SYNTHESIS OF SOME NEW PYRAZOLE DERIVATIVES AND THEIR ANTITUBERCULOSIS SCREENING

Thesis

Submitted in partial fulfilment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

by

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DECLARATION

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

Harikrishna. Nandam

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Place: NITK - Surathkal

Date:

CERTIFICATE

This is to *certify* that the Research Thesis entitled "**Synthesis of Some New Pyrazole Derivatives and Their Antituberculosis Screening**" submitted by **Mr. Harikrishna. Nandam (Register Number: 123006CY12P01)** as the record of the research work carried out by him, is *accepted as the Research Thesis submission* in partial fulfilment of the requirements for the award of degree of **Doctor of Philosophy.**

Dr. Arun M. Isloor
Research Guide
Date:

Chairman-DRPC

Date:


Dedicated to my
mother(Late)
Smt.RAJYALAKSHMI
& father and my
beloved family

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ABSTRACT

Antibiotics improve the better living life in the world for human as well as animals. Many types of bacteria are dramatically reduces illnesses and deaths caused by various infections. Therefore, it occupies great importance to discover newer, effective and safer drugs in the modern world. In the last few decades, the process of drug discovery program has undergone fundamental transformation to synthesize customs molecules and new chemical entities (NCEs). Organic synthesis approaches towards designing, innovation and low molecular chemical structures, which are easily available, biologically active, will definitely aid in combating the ailments prevailing universally. Although, the increasing cost for discovery of such molecules in terms of research and development, analysis, *in vitro* and *in vivo* studies for new sites were worries the pharmaceutical research. Newer heterocyclic compounds are being employed constantly in the hope of striking a proper perspective in combating the pathogen bacterial infections. A systemic investigation of this class of heterocyclic lead revealed that, pyrazole and its derivatives are well known nitrogen containing heterocyclic compounds occupy an important role in medicinal chemistry with wide variety of biological properties. Owing to this therapeutic degree of pyrazole and its derivatives, in the current research work, it has been planned to find out various potent heterocyclic moieties with pyrazole through active functional systems to form a new molecular framework. Accordingly, different libraries of pyrazole based compounds comprising of thiazole ($\mathbf{T_{1\text{-}12}}$), pyrazoline ($\mathbf{T_{13\text{-}27}}$), 1,4-dihydropyridine ($\mathbf{T_{28\text{-}45}}$), 1,3,4-oxadiazole ($\mathbf{T_{46\text{-}54}}$), [1,2,4]triazolo[3,4-*b*] [1,3,4]thiadiazole ($\mathbf{T_{55\text{-}63}}$), benzimidazole ($\mathbf{T_{64\text{-}79}}$) and trifluoromethylbenzyloxy derivatives ($\mathbf{T_{80\text{-}97}}$) have been designed and synthesized. Newly synthesized chemical derivatives were confirmed by various spectroscopic techniques *viz*. FT-IR, ^{1}H-NMR, ^{13}C-NMR, LC-MS and elemental analyses. Additionally, three dimension structures of few molecules were confirmed by single crystal X-ray diffraction (S-XRD) studies. Further, the target compounds were subjected to screen preliminary *in vitro* antitubercular, antibacterial and antifungal activities. The active molecules were identified and tested for their cytotoxicity studies against non-cancerous cells.

Keywords: Pyrazole, thiazole, pyrazoline, 1,4-dihydropyridine, 1,3,4-oxadiazole, [1,2,4]triazolo[3,4-*b*][1,3,4-thiadiazole], benzimidazole, trifluoromethylbenzyloxy derivatives, antitubercular activity, antibacterial activity, antifungal activity and cytotoxicity studies.

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SCHEMES

NOMENCLATURE

ABBREVIATIONS

AIDS	Acquired immuno deficiency syndrome
Anti-TB	Antitubercular
ATCC	American type culture collection
C. albicans	*Candida albicans*
CCDC	Cambridge crystallographic data centre
DNA	Deoxyribonucleic acid
DMF	*N,N*-Dimethylformammide
E. coli	*Escherichia coli*
EMB	Ethambutol
ESI-MS	Electro spray ionisation mass spectrometry
FDA	FOOD & DRUG ADMINISTRATION
FLZ	Fluconazole
FT-IR	Fourier transform-Infrared spectroscopy
GLP	Good laboratory practices
HIV	Human immuno deficiency virus
IND	Investigational new drug process
INH/INZ	Isoniazid
INN	Ciprofloxacin
KBr	Potassium bromide
LC-MS	Liquid chromatography-Mass spectroscopy
m. p	Melting point
MABA	Microplate alamar blue assay
MDR-TB	Multidrug resistant tuberculosis
MIC	Minimum inhibitory concentration
M. tb	*Mycobacterium tuberculosis*
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
M. smegmatis	*Mycobacterium smegmatis*
M. tuberculosis	*Mycobacterium tuberculosis*
NaH	Sodium hydride

NMR	Nuclear magnetic resonance
OLEDS	Organic light emitting diodes
ORTEP	OAK RIDGE thermal ellipsoid plot program
P. aeruginosa	*Pseudomonas aeruginosa*
P. Chrysogenum	*Penicillium chrysogenum*
PPA	Polyphosphoric acid
PZA	Pyrazinamide
QASR	Quantitative structure activity relationship
RIF	Rifampicin
RNA	Ribonucleic acid
RT	Room temperature
S. aureus	*Staphylococcus aureus*
S-XRD	Single crystal X-Ray diffraction
STM	Streptomycin
TB	Tuberculosis
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TMS	Tetramethylsilane
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
µM	Micromolar

mmol	Millimole
ppm	Parts per million
min	Minutes
sec	Seconds
h	Hour
%	Percentage
°C	Degree Celsius
K	Kelvin
Hz	Hertz
J	Coupling constant
MHz	Megahertz
<	Less than
>	Greater than
≥	Greater than or equal to
≤	Less than or equal to
Å	Angstrom units
±	Plus minus
m/z	Mass to charge ratio

CHAPTER 1

INTRODUCTION

1.1 HETEROCYCLIC CHEMISTRY

Heterocyclic compounds are organic compounds which have at least one hetero atom other than carbon within a ring skeleton. These hetero atoms could include oxygen, nitrogen or sulfur. In the recent years, they have acquired more importance in our daily life due to their varied pharmacological activities as well as other applications (Katritzky, 1985).

Many of the available vitamins, alkaloids, synthetic medicines and antibiotics are heterocyclic compounds. A large number of heterocyclic compounds occur in nature. Among these various elements of nature, the biological processes carry a variety of heterocyclic active compounds (Czarnik, 1996). Heterocyclic compounds are the major components of biological molecules such as Deoxyribonucleic acid (DNA) and Ribonucleic acid (RNA). DNA is the most essential macromolecule of life. Also, essential diet ingredients such as Pyridoxal (Vitamin B6), Niacin (Vitamin B3), Riboflavin (Vitamin B2), Thiamin (Vitamin B1) and Ascorbic acid (Vitamin C) are heterocyclic compounds. In addition, hemoglobin (oxygen carriers in the blood), chlorophyll and enzymes constitute many important known heterocyclic core structures (Remington, 1995). They are also present in a wide variety of drugs, many natural products, biomolecules and biologically active compounds, including antibacterial, antibiotic, antidiabetic, antidepressant, antifungal, anti-HIV, anti-inflammatory, antimalarial, antimicrobial, antitumor, antiviral, herbicidal, fungicidal and insecticidal agents. Many of the heterocycles possess important applications in material science such as analytical reagents, brightening agents, corrosion inhibitors, dyestuff, fluorescent sensor, information storage and plastics (Kozikowski, 1984). In addition, they have applications in supramolecular and polymer chemistry. These heterocyclic compounds have occupied great significance in new drug discovery. Owing to their biological activities, properties and applications, the synthesis of heterocyclic compounds has attracted great attention for a long time.

An interesting feature of many of the heterocyclic compounds is that, they can incorporate several functional groups either as constituents or as a part of the ring system itself. It is well-known that even a small modification in the molecular structure can alter their physiochemical properties as well as their biological characters. Both steric and electronic factors are responsible for the variation of

biological activity. Generally, a number of factors such as solubility, partition coefficient, hydrogen bonding, presence of hydrophilic and hydrophobic groups and many more play a crucial role in the selection of the lead molecule. Some of the exceptional properties of the heterocyclic compounds make it more intriguing for the medicinal chemist to develop a plethora of new molecules having pharmacological significance.

1.2 MEDICINAL CHEMISTRY

Medicinal chemistry is the branch of science that mainly deals with design, discovery, development of new biologically active molecules and their optimization at the molecular level which leads to invention of new drug molecules for the treatment of various diseases (Hughes *et al.* 2011). Human being has achieved a remarkable success in combating many of the fatal diseases which threatened the very existence of human race. Human have succeeded in complete eradication of certain types of diseases. However, the present global research sphere is engrossed in the invention of more suitable drugs that provide better activity and fewer side effects. Medicinal chemistry has made a remarkable change and progress in the last few decades. During the early stages of developments in medicinal chemistry scientists were more focused on isolation of medicinal agents found in phytochemistry. In the recent years, almost all the active drugs were synthesized by chemical synthesis. The incessant research in the field of medicinal chemistry has led to the discovery of many active compounds which have better therapeutic activity than the existing drugs. Though many effective drugs are available in the market to cure the most terrible and complicated diseases like cancer, hepatitis, tuberculosis, malaria and others, scientists are still investing efforts on a global level to find new drug targets and newer class of drugs which are more effective, lower cost, non-toxic in nature. New drugs are continuously discovered by molecular modification of active compounds with established activity. Modification of molecules can possibly result in augmenting the activity, minimizing the side effects and preventing the development of resistance by infectious microorganism.

Further, the development in medicinal chemistry field requires a combined contribution of experts from organic chemistry, analytical chemistry, molecular

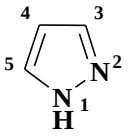
biology, pharmacology and biochemistry. The medicinal chemist must design and synthesize new molecules and also determine the interaction between designed molecule and their metabolic transformations. In the medicinal chemistry research, designing of the drug design plays a crucial role. The most important molecular design strategies in this direction are i) computer-aided drug design to find-out ligand-protein interaction (Aqvist *et al.* 1994) ii) molecular modification of the drug molecule (Lima and Barreiro, 2005) iii) hybridization of two active groups into a single molecular framework (Lazar *et al.* 2004) and iv) the random screening of different structural molecules for various activities (Cappoen *et al.* 2014), all these approaches are being considered as promising to develop effective drugs.

The process of drug development has evolved into an extremely complex procedure. The average drug takes about 12 years and $270 million from initial discovery to public usage. The drug development process involves 5 major steps. In step 1, discovery and developmental research begins in the laboratory. Step 2 involves preclinical research in which drugs undergo laboratory and animal testing to answer basic questions about safety. Before testing a drug in humans, researchers must find out whether it has the potential to cause any serious side effects to human health. The two types of preclinical research are *in vitro* and *in vivo* studies. However, these studies must provide detailed information on dosing and toxicity levels. After preclinical testing, researchers review their findings and decide whether the drug should be tested on human being or not. In step 3, the drugs are tested on people to make sure they are safe and effective. "Clinical research" refers to studies, or trials, that are done on people. In step 4, if a drug developer has evidence from the early tests and preclinical and clinical research that a drug is safe and effective for its intended use, the company can file an application to market the drug. Step 5 is Food and Drug Administration (FDA) should monitor the post-market data. Even though, clinical trials provide important information on a drug's efficacy and safety, it is impossible to have complete information about the safety of a drug at the time of approval. FDA reviews the reports of problems with prescription and over-the-counter drugs, and can decide to add cautions to the dosage or usage information, as well as other measures for more serious issues.

The scientific understanding of drug action is required to design a compound that will produce a specified therapeutic action. The contribution of medicinal chemistry is confined largely to the preparation of Active Pharmaceutical Ingredients (API) contains pyridines, quinolines, indoles, benzimidazoles, imidazoles, pyrazoles, thiazoles, pyrimidine ring structures and many more. Various pharmacophores containing the above heterocyclic moieties are reported to possess a wide range of biological properties such as antibacterial, anticancer, antifungal, anti-inflammatory, anticonvulsant, antiviral, antimalarial and antitubercular activities. Pyrazole is one of the important classes of heterocyclic chemistry due to its wide range of biological activities they possess, which are discussed in the following sections.

1.3 CHEMISTRY OF PYRAZOLES

Pyrazoles are five-membered two-nitrogen containing heterocyclic compound and they have the formula $C_3H_4N_2$ and are colorless liquid. Pyrazoles are aromatic compounds and have different tautomeric structures. Unsubstituted pyrazole can be represented in two tautomeric forms and for the pyrazole derivatives in which two carbon atoms neighboring the nitrogen atoms on the ring have different substituent, five tautomeric structures are possible. When substitutions are introduced on the first, third and fourth position of this ring, it is highly activated and readily reacts to give various moieties (Ansari *et al.* 2017).



Structure of Pyrazole

1.3.1 Review for various synthetic methods of pyrazole:

In early 1884, Knorr discovered the antipyretic action of pyrazole derivatives *i.e* antipyrine. This was the first discovery of pyrazole derivative used as medicinal agent. This stimulated the interest in pyrazole chemistry.

Many synthetic methods have been developed for the preparation of pyrazole derivatives. In general, pyrazoles are synthesized by the reaction of

(i) 1,3-Diketones with hydrazines,

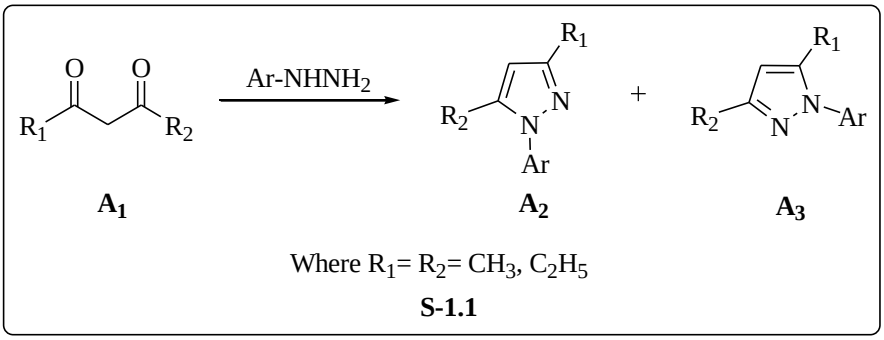
(ii) α,β-Unsaturated aldehydes and ketones reaction with different hydrazines,

(iii) Vilsmeier-Haack reaction of hydrazones,

(iv) Reaction of alkynes with diazo compound by 1,3-dipolar cycloaddition reaction.

***(i)* 1,3-Diketones with hydrazines**

First synthetic method for the synthesis of pyrazole (**S-1.1**) was explored by Knorr in 1883 from the reaction of 1,3-dicarbonyl compound $\mathbf{A_1}$ with aryl hydrazines to afford pyrazole derivatives $\mathbf{A_2}$ and $\mathbf{A_3}$. The condensation of symmetrical or unsymmetrical 1,3-diketones with aryl hydrazines in the presence of catalysts generally gives mixture of two regioisomers. The yields of the pyrazole isomers usually depend on the reaction conditions.



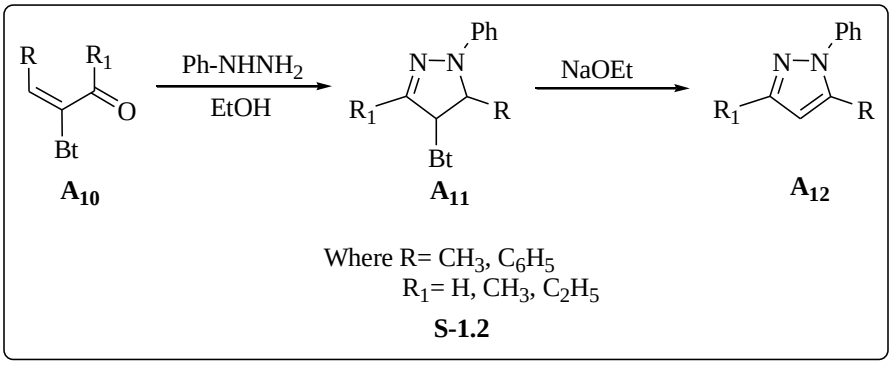
$\mathbf{A_1}$ $\mathbf{A_2}$ $\mathbf{A_3}$

Where $R_1 = R_2 = CH_3, C_2H_5$

S-1.1

***(ii)* α, β-Unsaturated aldehydes and ketones reaction with different hydrazines.**

Katritzky *et al.* (2001) prepared 1,3,5-trisubstituted pyrazole derivatives $\mathbf{A_{12}}$ (**S-1.2**) by the reaction of α- benzotriazolyl-α, β-unsaturated ketones $\mathbf{A_{10}}$ with hydrazines. During the reaction, pyrazoline derivatives $\mathbf{A_{11}}$ were produced and then oxidized to form target pyrazole derivatives $\mathbf{A_{12}}$ b**y** the elimination of benzotriazolyl group with sodium ethoxide.



$\mathbf{A_{10}}$ $\mathbf{A_{11}}$ $\mathbf{A_{12}}$

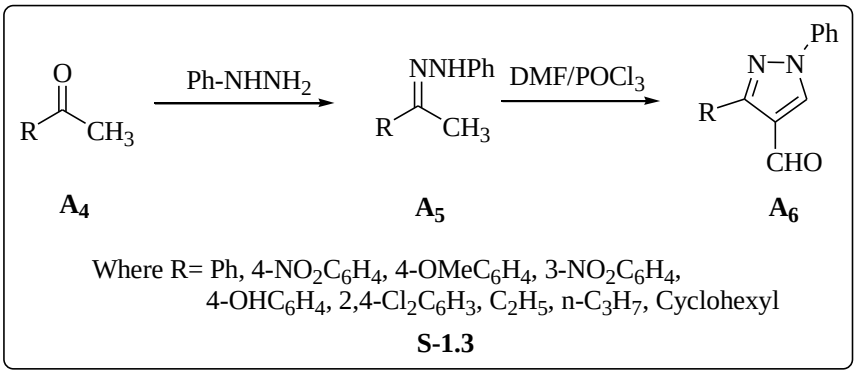
Where $R = CH_3, C_6H_5$

$R_1 = H, CH_3, C_2H_5$

S-1.2

(iii) Vilsmeier-Haack reaction of hydrazones

3-Aryl(alkyl)-1-phenyl-1*H*-pyrazole-4-carbaldehydes **A_6** were obtained *via* Vilsmeier-Haack reaction (**S-1.3**) of the appropriate intermediate hydrazone derivatives **A_5**, which are synthesized from aryl methyl ketone **A_4** with phenylhydrazine (Rathelot *et al.* 2002).



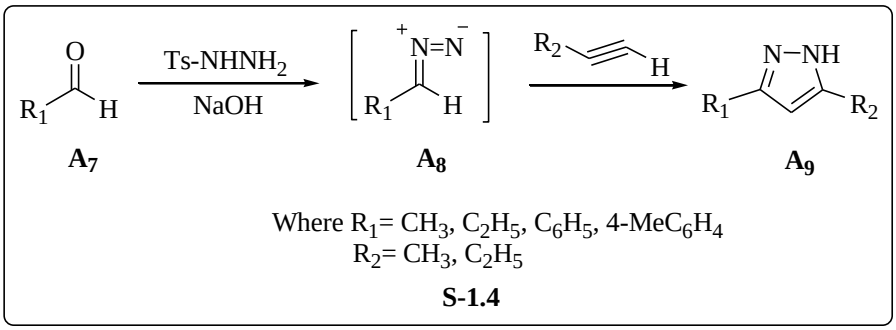
A_4 **A_5** **A_6**

Where R= Ph, 4-$NO_2C_6H_4$, 4-$OMeC_6H_4$, 3-$NO_2C_6H_4$, 4-OHC_6H_4, 2,4-$Cl_2C_6H_3$, C_2H_5, n-C_3H_7, Cyclohexyl

S-1.3

(iv) Reaction of alkynes with diazo compound by 1,3-dipolar cycloaddition reaction

3,5-Disubstituted pyrazoles (**S-1.4**) were synthesized *via* 1,3-dipolar cycloaddition of alkynes with diazo compounds by Aggarwal *et al.* (2003). Initially, diazo compound was reacted with *in situ* generated tosyl hydrazones **A_8** of aldehydes **A_7** by treating with sodium hydroxide, which then underwent cycloaddition with terminal alkynes to produce pyrazole derivatives **A_9**.



A_7 **A_8** **A_9**

Where R_1= CH_3, C_2H_5, C_6H_5, 4-MeC_6H_4
R_2= CH_3, C_2H_5

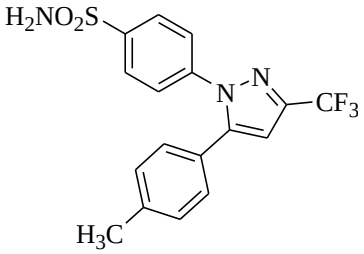
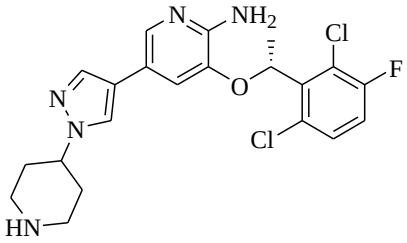
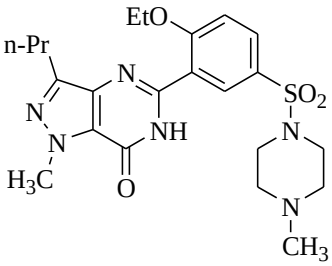
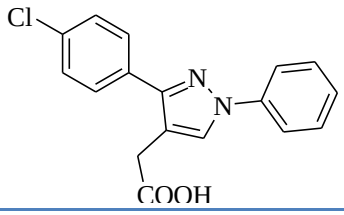
S-1.4

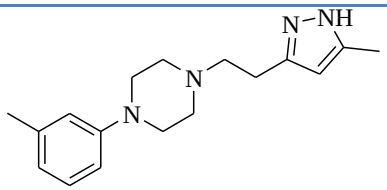
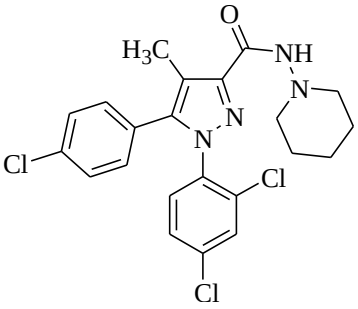
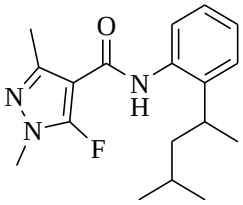
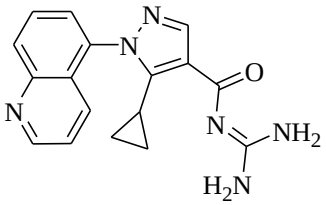
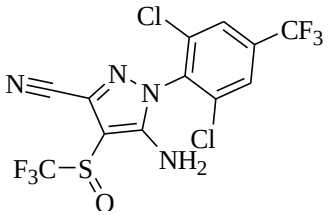
These reactions provide a high yield as well as purity of the desired product. Further, it is possible to synthesize new pyrazole derivatives containing different pharmacophores starting from simple molecules. Several books and reviews on pyrazole chemistry have been appeared. A review by Grimmett (1979) in "*Comprehensive Organic Chemistry"* gives deep insight into pyrazole chemistry.

1.4 BIOLOGICAL IMPORTANCE OF PYRAZOLE

A large number of structurally diverse compounds containing azole compounds are gaining more attention. Among azoles, pyrazoles are rarely found in nature. The difficulty in the formation of N–N bond by living organisms could be the one of the reason for this. A serial investigation of this class of heterocyclic compound revealed that pyrazole bearing different pharmacophore groups plays an enormous role. However, they exhibited numerous biological activities, including antibacterial (Holla *et al.* 2000), antitubercular (Chovatia *et al.* 2007), antiviral (Hashem *et al.* 2007), anticancer (Dhanya *et al.* 2009), antimicrobial and antioxidant (Vijesh *et al.* 2011) activities. Some of the pyrazole containing commercially available drugs are presented in **Table 1.1**.

Table 1.1 Pyrazole containing some of the commercially available drugs

Drug name	Structure	Therapeutic use
Celecoxib		NSAID
Crizotinib		Anticancer
Sildenafil		Erectile dysfunction
Lonazolac		Anti-inflammatory

Tolpiprazole		Anxiolytic
Rimonabant		Anti-obesity
Penflufen		Fungicide
Zoniporide		NHE-1 inhibitor
Fipronil		Insecticide

1.5 ANTIMICROBIALS AND THEIR IMPORTANCE

Antimicrobials are agents, which kill or suppress their multiplication or growth of harmful micro-organisms. Microbes are invisible to the naked eye and hence a powerful microscope is needed. Some of these microbes are useful, but some are harmful. Microbes are divided into three types. They are bacteria, virus and fungi. Viruses are the smallest and simplest life and are 10 to 100 times smaller than bacteria. The main difference between virus and bacteria is that viruses must have a living host like a plant or animal to multiply, while most bacteria can grow on non-living surfaces. Some bacteria are useful but all viruses are harmful. Bacteria are mainly of three shapes: little balls (-cocci), rods (-bacilli) and spiral (-boriella)

(**Figure 1.1**). Viruses can be rod-shaped, sphere-shaped or multisided. Some viruses look like tadpoles.

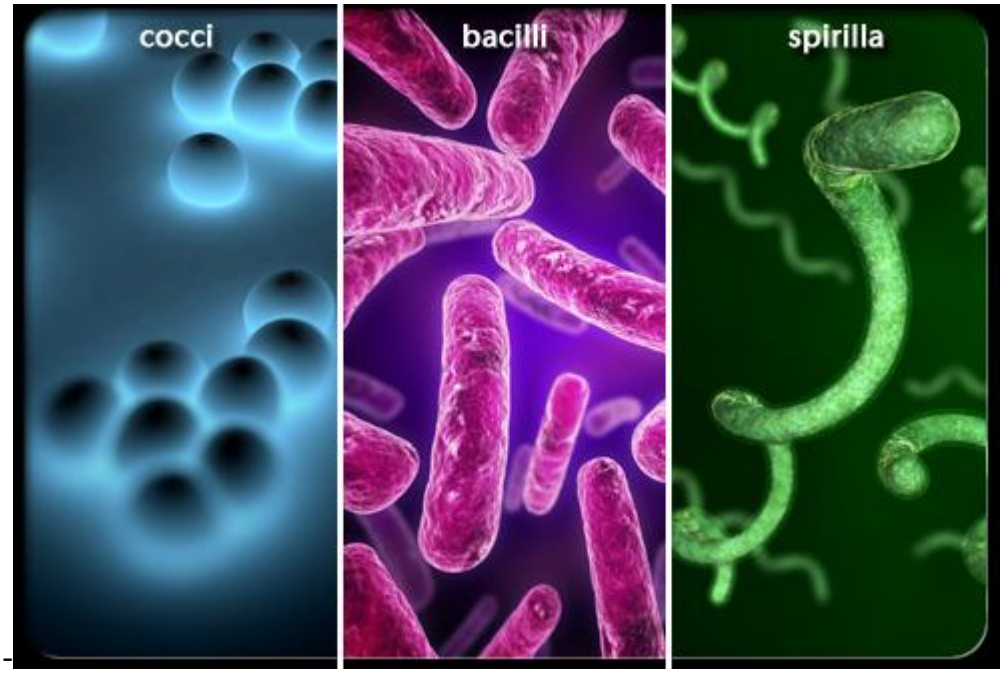


Figure 1.1 Shapes of bacteria (Little balls, rod type and spirals)
(Source: http://www.experiment.com)

1.5.1 History of antimicrobials

The history of antimicrobials begins with the observations of Pasteur and Koch, who discovered that, one type of bacteria could prevent the growth of another. In 1928, Sir Alexander Fleming, a Scottish biologist and Nobel laureate, observed that *Penicillium notatum*, a common mold, had destroyed *staphylococcus* bacteria in culture. Penicillin was isolated in 1939, and in 1944 Selman Waksman and Albert Schatz, American microbiologists, isolated Streptomycin and a number of other antibiotics from *Streptomyces griseus*. Penicillin came into clinical use in the 1940s, and it is found to be an outstanding agent in terms of safety and efficiency, led in the era of antimicrobial chemotherapy by saving the lives of many wounded soldiers during Second World War.

After the Second World War, the effort to find other novel antibiotic structures continued. This led to the discovery of Bacitracin (1945), Chloramphenicol (1947), Chlortetracycline (1948), Erythromycin (1952), Cycloserine (1955). Isoniazid was

found to be effective against human tuberculosis in 1952. Today, a large number of antibacterial agents are available and they have helped to control a vast majority of bacterial diseases

Antibacterial agents can be divided into two types based upon their effects on target cells. Substances that actually kill microorganisms are termed 'bactericidal'. Examples of bactericidal drugs are 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 and macrolides and others.

1.5.2 Mechanism of action on bacterial cell

Antimicrobial agents are classified functionally according to the manner in which they adversely affect a microorganism.

- **Inhibit cell wall synthesis:** A drug that targets cell walls can therefore selectively kill or inhibit bacterial organisms. The penicillins, cephalosporins, Bacitracin and Vancomycin are examples of this group of antimicrobials.
- **Inhibition of protein synthesis**: Antimicrobial agents inhibiting protein synthesis. Chloramphenicol, Erythromycin, Streptomycin, Tetracyclines are examples of this group of antimicrobials.
- **Inhibition of cell membrane**: Antimicrobial agents change the permeability of the cell membrane, causing a leakage of metabolic substrates essential to the life of the microorganism. Their action can be either bacteriostatic or bactericidal. Their examples include Colistin, Amphotericin B and Polymyxin B.
- **Inhibition of nucleic acid synthesis:** Antimicrobial agents interfere with metabolic processes (DNA and RNA replication and transcription) within the microorganism. Some drugs work by binding with components involved in the process DNA and RNA synthesis. Most of these agents are bacteriostatic. Some of the examples include the sulfonamides, Aminosalicylic acid (PAS) and Isoniazid (INZ).

- **Inhibition of other essential metabolites**: Other antibiotics act on selected cellular process essential for the survival of the bacterial pathogens. Sulfanilamides target and bind with dihydropteroate synthase, trimethophrim/ /inhibit dihydrofolate reductase; both of these enzymes are essential for the production of folic acid, a vitamin synthesized by bacteria, but not humans.

1.5.3 Most dangerous diseases caused by bacteria

Most of the bacteria are harmless and some of the bacteria are beneficial, few are pathogenic in nature. One of the most dangerous bacterial diseases is tuberculosis, infected by *Mycobacterium tuberculosis (Mtb)* strain. Few more pathogenic bacteria contribute to other globally important diseases, such as pneumonia, which can be caused by bacteria such as *Streptococcus* and *Pseudomonas*. Food borne illnesses can be caused by bacteria such as *Shigella*, *Campylobacter* and *Salmonella*. Pathogenic bacteria also cause infections such as tetanus, typhoid fever, diphtheria, syphilis and leprosy.

1.5.3.1 *Mycobacterium tuberculosis (M. tuberculosis)*

Tuberculosis (TB) is caused by *Mycobacterium tuberculosis* (**Figure 1.2**) and it most often affects the lungs. Tuberculosis can be preventable and curable disease. Infection occurs via aerosol and inhalation. A person needs to breathe in only a few of these germs to become infected. *M. tuberculosis* pathogenesis occurs in two stages. The first stage is an asymptomatic state that can persist for many years in the host. The second stage can show the immediate effect in the host, then the bacteria begins replicating and causing characteristic symptoms such as low fever, cough, fatigue and unexpected weight loss. If this infection is untreated, the disease eventually culminates in death. Over 50% of death among Human Immunodeficiency Virus (HIV) infected patients result from co-infection with *Mycobacterium tuberculosis.* This is due to the loss of immune system in the HIV infected patient. (Cole and Alzari. 2007) consequently, this loss of immune system activates the TB bacteria in turn causing death of tissue in the infected organs. The schematic representation of mechanism of action on bacteria is presented in **Figure 1.3.**

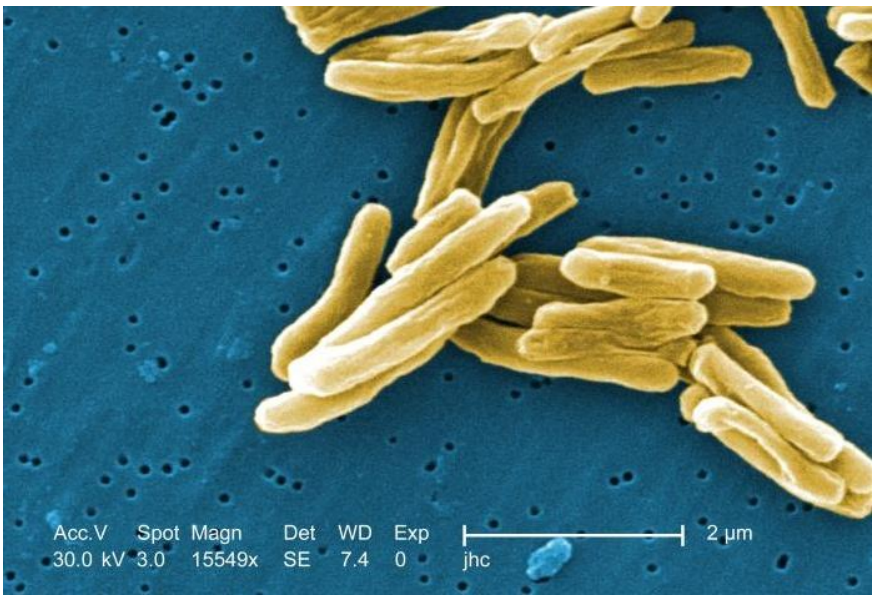


Figure 1.2 Scanning electron micrograph of *M. tuberculosis*

(Source: www.phil.cdc.gov)

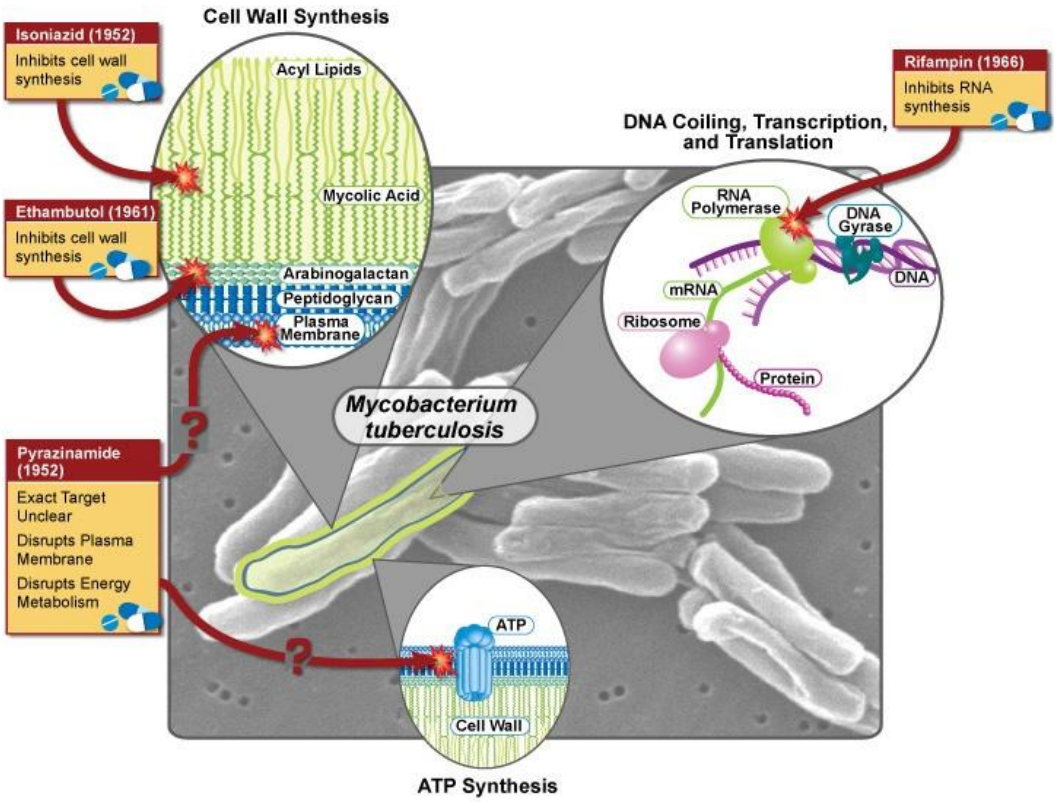


Figure 1.3 Schematic representation of mechanism of action on bacterial cell.

(Source: www.commons.wikimedia.org)

Mycobacterium tuberculosis is one of most dangerous pathogenic bacteria causing tuberculosis (TB). Tuberculosis is one of the rampant diseases worldwide. It causes sickness in more than 9.5 million people and is responsible for death of about 2 million people throughout the world every year as per the WHO report (Global tuberculosis report 2015). It is an aerobic disease and the sneeze and cough by the active TB person can affect and infect the hosts with the bacteria. More TB cases are in latent stage due to human immune system. It gets activated when the person loses their immune system due to HIV or other diseases (Cole and Alzari 2007). This is the reason, about 50 % of people die among HIV-AIDS infected patients. The development of anti-TB drugs are challengeable due to their mode of action and target site. The treatment of TB is complicated by the tendency for its etiological agent, predominantly *Mycobacterium tuberculosis*, to adopt a non-replicating persistent state (Wayne and Sohaskey 2001). It directly infects the lungs and splits every 15-20 hours. Moreover, it is 40-50 times slower than the other bacteria and its impact is not restricted only on lungs. It affects other parts of the body like blood, respiratory system and other diseases. Present treatment of tuberculosis is most inconvenient and involves multiple drugs in the treatment. Overall it takes an approximate span of 6-9 months for chemotherapy. Treatment followed as, initial two months with Isoniazid (INZ), Rifampicin (RIF), Pyrazinamide (PZA) and Ethambutol (EMB) followed by a continuation of treatment for four months with INZ and RIF (Dover and Coxon 2011). Due to its prolonged treatment, patients are suffering from many side effects such as gastro-intestinal (GI) problems, hepatoxicity and acute toxicity. Some of the available TB drugs are mentioned in **Figure 1.4** and some of the side effects are mentioned in **Table 1.2**.

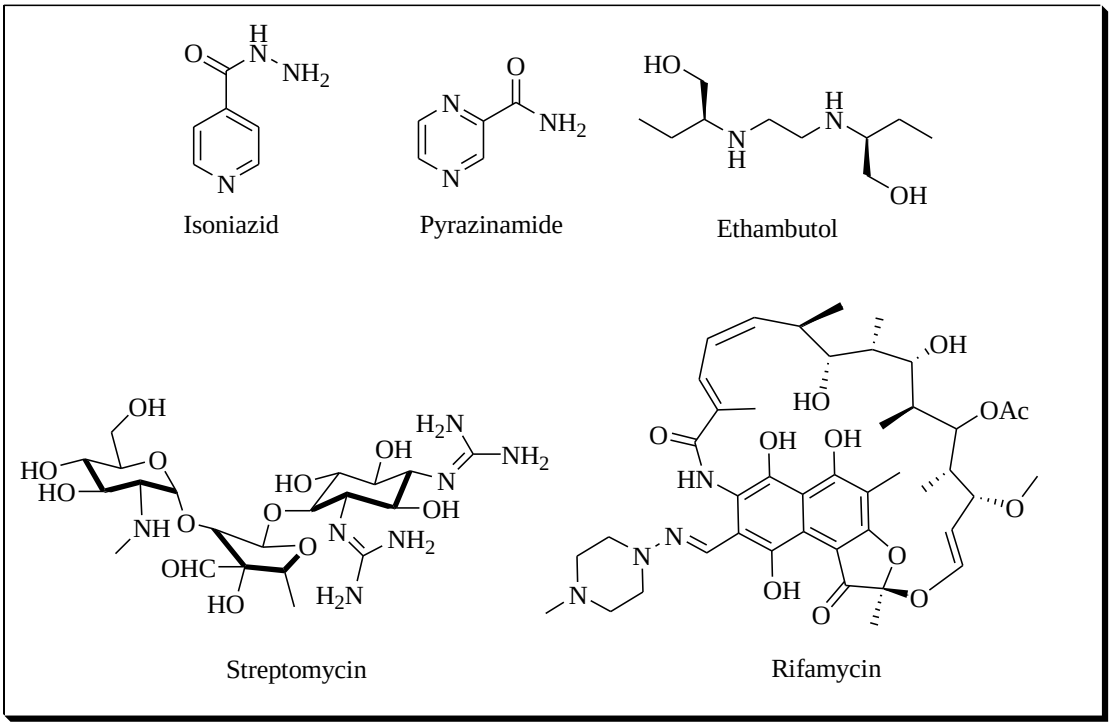


Figure 1.4 Some of the commercially available antitubercular drugs

Table 1.2 Side effects with available commercial TB drugs.

Drug name	**Sign and symptoms**
Pyrazinamide	Pain in large and small joints.
Isoniazid	Rashes, mild central nervous system effects, hepatitis, abnormal liver functions etc.
Ethambutol	Headache, stomach upset, or nausea/vomiting (symptoms of liver disease), vision change, weakness, severe stomach/ abdominal pain.
Rifampicin	Liver disease, stomach upset, heartburn, nausea, headache and dizziness.
Streptomycin	Ear damage, kidney damage.

1.5.3.2 *Staphylococcus aureus* (*S. aureus*)

Staphylococcus aureus is a Gram +ve coccal bacterium that is a member of the Firmicutes. It is frequently found in the human respiratory tract, nose and on the skin. Although, *S. aureus* is not always pathogenic, it is a common cause of skin infections (e.g., boils), respiratory diseases (e.g., sinusitis) and food poisoning. Commonly, pencillin is used for the treatment of *S. aureus* infection in most of the countries. However, penicillin resistance is extremely common, and first-line therapy is most commonly a penicillinase-resistant β-lactam antibiotic (e.g., Oxacillin or

Flucloxacillin). The emergence of antibiotic-resistant strains of *S. aureus* such as methicillin-resistant *S. aureus* (MRSA) is a global problem in medicinal chemistry.

1.5.3.3 *Mycobacterium smegmatis (M. smegmatis)*

Mycobacterium smegmatis is an acid-fast bacterial species in the phylum *Actinobateria* and the genus *Mycobacterium*. *M. smegmatis* is generally considered a non-pathogenic microorganism. However, in some very rare cases, it may lead to diseases. It is 3.0 to 5.0 µm long with a bacillus shape and can be stained by Ziehl-Neelsen method and the auramine-rhodamine fluorescent method. *M. smegmatis* is useful for the research analysis of other Mycobacteria species in laboratory experiments. *M. smegmatis* is commonly used in work on the mycobacterium species due to its being a "fast grower" and non-pathogenic. *M. smegmatis* is a simple model that is easy to work with, i.e., with a fast doubling time and only requires a bio-safety level-1 laboratory. The time and heavy infrastructure needed to work with pathogenic species prompted researchers to use *M. smegmatis* as a model for mycobacterial species. This species shares more than 2000 homologous genes with *M. tuberculosis* and shares the same peculiar cell wall structure of *M. tuberculosis* and other mycobacterial species. It is also capable of oxidizing carbon monoxide aerobically, as is the case with *M. tuberculosis.*

1.5.3.4 *Candida albicans (C. albicans)*

Candida albicans is a dimorohic fungus that grows both as yeast and filamentous cell. Systemic fungal infections including those by *C. albicans* have emerged as important causes of morbidity and mortality in immune compromised patients (e.g., AIDS, cancer chemotherapy and organ or bone marrow transplantation). It commonly occurs on mucous membranes in the mouth or vagina, but may affect a number of other regions. For example, higher prevalence of colonization of *C. albicans* was reported in young individuals with tongue piercing, in comparison to unpierced matched individuals. It then becomes suppressed by antibiotics. The infection is prolonged when the original sensitive strain is replaced by the antibiotic-resistant strain. Treatment of *C. albicans* is carried out with Nystatin, Amphotericin B, Fluconazole for systemic infections and Clotrimazole for skin infections.

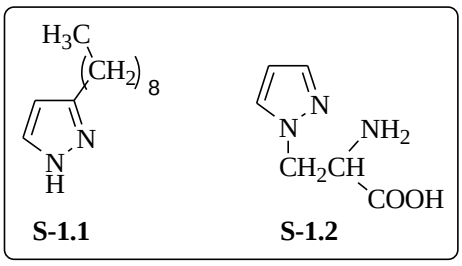
1.5.3.5 *Penicillium chrysogenum (P. chrysogenum)*

Penicillium chrysogenum is a species of fungus in the family of Trichocomaceae. It is commonly found in temperate and subtropical regions and can be found on salted food products, but it is more prevalent in indoor environments, especially in damp or water-damaged buildings. It was previously known as *Penicillium notatum*. It has rarely been reported as a cause of human disease. It is the source of several *β*-lactam antibiotics, most significantly Penicillin-C, Meleagrin, Chrysogine and Sorbicillin.

1.6 LITERATURE REVIEW

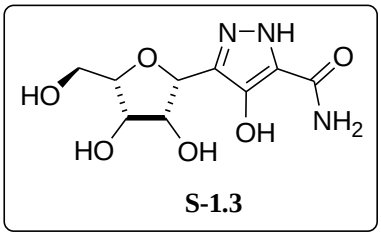
Pyrazole and its derivatives have been well known in pharmaceutical chemistry because of their wide spectrum of biological activities. In the following paragraphs carrying the literature review, the importance of pyrazole and its varied therapeutic uses have been explained.

Naturally occurring pyrazoles were isolated after 1950's. The first natural pyrazole, 3-*n*-nonylpyrazole (**S-1.1**), was obtained from a plant of the *piperaceae* family namely *Houttuynia Cordata.*This was obtained from tropical Asia and it showed antimicrobial activity. The other natural pyrazole derivative, *levo*-β-(1-pyrazolyl) alanine (**S-1.2**), was isolated from watermelon seeds (*Citrullus Vulgaris*) by Japanese researchers.



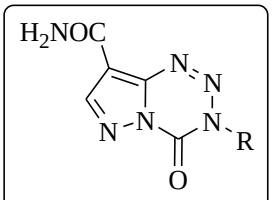
S-1.1 **S-1.2**

An azole class of C-nucleoside, 4-hydro-3-β-D-ribofuranosylpyrazole-5-carboxmide or pyrazofuran (**S-1.3**) was isolated by Buchanan *et al.* (1981) from the fermentation broth of *Streptomyces candidus.* This antibiotic has antiviral activity and also showed very good cytotoxicity.



S-1.3

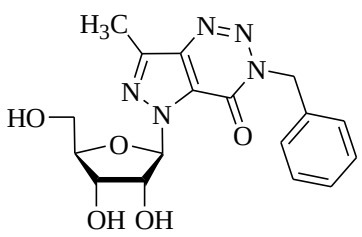
Cheng *et al.* (1986) have synthesized 3-(2-chloroethyl)-3,4-dihydro-4-oxopyrazolo[5,1-*d*]-1,2,3,5-tetrazine-8-carboxamide derivatives for their antineoplastic activity. Structure activity relationship explained that compound (**S-1.4**) showed excellent antineoplastic activity due to the presence of 2-chloroethyl functional group.



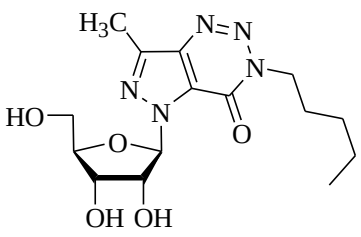
R= CH_2CH_2Cl, CH_3

S-1.4

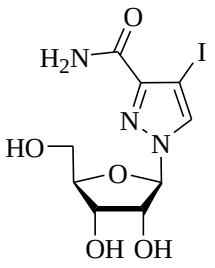
Manfredini *et al.* (1992) have synthesized several pyrazol-[4,3-*d*]-1,2,3-triazin-4-one ribonucleoside derivatives and performed for their *in vitro* antiviral and cytostatic activities. Majority of the synthesized compounds were devoid of any activity except compounds (**S-1.5** and **S-1.6**) have showed moderate cytostatic activity against T-cells. Compound (**S-1.7**) showed a selective activity against coxsackie B1.



S-1.5

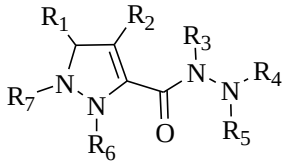


S-1.6



S-1.7

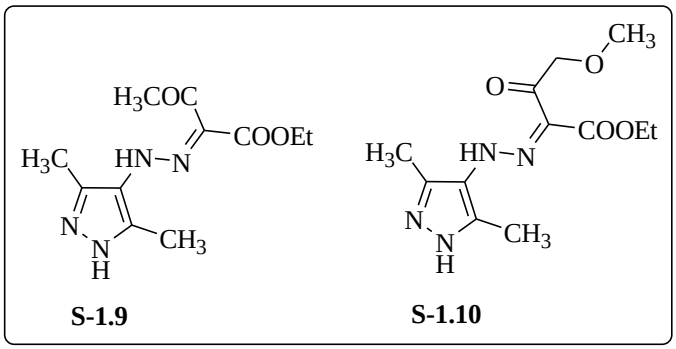
A series of pyrazole carboxylic acid hydrazide derivatives (**S-1.8**) were synthesized and screened for anti-inflammatory activity by Anshu and Madan (1995). All tested compounds showed very good activity against lipoxygenase enzyme.



R_1= R_2= R_3= H, Me
R_4= H, Me, *i*-Pr, Ph, 2,6-$Cl_2C_6H_3$,
R_5= H, Me, *i*-Pr, *t*-Bu, 2-ClPh, 4-ClPh, 4-tolyl, Ac, CHO
R_6= Me, -CH_2Ph, 2,6-$Cl_2C_6H_3$
R_7= H, Me, -CH_2Ph, COEt, Ac

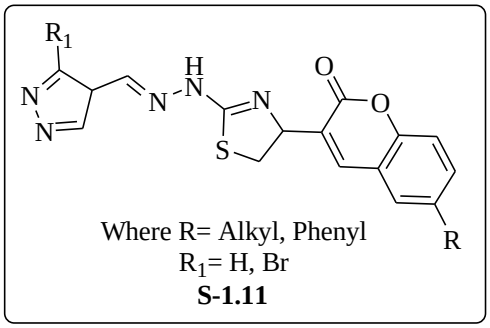
S-1.8

Kaymakcioglu and Rollas. (2002) developed potent pyrazole hydrazone derivatives and tested them against *Mycobacterium tuberculosis*. Among all the synthesized compounds, (**S-1.9** and **S-1.10**) showed 29 % and 28 % inhibition respectively for antitubercular activity.



S-1.9 **S-1.10**

A series of 4-(coumarino)-2-(4-arylhydrazono-3-methyl-5-oxo-2-pyrazolin-1-yl)thiazoles (**S-1.11**) were prepared by Hantszch condensation of 1-thiocarbamoyl-3-methyl-4-(arylhydrazono)-2-pyrazolin-5-one with 6-substituted-3-bromoacetyl coumarins by Kalluraya *et al.* (2004). Target compounds were tested for their antibacterial, antifungal, analgesic, anti-inflammatory and anthelmintic activity. Most of them showed significant antibacterial activity.

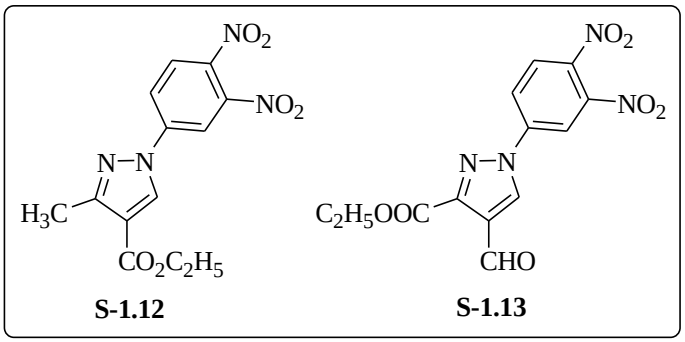


Where R= Alkyl, Phenyl
R_1= H, Br

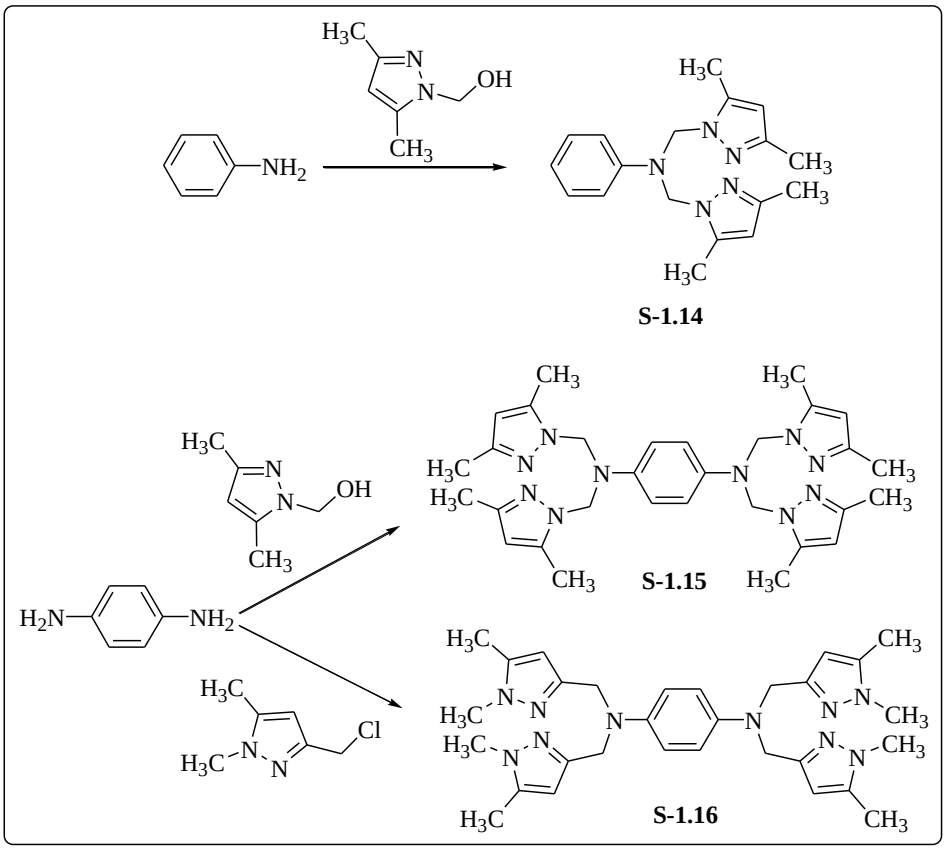
S-1.11

Sridhar *et al.* (2004) prepared a new 1*H*-pyrazole derivatives for their antimicrobial activities. Antibacterial activity tested against *Escherichia coli*, *Pseudomonas aeuroginosa*, *Enterobacter facecalis* and *Staphylococcus aureus.* Antifungal activity tested against *Alernarnia alternate*, *Bipolaris oryzae Curuvularia*

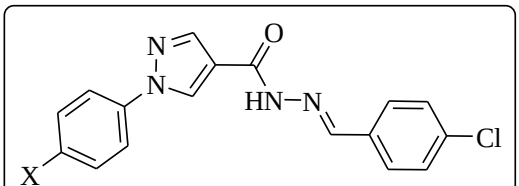
lunata, Fusaricom oxysperum and *Rhizochonia solani.* All compounds showed significant activity. Among all, compounds (**S-1.12** and **S-1.13**) showed good antibacterial and antifungal activity against tested microorganisms.



Three tripod pyrazole derivatives have been prepared by Bouabdallah *et al.* (2006). These derivatives were tested for their anticancer studies on two cell line murin mastocytoma (P815) and human laryngeal carcinome (Hep). Compound (**S-1.14** and **S-1.15**) showed promising cytotoxicity with IC_{50} 17.82 against P815 and IC_{50} 3.25 against Hep cell lines. More importantly, cytotoxicity of compound (**S-1.16**) against Hep cell lines is potent than standard Adriamycin.



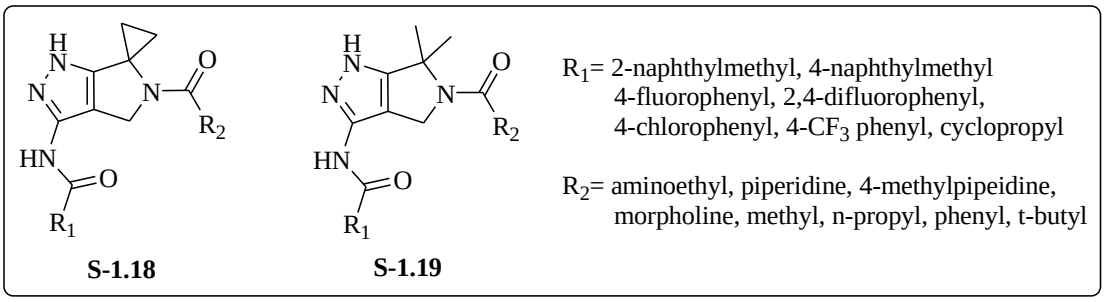
1-Substituted phenyl-N'-[(substituted phenyl)methylene]-1*H*-pyrazole-4-carbo-hydrazides derivatives (**S-1.17**) were synthesized and tested for Leishmanicidal and cytotoxic activity by Bernardino *et al.* (2006). Compounds with -NO_2 and -Cl substituted phenyl on pyrazole-1*H* enhance the activity and showed more potent on *L. chagasi* and *L.braziliensis* species.



X= NO_2, Cl

S-1.17

Brasca *et al.* (2007) reported a new class of tetrahydropyrrolo[3,4-*c*]pyrazole scaffolds (**S-1.18** and **S-1.19**) for CDK2/cyclin-A inhibitor. Most of the compounds exhibited potent CDK2/cyclin-A inhibitory activity. Additionally, majority of the compounds showed anticancer activity in the A2780 assay.

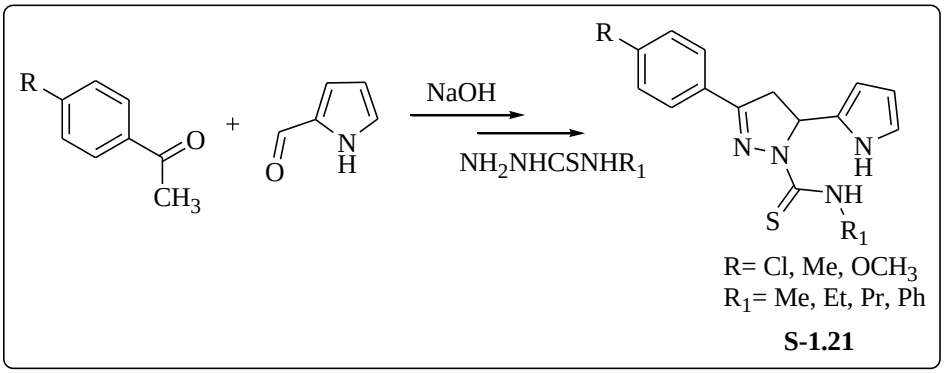


S-1.18 **S-1.19**

R_1= 2-naphthylmethyl, 4-naphthylmethyl
4-fluorophenyl, 2,4-difluorophenyl,
4-chlorophenyl, 4-CF_3 phenyl, cyclopropyl

R_2= aminoethyl, piperidine, 4-methylpipeidine,
morpholine, methyl, n-propyl, phenyl, t-butyl

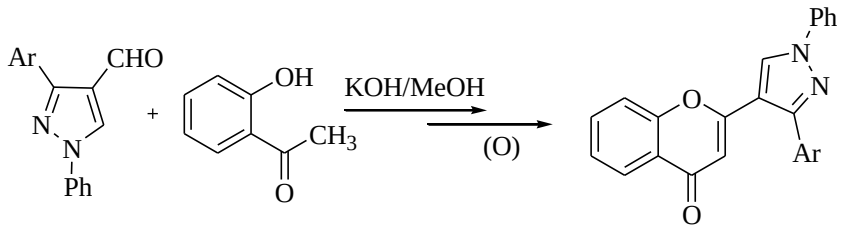
Gokhan-Kelekci *et al.* (2007) synthesized some new pyrazole derivatives (**S-1.20**) for their Alzheimer disease. Most of the synthesized compounds showed monoamine oxidase-B (MAO-B) inhibitors and anti-inflammatory agents. In addition, analgesic and ulcerogenic activities were also studied and showed promising equipotent ulcerogenic acitivity with standard Indomethacin.



R= Cl, Me, OCH_3
R_1= Me, Et, Pr, Ph

S-1.21

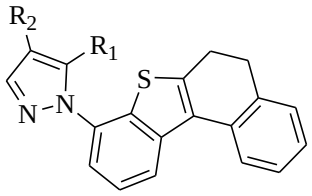
Prakash et al (2008) synthesized 2-(3-aryl-1-phenyl-1*H*-pyrazol-4-yl)-4*H*-chromen-4-ones from the reaction of pyrazole-4-carbaldehyde with 2-hydroxyacetophenone. All target compounds (**S-1.22**) were tested for their antifungal activity and consequently found to be a potent antifungal activity.



S-1.22

Ar= Ph, 4-tolyl, 4-anisyl, 4-ClC_6H_4, 4-BrC_6H_4, 4-$NO_2C_6H_4$, 4-FC_6H_4

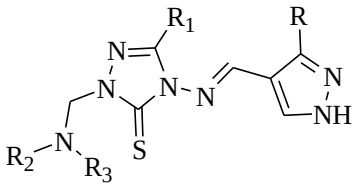
Rashad *et al.* (2008) developed and synthesized a new series of pyrazole derivatives and evaluated for their antiviral activity. Newly synthesized compounds (**S-1.23**) showed excellent antiviral activity against type-1 Herpes Simplex virus and hepatitis-A virus using plaque infective assay method.



S-1.23

R_1 = CN, R_2 = NH_2
R_1 = CN, R_2 = NHCSNHCOPh
R_1 = NH_2, R_2 = $COOC_2H_5$

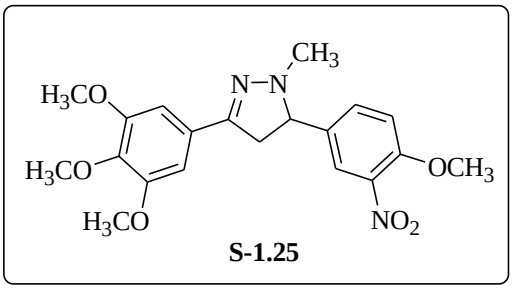
Isloor *et al.* (2009) developed a new series of pyrazole containing Schiff and Mannich bases (**S-1.24**). All target compounds were evaluated for antimicrobial activity. Few of the synthesized compounds were found to exhibit significant antimicrobial activity.



R= CH_3, C_6H_5, 4-ClC_6H_4, 4-$OMeC_6H_4$
R_1= CH_3, C_3H_7, C_6H_5
NR_2R_3 = N-methylpiperizine.

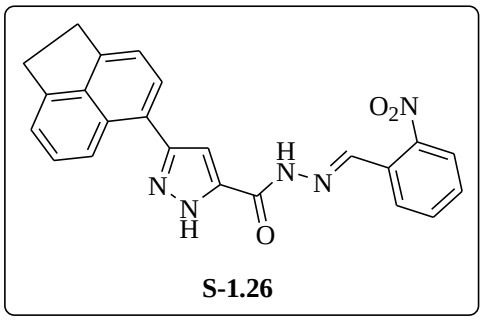
S-1.24

New pyrazole derivatives have been developed and synthesized for potential activity of ACE (Angiotensin I-Converting Enzyme) inhibitors by Bonesi *et al.*, (2010). All synthesized derivatives were confirmed by various spectral techniques. Among all synthesized compounds, chalcone derivative (**S-1.25**) exhibited excellent activity with IC_{50} value 0.123 mM.



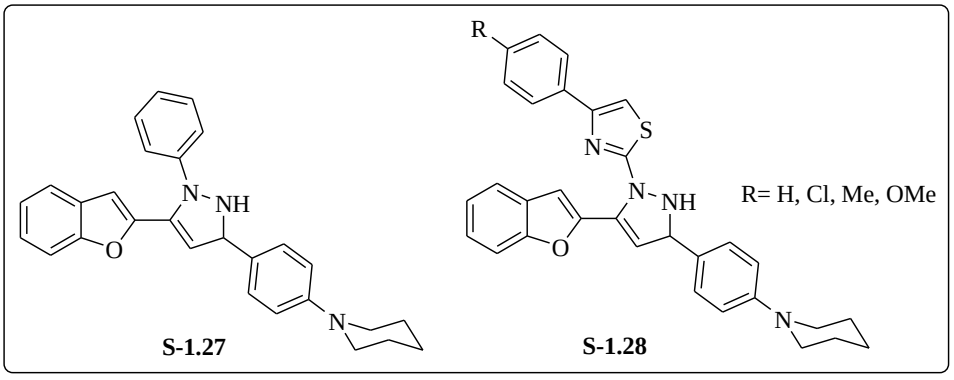
S-1.25

Shih *et al.* (2010) reported pyrazole based influenza agent as well as antiviral activity. Compound (**S-1.26**) showed both activities in sub-micromolar level and this moiety becomes a good influenza and antiviral agent in the future.



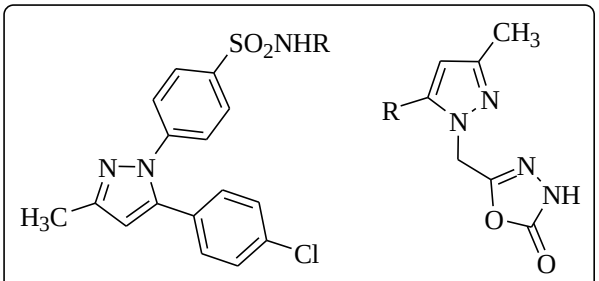
S-1.26

Hamad et al (2012) synthesized novel pyrazole-1-carbothioamide and pyrazole piperidine derivatives. Target compounds were evaluated for *in vitro* antibacterial and anti-inflammatory activity. Compounds (**S-1.27** and **S-1.28**) showed more than 70 % inhibition and these compounds may be promising anti-inflammatory agents in the future.



S-1.27 **S-1.28**

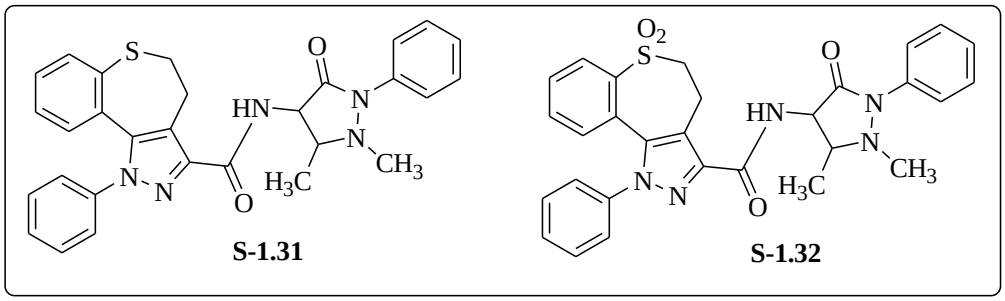
A novel series of pyrazole bearing benzenesulfinamide (**S-1.29**) and 1,3,4-oxadiazole (**S-1.30**) derivatives were synthesized by El-Moghazy *et al.* (2012). All target compounds were tested for *in vivo* anti-inflammatory activity by acute carrageenan-induced paw edema method. The active compounds (61.12-62.67 % inhibition of edema) were tested for ulcerogenic liability in rats and proved to be safer than standard Indomethacin.



S-1.29 **S-1.30**

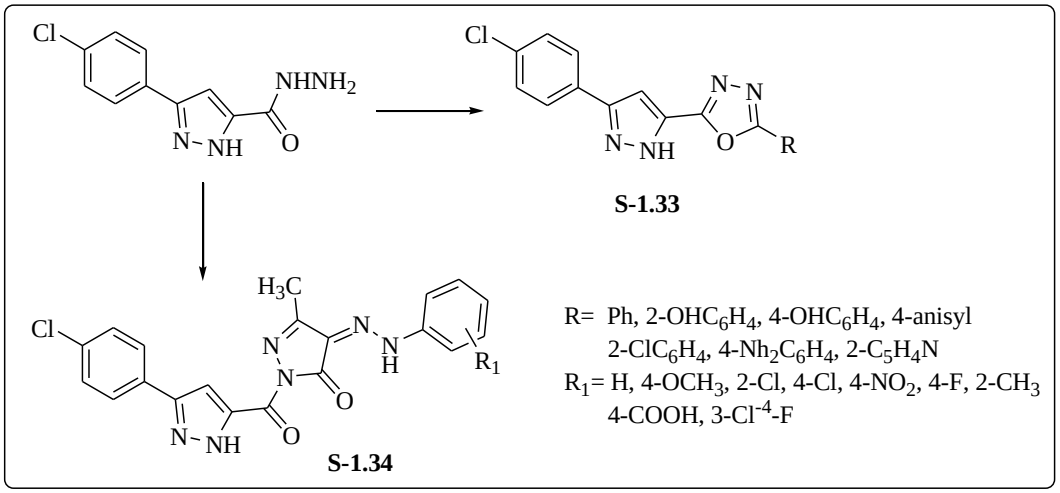
R= H, thiazolyl, Ph, Me, 4-chlorophenyl

Palanisamy and Kumaresan (2013) developed 4,5-dihydro-1*H*-[1]benzothiepino[5,4-*c*]pyrazole derivatives and tested for antimicrobial, antitubercular and antitumoral activity. Compounds (**S-1.31** and **S-1.32**) showed significant activity against *M. tuberculosis* (8.2 and 7.8 µM) and also showed highest antitumor activity of IC_{50} value 18 and 12 µM respectively.

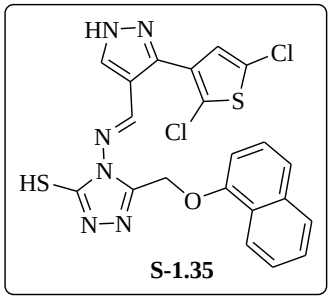


S-1.31 **S-1.32**

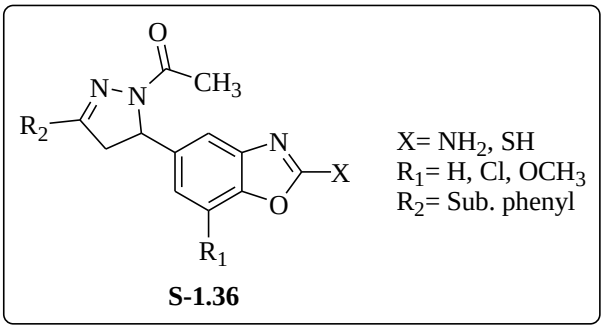
Horrocks *et al.* (2013) developed 1,3,4-oxadiazole (**S-1.33**) and 5-pyrazolinones (**S-1.34**) linked pyrazole as core moiety and tested for their *in vitro* antitubercular and antifungal activity. All the compounds showed excellent antitubercular activity against *M. tuberculosis* H_{37}Rv strain. The substitutions (-Cl and $-NO_2$) on oxadiazole increased the biological potency and hydroxyl substitution on phenyl ring showed poor antifungal activity.



Vijesh *et al.* (2013) synthesized a new series of 1,2,4-triazole and benzoxazole containing pyrazole derivatives. These derivatives found to be potent as antimicrobial and analgesic activity. Compound (**S-1.35**) showed significant analgesic activity and antimicrobial activity due to 2,5-dichlorothiophene substitution on pyrazole ring.

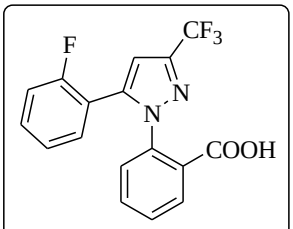


Two well-known pharmacophores pyrazole and benzoxazoles (**S-1.36**) were combined by Rana *et al.* (2014) and tested for their *in vitro* antitubercular screening against *Mycobacterium tuberculosis* $H_{37}Rv$. All synthesized compounds showed significant activity ranging between MIC value 0.625-25 µg/mL. Few compounds were found to be more active than tested standard anti-TB drug.



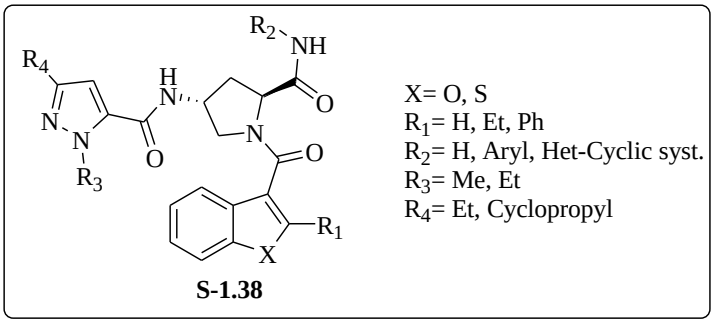
5-(2-Fluorophenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl] acid derivatives were synthesized from 2-fluoroacetophenone by Manojkumar *et al.* (2014). All compounds

have been screened for antibacterial activity with four bacterial strains using cup-plate method. Similarly, anthelmintic activity has been performed against *P. posthuma* and anti-inflammatory activity carried on carrageenan induced paw edema method. Compound (**S-1.37**) exhibited excellent antibacterial, anthelmintic and anti-inflammatory activity.



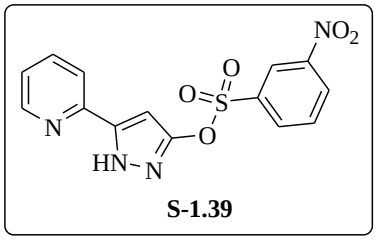
S-1.37

Benzofuran pyrrolidine pyrazole derivatives (**S-1.38**) were synthesized and proved as highly potent antitubercular agents by Kamsri *et al.* (2015). These derivatives are potent InhA inhibitors with IC_{50} values at nanomolar levels and led to the conclusion that core structure of these compounds are the key portion for binding in the InhA inhibitor.



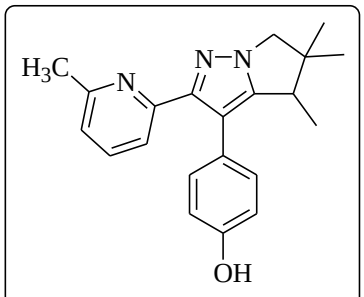
S-1.38

Pyrazole based pyridine-sulfonate derivatives were designed and synthesized for anti-HBV activity by Chuang *et al.* (2016). Structure activity relationship were established in HepG 2 2.2.15 cells. They found inhibition of HBV gene expression and viral DNA replication. Among all the compounds, compound (**S-1.39**) showed potent inhibitory activity with IC_{50} value of 9.19 µM and higher selectivity index value of 35.46.



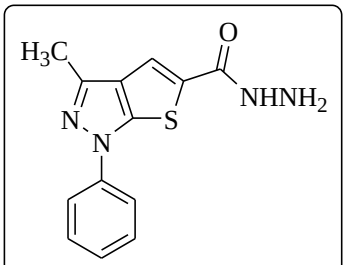
S-1.39

Eva *et al.* (2017) synthesized a series of 2,3,4-substituted 5,5-dimethyl-5,6-dihydro-4H-pyrrolo[1,2-b]pyrazoles (**S-1.40**) (DPPs) and evaluated for their ALK5 inhibition activity. The most potent compounds displayed submicromolar IC_{50} values for ALK5. In cells, the compounds caused dose-dependent dephosphorylation of SMAD2, a well-established substrate of ALK5. In addition, the compounds blocked translocation of SMAD2/3 to nuclei of cells stimulated with TGFβ and the protein remained predominantly in cytoplasm, further confirming their molecular target. Therefore, novel DPP derivatives proved to be active as ALK5 inhibitors.



S-1.40

Novel pyrazole containing hydrazone derivatives were synthesized by Pravin *et al.* (2017) and tested for their *in-vitro* antimicrobial activity. Some of the compounds (**S-1.41)** were more active against tested bacterial strain *V. cholera.*



S-1.41

1.7 IMPORTANT STRUCTURAL FEATURES OF PYRAZOLE DERIVATIVES

On the basis of literature review, the structure activity relationship of compounds with the structure represented in **Figure 1.5** indicated that the core pyrazole moiety is responsible for the activity of the compound. The structure activity relationship study was based on (i) a pyrazole ring, (ii) a linkage moiety (iii) hydrophobic moiety. Replacement of the pyrazole ring by either a naphthyl or a

phenyl ring resulted in the loss of activity. The activity of the compounds is also affected by the distance between the hydrophobic moiety and the basic nitrogen.

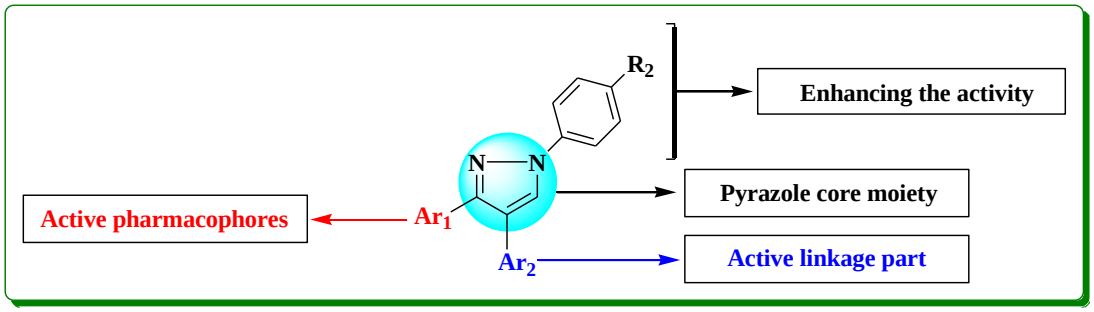


Figure 1.5 Structural features of pyrazole derivatives

1.8 SCOPE OF THE PRESENT WORK

The discovery and development of antimicrobial, also called "miracle drugs", has been significant leap/ breakout in the history of modern medicine. However, these magic bullets are losing their efficacy due to the development of antimicrobial resistance. Today, the infections caused by multi-drug resistant organisms have a higher morbidity and mortality, and the treatment is longer and more expensive.

Currently, a limited number of antibiotics are available to treat multidrug-resistant strains of infectious bacteria. In the drug discovery field, new antibiotics were discovered incessantly, but most of these agents failed to thrive in the commercial market owing to their side effects and toxicity. Thus, this justifies an urgent and continuous need for exploration and development of economical and more effective drug with higher bioactive potential and reduced side effects.

The greatest reward of the heterocyclic chemistry is to develop new pharmaceutically active and efficient compounds. Each step of the synthesis involves a chemical reaction; moreover the reagents and conditions need to be designed to give a good yield and pure compounds. The discovery of new methods and reagents grab the attention of chemists across the world. Optimization is where one or two starting materials are tested in the reaction under a wide variety of conditions of temperature, solvent, reaction time, and others, until the optimum conditions of the reaction of product, yield and purity are found.

Heterocyclic compounds, by virtue of their specific activity, could be employed in the treatment of infectious diseases. Review of literature indicated that,

pyrazole and its derivatives found a significant place in the development of pharmacologically important molecules. Keeping in view of these observations, it was planned to synthesize some new bio-active compounds carrying pyrazole moiety. Thus, in this research work, we are presenting the synthesis and preliminary antitubercular and antimicrobial evaluation of pyrazole analogues.

Tuberculosis (TB) is one of the dominant killer diseases and it causes huge number of human deaths in spite of the availability of more than 10 anti-TB drugs and the Bacille Calmette Guerin (BCG) anti-TB vaccine. The emergence of the extensively drug-resistant tuberculosis (XDR-TB) and multidrug-resistant tuberculosis (MDR-TB), against which the traditional anti-TB drugs showed limited efficacy, further caused serious problem in controlling TB. According to the WHO global Tuberculosis report 2014, globally 3.5 % of new and 20.5 % of previously treated TB cases were estimated to be multidrug-resistant. In view of this, a great deal of research work is being devoted to identify newer molecular entities which are active against the bacterial strains. Moreover, some of the currently available drugs have been shown to exhibit some side effects and toxicity.

The complexity and toxicity of the current TB drug regimes need an immediate attention. Similarly, the major problem of TB drug resistance demand immediate action. Therefore, new TB drugs are needed. However, the new TB drugs has to provide the users with the following,

a) Simpler and shorter, multi drug regimes for drug sensitive TB,
b) Shorter, more effective, less toxic and less expensive agents for drug resistant TB,
c) Short, simple, easily tolerable and safe regimes for latent TB,
d) Drugs with few drug interactions, so they can be safely provided to people with HIV (Ginsberg, 2010).

Therefore novel and effective drugs are the immediate need of present days to treatment of tuberculosis disease.

Based on the literature reports on the promising bactericidal activity exhibited by pyrazole system, it has been planned to design and synthesize new molecules with pyrazole containing various pharmacophores. The present work involves synthesis, characterization and biological studies of new derivatives of pyrazole carrying

thiazole, pyrazoline, 1,4-dihydropyridine, 1,3,4-oxadaizole, [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole, benzimidazole, trifluoromethylbenzyloxy derivatives.

1.9 OBJECTIVES OF THE PRESENT WORK

The main objectives of the research work are as follows:

- Design and synthesis of new pyrazole containing molecules based on the literature reports on structure activity relationship (SAR).
- Development of suitable purification methods like column chromatography and recrystallization techniques for the new pyrazole derivatives.
- Characterization of the newly synthesized molecules using different spectroscopic methods such as FT-IR, ^{1}H-NMR,^{13}C-NMR, Mass spectroscopy, elemental analysis and S-XRD crystallographic studies for selected compounds.
- *In vitro* antitubercular activity of the newly synthesized molecules. In addition, antibacterial and antifungal studies against various microorganisms.
- *In vitro* cytotoxicity studies of the active molecules against a normal cell line and to carry out the SAR studies to enhance the efficacy of the molecules.

The thesis comprises of seven chapters.

Chapter 1 deals with a brief introduction to pyrazole chemistry and its importance in the pharmaceutical division. A thorough literature survey on pyrazole based small bioactive molecules justifying the present research work is also included. On the basis of literature review, the scope and objectives of the current research work are drawn in this chapter.

In **chapter 2**, synthesis, characterization and antitubercular studies of some thiazole derivatives ($\mathbf{T_{1\text{-}12}}$) are presented. The detailed experimental procedures and analytical characterization data for intermediates and final compounds are presented. This chapter includes/presents a detailed antitubercular activity In addition, antimicrobial activity of the compounds against two bacterial and one fungal strain is discussed.

In **chapter 3**, synthesis, characterization and antitubercular studies of some 1'-(4-chlorophenyl) pyrazole containing 3,5-disubstituted pyrazoline derivatives **($\mathbf{T_{13\text{-}27}}$)**

are discussed. The *in vitro* cytotoxicity studies were performed for the active compounds and are included in this chapter.

In **chapter 4**, synthesis, characterization and antitubercular studies of some 4-chlorophenyl substituted pyrazole containing 1,4-dihydropyridine derivatives **(T_{28-45})** is presented. The chapter explains the *in vitro* antitubercular activity of the molecules against *Mycobacterium tuberculosis*.

C**hapter 5** presents the design, synthesis and characterization of a new series of pyrazole linked [1,3,4]oxadiazole-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole analogs **(T_{46-63})**. The details of the antitubercular, cytotoxicity studies are discussed in this chapter. In addition, antimicrobial activity of the compounds against two bacterial and two fungal strains are tested and discussed.

Similarly, in **Chapter 6**, synthesis, characterization and biological activity of some new pyrazole linked benzimidazoles and 2-chloro-6-trifluoromethyl benzyloxymethyl derivatives (**T_{64-97}**) are presented. The details of the antitubercular, antimicrobial and cytotoxicity studies are discussed.

Towards the end, **Chapter 7** summarizes the conclusions of present research work has been discussed in this chapter.

CHAPTER 2

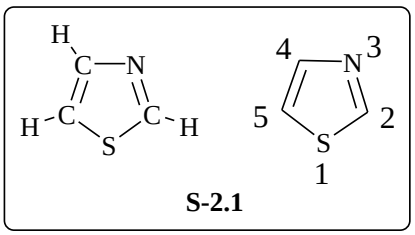
1,3,4-TRISUBSTITUTED PYRAZOLE BEARING 4-(CHROMEN-2-ONE) THIAZOLE: SYNTHESIS, CHARACTERIZATION AND ITS BIOLOGICAL STUDIES

Abstract

This chapter describes a detailed literature survey on 1,3-thiazole and its derivatives until August 2016. It includes the synthesis and biological importance of few of the previously reported 1,3-thiazole derivatives. The target pyrazole bearing thiazole derivatives were synthesized from 1,3-disubstituted-1H-pyrazole-4-carbaldehyde treated with thiosemicarbazide to give thiosemicarbazone intermediates. Which on reacting with 3-(bromoacetyl)-2H-chromen-2-one in ethanol media under reflux temperature to give 3-{2-[N′-(1,3-disubstituted-1H-pyrazol-4-yl-methylene)-hydrazino]-thiazol-4-yl}-chromen-2-one. Further, the synthesized thiazoles were characterized and screened for antitubercular, antibacterial and antifungal activities.

2.1 INTRODUCTION

Thiazole, or 1,3-thiazole is a heterocyclic compound that contains both sulfur and nitrogen had a large family of derivatives in medicinal chemistry. Thiazole is clear to pale yellow liquid with a pyridine-like odor and the molecular formula C_3H_3NS. Many of the natural products are available with thiazole as the core structure. The thiazole ring is notable as a component of the vitamin thiamine (B_1).

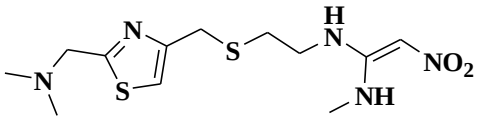
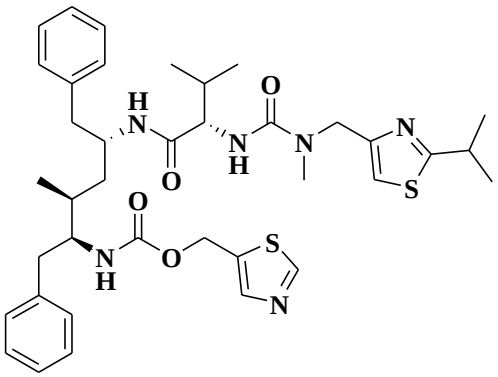
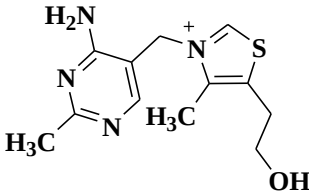
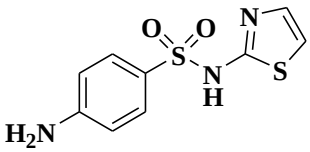
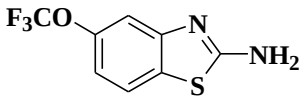


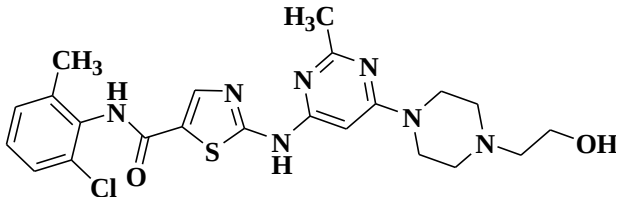
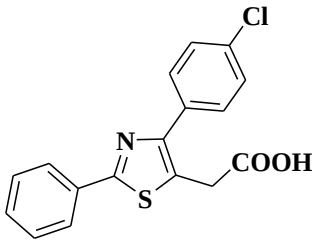
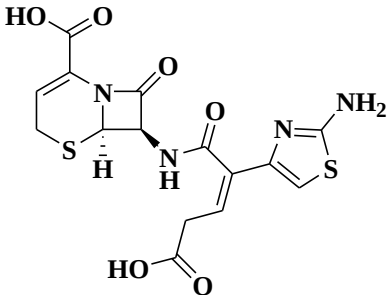
S-2.1

Thiazoles are members of the azoles that includes imidazoles, triazole and oxazoles. Thiazole can also be considered as a functional group. The interesting pharmacological properties of thiazole derivatives in relation to the various changes in the structure of these compounds are worth studying in order to synthesize high effective drug without any side effects. Commercial significant applications of thiazoles include pharmaceutical field, dyes and pesticides. Thifluzamide, Tricyclazole, and Thiabendazole are the commercially available as pesticides. Another widely used thiazole derivative is the non-steroidal anti-inflammatory drug Meloxicam. In recent years, thiazoles and their derivatives have attracted medicinal

chemists because of their varied biological activities such as, for the treatment of allergies (Hargrave *et al.* 1983), schizophrenia (Jaen *et al.* 1990), hypertension (Patt *et al.* 1992 and Bondock *et al.* 2007), HIV infections (Bell *et al.* 1995), fibrinogen receptor antagonists with antithrombotic activity (Badorc *et al.* 1997), inflammation (Sharma *et al.* 1998), hypnotics (Ergenc *et al.* 1999) and as new inhibitors of bacterial DNA gyrase B (Rudolph *et al.* 2001). Some of the commercially available thiazole containing drugs has been presented in **Table 2.1**.

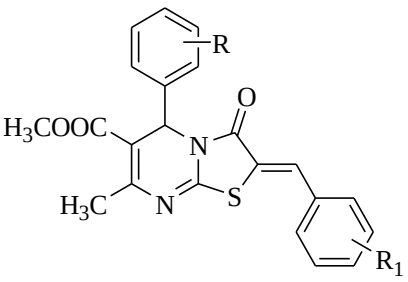
Table 2.1 Commercially available thiazole containing drugs

Drug name	Structure	Therapeutic use
Nizatidine		Peptic ulcer disease
Ritonavir		Antiviral
Thiamin		Vitamin B_1
Sulfathiazole		Antimicrobial
Riluzole		Anticonvulsant

Dasatinib		Tyrosine kinase inhibitor
Fentiazac		Anti-inflammatory
Ceftibuten		Antibiotic

Although, there are many new class of drugs with less toxicity as antimicrobial agents, their clinical efficacy in some invasive fungal infections, is not optimal (Vincent. 1999). In recent years, the widespread use of antimicrobial agents has resulted in the development of resistance to these drugs by pathogenic microorganisms, causing an increase in morbidity and mortality (Chandrakantha *et al.* 2010). Thus, an intense effort in antimicrobial drug discovery is still needed to develop more promising and effective active agents for use in the clinical arena. Keeping in view of this, it was planned to synthesis pyrazole bearing 4-(chromen-2-one) thiazole derivatives. The importance of thiazole derivatives on the earlier publications has been presented at below.

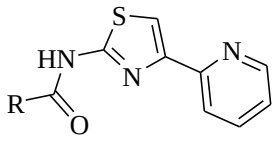
Birsen *et al.* (1999) synthesized a new series of 2-benzylidene-7-methyl-3-oxo-5-(substituted phenyl)-2,3-dihydro-5*H*-thiazolo[3,2-a]pyrimidine-6-carboxylic acid methyl esters from 1,2,3,4-tetrahydropyrimidine-2-thiones. All newly synthesized compounds (**S-2.2**) were confirmed by NMR and mass spectrometry and were tested for their anti-inflammatory activity. Some of the compounds were active at 100 mg/kg dose level when compared with Indomethacin.



R= 4-Br, 4-OCH_3, 4-CH_3, 2-F
R_1= H, 4-CH_3, 4-OCH_3, 4-Cl

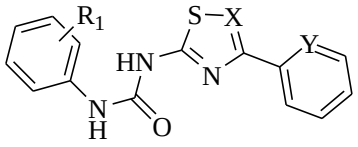
S-2.2

Novel thiazole derivatives were synthesized for a class of adenosine receptor antagonists based on a template approach by Jacqueline *et al.* (2001). Compounds (**S-2.3** and **S-2.4**) showed most promising adenosine A_1 receptor antagonist of the target compounds.



R= Ph, 4-ClPh, 4-IPh, 4-MePh, 4-OMePh, 3,4-Cl_2Ph, 3-ClPh, 4-NO_2Ph, cyclopentyl

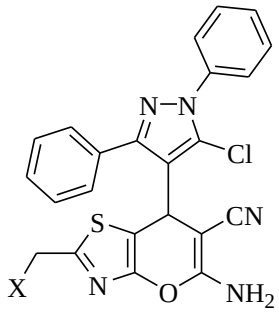
S-2.3



X= N, CH
Y= N, CH
R_1= H,4-OMePh, 2-OMePh

S-2.4

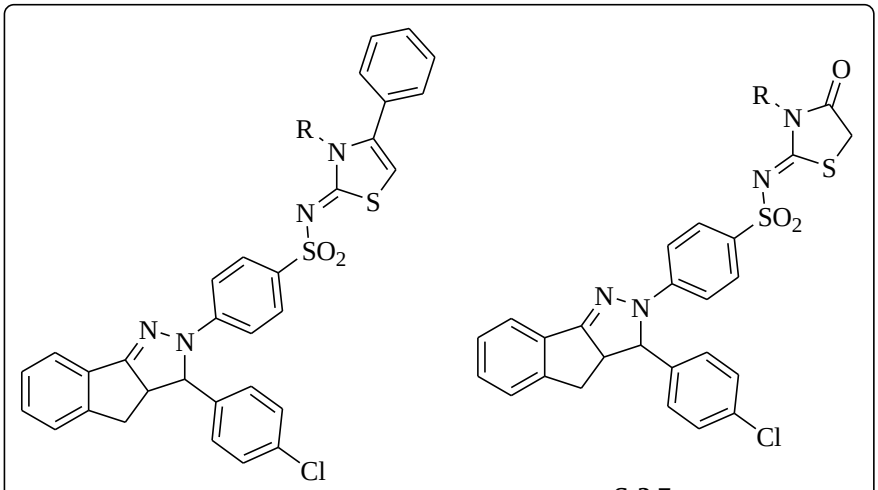
A novel thiazole derivatives have been synthesized by the multistep reaction from 2-(5-chloro-1,3-diphenyl-1*H*-pyrazol-4-yl-methylene)malononitrile by Fathy *et al.* (2006). Target compounds (**S-2.5**) were tested for their molluscicidal activity and thiazole fused derivatives were showed moderate molluscicidal activity.



S-2.5

X= CN, COOEt, $CONH_2$

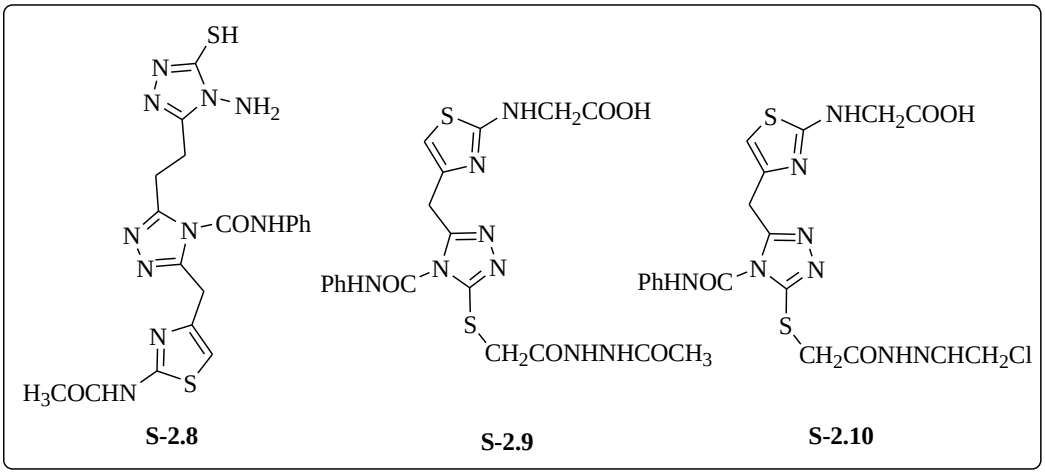
A broad spectrum of antitumor compounds was developed by Rostom (2006). He synthesized 3-(4-chlorophenyl)-[1,2-*c*]pyrazole substituted thiazolines (**S-2.6**) and thiazolidinone derivatives (**S-2.7**) and were confirmed by different spectral techniques. All compounds were tested for their NCI-*in vitro* disease-oriented antitumor activity and found that newly synthesized compounds exhibited broad spectrum antitumor activity.



S-2.6 **S-2.7**

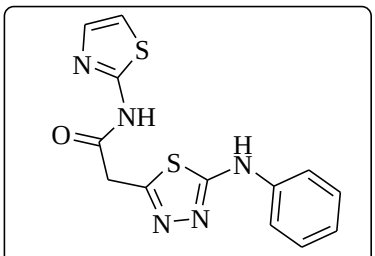
R= Phenyl, benzyl, benzoyl

Shiradkar *et al.* (2007) synthesized a new series of thiazolyl triazoles (**S-2.8**, **S-2.9** and **S-2.10**) from ethyl acetoacetate by microwave organic reaction enhancement method. These derivatives were tested for anti-TB activity and claimed that, the majority of the compounds have showed significant antitubercular activity and also antimicrobial activity. SAR explained that, S-alkylated compounds showed 97-100 % inhibition activity.



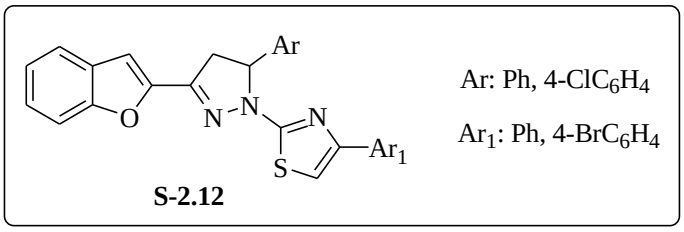
S-2.8 **S-2.9** **S-2.10**

Wahab *et al.* (2008) synthesized thiazolylmalonamide derivatives as a potent antihypertensive α-blocking activity. All the compounds were showed excellent to moderately active and among these whole series, compound (**S-2.11**) showed potent antihypertensive activity with low toxicity.



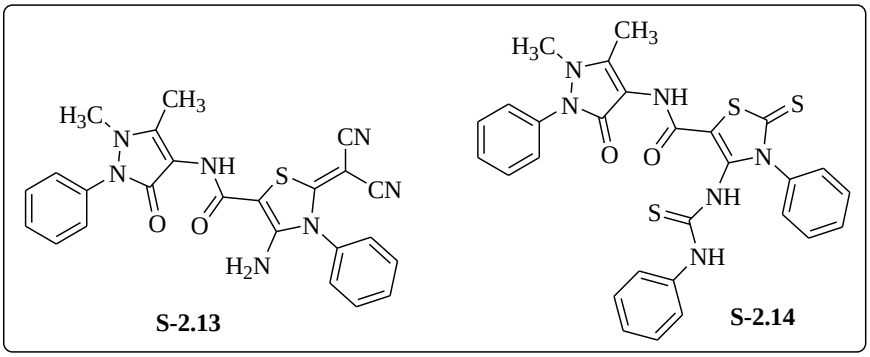
S-2.11

Novel series of 3-(benzofuran-2-yl)-4,5-dihydro-5-aryl-1-[4-(aryl)-1,3-thiazol-2-yl]-1*H*-pyrazole derivatives (**S-2.12**) were synthesized from 3-(benzofuran-2-yl)-5-(4-aryl)-4,5-dihydropyrazole-1-carbothioamides by Wahab *et al.* (2009). All the synthesized compounds were tested for their antibacterial and antifungal activities at 100 µg concentration. Some of the compounds have showed potent antimicrobial activity as compared to the tested standard drugs.



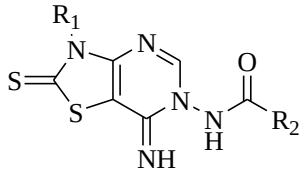
S-2.12

Rostom *et al.* (2009) synthesized a new series of thiazole linked pyrazole derivatives and were evaluated for their analgesic and anti-inflammatory activity using formalin-induced paw edema method and Diclofenac sodium was used as a standard. Two of the target compounds (**S-2.13** and **S-2.14**) have showed promising anti-inflammatory and analgesic activity.



S-2.13 **S-2.14**

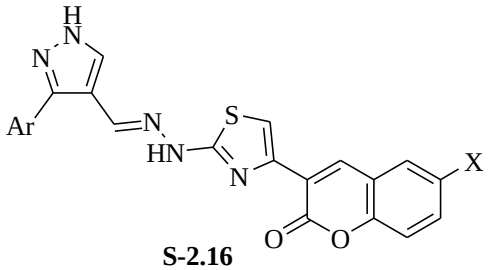
For the treatment of Parkinson's disease, Luthra *et al.* (2010) synthesized a new series of thiazolopyrimidine derivatives (**S-2.15**) and confirmed by different spectral techniques. All compounds were tested for their adenosine A_{2A} receptor antagonist and the binding affinities were evaluated using radio ligand binding assay on isolated membranes from stably transferred HEK293 cells. One of the compound showed a strongest $A_{2A}R$ binding affinity (*K*i value = 0.0038 nM) and selectivity is 737-fold more than A_1R.



S-2.15

R_1= C_2H_5, C_3H_5, C_3H_7, C_4H_9, Ph, C_6H_4I, CHPh
R_2= NH_2, 2-C_4H_3O

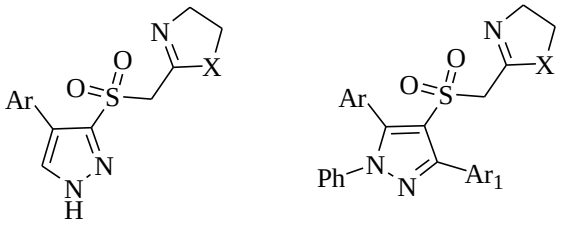
Vijesh *et al.* (2010) synthesized a novel series of 2,4-disubstituted thiazole linked pyrazole derivatives (**S-2.16**) from 3-aryl-1*H*-pyrazole-4-carbaldehyde thiosemicarbazones. All synthesized compounds were confirmed and evaluated for their antibacterial studies against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa.* The results revealed that compounds with 2,5-dichlorothiophene and 2,5-dichlorophenyl substituent at the third position of pyrazole have enhanced antibacterial activity.



S-2.16

Ar= Ph, 4-$CH_3C_6H_4$, 2,4-dichlorothiophene, 2,4-$Cl_2C_6H_3$
X= H, Br

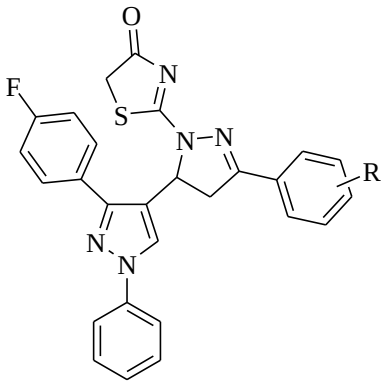
Padmaja *et al.* (2011) synthesized thiazolylsulfonylmethyl pyrazole derivatives (**S-2.17** and **S-2.18**) from *E*-styryl sulfonyl acetic acid methyl ester and tested for their antioxidant studies. Compounds with electron donating group showed good radical scavenging activity when compared to standard Ascorbic acid.



S-2.17 **S-2.18**

Ar= Ph, 4-tolyl, 4-ClPh
Ar_1= Ph, 4-anisyl, 4-ClPh
X= S, O

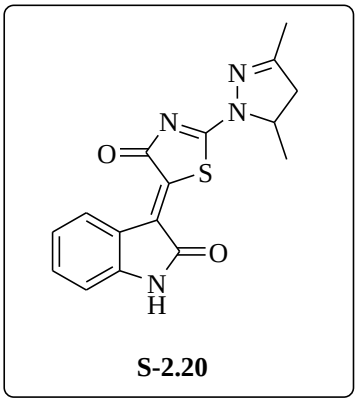
2-(5-(3-(4-Fluorophenyl)-1-phenyl-1*H*-pyrazol-4-yl)-3-(aryl)-4,5-dihydro-1*H*-pyrazol-1-yl)thiazol-4(5*H*)-ones (**S-2.19**) were synthesized and characterized by Desai *et al.* (2012). All the newly synthesized compounds were screened for *in vitro* antibacterial activity against Gram +ve bacteria *Staphylococcus aureus*, *Streptococcus pyogenes* and Gram -ve bacteria *Escherichia coli*, *Pseudomonas aeruginosa*. Antifungal activity against *Candida albicans*, *Aspergillus niger*, *Aspergillus clavatus*. The synthesized compounds showed potent inhibitory action against the test organisms.



S-2.19

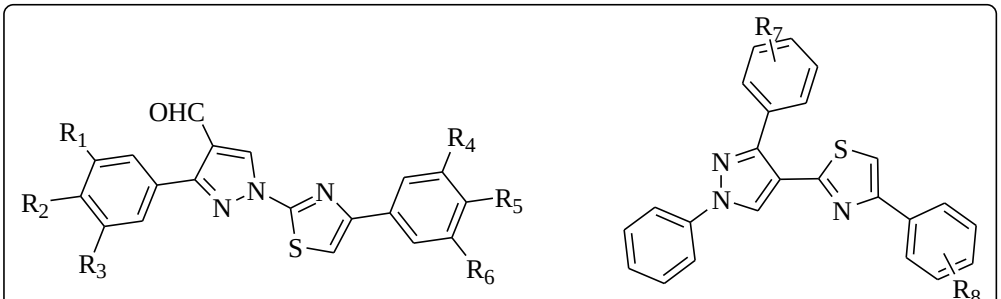
R= H, 2-Cl, 3-Cl, 4-Cl, 2-F, 3-F, 4-F, 2-NO_2, 3-NO_2, 4-NO_2, 2-OH, 3-OH, 4-OH, 2-Me, 3-Me, 4-Me, 2-OCH_3, 4-OCH_3

A novel class of 3-[2-(3,5-diaryl-4,5-dihydropyrazol-1-yl)-4-oxo-4,5-dihydro-1,3-thiazol-5-ylidene]-2,3-dihydro-1*H*-indol-2-ones have been synthesized and evaluated for their antitumor activity by Havrylyuk *et al.* (2012). Most of the synthesized compounds showed anticancer activity on leukemia, lung, CNS, ovarian, prostate, breast and colon cancer cell lines. Compound (**S-2.20**) showed potent activity with mean GI_{50} and TGI values of 0.071 µM and 0.76 µM, respectively.



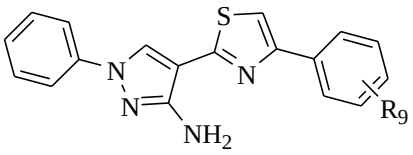
S-2.20

A new thiazole substituted pyrazole derivatives (**S-2.21**, **S-2.22** and **S-2.23**) have been prepared *via* Vilsmeier-Haack formylation and Hantzsch thiazole synthesis by Gaikwad *et al.* (2013). All synthesized compounds were tested for their antimicrobial activity and most of the derivatives showed potent antimicrobial activity. SAR was explained that, introduction of electron withdrawing group (F, Cl, Br, CF_3 and NO_2) on phenyl ring enhanced the activity of all tested organisms.



S-2.21

S-2.22

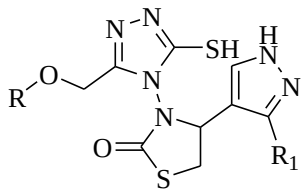


S-2.23

R_1, R_3, R_6= H, CF_3; R_2= H, Br, NO_2, Cl; R_4= H, CF_3, F, NO_2
R_5= H, OMe, NO_2, Br, Cl; R_7= F, Br; R_8= H, NO_2, Cl, Br, F, CH_3
R_9= H, F, Cl, Br, CH_3, 4-NO_2, 3-NO_2

Isloor *et al.* (2012) has developed thiazolidin-4-ones as potent anticancer agents. In this study, a new series of 2-(3-substituted-1*H*-pyrazol-4-yl)-3-(3-substituted-5-sulfanyl-1,2,4-triazol-4-yl)-1,3-thiazolidin-4-one (**S-2.24**) have been synthesized by cyclo-condensation reaction. The structures of all synthesized compounds were confirmed by various spectral techniques. Cytotoxicity studies have been studied by MTT assay in human breast cancer (MCF-7) cells and few

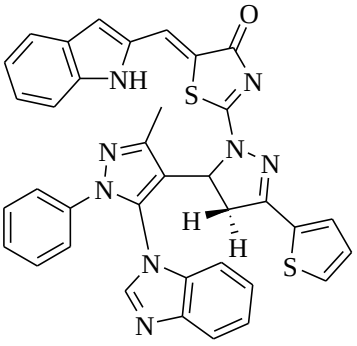
compounds showed significant dose-dependent cytotoxicity. Antioxidant studies were also performed by DPPH and ABTS-free radical scavenging assays indicated moderate antioxidant activity.



S-2.24

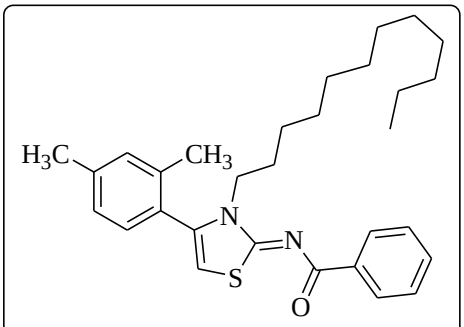
R= Ph, 2-$CH_3C_6H_4$, 4-$CH_3C_6H_4$, 1-naphthyl, 2-naphthyl
R_1= 4-ClC_6H_4, 4-FC_6H_4, 4-$OCH_3C_6H_4$

Based on molecular hybridization technique, Kalaria *et al.* (2014) developed new bipyrazolyl thiazolone hybrid derivatives. These compounds were tested for their antibacterial and antifungal activity. Compound (**S-2.25**) showed lowest IC_{50} value of 2.1 µM and it was the most antifungal activity as compared to all synthesized compounds. They stated that, higher activity of the compound is due to strong interactions such as hydrogen bond, π, π and π-cation interaction with the active sites of *E. coli* FabH (Binding energy ΔG_b = -52.27 kcal/mol).



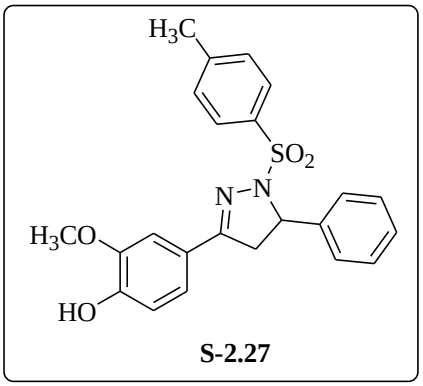
S-2.25

Zheng *et al.* (2015) synthesized 63 new thiazole library compounds by further structural modifications and investigated for their antimigration and antiinvasion activities. All synthesized compounds have demonstrated potent antimigration and antiinvasion activities via possible inhibition of fascin function. Compound (**S-2.26**) inhibited 50 % of cell migration at 24 nM. Moreover, the thiazole analogues showed strong antiangiogenesis activity.



S-2.26

A series of new 2-methoxy-4-(5-phenyl-4,5-dihyro-1*H*-pyrazol-3-yl)phenol derivatives were synthesized by Nayak *et al.* (2016). All the newly synthesized compounds were tested for their human MAO inhibitory activity. Compound (**S-2.27**) found to be a potent inhibitor of hMAO-B and it was found to be better than standard drug Moclobemide.



S-2.27

Although there are newer, less toxic antimicrobial agents that are available for clinical use, their clinical efficacy in some invasive fungal infections, is not optimal. Therefore novel, effective antimicrobials drugs are the immediate need of present days. Keeping this in view it was planned to synthesize some new 1,3,4-trisubstituted pyrazole bearing 4-(chromen-2-one) thiazoles. These compounds were evaluated for their antimicrobial property against *Mycobacterium tuberculosis* (antituberculosis bacteria), *Mycobacterium smegmatis*, *Staphylococcus aureus* (Gram +ve bacteria) and *Candida albicans* (fungi).

2.2 MATERIALS AND METHODS

The target compounds 3-{2-[N′-(1,3-disubstituted-1*H*-pyrazol-4-yl-methylene)-hydrazino]-thiazol-4-yl}-chromen-2-one (**T_{1-12}**) were synthesized by using the chemistry outlined in the **Scheme 2.1**. The basic pyrazole skeleton *i.e.* 1,3-

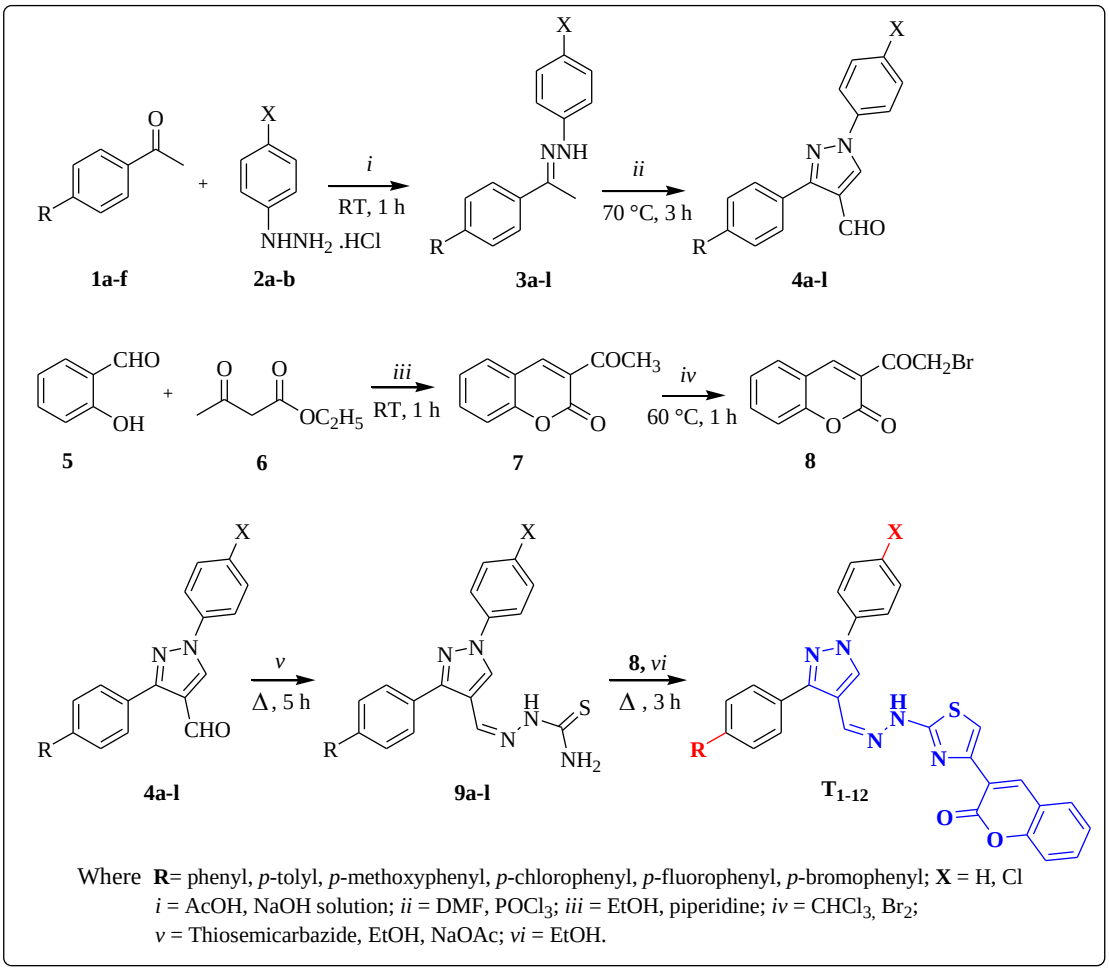
disubstituted-1*H*-pyrazole-4-carbaldehydes (**4a-l**) were synthesized by condensation of substituted acetophenone **1** with 4-substituted phenylhydrazine hydrochloride **2** in the presence of catalytic amount of sodium hydroxide solution in acetic acid media. Which on stirring for 1 h at ambient temperature, yielded the hydrazone derivative **3** in high yield. Further, on treatment with DMF/$POCl_3$ by the Vilsmeier-Haack reaction (Shetty and Bhagat, 2008) at 70 °C, gave the 1,3-disubstituted-1*H*-pyrazole-4-carbaldehydes (**4a-l**) in moderate to good yields from the corresponding hydrazone derivative **3**.

The other key starting material 3-(bromoacetyl)-2*H*-chromen-2-one **8** (Anklekar *et al.*, 2006**)** was prepared by the bromination of 3-acetyl-2*H*-chromen-2-one **7** in chloroform media, which was prepared by Knoevenagel condensation of salicylaldehyde **5** with ethyl acetoacetate **6** with piperidine as a base in ethanol media.

Target pyrazole bearing thiazole derivatives ($\mathbf{T_{1\text{-}12}}$) have been prepared as shown in **Scheme 2.1**. The 1,3-disubstituted-1*H*-pyrazole-4-carbaldehydes (**4a-l**) reacted with thiosemicarbazide to give thiosemicarbazones (**9a-l**) respectively. Which on reacting with 3-(bromoacetyl)-2*H*-chromen-2-one **8** in ethanol media, under reflux temperature to give 3-{2-[N′-(1,3-disubstituted-1*H*-pyrazol-4-yl-methylene)-hydrazino]-thiazol-4-yl}-chromen-2-one ($\mathbf{T_{1\text{-}12}}$) in reasonably good yields (85-96 %). The newly synthesized compounds were characterized by IR, NMR, mass spectra and C, H, N elemental analyses.

All the chemicals were purchased from Sigma-Aldrich, Merck and AVRA-India. Commercial grade solvents used for the reactions were distilled before use. Melting points were determined by open capillary method and were uncorrected. The IR spectra (in KBr pellet) were recorded on Perkin-Elmer FT-IR-4000-400 cm^{-1} spectrophotometer. NMR spectra were recorded on a Bruker Avance spectrometer (500, 400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-NMR) using tetramethylsilane (TMS) as internal standard. Chemical shift and coupling constants are recorded in units of δ (ppm) and Hz, respectively. The mass spectrum was recorded on an LC-MS Applied biosystems MDS SCIEX-API 4000 spectrometer. Elemental analysis was performed on a Flash EA 1112 series CHNS-O analyzer. The completion of the reaction was checked by thin layer chromatography (TLC) on silica gel coated,

readymade aluminium sheets (Merck F_{254}). The names of the structures were mentioned as per ChemDraw Ultra 7.0 software.



Scheme 2.1 Schematic synthetic route for pyrazole bearing thiazole derivatives

2.3 EXPERIMENTAL

The experimental protocols followed for the synthesis of compounds **4a-l**, **8** and **T_{1-12}** is given in the following section.

General procedure for the synthesis of 1,3-disubstituted-1*H*-pyrazole-4-carbaldehydes (4a-l)

A mixture of substituted acetophenone **1a-f** (0.01 mol) and 4-substituted phenylhydrazine hydrochloride **2a-b** (0.01 mol) in acetic acid (10 ml) was stirred with 10 % sodium hydroxide solution (0.5 ml) at an ambient temperature for 1 h. The

obtained hydrazone intermediate **3a-l** was filtered, washed with pre-chilled acetic acid (2 ml) to give off-white free flow solid. Yield: 90-96 %.

N,N-Dimethylformamide (DMF) (1.16 ml, 0.015 mol) was taken in a round bottom flask, which was cooled to -5 °C using ice-salt mixture and to this added phosphorous oxychloride ($POCl_3$) (2.8 ml, 0.03 mol) in about 1.5 h maintaining the temperature. The hydrazone intermediate **3a-l** (0.005 mol) was added at 0 °C and heated the reaction mass to 70 °C for 3 h. After the completion of reaction, resultant reaction mixture was cooled and poured into ice-chilled water. Neutralized the mass with 10 % sodium bicarbonate solution and the solid product was stirred for one hour at ambient temperature and filtered. Further, it was washed with distilled water and compounds were re-crystallized from methanol. Yield: 66-91 %.

1,3-Diphenyl-1*H*-pyrazole-4-carbaldehyde (4a). IR (KBr ν_{max} cm^{-1}): 3125 (C-H str), 1672 (C=O str), 1524 (C=N str), 1227 (C-O str).

1-Phenyl-3-(*p*-tolyl)-1*H*-pyrazole-4-carbaldehyde (4c). IR (KBr ν_{max} cm^{-1}): 3123 (C-H str), 1672 (C=O str), 1522 (C=N str), 1227 (C-O str); ^{1}H-NMR (500 MHz, $CDCl_3$, ppm): δ 2.43 (s, 3H, $-CH_3$), 7.30-7.32 (d, 2H, J = 7.9 Hz, Ar-H), 7.37-7.40 (t, 1H, J = 7.4 Hz, Ar-H), 7.49-7.52 (t, 2H, J = 7.9 Hz, Ar-H), 7.71-7.72 (d, 2H, J = 8.1 Hz, Ar-H), 7.78-7.80 (d, 2H, J = 7.5 Hz, Ar-H), 8.52 (s, 1H, pyrazole-5H), 10.05 (s, 1H, -CHO); ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): δ 185.29, 154.86, 139.31, 139.05, 130.82, 129.63, 129.45, 128.82, 128.45, 127.87, 122.45, 119.72, 21.34; MS: *m/z* = 263.2 (M+1), ANAL. Calcd. for $C_{17}H_{14}N_2O$; calcd: C, 77.84; H, 5.38; N, 10.68; found: C, 77.85; H, 5.38; N, 10.70.

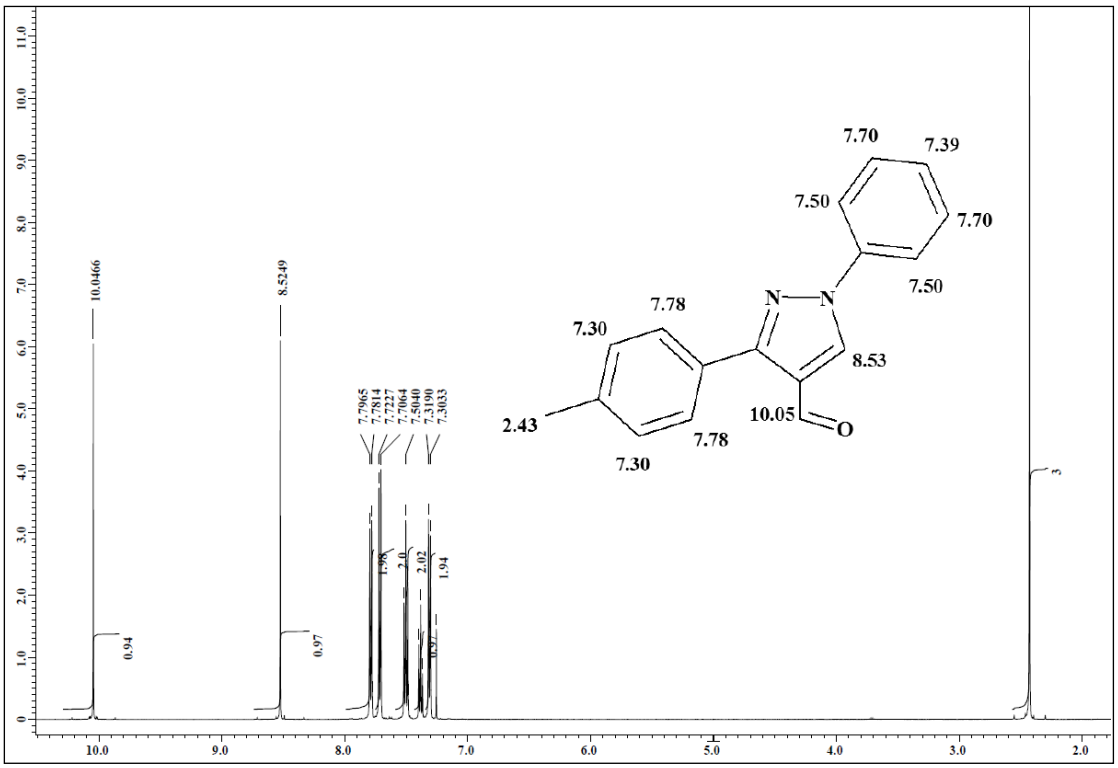


Figure 2.1 ^{1}H-NMR spectrum of compound **4c**

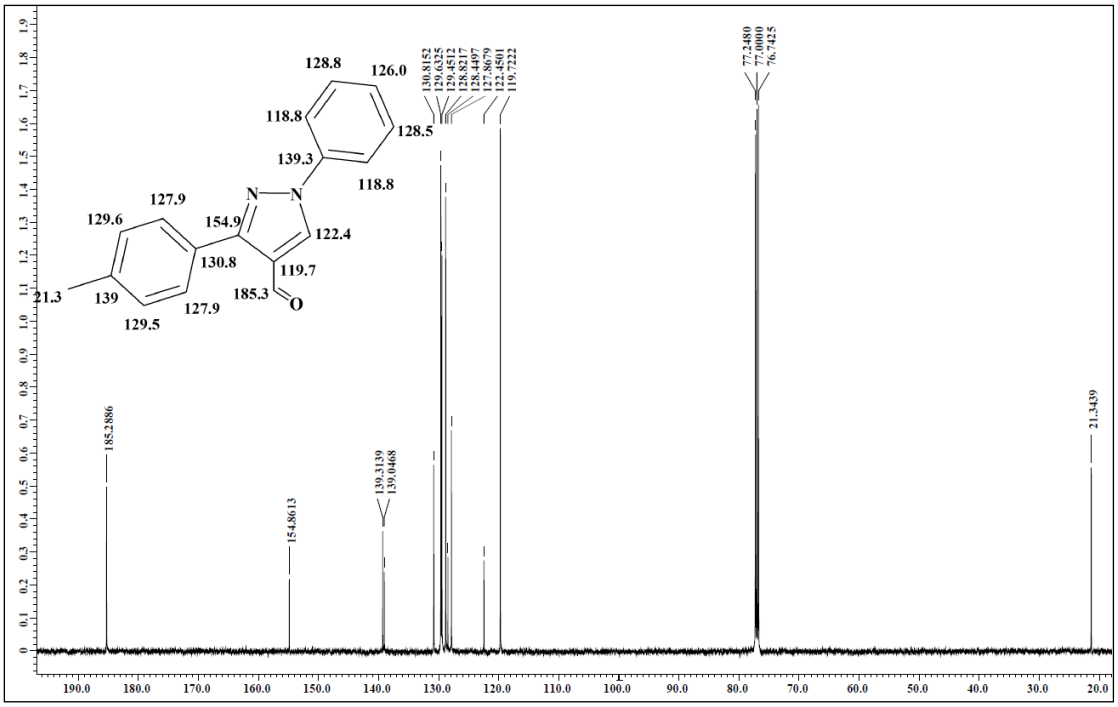


Figure 2.2 ^{13}C-NMR spectrum of compound **4c**

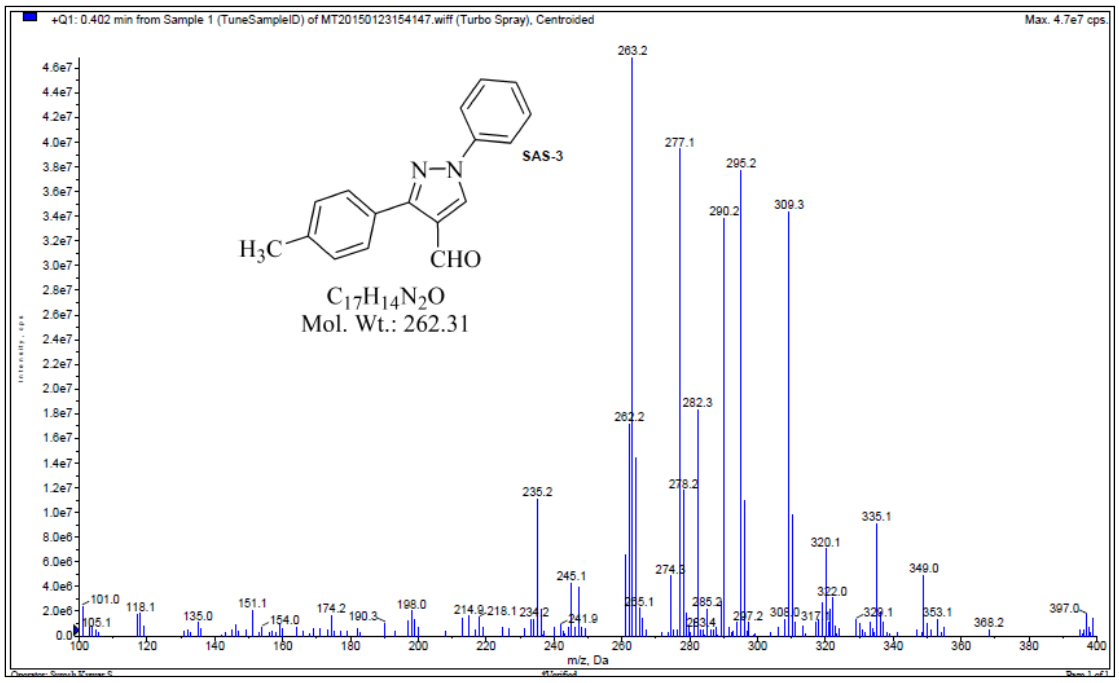


Figure 2.3 Mass spectrum of compound **4c**

Synthesis of 3-(2-bromoacetyl)-chromen-2-one (8)

A mixture of salicylaldehyde **5** (5.3 ml, 0.05 mol), ethyl acetoacetate **6** (6.37 ml, 0.05 mol) in the presence of catalytic amount of piperidine (5 % mol) in ethanol (20 ml) was stirred for 1 h at ambient temperature. The yellow colored precipitation was filtered and washed with ethanol to give 3-(2-acetyl)-chromen-2-one **7** as yellow colored solid having melting point of 121-122 °C (lit. range: 120-122 °C) (Pangal *et al.* 2014). The compound **7** was dissolved in 20 ml of chloroform and added an equimolar amount of bromine in 2 ml chloroform at ambient temperature. The reaction mixture was heated to 60 °C for 1 h and cooled to 20 °C to get the solid material. Obtained product was filtered and washed with pre-chilled chloroform to get white color solid having melting point range of 162-164 °C (Koelsch, 1950).

3-(2-Bromoacetyl)-chromen-2-one (8). Yield: 79 %; IR (KBr ν_{max} cm^{-1}): 3022 (C-H str), 1735 (C=O str), 1551 (C=C str), 1176 (C-O str); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 4.76 (s, 2H, -CH_2), 7.37-7.42 (t, 2H, J = 10.6, Ar-H), 7.69-7.71 (d, 2H, J = 7.2 Hz, Ar-H), 8.65 (s, 1H, Ar-H); MS: *m/z* = 266.9 (M-1).

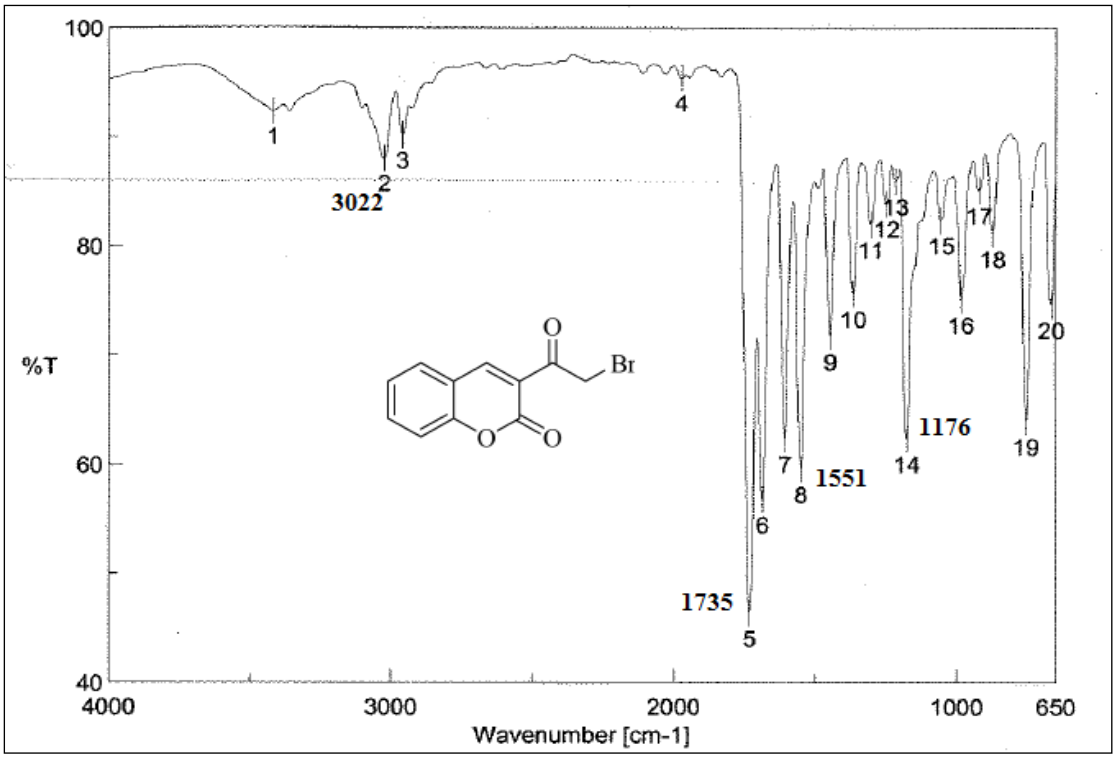


Figure 2.4 IR spectrum of compound **8**

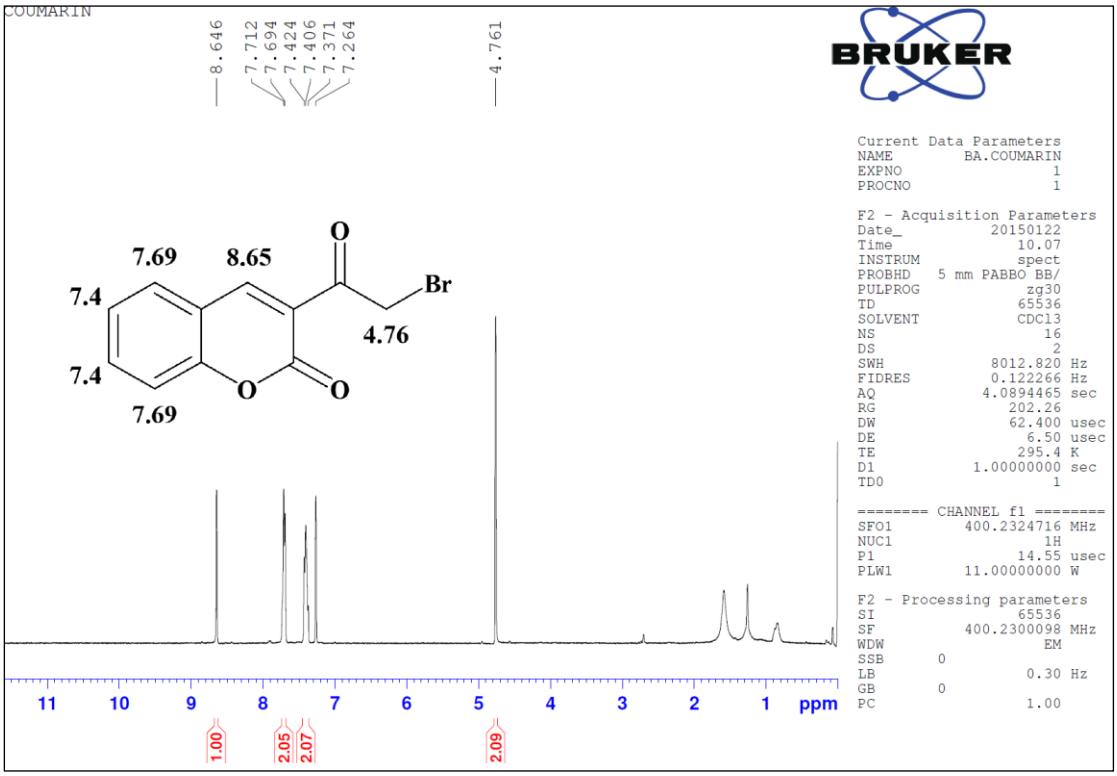


Figure 2.5 ^{1}H-NMR spectrum of compound **8**

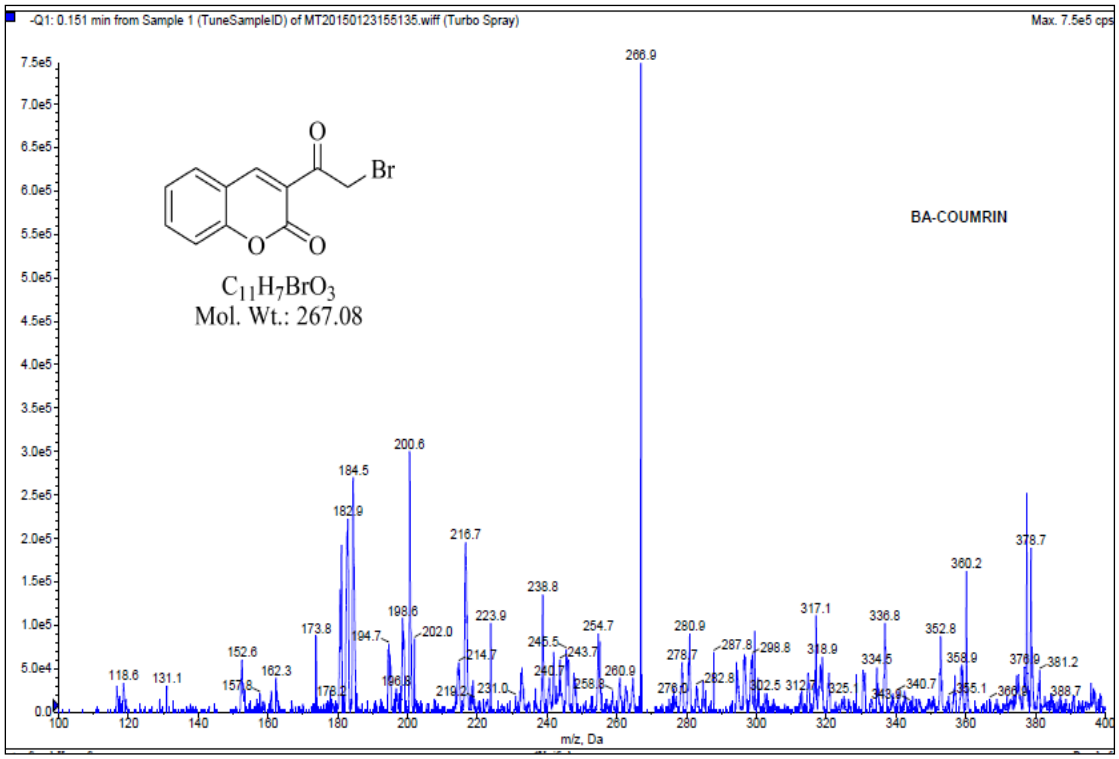


Figure 2.6 Mass spectrum of compound **8**

General method for the synthesis of 3-{2-[N′-(1,3-disubstituted-1*H*-pyrazol-4-yl-methylene)-hydrazino]-thiazol-4-yl}-chromen-2-one (T_{1-12}):

A mixture of 1,3-diphenyl-1*H*-pyrazole-4-carbaldehyde (**4a**) (2.48 g, 0.01 mol), thiosemicarbazide (0.91 g, 0.01 mol) in ethanol (10 ml) were stirred in the presence of sodium acetate (0.82 g, 0.01 mol) under reflux condition for 5 h. The reaction mass was cooled and quenched into water to get the semicarbazone intermediate (**9a**) as white solid. The compound (**9a**) (1.6 g, 0.005 mol) was taken in ethanol (10 ml) and added equimolar amount of 3-(2-bromoacetyl)-chromen-2-one **8** (1.3 g, 0.005 mol). The reaction mixture was heated at reflux temperature for 3 h and monitored by TLC [n-hexane : ethylacetate (4:1)]. The solid mass was cooled to room temperature and stirred for 0.5 h. The solid product was filtered, washed with pre-chilled ethanol (~10 °C) and dried to get 3-{2-[N′-(1,3-diphenyl-1*H*-pyrazol-4-yl-methylene)-hydrazino]-thiazol-4-yl}-chromen-2-one (T_1). The same procedure was followed for the synthesis of compounds T_{2-12}.

3-{2-[N′-(1,3-Diphenyl-1*H*-pyrazol-4-yl-methylene)-hydrazino]-thiazol-4-yl}-chromen-2-one (T_1). Yield: 92 %; IR (KBr ν_{max} cm^{-1}): 3377 (N-H str), 3143 (Ar-H str), 1726 (C=O str), 1633 (C=N str), 1501 (C=C str), 1215 (C-O str); ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): δ 7.36-7.40 (m, 2H, Ar-H), 7.44-7.50 (m, 2H, Ar-H), 7.52-7.56 (m, 4H, Ar-H), 7.60-7.63 (m, 1H, Ar-H), 7.76 (s, 1H, pyrazol-5H), 7.77-7.79 (d, 2H, J = 8.0 Hz, Ar-H), 7.85-7.87 (d, 1H, J = 7.8 Hz, chromen-2-one-5H), 7.98-8.00 (d, 2H, J = 8.4 Hz, Ar-H), 8.19 (s, 1H, -CH=N), 8.53 (s, 1H, chromen-2-one-4H), 8.92 (s, 1H, thiazole-5H), 12.03 (s, 1H, -NH); ^{13}C-NMR (100 MHz, DMSO-d_6, ppm): δ 167.89, 159.22, 152.75, 151.36, 144.31, 139.50, 138.60, 135.64, 132.69, 132.14, 130.06, 129.29, 126.06, 128.95, 128.13, 127.35, 125.17, 120.99, 119.66, 119.19, 117.33, 116.34, 110.89; MS: m/z = 490.1 (M+1), ANAL. Calcd. for $C_{28}H_{19}N_5O_2S$; calcd: C, 68.70; H, 3.91; N, 14.31; found: C, 68.65; H, 3.90; N, 14.28.

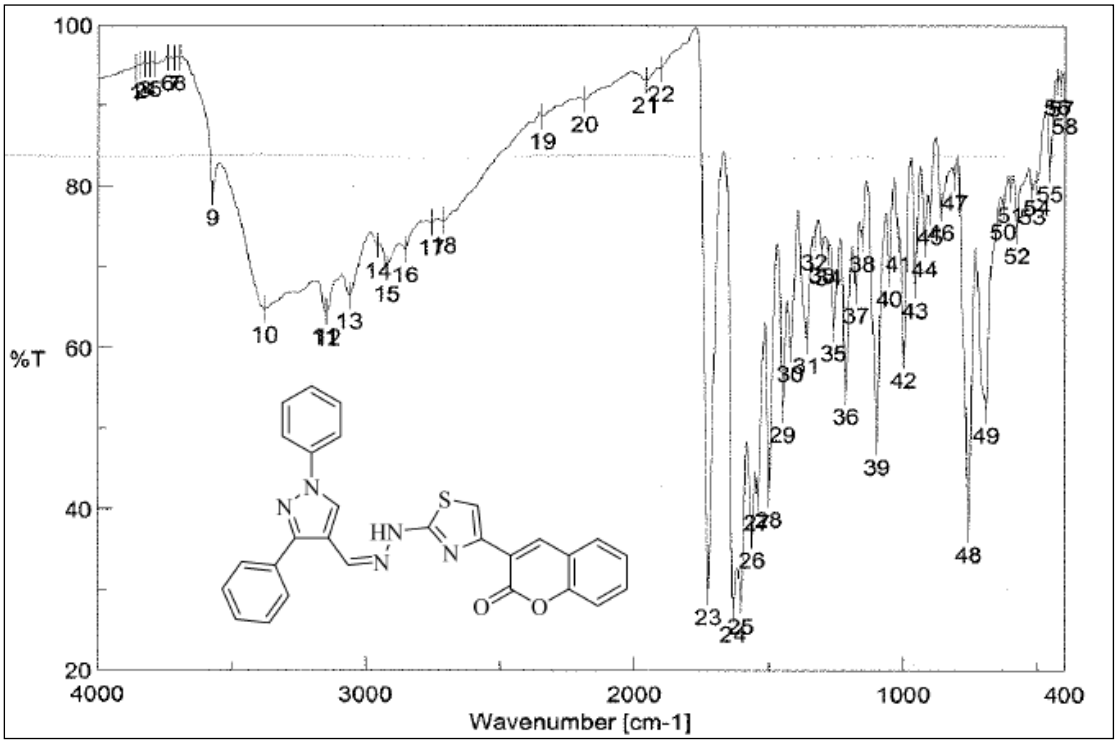


Figure 2.7 IR spectrum of compound **T_1**

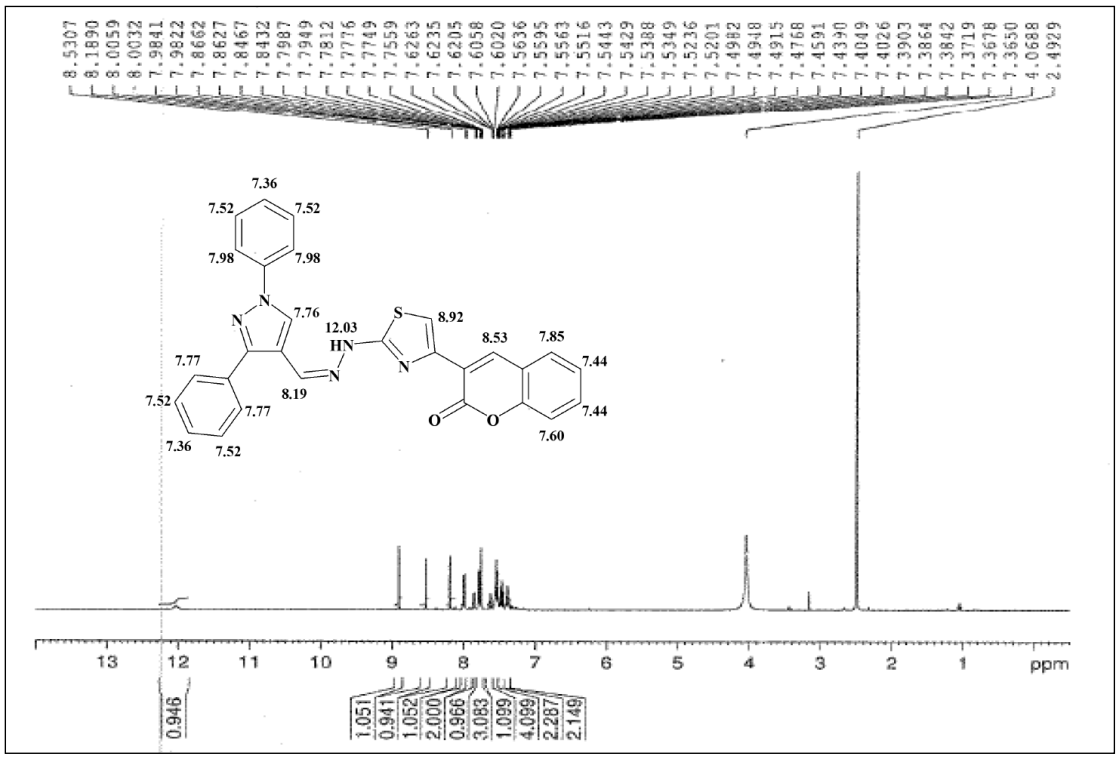


Figure 2.8 ^{1}H-NMR spectrum of compound **T_1**

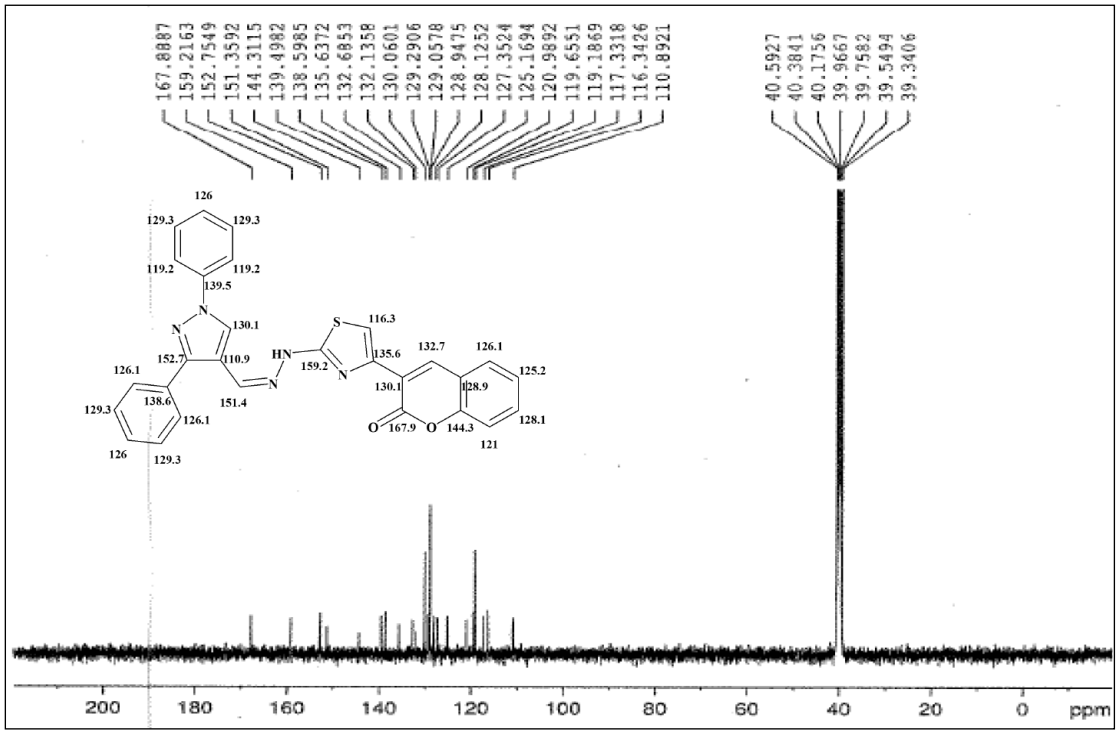


Figure 2.9 ^{13}C-NMR spectrum of compound **T_1**

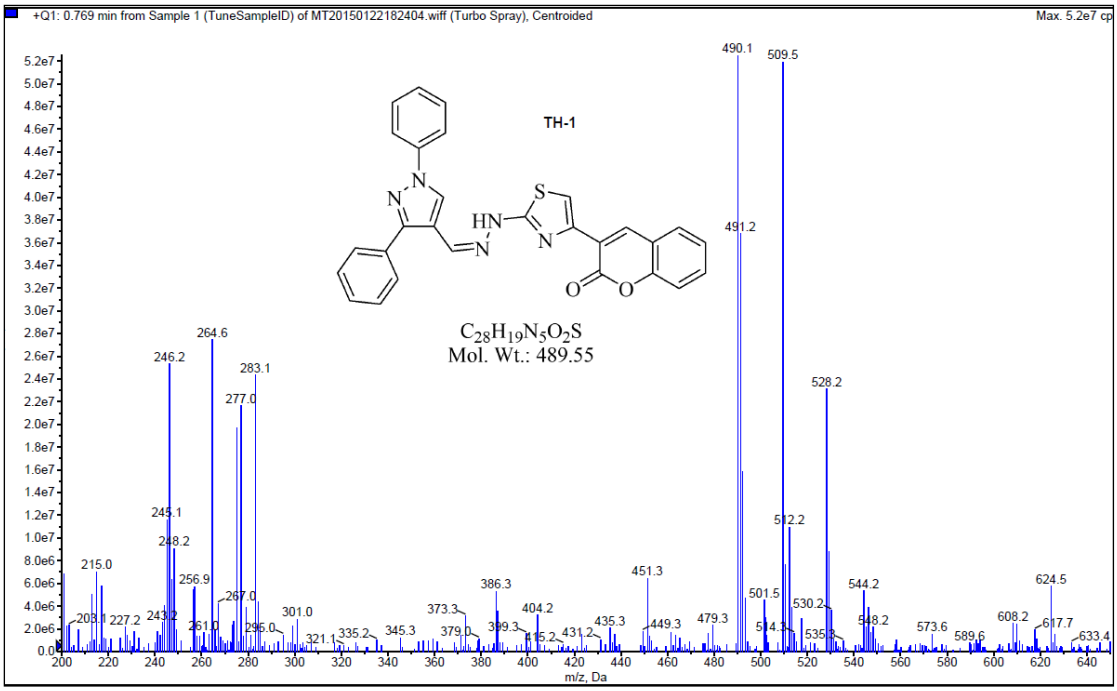


Figure 2.10 Mass spectrum of compound **T_1**

3-(2-{N′-[1-(4-Chlorophenyl)-3-phenyl-1*H*-pyrazol-4-yl-methylene)-hydrazino]-thiazol-4-yl}-chromen-2-one (T_2). Yield: 91 %; IR (KBr ν_{max} cm^{-1}): 3412 (N-H str), 3140 (Ar-H str), 1720 (C=O str), 1627 (C=N str), 1497 (C=C str), 1094 (C-O str); ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): δ 7.37-7.41 (t, 1H, J = 8.0 Hz, chromen-2-one-6H), 7.44-7.48 (m, 2H, Ar-H), 7.49-7.56 (m, 2H, Ar-H), 7.60-7.65 (m, 3H, Ar-H), 7.76-7.79 (m, 3H, Ar-H), 7.85-7.87 (d, 1H, J = 9.1 Hz, chromen-2-one-5H), 8.03-8.05 (d, 2H, J = 9.0 Hz, Ar-H), 8.18 (s, 1H, -CH=N), 8.53 (s, 1H, chromen-2-one-4H), 8.94 (s, 1H, thiazole-5H), 12.03 (s, 1H, -NH); ^{13}C-NMR (100 MHz, DMSO-d_6, ppm): δ 167.88, 159.24, 152.75, 151.65, 144.31, 138.63, 138.31, 135.43, 132.49. 132.16, 131.46, 126.97, 129.27, 129.10, 128.95, 128.24, 125.20, 120.96, 120.83, 119.64, 117.66, 116.34, 110.92; MS: *m/z* = 524.0 (M+1), ANAL. Calcd. for $C_{28}H_{18}ClN_5O_2S$; calcd: C, 64.18; H, 3.46; N, 13.37; found: C, 64.21; H, 3.45; N, 13.38.

3-{2-[N′-(1-Phenyl-3-*p*-tolyl-1*H*-pyrazol-4-yl-methylene)-hydrazino]-thiazol-4-yl}-chromen-2-one (T_3). Yield: 86 %; IR (KBr ν_{max} cm^{-1}): 3443 (N-H str), 3239 (Ar-H str), 1708 (C=O str), 1601 (C=N str), 1503 (C=C str), 1106 (C-O str); ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): δ 2.40 (s, 3H, -CH_3), 7.33-7.41 (m, 4H, Ar-H), 7.44-7.46

(d, 1H, *J* = 8.3 Hz, chromen-2-one-8H), 7.52-7.56 (t, 2H, *J* = 8.0 Hz, Ar-H), 7.60-7.65 (t, 1H, *J* = 8.6 Hz, chromen-2-one-7H), 7.67-7.69 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.76 (s, 1H, pyrazole-5H), 7.85-7.87 (d, 1H, *J* = 9.1 Hz, chromen-2-one-5H), 7.98-8.00 (d, 2H, *J* = 7.7 Hz, Ar-H), 8.18 (s, 1H, -CH=N), 8.53 (s, 1H, chromen-2-one-4H), 8.88 (s, 1H, thiazole-5H), 12.01 (s, 1H, -NH); ^{13}C-NMR (100 MHz, DMSO-d_6, ppm): δ 167.91, 159.23, 152.74, 151.38, 144.39, 139.51, 138.58, 138.41, 135.75, 132.13, 130.04, 129.82, 129.28, 128.79, 127.92, 127.28, 125.17, 121.00, 119.65, 119.15, 117.25, 116.34, 110.89, 21.37; MS: *m/z* = 502.2 (M-1), ANAL. Calcd. for $C_{29}H_{21}N_5O_2S$; calcd: C, 69.17; H, 4.20; N, 13.91; found: C, 69.20; H, 4.21; N, 13.92.

3-(2-{N′-[1-(4-Chlorophenyl)-3-*p*-tolyl-1*H*-pyrazol-4-yl-methylene)-hydrazino]-thiazol-4-yl}-chromen-2-one (T_4). Yield: 91 %; IR (KBr ν_{max} cm^{-1}): 3413 (N-H str), 3145 (Ar-H str), 1719 (C=O str), 1631 (C=N str), 1500 (C=C str), 1092 (C-O str); ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): δ 2.41 (s, 3H, -CH_3), 7.34-7.36 (d, 2H, *J* = 7.6 Hz, Ar-H), 7.39-7.41 (t, 1H, *J* = 7.6 Hz, chromen-2-one-6H), 7.45-7.47 (d, 1H, *J* = 8.4 Hz, chromen-2-one-8H), 7.59-7.61 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.63 (t, 1H, chromen-2-one-7H, merged), 7.67-7.69 (d, 2H, *J* = 7.2 Hz, Ar-H), 7.77 (s, 1H, pyrazole-5H), 7.85-7.87 (d, 1H, *J* = 7.6 Hz, chromen-2-one-5H), 8.03-8.05 (d, 2H, *J* = 7.6 Hz, Ar-H), 8.18 (s, 1H, -CH=N), 8.54 (s, 1H, chromen-2-one-4H), 8.91 (s, 1H, thiazole-5H), 12.03 (s, 1H, -NH); ^{13}C-NMR (100 MHz, DMSO-d_6, ppm): δ 167.88, 159.23, 152.77, 151.66, 144.32, 138.60, 138.55, 138.35, 135.57, 132.13, 131.36, 129.94, 129.67, 129.29, 128.80, 128.08, 125.18, 120.99, 120.79, 119.66, 117.57, 116.35, 110.93, 21.39; MS: *m/z* = 538.2 (M+1), ANAL. Calcd. for $C_{29}H_{20}ClN_5O_2S$; calcd: C, 64.74; H, 3.75; N, 13.02; found: C, 64.76; H, 3.75; N, 13.05.

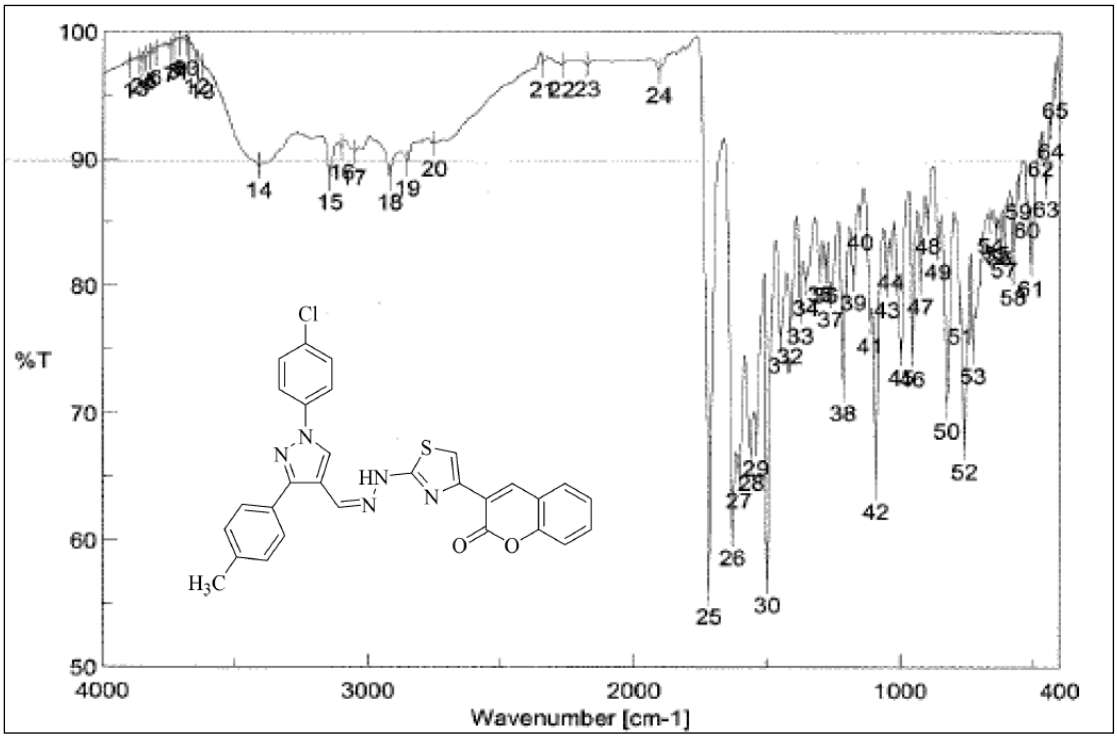


Figure 2.11 IR spectrum of compound **T_4**

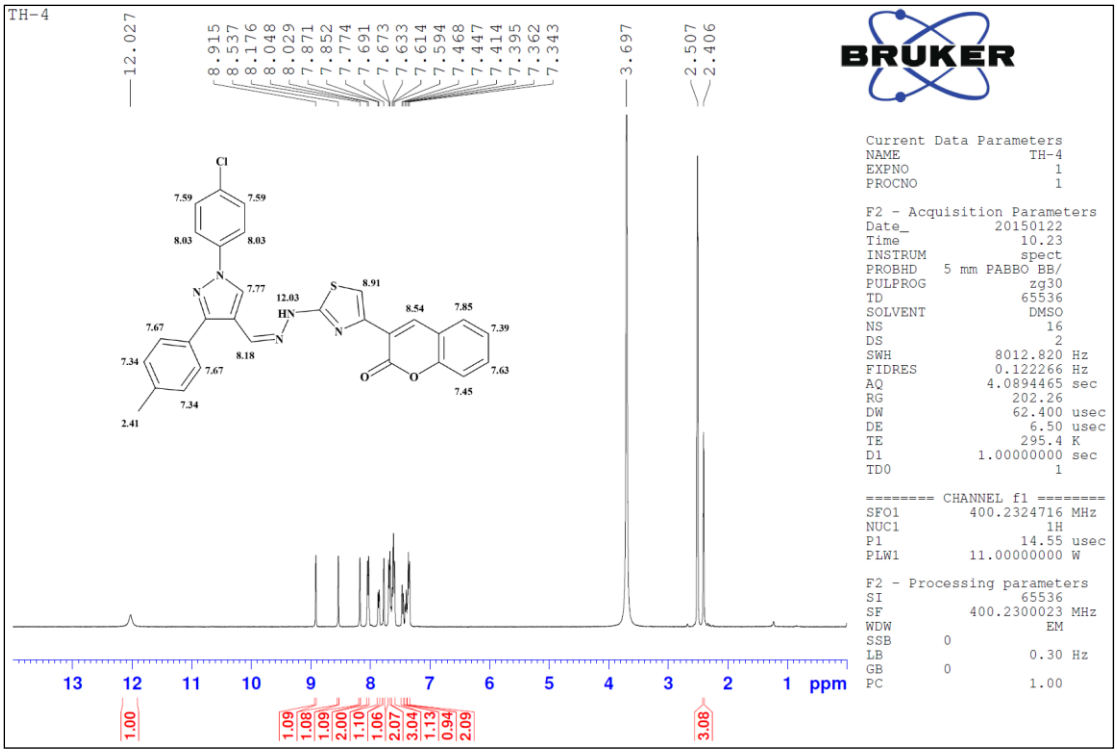


Figure 2.12 ^{1}H-NMR spectrum of compound **T_4**

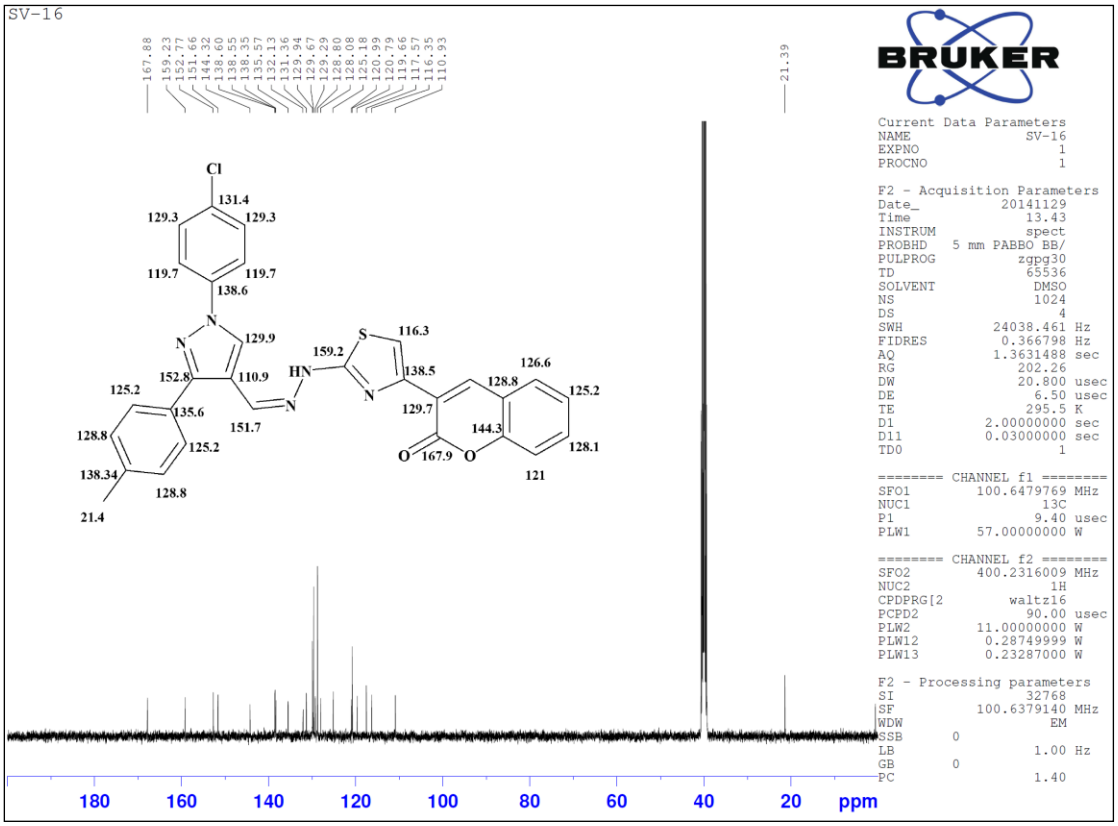


Figure 2.13 ^{13}C-NMR spectrum of compound **T_4**

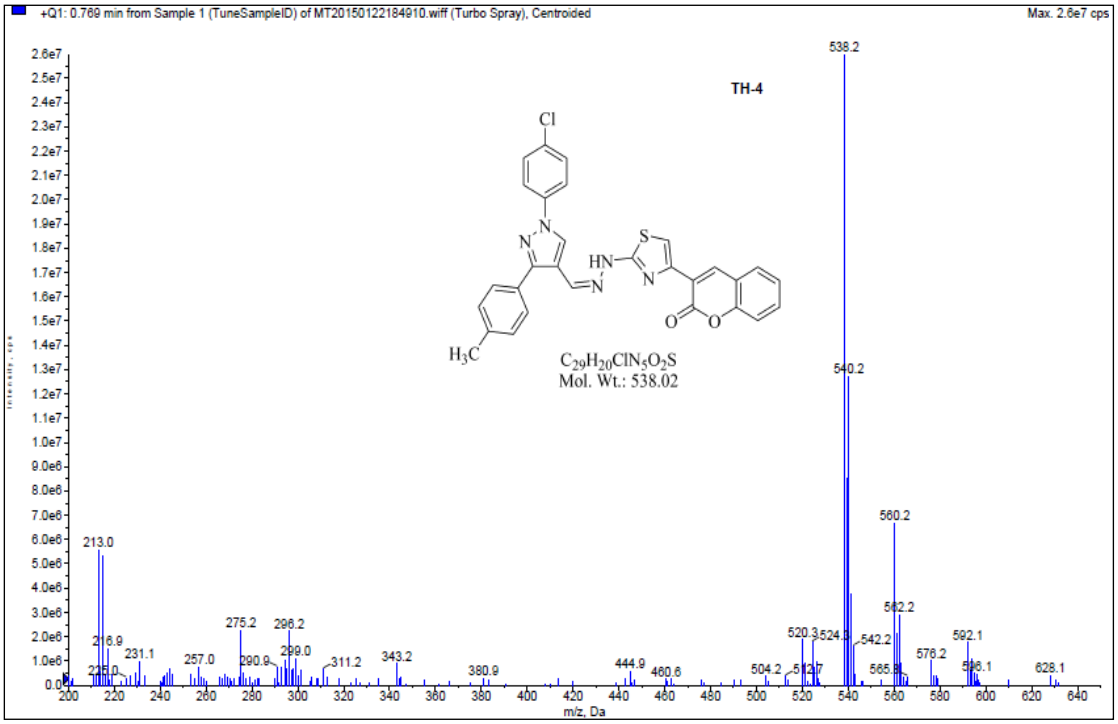


Figure 2.14 Mass spectrum of compound **T_4**

3-(2-{N′-[3-(4-Methoxyphenyl)-1-phenyl-1*H*-pyrazol-4-yl-methylene]-hydrazino}-thiazol-4-yl}-chromen-2-one (T_5). Yield: 85 %; IR (KBr ν_{max} cm^{-1}): 3426 (N-H str), 3142 (Ar-H str), 1719 (C=O str), 1607 (C=N str), 1500 (C=C str), 1106 (C-O str); ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): δ 3.84 (s, 3H, -OCH_3), 7.08-7.10 (d, 2H, J = 8.8 Hz, Ar-H), 7.34-7.41 (m, 2H, Ar-H), 7.44-7.46 (d, 1H, J = 8.3 Hz, chromen-2-one-8H), 7.51-7.55 (t, 2H, J = 8.0 Hz, Ar-H), 7.60-7.65 (t, 1H, J = 8.6 Hz, chromen-2-one-7H), 7.72-7.76 (m, 3H, Ar-H), 7.84-7.87 (d, 1H, J = 9.2 Hz, chromen-2-one-5H), 7.97-7.99 (d, 2H, J = 8.7 Hz, Ar-H), 8.17 (s, 1H, -CH=N), 8.53 (s, 1H, chromen-2-one-4H), 8.91 (s, 1H, thiazole-5H), 12.03 (s, 1H, -NH); ^{13}C-NMR (100 MHz, DMSO-d_6, ppm): δ 167.91, 159.98, 159.22, 152.75, 151.19, 144.31, 139.52, 138.84, 132.13, 130.26, 130.04, 129.29, 128.08, 127.21, 125.17, 125.07, 121.01, 119.66, 119.08, 117.05, 116.34, 114.48, 110.92, 55.73; MS: *m/z* = 520.3 (M+1), ANAL. Calcd. for $C_{29}H_{21}N_5O_3S$; calcd: C, 67.04; H, 4.07; N, 13.48; found: C, 67.11; H, 4.08; N, 13.46.

3-(2-{N′-[1-(4-Chlorophenyl)-3-(4-methoxyphenyl)-1*H*-pyrazol-4-yl-methylene]-hydrazino}-thiazol-4-yl}-chromen-2-one (T_6). Yield: 87 %; IR (KBr ν_{max} cm^{-1}): 3425 (N-H str), 3144 (Ar-H str), 1716 (C=O str), 1608 (C=N str), 1496 (C=C str), 1097 (C-O str); ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): δ 3.85 (s, 3H, -OCH_3), 7.08-7.10 (d, 2H, J = 7.6 Hz, Ar-H), 7.39-7.41 (t, 1H, J = 6.8 Hz, chromen-2-one-6H), 7.45-7.47 (d, 1H, J = 8.0 Hz, chromen-2-one-8H), 7.59-7.63 (m, 3H, Ar-H), 7.73-7.75 (d, 2H, J = 8.4 Hz, Ar-H), 7.77 (s, 1H, pyrazole-5H), 7.85-7.87 (d, 1H, J = 7.6 Hz, chromen-2-one-5H), 8.17 (s, 1H, -CH=N), 8.20-8.40 (d, 2H, J = 8.0 Hz, Ar-H), 8.54 (s, 1H, chromen-2-one-4H), 8.91 (s, 1H, thiazole-5H), 12.03 (s, 1H, -NH); ^{13}C-NMR (100 MHz, DMSO-d_6, ppm): δ 167.90, 160.08, 159.23, 152.77, 151.47, 144.32, 138.60, 138.37, 135.66, 132.14, 131.28, 130.29, 129.94, 129.29, 128.23, 125.18, 124.90, 121.01, 120.73, 119.67, 117.40, 116.35, 114.52, 110.95, 55.76; MS: *m/z* =555.3 (M+1), ANAL. Calcd. for $C_{29}H_{20}ClN_5O_3S$; calcd: C, 62.87; H, 3.64; N, 12.64; found: C, 62.88; H, 3.65; N, 12.63.

3-(2-{N′-[3-(4-Chlorophenyl)-1-phenyl-1*H*-pyrazol-4-yl-methylene]-hydrazino}-thiazol-4-yl}-chromen-2-one (T_7). Yield: 93 %; IR (KBr ν_{max} cm^{-1}): 3410 (N-H str), 3141 (Ar-H str), 1719 (C=O str), 1623 (C=N str), 1496 (C=C str), 1095 (C-O str); ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): δ 7.39 (m, 2H, Ar-H), 7.45-7.47 (d, 1H, J = 8.0

Hz, chromen-2-one-8H), 7.54-7.57 (t, 2H, *J* = 7.4 Hz, Ar-H), 7.60-7.61 (m, 3H, Ar-H), 7.77 (s, 1H, pyrazole-5H), 7.85-7.89 (t, 3H, *J* = 8.0 Hz, Ar-H), 7.98-8.00 (d, 2H, *J* = 7.6 Hz, Ar-H), 8.19 (s, 1H, -CH=N), 8.54 (s, 1H, chromen-2-one-4H), 8.93 (s, 1H, thiazole-5H), 12.05 (s, 1H, -NH); ^{13}C-NMR (100 MHz, DMSO-d_6, ppm): δ 167.89, 159.22, 152.78, 149.91, 139.42, 138.62, 135.46, 133.71, 132.16, 131.68, 130.73, 130.10, 129.30, 129.04, 127.48, 125.19, 121.01, 119.67, 119.22, 117.43, 116.36, 110.99; MS: *m/z* = 524.3 (M+1), ANAL. Calcd. for $C_{28}H_{18}ClN_5O_2S$; calcd: C, 64.18; H, 3.46; N, 13.37; found: C, 64.21; H, 3.46; N, 13.38.

3-(2-{N′-[1,3-Bis-(4-Chlorophenyl)-1*H*-pyrazol-4-yl-methylene]-hydrazino}-thiazol-4-yl}-chromen-2-one (T_8). Yield: 96 %; IR (KBr ν_{max} cm^{-1}): 3419 (N-H str), 3145 (Ar-H str), 1717 (C=O str), 1623 (C=N str), 1496 (C=C str), 1095 (C-O str); ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): δ 7.39-7.41 (t, 1H, *J* = 6.0 Hz, chromen-2-one-6H), 7.44-7.46 (d, 1H, *J* = 8.4 Hz, chromen-2-one-8H), 7.60-7.62 (m, 5H, Ar-H), 7.77 (s, 1H, pyrazole-5H), 7.86 (m, 3H, Ar-H), 8.01-8.04 (d, 2H, *J* = 8.4 Hz, Ar-H), 8.17 (s, 1H, -CH=N), 8.53 (s, 1H, chromen-2-one-4H), 8.96 (s, 1H, thiazole-5H), 12.05 (s, 1H, -NH); ^{13}C-NMR (100 MHz, DMSO-d_6, ppm): δ 167.84, 159.21, 152.77, 150.16, 144.32, 138.60, 138.22, 135.22, 133.81, 132.14, 131.55, 131.48, 130.73, 129.99, 129.28, 129.14, 129.05, 125.17, 120.99, 120.81, 119.65, 117.72, 116.35, 110.99; MS: *m/z* = 558.2 (M+1), ANAL. Calcd. for $C_{28}H_{17}Cl_2N_5O_2S$; calcd: C, 60.22; H, 3.07; N, 12.54; found: C, 60.19; H, 3.06; N, 12.55.

3-(2-{N′-[3-(4-Bromophenyl)-1-phenyl-1*H*-pyrazol-4-yl-methylene]-hydrazino}-thiazol-4-yl}-chromen-2-one (T_9). Yield: 88 %; IR (KBr ν_{max} cm^{-1}): 3427 (N-H str), 3153 (Ar-H str), 1720 (C=O str), 1608 (C=N str), 1499 (C=C str), 1099 (C-O str); ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): δ 7.39 (m, 2H, Ar-H), 7.45-7.47 (d, 1H, *J* = 7.6 Hz, chromen-2-one-8H), 7.54-7.55 (t, 2H, *J* = 6.8 Hz, Ar-H), 7.61-7.65 (t, 1H, *J* = 7.2 Hz, chromen-2-one-7H), 7.73-7.75 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.77 (s, 1H, pyrazole-5H), 7.81-7.83 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.85-7.87 (d, 1H, *J* = 6.8 Hz, chromen-2-one-5H), 7.98-8.00 (d, 2H, *J* = 7.2 Hz, Ar-H), 8.18 (s, 1H, -CH=N), 8.54 (s, 1H, chromen-2-one-4H), 8.93 (s, 1H, thiazole-5H), 12.06 (s, 1H, -NH); ^{13}C-NMR (100 MHz, DMSO-d_6, ppm): δ 166.80, 158.14, 151.70, 148.87, 143.24, 138.34, 137.54, 134.38, 131.07, 130.95, 130.88, 129.92, 129.02, 128.22, 127.98, 126.40, 124.10,

121.29, 119.92, 118.59, 118.14, 116.35, 115.28, 109.90; MS: *m/z* = 568.2 (M+1), ANAL. Calcd.for $C_{28}H_{18}BrN_5O_2S$; calcd: C, 59.16; H, 3.19; N, 12.32; found: C, 59.17; H, 3.20; N, 12.31.

3-(2-{N′-[3-(4-Bromophenyl)-1-(4-chlorophenyl)-1*H*-pyrazol-4-yl-methylene]-hydrazino}-thiazol-4-yl}-chromen-2-one (T_{10}). Yield: 85 %; IR (KBr ν_{max} cm^{-1}): 3418 (N-H str), 3145 (Ar-H str), 1717 (C=O str), 1624 (C=N str), 1496 (C=C str), 1097 (C-O str); ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): δ 7.37-7.41 (t, 1H, *J* = 8.0 Hz, chromen-2-one-6H), 7.44-7.46 (d, 1H, *J* = 8.2 Hz, chromen-2-one-8H), 7.60-7.65 (m, 3H, Ar-H), 7.72-7.74 (d, 2H, *J* = 8.6 Hz, Ar-H), 7.76 (s, 1H, pyrazole-5H), 7.79-7.81 (d, 2H, *J* = 8.6 Hz, Ar-H), 7.84-7.87 (d, 1H, *J* = 9.1 Hz, chromen-2-one-5H), 8.01-8.03 (d, 2H, *J* = 8.9 Hz, Ar-H), 8.16 (s, 1H, -CH=N), 8.53 (s, 1H, chromen-2-one-4H), 8.96 (s, 1H, thiazole-5H), 12.06 (s, 1H, -NH); ^{13}C-NMR (100 MHz, DMSO-d_6, ppm): δ 167.83, 159.20, 152.76, 150.21, 144.32, 138.59, 138.22, 135.19, 132.13, 131.97, 131.82, 131.56, 130.98, 129.99, 129.28, 129.14, 125.17, 122.48, 120.98, 120.82, 119.65, 117.70, 116.34, 111.00; MS: *m/z* = 602.2 (M+1), ANAL. Calcd. for $C_{28}H_{17}BrClN_5O_2S$; calcd: C, 55.78; H, 2.84; N, 11.62; found: C, 55.80; H, 2.85; N, 11.61.

3-(2-{N′-[3-(4-Fluorophenyl)-1-phenyl-1*H*-pyrazol-4-yl-methylene]-hydrazino}-thiazol-4-yl}-chromen-2-one (T_{11}). Yield: 90 %; IR (KBr ν_{max} cm^{-1}): 3414 (N-H str), 3138 (Ar-H str), 1723 (C=O str), 1605 (C=N str), 1501 (C=C str), 1099 (C-O str); ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): δ 7.38-7.40 (m, 4H, Ar-H), 7.45-7.47 (d, 1H, *J* = 8.4 Hz, chromen-2-one-8H), 7.54-7.56 (t, 2H, *J* = 7.6 Hz, Ar-H), 7.62-7.66 (t, 1H, *J* = 7.4 Hz, chromen-2-one-7H), 7.76 (s, 1H, pyrazole-5H), 7.87-7.88 (m, 3H, Ar-H), 7.98-8.00 (d, 2H, *J* = 7.2 Hz, Ar-H), 8.18 (s, 1H, -CH=N), 8.54 (s, 1H, chromen-2-one-4H), 8.92 (s, 1H, thiazole-5H), 12.04 (s, 1H, -NH); ^{13}C-NMR (100 MHz, DMSO-d_6, ppm): δ 167.92, 159.23, 152.78, 150.26, 144.34, 139.46, 138.61, 135.53, 131.13, 130.09, 129.30, 128.81, 127.39, 125.19, 121.02, 119.68, 119.17, 117.28, 116.36, 116.03, 115.81, 110.96; MS: *m/z* = 508.2 (M+1), ANAL. Calcd. for $C_{28}H_{18}FN_5O_2S$; calcd: C, 66.26; H, 3.57; N, 13.80; found: C, 66.31; H, 3.59; N, 13.82.

3-(2-{N′-[1-(4-Chlorophenyl)-3-(4-fluorophenyl)-1*H*-pyrazol-4-yl-methylene)-hydrazino]-thiazol-4-yl}-chromen-2-one (T_{12}). Yield: 92 %; IR (KBr ν_{max} cm^{-1}): 3417 (N-H str), 3173 (Ar-H str), 1719 (C=O str), 1623 (C=N str), 1497 (C=C str), 1094 (C-O str); ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): δ 7.35-7.41 (m, 3H, Ar-H), 7.44-7.46 (d, 1H, J = 8.3 Hz, chromen-2-one-8H), 7.59-7.61 (d, 2H, J = 6.6 Hz, Ar-H), 7.62-7.65 (t, 1H, J = 6.3 Hz, chromen-2-one-6H), 7.76 (s, 1H, pyrazole-5H), 7.84-7.88 (m, 3H, Ar-H,), 8.01-8.03 (d, 2H, J = 9.0 Hz, Ar-H), 8.15 (s, 1H, -CH=N), 8.53 (s, 1H, chromen-2-one-4H), 8.95 (s, 1H, thiazole-5H), 12.04 (s, 1H, -NH); ^{13}C-NMR (100 MHz, DMSO-d_6, ppm): δ 166.81, 158.30, 154.19, 151.70, 149.45, 143.29, 140.73, 137.77, 137.26, 134.31, 131.08, 130.41, 130.15, 130.06, 128.92, 124.11, 119.92, 119.73, 118.79, 116.51, 115.28, 114.98, 114.77, 109.91; MS: *m/z* = 542.1 (M+1), ANAL. Calcd. for $C_{28}H_{17}ClFN_5O_2S$; calcd: C, 62.05; H, 3.16; N, 12.92; found: C, 62.06; H, 3.16; N, 12.89.

2.4 PHARMACOLOGY

2.4.1 *In vitro* Antitubercular activity –Microplate Alamar Blue Assay (MABA) method

Antitubercular screening for the newly synthesized compounds $\mathbf{T_{1\text{-}12}}$ were determined by the Middle brook 7H9 broth against *Mycobacterium tuberculosis* of H_{37}Rv strain (ATCC-27294). The Minimum Inhibitory Concentration (MIC) of each synthesized compound was determined by the Microplate Alamar Blue Assay method (MABA). The lowest concentration of drug inhibits ≤ 99 % of bacterial population present at the beginning of the assay indicates the activity. This method is economical, non-toxic and shows good correlation with the BACTEC radiometric method (Lourenco *et al.* 2007). 200 µL of sterile de-ionized water was added to all outer perimeter wells of sterile 96 well plates to minimize the evaporation of medium in the test wells during incubation. The 96 well plates received 100 µL of the Middlebrook 7H9 broth and serial dilution of compounds was made directly on the plate. The final drug concentrations tested were 100-0.78 µg/mL. Plates were covered and sealed with the parafilm and incubated at 37 °C 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 indicated as bacterial growth. The MIC was defined as the lowest drug concentration, which prevented the color change from blue to pink. Pyrazinamide, Ethambutol and Streptomycin were used as standard drugs.

2.4.2 Antibacterial and antifungal studies

Antimicrobial screening of the newly synthesized compounds $\mathbf{T_{1\text{-}12}}$ were determined by the nutrient plate well diffusion method using a protocol explained elsewhere (Sathish *et al.* 2012). Antibacterial activity against 12 h old culture of Gram +ve *Staphylococcus aureus* (MTCC 3160) and Tuberculosis variant bacteria *Mycobacterium smegmatis* (MTCC 994) was determined by inhibition method. Antifungal activity of these compounds was also carried out against pathogenic fungi *Candida albicans* (MTCC 7253). All the bacterial and fungal cultures were purchased from the microbial type culture collection, IMTECH, Chandigarh, India and maintained the cultures as per the standard protocol. Nutrient agar plates were prepared and 100 µL of 0.5 McFarland standard of microbial culture was spread over agar medium. Using a sterile cork borer, 5 mm wells were made in the agar media. Working solution of the compounds was prepared in DMSO and 10 mg/mL solution was used for the test. Test compound volume of 50, 25 and 12.5 µL was transferred to separate wells in triplicates. Then agar plates were incubated in an incubator at 37 °C for 12 h and observed for the zone of inhibition. DMSO was used as negative control. Ciprofloxacin and Fluconazole were used as antibacterial and antifungal standards respectively. Inhibition zone was measured in millimeter using a scale.

All the synthesized thiazole compounds showed substantial inhibition of both bacteria and fungi, were tested further for the MIC. The Resazurin reduction method was used for determining the MIC value in 96 well microplates (Palomino *et al.* 2002, Driessche *et al.* 2014). The final drug concentrations tested were 100-0.78 µg/mL. All the compounds were dissolved in DMSO having a final stock concentration of 10 mg/mL. 50 µL of this stock solution was serially diluted to eight times and 50 µL of each serially diluted compound was added to microplate wells. All the microbial cultures were grown in nutrient agar to reach 0.5 McFarland concentrations and 50 µL of this culture was added to each well. Microplate contents were mixed well and incubated at room temperature for 12 h. After incubation, 10 µL of mixture from each

well was spread on the agar plate and checked for the Colony Forming Units (CFU). Further 30 µL of 0.1 % Resazurin solution was added to each well and incubated for another 24 h. Microplate well contents were observed for the change in color from blue to pink. Those wells that have microbes growing will change the blue Resazurin into pink color. The well which remains blue after 24 h of incubation indicates there are no microorganisms survived in the well, the minimum concentration where no microbial growth found are considered as MIC value.

2.5 RESULTS AND DISCUSSION

2.5.1 Chemistry

The target structures of the synthesized compounds were characterized by IR, NMR, mass spectral and elemental analyses. Formation of 1,3-disubstituted-1*H*-pyrazole-4-carbaldehydes **4a-l** was confirmed by recording their IR, ^{1}H-NMR, ^{13}C-NMR and MS spectrum. The IR data for compound **4c** was confirmed by the peak observed at 1672 cm^{-1} which is due to -CHO stretching of aldehyde group. Band at 1522 cm^{-1} is showing the presence of -C=N group. The ^{1}H-NMR spectrum of **4c** showed a singlet at δ 2.43 corresponding to the -CH_3 protons. A singlet at δ 10.05 is due to aldehyde proton of pyrazole moiety. Another singlet at δ 8.52 is due to pyrazole-5H proton. In ^{13}C-NMR spectrum, peak at 185.29 showed the presence of aldehyde carbon, other peaks for **4c** showed were listed in experimental section. The mass spectrum of **4c** showed a molecular ion peak at *m/z* = 263.2 (M+1), which is confirmed with the molecular formula $C_{17}H_{14}N_2O$. The characterization data of the synthesized compounds **4a-l** were presented in **Table 2.2**.

Table 2.2 Structural properties of the compounds **4a-l**

Compounds	R	X	M.F	M.Wt	M.p (° C)	Yield (%)
4a	H	H	$C_{16}H_{12}N_2O$	248.28	140-141	87
4b	H	Cl	$C_{16}H_{11}ClN_2O$	282.72	145-146	91
4c	CH_3	H	$C_{17}H_{14}N_2O$	262.31	121-123	88
4d	CH_3	Cl	$C_{17}H_{13}ClN_2O$	296.75	133-134	90
4e	OCH_3	H	$C_{17}H_{14}N_2O_2$	278.31	137-137	76
4f	OCH_3	Cl	$C_{17}H_{13}ClN_2O_2$	312.75	142-143	89
4g	Cl	H	$C_{16}H_{11}ClN_2O$	282.72	139-140	89
4h	Cl	Cl	$C_{16}H_{10}Cl_2N_2O$	317.17	177-178	85

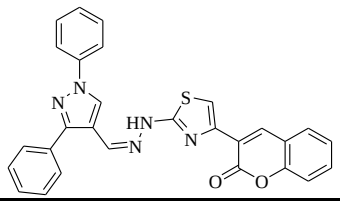
4i	Br	H	$C_{16}H_{11}BrN_2O$	327.18	139-140	69
4j	Br	Cl	$C_{16}H_{10}BrClN_2O$	361.62	156-157	79
4k	F	H	$C_{16}H_{11}FN_2O$	266.27	157-159	77
4l	F	Cl	$C_{16}H_{10}ClFN_2O$	300.71	204-205	66

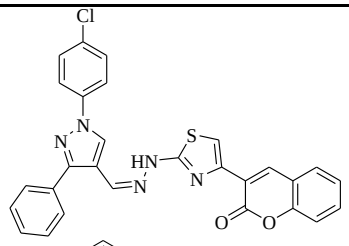
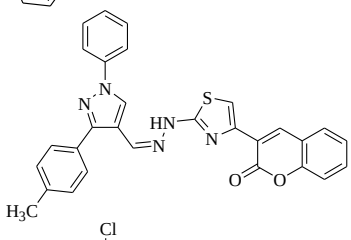
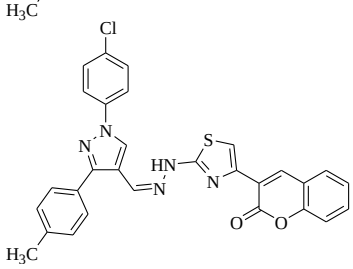
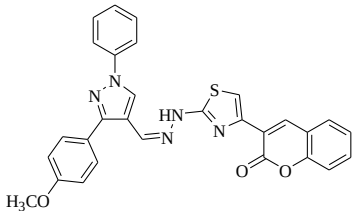
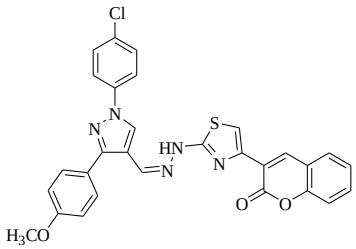
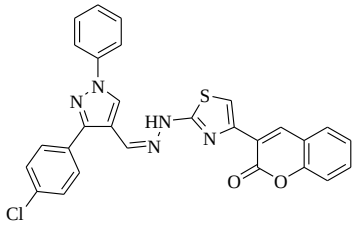
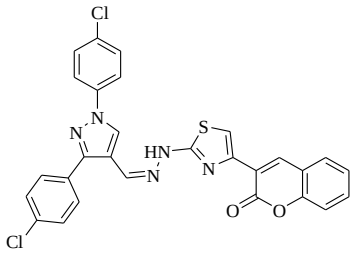
Formation of 3-{2-[N′-(1,3-disubstituted-1*H*-pyrazol-4-yl-methylene)-hydrazino]-thiazol-4-yl}-chromen-2-one (**T_{1-12}**) were confirmed by recording their IR, ^{1}H-NMR, ^{13}C-NMR and mass spectra. IR analysis of compound **T_1** showed the peak at 3412 cm^{-1}, which was due to the -NH group. Another absorption band at 3140 cm^{-1} was due to the -C-H stretching of the aromatic ring. The absorption band at 1720 cm^{-1} was due to the -C=O group, band at 1627 cm^{-1} due to the -C=N group, -C=C stretching was observed at 1497 cm^{-1} and an absorption band at 1094 cm^{-1} was due to the -C-O stretching, which confirmed the formation of compound **T_1**.

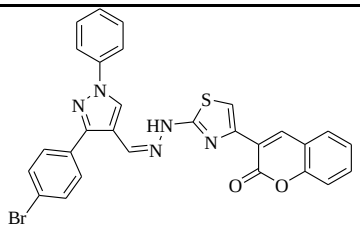
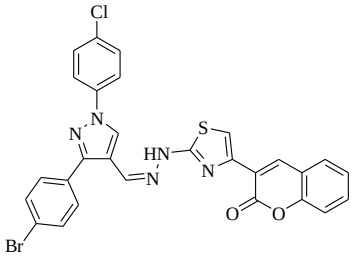
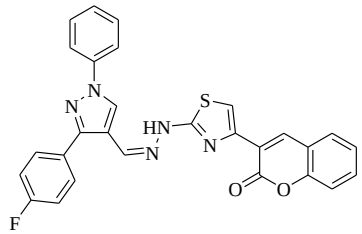
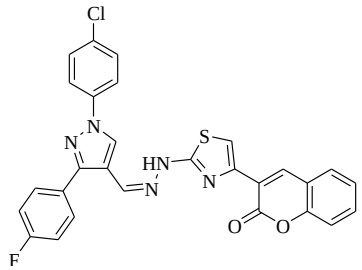
The ^{1}H-NMR spectrum of **T_1** in DMSO-d_6 solvent showed a singlet at δ 7.76 which was attributed to the thiazole-5H proton, another singlet attributed at δ 8.53 which was due to the chromen-2-one-4H proton. The characteristic peak of pyrazole-5H proton was observed as a singlet at δ 8.88 and the -NH proton was attributed at δ 12.03. The detailed ^{1}H-NMR resonances are summarized in the experimental section. The ^{13}C-NMR spectrum of compound **T_1** shows the peaks at 167.89, 159.22, 152.75, 151.36, 144.31, 139.50, 138.60, 135.64, 132.69, 132.14, 130.06, 129.29, 129.06, 128.95, 128.13, 127.35, 125.17, 120.99, 119.66, 119.19, 117.33, 116.34, 110.89.

The mass spectrum of **T_1** showed a molecular ion peak at *m/z* = 490.1 (M+1). This, in turn, confirmed the formation of a compound having the molecular formula $C_{28}H_{19}N_5O_2S$. The characterization data of the newly synthesized compounds **T_{1-12}** were presented in **Table 2.3**.

Table 2.3 Characterization data of the compounds **T_{1-12}**

Comp.	R/X	Structure	M. F/ M.Wt	M.P (°C)	Color & nature
T_1	H/H		$C_{28}H_{19}N_5O_2S$/ 489.55	188-190 (decom.)	Yellow solid

T_2	H/Cl		$C_{28}H_{18}ClN_5O_2S$/ 523.99	242-244 (decom.)	Yellow solid
T_3	CH_3/H		$C_{29}H_{21}N_5O_2S$/ 503.57	224-226 (decom.)	Yellow solid
T_4	CH_3/Cl		$C_{29}H_{20}ClN_5O_2S$/ 538.02	238-240 (decom.)	Yellow solid
T_5	OCH_3/H		$C_{29}H_{21}N_5O_3S$/ 519.57	225-227 (decom.)	Yellow solid
T_6	OCH_3/Cl		$C_{29}H_{20}ClN_5O_3S$/ 554.02	242-245 (decom.)	Yellow solid
T_7	Cl/H		$C_{28}H_{18}ClN_5O_2S$/ 523.99	243-245 (decom.)	Reddish yellow solid
T_8	Cl/Cl		$C_{28}H_{17}Cl_2N_5O_2S$/ 558.44	249-251 (decom.)	Yellow solid

T_9	Br/H		$C_{28}H_{18}BrN_5O_2S$/ 568.44	234-236 (decom.)	Reddish yellow solid
T_{10}	Br/Cl		$C_{28}H_{17}BrClN_5O_2S$/ 602.89	249-251 (decom.)	Yellow solid
T_{11}	F/H		$C_{28}H_{18}FN_5O_2S$/ 507.54	232-235 (decom.)	Reddish yellow solid
T_{12}	F/Cl		$C_{28}H_{17}ClFN_5O_2S$/ 541.98	252-255 (decom.)	Yellow solid

2.5.2. Biological results

The newly synthesized compounds **T_{1-12}** were tested for their antitubercular, antibacterial and antifungal activities. The MIC of the synthesized compounds against pathogenic bacteria *Mycobacterium tuberculosis* $H_{37}Rv$ was represented in **Figure 2.15**. All the compounds were tested at different concentrations ranging from 100-0.78 µg/mL against virulent strain *Mycobacterium tuberculosis* $H_{37}Rv$. Out of twelve compounds, six compounds have exhibited the activity with MIC ranging between 25-6.25 µg/mL. SAR relationship was explained for active compounds based on the MIC value. The compound **T_8** showed lowest MIC (6.25 µg/mL) among all other compounds and it was similarly active with the standard anti-TB drug Streptomycin (MIC value 6.25 µg/mL). The second lowest MIC (12.5 µg/mL) obtained for compound **T_3**, **T_5** and **T_{12}** against the tested microorganism and it was four-fold lesser

active than the anti-TB standard drug Pyrazinamide (MIC value 50 µg/mL). The compound $\mathbf{T_2}$ and $\mathbf{T_9}$ showed moderate activity with the MIC value 25 µg/mL. Other compounds were showed the least activity against *Mycobacterium tuberculosis*. This indicates that, 4-chlorophenyl substitution at 1,3-positions of pyrazole showed enhanced activity than the other compounds and it was proved for compound $\mathbf{T_8}$. Phenyl substitution with electron donating group on the third position of pyrazole ring was showed better antitubercular activity for compounds $\mathbf{T_3}$ and $\mathbf{T_5}$. By molecular modification of the present series of pyrazole bearing thiazole compounds may be useful as antitubercular agent after further investigations.

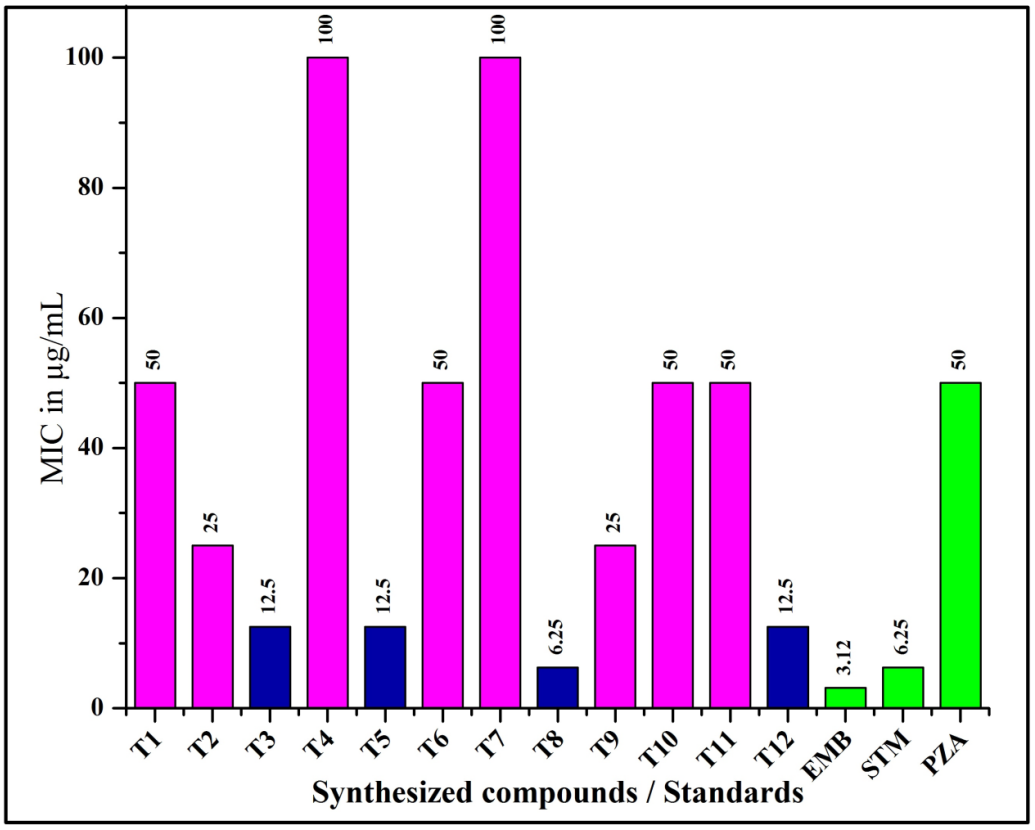


Figure 2.15 Minimum Inhibitory Concentration (MIC) of $\mathbf{T_{1\text{-}12}}$ against *Mycobacterium tuberculosis*

Zone of inhibition for all the compounds against *Mycobacterium smegmatis* was plotted in a bar diagram and represented in **Figure 2.16**. The **Figure 2.17** represents the zone of inhibition against *Staphylococcus aureus* (Gram +ve bacteria) at three different concentrations (1, 0.5 and 0.25 mg/mL) with a standard deviation of triplicates values. Antifungal zone of inhibition showed by the synthesized

compounds represented in **Figure 2.18**. Antibacterial standard (Ciprofloxacin, INN) and antifungal standard (Fluconazole, FLZ) were used as standards. Most of the compounds in this series showed good microbial inhibition for all the tested microorganisms such as *Mycobacterium smegmatis*, *Staphylococcus aureus* (Gram +ve bacteria) and *Candida albicans* (fungi) in well diffusion method. Compounds $\mathbf{T_2}$, $\mathbf{T_3}$, $\mathbf{T_8}$, $\mathbf{T_9}$ and $\mathbf{T_{11}}$ showed good zone of inhibition among all synthesized compounds with zone of inhibition up to 50 mm.

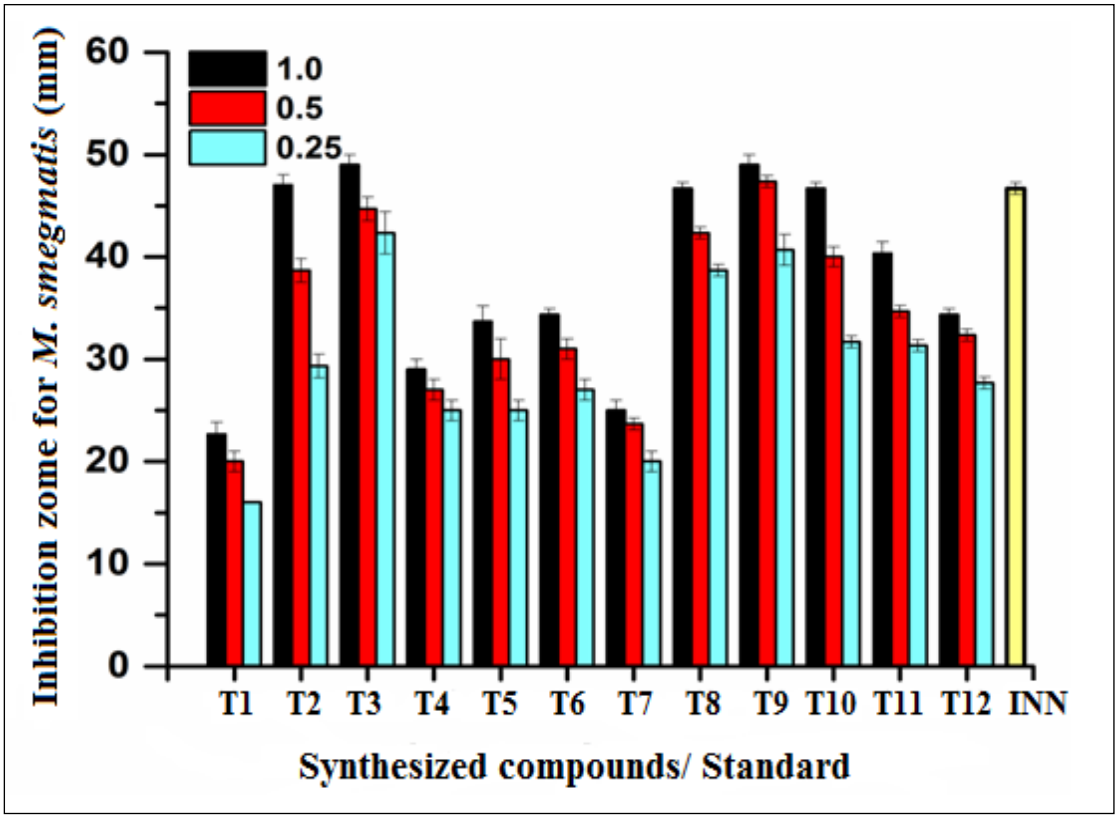


Figure 2.16 Anti-bacterial activity of pyrazole containing thiazole derivatives against *Mycobacterium smegmatis* (*M. smegmatis)* by the well diffusion method

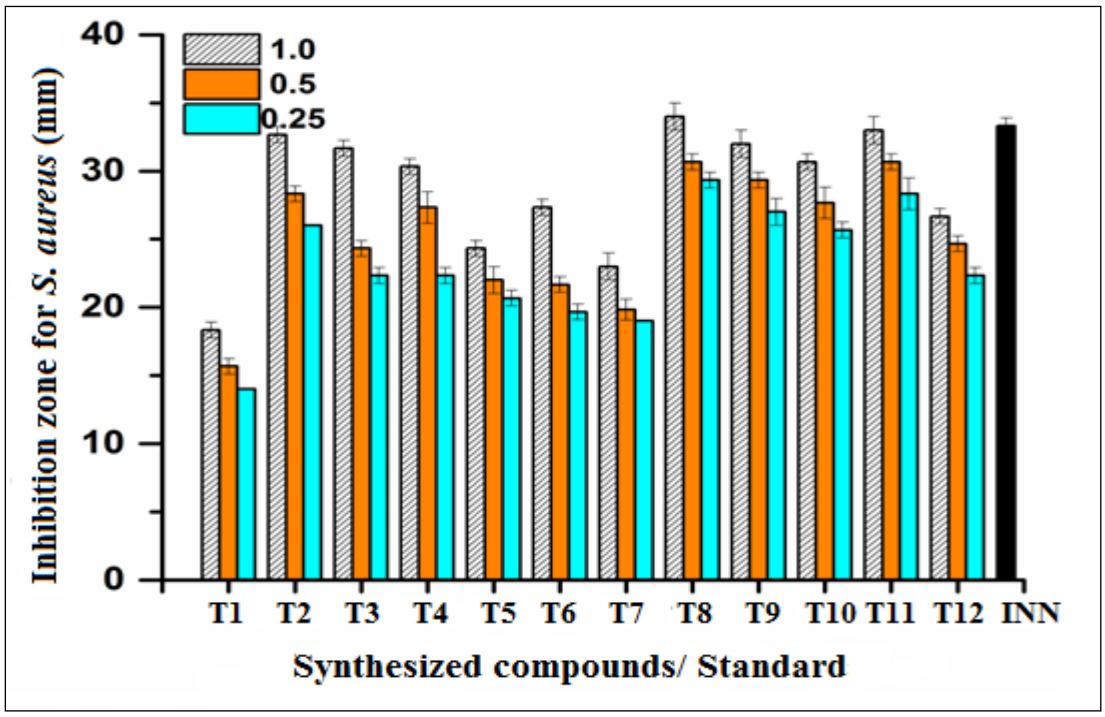


Figure 2.17 Anti-bacterial activity of pyrazole containing thiazole derivatives against *Staphylococcus aureus* (*S. aureus*) by the well diffusion method

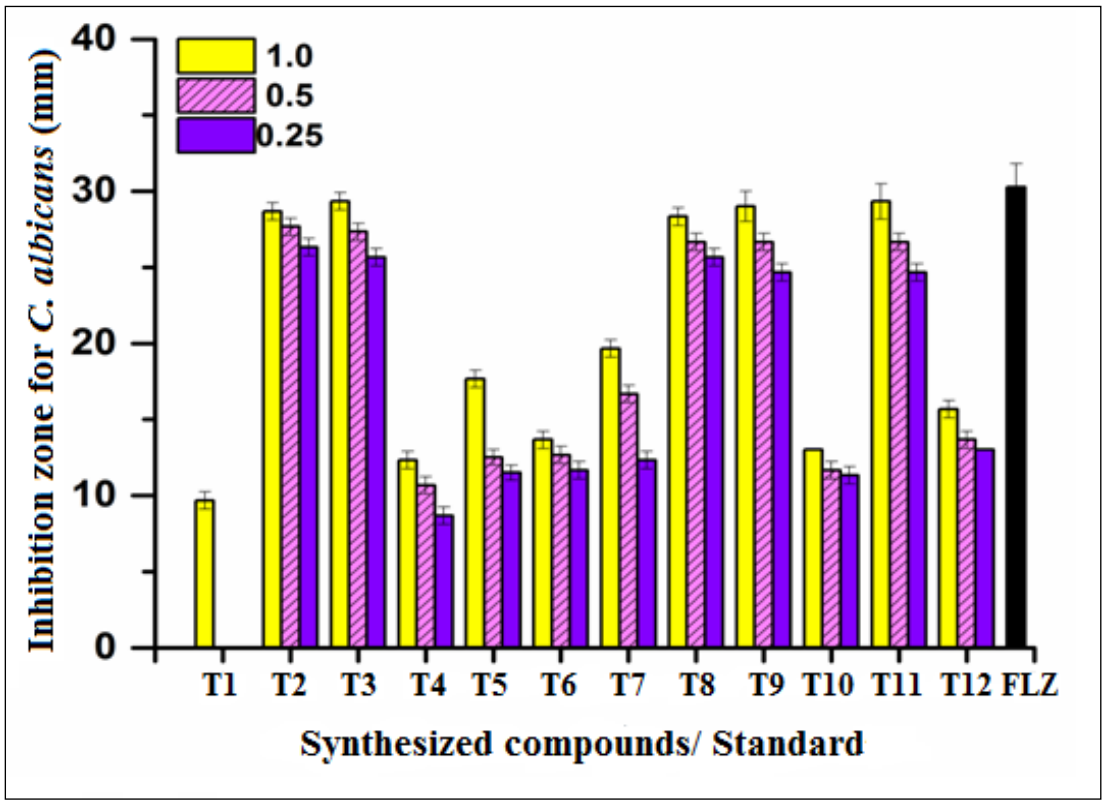


Figure 2.18 Anti-fungal activity of pyrazole containing thiazole derivatives against *Candida albicans* (*C. albicans*) by the well diffusion method

Structure activity relationship of all the synthesized compounds were explained based on the tested microorganisms. Among all the compounds, **T_2** has shown the best inhibition against all the tested microorganisms. This is due to the presence of 4-chlorophenyl at first position of the pyrazole ring. The presence of *p*-tolyl group (weak electron donating group) at third position of pyrazole has enhanced the activity of **T_3** against all the tested microorganisms. The compound **T_8** has showed significant inhibition against Gram +ve bacteria *Staphylococcus aureus*, which is due to the presence of 4-chlorophenyl group at first and third position of pyrazole ring respectively. The compound **T_9** has shown excellent inhibition on *Mycobacterium smegmatis*, which is due to the presence of 4-bromophenyl group at third position and phenyl group at first position of pyrazole ring. The compound **T_{10}** showed higher inhibition of bacteria and less for fungi indicating that, the compound may be a better antibacterial than antifungal due to the presence of 4-chlorophenyl and 4-bromophenyl at first and third position of pyrazole respectively. **T_{11}** has shown best inhibition on fungi *Candida albicans*, which is due to the presence of 4-fluorophenyl group at third position of the pyrazole ring. Among all, majority of the compounds were active against bacteria as well as fungi. This indicates that, the synthesized compounds are biologically active as antimicrobials. In the present series, *M. smegmatis* was inhibited to the maximum extent by most of the compounds.

MIC value of antimicrobial activity tested at different concentration from 100 to 0.78 µg/mL. MIC of **T_2** and **T_3** showed that, they are most active against bacteria *M. smegmatis* and fungi *C. albicans* with value of 15.6 µg/mL. The same compounds were showed moderate MIC value of 31.25 µg/mL against *S. aureus* (Gram +ve bacteria). MIC assay conducted for two bacteria and one fungi is represented in **Table 2.4**.

Table 2.4 Minimum Inhibitory Concentration (MIC) for the compounds **$T_{1\text{-}12}$**

Synthesized compounds	**MIC in µg/mL**		
	S. aureus	***M. smegmatis***	***C. albicans***
T_1	31.25	62.5	31.25
T_2	31.25	15.6	15.6
T_3	31.25	15.6	15.6
T_4	62.5	31.25	15.6
T_5	15.6	15.6	15.6

T_6	62.5	62.5	31.25
T_7	62.5	62.5	31.25
T_8	15.6	31.25	31.25
T_9	15.6	31.25	31.25
T_{10}	31.25	62.5	62.5
T_{11}	31.25	31.25	15.6
T_{12}	62.5	62.5	62.5
INN	<5	<5	--
FLZ	--	--	<10
Control	--	--	--

INN; antibacterial standard Ciprofloxacin; FLZ; anti-fungal standard Fluconazole; --: not detected inhibition; control; dimethylsulfoxide

2.6 CONCLUSIONS

A new series of 3-{2-[N′-(1,3-disubstituted-1*H*-pyrazol-4-yl-methylene)-hydrazino]-thiazol-4-yl}-chromen-2-one (**$T_{1\text{-}12}$**) derivatives were synthesized in good yields. They were characterized by IR, ^{1}H-NMR, ^{13}C-NMR, MS spectrometry and elemental analysis. Target compounds were investigated for their *in-vitro* antitubercular, antibacterial and anti-fungal activities and proved to be good antimicrobial compounds. Antitubercular activity exhibited least MIC value of 6.25 µg/mL. None of the compounds showed promising antitubercular activity. The compounds **T_2**, **T_3**, **T_8** and **T_9** exhibited the best inhibition against all the tested antibacterial and antifungal strains. The compound **T_{11}** has showed the excellent inhibition on fungi *Candida albicans* due to the presence of high electro-negativity of fluorine presence on phenyl group at third position of pyrazole. The compounds **T_2** and **T_3** showed the best MIC value against tested bacteria and fungi. The similarity between **T_2** and **T_3** is the pyrazole ring having phenyl substitution either at first or third position. Few of the synthesized compounds such as **T_2**, **T_3**, **T_8**, **T_9** and **T_{11}** have showed least MIC value (as low as 15.6 µg/mL) against most of the microorganisms. Most of the compounds showed a wide range of antimicrobial activity, which leads to conclude that, these compounds might be excellent antimicrobial compounds to combat with multidrug-resistant microorganisms.

CHAPTER 3

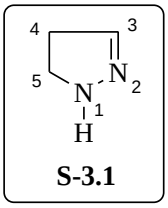
SYNTHESIS, ANTITUBERCULAR AND ANTIMICROBIAL ACTIVITY OF 1′-(4-CHLOROPHENYL) PYRAZOLE CONTAINING 3,5-DISUBSTITUTED PYRAZOLINE DERIVATIVES

Abstract

This chapter describes a detailed literature survey on pyrazoline derivatives up to August 2016. It includes the synthesis and biological importance of newly synthesized pyrazoline derivatives. The target pyrazole linked pyrazoline derivatives were synthesized by the condensation of 1,3-disubstituted-1H-pyrazole-4-carbaldehydes with substituted acetophenones to give chalcone intermediates. Which on reacting with hydrazine hydrate in ethanol media under reflux condition to give target 1'-(4-chlorophenyl)-5-(substituted aryl)-3'-(substituted aryl)-3,4-dihydro-2H, 1'H-[3,4']bipyrazolyl derivatives. Further, the synthesized bipyrazole derivatives were characterized and screened for their antitubercular, antibacterial and antifungal studies. Cytotoxicity studies were performed for active compounds against HeLa cell lines.

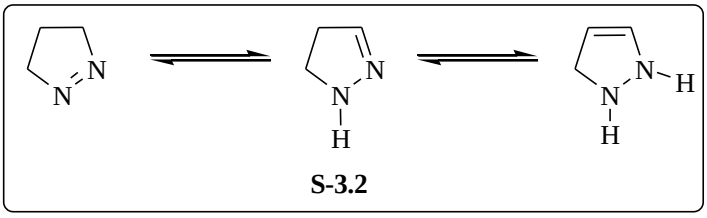
3.1 INTRODUCTION

Pyrazoline is an important monocyclic heterocycle containing two nitrogen atoms in a five-membered 1,2-diazole ring (**S-3.1**). Pyrazoline is an oil, having a boiling point of 114 °C and the molecular formula $C_3H_6N_2$.



S-3.1

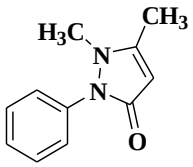
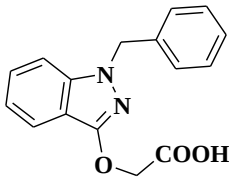
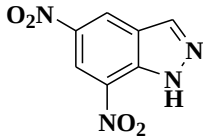
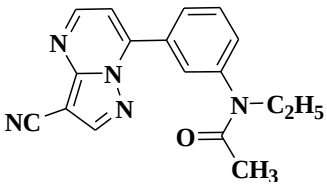
Pyrazoline or dihydropyrazole has three possible tautomeric forms *i.e.* Δ^1 -pyrazoline, Δ^2 -pyrazoline, Δ^3-pyrazoline presented in (**S-3.2**). Δ^2-Pyrazoline exhibits the monoimino character and hence more stable than the rest, even though all the three types have been synthesized. Fisher and Knovengel reported the formation of pyrazoline with an α,β-enone with hydrazine derivatives. These were synthesized by the reaction of acrolein with phenyl hydrazines. Later, Auwers proved the synthesis of the novel 1-phenyl-2-pyrazoline compound.

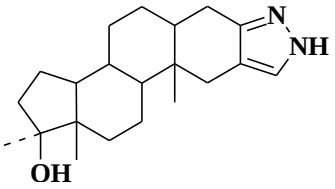
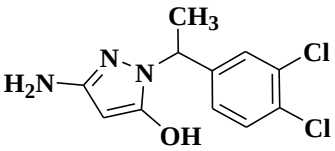
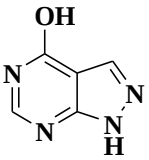


S-3.2

Pyrazoline derivatives represents one of the most desirable class of compounds with a wide variety of pharmacological activities *viz.*, antidiabetic (Garg and Singh 1969), antidepressant (Palaska *et al.* 2001, Abdel *et al.* 2009), antimicrobial (Chovatia *et al.* 2007, Vijesh *et al.* 2013), anti-inflammatory (Amir *et al.* 2008), anticancer (Dhanya *et al.* 2010, Isloor *et al.* 2012), antioxidant (Jois *et al.* 2014), antitubercular (Piyush *et al.* 2014 and Rana *et al.* 2014) and antifungal (Altinop *et al.* 2015). A systematic investigation of this class of compounds revealed that bipyrazole containing pharmacoactive agents plays an important role in medicinal chemistry. Literature survey revealed that, several synthetic protocols for the synthesis of these compounds and the presence of this core moiety in a molecule plays a key role in enhancing biological activities. Phenyl ring containing halogen and benzene sulfonamide substitutions at the first position of pyrazole have shown significant biological activities or enhance the biological activities of heterocyclic derivatives (Kuthiriya and Purohit *et al.* 2012, Ragab *et al.* 2013). Some of the pyrazole containing drugs were presented in **Table 3.1** for various therapeutic applications.

Table 3.1 Pyrazole containing some of the commercially available drugs

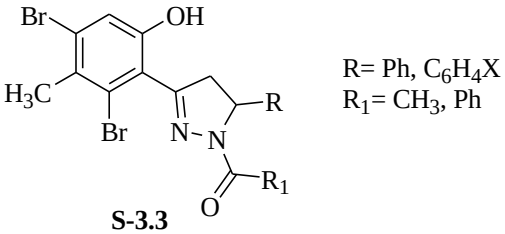
Drug name	Structure	Therapeutic use
Antipyrine		Analgesic
Bendazac		Anti-inflammatory
5,7-Dinitroindazole		Antibacterial
Zaleplon		Hypnotic/ sedative

Stanozolol		Androgenic/ anabolic
Muzolimine		Diuretic
Allopurinol		Treatment of gout

Tuberculosis remains as one of the leading infectious disease in the world, despite the availability of TB chemotherapy. This is further demonstrated by the fact that half a year of treatment with multiple drugs is needed. Treatment is also quite difficult due to the presence of metabolically silent, persistent or dormant bacteria within host lesions. While, there are many reasons for drug resistance, including the prescription of inadequate regimens, improper drug supply, and non-effective drugs, lengthy treatments are the major contributors. Many of the TB patients prematurely stop their therapy after an initial, rapid health improvement, thereby favoring the emergence of drug-resistant strains (Cole and Alzari, 2007).

Based on the above considerations and the need of effective anti-TB agents in the current scenario, it was planned to synthesize few biologically active pyrazoline derivatives. Some of the earlier reported potent pyrazoline derivatives with different biological activities were summarized below.

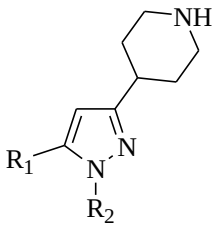
1-Acyl-3-(2-hydroxy-5-methyl-4,6-dibromophenyl)-5-(substituted phenyl)-2-pyrazoline derivatives (**S-3.3**) have been synthesized and characterized by Naik *et al.* (1999). These pyrazoline derivatives showed significant antibacterial activity when compared with the standard drug.



S-3.3

R= Ph, C_6H_4X
R_1= CH_3, Ph

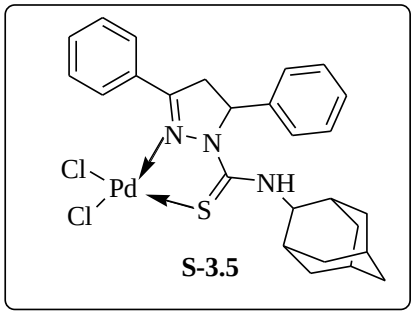
Tanitame *et al.* (2004) have synthesized pyrazole derivatives (**S-3.4**), which showed antibacterial activity and inhibition against DNA gyrase and topoisomerase IV. They found that, synthesized 5-[(E)-2-(5-chloroindol-3-yl)vinyl]pyrazole derivatives possesses potent antibacterial activity and selective inhibition against bacterial topoisomerases.



S-3.4

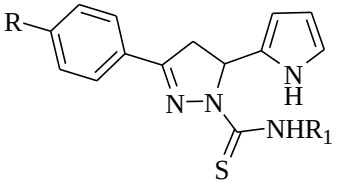
R_1= 4-$PhCH_2OPh$, 4-OCH_3Ph, 2-naphthyl
R_2= H, Me, 3-ClPh, CH_2Ph

A novel palladium (II) complexes of ^{1}N-substituted thiocarbamoyl-3,5-diphenyl-2-pyrazoline derivatives (**S-3.5**) were prepared by the condensation of benzaldehyde with acetophenones followed by cyclization with N^4-substituted thiosemicarbazides. The palladium (II) complexes of pyrazoline derivatives were characterized and tested for their *in vitro* antiamoebic activity by Asha *et al.* (2006). All the newly synthesized compounds were showed good *in vitro* antiamoebic activity.



S-3.5

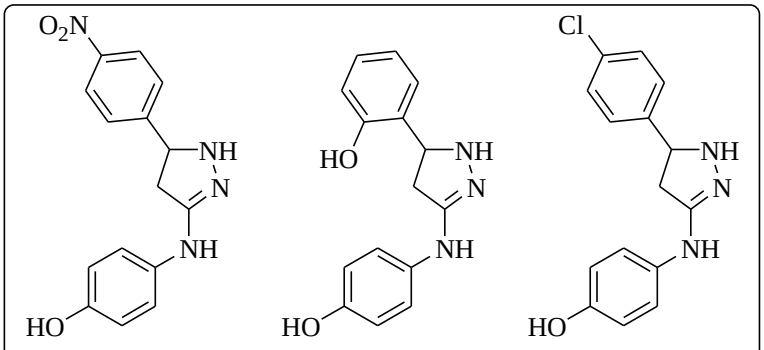
A new series of pyrazole derivatives (**S-3.6**) were synthesized by Nesrin *et al.* (2007). Synthesized compounds were tested for their dual monoamine oxidase (MAO) inhibitors, anti-inflammatory and analgesic activity. Most of the synthesized compounds showed promising activity against both MAO-A and MAO-B inhibition and also proved as anti-inflammatory and analgesic active agent.



S-3.6

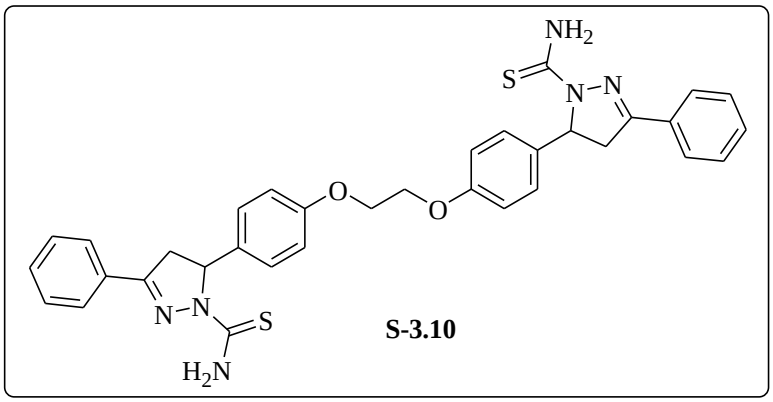
R= CH_3, Cl, OCH_3, C_2H_5
R_1= CH_3, C_2H_5, Ph, OCH_3

Sahu *et al.* (2008) synthesized a new series of pyrazoline derivatives and screened for their antimicrobial activity. Antibacterial activity was tested by Muller-Hinton agar plate method and antifungal activity was tested by Sabouraud dextrose agar plate method. Ciprofloxacin and Clotrimazole were used as standard drugs. All compounds showed significant antimicrobial activity. Further, all target compounds were tested for analgesic activity by tail flick method and anti-inflammatory activity by carrageenan induced rat paw edema method. Compounds (**S-3.7**, **S-3.8** and **S-3.9**) found to be potent antimicrobial, analgesic and anti-inflammatory agents.



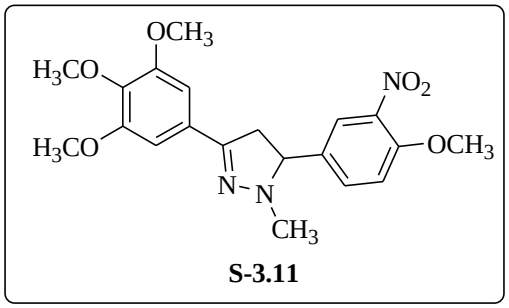
S-3.7 **S-3.8** **S-3.9**

A new series of bis(3-aryl)-4,5-dihydro-1*H*-pyrazoles-1-thiocarboxamides derivatives have been synthesized and tested for their anti-inflammatory activity by Barsoum *et al.* (2009). Compound (**S-3.10**) showed potent anti-inflammatory activity against carrageenan induced rat paw edema test.



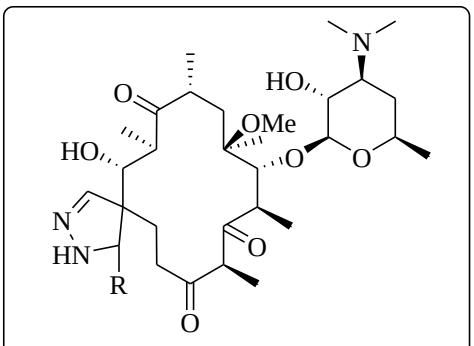
S-3.10

Novel disubstituted pyrazoline derivatives were prepared by Bonesi *et al.* (2010). These compounds were screened for their ACE-inhibitory activity and showed potential activity. One of the compound (**S-3.11**) showed ACE-inhibitory activity with IC_{50} 0.123 mM.



S-3.11

Both Erythromycin-susceptible and Erythromycin-resistance bacterial pyrazolinyl spiro ketolide derivatives (**S-3.12**) have been prepared and investigated by Hu *et al.* (2010). All the newly synthesized compounds were found to be more active than tested standard drug.

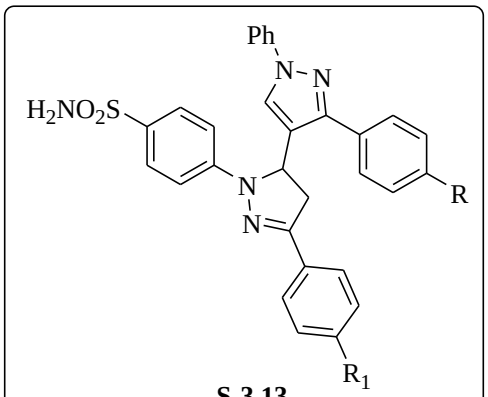


S-3.12

R= H, CH_3, $COOCH_3$, $COOC_2H_5$

A new series of sulfonamide derivatives containing pyrazoline (**S-3.13**) have been synthesized from chalcones with 4-hydrazinobenzenesulfonamide by Sharma *et*

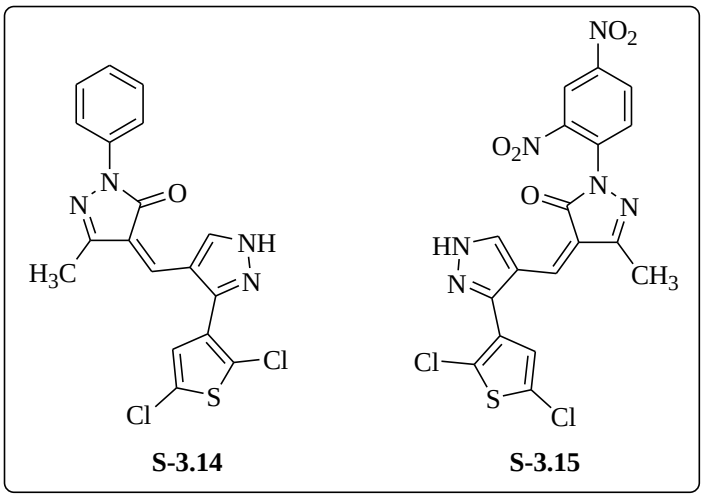
al. (2010). The synthesized compounds were confirmed and tested for their antibacterial activity against *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa* and *Escherichia coli*. Compound with fluoro and bromo substitution showed potent antibacterial activity.



S-3.13

R= H, CH_3, F, Br
R_1= H, CH_3, F, Br

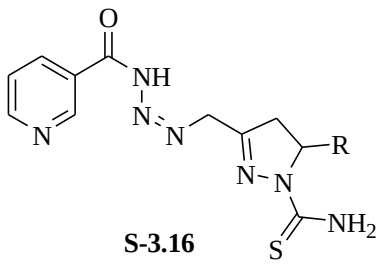
Vijesh *et al.* (2011) reported a new series of substituted pyrazolone derivatives by using various substituted carbaldehydes. These newly synthesized compounds were characterized by IR, NMR and mass spectra. New compounds were screened for their antimicrobial studies against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa*. Two compounds **S-3.14** and **S-3.15** having 2,5-dichlorothiophene substitution showed significant antibacterial activity against all tested microorganisms as compared to the standard drug Ceftriaxone.



S-3.14 **S-3.15**

Bindu *et al.* (2012) synthesized pyrazoline derivatives (**S-3.16**) from chalcone intermediates and were tested for their different activities. Resazurin microtiter assay

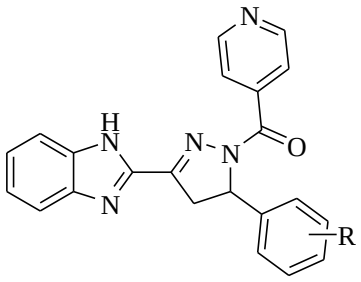
method used to identify the antitubercular activity using *Mycobacterium tuberculosis* $H_{37}Rv$ strain. The MIC of newly synthesized compounds showed potent anti-TB activity in the range of 1.25-6.25 µg/mL. And also showed the significant analgesic and anti-inflammatory activity.



S-3.16

R= 4-OMePh, 2-OHPh, Ph, 2-NH_{2-3}-MePh, 3-OH^{-4}-OMePh

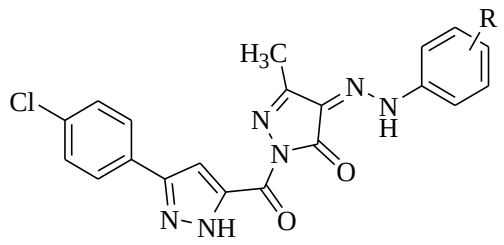
(3-1*H*-Benzo[*d*]imidazol-2-yl)-5-(substituted aryl)-4,5-dihydro-1*H*-pyrazol-1-yl)(pyridine-4-yl)methanone (**S-3.17**) were prepared from benzimidazolyl chalcones with Isoniazid have been reported by Desai *et al.* (2012). All compounds were confirmed by spectral techniques and were tested for antibacterial and antifungal activity. Few compounds were showed prominent results with MIC value 25 µg/mL.



S-3.17

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

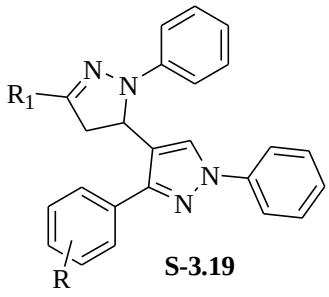
3-(4-Chlorophenyl)-4-substituted pyrazole derivatives (**S-3.18**) have been synthesized from ethyl-2-(arylhydrazono)-3-oxobutyrate with pyrazole acid hydrazide derivatives in moderate to good yields by Horrocks *et al.* (2013). These derivatives were screened for their antitubercular bacteria *Mycobacterium tuberculosis* and antifungal activity. Screening results suggested that, these compounds are promising antitubercular and antifungal agents.



S-3.18

R= H, 4-OCH_3, 2-Cl, 4-Cl, 4-NO_2, 4-F, 2-CH_3, 4-COOH, 3-Cl, 4-F, 3,4-Cl_2

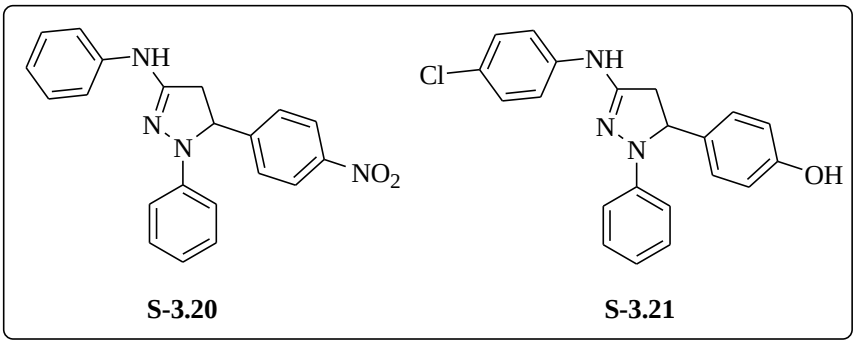
Shridhar *et al.* (2013) developed a new series of 3-aryl-4-(3-aryl-4,5-dihydro-1*H*-pyrazol-5-yl)-1-phenyl-1*H*-pyrazoles (**S-3.19**) from (E)-1-aryl-3-(3-aryl-1-phenyl-1*H*-pyrazol-4yl)prop-2-en-1-ones. All target compounds were evaluated for *in vitro* antibacterial activity against three bacterial strains, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* and *in vitro* antifungal activity against three pathogenic fungal strains *Aspergillus flavus*, *Chrysosporium keratinophilum* and *Candida albicans*. Some of the compounds were showed very good antibacterial and antifungal activity.



S-3.19

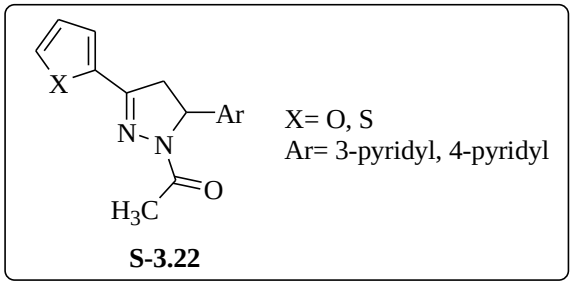
R= H, Cl, Br, OCH_3
R_1= Biphenyl, 4-$SCH_3C_6H_4$, 2,4-dichlorophenyl

Hipparagi and Mahesh (2013) have synthesized a series of 2-pyrazoline derivatives and were characterized by various spectral techniques. All newly synthesized derivatives were tested for their antitubercular activity. The purpose of this synthesis was to examine the molecular modification to identify antitubercular activity. Two compounds (**S-3.20** and **S-3.21**) showed significant activity as compared to the standard anti-TB drug.



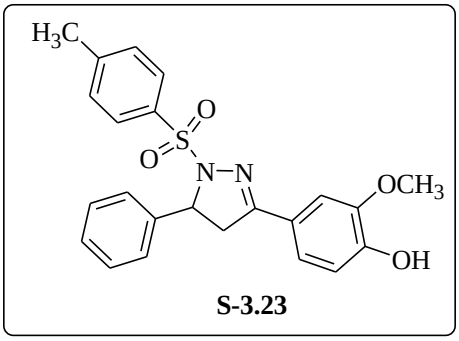
S-3.20 **S-3.21**

Pyrazoline derivatives (**S-3.22**) as COX-1, COX-2 and anti-inflammatory agents were developed and synthesized by Latif *et al.* (2015). All synthesized compounds have been evaluated for their *in vitro* COX-1 and COX-2 inhibitory and *in vitro* anti-inflammatory activity. All the compounds were active for their ulcerogenic liability when comparison with Celecoxib and Ibuprofen.



S-3.22

A new series of 2-methoxy-4-(5-phenyl-4,5-dihydro-1*H*-pyrazol-3-yl)phenol derivatives were developed for their human monoamine oxidase (MAO) inhibitory activity by Badavath *et al.* (2016). The majority of the compounds were reported to be selective and reversible towards hMAO-A. Compound (**S-3.23**) was found to be a potent inhibitor of hMAO-A with K_i = 0.06 ± 0.003 µM and was having selectivity index of 1.02 x 10^{-5}. Synthesized compound was far better than standard drug Moclobemide. Molecular docking studies were carried out to understand the crucial interactions responsible for selectivity and potency.



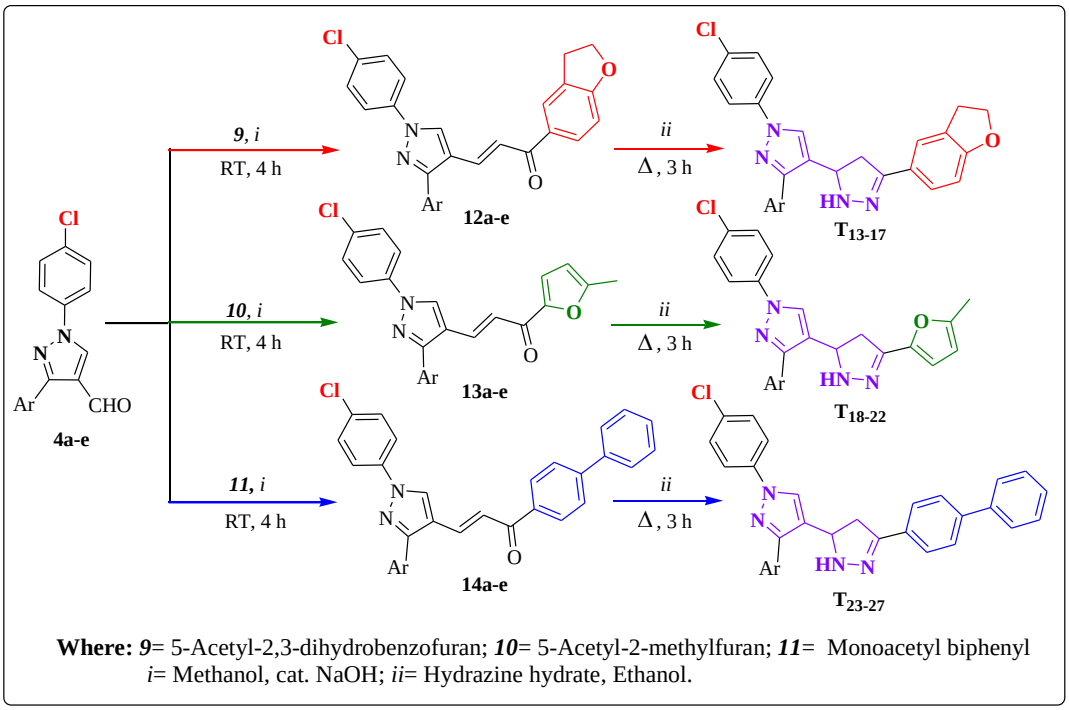
S-3.23

Based on the above considerations as well as the immediate need for the development of effective antitubercular drugs, a new series of 1′-(4-chlorophenyl) pyrazole containing pyrazoline derivatives was synthesized and its antimicrobial activity was evaluated. Antitubercular activity was performed against *Mycobacterium tuberculosis* $H_{37}Rv$ strain and antibacterial activity against *Mycobacterium smegmatis, Staphylococcus aureus* (Gram +ve bacteria) and antifungal activity against *Candida albicans.* Also, cytotoxicity studies against HeLa cell lines have been studied for active compounds. The key futures for anti-TB agents have determined that, the compound should be non-toxic, are specific for *Mycobacterium tuberculosis* and are bactericidal against non-replicating bacteria, features which are desirable in a new therapeutic for tuberculosis.

3.2 MATERIALS AND METHODS

The target compounds 1′-(4-chlorophenyl)-5-(2,3-dihydrobenzofuran-5-yl)-3′-(substituted aryl)-3,4-dihydro-2*H*, 1′*H*-[3,4′]bipyrazolyl (**T_{13-17}**), 1′-(4-chlorophenyl)-5-(5-methylfuran-2-yl)-3′-(substituted aryl)-3,4-dihydro-2*H*,1′*H*-[3,4′]bipyrazolyl (**T_{18-22}**) and 5-biphenyl-4-yl-1′-(4-chlorophenyl)-3′-(substituted aryl)-3,4-dihydro-2*H*,1′*H*-[3,4′]bipyrazolyl (**T_{23-27}**) were synthesized according to the steps outlined in **Scheme 3.1**. The basic pyrazole skeleton *i.e.* 1,3-disubstituted-1*H*-pyrazole-4-carbaldehydes **4a-e** were synthesized by the Vilsmeier-Haack reaction as per reported procedure as discussed in **Chapter 2**.

The other key starting materials, 5-acetyl-2,3-dihydrobenzofuran **9**, 2-acetyl-5-methylfuran **10** and 4-monoacetylbiphenyl **11** were prepared as per the reported literature (Alabaster *et al.* 1988) and confirmed by IR, NMR spectral data and melting point.



Scheme 3.1 Synthetic route for pyrazole bearing pyrazoline derivatives

All the chemicals were purchased from Sigma-Aldrich and Spectrochem-India. Melting points were determined by the open capillary method and were uncorrected. The IR spectra (in KBr pellet) were recorded on Perkin-Elmer FT-IR-4000-400 cm^{-1} spectrophotometer. NMR spectra were obtained on a Bruker Avance-400 spectrometer for ^{1}H-NMR and ^{13}C-NMR using tetramethylsilane (TMS) as the internal standard. Chemical shift and coupling constants are recorded in units of δ (ppm) and Hz, respectively. The mass spectrum was recorded on LC-MS Applied biosystems MDS SCIEX-API 4000 spectrometer. Elemental analysis was performed on a Flash EA 1112 series CHNS-O analyzer. The completion of the reaction was checked by thin layer chromatography (TLC) on silica gel coated, readymade aluminium sheets (Merck F_{254}). The names of the structures were mentioned as per ChemDraw Ultra 7.0 software.

3.3 EXPERIMENTAL

Experimental protocols followed for the synthesis of compounds **9**, **10**, **11** and **T_{13-27}** were given in the following section.

The synthetic procedure followed for the synthesis of compounds **4a-e** has been discussed in **Chapter 2**.

General procedure for the synthesis of 5-acetyl-2,3-dihydrobenzofuran (9)

Pre-chilled dichloromethane (DCM) (5 ml) was taken in a round bottom flask, added chloroacetylchloride (10.3 g, 0.091 mol) and charged with 2,3-dihydro benzofuran (10 g, 0.083 mol) at -5 to 0 °C. Powdered anhydrous aluminium chloride (12.2 g, 0.091 mol) was added in four equal parts at -5 to 0 °C. The temperature was maintained at 0 to 5 °C for 45 min. Further, the temperature was increased to 10-20 °C and again maintained for 45 min. The completion of the reaction was checked by TLC. After completion of the reaction, added 100 ml of DCM and quenched into ice cooled demineralized (DM) water. The organic layer was separated and washed with 100 ml of 5 % sodium bicarbonate solution. It was dried over anhydrous magnesium sulfate and distilled. Further 20 ml of isopropyl alcohol (IPA) was added to the oily mass and cooled to 0-5 °C. The compound was filtered and washed with pre-chilled IPA (10 ml) and dried at 50 °C. Yield: 12.0 g (89 %). M. p (°C): 62-63 (Reported M. p: 61-62 °C) (Alabaster *et al.* 1988). The same procedure was followed for the synthesis of compounds **10** and **11**.

5-Acetyl-2,3-dihydrobenzofuran (9). IR (KBr ν_{max} cm^{-1}): 2918 (C-H str, aliphatic), 1659 (C=O str), 1487 (C=C str), 1240 (C-O str); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 3.23-3.27 (t, 2H, CH_2, J = 8.4 Hz), 4.64-4.68 (t, 2H, CH_2, J = 8.4 Hz), 6.79-6.81 (d, 1H, Ar-H, J = 8.0 Hz), 7.79-7.81 (d, 1H, Ar-H, J = 7.6 Hz), 7.85 (s, 1H, Ar-H).

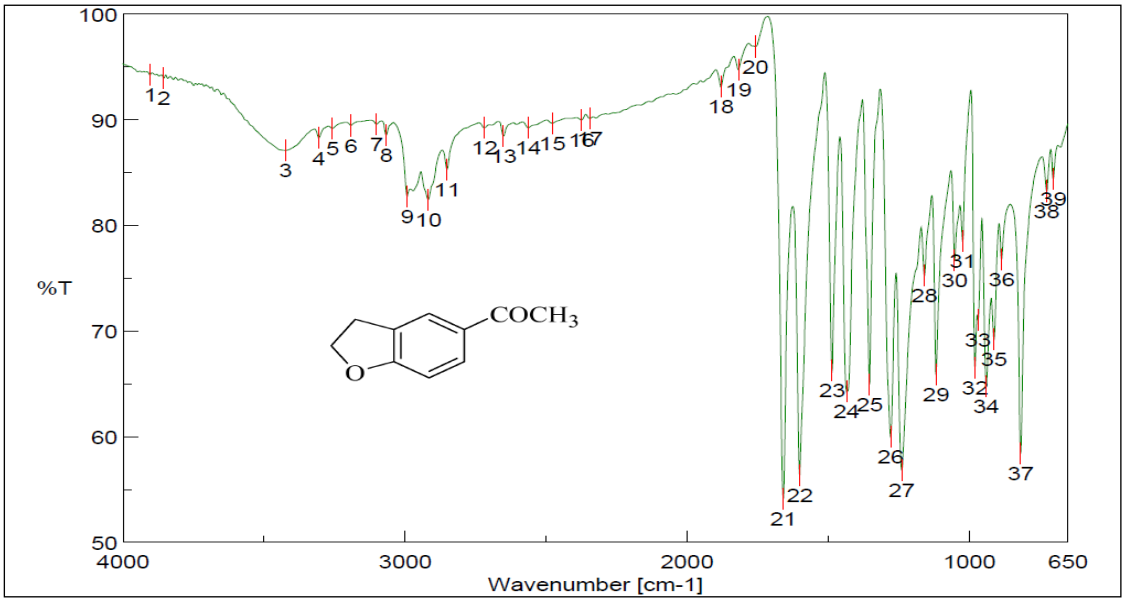


Figure 3.1 IR spectrum of compound **9**

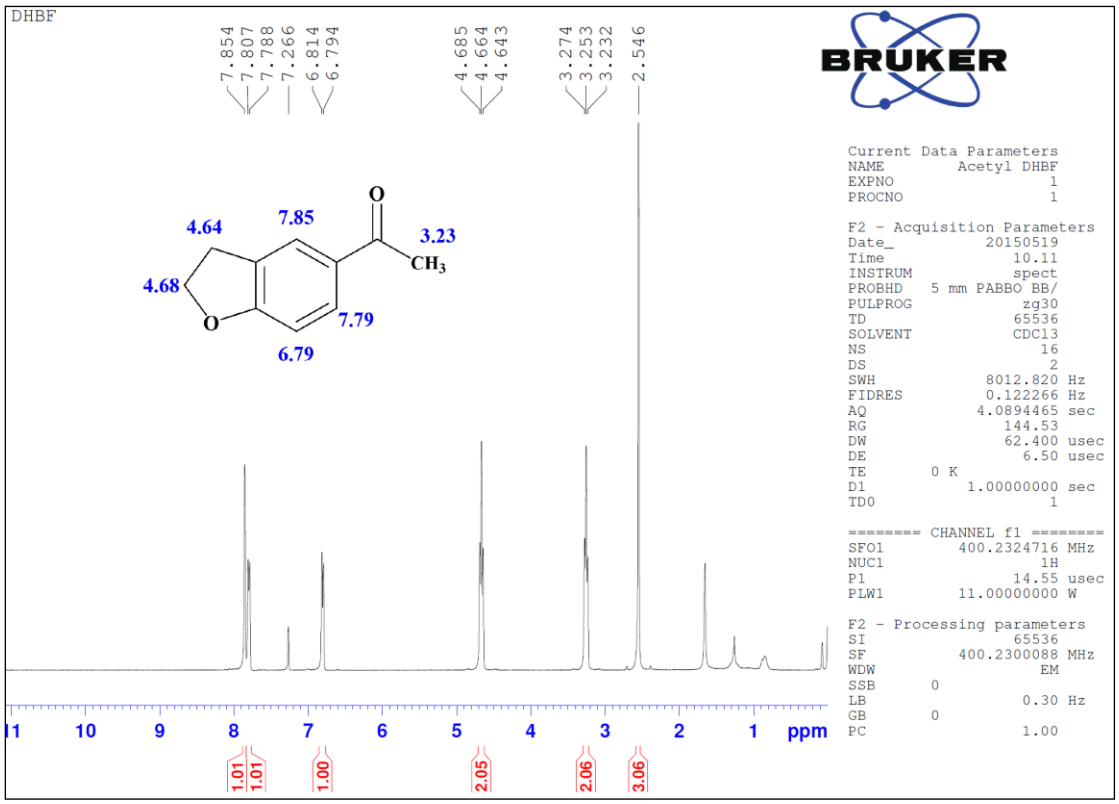


Figure 3.2 ^{1}H-NMR spectrum of compound **9**

4-Monoacetyl biphenyl (11). IR (KBr ν_{max} cm^{-1}): 1680 (C=O str), 1403 (C=C str); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 2.64 (s, 3H, -CH_3), 7.40-7.43 (t, 1H, Ar-H, J = 7.2 Hz), 7.46-7.50 (t, 2H, Ar-H, J = 7.4 Hz), 7.63-7.64 (d, 2H, Ar-H, J = 7.5 Hz), 7.68-7.70 (d, 2H, Ar-H, J = 8.3 Hz), 8.03-8.05 (d, 2H, Ar-H, J = 8.3 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): δ 26.7, 127.3, 128.3, 129.0, 135.9, 139.9, 145.8, 197.8.

General procedure for the synthesis of 1′-(4-chlorophenyl)-5-(substituted aryl)-3′-(substituted aryl)-3,4-dihydro-2*H*,1′*H*-[3,4′]bipyrazolyl (T_{13-17}), (T_{18-22}) and (T_{23-27}):

A mixture of 1,3-disubstituted-1*H*-pyrazole-4-carbaldehyde (**4a-e**) (0.01 mol), acetyl derivative (**9**, **10** and **11**) (0.01 mol) in ethanol (10 ml) were stirred in the presence of 10 % sodium hydroxide solution (2 ml) at ambient temperature for 5 h. The resultant yellow colored reaction mass was filtered and washed with 5 ml of ethanol, which resulted in chalcone intermediate (**12a-e**), (**13a-e**) and (**14a-e**) respectively in reasonably good yields (80-95 %). The chalcone intermediate (**12a-e**),

(**13a-e**) and (**14a-e**) (0.005 mol) was taken in ethanol (10 ml) and added excess amount of hydrazine hydrate (0.015 mol). The reaction mixture was heated at reflux temperature for 2 h and reaction was monitored by TLC [n-hexane : ethylacetate (4:1)]. The reaction mass was cooled to room temperature and stirred for 0.5 h. The solid product was filtered and washed with ethanol.

1′-(4-Chlorophenyl)-5-(2,3-dihydrobenzofuran-5-yl)-3′-phenyl-3,4-dihydro-2*H*,1′*H*-[3,4′]bipyrazolyl (T_{13}). Yield: 76 %; m.p. 156-157 °C; IR (KBr ν_{max} cm^{-1}): 3322 (N-H str), 3064 (Ar-H str), 2919 (C-H aliphatic str), 1607 (C=N str), 1497 (C=C str), 824 (C-Cl str); ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): δ 2.90-2.96 (t, 1H, H_A, J = 12.8 Hz), 3.19 (t, 2H, -CH_2), 3.42-3.49 (t, 1H, H_B, J = 13.6 Hz), 4.56 (t, 2H, -CH_2), 4.89-4.92 (t, 1H, H_X, J = 10.4 Hz), 6.77 (m, 1H, Ar-H), 7.33-7.36 (m, 2H, Ar-H), 7.43-7.56 (m, 6H, Ar-H), 7.77-7.95 (m, 4H, Ar-H), 8.62 (s, 1H, -NH); ^{13}C-NMR (100 MHz, DMSO-d_6, ppm): δ 160.5, 151.2, 138.8, 133.2, 130.7, 130.1, 129.9, 129.7, 129.1, 128.6, 128.4, 128.2, 128.0, 126.3, 122.9, 121.0, 120.2, 109.2, 71.7, 55.5, 29.3; MS: *m/z* = 441.2 (M+1), ANAL. Calcd. for $C_{26}H_{21}ClN_4O$; calcd: C, 70.82; H, 4.80; N, 12.71; found: C, 70.83; H, 4.80; N, 12.72.

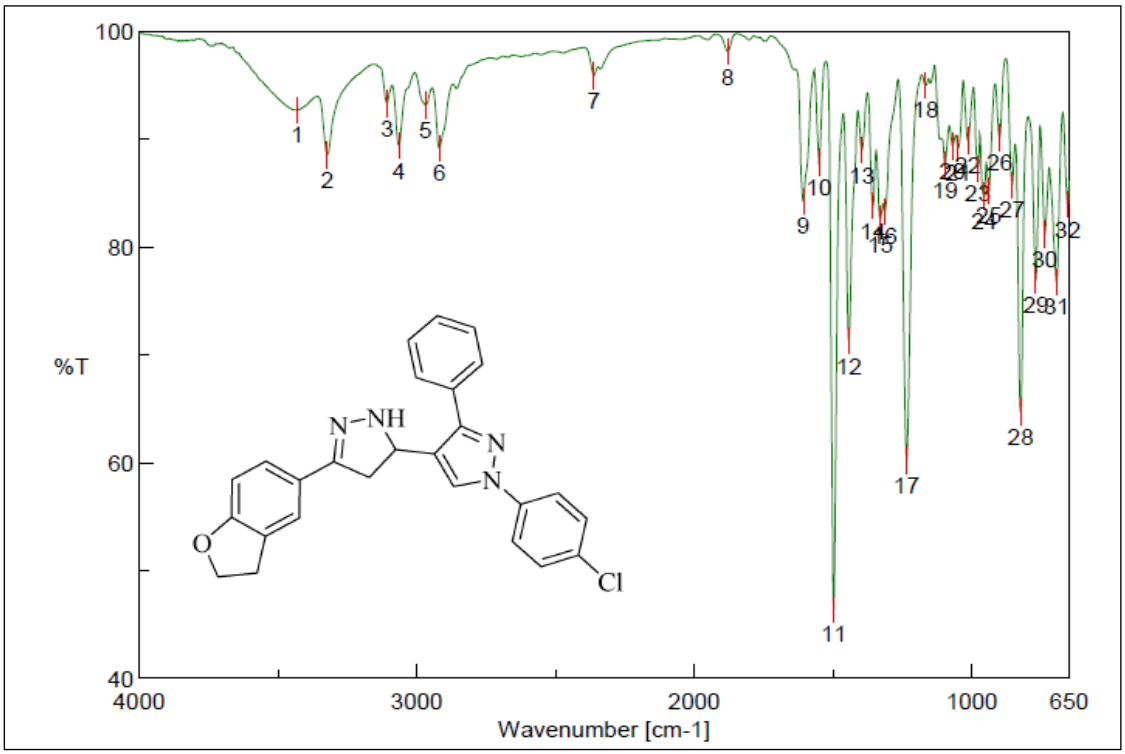


Figure 3.3 IR spectrum of compound **T_{13}**

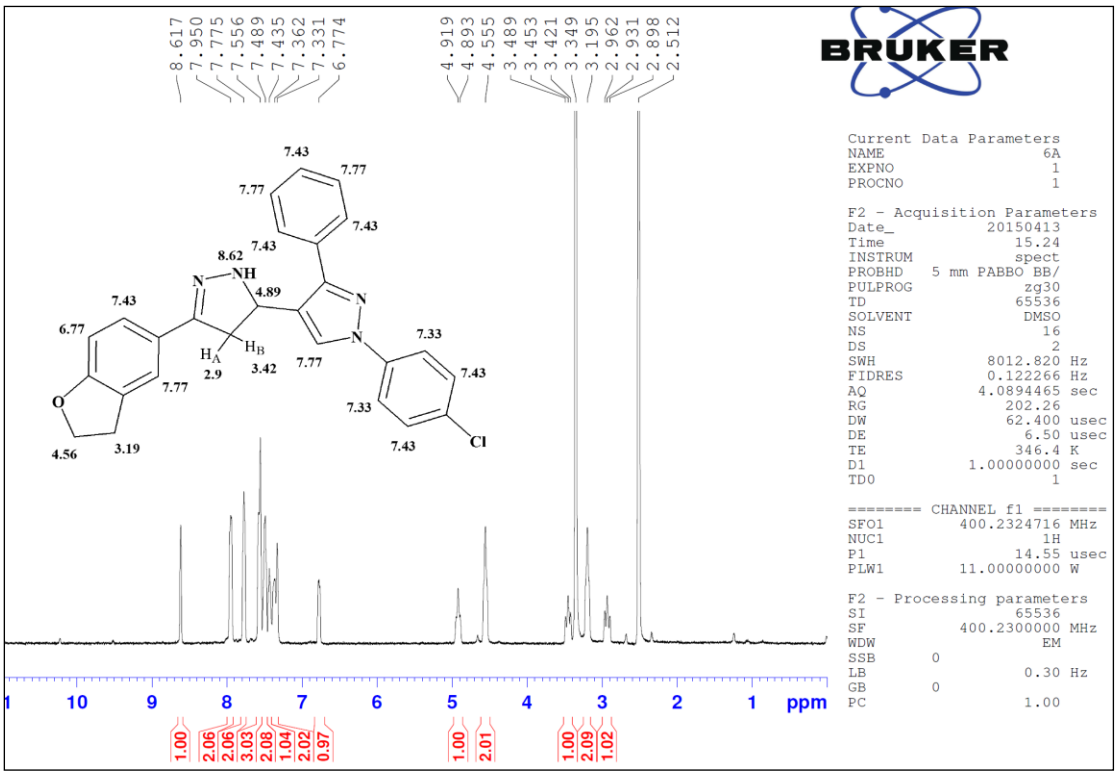


Figure 3.4 ^{1}H-NMR spectrum of compound **T_{13}**

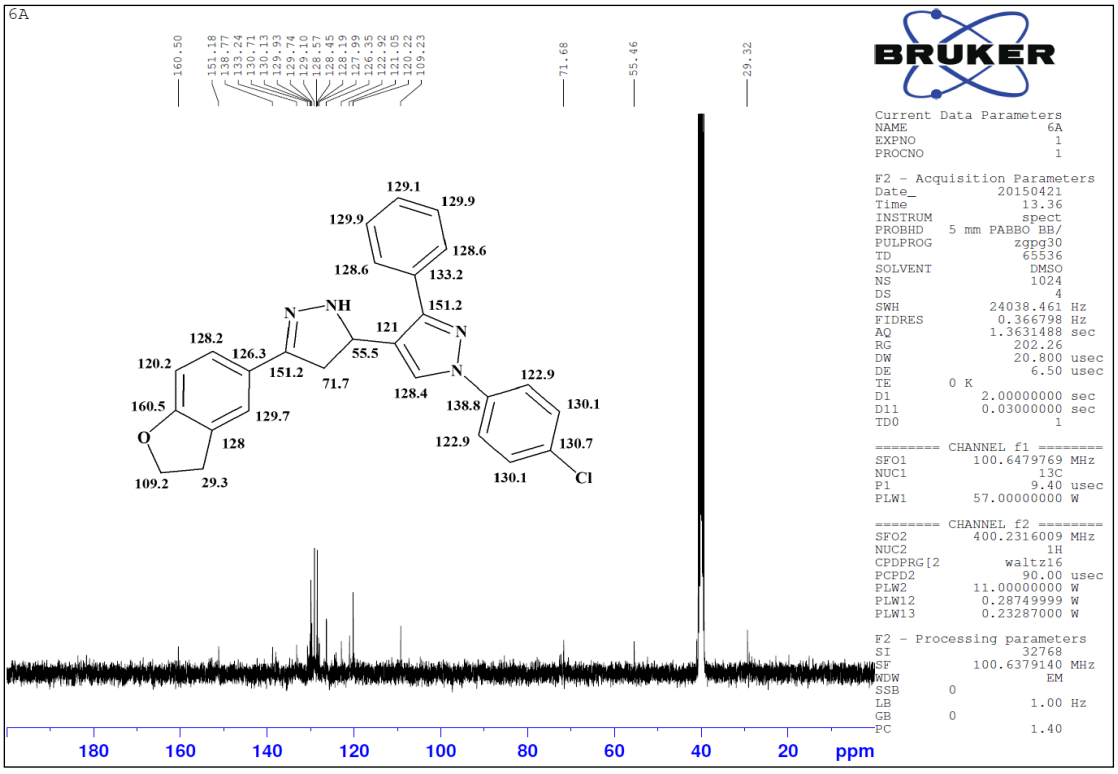


Figure 3.5 ^{13}C-NMR spectrum of compound **T_{13}**

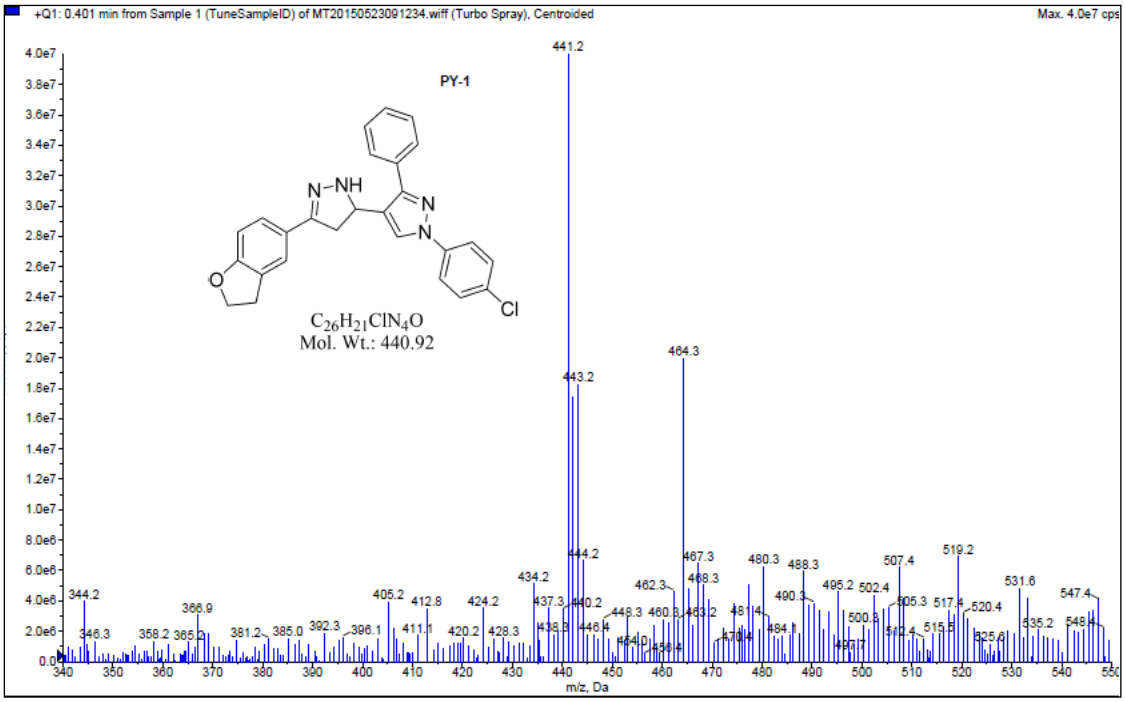


Figure 3.6 Mass spectrum of compound **T_{13}**

1′-(4-Chlorophenyl)-5-(2,3-dihydrobenzofuran-5-yl)-3′-*p*-tolyl-3,4-dihydro-2*H*,1′*H*-[3,4′]bipyrazolyl (T_{14}). Yield: 81 %; m.p. 151-153 °C; IR (KBr ν_{max} cm^{-1}): 3322 (N-H str), 3062 (Ar-H str), 2921 (C-H aliphatic str), 1610 (C=N str), 1497 (C=C str), 828 (C-Cl str); ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): δ 2.37 (s, 3H, -CH_3), 2.89-2.95 (t, 1H, H_A, J = 13.2 Hz), 3.19 (t, 2H, -CH_2), 3.36-3.46 (t, 1H, H_B, J = 12.4 Hz), 4.55 (t, 2H, -CH_2), 4.89-4.93 (t, 1H, H_X, J = 9.6 Hz), 6.76-6.77 (m, 1H, Ar-H), 7.30-7.36 (m, 4H, Ar-H), 7.55-7.67 (m, 5H, Ar-H), 7.92-7.94 (m, 2H, Ar-H), 8.58 (s, 1H, -NH); ^{13}C-NMR (100 MHz, DMSO-d_6, ppm): δ 160.5, 151.2, 150.3, 138.8, 137.9, 130.6, 130.4, 129.9, 129.7, 128.3, 128.2, 127.9, 126.4, 126.3, 124.0, 122.9, 120.1, 109.2, 71.7, 55.5, 29.3, 21.3; MS: *m/z* =455.2 (M+1), ANAL. Calcd. for $C_{27}H_{23}ClN_4O$; calcd: C, 71.28; H, 5.10; N, 12.31; found: C, 71.30; H, 5.11; N, 12.31.

1′-(4-Chlorophenyl)-5-(2,3-dihydrobenzofuran-5-yl)-3′-(4-methoxyphenyl)-3,4-dihydro-2*H*,1′*H*-[3,4′]bipyrazolyl (T_{15}). Yield: 69 %; m.p. 132-134 °C; IR (KBr ν_{max} cm^{-1}): 3309 (N-H str), 3070 (Ar-H str), 2921 (C-H aliphatic str), 1608 (C=N str), 1498 (C=C str), 830 (C-Cl str); ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): δ 2.89-2.95 (t, 1H, H_A, J = 13.0 Hz), 3.19 (t, 2H, -CH_2), 3.40-3.46 (t, 1H, H_B, J = 12.4 Hz), 3.81 (s,

3H, -OCH_3), 4.55 (t, 2H, -CH_2), 4.87-4.92 (t, 1H, H_X, *J* =10.4 Hz), 6.76-6.78 (m, 1H, Ar-H), 7.04-7.05 (m, 2H, Ar-H), 7.31 (s, 1H, Ar-H), 7.36-7.38 (m, 1H, Ar-H), 7.55 (b, 3H, Ar-H), 7.69-7.94 (m, 4H, Ar-H), 8.57 (s, 1H, -NH); ^{13}C-NMR (100 MHz, DMSO-d_6, ppm): δ 160.5, 159.7, 151.0, 150.3, 138.8, 130.5, 129.9, 129.7, 128.2, 127.8, 126.4, 126.3, 125.7, 123.8, 122.9, 120.1, 114.5, 109.2, 71.7, 55.6, 55.5, 29.3; MS: *m/z* =471.1 (M+1), ANAL. Calcd.for $C_{27}H_{23}ClN_4O_2$; calcd: C, 68.86; H, 4.92; N, 11.90; found: C, 68.90; H, 4.93; N, 11.91.

1′-(4-Chlorophenyl)-3′-(4-chlorophenyl)-5-(2,3-dihydrobenzofuran-5-yl)-3,4-dihydro-2*H*,1′*H*-[3,4′]bipyrazolyl (T_{16}). Yield: 75 %; m.p. 171-172 °C; IR (KBr ν_{max} cm^{-1}): 3315 (N-H str), 3062 (Ar-H str), 2928 (C-H aliphatic str), 1602 (C=N str), 1498 (C=C str), 831 (C-Cl str); ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): δ 2.89-2.95 (t, 1H, H_A, *J* = 12.8 Hz), 3.19 (t, 2H, -CH_2), 3.45-3.47 (m, 1H, H_B, *J* =11.6 Hz), 4.56 (t, 2H, -CH_2), 4.90-4.95 (t, 1H, H_X, *J* =9.8 Hz), 6.76-6.78 (d, 1H, Ar-H, *J* = 6.0 Hz), 7.33 (s, 1H, Ar-H), 7.36-7.37 (d, 1H, Ar-H, *J* = 6.4 Hz), 7.55-7.576 (m, 5H, Ar-H), 7.80-7.82 (m, 2H, Ar-H), 7.93-7.95 (d, 2H, Ar-H, *J* = 6.4 Hz), 8.62 (s, 1H, -NH); ^{13}C-NMR (100 MHz, DMSO-d_6, ppm): δ 160.5, 150.4, 149.9, 138.7, 133.3, 132.1, 130.9, 130.1, 130.0, 129.1, 128.3, 128.2, 126.4, 126.3, 124.2, 122.9, 120.3, 109.2, 71.7, 55.4, 29.3; MS: *m/z* =476.9 (M+1), ANAL. Calcd. for $C_{26}H_{20}Cl_2N_4O$; calcd: C, 65.59; H, 4.24; N, 11.79; found: C, 65.62; H, 4.25; N, 11.80.

1′-(4-Chlorophenyl)-5-(2,3-dihydrobenzofuran-5-yl)-3′-thiophene-2-yl-3,4-dihydro-2*H*,1′*H*-[3,4′]bipyrazolyl (T_{17}). Yield: 80 %; m.p. 140-141 °C; IR (KBr ν_{max} cm^{-1}): 3311 (N-H str), 3072 (Ar-H str), 2917 (C-H aliphatic str), 1602 (C=N str), 1497 (C=C str), 826 (C-Cl str); ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): δ 2.96-3.03 (t, 1H, H_A, *J* = 13.2 Hz), 3.25 (t, 2H, -CH_2), 3.51-3.58 (t, 1H, H_B, *J* = 13.2 Hz), 4.61 (t, 2H, -CH_2), 5.06-5.11 (t, 1H, H_X, *J* =9.8 Hz), 6.82-6.84 (m, 1H, Ar-H), 7.23-7.56 (m, 4H, Ar-H), 7.62-7.66 (m, 4H, Ar-H),7.95-7.97 (m, 2H, Ar-H), 8.64 (s, 1H, -NH); ^{13}C-NMR (100 MHz, DMSO-d_6, ppm): δ 160.5, 150.4, 145.7, 138.5, 135.3, 130.8, 130.0, 128.5, 128.2, 128.1, 126.7, 126.6, 126.4, 126.3, 123.6, 123.0, 120.1, 109.2, 71.7, 55.3, 29.3; MS: *m/z* =447.1 (M+1), ANAL. Calcd. for $C_{24}H_{19}ClN_4OS$; calcd: C, 64.49; H, 4.29; N, 12.94; found: C, 64.52; H, 4.31; N, 12.98.

1′-(4-Chlorophenyl)-5-(5-methylfuran-2-yl)-3′-phenyl-3,4-dihydro-2*H*,1′*H*-[3,4′]bipyrazolyl (T_{18}). Yield: 62 %; m.p. 144-145 °C; IR (KBr ν_{max} cm^{-1}): 3316 (N-H str), 3117 (Ar-H str), 2923 (C-H aliphatic str), 1594 (C=N str), 1499 (C=C str), 829 (C-Cl str); ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): δ 2.31 (s, 3H, -CH_3), 2.84-2.90 (t, 1H, H_A, J = 13.0 Hz), 3.31 (m, 1H, H_B), 4.89-4.95 (t, 1H, H_X, J =10.6 Hz), 6.17 (s, 1H, Ar-H), 6.50 (s, 1H, Ar-H), 7.44-7.76 (m, 8H, Ar-H), 7.94-7.95 (d, 2H, Ar-H, J = 6.0 Hz), 8.59 (s, 1H, -NH); ^{13}C-NMR (100 MHz, DMSO-d_6, ppm): δ152.9, 151.1, 147.3, 142.1, 138.7, 133.2, 130.7, 129.9, 129.1, 128.6, 128.4, 128.0, 123.7, 120.2, 110.9, 108.3, 55.2, 13.9; MS: *m/z* =403.2 (M+1), ANAL. Calcd. for $C_{23}H_{19}ClN_4O$; calcd: C, 68.57; H, 4.75; N, 13.91; found: C, 68.60; H, 4.76; N, 13.92.

1′-(4-Chlorophenyl)-5-(5-methylfuran-2-yl)-3′-*p*-tolyl-3,4-dihydro-2*H*,1′*H*-[3,4′]bipyrazolyl (T_{19}). Yield: 72 %; m.p. 206-208 °C; IR (KBr ν_{max} cm^{-1}): 3321 (N-H str), 3118 (Ar-H str), 2924 (C-H aliphatic str), 1590 (C=N str), 1500 (C=C str), 829 (C-Cl str); ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): δ 2.30 (s, 3H, -CH_3), 2.37 (s, 3H, -CH_3), 2.82-2.88 (t, 1H, H_A, J = 13.2 Hz), 3.36 (m, 1H, H_B), 4.89-4.94 (t, 1H, H_X, J =9.6 Hz), 6.17 (s, 1H, Ar-H), 6.50 (s, 1H, Ar-H), 7.30-7.65 (m, 7H, Ar-H), 7.92-7.94 (d, 2H, Ar-H, J = 7.6 Hz), 8.56 (s, 1H, -NH); ^{13}C-NMR (100 MHz, DMSO-d_6, ppm): δ 152.9, 147.3, 142.1, 138.8, 137.9, 130.6, 130.4, 129.9, 129.7, 128.3, 127.9, 123.6, 120.2, 110.9, 108.3, 55.2, 21.3, 13.9; MS: *m/z* =415.2 (M-1), ANAL. Calcd. for $C_{24}H_{21}ClN_4O$; calcd: C, 69.14; H, 5.08; N, 13.44; found: C, 69.16; H, 5.09; N, 13.46.

1′-(4-Chlorophenyl)-5-(5-methylfuran-2-yl)-3′-(4-methoxyphenyl)-3,4-dihydro-2*H*,1′*H*-[3,4′]bipyrazolyl (T_{20}). Yield: 79 %; m.p. 156-157 °C; IR (KBr ν_{max} cm^{-1}): 3317 (N-H str), 3121 (Ar-H str), 2927 (C-H aliphatic str), 1585 (C=N str), 1499 (C=C str), 831 (C-Cl str); ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): δ 2.30 (s, 3H, -CH_3), 2.82-2.89 (t, 1H, H_A, J = 12.4 Hz), 3.33 (m, 1H, H_B), 3.82 (s, 3H, -OCH_3), 4.90 (m, 1H, H_X), 6.17 (s, 1H, Ar-H), 6.50 (s, 1H, Ar-H), 7.05-7.92 (m, 9H, Ar-H), 8.54 (s, 1H, -NH); ^{13}C-NMR (100 MHz, DMSO-d_6, ppm): δ 159.7, 152.9, 150.9, 147.3, 142.1, 138.8, 130.5, 129.9, 129.7, 127.8, 125.6, 123.3, 120.1, 114.5, 110.9, 108.3, 55.6, 55.3, 13.9; MS: *m/z* =433.2 (M+1), ANAL. Calcd.for $C_{24}H_{21}ClN_4O_2$; calcd: C, 66.59; H, 4.89; N, 12.94; found: C, 66.65; H, 4.91; N, 12.96.

1′-(4-Chlorophenyl)-3′-(4-chlorophenyl)-5-(2,3-dihydrobenzofuran-5-yl)-3,4-dihydro-2*H*,1′*H*-[3,4′]bipyrazolyl (T_{21}). Yield: 77 %; m.p. 198-200 °C; IR (KBr ν_{max} cm^{-1}): 3318 (N-H str), 3119 (Ar-H str), 2924 (C-H aliphatic str), 1597 (C=N str), 1501 (C=C str), 830 (C-Cl str); ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): δ 2.30 (s, 3H, -CH_3), 2.82-2.89 (t, 1H, H_A, J = 13.2 Hz), 3.30 (m, 1H, H_B), 4.87-4.92 (t, 1H, H_X, J = 10.2 Hz), 6.17 (s, 1H, Ar-H), 6.50 (s, 1H, Ar-H), 7.32 (b, 2H, Ar-H), 7.45 (s, 1H, Ar-H), 7.56-7.80 (m, 4H, Ar-H), 7.93-7.95 (d, 2H, Ar-H, J = 7.2 Hz), 8.59 (s, 1H, -NH); ^{13}C-NMR (100 MHz, DMSO-d_6, ppm): δ 163.7, 161.3, 152.9, 150.2, 147.3, 142.2, 138.7, 130.8, 130.6, 130.5, 129.9, 129.7, 128.1, 123.6, 120.3, 116.1, 115.9, 110.9, 108.3, 55.1, 13.9; MS: *m/z* =435.0 (M-1), ANAL. Calcd. for $C_{23}H_{18}Cl_2N_4O$; calcd: C, 63.17; H, 4.15; N, 12.81; found: C, 63.20; H, 4.16; N, 12.83.

1′-(4-Chlorophenyl)-5-(2,3-dihydrobenzofuran-5-yl)-3′-thiophene-2-yl-3,4-dihydro-2*H*,1′*H*-[3,4′]bipyrazolyl (T_{22}). Yield: 60 %; m.p. 186-187 °C; IR (KBr ν_{max} cm^{-1}): 3312 (N-H str), 3125 (Ar-H str), 2919 (C-H aliphatic str), 1590 (C=N str), 1499 (C=C str), 830 (C-Cl str); ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): δ 2.31 (s, 3H, -CH_3), 2.84-2.91 (t, 1H, H_A, J = 13.0 Hz), 3.39-3.43 (t, 1H, H_B, J = 13.2 Hz), 5.00-5.05 (t, 1H, H_X, J = 10.0 Hz), 6.18 (s, 1H, Ar-H), 6.53 (s, 1H, Ar-H), 7.18 (b, 1H, Ar-H), 7.45-7.61 (m, 5H, Ar-H), 7.89-7.91 (d, 2H, Ar-H, J = 6.8 Hz), 8.56 (s, 1H, -NH); ^{13}C-NMR (100 MHz, DMSO-d_6, ppm): δ 163.6, 161.3, 152.9, 150.2, 147.3, 142.2, 138.7, 130.8, 130.6, 130.0, 129.7, 128.1, 123.6, 120.3, 116.1, 115.9, 110.9, 108.3, 55.3, 13.9; MS: *m/z* =409.1 (M+1), ANAL. Calcd. for $C_{21}H_{17}ClN_4OS$; calcd: C, 61.68; H, 4.19; N, 13.70; found: C, 61.70; H, 4.20; N, 13.72.

5-Biphenyl-4-yl-1′-(4-chlorophenyl)-3′-phenyl-3,4-dihydro-2*H*,1′*H*-[3,4′]bipyrazolyl (T_{23}). Yield: 88 %; m.p. 164-165 °C; IR (KBr ν_{max} cm^{-1}): 3312 (N-H str), 3073 (Ar-H str), 2921 (C-H aliphatic str), 1593 (C=N str), 1497 (C=C str), 830 (C-Cl str); ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): δ 2.97-3.03 (dd, 1H, H_A, J = 10.9 Hz), 3.49-3.56 (dd, 1H, H_B, J = 10.7 Hz), 4.97-5.03 (dt, 1H, Hx), 7.30-7.38 (m, 2H, Ar-H), 7.45-7.55 (m, 6H, Ar-H), 7.64-7.65 (d, 1H, Ar-H, J =3.3 Hz), 7.68 (s, 1H, pyrazole-5H), 7.70-7.71 (dd, 5H, Ar-H, J = 1.4 Hz), 7.80-7.82 (d, 2H, Ar-H, J = 8.5 Hz), 7.89-7.91 (d, 2H, Ar-H, J = 7.7 Hz), 8.61 (s, 1H, -NH); MS: *m/z* = 475.1 (M+1),

ANAL. Calcd.for $C_{30}H_{23}ClN_4$; calcd: C, 75.86; H, 4.88; N, 11.80; found: C, 75.90; H, 4.90; N, 11.90.

5-Biphenyl-4-yl-1′-(4-chlorophenyl)-3′-*p*-tolyl-3,4-dihydro-2*H*,1′*H*-[3,4′]bipyrazolyl (T_{24}). Yield: 84 %; m.p. 157-158 °C; IR (KBr ν_{max} cm^{-1}): 3318 (N-H str), 3066 (Ar-H str), 2926 (C-H aliphatic str), 1593 (C=N str), 1497 (C=C str), 830 (C-Cl str); ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): δ 2.37 (s, 3H, -CH_3), 2.98-3.04 (t, 1H, H_A, J = 13.2 Hz), 3.49-3.56 (t, 1H, H_B, J = 13.4 Hz), 4.97-5.02 (t, 1H, H_X, J = 10.4 Hz), 7.31-7.48 (m, 5H, Ar-H), 7.56-7.58 (d, 2H, Ar-H, J = 6.8 Hz), 7.62 (s, 1H, pyrazole-5H),7.69-7.72 (m, 8H, Ar-H),7.94-7.95 (d, 2H, Ar-H, J = 5.6 Hz), 8.62 (s, 1H, -NH); ^{13}C-NMR (100 MHz, DMSO-d_6, ppm): δ 151.2, 149.4, 140.0, 138.8, 137.9, 132.8, 130.6, 130.4, 129.9, 129.7, 129.5, 128.4, 128.0, 127.9, 127.2, 127.7, 126.6, 123.9, 120.2, 55.7, 21.3; MS: *m/z* =489.3 (M+1), ANAL. Calcd. for$C_{31}H_{25}ClN_4$; calcd: C, 76.14; H, 5.15; N, 11.46; found: C, 76.15; H, 5.16; N, 11.46.

5-Biphenyl-4-yl-1′-(4-chlorophenyl)-3′-(4-methoxyphenyl)-3,4-dihydro-2*H*,1′*H*-[3,4′]bipyrazolyl (T_{25}). Yield: 86 %; m.p. 160-162 °C; IR (KBr ν_{max} cm^{-1}): 3318 (N-H str), 3066 (Ar-H str), 2932 (C-H aliphatic str), 1610 (C=N str), 1496 (C=C str), 833 (C-Cl str); ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): δ 2.96-3.03 (dd, 1H, H_A, J = 10.7 Hz), 3.47-3.54 (dd, 1H, H_B, J = 10.7 Hz), 3.80 (s, 3H, -OCH_3), 4.93-4.99 (dt, 1H, Hx), 7.03-7.05 (d, 2H, Ar-H, J = 8.8 Hz), 7.36-7.38 (d, 1H, Ar-H, J = 7.4 Hz), 7.45-7.49 (t, 2H, Ar-H, J = 7.6 Hz), 7.53-7.55 (d, 2H, Ar-H, J = 8.9 Hz), 7.60-7.61 (d, 1H, Ar-H, J = 3.2 Hz), 7.68-7.70 (m, 8H, Ar-H), 7.91-7.93 (d, 2H, Ar-H, J = 8.9 Hz), 8.59 (s, 1H, -NH); ^{13}C-NMR (100 MHz, DMSO-d_6, ppm): δ 159.7, 151.0, 149.4, 140.1, 140.0, 138.8, 132.8, 130.5, 129.9, 129.8, 129.5, 128.0, 127.8, 127.2, 127.0, 126.6, 125.6, 123.7, 120.1, 114.5, 55.7, 55.6; MS: *m/z* = 503.3 (M-1), ANAL. Calcd.for $C_{31}H_{25}ClN_4O$; calcd: C, 73.73; H, 4.99; N, 11.09; found: C, 73.76; H, 4.99; N, 11.11.

5-Biphenyl-4-yl-1′-(4-chlorophenyl)-3′-(4-chlorophenyl)-3,4-dihydro-2*H*,1′*H*-[3,4′]bipyrazolyl (T_{26}). Yield: 80 %; m.p. 184-186 °C; IR (KBr ν_{max} cm^{-1}): 3315 (N-H str), 3058 (Ar-H str), 2927 (C-H aliphatic str), 1595 (C=N str), 1497 (C=C str), 833 (C-Cl str); ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): δ 2.98-3.04 (t, 1H, H_A, J = 12.8

Hz), 3.51-3.58 (t, 1H, H_B, J = 13.4 Hz), 4.98-5.04 (t, 1H, H_X, J = 10.6 Hz), 7.38-7.56 (m, 7H, Ar-H), 7.66 (s, 1H, pyrazole-5H), 7.72 (b, 6H, Ar-H), 7.81 (b, 2H, Ar-H), 7.95-7.97 (d, 2H, Ar-H, J = 7.2 Hz), 8.67 (s, 1H, -NH); MS: *m/z* = 509.6 (M+1), ANAL. Calcd. for $C_{30}H_{22}Cl_2N_4$; calcd: C, 70.73; H, 4.35; N, 11.00; found: C, 70.76; H, 4.37; N, 11.03.

5-Biphenyl-4-yl-1′-(4-chlorophenyl)-3′-thiophene-2-yl-3,4-dihydro-2*H*,1′*H*-[3,4′]bipyrazolyl (T_{27}). Yield: 83 %; m.p. 217-219 °C; IR (KBr ν_{max} cm^{-1}): 3307 (N-H str), 3075 (Ar-H str), 2919 (C-H aliphatic str), 1593 (C=N str), 1497 (C=C str), 831 (C-Cl str); ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): δ 3.00-3.07 (t, 1H, H_A, J =13.4 Hz), 3.55-3.62 (t, 1H, H_B, J = 13.2 Hz), 5.09-5.15 (t, 1H, H_X, J = 10.4 Hz), 7.19 (b, 1H, Ar-H), 7.38-7.64 (m, 8H, Ar-H), 7.72 (b, 6H, Ar-H), 7.91-7.92 (d, 2H, Ar-H, J = 6.4 Hz), 8.62 (s, 1H, -NH); ^{13}C-NMR (100 MHz, DMSO-d_6, ppm): δ 149.5, 145.7, 140.1, 140.0, 138.5, 135.2, 132.7, 130.8, 130.0, 129.5, 128.5, 128.2, 128.0, 127.2, 127.0, 126.8, 126.6, 123.5, 120.2, 55.5; MS: *m/z* = 479.2 (M-1), ANAL. Calcd.for $C_{28}H_{21}ClN_4S$; calcd: C, 69.92; H, 4.40; N, 11.65; found: C, 69.93; H, 4.40; N, 11.66.

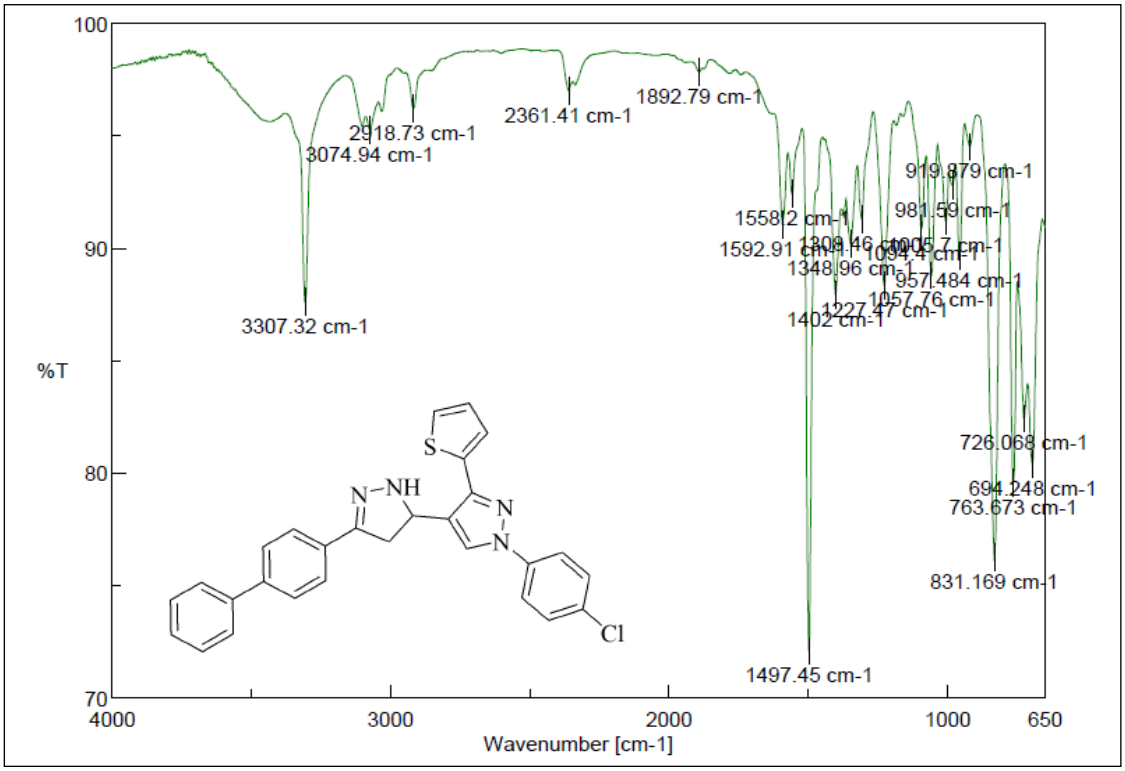


Figure 3.7 IR spectrum of compound **T_{27}**

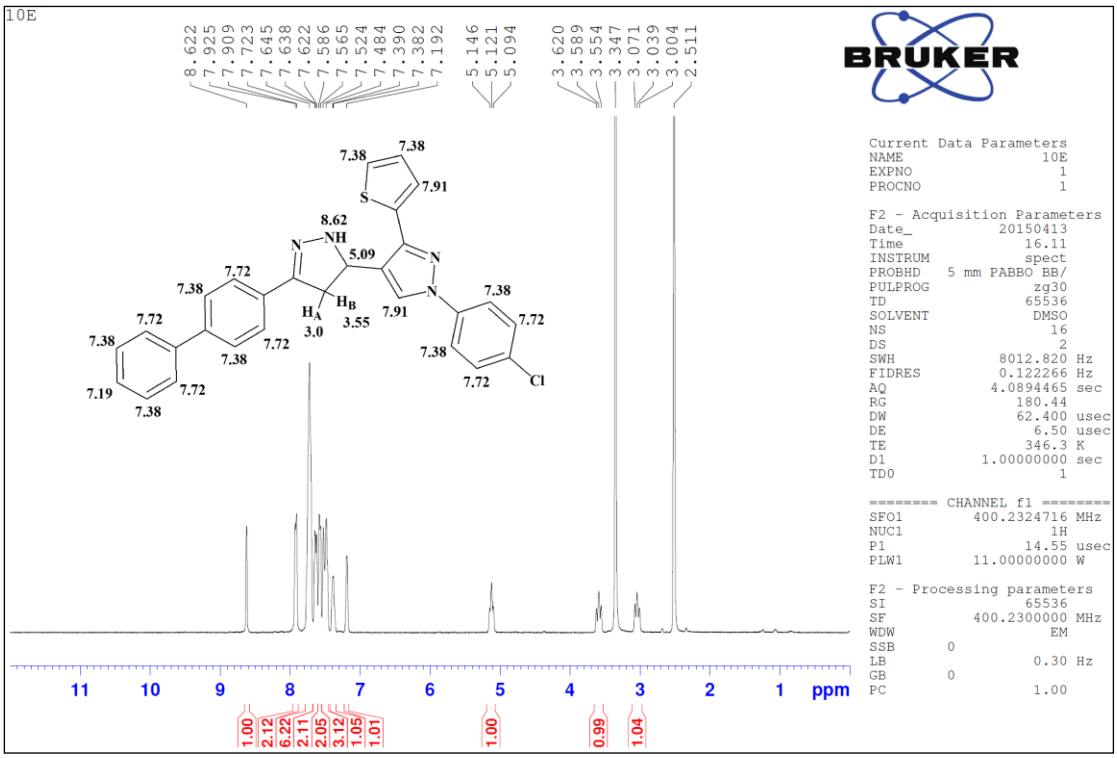


Figure 3.8 ^{1}H-NMR spectrum of compound $\mathbf{T_{27}}$

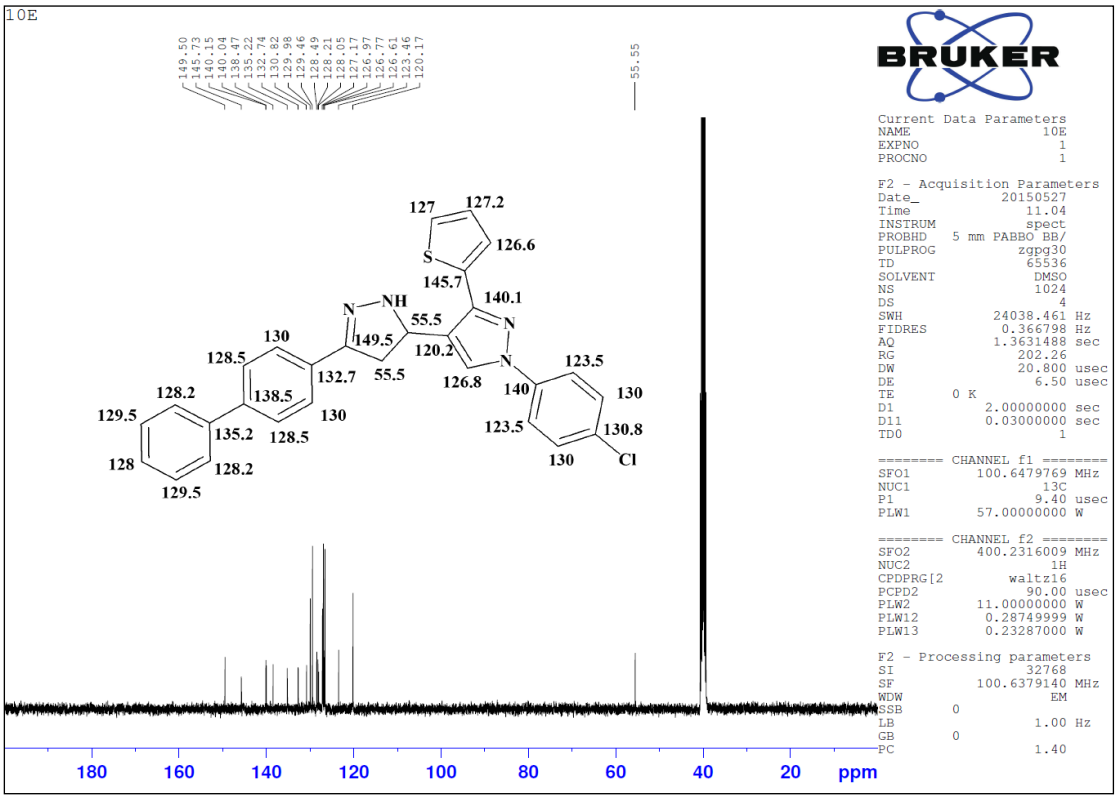


Figure 3.9 ^{13}C-NMR spectrum of compound $\mathbf{T_{27}}$

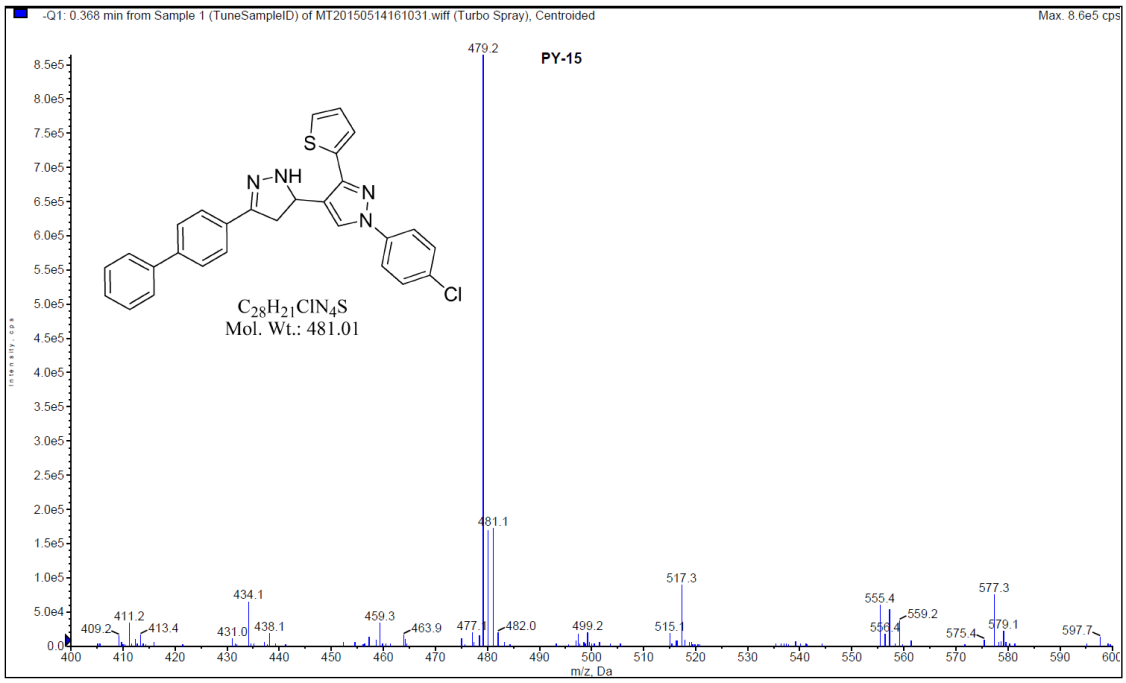


Figure 3.10 Mass spectrum of compound $\mathbf{T_{27}}$

3.4 PHARMACOLOGY

3.4.1 Antitubercular activity-Microplate Alamar Blue Assay (MABA) method

Antitubercular screening for the newly synthesized compounds $\mathbf{T_{13\text{-}27}}$ were determined by the Middle brook 7H9 broth against *Mycobacterium tuberculosis* of $H_{37}Rv$ strain (ATCC-27294) as explained in **Chapter 2**. The Minimum Inhibitory Concentration (MIC) of each synthesized compound was determined by the Microplate Alamar Blue Assay method (MABA). The final drug concentrations tested were 100–0.78 µg/mL. Ciprofloxacin (INN), Streptomycin (STM) and Pyrazinamide (PZA) were used as standard anti-TB drugs.

3.4.2 Antibacterial and antifungal activity

Antimicrobial screening for the newly synthesized compounds $\mathbf{T_{13\text{-}27}}$ were determined by MIC using Resazurin reduction method in 96 well microplates as explained in **Chapter 2**. In this work *Staphylococcus aureus* (MTCC 3160) and tuberculosis variant bacteria *Mycobacterium smegmatis* (MTCC 994) were used to study antibacterial activity. Antifungal activity of these compounds was carried out against pathogenic fungi *Candida albicans* (MTCC 7253). All the bacterial and fungal cultures were obtained from IMTECH, Chandigarh, India and maintained the cultures

as per the standard protocol. The wells, which remain blue after 24 hours of incubation indicates that, there is no microorganisms survived in the well, the minimum concentration where no microbial growth found are considered as MIC value.

3.4.3 Cytotoxicity studies

3.4.3.1 *In vitro* cell viability assay (MTT) and IC_{50} value determination

In vitro cytotoxicity study was carried out using HeLa cells. Cell lines were maintained in Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10 % heat-inactivated fetal bovine serum (FBS), 100 U/ml Penicillin, 100 µg/mL Streptomycin and 2.5 µg/mL Amphotericin-B solution, 200 mM L-Glutamine (HiMedia Labs, Mumbai, India). The cell lines were incubated at 37 °C in a humidified atmosphere of 95 % air, 5 % CO_2. Following 24 h of the incubation period, the adherent cells were detached using Trypsin-EDTA solution 1X/0.25 % (HiMedia Labs, Mumbai, India). Cell count was carried out using the Luna automated cell counter (Logos Biosystems, India) based on trypan blue dye exclusion method (Abdulla-Al-mamun *et al.* 2009).

200 µL cell suspension of the cell line was seeded in 96-well microplates (Corning®, USA) at a density of 25,000 cells/well and incubated for 24 h, after which the cells were exposed to different concentrations (100-500 µg/mL) of synthetic compounds for 24 h. Cells were seeded in triplicates and incubated in a CO_2 incubator (atmospheric with 5 % CO_2 and 37 °C temperature) (Loske *et al.* 1998). Treated cells were thereafter incubated with MTT (HiMedia Labs, Mumbai, India) for 3 h. The culture medium was aspirated and 100 µL dimethylsulfoxide (DMSO; Sigma-Aldrich, India) was added to each well. The untreated cells and Metformin treated cells were used as controls. Cell viability was determined by measuring the absorbance on a microplate reader at 570 nm. Viability was calculated as a percentage of viable cells at different test concentrations relative to the control (untreated) cells (% cell viability = (A_{570} of treated cells / A_{570} of control cells) ×100 %). DMSO served as vehicle control (0.1 % of final concentration). The concentration of synthetic compound that resulted in 50 % inhibition of cell growth was calculated as the half maximal inhibitory concentration (IC_{50}) by constructing a dose-response curve.

3.5 RESULTS AND DISCUSSIONS

3.5.1 Chemistry

The characterization data of the synthesized 1,3-disubstituted-1*H*-pyrazole-4-carbaldehydes (**4a-e**) are presented in **Table 3.2**.

Table 3.2 Physical data of the synthesized compounds **4a-e**

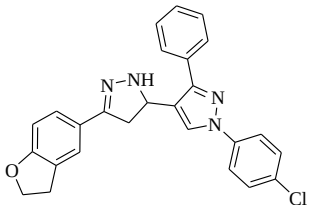
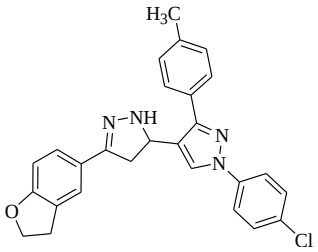
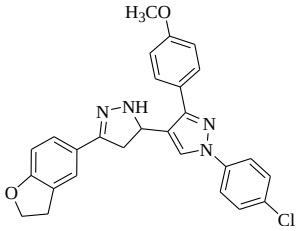
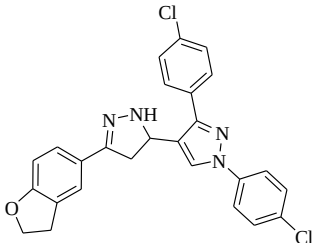
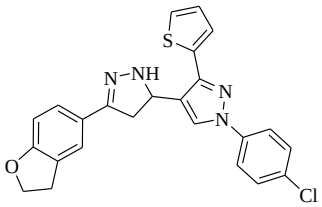
Compounds	Ar	M.Wt	M.F	M.p (°C)	Yield (%)
4a	C_6H_5	282.72	$C_{16}H_{11}ClN_2O$	145-146	91
4b	4-$CH_3C_6H_4$	296.5	$C_{17}H_{13}ClN_2O$	133-134	90
4c	4-$OMeC_6H_4$	312.75	$C_{17}H_{13}ClN_2O_2$	142-143	89
4d	4-ClC_6H_4	317.17	$C_{16}H_{10}Cl_2N_2O$	177-178	85
4e	2-Thiophene	288.75	$C_{14}H_9ClN_2OS$	140-140	82

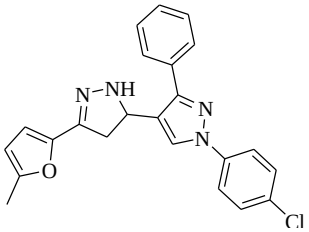
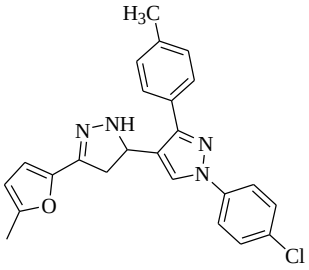
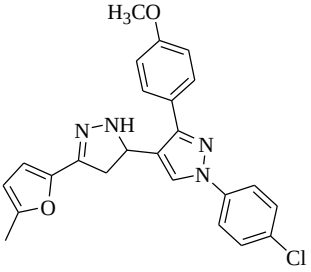
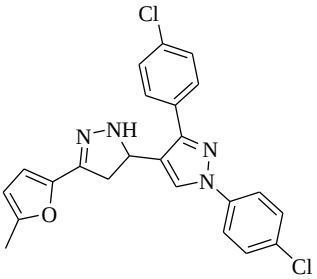
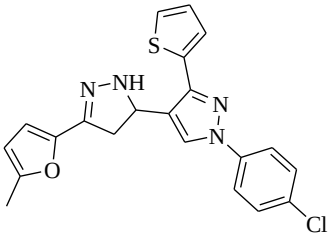
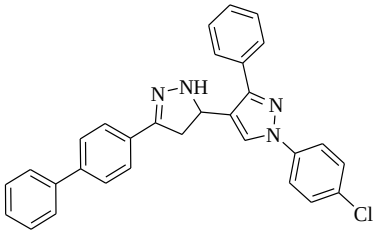
The structure of all the newly synthesized compounds was confirmed by recording FT-IR, NMR, mass spectral analyses. The IR data for compound **9** was confirmed by the peak at 1659 cm^{-1} which is due to the -C=O stretching of the acetyl group. The ^{1}H-NMR spectrum of **9** showed a singlet at δ 2.55 corresponds to -CH_3 protons. Two triplets at 3.23-3.27 and 4.64-4.68 is due to -CH_2 of dihydro benzofuran at the second and third position. A singlet appeared at δ 7.85 is due to 2,3-dihydrobenzofuran-4H, which is inconsistent with the molecular formula $C_{10}H_{10}O_2$.

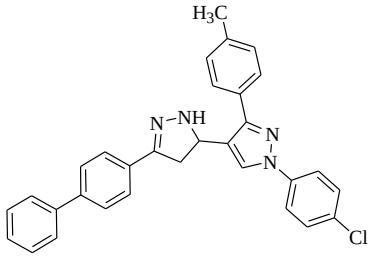
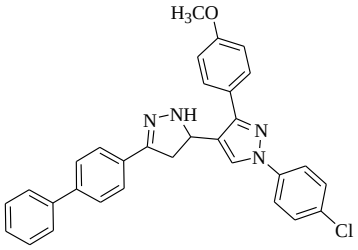
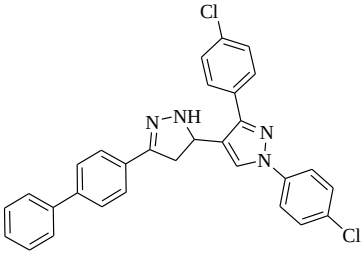
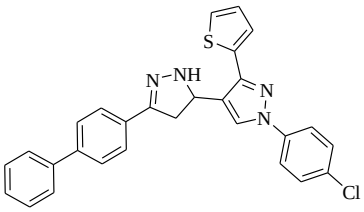
Formation of 1′-(4-chlorophenyl)-5-(2,3-dihydrobenzofuran-5-yl)-3′-(substituted aryl)-3,4-dihydro-2*H*,1′*H*-[3,4′]bipyrazolyl **T$_{13\text{-}27}$** were confirmed by recording their IR, ^{1}H-NMR, ^{13}C-NMR and mass spectra. IR analysis of compound **T$_{13}$** showed the peak at 3322 cm^{-1}, which was due to the NH group. Another absorption band at 3064 cm^{-1} was due to the -C-H stretching of the aromatic ring. The absorption band at 1607 cm^{-1} was due to the -C=N group, -C=C stretching was observed at 1497 cm^{-1}. The ^{1}H-NMR spectrum of **T$_{13}$** in DMSO-d_6 solvent showed a triplet at δ 2.90-2.96 which was attributed to H_A proton, and a triplet at δ 3.42-3.49 was due to the H_B proton of the pyrazoline ring. The characteristic peak of -NH proton was observed as a singlet at δ 8.62. The detailed ^{1}H-NMR resonances are summarized in the experimental section. The mass spectrum of **T$_{13}$** showed a molecular ion peak at *m/z* = 441.2 (M+1). This, in turn, confirmed the formation of a

compound having the molecular formula $C_{26}H_{21}ClN_4O$. The characterization data of the synthesized compounds **$T_{13\text{-}27}$** were presented in **Table 3.3**.

Table 3.3 Characterization data of the compounds **$T_{13\text{-}27}$**

Comp.	Ar	Structure	M. F/ M. wt.	Color & nature
T_{13}	C_6H_5		$C_{26}H_{21}ClN_4O$/ 440.92	White solid
T_{14}	4-$CH_3C_6H_4$		$C_{27}H_{23}ClN_4O$/ 454.95	Off-white solid
T_{15}	4-$OCH_3C_6H_4$		$C_{27}H_{23}ClN_4O$/ 470.95	Off-white solid
T_{16}	4-ClC_6H_4		$C_{26}H_{20}Cl_2N_4O$/ 475.37	white solid
T_{17}	2-Thiophene		$C_{24}H_{19}ClN_4OS$/ 446.95	White solid

T_{18}	C_6H_5		$C_{23}H_{19}ClN_4O$/ 402.88	White solid
T_{19}	4-$CH_3C_6H_4$		$C_{24}H_{21}ClN_4O$/ 416.90	Off-white solid
T_{20}	4-$OCH_3C_6H_4$		$C_{24}H_{21}ClN_4O_2$/ 432.9	Off-white solid
T_{21}	4-ClC_6H_4		$C_{23}H_{18}Cl_2N_4O$/ 437.32	Off-white solid
T_{22}	2-Thiophene		$C_{21}H_{17}ClN_4OS$/ 408.9	Off-white solid
T_{23}	C_6H_5		$C_{30}H_{23}ClN_4$/ 474.98	Off-white solid

T_{24}	4-$CH_3C_6H_4$		$C_{31}H_{25}ClN_4$/ 489.01	Off-white solid
T_{25}	4-$OCH_3C_6H_4$		$C_{31}H_{25}ClN_4O$/ 505.01	Off-white solid
T_{26}	4-ClC_6H_4		$C_{30}H_{22}Cl_2N_4$/ 509.43	white solid
T_{27}	2-Thiophene		$C_{28}H_{21}ClN_4S$/ 481.01	Off-white solid

3.5.2. Biological results

The MIC value of newly synthesized compounds against pathogenic bacteria *Mycobacterium tuberculosis* $H_{37}Rv$ was represented in **Figure 3.11**. All the compounds were tested against pathogenic strain *Mycobacterium tuberculosis* $H_{37}Rv$ at different concentrations ranging from 100 to 0.78 µg/mL. Most of the compounds exhibited the MIC value ranging between 50 to 1.56 µg/mL. All the compounds showed significant antitubercular activity due to the presence of 4-chlorophenyl substitution at the first position of pyrazole ring. Among all the synthesized derivatives, compound **T_{27}** showed most potent antitubercular activity with MIC value of 1.56 µg/mL. The enhanced activity of this compound was due to the presence of 2-thiophene group at the third position of the pyrazole ring and biphenyl at the third position of the pyrazoline ring. The compound found to be more active than the standard drug Ciprofloxacin (INN). The second lowest MIC value 6.25 µg/mL obtained for compound **T_{25}** was similarly active with the standard anti-TB drug

Streptomycin due to the presence of biphenyl present at the third position of pyrazoline and 4-methoxyphenyl at the third position of the pyrazole core moiety. The compound **T_{13}** also showed good MIC value (12.5 µg/mL) due to the presence of the phenyl group at the third position of pyrazole and 2,3-dihydrobenzofuran at the third position of pyrazoline ring. Other compounds **T_{16}**, **T_{17}**, **T_{18}**, **T_{19}**, **T_{20}** and **T_{21}** showed moderate activity with MIC value 25 µg/mL against tested *Mycobacterium tuberculosis.* This indicates that, most of the pyrazole containing pyrazoline compounds with 5-methylfuran substitution at third position is able to give moderate activity against *M. tuberculosis*. Compounds **T_{14}**, **T_{15}**, **T_{22}**, **T_{23}**, **T_{24}** and **T_{26}** were showed less activity against tested organisms with MIC value 50 µg/mL. This concludes that, the synthesized compounds are active as antitubercular agents. By molecular modification of the present series of pyrazole containing pyrazoline compounds may be useful as an antitubercular agent.

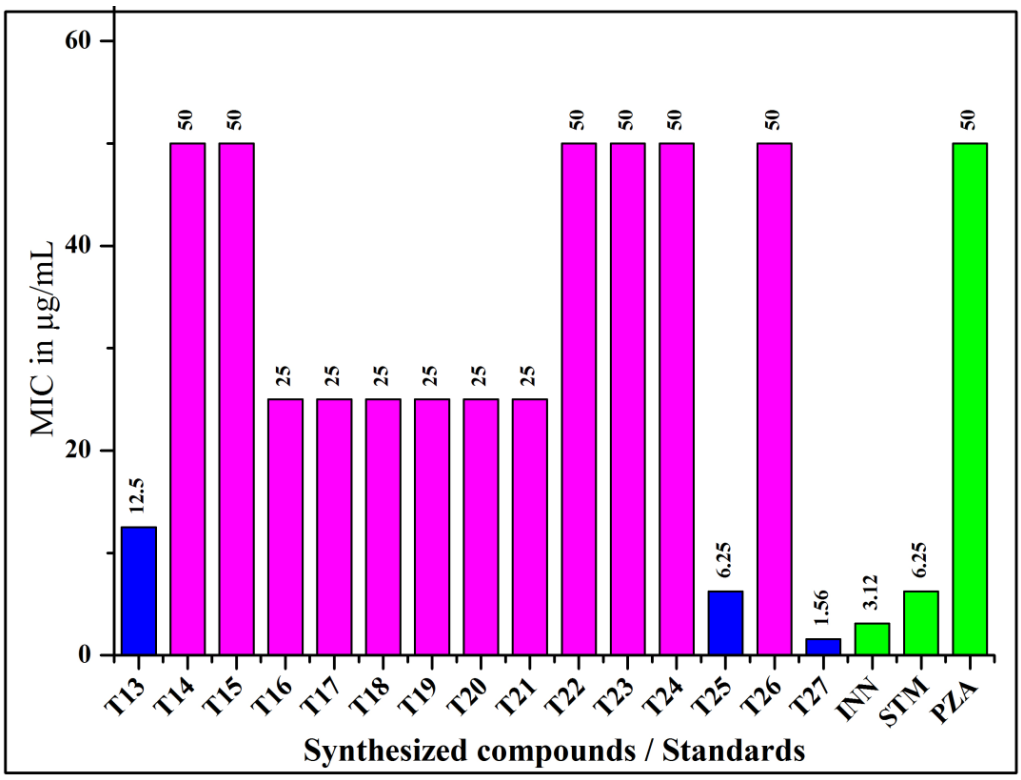


Figure 3.11 The MIC of **$T_{13\text{-}27}$** against *Mycobacterium tuberculosis.*

Minimum Inhibitory Concentration of antimicrobial activity of all the synthesized compounds **$T_{13\text{-}27}$** were screened against *Staphylococcus aureus* (Gram

+ve bacteria), *Mycobacterium smegmatis*, and *Candida albicans* (fungi) at different concentrations ranging from 500 to 3.9 µg/mL. Most of the compounds were exhibited the activity with MIC value ranging between 62.5 to 7.8 µg/mL. Structure activity relationship of the synthesized compound was explained based on MIC value. The compound $\mathbf{T_{27}}$ showed lowest MIC value against all tested organisms due to the presence of biphenyl at the third position of the pyrazoline ring. The second lowest MIC showed for compounds $\mathbf{T_{13}}$, $\mathbf{T_{25}}$ and $\mathbf{T_{26}}$ against the tested microorganism. The compound $\mathbf{T_{13}}$ is due to the presence of 2,3-dihydrobenzofuran at the third position of pyrazoline ring and a phenyl group, compound $\mathbf{T_{25}}$ is due to biphenyl at the third position of pyrazoline and 4-methoxyphenyl at the third position of pyrazole. Compound $\mathbf{T_{26}}$ is due to biphenyl substitution on pyrazoline at the third position and *p*-chlorophenyl at the third position of pyrazole ring. Other compounds $\mathbf{T_{16}}$, $\mathbf{T_{17}}$, $\mathbf{T_{19}}$, $\mathbf{T_{20}}$ and $\mathbf{T_{21}}$ showed moderate activity with MIC value 62.5 to 125 µg/mL against tested organisms. Antimicrobial results of target compounds $\mathbf{T_{13\text{-}27}}$ were tabulated in **Table 3.4**.

Table 3.4 MIC value of the synthesized compounds $\mathbf{T_{13\text{-}27}}$

Synthesized Compound	**MIC in µg/mL**		
	M. smegmatis	***S. aureus***	***C. albicans***
$\mathbf{T_{13}}$	15.6	15.6	31.25
$\mathbf{T_{14}}$	500	500	500
$\mathbf{T_{15}}$	500	500	500
$\mathbf{T_{16}}$	62.5	62.5	62.5
$\mathbf{T_{17}}$	62.5	125	62.5
$\mathbf{T_{18}}$	250	125	125
$\mathbf{T_{19}}$	62.5	125	62.5
$\mathbf{T_{20}}$	62.5	62.5	62.5
$\mathbf{T_{21}}$	62.5	62.5	62.5
$\mathbf{T_{22}}$	125	31.25	125
$\mathbf{T_{23}}$	125	125	125
$\mathbf{T_{24}}$	500	500	500
$\mathbf{T_{25}}$	15.6	15.6	31.25
$\mathbf{T_{26}}$	15.6	62.5	31.25
$\mathbf{T_{27}}$	7.8	15.6	31.25
INN	<5	<5	--
FLZ	--	--	<10
Control	--	--	--

INN; antibacterial standard Ciprofloxacin; FLZ; antifungal standard Fluconazole --: not detected inhibition; control; dimethylsulfoxide

The *in vitro* cytotoxicity study was carried out using HeLa cells at Stellixir Biotech Pvt. Ltd, Bangalore. The five compounds **T_{13}**, **T_{16}**, **T_{19}**, **T_{25}** and **T_{27}** which had the highest activity for antimicrobial and antituberculosis were tested for cytotoxicity against HeLa cells represented in **Figure 3.12**. The IC_{50} value of synthesized compounds found to be moderately effective for the HeLa cells. The control cells which are not treated with any compound have shown 100 % viability. One of the synthetic compound **T_{19}** has shown highest cytotoxicity (12.83 µg/mL) among tested compounds. The compounds having cytotoxicity below 50 µg/mL IC_{50} value are usually considered as toxic compounds.

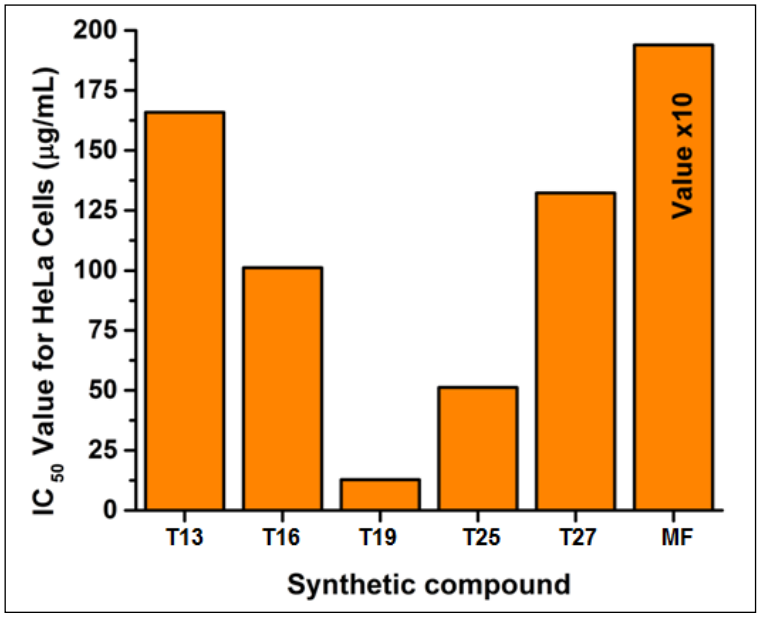


Figure 3.12 Cytotoxicity study of **T_{13}**, **T_{16}**, **T_{19}**, **T_{25}** and **T_{27}** with HeLa cell line

3.6 CONCLUSIONS

A new series of 1′-(4-chlorophenyl)-5-(aryl)-3′-(aryl)-3,4-dihydro-2*H*,1′*H*-[3,4′]bipyrazolyl **$T_{13\text{-}27}$** derivatives were synthesized in good yields. They were characterized by IR, ^{1}H-NMR, ^{13}C-NMR, MS spectrometry and elemental analyses. Target compounds have been investigated for their *in vitro* antimicrobial activity by MIC using Resazurin reduction method and antitubercular activity by Microplate Alamar Blue Assay method and proved to be very good antimicrobial and antitubercular agents. The results are consistent with specific substitution to the utility of tuberculosis chemotherapy and antimicrobial agents. The compounds **T_{27}** and **T_{25}**

showed the best screening results among all the synthesized compounds with biphenyl substitution at the third position of pyrazoline and 2-thiophene/4-methoxypheyl respectively. Compound **T_{13}** exhibited the good MIC value against antimicrobial and *M. tuberculosis* due to the presence of 2,3-dihydrobenzofuran at the third position of pyrazoline and phenyl substitution at the third position of pyrazole. The compound **T_{16}**, **T_{17}**, **T_{18}**, **T_{19}**, **T_{20}** and **T_{21}** have shown moderate activity on *Mycobacterium tuberculosis*. Newly synthesized pyrazole containing pyrazoline compounds might emerge as one of the antituberculosis agents.

CHAPTER 4

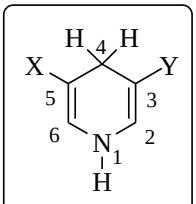
ANTITUBERCULAR AND ANTIMICROBIAL ACTIVITY OF NH_4VO_3 PROMOTED 1,4-DIHYDROPYRIDINE CONTAINING PYRAZOLE DERIVATIVES

Abstract

This chapter describes the importance of multicomponent reaction and detailed literature survey on 1,4-dihydropyridine and its derivatives until August 2016. It includes the various synthetic methods for 1,4-dihydropyridine derivatives and biological importance of few of the reported 1,4-dihydropyridine derivatives. The target molecule, pyrazole containing 1,4-dihydropyridine derivatives were synthesized by Hantzsch multicomponent reaction with 1,3-disubstituted-1H-pyrazole-4-carbaldehydes, ethyl and methyl ester of acetoacetic acid and ammonia to give target 4-(1,3-di substituted-1H-pyrazol-4-yl)-2,6-dimethyl-1,4-dihydro pyridine-3,5-di carboxylic acid di(ethyl/methyl)ester derivatives. Further, the synthesized 1,4-dihydropyridine derivatives were characterized and screened for antitubercular, antibacterial and antifungal studies.

4.1 INTRODUCTION

1,4-Dihydropyridine (1,4-DHP) is a six-membered heterocyclic ring containing nitrogen atom at first position. The most feasible position for substitution at fourth position exhibits various biological activities (Swarnalatha *et al.* 2011). Third and fifth position of 1,4-dihydropyridine by electron withdrawing substitutions such as -COR, -COOR, -CN and $-NO_2$ enhances their chemical stability. Whereas electron donating groups like $-SC_6H_5$ and $-OC_6H_5$ decreases the chemical stability.



S-4.1

Multicomponent reactions (MCR's) have gained wide applicability in the field of synthetic organic chemistry due to the increased efficiency of the reaction, short reaction time and decrease in the number of laboratory operations along with energy, quantities of solvent and chemicals used. Various methods have been proposed to synthesize 1,4-dihydropyridine derivatives. One-pot, multicomponent synthesis of symmetrically substituted 1,4-dihydropyridines were first reported by Arthur Hantzsch in 1882. Numerous literature data related to various attempts to improve

Hantzsch reaction using novel catalysts, novel reagents and green reaction conditions. Owing to the modest yield, several modifications to improve the efficiency of the reaction by this method have been developed including the use of novel catalysts such as metal triflates, ceric ammonium nitrate, Bu_4NHSO_4, TMS iodide, boronic acid, silica sulfuric acid, $Ba(NO_3)_2$ and PPA-SiO_2 (Aswin *et al.* 2012, Yang *et al.* 2013). Here, ammonium metavanadate used as catalyst and explained the role of catalyst to improve yield and reaction rate.

1,4-Dihydropyridines and their derivatives are an important class of bioactive molecules in the medicinal field (Stout and Meyers, 1982). They exhibit variety of biological activities such as cardiovascular, vasodilator, antitumor, neuroprotective, anti-inflammatory and antimicrobial agents (Kumar *et al.* 2011). Recently, 1,4-dihydropyridine derivatives were found to possess antiviral (Rand *et al.* 1986), antithrombotic (Sunkel *et al.* 1990), antitumor (Kawase *et al.* 2002), antitubercular (Kharkar *et al.* 2002), antidiabetic (Ogawa *et al.* 2003), hypnotic (Zheng *et al.* 2011), fungicidal and bactericidal (Siddiqui *et al.* 2013) activities.

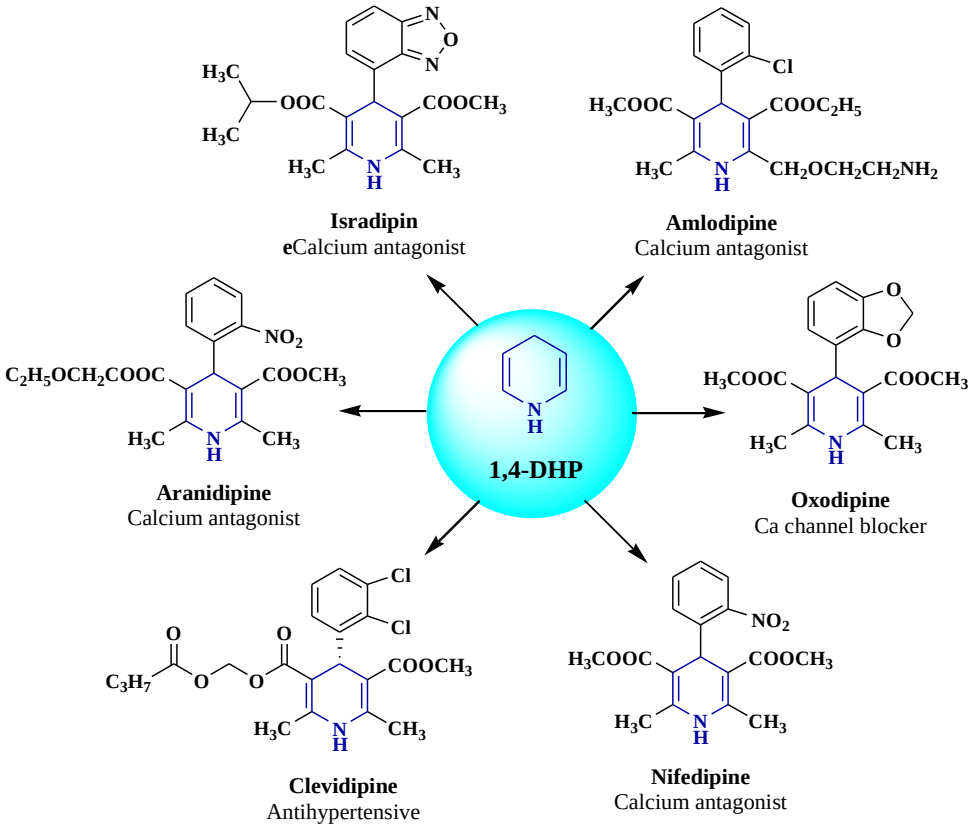
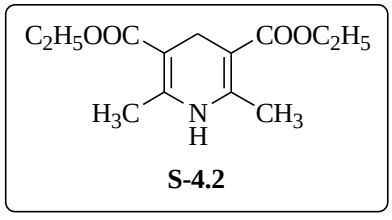


Figure 4.1 1,4-Dihydropyridine containing commercial drugs

1,4-Dihydropyridines have superfluous calcium channel blocking properties in the treatment of cardiovascular disorders (Triggle, 2007). 1,4-Dihydropyridine based commercially available drugs like Nifedipine, Amlodipine, Felodipine, Nimodipine, Nitrendipine, Nisoldipine, Nicardipine, Isradipine (Brown *et al.* 2000) exhibits selectivity for cerebral vasculature. Some of the 1,4-dihydropyridine based active pharmaceutical ingredients were presented in **Figure-4.1**.

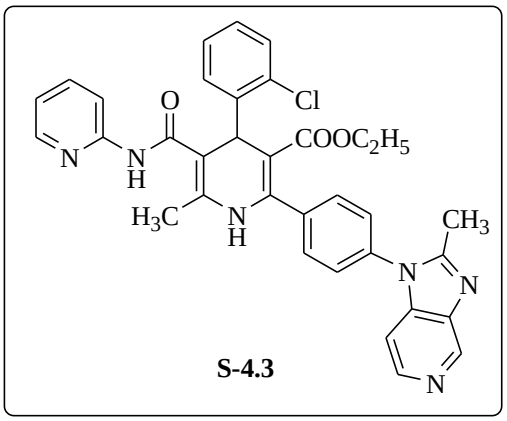
In recent years, the use of potent drugs is limited due to their unavoidable side effects. To overcome certain limitations, medicinal chemists have to put efforts to minimize these adverse effects that are directed to the discovery of high potency antimicrobial agents with reduced or no systemic adverse effects. Keeping in view of this and the importance of dihydropyridine derivatives a new methodology was developed to synthesize pyrazole containing 1,4-dihydropyridine derivatives. Further, the synthesized compounds were screened for their antitubercular and antimicrobial properties. Some of the earlier reported potent 1,4-dihydropyridine derivatives with different biological activities were summarized below.

Hantzsch (1882), first reported the synthesis of dialkyl 1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylates (**S-4.2**) by refluxing three component mixture of an aldehyde, a β-ketoester and aqueous ammonium hydroxide in ethanol media. Some modified procedures have been proposed and they involve the use of Knoevenagel adducts between the aldehyde and the ketoester.



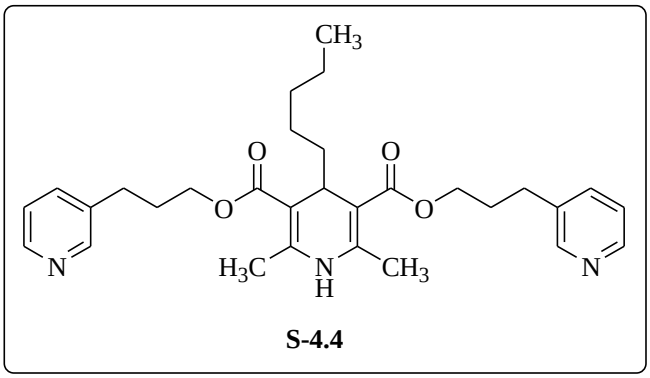
S-4.2

A novel series of 2-(4-heterocyclylphenyl)-1,4-dihydropyridines were prepared using Hantzsch synthesis by Cooper *et al.* (1992). All newly synthesized compounds were evaluated for antagonist activity against platelet activating factor (PAF). Compound (**S-4.3**) was found to be 33-fold active than standard WEB2086.



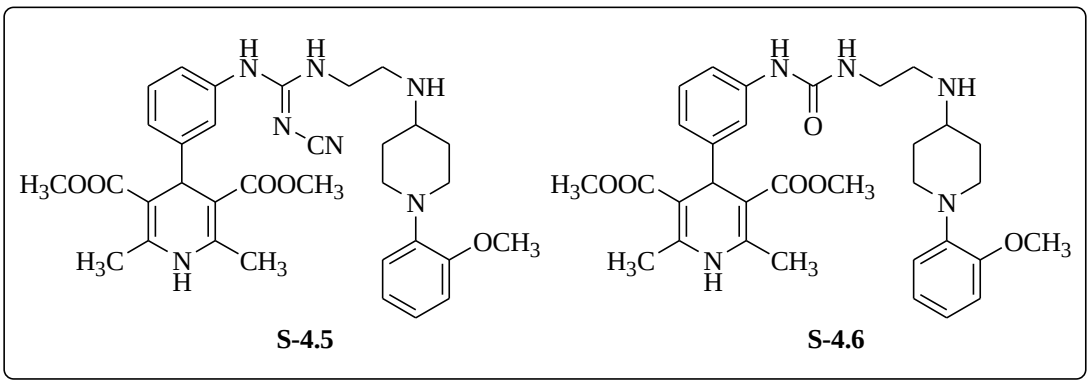
S-4.3

New pyridine linked 1,4-dihydropyridine derivatives were synthesized and their anticancer activity was performed by Tasaka *et al.* (2001). Anticancer activity study was done by using mice carrying P388 leukemia cell lines. Compound (**S-4.4**) showed active compound to overcome the multidrug resistance.



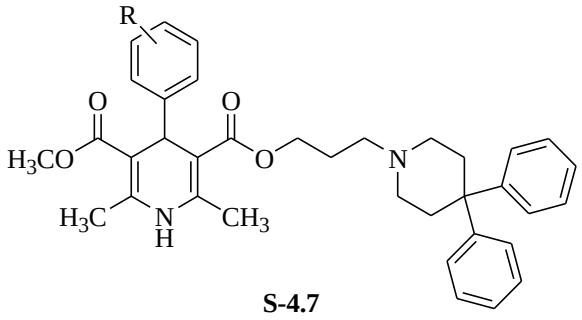
S-4.4

NPY_1 receptor antagonists were developed by Luo *et al.* (2004) by synthesis of new dihydropyridine derivatives (**S-4.5** and **S-4.6**). These derivatives were synthesized by a $ZnCl_2$ mediated reductive amination between primary amines and 4-arylpiperidones.



S-4.5 **S-4.6**

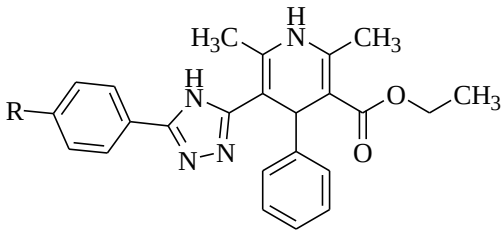
Zhou *et al.* (2005) synthesized a new series of 4-aryl-1,4-dihydropyridines (**S-4.7**) for anticancer activity. These cytotoxicity studies were mediated by P-glycoprotein for breast cancer. All compounds showed significant increased Vinblastine accumulation in MCF-7/adr cells. Eight compounds in this series demonstrated negligible calcium channel binding even at 2500 nM.



S-4.7

R= 3-NO_2, 4-OMe, 4-OEt, 4-NMe_2, 4-OBn, 4-COOH, 4-OH, 4-OCOMe, 4-O"Pr

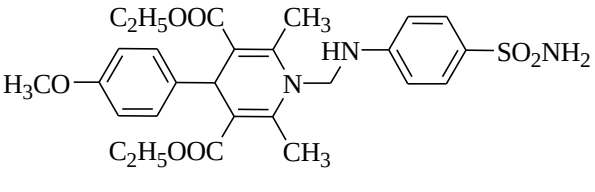
New 1,4-dihydropyridine derivatives were synthesized utilizing Hantzsch pyridine synthesis using ammonia, ethyl acetoacetate and benzaldehyde (Brijeshkunvar and Richa, 2007). All the compounds were confirmed by spectral techniques and processed for their analgesic and anti-inflammatory activity. Compounds (**S-4.8**) found to be equipotent activity with standard Piroxicam at a dose of 70 mg/kg.



S-4.8

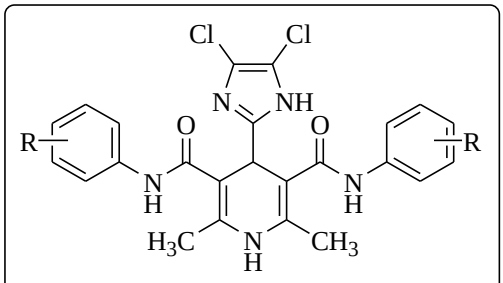
R= H, OCH_3, CH_3, OH, NH_2

1,4-Dihydropyridine containing sulfanilamide derivatives have developed and tested for antiulcer activity by Subudhi and Panda, (2009). They claimed that, compound (**S-4.9**) showed potent antiulcer activity due to methoxyphenyl substitution at fourth position of 1,4-dihydropyridine ring.



S-4.9

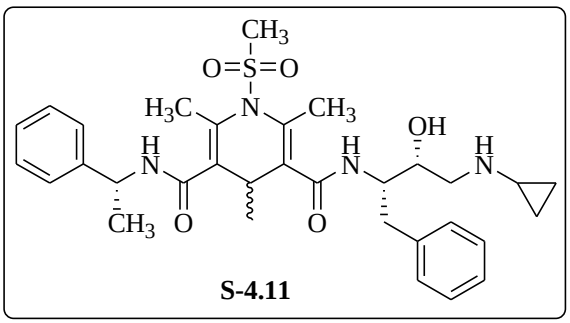
Fassihi *et al.* (2009) synthesized a novel 4-substituted imidazolyl-2,6-dimethyl-N^3, N^5-bisaryl-1,4-dihydropyridine-3,5-dicarboxamides. Synthesized compounds have tested for their antituberculosis activity against pathogenic bacteria *Mycobacterium tuberculosis* ($H_{37}Rv$) strain. Few compounds (**S-4.10**) showed similar antitubercular activity with standard Rifampicin with MIC value 2 µg/mL.



S-4.10

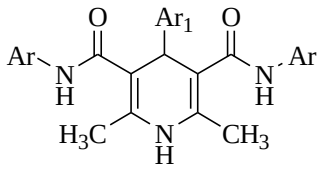
R= 3-Cl, 3-NO_2, 4-NO_2

Choi *et al.* (2010) investigated 1,4-dihydropyridine derivatives as BACE-1 inhibitors in Alzheimer's disease. They designed the structures based on computer-aided molecular docking. Several of the new dihydropyridine derivatives were synthesized and their BACE-1-inhibitory activities were evaluated using a cell-based, reporter gene assay system. Most of the 1,4-DHP analogs showed BACE-1-inhibitory activities with IC_{50} values in the range 8-30 µM. Compound (**S-4.11**) showed least IC_{50} value of 5 µM.



S-4.11

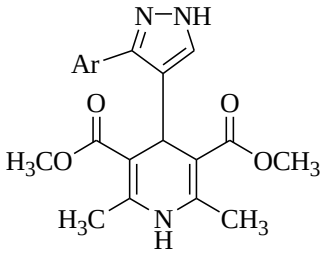
Sirisha *et al.* (2010) synthesized some new 4-substituted-2,6-dimethyl-3,5-bis-N-(heteroaryl)carbamoyl-1,4-dihydropyridine (**S-4.12**) derivatives. All newly synthesized compounds were tested for antituberculosis, antibacterial and anticancer activities. Compounds with 2-pyridyl and 6-methylpyridine-2-yl substitution showed more potent than anti-TB standard Pyrazinamide.



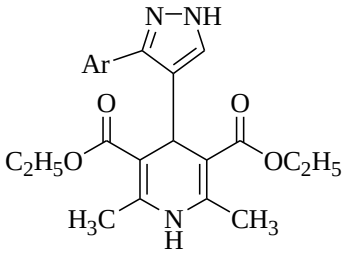
S-4.12

Ar= 5-chloropyridin-2-yl, 6-methylpyridin-2-yl
Ar_1= 2-imidazolyl, 2-pyridyl

Vijesh *et al.* (2011) synthesized two new series of 1,4-dihydropyridine derivatives containing pyrazole as core moiety (**S-4.13** and **S-4.14**). All target compounds were characterized and tested for their antimicrobial and antioxidant activity. The majority of the compounds found to be excellent antimicrobial and antioxidant activity.



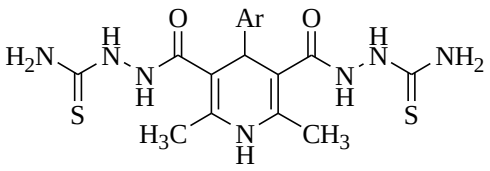
S-4.13



S-4.14

Ar= 2,4-dichlorophenyl, biphenyl, 4-thioanisyl, 4-anisyl, 2,5-dichlorothiophene

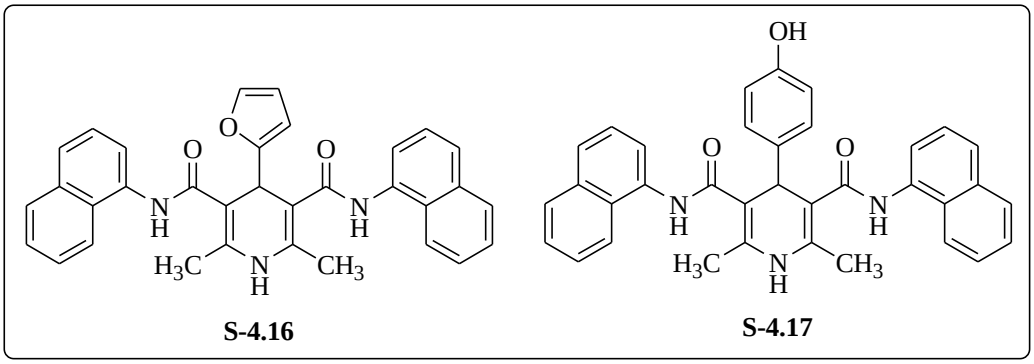
New thiosemicarbazide containing 1,4-dihydropyridine derivatives have synthesized started from the reaction of ethyl acetoacetate, aromatic aldehyde and ammonium hydroxide to get 1,4-dihydropyridine intermediate. Target thiosemicarbazide-1,4-DHP (**S-4.15**) derivatives were prepared by condensation of thiosemicarbazide with 1,4-dihydropyridine intermediate. All synthesized compounds were tested for their anticoagulant activity and found that, few compounds showed good anticoagulant activity (Surendrakumar *et al.* 2011).



S-4.15

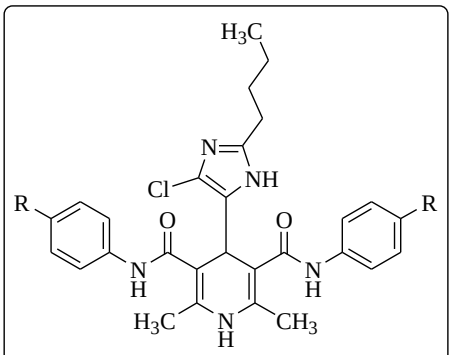
Ar= furyl, Ph, 4-ClC_6H_4, 4-OHC_6H_4, 4-$NO_2C_6H_4$, 4-$OCH_3C_6H_4$, 4-$(CH_3)_2NC_6H_4$

Srinivasarao and Lakshmi (2013) synthesized α-naphthayl amine binded 1,4-dihydropyridine derivatives for their antibacterial and antifungal activity. Compound (**S-4.16**) showed highest antibacterial activity than tested standard Ciprofloxacin and antifungal activity of compound (**S-4.17**) showed more potent than standard Clotrimazole.



S-4.16 **S-4.17**

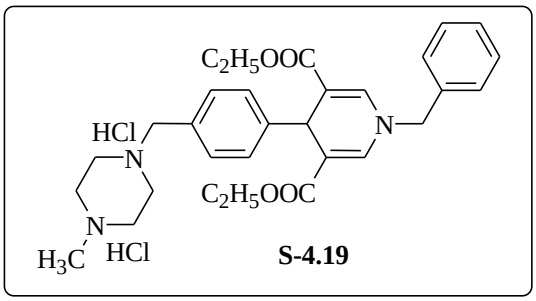
Desai *et al.* (2015) synthesized and characterized a new 1,4-dihydropyridine-3,5-dicarbamoyl derivative bearing an imidazole nucleus via Hantzsch multicomponent reaction. All compounds were screened for antitubercular activity against *Mycobacterium tuberculosis* strain. Among all compounds, compounds (**S-4.18**) showed more potent than standard with MIC value of 0.02 μg/mL and SI > 500. And also, these derivatives have displayed relatively low cytotoxicity.



S-4.18

R= F, NO_2

New 1,4-dihydropyridine (DHPs) analogues have been synthesized bearing changes at the C2/C6, C3/C5, C4 or N1 position. All compounds were tested for SIRT1 inhibitor, highlighting the involvement of the SIRT1/AMPK pathway in the action of DHPs. Compound (**S-4.19**) showed displayed antiproliferative effects in the range of 8-35 μM and increased H4K16 deacetylation. It indicates a possible role for SIRT1 activators in cancer therapy (Valente *et al.* 2016).



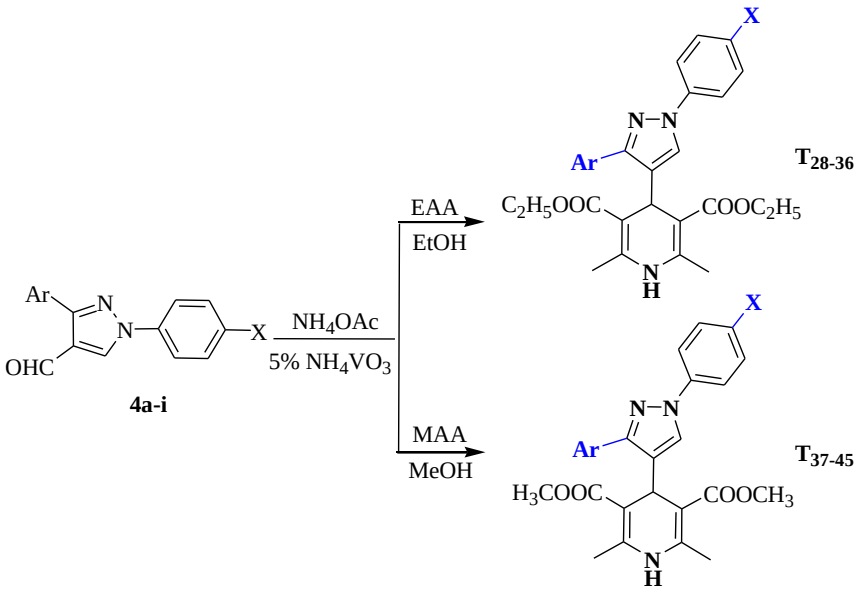
S-4.19

Based on the above literature survey as well as the immediate need for the development of effective antitubercular drugs, it was planned to synthesize a new series of 1,4-dihydropyridine derivatives. These compounds were evaluated for their antitubercular screening against *Mycobacterium tuberculosis* and antibacterial activity against *Mycobacterium smegmatis, Staphylococcus aureus* and antifungal activity against *Candida albicans.*

4.2. MATERIALS AND METHODS

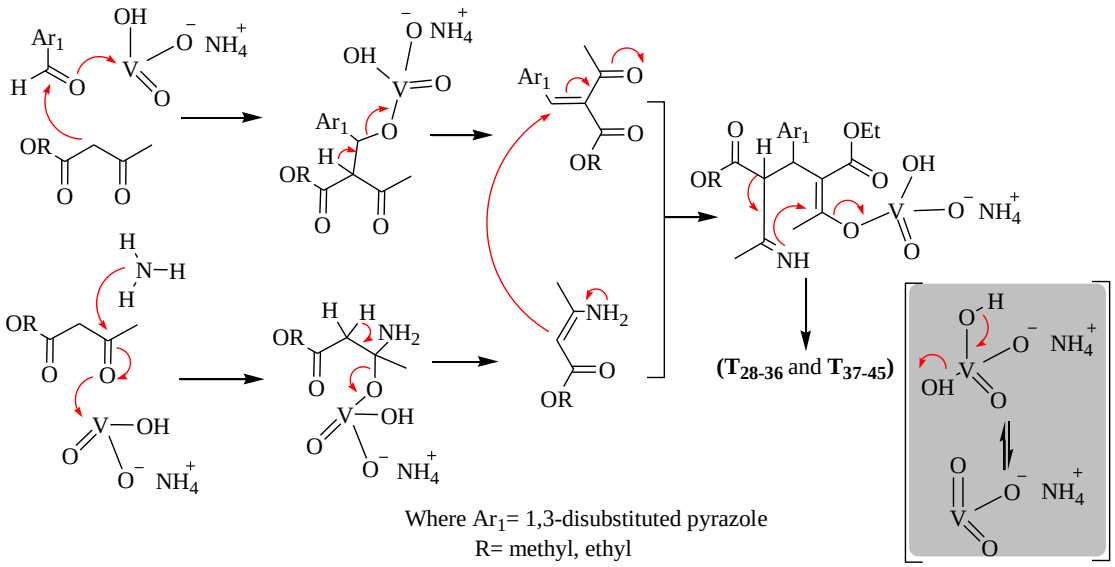
The versatile Hantzsch reaction was used to synthesize the target 1,4-dihydropyridine derivatives (**T_{28-36}** and **T_{37-45}**) by reacting ethyl acetoacetate or methyl acetoacetate with ammonium acetate in alcohol media with catalytic amount of ammonium metavanadate (5 % w/w). Ammonium metavanadate is the most common, non-expensive and an environmentally friendly inorganic acid in pharmaceutical industry. It is water soluble, mild and efficient catalyst. Ammonium metavanadate contains different oxidative states (+5, +4, +3 and +2) that are dependent on pH. It forms a stable $[VO_2(H_2O)_4]^+$ complex (Cruywagen *et al.* 1996). The logic behind this as a catalyst is, ease to formation of vanadium complex easily with aldehyde, moreover, it is a good dehydrating agent. Normally Hantzsch dihydropyridine will take more than 3 h for reaction conversion and also the yield of final compounds are not more than 70 % (Trivedi *et al.* 2011). The use of ammonium metavanadate helps to increase the reaction rate and yield achieved until 95 %. The reaction pathway and

mechanism has been described in **Scheme 4.1**. Newly synthesized compounds were characterized and confirmed by IR, NMR, mass spectral study and C, H, N elemental analyses.



Where Ar= 4-$CH_3C_6H_4$, 4-$OCH_3C_6H_4$, 4-ClC_6H_4, 4-BrC_6H_4, 3-BrC_6H_4, Biphenyl, 2-Thiophene,
X= H, Cl

Proposed pathway:



Where Ar_1= 1,3-disubstituted pyrazole
R= methyl, ethyl

Scheme 4.1 Synthetic route for the pyrazole bearing 1,4-dihydropyridine derivatives

All the chemicals were purchased from Sigma-Aldrich, Merck and Spectrochem-India. Commercial grade solvents used for the reactions were distilled before use. The melting points were determined by open capillary tube method and

were uncorrected. The IR spectra were recorded in KBr on Shimadzu FT-IR (4000-400 cm^{-1}) spectrophotometer. Bruker (300, 400 MHz) spectrometer was used to record ^{1}H-NMR and ^{13}C-NMR spectra ($CDCl_3$, DMSO-d_6) using TMS as internal standard. Chemical shift values were given in δ (ppm) scales. The mass spectrum was recorded on LC-MS Applied biosystems MDS SCIEX-API 4000 spectrometer. Single crystal XRD analysis for compound **T_{29}** was recorded by Bruker APEX-II diffractometer equipped with dual system CCD detector. Elemental analysis was performed on a Flash EA 1112 series CHNS-O analyzer. The completion of the reaction was checked by thin layer chromatography (TLC) on silica gel coated aluminium sheets (Merck F_{254}).

4.3 EXPERIMENTAL

The procedure for the synthesis of intermediate compounds **4a-i** has been discussed in **Chapter 2**.

General procedure for the synthesis of compounds ($T_{28\text{-}36}$):

A reaction mixture consisting of compound (**4a)** (1.48 g, 0.005 mol), ethyl acetoacetate (1.27 g, 0.01 mol), ammonium metavanadate (5 % w/w) and ammonium acetate (0.39 g, 0.005 mol) in ethanol (10 ml) were mixed in a round bottom flask. The reaction mixture was heated to 70 °C for 1 h. The completion of the reaction was confirmed by TLC [n-hexane : ethylacetate (9:1)]; the obtained solid was quenched into demineralized water and stirred for 30 min. Solid compound was filtered and washed with pre-chilled ethanol to get 4-[1-(4-Chlorophenyl)-3-*p*-tolyl-1*H*-pyrazol-4-yl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylicacid diethyl ester (**T_{28}**) in reasonably good yield. The same procedure was followed for the synthesis of compounds **$T_{29\text{-}36}$**.

The characterization details of the compounds were presented below.

4-[1-(4-Chlorophenyl)-3-*p*-tolyl-1*H*-pyrazol-4-yl]-2,6-dimethyl-1,4-dihydro pyridine-3,5-dicarboxylicacid diethylester (T_{28}):

IR (KBr ν_{max} cm^{-1}): 3329 (N-H str), 3102 (CH-aromatic str), 2980 (C-H str), 1688 (C=O str), 1645 (C=N str), 1535 (C=C str), 1209 (C-O str); ^{1}H-NMR (300 MHz, $CDCl_3$, ppm): δ 1.05-1.09 (t, J = 7.05 Hz, 6H, CH_3), 2.23 (s, 6H, CH_3), 2.39 (s, 3H, CH_3), 3.72-3.82 (m, 2H, CH_2), 3.94-4.08 (m, 2H, CH_2), 5.28 (s, 1H, dihydropyridine-

4H), 5.44 (s, 1H, NH), 7.21-7.23 (d, *J* = 7.8 Hz, 2H, ArH), 7.34-7.37 (d, *J* = 9.0 Hz, 2H, ArH), 7.60-7.63 (d, *J* = 8.7 Hz, 2H, ArH), 7.69 (s, 1H, pyrazole-5H), 7.69-7.72 (d, *J*= 9.3 Hz, 2H, ArH); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 167.5, 151.6, 143.3, 138.7, 137.2, 131.7, 131.2, 129.2, 129.0, 128.8, 128.6, 126.9, 119.8, 104.4, 59.7, 29.7, 21.3, 19.5, 14.3; MS: *m/z* = 519.9 (M+1), Anal. Calcd. for $C_{29}H_{30}ClN_3O_4$; C, 66.98; H, 5.81; N, 8.08; found: C, 67.01; H, 5.80; N, 8.09.

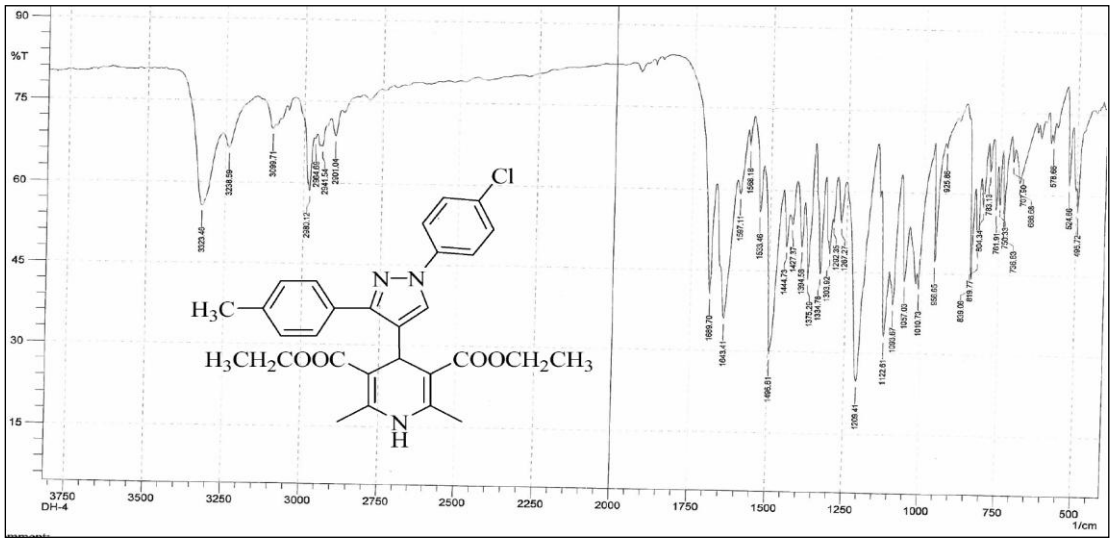


Figure 4.2 IR spectrum of compound **T_{28}**

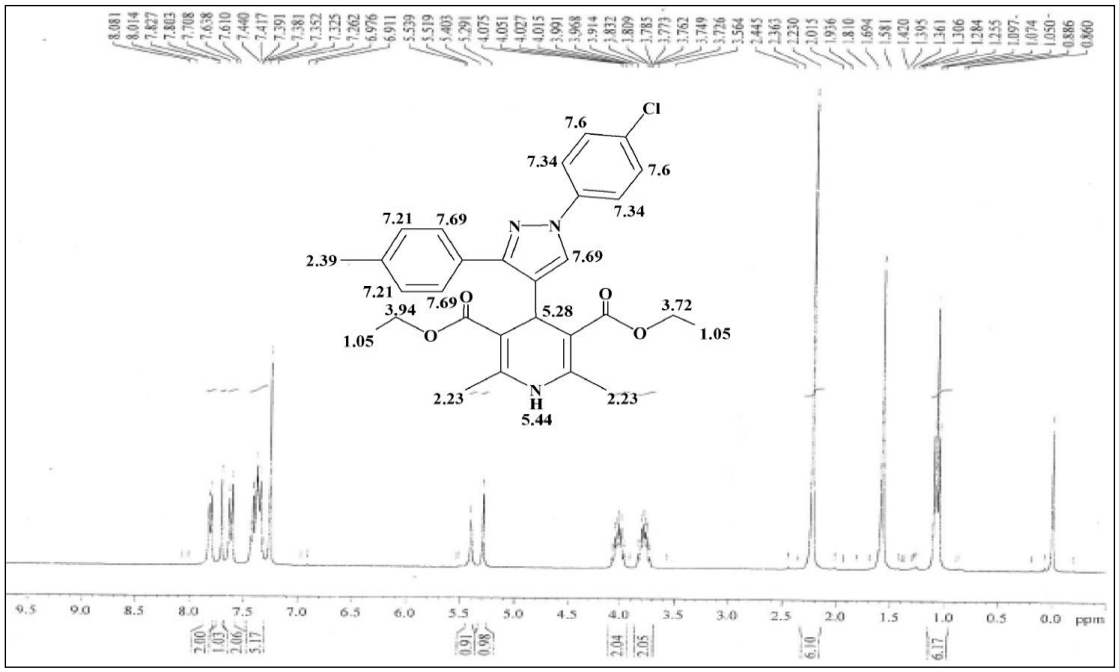


Figure 4.3 ^{1}H-NMR spectrum of compound **T_{28}**

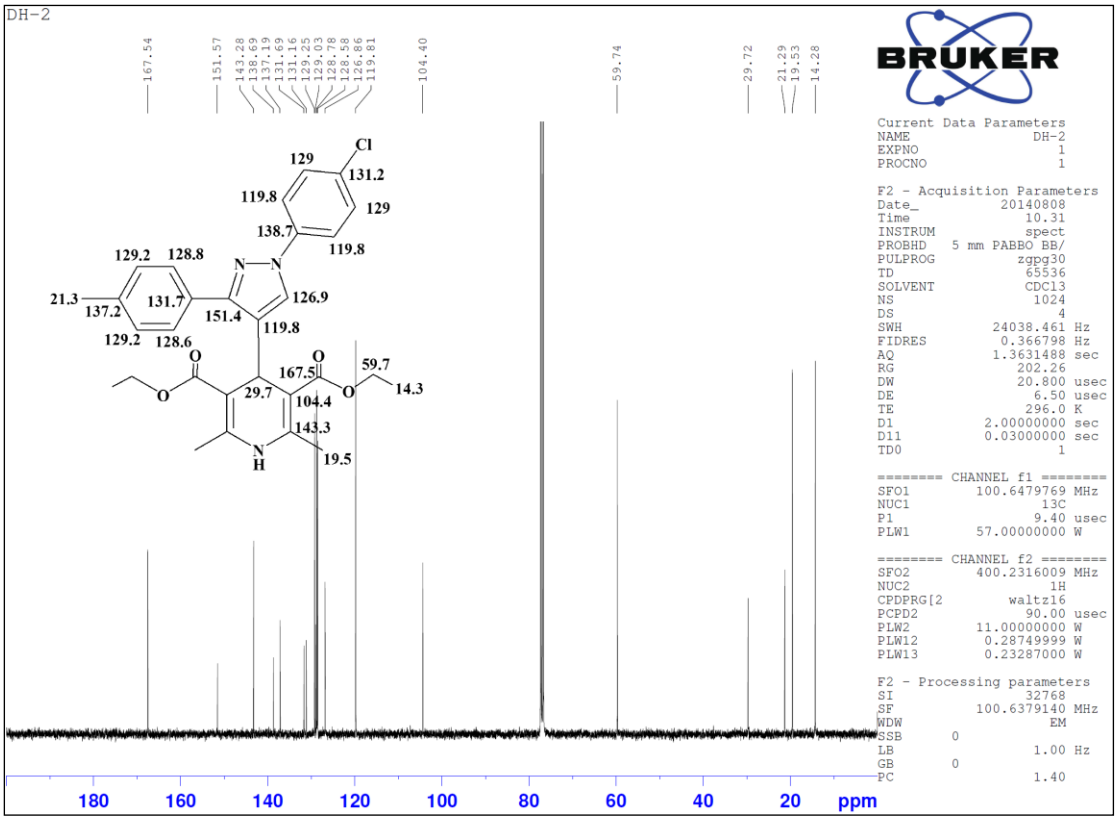


Figure 4.4 ^{13}C-NMR spectrum of compound **T_{28}**

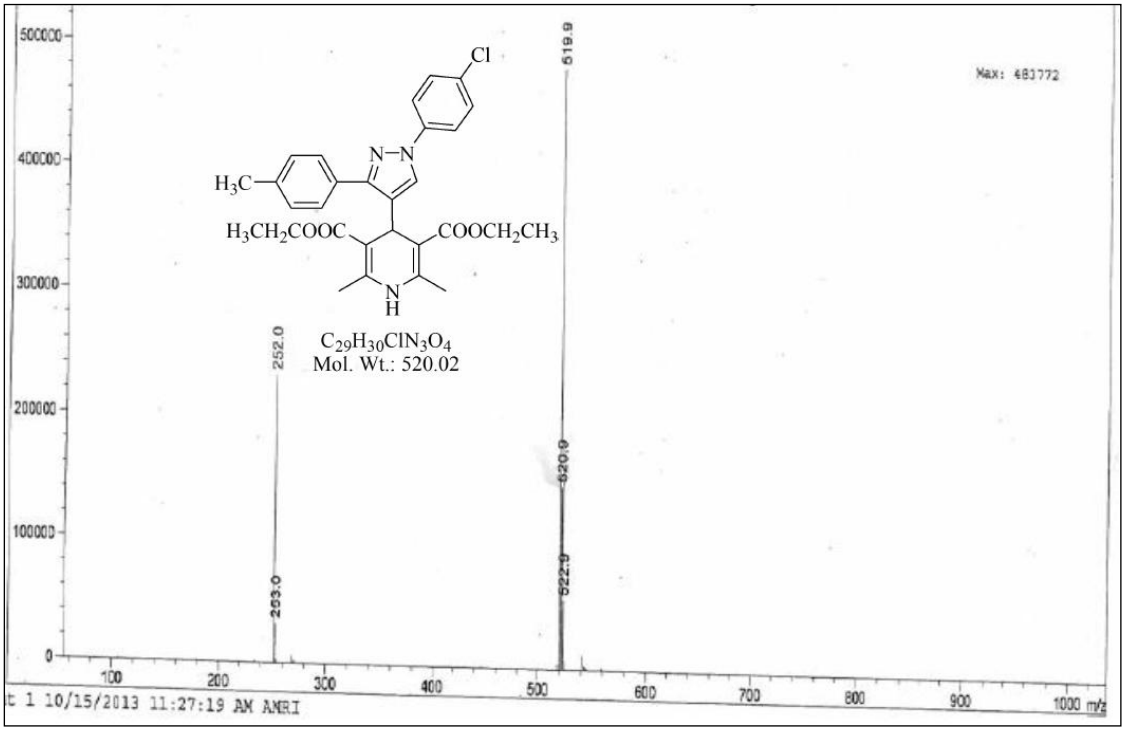


Figure 4.5 Mass spectrum of compound **T_{28}**

4-[1-(4-Chlorophenyl)-3-(4-methoxyphenyl)-1*H*-pyrazol-4-yl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylicacid diethylester (T_{29}):

IR (KBr ν_{max} cm^{-1}): 3329 (N-H str), 3084 (CH-aromatic str), 2980 (C-H str), 1688 (C=O str), 1643 (C=N str), 1528 (C=C str), 1207 (C-O str); ^{1}H-NMR (300 MHz, $CDCl_3$, ppm): δ 1.06-1.11 (t, J = 7.05 Hz, 6H, CH_3 on ester), 2.24 (s, 6H, CH_3), 3.76-3.83 (m, 2H, CH_2), 3.85 (s, 3H, OCH_3), 3.98-4.08 (m, 2H, CH_2), 5.26 (s, 1H, dihydropyridine-4H), 5.44 (s, 1H, NH), 6.94-6.97 (d, J = 8.7 Hz, 2H, ArH), 7.34-7.37 (d, J = 9.0 Hz, 2H, ArH), 7.60-7.63 (d, J = 9.0 Hz, 2H, ArH), 7.69 (s, 1H, pyrazole-5H), 7.74-7.77 (d, J = 8.7 Hz, 2H, ArH); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 167.5, 159.3, 151.3, 143.2, 138.7, 131.1, 130.1, 129.3, 128.9, 127.3, 126.8, 119.8, 113.4, 104.4, 59.8, 55.4, 29.8, 19.6, 14.3; MS: *m/z*= 535.9 (M+1), Anal. Calcd. for $C_{29}H_{30}ClN_3O_5$; C, 64.98; H, 5.64; N, 7.84; found: C, 65.01; H, 5.64; N, 7.84.

4-[1,3-Bis(4-Chlorophenyl)-1*H*-pyrazol-4-yl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylicacid diethylester (T_{30}):

IR (KBr ν_{max} cm^{-1}): 3323 (N-H str), 3100 (CH-aromatic str), 2980 (C-H str), 1690 (C=O str), 1643 (C=N str), 1533 (C=C str), 1209 (C-O str); ^{1}H-NMR (300 MHz, $CDCl_3$, ppm): δ 1.05-1.10 (t, J = 7.2 Hz, 6H, CH_3), 2.26 (s, 6H, CH_3), 3.74-3.85 (m, 2H, CH_2), 3.98-4.08 (m, 2H, CH_2), 5.26 (s, 1H, dihydropyridine-4H), 5.52 (s, 1H, NH), 7.36-7.42 (m, 4H, ArH), 7.59-7.62 (d, J = 8.7 Hz, 2H, ArH), 7.70 (s, 1H, pyrazole-5H), 7.83-7.86 (d, J = 8.4 Hz, 2H, ArH); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 167.4, 150.1, 143.3, 138.5, 133.6, 133.1, 131.5, 130.2, 129.3, 129.3, 128.1, 127.2, 119.9, 104.5, 59.8, 29.7, 19.6, 14.3; MS: *m/z* = 539.8 (M+1), Anal. Calcd. for $C_{28}H_{27}Cl_2N_3O_4$; C, 62.23; H, 5.04; N, 7.78; found: C, 62.24; H, 5.05; N, 7.80.

4-[3-(4-Bromophenyl)-1-(4-chlorophenyl)-1*H*-pyrazol-4-yl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylicacid diethylester (T_{31}):

IR (KBr ν_{max} cm^{-1}): 3325 (N-H str), 3100 (CH-aromatic str), 2980 (C-H str), 1690 (C=O str), 1643 (C=N str), 1533 (C=C str), 1209 (C-O str); ^{1}H-NMR (300 MHz, $CDCl_3$, ppm): δ 1.05-1.10 (t, J = 7.05 Hz, 6H, CH_3), 2.26 (s, 6H, CH_3), 3.74-3.84 (m, 2H, CH_2), 3.98-4.08 (m, 2H, CH_2), 5.26 (s, 1H, dihydropyridine-4H), 5.55 (s, 1H, NH), 7.35-7.38 (d, J = 8.7 Hz, 2H, ArH), 7.54-7.57 (d, J = 8.4 Hz, 2H, ArH), 7.59-7.62 (d, J = 8.7 Hz, 2H, ArH), 7.70 (s, 1H, pyrazole-5H), 7.77-7.80 (d, J = 8.4 Hz,

2H, ArH); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 167.7, 145.3, 143.4, 140.1, 136.4, 129.7, 129.4, 127.9, 127.3, 126.5, 126.3, 125.0, 119.0, 105.1, 60.0, 30.0, 19.8, 14.4; MS: *m/z* = 583.8 (M+1), Anal. Calcd. for $C_{28}H_{27}BrClN_3O_4$; C, 57.50; H, 4.65; N, 7.18; found: C, 57.51; H, 4.66; N, 7.21.

4-[3-(3-Bromophenyl)-1-phenyl-1*H*-pyrazol-4-yl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylicacid diethylester (T_{32}):

IR (KBr ν_{max} cm^{-1}): 3298 (N-H str), 3115 (CH-aromatic str), 2969 (C-H str), 1678 (C=O str), 1597 (C=N str), 1523 (C=C str), 1223 (C-O str); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 1.06-1.10 (t, *J* = 6.9 Hz, 6H, CH_3), 2.24 (s, 6H, CH_3), 3.76-3.86 (m, 2H, CH_2), 4.00-4.10 (m, 2H, CH_2), 5.26 (s, 1H, dihydropyridine-4H), 5.52 (s, 1H, NH), 7.23-7.27 (t, *J* = 7.2 Hz, 2H, ArH), 7.33-7.35 (d, *J* = 7.8 Hz, 2H, ArH), 7.56-7.58 (d, *J* = 7.5 Hz, 1H, ArH), 7.61-7.63 (d, *J* = 7.8 Hz, 2H, ArH), 7.70 (s, 1H, pyrazole-5H), 7.78-7.80 (d, *J* = 7.0 Hz, 1H, ArH), 7.99-8.01 (d, 1H, ArH); MS: *m/z* = 550.3 (M+1).

4-[3-(3-Bromophenyl)-1-(4-chlorophenyl)-1*H*-pyrazol-4-yl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylicacid diethylester (T_{33}):

IR (KBr ν_{max} cm^{-1}): 3306 (N-H str), 3100 (CH-aromatic str), 2948 (C-H str), 1698 (C=O str), 1644 (C=N str), 1495 (C=C str), 1213 (C-O str), 725 (C-Cl str); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 1.08-1.11 (t, *J* = 6.6 Hz, 6H, CH_3), 2.24 (s, 6H, CH_3), 3.80-3.88 (m, 2H, CH_2), 4.01-4.09 (m, 2H, CH_2), 5.26 (s, 1H, dihydropyridine-4H), 5.50 (s, 1H, NH), 7.28-7.31 (t, *J* = 6.6 Hz, 1H, ArH), 7.37-7.39 (d, *J* = 8.4 Hz, 2H, ArH), 7.48-7.50 (d, *J* = 7.6 Hz, 1H, ArH), 7.60-7.62 (d, *J* = 7.6 Hz, 2H, ArH), 7.72 (s, 1H, pyrazole-5H), 7.76-7.78 (d, *J* = 7.2 Hz, 1H, ArH), 7.99 (s, 1H, ArH); MS: *m/z* = 584.2 (M+1), Anal. Calcd. for $C_{28}H_{27}BrClN_3O_4$; C, 57.50; H, 4.65; N, 7.18; found: C, 57.53; H, 4.70; N, 7.21.

4-(3-Biphenyl-4-yl)-1-phenyl-1*H*-pyrazol-4-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylicacid diethylester (T_{34}):

IR (KBr ν_{max} cm^{-1}): 3362 (N-H str), 3065 (CH-aromatic str), 2978 (C-H str), 1688 (C=O str), 1651 (C=N str), 1530 (C=C str), 1206 (C-O str); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 1.05-1.08 (t, *J* = 6.8 Hz, 6H, CH_3), 2.25 (s, 6H, CH_3), 3.75-3.82 (m,

2H, CH_2), 4.00-4.08 (m, 2H, CH_2), 5.34 (s, 1H, dihydropyridine-4H), 5.56 (s, 1H, NH), 7.36-7.38 (d, *J* = 7.6 Hz, 3H, ArH), 7.45-7.49 (t, *J* = 7.4 Hz, 2H, ArH), 7.63-7.65 (m, 4H, ArH), 7.66-7.68 (d, *J* = 8.8 Hz, 2H, ArH), 7.72 (s, 1H, pyrazole-5H), 7.94-7.96 (d, *J* = 7.2 Hz, 1H, ArH). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 167.6, 151.0, 143.4, 141.1, 140.4, 138.6, 133.7, 131.3, 129.4, 129.3, 129.3, 128.9, 127.3, 127.1, 127.0, 126.7, 119.9, 104.4, 59.8, 29.8, 19.5, 14.3; MS: *m/z* = 582.3 (M+1), Anal. Calcd. for $C_{34}H_{32}ClN_3O_4$; C, 70.15; H, 5.54; N, 7.22; found: C, 70.18; H, 5.55; N, 7.22.

2,6-Dimethyl-4-(1-phenyl-3-thiophene-2-yl-1*H*-pyrazol-4-yl)-1,4-dihydropyridine-3,5-dicarboxylicacid diethylester (T_{35}):

IR (KBr ν_{max} cm^{-1}): 3337 (N-H str), 3102 (CH-aromatic str), 2978 (C-H str), 1678 (C=O str), 1614 (C=N str), 1532 (C=C str), 1204 (C-O str); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 1.02-1.05 (t, *J* = 7.0 Hz, 6H, CH_3), 2.31 (s, 6H, CH_3), 3.83-3.91 (m, 2H, CH_2), 3.99-4.07 (m, 2H, CH_2), 5.34 (s, 1H, dihydropyridine-4H), 5.55 (s, 1H, NH), 7.10-7.12 (m, 1H, ArH), 7.21-7.25 (t, *J* = 7.4 Hz, 1H, ArH), 7.29-7.30 (d, *J* = 4.8 Hz, 1H, ArH), 7.38-7.42 (t, *J* = 7.8 Hz, 2H, ArH), 7.66-7.68 (d, *J* = 7.6 Hz, 2H, ArH), 7.74 (s, 1H, pyrazole-5H), 7.75-7.76 (d, *J* =3.6 Hz,1H, ArH); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 167.7, 145.3, 143.4, 140.0, 136.3, 129.7, 129.4, 127.9, 127.3, 126.4, 126.3, 125.0, 119.0, 105.1, 60.0, 29.9, 19.8, 14.4; MS: *m/z*= 478.2 (M+1), Anal. Calcd. for $C_{26}H_{27}N_3O_4S$; C, 65.39; H, 5.70; N, 8.80; found: C, 65.41; H, 5.71; N, 8.81.

4-[1-(4-Chlorophenyl)-3-thiophene-2-yl-1*H*-pyrazol-4-yl]-2,6-dimethyl-1,4-dihydro pyridine-3,5-dicarboxylicacid diethylester (T_{36}):

IR (KBr ν_{max} cm^{-1}): 3331 (N-H str), 3103 (CH-aromatic str), 2976 (C-H str), 1678 (C=O str), 1616 (C=N str), 1537 (C=C str), 1206 (C-O str); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 1.01-1.05 (t, *J* = 7.0 Hz, 6H, CH_3), 2.30 (s, 6H, CH_3), 3.82-3.90 (m, 2H, CH_2), 3.99-4.07 (m, 2H, CH_2), 5.34 (s, 1H, dihydropyridine-4H), 5.62 (s, 1H, NH), 7.11 (m, 1H, ArH), 7.29-7.30 (d, *J* = 4.4 Hz, 1H, ArH), 7.35-7.37 (d, *J* = 8.0 Hz, 2H, ArH), 7.56-7.62 (d, *J* = 8.4 Hz, 2H, ArH), 7.70 (s, 1H, pyrazole-5H), 7.77 (m, 1H, ArH); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 167.5, 145.4, 143.2, 138.4, 135.8, 131.4, 129.9, 129.3, 127.6, 127.2, 126.4, 125.0, 119.9, 104.9, 59.9, 29.8, 19.6, 14.2;

MS: *m/z* = 512.2 (M+1), Anal. Calcd. for $C_{26}H_{26}ClN_3O_4S$; C, 60.99; H, 5.12; N, 8.21; found: C, 61.00; H, 5.12; N, 8.21.

General procedure for the synthesis compounds (T_{37-45}):

A reaction mixture consisting of compound (**4a)** (1.48 g, 0.005 mol), methyl acetoacetate (1.08 g, 0.01 mol), ammonium metavanadate (5 % w/w) and ammonium acetate (0.39 g, 0.005 mol) in methanol (10 ml) were mixed in a round bottom flask. The reaction mixture was heated to 60 °C for 1 h. The completion of the reaction was confirmed by TLC [n-hexane : ethylacetate (9:1)]; the obtained solid was quenched into DM water and stirred to get solid. Solid compound was filtered and washed with pre-chilled methanol to get 4-[1-(4-Chlorophenyl)-3-*p*-tolyl-1*H*-pyrazol-4-yl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylicacid dimethyl ester (**T_{37}**). The compound was used for further analysis without any purification. The same procedure was followed for the synthesis of compounds **T_{38-45}**.

The characterization details of the synthesized compounds were presented below.

4-[1-(4-Chlorophenyl)-3-*p*-tolyl-1*H*-pyrazol-4-yl]-2,6-dimethyl-1,4-dihydro pyridine-3,5-dicarboxylicacid dimethylester (T_{37}):

IR (KBr ν_{max} cm^{-1}): 3331 (N-H str), 3104 (CH-aromatic str), 2943 (C-H str), 1695 (C=O str), 1647 (C=N str), 1541 (C=C str), 1213 (C-O str); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 2.29 (s, 6H, CH_3), 2.41 (s, 3H, CH_3), 3.34 (s, 6H, CH_3), 5.26 (s, 1H, dihydropyridine-4H), 5.58 (s, 1H, NH), 7.25-7.27 (d, 2H, *J* = 6.8 Hz, ArH), 7.35-7.37 (d, 2H, *J* = 7.6 Hz, ArH), 7.60-7.62 (d, *J* = 7.6 Hz, 2H, ArH), 7.65 (s, 1H, pyrazole-5H), 7.72-7.74 (d, *J* = 7.2 Hz, 2H, ArH); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 167.8, 151.4, 143.5, 138.7, 137.1, 131.8, 131.2, 129.3, 129.2, 128.7, 128.5, 126.9, 119.9, 104.4, 50.7, 29.5, 21.3, 19.4; MS: *m/z* = 492.2 (M+1), Anal. Calcd. for $C_{27}H_{26}ClN_3O_4$; C, 65.92; H, 5.33; N, 8.54; found: C, 65.92; H, 5.34; N, 8.59.

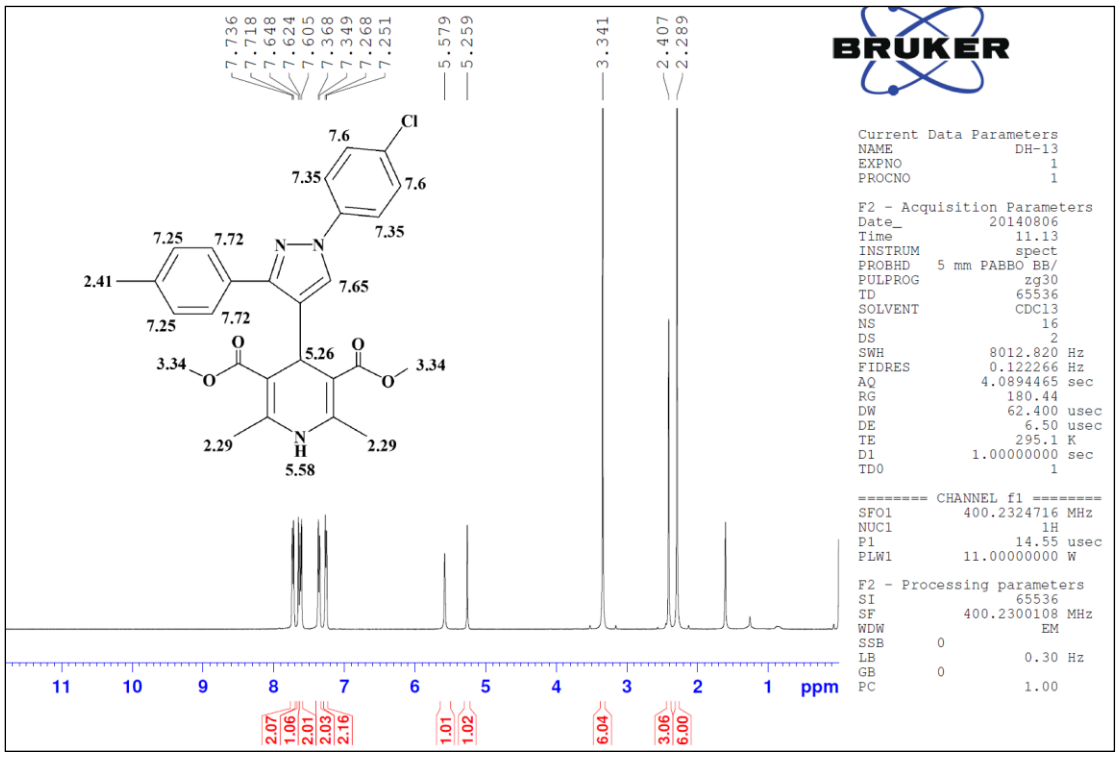


Figure 4.6 ^{1}H-NMR spectrum of compound **T_{37}**

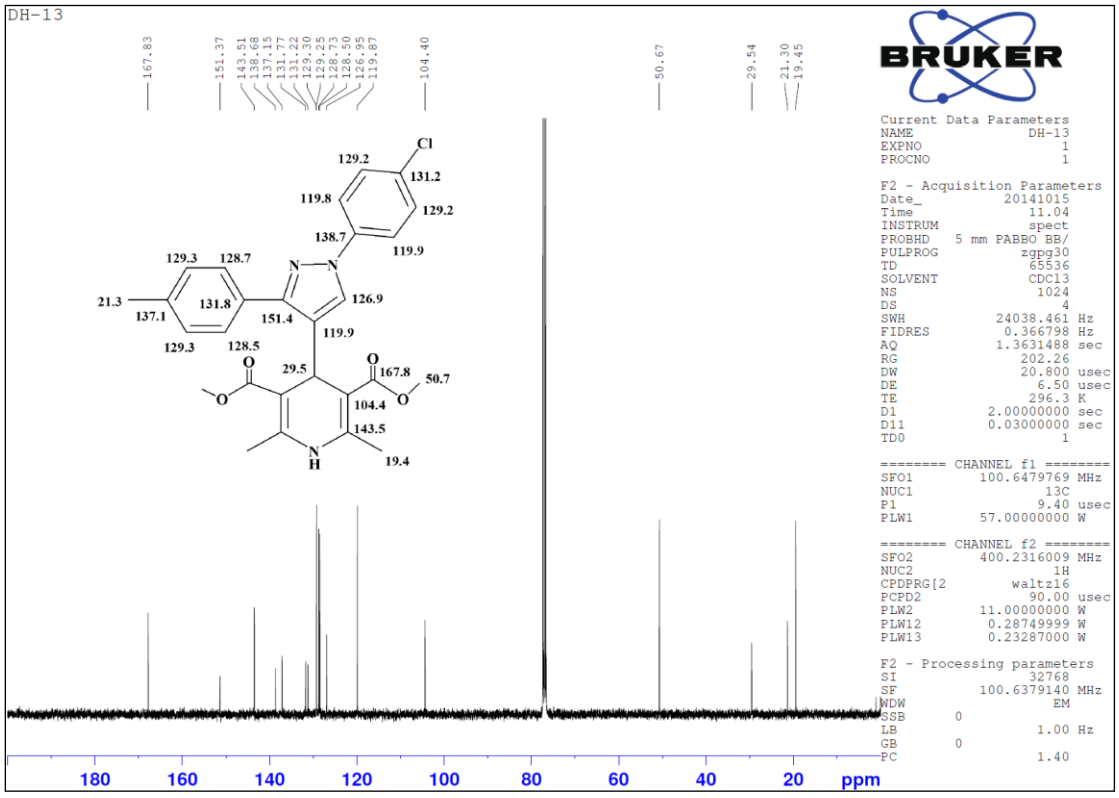


Figure 4.7 ^{13}C-NMR spectrum of compound **T_{37}**

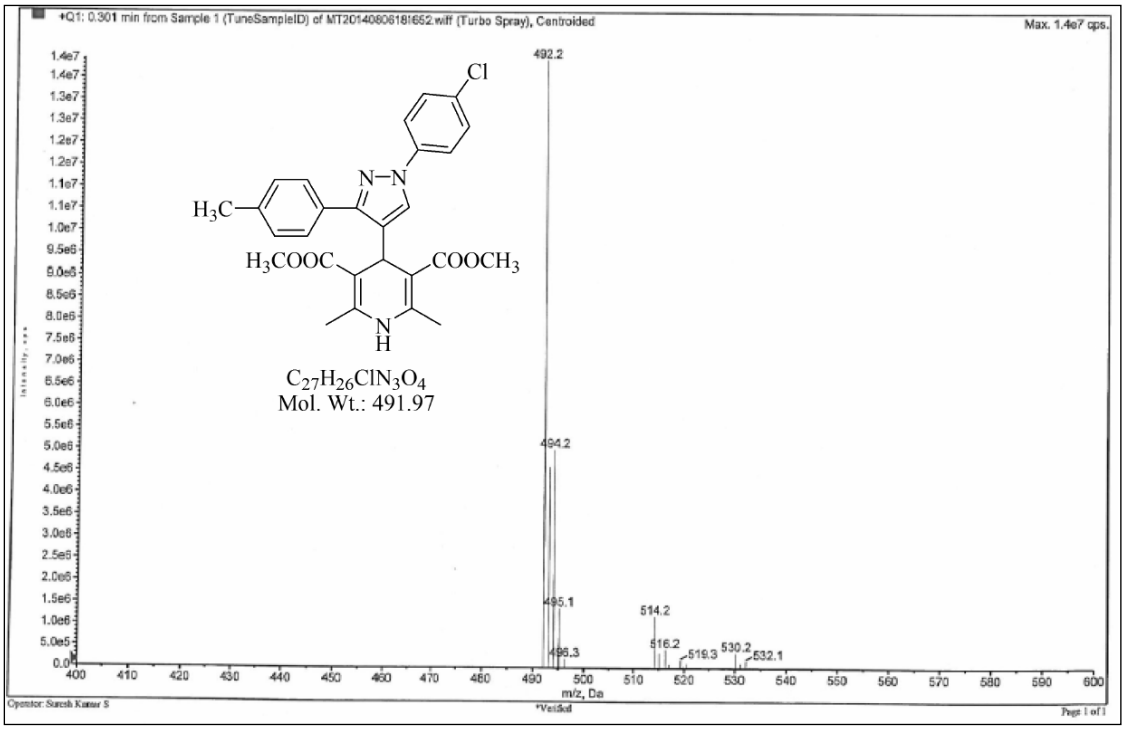


Figure 4.8 Mass spectrum of compound **T_{37}**

4-[1-(4-Chlorophenyl)-3-(4-methoxyphenyl)-1*H*-pyrazol-4-yl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylicacid dimethylester (T_{38}):

IR (KBr ν_{max} cm^{-1}): 3330 (N-H str), 2904 (C-H str), 1692 (C=O str), 1628 (C=N str), 1542 (C=C str), 1208 (C-O str); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 2.29 (s, 6H, CH_3), 3.37 (s, 6H, CH_3), 3.86 (s, 3H, OCH_3), 5.24 (s, 1H, dihydropyridine-4H), 5.56 (s, 1H, NH), 6.99-7.01 (d, 2H, J = 7.2 Hz, ArH), 7.35-7.37 (d, J = 7.2 Hz, 2H, ArH), 7.60-7.62 (d, J = 7.6 Hz, 2H, ArH), 7.65 (s, 1H, pyrazole-5H), 7.77-7.79 (d, J = 7.6 Hz, 2H, ArH); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 167.8, 159.3, 151.1, 143.5, 138.7, 131.2, 129.8, 129.2, 129.2, 127.3, 126.9, 119.8, 113.5, 104.4, 55.4, 50.7, 29.6, 19.5; MS: *m/z* = 507.3 (M+1), Anal. Calcd. for $C_{27}H_{26}ClN_3O_5$; C, 63.84; H, 5.16; N, 8.27; found: C, 63.83; H, 5.18; N, 8.30.

4-[1,3-Bis(4-chlorophenyl)-1*H*-pyrazol-4-yl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylicacid dimethylester (T_{39}):

IR (KBr ν_{max} cm^{-1}): 3318 (N-H str), 3104 (CH-aromatic str), 2943 (C-H str), 1697 (C=O str), 1645 (C=N str), 1539 (C=C str), 1211 (C-O str); ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): δ 2.25 (s, 6H, CH_3), 3.24 (s, 6H, CH_3), 5.09 (s, 1H,

dihydropyridine-4H), 7.50-7.53 (d, *J* = 8.8 Hz, 2H, ArH), 7.56-7.58 (d, *J* = 8.4 Hz, 2H, ArH), 7.86-7.89 (d, *J* = 8.8 Hz, 4H, ArH), 8.08 (s, 1H, pyrazole-5H), 8.87 (s, 1H, NH); MS: *m/z* = 512.2 (M+1), Anal. Calcd. for $C_{26}H_{23}Cl_2N_3O_4$; C, 60.95; H, 4.52; N, 8.20; found: C, 60.96; H, 4.52; N, 8.23.

4-[3-(4-Bromophenyl)-1-(4-chlorophenyl)-1*H*-pyrazol-4-yl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylicacid dimethylester (T_{40}):

IR (KBr ν_{max} cm^{-1}): 3323 (N-H str), 3102 (CH-aromatic str), 2943 (C-H str), 1697 (C=O str), 1643 (C=N str), 1539 (C=C str), 1211 (C-O str); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 2.31 (s, 6H, CH_3), 3.36 (s, 6H, CH_3), 5.23 (s, 1H, dihydropyridine-4H), 5.60 (s, 1H, NH), 7.36-7.38 (d, 2H, *J* = 8.0 Hz, ArH), 7.59-7.61 (m, 4H, ArH),7.66 (s, 1H, pyrazole-5H), 7.77-7.79 (d, *J* = 7.6 Hz, 2H, ArH). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 167.7, 150.0, 143.6, 138.5, 133.7, 131.6, 131.2, 130.2, 129.5, 129.3, 127.3, 121.6, 120.3, 120.0, 104.3, 50.7, 29.5, 19.5; MS: *m/z* = 556.3 (M+1), Anal. Calcd. for $C_{26}H_{23}BrClN_3O_4$; C, 56.08; H, 4.16; N, 7.55; found: C, 56.11; H, 4.19; N, 7.56.

4-[3-(3-Bromophenyl)-1-phenyl-1*H*-pyrazol-4-yl]-2,6-dimethyl-1,4-dihydro pyridine-3,5-dicarboxylicacid dimethylester (T_{41}):

IR (KBr ν_{max} cm^{-1}): 3318 (N-H str), 3104 (CH-aromatic str), 2943 (C-H str), 1697 (C=O str), 1643 (C=N str), 1537 (C=C str), 1211 (C-O str); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 2.29 (s, 6H, CH_3), 3.40 (s, 6H, CH_3), 5.24 (s, 1H, dihydropyridine-4H), 5.66 (s, 1H, NH), 7.23-7.27 (t, 1H, *J* =6.8 Hz, ArH), 7.31-7.35 (t, 1H, *J* = 7.6 Hz, ArH), 7.39-7.43 (t, 2H, *J* = 7.6 Hz, ArH), 7.49-7.51 (d,1H, *J* = 8.0 Hz, ArH), 7.65-7.67 (d, 2H, *J* = 7.6 Hz, ArH), 7.71 (s, 1H, pyrazole-5H), 7.79-7.81 (d, *J* = 7.6 Hz, 1H, ArH), 8.04 (s, 1H, ArH); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 167.7, 149.5, 143.8, 139.9, 137.0, 131.7, 130.3, 129.7, 129.3, 129.0, 127.4, 127.3, 126.3, 122.0, 119.0, 104.1, 50.7, 29.5, 19.4; MS: *m/z* = 522.1 (M+1), Anal. Calcd. for $C_{26}H_{24}BrN_3O_4$; C, 59.78; H, 4.63; N, 8.04; found: C, 60.00; H, 4.64; N, 8.09.

4-[3-(3-Bromophenyl)-1-(4-chlorophenyl)-1*H*-pyrazol-4-yl]-2,6-dimethyl-1,4-dihydro pyridine-3,5-dicarboxylicacid dimethylester (T_{42}):

IR (KBr ν_{max} cm^{-1}): 3323 (N-H str), 3121 (CH-aromatic str), 2945 (C-H str), 1684 (C=O str), 1597 (C=N str), 1501 (C=C str), 1225 (C-O str); ^{1}H-NMR (400 MHz,

$CDCl_3$, ppm): δ 2.30 (s, 6H, CH_3), 3.40 (s, 6H, CH_3), 5.23 (s, 1H, dihydropyridine-4H), 5.60 (s, 1H, NH), 7.31-7.35 (t, *J* = 8.0 Hz, 1H, ArH), 7.37-7.39 (d, 2H, *J* = 7.6 Hz, ArH), 7.50-7.52 (d, *J* = 8.0 Hz, 1H, ArH), 7.60-7.62 (d, *J* = 7.2 Hz, 2H, ArH), 7.68 (s, 1H, pyrazole-5H), 7.77-7.79 (d, 1H, *J* = 8.0 Hz, ArH), 8.02 (s, 1H, ArH); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 167.6, 149.9, 143.8, 138.5, 136.8, 131.7, 131.6, 130.4, 129.7, 129.4, 129.3, 127.3, 122.0, 120.1, 120.0, 104.1, 50.8, 29.5, 19.5; MS: *m/z*= 557.9 (M+1), Anal. Calcd. for $C_{26}H_{23}BrClN_3O_4$; C, 56.08; H, 4.16; N, 7.55; found: C, 56.11; H, 4.20; N, 7.59.

2,6-Dimethyl-4-(1-phenyl-3-thiophene-2-yl-1*H*-pyrazol-4-yl)-1,4-dihydropyridine-3,5-dicarboxylicacid dimethylester (T_{44}):

IR (KBr ν_{max} cm^{-1}): 3343 (N-H str), 3107 (CH-aromatic str), 2945 (C-H str), 1694 (C=O str), 1641 (C=N str), 1533 (C=C str), 1215 (C-O str); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 2.33 (s, 6H, CH_3), 3.41 (s, 6H, CH_3), 5.32 (s, 1H, dihydropyridine-4H), 5.60 (s, 1H, NH), 7.13-7.15 (t, 1H, ArH), 7.29-7.30 (d, *J* = 6.0 Hz, 1H, ArH), 7.32-7.33 (d, *J* = 4.8 Hz, 1H, ArH), 7.38-7.42 (t, *J* = 8.0 Hz, 2H, ArH), 7.66-7.69 (m, 3H, ArH), 7.71 (s, 1H, pyrazole-5H); ^{13}CNMR (100 MHz, $CDCl_3$): δ 168.0, 145.3, 143.7, 140.1, 136.3, 129.9, 129.7, 129.5, 129.4, 127.9, 127.3, 126.4, 126.1, 125.1, 119.1, 104.8, 50.9, 29.9, 19.6; MS: *m/z* = 449.52 (M+1), Anal. Calcd. for $C_{24}H_{23}N_3O_4S$; C, 64.13; H, 5.16; N, 9.35; found: C, 64.15; H, 5.20; N, 9.41.

4-[1-(4-Chlorophenyl)-3-thiophene-2-yl-1*H*-pyrazol-4-yl]-2,6-dimethyl-1,4-dihydro pyridine-3,5-dicarboxylicacid dimethylester (T_{45}):

IR (KBr ν_{max} cm^{-1}): 3341 (N-H str), 3105 (CH-aromatic str), 2943 (C-H str), 1678 (C=O str), 1614 (C=N str), 1535 (C=C str), 1215 (C-O str); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 2.33 (s, 6H, CH_3), 3.40 (s, 6H, CH_3), 5.31 (s, 1H, dihydropyridine-4H), 5.64 (s, 1H, NH), 7.15 (m, 1H, ArH), 7.33-7.34 (d, *J* = 5.2 Hz, 1H, ArH), 7.35-7.37 (d, *J* = 8.0 Hz, 2H, ArH), 7.60-7.62 (d, *J* = 7.6 Hz, 2H, ArH), 7.67 (m, 1H, ArH & 1H, pyrazole-5H); MS: *m/z* = 484.0 (M+1), Anal. Calcd. for $C_{24}H_{22}ClN_3O_4S$;C, 59.56; H, 4.58; N, 8.68; found: C, 59.61; H, 4.61; N, 8.72.

4.4 PHARMACOLOGY

4.4.1 Antitubercular activity

Antitubercular screening for the newly synthesized compounds $\mathbf{T_{28\text{-}36}}$ and $\mathbf{T_{37\text{-}45}}$ have determined by the Middle brook 7H9 broth against *Mycobacterium tuberculosis* of H_{37}Rv strain (ATCC-27294) as explained in **Chapter 2**. The Minimum Inhibitory Concentration (MIC) of each synthesized compound was determined by the Microplate Alamar Blue Assay method (MABA). The final drug concentrations tested were 100-0.78 µg/mL. Ethambutol, Streptomycin and Pyrazinamide have used as standard anti-TB drugs.

4.4.2 Antimicrobial activity

Antimicrobial activities of newly synthesized compounds ($\mathbf{T_{28\text{-}36}}$ and $\mathbf{T_{37\text{-}45}}$) were determined by Minimum Inhibitory Concentration (MIC) and were identified by using Resazurin reduction method in 96 well plate as explained in **Chapter 2**. *In vitro* antibacterial activity was performed against Gram +ve bacteria *Staphylococcus aureus* (MTCC 3160) and tuberculosis variant bacteria *Mycobacterium smegmatis* (MTCC 994) was determined. Antifungal studies of above compounds were carried out against *Candida albicans* (MTCC 7253) All the bacterial and fungal cultures were obtained from IMTECH, Chandigarh, India and maintained the cultures were maintained as per the standard protocol. The well, which remains blue after 24 hours of incubation indicates there are no microorganisms survived in the well, the minimum concentration where no microbial growth found are considered as MIC value.

4.5 RESULTS AND DISCUSSION

4.5.1 Chemistry

The characterization data of the synthesized 1,3-disubstituted-1*H*-pyrazole-4-carbaldehydes (**4a-i**) are presented in **Table 4.1**.

Table 4.1 Physical data of the synthesized compounds **4a-i**

Compounds	Ar	X	M. wt	M. F	M.p (°C)	Yield (%)
4a	4-$CH_3C_6H_4$	Cl	296.5	$C_{17}H_{13}ClN_2O$	133-134	90
4b	4-$OMeC_6H_4$	Cl	312.75	$C_{17}H_{13}ClN_2O_2$	142-143	89
4c	4-ClC_6H_4	Cl	317.17	$C_{16}H_{10}Cl_2N_2O$	177-178	85
4d	4-BrC_6H_4	Cl	361.62	$C_{16}H_{10}BrClN_2O$	156-157	79
4e	3-BrC_6H_4	H	327.18	$C_{16}H_{11}BrN_2O$	93-94	65
4f	3-BrC_6H_4	Cl	361.62	$C_{16}H_{10}BrClN_2O$	147-148	72
4g	Biphenyl	Cl	358.82	$C_{22}H_{15}ClN_2O$	207-208	76
4h	2-Thiophene	H	254.31	$C_{14}H_{10}N_2OS$	96-97	81
4i	2-Thiophene	Cl	288.75	$C_{14}H_9ClN_2OS$	140-140	82

Formation of 1,4-dihydropyridine derivatives (**$T_{28\text{-}36}$** and **$T_{37\text{-}45}$**) were confirmed by recording their IR, ^{1}H-NMR, ^{13}C-NMR and mass spectra. IR analysis of compound **T_{28}** showed the peak at 3329 cm^{-1} is due to the NH group on 1,4-dihydropyridine ring. The absorption band at 1688 cm^{-1} is due to the C=O stretching of ester group, which confirmed the formation of compound **T_{28}**. The ^{1}H-NMR spectrum of **T_{28}** showed a triplet of six protons at δ 1.05-1.09 which was attributed to the CH_3 protons of the ester group. A singlet of six protons attributed at δ 2.23 which is due to the CH_3 group on 1,4-dihydropyridine. The characteristic peak of 1,4-dihydropyridine-4H proton was observed as a singlet at δ 5.28 and the NH proton of 1,4-dihydropyridine attributed at δ 5.44. The other ^{1}H-NMR resonances are summarized in the experimental section. The mass spectrum of **T_{28}** showed a molecular ion peak at *m/z* = 519.9 (M+1). This, in turn, confirmed the formation of a compound having the molecular formula $C_{29}H_{30}ClN_3O_4$.

The ^{1}H-NMR spectrum of **T_{37}** showed a singlet of six protons at δ 2.29 which is due to the CH_3 group on 1,4-dihydropyridine. A singlet of six protons at δ 3.34 attributed to the CH_3 protons of the ester group. The singlet of characteristic proton pyrazole-5H was obtained at δ 7.65. The other ^{1}H-NMR resonances are summarized in the experimental section. The mass spectrum of **T_{37}** showed molecular ion peak at *m/z* = 492.2 (M+1), which is in agreement with the molecular formula $C_{27}H_{26}ClN_3O_4$. The characterization data of the synthesized 1,4-dihydropyridine derivatives are presented in **Table 4.2**.

Table 4.2 Structural properties of the target compounds $\mathbf{T_{28\text{-}36}}$ and $\mathbf{T_{37\text{-}45}}$

Compounds	Ar	X	M. wt	M. F	M.p (°C)	Yield (%)
$\mathbf{T_{28}}$	4-$CH_3C_6H_4$	Cl	520.02	$C_{29}H_{30}ClN_3O_4$	237-238	92
$\mathbf{T_{29}}$	4-$OCH_3C_6H_4$	Cl	536.02	$C_{29}H_{30}ClN_3O_5$	240-241	95
$\mathbf{T_{30}}$	4-ClC_6H_4	Cl	540.44	$C_{28}H_{27}Cl_2N_3O_4$	228-230	83
$\mathbf{T_{31}}$	4-BrC_6H_4	Cl	584.88	$C_{28}H_{27}BrClN_3O_4$	238-240	83
$\mathbf{T_{32}}$	3-BrC_6H_4	H	550.44	$C_{28}H_{28}BrN_3O_4$	198-199	81
$\mathbf{T_{33}}$	3-BrC_6H_4	Cl	584.88	$C_{28}H_{27}BrClN_3O_4$	147-148	87
$\mathbf{T_{34}}$	Biphenyl	Cl	582.08	$C_{34}H_{32}ClN_3O_4$	172-174	89
$\mathbf{T_{35}}$	2-Thiophene	H	477.57	$C_{26}H_{27}N_3O_4S$	195-196	84
$\mathbf{T_{36}}$	2-Thiophene	Cl	512.02	$C_{26}H_{26}ClN_3O_4S$	140-141	86
$\mathbf{T_{37}}$	4-$CH_3C_6H_4$	Cl	491.96	$C_{27}H_{26}ClN_3O_4$	243-244	89
$\mathbf{T_{38}}$	4-$OCH_3C_6H_4$	Cl	507.96	$C_{27}H_{26}ClN_3O_4$	235-236	88
$\mathbf{T_{39}}$	4-ClC_6H_4	Cl	512.38	$C_{26}H_{23}Cl_2N_3O_4$	234-235	81
$\mathbf{T_{40}}$	4-BrC_6H_4	Cl	556.83	$C_{26}H_{23}BrClN_3O_4$	248-248	80
$\mathbf{T_{41}}$	3-BrC_6H_4	H	522.39	$C_{26}H_{24}BrN_3O_4$	232-232	80
$\mathbf{T_{42}}$	3-BrC_6H_4	Cl	556.83	$C_{26}H_{23}BrClN_3O_4$	188-189	82
$\mathbf{T_{43}}$	Biphenyl	Cl	554.09	$C_{32}H_{29}ClN_3O_4$	200-201	88
$\mathbf{T_{44}}$	2-Thiophene	H	449.52	$C_{24}H_{23}N_3O_4S$	227-228	86
$\mathbf{T_{45}}$	2-Thiophene	Cl	483.96	$C_{24}H_{22}ClN_3O_4S$	215-216	86

4.5.2 X-ray diffraction

To understand the molecular interaction, compound $\mathbf{T_{29}}$ was selected for the single crystal X-ray diffraction study (**Figure 4.9**). The crystal structure of compound $\mathbf{T_{29}}$ (CCDC No. 1031058) was determined by X-ray crystallography. The details of data collection and structure refinement are listed in **Table 4.3**. All bond lengths and angles are in normal ranges (Allen *et al.* 1987). The molecular structure of compound $\mathbf{T_{29}}$ is composed of 1,4-dihydropyridine ring (C16-C18/N3/C19/C20) and pyrazole ring (C7-C9/N1/N2) which are nearly perpendicular. The planes between two rings make angles 87.27 (2)° in compound $\mathbf{T_{29}}$.

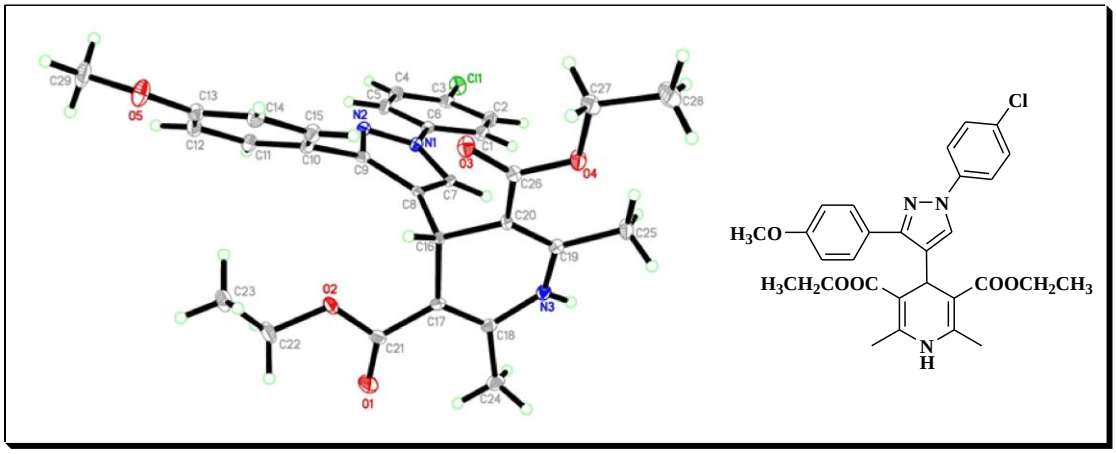


Figure 4.9 ORTEP diagram of compound **T_{29}**

Table 4.3 Details of data collection and structure refinement for compound **T_{29}**

Compound	**T_{29}**
Empirical formula	$C_{29}H_{29}ClN_3O_5$
Formula weight	535.00
Temperature (K)	100
Wavelength (A)	Mo Kα radiation, λ = 0.71073 Å
Crystal system, Space group	Monoclinic, *Cc*
Crystal	Colorless, needle
a (Å)	15.963 (3)
b (Å)	23.594 (4)
c (Å)	7.7722 (12)
β (°)	114.956 (5)°
Volume (Å^3)	2653.9 (8)
Z, D_{calc} (Mg m^{-3})	4, 1.339
Crystal size (mm)	0.84 × 0.37 × 0.21
θ Range for data (°)	2.8 – 30.6
Reflections collected/unique	35973/7097 [R_{int} = 0.103]
Goodness-of-fit on F^2	1.01
Final R indices[I > 2s(I)]	R1 = 0.0475, wR2 = 0.1052

4.5.3 Biological results

The MIC of newly synthesized compounds against *Mycobacterium tuberculosis* H_{37}Rv strain was represented in **Figure 4.10**. All the compounds were tested against pathogenic strain *Mycobacterium tuberculosis* H_{37}Rv at different concentrations ranging from 100 to 0.78 µg/mL. Antitubercular standard Ethambutol,

Streptomycin and Pyrazinamide were used as positive control for the comparison. Out of 18 synthesized compounds, 8 compounds have showed the MIC value ranging 12.5 to 3.12 µg/mL. Among all synthesized derivatives, compounds **T_{37}**, **T_{38}**, and **T_{44}** showed lowest MIC value of 3.12 µg/mL and equipotent with tested standard drug Ethambutol. The enhanced activity of compound **T_{37}** and **T_{38}** were due to electron donating group (4-methylphenyl and 4-methoxyphenyl) at third position of pyrazole core moiety and the presence of methyl ester at third and fifth position of 1,4-dihydropyridine. Another compound **T_{44}** was due to 2-thiophene group at the third position of the pyrazole ring and methyl ester at third and fifth position of 1,4-dihydropyridine enhancing the activity. The second lowest MIC value 6.25 µg/mL obtained for compounds **T_{36}** and **T_{45}** which is due to the presence of thiophene group at third position of pyrazole with ethyl ester or methyl ester at third and fifth position on 1,4-dihydropyridine. Other compounds **T_{28}**, **T_{29}** and **T_{43}** showed moderate activity with MIC value of 12.5 µg/mL against *Mycobacterium tuberculosis.*

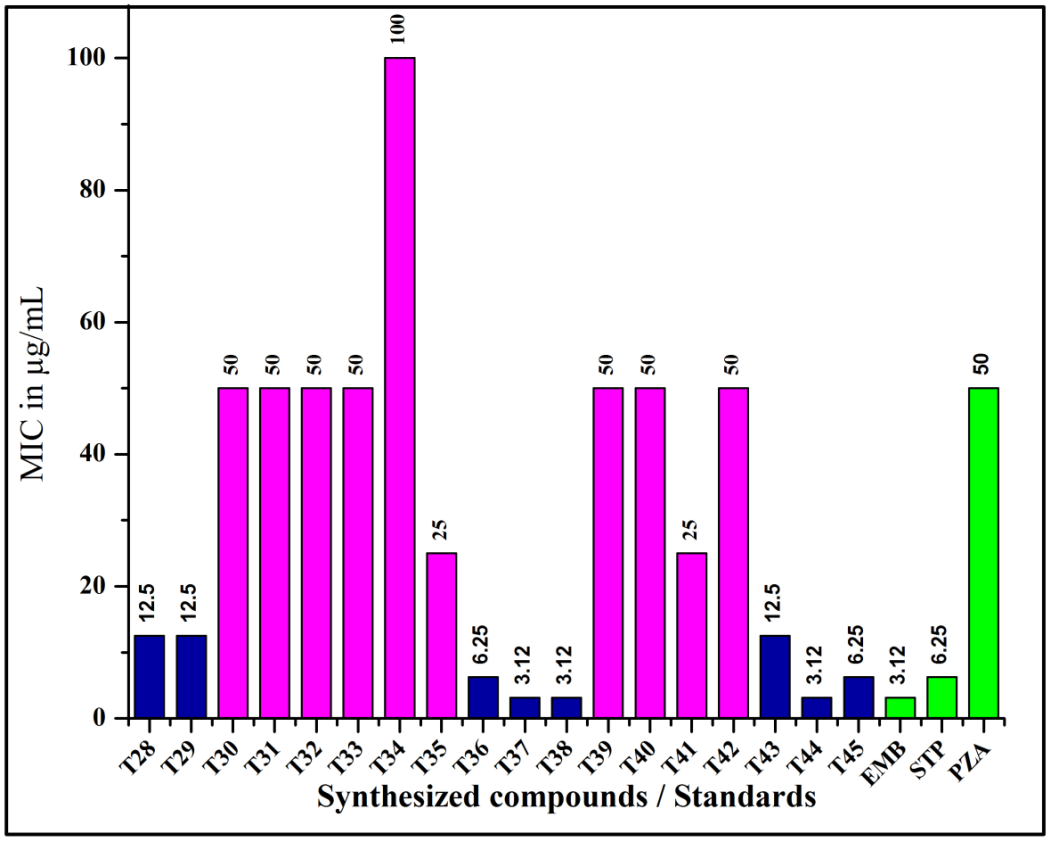


Figure 4.10 MIC of **$T_{28\text{-}45}$** against *Mycobacterium tuberculosis*

MIC value of antimicrobial activity of all the synthesized compounds $\mathbf{T_{28\text{-}45}}$ were screened against *Staphylococcus aureus*, *Mycobacterium smegmatis* and *Candida albicans* at different concentrations ranging 100 to 0.78 µg/mL was presented in **Table 4.4**. Ciprofloxacin used as antibacterial standard and Fluconazole used as antifungal standard. Few compounds, $\mathbf{T_{28}}$, $\mathbf{T_{29}}$, $\mathbf{T_{35}}$, $\mathbf{T_{36}}$, $\mathbf{T_{37}}$, $\mathbf{T_{38}}$, $\mathbf{T_{44}}$ and $\mathbf{T_{45}}$ were showed good to moderate MIC values at the range of 3.12 to 31.25 µg/mL for all the tested bacteria and fungi. This indicates that, compounds $\mathbf{T_{37}}$, $\mathbf{T_{38}}$, $\mathbf{T_{44}}$ and $\mathbf{T_{45}}$ have showed significantly active as antimicrobial as well as antitubercular. Hence, one can conclude that, pyrazole containing dihydropyridine derivatives were active as antimicrobial and antitubercular agents.

Table 4.4 MIC value of synthesized compounds $\mathbf{T_{28\text{-}45}}$

Compounds	**MIC in µg/mL**		
	Staphylococcus aureus	***Mycobacterium smegmatis***	***Candida albicans***
$\mathbf{T_{28}}$	31.25	15.6	31.25
$\mathbf{T_{29}}$	15.6	15.6	31.25
$\mathbf{T_{30}}$	62.5	62.5	62.5
$\mathbf{T_{31}}$	62.5	62.5	62.5
$\mathbf{T_{32}}$	62.5	62.5	62.5
$\mathbf{T_{33}}$	62.5	62.5	62.5
$\mathbf{T_{34}}$	125	62.5	62.5
$\mathbf{T_{35}}$	31.25	15.6	31.25
$\mathbf{T_{36}}$	15.6	15.6	31.25
$\mathbf{T_{37}}$	7.8	31.25	31.25
$\mathbf{T_{38}}$	7.8	31.25	31.25
$\mathbf{T_{39}}$	62.5	62.5	62.5
$\mathbf{T_{40}}$	62.5	62.5	62.5
$\mathbf{T_{41}}$	62.5	62.5	62.5
$\mathbf{T_{42}}$	62.5	62.5	62.5
$\mathbf{T_{43}}$	62.5	62.5	62.5
$\mathbf{T_{44}}$	7.8	31.25	31.25
$\mathbf{T_{45}}$	7.8	31.25	31.25
INN	3.9	3.9	--
FLZ	--	--	10
Control	--	--	--

INN; antibacterial standard Ciprofloxacin; FLZ; anti-fungal standard Fluconazole; --: not detected inhibition; control; dimethylsulfoxide

4.6 CONCLUSIONS

Catalytic promoted 1,4-dihydropyridine bearing pyrazole derivatives **T_{28-45}** were synthesized and characterized by IR, NMR, mass spectral study and C, H, N analysis. Target compounds were investigated for their *in vitro* antimicrobial and antitubercular activity by standard MIC method. Among the investigated samples, **T_{28}**, **T_{29}**, **T_{35}**, **T_{36}**, **T_{37}**, **T_{38}**, **T_{44}** and **T_{45}** have shown an excellent to moderate antibacterial and antitubercular agents. The compounds **T_{36}**, **T_{37}**, **T_{44}** and **T_{45}** against all the microorganisms were due to the presence of thiophene substitution at third position of pyrazole ring. The enhanced activity of compounds **T_{28}**, **T_{29}**, **T_{37}**, and **T_{38}** is due to the presence of electron donating group at third position of pyrazole ring.

With regards to the relationships between the structure of the heterocyclic scaffold and the detected antimicrobial and antitubercular properties, it showed varied pharmaceutical activity. Probably, in this case, the nature of the heterocyclic ring is not only the reason for activity. Moreover, the presence of different substituent causes a certain change of activity. It can be concluded that, the combination of heterocyclic moieties namely pyrazole and pyridine with different substitutions has enhanced the biological activity and hence they are ideally suited for further notifications to obtain more efficient antimicrobial and antitubercular compounds.

CHAPTER 5

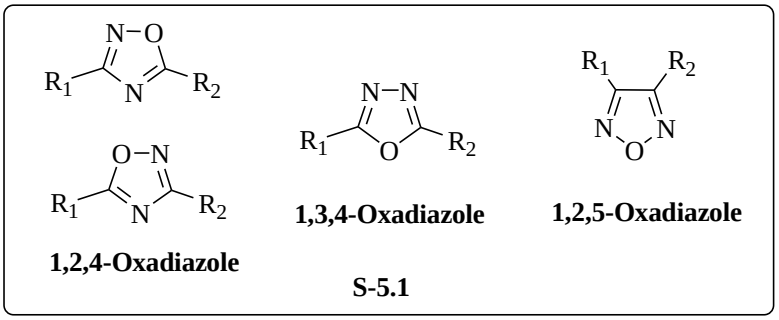
HIGHLY POTENT ANTITUBERCULAR AGENTS: SYNTHESIS OF PYRAZOLE LINKED [1,3,4]OXADIAZOLE AND [1,2,4]TRIAZOLO [3,4-*b*][1,3,4]THIADIAZOLE ANALOGS

Abstract

This chapter deals with the detailed literature survey on 1,3,4-oxadiazole and 1,3,4-thiadiazole derivatives until August 2016. Also, the detailed synthesis and biological importance of 1,3,4-oxadiazole and 1,3,4-thiadiazole derivatives have been explained. The target pyrazole containing 1,3,4-oxadiazole derivatives were synthesized from 1,3-disubstituted-1H-pyrazole-4-carboxylic acids with aryl hydrazide derivatives in phosphoryl chloride. Pyrazole containing 1,3,4-thiadiazole derivatives have been synthesized by reacting 1,3-disubstituted-1H-pyrazole-4-carboxylic acids with 4-amino-5-substitutedaryl-4H-[1,2,4]triazole-3-thiol in polyphosphoric acid. Further, the synthesized pyrazole containing oxadiazole and thiadiazole derivatives were characterized by different spectral analysis and evaluated for their antitubercular, antibacterial and antifungal studies. Cytotoxicity studies were performed for active compounds against normal cell lines. Few of the compounds were found to be biologically potential compounds.

5.1 INTRODUCTION

Oxadiazoles are the five-membered heteroaromatic compound containing two carbons, two nitrogen and one oxygen atom, and they exist in different regioisomeric forms. The replacement of two -CH= groups in furan ring structure by two nitrogen atoms (-N=) reduces the aromaticity of oxadiazole ring. Oxadiazoles have displayed interesting hydrogen bond acceptor properties. Oxadiazole exhibits different regioisomeric forms; two 1,2,4-isomers (if asymmetrically substituted), a 1,3,4-isomer, and a 1,2,5-isomer (**S-5.1**) (Bostrom *et al.* 2012).



S-5.1

Thiadiazole is a five-membered heterocyclic ring containing the hydrogen-binding domain, sulfur atom and two-electron donor nitrogen system (–N=C–S) that

exhibit a broad biological activity in the medicinal chemistry. Thiadiazoles occur in four isomeric forms such as 1,2,3-thiadiazole, 1,2,4-thiadiazole, 1,3,4-thiadiazole and 1,2,5-thiadiazoles, (**S-5.2**) (Gupta *et al.* 2005). Out of all isomers, 1,3,4-thiadiazole plays enormous biological activities and compounds containing thiadiazole nucleus have exhibit almost all types of biological properties.

1,2,3-Thiadiazole **1,2,4-Thiadiazole** **1,3,4-Thiadiazole** **1,2,5-Thiadiazole**

S-5.2

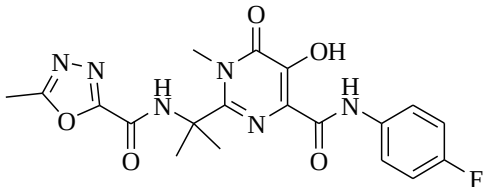
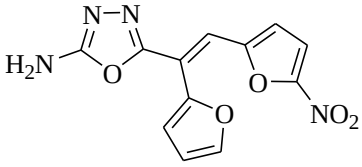
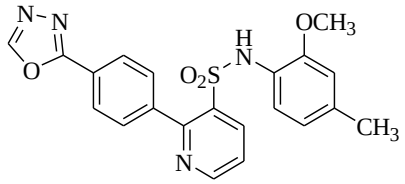
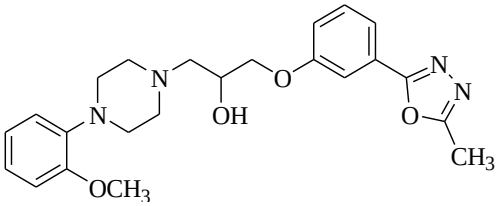
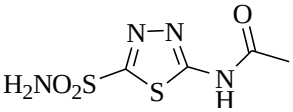
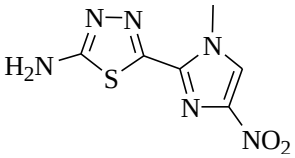
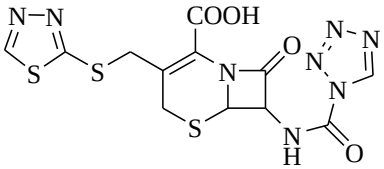
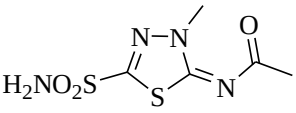
Among heterocyclic compounds, 1,3,4-oxadiazole is one of the important nucleus for drug discovery development. 1,3,4-Oxadiazole derivatives were found to exhibit diverse activities such as antiobesity (Lee *et al.* 2008), antitubercular (Ahsan *et al.* 2012), anti-inflammatory (Maddila *et al.* 2012), anticancer (Dawood *et al.* 2013), selective cyclooxygenase-2 inhibitor (Alegaon *et al.* 2014) and antiproliferative (Kamal *et al.* 2014) activities. Oxadiazole linked commercially available drugs are dominating the market due to their optoelectronic properties (Belavagi *et al.* 2015). Raltegravir is an important antiretroviral drug, Tiodazosin is an antihypertensive drug and Furamizole is an antibiotic under the class of 1,3,4-oxadiazole.

Thiadiazoles are an important class of heterocycles have shown their broad spectrum of biological activities. Various substituted thiadiazole compounds have been found to improved interesting activities such as antitubercular (Oruc *et al.* 2004), analgesic (Schenone *et al.* 2006), anti-hepatitis B viral (Tan *et al.* 2006, Liu *et al.* 2015) antimicrobial (Onkol *et al.* 2008), anticonvulsant (Yar and Akhter. 2009) activities. Thiadiazole derivatives associated with a large number of biological activities such as Acetazolamide, Methazolamide, Megazol and antibiotic Cefozopran. Acetazolamide is a class of diuretic drug occupies an essential drug in the list of WHO essential medicines (WHO model list of essential medicines, October 2013). Some of the commercially available oxadiazole and thiadiazole based drugs are presented in **Table 5.1**.

These azole classes of derivatives play an important role in medicinal chemistry with their broad pharmacological activities. Development of anti-TB drugs

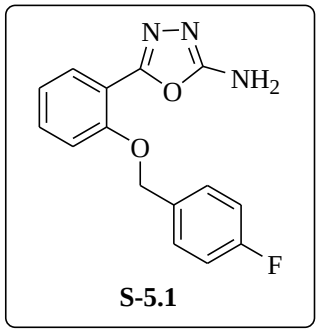
are challengeable due to their mode of action and target site. Present treatment of tuberculosis is most inconvenient and multidrugs were involved in the treatment.

Table 5.1 Commercially available oxadiazole and thiadiazole based drugs

Drug name	Structure	Therapeutic use
Raltegravir		Anti-HIV
Furamizole		Antibiotic
Zibotentan		Anticancer
Nesapidil		Vasodilator
Acetazolamide		Diuretic
Megazol		Protozoan agent
Cephazolin		Antibiotic
Methazolamide		Glaucoma agent

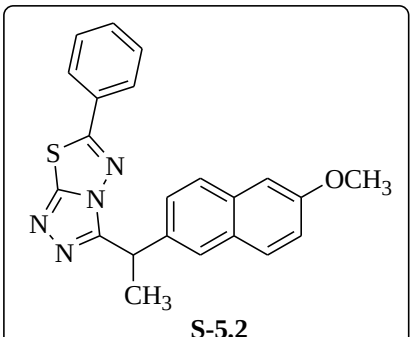
Some of the earlier reported potent oxadiazole and thiadiazole derivatives with different biological activities were summarized below.

Zarghi *et al.* (2005) synthesized 2-substituted-5-[2-benzyloxyphenyl]-1,3,4-oxadiazole. All newly synthesized compounds tested for their anticonvulsant activity. Anticonvulsant activity was determined by pentylene tetrazole (PTZ)-induced lethal convulsion and maximal electroshock (MES) tests. Compound (**S-5.1**) have showed prominent anticonvulsant activity.



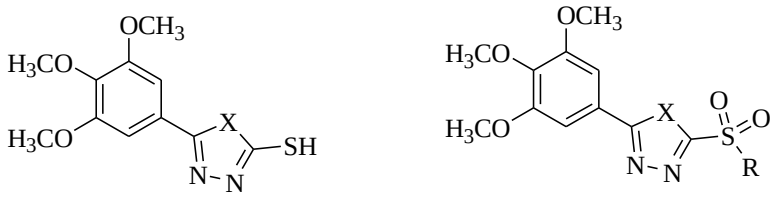
S-5.1

Amir *et al.* (2007) developed a novel series of aryl/alkyl isothiocyanates substituted 1,2,4-triazolo(3,4-*b*)-1,3,4-thiadiazole derivatives and were characterized and screened for their anti-inflammatory activity. Among all derivatives, compound (**S-5.2**) showed potent anti-inflammatory activity.



S-5.2

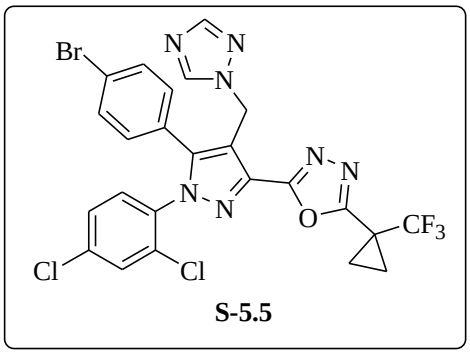
Antifungal activity of 5-(3,4,5-trimethoxyphenyl)-2-sulfonyl-1,3,4-thiadiazole and 5-(3,4,5-trimethoxyphenyl)-2-sulfonyl-1,3,4-oxadiazole derivatives were synthesized and charaterized by Chen and co-workers, (2007). The compounds 5-(3,4,5-trimethoxyphenyl)-2-sulfonyl-1,3,4-thiadiazole (**S-5.3**) and 5-(3,4,5-trimethoxyphenyl)-2-sulfonyl-1,3,4-oxadiazole (**S-5.4**) inhibited *in vitro* mycelia growth is EC_{50} ~ 50 % at 2.9-93.3 μg/mL against 10 kinds of fungi.



S-5.3 **S-5.4**

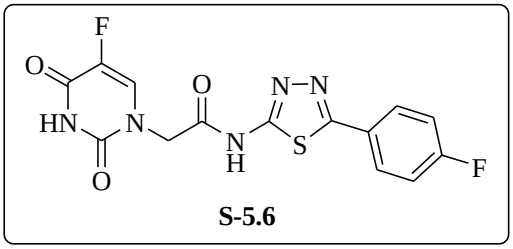
R = 2-FCH_2Ph, 3-FCH_2Ph, 4-FCH_2Ph, 2-$MeOCH_2Ph$, 3-$MeOCH_2Ph$, 4-$MeOCH_2Ph$, 3-NO_2CH_2Ph, 4-NO_2CH_2Ph, 2-$ClCH_2Ph$, 4-$ClCH_2PhN$, CH_2Ph, CH_3, CH_2CH_3, C_3H_7, CH_2CHCH_2, $CH_3CO_2CH_2$
X = O, S.

Lee *et al.* (2008) synthesized 1,3,4-oxadiazole linked biarylpyrazole derivatives and tested for cannabinoid (CB1) receptor binding affinity. 1,3,4-Oxadiazole and pyrazole ring led to CB1 antagonists with IC_{50} ~1 nM for the CB1 receptor binding. Among these analogues, compound (**S-5.5**) showed most potent antiobesity activity.



S-5.5

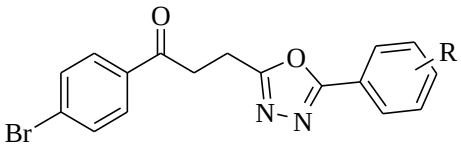
Zheng and co-workers, (2008) developed a new series of N1-acetylamino-(5-alkyl/aryl-1,3,4-thiadiazole-2-yl)-5-fluorouracil derivatives were evaluated for their anticancer activity. Anticancer studies were carried on A-549 (human lung cancer cell), Bcap-37 (human breast cancer cell) by MTT assay. Compound (**S-5.6**) with electron withdrawing group attached to benzene ring was found to have more potent antitumor inhibitory activity than 5-fluorouracil.



S-5.6

2-[3-(4-Bromophenyl)propan-3-one]-5-(substituted phenyl)-[1,3,4]-oxadiazoles (**S-5.7**) were synthesized from 3-(4-bromobenzoyl)propionic acid by

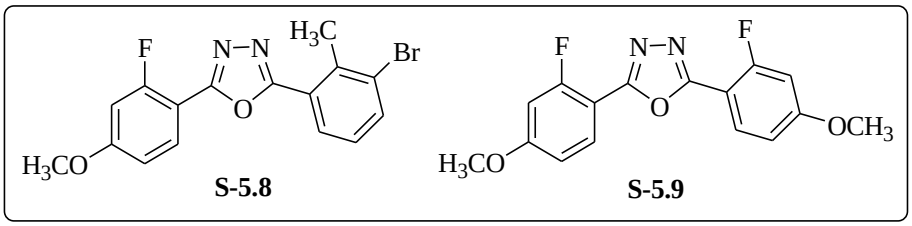
Husain and co-workers (2008). All target compounds were characterized and evaluated for their anti-inflammatory, antibacterial, ulcerogenic and analgesic activities. Target [1,3,4]oxadiazole derivatives were showed excellent activity in anti-inflammatory and analgesic activities and found to be less active in ulcerogenic activity.



S-5.7

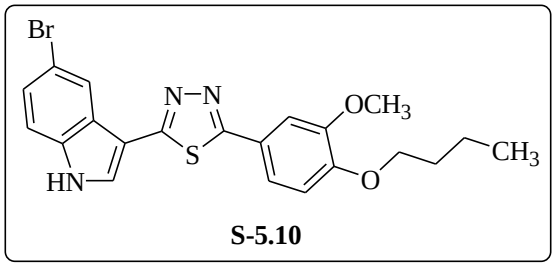
R= H, 2-Cl, 4-Cl, 2-OH, 4-NO_2, 4-F, 4-CH_3, 4-OCH_3, 3,4-$(OCH_3)_2$

Chandrakantha and group members, (2010) have reported a series of new 2-fluoro-4-methoxy binded 1,3,4-oxadiazole derivatives. All newly synthesized compounds were characterized and tested for their antibacterial and antifungal studies. Two [1,3,4]oxadiazole derivatives (**S-5.8** and **S-5.9**) showed potent antibacterial activity against *E.coli* and *P. aeruginosa* and antifungal activity against *C. albicans*.



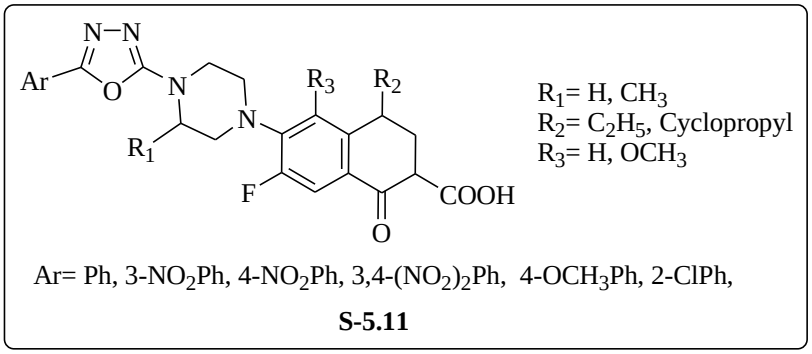
S-5.8 **S-5.9**

Kumar *et al.* (2010) synthesized 5-(3-indolyl)-1,3,4-thiadiazoles derivatives as anticancer agents. SAR explained that, substitution on 1,3,4-thiadiazole-2*H* plays a key role in enhancing the cytotoxic activity. Compound 2-(4-(benzyloxy)-5-(5-bromo-3-indolyl)-3-methoxyphenyl)-1,3,4-thiadiazole (**S-5.10**) with 4-benzyloxy-3-methoxyphenyl at the second position was found to be potent compound. Replacement of phenyl ring at second position with 4-(dimethylamino)phenyl, benzyl, 3,4-dimethoxyphenyl and 4-benzyloxy groups enhanced the antiproliferative activity.



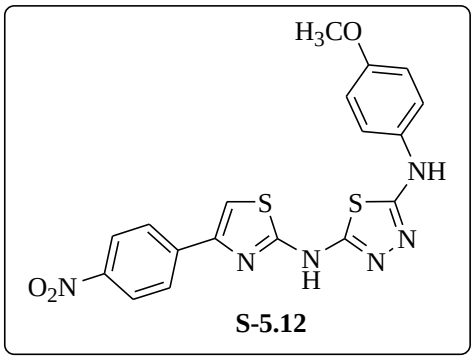
S-5.10

Kumar and co-workers, (2011) prepared a new series of 7-[4-(5-aryl-1,3,4-oxadiazole-2-yl)piperazinyl]quinoline derivatives (**S-5.11**) and confirmed by different spectral techniques. All compounds were tested for their antibacterial activity. The majority of the compounds showed significant antibacterial activity.



S-5.11

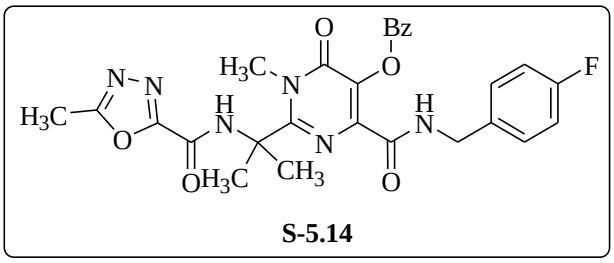
Siddiqui and Ahsan (2011) synthesized a new series of thiadiazole containing thiazolyl derivatives and characterized. All target compounds were evaluated for their anticonvulsant activity. Compound (**S-5.12)** found to be more active among all the compounds due to the nitro group attached to the phenyl ring adjacent to thiazole moiety and the removal or replacement of nitro function by a halogen moieties have decreased the activity.



S-5.12

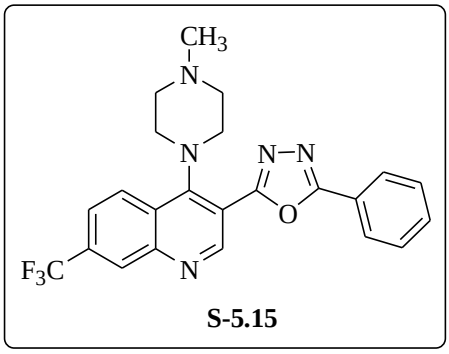
Wang *et al.* (2012) designed and synthesized a novel Raltegravir 1,3,4-oxadiazole derivatives to develop novel IN inhibitors with improved microsomal stability, while maintaining equal or better anti-HIV potency. Results found with

excellent to good anti-HIV activity for most of the compounds. The 5-hydroxyl modification showed significantly increase in the anti-HIV activity. The introduction of acyl at the fifth position was favorable for antiviral activity. Compound (**S-5.14**) was found to be most potent anti-HIV agents among synthesized compounds.



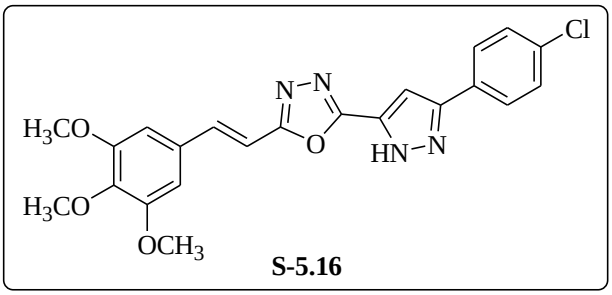
S-5.14

Garudachari *et al.* (2014) synthesized N-alkyl-3-(5-phenyl-1,3,4-oxadiazol-2-yl)-7-(trifluoromethyl)quinolin-4-amine derivatives. All target compounds were evaluated for their antibacterial activity against *M. smegmatis* and *P. aeruginosa*. Antifungal activity was also carried out on the fungi *C. albican* and *P. chrysogenum*. Compound (**S-5.15**) showed potent antimicrobial activity against all the tested microorganisms.



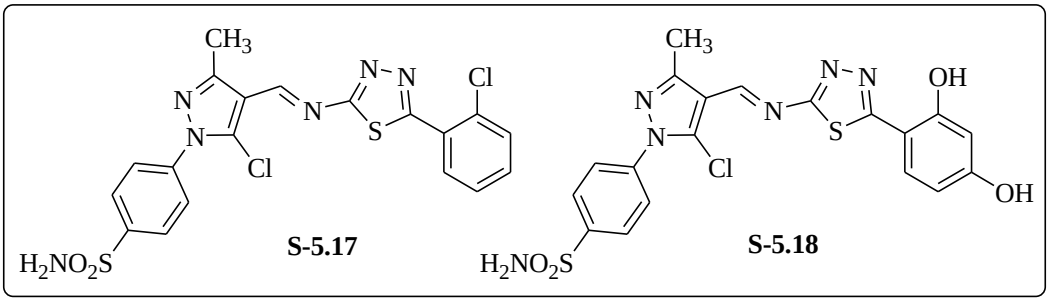
S-5.15

Kamal and co-workers, (2014) synthesized a number of pyrazole-oxadiazole derivatives and evaluated for their ability to function as antiproliferative agents on various human cancer cell lines. Compound (**S-5.16**) exhibited potent cytotoxicity with an IC_{50} value of 1.5 μM and inhibit tubulin polymerization with IC_{50} values of 1.3 μM. Molecular docking simulations determined the binding modes of these potent conjugates at Colchicine site of tubulin.



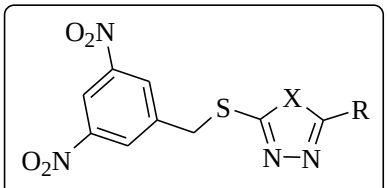
S-5.16

Alam *et al.* (2015) synthesized thiadiazole linked pyrazole benzenesulfonamides. All synthesized compounds were screened for their *in vivo* anti-inflammatory, analgesic and *in vitro* COX-II inhibition assay. Compounds (**S-5.17** and **S-5.18**) showed most significant *in vivo* anti-inflammatory properties with 72.33 and 71.17 % inhibition of the analgesic activity of 67.89 % and 71.37 % respectively. During the gastric ulceration study, selected compounds not exhibited any ulcerogenic effect on gastric mucosa.



S-5.17 **S-5.18**

Karabanovich and co-workers, (2016) have discovered and studied the structure-activity relationship of 5-substituted-2-[(3,5-dinitrobenzyl)sulfanyl]-1,3,4-oxadiazoles and 1,3,4-thiadiazoles (**S-5.20**) as a new class of antitubercular agents. The majority of the new derivatives found to be outstanding *in vitro* activity against *Mycobacterium tuberculosis* and exhibited minimum inhibitory concentration values as low as 0.03 μM (0.011–0.026 μg/mL). Furthermore, these derivatives exhibited low *in vitro* toxicities in four proliferating mammalian cell lines.

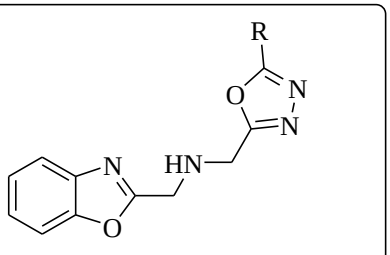


S-5.20

X= O, S

R= alkyl, aryl

Iyer *et al.* (2016) designed a series of 2,5-disubstituted-1,3,4-oxadiazole derivatives (**S-5.21**). The target derivatives were prepared from (benzo[*d*]oxazol-2-yl)methenamine and evaluated for *in vitro* radical scavenging properties and *ex vivo* anticoagulant activity. All compounds have shown moderate activity against radical scavenging experiments.



S-5.21

R= Ph, 2-$NO_2C_6H_4$, 3,5-$(NO_2)_2C_6H_3$, 2-$NH_2C_6H_4$, 4-$NH_2C_6H_4$, 2-OHC_6H_4, 4-OHC_6H_4, 3-C_5H_4N, H, CH_3, SH

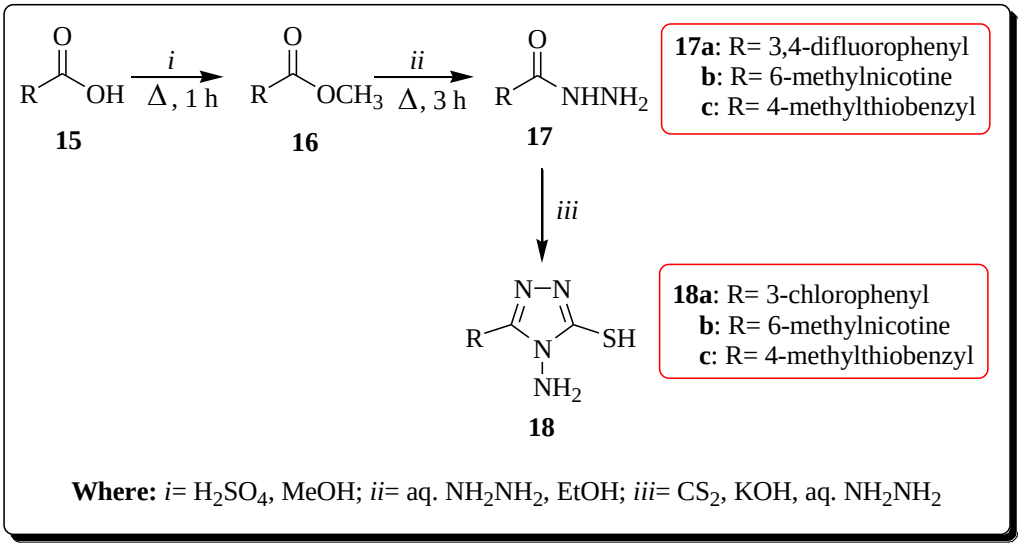
Based on the above considerations, it was planned to synthesize a new derivatives of 2-[1,3-(disubstituted)-1*H*-pyrazol-4-yl]-5-substituted aryl-[1,3,4]oxadiazoles and 6-[1,3-(disubstituted)-1*H*-pyrazol-4-yl]-3-substituted aryl-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles. Further, these compounds were evaluated for their antitubercular and antimicrobial activity. Antitubercular screening against *Mycobacterium tuberculosis* $H_{37}Rv$ strain and antimicrobial properties against *Mycobacterium smegmatis*, *Staphylococcus aureus*, *Candida albicans*, *Penicillium chrysogenum* and cytotoxicity studies were carried against non-cancerous Hela cell lines and Vero cell lines for active compounds.

5.2 MATERIAL AND METHODS

1,3-Disubstituted-1*H*-pyrazole-4-carbaldehydes **4a-c** were synthesized as discussed in **Chapter-2**.

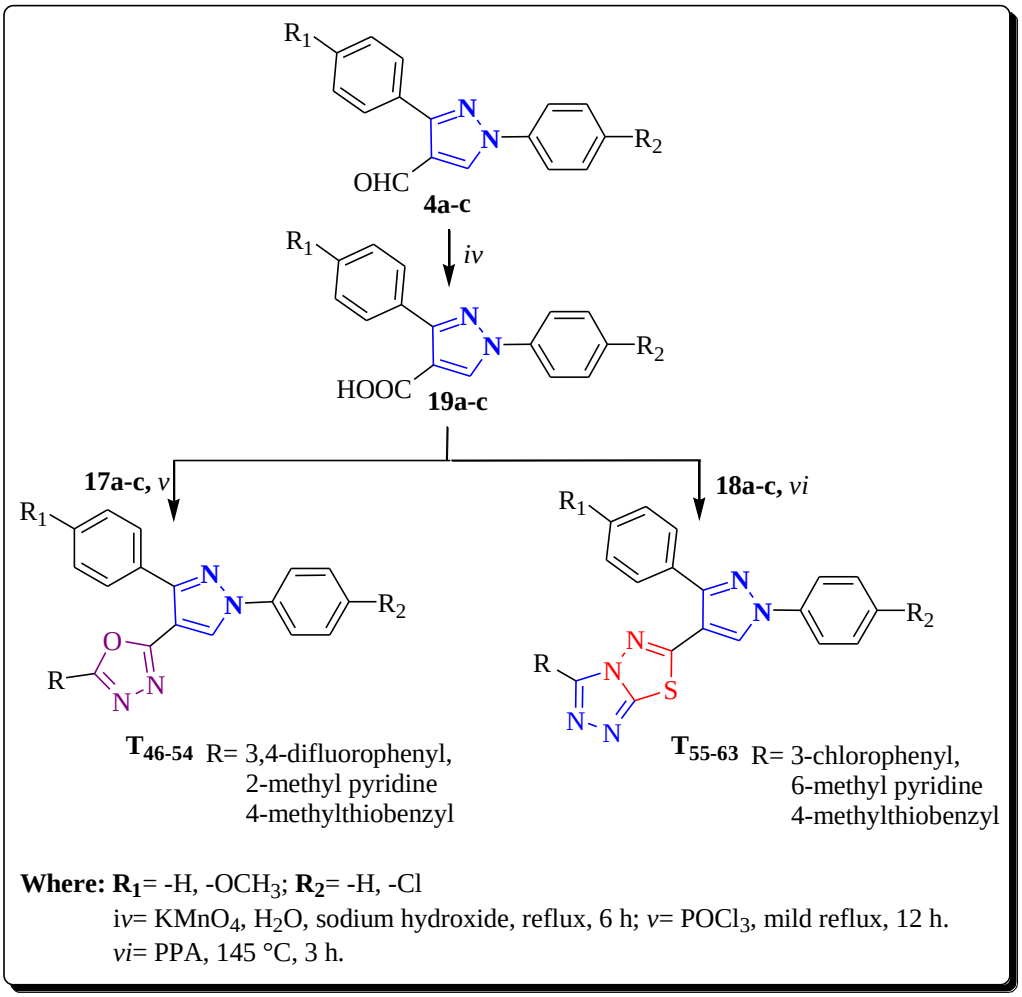
Two key starting materials, substituted acid hydrazides **17** and 4-amino-5-substituted aryl-4*H*-[1,2,4]triazole-3-thiols **18** were synthesized using the general procedure available in the literature (Sahoo *et al.* 2010, Pandeya *et al.* 2012). Substituted aryl acids **15** were esterified with methanol in presence of Conc. sulfuric acid. Then the ester derivatives **16** were treated with hydrazine hydrate solution at reflux temperature in ethanol media to get substituted acid hydrazides **17a-c**. Acid hydrazide derivatives **17a-c** were reacted with carbon disulfide in sodium ethoxide

solution and cyclised with hydrazine hydrate to get another key starting material 4-amino-5-substituted aryl-4*H*-[1,2,4]triazole-3-thiols **18a-c** and was presented in **Scheme-5.1**.



Scheme 5.1 Schematic presentation of acid hydrazides and [1,2,4]triazole derivatives

The key intermediates, 1,3-disubstituted-1*H*-pyrazole-4-carboxylic acids **19a-c** was obtained by oxidation of 1,3-disubstituted-1*H*-pyrazole-4-carbaldehydes **4a-c** with potassium permanganate at reflux temperature in distilled water at mild basic condition (Bratenko *et al.* 2001). The pyrazole acids **19a-c** were cyclized with substituted acid hydrazides **17a-c** in phosphoryl chloride ($POCl_3$) to get [1,3,4]oxadiazole derivatives $\mathbf{T_{46\text{-}54}}$ at reflux temperature for 12 h (Garudachari *et al.* 2014). Other series, [1,2,4]triazolo-[1,3,4]thiadiazole derivatives $\mathbf{T_{55\text{-}63}}$ were obtained from pyrazole acids **19a-c** with 4-amino-5-substituted aryl-4*H*-[1,2,4]triazole-3-thiols **18a-c** in polyphosphoric acid (PPA) at 145 °C for 3 h (Deng *et al.* 2012). Schematic representation has been presented in **Scheme-5.2** and yields of the products $\mathbf{T_{46\text{-}54}}$ and $\mathbf{T_{55\text{-}63}}$ were reasonably good. All 18 newly synthesized compounds were confirmed by IR, ^{1}H-NMR, ^{13}C-NMR, mass spectra and C, H, N elemental analysis.



Scheme 5.2 Schematic presentation of pyrazole containing oxadiazole-thiadiazole derivatives

All the chemicals were ordered from Sigma-Aldrich and Merck chemical company. Melting points were determined by open capillary tubes and were uncorrected. The FT-IR spectra were recorded on Perkin-Elmer FT-IR-4000-400 cm^{-1} spectrophotometer. Proton-NMR and Carbon-NMR spectra were recorded on a 400 MHz Bruker Avance-400 NMR spectrometer (400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-NMR). Chemical shift and coupling constants are recorded in units of δ (parts per million-ppm) and hertz (Hz), respectively with tetramethylsilane (TMS) as an internal standard. The mass spectrum was recorded on an LC-MS Applied biosystems MDS SCIEX-API 4000 spectrometer. Elemental analysis was performed on a Flash EA 1112 series CHNS-O analyzer. The reaction monitoring was checked by thin layer chromatography (TLC) (Merck F_{254}). The names of the structures were mentioned as per Chem Draw Ultra 8.0 software.

5.3 EXPERIMENTAL

The experimental protocols followed for the synthesis of compounds **18**, **19** and $\mathbf{T_{46\text{-}63}}$ were given in the following section.

Synthesis of substituted benzoates (16)

Substituted aryl acid **15** (0.1 mol) and the catalytic amount of sulfuric acid (preferably 2 drops) in methanol (10 ml) were stirred at ambient temperature. After being stirred for 15 min, the solution was heated to reflux temperature for 3 h. The reaction was monitored by TLC. After completion of the reaction, the mass was quenched into distilled water (20 ml). Further, the solid mass was extracted with ethyl acetate (2 x 20 ml) and the organic layer was washed with distilled water. The separated organic layer was dried over anhydrous sodium sulfate. The organic layer was collected and evaporated completely to get compound **16** in good yield. (Yield: 86-95 %).

Synthesis of substituted acid hydrazide (17a-c)

The ester derivative **16** (0.1 mol) and hydrazine hydrate (0.15 mol) were taken in ethanol (10 ml) and were stirred for 10 min at ambient temperature. The stirred solution was heated to reflux temperature and maintained for 2.5 h. After completion of the reaction, the excess of solvent was distilled out under reduced pressure (50 mbar). The solid obtained was filtered with fresh ethanol at 5 °C and washed with pre-chilled ethanol (2 ml) to get good yields of product **17** (Yield: 90-96 %).

General procedure for the synthesis of 4-amino-5-substituted aryl-4*H*-[1,2,4]triazole-3-thiol (18a-c).

The solution of acid hydrazide **17** (0.1 mol) in ethanol (25 ml), was cooled to 0 °C and added potassium hydroxide pellets (0.15 mol). After being stirred for 10 min at 0 °C, added carbon disulfide (0.15 mol) in about 30 min at 0°C. Thiol derivative was observed by the physical color change from wine red clear solution to yellow solid mass. Further, the reaction mass was maintained under the same condition for 2 h and the temperature was gradually increased to 25 °C. Added hydrazine hydrate (0.20 mol) and stirred the mass for 2 more hours at room temperature. Excess of the solvent was evaporated under reduced pressure (50 mbar) and added distilled water (5 ml). The reaction mass turns into a clear solution, and

further hydrazine hydrate (0.2 mol) added and refluxed the mass for 4 h. The progress of the reaction was monitored by TLC. After the completion of reaction, cooled the suspension to 25 °C and acidified the mass with Conc. HCl (pH: 1.0) to get the white color solid material. Filtered the solid material and washed with water (2 ml). Dried the material to get triazole derivative **18** in reasonable good yields. (Yield: 68-76 %).

General procedure for the synthesis of 1,3-disubstituted-1*H*-pyrazole-4-carboxylic acid (19a-c).

A suspension of 1,3-disubstituted-1*H*-pyrazole-4-carbaldehydes **4a-c** (0.1 mol) in demineralized water (250 ml), potassium permanganate (0.1 mol) and the catalytic amount of sodium hydroxide solution (pH: 8.0) were being stirred for 10 min at ambient temperature. The reaction mixture was heated at reflux temperature for 8 h. The progress of the reaction was monitored by TLC. After the completion of reaction, cooled the suspension to 25 °C and filtered the unwanted solid material. Filtrate solution was collected and acidified with Conc. HCl (pH: 0.8) to get the white color solid material. Filtered the solid material and washed with plenty of DM water. Dried the material to get 1,3-disubstituted-1*H*-pyrazole-4-carboxylic acid derivatives **19a-c** in reasonably good yields. (Yield: 81-89%).

General procedure for the synthesis of 2-(3,4-difluorophenyl)-5-(1,3-diphenyl-1*H*-pyrazol-4-yl)-[1,3,4]oxadiazole (T_{46}):

A suspension of 1,3-diphenyl-1*H*-pyrazole-4-carboxylic acid **19a** (2.64 g, 0.01 mol) and 3,4-difluoro benzoic acid hydrazide **17a** (1.72 g, 0.01 mol) in phosphoryl chloride (10 ml) were stirred for 10 min at 25 °C. The reaction mixture was heated at 105 °C for 12 h and the progress of the reaction was monitored by TLC, mobile phase [ethyl acetate : n-hexane (2:1)]. Further, the reaction mass was quenched into ice cold water and obtained solid mass was filtered. Washed the compound with plenty of water, dried and the compound was recrystallized using ethanol to get the target compound 2-(3,4-difluorophenyl)-5-(1,3-diphenyl-1*H*-pyrazol-4-yl)-[1,3,4]oxadiazole $\mathbf{T_{46}}$ in reasonable yield. The same procedure was followed for the synthesis of compounds $\mathbf{T_{47\text{-}54}}$.

2-(3,4-Difluorophenyl)-5-(1,3-diphenyl-1*H*-pyrazol-4-yl)-[1,3,4]oxadiazole (T_{46}). Yield: 78 %; m.p: 183-184 ^{o}C; IR (KBr) cm^{-1}: 1593 (C=N str), 1513 (C=C str), 1222

(C-O str); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.26-7.33 (m, 1H, Ar-H), 7.40-7.46 (m, 2H, ArH), 7.51-7.58 (m, 4H, Ar-H), 7.71-7.79 (m, 2H, Ar-H), 7.85 (dd, 2H, Ar-H, *J* = 7.6 Hz, 1.2 Hz), 7.91-7.94 (m, 2H, Ar-H), 8.75 (s, 1H, pyrazole-5H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 162.22, 157.36, 148.24, 139.00, 138.50, 134.13, 131.79, 129.89, 129.26, 129.26, 127.74, 123.56, 119.51, 118.62, 115.73, 108.49, 103.69, 102.02, 85.29; LC-MS: *m/z* = 401.2 (M+1); ANAL. Calcd. for $C_{23}H_{14}F_2N_4O$; calcd: C, 69.00; H, 3.52; N, 13.99; found: C, 69.02; H, 3.56; N, 14.01.

2-[1-(4-Chlorophenyl)-3-phenyl-1*H*-pyrazol-4-yl]-5-(3,4-difluorophenyl) [1,3,4]oxadiazole (T_{47}). Yield: 75 %; m.p: 170-171 ^{o}C; IR (KBr) cm^{-1}: 1599 (C=N str), 1514 (C=C str), 1220 (C-O str); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.34-7.61 (m, 6H, Ar-H), 7.74-7.92 (m, 6H, Ar-H), 8.58 (s, 1H, pyrazole-5H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 167.05, 162.24, 106.49, 112.92, 154.70, 152.27, 141.48, 139.69, 137.58, 133.33, 131.52, 129.83, 129.76, 120.59, 129.40, 129.04, 128.34, 128.06, 120.70; LC-MS: *m/z* = 435.2 (M+1); ANAL. Calcd. for $C_{23}H_{13}ClF_2N_4O$; calcd: C, 63.53; H, 3.01; N, 12.88; found: C, 63.56; H, 3.05; N, 12.90.

2-(3,4-Difluorophenyl)-5-[3-(4-methoxyphenyl)-1-phenyl-1*H*-pyrazol-4-yl]-[1,3,4]oxadiazole (T_{48}). Yield: 72 %; m.p: 146-147 ^{o}C; IR (KBr) cm^{-1}: 1593 (C=N str), 1514 (C=C str), 1222 (C-O str); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 3.91 (s, 3H, OCH_3), 7.05 (dd, 2H, Ar-H, *J* = 6.8 Hz, 2.0 Hz), 7.31-7.33 (m, 1H, Ar-H), 7.41 (t, 1H, Ar-H, *J* = 7.6 Hz), 7.55 (t, 2H, Ar-H, *J* = 7.6 Hz), 7.77-7.81 (m, 2H, Ar-H), 7.85 (d, 2H, Ar-H, *J* = 7.6 Hz), 7.89 (dd, 2H, Ar-H, *J* = 6.8 Hz, 2.0 Hz), 8.70 (s, 1H, pyrazole-5H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 162.10, 160.41, 160.35, 151.80, 139.11, 130.40, 129.69, 129.39, 127.65, 124.11, 123.50, 123.46, 123.41, 120.78, 118.55, 118.31, 116.30, 116.04, 105.90, 55.40; LC-MS: *m/z* = 431.3 (M+1); ANAL. Calcd. for $C_{24}H_{16}F_2N_4O_2$; calcd: C, 66.97; H, 3.75; N, 13.02; found: C, 67.01; H, 3.76; N, 13.03.

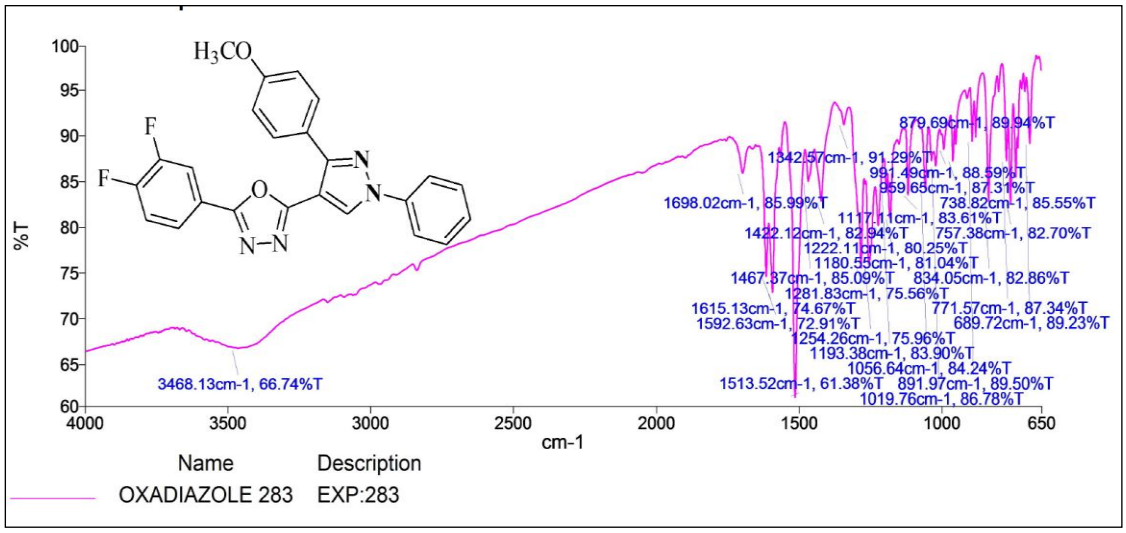


Figure 5.1 IR spectrum of compound **T_{48}**

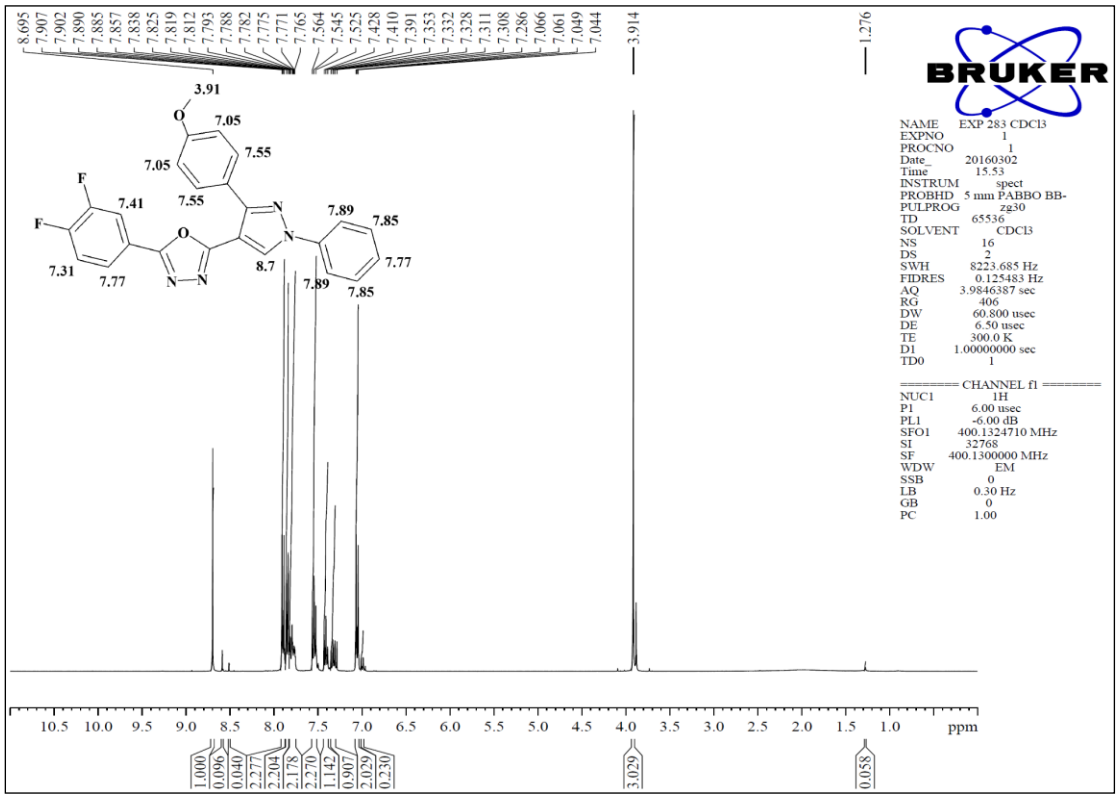


Figure 5.2 ^{1}H-NMR spectrum of compound **T_{48}**

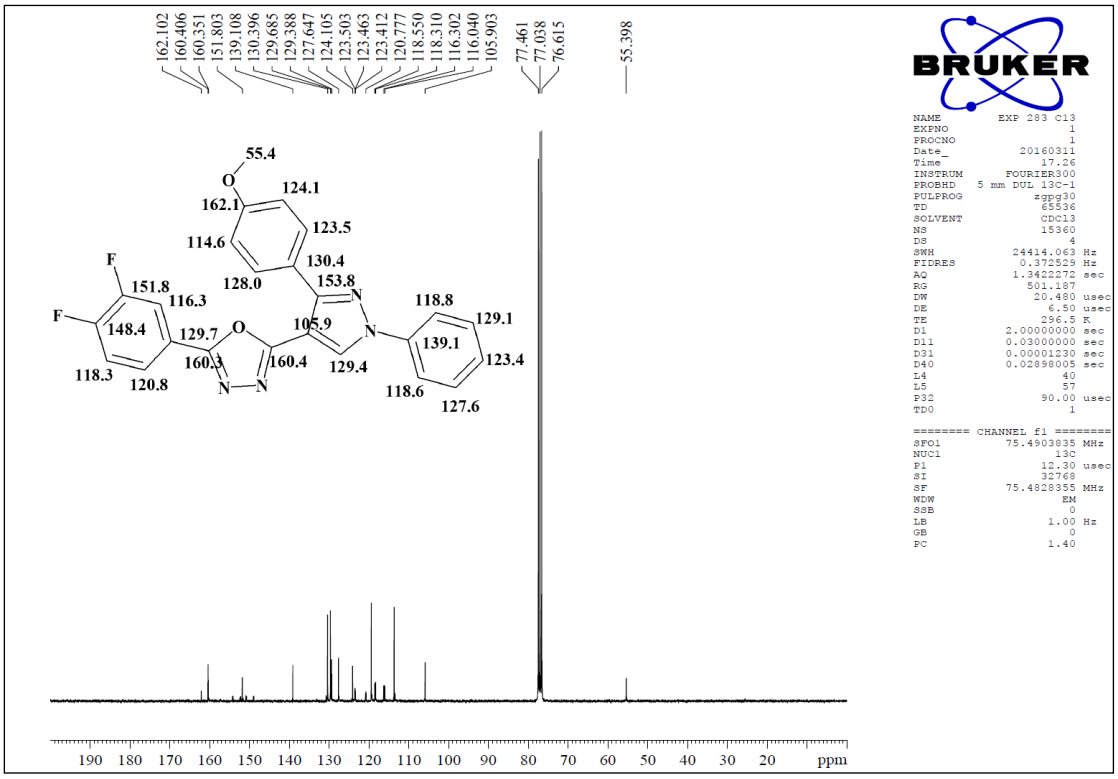


Figure 5.3 ^{13}C-NMR spectrum of compound **T$_{48}$**

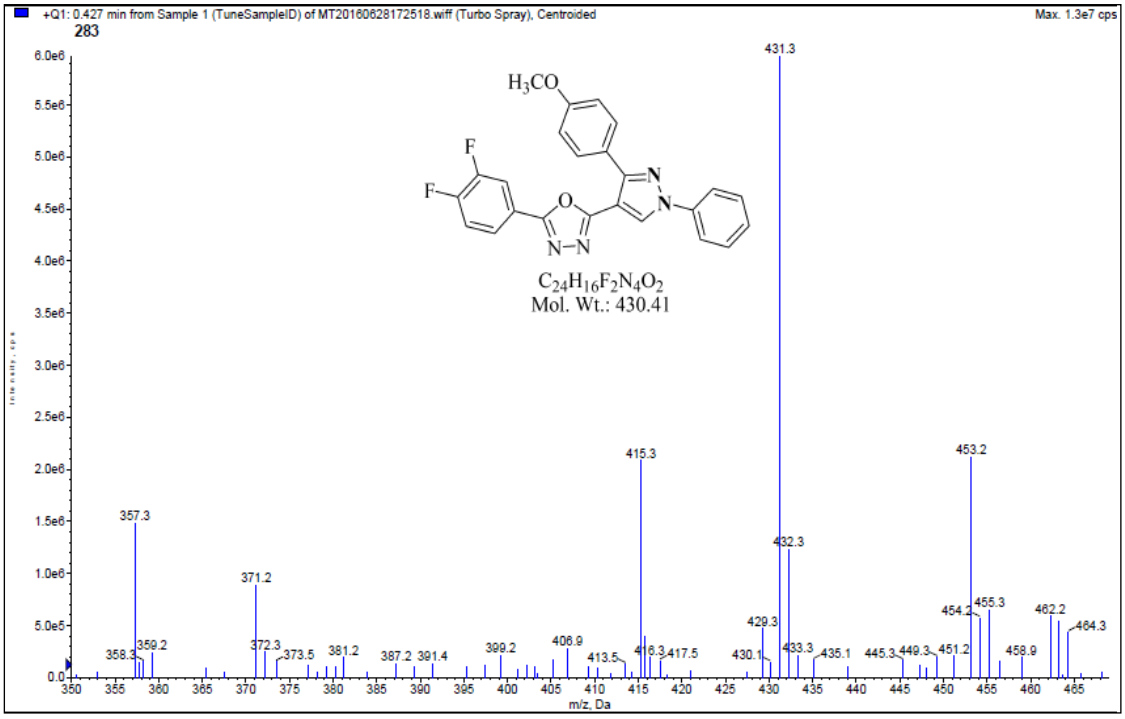


Figure 5.4 Mass spectrum of compound **T$_{48}$**

5-[5-(1,3-Diphenyl)-1*H*-pyrazol-4-yl)-[1,3,4]oxadiazol-2-yl]-2-methylpyridine (T$_{49}$). Yield: 79 %; m.p: 195-198 ^{o}C; IR (KBr) cm^{-1}: 3130 (Ar-H str), 1592 (C=N str), 1506 (C=C str), 1220 (C-O str); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 2.67 (s, 3H, CH_3), 7.32 (d, 1H, ArH, *J* = 8.0 Hz), 7.43 (d, 1H, ArH, *J* = 7.6 Hz), 7.51-7.58 (m, 4H, ArH), 7.86 (d, 2H, ArH, *J* = 8.0 Hz), 7.92 (d, 2H, ArH, *J* = 7.6 Hz), 8.19 (d, 2H, ArH, *J* = 8.0 Hz), 8.75 (s, 1H, pyrazole-5H), 9.02 (s, 1H, pyridine-2H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 162.08, 161.95, 160.13, 152.08, 147.12, 139.10, 134.30, 131.68, 129.71, 129.47, 129.24, 129.07, 128.30, 127.75, 123.54, 119.52, 117.58, 106.26, 24.73; LC-MS: *m/z* = 380.2 (M+1); ANAL. Calcd. for $C_{23}H_{17}N_5O$; calcd: C, 72.81; H, 4.52; N, 18.46; found: C, 72.86; H, 4.53; N, 18.50.

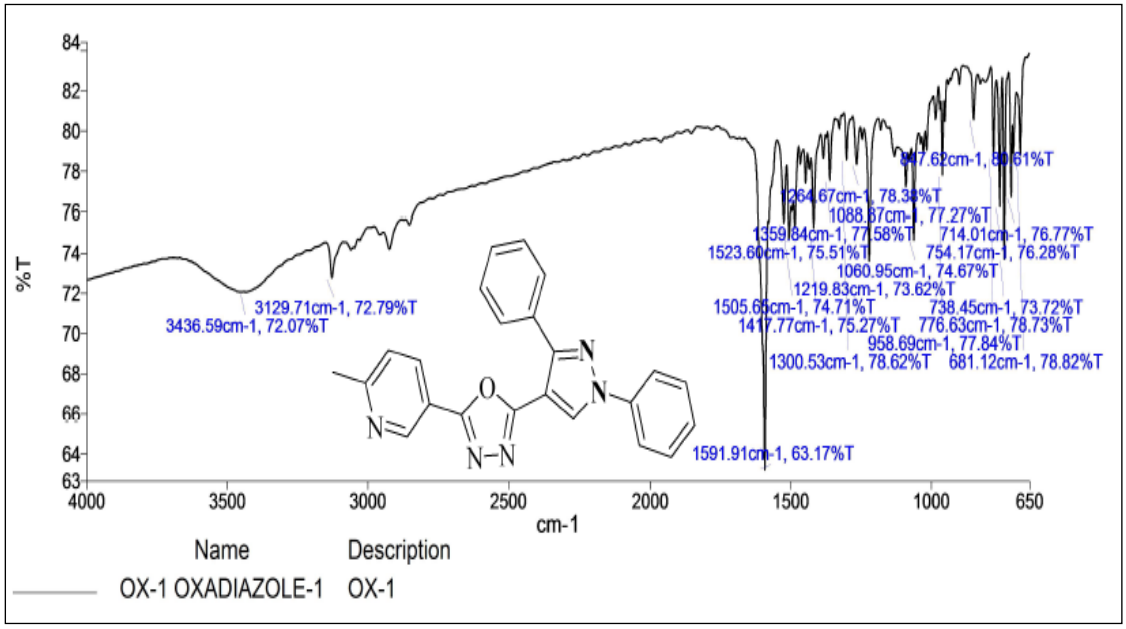


Figure 5.5 IR spectrum of compound **T$_{49}$**

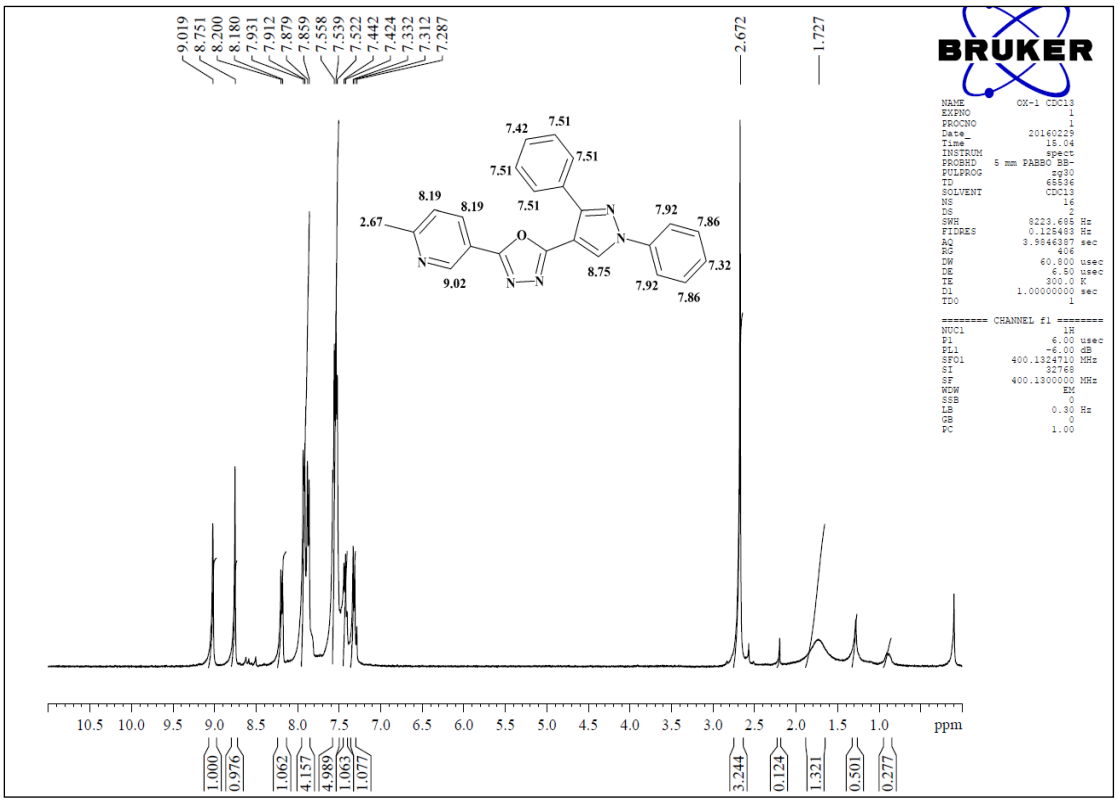


Figure 5.6 ^{1}H-NMR spectrum of compound **T_{49}**

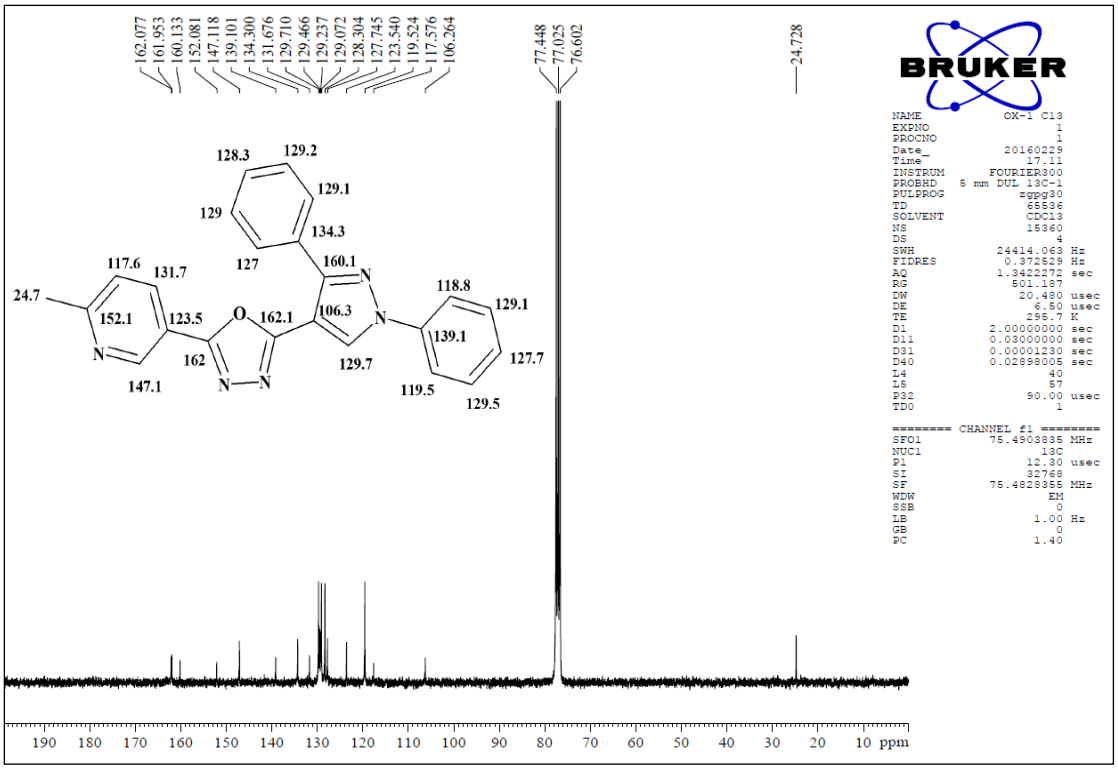


Figure 5.7 ^{13}C-NMR spectrum of compound **T_{49}**

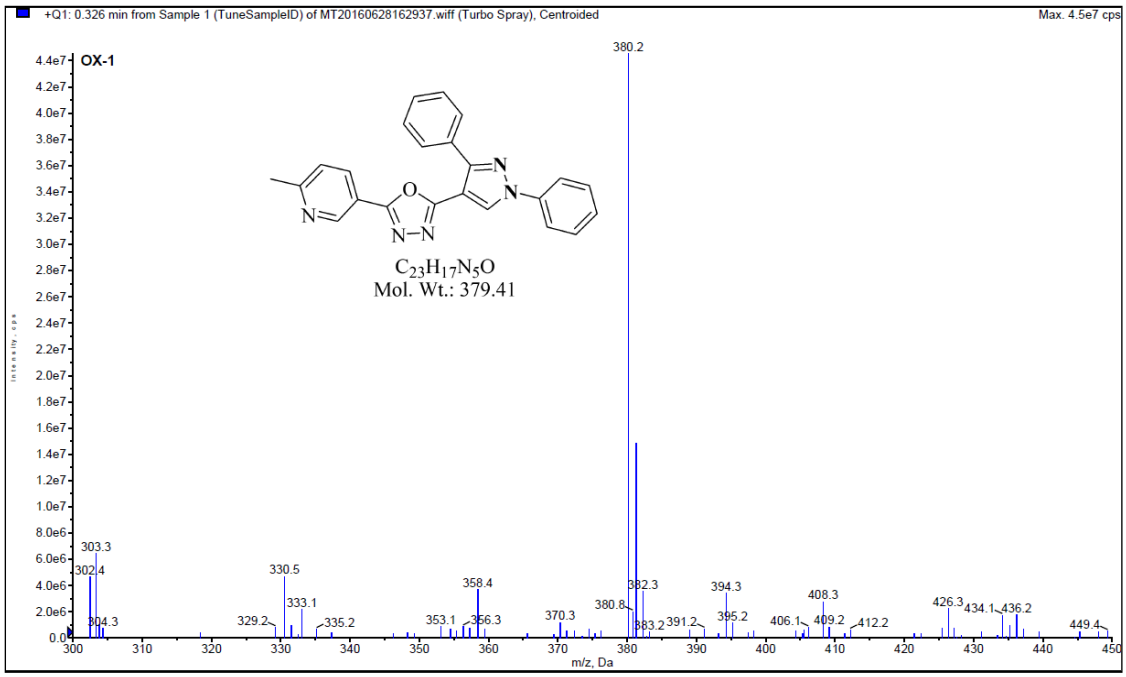


Figure 5.8 Mass spectrum of compound **T_{49}**

5-{5-[1-(4-Chlorophenyl)-3-phenyl-1*H*-pyrazol-4-yl)-[1,3,4]oxadiazol-2-yl]-2-methylpyridine (T_{50}). Yield: 72 %; m.p: 202-203 °C; IR (KBr) cm^{-1}: 3125 (Ar-H str), 1595 (C=N str), 1506 (C=C str), 1222 (C-O str); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 2.66 (s, 3H, -CH_3), 7.30-7.32 (d, 1H, Ar-H), 7.51-7.55 (m, 5H, Ar-H), 7.80-7.82 (d, 2H, Ar-H), 7.88-7.90 (d, 2H, Ar-H), 8.17-8.19 (d, 1H, Ar-H), 8.71 (s, 1H, pyrazole-5H), 9.00 (s, 1H, Ar-H); ^{13}C-NMR (100 MHz, DMSO-d_6, ppm): δ 162.2, 162.0, 160.0, 152.5, 147.0, 137.5, 134.5, 133.5, 131.5, 130.0, 129.5, 129.5, 129.0, 128.5, 123.5, 120.5, 117.5, 106.7, 24.7; LC-MS: *m/z* = 414.4 (M+1); ANAL. Calcd. for $C_{23}H_{16}ClN_5O$; calcd: C, 66.75; H, 3.90; N, 16.92; found: C, 66.75; H, 3.91; N, 16.96.

5-{5-[3-(4-Methoxyphenyl)-1-phenyl-1*H*-pyrazol-4-yl)-[1,3,4]oxadiazol-2-yl]-2-methylpyridine (T_{51}). Yield: 70 %; m.p: 167-169 °C; IR (KBr) cm^{-1}: 3129 (Ar-H str), 1593 (C=N str), 1513 (C=C str), 1221 (C-O str); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 2.68 (s, 3H, CH_3), 3.91 (s, 3H, OCH_3), 7.04-7.07 (m, 2H, Ar-H), 7.33 (d, 1H, Ar-H, J = 8.0 Hz), 7.39-7.43 (m, 1H, Ar-H), 7.53-7.57 (m, 2H, Ar-H), 7.85 (d, 2H, Ar-H, J = 7.6 Hz), 7.90 (d, 2H, Ar-H, J = 8.8 Hz), 8.21 (dd, 1H, Ar-H, J = 8.0 Hz, 2.0 Hz), 8.71 (s, 1H, pyrazole-5H), 9.08 (s, 1H, pyridine-2H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 162.05,

161.97, 160.39, 160.30, 151.82, 147.16, 139.13, 134.31, 130.37, 129.69, 129.46, 127.64, 124.08, 123.55, 119.47, 117.60, 113.73, 105.94, 55.40, 24.75; LC-MS: *m/z* = 410.5 (M+1); ANAL. Calcd. for $C_{24}H_{19}N_5O_2$; calcd: C, 70.40; H, 4.68; N, 17.10; found: C, 70.40; H, 4.70; N, 17.12.

2-(1,3-Diphenyl-1*H*-pyrazol-4-yl)-5-(4-methylsulfanyl benzyl)-[1,3,4]oxadiazole (T_{52}). Yield: 70 %; m.p: 145-147 °C; IR (KBr) cm^{-1}: 3110 (Ar-H str), 1610 (C=N str), 1507 (C=C str), 1228 (C-O str); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 2.56 (s, 3H, CH_3), 4.18 (s, 2H, CH_2), 7.28 (d, 2H, Ar-H, *J* = 8.4 Hz), 7.33 (d, 2H, Ar-H, *J* = 8.0 Hz), 7.45-7.51 (m, 4H, Ar-H), 7.58 (d, 2H, Ar-H, *J* = 8.8 Hz), 7.75 (d, 2H, Ar-H, *J* = 8.8 Hz), 7.79 (d, 2H, Ar-H, *J* = 8.4 Hz), 8.56 (s, 1H, pyrazole-5H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 164.72, 160.32, 152.12, 138.48, 137.60, 132.93, 131.34, 130.92, 130.42, 129.88, 129.54, 129.46, 129.31, 129.13, 128.42, 127.75, 121.20, 106.72, 31.26, 15.82; LC-MS: *m/z* = 425.3 (M+1); ANAL. Calcd. for $C_{25}H_{20}N_4OS$; calcd: C, 70.73; H, 4.75; N, 13.20; found: C, 70.82; H, 4.81; N, 13.36.

2-[1-(4-Chlorophenyl)-3-phenyl-1*H*-pyrazol-4-yl]-5-(4-methylsulfanyl benzyl)-[1,3,4]oxadiazole (T_{53}). Yield: 80 %; m.p: 139-141 °C; IR (KBr) cm^{-1}: 3114 (Ar-H str), 1611 (C=N str), 1506 (C=C str), 1228 (C-O str); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 2.51 (s, 3H, CH_3), 4.16 (s, 2H, CH_2), 7.19 (d, 2H, Ar-H, *J* = 8.4 Hz), 7.23 (d, 2H, Ar-H, *J* = 8.4 Hz), 7.40-7.46 (m, 3H, Ar-H), 7.48 (d, 2H, Ar-H, *J* = 8.8 Hz), 7.75 (d, 2H, Ar-H, *J* = 8.8 Hz), 7.79 (dd, 2H, Ar-H, *J* = 8.4 Hz, 1.6 Hz), 8.55 (s, 1H, pyrazole-5H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 164.68, 160.27, 151.99, 137.98, 137.62, 133.18, 131.34, 130.45, 129.77, 129.31, 129.34, 129.04, 128.82, 128.30, 126.93, 120.52, 106.69, 31.26, 15.80; LC-MS: *m/z* = 459.2 (M+1); ANAL. Calcd. for $C_{25}H_{19}ClN_4OS$; calcd: C, 65.42; H, 4.17; N, 12.21; found: C, 65.43; H, 4.18; N, 12.25.

2-[3-(4-Methoxyphenyl)-1-phenyl-1*H*-pyrazol-4-yl]-5-(4-methylsulfanylbenzyl)-[1,3,4]oxadiazole (T_{54}). Yield: 69 %; m.p: 109-111 °C; IR (KBr) cm^{-1}: 3113 (Ar-H str), 1609 (C=N str), 1512 (C=C str), 1227 (C-O str); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 2.51 (s, 3H, CH_3), 3.89 (s, 3H, OCH_3), 4.18 (s, 2H, CH_2), 6.93 (d, 2H, Ar-H, *J* = 8.8

Hz), 7.20-7.28 (m, 4H, Ar-H), 7.38 (t, 1H, Ar-H, *J* = 7.6 Hz), 7.52 (t, 2H, Ar-H, *J* = 7.6 Hz), 7.78-7.83 (m, 4H, Ar-H), 8.53 (s, 1H, pyrazole-5H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 164.50, 160.63, 160.17, 151.53, 139.15, 137.94, 130.56, 130.18, 129.63, 129.37, 129.27, 127.49, 126.90, 124.06, 119.38, 113.69, 106.04, 55.36, 31.26, 15.77; LC-MS: *m/z* = 455.3 (M+1); ANAL. Calcd. for $C_{26}H_{22}N_4O_2S$; calcd: C, 68.70; H, 4.88; N, 12.33; found: C, 68.75; H, 4.90; N, 12.36.

General procedure for the synthesis of 3-(3-Chlorophenyl)-6-(1,3-diphenyl-1*H*-pyrazol-4-yl)-[1,2,4]triazolo[3,4-*b*] [1,3,4]thiadiazole (T_{55}):

A suspension of 1,3-diphenyl-1*H*-pyrazole-4-carboxylic acid **19a** (2.64 g, 0.01 mol) and 4-amino-5-(3-chlorophenyl)-4*H*-[1,2,4]triazole-3-thiol **18a** (2.22 g, 0.01 mol) in polyphosphoric acid (10 ml) was stirred for 10 min at 25 °C. Further, the reaction mixture was stirred at 145 °C for 3 h and the completion of the reaction was monitored by TLC, mobile phase [ethyl acetate : n-hexane (2:1)]. After the completion of reaction, quenched the mass into ice cold water and obtained solid was filtered. Washed the compound with plenty of water, air dried and recrystallized using ethanol to get target compound **T_{55}** in reasonably good yield. The same procedure was followed to synthesis of compounds **$T_{56\text{-}63}$**.

3-(3-Chlorophenyl)-6-(1,3-diphenyl-1*H*-pyrazol-4-yl)-[1,2,4]triazolo[3,4-*b*] [1,3,4]thiadiazole (T_{55}). Yield: 76 %; m.p: 224-227 ^{o}C; IR (KBr) cm^{-1}: 3060 (Ar-H str), 1599 (C=N str), 1562 (C=C str), 1469 (C-N str), 755 (C-Cl str); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.45-7.67 (m, 7H, Ar-H), 7.78-7.88 (m, 5H, Ar-H), 8.18 (d, 1H, Ar-H, *J* = 6.8 Hz), 8.32 (s, 1H, Ar-H), 8.58 (s, 1H, pyrazole-5H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 159.44, 154.49, 152.39, 145.21, 138.86, 134.96, 130.84, 130.32, 130.19, 129.79, 129.59, 128.78, 128.70, 128.11, 127.28, 126.13, 124.40, 119.71, 112.43; LC-MS: *m/z* = 455.2 (M+1); ANAL. Calcd. for $C_{24}H_{15}ClN_6S$; calcd: C, 63.36; H, 3.32; N, 18.47; found: C, 63.42; H, 3.33; N, 18.51.

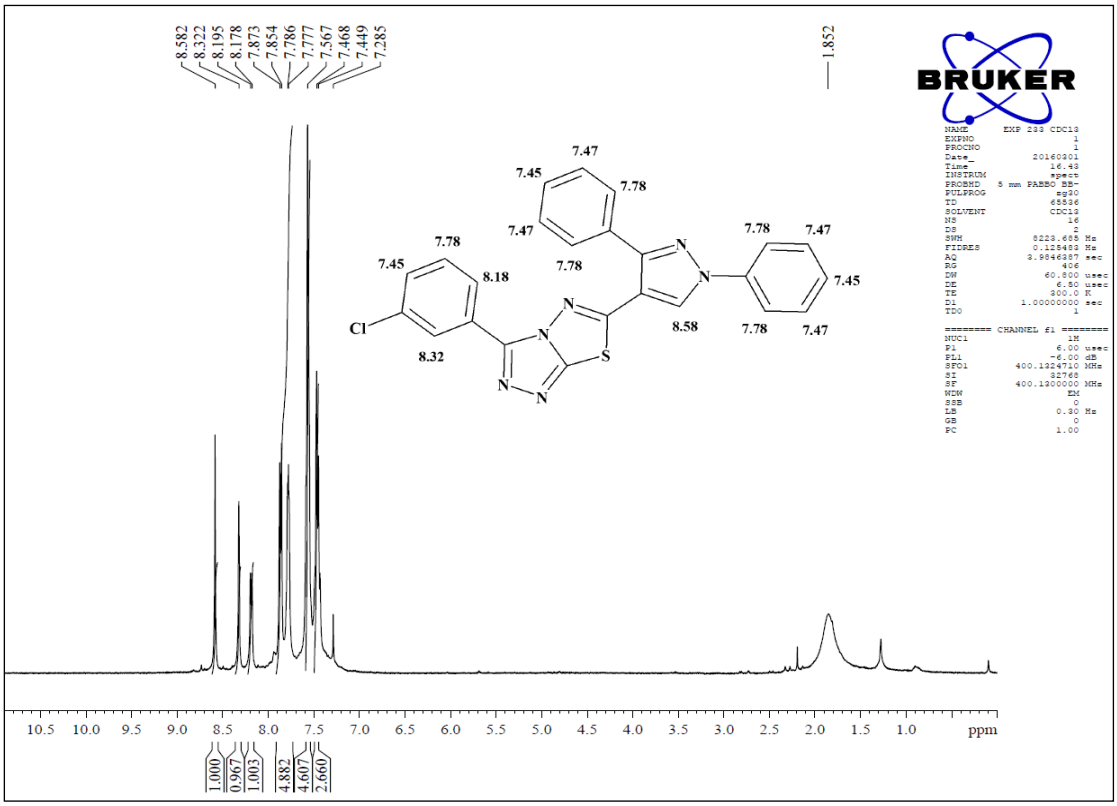


Figure 5.9 ^{1}H-NMR spectrum of compound **T_{55}**

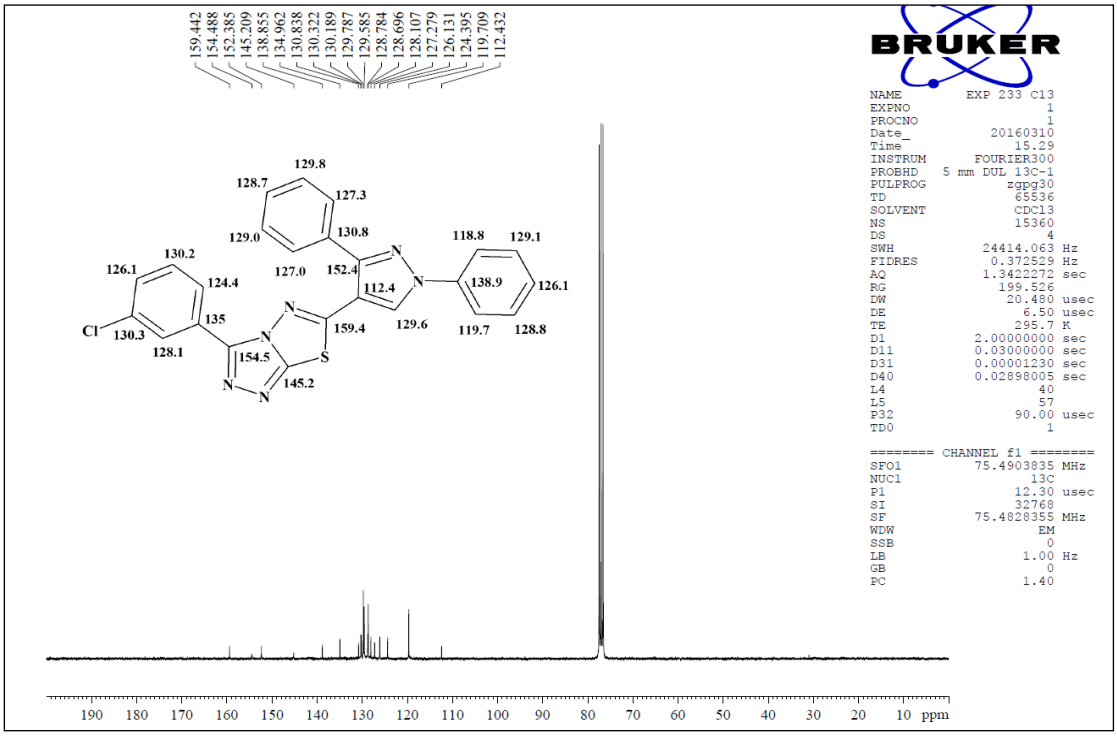


Figure 5.10 ^{13}C-NMR spectrum of compound **T_{55}**

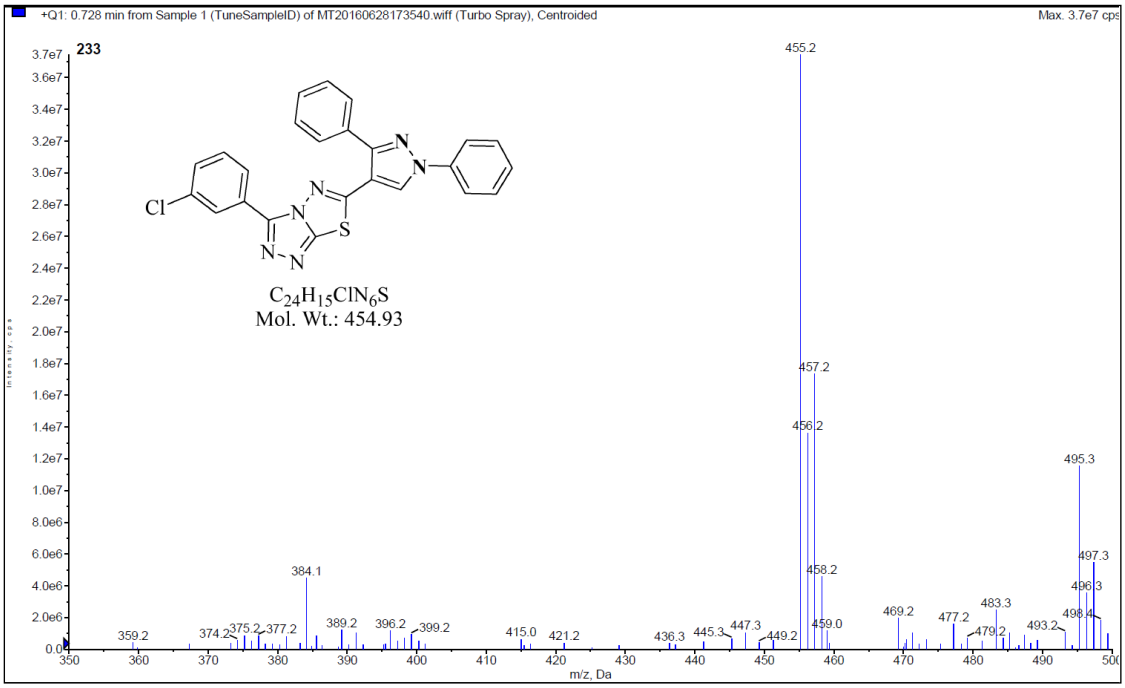


Figure 5.11 Mass spectrum of compound **T_{55}**

3-(3-Chlorophenyl)-6-(1-(4-chlorophenyl)-3-phenyl-1*H*-pyrazol-4-yl)-[1,2,4]triazolo[3,4-*b*] [1,3,4]thiadiazole (T_{56}).

IR (KBr ν_{max} cm^{-1}): Yield: 79 %; m.p: 182-184 °C; IR (KBr) cm^{-1}: 3060 (Ar-H str), 1598 (C=N str), 1565 (C=C str), 1468 (C-N str), 770 (C-Cl str); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.43-7.47 (m, 2H, Ar-H), 7.49-7.58 (m, 5H, Ar-H), 7.73-7.78 (m, 2H, Ar-H), 7.81-7.86 (m, 2H, Ar-H), 8.17 (d, 1H, Ar-H, *J* = 7.2 Hz), 8.31 (s, 1H, Ar-H), 8.56 (s, 1H, pyrazole-5H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 159.23, 154.46, 152.62, 145.23, 137.36, 134.97, 133.75, 130.60, 130.37, 130.21, 129.92, 129.54, 129.00. 128.74, 128.66, 127.22, 126.13, 124.40, 120.80, 112.76; LC-MS: *m/z* = 490.2 (M+1); ANAL. Calcd. for $C_{24}H_{14}Cl_2N_6S$; calcd: C, 58.90; H, 2.88; N, 17.17; found: C, 58.96; H, 2.91; N, 17.20.

3-(3-Chlorophenyl)-6-(3-(4-methoxyphenyl)-1-phenyl-1*H*-pyrazol-4-yl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (T_{57}). Yield: 72 %; m.p: 168-169 °C; IR (KBr) cm^{-1}: 3060 (Ar-H str), 1599 (C=N str), 1563 (C=C str), 1467 (C-N str), 756 (C-Cl str); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 3.92 (s, 3H, OCH_3), 6.99 (d, 1H, Ar-H, *J* = 8.8 Hz), 7.08 (d, 2H, Ar-H, *J* = 8.8 Hz), 7.37-7.47 (m, 2H, Ar-H), 7.50-7.57 (m, 2H, Ar-

H), 7.71 (d, 2H, Ar-H, *J* = 8.8 Hz), 7.81-7.88 (m, 2H, Ar-H), 8.21 (m, 1H, Ar-H), 8.34 (s, 1H, Ar-H), 8.57 (s, 1H, pyrazole-5H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 160.87, 152.26, 138.88, 134.94, 130.96, 130.74, 130.34, 130.20, 129.76, 129.61, 128.67, 128.01, 127.28, 126.16, 124.46, 119.66, 119.52, 114.14, 113.47, 112.32, 55.44; LC-MS: *m/z* = 485.2 (M+1); ANAL. Calcd. for $C_{25}H_{17}ClN_6OS$; calcd: C, 61.92; H, 3.53; N, 17.33; found: C, 61.96; H, 3.54; N, 17.40.

6-(1,3-Diphenyl-1*H*-pyrazol-4-yl)-3-(6-methylpyridin-3-yl)-[1,2,4]triazolo[3,4-*b*] [1,3,4]thiadiazole (T_{58}): Yield: 86 %; m.p: 222-224 ^{o}C; IR (KBr) cm^{-1}: 2927 (C-H str), 1601 (C=N str), 1563 (C=C str), 1519 (C-N str); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 2.69 (s, 3H, CH_3), 7.29-7.44 (m, 2H, Ar-H), 7.51-7.60 (m, 5H, Ar-H), 7.73 (d, 2H, Ar-H, *J* = 8.0 Hz), 7.85 (d, 2H, Ar-H, *J* = 8.0 Hz), 8.43 (dd, 1H, Ar-H, *J* = 8.0 Hz, 1.8 Hz), 8.64 (s, 1H, pyrazole-5H), 9.51 (s, 1H, pyridine-2H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 160.26, 159.65, 154.58, 152.44, 146.50, 144.21, 138.82, 133.93, 130.77, 129.77, 129.66, 129.38, 128.73, 128.59, 128.07, 123.45, 119.62, 119.48, 112.52, 24.50; LC-MS: *m/z* = 436.4 (M+1); ANAL. Calcd. for $C_{24}H_{17}N_7S$; calcd: C, 66.19; H, 3.93; N, 22.51; found: C, 66.26; H, 3.92; N, 22.53.

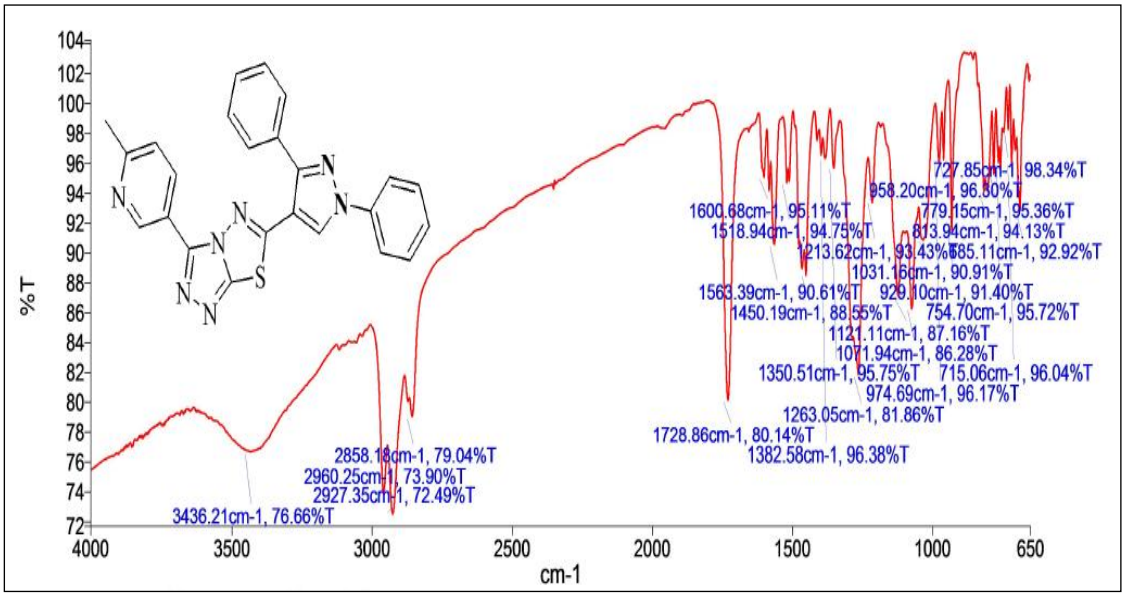


Figure 5.12 IR spectrum of compound **T_{58}**

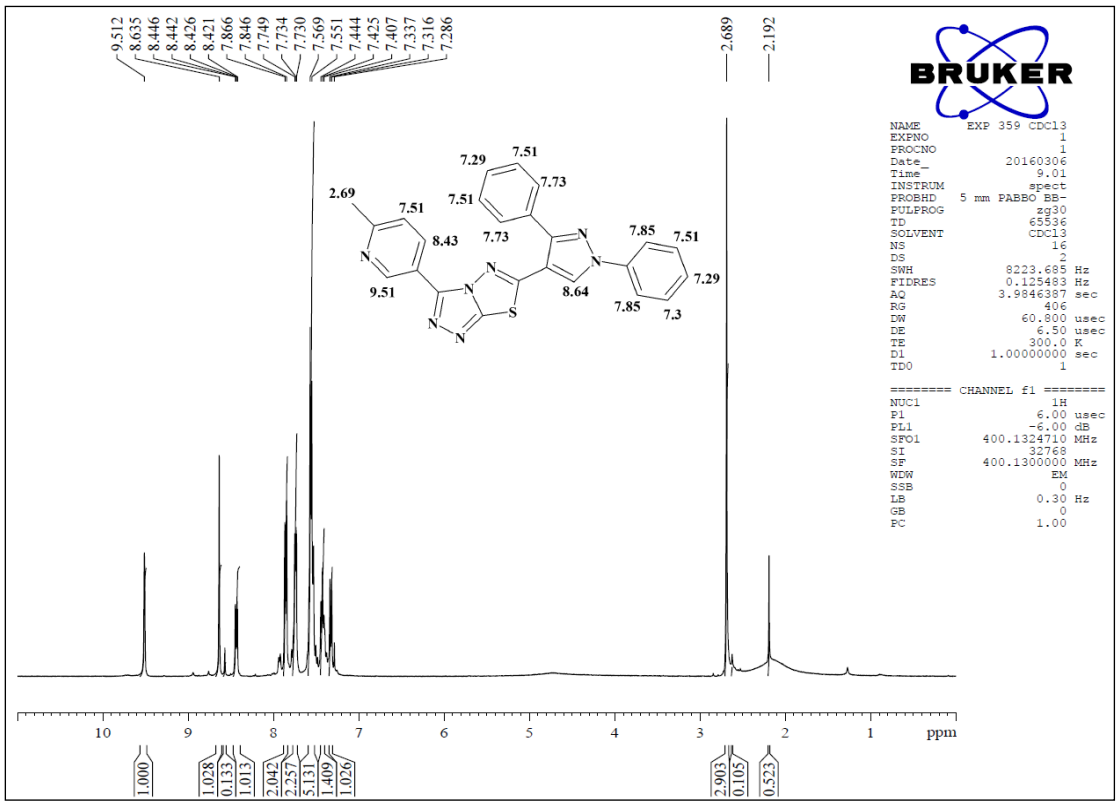


Figure 5.13 ^{1}H-NMR spectrum of compound **T_{58}**

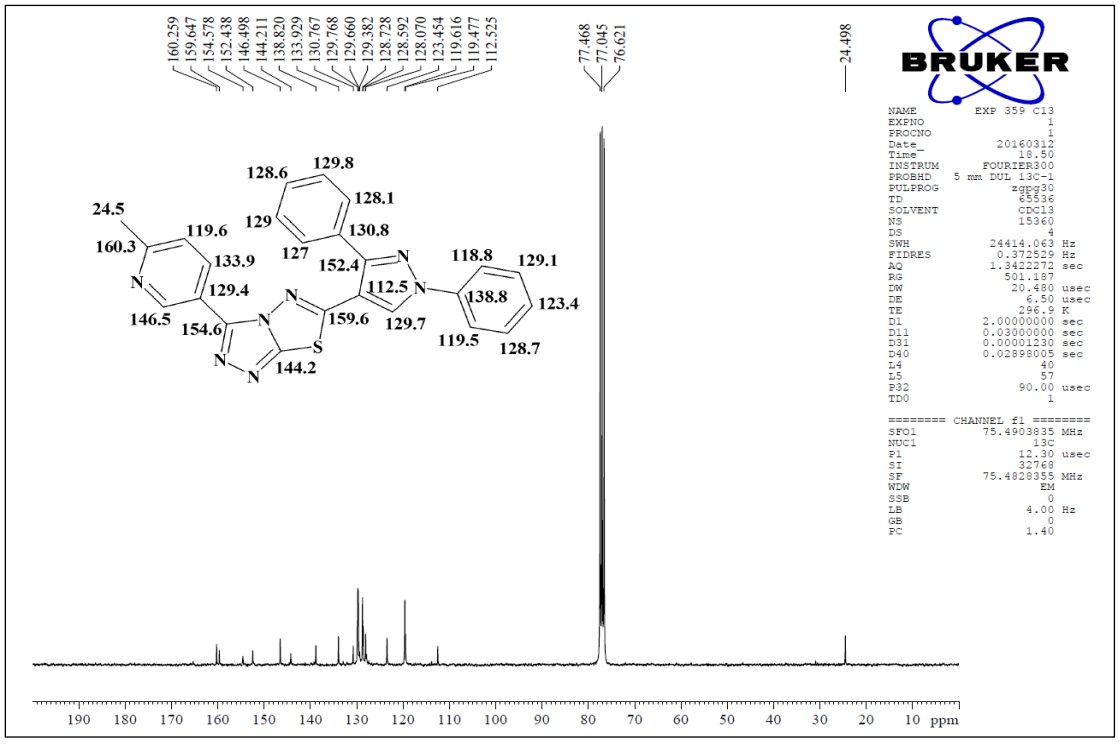


Figure 5.14 ^{13}C-NMR spectrum of compound **T_{58}**

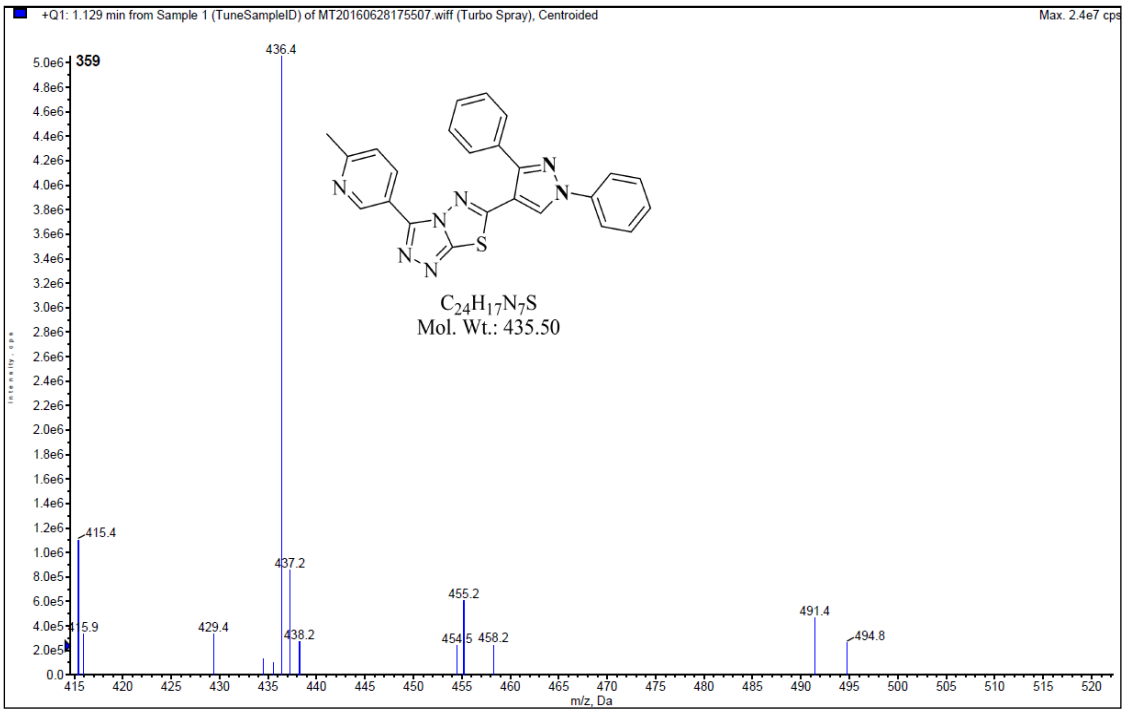


Figure 5.15 Mass spectrum of compound **T_{58}**

6-[1-(4-Chlorophenyl)-3-phenyl-1*H*-pyrazol-4-yl)-3-(6-methylpyridin-3-yl)-[1,2,4]triazolo[3,4-*b*] [1,3,4]thiadiazole (T_{59}): Yield: 80 %; m.p: 242-244 ^{o}C; IR (KBr) cm^{-1}: 2930 (C-H str), 1606 (C=N str), 1564 (C=C str), 1507 (C-N str), 770 (C-Cl str); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 2.69 (s, 3H, CH_3), 7.33 (d, 1H, Ar-H, *J* = 8.0 Hz), 7.41-7.58 (m, 4H, Ar-H), 7.72 (d, 2H, Ar-H, *J* = 8.0 Hz), 7.81 (d, 2H, Ar-H, *J* = 8.4 Hz), 8.43 (dd, 2H, Ar-H, *J* = 8.4 Hz, 2.0 Hz), 8.62 (s, 1H, pyrazole-5H), 9.52 (s, 1H, pyridine-2H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 160.38, 159.39, 154.56, 152.69, 144.28, 137.34, 133.88, 133.74, 130.52, 129.99, 129.92, 129.62, 128.80, 128.42, 127.98, 123.44, 120.72, 119.42, 112.91, 24.57; LC-MS: *m/z* = 470.3 (M+1); ANAL. Calcd. for $C_{24}H_{16}ClN_7S$; calcd: C, 61.34; H, 3.43; N, 20.86; found: C, 61.41; H, 3.45; N, 20.90.

6-[3-(4-Methoxyphenyl)-1-phenyl-1*H*-pyrazol-4yl]-3-(6-methylpyridin-3-yl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (T_{60}). Yield: 83 %; m.p: 210-212 ^{o}C; IR (KBr) cm^{-1}: 2927 (C-H str), 1605 (C=N str), 1563 (C=C str), 1503 (C-N str); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 2.51 (s, 3H, -CH_3), 3.87 (s, 3H, -OCH_3), 7.17-7.20 (t, 2H, Ar-H), 7.44-7.46 (t, 1H, Ar-H), 7.62-7.67 (m, 4H, Ar-H), 7.74-7.80 (m, 5H, Ar-H),

8.56 (s, 1H, pyrazole-5H); LC-MS: *m/z* = 466.1 (M+1); ANAL. Calcd. for $C_{26}H_{19}N_7OS$; calcd: C, 64.50; H, 4.11; N, 21.06; found: C, 64.66; H, 4.13; N, 21.13.

6-[1-(4-Chlorophenyl)3-phenyl-1*H*-pyrazol-4-yl]3-(4-methylsulfanyl benzyl)-[1,2,4]traizolo[3,4-*b*][1,3,4]thiadiazole (T_{62}). Yield: 71 %; m.p: 183-185 ^oC; IR (KBr) cm^{-1}: 2930 (C-H str), 1600 (C=N str), 1561 (C=C str), 1505 (C-N str), 769 (C-Cl str); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 2.53 (s, 3H, $-CH_3$), 4.22 (s, 2H, CH_2), 7.17-7.20 (t, 2H, Ar-H), 7.32-7.35 (m, 1H, Ar-H), 7.55-7.58 (m, 5H, Ar-H), 7.73-7.82 (m, 5H, Ar-H), 8.51 (s, 1H, pyrazole-5H); LC-MS: *m/z* = 515.3 (M+1); ANAL. Calcd. for $C_{26}H_{19}ClN_6S_2$; calcd: C, 60.63; H, 3.72; N, 16.32; found: C, 60.80; H, 3.78; N, 16.38.

6-[3-(4-Methoxyphenyl)-1-phenyl-1*H*-pyrazol-4yl]-3-(4-methylsulfanyl benzyl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (T_{63}). Yield: 76 %; m.p: 169-171 ^oC; IR (KBr) cm^{-1}: 2930 (C-H str), 1604 (C=N str), 1561 (C=C str), 1503 (C-N str); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 2.53 (s, 3H, $-CH_3$), 3.73 (s, 3H, $-OCH_3$), 4.19 (s, 2H, CH_2), 7.36-7.39 (m, 2H, Ar-H), 7.42-7.44 (t, 1H, Ar-H), 7.66-7.70 (m, 5H, Ar-H), 7.81-7.83 (m, 5H, Ar-H), 8.51 (s, 1H, pyrazole-5H); LC-MS: *m/z* = 510.1 (M-1), ANAL. Calcd. for $C_{27}H_{22}N_6OS_2$; calcd: C, 63.51; H, 4.34; N, 16.46; found: C, 63.60; H, 4.30; N, 16.49.

5.4 PHARMACOLOGY

5.4.1 Antitubercular activity by MABA method

Antitubercular screening for the newly synthesized compounds $\mathbf{T_{46\text{-}63}}$ were determined by the Middle brook 7H9 broth against *Mycobacterium tuberculosis* of $H_{37}Rv$ strain (ATCC-27294) as explained in **Chapter 2**. MIC value of each synthesized compound was determined by the Microplate Alamar Blue Assay method (MABA). Isoniazid, Ethambutol and Pyrazinamide were used as standard anti-TB drugs.

5.4.2 Antibacterial and antifungal activity

Antimicrobial screening for the newly synthesized compounds $\mathbf{T_{46\text{-}63}}$ were determined by MIC value using Resazurin reduction method in 96 well microplates as

explained in **Chapter 2**. In this work, *Staphylococcus aureus* (MTCC 3160) and tuberculosis variant bacteria *Mycobacterium smegmatis* (MTCC 994) were used to study antibacterial activity. Antifungal activity of these compounds was carried out against pathogenic fungi *Candida albicans* (MTCC 7253) and *Penicillium chrysogenum* (MTCC 6795). All the bacterial and fungal cultures were obtained from IMTECH, Chandigarh, India and the cultures were maintained as per the standard protocol. The wells, which remains blue after 24 hours of incubation indicates there are no microorganisms survived in the well, the minimum concentration where no microbial growth found are considered as MIC value.

5.4.3 Cytotoxicity studies

5.4.3.1 IC_{50} value determination for HeLa and Vero cell lines.

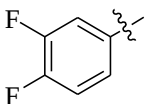
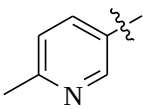
Vero (African green monkey kidney) cell line was procured from National Centre for Cell Sciences (NCCS), Pune, India. *In vitro* cytotoxicity of active compounds against *Mycobacterium tuberculosis* were tested for cytotoxicity against HeLa (Cervical cancer) and VERO (African green monkey kidney) cell lines. The high IC_{50} value and high selectivity index indicate the nontoxicity of the compound. The control cells which are not treated with any compound have shown 100 % viability. *In vitro* cytotoxicity study of active compounds was tested against HeLa and Vero cell lines as explained in **Chapter 3.**

5.5 RESULTS AND DISCUSSION

5.5.1 Chemistry

Physical properties data of compounds **17a-c**, **18a-c** and **4a-c**, **19a-c** were represented in **Table 5.2** and **Table 5.3** respectively.

Table 5.2 Physicochemical properties of the compounds **17a-c** and **18a-c**

Compounds	R	M. F	M. wt	M. p (°C)	Yield (%)
17a		$C_7H_6F_2N_2O$	172.13	144-146	92
17b		$C_7H_9N_3O$	151.17	178-179	96

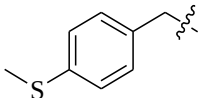
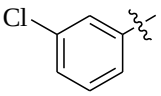
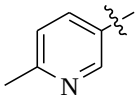
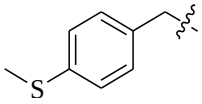
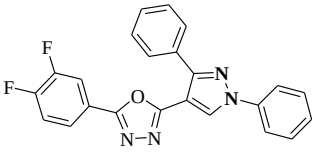
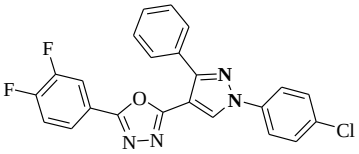
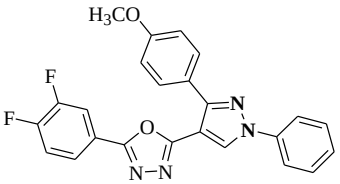
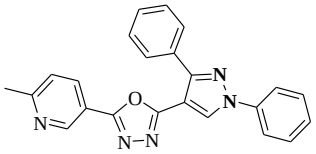
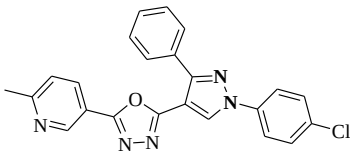
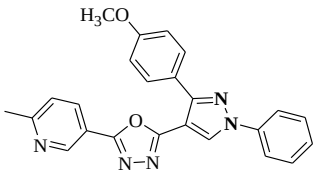
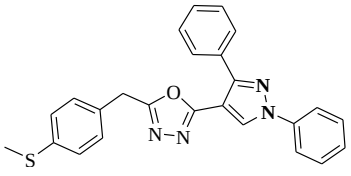
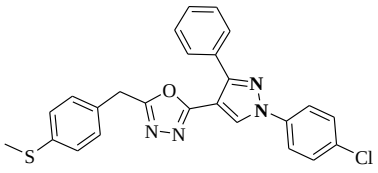
17c		$C_9H_{12}N_2OS$	196.27	121-123	90
18a		$C_8H_7ClN_4S$	226.69	133-134	76
18b		$C_8H_9N_5S$	207.26	137-137	75
18c		$C_{10}H_{12}N_2OS$	252.36	158-160	68

Table 5.3 Physicochemical properties of the compounds **4a-c** and **19a-c**

Compounds	R_1	R_2	M. F	M. wt	M. p (°C)	Yield (%)
4a	H	H	$C_{16}H_{12}N_2O$	248.28	144-146	87
4b	H	Cl	$C_{16}H_{11}ClN_2O$	282.72	145-146	91
4c	OCH_3	H	$C_{17}H_{14}N_2O_2$	278.31	137-137	76
19a	H	H	$C_{16}H_{12}N_2O_2$	264.28	201-203	86
19b	H	Cl	$C_{16}H_{11}ClN_2O_2$	298.72	198-199	89
19c	OCH_3	H	$C_{17}H_{14}N_2O_3$	294.31	205-206	81

Target compounds, $\mathbf{T_{46\text{-}54}}$ and $\mathbf{T_{55\text{-}63}}$ were confirmed by recording their FT-IR, ^{1}H-NMR, ^{13}C-NMR, mass spectrum and elemental analysis. The ^{1}H-NMR spectrum of $\mathbf{T_{46}}$ showed a characteristic singlet at 8.75 represents to pyrazole-5H. In ^{13}C-NMR spectrum of $\mathbf{T_{46}}$ showed at 162.22, 157.36, 148.24, 139.00, 138.50, 134.13, 131.79, 129.89, 129.26, 127.74, 123.56, 119.51, 118.62, 115.73, 108.49, 103.69, 102.02, 85.29. The mass spectrum of $\mathbf{T_{46}}$ showed a molecular ion peak at *m/z* = 401.2 (M+1); which is in consistent with the molecular formula $C_{23}H_{14}F_2N_4O$. The characterization data of the newly synthesized compounds $\mathbf{T_{46\text{-}54}}$ and $\mathbf{T_{55\text{-}63}}$ have been presented in **Table 5.4** and **Table 5.5** respectively.

Table 5.4 Structural properties of synthesized compounds **$T_{46\text{-}54}$**

Compounds	R	R_1	R_2	Structure	M. wt
T_{46}	3,4-$F_2C_6H_3$	H	H		400.38
T_{47}	3,4-$F_2C_6H_3$	H	Cl		434.83
T_{48}	3,4-$F_2C_6H_3$	OCH_3	H		430.41
T_{49}	6-$CH_3C_5H_3N$	H	H		379.41
T_{50}	6-$CH_3C_5H_3N$	H	Cl		413.86
T_{51}	6-$CH_3C_5H_3N$	OCH_3	H		409.44
T_{52}	4-$SCH_3C_6H_4CH_2$	H	H		424.52
T_{53}	4-$SCH_3C_6H_4CH_2$	H	Cl		458.96

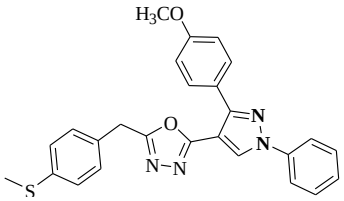
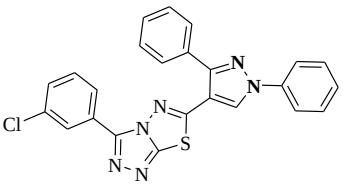
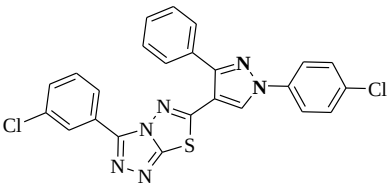
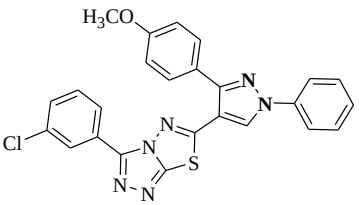
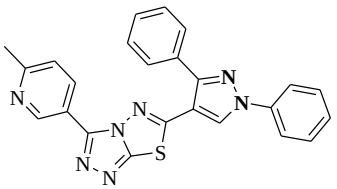
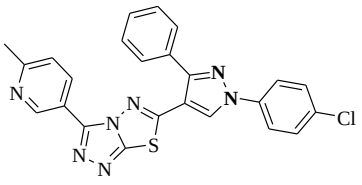
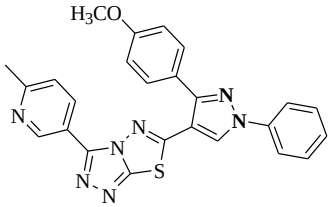
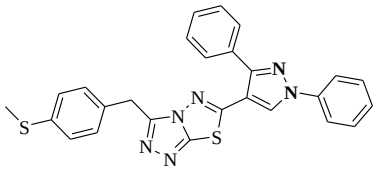
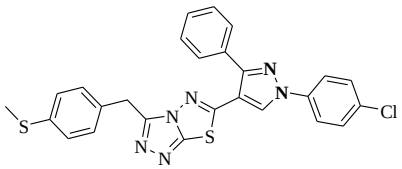
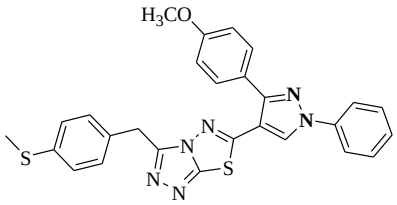
T_{54}	4-$SCH_3C_6H_4CH_2$	OCH_3	H		454.54

Table 5.5 Structural properties of synthesized compounds **$T_{55\text{-}63}$**

Compounds	R	R_1	R_2	Structure	M. wt
T_{55}	3-ClC_6H_4	H	H		454.93
T_{56}	3-ClC_6H_4	H	Cl		489.38
T_{57}	3-ClC_6H_4	OCH_3	H		484.96
T_{58}	6-$CH_3C_5H_3N$	H	H		435.50
T_{59}	6-$CH_3C_5H_3N$	H	Cl		469.95

T_{60}	6-$CH_3C_5H_3N$	OCH_3	H		465.53
T_{61}	4-$SCH_3C_6H_4CH_2$	H	H		480.61
T_{62}	4-$SCH_3C_6H_4CH_2$	H	Cl		515.05
T_{63}	4-$SCH_3C_6H_4CH_2$	OCH_3	H		510.63

5.5.2 Biological results

5.5.2.1 *In vitro* antitubercular activity

Antitubercular activity of newly synthesized compounds was tested against *Mycobacterium tuberculosis* $H_{37}Rv$ strain (ATCC-27294) and the same has been presented in **Figure 5.16**. The Minimum Inhibition Concentration assay is defined as the minimum concentration of a compound required to completely inhibit the bacterial growth. Anti-TB standards Isoniazid (INH), Ethambutol (EMB) and Pyrazinamide (PZA) were used for comparison.

The majority of the compounds showed the MIC values in the range between 0.78 to 50.0 µg/mL. The compounds **T_{49}**, **T_{50}**, **T_{51}**, **T_{59}** and **T_{60}** showed least MIC value at 0.78 µg/mL concentration against *Mycobacterium tuberculosis*. These five compounds were further screened at lower dilutions between 1.56 to $1.22x10^{-2}$ µg/mL using two-fold dilution method and found excellent activity with MIC value 0.39 µg/mL for compounds **T_{50}** and **T_{51}**. Three compounds, **T_{49}**, **T_{59}** and **T_{60}** were showed MIC value of 0.78 µg/mL. MIC value of 1.56 µg/mL showed for compounds **T_{53}** and

T_{58}. It indicates that, 7 out of 18 compounds have potent anti-TB activity (MIC ≤1.56 µg/mL) against *Mycobacterium tuberculosis.*

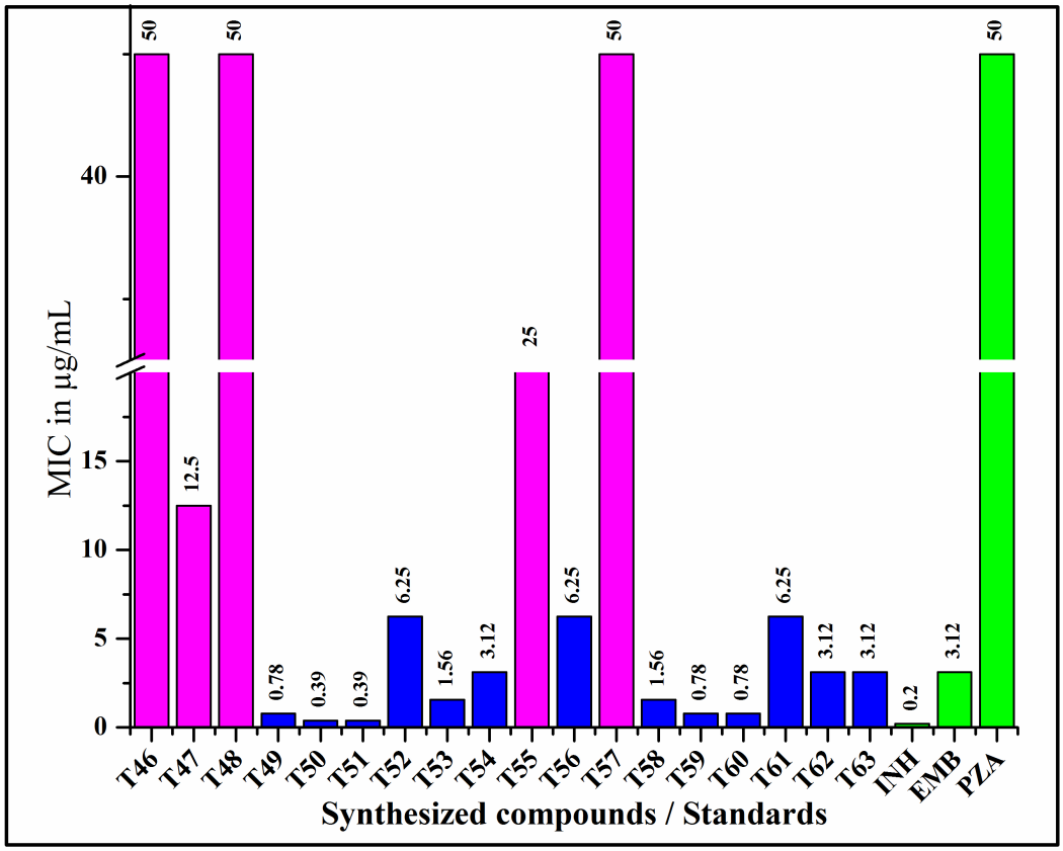


Figure 5.16 Minimum Inhibitory Concentration of **$T_{46\text{-}63}$** against *M. tuberculosis*

Structure activity relationship for compounds pyrazole-[1,3,4]oxadiazole and pyrazole-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole can be explained based on the following facts. Two key factors were identified for significance of the antituberculosis activity. The first factor is methylpyridine binds at the fifth position of [1,3,4]oxadiazole and third position of [1,2,4]triazolo[3,4-b][1,3,4]thiadiazole ring. Another key factor was 4-chlorophenyl substitution (*4-ClC_6H_4*) at first position of 1*H*-pyrazole showed significant change in the antitubercular activity. In previously published works, 4-chlorophenyl group on 1*H*-pyrazole exhibited enormous biological applications (Horrocks *et al.* 2013). Combinations of these two factors showed the excellent MIC value at 0.39 µg/mL for **T_{50}** and **T_{51}**. These two compounds were eight-fold active than that of anti-TB standard Ethambutol. Compounds **T_{49}**, **T_{59}** and **T_{60}** showed four-fold potent than the standard Ethambutol

with MIC value 0.78 µg/mL. Two more compounds **T_{53}** and **T_{58}** found to be two-fold more potent than the standard anti-TB drug Ethambutol. Other compounds, **T_{53}**, **T_{62}** and **T_{63}** showed similar activity with Ethambutol (MIC: 3.12 µg/mL), this is due to the presence of 4-methylsulfanylbenzyl (*-4-$SCH_3C_6H_4CH_2$*) at second position of [1,3,4]oxadiazole and third position of [1,2,4]triazolo[3,4-b][1,3,4]thiadiazole. Compounds **T_{52}**, **T_{56}** and **T_{61}** showed significant MIC value of 6.25 µg/mL. This information on structure activity relationship explored in the present study could be helpful in further structural modification and development of new antitubercular agents.

5.5.2.2 Antibacterial and antifungal activity

The *in vitro* antibacterial activity of the synthesized compounds **$T_{46\text{-}63}$** were carried out against *Mycobacterium smegmatis* (MTCC 994), *Staphylococcus aureus* (MTCC 3160) were screened for MIC of organism by Resazurin reduction method. Ciprofloxacin (INN) used as antibacterial standard. Minimum inhibitory concentration assay conducted at concentration 100 to 0.78 µg/mL. MIC of **T_{54}** showed the significant MIC value of 3.12 µg/mL against both bacteria *M. smegmatis* and *S. aureus*. It was similarly active as that of the standard drug Ciprofloxacin. Compound **T_{52}** and **T_{53}** showed MIC value 6.25 µg/mL against *M. smegmatis.* This indicates that the presence of 4-thiosulfanyl benzyl at fifth position of [1,3,4]oxadiazole playing key role to enhance the antibacterial activity. Gram +ve bacteria *S. aureus* showed significant activity at MIC value 6.25 µg/mL for **T_{47}**, **T_{50}**, **T_{52}**, **T_{53}**, **T_{58}**, **T_{59}**, **T_{62}** and **T_{63}**.

The *in vitro* antifungal activity of the synthesized compounds **$T_{46\text{-}63}$** were carried out against *Candida albicans* (MTTC 7253) and *Penicillium chrysogenum* (MTCC 6795) were screened for MIC by Resazurin reduction method. Fluconazole (FLZ) used as antifungal standard. Minimum inhibitory concentration assay conducted at concentration 100 to 0.78 µg/mL. None of the compound showed significant activity against fungi *C. albicans* and *P. chrysogenum.* This indicates that, these compounds were selectively for bacterial activity. Antibacterial and antifungal activity results have been represented in **Table 5.6**.

Table 5.6 Antibacterial and antifungal activity data of $\mathbf{T_{46\text{-}63}}$ by MIC method

Synthesized Compound	MIC in µg/mL			
	Mycobacterium smegmatis	***Staphylococcus aureus***	***Candida albicans***	***Penicillium chrysogenum***
$\mathbf{T_{46}}$	25	25	100	100
$\mathbf{T_{47}}$	6.25	6.25	100	50
$\mathbf{T_{48}}$	12.5	12.5	>100	50
$\mathbf{T_{49}}$	25	12.5	50	12.5
$\mathbf{T_{50}}$	6.25	6.25	50	50
$\mathbf{T_{51}}$	12.5	12.5	25	25
$\mathbf{T_{52}}$	6.25	6.25	50	50
$\mathbf{T_{53}}$	6.25	6.25	100	100
$\mathbf{T_{54}}$	3.12	3.12	50	50
$\mathbf{T_{55}}$	25	50	50	50
$\mathbf{T_{56}}$	25	25	50	25
$\mathbf{T_{57}}$	50	100	50	50
$\mathbf{T_{58}}$	12.5	6.25	25	25
$\mathbf{T_{59}}$	6.25	6.25	25	25
$\mathbf{T_{60}}$	25	12.5	50	50
$\mathbf{T_{61}}$	12.5	12.5	25	25
$\mathbf{T_{62}}$	12.5	6.25	50	50
$\mathbf{T_{63}}$	12.5	6.25	100	50
INN	3.12	3.12	--	--
FLZ	--	--	3.12	3.12
Control	--	--	--	--

INN; antibacterial standard Ciprofloxacin; FLZ; antifungal standard Fluconazole; --: not detected inhibition; control; dimethylsulfoxide

The above results indicates that, pyrazole bearing oxadiazole and thiadiazole derivatives are prominent to enhance the antitubercular and antibacterial activity.

5.5.2.3 Cytotoxicity studies

Following derivatives were found to be excellent in terms of their antimicrobial properties ($\mathbf{T_{49}}$, $\mathbf{T_{50}}$, $\mathbf{T_{51}}$, $\mathbf{T_{53}}$, $\mathbf{T_{58}}$, $\mathbf{T_{59}}$ and $\mathbf{T_{60}}$) against tubercular causing *M. tuberculosis* $H_{37}Rv$ strain. Furthermore, cytotoxicity of these compounds against cervical cancer-HeLa cell lines and Vero cell lines showed that, none of the active compounds showed toxicity. Out of seven active compounds, $\mathbf{T_{51}}$ showed the least toxicity with IC_{50} value 311/162 µg/mL followed by highest selectivity index 797.4/415.4 respectively against Hela and Vero cell line. The compounds with IC_{50} less than 50 µg/mL considered as toxic in nature. The graphical representation of the IC_{50} value against HeLa and Vero cell lines were showed in **Figure 5.17**. The

comparison values between CLogP, MIC, IC_{50} and SI were presented in **Table 5.7**. The enhanced activity is due to the presence of electron donating group on phenyl group at third position of pyrazole.

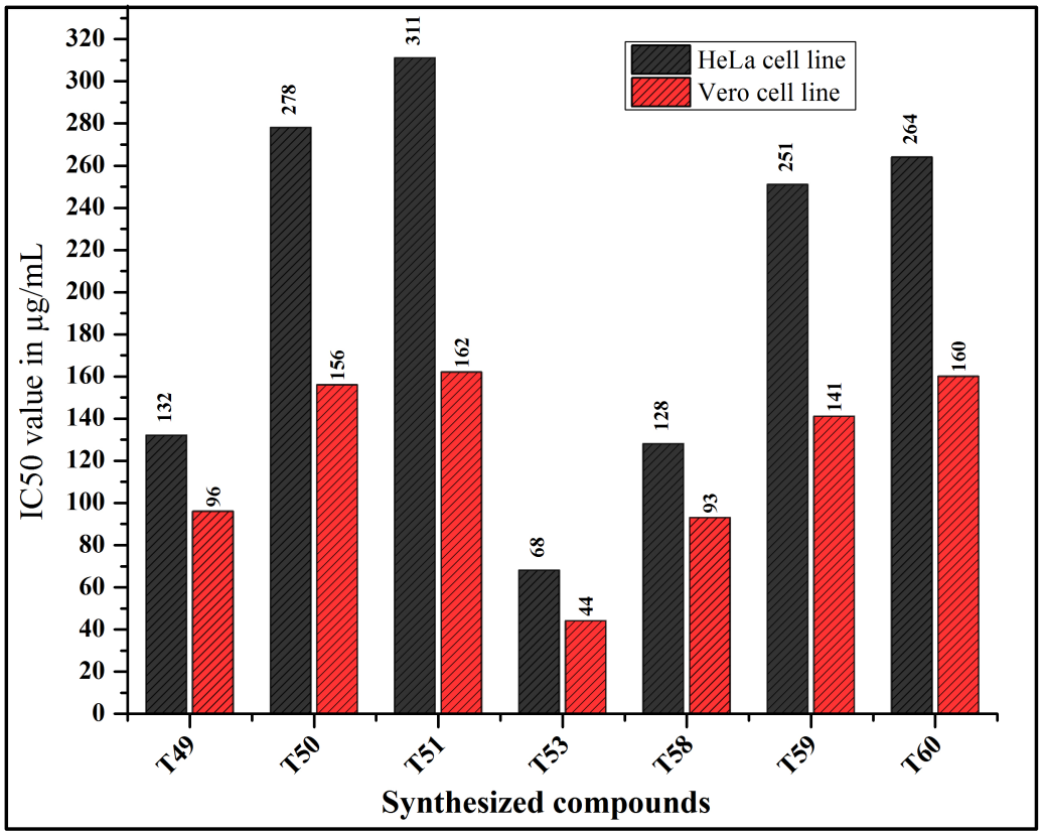


Figure 5.17 Cytotoxicity of **T_{49}, T_{50}, T_{51}, T_{53}, T_{58}, T_{59}** and **T_{60}** against HeLa/Vero cell lines

Table 5.7 Selectivity Index (SI) on HeLa and Vero cell lines against *M.tb* $H_{37}Rv$

Compounds	M. F	CLogP	*Mtb* $H_{37}Rv$ (µg/mL)	HeLa cell line		Vero cell line	
				IC_{50} (µg/mL)	SI	IC_{50} (µg/mL)	SI
T_{49}	379.41	3.49	0.78	132	169.2	96	123.1
T_{50}	413.86	4.23	0.39	278	712.8	156	400
T_{51}	409.44	3.45	0.39	311	797.4	162	415.4
T_{53}	458.96	5.59	1.56	68	43.6	44	28.2
T_{58}	435.5	3.91	1.56	128	82.1	93	59.6
T_{59}	469.95	4.70	0.78	251	321.8	141	180.8
T_{60}	465.53	3.88	0.78	264	338.5	160	205.1

CLogP calculated by ChemDraw version 8.0; Inhibitory concentration (IC_{50}) calculated against HeLa/Vero cells; Selectivity Index (SI) is the ratio of cytotoxicity IC_{50} (µg/mL) to *in vitro M.tb* $H_{37}Rv$ expressed in MIC (µg/mL)

5.6 CONCLUSIONS

Total 18 new biheterocyclic derivatives, 2-[1,3-(disubstituted)-1*H*-pyrazol-4-yl]-5-substituted aryl-[1,3,4]oxadiazole **T_{46-54}** and 6-[1,3-(disubstituted)-1*H*-pyrazol-4-yl]-3-substituted aryl-[1,2,4]triazolo[3,4-*b*][1,3,4] thiadiazole **T_{55-63}** scaffolds were synthesized. They were characterized by FT-IR, ^{1}H-NMR, ^{13}C-NMR and mass spectrometry. Target compounds were investigated for their *in-vitro* antitubercular and antimicrobial activities by minimum inhibition concentration and proved to be very good active compounds. The compounds **T_{50}** and **T_{51}** exhibited the best MIC value of 0.39 µg/mL, compounds **T_{49}**, **T_{59}** and **T_{60}** showed MIC value of 0.78 µg/mL and compounds **T_{53}** and **T_{58}** showed MIC value 1.56 µg/mL against *Mycobacterium tuberculosis.* The above five compounds were highly potent than tested anti-TB standards Ethambutol and Pyrazinamide. Antibacterial activity of compound **T_{54}** showed MIC value at 3.12 µg/mL. This MIC value is equal to the antibacterial standard Ciprofloxacin. Structure activity relationship reveals that, the enhanced activity is due to the presence of methylpyridine at second position of [1,3,4]oxadiazole and [1,3,4]thiadiazole pharmacophore. It can be concluded that, these biheterocyclic compounds are more promising antitubercular drugs in the future.

CHAPTER 6

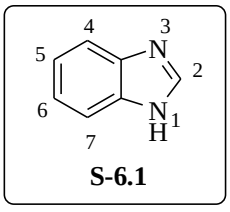
BENZIMIDAZOLE AND TRIFLUOROMETHYL BENZYLOXYMETHYL CONTAINING PYRAZOLE DERIVATIVES: SYNTHESIS, CHARACTERIZATION, ANTITUBERCULAR AND ANTIMICROBIAL STUDIES

Abstract

This chapter deals with detailed literature survey on benzimidazole and trifluoromethyl pyrazole derivatives until August 2016. Also, the detailed synthesis and biological importance of benzimidazole and trifluoromethyl substituted derivatives have been explained. The target benzimidazole containing pyrazole derivatives were synthesized by the reaction of 1,3-disubstituted-1H-pyrazole-4-carbaldehyde with 4-substituted-o-phenylenediamines under reflux condition. In the other series, trifluoromethyl substituted derivatives were synthesized from 6-trifluoromethyl-2-chlorobenzylbromide by treating with (1,3-disubstituted-1H-pyrazol-4-yl)-methanol in alkaline media at ambient temperature. Further, the synthesized pyrazole containing benzimidazole and trifluoromethyl substituted derivatives were characterized by spectral techniques and screened for their antitubercular, antibacterial and antifungal studies. Cytotoxicity studies were performed for active compounds against normal cell lines.

6.1 INTRODUCTION

Benzimidazole is a heterocyclic bicyclic organic compound. This bicyclic compound having imidazole ring (containing two nitrogen atoms at non-adjacent positions) fused to the benzene ring. The most prominent benzimidazole compound in nature is *N*-ribosyl-dimethyl benzimidazole, which serves as an axial ligand for cobalt in vitamin B12 (Barker *et al.* 1960).



S-6.1

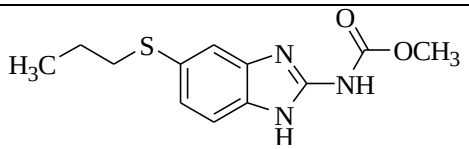
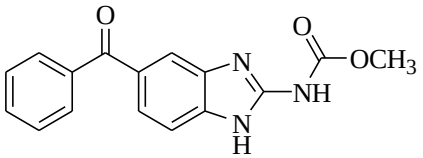
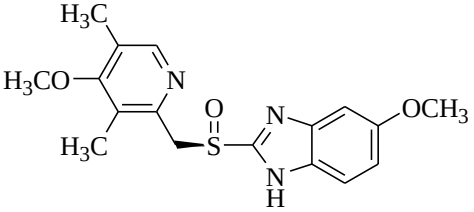
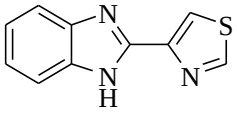
Benzimidazole is a white to slightly beige solid, melting at 172 °C, sparingly soluble in water and freely soluble in ethanol. Their derivatives are associated with various types of pharmacokinetic and pharmacodynamic properties. Benzimidazole nucleus is one of the bioactive heterocyclic compounds that exhibit a wide range of biological activities. Since last few decades, they have been used in various medicinal compounds. For example, Miconazole (antifungal), Imiquimod (anticancer), Albendazole (antihelminthic), Triclabendazole (anthelmintic) and so on. Literature

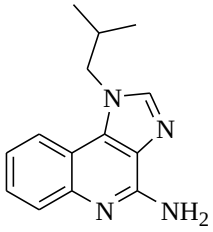
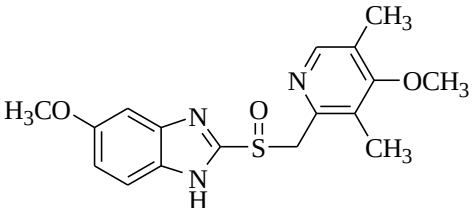
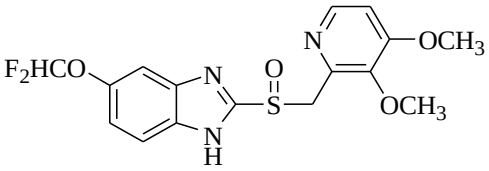
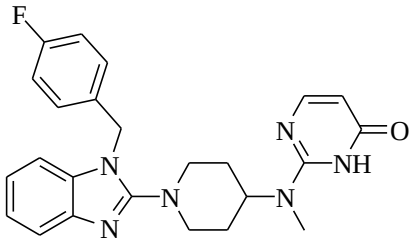
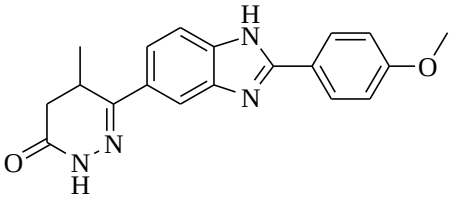
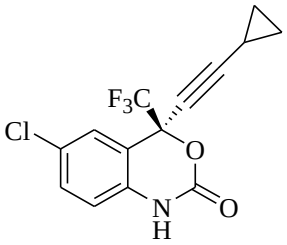
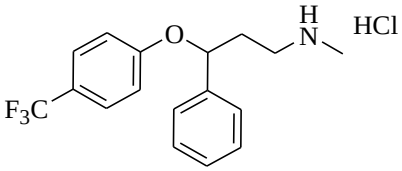
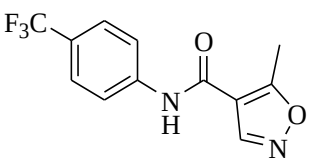
survey reveals that the various derivatives of benzimidazole have been synthesized for their different pharmacological activities. Some of the reported compounds were found to possess anti-HIV (Demirayak *et al.* 2002), antiviral (Kristina *et al.* 2007), antiprotozoal (Gomez *et al.* 2008), antimicrobial (Ansari and Lal 2009), anticancer (Shaharyar *et al.* 2010), antihypertensive (Zhang *et al.* 2015) and antitubercular (Ramprasad *et al.* 2015) activities.

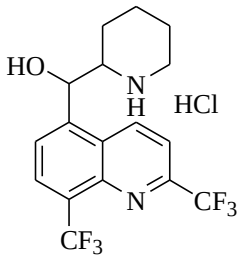
Fluorine chemistry exhibits important pharmacological activities due to its unique stereoelectronic properties. The reason behind choosing this work was to develop novel pyrazole derivatives having a fluorine atom. Moreover, since past three to four decades, research on fluorine containing molecules is rapidly increasing. Trifluoromethyl containing pyrazoles exhibits significant biological activities *viz.* antiinfuenza (Secor and DeBardeleben 1971), COX-2 inhibitor (Penning *et al.* 1997), antitumor (Abdou *et al.* 2004), insecticidal (Lahm *et al.* 2005), antitubercular (Pedro *et al.* 2008), analgesic and anti-inflammatory (Sauzem *et al.* 2008), antipyretic and antioxidant (Pasin *et al.* 2010) and antimicrobial (Garudachari *et al.* 2013) activities.

Some of the commercially available benzimidazole containing drugs has been tabulated in **Table 6.1**.

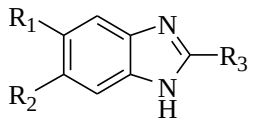
Table 6.1 Commercially available benzimidazole and trifluoromethyl containing drugs

Drug name	Structure	Therapeutic use
Albendazole		Antiparasitic
Mebendazole		Antiparasitic
Esomeprazole		Proton pump inhibitor
Thiabendazole		Fungicide

Imiquimod		Anticancer
Omeprazole		Antiulcer
Pantoprazole		Antiulcer
Telmisartan		Antihistamine
Pimobendan		Vasodilator
Efavirenz		Anti-HIV
Fluoxetine HCl		Antidepressant
Leflunomide		Antirheumatic

Mefloquine HCl		Antimalarial

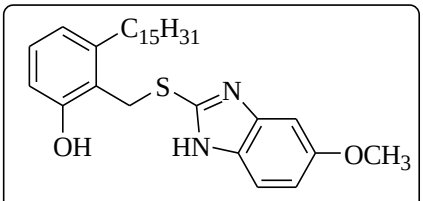
Benzimidazole derivatives (**S-6.1**) have been synthesized and tested for *in vitro* analysis against the protozoa *Giardia lamblia, Entamoeba histolytic* and the helminth *Trichinella spiralis* (Valdez *et al.* 2002). Results indicated that these derivatives were more active as antiprotozoal agents than standard drugs Metronidazole and Albendazole. Structure activity relationship explains that, compounds with 2-methoxycarbonylamino substitution have decreased the antiparasitic activity.



S-6.1

R_1= H, Cl
R_2= H, Cl
R_3= H, CH_3, NH_2, $NHCOCH_3$, SH, SCH_3

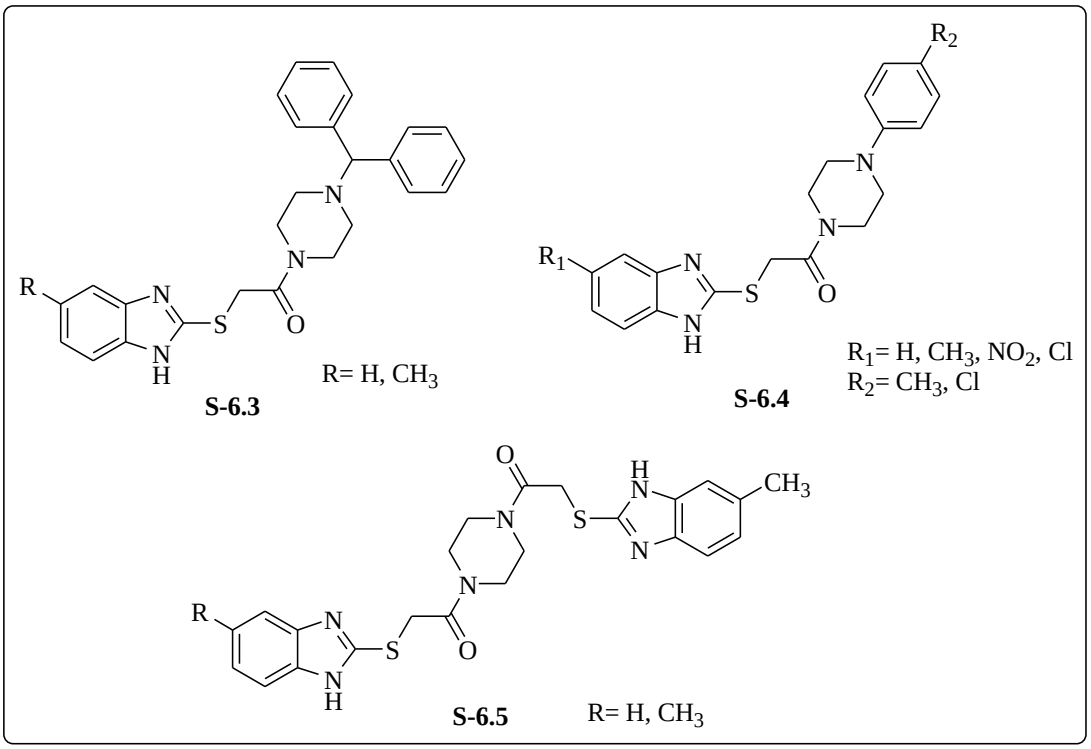
Paramashivappa *et al.* (2003) have developed a new series of 2-[[2-alkoxy-6-pentadecylphenyl)methyl]thio]-1*H*-benzimidazoles, benzothiazoles and benzoxazoles from anacardic acid and investigated for their ability to inhibit human cyclooxygenase-2 enzyme (COX-2). The active compounds were screened for cyclooxygenase-1 (COX-1) inhibition and found that compound (**S-6.2**) was showed selective towards COX-2 inhibition compared to COX-1 inhibition.



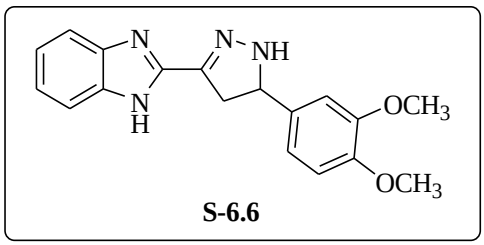
S-6.2

The 1*H*-benzimidazole containing piperazine derivatives have synthesized and evaluated for their *in vitro* antiparasitic activity against *T. spiralis* as well as their *in vivo* antinematode activity against *S. obvelata* (Mavrova *et al.*, 2006). The *in vitro*

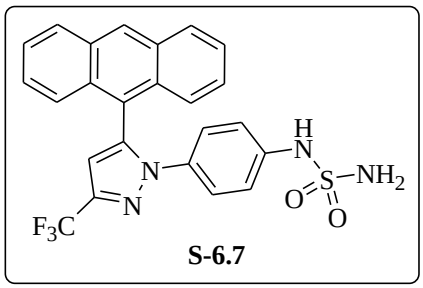
antiparasitic activity showed that most of the target compounds exhibit higher activity than tested standard Albendazole. Some of the compounds (**S-6.3**, **S-6.4** and **S-6.5**) demonstrated 96.0 %, 98.2 % and 100 % activities at a dose of 200 µg/mL after 48 h. Some other compounds were found most active with 76 %, 73 % and 77 % against *S. obvelata*.



Two new series of 2-{5-[(substituted) phenyl]-4,5-dihydro-1*H*-3-pyrazolyl}-1*H*-benzoimidazole and 2-{5-[(substituted)phenyl]-1-phenyl-4,5-dihydro-1*H*-3-pyrazolyl}-1*H*-benzimidazole have been prepared by Shaharyar *et al.* (2010). All compounds were screened at the National Cancer Institute (NCI), USA for anticancer activity. Compound 2-[5-(3,4-dimethoxyphenyl)-1-phenyl- 4,5-dihydro-1*H*-3-pyrazolyl]-1*H*-benzimidazole (**S-6.6**) was found to be the most active among the series.

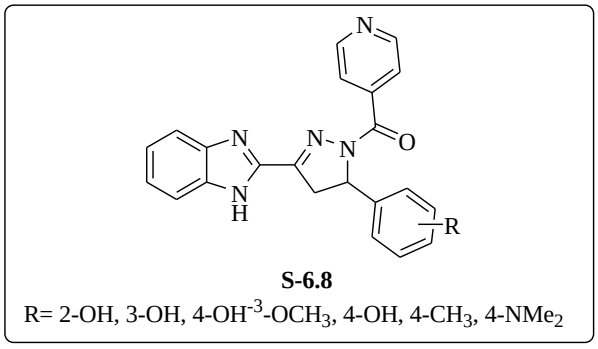


Celecoxib-based library compounds were synthesized by Chiu *et al.* (2012). Newly modified derivatives were tested against a panel of *Staphylococcus* pathogens and different strains of Methicillin-resistant *Staphylococcus aureus* (MRSA) and identified the compound (**S-6.7**) as the lead agent with high antibacterial potency.



S-6.7

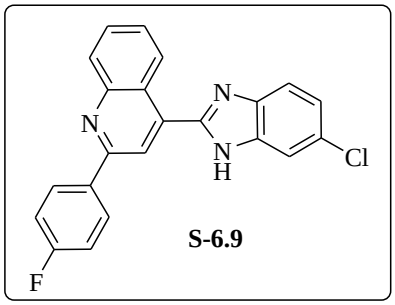
Desai *et al.* (2012) synthesized a series of benzimidazole containing pyrazole derivatives (**S-6.8**) by the reaction of benzimidazolyl chalcones with anti-TB standard drug Isoniazid. The newly synthesized compounds have evaluated for their antimicrobial activity by serial dilution method. Few compounds exhibited MIC value of 25 µg/mL against tested bacterial strains.



S-6.8

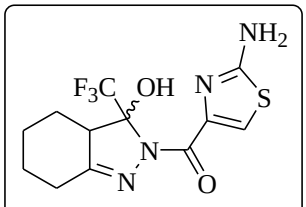
R= 2-OH, 3-OH, 4-OH^{-3}-OCH_3, 4-OH, 4-CH_3, 4-NMe_2

Two new series of benzimidazole incorporated quinoline derivatives have been synthesized from substituted anilines by Doebner reaction and Pfitzinger reactions (Garudachari *et al.* 2012). All target compounds were evaluated for their *in vitro* antimicrobial activity by well plate method. Compound (**S-6.9**) found to be most potent antifungal agent among the synthesized compounds.



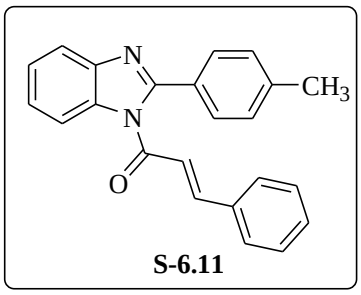
S-6.9

Rosa and group members, (2013) describe the selection and optimization of a chemical series active in both a full-length and a fragment-based Huntington's disease (HD) assay. They identified a series of compounds bearing a 3-hydroxy-3-trifluoromethylpyrazole moiety as able to revert the toxicity induced by full-length mutant Htt by up to 50 %. A chemical exploration around the series led to the identification of compound (**S-6.10**) which demonstrated to be active in Htt171–82Q rat primary striatal neuron assay and a PC12-Exon-1 based assay. These studies provide the strong rationale for further testing the potential benefits of 3-hydroxy-3-trifluoromethylpyrazoles in treating Huntington's disease.



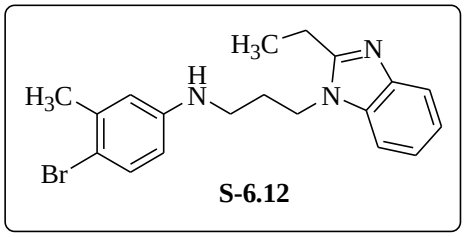
S-6.10

Kalalbandi *et al.* (2014) synthesized a novel series of 1-[(2E)-3-phenylprop-2-enoyl]-1H-benzimidazole derivatives. Newly synthesized compounds were evaluated for antitubercular activity by Microplate Alamar Blue Assay method. Compound with electron donating group at C-2 carbon showed more potent in inhibiting *Mycobacterium tuberculosis.* Compound (**S-6.11**) was found to be an excellent activity with IC_{50} < 10 µg/mL.



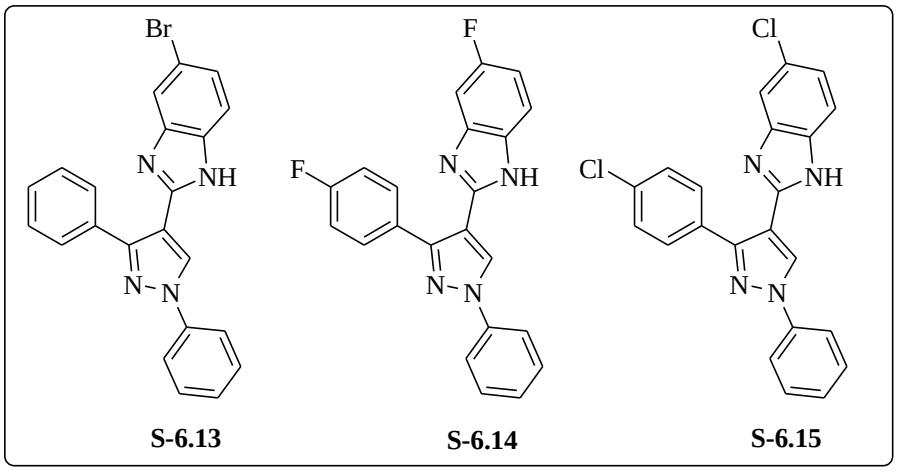
S-6.11

Chandrasekera *et al.* (2015) evaluated phenoxy alkyl benzimidazoles as antitubercular agents. They synthesized 2-ethyl-1-(3-phenoxypropyl)-1*H*-benzo[*d*]imidazole derivatives and the structure-activity relationship was explained with respect to the biological activity. Compound (**S-6.12**) showed most potent with the sub-micromolar level at 52 µM against *Mycobacterium tuberculosis.* Active compounds had moderate to high permeability in MDCK cells.



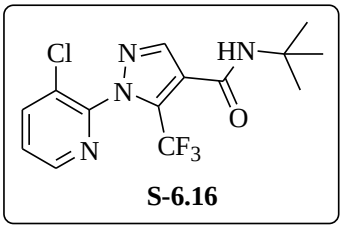
S-6.12

Library series of pyrazole containing benzimidazole hybrids have been developed by Reddy and co-workers, (2015) and screened for antiproliferative activity against human tumor cell lines - lung (A549), breast (MCF-7), and cervical (HeLa). Compounds, specifically (**S-6.13**, **S-6.14** and **S-6.15**) showed potent inhibition against all tested cell lines with IC50 values in the range of 0.83-1.81 μM.



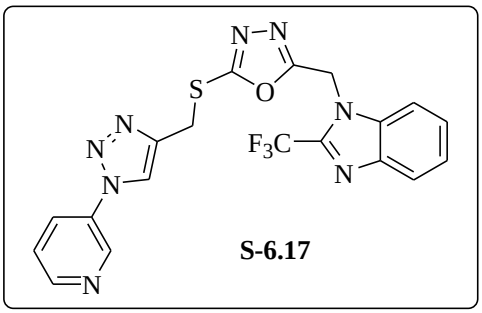
S-6.13 **S-6.14** **S-6.15**

A novel series of 1-(3-chloropyridin-2-yl)-*N*-substituted-5-(trifluoromethyl)-pyrazole carboxamide derivatives were synthesized and characterized by Wu and group members, (2015). All the target compounds were tested *in vitro* antimicrobial activities. The preliminary bioassays indicated that compound (**S-6.16**) exhibited excellent activity against *Xanthomonas oryzae* (94.9 % and 84.9 %) at different concentrations (200 and 100 μg/mL), which was higher than the tested standard Bismerthiazol (94.6 % and 64.0 %), respectively. At the same time, most of the compounds exhibited moderate antifungal activities.



S-6.16

Tantray and co-workers, (2016) developed a series of benzimidazole based 1,3,4-oxadiazole-1,2,3-triazole derivatives for in vitro GSK-3β inhibitory activity. Some of the compounds exhibited sub-micromolar IC50 values and were examined further for antidepressant activity by forced swin test (FST) and tail suspension test (TST) models in Wistar rats. Among all compounds, (**S-6.17**) found to be more active as antidepressant with IC50 value 0.15μM. Molecular docking studies were also performed against GSK-3β to gain an understanding of their binding interactions.



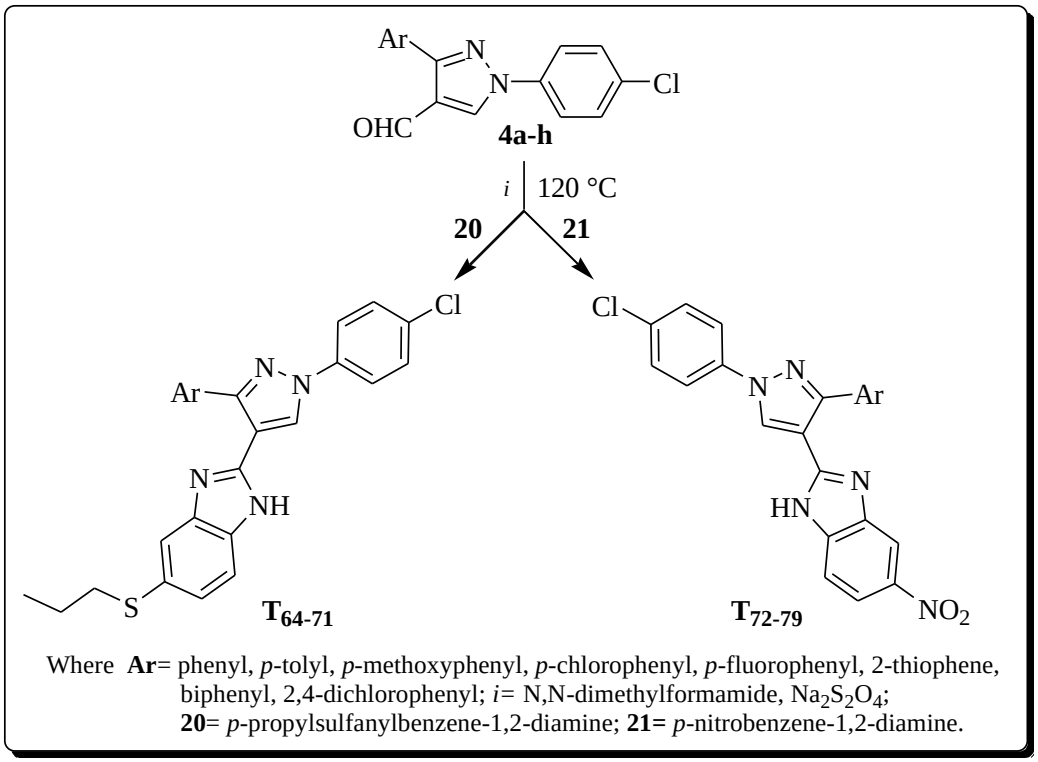
S-6.17

Based on the above considerations, it is clear that, benzimidazole and trifluoromethyl substituted derivatives have wide applications in the medicinal chemistry field. In view of this, it was planned to synthesize pyrazole containing benzimidazole and trifluoromethyl derivatives and to study its antitubercular activities. Antitubercular activity was performed against *Mycobacterium tuberculosis* $H_{37}Rv$ strain and antibacterial activity against *Mycobacterium smegmatis*, *Staphylococcus aureus* (Gram+ve bacteria) and antifungal activity against *Candida albicans* and *Penicillium chrysogenum*. Also, cytotoxicity studies against HeLa and Vero cell lines have been studied for active compounds to identify the toxicity of the active compounds.

6.2. MATERIALS AND METHODS

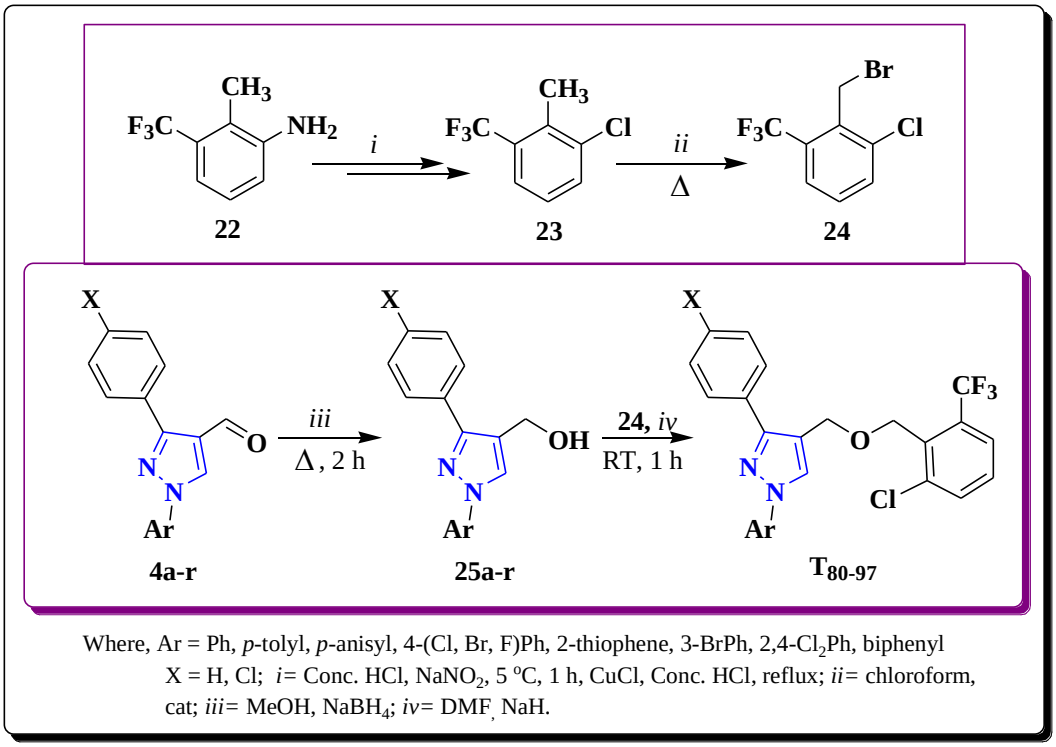
Compounds $\mathbf{T_{64\text{-}71}}$ and $\mathbf{T_{72\text{-}79}}$ were prepared from 1,3-disubstuituted-1*H*-pyrazole-4-carbaldehydes **4a-h** with 4-propylsulfanyl-*o*-phenylenediamine **20** and 4-nitro-*o*-phenylenediamine **21** respectively in presence of sodium dithionite ($Na_2S_2O_4$) in N,N-dimethylformamide (DMF) media at reflux temperature for 3 h. The reaction mass was quenched in purified water and the solid obtained was filtered. The crude product was purified by recrystallized in ethanol to get 2-(1,3-disubstituted-*1H*-

pyrazol-4-yl)-5-substituted-*1H*-benzimidazole derivatives **T_{64-79}**. The synthetic scheme was outlined in **Scheme 6.1**.



Scheme 6.1 Schematic presentation of benzimidazole derivatives **T_{63-79}**

In the other series, 1,3-disubstitutedphenyl-1*H*-pyrazole-4-carbaldehydes **4a-r** was reduced to (1,3-disubstitutedphenyl-1*H*-pyrazol-4-yl)-methanol **25a-r** with sodium borohydride in methanol media. Compound **25a-r** was reacted with 6-trifluoromethyl-2-chloro-benzylbromide **24** in *N*,*N*-dimethylformamide (DMF) media with sodium hydride to gave 4-(2-chloro-6-trifluoromethylbenzyloxymethyl)-1,3-disubstituted-1*H*-pyrazole **T_{80-97}** in reasonable yields and the synthetic scheme was outlined in **Scheme 6.2**. The newly synthesized compounds **T_{63-79}** and **T_{80-97}** were characterized by IR, NMR, mass spectra and C, H, N elemental analysis.



Scheme 6.2 Schematic presentation of trifluoromethyl derivatives **T_{80-97}**

All raw materials and solvents were procured from TCI-India, Sigma-Aldrich and Merck-India. Melting points were determined by the open capillary method and were uncorrected. The FT-IR spectra were recorded on PerkinElmer FT-IR-4000-400 spectrophotometer. ^{1}H-NMR and ^{13}C-NMR were recorded on a Bruker Avance 400/300 spectrometer using Me_4Si as an internal standard. Coupling constants and chemical shift are recorded in units of Hz and ppm, respectively. Applied Biosystems MDS SCIEX-API 4000 spectrometer was used for detection of molecular ion peak. Single crystal XRD for compounds **25c**, **T_{80}**, **T_{83}**, **T_{86}** and **T_{88}** was recorded by Bruker APEX-II diffractometer equipped with dual system CCD detector. Monochromatic Mo-Ka radiation (l ¼ 0.71073 Å) was used for the measurement. Flash EA 1112 series CHN-S analyzer was used to calculate elemental analysis. In-process reaction monitoring was checked by TLC on Merck F_{254} readymade plates. The names of all compounds were mentioned as per ChemDraw Ultra 8.0.

6.3 EXPERIMENTAL

The experimental protocols followed for the synthesis of compounds **T_{64-79}** and **T_{80-97}** were given in the following section.

The synthetic procedure for the synthesis of intermediate compounds **4a-r** has been discussed in **Chapter 2**.

General procedure for the synthesis of 2-[1-(4-Chlorophenyl)-3-phenyl-1*H*-pyrazol-4-yl]-5-propylsulfanyl-1*H*-benzimidazole (T_{64}):

1-(4-Chlorophenyl)-3-phenyl-1*H*-pyrazole-4-carbaldehyde **4a** (2.83 g, 0.01 mol) in DMF (5 ml) was stirred with sodiumdithionite (2.61 g, 0.015 mol) and 4-propylsulfanylbenzene-1,2-diamine **20** (1.82 g, 0.01 mol) at 25 °C for 15 min. The reaction mixture was heated to 120 °C and stirred for 3 h. Completion of the reaction was confirmed by monitoring the TLC [n-hexane : ethyl acetate (8:2)]. Further, the reaction mass was quenched into distilled water and stirred for 15 more minutes. The solid obtained was filtered, washed with purified water. Crude product was recystallized with ethanol. Same procedure has been followed for synthesis of target compounds (**T_{65-79}**).

2-[1-(4-Chlorophenyl)-3-phenyl-1*H*-pyrazol-4-yl]-5-propylsulfanyl-1*H*-benzimidazole (T_{64}). Yield: 82 %; m. p.: 132-133 °C; ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): δ 0.98 (t, 3H, -CH_3, J = 7.0 Hz), 1.58 (m, 2H, -CH_2), 2.95 (t, 2H, -CH_2, J = 7.2 Hz), 7.24 (s, 1H, Ar-H), 7.44 (m, 3H, Ar-H), 7.55 (d, 2H, Ar-H, J = 7.2 Hz), 7.67 (d, 2H, Ar-H, J = 7.8 Hz), 7.87 (d, 2H, Ar-H, J = 7.8 Hz), 8.01 (d, 2H, Ar-H, J = 8.2 Hz), 9.12 (s, 1H, pyrazole-5H); LC-MS: *m/z* = 446.2 (M+1), ANAL. Calcd. for $C_{25}H_{21}ClN_4S$; calcd: C, 67.48; H, 4.76; N, 12.59; found: C, 67.61; H, 4.80; N, 12.65.

2-[1-(4-Chlorophenyl)-3-*p*-tolyl-1*H*-pyrazol-4-yl]-5-propylsulfanyl-1*H*-benzimidazole (T_{65}). Yield: 79 %; m. p.: 138-140 °C; ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): δ 1.00 (t, 3H, CH_3, J = 7.2 Hz), 1.58 (m, CH_2, 2H), 2.31 (s, 3H, CH_3), 2.94 (t, 2H, CH_2, J = 7.2 Hz), 7.20-7.25 (m, 3H, Ar-H), 7.54 (m, 2H, Ar-H), 7.63 (d, 2H, Ar-H, J = 8.8 Hz), 7.71 (d, 2H, Ar-H, J = 8.0 Hz), 7.97 (d, 2H, Ar-H, J = 8.8 Hz), 9.09 (s, 1H, pyrazole-5H); ^{13}C-NMR (75 MHz, DMSO-d_6): δ 151.2, 146.3, 138.4, 138.2, 137.9, 131.5, 131.1, 130.1, 129.5, 129.3, 128.5, 125.1, 120.6, 116.3, 115.8, 112.7, 36.5 (CH_2), 22.5, 21.3, 13.56 (CH_3); LC-MS: *m/z* = 460.1 (M+1), ANAL. Calcd. for $C_{26}H_{23}ClN_4S$; calcd: C, 68.03; H, 5.05; N, 12.21; found: C, 68.06; H, 5.06; N, 12.28.

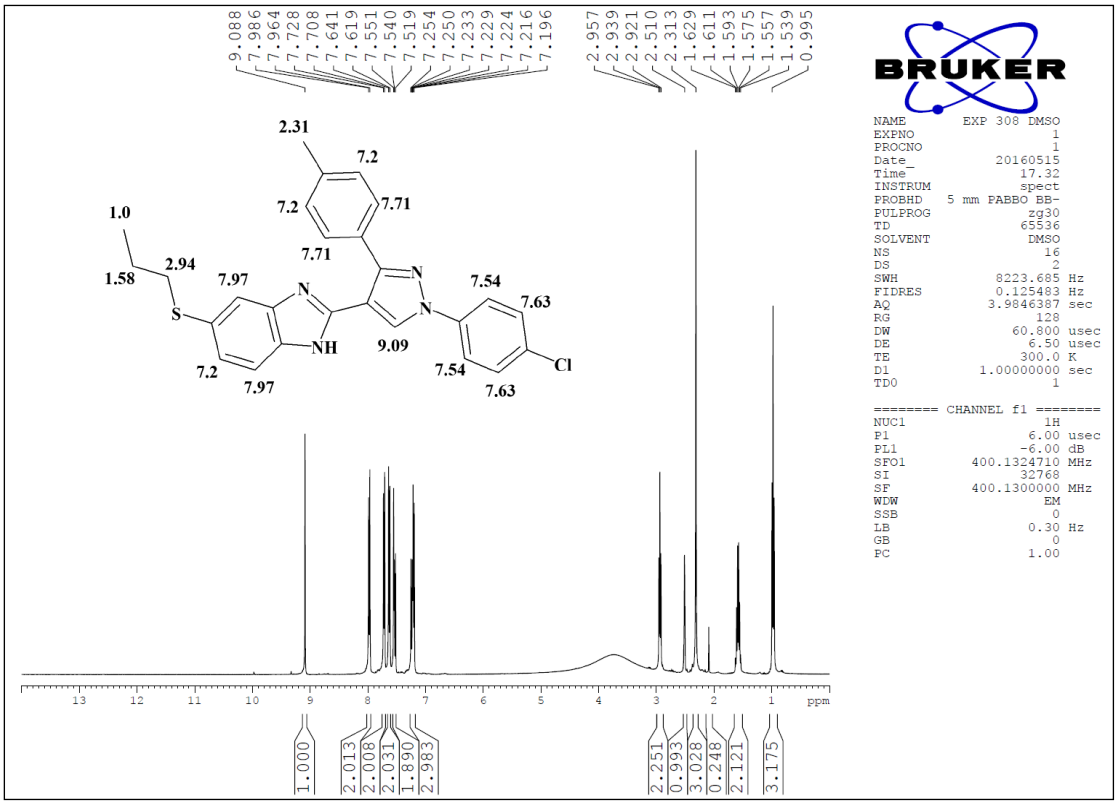


Figure 6.1 ^{1}H-NMR spectrum of compound $\mathbf{T_{65}}$

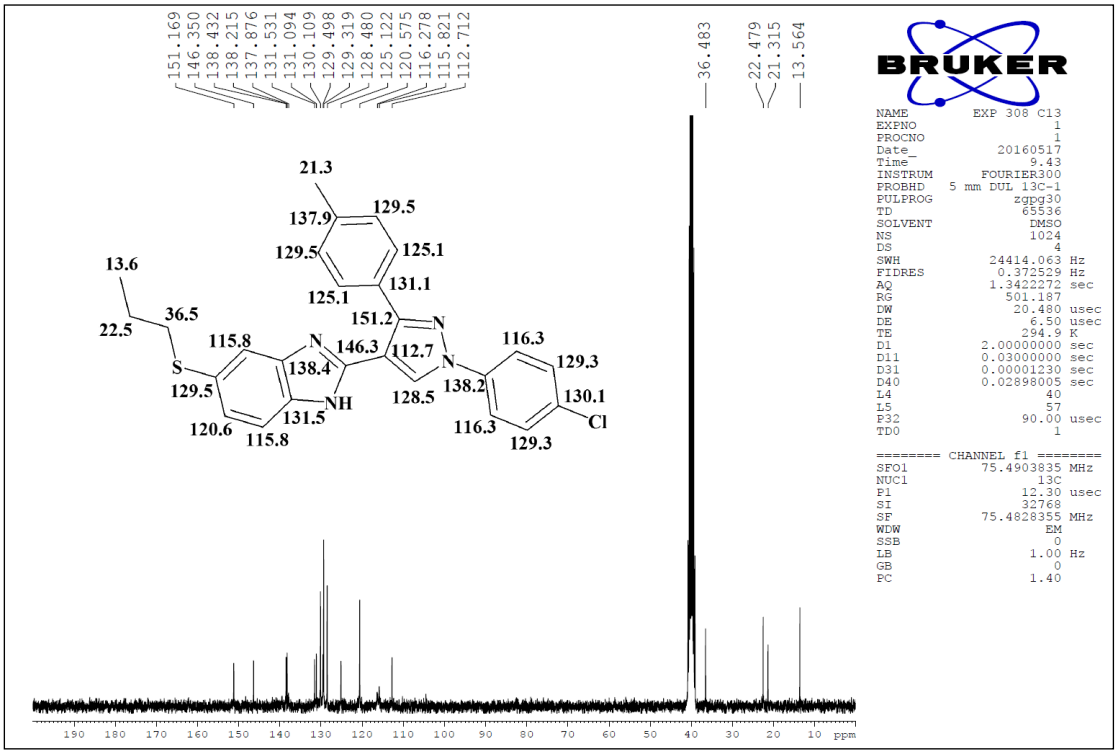


Figure 6.2 ^{13}C-NMR spectrum of compound $\mathbf{T_{65}}$

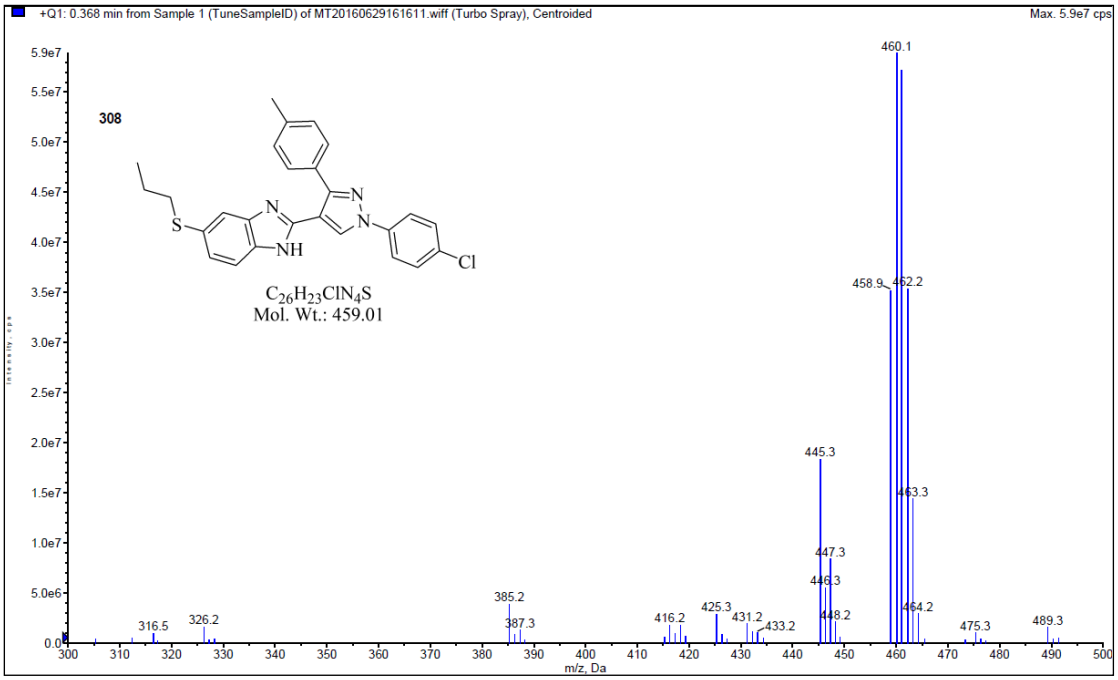


Figure 6.3 Mass spectrum of compound **T_{65}**

2-[1-(4-Chlorophenyl)-3-(4-methoxyphenyl)-1*H*-pyrazol-4-yl]-5-propylsulfanyl-1*H*-benzimidazole (T_{66}). Yield: 87 %; m. p.: 155-156 °C; ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): δ 0.98 (t, 3H, CH_3, J = 7.2 Hz), 1.58 (m, CH_2, 2H), 2.51 (2.93 (t, 2H, CH_2, J = 7.2 Hz), 3.79 (s, 3H, OCH_3), 6.98 (d, 2H, Ar-H, J = 8.4 Hz), 7.07 (d, 1H, Ar-H, J = 8.4 Hz), 7.25 (dd, 1H, Ar-H, J = 1.2 Hz, 8.4 Hz), 7.53-7.56 (m, 2H, Ar-H), 7.64 (dd, 2H, Ar-H, J = 2.0 Hz, 8.8 Hz), 7.90-8.04 (m, 3H, Ar-H), 9.08 (s, 1H, pyrazole-5H); ^{13}C-NMR (75 MHz, DMSO-d_6): δ 160.1, 151.0, 146.3, 138.2, 135.7, 131.5, 131.1, 130.5, 129.9, 129.5, 129.3, 125.2, 124.6, 122.5, 121.2, 120.6, 114.2, 112.2, 55.61 (OCH_3), 36.39 (CH_2), 22.46 (CH_2), 13.57 (CH_3); LC-MS: m/z = 475.2 (M+1), ANAL. Calcd. for $C_{26}H_{23}ClN_4OS$; calcd: C, 65.74; H, 4.88; N, 11.79; found: C, 65.82; H, 4.92; N, 11.86.

2-[1,3-Bis-(4-Chlorophenyl)-1*H*-pyrazol-4-yl]-5-propylsulfanyl-1*H*-benzimidazole (T_{67}). Yield: 90 %; m. p.: 194-196 °C; ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): δ 0.98 (t, 3H, CH_3, J = 7.2 Hz), 1.59 (sextet, 2H, CH_2, J = 7.2 Hz), 2.96 (t, 2H, CH_2, J = 7.2 Hz), 7.27 (dd, 1H, Ar-H, J = 8.4 Hz, 1.6 Hz), 7.51 (d, 2H, Ar-H, J = 8.4 Hz), 7.56 (d, 2H, Ar-H, J = 8.8 Hz), 7.66 (d, 2H, Ar-H, J = 8.8 Hz), 7.91 (d, 2H, Ar-H, J = 8.4 Hz), 7.99 (d, 2H, Ar-H, J = 8.8 Hz), 9.16 (s, 1H, pyrazole-5H); ^{13}C-

NMR (75 MHz, DMSO-d_6): δ 150.0, 145.7, 138.6, 138.1, 137.1, 136.8, 133.9, 131.9, 131.6, 131.1, 130.5, 130.2, 130.0, 128.9, 125.4, 120.8, 115.8, 112.2, 36.28, 22.4, 13.6; LC-MS: *m/z* = 479.2 (M+1), ANAL. Calcd. for $C_{25}H_{20}Cl_2N_4S$; calcd: C, 62.63; H, 4.20; N, 11.69; found: C, 62.70; H, 4.25; N, 11.76.

2-[1-(4-Chlorophenyl)-3-(4-fluorophenyl)-1*H*-pyrazol-4-yl]-5-propylsulfanyl-1*H*-benzimidazole (T_{68}). Yield: 68 %; m. p.: 155-157 °C; ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): δ 0.98 (t, 3H, CH_3, *J* = 7.2 Hz), 1.58 (m, CH_2, 2H), 2.95 (t, 2H, CH_2, *J* = 7.2 Hz), 7.23-7.28 (m, 3H, Ar-H), 7.55 (d, 2H, Ar-H, *J* = 10.0 Hz), 7.64 (d, 2H, Ar-H, *J* = 8.8 Hz), 7.90-7.99 (m, 4H, Ar-H), 9.14 (s, 1H, pyrazole-5H); ^{13}C-NMR (75 MHz, DMSO-d_6): δ 164.5, 161.2, 150.3, 145.9, 138.8, 138.1, 137.1, 131.8, 131.4, 130.9, 130.8, 130.2, 129.9, 128.7, 125.4, 120.7, 115.9, 115.6, 112.1, 36.35 (CH_2), 22.44 (CH_2), 13.56 (CH_3); LC-MS: *m/z* = 463.9 (M+1), ANAL. Calcd. for $C_{25}H_{20}ClFN_4S$; calcd: C, 64.86; H, 4.35; N, 12.10; found: C, 64.96; H, 4.41; N, 12.18.

2-[1-(4-Chlorophenyl)-3-thiophene-2-yl-1*H*-pyrazol-4-yl]-5-propylsulfanyl-1*H*-benzimidazole (T_{69}). Yield: 71 %; m. p.: 129-130 °C; ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): δ 0.99 (t, 3H, CH_3, *J* = 7.2 Hz), 1.59 (sextet, 2H, CH_2, *J* = 7.2 Hz), 2.97 (t, 2H, CH_2, *J* = 7.2 Hz), 7.16 (dd, 1H, Ar-H, *J* = 5.2 Hz, 3.6 Hz), 7.28 (1H, dd, Ar-H, *J* = 8.4 Hz, 1.6 Hz), 7.59-7.63 (m, 3H, Ar-H), 7.68 (dd, 2H, Ar-H, *J* = 6.8 Hz, 2.0 Hz), 7.95 (dd, 2H, Ar-H, *J* = 6.8 Hz, 2.0 Hz), 8.32 (dd, 1H, Ar-H, *J* = 3.2 Hz, 1.2 Hz), 9.15 (s, 1H, pyrazole-5H); ^{13}C-NMR (75 MHz, DMSO-d_6): δ 146.0, 145.9, 138.0, 134.4, 131.8, 131.3, 130.3, 129.8, 128.9, 128.3, 127.4, 125.4, 120.7, 112.0, 36.5, 22.5, 13.6; LC-MS: *m/z* = 451.1 (M+1), ANAL. Calcd. for $C_{23}H_{19}ClN_4S_2$; calcd: C, 61.25; H, 4.25; N, 12.42; found: C, 61.33; H, 4.25; N, 12.45.

2-[3-Biphenyl-4-yl-1-(4-chlorophenyl)-1*H*-pyrazol-4-yl]-5-propylsulfanyl-1*H*-benzimidazole (T_{70}). Yield: 85 %; m. p.: 118-119 °C; ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): δ 0.98 (t, 3H, CH_3, *J* = 7.2 Hz), 1.58 (sextet, 2H, CH_2, *J* = 7.2 Hz), 2.95 (t, 2H, CH_2, *J* = 7.2 Hz), 7.26 (dd, 1H, Ar-H, *J* = 8.4 Hz, 1.6 Hz), 7.39 (t, 1H, Ar-H, *J* = 7.6 Hz), 7.49 (t, 2H, Ar-H, *J* = 7.6 Hz), 7.55-7.58 (m, 2H, Ar-H), 7.66 (d, 2H, Ar-H, *J* = 8.8 Hz), 7.77 (dd, 4H, Ar-H, *J* = 8.8 Hz, 2.0 Hz), 7.96 (d, 2H, Ar-H, *J* = 8.0 Hz), 8.01 (d, 2H, Ar-H, *J* = 8.8 Hz), 9.16 (s, 1H, pyrazole-5H); ^{13}C-NMR (75 MHz,

DMSO-d_6): δ 150.7, 146.2, 140.7, 140.0, 138.2, 131.7, 131.5, 131.4, 130.2, 129.7, 129.1, 128.1, 127.1, 125.3, 120.7, 116.2, 115.8, 112.6, 36.4, 22.5, 13.6; LC-MS: *m/z* = 515.2 (M+1), ANAL. Calcd. for $C_{31}H_{25}ClN_4S$; calcd: C, 71.45; H, 4.84; N, 10.75; found: C, 71.55; H, 4.85; N, 10.81.

2-[1-(4-Chlorophenyl)-3-(2,4-dichlorophenyl)-1*H*-pyrazol-4-yl]-5-propylsulfanyl-1*H*-benzimidazole (T_{71}). Yield: 77 %; m. p.: 104-105 °C; ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): δ 0.98 (t, 3H, CH_3, *J* = 7.2 Hz), 1.59 (m, CH_2, 2H), 2.92 (t, 2H, CH_2, *J* = 7.2 Hz), 7.20 (dd, 1H, ArH, *J* = 2.0 Hz, 8.4 Hz), 7.46-7.49 (m, 3H, ArH), 7.55 (dd, 1H, ArH, *J* = 2.0 Hz, 8.4 Hz), 7.65 (dd, 3H, ArH, *J* = 2.0 Hz, 9.2 Hz), 7.72 (d, 1H, ArH, *J* = 2.0 Hz), 7.96 (d, 1H, ArH, *J* = 9.2 Hz), 9.20 (s, 1H, pyrazole-5H); ^{13}C-NMR (75 MHz, DMSO-d_6): δ 148.6, 145.8, 138.1, 134.8, 134.5, 134.0, 133.9, 131.9, 131.1, 131.0, 130.3, 129.8, 129.7, 129.6, 129.4, 128.8, 127.9, 125.1, 120.8, 114.6, 36.36 (CH_2), 22.44 (CH_2), 13.56 (CH_3); LC-MS: *m/z* = 522.3 (M+1), ANAL. Calcd. for $C_{25}H_{19}Cl_3N_4S$; calcd: C, 58.43; H, 3.73; N, 10.90; found: C, 58.50; H, 3.75; N, 10.96.

2-[1-(4-Chlorophenyl)-3-phenyl-1*H*-pyrazol-4-yl]-5-nitro-1*H*-benzimidazole (T_{72}). Yield: 86 %; m. p.: 155-156 °C; ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): δ 7.28 (s, 1H, Ar-H), 7.41 (m, 3H, Ar-H), 7.48 (d, 2H, Ar-H, *J* = 8.0 Hz), 7.68 (d, 2H, Ar-H, *J* = 7.0 Hz), 7.82 (d, 2H, Ar-H, *J* =7.2 Hz), 8.12 (d, 2H, Ar-H, *J* = 7.8 Hz), 9.16 (s, 1H, pyrazole-5H); ^{13}C-NMR (75 MHz, DMSO-d_6, ppm): δ 151.2, 155.5, 150.2, 144.8, 140.8, 132.9, 131.6, 130.1, 129.3, 129.1, 128.7, 128.2, 127.5, 121.6, 119.0, 113.2, 112.1, 111.6; LC-MS: *m/z* = 415.1 (M-1), ANAL. Calcd. for $C_{22}H_{14}ClN_5O_2$; calcd: C, 63.54; H, 3.39; N, 16.84; found: C, 63.58; H, 4.00; N, 16.89.

2-[1-(4-Chlorophenyl)-3-*p*-tolyl-1*H*-pyrazol-4-yl]-5-nitro-1*H*-benzimidazole (T_{73}). Yield: 86 %; m. p.: 165-167 °C; ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): δ 2.35 (s, 3H, CH_3), 7.25 (d, 2H, Ar-H, *J* = 8.0 Hz), 7.65 (d, 2H, Ar-H, *J* = 8.8 Hz), 7.73 (d, 3H, Ar-H, *J* = 8.0 Hz), 7.99 (d, 2H, Ar-H, *J* = 8.8 Hz), 8.13 (dd, 1H, Ar-H, *J* = 2.0 Hz, 8.8 Hz), 8.45 (s, 1H, Ar-H), 9.18 (s, 1H, pyrazole-5H); ^{13}C-NMR (75 MHz, DMSO-d_6): δ 151.4, 143.0, 139.0, 138.7, 138.2, 137.8, 131.7, 131.5, 130.5, 130.2, 129.5, 129.4, 128.9, 128.7, 120.7, 118.6, 118.3, 112.5, 21.51 (CH_3); LC-MS: *m/z* = 428.0 (M-1),

ANAL. Calcd. for $C_{23}H_{16}ClN_5O_2$; calcd: C, 64.26; H, 3.75; N, 16.29; found: C, 64.33; H, 3.80; N, 16.35.

2-[1-(4-Chlorophenyl)-3-(4-methoxyphenyl)-1*H*-pyrazol-4-yl]-5-nitro-1*H*-benzimidazole (T_{74}). Yield: 88 %; m. p.: 149-151 °C; ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): δ 3.80 (s, 3H, OCH_3), 7.00 (d, 2H, Ar-H, J = 8.8 Hz), 7.65 (d, 2H, Ar-H, J = 8.8 Hz), 7.74 (d, 1H, Ar-H, J = 8.8 Hz), 7.81 (d, 2H, Ar-H, J = 8.8 Hz), 7.98 (d, 2H, Ar-H, J = 8.8 Hz), 8.13 (dd, 1H, Ar-H, J = 2.0 Hz, 8.8 Hz), 8.45 (s, 1H, Ar-H), 9.16 (s, 1H, pyrazole-5H); ^{13}C-NMR (75 MHz, DMSO-d_6): δ 160.2, 151.2, 150.9, 143.1, 143.0, 138.2, 131.7, 131.5, 130.9, 130.2, 130.1, 124.6, 120.8, 120.7, 118.3, 114.2, 113.8, 112.3, 55.65 (OCH_3); LC-MS: m/z = 444.0 (M-1), ANAL. Calcd. for $C_{23}H_{16}ClN_5O_3$; calcd: C, 61.96; H, 3.62; N, 15.71; found: C, 62.02; H, 3.68; N, 15.80. 64.26; H, 3.75; N, 16.29; found: C, 64.33; H, 3.80; N, 16.35.

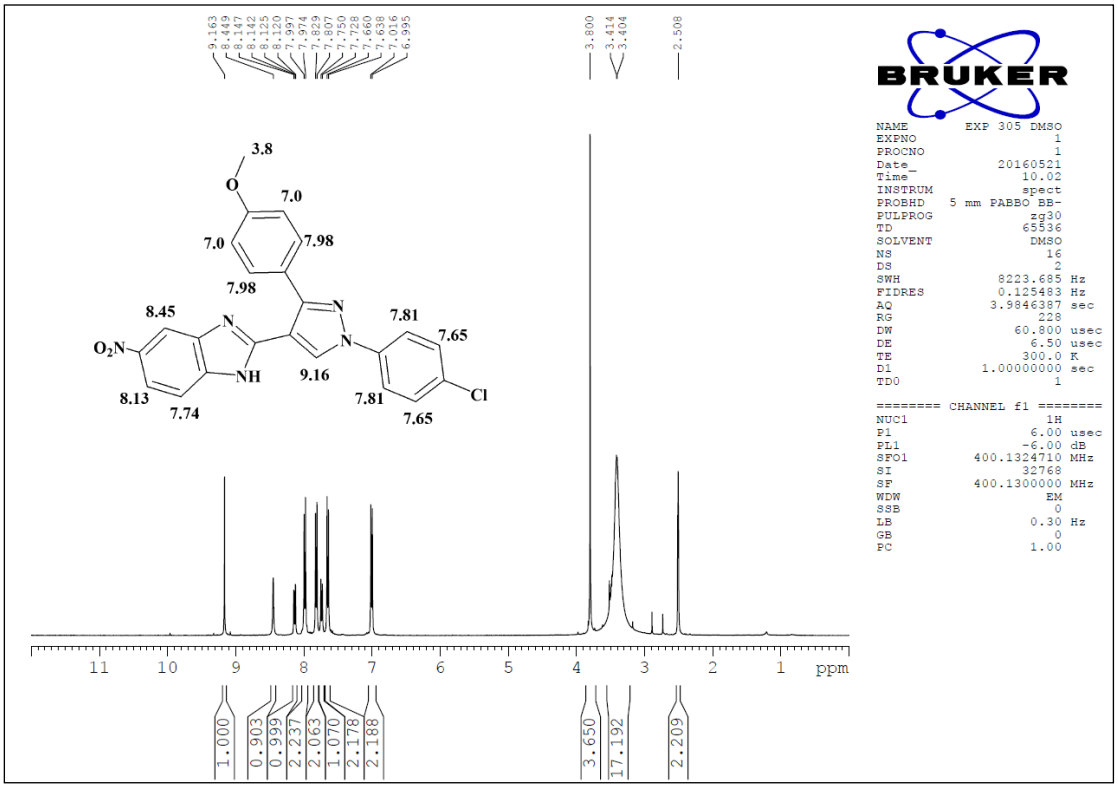


Figure 6.4 ^{1}H-NMR spectrum of compound **T_{74}**

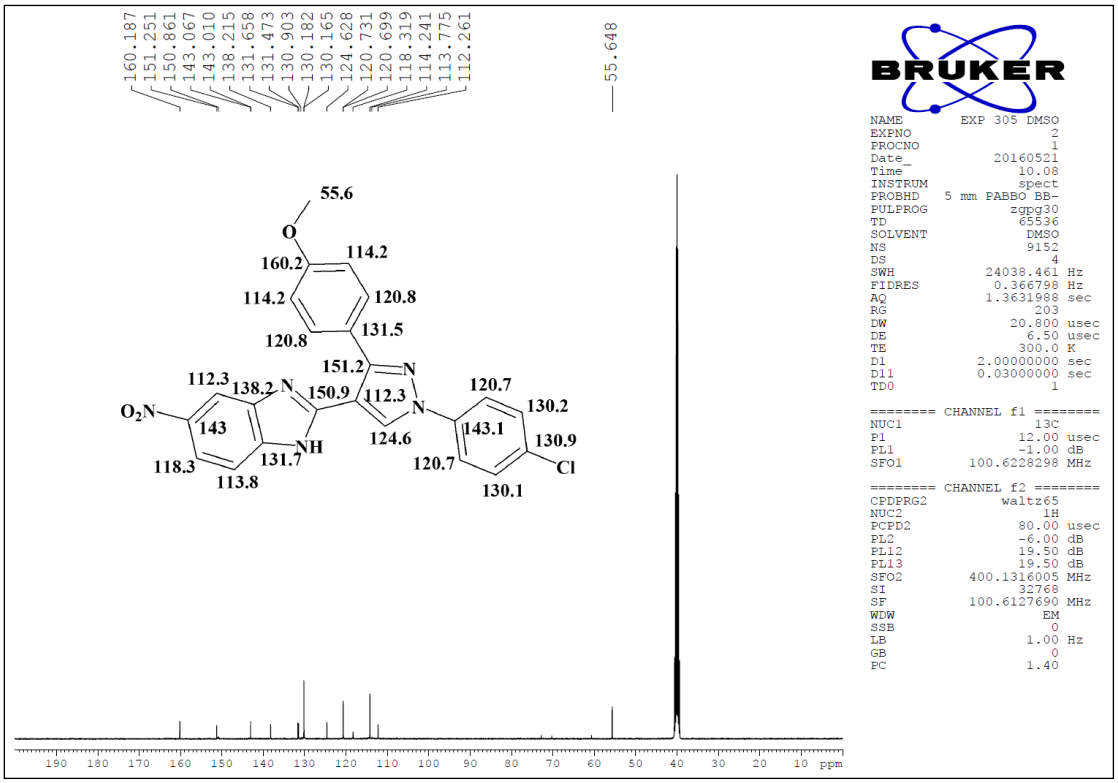


Figure 6.5 ^{13}C-NMR spectrum of compound **T_{74}**

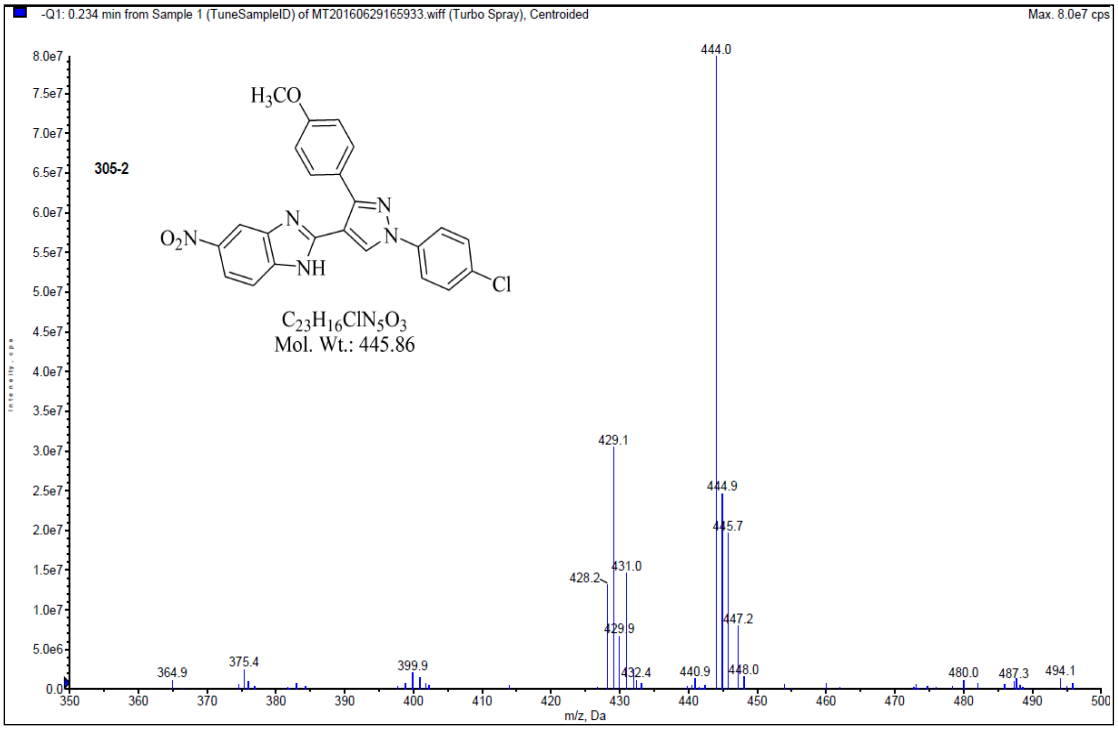


Figure 6.6 Mass spectrum of compound **T_{74}**

2-[1,3-Bis-(4-Chlorophenyl)-1*H*-pyrazol-4-yl]-5-nitro-1*H*-benzimidazole (T_{75}). Yield: 83 %; m. p.: 151-152 °C; ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): ^{1}H-NMR (400 MHz, DMSO-d_6): δ 7.50-7.54 (m, 4H, Ar-H), 7.58 (s, 1H, Ar-H), 7.62-7.92 (m, 5H, Ar-H), 7.95 (m, 1H, Ar-H), 8.50 (m, 1H, Ar-H), 9.22 (s, 1H, pyrazole-5H); ^{13}C-NMR (75 MHz, DMSO-d_6): δ 150.2, 143.0, 142.8, 138.0, 134.1, 134.0, 132.0, 131.7, 131.6, 131.1, 130.8, 130.7, 130.3, 130.2, 128.9, 128.8, 121.0, 112.6; LC-MS: *m/z* = 449.9 (M-1), ANAL. Calcd.for $C_{22}H_{13}Cl_2N_5O_2$; calcd: C, 58.68; H, 2.91; N, 15.55; found: C, 58.74; H, 2.92; N, 15.60.

2-[1-(4-Chlorophenyl)-3-(4-fluorophenyl)-1*H*-pyrazol-4-yl]-5-nitro-1*H*-benzimidazole (T_{76}). Yield: 76 %; m. p.: 153-156 °C; ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): ^{1}H-NMR (400 MHz, DMSO): δ 7.28-7.32 (m, 2H, Ar-H), 7.66-7.68 (m, 2H, Ar-H), 7.74 (s, 1H, Ar-H), 7.94-7.99 (m, 4H, Ar-H), 8.13 (m, 1H, Ar-H), 8.45 (s, 1H, Ar-H), 9.21 (s, 1H, pyrazole-5H); ^{13}C-NMR (75 MHz, DMSO-d_6): δ 164.5, 161.3, 158.1, 150.5, 143.0, 138.1, 131.9, 131.6, 131.2, 131.1, 130.2, 128.8, 128.7, 120.8, 118.67, 115.9, 115.6, 112.5; LC-MS: *m/z* = 432.4 (M-1), ANAL. Calcd.for $C_{22}H_{13}ClFN_5O_2$; calcd: C, 60.91; H, 3.02; N, 16.14; found: C, 60.97; H, 3.05; N, 16.20.

2-[1-(4-Chlorophenyl)-3-thiophene-2-yl-1*H*-pyrazol-4-yl]-5-nitro-1*H*-benzimidazole (T_{77}). Yield: 75 %; m. p.: 160-161 °C; ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): δ 7.18 (dd, 1H, Ar-H, *J* = 4.8 Hz, 3.6 Hz), 7.26 (1H, dd, Ar-H, *J* = 8.4 Hz, 1.6 Hz), 7.51-7.54 (m, 3H, Ar-H), 7.77 (dd, 2H, Ar-H, *J* = 7.8 Hz, 1.8 Hz), 7.87 (dd, 2H, Ar-H, *J* = 7.8 Hz, 2.0 Hz), 8.18 (dd, 1H, Ar-H, *J* = 3.2 Hz, 1.8 Hz), 9.18 (s, 1H, pyrazole-5H); ^{13}C-NMR (75 MHz, DMSO-d_6): δ 148.1, 146.8, 139.6, 135.1, 132.8, 131.0, 130.5, 129.9, 129.6, 128.5, 127.6, 126.2, 121.7, 114.2; LC-MS: *m/z* = 421.0 (M-1), ANAL. Calcd.for $C_{20}H_{12}ClN_5O_2S$; calcd: C, 56.94; H, 2.87; N, 16.60; found: C, 57.02; H, 2.91; N, 16.65.

2-[3-Biphenyl-4-yl-1-(4-chlorophenyl)-1*H*-pyrazol-4-yl]-5-nitro-1*H*-benzimidazole (T_{78}). Yield: 87 %; m. p.: 149-151 °C; ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): δ 7.28 (dd, 1H, Ar-H, *J* = 9.0 Hz, 2.0 Hz), 7.41 (t, 1H, Ar-H, *J* = 7.8 Hz), 7.51 (t, 2H, Ar-H, *J* = 7.8 Hz), 7.54-7.57 (m, 2H, Ar-H), 7.61 (d, 2H, Ar-H, *J* = 8.6

Hz), 7.71 (dd, 4H, Ar-H, *J* = 8.6 Hz, 2.0 Hz), 7.91 (d, 2H, Ar-H, *J* = 8.0 Hz), 8.11 (d, 2H, Ar-H, *J* = 8.8 Hz), 9.18 (s, 1H, pyrazole-5H); LC-MS: *m/z* = 479.9 (M-1), ANAL. Calcd.for $C_{28}H_{18}ClN_5O_2$; calcd: C, 68.36; H, 3.69; N, 14.24; found: C, 68.41; H, 3.71; N, 14.26.

2-[1-(4-Chlorophenyl)-3-(2,4-dichlorophenyl)-1*H*-pyrazol-4-yl]-5-nitro-1*H*-benzimidazole (T_{79}). Yield: 88 %; m. p.: 158-160 °C; ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): δ 7.26 (dd, 1H, ArH, *J* = 2.4 Hz, 8.0 Hz), 7.42-7.46 (m, 3H, ArH), 7.63 (dd, 1H, ArH, *J* = 2.2 Hz, 8.0 Hz), 7.65 (dd, 3H, ArH, *J* = 2.0 Hz, 9.0 Hz), 7.77 (d, 1H, ArH, *J* = 6.8 Hz), 7.91 (d, 1H, ArH, *J* = 8.6 Hz), 9.18 (s, 1H, pyrazole-5H); ^{13}C-NMR (75 MHz, DMSO-d_6): δ 147.2, 143.8, 138.3, 134.7, 134.5, 134.2, 134.0, 131.8, 131.6, 131.1, 130.3, 130.0, 129.8, 129.6, 129.4, 128.5, 127.8, 125.0, 119.2, 111.6; LC-MS: *m/z* = 491.2 (M-1), ANAL. Calcd.for $C_{22}H_{12}Cl_3N_5O_2$; calcd: C, 54.51; H, 2.50; N, 14.45; found: C, 54.62; H, 2.52; N, 14.51.

General procedure for the synthesis of 6-Trifluoromethyl-2-chloro toluene (23)

A mixture of 3-trifluoromethyl-2-methyl aniline **22** (10 g, 0.057 mol) and conc. HCl (13.5 ml) in demineralized (DM) water (20 ml) was stirred at -5 °C. The pre-prepared sodium nitrite solution (4.8 g in 8 ml DM water) was added for 90 min at -5 °C and stirred for 1 h. The reaction mass was filtered and was cooled to -5 °C. The chilled mass was then quenched into a mixture of cuprous chloride (4.6 g, 0.046 mol), conc. HCl (14 ml) at reflux temperature. The progress of the reaction was checked by TLC and the mass was cooled to ambient temperature. Further, the compound was extracted with dichloromethane (3 x 10 ml) and washed with 10 % sodium hydroxide solution. The organic layer was separated and dried over anhydrous sodium sulfate. The excess solvent was distilled off and the product was purified by fractional distillation (Vapor temp.: 38-40 °C at 10 mbar). The product was colorless liquid. Yield: 79 %.

6-Trifluoromethyl-2-chloro toluene (23). ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): δ 2.40 (s, 3H, -CH_3), 7.38-7.42 (t, 1H, Ar-H, *J* = 7.9 Hz), 7.63-7.65 (d, 1H, Ar-H, *J* = 7.6 Hz), 7.71-7.73 (d, 1H, Ar-H, *J* = 7.9 Hz); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 136.07, 134.35, 133.71, 130.21, 129.92, 129.63, 129.34, 128.36, 128.21, 125.64, 125.16, 125.10, 122.92, 16.24; LC-MS: *m/z* = 194 (M+1).

General procedure for the synthesis of 6-trifluoromethyl-2-chlorobenzylbromide (24)

A mixture of 6-trifluoromethyl-2-chloro toluene **23** (10 g, 0.051 mol), N-bromosuccinimide (11 g, 0.062 mol) and the catalytic amount of m-chloroperbenzoic acid (5 % w/w) in chloroform was stirred at room temperature. It was exposed with 160-watt mercury lamp throughout the reaction and the reaction mass was heated to reflux temperature for 2 h. The progress of the reaction was monitored by TLC [n-hexane : ethylacetate (4:1)]. The solid mass was cooled to 0 °C and the unwanted solid was removed by filtration. The filtrate solution was washed with sodium metabisulfite solution (14 % w/w in 20 ml DM water). The organic layer was separated and washed with DM water (20 ml). The organic layer was collected and dried over anhydrous sodium sulfate. The solvent was distilled under vacuum and the crude product was distilled by fractional distillation. The product obtained was colorless liquid (Vapor temp.: 80-82 °C at 10 mbar). Yield: 76 %.

6-Trifluoromethyl-2-chloro benzylbromide (24). ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 4.73 (s, 2H, -CH_2), 7.37-7.41 (t, 1H, Ar-H, J = 8.0 Hz), 7.60-7.64 (t, 2H, Ar-H, J = 7.6 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 137.53, 133.91, 130.71, 129.56, 125.07, 125.01, 124.85, 122.12, 25.23; DEPT-135 (100 MHz, $CDCl_3$): δ 133.92, 129.57, 125.08, 25.22 (-ve peak); LC-MS: m/z = 274.2 (M+1).

General procedure for the synthesis of (1,3-disubstituted-1*H*-pyrazol-4-yl)methanol (25a-r):

1,3-Diphenyl-1*H*-pyrazole-4-carbaldehyde **4a** (2.48 g, 0.01 mol) in methanol (20 ml) was stirred at ambient temperature. To this, added freshly prepared sodium borohydride (0.42 g, 0.011 mol.) in DM water (4 ml) in four equal lots and the progress of the reaction was monitored by TLC [n-hexane : ethylacetate (8:2)]. The reaction mixture was distilled off and added DM water (10 ml) and extracted with DCM (2 x 20 ml). The organic layer was washed with DM water and dried over anhydrous sodium sulfate. The organic layer was evaporated to get (1,3-diphenyl-1*H*-pyrazol-4-yl)methanol **25a** as an off-white crystalline solid. The solid compound **25a** was converted to the next step without any purification. The same procedure has been followed for the synthesis of compounds **25b-r**.

(1,3-Diphenyl-1*H*-pyrazol-4-yl)methanol (25a): ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 4.60-4.62 (d, 2H, J = 5.6 Hz, -CH_2), 5.05-5.07 (t, 1H, J = 5.7 Hz, -OH), 7.28-7.30 (d, 2H, J = 7.8 Hz, Ar-H), 7.32-7.34 (t, 2H, J = 7.4 Hz, Ar-H), 7.45-7.51 (t, 4H, J = 8.1 Hz, Ar-H), 7.71-7.72 (d, 2H, J = 8.0 Hz, Ar-H), 7.87 (s, 1H, pyrazole-5H), ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): δ 157.3, 140.7, 138.3, 135.2, 129.2, 128.8, 128.5, 127.0, 126.9, 119.2, 117.2, 55.6; MS: *m/z* = 250.9 (M+1).

General procedure for the synthesis of 1,3-disubstituted-4-(2-chloro-6-trifluoromethylbenzyloxymethyl)-1*H*-pyrazole (T_{80-97}):

(1,3-Diphenyl-1*H*-pyrazol-4-yl)methanol **25a** (2.5 g, 0.01 mol) in dry DMF (5 ml) was stirred at 25 °C for 15 min. and charged with 60 % sodium hydride (0.29 g, 0.012 mol, 100 % basis) and 6-trifluoromethyl-2-chlorobenzylbromide **24** (2.74 g, 0.01 mol) at ambient temperature and stirred for 1 h. The reaction mass was turned to a viscous mass of wine red color. The completion of the reaction was monitored by TLC [n-hexane : ethylacetate (8:2)]. The unwanted solid was filtered off and the pale red colored filtrate solution was collected. Further the filtrate mass was quenched into 50 ml of distilled water and stirred for 15 min. The solid obtained was filtered, washed with distilled water and dried. The obtained product was recrystallized from DMF/methanol mixture to get pure 4-(2-Chloro-6-trifluoromethylbenzyloxymethyl)-1,3-diphenyl-1*H*-pyrazole (**T_{80}**). The same procedure has been followed for the preparation of target compounds (**T_{81-97}**).

4-(2-Chloro-6-trifluoromethylbenzyloxymethyl)-1,3-diphenyl-1*H*-pyrazole (**T_{80}**). Yield: 76 %; FT-IR (KBr, cm^{-1}): 1657 (C=N str), 1501 (C=C str), 1312 (C-N str), 1121 (C-F str), 752 (C-Cl str); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 4.70 (s, 2H, -CH_2), 4.84 (s, 2H, -CH_2), 7.29-7.45 (m, 7H, ArH), 7.62-7.88 (*b*, 6H, ArH), 8.01 (s, 1H, pyrazole-5H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 152.51, 140.01, 138.20, 133.66, 132.84, 131.86, 131.56, 129.49, 129.41, 128.88, 128.55, 128.08, 128.06, 126.46, 125.06, 124.76, 124.71, 119.15, 117.35, 65.50, 64.10; LC-MS: *m/z* = 443.8 (M+1); calculated for $C_{24}H_{18}ClF_3N_2O$; C, 65.09; H, 4.10; N, 6.33; Found: C, 66.02; H, 4.11; N, 6.35.

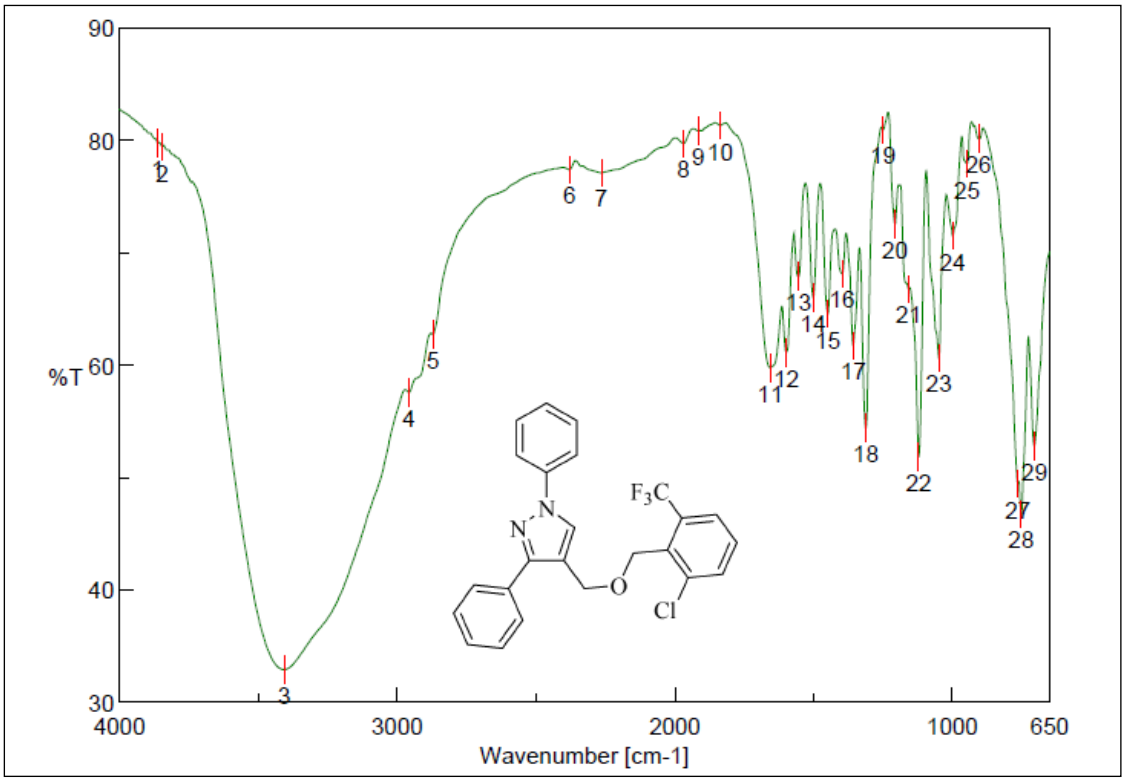


Figure 6.7 IR spectrum of compound **T_{80}**

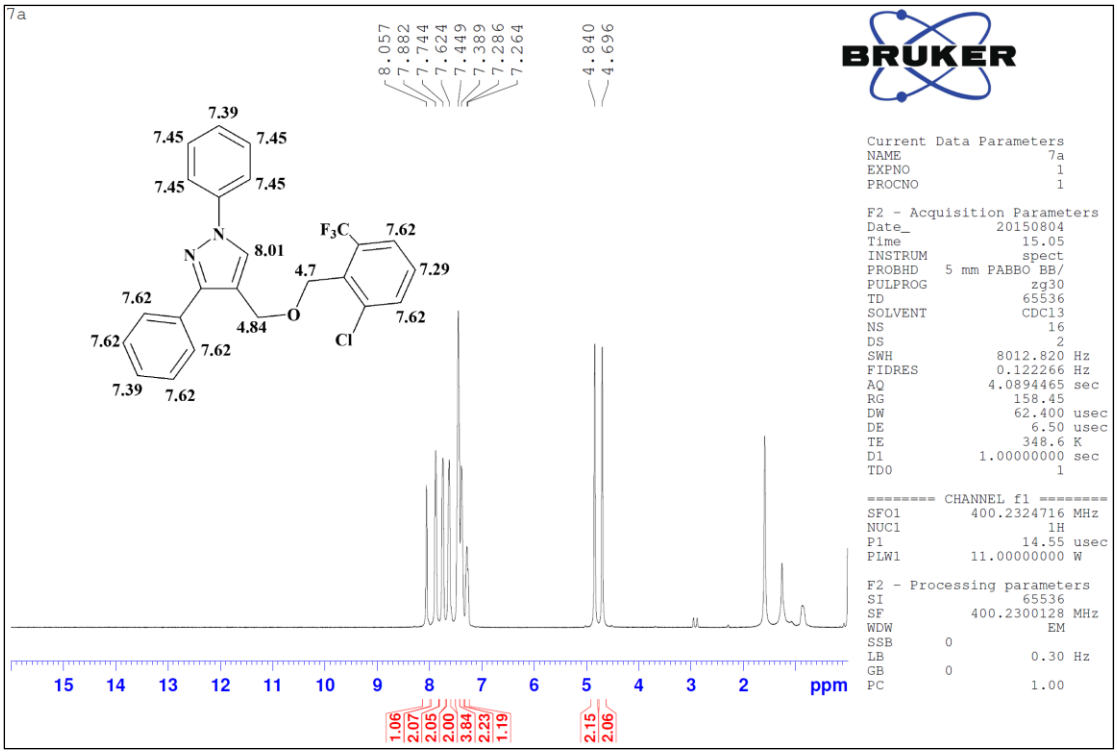


Figure 6.8 ^{1}H-NMR spectrum of compound **T_{80}**

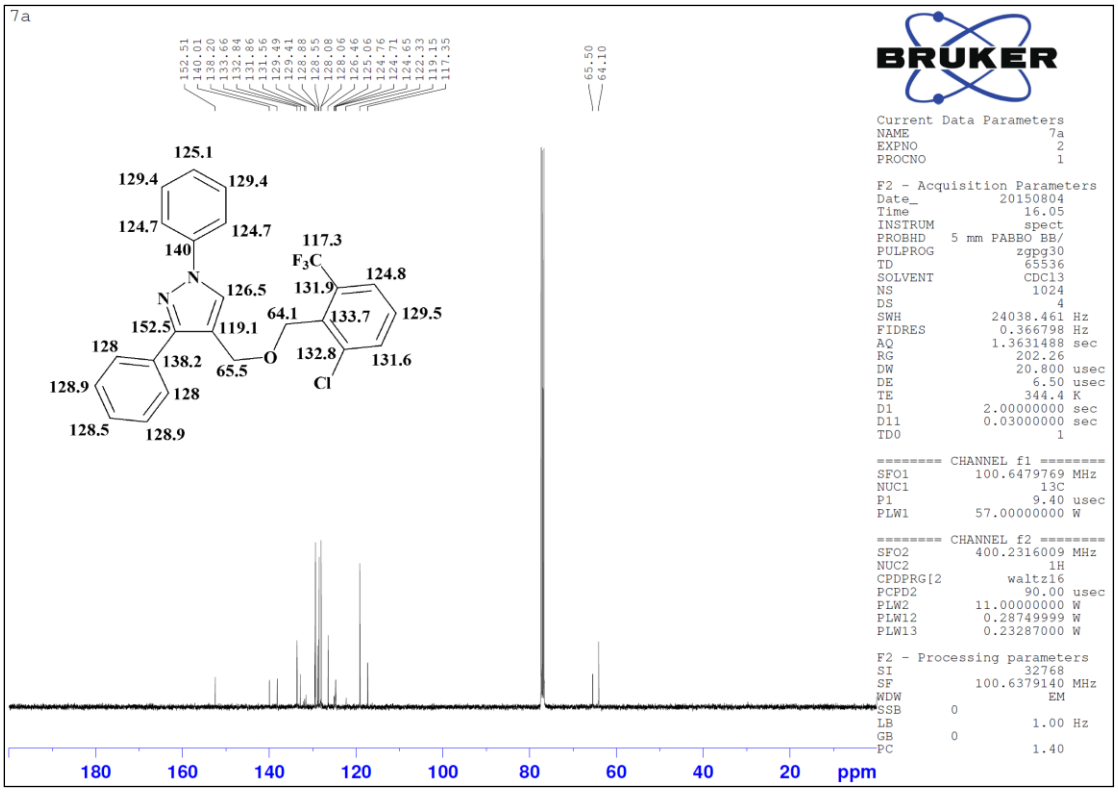


Figure 6.9 ^{13}C-NMR spectrum of compound **T_{80}**

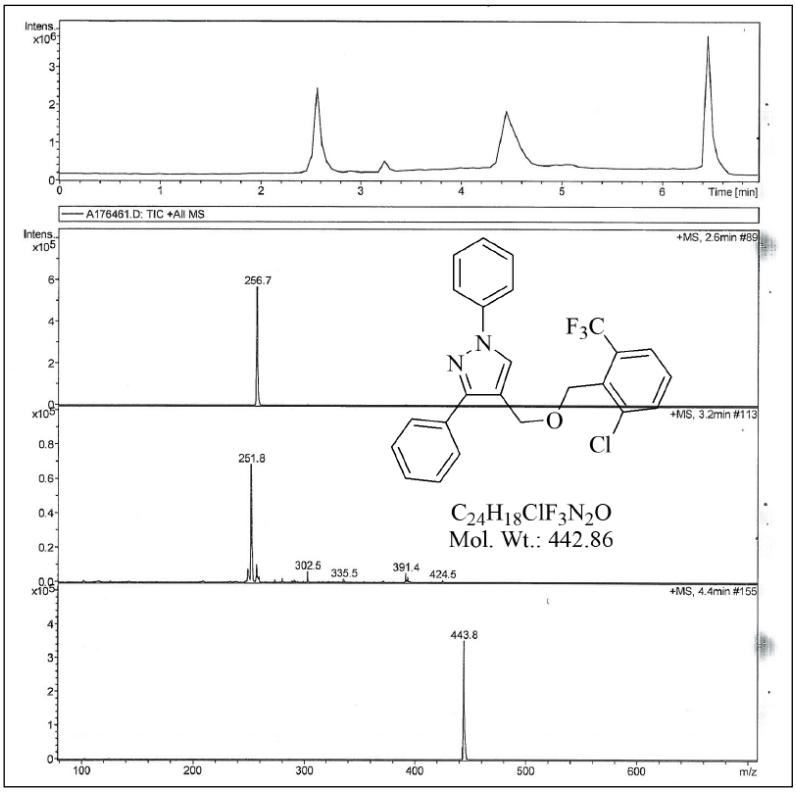


Figure 6.10 Mass spectrum of compound **T_{80}**

1-(4-Chlorophenyl)-4-(2-chloro-6-trifluoromethylbenzyloxymethyl)-3-phenyl-1*H*-pyrazole (T_{81}). Yield: 82 %; FT-IR (KBr, cm^{-1}): 1652 (C=N str), 1499 (C=C str), 1309 (C-N str), 1130 (C-F str), 741 (C-Clstr); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 4.71 (s, 2H, -CH_2), 4.87 (s, 2H, -CH_2), 7.40-7.50 (m, 6H, ArH), 7.65-7.67 (dd, 2H, *J* = 7.7 Hz, ArH), 7.71-7.73 (d, 2H, *J* = 8.7 Hz, ArH), 7.88-7.89 (d, 2H, *J* = 7.3 Hz, ArH), 8.05 (s, 1H, pyrazole-5H); LC-MS: *m/z* = 478.0 (M+1); calculated for $C_{24}H_{17}Cl_2F_3N_2O$; C, 60.39; H, 3.59;N, 5.87; Found: C, 60.42; H, 3.60; N, 5.89.

4-(2-Chloro-6-trifluoromethylbenzyloxymethyl)-1-phenyl-3-*p*-tolyl-1*H*-pyrazole (T_{82}). Yield: 82 %; FT-IR (KBr, cm^{-1}): 1653 (C=N str), 1504 (C=C str), 1307 (C-N str), 1124 (C-F str), 752 (C-Cl str); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 2.43 (s, 3H, -CH_3), 4.71 (s, 2H, -CH_2), 4.86 (s, 2H, -CH_2), 7.27-7.32 (m, 3H, ArH), 7.39-7.49 (m, 3H, ArH), 7.64-7.67 (dd, 2H, *J* = 7.9 Hz, ArH), 7.76-7.78 (d, 2H, *J* =8.2 Hz, ArH), 7.79-7.81 (d, 2H, *J* = 8.0 Hz, ArH), 8.06 (s, 1H, pyrazole-5H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 152.55, 140.06, 138.21, 137.85, 133.65, 129.99, 129.47, 129.39, 129.26, 128.80, 127.97, 126.36, 124.81, 124.76, 124.70, 124.64, 119.12, 117.21, 65.46, 64.16, 21.33; LC-MS: *m/z* = 457.5 (M+1); calculated for $C_{25}H_{20}ClF_3N_2O$; C, 65.72; H, 4.41; N, 6.13; Found: C, 65.74; H, 4.44; N, 6.16.

1-(4-Chlorophenyl)-4-(2-chloro-6-trifluoromethylbenzyloxymethyl)-3-*p*-tolyl-1*H*-pyrazole (T_{83}). Yield: 86 %; FT-IR (KBr, cm^{-1}): 1648 (C=N str), 1498 (C=C str), 1316 (C-N str), 1124 (C-F str), 745 (C-Cl str); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 2.43 (s, 3H, -CH_3), 4.70 (s, 2H, -CH_2), 4.86 (s, 2H, -CH_2), 7.28-7.29 (d, 2H, *J* = 3.8 Hz, ArH), 7.39-7.45 (m, 3H, ArH), 7.64-7.67 (dd, 2H, *J* = 7.6 Hz, 8.2 Hz, ArH), 7.69-7.73 (m, 2H, ArH), 7.76-7.78 (d, 2H, *J* = 8.0 Hz, ArH), 8.03 (s, 1H, pyrazole-5H); LC-MS: *m/z* = 491.1 (M+1); calculated for $C_{25}H_{19}Cl_2F_3N_2O$; C, 61.11; H, 3.90; N, 5.70; Found: C, 61.15; H, 3.91; N, 5.71.

4-(2-Chloro-6-trifluoromethylbenzyloxymethyl)-3-(4-methoxyphenyl)-1-phenyl-1*H*-pyrazole (T_{84}). Yield: 78 %; FT-IR (KBr, cm^{-1}): 1507 (C=C str), 1311 (C-N str), 1119 (C-F str), 746 (C-Cl str); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 3.86 (s, 3H, -OCH_3), 4.67 (s, 2H, -CH_2), 4.83 (s, 2H, -CH_2), 6.97-6.99 (d, 2H, *J* = 8.0 Hz, ArH), 7.26-7.29 (t, 1H, *J* = 6.8 Hz, ArH), 7.37-7.40 (t, 1H, *J* = 7.8 Hz, ArH), 7.62-7.64 (d,

2H, *J* = 7.6 Hz, ArH), 7.73-7.75 (d, 2H, *J* =7.6 Hz, ArH), 7.81-7.83 (d, 2H, *J* = 8.0 Hz, ArH), 8.03 (s, 1H, pyrazole-5H); LC-MS: *m/z* = 473.2 (M+1); calculated for $C_{25}H_{20}ClF_3N_2O_2$; C, 63.50; H, 4.26; N, 5.92; Found: C, 63.52; H, 4.28; N, 5.94.

1-(4-Chlorophenyl)-4-(2-chloro-6-trifluoromethylbenzyloxymethyl)-3-(4-methoxyphenyl)-1*H*-pyrazole (T_{85}). Yield: 75 %; FT-IR (KBr, cm^{-1}): 1655 (C=N str), 1500 (C=C str), 1308 (C-N str), 1131 (C-F str), 745 (C-Cl str); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 3.88 (s, 3H, $-OCH_3$), 4.68 (s, 2H, $-CH_2$), 4.84 (s, 2H, $-CH_2$), 6.98-7.00 (d, 2H, *J* = 8.4 Hz, ArH), 7.38-7.43 (m, 3H, ArH), 7.63-7.66 (dd, 2H, *J* = 7.6 Hz, 8.0 Hz, ArH), 7.68-7.70 (d, 2H, *J* =8.4 Hz, ArH), 7.80-7.82 (d, 2H, *J* = 8.8 Hz, ArH), 8.01 (s, 1H, pyrazole-5H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 159.74, 152.63, 138.55, 138.16, 133.65, 133.59, 131.68, 129.51, 129.46, 129.32, 128.61, 125.23, 124.77, 124.71, 120.06, 117.39, 114.01, 65.50, 64.06, 55.32; LC-MS: *m/z* = 507.7 (M+1); calculated for $C_{25}H_{19}Cl_2F_3N_2O$; C, 59.19; H, 3.77; N, 5.52; Found: C, 59.23; H, 3.78; N, 5.54.

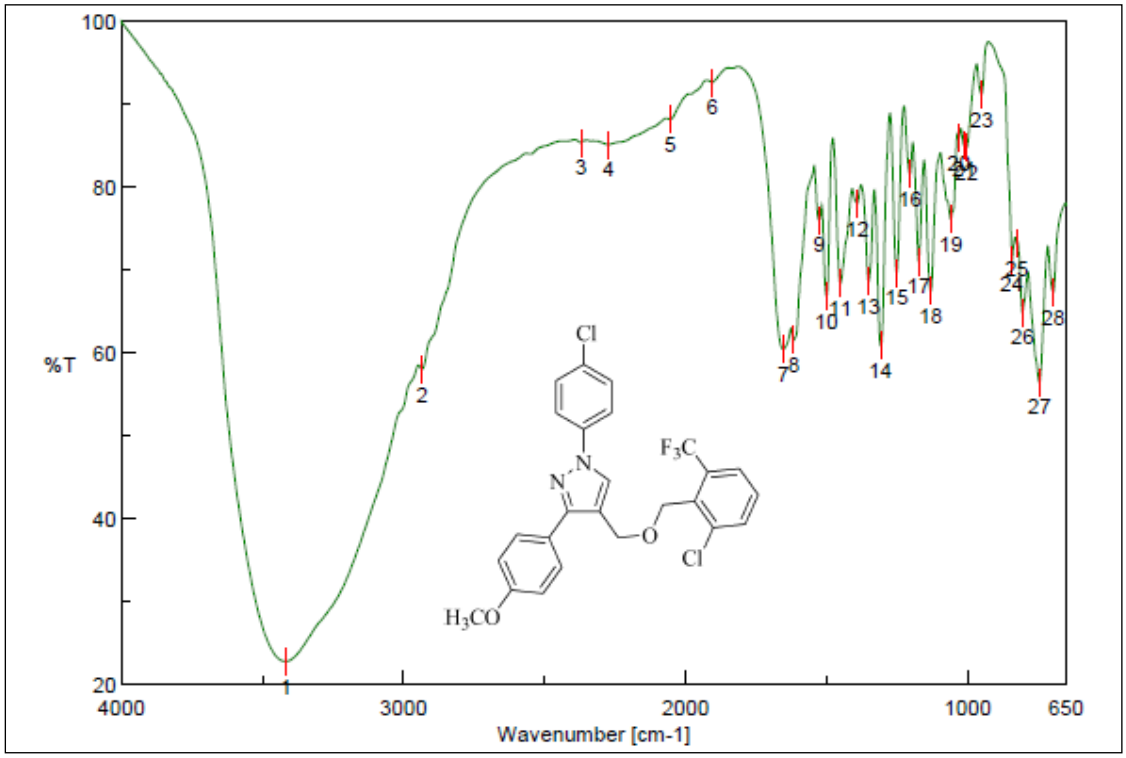


Figure 6.11 IR spectrum of compound **T_{85}**

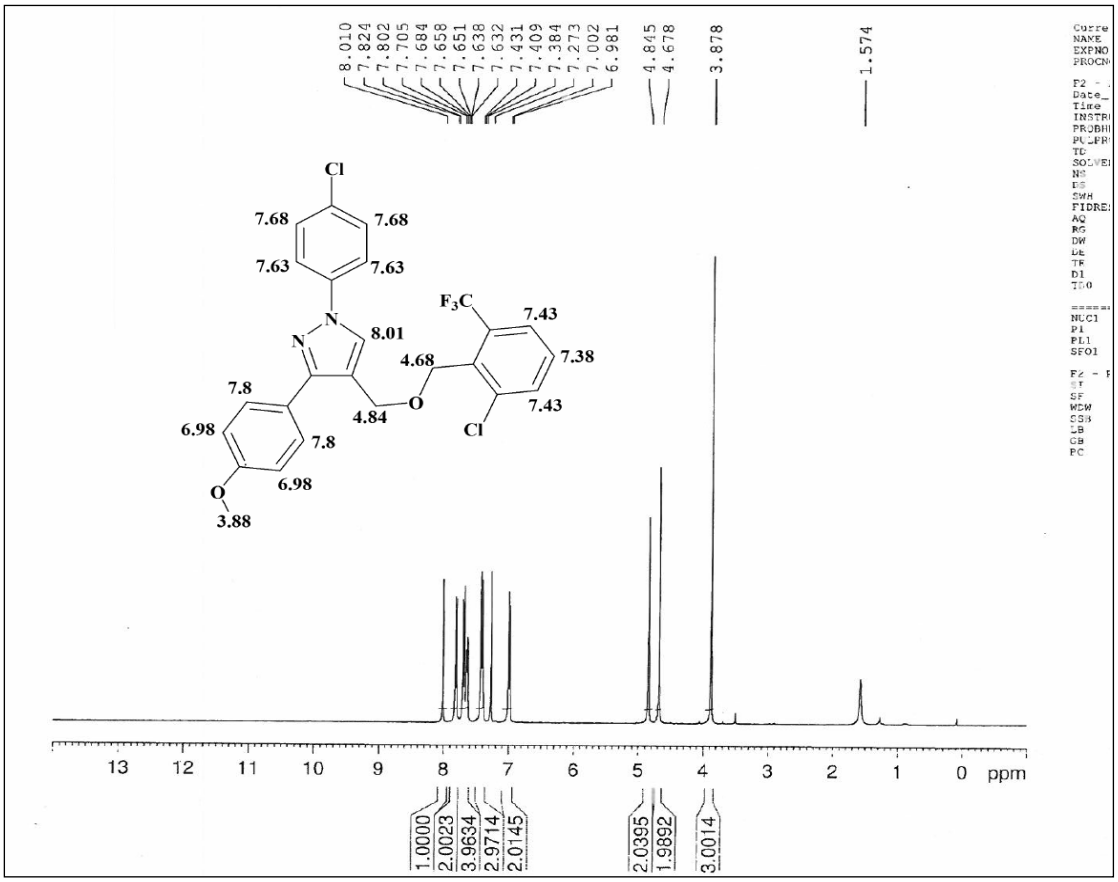


Figure 6.12 ^{1}H-NMR spectrum of compound **T_{85}**

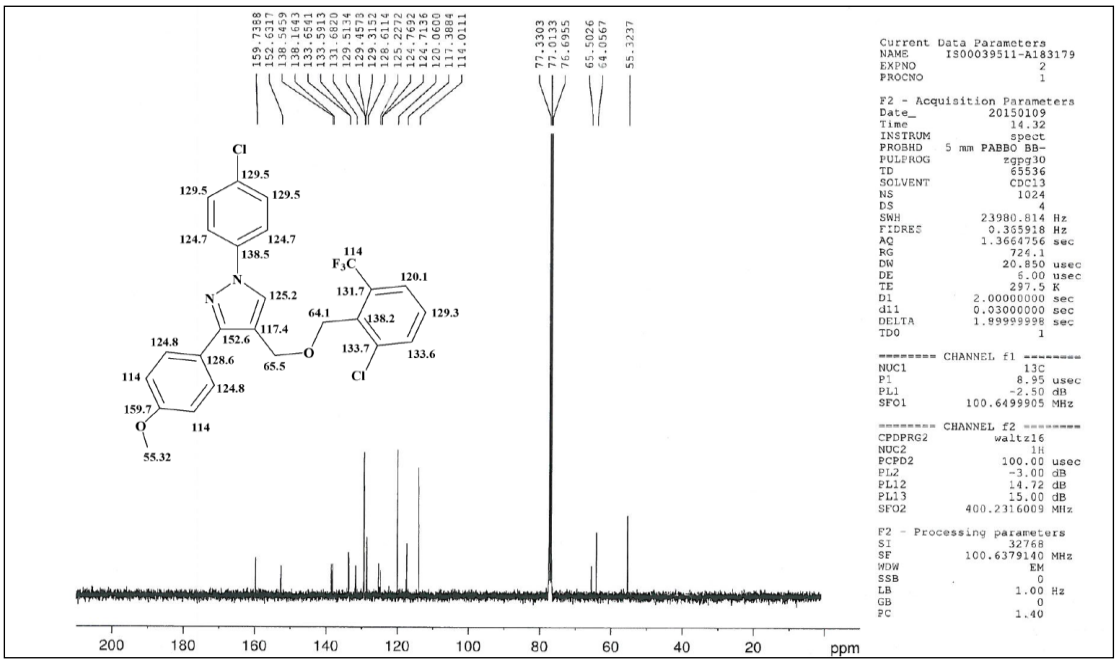


Figure 6.13 ^{13}C-NMR spectrum of compound **T_{85}**

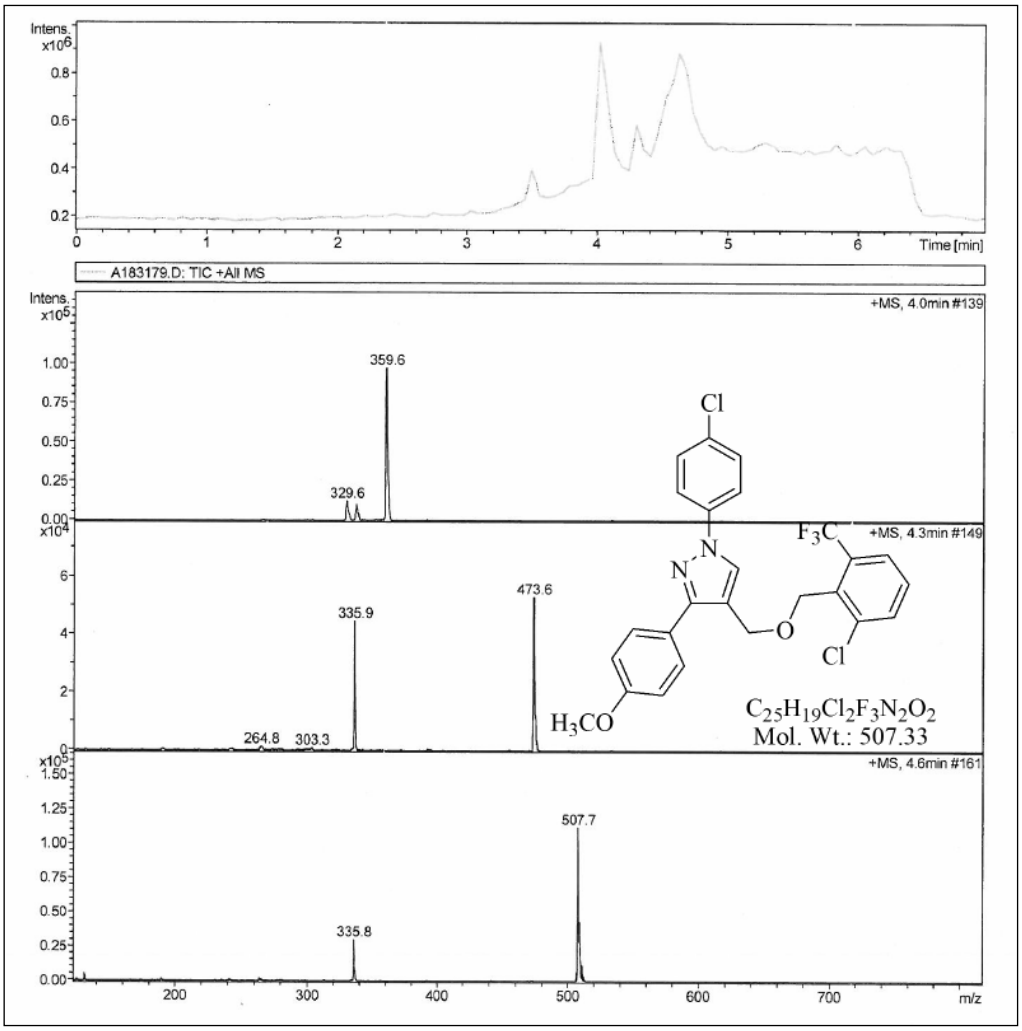


Figure 6.14 Mass spectrum of compound **T_{85}**

3-(4-Chlorophenyl)-4-(2-chloro-6-trifluoromethylbenzyloxymethyl)-1-phenyl-1*H*-pyrazole (T_{86}). Yield: 88 %; FT-IR (KBr, cm^{-1}): 1653 (C=N str), 1500 (C=C str), 1310 (C-N str), 1131 (C-F str), 746 (C-Cl str); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 4.68 (s, 2H, -CH_2), 4.85 (s, 2H, -CH_2), 7.29-7.33 (t, 1H, *J* = 7.4 Hz, ArH), 7.39-7.45 (m, 3H, ArH), 7.45-7.49 (t, 2H, *J* = 8.0 Hz, ArH), 7.64-7.66 (dd, 2H, *J* =8.0 Hz, 8.0 Hz, ArH), 7.74-7.76 (d, 2H, *J* = 7.6 Hz, ArH), 7.86-7.87 (d, 2H, *J* = 6.8 Hz, ArH), 8.06 (s, 1H, pyrazole-5H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 151.47, 139.85, 138.14, 134.02, 133.68, 133.49, 131.52, 131.30, 129.57, 129.45, 129.31, 129.14, 128.72, 126.63, 124.80, 124.74, 119.16, 117.19, 65.47, 63.86; LC-MS: *m/z* = 477.7 (M+1); calculated for $C_{24}H_{17}Cl_2F_3N_2O$; C, 60.39; H, 3.59; N, 5.87; Found: C, 60.40; H, 3.60; N, 5.87.

1,3-Bis-(4-chlorophenyl)-4-(2-chloro-6-trifluoromethylbenzyloxymethyl)-1*H*-pyrazole (T_{87}). Yield: 92 %; FT-IR (KBr, cm^{-1}): 1653 (C=N str), 1498 (C=C str), 1311 (C-N str), 1119 (C-F str), 743 (C-Cl str); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 4.67 (s, 2H, -CH_2), 4.85 (s, 2H, -CH_2), 7.43 (m, 5H, ArH), 7.66-7.70 (m, 4H, ArH), 7.83-7.85 (d, 2H, *J* = 6.0 Hz, ArH), 8.03 (s, 1H, pyrazole-5H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 151.73, 138.38, 138.13, 134.21, 133.70, 133.41, 132.08, 131.05, 129.63, 129.55, 129.29, 128.97, 128.78, 124.83, 120.20, 117.64, 65.54, 63.80; LC-MS: *m/z* = 511.7 (M+1); calculated for $C_{24}H_{16}Cl_3F_3N_2O$; C, 56.33; H, 3.15; N, 5.47; Found: C, 56.34; H, 3.16; N, 5.48.

4-(2-Chloro-6-trifluoromethylbenzyloxymethyl)-3-(4-fluorophenyl)-1-phenyl-1*H*-pyrazole (T_{88}). Yield: 78 %; FT-IR (KBr, cm^{-1}): 1641 (C=N str), 1504 (C=C str), 1315 (C-N str), 1125 (C-F str), 757 (C-Cl str); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 4.69 (s, 2H, -CH_2), 4.86 (s, 2H, -CH_2), 7.13-7.17 (t, 2H, *J* = 8.7 Hz, ArH), 7.30-7.33 (t, 1H, *J* = 7.4 Hz, ArH), 7.35-7.50 (m, 3H, ArH), 7.60-7.66 (dd, 2H, *J* = 7.6 Hz, ArH), 7.75-7.77 (d, 2H, *J* =8.4 Hz, ArH), 7.88-7.90 (d, 1H, *J* = 5.6 Hz, ArH), 7.91-7.91 (d, 1H, *J* = 3.2 Hz, ArH), 8.07 (s, 1H, pyrazole-5H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 164.11, 161.65, 151.75, 139.91, 138.15, 133.67, 133.55, 129.86, 129.78, 129.55, 129.43, 129.01, 128.97, 128.93, 126.54, 124.79, 124.74, 119.12, 117.05, 115.56, 115.34, 65.45, 63.92; LC-MS: *m/z* = 461.1 (M+1); calculated for $C_{24}H_{17}ClF_4N_2O$; C, 62.55; H, 3.72; N, 6.08; Found: C, 62.55; H, 3.73; N, 6.09.

1-(4-Chlorophenyl)-4-(2-chloro-6-trifluoromethylbenzyloxymethyl)-3-(4-fluorophenyl)-1*H*-pyrazole (T_{89}). Yield: 80 %; FT-IR (KBr, cm^{-1}): 1601 (C=N str), 1502 (C=C str), 1313 (C-N str), 1113 (C-F str), 746 (C-Cl str); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 4.67 (s, 2H, -CH_2), 4.85 (s, 2H, -CH_2), 7.13-7.17 (t, 2H, *J* = 8.8 Hz, ArH), 7.42-7.45 (m, 3H, ArH), 7.65-7.67 (dd, 2H, *J* = 8.0 Hz, 8.0 Hz, ArH), 7.69-7.71 (d, 2H, *J* = 8.9 Hz, ArH), 7.85-7.90 (dd, 2H, *J* = 5.4 Hz, ArH), 8.03 (s, 1H, pyrazole-5H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 164.18, 161.72, 151.99, 138.43, 138.13, 133.67, 133.46, 131.98, 129.86, 129.78, 129.59, 129.52, 128.84, 128.71, 125.03, 124.82, 124.76, 120.15, 117.50, 115.62, 115.41, 65.51, 63.84; LC-MS: *m/z* = 495.1 (M+1); calculated for $C_{24}H_{16}Cl_2F_4N_2O$; C, 58.20; H, 3.26; N, 5.66; Found: C, 58.21; H, 3.27; N, 5.68.

3-(4-Bromophenyl)-4-(2-chloro-6-trifluoromethylbenzyloxymethyl)-1-phenyl-1*H*-pyrazole (T_{90}). Yield: 91 %; FT-IR (KBr, cm^{-1}): 1656 (C=N str), 1502 (C=C str), 1304 (C-N str), 1127 (C-F str), 753 (C-Cl str); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 4.68 (s, 2H, -CH_2), 4.86 (s, 2H, -CH_2), 7.30-7.34 (t, 1H, *J* = 7.4 Hz, ArH), 7.40-7.44 (t, 1H, *J* = 8.0 Hz, ArH), 7.46-7.50 (t, 2H, *J* = 7.8 Hz, ArH), 7.57-7.60 (d, 2H, *J* = 8.4 Hz, ArH), 7.65-7.67 (dd, 2H, *J* = 7.6 Hz, 8.0 Hz, ArH), 7.74-7.76 (d, 2H, *J* =8.4 Hz, ArH), 7.80-7.83 (d, 2H, *J* = 8.0 Hz, ArH), 8.07 (s, 1H, pyrazole-5H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 151.47, 139.86, 138.14, 133.68, 133.48, 131.77, 131.67, 131.53, 129.61, 129.57, 129.45, 129.16, 126.65, 125.04, 124.86, 124.80, 124.75, 122.30, 119.18, 117.21, 65.46, 63.86; LC-MS: *m/z* = 521.1 (M+1); calculated for $C_{24}H_{17}BrClF_3N_2O$; C, 55.25; H, 3.28; N, 5.37; Found: C, 55.27; H, 3.30; N, 5.38.

3-(4-Bromophenyl)-1-(4-chlorophenyl)-4-(2-chloro-6-trifluoromethyl benzyloxymethyl)-1*H*-pyrazole (T_{91}). Yield: 87 %; FT-IR (KBr, cm^{-1}): 1651 (C=N str), 1500 (C=C str), 1317 (C-N str), 1119 (C-F str), 749 (C-Clstr); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 4.69 (s, 2H, -CH_2), 4.86 (s, 2H, -CH_2), 7.13-7.17 (t, 2H, *J* = 8.7 Hz, ArH), 7.30-7.33 (t, 1H, *J* = 7.4 Hz, ArH), 7.40-7.44 (t, 1H, *J* = 8.0 Hz, ArH), 7.46-7.50 (t, 2H, *J* = 7.9 Hz, ArH), 7.64-7.67 (dd, 2H, *J* = 7.8 Hz, 8.0 Hz, ArH), 7.75-7.77 (d, 2H, *J* =7.8 Hz, ArH), 7.88-7.90 (d, 1H, *J* = 5.6 Hz, ArH), 7.91-7.92 (d, 1H, *J* = 5.6 Hz, ArH), 8.07 (s, 1H, pyrazole-5H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 151.72, 138.38, 138.12, 133.68, 133.42, 132.10, 131.83, 131.72, 131.52, 129.61, 129.58, 129.54, 129.32, 128.97, 124.82, 124.77, 122.48, 120.20, 117.67, 65.55, 63.79; LC-MS: *m/z*= 557.1 (M+1); calculated for $C_{24}H_{16}BrCl_2F_3N_2O$; C, 51.83; H, 2.90; N, 5.04; Found: C, 51.84; H, 2.90; N, 5.04.

4-(2-Chloro-6-trifluoromethylbenzyloxymethyl)-1-phenyl-3-thiophen-2-yl-1*H*-pyrazole (T_{92}). Yield: 74 %; FT-IR (KBr, cm^{-1}): 1651 (C=N str), 1508 (C=C str), 1352 (C-N str), 1121 (C-F str), 755 (C-Cl str); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 4.79 (s, 2H, -CH_2), 4.86 (s, 2H, -CH_2), 7.12-7.14 (t, 1H, *J* = 4.3 Hz, ArH), 7.31-7.43 (m, 3H, ArH), 7.45-7.49 (t, 2H, *J* = 7.8 Hz, ArH), 7.52-7.53 (d, 1H, *J* = 3.4 Hz, ArH), 7.65-7.63 (d, 2H, *J* =8.1 Hz, ArH), 7.74-7.76 (d, 2H, *J* = 8.0 Hz, ArH), 8.04 (s, 1H, pyrazole-5H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 146.96, 139.77, 138.51, 138.17, 135.01, 133.64, 131.84, 129.47, 129.41, 128.55, 127.57, 126.51, 126.25, 125.46,

124.75, 124.70, 119.09, 117.06, 65.46, 64.06; LC-MS: *m/z* = 449.1 (M+1); calculated for $C_{22}H_{16}ClF_3N_2OS$; C, 58.86; H, 3.59; N, 6.24; Found: C, 58.87; H, 3.60; N, 6.25.

1-(4-Chlorophenyl)-4-(2-chloro-6-trifluoromethylbenzyloxymethyl)-3-thiophen-2-yl-1*H*-pyrazole (T_{93}). Yield: 67 %; FT-IR (KBr, cm^{-1}): 1656 (C=N str), 1502 (C=C str), 1310 (C-N str), 1132 (C-F str), 774 (C-Cl str); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 4.77 (s, 2H, -CH_2), 4.85 (s, 2H, -CH_2), 7.11-7.13 (t, 1H, *J* = 4.4 Hz, ArH), 7.35-7.43 (m, 4H, ArH), 7.50-7.51 (d, 1H, *J* = 3.2 Hz, ArH), 7.63-7.65 (d, 2H, *J* = 8.0 Hz, ArH), 7.67-7.69 (d, 2H, *J* = 8.8 Hz, ArH), 7.99 (s, 1H, pyrazole-5H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 147.19, 138.29, 138.15, 134.72, 133.66, 133.57, 131.93, 131.54, 129.53, 129.50, 128.39, 127.64, 126.42, 125.66, 124.79, 124.73, 122.30, 120.13, 117.52, 65.56, 64.02; LC-MS: *m/z* = 483.3 (M+1), Calculated for $C_{22}H_{15}Cl_2F_3N_2OS$; C, 54.67; H, 3.13; N, 5.80; Found: C, 54.71; H, 3.14; N, 5.81.

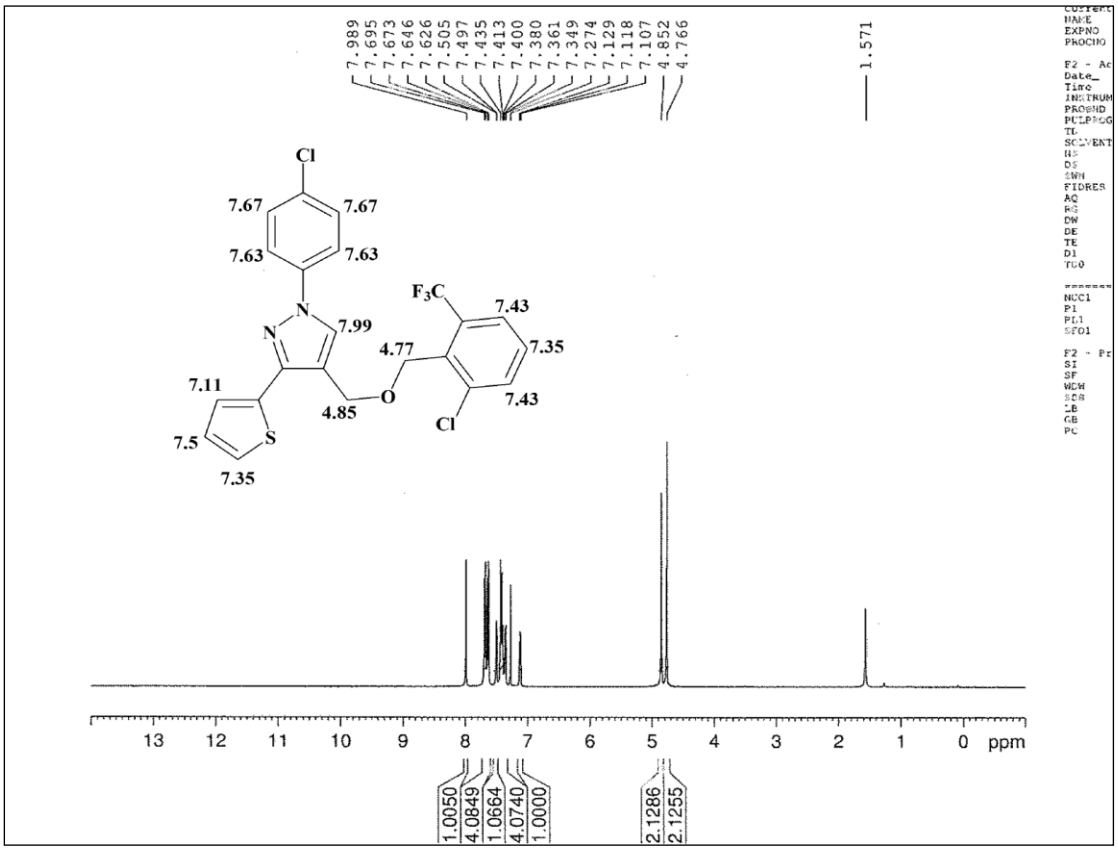


Figure 6.15 ^{1}H-NMR spectrum of compound **T_{93}**

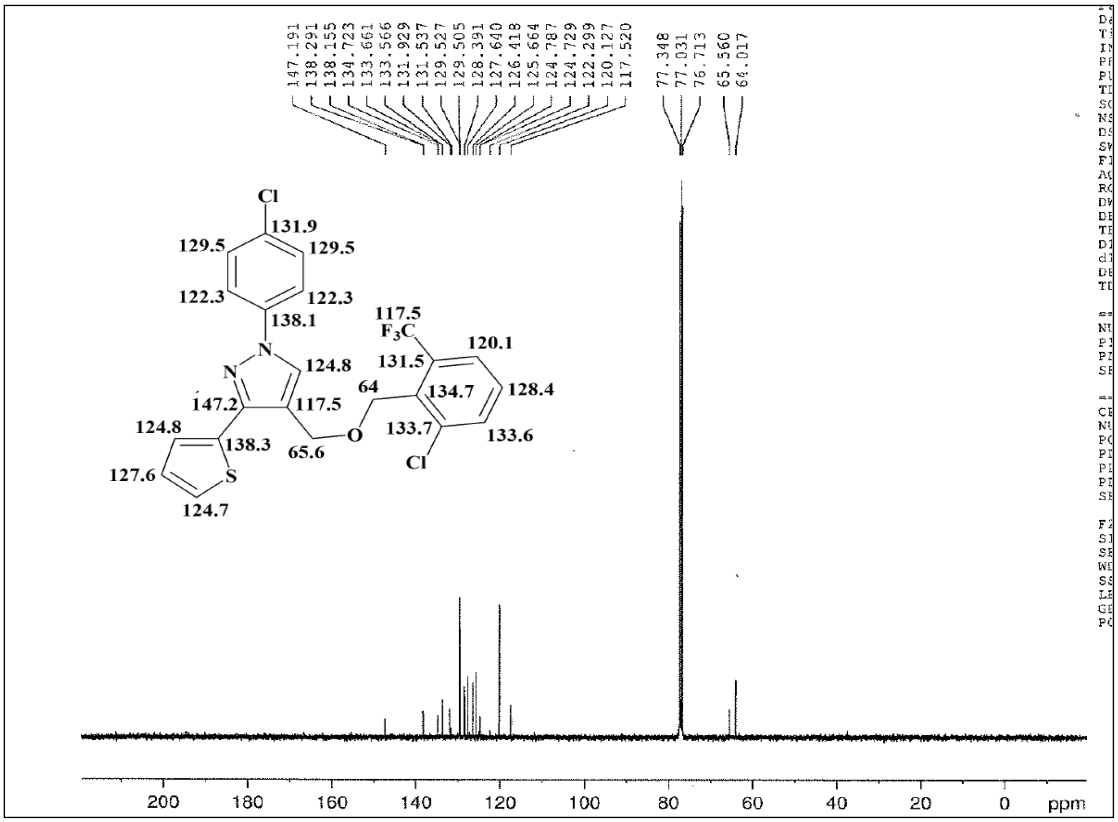


Figure 6.16 ^{13}C-NMR spectrum of compound **T_{93}**

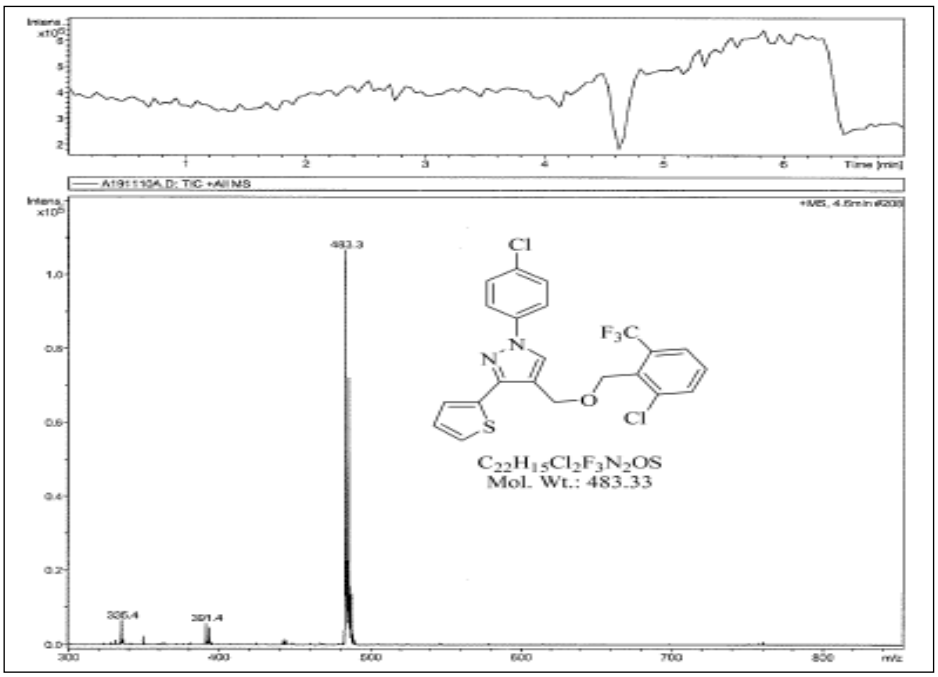


Figure 6.17 Mass spectrum of compound **T_{93}**

3-(3-Bromophenyl)-4-(2-chloro-6-trifluoromethylbenzyloxymethyl)-1-phenyl-1*H*-pyrazole (T_{94}). Yield: 88 %; FT-IR (KBr, cm^{-1}): 1647 (C=N str), 1507 (C=C str), 1316 (C-N str), 1118 (C-F str), 748 (C-Cl str); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 4.70 (s, 2H, -CH_2), 4.86 (s, 2H, -CH_2), 7.30-7.34 (t, 2H, *J* = 7.8 Hz, ArH), 7.38-7.42 (t, 1H, *J* = 8.0 Hz, ArH), 7.45-7.53 (m, 3H, ArH), 7.63-7.66 (dd, 2H, *J* = 8.0 Hz, 8.0 Hz, ArH), 7.74-7.76 (d, 2H, *J* = 7.6 Hz, ArH), 7.83-7.85 (d, 1H, *J* = 7.6 Hz, ArH), 8.06 (s, 1H, ArH), 8.07 (s, 1H, pyrazole-5H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 151.07, 139.83, 138.19, 134.89, 133.67, 131.04, 130.67, 130.07, 129.54, 129.47, 129.10, 126.75, 126.71, 124.72, 122.74, 119.21, 117.47, 65.56, 63.88; LC-MS: *m/z* = 523.1 (M+1); calculated for $C_{24}H_{17}BrClF_3N_2O$; C, 55.25; H, 3.28; N, 5.37; Found: C, 55.28; H, 3.30; N, 5.40.

3-(3-Bromophenyl)-1-(4-chlorophenyl)-4-(2-chloro-6-trifluoromethyl benzyloxymethyl)-1*H*-pyrazole (T_{95}). Yield: 81 %; FT-IR (KBr, cm^{-1}): 1598 (C=N str), 1504 (C=C str), 1313 (C-N str), 1121 (C-F str), 744 (C-Clstr); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 4.70 (s, 2H, -CH_2), 4.87 (s, 2H, -CH_2), 7.31-7.35 (t, 1H, *J* = 7.8 Hz, ArH), 7.40-7.46 (m, 3H, ArH), 7.53-7.55 (d, 1H, *J* = 8.0 Hz, ArH), 7.64-7.67 (dd, 2H, *J* = 7.6 Hz, 8.0 Hz, ArH), 7.70-7.72 (d, 2H, *J* = 8.7 Hz, ArH), 7.81-7.83 (d, 1H, *J* = 7.7 Hz, ArH), 8.04 (s, 1H, ArH), 8.05 (s, 1H, pyrazole-5H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 151.15, 138.35, 138.16, 134.65, 133.67, 133.45, 132.15, 131.19, 130.65, 130.10, 129.55, 128.89, 126.70, 124.79, 122.77, 120.22, 117.93, 65.64, 63.81; LC-MS: *m/z* = 557.1 (M+1), Calculated for $C_{24}H_{16}BrCl_2F_3N_2O$; C, 51.83; H, 2.90; N, 5.04; Found: C, 51.84; H, 2.90; N, 5.06.

1-(4-Chlorophenyl)-4-(2-chloro-6-trifluoromethylbenzyloxymethyl)-3-(2,4-dichlorophenyl)-1*H*-pyrazole (T_{96}). Yield: 91 %; FT-IR (KBr, cm^{-1}): 1655 (C=N str), 1500 (C=C str), 1314 (C-N str), 1113 (C-F str), 747 (C-Cl str); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 4.56 (s, 2H, -CH_2), 4.73 (s, 2H, -CH_2), 7.33-7.35 (t, 1H, *J* = 4.1 Hz, ArH), 7.38-7.47 (m, 3H, ArH), 7.52-7.54 (dd, 2H, *J* = 4.0 Hz, ArH), 7.61-7.63 (d, 2H, *J* = 8.0 Hz, ArH), 7.66-7.68 (d, 2H, *J* = 7.0 Hz, ArH), 8.0692 (s, 1H, pyrazole-5H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 164.18, 161.72, 151.99, 138.43, 138.13, 133.67, 133.46, 131.98, 129.86, 129.78, 129.59, 129.52, 128.84, 128.71, 125.03, 124.82, 124.76, 120.15, 117.50, 115.62, 115.41, 65.51, 63.84; LC-MS: *m/z* = 547.1 (M+1);

calculated for $C_{24}H_{15}Cl_4F_3N_2O$; C, 52.78; H, 2.77; N, 5.13; Found: C, 52.80; H, 2.78; N, 5.15.

3-Biphenyl-4-yl-1-(4-Chlorophenyl)-4-(2-chloro-6-trifluoromethyl benzyloxymethyl)-1*H*-pyrazole (T_{97}). Yield: 77 %; FT-IR (KBr, cm^{-1}): 1656 (C=N str), 1498 (C=C str), 1311 (C-N str), 1121 (C-F str), 743 (C-Cl str); ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 4.75 (s, 2H, -CH_2), 4.89 (s, 2H, -CH_2), 7.39-7.51 (m, 6H, ArH), 7.65-7.74 (m, 7H, ArH), 796-7.98 (d, 2H, *J* = 8.0 Hz, ArH), 8.06 (s, 1H, pyrazole-5H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 152.39, 140.97, 140.86, 138.52, 138.18, 133.68, 131.90, 131.58, 129.55, 129.51, 128.80, 128.39, 127.38, 127.33, 127.12, 124.74, 120.17, 117.86, 65.57, 64.04; LC-MS: *m/z* = 553.9 (M+1); calculated for $C_{30}H_{21}Cl_2F_3N_2O$; C, 65.11; H, 3.82; N, 5.06; Found: C, 65.15; H, 3.84; N, 5.08.

6.4 PHARMACOLOGY

6.4.1 Antitubercular activity

Antitubercular screening for the newly synthesized compounds $T_{64\text{-}79}$ were stored in the chemical repository at RT as solid powder until solubilization. Compounds were solubilized in DMSO to a final concentration of 10 mM, aliquotted into matrix tubes and stored at -20 °C. Two-fold serial dilutions in DMSO were prepared and reformatted into 96-well polypropylene, V-bottom assay plates. The MIC of compound was determined by measuring bacterial growth after 5 d in the presence of test compounds. Compounds were prepared as 10-point two-fold serial dilutions in DMSO and diluted into 7H9-Tw-OADC medium in 96-well plates with a final DMSO concentration of 2 %. The highest concentration of compound was 20 µM. Each plate included assay controls for background (medium/DMSO only, no bacterial cells), zero growth (2 µM Rifampicin) and maximum growth (DMSO only), as well as a Rifampicin dose response curve. Plates were inoculated with *M. tuberculosis* and incubated for 5 days: growth was measured by OD_{590} and fluorescence (Ex 560/ Em 590). Growth was calculated separately for OD_{590} and RFU. Dose response curves were plotted as % growth and fitted using the Levenberg-Marquardt algorithm. The concentration that resulted in 90 % inhibition of growth was determined where appropriate (IC_{90}). RFU data were used for quality control

only. All values were converted into µg/mL from µM based on molecular weight of the synthesized compound.

Anti-TB activity of **T_{80-97}** were determined by the Middle brook 7H9 broth against *Mycobacterium tuberculosis* of H_{37}Rv strain (ATCC-27294) as explained in **Chapter 2**. The MIC value of each synthesized compound was determined by the MABA method. Isoniazid, Ethambutol and Pyrazinamide were used as standard anti-TB drugs.

6.4.2 Antibacterial and antifungal activity

MIC value for antibacterial and antifungal activity for the target compounds **T_{64-79}** and **T_{80-97}** were determined by Resazurin reduction method in 96 well plate as explained in **Chapter 2**. In this work *Staphylococcus aureus* (MTCC 3160) and *Mycobacterium smegmatis* (MTCC 994) were used to study antibacterial activity and *Candida albicans* (MTCC 7253) and *Penicillium chrysogenum* (MTCC 6795) were used to study antifungal activity. All the microorganisms were obtained from IMTECH, Chandigarh, India and were maintained as per the standard protocol. The well, which remains blue after 24 h of incubation indicates there are no microorganisms survived in the well, the minimum concentration where no microbial growth found are considered as MIC value.

6.4.3 Cytotoxicity studies

6.4.3.1 IC_{50} value determination for HeLa and Vero cell lines

Vero (African green monkey kidney) cell line was procured from National Centre for Cell Sciences (NCCS), Pune, India. *In vitro* cytotoxicity of active compounds against *Mycobacterium tuberculosis* were tested for cytotoxicity against HeLa (Cervical cancer) and VERO (African green monkey kidney) cell lines. The high IC_{50} value and high selectivity index indicate the nontoxicity of the compound. The control cells which are not treated with any compound have shown 100 % viability. *In vitro* cytotoxicity study of active compounds have tested against HeLa and Vero cell lines as explained in **Chapter 3**.

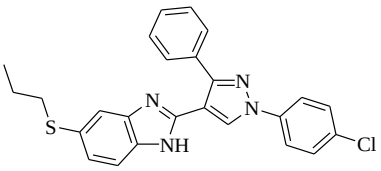
6.5 RESULTS AND DISCUSSION

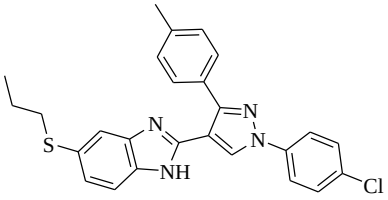
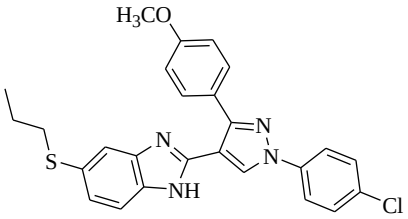
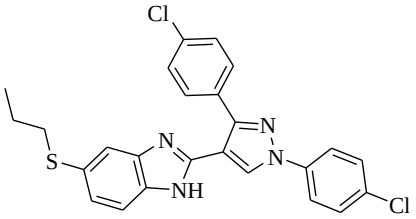
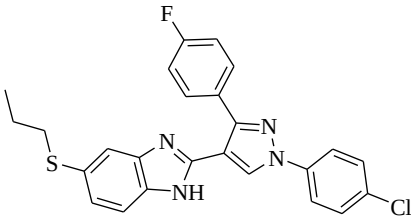
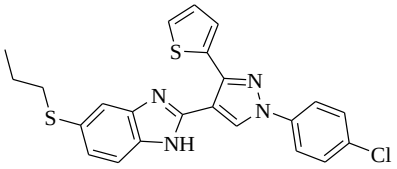
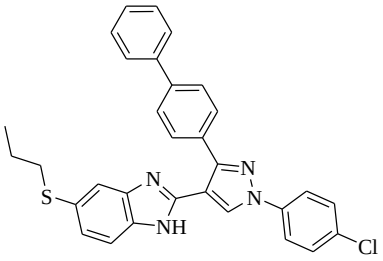
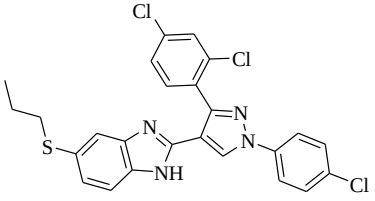
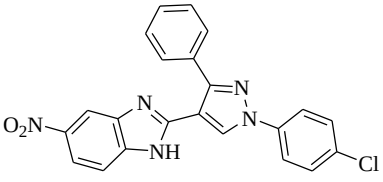
6.5.1 Chemistry

Target compounds **T_{64-79}** and **T_{80-97}** were confirmed by various spectroscopic techniques. The ^{1}H-NMR spectrum of **T_{64}** showed a triplet at δ 0.98 corresponding to the -CH_3 protons. Sextet obtained at δ 1.58 corresponding to -CH_2 protons and a triplet obtained at δ 2.95 corresponding to -CH_2 proton. A singlet obtained at δ 9.12 represents to pyrazole-5*H* proton. The mass spectrum of **T_{64}** observed a molecular ion peak at 446.2 (M+1), which is inconsistent with the molecular formula $C_{25}H_{21}ClN_4S$. The physical data of the all the synthesized compounds **T_{64-79}** were presented in **Table 6.2**.

In the other series, the formation of 4-(2-chloro-6-trifluoro methyl benzyloxy methyl)-1,3-disubstituted-1*H*-pyrazole **T_{80-97}** were confirmed by spectroscopic analysis. IR analysis of compound **T_{80}** showed the peak at 1657 cm^{-1}, which is due to the C=N group and C=C stretching was observed at 1501 cm^{-1}. A sharp C-F absorption band appeared at 1121 cm^{-1} and C-Cl stretching peak was observed at 752 cm^{-1}, which confirmed the formation of compound **T_{80}**. The ^{1}H-NMR spectrum of **T_{80}** in $CDCl_3$ solvent showed two singlets at δ 4.70 and 4.84, which were attributed due to -CH_2 groups adjacent to ether link. The aromatic protons were observed in the range of δ 7.29-7.88. A singlet attributed at δ 8.06, which is due to the pyrazole-5*H* proton. The mass spectrum of **T_{80}** showed a molecular ion peak at 443.8 (M+1). This, in turn, confirmed the formation of a compound having the molecular formula $C_{24}H_{18}ClF_3N_2O$. The physical data of the all the synthesized compounds **T_{80-97}** were presented in **Table 6.3**.

Table 6.2 Structural properties of the compounds **T_{64-79}**

Comp.	Ar	R	Structure	M. F/M. wt
T_{64}	C_6H_5	-$S(CH_2)_2CH_3$		$C_{25}H_{21}ClN_4S$/ 444.98

T_{65}	4-$CH_3C_6H_4$	-$S(CH_2)_2CH_3$		$C_{26}H_{13}ClN_4S$/ 459.01
T_{66}	4-$OCH_3C_6H_4$	-$S(CH_2)_2CH_3$		$C_{26}H_{23}ClN_4OS$/ 475.01
T_{67}	4-ClC_6H_4	-$S(CH_2)_2CH_3$		$C_{25}H_{20}Cl_2N_4S$/ 479.42
T_{68}	4-FC_6H_4	-$S(CH_2)_2CH_3$		$C_{25}H_{20}ClFN_4S$/ 462.97
T_{69}	2-Thiophene	-$S(CH_2)_2CH_3$		$C_{23}H_{19}ClN_4S_2$/ 451.01
T_{70}	Biphenyl	-$S(CH_2)_2CH_3$		$C_{31}H_{25}ClN_4S$/ 521.08
T_{71}	2,4-$Cl_2C_6H_3$	-$S(CH_2)_2CH_3$		$C_{25}H_{19}Cl_3N_4S$/ 513.87
T_{72}	C_6H_5	-NO_2		$C_{22}H_{14}ClN_5O_2$/ 415.83

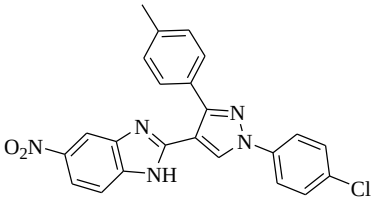
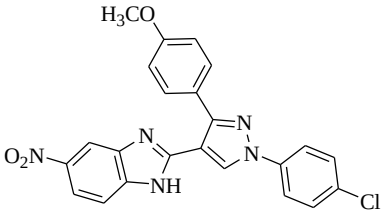
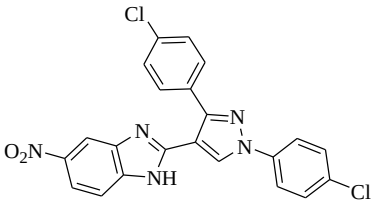
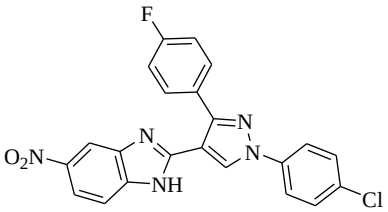
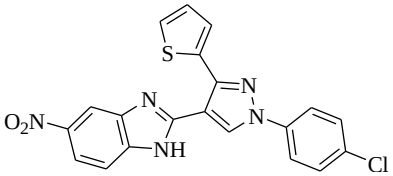
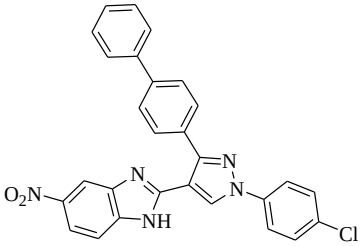
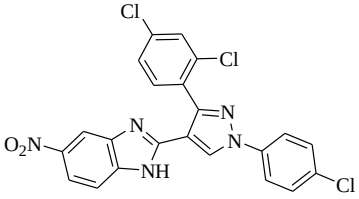
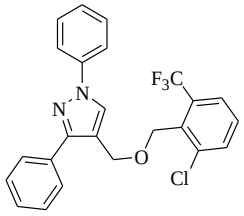
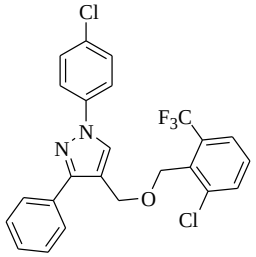
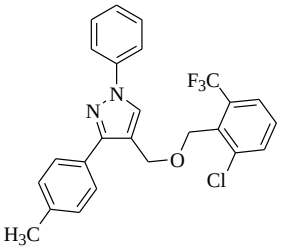
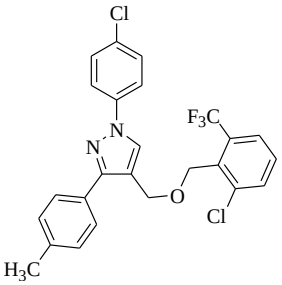
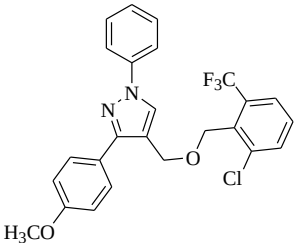
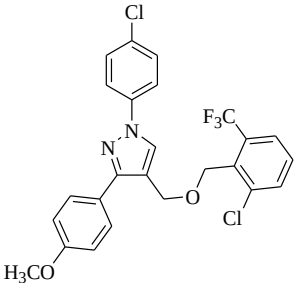
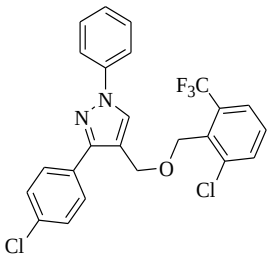
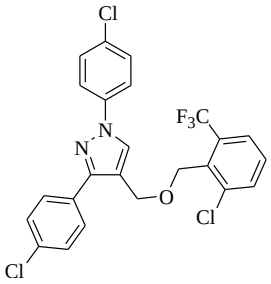
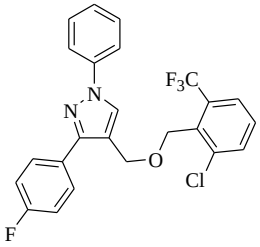
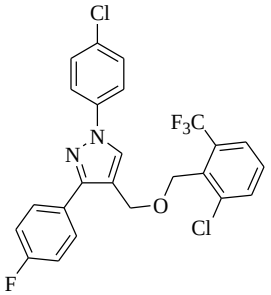
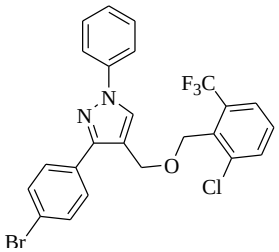
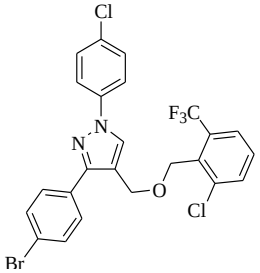
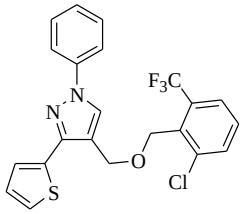
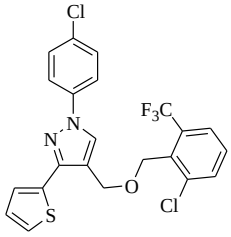
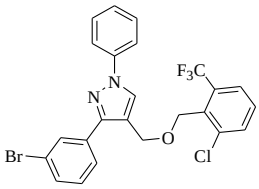
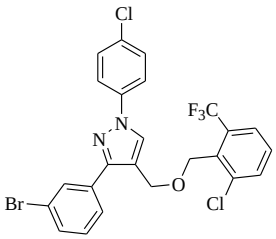
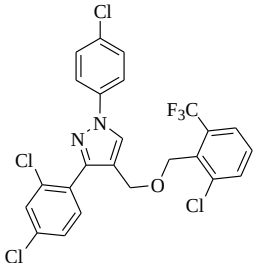
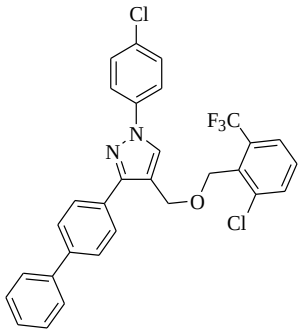
T_{73}	4-$CH_3C_6H_4$	-NO_2		$C_{23}H_{16}ClN_5O_2$/ 429.86
T_{74}	4-$OCH_3C_6H_4$	-NO_2		$C_{23}H_{16}ClN_5O_3$/ 445.86
T_{75}	4-ClC_6H_4	-NO_2		$C_{22}H_{13}Cl_2N_5O_2$/ 450.28
T_{76}	4-FC_6H_4	-NO_2		$C_{22}H_{13}ClFN_5O_2$/ 433.82
T_{77}	2-Thiophene	-NO_2		$C_{20}H_{12}ClN_5O_2S$/ 421.86
T_{78}	Biphenyl	-NO_2		$C_{28}H_{18}ClN_5O_2$/ 491.93
T_{79}	2,4-$Cl_2C_6H_3$	-NO_2		$C_{22}H_{12}Cl_3N_5O_2$/ 484.72

Table 6.3 Structural properties of the compounds $\mathbf{T_{80\text{-}97}}$

Comp.	Ar/X	Structure	M. F/ M. wt	M. p (°C)
$\mathbf{T_{80}}$	Ph/H		$C_{24}H_{18}ClF_3N_2O$/ 442.86	123-124
$\mathbf{T_{81}}$	Ph/Cl		$C_{24}H_{17}Cl_2F_3N_2O$/ 477.31	119-121
$\mathbf{T_{82}}$	4-$CH_3C_6H_4$/H		$C_{25}H_{20}ClF_3N_2O$/ 456.89	118-119
$\mathbf{T_{83}}$	4-$CH_3C_6H_4$/Cl		$C_{25}H_{19}Cl_2F_3N_2O$/ 491.33	136-138
$\mathbf{T_{84}}$	4-$OCH_3C_6H_4$/H		$C_{25}H_{20}ClF_3N_2O_2$/ 472.89	118-119
$\mathbf{T_{85}}$	4-$OCH_3C_6H_4$/Cl		$C_{25}H_{19}Cl_2F_3N_2O_2$/ 507.33	104-105

T_{86}	4-ClC_6H_4/H		$C_{24}H_{17}Cl_2F_3N_2O$/ 477.31	102-103
T_{87}	4-ClC_6H_4/Cl		$C_{24}H_{16}Cl_3F_3N_2O$/ 511.75	104-105
T_{88}	4-FC_6H_4/H		$C_{24}H_{17}ClF_4N_2O$/ 460.85	110-111
T_{89}	4-FC_6H_4/Cl		$C_{24}H_{16}Cl_2F_4N_2O$/ 495.30	144-145
T_{90}	4-BrC_6H_4/H		$C_{24}H_{17}BrClF_3N_2O$/ 521.76	111-112
T_{91}	4-BrC_6H_4/Cl		$C_{24}H_{16}BrCl_2F_3N_2O$/ 556.20	102-104

T_{92}	2-Thiophene/H		$C_{22}H_{16}ClF_3N_2OS$/ 448.89	96-98
T_{93}	2-Thiophene/Cl		$C_{22}H_{15}Cl_2F_3N_2OS$/ 483.33	113-114
T_{94}	3-BrC_6H_4/H		$C_{24}H_{17}BrClF_3N_2O$/ 521.76	84-85
T_{95}	3-BrC_6H_4/Cl		$C_{24}H_{16}BrCl_2F_3N_2O$/ 556.20	93-94
T_{96}	2,4-$Cl_2C_6H_3$/Cl		$C_{24}H_{15}Cl_4F_3N_2O$/ 546.20	101-103
T_{97}	Biphenyl/Cl		$C_{30}H_{21}Cl_2F_3N_2O$/ 553.40	144-145

6.5.2 Single crystal X-ray crystallography studies

To understand the molecular interaction, single crystal X-ray diffraction study was performed for few selected compounds. The crystal structure of intermediate compound **25c** (CCDC: 1415708) was determined by X-ray crystallography. The details of data collection and structure refinement are listed in **Table 6.4**. The crystal structure of compounds $\mathbf{T_{80}}$, $\mathbf{T_{83}}$, $\mathbf{T_{86}}$ and $\mathbf{T_{88}}$ (CCDC: 1415672, 1415692, 1415693 and 1415702 respectively) were determined by X-ray crystallography. The details of data collection and structure refinement are listed in **Table 6.5**. All bond lengths and angles are within normal ranges. ORTEP diagrams were presented in **Figure 6.18** and **Figure 6.19** respectively.

Table 6.4 Crystal data and measurement details of compound **25c**

Compound	**25c**
Chemical formula	$C_{17}H_{16}N_2O$
Formula wt	264.32
Crystal system, space group	Monoclinic, $P2_1/c$
Temp. (K)	150
***a, b, c* (Å)**	8.5940 (3), 16.9419 (6), 9.7701 (3)
***Angle α,β, γ*(°)**	90.00, 98.350, 90.00
***Volume* (Å^3)**	1407.43 (8)
Z	4
Radiation type	Mo *K*α
μ (mm^{-1})	0.08
Crystal size (mm)	0.55 × 0.27 × 0.12
T_{min}, T_{max}	0.958, 0.990
No. of measured, independent and observed [$I>2\sigma(I)$] reflections	48069, 8383, 6477
R_{int}	0.036
$R[F^2>2\sigma(F^2)]$, $wR(F^2)$, S	0.046, 0.134, 1.00
No. of reflections	8383
No. of parameters	186
$\Delta\rho_{max}$, $\Delta\rho_{min}$ (e Å^{-3})	0.50, - 0.27

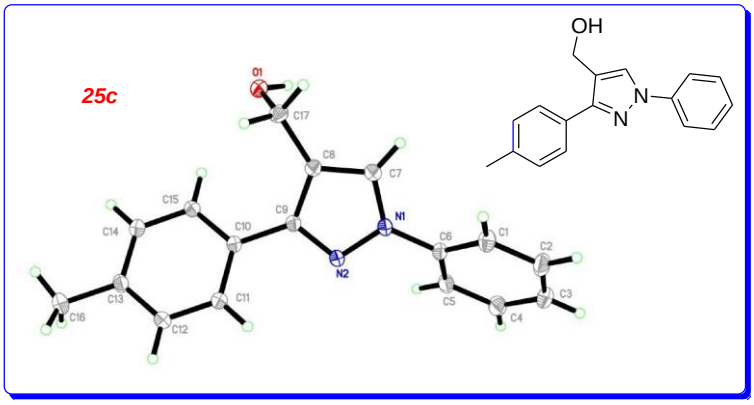


Figure 6.18 ORTEP diagram of compound **25c**

Table 6.5 Crystal data and measurement details of compound $\mathbf{T_{80}}$, $\mathbf{T_{83}}$, $\mathbf{T_{86}}$ and $\mathbf{T_{88}}$

Compounds	$\mathbf{T_{80}}$	$\mathbf{T_{83}}$	$\mathbf{T_{86}}$	$\mathbf{T_{88}}$
Chemical formula	$C_{24}H_{18}ClF_3N_2O$	$C_{25}H_{19}Cl_2F_3N_2O$	$C_{24}H_{17}Cl_2F_3N_2O$	$C_{24}H_{17}ClF_4N_2O$
Formula wt	442.85	491.32	477.30	460.85
Crystal system, space group	Orthorhombic, *Iba*2	Triclinic, $P2_1/c$	Monoclinic, $P2_1/c$	Monoclinic, $P2_1/c$
Temp. (K)	150	296	150	150
***a, b, c* (Å)**	14.719 (2), 39.483 (7), 7.0507 (11)	7.3094 (4), 12.4709 (6), 14.1256 (7)	11.8061 (14), 20.161 (2), 9.2474 (10)	20.3709 (8), 14.4610 (6), 7.0590 (3)
Angle α,	90.00	108.737	90.00	90.00
ß,	90.00	98.380	104.167	92.535
***γ*(°)**	90.00	102.785	90.00	90.00
***Volume* (Å^3)**	4097.5 (11)	1155.91 (10)	2134.2 (4)	2077.43 (15)
Radiation type	Mo *K*α	Mo *K*α	Mo *K*α	Mo *K*α
Crystal size (mm)	0.68 × 0.29 × 0.13	0.61 × 0.55 × 0.51	0.57 × 0.09 × 0.05	0.65 × 0.28 × 0.15
T_{min}, T_{max}	0.81, 0.99	0.76, 0.85	0.825, 0.984	0.91, 0.96
No. of measured, independent and observed [$I>2\sigma(I)$] reflections	41426, 3614, 3390	28017, 3984, 3677	19915, 4850, 3641	118170, 9119, 8203
R_{int}	0.063	0.021	0.058	0.037
$R[F^2> 2\sigma(F^2)]$, $wR(F^2)$, S	0.046, 0.105, 1.14	0.046, 0.128, 1.03	0.059, 0.185, 1.02	0.107, 0.259, 1.16
No. of reflections	3614	3984	4850	9119
No. of parameters	333	299	289	578
$\Delta\rho_{max}$, $\Delta\rho_{min}$ (e Å^{-3})	0.23, − 0.24	0.34, − 0.50	1.11, − 0.54	1.10, − 0.62

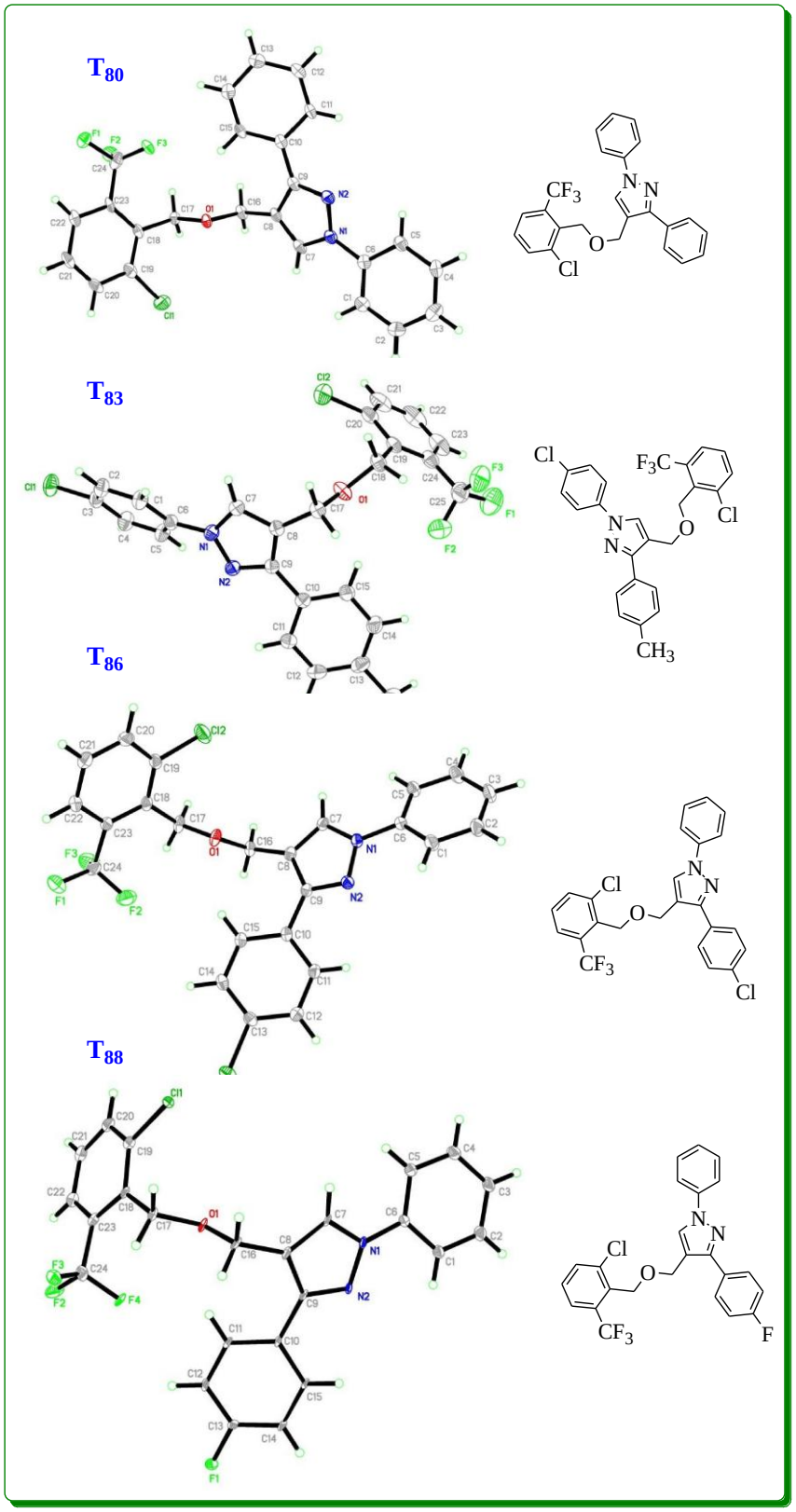


Figure 6.19 ORTEP diagrams of compound **T_{80}**, **T_{83}**, **T_{86}** and **T_{88}**

6.5.3 BIOLOGICAL RESULTS

6.5.3.1 *In vitro* antitubercular activity

Antitubercular activity of newly synthesized compounds ($\mathbf{T_{64\text{-}79}}$) was tested against *Mycobacterium tuberculosis* H_{37}Rv strain (ATCC-27294) has been presented in **Figure 6.20**. The Minimum Inhibition Concentration assay is defined as the minimum concentration of a compound required to completely inhibit the bacterial growth. Anti-TB standards Isoniazid (INH), and Ethambutol (EMB) were used for comparison.

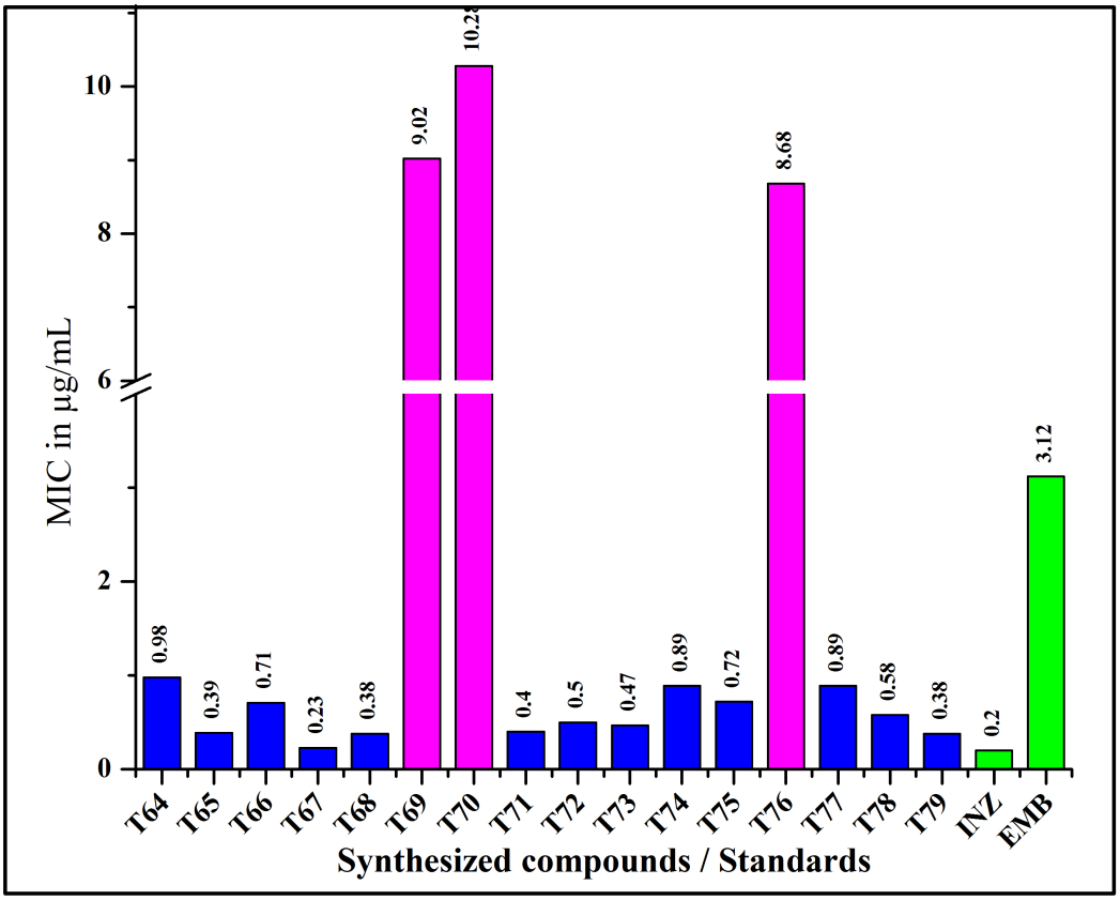


Figure 6.20 MIC values of target compounds $\mathbf{T_{64\text{-}79}}$ against *M. tuberculosis*

Structure activity relationship between benzimidazole with thiopropyl / nitro groups was identified in the first benzimidazole series. Out of eighteen new derivatives, fifteen compounds are more potent (MIC ≤1.0 µg/mL) than the standard anti-TB drug Ethambutol (EMB). Among fifteen compounds, $\mathbf{T_{67}}$ showed potent antitubercular activity with a MIC value of 0.23 µg/mL, it was equipotent with standard Isoniazid (INZ). This is because of 4-chlorophenyl on pyrazole at first and

third positions and thiopropyl substitution on the fifth position on benzimidazole ring. Electron donating groups at the third position on pyrazole was enhancing the antitubercular activity. 2,4-Dichlorophenyl substitution at third position showed excellent activity irrespective of the functional group at fifth position on benzimidazole. They exhibited MIC values 0.40 and 0.38 µg/mL for thiopropyl and nitro group respectively on benzimidazole-*5H*. These fifteen compounds were ≥ three-fold active than that of tested anti-TB standard Ethambutol (EMB).

In the other series, compounds $\mathbf{T_{80\text{-}97}}$ were tested against *Mycobacterium tuberculosis* $H_{37}Rv$ strain (ATCC-27294) has been presented in **Figure 6.21**. Anti-TB standards Isoniazid (INH), Ethambutol (EMB) and Pyrazinamide (PZA) were used as anti-TB standards.

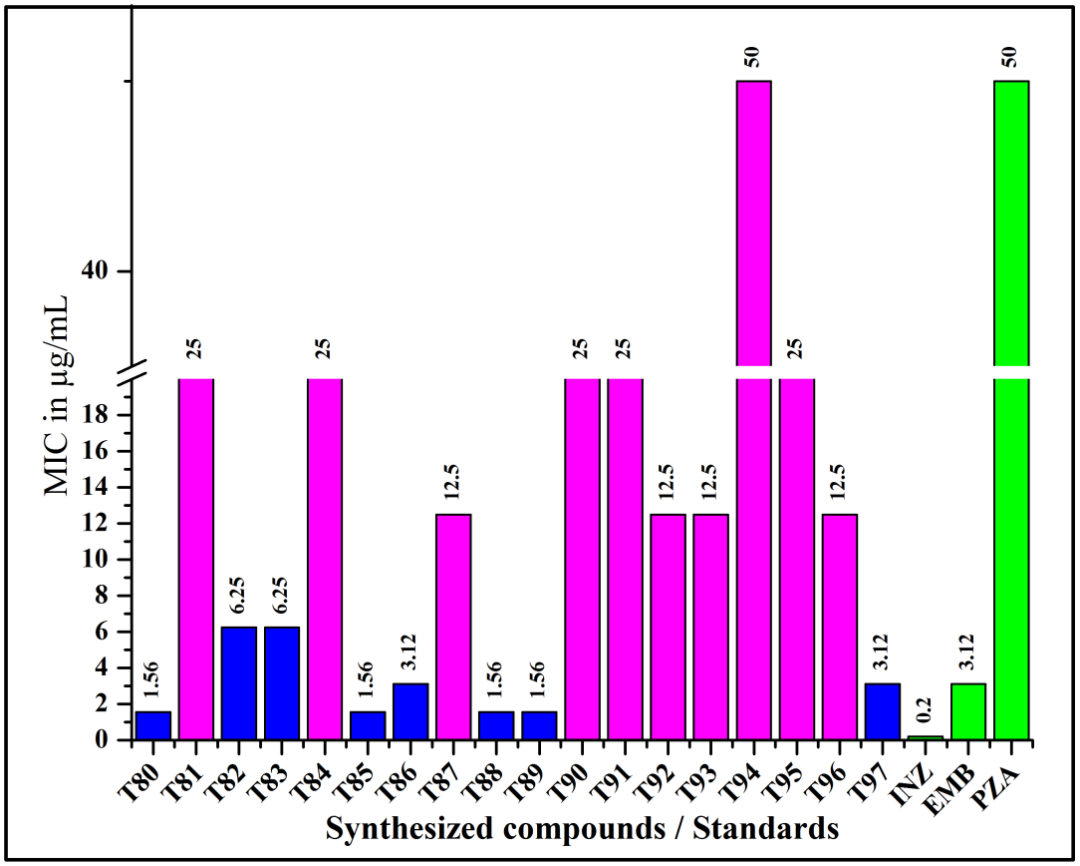


Figure 6.21 MIC values of target compounds $\mathbf{T_{80\text{-}97}}$ against *M. tuberculosis*

All the compounds ($\mathbf{T_{80\text{-}97}}$) showed moderate to excellent antitubercular activity, with MIC value ranging between 50 to 1.56 µg/mL, which are more potent than that of standard anti-TB drug Pyrazinamide. Among the synthesized compounds

T_{80}, **T_{85}**, **T_{88}** and **T_{89}** showed lowest MIC value of 1.56 µg/mL and were found to be two-fold more active than that of standard first-line anti-TB drug Ethambutol. The enhanced activity of compound **T_{80}** was due to the presence of phenyl substitution on pyrazole-3*H* and compound **T_{85}** was because of electron donating group present on pyrazole-3*H*. 4-Fluorophenyl substitution has enhanced the *M. tuberculosis* activity for compounds **T_{88}** and **T_{89}**. The second lowest MIC value of 3.12 µg/mL obtained for compounds **T_{86}** and **T_{97}** and compounds **T_{82}** and **T_{83}** have shown MIC value of 6.25 µg/mL were almost similarly active as that of the standard anti-TB drug Ethambutol.

This concludes that, modification of the pyrazole containing benzimidazole and trifluoromethyl substitution is able to give better activity against *Mycobacterium tuberculosis*.

6.5.3.2 Antibacterial and antifungal activity

Antibacterial activity of the synthesized compounds **$T_{64\text{-}79}$** were carried out against *Mycobacterium smegmatis* (MTCC 994), Gram +ve bacteria *Staphylococcus aureus* (MTCC 3160) and antifungal activity on *Candida albicans* (MTTC 7253) and *Penicillium chrysogenum* (MTCC 6795) were screened for MIC by resazurin reduction method. Antibacterial standard Ciprofloxacin (INN) and antifungal standard Fluconazole (FLZ) were used. Minimum inhibitory concentration assay conducted at the concentration between 100 to 0.78 µg/mL were represented in **Table 6.6**. Tested bacteria, *Mycobacterium smegmatis* and *Staphylococcus aureus* have been identified the least MIC value for most of the target derivatives. Compounds **T_{64}**, **T_{65}**, **T_{67}**, **T_{68}**, **T_{71}**, **T_{73}**, **T_{74}** and **T_{79}** are showed good activity with a MIC value of 1.56 µg/mL, which are two-fold more active than the tested standard Ciprofloxacin. Antifungal activity was tested against *Candida albicans* and *Penicillium chrysogenum* showed least MIC value of 3.12 µg/mL for **T_{67}**, **T_{68}**, **T_{73}** and **T_{77}**. These four compounds are similarly active as that of antifungal standard Fluconazole. Other compounds **T_{65}**, **T_{69}**, **T_{74}**, **T_{75}** and **T_{78}** showed significant activity against tested fungal strains.

Table 6.6 MIC value of $\mathbf{T_{64\text{-}79}}$ against bacterial and fungal strains

Synthesized Compound	MIC in µg/mL			
	M. smegmatis	***S. aureus***	***C. albicans***	***P. chrysogenum***
$\mathbf{T_{64}}$	1.56	1.56	12.5	6.25
$\mathbf{T_{65}}$	1.56	1.56	6.5	12.5
$\mathbf{T_{66}}$	3.12	3.12	12.5	12.5
$\mathbf{T_{67}}$	1.56	1.56	3.12	3.12
$\mathbf{T_{68}}$	1.56	1.56	3.12	3.12
$\mathbf{T_{69}}$	12.5	25	12.5	6.25
$\mathbf{T_{70}}$	25	25	25	12.5
$\mathbf{T_{71}}$	1.56	1.56	25	25
$\mathbf{T_{72}}$	3.12	3.12	12.5	6.25
$\mathbf{T_{73}}$	1.56	1.56	3.12	3.12
$\mathbf{T_{74}}$	1.56	1.56	6.25	12.5
$\mathbf{T_{75}}$	6.25	3.12	6.25	6.25
$\mathbf{T_{76}}$	50	50	25	25
$\mathbf{T_{77}}$	6.25	6.25	3.12	3.12
$\mathbf{T_{78}}$	3.12	3.12	6.25	6.25
$\mathbf{T_{79}}$	1.56	1.56	25	25
INN	3.12	3.12	--	--
FLZ	--	--	3.12	3.12
Control	--	--	--	--

INN; antibacterial standard Ciprofloxacin; FLZ; antifungal standard Fluconazole; --: not detected inhibition; control; dimethylsulfoxide

In the other series, MIC value of newly synthesized compounds $\mathbf{T_{80\text{-}97}}$ has been presented in **Table 6.7**. All the compounds were tested at 100 to 0.78 µg/mL concentration by serial dilution method. Compounds $\mathbf{T_{80}}$ and $\mathbf{T_{97}}$ were showed good activity against tested organisms and identified the least MIC value of 3.12 µg/mL, which are equipotent with standard Ciprofloxacin and Fluconazole. Compounds $\mathbf{T_{88}}$ and $\mathbf{T_{89}}$ were showed MIC value of 3.12 µg/mL specifically against *Mycobacterium smegmatis* and *Staphylococcus aureus* bacteria. The majority of the compounds showed significant activity with MIC value 6.25-25 µg/mL for bacterial and fungal strains.

Table 6.7: MIC value of $\mathbf{T_{80\text{-}97}}$ against bacterial and fungal strains

Synthesized Compound	MIC in µg/mL			
	M. Smegmatis	***S.aureus***	***C.albicans***	***P. chrysogenum***
$\mathbf{T_{80}}$	3.12	3.12	3.12	3.12
$\mathbf{T_{81}}$	25	50	50	50
$\mathbf{T_{82}}$	6.25	6.25	6.25	12.5
$\mathbf{T_{83}}$	6.25	6.25	6.25	6.25
$\mathbf{T_{84}}$	25	50	50	25
$\mathbf{T_{85}}$	6.25	6.25	6.25	6.25
$\mathbf{T_{86}}$	6.25	12.5	12.5	6.25
$\mathbf{T_{87}}$	12.5	12.5	12.5	12.5
$\mathbf{T_{88}}$	3.12	3.12	6.25	6.25
$\mathbf{T_{89}}$	3.12	3.12	6.25	3.12
$\mathbf{T_{90}}$	50	50	50	50
$\mathbf{T_{91}}$	50	50	50	50
$\mathbf{T_{92}}$	12.5	6.25	6.25	6.25
$\mathbf{T_{93}}$	12.5	12.5	12.5	12.5
$\mathbf{T_{94}}$	25	50	100	50
$\mathbf{T_{95}}$	25	50	100	100
$\mathbf{T_{96}}$	6.25	12.5	12.5	12.5
$\mathbf{T_{97}}$	3.12	3.12	3.12	3.12
INN	3.12	3.12	--	--
FLZ	--	--	3.12	3.12
Control	--	--	--	--

INN; antibacterial standard Ciprofloxacin; FLZ; antifungal standard Fluconazole; --: not detected inhibition; control; dimethylsulfoxide

6.5.4 Cytotoxicity studies

The *in vitro* cytotoxicity study was carried out using HeLa and Vero cell lines. The active target compounds, which had the highest activity for antituberculosis were tested for cytotoxicity with cervical cancer-HeLa cell line and Vero cell line was represented in **Figure 6.22**. The IC_{50} values of target compounds were identified as more effective against tested HeLa and Vero cells. Out of tested compounds, $\mathbf{T_{85}}$ showed lowest cytotoxicity (IC_{50} is 440 µg/mL) of tested compounds.

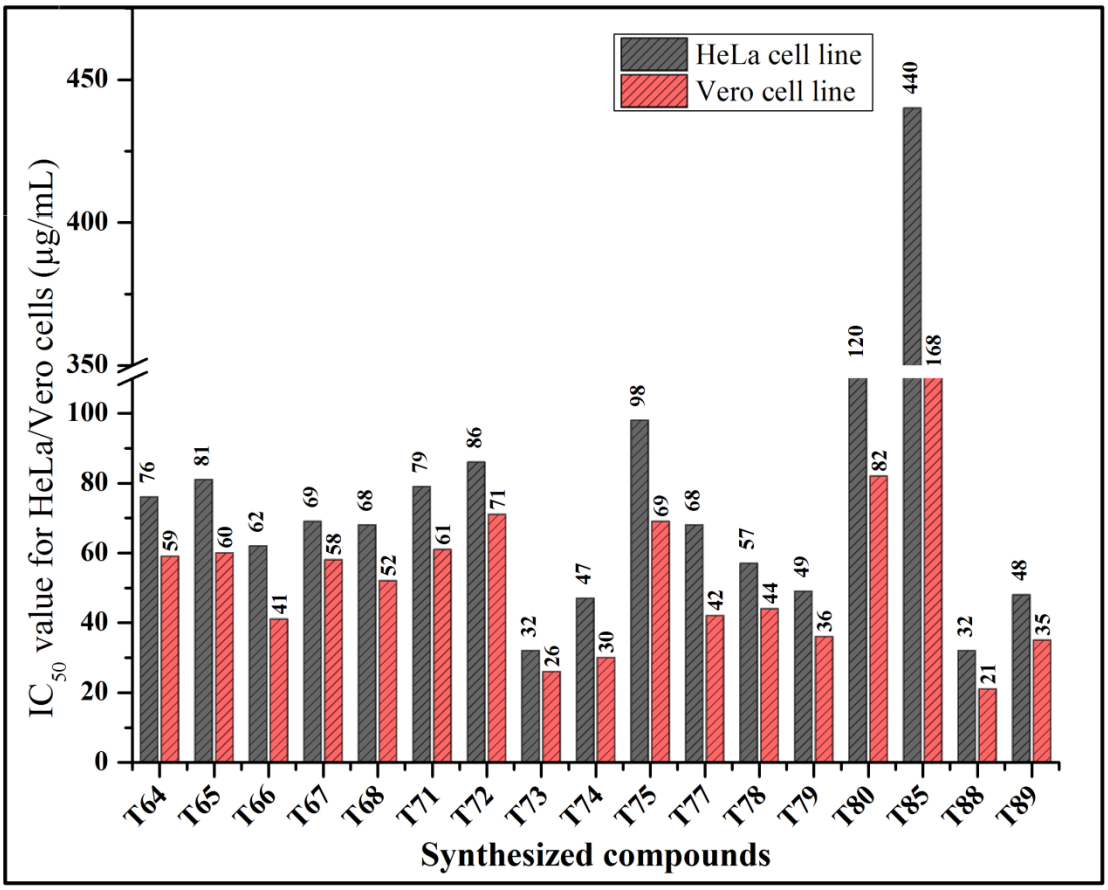


Figure 6.22 Cytotoxicity studies of active compounds with HeLa and Vero cell lines

6.5.5 Structure-activity relationship of pyrazole derivatives

Excellent TB activity found when these pyrazole derivatives were tested against tubercular causing *M. tuberculosis* $H_{37}Rv$ strain. Furthermore, cytotoxicity of these compounds against cervical cancer-HeLa cell lines showed moderate to excellent inhibition. Out of the leading compounds, **T_{85}** showed good cytotoxicity (IC_{50}: 440 µg/mL) with selectivity index 282.1. The comparison values between CLogP, MIC, IC_{50} and SI were presented in **Table 6.8**. The enhanced activity is because of electron donating group present on phenyl group at pyrazole-3*H*.

Table 6.8 Selectivity Index (SI) on HeLa and Vero cell lines against *M.tb* $H_{37}Rv$

Compounds	M. F	CLogP	*Mtb* $H_{37}Rv$ (µg/mL)	HeLa cell line		Vero cell line	
				IC_{50} (µg/mL)	SI	IC_{50} (µg/mL)	SI
T_{64}	444.98	7.82	0.98	76	77.6	59	60.2
T_{65}	459.01	8.32	0.39	81	207.7	60	153.8
T_{66}	475.01	7.74	0.71	62	87.3	41	57.7
T_{67}	479.42	8.53	0.23	83	360.8	77	334.8
T_{68}	462.97	7.96	0.38	68	178.9	52	136.8
T_{71}	513.87	8.99	0.40	79	197.5	61	152.5
T_{72}	415.83	5.96	0.50	86	172	71	142
T_{73}	429.86	6.46	0.47	32	68.1	26	55.3
T_{74}	445.86	5.88	0.89	47	52.8	30	33.7
T_{75}	450.28	6.67	0.72	98	136.1	69	95.8
T_{77}	421.86	5.81	0.89	68	76.4	42	47.2
T_{78}	491.93	7.84	0.58	57	98.3	44	75.9
T_{79}	484.72	7.13	0.38	49	128.9	36	94.7
T_{80}	442.86	6.75	1.56	120	76.9	82	52.6
T_{85}	507.33	7.51	1.56	440	282.1	168	107.7
T_{88}	460.85	6.91	1.56	32	20.6	21	13.46
T_{89}	495.30	7.69	1.56	48	30.8	35	22.4

CLogP calculated by ChemDraw version 8.0; Inhibitory concentration (IC_{50}) calculated against HeLa/Vero cells; Selectivity Index (SI) is the ratio of cytotoxicity IC_{50} (µg/mL) to *in vitro M.tb*$H_{37}Rv$ expressed in MIC (µg/mL).

6.6 CONCLUSIONS

A new series of 2-(1,3-disubstituted-*1H*-pyrazol-4-yl)-5-substituted-*1H*-benzimidazoles $\mathbf{T_{64\text{-}79}}$ and 4-(2-chloro-6-trifluoromethylbenzyloxymethyl)-1,3-disubstituted phenyl-1*H*-pyrazoles $\mathbf{T_{80\text{-}97}}$ were synthesized and were characterized by FT-IR, NMR, Mass spectroscopy and CHN-S elemental analyses. Target compounds were screened for their antimicrobial activity using MIC method, antitubercular activity was carried by MABA method and confirmed that, the newly synthesized compounds were good antibacterial, antifungal and antitubercular agents. Almost all the compounds in benzimidazole derivatives have shown excellent activity against *Mycobacterium tuberculosis.* Among all these derivatives, $\mathbf{T_{67}}$ showed most potent antitubercular activity with a MIC value of 0.23 µg/mL, it was equipotent with

standard Isoniazid (INZ). Compounds **T_{64}**, **T_{65}**, **T_{67}**, **T_{68}**, **T_{71}**, **T_{73}**, **T_{74}** and **T_{79}** are showed good activity against bacteria with a MIC value of 1.56 µg/mL and **T_{67}**, **T_{68}**, **T_{73}** and **T_{77}** against fungal strains. In the other series, MIC value of 1.56 µg/mL for **T_{80}**, **T_{85}**, **T_{88}** and **T_{89}** for tuberculosis bacteria *Mycobacterium tuberculosis*. In addition, all newly synthesized compounds were tested for antimicrobial activity and proved that these compounds were active as an antimicrobial agent. Cytotoxicity studies were carried for the most active compounds, and the best cytotoxicity value was obtained for compound **T_{85}** against cervical cancer-HeLa cell line with IC_{50} value 440 µg/mL and Vero cell line with IC_{50} value 88 µg/mL. It concludes that, majority of the compounds showed good activity against *Mycobacterium tuberculosis* and good cytotoxicity with higher selectivity index. Hence, target pyrazole containing benzimidazole/trifluoromethyl derivatives can be tested further for lead molecule for antitubercular and antimicrobial drugs.

CHAPTER 7

SUMMARY AND CONCLUSIONS

Abstract

This chapter presents the brief summary and conclusions of the research work. In addition, the scope of future work is deliberated.

7.1 SUMMARY

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

Literature survey on pyrazole based molecules reveals its significance in the field of medicinal chemistry. Hence, in the present study, pyrazole moiety was chosen as the core structural and various pharmacophores such as thiazole, pyrazoline, 1,4-dihydropyridine, 1,3,4-oxadiazole and thiadiazole, benzimidazole and trifluoromethyl benzyloxy substituted derivatives were incorporated on this core moiety, with the hope of getting enhanced anti-TB activity.

Accordingly, the following five new series of target compounds were synthesized through multistep organic synthetic routes.

a) Pyrazole carrying thiazole derivatives (**T_{1-12}**).
b) Pyrazole carrying pyrazoline derivatives with different substitutions (**T_{13-27}**).
c) 1,4-Dihydropyridine derivatives on the fourth position of pyrazole moiety (**T_{28-45}**).
d) Pyrazole containing 1,3,4-oxadiazole and [1,2,4]triazolo[3,4-*b*] [1,3,4]thiadiazole derivatives (**T_{46-63}**).
e) Pyrazole linked benzimidazole and trifluoromethyl benzyloxy substituted derivatives (**T_{64-97}**).

The newly synthesized compounds were purified by recrystallization or column chromatography techniques and their synthetic methods were established. The structures of new intermediates and target compounds were confirmed by various spectral techniques. Further, X-ray crystallographic study was carried out for few compounds in order to elucidate their final structure. All the target compounds were screened for their *in vitro* antitubercular study following MABA methodology, by taking Isoniazid, Ethambutol, Ciprofloxacin, Streptomycin and Pyrazinamide as

standard anti-TB drugs. Based on the results of *in vitro* studies showed that among 97 compounds, *In vitro* assay results showed that 6 compounds showed most active anti-TB activity against *Mycobacterium tuberculosis* $H_{37}Rv$ with MIC value of ≤0.39 µg/mL, 9 compounds with MIC value of 0.79 µg/mL and another 10 compounds with MIC value of 1.56 µg/mL. Some of the selected compounds were taken for cytotoxicity studies to check their toxicity using MTT assay against non-cancerous cell lines. In addition, *in vitro* antimicrobial screening studies were carried out with bacterial strains, *M. smegmatis* and *S. aureus* fungal strain *C. albicans.*

7.2 CONCLUSIONS

The following conclusions have been drawn from the present research work.

- Newly designed compounds **T_{1-97}** were successfully designed and synthesized. Their synthetic methods, as well as purification techniques, were established.
- Structures of newly synthesized compounds have been established using FT-IR, ^{1}H NMR, ^{13}C NMR and mass spectroscopy followed by elemental analysis.
- Single crystal XRD studies established the 3D structure of the compounds **25c**, **T_{29}**, **T_{80}**, **T_{83}**, **T_{86}** and **T_{88}**.
- The *in vitro* antitubercular study by MABA method results indicated that some of the new pyrazole containing thiazole, pyrazoline, dihydropyridine, oxadiazole/thiadiazole, benzimidazole and trifluoromethyl benzyloxy substituted derivatives displayed better activity when compared to the standard drugs.
- The *in vitro* cytotoxicity studies of the potent compounds against noncancerous cells revealed that these compounds are not toxic to normal cells, thus signifying their suitability for further drug development.
- The compounds **T_{50}**, **T_{51}**, **T_{65}**, **T_{67}**, **T_{68}** and **T_{79}** have exhibited most potent anti-TB activity with MIC value ≤0.39 µg/mL and were more potent than standard drugs EMB, INN, STP and PZA. In addition, 9 compounds **T_{49}**, **T_{59}**, **T_{60}**, **T_{66}**, **T_{71}**, **T_{72}**, **T_{73}**, **T_{75}** and **T_{78}** showed activity with MIC of 0.40-0.79 µg/mL. Another 10 compounds, **T_{27}**, **T_{53}**, **T_{58}**, **T_{64}**, **T_{74}**, **T_{77}**, **T_{80}**, **T_{85}**, **T_{88}** and **T_{89}** were displayed significant activity with MIC of 0.80-1.56 µg/mL. Further, the

cytotoxicity study revealed the non-toxic nature of the active molecules to noncancerous cells.

- Among all synthesized derivatives pyrazole linked nitro/thiopropyl substituted benzimidazole derivatives displayed most potent anti-TB activity. 4-Chlorophenyl substitution on the first position of pyrazole ring playing a key role to enhance the activity of most of the synthesized compounds.
- *In vitro* antibacterial results showed significant activity of compounds **T_{54}**, **T_{80}**, **T_{88}**, **T_{89}** and **T_{97}** with MIC value of 3.12 µg/mL against tested bacterial strains and these are promising antibacterial agents. *In vitro* antifungal activity exhibited significant activity for compounds **T_{80}** and **T_{97}** with MIC of 3.12 µg/mL.
- A combination of pyrazole with certain other biologically important heterocyclic entities in a single framework has enhanced the biological activities. Hence, they are ideally suited for further modification to obtain more efficient antitubercular and antimicrobial agents.

7.3 SCOPE FOR FUTURE WORK

- Based on results of anti-TB activity, 25 molecules showed good inhibition against *Mycobacterium tuberculosis* and these molecules are non toxic to normal cell. These molecules can be considered as potent molecules and could be taken for further *in vivo* and enzymatic studies for the drug development.
- Antitubercular and antimicrobial 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).
- Further structural modification of the active structural backbone in order to improve the potency and to explore in detail about the anti-TB behavior of the resulting structures in biological surroundings could be a good scope in the future.

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LIST OF PUBLICATIONS

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N. Harikrishna., Arun M. Isloor., K. Ananda., Abdulrahman Obaid. and Hoong-Kun Fun. (2015). "1,3,4-Trisubstituted pyrazole bearing 4-(chromen-2-one) thiazole: Synthesis, characterization and its biological studies." *RSC Adv.*, 5, 43648-43659. **Impact factor: 3.289.**

N. Harikrishna., Arun M. Isloor., K. Ananda., Abdulrahman Obaid and Hoong-Kun Fun. (2016). "Synthesis, and antitubercular and antimicrobial activity of 1'-(4-chlorophenyl)pyrazole containing 3,5-disubstituted pyrazoline derivatives." *New J. Chem.*, 40, 73-76. **Impact factor: 3.277.**

N. Harikrishna., Arun M. Isloor., K. Ananda., Hazem A. Ghabbour, Joazaizulfazli Jamalis. and H-K Fun. "Antitubercular and antimicrobial activity of NH_4VO_3 promoted 1,4-dihydropyridine containing 1,3,4-trisubstituted pyrazole." *Lett. Drug Design & Discovery,* Accepted manuscript, In-press. **Impact factor: 0.974.**

N. Harikrishna., Arun M. Isloor., K. Ananda., Tanya Parish., Hazem. A. Ghabbour., C. K. Quah and H-K. Fun. "2-Chloro-6-trifluoromethylbenzyloxymethyl containing 1,3,4-trisubstituted pyrazole: Synthesis, characterization, antitubercular and antimicrobial studies." *Eur. J. Med. Chem.*, Communicated on March 2017.

N. Harikrishna., Arun M. Isloor., Tanya Parish., Joazaizulfazli Jamalis. and Sandeep. "Highly potent antitubercular agents: Synthesis of pyrazole linked [1,3,4]oxadiazole and [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole analogs." *Eur. J. Med. Chem*., Communicated on March 2017.

N. Harikrishna., Arun M. Isloor., K. Ananda., Tanya Parish. and Joazaizulfazli. Jamalis. "1′-(4-Chlorophenyl)pyrazole containing benzimidazole derivatives: Synthesis, characterization and their antitubercular activity." *Eur. J. Med. Chem*., Communicated on April 2017.

Research papers presented in national / international conferences

Poster presented on research paper titled "Synthesis, characterization of some new 1,3,4-trisubstituted pyrazole containing 1,4-dihydropyridine derivatives and its biological studies." *National conference - 10thMid year CRSI-India*, NIT-Trichy, July 23-25, 2015, Pg. No. 90.

Poster presented on research paper titled "1'-(4-Chlorophenyl) pyrazole containing 3,5-disubstituted pyrazoline derivatives: Synthesis, characterization and its antitubercular studies." *International conference – ICRAMCS 2016*, Jhansi, March 2-5, 2016, Pg. No.66.

CURRICULUM VITAE

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Educational Background:

Ph.D (Medicinal organic chemistry) (Jan-2013 to Oct-2016): Title of the thesis - "Synthesis of some new pyrazole derivatives and their antituberculosis screening." Under the guidance of **Dr. Arun M. Isloor,** Department of Chemistry, NITK Surathkal, Karnataka.

- **M.Sc** (Organic Chemistry) (June 2001 to April 2003) from **Acharya Nagarjuna University**, first class with 62%.
- **B.Sc** (Mathematics, Physics, Chemistry) (July 1997 to April 2000) from A.J. Kalasala, Machilipatnam, Acharya Nagarjuna University, First class with 69%.

Industrial Experience:

- Working as Asst. Manager, *Research & Development* in **SeQuent Scientific Limited, Mangalore** from Dec 2010 to till date.
- Worked as Research executive, *Research & Development* in **Orchid Chemical & Pharmaceuticals Limited, Chennai** from Jul 2008 to Dec 2010**.**
- Worked as Jr. Scientist, *Research & Development* in **Virchow Laboratories Limited, Hyderabad** from Apr 2004 to Jul 2008**.**

Job Profile:

The work involved designing and synthesis of novel biologically active organic molecules and pharmacological properties.

Job Responsibilities:

- Handling of projects independently/ under the supervision of team leader.
- Stabilizing and synthesizing of new Scaffolds and making Libraries on it, Training fresh chemists and coordinating the lab.
- Handling of reactions from milligram to kilogram scale synthesis.
- Process development, process optimization in R&D scale and scale up from R&D scale to pilot scale, finally to production scale with cost effectively.
- Characterization of molecules by using ^{1}H NMR, ^{13}C NMR, MS, HPLC, LC-MS, UV, FT-IR and GC.
- Preparation of Technology Transfer Document report and MDD reports.

Technical Skills:

- Literature search for the preparation, properties and biological activity of organic compounds.
- Oxidations using $KMnO_4$, OsO_4, Swern oxidation, PCC, Dess–Martin periodinane, sodium chlorite and MnO_2.
- Reductions using LAH, $NaBH_4$, DIBAL-H, BH_3-THF, $NaBH(OAC)_3$ $NaCNBH_3$, Pd/C, Raney Ni, $Pd(OAC)_2$ in CH_3COOH.
- Generation of enolates using LDA, *n*-BuLi, NaH, LiHMDS and NaHMDS.
- Reactions handled Click, Vilsmeier-Haack, Buchwald, Suzuki coupling, Sonogashira coupling and Heck Reactions.
- Halogenations, esterification, hydrolysis, methylation, demethylation, acetylation, benzylation, debenzylation, peptide coupling reactions and silylation reactions by using different reaction conditions.

Analytical techniques:

- Characterization of molecules by using ^{1}H NMR, ^{13}C NMR, MS, HPLC, LC-MS, UV, GC and SEC.

Projectes handled:

- Oseltamivir phosphate
- Sofosbuvir
- Praziquantel
- Succinyl choline chloride
- Citicoline sodium
- L-Selenomethionine
- Cetirizine hydrochloride
- Clopidogrel bisulfate (Form-1)
- Venlafaxine hydrochloride
- Levofloxacin hemihydrates
- Sumatriptan succinate
- Zolmitriptan

Computer Awareness:

- MS Office, ISIS Draw, Chemdraw, SAP, MDL Cross fire and Scifinder.

Instruments Handled:

- Auto clave (high-pressure reactions)
- Microwave (biotage)
- Flash column chromatography
- UV-Vis Spectrophotometer
- Bruker Alpha FT-IR Spectrometer

Publications:

N. Harikrishna., Arun M. Isloor., K. Ananda., Abdulrahman Obaid. and Hoong-Kun Fun. (2015). "1,3,4-Trisubstituted pyrazole bearing 4-(chromen-2-one) thiazole: Synthesis, characterization and its biological studies." *RSC Adv*., 5, 43648-43659.

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N. Harikrishna., Arun M. Isloor., K. Ananda., Tanya Parish., Hazem. A. Ghabbour., C. K. Quah and H-K. Fun. "2-Chloro-6-trifluoromethylbenzyloxymethyl containing 1,3,4-trisubstituted pyrazole: Synthesis, characterization, antitubercular and antimicrobial studies."*Eur. J. Med. Chem*., Under review.

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N. Harikrishna., Arun M. Isloor., K. Ananda., Tanya Parish. and Joazaizulfazli. Jamalis. "1′-(4-Chlorophenyl)pyrazole containing benzimidazole derivatives: Synthesis, characterization and their antitubercular activity." Manuscript prepared.

Papers presented in national / international conference

Poster presented on research paper titled "Synthesis, characterization of some new 1,3,4-trisubstituted pyrazole containing 1,4-dihydropyridine derivatives and its biological studies." *National conference - 10thMid year CRSI-India,* NIT-Trichy, July 23-25, 2015, Pg. No. 90.

Poster presented on research paper titled "1'-(4-Chlorophenyl) pyrazole containing 3,5-disubstituted pyrazoline derivatives: Synthesis, characterization and its antitubercular studies." *International conference – ICRAMCS 2016,* Jhansi, March 2-5, 2016, Pg. No.66.

Declaration:

Hereby I declared that the above information furnished by me is true best of my knowledge.

Yours truly,

(Nandam. Harikrishna)

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