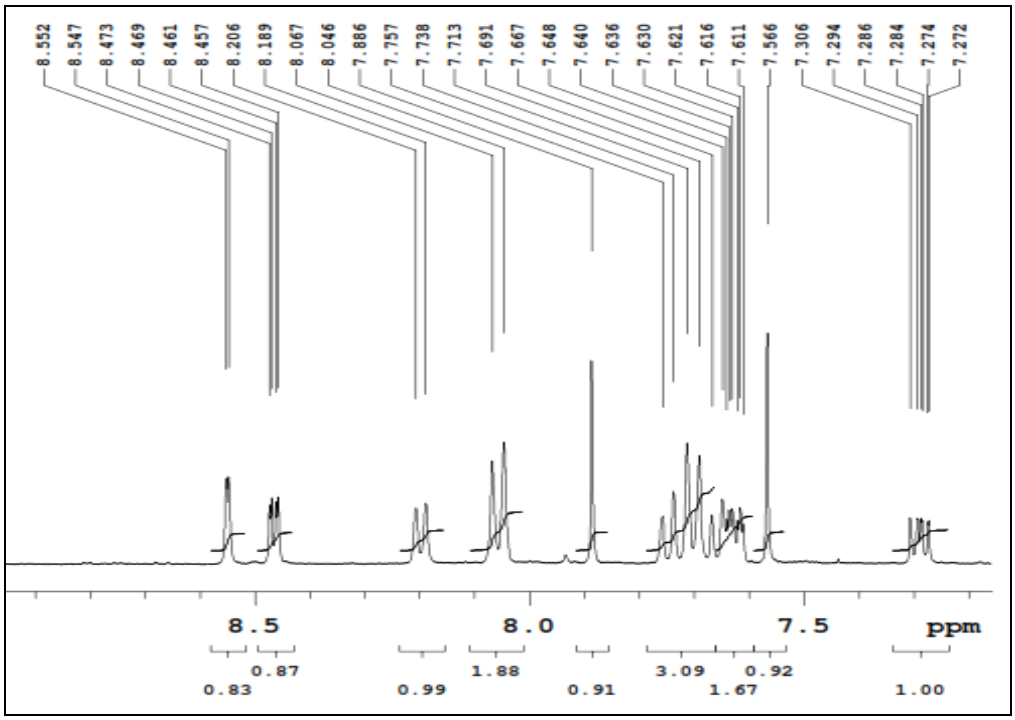


Figure 5.15 ^{1}H NMR of spectrum of **P73** (expanded aromatic region).

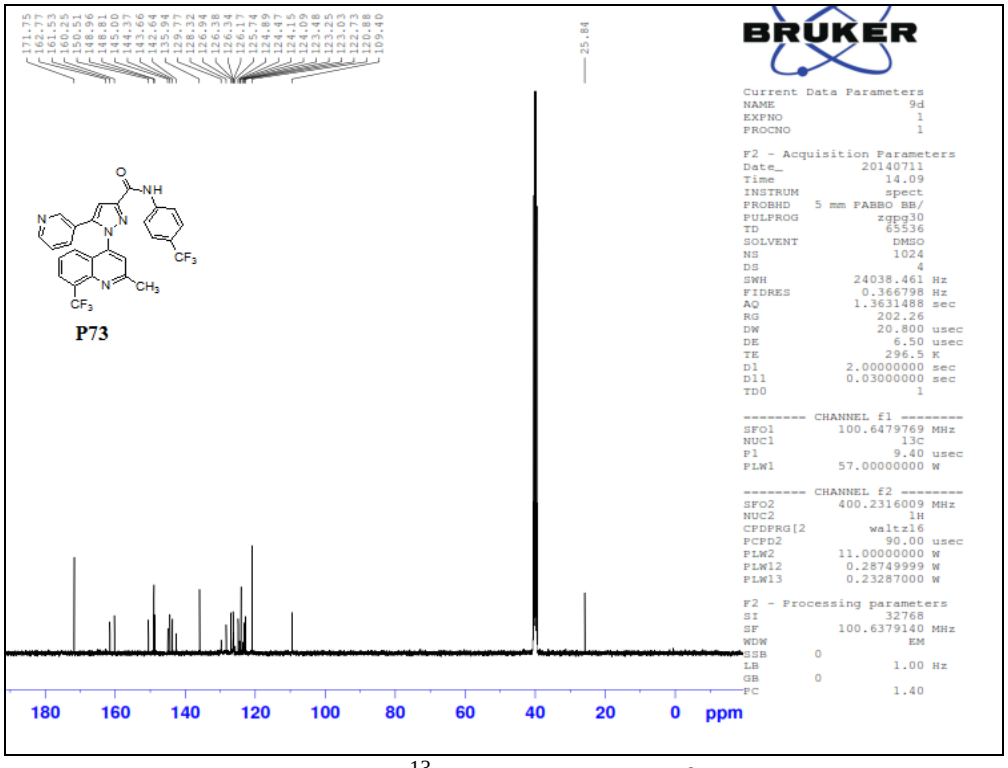


Figure 5.16 ^{13}C NMR spectrum of **P73.**

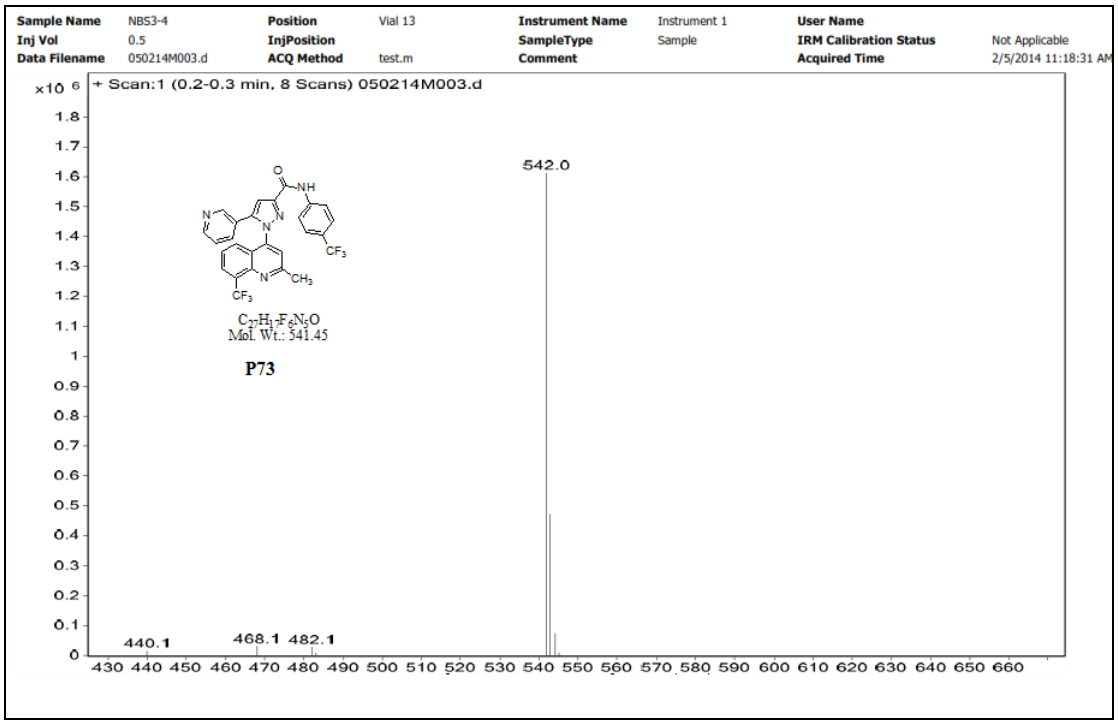


Figure 5.17 Mass spectrum of **P73.**

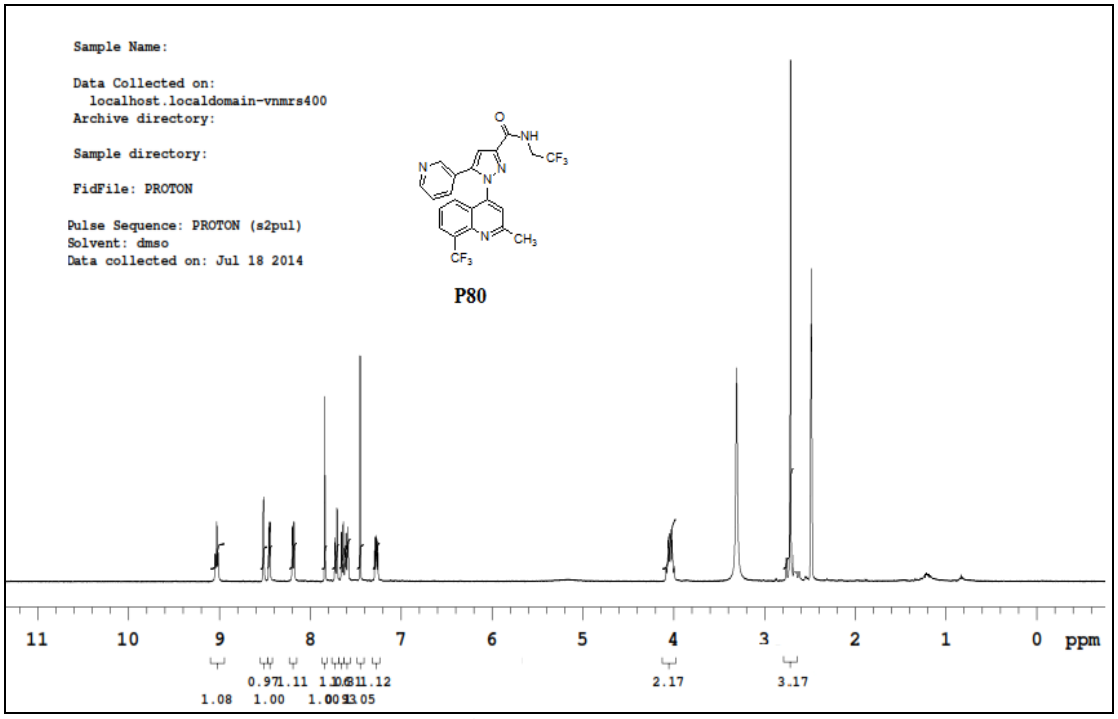


Figure 5.18 ^{1}H NMR spectrum of **P80.**

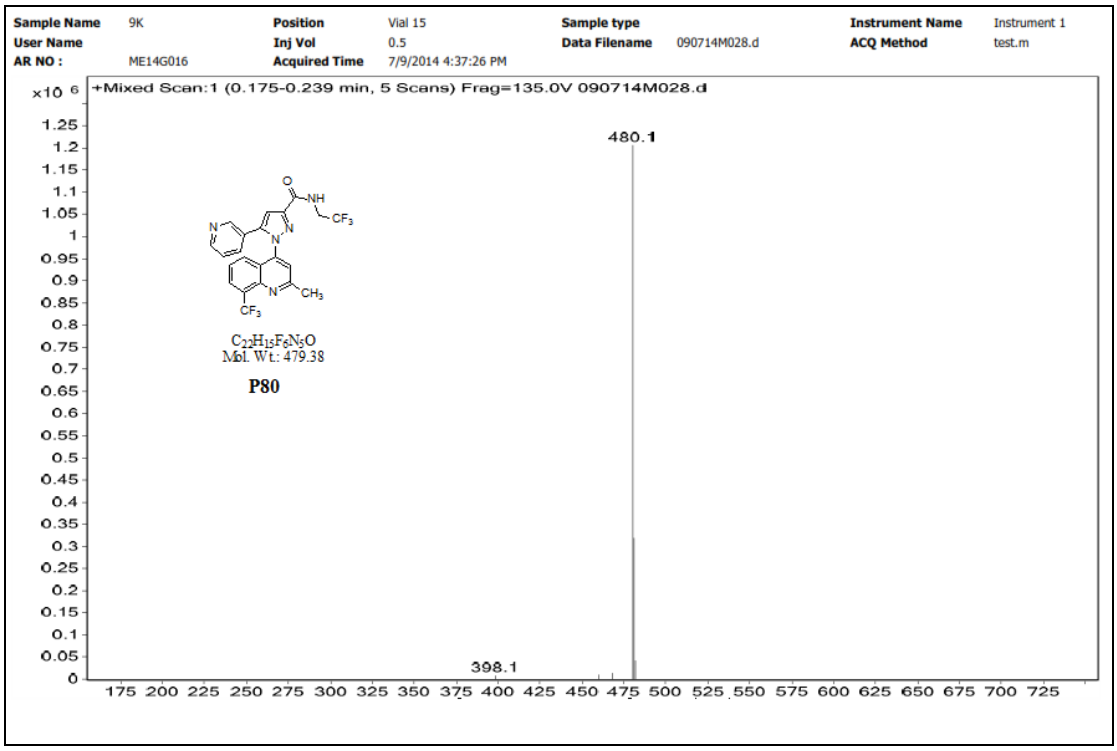


Figure 5.19 Mass spectrum of **P80.**

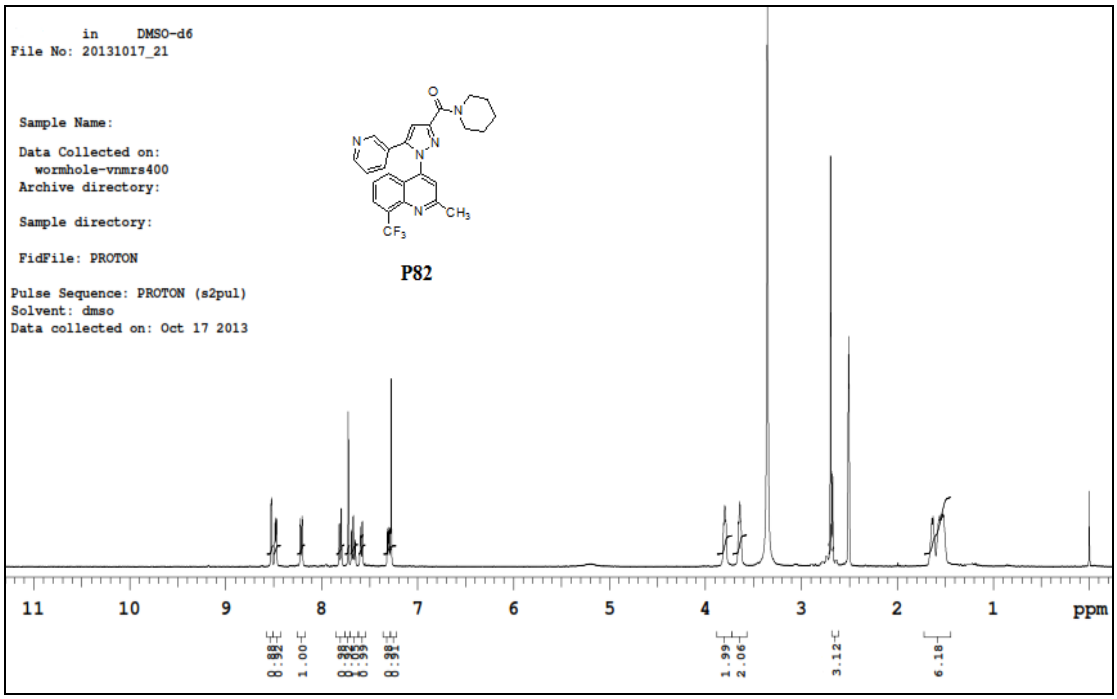


Figure 5.20 ^{1}H NMR spectrum of **P82.**

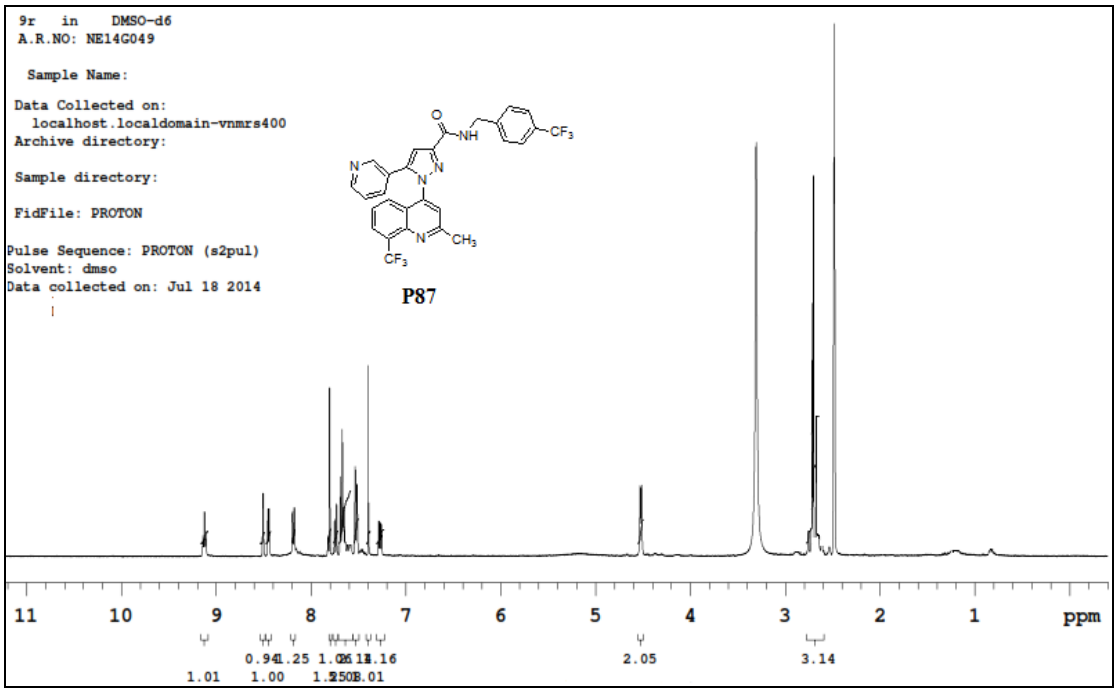


Figure 5.21 ^{1}H NMR spectrum of **P87.**

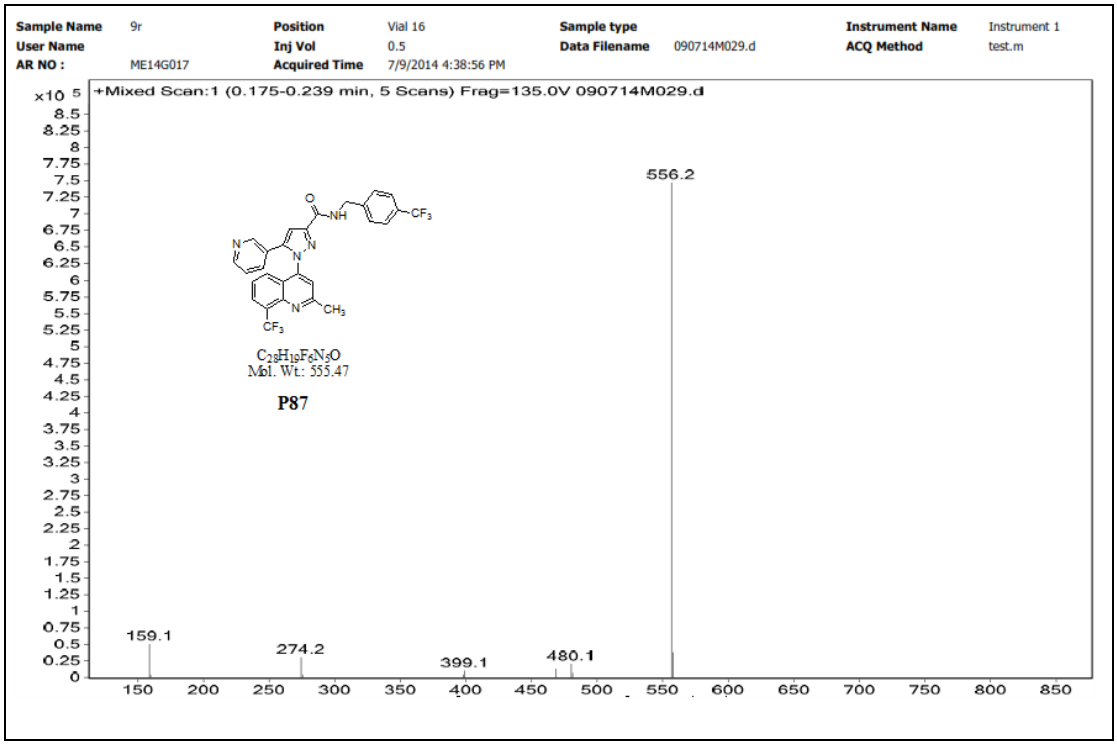


Figure 5.22 Mass spectrum of **P87.**

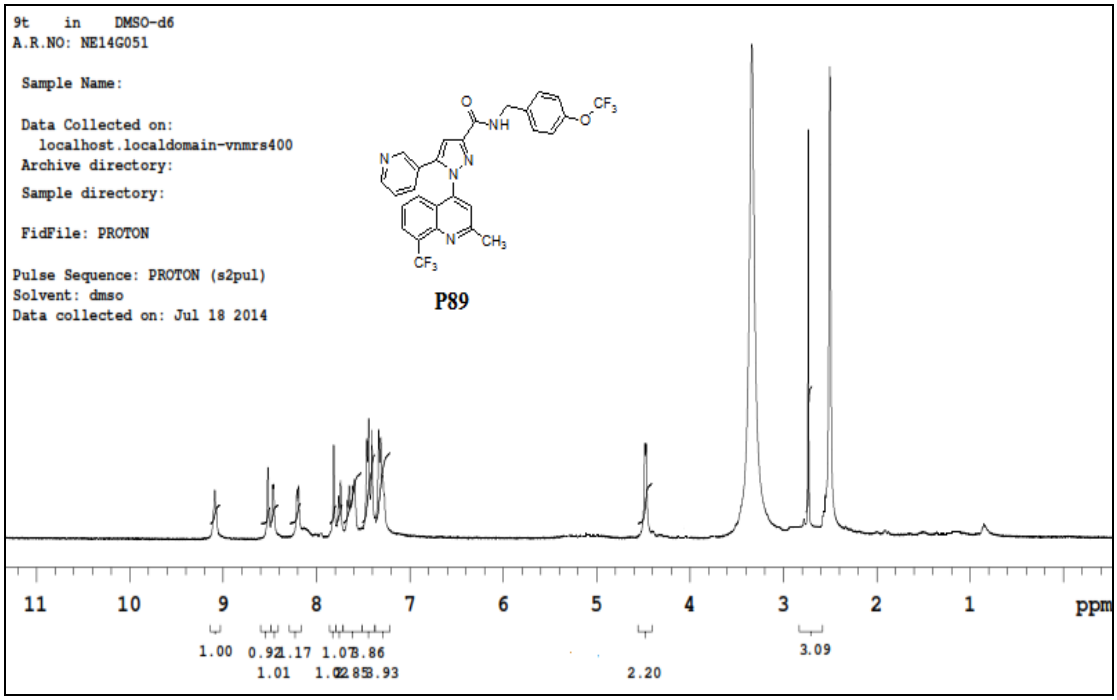


Figure 5.23 ^{1}H NMR spectrum of **P89.**

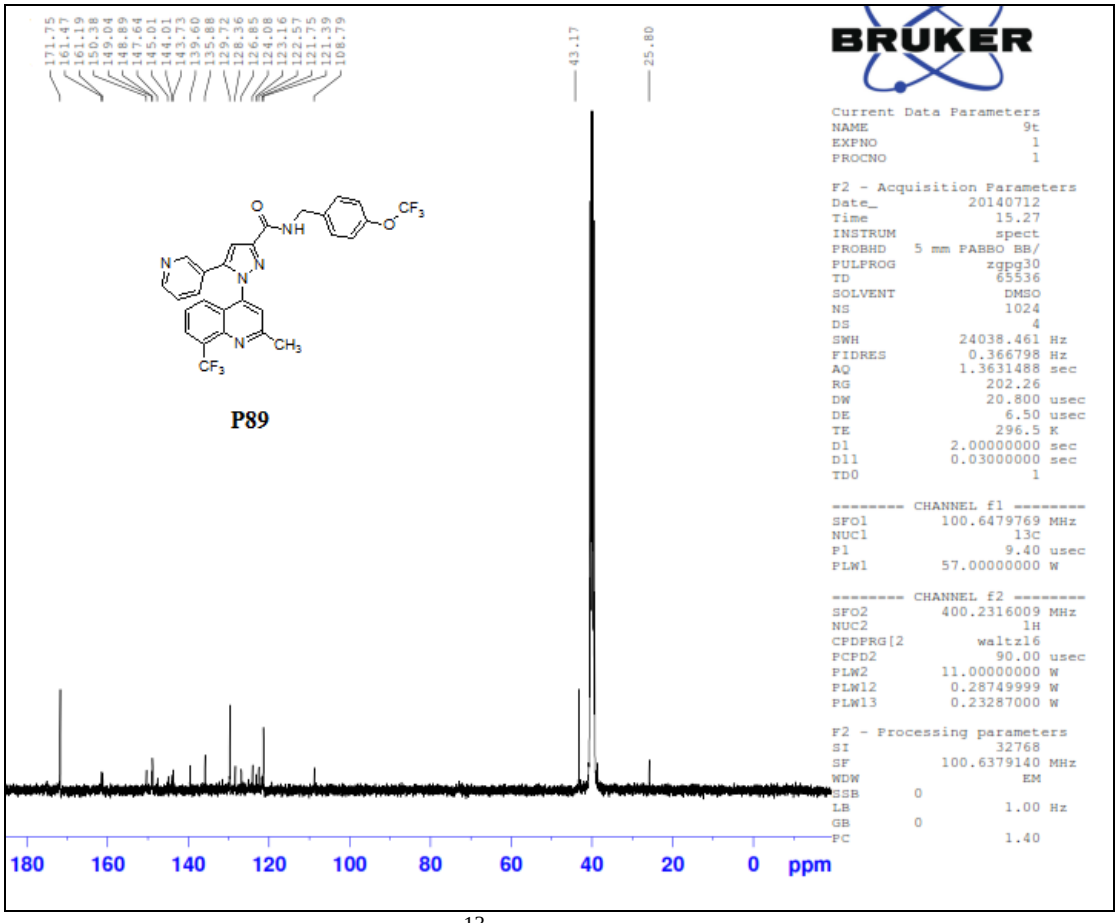


Figure 5.24 ^{13}C NMR spectrum of **P89.**

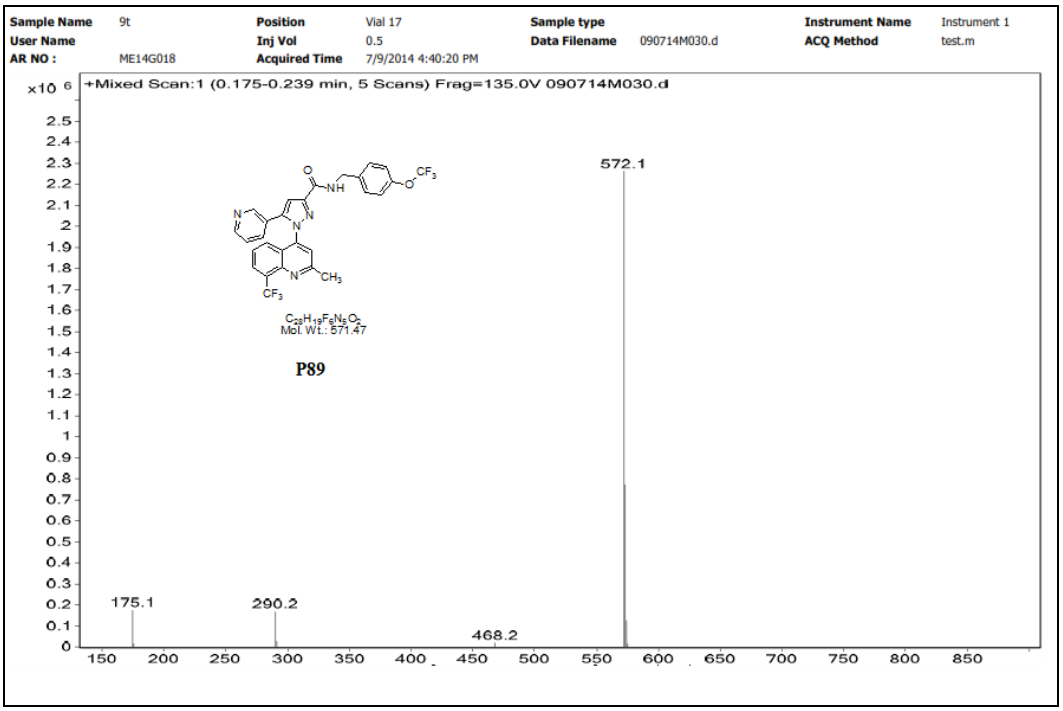


Figure 5.25 Mass spectrum of **P89.**

CHAPTER 6

SYNTHESIS AND BIOLOGICAL ACTIVITY OF NEW CHLOROPHENYL-1*H*-(PYRAZOL-4-YL)METHYLENE ISONICOTINOHYDRAZIDE DERIVATIVES

Abstract

This chapter explains the design and synthesis of a novel series of INH-pyrazole analogs with structural characterization of all the newly formed intermediates and final molecules. It also includes a comprehensive discussion on in vitro antiTB and antibacterial results of all the final compounds.

6.1 INTRODUCTION

In recent days, development of new drugs by molecular modification of a lead compound or already existing drug with an established activity has become an active area of research in medicinal chemistry. Such a molecular modification can possibly results in enhancement of the activity and sometimes may also help in reducing the toxicity of the molecule. This approach of molecular modification has become a promising strategy to design and development of potent antiTB agents as well.

INH is one of the most studied antiTB drugs. It is a potent first-line antiTB drug; a pro-drug triggered via oxidation that forms an adduct with Nicotinamide adenine dinucleotide (NAD (+)) to inhibit NADH dependent targets of MTB bacillus, such as the enoyl-acyl carrier protein reductase (InhA) (Zhang et al. 1992; Zhao et al. 2006; Nguyen et al. 2001). But the major drawback of INH is its failure against the treatment of MDR-TB, especially among patients infected with HIV. The recent studies indicate that the incorporation of lipophilic moieties into the framework of INH can increase permeation of the drug into the tissues of the mammalian host and into the waxy cell wall of the bacterium (Maccari et al. 2002; Manjashetty et al. 2011; Ramani et al. 2012). The functionalization of the INH is possible via its reactive hydrazide group which can easily react with carbonyl compounds to form hydrazones or undergo cyclization reactions. LL-3858 (**I**) (**Figure 6.1**) is one such INH derivative (developed by Lupin Limited) which shows activity against both drug sensitive and MDR-TB (Arora et al. 2004; Kumar et al. 2014) and EMB is in the initial stages of phase II clinical trial for the treatment of tuberculosis. Also, a few other hydrazone derivatives of INH (**II - IV**) showed promising antiTB activity (Kumar et al. 2010; Sriram et al. 2005). Further, some of the literature reports suggested INH analogs containing pyrazole unit (**V - VII**) establish promising antiTB agents (Pandit and Dodiya, 2013; Aragade et al. 2013; Horrocks et al. 2013).

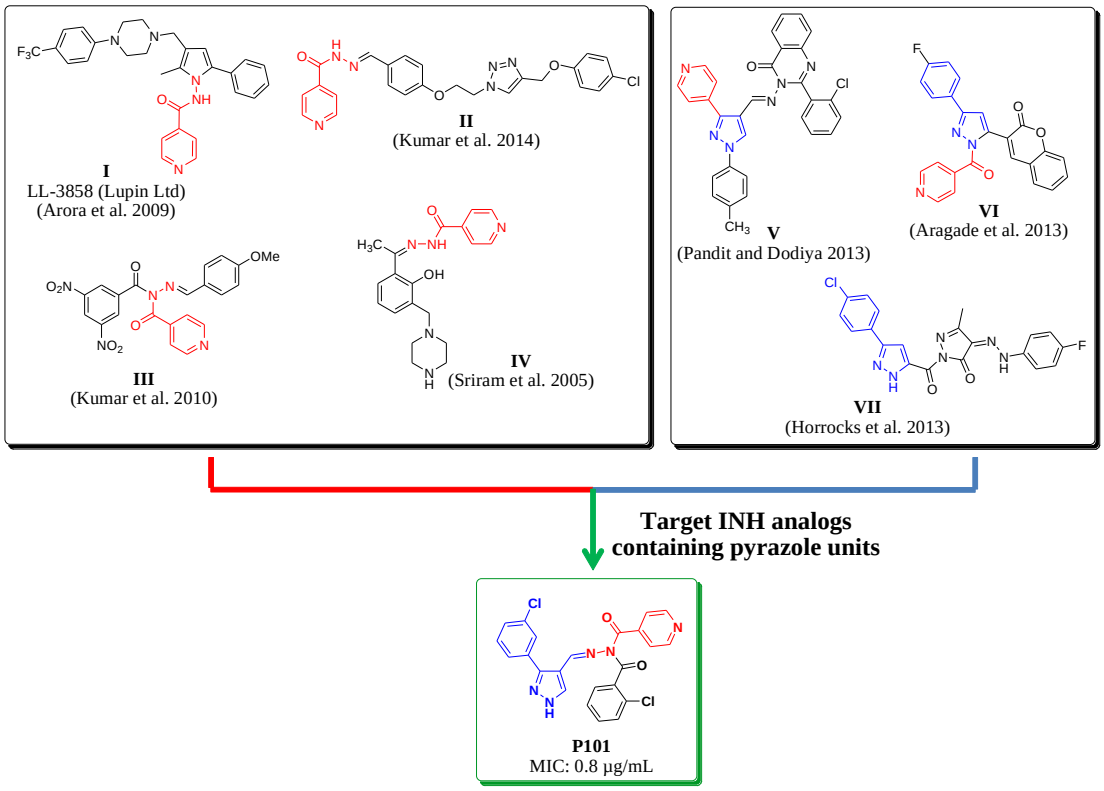


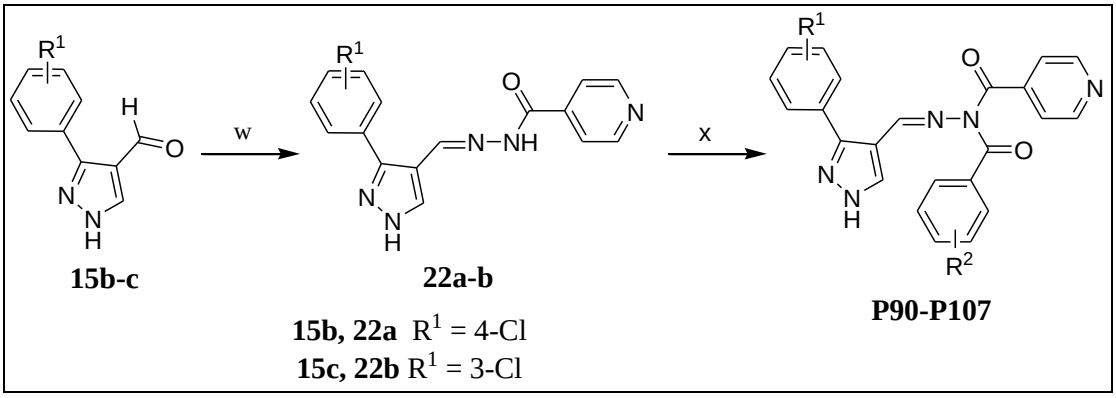
Figure 6.1 Some representative INH and pyrazole containing potent antiTB analogs. The target INH-pyrazole analog **P101** exhibited the highest antiTB activity among the synthesized compounds.

In view of these facts, we envisaged that re-engineering of INH nucleus by replacing the five-member sugar of NAD (+) by active 3,4-disubstituted pyrazole pharmacophore could deliver scaffolds with diverse structural features and a better antiTB activity. So, we have designed and synthesized a new class of INH derivatives containing active pyrazole unit and evaluated their antimycobacterial activity against MTB strain. Additionally, the compounds were screened against three common pathogenic bacterial strains as well.

6.2 CHEMISTRY

The synthetic route of new isonicotinohydrazide based pyrazole derivatives (**P90-P107**) is represented in **Scheme 6.1**. The hydrazone scaffolds (**22a-b**) were synthesized in good yield by refluxing a mixture of **15b-c** and isonicotinohydrazide in the presence of catalytic amount of sulphuric acid (Vijesh et al. 2013). The target molecules (**P90-P107**) were synthesized by coupling intermediates **22a-b** with different aromatic acids using the typical acid-amine coupling agents, *N*-(3-

dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC) and 1-hydroxybenzotriazole hydrate (HOBt) in presence of 4-dimethylaminopyridine (DMAP) as the base at RT. The compounds were purified using appropriate methods like recrystallization and column chromatography. The structural details of the target compounds (**P90-P107**) are presented in **Table 6.1**.



Scheme 6.1. Synthetic route for the preparation of target compounds (**P90-P107**). Reagents and conditions: w) Isonicotinohydrazide, ethanol, cat. sulfuric acid, 80 °C, 4 h; x) Aromatic acid derivative, EDC, HOBt, DMAP, DCM, RT, 16 h.

6.3 EXPERIMENTAL

6.3.1 Materials and methods

Refer section **2.3.1**

6.3.2 Synthesis

Synthesis of *N*′-((3-(aryl)-1*H*-pyrazol-4-yl)methylene)isonicotinohydrazide (22a-b)

***N*′-((3-(4-chlorophenyl)-1*H*-pyrazol-4-yl)methylene)isonicotinohydrazide (22a):** An equimolar mixture of 3-(4-chlorophenyl)-1*H*-pyrazole-4-carbaldehyde **15b** (4.0 g, 0.019 mol) and INH (2.65 g, 0.019 mol) was taken in ethanol (40 mL). A catalytic amount of concentrated sulphuric acid was added and the reaction mixture was refluxed at 80 ºC for 4 h, then kept at RT overnight. The resultant solid was filtered, washed with chilled ethanol, dried and recrystallized from ethanol-dioxane (1:2) mixture to afford the pure compound **22a**. Yield 5.5 g (87%); Off white solid; mp 241-242 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 13.49 (brs, 1H, NH), 11.79 (brs, 1H, NH), 8.75 (m, 2H, ArH), 8.49 (s, 1H, pyrazole-CH), 8.15 (s, 1H, CH), 7.78 (dd, 2H, ArH, *J* = 4.4 Hz, *J* = 1.6 Hz), 7.66 (d, 2H, ArH, *J* = 8.8 Hz), 7.56 (d, 2H, ArH, *J* =

8.4 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 161.47, 150.75, 144.79, 143.31, 140.98, 134.31, 131.23, 129.48, 128.87, 128.23, 121.91, 114.35; ESI-MS (*m/z*) 326.1 $(M+H)^+$; Anal. calculated for $C_{16}H_{12}ClN_5O$; C, 58.99; H, 3.71; N, 21.50. Found: C, 58.95; H, 3.73; N, 21.54.

***N'*-((3-(3-chlorophenyl)-1*H*-pyrazol-4-yl)methylene)isonicotinohydrazide (22b):** This compound was synthesized according to the above mentioned procedure by treating **15c** (4.0 g, 0.019 mol) with INHe (2.65 g, 0.019 mol) using catalytic amount of concentrated sulphuric acid in ethanol (40 mL). Yield 5.2 g (82%); Off white solid; mp 239-240 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 13.47 (brs, 1H, NH), 11.82 (brs, 1H, NH), 8.78 (s, 2H, ArH), 8.52 (s, 1H, pyrazole-CH), 8.18 (s, 1H, ArH), 7.80 (s, 2H, ArH), 7.68 (s, 2H, ArH), 7.60 (s, 2H, ArH); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 161.46, 150.77, 144.78, 143.27, 141.08, 134.20, 133.74, 130.50, 129.28, 128.33, 121.89, 114.36; ESI-MS (*m/z*) 326.1 $(M+H)^+$; Anal. calculated for $C_{16}H_{12}ClN_5O$; C, 58.99; H, 3.71; N, 21.50. Found: C, 59.04; H, 3.69; N, 21.55.

General procedure for the synthesis of the final compounds (P90-P107)

The acid derivative (0.1g) was dissolved in dry DCM (2 mL) with stirring. To this mixture EDC (1.1 eq), HOBt (0.3 eq) and DMAP (0.3 eq) were added and the resulting clear solution was stirred for 10 minutes at RT. Then, the amine derivative **22a-b** (1.0 eq) was added and the mixture was stirred for additional 16 h at RT; completion of the reaction was confirmed by TLC. The reaction mass was quenched with cold water and extracted with DCM (3 x 25 mL). The organic layer was separated, washed with brine solution (2 x 25 mL), dried over anhydrous sodium sulfate and concentrated under reduced pressure. The crude residue thus obtained was purified over silica gel (60-120 mesh size) column chromatography eluted with EtOAc/ hexane (1:1) to give pure final derivatives (**P90-P107**).

***N'*-((3-(4-chlorophenyl)-1*H*-pyrazol-4-yl)methylene)-*N*-(4-fluorobenzoyl) isonicotinohydrazide (P90):** Yield 0.12 g (88%); Off white solid; mp 226-227 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 12.12 (s, 1H, NH), 8.95 (s, 1H, ArH), 8.78 (d, 2H, ArH, J = 4.8 Hz), 8.54 (s, 1H, pyrazole-CH), 7.48-7.93 (m, 8H, ArH), 7.21 (s, 2H, J = 8.3 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 164.61, 161.83, 161.01, 150.85, 143.83, 141.26, 140.68, 134.77, 131.55, 131.21, 128.93, 126.86, 125.54,

121.94, 119.32, 116.20, 115.91; ESI-MS (*m/z*) 448.2 $(M+H)^+$; Anal. calculated for $C_{23}H_{15}ClFN_5O_2$; C, 61.68; H, 3.38; N, 15.64. Found: C, 61.64; H, 3.39; N, 15.66.

Table 6.1 Substitution pattern of final compounds **P90-P107**.

Compound	R^1	R^2	LogP/CLogP[a]
P90	4-Cl	4-F	4.79/4.12
P91	4-Cl	4-Cl	5.19/4.69
P92	4-Cl	2-Cl	5.19/4.69
P93	4-Cl	4-NO_2	--/3.72
P94	4-Cl	4-CH_3	5.12/4.47
P95	4-Cl	4-OCH_3	4.50/3.89
P96	4-Cl	H	4.63/3.98
P97	4-Cl	3,4-dimethoxy	4.38/3.63
P98	4-Cl	4-CH_2-Cl	5.28/4.54
P99	3-Cl	4-F	4.79/4.12
P100	3-Cl	4-Cl	5.19/4.69
P101	3-Cl	2-Cl	5.19/4.69
P102	3-Cl	4-NO_2	--/3.72
P103	3-Cl	4-CH_3	5.12/4.47
P104	3-Cl	4-OCH_3	4.50/3.89
P105	3-Cl	H	4.63/3.98
P106	3-Cl	3,4-dimethoxy	4.38/3.63
P107	3-Cl	4-CH_2-Cl	5.28/4.54

[a] Obtained from ChemDraw Ultra 8.0 software.

***N*-(4-chlorobenzoyl)-*N'*-((3-(4-chlorophenyl)-1*H*-pyrazol-4-yl)methylene) isonicotinohydrazide (P91):** Yield 0.13 g (91%); Off white solid; mp 242-243 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 12.09 (s, 1H, NH), 8.94 (s, 1H, ArH), 8.78 (d, 2H, ArH, J = 4.8 Hz), 8.54 (s, 1H, pyrazole-CH), 8.15 (s, 2H, J = 8.3 Hz), 7.49-7.85 (m, 8H, ArH); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 164.58, 161.84, 150.86, 143.86, 141.02, 140.71, 138.08, 133.32, 132.86, 131.24, 130.09, 129.68, 129.10, 128.44, 128.34, 122.36, 120.24; ESI-MS (*m/z*) 465.3 $(M+H)^+$; Anal. calculated for $C_{23}H_{15}Cl_2N_5O_2$; C, 59.50; H, 3.26; N, 15.08. Found: C, 59.45; H, 3.28; N, 15.10.

***N*-(2-chlorobenzoyl)-*N'*-((3-(4-chlorophenyl)-1*H*-pyrazol-4-yl)methylene) isonicotinohydrazide (P92):** Yield 0.12 g (86%); Off white solid; mp 236-237 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 12.11 (s, 1H, NH), 8.95 (s, 1H, ArH), 8.79 (d, 2H, ArH, *J* = 4.8 Hz), 8.54 (s, 1H, pyrazole-CH), 7.37-7.86 (m, 10H, ArH); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 165.16, 161.89, 154.09, 150.85, 141.04, 140.70, 134.34, 133.79, 132.74, 131.14, 131.02, 130.41, 130.19, 130.04, 129.82, 128.34, 127.68, 121.92, 120.22; ESI-MS (*m/z*) 465.1 $(M+H)^+$; Anal. calculated for $C_{23}H_{15}Cl_2N_5O_2$; C, 59.50; H, 3.26; N, 15.08. Found: C, 59.54; H, 3.27; N, 15.11.

***N'*-((3-(4-chlorophenyl)-1*H*-pyrazol-4-yl)methylene)-*N*-(4-nitrobenzoyl) isonicotinohydrazide (P93):** Yield 0.11 g (78%); Off white solid; mp 205-206 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 12.09 (s, 1H, NH), 8.94 (s, 1H, ArH), 8.78 (d, 2H, ArH, *J* = 5.2 Hz), 8.54 (s, 1H, pyrazole-CH), 8.36 (d, 2H, ArH, *J* = 7.8 Hz), 7.38-7.98 (m, 8H, ArH); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 166.21, 161.86, 152.11, 150.84, 141.06, 140.67, 140.28, 134.33, 130.37, 130.18, 129.66, 128.53, 127.67, 122.28, 121.92, 121.45, 120.22; ESI-MS (*m/z*) 475.3 $(M+H)^+$; Anal. calculated for $C_{23}H_{15}ClN_6O_2$; C, 58.17; H, 3.18; N, 17.70. Found: C, 58.20; H, 3.20; N, 17.74.

***N'*-((3-(4-chlorophenyl)-1*H*-pyrazol-4-yl)methylene)-*N*-(4-methylbenzoyl) isonicotinohydrazide (P94):** Yield 0.12 g (88%); Off white solid; mp 243-244 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 12.08 (s, 1H, NH), 8.90 (s, 1H), 8.78 (d, 2H, *J* = 5.7 Hz), 8.54 (s, 1H, pyrazole-CH), 8.05 (d, 2H, *J* = 8.3 Hz), 7.79-7.84 (m, 4H, ArH), 7.58 (d, 2H, *J* = 8.3 Hz), 7.41 (d, 2H, *J* = 8.3 Hz), 2.42 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 164.54, 161.86, 153.83, 145.01, 142.59, 140.64, 134.39, 132.14, 131.59, 130.88, 130.51, 129.32, 128.74, 128.57, 127.89, 122.47, 119.21, 24.03; ESI-MS (*m/z*) 444.2 $(M+H)^+$; Anal. calculated for $C_{24}H_{18}ClN_5O_2$; C, 64.94; H, 4.09; N, 15.78. Found: C, 64.99; H, 4.11; N, 15.81.

***N'*-((3-(4-chlorophenyl)-1*H*-pyrazol-4-yl)methylene)-*N*-(4-methoxybenzoyl) isonicotinohydrazide (P95):** Yield 0.13 g (89%); Off white solid; mp 232-233 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 12.09 (s, 1H, NH), 8.91 (s, 1H), 8.78 (d, 2H, *J* = 5.7 Hz), 8.55 (s, 1H, pyrazole-CH), 8.01 (d, 2H, *J* = 8.3 Hz), 7.81 (d, 2H, ArH, *J* = 7.8 Hz), 7.01-7.41 (m, 6H, ArH), 3.75 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 165.18, 164.53, 161.87, 150.84, 143.61, 141.15, 140.77, 134.63, 131.60, 130.54,

129.21, 127.95, 127.37, 122.44, 121.95, 117.95, 56.36; ESI-MS (*m/z*) 460.4 $(M+H)^+$; Anal. calculated for $C_{24}H_{18}ClN_5O_3$; C, 62.68; H, 3.95; N, 15.23. Found: C, 62.72; H, 3.97; N, 15.26.

***N*-benzoyl-*N'*-((3-(4-chlorophenyl)-1*H*-pyrazol-4-yl)methylene)isonicotino hydrazide (P96):** Yield 0.12 g (92%); Off white solid; mp 224-225 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 12.08 (s, 1H, NH), 8.91 (s, 1H), 8.78 (d, 2H, *J* = 4.8 Hz), 8.54 (s, 1H, pyrazole-CH), 8.11 (d, 2H, *J* = 8.3 Hz), 7.38-7.89 (m, 9H, ArH); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 165.95, 161.88, 153.11, 150.84, 142.28, 140.88, 134.31, 133.97, 132.77, 132.09, 130.03, 131.13, 129.76, 129.14, 128.54, 128.25, 127.38, 122.12, 119.23; ESI-MS (*m/z*) 430.3 $(M+H)^+$; Anal. calculated for $C_{23}H_{16}ClN_5O_2$; C, 64.26; H, 3.75; N, 16.29. Found: C, 64.30; H, 3.76; N, 16.28.

***N'*-((3-(4-chlorophenyl)-1*H*-pyrazol-4-yl)methylene)-*N*-(3,4-dimethoxybenzoyl) isonicotinohydrazide (P97):** Yield 0.12 g (81%); Off white solid; mp 215-216 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 12.07 (s, 1H, NH), 8.89 (s, 1H), 8.78 (s, 2H), 8.55 (s, 1H, pyrazole-CH), 7.59-7.95 (m, 8H, ArH), 7.16 (d, 1H, *J* = 7.4 Hz), 3.88 (s, 3H, CH_3), 3.83 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 164.58, 161.86, 154.13, 150.85, 148.62, 141.37, 139.02, 134.69, 131.75, 131.00, 130.49, 129.26, 127.32, 122.52, 121.93, 118.73, 114.86, 111.43, 56.35, 56.15; ESI-MS (*m/z*) 490.1 $(M+H)^+$; Anal. calculated for $C_{25}H_{20}ClN_5O_4$; C, 61.29; H, 4.11; N, 14.30. Found: C, 61.33; H, 4.09; N, 14.27.

***N*-(4-(chloromethyl)benzoyl)-*N'*-((3-(4-chlorophenyl)-1*H*-pyrazol-4-yl)methylene) isonicotinohydrazide (P98):** Yield 0.11 g (74%); Off white solid; mp 238-239 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 12.12 (s, 1H, NH), 8.92 (s, 1H, ArH), 8.77 (s, 2H, ArH), 8.56 (s, 1H, pyrazole-CH), 8.12 (d, 2H, ArH, *J* = 7.4 Hz), 7.81-8.11 (m, 4H, ArH), 7.47-7.66 (m, 4H, ArH), 4.87 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 165.52, 161.84, 150.84, 143.02, 141.25, 132.31, 131.10, 130.53, 129.23, 129.06, 128.75, 128.07, 121.96, 119.30, 45.68; ESI-MS (*m/z*) 479.0 $(M+H)^+$; Anal. calculated for $C_{20}H_{13}F_3N_4O_2$; C, 60.26; H, 3.58; N, 14.64. Found: C, 60.27; H, 3.59; N, 14.67.

***N'*-((3-(3-chlorophenyl)-1*H*-pyrazol-4-yl)methylene)-*N*-(4-fluorobenzoyl) isonicotinohydrazide (P99):** Yield 0.12 g (86%); Off white solid; mp 285-286 °C; ^{1}H

NMR (400 MHz, DMSO-d_6) δ ppm 12.13 (s, 1H, NH), 8.96 (s, 1H, ArH), 8.80 (s, 2H, ArH), 8.56 (s, 1H, pyrazole-CH), 8.26 (s, 2H, ArH), 7.46-7.86 (m, 8H, ArH); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 164.59, 161.82, 161.02, 150.86, 143.76, 141.22, 140.72, 135.19, 135.10, 134.79, 131.11, 130.30, 129.23, 121.94, 119.25, 116.15, 115.93; ESI-MS (*m/z*) 448.3 $(M+H)^+$; Anal. calculated for $C_{23}H_{15}ClFN_5O_2$; C, 61.68; H, 3.38; N, 15.64. Found: C, 61.62; H, 3.39; N, 15.63.

***N*-(4-chlorobenzoyl)-*N'*-((3-(3-chlorophenyl)-1*H*-pyrazol-4-yl)methylene) isonicotinohydrazide (P100):** Yield 0.12 g (85%); Off white solid; mp 260-261 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 12.09 (s, 1H, NH), 8.93 (s, 1H, ArH), 8.78 (d, 2H, ArH, J = 4.4 Hz), 8.54 (s, 1H, pyrazole-CH), 7.27-7.88 (m, 10H, ArH); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 164.56, 161.84, 150.85, 143.83, 141.05, 140.72, 137.97, 134.71, 133.92, 132.57, 130.85, 130.26, 129.19, 129.11, 128.43, 128.28, 124.87, 122.40, 120.23; ESI-MS (*m/z*) 465.0 $(M+H)^+$; Anal. calculated for $C_{23}H_{15}Cl_2N_5O_2$; C, 59.50; H, 3.26; N, 15.08. Found: C, 59.47; H, 3.28; N, 15.09.

***N*-(2-chlorobenzoyl)-*N'*-((3-(3-chlorophenyl)-1*H*-pyrazol-4-yl)methylene) isonicotinohydrazide (P101):** Yield 0.11 g (80%); Off white solid; mp 190-191 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 12.15 (s, 1H, NH), 8.95 (s, 1H, ArH), 8.80 (s, 2H, ArH), 8.54 (s, 1H, pyrazole-CH), 7.56-7.82 (m, 10H, ArH); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 165.15, 161.90, 154.06, 150.86, 141.01, 140.67, 134.88, 133.31, 132.73, 131.04, 130.79, 130.47, 130.00, 129.22, 127.69, 121.94, 120.24; ESI-MS (*m/z*) 465.1 $(M+H)^+$; Anal. calculated for $C_{23}H_{15}Cl_2N_5O_2$; C, 59.50; H, 3.26; N, 15.08. Found: C, 59.53; H, 3.28; N, 15.10.

***N'*-((3-(3-chlorophenyl)-1*H*-pyrazol-4-yl)methylene)-*N*-(4-nitrobenzoyl) isonicotinohydrazide (P102):** Yield 0.11 g (73%); Off white solid; mp 193-194 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 12.09 (s, 1H, NH), 8.94 (s, 1H, ArH), 8.79 (d, 2H, ArH, J = 4.8 Hz), 8.54 (s, 1H, pyrazole-CH), 8.36 (d, 2H, J = 8.4 Hz), 7.28-7.99 (m, 8H, ArH); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 166.22, 161.86, 152.06, 150.84, 141.08, 140.66, 140.31, 134.35, 133.97, 130.83, 130.18, 128.98, 128.75, 127.77, 127.67, 122.29, 121.94, 121.43, 120.21; ESI-MS (*m/z*) 475.2 $(M+H)^+$; Anal. calculated for $C_{23}H_{15}ClN_6O_2$; C, 58.17; H, 3.18; N, 17.70. Found: C, 58.21; H, 3.29; N, 17.67.

***N'*-((3-(3-chlorophenyl)-1*H*-pyrazol-4-yl)methylene)-*N*-(4-methylbenzoyl) isonicotinohydrazide (P103):** Yield 0.11 g (78%); Off white solid; mp 214-215 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 12.12 (s, 1H, NH), 8.89 (s, 1H), 8.77 (d, 2H, ArH, *J* = 5.0 Hz), 8.55 (s, 1H, pyrazole-CH), 7.40-8.05 (m, 10H, ArH), 2.42 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 164.55, 161.84, 150.84, 142.96, 141.86, 141.22, 140.65, 134.66, 134.29, 131.52, 130.32, 130.13, 129.27, 128.78, 127.89, 127.74, 125.64, 121.91, 25.03; ESI-MS (*m/z*) 444.3 $(M+H)^+$; Anal. calculated for $C_{24}H_{18}ClN_5O_2$; C, 64.94; H, 4.09; N, 15.78. Found: C, 64.91; H, 4.08; N, 15.81.

***N'*-((3-(3-chlorophenyl)-1*H*-pyrazol-4-yl)methylene)-*N*-(4-methoxybenzoyl) isonicotinohydrazide (P104):** Yield 0.11 g (82%); Off white solid; mp 221-222 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 12.12 (s, 1H, NH), 8.90 (s, 1H), 8.78 (d, 2H, *J* = 5.2 Hz), 8.55 (s, 1H, pyrazole-CH), 7.27-7.98 (m, 10H, ArH), 3.74 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 164.53, 161.87, 154.17, 152.73, 150.84, 149.87, 148.61, 141,15, 140.77, 133.97, 133.67 131.60, 131.14, 129.75, 128.69, 127.95, 127.37, 122.43, 121.96, 118.79, 56.35; ESI-MS (*m/z*) 460.1 $(M+H)^+$; Anal. calculated for $C_{24}H_{18}ClN_5O_3$; C, 62.68; H, 3.95; N, 15.23. Found: C, 62.71; H, 3.94; N, 15.25.

***N*-benzoyl-*N'*-((3-(3-chlorophenyl)-1*H*-pyrazol-4-yl)methylene)isonicotino hydrazide (P105):** Yield 0.11 g (85%); Off white solid; mp 230-231 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 12.09 (s, 1H, NH), 8.92 (s, 1H), 8.77 (d, 2H, ArH, *J* = 4.3 Hz), 8.54 (s, 1H, pyrazole-CH), 8.12 (d, 2H, ArH, *J* = 7.4 Hz), 7.46-7.82 (m, 9H, ArH); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 165.94, 161.89, 153.16, 150.85, 141.06, 140.76, 134.06, 133.96, 133.53, 131.91, 131.33, 131.13, 131.05, 129.83, 129.03, 128.85, 128.76, 128.06, 121.96, 119.29; ESI-MS (*m/z*) 430.1 $(M+H)^+$; Anal. calculated for $C_{23}H_{16}ClN_5O_2$; C, 64.26; H, 3.75; N, 16.29. Found: C, 64.29; H, 3.72; N, 16.27.

***N'*-((3-(3-chlorophenyl)-1*H*-pyrazol-4-yl)methylene)-*N*-(3,4-dimethoxybenzoyl) isonicotinohydrazide (P106):** 0.11 g (75%); Off white solid; mp 219-220 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 12.09 (s, 1H, NH), 8.90 (s, 1H), 8.77 (d, 2H, *J* = 5.7 Hz), 8.55 (s, 1H, pyrazole-CH), 7.46-7.95 (m, 8H, ArH), 7.18 (d, 1H, *J* = 8.8 Hz), 3.88 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm

164.57, 161.87, 154.17, 150.84, 148.60, 142.84, 141.38, 139.10, 134.72, 133.88, 131.76, 131.75, 130.55, 129.27, 127.33, 125.26, 121.91, 118.71, 114.87, 111.43, 56.35, 56.16; ESI-MS (*m/z*) 490.4 $(M+H)^+$; Anal. calculated for $C_{25}H_{20}ClN_5O_4$; C, 61.29; H, 4.11; N, 14.30. Found: C, 61.33; H, 4.08; N, 14.31.

***N*-(4-(chloromethyl)benzoyl)-*N'*-((3-(3-chlorophenyl)-1*H*-pyrazol-4-yl)methylene) isonicotinohydrazide (P107):** Yield 0.11 g (72%); Off white solid; mp 239-240 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 12.10 (s, 1H, NH), 8.91 (s, 1H), 8.77 (d, 2H, *J* = 4.8 Hz), 8.56 (s, 1H, pyrazole-CH), 7.78-8.01 (m, 4H, ArH), 7.27-7.47 (m, 6H, ArH), 4.86 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 165.51, 161.84, 150.84, 143.05, 141.26, 139.44, 133.91, 132.56, 130.53, 129.23, 129.05, 128.74, 128.11, 127.75, 127.39, 125.82, 121.95, 119.34, 45.69; ESI-MS (*m/z*) 479.0 $(M+H)^+$; Anal. calculated for $C_{20}H_{13}F_3N_4O_2$; C, 60.26; H, 3.58; N, 14.64. Found: C, 60.30; H, 3.59; N, 14.62.

6.4 PHARMACOLOGY

AntiTB studies, cytotoxicity studies and molecular docking studies were performed as discussed in the **Section 2.4** and antibacterial studies were performed as discussed in the **Section 3.4.**

6.5 RESULTS AND DISCUSSION

6.5.1 Chemistry

In the ^{1}H NMR spectrum of **22a** a new singlet signal is appeared at δ 11.79 ppm which corresponds to the -NH of pyrazole ring whereas the -NH proton of isonicotinohydrazide resonated at 13.49 ppm (**Figure 6.2**). The signals due to the aromatic protons also are in agreement with the structure of **22a**. The ESI-MS of displayed a molecular ion peak at (*m/z*) 326.1 $(M+H)^+$ which is in agreement with its molecular formula ($C_{16}H_{12}ClN_5O$). The ^{1}H NMR spectrum of **P91** shows the pyrazole NH signal at δ 12.12 ppm and the signal due to the -NH of isonicotinohydrazide was absent (**Figure 6.3**). Also, the number of signals in the aromatic region is in well agreement with its structure. Furthermore, the ESI mass spectrum shows the molecular ion peak at (*m/z*) 465.3 $(M+H)^+$, which confirms its molecular formula ($C_{23}H_{15}Cl_2N_5O_2$) **(Figure 6.4**).

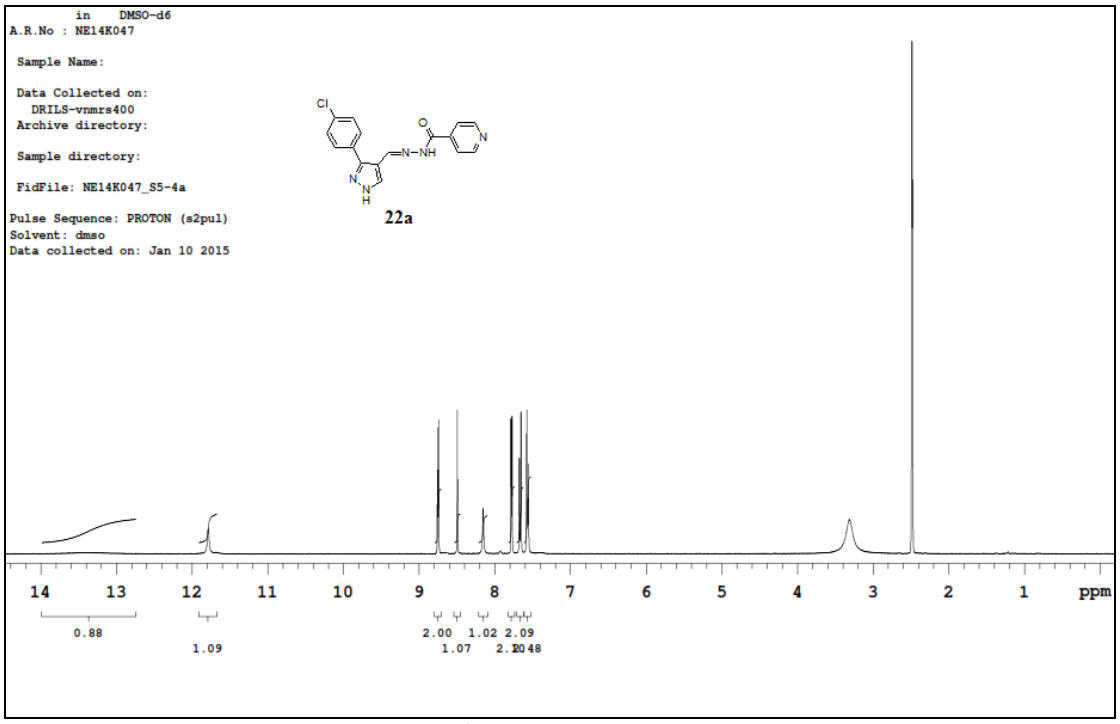


Figure 6.2 ^{1}H NMR spectrum of **22a.**

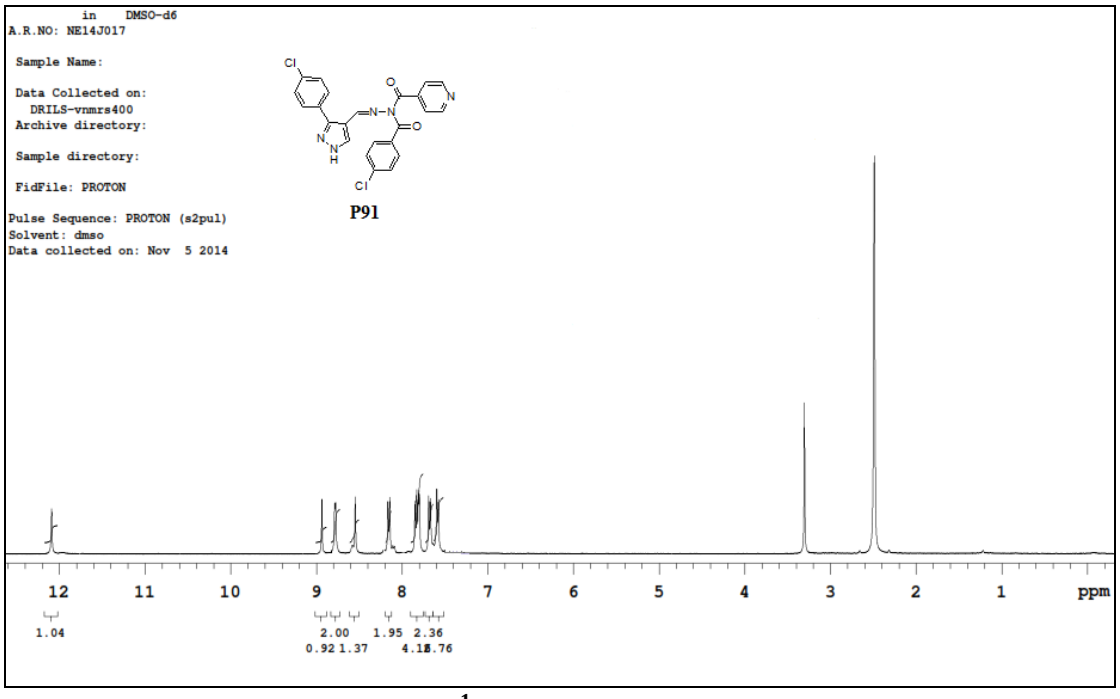


Figure 6.3 ^{1}H NMR spectrum of **P91.**

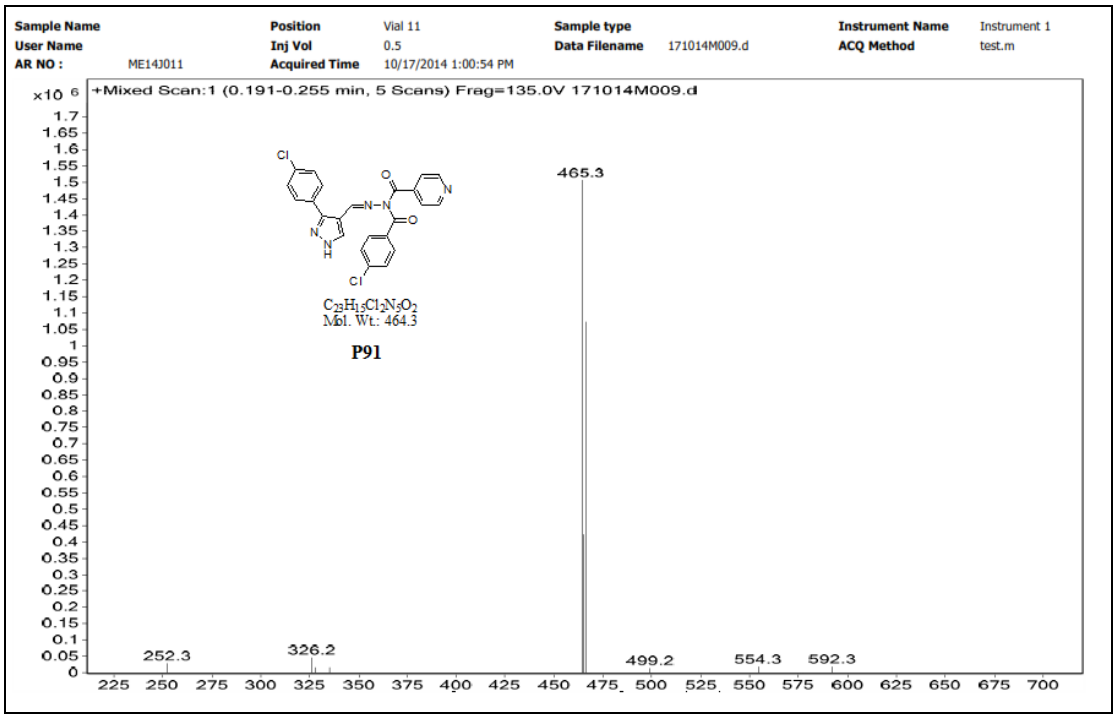


Figure 6.4 Mass spectrum of **P91.**

6.5.2 Antitubercular studies

The results of the *in vitro* antimycobacterial screening are given in **Figure 6.5**. Four compounds namely **22b, P99, P100** and **P101** displayed significant inhibition activity with a MIC of 1.6 µg/mL. Among these, **P101** is the most potent molecule with a MIC of 0.8 µg/mL which is comparable with the MIC value of standard drug INH, whereas the inhibition activity of compounds **22b, P99** and **P100** is much greater (in terms of MIC value) than that of standard drug, EMB. The 3-(chlorophenyl)-1*H*-pyrazole scaffolds, **22a** and **22b**, differ in their structure only in terms of the position of the chloro substituent on the phenyl ring. However, only compound **22b** with a 3-Cl substitution showed a significant activity, whereas compound **22a** with a 4-chloro substitution displayed a moderate activity with a MIC of 12.5 µg/mL. This result clearly suggests that the chloro substitution at the meta position enhances the antiTB activity (**Figure 6.6**). Further, the active target compounds (**P99, P100** and **P101)** are the derivatives of scaffold **22b**. It is worth mentioning that the inhibition activity of all the target compounds derived from scaffold **22b**, except **P107**, is either the same or higher than that of their respective analogues derived from scaffold **22a**. That is, compounds **P99-P106** are either equipotent or more potent than compounds **P90-97** respectively. Among molecules

derived from **22a**, compound **P98** showed a moderate activity with a MIC of 6.25 µg/mL whereas rest of the molecules did not show a promising activity. The substitution of a halogen (F or Cl) at $\mathbf{R^2}$ enhances the inhibition activity of the molecules which is evident from the observation that all three active target molecules **P99, P100** and **P101** contain a halide substitution at $\mathbf{R^2}$. Compound **P101** with a 2-chloro substitution is twofold more potent than **P100** which has a 4-chloro substitution at $\mathbf{R^2}$. This observation signifies the effect of the position of the halide substitution on the activity. However the nature of the halogen (F or Cl) has no effect on the inhibition activity, both fluoro and chloro substituted derivatives (**P99** and **P100** respectively) are equipotent (MIC = 1.6 µg/mL). But this structure-activity trend was not observed in the case of halogen substituted target molecules (**P90-P92**) derived from scaffold **22a**. The chloro substituted derivative (**P91**) was more active than the fluoro substituted one (**P90**) whereas both 2- and 4-chloro substituted derivatives (**P91** and **P92** respectively) showed a similar activity (MIC = 25 µg/mL). The electron releasing groups such as CH_3, -OCH_3 and 3,4-dimethoxy at $\mathbf{R^2}$ failed to enhance the inhibition activity of the molecules whereas, molecules **P98** and **P107** which contain an electron withdrawing nitro group at $\mathbf{R^2}$ showed moderate activity (**Figure 6.6**).

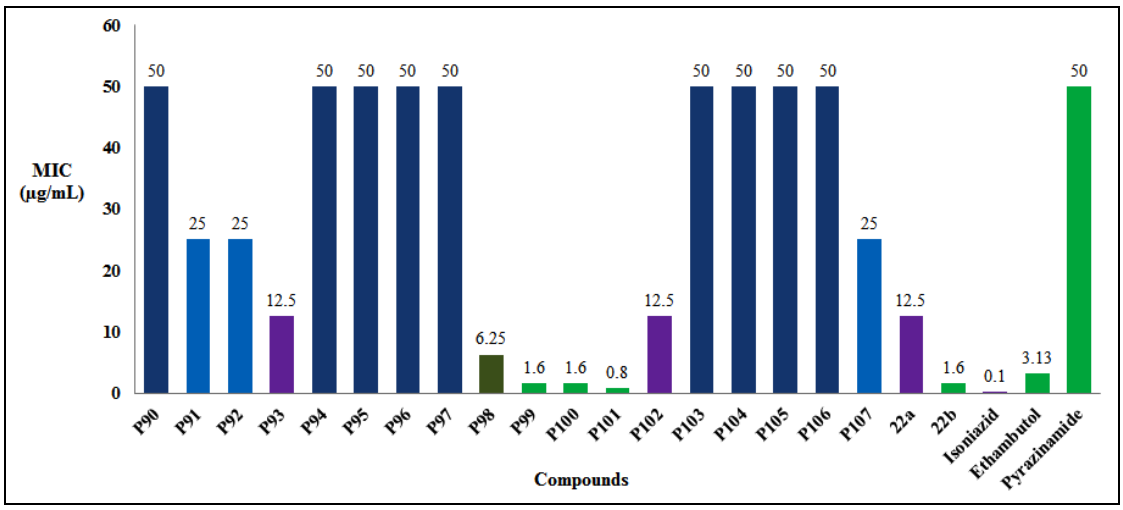


Figure 6.5. AntiTB activities of compounds **P90-P107** and **22a-b**.

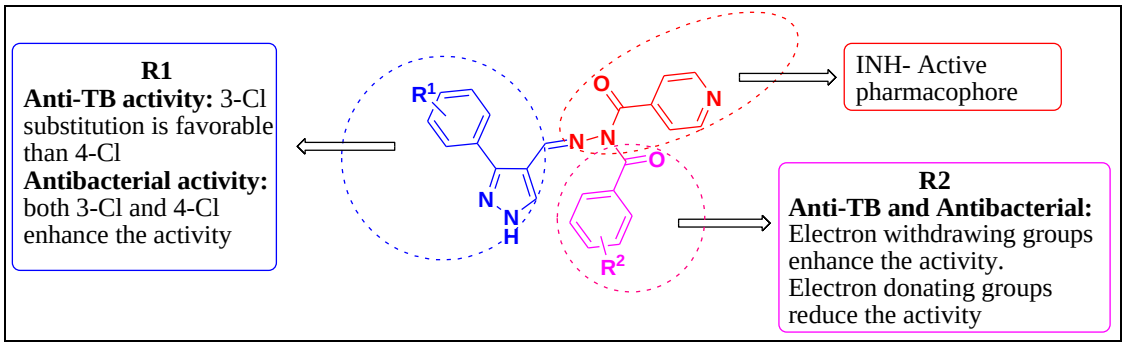


Figure 6.6. The structure-activity relationship in the target compounds.

6.5.3 Cytotoxicity studies

The *in vitro* cytotoxicity studies of the active compounds with MTB MIC ≤ 6.25 µg/mL were carried out by MTT assay against a normal Vero cell line. The results of the cytotoxicity study are presented in **Table 6.2**. All active antiTB compounds (**22b, P98, P99, P100** and **P101**) exhibited low toxicity between 10.51 to 26.32 % at a concentration of 65 µg/mL. Further, the high SI of these derivatives (>160) clearly implies the suitability of the compounds for drug development as new antiTB agents.

Table 6.2. Cytotoxicity of active antiTB derivatives.

Compound	Percentage Inhibition[a]	IC_{50} (µg/mL)	SI[b]
P98	12.01±1.0	>1000	>160
P99	17.83±2.4	>1000	>625
P100	10.51±2.5	>1000	>625
P101	14.94±4.4	513.33	641.6
22b	26.32±3.9	>1000	>625

[a] Percentage inhibition at 62.5 µg/mL.
[b] Selectivity index (*in vitro*) = IC_{50} against Vero cells/MIC against *M. tuberculosis*.

6.5.4 Molecular docking studies

The docking score of the docked ligands against InhA protein were ranged between -8.56 to -11.61 (**Table 6.3**) which are comparable with that of the standard ligand (-12.51). The ligands showed one or more hydrogen bonding interaction with the amino acid residues of InhA and other type of interactions as well. The docking score of the most active compound of the series, **P101** was found to be -10.58 which showed H-bond interaction with Gly 104 and nitrogen atom of pyrazole ring. Additionally, a π-π interaction also observed between Tyr 158 and the pyridine ring

(**Figure 6.7**). The ligands were found to interact well also with the CYP121 protein framework. All docked ligands showed at least of three interactions with the amino acid residues (**Figure 6.8**). Even in case of TMPK, the ligands showed good interaction (**Figure 6.9**) and the docking score was found to be in the range -8.06 to -8.32 except for **22b-**conf 2 (-4.70).

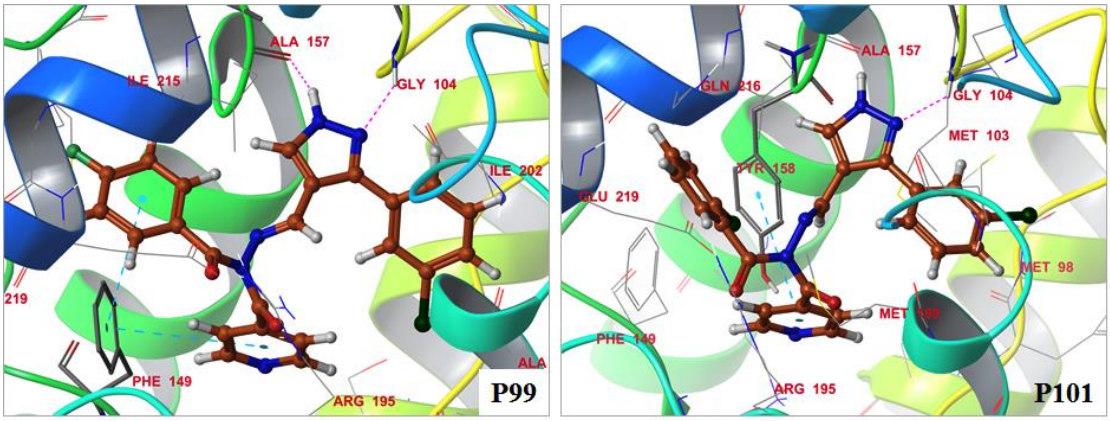


Figure 6.7 The docking pose of the compounds **P99** and **P101** in the active pocket of InhA.

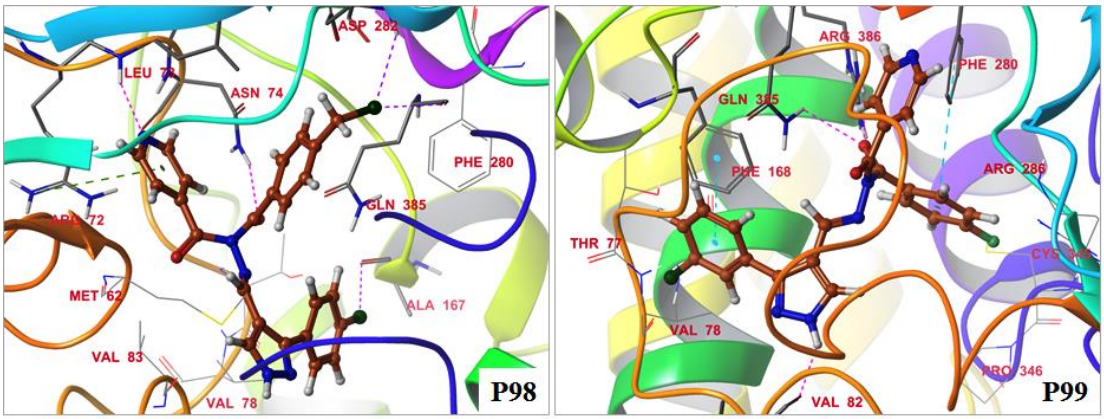


Figure 6.8 The docking pose of the compounds **P98** and **P99** in the active pocket of CYP121.

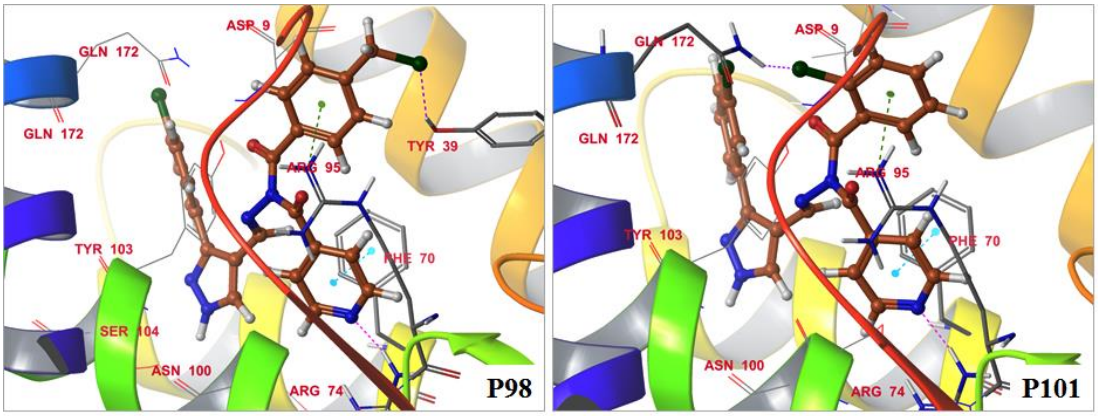


Figure 6.9 The docking pose of the compounds **P98** and **P101** in the active pocket of TMPK.

Table 6.3 Docking results of the active antiTB compounds

Compound ID	InhA		CYP121		TMPK	
	Docking score	**Amino acids that interacted with ligands**	**Docking score**	**Amino acids that interacted with ligands**	**Docking score**	**Amino acids that interacted with ligands**
Standard Ligand	-12.51	Tyr 158, Phe 149	-10.38	Gln 385, Val 228, Asn 85	-13.12	Asn 100, Tyr 103, Arg 74, Arg 95,
P98	-9.63	Phe 149, Tyr 158	-8.03	Arg 72, Asn 74, Leu 73, Asp 282, Gln 385, Ala 167	-8.25	Arg 74, Arg 95, Phe 70, Thr 39
P99	-10.93	Phe 149, Gly 104, Ala 157	-6.49	Phe 280, Arg 386, Gln 385, Phe 168, Val 82	-8.32	Arg 74, Arg 107, Phe 70

P100	-11.61	Gly 104	-8.56	Asp 282, Asn 74, Leu 73	-8.32	Arg 74, Arg 95
P101	-10.58	Gly 104, Tyr158	-6.90	Phe 168, Gln 385, Arg 386	-8.25	Arg 74, Arg 95, Phe 70, Gln 172,
22b (conf-1)	-8.69	Phe 149, Tyr 158	-7.01	Trp 182, Gln 385, Phe 168	-8.06	Arg 95, Asn 100,
22b (conf-2)	-8.56	Ala 157, Tyr 158	-5.97	Thr 77, Val 78, Phe 168	-4.70	Arg 95, Arg 100

6.5.5 Antibaterial studies

The results of the antibacterial activities of all the final compounds (**P90-P107** and **22a-b)** are presented in **Table 6.4**. Three compounds **P91, P100** and **22b** exhibited significant broad spectrum of activity against all the tested strains whereas other nine compounds **P90, P92, P93, P94, P98, P99, P101, P102** and **P107** showed moderate activity. It is worth mentioning that all these active molecules, except **P94**, carry an electron withdrawing substitution at $\mathbf{R^2}$. The variation in the position of chloro substitution at $\mathbf{R^1}$ did not affect the antibacterial activity of the target compounds. However, 3-chloro substituted intermediate **22b** is more active than its 4-chloro analogue **22a**, which is quite similar to the activity trend seen in their antiTB study. It is interesting to note that the lead derivatives of the series, **P91** and **P100** have a 4-chloro substitution at $\mathbf{R^2}$ whereas their 2-chloro analogues (**P92** and **P101** respectively) showed only moderate activity which reveals the significant contribution of the 4-chloro substituent towards the antibacterial activity. Other electron withdrawing substituents like NO_2 group (in **P93** and **P102**), also enhanced the antibacterial activity of the compounds. Thus, 3,4-disubstituted final derivatives with a chlorophenyl substitution on the pyrazole ring and an electron withdrawing group at

R^2 (a 4-chloro group in particular) are promising antibacterial agents and are best suited for further lead optimization.

Table 6.4 Antibacterial activity of target compounds **P90-P107** and **22a-b**.

Compound	Zone of inhibition (mm)					
	Staphylococcus aureus		***Escherichia coli***		***Pseudomonas aeruginosa***	
Conc. in µg/mL	75	50	75	50	75	50
Control	00	00	00	00	00	00
Ciprofloxacin	26±0.1	21±0.2	32±0.2	27±0.2	21±0.2	18±0.1
P90	17±0.2	14±0.4	19±0.3	17±0.3	17±0.1	14±0.1
P91	**24±0.2**	**21±0.2**	**24±0.3**	**19±0.3**	**21±0.1**	**18±0.2**
P92	18±0.3	14±0.2	19±0.2	16±0.4	19±0.1	15±0.3
P93	19±0.4	17±0.1	20±0.3	18±0.3	17±0.2	15±0.3
P94	20±0.1	17±0.1	14±0.4	12±0.2	15±0.1	11±0.2
P95	12±0.1	10±0.3	09±0.3	07±0.2	13±0.4	10±0.4
P96	11±0.1	09±0.2	--	--	12±0.3	10±0.3
P97	10±0.2	08±0.2	09±0.2	06±0.2	11±0.2	09±0.1
P98	16±0.3	13±0.2	19±0.4	15±0.1	20±0.2	18±0.2
P99	16±0.2	13±0.4	14±0.3	12±0.2	17±0.2	13±0.2
P100	**23±0.2**	**18±0.2**	**25±0.3**	**21±0.2**	**21±0.3**	**19±0.3**
P101	15±0.2	13±0.3	18±0.1	14±0.4	16±0.1	12±0.1
P102	17±0.3	14±0.2	15±0.1	13±0.3	14±0.1	11±0.2
P103	12±0.3	10±0.3	11±0.3	08±0.4	10±0.2	08±0.3
P104	10±0.2	07±0.4	11±0.1	09±0.3	14±0.1	11±0.4
P105	13±0.4	11±0.1	--	--	--	--
P106	10±0.3	08±0.3	--	--	--	--
P107	17±0.3	14±0.3	16±0.4	14±0.3	20±0.3	18±0.3
22a	19±0.3	16±0.2	16±0.4	14±0.2	18±0.4	14±0.3
22b	**23±0.2**	**18±0.1**	**24±0.2**	**20±0.1**	**19±0.3**	**15±0.2**

--: Bacterial resistant; Control: dimethylsulfoxide

6.6 CONCLUSIONS

- In conclusion, we demonstrated the design, synthesis, characterization and studies on antiTB and antibacterial activity of novel series of INH-pyrazole analogs.
- Four derivatives (**P99, P100, P101** and **22b)** were identified as highly potent antiTB agents (MIC = 1.56 µg/mL) and are more potent than standard drug ethambutol. The activity of derivative **P101** (MIC = 0.8 µM) is comparable with that of the potent first-line drug INH.
- The *in vitro* cytotoxicity study against normal Vero cell line revealed low toxicity level and high selectivity index (>160) of the active molecules.
- The structure-activity relationship revealed that the 3-chlorophenyl-1*H*-pyrazole derivatives of INH which contain an electron withdrawing halide substitution at $\mathbf{R^2}$ are the promising lead molecules for the development of new antiTB agents.
- The molecular docking study against InhA domain further revealed the favorable interactions of the active molecules with amino acid residues of the enzyme.
- Also, three compounds **P91**, **P100** and **22b** showed significant antibacterial activity.

Appendix 6.1

Representative ^{1}H NMR, ^{13}C NMR and ESI-MS spectra of final compounds

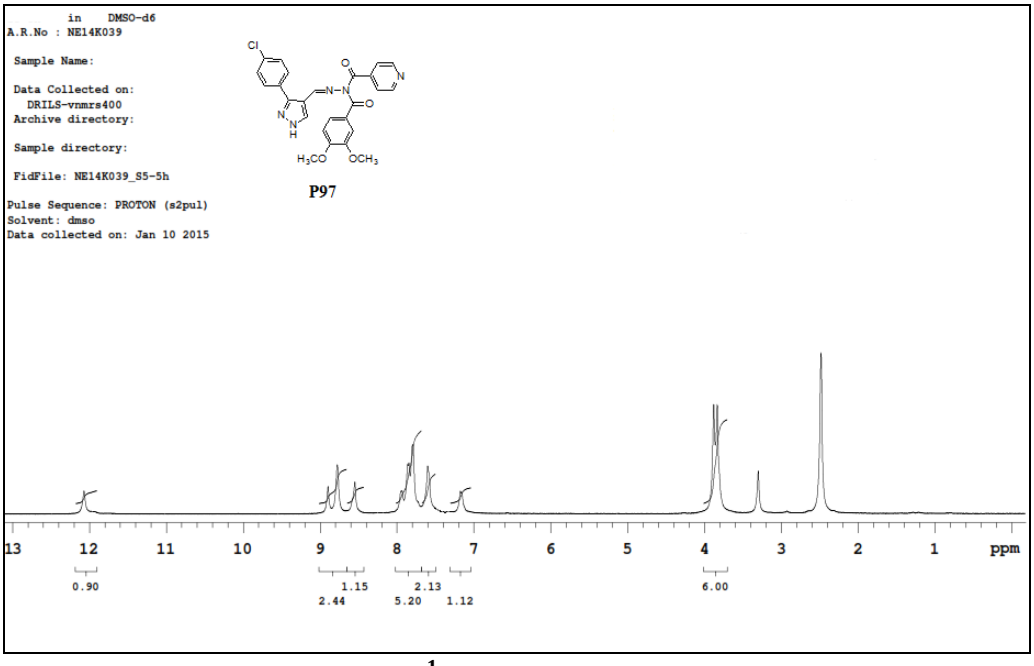


Figure 6.10 ^{1}H NMR spectrum of **P97.**

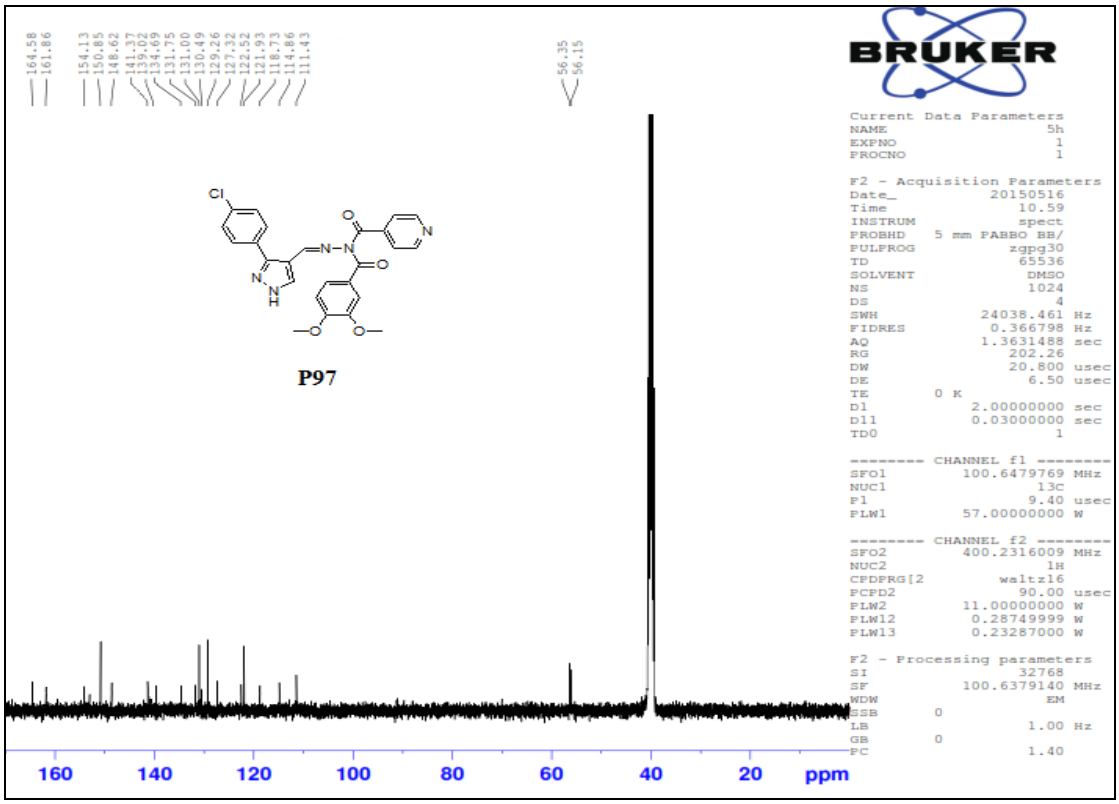


Figure 6.11 ^{13}C NMR spectrum of **P97.**

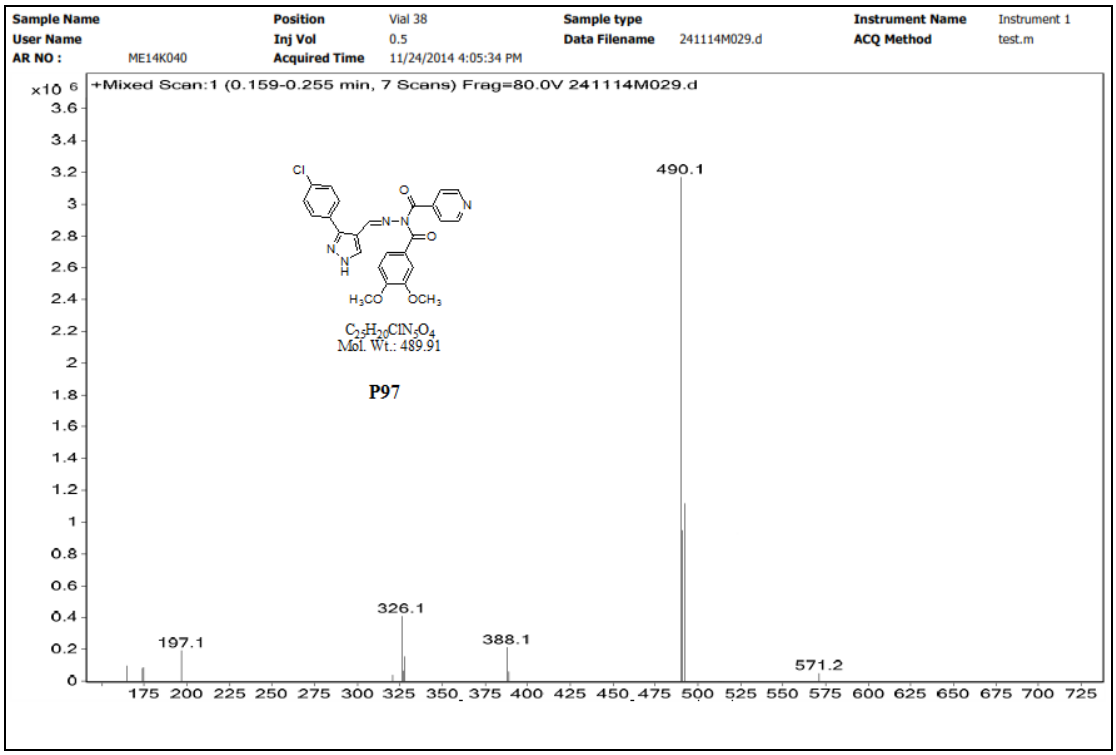


Figure 6.12 Mass spectrum of **P97.**

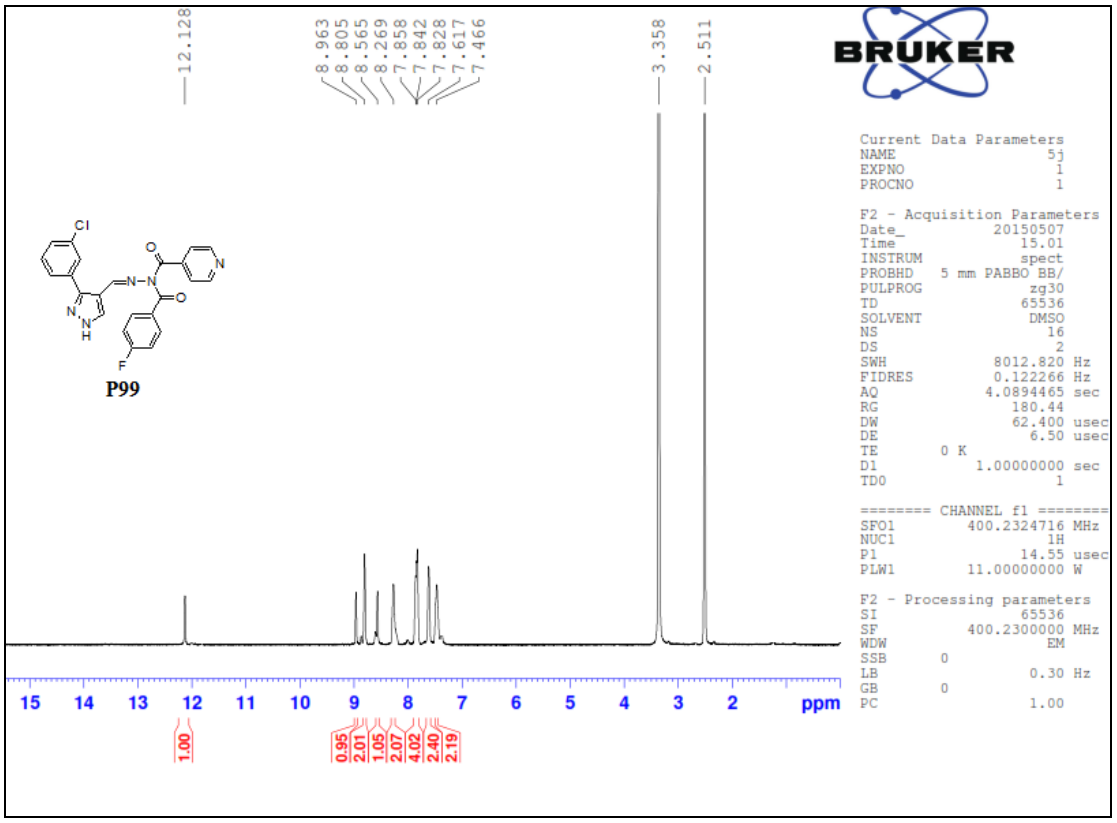


Figure 6.12 ^{1}H NMR spectrum of **P99.**

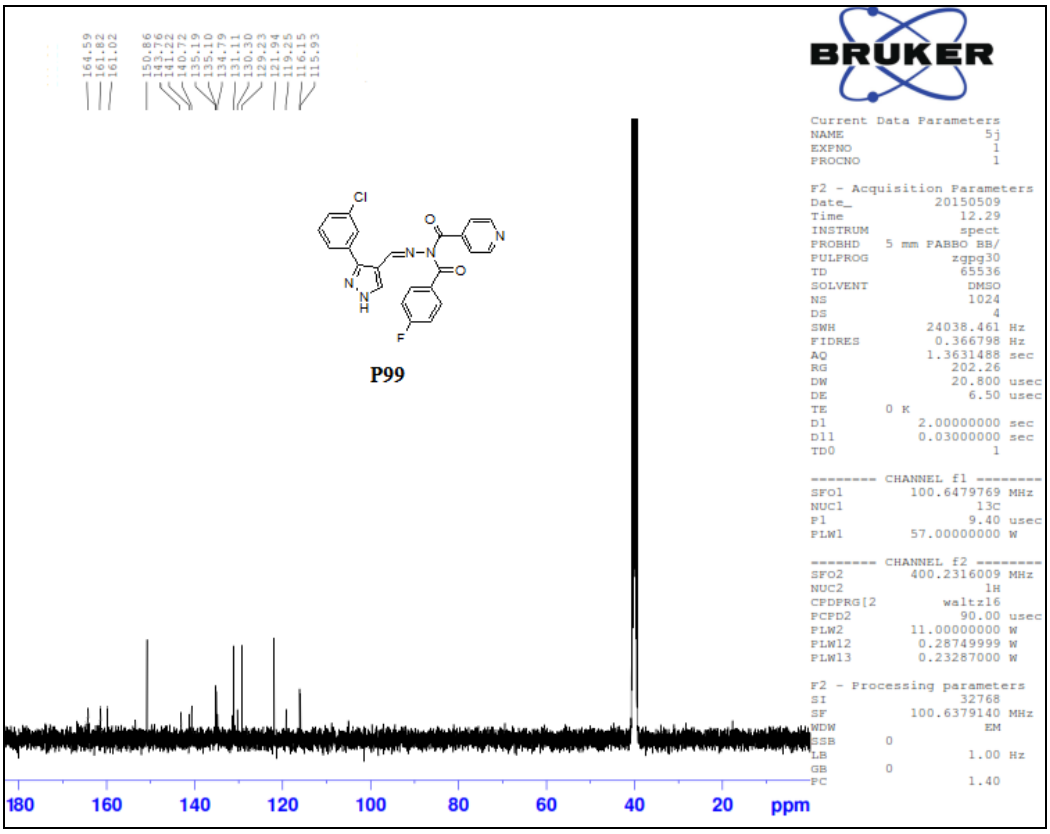


Figure 6.13 ^{13}C NMR spectrum of **P99.**

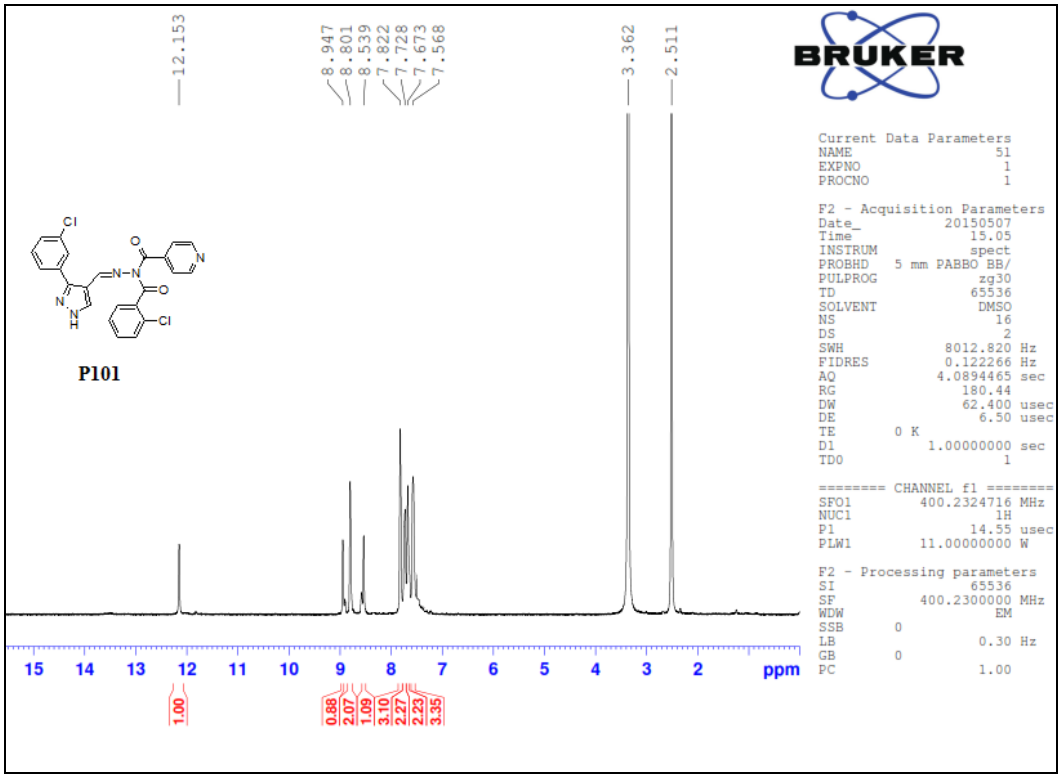


Figure 6.14 ^{1}H NMR spectrum of **P101.**

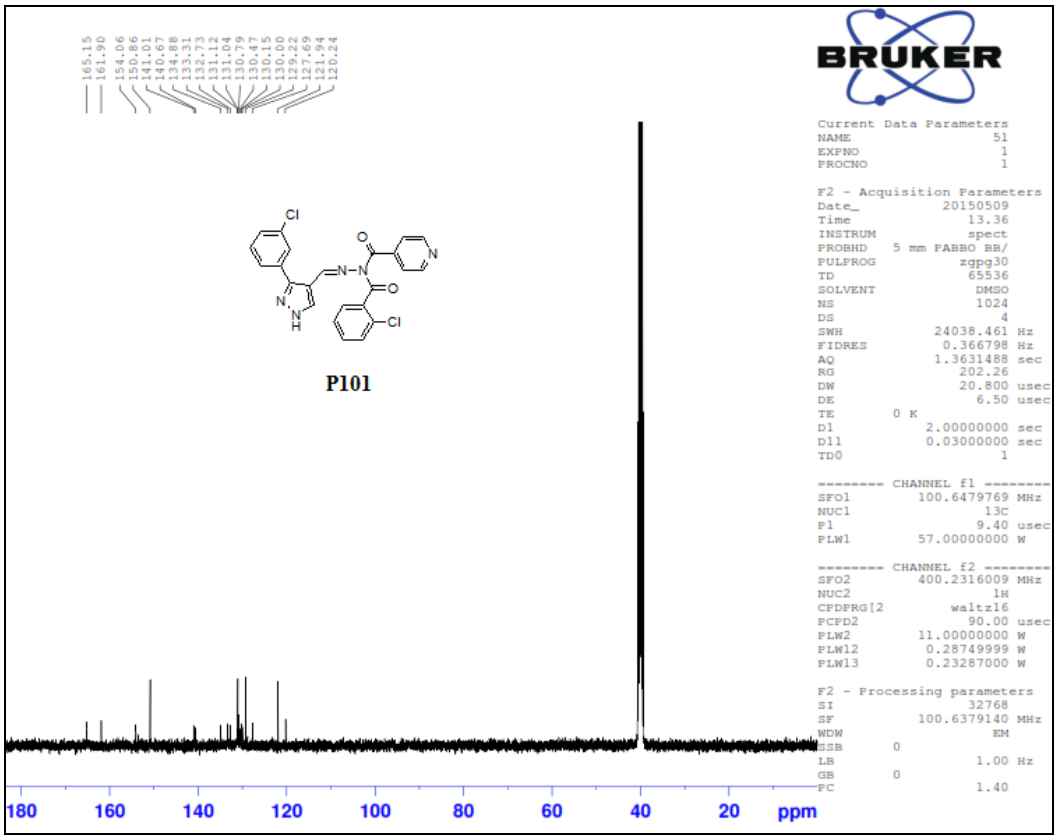


Figure 6.15 ^{13}C NMR spectrum of **P101.**

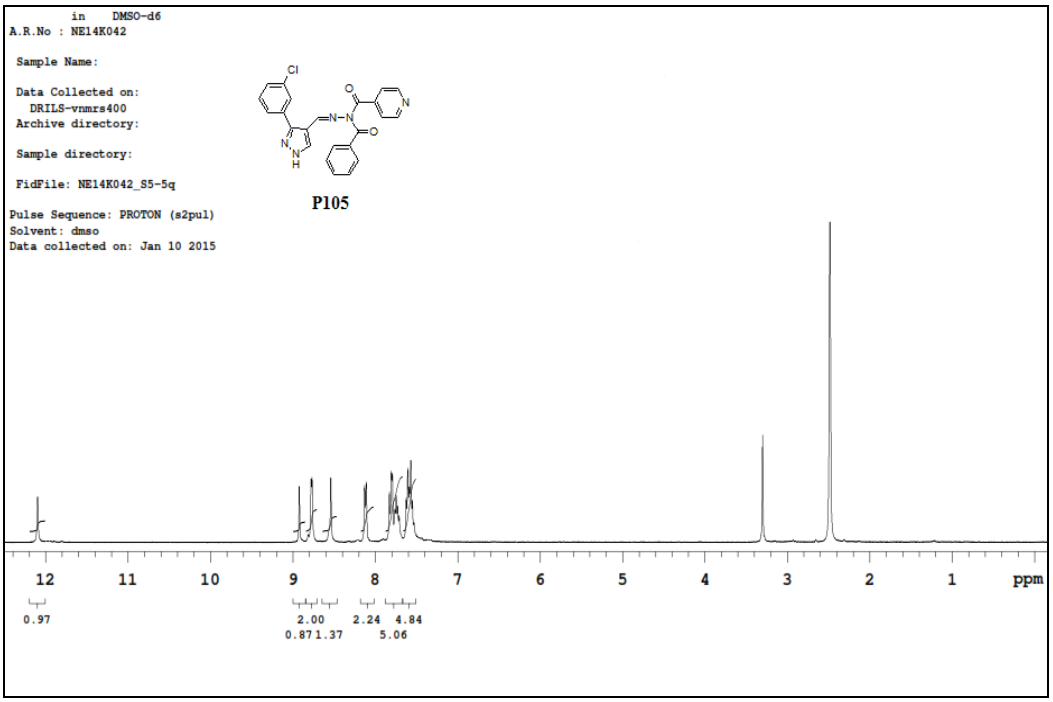


Figure 6.16 ^{1}H NMR spectrum of **P105.**

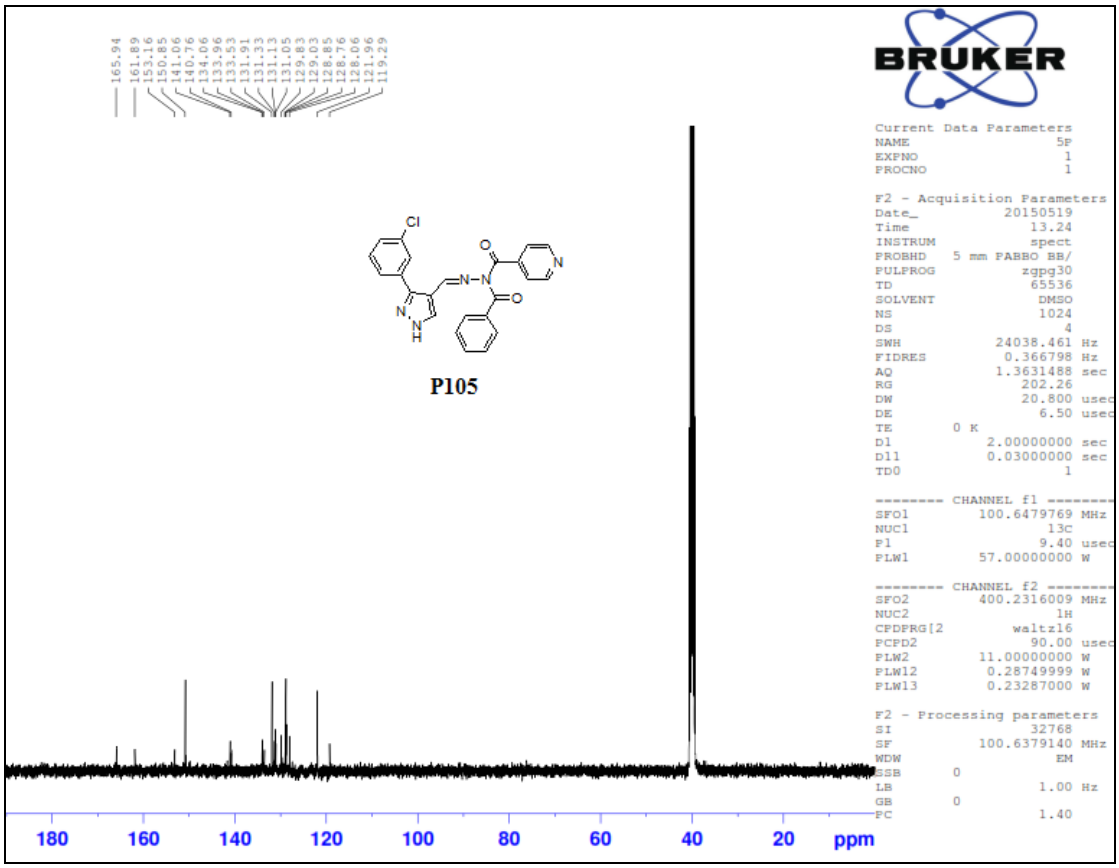


Figure 6.17 ^{13}C NMR spectrum of **P105.**

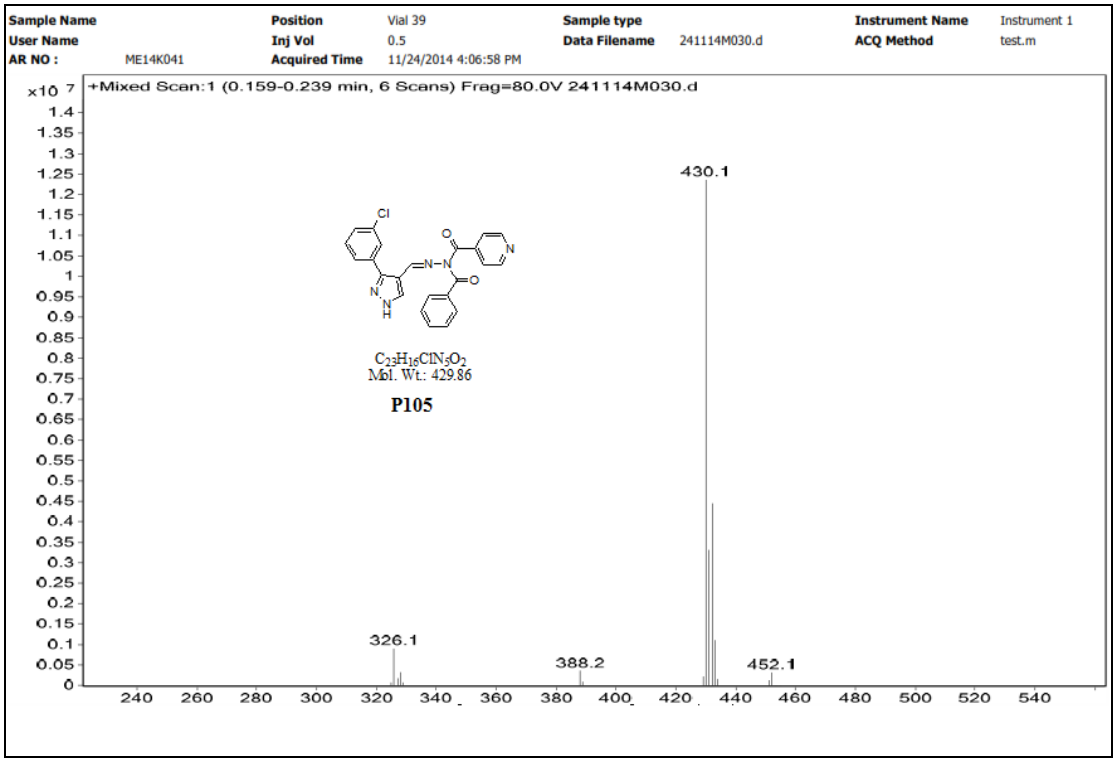


Figure 6.18 Mass spectrum of **P105.**

CHAPTER 7

SUMMARY AND CONCLUSIONS

Abstract

This chapter presents a brief summary and conclusions of the current research work. Further, the scope for future work is discussed.

7.1 SUMMARY

Nowadays, world has conquered with many deadly infectious diseases and advanced drug discovery has immensely brought down the mortality rate to some extent. However diseases caused by various infectious agents like TB, HIV, pneumonia, typhoid, H1N1, dengue and malaria are matters of big concern at present. Further, emerging antimicrobial resistance has created a major public health dilemma, compounded by a dearth of new antimicrobial options. In addition, the alarming rates of reemerging microbial threats coupled with increasing antimicrobial resistance, particularly in regard to multi drug-resistant mycobacterium and various other bacteria are major concerns to the public health as well as scientific communities worldwide. These developments have emphasized the pressing need for new, more effective and safe antimycobacterial and antibacterial agents; which in turn has opened up a new area of research for the scientists.

The present research work, involving design, synthesis and evaluation of antitubercular activities of NCEs was centered on pyrazole derivatives. Based on the literature on structure-activity relationship, different series of pyrazole based NCEs conatining 1,2,3-triazole (**P1-P24**), 1,3,4-oxadiazole (**P25-P48**), quinoline (**P49-P89)** or isoniazid (**P90-107**) pharamacophores were designed. The newly designed molecules were synthesized using appropriate synthetic routes. Further, the synthetic methods were optimized for different derivatives. Appropriate purification methods were developed in order to achieve spectral grade purity. Their structures were confirmed by ^{1}H and ^{13}C NMR, and mass spectral studies. Selected compounds were subjected to X-ray crystallographic studies for further confirmation of their structures. Also, their structures were established by elemental, i.e. CHN analysis.

The target compounds were evaluated for their *in vitro* antimycobacterial activity against MTB $H_{37}Rv$ strain and antibacterial screening against three common infectious bacterial strains of *S. aureus*, *P. aeruginosa* and *E. coli*. Furthermore, all the active antiTB target molecules were subjected to cytotoxicity study against benign non-cancerous cells. Finally, in order to understand binding affinity and interactions

of these molecules with the proteins, the *in silico* molecular modeling studies of these active derivatives were carried out against three important enzymes (InhA, CYP121, TMPK) of MTB.

7.2 CONCLUSIONS

The conclusions of present research study are as follows:

- Five different types of new chemical entities (NCEs) centered on pyrazole were successfully designed. While designing, important pharmacophoric elements such as 1,2,3-triazole, 1,3,4-oxadiazole, quinoline and isoniazid moieties were incorporated.
- The synthetic methods for the preparation of the NCEs were developed and the reaction conditions were optimized.
- The purification methods were established for the newly synthesized pyrazole derivatives.
- Structures of new compounds were established using ^{1}H NMR, ^{13}C NMR and mass spectrometry, followed by elemental analysis. Structures of selected new compounds were confirmed by X-ray crystallographic studies.
- All the final target molecules were evaluated for their *in vitro* antitubercular and antibacterial activities.
- In the case of pyrazole-1,2,3-triazole hybrids (**P1-P24**), five compounds (**P1, P2, P4, P6** and **P7**) showed good level of antitubercular properties (MIC $\leq$ 6.25 µg/mL). Further, derivatives **P1**, **P3**, **P4**, **P5**, **P12** and **P23** exhibited significant antibacterial activity against the tested bacterial strains.
- With regard to the relationship between the structure of pyrazole-1,2,3-triazole hybrids and the detected antiTB and antimicrobial properties, it can be concluded that the chlorophenyl substituted pyrazole scaffolds are ideally suited for further structural modifications to obtain efficient antibacterial leads.
- In the substituted 1,3,4-oxadiazole series **(P25-P48)** it was observed that 4-chlorophenyl substituted pyrazole derivatives are better antitubercular agents than the corresponding pyridine and 4-methoxyphenyl substituted derivatives. One of the derivative **P38** was the most active molecule with a MIC of 1.56 µg/mL and is two-fold stronger than the standard ethambutol. The antibacterial results on the

final compounds showed that, three compounds **P25, P26** and **P31** exhibit promising activity.

- In the pyrazole-quinoline containing methylene hydrazide series **(P49-P69)**, four compounds (**P50**, **P51**, **P58** and **P63)** have shown to possess moderate antitubercular activity (MIC = 12.5 µg/mL). In the antibacterial studies, four compounds (**P50-P52** and **P55**) showed substantial inhibition activity against the tested bacterial strains. It was found that the compounds with 8-trifluoromethyl quinoline substituted derivatives are promising lead molecules for further drug development.
- In the 8-trifluoromethyl quinoline containing pyrazole-3-carboxamide series **(P70-P89)** two compounds, **P80** and **P89** emerged as lead molecules against MTB with a MIC of 3.13 µg/mL. On the basis of observed activity profile it was established that hybrid amides with less sterically hindered substituents on the amide nitrogen exhibit enhanced antitubercular and antibacterial activity.
- The antitubercular activity of novel series of isonicotinohydrazide analogs of pyrazole series **(P90-P107)** showed that four derivatives (**P99, P100, P101** and **22b)** are potent antitubercular agents (MIC = 1.56 µg/mL), which are more potent than standard drug ethambutol. The activity of derivative **P101** (MIC = 0.8 µM) is comparable with that of the potent first-line drug isoniazid. Also, three compounds **P91**, **P100** and **22b** showed significant antibacterial activity.
- Cytotoxicity study on the active antiTB derivatives also suggested that the compounds are nontoxic against the normal cells.
- *In silico* molecular docking studies of the active antiTB compounds against three enzymes of *M. tuberculosis* further suggests that the good binding affinity of these targets against MTB. And this may be the reason for the observed activity of the target derivatives.
- Among the five newly designed pyrazole series, ten compounds **P7, P38, P39, 10b, P80, P89, P99, P100, P101** and **22b** are found to be potent mycobacterium inhibitors with low toxicity profile and hence are promising leads for further drug development.

The present research study has mainly focused on the synthesis, characterization and investigation of antitubercularar and antibacterial activities of

some new pyrazole derivatives. It is interesting to note that 20-30% of the target molecules were emerged to be a promising antitubercular leads with low toxicity profile and good binding affinity with the enzymes of MTB. Also, nearly 60-70% of the targeted compounds were found to be moderately active against at least one of the pathogenic strains, used in the present investigation. From the study, it can be concluded that pyrazole derivatives are potent and reliable scaffolds in search for new antitubercular and antibacterial drugs.

7.3 SCOPE FOR FUTURE WORK

- All the newly synthesized compounds which showed promising *in vitro* antiTB activity can be further subjected to *in vivo* studies.
- Antitubercular and antibacterial activity of the compounds synthesized in the present work has been tested only against normal bacteria. Further, the activity of these newly synthesized compounds can be extended to multidrug-resistant bacterial strains such as Multidrug-resistant tuberculosis (MDR-TB), Methicillin resistant *Staphylococcus aureus* (MRSA), Vancomycin-resistant *Staphylococcus aureus* (VRSA),etc.
- The SAR details of information derived in the present study could be employed in computer aided drug design (CADD)/QSAR studies to develop new pyrazole based lead molecules.

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LIST OF PUBLICATIONS

Papers published/communicated in international journals

1. **Nagabhushana Nayak,** Jurupula Ramprasad and Udayakumar Dalimba (2015). "New INH-pyrazole analogs: Design, synthesis and evaluation of antitubercular and antibacterial activity." *Bioorg. Med. Chem. Lett.*, 25, 5540-5545.

2. **Nagabhushana Nayak,** Jurupula Ramprasad, Udayakumar Dalimba, Perumal Yogeeswari, Dharmarajan Sriram, H.S. Santosh Kumar, S.K. Peethambar, Rajeshwara Achur (2016). "Synthesis of new pyrazole-triazole hybrids by click reaction using a green solvent and evaluation of their antitubercular and antibacterial activity." *Res. Chem. Intermed.*, 42, 3721-3741.

3. **Nagabhushana Nayak,** Jurupula Ramprasad, Udayakumar Dalimba (2015). "Design, synthesis and biological evaluation of new 8-trifluoromethylquinoline containing pyrazole-3-carboxamide derivatives." *J. Heterocycl. Chem.*, DOI 10.1002/jhet.2564.

4. **Nagabhushana Nayak,** Jurupula Ramprasad, Udayakumar Dalimba, Perumal Yogeeswari, Dharmarajan Sriram (2016). "Synthesis and antimycobacterial screening of new *N*-(4-(5-aryl-3-(5-methyl-1,3,4-oxadiazol-2-yl)-1*H*-pyrazol-1-yl)phenyl)-4-amide derivatives." *Chin. Chem. Lett.*, 27, 365-367.

5. **Nagabhushana Nayak,** Jurupula Ramprasad, Udayakumar Dalimba (2016). "Synthesis and antitubercular and antibacterial activity of some active fluorine containing quinoline-pyrazole hybrid derivatives." *J. Fluorine Chem.*, 183, 59-68.

Research papers presented in national/international conferences

1. **Nagabhushana Nayak**, Ramaprasad Jurupula, Udayakumar Dalimba (2014). "Synthesis, characterization and antimycobacterial activity studies of some novel pyrazoles containing triazole moiety." International Symposium on Chemical Biology - Drug Discovery Programme. University of Mysore, Karnataka, India. January 9-10, 2014.

2. **Nagabhushana Nayak**, Jurupula Ramprasad, Udayakumar Dalimba, Perumal Yogeeswari, Dharmarajan Sriram (2014). "Synthesis, characterization and antimycobacterial activity studies of some new 8-trifluoromethyl-quinoline and pyrazole hybrid derivatives." 13th Eurasia Conference on Chemical Sciences. Indian Institute of Science, Bangalore, India. December 14-18, 2014.

3. **Nagabhushana Nayak**, Jurupula Ramprasad, Udayakumar Dalimba (2016). "Synthesis and antitubercular study of a series of new quinoline-pyrazole hybrid derivatives." National Conference on Recent Trends in Chemical Sciences (NCRTCS)-2016. Department of Chemistry, Manipal Institute of Technology, Manipal, Karnataka, India. January 11-12, 2016.

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CAREER OBJECTIVE

To be a part of creative, growth oriented, value based education.

ACADEMIC QUALIFICATIONS

08/2012-01/2016: **Doctor of Philosophy (PhD) in Medicinal Chemistry** under the guidance of **Dr. Udayakumar D.,** at Department of Chemistry, National Institute of Technology Karnataka, Surathkal, India. Title of thesis - "**Synthesis, Characterization and Antitubercular Studies of some Pyrazole based Molecules**."

08/2004-04/2006: **Master of Science (MSc) Applied Chemistry** at Mangalore University, Mangalore, India in First Division with Distinction.

06/2001-04/2004: **Bachelor of Science (BSc)** at Poorna Prajna College, Udupi, Karnataka, India in First Division with Distinction.

EXPERIENCE SUMMARY

Work experience

- Worked as a Research Associate in the area of Synthetic Organic Chemistry at **Syngene International Ltd. (A Biocon Company)**, Bangalore, India, from **May 2006 to August 2012 (6 years and 2 months)**.

- Currently working as a Senior Research Associate in the area of Synthetic Organic Chemistry at **Kemio Solutions Pvt Ltd,** Bangalore, India, from **January 2016.**

Project experience

- MSc project on "Synthesis of some oxazole and thiazole derivatives" at Synthetic Organic Chemistry Laboratory, **Syngene International Ltd. (A Biocon Company)**, Bangalore, India, from **December 2005 to January 2006** under the guidance of Dr. Boja Poojary, Department of Chemistry, Mangalore University. Mangalore and Shashidhar N. L., Senior scientific manager, **Syngene International Ltd. (45 days).**

Job profile

The work involved designing and synthesis of complex organic molecules of pharmacological and agrochemical interest using reported or novel reaction schemes.

- Involved in developing the synthetic routes for building up various heterocyclic ring systems.
- Having very good experience in handling hazardous reagents and in performing sensitive reactions.
- Having good knowledge in interpreting spectroscopic data.
- Having good experience in various types of purification techniques like column chromatography, Preparative HPLC, recrystallization etc.

Experimental skills:

- Multistep synthesis of heterocyclic molecules via Aldol condensation, Wittig reaction, Click chemistry protocols, Palladium catalyzed cross coupling reactions viz. Suzuki, Sonogashira and Heck coupling reactions, Oxidations (using potassium permanganate, Swern oxidation etc.), Reductions (using CBS reagent, sodium borohydride, lithium aluminium hydride, DIBAL-H, Pd/C, Raney-nickel, Fe/ammoniumchloride, Sn/HCl etc.).
- Acquired particular competence in providing building block for construction of combinatorial libraries.
- Handled hazardous reagents viz. KCN (up to 50g), hazardous/flammable reagents like Borane dimethyl sulphide, BBr_3, nBuLi, Sodium metal etc. and air/moisture sensitive reactions.
- Microwave and Autoclave reaction under various condition and different solvents.
- Grignard reactions.
- Good experience in combinatorial library synthesis (milligram scale) as well as in bulk (kilogram) scale reactions.

Instrumentation skills

Hands-on experience in handling and usage of Single Crystal X-Ray diffractometer (Bruker Apex-II Smart Duo), Bruker Alpha-ATR, UV-visible and Fluorescence spectrometers, Agilent technologies-GPLC, Agilent technologies-UPLC, Biotage column machine, Biotage microwave reactor etc.

Computer skills

SHELX SC-XRD Software, Schrödinger Docking software, Mestrenova NMR software, Origin, EndNote, MS Office, ChemDraw, Chemsketch, Reaxys, SciFinder etc.

Hobbies

- Volleyball, Swimming Cricket, Hockey (National game of India) and Kabaddi.
- Reading, Travelling, Music and Cooking.

Field of interests

Organic Synthesis, Green Chemistry, Medicinal Chemistry, Heterocyclic Chemistry

PUBLICATIONS

Papers published/communicated in international journals

1. **Nagabhushana Nayak,** Jurupula Ramprasad, Udayakumar Dalimba* "New INH-pyrazole analogs: Design, synthesis and evaluation of antitubercular and antibacterial activity." **Bioorganic & Medicinal Chemistry Letters**, **2015,** *25*, 5540-5545.
2. **Nagabhushana Nayak,** Jurupula Ramprasad, Udayakumar Dalimba*, Perumal Yogeeswari, Dharmarajan Sriram, H.S. Santosh Kumar, S.K. Peethambar, Rajeshwara Achur "Synthesis of new pyrazole-triazole hybrids by click reaction using a green solvent and evaluation of their antitubercular and antibacterial activity." **Research on Chemical Intermediates**, **2016**, *42*, 3721-3741.
3. **Nagabhushana Nayak,** Jurupula Ramprasad, Udayakumar Dalimba* "Design, synthesis and biological evaluation of new 8-trifluoromethylquinoline containing pyrazole-3-carboxamide derivatives." **Journal of Heterocyclic Chemistry**, **2015**, DOI: 10.1002/jhet.2564.
4. **Nagabhushana Nayak,** Jurupula Ramprasad, Udayakumar Dalimba*, Perumal Yogeeswari, Dharmarajan Sriram (2016). "Synthesis and antimycobacterial screening of new *N*-(4-(5-aryl-3-(5-methyl-1,3,4-oxadiazol-2-yl)-1*H*-pyrazol-1-yl)phenyl)-4-amide derivatives." **Chinese Chemical Letters**, **2016**, *27*, 365-367.
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6. Jurupula Ramprasad, **Nagabhushana Nayak,** Udayakumar Dalimba*, Perumal Yogeeswari, Dharmarajan Sriram "Ionic liquid promoted one-pot synthesis of thiazole-imidazo[2,1-*b*][1,3,4]thiadiazole hybrids and their antitubercular activity." **MedChemComm**, **2016**, *7*, 338-344.
7. Jurupula Ramprasad, **Nagabhushana Nayak,** Udayakumar Dalimba*, Perumal Yogeeswari, Dharmarajan Sriram, S.K. Peethambar, Rajeshwara Achur, H.S. Santosh Kumar "Synthesis and biological evaluation of new imidazo[2,1-*b*][1,3,4]thiadiazole-benzimidazole derivatives." **European Journal of Medicinal Chemistry**, **2016**, *95*, 49-63.
8. Jurupula Ramprasad, **Nagabhushana Nayak,** Udayakumar Dalimba* "Design of new phenothiazine-thiadiazole hybrids via molecular hybridization approach for the development of potent antitubercular agents." **European Journal of Medicinal Chemistry**, **2015**, *106*, 75-84.
9. Jurupula Ramprasad, **Nagabhushana Nayak,** Udayakumar Dalimba*, Perumal Yogeeswari, Dharmarajan Sriram "One-pot synthesis of new triazole-Imidazo[2,1-*b*][1,3,4]thiadiazole hybrids via click chemistry and evaluation of their antitubercular activity." **Bioorganic & Medicinal Chemistry Letters**, **2015**, *25*, 4169–4173.

Papers presented in international conference:

1. **Nagabhushana Nayak**, Ramaprasad Jurupula, Udayakumar Dalimba* "Synthesis, characterization and antimycobacterial activity studies of some novel pyrazoles containing triazole moiety." International Symposium on Chemical Biology - Drug Discovery Programme. University of Mysore, Karnataka, India. January 9-10, 2014.
2. **Nagabhushana Nayak**, Jurupula Ramprasad, Udayakumar Dalimba*, Perumal Yogeeswari, Dharmarajan Sriram "Synthesis, characterization and antimycobacterial activity studies of some new 8-trifluoromethyl-quinoline and pyrazole hybrid derivatives." 13th Eurasia Conference on Chemical Sciences. Indian Institute of Science, Bangalore, India. December14-18, 2014.
3. **Nagabhushana Nayak**, Jurupula Ramprasad, Udayakumar Dalimba* "Synthesis and antitubercular study of a series of new quinoline-pyrazole hybrid derivatives." National Conference on Recent Trends in Chemical Sciences (NCRTCS)-2016. Department of Chemistry, Manipal Institute of Technology, Manipal, Karnataka, India. January 11-12, 2016.
4. Jurupula Ramprasad, **Nagabhushana Nayak**, Udayakumar Dalimba*, Perumal Yogeeswari, Dharmarajan Sriram "Imidazo[2,1-*b*][1,3,4]thiadiazole-benzimidazole derivatives as potent antitubercular agents." 13th Eurasia Conference on Chemical Sciences. Indian Institute of Science, Bangalore, India. December 14-18, 2014.
5. Jurupula Ramprasad, **Nagabhushana Nayak**, Udayakumar Dalimba (2016). "New PZA-imidazo[2,1-*b*][1,3,4]thiadiazole hybrids: Synthesis and evaluation of antitubercular activity." National Conference on Recent Trends in Chemical Sciences (NCRTCS)-2016. Department of Chemistry, Manipal Institute of Technology, Manipal, Karnataka, India. January 11-12, 2016.

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Mother's Name	: Ramadevi
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Permanent Address	Srinilaya, Kodugudde, Shirva Post, Udupi Taluk and District, Karnataka, India-574116.

Qualities with me

- A strong candidature with excellent problem solving skills.
- Strong verbal and written communication skills.
- Professional attitude, self-motivated and self-starter.
- Quick learner and excellent team player, ability to meet tight deadlines and work under pressure.

I, hereby declare that the above-furnished details are true to the best of my knowledge.

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