SYNTHESIS, CHARACTERIZATION AND STUDIES ON ANTITUBERCULAR ACTIVITY OF SOME 1,3,4-THIADIAZOLE BASED MOLECULES

Thesis

Submitted in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

by

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June, 2016

DECLARATION

By the Ph.D. Research Scholar

I hereby *declare* that the thesis entitled **"Synthesis, characterization and studies on antitubercular activity of some 1,3,4-thiadiazole based molecules"** which is being submitted to the National Institute of Technology Karnataka, Surathkal in partial fulfillment of the requirements for the award of the Degree of **Doctor of Philosophy** in Chemistry is a *bonafide report of the research work carried out by me.* The material contained in this thesis has not been submitted to any University or Institution for the award of any degree.

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CERTIFICATE

This is to *certify* that the thesis entitled "**Synthesis, characterization and studies on antitubercular activity of some 1,3,4-thiadiazole based molecules**" submitted by **Mr. J Ramprasad (Register Number: 123004CY12F06)** as the record of the research work carried out by him is *accepted as the Research Thesis submission* in partial fulfillment of the requirements for the award of degree of **Doctor of Philosophy.**

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Chairman-DRPC
Date:

Dedicated

to Lord Siva and my family

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ABSTRACT

In the last few decades, the process of drug discovery program has undergone a fundamental transformation, thanks to the integrated approach of chemists and biologists resulting in the development of new chemical entities (NCEs). Although the increasing costs of production, research and development worries the pharmaceutical industry, synthetic organic approach towards designing innovative and low molecular weight chemical structures, which are also biologically active, will definitely aid in combating the ailments prevailing universally. Such endeavours have led us to domain of heterocyclic chemistry and thiadiazole is one such frequently encountered heterocycle which has proven itself with a diverse range of biological activities. Owing to this therapeutic degree of thiadiazole and its derivatives, in the current work, it has been planned to integrate various potent heterocyclic units with the thiadiazole skeleton to form a new molecular framework. Accordingly, five different libraries of thiadiazole based compounds comprising of benzimidazole (**T1-T29**), 1,2,3-triazole (**T30-T49**), thiazole (**T50-T72**), phenothiazine derivatives (**T73-T93**) and pyrazinamide (**T94-T111**) have been successfully synthesized through multistep organic synthetic protocols. Derivatives **T1-T72** and **T93-T111** were synthesized with a pharmacophore substitution at position-5 of imidazo[2,1-*b*] [1,3,4]thiadiazole ring. Derivatives of **T73-T93** were synthesized by the reaction of 1,3,4-thiadiazole core with substituted phenothiazine as the pharmacophore unit. The chemical structures of the prepared molecules were established by various spectroscopic techniques viz. ^{1}H NMR, ^{13}C NMR, ESI-MS and elemental analyses. Additionally, 3-dimensional structures of a few molecules were confirmed by single crystal X-ray diffraction (SCXRD) studies. Further, the synthesized title compounds were subjected to preliminary *in vitro* antitubercular and antibacterial screening. The active molecules in each series were identified and tested for their toxicity on the benign noncancerous cells. The *in silico* molecular modeling studies of these active derivatives were also carried out.

Keywords: Imidazo[2,1-*b*][1,3,4]thiadiazole, 1,3,4-thiadiazole, benzimidazole, 1,2,3-triazole, thiazole, phenothiazine, pyrazinamide, antitubercular activity, antibacterial activity, cytotoxicity studies, *in silico* molecular modeling.

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NOMENCLATURE

ABBREVIATIONS

AIDS	Acquired immuno deficiency syndrome
AntiTB	Antitubercular
ATCC	American type culture collection
B. subtilis	*Bacillus subtilis*
CCDC	Cambridge crystallographic data centre
DNA	Deoxyribonucleic acid
E. coli	*Escherichia coli*
EMB	Ethambutol
ESI-MS	Electrospray ionisation mass spectrometry
FDA	Food and drug administration
GLP	Good laboratory practices
HIV	Human immuno deficiency virus
IL	Ionic liquid
IND	Investigational new drug process
INH	Isoniazid
ITD	Imidazo[2,1-*b*][1,3,4]thiadiazole
m.p	Melting point
MABA	Microplate alamar blue assay
MDR-TB	Multidrug resistant tuberculosis
MIC	Minimum inhibitory concentration
Mtb	*Mycobacterium tuberculosis*
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NMR	Nuclear magnetic resonance
OLEDS	Organic light emitting diodes
ORTEP	Oak ridge thermal ellipsoid plot program
P. aeruginosa	*Pseudomonas aeruginosa*
PDB	Protein data bank
PPA	Polyphosphoric acid
QASR	Quantitative structure activity relationship

RIF	Rifampicin
RNA	Ribonucleic acid
RT	Room temperature
S. aureus	*Staphylococcus aureus*
SCXRD	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	hours
%	Percent
°C	Degree Celsius
K	Kelvin

h	Hour
Hz	Hertz
MHz	Megahertz
<	Less than
>	Greater than
≥	greater than or equal to
Å	Angstrom
±	Plus minus
m/z	Mass to charge ratio

CHAPTER 1

INTRODUCTION

Abstract

This chapter gives a brief introduction to medicinal and heterocyclic chemistry. It also covers a brief description on biological importance of various thiadiazole derivatives. Further, it deals with a brief note on tuberculosis and treatment procedures against Mycobacterium tuberculosis. In the end, the broad objectives of the present work have been highlighted.

1.1 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. The development in this 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 determine the interaction between designed molecule and proteins (biological macromolecules), explain the structure-activity relationship, determine their absorption and distribution throughout the body, and evaluate their metabolic transformations. In the medicinal chemistry research, designing of the drug moieties plays a crucial role. The most important molecular design strategies in this direction are i) structural modification of the known drug molecule (Lima and Barreiro, 2005) ii) computer-aided drug design based on the study on ligand-protein interaction (Åqvist el al. 1994) iii) hybridization of two active pharmacophoric units into a single molecular framework (Lazar et al. 2004) and iv) the random screening of different structural units and proceeding in an observed window of various activity (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 12 years and $270 million from initial discovery to public usage. The drug development process involves 5 steps. In step 1, discovery and development 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 serious side effects (toxicity) to human health. The two types of preclinical research are *in vitro* and *in vivo* studies. Food and Drug Administration (FDA) requires researchers to use good laboratory practices (GLP), defined in medical product development regulations, for preclinical laboratory studies. Usually, preclinical studies are not very large. 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 in people. In step 3 the drugs are tested on people to make sure they are safe and effective. While preclinical research answers basic questions about a drug's safety, it is not a substitute for studies of ways the drug will interact with the human body. "Clinical research" refers to studies, or trials, that are done in people. As the developers design the clinical study, they will consider what they want to accomplish for each of the different clinical research phases and begin the Investigational new drug process (IND), a process they must go through before clinical research begins. 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. The FDA review team thoroughly examines all submitted data on the drug and makes a decision to approve or not to approve it. Step 5 is FDA Post-Market Safety Monitoring. FDA monitors all drug and device safety once products are available for use by the public. 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. Despite the rigorous steps in the process of drug development, limitations exist. Therefore, the true picture of a product's safety actually evolves over the months and even years that make up a product's lifetime in the marketplace. FDA reviews 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.

1.2 HETEROCYCLIC CHEMISTRY

Heterocyclic chemistry is the branch of chemistry which deals with synthesis, characterization and application of heterocycles (Hauptmann, 2003). Heterocyclic compounds are the organic compounds which contain one or more rings

with at least one atom (the heteroatom) being an element other than carbon atom, most frequently oxygen, nitrogen or sulphur.

Heterocyclic compounds are the major components of biological molecules such as DNA and RNA. DNA is the most essential macromolecule of life. Nucleotides are derivatives of pyrimidine and purine ring structures. Essential diet ingredients such as thiamin, riboflavin, nicotinamide, pyridoxal and two of the essential amino acids, viz. tryptophan and histidine belong to the family of heterocyclic compounds. In addition haemoglobin (oxygen carriers in the blood), chlorophyll and enzymes constitute many important known heterocyclic core structures (Remington, 1995). Heterocycles are present in a wide variety of drugs, vitamins, many natural products, biomolecules and biologically active compounds, including antitumor, antibiotic, anti-inflammatory, antidepressant, antimalarial, anti-HIV, antimicrobial, antibacterial, antifungal, antiviral, antidiabetic, herbicidal, fungicidal and insecticidal agents. Many of the heterocycles possess important applications in material science such as dye stuff, fluorescent sensor, brightening agents, information storage, plastics, corrosion inhibitors and analytical reagents. In addition, they have applications in supramolecular and polymer chemistry. Conjugated molecules and polymers derived from aromatic heterocyclic system are of particular interest because these are found to be potential materials for the fabrication of several optoelectronic devices such as organic photovoltaic cells, organic light-emitting diodes (OLEDs), organic field effect transistors, elechrochromic devices etc.

An interesting feature of many 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, bioisosterism, presence of hydrophilic and hydrophobic groups etc. play a major role in the selection of the lead molecule. Some of the interesting properties about heterocyclic compounds make it more passionate for the medicinal chemist to develop variety of new molecules having pharmacological importance.

1) Availability of the low cost raw materials

2) Synthetic feasibility

3) Chemical stability

4) Capacity to incorporate functional groups either as constituents or as part of the ring system.

1.3 CHEMISTRY OF THIADIAZOLE

The thiadiazole nucleus is one of the most important and well-known heterocyclic system. There are mainly four isomers of thiadiazole, that is 1,2,3-thiadiazole, 1,2,5-thiadiazole, 1,2,4-thiadiazole and 1,3,4-thiadiazole (**figure 1.1**). Thiadiazole is a 5-membered aromatic ring system containing a sulfur atom and two nitrogen atoms. The current research on 1,3,4-thiadiazole has been explored more than other isomers. The 1,3,4-thiadiazole ring has reasonably high aromaticity and is a very weak base because of the inductive effect of the sulfur atom. The ring cleaves in presence of aqueous base and is moderately stable in acid solutions. The ring is very electron deficient because of the electron-withdrawing effect of the nitrogen atoms and quite inert toward electrophilic substitution but nucleophilic attack on the ring is susceptible. When substitutions are introduced on the 2^{nd} and 5^{th} position of this ring, it is extremely activated and readily reacts to give various moieties.

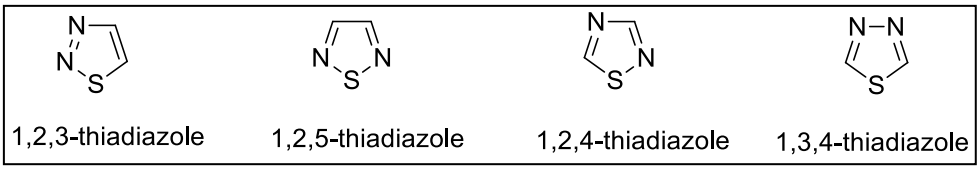
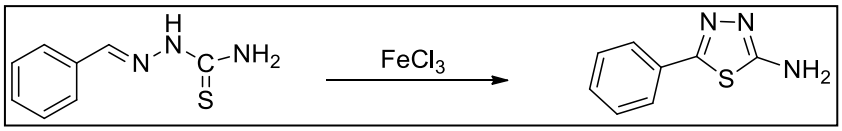


Figure 1.1 Isomers of thiadiazole.

1.3.1 Review for various synthetic methods of 1,3,4-thiadiazoles

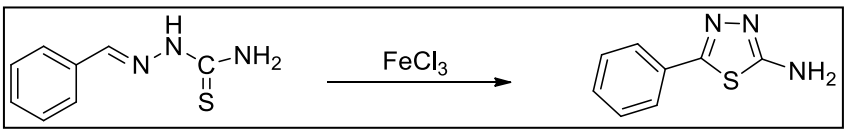
A number of synthetic methods have been reported in the literature to prepare a variety of 1,3,4-thiadiazole derivatives as explained below.

Young and Eyre (1901) reported the synthesis of 5-phenyl-1,3,4-thiadiazol-2-amine by using benzylidene thiosemicarbazide and ferric chloride (**scheme 1.1**). Later a large number of 5-substituted 2-amino-1,3,4-thiadiazoles have been prepared by using this procedure.



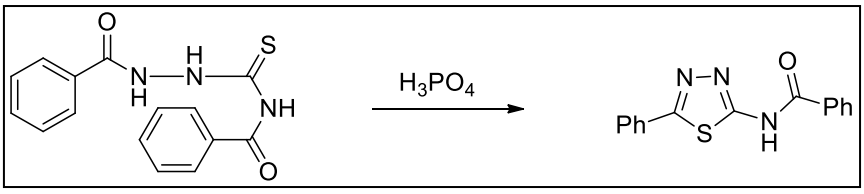
Scheme 1.1

Guha (1922) developed a method for the synthesis of 2-amino-5-mercapto-1,3,4-thiadiazole which involves the reaction of thiosemicarbazide with carbon disulphide and KOH (**scheme 1.2**).



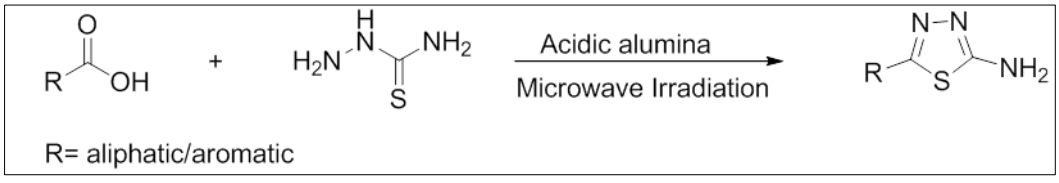
Scheme 1.2

Hoggarth (1949) prepared a number of 2-amino-5-aryl-1,3,4-thiadiazoles using phosphoric acid as the dehydrating agent. An example of smooth cyclization of 1,4-dibenzoylthiosemicarbazide to give *N*-(5-phenyl-1,3,4-thiadiazol-2yl)benzamide is presented in **scheme 1.3**.



Scheme 1.3

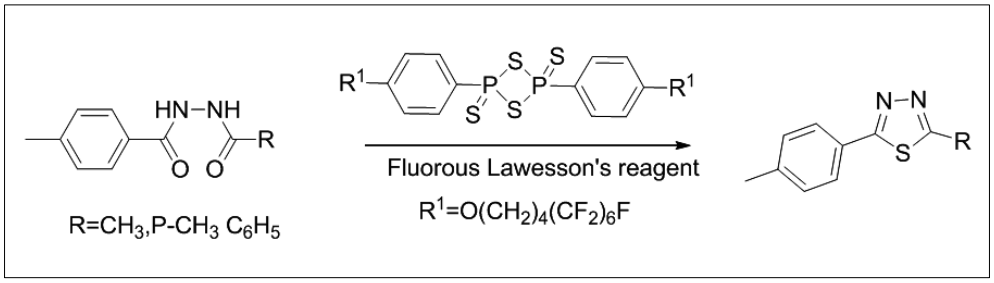
Kidwai et al. (2000) synthesized 5-substituted-2-amino-1,3,4-thiadiazoles within 40–80 sec from a mixture of alkyl/aryl substituted acid, thiosemicarbazide and acidic alumina using microwave irradiation condition (**scheme 1.4**).



Scheme 1.4

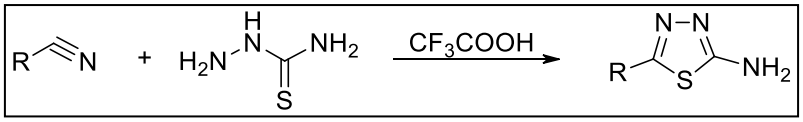
Kaleta et al. (2006) reported the synthesis of 1,3,4-thiadiazoles by thionation of *N,N'*-acylhydrazines using a fluorous derivative of the Lawesson's reagent (**scheme**

1.5). THF was the solvent used and the reaction was carried out at 55 ^{o}C for 6 h. The product was isolated easily by simple filtration of the reaction mixture.



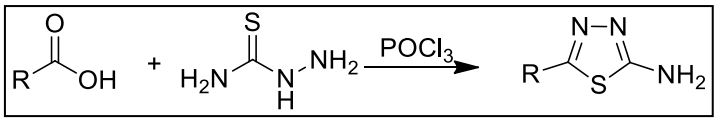
Scheme 1.5

Sancak et al. (2007) synthesized 5-subsituted 2-amino-1,3,4-thiodiazole using aromatic nitrile and thiosemicarbazide in trifloroaceticacid (**scheme 1.6**).



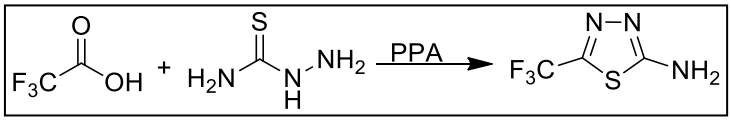
Scheme 1.6

Tu et al. (2008) reported the synthesis of 5-substituted-1,3,4-thiadiazol-2-amine from a mixture of aromatic acid, *N*-aminothiourea and $POCl_3$ under heating (75 ^{o}C for 30 min conditions (**scheme 1.7**).



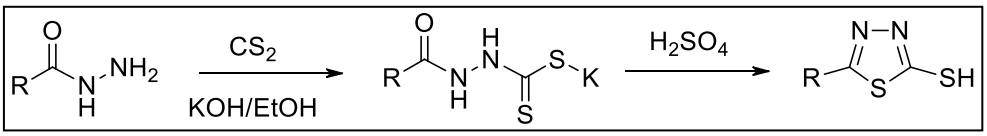
Scheme 1.7

Li and Chen (2008) reported the synthesis of 2-amino-5-trifluoromethyl-1,3,4-thiadiazole from trifluoroaceticacid and thiosemicarbazide in polyphosphoric acid (PPA) at 110 $^{\circ}$C for about 8 h (**scheme 1.8**).



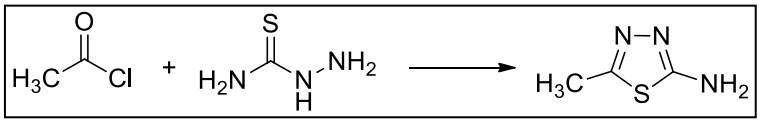
Scheme 1.8

Wei et al. (2009) and Kadi et al. (2010) reported a general synthetic method to prepare dithiocarbazate and 1,3,4-thiadiazole starting from an acylhydrazide (**scheme 1.9**).



Scheme 1.9

Schuttelkopf et al. (2010) reported the synthesis of 5-methyl-1,3,4-thiadiazole-2-amine by treating acetyl chloride with thiosemicarbazide at RT for 4h (**scheme 1.10**).



Scheme 1.10

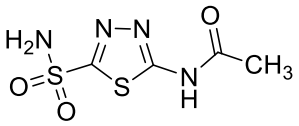
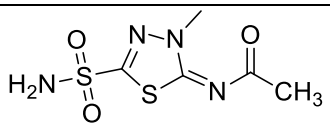
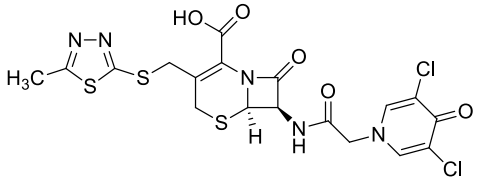
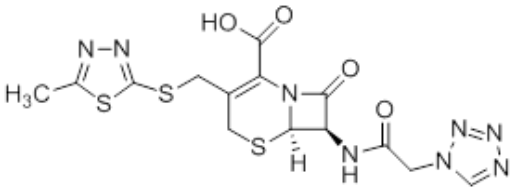
1.4 THIADIAZOLE AND ITS APPLICATIONS

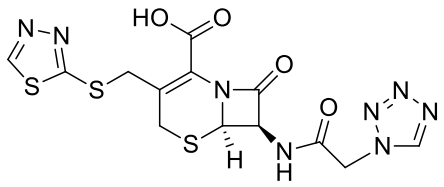
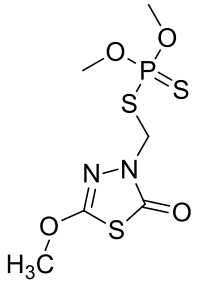
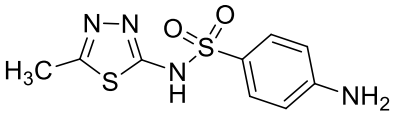
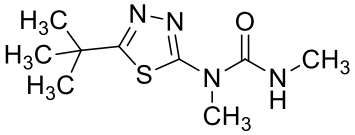
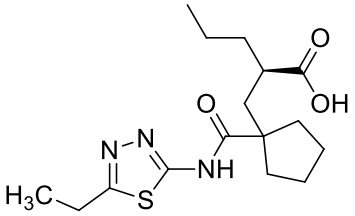
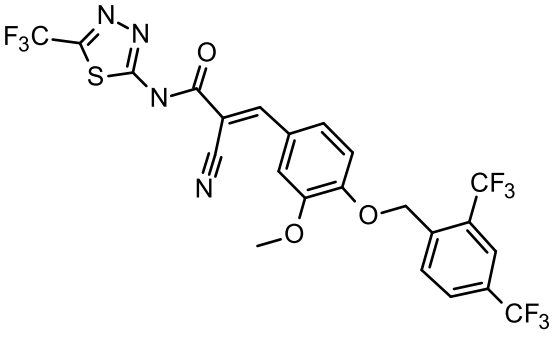
The thiadiazole derivatives exhibit a wide variety of applications in pharmaceutical, agricultural, and materials chemistry. There are several reports in the literature describing the various biological activities of 1,3,4-thiadiazole derivatives and the most relevant and recent studies have revealed that 1,3,4-thiadiazole derivatives have a broad spectrum of pharmacological categories such as antimicrobial (Gadad et al. 2000; Foroumadi et al. 2005; Khalaj et al. 2011), anti-inflammatory (Kadi et al. 2007; Jadhav et al. 2008, antitumor (Karki et al. 2011), antiviral (El-Emam et al. 2004), antitubercular agents (Alegaon et al. 2012), anticonvulsants (Jatav et al. 2008), antidepressant and anxiolytic (Clerici et al. 2001), antihypertensive (Hasui et al. 2011) activities.

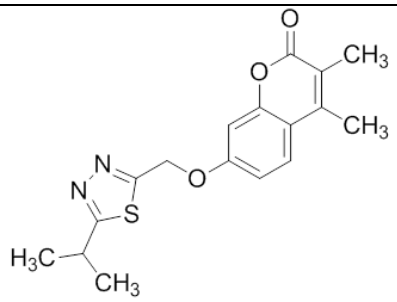
The most important thiadiazole containing drug is acetazolamide, the very well-known carbonic anhydrase inhibitor, being used in treatment of glaucoma (Kaur et al. 2002), high-altitude illness (Luks et al.2010), epileptic seizures (Wolf, 2011) idiopathic intracranial hypertension (Rangwala and Liu, 2007), hemiplegic migraine (Russell and Ducros, 2011), cystinuria (Tiselius, 2010), obstructive sleep apnea

(Jalandhara et al. 2009), congenital myasthenic syndromes (Schara and Lochmuller, 2008), etc. 1,3,4-Thiadiazoles also exhibit high potential as pesticides in the fields of herbicides, fungicides, insecticides, bactericides, and even plant-growth regulators (Gilden et al. 2010). In addition, the electron-deficient 1,3,4-thiadiazole core has good electron-accepting ability and it exhibits good thermal and chemical stability. Hence thiadiazoles are promising materials in the field of optics and electrochemistry. Also, applications are much focused on their charge-transporting capacity, photoluminescence, photoconductivity, mesomorphism to obtain liquid crystals, anticorrosive activity etc. Some of the prominent thiadiazole containing commercial drugs available in the market are tabulated below.

Table 1.1 Thiadiazole containing drugs and their application.

Drug name	structure	Biological importance
Acetazolamide		It is used to treat glaucoma upset stomach, headache, shortness of breath, dizziness.
Methazolamide		It is also a carbonic anhydrase inhibitor.
Cefazedone		It is a semi synthetic first generation cephalosporin antibiotic inhibiting cell wall biosynthesis.
Cefazolin		It is mainly used to treat bacterial infections of the skin. It can also be used to treat moderately severe bacterial infections

Ceftezole		It is a class of cephalosporin antibiotic similar to cefazolin. It is found to be a broad-spectrum antibiotic.
Methidathion		It is an organophosphate insecticide
Sulfamethizole		It is mainly used to treat Gram-Negative, Gram-Positive bacterial infections and urinary tract infections.
Tebuthiuron		It is a nonselective broad spectrum herbicide of the urea class.
UK-414,495		It is a drug developed by Pfizer for the treatment of female sexual arousal disorder.
XCT790		It is a potent and selective inverse agonist ligand of the estrogen-related receptor alpha.

Atibeprone		It is an antidepressant which was developed in the mid-1990s.

1.5 ANTIMICROBIALS AND THEIR IMPORTANCE

Microbes are invisible to the naked eye and hence a powerful microscope is needed to see them. These are everywhere around us, in our food, inside us, on us, in our homes, in the air we breathe. For example our skin, mouth and the intestines are sheltered in millions of individual micro-organisms. Some of these microbes are mostly useful, but some are harmful. In one single teaspoon of garden soil, there are over 100,000 microbes. Microbes are divided into three types. They are bacteria, viruses and fungi.Viruses are the smallest and simplest life form known. They are 10 to 100 times smaller than bacteria. The biggest difference between viruses 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. There are some useful bacteria but all viruses are harmful. Bacteria are mainly of three shapes (**figure 1.2**) spiral (boriella), little balls (-cocci) and rods (-bacilli). Viruses, however, are not cells. They consist of one or more molecules of DNA or RNA, which contain the viruses. Viruses can be rod-shaped, sphere-shaped, or multisided. Some viruses look like tadpoles. Antimicrobials are agents that kills or suppress their multiplication or growth of harmful micro-organisms.

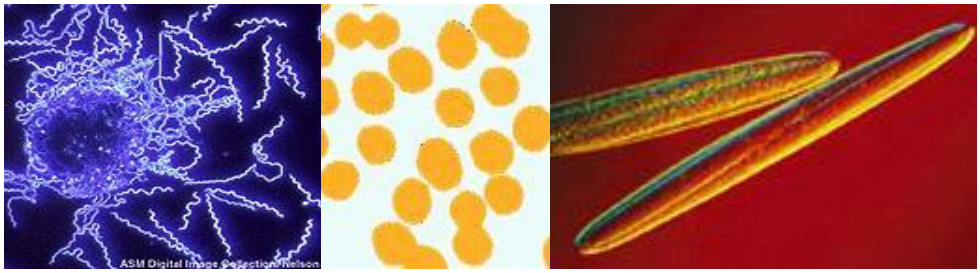


Figure 1.2 Shapes of bacteria (spiral, little balls and rods)

(Source: http://www.microbeworld.org/)

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. They did not know at that time that the reason one bacterium failed to grow was that the other bacterium was producing an antibiotic. Technically, antibiotics are only those substances that are produced by one microorganism that kill, or prevent the growth, of another microorganism. 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 World War II.

After the Second World War, the effort continued to find other novel antibiotic structures. This led to the discovery of a peptide with an antibiotic activity e.g. bacitracin (1945), chloramphenicol (1947), chlortetracycline (1948), the microcline antibiotics e.g. erythromycin (1952), the cyclic peptide antibiotics e.g. cycloserine (1955), and in 1955 the first example of a second major group of cephalosporin C. As far as synthetic agents were concerned, isoniazid (a pyridine hydrazide structure) was found to be effective against human tuberculosis in 1952, and in 1962 nalidixic acid (the first of the quinolone antibacterial agents) was discovered. A second generation of this class of drugs was introduced in 1987 with ciprofloxacin. Many antibacterial agents are now available and the vast majority of bacterial diseases have been brought under control (e.g. syphilis, tuberculosis, typhoid, bubonic plague, leprosy, diphtheria, gas gangrene, tetanus, gonorrhea etc).

Gram staining is a method of differentiating bacterial species into two large groups (Gram-positive and Gram-negative). It is based on the chemical and physical properties of their cell walls. Primarily, it detects peptidoglycan, which is present in a thick layer in Gram-positive bacteria. A Gram-positive results in a purple/blue color with crystal violet while a Gram-negative results in a pink/red color with safranin. The Gram stain is almost always the first step in the identification of a bacterial

organism, and is the default stain performed by laboratories over a sample when no specific culture is referred. It is a valuable diagnostic tool in both clinical and research laboratories.

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

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 (**figure 1.3**).

- **Inhibit cell wall synthesis:** Some interfere with the synthesis of the bacterial cell wall. This results in cell lysis because the contents of the bacterial cell are hypertonic and therefore under high osmotic pressure. A weakening of the cell wall causes the cell to rupture, spill its contents, and be destroyed. The penicillins, cephalosporins, bacitracin and vancomycin are examples of this group of antimicrobials.

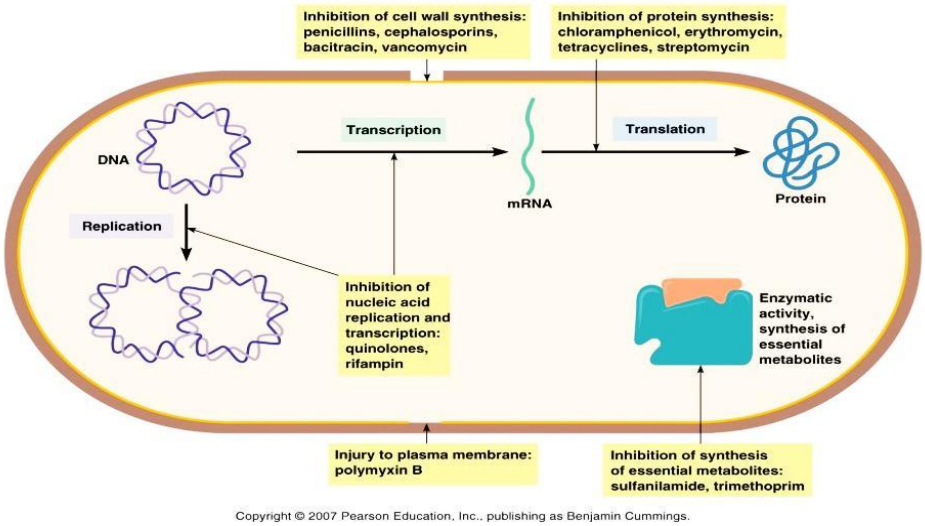


Figure 1.3 Schematic representation of mechanism of action on bacterial cell (Source: http://www.cs.stedwards.edu)

- **Inhibition of protein synthesis**: A second group of antimicrobial agents inhibiting protein synthesis. The chloramphenicol, erythromycin, streptomycin, tetracyclines are examples of this group of antimicrobials.
- **Injury to plasma membrane**: A third group of 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. Examples include amphotericin B and polymyxin B.
- **Inhibit synthesis of essential metabolites:** A fourth group of antimicrobial agents interfere with metabolic processes (DNA replication and transcription) within the microorganism. Most of these agents are bacteriostatic. Examples include the sulfonamides, aminosalicylic acid (PAS) and isoniazid (INH).
- **Inhibition of the synthesis of essential metabolites**: Generally sulfas and trimethoprim functions by this way. They interfere with the pathway on which bacteria synthesize folic acid. Since humans produce folic acid by a different pathway, these drugs have less effect on human cells.

1.5.3 Some important diseases caused by bacteria

Most of the bacteria are harmless and some of bacteria are beneficial, several are pathogenic. One of the dangerous bacterial diseases is tuberculosis, infected by the *Mycobacterium tuberculosis (Mtb).* Pathogenic bacteria contribute to other globally important diseases, such as pneumonia, which can be caused by bacteria such as Streptococcus and Pseudomonas, and foodborne illnesses, which 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*

Tuberculosis (TB) is caused by *Mtb* (**figure 1.4**) that most often affect the lungs. Tuberculosis is curable and preventable. TB is spread from person to person through the air. When people with lung TB cough, sneeze or spit, they push the TB germs into the air. A person needs to breathe in only a few of these germs to become infected. TB never develops symptoms because the bacteria can live in an inactive form in the body. But if the immune system weakens, such as in people with HIV, TB

bacteria can become active. In their active state, TB bacteria cause death of tissue in the organs they infect. Active TB disease can be cause death if the treatment is not given.

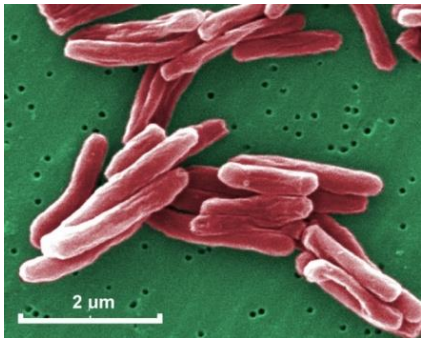


Figure 1.4 Scanning electron micrograph of *Mtb*

(Source: www.microbiologyinpictures.com)

TB is the second disease after HIV/AIDS as the greatest killer worldwide due to a single infectious agent. According to the report released from world health organization (WHO) in 2014, 8.6 million people fell sick with TB and 1.3 million died from TB. Over 95% of TB deaths occur in low- and middle-income countries. In 2012, an estimated 5,30,000 children became ill with TB and 74,000 HIV-negative children died of TB. However persons with compromised immune systems, such as people living with HIV, malnutrition or diabetes, or people who use tobacco, have a much higher risk of falling ill. When a person develops active TB (disease), the symptoms like cough, fever, night sweats, weight loss etc are seen. This can lead to delays in seeking care, and results in transmission of the bacteria to others. People ill with TB can infect up to 10-15 other people through close contact over the course of a year. Many countries still rely on a long-used method called sputum smear microscopy to diagnose TB. TB disease can be treated by taking several drugs for 6 to 9 months. There are 10 drugs currently approved by the FDA for treating TB. The first-line anti-TB drugs are isoniazid (INH), rifampin (RIF), ethambutol (EMB) and pyrazinamide (PZA). Isoniazid, rifampicin, pyrazinamide, and ethambutol for two months then isoniazid and rifampicin alone for a further four months. These drugs are known as the first line tuberculosis drugs (**figure 1.5**). The second line drugs are only used to treat disease that is resistant to first line therapy (i.e., for extensively drug-resistant tuberculosis (XDR-TB) or multi drug-resistant tuberculosis (MDR-TB). The

second line drugs are less preferred instead of first-line for one of three possible reasons: it may be less effective than the first-line drugs (e.g., *p*-aminosalicylic acid); or, it may have toxic side-effects (e.g., cycloserine); or it may be effective, but unavailable in many developing countries (e.g., fluoroquinolones). All the antitubercular medicines show some or other side effects. Some of the side effects are mentioned in the **table 1.2**.

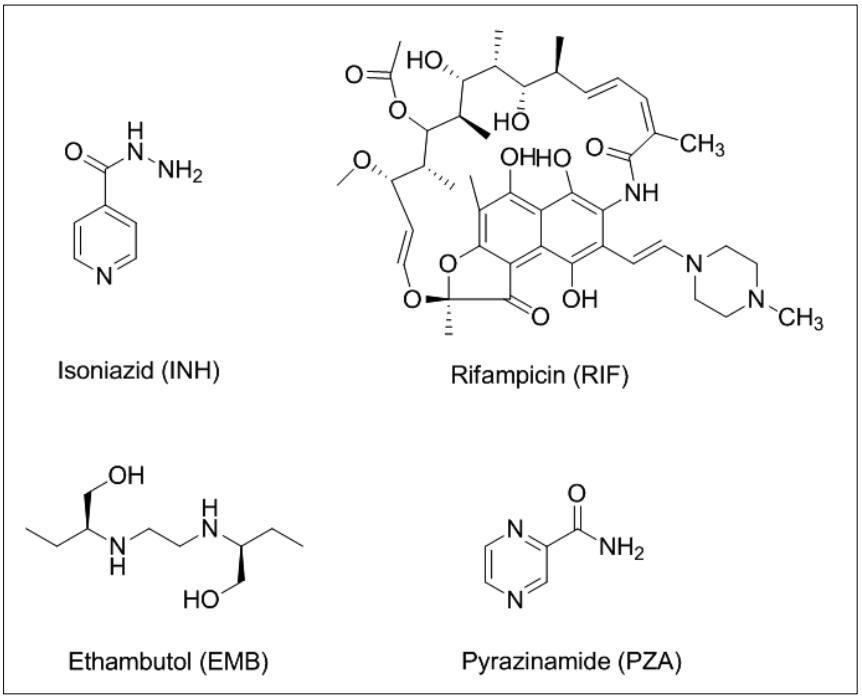


Figure 1.5 Antitubercular drugs available in the market.

Table 1.2 side effects with available 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-positive bacterium. It is frequently found in the human respiratory tract and on the skin. Although *S. aureus* is not always pathogenic, it is a common cause of skin infections (e.g. boils), respiratory disease (e.g. sinusitis), and food poisoning. The treatment of choice for *S. aureus* infection is penicillin; in most countries, however, penicillin resistance is extremely common, and first-line therapy is most commonly a penicillinase-resistant β-lactam antibiotic (for example, oxacillin or flucloxacillin). Combination therapy with gentamicin may be used to treat serious infections, such as endocarditis, but its use is controversial because of the high risk of damage to the kidneys. The duration of treatment depends on the site of infection and on severity.

1.5.3.3 *Streptococcus pneumonia (S. pneumonia)*

It is a Gram-positive bacterium. The bacterium can cause pneumonia. Pneumonia is a lung disease. It is a normal inhabitant of the human upper respiratory tract and can spread to the blood, lungs, middle ear, or nervous system. It is currently the leading cause of invasive bacterial disease in children and the elderly. *S. pneumoniae* is also called pneumococcus. Pneumococcus is spread through contact with people who are ill or who carry the bacteria in their throat. Pneumococcus disease is treated with antibiotics. However, many types of pneumococcus bacteria have become resistant to some of the antibiotics used to treat these infections. Resistance to penicillin and other antibiotics is common. Antibiotic treatment for pneumococcal infections in general includes 'broad-spectrum' antibiotics until results of antibiotic sensitivity testing are available.

1.5.3.4 *Pseudomonas aeruginosa (P. aeruginosa)*

It is a Gram-negative, aerobic, rod shaped bacterium. It is a common bacterium that can cause disease in animals, including humans. It is found in soil, water, skin, and most man-made environments throughout the world. This bacterium leads to cause infections commonly involve pulmory infections, burn wound infections, external otisis and eye infections. Treatment for this disease by using combine effective antibiotics (e.g., amino glycoside and *β*-lactum antibiotics) frequently required.

1.5.3.*5 Salmonella typhimurium (S. typhimurium)*

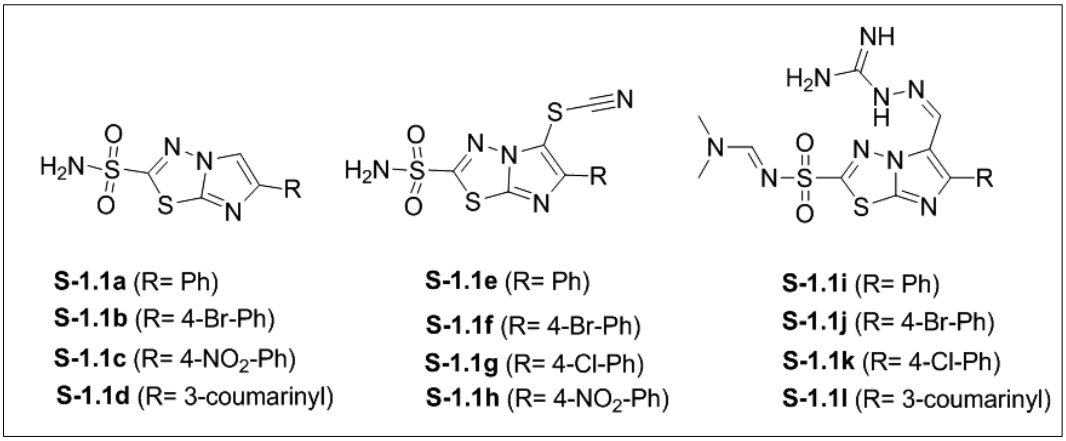
Salmonella is a Gram-negative, a cylindrical rod of size bacterium which causes typhoid fever, with symptoms such as gradually increasing fever, malaise, headache, myalgias, and anorexia, which persist for a week or longer. Infection is caused by consuming contaminated food or drinks. Antibiotics are required to treat typhoid fever such as chloramphenicol, ampicillin, trimethoprim-sulfamethoxazole.

1.5.3.*6 Escherichia coli (E.coli)*

It is a Gram-negative, rod-shaped bacterium. *E. coli* bacteria normally live in the intestines of people and animals. Most *E. coli* are harmless and actually are an important part of a healthy human intestinal tract. However, some *E. coli* are pathogenic, meaning they can cause illness, either diarrhea or illness outside of the intestinal tract. The types of *E. coli* that can cause diarrhea can be transmitted through contaminated water or food, or through contact with animals or persons. The treatment is the assessment of dehydration and replacement of fluid and electrolytes. A semisynthetic rifamycin derivative is an effective and well-tolerated antibacterial for the management of adults with non-invasive traveller's diarrhoea.

1.6 LITERATURE REVIEW

Gadad et al. (2000) evaluated antibacterial activity of various 6-arylimidazo [2,1-*b*][1,3,4]thiadiazole-2-sulfonamide/*N*-(dimethylamino)sulphonamides and their 5-thiocyanato and 5-guanylhydrazone derivatives against *E. coli*, *S. aureus*, *Salmonella typhi*, *P. aeruginosa* and *Pneumococci*. **S-1.1a-d** were inactive against *S. aureus* and *P. aeruginosa*, however they were moderately active against *E. coli*, *Salmonella typhi* and *Pneumococci*, while their 5-thiocyanato (**S-1.1e-h**) and 5-guanylhydrazone derivatives (**S-1.1i-l**) were significantly active against *E. coli* and *S. aureus*. **S-1.1e-h** were reported to be equipotent in activity compared to sulfamethoxazole against *E. coli* and *S. aureus*. The presence of thiocyanato and guanylhydrazone groups at 5-position overall enhanced the antibacterial spectrum of these series of compounds. However the compounds were reported to be less active than norfloxacin.

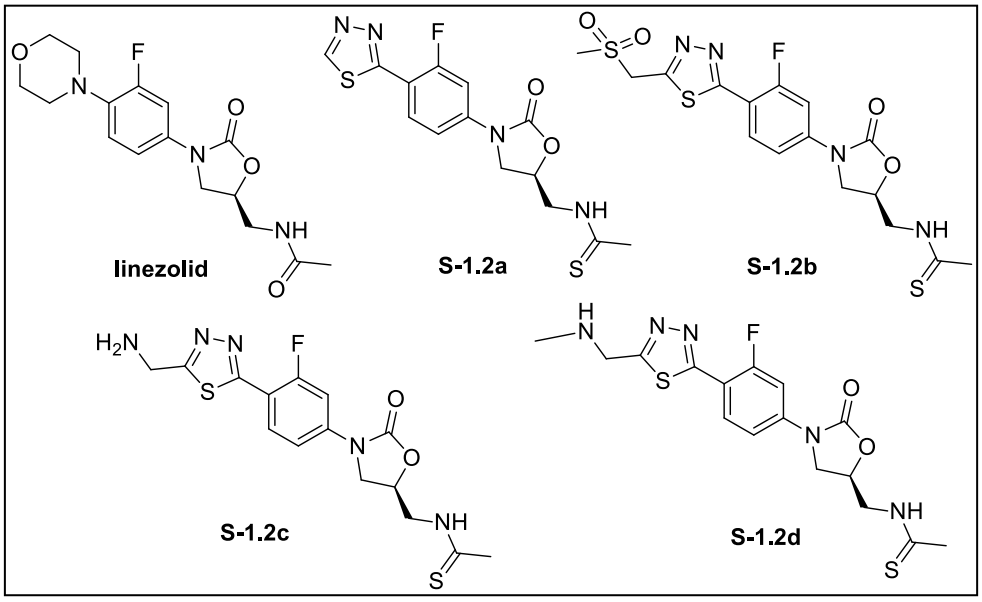


S-1.1a (R= Ph)
S-1.1b (R= 4-Br-Ph)
S-1.1c (R= 4-NO_2-Ph)
S-1.1d (R= 3-coumarinyl)

S-1.1e (R= Ph)
S-1.1f (R= 4-Br-Ph)
S-1.1g (R= 4-Cl-Ph)
S-1.1h (R= 4-NO_2-Ph)

S-1.1i (R= Ph)
S-1.1j (R= 4-Br-Ph)
S-1.1k (R= 4-Cl-Ph)
S-1.1l (R= 3-coumarinyl)

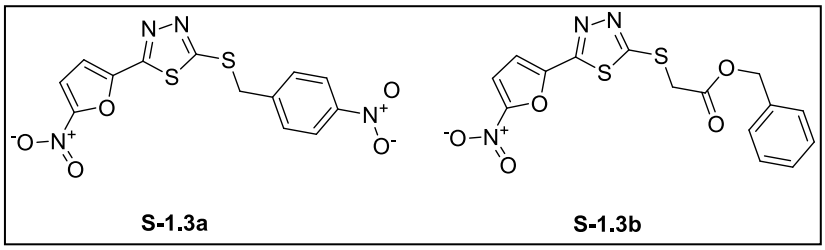
Thomasco et al. (2003) reported the synthesis of 1,3,4-thiadiazolyl ring by replacement of the morpholine c-ring of linezolid with 1,3,4-thiadiazolyl ring and these compounds were screened for antibacterial activity against both Gram-positive and gram-negative organisms. Conversion of the C5 acetamide group to a thioacetamide further increases the potency of these compounds. Compounds **S-1.2a-d** were extremely potent against both Gram-positive and Gram-negative organisms.



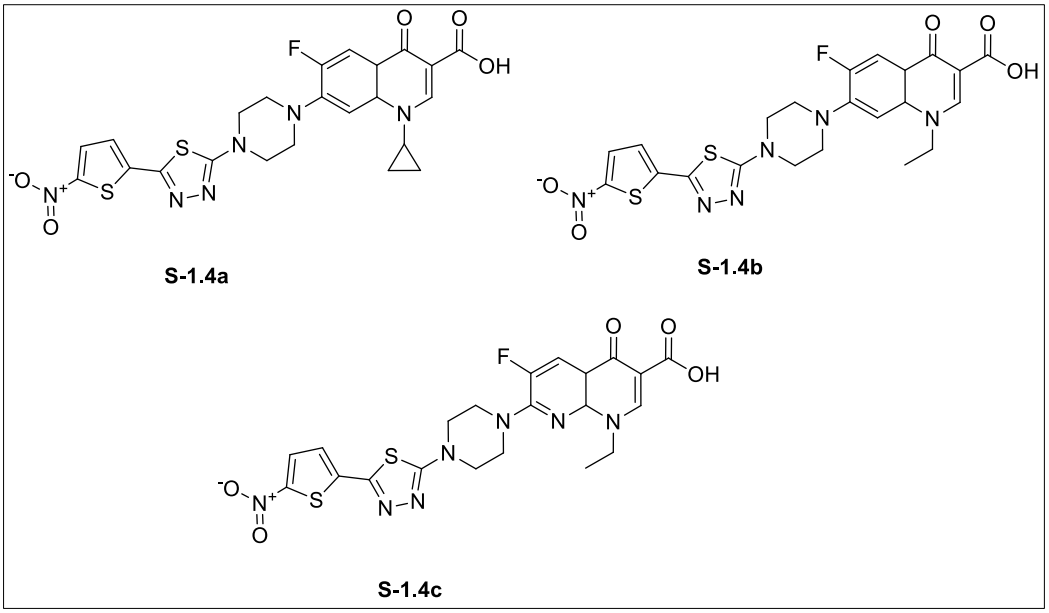
linezolid **S-1.2a** **S-1.2b** **S-1.2c** **S-1.2d**

Foroumadi et al. (2003) synthesized 2-(5-nitro-2-furyl) and 2-(1-methyl-5-nitro-l*H*-imidazol-2-yl)-1,3,4-thiadiazole derivatives and tested their *in vitro* antiTB against *Mtb*. The data was compared with the standard drug rifampin at 0.031 µg/mL concentration which showed 97% inhibition. Compounds with 2-(4-nitrobenzylthio)-5-(5-nitrofuran-2-yl)-1,3,4-thiadiazole substitution (**S-1.3a**) showed the highest

activity against *Mtb* with MIC of 3.13 µg/mL. Among nitro furan derivatives compound **S-1.3b** was found to be the most active (MIC = 0.78 µg/mL) molecule.

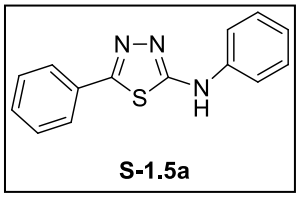


Foroumadi et al. (2003) synthesized a series of *N*-[5-(5-nitro-2-thienyl)-1,3,4-thiadiazole-2-yl]piperazinyl quinolones and evaluated their *in vitro* antibacterial activity. The antibacterial activity of synthesized compounds **S-1.4a**, **S-1.4b** and **S-1.4c** was compared with standard drugs ciprofloxacin, norfloxacin and enoxacin against some Gram-positive (*S. aureus, Staphylococcus epidermidis and B. subtilis*) and Gram-negative (*E. coli, Klebsiella pneumoniae, P. aeruginosa and Enterobacter cloacae*) bacteria using conventional agar dilution procedure. The antibacterial data showed that compounds **S-1.4a**, **S-1.4b**, and **S-1.4c** have a better inhibition activity against tested Gram-positive bacteria than the reference quinolones.



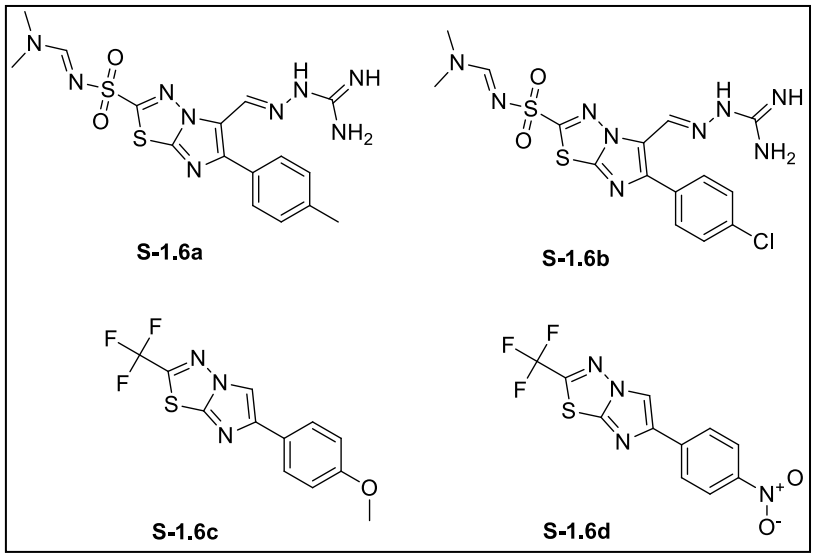
Oruç et al. (2004) synthesized a series of 2,5-disubstituted-1,3,4-thiadiazoles derivatives and screened for their antiTB against *Mtb* H37Rv. Among the screened

compounds, 2-phenylamino-5-(4-fluorophenyl)-1,3,4-thiadiazole (**S-1.5a**) showed the highest inhibitory activity.



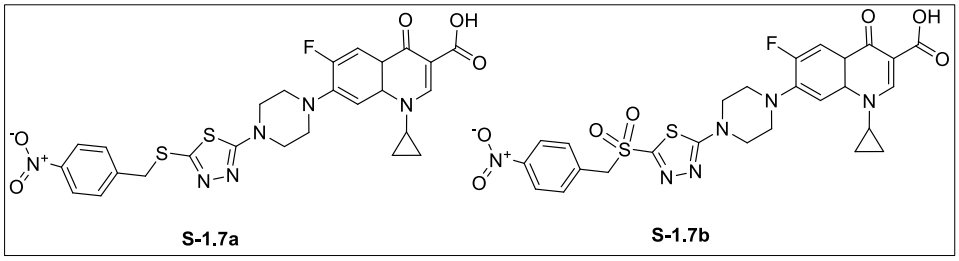
S-1.5a

Gadad et al. (2004) reported the synthesis of a series of 2-sulfonamido/trifluoromethyl-6-(4-substituted aryl/heteroaryl) imidazo[2,1-*b*][1,3,4] thiadiazole derivatives. These compounds were screened for their preliminary *in vitro* antiTB activity against *Mtb* H37Rv strain. The results revealed the moderate anti-tubercular activity of compounds **S-1.6a-d** with percentage inhibition of 43, 58, 31 and 41, respectively, with a MIC of > 6.25 μg/mL.

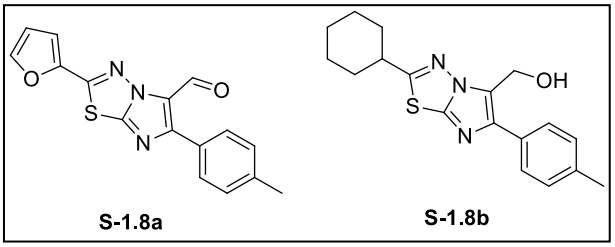


S-1.6a **S-1.6b** **S-1.6c** **S-1.6d**

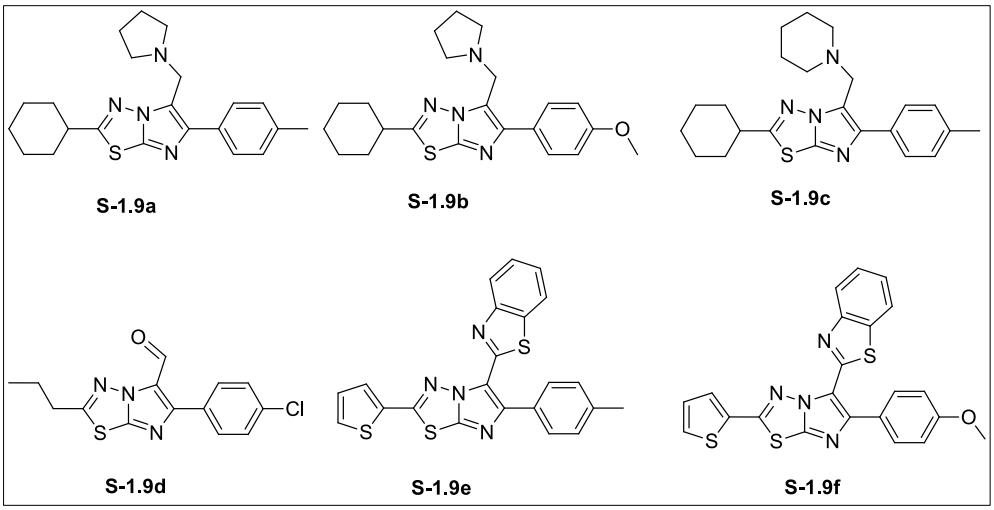
Foroumadi et al. (2005) synthesized a series of N-(5-benzylthio-1,3,4-thiadiazol-2-yl) and N-(5-benzylsulfonyl-1,3,4-thiadiazol-2-yl) derivatives of piperazinyl quinolones and evaluated their antibacterial activity against Gram-positive and Gram-negative microorganisms. Compound **S-1.7a** exhibited significant *in vitro* antibacterial activity against Gram-positive bacteria, with MIC of 0.5, 0.03, and 0.5 μg/mL against *S. aureus*, *S. epidermidis*, and *B. subtilis*, respectively. The structural activity relationship (SAR) of this series indicates that both the structure of the benzyl unit and the S or SO_2 linker dramatically impact antibacterial activity.



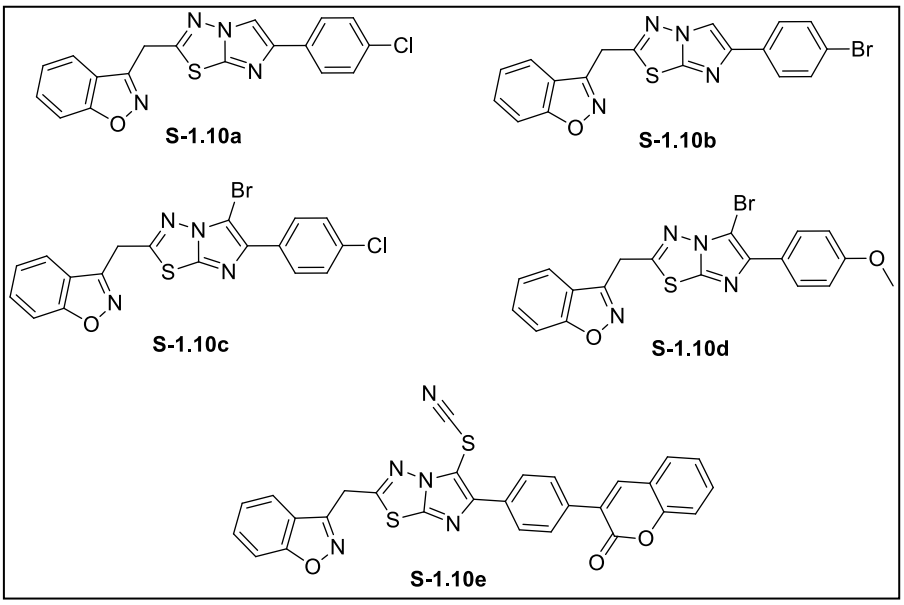
Kolavi et al. (2006) synthesized a series of 2,6-disubstituted and 2,5,6-trisubstituted imidazo[2,1-*b*][1,3,4]thiadiazoles and the compounds were screened for *in vitro* antiTB activity against *Mtb* H37Rv. Among the tested compounds **S-1.8a** and **S-1.8b** have shown the highest (100%) inhibitory activity.



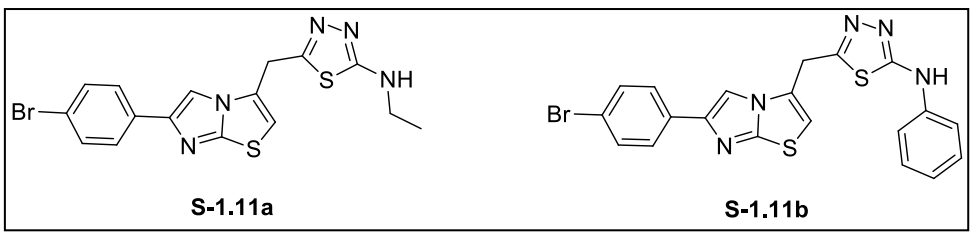
Hegde et al. (2006) synthesized a series of 2,6-disubstituted imidazo[2,1-*b*][1,3,4]thiadiazoles and their new mannich bases and novel benzothiazole derivatives were also synthesized. All the compounds were screened for their antiTB against *Mtb* H37Rv and antibacterial activity against *E. coli*. Among the tested compounds mannich bases **S-1.9a**, **S-1.9b**, **S-1.9c** and 5-carbaldehyde derivative **S-1.9d** have shown excellent inhibition (99, 99, 97 and 95%, respectively) against *Mtb*.



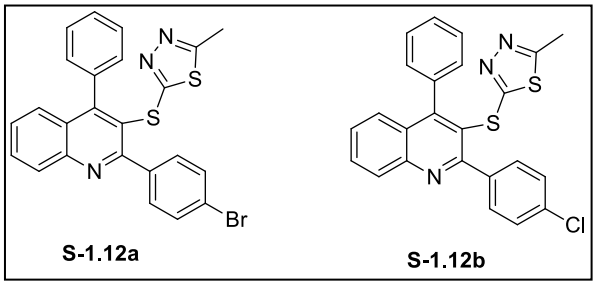
Lamani et.al (2009) synthesized novel methylene bridged benzisoxazolyl imidazo[2,1-*b*][1,3,4]thiadiazoles and screened them for antibacterial activity. The investigation revealed that some of the tested compounds exhibit moderate to good bacterial inhibition. Particularly, compounds **S-1.10a**, **S-1.10b**, **S-1.10c**, **S-1.10d** and **S-1.10e** have shown very good activity against *B. subtilis* and *E. coli*. Compound **S-1.10b** exhibited very good activity against all the bacterial strains, however compounds **S-1.10b** and **S-1.10d** are highly active against *E. coli*-ATCC 35218 when compared to ampicillin. The high activity is attributed to the presence of electron withdrawing chloro and bromo functional groups.



Guzeldemirci and Kucukbasmaci (2009) synthesized 2-alkyl/arylamino-5-((6-(4-bromophenyl)imidazo[2,1-*b*]thiazol-3-yl)methyl)-1,3,4-thiadiazoles. These were tested for their antibacterial activity against *S. aureus*, *P. aeruginosa* and *E. coli*. **S-1.11a** showed the highest activity against *E. coli*. Synthesized compounds were also screened for *in vitro* antiTB activity against *Mtb* H37Rv and **S-1.11b** showed 16% inhibition.

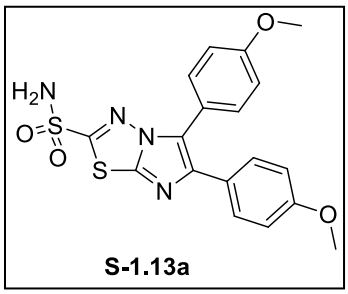


Chitra et al. (2011) synthesized 3-heteroarylthioquinoline derivatives of 1,3,4-thiadiazole and screened them for *in vitro* antiTB activity against *Mtb* H37Rv strain. Compounds 2-[2-(4-bromophenyl)-4-phenyl-3-quinolyl]sulfanyl-5-methyl-1,3,4-thiadiazole (**S-1.12a**) and 2-[2-(4-chlorophenyl)-4-phenyl-3-quinolyl]sulfanyl-5-methyl-1,3,4-thiadiazole (**S-1.12b**) were found to be the most active compounds with MIC of 3.2 and 3.5 μM respectively against *Mtb*. The *in vitro* cytotoxic effects against mouse fibroblasts (NIH 3T3) were evaluated for **S-1.12a** and **S-1.12b**, which displayed no toxic effects (IC_{50} > 1000 μM).



S-1.12a **S-1.12b**

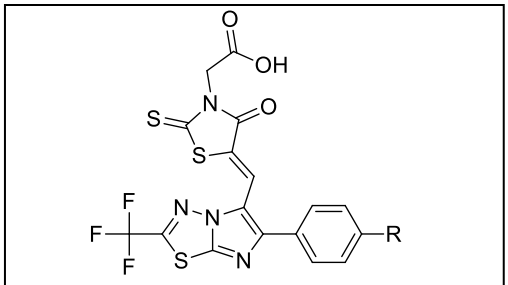
Palkar et al. (2012) reported a novel series of 2-substituted-5,6-diarylsubstituted imidazo[2,1-*b*][1,3,4]thiadiazoles. All the title compounds were tested for their *in vitro* antiTB activity against *Mtb* H37Rv. Among the synthesized compounds, **S-1.13a** exhibited excellent antitubercular activity with MIC of 1.25 μg/mL and comparable with reference drugs. Further, some title compounds were also assessed for their cytotoxic activity (IC_{50}) against in a mammalian vero cell line using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The results reveal that these compounds exhibit antitubercular activity at non-cytotoxic concentrations.



S-1.13a

Alegaon et al. (2012) reported synthesis of novel imidazo[2,1-*b*][1,3,4] thiadiazole carrying rhodanine-3-acetic acid and evaluated for their *in vitro* antiTB

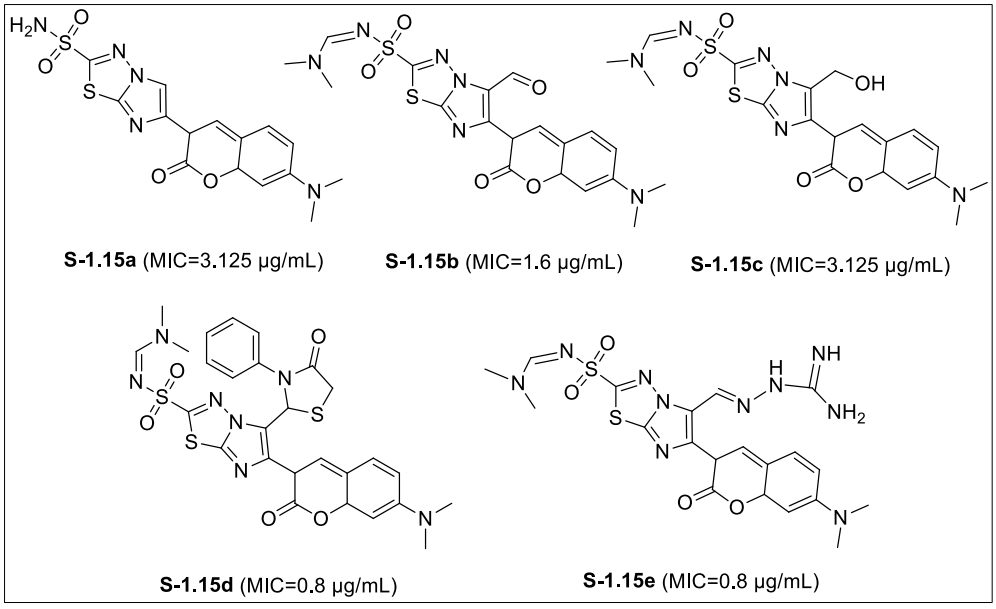
against *Mtb* H37Rv. Moreover, various drug-likeness properties of new compounds were predicted. Seven compounds from the series exhibited good activity with MIC in range 3.12–1.56 µg/ml. AntiTB activity study suggests that compounds (**S-1.14a-d**) may serve as promising lead scaffolds for further generation of new antiTB agents.



S-1.14a-d

Where a: R=CH_3, b: R= Br, c: R= F, d: R= OCH_3

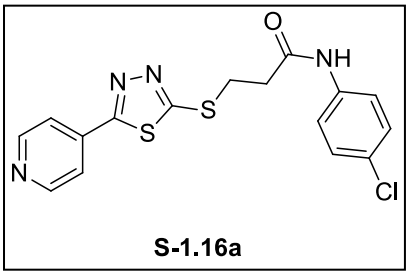
Joshi et al. (2013) synthesized series of 2,5,6-trisubstituted imidazo[2,1-*b*][1,3,4]thiadiazoles and screened for antiTB activity against *Mtb H37Rv* strain and antibacterial activity against *S. aureus*, *B. subtilis*, *Vibrio cholerae* and *E. coli*. Some compounds exhibited significant antibacterial and antitubercular activities. Compounds **S-2.15a-e** were emerged as the most active molecules, which showed significant *in vitro* antiTB activity.



S-1.15a (MIC=3.125 µg/mL) **S-1.15b** (MIC=1.6 µg/mL) **S-1.15c** (MIC=3.125 µg/mL)

S-1.15d (MIC=0.8 µg/mL) **S-1.15e** (MIC=0.8 µg/mL)

Mahajan and Dhawale (2015) reported the synthesis of pyridinyl-thiadiazole derivatives and evaluated for antiTB activity against *Mtb*. Among the screened compounds, *N*-(4-chlorophenyl)-3-((5-(pyridin-4-yl)-1,3,4-thiadiazol-2-yl)thio)-propanamide(**S-1.16a**) showed the highest inhibitory activity with MIC of 0.07 μM.



S-1.16a

1.7 SCOPE OF THE PRESENT WORK

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 20 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 show limited efficacy, further cause serious problem in TB control. 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. This translates into an estimated 4,80,000 people having developed MDR-TB in 2013. Thus there is an emergent need to develop more effective drugs with minimum side effects to treat XDR-TB and MDR-TB. 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. Cost of the drug happens to be another factor that makes it difficult for the public to afford the present drugs available in the market. Moreover, some of the currently available drugs have been shown to exhibit some side effects and toxicity. Based on the literature reports on the promising bactericidal activity exhibited by 1,3,4-thiadiazole system, it has been planned to design and synthesize new molecules with 1,3,4-thiadiazole containing pharmacophore.

1.8 OBJECTIVES

- To design new thiadiazole containing molecules based on the literature reports on structure-activity relationship.
- To synthesize the designed molecules by using multistep organic synthetic protocols.
- To develop suitable purification methods like column chromatography and recrystallization techniques for the new thiadiazole derivatives.
- To characterize the new molecules using spectroscopic methods (^{1}H NMR, ^{13}C NMR, Mass spectroscopy) and elemental analysis.
- X-ray crystallographic studies of selected compounds for elucidation of final three-dimensional structure.
- To study *in vitro* antitubercular activities of synthesized molecules.
- To carry out *in vitro* cytotoxicity studies of the active molecules on normal cell line.
- To carry out the structural activity relationship studies to enhance the efficacy of the molecules.
- To study the mode of binding of the molecules with the receptor using molecular docking simulations.

In conclusion, in the present research work, it has been planned to design, synthesize and characterize new molecules with 1,3,4-thiadiazole as core heterocyclic nucleus so as to develop new lead molecules that may find future application in fighting against tuberculosis bacteria.

CHAPTER 2

SYNTHESIS AND ANTITUBERCULAR ACTIVITY OF NEW IMIDAZO[2,1-*b*] [1,3,4]THIADIAZOLE-BENZIMIDAZOLE DERIVATIVES

Abstract

This chapter describes the design and synthesis of new imidazo[2,1-b][1,3,4]thiadiazole-benzimidazole hybrids. It also explains the experimental protocols followed for the synthesis of the target molecules and their characterization using various spectral techniques followed by their antimycobacterial and antibacterial screening studies.

2.1 INTRODUCTION

The Imidazo[2,1-*b*][1,3,4]thiadiazole (ITD) moiety is an important class of heterocyclic compounds (Khazi et al. 2011) in medicinal chemistry research. There are several reports available in the literature describing ITD derivatives for their various biological activities. The most relevant and recent studies revealed that these molecules exhibit antimicrobial (Lamani et al. 2009; Alagawadi et al. 2011; Chandrakantha et al. 2014), inflammatory (Jadhav et al. 2008), anti-anticonvulsant (Farghaly et al. 2014), antituberculosis (Gadad et al. 2004; Palkar et al. 2012; Joshi et al. 2013), anticancer (Kamal et al. 2014) and antihyperlipidemic (Patel et al. 2013) activities. A few ITD derivatives carrying other active heterocyclic pharacophores particularly at position-5 (V, VI) have been found to possess good activity against *Mtb* H_{37}Rv strain (Kolavi et al. 2006; Hegde et al. 2006). For instance, Alegaon et al. (2012) reported the synthesis and antitubercular evaluation of a series of ITD derivatives carrying different heterocyclic moieties at position-5 (VII).

On the other hand, benzimidazole and its derivatives are finding great importance in medicinal chemistry research due to their important biological actions as well as their synthetic applications (Bansal and Silakari 2012). Benzimidazole is a core structural moiety found in some of the important drugs like albendazole (I), mebendazole (II), thiabendazole (III), rabeprazole (IV) etc (**figure 2.1**). A few recent reports demonstrated the promising antiTB activity of benzimidazole derivatives. For example, a series of pyrido[1,2-*a*]benzimidazole based molecules (VIII) exhibit excellent bactericidal activity, with several of them having MIC_{MABA} values lower than 1µg/mL (Pieroni et al. 2011).

Further, Stanley et al. (2012) identified a novel benzimidazole compound, *N*-(2,4-dichlorobenzyl)-1-propyl-1*H*-benzo[*d*]imidazol-5-amine (IX), which targets

mycobacterial membrane protein large 3 (MmpL3). Also, a library of novel trisubstituted benzimidazoles were developed through rational drug design by Kumar et al. (2010) and some of these compounds (X, XI) exhibited promising MIC values in the range of 0.5-6.1μg/mL against *Mtb* $H_{37}Rv$ strain **figure 2.2**.

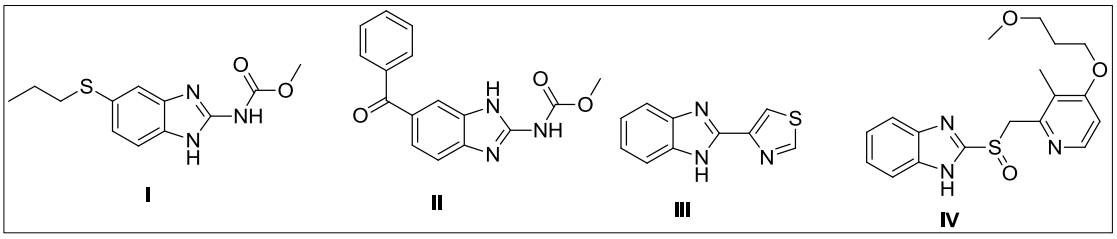


Figure 2.1 Representative benzimidazole based drug molecules (I-IV).

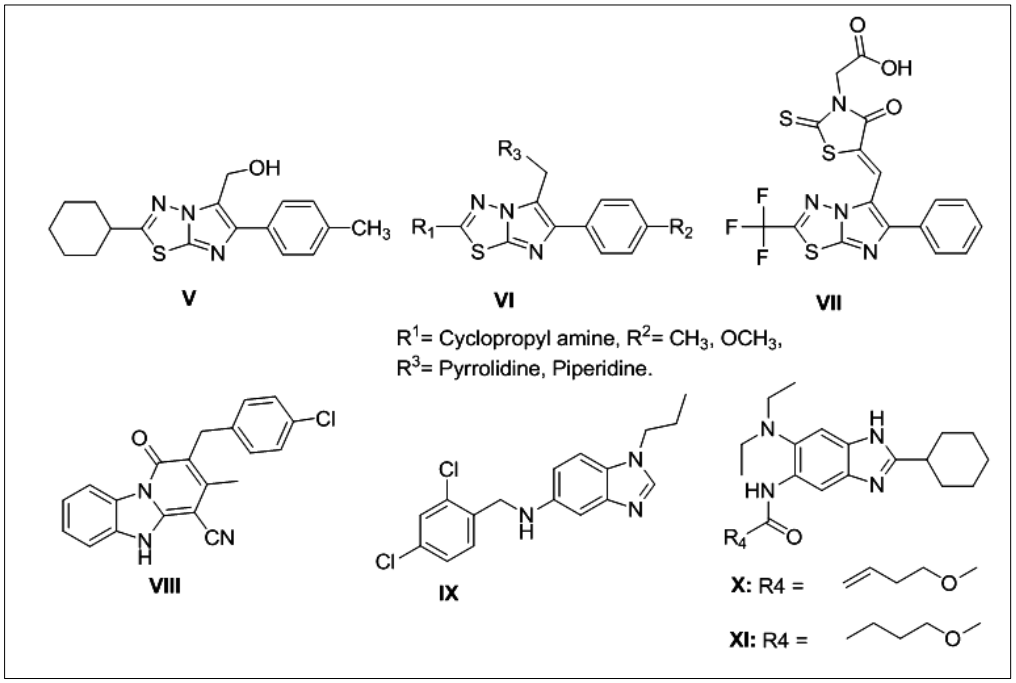


Figure 2.2 ITD and benzimidazole based antitubercular agents (V-XI).

Based on detailed literature reports on the promising bactericidal activity exhibited by ITD and benzimidazole systems (**figure 2.3**), we envisaged to amalgamate these two structural units in a single molecular frame and to explore the effects of this structural amalgamation towards their antiTB activity (Viegas-Júnior et al. 2007; Lazar et al. 2004). Hence we synthesized a series of ITD derivatives containing benzimidazole unit and evaluated their antiTB screening.

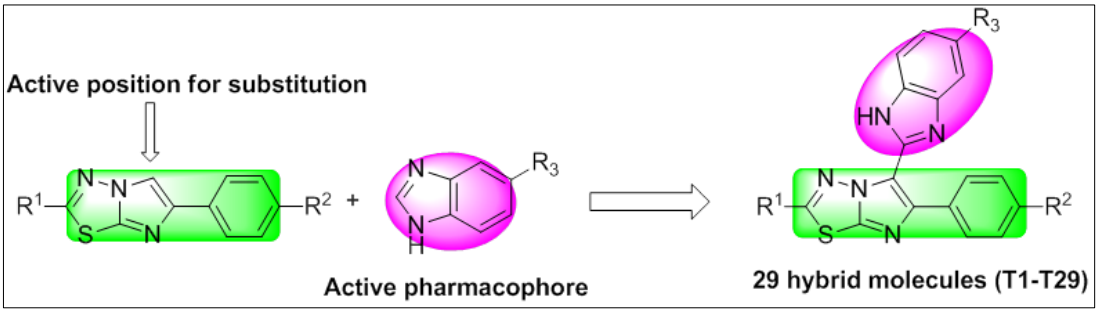
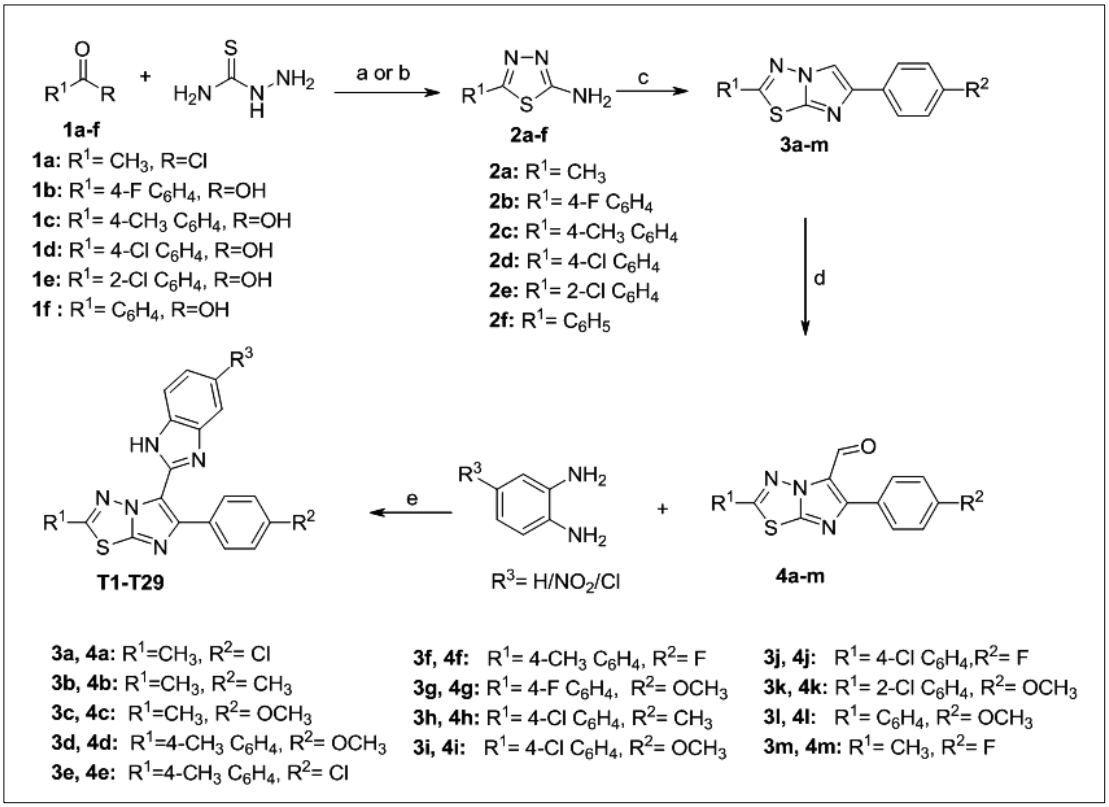


Figure 2.3 Design of new ITD-benzimidazole derivatives

2.2 CHEMISTRY

Twenty nine new 2-subsituted-6-arylimidazo[2,1-*b*][1,3,4]thiadiazole derivatives (**T1-T29**) were synthesized according to the synthetic route presented in **scheme 2.1**. One of the key intermediates, 5-methyl-1,3,4-thiadiazol-2-amine (**2a)** was synthesized by treating acetyl chloride with thiosemicarbazide using the reported procedure (Schüttelkopf et al. 2010) with a modification of the work up method (After completion of the reaction, reaction mixture is cooled to 0 ^{o}C and added 50 % NaOH (aqueous, cooled solution). The obtained solid is filtered off to get pure compound) to get a better yield (80 % as against the reported yield of 23 %) of the product (without column chromatography purification). Other intermediate compounds, 5-aromatic-1,3,4-thiadiazole-2-amines (**2b-f**) were synthesized by treating the corresponding aromatic acid with thiosemicarbazide in the presence of phosphorous oxychloride (Tu et al. 2008). The 2-subsituted-6-arylimidazo[2,1-*b*][1,3,4]thiadiazole derivatives (**3a–m**) were synthesized by the reaction between **2a-f** and the corresponding substituted *α*-halo aryl ketone under heating conditions.

In the next step, compounds **3a–m** were subjected to Vilsmeier–Haack formylation reaction to afford 2-substituted-6-arylimidazo[2,1-*b*][1,3,4]thiadiazole-5-carbaldehydes **(4a–m),** the structures of which were confirmed by spectral analysis. Finally, the target compounds, 2-(2-substituted-6-phenylimidazo[2,1-*b*][1,3,4] thiadiazol-5-yl)-1*H*-benzimidazoles (**T1-T29**), were synthesized by treating compounds **4a–m** with different substituted *o*-phenylenediamines in the presence sodium meta bisulfite ($Na_2S_2O_5$) under heating conditions using *N*,*N*-dimethyl formamide (DMF) as a solvent (Li et al. 2011; MacPherson et al. 2010).



Scheme 2.1 Synthesis of ITD-benzimidazole derivatives (**T1-T29)**. Reagents and conditions a) Acetyl chloride, 0 ˚C - RT, 3 h; b) Substituted aromatic acid, $POCl_3$, 75 ˚C, 30 min; c) Phenacyl bromide (R^2= Cl, CH_3, OCH_3, F), ethanol, 80-85 ˚C, 24 h; d) DMF, $POCl_3$, 60 ˚C, 6h; e) sodium meta bisulfite, DMF, 120 ˚C, 3h.

Table 2.1 Substitution pattern, yield and solubility of target compounds (**T1-T29**).

Product	R^1	R^2	R^3	log *P*/*C* log *P*[a]	Yield (%)
T1	4-$CH_3C_6H_4$	OCH_3	H	6.46/6.17	78
T2	4-$CH_3C_6H_4$	OCH_3	NO_2	-/6.01	73
T3	4-$CH_3C_6H_4$	OCH_3	Cl	7.02/6.89	76
T4	4-ClC_6H_4	CH_3	H	7.14/6.87	74
T5	4-ClC_6H_4	CH_3	NO_2	-/6.72	72
T6	4-ClC_6H_4	CH3	Cl	7.70/7.60	76
T7	2-ClC_6H_4	OCH_3	NO_2	-/5.98	78
T8	C_6H_5	OCH_3	NO_2	-/5.51	74
T9	4-$CH_3C_6H_4$	F	H	6.74/6.30	77
T10	4-$CH_3C_6H_4$	F	NO_2	-/6.15	74

T11	4-ClC_6H_4	OCH_3	H	6.53/6.38	72
T12	4-ClC_6H_4	F	H	6.81/6.52	79
T13	4-FC_6H_4	OCH_3	H	6.13/5.81	77
T14	4-FC_6H_4	OCH_3	NO_2	-/5.66	78
T15	4-FC_6H_4	OCH_3	Cl	6.69/6.54	74
T16	4- $CH_3C_6H_4$	Cl	H	7.14/6.87	78
T17	4- $CH_3C_6H_4$	Cl	NO_2	-/6.72	76
T18	4- $CH_3C_6H_4$	Cl	Cl	7.70/7.60	79
T19	CH3	OCH_3	H	4.59/4.07	82
T20	CH3	OCH_3	NO_2	-/3.91	86
T21	CH_3	F	H	4.87/4.21	80
T22	CH_3	F	NO_2	-/4.05	82
T23	CH_3	F	Cl	5.43/4.93	79
T24	CH_3	Cl	H	5.27/4.78	86
T25	CH_3	Cl	NO_2	-/4.62	87
T26	CH_3	Cl	Cl	5.83/5.50	78
T27	CH_3	CH_3	H	5.20/4.56	88
T28	CH_3	CH_3	NO_2	-/4.40	84
T29	CH_3	CH_3	Cl	5.76/5.28	82

[a]Obtained from Chemdraw ultra 12.0 software;

Note: Over all yield of compound **T1** is 43.92 %

2.3 EXPERIMENTAL

2.3.1 Materials and instruments

The required chemicals and solvents were procured from Sigma Aldrich (Germany), Merck (India) and Spectrochem Chemicals Pvt.Ltd. All the solvents were distilled and dried before usage. The progress of the reaction was monitored by TLC using pre coated aluminum sheets with 60 F254 silica gel (Merck KGaA). Melting point of the synthesized compounds was recorded by a Stuart SMP3 melting point (m.p) apparatus. ^{1}H NMR spectra of the intermediates and final compounds were recorded using a Bruker 400 MHz NMR spectrometer using TMS as internal standard. ^{13}C NMR spectra of the compounds were recorded using a Bruker 100 MHz

NMR spectrometer. Elemental analysis was carried out using a Thermo Electron Corporation EA-112 series C, H, N, S analyzer. Mass spectra were recorded using a Waters micro mass Q-Tofmicro spectrometer with an ESI source. X-ray intensity data for compounds **4d** and **T27** was collected at room RT using a Bruker smart Apex Duo single crystal x-ray diffractometer equipped with dual system (compact copper micro-porous source plus molybdenum scaled tube source) CCD detector. Monochromatic Mo- Kα radiation (λ=0.71073 Å) was used for the measurement. Infrared spectra of the compounds were recorded on a Jasco FTIR 4200 spectrometer.

2.3.2 Synthesis

Synthesis of 5-amino-2-methyl-1,3,4-thiadiazole (2a): To the thiosemicarbazide (15.0 g, 164.58 mmol), acetyl chloride (28.5 mL, 329.16 mmol) was added slowly and the mixture was stirred for 4 h at RT. To the reaction mixture ice cold water was added and the solid obtained, solid filtered using buckner flask. The obtained solid was made slurry with ice cold water. To the above slurry a solution of 50 % NaOH was added till the pH of the solution becomes basic and solid obtained was filtered off and dried under vacuum to get the compound **2a** as white solid. Yield: 14.4 g, 76 %; m.p: 269–270 °C; FTIR (ATR, cm^{-1}): 3288, 3105, 2925, 1614, 689; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 13.05 (br s, 2H, NH_2), 2.18 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 165.7, 148.8, 10.9; ESI-MS (*m/z*) = 116.02 $(M+H)^+$; calculated for $C_3H_5N_3S$; C, 31.9; H, 4.38; N, 36.49; S, 27.84. Found: C, 31.89; H, 4.41; N, 36.42; S, 27.44.

General procedure for the synthesis of 5-subsituted-1,3,4-thiadiazole-2-yl amines (**2b-f**).

5-(4-Fluorophenyl)-1,3,4-thiadiazol-2-amine (2b): A mixture of 4-fluoro benzoic acid (5.0 g, 35.70 mmol), thiosemicarbazide (3.25 g, 35.70 mmol) and $POCl_3$ (9.3 mL) was heated to 75 °C and maintained same temperature for 30min under stirring. The reaction mixture was cooled to RT, water (55 ml) was added and refluxed for 4 h. After cooling, the mixture was basified with 50 % NaOH to pH 8 by the drop wise addition under stirring. The obtained solid was filtered and recrystallized from ethanol to give the target compound **2b** as a colorless solid. Yield: 5.69 g, 82 %; m.p: 230-231 °C; FTIR (ATR, cm^{-1}): 3346, 3251, 2934, 1633, 1063, 683; ^{1}H NMR (400 MHz,

DMSO-d_6) δ (ppm): 7.29–7.33 (t, J = 8.77 Hz, 2H, Ar-H), 7.41 (s, 2H), 7.79–7.83 (dd, J = 5.46 Hz, 8.59 Hz, 2H, Ar-H); ESI-MS (*m/z*) = 196.4 $(M+H)^+$; calculated for $C_8H_6FN_3S$; C, 49.22; H, 3.10; N, 21.52; S, 16.43. Found: C, 49.20; H, 3.11; N, 21.52; S, 16.44.

5-(4-Methylphenyl)-1,3,4-thiadiazol-2-amine (2c): Compound **2c** was synthesized by following the above procedure with 4-methyl benzoic acid (5.0 g, 36.76 mmol), thiosemicarbazide (3.35 g, 36.76 mmol) and $POCl_3$ (9.3 mL). White solid; yield: 6.31 g, 90 %; m.p: 213–214 °C; FTIR (ATR, cm^{-1}): 3278, 3103, 2955, 1611, 690; ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.33 (s, 3H), 7.25–7.27 (d, J = 8.00 Hz, 2H, Ar-H), 7.36 (s, 2H), 7.63–7.65 (d, J = 8.12 Hz, 2H, Ar-H); ESI-MS (*m/z*) = 192.2 $(M+H)^+$; calculated for $C_9H_9N_3S$; C, 56.22; H, 4.74; N, 21.97; S, 16.77. Found: C, 56.20; H, 4.74; N, 21.90; S, 16.74.

5-(4-Chlorophenyl)-1,3,4-thiadiazol-2-amine (2d): The above procedure was followed for 4-chloro benzoic acid (5.0 g, 32.05 mmol), thiosemicarbazide (2.92 g, 32.05 mmol) and $POCl_3$ (9.3 mL) to afford compound **2d** as White solid. Yield: 5.20 g, 77 %; m.p: 210–211 °C; FTIR (ATR, cm^{-1}): 3258, 3093, 1633, 751, 684; ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.47 (s, 2H), 7.52–7.54 (d, J = 6.55 Hz, 2H, Ar-H), 7.76–7.78 (d, J = 6.24 Hz, 2H, Ar-H), ESI-MS (*m/z*) = 212.6 $(M+H)^+$; calculated for $C_8H_6ClN_3S$; C, 45.39; H, 2.86; N, 19.85; S, 15.15. Found: C, 45.30; H, 2.88; N, 19.90; S, 15.14.

5-(2-Chlorophenyl)-1,3,4-thiadiazol-2-amine (2e): Compound **2e** was synthesized by following the above procedure for the 2-chloro benzoic acid (5.0 g, 32.05 mmol), thiosemicarbazide (2.92 g, 32.05 mmol) and $POCl_3$ (9.3 mL). Yield: 5.41 g, 80 % (white solid); m.p: 220–221 °C; FTIR (ATR, cm^{-1}): 3269, 3098, 1636, 748, 694; ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.97–8.01 (m, 2H, Ar-H), 7.57–7.60 (m, 1H, Ar-H), 7.44–7.47 (m, 1H, Ar-H), 7.42 (s, 2H), ESI-MS (*m/z*) 212.10 $(M+H)^+$: calculated for $C_8H_6ClN_3S$; C, 45.39; H, 2.86; N, 19.85; S, 15.15. Found: C, 45.31; H, 2.89; N, 19.85; S, 15.16.

5-Phenyl-1,3,4-thiadiazol-2-amine (2f): Compound **2f** was synthesized by following the above procedure for benzoic acid (5.0 g, 40.32 mmol), thiosemicarbazide (3.67 g,

40.32 mmol) and $POCl_3$ (9.3 mL). Yield: 5.56 g, 78.0 % (white solid); m.p: 224–225 °C; FTIR (ATR, cm^{-1}): 3288, 3100, 1611, 690; ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.40 (s, 2H), 7.43–7.49 (dd, *J* = 6.38 Hz, 14.00 Hz, 2H, Ar-H), 7.74–7.76 (d, *J* = 6.94 Hz, 2H, Ar-H), ESI-MS (*m/z*) = 178.3 $(M+H)^+$; calculated for $C_8H_7N_3S$; C, 54.22; H, 3.98; N, 23.71; S, 18.09. Found: C, 54.21; H, 3.90; N, 23.80; S, 18.10.

General procedure for the synthesis of 2-subsituted-6-arylimidazo[2,1-*b*][1,3, 4]thiadiazoles (**3a-m**).

6-(4-Chlorophenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazole (3a): A mixture of **2a** (2 g, 17.39 mmol) and 4-chloro phenacyl bromide (4.032 g, 17.39 mmol) was refluxed in dry ethanol (20 mL) for 24 h. The excess of solvent was removed under reduced pressure and the solid hydrobromide salt was suspended in water, and neutralized by the aqueous sodium carbonate solution to get free base. It was then filtered, washed with water, dried, and recrystallized from ethanol to get compound **3a** as light yellow solid. Yield: 3.4 g, 80 %; m.p: 184-185 °C; FTIR (ATR, cm−1): 3066, 2938, 1592, 1504, 1466, 843, 741, 682; ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 2.72 (s, 3H), 7.37 (dd, *J* = 2.0, 6.8 Hz, 2H, Ar-H),7.74 (dd, *J* =1.8, 6.6 Hz, 2H, Ar-H), 7.94 (s, 1H, H-5 imidazole), ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 17.86, 102.29, 126.24, 128.87, 132.48, 1 33.09, 144.93, 145.86, 159.59; ESI-MS= (*m/z*) 249.9 $(M+H)^+$; calculated for $C_{11}H_8ClN_3S$; C, 52.91; H, 3.23; N, 16.83; S, 12.84. Found: C, 52.98; H, 3.28; N, 16.92; S, 12.54.

Compounds **3b-m** were synthesized by following the above procedure by reacting of 5-subsituted-1,3,4-thiadiazole-2-yl amine (2g) with same equivalent of corresponding phenacyl bromide derivative. The structural characterization data for compounds **3b-m** are given below.

2-Methyl-6-(4-methylphenyl)imidazo[2,1-*b*][1,3,4]thiadiazole (3b): Yellow solid, yield: 3.26 g, 82.0 %; m.p: 211-212 °C; FTIR (ATR, cm^{-1}): 3314, 1696, 1601, 1460, 1259, 1157, 948, 751, 704; ^1H NMR (400 MHz, $CDCl_3$): δ 2.37(s, CH_3), 2.71 (s, CH_3), 7.21 (d, *J* =7.9 Hz, 2H, Ar-H),7.70 (d, *J* = 7.9 Hz, 2H, Ar-H) 7.92 (s, H-5 imidazole, 1H), ^{13}C NMR (100 MHz, CDCl3) δ (ppm): 17.82, 21.26, 108.76, 124.94, 129.41, 131.14, 137.27, 145.53, 146.17, 159.14; ESI-MS (*m/z*) = 230.0 $(M+H)^+$;

calculated for $C_{12}H_{11}N_3S$; C, 62.86; H, 4.84; N, 18.33; S, 13.98. Found: C, 62.98; H, 4.78; N, 18.40; S, 14.02.

6-(4-Methoxyphenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazole (3c): Yellow solid, yield: 3.44 g, 81 %; m.p: 192-193 °C; FTIR (ATR, cm^{-1}): 3043, 2935, 1540, 1462, 1239, 1023, 830, 670; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 2.70 (s, CH_3), 3.84 (s, 3H, OCH_3), 6.95-6.97 (m, 2H, ArH),7.73(d, J = 8.8 Hz, 2H, ArH),7.871 (s, H-5 imidazole, 1H), ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 17.52, 54.60, 109.77, 125.65, 128.42, 131.15, 132.78, 145.23, 146.20, 159.40; ESI-MS (*m/z*) = 245.9 $(M+H)^+$; calculated for $C_{12}H_{11}N_3OS$; C, 58.76; H, 4.52; N, 17.13; S, 13.07. Found: C, 58.86; H, 4.60; N, 17.12; S, 13.14.

6-(4-Methoxyphenyl)-2-(4-methylphenyl)imidazo[2,1-*b*][1,3,4]thiadiazole (3d): Yellow solid, yield: 2.62 g, 78.0 %; m.p:177-178 °C; FTIR (ATR, cm^{-1}): 3042, 2934, 1540, 1461, 1234, 1023, 830, 671; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 2.43 (s, 3H, CH_3), 3.85 (s, 3H, OCH_3), 6.96 (d, J = 8.8 Hz, 2H, ArH),7.29 (d, J = 22.82 Hz, 2H, Ar-H), 7.75-7.78 (m, 4H, Ar-H), 7.94 (s, imidazole-H, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 21.57, 55.34, 108.35, 114.17, 126.37, 126.61, 126.72, 127.60, 129.95, 142.18, 144.97, 146.39, 159.26, 161.28 ; ESI-MS (*m/z*) = 322.0 $(M+H)^+$; calculated for $C_{18}H_{15}N_3OS$; C, 67.27; H, 4.70; N, 13.07; S, 9.98. Found: C, 67.15; H, 4.75; N, 13.10; S, 10.01.

6-(4-Chlorophenyl)-2-*p*-tolylimidazo[2,1-*b*][1,3,4]thiadiazole (3e): Off white solid, yield: 2.72 g, 80 %; m.p: 234-235 °C; FTIR (ATR, cm^{-1}): 3068, 2924, 1592, 1511, 1474, 840, 729, 670; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 2.45 (s, CH_3), 7.38 (m, 2H, Ar-H), 7.74 (dd, J =2.0, 6.8 Hz, 2H, Ar-H), 7.96 (s, 1H, imidazole-H); ESI-MS (*m/z*) = 326.0 $(M+H)^+$; calculated for $C_{17}H_{12}ClN_3S$; C, 62.67; H, 3.71; N, 12.90; S, 9.84. Found: C, 62.65; H, 3.75; N, 12.85; S, 9.86.

6-(4-Fluorophenyl)-2-*p*-tolylimidazo[2,1-*b*][1,3,4]thiadiazole (3f): White solid, yield: 2.55 g, 79.0 %; m.p: 228-230 °C; FTIR (ATR, cm^{-1}): 3067, 2924, 1539, 1464, 1062, 827, 671; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 2.42 (s, 3H, CH_3),7.431 (d, J =8.4Hz, 2H, Ar-H), 7.58-7.60 (m, 2H, Ar-H), 7.95 (d, J = 7.9 Hz, Ar-H), 8.12-8.15 (m, 2H, Ar-H),7.98 (s, 1H, imidazole-H); ESI-MS (*m/z*) = 310.1 $(M+H)^+$; calculated

for $C_{17}H_{12}FN_3S$; C, 66.00; H, 3.91; N, 13.58; S, 10.36. Found: C, 66.12; H, 3.95; N, 13.54; S, 10.41.

2-(4-Fluorophenyl)-6-(4-methoxyphenyl)imidazo[2,1-*b*][1,3,4]thiadiazole (3g): Light yellow solid, yield: 2.47 g, 75 %; m.p: 203-204 ˚C; FTIR (ATR, cm^{-1}): 3063, 2935, 1539, 1463, 1243, 1066, 1022, 828, 671; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 3.810 (s, 3H, OCH_3), 7.22-7.30 (m, 4H, ArH), 7.64-7.68 (m, 2H, ArH), 7.85 (d, *J* = 7.4 Hz, 2H, Ar-H), 7.92 (s, imidazole-H). ESI-MS (*m/z*) = 326.10 $(M+H)^+$; calculated for $C_{17}H_{12}FN_3OS$; C, 62.76; H, 3.72; N, 12.91; S, 9.86. Found: C, 62.66; H, 3.75; N, 12.94; S, 9.81.

2-(4-Chlorophenyl)-6-p-tolylimidazo[2,1-*b*][1,3,4]thiadiazole (3h): Yellow solid, yield: 2.40 g,78.2 %; m.p: 239-240 ˚C; FTIR (ATR, cm^{-1}): 3087, 2919, 1593, 1513, 1460, 828, 761, 668; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 2.34 (s, 3H, CH_3), 7.21 (d, *J* = 7.6Hz, 2H, Ar-H), 7.30 (m, 2H, Ar-H), 7.70-7.66 (m, 4H, Ar-H), 7.94 (s, imidazole-H, 1H); ESI-MS (*m/z*) = 326.04 $(M+H)^+$; calculated for $C_{17}H_{12}ClN_3S$; C, 62.67; H, 3.71; N, 12.90; S, 9.84. Found: C, 62.66; H, 3.73; N, 12.84; S, 9.83.

2-(4-Chlorophenyl)-6-(4-methoxyphenyl)imidazo[2,1-*b*][1,3,4]thiadiazole (3i): Yellow solid, yield: 2.54 g,79.0 %; m.p: 159-160 ^{0}C; FTIR (ATR, cm^{-1}): 3067, 2934, 1539, 1462, 1239, 1023, 828, 758, 671; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 3.81 (s, 3H, CH3), 7.23-7.33 (m, 4H, Ar-H), 7.66-7.75 (m, 2H, Ar-H),7.96 (d, *J* = 7.9 Hz, 2H, Ar-H),7.93 (s, imidazole-H); ESI-MS (*m/z*) = 342.0 $(M+H)^+$; calculated for $C_{17}H_{12}ClN_3OS$; C, 59.73; H, 3.54; N, 12.29; S, 9.38. Found: C, 59.76; H, 3.54; N, 12.32; S, 9.33.

2-(4-Chlorophenyl)-6-(4-fluorophenyl)imidazo[2,1-*b*][1,3,4]thiadiazole (3j): White solid, yield: 2.52 g, 81.2 %; m.p: 225-226 ˚C; FTIR (ATR, cm^{-1}): 3066, 2925, 1594, 1503, 1474, 1087, 839, 729, 670; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.45 (d, *J* = 8.3 Hz, 2H, Ar-H), 7.66-7.70 (m, 4H, Ar-H), 8.09 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.96 (1H, s, imidazole-H); ESI-MS (*m/z*) = 330.0 $(M+H)^+$; calculated for $C_{16}H_9ClFN_3S$; C, 58.27; H, 2.75; N, 12.74; S, 9.72. Found: C, 58.26; H, 2.76; N, 12.72; S, 9.73.

2-(2-Chlorophenyl)-6-(4-methoxyphenyl)imidazo[2,1-*b*][1,3,4]thiadiazole (3k): Off white solid, yield: 2.30 g, 75.0 %; m.p: 213-214 °C; FTIR (ATR, cm^{-1}): 3068, 2921, 1594, 1503, 1476, 1245, 1024, 840, 752, 670; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 3.81 (s, 3H, OCH_3), 7.02 (d, J = 8.3 Hz, 2H, Ar-H),7.62 (d, J = 6.6 Hz, 1H, Ar-H), 7.67 (s, 1H, Ar-H), 7.71 (d, J = 7.5Hz, 1H, Ar-H,),7.84 (d, J =8.7 Hz, 1H, Ar-H), 8.67 (d, J = 8.3Hz, 2H, Ar-H), 7.97 (s, 1H, imidazole-H); ESI-MS (*m/z*) 342.0 (M+H)+; calculated for $C_{17}H_{13}N_3OS$; C, 59.73; H, 3.54; N, 12.29; S, 9.38. Found: C, 59.69; H, 3.56; N, 12.26; S, 9.40.

6-(4-Methoxyphenyl)-2-phenylimidazo[2,1-*b*][1,3,4]thiadiazole (3l): White solid, yield: 2.84 g, 82.0 %; m.p: 219-220 °C; FTIR (ATR, cm^{-1}): 3066, 2924, 1593, 1503, 1465, 1244, 1028, 840, 668; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 3.79 (s, 3H, OCH_3), 7.02 (d, J = 8.3 Hz, 2H, Ar-H), 7.32-7.42 (m, 3H, Ar-H), 7.43–7.49 (m, 2H), 7.61 (d, J = 8.8 Hz, 2H) 7.87 (s, 1H, imidazole-H); ESI-MS (*m/z*) = 308.10 $(M+H)^+$; calculated for $C_{17}H_{13}N_3OS$; C, 66.43; H, 4.26; N, 13.67; S, 10.43. Found: C, 66.45; H, 4.24; N, 13.66; S, 10.42.

6-(4-Fluorophenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazole (3m): Light yellow solid, yield: 2.56 g, 79.0 %; m.p:179-180 °C; FTIR (ATR, cm^{-1}): 3068, 2926, 1597, 1538, 1470, 1064, 834, 670; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 2.73 (s, 3H),7.38 (m, 2H, Ar-H),7.741 (d, J = 7.6Hz, 2H, Ar-H), 7.96 (s, 1H, H-5 imidazole); ESI-MS (*m/z*) = 234.1 $(M+H)^+$; calculated for $C_{11}H_8FN_3S$; C, 56.64; H, 3.46; N, 18.01; S, 13.75. Found: C, 56.66; H, 3.44; N, 18.06; S, 13.72.

General procedure for the preparation of 2-subsituted-6-arylimidazo[2,1-*b*][1,3,4]thiadiazole-5-carbaldehydes (4 a-m).

6-(4-Chlorophenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazole-5-carbaldehyde (4a): Vilsmeier-Haack salt was synthesized by adding $POCl_3$ (1.5 mL, 16.06 mmol) drop-wisely to a dry RB containing DMF (1.23 mL, 16.06 mmol) maintaining temperature at 0-5 °C under N_2 atmosphere. Later, a solution of **3a** (2.0 g, 8.032 mmol) in 20 mL of DMF was added to the resulting complex at a stretch. The resulting solution was stirred at 60 °C for about 6 h. The container was cooled to RT and quenched to ice cold water while stirring. The solid obtained was filtered, washed

with excess of water and then purified by column chromatographic technique using EtOAc and hexane (3:7) system to give compound **4a** as white solid. Yield:1.9 g, 85 %, m.p: 174-175 °C; FTIR (ATR, cm^{-1}): 3066, 2924, 1679, 1592, 1504, 1479, 843, 728, 671; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 2.85 (3H, CH_3), 7.48 (dd, J =1.7, 6.6Hz, 2H, Ar-H),7.82 (d, J =8.3Hz, 2H, Ar-H),10.35 (s, CHO, 1H), ^{13}CNMR (100MHz, $CDCl_3$) δ(ppm): 18.09, 124.04, 129.09, 130.28, 130.76, 136.05, 151.20, 154.51, 162.28, 177.18; ESI-MS (*m/z*) = 277.9 $(M+H)^+$; calculated for $C_{12}H_8ClN_3OS$; C, 51.90; H, 2.90; N, 15.13; S, 11.55. Found: C, 51.86; H, 2.88; N, 15.15; S, 11.44.

Compounds **4b-m** were synthesized by following the above procedure and structural characterization data for the compounds are given below

2-Methyl-6-(4-methylphenyl)imidazo[2,1-*b*][1,3,4]thiadiazole-5-carbaldehyde (4b): Light yellow solid, yield: 1.84 g, 82.2 %; m.p: 145-146 °C; FTIR (ATR, cm^{-1}): 3068, 2924, 1678, 1592, 1504, 1478, 842, 670; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 2.48(s, CH_3), 2.84 (s, CH_3), 7.28 (d, J =7.9Hz, 2H, Ar-H),7.71 (d, J =7.9 Hz, 2H, Ar-H) 10.00 (s, CHO, 1H),^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) : 18.08, 21.41, 123.98, 129.06, 129.44, 129.63, 140.10, 151.45, 156.76, 161.85, 177.61; ESI-MS (*m/z*) = 257.9 $(M+H)^+$; calculated for $C_{13}H_{11}N_3OS$; C, 60.68; H, 4.31; N, 16.33; S, 12.46. Found: C, 60.66; H, 4.31; N, 16.35; S, 12.44.

6-(4-Methoxyphenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazole-5-carbaldehyde (4c): Off white solid, yield: 1.89 g, 85%; m.p:161-162 °C; FTIR (ATR, cm^{-1}): 3044, 2935, 1679, 1540, 1462, 1238, 1024, 830, 671; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 2.84 (s, 3H, CH_3), 3.88 (s, 3H, OCH_3), 7.02-7.04 (m, 2H, Ar-H), 7.7-7.8 (m, 2H, Ar-H), 9.99 (s, 1H, CHO); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 17.82, 55.60, 109.97, 125.95, 128.62, 131.25, 133.1, 145.83, 146.60, 159.70, 177.41; ESI-MS (*m/z*) = 274.0 $(M+H)^+$; calculated for $C_{13}H_{11}N_3O_2S$; C, 57.13; H, 4.06; N, 15.37; S, 11.73. Found: C, 57.16; H, 4.01; N, 15.35; S, 11.74.

6-(4-Methoxyphenyl)-2-(4-methylphenyl)imidazo[2,1-*b*][1,3,4]thiadiazole-5-carbaldehyde (4d): Light yellow solid, yield: 1.73 g, 80 %; m.p: 195-196 °C; FTIR (ATR, cm^{-1}): 3043, 2935, 1679, 1540, 1462, 1238, 1024, 830, 671; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 2.45 (s, 3H, CH_3), 3.89 (s, 3H, OCH_3), 7.03-7.05 (m, 2H, Ar-

H), 7.33 (d, *J* =7.9Hz, 2H, Ar-H) ,7.85-7.91 (m, 4H, Ar-H), 10.10 (s, 1H, CHO), ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 21.65, 55.44, 114.28, 123.71, 124.91, 126.82, 127.14, 130.06, 130.55, 143.06, 150.45, 156.02, 161.10, 163.85, 177.44; ESI-MS (*m/z*) = 350.0 $(M+H)^+$; calculated for $C_{19}H_{15}N_3O_2S$; C, 65.31; H, 4.33; N, 12.03; S, 9.18. Found: C, 65.28; H, 4.32; N, 12.05; S, 9.20.

6-(4-Chlorophenyl)-2-p-tolylimidazo[2,1-b][1,3,4]thiadiazole-5-carbaldehyde (4e): Light brown solid, yield: 1.69 g, 78 %; m.p: 209-210 °C; FTIR (ATR, cm^{-1}): 3068, 2924, 1679, 1592, 1504, 1476, 840, 729, 669; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 2.48 (s, 3H, CH_3) ,7.39 (m, 2H, Ar-H), 7.05-7.08 (m, 2H, Ar-H), 7.50 (d, *J* = 7.5 Hz, 2H, Ar-H),7.75 (d, *J* = 8.72 Hz 2H, Ar-H),10.18 (s, 1H,CHO); ESI-MS (*m/z*) = 354.0 $(M+H)^+$; calculated for $C_{18}H_{12}ClN_3OS$; C, 61.10; H, 3.42; N, 11.88; S, 9.06. Found: C, 61.12; H, 3.42; N, 11.90; S, 9.09.

6-(4-Fluorophenyl)-2-*p*-tolylimidazo[2,1-*b*][1,3,4]thiadiazole-5-carbaldehyde (4f): White solid, yield: 1.78 g, 82.0 %; m.p: 193-194 °C; FTIR (ATR, cm^{-1}): 3066, 2925, 1678, 1539, 1462, 1061, 827, 671; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 2.46 (s, 3H, CH_3), 7.53(m, 2H, Ar-H), 7.59 (d, *J* =8.4Hz, 2H, ArH), 7.98 (d, *J* =7.8Hz, 2H, ArH), 8.14-8.17 (m, 2H, ArH),10.25 (s, CHO); ESI-MS (*m/z*) = 338.1 $(M+H)^+$; calculated for $C_{18}H_{12}FN_3OS$; C, 64.08; H, 3.59; N, 12.46; S, 9.50. Found: C, 64.12; H, 3.52; N, 12.45; S, 9.56.

2-(4-Fluorophenyl)-6-(4-methoxyphenyl)imidazo[2,1-*b*][1,3,4]thiadiazole-5-carbaldehyde (4g): Light yellow solid, yield: 1.78 g, 82.0 %; m.p: 189-190 °C; FTIR (ATR, cm^{-1}): 3043, 2935, 1677, 1539, 1463, 1244, 1062, 1023, 828, 671; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 3.82 (s, 3H, OCH_3), 7.26-7.30 (m, 4H, ArH), 7.66-7.70 (m, 2H, ArH), 7.87 (d, *J* = 7.9 Hz, 2H, Ar-H), 10.21 (s, 1H, CHO); ESI-MS (*m/z*) = 354.0 $(M+H)^+$; calculated for $C_{18}H_{12}FN_3O_2S$; C, 61.18; H, 3.42; N, 11.89; S, 9.07. Found: C, 61.22; H, 3.39; N, 11.89; S, 9.06.

2-(4-Chlorophenyl)-6-*p*-tolylimidazo[2,1-*b*][1,3,4]thiadiazole-5-carbaldehyde (4h): Light yellow solid, yield: 1.69 g, 78.0 % ; m.p: 207-208 °C; FTIR (ATR, cm^{-1}): 3087, 2918, 1678, 1592, 1512, 1459, 828, 760, 668; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 2.35 (s, 3H, CH_3), 7.21 (m, 2H, Ar-H), 7.32 (m, 2H, Ar-H), 7.68-7.72 (m, 4H,

Ar-H), 10.10 (s, 1H, CHO); ESI-MS (*m/z*) = 354.0 $(M+H)^+$; calculated for $C_{18}H_{12}ClN_3OS$; C, 61.10; H, 3.42; N, 11.88; S, 9.06. Found: C, 61.25; H, 3.42; N, 11.91; S, 9.16.

2-(4-Chlorophenyl)-6-(4-methoxyphenyl)imidazo[2,1-*b*][1,3,4]thiadiazole-5-carbaldehyde (4i): White solid, yield:1.71 g, 79.2 %; m.p: 196-197 °C; FTIR (ATR, cm^{-1}): 3043, 2935, 1679, 1539, 1462, 1239, 1023, 828, 758, 671; ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 3.821 (s, 3H, CH_3), 7.25-7.34 (m, 4H, Ar-H), 7.68-7.78 (m, 2H, Ar-H), 7.98 (d, *J* = 7.4 Hz, 2H, Ar-H), 10.22 (s, 1H, CHO); ESI-MS (*m/z*) = 370.1 $(M+H)^+$; calculated for $C_{18}H_{12}ClN_3O_2S$; C, 58.46; H, 3.27; N, 11.36; S, 8.67. Found: C, 58.45; H, 3.25; N, 11.35; S, 8.66.

2-(4-Chlorophenyl)-6-(4-fluorophenyl)imidazo[2,1-b][1,3,4]thiadiazole-5-carbaldehyde (4j): Off white solid, yield: 1.66 g, 76.6 %; m.p: 189-190 °C; FTIR (ATR, cm^{-1}): 3068, 2921, 1676, 1594, 1503, 1476, 1088, 839, 729, 668; ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.48 (d, *J* = 8.6 Hz, 2H, Ar-H), 7.72-7.68 (m, 4H, Ar-H), 8.12 (d, *J* = 8.3 Hz, 2H, Ar-H), 10.20 (s, 1H, CHO); ESI-MS (*m/z*) = 358.1 $(M+H)^+$; calculated for $C_{17}H_9ClFN_3OS$; C, 57.07; H, 2.54; N, 11.74; S, 8.96. Found: C, 57.15; H, 2.52; N, 11.71; S, 8.96.

2-(2-Chlorophenyl)-6-(4-methoxyphenyl)imidazo[2,1-*b*][1,3,4]thiadiazole-5-carbaldehyde (4k): Off white solid, yield: 1.69 g, 78.2%; m.p: 178-180 °C; FTIR (ATR, cm^{-1}): 3068, 2921, 1676, 1594, 1503, 1244, 1028, 1476, 839, 752, 671; ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 3.81 (s, 3H, OCH_3), 7.026 (d, *J* =8.7 Hz, 2H, Ar-H), 7.623 (d, *J* = 6.6 Hz, 1H, Ar-H),7.67 (s, 1H, Ar-H) 7.72 (d, *J* = 8.4 Hz, 1H, Ar-H),7.84 (d, *J* = 8.5 Hz, 1H, Ar-H), 8.70 (d, *J* = 8.3 Hz, 2H, Ar-H), 10.28 (s, 1H, CHO). ESI-MS (*m/z*) = 370.1 $(M+H)^+$; calculated for $C_{18}H_{12}ClN_3O_2S$; C, 58.46; H, 3.27; N, 11.36; S, 8.67. Found: C, 58.44; H, 3.30; N, 11.41; S, 8.68.

6-(4-Methoxyphenyl)-2-phenylimidazo[2,1-*b*][1,3,4]thiadiazole-5-carbaldehyde (4l): White solid, yield: 1.63 g, 75.0 %; m.p: 179-180 °C; FTIR (ATR, cm^{-1}): 3067, 2925, 1678, 1594, 1503, 1464, 1244, 1028, 1476, 839, 668; ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 3.81 (s, 3H, OCH_3), 7.10 (d, *J* =8.3 Hz, 2H, Ar-H), 7.32-7.42 (m, 3H, Ar-H), 7.45–7.52 (m, 2H, ArH), 7.63 (d, *J* = 8.8 Hz, 2H),10.18 (s, 1H, CHO);

ESI-MS (*m/z*) = 336.10 $(M+H)^+$; calculated for $C_{18}H_{13}N_3O_2S$; C, 64.46; H, 3.91; N, 12.53; S, 9.56. Found: C, 64.50; H, 3.90; N, 12.46; S, 9.58.

6-(4-Fluorophenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazole-5-carbaldehyde (4m): Light yellow solid, yield: 1.81 g, 81.1 %; m.p:169-170 °C; FTIR (ATR, cm^{-1}): 3068, 2926, 1678, 1597, 1538, 1470, 1064, 834, 670; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 2.76 (s, 3H, CH_3), 7.40 (m, 2H, Ar-H),7.76 (d, *J* = 8.6 Hz, 2H, Ar-H),10.25 (s, CHO, 1H); ESI-MS (*m/z*) = 262.0 $(M+H)^+$; calculated for $C_{12}H_8FN_3OS$; C, 55.16; H, 3.09; N, 16.08; S, 12.27. Found: C, 55.20; H, 3.10; N, 16.06; S, 12.21.

General procedure for the synthesis of target molecules **T1-T29** and their spectral data.

2-(6-(4-Methoxyphenyl)-2-*p*-tolylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)-1*H*-benzo [*d*]imidazole (T1): To a stirred solution of o-phenylenediamine (0.046 g, 0.429 mmol) in DMF (3 mL) under nitrogen atmosphere, compound **4d** (0.15 g, 0.0429 mmol) was added and stirred for 5 min after that sodium met bisulfite (0.122 g, 0.644 mmol) was added. The reaction mixture was then heated to 120 °C and maintained same temperature for 3 h. Reaction was monitored with TLC and once starting material was completely consumed the solvent was removed by under vacuum. The solid obtained was dissolved in EtOAc (10 mL) and washed with water (10 mL). The aqueous layer was extracted with EtOAc (3 x 10 mL). The organic extracts were combined, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuum. The crude product was purified by flash column chromatography eluting with methanol/chloroform (1:9) to give **T1** as off white solid. FTIR (ATR, cm^{-1}): 3352, 3077, 2938, 1603, 1563, 1466, 1244, 1026, 827, 691; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.41 (s, 3H, CH_3) , 3.81(s, 3H, OCH_3) , 7.30 (dd, *J* = 3.0, 6.1 Hz, 2H, Ar-H), 7.43 (d, *J* = 8.3 Hz, 2H, Ar-H), 7.48 (d, *J* = 8.5 Hz, 2H, Ar-H), 7.70-7.72 (m, 2H, Ar-H), 7.96 (d, *J* = 8.3 Hz, 2H, Ar-H), 8.12 (d, *J* = 8.6 Hz, 2H, Ar-H), 11.6-14.2 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 21.60, 55.52, 115.70, 123.21, 127.02, 128.80, 129.86, 132.77, 133.26, 143.23, 144.43, 145.83, 160.7, 163.21; ESI-MS (*m/z*) = 438.1 $(M+H)^+$; calculated for $C_{25}H_{19}N_5OS$; C, 68.63; H, 4.38; N, 16.01; S, 7.33. Found: C, 68.54; H, 4.40; N, 16.10; S, 7.32.

Compounds **T2-T29** were synthesized by following the above procedure by reacting 0.15 g of imidazo[2,1-*b*][1,3,4]thiadiazole-5-carbaldehyde (**4a-m**) with same equivalent of corresponding substituted o-phenylenediamine.

2-(6-(4-Methoxyphenyl)-2-*p*-tolylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)-5-nitro-1*H*-benzo[*d*]imidazole (T2): Yellow solid. FTIR (ATR, cm^{-1}): 3348, 3073, 2934, 1604, 1517, 1457, 1244, 1024, 836, 681; ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 2.42 (s, 3H, CH_3), 3.81 (s, 3H, OCH_3), 7.01 (d, *J* = 8.5 Hz, 2H, Ar-H), 7.45 (2H, d, *J* = 7.8 Hz, ArH), 7.86 (d, *J* = 8.9 Hz, 1H, Ar-H),8.00-8.20 (m, 4H, Ar-H), 8.612 (d, *J* = 1.8Hz, 1H, Ar-H), 13.10- 13.29 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 21.69, 55.82, 114.24, 115.12, 123.39, 126.51, 127.19, 127.28, 127.46, 130.74, 142.90, 143.08, 143.63, 146.90, 146.09, 159.72, 162.54; ESI-MS (*m/z*) = 483.1 $(M+H)^+$; calculated for $C_{25}H_{18}N_6O_3S$; C, 62.23; H, 3.76; N, 17.42; S, 6.65. Found: C, 62.25; H, 3.78; N, 17.40; S, 6.67.

5-Chloro-2-(6-(4-methoxyphenyl)-2-*p*-tolylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)-1*H*-benzo[*d*]imidazole (T3): Green solid. FTIR (ATR, cm^{-1}): 3353, 3044, 2935, 1540, 1462, 1238, 1024, 828, 751, 691; ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 2.41 (s, 3H, CH_3), 3.78 (s, 3H, OCH_3), 6.98 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.30 (dd, J=1.7, 8.3Hz, 1H, Ar-H), 7.43 (d, *J* = 8.3 Hz, 2H, Ar-H), 7.71 (d, *J* = 8.8 Hz, 1H, Ar-H),7.76 (s, 1H, Ar-H), 7.96 (d, *J* = 8.3 Hz, 2H, Ar-H), 8.01 (d, *J* = 8.8 Hz, 2H, Ar-H), 12.4-13.6 (s, NH, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): 21.59, 55.62, 114.14, 114.22, 123.19, 126.31, 127.09, 127.28, 127.46, 129.54, 130.54, 143.08, 143.53, 145.60, 146.09, 159.82, 162.64; ESI-MS (*m/z*) 472.0 $(M+H)^+$; calculated for $C_{25}H_{18}ClN_5OS$; C, 63.62; H, 3.84; N, 14.84; S,6.79. Found: C, 63.52; H, 3.82; N, 14.86; S, 6.74.

2-(2-(4-Chlorophenyl)-6-*p*-tolylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)-1*H*-benzo[*d*]imidazole (T4): Light yellow solid. FTIR (ATR, cm^{-1}): 3353, 3072, 2936, 1596, 1518, 1459, 828, 756, 693; ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 2.34 (s, 3H, CH_3) ,7.21 (d, *J* = 7.6 Hz, 2H, Ar-H),7.30 (m, 2H, Ar-H), 7.70-7.68 (m, 4H, Ar-H), 7.908 (d, *J* = 7.6 Hz, 2H, Ar-H), 8.06 (d, *J* = 8.0 Hz, 2H, Ar-H), 12.88 (1H, br, NH), ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 21.33, 115.11, 123.06, 127.96, 128.70, 129.29, 129.34, 130.12, 131.04, 137.35, 138.11, 141.78, 145.66, 146.02, 161.44; ESI-

MS (*m/z*) = 442.0 $(M+H)^+$; calculated for $C_{24}H_{16}ClN_5S$; C, 65.23; H, 3.65; N, 15.85; S,7.26. Found: C, 65.25; H, 3.67; N, 15.83; S, 7.24.

2-(2-(4-Chlorophenyl)-6-*p*-tolylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)-5-nitro-1*H*-benzo[*d*]imidazole (T5): Yellow solid. FTIR (ATR, cm^{-1}): 3348, 3072, 2934, 1596, 1517, 1462, 827, 754, 682; ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 2.34 (s, 3H, CH_3), 7.21 (m, 2H, ArH), 7.30 (m, 2H, ArH), 7.70-7.66 (m, 4H, ArH), 8.18 (d, 1H, *J* = 8.5 Hz), 8.26 (d, *J* = 7.6 Hz, 1H, ArH), 8.55 (s, 1H, ArH), 12.91 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): 21.33, 110.5, 116.2, 118.4, 128.70, 129.29, 130.34, 130.72, 131.04, 137.35, 138.11, 139.8, 141.78, 142.8, 145.86, 145.2, 146.12, 161.54; ESI-MS (*m/z*) = 487.0 $(M+H)^+$; calculated for $C_{24}H_{15}ClN_6O_2S$; C, 59.20; H, 3.10; N, 17.26; S,6.59. Found: C, 69.10; H, 3.10; N, 17.20; S, 6.48.

5-Chloro-2-(2-(4-chlorophenyl)-6-*p*-tolylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)-1*H*-benzo[*d*]imidazole (T6): Green solid. FTIR (ATR, cm^{-1}): 3351, 3084, 2936, 1598, 1463, 827, 756, 693; ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 2.34 (s, 3H, CH_3), 7.21 (m, 2H, 4H), 7.27 (d, *J* = 8.5 Hz, 1H, Ar-H), 7.30 (m, 2H, Ar-H), 7.64 (d, *J* = 7.4 Hz, 1H, Ar-H), 7.66-7.70 (m, 4H, Ar-H), 7.72 (s, 1H, Ar-H),12.91 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 21.33, 115.8, 116.2, 124.1, 128.70, 129.20, 129.29, 130.34, 130.72, 131.04, 137.35, 138.11, 140.3, 141.78, 145.86, 145.2, 146.12, 161.54; ESI-MS (*m/z*) = 476.0 $(M+H)^+$; calculated for $C_{24}H_{15}Cl_2N_5S$; C, 60.51; H, 3.17; N, 14.88; S,6.73. Found: C, 60.55; H, 3.18; N, 14.90; S, 6.70.

2-(2-(2-Chlorophenyl)-6-(4-methoxyphenyl)imidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)-5-nitro-1*H*-benzo[d]imidazole (T7): Light yellow solid. FTIR (ATR, cm^{-1}): 3351, 3068, 2921, 1594, 1517, 1503, 1463, 1244, 1028, 838, 752, 681; ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 3.81 (s, 3H, OCH_3), 7.02 (d, *J* = 8.3 Hz, 2H, Ar-H), 7.62 (d, *J* = 6.6 Hz, 1H, Ar-H), 7.67 (s, 1H, Ar-H), 7.70 (d, *J* = 7.5 Hz, 1H, Ar-H), 7.84 (d, *J* = 8.7 Hz, 1H, Ar-H), 8.07 (d, *J* = 8.3 Hz, 2H, Ar-H), 8.18 (d, *J* = 8.4 Hz, 1H, Ar-H), 8.26 (d, *J* =7.5Hz, 1H, Ar-H), 8.58 (s, 1H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 55.82, 114.24, 115.12, 127.0, 129.0, 130.6, 131.6, 132.5, 134.02, 142.90, 143.08, 143.63, 146.09, 146.90, 159.72; ESI-MS (*m/z*) = 503.0 $(M+H)^+$; calculated

for $C_{24}H_{15}ClN_6O_3S$; C, 57.32; H, 3.01; N, 16.71; S,6.38. Found: C, 57.34; H, 3.08; N, 16.75; S, 6.35.

2-(6-(4-Methoxyphenyl)-2-phenylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)-5-nitro-1*H*-benzo[*d*]imidazole (T8): Light yellow solid. FTIR (ATR, cm^{-1}): 3351, 3078, 2935, 1563, 1519, 1466, 1248, 1028, 833, 683; ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 3.78 (s, 3H, OCH_3) , 7.10 (d, J = 8.8 Hz, 2H, Ar-H), 7.34-7.45 (m, 3H, Ar-H), 7.58-7.60 (m, 2H, Ar-H), 7.91 (d, J = 8.4 Hz, 2H, Ar-H), 8.18 (d, J =8.5 Hz, 1H, Ar-H), 8.26 (d, J = 7.6 Hz, 1H, Ar-H),8.55 (s, 1H, Ar-H),12.91 (s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 55.59, 114.62, 115.44, 115.65, 123.18, 126.37, 129.43, 126.91, 132.45, 144.12, 142.70, 146.20, 160.74, 162.32; ESI-MS (*m/z*) = 469.1 $(M+H)^+$; calculated for $C_{14}H_{16}N_6O_3S$; C, 61.53; H, 3.44; N, 17.94; S,6.84. Found: C, 61.60; H, 3.45; N, 17.96; S, 6.85.

2-[6-(4-Fluorophenyl)-2-(4-methylphenyl)imidazo[2,1-*b*][1,3,4]thiadiazol-5-yl]-1*H*-benzimidazole (T9): Yellow solid. FTIR (ATR, cm^{-1}): 3353, 3077, 2934, 1602, 1538, 1464, 1061, 827, 688; ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 2.50 (s, 3H, CH_3), 7.23-7.33 (m, 4H, Ar-H),7.43 (d, J = 8.4 Hz, 2H, Ar-H), 7.66-7.76 (m, 2H, Ar-H), 7.96 (d, J = 7.9 Hz, 2H, Ar-H), 8.12-8.15 (m, 2H, Ar-H), 12.82 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 21.59, 115.70, 123.55, 127.05, 127.50, 130.12, 130.47, 141.81, 143.13, 145.58, 161.25, 162.85, 163.69; ESI-MS (*m/z*) = 426.0 $(M+H)^+$; calculated for $C_{24}H_{16}FN_5S$; C, 67.75; H, 3.79; N, 16.46; S,7.54. Found: C, 67.70; H, 3.81; N, 16.50; S, 7.52.

2-(6-(4-Fluorophenyl)-2-*p*-tolylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)-5-nitro-1*H*-benzo[*d*]imidazole (T10): Light yellow solid. FTIR (ATR, cm^{-1}): 3351, 3068, 2926, 1597, 1538, 1475, 1064, 834, 687; ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 2.42 (s, 3H, CH_3), 7.29 (t, J = 17.5 Hz, 2H, Ar-H), 7.39-7.46 (m, 2H, Ar-H), 7.83-07.91 (m, 2H, Ar-H), 8.301 (d, J = 10.1 Hz, 2H, Ar-H), 8.20 (m, 2H, Ar-H), 8.60 (s, 1H, Ar-H), 13.29 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 21.62, 114.58, 115.44, 115.65, 126.91, 127.02, 127.63, 130.21, 130.53, 130.95, 143.31, 161.45, 163.90; ESI-MS (*m/z*) = 468.9 $(M-H)^+$; calculated for $C_{24}H_{15}FN_6O_2S$; C, 61.27; H, 3.21; N, 17.86; S,6.82. Found: C, 61.25; H, 3.23; N, 17.82; S, 6.80.

2-[2-(4-Chlorophenyl)-6-(4-methoxyphenyl)imidazo[2,1-*b*][1,3,4]thiadiazol-5-yl]-1*H*-benzimidazole (T11): Light yellow solid. FTIR (ATR, cm^{-1}): 3350, 3077, 2937, 1608, 1538, 1437, 1028, 826, 751, 689; ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 3.78 (s, 3H, CH_3), 6.98 (d, J = 8.8 Hz, 2H, Ar-H), 7.2-7.4 (m, 2H, Ar-H), 7.694 (d, J = 8.8 Hz, 4H, Ar-H), 8.02-8.10 (m, 4H, Ar-H), 12.77 (s, NH, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 55.63, 114.8, 126.35, 123.06, 128.73, 129.27, 129.41, 130.12, 137.30,141.78, 145.50, 145.66, 159.80, 161.18; ESI-MS (*m/z*) = 458.0 $(M+H)^+$; calculated for $C_{24}H_{16}ClN_5S$; C, 62.95; H, 3.52; N, 15.29; S, 7.00. Found: C, 62.90; H, 3.53; N, 15.50; S, 6.90.

2-[2-(4-Chlorophenyl)-6-(4-fluorophenyl)imidazo[2,1-*b*][1,3,4]thiadiazol-5-yl]-1*H*-benzimidazole (T12): Light yellow solid. FTIR (ATR, cm^{-1}): 3351, 3068, 2936, 1616, 1537, 1467, 1068, 837, 748, 693; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.24-7.29 (m, 4H, Ar-H), 7.70 (d, J = 8.4 Hz, 4H, Ar-H), 8.10-8.17 (m, 4H, Ar-H), 12,83 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 115.50, 115.72, 123.12, 128.63, 129.33, 130.10, 130.20, 130.28, 137.43, 144.80, 145.71, 161.30, 161.68, 163.78; ESI-MS (*m/z*) = 446.0 $(M+H)^+$; calculated for $C_{23}H_{13}ClFN_5S$; C, 61.95; H, 2.94; N, 15.71; S,7.19. Found: C, 61.90; H, 2.96; N, 15.65; S, 7.20.

2-(2-(4-Fluorophenyl)-6-(4-methoxyphenyl)imidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)-1*H*-benzo[*d*]imidazole (T13): Off white solid. FTIR (ATR, cm^{-1}): 3349, 3073, 2937, 1611, 1540, 1467, 1244, 1061, 1028, 827, 686; ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 3.79 (s, 3H, OCH_3),6.97 (d, J = 8.4 Hz, 2H, Ar-H), 7.3-7.5 (m, 2H, Ar-H), 7.69-7.72 (m, 4H, Ar-H), 8.04-8.08 (m, 4H, Ar-H), 12.76 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 55.73, 114.7, 126.45, 123.16, 127.63, 129.37, 129.21, 130.32, 142.98, 145.70,145.66, 159.80, 161.18, 165.20; ESI-MS (*m/z*) = 442.1 $(M+H)^+$; calculated for $C_{24}H_{16}CFN_5S$; C, 65.29; H, 3.65; N, 15.86; S,7.26. Found: C, 64.90; H, 3.68; N, 15.60; S, 7.20.

2-(2-(4-Fluorophenyl)-6-(4-methoxyphenyl)imidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)-5-nitro-1*H*-benzo[*d*]imidazole (T14): Yellow solid. FTIR (ATR, cm^{-1}): 3350, 3077, 2936, 1603, 1518, 1464, 1244, 1071, 1028, 831, 691; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 3.81 (s, 3H, OCH_3),7.02 (d, J = 8.8 Hz, 2H, Ar-H),7.46 (d, J = 7.4 Hz, 2H,

Ar-H), 7.86 (d, *J* = 8.8 Hz, 2H, Ar-H), 8.00-8.20 (m, 4H, Ar-H), 8.61 (s, 1H, Ar-H), 13.10-13.293 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 55.73, 114.7, 115.32, 123.19, 126.45, 123.16, 127.63, 129.37, 129.21, 130.32, 134.31, 142.98, 145.70, 145.66, 159.80, 161.18, 165.20; ESI-MS (*m/z*) = 487.1 $(M+H)^+$; calculated for $C_{24}H_{15}FN_6O_3S$; C, 59.25; H, 3.11; N, 17.28; S, 6.59. Found: C, 59.22; H, 3.12; N, 17.26; S, 6.62.

5-Chloro-2-(2-(4-fluorophenyl)-6-(4-methoxyphenyl)imidazo[2,1-*b*][1,3,4] thiadiazol-5-yl)-1*H*-benzo[*d*]imidazole (T15): Light brown solid. FTIR (ATR, cm^{-1}): 3351, 3078, 2937, 1601, 1538, 1463, 1068, 1024, 1028, 751, 686; ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 3.84 (s, 3H, OCH_3), 6.99 (d, *J* = 8.4 Hz, 2H, Ar-H), 7.30 (dd, *J* = 1.7, 8.3 Hz, 1H, Ar-H), 7.43 (m, 2H, Ar-H),7.71 (d, *J* =8.8 Hz, 1H, Ar-H),7.76 (s, 1H, Ar-H), 7.96 (d, *J* = 8.3 Hz, 2H, Ar-H), 8.01 (m, 2H, Ar-H), 12.4-13.6 (s, 1H, br); ^{13}C NMR (100 MHz, DMSO-d_6): 55.42, 114.24, 114.32, 123.29, 126.61, 127.19, 127.56, 129.94, 131.44, 143.08, 143.53, 145.70, 146.09, 159.92, 162.64, 165.20; ESI-MS (*m/z*) = 476.1 $(M+H)^+$; calculated for $C_{24}H_{15}ClFN_5OS$; C, 60.57; H, 3.18; N, 14.72; S,6.74. Found: C, 60.48; H, 3.20; N, 14.68; S, 3.20.

2-(6-(4-Chlorophenyl)-2-p-tolylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)-1*H*-benzo[*d*] imidazole (T16): Light yellow solid. FTIR (ATR, cm^{-1}): 3348, 3068, 2924, 1592, 1534, 1476, 840, 727, 689; ^{1}H NMR (400 MHz, DMSO-d_6) δ(ppm): 2.41 (s, 3H, CH_3), 7.30 (dd, *J* = 3.0, 6.1 Hz, 2H, Ar-H), 7.40 (d, *J* = 8.3 Hz, 2H, Ar-H),7.48 (d, *J* = 8.7 Hz, 2H, Ar-H), 7.70-7.72 (m, 2H, Ar-H), 7.96 (d, *J* = 8.3 Hz, 2H, Ar-H), 8.10 (d, *J* = 8.3 Hz, 2H, Ar-H), 11.6-14.2 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ(ppm): 21.60, 115.60, 123.21, 127.02, 127.55, 128.80, 129.76, 130.57, 132.77, 133.26, 143.23, 144.43, 145.83, 163.21; ESI-MS (*m/z*) = 442.0 $(M+H)^+$; calculated for $C_{24}H_{16}ClN_5S$; C, 65.23; H, 3.65; N, 18.44; S, 7.26. Found: C, 65.18; H, 3.68; N, 18.46; S, 7.28.

2-(6-(4-Chlorophenyl)-2-*p*-tolylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)-5-nitro-1*H*-benzo[*d*]imidazole (T17): Yellow solid. FTIR (ATR, cm^{-1}): 3351, 3068, 2924, 1592, 1534, 1518, 1476, 837, 748, 686; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.41(s, 3H, CH_3) ,7.43 (d, *J* = 7.9 Hz, 2H, Ar-H), 7.49 (d, *J* = 8.3 Hz, 2H, Ar-H), 7.72 (d, *J* = 7.5 Hz, 1H, Ar-H), 7.98 (d, *J* = 7.9 Hz, 2H, Ar-H), 8.10 (d, *J* = 8.4 Hz, 2H, Ar-H),

8.26 (d, *J* = 7.5 Hz, 1H, Ar-H), 8.58 (s, 1H, Ar-H), 12.6-14.2 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 21.39, 112.64, 113.32, 123.19, 126.31, 127.29, 127.38, 127.86, 130.44, 134.31, 142.6, 143.18, 143.33, 145.30, 146.29, 160.32; ESI-MS (*m/z*) = 487.0 $(M+H)^+$; calculated for $C_{24}H_{15}ClN_6O_2S$; C, 59.20; H, 3.10; N, 17.26; S, 6.59. Found: C, 59.22; H, 3.12; N, 17.26; S, 6.60.

5-chloro-2-(6-(4-chlorophenyl)-2-*p*-tolylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)-1*H*-benzo[*d*]imidazole (T18): Light yellow solid. FTIR (ATR, cm^{-1}): 3351, 3073, 2935, 1598, 1540, 1464, 827, 693; ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 2.416 (s, 3H, CH_3),7.31 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.43 (d, *J* = 7.9 Hz, 2H, Ar-H), 7.49 (d, *J* = 8.3 Hz, 2H, Ar-H), 7.72 (d, *J* = 7.9 Hz, 1H, Ar-H), 7.77 (s, 1H, Ar-H), 7.98 (d, *J* = 7.9 Hz, 2H, Ar-H), 8.10 (d, *J* = 8.4 Hz, 2H, Ar-H), 12.6-13.1(s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 21.49, 112.14, 113.12, 123.09, 126.21, 127.19, 127.38, 127.56, 129.44, 130.44, 134.31, 143.08, 143.43, 145.50, 146.19, 160.82; ESI-MS (*m/z*) = 476.0 $(M+H)^+$; calculated for $C_{24}H_{15}ClN_6O_2S$; C, 59.20; H, 3.10; N, 17.26; S, 6.59. Found: C, 59.22; H, 3.12; N, 17.26; S, 6.60.

2-(6-(4-Methoxyphenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)-1*H*-benzo [*d*]imidazole (T19): White solid. FTIR (ATR, cm^{-1}): 3273, 3071, 2965, 1592, 1494, 1437, 1248, 1028, 835, 691; ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 2.78 (s, 3H, CH_3), 3.75 (s, 3H, OCH_3), 6.94 (d, *J* = 8.8 Hz, 2H, Ar-H),7.25 (m, 2H, Ar-H),7.58 (d, *J* = 7 Hz, 1H, Ar-H), 7.70 (d, *J* = 7.4 Hz, 1H, Ar-H), 7.91 (d, *J* = 8.8 Hz, 2H, Ar-H),12.73 (s, 1H, NH) ^{13}C NMR (100 MHz, DMSO-d_6): 18.07, 55.60, 114.19, 114.33, 119.69, 126.56, 129.10, 142.23, 144.99, 146.12, 159.61, 161.93; ESI-MS (*m/z*) = 362.0 $(M+H)^+$; calculated for $C_{19}H_{15}N_5OS$; C, 63.14; H, 4.18; N, 19.38; S,8.87. Found: C, 63.10; H, 4.20; N, 19.40; S, 8.85.

2-(6-(4-Methoxyphenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)-5-nitro-1*H*-benzo[*d*]imidazole (T20): yellow solid. FTIR (ATR, cm^{-1}): 3273, 3068, 2965, 1604, 1518, 1497, 1436, 1246, 1024, 836, 687; ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 2.78 (s, 3H, CH_3), 3.78 (s, 3H, OCH_3), 7.913 (d, *J* = 8.8 Hz, 2H, Ar-H),7.58-7.60 (m, 2H, Ar-H), 8.18 (d, *J* = 8.5 Hz, 1H, Ar-H), 8.26 (d, *J* =7.6Hz, 1H, Ar-H), 8.55(s, 1H, Ar-H), 12.91 (s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 18.22,

55.60, 114.52, 123.08, 127.37, 129.43, 132.35, 144.12, 142.8, 146.20, 160.7, 162.42; ESI-MS (*m/z*) = 407.0 $(M+H)^+$; calculated for $C_{19}H_{14}N_6O_3S$; C, 56.15; H, 3.47; N, 20.68; S,7.89. Found: C, 56.10; H, 3.45; N, 20.70; S, 7.85.

2-(6-(4-Fluorophenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)-1*H*-benzo[*d*] imidazole (T21): White solid. FTIR (ATR, cm^{-1}): 3273, 3074, 2964, 1597, 1538, 1437, 1062, 834, 687; ^1H NMR (DMSO-d_6, 400 MHz) δ (ppm): 2.804 (s, 3H, CH_3),7.22 (d, 2H, ArH, *J* =8.8Hz), 7.26-7.28 (m, 2H, Ar-H) , 7.67(dd, *J* = 5.6, 3.2 Hz, 2H, Ar-H), 8.04 (dd, *J* =8.4 , 6.0 Hz, 2H, Ar-H) , 12.82 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 18.10, 115.09, 115.52, 115.74, 122.99, 129.87, 129.95, 130.52, 130.55, 141.87, 144.04, 146.42, 161.17, 163.61; ESI-MS (*m/z*) = 350.08 $(M+H)^+$; calculated for $C_{18}H_{12}FN_5S$; C, 61.88; H, 3.46; N, 20.04; S, 9.18. Found: C, 61.76; H, 3.48; N, 21.06; S, 9.16.

2-(6-(4-Fluorophenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)-5-nitro-1*H*-benzo[*d*]imidazole (T22): Yellow solid. FTIR (ATR, cm^{-1}): 3347, 3077, 2935, 1603, 1518, 1464, 1092, 831, 690; ^1H NMR (DMSO-d_6, 400 MHz) δ (ppm): 2.82 (s, 3H, CH_3), 7.68 (dd, *J* = 2.0, 6.8 Hz, 2H, Ar-H), 7.70 (d, *J* = 7.6 Hz, 1H, Ar-H), 8.06 (dd, *J* = 3.2, 5.6 Hz, 2H, Ar-H), 8.26 (d, *J* =7.6 Hz, 1H, Ar-H), 8.58 (s, 1H, Ar-H), 12.89 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 18.15, 115.19, 123.19, 129.87, 129.97, 130.52, 138.55, 141.77, 144.04, 146.82, 161.27, 163.61; ESI-MS (*m/z*) = 395.1 $(M+H)^+$; calculated for $C_{18}H_{11}FN_6O_2S$; C, 54.82; H, 2.81; N, 21.31;S,8.13. Found: C, 54.80; H, 2.83; N, 21.32; S, 8.11.

5-Chloro-2-(6-(4-fluorophenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)-1*H*-benzo[*d*]imidazole (T23): Light green solid. FTIR (ATR, cm^{-1}): 3352, 3071, 2937, 1603, 1478, 1437, 1071, 837, 752, 690; ^1H NMR (DMSO-d_6, 400 MHz) δ (ppm): 2.80 (s, 3H, CH_3), 7.28 (dd, *J* =1.8, 8.8 Hz, 1H, Ar-H), 7.45 (d, *J* = 8.3 Hz, 2H, Ar-H), 7.64 (d, *J* = 8.7 Hz, 1H, Ar-H), 7.72 (s, 1H, Ar-H), 8.09 (d, *J* = 8.0 Hz, 2H, Ar-H), 12.83 (s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 18.25, 115.0, 115.7, 123.62, 127.48, 129.81, 121.63, 131.29, 132.89, 133.27, 138.90, 142.1, 143.44, 144.28, 158.9, 162.97; ESI-MS (*m/z*) = 383.9 $(M+H)^+$; calculated for $C_{18}H_{11}ClFN_5S$; C, 56.33; H, 2.89; N, 18.25; S, 8.35. Found: C, 56.31; H, 2.90; N, 18.19; S, 8.31.

2-(6-(4-Chlorophenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)-1*H*-benzo[*d*] imidazole (T24): Off white solid. FTIR (ATR, cm^{-1}): 3272, 3070, 2965, 1590, 1495, 1436, 835, 751, 690; ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 2.81 (s, 3H, CH_3), 7.23 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.28-7.30 (m, 2H, Ar-H), 7.69 (dd, *J* =1.8, 8.8Hz, 2H, Ar-H), 8.06 (dd, *J* =6.0, 8.4 Hz, 2H, Ar-H), 12.83 (s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 18.20, 115.09, 123.29, 129.89, 129.95, 130.53, 130.75, 134.2, 141.77, 144.14, 146.32, 161.13; ESI-MS (*m/z*) = 366.08 $(M+H)^+$; calculated for $C_{18}H_{12}ClN_5S$; C, 59.09; H, 3.31; N, 19.14; S, 8.76. Found: C, 59.06; H, 3.33; N, 19.12; S, 8.74.

2-(6-(4-Chlorophenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)-5-nitro-1*H*-benzo[*d*]imidazole (T25): Yellow solid. FTIR (ATR, cm^{-1}): 3278, 3070, 2968, 1591, 1496, 1434, 834, 758, 689; ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 2.83 (s, 3H, CH_3),7.68 (dd, *J* = 1.9, 7.6 Hz, 2H, Ar-H) ,7.70 (d, *J* = 7.5Hz, 1H, Ar-H), 8.08 (dd, *J* = 1.8, 8.8 Hz, 2H, Ar-H), 8.26 (d, *J* = 7.6 Hz, 1H, Ar-H), 8.58 (s, 1H, Ar-H), 12.85 (s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 18.16, 115.96, 123.42, 127.58, 129.21, 121.93, 130.83, 132.89, 133.27, 145.14, 146.28, 162.96; ESI-MS (*m/z*) = 411.0 $(M+H)^+$; calculated for $C_{18}H_{11}ClN_6O_2S$; C, 52.62; H, 2.70; N, 20.46; S, 7.80. Found: C, 52.55; H, 2.71; N, 20.48; S, 7.82.

5-Chloro-2-(6-(4-chlorophenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)-1*H*-benzo[*d*]imidazole (T26): Light green solid. FTIR (ATR, cm^{-1}): 3275, 3069, 2965, 1592, 1497, 837, 753, 690; ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 2.81 (s, 3H, OCH_3), 7.29 (dd, J =8.7, 1.7Hz, 1H, ArH), 7.46 (d, *J* = 8.3Hz, 2H, ArH), 7.67 (d, *J* = 8.3Hz, 1H, ArH), 7.73 (s, 1H, ArH), 8.00 (d, *J* = 8.3Hz, 2H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 18.15, 114.96, 123.32, 127.38, 128.77, 129.20, 129.73, 130.89, 132.79, 133.17, 143.14, 144.18, 146.87, 162.94; ESI-MS (*m/z*) = 400.0 $(M+H)^+$; calculated for $C_{18}H_{11}Cl_2N_5S$; C, 54.01; H, 2.77; N, 17.50; S, 8.01. Found: C, 54.02; H, 2.80; N, 17.52; S, 8.04.

2-(2-Methyl-6-*p*-tolylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)-1*H*-benzo[*d*]imidazole (T27): Off white solid. FTIR (ATR, cm^{-1}): 3273, 3069, 2964, 1590, 1497, 1437, 838, 751, 691; ^{1}H NMR (400 MHz, DMSO-d_6) δ(ppm): 2.30 (s, 3H, CH_3), 2.89 (s, 3H,

CH_3), 7.18 (d, *J* =8.4 Hz, 2H, Ar-H),7.26 (dd, *J*=3.1, 6.2 Hz, 2H, Ar-H),7.65 (m, 2H, Ar-H),7.80 (d, *J* =8.4 Hz, 2H, Ar-H),12.76 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 18.07, 21.29, 114.82, 122.88, 127.67, 129.33, 131.25, 137.78, 142.09, 145.09, 146.22, 162.15; ESI-MS (*m/z*) = 346.0 $(M+H)^+$; calculated for $C_{19}H_{15}N_5S$; C, 66.07; H, 4.38; N, 20.27; S, 9.28. Found: C, 66.12; H, 4.40; N, 20.25; S, 9.25.

2-(2-Methyl-6-*p*-tolylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)-5-nitro-1*H*-benzo[*d*] imidazole (T28): Yellow solid. FTIR (ATR, cm^{-1}): 3271, 3068, 2924, 1598, 1518, 1438, 842, 688; ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 2.30 (s, 3H, CH_3), 2.78 (s, 3H, CH_3), 7.26 (dd, *J* =3.1, 6.2 Hz, 1H, Ar-H), 7.29 (d, *J* = 8.7 Hz, 1H, Ar-H), 7.65 (m, 2H, Ar-H), 8.12 (d, *J* = 7.4 Hz, 1H, Ar-H), 8.26 (d, *J* = 8.4 Hz, 1H, Ar-H), 8.68 (s, 1H, Ar-H), 12.90 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 18.12, 21.30, 114.62, 122.98, 127.27, 129.43, 131.35, 137.78, 145.12, 142.8, 146.30, 162.32; ESI-MS (*m/z*) = 391.08 $(M+H)^+$; calculated for $C_{19}H_{14}N_6O_2S$; C, 58.45; H, 3.61; N, 21.53; S, 8.21. Found: C, 58.29; H, 3.62; N, 21.55; S, 8.23.

5-Chloro-2-(2-methyl-6-*p*-tolylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)-1*H*-benzo[*d*] imidazole (T29): Light green solid. FTIR (ATR, cm^{-1}): 3273, 3070, 2965, 1590, 1498, 1435, 835, 751, 691; ^{1}H NMR (DMSO-d_6, 400 MHz) δ(ppm): 2.30 (s, 3H, CH_3), 2.79(s, 3H, CH_3), 7.01 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.49 (d, *J* = 8.3 Hz, 2H, Ar-H), 8.10 (d, *J* = 8.4 Hz, 1H, Ar-H), 8.26 (d, *J* = 7.6 Hz, 1H, Ar-H), 8.58 (s, 1H, Ar-H), 12.94 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 18.16, 21.35, 114.98, 122.42, 127.48, 129.61, 121.93, 130.89, 132.79, 133.17, 143.14, 144.18, 151.2, 152.3, 162.94; ESI-MS (*m/z*) = 380.0 $(M+H)^+$; calculated for $C_{19}H_{14}ClN_5S$; C, 66.07; H, 3.71; N, 18.44; S, 8.44. Found: C, 66.06; H, 3.70; N, 18.46; S, 8.40.

2.4 PHARMACOLOGY

2.4.1 Antitubercular screening

All the compounds and standard drugs were prepared two fold dilutions (50.0, 25.0, 12.5, 6.25, 3.13, 1.56, 0.78 and 0.4 μg/mL) of all and incorporated in Middle broke 7H11 agar medium with OADC (oleic acid, albumin, dextrose and catalase; Difco) Growth Supplement adjusted to 1 mg/mL (wet weight) in Tween 80 (0.05%) saline diluted to 10^{-2} to give a concentration of $\sim 10^7$ cfu/mL. A 5 μL amount of

bacterial suspension was spotted into 7H11 agar tubes containing 10-fold serial dilutions of drugs per mL. The tubes were incubated at 37 °C, and final readings were recorded after 28 days. This method is similar to that recommended by the National Committee for Clinical Laboratory Standards for the determination of MIC in triplicate.

2.4.2 Antibacterial screening

All bacterial strains were maintained on nutrient agar medium at 37 °C. The cultures were inoculated in fresh 10 mL Nutrient Broth to yield an initial suspension of approximately 10–100 cfu/mL. All broths were then incubated statically at 37 °C for 18–24 h. Susceptibility of the test organism to the organic compound was determined by well plate technique. The bacterial suspensions were serially diluted in saline and 0.1 ml from the appropriate dilution was spread on nutrient agar. The wells were dug in each petri plate by sterilized cork borer. The compounds were dissolved in DMSO and appropriate dilutions were made (1 mg/mL and 0.5 mg/mL). Each experiment was carried out in triplicate. The same procedure was repeated for all microorganisms. After the inoculation of the organism and compound, the petri plates were incubated for 18 h at 37 °C. The diameter of zone of inhibition was measured and the values for DMSO solvent were subtracted to get the actual values.

2.4.3 *In vitro* cytotoxicity studies

Vero (African green monkey kidney) cell line was procured from National Centre for Cell Sciences (NCCS), Pune, India. Stock cells were cultured in MEM supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 μg/ml) and amphotericin B (5 μg/ml) in a humidified atmosphere of 5% CO_2 at 37 °C until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 cm^2 culture flasks and all experiments were carried out in 96 microtitre plates (Tarsons India Pvt. Ltd., Kolkata, India). For cytotoxicity studies, each weighed test drugs were separately dissolved in distilled DMSO and volume was made up with MEM supplemented with 2% inactivated FBS to obtain a stock solution of 1 mg/ml concentration and sterilized by filtration. Serial two fold dilutions were prepared from this for carrying out MTT assay. The monolayer cell culture was

trypsinized and the cell count was adjusted to 1.0 x 10^5 cells/ml using MEM containing 10% FBS. To each well of the 96 well microtitre plate, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100 μL of different test concentrations of test drugs were added on to the partial monolayer in microtitre plates. The plates were then incubated at 37 °C for 3 days in 5% CO_2 atmosphere, and microscopic examination was carried out and observations were noted every 24 h interval. After 72 h, the drug solutions in the wells were discarded and 50 μl of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 h at 37 °C in 5% CO_2 atmosphere. The supernatant was removed and 100 μL of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm.

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

2.4.4 Molecular docking studies

The crystal structure of InhA (PDB code: 1P44) from species of *Mtb* for docking explorations were taken from Protein Data Bank. The protein was initially subjected to various processes such as removal of water molecules and removal of heteroatoms etc., using the Protein Preparation Wizard of Schrödinger 2015. The molecules were sketched using Maestro panel of Schrodinger and were subjected for all possible conformation generation using LigPrep module. These molecules were docked to the active site of protein using extra precision (XP) mode of Glide.

2.5 RESULTS AND DISCUSSION

2.5.1 Chemistry

The formation of compounds **3a-m** was confirmed by ^{1}H NMR, ^{13}C NMR and mass spectral analysis. For instance, the ^{1}H NMR spectrum of compound **3b** (**figure 2.16**) showed a singlet with one proton at δ 7.92 ppm, which indicates the presence of imidazole ring proton (C_5-H). Further, the spectrum displayed singlets at δ (CDCl3, 400 MHz) 2.38 and 2.71 ppm representing the methyl protons on phenyl and 1,3,4-

thiadiazole rings, respectively. Also, its mass spectrum showed molecular ion peak at *m/z* 230.0 (**figure 2.6)**, which corresponds to M+1 peak of the molecule. In the ^{1}H NMR spectrum of **4c (figure 2.29)**, the singlet peak due to imidazole ring proton (C_5-H) disappeared whereas a new singlet appeared at δ 10 ppm confirming the presence of aldehyde (-CHO) group. Also, its mass spectrum showed molecular ion peak at *m/z* 257.9, which corresponds to M+1 peak of the molecule. The ^{1}H NMR spectrum of **4d** (**figure 2.31**) shows two singlets at 2.5 (aromatic methyl) and 3.9 ppm (aromatic methoxy) which indicates the presence of methyl and methoxy groups in the molecule, respectively along with other characteristic signals. Single crystals of **4d** were analyzed by single crystal X-ray diffractometer to confirm its three dimensional structure. Finally, the target compounds, 2-(2-substituted-6-phenylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)-1*H*-benzimidazole (**T1-T29**), were synthesized by treating compounds **4a–m** with different substituted o-phenylenediamines in the presence $Na_2S_2O_5$ under heating conditions using DMF as a solvent. The structural features and physical constant of compounds **T1-T29** are given in **table 2.1**. The chemical structure of the target compounds were confirmed by spectral analysis and elemental data. The ^{1}H NMR spectrum of **T27** (**figure 2.4**) showed a broad singlet at δ 12.8 ppm due to NH proton of benzimidazole ring. Further, singlets at δ 2.9 and δ 2.3 ppm indicate the methyl protons of 1,3,4-thiadiazole and phenyl rings, respectively. In addition, the spectrum displayed two doublets at δ 7.18 and 7.8 ppm, multiplet in the region of δ 7.65 ppm and double of doublet in the region of δ 7.26 ppm due to aromatic (phenyl) protons. Also, its mass spectrum (**figure 2.6**) showed molecular ion peak at *m/z* 346.0, which corresponds to M+1 peak of the molecule and is in agreement with its molecular formula $C_{19}H_{15}N_5S$. The three dimensional structure of **T27** was evidenced by single crystal X-ray diffraction (SCXRD) study. The spectral and elemental analysis data of all target compounds are given in the experimental part.

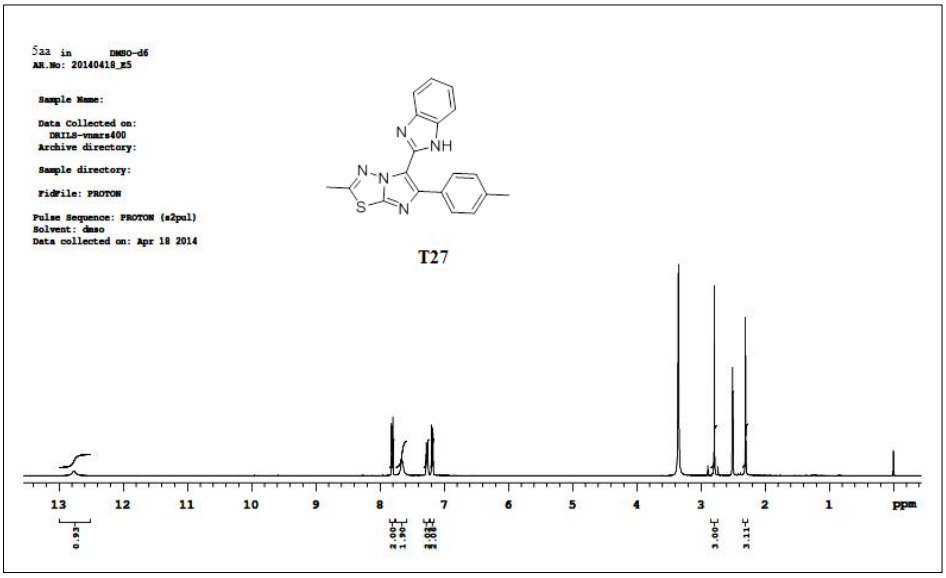


Figure 2.4 ^{1}H NMR spectrum of **T27**

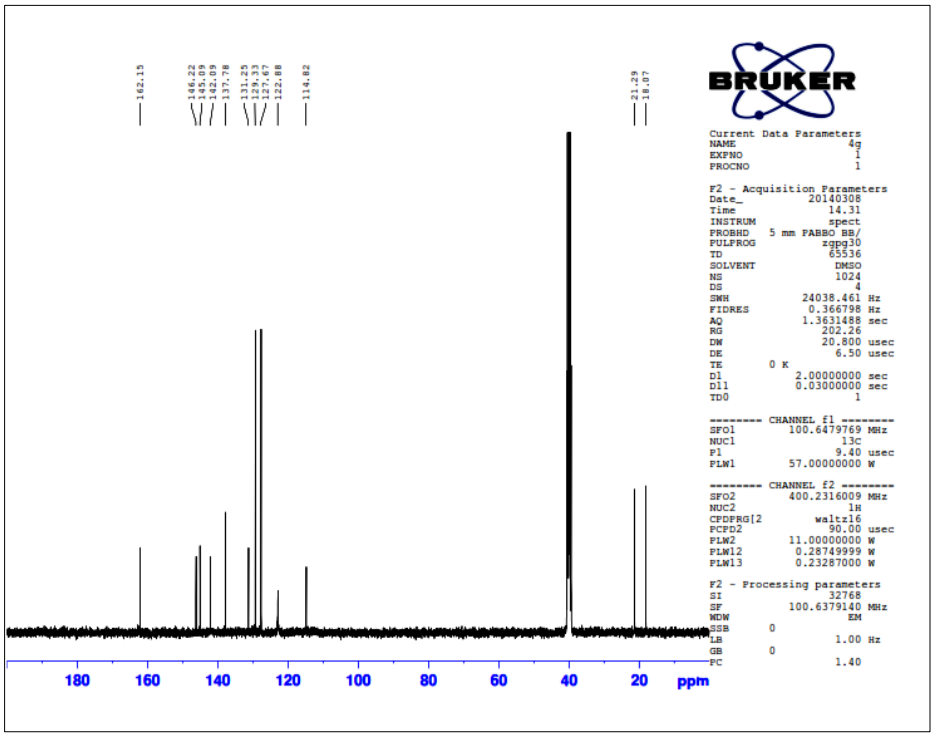


Figure 2.5 ^{13}C NMR spectrum of **T27**

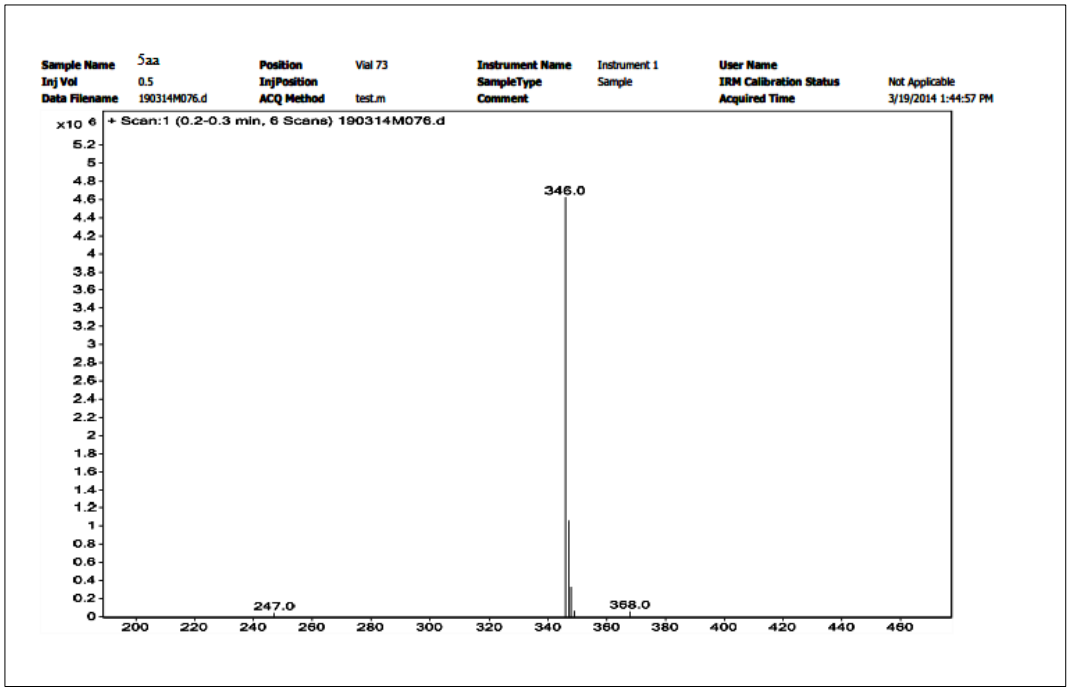
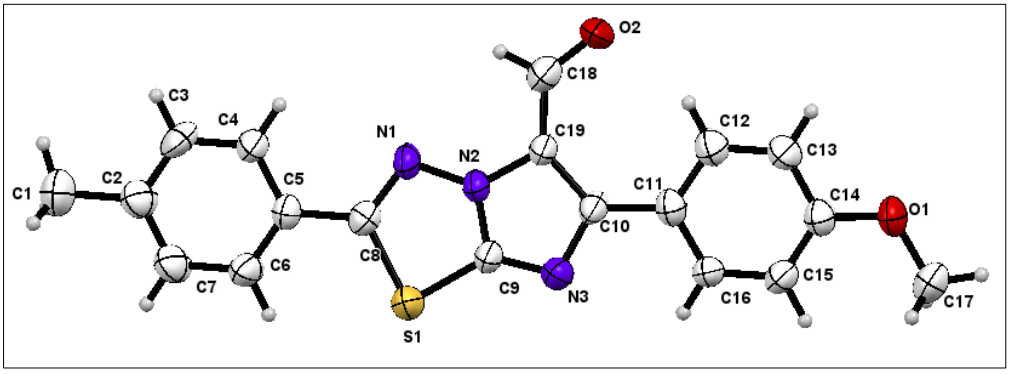


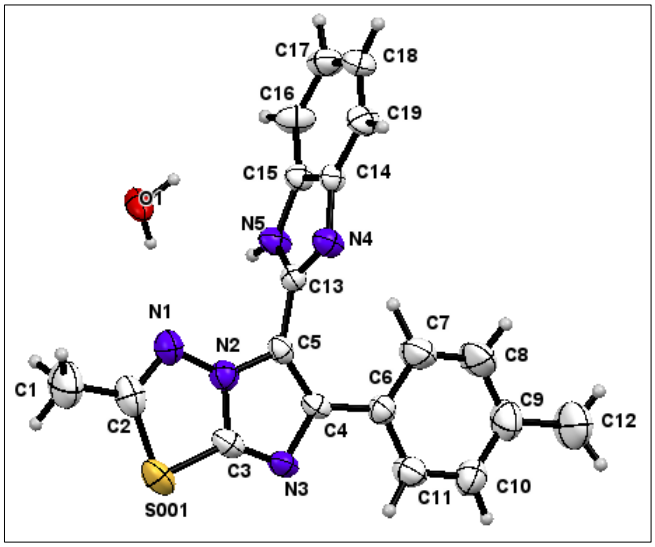
Figure 2.6 Mass spectrum of **T27**

2.5.2 Single crystal X-ray crystallography studies

Single crystals of **4d** and **T27** were grown from a solvent mixture of methanol and chloroform (1:1) by the slow evaporation of solvent mixture at RT. Compounds (**4d** and **T27**) crystallized in the monoclinic system with P 2_1/n space group. The crystal structure of the compounds is shown in **figure 2.7**. The crystal data and measurement details for compounds **4d** and **T27** are given in **table 2.2**.



(a)



(b)

Figure 2.7 ORTEP diagram showing the X-ray crystal structure of compounds a) **4d** and b) **T27**.

Table 2.2 Crystal data and measurement details for compounds **4d** and **T27**.

Parameters	**Crystal data of 4d**	**Crystal data of T27**
Empirical formula	$C_{19}H_{15}N_3O_2S$	$C_{19}H_{15}N_5S.H_2O$
Formula weight	349.41	363.45
Crystal system	Monoclinic	Monoclinic
Space group	P 2_1/n	P 2_1/n
a (Å)	7.1761(7)	7.1846(5)
b (Å)	19.468(17)	22.9252(13)
c (Å)	12.022(11)	11.0542(6)
Volume (Å^3)	1611.2(3)	1820.72(19)
Angle α, β, γ	90, 106.408(6), 90	90, 90.087(3), 90
Z	4	4
F_{000}	728	760
μ (mm−1)	0.219	0.196
Temperature (T)	296k	296k
Radiation wavelength (Å)	0.71073	0.71073
Radiation type	Mo Kα	Mo Kα

Radiation source	Mo	Mo
CCDC number	1032180	1032193

2.5.3 *In vitro* antimycobacterial activity

The synthesized ITD-benzimidazole hybrids (**T1-T29**) were screened against *Mtb* H37Rv (ATCC27294) by agar dilution method to evaluate their antimycobacterial activity in terms of MIC values. The MIC is defined as the minimum concentration of compound required to completely inhibit the bacterial growth. The MIC values in µg/mL of **T1-T29** along with those of standard drugs for comparison are presented in **figure 2.8**, which shows that the values are in the range 3.125-50.0 µg/mL. It is evident that among the twenty nine compounds, seven compounds namely **T3**, **T4**, **T12**, **T16**, **T18**, **T25** and **T27** showed potent antiTB activity with MIC of 3.125 µg/mL and are equipotent with the standard Ethambutol. Four other compounds (**T5**, **T10**, **T19** and **T21**) showed promising activity against *Mtb* with MIC of 6.25 µg/mL.

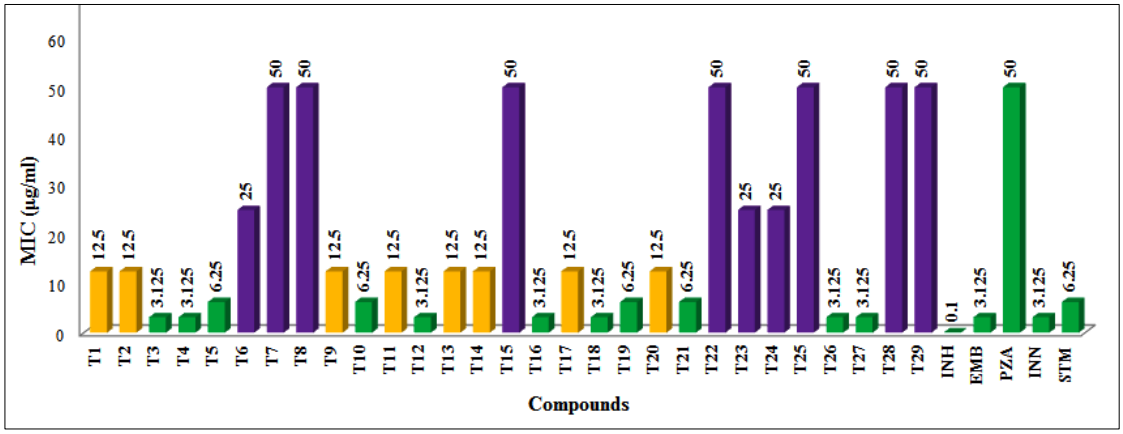


Figure 2.8 Antitubercular activity of **T1-T29** against *M. tuberculosis* H37RV (INH: Isoniazid; EMB: Ethambutol; PZA: Pyrazinamide; INN: Ciprofloxacin STM: Streptomycin).

The nature of the substituents on ITD (R^1/R^2) and benzimidazole (R^3) rings was found to affect the activity of these compounds. The nitro group on benzimidazole ring substantially decreased the antiTB activity as can be seen in case of compounds **T7**, **T8**, **T22**, **T25** and **T28** which showed an MIC value of 50 µg/mL. Whereas compounds with unsubstituted or chlorosubstituted benzimidazole rings showed excellent to moderate activity. Though two compounds (**T26** and **T27**) with a

methyl substitution at position-2 of the ITD ring exhibited significant activity, replacing the methyl group with a 4-substituted phenyl group (4-tolyl or 4-chlorophenyl) was found to be effective in enhancing anti-TB activity. This is evident from the fact that compounds with nitrobenzimidazole substitution also have shown moderate activity when a methyl group is replaced with a 4-substituted phenyl group. For instance, compounds **T5** and **T10** showed improved activity (MIC = 6.25 μg/mL) when compared to the activity of compounds **T22** and **T28** (MIC = 50 μg/mL), respectively. This information on structure-activity relationship explored in the present study could be helpful in further structural modification and development of new ITD-benzimidazole hybrids as potent antitubercular agents.

2.5.4 *In vitro* antibacterial activity

The *in vitro* antibacterial activity of synthesized compounds **T1-T29** were determined using Muller Hinton Agar method (Arthington-Skaggs et al. 2000; Rocha et al. 1995). These compounds were screened against *S. aureus*, *E. coli* and *P. aeruginosa.* The compounds were dissolved in DMSO and appropriate dilutions were made (1 mg/mL and 0.5 mg/mL). STM was taken as the standard drug for the screening. The result showed that among the tested compounds, **T9** exhibit good activity against all the tested bacterial strains at concentrations of 1 and 0.5 mg/mL, whereas compounds **T23** and **T29** are moderately active against all these microbial strains. The results of the antibacterial screening are summarized in **table 2.3**, where in, the figure represents the zone of inhibition (mm).

Table 2.3 Antibacterial activity of target compounds (**T1-T29**).

Entry	***E. coli***		***S. aureus***		***P. aeruginosa***	
Cocn. (mg/ml)	1	0.5	1	0.5	1	0.5
Control	0	0	0	0	0	0
STM	18.6±0.2	14.3±0.1	16.8±0.2	12.5±0.2	16.7±0.2	13.6±0.2
T1	–	–	–	–	–	–
T2	–	–	–	–	–	–
T3	04±0.1	02±0.2	06±0.1	03±0.2	07±0.1	03±0.2
T4	05±0.2	03±0.1	04±0.1	01±0.1	04±0.2	01±0.1
T5	–	–	–	–	–	–
T6	04±0.1	02±0.4	03±0.1	03±0.1	04±0.1	03±0.1

T7	–	–	–	–	–	–
T8	04±0.4	03±0.3	04±0.2	02±0.1	05±0.1	03±0.2
T9	13±0.5	09±0.5	14±0.1	11±0.2	13±0.1	10±0.2
T10	04±0.5	02±0.1	04±0.1	02±0.2	03±0.2	02±0.2
T11	03±0.2	01±0.1	03±0.1	02±0.2	02±0.2	01±0.2
T12	05±0.1	03±0.2	07±0.1	03±0.2	08±0.1	05±0.2
T13	–	–	–	–	–	–
T14	05±0.3	03±0.4	05±0.1	03±0.2	06±0.1	03±0.2
T15	05±0.2	01±0.1	04±0.1	01±0.1	02±0.1	01±0.1
T16	04±0.2	02±0.2	03±0.1	02±0.1	06±0.1	03±0.2
T17	–	–	–	–	–	–
T18	04±0.1	02±0.2	05±0.2	03±0.1	03±0.1	02±0.1
T19	–	–	–	–	–	–
T20	–	–	–	–	–	–
T21	04±0.1	03±0.1	02±0.1	01±0.2	03±0.2	02±0.3
T22	04±0.3	02±0.4	03±0.1	02±0.2	01±0.1	03±0.2
T23	11±0.2	08±0.2	09±0.4	06±0.6	13±0.1	10±0.2
T24	04±0.2	03±0.1	04±0.2	03±0.3	05±0.2	03±0.1
T25	06±0.1	04±0.2	09±0.1	07±0.2	08±0.1	05±0.2
T26	04±0.1	02±0.4	03±0.2	02±0.1	04±0.1	01±0.1
T27	05±0.1	02±0.2	05±0.2	03±0.1	03±0.1	02±0.1
T28	–	–	–	–	–	–
T29	08±0.1	05±0.2	10±0.1	07±0.2	09±0.1	06±0.2
−: inhibition not detected; control: dimethylsulfoxide.						

2.5.5 *In vitro* cytotoxicity studies

The *in vitro* cytotoxicity of the active compounds (MIC ≤6.25 μg/mL against *Mtb*) were evaluated by MTT assay (Gundersen et al. 2002) against Vero cell line. The graphical representation of the cell growth inhibition of the compounds at a concentration of 62.5 μg/mL is shown in **figure 2.9**. Some of the most potent antitubercular compounds **T18**, **T19** and **T27** exhibited very low toxicity of 7.4, 7.1 and 5.03 %, respectively. Also, none of the other active compounds are toxic to the normal cells thus proving the lack of general cellular toxicity.

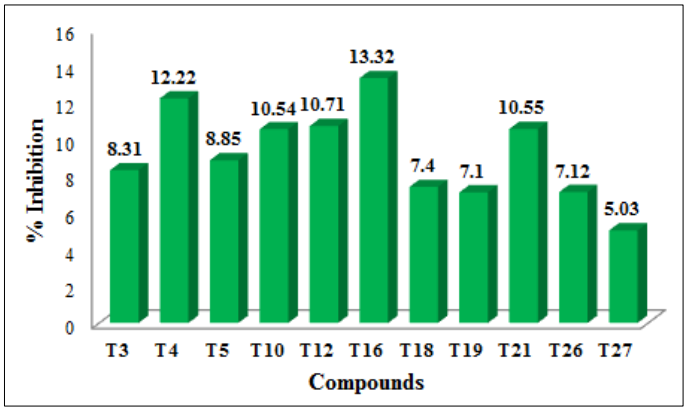


Figure 2.9 The graphical representation of the cell growth inhibition of the compounds at a concentration of 62.5 µg/mL against vero cell

2.5.6 Molecular docking studies

11 Compounds (**T3**, **T4**, **T5**, **T10**, **T12**, **T16**, **T18**, **T19**, **T21**, **T26**, **and T27**) which showed promising antiTB activity (MIC ≤ 6.25 µg/mL) were taken for molecular docking studies to check the binding interactions with the enzyme. The molecules were docked with in the active sites of enoyl-acyl carrier protein reductase (InhA, Protein code: 1P44) using Glide 6.6 (Schrodinger, 2015-1) package. The ligands from the crystal structure of the enzyme-ligand complexes were rebuilt and redocked to validate the docking procedure. InhA is one of the key enzymes involved in the type II fatty acid biosynthesis pathway. Tyr 158 is an important amino acid residue which interacts with the long chain fatty acyl substrates required for the synthesis of mycolic acids in the mycobacteria (Jena et al. 2014; Matviiuk et al. 2013).

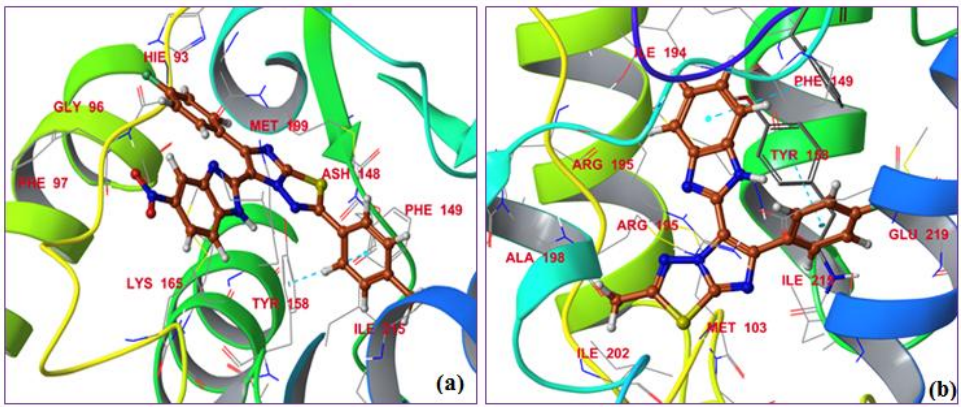


Figure 2.10 The docking poses of some active compounds with target enzyme InhA (a) **T10** with InhA; (b) **T21** with InhA.

Most of the active compounds showed interaction with Tyr 158. Compound **T10** showed *pi-pi* stacking interaction with Tyr 158. Whereas compound **T21** showed *pi-pi* stacking interactions with Phe 149 and Tyr 158. The docking score of all the active molecules and details of interacting amino acid residues are given in the **table 2.4**. The docking images of **T10** and **T21** are shown in **figure 2.10**.

Table 2.4 Docking scores of the compounds with the target enzyme (1P44) and details of the interacting amino acid residues.

Compounds	Docking score	Interacting amino acid residues
T16	-9.049	Gly 104
T12	-8.138	Phe 149
T10	-7.619	Tyr 158
T18	-7.484	Tyr 158
T3	-7.296	Tyr 158
T4	-7.10	-
T21	-7.047	Tyr 158, Phe 149
T27	-6.93	Phe 149
T26	-6.844	Tyr 158
T19	-6.202	Phe 149
T5	-6.14	Tyr 158

2.6 CONCLUSIONS

- A series of twenty nine 2-(imidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)-1*H*-benzimidazole derivatives were designed and synthesized.
- These compounds were characterized by ^{1}H NMR, ^{13}C NMR, mass spectroscopic techniques, elemental analysis and compounds **4d** and **T27** were confirmed by SCXRD studies.
- Among the tested compounds, seven compounds (**T3**, **T4**, **T12**, **T16**, **T18**, **T26** and **T27)** exhibited significant activity against the growth of *Mtb* with MIC of 3.125 μg/mL.

- The structure-activity relationships (SAR) reveal that the various substituents on ITD and benzimidazole rings affect significantly the antiTB activity of these compounds.
- Compounds with a chloro or an unsubstituted benzimidazole ring exhibited better activity when compared with that of nitro substituted derivatives. Further, phenyl group with 4-methyl or 4-chloro substituent at position-2 of ITD ring enhanced the activity.
- Further, these compounds were screened also for their antibacterial activity. Among the tested compounds **T9** showed good activity and compound **T23** and **T29** showed moderate activity against the tested bacterial strains.
- In the cytotoxicity study, the active antitubercular compounds exhibited very low toxicity against a normal cell line.
- Further, molecular docking studies showed that these molecules could be good inhibitors of InhA.
- In conclusion, the new ITD-benzimidazole hybrids (**T1-T29**) show remarkable selectivity against *Mtb* H37Rv strain and interestingly, most derivatives (19 compounds) of the series exhibited MIC of 12.5 μg/mL or less.

Appendix 2.1

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

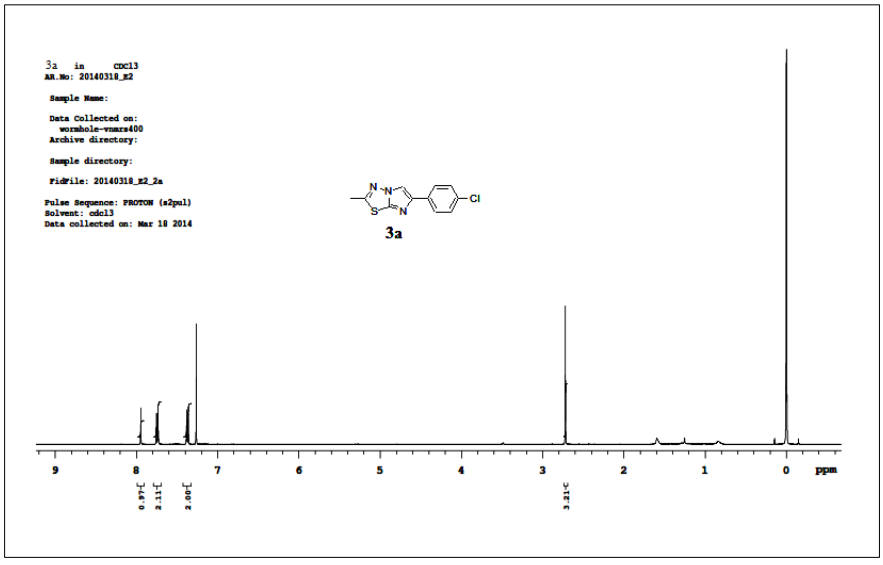


Figure 2.11 ^{1}H NMR spectrum of **3a**

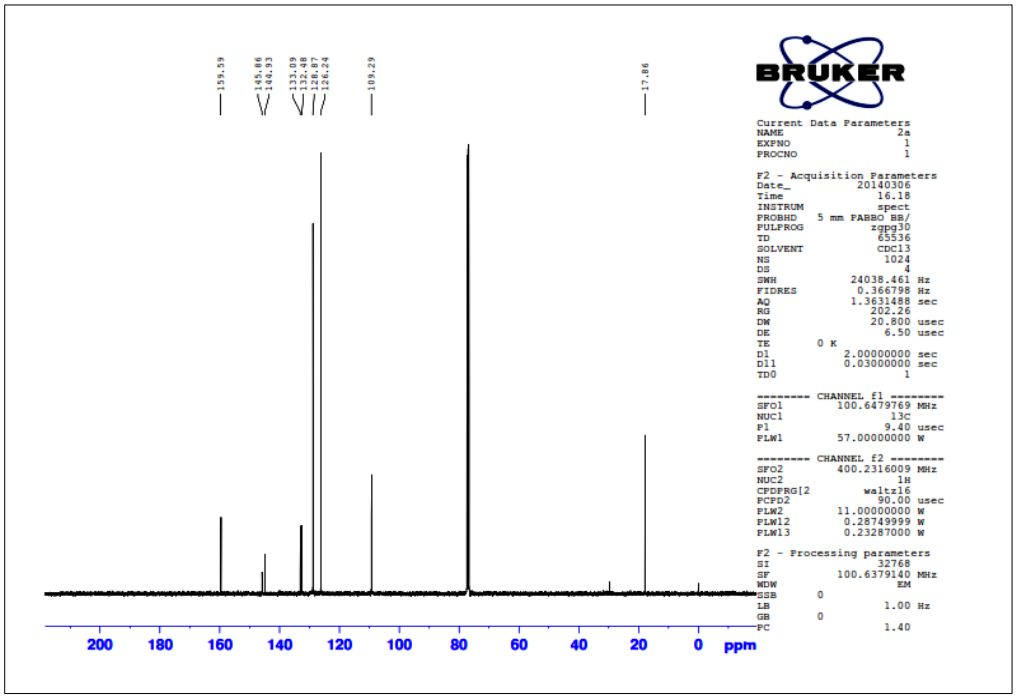


Figure 2.12 ^{13}C NMR spectrum of **3a**

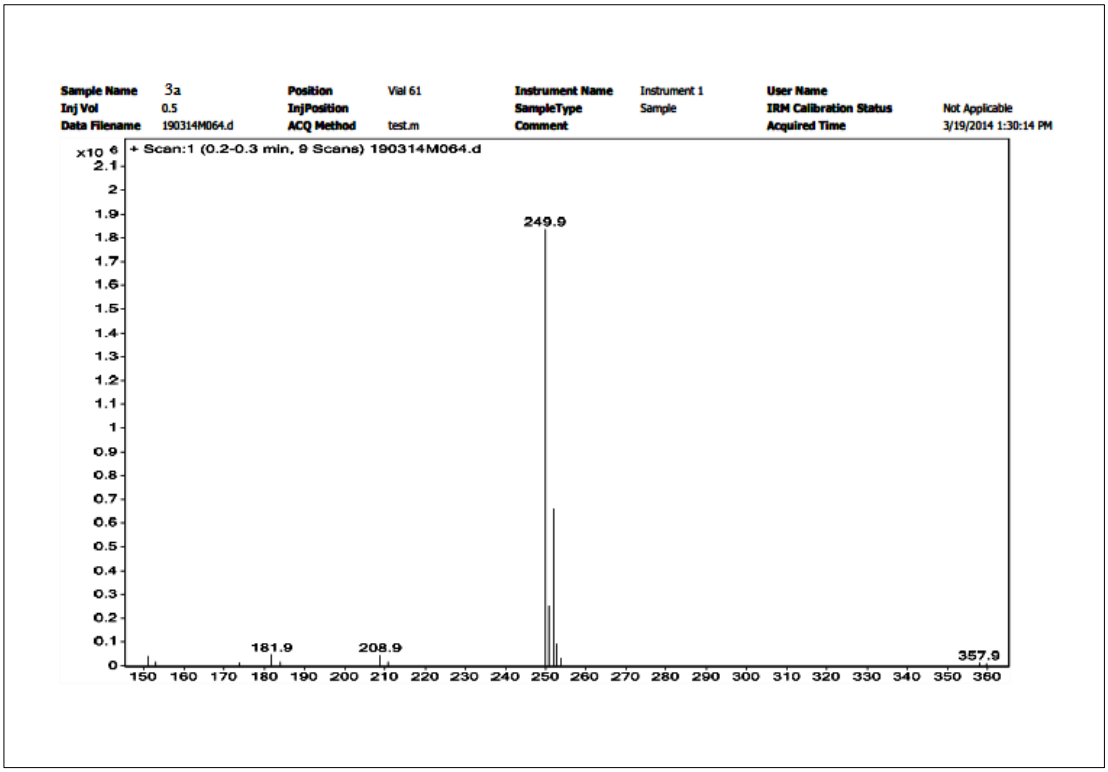


Figure 2.13 Mass spectrum of **3a**

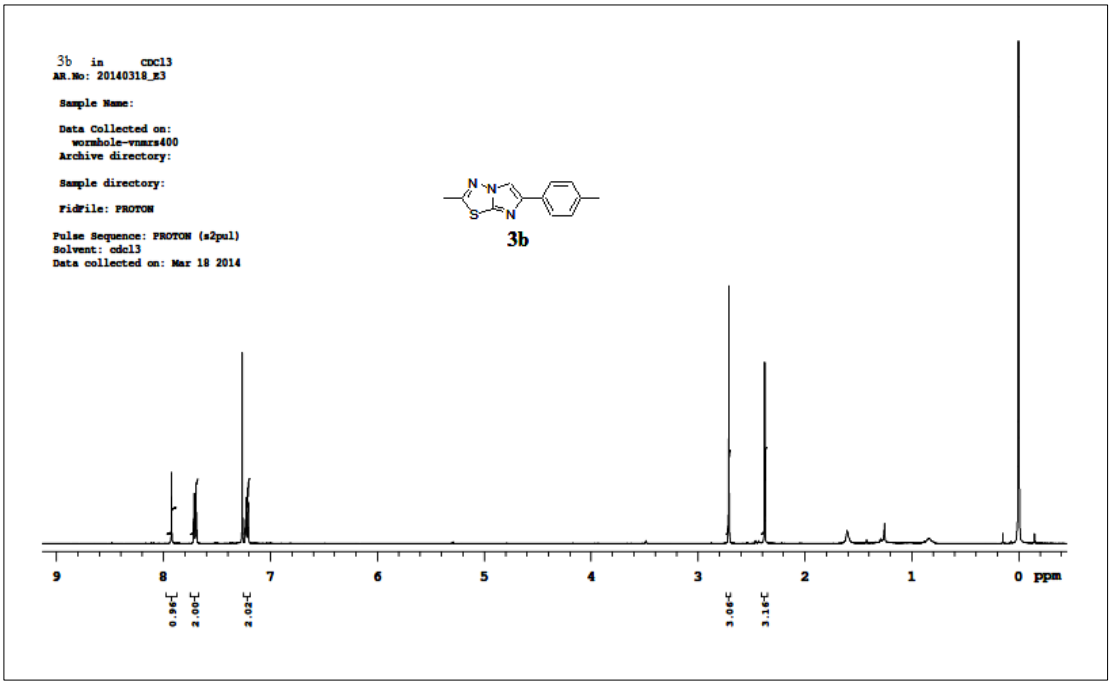


Figure 2.14 ^{1}H NMR spectrum of **3b**

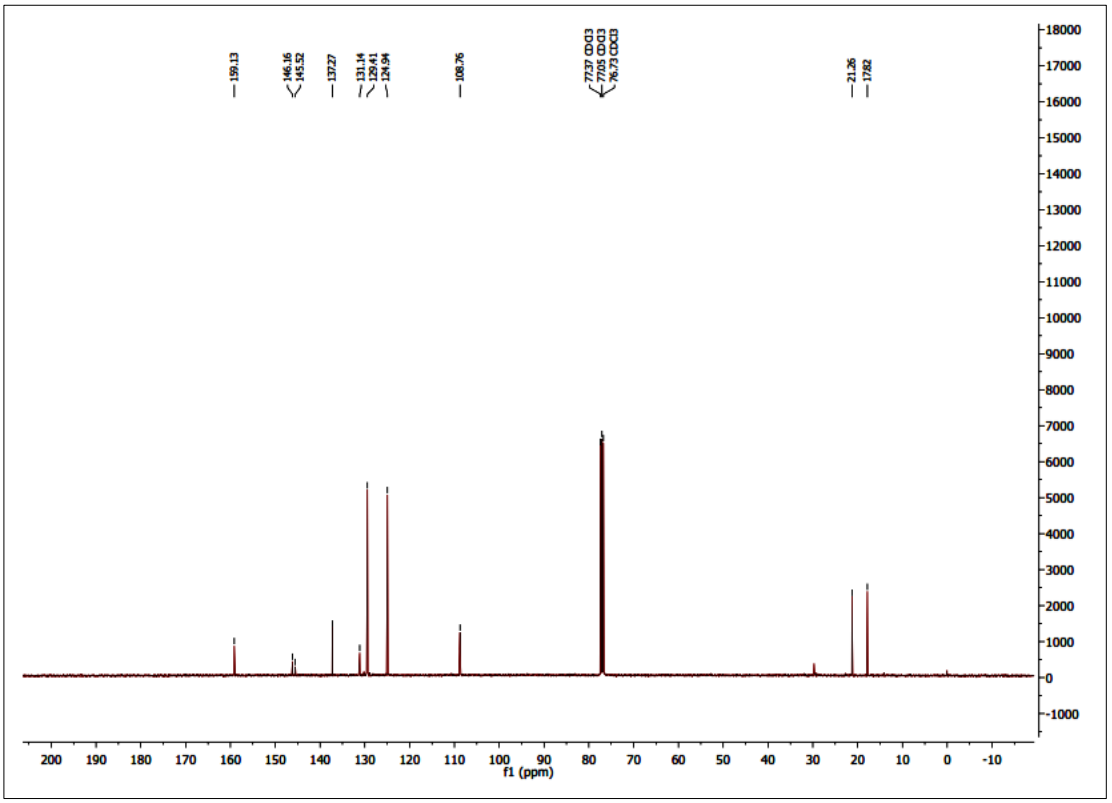


Figure 2.15 ^{13}C NMR spectrum of **3b**

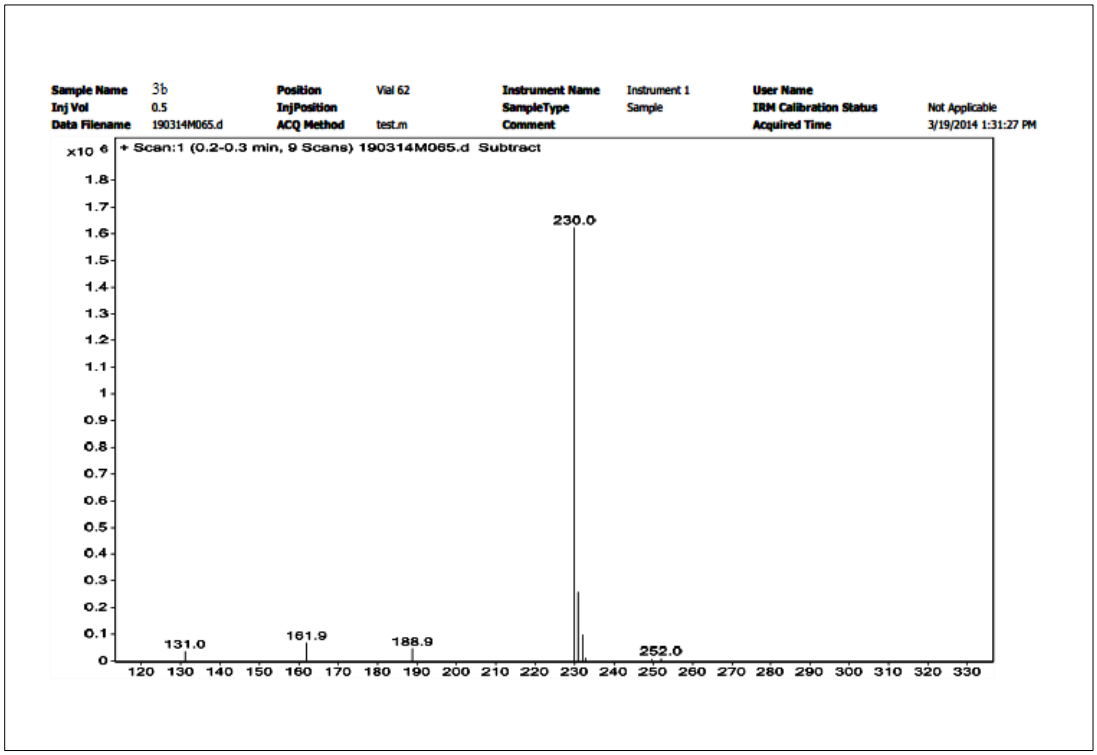


Figure 2.16 Mass spectrum of **3b**

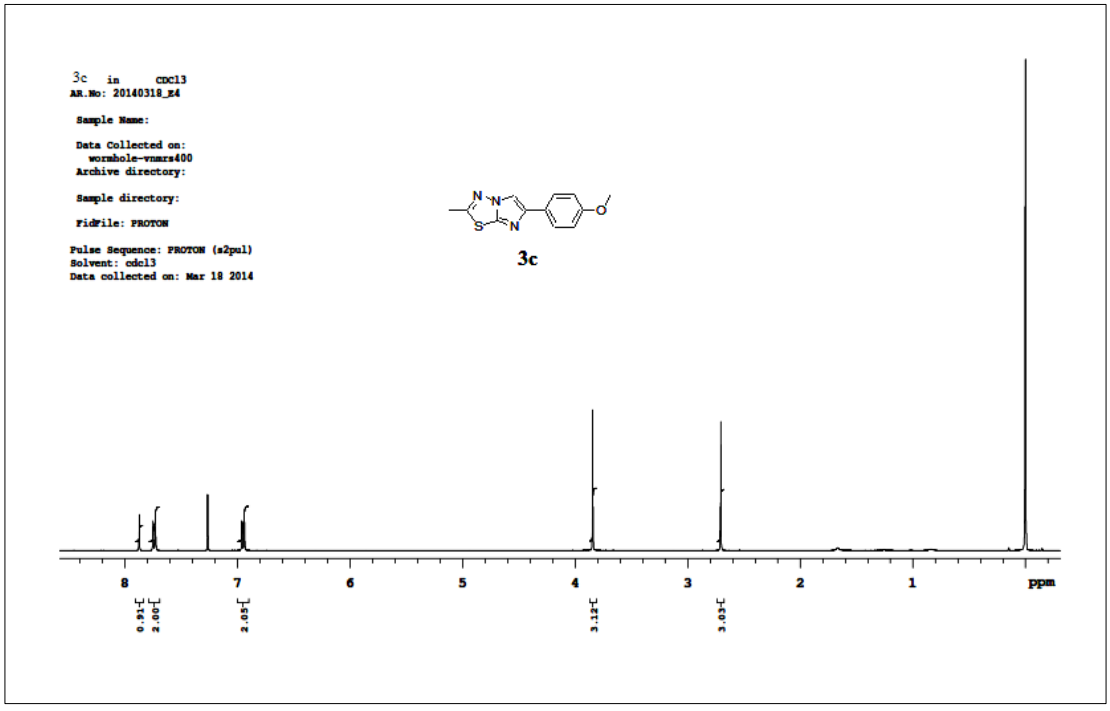


Figure 2.17 ^{1}H NMR spectrum of **3c**

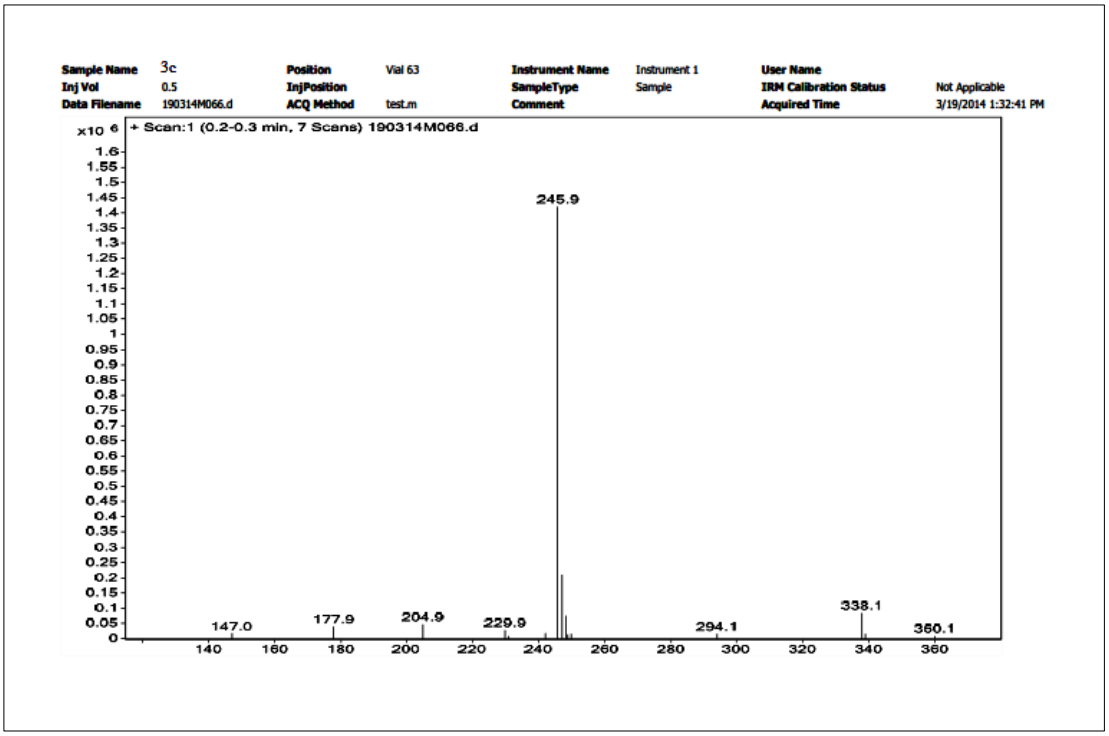


Figure 2.18 Mass spectrum of **3c**

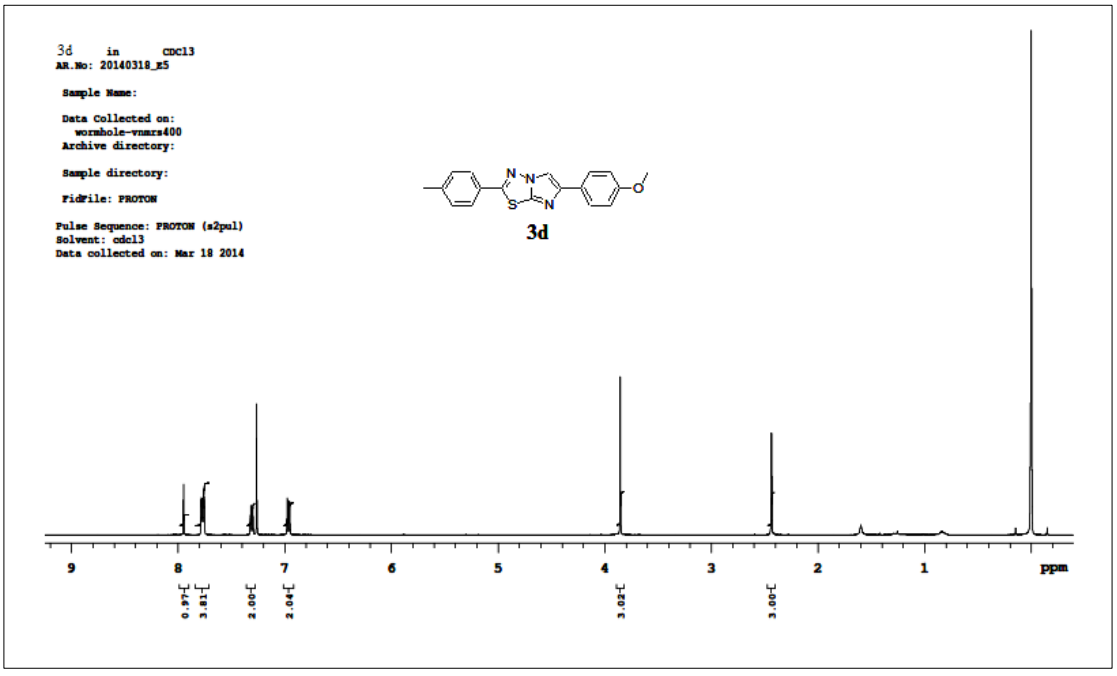


Figure 2.19 ^{1}H NMR spectrum of **3d**

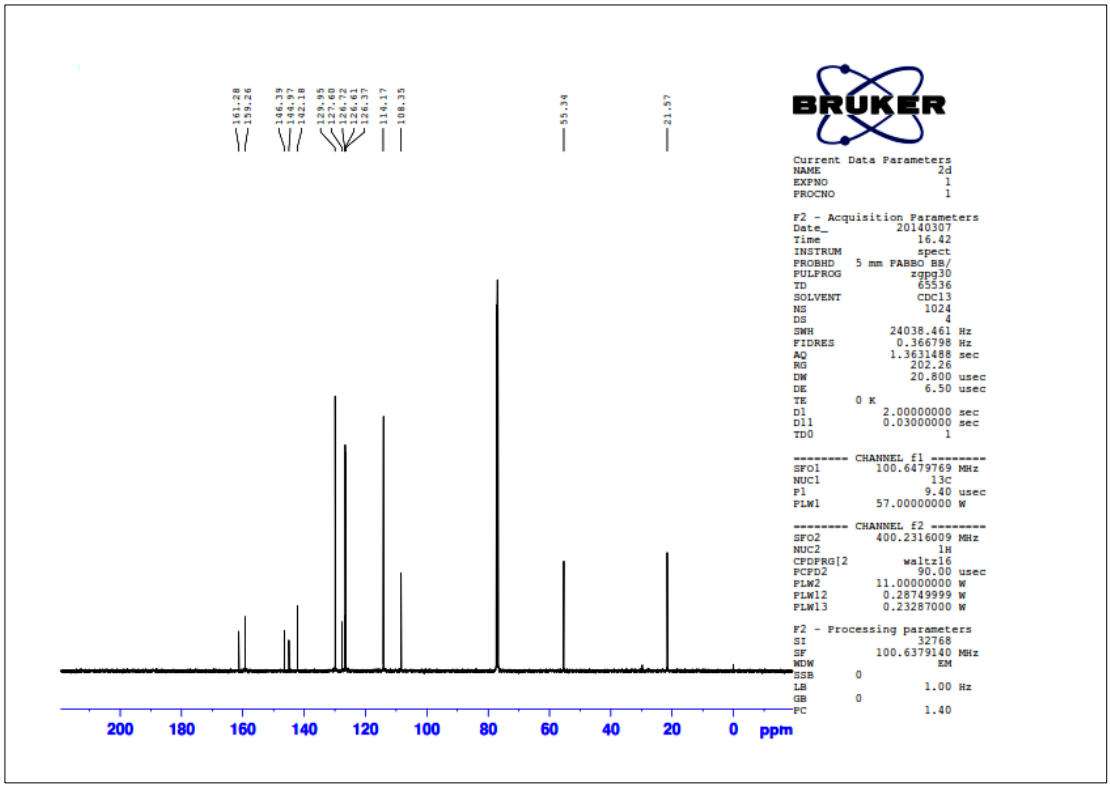


Figure 2.20 ^{13}C NMR spectrum of **3d**

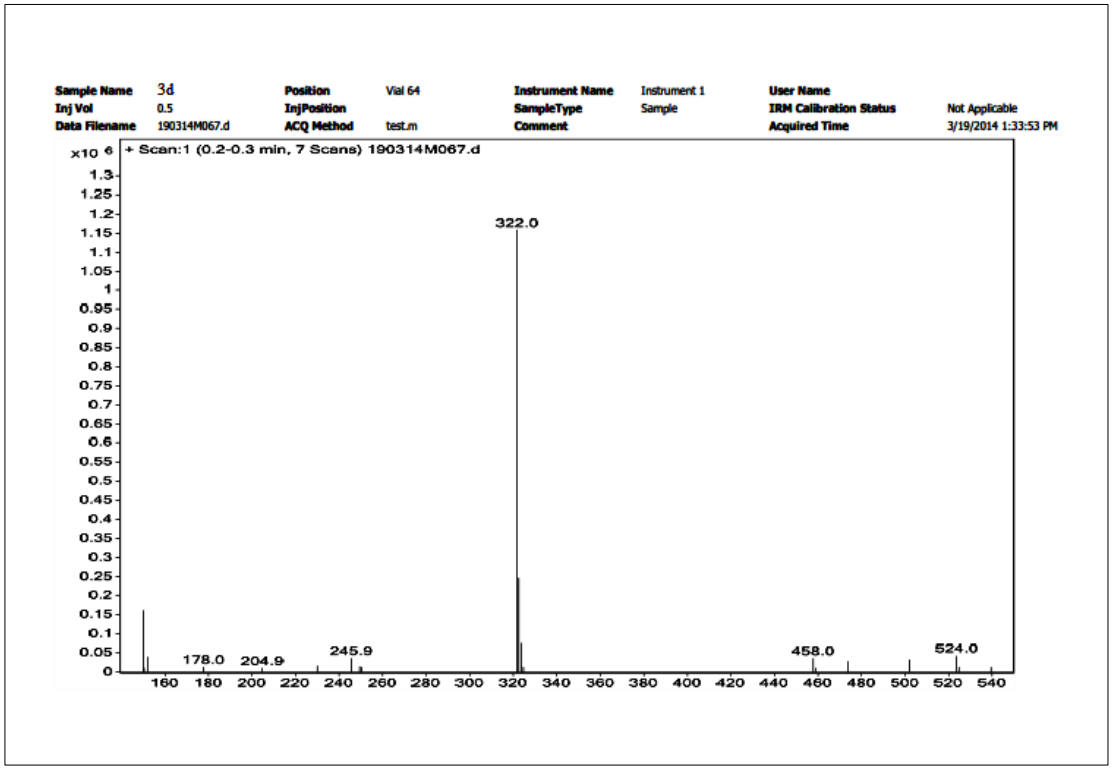


Figure 2.21 Mass spectrum of **3d**

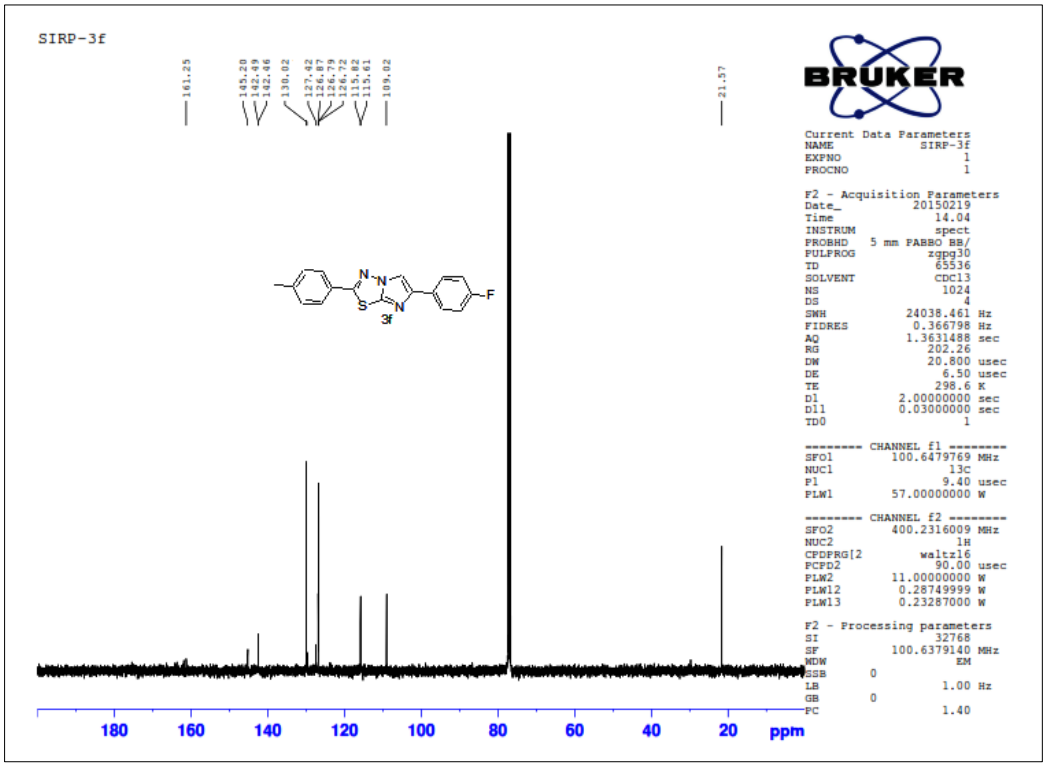


Figure 2.22 Mass spectrum of **3f**

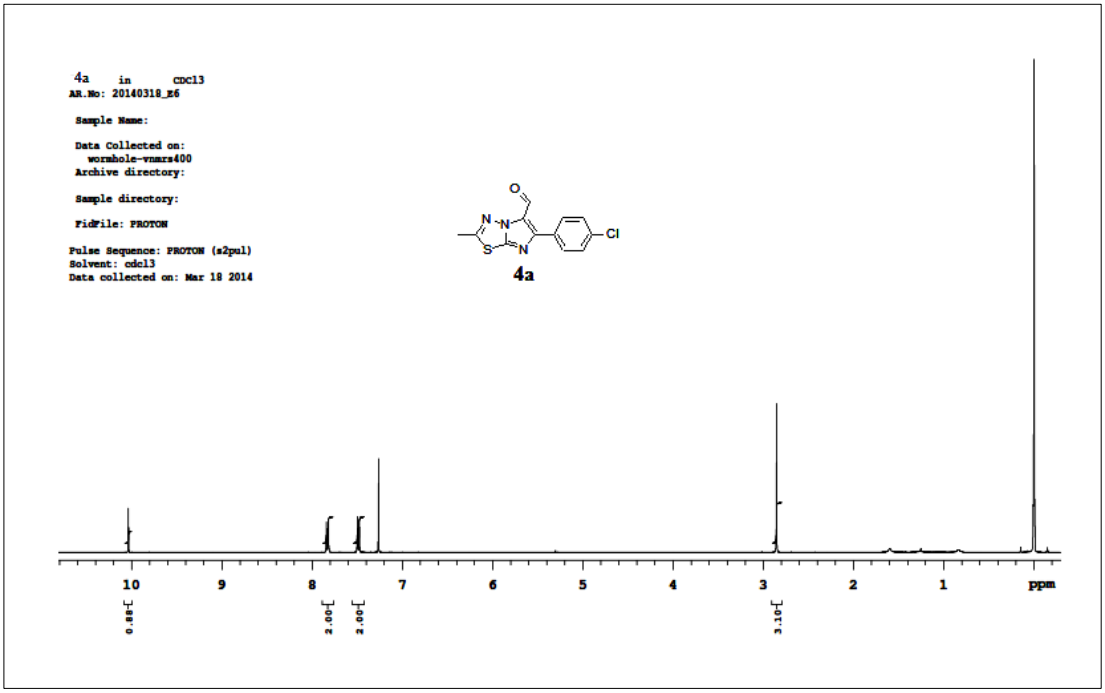


Figure 2.23 ^{1}H NMR spectrum of **4a**

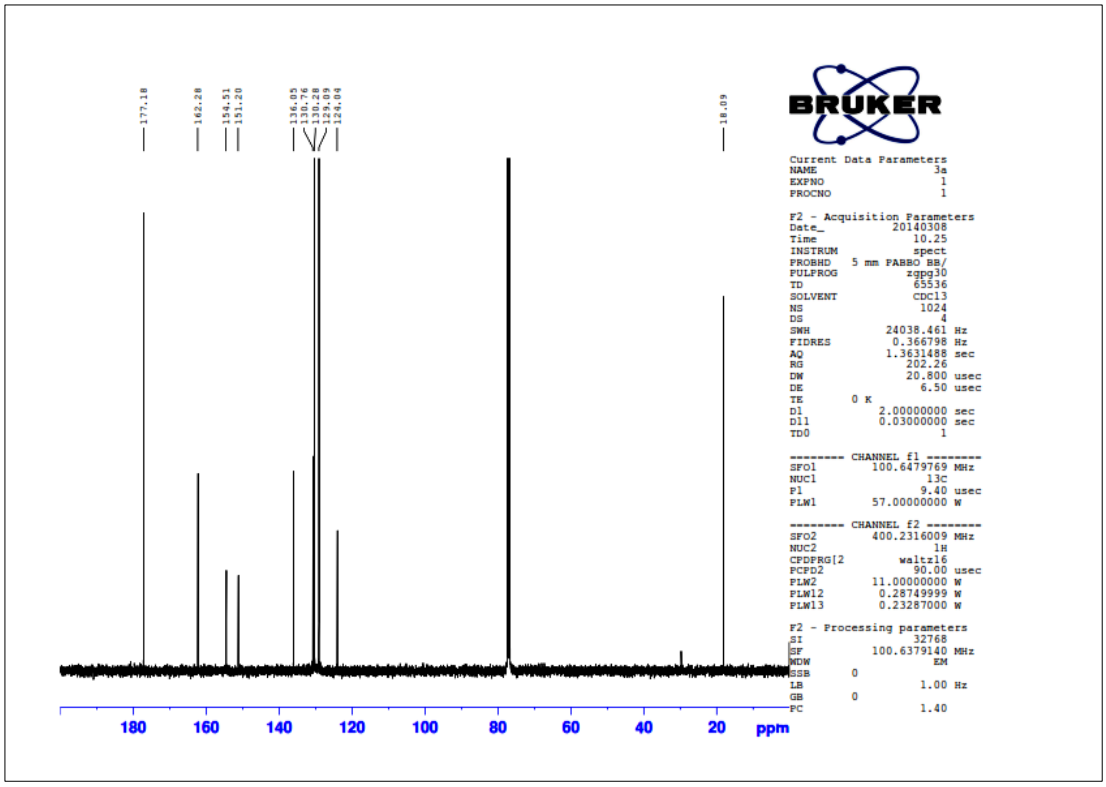


Figure 2.24 ^{13}C NMR spectrum of **4a**

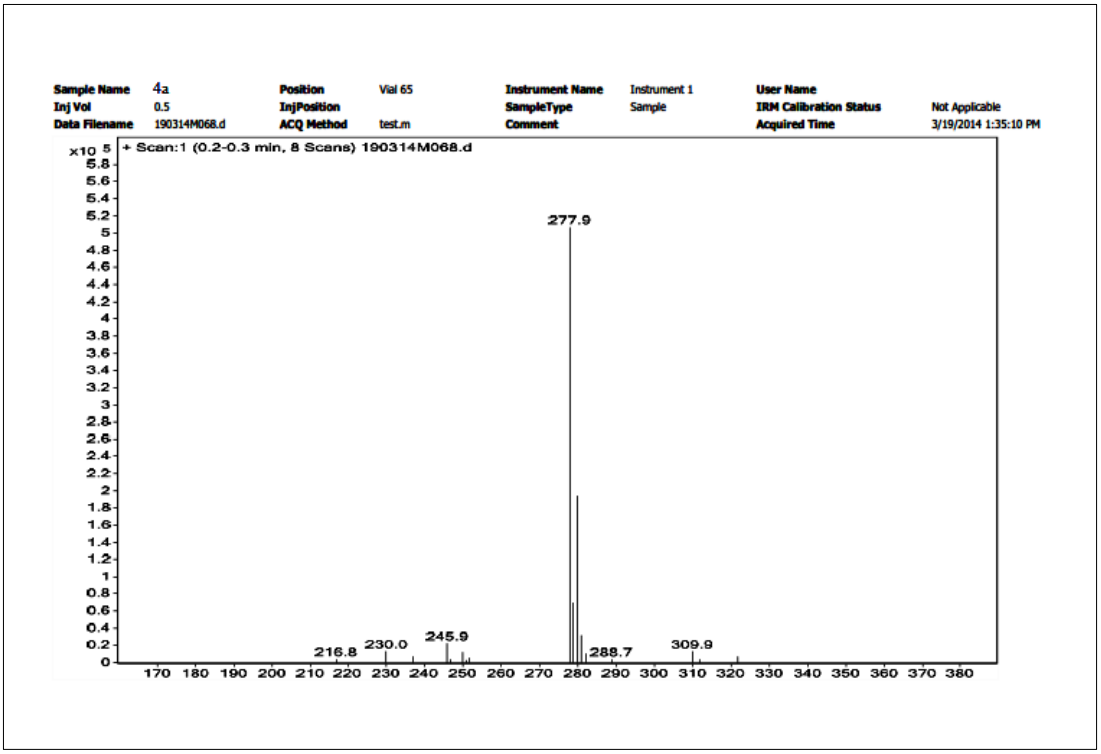


Figure 2.25 Mass spectrum of **4a**

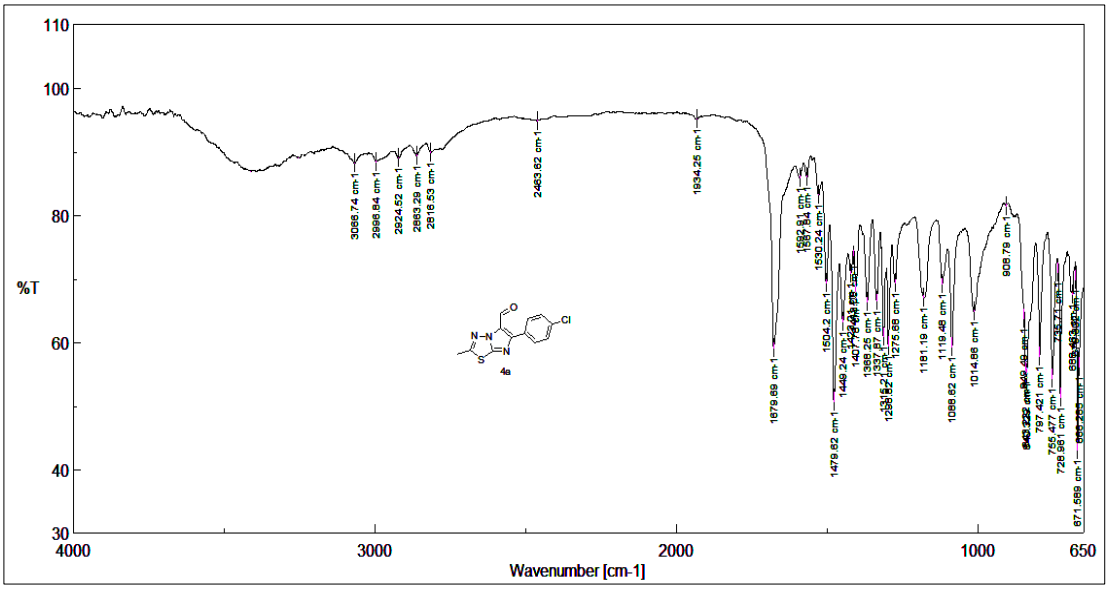


Figure 2.26 FTIR spectrum of **4a**

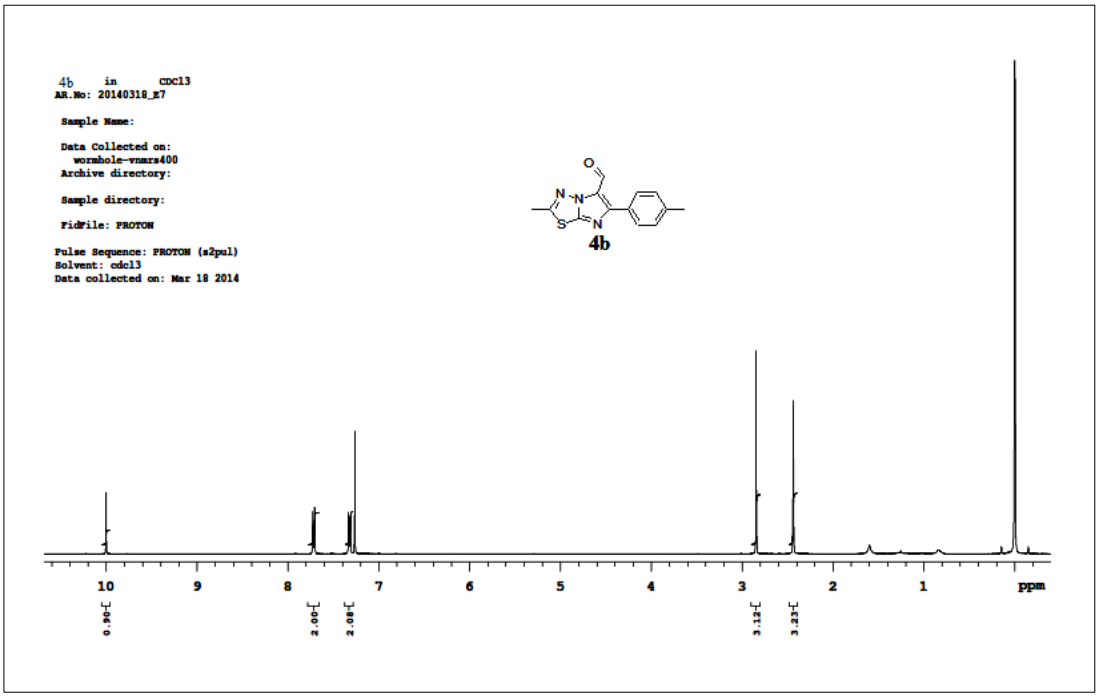


Figure 2.27 ^{1}H NMR spectrum of **4b**

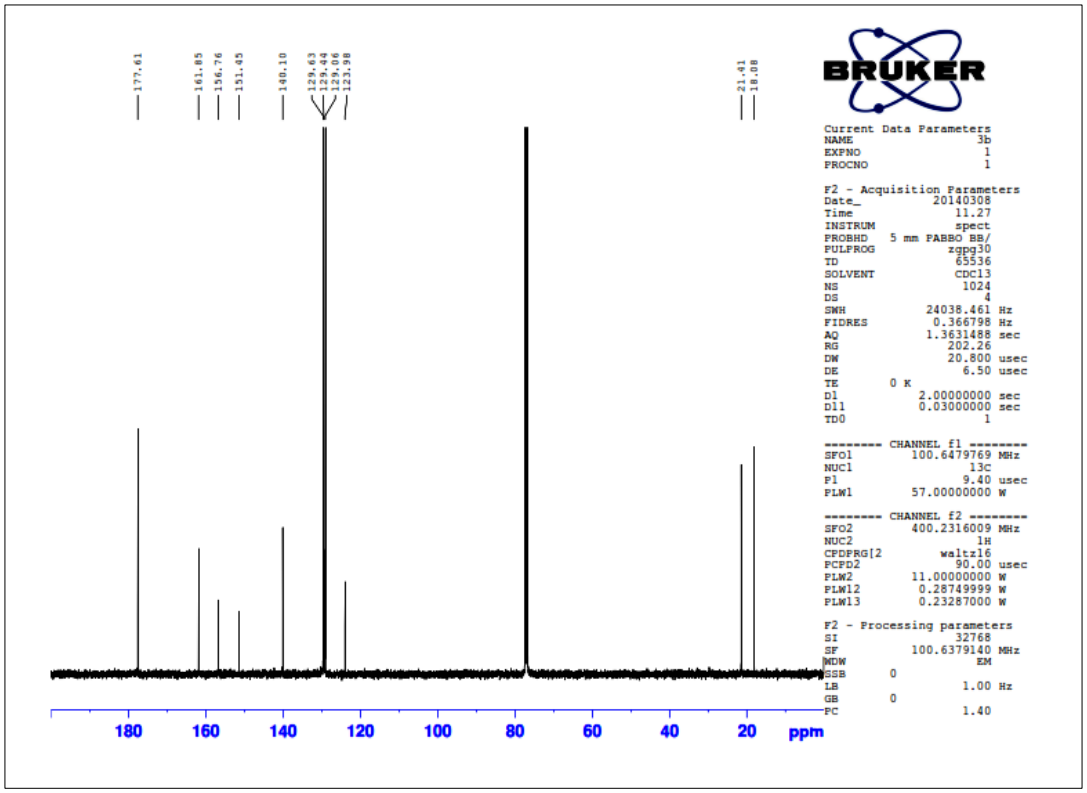


Figure 2.28 ^{13}C NMR spectrum of **4b**

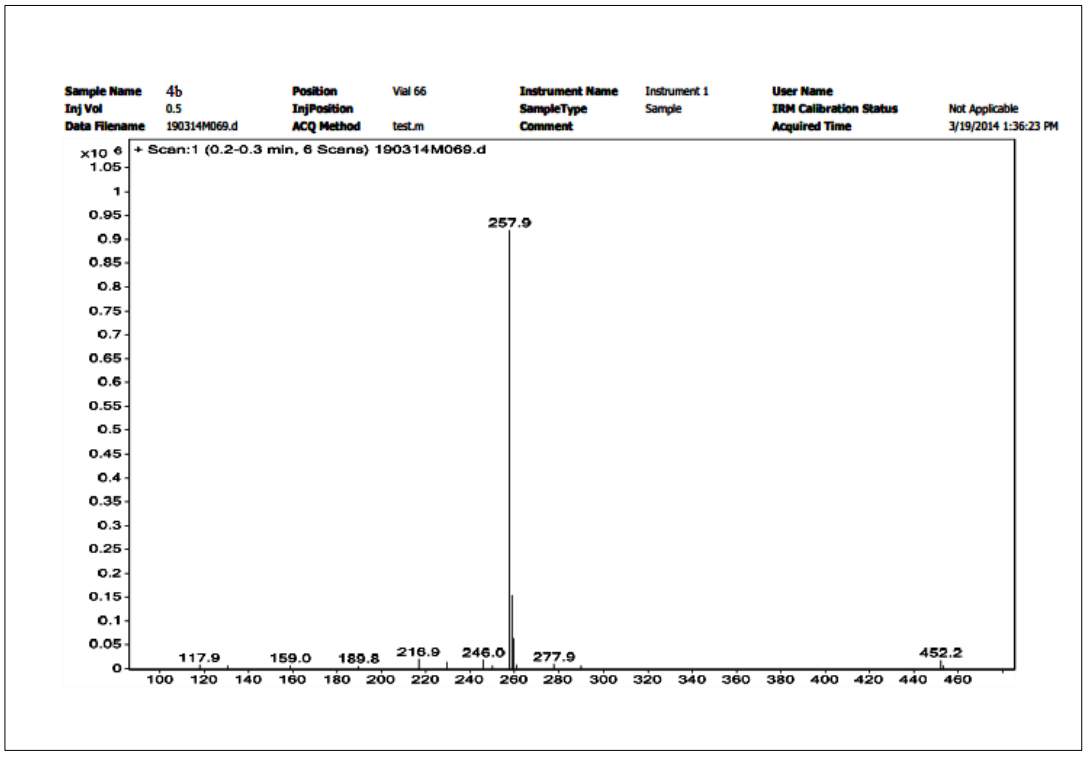


Figure 2.29 Mass spectrum of **4b**

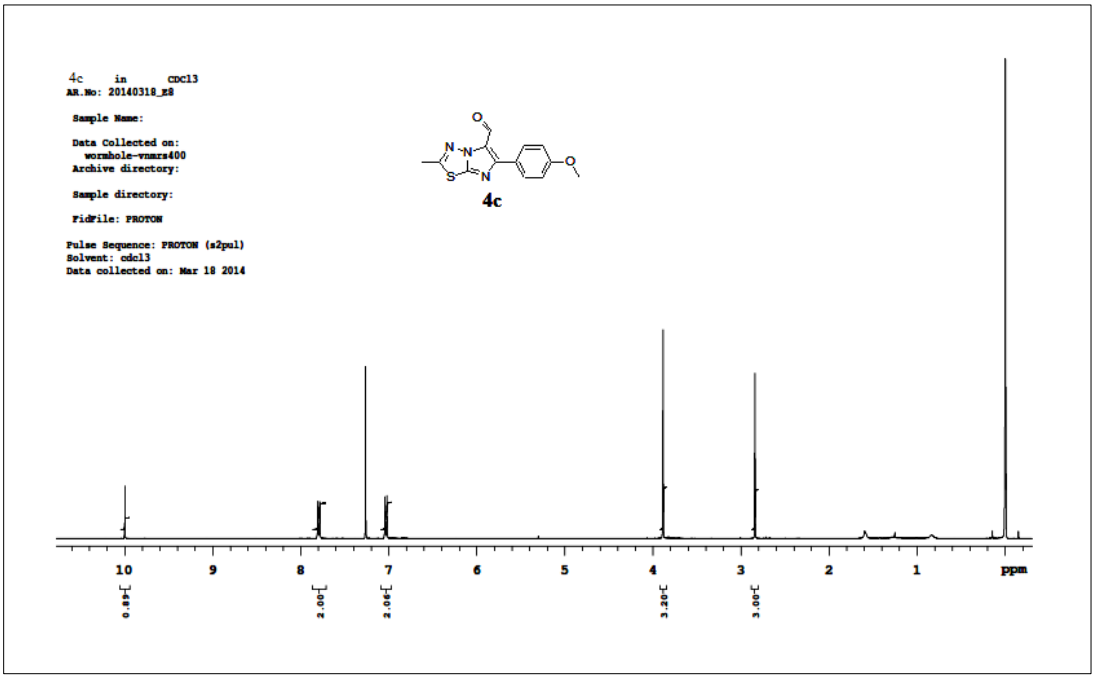


Figure 2.30 ^{1}H NMR spectrum of **4c**

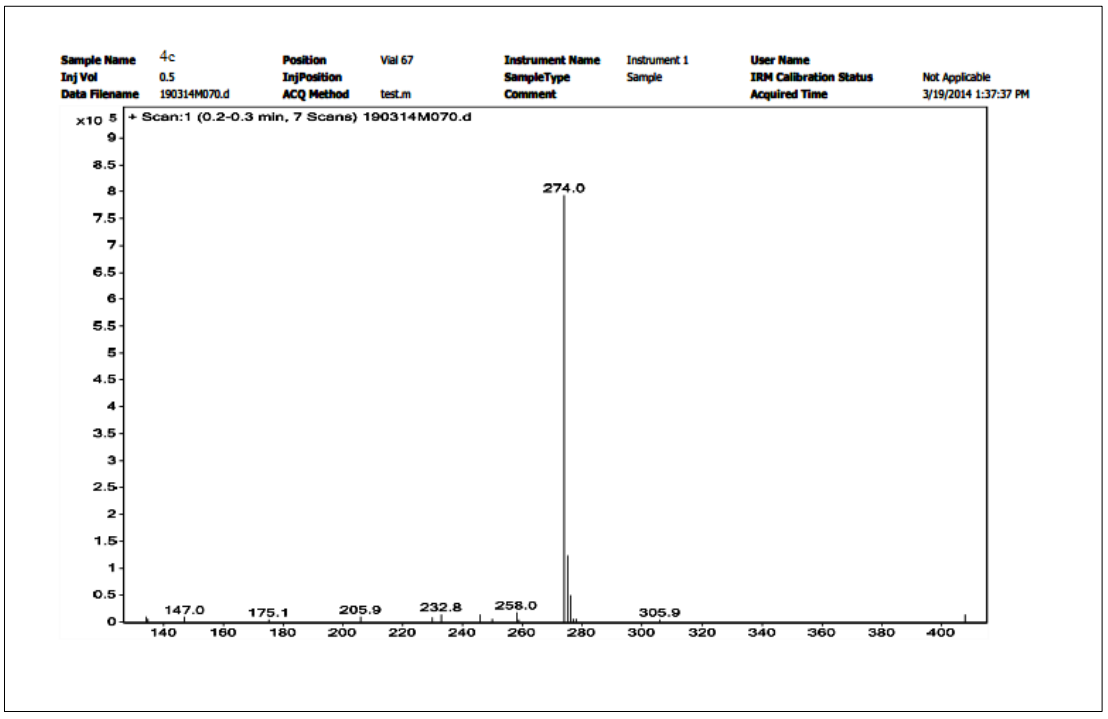


Figure 2.31 Mass spectrum of **4c**

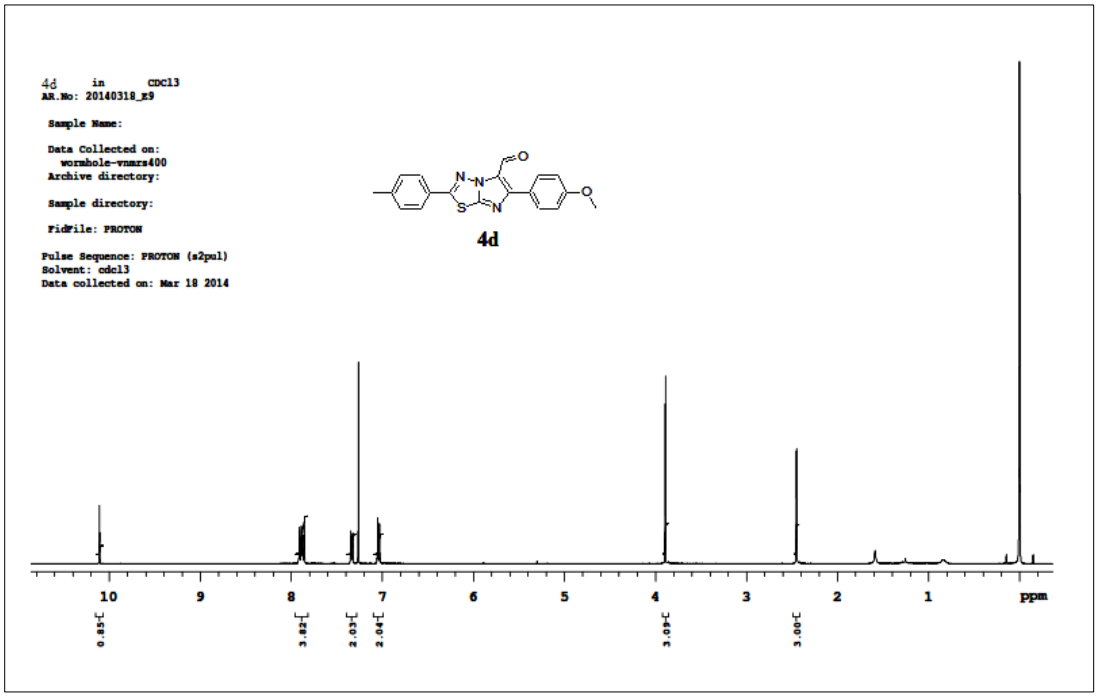


Figure 2.32 ^{1}H NMR spectrum of **4d**

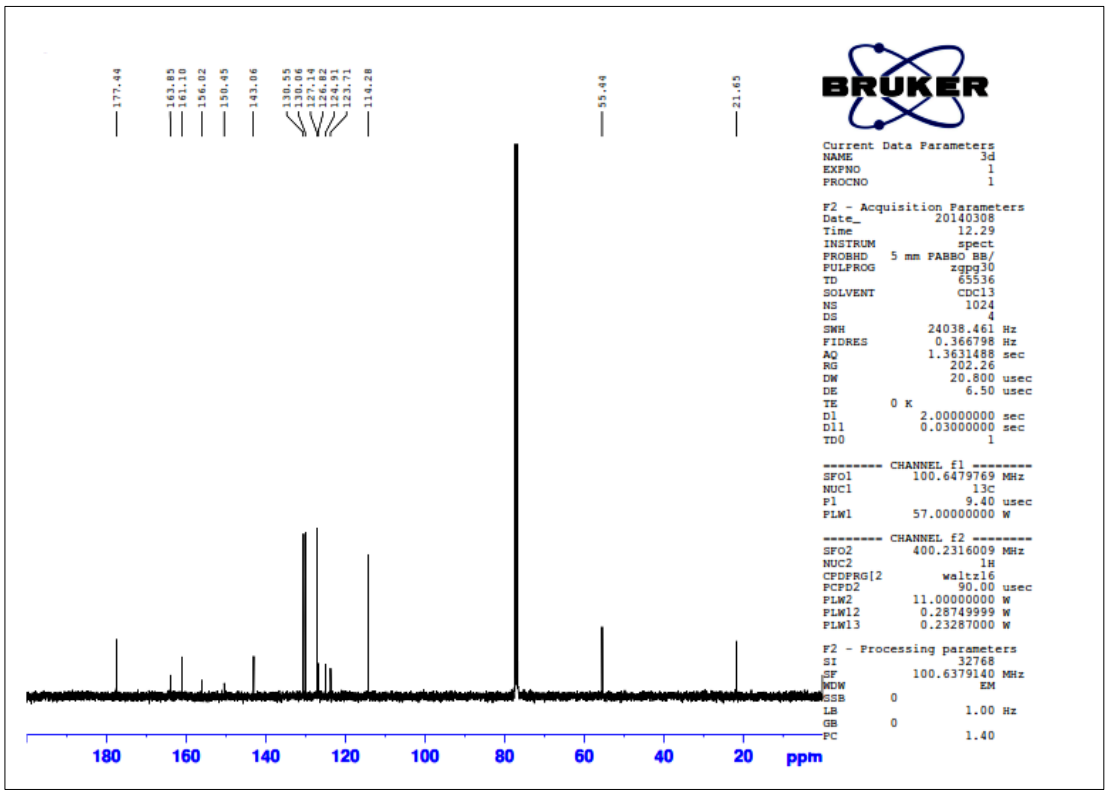


Figure 2.33 ^{13}C NMR spectrum of **4d**

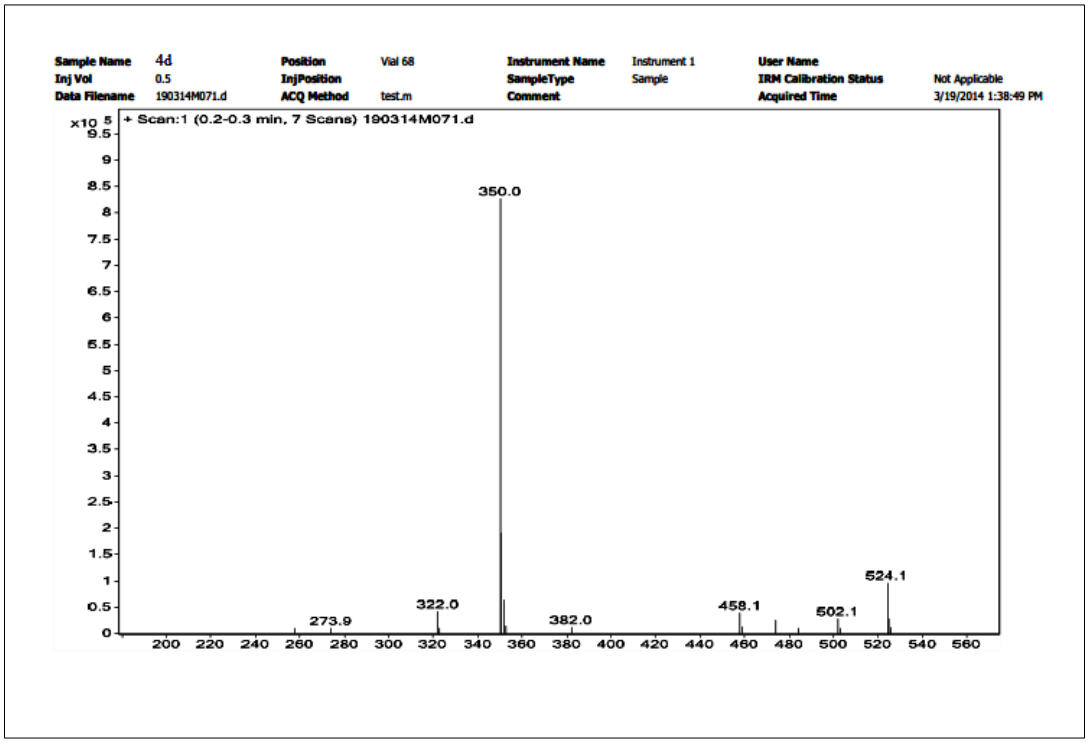


Figure 2.34 Mass spectrum of **4d**

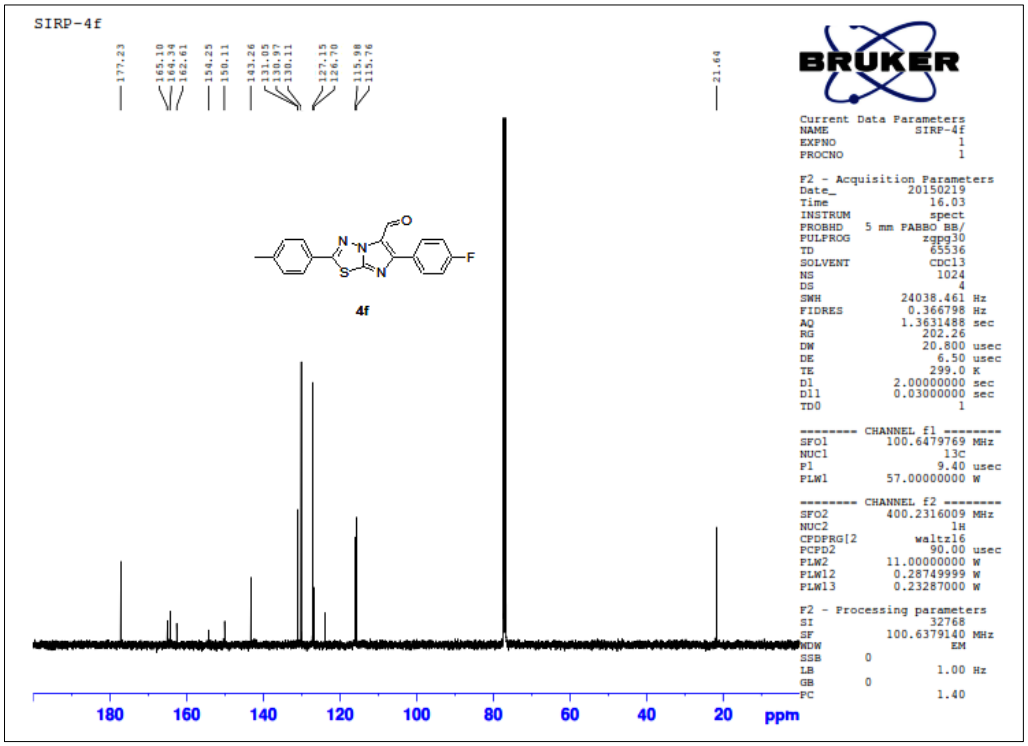


Figure 2.35 ^{13}C NMR spectrum of **4f**

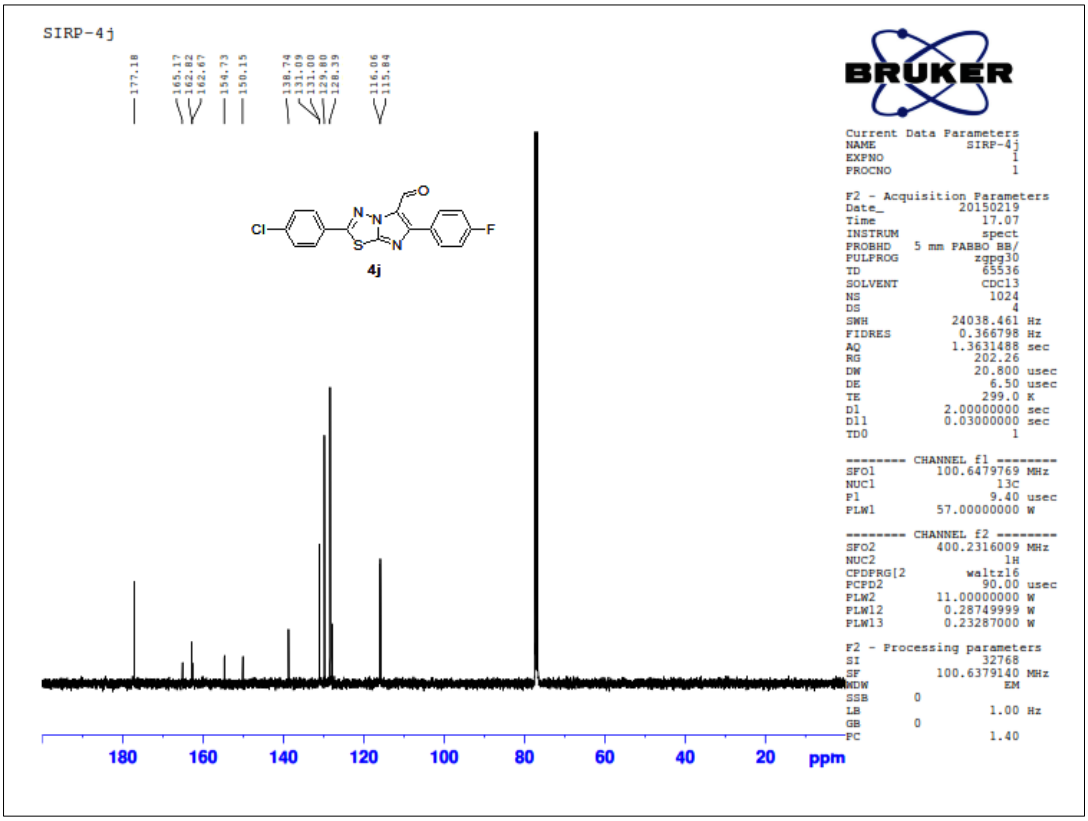


Figure 2.36 ^{13}C NMR spectrum of **4j**

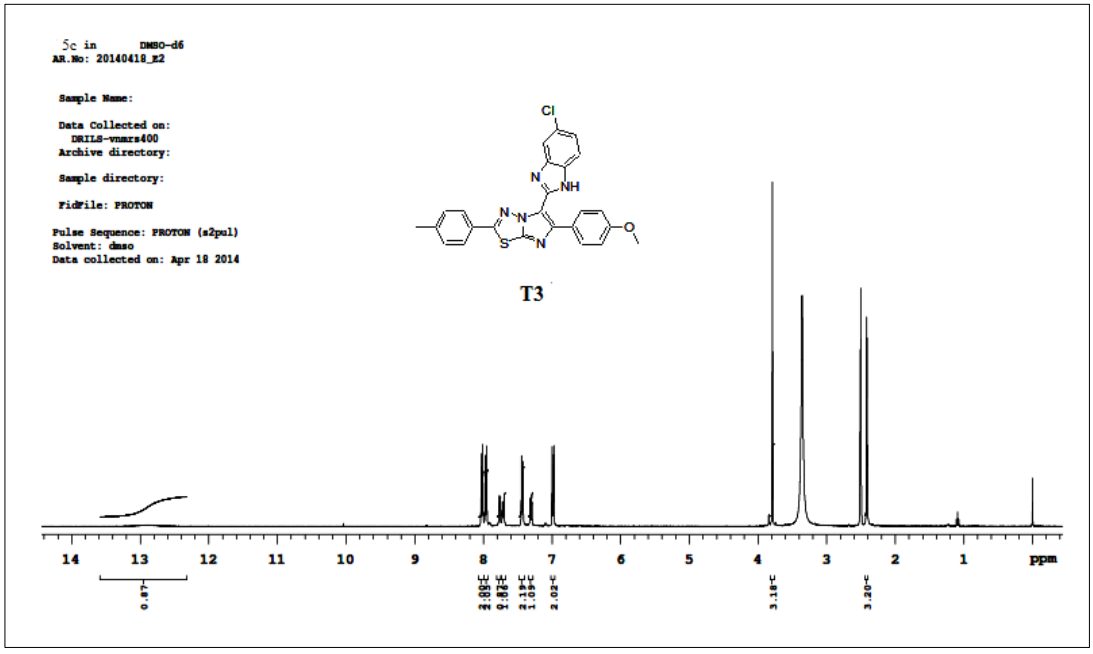


Figure 2.37 ^{1}H NMR spectrum of **T3**

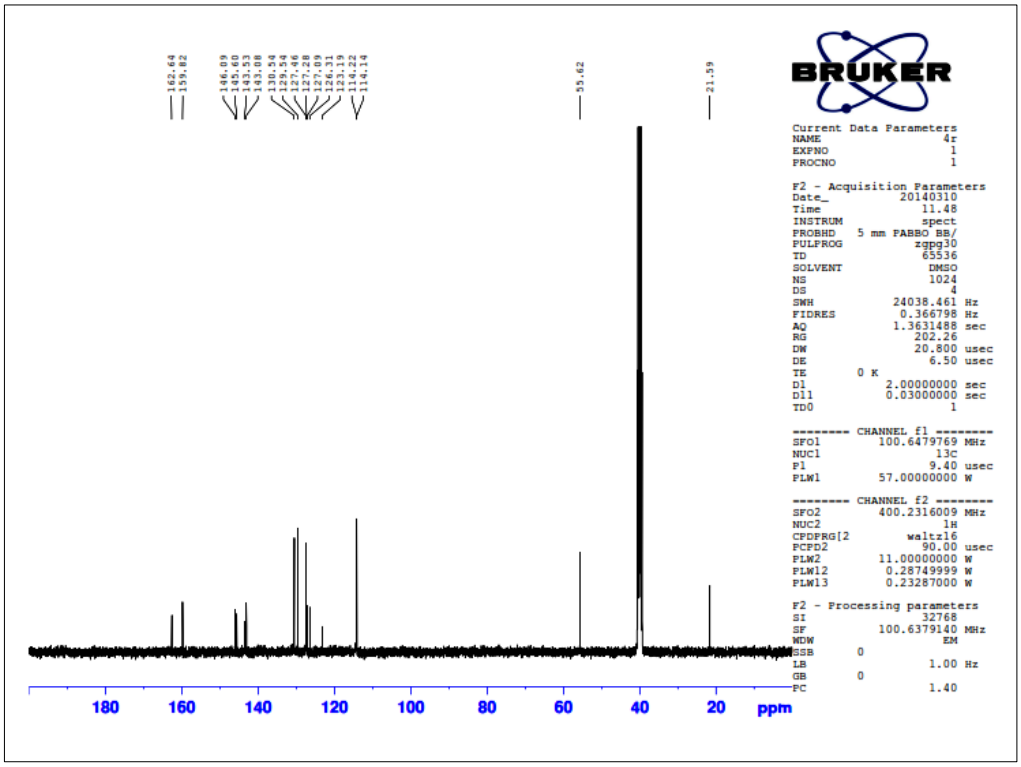


Figure 2.38 ^{13}C NMR spectrum of **T3**

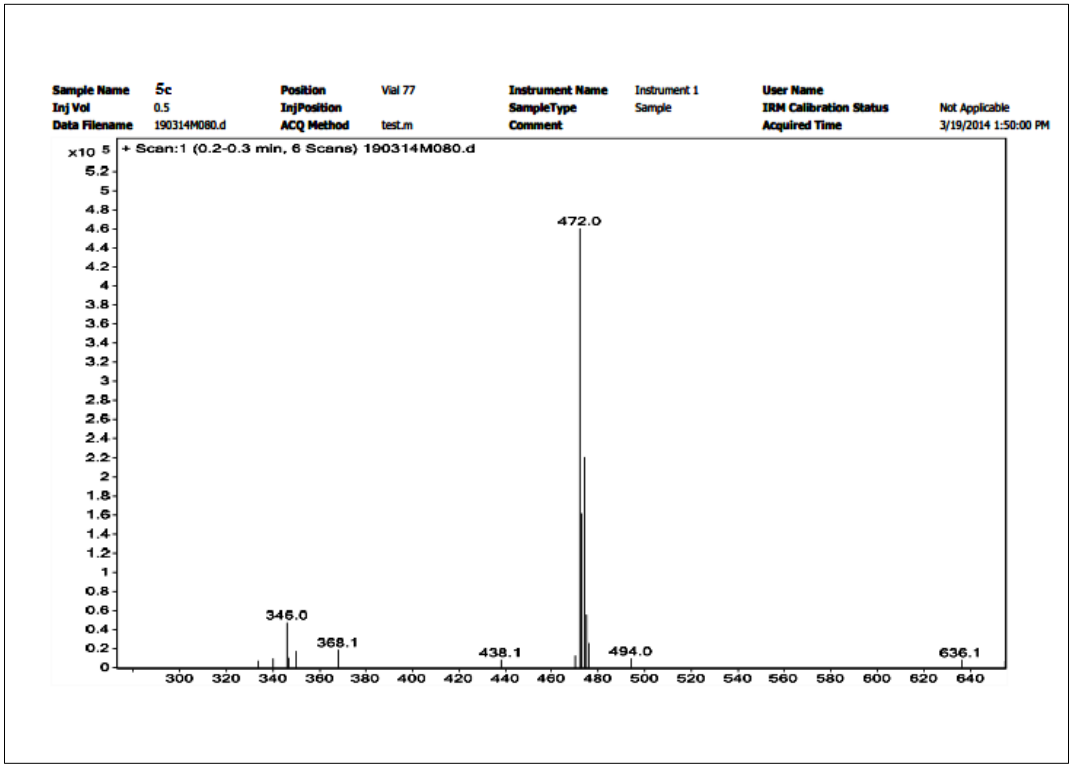


Figure 2.39 Mass spectrum of **T3**

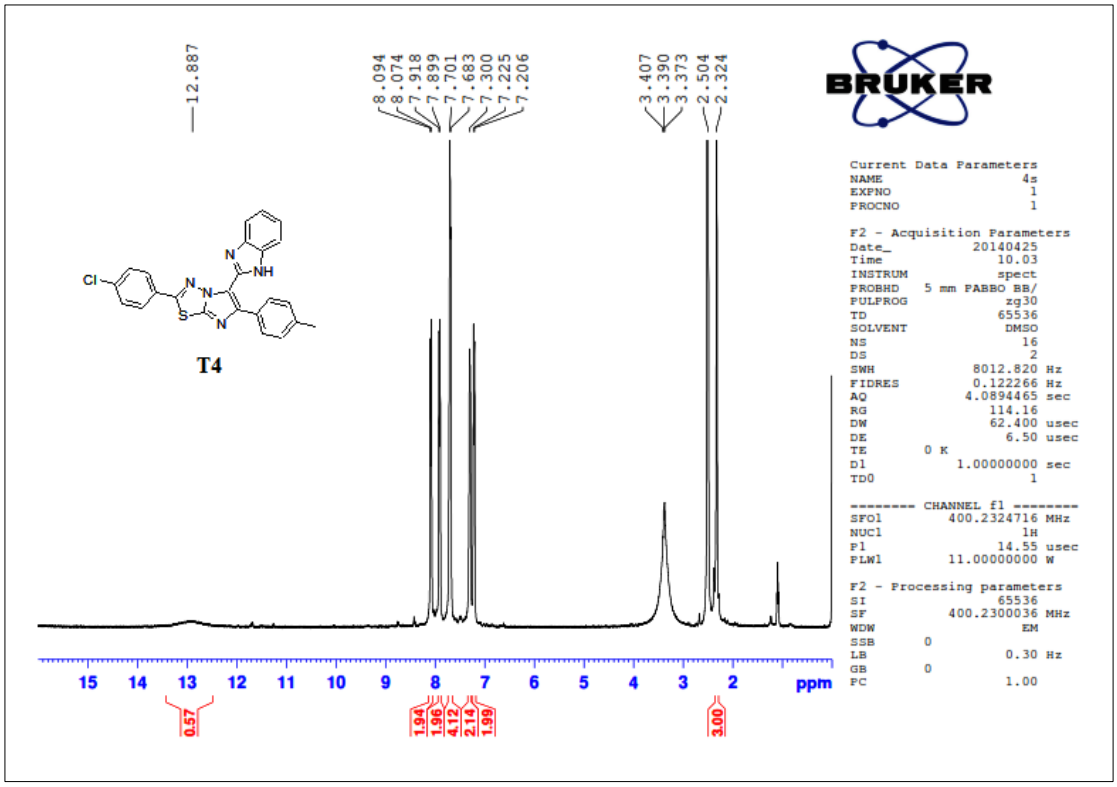


Figure 2.40 ^{1}H NMR spectrum of **T4**

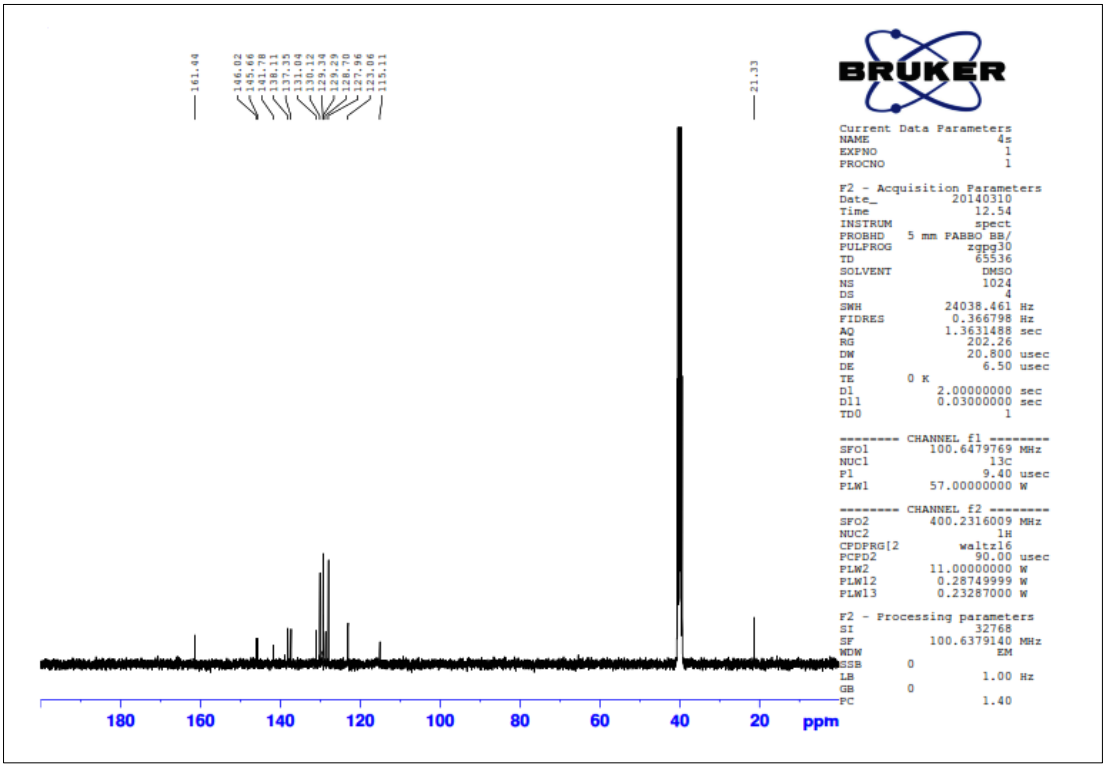


Figure 2.41 ^{13}C NMR spectrum of **T4**

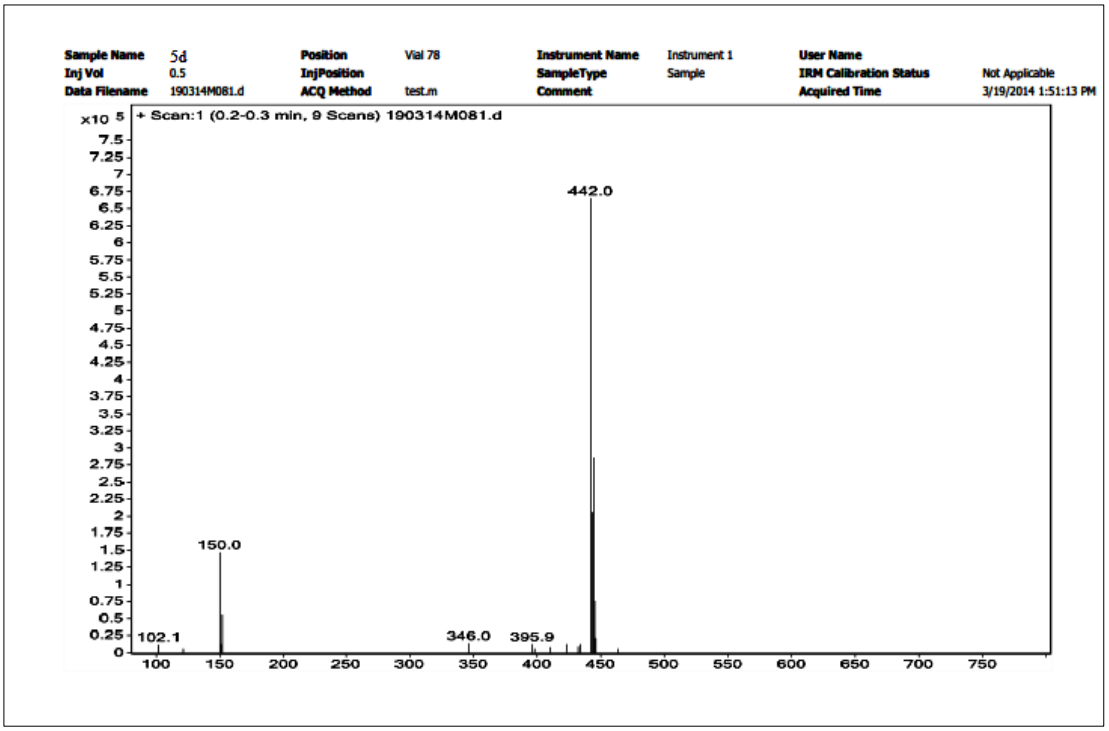


Figure 2.42 Mass spectrum of **T4**

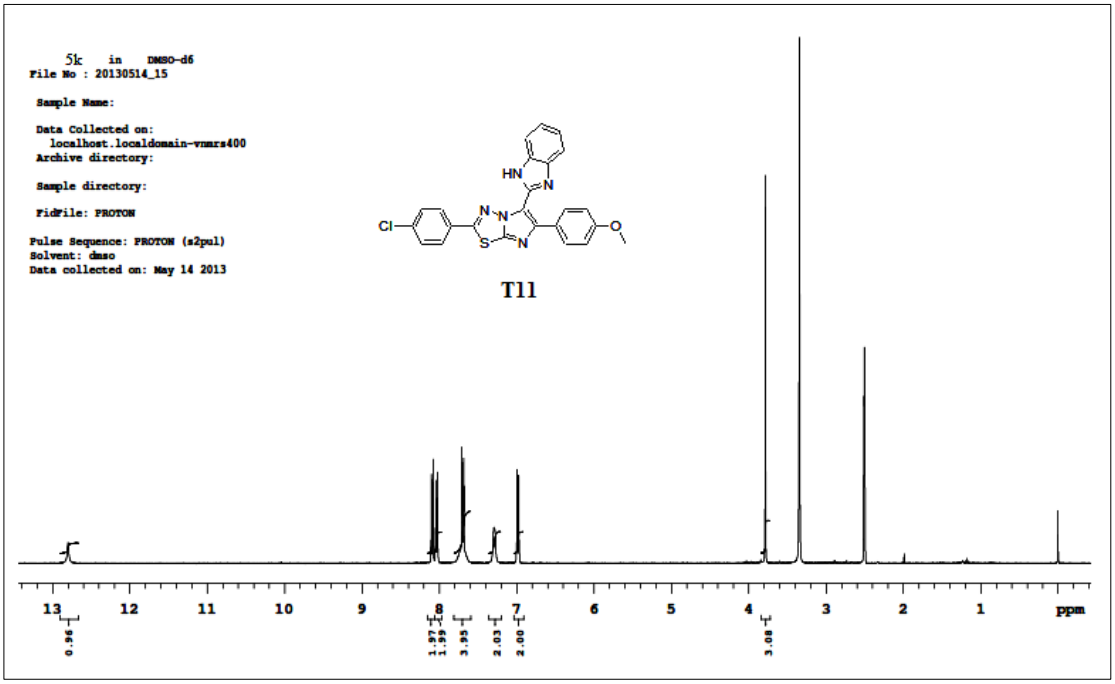


Figure 2.43 ^{1}H NMR spectrum of **T11**

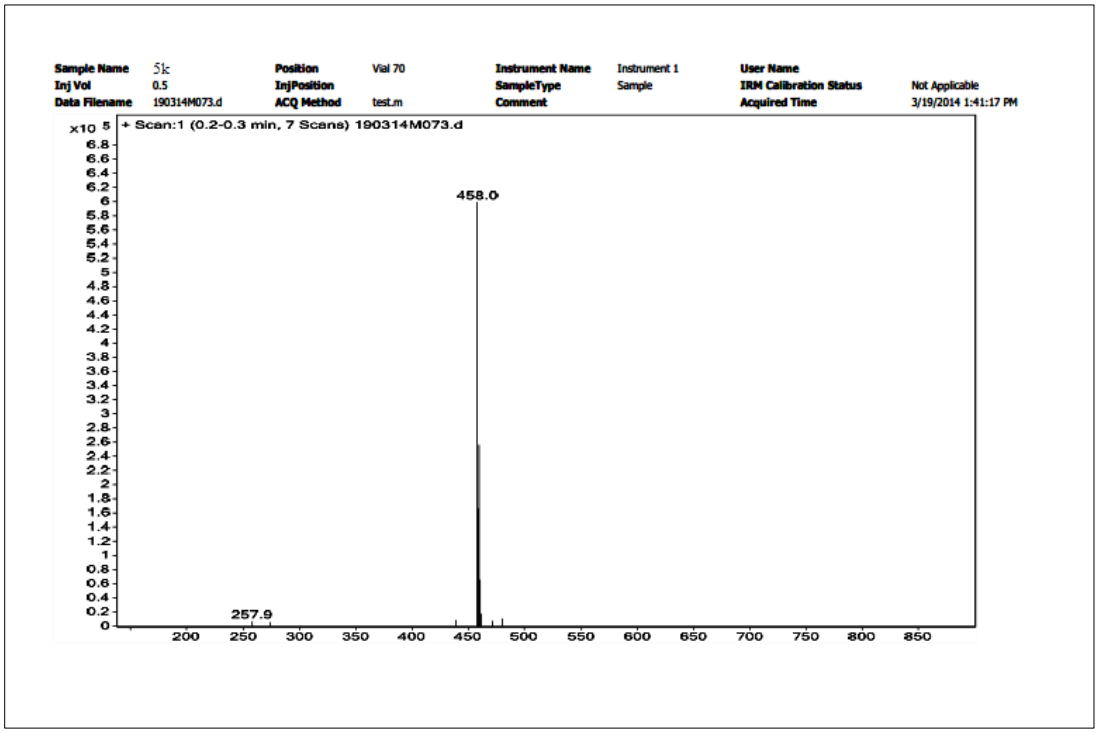


Figure 2.44 Mass spectrum of **T11**

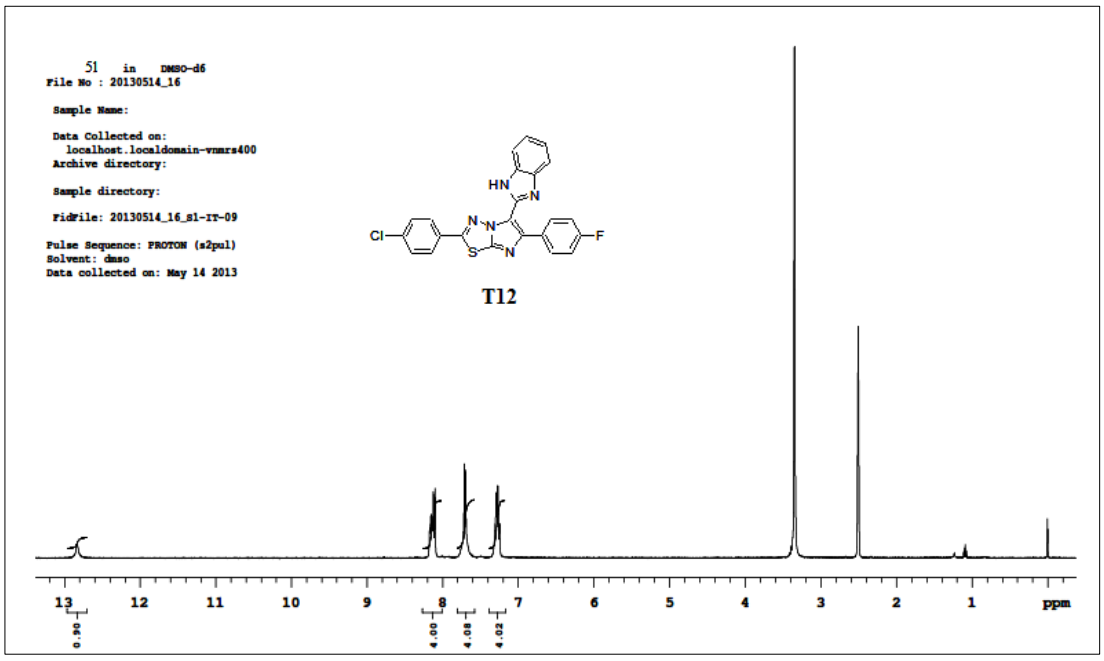


Figure 2.45 ^{1}H NMR spectrum of **T12**

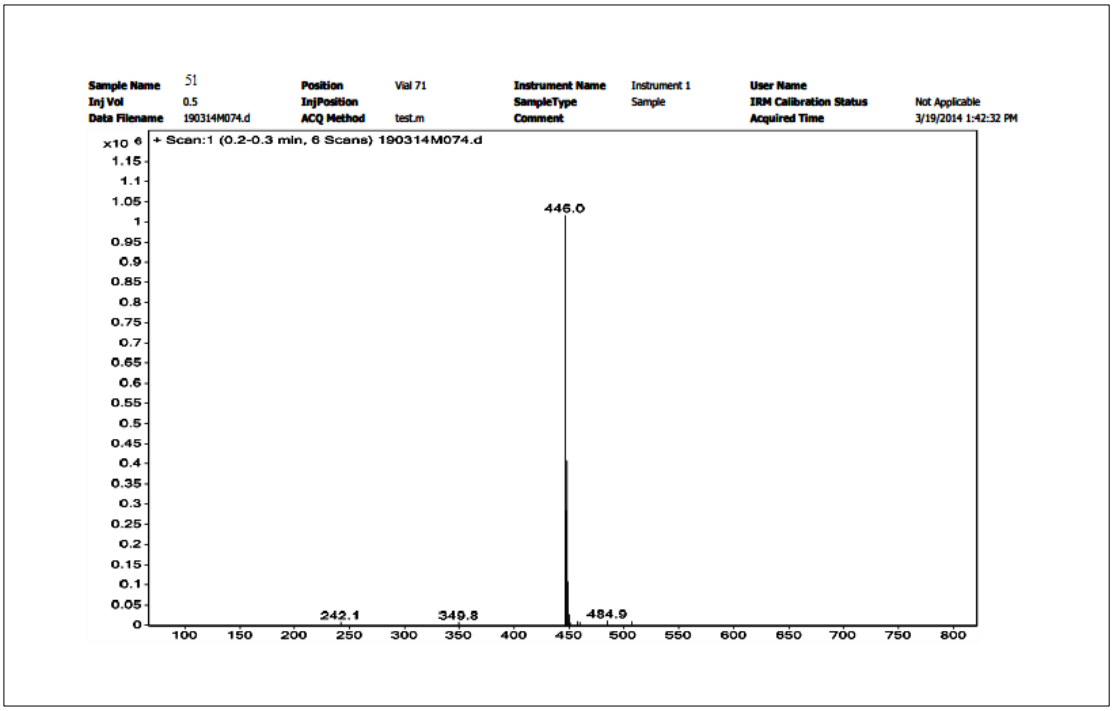


Figure 2.46 Mass spectrum of **T12**

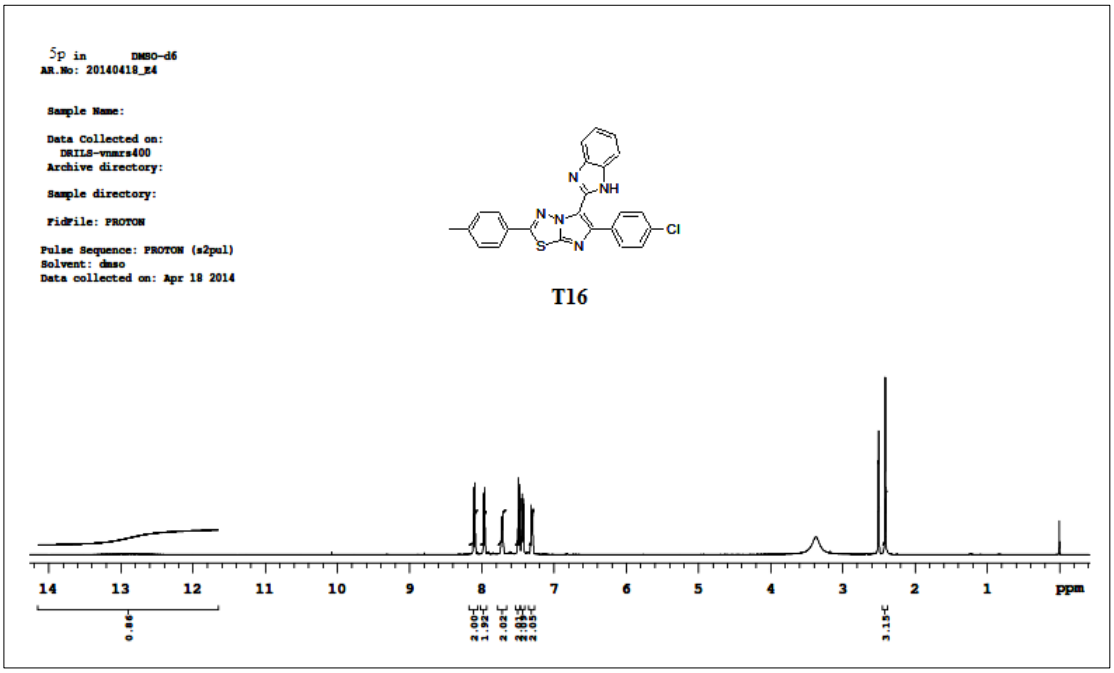


Figure 2.47 ^{1}H NMR spectrum of **T16**

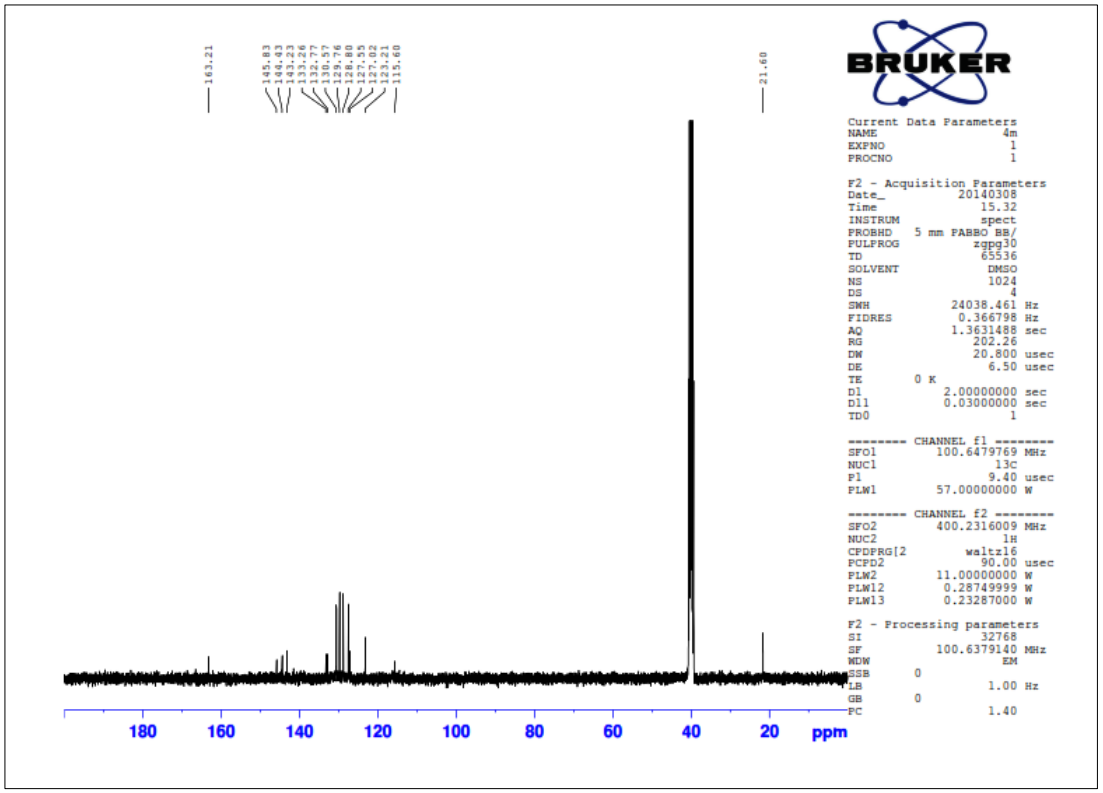


Figure 2.48 ^{13}C NMR spectrum of **T16**

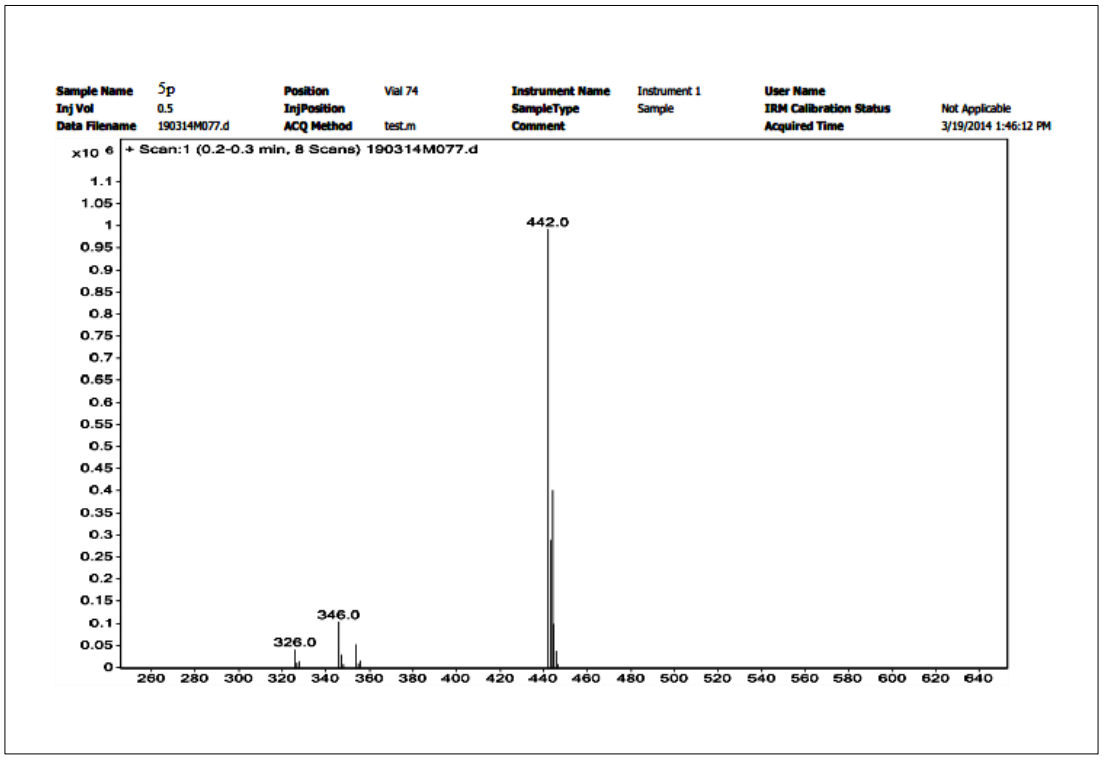


Figure 2.49 Mass spectrum of **T16**

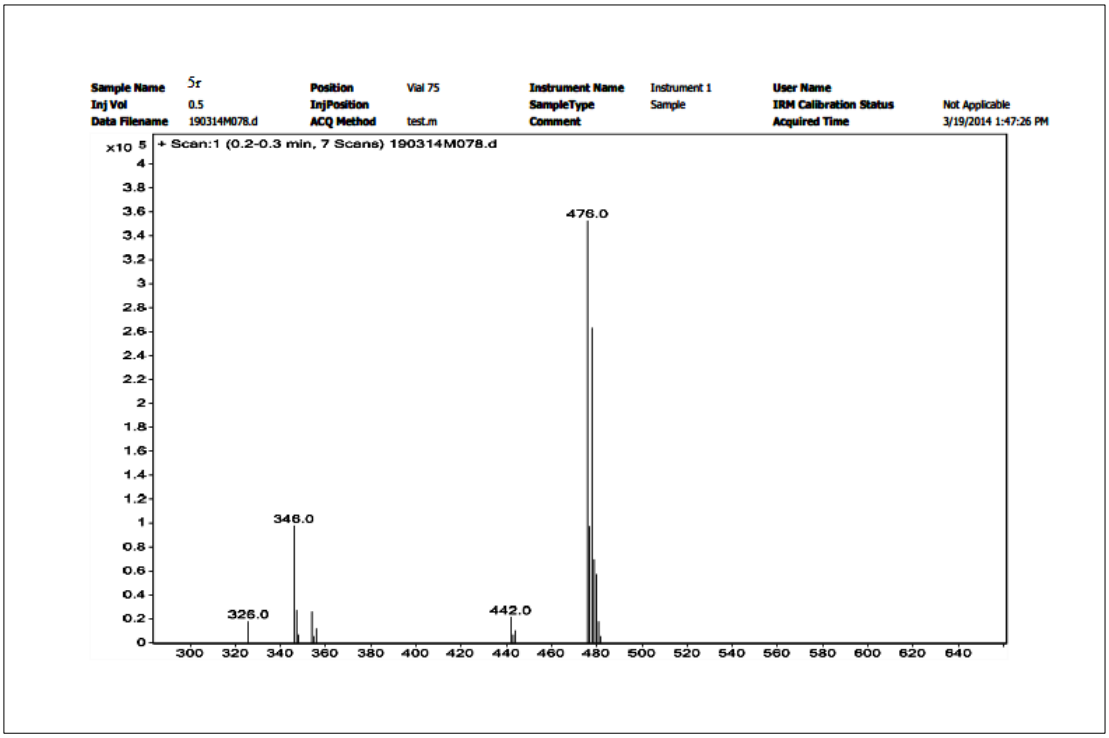


Figure 2.50 Mass spectrum of **T18**

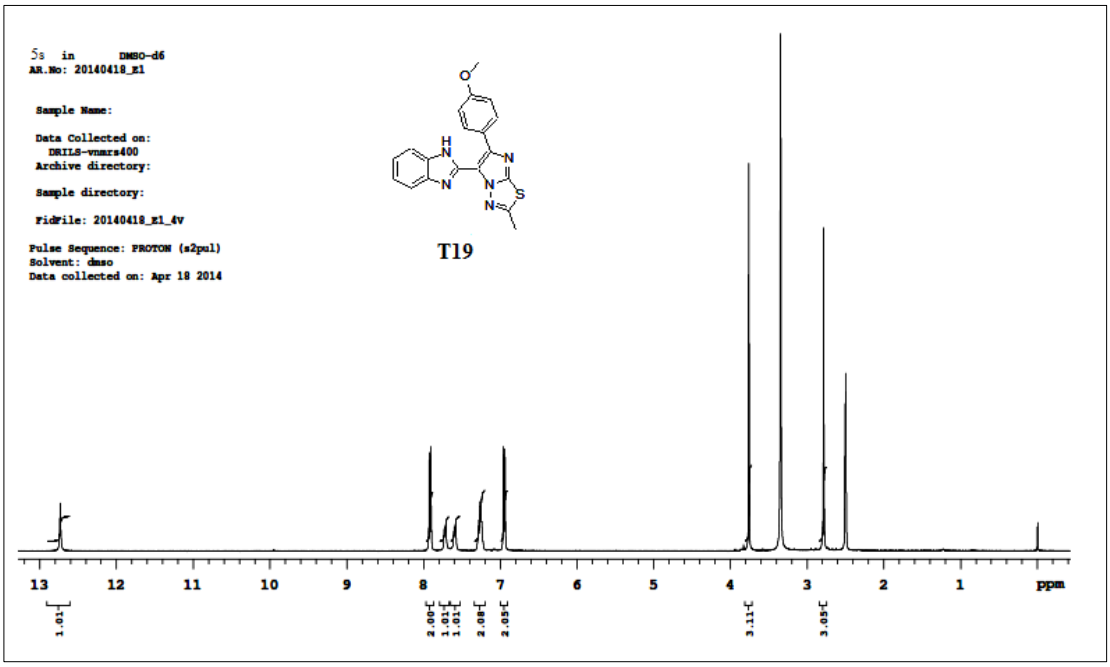


Figure 2.51 ^{1}H NMR spectrum of **T19**

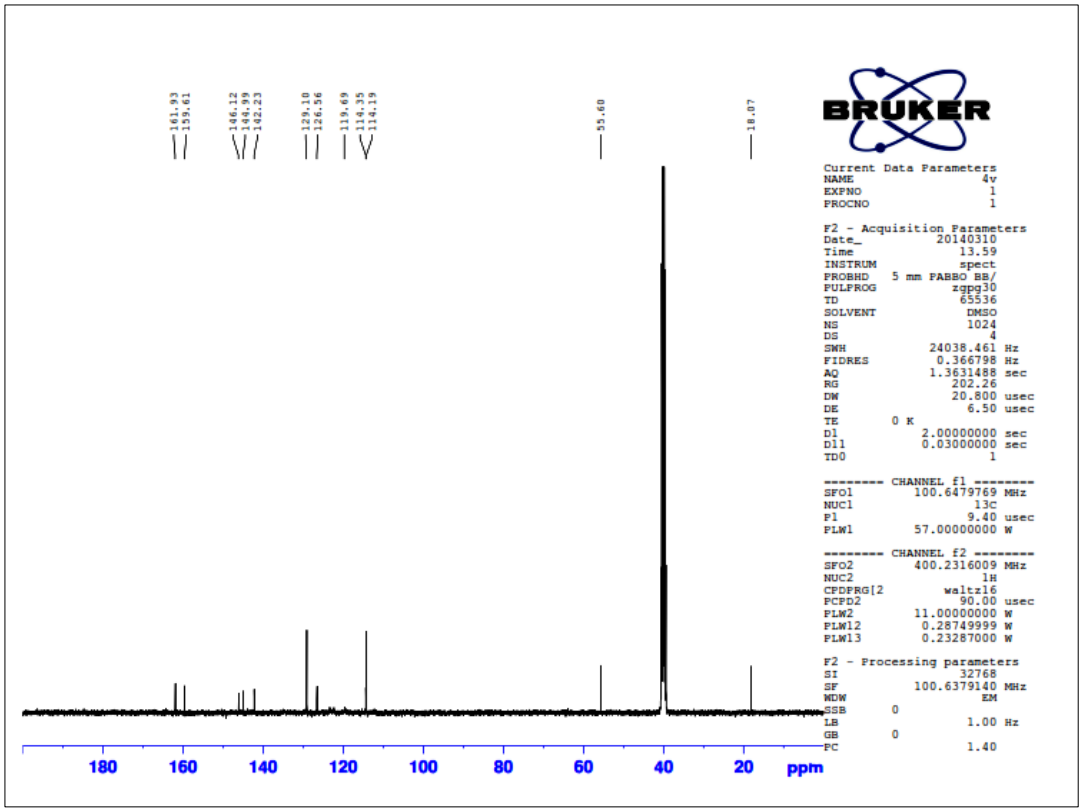


Figure 2.52 ^{13}C NMR spectrum of **T19**

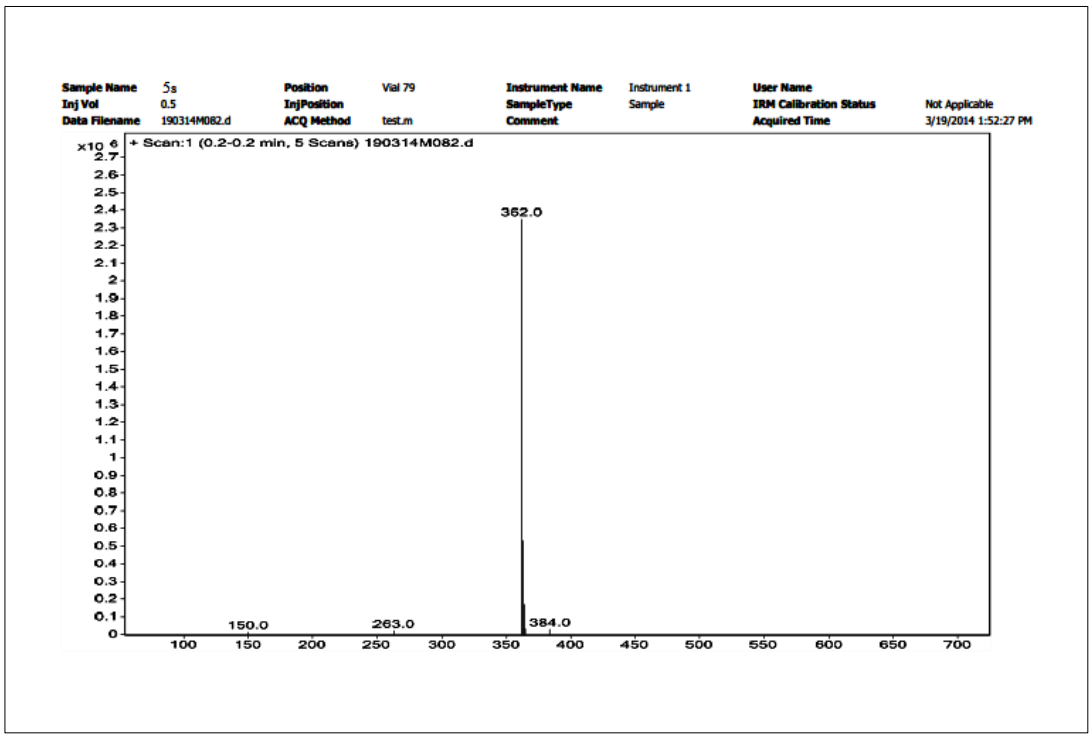


Figure 2.53 Mass spectrum of **T19**

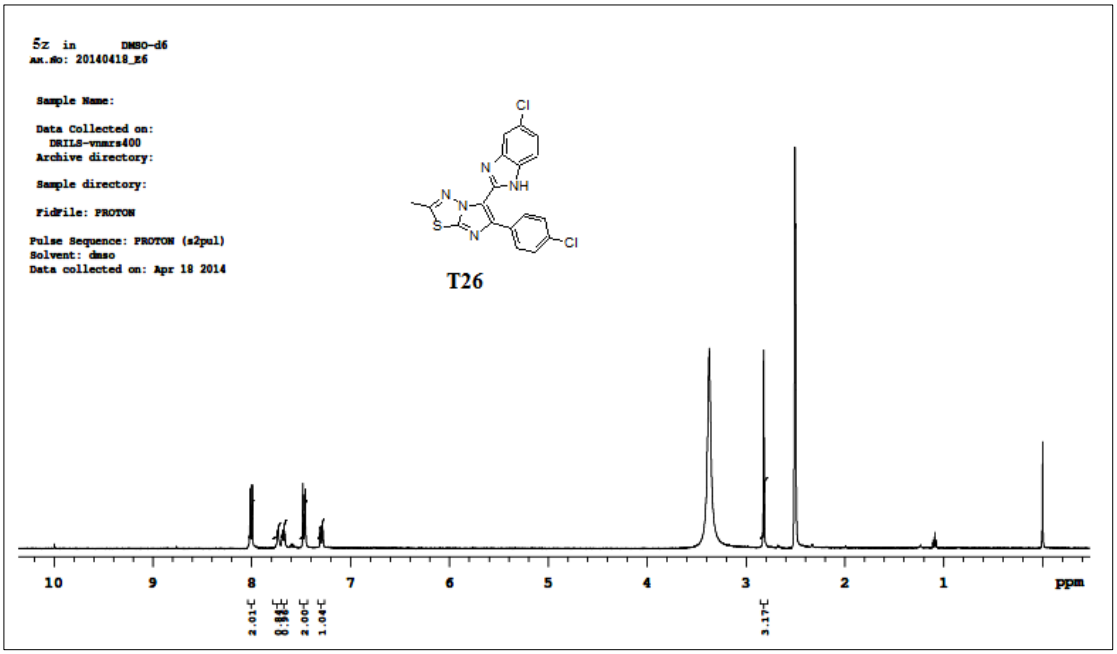


Figure 2.54 ^{1}H NMR spectrum of **T26**

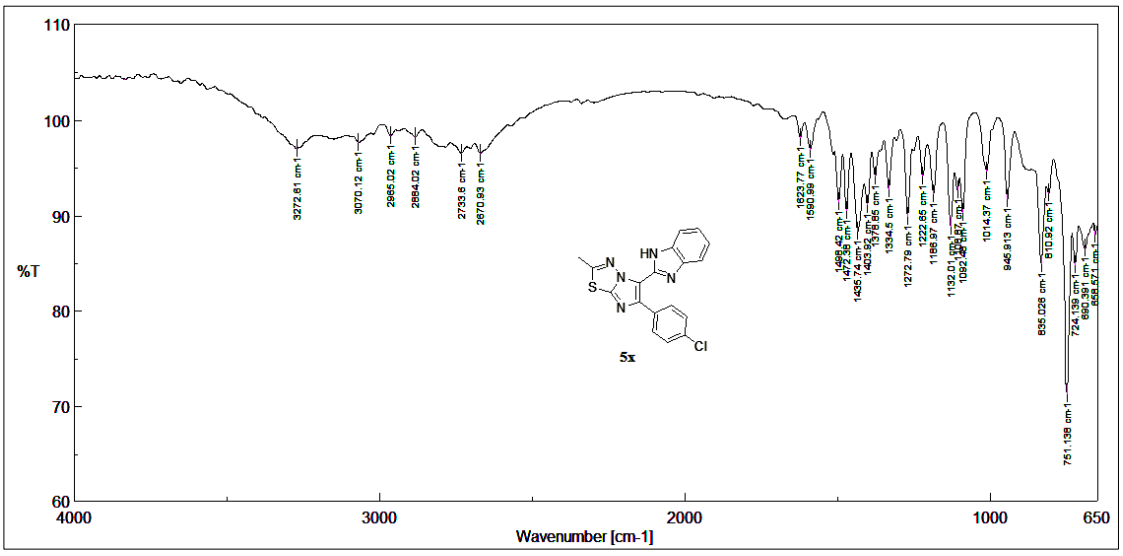


Figure 2.55 FTIR spectrum of **T29**

CHAPTER 3

SYNTHESIS AND ANTITUBERCULAR SCREENING OF NEW 1,2,3-TRIAZOLE - IMIDAZO [2,1-*b*][1,3,4]THIADIAZOLE HYBRIDS

Abstract

This chapter describes design and synthesis of new imidazo[2,1-b][1,3,4]thiadiazole carrying 1,2,3-triazole hybrid analogs. It also discusses their characterization by various spectral techniques followed by their antitubercular screening studies.

3.1 INTRODUCTION

1,2,3-Triazole and its derivatives find great importance in medicinal chemistry research due to their important biological actions in addition to their synthetic applications (Thirumurugan et al. 2013; Agalave et al. 2011). Triazole is a core structural moiety in some of the important drugs like tazobactam (I), cefatrizine (II), carboxyamidotriazole (III) and rufinamide (IV). There are several reports available in the literature which describe various biological activities of 1,2,3-triazole derivatives. The most significant and current studies have demonstrated that these derivatives possess a broad spectrum of pharmacological activities such as antibacterial (Thomas et al. 2011), inflammatory (Shafi et al. 2012), anticonvulsant (Ulloora et al. 2013), anticancer (Singh et al. 2012) and in particular antituberculosis (Addla et al 2014a; Yempala et al. 2014; Shanmugavelan et al. 2011) activity.

A few recent reports demonstrated the promising antiTB activity of 1,2,3-triazole derivatives. For example, a series of (*E*)-*N*1-[(1-aryl)-1*H*-1,2,3-triazole-4-yl)methylene]isonicotinoyl hydrazide (IX) based molecules exhibit outstanding antitubercular activity, several of them possess MIC value in the range 2.5 - 0.62 µg/mL (Boechat et al. 2011). Triazole based antitubercular agent I-A09 (X), which is presently under preclinical trials, is viewed as a promising class of pharmacophore to provide effective drug candidates (Zhou et al. 2010). Further, Patpi et al. (2012) designed a set of 1,2,3-triazole based hybrid molecules following the molecular hybridization strategy and among these, compounds XI and XII exhibited excellent antiTB activity with MIC of 0.78 µg/mL. Both imidazole and thiadiazole moieties are key scaffolds in medicinal chemistry and several drugs containing these moieties are available in the market. For instance megazol (V) which acts as antiparasitic, contain both 1,3,4-thiadiazole and imidazole rings. Imidazole containing PA-824 (nitro imidazopyran) is potent against both replicating and hypoxic, non-replicating *Mtb*.

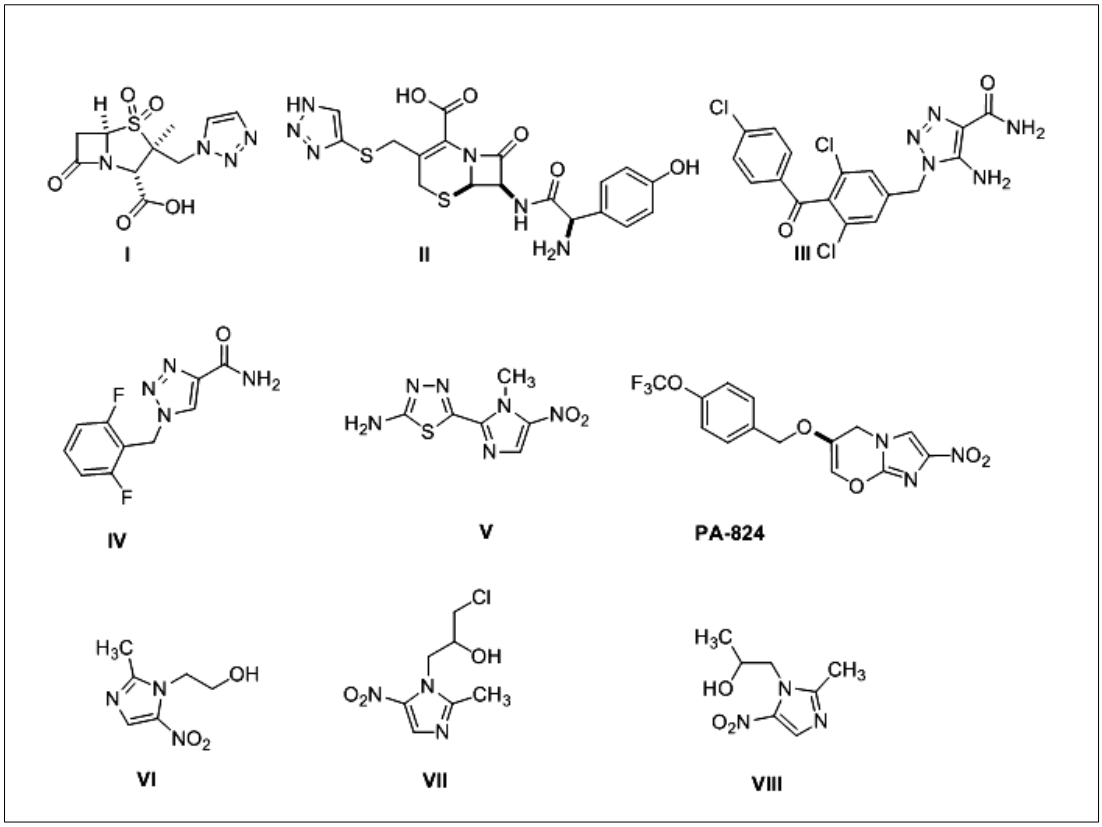


Figure 3.1 Representative imidazole, thiadiazole and 1,2,3-triazole based drug candidates.

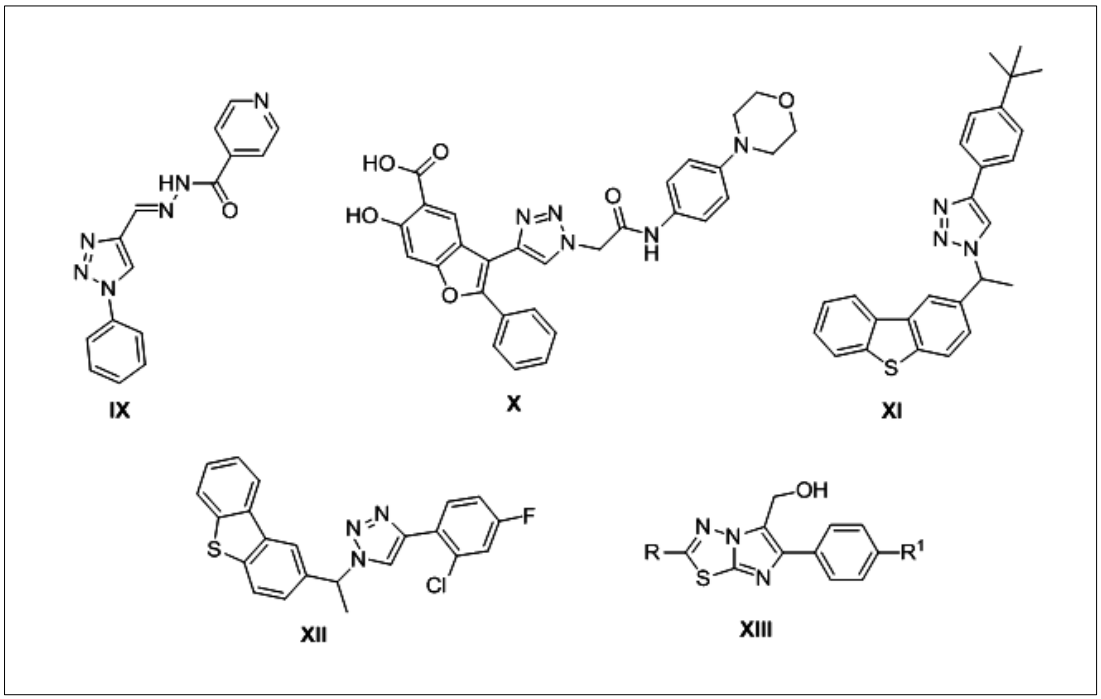


Figure 3.2 Some ITD and 1,2,3-triazole based antitubercular agents.

Further, it is the core moiety in some important anti-bacterial drugs like metronidazole (VI), ornidazole (VII) and sacnidazole (VIII). However, the amalgamation of these two molecular entities as ITD ring resulted an active pharmacophore possessing a broad spectrum of pharmacological activity (refer section 2.1).

Kolavi et al. (2006) reported the synthesis and antiTB evaluation of a series of ITD carrying a hydroxymethyl group at position-5 (XIII) which exhibited prominent inhibition activity with MIC of 6.25 µg/mL. Thus, the promising antitubercular activity exhibited by ITD and 1,2,3-triazole systems prompted us to integrate these two pharmacophores to a single molecular framework and to explore the effects of this molecular hybridization towards their antitubercular biological evaluation.

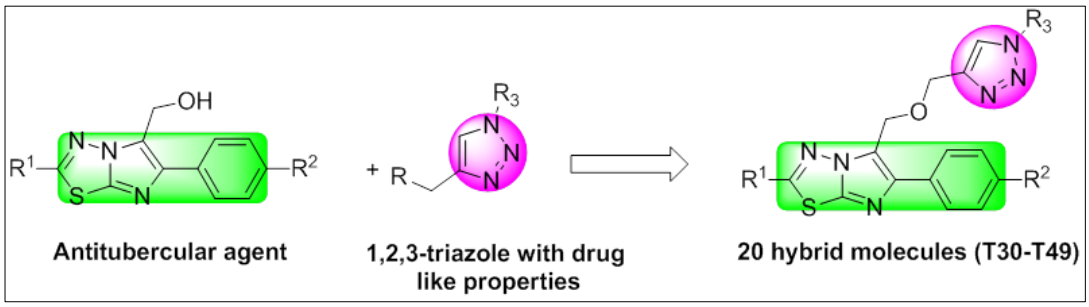
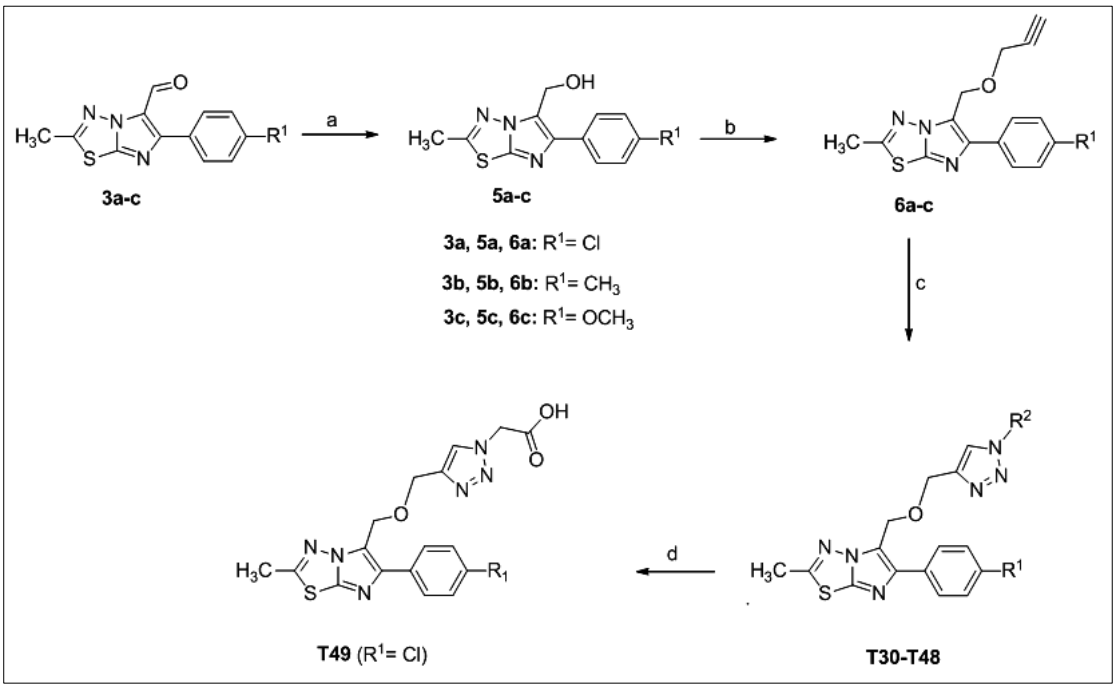


Figure 3.3 Design of new ITD-1,2,3-triazole derivatives

3.2 CHEMISTRY

The synthetic route of new 1,2,3-triazole-ITD hybrids (**T30-T49**) is presented in **scheme 3.1**. The key intermediates, 6-aryl-2-methylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)methanols (**5a-c**) were synthesized by reducing the corresponding aldehyde intermediate (**3a-c)**, using sodium borohydride ($NaBH_4$) as the reducing agent. Intermediates **5a-c** were then treated with propargyl bromide in the presence of sodium hydride to yield 6-aryl-2-methyl-5-((prop-2-ynyloxy)methyl)imidazo[2,1-*b*][1,3,4]thiadiazoles (**6a-c**). Finally, the target compounds (**T30-T48**) were synthesized by following Huisgen 1,3-dipolar cycloaddition reaction (click reaction) in which alkyne intermediates (**6a-c**) were treated with different substituted alkyl bromides in the presence of sodium azide (Odlo et al. 2007). The ester group in compound **T42** was hydrolysed using lithium

hydroxide (LiOH) to get the target compound **T49**. The substitution pattern, yield and solubility of target compounds were presented in **table 3.1**.



Scheme 3.1 Synthesis of ITD-1,2,3-triazole hybrid derivatives (**T30-T49**). Reagents and conditions a) $NaBH_4$, MeOH, 0 °C to RT, 4h; b) Propargyl bromide, NaH, THF, 0 °C to RT, 4h; c) R^2Br, NaN_3, Sodium ascorbate, Copper (II) sulphate, *t*-BuOH and water, RT, 24h; d) $LiOH.H_2O$, THF, MeOH, Water, RT, 5hr.

Table 3.1 Substitution pattern, yield and solubility of target compounds (**T30-T49**).

Product	**R^1**	**R^2**	**log *P*/*C* log *P*[a]**	**Yield (%)**
T30	OCH_3	CH_2-COOEt	3.51/1.65	91
T31	OCH_3	CH_2-CH_3	3.98/1.55	93
T32	OCH_3	CH_2-CN	3.39/0.49	89
T33	OCH_3	2-Fluorobenzyl	5.54/2.93	91
T34	OCH_3	4- Fluorobenzyl	5.54/2.93	94
T35	OCH_3	CH_2-C_6H_5	5.38/2.79	93
T36	CH_3	CH_2-COOEt	4.13/2.14	87
T37	CH_3	CH_2-CH_3	4.6/2.04	90
T38	CH_3	CH_2-CN	4.00/0.98	92

T39	CH_3	2- Fluorobenzyl	6.15/3.42	87
T40	CH_3	4- Fluorobenzyl	6.15/3.42	90
T41	CH_3	CH_2-C_6H_5	5.99/3.28	92
T42	Cl	CH_2-COOEt	4.2/2.35	86
T43	Cl	CH_2-CH_3	4.67/2.25	88
T44	Cl	$CH(CH_3)_2$	4.99/2.56	87
T45	Cl	CH_2-CN	4.07/1.19	90
T46	Cl	2- Fluorobenzyl	6.22/3.63	85
T47	Cl	4- Fluorobenzyl	6.22/3.63	88
T48	Cl	CH_2-C_6H_5	6.06/3.49	92
T49	Cl	CH_2-COOH	3.6/1.492	82

[a]Obtained from Chemdraw ultra 12.0 software

Note: Over all yield of compound **T30** is 64.06 %

3.3 EXPERIMENTAL

3.3.1 Materials and instruments (Refer section 2.3.1)

3.3.2 Synthesis

General procedure for the synthesis of 6-aryl-2-methylimidazo[2,1-*b*][1,3,4]-thiadiazole-5-methanol (**5 a-c**).

(6-(4-Chlorophenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)methanol (5a): To a solution of **3a** (1.3 g, 4.69 mmol**)** in methanol at 0 ^oC under nitrogen atmosphere sodium borohydride (0.266g, 7.039 mmol) was added in portions and the mixture was allowed to attain the RT. The solution was then stirred for 4h. After completion of the reaction (as monitored by TLC), the mixture was poured into ice cold water and stirred for 10 min. The solid obtained was filtered, washed with cold methanol, dried and recrystallized from ethanol to get desired product **5a** as white solid. Yield: 1.20 g, 92 %, m.p: 166-167 ^oC; ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.713-7.740 (m, 2H), 7.432-7.405 (m, 2H), 5.035 (s, 2H,C5-CH_2-O of imidazole), 2.748 (s, 3H, CH_3) ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 160.26, 144.82, 143.00, 133.65, 132.43, 128.89, 128.68, 122.38, 54.11, 17.90; ESI-MS (*m/z*) = 280.1 $(M+H)^+$; calculated for $C_{12}H_{10}ClN_3O_2S$; C, 51.52; H, 3.60; N, 15.02; S, 11.46. Found: C, 51.45; H, 3.66; N, 15.16; S, 11.36.

Compounds **5b** and **5c** were synthesized by following the above procedure

(2-Methyl-6-p-tolylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)methanol (5b): Off white solid. Yield: 1.22 g, 93 %, m.p: 174-175 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.655 (d, *J* =8.4 Hz, 2H, Ar-H), 7.265-7.245 (m, 2H), 5.046 (s, 2H, C5-CH_2-O of imidazole), 2.724 (s, 3H, CH_3), 2.394 (s, 3H, CH_3) ^{13}C NMR (100 MHz, CDCl3) δ (ppm): 159.73, 144.57, 144.21, 137.51, 131.17, 129.40, 127.37, 121.98, 54.26, 21.27, 17.84; ESI-MS (*m/z*) = 260.0 $(M+H)^+$; calculated for $C_{13}H_{13}N_3OS$; C, 60.21; H, 5.05; N, 16.20; S, 12.36. Found: C, 60.15; H, 4.98; N, 16.16; S, 12.26.

(6-(4-Methoxyphenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)methanol (5c): Yellow solid. Yield: 1.25 g, 96 %; m.p: 162-163 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.755 (d, *J* = 7.6 Hz, 2H, Ar-H), 7.02-7.04 (m, 2H, Ar-H), 5.056 (s, 2H,C_5-CH_2-O of imidazole), 3.87 (s, 3H, OCH_3), 2.84 (s, 3H, CH_3), ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 159.93, 144.67, 144.31, 137.71, 131.27, 129.60, 127.47, 122.18, 55.40, 54.36, 17.82; ESI-MS (*m/z*) =276.0 $(M+H)^+$; calculated for $C_{13}H_{13}N_3O_2S$; C, 56.71; H, 4.76; N, 15.26; S, 11.65. Found: C, 56.75; H, 4.78; N, 15.16; S, 11.46.

General procedure for the synthesis of 6-Aryl-2-methyl-5-((prop-2-ynyloxy)methyl) imidazo [2,1-*b*][1,3,4]thiadiazole (**6a-c)**

6-(4-Chlorophenyl)-2-methyl-5-((prop-2-ynyloxy)methyl)imidazo[2,1-*b*][1,3,4] thiadiazole (6a): To a stirred solution of compound **5a** (1.2 g, 4.36 mmol) in THF at 0 ^{0}C under nitrogen atmosphere, sodium hydride (0.373 g, 8.72 mmol, 60% suspension with oil) was added and the reaction mixture was stirred at same temperature for 10 min. Then propargyl bromide (0.717g, 6.54 mmol) was added with stirring and the reaction mixture was allowed to attain the RT and stirred for 4h. After the completion of the reaction (as monitored by TLC), ice cold water was added to the reaction mass. The solid product separated was filtered and washed with n-hexane to get compound **6a** as yellow solid, Yield: 1.31 g, 96 %; m.p: 116-117 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.776 (d, *J* = 8.4 Hz, 2H, Ar-H), 7.4085 (d, *J* = 8.4 Hz, 2H, Ar-H), 4.930 (s, CH_2), 4.312 (d, *J* =2.8 Hz, 2H), 2.732 (s, 3H, CH_3), 2.502 (t, *J* = 4.8 Hz, 1H, acetylene CH); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 160.01, 145.14,

144.57, 133.67, 132.45, 128.79, 119.62, 79.23, 75.13, 60.39, 57.58, 17.95; ESI-MS = (*m/z*) 318.0 $(M+H)^+$; calculated for $C_{15}H_{12}ClN_3OS$; C, 56.69; H, 3.81; N, 13.22; S, 10.09. Found: C, 56.65; H, 3.80; N, 13.16; S, 10.16.

Compounds **6b** and **6c** were synthesized by following the above procedure

2-Methyl-5-((prop-2-ynyloxy)methyl)-6-*p*-tolylimidazo[2,1-*b*][1,3,4]thiadiazole (6b): yellow solid. Yield: 1.30 g, 95%; m.p: 132-133 ^oC; ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.71 (d, J = 8.4 Hz, 2H, Ar-H), 7.25 (d, J = 6.8 Hz, 2H, Ar-H), 4.942 (s, CH_2), 4.30 (d, J = 2.0 Hz, 2H), 2.73 (s, 3H, CH_3), 2.48 (t, J = 4.4 Hz, 1H, acetylene CH), 2.39 (s, 3H, CH_3); ^{13}C NMR (100 MHz, CDCl3) δ (ppm): 159.58, 145.80, 144.93, 137.56, 131.12, 129.33, 127.48, 119.13, 79.41, 74.93, 60.63, 57.51, 21.28, 17.91; ESI-MS (*m/z*) = 298.0 $(M+H)^+$; calculated for $C_{16}H_{15}N_3OS$; C, 64.62; H, 5.08; N, 14.13; S, 10.78. Found: C, 64.52; H, 5.10; N, 14.16; S, 14.01.

6-(4-Methoxyphenyl)-2-methyl-5-((prop-2-ynyloxy)methyl)imidazo[2,1-*b*][1,3,4] thiadiazole (6c): yellow solid. Yield: 1.33 g, 98 %; m.p: 122-123 ^oC; ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.724 (d, J = 7.4 Hz, 2H, Ar-H), 7.25-7.27 (m 2H, Ar-H), 4.952 (s, CH2), 4.312 (d, J = 2.4 Hz, 2H), 3.89 (s, 3H, OCH_3), 2.744 (s, 3H, CH_3), 2.505 (t, J = 4.4 Hz, 1H, acetylene CH); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 160.18, 145.90, 144.93, 137.56, 131.12, 129.33, 127.58, 119.23, 79.61, 74.93, 60.73, 57.51, 54.27, 17.94; ESI-MS (*m/z*) = 314.0 $(M+H)^+$; calculated for $C_{16}H_{15}N_3O_2S$; C, 61.32; H, 4.82; N, 13.41; S, 10.23. Found: C, 61.34; H, 4.78; N, 13.43; S, 10.18.

General procedure for synthesis of 1,2,3-triazole-imidazo[2,1-*b*][1,3,4]thiadiazole derivatives (**T30-T48**).

Ethyl 2-(4-(((6-(4-methoxy phenyl)-2-methyl imidazo[2,1-*b*][1,3,4]thiadiazol-5-yl) methoxy) methyl)-1*H*-1,2,3-triazol-1-yl)acetate (T30): A mixture of compound **6c** (0.2 g, 0.638 mmol), ethyl bromoacetate (0.158 g, 0.958mmol) and sodium azide (0.062 g, 0.958 mmol) was taken in 1:1 mixture of *t*-BuOH and water (4 mL). To this mixture sodium ascorbate (10 mol %) and copper (II) sulfate (5 mol %) were added consecutively and the reaction mixture was stirred at room temperature for 24 h. Then, the reaction mixture was diluted with ice cold water (15 mL) and 10 % aqueous ammonia (2 mL) was added to increase the solution pH to 8.5. To this solution, EAA

was added and the resulting mixture was filtered through celite bed under vacuum. The organic layer was separated and concentrated under reduced pressure. The crude product was purified by column chromatography to get compound **T30** as light yellow solid. Yield: 91 %; m.p: 123-124 $^{\circ}$C; ^{1}H NMR (400 MHz,$CDCl_3$) δ (ppm): 7.72 (s, 1H, Ar-H), 7.62 (d, J = 7.4 Hz, 2H, Ar-H), 7.25 (d, J = 7.4 Hz, 2H, Ar-H), 5.18 (s, 2H, CH_2), 4.84 (s, 2H, CH_2), 4.70 (s, 2H), 4.25 (q, J = 6.0 Hz, 2H), 3.83 (s, 3H, OCH_3), 2.76 (s, 3H, CH_3), 1.28 (t, 3H, J =6.0Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 164.2, 161.81, 144.79, 143.73, 143.10, 133.23, 132.74, 129.25, 128.88, 126.21, 120.75, 63.60, 62.43, 60.17, 54.30, 50.85, 17.85, 14.57; ESI-MS (*m/z*) = 443.1 $(M+H)^+$; Anal. calcd. for $C_{20}H_{22}N_6O_4S$; calcd: C, 54.29; H, 5.01; N, 18.99; S, 7.25. Found: C, 54.20; H, 4.99; N, 18.92; S, 7.20.

Compounds **T31-T48** were synthesized according to above procedure and structural characterization data of the compounds are given below.

5-(((1-Ethyl-1*H*-1,2,3-triazol-4-yl)methoxy)methyl)-6-(4-methoxyphenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazole (T31): Light yellow solid. Yield: 93 %; m.p: 170-171 $^{\circ}$C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm):7.65 (d, 2H, J =7.4 Hz, Ar-H), 7.46 (s, 1H, Ar-H), 7.22-7.25 (m, 2H, Ar-H), 4.93 (s, 2H, CH_2), 4.78 (s, 2H, CH_2), 4.37 (q, J = 7.6 Hz, 2H), 3.87 (s, 3H, OCH_3), 2.74 (s, 3H ,CH_3), 1.54 (t, J = 7.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 159.61, 158.71, 145.46, 144.98, 144.82, 131.35, 127.42, 121.96, 119.58, 114.21, 63.94, 61.17, 54.35, 45.36, 17.92, 15.35; ESI-MS: *(m/z)* 385.2 $(M+H)^+$; Anal. calcd. for $C_{18}H_{20}N_6O_2S$; calcd: C, 56.23; H, 5.24; N, 21.86; S, 8.34. Found: C, 56.25; H, 5.25; N, 21.82; S, 8.30.

2-(4-(((6-(4-Methoxyphenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl) methoxy)methyl)-1*H*-1,2,3-triazol-1-yl)acetonitrile (T32): White solid. Yield: 89 %; m.p: 167-168 $^{\circ}$C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.76 (s, 1H, Ar-H), 7.70 (d, 2H, J = 8.8 Hz), 6.99 (d, J =8.8 Hz, 2H, Ar-H), 5.43 (s, 2H), 4.94 (s, 2H, OCH_2), 4.81 (s, 2H, OCH_2), 3.86 (s, 3H, OCH_3), 2.77 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 159.68, 159.42, 146.55, 145.33, 144.86, 128.78, 126.67, 122.86, 114.17, 112.51, 63.33, 61.29, 55.37, 37.43, 17.91; ESI-MS (*m/z*) = 396.0 $(M+H)^+$;

Anal. calcd. for $C_{18}H_{17}N_7O_2S$; calcd: C, 54.67; H, 4.33; N, 24.79; S, 8.11. Found: C, 54.55; H, 4.36; N, 24.86; S, 8.12.

5-(((1-(2-Fluorobenzyl)-1*H*-1,2,3-triazol-4-yl)methoxy)methyl)-6-(4-methoxy phenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazole (T33): Light brown syrup. yield: 91 %; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.62 (d, *J* = 7.6 Hz, 2H, Ar-H), 7.54 (s, 1H, Ar-H), 7.24-7.31 (m, 2H, Ar-H), 7.215 (d, *J* = 8.4 Hz, 2H, Ar-H), 7.08-7.14 (m, 2H, Ar-H), 5.56 (s, 2H), 4.91 (s, 2H), 4.76 (s, 2H), 3.80 (s, 3H, OCH_3), 2.73 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 160.93, 159.74, 158.61, 158.37, 144.56, 144.38, 143.90, 126.47, 123.94, 123.80, 121.81, 120.92, 118.46, 114.80, 114.69, 113.37, 62.93, 60.28, 53.34, 46.70, 17.54; ESI-MS (*m/z*) = 465.0 $(M+H)^+$; Anal. calcd. for $C_{23}H_{21}FN_6O_2S$; calcd: C, 59.47; H, 4.56; N, 18.09; S, 6.90. Found: C, 59.40; H, 4.55; N, 18.10; S, 6.88.

5-(((1-(4-Fluorobenzyl)-1*H*-1,2,3-triazol-4-yl)methoxy)methyl)-6-(4-methoxy phenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazole (T34): Off white solid. Yield: 94 %; m.p: 100-101 $^\circ$C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.69 (d, *J* = 7.4 Hz, 2H, Ar-H), 7.62 (s, 1H, Ar-H), 7.37 (d, *J* = 7.4 Hz, 2H, Ar-H), 7.25-7.27 (m, 2H, Ar-H), 7.04-7.07 (m, 2H), 5.49 (s, 2H, CH_2), 4.92 (s, 2H, CH_2), 4.77 (s, 2H, CH_2), 3.84 (s, 3H, OCH_3), 2.73 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 164.18, 161.76, 160.21, 159.98, 146.43, 145.12, 144.40, 132.61, 131.25, 123.17, 128.79, 122.34, 119.97, 116.28, 116.07, 114.28, 63.81, 61.06, 55.39, 53.44, 17.91; ESI-MS (*m/z*) = 465.0 $(M+H)^+$; Anal. calcd. for $C_{23}H_{21}FN_6O_2S$; calcd: C, 59.47; H, 4.56; N, 18.09; S, 6.09. Found: C, 59.50; H, 4.52; N, 18.16; S, 6.02.

5-(((1-Benzyl-1*H*-1,2,3-triazol-4-yl)methoxy)methyl)-6-(4-methoxyphenyl)-2-methyl imidazo [2,1-*b*][1,3,4]thiadiazole (T35): Off white solid. Yield: 93 %; m.p: 115-116 $^\circ$C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.697 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.44 (s, 1H, Ar-H), 7.26-7.28 (m, 3H, Ar-H), 7.26 (d, *J* = 1.8 Hz, 2H, Ar-H), 6.96 (d, *J* = 8.8 Hz, 2H, Ar-H), 5.51 (s, 2H, CH_2), 4.91 (s, 2H, Ar-H), 4.78 (s, 2H, CH_2), 3,85 (s, 3H, OCH_3), 2.74 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 159.34, 145.44, 145.38, 144.72, 134.54, 129.12, 128.76, 128.11, 126.74, 122.53, 119.08, 114.09, 63.78, 61.21, 55.32, 54.16, 17.87; ESI-MS (*m/z*) = 447.0 $(M+H)^+$; Anal.

calcd. for $C_{23}H_{22}N_6O_2S$; calcd: C, 61.87; H, 4.97; N, 18.82; S, 7.18. Found: C, 61.85; H, 4.95; N, 18.85; S, 7.15.

Ethyl2-(4-(((2-methyl-6-*p*-tolylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)methoxy) methyl)-1*H*-1,2,3-triazol-1-yl)acetate (T36): White solid. Yield: 87 %; m.p: 157-158 $^{\circ}$C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.60-7.78 (m, 3H, Ar-H), 7.20-7.31 (m, 2H, Ar-H), 5.13 (s, 2H, CH_2), 4.94 (s, 2H, Ar-H), 4.26 (q, *J* = 6.8 Hz, 2H), 2.75 (s, 3H, CH_3), 2.39 (s, 3H, CH_3), 1.29 (t, *J* = 6.8Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 166.18, 159.55, 145.61, 145.44, 144.84, 137.47, 131.24, 129.37, 127.43, 124.01, 119.54, 63.70, 62.43, 61.17, 50.85, 21.26, 17.91, 14.07; ESI-MS (*m/z*) = 427.1 $(M+H)^+$; Anal. calcd. for $C_{20}H_{22}N_6O_3S$; calcd: C, 56.32; H, 5.20; N, 19.70; S, 7.52. Found: C, 56.22; H, 5.25; N, 19.72; S, 7.55.

5-(((1-Ethyl-1*H*-1,2,3-triazol-4-yl)methoxy)methyl)-2-methyl-6-*p*-tolylimidazo [2,1-*b*][1,3,4] thiadiazole (T37): Brown syrup. Yield: 90 %; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm):7.64 (d, 2H, *J* = 8.4 Hz), 7.48 (s, 1H, Ar-H), 7.22-7.25 (m, 2H, Ar-H), 4.93(s, 2H, CH_2), 4.78 (s, 2H, CH2), 4.37 (q, *J* = 7.6 Hz, 2H), 2.74 (s, 3H ,CH_3), 2.38 (s, 3H, CH3), 1.53 (t, *J* =7.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 159.51, 145.56, 144.98, 144.82, 137.48, 131.25, 129.35, 127.42, 121.86, 119.58, 63.84, 61.16, 45.26, 21.27, 17.92, 15.45; ESI-MS (*m/z*) = 369.0 $(M+H)^+$; Anal. calcd. for $C_{18}H_{20}N_6OS$; calcd: C, 58.68; H, 5.47; N, 22.81; S, 8.70. Found: C, 58.70; H, 5.45; N, 22.80; S, 8.68.

2-(4-(((2-Methyl-6-*p*-tolylimidazo[2,1-b][1,3,4]thiadiazol-5-yl)methoxy)methyl)-1*H*-1,2,3-triazol-1-yl) acetonitrile (T38): Off white solid. Yield: 92 %; m.p: 140-141 $^{\circ}$C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.75 (s, 1H, Ar-H), 7.70 (d, *J* = 7.4 Hz, 2H, Ar-H), 6.99 (d, *J* = 8.0 Hz, 2H, Ar-H), 5.42 (s, 2H), 4.93 (s, 2H, OCH_2), 4.78 (s, 2H, CH_2), 2.77 (s, 3H, CH_3), 2.38 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 159.58, 146.45, 145.33, 144.76, 138.56, 128.68, 126.47, 123.16, 116.21, 114.17, 112.51, 63.33, 61.29 , 37.43, 20.23, 17.91; ESI-MS (*m/z*) = 380.0 $(M+H)^+$; Anal. calcd. for $C_{18}H_{17}N_7OS$; calcd: C, 56.98; H, 4.52; N, 25.84; S, 8.45. Found: C, 56.85; H, 4.46; N, 25.86; S, 8.48.

5-(((1-(2-fluorobenzyl)-1*H*-1,2,3-triazol-4-yl)methoxy)methyl)-2-methyl-6-*p*-tolyl imidazo [2,1-*b*] [1,3,4] thiadiazole (T39): White solid. Yield: 87 %; m.p: 102-103 $^{\circ}$C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.62 (d, *J* = 7.6 Hz, 2H, Ar-H), 7.54 (s, 1H, Ar-H), 7.31-7.35 (m, 1H, Ar-H), 7.24-7.28 (m, 1H, Ar-H), 7.21 (d, *J* = 8.4 Hz), 7.08-7.14 (m, 2H, Ar-H), 5.56 (s, 2H), 4.91 (s, 2H), 4.76 (s, 2H), 2.73 (s, 3H, CH_3), 2.38 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 160.73, 158.51, 158.27, 144.56, 144.38, 143.80, 136.45, 128.31, 126.37, 123.84, 123.80, 121.71, 120.92, 118.46, 114.90, 114.69, 62.73, 60.18, 46.60, 20.23, 16.84; ESI-MS (*m/z*) = 449.0 $(M+H)^+$; Anal. calcd for $C_{23}H_{21}FN_6OS$; calcd: C, 61.59; H, 4.72; N, 18.74; S, 7.15. Found: C, 61.54; H, 4.75; N, 18.70; S, 7.18.

5-(((1-(4-Fluorobenzyl)-1*H*-1,2,3-triazol-4-yl)methoxy)methyl)-2-methyl-6-(*p*-tolyl)imidazo[2,1-*b*][1,3,4]thiadiazole (T40): Off white solid. Yield: 90 %; m.p: 129-130 $^{\circ}$C; ^{1}H NMR (400 MHz,$CDCl_3$) δ (ppm): 7.68 (d, *J* = 8.4 Hz, 2H, Ar-H), 7.52 (s, 1H, Ar-H), 7.36 (d, *J* = 8.4 Hz, 2H, Ar-H), 7.24-7.26 (m, 2H, Ar-H), 7.03-7.07 (m, 2H), 5.48 (s, 2H, CH_2), 4.91 (s, 2H, CH_2), 4.76 (s, 2H, CH_2), 2.73 (s, 3H, CH_3), 2.34 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 164.18, 161.76, 159.98, 145.43, 145.12, 144.30, 138.68, 132.51, 131.15, 123.07, 128.79, 128.65, 122.34, 119.97, 116.28, 116.07, 63.81, 61.06, 53.44, 21.45, 17.91; ESI-MS (*m/z*) = 449.0 $(M+H)^+$; Anal. calcd for $C_{23}H_{21}FN_6OS$; calcd: C, 61.59; H, 4.72; N, 18.74; S, 7.15. Found: C, 61.55; H, 4.80; N, 18.66; S, 7.12.

5-(((1-Benzyl-1*H*-1,2,3-triazol-4-yl)methoxy)methyl)-2-methyl-6-*p*-tolylimidazo [2,1-*b*][1,3,4] thiadiazole (T41): White solid. Yield: 92 %; mp: 124-125 $^{\circ}$C; ^{1}H NMR (400 MHz,$CDCl_3$) δ (ppm): 7.68 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.45 (s, 1H, Ar-H), 7.25-7.28 (m, 3H, Ar-H), 7.16-7.18 (m, 2H, Ar-H), 6.97 (d, *J* = 8.8 Hz, 2H, Ar-H), 5.52 (s, 2H, CH_2), 4.91 (s, 2H, Ar-H), 4.78 (s, 2H, CH_2), 2.74 (s, 3H, CH_3), 2.342 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 145.44, 145.38, 144.72, 138.4, 134.54, 129.12, 128.76, 128.11, 126.74, 122.53, 119.08, 114.09, 63.78, 61.21, 55.32, 21.45, 17.87; ESI-MS: *(m/z)* = 431.3 $(M+H)^+$; Anal. calcd for $C_{23}H_{22}N_6OS$; calcd: C, 64.16; H, 5.15; N, 19.52; S, 7.45. Found: C, 64.15; H, 5.16; N, 19.85; S, 7.39.

Ethyl2-(4-(((6-(4-chlorophenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl) methoxy)methyl)-1*H*-1,2,3-triazol-1-yl) acetate (T42): Light yellow solid. Yield: 86 %; m.p: 157-158 °C; ^{1}H NMR (400 MHz,$CDCl_3$) δ (ppm): 7.82 (s, 1H, Ar-H), 7.62 (d, *J* = 8.4 Hz, 2H, Ar-H), 7.36 (d, *J* = 8.4 Hz, 2H, Ar-H), 5.18 (s, 2H, CH_2), 4.84 (s, 2H, CH_2), 4.70 (s, 2H), 4.25 (q, *J* = 6.8 Hz, 2H), 2.76 (s, 3H, CH_3), 1.29 (t, 3H, *J* = 6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 165.2, 161.91, 144.79, 143.73, 142.98, 133.23, 132.64, 129.25, 128.88, 126.21, 120.75, 63.60, 62.43, 60.17, 50.85, 17.91, 14.57; ESI-MS (*m/z*) = 447.1 $(M+H)^+$; Anal. calcd for $C_{19}H_{19}ClN_6O_3S$; calcd: C, 51.06; H, 4.29; N, 18.80; S, 7.17. Found: C, 51.12; H, 4.25; N, 18.72; S, 7.19

6-(4-Chlorophenyl)-5-(((1-ethyl-1*H*-1,2,3-triazol-4-yl)methoxy)methyl)-2-methyl imidazo[2,1-*b*][1,3,4] thiadiazole (T43): Brown solid. Yield: 88 %; m.p: 128-129 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.71 (d, *J* = 8 Hz, 2H, Ar-H), 7.51 (s, 1H, Ar-H), 7.38 (d, *J* = 8.4 Hz, 2H, Ar-H), 4.93 (s, 2H), 4.79 (s, CH2), 4.40 (q, *J* = 7.6 Hz, 2H), 2.74 (s, 3H, CH_3), 1.55 (t, *J* = 7.6 Hz, 3H, Ethyl); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 159.99, 145.04, 144.78, 144.28, 133.58, 132.53, 128.79, 128.68, 121.87, 120.10, 63.88, 60.98, 45.31, 17.94, 14.46; ESI-MS (*m/z*) = 389.0 $(M+H)^+$; Anal. calcd for $C_{17}H_{17}ClN_6OS$; calcd: C, 52.51; H, 4.41; N, 21.61; S, 8.25. Found: C, 52.62; H, 4.46; N, 21.66; S, 8.21.

6-(4-Chlorophenyl)-5-(((1-isopropyl-1*H*-1,2,3-triazol-4-yl)methoxy)methyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazole (T44): Brown solid. Yield: 87 %; m.p: 100-101 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm):7.72 (d, *J* =7.6 Hz), 7.52 (s, 1H, Ar-H), 7.39 (d, *J* = 8 Hz, 2H, Ar-H), 4.94 (s, 2H, Ar-H), 4.83-4.81 (m, 1H, Ar-H), 4.79 (s, 2H, CH_2), 2.74 (s, 3H, CH_3), 1.56 (d, *J* = 6.4 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 159.89, 145.04, 144.68, 144.38, 133.58, 132.43, 128.79, 128.48, 122.17, 120.10 , 63.88, 60.98, 54.8, 22.3, 17.94; ESI-MS (*m/z*) = 403.2 $(M+H)^+$; Anal. calcd for $C_{18}H_{19}ClN_6OS$; calcd: C, 53.66; H, 4.75; N, 20.86; S, 7.96. Found: C, 53.62; H, 4.76; N, 20.76; S, 7.91.

2-(4-(((6-(4-Chlorophenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl) methoxy)methyl)-1*H*-1,2,3-triazol-1-yl) acetonitrile (T45): Yellow solid. Yield: 90 %; m.p: 122-123 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.77 (s, 1H, Ar-H), 7.72

(d, *J* =8.4 Hz, 2H, Ar-H), 7.40 (d, *J* = 8.4 Hz, 2H, Ar-H), 5.328 (s, 2H), 4.94 (s, 2H), 4.81 (s, 2H, Ar-H), 2.75 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm):160.27, 146.46, 145.15, 133.71, 132.46, 128.89, 128.68, 122.79, 119.78, 112.43, 63.49, 61.20, 37.49, 17.96; ESI-MS (*m/z*) = 400.2 $(M+H)^+$; Anal. calcd for $C_{17}H_{14}ClN_7OS$; calcd: C, 51.06; H, 3.53; N, 24.52; S, 8.02. Found: C, 51.05; H, 3.56; N, 24.76; S, 8.11.

5-(((1-(2-Fluorobenzyl)-1*H*-1,2,3-triazol-4-yl)methoxy)methyl)-6-(4-chloro phenyl)-2-methyl imidazo[2,1-*b*][1,3,4]thiadiazole (T46): Light yellow solid**.** Yield: 85 %; mp: 113-114 $^{\circ}$C; ^1H NMR (400 MHz,$CDCl_3$) δ (ppm):7.71 (d, *J* = 8.4 Hz, 2H, Ar-H), 7.55 (s, 1H, Ar-H), 7.24-7.38 (m, 1H, Ar-H), 7.23 (d, *J* = 8.4 Hz, 2H, Ar-H), 7.08-7.14(m, 2H, Ar-H), 5.57 (s, 2H), 4.92 (s, 2H), 4.77 (s, 2H), 2.73 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 160.63, 158.51, 158.37, 144.56, 144.28, 143.70, 134.35, 128.31, 126.47, 123.84, 123.60, 121.61, 120.92, 118.46, 114.90, 114.70, 62.73, 60.18, 46.60, 17.24; ESI-MS (*m/z*) = 469.0 $(M+H)^+$; Anal. calcd for $C_{22}H_{18}ClFN_6OS$; calcd: C, 56.35; H, 3.87; N, 17.92; S, 6.84. Found: C, 56.34; H, 3.85; N, 17.79; S, 6.78.

5-(((1-(4-Fluorobenzyl)-1*H*-1,2,3-triazol-4-yl)methoxy)methyl)-6-(4-chloro phenyl)-2-methyl imidazo[2,1-*b*][1,3,4]thiadiazole (T47): White solid. Yield: 88 %; mp: 156-157 $^{\circ}$C; ^1H NMR (400 MHz,$CDCl_3$) δ (ppm): 7.68 (d, *J* = 8.0 Hz), 7.42 (s, 1H, Ar-H), 7.35 (d, *J* = 8.0 Hz, Ar-H), 7.24-7.27 (m, 2H, Ar-H), 7.03-7.07 (m, 2H), 5.47 (s, 2H, CH_2), 4.90 (s, 2H, CH_2), 4.76 (s, 2H, CH_2), 2.73 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 164.13, 161.66, 159.98, 145.33, 145.02, 144.30, 133.58, 132.51, 130.05, 129.97, 128.79, 128.65, 122.34, 119.97, 116.28, 116.07, 63.81, 61.06, 53.44, 17.91; ESI-MS (*m/z*) = 469.0 $(M+H)^+$; Anal. calcd for $C_{22}H_{18}ClFN_6OS$; calcd: C, 56.35; H, 3.87; N, 17.92; S, 6.84. Found: C, 56.45; H, 3.86; N, 17.96; S, 6.82.

5-(((1-Benzyl-1*H*-1,2,3-triazol-4-yl)methoxy)methyl)-6-(4-chlorophenyl)-2-methyl imidazo [2,1-*b*][1,3,4]thiadiazole (T48): Light yellow solid. Yield: 92 %; m.p: 101-102 $^{\circ}$C; ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.68 (d, *J* = 8.4 Hz, 2H, Ar-H), 7.46 (s, 1H), 7.27-7.29 (m, 3H, Ar-H), 7.16-7.18 (m, 2H, Ar-H), 6.97 (d, *J* = 8.4 Hz, 2H, Ar-H), 5.52 (s, 2H, CH_2), 4.92 (s, 2H, Ar-H), 4.78 (s, 2H, CH2), 2.75 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 146.44, 145.78, 144.62, 135.62, 134.54, 129.22,

128.76, 128.21, 126.74, 122.43, 119.08, 114.19, 63.78, 61.31, 55.42, 17.97; ESI-MS: (*m/z*) = 451.2 $(M+H)^+$; Anal. calcd for $C_{22}H_{19}ClN_6OS$; calcd: C, 58.60; H, 4.25; N, 18.64; S, 7.11. Found: C, 58.62; H, 4.26; N, 18.65; S, 7.12.

2-(4-(((6-(4-Chlorophenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl) methoxy)methyl)-1*H*-1,2,3-triazol-1-yl)acetic acid (T49): Compound **T42** (0.1g, 0.224 mmol) was dissolved in methanol and THF mixture (1:1) and LiOH (4.48 mmol, dissolved in water) added at 0 °C and the contents were stirred for 5 h at RT. After the completion of the reaction (as monitored by TLC), reaction mass was concentrated under reduced pressure. The reaction mass was then diluted with water and glacial acetic acid was added to bring down the solution pH to 5. The solid separated was filtered under vacuum. The crude product was recrystallized from a mixture of DMF and water system to get compound **T49** as yellow solid. Yield: 82 %; m.p: 194-195 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 13.2-13.6 (s, 1H, COOH), 8.14 (s, 1H, Ar-H), 7.72 (d, *J* = 8.4 Hz, 2H, Ar-H), 7.47 (d, *J* = 8.4 Hz, 2H, Ar-H), 5.28 (s, 2H, CH_2), 4.85 (s, 2H, CH_2), 4.70 (s, 2H), 2.76 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 169.07, 161.91, 144.89, 143.83, 142.96, 133.21, 132.54, 129.15, 128.78, 126.11, 120.65, 63.30, 60.68, 50.95, 18.05; ESI-MS (m/z) = 419.0 $(M+H)^+$; Anal. calcd for $C_{17}H_{15}ClN_6O_3S$; calcd: C, 48.75; H, 3.61; N, 20.06; S, 7.66. Found: C, 48.62; H, 3.66; N, 20.16; S, 7.62.

3.4 PHARMACOLOGY

3.4.1 Experimental protocol for TB screening (Refer section 2.4.1)

3.4.2 Antibacterial screening

The compounds were screened for antibacterial activity using the disc diffusion method by measuring the zone of inhibition. Bacterial strains of *S. aureus*, *P. aeruginosa* and *E. coli* were cultivated in Brain heart infusion agar medium for 24 h. The culture suspensions were prepared and adjusted by comparing against 0.5 McFarland turbidity standard tubes. The hollow tube of 5 mm diameter was taken and heated. Pressed it on above inoculated agar plate and removed it immediately by making a well in the plate. All The compounds were dissolved in DMSO and appropriate dilutions were made (75 and 50 µg/mL) and added to respective wells. DMSO was used as a solvent and as control. After the inoculation of organism and

compound, the petri plates were incubated for 18-24 h at 37 °C. Inhibition zones formed on the medium were evaluated in millimeter (mm). The negative solvent control (DMSO) did not show any antimicrobial activity. Studies were performed in triplicate and the average reading was taken. Inhibition zones were compared with those of the reference discs.

3.4.3 *In vitro* cytotoxicity studies

The NIH 3T3 mouse embryonic fibroblasts cell line was procured from NCCS, Pune, India. The cell lines were maintained in 96 wells micro titer plate containing MEM media supplemented with 10% heat inactivated fetal calf serum (FCS), containing 5% of mixture of gentamicin (10µg), penicillin (100 Units/ml) and streptomycin (100µg/ml) in presence of 5% CO_2 at 37 °C for 48-72 hours. For cytotoxicity studies, each weighed test drugs were separately dissolved in distilled DMSO to obtain a stock solution of 1 mg/ml concentration and sterilized by filtration. Serial two fold dilutions were prepared from this for carrying out MTT assay. The cells were then exposed to different concentrations of drug and kept for incubation for 48 h. The control group contains only DMSO. After the incubation, stock solution of MTT (20 µL, 5mg/mL in sterile PBS) was added to each well and cells were incubated for additional 2 - 3 h at 5% CO_2 atmosphere. After careful removal of incubation medium from the incubator, 100 µL of DMSO was added and the plates were gently shaken to re-suspend formed formazan and waited for few minutes to form a homogenized color. The suspension was placed on a micro vibrator for 5 min, and absorbances of wells containing cells and blanks were recorded at 490 nm. The experiment was executed in triplicate. The mean of the absorbance of wells was calculated with the same treatment after subtracting of blank absorbance. The results were normalized by considering control wells as 100% (maximum absorbance obtained), expressing then the results as percentage of controls. Percentage of growth inhibition was calculated from below equation.

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

3.4.4 Molecular docking studies (Refer section 2.4.4)

3.5 RESULTS AND DISCUSSION

3.5.1 Chemistry

The structures of newly synthesized intermediates and target compounds were confirmed by ^{1}H NMR, ^{13}C NMR, mass spectral analysis and elemental analysis. Intermediate **3a** displayed aldehyde peak along with other characteristic peaks in their NMR spectra. For instance, the formyl group in **3a** resonated as a singlet at δ 10.3 ppm in its ^{1}H NMR spectrum whereas in the ^{13}C NMR spectrum the aldehyde signal appeared at δ 177 ppm. In the ^{1}H NMR spectrum of **5a (figure 3.13)**, the singlet peak due to aldehyde group disappeared whereas a new singlet peak appeared at δ 5.04 ppm due to alcoholic group thus confirming reduction of the formyl group to the hydroxymethyl group. Further, the methylene carbon (–CH_2OH) of **5a** appeared at δ 54.1 ppm in the ^{13}C NMR spectrum (**figure 3.14**), whereas its mass spectrum showed molecular ion peak (M+1 peak) at *(m/z)* 277.9 thus confirming its molecular mass (**figure 3.15**). The ^{1}H NMR spectrum of **6a** (**figure 3.18**) displayed a doublet at δ 4.31 ppm and a triplet at δ 2.50 ppm corresponding to CH_2 and CH protons of propargyl group, respectively. Whereas, its ^{13}C NMR spectrum showed peaks at δ 79.2, 75.1 and 60.4 ppm corresponding to two alkyne carbons and a methylene carbon, respectively (**figure 3.19**). Successful conversion of alkynes **6a-c** to 1,2,3-triazoles **T30-T48** was confirmed by the spectral analysis. For instance, the ^{1}H NMR spectrum of **T43** showed a singlet at δ 7.5 ppm due to –CH proton of triazole ring whereas the quartet at δ 4.40 ppm and the triplet at δ 1.55 ppm correspond to methylene and methyl protons of N-ethyl group respectively (**figure 3.4**). The methylene protons of –CH_2-O-CH_2- bridge appeared as two separate singlets at δ 4.93 and 4.79 ppm in which the singlet at slightly higher chemical shift corresponds to the methylene group attached to the 1,2,3-triazole ring. In addition the spectrum displayed a singlet at δ 2.75 ppm due to methyl group present at position-2 of the ITD ring. The ^{13}C NMR spectrum of **T43** displayed all characteristic peaks corresponding to its molecular structure; the two methylene carbons of –CH_2-O-CH_2- bridge appeared at δ 63.88 and 60.98 ppm whereas peaks at δ 45.3 and 15.5 ppm represent methylene and methyl carbons of the ethyl group respectively (**figure 3.5**).

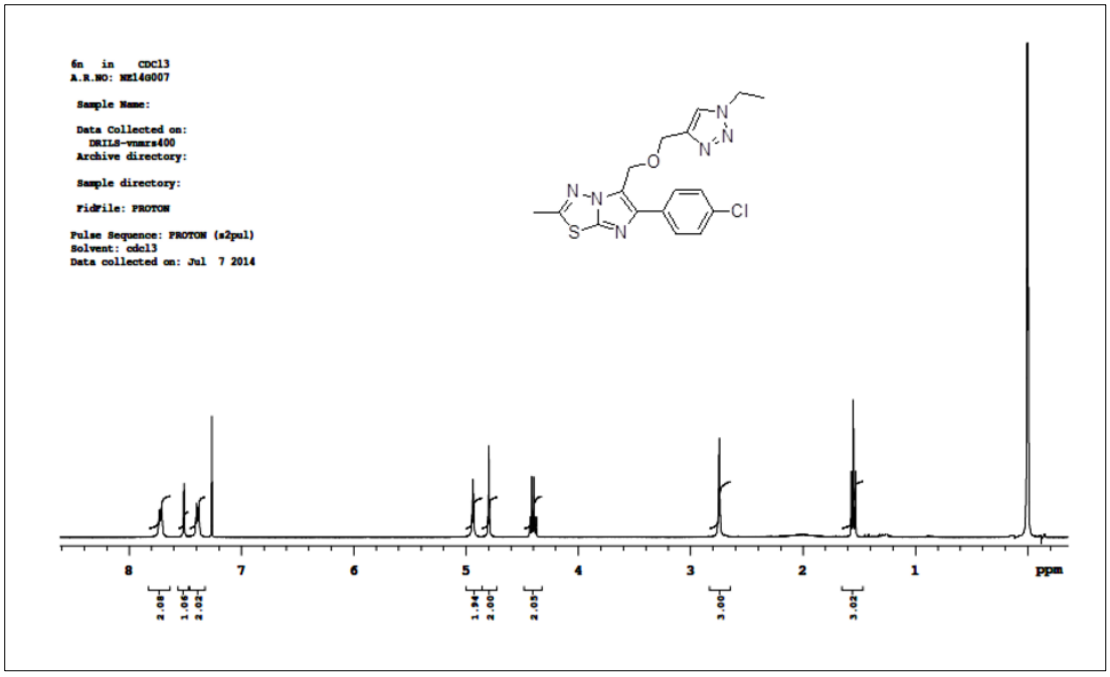


Figure 3.4 ^{1}H NMR spectrum of **T43**

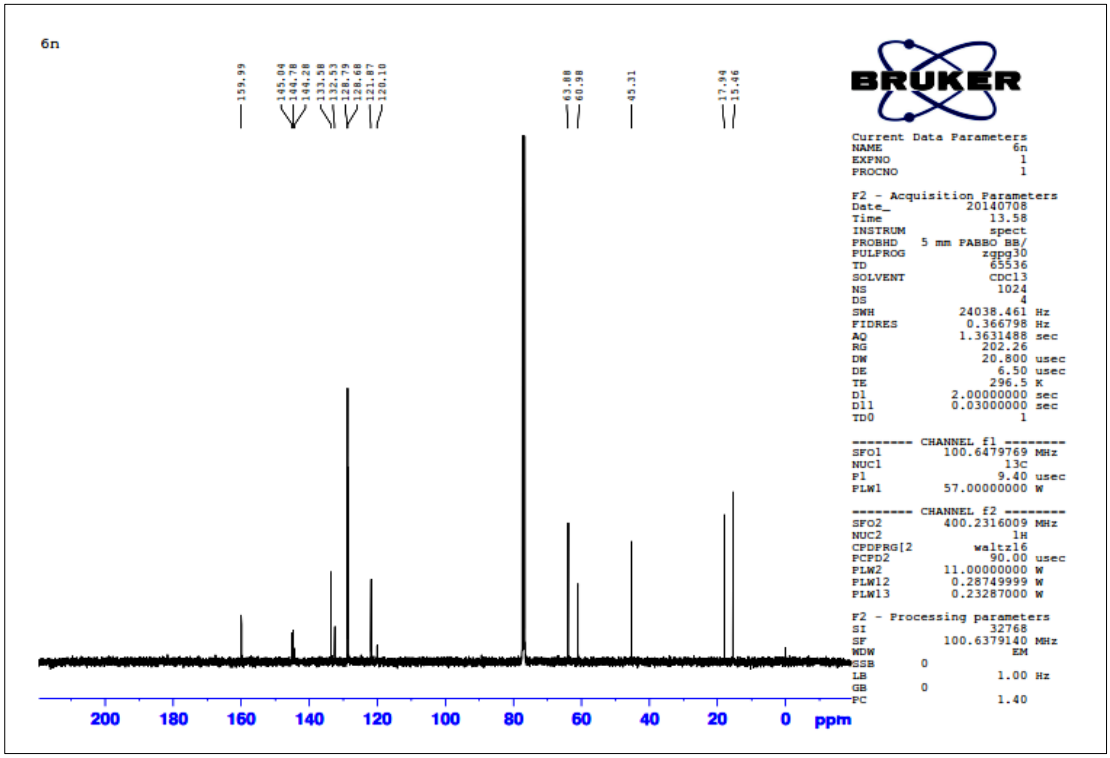


Figure 3.5 ^{13}C NMR spectrum of **T43**

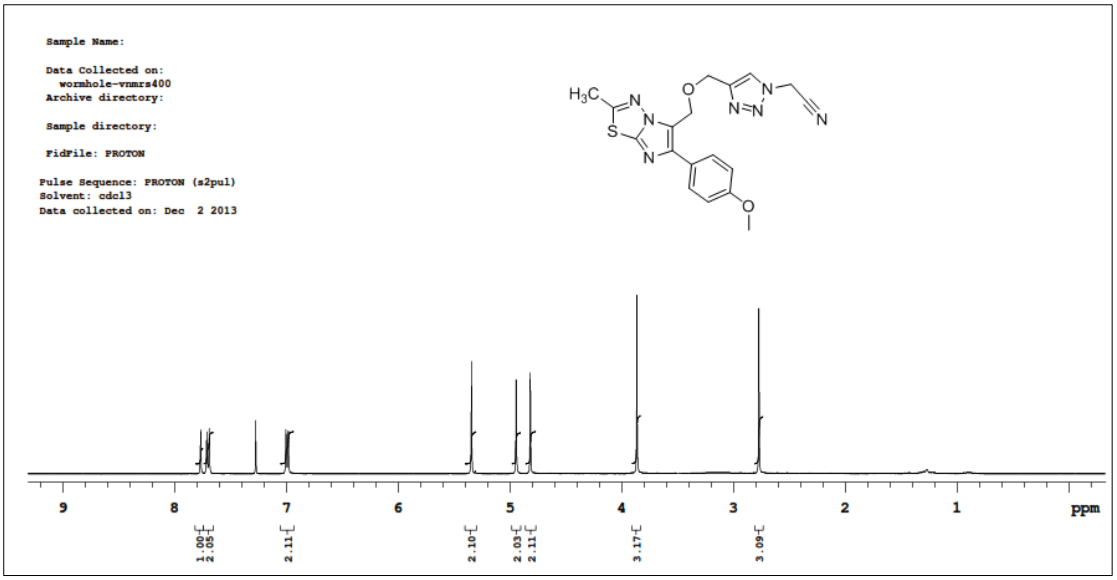


Figure 3.6 ^{1}H NMR spectrum of **T32**

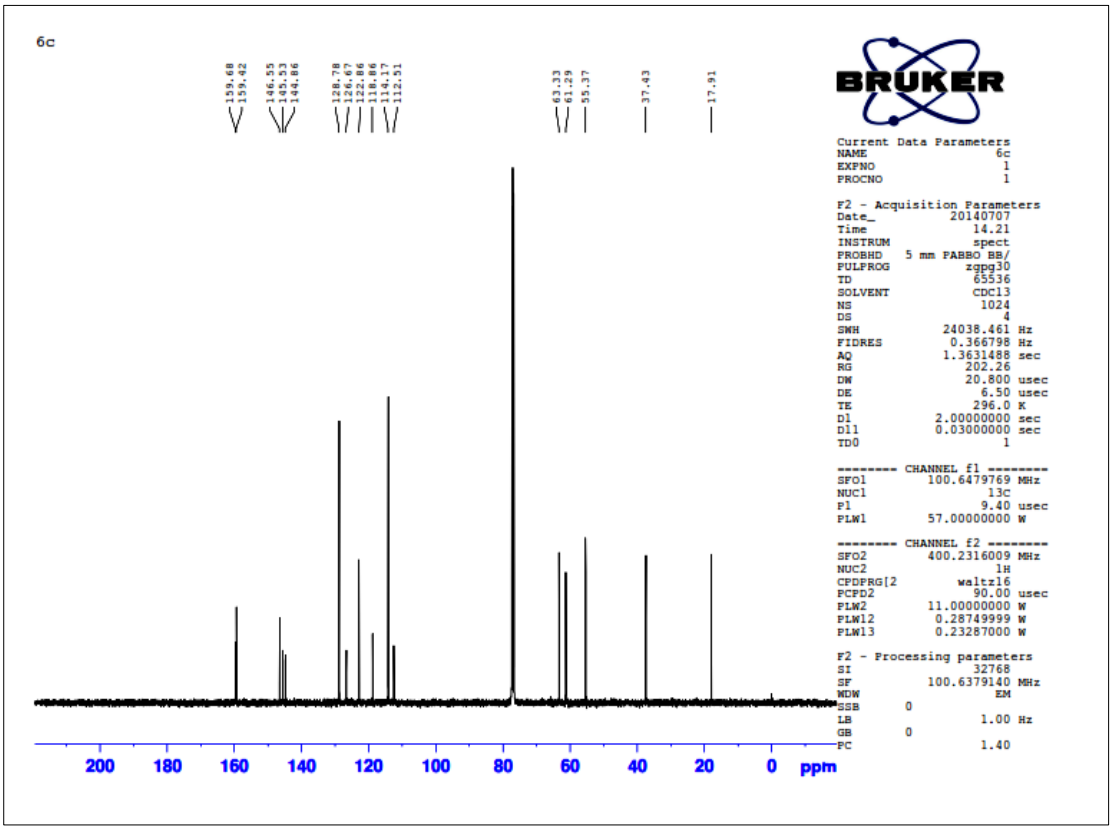


Figure 3.7 ^{13}C NMR spectrum of **T32**

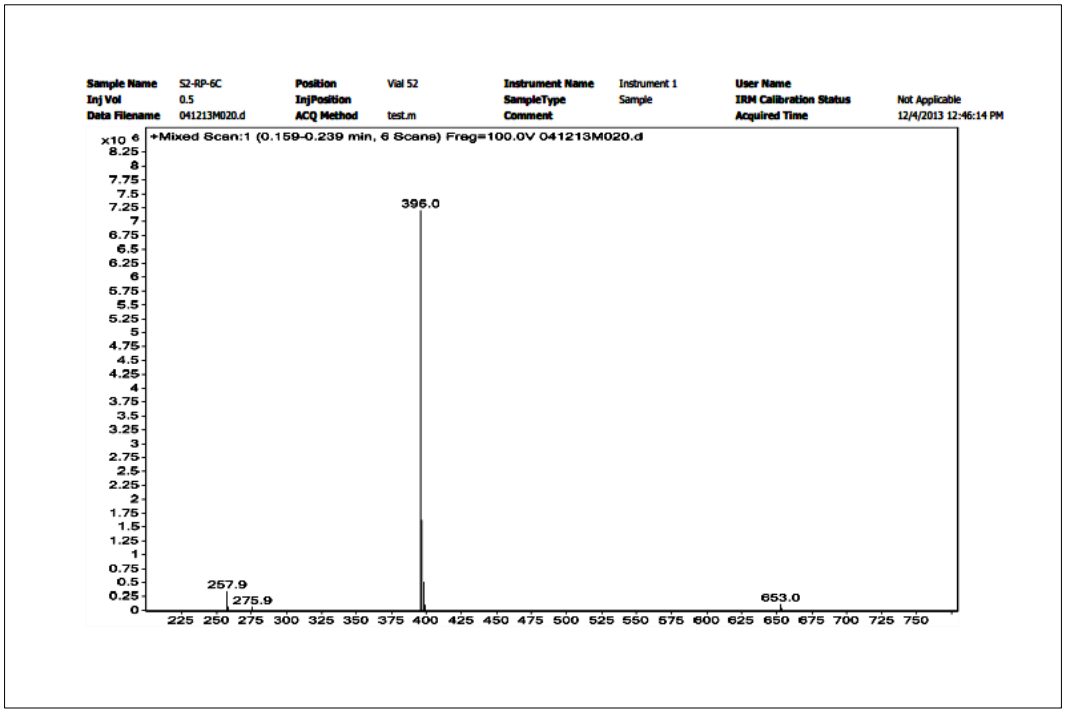


Figure 3.8 Mass spectrum of **T32**

Appearance of a broad singlet in the region δ 13.2-13.6 ppm in the ^{1}H NMR spectrum of **T49** confirms the presence of a carboxylic acid group in the molecule. The characterization data of all target compounds are given in the experimental part while their substitution pattern, yield and solubility of target compounds are tabulated in **table 3.1**.

3.5.2 Single crystal X-ray crystallography studies

The three dimensional structure of one of the target compounds, **T32** was evidenced by SCXRD studies. Single crystal of **T32** was grown from methanol and chloroform (1:1) solvent mixture by the slow evaporation of solvent at RT. A crystal of suitable size was mounted and single-crystal data was collected at RT. The molecule crystallises in the triclinic system with P-1 space group. The crystal structure (ORTEP diagram) of the compound is shown in **figure 3.9**. The crystal data and measurement details for compound **T32** are given in **table 3.2**.

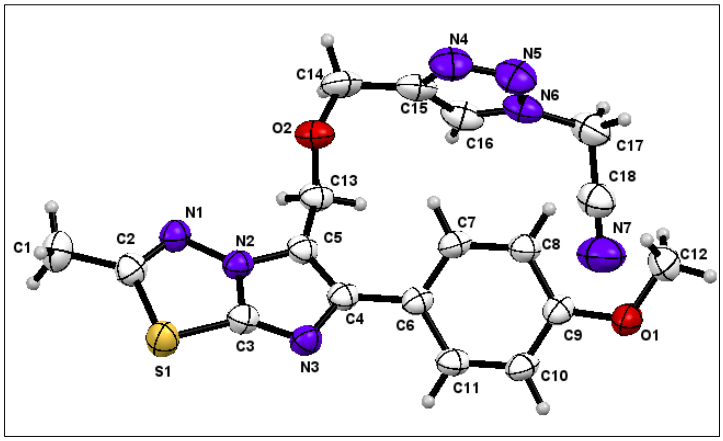


Figure 3.9 ORTEP diagram showing the X-ray crystal structure of compounds **T32**.

Table 3.2 Crystal data and measurement details for compound **T32**.

Parameters	**Crystal data**
Empirical formula	$C_{18}H_{17}N_7O_2S$
Formula weight	395.45
Crystal system	Triclinic
Space group	P-1
a (Å)	7.664(3)
b (Å)	8.644(3)
c (Å)	28.420(10)
Volume ($Å^3$)	1790.3(11)
Angle α, β, γ	85.80(2), 89.38(2), 72.46(19)
Z	4
F_{000}	824
μ (mm−1)	0.213
Temperature (T)	296k
Radiation wavelength (Å)	0.71073
Radiation type	Mo Kα
Radiation source	Mo
CCDC number	1059072

3.5.3 *In vitro* antimycobacterial activity

All the target molecules (**T30T49**) were screened against *Mtb* H37Rv (ATCC27294) using agar dilution method. The MIC values in μg/mL of **T30-T49** along with those of standard drugs for comparison are presented in **figure 3.10**. The MIC values are in the range 3.125 - 50.0 μg/mL. It is evident that among twenty compounds, **T35** and **T43** show potent antiTB activity with MIC of 3.125 μg/mL. The MIC of these two compounds is comparable with that of the standard drug, ethambutol. Compound **T45** showed moderate inhibition activity with MIC of 6.25 μg/mL. The nature of the substituent on ITD (R^1) and 1,2,3-triazole (R^2) rings affect the activity of the compounds. Most of the chloro or methoxy substituted derivatives exhibited superior activity than their methyl substituted analogues. All the methyl substituted derivatives (**T36-T41**) showed MIC ≥ 25 μg/mL irrespective of the nature of the substituent at R^2 whereas a few methoxy and chloro analogues exhibited lower MIC values (3.125 - 12.5 μg/mL). This general SAR signifies the contribution of chloro/methoxy substituent (R^1) towards the inhibition activity of the molecules. The presence of ethyl or benzyl groups on the 1,2,3-triazole ring enhances the inhibition activity of the molecules, which is evident by the significant activity shown by compounds **T43** and **T35**. Further, methyl analogues (**T37** and **T41**) with these substituents also exhibited moderate activity (MIC= 25 μg/mL). Other substituents like CH_2CN, CH_2COOEt and 4-fluorobenzyl on the 1,2,3-triazole ring also contributed in enhancing the activity of the molecules. The 4-fluorobenzyl derivatives (**T34**, **T40** and **T47**) are either equipotent or two fold more potent than respective 2-fluorobenzyl analogues (**T33**, **T39** and **T46**) which reveals the dependence of the activity on the position of the fluoro substitution. The hydrolysis of the ester functionality on the 1,2,3-triazole ring (**T42**, MIC = 12.5 μg/mL) to a carboxylic acid group substantially decreased the potency of the molecule (**T49**, MIC = 50 μg/mL). The substitution of different groups at R^1 and R^2 affect the lipophilicity of the molecules (**table 3.1**). However, did not observe a general relationship between lipophilicity and the activity of the compounds. Nevertheless, it is interesting to compare the log*P*/*C*log*P* values of the active molecules and to account for the activity based on their lipophilicity. The *C*log*P* values of the active molecules are in the range 2.2-2.9 with log*P* values in the range 4-5.5. Though ethyl and benzyl substituents (R^2)

are found to enhance the activity, ethyl derivative **T31** and benzyl derivative **T48** showed only a moderate activity (MIC = 25 µg/mL) which could be rationalised based on the lower *C*log*P* value for **T31** and a higher value for **T48**.

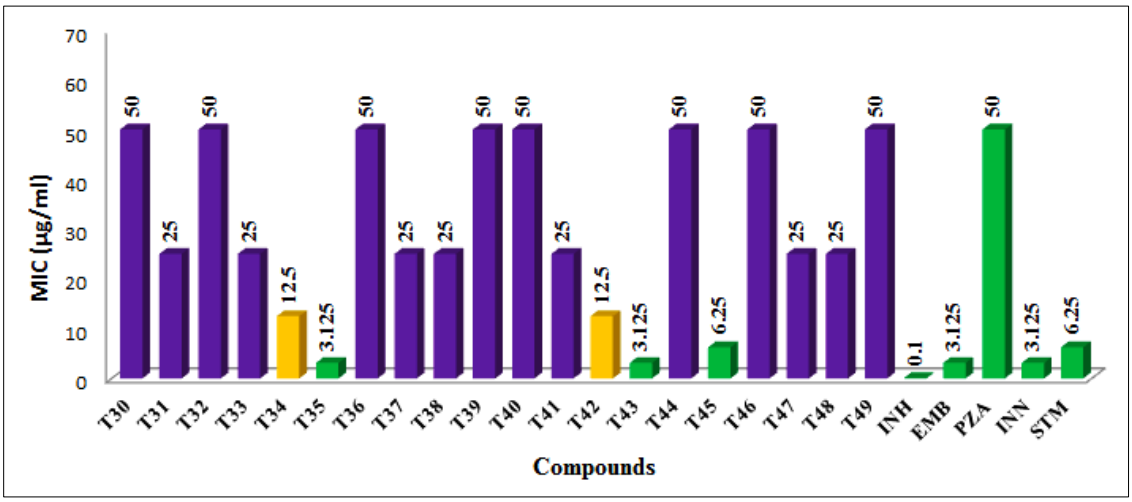


Figure 3.10 AntiTB activity of **T30-T48** and **T49** against *Mtb* H37RV.

A similar relationship between lipophilicity and activity was observed in other active derivatives as well which contain substituents like CH_2COOEt and 4-fluorobenzyl group. Only those derivatives (viz **T34** and **T42**), whose lipophilicity falls in the above mentioned values have shown good activity whereas their analogs with higher (**T47**) or lower (**T48**) values have shown relatively a lower activity. So it may be concluded that in addition to the structural features, which play a major role, the lipophilicity factor also influences the inhibition activity of the molecules. This information on structure/lipophilicity-activity relationship explored in the present study could be helpful in further structural modification and development of new 1,2,3-triazole-ITD hybrids as potent antitubercular agents.

3.5.4 *In vitro* antibacterial activity

The *in vitro* antibacterial activity of synthesized compounds **T30-T49** was tested using the disc diffusion method (Isenberg, 1992) and zone of inhibition was measured in mm. All the compounds were screened against three bacterial strains viz. *S. aureus*, *P. aeruginosa* and *E. coli* using ciprofloxacin as the standard drug. The compounds were dissolved in DMSO with two concentrations (75 µg/mL and 50

µg/mL). Compounds **T43** and **T45** demonstrated significant inhibition activity (**table 3.3**) against all three bacterial strains at both concentrations.

Table 3.3 Antibacterial activity of target compounds (**T30-T49**).

Compounds	***E. coli***		***S. aureus***		***P. aeruginosa***	
Cocn. in µg/ml	75	50	75	50	75	50
T30	-	-	10±0.1	08±0.3	12±0.1	10±0.2
T31	14±0.2	12±0.3	14±0.3	10±0.3	12±0.2	10±0.4
T32	12±0.1	10±0.1	-	-	12±0.1	10±0.1
T33	-	-	-	-	13±0.2	10±0.4
T34	14±0.4	12±0.4	-	-	12±0.3	08±0.1
T35	16±0.3	13±0.2	17±0.1	14±0.3	10±0.3	08±0.4
T36					12±0.1	10±0.2
T37	-	-	10±0.3	-	17±0.2	10±0.1
T38	12±0.4	10±0.3	-	-	12±0.1	10±0.2
T39	14±0.1	-	-	-	10±0.2	-
T40	-	-	-	-	12±0.1	08±0.1
T41	10±0.1	09±0.4	-	-	12±0.2	08±0.3
T42	14±0.4	11±0.2	16±0.1	12±0.1	14±0.3	08±0.1
T43	24±0.3	19±0.2	33±0.2	26±0.2	18±0.1	14±0.2
T44	13±0.4	10±0.3	-	-	12±0.3	10±0.1
T45	22±0.1	18±0.4	30±0.1	28±0.2	15±0.2	12±0.1
T46	08±0.1	04±0.2	08±0.2	06±0.2	06±0.1	04±0.2
T47	10±0.2	08±0.1	07±0.1	04±0.1	12±0.2	10±0.1
T48	-	-	–	–	10±0.2	08±0.2
T49	14±0.1	10±0.2	08±0.2	05±0.1	14±0.2	10±0.1
Control	00	00	00	00	00	00
INN	32±0.2	27±0.2	26±0.1	21±0.2	21±0.2	18±0.1
INN: Ciprofloxacin; –: inhibition not detected; control: DMSO						

3.5.5 *In vitro* cytotoxicity

The *in vitro* cytotoxicity of the active compounds (MIC ≤12.5 µg/mL) were evaluated by MTT assay against NIH 3T3 mouse embryonic fibroblasts cell line. The graphical representation of the cell growth inhibition by the compounds at a concentration of 50 µg/mL is shown in **figure 3.11**. The compounds did not show any toxicity to the cell line signifying the lack of general cellular toxicity.

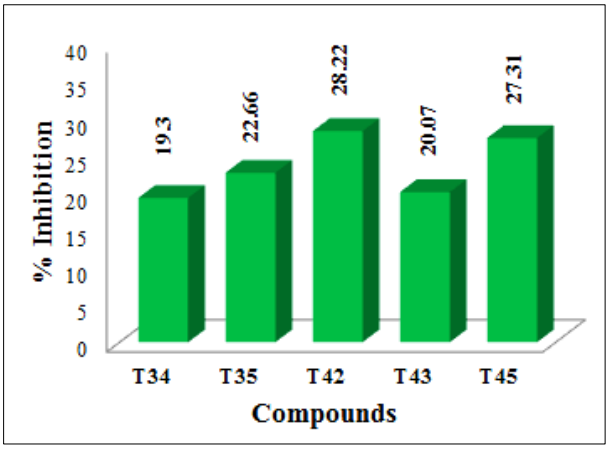


Figure 3.11 Growth inhibition activity of active compounds (at a concentration of 50 µg/mL) against NIH 3T3 cell line.

3.5.6 Molecular docking studies

Compounds which showed promising antitubercular activity (MIC ≤ 6.25 µg/mL) were taken for molecular docking studies to check the binding interactions with the enzyme. The molecules were docked with in the active sites of InhA (PDB code: 1P44) using Glide 6.6 (Schrodinger, 2015-1) package. The ligands from the crystal structure of the enzyme-ligand complexes were rebuilt and redocked to validate the docking procedure. The docking poses of molecules **T35** and **T43** are shown in **figure 3.12**. Compound **T35** showed the docking score of -8.346 and exhibited *pi-pi* stacking interaction with residues Phe 149. The most potent antiTB compound **T43** with a docking score of -8.248 showed *pi-pi* stacking interaction with residues Phe 149. The active compound **T45** with MIC of 6.25 µg/mL has shown interaction with amino acid residue Tyr 158.

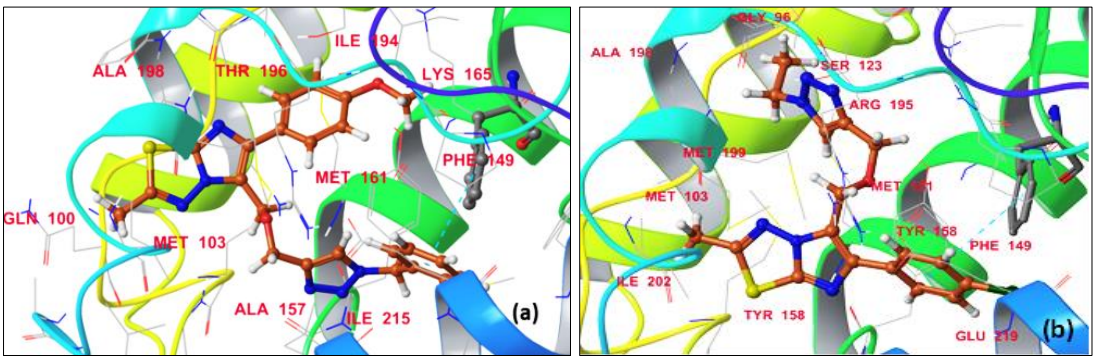


Figure 3.12 The docking poses of some active compounds with target enzyme InhA (a) **T35** with InhA; (b) **T43** with InhA.

3.6 CONCLUSION

- A series of twenty new 1,2,3-triazole-ITD hybrids (**T30-T49**) were designed by molecular hybridization approach and were synthesized using a click chemistry reaction.
- These compounds were characterized by ^{1}H NMR, ^{13}C NMR, mass spectroscopic techniques and elemental analysis.
- In the antitubercular screening, compounds **T35** and **T43** exhibited significant activity against the growth of *Mtb* with MIC of 3.125 µg/mL. A moderate activity with MIC of 6.25 µg/mL was observed for compound **T45**.
- The SAR revealed that the 4-chlorophenyl group contributes significantly in enhancing the inhibition activity of the molecules.
- Further, substituents like ethyl, benzyl, 4-fluorobenzyl, CH_2CN and CH_2COOEt on the 1,2,3-triazole ring enhance the antiTB activity.
- Further, molecular docking studies showed that these molecules are good inhibitors of InhA.
- Also, none of the active molecules is toxic to a normal cell line. Hence, these compounds with significant antiTB activity could serve as promising lead molecules for further generation of potent antiTB agents.

Appendix 3.1

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

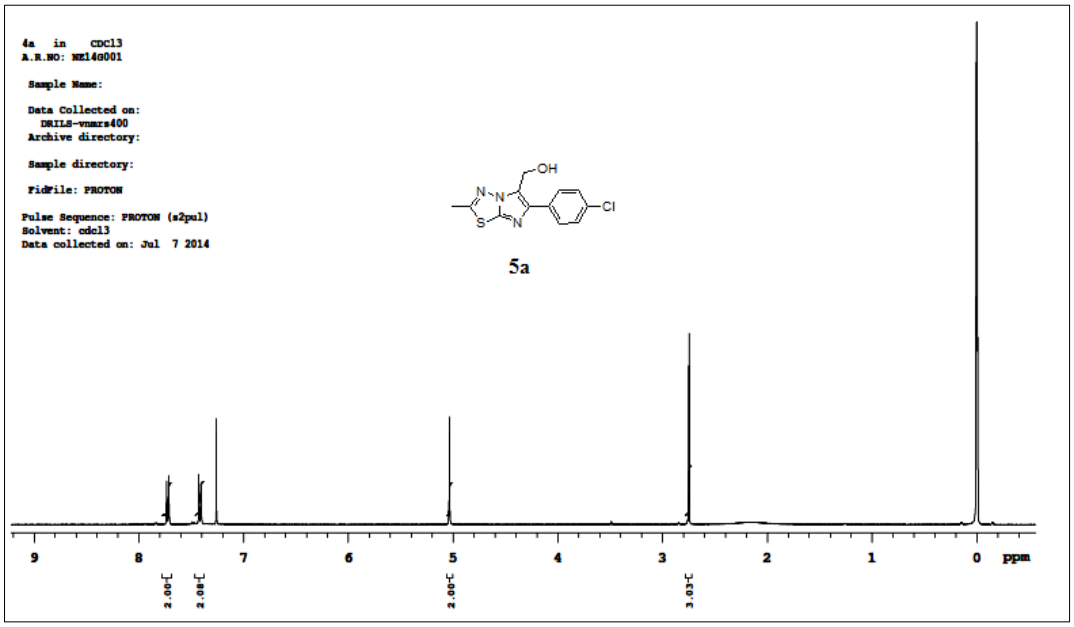


Figure 3.13 ^{1}H NMR spectrum of **5a**

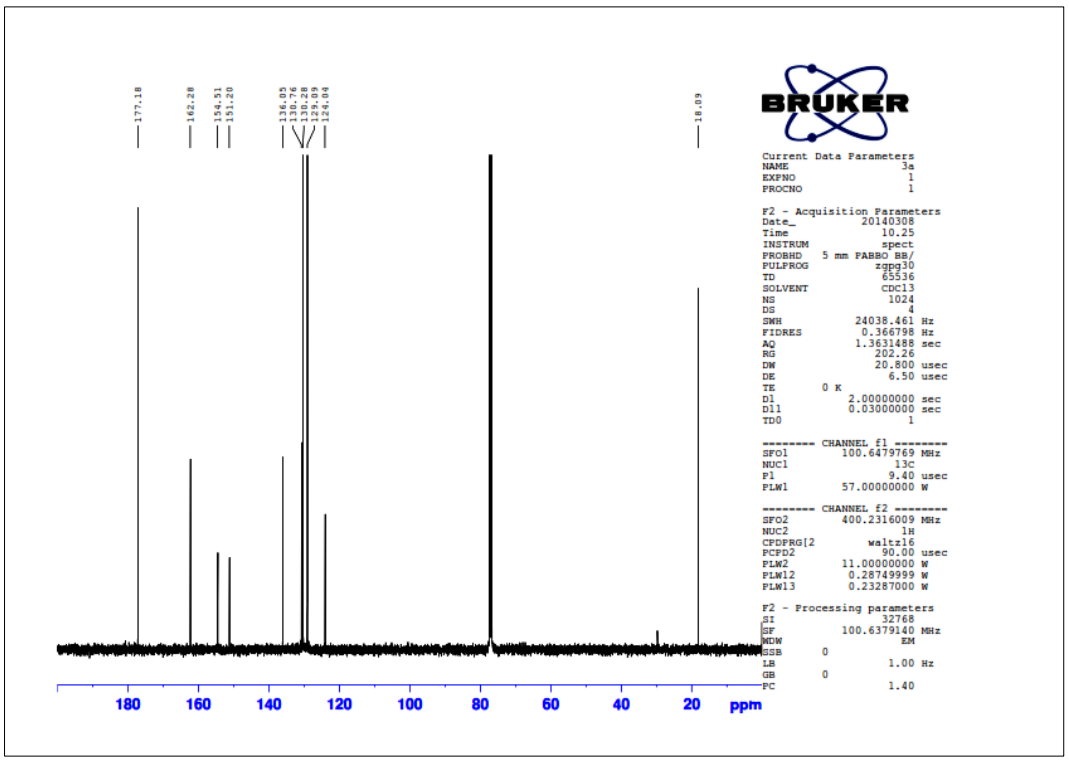


Figure 3.14 ^{13}C NMR spectrum of **5a**

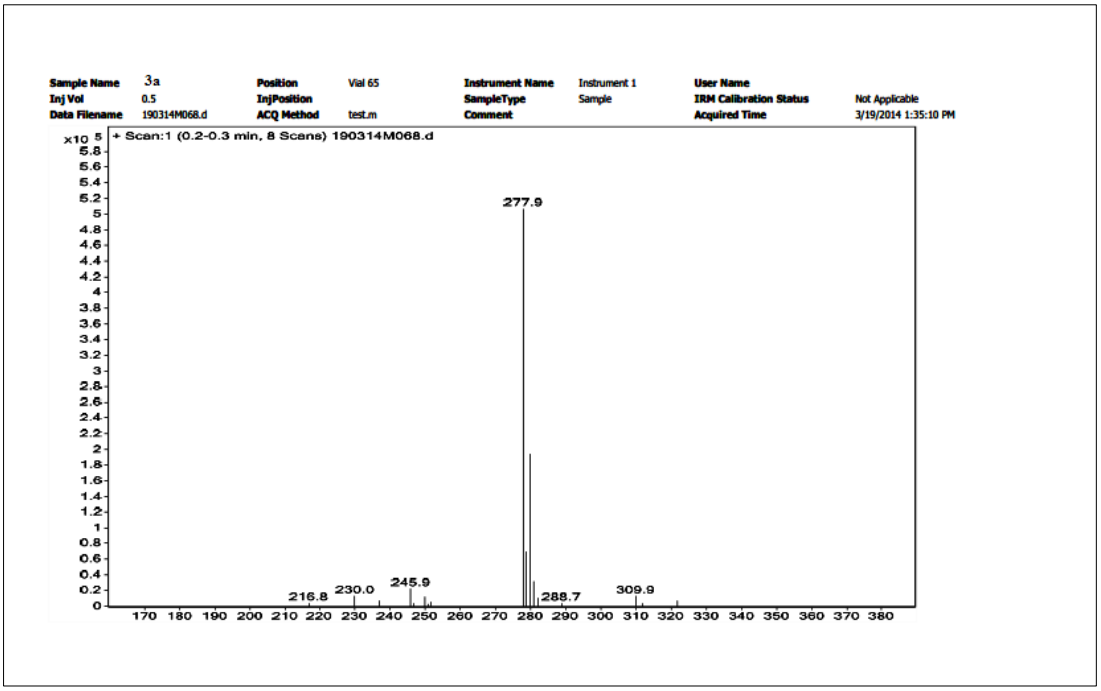


Figure 3.15 Mass spectrum of **5a**

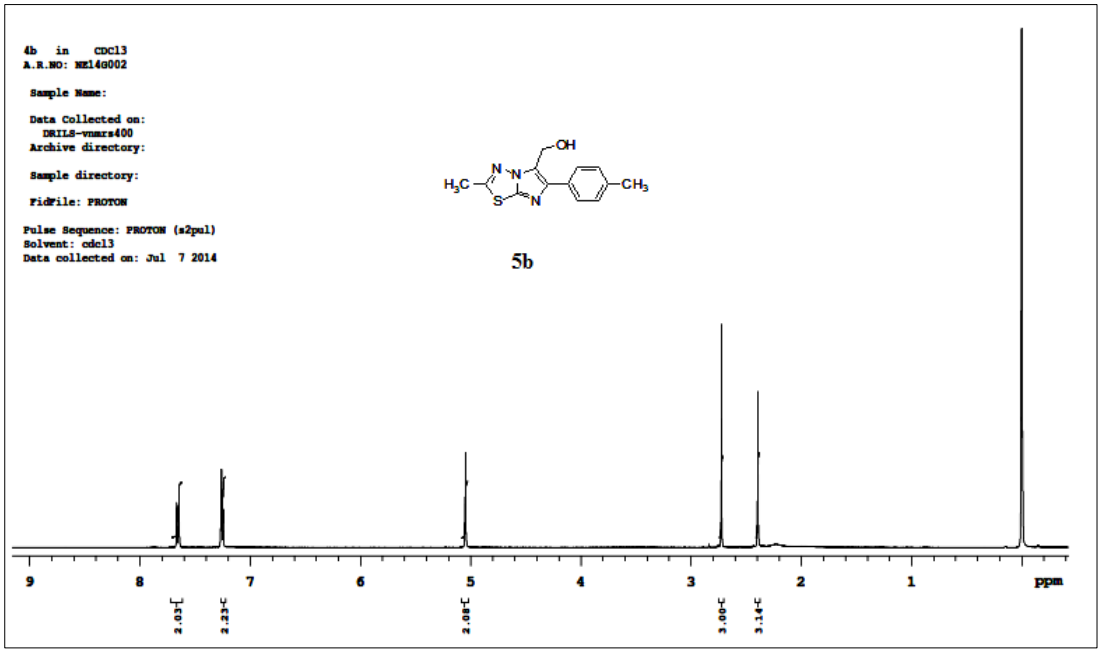


Figure 3.16 ^{1}H NMR spectrum of **5b**

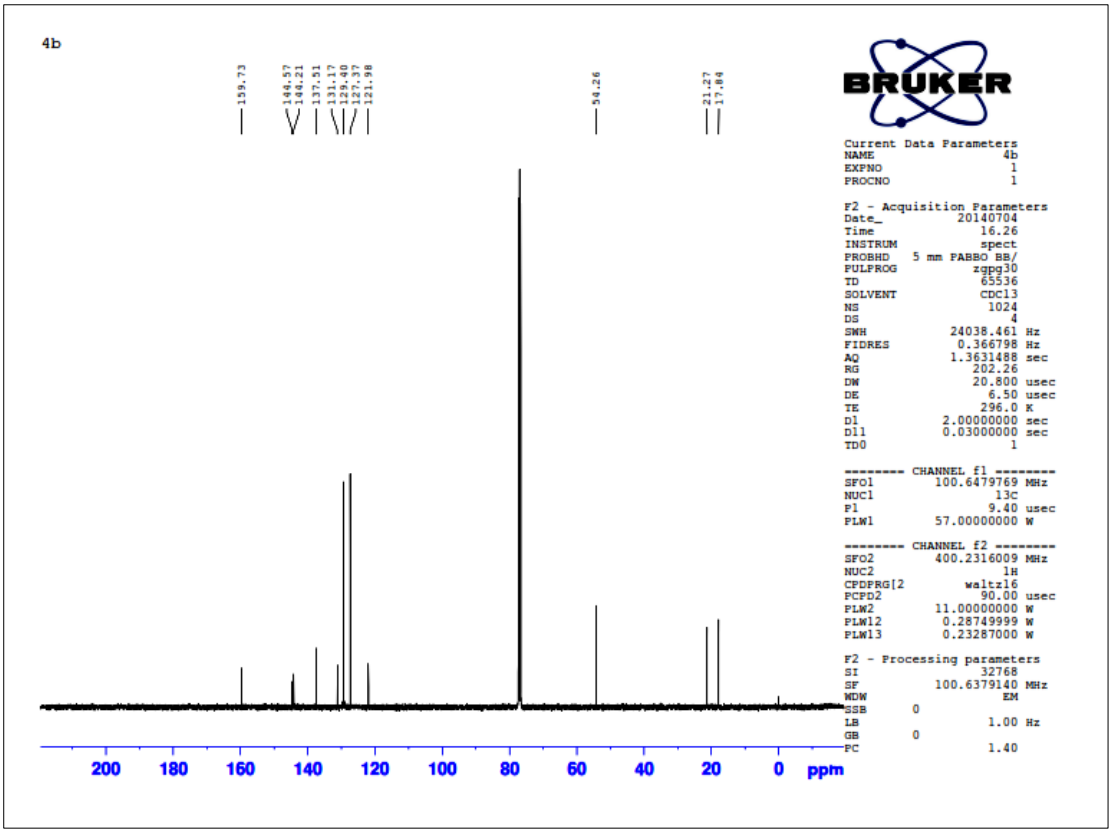


Figure 3.17 ^{13}C NMR spectrum of **5b**

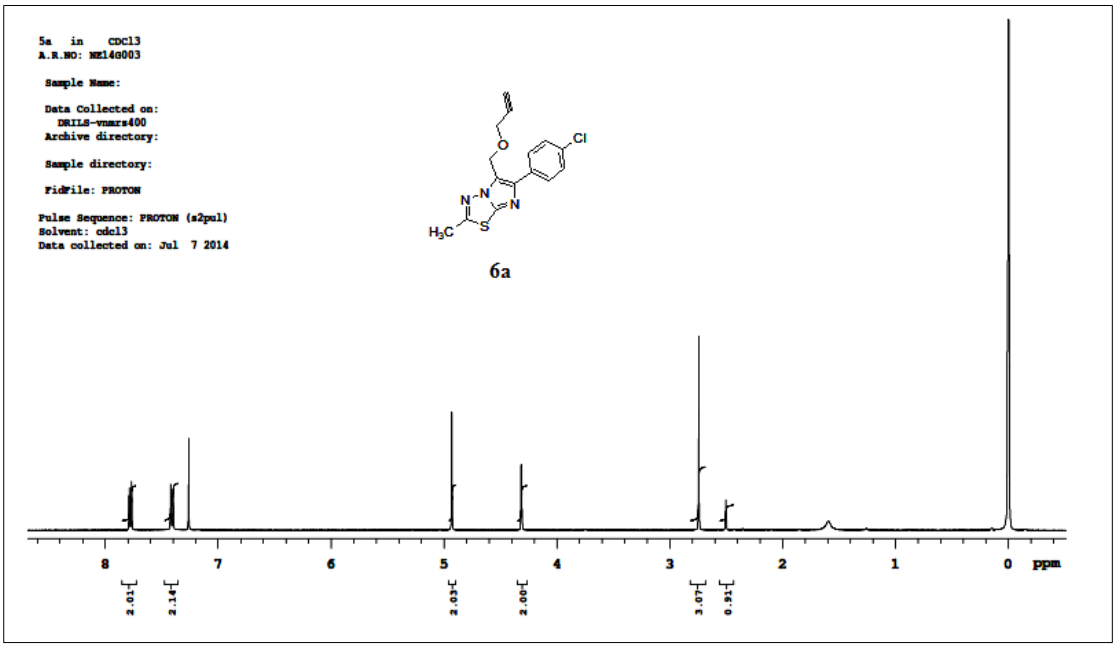


Figure 3.18 ^{1}H NMR spectrum of **6a**

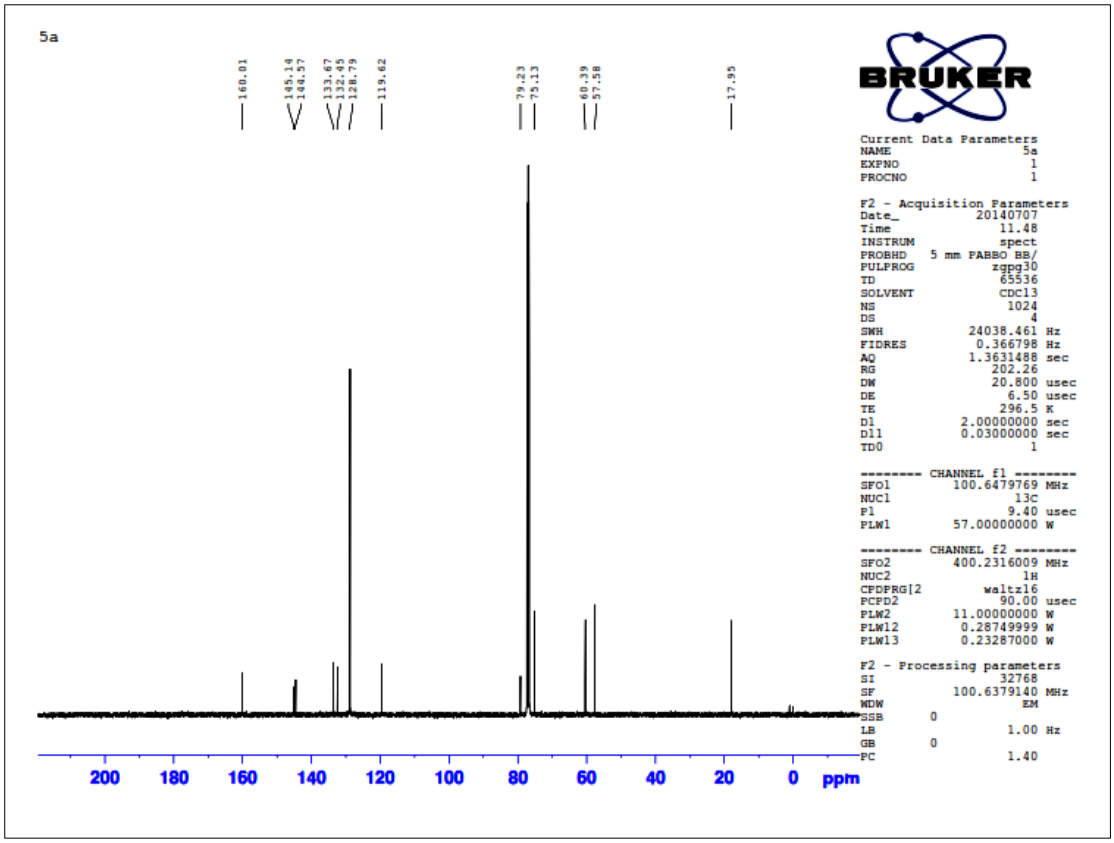


Figure 3.19 ^{13}C NMR spectrum of **6a**

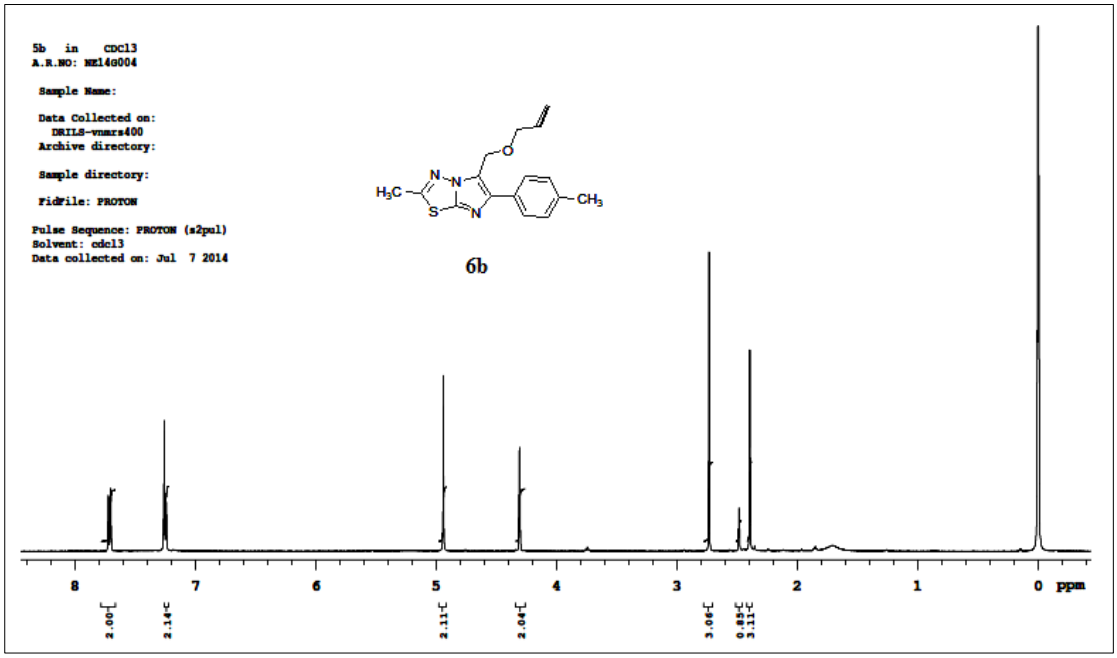


Figure 3.20 ^{1}H NMR spectrum of **6b**

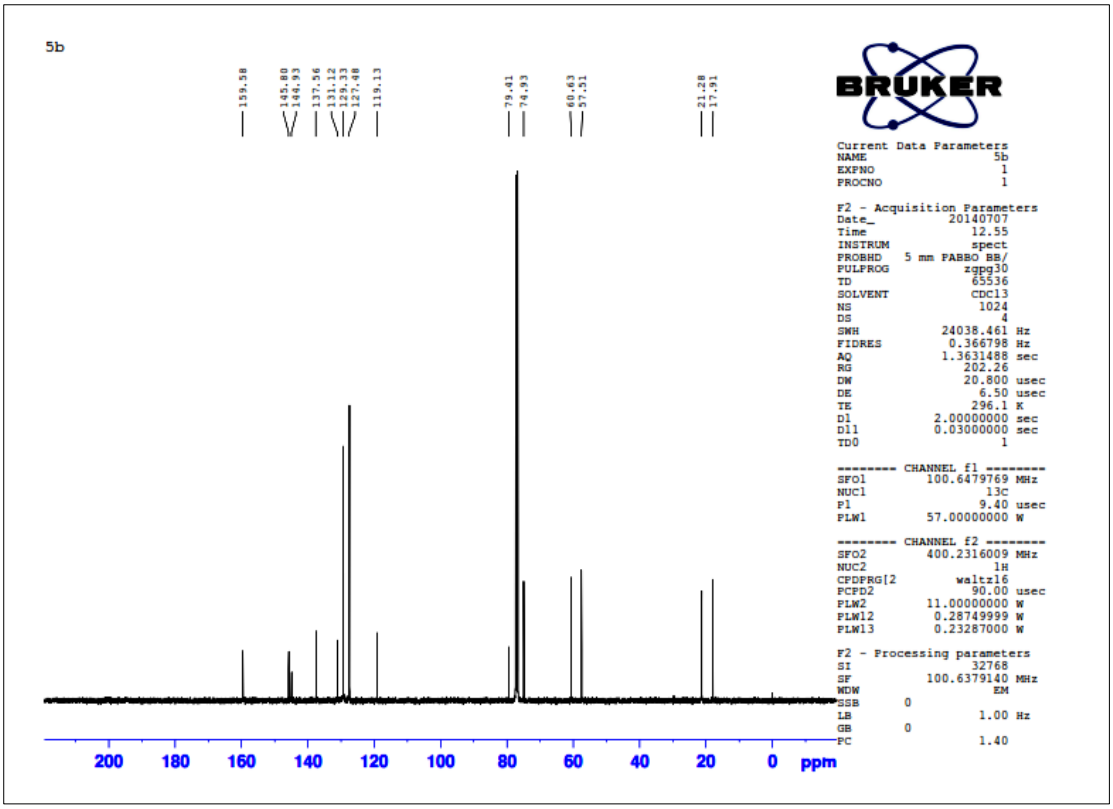


Figure 3.21 ^{13}C NMR spectrum of **6b**

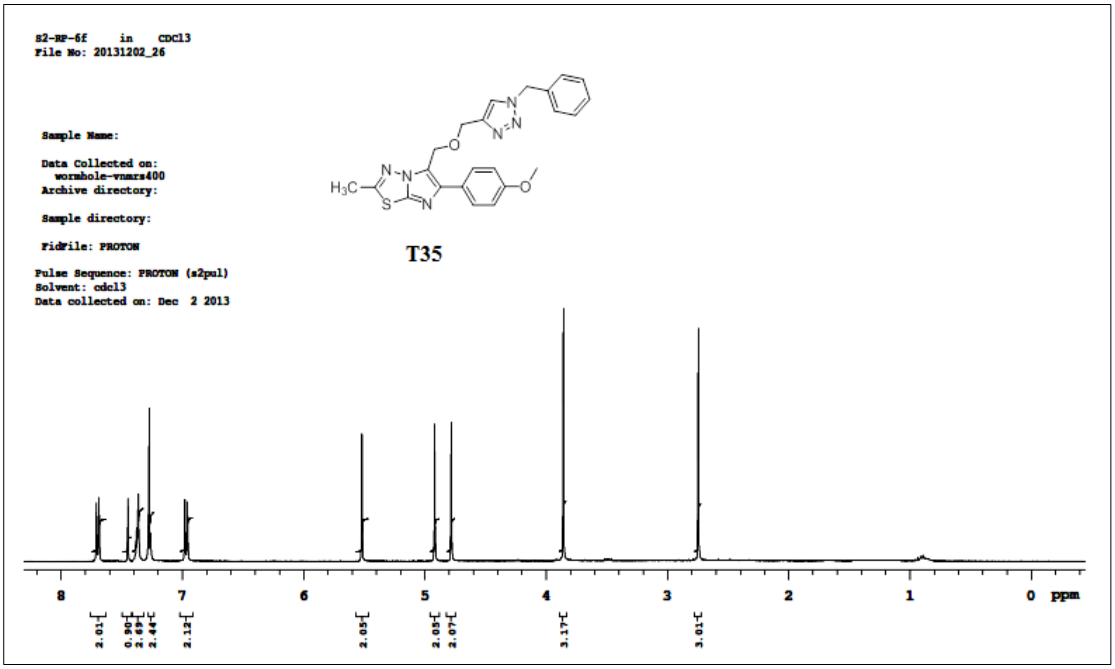


Figure 3.22 ^{1}H NMR spectrum of **T35**

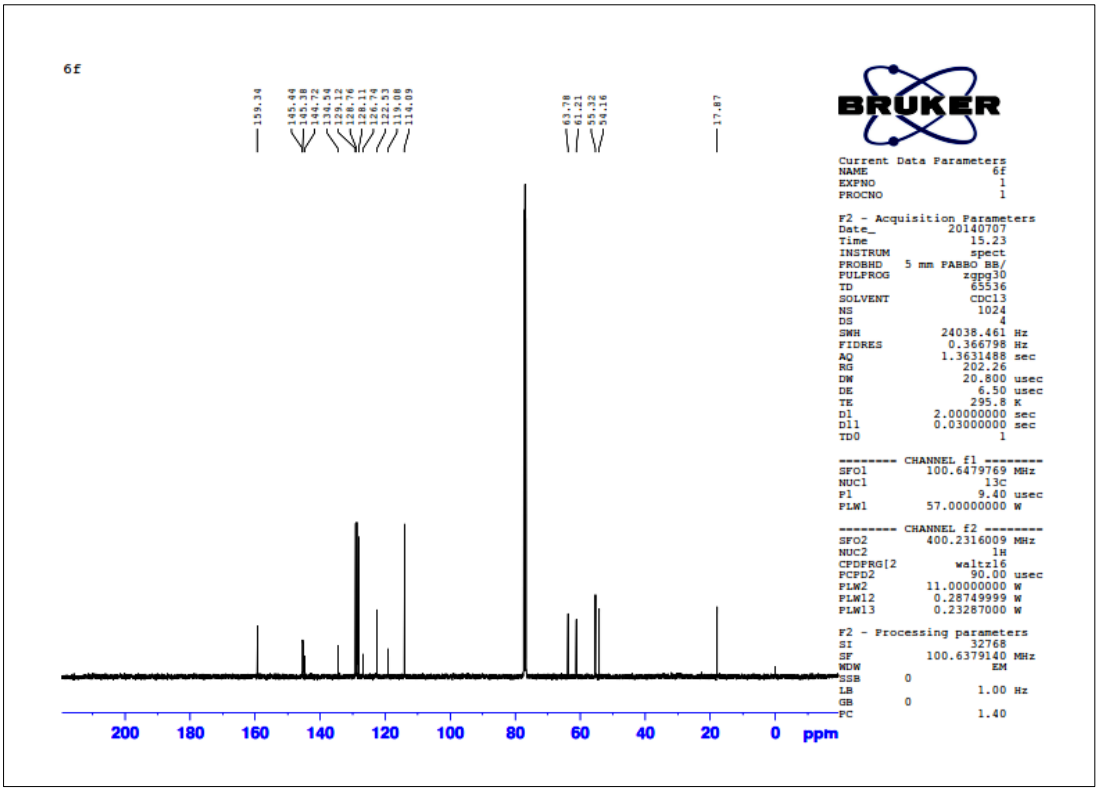


Figure 3.23 ^{13}C NMR spectrum of **T35**

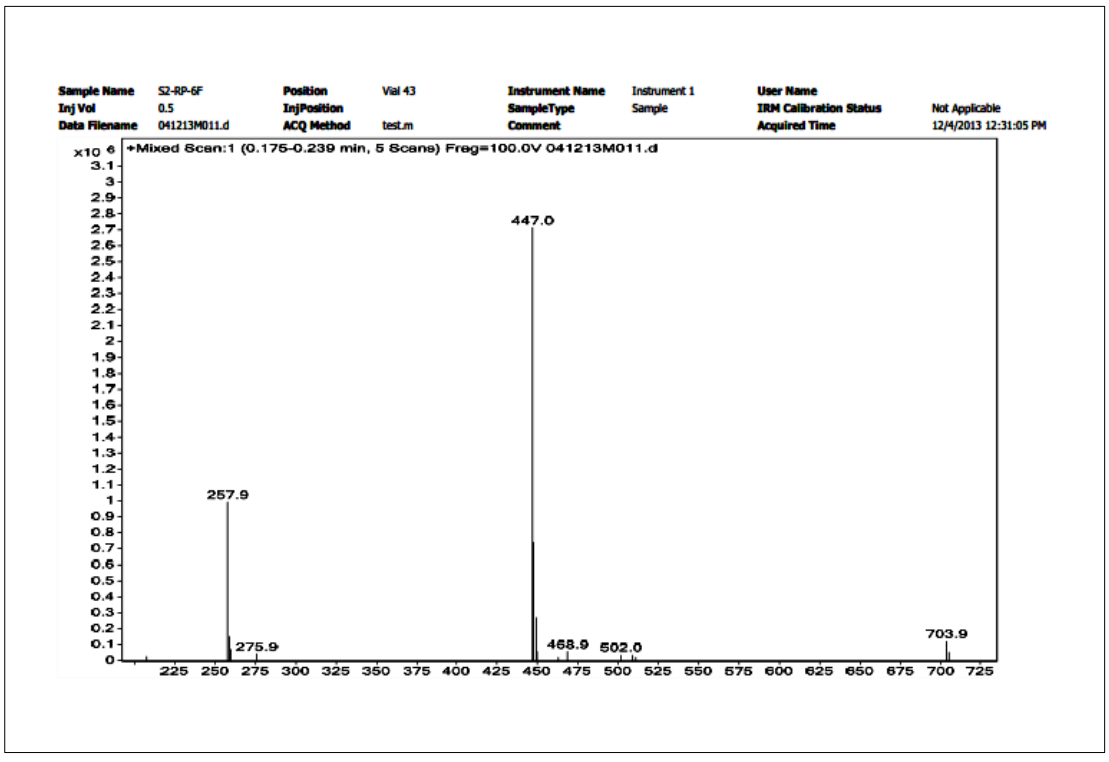


Figure 3.24 Mass spectrum of **T35**

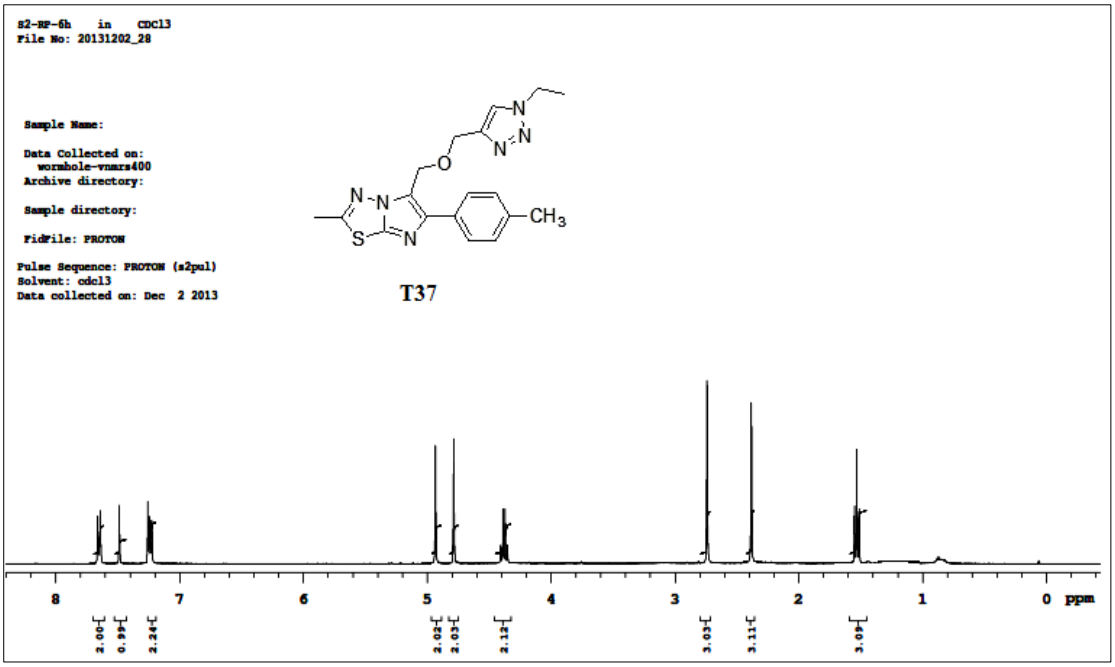


Figure 3.25 ^{1}H NMR spectrum of **T37**

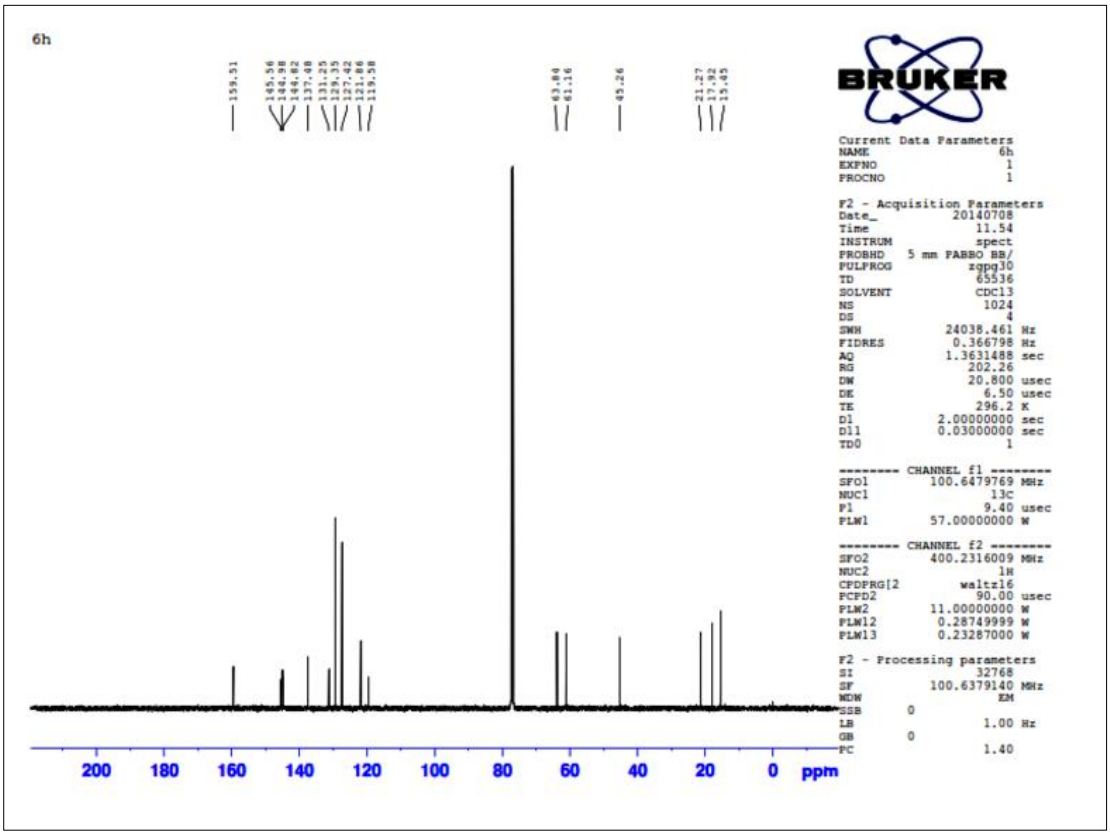


Figure 3.26 ^{13}C NMR spectrum of **T37**

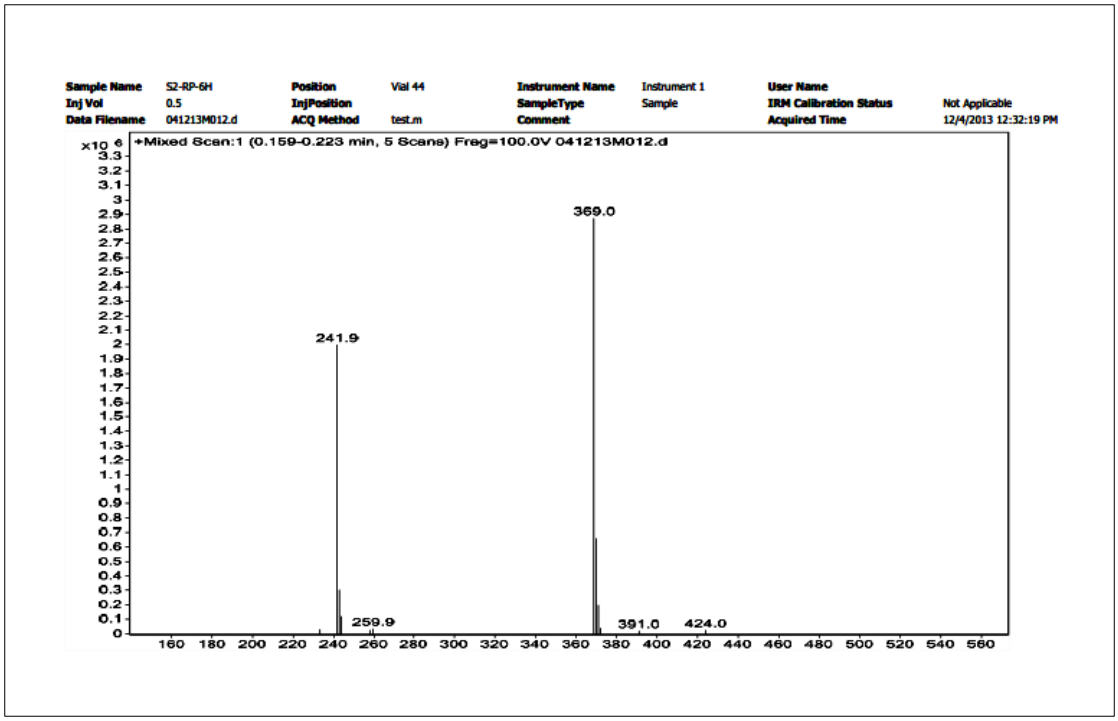


Figure 3.27 Mass spectrum of **T37**

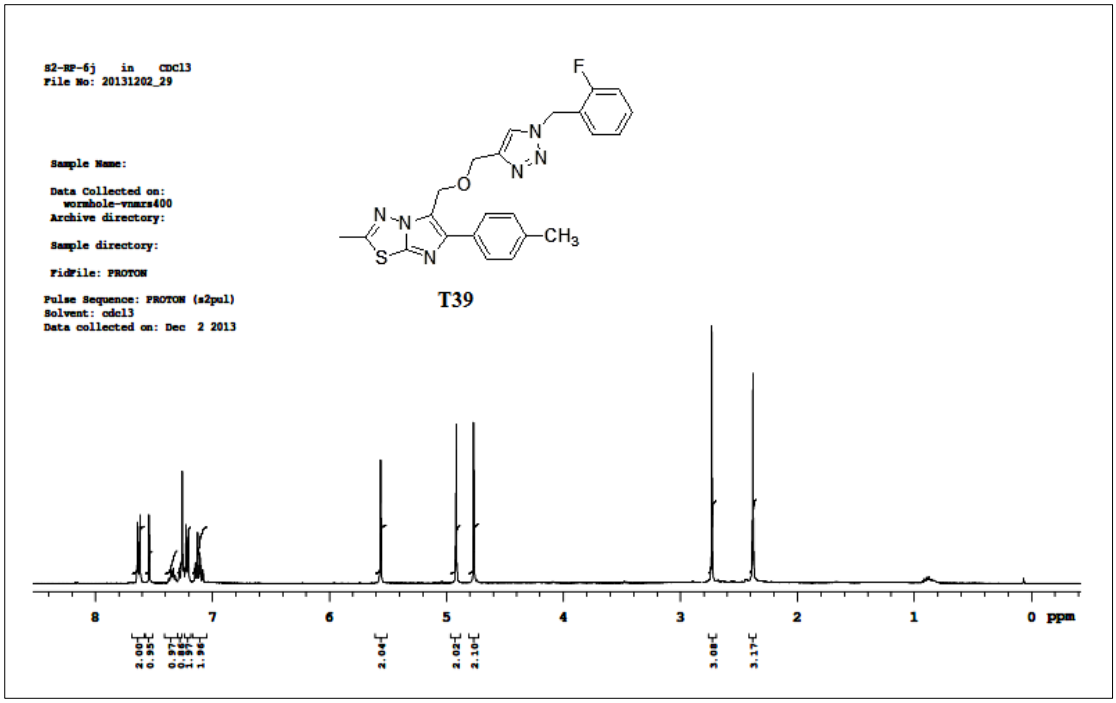


Figure 3.28 ^{1}H NMR of spectrum of **T39**

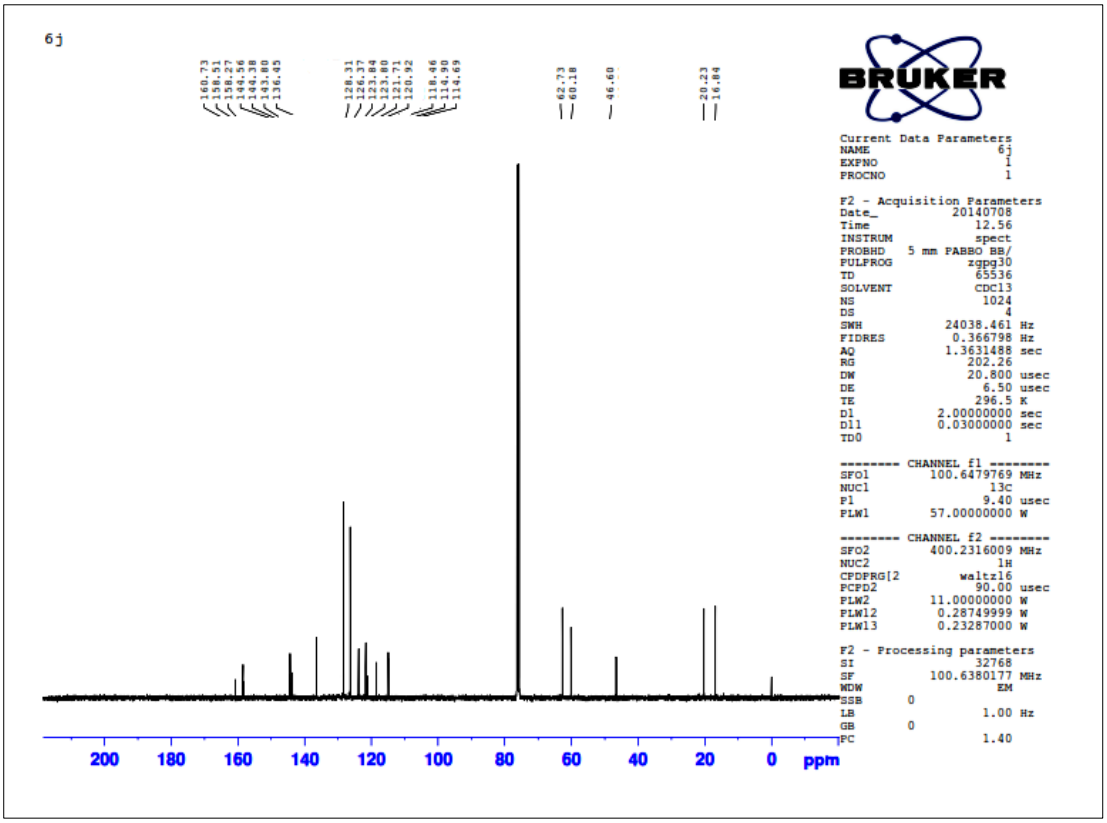


Figure 3.29 ^{13}C NMR spectrum of **T39**

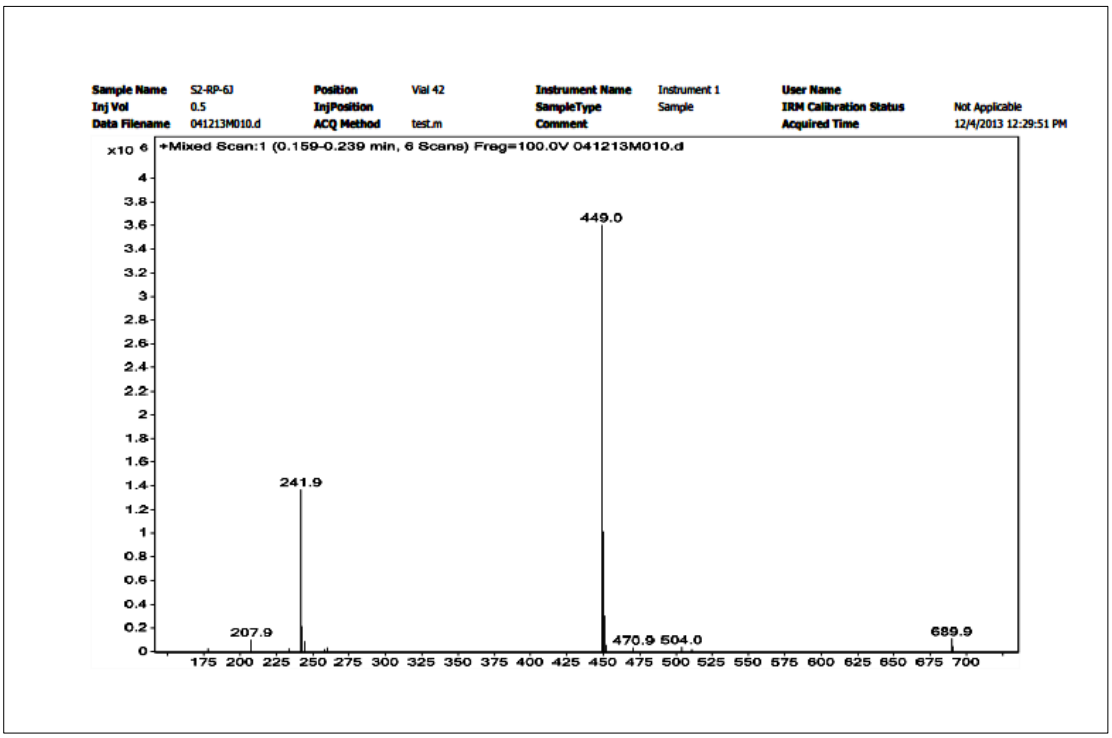


Figure 3.30 Mass spectrum of **T39**

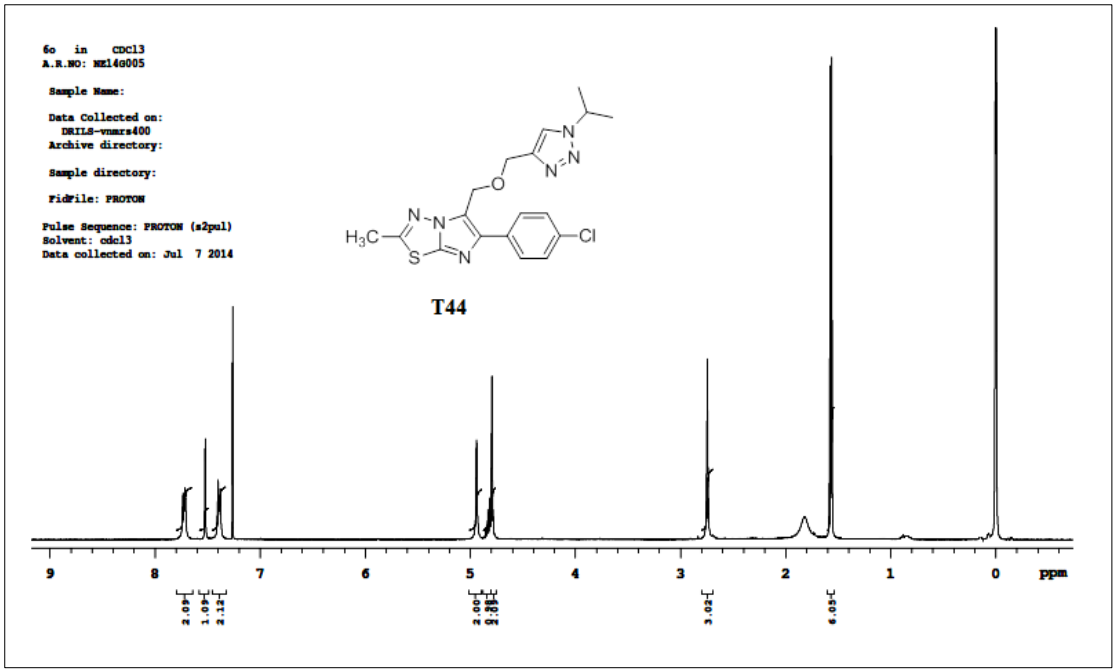


Figure 3.31 ^{1}H NMR spectrum of **T44**

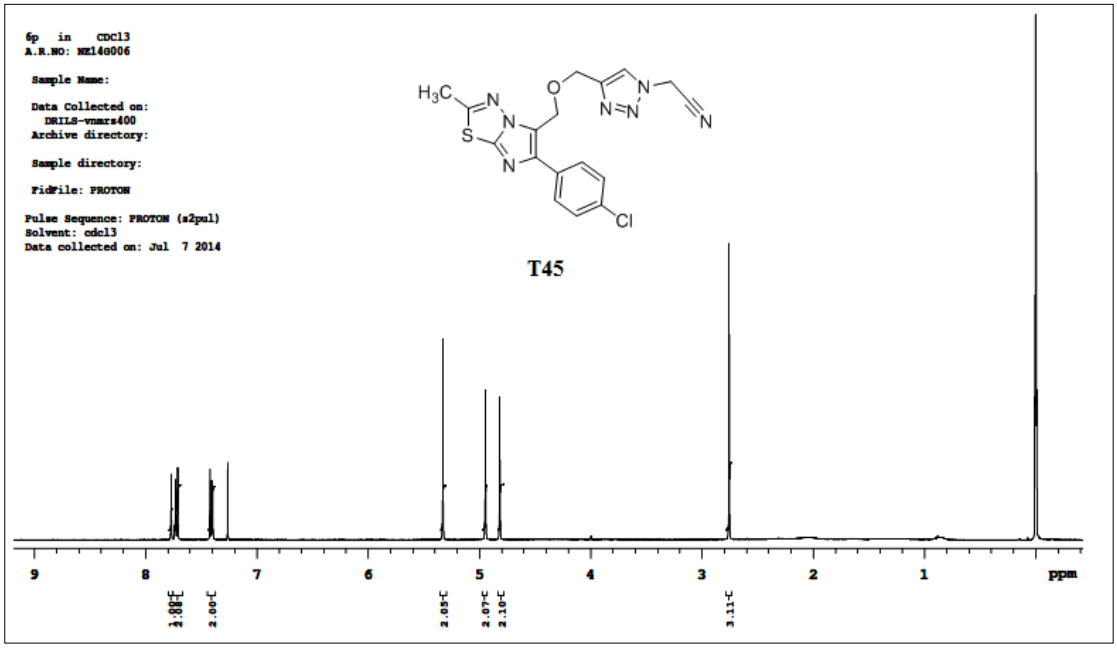


Figure 3.32 ^{1}H NMR spectrum of **T45**

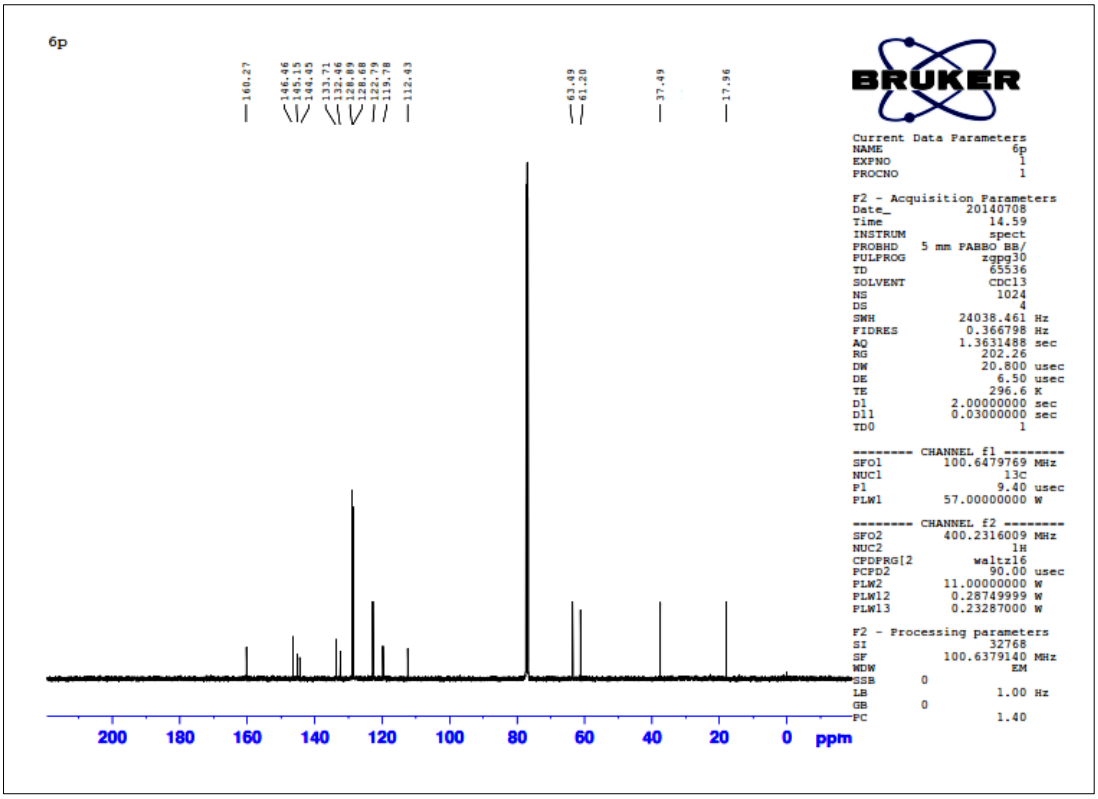


Figure 3.33 ^{13}C NMR spectrum of **T45**

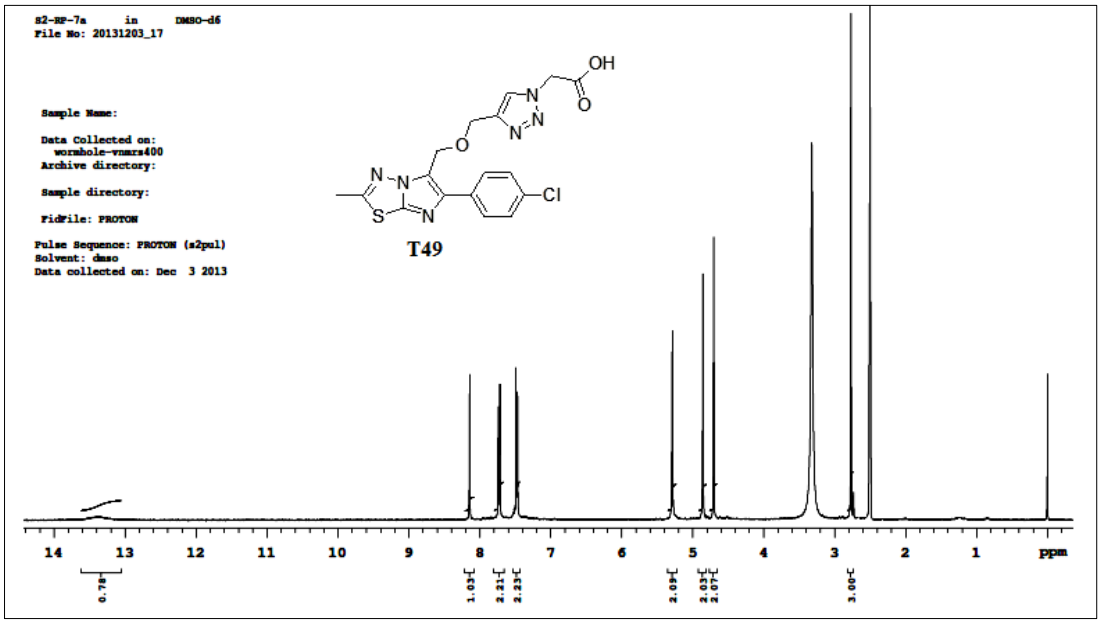


Figure 3.34 ^{1}H NMR spectrum of **T49**

CHAPTER 4

IONIC LIQUID PROMOTED ONE-POT SYNTHESIS OF THIAZOLE- IMIDAZO [2,1-*b*][1,3,4]THIADIAZOLE HYBRIDS AND THEIR ANTITUBERCULAR ACTIVITY

Abstract

In this chapter, an efficient and facile synthetic route for the synthesis of thiazole-imidazo[2,1-b][1,3,4]thiadiazole hybrid derivatives has been elucidated. A detailed experimental protocol along with thorough analytical data of the molecules are presented in the chapter. It also comprises of in vitro antimycobacterial and antibacterial evaluation of all the target molecules.

4.1 INTRODUCTION

Thiazole derivatives are being considered as important pharmacophores in the development new antiTB leads against *Mtb* H_{37}Rv strain (Makam and Kannan, 2014; Meissner et al. 2013). In addition, the thiazole based molecules (Pitta et al. 2015; Garella et al. 2013) exhibit low toxic level which is evident also from the safety profile of some of the marketed drugs like nitazoxanide, tizoxanide, aztreonam, meloxicam and relutex. Further, some thiazolylhydrazone derivatives (I-III) exhibited remarkable growth inhibitory activity against *Mtb* (Makam et al. 2013; Shaikh et al. 2014). For instance, Ozadali et al. (2014) synthesized a series of thiazolyl hydrazone derivatives, among which compound III exhibited excellent activity with MIC_{MABA} of 1.03 μM. The ITD scaffold is a promising heterocyclic active pharmacophore possessing a broad spectrum of pharmacological activity (refer section 2.1). In our earlier studies (chapter 1 and 3), incorporated benzimidazole and 1,2,3-triazole moieties at position-5 of the ITD ring and evaluated the antitubercular activity of the hybrid molecules. Interestingly, most of the compounds exhibited significant activity against *Mtb* H37Rv strain. These results motivated us to incorporate other pharmacophores at position-5 of the ITD ring and to investigate the effect of the structural modification on the antitubercular activity of the molecules.

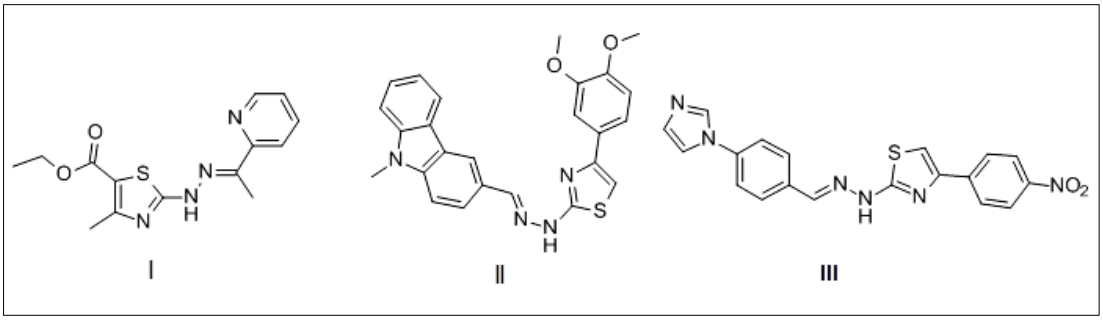


Figure 4.1 Thiazolylhydrazone based active antitubercular agents (I – III).

Further, the molecular design strategy which involves the hybridization of two active pharmacophores into a single molecular framework has become one of most promising approaches to develop potent antiTB agents. In this direction, we envisaged the integration of substituted thiazoles with the core ITD ring at position-5 of the ITD core through an amine-imine linkage. The target compounds, 1-((6-phenyl imidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)methylene)-2-(4-phenylthiazol-2-yl)hydrazines (**T50-T72**) were synthesized by a one-pot three-component synthesis in an IL medium.

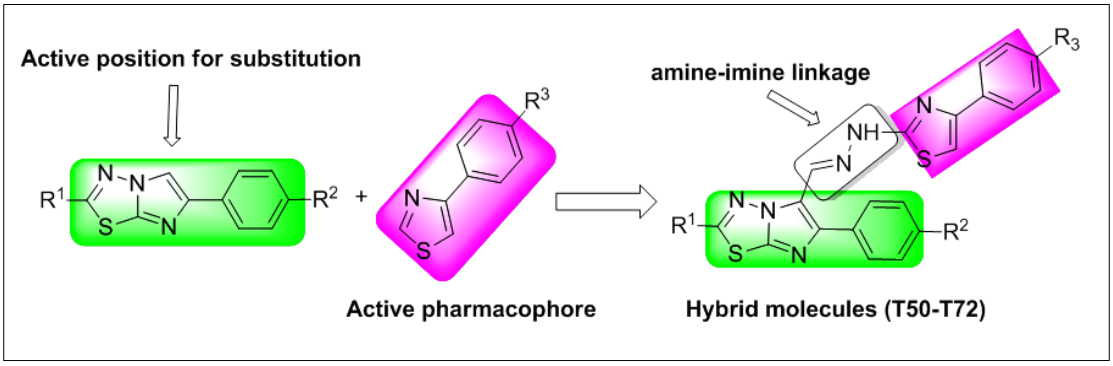
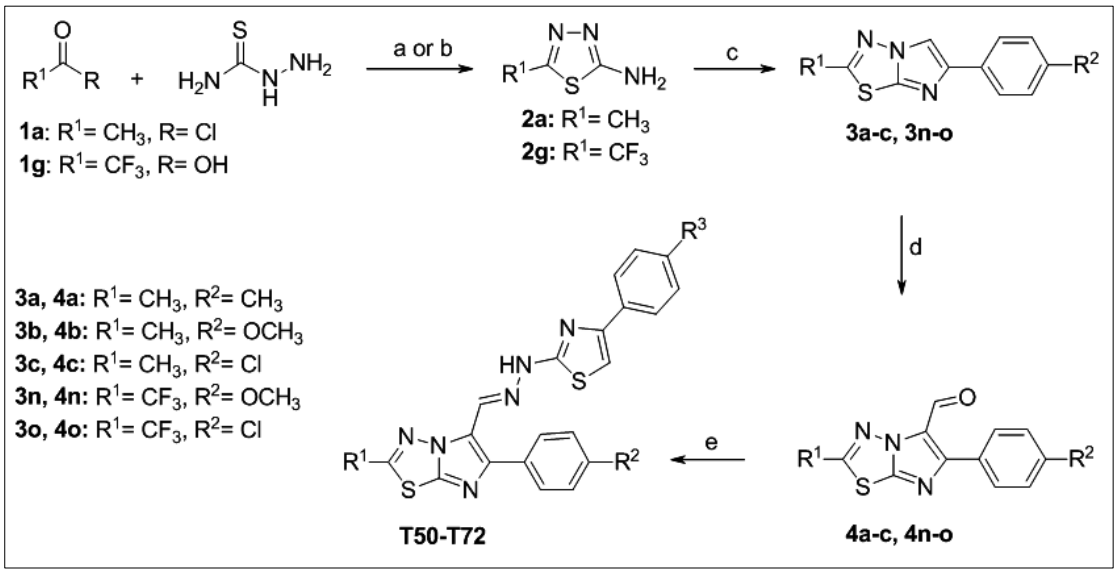


Figure 4.2 Design of new 1-((6-phenylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl) methylene)-2-(4-phenylthiazol-2-yl)hydrazines (**T50-T72**) derivatives.

ILs unlike conventional organic solvents are non-volatile, green, non-hazardous, easy to handle and thermally durable (Dupont et al. 2002; H. Davis and James, 2004). Ionic liquids, particularly based on *N*-alkyl-3-methylimidazolium cation have exhibited great importance in modern heterocyclic chemistry and these are considered as substitution for conventional organic solvents (Yadav et al. 2013; Noei and Khosropour, 2013). So, 1-butyl-3-methylimidazolium bromide ([Bmim]Br), which is one of the widely used ILs, was employed in the present study. The one-pot reaction was carried out between three-components viz 2,6-disubstituted imidazo[2,1-*b*][1,3,4]thiadiazole-5-carbaldehyde, thiosemicarbazide and 4-substituted phenacyl bromide. This method is highly atom efficient and there is no formation of by-products. Hence, the pure product was isolated straightaway (without column purification procedure) from the reaction system.

4.2 CHEMISTRY

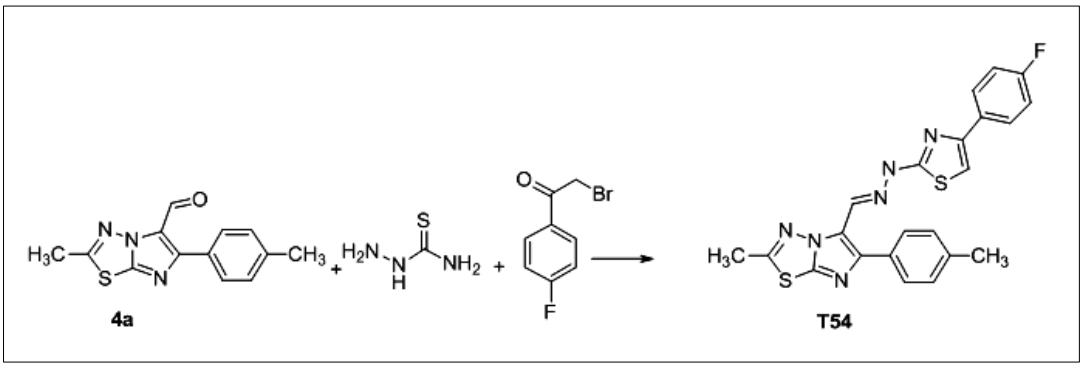
One of the key intermediate 5-trifluoro methyl-1,3,4-thiadiazole-2-amine (**2g**) was synthesized according to the reported procedure (Li and Chen, 2008). Compounds (**3a–c, 3n-o)** were synthesized by the reaction between **2a, 2g** and the corresponding substituted *α*-haloarylketone at 80-85 °C for 24 h. In the next step, these compounds **(3a–c, 3n-o)** were subjected to the Vilsmeier–Haack formylation reaction to afford 2-substituted-6-arylimidazo[2,1-*b*][1,3,4]thiadiazole-5-carbaldehydes (**4a–c, 4n-o**) (Alegaon et al. 2012). For the synthesis of target molecules, a one pot synthetic protocol (**scheme 4.1**) was employed instead of conventional two step procedure (Ozadali et al. 2014) which generally takes a longer duration for the isolation of the final products.



Scheme 4.1 Synthesis of thiazole-ITD hybrids (**T50-T72**). Reagents and conditions a) Acetyl chloride, 0 °C - RT, 3 h; b) Polyphosphoric acid, 110 °C, 8h; c) Phenacyl bromide, ethanol, 80-85 °C, 24 h; d) DMF, $POCl_3$, 60 °C, 6h; e) Thiosemicarbazide, substituted phenacyl bromide, [Bmim]Br and ethanol, 80 °C, 30-45 min.

The solvent system, temperature and reaction time for the one-pot three-component protocol was optimized so as to get a good yield of the product. For this, the one-pot reaction of 2-methyl-6-(4-methylphenyl)imidazo[2,1-*b*][1,3,4]thiadiazole-5-carbaldehyde (**4a**) with thiosemicarbazide and 4-flouro phenacyl bromide (**scheme 4.2**) was investigated under different reaction conditions as presented in **table 4.1**. Among these, the reaction in a mixture of [Bmim]Br and ethanol at 80 °C resulted in

a better product (**T54**) yield. Also, the reaction under this condition was complete in a shorter duration of 30 min. The optimized reaction condition was applied for the reaction of all five aldehydes (**4a–c, 4n-o**) with thiosemicarbazide and different phenacyl bromides. The corresponding derivatives (**T50-T72**) were isolated in good yields. Substitution pattern, yield and solubility of target compounds (**T50-T72**) are given in **table 4.2**.



Scheme 4.2 Three component one-pot synthesis of **T54**. This reaction was taken as a model to optimize the reaction conditions for the one-pot synthesis of target molecules **T50-T72**.

Table 4.1 Optimization of reaction conditions for the one-pot synthesis of **T54**.

Entry	Solvent system	T (° C)	Time (h)	Isolated yield (%)
1	Ethanol	r.t.	18	-
2	Ethanol	50	18	10
3	Ethanol	80	18	15
4	[Bmim]Br and ethanol	r.t.	12	40
5	[Bmim]Br and ethanol	50	02	62
6	[Bmim]Br and ethanol	80	0.5	90

Table 4.2 Substitution pattern, yield and solubility of target compounds (**T50-T72**).

Product	R^1	R^2	R^3	log *P*/*C* log P^a	Time (min)	Yield (%)
T50	CH_3	CH_3	CH_3	8.06/5.70	45	85
T51	CH_3	CH_3	OCH_3	7.45/5.21	40	88
T52	CH_3	CH_3	Cl	7.18/4.74	30	90
T53	CH_3	CH_3	NO_2	-/4.95	30	89

T54	CH_3	CH_3	F	7.73/5.34	30	90
T55	CH_3	OCH_3	CH_3	7.45/5.21	40	84
T56	CH_3	OCH_3	OCH_3	6.83/4.72	40	80
T57	CH_3	OCH_3	Cl	7.52/5.42	30	85
T58	CH_3	OCH_3	NO_2	-/4.46	30	88
T59	CH_3	OCH_3	F	8.15/5.87	30	85
T60	CH_3	Cl	CH_3	8.13/5.91	30	83
T61	CH_3	Cl	OCH_3	7.52/5.43	30	87
T62	CH_3	Cl	Cl	7.26/5.07	30	94
T63	CH_3	Cl	F	7.8/5.68	30	88
T64	CF_3	OCH_3	CH_3	8.35/5.59	45	82
T65	CF_3	OCH_3	OCH_3	7.74/5.11	45	82
T66	CF_3	OCH_3	Cl	7.47/4.63	35	82
T67	CF_3	OCH_3	F	8.02/5.24	35	84
T68	CF_3	Cl	CH_3	9.03/6.29	35	88
T69	CF_3	Cl	OCH_3	8.42/5.81	35	86
T70	CF_3	Cl	Cl	8.16/5.33	30	94
T71	CF_3	Cl	NO_2	4.9/5.54	30	96
T72	CF_3	Cl	F	8.71/5.94	30	95

[a]Obtained from Chemdraw ultra 12.0 software

Note: Over all yield of compound **T52** is 60.9 %

4.3 EXPERIMENTAL

4.3.1 Materials and instruments (refer section 2.3.1)

4.3.2 Synthesis

General procedure for the synthesis of target molecules (T50-T72): A mixture of **4 a-c**, **4 n-o** (1 mmol), thiosemicarbazide (1 mmol) and the corresponding phenacyl bromide (1 mmol) was taken in ethanol (10 mL) in a dry 50 mL round bottom flask. To this mixture [Bmim]Br (2 mL) was added and the resulting mixture was stirred at 80 °C for 30-45 min. After the completion of the reaction (as monitored by TLC), water (10 mL) was added to the reaction mixture. The separated solid was filtered off and washed with water. The product was then recrystallized from ethanol.

1-((2-Methyl-6-*p*-tolylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)methylene)-2-(4-*p*-tolyl thiazol-2-yl)hydrazine (T50): Light yellow solid; m.p. 241-242 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.28 (s, 1H, -NH), 8.43 (s, 1H, -CH), 8.32 – 8.24 (m, 2H, Ar-H), 8.15 – 8.07 (m, 2H, Ar-H), 7.86 – 7.79 (m, 2H, Ar-H), 7.74 (s, 1H, Ar-H), 7.33 (d, J = 7.9 Hz, 2H, Ar-H), 2.81 (s, 3H, CH_3), 2.39 (s, 3H, CH_3), 2.32 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 168.8, 161.7, 149.8, 147.3, 146.7, 145.3, 142.1, 138.0, 131.3, 131.0, 129.5, 128.4, 126.8, 123.6, 119.2, 105.3, 21.3, 21.2, 18.2; ESI-MS (*m/z*) = 445.4 $(M+H)^+$; anal. calcd (%) for $C_{23}H_{20}N_6S_2$: C, 62.14; H, 4.53; N, 18.90; S, 14.43. Found: C, 62.08; H, 4.58; N, 18.95; S, 14.38.

1-(4-(4-Methoxyphenyl)thiazol-2-yl)-2-((2-methyl-6-*p*-tolylimidazo[2,1-*b*][1,3,4] thiadiazol-5-yl)methylene)hydrazine (T51): Yellow solid; m.p. 239-240 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.13 (s, 1H, -NH), 8.41 (s, 1H, -CH), 7.83-7.78 (m, 2H, Ar-H), 7.74 (s, 1H, Ar-H), 7.50-7.42 (m, 2H, Ar-H), 7.38 (d, J = 7.8Hz, 2H, Ar-H), 7.34-7.28 (m, 2H, Ar-H), 3.73 (s, 3H, OCH_3), 2.81 (s, 3H, CH_3), 2.39 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 168.5, 161.5, 150.2, 147.1, 145.1, 138.0, 130.2, 130.9, 129.5, 128.4, 128.1, 127.8, 119.2, 116.1, 115.8, 104.2, 55.7, 21.3, 18.2; ESI-MS (*m/z*) = 461.1 $(M+H)^+$; anal. calcd (%) for $C_{23}H_{20}N_6OS_2$: C, 59.98; H, 4.38; N, 18.25; S, 13.92. Found: C, 60.04; H, 4.36; N, 18.28; S, 13.98.

1-(4-(4-Chlorophenyl)thiazol-2-yl)-2-((2-methyl-6-*p*-tolylimidazo[2,1-*b*][1,3,4] thiadiazol-5-yl)methylene)hydrazine (T52): Light green solid; m.p. 249-250 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.13 (s, 1H, -NH), 8.43 (s, 1H, -CH), 7.92 – 7.78 (m, 4H, Ar-H), 7.33 – 7.16 (m, 5H, Ar-H), 2.81 (s, 3H, CH_3), 2.37 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 168.5, 161.5, 148.2, 146.3, 144.1, 137.0, 131.3, 130.7, 129.3, 128.5, 128.0, 127.9, 119.2, 116.0, 115.8, 103.2, 21.3, 18.2; ESI-MS (*m/z*) = 464.0 $(M+H)^+$; anal. calcd (%) for $C_{22}H_{17}ClN_6S_2$: C, 56.83; H, 3.68; N, 18.07; S, 13.79. Found: C, 56.88; H, 3.66; N, 18.10; S, 13.78.

2-Methyl-5-((2-(4-(4-nitrophenyl)thiazol-2-yl)hydrazono)methyl)-6-(*p*-tolyl) imidazo[2,1-*b*][1,3,4]thiadiazole (T53): Yellow solid; m.p. 257-258 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.28 (s, 1H, -NH), 8.43 (s, 1H, -CH), 8.32 – 8.24 (m, 2H, Ar-H), 8.15 – 8.07 (m, 2H, Ar-H), 7.86 – 7.79 (m, 2H, Ar-H), 7.74 (s, 1H,

Ar-H), 7.33 (d, *J* = 7.9 Hz, 2H, Ar-H), 2.81 (s, 3H, CH_3), 2.39 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 168.8, 161.7, 148.8, 147.3, 146.6, 145.3, 141.0, 138.0, 131.3, 131.0, 129.5, 128.4, 126.8, 124.5, 119.2, 109.3, 21.3, 18.2; ESI-MS (*m/z*) = 475.90 $(M+H)^+$; anal. calcd (%) for $C_{12}H_{17}N_7O_2S_2$: C, 55.56; H, 3.60; N, 20.62; S, 13.49. Found: C, 55.49; H, 3.58; N, 20.55; S, 13.38.

1-(4-(4-Fluorophenyl)thiazol-2-yl)-2-((2-methyl-6-*p*-tolylimidazo[2,1-*b*][1,3,4] thiadiazol-5-yl) methylene)hydrazine (T54): Off white solid; m.p. 244-246 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.14 (s, 1H, -NH), 8.41 (s, 1H, -CH), 7.93 – 7.81 (m, 4H, Ar-H), 7.31 (t, *J* = 3.9 Hz, 3H, Ar-H), 7.24 (t, *J* = 8.9 Hz, 2H, Ar-H), 2.80 (s, 3H, CH_3), 2.38 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 168.5, 161.7, 149.3, 147.2, 145.1, 138.0, 131.2, 130.9, 129.5, 128.4, 128.0, 128.0, 119.2, 116.0, 115.8, 104.2, 21.3, 18.2; ESI-MS (*m/z*) = 449.0 $(M+H)^+$; anal. calcd (%) for $C_{12}H_{17}FN_6S_2$: C, 58.91; H, 3.82; N, 18.74; S, 14.30. Found: C, 58.89; H, 3.78; N, 18.50; S, 14.32.

1-((6-(4-Methoxyphenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl) methylene)-2-(4-*p*-tolyl thiazol-2-yl)hydrazine (T55): Brown solid; m.p. 236-237 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.24 (s, 1H, -NH), 8.41 (s, 1H, -CH), 7.92 – 7.79 (m, 4H, Ar-H), 7.34 – 7.18 (m, 5H, Ar-H), 3.84 (s, 3H, OCH_3), 2.80 (s, 3H, CH_3), 2.38 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 168.5, 161.7, 149.3, 147.2, 145.1, 138.0, 131.2, 130.9, 129.5, 128.4, 128.0, 127.9, 119.2, 116.0, 115.8, 104.2, 55.8, 21.3, 18.2; ESI-MS (*m/z*) = 461.2 $(M+H)^+$; anal. calcd (%) for $C_{23}H_{20}N_6OS_2$: C, 59.98; H, 4.38; N, 18.25; S, 13.92. Found: C, 59.91; H, 4.39; N, 18.30; S, 13.88.

1-((6-(4-Methoxyphenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl) methylene)-2-(4-(4-methoxyphenyl)thiazol-2-yl)hydrazine (T56): Light brown solid; m.p. 243-245 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.29 (s, 1H, -NH), 8.42 (s, 1H, -CH), 7.84 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.78 (d, *J* = 7.6 Hz, 2H, Ar-H), 7.19 (s, 1H, Ar-H), 7.11 (d, *J* = 7.4 Hz, 2H, Ar-H), 6.97 (d, *J* = 9.2Hz, 2H, Ar-H), 3.85 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 168.3, 160.1, 159.3, 144.3, 149.9, 147.1, 146.7, 130.4, 130.0, 127.4, 127.3, 119.7, 118.8,

115.3, 114.6, 102.5, 55.7, 55.6, 18.2; ESI-MS (*m/z*) = 477.4 $(M+H)^+$; anal. calcd (%) for $C_{23}H_{20}N_6O_2S_2$: C, 57.97; H, 4.23; N, 17.63; S, 13.46. Found: C, 57.91; H, 4.29; N, 17.40; S, 13.58.

1-(4-(4-Chlorophenyl)thiazol-2-yl)-2-((6-(4-methoxyphenyl)-2-methylimidazo[2,1-*b*][1,3,4] thiadiazol-5-yl)methylene)hydrazine (T57): Off white solid; m.p. 203-204 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.09 (s, 1H, -NH), 8.40 (s, 1H, -CH), 7.94 – 7.80 (m, 4H, Ar-H), 7.50 – 7.42 (m, 2H, Ar-H), 7.39 (s, 1H, Ar-H), 7.10 – 7.00 (m, 2H, Ar-H), 3.82 (s, 3H, OCH_3), 2.79 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 168.6, 161.5, 159.7, 149.4, 147.0, 145.1, 133.8, 132.4, 131.2, 129.9, 129.0, 127.7, 126.3, 118.8, 114.3, 105.1, 55.7, 18.1; ESI-MS (m/z) = 481.0 (M+H)+; anal. calcd (%) for $C_{22}H_{17}ClN_6OS_2$: C, 54.94; H, 3.56; N, 17.47; S, 13.33. Found: C, 54.89; H, 3.58; N, 17.50; S, 13.32.

1-((6-(4-Methoxyphenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)methylene) -2-(4-(4-nitro phenyl)thiazol-2-yl)hydrazine (T58): Yellow solid; m.p. 247-248 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.22 (s, 1H, -NH), 8.41 (s, 1H, -CH), 8.31 – 8.23 (m, 2H, Ar-H), 8.15 – 8.06 (m, 2H, Ar-H), 7.92 – 7.84 (m, 2H, Ar-H), 7.71 (s, 1H, Ar-H), 7.11 – 7.02 (m, 2H, Ar-H), 3.83 (s, 3H, OCH_3), 2.80 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 168.8, 161.6, 159.7, 148.8, 147.1, 146.6, 145.0, 141.0, 131.3, 129.9, 126.7, 126.2, 124.5, 118.7, 114.3, 109.2, 55.7, 18.1; ESI-MS (*m/z*) = 492.2 $(M+H)^+$; anal. calcd (%) for $C_{22}H_{17}N_7O_3S_2$: C, 53.76; H, 3.49; N, 19.95; S, 13.05. Found: C, 53.80; H, 3.48; N, 19.80; S, 13.12.

1-(4-(4-Fluorophenyl)thiazol-2-yl)-2-((6-(4-methoxyphenyl)-2-methylimidazo[2,1-*b*][1,3,4] thiadiazol-5-yl)methylene)hydrazine (T59): Green solid; m.p. 239-240 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.12 (s, 1H, -NH), 8.41 (s, 1H, -CH), 7.84 – 7.70 (m, 4H, Ar-H), 7.40 – 7.32 (m, 2H, Ar-H), 7.38 (s, 1H, Ar-H), 7.10 – 7.00 (m, 2H, Ar-H), 3.78 (s, 3H, OCH_3), 2.79 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 168.6, 162.5, 159.7, 149.4, 147.0, 146.1, 133.8, 132.4, 130.2, 127.9, 129.0, 127.7, 126.3, 118.8, 115.3, 105.1, 55.6, 18.1; ESI-MS (*m/z*) = 465.2 $(M+H)^+$; anal. calcd (%) for $C_{22}H_{17}FN_6OS_2$: C, 56.88; H, 3.69; N, 18.09; S, 13.81. Found: C, 56.82; H, 3.68; N, 18.10; S, 13.91.

1-((6-(4-Chlorophenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)methylene)-2-(4-*p*-tolylthiazol-2-yl)hydrazine (T60): Yellow solid; m.p. 249-250 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.18 (s, 1H, -NH), 8.41 (s, 1H, -CH), 8.21 – 8.18 (m, 2H, Ar-H), 8.15 – 8.06 (m, 2H, Ar-H), 7.82 – 7.74 (m, 2H, Ar-H), 7.71 (s, 1H, Ar-H), 7.11 – 7.02 (m, 2H, Ar-H), 2.82 (s, 3H, CH_3), 2.38 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 168.8, 162.6, 159.7, 148.8, 147.1, 146.6, 145.0, 141.0, 131.3, 129.9, 126.8, 126.2, 125.1, 118.7, 114.3, 109.2, 21.3, 18.1; ESI-MS (*m/z*) = 465.3 $(M+H)^+$; anal. calcd (%) for $C_{22}H_{17}ClN_6S_2$: C, 56.83; H, 3.68; N, 18.07; S, 13.79. Found: C, 56.81; H, 3.62; N, 18.12; S, 13.71.

1-((6-(4-Chlorophenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)methylene)-2-(4-(4-methoxyphenyl)thiazol-2-yl)hydrazine (T61): Light yellow solid; m.p. 201-202 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.22 (s, 1H, -NH), 8.41 (s, 1H, -CH), 8.31 – 8.23 (m, 2H, Ar-H), 8.10 – 8.06 (m, 2H, Ar-H), 7.82 – 7.74 (m, 2H, Ar-H), 7.71 (s, 1H, Ar-H), 7.11 – 7.02 (m, 2H, Ar-H), 3.83 (s, 3H, OCH_3), 2.80 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 168.8, 161.6, 159.7, 148.8, 147.1, 146.5, 145.0, 142.0, 131.4, 129.9, 126.79, 126.2, 124.5, 118.7, 114.3, 109.2, 55.7, 18.2; ESI-MS (*m/z*) = 481.2 $(M+H)^+$; anal. calcd (%) for $C_{22}H_{17}ClN_6OS_2$: C, 54.94; H, 3.56; N, 17.47; S, 13.33. Found: C, 54.91; H, 3.52; N, 17.42; S, 13.31.

1-((6-(4-Chlorophenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)methylene)-2-(4-(4-chlorophenyl)thiazol-2-yl)hydrazine (T62): Orange solid; m.p. 235-236 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.27 (s, 1H, -NH), 8.44 (s, 1H, -CH), 8.08 – 8.01 (m, 2H, Ar-H), 7.91 – 7.83 (m, 2H, Ar-H), 7.61 – 7.54 (m, 2H, Ar-H), 7.51 – 7.39 (m, 3H, Ar-H), 2.81 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 168.4, 162.2, 149.4, 147.0, 143.4, 133.8, 133.0, 132.9, 132.4, 130.8, 130.1, 129.0, 128.8, 127.7, 119.7, 105.2, 18.1; ESI-MS (*m/z*) = 484.80 $(M+H)^+$; anal. calcd (%) for $C_{21}H_{14}Cl_2N_6O_3S_2$: C, 51.96; H, 2.91; N, 17.31; S, 13.21. Found: C, 51.88; H, 2.94; N, 17.38; S, 13.22.

1-((6-(4-Chlorophenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)methylene)-2-(4-(4-fluorophenyl)thiazol-2-yl)hydrazine (T63): Yellow solid; m.p. 246-248 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.29 (s, 1H, -NH), 8.43 (s, 1H, -CH), 7.91–

7.82 (m, 2H, Ar-H), 7.71 – 7.63 (m, 2H, Ar-H), 7.58 – 7.54 (m, 2H, Ar-H), 7.48 – 7.39 (m, 3H, Ar-H), 2.81 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 168.3, 160.8, 148.4, 146.6, 145.2, 139.3, 133.7, 132.3, 131.5, 131.4, 129.0, 128.8, 127.7, 122.7, 122.1, 119.7, 105.3, 18.1; ESI-MS (*m/z*) = 469.2 $(M+H)^+$; anal. calcd (%) for $C_{21}H_{14}ClFN_6S_2$: C, 53.78; H, 3.01; N, 17.92; S, 13.68. Found: C, 53.71; H, 3.05; N, 17.98; S, 13.62.

1-((6-(4-Methoxyphenyl)-2-(trifluoromethyl)imidazo[2,1-*b*][1,3,4]thiadiazol-5-yl) methylene)-2-(4-*p*-tolylthiazol-2-yl)hydrazine (T64): Light yellow solid; m.p. 238-239 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.25 (s, 1H, -NH), 8.43 (s, 1H, -CH), 7.86 (d, *J* = 8.4 Hz, 2H, Ar-H), 7.781 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.197 (s, 1H, Ar-H), 7.11-7.10 (m, 2H, Ar-H), 6.98 (d, *J* = 9.2Hz, 2H, Ar-H), 3.84 (s, 3H, OCH_3), 2.38 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 168.3, 160.1, 159.3, 149.9, 147.1, 146.7, 143.2, 130.4, 130.0, 127.4, 127.3, 125.8, 123.5, 119.7, 114.6, 114.4, 102.5, 55.7, 21.4; ESI-MS (*m/z*) = 515.2 $(M+H)^+$; anal. calcd (%) for $C_{23}H_{17}F_3N_6OS_2$: C, 53.69; H, 3.33; N, 16.33; S, 12.46. Found: C, 53.61; H, 3.29; N, 16.38; S, 12.42.

1-((6-(4-Methoxyphenyl)-2-(trifluoromethyl)imidazo[2,1-*b*][1,3,4]thiadiazol-5-yl) methylene)-2-(4-(4-methoxy phenyl) thiazol-2-yl) hydrazine (T65): Yellow solid; m.p. 232-233 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.42 (s, 1H, -CH), 7.86 (d, *J* = 9.2 Hz, 2H, Ar-H), 7.78 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.19 (s, 1H, Ar-H), 7.11 (d, *J* = 8.8 Hz, 2H, Ar-H), 6.97 (d, *J* = 9.2Hz, 2H, Ar-H), 3.84 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 168.3, 160.1, 159.3, 149.9, 147.2, 146.7, 130.4, 130.0, 127.5, 127.4, 125.8, 119.7, 114.6, 114.4, 102.5, 55.7, 55.6; ESI-MS (*m/z*) = 530.90 $(M+H)^+$; anal. calcd (%) for $C_{23}H_{17}F_3N_6O_2S_2$: C, 52.07; H, 3.23; N, 15.84; S, 12.09. Found: C, 51.98; H, 3.24; N, 15.78; S, 12.12.

1-(4-(4-Chlorophenyl)thiazol-2-yl)-2-((6-(4-methoxyphenyl)-2-(trifluoromethyl) imidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)methylene)hydrazine (T66): Light brown solid; m.p. 236-237 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.25 (s, 1H, -NH), 8.43 (s, 1H, -CH), 7.91 – 7.81 (m, 4H, Ar-H), 7.52 – 7.42 (m, 3H, Ar-H), 7.16 – 7.07 (m, 2H, Ar-H), 3.85 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 168.5,

160.1, 149.5, 147.1, 146.7, 133.8, 132.4, 130.2, 130.0, 129.0, 127.7, 125.8, 119.7, 114.6, 105.4, 55.7**;** ESI-MS (*m/z*)= 534.80 $(M+H)^+$; anal. calcd (%) for $C_{22}H_{14}ClF_3N_6OS_2$: C, 49.39; H, 2.64; N, 15.71; S, 11.99. Found: C, 49.40; H, 2.66; N, 15.78; S, 11.92.

1-(4-(4-Fluoro phenyl) thiazol-2-yl)-2-((6-(4-methoxy phenyl)-2-(trifluoromethyl) imidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)methylene)hydrazine (T67): Yellow solid; m.p. 267-268 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.14 (s, 1H, -NH), 8.41 (s, 1H, -CH), 7.84 (m, 2H, Ar-H), 7.61 (d, *J* = 8.4 Hz, 2H, Ar-H), 7.52 – 7.42 (m, 2H, Ar-H), 7.35 (s, 1H, Ar-H), 7.26 – 7.18 (m, 2H, Ar-H), 3.80 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 168.4, 159.1, 149.5, 148.1, 146.8, 133.9, 132.4, 130.3, 130.0, 129.1, 126.7, 125.8, 120.1, 114.6, 105.4, 55.7; ESI-MS (*m/z*) = 519.4 $(M+H)^+$; anal. calcd (%) for $C_{22}H_{14}F_4N_6OS_2$: C, 50.96; H, 2.72; N, 16.21; S, 12.37. Found: C, 50.90; H, 2.78; N, 16.31; S, 12.32.

1-((6-(4-Chlorophenyl)-2-(trifluoromethyl)imidazo[2,1-*b*][1,3,4]thiadiazol-5-yl) methylene)-2-(4-*p*-tolylthiazol-2-yl)hydrazine (T68): Yellow solid; m.p. 264-265 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.29 (s, 1H, -NH), 8.45 (s, 1H, -CH), 7.87-7.81 (m, 2H, Ar-H), 7.76 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.61 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.47 – 7.43 (m, 3H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 168.5, 160.1, 149.5, 147.1, 146.7, 143.2, 133.8, 132.4, 130.2, 130.0, 129.0, 127.7, 123.4, 125.8, 119.7, 114.6, 105.4, 22.3; ESI-MS (*m/z*) = 519.3 $(M+H)^+$: anal. calcd (%) for $C_{22}H_{14}ClF_3N_6S_2$: C, 50.92; H, 2.72; N, 16.19; S, 12.36. Found: C, 50.82; H, 2.68; N, 16.18; S, 12.44.

1-((6-(4-Chlorophenyl)-2-(trifluoromethyl)imidazo[2,1-*b*][1,3,4]thiadiazol-5-yl) methylene)-2-(4-(4-methoxyphenyl)thiazol-2-yl)hydrazine (T69): Yellow solid; m.p. 228-229 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.29 (s, 1H, -NH), 8.45 (s, 1H, -CH), 7.97 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.86 (d, *J* = 8.4 Hz, 2H, Ar-H), 7.61 (d, *J* = 8.4 Hz, 2H, Ar-H), 7.47 – 7.43 (m, 3H, Ar-H), 3.82(s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 168.5, 160.1, 149.5, 147.1, 146.7, 144.2, 133.8, 132.4, 130.2, 130.0, 129.0, 127.7, 125.8, 123.2 119.7, 114.6, 105.4, 55.7; ESI-MS (*m/z*) =

535.20 $(M+H)^+$; anal. calcd (%) for $C_{24}H_{14}ClF_3N_6OS_2$: C, 49.39; H, 2.64; N, 15.71; S, 11.99. Found: C, 49.42; H, 2.68; N, 15.68; S, 11.94.

1-((6-(4-Chlorophenyl)-2-(trifluoromethyl)imidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)methylene)-2-(4-(4-chlorophenyl)thiazol-2-yl)hydrazine (T70): Light Yellow solid; m.p. 240-241 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.29 (s, 1H, -NH), 8.45 (s, 1H, -CH), 7.97 (d, J = 8.4Hz, 2H, Ar-H), 7.86 (d, J = 8.8 Hz, 2H, Ar-H), 7.61 (d, J =8.8Hz, 2H, Ar-H), 7.47 – 7.43 (m, 3H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 168.3, 160.8, 149.7, 146.6, 145.2, 133.7, 132.3, 131.5, 131.5, 130.2, 129.6, 129.1, 128.0, 127.9, 120.7, 116.0, 115.8, 104.5; ESI-MS (m/z) = 539.10 $(M+H)^+$; anal. calcd (%) for $C_{21}H_{11}Cl_2F_3N_6S_2$: C, 46.76; H, 2.06; N, 15.58; S, 11.89. Found: C, 46.66; H, 2.08; N, 15.68; S, 11.92.

1-((6-(4-Chlorophenyl)-2-(trifluoromethyl)imidazo[2,1-b][1,3,4]thiadiazol-5-yl) methylene)-2-(4-(4-nitro phenyl)thiazol-2-yl)hydrazine (T71): Yellow solid; m.p. 261-262 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.30 (s, 1H, -NH), 8.46 (s, 1H, -CH), 7.96 -7.90 (m, 2H, Ar-H, Ar-H), 7.86 (d, J = 8.4 Hz, 2H, Ar-H), 7.58 (d, J =8.6 Hz, 2H, Ar-H), 7.45 – 7.41 (m, 2H, Ar-H), 7.35 (s, 1H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 168.4, 160.2, 148.4, 147.2, 146.3, 134.2, 131.4, 130.3, 130.0, 129.0, 128.2, 127.7, 124.3, 120.7, 115.8, 114.6, 105.4; ESI-MS (m/z) = 550.20 $(M+H)^+$; anal. calcd (%) for $C_{21}H_{11}ClF_3N_7O_2S_2$: C, 45.86; H, 2.02; N, 17.83; S, 11.66. Found: C, 45.76; H, 2.04; N, 17.85; S, 11.72.

1-((6-(4-Chlorophenyl)-2-(trifluoromethyl)imidazo[2,1-*b*][1,3,4]thiadiazol-5-yl) methylene)-2-(4-(4-fluorophenyl)thiazol-2-yl)hydrazine (T72): Yellow solid; m.p. 263-264 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.24 (s, 1H, -NH), 8.45 (s, 1H), 8.01 – 7.94 (m, 2H), 7.92 – 7.84 (m, 2H), 7.61 (d, J = 8.6 Hz, 2H), 7.35 (s, 1H), 7.24 (t, J = 8.9 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 168.3, 160.8, 149.7, 146.6, 145.2, 133.7, 132.3, 131.5, 131.5, 130.2, 129.6, 129.1, 128.0, 127.9, 120.7, 116.0, 115.8, 104.5; ESI-MS (m/z) = 523.10 $(M+H)^+$; anal. calcd (%) for $C_{21}H_{11}ClF_4N_6S_2$: C, 48.23; H, 2.12; N, 16.07; S, 12.26. Found: C, 48.20; H, 2.13; N, 16.10; S, 12.32.

4.4 PHARMACOLOGY (refer sections 2.4.1, 3.4.2, 3.4.3, 2.4.4**)**

4.5 RESULTS AND DISCUSSION

4.5.1 Chemistry

The target molecules were analyzed using spectroscopic techniques to confirm their structure. For instance, the ^{1}H NMR spectrum of compound **T54** showed a singlet with one proton at δ 12.14 ppm due to the -NH proton (**figure 4.3**). Another singlet at δ 8.41 ppm is due to the -CH proton of the imine (CH=N-) linkage. The singlets at δ 2.80 and 2.38 ppm represent the methyl protons on the 1,3,4-thiadiazole and phenyl rings respectively. The spectrum displayed a multiplet in the region δ 7.93 – 7.81 ppm and two triplets at δ 7.31 and 7.24 ppm due to the aromatic protons. Also, its mass spectrum showed a molecular ion peak at m/z 449.0, which corresponds to M+1 peak of the molecule. The ^{1}H and ^{13}C NMR spectral data along with the elemental data of all the target molecules are given in the experimental section.

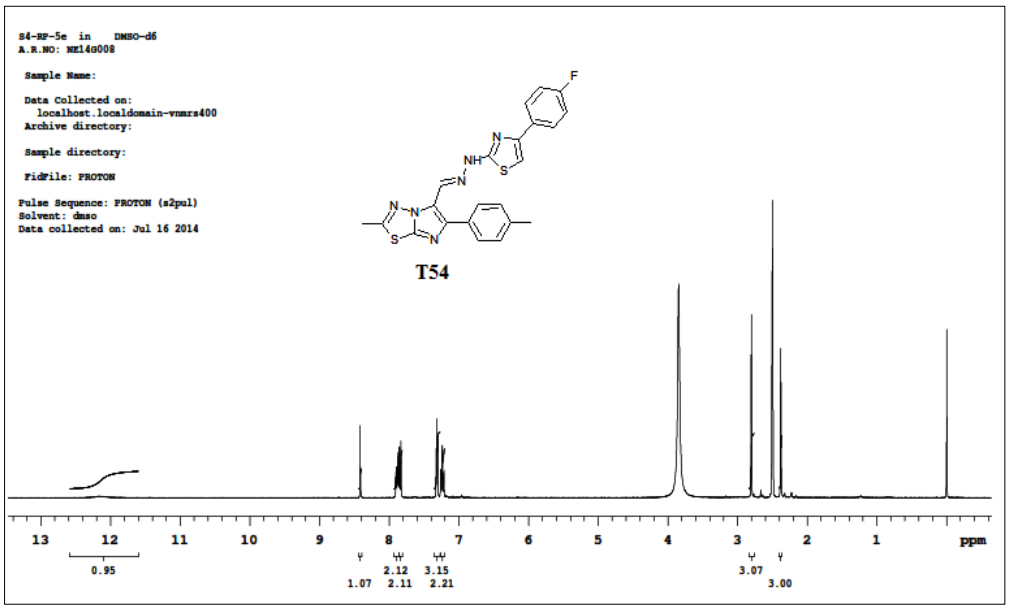


Figure 4.3 ^{1}H NMR spectrum of **T54**

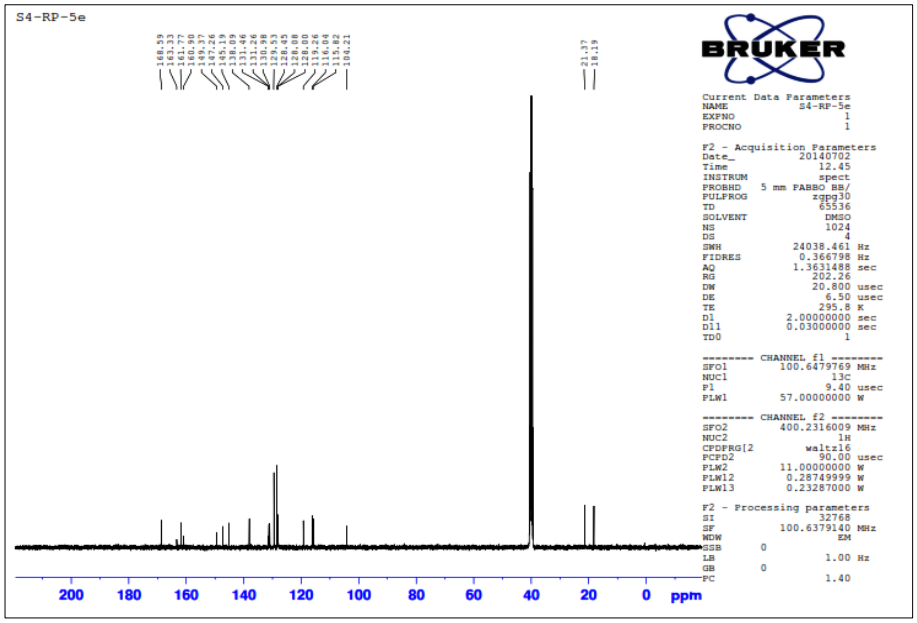


Figure 4.4 ^{13}C NMR spectrum of **T54**

4.5.2 *In vitro* antimycobacterial activity

The target compounds (**T50-T72**) were screened against *Mtb* H37Rv (ATCC27294) using the agar dilution method to evaluate their antimycobacterial activity in terms of MIC values. The MIC values of **T50-T72** along with those of standard drugs for comparison are presented in **figure 4.5**. Compound, 1-((6-(4-chlorophenyl)-2-(trifluoromethyl)imidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)methylene)-2-(4-*p*-tolyl thiazol-2yl)hydrazine (**T68**) is the most active derivative of the series and its MIC (3.125 µg/mL) is lower than those of standard drugs, ethambutol and ciprofloxacin. Additionally, compounds **T54**, **T58**, **T69** and **T72** with MIC values 6.25 (13.94 µM), 6.25 (12.72 µM), 6.25 (11.70 µM) and 6.25 µg/mL (11.97 µM) respectively are more active than ethambutol (15.32 µM). The SAR reveals that the various substituents (R^1/R^2) on the ITD ring affect significantly the antitubercular activity of the compounds. The trifluoromethyl substitution at position-2 (R^1) was found to enhance the activity of the molecules. All the trifluoromethyl substituted derivatives except **T66**, showed lower MIC values than their methyl analogs. That is to say, compounds **T64**, **T65**, **T67**, **T68**, **T69**, **T70** and **T72** are more active than compounds **T55**, **T56**, **T59**, **T60**, **T61**, **T62** and **T63** respectively. Among trifluoromethyl substituted derivatives, the 4-chlorophenyl substituted (*i.e* R^2 = Cl)

compounds (**T68**, **T69**, **T70** and **T72**) showed better activity than the respective *p*-methoxyphenyl (*i.e* $R^2 = OCH_3$) analogs (**T64**, **T65**, **T66** and **T67**). A similar SAR was observed with most of the methyl (R^1) substituted derivatives (**T55** – **T63**) as well. Though the substituents (R^3) on the thiazole ring affect remarkably the activity of the compounds, we did not observe a uniform trend in the SAR. However, compounds with methyl, methoxy, fluoro or nitro substituents on the thiazole ring showed significant activity. Hence, the ITD ring with a trifluoromethyl group at position-2 and a *p*-chlorophenyl substituent at position-6 could be an active core for further structural modification, particularly by introducing different pharmacophoric units at position-5 of the core structure, in order to develop potent antiTB agents.

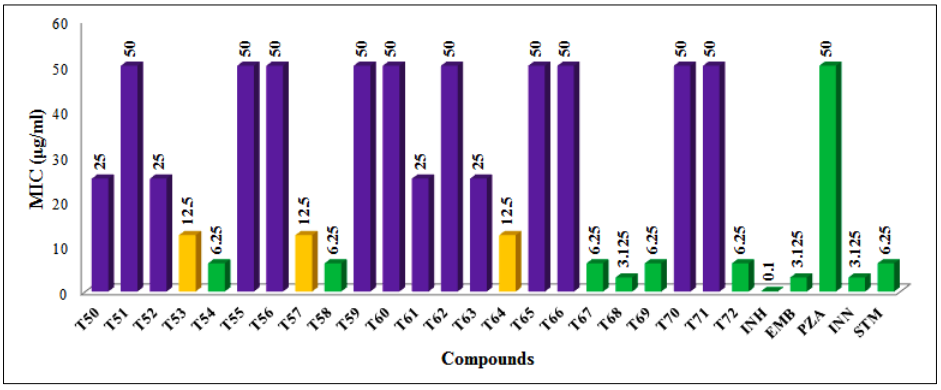


Figure 4.5 AntiTB activity of **T50-T72** against *Mtb* H37RV

4.5.3 *In vitro* antibacterial activity

The *in vitro* antibacterial activity of compounds **T50-T72** was tested using disc diffusion method and zone of inhibition (mm) was measured. All the compounds were screened against three bacterial strains *viz. S. aureus, P. aeruginosa and E. coli.* The compounds were dissolved in DMSO with two concentrations (75 µg/mL and 50 µg/mL). Ciprofloxacin was taken as the standard drug. Compound **T72** demonstrated significant inhibition activity against all three tested bacterial strains at both concentrations, while compounds **T53** and **T57** showed moderate activity against these bacterial strains. The antibacterial screening results are displayed in **table 4.3**. It can be seen that the molecules show a better inhibition activity against *Mtb* H37Rv when compared to their activity against the other tested bacterial strains. So it may be concluded that these molecules are somewhat selective in their inhibition action against *Mtb* H37Rv strain.

Table 4.3 Antibacterial activity of target compounds (**T50-T72**)

Compounds	***E. coli***		***S. aureus***		***P. aeruginosa***	
Cocn. in µg/ml	75	50	75	50	75	50
T50	08±0.1	04±0.1	12±0.1	07±0.4	13±0.4	12±0.1
T51	10±0.2	07±0.2	07±0.1	04±0.1	12±0.1	10±0.1
T52	09±0.1	06±0.1	10±0.2	07±0.1	–	–
T53	19±0.4	16±0.1	18±0.4	13±0.1	17±0.4	13±0.3
T54	11±0.4	06±0.2	–	–	–	–
T55	12±0.3	09±0.1	13±0.4	09±0.2	–	–
T56	16±0.1	14±0.3	–	–	14±0.2	12±0.1
T57	20±0.1	17±0.1	18±0.4	10±0.3	17±0.3	14±0.3
T58	12±0.3	11±0.3	–	–	–	–
T59	10±0.4	08±0.1	12±0.2	10±0.1	–	–
T60	09±0.1	07±0.3	–	–	12±0.3	10±0.2
T61	10±0.3	06±0.1	07±0.2	05±0.2	15±0.3	12±0.3
T62	11±0.2	09±0.3	11±0.2	09±0.2	11±0.2	07±0.1
T63	14±0.3	11±0.3	10±0.2	07±0.2	04±0.2	02±0.1
T64	12±0.2	08±0.3	08±0.2	05±0.4	11±0.1	09±0.1
T65	07±0.3	06±0.2	–	–	04±0.2	02±0.2
T66	08±0.1	04±0.2	–	–	11±0.2	07±0.1
T67	10±0.2	08±0.1	08±0.1	05±0.1	12±0.1	08±0.3
T68	12±0.1	07±0.2	11±0.1	08±0.4	11±0.1	07±0.2
T69	14±01	11±0.1	07±0.2	04±0.2	09±0.2	07±0.1
T70	13±0.2	09±0.1	13±0.1	09±0.2	12±0.4	10±0.2
T71	16±0.3	12±0.2	11±0.1	08±0.1	12±0.1	10±0.4
T72	24±0.4	19±0.2	20±0.1	17±0.1	18±0.2	14±0.1
Control	00	00	00	00	00	00
INN	32±0.2	27±0.2	26±0.1	21±0.2	21±0.2	18±0.1
INN: Ciprofloxacin; –: inhibition not detected; control: DMSO						

4.5.4 *In vitro* cytotoxicity studies

The *in vitro* cytotoxicity of the active compounds (MIC ≤ 12.5 µg/mL against *Mtb*) was evaluated against NIH 3T3 mouse embryonic fibroblasts cell line using MTT assay. The graphical representation of the cell growth inhibition by the compounds at a concentration of 50 µg/mL is shown in **figure 4.6**. It can be seen that none of the active compounds are toxic to the normal cells thus proving the lack of general cellular toxicity.

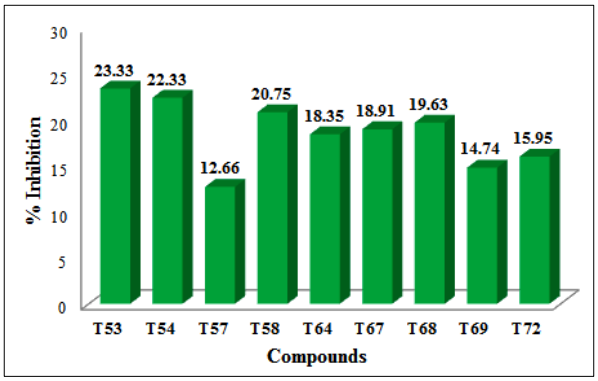


Figure 4.6 Growth inhibition activity of active compounds (at a concentration of 50 µg/mL) against NIH 3T3 cell line.

4.5.5 Molecular docking studies

In order to gain an insight about the mechanism of action of the new thiazole-ITD hybrids, the active molecules were subjected to molecular docking studies against InhA enzyme of *Mtb*, which have been validated as effective antiTB targets (Ozadali et al. 2014).

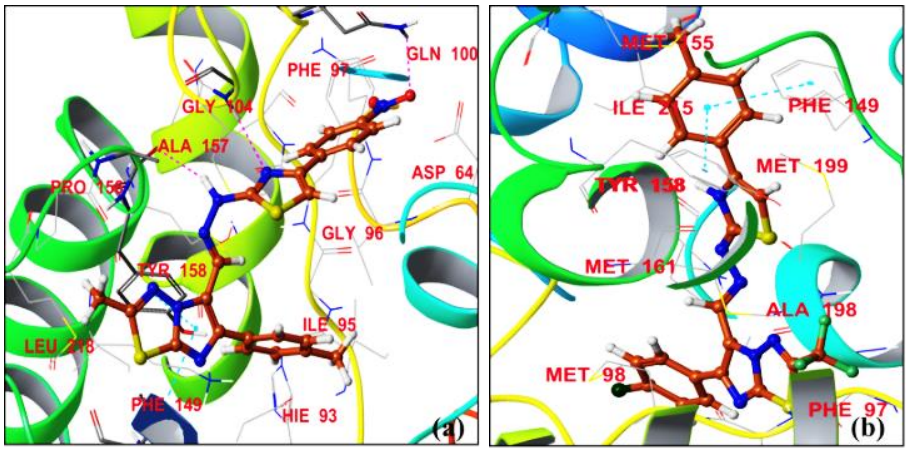


Figure 4.7 The docking poses of active compounds (a) **T53** with InhA; (b) **T68** with target enzyme InhA.

Further, Isoniazid (the first line antiTB drug) acts by inhibiting InhA. Recent studies on thiazolylhydrazone and 1,3,4-thiadiazole derivatives with InhA revealed good binding interaction of these molecules with amino acid residue of the enzyme (McLean et al. 2002; Joshi et al. 2015). InhA is one of the key enzymes involved in the type II fatty acid biosynthesis pathway. The ligands from the crystal structure of the enzyme-ligand complexes were rebuilt and redocked to validate the docking procedure. The docking poses of molecules **T53** and **T68** are shown in **figure 4.7**. Compound **T53** showed the highest docking score of -8.89 and exhibited H-bond interactions with residues Ala 157, Gly 104 (back bone) and Gln 100 (side chain) as well as *pi-pi* stacking interaction with residues Phe 149 and Tyr 158. The most potent antiTB compound **T68** with a docking score of -7.23 showed *pi-pi* stacking interaction with residues Phe 149 and Tyr 158. It is interesting to note that most of the active compounds have shown interaction with amino acid residue Tyr 158. The docking score of all the active molecules and details of interacting amino acid residues are given in **table 4.4.**

Table 4.4 Docking score of the active compounds and list of interacting amino acid residues.

Compounds	**Docking score**	**Interacting amino acid residues**
T53	-8.89	Phe 149, Tyr 158, Ala 157, Gly 104
T54	-7.98	Glu 219, Tyr 158
T57	-7.9	Ala 157, Gly 104, Phe 149, Tyr 158
T58	-8.11	Ala 157, Gly 104, Phe 149, Tyr 158
T64	-6.71	Phe 149
T67	-8.44	Phe 149
T68	-7.23	Phe 149, Tyr 158
T69	-7.43	Phe 149
T72	-6.28	-

4.6 Conclusions

- We synthesized a series of 1-((6-phenylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl) methylene)-2-(4-phenylthiazol-2-yl)hydrazine derivatives via one-pot three-

component approach using an ionic liquid ([Bmim]Br), with excellent product yields.

- The antTB screening of the molecules revealed that derivative **T68** is the most active molecule with an MIC of 3.125 μg/mL.
- The inhibition activity of this molecule is higher than that of the standard drugs Ethambutol and Ciprofloxacin. Other four derivatives, **T54**, **T57**, **T69** and **T72** showed better inhibitory activity than ethambutol.
- The SAR revealed that the ITD ring with a trifluoromethyl group at position-2 and a *p*-chlorophenyl substituent at position-6 could be an active core for further structural modification in order to develop potent antiTB agents.
- Further, none of the active molecules are toxic to a normal cell line which signifies the lack of general cellular toxicity. Also, these molecules are somewhat selective in their inhibition action against *Mtb* H37Rv strain.
- The molecular docking studies revealed the strong interaction of the active molecules with the target enzyme InhA. Active compounds **T53, T54, T57, T58** and **T68** showed interaction with amino acid Tyr 158 which is an important residue to interact with the long chain fatty acyl substrates required for the synthesis of mycolic acids in the mycobacteria.
- Hence, these compounds with significant anti-TB activity could serve as promising lead molecules for further development of potent antitubercular agents.

Appendix 4.1

The ^{1}H NMR, ^{13}C NMR and mass spectra of selected compounds are given below.

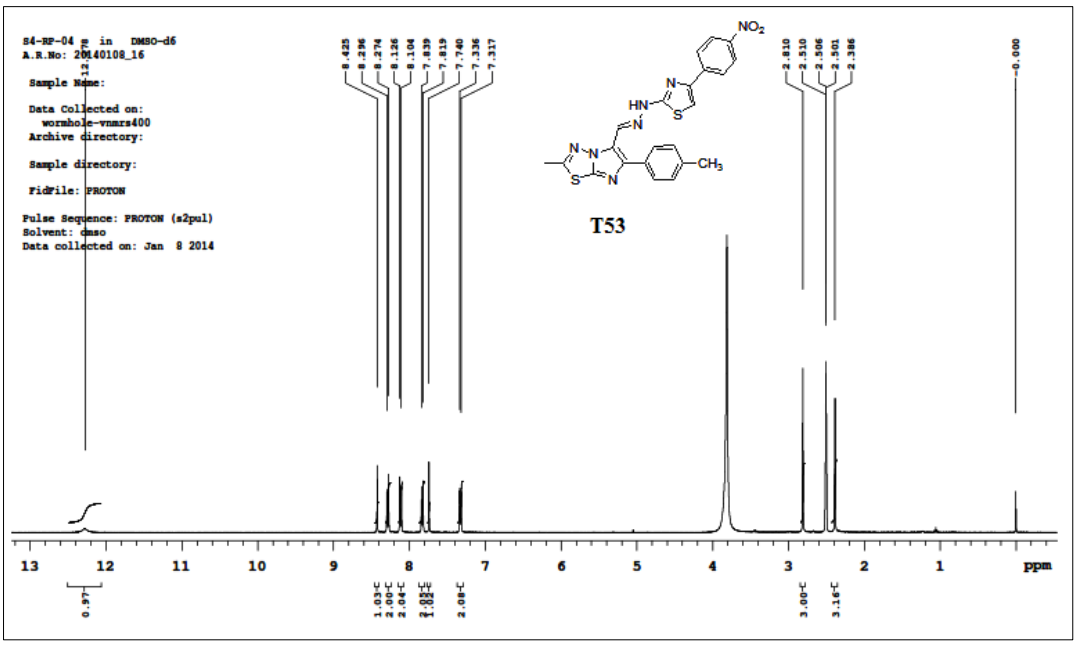


Figure 4.8 ^{1}H NMR spectrum of **T53**

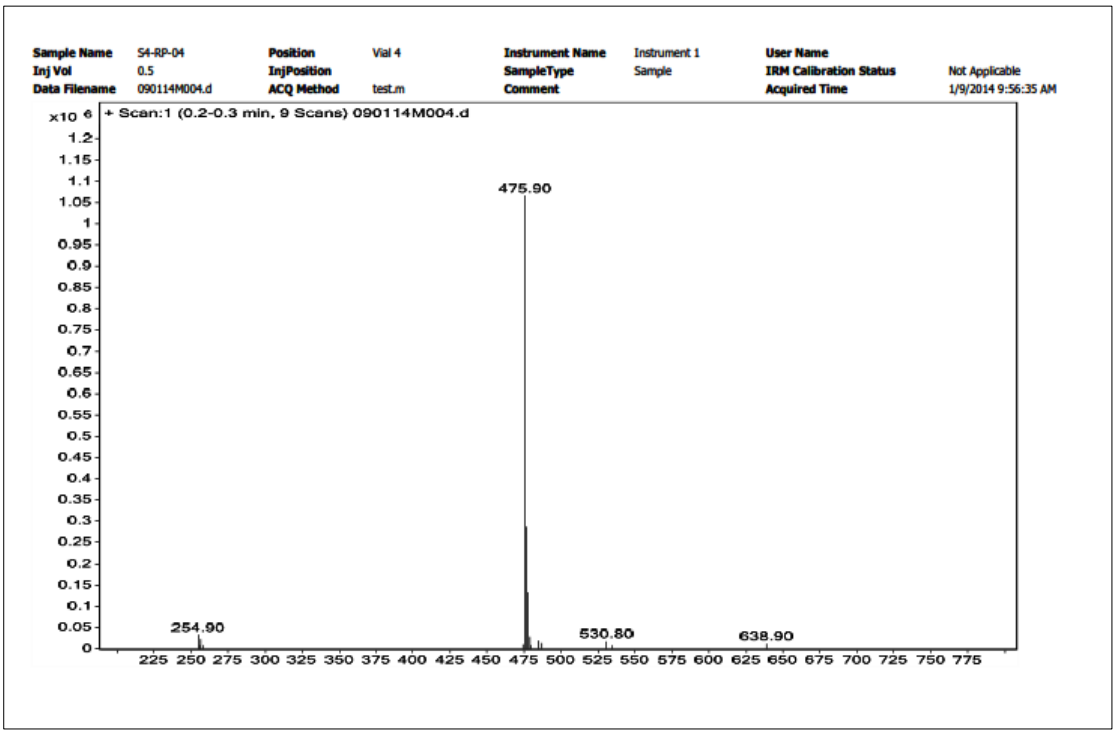


Figure 4.9 Mass spectrum of **T53**

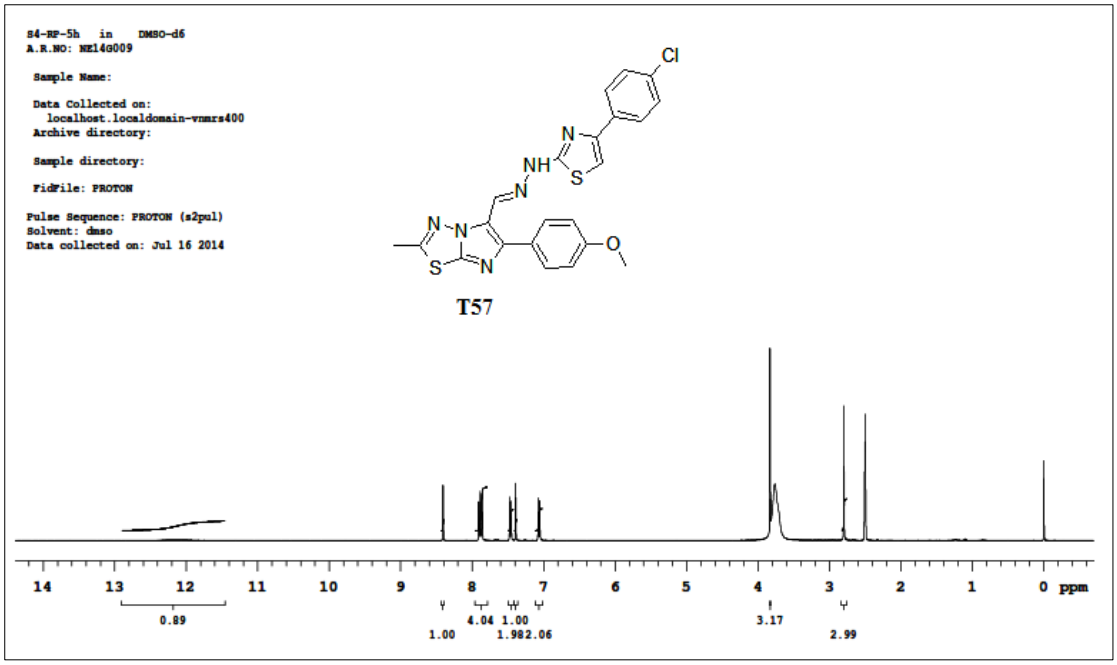


Figure 4.10 ^{1}H NMR spectrum of **T57**

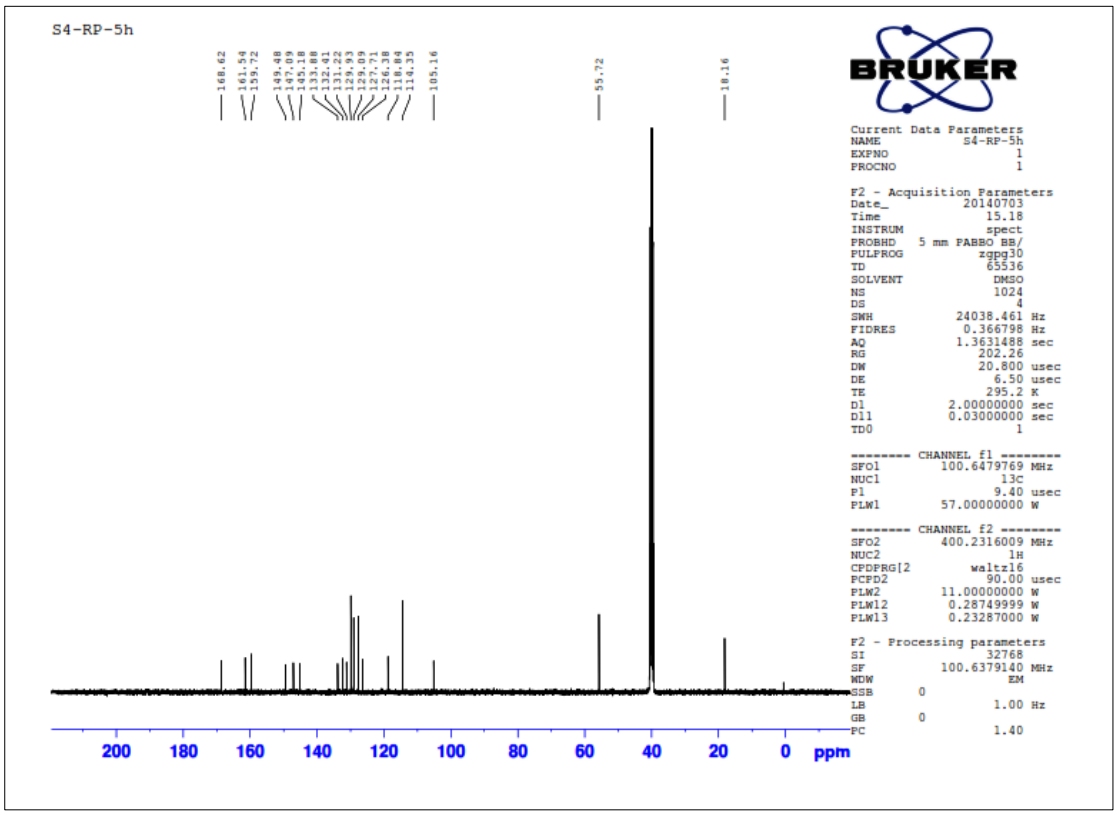


Figure 4.11 ^{13}C NMR spectrum of **T57**

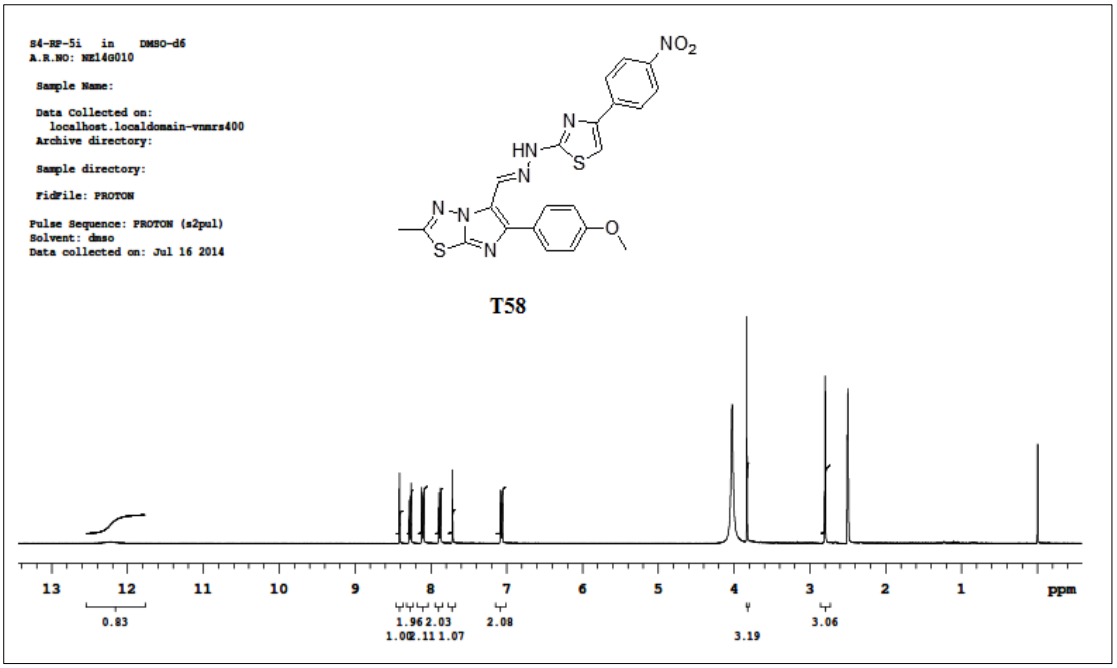


Figure 4.12 ^{1}H NMR spectrum of **T58**

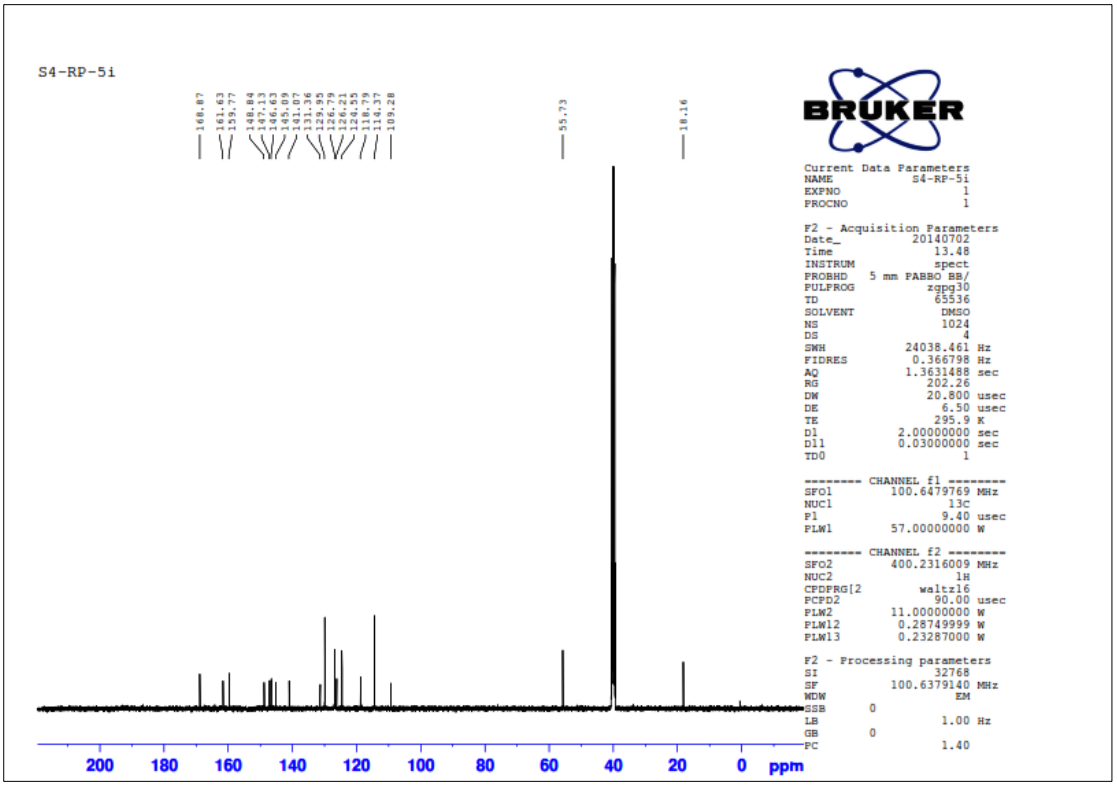


Figure 4.13 ^{13}C NMR spectrum of **T58**

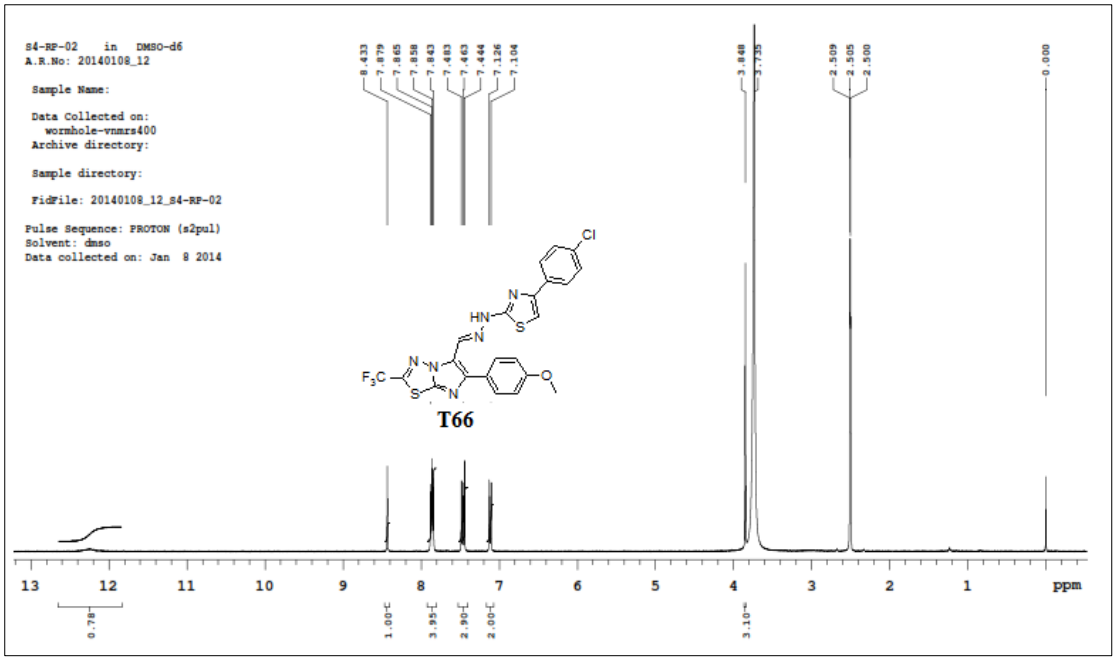


Figure 4.14 ^{1}H NMR spectrum of **T66**

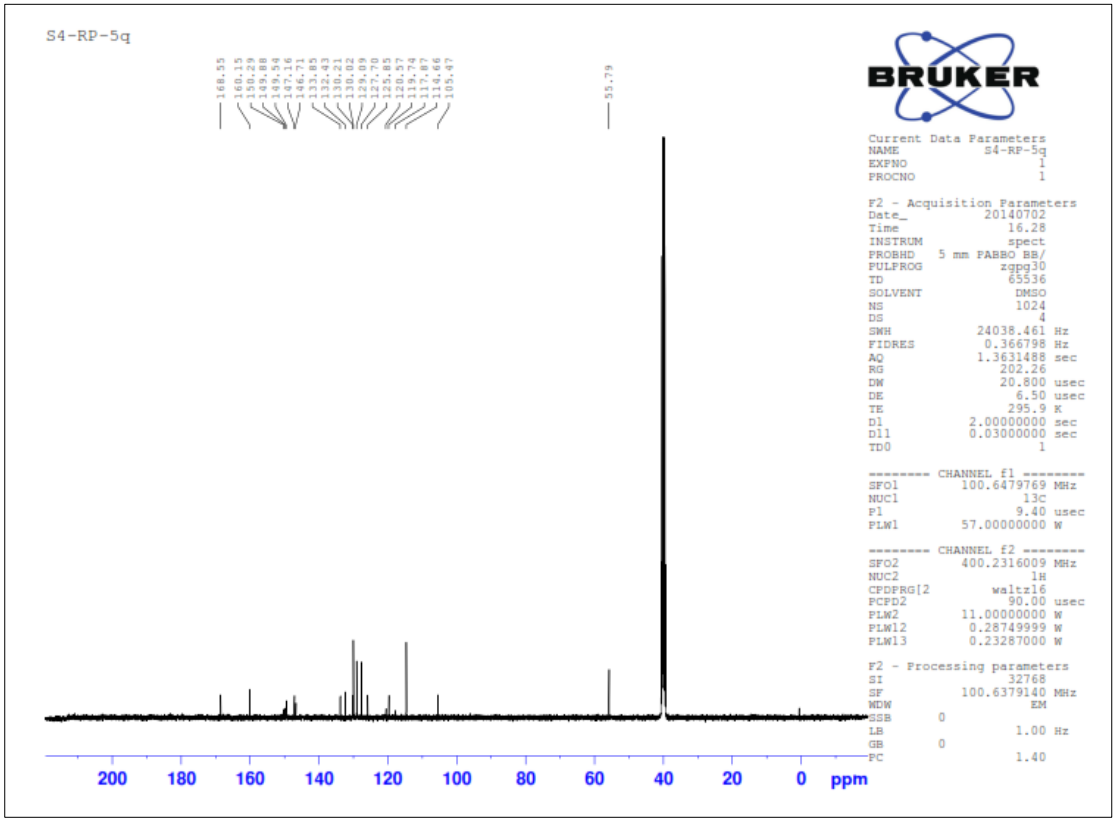


Figure 4.15 ^{13}C NMR spectrum of **T66**

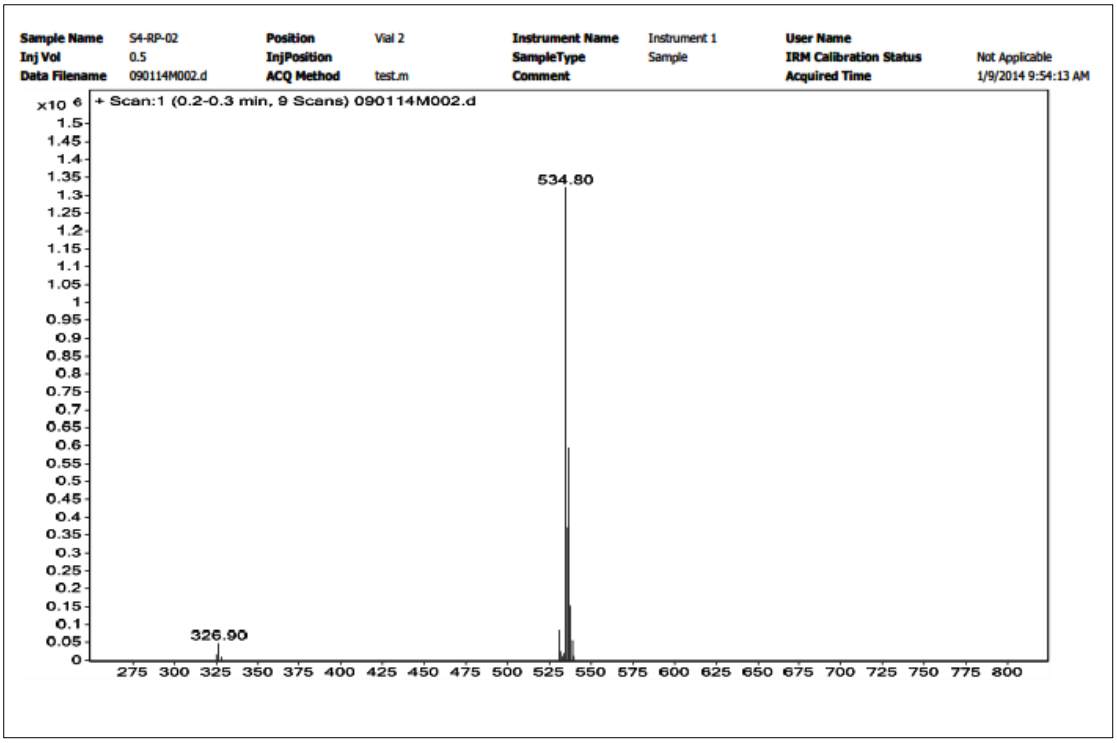


Figure 4.16 Mass spectrum of **T66**

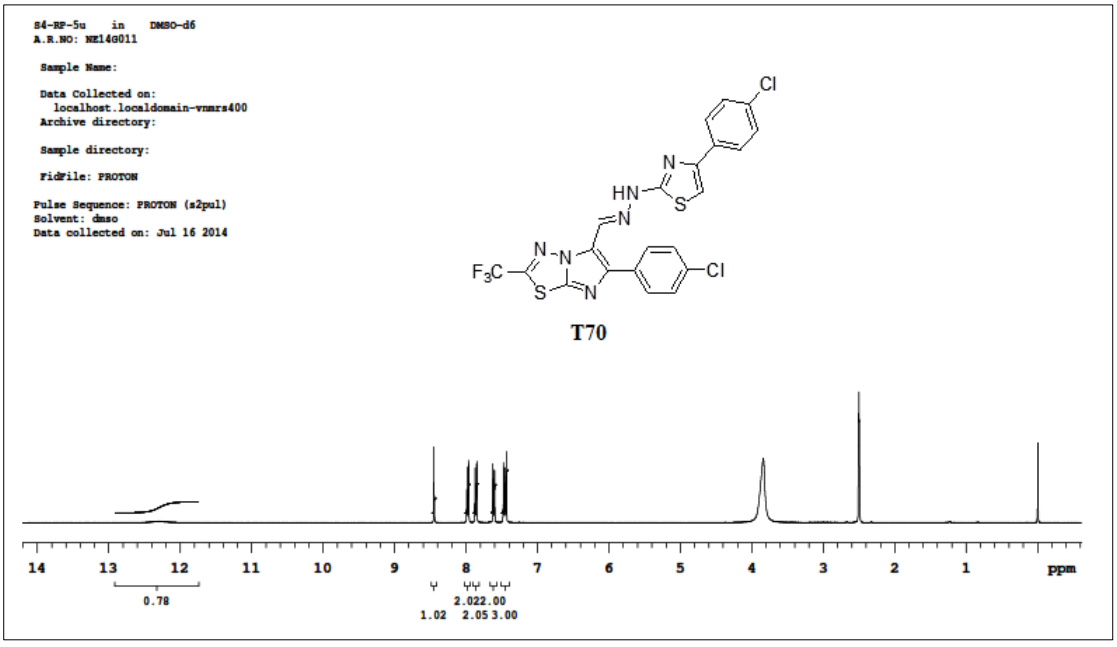


Figure 4.17 ^{1}H NMR spectrum of **T70**

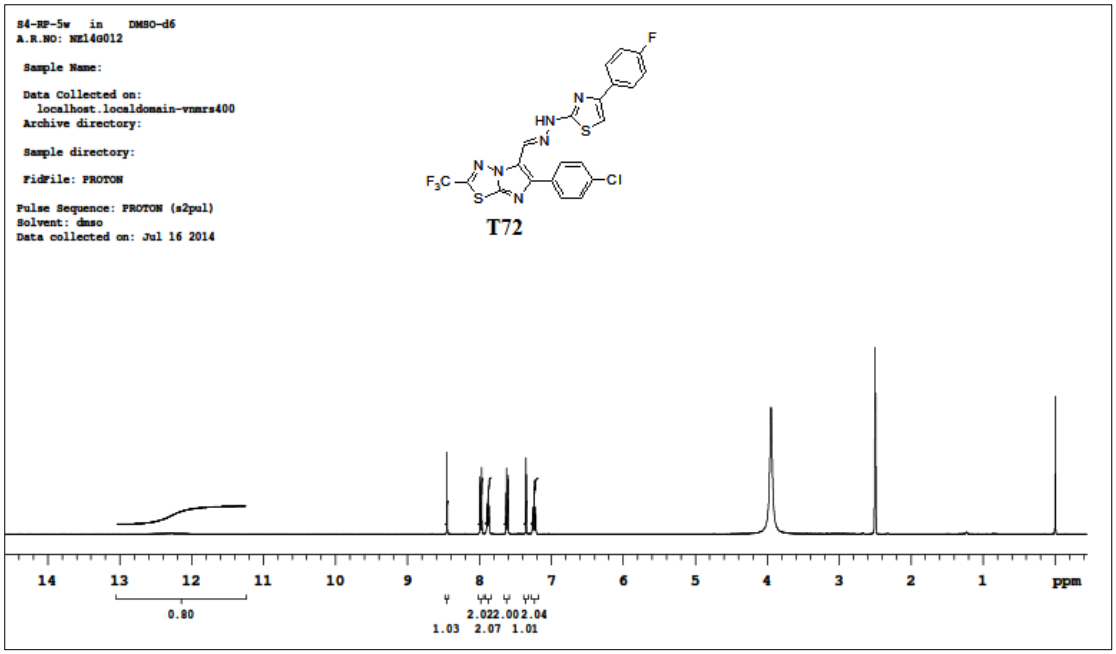


Figure 4.18 ^{1}H NMR of spectrum **T72**

CHAPTER 5

DESIGN OF NEW PHENOTHIAZINE-THIADIAZOLE HYBRIDS VIA MOLECULAR HYBRIDIZATION APPROACH FOR THE DEVELOPMENT OF POTENT ANTITUBERCULAR AGENTS

Abstract

This chapter describes the synthesis of a series of 1,3,4-thiadiazole based compounds bearing phenothiazine moiety and the in vitro antitubercular and antibacterial activity of these compounds. A detailed experimental procedure and analytical data for all the intermediates and final compounds are incorporated.

5.1 INTRODUCTION

The *in vitro* antiTB activity of phenothiazines is well-known for many years (Ordway et al. 2003; Bettencourt et al. 2000). In clinical trials, thioridazine (I) (**figure 5.1**) is being used in combination with linezolid and moxifloxacin as front-line drug in combinatorial therapeutic approaches for the treatment of *Mtb* infection (Abbate et al. 2012). Chlorpromazine (II) is effective against virulent *Mtb* strain H37Rv in cultured human macrophage model of infection and it is described as synergistic with both INH and RIF (Crowle et al. 1992). A recent report demonstrated the significant antiTB activity of a series of 4,5-dihydro-1*H*-phenothiazine containing pyrazolo[3,4-*d*]pyrimidines (III). One of the derivatives was more potent (with MIC_{MABA} value of 0.025µg/mL) than the standard drug isoniazid (Siddiqui et al. 2014). Further, several *N*-substituted phenothiazine derivatives demonstrated promising antitubercular activity (He et al. 2015; Addla et al. 2014b; Bate et al. 2007; Kunciw et al. 2012; Bansode et al. 2009).

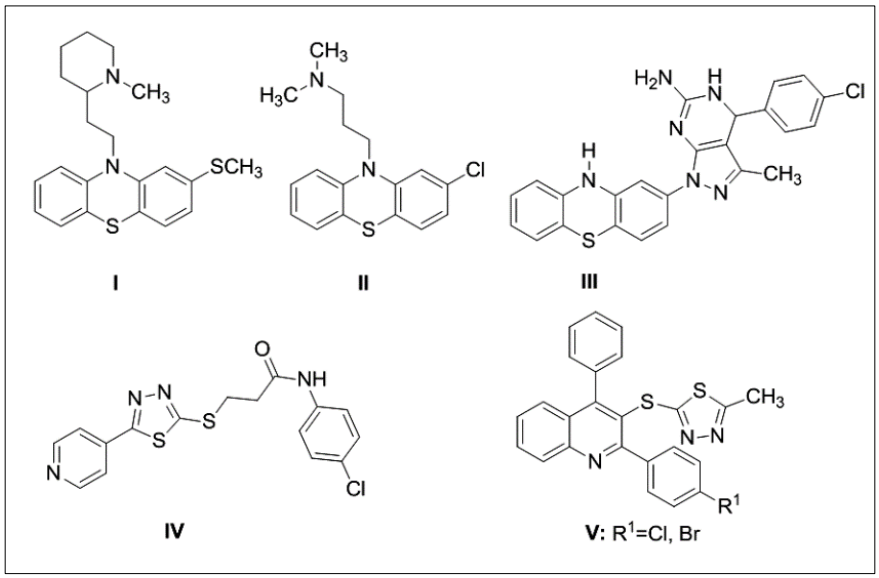


Figure 5.1 Representative phenothiazine and 1,3,4-thiadiazole based antitubercular agents.

On the other hand, 1,3,4-thiadiazole derivatives exhibited promising antitubercular activity (Foroumadi et al. 2003). For example, 3-heteroarylthioquinoline derivatives (Chitra et al. 2011) of 1,3,4-thiadiazole demonstrated MIC of ~3.5 μM against *Mtb* (IV) whereas a pyridinyl-thiadiazole derivative (V) exhibited MIC of 0.07 μM (Mahajan et al. 2015) (**figure 5.1**).

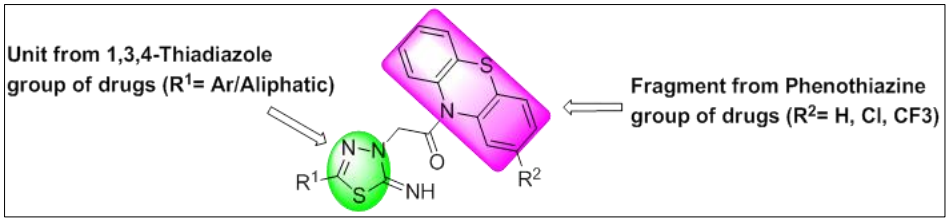
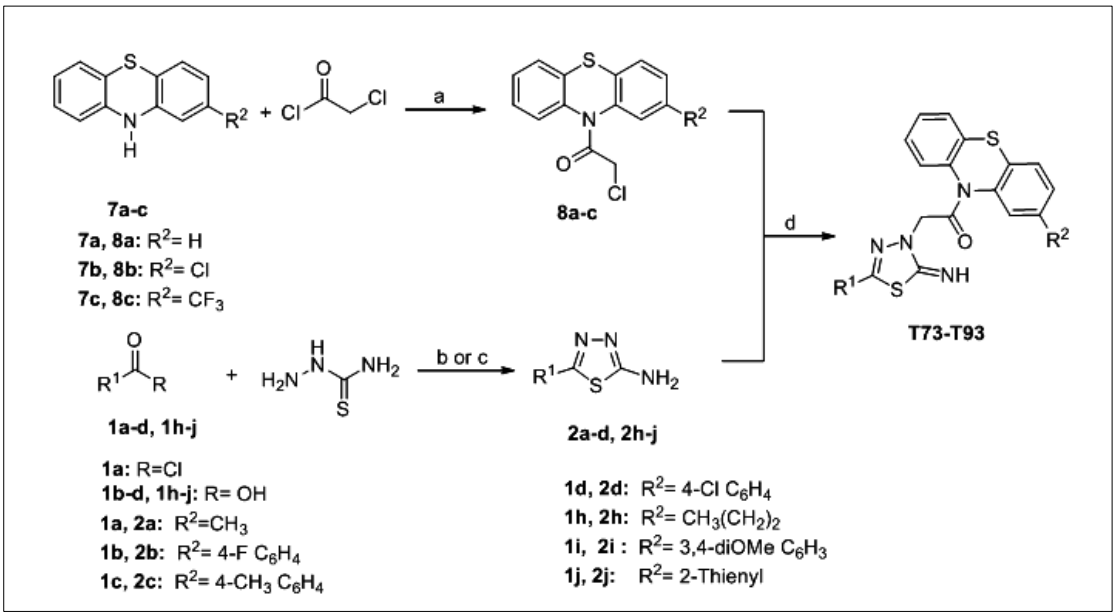


Figure 5.2 Design strategy of hybrid molecules (**T73-T93**).

In view of these facts on promising antimycobacterial activity of 1,3,4-thiadiazole and phenothiazine derivatives, we envisaged the incorporation of these two molecular units in to a single molecular framework and synthesized a new series of hybrid derivatives (**T73-T93**).

5.2 CHEMISTRY

The target phenothiazine-thiadiazole hybrids were synthesized according to the synthetic route presented in **scheme 5.1**.



Scheme 5.1 Synthesis of 1-(2-imino-1,3,4-thiadiazol-3(2*H*)-yl)-2-(10*H*-pheno thiazin-10-yl) ethanone derivatives (**T73-T93)**. Reagents and conditions a) toulene, reflux, 6

h; b) Acetyl chloride (**1a**), 0 °C - RT, 3 h; c) Substituted aromatic carboxylic acid (**1b-d, 1h-j**), $POCl_3$, 75 °C, 30 min; d) Ethanol, 80-85 °C, 17h.

2-Subsituted-1-(2-chloro-10*H*-phenothiazin-10-yl)ethanones (**8a-c)** were synthesized by the reaction of substituted phenothiazines (**7a-c)** with chloroacetyl chloride under reflux conditions. Other 5-aryl/propyl-1,3,4-thiadiazole-2-amines (**2b-d, 2h-i**) were synthesized by treating the corresponding aromatic acid (**1b-d, 1h-i**) with thiosemicarbazide in the presence of $POCl_3$. The target compounds, 1-(2-imino-1,3,4-thiadiazol-3(2*H*)-yl)-2-(10*H*-phenothiazin-10-yl)ethanone derivatives (**T73-T93**) were synthesized by the reaction between compounds **2a-d, 2h-j** and **8a-c** in ethanol under reflux conditions.

Table 5.1 Substitution pattern, yield and solubility of target compounds (**T73-T93**).

Product	**R^1**	**R^2**	**log *P*/*C* log *P*[a]**	**Yield (%)**
T73	CH_3	H	3.87/3.67	82
T74	CH_3	Cl	4.43/4.54	85
T75	CH_3	CF_3	4.79/4.83	88
T76	4-ClC_6H_4	H	6.33/5.98	88
T77	4-ClC_6H_4	Cl	6.89/6.85	90
T78	4-ClC_6H_4	CF_3	7.25/7.14	92
T79	4-$CH_3C_6H_4$	H	6.26/5.77	85
T80	4-$CH_3C_6H_4$	Cl	6.81/6.64	88
T81	4-$CH_3C_6H_4$	CF_3	7.18/6.93	91
T82	4-FC_6H_4	H	5.93/5.41	92
T83	4-FC_6H_4	Cl	6.49/6.28	95
T84	4-FC_6H_4	CF_3	6.85/6.57	88
T85	$CH_3(CH_2)_2$	H	4.94/4.73	82
T86	$CH_3(CH_2)_2$	Cl	5.50/5.60	88
T87	$CH_3(CH_2)_2$	CF_3	5.86/5.89	89
T88	3,4-diOMe C_6H_3	H	5.52/4.93	79
T89	3,4-diOMe C_6H_3	Cl	6.07/5.80	81
T90	3,4-diOMe C_6H_3	CF_3	6.44/6.09	85

T91	2-Thienyl	H	5.17/5.13	88
T92	2-Thienyl	Cl	6.31/6.00	89
T93	2-Thienyl	CF_3	6.67/6.28	87

[a]Obtained from Chemdraw ultra 12.0 software;

Note: Over all yield of compound **T73** is 63.86 %

5.3 EXPERIMENTAL

5.3.1 Materials and instruments (Refer section 2.3.1)

5.3.2 Synthesis

2-Chloro-1-(10*H*-phenothiazin-10-yl)ethanone (8a): To the solution of 10*H*-phenothiazine **7a** (5.0 g, 25.11 mmol) in toluene (75 mL) was cooled to 0 °C. To the above solution chloroacetyl chloride (3.0 mL, 37.6 mmol) was added and RT was heated at 80 °C for 12 h. The reaction mixture was allowed cool to room temperature, concentrated under reduced pressure (< 45 °C) and to the crude material water (50 mL) was added and extracted with dichloromethane (2 × 50 mL). Organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed to get compound **8a** as off white solid. Yield: 6.56 g, 95 %; m.p: 114-115 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm):7.52 (s, 2H, Ar-H), 7.40 (s, 2H, Ar-H), 7.29 (d, J = 5.7 Hz, 2H, Ar-H), 7.21 (d, J = 6.3 Hz, 2H, Ar-H), 4.12 (s, 2H, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 165.5, 137.9, 133.1, 128.1, 127.4, 127.3, 126.5, 41.8; ESI-MS (*m/z*) = 276.1 $(M+H)^+$; calculated for $C_{14}H_{10}ClNOS$; C, 60.98; H, 3.66; N, 5.08; S, 11.63. Found: C, 60.87; H, 3.68; N, 5.10; S, 11.65.

2-Chloro-1-(2-chloro-10*H*-phenothiazin-10-yl)ethanone (8b): White solid. Yield: 6.0 g, 90 %; m.p: 118-119 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.58 (s, 1H, Ar-H), 7.46 (s, 1H, Ar-H), 7.39 (s, 1H, Ar-H), 7.31 (d, J = 3.4 Hz, 2H, Ar-H), 7.21 (d, J = 14.9 Hz, 2H, Ar-H), 4.16 (d, J = 12.5 Hz, 1H, CH), 4.08 (d, J = 12.5 Hz, CH); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm):165.4, 139.2, 137.5, 132.4, 132.3, 131.9, 129.7, 128.6, 128.3, 128.2, 127.9, 127.4, 127.3, 43.2; ESI-MS (*m/z*) = 310.1 $(M+H)^+$; calculated for $C_{14}H_9Cl_2NOS$; C, 54.21; H, 2.92; N, 4.52; S, 10.34. Found: C, 54.18; H, 2.93; N, 4.55; S, 10.35.

2-Chloro-1-(2-(trifluoromethyl)-10*H*-phenothiazin-10-yl)ethanone (8c): White solid. Yield: 6.16 g, 96 %; m.p: 110-111 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.84 (s, 1H, Ar-H), 7.54 – 7.38 (m, 4H, Ar-H), 7.33 (s, 1H, Ar-H), 7.27 (s, 1H, Ar-H), 4.18 (d, J = 12.5 Hz, 1H, CH), 4.06 (d, J = 12.5 Hz, 1H, CH); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 165.4, 138.0, 137.1, 128.9, 128.3, 128.2, 127.9, 126.1, 125.2, 124.1, 123.9, 41.5; ESI-MS (*m/z*) = 344.1 $(M+H)^+$; calculated for $C_{15}H_9ClF_3NOS$; C, 52.41; H, 2.64; N, 4.07; S, 9.33. Found: C, 52.38; H, 2.63; N, 4.05; S, 9.35.

5-Propyl-1,3,4-thiadiazol-2-amine (2h): White solid. Yield: 6.33 g, 78 %; m.p: 209-210 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 6.82 (br s, 2H), 2.61 (t, J = 7.6 Hz, 2H), 1.61 (sext, J = 7.6 Hz, 2H), 0.92 (t, J = 7.6 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 165.7, 148.8, 31.4, 21.7, 13.3; ESI-MS (*m/z*) = 144.1 $(M+H)^+$; calculated for $C_5H_9N_3S$; C, 41.93; H, 6.33; N, 29.34; S, 22.39. Found: C, 41.89; H, 6.35; N, 29.22; S, 22.44.

5-(3,4-Dimethoxyphenyl)-1,3,4-thiadiazol-2-amine (2i): White solid. Yield: 5.53 g, 85 %; m.p: 213-214 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.65–8.69 (m, 3H, Ar-H), 7.61 (br s, 2H), 3.71 (s, 3H, OCH_3), 3.70 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 168.7, 154.1, 144.1, 132.8, 125.5, 118.4, 115.8, 108.1, 55.8, 55.2; ESI-MS (*m/z*) = 238.1 $(M+H)^+$; calculated for $C_{10}H_{11}N_3O_2S$; C, 50.62; H, 4.67; N, 17.71; S, 13.51. Found: C, 50.72; H, 4.66; N, 17.75; S, 13.54.

5-(Thiophen-2-yl)-1,3,4-thiadiazol-2-amine (2j): White solid. Yield: 6.57 g, 92 %; m.p: 175-176 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.70 – 7.64 (m, 2H, Ar-H), 7.63 – 7.57 (m, 1H, Ar-H), 7.34 (br s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 169.3, 154.3, 134.9, 131.6, 133.7, 127.4; ESI-MS (*m/z*) = 183.9 $(M+H)^+$; calculated for $C_6H_5N_3S_2$; C, 39.32; H, 2.75; N, 22.93; S, 35.00. Found: C, 39.29; H, 2.74; N, 22.89; S, 35.03.

General Procedure for the Synthesis of derivatives (T73-T93): A mixture of 5-subsituted 2-amino-1,3,4-thiadiazole (**2a-d, 2h-j**) (0.2 g, 1.0 mmol) and 2-chloro-1-(2-substituted)-10*H*-phenothiazin-10-yl)ethanone (**8a-c**) (1.0 mmol) in ethanol was stirred at 80-85 °C for 17 h. After the completion of reaction (as confirmed by TLC),

the reaction mass was cooled to room temperature and then concentrated under reduced pressure. The obtained crude product was basified with aqueous 10% Na_2CO_3 solution. To the above solution ethyl acetate (20 mL) was added, the organic layer was separated, washed with brine solution (2 × 10 mL), dried over anhydrous $Na_2S_2O_4$ and concentrated under reduced pressure. The crude residue was purified over silica gel column chromatography (100-200 mesh) eluted with methanol and dichloromethane (0.5: 9.5) to give pure products **T73-T93**.

2-(2-Imino-5-methyl-1,3,4-thiadiazol-3(2*H*)-yl)-1-(10*H*-phenothiazin-10-yl) ethanone (T73): White solid. Yield: 0.647 g; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.16 (br s, 1H, NH, D_2O exchangeable proton), 7.68 (s, 2H, Ar-H), 7.56 (d, J = 7.3 Hz, 2H, Ar-H), 7.40 (t, J = 7.2 Hz, 2H, Ar-H), 7.32 (t, J = 7.3 Hz, 2H, Ar-H), 4.74 (s, 2H), 2.20 (s, 3H, CH_3); ^{1}H NMR (400 MHz, D_2O) δ 7.61 (s, 2H), 7.53 (d, J = 7.4 Hz, 2H), 7.36 (t, J = 7.3 Hz, 2H), 7.29 (t, J = 7.4 Hz, 2H), 4.68 (s, 2H), 2.15 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 165.9, 137.9, 132.5, 128.4, 127.9, 127.8, 127.5, 124.7, 49.3, 17.08; ESI-MS (*m/z*) = 355.1 $(M+H)^+$; anal. calcd (%) for $C_{17}H_{14}N_4OS_2$: C, 57.61; H, 3.98; N, 15.81; S, 18.09. Found: C, 57.42; H, 4.02; N, 15.78; S, 18.12.

1-(2-Chloro-10*H*-phenothiazin-10-yl)-2-(2-imino-5-methyl-1,3,4-thiadiazol-3(2*H*)yl)ethanone (T74): Off-white solid. Yield: 0.573 g; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.95 (br s, 1H, NH), 7.78 (s, 1H, Ar-H), 7.70 (d, J = 7.5 Hz, 1H, Ar-H), 7.57 (d, J = 8.3 Hz, 2H, Ar-H), 7.45 – 7.37 (m, 2H, Ar-H), 7.34 (t, J = 7.6 Hz, 1H, Ar-H), 4.84 (s, 1H, CH), 4.62 (s, 1H, CH), 2.18 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 168.3, 141.6, 140.3, 138.2, 137.7, 134.5, 129.3, 128.3, 127.6, 125.7, 125.1, 124.4, 122.2, 121.2, 49.7, 17.1; ESI-MS (*m/z*) = 389.1 $(M+H)^+$; anal. calcd (%) for $C_{17}H_{13}ClN_4OS_2$: C, 52.50; H, 3.37; N, 14.41; S, 16.49. Found: C, 52.48; H, 3.40; N, 14.38; S, 16.52.

2-(2-Imino-5-methyl-1,3,4-thiadiazol-3(2*H*)-yl)-1-(2-(trifluoromethyl)-10*H*-phenothiazin-10-yl)ethanone (T75): Light brown solid. Yield: 0.645 g; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.98 (br s, 1H, NH), 7.84 (d, J = 8.0 Hz, 1H, Ar-H), 7.68 (dd, J = 22.2, 7.5 Hz, 3H, Ar-H), 7.52 – 7.35 (m, 3H, Ar-H), 4.74 (s, 2H, CH_2),

2.46 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 167.4, 143.9, 141.81, 138.87, 137.73, 129.9, 128.33, 127.6, 127.04, 125.77, 125.12, 124.84, 122.29, 116.02, 52.79, 17.6; ESI-MS (*m/z*) = 423.1 $(M+H)^+$; anal. calcd (%) for $C_{18}H_{13}F_3N_4OS_2$: C, 51.18; H, 3.10; N, 13.26; S, 15.18. Found: C, 51.22; H, 3.12; N, 13.28; S, 15.22.

2-(5-(4-Chlorophenyl)-2-imino-1,3,4-thiadiazol-3(2*H*)-yl)-1-(10*H*-phenothiazin-10-yl)ethanone (T76): White solid. Yield: 0.375 g; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.01 (br s, 1H, NH), 7.78 (d, *J* = 7.5 Hz, 4H, Ar-H), 7.61 (d, *J* = 7.7 Hz, 4H, Ar-H), 7.46 (s, 2H, Ar-H), 7.38 (s, 2H, Ar-H), 5.27 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 164.1, 137.4, 137.3, 137.0, 136.9, 130.0, 129.6, 128.6, 128.1, 127.3, 52.8; ESI-MS (*m/z*) = 451.1 $(M+H)^+$; anal. calcd (%) for $C_{22}H_{15}ClN_4OS_2$: C, 58.59; H, 3.35; N, 12.42; S, 14.22. Found: C, 58.52; H, 3.32; N, 12.48; S, 14.25.

1-(2-Chloro-10*H*-phenothiazin-10-yl)-2-(5-(4-chlorophenyl)-2-imino-1,3,4-thiadiazol-3(2*H*)-yl)ethanone (T77): Light brown solid. Yield: 0.412 g; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.02 (br s, 1NH), 7.82-7.75 (m, 4H), 7.60 (d, *J* = 7.8 Hz, 4H), 7.45 (m, 3H), 5.28 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 163.5, 146.8, 146.7, 145.8, 144.7, 142.3, 141.5, 137.6, 137.5, 137.2, 130.1, 129.0, 126.5, 53.9; ESI-MS (*m/z*) = 484.9 $(M+H)^+$; anal. calcd (%) for $C_{22}H_{14}Cl_2N_4OS_2$: C, 54.44; H, 2.91; N, 11.54; S, 13.21. Found: C, 54.48; H, 2.91; N, 11.56; S, 13.23.

2-(5-(4-Chlorophenyl)-2-imino-1,3,4-thiadiazol-3(2*H*)-yl)-1-(2-(trifluoromethyl)-10*H*-phenothiazin-10-yl)ethanone (T78): Light brown solid. Yield: 0.451 g; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.08 (br s, 1NH), 7.79 (s, 1H), 7.68 (d, *J* = 7.8 Hz, 2H), 7.60-7.54 (m, 5H), 7.45 (d, *J* = 7.4 Hz, 2H), 7.40 (s, 1H, Ar-H), 4.83 (s, 1H), 4.70 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 166.15, 138.2, 135.1, 129.7, 129.4, 129.3, 128.7 128.5, 128.3, 127.5, 124.4, 124.3, 124.2, 122.9, 49.84; ESI-MS (*m/z*) = 519.1 $(M+H)^+$; anal. calcd (%) for $C_{23}H_{14}ClF_3N_4OS_2$: C, 53.23; H, 2.72; N, 10.80; S, 12.36. Found: C, 53.33; H, 2.71; N, 10.79; S, 12.40.

2-(2-Imino-5-p-tolyl-1,3,4-thiadiazol-3(2*H*)-yl)-1-(10*H*-phenothiazin-10-yl) ethanone (T79): Off-white solid. Yield: 0.382 g; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.78 (s, 1H), 7.73 (s, 2H), 7.58 (d, *J* = 6.5 Hz, 2H), 7.48 (d, *J* = 6.7 Hz, 2H),

7.41 (s, 2H), 7.33 (s, 2H), 7.28 (d, *J* = 6.4 Hz, 2H), 4.96 (s, 2H), 2.33 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 165.5, 141.0, 137.8, 132.5, 130.2, 128.5, 127.9, 127.5, 127.3, 126.07, 50.50, 21.42; ESI-MS (*m/z*) = 431.1 $(M+H)^+$; anal. calcd (%) for $C_{23}H_{18}N_4OS_2$: C, 64.16; H, 4.21; N, 13.01; S, 14.90. Found: C, 64.20; H, 4.19; N, 13.10; S, 14.88.

1-(2-Chloro-10*H*-phenothiazin-10-yl)-2-(2-imino-5-(p-tolyl)-1,3,4-thiadiazol-3(2*H*) yl)ethanone (T80): Light yellow solid. Yield: 0.426 g; ^{1}H NMR (400 MHz, DMSO) δ 8.45 (br s, NH), 7.82 (s, 1H, Ar-H), 7.73-7.69 (m, 2H, Ar-H), 7.60 (d, *J* = 7.4 Hz, 2H), 7.48 (d, *J* = 7.3 Hz, 2H), 7.41 (s, 2H), 7.25 – 7.17 (m, 2H 96 (s, 2H), 2.38 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 165.7, 141.6, 139.1, 136.8, 135.4, 136.7, 135.5, 130.0, 129.3, 129.0, 128.4, 127.9, 126.3, 125.8, 125.4, 124.6, 122.6, 121.2, 51.2, 21.33; ESI-MS (*m/z*) = 464.1 $(M+H)^+$; anal. Calcd (%) for $C_{23}H_{17}ClN_4OS_2$: C, 59.41; H, 3.69; N, 12.05; S, 13.79. Found: C, 59.43; H, 3.70; N, 12.08; S, 13.80.

2-(2-Imino-5-(p-tolyl)-1,3,4-thiadiazol-3(2*H*)-yl)-1-(2-(trifluoromethyl)-10*H*-phenothiazin-10-yl)ethanone (T81): Yellow solid. Yield: 0.474 g; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.38 (br s, NH, 1H), 7.92 (s, 1H, Ar-H), 7.71 (t, *J* = 6.1 Hz, 2H), 7.66 – 7.58 (m, 4H, Ar-H), 7.40 (d, *J* = 7.4 Hz, 2H, Ar-H), 7.31 (dd, *J* = 14.23, 7.4 Hz, 2H, Ar-H), 4.83 (s, 1H), 4.70 (s, 1H), 2.35 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 167.7, 143.9, 142.81, 140.1, 137.7, 136.4, 131.0, 129.9, 129.0, 128.3, 127.6, 127.0, 126.2, 125.7, 125.1 , 124.8, 122.2, 116.0, 52.7, 21.3; ESI-MS (*m/z*) = 499.1 $(M+H)^+$; anal. calcd (%) for $C_{24}H_{17}F_3N_4OS_2$: C, 57.82; H, 3.44; N, 11.24; S, 12.86. Found: C, 57.78; H, 3.48; N, 11.22; S, 12.90.

2-(5-(4-Fluorophenyl)-2-imino-1,3,4-thiadiazol-3(2*H*)-yl)-1-(10*H*-phenothiazin-10-yl)ethanone (T82): Light brown solid. Yield: 0.409 g; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.35 (br s, 1H, NH), 7.71 (s, 2H, Ar-H), 7.63 – 7.54 (m, 4H, Ar-H), 7.40 (t, *J* = 7.6 Hz, 2H, Ar-H), 7.31 (dd, *J* = 16.3, 7.8 Hz, 4H, Ar-H), 4.88 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 166.1, 162.8, 156.4, 137.7, 129.3, 128.4, 128.2, 127.6, 125.86, 122.3, 114.6, 50.21; ESI-MS (*m/z*):= 435.1 $(M+H)^+$; anal. calcd

(%) for $C_{22}H_{15}FN_4OS_2$: C, 60.81; H, 3.48; N, 12.89; S, 14.76. Found: C, 60.77; H, 3.50; N, 12.88; S, 14.70.

1-(2-Chloro-10*H*-phenothiazin-10-yl)-2-(5-(4-fluorophenyl)-2-imino-1,3,4-thiadiazol-3(2*H*)-yl)ethanone (T83): Brown solid. Yield: 0.456 g; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.35 (br s, 1H, NH), 7.82 (s, 1H, Ar-H), 7.73 (s, 1H, Ar-H), 7.64 – 7.53 (m, 3H), 7.46-7.35 (m , 3H, Ar-H), 7.31 (dd, *J* = 15.7, 7.0 Hz, 3H), 5.01 (s, 1H), 4.77 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 166.0, 164.67, 159.91, 159.24, 158.90, 149.25, 139.1, 132.2, 129.57, 128.65, 127.9, 127.5, 127.46, 117.3, 116.7, 49.8; ESI-MS (*m/z*) = 469.1 $(M+H)^+$; anal. calcd (%) for $C_{22}H_{14}ClFN_4OS_2$: C, 56.35; H, 3.01; N, 11.95; S, 13.68. Found: C, 56.28; H, 3.00; N, 11.93; S, 13.70.

2-(5-(4-Fluorophenyl)-2-imino-1,3,4-thiadiazol-3(2*H*)-yl)-1-(2-(trifluoromethyl)-10*H*-phenothiazin-10-yl)ethanone (T84): Yellow solid. Yield: 0.453 g; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.33 (br s, 1H, NH), 8.03 (s, 1H, Ar-H), 7.78 (d, *J* = 8.2 Hz, 2H, Ar-H), 7.65 (d, *J* = 6.9 Hz, 1H, Ar-H), 7.60 (dd, *J* = 8.9, 5.3 Hz, 3H, Ar-H), 7.45 (dd, *J* = 14.2, 7.6 Hz, 1H, Ar-H), 7.38 (dd, *J* = 13.0, 6.3 Hz, 1H), 7.34 – 7.26 (m, 2H, Ar-H), 5.06 (s, 1H), 4.75 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 166.1, 162.22, 138.3, 137.1, 129.3, 128.76, 128.5, 128.3, 128.2, 128.15, 124.3, 116.8, 116.6, 49.8; ESI-MS (*m/z*) = 503.1 $(M+H)^+$; anal. calcd (%) for $C_{23}H_{14}F_4N_4OS_2$: C, 54.97; H, 2.81; N, 11.15; S, 12.76. Found: C, 54.88; H, 2.81; N, 11.16; S, 12.66.

2-(2-Imino-5-propyl-1,3,4-thiadiazol-3(2*H*)-yl)-1-(10*H*-phenothiazin-10-yl) ethanone (T85): Off-white solid. Yield: 0.432 g; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.68 (s, 2H, Ar-H), 7.63 (d, *J* = 6.9 Hz, 2H, Ar-H), 7.45 (s, 2H, Ar-H), 7.38 (s, 2H, Ar-H), 5.84 (s, 1H), 4.92 (s, 1H), 2.80 (t, *J* = 7.2 Hz, 2H), 1.61 (sextet, *J* = 7.2 Hz, 2H), 0.88 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm):169.01, 163.5, 158.3, 158.1, 128.7, 128.6, 128.5, 128.3, 128.2, 53.3, 31.5, 21.7, 13.4; ESI-MS (*m/z*) = 383.1 $(M+H)^+$; anal. calcd (%) for $C_{20}H_{14}N_4OS_3$: C, 56.85; H, 3.34; N, 13.26; S, 22.77. Found: C, 56.94; H, 3.36; N, 13.24; S, 22.69.

1-(2-Chloro-10*H*-phenothiazin-10-yl)-2-(2-imino-5-propyl-1,3,4-thiadiazol-3(2*H*)yl)ethanone (T86): Light yellow solid. Yield: 0.504 g; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.21 (br s, 1H, NH), 7.79 (s, 1H, Ar-H), 7.70 (d, J = 7.6 Hz, 2H), 7.63 – 7.54 (m, 2H, Ar-H), 7.19 (d, J = 7.6 Hz, 2H), 5.08 (s, 1H), 4.85 (s, 1H), 2.77 (t, J = 7.8 Hz, 2H), 1.54 (sextet, J = 7.8 Hz, 2H), 0.89 (t, J = 7.8 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 168.2, 163.8, 159.3, 141.6, 138.5, 134.5, 130.2, 128.3, 127.9, 126.7, 125.4, 124.5, 122.31, 121.23, 49.5, 31.3, 21.4, 13.2; ESI-MS (*m/z*) = 417.1 $(M+H)^+$; anal. calcd (%) for $C_{19}H_{17}ClN_4OS_2$: C, 54.73; H, 4.11; N, 13.44; S, 15.38. Found: C, 54.62; H, 4.14; N, 13.39; S, 15.44.

2-(2-Imino-5-propyl-1,3,4-thiadiazol-3(2*H*)-yl)-1-(2-(trifluoromethyl)-10*H*-phenothiazin-10-yl)ethanone (T87): Light brown solid. Yield: 0.560 g; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.21 (s, 1H, Ar-H), 7.89 – 7.73 (m, 2H, Ar-H), 7.73 – 7.54 (m, 3H, Ar-H), 7.52 – 7.29 (m, 2H, Ar-H), 4.80 (s, 2H, CH_2), 2.79 (t, J = 7.3 Hz, 2H), 1.57 (sextet, J = 7.2 Hz, 2H), 0.89 (t, J = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 168.3, 163.4, 159.4, 144.9, 138.5, 137.7, 136.82, 133.5, 132.3, 125.5, 125.0, 124.3, 122.2, 116.0, 50.8, 31.2, 22.1, 13.5; ESI-MS (*m/z*) = 451.1 $(M+H)^+$; anal. calcd (%) for $C_{20}H_{17}F_3N_4OS_2$: C, 53.32; H, 3.80; N, 12.44; S, 14.24. Found: C, 53.38; H, 3.81; N, 12.45; S, 14.26.

2-(5-(3,4-Dimethoxyphenyl)-2-imino-1,3,4-thiadiazol-3(2*H*)-yl)-1-(10*H*-pheno thiazin-10-yl)ethanone (T88): White solid. Yield: 0.317 g; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.20 (s, 1H), 7.69 (d, J = 17.4 Hz, 2H, Ar-H), 7.60 – 7.53 (m, 2H, Ar-H), 7.40 (t, J = 7.0 Hz, 2H, Ar-H), 7.32 (t, J = 7.1 Hz, 2H, Ar-H), 7.12 (s, 1H, Ar-H), 7.06 – 6.97 (m, 2H, Ar-H), 4.86 (s, 2H), 3.78 (s, 6H, dimethoxy); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 169.2, 159.0, 158.7, 149.4, 145.3, 141.9, 136.7, 132.7, 132.6, 128.4, 127.8, 127.5, 119.7, 112.2, 108.0, 56.1, 56.0, 49.8; ESI-MS (*m/z*) = 477.2 $(M+H)^+$; anal. calcd (%) for $C_{24}H_{20}N_4O_3S_2$: C, 60.49; H, 4.23; N, 11.76; S, 13.46. Found: C, 60.52; H, 4.23; N, 11.78; S, 13.50.

1-(2-Chloro-10*H*-phenothiazin-10-yl)-2-(5-(3,4-dimethoxyphenyl)-2-imino-1,3,4-thiadiazol-3(2*H*)-yl)ethanone (T89): Off-white solid. Yield: 0.348 g; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.19 (s, 1H), 7.98 (s, 1H, Ar-H), 7.74 (d, J = 7.4 Hz, 2H,

Ar-H), 7.64 (d, *J* = 7.2 Hz, 1H, Ar-H), 7.57 – 7.51 (m, 1H, Ar-H), 7.46 (t, *J* = 7.6 Hz, 1H, Ar-H), 7.32 (t, *J* = 7.3 Hz, 1H, Ar-H), 7.21 (s, 1H, Ar-H), 7.04 – 6.95 (m, 2H, Ar-H), 4.98 (s, 1H), 4.69 (s, 1H), 3.74 (s, 6H, dimethoxy); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 169.6, 161.8, 155.8, 153.1, 150.6, 141.6, 137.7, 134.5, 129.3, 128.2, 127.6, 125.7, 125.1, 124.4, 122.2, 121.2, 118.4, 115.8, 107.9, 56.8, 56.5, 50.3; ESI-MS (*m/z*) = 511.1 $(M+H)^+$; anal. Calcd (%) for $C_{24}H_{19}ClN_4O_3S_2$: C, 56.41; H, 3.75; N, 10.96; S, 12.55. Found: C, 56.38; H, 3.74; N, 10.97; S, 12.49.

2-(5-(3,4-Dimethoxyphenyl)-2-imino-1,3,4-thiadiazol-3(2*H*)-yl)-1-(2-(trifluoro methyl)-10*H*-phenothiazin-10-yl)ethanone (T90): Brown solid. Yield: 0.390 g; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.25 (br s, 1H, NH), 8.05 (s, 1H, Ar-H), 7.79 (d, *J* = 8.2 Hz, 2H, Ar-H), 7.66 (d, *J* = 7.1 Hz, 1H, Ar-H), 7.64 – 7.57 (m, 1H, Ar-H), 7.46 (t, *J* = 7.0 Hz, 1H, Ar-H), 7.37 (t, *J* = 7.3 Hz, 1H, Ar-H), 7.15 – 7.08 (m, 1H, Ar-H), 7.04 – 6.97 (m, 2H, Ar-H), 5.07 (s, 1H, CH), 4.71 (s, 1H, CH), 3.78 (s, 6H, dimethoxy); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 169.7, 159.8, 155.84 (s), 153.1, 150.6, 143.9, 137.7, 137.7, 133.8, 131.0, 125.9, 125.5, 125.03, 124.04, 122.2, 118.4, 116.0, 115.8, 107.9, 56.2, 55.9, 49.9; ESI-MS (*m/z*) = 545.1 $(M+H)^+$; anal. calcd (%) for $C_{25}H_{19}F_3N_4O_3S_2$: C, 55.14; H, 3.52; N, 10.29; S, 11.78. Found: C, 55.10; H, 3.56; N, 10.22; S, 11.80.

2-(2-Imino-5-(thiophen-2-yl)-1,3,4-thiadiazol-3(2*H*)-yl)-1-(10*H*-phenothiazin-10-yl)ethanone (T91): Light brown solid. Yield: 0.405 g; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.33 (br s, 1H, NH), 7.72 (s, 2H, Ar-H), 7.68 (dd, *J* = 5.1, 1.1 Hz, 1H, Ar-H), 7.57 (d, *J* = 7.7 Hz, 2H, Ar-H), 7.39 (dd, *J* = 10.9, 4.4 Hz, 2H, Ar-H), 7.32 (dd, *J* = 8.4, 4.6 Hz, 3H, Ar-H), 7.11 (dd, *J* = 5.1, 3.7 Hz, 1H, Ar-H), 4.84 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 165.9, 137.9, 129.0, 128.9, 128.7, 128.5, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5,127.4, 49.7; ESI-MS (*m/z*) = 423.1 $(M+H)^+$; anal. calcd (%) for $C_{20}H_{14}N_4OS_3$: C, 56.85; H, 3.34; N, 13.26; S, 22.77. Found: C, 56.94; H, 3.36; N, 13.24; S, 22.69.

1-(2-Chloro-10*H*-phenothiazin-10-yl)-2-(2-imino-5-(thiophen-2-yl)-1,3,4-thiadiazol-3(2*H*)-yl)ethanone (T92): Off-white solid. Yield: 0.443 g; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.31 (br s, 1H, NH), 7.82 (s, 1H, Ar-H), 7.79 (d, *J* = 6.4

Hz, 2H, Ar-H), 7.68 – 7.64 (m, 2H, Ar-H), 7.62 – 7.56 (m, 2H, Ar-H), 7.33 (d, *J* = 6.4 Hz, 1H, Ar-H), 7.16 (t, *J* = 5.4 Hz, 2H), 5.03 (s, 1H, CH), 4.82 (s, 1H, CH); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 166.2, 148.3, 145.8, 141.6, 137.7, 134.5, 133.5, 130.0, 129.3, 128.3, 127.6, 127.4, 127.2, 125.7, 125.1, 124.4, 122.2, 121.2, 50.7; ESI-MS (*m/z*) = 456.9 $(M+H)^+$; anal. calcd (%) for $C_{20}H_{13}ClN_4OS_3$: C, 52.56; H, 2.87; N, 12.26; S, 21.05. Found: C, 52.63; H, 2.88; N, 12.30; S, 20.98.

2-(2-Imino-5-(thiophen-2-yl)-1,3,4-thiadiazol-3(2*H*)-yl)-1-(2-(trifluoromethyl)-10*H*-phenothiazin-10-yl)ethanone (T93): Off-white solid. Yield: 0.465 g, 87 %; m.p: 108-109 $^{\circ}$C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.31 (br s, 1H, NH), 8.04 (s, 1H, Ar-H), 7.79 (d, *J* = 8.2 Hz, 2H, Ar-H), 7.70 – 7.64 (m, 2H, Ar-H), 7.63 – 7.57 (m, 1H, Ar-H), 7.45 (dd, *J* = 11.5, 3.8 Hz, 1H, Ar-H), 7.37 (t, *J* = 7.1 Hz, 1H, Ar-H), 7.31 (d, *J* = 2.7 Hz, 1H, Ar-H), 7.11 (dd, *J* = 5.0, 3.7 Hz, 1H, Ar-H), 5.02 (s, 1H), 4.75 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 167.1, 148.3, 146.8, 143.9, 137.7, 133.4, 130.9, 128.4, 127.6, 127.4, 127.1, 125.7, 125.1, 124.8, 122.2, 116.02, 50.7; ESI-MS (*m/z*) = 491.1 $(M+H)^+$; anal. calcd (%) for $C_{21}H_{13}F_3N_4OS_3$: C, 51.42; H, 2.67; N, 11.42; S, 19.61. Found: C, 51.50; H, 2.63; N, 11.42; S, 19.62.

5.4 PHARMACOLOGY (Experimental procedure for antibacterial, *In vitro* cytotoxicity and molecular docking studies are discussed in 3.4.2, 2.4.3 and 2.4.4 respectively).

5.4.1 Antitubercular screening

The antimycobacterial activities of compounds were assessed against *M. tuberculosis* using microplate alamar blue assay (MABA). This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric method. Briefly, 200 µL of sterile deionized water was added to all outer perimeter wells of sterile 96 wells plate to minimized evaporation of medium in the test wells during incubation. The 96 wells plate received 100 µL of the Middlebrook 7H9 broth and serial dilution of compounds were made directly on plate. The final drug concentrations tested were 50 to 0.8 µg/mL. Plates were covered and sealed with parafilm and incubated at 37 ºC for five days. After this time, 25 µL of freshly prepared 1:1 mixture of alamar blue reagent and 10% tween 80 was added to the plate and incubated for 24 h. A blue color in the well was interpreted as no

bacterial growth, and pink color was scored as growth. The MIC was defined as lowest compound/drug concentration which prevented the color change from blue to pink.

5.5 RESULTS AND DISCUSSION

5.5.1 Chemistry

The structure of the target molecules (**T73-T93)** was confirmed by spectral (^{1}H NMR, ^{13}C NMR, ESI-MS) and elemental analysis. For instance, the ^{1}H NMR spectrum of compound **T73** showed a broad singlet with one proton at δ 8.16 ppm due to the imine (C=NH) proton and another singlet at δ 2.20 ppm due to methyl protons of the 1,3,4- thiadiazole ring. The singlet at δ 4.74 ppm corresponds to the CH_2 group (**figure 5.3)**. The broad NH at δ 8.16 ppm was disappeared upon D_2O exchange (**figure 5.4**). Also, its mass spectrum showed the molecular ion peak at m/z 355.1, which corresponds to M +1 peak of the molecule and is in agreement with its molecular formula $C_{17}H_{14}N_4OS_2$. Substitution pattern, yield and solubility of target compounds (**T73-T93**) are tabulated in **table 5.1**.

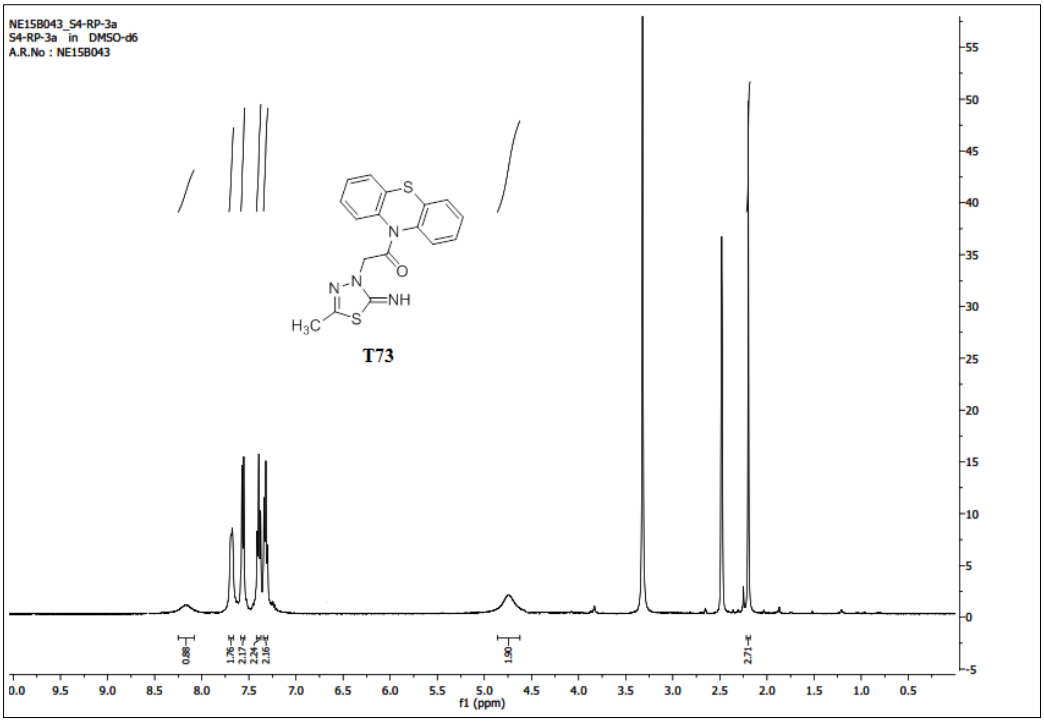


Figure 5.3 ^{1}H NMR spectrum of **T73**

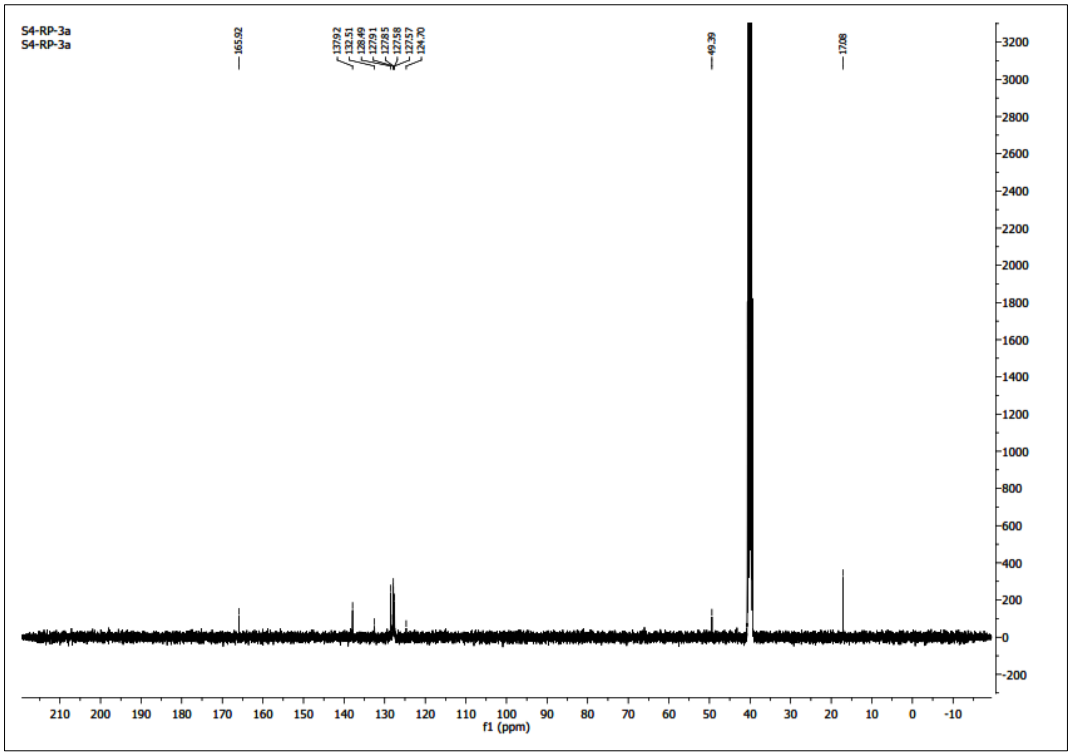


Figure 5.4 ^{13}C NMR spectrum of **T73**

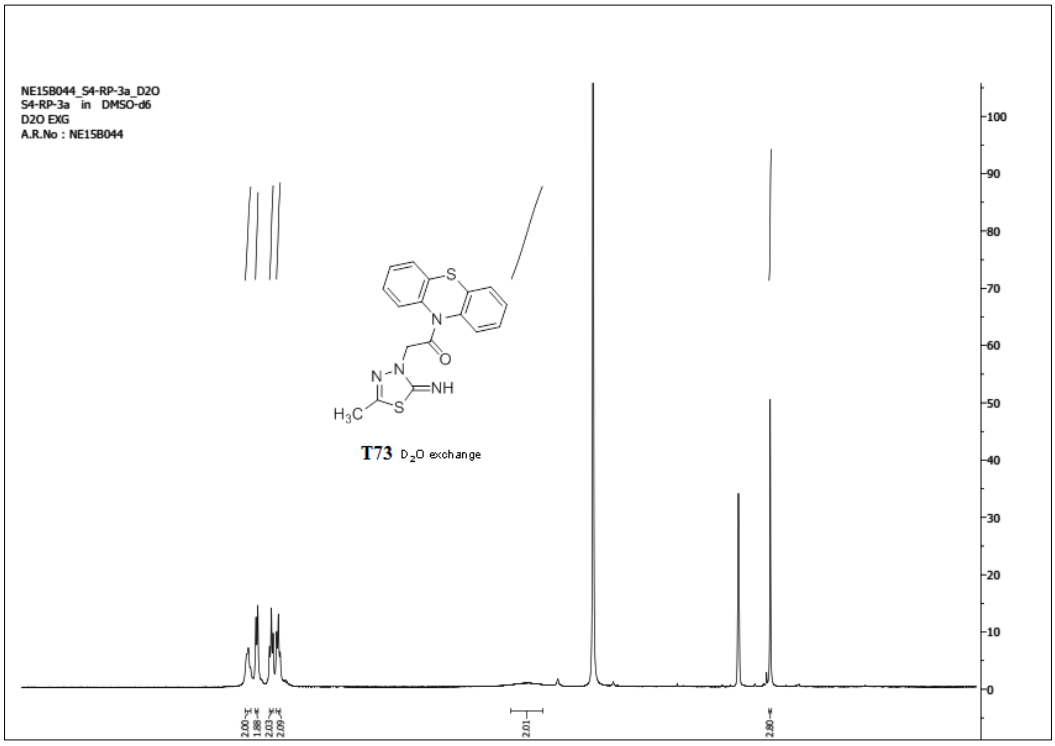


Figure 5.5 D_2O exchange spectrum of **T73**

5.5.2 *In vitro* antimycobacterial activity

All the target derivatives (**T73-T93**) were screened against *Mtb* H37Rv (ATCC27294) using MABA method (Franzblau et al. 1998) and their antimycobacterial activity was evaluated in terms of MIC values. The MIC values in μg/mL of **T73-T93** along with those of standard drugs for comparison are presented in **figure 5.7**. The MIC values of the compounds are in the range of 0.8-50 μg/mL. Interestingly, eleven compounds of the series showed significant inhibitory activity (MIC ≤ 3.125 μg/mL) among which eight compounds exhibited MIC ≤ 1.6 μg/mL (**figure 5.6**). The MIC values of these compounds are comparable with those of the standard drugs ethambutol and ciprofloxacin.

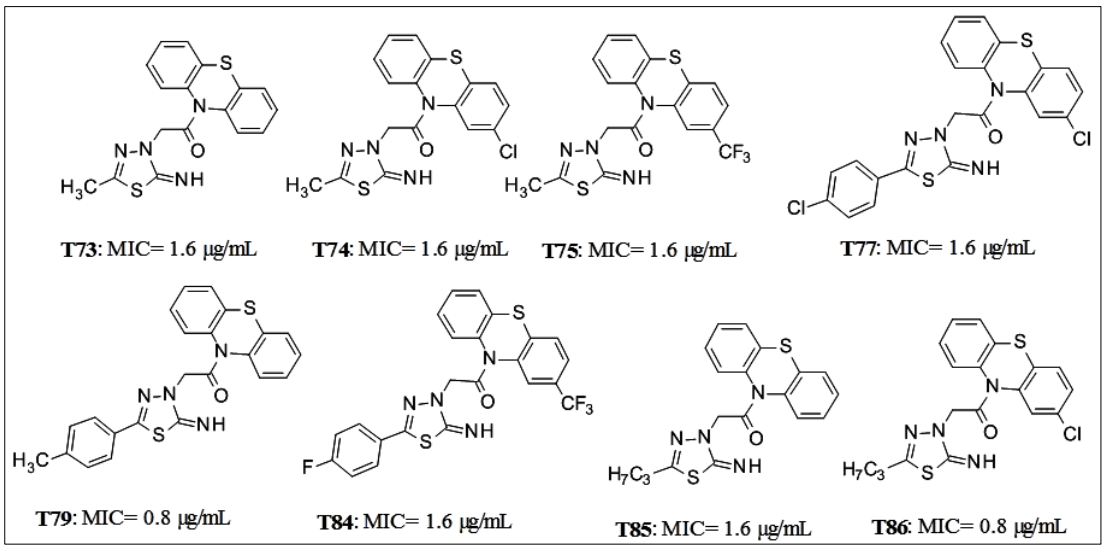


Figure 5.6 Highest inhibitory compounds (MIC ≤ 1.6 μg/mL) against *Mtb* among the synthesized series.

Compounds **T79** and **T86** which contain 4-methylphenyl and *n*-propyl groups respectively on the thiadiazole ring emerged as the most potent leads with a MIC of 0.8 μg/mL and are more potent than standard drugs EMB and INN. The nature of the substituents on the phenothiazine (R^2) and 1,3,4-thiadiazole (R^1) rings affected the activity of the molecules. The presence of a methyl (compounds **T73-T75**) or *n*-propyl (compounds **T85-T87**) group on the 1,3,4-thiadiazole ring substantially increased the antitubercular activity regardless of the nature of the substituent at R^2 (H, Cl or CF_3). Among derivatives which contain an unsubstituted phenothiazine ring

(R^2=H), those with a 4-methylphenyl (**T79**) or 4-fluorophenyl (**T82**) substituent at R^1 displayed substantial activity. In the case of chloro substituted (R^2= Cl) derivatives, a 4-chlorophenyl (**T77**) or 4-fulorophenyl (**T83**) substituent at R^1 enhanced the activity. Among trifluoromethyl substituted analogs, only one compound (**T84**) with a 4-fluoro phenyl substituent at R^2 showed promising activity.

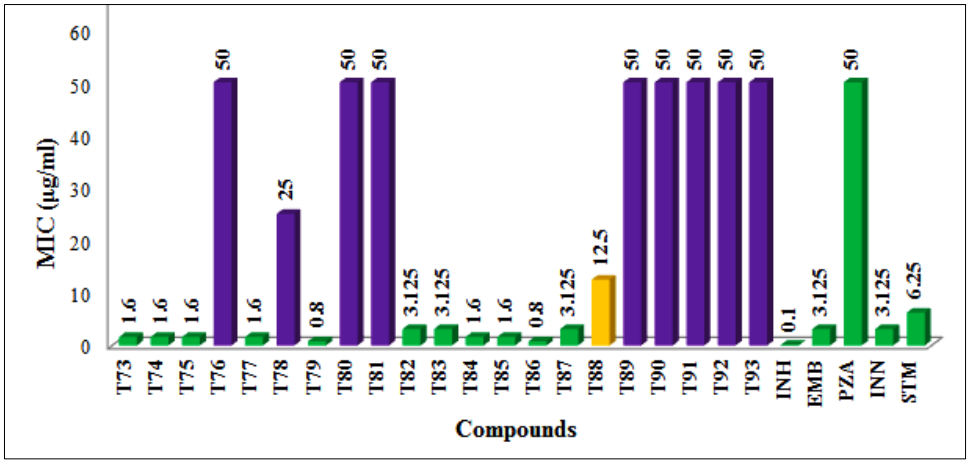


Figure 5.7 AntiTB activity of **T73-T93** against *Mtb* H37RV

The presence of electron rich groups such as 3,4-dimethoxy phenyl or thienyl at R^1 failed to enhance the inhibition activity of the molecules (**T88-T93**). This is evident from the observation that except compound **T88** which showed a MIC of 12.5 µg/mL, all other molecules (**T89-T93**) were only moderately active. Further, an alkyl/substituted (4-methyl/4-Cl/4-F) phenyl group on the 1,3,4-thiadiazole ring and a chlorosubstituted/unsubstituted phenothiazine ring serve to enhance the antitubercular activity of the hybrid derivatives. The structure activity relationship suggest that the phenothiazine-thiadiazole core could be considered as a promising structural unit for the development of new antiTB agents.

5.5.3 *In vitro* antibacterial activity

The *in vitro* antibacterial activity of synthesized compounds **T73-T93** was tested using the disc diffusion method. All the compounds were screened against three bacterial strains viz. *S. aureus, P. aeruginosa* and *E. coli* using ciprofloxacin as the standard drug. The compounds were dissolved in DMSO with two concentrations (75 µg/mL and 50 µg/mL). Compounds **T73**, **T79** and **T88** demonstrated significant inhibition activity (**table 5.2**) against all three bacterial strains at both concentrations.

It is interesting to note that all three derivatives contain an unsubstituted phenothiazine ring (R^1=H) and an electron rich substitution (R^2) on the 1,3,4-thiadiazole ring.

Table 5.2 Antibacterial activity of target compounds (**T73-T93**).

Compounds	***E. coli***		***S. aureus***		***P. aeruginosa***	
Cocn. in µg/ml	75	50	75	50	75	50
T73	25±0.1	20±0.4	26±0.2	22±0.3	25±0.4	20±0.1
T74	16±0.2	14±0.3	13±0.3	10±0.3	13±0.2	09±0.4
T75	20±0.1	15±0.1	17±0.1	14±0.2	15±0.1	12±0.1
T76	10±0.2	08±0.2	09±0.3	07±0.3	10±0.2	07±0.4
T77	15±0.4	12±0.4	17±0.2	15±0.1	20±0.3	15±0.1
T78	12±0.1	11±0.2	13±0.1	11±0.2	14±0.2	09±0.2
T79	27±0.3	24±0.2	21±0.2	16±0.2	11±0.1	09±0.2
T80	09±0.3	07±0.4	12±0.3	10±0.1	07±0.2	04±0.1
T81	13±0.4	11±0.4	11±0.1	09±0.2	09±0.1	06±0.2
T82	11±0.1	08±0.2	08±0.2	05±0.3	12±0.2	08±0.2
T83	12±0.4	07±0.2	09±0.1	05±0.3	10±0.1	07±0.1
T84	11±0.1	09±0.4	10±0.4	07±0.2	13±0.2	07±0.3
T85	14±0.4	11±0.2	16±0.1	12±0.1	15±0.3	11±0.1
T86	12±0.1	08±0.4	10±0.3	08±0.2	12±0.2	10±0.2
T87	09±0.4	06±0.3	11±0.4	07±0.2	07±0.3	03±0.1
T88	16±0.3	13±0.2	21±0.1	18±0.3	25±0.1	20±0.4
T89	08±0.1	04±0.2	08±0.2	06±0.2	06±0.1	04±0.2
T90	10±0.2	08±0.1	07±0.1	04±0.1	09±0.2	06±0.1
T91	07±0.3	06±0.2	−	−	04±0.2	02±0.2
T92	08±0.1	04±0.2	−	−	11±0.2	07±0.1
T93	10±0.2	08±0.1	08±0.1	05±0.1	12±0.1	08±0.3
Control	00	00	00	00	00	00
INN	32±0.2	27±0.2	26±0.1	21±0.2	21±0.2	18±0.1
INN: Ciprofloxacin; −: inhibition not detected; control: DMSO						

5.5.4 *In vitro* cytotoxicity studies

The cytotoxicity of the potent compounds was evaluated against vero (African Green Monkey Kidney) cell lines using the MTT assay (Gundersen et al. 2002). The graphical representation of the cell growth inhibition by the compounds at a concentration of 62.5 µg/mL is shown in **figure 5.8**. The compounds did not show any toxicity to the cell line signifying the lack of general cellular toxicity.

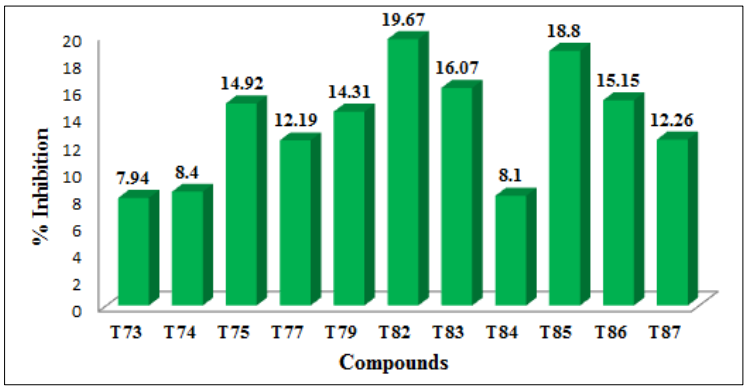


Figure 5.8 Growth inhibition activity of active compounds (at a concentration of 62.5 µg/mL) against vero cell line.

5.5.5 Molecular docking studies

In order to further understand the mechanism of action of the new phenothiazine-1,3,4-thiadiazole hybrids, the active molecules were subjected to molecular docking studies with InhA of *Mtb,* which have been validated as effective antiTB targets. The docking poses of molecules **T79** and **T84** are shown in **figure 5.9**.

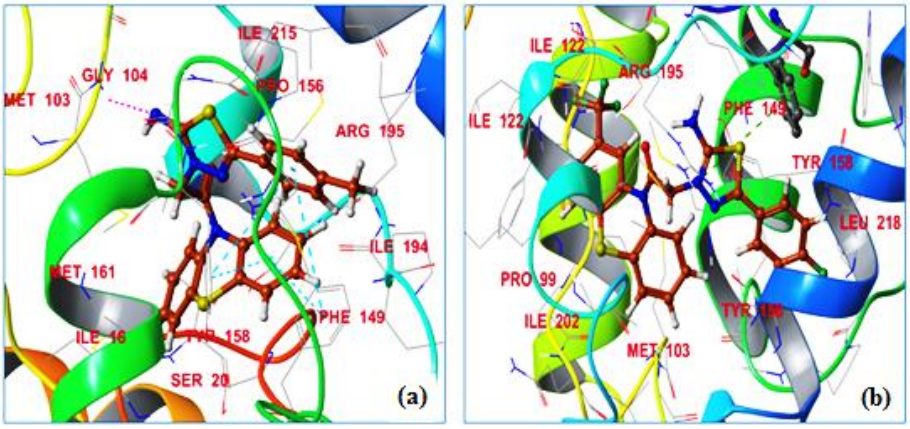


Figure 5.9 Three dimensional docking pose showing the interaction of some active antiTB compounds (a) **T79** with InhA; (b) **T84** with InhA with the target enzymes, InhA.

The docking score of all the active molecules and details of interacting amino acid residues are given in the **table 5.3.** The target molecules, as they contain 4 or 5 aromatic rings, showed strong *pi-pi* stacking interactions in addition to hydrogen bonding interaction with the target enzyme. Most of the active compounds interacted with Phe 149 residue of 1P44. For instance, both **T79** and **T86** (the most active compounds of the series) showed *pi-pi* stacking interaction with these amino acid residues. Compound **T79** with a docking score of -8.98 displayed additional interactions *viz* a *pi-pi* stacking interaction with Tyr 158 and an H-bonding interaction with Gly 104. It is interesting to note that the two phenyl rings of **T79** interacted simultaneously with both Phe 149 and Tyr 158 residues (**figure 5.9 a**) which ensures the stronger binding of the compound with the target enzyme. Compound **T84**, which showed an MIC of 1.6 μg/mL, displayed the highest docking score (-9.37) with target enzyme. These results suggest that the strong *pi-pi* stacking interaction of the active molecules with the target enzyme could be responsible for their significant inhibition activity against the MTB strain. The presence of a *pi-pi* stacking interaction was observed also in the case of isoniazid (INH), which is a first line antiTB drug. It is known that INH acts by inhibiting InhA and shows a *pi-pi* stacking interaction with Phe 149 residue of the enzyme. Hence, the *MTB* inhibition and docking studies signifies that the active phenothiazine-thiadiazole hybrids are suitable for the development new antiTB agents.

Table 5.3 Docking scores of the compounds with the target enzymes (1P44) and details of the interacting amino acid residues.

Compounds	Docking score	Interacting amino acid residues
T73	-8.17	Phe 149
T74	-8.10	Phe 149
T75	-9.01	Tyr 158
T77	-8.59	Tyr 158, Ile 194
T79	-8.98	Gly 104, Phe 149, Tyr 158
T82	-9.12	Ile 154, Tyr 158
T83	-8.69	Phe 149
T84	-9.38	Phe 149

T85	-8.03	Phe 149
T86	-8.41	Phe 149
T87	-9.27	Tyr 158

5.6 CONCLUSIONS

- We have designed a series of 2-(2-imino-1,3,4-thiadiazol-3(2*H*)-yl)-1-(10*H*-phenothiazin-10-yl)ethanone derivatives (**T73-T93)** following the molecular hybridization approach.
- The antiTB assay against *Mtb* H37Rv revealed the significant inhibition activity of the molecules. Interestingly, eleven compounds of the series showed remarkable inhibitory activity with MIC ≤ 3.125 μg/mL.
- Compounds **T79** and **T85** emerged as the most potent leads with MIC of 0.8 μg/mL which suggests that the phenothiazine-thiadiazole core could be considered as a new active structural unit for the development of antiTB leads.
- The structure-activity relationship revealed that alkyl (methyl/n-propyl) or substituted (4-methyl/4-Cl/4-F) phenyl groups on the 1,3,4-thiadiazole ring enhance the inhibition activity of the compounds.
- Also, compounds containing chloro/unsubstituted phenothiazine ring exhibited remarkable antiTB activity.
- Further, the cytotoxicity study revealed the nontoxic nature of the active molecules against a normal Vero cell line.
- The molecular docking study demonstrated that strong *pi-pi* stacking interaction of the active molecules with the target enzymes could be responsible for their significant inhibition activity against MTB strain. These results suggest that the molecules are promising leads for further studies and development of new antiTB agents.

Appendix 5.1

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

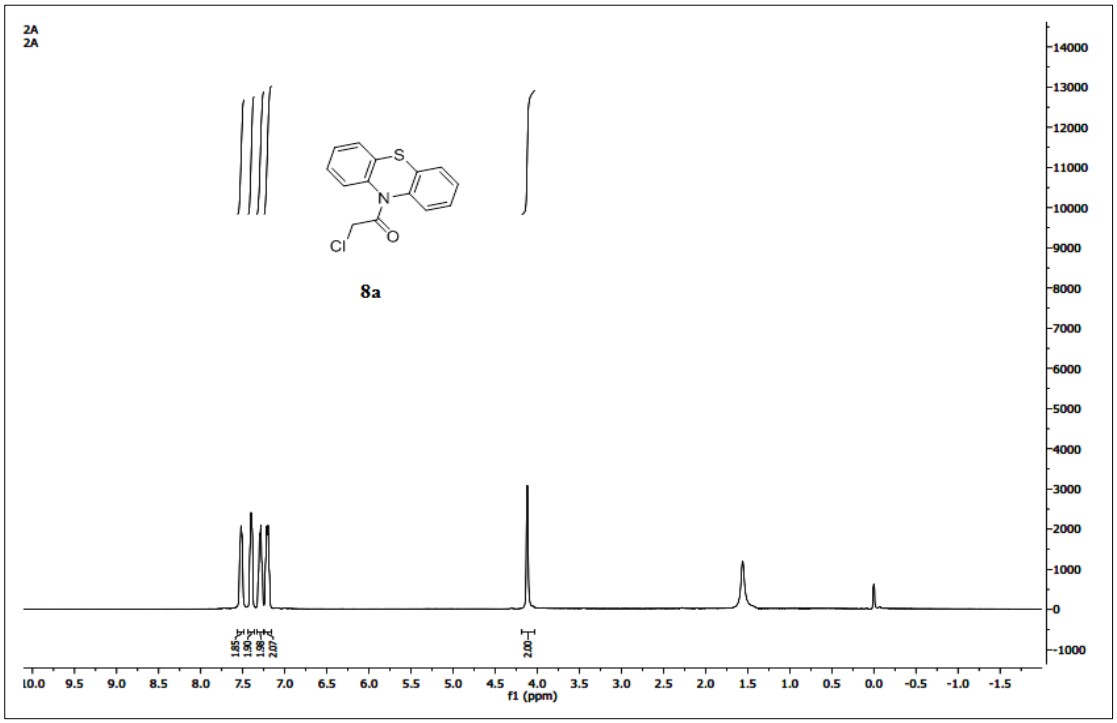


Figure 5.10 ^{1}H NMR spectrum of **8a**

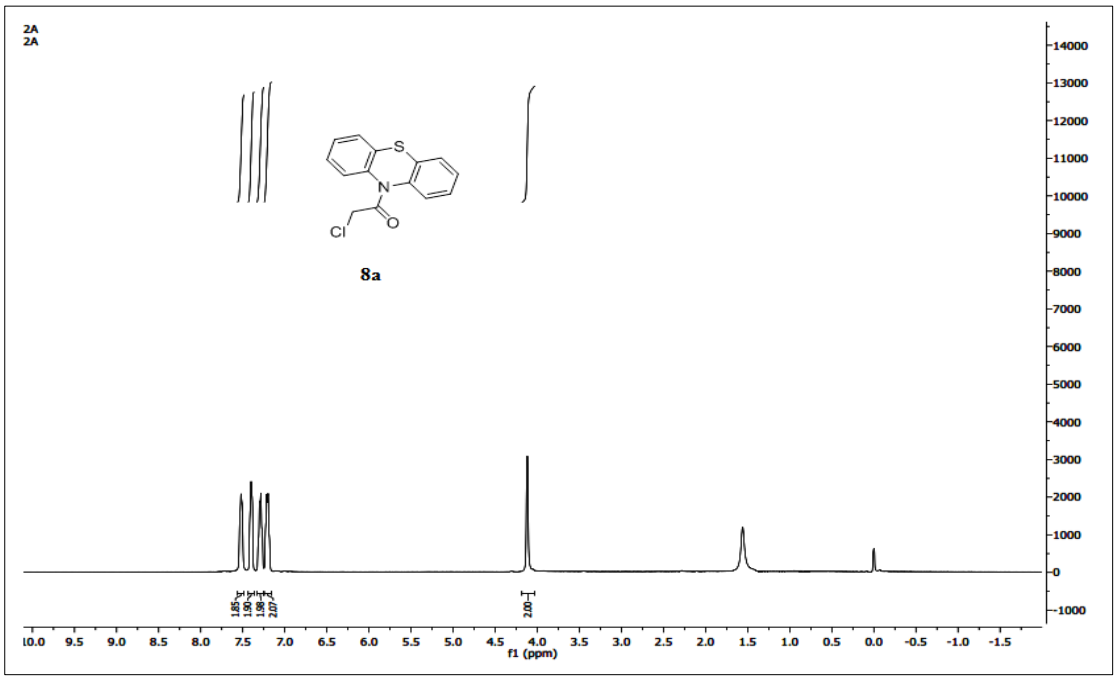


Figure 5.11 ^{13}C NMR spectrum of **8a**

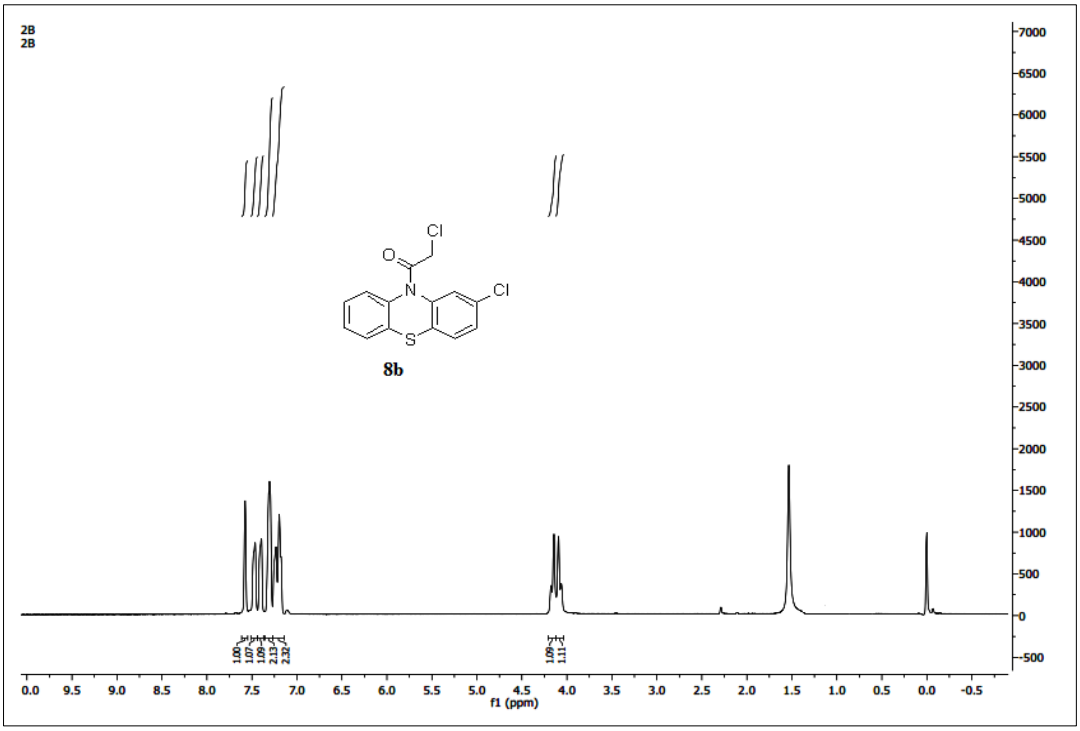


Figure 5.12 ^{1}H NMR spectrum of **8b**

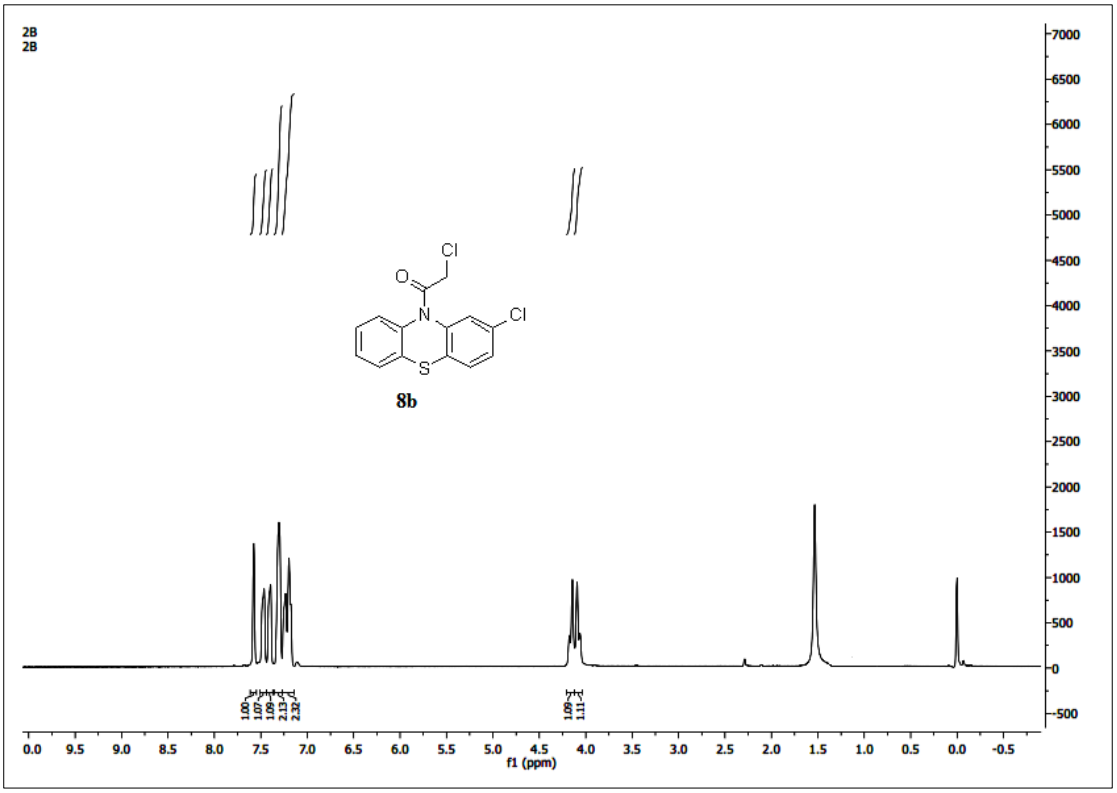


Figure 5.13 ^{13}C NMR spectrum of **8b**

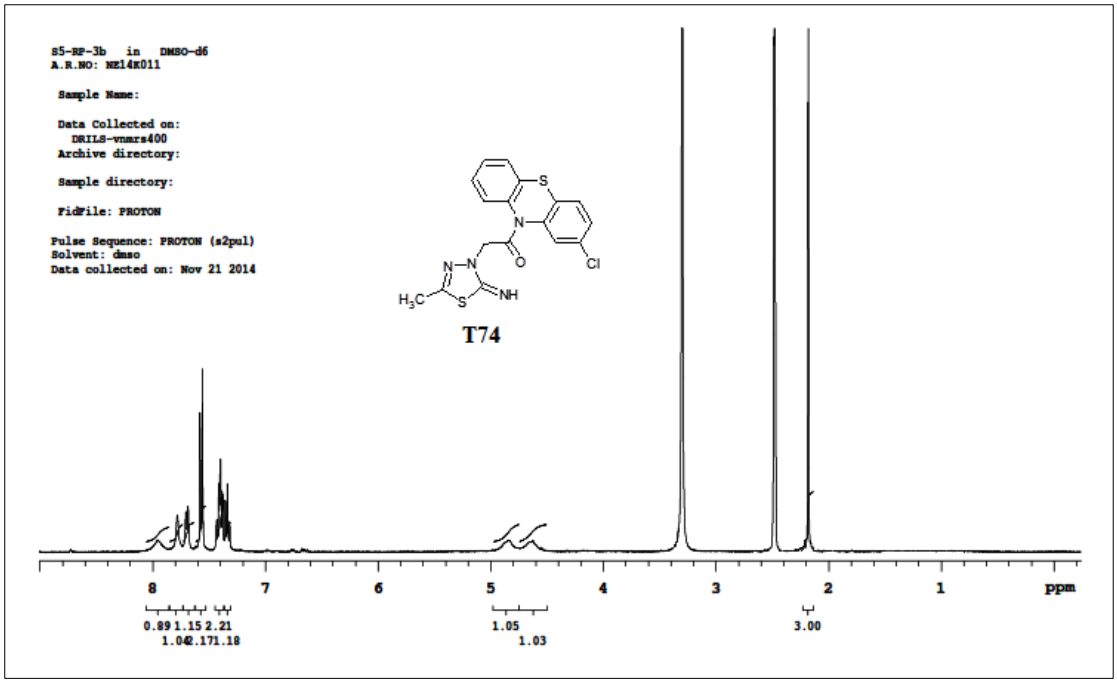


Figure 5.14 ^{1}H NMR spectrum of **T74**

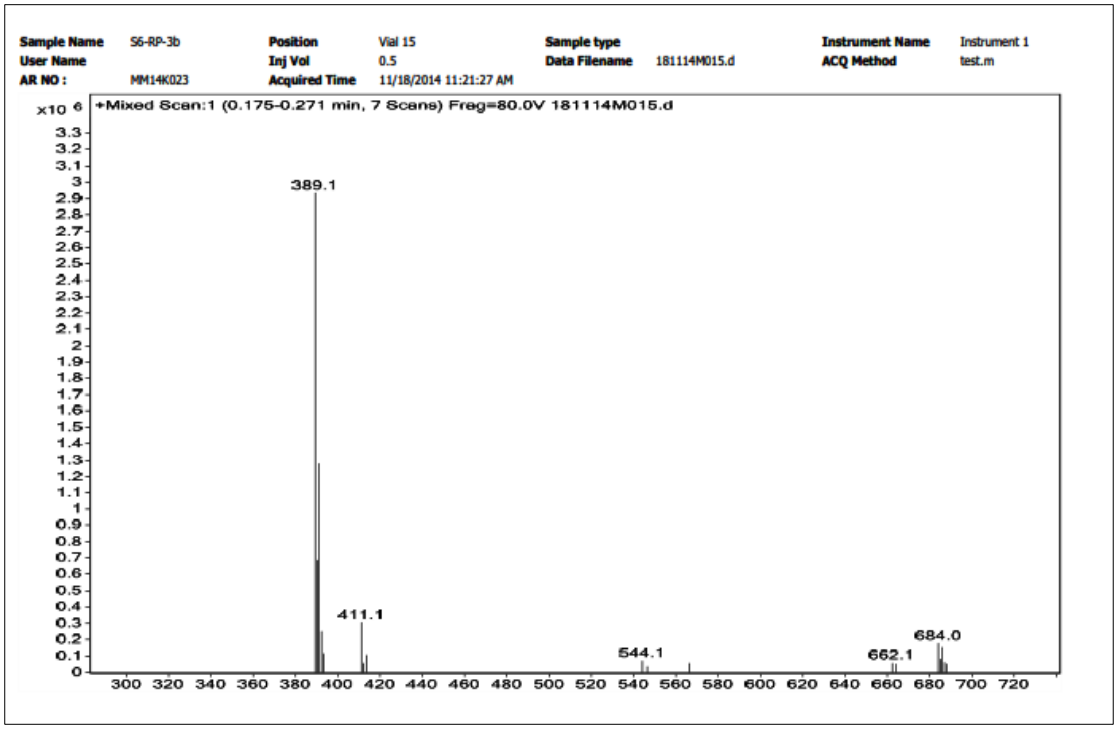


Figure 5.15 Mass spectrum of **T74**

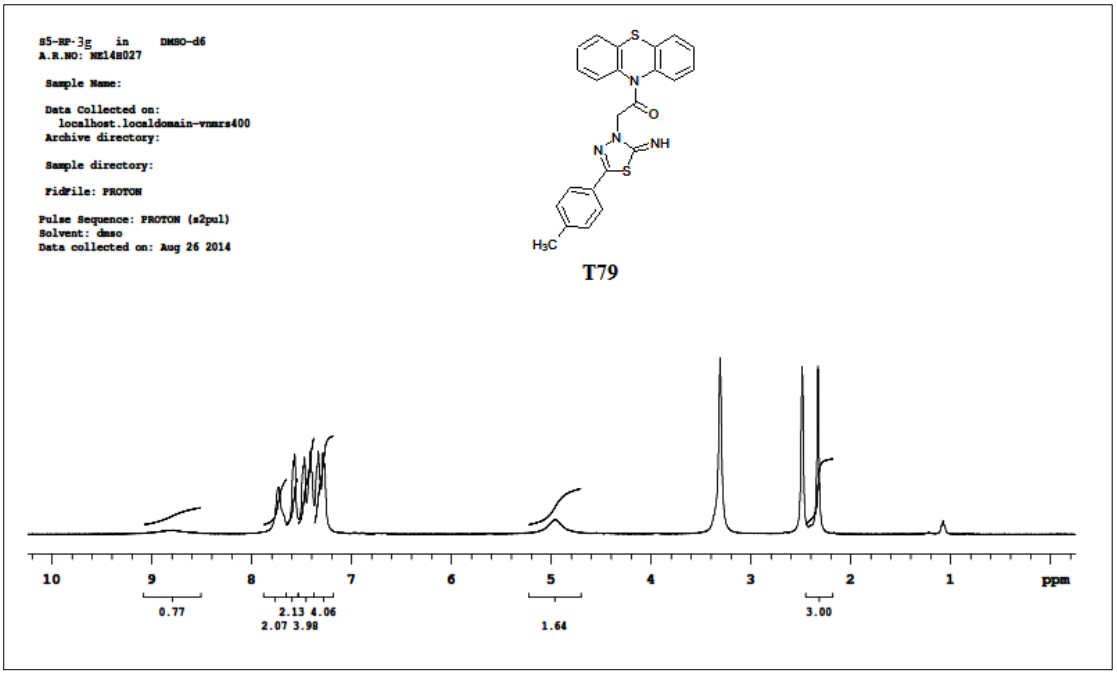


Figure 5.16 ^{1}H NMR spectrum of **T79**

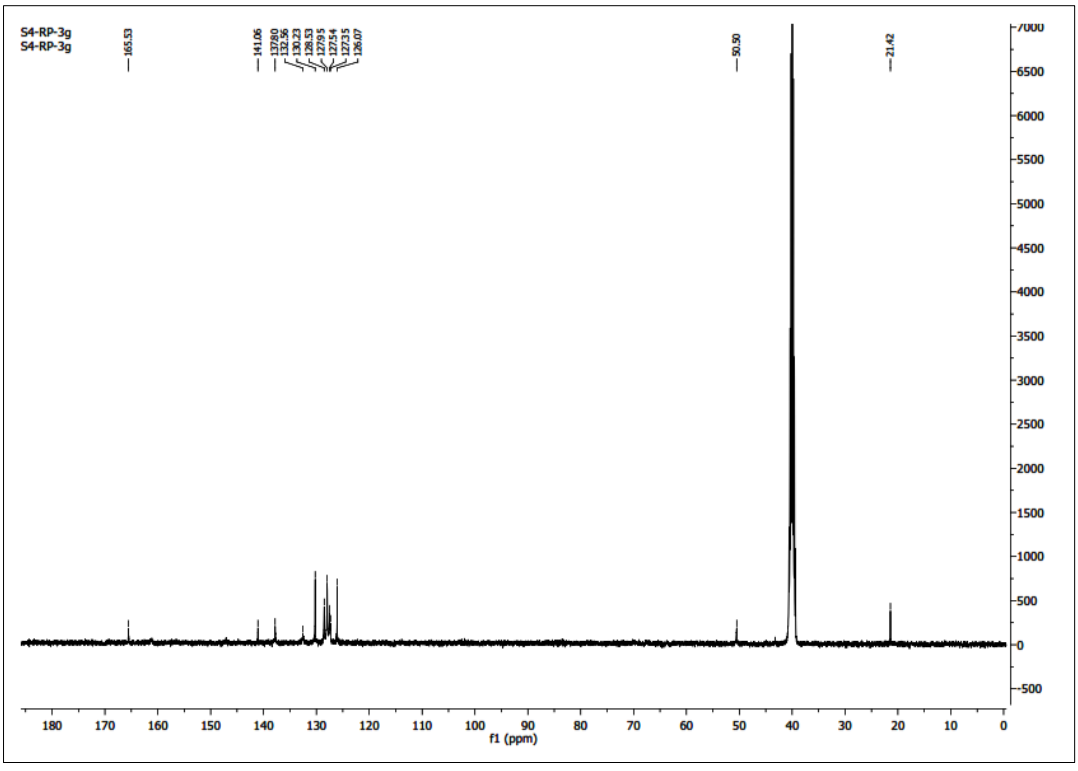


Figure 5.17 ^{13}C NMR spectrum of **T79**

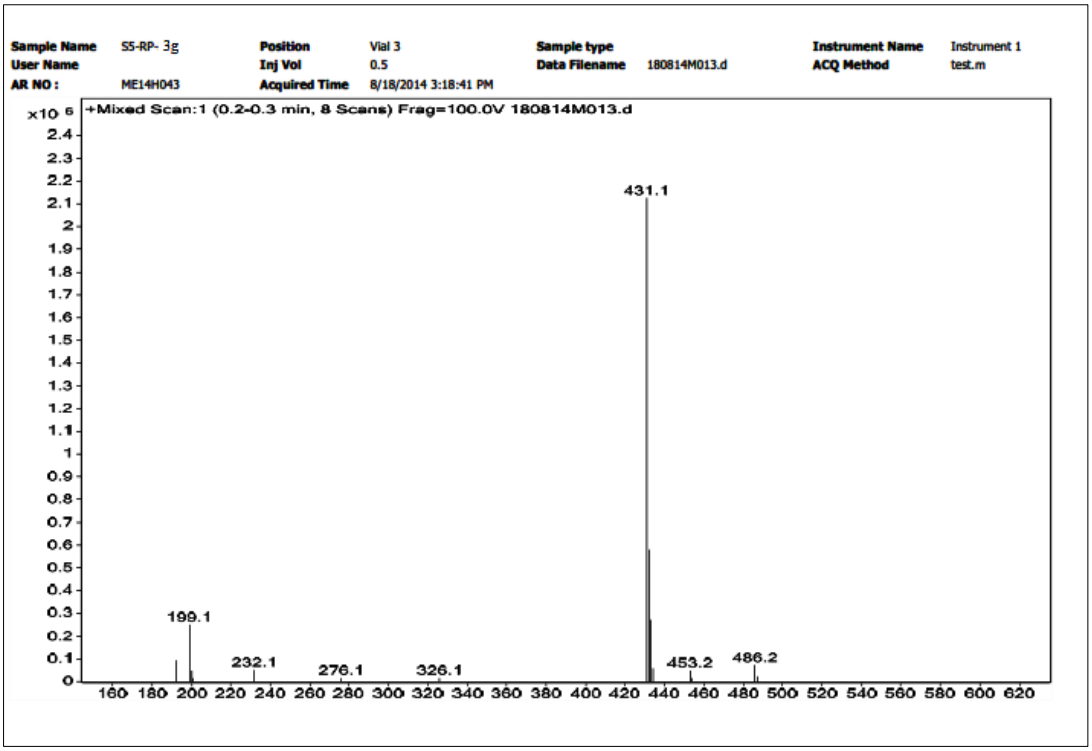


Figure 5.18 MS spectrum of **T79**

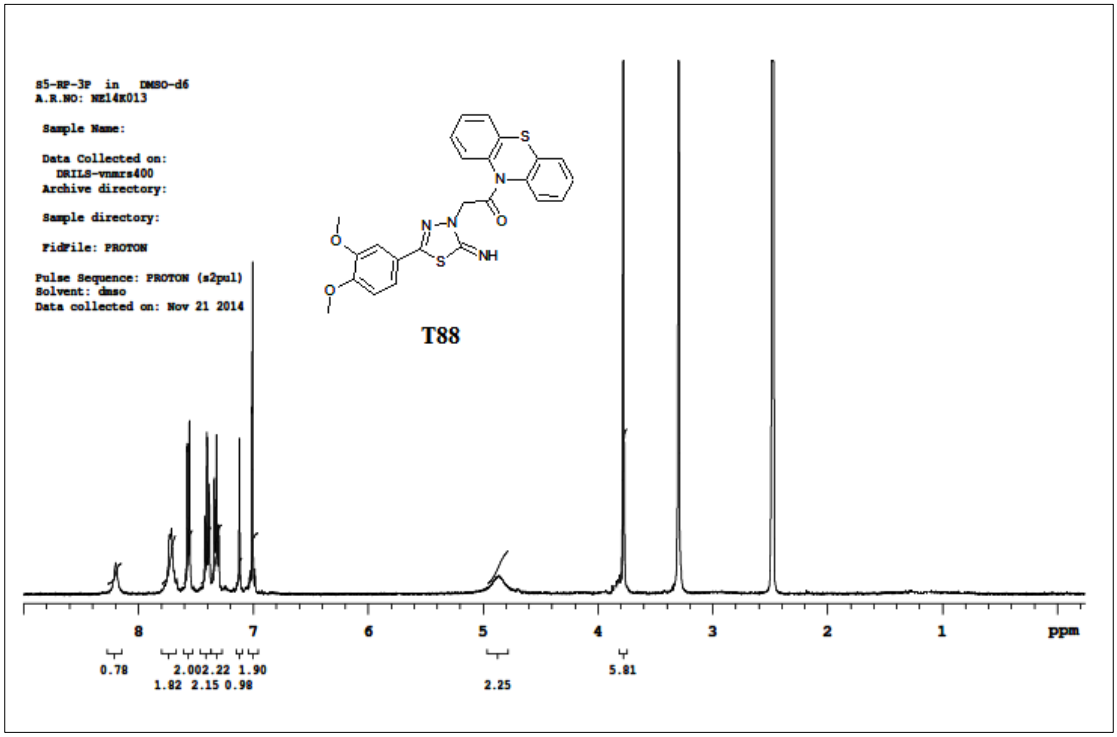


Figure 5.19 ^{1}H NMR spectrum of **T88**

CHAPTER 6

NEW PYRAZINAMIDE-IMIDAZO[2,1-*b*] [1,3,4]THIADIAZOLE HYBRIDS: SYNTHESIS AND EVALUATION OF ANTITUBERCULAR AND ANTIBACTERIAL ACTIVITY

Abstract

This chapter elucidates the design and synthesis of new imidazo[2,1-b][1,3,4]thiadiazole carrying pyrazinamide hybrid molecules along with structural characterization of all the newly formed intermediates and final molecules. In vitro antimycobacterial and antibacterial studies of all the title compounds also are presented in the chapter.

6.1 INTRODUCTION

In the present study, we have modified the structure of PZA by connecting it with an ITD core through an imine linkage. Pyrazinamide is a well-known drug which in combination with isoniazid and rifampicin is used for the treatment of *Mtb* (Baumann and Baxendale, 2013). The active form of the drug, pyrazinoic acid inhibits the enzyme fatty acid synthase (FAS) I, which is required by the bacterium to synthesize fatty acids. It has also been suggested that the accumulation of pyrazinoic acid disrupts membrane potential and interferes with energy production, necessary for survival of *Mtb* (Zhang et al. 2003; Zimhony et al. 2000). Some recent reports demonstrated that pyrazine-2-carbohydrazide derivatives (**figure 6.1**, III and IV) exhibit significant antimycobacterial activity (Abdel-Aziz et al. 2010; Vergara et al 2009).

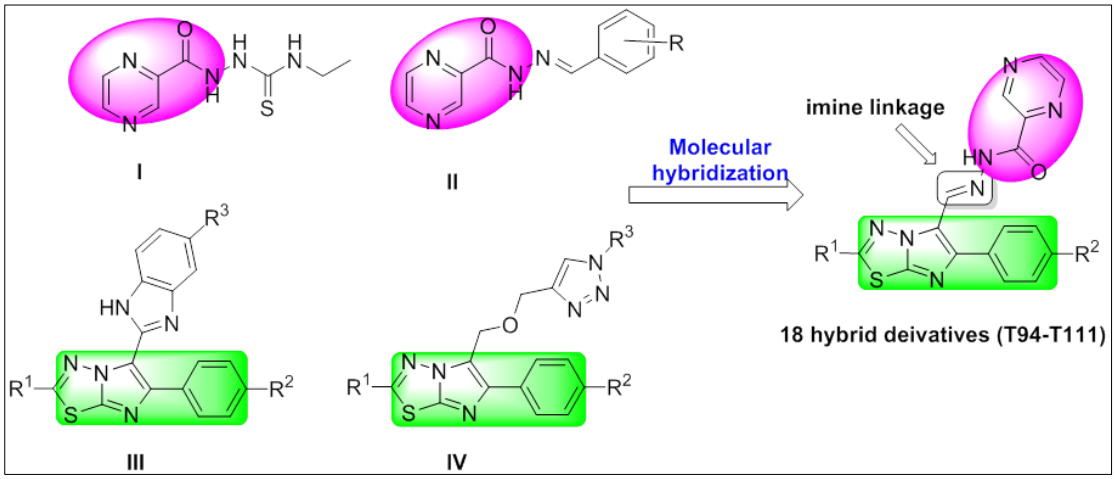
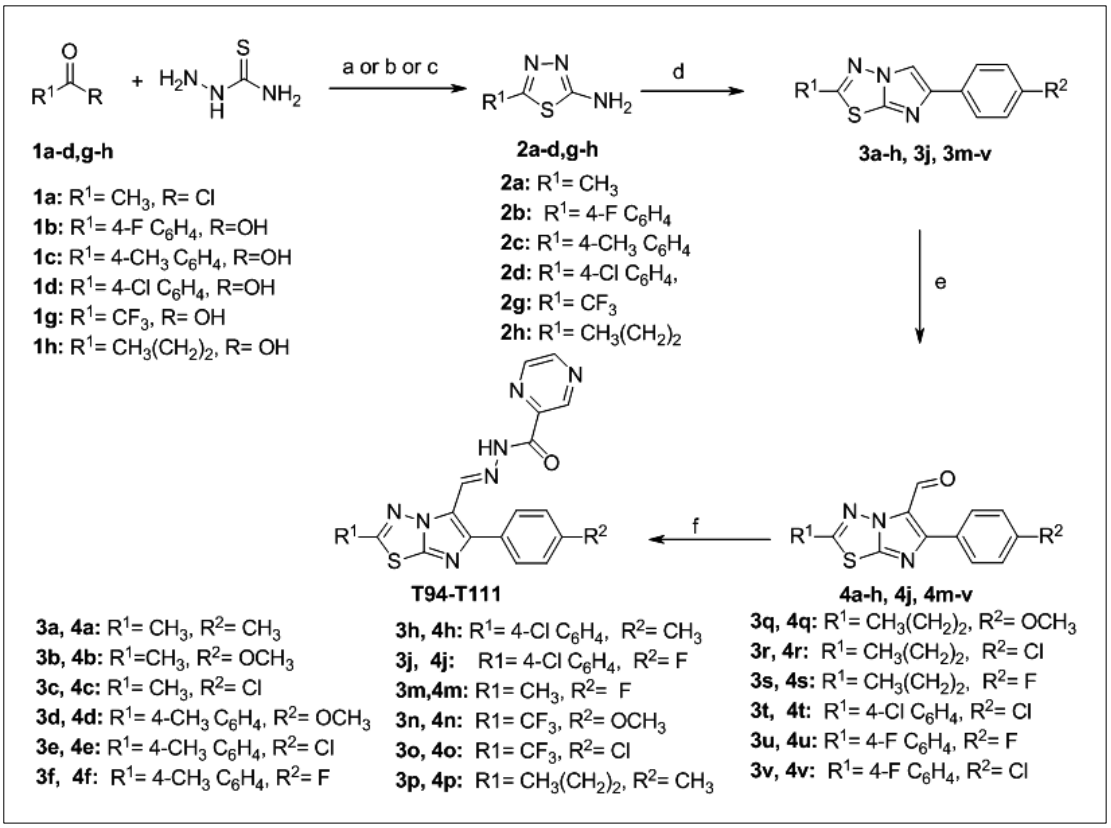


Figure 6.1 Designing strategy of targets molecules (**T94-T111**).

6.2 CHEMISTRY

The target molecules were synthesized according to the synthetic protocol given in **Scheme 6.1**. Intermediates, 5-substituted-1,3,4-thiadiazole-2-amines (**2a-d, g-h**) were synthesized according to the reported procedure (Li and Chen, 2008). The 2-subsituted-6-arylimidazo[2,1-*b*][1,3,4]thiadiazole derivatives (**3a–h, 3j, 3m-v**) were synthesized by the reaction between 5-substituted-1,3,4-thiadiazol-2-amine (**2a-d, g-h**) and the corresponding substituted α-halo aryl ketone under thermal conditions using ethanol as solvent. The Vilsmeier–Haack formylation of intermediates (**3a–h, 3j, 3m-v)** yielded 2-substituted-6-arylimidazo[2,1-*b*][1,3,4]thiadiazole-5-carb aldehydes **(4a–h, 4j, 4m-v)**. These aldehydes are then reacted with pyrazine-2-carbrohydrazide to get the target molecules (**T94-T111)**.



Scheme 6.1 Synthesis of ITD and pyrazine-2-carbohyrazide hybrids derivatives (**T94-T111**). Reagents and conditions a) Acetyl chloride, 0 °C - RT, 3 h; b) Polyphosphoric acid, 110 °C, 8h; c) Substituted aromatic acid, $POCl_3$, 75 °C, 30 min; d) Phenacyl bromide, ethanol, 80-85 °C, 24 h; e) DMF, $POCl_3$, 60° C, 6h; f) Pyrazine-2-carbohydrazide, ethanol, Conc.H_2SO_4, 10-15 min.

Table 6.1 Substitution pattern, yield and solubility of target compounds (**T94-T111)**.

Product	R^1	R^2	log*P*/*C*log*P*	Yield (%)
T94	CH_3	CH_3	3.45/1.23	93
T95	CH_3	OCH_3	2.84/0.74	91
T96	CH_3	Cl	3.52/1.45	95
T97	CH_3	F	3.12/0.89	96
T98	$CH_3(CH_2)_2$	CH_3	4.44/2.3	89
T99	$CH_3(CH_2)_2$	OCH_3	3.83/1.81	90
T100	$CH_3(CH_2)_2$	Cl	4.51/2.51	93
T101	$CH_3(CH_2)_2$	F	4.11/1.94	96
T102	4-$CH_3C_6H_4$	OCH_3	4.71/2.85	92
T103	4-$CH_3C_6H_4$	Cl	5.39/3.55	95
T104	4-$CH_3C_6H_4$	F	4.99/2.98	89
T105	4-ClC_6H_4	CH_3	5.39/3.55	92
T106	4-ClC_6H_4	Cl	5.46/3.76	96
T107	4-ClC_6H_4	F	5.06/3.19	89
T108	4-FC_6H_4	Cl	5.06/3.19	91
T109	4-FC_6H_4	F	4.66/2.62	89
T110	CF_3	OCH_3	3.74/1.12	86
T111	CF_3	Cl	4.42/1.84	89

[a]Obtained from Chemdraw ultra 12.0 software.

Note: Over all yield of compound **T96** is 49.88 %

6.3 EXPERIMENTAL

6.3.1 Materials and instruments (Refer section 2.3.1)

6.3.2 Synthesis

2-Propyl-6-(*p*-tolyl)imidazo[2,1-*b*][1,3,4]thiadiazole (3p): White solid. yield: 1.52 g, 85 %; m.p: 125-126 $^{\circ}$C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 7.94 (s, 1H, H-5 imidazole) 7.82 (d, J = 7.4 Hz, 2H, Ar-H), 7.01 (d, J = 8.4 Hz, 2H, Ar-H), 3.08 (t, J = 7.6 Hz, 2H, CH_2), 2.34 (s, 3H, CH_3), 1.80 (sextet, J = 7.6 Hz, 2H, CH_2), 1.01 (t, J = 7.6 Hz, 3H, CH_3); ESI-MS (*m/z*) = 258.1 $(M+H)^+$; calculated for $C_{14}H_{15}N_3S$; C, 65.34; H, 5.87; N, 16.33; S, 12.46. Found: C, 65.32; H, 5.79; N, 16.32; S, 12.44.

6-(4-Methoxyphenyl)-2-propylimidazo[2,1-*b*][1,3,4]thiadiazole (3q): Yellow solid. yield: 1.55 g, 82 %; m.p: 115-116 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 7.95 (s, 1H, H-5 imidazole), 7.83 (d, *J* = 7.6 Hz, 2H, Ar-H), 6.98 (d, *J* = 8.4 Hz, 2H, Ar-H), 3.80 (s, 3H, OCH_3), 3.10 (t, *J* = 6.6 Hz, 2H, CH_2), 1.82 (sextet, *J* = 6.6 Hz, 2H, CH_2), 0.98 (t, *J* = 6.6 Hz, 3H, CH_3); ESI-MS (*m/z*) = 273.1 (M+H)+; calculated for $C_{14}H_{15}N_3OS$; C, 61.51; H, 5.53; N, 15.37; S, 11.73. Found: C, 61.48; H, 5.55; N, 15.36; S, 11.80.

(6-(4-Chlorophenyl)-2-propylimidazo[2,1-*b*][1,3,4]thiadiazole (3r): Brown solid. yield: 1.51 g, 78 %; m.p: 124-125 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 7.94 (s, 1H, H-5 imidazole), 7.98 (d, *J* = 7.4 Hz, 2H, Ar-H), 7.38 (d, *J* = 8.4 Hz, 2H, Ar-H), 3.14 (t, *J* = 8.3 Hz, 2H, CH_2), 1.83 (sextet, *J* = 8.3 Hz, 2H, CH_2), 1.04 (t, *J* = 8.3 Hz, 3H, CH_3); ESI-MS *(m/z)* = 277.9 $(M+H)^+$; calculated for $C_{13}H_{12}ClN_3S$; C, 56.21; H, 4.35; N, 15.13; S, 11.54. Found: C, 56.25; H, 4.35; N, 15.10; S, 11.54.

6-(4-Fluorophenyl)-2-propylimidazo[2,1-*b*][1,3,4]thiadiazole (3s): Yellow solid. yield: 1.46 g, 80 %; mp: 109-110 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 7.96 (s, 1H, *H*-5 imidazole), 8.03 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.22-7.19 (m, 2H, Ar-H), 3.15 (t, *J* = 7.8 Hz, 2H, CH_2), 1.85 (sextet, *J* = 7.8 Hz, 2H, CH_2), 1.01 (t, *J* = 7.8 Hz, 3H, CH_3); ESI-MS (*m/z*) = 261.9 $(M+H)^+$; calculated for $C_{13}H_{12}FN_3S$; C, 59.75; H, 4.63; N, 16.08; S, 12.27. Found: C, 59.68; H, 4.65; N, 16.10; S, 12.22.

2,6-Bis(4-chlorophenyl)imidazo[2,1-*b*][1,3,4]thiadiazole (3t): Brown solid. yield: 1.29 g, 79 %; m.p: 220-221 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 8.01 (d, *J* = 8.6 Hz, 4H), 7.96 (s, 1H, H-5 imidazole), 7.72 (d, *J* = 8.7 Hz, 2H, Ar-H), 7.45 (d, *J* = 7.6 Hz, 2H, Ar-H); ESI-MS (*m/z*) = 345.9 $(M+H)^+$; calculated for $C_{16}H_9Cl_2N_3S$; C, 55.50; H, 2.62; N, 12.14; S, 9.26. Found: C, 55.45; H, 2.63; N, 12.15; S, 9.28.

2,6-Bis(4-fluorophenyl)imidazo[2,1-*b*][1,3,4]thiadiazole (3u): White solid. Yield: 1.35 g, 83 %; m.p: 230-231 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm):7.97 (s, 1H, H-5 imidazole), 7.88 -7.72 (m, 4H, Ar-H), 7.58 (d, *J* = 5.6 Hz, 2H, Ar-H), 7.46 (d, *J* = 6.8 Hz, 2H, Ar-H); ESI-MS (*m/z*) = 314.1 (M+H)+; calculated for $C_{16}H_9F_2N_3S$; C, 61.33; H, 2.90; N, 13.41; S, 10.23. Found: C, 61.35; H, 2.93; N, 13.45; S, 10.25.

6-(4-Chlorophenyl)-2-(4-fluorophenyl)imidazo[2,1-b][1,3,4]thiadiazole (3v): White solid. Yield: 1.43 g, 85 %; m.p: 223-224 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm):7.98 (s, 1H, H-5 imidazole), 7.75-7.68 (m, 4H, Ar-H), 7.45 (d, J = 8.8 Hz, 2H, Ar-H), 7.34 – 7.28 (m, 2H, Ar-H); ESI-MS (*m/z*) = 330.1 $(M+H)^+$; calculated for $C_{16}H_9ClFN_3S$; C, 58.27; H, 2.75; N, 12.74; S, 9.72. Found: C, 58.22; H, 2.76; N, 12.75; S, 9.74.

6-(4-methoxy phenyl)-2-(trifluoromethyl)imidazo[2,1-*b*][1,3,4]thiadiazole (**3n**) and 6-(4-chlorophenyl)-2-(trifluoromethyl)imidazo[2,1-*b*][1,3,4]thiadiazole (**3o**) are prepared according to the reported procedure (Alegaon et al. 2012).

2-Propyl-6-(*p*-tolyl)imidazo[2,1-*b*][1,3,4]thiadiazole-5-carbaldehyde (4p): White solid. Yield: 0.975 g, 88 %; m.p: 109-110 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 10.00 (s, 1H, CHO) 7.88 (d, *J* = 7.8 Hz, 2H, Ar-H), 7.16 (d, *J* = 7.4 Hz, 2H, Ar-H), 3.10 (t, *J* = 7.5 Hz, 2H, CH_2), 2.35 (s, 3H, CH_3), 1.82 (sextet, *J* = 7.5 Hz, 2H, CH_2), 1.01 (t, *J* = 7.5 Hz, 3H, CH_3); ESI-MS (*m/z*) = 285.9 $(M+H)^+$; calculated for $C_{15}H_{15}N_3OS$; C, 63.13; H, 5.30; N, 14.73; S, 11.24. Found: C, 63.22; H, 5.31; N, 14.72; S, 11.24.

6-(4-Methoxyphenyl)-2-propylimidazo[2,1-*b*][1,3,4]thiadiazole-5-carbaldehyde (4q): Light brown solid. Yield: 0.874 g, 79 %; m.p: 109-110 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 10.10 (s, 1H, CHO), 7.88 (d, *J* = 8.4 Hz, 2H, Ar-H), 7.02-6.97 (m, 2H, Ar-H), 3.82 (s, 3H, OCH_3), 3.11 (t, *J* = 6.4 Hz, 2H, CH_2), 1.85 (sextet, *J* = 6.4 Hz, 2H, CH_2), 1.03 (t, *J* = 6.4 Hz, 3H, CH_3); ESI-MS (*m/z*) = 302.1 $(M+H)^+$; calculated for $C_{15}H_{15}N_3O_2S$; C, 59.78; H, 5.02; N, 13.94; S, 10.64. Found: C, 59.81; H, 5.05; N, 13.92; S, 10.65.

6-(4-Chlorophenyl)-2-propylimidazo[2,1-*b*][1,3,4]thiadiazole-5-carbaldehyde (4r): White solid. Yield: 0.935 g, 85 %; m.p: 106-107 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 10.18 (s, 1H, CHO), 8.01 (d, *J* = 7.5 Hz, 2H, Ar-H), 7.42 (d, *J* = 8.8 Hz, 2H, Ar-H), 3.15 (t, *J* = 7.6 Hz, 2H, CH_2), 1.85 (sextet, *J* = 7.6 Hz, 2H, CH_2), 1.05 (t, *J* = 7.6 Hz, 3H, CH_3); ESI-MS (*m/z*) = 306.1 $(M+H)^+$; calculated for $C_{14}H_{12}ClN_3OS$; C, 54.99; H, 3.96; N, 13.74; S, 10.49. Found: C, 54.95; H, 4.33.945; N, 13.70; S, 10.54.

6-(4-Fluorophenyl)-2-propylimidazo[2,1-*b*][1,3,4]thiadiazole-5-carbaldehyde (4s): Yellow solid. Yield: 0.985 g, 89 %; m.p: 100-101 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 10.24 (s, 1H, CHO), 8.13 (d, J = 8.4 Hz, 2H, Ar-H), 7.34 (d, J = 8.5 Hz, 2H, Ar-H), 3.16 (t, J = 7.6 Hz, 2H, CH_2), 1.85 (sextet, J = 7.6 Hz, 2H, CH_2), 1.05 (t, J = 7.6 Hz, 3H, CH_3); ESI-MS (m/z) = 290.1 $(M+H)^+$; calculated for $C_{14}H_{12}FN_3OS$; C, 58.12; H, 4.18; N, 14.52; S, 11.08. Found: C, 58.08; H, 4.15; N, 14.50; S, 11.10.

2,6-Bis(4-chlorophenyl)imidazo[2,1-*b*][1,3,4]thiadiazole-5-carbaldehyde (4t): Red solid. Yield: 0.916 g, 85 %; m.p: 202-203 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 10.28 (s, 1H, CHO), 7.98-8.02 (m, 4H, Ar-H), 7.80 (d, J = 7.6 Hz, 2H, Ar-H), 7.55 (d, J = 8.4 Hz, 2H, Ar-H); ESI-MS (m/z) = 373.2 $(M+H)^+$; calculated for $C_{17}H_9Cl_2N_3OS$; C, 54.56; H, 2.42; N, 11.23; S, 8.57. Found: C, 54.45; H, 2.43; N, 11.25; S, 8.58.

2,6-Bis(4-fluorophenyl) imidazo[2,1-*b*][1,3,4] thiadiazole-5-carbaldehyde (4u): White solid. Yield: 0.958 g, 88 %; m.p: 230-231 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 10.29 (s, 1H, CHO), 7.92 – 7.89 (m, 4H, Ar-H), 7.68 (d, J = 7.6 Hz, 2H, Ar-H), 7.56 (d, J = 7.4 Hz, 2H, Ar-H); ESI-MS (*m/z*) = 341.9 $(M+H)^+$; calculated for $C_{17}H_9F_2N_3OS$; C, 59.82; H, 2.66; N, 12.31; S, 9.39. Found: C, 59.88; H, 2.63; N, 12.35; S, 9.40.

6-(4-Chlorophenyl)-2-(4-fluorophenyl)imidazo[2,1-*b*][1,3,4]thiadiazole-5-carbaldehyde (4v): ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): Yellow solid. Yield: 0.922 g, 85 %; m.p: 210-211 °C; 10.27 (s, 1H, CHO), 7.95-7.88 (m, 4H, Ar-H), 7.59 – 7.55 (m, 2H, Ar-H), 7.44 – 7.40 (m, 2H, Ar-H); ESI-MS (*m/z*) = 358.1 $(M+H)^+$; calculated for $C_{17}H_9ClFN_3OS$; C, 57.07; H, 2.54; N, 11.74; S, 8.96. Found: C, 57.12; H, 2.56; N, 11.75; S, 8.94.

6-(4-methoxyphenyl)-2-(trifluoromethyl)imidazo[2,1-*b*][1,3,4]thiadiazole-5-carbaldehyde (**4q**) and 6-(4-chloro phenyl)-2-(trifluoromethyl)imidazo[2,1-*b*] [1,3,4] thiadiazole-5-carbaldehyde (**4r**) prepared according to the reported procedure (Alegaon et al. 2012).

General procedure for the synthesis of target molecules T94-T111: A mixture of imidazo[2,1-*b*][1,3,4]thiadiazole-5-carbaldehyde (0.2g, 1 mmol) and pyrazine-2-carbohydrazide (1 mmol) was taken in ethanol (10 mL) in a dry 50 mL RBF. To the above mixture catalytic amount of conc. H_2SO_4 was added and the resulting mixture was stirred at room RT for 10-15 minutes. After the completion of the reaction (as monitored by TLC), water (10 mL) was added to the reaction mixture and the solid separated was filtered off and washed with water. The product was then recrystallized from ethanol.

(*E*)-*N*'-((2-methyl-6-(*p*-tolyl)imidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)methylene) pyrazine-2-carbohydrazide (T94): White solid. mp: 239-240°C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 12.37 (br s, 1H, NH), 9.25 (d, *J* = 1.3 Hz, 1H, Ar-H), 9.00 (s, 1H, Ar-H), 8.90 (d, *J* = 2.4 Hz, 1H, Ar-H), 8.76 (dd, *J* = 2.4 Hz, 1.5, 1H, Ar-H), 7.76 (d, *J* = 8.1 Hz, 2H, Ar-H), 7.29 (d, *J* = 7.9 Hz, 2H, Ar-H), 2.79 (s, 3H, CH_3), 2.35 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz, δ in ppm): 161.5, 159.6, 148.7, 148.3, 148.2, 145.2, 144.5, 143.7, 139.5, 138.2, 130.9, 129.6, 128.7, 119.20, 18.15, 21.33; ESI-MS (m/z) = 378.1 $(M+H)^+$; calculated for $C_{18}H_{15}N_7OS$; C, 57.28; H, 4.01; N, 25.98; S, 8.50. Found: C, 57.18; H, 4.08; N, 26.01; S, 8.54.

(*E*)-*N*'-((6-(4-methoxyphenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl) methylene)pyrazine-2-carbohydrazide (T95): Off whit solid. m.p: 224-225 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 12.36 (br s, 1H, NH), 9.25 (s, 1H, CH), 9.00 (s, 1H, Ar-H), 8.89 (d, *J* = 2.4 Hz, 2H, Ar-H), 7.75 (d, *J* = 7.6 Hz, 2H, Ar-H), 7.30 (d, *J* = 8.8 Hz, 2H, Ar-H), 3.83 (s, 3H, OCH_3), 2.73 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz, δ in ppm): 162.5, 159.4, 149.1, 148.3, 147.6, 145.2, 144.5, 143.7, 139.5, 138.2, 131.4, 129.6, 128.7, 119.20, 56.0, 18.15; ESI-MS (*m/z*) = 394.1 (M+H)+; calculated for $C_{18}H_{15}N_7O_2S$; C, 54.95; H, 3.84; N, 24.92; S, 8.15. Found: C, 54.98; H, 3.88; N, 24.91; S, 8.14.

(*E*)-*N*'-((6-(4-chlorophenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl) methylene) pyrazine-2-carbohydrazide (T96): Yellow solid. m.p: 228-229 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 12.52 (br s, 1H, NH), 9.28 (s, 1H, CH), 9.11 (s, 1H, Ar-H), 8.92 (d, *J* = 6.4 Hz, 2H, Ar-H), 7.80 (d, *J* = 7.6 Hz, 2H, Ar-H), 7.44 (d,

J = 8.8 Hz, 2H, Ar-H), 2.73 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz, δ in ppm): 162.5, 159.4, 149.1, 148.3, 147.6, 145.2, 144.5, 143.7, 139.5, 138.2, 131.4, 129.6, 128.7, 120.5, 18.5; ESI-MS (*m/z*) = 397.9 (M+H)+; calculated for $C_{17}H_{12}ClN_7OS$; C, 51.32; H, 3.04; N, 24.64; S, 8.06. Found: C, 51.35; H, 3.08; N, 24.58; S, 8.10.

(*E*)-*N*'-((6-(4-fluorophenyl)-2-methylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl) methylene) pyrazine-2-carbohydrazide (T97): Yellow solid. m.p: 238-239 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 12.53 (br s, 1H, NH), 9.28 (s, 1H, CH), 9.13 (s, 1H, Ar-H), 8.95 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.82 (d, *J* = 7.6 Hz, 2H, Ar-H), 7.44 (d, *J* = 8.4 Hz, 2H, Ar-H), 2.75 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz, δ in ppm): 161.9, 159.6, 148.5, 148.2, 146.8, 145.1, 144.5, 143.7, 139.2, 130.9, 130.2, 119.4, 115.9, 115.7, 18.1; ESI-MS (*m/z*) = 382.2 (M+H)+; calculated for $C_{17}H_{12}FN_7OS$; C, 53.54; H, 3.17; N, 25.71; S, 8.41. Found: C, 53.55; H, 3.18; N, 25.70; S, 8.40.

(*E*)-*N*'-((2-propyl-6-(*p*-tolyl)imidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)methylene) pyrazine-2-carbohydrazide (T98): Off white solid. m.p: 200-201 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 12.32 (br s, 1H, NH), 9.19 (s, 1H, CH), 8.98 (s, 1H, Ar-H), 8.88 – 8.76 (m, 2H, Ar-H), 7.82 (d, *J* = 7.6 Hz, 2H), 7.01 (d, *J* = 8.8 Hz, 2H, Ar-H), 3.08 (t, *J* = 7.4 Hz, 2H, CH_2), 2.35 (s, 3H, CH_3), 1.82 (sextet, *J* = 7.4 Hz, 2H, CH_2), 1.01 (t, *J* = 7.3 Hz, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz, δ in ppm): 164.3, 160.0, 156.8, 151.7, 148.5, 146.2, 144.6, 141.6, 140.7, 135.1, 129.8, 126.8, 115.1, 114.39, 33.1, 23.5, 21.3, 13.4; ESI-MS (*m/z*) = 406.1 (M+H)+; calculated for $C_{20}H_{19}N_7OS$; C, 59.24; H, 4.72; N, 24.18; S, 7.91. Found: C, 59.15; H, 4.75; N, 24.20; S, 7.94.

(*E*)-*N*'-((6-(4-methoxyphenyl)-2-propylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl) methylene)pyrazine-2-carbohydrazide (T99): Yellow solid. m.p: 291-292 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 12.38 (br s, 1H, NH), 9.25 (s, 1H, CH), 9.00 (s, 1H, Ar-H), 8.90 (d, *J* = 2.1 Hz, 1H, Ar-H), 8.76 (s, 1H, Ar-H), 7.87 (d, *J* = 8.6 Hz, 2H, Ar-H), 7.03 (d, *J* = 8.7 Hz, 2H, Ar-H), 3.81 (s, 3H, OCH_3), 3.08 (t, *J* = 7.4 Hz, 2H), 1.80 (sextet, *J* = 7.4 Hz, 2H), 1.01 (t, *J* = 7.4 Hz, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz, δ in ppm): 165.3, 161.0, 157.7, 151.8, 148.6, 146.2, 145.6, 141.6, 140.7, 136.2, 129.8, 126.8, 115.1, 114.39, 55.6, 33.2, 23.8, 13.5; ESI-MS (*m/z*)= 422.2

(M+H)+; calculated for $C_{20}H_{19}N_7O_2S$; C, 56.99; H, 4.54; N, 23.26; S, 7.61. Found: C, 57.05; H, 4.55; N, 23.20; S, 7.64.

(*E*)-*N*'-((6-(4-chlorophenyl)-2-propylimidazo[2,1-b][1,3,4]thiadiazol-5-yl) methylene)pyrazine-2-carbohydrazide (T100): Yellow solid. m.p: 216-217 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 12.48 (br s, 1H, NH), 9.27 (s, 1H, CH), 9.03 (s, 1H, Ar-H), 8.93- 8.84 (m, 2H, Ar-H), 8.03 (d, J = 6.4 Hz, 2H, Ar-H), 7.34-7.27 (m, 2H, Ar-H), 3.12 (t, J = 7.3 Hz, 2H, CH_2), 1.83 (sextet, J = 7.3 Hz, 2H, CH_2), 1.04 (t, J = 7.4 Hz, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz, δ in ppm): 166.3, 158.7, 152.8, 148.6, 146.7, 145.6, 141.4, 140.7, 136.2, 134.4, 132.7, 130.0, 129.1, 115.1, 33.4, 22.8, 13.5; ESI-MS (*m/z*) = 426.1(M+H)+; calculated for $C_{19}H_{16}ClN_7OS$; C, 53.58; H, 3.79; N, 23.02; S, 7.53. Found: C, 53.55; H, 3.75; N, 23.10; S, 7.54.

(*E*)-*N*'-((6-(4-fluorophenyl)-2-propylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl) methylene)pyrazine-2-carbohydrazide (T101): Light brown solid. m.p: 201-202 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 12.47 (br s, 1H, NH), 9.28 (s, 1H, CH), 9.05 (s, 1H, Ar-H), 8.94 (s, 1H, Ar-H), 8.80 (s, 1H, Ar-H), 8.03 (s, 2H, Ar-H), 7.34 (s, 2H, Ar-H), 3.12 (t, J = 7.6 Hz, 2H, CH_2), 1.83 (sextet, J = 7.6 Hz, 2H, CH_2), 1.04 (t, J = 7.6 Hz, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz, δ in ppm): 166.4, 163.8, 159.6, 148.2, 148.1, 146.7, 145.1, 144.5, 143.7, 139.2, 131.0, 130.9, 119.4, 115.9, 115.7, 33.5, 22.4, 13.7; ESI-MS (*m/z*) = 410.2 (M+H)+; calculated for $C_{19}H_{16}FN_7OS$; C, 55.74; H, 3.94; N, 23.95; S, 7.83. Found: C, 55.75; H, 3.95; N, 23.90; S, 7.84.

(*E*)-*N*'-((6-(4-methoxyphenyl)-2-(*p*-tolyl)imidazo[2,1-*b*][1,3,4]thiadiazol-5-yl) methylene)pyrazine-2-carbohydrazide (T102): Yellow solid. m.p: 249-250 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 12.34 (br s, 1H, NH), 9.18 (s, 1H, CH), 9.01 (s, 1H, Ar-H), 8.72 (d, J = 7.4 Hz, 2H, Ar-H), 7.90-7.85 (m, 4H, Ar-H), 7.44-7.35 (m, 2H, Ar-H), 7.23 (d, J = 6.8 Hz, 2H, Ar-H), 3.89 (s, 3H, OCH_3), 2.45 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz, δ in ppm): 164.4, 161.0, 157.7, 148.6, 146.2, 145.6, 141.6, 140.7, 140.1, 136.2, 131.0, 130.3, 128.7, 126.8, 12, 6.0, 115.1, 114.3, 56.0, 21.7; ESI-MS (*m/z*) = 470.1(M+H)+; calculated for $C_{24}H_{19}N_7O_2S$; C, 61.39; H, 4.08; N, 20.88; S, 6.83. Found: C, 61.40; H, 4.10; N, 20.90; S, 6.84.

(*E*)-*N*'-((6-(4-chlorophenyl)-2-(p-tolyl)imidazo[2,1-*b*][1,3,4]thiadiazol-5-yl) methylene)pyrazine-2-carbohydrazide (T103): Yellow solid. m.p: 299-300 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 12.51 (br s, 1H, NH), 9.29 (s, 1H, CH), 9.12 (s, 1H, Ar-H), 8.92 (s, 1H, Ar-H), 8.78 (s, 1H, Ar-H), 8.06 (s, 2H, Ar-H), 7.89 (s, 2H, Ar-H), 7.49 (d, *J* = 40.0 Hz, 4H, Ar-H), 2.41 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz, δ in ppm): 164.4, 157.75, 148.6, 146.2, 145.6, 141.6, 140.7, 140.1, 136.2, 134.4, 132.7, 131.0, 130.2, 129.11, 128.79, 126.23, 115.13, 21.13; ESI-MS (*m/z*) = 475.4 (M+H)+; calculated for $C_{23}H_{16}ClN_7OS$; C, 58.29; H, 3.40;N, 20.69; S, 6.77; S, 6.83. Found: C, 58.30; H, 3.41; N, 20.70; S, 6.74.

(*E*)-*N*'-((6-(4-fluorophenyl)-2-(p-tolyl)imidazo[2,1-*b*][1,3,4]thiadiazol-5-yl) methylene) pyrazine-2-carbohydrazide (T104): Light yellow solid. m.p: 292-293 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 12.54 (br s, 1H, NH), 9.22 (s, 1H, CH), 9.02 (s, 1H, Ar-H), 8.86 (d, *J* = 8.6 Hz, 2H, Ar-H), 7.75 – 7.67 (m, 2H, Ar-H), 7.53 – 7.48 (m, 4H, Ar-H), 7.38 – 7.27 (m, 2H, Ar-H), 2.37 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz, δ in ppm): 164.3, 157.7, 148.6, 146.2, 145.6, 141.6, 140.7, 140.1, 136.2, 131.5, 131.09, 128.7, 126.23, 116.86, 115.13, 21.13; ESI-MS (*m/z*) = 458.1 (M+H)+; calculated for $C_{23}H_{16}FN_7OS$; C, 60.38; H, 3.53; N, 21.43; S, 7.01. Found: C, 60.40; H, 3.51; N, 21.40; S, 7.04.

(*E*)-*N*'-((2-(4-chlorophenyl)-6-(*p*-tolyl)imidazo[2,1-b][1,3,4]thiadiazol-5-yl) methylene)pyrazine-2-carbohydrazide (T105): Yellow solid. m.p: 297-298 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 12.50 (br s, 1H, NH), 9.28 (s, 1H, CH), 9.03 (s, 1H, Ar-H), 8.88 (d, *J* = 7.4 Hz, 2H, Ar-H), 7.74 (d, *J* = 8.6 Hz, 2H, Ar-H), 7.60-7.55 (m, 4H, Ar-H), 7.37 (d, *J* = 8.5 Hz, 2H, Ar-H), 2.34 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz, δ in ppm): 164.4, 157.7, 148.6, 146.2, 145.6, 141.6, 140.7, 137.8, 136.3, 133.7, 129.6, 129.4, 128.9, 127.4, 127.2, 115.1, 21.13; ESI-MS (*m/z*) = 474.1 (M+H)+; calculated for $C_{23}H_{16}ClN_7OS$; C, 58.29; H, 3.40; N, 20.69; S, 6.77. Found: C, 58.30; H, 3.41; N, 20.70; S, 6.74.

(*E*)-*N*'-((2,6-bis(4-chlorophenyl)imidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)methylene) pyrazine-2-carbohydrazide (T106): Light yellow solid. m.p: 299-300 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 12.51 (br s, 1H, NH), 9.29 (s, 1H, CH), 9.12 (s, 1H,

Ar-H), 8.92 (d, *J* = 2.4 Hz, 1H, Ar-H), 8.78 (s, 1H, Ar-H), 8.02 (dd, *J* = 14.2, 8.6 Hz, 4H, Ar-H), 7.72 (d, *J* = 8.7 Hz, 2H, Ar-H), 7.55 (d, *J* = 8.6 Hz, 2H, Ar-H); ^{13}C NMR (DMSO-d_6, 100 MHz, δ in ppm): 164.4, 158.3, 149.6, 146.7, 144.5, 140.6, 140.3, 136.3, 134.4, 133.7, 132.4, 130.1, 129.5, 128.9, 127.4, 115.13; ESI-MS (*m/z*) = 494.1 (M+H)+; calculated for $C_{22}H_{13}Cl_2N_7OS$; C, 53.45; H, 2.65; N, 19.83; S, 6.49. Found: C, 53.40; H, 2.64; N, 19.85; S, 6.45.

(*E*)-*N*'-((2-(4-chlorophenyl)-6-(4-fluorophenyl)imidazo[2,1-*b*][1,3,4] thiadiazol-5-yl) methylene)pyrazine-2-carbohydrazide (T107): Brown solid. mp: 291-292 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 12.55 (br s, 1H, NH), 9.32 (s, 1H, CH), 9.13 (s, 1H, Ar-H), 8.95 (s, 1H, Ar-H), 8.81 (s, 1H, Ar-H), 8.06 (s, 4H, Ar-H), 7.76 (d, *J* = 8.2 Hz, 2H, Ar-H), 7.37 (s, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz, δ in ppm): 164.3, 157.7, 148.6, 146.2, 145.6, 141.6, 140.7, 136.3, 133.8, 131.5, 128.9, 127.2, 115.2, 115.1; ESI-MS (*m/z*) = 478.1(M+H)+; calculated for $C_{22}H_{13}ClFN_7OS$; C, 55.29; H, 2.74; N, 20.52; S, 6.71. Found: C, 55.30; H, 2.74; N, 20.48; S, 6.75.

(*E*)-*N*'-((6-(4-chlorophenyl)-2-(4-fluorophenyl)imidazo[2,1-*b*][1,3,4]thiadiazol-5-yl) methylene)pyrazine-2-carbohydrazide (T108): Light yellow solid. m.p: 298-299 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 12.50 (br s, 1H, NH), 9.28 (s, 1H, Ar-H), 8.84 (s, 1H, Ar-H), 8.80 (s, 1H, Ar-H), 8.72 (s, 1H, Ar-H), 7.66 – 7.62 (m, 2H, Ar-H), 7.59 – 7.55 (m, 2H, Ar-H), 7.75 (d, *J* = 7.6 Hz, 2H, Ar-H), 7.44 – 7.40 (m, 2H, Ar-H); ^{13}C NMR (DMSO-d_6, 100 MHz, δ in ppm): 166.6, 164.4, 157.7, 148.6, 146.2, 145.6, 141.6, 140.7, 136.2, 134.4, 132.7, 130.2, 129.3, 128.3, 117.4, 115.2, 115.1; ESI-MS (m/z) = 478.1(M+H)+; calculated for $C_{22}H_{13}ClFN_7OS$; C, 55.29; H, 2.74; N, 20.52; S, 6.71. Found: C, 55.32; H, 2.76; N, 20.45; S, 6.74.

(*E*)-*N*'-((2,6-bis(4-fluorophenyl)imidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)methylene) pyrazine-2-carbohydrazide (T109): Yellow solid. m.p: 300-301 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 12.50 (br s, 1H, NH), 9.32 (s, 1H, CH), 8.94 (s, 1H, Ar-H), 8.79 (d, *J* = 8.8 Hz, 2H), 7.79 – 7.72 (m, 4H), 7.53 – 7.44 (m, 4H); ^{13}C NMR (DMSO-d_6, 100 MHz, δ in ppm): 166.6, 164.3, 157.7, 148.6, 146.2, 145.63, 141.6, 140.7, 136.2, 131.5, 129.3, 129.2, 113.5,117.6, 114.3, 115.1; ESI-MS (*m/z*) = 462.2

(M+H)+; calculated for $C_{22}H_{13}F_2N_7OS$; C, 57.26; H, 2.84; N, 21.25; S, 6.95. Found: C, 57.32; H, 2.86; N, 21.30; S, 6.94.

(*E*)-*N*'-((6-(4-methoxyphenyl)-2-(trifluoromethyl)imidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)methylene) pyrazine-2-carbohydrazide (T110): White solid. mp: 199-200 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 12.52 (br s, 1H, NH), 9.25 (s, 1H, CH), 9.08 (s, 1H, Ar-H), 8.92 (d, *J* = 2.1 Hz, 1H, Ar-H), 8.76 (s, 1H, Ar-H), 7.87 (d, *J* = 8.6 Hz, 2H, Ar-H), 7.03 (d, *J* = 7.6 Hz, 2H, Ar-H), 3.84 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6, 100 MHz, δ in ppm): 160.7, 158.3, 148.7, 146.2, 145.6, 141.6, 140.7, 136.2, 130.0, 129.8, 126.9, 121.8, 115.6, 115.1, 55.3; ESI-MS (*m/z*) = 448.1(M+H)+; calculated for $C_{18}H_{12}F_3N_7O_2S$; C, 48.32; H, 2.70; N, 21.92;S, 7.17. Found: C, 48.29; H, 2.76; N, 21.93; S, 7.14.

(*E*)-*N*'-((6-(4-chlorophenyl)-2-(trifluoromethyl)imidazo[2,1-*b*][1,3,4]thiadiazol-5-yl) methylene) pyrazine-2-carbohydrazide (T111): Off white solid. m.p: 240-241 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 12.53 (br s, 1H, NH), 9.26 (d, *J* = 1.3 Hz, 1H, CH), 9.10 (s, 1H, Ar-H), 8.92 (d, *J* = 2.4 Hz, 1H, Ar-H), 8.77 (dd, *J* = 2.3, 1.5 Hz, 1H, Ar-H), 8.00 (d, *J* = 8.6 Hz, 2H, Ar-H), 7.58 (d, *J* = 8.6 Hz, 2H, Ar-H); ^{13}C NMR (DMSO-d_6, 100 MHz, δ in ppm): 161.2, 158.7, 148.3, 146.1, 145.4, 141.5, 140.7, 136.1, 134.6, 132.7, 131.0, 129.5, 129.1, 121.8, 115.6; ESI-MS (*m/z*) = 452.1 (M+H)+; calculated for $C_{17}H_9ClF_3N_7OS$; C, 45.19; H, 2.01; N, 21.70; S, 7.10 Found: C, 45.21; H, 2.06; N, 21.73; S, 7.08.

6.4 PHARMACOLOGY (Experimental procedure for *in vitro* antitubercular, antibacterial, *in vitro* cytotoxicity and molecular docking studies are discussed in section 4.4.1, 3.4.2, 3.4.3 and 2.4.4 respectively).

6.5 RESULTS AND DISCUSSION

6.5.1 Chemistry

The structure of the target molecules (**T94-T111)** was confirmed by spectral (^{1}H NMR, ^{13}C NMR, ESI-MS) and elemental analysis. For instance, the ^{1}H NMR spectrum of compound **T94** showed a broad singlet with one proton at δ 12.37 ppm due to the NH proton and two more singlets at δ 9.25 and 9.00 ppm due to hydrazone CH and C_3-H of pyrazine respectively.

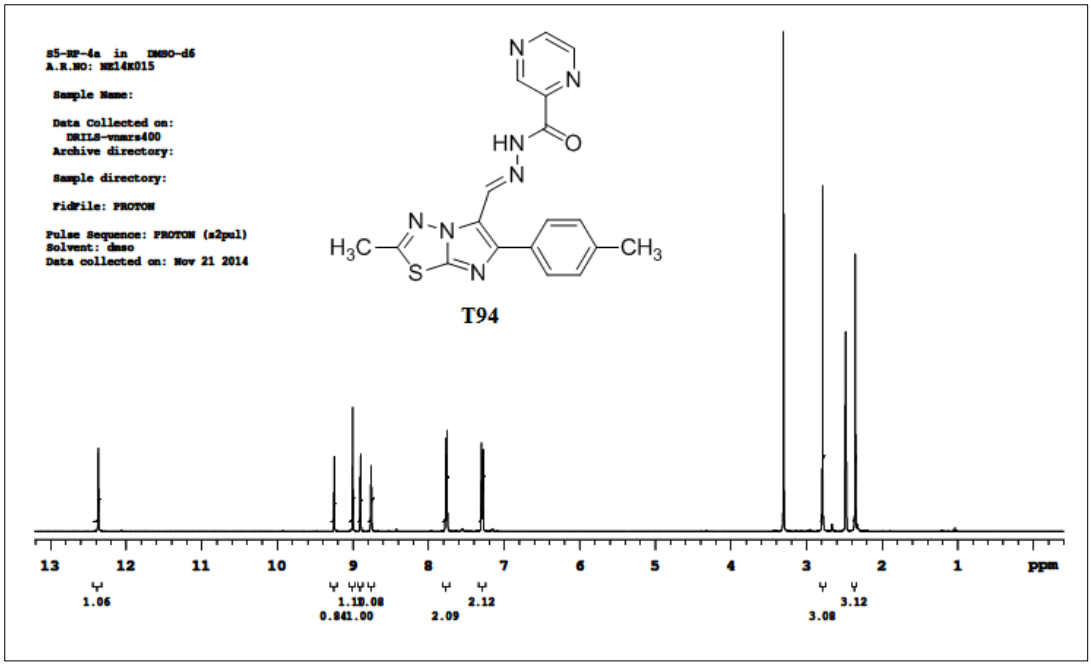


Figure 6.2 ^{1}H NMR Spectrum of **T94**

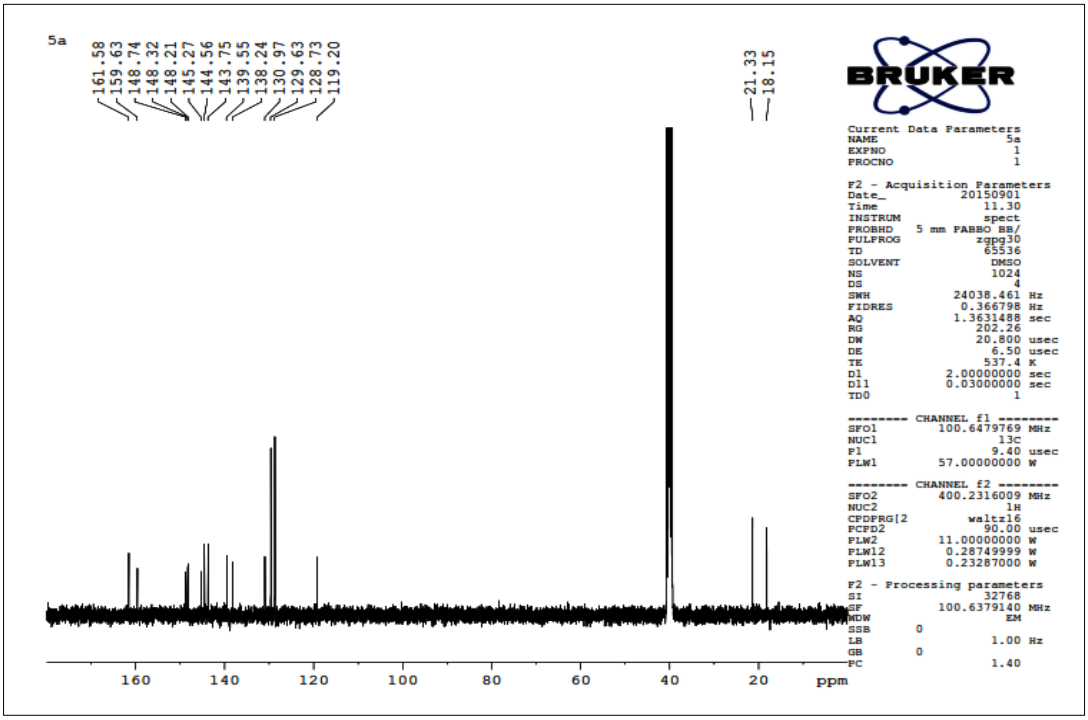


Figure 6.3 ^{13}C NMR Spectrum of **T94**

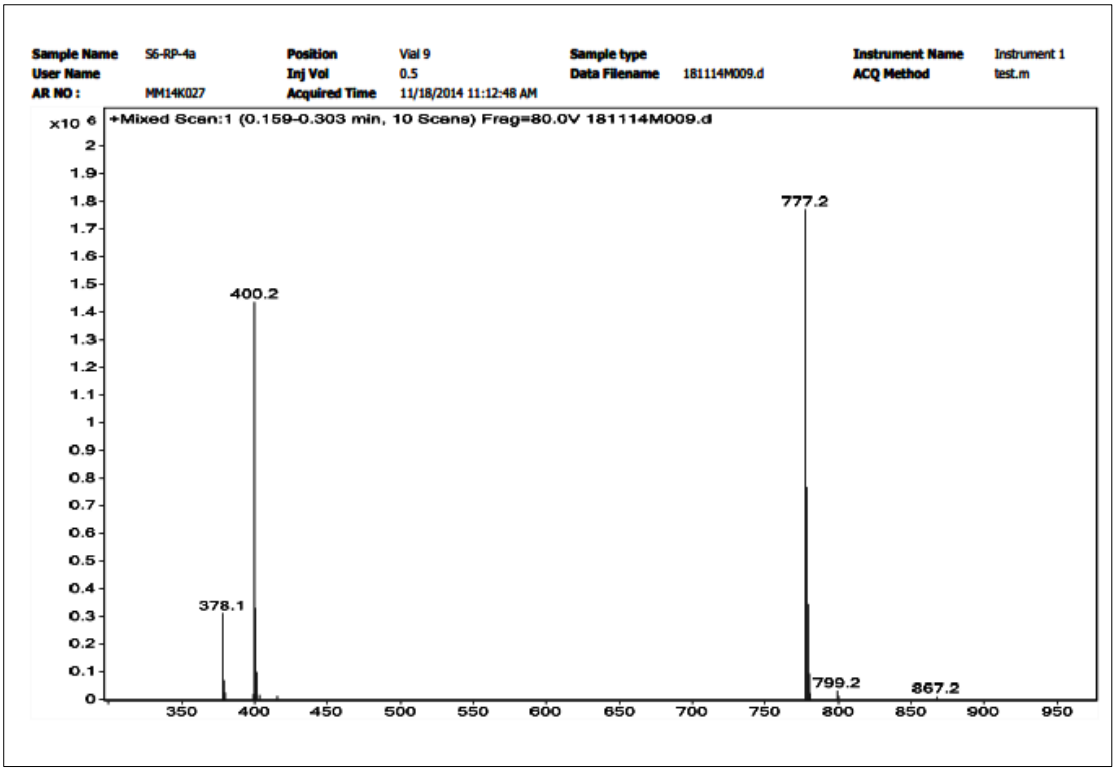


Figure 6.4 Mass Spectrum of **T94**

Compound **T94** also displayed one doublet at 8.90 ppm and doublet of doublet (dd) at 8.76 ppm due to C_5-H and C_6-H of pyrazine protons respectively (**figure 6.2**). Also, its mass spectrum (**figure 6.4**) showed a molecular ion peak at *m/z* 378.1, which corresponds to M+1 peak of the molecule and is in agreement with its molecular formula $C_{18}H_{15}N_7OS$. Substitution pattern, yield and solubility of target compounds **T94-T111** are given in **table 6.1.**

6.5.2 Single crystal X-ray crystallography studies

Single crystals of **T96** and **T97** were grown from their solutions in methanol, by the slow evaporation of the solvent at ambient temperature. A suitable crystal was mounted and the single-crystal data was collected at room temperature. The crystal structure were solved and refined by direct methods using SHELXL-2013 package. The hydrogens were fixed in geometrically calculated positions and refined isotropically. The compounds (**T96** and **T97)** crystallized in the monoclinic system with P 2/c space group. The crystal structures (ORTEP diagram) of the compounds are given in **figure 6.5** and crystal data are given in **table 6.2**.

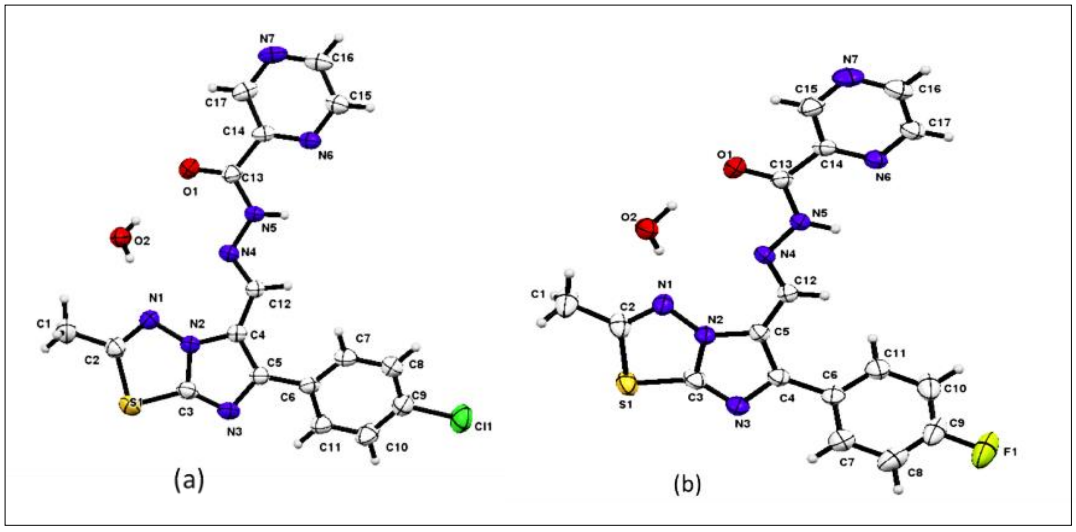


Figure 6.5 ORTEP diagram showing the X-ray crystal structure of compounds (a) **T96** and (b) **T97**.

Table 6.2 Crystal data and measurement details for compounds **T96** and **T97**.

Parameters	**Crystal data of T96**	**Crystal data of T97**
Empirical formula	$C_{17}H_{12}ClN_7OS.H_2O$	$C_{17}H_{12}FN_7OS.H_2O$
Formula weight	415.86	399.41
Crystal system	Monoclinic	Monoclinic
Space group	P 2/c	P 2/c
a (Å)	25.4649(19)	25.4405(13)
b (Å)	11.3125(7)	10.9625(5)
c (Å)	13.2219(9)	13.1371(6)
Volume ($Å^3$)	3707.14	3582.7(3)
Angle α, β, γ	90, 103.271(5), 90	90, 102.078(3), 90
Z	8	8
F_{000}	1712.0	1648.0
μ (mm−1)	0.349	0.221
Temperature (T)	296k	296k
Radiation wavelength (Å)	0.71073	0.71073
Radiation type	Mo Kα	Mo Kα
Radiation source	Mo	Mo
R-Factor (%)	3.41	4.38
CCDC number	1431975	1431976

6.5.3 *In vitro* antimycobacterial activity

All the target derivatives (**T94-T111**) were screened against *Mtb* H37Rv (ATCC27294) using MABA method. The MIC values in µg/mL of **T94-T111** along with those of standard drugs for comparison are presented in **figure 6.6**. The MIC values of the compounds are in the range 12.5-50 µg/mL. Six compounds *viz*. **T97**, **T101**, **T104**, **T107**, **T108** and **T109** showed significant inhibitory activity with MIC of 12.5 µg/mL. Other six compounds (**T96**, **T100**, **T103**, **T105**, **T106** and **T111**) showed moderate activity with MIC of 25 µg/mL. It is interesting to note that the inhibitory activity of these twelve molecules is superior to that of the pyrazinamide (MIC = 50 µg/mL). Hence it can be concluded that hybridization of the ITD ring with the pyrazinamide core serves to enhance the inhibition activity of the hybrid molecules. The nature of the substituents at R^1 and R^2, particularly at R^2, was found to affect the activity of these compounds. It is interesting to note that all chloro and fluoro substituted (R^2) derivatives are more active than their methyl or methoxy analogs irrespective of the nature of the substituent at R^1. Among fluoro and chloro substituted derivatives, most of the fluoro compounds showed better activity than their chloro analogs. Similarly, it was observed that the inhibition activity increases with a fluoro/chloro substitution at R^2 as well. Hence, the PZA-ITD hybrids with a fluoro/chloro substitution at R^1 and R^2 are promising leads for the development of potent antiTB agents.

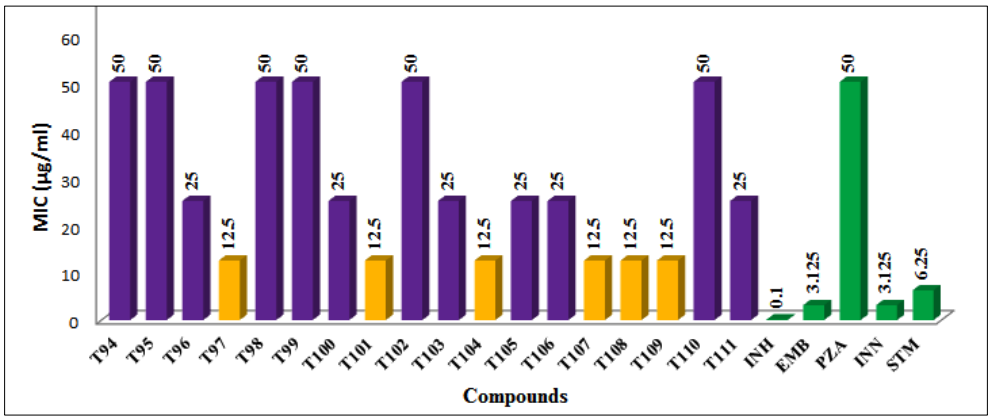


Figure 6.6 AntiTB activity of **T94-T111** against *Mtb* H37RV

6.5.4 *In vitro* antibacterial activity

The *in vitro* antibacterial activity of synthesized compounds **T94-T111** were tested using disc diffusion method (Isenberg, 1992) by screening the compounds

against *S. aureus*, *P. aeruginosa* and *E. coli*. The compounds were dissolved in DMSO with two concentrations (75 µg/mL and 50 µg/mL). Ciprofloxacin was taken as standard drug. Compounds **T107, T108** and **T109** demonstrated good inhibition activity against all the tested bacterial strains at both concentrations (75 and 50 µg/mL). Interestingly, these compounds contain a halogen group (Cl/F) both at R^1 and R^2. Also, other three fluoro derivatives viz. **T97**, **T101**, and **T104** showed moderate activity which signifies the contribution of the fluoro group towards the inhibition activity. The antibacterial screening results are displayed in **table 6.3**.

Table 6.3 Antibacterial activity of target compounds (**T94-T111**)

Compounds	***E. coli***		***S. aureus***		***P. aeruginosa***	
Cocn. in µg/ml	75	50	75	50	75	50
T94	12±0.1	10±0.2	12±0.2	08±0.3	14±0.4	10±0.2
T95	10±0.1	08±0.1	09±0.3	07±0.3	13±0.2	09±0.2
T96	15±0.2	11±0.2	13±0.1	10±0.2	16±0.1	12±0.1
T97	18±0.1	15±0.1	19±0.3	14±0.3	15±0.2	13±0.2
T98	13±0.2	10±0.2	14±0.2	09±0.1	11±0.2	08±0.2
T99	14±0.1	11±0.2	13±0.1	11±0.2	15±0.2	11±0.3
T100	12±0.3	08±0.1	09±0.2	06±0.2	09±0.1	06±0.3
T101	17±0.3	13±0.3	15±0.3	11±0.1	13±0.3	11±0.1
T102	08±0.1	05±0.3	08±0.2	05±0.3	07±0.1	04±0.2
T103	13±0.2	11±0.2	12±0.1	10±0.2	14±0.1	12±0.1
T104	23±0.4	19±0.1	18±0.4	14±0.2	17±0.3	13±0.1
T105	12±0.4	08±0.1	11±0.1	08±0.1	13±0.2	11±0.2
T106	15±0.4	11±0.1	16±0.1	12±0.1	16±0.2	13±0.2
T107	25±0.4	21±0.2	23±0.4	17±0.2	15±0.1	12±0.2
T108	26±0.3	22±0.3	22±0.1	18±0.3	18±0.3	16±0.1
T109	25±0.4	21±0.2	23±0.4	17±0.2	16±0.1	14±0.2
T110	11±0.2	07±0.3	07±0.1	04±0.1	12±0.4	08±0.1
T111	12±0.3	08±0.1	06±0.3	05±0.1	13±0.1	11±0.4
Control	00	00	00	00	00	00

INN	32±0.2	27±0.2	26±0.1	21±0.2	21±0.2	18±0.1
INN: Ciprofloxacin; −: inhibition not detected; control: DMSO						

6.5.5 *In vitro* cytotoxicity studies

The potent compounds (MIC 12.5 µg/mL) were taken for MTT assay to check their toxicity against normal cells of NIH 3T3 mouse embryonic fibroblasts (Gundersen et al. 2002). The cell growth inhibition of potent compounds at a concentration of 50 µg/mL is shown in **Figure 4.7**. The compounds did not show any toxicity to NIH 3T3 mouse embryonic fibroblasts cells demonstrating the lack of general cellular toxicity.

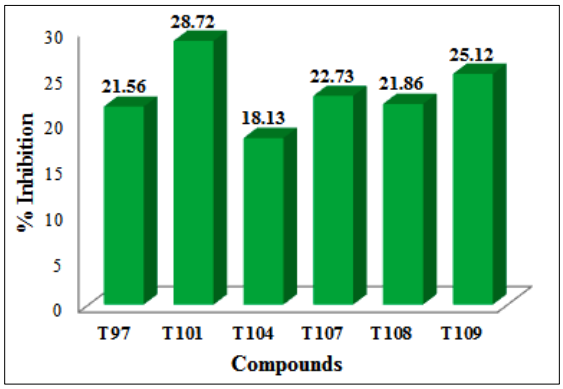


Figure 6.7 Growth inhibition activity of active compounds (at a concentration of 50 µg/mL) against NIH 3T3 cell line.

6.5.6 Molecular docking studies

Molecular docking studies of the new PZA-ITD derivatives were performed with InhA of *Mtb* as the target enzyme, which is validated as an effective anti-TB target (Joshi et al. 2015; Matviiuk et al. 2013). The molecules were docked within the active site of InhA (PDB code: 1P44) using Glide 6.6 package (Schrodinger, 2015-1). Compounds **T104**, **T105**, **T108** and **T109** showed very good docking score of -10.39, -9.70, -10.76 and -11.28 respectively. The docking poses of molecules **T104**, **T105**, **T108** and **T109** are shown in **figure 6.8**. Compounds **T104**, **T105** and **T108** showed a similar type of interactions with Gly 104, Phe 149 and Tyr 158 residues of the enzyme. Compound **T109** with the highest docking score showed interactions with Gly 104, Phe 149 and Tyr 158. It is known that PZA interacts with Try 158 whereas isoniazid (INH) makes a *pi-pi* interaction with Phe 149 residues of Inha. Atleast one

of these two interactions was common in all the target molecules. In addition, as the target molecules contain 4 or 5 aromatic rings the pi-pi interaction with the target enzyme is quite strong. For instance two aromatic rings of **T104**, **T105** and **T108** make simultaneous *pi-pi* interactions (**figure 6.8** a, b and c respectively) with Phe 149 residue of the target enzyme. The strong *pi-pi* interaction in combination with hydrogen bonding interactions ensures the robust binding between the target molecules and the enzyme, which could be responsible for the significant inhibition activity of these molecules against the *MTB* strain. The docking score of all the molecules and details of interacting amino acid residues are given in **table 6.4**.

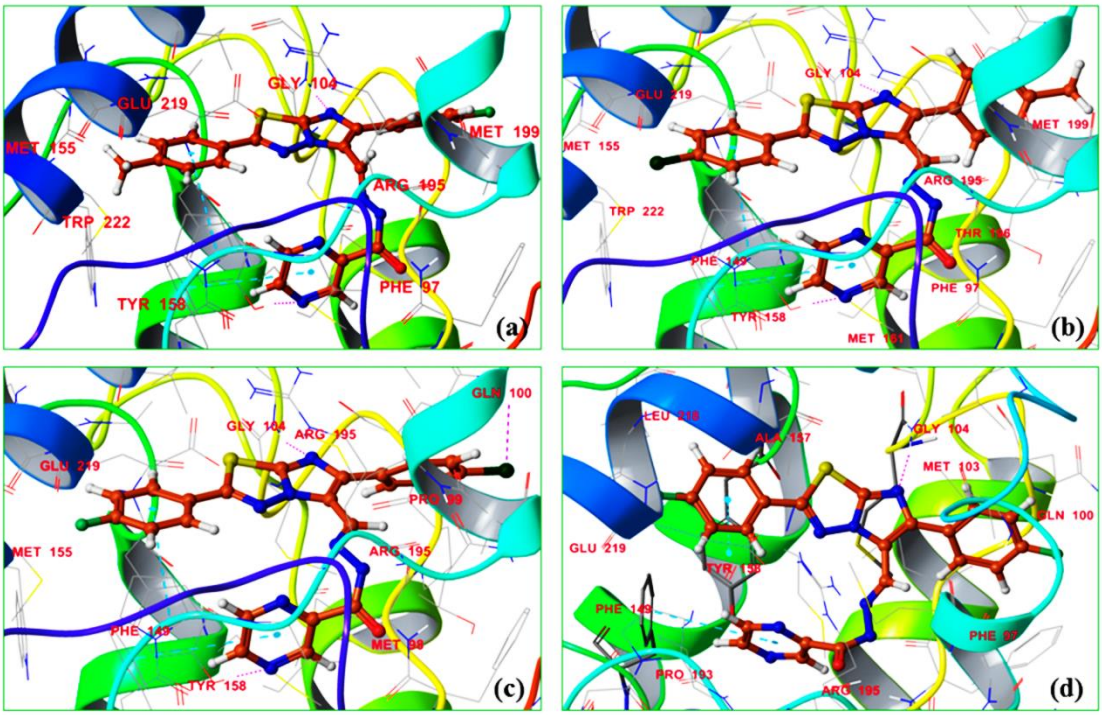


Figure 6.8 The docking poses of some new PZA-ITD hybrids with target enzyme InhA (a) **T104**; (b) **T105** (c) **T108**; (d) **T109**

Table 6.4 Docking scores of the compounds with the target enzyme (1P44) and details of the interacting amino acid residues.

Compounds	Docking score	Interacting amino acid residues
T94	-6.05	Tyr 158
T95	-9.33	Tyr 158
T96	-9.01	Phe 149, Tyr 158
T97	-8.802	Phe 149, Tyr 158

T98	-9.852	Phe 149, Tyr 158
T99	-7.803	Tyr 158
T100	-8.98	Tyr 158
T101	-9.495	Tyr 158
T102	-9.486	Tyr 158, Gly104, Gln100
T103	-9.12	Tyr 158, Gly104,
T104	-10.389	Gly 104, Phe 149, Tyr 158
T105	-9.70	Gly 104, Phe 149, Tyr 158
T106	-9.155	Phe 149, Tyr 158, Gly104
T107	-9.166	Phe 149, Tyr 158, Gly104
T108	-10.758	Gly 104, Phe 149, Tyr 158, Gln 100
T109	-11.282	Gly 104, Phe 149, Tyr 158
T111	-8.784	Phe 149, Tyr 158

6.6 CONCLUSIONS

- A new library of ITD and pyrazinamide hybrid derivatives (**T94-T111**) were designed via molecular hybridization approach and were synthesized with excellent yields.
- All the compounds were characterized using ^{1}H NMR, ^{13}C NMR, mass spectral data and CHN elemental analysis. The structures of compounds **T96** and **T97** were confirmed by single crystal x-ray studies.
- The target compounds were evaluated for their *in vitro* inhibition activity against *Mtb* $H_{37}Rv$ (*Mtb*) and antibacterial activity against *S. aureus*, *P. aeruginosa* and *E. coli.*
- Among 18 new derivatives, 12 compounds are more potent than the standard antiTB drug PZA. The SAR revealed that 4-fluorophenyl and 4-chlorophenyl substituents on the ITD ring substantially enhance both antimycobacterial and antibacterial activity of the compounds.
- Further, the active compounds are not toxic to normal cells of NIH 3T3 mouse embryonic fibroblasts.

- The molecular docking study of the active compounds was carried out against the InhA enzyme of *Mtb* to comprehend the binding interactions between the molecules and the amino acid residues of the enzyme.

Appendix 2.1

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

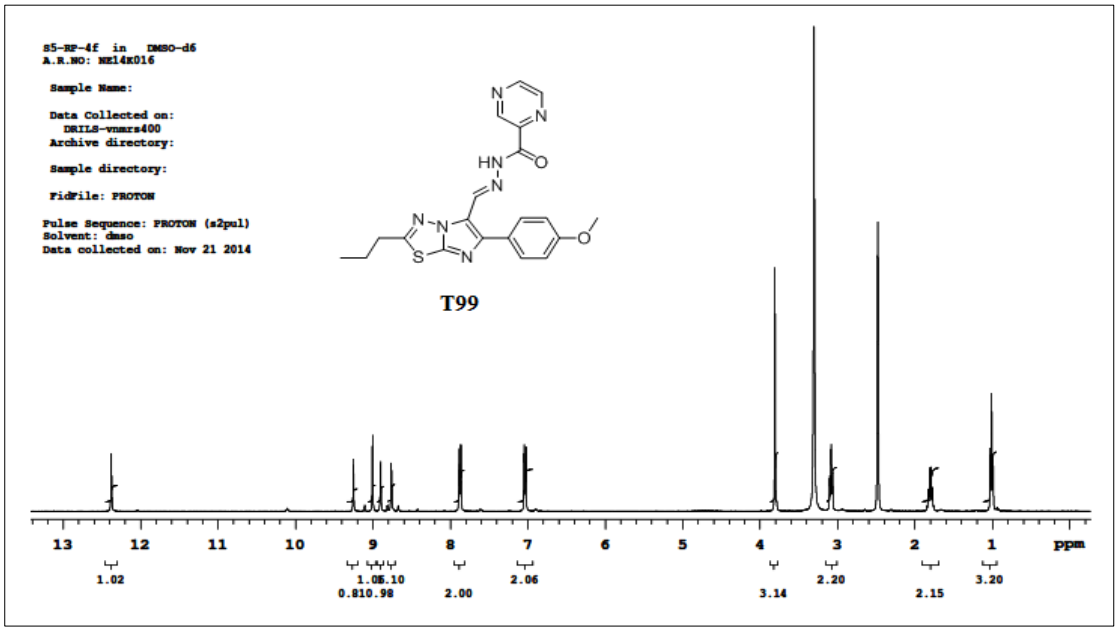


Figure 6.9 ^{1}H NMR spectrum of **T99**

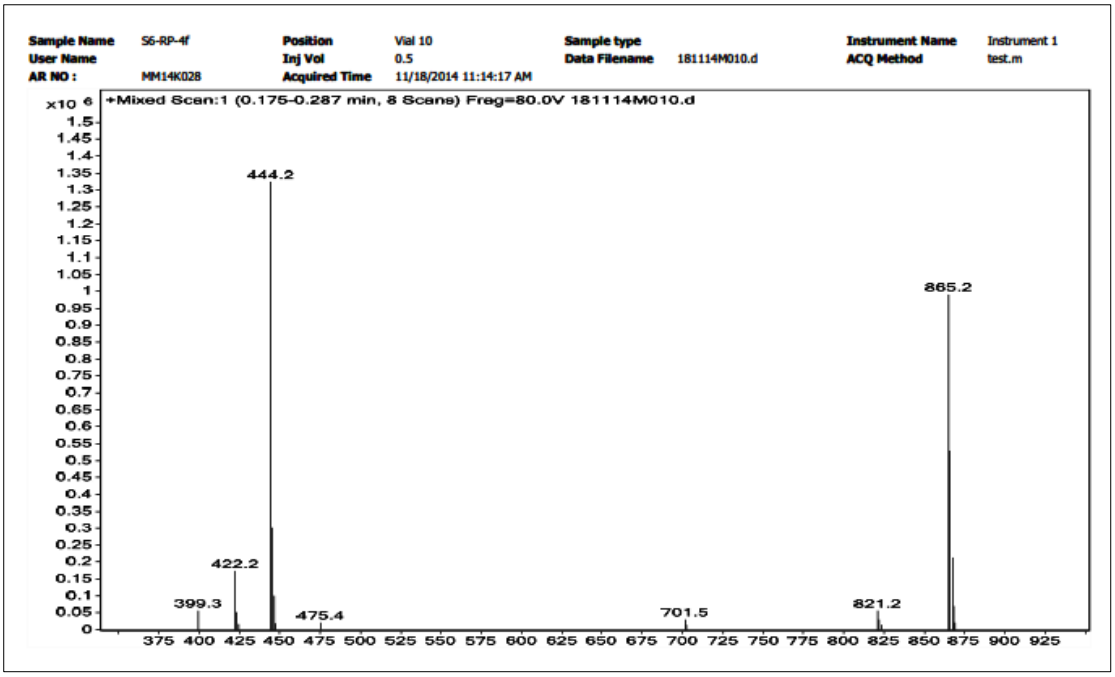


Figure 6.10 Mass spectrum of **T99**

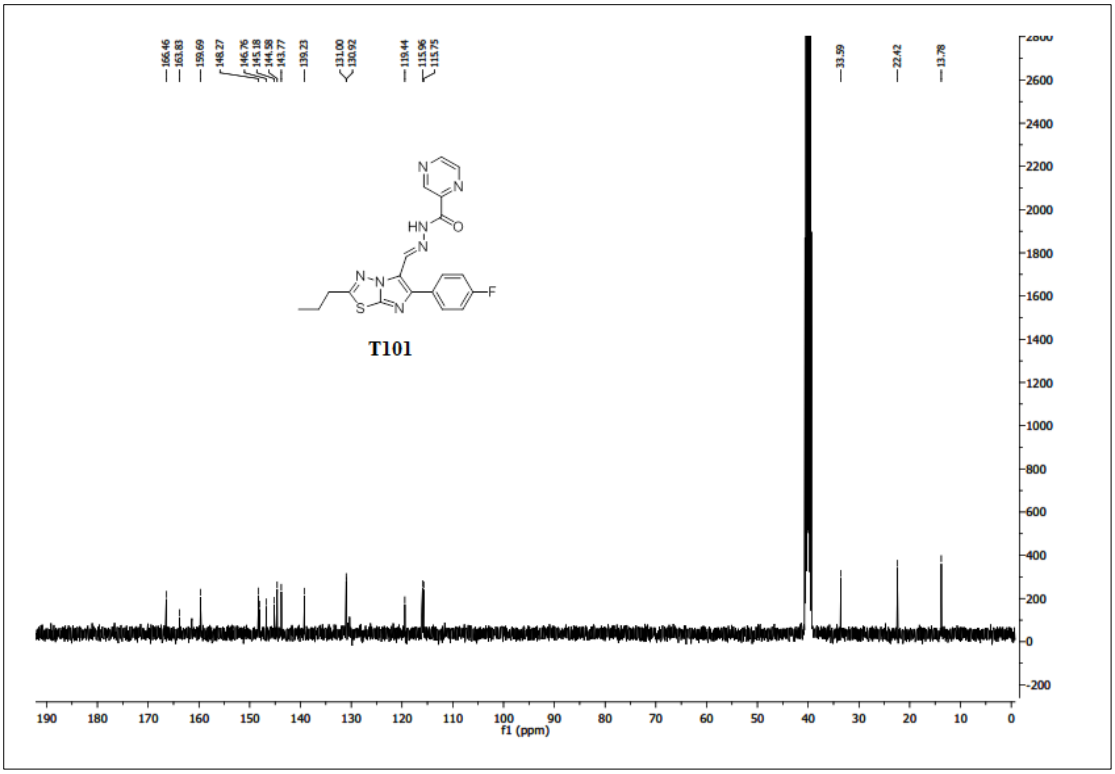


Figure 6.11 ^{13}C NMR spectrum of **T101**

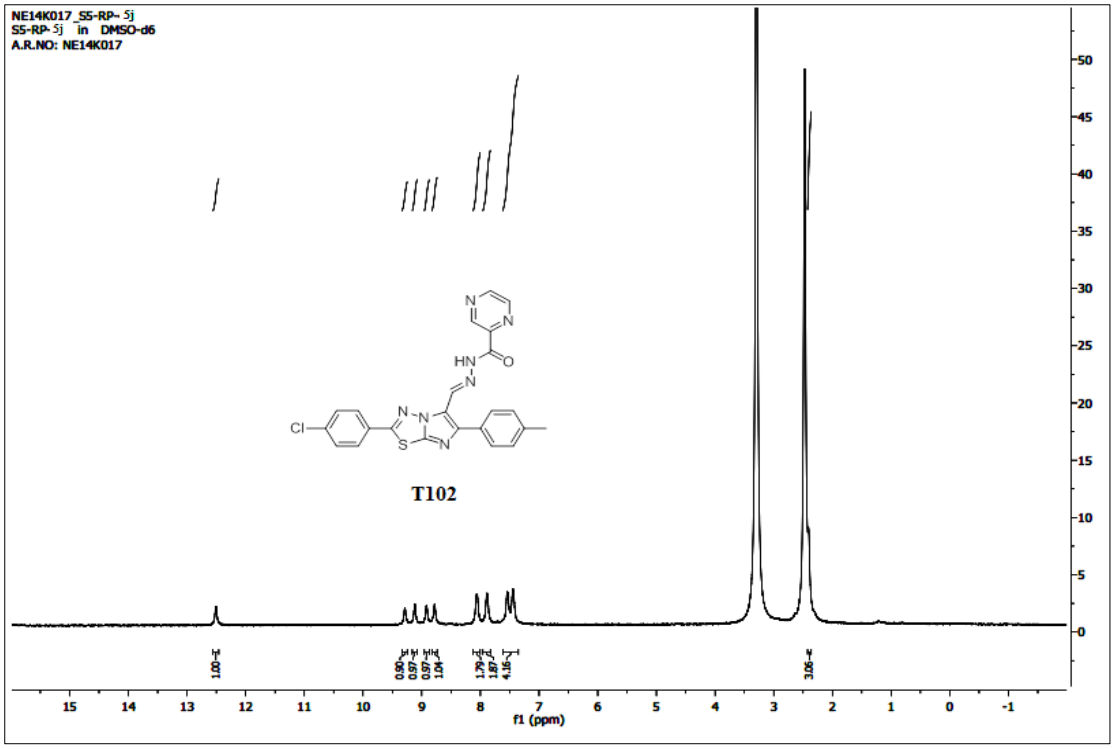


Figure 6.12 ^{1}H NMR spectrum of **T102**

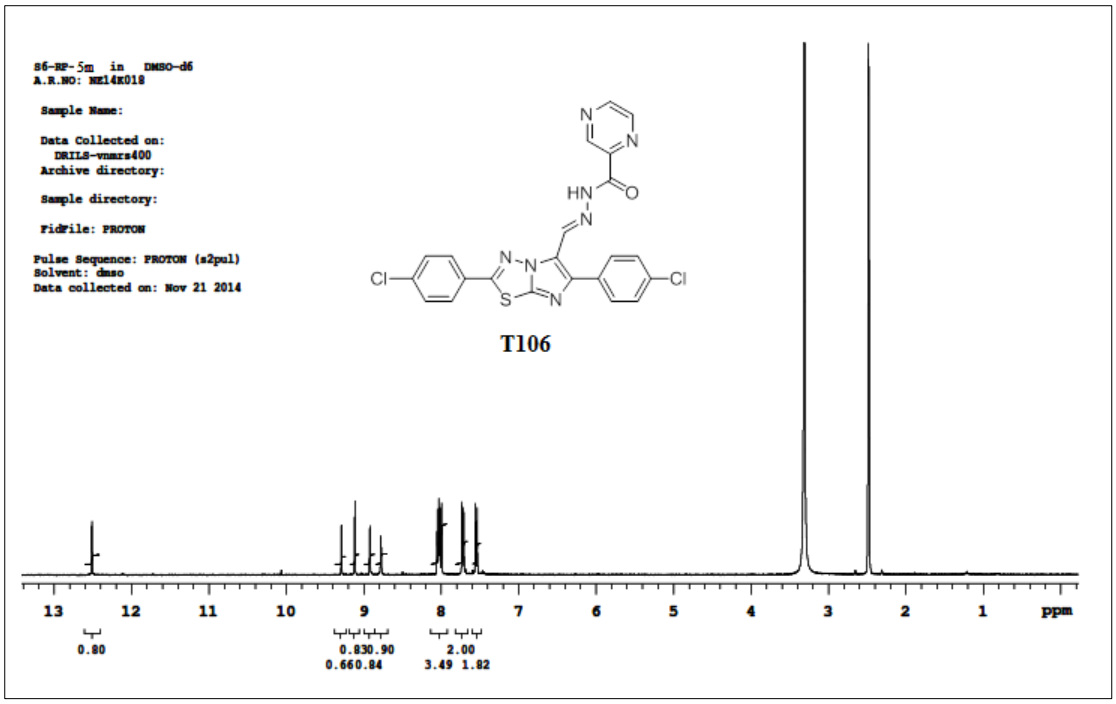


Figure 6.13 ^{1}H NMR spectrum of **T106**

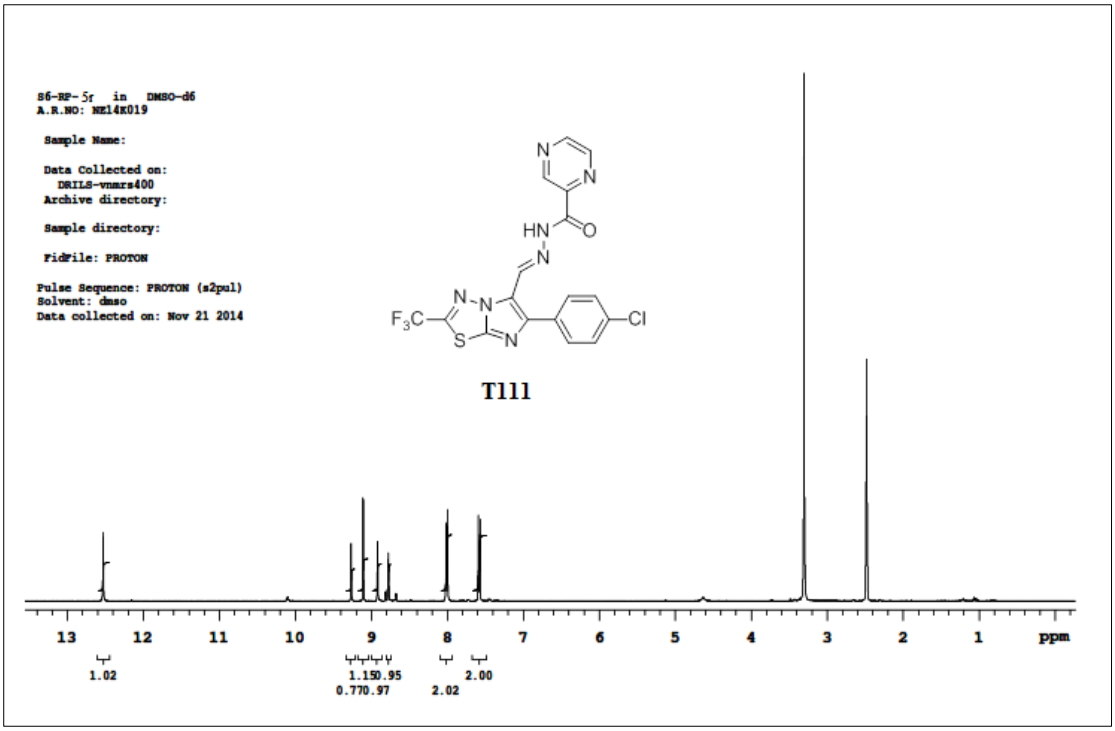


Figure 6.14 ^{1}H NMR spectrum of **T111**

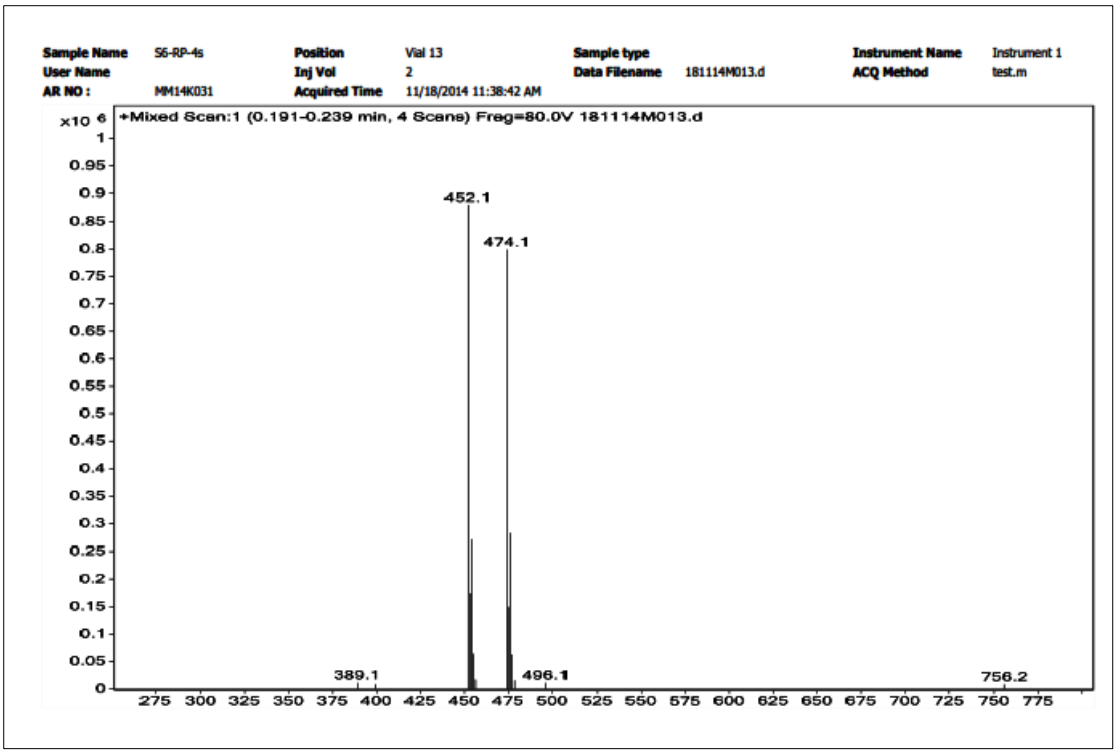


Figure 6.15 Mass spectrum of **T111**

CHAPTER 7

SUMMARY AND CONCLUSIONS

Abstract

This chapter presents the brief summary and conclusions of the research work. Additionally the scope for future work is deliberated.

7.1 SUMMARY

Literature survey on thiadiazole based molecules reveals its significance in the field of medicinal chemistry. Hence, in the present study 1,3,4-thiadiazole/Imidazo[2,1-*b*][1,3,4]thiadiazole unit was chosen as the core structural unit and various pharmacophores such as benzimidazole, 1,2,3-triazole, thiazole, phenothiazine and pyrazinamides were incorporated in to this core moiety, with the hope of getting enhanced AntiTB activity.

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

(i) Imidazo[2,1-*b*][1,3,4]thiadiazole carrying benzimidazole derivative at position-5 (**T1-T29**)

(ii) Imidazo[2,1-*b*][1,3,4]thiadiazole carrying 1,2,3-triazole derivative at position-5 (**T30-T49**)

(iii) Thiazole derivatives on position-5 of the Imidazo[2,1-*b*][1,3,4]thiadiazole ring (**T50-T72**)

(iv) Thiadiazole containing phenothiazine derivatives (**T73-T93**)

(v) Pyrazinamide derivatives at position-5 of the Imidazo[2,1-*b*][1,3,4]thiadiazole ring (**T94-T111**)

The newly synthesized compounds were purified by recrystallization and/or column chromatography techniques and their synthetic methods were established. The structures of new intermediates and target compounds were confirmed by various spectral and elemental analysis studies. Further, X-ray crystallographic study was carried out for few compounds in order to elucidate their final structure. All the final target compounds were screened for their *in vitro* antitubercular study following agar dilution/MABA methodologies, by taking isoniazid, ethambutol, ciprofloxacin, streptomycin and pyrazinamide as standard drugs. Based on the results of *in vitro* studies showed that among 111 compounds, 21 compounds showed excellent activity with MIC of $\leq$ 3.125 μg/mL and 10 compounds showed MIC of 6.25 μg/mL. Potent

compounds against *Mtb* H37Rv were taken for cytotoxicity studies to check their toxicity using MTT assay against non-cancerous cells. In addition *in vitro* antibacterial screening studies were carried out with three bacterial strains such as *E. coli, S. aureus and P. aeruginosa.* Also, *in silico* molecular docking studies were carried out to understand binding interaction with active molecules against enzyme of enoyl-acyl carrier protein reductase (InhA) of *Mtb*.

7.2 CONCLUSIONS

Following important conclusions have been drawn from the present research work.

- Newly designed compounds **T1-T111** were successfully synthesized. Their synthetic methods as well as purification techniques were established and their structures were confirmed by various spectral studies. SCXRD studies established the three dimensional structure of compounds **4d**, **T27**, **T32**, **T96** and **T97**.
- The *in vitro* antituberculosis study by agar dilution/MABA method results indicated that some of the new thiadiazole containing benzimidazole, 1,2,3-triazole, thiazole, phenothiazine and pyrazinamide derivatives displayed better inhibition 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 **T79** (R^1= methyl phenyl, R^2=H) and **T86** (R^1= *n*-propyl, R^2= Cl) are showed most potent leads with a MIC of 0.8 μg/mL and are more potent than standard drugs EMB and INN. In addition, 6 other compounds (**T73, T74, T75, T77, T84** and **T85)** displayed significant activity with a MIC of 1.6 μg/mL whereas another set of 13 derivatives (**T3**, **T4**, **T12**, **T16**, **T18**, **T26**, **T27, T35, T43, T68, T82, T83, T87)** showed a MIC of 3.125 μg/mL. Further, the cytotoxicity study revealed the nontoxic nature of the active molecules to noncancerous cells.
- Among derivatives which contain an unsubstituted phenothiazine ring (R^2=H), those with a 4-methylphenyl (**T79**) or 4-fluorophenyl (**T82**) substituent at R^1 displayed substantial activity. In the case of chloro substituted (R^2= Cl)

derivatives, a 4-chlorophenyl (**T77**) or 4-fulorophenyl (**T83**) substituent at R^1 enhanced the activity. Among trifluoromethyl substituted analogs, only one compound (**T84**) with a 4-fluoro phenyl substituent at R^2 showed promising activity.

- *In vitro* antibacterial results showed that compounds **T43**, **T45**, **T72**, **T73**, **T79**, **T88**, **T107**, **T108** and **T109** with good inhibition against three bacterial strains are promising leads as antibacterial agents.
- The molecular docking studies revealed the strong interaction of the active molecules with the target enzyme of InhA. Most of the derivatives showed interaction with amino acid Tyr 158 which is an important residue to interact with the long chain fatty acyl substrates required for the synthesis of mycolic acid in the mycobacteria.
- Hence, these compounds with significant antiTB activity could serve as promising lead molecules for further development of potent antiTB agents.

7.3 SCOPE FOR FUTURE WORK

- Based on results of antiTB activity, 21 molecules showed good inhibition against *Mtb* and these molecules are not toxic to normal cell. These molecules can be considered as lead molecules and could be taken for further *in vivo* and enzymatic studies for the drug development.
- Antitubercular and antibacterial activity of the compounds synthesized in the present work has been tested only against normal bacteria. Further, the activity of these newly synthesized compounds can be extended to multidrug-resistant bacterial strains such as Multidrug-resistant tuberculosis (MDR-TB), Methicillin resistant *Staphylococcus aureus* (MRSA), Vancomycin-resistant *Staphylococcus aureus* (VRSA), etc.
- Further structural modification of the active structural backbone in order to improve the potency and to explore in detail about the antiTB behaviour of the resulting structures in biological surroundings could be a good scope in the future.

- The SAR details of information derived in the present study could be employed in computer aided drug design (CADD)/ QSAR studies to develop new 1,3,4-thiadiazoles based lead molecules.

REFERENCES

Abbate, E., Vescovo, M., Natiello, M., Cufré, M., García, A., Montaner, P.G., Ambroggi, M., Ritacco, V. and van Soolingen, D. (2012). "Successful alternative treatment of extensively drug-resistant tuberculosis in Argentina with a combination of linezolid, moxifloxacin and thioridazine." *J. Antimicrob. Chemother.*, 67(2), 473-477.

Abdel-Aziz, M. and Abdel-Rahman, H.M. (2010). "Synthesis and antimycobacterial evaluation of some pyrazine-2-carboxylic acid hydrazide derivatives." *Eur. J. Med. Chem.*, 45, 3384-3388.

Addla, D., Jallapally, A., Gurram, D., Yogeeswari, P., Sriram, D. and Kantevari, S. (2014a). "Design, synthesis and evaluation of 1,2,3-triazole-adamantylacetamide hybrids as potent inhibitors of *Mycobacterium tuberculosis*." *Bioorg. Med. Chem. Lett.*, 24 (8), 1974-1979.

Addla, D., Jallapally, A., Gurram, D., Yogeeswari, P., Sriram, D. and Kantevari, S. (2014b). "Rational design, synthesis and antitubercular evaluation of novel 2-(trifluoromethyl) phenothiazine-[1,2,3]triazole hybrids." *Bioorg. Med. Chem. Lett.*, 24 (1), 233-236.

Agalave, S.G., Maujan, S.R. and Pore, V.S. (2011). "Click chemistry: 1,2,3-triazoles as pharmacophores." *Chem. Asian J.*, 6 (10), 2696-2718.

Alagawadi, K.R. and Alegaon, S.G. (2011). "Synthesis, characterization and antimicrobial activity evaluation of new 2,4-Thiazolidinediones bearing imidazo[2,1-*b*][1,3,4]thiadiazole moiety." *Arabian J. Chem.*, 4 (4), 465-472.

Alegaon, S.G., Alagawadi, K.R., Sonkusare, P.V., Chaudhary, S.M., Dadwe, D.H. and Shah, A.S. (2012). "Novel imidazo[2,1-*b*][1, 3, 4]thiadiazole carrying rhodanine-3-acetic acid as potential antitubercular agents." *Med. Chem. Lett.*, 22, 1917-1921.

Alwan, W.S., Karpoormath, R., Palkar, M.B., Patel, H.M., Rane, R.A., Shaikh, M.S., Kajee, A. and Mlisana, K.P. (2015). "Novel imidazo[2,1-*b*][1,3,4]thiadiazoles as

promising antifungal agents against clinical isolate of *Cryptococcus neoformans*." *Eur. J. Med. Chem.,* 95, 514-525.

Andrews, J.M. (2001). "Determination of minimum inhibitory concentrations." *J. Antimicrob. Chemother.,* 48, 5-16.

Åqvist, J., Medina, C. and Samuelsson, J.-E. (1994). "A new method for predicting binding affinity in computer-aided drug design." *Protein eng.,* 7(3), 385-391.

Arthington-Skaggs, B.A., Motley, M., Warnock, D.W. and Morrison, C.J. (2000). "Comparative evaluation of PASCO and national committee for clinical laboratory standards M27-A broth microdilution methods for antifungal drug susceptibility testing of yeasts." *J. Clin.Microbiol.,* 38 (6), 2254-2260.

Bansal, Y. and Silakari, O. (2012). "The therapeutic journey of benzimidazoles: A review." *Bioorg. Med. Chem.,* 20 (21), 6208-6236.

Bansode, T.N., Shelke, J.V. and Dongre, V.G. (2009). "Synthesis and antimicrobial activity of some new *N*-acyl substituted phenothiazines." *Eur. J. Med. Chem.,* 44(12), 5094-5098.

Bate, A.B., Kalin, J.H., Fooksman, E.M., Amorose, E.L., Price, C.M., Williams, H.M., Rodig, M.J., Mitchell, M.O., Cho, S.H. and Wang, Y. (2007). "Synthesis and antitubercular activity of quaternized promazine and promethazine derivatives." *Bioorg. Med. Chem. Lett.,* 17 (5), 1346-1348.

Baumann, M. and Baxendale, I.R. (2013). "An overview of the synthetic routes to the best selling drugs containing 6-membered heterocycles." *Beilstein J. Org. Chem*., 9, 2265-2319.

Bettencourt, M.V., Bosne-David, S. and Amaral, L. (2000). "Comparative *in vitro* activity of phenothiazines against multidrug-resistant *Mycobacterium tuberculosis*." *Int. J. Antimicrob.,* 16(1), 69-71.

Boechat, N., Ferreira, V.F., Ferreira, S.B., Ferreira, M.d.L.G., da Silva, F.d.C., Bastos, M.M., Costa, M.d.S., Lourenço, M.C.S., Pinto, A.C. and Krettli, A.U. (2011). "Novel

1,2,3-triazole derivatives for use against *Mycobacterium tuberculosis* H37Rv (ATCC 27294) strain." *J. Med. Chem.,* 54(17), 5988-5999.

Cappoen, D., Claes, P., Jacobs, J., Anthonissen, R., Mathys, V., Verschaeve, L., Huygen, K. and Kimpe, N.D. (2014). "1, 2, 3, 4, 8, 9, 10, 11-Octahydrobenzo [j] phenanthridine-7, 12-diones as New Leads against Mycobacterium tuberculosis." *J. Med. Chem.,* 57(7), 2895-2907.

Chandrakantha, B., Isloor, A.M., Shetty, P., Fun, H.K. and Hegde, G. (2014). "Synthesis and biological evaluation of novel substituted 1, 3, 4-thiadiazole and 2,6-di aryl substituted imidazo[2,1-*b*][1,3,4]thiadiazole derivatives." *Eur. J. Med. Chem.,* 71, 316-323.

Chitra, S., Paul, N., Muthusubramanian, S., Manisankar, P., Yogeeswari, P. and Sriram, D. (2011). "Synthesis of 3-heteroarylthioquinoline derivatives and their *in vitro* antituberculosis and cytotoxicity studies." *Eur. J. Med. Chem.,* 46 (10), 4897-4903.

Clerici, F., Pocar, D., Guido, M., Loche, A., Perlini, V. and Brufani, M. (2001). "Synthesis of 2-amino-5-sulfanyl-1, 3, 4-thiadiazole derivatives and evaluation of their antidepressant and anxiolytic activity." *J. Med. Chem.,* 44 (6), 931-936.

Crowle, A.J., Douvas, G.S. and May, M.H. (1992). "Chlorpromazine: A Drug Potentially Useful for Treating Mycobacterial Infections." *Chemotherapy,* 38 (6), 410-419.

Davis, J.H. (2004). "Task-specific ionic liquids." *Chem. Lett.*, 33 (9), 1072-1077.

Dupont, J., de Souza, R.F. and Suarez, P.A. (2002). "Ionic liquid (molten salt) phase organometallic catalysis." *Chem. Rev.,* 102 (10), 3667-3692.

El-Emam, A. A., Al-Deeb, O. A., Al-Omar, M. and Lehmann, J. (2004). "Synthesis, antimicrobial, and anti-HIV-1 activity of certain 5-(1-adamantyl)-2-substituted thio-1,3,4-oxadiazoles and 5-(1-adamantyl)-3-substituted aminomethyl-1,3,4- oxadiazoline -2-thiones." *Bioorg. Med. Chem.,* 12 (19), 5107-5113.

Farghaly, A.-R., Esmail, S., Abdel-Zaher, A., Abdel-Hafez, A. and El-Kashef, H. (2014). "Synthesis and anticonvulsant activity of some new pyrazolo[3,4-*b*]pyrazines and related heterocycles." *Bioorg. Med. Chem.,* 22, 2166-2175.

Foroumadi, A., Emami, S., Hassanzadeh, A., Rajaee, M., Sokhanvar, K., Moshafi, M. H. and Shafiee, A. (2005). "Synthesis and antibacterial activity of N-(5-benzylthio-1,3,4-thiadiazol-2-yl) and N-(5-benzylsulfonyl-1,3,4-thiadiazol-2-yl)piperazinyl quinolone derivatives." *Bioorg. Med. Chem. Lett.*, 15(20), 4488-4492.

Foroumadi, A., Emami, S., Hassanzadeh, A., Rajaee, M., Sokhanvar, K., Moshafi, M. H. and Shafiee, A. (2005). "Synthesis and antibacterial activity of *N*-(5-benzylthio-1, 3,4-thiadiazol-2-yl) and N-(5-benzylsulfonyl-1,3,4-thiadiazol-2-yl)piperazinyl quinolone derivatives." *Bioorg. Med. Chem. Lett.*, 15 (20), 4488-4492.

Foroumadi, A., Kiani, Z. and Soltani, F. (2003). "Antituberculosis agents VIII: Synthesis and *in vitro* antimycobacterial activity of alkyl α-[5-(5-nitro-2-thienyl)-1,3,4-thiadiazole-2-ylthio]acetates." *Farmaco,* 58 (11), 1073-1076.

Foroumadi, A., Kiani, Z. and Soltani, F. (2003). "Antituberculosis agents VIII: Synthesis and in vitro antimycobacterial activity of alkyl α-[5-(5-nitro-2-thienyl)-1,3,4-thiadiazole-2-ylthio]acetates." *Farmaco,* 58(11), 1073-1076.

Foroumadi, A., Mansouri, S., Kiani, Z. and Rahmani, A. (2003). "Synthesis and in vitro antibacterial evaluation of *N*-[5-(5-nitro-2-thienyl)-1,3,4-thiadiazole-2-yl] piperazinyl quinolones." *Eur. J. Med. Chem.*, 38 (9), 851-854.

Franzblau, S.G., Witzig, R.S., McLaughlin, J.C., Torres, P., Madico, G., Hernandez, A., Degnan, M.T., Cook, M.B., Quenzer, V.K. and Ferguson, R.M. (1998). "Rapid, low-technology MIC determination with clinical *Mycobacterium tuberculosis* isolates by using the microplate Alamar Blue assay." *J. Clin. Microbiol.,* 36, 362-366.

Gadad, A. K., Mahajanshetti, C. S., Nimbalkar, S. and Raichurkar, A. (2000). "Synthesis and antibacterial activity of some 5-guanylhydrazone/thiocyanato-6-arylimidazo[2,1-*b*][1,3,4]thiadiazole-2-sulfonamide derivatives." *Eur. J. Med. Chem.*, 35 (9), 853-857.

Gadad, A. K., Noolvi, M. N., and Karpoormath, R. V. (2004). "Synthesis and anti-tubercular activity of a series of 2-sulfonamido/trifluoromethyl-6-substituted imidazo [2,1-*b*][1,3,4]thiadiazole derivatives." *Bioorg. Med. Chem.*, 12 (21), 5651-5659.

Garella, D., Borretto, E., Di Stilo, A., Martina, K., Cravotto, G. and Cintas, P. (2013). "Microwave-assisted synthesis of *N*-heterocycles in medicinal chemistry." *Med. Chem. Commun.*, 4 (10), 1323-1343.

Gilden, R.C., Huffling, K. and Sattler, B. (2010). "Pesticides and health risks." *J. Obstet. Gynecol. Neonatal Nurs.* 39(1), 103-110.

Guha, P.C. (1922). "Constitution of the so-called dithio-urazole of martin freund. II. New methods of synthesis, isomerism and poly-derivatives." *J. Am. Chem. Soc.*, 44(7), 1510-1517.

Gundersen, L.-L., Nissen-Meyer, J. and Spilsberg, B. (2002). "Synthesis and antimycobacterial activity of 6-arylpurines: the requirements for the *N*-9 substituent in active antimycobacterial purines." *J. Med. Chem.*, 45 (6), 1383-1386.

Güzeldemirci, N. U. and Küçükbasmacı, Ö. (2010). "Synthesis and antimicrobial activity evaluation of new 1,2,4-triazoles and 1,3,4-thiadiazoles bearing imidazo[2,1-*b*]thiazole moiety." *Eur. J. Med. Chem.*, 45(1), 63-68.

Haider, S., Alam, M.S. and Hamid, H. (2015). "1,3,4-Thiadiazoles: A potent multi targeted pharmacological scaffold." *Eur. J. Med. Chem.*, 92, 156-177.

Hasui, T., Matsunaga, N., Ora, T., Ohyabu, N., Nishigaki, N., Imura, Y., Igata, Y., Matsui, H., Motoyaji, T. and Tanaka, T. (2011). "Identification of benzoxazin-3-one derivatives as novel, potent, and selective nonsteroidal mineralocorticoid receptor antagonists." *J. Med. Chem.*, 54 (24), 8616-8631.

Hauptmann, E.T. (2003). "The chemistry of heterocycles. Structure, reactions, syntheses and applications." *Wiely-vch GmbH & Co. KGaA, Weinheim*.

He, C.-X., Meng, H., Zhang, X., Cui, H.-Q. and Yin, D.-L. (2015). "Synthesis and bio evaluation of phenothiazine derivatives as new anti-tuberculosis agents." *Chin. Chem. Lett.*, 26(8), 951-954.

Hegde, V. S., Kolavi, G. D., Lamani, R. S. and Khazi, I. A. M. (2006). "Mannich bases and novel benzothiazole derivatives of imidazo [2,1-*b*][1,3,4] thiadiazoles and their biological evaluation." *J. Sulfur Chem.*, 27(6), 553-569.

Hoggarth, E. (1949). "Compounds related to thiosemicarbazide. Part II. 1-Benzoylthiosemicarbazides." *J. Chem. Soc.,* 1163-1167.

Hu, Y., Li, C.-Y., Wang, X.-M., Yang, Y.-H. and Zhu, H.-L. (2014). "1,3,4-Thiadiazole: Synthesis, Reactions, and Applications in Medicinal, Agricultural, and Materials Chemistry." *Chem. Rev.,* 114 (10), 5572-5610.

Isenberg, H.D (1992). "Clinical microbiology procedures handbook." *American society for microbiology*, Volume 1 **(**Washington, D.C.,).

Jadhav, V.B., Kulkarni, M.V., Rasal, V.P., Biradar, S.S. and Vinay, M.D. (2008). "Synthesis and anti-inflammatory evaluation of methylene bridged benzofuranyl imidazo[2,1-*b*][1,3,4]thiadiazoles." *Eur. J. Med. Chem.*, 43 (8), 1721-1729.

Jalandhara, N.B., Patel, A., Arora, R.R. and Jalandhara, P. (2009). "Obstructive sleep apnea: a cardiopulmonary perspective and medical therapeutics." *Am. J. Ther.,* 16 (3), 257-263.

Jatav, V., Mishra, P., Kashaw, S. and Stables, J. (2008). "CNS depressant and anticonvulsant activities of some novel 3-[5-substituted 1,3,4-thiadiazole-2-yl]-2-styryl quinazoline-4 (3*H*)-ones." *Eur. J. Med. Chem.*, 43 (9), 1945-1954.

Jena, L., Waghmare, P., Kashikar, S., Kumar, S. and Harinath, B.C. (2014). "Computational approach to understanding the mechanism of action of isoniazid, an anti-TB drug." *Int. J. Mycobacteriol*., 3(4), 276-282.

Joshi, S., Manish, K. and Badiger, A. (2013). "Synthesis and evaluation of antibacterial and antitubercular activities of some novel imidazo [2,1-*b*][1,3,4]

thiadiazole derivatives." *Med. Chem. Res*., 22 (2), 869-878.

Joshi, S.D., More, U.A., Koli, D., Kulkarni, M.S., Nadagouda, M.N. and Aminabhavi, T.M. (2015). "Synthesis, evaluation and *in silico* molecular modeling of pyrroyl-1,3,4-thiadiazole inhibitors of InhA." *Bioorg. Chem.*, 59, 151-167.

Kadi, A. A., El-Brollosy, N. R., Al-Deeb, O. A., Habib, E. E., Ibrahim, T. M. and El-Emam, A. A. (2007). "Synthesis, antimicrobial, and anti-inflammatory activities of novel 2-(1-adamantyl)-5-substituted-1,3,4-oxadiazoles and 2-(1-adamantylamino)-5-substituted-1,3,4-thiadiazoles." *Eur. J. Med. Chem.*, 42 (2), 235-242.

Kaleta, Z., Makowski, B.T., Soós, T. and Dembinski, R. (2006). "Thionation using fluorous Lawesson's reagent." *Org. Lett.*, 8 (8), 1625-1628.

Kamal, A., Reddy, V.S., Santosh, K., Bharath Kumar, G., Shaik, A.B., Mahesh, R., Chourasiya, S.S., Sayeed, I.B. and Kotamraju, S. (2014). "Synthesis of imidazo[2,1-*b*][1,3,4]thiadiazole-chalcones as apoptosis inducing anticancer agents." *Med. Chem. Commun.*, 5, 1718-1723.

Kantevari, S., Yempala, T., Surineni, G., Sridhar, B., Yogeeswari, P. and Sriram, D. (2011). "Synthesis and antitubercular evaluation of novel dibenzo[*b, d*]furan and 9-methyl-9H-carbazole derived hexahydro-2H-pyrano[3,2-*c*]quinolines via Povarov reaction." *Eur. J. Med. Chem.*, 46 (10), 4827-4833.

Karki, S. S., Panjamurthy, K., Kumar, S., Nambiar, M., Ramareddy, S. A., Chiruvella, K. K. and Raghavan, S. C. (2011). "Synthesis and biological evaluation of novel 2-aralkyl-5-substituted-6-(4′-fluorophenyl)-imidazo[2,1-*b*][1,3,4]thiadiazole derivatives as potent anticancer agents." *Eur. J. Med. Chem.*, 46 (6), 2109-2116.

Kaur, I.P., Smitha, R., Aggarwal, D. and Kapil, M. (2002). "Acetazolamide: future perspective in topical glaucoma therapeutics." *Int. J. Pharm.* 248 (1), 1-14.

Khalaj, A., Nakhjiri, M., Negahbani, A. S., Samadizadeh, M., Firoozpour, L., Rajabalian, S., Samadi, N., Faramarzi, M. A., Adibpour, N. and Shafiee, A. (2011). "Discovery of a novel nitroimidazolyl–oxazolidinone hybrid with potent anti Gram-positive activity: Synthesis and antibacterial evaluation." *Eur. J. Med. Chem.*, 46 (1),

65-70.

Khazi, I.A.M., Gadad, A.K., Lamani, R.S. and Bhongade, B.A. (2011). "Chemistry of imidazo [2,1-*b*][1,3,4] thiadiazoles." *Tetrahedron,* 67(19), 3289-3316.

Kidwai, M., Misra, P., Bhushan, K. R. and Dave, B. (2000). "A novel route to 1, 2, 4-triazoles." *Synth. Commun.,* 30 (16), 3031-3040.

Kolavi, G., Hegde, V. and Gadad, P. (2006). "Synthesis and evaluation of antitubercular activity of imidazo [2,1-*b*][1,3,4] thiadiazole derivatives." *Bioorg. Med. Chem.,* 14 (9), 3069-3080.

Kolavi, G., Hegde, V. and Gadad, P. (2006). "Synthesis and evaluation of antitubercular activity of imidazo [2,1-*b*][1,3,4] thiadiazole derivatives." *Bioorg. Med. Chem.,* 14 (9), 3069-3080.

Kumar, D., Maruthi Kumar, N., Chang, K.-H. and Shah, K. (2010). "Synthesis and anticancer activity of 5-(3-indolyl)-1,3,4-thiadiazoles." *Eur. J. Med. Chem.,* 45(10), 4664-4668.

Kumar, K., Awasthi, D., Lee, S.-Y., Zanardi, I., Ruzsicska, B., Knudson, S., Tonge, P.J., Slayden, R.A. and Ojima, I. (2010). "Novel Trisubstituted Benzimidazoles, Targeting Mtb FtsZ, as a New Class of Antitubercular Agents." *J. Med. Chem.,* 54, 374-381.

Kunciw, D.L., Liechty, J.J., Mitchell, M.O., Wan, B. and Franzblau, S.G. (2012). "Structural requirements for the antitubercular quaternized triflupromazine pharmacophore." *Bioorg. Med. Chem. Lett.,* 22 (17), 5679-5680.

Lamani, R.S., Shetty, N.S., Kamble, R.R. and Khazi, I.A.M. (2009). "Synthesis and antimicrobial studies of novel methylene bridged benzisoxazolyl imidazo [2,1-*b*][1,3,4] thiadiazole derivatives." *Eur. J. Med. Chem.,* 44 (7), 2828-2833.

Lazar, C., Kluczyk, A., Kiyota, T. and Konishi, Y. (2004). "Drug evolution concept in drug design: 1. Hybridization method." *J. Med. Chem.,* 47 (27), 6973-6982.

Li, K. and Chen, W. (2008). "A new convenient way to synthesize 1, 3, 4-thiadiazol-2-yl urea derivatives under microwave irradiation." *Heteroat. Chem.,* 19 (6), 621-629.

Li, P., Shi, L., Yang, X., Yang, L., Chen, X.-W., Wu, F., Shi, Q.-C., Xu, W.-M., He, M., Hu, D.-Y. and Song, B.-A. (2014). "Design, synthesis, and antibacterial activity against rice bacterial leaf blight and leaf streak of 2,5-substituted-1,3,4-oxadiazole/thiadiazole sulfone derivative." *Bioorg. Med. Chem. Lett.,* 24(7), 1677-1680.

Li, Y., Tan, C., Gao, C., Zhang, C., Luan, X., Chen, X., Liu, H., Chen, Y. and Jiang, Y. (2011). "Discovery of benzimidazole derivatives as novel multi-target EGFR, VEGFR-2 and PDGFR kinase inhibitors." *Bioorg. Med. Chem.,* 19, 4529-4535.

Lima, L.M. and Barreiro, E.J. (2005). "Bioisosterism: a useful strategy for molecular modification and drug design." *Curr. Med. Chem.,* 12(1), 23-49.

Luks, A.M., McIntosh, S.E., Grissom, C.K., Auerbach, P.S., Rodway, G.W., Schoene, R.B., Zafren, K. and Hackett, P.H. (2010). "Wilderness Medical Society consensus guidelines for the prevention and treatment of acute altitude illness." *Wilderness Environ. Med.,* 21(2), 146-155.

MacPherson, I.S., Kirubakaran, S., Gorla, S.K., Riera, T.V., D'Aquino, J.A., Zhang, M., Cuny, G.D. and Hedstrom, L. (2010). "The Structural Basis of Cryptosporidium-Specific IMP Dehydrogenase Inhibitor Selectivity." *J. Am. Chem. Soc.,* 132, 1230-1231.

Mahajan, N.S. and Dhawale, S.C. (2015). "Linked pyridinyl-thiadiazoles: Design and synthesis as potential candidate for treatment of XDR and MDR tuberculosis." *Eur. J. Med. Chem.,* 102, 243-248.

Makam, P. and Kannan, T. (2014). "2-Aminothiazole derivatives as antimycobacterial agents: Synthesis, characterization, *in vitro* and *in silico* studies." *Eur. J. Med. Chem.,* 87, 643-656.

Makam, P., Kankanala, R., Prakash, A. and Kannan, T. (2013). "2-(2-Hydrazinyl) thiazole derivatives: Design, synthesis and *in vitro* antimycobacterial studies." *Eur. J. Med. Chem.,* 69, 564-576.

Matviiuk T., Rodriguez F., Saffon N., Mallet-Ladeira S., Gorichko M., Ribeiro A. L. d. J. L., Pasca M. R., Lherbet C., Voitenko Z. and Baltas M. (2013). "Design, chemical synthesis of 3-(9*H*-fluoren-9-yl) pyrrolidine-2,5-dione derivatives and biological activity against enoyl-ACP reductase (InhA) and *Mycobacterium tuberculosis*. " *Eur. J. Med. Chem.,* 70, 37-48.

Meissner, A., Boshoff, H. I., Vasan, M., Duckworth, B.P., Barry Iii, C.E. and Aldrich, C.C. (2013). "Structure–activity relationships of 2-aminothiazoles effective against *Mycobacterium tuberculosis*." *Bioorg. Med. Chem.,* 21(21), 6385-6397.

Noei, J. and Khosropour, A.R. (2013). "A novel process for the synthesis of 3,5-diaryl-1,2,4-thiadiazoles from aryl nitriles." *Tetrahedron Lett.,* 54(1), 9-11.

Odlo, K., Høydahl, E.A. and Hansen, T.V. (2007). "One-pot synthesis of 1, 4-disubstituted 1, 2, 3-triazoles from terminal acetylenes and *in situ* generated azides." *Tetrahedron Lett.,* 48(12), 2097-2099.

Ordway, D., Viveiro, M., Leandro C., Bettencourt, R., Almeida, J., Martins, M., Kristiansen, J.E., Molnar, J. and Amaral, L. (2003). "Clinical concentrations of thioridazine kill intracellular multidrug-resistant *Mycobacterium tuberculosis*." *Antimicrob. Agents Chemother.,* 47 (3), 917-922.

Oruç, E. E., Rollas, S., Kandemirli, F., Shvets, N. and Dimoglo, A. S. (2004). " 1,3,4-Thiadiazole derivatives. Synthesis, structure elucidation, and structure-antituberculosis activity relationship investigation." *J. Med. Chem.,* 47 (27), 6760-6767.

Ozadali, K., Unsal Tan, O., Yogeeswari, P., Dharmarajan, S. and Balkan, A. (2014). "Synthesis and antimycobacterial activities of some new thiazolylhydrazone derivatives." *Bioorg. Med. Chem. Lett.,* 24 (7), 1695-1697.

Palkar, M. B., Noolvi, M. N., Maddi, V. S., Ghatole, M. and Nargund, L. G. (2012). "Synthesis, spectral studies and biological evaluation of a novel series of 2-substituted-5, 6-diarylsubstituted imidazo [2, 1-b][1, 3, 4]-thiadiazole derivatives as possible anti-tubercular agents." *Med. Chem. Res*., 21 (7), 1313-1321.

Patel, H.M., Noolvi, M. N., Goyal, A. and Thippeswamy, B. (2013). "2,5,6-Trisubstituted imidazo [2,1-*b*][1,3,4]thiadiazoles: Search for antihyperlipidemic agents." *Eur. J. Med. Chem.*, 65, 119-133.

Patpi, S.R., Pulipati, L., Yogeeswari, P., Sriram, D., Jain, N., Sridhar, B., Murthy, R., Kalivendi, S.V. and Kantevari, S. (2012). "Design, synthesis, and structure–activity correlations of novel dibenzo [b, d] furan, dibenzo [*b, d*] thiophene, and *N*-methylcarbazole clubbed 1,2,3-triazoles as potent inhibitors of *Mycobacterium tuberculosis*." *J. Med. Chem.,* 55(8), 3911-3922.

Pieroni, M., Tipparaju, S.K., Lun, S., Song, Y., Sturm, A.W., Bishai, W.R. and Kozikowski, A.P. (2011). "Pyrido[1,2-*a*]benzimidazole-Based Agents Active Against Tuberculosis (TB), Multidrug-Resistant (MDR) TB and Extensively Drug-Resistant (XDR) TB." *Med. Chem. Commun.,* 6 (2), 334-342.

Pitta, E., Tsolaki, E., Geronikaki, A., Petrovic, J., Glamoclija, J., Sokovic, M., Crespan, E., Maga, G., Bhunia, S.S. and Saxena, A.K. (2015). "4-Thiazolidinone derivatives as potent antimicrobial agents: microwave-assisted synthesis, biological evaluation and docking studies." *Med. Chem. Commun.,* 6(2), 319-326.

Rangwala, L.M. and Liu, G.T. (2007). "Pediatric idiopathic intracranial hypertension." *Surv. Ophthalmol.,* 52(6), 597-617.

Remington, M.D. (1995). "The science and practice of pharmacy." 19th ed. *Pennsylvania: MACK Publishing Company*.

Rocha, L., Marston, A., Potterat, O., Kaplan, M.A.C., Stoeckli-Evans, H. and Hostettmann, K. (1995). "Antibacterial phloroglucinols and flavonoids from Hypericum brasiliense." *Phytochemistry,* 40(5), 1447-1452.

Romagnoli, R., Baraldi, P.G., Prencipe, F., Balzarini, J., Liekens, S. and Estévez, F. (2015). "Design, synthesis and antiproliferative activity of novel heterobivalent hybrids based on imidazo[2,1-*b*][1,3,4]thiadiazole and imidazo[2,1-*b*][1,3]thiazole scaffolds." *Eur. J. Med. Chem.*, 101, 205-217.

Russell, M.B. and Ducros, A. (2011). "Sporadic and familial hemiplegic migraine: pathophysiological mechanisms, clinical characteristics, diagnosis, and management." *Lancet Neurol.*, 10 (5), 457-470.

Sancak, K., Ünver, Y. and Er, M. (2007). "Synthesis of 2-Acylamino, 2- Aroylamino and Ethoxycarbonyl Imino-1,3,4-thiadiazoles as Antitumor Agents." *Turk. J. Chem.*, 31(2), 125-134.

Schara, U. and Lochmüller, H. (2008)."Therapeutic strategies in congenital myasthenic syndromes." *Neurotherapeutics*, 5(4), 542-547.

Schüttelkopf, A.W., Gros, L., Blair, D.E., Frearson, J.A., van Aalten, D.M. and Gilbert, I.H. (2010). "Acetazolamide-based fungal chitinase inhibitors." *Bioorg. Med. Chem.*, 18(23), 8334-8340.

Shafi, S., Mahboob Alam, M., Mulakayala, N., Mulakayala, C., Vanaja, G., Kalle, A.M., Pallu, R. and Alam, M.S. (2012). "Synthesis of novel 2-mercapto benzothiazole and 1,2,3-triazole based bis-heterocycles: Their anti-inflammatory and anti-nociceptive activities." *Eur. J. Med. Chem.*, 49, 324-333.

Shaikh, M.S., Palkar, M.B., Patel, H.M., Rane, R.A., Alwan, W.S., Shaikh, M.M., Shaikh, I.M., Hampannavar, G.A. and Karpoormath, R. (2014). "Design and synthesis of novel carbazolo–thiazoles as potential anti-mycobacterial agents using a molecular hybridization approach." *RSC. Adv.*, 4 (107), 62308-62320.

Shanmugavelan, P., Nagarajan, S., Sathishkumar, M., Ponnuswamy, A., Yogeeswari, P. and Sriram, D. (2011). "Efficient synthesis and in vitro antitubercular activity of 1,2,3-triazoles as inhibitors of *Mycobacterium tuberculosis*." *Bioorg. Med. Chem. Lett.*, 21(24), 7273-7276.

Siddiqui, A.B., Trivedi, A.R., Kataria, V.B. and Shah, V.H. (2014). "4,5-Dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidine containing phenothiazines as antitubercular agents." *Bioorg. Med. Chem. Lett.*, 24 (6), 1493-1495.

Singh, P., Raj, R., Kumar, V., Mahajan, M.P., Bedi, P.M.S., Kaur, T. and Saxena, A.K. (2012). "1,2,3-Triazole tethered *β*-lactam-Chalcone bifunctional hybrids: Synthesis and anticancer evaluation." *Eur. J. Med. Chem.*, 47, 594-600.

Stanley, S.A., Grant, S.S., Kawate, T., Iwase, N., Shimizu, M., Wivagg, C., Silvis, M., Kazyanskaya, E., Aquadro, J., Golas, A., Fitzgerald, M., Dai, H., Zhang, L. and Hung, D.T. (2012). "Identification of novel inhibitors of M. tuberculosis growth using whole cell based high-throughput screening." *ACS Chem. Biol.*, 7, 1377-1384.

Tang, Z.-l., Xia, Z.-w., Chang, S.-h. and Wang, Z.-x. (2015). "Synthesis and fungicidal activity of novel 2-aryl-3-(1,3,4-thiadiazolyl)-6(8)-methyl-1,3-benzo-xazines." *Bioorg. Med. Chem. Lett.*, 25 (16), 3378-3381.

Thirumurugan, P., Matosiuk, D. and Jozwiak, K. (2013). "Click chemistry for drug development and diverse chemical-biology applications." *Chem. Rev.*, 113 (7), 4905-4979.

Thomas, K.D., Adhikari, A.V., Chowdhury, I.H., Sumesh, E. and Pal, N.K. (2011). "New quinolin-4-yl-1,2,3-triazoles carrying amides, sulphonamides and amido-piperazines as potential antitubercular agents." *Eur. J. Med. Chem.*, 46 (6), 2503-2512.

Thomasco, L.M., Gadwood, R.C., Weaver, E.A., Ochoada, J.M., Ford, C.W., Zurenko, G.E., Hamel, J.C., Stapert, D., Moerman, J.K. and Schaadt, R.D. (2003). "The synthesis and antibacterial activity of 1,3,4-thiadiazole phenyl oxazolidinone analogues." *Bioorg. Med. Chem. Lett.* 13 (23), 4193-4196.

Tiselius, H.-G. (2010). "New horizons in the management of patients with cystinuria." *Curr. Opin. Urol.*, 20(2), 169-173.

Tu, G., Li, S., Huang, H., Li, G., Xiong, F., Mai, X., Zhu, H., Kuang, B. and Xu, W. F. (2008). "Novel aminopeptidase *N* inhibitors derived from 1,3,4-thiadiazole

scaffold." *Bioorg. Med. Chem.*, 16 (14), 6663-6668.

Ulloora, S., Shabaraya, R. and Adhikari, A.V. (2013). "Facile synthesis of new imidazo[1,2-*a*]pyridines carrying 1,2,3-triazoles via click chemistry and their antiepileptic studies." *Bioorg. Med. Chem. Lett.*, 23 (11), 3368-3372.

Vergara, F.M., Lima, C.H.d.S., Maria das Graças, M.d.O., Candéa, A.L., Lourenço, M.C., Ferreira, M.d.L., Kaiser, C.R. and de Souza, M.V. (2009). "Synthesis and antimycobacterial activity of *N′*-[(*E*)-(monosubstituted-benzylidene)]-2-pyrazine carbohydrazide derivatives." *Eur. J. Med. Chem.*, 44, 4954-4959

Viegas-Júnior, C., Danuello, A., da Silva Bolzani, V., Barreiro, E.J. and Fraga, C.A.M. (2007). "Molecular hybridization: a useful tool in the design of new drug prototypes." *Curr. Med. Chem.*, 14(17), 1829-1852.

Wei, M.-X., Feng, L., Li, X.-Q., Zhou, X.-Z. and Shao, Z.-H. (2009). "Synthesis of new chiral 2, 5-disubstituted 1,3,4-thiadiazoles possessing γ-butenolide moiety and preliminary evaluation of *in vitro* anticancer activity." *Eur. J. Med. Chem.*, 44(8), 3340-3344.

Wolf, P. (2011). "Acute drug administration in epilepsy: a review." *CNS Neurosci. Ther.*, 17(5), 442-448.

World Health Organization, Global Tuberculosis Report, 2014.

Yadav, A.K., Dhakad, P. and Sharma, G.R. (2013). "An efficient ionic liquid mediated synthesis of substituted 5*H*[1,3]-thiazolo[2,3-*b*]quinazoline-3,5-(2*H*)-dione and 5*H*-thiazolo[2,3-*b*]quinazolin-5-one." *Tetrahedron Lett.*, 54(45), 6061-6063.

Yempala, T., Sridevi, J.P., Yogeeswari, P., Sriram, D. and Kantevari, S. (2014). "Rational design and synthesis of novel dibenzo[b,d]furan-1,2,3-triazole conjugates as potent inhibitors of Mycobacterium tuberculosis." European Journal of Medicinal Chemistry, 71, 160-167.

Young, G. and Eyre, W. (1901). "III. Oxidation of benzalthiosemicarbazone." *J. Chem. Soc., Trans.*, 79, 54-60.

Zhang, Y., Wade, M.M., Scorpio, A., Zhang, H. and Sun, Z. (2003). “Mode of action of pyrazinamide: disruption of Mycobacterium tuberculosis membrane transport and energetics by pyrazinoic acid.” *J. Antimicrob. Chemother.*, 52, 790-795.

Zhou, B., He, Y., Zhang, X., Xu, J., Luo, Y., Wang, Y., Franzblau, S.G., Yang, Z., Chan, R.J. and Liu, Y. (2010). "Targeting mycobacterium protein tyrosine phosphatase B for antituberculosis agents." *Proc. Natl. Acad. Sci.*, 107(10), 4573-4578.

Zimhony, O., Cox, J.S., Welch, J.T., Vilchèze, C. and Jacobs W.R. (2000). "Pyrazinamide inhibits the eukaryotic-like fatty acid synthetase I (FASI) of *Mycobacterium tuberculosis*." *Nat. Med.*, 6, 1043-1047.

LIST OF PUBLICATIONS

Papers published/communicated in international journals

1. **Jurupula Ramprasad**, Nagabhushana Nayak, Udayakumar Dalimba, Perumal Yogeeswari, Dharmarajan Sriram, S K Peethambar, Rajeshwara Achur, H S Santosh Kumar (2015). "Synthesis and biological evaluation of new imidazo[2,1-*b*][1,3,4]thiadiazole-benzimidazole derivatives." *Eur. J. Med. Chem.*, 95, 49-63.

2. **Jurupula Ramprasad**, Nagabhushana Nayak, Udayakumar Dalimba, Perumal Yogeeswari, Dharmarajan Sriram (2015). "One-pot synthesis of new triazole-imidazo[2,1-*b*][1,3,4]thiadiazole hybrids via click chemistry and evaluation of their antitubercular activity." *Bioorg. Med. Chem. Lett.,* 25 (19), 4169-4173.

3. **Jurupula Ramprasad**, Nagabhushana Nayak, Udayakumar Dalimba (2015). "Design of new phenothiazine-thiadiazole hybrids via molecular hybridization approach for the development of potent antitubercular agents." *Eur. J. Med. Chem.*, 106, 75-84.

4. **Jurupula Ramprasad**, Nagabhushana Nayak, Udayakumar Dalimba, Perumal Yogeeswari, Dharmarajan Sriram (2015). "Ionic liquid promoted one-pot synthesis of Thiazole imidazo[2,1-*b*][1,3,4]thiadiazole hybrids and their antitubercular activity." *Med.Chem. Commun.,* DOI: 10.1039/c5md00346f.

5. **Jurupula Ramprasad**, Nagabhushana Nayak, Udayakumar Dalimba (2015). "New PZA-imidazo[2,1-*b*][1,3,4]thiadiazole hybrids: Synthesis and evaluation of antitubercular and antibacterial activity." *communicated to J. Saudi Chem. Soc., (November 2015).*

Research papers presented in national/international conferences

1. **Jurupula Ramprasad**, Nagabhushana Nayak, Udayakumar Dalimba, Perumal Yogeeswari, Dharmarajan Sriram. "Imidazo[2,1-*b*][1,3,4]thiadiazole-benzimidazole derivatives as potent antitubercular agents." *International Conference on chemical sciences* (Eurassia-13), Indian institute of science, Bangalore, December 14-18, 2014.

2. **Jurupula Ramprasad**, Nagabhushana Nayak, Udayakumar Dalimba (2016). "New PZA-imidazo[2,1-*b*][1,3,4]thiadiazole hybrids: Synthesis and evaluation of antitubercular activity." National conference on recent trends in chemical sciences (NCRTCS), Manipal institute of technology-Manipal, January 11-12, 2016.

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Educational Background:

- **Ph.D** (Synthetic Organic chemistry) (Jan-2013 to Jan-2016): Title of thesis - "Synthesis, characterization and studies on antitubercular activity of some 1,3,4-Thiadiazole based molecules." Under the guidance of **Dr. Udayakumar D.,** Department of Chemistry, **NITK Surathkal**, Karnataka.
- **M.Sc** (Organic Chemistry) (July-2005 to April 2007) from **NIT Warangal**, Distinction with 8.06/10 CGPA.
- **B.Sc** (Biotechnology, Botany, Chemistry) (July-2001 to April 2004) from S.R & B.G.N.R Degree College (**Kakatiya University**) Khammam, First class with 70%.

Industrial Experience:

- As a Scientist, *Pharmaceutical division* in **Biocon limited** in Bangalore from Aug-2010 to Dec-2012.
- As a Junior Research Associate**,** *Medicinal Chemistry Division* in **Jubilant biosys *Drug discovery center*** in Bangalore from Aug – 2009 to Aug-2010**.**
- As a Senior Project chemist, *Medicinal Chemistry Division* in **GVK Bio Sciences Pvt. Ltd.,** from Sep – 2007 to Aug-2009.

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.
- 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.
- Documentation like process development reports and technology transfer Preparations.
- Synthesis of biologically active compounds for Structure activity Relationships in different therapeutic areas.
- Stabilizing and synthesizing of new Scaffolds and making Libraries on it, Training fresh chemists and coordinating the lab.

Technical Skills:

- Literature search for the preparation, properties and biological activity of organic compounds.
- Oxidations using $KMnO_4$, OsO_4, Swern oxidation, PCC, PDC, 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 and PtO_2 .
- 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
- Amine protection & deportation reactions.

Analytical techniques:

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

Computer Awareness:

- MS Office, ISIS Draw, Chemdraw, MDL Cross fire, Scifinder and Reaxys.
- Schrodinger Glide, version 6.6.

Instruments Handled:

- Single crystal X-ray diffractometer (Bruker Apex duo)
- Auto clave (high-pressure reactions)
- Microwave (biotage)
- Lyophilizer
- Combiflash (Biotage)
- Flash column chromatography
- Combinatorial synthesizer
- UV-Vis Spectrophotometer
- Bruker Alpha FT-IR Spectrometer

Publications:

1. **Jurupula Ramprasad**, Nagabhushana Nayak, Udayakumar Dalimba, Perumal Yogeeswari, Dharmarajan Sriram, S K Peethambar, Rajeshwara Achur, H S Santosh Kumar (2015). "Synthesis and biological evaluation of new imidazo[2,1-*b*][1,3,4]thiadiazole-benzimidazole derivatives." *Eur. J. Med. Chem.*, 95, 49-63.
2. **Jurupula Ramprasad**, Nagabhushana Nayak, Udayakumar Dalimba, Perumal Yogeeswari, Dharmarajan Sriram (2015). "One-pot synthesis of new triazole-Imidazo[2,1-*b*][1,3,4]thiadiazole hybrids via click chemistry and evaluation of their antitubercular activity." *Bioorg. Med. Chem. Lett.*, 25 (19), 4169-4173.
3. **Jurupula Ramprasad**, Nagabhushana Nayak, Udayakumar Dalimba (2015). "Design of new phenothiazine-thiadiazole hybrids via molecular hybridization approach for the development of potent antitubercular agents." *Eur. J. Med. Chem.*, 106, 75-84.
4. **Jurupula Ramprasad**, Nagabhushana Nayak, Udayakumar Dalimba, Perumal Yogeeswari, Dharmarajan Sriram (2015). "Ionic liquid promoted one-pot synthesis of Thiazole imidazo[2,1-*b*][1,3,4]thiadiazole hybrids and their antitubercular activity." *Med.Chem. Commun.*, DOI: 10.1039/c5md00346f.
5. Nagabhushana Nayak, **Jurupula Ramprasad**, Udayakumar Dalimba (2015). "New INH–pyrazole analogs: Design, synthesis and evaluation of antitubercular and antibacterial activity." *Bioorg. Med. Chem. Lett.*, 25(23),

5540-5545.

6. Nagabhushana Nayak, **Jurupula Ramprasad,** Udayakumar Dalimba, Perumal Yogeeswari, Dharmarajan Sriram, H.S. Santosh Kumar, S.K. Peethambar, Rajeshwara Achur (2015). "Synthesis of new pyrazole-triazole hybrids by click reaction using a green solvent and evaluation of their antitubercular and antibacterial activity." *Res. Chem. Intermed.*, DOI: 10.1007/s11164-015-2241-9.
7. Nagabhushana Nayak, **Jurupula Ramprasad,** Udayakumar Dalimba (2015). "Design, synthesis and biological evaluation of new 8-trifluoromethylquinoline containing pyrazole-3-carboxamide derivatives." *J. Heterocycl. Chem.*, DOI: 10.1002/jhet.2564.
8. Nagabhushana Nayak, **Jurupula Ramprasad,** Udayakumar Dalimba, Perumal Yogeeswari, Dharmarajan Sriram (2016). "Synthesis and antimycobacterial screening of new *N*-(4-(5-aryl-3-(5-methyl-1,3,4-oxadiazol-2-yl)-1*H*-pyrazol-1-yl)phenyl)-4-amide derivatives." *Chin. Chem. Lett.* DOI: 10.1016/j.cclet. 2016.01.015.
9. Nagabhushana Nayak, **Jurupula Ramprasad**, Udayakumar Dalimba (2015). "Synthesis and antitubercular and antibacterial activity of some active fluorine containing quinoline-pyrazole hybrid derivatives." Communicated to *J. Fluorine Chem.*,
10. **Jurupula Ramprasad**, Nagabhushana Nayak, Udayakumar Dalimba (2015). "New PZA-imidazo[2,1-*b*][1,3,4]thiadiazole hybrids: Synthesis and evaluation of antitubercular and antibacterial activity." communicated to *J. Saudi Chem. Soc.*,

Papers presented in national/international conference

1. **Jurupula Ramprasad,** Nagabhushana Nayak, Udayakumar Dalimba, Perumal Yogeeswari, Dharmarajan Sriram (2014). "Imidazo[2,1-*b*][1,3,4]thiadiazole-benzimidazole derivatives as potent antitubercular agents." 13th Eurasia Conference on Chemical Sciences. Indian Institute of Science, Bangalore, India. December 14-18, 2014.

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

Declaration:

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

Yours truly,

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