Design and Synthesis of Novel Anti-tubercular Compounds

THESIS

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by

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CERTIFICATE

This is to certify that the thesis entitled "**Design and Synthesis of Novel Anti-tubercular Compounds**" and submitted by **S. GANESH** ID No. **2011PHXF021H** for award of Ph.D. of the Institute embodies original work done by him under my supervision.

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Abstract

M. tuberculosis, the etiological agent of TB in humans is estimated to claim two million deaths annually. Although the existing drugs possess immense value in controlling disease to some extent, have several shortcomings. As drug discovery efforts are increasingly becoming rational, focusing at different target enzymes and identification of appropriate targets becomes fundamental pre-requisite.

In the present study we focused on achieving promising anti-tubercular compounds by design, synthesis and anti-mycobacterial evaluation of compounds based on reported promising anti-tubercular agents. To explore the possible target for action we subjected the synthesized compounds for various *M. tuberculosis* enzymes, namely *M. tuberculosis* PS, ADH and LAT.

In the present work, seven series of compounds (total 225 compounds) were designed and synthesized by simple and commercially feasible methods. Compound **IT_25** (*N*-(4-bromophenyl)-6-methylimidazo[2,1-*b*]thiazole-5-carboxamide) emerged as the most active in inhibiting *M. tuberculosis* PS enzyme having an IC_{50} of 0.69±04 µM, while compound **PR_31** (*N*-(2,5-dimethylphenyl)-2-phenyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*] pyrimidin-4-amine) was found to be the most active compound with *M. tuberculosis* ADH IC_{50} of 5.82 µM and compound **PZ_11** (4-(2-chlorobenzylidene)-1-phenylpyrazolidine-3,5-dione) was found to be the most active compound with *M. tuberculosis* LAT IC_{50} of 11.45 µM.

Overall, compound **PR_31** (*N*-(2,5-dimethylphenyl)-2-phenyl-5,6,7,8-tetrahydrobenzo [4,5]thieno[2,3-*d*]pyrimidin-4-amine) emerged as the most potent molecule displaying *M. tuberculosis* MIC of 2.02 µM.

The safety profile of synthesized compounds was evaluated by checking their *in vitro* cytotoxicity against RAW 264.7 cell line (mouse leukemic monocyte macrophage) MTT assay.

We believe that the present class of inhibitors reported, the potency, selectivity and no cytotoxicity with few compounds thus emerged as valid leads for further chemical optimization as novel potential anti-tubercular agents.

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List of Abbreviations

µg	:	Microgram
µM	:	Micromolar
^{13}C NMR	:	Carbon Nuclear Magnetic Resonance
^{1}H NMR	:	Proton Nuclear Magnetic Resonance
ADH	:	Alanine dehydrogenase
ATP	:	Adenosine Triphosphate
$CDCl_3$	:	Chloroform deuterated
CFU	:	Colony-forming unit
d	:	Doublet
DCM	:	Dichloromethane
DIPEA	:	*N*,*N*-Diisopropylethylamine
DMF	:	*N*,*N*-Dimethylformamide
DMSO-d_6	:	Dimethyl sulphoxide deuterated
DNA	:	Deoxyribonucleic acid
DOTS	:	Directly Observed Treatment, Short course
DSF	:	Differential Scanning Fluorimeter
EBA	:	Early Bactericidal Activity
EDCI	:	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
EMA	:	European Medicines Agency
EMB	:	Ethambutol
EU	:	European Union
FAD	:	Flavin adenine dinucleotide
FDA	:	Food and Drug Administration
HIV	:	Human Immunodeficiency Virus
HOBt	:	Hydroxybenzotriazole
HTS	:	High throughput screening
IC_{50}	:	Half Maximal Inhibitory Concentration
INH	:	Isoniazid
J	:	Coupling constant
KM	:	Kanamycin
LAT	:	Lysine aminotransferase

LCMS	:	Liquid chromatography–Mass Spectrometry
LJ medium	:	Lowenstein–Jensen medium
m	:	Multiplet
M.P.	:	Melting point
MDR-TB	:	Multidrug-Resistant *Mycobacterium tuberculosis*
mg	:	Milligram
MIC	:	Minimum Inhibitory Concentration
mL	:	Milliliter
mmol	:	Millimole
MTT	:	(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NADH	:	Nicotinamide Adenine Dinucleotide
NIAID	:	National Institute of Allergy and Infectious Diseases
nM	:	Nanomolar
OADC	:	Oleic Albumin Dextrose Catalase
PDB	:	Protein Data Bank
ppm	:	Parts per million
PS	:	Pantothenate synthetase
PTSA	:	*p*-Toluenesulfonic acid
PZA	:	Pyrazinamide
RB flask	:	Round bottom flask
RMP	:	Rifampicin
RNA	:	Ribonucleic acid
rRNA	:	Ribosomal Ribonucleic acid
rt	:	Room temperature
s	:	Singlet
SAR	:	Structure Activity Relationship
SM	:	Streptomycin
t	:	Triplet
TAACF	:	Tuberculosis Antimicrobial Acquisition and Coordinating Facility
TB	:	Tuberculosis
TDR-TB	:	Totally Drug-Resistant *Mycobacterium tuberculosis*
TEA	:	Triethylamine
TFA	:	Trifluoroacetic acid

THF	:	Tetrahydrofuran
TLC	:	Thin-layer chromatography
T_m	:	Melting temperature
TMS	:	Tetramethylsilane
US	:	United States
WHO	:	World Health Organisation
XDR-TB	:	Extensively Drug-Resistant *Mycobacterium tuberculosis*
XP	:	Extra Precision
δ	:	Chemical shift

Introduction

Chapter 1

"The captain of all these men of death that came against him to take him away, was the consumption, for it was that brought him down to the grave"

John Bunyan (1680)

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* (*M. tuberculosis*) is an ancient foeman of humanity, being considered as leading cause of death since the start of last century [Wong E.B., *et al*., 2013; Khalid H.J., *et al*., 2012]. Among all the infectious diseases known to mankind TB hangs on the deadliest [Ducati R.G., *et al*., 2006]. This wasting disease has been wiping out humankind throughout known history from the time immemorial. Despite the fact that many other diseases like cholera, plague and smallpox have destructed the lives of lakhs of people, they demised in a short period of time, but *M. tuberculosis* has been ever present [Sharma S.K., *et al*., 2013]. During 18^{th} and 19^{th} century, TB reached epidemic in Europe and North America and consumed millions of lives by earning the sobriquet "Captain among these men of death [Daniel T.M., *et al*., 2006]. TB as an infection of respiratory tract attack lungs primarily, and if not treated properly diffuses to other parts of the body except hair and nail [Pal R., *et al*., 2014].

TB develops in two different ways: 1) from a recent infection caused by inhalation of aerosol containing TB bacilli and 2) energising of dormant tubercle bacilli which is already present in the body for years or decades. The present scenario of two billion TB cases comprises both individuals with new exogenous disease and old reactivated endogenous disease. The risk of extra pulmonary TB is very high in patients with active HIV infection [Backer A.D., *et al*., 2006].

According to World Health Organization (WHO), 2 billion people are infected with latent *M. tuberculosis*, which is responsible for 8 to 10 million new cases of TB and 2 million deaths annually throughout the globe. In 2013, an estimated 9.0 million people developed new TB cases of which 11,00,000 (13%) people were HIV positive. Among the new TB cases 5% population belongs to age group of 0-14 years [Jeon D., *et al*., 2014]. Worldwide in 2013, estimations revealed that 4,50,000 people developed MDR-TB and there were estimated

1,70,000 deaths form MDR-TB. In 2012, 8.6 million people developed TB and 1.4 million people died of TB (including 4,30,000 deaths from TB among HIV-positive people). It included 0.5 million women deaths making TB one of the top killers of women worldwide. The majority of TB cases in 2011 occurred in Asia (59%), Africa (26%) and more than 50% of all deaths occurred in Asia alone (**Figure 1.1**) [WHO Global Tuberculosis report - 2014].

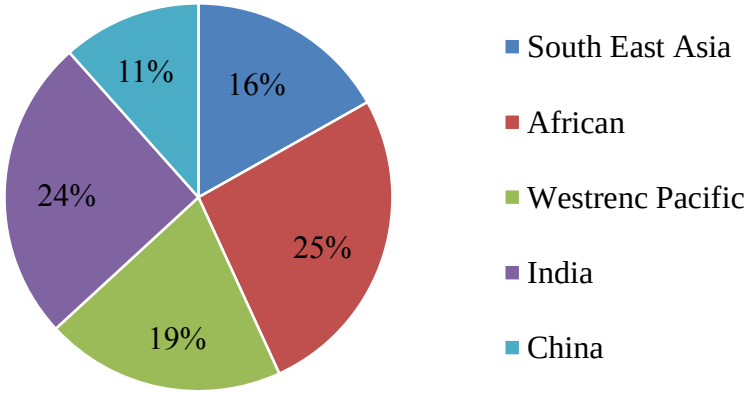


Figure 1.1: Worldwide majority of TB cases [WHO Global Tuberculosis report - 2014]

The burden of this dreadful disease can be measured in terms of:

1. Incidence (number of new and relapse cases of TB in particular time period)

2. Prevalence (number of cases of TB at a given point of time)

3. Mortality (number of deaths caused by TB in a given time period)

TB is a major public health crisis in the second most populated country India. The incidence of new TB cases in India is higher than any other country. Almost 40% of the total population in India are latently infected with *M. tuberculosis* and accounts for one-fourth of the global TB cases [WHO Global Tuberculosis report 2013]. 2014 WHO Global tuberculosis report reveals that India along with Nigeria constitutes one third global TB deaths. In 2012, out of estimated 8.6 million new TB global incidences 2.3 million cases were estimated to occur in India alone [TB India 2014-Annual Status Report].

With the discovery of isoniazid (1952), pyrazinamide (1952), ethambutol (1961) and rifampin (1966) the TB drug discovery had flourished in the middle decades of last century (**Figure 1.2** and **Table 1.1**) and the commencement of combination chemotherapy led TB from incurable scourge to curable illness by drastically reducing mortality rate and number of incident cases [Diacon A.H., *et al.*, 2009]. The blooming results of combination therapy

throve expectations of total eradication of TB. As expected TB incidence and mortality rates declined, but this was limited to developed countries. Whereas, in case of poor and developing countries, it increased and prevailed owing to improper treatment options, poor surveillance and also TB co-infection with HIV. In the last 2 decades of 20th century, development of drug resistance and HIV/AIDS co-infection has fuelled the TB, as a result the bountiful blazes of TB has fired millions lives across the globe and has been continuing steadily [Tripathi R.P., *et al.*, 2012].

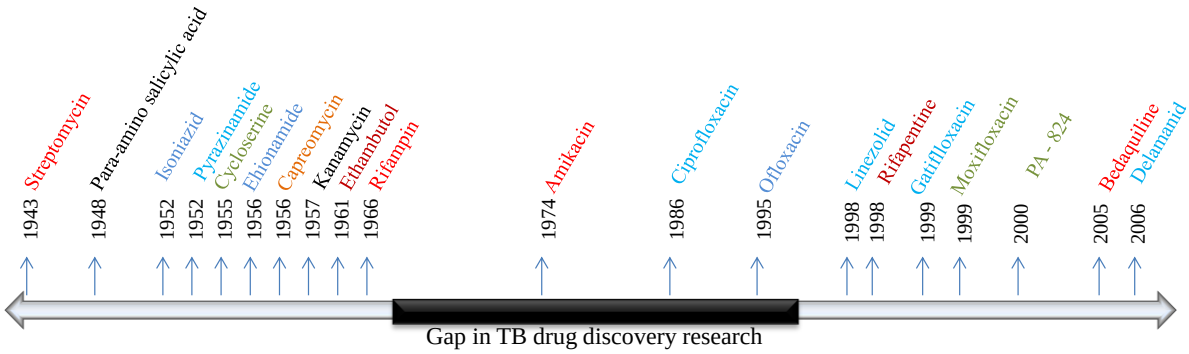


Figure 1.2: Chronicle to TB drug discovery [Wong E.B., *et al.*, 2013]

1.1. A chronicle to TB

Even though TB has been recorded in history of Egyptian civilisation (3400 BC), the earliest reference was found in ancient Indian Holy Scripture "The Vedas", where TB was referred as "Yakshma" (meaning of Wasting disease) written between 1500 and 700 BC [Dhillon M.S., *et al.*, 2001]. In classical Greece, TB was called as "Phthisis". Hippocrates wrote in his book of epidemics "Consumption was the most considerable of the diseases which then prevailed and only one which proved fatal to many persons". TB was first believed to be contagious by Fracastorius (1443-1553) and Thomas Willis first documented the clinical presentation in his treatise *Pthisilogica.* However the name TB has been ascribed to Laennec in the 1800's [Backer A.D., *et al.*, 2006]. On 24th March 1882, Robert Koch announced the discovery of the tubercle bacillus as the causative agent of the disease at Berlin Physiological Society [Daniel T.M., *et al.*, 2006]. Immortalising the centennial of this great discovery, WHO commenced celebrating 24th March as "World TB Day" globally [Herbert N., *et al.*, 2014]. Different means of TB regimens were developed from time to time. In the year 1921, a French bacteriologist Calmette together with Guerin created the Bacilli Calmette-Guerin (BCG) vaccine. Even though relatively ineffective, it is the only vaccine approved by WHO available today for the prevention of TB. TB was not epidemic until the second half of the

19th century; ensuing with migration caused by industrialization, migrations from high prevalence nations to developed ones; immigrants on their return to home country could "bring back" tubercle bacilli and diffusing of the infection [Backer A.D., *et al*., 2006; Jeon D., *et al*., 2014]. Since then TB incidence has been progressively increased till mid decades of last century.

In last decades of 20th century the emergence of HIV/AIDS pandemic (1980), drug resistance which is a result of anthropic mistakes such as improper treatment, patient's non-compliance and poor surveillance led to resumption of consumption [Arcuri H.A., *et al*., 2011]. The resurged TB unfolded its paw during 1990s and TB was recognised by WHO as a global public health problem in 1990. The fatal conditions even dragged WHO to announce TB as a "Global Public Health Emergency" in 1993, and it was the only disease so far warranted by this designation [Akgun H., *et al*., 2012; Ducati R., *et al.*, 2006].

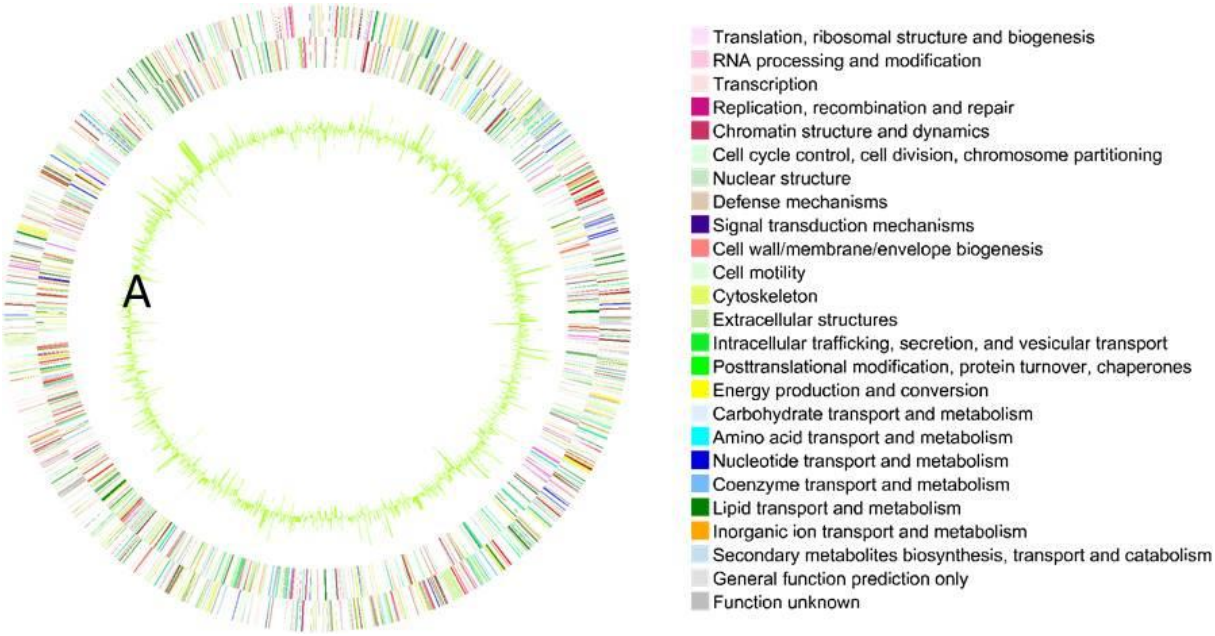
Table 1.1: Group name and mechanism of action of first and second line anti-TB agents

Name of the group	Drug*	Mechanism of action
First line oral agents	**Isoniazid**	Inhibition of Mycolic acid biosynthesis
	Rifampin	Inhibition of RNA synthesis
	Pyrazinamide	Disruption of electron transport across the membrane
	Ethambutol	Arabinogalactone synthesis inhibitor
Second line Injectable anti-TB drugs	Kanamycin	Protein Synthesis Inhibitor
	Amikacin	Protein Synthesis Inhibitor
	Capreomycin	Protein Synthesis Inhibitor
Second line Fluoroquinolones	Levofloxacin	Inhibition of DNA gyrase
	Gatifloxacin	Inhibition of DNA gyrase
	Ofloxacin	Inhibition of DNA gyrase
	Ciprofloxacin	Inhibition of DNA gyrase
	Moxifloxacin	Inhibition of DNA gyrase
Second line (oral bacteriostatic) anti-TB drugs	**Ethionamide**	Cell wall synthesis inhibitor
	Prothionamide	Cell wall synthesis inhibitor
	Cycloserine	Inhibition of peptidoglycan synthesis
	***p*-Aminosalicylic acid**	Inhibition of folic acid and Iron metabolism

*Drugs in bold letters are FDA-approved for use in TB therapy [Wong E.B., *et al*., 2013].

1.2. *M. tuberculosis*: An overview

The bacterium *M. tuberculosis* is the one of the cleverest bacteria. It has many unique properties compared to other microorganisms [Brennan P., *et al*., 2003]. *M. tuberculosis* has rigid cell wall which prevents the penetration of many drugs [Dobrikov G.M., *et al*., 2013]. The genome sequence of *M. tuberculosis* is one of the first complete genomes to be sequenced, and was decoded in 1998 by Cole and co-workers [Cole S., *et al.*, 1998]. It consists of high guanine-cytosine content, and comprises 44,11,529 base pairs, and contain around 4,000 genes (**Figure 1.3**). The H37Rv strain of *M. tuberculosis* was isolated in 1905, since then it has been found extensively in many applications related to biomedical research.



A = guanine + cytosine content. (Source: http://www.cmbi.kun.nl/MGV)

Figure 1.3: Genome of *M. tuberculosis* [Cole S., *et al*., 1998]

1.3. Drug resistance in TB

Drug resistance in TB therapy is not an immediate past, as the strains of *M. tuberculosis* that were resistant to streptomycin were observed soon after its introduction for TB treatment in 1944 [Zhang Y., *et al*., 2009; Keshavjee S., *et al*., 2012]. Today resistance to all the available anti-tubercular drugs have been found in different parts of the world. The most important factors causing drug resistance is incomplete and inadequate treatment procedures (**Figure 1.4**) and it emerges mostly where TB control programmes are feeble [Black P.A., *et al*., 2014].

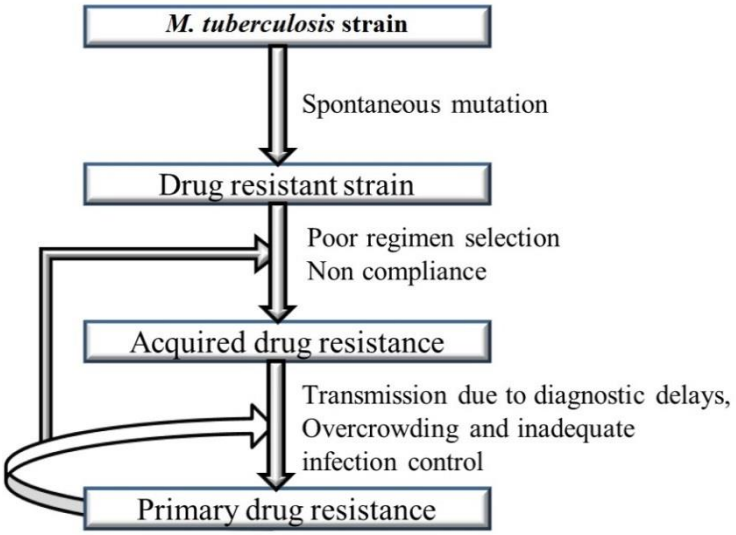


Figure 1.4: Concepts in the development of drug resistant TB [Zhang Y., *et al.*, 2009]

1.3.1. Intrinsic and acquired drug resistance

Intrinsic drug resistance pertains to the inborn ability of a bacterium to resist the functioning of a particular drug through its inherent structural properties and functions [Karakoshis P.C., *et al.*, 2008]. In case of *M. tuberculosis*, intrinsic drug resistance has been ascribed to its unique cell wall structure. The cell wall of *M. tuberculosis* built with mycolic acids which are high molecular weight α-alkyl, β-hydroxy fatty acids covalently bonded to arabinogalactan, and forms a rich hydrophobic barrier responsible for resistance to certain hydrophilic antibiotics [Michalska K., *et al.*, 2013; Karakoshis P.C., *et al.*, 2008]. Apart from hydrophobic cell wall, *M. tuberculosis* also possesses β-lactamase enzyme which shows intrinsic resistance to β-lactam antibiotics [Kolyva A., *et al.*, 2009; Dover L.G., *et al.*, 2011]. These enzymes opens the β-lactam ring of the antibiotic there by altering the chemical structure of the drug, and this causes either failing to reach the target of action or no intended action at the target site. Bacterial efflux mechanism also plays an important role in intrinsic resistance [Pal R., *et al.*, 2014; Webber M.A., *et al.*, 2003].

Acquired drug resistance happens when *M. tuberculosis* obtains the power to resist the activity of a particular antimicrobial agent to which it was previously sensitive [Ducati R., *et al.*, 2006]. The spontaneous mutations in chromosomal gene of *M. tuberculosis*, is the cause of acquired drug resistance [Zhang Y., *et al.*, 2009]. The rate of genetic mutations in *M. tuberculosis* leading to drug resistance varies between ~10^{-5} to ~10^{-6} organisms for isoniazid and 10^{-7} to ~10^{-8} for rifampin.

1.3.2. Multidrug-resistant TB (MDR-TB)

MDR-TB can be delineated as simultaneous resistance to at least isoniazid and rifampicin, with resistance to other anti-tubercular drugs [Lechartier B., *et al*., 2014; Moraski G.C., *et al*., 2012]. This situation is a consequence of inappropriate chemotherapy which is defined as the use of monotherapy (single drug administration) [Petrini B., *et al*., 1999], use of inappropriate combination of TB drugs and short treatment periods resultant of patients non-compliance [Trauner A., *et al.*, 2014]. In these situations the *M. tuberculosis* pathogen would be exposed to sub-lethal anti-bacterial conditions, which favours the growth of resistant bacilli among an originally drug susceptible pathogenic population [Ducati R., *et al*., 2006].

Globally in 2012, an estimated 4,50,000 people developed MDR-TB and 1,70,000 died of this drug-resistant strain [WHO MDR-TB fact sheet 2013]. India, China and Russian federation contribute more than half of the world's cases of MDR-TB [Zignol M., *et al*., 2012]. The "Global Plan to Stop TB" imagines that in order to proceed towards universal access, about one million MDR-TB patients need to be put on treatment in between 2011-2015 (**Figure 1.5**) [WHO MDR-TB fact sheet 2013]. An estimated 0.5 million cases of MDR-TB arise each year among both primary and acquired drug resistance cases [WHO Global Tuberculosis report-2014].

In general, *M. tuberculosis* resistant strains are less in number in regions where there was less availability of drugs to fight disease, because non treated patients either die or become chronic bacilli disseminators, but their infecting bacteria usually do not develop any kind of drug resistance. However, among the nations with a greater and ready availability of anti-tubercular regimen (developed countries), the drug resistance rates were observed to be higher although having less TB incident cases [Ducati R., *et al.*, 2006]. MDR-TB was much more difficult and pricey to treat than fully drug susceptible TB, and also the cure rate is found to be less (50-60%) compared to cure rate of (94-97%) patients with drug sensitive TB [Kwon Y.S., *et al.*, 2014].

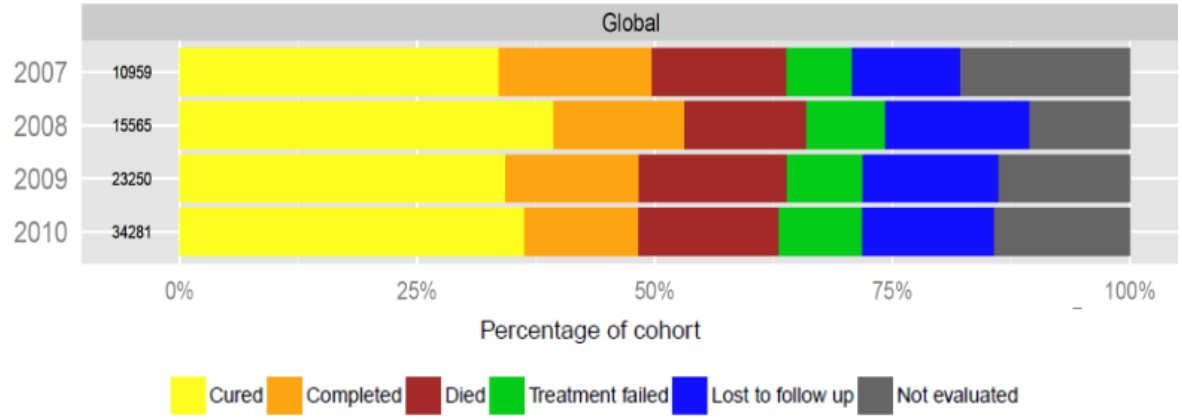


Figure 1.5: Worldwide treatment outcomes (2007-2010) for MDR-TB patients started on treatment [WHO MDR-TB 2013 update]

1.3.3. Extensively drug-resistant TB (XDR-TB)

Inappropriate, poor and partial management of MDR-TB cases resulted in a new type of drug resistant TB, well known as extensively drug-resistant TB. XDR-TB can be defined as MDR-TB, energised by acquiring an additional resistance to any fluoroquinolones and any of second line anti-tubercular injectable drugs e.g. amikacin, kanamycin or capreomycin [Migliori G.B., *et al.*, 2007; Fauci A.S., *et al.*, 2008; Shah N., *et al.*, 2007]. A total of 92 countries have reported at least one XDR-TB case by September 2013. And on an average, 9.6% of MDR-TB cases were found to be XDR-TB [WHO MDR-TB fact sheet 2013].

1.3.4. RR-TB, XXDR-TB and TDR-TB

The emergence of human immuno deficiency virus (HIV)/acquired immuno deficiency syndrome (AIDS) fuelled the resurgence of TB globally [Ginsberg A.M., *et al.*, 2009]. The last decade of previous century has witnessed the reappearance of drug-resistant TB with MDR-TB arising as a big threat to TB community along with the rising cases of XDR-TB. Recently, some parts of the world have reported the cases of extremely drug-resistant TB (XXDR-TB) and totally drug-resistant TB (TDR-TB) also called as super XDR-TB [Loewenberg S., *et al.*, 2012; Migliori G.B., *et al.*, 2007]. XXDR-TB can be defined as the isolates of *M. tuberculosis* resistant to all first-line and second-line available anti-tubercular drugs in addition to other drugs rifabutin, thiacetazone, clofazamine, dapsone and clarithromycin [Sharma S.K., *et al.*, 2013]. Rifampicin-resistant TB (RR-TB) is caused by *M. tuberculosis* strains resistant to rifampicin, with or without resistance to other drugs. Both MDR-TB and XDR-TB are forms of RR-TB.

The strain of *M. tuberculosis* which is resistant to all first line and second line licenced anti-tubercular drugs is defined as totally drug resistant TB (TDR-TB) [Sharma S.K., *et al*., 2013]. The clinical isolates of TDR-TB were observed in Italy for the first time in 2003 [Migliori G., *et al.*, 2007], next in Iran [Velayati A.A., *et al.*, 2009] and now it has been reported from India that there were TB patients who did not respond to any of the anti-tubercular regimens [Udwadia Z., *et al.*, 2013; Udwadia Z., *et al.*, 2012]. Though it is not yet prevalent, chances are bright to through a challenge of TDR-TB to researchers round the globe.

1.4. Current therapy for TB

1.4.1. Treatment for drug susceptible-TB

The current six months standard treatment is the result of a series of intensive trials conducted over 20 years by the British Medical Research Council [Fox W., *et al*., 1999]. Current TB therapy consists of isoniazid, ethambutol, rifampicin and pyrazinamide for two months followed by isoniazid and rifampicin for four months (**Table 1.2**) [Wong E.B., *et al*., 2013; Yee D., *et al.*, 2003]. This standard drug TB therapy is lengthy as patients have to take the drugs for six months and often leads to patient's non-adherence [Yew W.W., *et al*., 2011; Trauner A., *et al.*, 2014]. In this situation an incomplete treatment results in development of drug resistance. To confront this situation, WHO promoted a program known as "Directly Observed Treatment-Short course (DOTS)" [Pieroni M., *et al*., 2014; Chan B., *et al*., 2013; Hegymegi B.B., *et al*., 2008]. In this type of treatment there is a direct observation by trained personnel on patients undergoing treatment. DOTS therapy has proven to be one of the most cost effective health interventions available today around the globe [Villemagne B., *et al*., 2012].

Table 1.2: WHO recommended regimen for drug-susceptible TB [Wong E.B., *et al*., 2013]

Drug	Daily dose	Duration	Defects
Isoniazid	5 mg/kg*	24 weeks	Peripheral neuropathy, lupus-like syndrome
Rifampin	10 mg/kg**	24 weeks	Orange discoloration of secretions, fever, hepatitis
Pyrazinamide	25 mg/kg	8 weeks	Hepatitis, arthritis
Ethambutol	15 mg/kg	8 weeks	Optic neuritis

*****Maximum daily dose 300 mg ******maximum daily dose 600 mg

1.4.2. Treatment for drug resistant-TB

Drug resistant TB can be largely cured with the appropriate combination and rational use of available anti-tubercular drugs [Caminero J., *et al*., 2006]. For the treatment of MDR-TB, WHO recommends the use of DOTS-Plus therapy, which includes drugs used in DOTS therapy plus second line TB drugs (**Figure 1.6**) [Ahuja S.D., *et al*., 2012].

Composition of MDR-TB drug regimen [Lange C., *et al*., 2014; Van Deun A., *et al*., 2010]:

1. Choose, if possible:
    a) Injectible second line drug (e.g. Amikacin)
    b) Later generation fluoroquinolone (e.g. Levofloxacin)
    c) Ethionamide or prothionamide
    d) Cycloserine or terizidone
2. Choose at least 4 drugs (it is unclear whether all patients with MDR-TB/XDR-TB should be treated with pyrazinamide)
3. Choose group five drugs only if needed to sum up to at least four active drugs
4. Treatment for a total of 24 months with an intensive phase of 8 months
5. Prolongation of duration of therapy should be considered based on treatment success

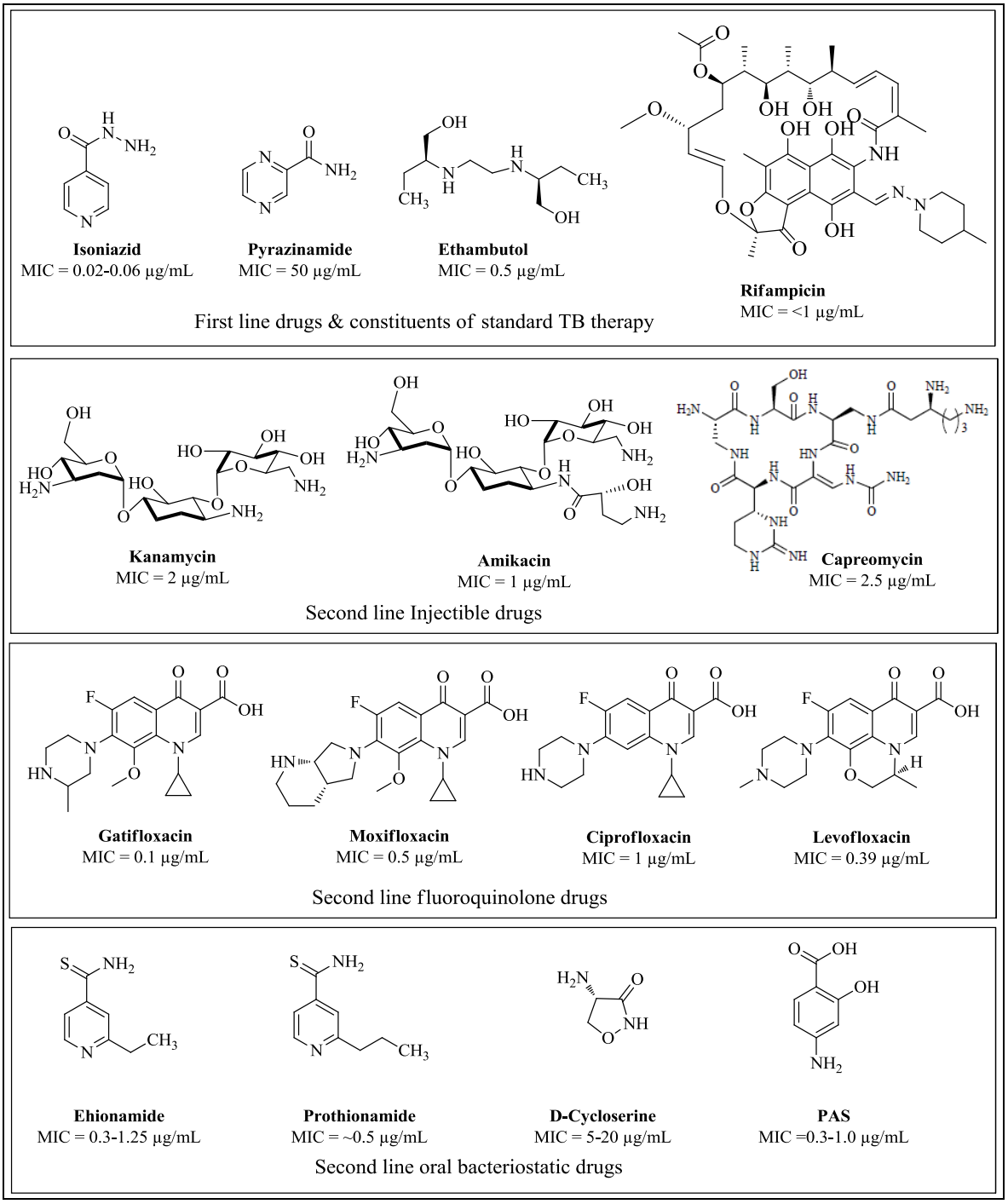


Figure 1.6: Structures of first line and second line anti-TB drugs with MIC values.

Recently USFDA has approved bedaquiline (TMC-207) for the treatment of MDR-TB in adults [Koul A., *et al.*, 2007; Koul A., *et al.*, 2008].

The introduction of TMC-207 to standard therapy for MDR-TB significantly reduced the treatment time [Diacon A.H., *et al.*, 2009]. WHO and the US Centre for Disease Control and Prevention (CDC) recently suggested that, bedaquiline may be used for treatment of MDR-

TB in adults when an effective treatment regimen is not available. However there are safety issues with this drug, as it showed an increased risk of death and QT prolongation [Mase S., *et al*., 2013]. SIRTURO (bedaquiline fumarate) a bedaquiline drug for oral administration is available as 100 mg tablets. SIRTURO has received conditional approval from EMA in March 2014 to market in EU region for the treatment of MDR-TB. Each tablet contains 120.89 mg of bedaquiline fumarate, which is equal to 100 mg of bedaquiline. SIRTURO should only be used in combination with at least three other drugs to which the patient's MDR-TB isolate is susceptible *in vitro*.

While treating XDR-TB, drugs can be chosen with a stepwise selection on the basis of safety and efficacy. There are new drugs (PA-824, OPC-67683 and TMC-207) and novel regimens (PA-824-Moxifloxacin-Pyrazinamide (PaMZ) and NC-003) for treating drug resistant TB are now available. Their mechanism of action and properties are discussed in next chapter (Chapter 2).

1.4.3. Treatment for latent-TB infection

Latent-TB infection (LTBI) is the presence of *M. tuberculosis* organisms without symptoms or radiographic evidence of active disease [Menzies D., *et al*., 2011]. Who needs LTBI testing? LTBI testing is very mandatory for: a) health care workers, b) close contacts of infectious TB patients, and c) frequent travellers to abroad.

For treating LTBI there are few regimens used based on the results of drug susceptibility testing [Panickar J.R., *et al*., 2007].

1. Nine months isoniazid therapy: In this therapy, patient will be given a daily dose of isoniazid for nine months. A minimum of 270 doses must be administered within this period. This therapy was found to be safer, but the only problem is length of treatment.

2. Four months rifampicin therapy: Though this therapy is shorter than isoniazid therapy, it cannot be recommended for routine use until the reviewing of regular results of the efficacy trial. This therapy requires direct observation treatment.

3. Two months rifampicin–pyrazinamide therapy: In this therapy, a combination of rifampicin–pyrazinamide will be given to patients for two months. Due to severe hepatic injury and death, this regimen was not recommended.

4. On 9th December 2011, CDC released the recommendations on the use of new treatment regimen for LTBI. CDC has recommended a 12-dose regimen; the regimen is a combination of INH and RMP given in 12 once weekly doses under directly observed treatment. This 12-dose regimen is very effective which reduces the required treatment for LTBI from 270 daily doses over 9 months to 12-once weekly doses given over 3 months [http://www.cdc.gov/MMWR/PDF/rr/rr4906.pdf].

Literature Review

Chapter 2

2.1. Classification and confinement of current anti-TB drugs

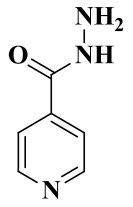
Current anti-TB drugs can be classified as inhibitors of: a) cell wall synthesis (isoniazid, ethambutol, ethionamide and cycloserine), b) protein synthesis (amikacin, kanamycin & capreomycin), c) nucleic acid synthesis (rifampin and quinolones), and d) electron transport across the bacterial membrane (pyrazinamide) [Zhang Y., *et al*., 2005]. Although the success rates are as high as 95-98% in drug susceptible TB, once the bacterium acquires drug resistance (MDR-TB & XDR-TB) these drugs are unable to cure TB completely. The bacterial sub populations although drug-susceptible, can display phenotypic drug resistance in response to altered environmental signals [Mak P.A., *et al*., 2012]. The strains of *M. tuberculosis* resistant to all the above said drugs have been isolated from clinical isolates of different stages of TB-infected patients. Therefore the need for novel drugs possessing different mechanisms of action to kill different bacterial sub populations is ineluctable. The current anti-tubercular regimen, their mode of action and treatment confinement has been briefed as follows:

2.1.1. Inhibitors cell wall synthesis

Isoniazid

Isoniazid also known as isonicotinylhydrazide is the most widely used first line anti-tubercular orally active drug [Vilcheze C., *et al*., 2011]. It is a prodrug activated by 'catalase peroxidase' enzyme (*KatG*) [Matsumoto M., *et al*., 2007; Murillo A., *et al*., 2007] and active against growing tubercle bacilli, but not active against non-replicating bacilli. The primary target of isoniazid inhibition is "enoyl acyl carrier protein reductase (InhA)" enzyme [Tonge P.J., *et al*., 2007]. InhA involves in elongation of fatty acids in mycolic acid synthesis. *KatG* activates isoniazid to produce a range of highly reactive species which then attacks multiple targets; one such reactive species isonicotinic-acyl anion or radical reacts with NAD(H) to form an isoniazid-NAD adduct, and then attacks InhA. Recent research shows that besides InhA it also attacks DfrA ('dihyrofolate reductase' involved in DNA synthesis) [Zhang Y., *et al*., 2009].

Because of its wide use, resistance to isoniazid has been seen more frequently among clinical isolates of *M. tuberculosis* infected patients. Resistance to isoniazid occurs due to the mutations in *KatG* gene; as a result the ability of catalase peroxidase to activate isoniazid prodrug reduces (**Table 2.1**). Hepatitis, lupus-like syndrome, peripheral neuropathy and drug-drug interactions are major adverse reactions of isoniazid [Vilcheze C., *et al*., 2011].

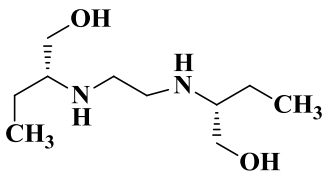


Isoniazid

Ethambutol

Ethambutol (Ethylene diamino-di-1-butanol) is a first line anti-tubercular drug, together with isoniazid, rifampicin and pyrazinamide constitutes short course for the treatment of drug sensitive TB. It interferes with the biosynthesis of cell wall of *M. tuberculosis*. Ethambutol inhibits the enzyme arabinoyl transferase which is needed for the synthesis of arabinogalactan. By inhibiting the synthesis of arabinogalactan (the chief constituent of bacterial cell wall) leads to increased permeability of the bacterial cell wall occurs. S, S (dextro) form of ethambutol is 600 times more active than R, R-isomer [Yendapally R., *et al*., 2008; Tripathi R.P., *et al*., 2012].

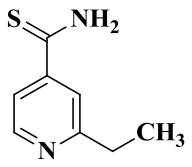
The enzyme arabinoyl transferase encoded by the gene *embB* involved in the synthesis of arabinogalactan has been proposed as the target of ethambutol in *M. tuberculosis* [Hasan S., *et al*., 2006]. Resistance to ethambutol is generally associated with mutations in the *embCAB* operon, in particular *embB* and occasionally *embC*. Some inconsistent reports revealed that one quarter of all ethambutol resistant *M. tuberculosis* isolates do not harbour mutations in any of the above named genes, suggesting further studies needed to investigate possible mechanism of ethambutol resistance [Escuyer V.E., *et al*., 2001].



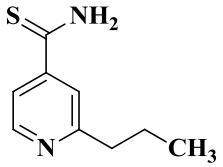
Ethambutol

Ethionamide and prothionamide

Ethionamide, 2-ethylisothionicotinamide a synthetic compound structurally related to isoniazid is bactericidal against *M. tuberculosis*. Like isoniazid, ethionamide is also a prodrug requiring activation by the monooxygenase EthA/EtaA [Vannelli D., *et al*., 2002; Debarber A.E., *et al*., 2000]. EtaA/EthA is a flavin adenosine dinucleotide (FAD) containing enzyme that oxidises ethionamide to the corresponding S-oxide. Similar to isoniazid, ethionamide inhibits mycolic acid synthesis by binding to the enzyme InhA. Protionamide is almost similar to ethionamide with regard to structure and activity [Yew W.W., *et al*., 2011]. Resistance to ethionamide is because of mutations in genes *ethA* or *inhA* [Vilcheze C., *et al*., 2008; Baulard A.R., *et al*., 2000]. The various genes involved in drug resistance are presented in **Table 2.1**.



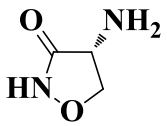
Ethionamide



Prothionamide

Cycloserine

It is a structural analog of the amino acid D-alanine, which inhibits the synthesis of cell wall of mycobacteria by blocking the action of D-alanine racemase and D-alanine: D-alanine ligase [Strych U., *et al*., 2001]. Cycloserine possesses activity against a wide range of bacteria [Otten H., *et al.*, 1998], and inhibits *M. tuberculosis* at concentrations of 5-20 μg/mL [David H.L., *et al.*, 1969]. Cycloserine produces side effects in the central nervous system that could also generate psychotic states with suicidal tendencies and epileptic convulsion [Cacers N.E., *et al*., 1997]. Resistance to cycloserine is due to overexpression of AlrA and Dd1 [Feng Z., *et al*., 2003].



D-Cycloserine

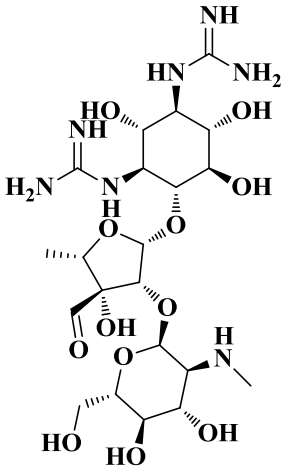
Table 2.1: *M. tuberculosis* genes associated drug resistance [Wong E.B., *et al.*, 2013]

Drug	Effect on bacterial cell	*M. tuberculosis* gene	Role of gene product
		katG	Catalase/peroxidase
Isoniazid	Bactericidal	*inhA*	Enoyl reductase
		aphC	Alkyl hydroperoxide reductase
Rifampicin	Bactericidal	*rpoB*	β-subunit of RNA polymerase
Pyrazinamide	Bactericidal	*pncA*	Pyrazinamidase/nicotinamidase
Ethambutol	Bacteriostatic	*embB*	Arabinosyl transferase
		rpsL	S12 ribosomal protein
Streptomycin	Bacteriostatic	*rrs*	16S rRNA
		gidB	7-methylguanosine methyltransferase
Fluoroquinolones	Bactericidal	*gyrA/gyrB*	DNA gyrase
Kanamycin/ Amikacin	Bactericidal	*rrs*	16S rRNA
Ehionamide	Bacteriostatic	*inhA*	Enoyl reductase
p-aminosalicylic acid	Bacteriostatic	*thyA*	Thymidylate synthase A

2.1.2. Inhibitors of protein synthesis

Streptomycin

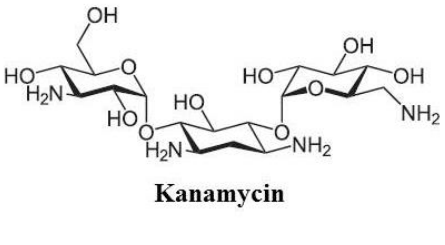
Streptomycin, the first antibiotic drug used against TB was derived from the actinobacterium *Streptomyces griseus*. The MIC value of streptomycin is 1 μg/mL with a half-life of 5-7 h. Because of its poor absorption through gastrointestinal tract, the mode of administration is intramuscular and very occasionally by intrathecal route [Tripathi R.P., *et al*., 2012]. Streptomycin acts as inhibitor of protein synthesis by binding to the S12 protein of the 30S subunit of the bacterial ribosome and interfering with the binding of formyl-methionyl-tRNA to the 30S subunit of the ribosome. Although resistance to streptomycin has become less common due the wider use of ethambutol as fourth drug in the WHO standard regimen, owing to its side effects it is not used currently in TB chemotherapy. Streptomycin exhibits toxic manifestations on peripheral and central nervous system at higher doses and leads to hypersensitivity reactions.



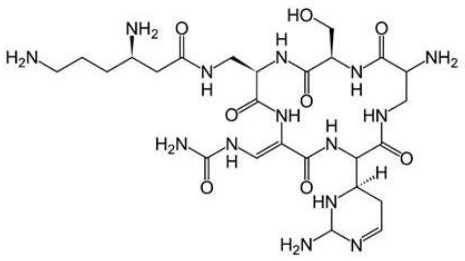
Streptomycin

Kanamycin, amikacin and capreomycin

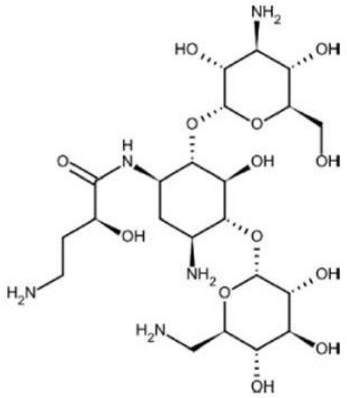
Kanamycin and its derivative amikacin belong to aminoglycoside family of drug and are inhibitors of protein synthesis. These drugs target the 30S subunit of ribosome [Alangaden G.J., *et al*., 1998; Suzuki Y., *et al*., 1998] in *M. tuberculosis* strain. Capreomycin is a macrocyclic polypeptide, like streptomycin and kanamycin it modifies the ribosomal structure at 16S RNA there by inhibits protein synthesis [Maus C.E., *et al*., 2005; Johansen S., *et al*., 2006]. *M. tuberculosis* resistant to kanamycin and capreomycin has been associated with mutations in the *rrs* gene encoding 16S rRNA [Alangaden G.J., *et al*., 1998].



Kanamycin



Capreomycin

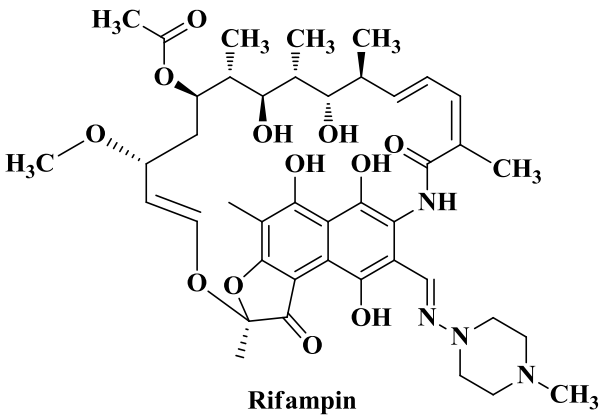


Amikacin

2.1.3. Inhibitors of nucleic acid synthesis

Rifampin

Introduction of rifampin into the standard anti-TB regimen reduced the duration of treatment from 18 months to 9 months. Higher doses of newly emerged drug rifapentine, has the potential to further reduce the duration of TB treatment, although additional studies are needed to evaluate maximum tolerated dose of this drug [Ginsberg A.M., *et al*., 2009]. The mode of action of rifampin is inhibition of DNA-dependent RNA polymerase. Rifampin binds to β-subunit of the enzyme RNA polymerase, an enzyme necessary for RNA synthesis, thus preventing transcription to RNA and subsequent translation to proteins. Resistance to rifampin is a result of mutations in the *rpoB* gene, which encodes the β-subunit of RNA polymerase.

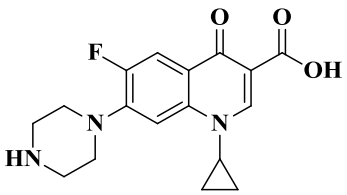


Rifampin

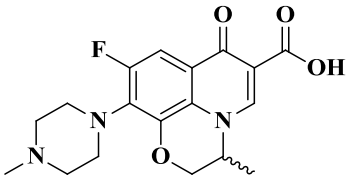
Fluoroquinolones

The fluoroquinolones, moxifloxacin, gatifloxacin, ciprofloxacin and levofloxacin are most important bactericidal antibiotics and have broad spectrum activity. They are active against both gram-positive and gram-negative bacteria. Gatifloxacin and moxifloxacin are under phase III clinical evaluation aiming at better TB treatment.

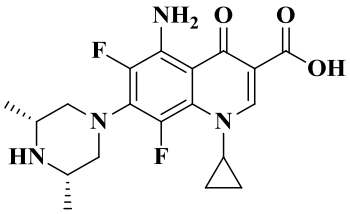
Fluoroquinoles inhibits both ATP dependent DNA gyrase (topoisomerase II) as well as ATP dependent topoisomerase IV [Takei M., *et al*., 2001; Kato J., *et al*., 1990]. Fluoroquinolones blocks the movement of replication works and transcription complexes [Drlica K., *et al*., 2003]. Resistance to fluoroquinolones in *M. tuberculosis* is due to mutations in the conserved quinolone resistant-determining region of gyrA and gyrB involved in the interaction between the drug and DNA gyrase [Aubry A., *et al*., 2006].



Ciprofloxacin



Ofloxacin (R/S)
S: Levofloxacin

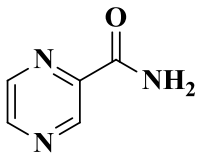


Sparfloxacin

2.1.4. Inhibitors of electron transport across the bacterial membrane

Pyrazinamide

Although anti-bacterial activity of pyrazinamide is inferior to that of rifampin and isoniazid, it is still part of WHO recommended standard TB therapy. This can be attributed to its unique role in shortening TB treatment from previous 9-12 months to 6 months. Pyrazinamide kills the semi-dormant population of bacilli residing within an acidic environment. Like isoniazid, pyrazinamide is a prodrug and it requires activation to its active form pyrazinoic acid by the enzyme pyrazinamidase/nicotinamidase [Silva A.D., *et al*., 2011].



Pyrazinamide

The anti-tubercular activity of pyrazinamide has been ascribed to disruption of electron transport across the membrane. The pyrazinamide resistance in *M. tuberculosis* is due to mutations in the *pncA*, which creates defect in the functioning of pyrazinamidase. Hyperuricemia, gouty arthritis and rarely nephritis are major adverse reactions observed with Pyrazinamide [Jureen P., *et al.*, 2008].

As an overview of all these drugs, in **Figure 2.1**, a pictorial representation of various mode of action has been delineated.

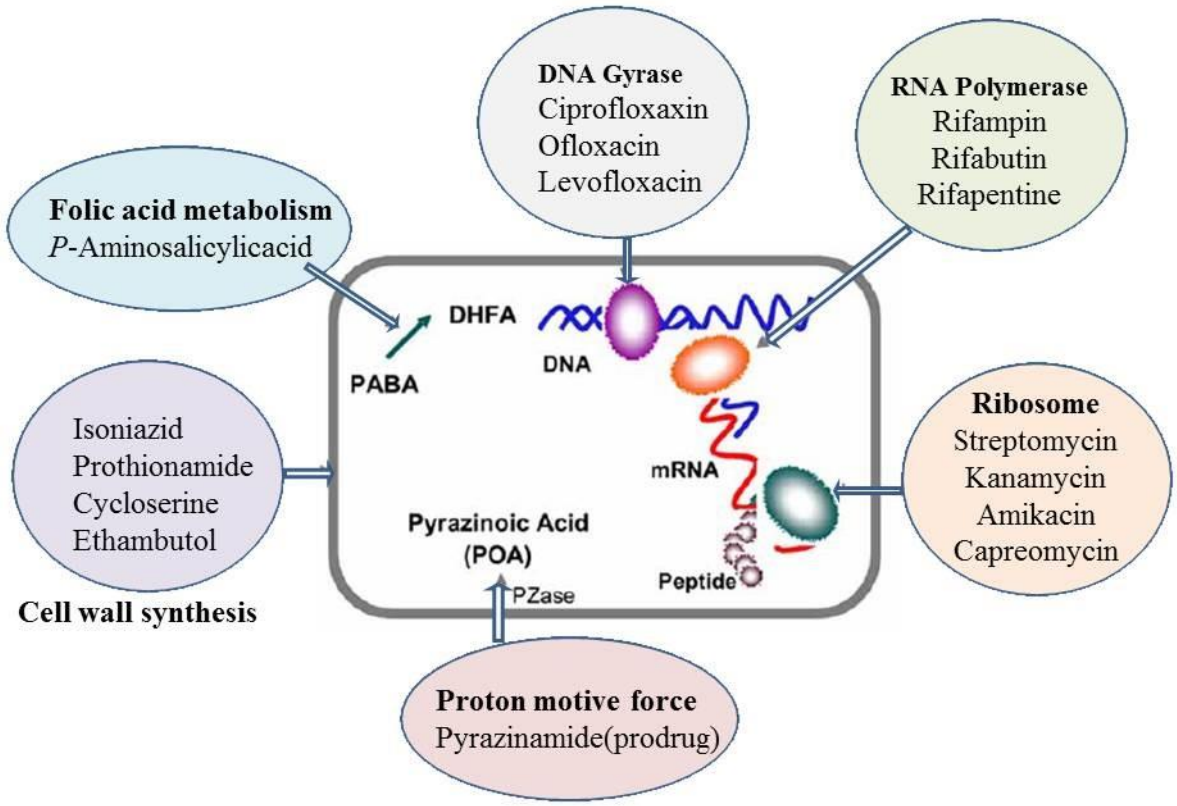


Figure 2.1: Current anti-TB drugs and their site of action [Laurenzi M., *et al*., 2007]

2.2. Anti-TB drug discovery-pipeline

The portfolio of anti-TB agents currently in research and development is usually referred as TB drug-pipeline [Laurenzi M., *et al*., 2007]. The current decade blossoms with a promising anti-TB drug pipeline, with several promising drugs targeting various *M. tuberculosis* terminating sites in different stages of development (**Figure 2.2**). There are few drug candidates in various stages of development are also in market (e.g., Deltyba, Situro) before finishing complete process of phase III trials. Also novel drug combinations which intend to combat drug resistant TB and reduce the duration of therapy are in pipeline. The various phases of clinical trials in drug discovery pipeline are presented in **Table 2.2**.

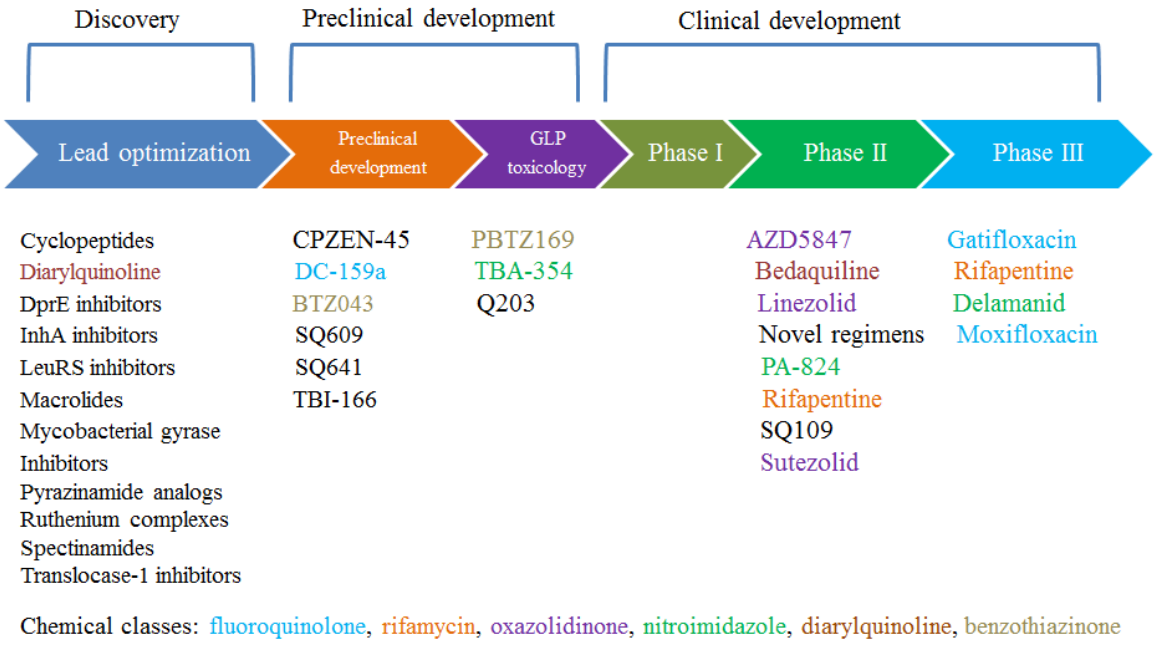


Figure 2.2: New TB drugs in development pipeline [WHO Global Tuberculosis report-2014]

According to WHO Global Tuberculosis report, currently there are four drugs in phase III clinical trials, seven drugs and one novel regimen in phase II clinical studies and many new individual drug candidates and class of molecules are in lead optimisation and preclinical development.

Table 2.2: Phases in clinical trials [Lienhardt C., *et al*., 2010]

Phase trial	Description
Phase I trials	Preliminary studies to ascertain the metabolism and pharmacologic actions of drugs in humans, the side effects versus quantity of doses, and to gain early evidence of effectiveness; may include healthy participants or patients.
Phase II trials	Controlled clinical studies conducted to evaluate the effectiveness of the drug for a particular indication or indications in patients with the disease or condition under study and to determine the common short-term side effects and risks. This phase can also be used to establish dose ranges and dose-response relationships.
Phase IIa	Addresses dose and dose-response with limited numbers of participants (typically 30-50)
Phase IIb	Addresses risks and efficacy with large number of participants (typically 200-500)
Phase III trials	Expanded controlled and uncontrolled trials conducted after preliminary evidence suggesting effectiveness of the drug has been obtained, that are intended to gather additional information to evaluate the overall benefit-risk relationship of the drug and provide adequate basis for physician labelling based on established short and long-term safety and efficacy of the drug.
Phase IV trials	Post marketing studies to delineate additional information, including the drug's risks, benefits, and optimal use in populations.

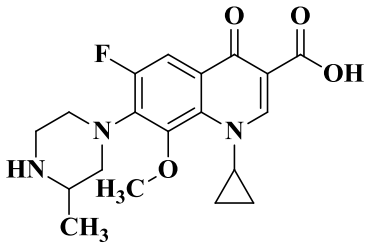
2.2.1. Current drugs in phase III clinical trials

2.2.1.1. Fluoroquinolones

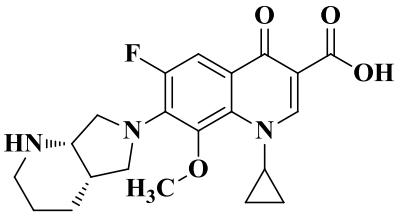
The two promising fluoroquinolone drug candidates gatifloxacin and moxifloxacin are presently in phase III clinical studies. Fluoroquinolones are the backbone of treatment for MDR-TB and their potential has been tested in many studies [Falzon D., *et al*., 2013; Johnston J.C., *et al*., 2009]. They are also capable in reducing the treatment duration in drug susceptible TB [Rustomjee R., *et al*., 2008; Conde M.B., *et al*., 2009; Dorman S.E., *et al*., 2009]. Recent studies showed that these drugs possess potent activity against *M. tuberculosis* than the other members of this class including ofloxacin [Hu Y., *et al*., 2003].

In 1999, FDA approved gatifloxacin for the treatment of patients with bronchitis, pneumonia and various infections including those of the urinary tract, kidneys and skin. Presently OFLOTUB consortium is conducting gatifloxacin clinical development programme. In phase II studies conducted in Durban (South Africa) the newly diagnosed patients were treated with four drug regimen comprising isoniazid, rifampin and pyrazinamide in combination with gatifloxacin for first two months (OFLOTUB phase II surrogate marker study). The result of the study shows that when substituted in place of ethambutol in standard TB therapy, both moxifloxacin and gatifloxacin killed *M. tuberculosis* significantly faster than the control or ofloxacin based regimens. This supports that introduction of these fluoroquinolones in place of ethambutol in standard therapy may reduce treatment duration by one or two months. The consortium is continuing the evaluation of gatifloxacin substituted regimen versus standard 6 months treatment in phase III design [Laurenzi M., *et al*., 2007].

Moxifloxacin, a fourth generation synthetic fluoroquinolone developed by Bayer AG, was marketed worldwide under the brand names of Avelox and Avelon for oral treatment [www. Avelox.com]. In 1999, moxifloxacin hydrochloride (Avelox) was approved by USFDA for use in US. During 2005, in association with TB-alliance, Bayer started further exploration of moxifloxacin. The studies at John Hopkins University, used mice models where the infected mice were treated for one month with sparfloxacin, clinafloxacin, moxifloxacin or isoniazid [Ji B., *et al*., 1998] and it was found that moxifloxacin had greatest bactericidal activity as compared to isoniazid. Another study suggested that moxifloxacin also had potent sterilizing activity [Kwon Y.S., *et al*., 2014].



Gatifloxacin



Moxifloxacin

In vitro activities of gatifloxacin and moxifloxacin are better than the older fluoroquinolones ciprofloxacin and ofloxacin [Rodriguez J., *et al*., 2001] and their MICs against *M. tuberculosis* H37Rv are as follows [Villemagne B., *et al*., 2012]:

Drug	MIC	MIC_{90}
Gatifloxacin	0.12-0.25 µg/mL	0.007-0.12 µg/mL
Moxifloxacin	0.18-0.5 µg/mL	0.031-0.12 µg/mL

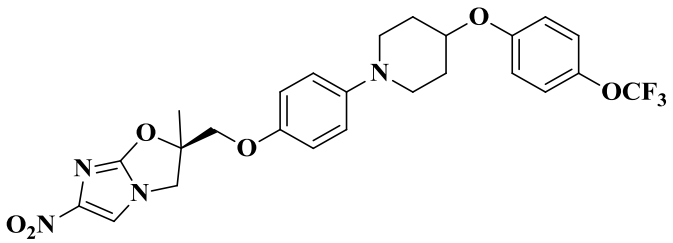
In a murine model of TB, moxifloxacin (100 mg/kg), gatifloxacin (100 mg/kg) and isoniazid (25 mg/kg) had similar activities after 4 weeks of treatment [Alvirez-Freites E.J., *et al*., 2002]. In *M. tuberculosis* infected mice replacement of INH (25 mg/kg) with moxifloxacin (100 mg/kg) in the standard RMP/INH/PZA 6 months regimen shortened the duration of therapy by upto 2 months and no relapse was observed 3 months after the end of the treatment [Nuermberger E.L., *et al*., 2004]. Moxifloxacin was tested safe and well tolerated in a long-term administration at 400 mg once daily [Codecasa L.R., *et al*., 2006].

2.2.1.2. Delamanid

Delamanid (OPC-67683) is a nitroimidazooxazole derivative. This new drug has received approval by European Medicines Agency (EMA) for the treatment of MDR-TB in November 2013. Like isoniazid and pyrazinamide, delamanid also a prodrug which is activated by the enzyme deazaflavin dependent nitroreductase (Rv3547). OPC-67683 acts by inhibiting the synthesis of *M. tuberculosis* cell wall components methoxy mycolic acid and ketomycolic acid [Xavier A.S., *et al*., 2014].

Unlike, first-line anti-tubercular drugs which should be taken on empty stomach, delamanid is advised to take along with food. After oral administration the maximum concentration is observed at 4-5 h. The half-life is 38 h after drug discontinuation. During *in vitro* studies, it

showed good anti-bacterial activity against drug sensitive and drug resistant strains of *M. tuberculosis* with observed MIC of an extremely lower range of 0.006-0.024 µg/mL. In one study, delamanid was tested on HIV negative MDR patients along with WHO standard therapy for 2 months. The results showed that higher sputum culture conversion rates were observed in the treatment group compared to patients on placebo and background regimen [Gler M.T., *et al*., 2012].



Delamanid

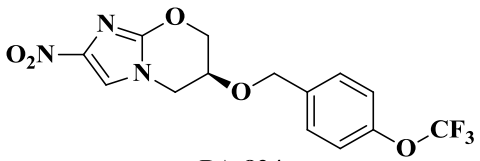
In clinical studies, delamanid showed no significant interactions with other drugs such as lopinavir, tenofovir and efavirenz. This shows that delamanid can be combined with other anti-tubercular drugs as there are no adverse drug-drug interactions. Presently delamanid is marketed as Deltyba, 50 mg tablet used as a part of an appropriate combination for pulmonary MDR-TB in adults for whom current approved regimen fails to combat [Xavier A.S., *et al*., 2014].

2.2.2. Current drugs in phase II clinical trials

2.2.2.1. PA-824: Nitroimidazole derivative

PA-824 is a new potential drug in clinical development pipeline developed by TB-alliance (**Table 2.3**). It has entered phase-I studies in 2005 and after successfully completing phase-I trials it is now in phase-II clinical trials. PA-824 is capable of treating drug sensitive as well as MDR/XDR-TB [Laurenzi M., *et al*., 2007; Obrien R.J., *et al*., 2005]. PA-824 is also a prodrug like isoniazid and requires the activation of aromatic nitro group by F420-dependent mechanism [Yew W.W., *et al*., 2011]. PA-824 inhibits both protein and lipid synthesis but does not affect nucleic acid synthesis. It undergoes nitro reduction producing highly reactive intermediates which then reacts with multiple targets inside the bacterial cell. *In vitro* studies indicated that PA-824 was active at MIC similar to that of isoniazid [Lenaerts A.J., *et al*., 2005]. Further *in vitro* studies with anaerobic culture models indicated that PA-824 has activity against non-replicating bacilli [Stover C.K., *et al*., 2000]. It also showed activity

against strains with known resistance to standard TB treatment [Tyagi S., *et al*., 2005]. PA-824 has been observed to kill bacteria in two distinct mechanisms: i) by interfering with the synthesis of ketomycolate which is an essential component of the mycobacterial cell wall, and ii) by acting as a nitric oxide donor and causing respiratory poising [Singh R., *et al*., 2008]. Mutations in the gene encoding the F420 enzyme are responsible for few instances of drug resistance identified *in vitro* [Manjunatha U.H., *et al*., 2006].

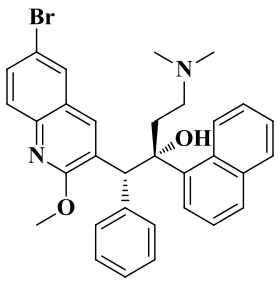


PA-824

A charging up role of PA-824 has been identified in novel drug combinations which appear to outperform standard treatment in both the murine model and in a human EBA trial. The novel combination PaMZ has showed superior bactericidal and sterilizing activity in murine model [Wong E.B., *et al*., 2013]. The results are far better when compared to the standard four drug combination therapy. The mice were treated with above combination for 4 months and then monitored for relapse; encouragingly mice treated with PaMZ were all cured just with four months of treatment. These results encouraged to create novel combinations for better regimens.

2.2.2.2. Bedaquiline

Bedaquiline (Trade name: SITURO; code name: TMC-207) belongs to new class of drugs, the diarylquinolines discovered by Janssen Pharmaceutica. It was approved on 28th December 2012 by the FDA, and a drug of novel class to be approved over 40 years [Chan B., *et al*., 2013]. Mechanism of action of TMC-207 is inhibition of mycobacterial ATP synthase resulting in decreased ATP levels and pH imbalance in the organism. The bactericidal bedaquiline has an unusual long half-life of >24 hours in human [Andries K., *et al*., 2005].



Bedaquiline

Table 2.3: New anti-TB drugs, stage of development and their targets [Yew W.W., *et al*., 2011]

Drug	Class	Company	Stage of development	Target
Moxifloxacin	Fluoroquinolone	Bayer	III	DNA gyrase
Gatifloxacin	Fluoroquinolone	BMS	III	DNA gyrase
OPC-67683	Nitroimidazo-oxazole	Otsuka	III	Unknown
Rifapentine	Rifamycin	Sanofi-Aventis	III	RNA polymerase
PA-824	Nitroimidazo-oxazine	TB-alliance	II	Unknown
Linezolid	Oxazolidinone	Pfizer	II	Ribosomal initition complex
TMC-207	Diarylquinoline	Tibotec/Janssen	II	ATP synthase
PNU-100480	Oxazolidinone	Pfizer	II	Ribosomal initition complex
AZD-5847	Oxazolidinone	Astrazeneca	II	Unknown
SQ-109	Diethylamine	Sequella	II	Unknown

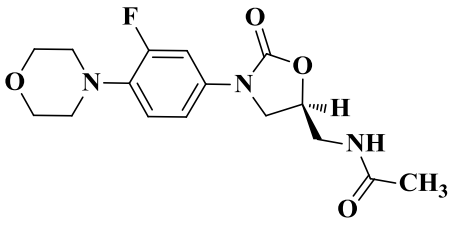
2.2.2.3. Oxazolidinones (Linezolid, sutezolid and AZD 5847)

Oxazolidinones possess a broad spectrum of antibiotic activity covering anaerobic and gram positive aerobic bacteria as well as mycobacteria. Oxazolidinones inhibit the protein synthesis by binding to 23S RNA in the 50S ribosomal subunit of *M. tuberculosis* [Zhang Y., *et al*., 2005].

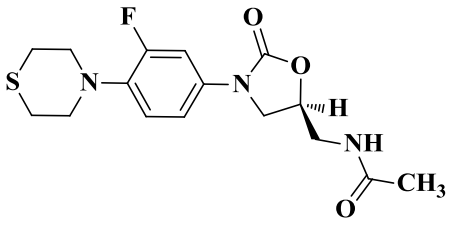
Linezolid was approved in the year 2000 for the treatment of drug resistant gram-positive bacterial infections (WHO Global Tuberculosis report-2013). It was originally developed for resistant Gram positive organisms and commonly used to treat methicillin resistant *Staphylococcus aureus* [Wong E.B., *et al*., 2013]. It has good anti-mycobacterial activity *in vitro* and is used off-label in combination regimens for the treatment of MDR-TB, but its efficacy is unclear. Serious adverse effects such as peripheral and optic neuropathies, thrombocytopenia and anaemia have been reported with the use of linezolid.

Therefore trials were conducted to evaluate the efficacy at lower doses (600 or 300 mg/day) compared with that of standard doses (1200 mg/day). In recent trial patients with XDR-TB were given lower doses (300-600 mg/day), 87% of all patients achieved negative conversion

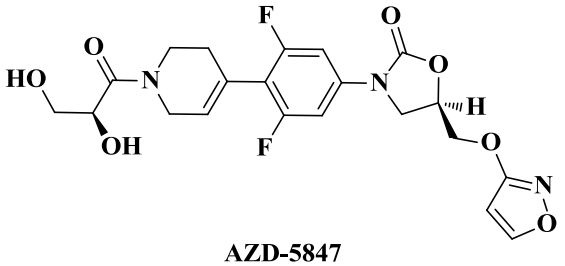
within 6 months [Lee M., *et al*., 2012]. But four patients acquired linezolid resistance during treatment. Three of them received only 300 mg/day of linezolid. Thus additional studies are needed to determine the potential efficient doses of this drug, while preventing the side effects [Chang K.C., *et al*., 2012; Bolhuis M.S., *et al*., 2013].



Linezolid



Sutezolid



AZD-5847

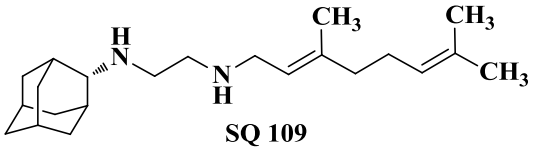
Sutezolid (PNU-100480), a new oxazolidinone developed by Pfizer is a close analogue of linezolid and demonstrated better activity *in vitro* than linezolid [Cynamon M.H., *et al*., 1999]. Pharmacokinetic data shows that sutezolid converts into sulfone and sulfoxide metabolites, the sulfoxide metabolite is more active and reaches four times higher in concentration than parent compound [Barbachyn M.R., *et al*., 1996]. Sutezolid has been tested in an EBA study at doses of either 600 mg twice a day or 1200 mg once a day. Results express that sutezolid led to significant reduction in log colony forming units (CFU) compared with both dosage options. Mouse model studies showed that addition of sutezolid to current first line TB drugs improved the bactericidal activity. It also gave better results when used in combination with moxifloxacin and pyrazinamide. These results suggest that sutezolid has the potential to reduce the treatment duration in both drug susceptible and drug resistant TB. Sutezolid is currently in phase-II clinical trials [Balasubramanian V., *et al*., 2014].

AZD 5847 (Posizolid) is another oxazolidinone drug in pipeline developed by AstraZeneca for the treatment of pulmonary TB [Wookey A., *et al*., 2004]. AZD 5847 binds to 50S

ribosomal subunit and blocks initiation of protein synthesis [Kwon Y.S., *et al*., 2014]. It is active against extracellular, intracellular and slowly and rapidly dividing mycobacteria in mouse models. In toxicological testing AZD 5847 has only minor haematological effects (decrease in RBCs and WBCs) but has no effect on bone marrow. Presently AZD 5847 is in phase II clinical studies [Wong E.B., *et al*., 2013].

2.2.2.4. SQ 109

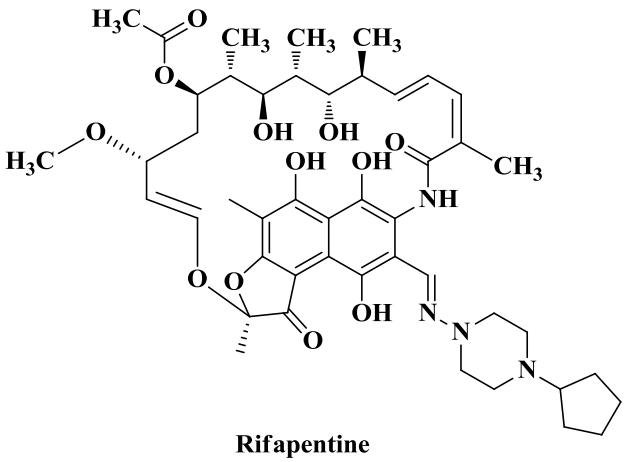
SQ 109 is a derivative of ethambutol and has activity against both drug sensitive and drug resistant-TB by targeting MmpL3 in *M. tuberculosis* their by causing inhibition of protein synthesis [Sacksteder K.A., *et al*., 2012]. The replacement of ethambutol with SQ 109 in standard regimen increased the efficacy in mouse model [Nikonenko B.V., *et al*., 2007]. It has MIC of 0.16-0.64 µg/mL and no cross resistance with ethambutol. SQ109 has synergetic effects with TMC-207 and favourable interactions with sutezolid *in vitro* [Reddy V.M., *et al*., 2010; Reddy V.M., *et al*., 2012]. In phase-I studies SQ 109 was proved to be safe and well tolerated in single doses up to 300 mg and currently it is in phase-II clinical studies [Sacksteder K.A., *et al*., 2012].



SQ 109

2.2.2.5. Rifapentine

Rifapentine, (cyclopentyl rifampin) is a long acting derivative of rifamycin which inhibits DNA-dependent RNA polymerase. Rifapentine exhibits bactericidal activity against both intracellular and extracellular *M. tuberculosis* organisms and has a better pharmacokinetic profile in mice. Human arylacetamide deacetylase metabolizes rifapentine into its major metabolite 25-O-deacetylrifapentine and it retains activity. The half-life and protein binding (13-14 hours and 97%) of rifapentine is much longer than rifampin (2-3 hours and 85%) [Chan J.G.Y., *et al*., 2014]. Rifapentine is marketed as PRIFTIN for oral administration and it contains 150 mg of active ingredient per tablet.



Rifapentine

2.2.2.6. Novel regimens in phase II trials

As the cleverest bacterium *M. tuberculosis* has been improving its resistance to anti-tubercular drugs day by day, there should be combination of drugs needed to invade *M. tuberculosis* rather than single potent compound. Apart from the potential individual anti-tubercular compounds and standard regimens, a search for combinations has been continuing for years. Recently there has been a set of novel combination of drugs which are efficient in simplifying TB therapy by reducing the treatment complexities such as long duration, acquired drug resistance and increased relapse rates etc., are in phase II clinical trials.

Current novel combinations of drugs in phase II clinical studies are:

NC-001: Moxifloxacin, PZA and PA-824

NC-002: Same regimen NC-001, testing in a two month trial.

NC-003: Bedaquiline, PA-824, clofazimine and PZA.

MAMS-TB-01: INH, RMP, PZA, EMB, moxifloxacin and SQ-109 [Zumla A., *et al*., 2014].

2.3. SAR and drug optimisation

2.3.1. SAR of rifamycins

Rifampicin is the first drug from the family of rifamycins. Although it is one of the most used first line anti-TB drug to treat drug sensitive TB, its usage was limited in case of drug resistant bacterial strains. In case of drug susceptible TB, rifampicin has to be taken for six months, such a long time leads to patient's not adherence which implified failing of treatment

as well as development of drug resistance. To overcome these difficulties rifampicin was structurally modified to get better compound which showed better activity for both drug susceptible and drug resistant TB strains and also to shorten present long treatment time. Rifabutine and rifapentine, the structural analogues of rifampicin are present generation rifamycins possessing high efficacy than rifampicin. Rifapentine is presently in phase III trials, the studies conducted in phase II shows that though it is more potent than rifampicin and active against MDR-TB, it possesses few side effects which was explained in previous section. There is a scope to develop even more potent compounds than rifapentine by thorough research on SAR of rifamycins core structure.

Based on the extensive research work on rifamycin scaffold SAR analysis shows that (**Figure 2.3**) [Aristoff P.A., *et al*., 2010]:

- Appropriate modifications are allowed at C3, C4 and C25 positions.
- Modifications at naphthalene group are possible without altering C8-hydroxyl group.
- The hydroxyl groups at C8, C21 and C23 must be present in order to retain its activity.
- Ring closure between C3 and C4 positions are more tolerated.

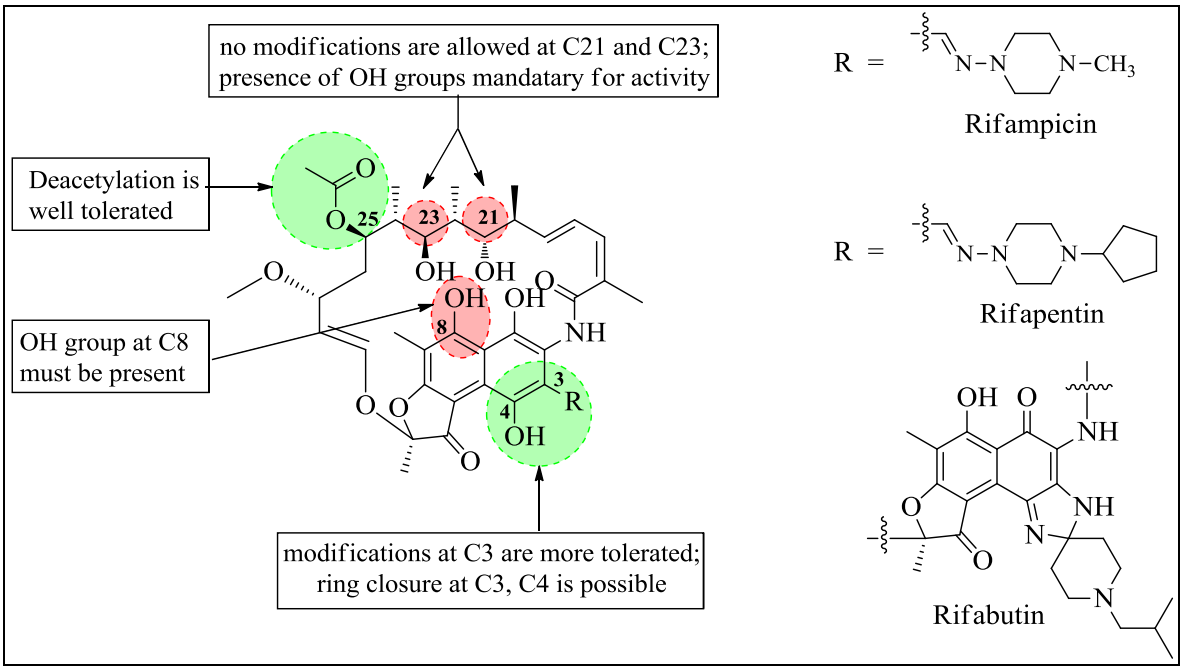


Figure 2.3: SAR of rifamycins

2.3.2. SAR of oxazolidinones

The three oxazolidinone drug candidates namely linezolid, sutezolid and AZD-5847 are presently in phase-II clinical trials. Linezolid was first drug in this class, which possessed activity (MIC<1µg/mL) against both drug sensitive and drug resistant forms of TB [Barbachyn M.R., *et al.*, 2003; Tokuyama R., *et al.*, 2001]. It was associated with few side effects due to which it cannot be used for long time therapy especially in patients infected with HIV. The next drug in this class is sutezolid, which is obtained by replacing oxygen atom in morpholine ring of linezolid with sulphur atom. The SAR worked well and increased the activity of sutezolid (MIC = 0.125 µg/mL) than parent linezolid. Sutezolid was well tolerated than linezolid at a dosage of 1000 mg [Wallis R.S., *et al.*, 2011]. Another oxazolidinone drug AZD-5847 was obtained by few modifications on core structure: i) replacement of morpholine ring with (S)-1-(5,6-dihydropyridin-1(2*H*)-yl)-2,3-dihydroxypropan-1-one, ii) di-substitution of fluorine atom on aromatic ring, and iii) replacement of acylamino group with isoxazol-3-yloxy (**Figure 2.4**). Recent literature showed that its *in vitro* efficacy was similar to that of linezolid (MIC against H37Rv = 1µg/mL) and it is currently in phase-IIa trials [Balasubramanian V., *et al.*, 2014].

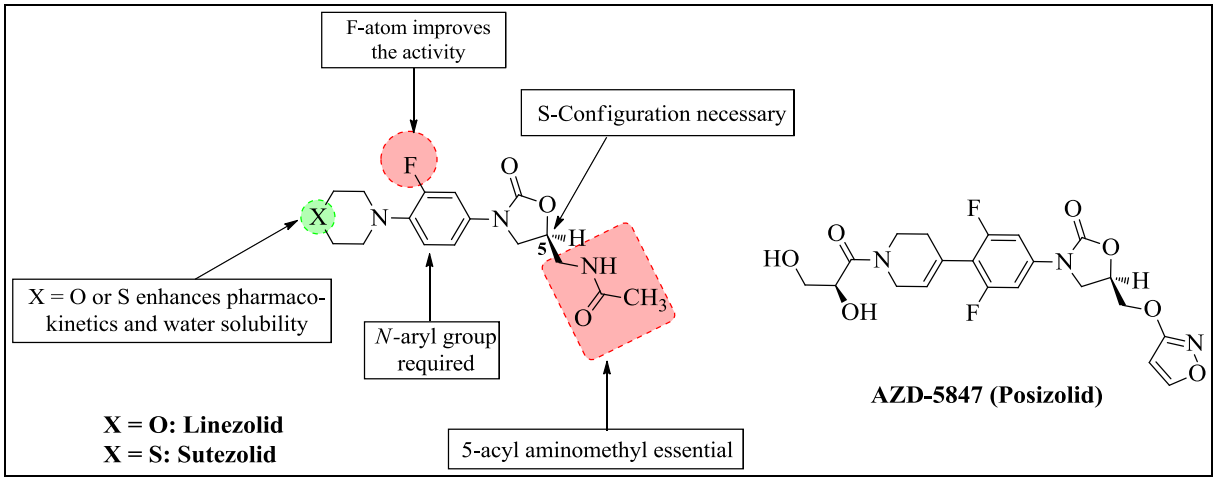


Figure 2.4: SAR of oxazolidinones

- S-configuration at 5th position of oxazolidinone ring is required for activity (**Figure 2.4**);
- Presence of acylaminomethyl at 5th position is necessary (sutezolid has better MIC than AZD-5847 which doesn't have acylaminomethyl group);
- Electron withdrawing substitutions on aromatic ring allowed.

2.3.3. SAR of fluoroquinolones

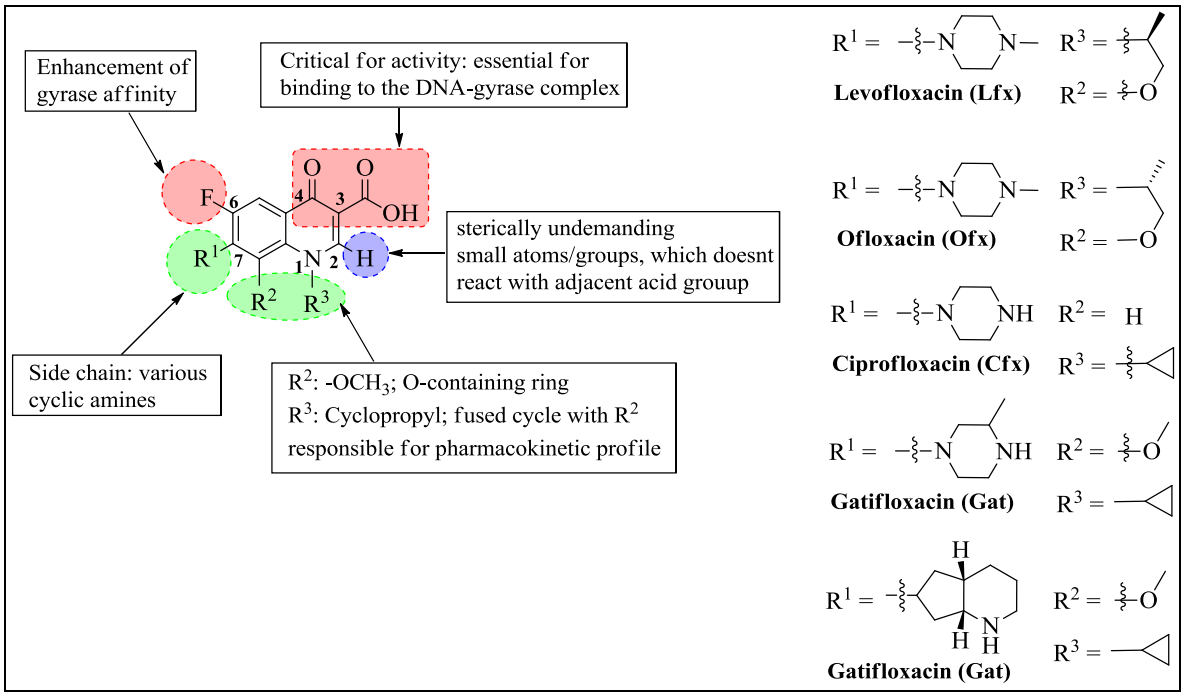


Figure 2.5: SAR of fluoroquinolones

As delineated in **Figure 2.5**, the SAR of fluoroquinolones could be summarized as,

- The presence of carboxylic acid group at C3 and carbonyl group at C4 are responsible and essential for the binding with DNA gyrase, hence no modifications are allowed at these positions.
- The suitable groups at C2 are small atoms, H-atom is preferred. Large groups causes steric crowd which may interfere with adjacent carboxylic acid group (**Figure 2.5**).
- The fluorine atom at C6 is essential for the activity, increases affinity for the targeted enzyme
- Modifications at C7 position are allowed, substituents at this position responsible for both pharmacokinetic profile and activity. Best groups are cyclic five or six membered rings containing nitrogen atoms.

2.3.4. SAR of nitroimidazoles

The interesting point in this class of compounds is that, they have the ability to inhibit anaerobic bacteria; hence they can be used for treating latent TB infection. CGI-17341 is the most active compound in this class, but due to its mutagenicity it has not developed [Ashtekar

D.R., *et al*., 1993]. Further studies showed that this problem could be overcome by introduction of side chain at 2nd position of oxazole ring. Based on these SAR findings new series of nitroimidazoles were developed.

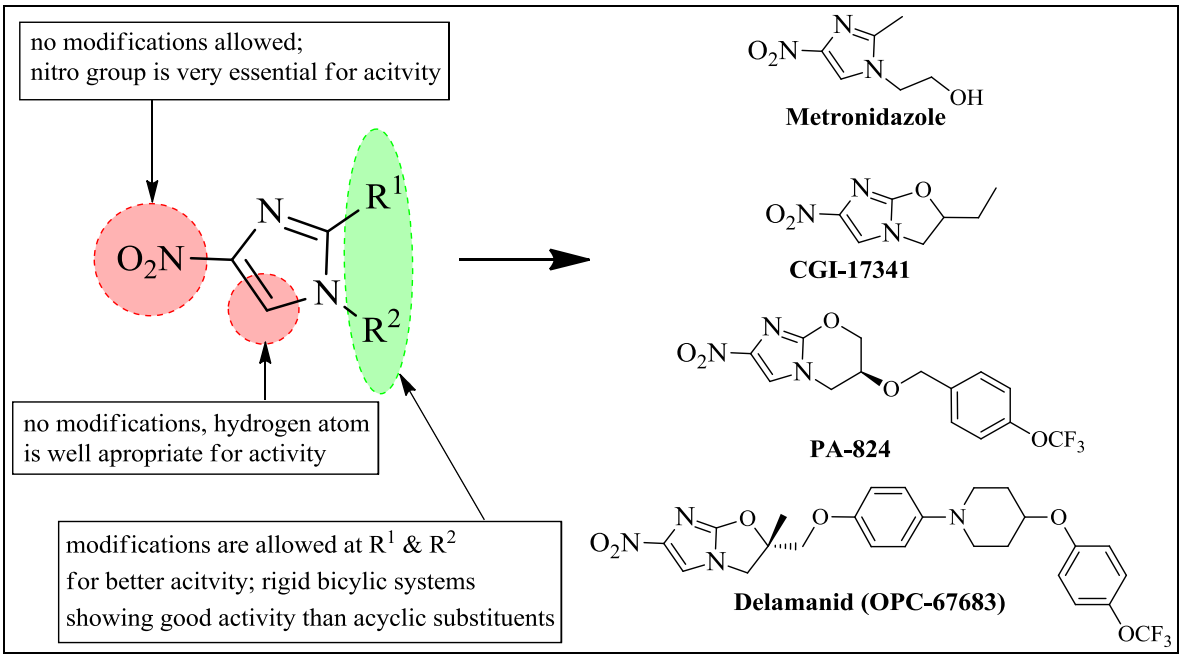
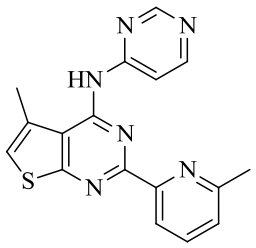


Figure 2.6: SAR of nitroimidazoles

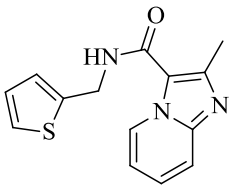
- The nitro group on imidazole nucleus is essential for anti-bacterial activity (**Figure 2.6**);
- Rigid bicyclic system is required, open ring compounds are not active;
- Replacement of oxygen atom in bicyclic system with methylene group causes loss of activity, whereas sulphur and nitrogen atoms are tolerates.

2.4. Other promising anti-TB drugs

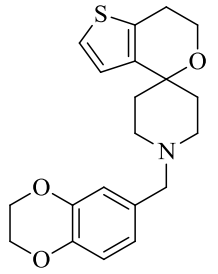
To invade the growing TB infection, researchers at GlaxoSmithKline and others have reported most promising drug families, from high throughput screening of their corporate compound libraries (>2 × 10^6 chemical entities) against mycobacteria. The most effective drugs with their MICs and code numbers are presented below [Ballell L., *et al.*, 2013].



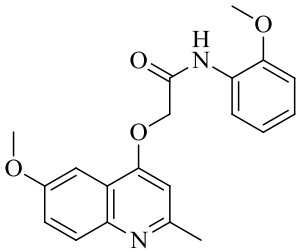
GSK163574A
MIC H37Rv: 0.76 μM



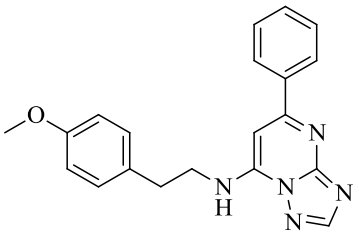
GSK1829820A
MIC H37Rv: 0.19 μM



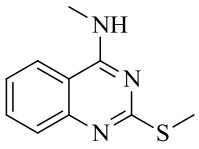
GSK2200150A
MIC H37Rv: 0.38 μM



GSK358607A
MIC H37Rv: 0.70 μM

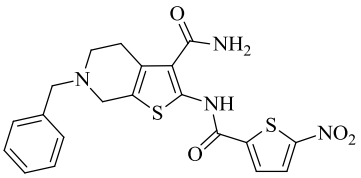


GSK888636A
MIC H37Rv: 0.94 μM

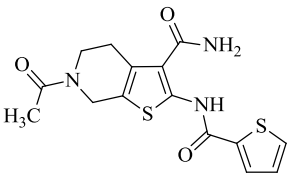


GSK353069A
MIC H37Rv: 0.13 μM

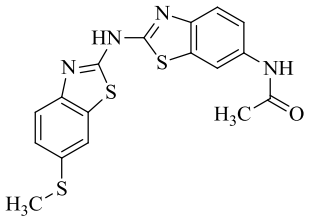
Robert C Reynolds and co-workers [Reynolds R.C., *et al.*, 2012] reported high throughput screening results of a total of 25,671 compounds against *M. tuberculosis* in a single dose assay at a concentration of 10 μg/mL. Out of these, 1,329 compounds were found to be active based on their ability to inhibit growth of organism by ≥ 85%. These active compounds were further evaluated in a dose-response format against *M. tuberculosis* where 584 compounds possessed *M. tuberculosis* IC_{90} values of < 10 μg/mL. The structures of most potent and non-cytotoxic compounds are shown below:



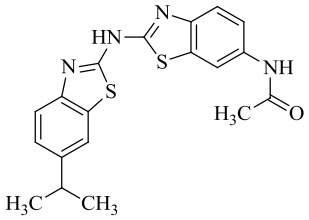
IC_{90} = 0.5 µg/mL
SI = 12



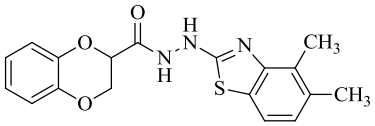
IC_{90} = 3.2 µg/mL
SI = 13



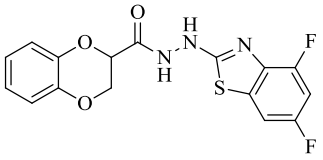
IC_{90} = <0.2 µg/mL
SI > 172



IC_{90} = 2.0 µg/mL
SI = 8.8

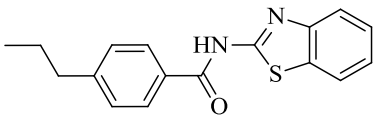


IC_{90} = 0.9 µg/mL
SI = 14

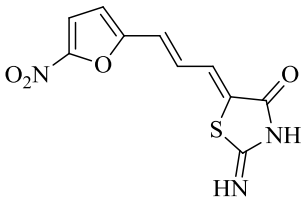


IC_{90} = 3.3 µg/mL
SI > 12

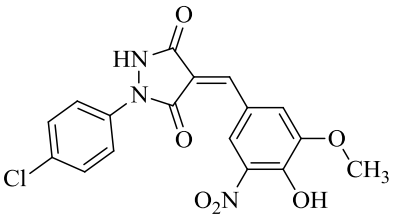
Catherine Vilcheze *et al*., [Vilcheze C., *et al*., 2011] were screened 300 compounds against *M. tuberculosis* H37Rv cell line. Out of these compounds, 11 compounds were found to be active by inhibiting the growth of *M. tuberculosis.* Out of which, four compounds (CD 13, CD39, CD59 and CD113) showed MICs below 10 µM are presented below.



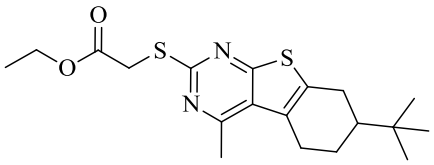
CD13
MIC H37Rv = 8.5 µM



CD39
MIC H37Rv = 9.0 µM

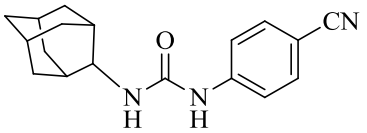


CD59
MIC H37Rv = 1.5 µM

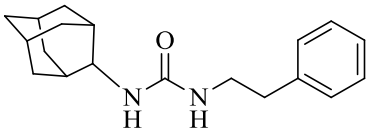


CD117
MIC H37Rv = 1.0 µM

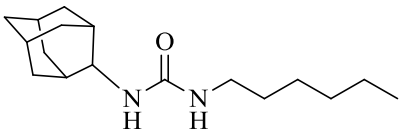
Joshua R. Brown and co-workers, reported synthesis of adamantyl urea derivatives and evaluated for *in vitro* anti-mycobacterial activity against *M. tuberculosis*. Among the synthesized compounds the following compounds showed MICs of < 1 µg/mL [Brown J.R., *et al*., 2011].



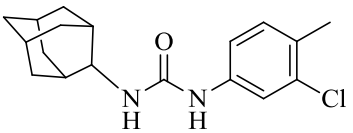
MTB MIC = 0.4 µg/mL



MTB MIC = 0.01 µg/mL

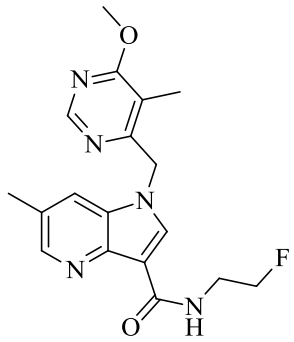


MTB MIC = 0.01 µg/mL

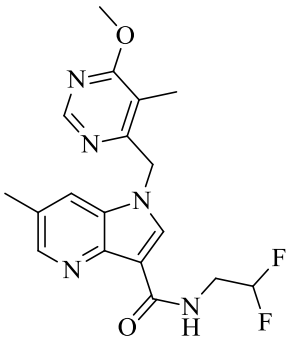


MTB MIC = 0.02 µg/mL

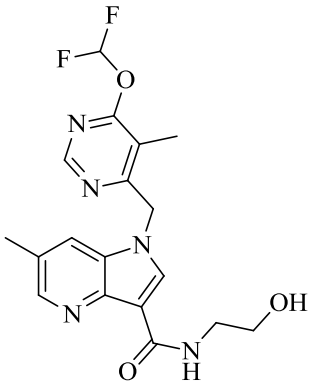
Pravin S. Shirude and team have recently reported synthesis of 1,4-azaindoles and evaluated for *in vitro* anti-mycobacterial activity against *M. tuberculosis* H37Rv. Among a total of 37 molecules, the most active molecules have the following structures [Shirude P.S., *et al.*, 2012].



MTB MIC = 0.39 µM

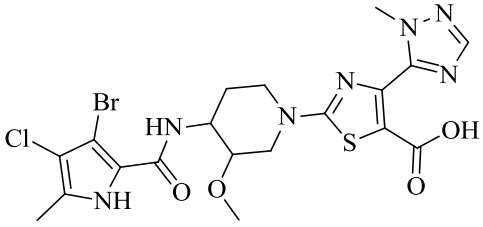


MTB MIC = 0.39 µM



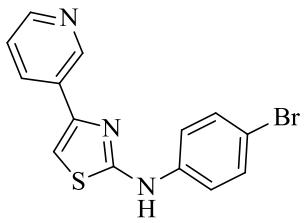
MTB MIC = 0.39 µM

David E. Ehmann and Sushmita, [Ehmann D.E., *et al.*, 2014] reported the following molecule as potential inhibitor for bacterial DNA topoisomerase with MIC of 0.25 µM. DNA topoisomerases are the essential enzymes for bacterial survival as they involve in the regulation of DNA over winding or underwinding.



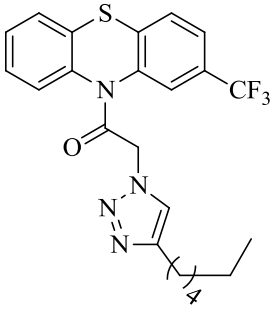
MTB MIC = 0.25 µM

Parameshwar M and Tharanikkarasu K, have published synthesis of 2-aminothiazoles starting with substituted acetophenones and evaluated for their anti-mycobacterial activity against *M. tuberculosis* H37Rv. Among the reported 34 molecules, compound **7m** emerged as the most active compound with MIC of 6.25 µM. Compound **7m** also possessed, three H-acceptors, one H-donor, molecular weight of 332.2, logP of 4.34 and three freely rotatable bonds making zero violations to Lipinski rule [Parameshwar M., *et al.*, 2014].

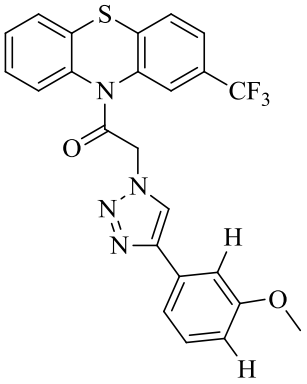


Compound **7m**
MTB MIC = 6.25 µM

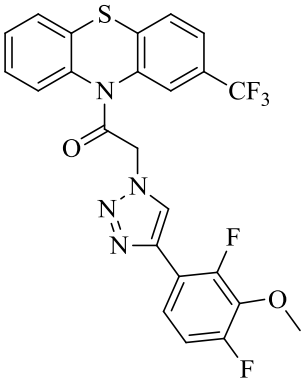
Dinesh Addla and his co-workers [Addla D., *et al.*, 2014] synthesized 2-(trifluoromethyl) phenothiazine-[1,2,3]triazole hybrids from 2-(trifluoromethyl)-10H-phenothiazine in three steps and evaluated for their anti-tubercular activity. Among the 18 molecules, the following molecules emerged as the most active compounds by inhibiting *M. tuberculosis* H37Rv with MICs of 6.25 µg/mL.



MTB MIC = 6.25 µg/mL



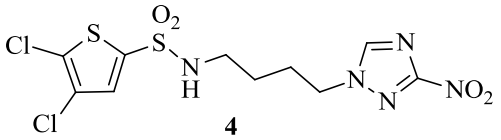
MTB MIC = 6.25 µg/mL



MTB MIC = 6.25 µg/mL

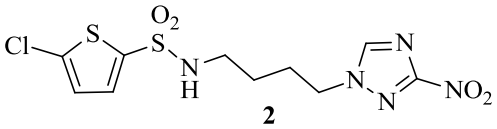
Recently, Maria V. Papadopoulou *et al.*, [Papadopolou M.V., *et al.*, 2014] have reported anti-mycobacterial screening results of nitrotriazole and imidazole based amides and sulphonamides. Among the series of molecules, top two active molecules had the below structures. 4,5-Dichloro-*N*-(4-(3-nitro-1*H*-1,2,4-triazol-1-yl)butyl)thiophene-2-sulfonamide (**4**) and 5-chloro-*N*-(4-(3-nitro-1*H*-1,2,4-triazol-1-yl)butyl)thiophene-2-sulfonamide (**2**) demonstrated 50% inhibitory concentration (IC_{50}), IC_{90} and MIC values of 0.38, 0.43 and

1.56 µM (compound **4**), 0.57, 0.98 and 3.13 µM (compound **2**) respectively. These molecules could acts as potential leads for further development.



4

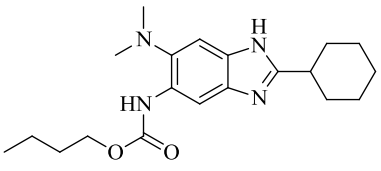
IC_{50} = 0.38 µM
IC_{90} = 0.43 µM
MTB MIC = 1.56 µM



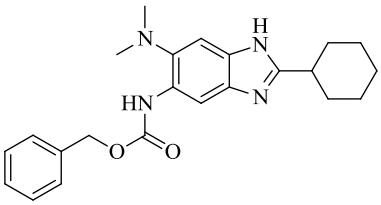
2

IC_{50} = 0.57 µM
IC_{90} = 0.98 µM
MTB MIC = 3.13 µM

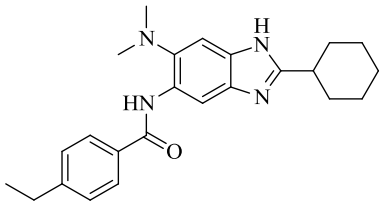
Divya Aswathi and Iwao Ojima, reported synthesis and *M. tuberculosis* H37Rv screening results of 63 new trisubstituted benzimidazoles. The synthesized molecules were studied their SAR, based on the screening data of 587 compounds against H37Rv cell line. Out of these 587 compounds, 81 hit compounds have been found to be possessing MIC less than 5 µg/mL. Further screening at lower concentrations revealed 11 hit compounds possessing MIC of 0.39-6.1 µg/mL. Based on top most four molecules, SAR was built and further synthesized 63 more molecules. The compounds that emerged as the most active are represented as follows [Aswathi D., *et al.,* 2013]:



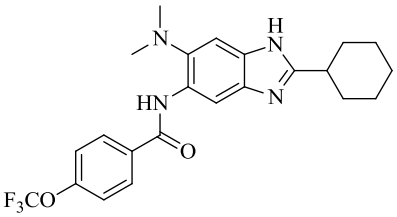
MTB MIC = 0.06 µg/mL



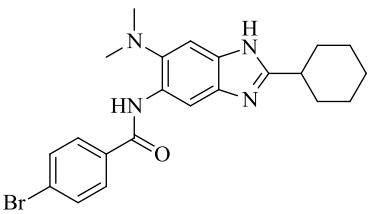
MTB MIC = 0.16 µg/mL



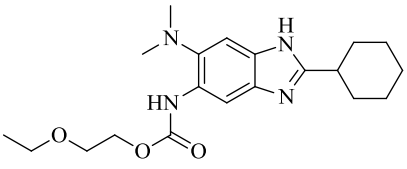
MTB MIC = 0.16 µg/mL



MTB MIC = 0.16 µg/mL

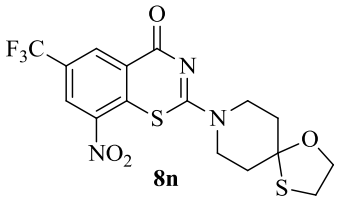


MTB MIC = 0.31 µg/mL



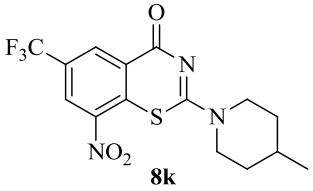
MTB MIC = 0.31 µg/mL

Chao Gao, et al., studied the anti-mycobacterial activity of *N*-alkyl and heterocycles substituted 1,3-benzothiazin-4-one derivatives. Among the compounds, compound **8o**, which contained an azaspirodithiolane group, showed MIC of 0.0001 µM against *M. tuberculosis* H37Rv, and it was also non-toxic to Vero cells. SAR analysis evinced that extended or branched alkyl chain could enhance the potency of *N*-alkyl substituted 1,3-benzothiazin-4-ones [Gao C., *et al.,* 2013].



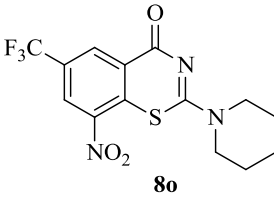
8n

MTB MIC = 0.002 μM



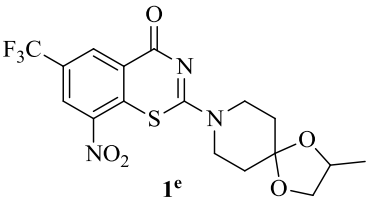
8k

MTB MIC = 0.08 μM



8o

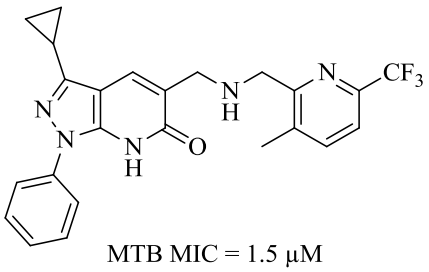
MTB MIC = 0.001 μM



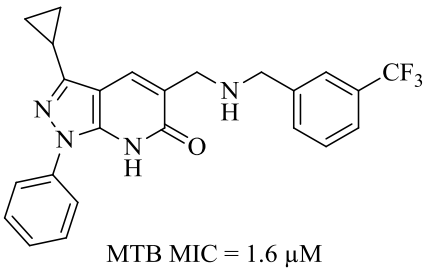
1[e]

MTB MIC = 0.002 μM

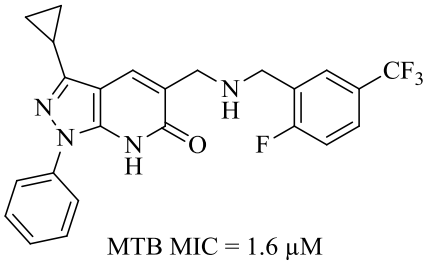
Manoranjan Panda *et al.*, from AstraZeneca-India, published the synthesis and anti-mycobacterial activity of pyrazolopyridones. The most active compounds from their study have the following structures [Panda M., *et al.*, 2014].



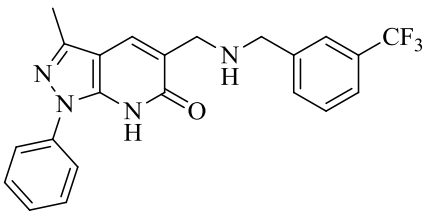
MTB MIC = 1.5 μM



MTB MIC = 1.6 μM

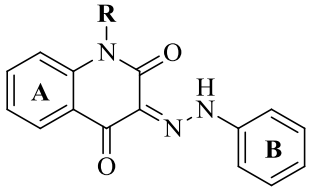


MTB MIC = 1.6 μM



MTB MIC = 3.1 μM

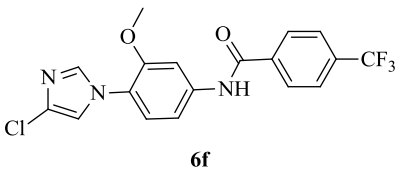
Atul Manvar *et al.*, [Manvar A., *et al.*, 2013] synthesized 79 new quinolylhydrazides and evaluated for anti-tubercular activity. Out of these 79 compounds, 40 compounds exhibited MICs of <6.25 µg/mL against *M. tuberculosis* H37Rv strains.



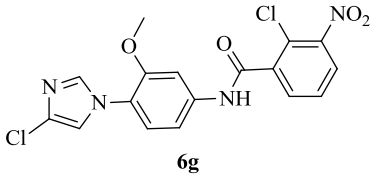
Compounds **4** to **44** MIC <6.25 µg/mL

R = H and CH_3; ringa A, B = various *o,m,p*- and mono, di-substituted benzenes

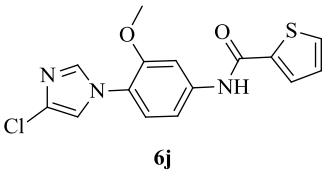
Pakkath Karuvalam Ranjith and co-workers [Ranjith P.K., *et al.*, 2014], reported synthesis and anti-tubercular activity of *N*-(4-(4-chloro-1*H*-imidazol-1-yl)-3-methoxyphenyl)amide/sulphonamide derivatives. Among the amides (**6a-o**), three molecules **6f**, **6g** and **6j** were found to be more active with *M. tuberculosis* MIC (H37Rv) of 1 µg/mL and among the sulphonamides (**7a-o**) three molecules **7d**, **7f** and **7h** were found to be more active with *M. tuberculosis* MIC (H37Rv) of 1 µg/mL.



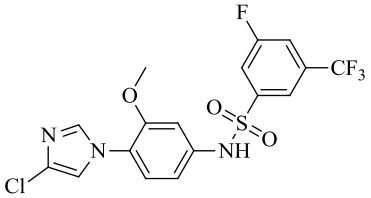
6f



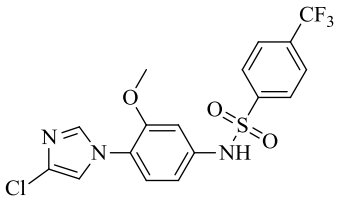
6g



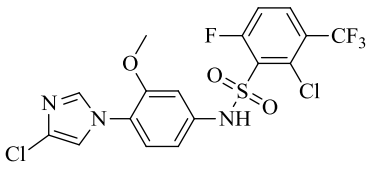
6j



7d



7f



7h

Objectives and Plan of Work

Chapter 3

3.1. Objective

After in-depth literature review of existing and new promising anti-tubercular drugs, we concluded that lot more work can be done in developing better anti-tubercular agents having superior qualities over the existing ones in terms of potency against drug resistant bacteria.

Hence the main objectives of the proposed research are as follows:

1. To design molecules based on reported anti-tubercular leads.

2. To synthesize the designed molecules.

3. To undertake *in vitro* anti-mycobacterial screening of the synthesized compounds against *M. tuberculosis.*

4. To evaluate the inhibitory potency of the synthesized compounds by *in vitro* enzyme inhibition assays.

5. To evaluate *in vitro* cytotoxicity of the synthesized compounds.

6. To study the protein inhibitor interaction using biophysical methods.

3.2. Plan of work

The plan of work was classified into following categories:

3.2.1. Design of anti-tubercular agents

For designing the new anti-tubercular agents we followed two approaches:

1. *Molecular hybridisation strategy*

2. *Molecular derivatization strategy*

3.2.2. Synthesis and characterization of designed molecules

Synthesis: The molecules designed with either of the above approaches were taken up for synthesis in our laboratory using previously reported methodologies available in literature for structurally related molecules. Wherever possible we carried out reactions using microwave assisted methods for less exposure of hazardous chemicals/vapours to the environment. Most of the synthesized molecules were purified by trituration, recrystallization techniques and flash chromatography with lesser amount of solvents for eco-friendly conditions.

Characterization: characterization of the synthesized compounds were carried out by ^{1}H NMR, ^{13}C NMR, LC-MS and elemental analyses.

3.2.3. *In vitro* anti-mycobacterial activity against *M. tuberculosis* H37Rv

The *in vitro* anti-mycobacterial screening of the synthesized compounds were carried out against *M. tuberculosis* H37Rv bacteria. This test was performed using micro plate alamar blue assay (MABA) method.

3.2.4. Enzymatic evaluation of synthesized compounds

The synthesized compounds were subjected to various enzyme inhibition studies namely *M. tuberculosis* pantothenate synthetase (PS), *M. tuberculosis* alanine dehydrogenase (ADH) and *M. tuberculosis* lysine amino transferase (LAT).

3.2.5. *In vitro* cytotoxicity screening

The synthesized compounds were evaluated *in vitro* for their cytotoxic activity using 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) reduction assay method.

3.2.6. To study the protein interaction and stability using biophysical method

The enzyme interaction and stability of representative compounds were assessed biophysically by performing differential scanning fluorimetry experiment.

Materials and Methods

Chapter 4

4.1. Design of the molecules

For design of the new anti-TB molecules we followed two approaches:

1. *Molecular hybridisation strategy*: It is a strategy of rational design of new ligands or prototypes based on the recognition of pharmacophoric sub-units in the molecular structure of two or more known bioactive derivatives which, through the adequate fusion of the sub-units, led to the design of new hybrid architectures that maintained pre-selected characteristics of the original templates.

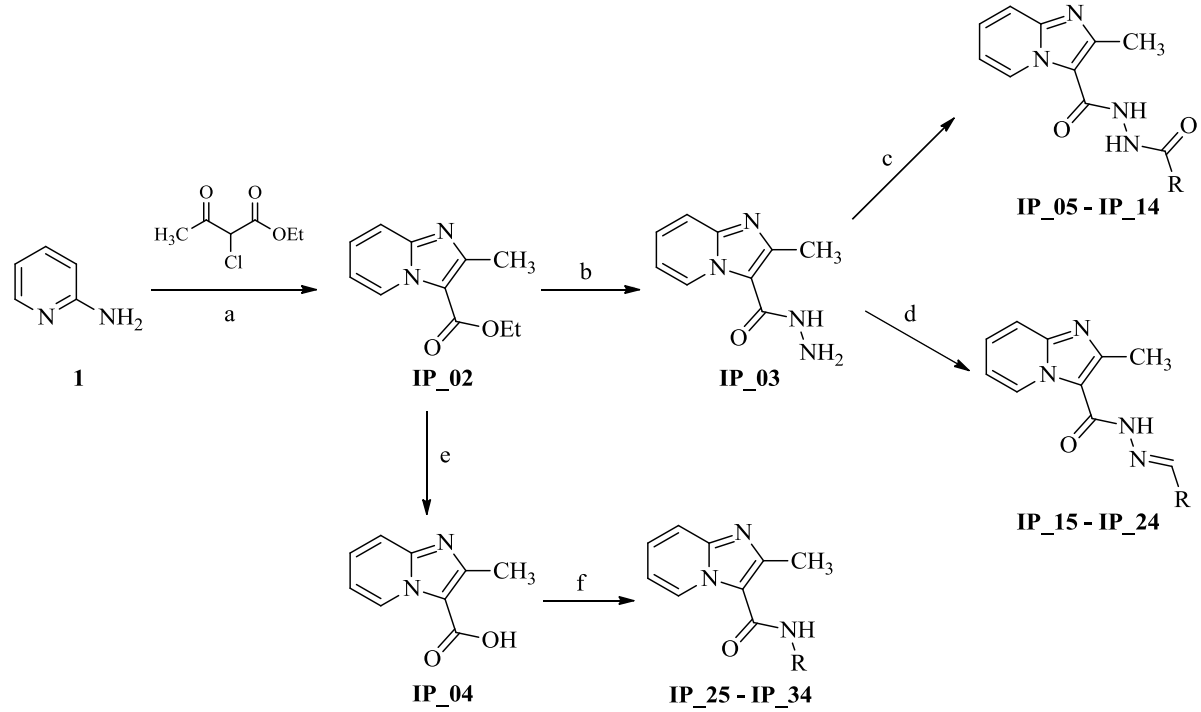
2. *Molecular derivatization strategy*: A library was designed based on our previous research experience in TB, in an effort to improve the potency of reported anti-tubercular compounds. We utilized these reported potent molecules as structural framework to construct a library for developing strong SAR.

4.2. Chemistry and methodology

Reagents and solvents obtained from commercial sources were used without further purification. All the reactions were monitored by thin layer chromatography (TLC) on silica gel 40 F_{254} (Merck, Darmstadt, Germany) coated on aluminium plates. All ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AM-400/300 MHz and 100/75 MHz spectrometer, Bruker Bio Spin Corp., Germany. Chemical shifts are reported in parts per million (ppm) using tetramethyl silane (TMS) as an internal standard. Compounds were purified by Biotage Isolera flash chromatography. Temperatures were reported in degrees celsius and are uncorrected. Compounds were analysed for C, H, N using Elementar and analytical results obtained were found within ±0.4% of the calculated values for the formula shown. Molecular weights of the synthesized compounds were checked by Shimadzu, LCMS-2020 and the method used was electron spray ionisation (ESI-MS) method.

4.2.1. Synthesis of the designed molecules

Scheme – 1: Synthesis of 2-methylimidazo[1,2-a]pyridine-3-carboxylic acid derivatives as novel anti-tubercular agents



Reagents & Conditions: a: 1,2-Dimethoxyethane, 90 °C, 6 h; b: 35% $N_2H_4.H_2O$, Ethanol, reflux, 3 h; c: RCOOH, EDCI, HOBt, Et_3N, CH_2Cl_2, 0 °C - rt, 3 h; d: RCHO, Ethanol, reflux, 3 to 60 min; e: LiOH, Ethanol/H_2O (1:1), rt, 6 h; f: RNH_2, EDCI, HOBt, Et_3N, CH_2Cl_2, 0 °C - rt, 4 h.

Figure 4.1: Synthetic protocol utilized for the synthesis of compounds **IP_05 – IP_34**

Scheme – 2: Synthesis of 6-methylimidazo[2,1-b]- thiazole-5-carboxylic acid derivatives as novel anti-tubercular agents

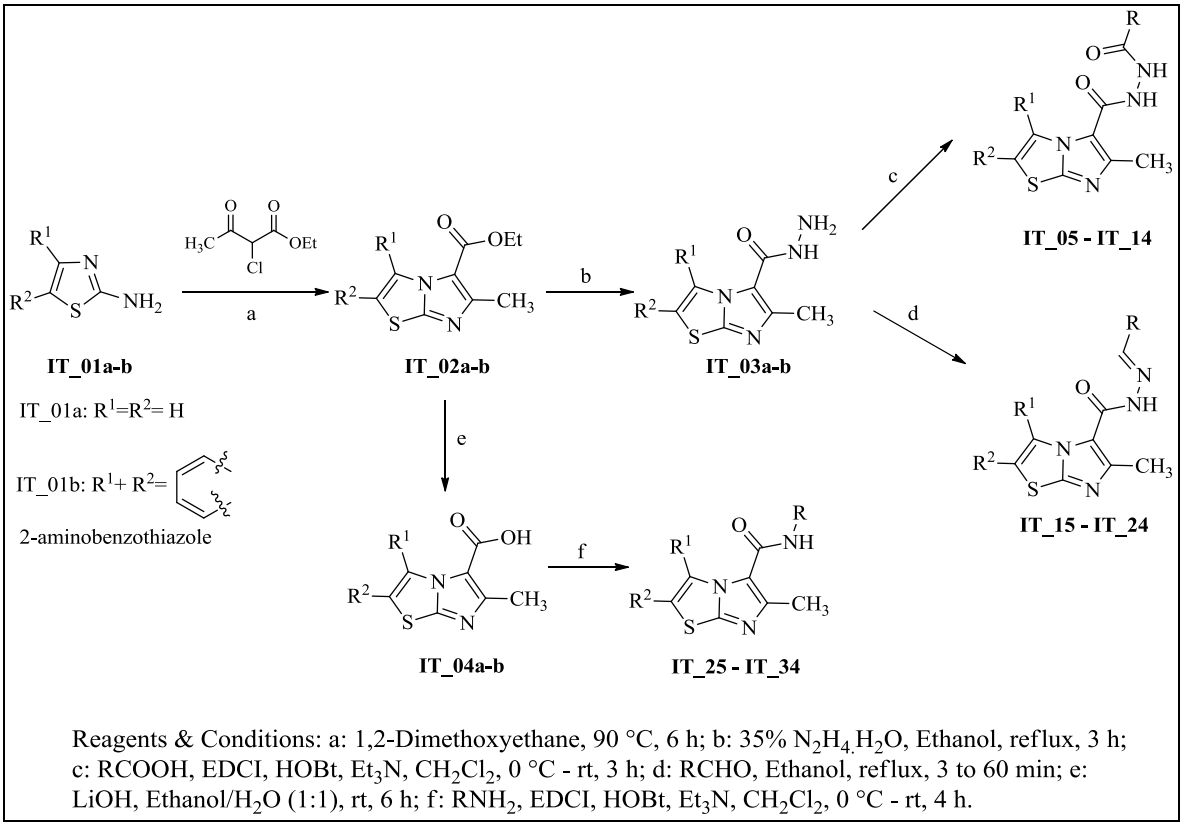


Figure 4.2: Synthetic protocol utilized for the synthesis of molecules **IT_05 – IT_34**

Scheme – 3: Synthesis of 5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4-amine derivatives as novel anti-tubercular agents

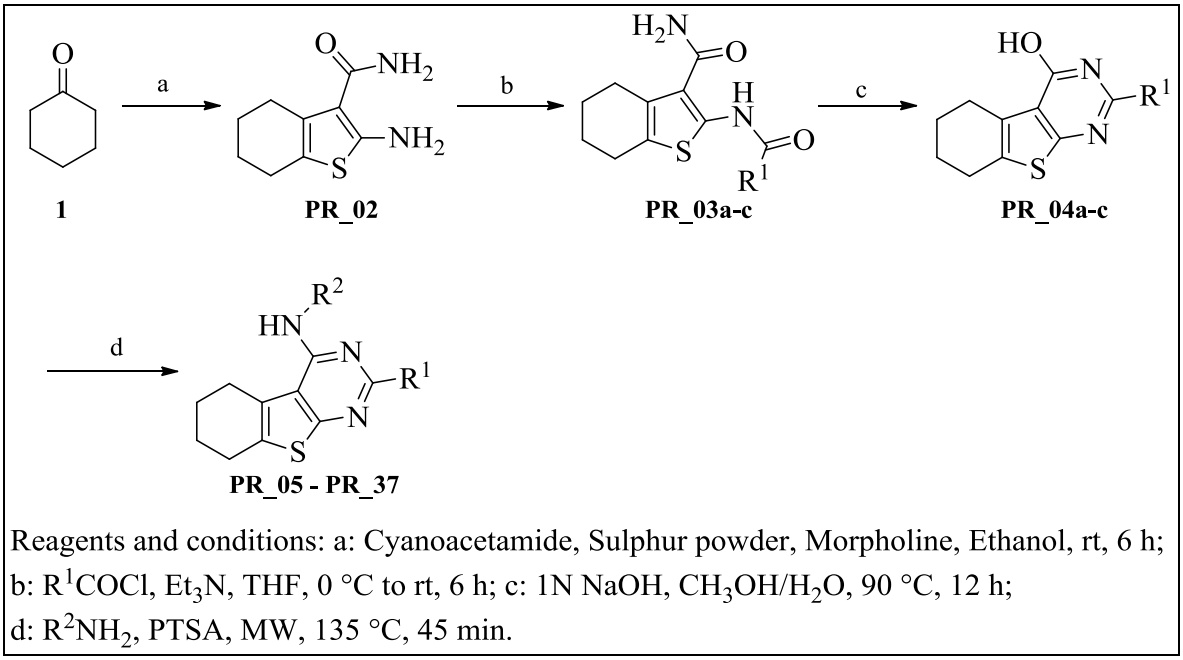


Figure 4.3: Synthetic protocol utilized for the synthesis of molecules **PR_05 – PR_37**

Scheme – 4: Synthesis of 3-phenyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridine derivatives as novel anti-tubercular agents

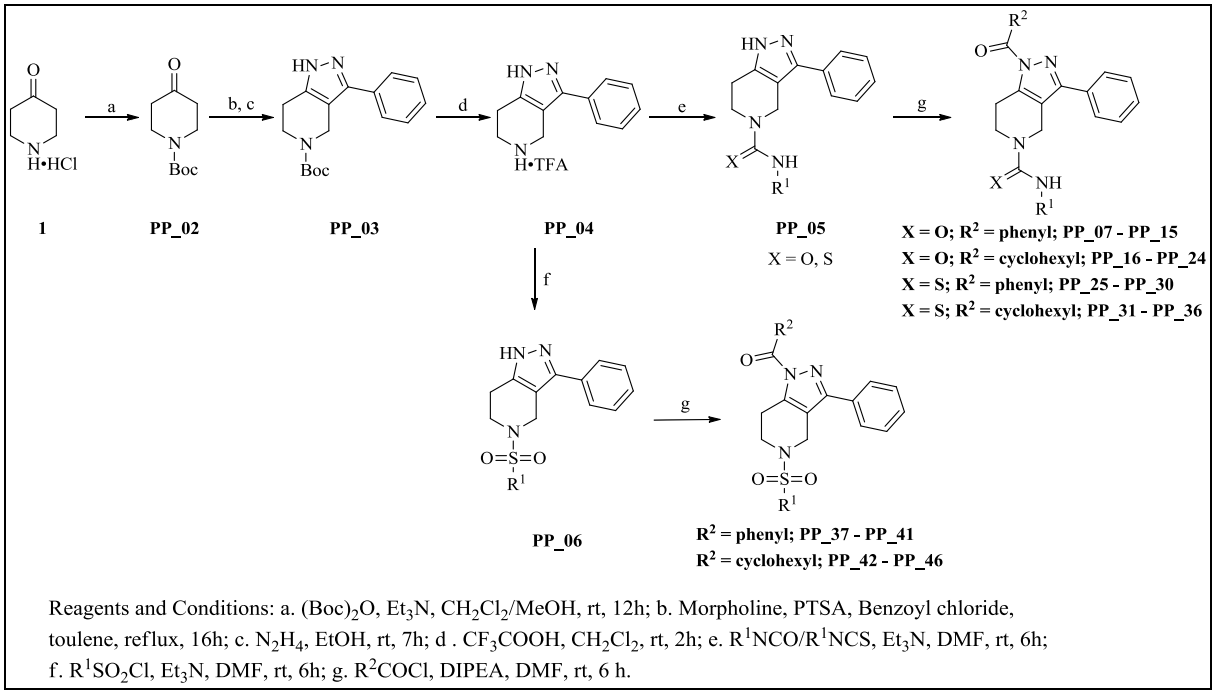


Figure 4.4: Synthetic protocol utilized for the synthesis of compounds **PP_07 – PP_46**

Scheme – 5: Synthesis of tetrahydrothieno[2,3-c]pyridine-3-carboxamide derivatives as novel anti-tubercular agents

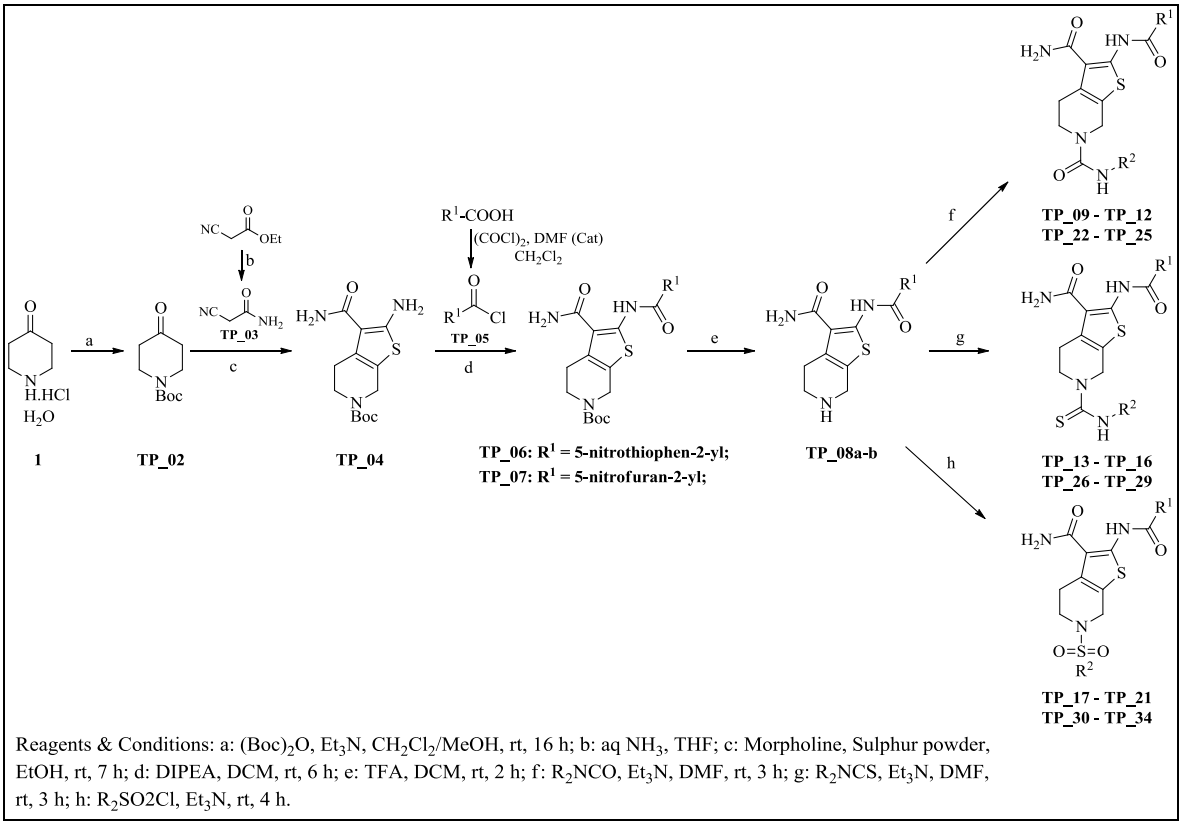


Figure 4.5: Synthetic protocol utilized for the synthesis of compounds **TP_09 – TP_34**

Scheme – 6: Synthesis of pyrazolidine-3,5-dione derivatives as novel anti-tubercular agents

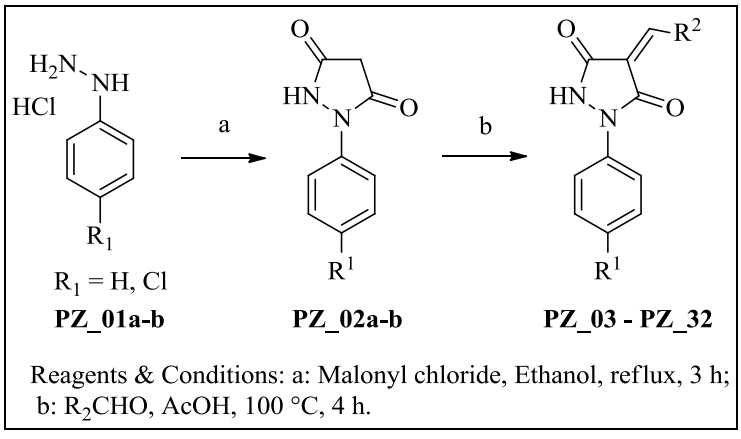


Figure 4.6: Synthetic protocol utilized for the synthesis of compounds **PZ_03 – PZ_32**

Scheme – 7: Synthesis of 2-iminothiazolidine-4-ones as novel anti-tubercular agents

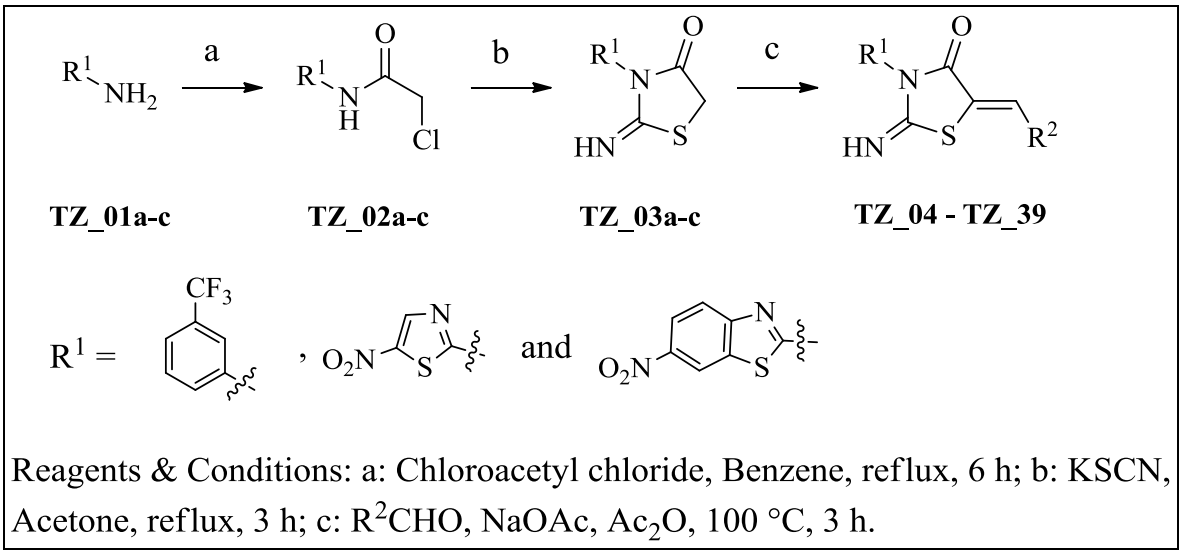


Figure 4.7: Synthetic protocol utilized for synthesis of compounds **TZ_04 – TZ_39**

4.3. Biological screening

4.3.1. *In vitro* anti-mycobacterial screening

***In vitro M. tuberculosis* by MABA assay**

All the synthesized compounds were evaluated for anti-mycobacterial screening as per previously reported procedure [Franzblau S.G. *et al.*, 1998]. Briefly, the inoculum was prepared from fresh LJ medium re-suspended in 7H9-S medium (7H9 broth, 0.1% casitone, 0.5% glycerol, supplemented oleic acid, albumin, dextrose, and catalase [OADC]), adjusted to a McFarland tube No. 1, and diluted 1:20; 100 µl was used as inoculum. Each drug stock solution was thawed and diluted in 7H9-S at four-fold the final highest concentration tested. Serial two-fold dilutions of each drug were prepared directly in a sterile 96-well microtiter plate using 100 µl 7H9-S. A growth control containing no antibiotic and a sterile control were also prepared on each plate. Sterile water was added to all perimetre wells to avoid evaporation during the incubation. The plate was covered, sealed in plastic bags and incubated at 37 °C in normal atmosphere. After 7 days incubation, 30 µL of alamar blue solution was added to each well, and the plate was re-incubated overnight. A change in colour from blue (oxidised state) to pink (reduced) indicated the growth of bacteria, and the MIC was defined as the lowest concentration of drug that prevented this change in colour. Each reaction was carried out in triplicates.

4.3.2. Cytotoxicity

All the synthesized compounds were further examined for cytotoxicity in a RAW 264.7 cell line at concentrations of 50 and/or 100 µM. After 48 h of exposure, viability was assessed on the basis of cellular conversion of MTT into a formazan product using the Promega Cell Titer 96 non-radioactive cell proliferation assay [Gerlier D. and Thomasset N., 1986]. We selected this macrophage cell line to test the toxicity as naturally *M. tuberculosis* resides inside the macrophages and the drug molecules should not possess any toxicity against these macrophages. The RAW 264.7 cells were grown in RPMI medium supplemented with 10 % fetal bovine serum (FBS), 10,000 units' penicillin and 10 mg streptomycin per ml in T25 flasks to attain 80-90 % confluence. Cells were scraped and seeded into wells as 5,000 cells per well in poly-L-lysine coated plates. The microtiter plates were incubated at 37 °C, 5 % CO_2, 95 % air and 100 % relative humidity for 24 h prior to addition of synthesized compounds. Each compound at 50 and/or 100 µM concentration was then added to cells and incubated at 37 °C for 72 h; later 10 µL of 10 mg/ml concentration of MTT was added and incubated for 3 h at 37 °C. At the end of incubation formazan crystals were formed, the media from microtiter plates were removed. Later, the bound crystals were subsequently dissolved by adding 100 µL DMSO. Absorbance was then read on plate reader at a wavelength of 595 nm. The percent growth was calculated for each well relative to the control wells. The percentage inhibition was calculated from the following formula:

$$\text{Percentage inhibition} = \frac{100 - \text{mean OD sample}}{\text{mean OD day 0}}$$

4.3.3. *M. tuberculosis* Alanine dehydrogenase (ADH) assay

To each well of a 96-well plate, reaction mixture consisted of 125 mM glycine/KOH (pH 10.2), 100 mM L-alanine, 1.25 mM NAD^+ and 6.026 pM of *M. tuberculosis* ADH in a final volume of 200 µL diluted in 125 mM glycine/KOH (pH 10.2). Compounds were then added to plates. Reaction was initiated with the addition of 10 µL of enzyme diluted in buffer. Enzymatic activity was measured by the rate of production of NADH that accompanies the conversion of alanine to pyruvate by oxidative deamination. The reaction components, except *M. tuberculosis* ADH, were mixed in the well and the background reaction was measured; *M. tuberculosis* ADH was then added and the reaction kinetics was monitored. All

measurements were performed at 340 nm with heat-controlled Perkin Elmer Victor V3 spectrophotometer [Tripathi S.M., *et al*., 2008].

4.3.4. *M. tuberculosis* Lysine aminotransferase (LAT) assay

To each well of a 96-well plate, reaction mixture consisted of 1 mM L-lysine-HCl, 1 mM α-ketoglutarate and 15 μM of pyridoxal-5'-phosphate and 1.25 pM of *M. tuberculosis* LAT in a final volume of 200 μL diluted in 200 mM phosphate buffer (pH 7.2). Compounds were then added to plates. The reaction was initiated with the addition of 10 μL of *M. tuberculosis* LAT, diluted in buffer. The mixture was incubated at 37 °C for 1 h. The reaction was terminated by addition of 10% trichloroacetic acid in ethanol. Piperideine 6-carboxylate (P6C) was detected by measuring the colour intensity of its adduct with 2-aminobenzaldehyde spectroscopically at 465 nm. The reaction components except *M. tuberculosis* LAT were mixed in the well and the background reaction was measured; *M. tuberculosis* LAT was then added and the reaction kinetics was monitored. Reactions were carried out at 37 °C in a heat-controlled Perkin Elmer Victor V3 Spectrophotometer. One LAT unit (1 U) is the activity which produces 1 μM of P6C per min under these conditions [Tripathi S.M., *et al.*, 2006].

4.3.5. *M. tuberculosis* Pantothenate synthetase (PS) assay

The *M. tuberculosis* PS gene (Rv3602c) encoding the pantothenate synthetase was cloned and transformed into BL21 (DE3) cells and the expression of the protein was performed as reported in literature. For the assay, in a 96-well plate, 60 μL of PS reagent mix containing NADH, pantoic acid, β-alanine, ATP, phosphoenolpyruvate, $MgCl_2$, myokinase, pyruvate kinase, and lactate dehydrogenase in buffer was added. Compounds were then added to plates in 1-μL volumes. The reaction was initiated with the addition of 39 μL of PS, diluted in buffer. The test plate was immediately transferred to a microplate reader, and absorbance was measured at 340 nm every 12 sec for 120 sec. Percentage inhibition was calculated using following formula [Zheng R., *et al.*, 2001].

$$\%\text{Inhibition}=100 \times \frac{\text{1-compound rate-background rate}}{\text{full reaction rate-background rate}}$$

4.4. Biophysical characterization using DSF experiment

The transition from native form to denatured form was measured as a function of increase in temperature using the fluorescence of an environmentally-sensitive dye called sypro orange. The DSF study was performed as per the standard protocols [Niesen F.H., *et al*., 2007]. The real time PCR instrument (Bio-Rad iCycler5) was programmed to equilibrate the samples at 25°C for 3 min and increased temperature upto 95°C, taking fluorescence reading at every 0.1°C rise using a LED/Photodiode set matched to the dye excitation and emission wavelengths. The melting points of the protein and protein with ligand were obtained as the lowest point of first derivative plot and calculated by the software included with the instrument. In a 96-well PCR plate, 20 µl of reaction was carried by combining 10 µl of protein solution (75-90 µg/ml) in analysis buffer, 2 µl of 5X dye (diluted from 5000X stock with the sub stock of 50X) and 1.6 µl of ligand solution diluted from its subsequent stock solutions.

5.1a. Design, synthesis and biological evaluation of 2-methylimidazo[1,2-*a*]pyridine-3-carboxylic acid derivatives as novel anti-tubercular agents

5.1a.1. Design of the molecules

To speed up the process of better anti-TB drug discovery process, recently many non-profit organisations/institutes and commercial organisations have been working together. They are providing preliminary screening results of thousands of compounds, to allow the researchers worldwide to utilize the high quality screening data for developing new leads and potential anti-TB agents. To fuel the open source drug discovery, recently researchers at GSK reported their compound's (~20,000,00) *M. tuberculosis* screening data [Ballell L., *et al*., 2013]. One of the most active compounds **GSK358607A** (2,6-dimethyl-*N*-(thiophen-2-ylmethyl)imidazo[1,2-*a*]pyridine-3-carboxamide) (**Figure 5.1**) was taken as lead compound in the present study to generate a library of 30 molecules (**Table 5.10**).

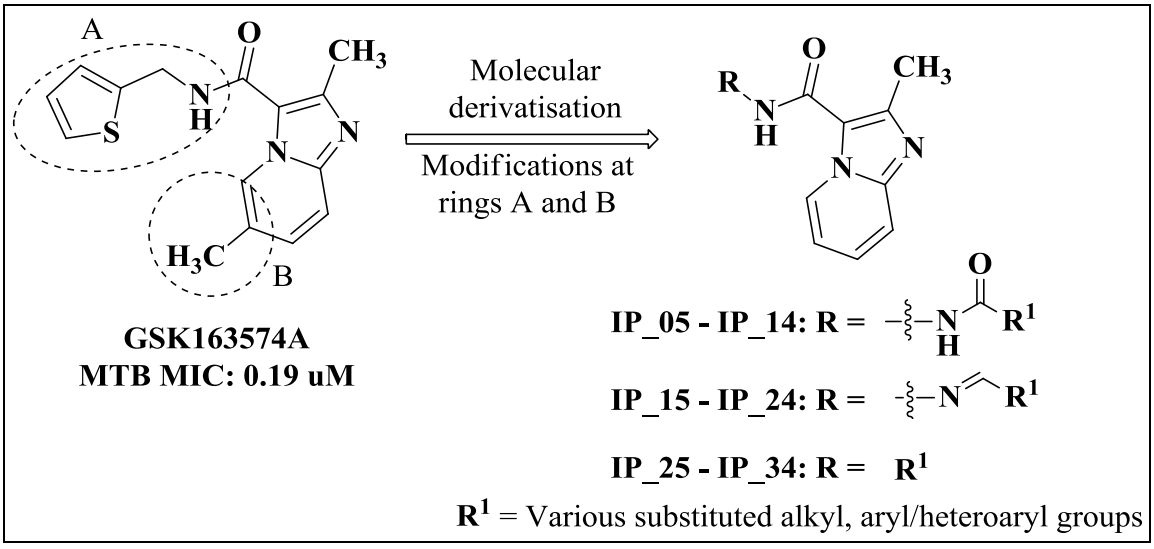


Figure 5.1: Structure of lead molecule for the generation of compounds **IP_05 – IP_34**

5.1a.2. Experimental procedures utilized for the synthesis of IP_05 – IP_34

The target molecules were synthesized by following a three step synthetic protocol (**Figure 4.1**), wherein the first step of the reaction was of 2-aminopyridine (**1**) with 2-chloroethylacetoacetate in ethanol under reflux conditions to yield the bicyclic compound –

"ethyl 2-methylimidazo[1,2-*a*]pyridine-3-carboxylate" (**IP_02**) (**Figure 5.2**) in good yield. In the next step, two types of reactions were carried out on ester group, one was the conversion of ester group into carboxylic acid (**IP_04**) [Kummerle A.E., *et al*., 2012] using LiOH in ethanol/H_2O (1:1), and the other was the direct conversion of ester into acid hydrazide (**IP_03**) using 35% aqueous solution of hydrazine hydrate in ethanol under reflux conditions. Further the 2-methylimidazo[1,2-*a*]pyridine-3-carbohydrazide (**IP_03)** on reaction with various substituted aromatic/aliphatic carboxylic acids (**Table 5.1**) in presence of coupling agents EDCI, and HOBt produced the double amides (**IP_05** – **IP_14**). Reaction of compound **IP_03** with various substituted aldehydes in ethanol reflux conditions produced the acid hydrazones (**IP_15** – **IP_24**) in excellent yields. This reaction was faster in presence of catalytic amount of con. H_2SO_4, as it formed the final molecules **IP_16**, **IP_21**, and **IP_23** in less than 10 minutes and for others the reaction time ranged from 30 to 60 minutes. During the reaction we observed the formation of desired product as a solid then reaction mixture was filtered directly and washed with distilled water, cold ethanol and hexanes to obtain pure products without further purification steps. In the case of simple amides, 2-methylimidazo[1,2-*a*]pyridine-3-carboxylic acid (**IP_04**) was treated with substituted aromatic/aliphatic primary amines in presence of peptide coupling agent EDCI to produce final compounds (**IP_25 – IP_34**).

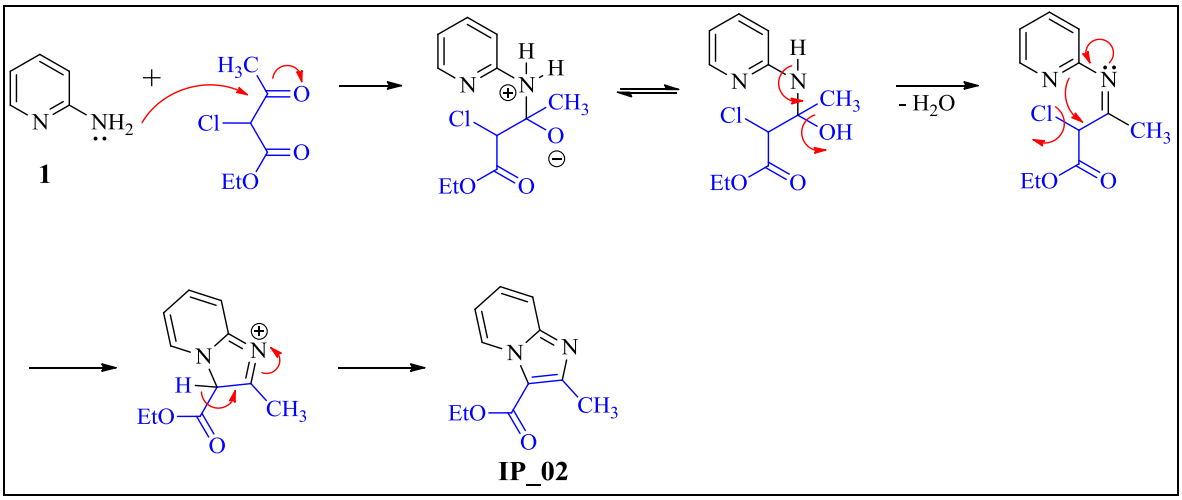
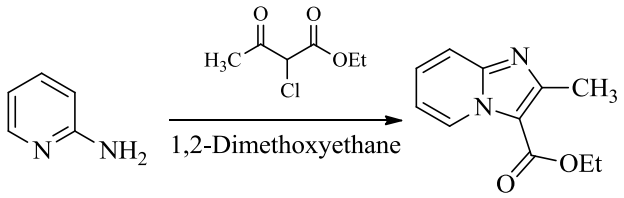


Figure 5.2: Mechanism of conversion of compound **1** to **IP_02**

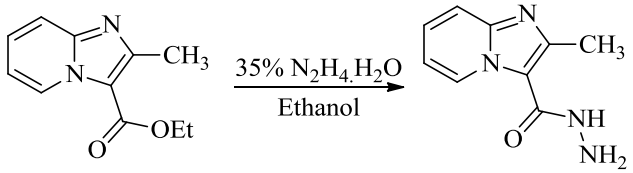
Preparation of ethyl 2-methylimidazo[1,2-*a*]pyridine-3-carboxylate (IP_02)



1 **IP_02**

2-Aminopyridine (4.00 g, 42.50 mmol) and 2-chloroethylacetoacetate (7.08 mL, 51.00 mmol) were taken in 1,2-dimethoxyethane (40 mL) and heated at 90 °C for 6 h. The reaction mixture was concentrated under reduced pressure, diluted with EtOAc (80 mL), washed the organic layer with H_2O (3 × 30 mL). The separated organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to get crude compound. The crude compound was purified by column chromatography using 15% EtOAc in hexanes as eluant to get ethyl 2-methylimidazo[1,2-*a*]pyridine-3-carboxylate (**IP_02**) (5.40 g, 62%) as an off-white solid. ESI-MS showed 205 $[M+H]^+$ and carried to next step.

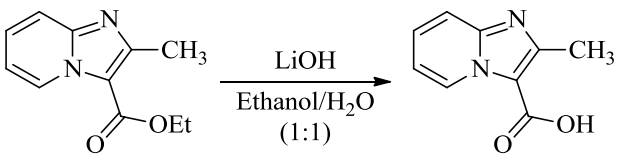
Preparation of 2-methylimidazo[1,2-*a*]pyridine-3-carbohydrazide (IP_03)



IP_02 **IP_03**

To the stirred solution of ethyl 2-methylimidazo[1,2-*a*]pyridine-3-carboxylate (**IP_02)** (2.70 g) in ethanol (30 mL) was added 35% aqueous solution of $N_2H_4.H_2O$ (25 mL) and refluxed for 3 h. The reaction mixture was concentrated to half volume and cooled on ice bath, the solids formed were filtered and dried in vacuum oven to get 2-methylimidazo[1,2-*a*]pyridine-3-carbohydrazide (**IP_03**) (2.25 g, 89%) as an off-white solid. ESI-MS showed 191 $[M+H]^+$.

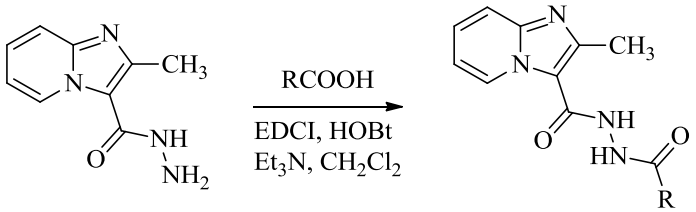
Preparation of 2-methylimidazo[1,2-*a*]pyridine-3-carboxylic acid (IP_04)



IP_02 **IP_04**

To the stirred solution of ethyl 2-methylimidazo[1,2-*a*]pyridine-3-carboxylate (**IP_02)** (2.00 g) in ethanol/H_2O (1:1) (30 mL) was added LiOH (4.00 g) and stirred at room temperature for 4 h. The reaction mixture was concentrated to half volume, and added 6N HCl at 0 °C till the reaction mixture turned to pH ~ 6, the solids formed were filtered and dried in vacuum oven to get 2-methylimidazo[1,2-*a*]pyridine-3-carboxylic acid (**IP_04**) (1.53 g, 88%) as an off-white solid. ESI-MS showed 177 $[M+H]^+$.

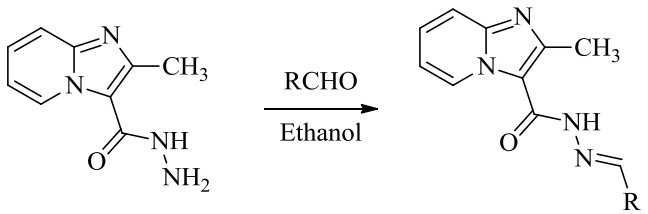
General procedure for the synthesis of final molecules (IP_05 – IP_14)



IP_03 **IP_05 - IP_14**

To the stirred solution of various substituted carboxylic acids (**Table 5.1**) (1.0 equiv), EDCI (1.2 equiv), HOBt (1.2 equiv) and Et_3N (2.5 equiv) in CH_2Cl_2 at 0 °C, was added compound **IP_03** (1.05 equiv) and allowed to stir at room temperature for 3 h. The reaction mixture was diluted with CH_2Cl_2 and washed with H_2O and the separated organic layer was concentrated under reduced pressure, purified by column chromatography using EtOAc/hexanes as eluant.

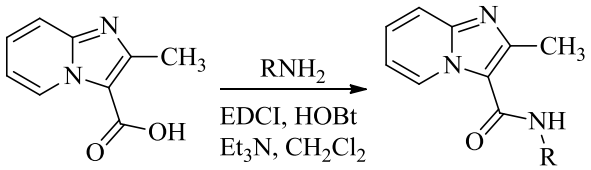
General procedure for the synthesis of final molecules (IP_15 – IP_24)



IP_03 **IP_05 - IP_14**

2-Methylimidazo[1,2-*a*]pyridine-3-carbohydrazide (**IP_03)** (1.0 equiv)**,** aldehyde (1.1 equiv), conc. H_2SO_4 (cat) were taken in ethanol and refluxed for 3 minutes to 1 h. The formed solids were filtered, dried and triturated with CH_2Cl_2/hexanes to get pure products.

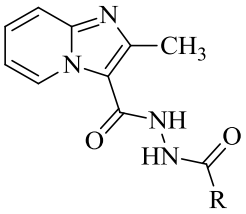
General procedure for the synthesis of final molecules (IP_25 – IP_34)



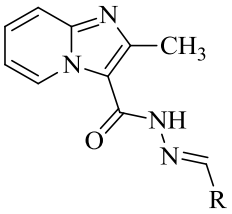
IP_04 **IP_25 - IP_34**

To the stirred solution of **IP_04** (1.0 equiv), EDCI (1.2 equiv), HOBt (1.2 equiv) and Et_3N (2.5 equiv) in CH_2Cl_2 at 0 °C, was added various substituted primary amines (**Table 5.1**) (1.05 equiv) and allowed stir at room temperature for 4 h. The reaction mixture was diluted with CH_2Cl_2 and washed with H_2O and the separated organic layer was concentrated under reduced pressure to get crude compound. The crude product was purified by column chromatography using EtOAc/hexanes as eluant.

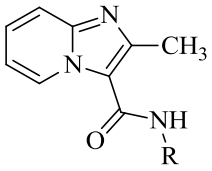
Table 5.1: Physiochemical properties of the synthesized compounds **IP_05 – IP_34**



IP_05 - IP_14



IP_15 - IP_24



IP_25 - IP_34

Compd	R	Yield (%)	M.P. (°C)	Molecular formula	Molecular weight
IP_05	Phenyl	81	180-181	$C_{16}H_{14}N_4O_2$	294.31
IP_06	Naphthyl	79	260-261	$C_{20}H_{16}N_4O_2$	344.37
IP_07	Cyclohexyl	88	251-252	$C_{16}H_{20}N_4O_2$	300.36
IP_08	2-Furyl	69	186-187	$C_{14}H_{12}N_4O_3$	284.27
IP_09	3-Nitrophenyl	76	220-221	$C_{16}H_{13}N_5O_4$	339.31
IP_10	3,5-Dinitrophenyl	80	138-139	$C_{16}H_{12}N_6O_6$	384.30
IP_11	2,4-Dichlorophenyl	87	251-252	$C_{16}H_{12}Cl_2N_4O_2$	363.30
IP_12	4-Tolyl	74	189-190	$C_{17}H_{16}N_4O_2$	308.33
IP_13	2-Methoxyphenyl	69	162-163	$C_{17}H_{16}N_4O_3$	324.33

Contd

Compd	R	Yield (%)	M.P. (°C)	Molecular formula	Molecular weight
IP_14	4-Phenoxyphenyl	89	142-143	$C_{22}H_{18}N_4O_3$	386.40
IP_15	Phenyl	84	184-186	$C_{16}H_{14}N_4O$	278.31
IP_16	4-Bromophenyl	90	274-275	$C_{16}H_{13}BrN_4O$	357.20
IP_17	4-Fluorophenyl	88	250-252	$C_{16}H_{13}FN_4O$	296.30
IP_18	4-Trifluromethylphenyl	76	223-224	$C_{17}H_{13}F_3N_4O$	346.31
IP_19	4-Nitrophenyl	92	268-269	$C_{16}H_{13}N_5O_3$	323.31
IP_20	4-Hydroxyphenyl	87	277-278	$C_{16}H_{14}N_4O_2$	294.31
IP_21	4-Methoxyphenyl	93	201-202	$C_{17}H_{16}N_4O_2$	308.33
IP_22	4-Benzyloxyphenyl	90	172-173	$C_{23}H_{20}N_4O_2$	384.33
IP_23	3,4,5-Trimethoxyphenyl	91	206-207	$C_{19}H_{20}N_4O_4$	368.39
IP_24	4-Tolyl	82	238-240	$C_{17}H_{16}N_4O$	292.24
IP_25	Phenyl	78	184-185	$C_{15}H_{13}N_3O$	251.28
IP_26	Benzyl	82	>300	$C_{16}H_{15}N_3O$	265.31
IP_27	Phenethyl	84	120-121	$C_{17}H_{17}N_3O$	279.34
IP_28	Cyclohexyl	76	155-156	$C_{15}H_{19}N_3O$	257.33
IP_29	2-Pyridyl	69	163-164	$C_{14}H_{12}N_4O$	252.27
IP_30	2-Furanylmethyl	63	141-142	$C_{14}H_{13}N_3O_2$	255.27
IP_31	4-Bromophenyl	81	155-156	$C_{15}H_{12}BrN_3O$	330.18
IP_32	4-Chlorophenyl	83	191-192	$C_{15}H_{12}ClN_3O$	285.73
IP_33	3-Trifluromethylphenyl	72	152-153	$C_{16}H_{12}F_3N_3O$	319.28
IP_34	4-Ethoxyphenyl	81	160-161	$C_{17}H_{17}N_3O_2$	295.34

5.1a.3. Characterization of the synthesized molecules

***N'*-Benzoyl-2-methylimidazo[1,2-*a*]pyridine-3-carbohydrazide (IP_05):** To the stirred solution of benzoic acid (0.4 g, 3.27 mmol), in CH_2Cl_2 at 0 °C was added EDCI (0.76 g, 3.92 mmol), HOBt (0.53 g, 3.92 mmol) and Et_3N (1.02 mL, 7.19 mmol) stirred for few minutes then was added 2-methylimidazo[1,2-*a*]pyridine-3-carbohydrazide (0.69 g, 3.60 mmol), and allowed stir at rt for 3 h, The reaction mixture was diluted with CH_2Cl_2 and washed with H_2O and the separated organic layer was concentrated under reduced pressure, purified by column chromatography using 25% EtOAc/hexanes as eluant. Yield: 81%; m.p. 180–181 °C;

MS(ESI) *m/z* 295 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.33 (s, 2H), 8.34 (d, *J* = 8.4 Hz, 1H), 8.10 (d, *J* = 8.0 Hz, 2H), 7.72–7.54 (m, 5H), 7.34 (t, *J* = 8.0 Hz, 1H), 2.61 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.7, 170.8, 153.6, 149.1, 136.8, 132.5, 126.6, 125.6(2C), 124.5, 121.4(2C), 120.6, 118.4, 116.1, 18.0. Anal. calcd for $C_{16}H_{14}N_4O_2$: C, 65.30; H, 4.79; N, 19.04% Found C, 65.33; H, 4.89; N, 19.11%.

***N′*-(1-Naphthoyl)-2-methylimidazo[1,2-*a*]pyridine-3-carbohydrazide (IP_06):** Yield: 79%; m.p. 260–261 °C; MS(ESI) *m/z* 345 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.44 (s, 2H), 8.55 (d, *J* = 8.8 Hz, 1H), 8.19 (d, *J* = 8.4 Hz, 1H), 7.90–7.72 (m, 3H), 7.63–7.54 (m, 4H), 7.36 (t, *J* = 8.4 Hz, 1H), 7.29 (t, *J* = 8.4 Hz, 1H), 2.67 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.9, 167.8, 152.6, 150.1, 136.4, 133.4, 132.4, 130.6, 129.4, 128.4, 127.2, 126.9, 126.0, 125.6, 125.1, 124.2, 120.6, 119.2, 117.9, 19.2. Anal. calcd for $C_{20}H_{16}N_4O_2$: C, 69.76; H, 4.68; N, 16.27% Found C, 69.83; H, 4.72; N, 16.31%.

***N′*-(Cyclohexanecarbonyl)-2-methylimidazo[1,2-*a*]pyridine-3-carbohydrazide (IP_07):** Yield: 88%; m.p. 251–252 °C; MS(ESI) *m/z* 301 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.51 (s, 1H, NH), 10.42 (s, 1H, NH), 8.44 (d, *J* = 8.4 Hz, 1H, Ar), 7.72 (d, *J* = 8.4 Hz, 1H, Ar), 7.27 (t, *J* = 8.0 Hz, 1H, Ar), 7.02 (t, *J* = 8.4 Hz, 1H, Ar), 2.58 (s, 3H, CH_3), 2.22–2.19 (m, 1H, CH), 1.71–1.43 (m, 10H, $(CH_2)_5$); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.1, 166.8, 151.6, 149.6, 133.4, 130.4, 127.4, 123.2, 118.8, 48.3, 27.9(2C), 26.3(2C), 26.1, 18.2. Anal. calcd for $C_{16}H_{20}N_4O_2$: C, 63.98; H, 6.71; N, 18.65% Found C, 63.99; H, 6.73; N, 18.71%.

***N′*-(Furan-2-carbonyl)-2-methylimidazo[1,2-*a*]pyridine-3-carbohydrazide (IP_08):** Yield: 69%; m.p. 186–187 °C; MS(ESI) *m/z* 285 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.53 (s, 2H, NH), 8.39 (d, *J* = 8.8 Hz, 1H, Ar), 8.22 (d, *J* = 8.8 Hz, 1H, Ar), 7.83–7.72 (m, 3H, Ar), 7.58–7.40 (m, 2H, Ar), 2.58 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.9, 169.2, 157.6, 153.1, 142.4, 139.4, 136.6, 135.2, 133.9, 128.6, 126.0, 123.3, 118.8, 19.1. Anal. calcd for $C_{14}H_{12}N_4O_3$: C, 59.15; H, 4.25; N, 19.71% Found C, 59.23; H, 4.32; N, 19.91%.

2-Methyl-*N′*-(3-Nitrobenzoyl)imidazo[1,2-*a*]pyridine-3-carbohydrazide (IP_09): Yield: 76%; m.p. 220–221 °C; MS(ESI) *m/z* 340 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.62 (s, 2H, NH), 8.91 (s, 1H, Ar), 8.32 (d, *J* = 8.4 Hz, 1H, Ar), 8.10–7.90 (m, 2H, Ar), 7.81 (t, *J* = 8.4 Hz, 1H, Ar), 7.72–7.60 (m, 3H, Ar), 2.58 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.2, 170.4, 166.6, 154.1, 145.7, 136.4, 135.6, 134.6, 133.9, 129.4, 127.4, 125.6, 123.3,

120.6, 118.8, 19.8. Anal. calcd for $C_{16}H_{13}N_5O_4$: C, 56.64; H, 3.86; N, 20.64% Found C, 56.73; H, 3.92; N, 20.71%.

***N′*-(3,5-Dinitrobenzoyl)-2-methylimidazo[1,2-*a*]pyridine-3-carbohydrazide (IP_10):** Yield: 80%; m.p. 138–139 °C; MS(ESI) *m/z* 385 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.10 (s, 2H, NH), 9.21 (s, 2H, Ar), 8.91 (s, 1H, Ar), 8.39 (d, *J* = 8.8 Hz, 1H, Ar), 7.72–7.54 (m, 2H, Ar), 7.30 (d, *J* = 8.4 Hz, 1H, Ar), 2.55 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.6, 171.4, 165.2, 156.2, 148.3(2C), 138.3, 137.1, 132.4(2C), 128.3, 126.6, 124.2, 119.2, 117.6, 20.5. Anal. calcd for $C_{16}H_{12}N_6O_6$: C, 50.01; H, 3.15; N, 21.87% Found C, 50.03; H, 3.22; N, 21.91%.

***N′*-(2,4-Dichlorobenzoyl)-2-methylimidazo[1,2-*a*]pyridine-3-carbohydrazide (IP_11):** Yield: 87%; m.p. 251–252 °C; MS(ESI) *m/z* 363 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.57 (s, 1H, NH), 10.06 (s, 1H, NH), 8.97 (d, *J* = 6.8 Hz, 1H, Ar), 7.76 (s, 1H, Ar), 7.71–7.57 (m, 3H, Ar), 7.43 (t, *J* = 7.2 Hz, 1H, Ar), 7.07 (t, *J* = 6.8 Hz, 1H, Ar), 2.67 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.9, 160.5, 146.7, 145.5, 135.3, 133.3, 131.7, 130.7, 129.5, 127.4, 127.0, 126.9, 116.2, 114.2, 113.3, 15.6. Anal. calcd for $C_{16}H_{12}Cl_2N_4O_2$: C, 52.91; H, 3.33; N, 15.43% Found C, 52.94; H, 3.49; N, 15.48%.

2-Methyl-*N′*-(4-methylbenzoyl)imidazo[1,2-*a*]pyridine-3-carbohydrazide (IP_12): Yield: 74%; m.p. 189–190 °C; MS(ESI) *m/z* 309 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.56 (s, 2H, NH), 8.53 (d, *J* = 9.2 Hz, 1H, Ar), 8.01–7.74 (m, 3H, Ar), 7.63 (d, *J* = 8.4 Hz, 2H, Ar), 7.47 (t, *J* = 8.0 Hz, 1H, Ar), 7.36 (t, *J* = 8.0 Hz, 1H, Ar), 2.60 (s, 3H, CH_3), 2.41 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.8, 169.2, 158.6, 152.1, 137.3, 136.3, 134.6(2C), 132.4, 127.6(2C), 126.2, 123.6, 120.4, 118.2, 22.5, 17.9. Anal. calcd for $C_{17}H_{16}N_4O_2$: C, 66.22; H, 5.23; N, 18.17% Found C, 66.28; H, 5.29; N, 18.29%.

***N′*-(2-Methoxybenzoyl)-2-methylimidazo[1,2-*a*]pyridine-3-carbohydrazide (IP_13):** Yield: 69%; m.p. 162–163 °C; MS(ESI) *m/z* 325 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.72 (s, 2H, NH), 8.39 (d, *J* = 8.8 Hz, 1H, Ar), 7.99–7.81 (m, 3H, Ar), 7.69–7.54 (m, 3H, Ar), 7.39 (t, *J* = 8.4 Hz, 1H, Ar), 3.96 (s, 3H, OCH_3), 2.61 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.8, 167.6, 157.4, 151.9, 144.7, 139.7, 136.2, 134.2, 130.6, 127.4, 125.4, 123.5, 121.6, 119.5, 117.9, 61.2, 18.3. Anal. calcd for $C_{17}H_{16}N_4O_3$: C, 62.95; H, 4.97; N, 17.27% Found C, 62.98; H, 5.02; N, 17.34%.

2-Methyl-*N′*-(4-phenoxybenzoyl)imidazo[1,2-*a*]pyridine-3-carbohydrazide (IP_14): Yield: 89%; m.p. 142–143 °C; MS(ESI) *m/z* 387 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.71 (s, 2H, NH), 8.61 (d, *J* = 8.8 Hz, 1H, Ar), 7.92–7.81 (m, 4H, Ar), 7.69 (d, *J* = 8.0 Hz, 2H, Ar), 7.54–7.36 (m, 4H, Ar), 7.33–7.20 (m, 2H, Ar), 2.66 (s, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6) δ 172.4, 170.3, 168.5, 160.6, 158.1, 155.4, 144.9, 141.4, 136.6, 134.5, 133.2(2C), 132.9, 132.1(2C), 130.2(2C), 128.5, 126.4, 121.6, 119.6, 18.7. Anal. calcd for $C_{22}H_{18}N_4O_3$: C, 68.38; H, 4.70; N, 14.50% Found C, 68.44; H, 4.79; N, 14.58%.

***N′*-Benzylidene-2-methylimidazo[1,2-*a*]pyridine-3-carbohydrazide (IP_15):** 2-methylimidazo[1,2-*a*]pyridine-3-carbohydrazide (0.4 g, 2.10 mmol), benzaldehyde (0.23 mL, 2.31 mmol), conc. H_2SO_4 (3 drops) were taken in Ethanol (7 mL) and refluxed for 30 minutes. The solids in the reaction mixture were filtered, washed with H_2O, cold ethanol, hexanes and dried in vacuum oven to get (0.49 g, 84%) title compound **IP_15**. m.p. 184–185 °C; MS(ESI) *m/z* 279 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.33 (s, 1H, NH), 8.91 (s, 1H, CH), 8.21 (d, *J* = 8.4 Hz, 1H, Ar), 7.99 (d, *J* = 8.4 Hz, 2H, Ar), 7.63–7.44 (m, 5H, Ar), 7.27 (t, *J* = 8.0 Hz, 1H, Ar), 2.63 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.6, 158.8, 154.2, 144.9, 134.8, 132.9, 129.6(2C), 127.6, 124.3(2C), 123.2, 119.4, 117.8, 116.1, 17.1. Anal. calcd for $C_{16}H_{14}N_4O$: C, 69.05; H, 5.07; N, 20.13% Found C, 69.13; H, 5.12; N, 20.19%.

***N′*-(4-Bromobenzylidene)-2-methylimidazo[1,2-*a*]pyridine-3-carbohydrazide (IP_16):** Yield: 90%; m.p. 274–275 °C; MS(ESI) *m/z* 357 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.06 (s, 1H, NH), 9.03 (s, 1H, CH), 8.31 (d, *J* = 9.2 Hz, 1H, Ar), 8.03–7.72 (m, 3H, Ar), 7.63 (d, *J* = 8.4 Hz, 2H, Ar), 7.47 (t, *J* = 8.0 Hz, 1H, Ar), 7.29 (t, *J* = 8.4 Hz, 1H, Ar), 2.56 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.5, 162.8, 152.4, 146.2, 136.4, 135.8, 133.2(2C), 128.4(2C), 126.9, 125.2, 124.4, 120.6, 118.2, 19.2. Anal. calcd for $C_{16}H_{13}BrN_4O$: C, 53.80; H, 3.67; N, 15.68% Found C, 53.93; H, 3.72; N, 15.79%.

***N′*-(4-Fluorobenzylidene)-2-methylimidazo[1,2-*a*]pyridine-3-carbohydrazide (IP_17):** Yield: 88%; m.p. 250–251 °C; MS(ESI) *m/z* 297 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.12 (s, 1H, NH), 8.78 (s, 1H, CH), 8.26 (d, *J* = 8.8 Hz, 1H, Ar), 7.92 (d, *J* = 8.4 Hz, 2H, Ar), 7.81–7.72 (m, 2H, Ar), 7.54 (d, *J* = 8.8 Hz, 2H, Ar), 7.39 (t, *J* = 8.0 Hz, 1H, Ar), 2.62 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.2, 163.5, 156.3, 150.3, 144.7, 138.3, 136.3, 135.3(2C), 133.5, 129.7(2C), 125.4, 123.3, 119.6, 18.8. Anal. calcd for $C_{16}H_{13}FN_4O$: C, 64.86; H, 4.42; N, 18.91% Found C, 64.93; H, 4.52; N, 18.99%.

2-Methyl-*N′*-(4-(Trifluoromethyl)benzylidene)imidazo[1,2-*a*]pyridine-3-carbohydrazide (IP_18): Yield: 76%; m.p. 223–224 °C; MS(ESI) *m/z* 347 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.70 (s, 1H, NH), 8.91 (d, *J* = 8.0 Hz, 1H, Ar), 8.40 (s, 1H, CH), 7.94 (d, *J* = 8.4 Hz, 2H, Ar), 7.81 (d, *J* = 8.4 Hz, 2H, Ar), 7.63 (d, *J* = 8.0 Hz, 1H, Ar), 7.42 (t, *J* = 8.0 Hz, 1H, Ar), 7.06 (t, *J* = 8.0 Hz, 1H, Ar), 2.58 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.3, 152.5, 151.6, 150.0, 146.2, 142.6, 141.9, 128.9(2C), 127.8, 124.7(2C), 122.3, 117.8, 116.8, 113.7, 17.8. Anal. calcd for $C_{17}H_{13}F_3N_4O$: C, 58.96; H, 3.78; N, 16.18% Found C, 58.99; H, 3.82; N, 16.29%.

2-Methyl-*N′*-(4-nitrobenzylidene)imidazo[1,2-*a*]pyridine-3-carbohydrazide (IP_19): Yield: 92%; m.p. 268–269 °C; MS(ESI) *m/z* 324 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.82 (s, 1H, NH), 8.62 (s, 1H, CH), 8.55 (d, *J* = 8.8 Hz, 1H, Ar), 8.23 (d, *J* = 8.8 Hz, 2H, Ar), 7.94 (d, *J* = 8.4 Hz, 2H, Ar), 7.54–7.42 (m, 2H, Ar), 7.09 (t, *J* = 8.4 Hz, 1H, Ar), 2.61 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.3, 162.3, 158.8, 148.2, 144.7, 137.6, 136.3, 134.2, 129.3(2C), 126.6, 123.5(2C), 121.5, 119.1, 16.9. Anal. calcd for $C_{16}H_{13}N_5O_3$: C, 59.44; H, 4.05; N, 21.66% Found C, 59.49; H, 4.12; N, 21.69%.

***N′*-(4-Hydroxybenzylidene)-2-methylimidazo[1,2-*a*]pyridine-3-carbohydrazide (IP_20):** Yield: 87%; m.p. 277–278 °C; MS(ESI) *m/z* 295 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.22 (s, 1H, NH), 9.61 (s, 1H, Ar), 8.71 (s, 1H, CH), 8.58 (d, *J* = 8.4 Hz, 1H, Ar), 7.93 (d, *J* = 8.8 Hz, 2H, Ar), 7.72–7.54 (m, 2H, Ar), 7.04 (d, *J* = 8.4 Hz, 2H, Ar), 6.99 (t, *J* = 8.4 Hz, 1H, Ar), 2.56 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.5, 166.8, 156.3, 144.9, 142.9, 136.4, 133.2, 130.9, 124.2(2C), 123.9, 120.5(2C), 117.5, 116.1, 18.1. Anal. calcd for $C_{16}H_{14}N_4O_2$: C, 65.30; H, 4.79; N, 19.04% Found C, 65.40; H, 4.82; N, 19.09%.

***N′*-(4-Methoxybenzylidene)-2-methylimidazo[1,2-*a*]pyridine-3-carbohydrazide (IP_21):** Yield: 93%; m.p. 201–202 °C; MS(ESI) *m/z* 309 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.92 (s, 1H, NH), 8.49 (s, 1H, CH), 8.39 (d, *J* = 8.4 Hz, 1H, Ar), 7.89 (d, *J* = 8.4 Hz, 2H, Ar), 7.80 (d, *J* = 7.6 Hz, 1H, Ar), 7.69–7.32 (m, 4H, Ar), 3.94 (s, 3H, OCH_3), 2.56 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5, 166.8, 156.3, 144.9, 142.9, 136.4, 133.2, 130.9, 124.2(2C), 123.9, 120.5(2C), 117.5, 116.1, 63.7, 18.1. Anal. calcd for $C_{17}H_{16}N_4O_2$: C, 66.22; H, 5.23; N, 18.17% Found C, 66.26; H, 5.27; N, 18.29%.

***N′*-(4-(Benzyloxy)benzylidene)-2-methylimidazo[1,2-*a*]pyridine-3-carbohydrazide (IP_22):** Yield: 90%; m.p. 172–173 °C; MS(ESI) *m/z* 385 $[M+H]^+$; ^{1}H NMR (400 MHz,

DMSO-d_6) δ 10.72 (s, 1H, NH), 8.61 (s, 1H, CH), 8.52 (d, *J* = 8.8 Hz, 1H, Ar), 8.12–7.82 (m, 6H, Ar), 7.72 (d, *J* = 7.6 Hz, 2H, Ar), 7.67–7.45 (m, 3H, Ar), 7.36 (t, *J* = 8.4 Hz, 1H, Ar), 5.22 (s, 2H, CH_2), 2.62 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.4, 164.3, 154.1, 148.5, 138.9, 135.2, 134.1, 133.6, 133.0, 130.9, 130.4(2C), 128.5(2C), 127.2, 126.6, 125.1(2C), 123.3(2C), 121.5, 118.7, 16.9. Anal. calcd for $C_{23}H_{20}N_4O_2$: C, 71.86; H, 5.24; N, 14.57% Found C, 71.96; H, 5.34; N, 14.60%.

2-Methyl-*N'*-(3,4,5-trimethoxybenzylidene)imidazo[1,2-*a*]pyridine-3-carbohydrazide (IP_23): Yield: 91%; m.p. 206–207 °C; MS(ESI) *m/z* 369 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.92 (s, 1H, NH), 8.47–8.35 (m, 2H, Ar), 7.62 (d, *J* = 8.4 Hz, 1H, Ar), 7.36 (s, 2H, Ar), 7.22–7.15 (m, 2H, Ar), 3.94 (s, 9H, $(OCH_3)_3$), 2.55 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.3, 166.1, 152.6, 149.2, 146.6, 144.7, 141.5, 137.4, 136.2, 134.3, 133.9, 132.8, 125.1(2C), 119.1, 63.9(2C), 63.0, 17.8. Anal. calcd for $C_{19}H_{20}N_4O_4$: C, 61.95; H, 5.47; N, 15.21% Found C, 62.01; H, 5.53; N, 15.29%.

2-Methyl-*N'*-(4-methylbenzylidene)imidazo[1,2-*a*]pyridine-3-carbohydrazide (IP_24): Yield: 82%; m.p. 238–239 °C; MS(ESI) *m/z* 293 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.88 (s, 1H, NH), 8.54 (s, 1H, CH), 8.41 (d, *J* = 8.0 Hz, 1H, Ar), 7.81 (d, *J* = 8.4 Hz, 2H, Ar), 7.74 (d, *J* = 7.2 Hz, 1H, Ar), 7.64–7.48 (m, 3H, Ar), 7.24 (t, *J* = 7.6 Hz, 1H, Ar), 2.58 (s, 3H, CH_3), 2.29 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.4, 163.8, 152.4, 142.6, 139.5, 135.2, 132.9, 129.5, 125.6, 123.7(2C), 121.5(2C), 119.5, 118.1, 22.5, 17.7. Anal. calcd for $C_{17}H_{16}N_4O$: C, 69.85; H, 5.52; N, 19.17% Found C, 69.96; H, 5.67; N, 19.29%.

2-Methyl-*N*-phenylimidazo[1,2-*a*]pyridine-3-carboxamide (IP_25): To the stirred solution of 2-methylimidazo[1,2-*a*]pyridine-3-carboxylic acid (0.30 g, 1.70 mmol), in CH_2Cl_2 at 0 °C was added EDCI (0.39 g, 2.04 mmol), HOBt (0.27 g, 2.04 mmol), and Et_3N (0.53 mL, 3.74 mmol) stirred for few minutes then was added aniline (0.17 mL, 1.87 mmol) and allowed stir at room temperature for 4 h, The reaction mixture was diluted with CH_2Cl_2 and washed with H_2O and the separated organic layer was concentrated under reduced pressure, purified by column chromatography using 20% EtOAc/Hexanes as eluant to get (0.33 g, 78%) title compound **IP_25**. m.p. 184–185 °C; MS(ESI) *m/z* 252 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.91 (s, 1H, NH), 8.50 (d, *J* = 8.4 Hz, 1H, Ar), 7.91–7.72 (m, 3H, Ar), 7.53–7.26 (m, 5H, Ar), 2.67 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.6, 164.9, 156.3, 141.1, 135.1, 133.0, 132.4, 132.0, 127.4, 126.1, 125.6, 123.3, 120.8, 119.1, 18.1. Anal. calcd for $C_{15}H_{13}N_3O$: C, 71.70; H, 5.21; N, 16.72% Found C, 71.78; H, 5.38; N, 16.79%.

***N*-Benzyl-2-methylimidazo[1,2-*a*]pyridine-3-carboxamide (IP_26):** Yield: 82%; m.p. >300 °C; MS(ESI) *m/z* 266 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.42 (d, *J* = 9.6 Hz, 1H), 7.56 (d, *J* = 9.6 Hz, 1H), 7.39–7.29 (m, 5H), 6.91–6.82 (m, 3H), 4.72 (d, *J* = 5.6 Hz, 2H), 2.68 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.2, 163.6, 154.7, 144.6, 136.3, 130.9(2C), 128.4(2C), 127.0, 124.4, 121.9, 121.3, 120.4, 50.4, 17.8. Anal. calcd for $C_{16}H_{15}N_3O$: C, 72.43; H, 5.70; N, 15.84% Found C, 72.49; H, 5.68; N, 15.92%.

2-Methyl-*N*-phenethylimidazo[1,2-*a*]pyridine-3-carboxamide (IP_27): Yield: 84%; m.p. 120–121 °C; MS(ESI) *m/z* 280 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.03 (s, 1H, NH), 8.62 (d, *J* = 8.0 Hz, 1H, Ar), 7.69–7.48 (m, 6H, Ar), 7.38–7.27 (m, 2H, Ar), 3.44 (t, *J* = 8.4 Hz, 2H, CH_2), 2.76 (t, *J* = 8.4 Hz, 2H, CH_2), 2.56 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.2, 164.8, 156.9, 144.6, 134.6, 132.2(2C), 127.9(2C), 127.3, 125.7, 121.4, 120.5, 119.6, 44.4, 38.4, 18.9. Anal. calcd for $C_{17}H_{17}N_3O$: C, 73.10; H, 6.13; N, 15.04% Found C, 73.19; H, 6.18; N, 15.12%.

***N*-Cyclohexyl-2-methylimidazo[1,2-*a*]pyridine-3-carboxamide (IP_28):** Yield: 76%; m.p. 155–156 °C; MS(ESI) *m/z* 258 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.93 (s, 1H, NH), 8.58 (d, *J* = 8.0 Hz, 1H, Ar), 7.89–7.74 (m, 2H, Ar), 7.12 (t, *J* = 8.0 Hz, 1H, Ar), 3.39–3.31 (m, 1H, CH), 2.58 (s, 3H, CH_3), 1.68–1.11 (m, 10H, $(CH_2)_5$); ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.8, 160.8, 155.3, 142.6, 133.9, 129.3, 123.3, 120.4, 49.5, 36.0(2C), 27.4(2C), 26.9, 17.1. Anal. calcd for $C_{15}H_{19}N_3O$: C, 70.01; H, 7.44; N, 16.33% Found C, 70.11; H, 7.48; N, 16.42%.

2-Methyl-*N*-(pyridin-2-yl)imidazo[1,2-*a*]pyridine-3-carboxamide (IP_29): Yield: 69%; m.p. 163–164 °C; MS(ESI) *m/z* 253 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.97 (s, 1H, NH), 8.63 (d, *J* = 8.0 Hz, 1H, Ar), 8.11 (d, *J* = 8.0 Hz, 1H, Ar), 7.81–7.64 (m, 3H, Ar), 7.27–7.02 (m, 3H, Ar), 2.61 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.8, 161.1, 158.6, 144.7, 139.3, 136.7, 135.0, 129.4, 127.8, 124.3, 121.5, 120.6, 118.9, 17.7. Anal. calcd for $C_{14}H_{12}N_4O$: C, 66.65; H, 4.79; N, 22.21% Found C, 66.68; H, 4.88; N, 22.29%.

***N*-(Furan-2-ylmethyl)-2-methylimidazo[1,2-*a*]pyridine-3-carboxamide (IP_30):** Yield: 63%; m.p. 141–142 °C; MS(ESI) *m/z* 256 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.64 (s, 1H, NH), 8.49 (d, *J* = 8.4 Hz, 1H, Ar), 7.72–7.58 (m, 3H, Ar), 7.44 (d, *J* = 8.0 Hz, 1H, Ar), 7.38–7.20 (m, 2H, Ar), 5.02 (d, *J* = 8.0 Hz, 2H, CH_2), 2.56 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.9, 161.1, 157.6, 145.4, 139.4, 132.4, 130.5, 128.4, 126.1, 120.3,

119.6, 118.8, 42.1, 18.4. Anal. calcd for $C_{14}H_{13}N_3O_2$: C, 65.87; H, 5.13; N, 16.46% Found C, 65.93; H, 5.22; N, 16.51%.

***N*-(4-Bromophenyl)-2-methylimidazo[1,2-*a*]pyridine-3-carboxamide (IP_31):** Yield: 81%; m.p. 155–156 °C; MS(ESI) *m/z* 330 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.07 (s, 1H, NH), 8.64 (d, *J* = 8.4 Hz, 1H, Ar), 7.81 (d, *J* = 9.2 Hz, 2H, Ar), 7.69–7.54 (m, 4H, Ar), 7.18 (t, *J* = 8.0 Hz, 1H, Ar), 2.62 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.4, 158.5, 149.9, 139.6, 137.3, 133.6(2C), 132.4, 129.6, 127.6, 125.9(2C), 121.5, 119.8, 18.7. Anal. calcd for $C_{15}H_{12}BrN_3O$: C, 54.56; H, 3.66; N, 12.73% Found C, 54.63; H, 3.72; N, 12.79%.

***N*-(4-Chlorophenyl)-2-methylimidazo[1,2-*a*]pyridine-3-carboxamide (IP_32):** Yield: 83%; m.p. 191–192 °C; MS(ESI) *m/z* 286 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.27 (s, 1H, NH), 8.67 (d, *J* = 8.4 Hz, 1H, Ar), 7.90 (d, *J* = 9.2 Hz, 2H, Ar), 7.68 (d, *J* = 9.2 Hz, 2H, Ar), 7.63–7.56 (m, 2H, Ar), 7.21 (t, *J* = 8.4 Hz, 1H, Ar), 2.64 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.6, 160.4, 151.2, 139.3, 138.2, 134.4(2C), 133.6, 127.4, 126.2(2C), 124.8, 121.9, 120.4, 18.9. Anal. calcd for $C_{15}H_{12}ClN_3O$: C, 63.05; H, 4.23; N, 14.71% Found C, 63.13; H, 4.32; N, 14.78%.

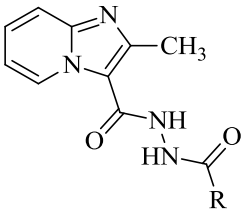
2-Methyl-*N*-(3-(trifluoromethyl)phenyl)imidazo[1,2-*a*]pyridine-3-carboxamide (IP_33): Yield: 72%; m.p. 152–153 °C; MS(ESI) *m/z* 320 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.21 (s, 1H, NH), 8.54 (d, *J* = 8.4 Hz, 1H, Ar), 8.17 (s, 1H, Ar), 7.78–7.39 (m, 5H, Ar), 7.18 (t, *J* = 8.4 Hz, 1H, Ar), 2.66 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.9, 160.2, 156.4, 142.5, 138.4, 135.2, 133.9, 130.6, 128.3, 126.4, 125.6, 124.4, 123.9, 121.5, 120.9, 16.9. Anal. calcd for $C_{16}H_{12}F_3N_3O$: C, 60.19; H, 3.79; N, 13.16% Found C, 60.28; H, 3.88; N, 13.29%.

***N*-(4-Ethoxyphenyl)-2-methylimidazo[1,2-*a*]pyridine-3-carboxamide (IP_34):** Yield: 81%; m.p. 160–161 °C; MS(ESI) *m/z* 296 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.47 (s, 1H, NH), 8.58 (d, *J* = 8.4 Hz, 1H, Ar), 7.72 (d, *J* = 8.8 Hz, 2H, Ar), 7.58–7.49 (m, 2H, Ar), 7.42–7.26 (m, 3H, Ar), 4.19 (q, *J* = 7.2 Hz, 2H, CH_2), 2.68 (s, 3H, CH_3), 1.38 (t, *J* = 7.2 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.4, 158.5, 149.9, 139.6, 137.3, 133.6(2C), 132.4, 129.6, 127.6, 125.9(2C), 121.5, 119.8, 69.1, 18.7, 16.2. Anal. calcd for $C_{17}H_{17}N_3O_2$: C, 69.14; H, 5.80; N, 14.23% Found C, 69.23; H, 5.92; N, 14.39%.

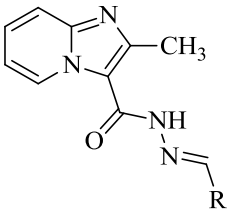
5.1a.4. *In vitro M. tuberculosis* screening, *M. tuberculosis* PS enzyme inhibition assay and cytotoxicity studies of the synthesized molecules

All the synthesized compounds were first screened for their *in vitro* anti-tubercular activity against *M. tuberculosis* H37Rv using MABA method. Isoniazid, ethambutol, and **GSK358607A** were used as reference compounds for comparison. All the synthesized derivatives were also screened for their *in vitro M. tuberculosis* PS inhibitory potency as steps towards hit optimization. Compounds showing *M. tuberculosis* MICs <25 µM were also tested for *in vitro* cytotoxicity against RAW 264.7cells at 50 µM concentration using MTT assay, all the results are presented in **Table 5.2**.

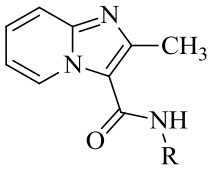
Table 5.2: *In vitro* biological evaluation of the synthesized compounds **IP_05** – **IP_34**



IP_05 - IP_14



IP_15 - IP_24



IP_25 - IP_34

Compd	R	MTB PS IC_{50} in µM	MTB[a] MIC in µM	Cytotoxicity[b] at 50 µM % inhibition
IP_05	Phenyl	3.54±0.18	42.37	NT
IP_06	Naphthyl	1.90±0.12	4.53	28.42
IP_07	Cyclohexyl	7.70±0.67	10.38	31.67
IP_08	2-Furyl	8.93±0.53	10.96	16.76
IP_09	3-Nitrophenyl	9.20±0.96	36.76	NT
IP_10	3,5-Dinitrophenyl	6.48±0.26	16.23	40.62
IP_11	2,4-Dichlorophenyl	5.13±0.24	17.22	20.12
IP_12	4-Tolyl	3.35±0.32	80.9	NT
IP_13	2-Methoxyphenyl	8.21±0.42	19.23	18.96
IP_14	4-Phenoxyphenyl	7.22±0.29	32.30	NT
IP_15	Phenyl	4.86±0.61	179.2	NT

Contd

Compd	R	MTB PS IC_{50} in µM	MTB[a] MIC in µM	Cytotoxicity[b] at 50 µM % inhibition
IP_16	4-Bromophenyl	4.43±0.12	35.01	NT
IP_17	4-Fluorophenyl	3.77±0.08	21.04	20.94
IP_18	4-Trifluromethylphenyl	8.18±0.14	9.01	24.56
IP_19	4-Nitrophenyl	>25	38.58	NT
IP_20	4-Hydroxyphenyl	7.49±0.22	21.19	19.42
IP_21	4-Methoxyphenyl	6.37±0.12	80.91	NT
IP_22	4-Benzyloxyphenyl	5.37±0.36	32.47	NT
IP_23	3,4,5-Trimethoxyphenyl	7.05±0.47	67.75	NT
IP_24	4-Tolyl	7.46±0.45	21.33	16.66
IP_25	Phenyl	12.83±0.19	187.9	NT
IP_26	Benzyl	2.74±0.05	23.50	24.50
IP_27	Phenethyl	5.77±0.03	89.29	NT
IP_28	Cyclohexyl	1.99±0.01	96.90	NT
IP_29	2-Pyridyl	>25	98.81	NT
IP_30	2-Furanylmethyl	2.81±0.02	24.41	20.70
IP_31	4-Bromophenyl	6.95±0.34	37.88	NT
IP_32	4-Chlorophenyl	2.60±0.03	87.4	NT
IP_33	3-Trifluromethylphenyl	7.01±0.04	78.1	NT
IP_34	4-Ethoxyphenyl	>25	42.23	NT
Isoniazid		>25	0.72	NT
Ethambutol		>25	7.64	NT
GSK358607A		8.12±0.03	0.19	NT

IC_{50}, 50% inhibitory concentration; MTB, *Mycobacterium tuberculosis*; MIC, minimum inhibitory concentration; [a]*In vitro* activity against MTB H37Rv; [b]Against RAW 264.7 cells; NT, not tested.

5.1a.5. SAR and discussion

All the synthesized compounds showed activity against *M. tuberculosis* with MIC ranging from 4.53 to 98.81 µM. Seven compounds (**IP_06** – **IP_08**, **IP_10** – **IP_11**, **IP_13** and **IP_18**) inhibited *M. tuberculosis* with MIC of <20 µM. Compound **IP_06** was found to be the most active compound *in vitro* with MIC of 4.53 µM and it was more potent than ethambutol (MIC 7.64 µM). All the synthesized compounds were less potent than standard antitubercular compounds like isoniazid and GSK lead compound. With respect to SAR, the order of activity was double amides (**IP_05** – **IP_14**) showed better activity followed by acid hydrazones (**IP_15** – **IP_24**) and amides (**IP_25** – **IP_34**). Among double amides, replacement of phenyl ring (**IP_05**) with napthyl ring (**IP_06**) enhanced (~10 times) the potency. Conversion of phenyl to cyclohexyl (**IP_07**) and furanyl ring (**IP_08**) yielded four times more potent compounds. Introduction of nitro, chloro, methoxy and benzyloxy groups on phenyl ring enhanced the activity, whereas 4-methyl group (**IP_12**) was found to be detrimental. In case of acid hydrazones, compound with 4-trifluoromethyl phenyl substituent (**IP_18**) showed good potency indicated by its MIC of 9.01 µM. In the case of amides, replacement of phenyl ring (**IP_25**) with benzyl group (**IP_26**) enhanced potency up to eight times, but further enlargement with phenylethyl group (**IP_27**) reduced the activity.

In order to evaluate the mode of action, the compounds were screened for *M. tuberculosis* enzyme inhibitory assay. In the initial screening at 25 µM, twenty seven compounds showed more than 50% inhibition against *M. tuberculosis* PS and were further studied for IC_{50} measurements. Compounds showed IC_{50} in the range of 1.90±0.12 µM to 9.20±0.96 µM. Compound *N'*-(1-naphthoyl)-2-methylimidazo[1,2-*a*]pyridine-3-carbohydrazide (**IP_06**) emerged as the most active compound with an IC_{50} of 1.90±0.12 µM. Further to support the activity we performed docking for these compounds. Compound **IP_06** showed highest docking score of -8.60 kcal/mol which correlates well with its potency in the enzyme assay (**Figure 5.3**).

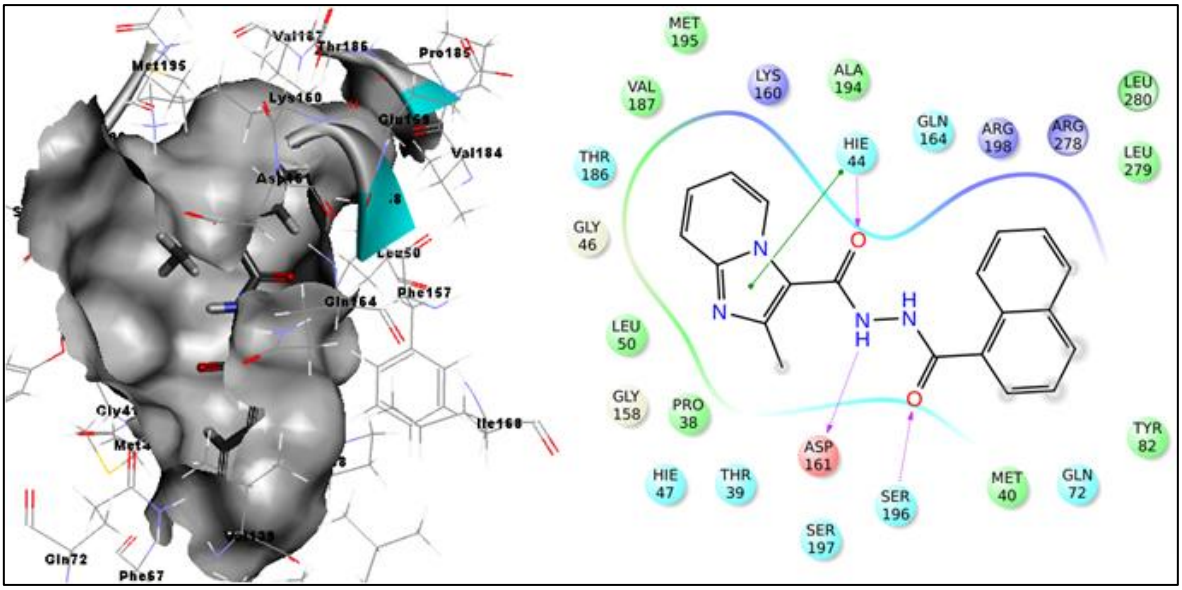


Figure 5.3: Binding pose and interaction pattern of most active compound **IP_06** with the *M. tuberculosis* PS protein

5.1a.6. Evaluation of protein interaction and stability using biophysical characterization experiment

The binding affinity of the most potent derivative was evaluated by measuring the thermal stability of the protein-ligand complex using the biophysical differential scanning fluorimetry (DSF), which measure the thermal stability of a target protein and a subsequent increase in protein melting temperature indicate binding of a ligand to the protein. Protein complexes with ligand were heated from 25 to 95 °C in steps of 0.1 °C in the presence of a dye called sypro orange. The fluorescence increased when the protein interacted with hydrophobic residues. Positive shift of T_m corresponding to native protein indicated that stability was increased due to inhibitor binding. The curves obtained in this are depicted in **Figure 5.4**. The protein *M. tuberculosis* PS showed a melting temperature of 49.20 °C, whereas with compound **IP_06** the corresponding T_m was found to be 51 °C. The difference in the T_m indicated the stability of the native protein when it was bound with inhibitor.

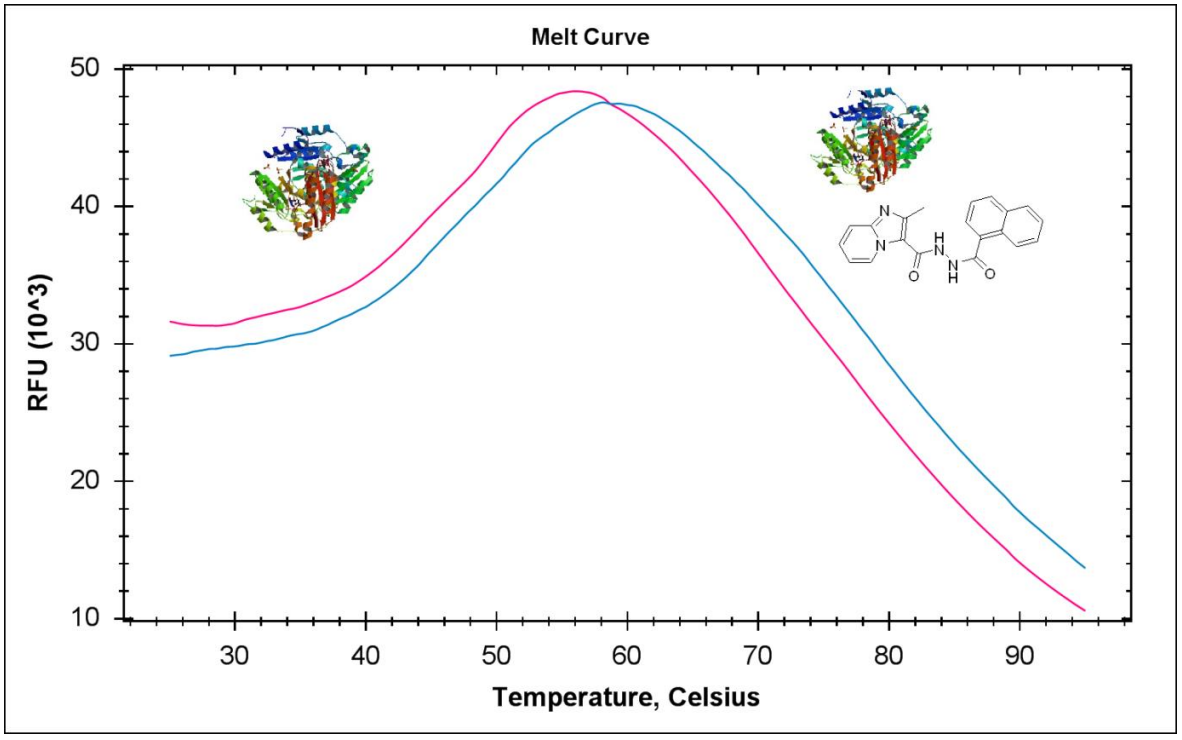


Figure 5.4: DSF experiment for compound **IP_06** (protein-ligand complex, blue) showing an increase in the thermal shift of 1.8 °C when compared to the native PS protein (red)

5.1a.7. Highlights of the study

In summary, we identified and synthesized a novel lead 2-methylimidazo[1,2-*a*]pyridine-3-carboxylic acid derivatives from a reported anti-tubercular compound **GSK358607A**. Many of the compounds showed potent *M. tuberculosis* PS inhibition and *M. tuberculosis* MIC. Compound **IP_06** (*N'*-(1-naphthoyl)-2-methylimidazo[1,2-*a*]pyridine-3-carbohydrazide) was found to be the most active compound with *M. tuberculosis* PS IC_{50} of 1.90±0.12 µM and inhibited drug sensitive *M. tuberculosis* with MIC of 4.53 µM.

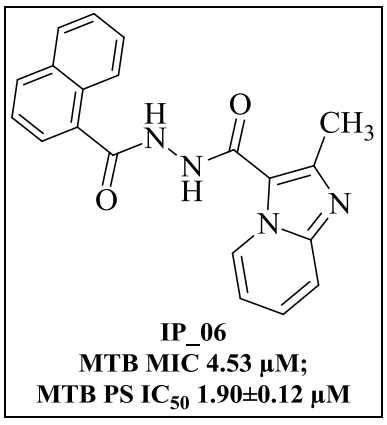


Figure 5.5: Chemical structure and biological activity of the most active compound **IP_06**

5.1b. Synthesis and biological evaluation of 6-methylimidazo[2,1-*b*]thiazole-5-carboxylic acid and 2-methylbenzo[*d*]imidazo[2,1-*b*]thiazole-3-carboxylic acid derivatives as novel anti-tubercular agents

5.1b.1. Design of the molecules

In our previous series of molecules *i.e.*, 2-methylimidazo[1,2-*a*]pyridine-3-carboxylic acid derivatives, the most active compound **IP_06** was found to be valid lead for further development owing to its good *in vitro* anti-mycobacterial activity, *in vitro M. tuberculosis* PS inhibitory activity and less cytotoxicity (**Figure 5.6**). The binding mode and interaction pattern of compound **IP_06** in the active site cavity of *M. tuberculosis* PS has suggested that compounds with bicyclic rings of: i) imidazole-thiazole in place of imidazole-pyridine for increased H-bonding there by strong interactions or ii) imidazole-benzothiazole in place of imidazole-pyridine for increased π- π static interactions.

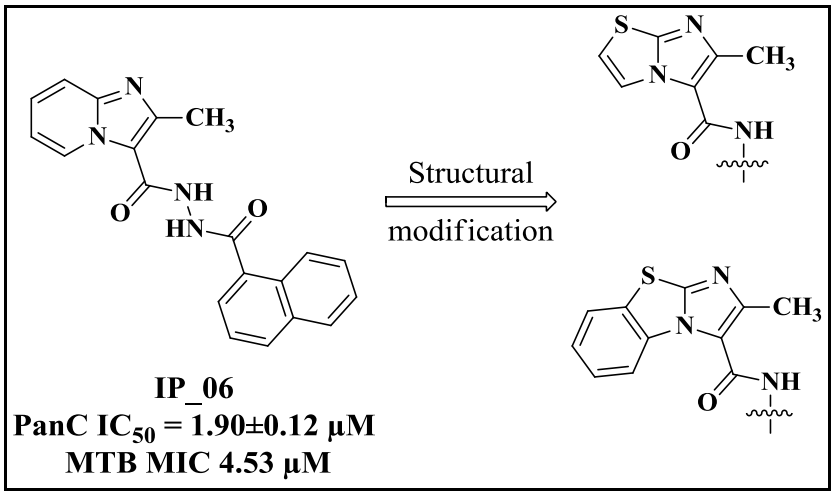


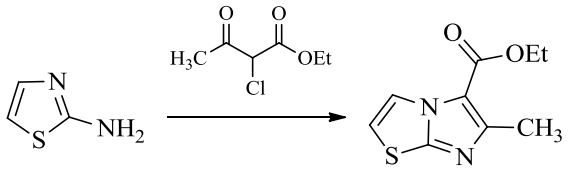
Figure 5.6: Structure modification of lead molecule to generate derivatives **IT_05 – IT_34**

5.1b.2. Experimental procedures utilized for the synthesis of IT_05 – IT_34

The target molecules were synthesized by following a three step synthetic protocol (**Figure 4.2**), wherein the first step of the reaction was of 2-aminothiazole (**IT_01a**)/2-aminobenzothiazole (**IT_01b**) with 2-chloroethylacetoacetate in 1,2-dimethoxyethane at 90 °C to yield the bicyclic compound **IT_02a / IT_02b**. In the next step, two types of reactions were carried out on ester group, one was the conversion of ester group into carboxylic acid (**IT_04a/IT_04b**) using LiOH in ethanol/H_2O (1:1), and the other was the direct conversion of ester into acid hydrazide (**IT_03a/IT_03b**) using 35% aqueous solution of hydrazine

hydrate in ethanol under reflux conditions. Reaction of compound **IT_03a** with various substituted aromatic carboxylic acids (**Table 5.3**) in presence of coupling agents EDCI and HOBt produced the compounds **IT_05** – **IT_09**, whereas compound **IT_03b** produced **IT_10** – **IT_14**. In the next step, compound **IT_03a/IT_03b** on reaction with various substituted aldehydes in ethanol reflux conditions produced the acid hydrazones (**IT_15** – **IT_24**) respectively in excellent yields. During the reaction, we observed the formation of desired product as a solid and then reaction mixture was filtered directly and washed with distilled water, cold ethanol and hexane to obtain pure products without further purification steps. In the case of simple amides, compound **IT_04a/IT_04a** was treated with substituted aromatic/aliphatic primary amines in presence of peptide coupling agent EDCI to produce final compounds (**IT_25** – **IT_34**) [Samala G., *et al*., 2014].

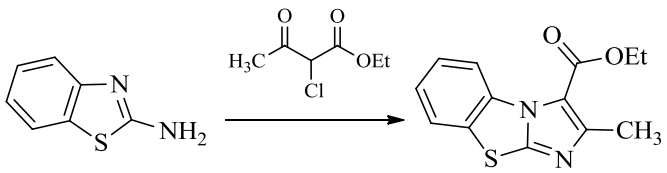
Preparation of ethyl 6-methylimidazo[2,1-*b*]thiazole-5-carboxylate (IT_02a)



IT_01a **IT_02a**

2-Aminothiazole (3.00 g, 29.99 mmol) and 2-chloroethylacetoacetate (4.96 mL, 35.99 mmol) were taken in 1,2-dimethoxyethane (30 mL) and heated at 90 °C for 6 h. The reaction mixture was concentrated under reduced pressure, diluted with EtOAc (80 mL), washed the organic layer with H_2O (3 × 30 mL). The separated organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to get crude compound. The crude compound was purified by column chromatography using 20% EtOAc in hexanes as eluant to get ethyl 6-methylimidazo[2,1-*b*]thiazole-5-carboxylate (**IT_02a**) (5.20 g, 82%) as an off-white solid. ESI-MS showed 211 $[M+H]^+$ and carried to next step.

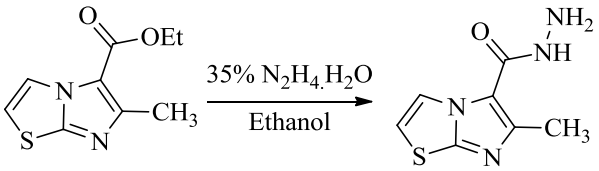
Preparation of ethyl 2-methylbenzo[*d*]imidazo[2,1-*b*]thiazole-3-carboxylate (IT_02b)



IT_01b **IT_02b**

2-Aminobenzothiazole (3.00 g, 19.97 mmol) and 2-chloroethylacetoacetate (3.30 mL, 23.96 mmol) were taken in 1,2-dimethoxyethane (30 mL) and heated at 90 °C for 6 h. The reaction mixture was concentrated under reduced pressure, diluted with EtOAc (80 mL), washed the organic layer with H_2O (3 × 30 mL). The separated organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to get crude compound. The crude compound was purified by column chromatography using 25% EtOAc in hexanes as eluant to get ethyl 2-methylbenzo[*d*]imidazo[2,1-*b*]thiazole-3-carboxylate (**IT_02b**) (4.32 g, 83%) as an off-white solid. ESI-MS showed 261 $[M+H]^+$ and carried to next step.

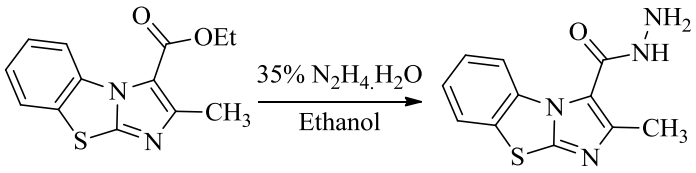
Preparation of 6-methylimidazo[2,1-*b*]thiazole-5-carbohydrazide (IT_03a)



IT_02a **IT_03a**

To the stirred solution of ethyl 6-methylimidazo[2,1-*b*]thiazole-5-carboxylate (**IT_02a)** (5.20 g) in ethanol (40 mL) was added 35% aqueous solution of $N_2H_4.H_2O$ (40 mL) and refluxed for 3 h. The reaction mixture was concentrated to half volume and cooled on ice bath, the solids formed were filtered and dried in vacuum oven to get 6-methylimidazo[2,1-*b*]thiazole-5-carbohydrazide (**3a**) (4.30 g, 89%) as an off-white solid. ESI-MS showed 197 $[M+H]^+$.

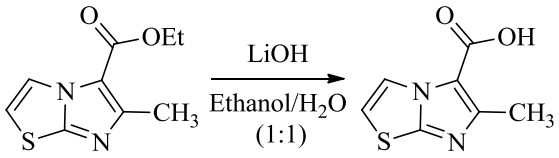
Preparation of 2-methylbenzo[*d*]imidazo[2,1-*b*]thiazole-3-carbohydrazide (IT_03b)



IT_02b **IT_03b**

To the stirred solution of ethyl 2-methylbenzo[*d*]imidazo[2,1-*b*]thiazole-3-carboxylate (**IT_02b)** (4.32 g) in ethanol (40 mL) was added 35% aqueous solution of $N_2H_4.H_2O$ (35 mL) and refluxed for 3 h. The reaction mixture was concentrated to half volume and cooled on ice bath, the solids formed were filtered and dried in vacuum oven to get 2-methylbenzo[*d*]imidazo[2,1-*b*]thiazole-3-carbohydrazide (**IT_03b**) (3.69 g, 90%) as an off-white solid. ESI-MS showed 247 $[M+H]^+$.

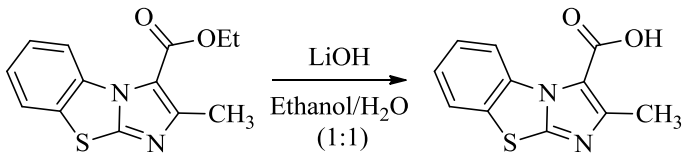
Preparation of 6-methylimidazo[2,1-*b*]thiazole-5-carboxylic acid (IT_04a)



IT_02a **IT_04a**

To the stirred solution of ethyl 6-methylimidazo[2,1-*b*]thiazole-5-carboxylate (**IT_02a)** (3.00 g) in ethanol/H_2O (1:1) (30 mL) was added LiOH (4.00 g) and stirred at room temperature for 4 h. The reaction mixture was concentrated to half volume, and added 6N HCl at 0 °C till the reaction mixture turned to pH ~ 6, the solids formed were filtered and dried in vacuum oven to get 6-methylimidazo[2,1-*b*]thiazole-5-carboxylic acid (**IT_04a**) (2.10 g, 80%) as an off-white solid. ESI-MS showed 183 $[M+H]^+$ and carried to next step.

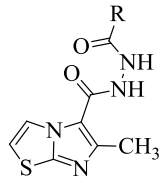
Preparation of 2-methylbenzo[*d*]imidazo[2,1-*b*]thiazole-3-carboxylic acid (IT_04b)



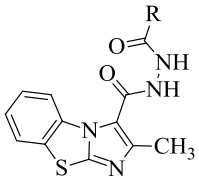
IT_02b **IT_04b**

To the stirred solution of ethyl 2-methylbenzo[*d*]imidazo[2,1-*b*]thiazole-3-carboxylate (**IT_02b)** (3.00 g) in ethanol/H_2O (1:1) (30 mL) was added LiOH (4.00 g) and stirred at room temperature for 4 h. The reaction mixture was concentrated to half volume, and added 6N HCl at 0 °C till the reaction mixture turned to pH ~ 6, the solids formed were filtered and dried in vacuum oven to get 2-methylbenzo[*d*]imidazo[2,1-*b*]thiazole-3-carboxylic acid (**IT_04b**) (2.05 g, 76%) as an off-white solid. ESI-MS showed 233 $[M+H]^+$ and carried to next step.

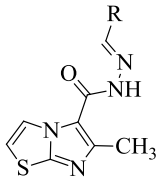
Table 5.3: Physiochemical properties of the synthesized compounds **IT_05 – IT_34**



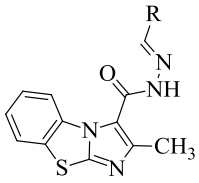
IT_05 - IT_09



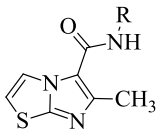
IT_10 - IT_14



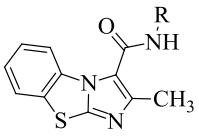
IT_15 - IT_19



IT_20 - IT_24



IT_25 - IT_29



IT_30 - IT_34

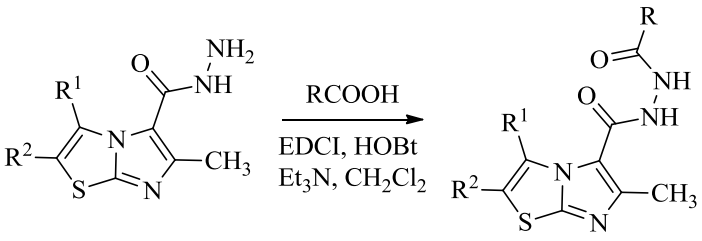
Compd	R	Yield (%)	M.P. (°C)	Molecular formula	Molecular weight
IT_05	Phenyl	81	166-167	$C_{14}H_{12}N_4O_2S$	300.34
IT_06	4-Tolyl	79	171-172	$C_{15}H_{14}N_4O_2S$	314.36
IT_07	4-Phenoxyphenyl	88	160-161	$C_{20}H_{16}N_4O_3S$	392.43
IT_08	1-Naphthyl	69	162-163	$C_{18}H_{14}N_4O_2S$	350.39
IT_09	Cyclohexyl	76	177-178	$C_{14}H_{18}N_4O_2S$	306.38
IT_10	Phenyl	80	260-261	$C_{18}H_{14}N_4O_2S$	350.39
IT_11	4-Tolyl	87	270-271	$C_{19}H_{16}N_4O_2S$	364.42
IT_12	4-Phenoxyphenyl	74	214-215	$C_{24}H_{18}N_4O_3S$	442.49
IT_13	1-Naphthyl	69	260-261	$C_{22}H_{16}N_4O_2S$	400.45
IT_14	Cyclohexyl	89	241-242	$C_{18}H_{20}N_4O_2S$	356.44
IT_15	4-Bromophenyl	72	269-270	$C_{14}H_{11}BrN_4OS$	363.33
IT_16	4-Trifluromethylphenyl	90	153-154	$C_{15}H_{11}F_3N_4OS$	352.33
IT_17	Phenyl	88	151-152	$C_{14}H_{12}N_4OS$	284.34
IT_18	3,4,5-Trimethoxyphenyl	76	218-219	$C_{17}H_{18}N_4O_4S$	374.41
IT_19	4-*N*,*N*-dimethylphenyl	92	119-120	$C_{16}H_{17}N_5OS$	327.40
IT_20	4-Bromophenyl	87	252-253	$C_{18}H_{13}BrN_4OS$	413.19
IT_21	4-Trifluromethylphenyl	93	271-272	$C_{19}H_{13}F_3N_4OS$	402.39
IT_22	Phenyl	90	246-247	$C_{18}H_{14}N_4OS$	334.39
IT_23	3,4,5-Trimethoxyphenyl	91	249-250	$C_{21}H_{20}N_4O_4S$	424.47
IT_24	4-*N*,*N*-dimethylphenyl	82	256-257	$C_{20}H_{19}N_5OS$	377.46
IT_25	4-Bromophenyl	78	213-214	$C_{13}H_{10}BrN_3OS$	336.21
IT_26	Phenyl	82	109-110	$C_{13}H_{11}N_3OS$	257.31

Contd

Compd	R	Yield (%)	M.P. (°C)	Molecular formula	Molecular weight
IT_27	4-Ethoxyphenyl	84	120-121	$C_{15}H_{15}N_3O_2S$	301.36
IT_28	Benzyl	76	141-142	$C_{14}H_{13}N_3OS$	271.34
IT_29	Cyclohexyl	69	146-147	$C_{13}H_{17}N_3OS$	263.63
IT_30	4-Bromophenyl	63	255-256	$C_{17}H_{12}BrN_3OS$	386.27
IT_31	Phenyl	81	200-201	$C_{17}H_{13}N_3OS$	307.37
IT_32	4-Ethoxyphenyl	83	245-246	$C_{19}H_{17}N_3O_2S$	351.42
IT_33	Benzyl	72	216-217	$C_{18}H_{15}N_3OS$	321.40
IT_34	Cyclohexyl	81	243-244	$C_{17}H_{19}N_3OS$	313.42

5.1b.3. Characterization of the synthesized molecules

General procedure for the synthesis of final molecules (IT_05 – IT_09 and **IT_10 – IT_14)**



IT_03a-b **IT_05 - IT_14**

R^1=R^2 = H: 2-aminothiazole

R^1+ R^2 =

2-aminobenzothiazole

To the stirred solution of R-COOH (1.0 equiv), in CH_2Cl_2 at 0 °C was added EDCI (1.2 equiv), HOBt (1.2 equiv) and Et_3N (2.0 equiv), stirred for few minutes and was added 6-methylimidazo[2,1-*b*]thiazole-5-carbohydrazide (for **IT_05 – IT_09**)/2-methylbenzo[*d*] imidazo[2,1-*b*]thiazole-3-carbohydrazide (for **IT_10 – IT_14**) (1.2 equiv), and allowed to stir at room temperature for 3 h. The reaction mixture was diluted with CH_2Cl_2 and washed with H_2O and the separated organic layer was concentrated under reduced pressure, purified by column chromatography.

***N′*-Benzoyl-6-methylimidazo[2,1-*b*]thiazole-5-carbohydrazide (IT_05):** To the stirred solution of benzoic acid (0.4 g, 3.27 mmol), in CH_2Cl_2 at 0 °C was added EDCI (0.76 g, 3.92 mmol), HOBt (0.53 g, 3.92 mmol), and Et_3N (1.02 mL, 7.19 mmol) stirred for few minutes then was added 6-methylimidazo[2,1-*b*]thiazole-5-carbohydrazide (0.71 g, 3.60 mmol), and allowed stir at room temperature for 3 h. The reaction mixture was diluted with CH_2Cl_2 and washed with H_2O and the separated organic layer was concentrated under reduced pressure, purified by column chromatography using 25% EtOAc/hexanes as eluant. Yield: 81%; m.p. 166–167 °C; MS(ESI) *m/z* 301 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.48 (s, 1H), 8.49 (d, *J* = 8.0 Hz, 1H), 7.92–7.78 (m, 3H), 7.54–7.46 (m, 3H), 7.30 (d, *J* = 8.0 Hz, 1H), 2.60 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.3, 161.8, 153.4, 145.6, 142.2, 139.7, 137.9, 133.1, 127.6 (2C), 126.3(2C), 119.2, 17.8. Anal. calcd for $C_{14}H_{12}N_4O_2S$: C, 55.99; H, 4.03; N, 18.65% Found C, 56.03; H, 4.12; N, 18.71%.

6-Methyl-*N′*-(4-methylbenzoyl)imidazo[2,1-*b*]thiazole-5-carbohydrazide (IT_06): Yield: 79%; m.p. 171–172 °C; MS(ESI) *m/z* 315 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.36 (s, 1H), 8.50 (d, *J* = 8.0 Hz, 1H), 7.99–7.81 (m, 3H), 7.56 (d, *J* = 7.6 Hz, 2H), 7.29 (d, *J* = 8.0 Hz, 1H), 2.61 (s, 3H), 2.39 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.9, 162.3, 154.6, 146.2, 141.3, 140.4, 138.5, 133.6, 128.4 (2C), 126.9(2C), 120.6, 20.9, 18.2. Anal. calcd for $C_{15}H_{14}N_4O_2S$: C, 57.31; H, 4.49; N, 17.82% Found C, 57.43; H, 4.52; N, 17.91%.

6-Methyl-*N′*-(4-phenoxybenzoyl)imidazo[2,1-*b*]thiazole-5-carbohydrazide (IT_07): Yield: 88%; m.p. 160–161 °C; MS(ESI) *m/z* 393 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.63 (s, 1H), 8.51 (d, *J* = 8.0 Hz, 1H), 7.91 (d, *J* = 7.6 Hz, 2H), 7.76–7.63 (m, 3H), 7.56–7.45 (m, 3H), 7.42–7.30 (m, 3H), 2.58 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.9, 164.4, 160.3, 154.6, 151.7, 144.0, 139.6, 136.3, 135.1, 129.4 (2C), 127.1(2C), 124.9, 124.2 (2C), 122.4(2C), 119.1, 18.0. Anal. calcd for $C_{20}H_{16}N_4O_3S$: C, 61.21; H, 4.11; N, 14.28% Found C, 61.33; H, 4.20; N, 14.31%.

***N′*-(1-Naphthoyl)-6-methylimidazo[2,1-*b*]thiazole-5-carbohydrazide (IT_08):** Yield: 69%; m.p. 162–163 °C; MS(ESI) *m/z* 351 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.63 (s, 1H), 9.31 (d, *J* = 8.0 Hz, 1H), 8.47 (d, *J* = 8.0 Hz, 1H), 7.81–7.72 (m, 2H), 7.68–7.54 (m, 3H), 7.47–7.32 (m, 3 H), 2.61 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.0, 166.2, 162.0, 152.4, 149.7, 139.0, 137.3, 134.2, 133.9, 133.0, 129.4, 128.3, 126.4, 125.7, 125.1, 124.4, 119.3, 17.2. Anal. calcd for $C_{18}H_{14}N_4O_2S$: C, 61.70; H, 4.03; N, 15.99% Found C, 61.73; H, 4.09; N, 16.09%.

***N′*-(Cyclohexanecarbonyl)-6-methylimidazo[2,1-*b*]thiazole-5-carbohydrazide (IT_09):** Yield: 76%; m.p. 177–178 °C; MS(ESI) *m/z* 307 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.54 (s, 1H), 8.61 (d, *J* = 8.0 Hz, 1H), 7.92 (s, 1H), 7.32 (d, *J* = 8.0 Hz, 1H), 2.56 (s, 3H), 2.18–2.14 (m, 1H), 1.78–1.50 (m, 10H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 174.2, 164.8, 160.2, 148.3, 144.8, 138.4, 118.2, 47.9, 31.3(2C), 26.6(3C), 16.9. Anal. calcd for $C_{14}H_{18}N_4O_2S$: C, 54.88; H, 5.92; N, 18.29% Found C, 54.93; H, 5.98; N, 18.39%.

***N′*-Benzoyl-2-methylbenzo[*d*]imidazo[2,1-*b*]thiazole-3-carbohydrazide (IT_10):** Yield: 80%; m.p. 260–261 °C; MS(ESI) *m/z* 351 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.44 (s, 1H), 8.09–7.81 (m, 3H), 7.74–7.63 (m, 4H), 7.56–7.44 (m, 3H), 2.63 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.2, 163.5, 158.6, 149.3, 143.6, 142.7, 138.6, 136.7, 134.2, 129.6, 128.0 127.8 (2C), 126.4(2C), 124.6, 120.4, 17.8. Anal. calcd for $C_{18}H_{14}N_4O_2S$: C, 61.70; H, 4.03; N, 15.99% Found C, 61.73; H, 4.12; N, 16.11%.

2-Methyl-*N′*-(4-methylbenzoyl)benzo[*d*]imidazo[2,1-*b*]thiazole-3-carbohydrazide (IT_11): Yield: 87%; m.p. 270–271 °C; MS(ESI) *m/z* 365 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.08–7.92 (m, 3H), 7.86 (d, *J* = 8.0 Hz, 2H), 7.72–7.64 (m, 2H), 7.54–7.39 (m, 3H), 2.60 (s, 3H), 2.42 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.6, 163.2, 155.8, 146.9, 140.4, 138.4, 137.3, 133.8, 129.6 (2C), 128.3, 127.4(2C), 126.5, 124.7, 124.0, 119.7, 22.3, 17.8. Anal. calcd for $C_{19}H_{16}N_4O_2S$: C, 62.62; H, 4.43; N, 15.37% Found C, 62.63; H, 4.52; N, 15.41%.

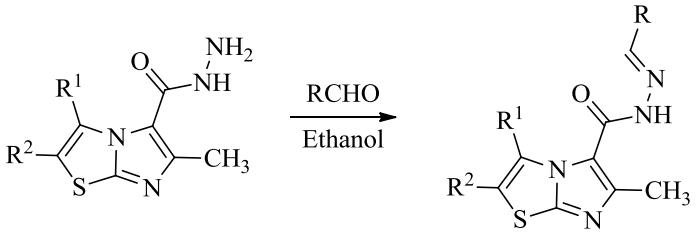
2-Methyl-*N′*-(4-phenoxybenzoyl)benzo[*d*]imidazo[2,1-*b*]thiazole-3-carbohydrazide (IT_12): Yield: 74%; m.p. 214–215 °C; MS(ESI) *m/z* 443 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10–7.96 (m, 4H), 7.90–7.74 (m, 3H), 7.63 (d, *J* = 8.0 Hz, 2H), 7.60 (s, 1H), 7.56–7.45 (m, 5H), 2.62 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.4, 165.6, 162.5, 157.4, 153.0, 145.5, 140.3, 137.4, 136.2, 133.2, 128.9 (2C), 127.4(2C), 126.6, 125.7, 124.9(2C), 124.2, 123.9(2C), 121.4, 117.9, 17.1. Anal. calcd for $C_{24}H_{18}N_4O_3S$: C, 65.14; H, 4.10; N, 12.66% Found C, 65.23; H, 4.20; N, 12.71%.

***N′*-(1-Naphthoyl)-2-methylbenzo[*d*]imidazo[2,1-*b*]thiazole-3-carbohydrazide (IT_13):** Yield: 69%; m.p. 260–261 °C; MS(ESI) *m/z* 401 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.28 (d, *J* = 8.0 Hz, 1H), 8.04–7.87 (m, 4H), 7.81–7.74 (m, 4H), 7.66–7.53 (m, 4H), 2.59 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.3, 167.1, 163.5, 155.7, 150.4, 139.5, 138.2, 136.4, 135.6, 133.6, 129.4, 129.2, 128.6, 127.4, 126.4, 126.2, 125.8, 125.0, 124.8, 124.3,

117.8, 17.2. Anal. calcd for $C_{22}H_{16}N_4O_2S$: C, 65.98; H, 4.03; N, 13.99% Found C, 66.03; H, 4.09; N, 14.09%.

***N'*-(Cyclohexanecarbonyl)-2-methylbenzo[*d*]imidazo[2,1-*b*]thiazole-3-carbohydrazide (IT_14):** Yield: 89%; m.p. 241–242 °C; MS(ESI) *m/z* 357 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.07–7.96 (m, 3H), 7.69–7.58 (m, 3H), 2.58 (s, 3H), 2.16–2.11 (m, 1H), 1.76–1.51 (m, 10H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.9, 163.3, 162.7, 152.2, 139.4, 137.6, 126.8, 125.4, 124.7, 124.1, 119.4, 47.2, 31.6(2C), 25.9(3C), 16.7. Anal. calcd for $C_{18}H_{20}N_4O_2S$: C, 60.65; H, 5.66; N, 15.72% Found C, 60.73; H, 5.68; N, 15.89%.

General procedure for the synthesis of final molecules (IT_15 – IT_24)



IT_03a-b **IT_15 - IT_24**

R^1=R^2= H: 2-aminothiazole

R^1+R^2= 

2-aminobenzothiazole

To the stirred solution of 6-methylimidazo[2,1-*b*]thiazole-5-carbohydrazide (**IT_03a**) (for **IT_15** – **IT_19**)/2-methylbenzo[*d*]imidazo[2,1-*b*]thiazole-3-carbohydrazide (**IT_03b)** (for **IT_20** – **IT_24**) (1.0 equiv), aldehyde (1.1 equiv), conc. H_2SO_4 (cat) were taken in ethanol and refluxed for 1 h. The formed solids were filtered, dried and triturated with CH_2Cl_2/hexanes to get pure products.

***N'*-(4-Bromobenzylidene)-6-methylimidazo[2,1-*b*]thiazole-5-carbohydrazide (IT_15):** 6-methylimidazo[2,1-*b*]thiazole-5-carbohydrazide (0.4 g, 2.03 mmol), 4-bromobenzaldehyde (0.22 mL, 2.23 mmol), conc. H_2SO_4 (3 drops) were taken in ethanol (7 mL) and refluxed for 30 minutes. The solids in the reaction mixture were filtered, washed with H_2O, cold ethanol, hexanes and dried in vacuum oven to get (0.54 g, 72%) title compound **IT_15**. m.p. 269–270 °C; MS(ESI) *m/z* 364 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.61 (s, 1H), 8.32 (d, *J* = 7.2 Hz, 1H), 7.81 (d, *J* = 7.6 Hz, 2H), 7.68–7.49 (m, 3H), 7.29 (d, *J* = 7.6 Hz, 1H), 2.62 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.3, 162.1, 149.8, 146.1, 141.4, 130.6, 128.7,

127.4(2C), 125.4(2C), 124.1, 118.6, 17.3. Anal. calcd for $C_{14}H_{11}BrN_4OS$: C, 46.29; H, 3.05; N, 15.42% Found C, 46.33; H, 3.09; N, 15.51%.

6-Methyl-*N′*-(4-(trifluoromethyl)benzylidene)imidazo[2,1-*b*]thiazole-5-carbohydrazide (IT_16): Yield: 90%; m.p. 153–154 °C; MS(ESI) *m/z* 353 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.63 (s, 1H), 8.49 (d, *J* =7.6 Hz, 1H), 7.74 (d, *J* =7.6 Hz, 2H), 7.63 (d, *J* =7.6 Hz, 2H), 7.48 (s, 1H), 7.30 (d, *J* =7.6 Hz, 1H), 2.60 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.4, 162.1, 148.9, 146.5, 137.6, 133.3, 130.6, 129.1, 127.3(2C), 126.2(2C), 124.4, 118.6, 17.4. Anal. calcd for $C_{15}H_{11}F_3N_4OS$: C, 51.13; H, 3.15; N, 15.90% Found C, 51.23; H, 3.19; N, 15.96%.

***N′*-Benzylidene-6-methylimidazo[2,1-*b*]thiazole-5-carbohydrazide (IT_17):** Yield: 88%; m.p. 151–152 °C; MS(ESI) *m/z* 285 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.34 (s, 1H), 8.52 (d, *J* = 8.1 Hz, 2H), 8.12 (s, 1H), 7.69–7.36 (m, 5H), 2.64 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.4, 164.9, 152.9, 148.3, 139.4, 129.8, 128.3, 124.9(2C), 124.0(2C), 123.7, 119.1, 18.9. Anal. calcd for $C_{14}H_{12}N_4OS$: C, 59.14; H, 4.25; N, 19.70% Found C, 59.33; H, 4.29; N, 19.91%.

6-Methyl-*N′*-(3,4,5-trimethoxybenzylidene)imidazo[2,1-*b*]thiazole-5-carbohydrazide (IT_18): Yield: 76%; m.p. 218–219 °C; MS(ESI) *m/z* 375 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.45 (s, 1H), 8.21 (s, 1H), 8.01 (d, *J* = 8.0 Hz, 1H), 7.35 (d, *J* = 8.0 Hz, 1H), 6.99 (s, 2H), 3.88 (s, 6H), 3.70 (s, 3H), 2.52 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.5, 161.9, 153.3(2C), 147.4, 143.1, 139.0, 129.6, 121.1, 117.6, 113.6, 104.1(2C), 60.1, 55.8(2C), 15.7. Anal. calcd for $C_{17}H_{18}N_4O_4S$: C, 54.53; H, 4.85; N, 14.96% Found C, 54.63; H, 4.92; N, 14.98%.

***N′*-(4-(Dimethylamino)benzylidene)-6-methylimidazo[2,1-*b*]thiazole-5-carbohydrazide (IT_19):** Yield: 92%; m.p. 119–120 °C; MS(ESI) *m/z* 328 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.55 (s, 1H), 8.46 (d, *J* = 8.0 Hz, 1H), 7.56–7.47 (m, 3H), 7.29 (d, *J* = 8.0 Hz, 1H), 7.11 (d, *J* = 8.0 Hz, 2H), 3.15 (s, 6H), 2.62 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.4, 162.5, 156.3, 146.2, 143.8, 141.4, 136.3, 129.3(2C), 125.1, 120.4, 115.2(2C), 44.1(2C), 17.3. Anal. calcd for $C_{16}H_{17}N_5OS$: C, 58.70; H, 5.23; N, 21.39% Found C, 58.73; H, 5.29; N, 21.51%.

***N′*-(4-Bromobenzylidene)-2-methylbenzo[*d*]imidazo[2,1-*b*]thiazole-3-carbohydrazide (IT_20):** Yield: 87%; m.p. 252–253 °C; MS(ESI) *m/z* 414 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.58 (s, 1H), 8.04–7.81 (m, 3H), 7.71 (d, *J* = 7.6 Hz, 2H), 7.64–7.51 (m, 4H), 2.60 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.7, 163.3, 160.8, 144.0, 139.5, 137.3, 136.8, 135.4, 133.0, 130.7(2C), 129.0, 128.6(2C), 126.7, 123.4, 120.4, 17.4. Anal. calcd for $C_{18}H_{13}BrN_4OS$: C, 52.31; H, 3.17; N, 13.56% Found C, 52.33; H, 3.29; N, 13.71%.

2-Methyl-*N′*-(4-(trifluoromethyl)benzylidene)benzo[*d*]imidazo[2,1-*b*]thiazole-3-carbohydrazide (IT_21): Yield: 93%; m.p. 271–272 °C; MS(ESI) *m/z* 403 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.58 (s, 1H), 8.09–7.94 (m, 3H), 7.76 (d, *J* =8.0 Hz, 2H), 7.65–7.48 (m, 4H), 2.62 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.6, 163.0, 156.6, 140.1, 138.4, 137.1, 134.3, 131.8, 129.5, 128.2, 128.9(2C), 128.3, 127.5(2C), 126.6, 123.9, 119.1, 16.9. Anal. calcd for $C_{19}H_{13}F_3N_4OS$: C, 56.71; H, 3.26; N, 13.92% Found C, 56.83; H, 3.29; N, 14.06%.

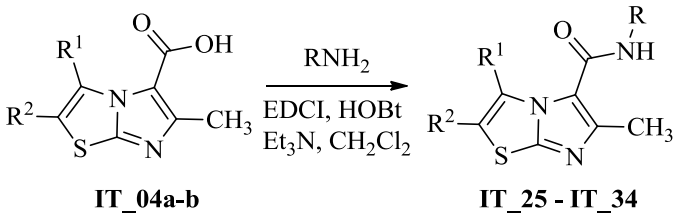
***N′*-Benzylidene-2-methylbenzo[*d*]imidazo[2,1-*b*]thiazole-3-carbohydrazide (IT_22):** Yield: 90%; m.p. 246–247 °C; MS(ESI) *m/z* 335 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.55 (d, *J* = 8.0 Hz, 1H), 8.02–7.91 (m, 3H), 7.78 (d, *J* = 8.0 Hz, 2H), 7.65–7.51 (m, 5H), 2.62 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.3, 162.6, 158.9, 146.2, 139.0, 137.5, 136.8, 134.5, 133.0, 128.4(2C), 126.2, 125.7(2C), 124.2, 123.4, 119.6, 17.4. Anal. calcd for $C_{18}H_{14}N_4OS$: C, 64.65; H, 4.22; N, 16.75% Found C, 64.73; H, 4.29; N, 16.91%.

2-Methyl-*N′*-(3,4,5-trimethoxybenzylidene)benzo[*d*]imidazo[2,1-*b*]thiazole-3-carbohydrazide (IT_23): Yield: 91%; m.p. 249–250 °C; MS(ESI) *m/z* 425 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.56 (s, 1H), 7.99–7.90 (m, 2H), 7.54–7.42 (m, 3H), 7.21 (s, 2H), 3.96 (s, 9H), 2.60 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.6, 162.9, 160.2, 150.3(2C), 146.4, 142.6, 139.3, 136.2, 135.1, 134.6, 129.8, 126.3, 124.6, 121.4, 114.6(2C), 62.1, 60.6(2C), 17.3. Anal. calcd for $C_{21}H_{20}N_4O_4S$: C, 59.42; H, 4.75; N, 13.20% Found C, 59.63; H, 4.82; N, 13.38%.

***N′*-(4-(Dimethylamino)benzylidene)-2-methylbenzo[*d*]imidazo[2,1-*b*]thiazole-3-carbohydrazide (IT_24):** Yield: 82%; m.p. 256–257 °C; MS(ESI) *m/z* 328 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.79 (s, 1H), 8.61 (s, 1H), 8.01–7.90 (m, 2H), 7.69–7.54 (m, 4H), 7.15 (d, *J* = 8.0 Hz, 2H), 3.13 (s, 6H), 2.61 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.3, 161.1, 158.4, 153.6, 146.5, 137.8, 135.1, 133.8, 130.4, 128.2(2C), 126.0, 125.3, 124.6,

122.4(2C), 118.1, 43.2(2C), 16.9. Anal. calcd for $C_{20}H_{19}N_5OS$: C, 63.64; H, 5.07; N, 18.55% Found C, 63.73; H, 5.19; N, 18.71%.

General procedure for the synthesis of final molecules (IT_25 – IT_34)



R^1=R^2= H: 2-aminothiazole

R^1+R^2= 

2-aminobenzothiazole

To the stirred solution of 6-methylimidazo[2,1-*b*]thiazole-5-carboxylic acid (for **IT_25 – IT_29**)/ 2-methylbenzo[*d*]imidazo[2,1-*b*]thiazole-3-carboxylic acid (for **IT_30 – IT_34**) (1.0 equiv), in CH_2Cl_2 at 0 °C was added EDCI (1.2 equiv), HOBt (1.2 equiv) and Et_3N (2.0 equiv) stirred for few minutes then was added R-NH_2 (1.2 equiv) and allowed to stir at room temperature for 3 h, the reaction mixture was diluted with CH_2Cl_2 and washed with H_2O and the separated organic layer was concentrated under reduced pressure, purified by column chromatography using EtOAc/hexanes as eluant.

***N*-(4-Bromophenyl)-6-methylimidazo[2,1-*b*]thiazole-5-carboxamide (IT_25):** To the stirred solution of 6-methylimidazo[2,1-*b*]thiazole-5-carboxylic acid (0.4 g, 2.19 mmol), in CH_2Cl_2 at 0 °C was added EDCI (0.50 g, 2.64 mmol), HOBt (0.36 g, 2.64 mmol) and Et_3N (0.62 mL, 4.38 mmol) stirred for few minutes then was added 4-bromoaniline (0.45 g, 2.64 mmol), and allowed stir at room temperature for 3 h, The reaction mixture was diluted with CH_2Cl_2 and washed with H_2O and the separated organic layer was concentrated under reduced pressure, purified by column chromatography using 40% EtOAc/hexanes as eluant. Yield: 78%; m.p. 213–214 °C; MS(ESI) *m/z* 335 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.70 (s, 1H), 8.57 (d, *J* = 8.0 Hz, 1H), 7.81 (d, *J* = 8.4 Hz, 2H), 7.67 (d, *J* = 8.0 Hz, 2H), 7.29 (d, *J* = 7.6 Hz, 1H), 2.61 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.5, 162.3, 143.2, 141.7, 133.9, 132.8, 130.2, 128.3, 126.5(2C), 121.4, 118.4, 16.9. Anal. calcd for $C_{13}H_{10}BrN_3OS$: C, 46.44; H, 3.00; N, 12.50% Found C, 46.53; H, 3.12; N, 12.71%.

6-Methyl-*N*-phenylimidazo[2,1-*b*]thiazole-5-carboxamide (IT_26): Yield: 82%; m.p. 109–110 °C; MS(ESI) *m/z* 258 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.21 (s, 1H), 8.52 (d, *J* = 8.0 Hz, 1H), 7.74–7.56 (m, 5H), 7.24 (d, *J* = 8.0 Hz, 1H), 2.64 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.9, 156.3, 146.5, 138.0, 132.2, 129.4, 127.7 (2C), 126.2, 124.4(2C), 119.4, 16.9. Anal. calcd for $C_{13}H_{11}N_3OS$: C, 60.68; H, 4.31; N, 16.33% Found C, 60.73; H, 4.42; N, 16.51%.

***N*-(4-Ethoxyphenyl)-6-methylimidazo[2,1-*b*]thiazole-5-carboxamide (IT_27):** Yield: 84%; m.p. 120–121 °C; MS(ESI) *m/z* 302 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.34 (s, 1H), 8.49 (d, *J* = 8.0 Hz, 1H), 7.62 (d, *J* = 7.6 Hz, 2H), 7.32 (d, *J* = 7.6 Hz, 1H), 7.02 (d, *J* = 8.0 Hz, 2H), 4.12 (q, *J* = 7.2 Hz, 2H), 2.63 (s, 3H), 1.38 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.2, 160.2, 158.4, 145.3, 137.4, 134.6, 126.3(2C), 123.2, 119.4(2C), 118.1, 71.3, 17.2, 15.8. Anal. calcd for $C_{15}H_{15}N_3O_2S$: C, 59.78; H, 5.02; N, 13.94% Found C, 59.93; H, 5.12; N, 14.11%.

***N*-Benzyl-6-methylimidazo[2,1-*b*]thiazole-5-carboxamide (IT_28):** Yield: 76%; m.p. 141–142 °C; MS(ESI) *m/z* 272 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.45 (s, 1H), 8.52 (d, *J* = 8.0 Hz, 1H), 7.51–7.38 (m, 5H), 7.36 (d, *J* = 8.0 Hz, 1H), 4.23 (s, 2H), 2.62 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.4, 160.6, 144.4, 142.3, 137.3, 136.2, 127.4(2C), 126.5(2C), 125.9, 119.4, 51.3, 16.8. Anal. calcd for $C_{14}H_{13}N_3OS$: C, 61.97; H, 4.83; N, 15.49% Found C, 62.03; H, 4.92; N, 15.61%.

***N*-Cyclohexyl-6-methylimidazo[2,1-*b*]thiazole-5-carboxamide (IT_29):** Yield: 69%; m.p. 146–147 °C; MS(ESI) *m/z* 264 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.27 (s, 1H), 8.49 (d, *J* = 7.6 Hz, 1H), 7.31 (d, *J* = 7.6 Hz, 1H), 3.34–3.28 (m, 1H), 2.61 (s, 3H), 1.71–1.63 (m, 4H), 1.48–1.24 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.7, 158.3, 146.2, 142.3, 136.2, 119.4, 61.3, 34.2(2C), 27.3, 25.2(2C), 16.2. Anal. calcd for $C_{13}H_{17}N_3OS$: C, 59.29; H, 6.51; N, 15.96% Found C, 59.43; H, 6.64; N, 16.11%.

***N*-(4-Bromophenyl)-2-methylbenzo[*d*]imidazo[2,1-*b*]thiazole-3-carboxamide (IT_30):** Yield: 63%; m.p. 255–256 °C; MS(ESI) *m/z* 386 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.71 (s, 1H), 8.03–7.90 (m, 2H), 7.83 (d, *J* = 7.2 Hz, 2H), 7.69–7.54 (m, 4H), 2.59 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.7, 160.2, 156.4, 138.4, 136.9, 133.6, 132.2, 131.5, 130.3(2C), 128.2, 126.5, 126.1, 123.3(2C), 119.5, 15.6. Anal. calcd for $C_{17}H_{12}BrN_3OS$: C, 52.86; H, 3.13; N, 10.88% Found C, 52.93; H, 3.22; N, 10.99%.

2-Methyl-*N*-phenylbenzo[*d*]imidazo[2,1-*b*]thiazole-3-carboxamide (IT_31): Yield: 81%; m.p. 200–201 °C; MS(ESI) *m/z* 308 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.35 (s, 1H), 8.02 (d, *J* = 7.2 Hz, 1H), 7.72–7.63 (m, 3H), 7.58 (d, *J* = 7.6 Hz, 2H), 7.47–7.35 (m, 3H), 2.62 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.4, 159.5, 156.2, 139.4, 137.6, 136.1, 135.6, 134.2, 128.8(2C), 127.4, 127.0, 126.5, 125.3(2C), 120.4, 16.4. Anal. calcd for $C_{17}H_{13}N_3OS$: C, 66.43; H, 4.26; N, 13.67% Found C, 66.70; H, 4.42; N, 13.81%.

***N*-(4-Ethoxyphenyl)-2-methylbenzo[*d*]imidazo[2,1-*b*]thiazole-3-carboxamide (IT_32):** Yield: 83%; m.p. 245–246 °C; MS(ESI) *m/z* 352 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.34 (s, 1H), 8.09 (d, *J* = 6.8 Hz, 1H), 8.04 (d, *J* = 6.8 Hz, 1H), 7.77–7.63 (m, 4H), 7.22 (d, *J* = 7.6 Hz, 2H), 4.14 (q, *J* = 7.2 Hz, 2H), 2.61 (s, 3H), 1.36 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.6, 161.3, 156.5, 152.4, 139.3, 136.1, 134.2, 133.6, 130.3, 127.2, 124.4, 122.4(2C), 119.9, 119.4(2C), 69.4, 16.6, 15.4. Anal. calcd for $C_{19}H_{17}N_3O_2S$: C, 64.94; H, 4.88; N, 11.96% Found C, 64.99; H, 4.92; N, 12.09%.

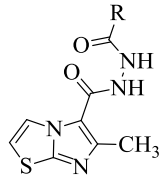
***N*-Benzyl-2-methylbenzo[*d*]imidazo[2,1-*b*]thiazole-3-carboxamide (IT_33):** Yield: 72%; m.p. 216–217 °C; MS(ESI) *m/z* 322 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.33 (s, 1H), 8.10–8.03 (m, 2H), 7.69–7.42 (m, 7H), 4.19 (s, 2H), 2.63 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.5, 160.5, 158.9, 140.1, 138.6, 136.8, 135.2, 129.1(2C), 127.4, 126.5(2C), 125.1, 123.5, 123.0, 119.4, 52.2, 16.9. Anal. calcd for $C_{18}H_{15}N_3OS$: C, 67.27; H, 4.70; N, 13.07% Found C, 67.33; H, 4.82; N, 13.21%.

***N*-Cyclohexyl-2-methylbenzo[*d*]imidazo[2,1-*b*]thiazole-3-carboxamide (IT_34):** Yield: 81%; m.p. 243–244 °C; MS(ESI) *m/z* 314 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (d, *J* = 6.8 Hz, 1H), 8.01 (d, *J* = 6.8 Hz, 1H), 7.67–7.55 (m, 3H), 3.33–3.28 (m, 1H), 2.63 (s, 3H), 1.74–1.66 (m, 4H), 1.51–1.27 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.5, 162.8, 158.4, 137.2, 135.9, 135.2, 127.5, 126.3, 126.0, 122.2, 60.3, 34.6(2C), 27.8, 26.0(2C), 16.9. Anal. calcd for $C_{17}H_{19}N_3OS$: C, 65.15; H, 6.11; N, 13.41% Found C, 65.23; H, 6.14; N, 13.61%

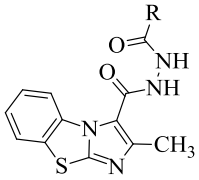
5.1b.4. *In vitro M. tuberculosis* screening, *M. tuberculosis* PS enzyme inhibition assay and cytotoxicity studies of the synthesized molecules

All the synthesized compounds were screened for their *in vitro* anti-tubercular activity against *M. tuberculosis* H37Rv using MABA method. Isoniazid, ethambutol, and **GSK358607A** were used as reference compounds for comparison. All the synthesized derivatives were also screened for their *in vitro M. tuberculosis* PS inhibitory potency and *in vitro* cytotoxicity against RAW 264.7cells at 50 µM concentration using MTT assay and all the results are tabulated in **Table 5.4**.

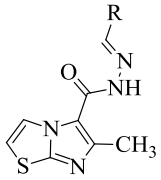
Table 5.4: *In vitro* biological evaluation of synthesized compounds **IT_05 – IT_34**



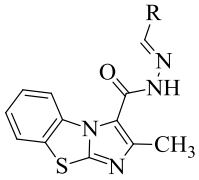
IT_05 - IT_09



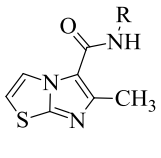
IT_10 - IT_14



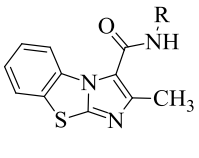
IT_15 - IT_19



IT_20 - IT_24



IT_25 - IT_29



IT_30 - IT_34

Compd	R	MTB PS IC_{50} in µM	MTB MIC in µM[a]	Cytotoxicity[b] at 50 µM % inhibition
IT_05	Phenyl	5.31±0.12	80.24	10.34
IT_06	4-Tolyl	4.99±0.21	2.48	39.25
IT_07	4-Phenoxyphenyl	1.23±0.30	7.96	2.98
IT_08	1-Naphthyl	0.64±0.10	35.67	27.47
IT_09	Cyclohexyl	5.38±0.09	40.80	31.19
IT_10	Phenyl	1.10±0.04	35.67	29.71
IT_11	4-Tolyl	5.83±0.24	17.15	53.01
IT_12	4-Phenoxyphenyl	0.53±0.13	7.06	1.40
IT_13	1-Naphthyl	1.39±0.18	31.21	32.03
IT_14	Cyclohexyl	2.91±0.11	35.07	45.41
IT_15	4-Bromophenyl	1.20±0.16	68.81	22.23
IT_16	4-Trifluromethylphenyl	0.67±0.18	17.74	12.84
IT_17	Phenyl	0.58±0.19	116.06	23.12

Contd

Compd	R	MTB PS IC_{50} in µM	MTB MIC in µM[a]	Cytotoxicity[b] at 50 µM % inhibition
IT_18	3,4,5-Trimethoxyphenyl	5.61±0.27	66.77	1.17
IT_19	4-*N*,*N*-dimethylphenyl	2.50±0.13	76.36	6.75
IT_20	4-Bromophenyl	1.02±0.22	30.25	10.06
IT_21	4-Trifluromethylphenyl	5.31±0.11	31.06	16.11
IT_22	Phenyl	2.15±0.08	9.35	34.57
IT_23	3,4,5-Trimethoxyphenyl	2.07±0.20	29.45	16.18
IT_24	4-*N*,*N*-dimethylphenyl	1.46±0.12	4.13	12.06
IT_25	4-Bromophenyl	0.69±0.13	2.32	32.32
IT_26	Phenyl	0.74±0.21	12.14	14.07
IT_27	4-Ethoxyphenyl	5.83±0.11	20.74	58.69
IT_28	Benzyl	1.06±0.14	11.52	12.67
IT_29	Cyclohexyl	2.00±0.16	11.85	32.10
IT_30	4-Bromophenyl	0.52±0.24	16.18	20.87
IT_31	Phenyl	1.03±0.11	40.67	33.21
IT_32	4-Ethoxyphenyl	2.10±0.09	83.9	52.73
IT_33	Benzyl	0.84±0.21	38.89	6.62
IT_34	Cyclohexyl	1.02±0.11	19.94	13.39
Isoniazid		>25	0.72	NT
Ethambutol		>25	7.64	NT

IC_{50}, 50% inhibitory concentration; MTB, *Mycobacterium tuberculosis*; MIC, minimum inhibitory concentration; [a]*In vitro* activity against MTB H37Rv; [b]Against RAW 264.7 cells; NT, not tested.

5.1b.5. SAR and discussion

All the synthesized compounds showed activity against *M. tuberculosis* with MIC ranging from 2.32 to 83.9 µM. Thirteen compounds (**IT_06** – **IT_07**, **IT_11** – **IT_12**, **IT_16**, **IT_22**, **IT_24** – **IT_26**, **IT_28** – **IT_30** and **IT_34**) inhibited *M. tuberculosis* with MIC of <20 µM, out of which six compounds (**IT_06** – **IT_07**, **IT_12**, **IT_22** and **IT_24** – **IT_25**) inhibited *M. tuberculosis* with MIC of <10 µM. Four compounds (**IT_06**, **IT_12** and **IT_24** – **IT_25**) were found to be better than ethambutol. Compound **IT_25** was found to be the most active compound *in vitro* with MICs of 2.32 µM and it was more potent than ethambutol (MIC 7.64

μM). When compared to lead compound (**IP_06**), three compounds (**IT_06**, **IT_24** and **IT_25**) were showing better *M. tuberculosis* MICs.

With respect to SAR, the compounds containing thiazole ring (**IT_05** – **IT_09**, **IT_15** – **IT_19** and **IT_25** – **IT_29**) showed better activities than compounds with benzothiazole ring (**IT_09** – **IT_14**, **IT_20** – **IT_24** and **IT_30** – **IT_34**). The order of activity was carbohydrazides (**IT_05** – **IP_09**) showed better activity followed by acid hydrazones (**IT_25** – **IP_29**) and benzothiazole ring containing acid hydrazones (**IT_20** – **IT_24**). Among thiazole ring containing carbohydrazides, replacement of phenyl ring (**IT_05**) with 4-tolyl ring (**IP_06**) enhanced the activity 40-fold from 80.24 μM to 2.48 μM, while replacement with 4-phenoxyphenyl ring (**IT_07**) increased the activity to 7.96 μM, whereas replacement with 1-naphthyl ring (**IT_08**) showed MIC of 35.67 μM.

All the synthesized compounds showed activity against *M. tuberculosis* PS with IC_{50} ranging from 0.52±0.24 to 5.83±0.24 μM. Fourteen compounds inhibited *M. tuberculosis* with MIC of <10 μM. Compounds **IT_06** and **IT_25** were found to be the most active compounds *in vitro* with MIC of 0.78 μM. All the compounds showed *M. tuberculosis* PS IC_{50} in the range of 0.52 to 5.83 μM. Compound **IT_30** (*N*-(4-bromophenyl)-2-methylbenzo[*d*]imidazo[2,1-*b*]thiazole-3-carboxamide) emerged as the most active compound with an IC_{50} of 0.52±0.24 μM, which is four times better than the most active compound of previous series **IP_06**.

5.1b.6. Evaluation of protein interaction and stability using biophysical characterization experiment

The binding affinity of the most potent derivative was evaluated by measuring the thermal stability of the protein-ligand complex using the biophysical technique, which measures the thermal stability of a target protein and a subsequent increase in protein melting temperature indicating binding of a ligand to the protein. Protein in complex with ligand was heated from 25 to 95 °C in steps of 0.1 °C in the presence of a dye called sypro orange. The fluorescence increased when the protein interacted with hydrophobic residues. The curves obtained in this study are depicted in **Figure 5.7**. The protein *M. tuberculosis* PS showed a melting temperature of 45.60 °C, whereas with compound **IT_08** showed corresponding T_m at 47.80 °C. The difference in the T_m indicated the stability of the native protein when it was bound with inhibitor **IT_08**.

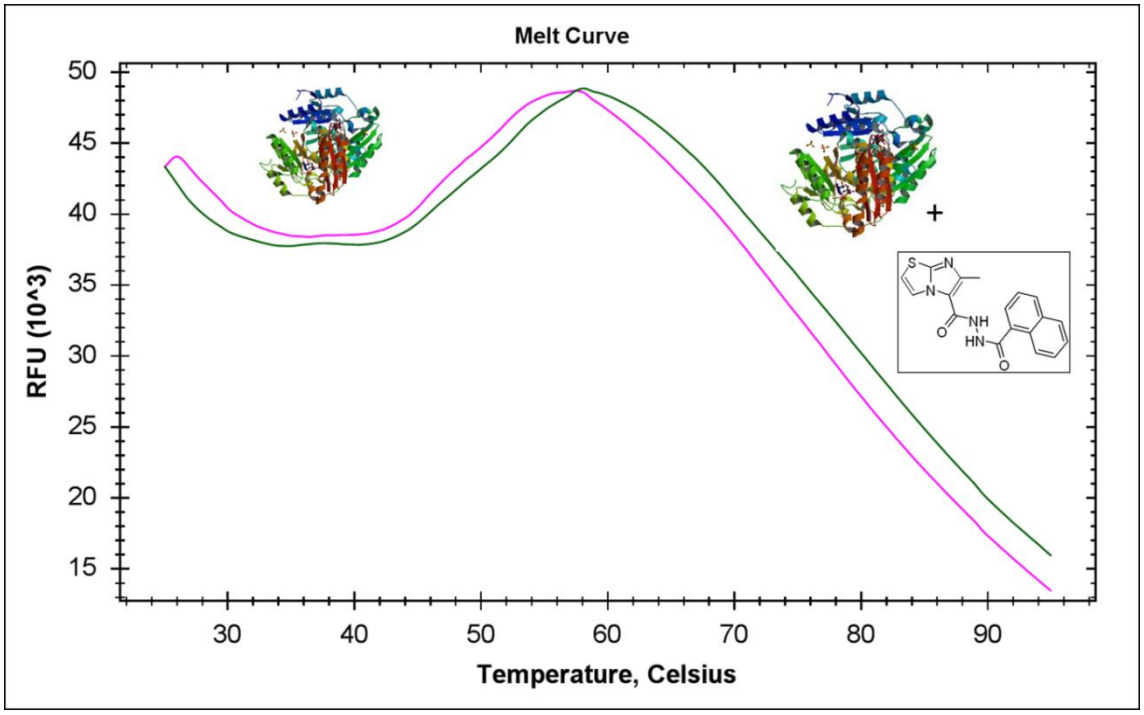


Figure 5.7: DSF experiment for compound **IT_08** (protein-ligand complex, green) showing an increase in the thermal shift of 2.2 °C when compared to the native PS protein (red)

5.1b.7. Highlights of the study

Based on the activity results of our previous work, we anticipated that replacing 2-aminopyridine with heterocyclic amines would lead to increase in activity. The most active compound from the previous series was (**IP_06**) taken as lead molecule for further extension of library. We decided to synthesis molecules starting with 2-aminothiazole and 2-aminobenzothiazole. As expected, we found nine molecules exhibiting greater *M. tuberculosis* MIC than lead molecule. The most active compound **IT_25** (**Figure 5.8**) showed *M. tuberculosis* MIC of 2.32 µM, which was two times more than lead molecule.

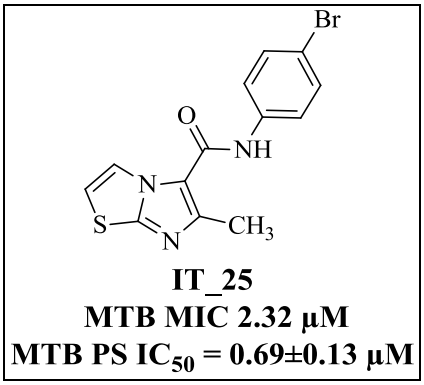


Figure 5.8: Chemical structure and biological activity of the most active compound **IT_25**

5.2. Design and synthesis of 5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-amine derivatives as novel anti-tubercular agents

5.2.1. Design of the molecules

Recently Lluis Ballell and co-workers reported high-throughput screening (HTS) results of GSK library. They subjected around 2 million molecules from GSK collection to HTS and found 62,000 hits after first stage of screening. After crossing their five different stages of screening, seven chemical families were reported [Ballell L., *et al.*, 2013] as most promising anti-tubercular agents. Based on the availability of starting materials and synthetic feasibility, we selected one of the most active compounds **GSK163574A** (5-methyl-2-(6-methylpyridin-2-yl)-*N*-(pyrimidin-4-yl)thieno[2,3-*d*]pyrimidin-4-amine) (**Figure 5.9**) as lead compound. To investigate the possible target enzyme and to study SAR, we synthesized a total of 33 derivatives

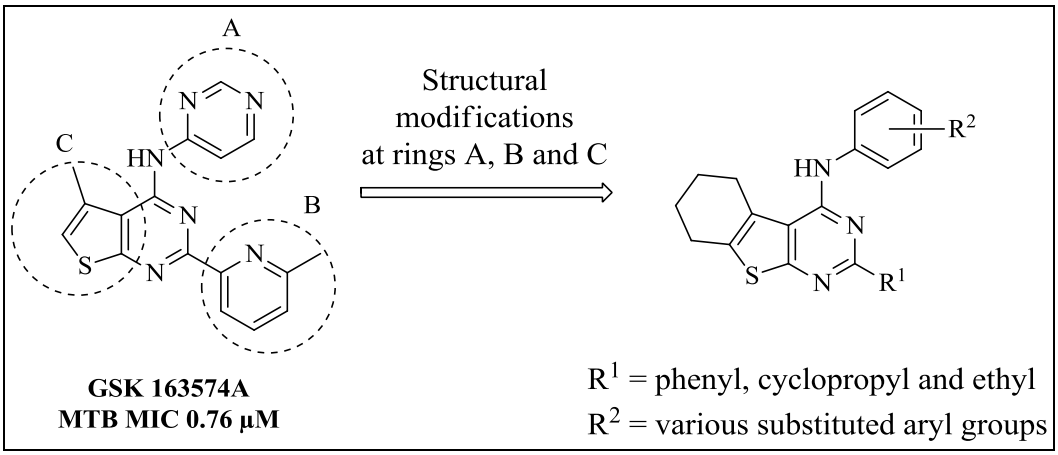


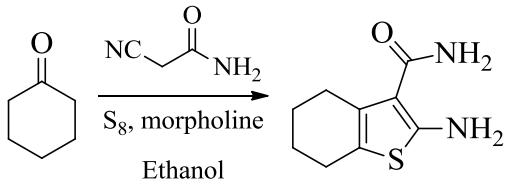
Figure 5.9: Structure of lead molecule **GSK 163574A**

5.2.2. Experimental procedures utilized for the synthesis PR_05 – PR_37

A library of thirty three molecules was synthesized by following four step synthetic protocol as shown in **Figure 4.3**. In first step, commercially available cyclohexanone (**1**) was converted to 2-amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxamide (**PR_02**) using cyanoacetamide and elemental sulphur following Gewald reaction. In the next step, *N*-acylation was carried out under basic conditions using benzoyl chloride, propionyl chloride and cyclopropylcarbonyl chloride to get three different *N*-acylated compounds (**PR_03a-c**). In the third step, cyclisation was achieved under basic, reflux conditions by treating with aqueous NaOH, to yield tricyclic compound (**4a-c**) [Dodic N., *et al.*, 2004]. Finally, each of

the compounds (**PR_04a-c)** was treated with eleven different substituted primary amines (**Table 5.5**) under microwave conditions at high temperature to afford compounds (**PR_05 – PR_37**).

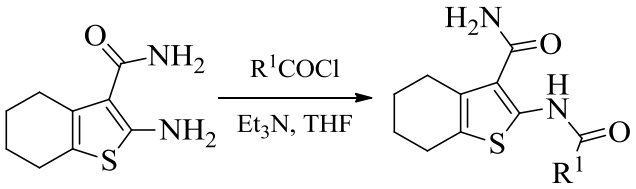
Preparation of 2-amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxamide (PR_02)



1 **PR_02**

To the stirred solution of compound **1** (3.0 g, 30.56 mmol), 2-cyanoacetamide (2.56 g, 30.56 mmol), sulphur powder (0.97 g, 30.56 mmol) in ethanol (40 mL) was added morpholine (5.31 mL, 61.11 mmol) and stirred the reaction mixture at room temperature for 6 h. The reaction mixture was concentrated, diluted with EtOAc and washed the organic layer with H_2O (2 × 30 mL). The separated organic layer was dried over anhydrous Na_2SO_4, evaporated and purified by column chromatography to get compound **PR_02** (5.40 g, 90%) as a light yellow solid. ESI-MS found 197 $[M+H]^+$ and carried to next step.

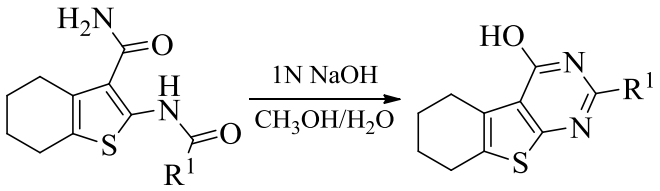
General procedure for the preparation of PR_03a-c



PR_02 **PR_03a-c**

To the stirred solution of compound **PR_02** (1.0 equiv) in CH_2Cl_2 at 0 °C was added Et_3N (2.0 equiv) followed by R^1COCl (1.2 equiv) and allowed to stir at room temperature for 6 h. The reaction mixture was diluted with CH_2Cl_2 and washed with sat $NaHCO_3$, H_2O and dried over anhydrous Na_2SO_4 and evaporated *in vacuo* to get compound **PR_03a-c** as an off-white solid.

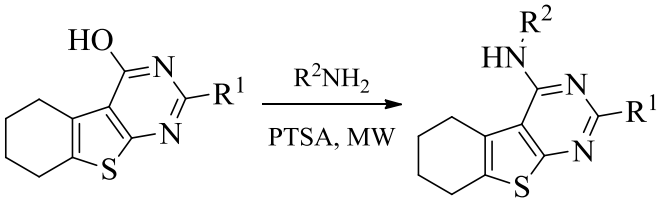
General procedure for the preparation of PR_04a-c



PR_03a-c **PR_04a-c**

To a solution of compound **PR_03a-c** in MeOH (2.0 vol) was added a solution of 1N NaOH (10.0 vol) and the mixture was refluxed for 3 h. Then the mixture was poured into water and neutralised with conc. HCl to give a precipitate which was filtered and washed with water and dried to obtain desired compound.

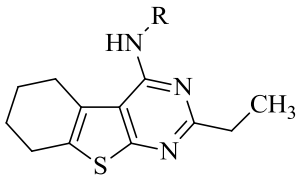
General procedure for the synthesis of PR_05 – PR_37



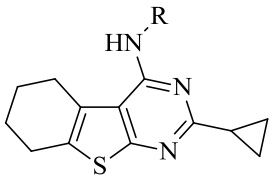
PR_04a-c **PR_05 - PR_37**

The mixture of compound **PR_04a/b/c** (1.0 equiv), substituted primary amines (**Table 5.5**) (R^2NH_2) (1.1 equiv) and *p*-toluenesulfonic acid (catalytic) were taken in methanol (3 volumes) and subjected to microwave irradiation (temperature 135 °C, pressure & power automatic) for 45 minutes. Ice water was added to the reaction mixture and the obtained solids were filtered, washed with water, cold ethanol and hexanes to get final compounds.

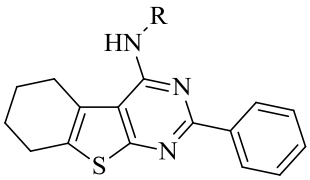
Table 5.5: Physiochemical properties of the synthesized compounds **PR_05** – **PR_37**



PR_05 - PR_15



PR_16 - PR_26



PR_27 - PR_37

Compd	R	Yield (%)	M.P. (°C)	Molecular formula	Molecular weight
PR_05	Phenyl	79	170-171	$C_{18}H_{19}N_3S$	309.43
PR_06	Benzyl	79	180-181	$C_{19}H_{21}N_3S$	323.46
PR_07	Phenethyl	91	191-192	$C_{20}H_{23}N_3S$	337.48
PR_08	2,4-Dimethylphenyl	69	141-142	$C_{20}H_{23}N_3S$	337.48
PR_09	2,5-Dimethylphenyl	76	196-197	$C_{20}H_{23}N_3S$	337.48
PR_10	2,6-Dimethylphenyl	84	201-202	$C_{20}H_{23}N_3S$	337.48
PR_11	4-Tolyl	87	182-183	$C_{19}H_{21}N_3S$	323.46
PR_12	4-Methoxyphenyl	74	243-244	$C_{19}H_{21}N_3OS$	339.45
PR_13	4-Bromophenyl	78	290-291	$C_{18}H_{18}BrN_3S$	388.32
PR_14	4-Chlorophenyl	83	279-280	$C_{18}H_{18}ClN_3S$	343.87
PR_15	4-Flurophenyl	84	264-265	$C_{18}H_{18}FN_3S$	327.42
PR_16	Phenyl	91	190-191	$C_{19}H_{19}N_3S$	321.44
PR_17	Benzyl	85	198-199	$C_{20}H_{21}N_3S$	335.47
PR_18	Phenethyl	78	204-205	$C_{21}H_{23}N_3S$	349.49
PR_19	2,4-Dimethylphenyl	92	213-214	$C_{21}H_{23}N_3S$	349.49
PR_20	2,5-Dimethylphenyl	87	222-223	$C_{21}H_{23}N_3S$	349.49
PR_21	2,6-Dimethylphenyl	80	116-117	$C_{21}H_{23}N_3S$	349.49
PR_22	4-Tolyl	93	218-219	$C_{20}H_{21}N_3S$	335.47
PR_23	4-Methoxyphenyl	91	209-210	$C_{20}H_{21}N_3OS$	351.47
PR_24	4-Bromophenyl	71	234-235	$C_{19}H_{18}BrN_3S$	400.34
PR_25	4-Chlorophenyl	78	225-226	$C_{19}H_{18}ClN_3S$	355.88
PR_26	4-Flurophenyl	80	236-237	$C_{19}H_{18}FN_3S$	339.43

Contd

Compd	R	Yield (%)	M.P. (°C)	Molecular formula	Molecular weight
PR_27	Phenyl	84	174-175	$C_{22}H_{19}N_3S$	357.47
PR_28	Benzyl	72	184-185	$C_{23}H_{21}N_3S$	371.50
PR_29	Phenethyl	70	190-191	$C_{24}H_{23}N_3S$	385.52
PR_30	2,4-Dimethylphenyl	79	196-197	$C_{24}H_{23}N_3S$	385.52
PR_31	2,5-Dimethylphenyl	81	156-157	$C_{24}H_{23}N_3S$	385.52
PR_32	2,6-Dimethylphenyl	64	150-151	$C_{24}H_{23}N_3S$	385.52
PR_33	4-Tolyl	72	190-191	$C_{23}H_{21}N_3S$	371.50
PR_34	4-Methoxyphenyl	68	180-181	$C_{23}H_{21}N_3OS$	387.50
PR_35	4-Bromophenyl	88	210-211	$C_{22}H_{18}BrN_3S$	436.47
PR_36	4-Chlorophenyl	79	214-215	$C_{22}H_{18}ClN_3S$	391.92
PR_37	4-Flurophenyl	90	222-223	$C_{22}H_{18}FN_3S$	375.46

5.2.3. Characterization of the synthesized molecules

2-Ethyl-*N*-phenyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-amine (PR_05): To the mixture of 2-ethyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-ol (**PR_04a**) (0.3 g, 1.28 mmol), aniline (0.13 mL, 1.41 mmol) and PTSA (cat) was added methanol (0.9 mL) and subjected to microwave irradiation (temperature 135 °C, pressure & power automatic) for 45 minutes. Ice water was added to the reaction mixture and the obtained solids were filtered, washed with water, cold ethanol and hexanes to get title compound **PR_05** (0.31 g, 79%) as an off-white solid. m.p. 170–171 °C; MS(ESI) *m/z* 310 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.24 (s, 1H), 7.92 (d, *J* = 7.6 Hz, 2H), 7.81–7.74 (m, 3H), 3.11 (q, *J* = 7.2 Hz, 2H), 3.06 (t, *J* = 7.2 Hz, 2H), 2.83 (t, *J* = 7.2 Hz, 2H), 1.86 (t, *J* = 6.8 Hz, 4H), 1.29 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 174.8, 158.4, 146.1, 140.3, 139.8, 133.3(2C), 129.0, 128.4, 124.3(2C), 118.4, 33.4, 25.2, 24.7, 24.1(2C), 12.5. Anal. calcd for $C_{18}H_{19}N_3S$: C, 69.87; H, 6.19; N, 13.58% Found C, 69.98; H, 6.29; N, 13.66%.

***N*-Benzyl-2-ethyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-amine (PR_06):** Yield: 79%; m.p. 180–181 °C; MS(ESI) *m/z* 324 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.66 (s, 1H), 7.56–7.48 (m, 5H), 4.41 (s, 2H), 3.13 (q, *J* = 7.2 Hz, 2H), 3.03 (t, *J* = 6.8 Hz, 2H), 2.84 (t, *J* = 7.2 Hz, 2H), 1.88 (t, *J* = 6.8 Hz, 4H), 1.25 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.9, 160.4, 146.5, 141.2, 138.6, 133.0, 130.3(2C), 128.4, 126.6(2C),

119.6, 49.3, 33.1, 24.8, 24.3, 23.4(2C), 12.3. Anal. calcd for $C_{19}H_{21}N_3S$: C, 70.55; H, 6.54; N, 12.99% Found C, 70.68; H, 6.69; N, 13.06%.

2-Ethyl-*N*-phenethyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-amine (PR_07): Yield: 91%; m.p. 191–192 °C; MS(ESI) *m/z* 338 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.31 (s, 1H), 7.60–7.49 (m, 5H), 3.49 (s, 2H), 3.11–2.96 (m, 4H), 2.89–2.74 (m, 4H), 1.83 (t, *J* = 6.8 Hz, 4H), 1.23 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.8, 160.1, 144.9, 139.3, 136.4, 128.5(2C), 127.3, 125.3(2C), 123.4, 118.2, 48.1, 36.3, 34.6, 25.2, 24.3, 23.8(2C), 13.2. Anal. calcd for $C_{20}H_{23}N_3S$: C, 71.18; H, 6.87; N, 12.45% Found C, 71.28; H, 6.99; N, 12.58%.

***N*-(2,4-Dimethylphenyl)-2-ethyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-amine (PR_08):** Yield: 69%; m.p. 141–142 °C; MS(ESI) *m/z* 338 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.34 (s, 1H), 7.63 (s, 1H), 7.38 (d, *J* = 7.2 Hz, 1H), 7.18 (d, *J* = 7.2 Hz, 1H), 3.09–2.94 (m, 4H), 2.81 (t, *J* = 6.8 Hz, 2H), 2.43 (s, 3H), 2.36 (s, 3H), 1.88 (t, *J* = 6.8 Hz, 4H), 1.24 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.6, 157.3, 146.8, 138.3, 137.5, 136.4, 133.3, 129.3, 127.4, 124.3, 119.5, 117.3, 34.2, 24.9, 24.3, 23.8(2C), 22.2, 20.9, 12.6. Anal. calcd for $C_{20}H_{23}N_3S$: C, 71.18; H, 6.87; N, 12.45% Found C, 71.28; H, 6.99; N, 12.56%.

***N*-(2,5-Dimethylphenyl)-2-ethyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-amine (PR_09):** Yield: 76%; m.p. 196–197 °C; MS(ESI) *m/z* 338 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.63–7.54 (m, 2H), 7.24 (s, 1H), 7.18 (d, *J* = 6.8 Hz, 1H), 3.14 (t, *J* = 6.8 Hz, 2H), 2.87–2.80 (m, 4H), 2.41 (s, 3H), 2.32 (s, 3H), 1.89 (t, *J* = 6.8 Hz, 4H), 1.26 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.4, 156.1, 145.9, 137.8, 136.5, 136.2, 133.1, 127.1, 125.3, 123.9, 120.4, 118.6, 34.4, 25.1, 24.6, 24.2(2C), 23.4, 20.7, 13.0. Anal. calcd for $C_{20}H_{23}N_3S$: C, 71.18; H, 6.87; N, 12.45% Found C, 71.26; H, 6.94; N, 12.61%.

***N*-(2,6-Dimethylphenyl)-2-ethyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-amine (PR_10):** Yield: 84%; m.p. 201–202 °C; MS(ESI) *m/z* 338 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.21 (s, 1H), 7.31 (d, *J* = 6.8 Hz, 2H), 7.09 (t, *J* = 6.4 Hz, 1H), 3.08 (t, *J* = 6.8 Hz, 2H), 2.90–2.82 (m, 4H), 2.31 (s, 6H), 1.86 (t, *J* = 6.8 Hz, 4H), 1.28 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.0, 152.4, 144.9, 136.9, 137.5(2C), 133.8(2C), 131.1, 129.4, 124.4, 119.3, 34.6, 25.3, 24.9(2C), 24.5(2C), 21.2, 12.7. Anal. calcd for $C_{20}H_{23}N_3S$: C, 71.18; H, 6.87; N, 12.45% Found C, 71.24; H, 6.90; N, 12.64%.

2-Ethyl-*N*-(*p*-tolyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-amine (PR_11): Yield: 87%; m.p. 182–183 °C; MS(ESI) *m/z* 325 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.91 (s, 1H), 7.46 (d, *J* = 7.6 Hz, 2H), 7.36 (d, *J* = 7.6 Hz, 2H), 3.12 (t, *J* = 7.2 Hz, 2H), 2.96–2.87 (m, 4H), 2.46 (s, 3H), 1.87 (t, *J* = 6.8 Hz, 4H), 1.29 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.2, 156.1, 147.3, 137.6, 136.8, 133.9, 129.5(2C), 126.8, 122.6(2C), 120.3, 34.5, 26.0, 24.5(2C), 23.2, 20.5, 13.2. Anal. calcd for $C_{19}H_{21}N_3S$: C, 70.55; H, 6.54; N, 12.99% Found C, 70.64; H, 6.60; N, 13.04%.

2-Ethyl-*N*-(4-methoxyphenyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-amine (PR_12): Yield: 74%; m.p. 243–244 °C; MS(ESI) *m/z* 340 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.46 (s, 1H), 7.54 (d, *J* = 7.6 Hz, 2H), 7.42 (d, *J* = 7.6 Hz, 2H), 3.96 (s, 3H), 3.08 (t, *J* = 6.8 Hz, 2H), 2.99–2.88 (m, 4H), 1.91 (t, *J* = 6.8 Hz, 4H), 1.30 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.9, 158.0, 142.4, 139.1, 134.6, 132.6, 131.4, 127.3(2C), 124.9(2C), 119.9, 61.2, 33.3, 25.6, 24.8(2C), 23.8, 12.7. Anal. calcd for $C_{19}H_{21}N_3OS$: C, 67.23; H, 6.24; N, 12.38% Found C, 67.34; H, 6.33; N, 12.44%.

***N*-(4-Bromophenyl)-2-ethyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-amine (PR_13):** Yield: 78%; m.p. 290–291 °C; MS(ESI) *m/z* 389 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.36 (s, 1H), 7.56 (d, *J* = 8.0 Hz, 2H), 7.48 (d, *J* = 8.0 Hz, 2H), 3.13 (t, *J* = 6.8 Hz, 2H), 2.89 (t, *J* = 6.8 Hz, 2H), 2.74 (t, *J* = 6.8 Hz, 2H), 1.86 (t, *J* = 7.2 Hz, 4H), 1.28 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 174.4, 156.7, 146.3, 140.1, 138.3, 136.5, 134.3, 130.4(2C), 126.3(2C), 120.1, 34.2, 26.1, 25.3(2C), 24.6, 13.2. Anal. calcd for $C_{18}H_{18}BrN_3S$: C, 55.67; H, 4.67; N, 10.82% Found C, 55.74; H, 4.73; N, 10.94%.

***N*-(4-Chlorophenyl)-2-ethyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-amine (PR_14):** Yield: 83%; m.p. 279–280 °C; MS(ESI) *m/z* 344 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.32 (s, 1H), 7.60 (d, *J* = 8.0 Hz, 2H), 7.49 (d, *J* = 8.0 Hz, 2H), 3.12 (t, *J* = 6.8 Hz, 2H), 2.91 (t, *J* = 6.8 Hz, 2H), 2.77 (t, *J* = 6.8 Hz, 2H), 1.89 (t, *J* = 7.2 Hz, 4H), 1.32 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 176.4, 155.4, 147.6, 141.3, 139.0, 136.2, 131.2(2C), 127.1(2C), 124.3, 120.9, 34.6, 27.3, 26.0(2C), 24.9, 13.9. Anal. calcd for $C_{18}H_{18}ClN_3S$: C, 62.87; H, 5.28; N, 12.22% Found C, 62.94; H, 5.33; N, 12.34%.

2-Ethyl-*N*-(4-fluorophenyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-amine (PR_15): Yield: 84%; m.p. 264–265 °C; MS(ESI) *m/z* 328 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.13 (s, 1H), 7.63 (d, *J* = 8.0 Hz, 2H), 7.11 (d, *J* = 8.0 Hz, 2H), 3.12 (t, *J* = 7.2

Hz, 2H), 2.81 (t, *J* = 7.2 Hz, 2H), 2.76 (t, *J* = 6.8 Hz, 2H), 1.90 (t, *J* = 7.2 Hz, 4H), 1.21 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.3, 154.7, 136.3, 132.4, 131.9, 128.7(3C), 126.4, 122.1(2C), 114.5, 30.9, 24.9, 22.2, 21.9, 20.5, 12.1. Anal. calcd for $C_{18}H_{18}FN_3S$: C, 66.03; H, 5.54; N, 12.83% Found C, 66.14; H, 5.63; N, 12.94%.

2-Cyclopropyl-*N*-phenyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-amine (PR_16): Yield: 91%; m.p. 190–191 °C; MS(ESI) *m/z* 322 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.64 (s, 1H), 7.82 (d, *J* = 7.6 Hz, 2H), 7.61–7.54 (m, 3H), 3.06 (t, *J* = 7.2 Hz, 2H), 2.80 (t, *J* = 7.2 Hz, 2H), 1.84 (t, *J* = 6.8 Hz, 4H), 1.56–1.51 (m, 1H), 1.29–1.11 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.8, 156.2, 144.0, 139.3, 136.4, 128.9(2C), 128.1, 126.5, 123.6(2C), 119.4, 25.4, 24.9, 22.4(2C), 13.3, 10.2(2C). Anal. calcd for $C_{19}H_{19}N_3S$: C, 70.99; H, 5.96; N, 13.07% Found C, 71.08; H, 6.04; N, 13.16%.

***N*-Benzyl-2-cyclopropyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-amine (PR_17):** Yield: 85%; m.p. 198–199 °C; MS(ESI) *m/z* 336 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.53 (s, 1H), 7.58–7.49 (m, 5H), 4.43 (s, 2H), 3.04 (t, *J* = 7.2 Hz, 2H), 2.86 (t, *J* = 7.2 Hz, 2H), 1.85 (t, *J* = 6.8 Hz, 4H), 1.54–1.49 (s, 1H), 1.27–1.14 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.9, 159.4, 146.3, 139.4, 137.9, 136.3, 128.3(2C), 126.1, 125.8(2C), 117.9, 47.8, 24.6, 24.0, 23.6(2C), 12.9. 9.99 (2C). Anal. calcd for $C_{20}H_{21}N_3S$: C, 71.61; H, 6.31; N, 12.53% Found C, 71.68; H, 6.39; N, 12.66%.

2-Cyclopropyl-*N*-phenethyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-amine (PR_18): Yield: 78%; m.p. 204–205 °C; MS(ESI) *m/z* 350 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.31 (s, 1H), 7.74 (t, *J* = 7.2 Hz, 2H), 7.62–7.54 (m, 3H), 3.47 (s, 2H), 3.13–2.97 (m, 4H), 2.83 (t, *J* = 6.8 Hz, 2H), 1.86 (t, *J* = 6.8 Hz, 4H), 1.56–1.50 (m, 1H), 1.26–1.16 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.4, 159.6, 144.7, 138.6, 137.2, 129.2(2C), 128.1, 126.3(2C), 125.3, 117.4, 47.3, 36.9, 25.4, 24.7, 24.2(2C), 13.0, 10.3(2C). Anal. calcd for $C_{21}H_{23}N_3S$: C, 72.17; H, 6.63; N, 12.02% Found C, 72.28; H, 6.69; N, 12.08%.

2-Cyclopropyl-*N*-(2,4-dimethylphenyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-amine (PR_19): Yield: 92%; m.p. 213–214 °C; MS(ESI) *m/z* 350 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.36 (s, 1H), 7.49 (s, 1H), 7.36 (d, *J* = 7.2 Hz, 1H), 7.12 (d, *J* = 7.2 Hz, 1H), 3.09 (d, *J* = 7.2 Hz, 1H), 2.94 (d, *J* = 7.2 Hz, 1H), 2.41 (s, 3H), 2.37 (s, 3H), 1.85 (t, *J* = 6.8 Hz, 4H), 1.52–1.48 (m, 1H), 1.25–1.11 (m, 4H); ^{13}C NMR (100 MHz,

DMSO-d_6) δ 172.6, 154.3, 144.3, 139.4, 138.1, 136.3, 132.8, 129.3, 128.2, 126.6, 125.4, 119.6, 25.8, 25.0, 24.6(2C), 24.3, 23.8, 12.6, 9.3(2C). Anal. calcd for $C_{21}H_{23}N_3S$: C, 72.17; H, 6.63; N, 12.02% Found C, 72.28; H, 6.79; N, 12.06%.

2-Cyclopropyl-*N*-(2,5-dimethylphenyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-amine (PR_20): Yield: 87%; m.p. 222–223 °C; MS(ESI) *m/z* 350 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.46 (s, 1H), 7.45 (d, *J* = 7.6 Hz, 1H), 7.39–7.31 (m, 2H), 3.11 (d, *J* = 7.2 Hz, 1H), 2.97 (d, *J* = 7.2 Hz, 1H), 2.44 (s, 3H), 2.39 (s, 3H), 1.88 (t, *J* = 7.2 Hz, 4H), 1.53–1.47 (m, 1H), 1.26–1.14 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.9, 155.2, 145.1, 138.8, 138.1, 135.4, 133.2, 129.2, 127.6, 126.2, 126.0, 120.0, 25.6, 25.3, 24.9(2C), 24.6, 22.2, 11.8, 9.1(2C). Anal. calcd for $C_{21}H_{23}N_3S$: C, 72.17; H, 6.63; N, 12.02% Found C, 72.26; H, 6.72; N, 12.09%.

2-Cyclopropyl-*N*-(2,6-dimethylphenyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-amine (PR_21): Yield: 80%; m.p. 116–117 °C; MS(ESI) *m/z* 350 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.42 (d, *J* = 7.6 Hz, 2H), 7.27–7.19 (m, 2H), 3.04 (d, *J* = 7.2 Hz, 1H), 2.94 (d, *J* = 7.2 Hz, 1H), 2.33 (s, 6H), 1.91 (t, *J* = 7.2 Hz, 4H), 1.51–1.46 (m, 1H), 1.24–1.12 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.9, 153.6, 146.3, 138.4, 136.3, 133.1, 130.2(2C), 129.5(2C), 126.2, 120.4, 25.2, 24.7, 23.8(2C), 21.4(2C), 11.6, 9.0(2C). Anal. calcd for $C_{21}H_{23}N_3S$: C, 72.17; H, 6.63; N, 12.02% Found C, 72.26; H, 6.72; N, 12.09%.

2-Cyclopropyl-*N*-(*p*-tolyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-amine (PR_22): Yield: 93%; m.p. 218–219 °C; MS(ESI) *m/z* 336 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.36 (s, 1H), 7.42 (d, *J* = 8.0 Hz, 2H), 7.33 (d, *J* = 8.0 Hz, 2H), 3.08 (t, *J* = 7.6 Hz, 2H), 2.98 (t, *J* = 7.6 Hz, 2H), 2.41 (s, 3H), 1.84 (t, *J* = 7.2 Hz, 4H), 1.52–1.47 (m, 1H), 1.23–1.08 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.5, 154.3, 146.3, 138.4, 137.3, 131.8, 128.7(2C), 127.3, 123.6(2C), 116.1, 26.1, 25.3(2C), 24.6, 22.3, 12.3, 9.3(2C). Anal. calcd for $C_{20}H_{21}N_3S$: C, 71.61; H, 6.31; N, 12.53% Found C, 71.66; H, 6.40; N, 12.64%.

2-Cyclopropyl-*N*-(4-methoxyphenyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-amine (PR_23): Yield: 91%; m.p. 209–210 °C; MS(ESI) *m/z* 352 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.39 (s, 1H), 7.63 (d, *J* = 7.6 Hz, 2H), 7.48 (d, *J* = 7.6 Hz, 2H), 4.06 (s, 3H), 3.06 (t, *J* = 7.2 Hz, 2H), 2.91 (t, *J* = 7.2 Hz, 2H), 1.87 (t, *J* = 6.8 Hz, 4H), 1.48–1.43 (m, 1H), 1.26–1.05 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.8, 156.3, 155.4, 144.3,

138.3, 135.2, 128.2, 126.4(2C), 124.2(2C), 123.2, 61.9, 25.2, 24.4(2C), 23.4, 12.4, 9.7(2C). Anal. calcd for $C_{20}H_{21}N_3OS$: C, 68.35; H, 6.02; N, 11.96 % Found C, 68.39; H, 6.13; N, 12.04%.

***N*-(4-Bromophenyl)-2-cyclopropyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-amine (PR_24):** Yield: 71%; m.p. 234–235 °C; MS(ESI) *m/z* 400 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.42 (s, 1H), 7.81 (d, *J* = 7.6 Hz, 2H), 7.54 (d, *J* = 7.6 Hz, 2H), 3.01 (t, *J* = 7.2 Hz, 2H), 2.81 (t, *J* = 7.2 Hz, 2H), 1.89 (t, *J* = 7.2 Hz, 4H), 1.53–1.44 (m, 1H), 1.27–1.08 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.6, 154.6, 145.9, 140.3, 136.9, 134.6(2C), 132.0, 124.3(2C), 120.4, 118.6, 25.5, 24.7(2C), 24.1, 11.6, 10.1(2C). Anal. calcd for $C_{19}H_{18}BrN_3S$: C, 57.00; H, 4.53; N, 10.50% Found C, 57.04; H, 4.63; N, 10.64%.

***N*-(4-Chlorophenyl)-2-cyclopropyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-amine (PR_25):** Yield: 78%; m.p. 225–226 °C; MS(ESI) *m/z* 356 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.63 (s, 1H), 7.77 (d, *J* = 7.6 Hz, 2H), 7.56 (d, *J* = 7.6 Hz, 2H), 3.05 (t, *J* = 7.2 Hz, 2H), 2.88 (t, *J* = 7.2 Hz, 2H), 1.87 (t, *J* = 7.2 Hz, 4H), 1.52–1.46 (m, 1H), 1.25–1.06 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.7, 155.5, 146.4, 139.4, 135.3, 132.3(2C), 130.1, 128.5, 126.7(2C), 119.3, 24.9, 24.3(2C), 23.5, 11.7, 9.4(2C). Anal. calcd for $C_{19}H_{18}ClN_3S$: C, 64.12; H, 5.10; N, 11.81% Found C, 64.24; H, 5.23; N, 11.94%.

2-Cyclopropyl-*N*-(4-fluorophenyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-amine (PR_26): Yield: 80%; m.p. 236–237 °C; MS(ESI) *m/z* 340 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.35 (s, 1H), 7.56 (d, *J* = 7.6 Hz, 2H), 7.44 (d, *J* = 7.6 Hz, 2H), 3.10 (t, *J* = 7.2 Hz, 2H), 2.92 (t, *J* = 7.2 Hz, 2H), 1.88 (t, *J* = 7.2 Hz, 4H), 1.49–1.45 (m, 1H), 1.23–1.10 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.4, 158.4, 149.6, 144.2, 137.2, 136.7, 128.3, 126.1(2C), 124.7(2C), 121.3, 25.4, 24.3, 24.0(2C), 11.6, 10.3(2C). Anal. calcd for $C_{19}H_{18}FN_3S$: C, 67.23; H, 5.35; N, 12.38% Found C, 67.34; H, 5.63; N, 12.44%.

***N*,2-Diphenyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-amine (PR_27):** Yield: 84%; m.p. 174–175 °C; MS(ESI) *m/z* 358 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.37 (d, *J* = 7.6 Hz, 2H), 7.82–7.69 (m, 6H), 7.54–7.42 (m, 3H), 3.03 (t, *J* = 7.6 Hz, 2H), 2.85 (t, *J* = 7.2 Hz, 2H), 1.87 (t, *J* = 7.2 Hz, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.4, 155.2, 147.5, 142.7, 139.2, 137.4, 133.2, 128.1(2C), 127.3(2C), 126.0, 125.2, 124.6(2C), 120.8(2C), 117.2, 25.6, 24.3, 23.6(2C). Anal. calcd for $C_{22}H_{19}N_3S$: C, 73.92; H, 5.36; N, 11.75% Found C, 73.99; H, 5.63; N, 12.04%.

***N*-Benzyl-2-phenyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-amine (PR_28):** Yield: 72%; m.p. 184–185 °C; MS(ESI) *m/z* 372 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.36 (s, 1H), 7.81 (d, *J* = 7.2 Hz, 2H), 7.67–7.56 (m, 3H), 7.48–7.32 (m, 5H), 4.42 (s, 2H), 3.08 (t, *J* = 7.2 Hz, 2H), 2.89 (t, *J* = 7.2 Hz, 2H), 1.84 (t, *J* = 7.2 Hz, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.9, 156.6, 146.3, 141.3, 138.8, 137.2, 133.6, 127.7(2C), 127.1(2C), 125.8, 125.3, 124.3(2C), 121.2(2C), 118.4, 45.2, 24.9, 24.1, 23.2(2C). Anal. calcd for $C_{23}H_{21}N_3S$: C, 74.36; H, 5.70; N, 11.31% Found C, 74.49; H, 5.83; N, 11.44%.

***N*-Phenethyl-2-phenyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-amine (PR_29):** Yield: 70%; m.p. 190–191 °C; MS(ESI) *m/z* 386 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.43 (s, 1H), 8.11 (d, *J* = 7.2 Hz, 2H), 7.77–7.68 (m, 4H), 7.62–7.48 (m, 4H), 3.46 (t, *J* = 6.8 Hz, 2H), 3.04 (t, *J* = 6.8 Hz, 2H), 2.89–2.65 (m, 4H), 1.87 (t, *J* = 6.8 Hz, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.2, 158.3, 144.6, 140.3, 139.2, 137.6, 134.2, 127.9(2C), 127.2(2C), 126.2, 125.5, 124.1(2C), 122.1(2C), 119.3, 46.1, 36.9, 24.7, 23.8, 23.4(2C). Anal. calcd for $C_{24}H_{23}N_3S$: C, 74.77; H, 6.01; N, 10.90% Found C, 74.89; H, 6.13; N, 11.04%.

***N*-(2,4-Dimethylphenyl)-2-phenyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-amine (PR_30):** Yield: 79%; m.p. 196–197 °C; MS(ESI) *m/z* 386 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.16 (s, 1H), 7.84 (d, *J* = 7.2 Hz, 2H), 7.72–7.66 (m, 3H), 7.56 (s, 1H), 7.49–7.41 (m, 2H), 3.03 (d, *J* = 6.8 Hz, 2H), 2.91 (d, *J* = 6.8 Hz, 2H), 2.43 (s, 3H), 2.34 (s, 3H), 1.82 (t, *J* = 6.8 Hz, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 174.9, 155.3, 145.7, 138.6, 137.4, 136.7, 133.9, 130.3, 129.6, 128.6, 127.3, 126.6(2C), 125.9, 125.1, 123.5(2C), 118.4, 25.3, 24.7, 24.2(2C), 23.1, 22.2. Anal. calcd for $C_{24}H_{23}N_3S$: C, 74.77; H, 6.01; N, 10.90% Found C, 74.88; H, 6.09; N, 11.06%.

***N*-(2,5-dimethylphenyl)-2-phenyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-amine (PR_31):** Yield: 81%; m.p. 156–157 °C; MS(ESI) *m/z* 386 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.37 (s, 1H), 7.85 (d, *J* = 7.2 Hz, 2H), 7.68–7.58 (m, 3H), 7.53–7.47 (m, 3H), 3.04 (d, *J* = 6.8 Hz, 2H), 2.92 (d, *J* = 6.8 Hz, 2H), 2.41 (s, 3H), 2.33 (s, 3H), 1.82 (t, *J* = 6.8 Hz, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.7, 156.6, 146.3, 139.5, 138.7, 136.8, 134.8, 133.9, 133.0, 130.6, 128.6(2C), 127.9(2C), 125.7, 123.1, 120.4, 119.2, 25.9, 24.8, 24.2(2C), 23.1, 21.4. Anal. calcd for $C_{24}H_{23}N_3S$: C, 74.77; H, 6.01; N, 10.90 % Found C, 74.86; H, 6.12; N, 10.99%.

***N*-(2,6-Dimethylphenyl)-2-phenyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-amine (PR_32):** Yield: 64%; m.p. 150–151 °C; MS(ESI) *m/z* 386 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.37 (d, *J* = 7.2 Hz, 2H), 7.87–7.69 (m, 3H), 7.63 (d, *J* = 7.2 Hz, 2H), 7.58–7.53 (m, 2H), 3.06 (d, *J* = 6.8 Hz, 2H), 2.91 (d, *J* = 6.8 Hz, 2H), 2.31 (s, 6H), 1.86 (t, *J* = 6.8 Hz, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.4, 154.3, 145.8, 139.0, 137.1, 135.8, 133.6(2C), 132.6, 130.6(2C), 129.3(2C), 128.7(2C), 128.2, 126.7, 121.3, 24.7, 24.3, 23.4(2C), 19.9(2C). Anal. calcd for $C_{24}H_{23}N_3S$: C, 74.77; H, 6.01; N, 10.90% Found C, 74.86; H, 6.12; N, 11.01%.

2-Phenyl-*N*-(*p*-tolyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-amine (PR_33): Yield: 72%; m.p. 190–191 °C; MS(ESI) *m/z* 372 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.43 (s, 1H), 8.26 (d, *J* = 7.2 Hz, 2H), 7.82–7.74 (m, 3H), 7.67–7.56 (m, 4H), 3.03 (t, *J* = 7.2 Hz, 2H), 2.89 (t, *J* = 7.2 Hz, 2H), 2.40 (s, 3H), 1.83 (t, *J* = 6.8 Hz, 4H), 1.52–1.47 (m, 1H), 1.23–1.08 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.7, 155.4, 144.8, 139.2, 138.0, 136.3, 134.2, 130.4(2C), 129.3(2C), 127.8(2C), 127.1, 126.3(2C), 120.4, 117.9, 25.4, 24.7(2C), 23.8, 22.5. Anal. calcd for $C_{23}H_{21}N_3S$: C, 74.36; H, 5.70; N, 11.31 % Found C, 74.46; H, 5.80; N, 11.44%.

***N*-(4-Methoxyphenyl)-2-phenyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-amine (PR_34):** Yield: 68%; m.p. 180–181 °C; MS(ESI) *m/z* 388 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (d, *J* = 7.6 Hz, 2H), 7.83 (d, *J* = 7.6 Hz, 2H), 7.68–7.60 (m, 6H), 3.96 (s, 3H), 3.12 (t, *J* = 7.2 Hz, 2H), 2.96 (t, *J* = 7.2 Hz, 2H), 1.89 (t, *J* = 6.8 Hz, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.9, 158.5, 156.5, 146.1, 139.6, 138.3, 136.2, 134.7, 130.6(2C), 129.1(2C), 127.8, 127.2(2C), 124.7(2C), 121.1, 60.3, 25.1, 24.3(2C), 22.8. Anal. calcd for $C_{23}H_{21}N_3OS$: C, 71.29; H, 5.46; N, 10.84 % Found C, 71.38; H, 5.62; N, 10.94%.

***N*-(4-Bromophenyl)-2-phenyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-amine (PR_35):** Yield: 88%; m.p. 210–211 °C; MS(ESI) *m/z* 436 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.91 (s, 1H), 8.31 (d, *J* = 6.8 Hz, 2H), 7.87 (d, *J* = 6.8 Hz, 2H), 7.62–7.56 (m, 5H), 3.14 (t, *J* = 6.4 Hz, 2H), 2.87 (t, *J* = 6.4 Hz, 2H), 1.83 (t, *J* = 6.4 Hz, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.3, 157.2, 152.8, 144.6, 139.7, 136.7, 135.1, 129.2(2C), 127.8(2C), 127.0, 126.1(2C), 125.3, 124.2(2C), 118.0, 25.1, 24.4, 23.8(2C). Anal. calcd for $C_{22}H_{18}BrN_3S$: C, 60.55; H, 4.16; N, 9.63% Found C, 60.69; H, 4.29; N, 9.74%.

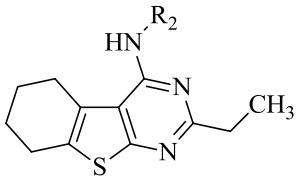
***N*-(4-Chlorophenyl)-2-phenyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-amine (PR_36):** Yield: 79%; m.p. 214–215 °C; MS(ESI) *m/z* 392 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.33–8.28 (m, 3H), 7.81 (d, *J* = 7.2 Hz, 2H), 7.53–7.48 (m, 5H), 3.13 (t, *J* = 6.8 Hz, 2H), 2.81 (t, *J* = 6.8 Hz, 2H), 1.87 (t, *J* = 6.8 Hz, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.3, 156.1, 146.4, 141.3, 138.9, 137.8, 134.0, 129.2(2C), 128.3(2C), 127.3, 126.6(2C), 125.3, 124.2(2C), 118.7, 25.3, 24.8, 24.1(2C). Anal. calcd for $C_{22}H_{18}ClN_3S$: C, 67.42; H, 4.63; N, 10.72% Found C, 67.49; H, 4.69; N, 10.84%.

***N*-(4-Fluorophenyl)-2-phenyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-amine (PR_37):** Yield: 90%; m.p. 222–223 °C; MS(ESI) *m/z* 376 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.21 (s, 1H), 8.30 (d, *J* = 6.8 Hz, 2H), 7.85 (d, *J* = 6.8 Hz, 2H), 7.60–7.54 (m, 5H), 3.10 (t, *J* = 6.4 Hz, 2H), 2.85 (t, *J* = 6.4 Hz, 2H), 1.84 (t, *J* = 6.4 Hz, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.7, 156.6, 151.4, 144.2, 138.4, 137.2, 134.4, 129.4(2C), 127.1(2C), 126.5, 125.2(2C), 124.1, 123.4(2C), 118.4, 25.6, 24.7, 24.3(2C). Anal. calcd for $C_{22}H_{18}FN_3S$: C, 70.38; H, 4.83; N, 11.19% Found C, 70.49; H, 4.99; N, 11.24%.

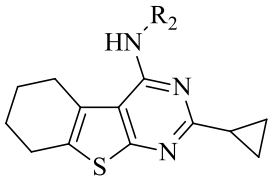
5.2.4. *In vitro M. tuberculosis* screening, *M. tuberculosis* ADH enzyme inhibition assay and cytotoxicity studies of the synthesized molecules

All the synthesized compounds were screened for their *in vitro* anti-tubercular activity against *M. tuberculosis* H37Rv using MABA method. All the synthesized compounds were also screened for their *in vitro M. tuberculosis* ADH inhibitory potency and *in vitro* cytotoxicity against RAW 264.7 cells at 50 μM concentration using MTT assay and the results are tabulated in **Table 5.6**.

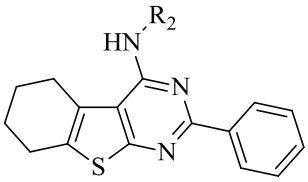
Table 5.6: *In vitro* biological evaluation of the synthesized compounds **PR_05 – PR_37**



PR_05 - PR_15



PR_16 - PR_26



PR_27 - PR_37

Compd	R	MTB ADH IC_{50} in µM	MTB MIC in µM[a]	Cytotoxicity[b] at 50 µM % inhibition
PR_05	Phenyl	7.27	20.20	7.82
PR_06	Benzyl	6.03	38.64	7.05
PR_07	Phenethyl	1.82	37.04	25.23
PR_08	2,4-Dimethylphenyl	7.41	74.08	9.65
PR_09	2,5-Dimethylphenyl	27.18	74.08	29.89
PR_10	2,6-Dimethylphenyl	5.02	18.52	26.54
PR_11	4-Tolyl	3.26	77.29	15.62
PR_12	4-Methoxyphenyl	4.68	73.65	15.18
PR_13	4-Bromophenyl	6.54	32.19	12.56
PR_14	4-Chlorophenyl	4.94	18.18	15.62
PR_15	4-Flurophenyl	6.05	38.18	13.98
PR_16	Phenyl	36.37	9.72	8.80
PR_17	Benzyl	4.23	18.63	23.05
PR_18	Phenethyl	17.03	35.77	17.45
PR_19	2,4-Dimethylphenyl	9.00	71.53	23.88
PR_20	2,5-Dimethylphenyl	2.07	35.77	16.34
PR_21	2,6-Dimethylphenyl	7.55	17.88	13.51
PR_22	4-Tolyl	13.43	74.52	16.25
PR_23	4-Methoxyphenyl	>25	17.78	34.40
PR_24	4-Bromophenyl	8.93	15.61	33.39
PR_25	4-Chlorophenyl	11.12	8.78	15.49

Contd

Compd	R	MTB ADH IC_{50} in µM	MTB MIC in µM[a]	Cytotoxicity[b] at 50 µM % inhibition
PR_26	4-Flurophenyl	8.91	36.83	4.82
PR_27	Phenyl	11.37	4.36	7.15
PR_28	Benzyl	9.68	33.65	26.52
PR_29	Phenethyl	5.60	64.85	24.58
PR_30	2,4-Dimethylphenyl	9.96	16.21	7.97
PR_31	2,5-Dimethylphenyl	5.82	2.02	3.23
PR_32	2,6-Dimethylphenyl	8.82	8.11	25.46
PR_33	4-Tolyl	7.97	33.65	41.14
PR_34	4-Methoxyphenyl	8.72	16.13	28.19
PR_35	4-Bromophenyl	8.70	28.64	20.35
PR_36	4-Chlorophenyl	2.72	63.79	20.80
PR_37	4-Flurophenyl	12.18	33.29	33.41
Isoniazid		NT	0.72	NT
Ethambutol		NT	7.64	NT
GSK16374A		13.23	0.76	NT

IC_{50}, 50% inhibitory concentration; MTB, *Mycobacterium tuberculosis*; MIC, minimum inhibitory concentration; [a]*In vitro* activity against MTB H37Rv; [b]Against RAW 264.7 cells, NT, Not tested.

5.2.5. SAR and discussion

All the synthesized compounds showed activity against *M. tuberculosis* with MIC ranging from 2.02 to 77.29 µM. Out of synthesized compounds, twelve compounds (**PR_14**, **PR_16** – **PR_17**, **PR_21**, **PR_23** – **PR_25**, **PR_27**, **PR_30** – **PR_32** and **PR_34**) inhibited *M. tuberculosis* with MIC of <20 µM. Five compounds (**PR_16**, **PR_25**, **PR_27**, and **PR_31** – **PR_32**) inhibited *M. tuberculosis* with MIC of <10 µM. Compound **PR_31** was found to be the most active compound with *in vitro* MIC of 2.02 µM. Two compound **PR_27** and **PR_31** were more potent than ethambutol.

With respect to SAR, the compounds containing phenyl ring (**PR_27** – **PR_37**) showed better activities than compounds with ethyl group (**PR_05** – **PR_15**) or cyclopropyl group (**PR_16** – **PR_26)**. Among the compounds, in general compounds (**PR_13** – **PR_15**, **PR_24** – **PR_26** and **PR_35** **PR_36**) with electron withdrawing substitutions on phenyl ring at R^2 position

showed better activity compared to compounds (**PR_08 PR_12**, **PR_19** – **PR_23** and **PR_30** – **PR_34**) with electron donating groups. In order to evaluate the mechanism of activity, the compounds were evaluated for various *M. tuberculosis* enzymes. All the synthesized compounds showed activity against *M. tuberculosis* ADH with IC_{50} ranging from 1.82 to 27.18 µM. Twenty five compounds inhibited *M. tuberculosis* ADH with IC_{50} of <10 µM. Compound **PR_07** was found to be the most potent ADH inhibitor with IC_{50} of 1.82 µM.

5.2.6. Evaluation of protein interaction and stability using biophysical characterization experiment

The binding affinity of the most potent derivative **PR_07** was evaluated by measuring the thermal stability of the protein-ligand complex using DSF. Protein complexes with ligand were heated from 25 to 95 °C in steps of 0.1 °C in the presence of sypro orange. The fluorescence increased when the protein interacted with hydrophobic residues. The curves obtained in this are depicted in **Figure 5.10**. The protein *M. tuberculosis* ADH showed a melting temperature of 50.20 °C, whereas with compound **PR_07** the corresponding T_m was found to be 51.60 °C. The difference in the T_m indicated the stability of the native protein when it was bound with this compound.

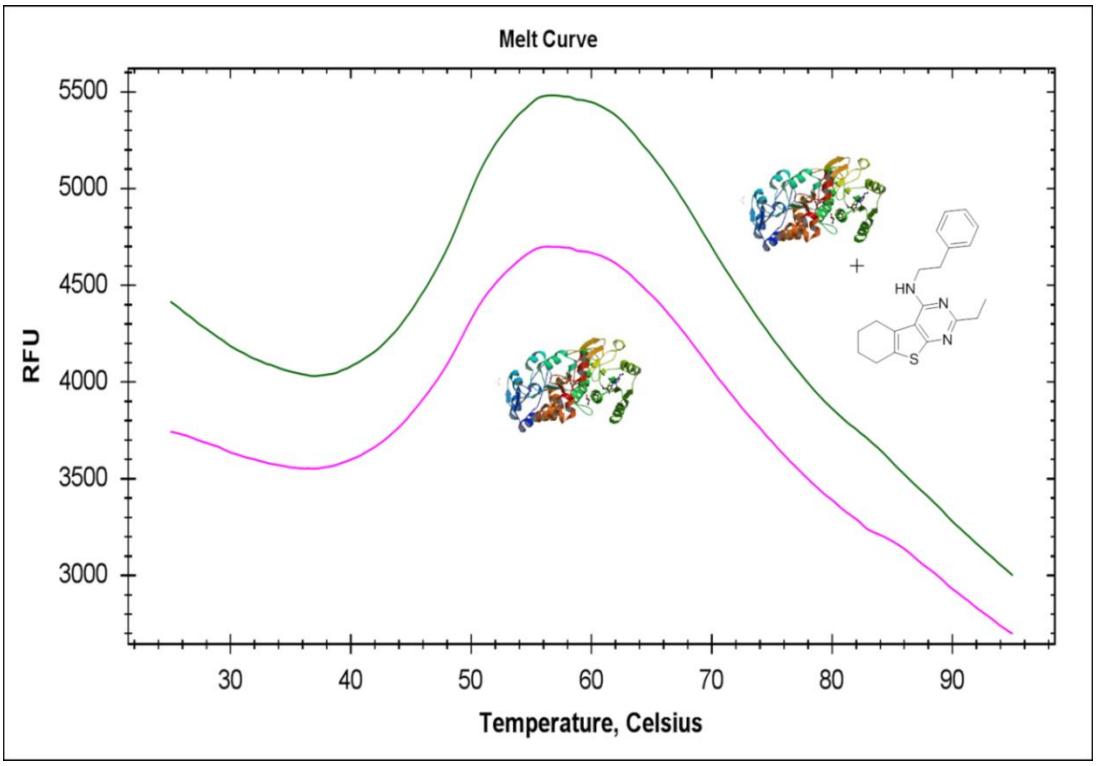


Figure 5.10: DSF experiment for compound **PR_07** (protein-ligand complex, green) showing an increase in the thermal shift of 1.4 °C when compared to the native ADH protein (red)

5.2.7. Highlights of the study

In summary, we identified a novel lead and synthesized derivatives from a reported anti-tubercular compound **GSK163574A**. Twenty five out of thirty three compounds showed *M. tuberculosis* ADH inhibition with IC_{50} <10 µM and many of them showed good *M. tubercular* MICs. Compound **PR_31** (*N*-(2,5-dimethylphenyl)-2-phenyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-amine) was found to be the most active compound with *M. tuberculosis* ADH IC_{50} of 5.82 µM and inhibited drug sensitive *M. tuberculosis* with MIC of 2.02 µM (**Figure 5.11**).

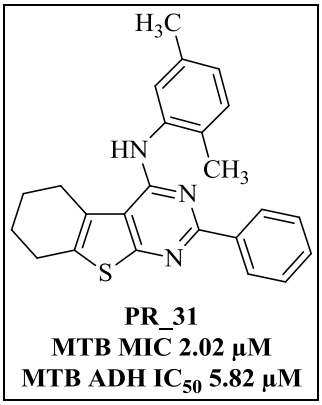


Figure 5.11: Chemical structure and biological activity of the most active compound **PR_31**

5.3. Synthesis of 3-phenyl-4,5,6,7-tetrahydro-1*H*-pyrazolo[4,3-*c*]pyridine derivatives as novel *M. tuberculosis* pantothenate synthetase inhibitors

5.3.1. Design of the molecules

In literature there were reports on fused pyrazole ring containing compounds as potential anti-tubercular agents [Vimal P.I., *et al*., 2010; Hardesh M.K., *et al*., 2013]. Recently Prashant Aragade and co-workers reported pyrazolocoumarins derivatives as potential anti-tubercular agents [Aragade P., *et al*., 2013]. From our experience in anti-tubercular drug discovery, we decided to take the most active compound among these reported derivatives as initial lead molecule and replaced of coumarin ring at 5th position of pyrazole ring with fused tetrahydropyridine ring (**Figure 5.12**). Based on the availability of starting materials and synthetic feasibility we performed modification at rings A, B and also synthesized urea, thiourea and sulphonamide derivatives using nitrogen atom of fused pyrazolopiperidine ring to generate a library of molecules.

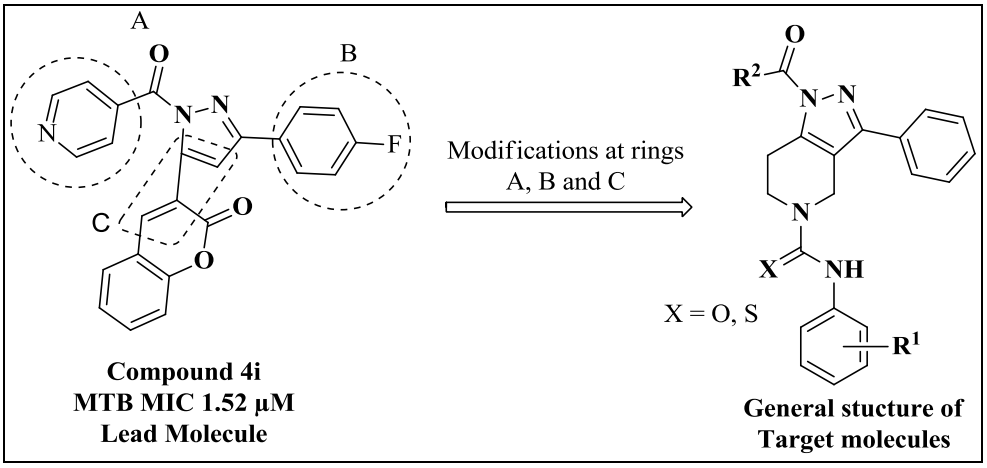


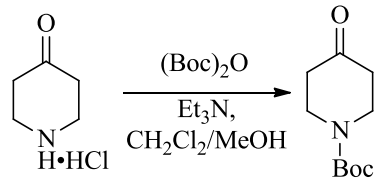
Figure 5.12: Structure of Lead molecule (**4i**) for the synthesis of compounds **PP_07 – PP_46**

5.3.2. Experimental procedures utilized for the synthesis of PP_07 – PP_46

The target molecules were synthesized in five steps (**Figure 4.4**), starting with commercially available, less expensive, 4-piperidone hydrochloride salt (**1**). Initially the amine was protected by *t*-butyloxycarbonyl (Boc) protecting group to get 4-*N*-Boc-piperidone (**PP_02**), and was subjected to stark-enamine reaction conditions using morpholine, *p*-toluenesulfonic acid (catalytic) and benzoyl chloride to produce 1,3-dicarbonyl intermediate; which was then treated *in situ* with hydrazine hydrate to get pyrazole ring (**PP_03**) [Ye X.M., *et al*., 2010].

Compound **PP_03** was then deprotected using trifluoroacetic acid to get compound **PP_04**. Under mild conditions the more nucleophilic amine of aliphatic ring was reacted selectively with various substituted isocyanates, isothiocyanates and arylsulphonyl halides using DIPEA as base and DMF as solvent at room temperature to yield corresponding urea, thiourea and sulphonamides (**PP_05a-b** and **PP_06**). The free amino group of pyrazole ring was finally treated with benzoyl/cyclohexanecarbonyl chloride using DIPEA as base to get target compounds **PP_07 – PP_46**.

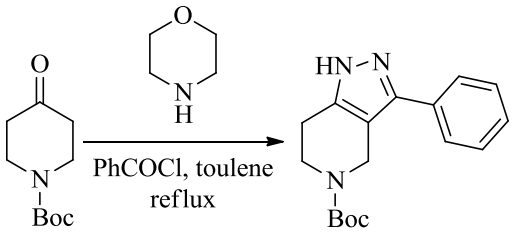
Preparation of *tert*-butyl 4-oxopiperidine-1-carboxylate (PP_02)



1 **PP_02**

Et_3N (26.45 mL, 185.17 mmol) was added dropwise to a stirred solution of compound **1** (10.0 g, 74.07 mmol) in CH_2Cl_2 (100 mL) and MeOH (10 mL) at 0 °C, then $(Boc)_2O$ (19.37 mL, 88.88 mmol) was added drop wise to the reaction mixture at same temperature and allowed to stir at room temperature for 16 h. The reaction mixture was diluted with H_2O (100 mL) and extracted with CH_2Cl_2 (3 X 200 mL). The separated organic layer was concentrated under reduced pressure and the crude residue was washed with hexanes to get compound **PP_02** (11.5 g, 78%) as an off-white solid. ESI-MS found 200 $[M+H]^+$and carried to next step.

Preparation of *tert* -butyl 3-phenyl-6,7-dihydro-1*H*-pyrazolo[4,3-*c*]pyridine-5(4*H*)-carboxylate (PP_03)

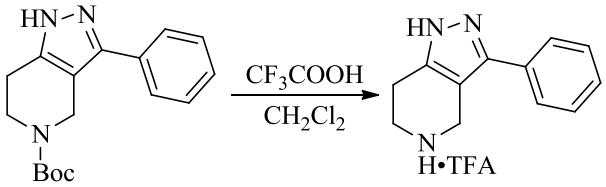


PP_02 **PP_03**

In a 100 mL round-bottom flask equipped with a Dean-stark trap, a reflux condenser and an internal thermocouple, compound **PP_02** (1.0 g, 4.63 mmol), toluene (10 mL), morpholine (0.42 mL, 4.63 mmol), and *p*-toluenesulfonic acid (catalytic) were added sequentially. The

reaction solution was refluxed under N_2 atmosphere for 16 h. The solvent was evaporated and the crude residue was dissolved in CH_2Cl_2 (20 mL) and then Et_3N (1.07 mL, 7.53 mmol) was added at 0 °C, and under N_2 atmosphere a solution of benzoyl chloride (0.58 mL, 5.02 mmol) in CH_2Cl_2 (10 mL) was added over 10 min. The ice bath was then removed and the reaction solution was stirred at room temperature for 4 h. All the volatile solvents were removed *in vacuo* and the residue was dissolved in ethanol at 0 °C, and hydrazine hydrate (0.25 mL, 7.53 mmol) was then added over 5 min (exothermic reaction) and was stirred at room temperature for 16 h. The reaction mixture was concentrated under reduced pressure and the crude residue was purified by column chromatography to get compound **PP_03** (1.34 g, 90%) as an off-white solid. ESI-MS found 300 $[M+H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.45 (s, 1H), 7.5–7.2 (m, 5H), 4.80 (s, 2H), 3.90 (t, J = 7.8 Hz, 2H), 2.81 (t, J = 7.8 Hz, 2H), 1.40 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.5, 147.6, 143.5, 136.6(2C), 132.7, 129,3(2C), 127.3, 117.5, 81.7, 43.9, 36.9, 31.5(3C), 27.7; Anal. calcd for $C_{17}H_{21}N_3O_2$: C, 68.20; H, 7.07; N, 14.04% Found C, 68.24; H, 7.09; N, 14.13%.

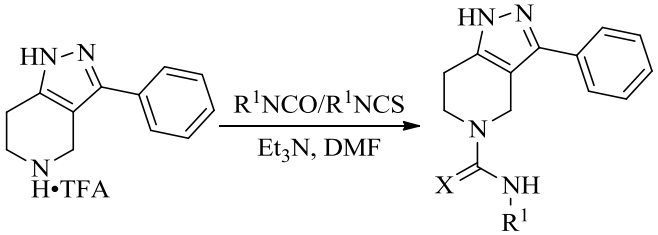
Preparation of 3-phenyl-4,5,6,7-tetrahydro-1*H*-pyrazolo[4,3-*c*]pyridine (PP_04)



PP_03 **PP_04**

CF_3COOH (0.65 mL, 8.36 mmol) was added dropwise to a stirred solution of compound **PP_03** (0.6 g, 1.67 mmol) in CH_2Cl_2 (10 mL) at 0 °C, and allowed to stir at room temperature for 4 h. The reaction mixture was concentrated under reduced pressure and the crude residue was washed with hexanes and diethyl ether to get compound **PP_04** (0.45 g, 91%) as an off-white solid. ESI-MS showed 200 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) 7.38–7.10 (m, 5H), 4.22 (s, 2H), 3.45 (t, J = 7.2 Hz, 2H), 2.61 (t, J = 7.8 Hz, 2H).

Preparation of PP_05a-b

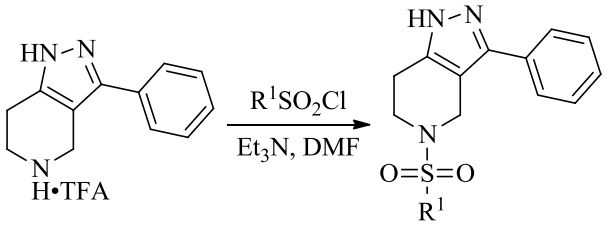


PP_04

X = O: PP_05a
X = S: PP_05b

Substituted arylisocyanate/arylisothiocyanate (**Table 5.7**) (1.20 equiv) was added to the stirred solution of compound **PP_04** (1.0 equiv) and Et_3N (2.5 equiv) in DMF at 0 °C under N_2 atmosphere, and allowed to stir at room temperature for 6 h. The reaction mixture was diluted with EtOAc and washed with brine solution, the separated organic layer was concentrated under reduced pressure and the crude residue was purified by column chromatography (EtOAc/Hexanes) to get compounds **PP_05a-b**.

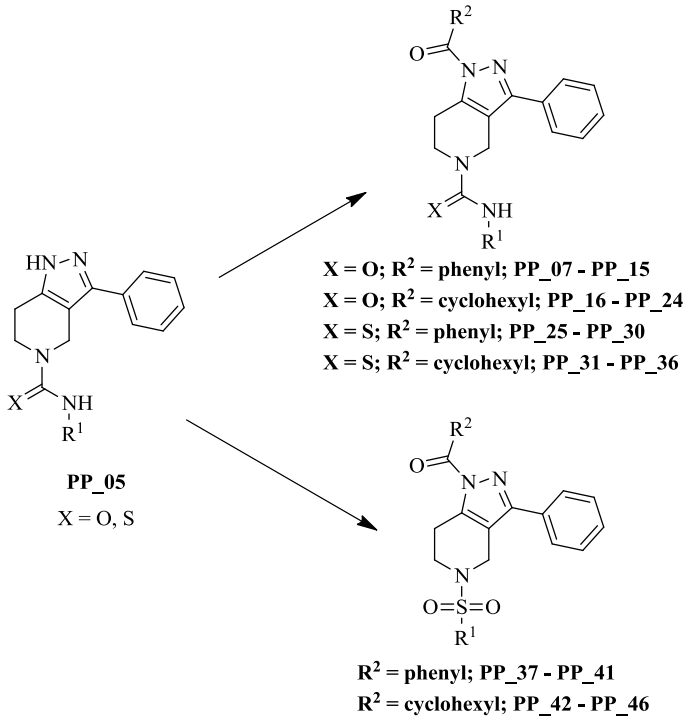
Preparation of PP_06



PP_04 **PP_06**

Substituted arylsulphonyl halide (**Table 5.7**) (1.20 equiv) was added to the stirred solution of compound **PP_04** (1.0 equiv) and Et_3N (2.5 equiv) in DMF at 0 °C under N_2 atmosphere, and allowed to stir at room temperature for 6 h. The reaction mixture was diluted with EtOAc and washed with H_2O, the separated organic layer was concentrated under reduced pressure and the crude residue was purified by column chromatography (EtOAc/Hexanes) to get compounds **PP_06**.

Preparation of PP_07 – PP_15, PP_25 – PP_30 and PP_37 – PP_41

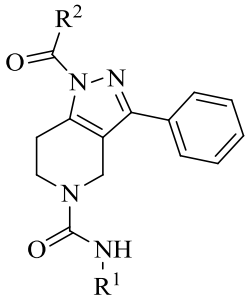


Benzoyl chloride (1.20 equiv) was added to the stirred solution of compound **PP_05**/compound **PP_06** (1.00 equiv) and DIPEA (2.20 equiv) in DMF at 0 °C under N_2 atmosphere, and allowed to stir at room temperature for 12 h. The reaction mixture was quenched with H_2O, extracted with EtOAc and washed the EtOAc layer with brine solution. The separated organic layer was concentrated under reduced pressure and the crude residue was purified by column chromatography (EtOAc/Hexanes) to get compounds **PP_07 – PP_15**, **PP_25 – PP_30** and **PP_37 – PP_41**.

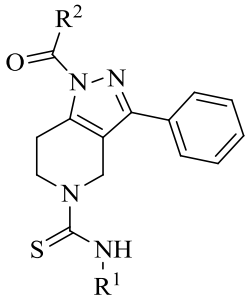
Preparation of PP_16 – PP_24, PP_31 – PP_36 and PP_42 – PP_46

Cyclohexanecarbonyl chloride (1.20 equiv) was added to the stirred solution of compound **PP_05a-b**/compound **PP_06** (1.00 equiv) and DIPEA (2.20 equiv) in DMF at 0 °C under N_2 atmosphere, and allowed to stir at room temperature for 12 h. The reaction mixture was quenched with H_2O, extracted with EtOAc and washed the EtOAc layer with brine solution. The separated organic layer was concentrated under reduced pressure and the crude residue was purified by column chromatography (EtOAc/Hexanes) to get compounds **PP_16 – PP_24**, **PP_31 – PP_36** and **PP_42 – PP_46**.

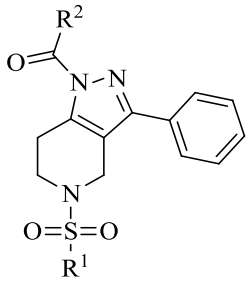
Table 5.7: Physiochemical properties of the synthesized compounds **PP_07** – **PP_46**



PP_07 - PP_24



PP_25 - PP_36



PP_37 - PP_46

Compd	R^1	R^2	Yield (%)	M.P. (°C)	Molecular formula	Molecular weight
PP_07	4-Bromophenyl	Phenyl	78	167-169	$C_{26}H_{21}BrN_4O_2$	500.37
PP_08	4-Chlorophenyl	Phenyl	85	160-162	$C_{26}H_{21}ClN_4O_2$	456.92
PP_09	4-Nitrophenyl	Phenyl	75	172-175	$C_{26}H_{21}N_5O_4$	467.48
PP_10	4-Acetylphenyl	Phenyl	64	166-168	$C_{28}H_{24}N_4O_3$	464.52
PP_11	4-Tolyl	Phenyl	66	163-165	$C_{27}H_{24}N_4O_2$	436.51
PP_12	4-Ethoxyphenyl	Phenyl	68	155-158	$C_{28}H_{26}N_4O_3$	466.53
PP_13	1-Naphthyl	Phenyl	85	185-187	$C_{30}H_{24}N_4O_2$	472.54
PP_14	Benzyl	Phenyl	64	176-178	$C_{27}H_{24}N_4O_2$	436.51
PP_15	Isopropyl	Phenyl	60	144-146	$C_{23}H_{24}N_4O_2$	388.46
PP_16	4-Bromophenyl	Cyclohexyl	84	173-176	$C_{26}H_{27}BrN_4O$	507.42
PP_17	4-Chlorphenyl	Cyclohexyl	92	160-162	$C_{26}H_{27}ClN_4O_2$	462.97
PP_18	4-Nitrophenyl	Cyclohexyl	62	157-159	$C_{26}H_{27}N_5O_4$	473.52
PP_19	4-Acetylphenyl	Cyclohexyl	78	169-171	$C_{28}H_{30}N_4O_3$	470.56
PP_20	4-Tolyl	Cyclohexyl	88	171-172	$C_{27}H_{30}N_4O_2$	442.55
PP_21	4-Methoxyphenyl	Cyclohexyl	76	147-149	$C_{27}H_{30}N_4O_3$	458.55
PP_22	4-Ethoxyphenyl	Cyclohexyl	64	177-178	$C_{28}H_{32}N_4O_3$	472.58
PP_23	1-Naphthyl	Cyclohexyl	74	184-186	$C_{30}H_{30}N_4O_2$	478.58
PP_24	Benzyl	Cyclohexyl	69	176-178	$C_{27}H_{30}N_4O_2$	442.55
PP_25	4-Chlorphenyl	Phenyl	75	176-178	$C_{26}H_{21}ClN_4OS$	472.99
PP_26	4-Fluorophenyl	Phenyl	81	176-177	$C_{26}H_{21}FN_4OS$	456.14

Contd

Compd	R^1	R^2	Yield (%)	M.P. (°C)	Molecular formula	Molecular weight
PP_27	4-Tolyl	Phenyl	69	189-190	$C_{27}H_{24}N_4OS$	452.57
PP_28	4-Methoxyphenyl	Phenyl	73	169-172	$C_{27}H_{24}N_4O_2S$	468.57
PP_29	Benzyl	Phenyl	61	180-181	$C_{27}H_{24}N_4OS$	452.57
PP_30	Allyl	Phenyl	84	184-186	$C_{23}H_{22}N_4OS$	402.51
PP_31	4-Chlorphenyl	Cyclohexyl	67	192-195	$C_{26}H_{27}ClN_4OS$	479.04
PP_32	4-Fluorophenyl	Cyclohexyl	62	180-182	$C_{26}H_{27}FN_4OS$	462.58
PP_33	4-Nitrophenyl	Cyclohexyl	74	178-180	$C_{26}H_{27}N_5O_3S$	489.58
PP_34	4-Tolyl	Cyclohexyl	75	170-174	$C_{27}H_{30}N_4OS$	458.62
PP_35	4-Methoxyphenyl	Cyclohexyl	69	185-188	$C_{27}H_{30}N_4O_2S$	474.62
PP_36	Benzyl	Cyclohexyl	61	189-192	$C_{27}H_{30}N_4OS$	458.62
PP_37	4-Fluorophenyl	Phenyl	71	193-195	$C_{25}H_{20}FN_3O_3S$	461.51
PP_38	4-Nitrophenyl	Phenyl	64	187-189	$C_{25}H_{20}N_4O_5S$	488.52
PP_39	4-Acetylphenyl	Phenyl	72	176-179	$C_{27}H_{23}N_3O_4S$	485.55
PP_40	4-Tolyl	Phenyl	81	120-123	$C_{26}H_{23}N_3O_3S$	457.54
PP_41	Thiophen-2-yl	Phenyl	75	221-223	$C_{23}H_{19}N_3O_3S_2$	449.55
PP_42	4-Fluorophenyl	Cyclohexyl	67	192-195	$C_{25}H_{26}FN_3O_3S$	467.56
PP_43	4-Nitrophenyl	Cyclohexyl	81	192-193	$C_{25}H_{26}N_4O_5S$	494.56
PP_44	4-Acetylphenyl	Cyclohexyl	84	206-208	$C_{27}H_{29}N_3O_4S$	491.60
PP_45	4-Tolyl	Cyclohexyl	69	194-196	$C_{26}H_{29}N_3O_3S$	463.51
PP_46	Thiophen-2-yl	Cyclohexyl	75	210-214	$C_{23}H_{25}N_3O_3S_2$	455.59

5.3.3. Characterization of the synthesized molecules

^{1}H NMR, ^{13}C NMR and mass spectral analyses of all the molecules were in full agreement with the proposed molecules.

1-Benzoyl-*N*-(4-bromophenyl)-3-phenyl-6,7-dihydro-1*H*-pyrazolo[4,3-*c*]pyridine-5(4*H*)-carboxamide (PP_07): Yield: 78%, m.p. 167–169 °C; MS(ESI) m/z 501 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.40 (s, 1H), 7.89 (d, *J* = 7.6 Hz, 2H), 7.50–7.25 (m, 12H), 4.70 (s, 2H), 3.82 (t, *J* = 8.0 Hz, 2H), 3.10 (t, *J* = 7.6 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.2, 159.4, 145.6, 144.6(2C), 134.9, 134.3, 133.7, 132.2, 131.4, 129.2, 128.3(2C), 127.1,

126.6(2C), 125.0, 124.2, 124.0, 123.4(2C), 122.7, 118.9, 54.7, 48.4, 29.6. Anal. calcd for $C_{26}H_{21}BrN_4O_2$: C, 62.28; H, 4.22; N, 11.17% Found C, 62.31; H, 4.26; N, 11.21%.

1-Benzoyl-*N*-(4-chlorophenyl)-3-phenyl-6,7-dihydro-1*H*-pyrazolo[4,3-*c*]pyridine-5(4*H*)-carboxamide (PP_08): Yield: 85%, m.p. 160–162 °C; MS(ESI) m/z 457 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.90 (s, 1H), 7.90 (d, *J* = 8.0 Hz, 2H), 7.78 (d, *J* = 8.4 Hz, 2H), 7.60–7.24 (m, 10H), 4.75 (s, 2H), 3.85 (t, *J* = 8.0 Hz, 2H), 3.21 (t, *J* = 8.0 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.2, 160.0, 145.2, 144.9, 141.3, 135.4(2C), 132.6, 131.9, 130.6, 130.1, 129.6(2C), 128.4, 128.5, 127.9, 127.4, 126.4, 126.0, 125.3(2C), 123.9, 119.6, 54.9, 48.6, 29.9. Anal. calcd for $C_{26}H_{21}ClN_4O_2$: C, 68.34; H, 4.63; N, 12.26% Found C, 68.36; H, 4.69; N, 12.34%.

1-Benzoyl-*N*-(4-nitrophenyl)-3-phenyl-6,7-dihydro-1*H*-pyrazolo[4,3-*c*]pyridine-5(4*H*)-carboxamide (PP_09): Yield: 75%, m.p. 172–175 °C; MS(ESI) m/z 468 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.45 (s, 1H), 8.16 (d, *J* = 12.0 Hz, 2H), 8.03 (d, *J* = 10.0 Hz, 2H), 7.74–7.46 (m, 10H), 4.82 (s, 2H), 3.87 (t, *J* = 6.8 Hz, 2H), 3.26 (t, *J* = 7.2 Hz, 2H); ^{13} C NMR (75 MHz, DMSO-d_6) δ 167.2, 154.4, 150.2, 147.2, 142.6, 141.1, 132.8, 132.3, 131.4, 131.1, 129.2(2C), 129.0(2C), 128.0(2C), 127.1(2C), 124.6(2C), 118.6(2C), 116.6, 41.3, 40.8, 25.2. Anal. calcd for $C_{26}H_{21}N_5O_4$: C, 66.80; H, 4.53; N, 14.98% Found C, 66.88; H, 4.58; N, 14.91%.

***N*-(4-Acetylphenyl)-1-benzoyl-3-phenyl-6,7-dihydro-1*H*-pyrazolo[4,3-*c*]pyridine-5(4*H*)-carboxamide (PP_10):** Yield: 64%, m.p. 166–168 °C; MS(ESI) m/z 465 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.48 (bs, 1H), 7.63 (d, *J* = 6.8 Hz, 2H), 7.42–7.15 (m, 12H), 4.28 (s, 2H), 3.45 (t, *J* = 7.6 Hz, 2H), 3.05 (t, *J* = 7.6 Hz, 2H), 2.46 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 187.6, 172.4, 164.4, 143.6, 142.6, 141.9, 136.7, 136.4, 135.7, 134.2(2C), 132.9, 129.1(2C), 128.6, 128.3, 127.6(2C), 127.1, 126.9, 126.4, 124.7, 122.1, 120.3, 56.9, 45.7, 29.1, 25.9: Anal. calcd for $C_{28}H_{24}N_4O_3$: C, 72.40; H, 5.21; N, 12.06% Found C, 72.45, H, 5.24, N, 12.09%.

1-Benzoyl-3-phenyl-*N*-*p*-tolyl-6,7-dihydro-1*H*-pyrazolo[4,3-*c*]pyridine-5(4*H*)-carboxamide (PP_11): Yield: 66%, m.p. 163–165 °C; MS(ESI) m/z 437 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.45 (bs, 1H), 7.89–7.21 (m, 14H) ,4.92 (s, 2H), 3.45 (t, *J* = 8.0 Hz, 2H), 3.15 (t, *J* = 7.6 Hz, 2H), 2.40 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.6, 162.4, 139.8, 139.7, 138.6, 137.6, 137.8, 136.8, 134.8, 134.6(2C), 133.8, 133.0, 131.8, 130.8, 127.8,

126.7(2C), 125.1, 124.8(2C), 124.1, 119.9, 56.8, 44.7, 31.7, 25.2; Anal. calcd for $C_{27}H_{24}N_4O_2$: C, 74.29; H, 5.54; N, 12.84% Found C, 74.34; H, 5.59; N, 12.88%.

1-Benzoyl-*N*-(4-ethoxyphenyl)-3-phenyl-6,7-dihydro-1*H*-pyrazolo[4,3-*c*]pyridine-5(4*H*)-carboxamide (PP-12): Yield: 68%, m.p. 155–158 °C; MS(ESI) m/z 467 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65–7.30 (m, 15H), 4.70 (s, 2H), 4.21–4.18 (m, 2H), 3.45 (t, *J* = 8.0 Hz, 2H), 2.95 (t, *J* = 7.2 Hz, 2H), 1.42 (t, *J* = 7.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 167.4, 158.3, 153.9, 144.6, 144.2, 142.6, 137.9, 136.6, 135.6, 133.4, 132.6(2C), 130.3(2C), 131.1, 128.9, 128.2, 126.7, 125.6, 124.3(2C), 121.9, 116.3, 66.3, 56.3, 47.3, 27.8, 16.9. Anal. calcd for $C_{28}H_{26}N_4O_3$: C, 72.09; H, 5.62; N, 12.01% Found C, 72.12; H, 5.66; N, 12.18%.

1-Benzoyl-*N*-(naphthalen-1-yl)-3-phenyl-6,7-dihydro-1*H*-pyrazolo[4,3-*c*]pyridine-5(4*H*)-carboxamide (PP_13): Yield: 85%, m.p. 185–187 °C; MS(ESI) m/z 473 $[M+H]^+$; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.92 (s, 1H), 8.06 (t, *J* = 7.5 Hz, 2H), 7.93–7.39 (m, 15H), 4.78 (s, 2H), 3.95 (t, *J* = 7.6 Hz, 2H), 3.12 (t, *J* = 8.0 Hz, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 167.3, 156.3, 150.2, 142.8, 135.3, 133.7, 132.8, 132.4, 131.5, 131.1, 129.6, 129.1(2C), 128.9(2C), 128.0(2C), 127.9(2C), 127.0(2C), 125.8, 125.5, 125.2, 123.5, 123.3, 117.0, 41.3, 40.6, 25.3. Anal. Calcd. for $C_{30}H_{24}N_4O_2$: C, 76.25; H, 5.12; N, 11.86 Found C, 76.29; H, 5.16; N, 11.90%.

1-Benzoyl-*N*-benzyl-3-phenyl-6,7-dihydro-1*H*-pyrazolo[4,3-*c*]pyridine-5(4*H*)-carboxamide (PP_14): Yield: 64%, m.p. 176–178 °C; MS(ESI) m/z 437 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.45 (t, 1H), 7.65–7.18 (m, 15H), 4.92 (s, 2H), 4.65 (d, *J* = 6.8 Hz, 2H), 3.75 (t, *J* = 8.0 Hz, 2H), 2.96 (t, *J* = 7.6 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.4, 159.5, 138.5, 138.0, 137.4, 136.3, 135.9, 133.8, 133.0, 132.3(2C), 131.8, 129.0, 127.5, 126.4, 123.8, 122.4(2C), 121.6, 121.0(2C), 120.6, 120.0, 51.8, 43.7, 39.3, 22.5. Anal. calcd for $C_{27}H_{24}N_4O_2$: C, 74.29; H, 5.54; N, 12.84% Found C, 74.36; H, 5.52; N, 12.92%.

1-Benzoyl-*N*-isopropyl-3-phenyl-6,7-dihydro-1*H*-pyrazolo[4,3-*c*]pyridine-5(4*H*)-carboxamide (PP_15): Yield: 60%, m.p. 144–146 °C; MS(ESI) m/z 389 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86–7.58 (m, 10H), 4.42 (s, 2H), 4.23 (m, 1H), 3.65 (t, *J* = 7.2 Hz, 2H), 3.06 (t, *J* = 7.6 Hz, 2H); 1.38 (d, *J* = 9.6 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.4, 158.3, 142.7, 142.2, 141.7, 140.1, 133.5, 133.1, 131.7, 130.9(2C), 130.3, 130.0, 127.5, 126.9, 126.6, 125.1, 51.3, 43.9, 40.5, 29.7, 21.6(2C). Anal. calcd for $C_{23}H_{24}N_4O_2$: C, 71.11; H, 6.23; N, 14.42% Found C, 71.21; H, 6.24; N, 14.52%.

***N*-(4-Bromophenyl)-1-(cyclohexanecarbonyl)-3-phenyl-6,7-dihydro-1*H*-pyrazolo[4,3-*c*]pyridine-5(4*H*)-carboxamide (PP_16):** Yield: 84%, m.p. 173–174 °C; MS(ESI) m/z 507 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89 (d, *J* = 9.6 Hz, 2H), 7.76 (d, *J* = 9.6 Hz, 2H), 7.62–7.48 (m, 6H), 4.84 (s, 2 H), 3.88 (t, *J* = 7.6 Hz, 2H), 3.52 (t, *J* = 8.0 Hz, 2H), 2.36–1.25 (m, 11H): ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.5, 165.6, 144.7, 142.9, 132.3, 131.3, 128.9(2C), 127.5, 126.4(2C), 122.5(2C), 121.9(2C), 120.2, 119.4, 60.1, 49.4, 28.6(2C), 25.8(2C), 23.8, 23.4, 22.5. Anal. Calcd. for $C_{26}H_{27}BrN_4O$: C, 61.54; H, 5.36; N, 11.04% Found C, 61.60; H, 5.40; N, 11.12%.

***N*-(4-Chlorophenyl)-1-(cyclohexanecarbonyl)-3-phenyl-6,7-dihydro-1*H*-pyrazolo[4,3-*c*]pyridine-5(4*H*)-carboxamide (PP_17):** Yield: 92%, m.p. 160–162 °C; MS(ESI) m/z 463 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (d, *J* = 10.4 Hz, 2H), 7.86 (d, *J* = 9.6 Hz, 2H), 7.68–7.46 (m, 5H), 6.52 (s, 1H) 4.74 (s, 2H), 3.82 (t, *J* = 7.6 Hz, 2H), 3.42 (t, *J* = 8.0 Hz, 2H), 2.25–1.25 (m, 11H): ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.6, 151.7, 145.6, 143.7, 131.6, 130.8, 129.4(2C), 125.7, 121.6(2C), 121.0(2C), 119.9(2C), 119.2, 117.9, 61.5, 48.6, 29.3(2C), 26.2(2C), 24.9, 24.4, 23.4. Anal. calcd for $C_{26}H_{27}ClN_4O_2$: C, 67.45; H, 5.88; N, 12.10% Found C, 67.54; H, 5.93; N, 12.23%.

1-(Cyclohexanecarbonyl)-*N*-(4-nitrophenyl)-3-phenyl-6,7-dihydro-1*H*-pyrazolo[4,3-*c*]pyridine-5(4*H*)-carboxamide (PP_18): Yield: 62%, m.p. 157–159 °C; MS(ESI) m/z 474 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98–7.42 (m, 10H), 4.84 (s, 2H), 3.95 (t, *J* = 7.2 Hz, 2H), 3.08 (t, *J* = 7.6 Hz, 2H), 2.30 (s, 1H), 2.05–1.25 (m, 10H): ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.4, 166.6, 154.7, 153.7, 143.6, 136.8, 132.5(2C), 124.8, 123.7(2C), 122.6(2C), 121.2(2C), 119.2, 117.9, 59.6, 38.8, 26.3(2C), 25.8, 26.9(2C), 23.9, 22.1. Anal. calcd for $C_{26}H_{27}N_5O_4$: C, 65.95; H, 5.75; N, 14.79% Found C, 65.99; H, 5.83; N, 14.88%.

***N*-(4-Acetylphenyl)-1-(cyclohexanecarbonyl)-3-phenyl-6,7-dihydro-1*H*-pyrazolo[4,3-*c*]pyridine-5(4*H*)-carboxamide (PP_19):** Yield: 78%, m.p. 169–171 °C; MS(ESI) m/z 471 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (d, *J* = 9.6 Hz, 2H), 7.72 (d, *J* = 9.2 Hz, 2H), 7.56–7.44 (m, 5H), 6.75 (s, 1H), 4.79 (s, 2H), 3.82 (t, *J* = 7.6 Hz, 2H), 3.32 (t, *J* = 7.6 Hz, 2H), 2.55 (s, 3H), 2.16–1.21 (m, 11H): ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.3, 164.3, 154.3, 148.6, 145.6, 136.5, 133.5(2C), 128.4, 125.7(2C), 125.4, 124.8(2C), 122.7(2C), 121.6, 120.4, 58.5, 46.5, 37.6, 35.2, 28.4, 26.5(2C), 24.8, 24.3, 21.6. Anal. calcd for $C_{28}H_{30}N_4O_3$: C, 71.47; H, 6.43; N, 11.91% Found C, 71.52; H, 6.48; N, 11.85%.

1-(Cyclohexanecarbonyl)-3-phenyl-*N*-*p*-tolyl-6,7-dihydro-1*H*-pyrazolo[4,3-*c*]pyridine-5(4*H*)-carboxamide (PP_20): Yield: 88%, m.p. 171–172 °C; MS(ESI) m/z 443 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89 (d, *J* = 10.8 Hz, 2H), 7.72 (d, *J* = 9.6 Hz, 2H), 7.78–7.52 (m, 5H), 6.78 (s, 1H) 4.59 (s, 2H), 3.82 (t, *J* = 7.6 Hz, 2H), 3.18 (t, *J* = 8.0 Hz, 2H), 2.39 (s, 3H), 2.26 (s, 1H), 2.05–1.25 (m, 10H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 182.6, 161.3, 155.9, 151.6, 141.5, 139.5, 133.7(2C), 125.6, 124.6(2C), 124.4, 122.6, 121.9(2C), 118.5, 118.0, 58.9, 39.5, 34.9, 27.6(2C), 28.8, 26.5(2C), 24.3, 23.4. Anal. calcd for $C_{27}H_{30}N_4O_2$: C, 73.28; H, 6.83; N, 12.66% Found C, 73.32; H, 6.92; N, 12.69%.

1-(Cyclohexanecarbonyl)-*N*-(4-methoxyphenyl)-3-phenyl-6,7-dihydro-1*H*-pyrazolo[4,3-*c*]pyridine-5(4*H*)-carboxamide (PP_21): Yield: 76%, m.p. 147–149 °C; MS(ESI) m/z 459 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (d, *J* = 11.2 Hz, 2H), 7.76 (d, *J* = 9.6 Hz, 2H), 7.75–7.49 (m, 5H), 6.75 (s, 1H) 4.76 (s, 2H), 3.95 (t, *J* = 8.0 Hz, 2H), 3.90 (s, 3H), 3.38 (t, *J* = 6.8 Hz, 2H), 2.05–1.25 (m, 11H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 184.5, 160.3, 156.3, 152.2, 146.1, 141.5, 135.6(2C), 129.4, 128.6(2C), 125.4, 124.6, 123.2(2C), 119.5, 117.9, 57.6, 49.9, 38.4, 34.0, 29.4, 27.9(2C), 25.9, 24.3, 23.4. Anal. calcd for $C_{27}H_{30}N_4O_3$: C, 70.72; H, 6.59; N, 12.22% Found C, 70.76; H, 6.66; N, 12.25%.

1-(Cyclohexanecarbonyl)-*N*-(4-ethoxyphenyl)-3-phenyl-6,7-dihydro-1*H*-pyrazolo[4,3-*c*]pyridine-5(4*H*)-carboxamide (PP_22): Yield: 64%, m.p. 177–178 °C; MS(ESI) m/z 473 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05–7.56 (m, 9H), 4.69 (s, 2H), 4.11–4.03 (m, 2H), 3.68 (t, *J* = 8.4 Hz, 2H), 3.12 (t, *J* = 8.0 Hz, 2H), 2.41 (s, 1H), 2.45–1.25 (m, 10H), 1.39 (t, *J* = 8.0 Hz, 3H): ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.9, 164.7, 155.4, 149.6, 147.5, 140.1, 134.2(2C), 125.2, 124.7(2C), 123.6, 123.0, 121.4(2C), 118.2, 115.7, 60.5, 54.9, 48.9, 39.6, 33.8, 27.7, 26.3(2C), 24.9(2C), 21.9. Anal. calcd for $C_{28}H_{32}N_4O_3$: C, 71.16; H, 6.83; N, 11.86% Found C, 71.21; H, 6.91; N, 11.92%.

1-(Cyclohexanecarbonyl)-*N*-(naphthalen-1-yl)-3-phenyl-6,7-dihydro-1*H*-pyrazolo[4,3-*c*]pyridine-5(4*H*)-carboxamide (PP_23): Yield: 74%, m.p. 184–186 °C; MS(ESI) m/z 478 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05–7.15 (m, 13H), 4.66 (s, 2H), 3.72 (t, *J* = 7.6 Hz, 2H), 3.03 (t, *J* = 8.0 Hz, 2H), 2.19–1.30 (m, 11H): ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.8, 166.6, 145.6, 141.7, 138.7, 136.6, 135.2(2C), 134.3(2C), 131.6, 126.3, 126.0, 125.8, 124.6(2C), 123.4, 122.7(2C), 120.6, 119.5, 60.6, 47.2, 29.4(2C), 28.6(2C), 26.3, 24.6, 24.1. Anal. calcd for $C_{30}H_{30}N_4O_2$: C, 75.29; H, 6.32; N, 11.71% Found C, 75.32; H, 6.29; N, 11.82%.

***N*-Benzyl-1-(cyclohexanecarbonyl)-3-phenyl-6,7-dihydro-1*H*-pyrazolo[4,3-*c*]pyridine-5(4*H*)-carboxamide (PP_24):** Yield: 69%, m.p. 176–178 °C; MS(ESI) m/z 443 $[M+H]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 7.98–7.28 (m, 10H), 6.78 (bs, 1H), 4.82 (s, 2H), 4.64 (d, 2H, *J* = 8.8 Hz), 3.66 (t, *J* = 7.6 Hz, 2H), 3.21 (t, *J* = 8.0 Hz, 2H), 2.34 (m, 1H), 2.19–1.30 (m, 10H): ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.6, 162.9, 149.3, 144.5, 139.5, 138.6, 137.6, 136.4(2C), 124.9, 124.2, 123.6, 121.9, 120.4(2C), 119.6, 118.4, 59.5, 55.4, 34.5, 28.5(2C), 28.4(2C), 27.4, 23.6, 22.5. Anal. Calcd. for $C_{27}H_{30}N_4O_2$: C, 73.28; H, 6.83; N, 12.66% Found C, 73.43; H, 6.92; N, 12.75%.

1-Benzoyl-*N*-(4-chlorophenyl)-3-phenyl-6,7-dihydro-1*H*-pyrazolo[4,3-*c*]pyridine-5(4*H*)-carbothioamide (PP_25): Yield: 75%, m.p. 176–178 °C; MS(ESI) m/z 473 $[M+H]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 9.28 (bs, 1H), 8.05–7.40 (m, 14H), 4.95 (s, 2H), 3.82 (t, *J* = 7.6 Hz, 2H), 3.12 (t, *J* = 7.6 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.3, 163.9, 144.3, 144.0, 143.5, 141.8, 138.9, 137.8, 136.5, 135.9, 135.5, 134.9, 134.2, 133.9, 132.9, 129.6(2C), 129.1, 128.6, 127.6(2C), 126.7, 125.8, 59.6, 49.9, 27.3. Anal. calcd for $C_{26}H_{21}ClN_4OS$: C, 66.02; H, 4.48; N, 11.85% Found C, 66.12; H, 4.54; N, 11.89%.

1-Benzoyl-*N*-(4-fluorophenyl)-3-phenyl-6,7-dihydro-1*H*-pyrazolo[4,3-*c*]pyridine-5(4*H*)-carbothioamide (PP_26): Yield: 81%, m.p. 176–177 °C; MS(ESI) m/z 473 $[M+H]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 8.28 (s, 1H), 7.92 (d, *J* = 8.0 Hz, 2H), 7.80 (d, *J* = 8.4 Hz, 2H), 7.62–7.35 (m, 10H), 4.85 (s, 2H), 3.92 (t, *J* = 7.2 Hz, 2H), 3.06 (t, *J* = 7.6 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.7, 159.6, 153.7, 144.3, 144.1, 143.7, 142.5, 139.9, 138.6, 135.9, 134.4, 133.8, 132.9, 132.3, 131.9, 130.7(2C), 129.6, 129.0, 128.5(2C), 126.3, 124.8, 61.6, 52.7, 26.9. Anal. calcd for $C_{26}H_{21}FN_4OS$: C, 68.40; H, 4.64; N, 12.27% Found C, 68.54; H, 4.68; N,12.31%.

1-Benzoyl-3-phenyl-*N*-*p*-tolyl-6,7-dihydro-1*H*-pyrazolo[4,3-*c*]pyridine-5(4*H*)-carbothioamide (PP_27): Yield: 69%, m.p. 189–190 °C; MS(ESI) m/z 453 $[M+H]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 7.85–7.21 (m, 13H), 7.18 (d, *J* = 7.6 Hz, 2H), 4.85 (s, 2H), 3.58 (t, *J* = 7.6 Hz, 2H), 2.95 (t, *J* = 7.6 Hz, 2H), 2.52 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.4, 165.7, 138.9, 137.4, 137.0, 136.5, 136.0, 134.9, 134.3, 134.1(2C), 131.8, 130.0, 129.8, 127.4, 127.0, 125.9(2C), 123.8(2C), 123.4, 122.1, 118.8, 58.9, 46.8, 29.5, 22.8. Anal. calcd for $C_{27}H_{24}N_4OS$: C, 71.65; H, 5.35; N, 12.38% Found C, 71.73; H, 5.40; N, 12.45%.

1-Benzoyl-*N*-(4-methoxyphenyl)-3-phenyl-6,7-dihydro-1*H*-pyrazolo[4,3-*c*]pyridine-5(4*H*)-carbothioamide (PP_28): Yield: 73%, m.p. 169–172 °C; MS(ESI) m/z 469 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.08 (s, 1H), 7.72–6.98 (m, 14H), 4.75 (s, 2H), 4.05 (s, 3H) 3.82 (t, *J* = 7.6 Hz, 2H), 2.89 (t, *J* = 7.2 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.6, 163.7, 156.8, 142.6, 142.2, 141.6, 141.0, 139.2, 138.4, 137.8, 133.9, 132.6, 129.3(2C), 129.1, 128.6, 127.0, 126.5, 124.9, 122.9(2C), 120.9, 119.4, 60.9, 52.7, 44.1, 23.4. Anal. calcd for $C_{27}H_{24}N_4O_2S$: C, 69.21; H, 5.16; N, 11.96% Found C, 69.24; H, 5.19; N, 12.04%.

1-Benzoyl-*N*-benzyl-3-phenyl-6,7-dihydro-1*H*-pyrazolo[4,3-*c*]pyridine-5(4*H*)-carbothioamide (PP_29): Yield: 61%, m.p. 180–181 °C; MS(ESI) m/z 453 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.15 (t, *J* = 7.6 Hz, 1H), 7.69–7.28 (m, 15H), 4.89 (s, 2H), 4.45 (d, *J* = 6.8 Hz, 2H), 3.85 (t, *J* = 7.6 Hz, 2H), 2.88 (t, *J* = 7.6 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.4, 155.5, 139.5, 136.9, 135.4, 134.9, 134.0, 133.8, 133.2, 132.8(2C), 1320, 128.9, 127.8, 127.3, 125.9, 125.4(2C), 124.4, 121.8(2C), 120.8, 119.5, 55.8, 43.5, 39.9, 20.9. Anal. calcd for $C_{27}H_{24}N_4OS$: C, 71.65; H, 5.35; N, 12.38% Found C, 71.69; H, 5.39; N, 12.41%.

***N*-Allyl-1-benzoyl-3-phenyl-6,7-dihydro-1*H*-pyrazolo[4,3-*c*]pyridine-5(4*H*)-carbothioamide (PP_30):** Yield: 84%, m.p. 184–186 °C; MS(ESI) m/z 403 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99–7.16 (m, 11H), 6.02 (m, 1H), 5.23 (m, 2H), 4.79 (s, 2H), 4.38 (m, 2H), 3.96 (t, *J* = 8.0 Hz, 2H), 2.95 (t, *J* = 7.6 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.5, 165.7, 140.7, 140.2, 133.5, 132.9, 131.9, 130.5(2C), 130.3, 129.5, 127.5, 126.5(2C), 126.2, 125.6, 124.5, 119.1, 114.3, 51.3, 45.9, 43.2, 21.6. Anal. calcd for $C_{23}H_{22}N_4OS$: C, 68.63; H, 5.51; N, 13.92% Found C, 68.69; H, 5.56; N, 13.99%.

***N*-(4-Chlorophenyl)-1-(cyclohexanecarbonyl)-3-phenyl-6,7-dihydro-1*H*-pyrazolo[4,3-*c*]pyridine-5(4*H*)-carbothioamide (PP_31):** Yield: 67%, m.p. 192–195 °C; MS(ESI) m/z 479 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.05 (s, 1H), 8.10 (d, *J* = 7.2 Hz, 2H), 7.92 (d, *J* = 7.2 Hz, 2H), 8.12–7.45 (m, 5H), 4.95 (s, 2H), 3.88 (t, *J* = 7.6 Hz, 2H), 3.02 (t, *J* = 7.2 Hz, 2H); 2.18–1.28 (m, 11H): ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.5, 172.6, 152.6, 143.7, 143.4, 141.6, 137.5, 137.2, 136.3, 132.7, 131.9, 128.5, 124.3(2C), 123.7, 119.3, 116.7, 59.2, 54.6, 31.6, 26.6(2C), 26.1(2C), 24.3, 22.5. Anal. calcd for $C_{26}H_{27}ClN_4OS$: C, 65.19; H, 5.68; N, 11.70% Found C, 65.27; H, 5.76; N, 11.79%.

1-(Cyclohexanecarbonyl)-*N*-(4-fluorophenyl)-3-phenyl-6,7-dihydro-1*H*-pyrazolo[4,3-*c*]pyridine-5(4*H*)-carbothioamide (PP_32): Yield: 62%, m.p. 180–182 °C; MS(ESI) m/z 463 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (s, 1H), 7.92 (d, *J* = 8.4 Hz, 2H), 7.85 (d, *J* = 8.4 Hz, 2H), 7.62–7.35 (m, 5H), 4.75 (s, 2H), 3.85 (t, *J* = 7.6 Hz, 2H), 3.06 (t, *J* = 6.8 Hz, 2H), 2.31(m, 1H), 2.11–1.28 (m, 10H): ^{13}C NMR (100 MHz, $CDCl_3$) δ 184.7, 179.7, 151.8, 145.6, 144.6, 142.2, 140.9, 139.4, 136.4, 133.3, 131.6, 127.3, 124.7(2C), 123.4, 121.3(2C), 59.8, 58.5, 32.5, 27.8(2C), 26.5(2C), 25.6, 23.8. Anal. calcd for $C_{26}H_{27}FN_4OS$: C, 67.51; H, 5.88; F, 4.11; N, 12.11% Found C, 67.60; H, 5.93; N, 12.22%.

1-(Cyclohexanecarbonyl)-*N*-(4-nitrophenyl)-3-phenyl-6,7-dihydro-1*H*-pyrazolo[4,3-*c*]pyridine-5(4*H*)-carbothioamide (PP_33): Yield: 74%, m.p. 178–180 °C; MS(ESI) m/z 490 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98–7.42 (m, 10H), 4.84 (s, 2H), 3.95 (t, *J* = 7.2 Hz, 2H), 3.08 (t, *J* = 7.6 Hz, 2H), 2.30 (s, 1H), 2.05–1.25 (m, 10H): ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.4, 176.3, 153.4, 142.8, 141.6, 140.4, 139.4, 138.3, 136.5, 135.3, 129.9, 127.5, 123.9(2C), 122.5, 120.3, 119.5, 61.5, 49.6, 32.3, 27.4(2C), 25.7(2C), 25.6, 22.9. Anal. calcd for $C_{26}H_{27}N_5O_3S$: C, 63.78; H, 5.56; N, 14.30%. Found C, 63.83; H, 5.62; N, 14.41%.

1-(Cyclohexanecarbonyl)-3-phenyl-*N*-*p*-tolyl-6,7-dihydro-1*H*-pyrazolo[4,3-*c*]pyridine-5(4*H*)-carbothioamide (PP_34): Yield: 75%, m.p. 170–174 °C; MS(ESI) m/z 459 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.92–7.35 (m, 9H), 7.20 (s, 1H), 4.93 (s, 2H), 3.71 (t, *J* = 6.8 Hz, 2H), 2.86 (t, *J* = 7.2 Hz, 2H), 2.49 (s, 3H), 2.24–1.35 (m, 11H): ^{13}C NMR (100 MHz, DMSO-d_6) δ 186.7, 166.7, 148.4, 143.9, 142.4, 139.9, 137.2, 136.9, 134.7, 133.9, 132.3, 132.0, 128.9, 125.7(2C), 123.4, 120.7(2C), 56.2, 45.0, 33.3, 27.7(2C), 25.0(2C), 24.3, 22.7. Anal. calcd for $C_{27}H_{30}N_4OS$: C, 70.71; H, 6.59; N, 12.22% Found C, 70.78; H, 6.69; N, 12.26%.

1-(Cyclohexanecarbonyl)-*N*-(4-methoxyphenyl)-3-phenyl-6,7-dihydro-1*H*-pyrazolo[4,3-*c*]pyridine-5(4*H*)-carbothioamide (PP_35): Yield: 69%, m.p. 185–188 °C; MS(ESI) m/z 475 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.12 (s, 1H), 7.92–7.38 (m, 9H), 4.85 (s, 2H), 4.18 (s, 3H) 3.69 (t, *J* = 7.6 Hz, 2H), 2.95 (t, *J* = 8.0 Hz, 2H), 2.35 (m, 1H), 2.05–1.25 (m, 10H): ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.2, 163.3, 143.9, 141.4, 139.3, 138.6, 137.4, 136.5, 133.5(2C), 132.7, 131.6, 130.7, 129.6(2C), 124.1(2C), 123.7, 122.7(2C), 62.1, 57.9, 36.1, 31.3, 27.6(2C), 25.9(2C), 24.5, 24.3. Anal. calcd for $C_{27}H_{30}N_4O_2S$: C, 68.33; H, 6.37; N, 11.80%. Found C, 68.45; H, 6.46; N, 11.92%.

***N*-Benzyl-1-(cyclohexanecarbonyl)-3-phenyl-6,7-dihydro-1*H*-pyrazolo[4,3-*c*]pyridine-5(4*H*)-carbothioamide (PP_36):** Yield: 61%, m.p. 189–192 °C; MS(ESI) m/z 459 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.15 (bs, 1H), 7.81–7.42 (m, 10H), 4.79 (s, 2H), 4.39 (d, *J* = 7.2 Hz, 2H), 3.99 (t, *J* = 7.6 Hz, 2H), 3.08 (t, *J* = 8.0 Hz, 2H), 2.25–1.35 (m, 11H): ^{13}C NMR (100 MHz, DMSO-d_6) δ 176.8, 162.7, 142.9, 140.7, 138.7, 137.2, 135.3, 134.7, 133.9, 132.6, 131.4, 129.7, 124.3(2C), 123.7, 121.4(2C), 58.7, 56.1, 36.6, 34.6, 27.4(2C), 26.1(2C), 25.2, 23.2. Anal. calcd for $C_{27}H_{30}N_4OS$: C, 70.71; H, 6.59; N, 12.22%. Found C, 71.01; H, 6.68; N, 12.54%.

(5-(4-Fluorophenylsulfonyl)-3-phenyl-4,5,6,7-tetrahydro-1*H*-pyrazolo[4,3-*c*]pyridin-1-yl)(phenyl)methanone (PP_37): Yield: 71%, m.p. 193–195 °C; MS(ESI) m/z 462 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.02–7.91 (m, 4H), 7.7–7.42 (m, 10H), 4.45 (s, 2H), 3.55 (t, *J* = 7.6 Hz, 2H), 3.20 (t, *J* = 7.6 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.8, 167.1, 163.7, 150.8, 141.7, 133.5, 133.0, 132.3, 131.7(2C), 130.3, 130.2, 129.3, 129.0(2C), 128.0(2C), 127.2(2C), 116.8, 116.5, 115.0, 43.1, 42.9, 25.5. Anal. calcd for $C_{25}H_{20}FN_3O_3S$: C, 65.06; H, 4.37; N, 9.10% Found C, 65.12; H, 4.41; N, 9.16%.

(5-(4-Nitrophenylsulfonyl)-3-phenyl-4,5,6,7-tetrahydro-1*H*-pyrazolo[4,3-*c*]pyridin-1-yl)(phenyl)methanone (PP_38): Yield: 64%, m.p. 187–189 °C; MS(ESI) m/z 489 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (d, *J* = 9.2 Hz, 2H), 7.78 (d, *J* = 8.4 Hz, 2H), 7.72–7.39 (m, 10H), 4.86 (s, 2H), 3.95 (t, *J* = 8.0 Hz, 2H), 3.28 (t, *J* = 8.0 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.3, 144.8, 143.6, 143.1(2C), 139.8, 133.5, 132.2, 131.7, 130.6, 129.5, 129.2, 128.7(2C), 127.6, 127.0, 125.8, 124.9, 122.8(2C), 120.4, 120.8, 55.9, 42.8, 23.4. Anal. calcd for $C_{25}H_{20}N_4O_5S$: C, 61.47; H, 4.13; N, 11.47% Found C, 61.54; H, 4.19; N, 11.54%.

1-(4-(1-Benzoyl-3-phenyl-6,7-dihydro-1*H*-pyrazolo[4,3-*c*]pyridin-5(4*H*)-ylsulfonyl)phenyl)ethanone (PP_39): Yield: 72%, m.p. 176–179 °C; MS(ESI) m/z 486 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83–6.96 (m, 14H), 4.68 (s, 2H), 3.65 (t, *J* = 7.6 Hz, 2H), 3.05 (t, *J* = 8.0 Hz, 2H), 2.52 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 189.9, 168.5, 143.6, 142.9, 142.2, 139.9, 138.4, 137.9, 136.7, 134.7, 133.9, 132.6(2C), 131.6, 129.6, 127.6(2C), 126.6, 125.2, 124.6, 124.0, 123.1, 122.3, 57.9, 46.3, 29.5, 22.5. Anal. calcd for $C_{27}H_{23}N_3O_4S$: C, 66.79; H, 4.77; N, 8.65% Found C, 66.84; H, 4.79; N, 8.69%.

Phenyl(3-phenyl-5-tosyl-4,5,6,7-tetrahydro-1*H*-pyrazolo[4,3-*c*]pyridin-1-yl)methanone (PP_40): Yield: 81%, m.p. 120–123 °C; MS(ESI) m/z 458 $[M+H]^+$; ^{1}H NMR (400 MHz,

$CDCl_3$) δ 7.99–7.21 (m, 14H), 4.62 (s, 2H), 3.65 (t, *J* = 7.6 Hz, 2H), 3.09 (t, *J* = 8.0 Hz, 2H), 2.42 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.4, 137.9, 137.4, 136.8, 136.5, 136.0, 133.9, 133.1, 131.4(2C), 129.8, 129.2, 128.8, 127.5, 126.7, 125.5(2C), 124.6(2C), 122.5, 121.5, 119.9, 59.5, 48.8, 31.5, 23.4. Anal. calcd for $C_{26}H_{23}N_3O_3S$: C, 68.25; H, 5.07; N, 9.18% Found C, 68.30; H, 5.11; N, 9.21%.

Phenyl(3-phenyl-5-(thiophen-2-ylsulfonyl)-4,5,6,7-tetrahydro-1*H*-pyrazolo[4,3-*c*]pyridin-1-yl)methanone (PP_41): Yield: 75%, m.p. 221–223 °C; MS(ESI) m/z 450 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79–7.01 (m, 13H), 4.22 (s, 2H), 3.25 (t, *J* = 7.6 Hz, 2H), 2.86 (t, *J* = 7.6 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.2, 146.9, 145.3, 139.3, 138.7, 137.4, 136.7, 131.8, 131.5, 130.5, 128.4, 126.6, 125.7, 124.3, 122.8, 121.9, 120.4, 119.6, 119.2, 118.6, 56.7, 45.9, 23.6. Anal. Calcd. for $C_{23}H_{19}N_3O_3S_2$: C, 61.45; H, 4.26; N, 9.35% Found C, 61.50; H, 4.30; N, 9.40%.

Cyclohexyl(5-(4-fluorophenylsulfonyl)-3-phenyl-4,5,6,7-tetrahydro-1*H*-pyrazolo[4,3-*c*]pyridin-1-yl)methanone (PP_42): Yield: 62%, m.p. 186–188 °C; MS(ESI) m/z 468 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.02–7.38 (m, 9H), 4.56 (s, 2H), 3.65 (t, *J* = 8.4 Hz, 2H), 3.08 (t, *J* = 8.0 Hz, 2H), 2.19–1.16 (m, 11H): ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.8, 148.1, 140.6, 138.8, 137.5, 136.4(2C), 135.8, 134.8, 133.7, 130.7, 126.6, 123.2(2C), 119.5, 118.9, 62.5, 39.2, 34.5, 29.2(2C), 25.7(2C), 24.4, 22.3. Anal. calcd for $C_{25}H_{26}FN_3O_3S$: C, 64.22; H, 5.60; N, 8.99%. Found 64.32; H, 5.67; N, 9.20%.

Cyclohexyl(5-(4-nitrophenylsulfonyl)-3-phenyl-4,5,6,7-tetrahydro-1*H*-pyrazolo[4,3-*c*]pyridin-1-yl)methanone (PP_43): Yield: 81%, m.p.192–193 °C; MS(ESI) m/z 495 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93–7.29 (m, 9H), 4.76 (s, 2H), 3.88 (t, *J* = 7.6 Hz, 2H), 3.31 (t, *J* = 8.0 Hz, 2H), 2.25–1.30 (m, 11H): ^{13}C NMR (100 MHz, $CDCl_3$) δ 182.6, 142.6, 139.6, 138.4, 136.3, 134.6(2C), 133.5, 132.7, 132.2, 129.6, 127.8, 121.6(2C), 120.7, 119.7, 59.6, 45.2, 33.6, 28.6(2C), 26.9(2C), 25.7, 24.3. Anal. calcd for $C_{25}H_{26}N_4O_5S$: C, 60.71; H, 5.30; N, 11.33%. Found C. 60.83; H, 5.36; N, 11.39%.

1-(4-(1-(Cyclohexanecarbonyl)-3-phenyl-6,7-dihydro-1*H*-pyrazolo[4,3-*c*]pyridin-5(4*H*)-ylsulfonyl)phenyl)ethanone (PP_44): Yield: 84%, m.p. 206–208 °C; MS(ESI) m/z 492 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78–6.92 (m, 9H), 4.72 (s, 2H), 3.75 (t, *J* = 8.0 Hz, 2H), 3.16 (t, *J* = 7.6 Hz, 2H), 2.49 (s, 3H) 2.21–1.28 (m, 11H): ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.4, 171.5, 148.5, 139.6, 138.3, 137.5, 136.7(2C), 135.7, 134.6, 131.4, 127.5, 126.2,

122.5, 120.1, 119.8, 118.4, 63.4, 51.6, 49.6, 32.7, 28.3(2C), 27.4(2C), 24.8, 21.9. Anal. calcd for $C_{27}H_{29}N_3O_4S$: C, 65.97; H, 5.95; N, 8.55%. Found C, 66.02; H, 5.99; N, 8.64%

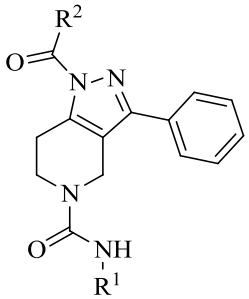
Cyclohexyl(3-phenyl-5-tosyl-4,5,6,7-tetrahydro-1*H*-pyrazolo[4,3-*c*]pyridin-1-yl)methanone (PP_45): Yield: 69%, m.p. 194–196 °C; MS(ESI) m/z 464 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86–7.34 (m, 9H), 4.85 (s, 2H), 3.75 (t, *J* = 8.0 Hz, 2H), 3.12 (t, *J* = 7.6 Hz, 2H), 2.56 (s, 3H), 2.25–1.32 (m, 11H): ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.9, 146.5, 139.4, 136.9, 133.4, 132.3(2C), 130.3, 129.7(2C), 127.8, 126.3(2C), 122.7(2C), 121.7, 119.5, 64.1, 38.8, 35.7, 30.3(2C), 26.8(2C), 25.8, 23.6. Anal. calcd for $C_{26}H_{29}N_3O_3S$: C, 67.36; H, 6.31; N, 9.06%. Found C, 67.43; H, 6.39; N, 9.16%.

Cyclohexyl(3-phenyl-5-(thiophen-2-ylsulfonyl)-4,5,6,7-tetrahydro-1*H*-pyrazolo[4,3-*c*]pyridin-1-yl)methanone (PP_46): Yield: 75%, m.p. 210–214 °C; MS(ESI) m/z 456 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69–6.98 (m, 8H), 4.42 (s, 2H), 3.32 (t, *J* = 7.6 Hz, 2H), 2.98 (t, *J* = 8.0 Hz, 2H), 2.30 (m, 1H), 2.22–1.31 (m, 10H): ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.6, 145.6, 138.3, 136.7, 131.8, 129.6, 125.7, 124.7, 123.6, 122.6, 121.4, 120.4, 119.2, 118.6, 56.9, 46.9, 31.3(2C), 27.8(2C), 26.7, 24.5, 23.6. Anal. calcd for $C_{23}H_{25}N_3O_3S_2$: C, 60.63; H, 5.53; N, 9.22%. Found C, 60.73; H, 5.59; N, 9.31%.

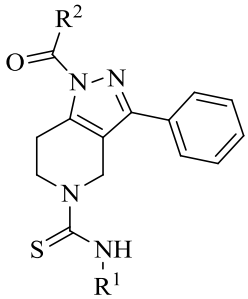
5.3.4. *In vitro M. tuberculosis* screening, *M. tuberculosis* PS enzyme inhibition assay and cytotoxicity studies of the synthesized molecules

All the synthesized compounds were screened for their *in vitro* anti-tubercular activity against *M. tuberculosis* H37Rv using MABA method. Isoniazid and ethambutol were used as reference compounds for comparison. All the synthesized derivatives were also screened for their *in vitro M. tuberculosis* PS inhibitory potency and *in vitro* cytotoxicity against RAW 264.7 cells at 50 μM concentration using MTT assay and the results are tabulated in **Table 5.8**.

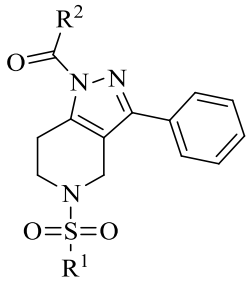
Table 5.8: *In vitro* biological evaluation of the synthesized derivatives **PP_07 – PP_46**



PP_07 - PP_24



PP_25 - PP_36



PP_37 - PP_46

Compd	R^1	R^2	% Inhibition of MTB PS at 100 µM (IC_{50})	MTB MIC in µM[a]	Cytotoxicity[b] at 50 µM % inhibition
PP_07	4-Bromophenyl	Phenyl	60.6 (82.1±2.3)	99.7	42.0
PP_08	4-Chlorophenyl	Phenyl	40.2	109.43	4.9
PP_09	4-Nitrophenyl	Phenyl	95.7 (21.8±0.8)	26.7	0
PP_10	4-Acetylphenyl	Phenyl	43.7	>107.6	12.8
PP_11	4-Tolyl	Phenyl	49.4	114.1	57.1
PP_12	4-Ethoxyphenyl	Phenyl	47.2	>107.1	0
PP_13	1-Naphthyl	Phenyl	89.8 (38.2±1.7)	48.8	4.7
PP_14	Benzyl	Phenyl	46.2	114.1	0.7
PP_15	Isopropyl	Phenyl	40.9	128.7	10.6
PP_16	4-Bromophenyl	Cyclohexyl	50.5	98.54	0
PP_17	4-Chlorphenyl	Cyclohexyl	54.7	108.0	4.0
PP_18	4-Nitrophenyl	Cyclohexyl	50.8	52.79	14.9
PP_19	4-Acetylphenyl	Cyclohexyl	51.4	108.1	22.5
PP_20	4-Tolyl	Cyclohexyl	40.8	56.49	15.1
PP_21	4-Methoxyphenyl	Cyclohexyl	38.7	109.0	52.5
PP_22	4-Ethoxyphenyl	Cyclohexyl	48.5	105.8	4.9
PP_23	1-Naphthyl	Cyclohexyl	50.9	52.19	4.1
PP_24	Benzyl	Cyclohexyl	49.1	54.51	35.7
PP_25	4-Chlorphenyl	Phenyl	37.7	105.7	0

Contd

Compd	R^1	R^2	% Inhibition of MTB PS at 100 µM (IC_{50})	MTB MIC in µM[a]	Cytotoxicity[b] at 50 µM % inhibition
PP_26	4-Fluorophenyl	Phenyl	43.5	109.5	0.5
PP_27	4-Tolyl	Phenyl	23.9	>110.4	0
PP_28	4-Methoxyphenyl	Phenyl	10.2	106.7	0
PP_29	Benzyl	Phenyl	52.1	110.4	0
PP_30	Allyl	Phenyl	41.7	>124.22	6
PP_31	4-Chlorphenyl	Cyclohexyl	56.3	109.0	51.1
PP_32	4-Fluorophenyl	Cyclohexyl	49.2	105.3	58.4
PP_33	4-Nitrophenyl	Cyclohexyl	47.3	53.5	0
PP_34	4-Tolyl	Cyclohexyl	50.5	51.07	27.8
PP_35	4-Methoxyphenyl	Cyclohexyl	50.6	101.7	9.5
PP_36	Benzyl	Cyclohexyl	51.4	108.0	20.6
PP_37	4-Fluorophenyl	Phenyl	77.0 (38.6±0.9)	54.17	0
PP_38	4-Nitrophenyl	Phenyl	39.7	25.58	18.6
PP_39	4-Acetylphenyl	Phenyl	78.5 (39.1±2.2)	51.49	5.3
PP_40	4-Tolyl	Phenyl	9.0	109.31	0
PP_41	Thiophen-2-yl	Phenyl	48.7	55.61	6.8
PP_42	4-Fluorophenyl	Cyclohexyl	46.8	53.5	1.4
PP_43	4-Nitrophenyl	Cyclohexyl	50.2	50.5	8.3
PP_44	4-Acetylphenyl	Cyclohexyl	39.2	50.8	11.5
PP_45	p-Tolyl	Cyclohexyl	41.6	53.9	0
PP_46	Thiophen-2-yl	Cyclohexyl	52.0	54.9	8.1
Isoniazid			NT	0.72	NT
Ethambutol			NT	7.64	NT

C_{50}, 50% inhibitory concentration; MTB, *Mycobacterium tuberculosis*; MIC, minimum inhibitory concentration; [a]*In vitro* activity against MTB H37Rv; [b]Against RAW 264.7 cells; NT, Not tested.

5.3.5. SAR and discussion

With respect to SAR, we prepared twenty benzoyl and twenty cyclohexanecarbonyl derivatives at 1st position of 4,5,6,7-tetrahydro-1*H*-pyrazolo[4,3-*c*]pyridine scaffold (**R^2**). Phenyl group substituted compounds showed better activity than cyclohexyl derivatives against *M. tuberculosis*. We did modification at 5th position by preparing urea (**PP_07 – PP_24**), thiourea (**PP_25 – PP_36**) and sulphonamides (**PP_37 – PP_46**). In general the order of activity was follows urea > sulfonamides > thiourea. Among the urea derivatives, (sub) phenyl, naphthyl, benzyl & alkyl derivatives were prepared.

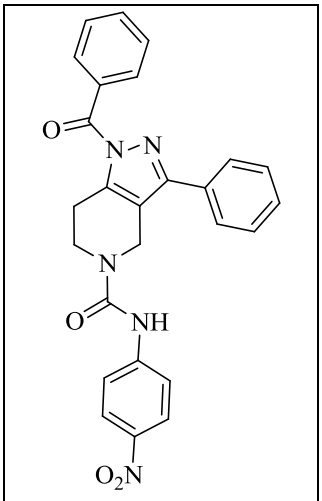
Among the forty compounds screened seventeen compounds showed activity against *M. tuberculosis* with MIC ≤ 60 µM. Two compounds (**PP_09**, and **PP_38**) inhibited *M. tuberculosis* with MIC of < 30 µM. Compound **PP_38** ((5-(4-Nitrophenylsulfonyl)-3-phenyl-4,5,6,7-tetrahydro-1*H*-pyrazolo[4,3-*c*]pyridin-1-yl)(phenyl)methanone) was found to be the most active compound *in vitro* with MIC of 25.58 µM against log-phase culture of *M. tuberculosis*. Structural changes at N-1 position did not affect activity appreciably, whereas sulfonamides at N-5 position showed better activity. Compounds with electron withdrawing substitutions on phenyl ring (**PP_09**, **PP_18**, **PP_37 – PP_39** and **PP_42 – PP_44**) at N-5 position showed better activity than that of electron donating groups. In the mechanistic studies, compounds were evaluated against various *M. tuberculosis* enzymes.

All the synthesized compounds at 100 µM, showed inhibition ranged from 9.0-95.7%. Seventeen compounds showed >50% inhibition against *M. tuberculosis* PS and compounds which showed >60% inhibition were further selected for IC_{50} estimation. Among these five compounds (**PP_07**, **PP_09**, **PP_13**, **PP_37** and **PP_39**) showed IC_{50} in the range of 21.8±0.8 to 82.1±2.3 µM. Compound **PP_09** (1-Benzoyl-*N*-(4-nitrophenyl)-3-phenyl-6,7-dihydro-1*H*-pyrazolo[4,3-*c*]pyridine-5(4*H*)-carboxamide) was found to be the most potent compound with IC_{50} of 21.8 ± 0.8 µM. All the compounds were tested for *in vitro* cytotoxicity against RAW 264.7 cells at 50 µM concentration by MTT assay. The most promising anti-TB compound **PP_09** was devoid of cytotoxicity at 50 µM [0% inhibition].

5.3.6. Highlights of the study

In the present study, we report design, synthesis and biological evaluation of forty 3-phenyl-4,5,6,7-tetrahydro-1*H*-pyrazolo[4,3-*c*]pyridine derivatives against *M. tuberculosis* PS as well as drug sensitive *M. tuberculosis* strains. Compound **PP_09 (**1-Benzoyl-*N*-(4-nitrophenyl)-3-

phenyl-6,7-dihydro-1*H*-pyrazolo[4,3-*c*]pyridine-5(4*H*)-carboxamide) was found to be the most active compound with IC_{50} of 21.8±0.8 µM against *M. tuberculosis* PS, inhibited drug sensitive *M. tuberculosis* with MIC of 26.7 µM and was non-cytotoxic at 50 µM (**Figure 5.13**).



PP_09
MTB PS IC_{50} 21.8±0.8 µM
MTB MIC 26.7 µM

Figure 5.13: Chemical structure and biological activity of the most active compound **PP_09**

5.4. Design and synthesis of tetrahydrothieno[2,3-*c*]pyridine-3-carboxamide derivatives as novel anti-tubercular agents

We attempted to design lead molecule using molecular hybridization strategy and synthesized twenty six 2,6-disubstituted 4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine-3-carboxamide derivatives from piperidin-4-one by six step synthesis. Synthesized compounds were evaluated for *M. tuberculosis* PS inhibition, *in vitro* drug sensitive *M. tuberculosis* inhibition and cytotoxicity.

5.4.1. Design of lead molecule

Molecular hybridization is a strategy of rational design of new ligands or prototypes based on the recognition of pharmacophoric sub-units in the molecular structure of two or more known bioactive derivatives, which through adequate fusion lead to the design of new hybrid architectures that maintain pre-selected characteristics of the original templates.

The most active compound from our first series of molecules against *M. tuberculosis* PS was **PP_09** (1-Benzoyl-*N*-(4-nitrophenyl)-3-phenyl-6,7-dihydro-1*H*-pyrazolo[4,3-*c*]pyridine-5(4*H*)-carboxamide) with IC_{50} 21.8 µM and a reported anti-TB compound **SID 92097880** (6-acetyl-2-(thiophene-2-carboxamido)-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine-3-carboxamide) [Reynolds R.C., *et al*., 2012] with *M. tuberculosis* MIC of 9.15 µM were taken and subjected to molecular hybridisation strategy. After combining the pharmocophoric areas from both the compounds, the resulted compound was taken as lead molecule (**Figure 5.14**).

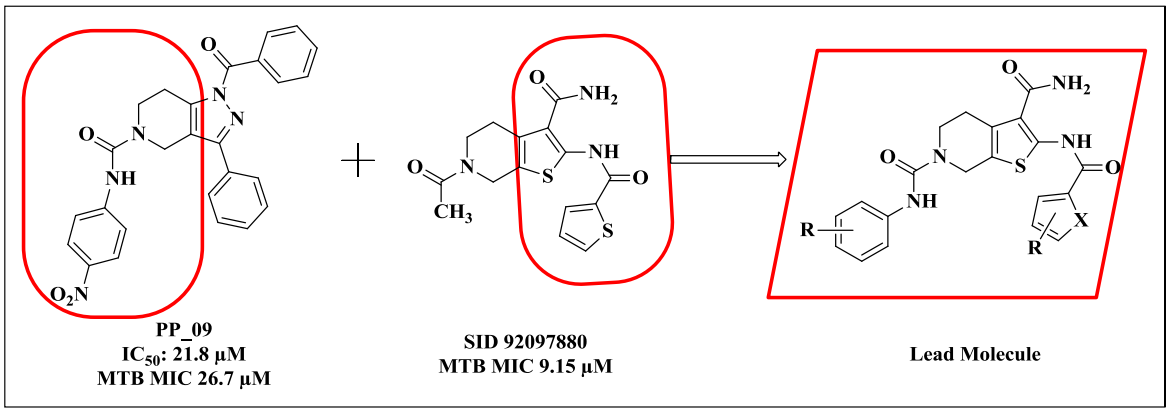


Figure 5.14: Designing of lead molecule by molecular hybridization strategy

5.4.2. Experimental procedures utilized for the synthesis of TP_09 – TP_34

The designed target molecules were synthesized by following six step synthetic protocol (**Figure 4.5**). Starting with commercially available and less expensive 4-piperidone hydrochloride monohydrate (**1**), in the first step we protected the amine group of starting material with Boc-protection. Here we used the combination of methanol/dichloromethane as reaction solvents in order to achieve greater yields. Then the obtained *N*-Boc-piperidone (**TP_02**) was treated with cyanoacetamide (**TP_03**), sulphur powder and morpholine in ethanol (Gewald reaction, **Figure 5.15**) to yield 2-aminothiophene derivative (**TP_04**). Here our initial plan was to synthesize compound **TP_04**, in linear synthesis fashion using ethylcyanoacetate. *i.e.,* reaction of compound **TP_02** with ethylcyanoacetate, sulphur powder, morpholine under Gewald reaction conditions to produce the intermediate "6-*tert*-butyl 3-ethyl 2-amino-4,5-dihydrothieno[2,3-*c*]pyridine-3,6(7*H*)-dicarboxylate"; later conversion of ester to amide to achieve compound **TP_04**. Here we succeeded in synthesising "6-*tert*-butyl 3-ethyl 2-amino-4,5-dihydrothieno[2,3-*c*]pyridine-3,6(7*H*)-dicarboxylate", but we were unable convert ester group to amide. This might be due to prevention of the attack by nucleophile (NH_3) on ester by the steric bulkiness of Boc-protected six membered ring. Hence we followed the convergent synthesis; wherein we reacted the ethylcyanoacetate with ammonia to produce cyanoacetamide (**TP_03**). Then treating cyanoacetamide with compound **TP_02** under Gewald reaction conditions produced compound **TP_04**. The 2-amino group of compounds **TP_04** has reacted with 5-nitro furan/thiophen-2-caryboxylic acid chlorides **TP_05a-b** to yield compounds **TP_06** and **TP_07**. Here we converted the carboxylic group into acid chloride using oxalyl chloride, since the attempts to couple the carboxylic acid group with the amine using coupling agents (EDCI/HOBt and HATU) failed to obtain desired product. Then the deprotection of Boc-group using trifluoroacetic acid yielded free secondary amino function **TP_08a-b**; followed by reaction with various substituted arylisocyantes, arylisothiocyanates and arylsulphonyl chloride (**Table 5.9**) to yield corresponding urea, thiourea and sulphonyl chloride derivatives (**TP_09 to TP_34**).

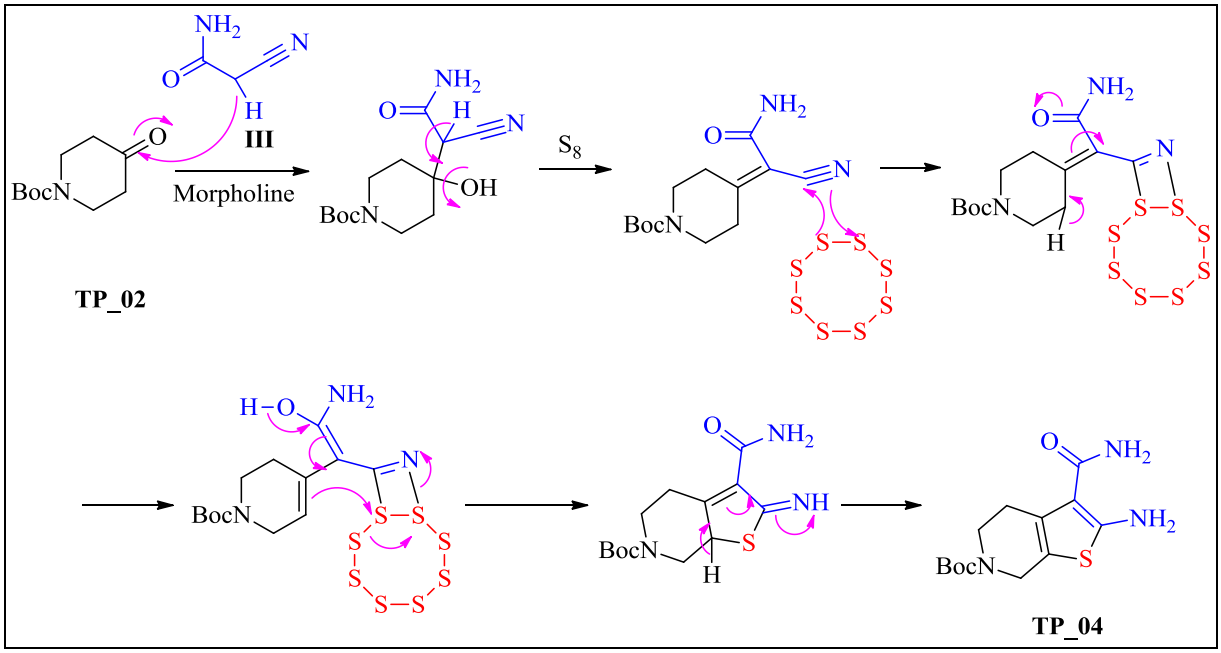
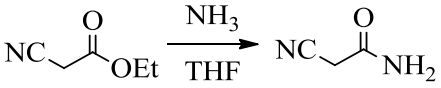


Figure 5.15: Mechanism of conversion of compound **TP_02** to **TP_04** (Gewald reaction)

Preparation of *tert*-butyl 4-oxopiperidine-1-carboxylate (TP_02)

Et_3N (52.90 mL, 370.34 mmol) was added dropwise to a stirred solution of compound **1** (20.0 g, 148.14 mmol) in CH_2Cl_2 (250 mL) and MeOH (25 mL) at 0 °C, then $(Boc)_2O$ (38.74 mL, 177.76 mmol) was added drop wise to the reaction mixture at same temperature and allowed to stir at room temperature for 16 h. The reaction mixture was diluted with H_2O (200 mL) and extracted with CH_2Cl_2 (3 × 300 mL). The separated organic layer was concentrated under reduced pressure and the crude residue was washed with hexanes to get compound **TP_02** (21.6 g, 73%) as an off-white solid. ESI-MS found 200 $[M+H]^+$and carried to next step.

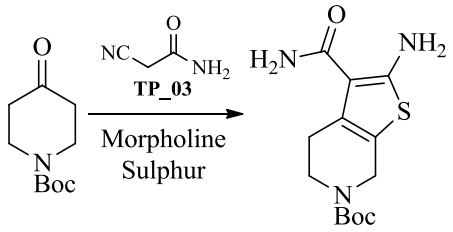
Preparation of 2-cyanoacetamide (TP_03)



TP_03

To the stirred solution of ethylcyanoacetate (3.0 g) in THF (10 mL) was added aqueous ammonia (15.0 mL), and allowed to stir at room temperature in a closed vessel for 2 h. The reaction mixture was concentrated under reduced pressure and obtained solids were filtered, dried in vacuum oven to get 2-cyanoacetamide (2.1 g, 93%) as white solid. ESI-MS found 85 $[M+H]^+$.

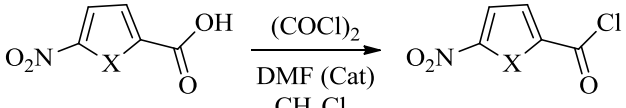
Preparation of *tert*-butyl 2-amino-3-carbamoyl-4,5-dihydrothieno[2,3-*c*]pyridine-6(7*H*)-carboxylate (TP_04)



TP_02 **TP_04**

To the stirred solution of compound **TP_02** (3.0 g, 15.07 mmol), 2-cyanoacetamide (1.51 g, 18.09 mmol), sulphur powder (1.06 g, 15.07 mmol) in ethanol (40 mL) was added morpholine (3.21 mL, 33.15 mmol) and stirred the reaction mixture at room temperature for 9 h. The reaction mixture was concentrated, diluted with EtOAc and washed the organic layer with H_2O (2 × 30 mL). The separated organic layer was dried over anhydrous Na_2SO_4, evaporated and purified by column chromatography to get compound **TP_04** (3.67 g, 82%) as light yellow solid. ESI-MS found 298 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.25 (s, 2H), 7.90 (s, 2H), 4.32 (s, 2H), 3.51 (t, J = 6.8 Hz, 2H), 3.04 (t, J = 6.8 Hz, 2H).

General procedure for the preparation of compound TP_05

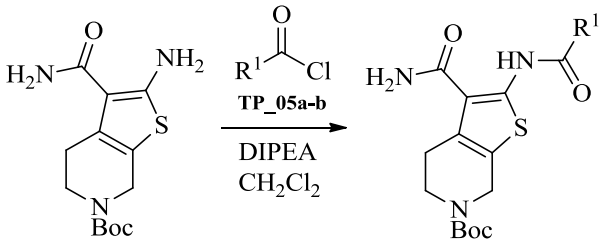


X = O,S

X = O: TP_05a
X = S: TP_05b

5-Nitrothiophene-2-carboxylic acid (2.0 g) was taken in a 100 mL single neck RB flask equipped with N_2-inlet, to this was added CH_2Cl_2 (30 mL), oxalyl chloride (6.0 mL, 3 vol) at 0 °C and stirred for few minutes. Then DMF (few drops) was added dropwise at same temperature until evolution of bubbles in the reaction mixture was ceased, and allowed to stir at room temperature for 3 h. The reaction mixture was concentrated and re-dissolved in dry CH_2Cl_2 under inert atmosphere and used in next step.

General procedure for the preparation of compound TP_06/TP_07

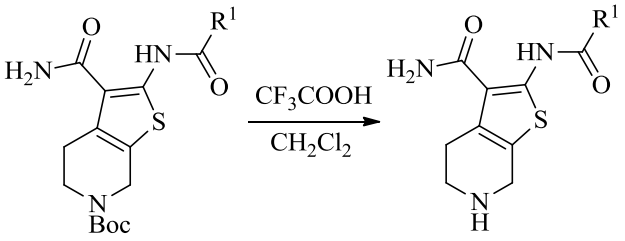


TP_04

TP_06: R^1 = 5-nitrothiophen-2-yl
TP_07: R^1 = 5-nitrofuran-2-yl

To the stirred solution of compound **TP_04** (1.00 equiv), in CH_2Cl_2 at 0 °C, was added DIPEA (2.50 equiv) stirred for few minutes and then added compound **TP_05** (1.20 equiv) dropwise under N_2 atmosphere at same temperature, and allowed to stir at room temperature for 6 h. The reaction mixture was diluted with CH_2Cl_2 and washed with H_2O, the separated organic layer was dried over anhydrous Na_2SO_4 and evaporated *in vacuo*. The obtained product was purified by column chromatography using EtOAc/Hexanes as eluant.

General procedure for the preparation of compounds TP_08a-b

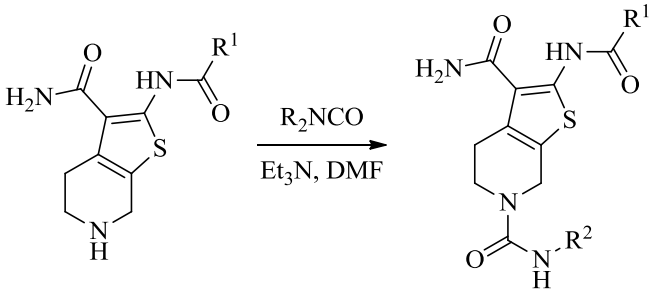


TP_06: R^1 = 5-nitrothiophen-2-yl;
TP_07: R^1 = 5-nitrofuran-2-yl;

TP_08a-b

To the stirred solution of compound **TP_06/TP_07** in CH_2Cl_2 at 0 °C under N_2 atmosphere, was added CF_3COOH (2 vol) and allowed to stir at room temperature for 2 h. The reaction mixture was concentrated to dryness and the obtained solids were washed with hexanes to afford compound **TP_08a-b** respectively.

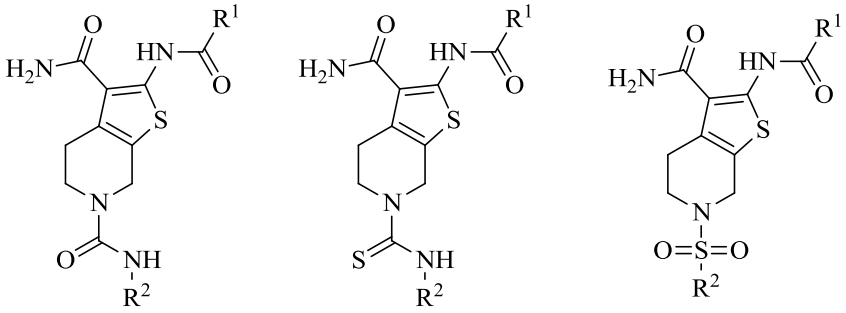
General procedure for the synthesis of final compounds TP_09 – TP_12, TP_22 – TP_25



TP_08a-b **TP_09 - TP_12 TP_22 - TP_25**

To the stirred solution of compound **TP_08a** (for **TP_09** – **TP_12**)/ **TP_08b** (for **TP_22** – **TP_25**) in DMF at 0 °C under N_2 atmosphere, was added Et_3N (2.0 equiv) followed by addition of arylisocyanate (1.2 equiv) and allowed to stir at room temperature for 3 h. The reaction mixture was diluted with EtOAc and washed with brine solution and H_2O. The separated organic layer was dried over anhydrous Na_2SO_4 and evaporated *in vacuo*, the obtained product was purified by column chromatography using EtOAc/Hexanes as eluant.

Table 5.9: Physiochemical properties of the synthesized compounds **TP_09** – **TP_34**



TP_09 - TP_12 TP_22 - TP_25 **TP_13 - TP_16 TP_26 - TP_29** **TP_17 - TP_21 TP_30 - TP_34**

Compd	R^1	R^2	Yield (%)	M.P. (°C)	Molecular formula	Molecular weight
TP_09	5-Nitrothiophen-2-yl	4-Chlorophenyl	82	210-211	$C_{20}H_{16}ClN_5O_5S_2$	505.95
TP_10	5-Nitrothiophen-2-yl	4-Nitrophenyl	79	234-235	$C_{20}H_{16}N6O_7S_2$	516.51
TP_11	5-Nitrothiophen-2-yl	Benzyl	81	223-224	$C_{21}H_{19}N_5O_5S_2$	485.08
TP_12	5-Nitrothiophen-2-yl	4-Methoxyphenyl	69	169-170	$C_{21}H_{19}N_5O_6S_2$	501.54

Contd

Compd	R^1	R^2	Yield (%)	M.P. (°C)	Molecular formula	Molecular weight
TP_13	5-Nitrothiophen-2-yl	4-Fluorophenyl	76	184-185	$C_{20}H_{16}FN_5O_4S_3$	505.56
TP_14	5-Nitrothiophen-2-yl	4-Chlorophenyl	64	218-219	$C_{20}H_{16}ClN_5O_4S_3$	522.02
TP_15	5-Nitrothiophen-2-yl	4-Nitrophenyl	79	225-226	$C_{20}H_{16}N_6O_6S_3$	532.58
TP_16	5-Nitrothiophen-2-yl	Benzyl	67	163-164	$C_{21}H_{19}N_5O_4S_3$	501.61
TP_17	5-Nitrothiophen-2-yl	4-Fluorophenyl	67	230-231	$C_{19}H_{15}FN_4O_6S_3$	510.54
TP_18	5-Nitrothiophen-2-yl	4-Bromophenyl	74	222-223	$C_{19}H_{15}BrN_4O_6S_3$	571.44
TP_19	5-Nitrothiophen-2-yl	4-Nitrophenyl	78	228-229	$C_{19}H_{15}N_5O_8S_3$	537.55
TP_20	5-Nitrothiophen-2-yl	4-Acetylphenyl	81	243-244	$C_{21}H_{18}N_4O_7S_3$	534.51
TP_21	5-Nitrothiophen-2-yl	4-Methoxyphenyl	63	213-214	$C_{20}H_{18}N_4O_7S_3$	522.57
TP_22	5-Nitrofuran-2-yl	4-Chlorophenyl	71	228-229	$C_{20}H_{16}ClN_5O_6S$	489.89
TP_23	5-Nitrofuran-2-yl	4-Nitrophenyl	66	246-247	$C_{20}H_{16}N_6O_8S$	500.44
TP_24	5-Nitrofuran-2-yl	Benzyl	67	171-172	$C_{21}H_{19}N_5O_6S$	469.47
TP_25	5-Nitrofuran-2-yl	4-Methoxyphenyl	74	189-190	$C_{21}H_{19}N_5O_7S$	485.47
TP_26	5-Nitrofuran-2-yl	4-Fluorophenyl	70	215-216	$C_{20}H_{16}FN_5O_5S_2$	489.50
TP_27	5-Nitrofuran-2-yl	4-Chlorophenyl	62	236-237	$C_{20}H_{16}ClN_5O_5S_2$	505.95
TP_28	5-Nitrofuran-2-yl	4-Nitrophenyl	69	252-253	$C_{20}H_{16}N6O_7S_2$	516.51
TP_29	5-Nitrofuran-2-yl	Benzyl	64	180-181	$C_{21}H_{19}N_5O_5S_2$	485.54
TP_30	5-Nitrofuran-2-yl	4-Fluorophenyl	74	219-220	$C_{19}H_{15}FN_4O_7S_2$	494.47
TP_31	5-Nitrofuran-2-yl	4-Bromophenyl	76	226-227	$C_{19}H_{15}BrN_4O_7S_2$	555.38
TP_32	5-Nitrofuran-2-yl	4-Nitrophenyl	79	231-232	$C_{19}H_{15}N_5O_9S_2$	521.48
TP_33	5-Nitrofuran-2-yl	4-Acetylphenyl	72	245-246	$C_{21}H_{18}N_4O_8S_2$	518.52
TP_34	5-Nitrofuran-2-yl	4-Methoxyphenyl	74	204-205	$C_{20}H_{18}N_4O_8S_2$	506.51

5.4.3. Characterization of the synthesized molecules

6-(4-Chlorophenylcarbamoyl)-2-(5-nitrothiophene-2-carboxamido)-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine-3-carboxamide (TP_09): Yield: 82%; m.p. 210–211 °C; MS(ESI) *m/z* 506 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.21 (s, 1H), 11.23 (s, 1H),

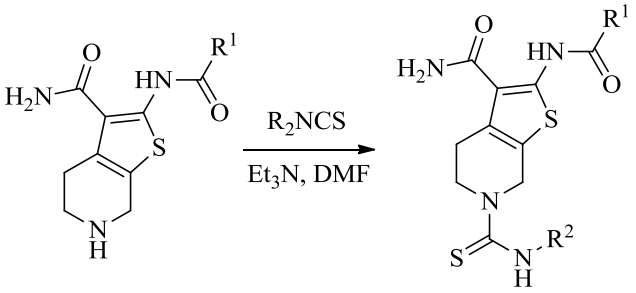
8.31 (d, *J* = 7.6 Hz, 1H), 8.10 (d, *J* = 7.2 Hz, 1H), 7.90 (d, *J* = 8.0 Hz, 2H), 7.81–7.72 (m, 4H), 4.66 (s, 2H), 3.91 (t, *J* = 6.8 Hz, 2H), 3.13 (t, *J* = 6.8 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 179.1, 172.8, 165.2, 162.9, 150.3, 148.4, 139.6, 136.9, 135.6, 134.4, 132.6(2C), 130.4, 129.5(2C), 124.5, 116.6, 49.9, 44.6, 21.9. Anal calcd for: $C_{20}H_{16}ClN_5O_5S_2$: C, 47.48; H, 3.19; N, 13.84 % Found C, 47.66; H, 3.24; N, 13.92%.

6-(4-Nitrophenylcarbamoyl)-2-(5-nitrothiophene-2-carboxamido)-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine-3-carboxamide (TP_10): Yield: 79%; m.p. 234–235 °C; MS(ESI) *m/z* 517 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.42 (s, 1H), 11.26 (s, 1H), 8.41 (d, *J* = 7.2 Hz, 1H), 8.22 (d, *J* = 7.2 Hz, 1H), 7.87–7.69 (m, 6H), 4.72 (s, 2H), 4.03 (t, *J* = 6.8 Hz, 2H), 3.31 (t, *J* = 6.8 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 180.1, 171.4, 165.2, 164.9, 156.3, 146.4, 142.6, 139.9, 137.6, 133.4, 132.6(2C), 129.9, 129.1(2C), 127.4, 109.6, 47.9, 42.3, 20.1. Anal calcd for: $C_{20}H_{16}N6O_7S_2$: C, 46.51; H, 3.12; N, 16.27 % Found C, 46.66; H, 3.24; N, 16.33%.

6-(Benzylcarbamoyl)-2-(5-nitrothiophene-2-carboxamido)-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine-3-carboxamide (TP_11): Yield: 81%; m.p. 223–224 °C; MS(ESI) *m/z* 486 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.42 (s, 1H), 11.62 (s, 1H), 8.31 (d, *J* = 7.2Hz, 1H), 8.13 (d, *J* = 7.2 Hz, 1H), 7.81–7.63 (m, 7H), 5.11 (s, 2H), 4.63 (s, 2H), 4.03 (t, *J* = 6.8 Hz, 2H), 3.01 (t, *J* = 6.8 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.3, 172.8, 166.2, 163.9, 158.3, 152.5, 147.2, 142.6, 133.9, 135.5(2C), 133.4, 130.3(2C), 128.4, 126.9, 116.7, 52.4, 46.9, 42.6, 19.2. Anal calcd for: $C_{21}H_{19}N_5O_5S_2$: C, 51.95; H, 3.94; N, 14.42 % Found C, 52.00; H, 3.97; N, 14.54%.

6-(4-Methoxyphenylcarbamoyl)-2-(5-nitrothiophene-2-carboxamido)-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine-3-carboxamide (TP_12): Yield: 69%; m.p. 169–170 °C; MS(ESI) *m/z* 502 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.32 (s, 1H), 11.54 (s, 1H), 8.22 (d, *J* = 7.6 Hz, 1H), 8.08 (d, *J* = 7.2 Hz, 1H), 7.83 (d, *J* = 7.6 Hz, 2H), 7.77 (s, 2H), 7.72 (d, *J* = 8.0 Hz, 2H), 4.58 (s, 2H), 4.03 (t, *J* = 6.8 Hz, 2H), 3.94 (s, 3H), 3.21 (t, *J* = 6.8 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 181.1, 177.8, 164.2, 161.9, 158.3, 154.5, 148.2, 141.6, 136.9, 136.0, 133.4, 132.3(2C), 132.4 (2C), 128.5, 118.5, 56.4, 47.9, 43.6, 21.0. Anal calcd for: $C_{21}H_{19}N_5O_6S_2$: C, 50.29; H, 3.82; N, 13.96 % Found C, 50.33; H, 3.91; N, 13.99%.

General procedure for the synthesis of final compounds TP_13 – TP_16, TP_26 – TP_29



TP_08a-b **TP_13 - TP_16** **TP_26 - TP_29**

To the stirred solution of compound **TP_08a** (for **TP_13** – **TP_16**)/ **TP_08b** (for **TP_26** – **TP_29**) in DMF at 0 °C under N_2 atmosphere, was added Et_3N (2.0 equiv) followed by addition of arylisothiocyanate (1.2 equiv) and allowed to stir at room temperature for 3 h. The reaction mixture was diluted with EtOAc and washed with brine solution and H_2O. The separated organic layer was dried over anhydrous Na_2SO_4 and evaporated *in vacuo,* the obtained product was purified by column chromatography using EtOAc/Hexanes as eluant.

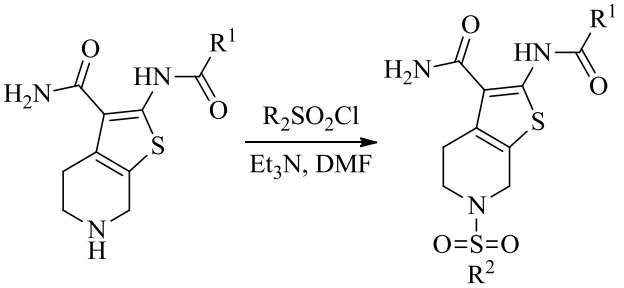
6-(4-Fluorophenylcarbamothioyl)-2-(5-nitrothiophene-2-carboxamido)-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine-3-carboxamide (TP_13): Yield: 76%; m.p. 184–185 °C; MS(ESI) *m/z* 506 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.43 (s, 1H), 8.31 (d, *J* = 7.2 Hz, 1H), 8.03 (d, *J* = 7.2 Hz, 1H), 7.91 (d, *J* = 8.0 Hz, 2H), 7.87 (d, *J* = 8.0 Hz, 2H), 7.81–7.74 (m, 3H), 4.82 (s, 2H), 3.89 (t, *J* = 7.2 Hz, 2H), 3.04 (t, *J* = 7.2 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 180.8, 180.0, 171.9, 164.1, 160.9, 158.2, 146.2, 142.4, 141.5, 139.4, 134.3(2C), 133.8, 130.0(2C), 129.0, 116.1, 47.3, 43.2, 22.3. Anal calcd for: $C_{20}H_{16}FN_5O_4S_3$: C, 47.51; H, 3.19; N, 13.85 % Found C, 47.61; H, 3.23; N, 13.88%.

6-(4-Chlorophenylcarbamothioyl)-2-(5-nitrothiophene-2-carboxamido)-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine-3-carboxamide (TP_14): Yield: 64%; m.p. 218–219 °C; MS(ESI) *m/z* 523 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.54 (s, 1H), 8.29 (d, *J* = 7.2 Hz, 1H), 8.16 (d, *J* = 7.2 Hz, 1H), 7.86 (d, *J* = 8.0 Hz, 2H), 7.83–7.74 (m, 5H), 4.72 (s, 2H), 3.96 (t, *J* = 7.2 Hz, 2H), 3.11 (t, *J* = 7.2 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 178.8, 177.1, 168.8, 161.1, 159.3, 148.2, 144.2, 143.4, 141.9, 138.4, 136.3(2C), 133.2, 132.7(2C), 126.0, 116.5, 49.3, 42.3, 21.0. Anal calcd for: $C_{20}H_{16}ClN_5O_4S_3$: C, 46.02; H, 3.09; N, 13.42 % Found C, 46.09; H, 3.11; N, 13.49%.

6-(4-Nitrophenylcarbamothioyl)-2-(5-nitrothiophene-2-carboxamido)-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine-3-carboxamide (TP_15): Yield: 79%; m.p. 225–226 °C; MS(ESI) *m/z* 533 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.38 (s, 1H), 11.22 (s, 1H), 8.37 (d, *J* = 7.2 Hz, 1H), 8.18 (d, *J* = 7.2 Hz, 1H), 8.01 (d, *J* = 7.6 Hz, 2H), 7.83–7.72 (m, 4H), 4.69 (s, 2H), 3.94 (t, *J* = 6.8Hz, 2H), 3.23 (t, *J* = 6.8 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 179.8, 178.9, 168.2, 161.9, 158.3, 148.2, 143.6, 141.9, 138.9, 136.4, 135.9(2C), 134.1, 133.9(2C), 127.0, 120.6, 50.2, 45.0, 22.5. Anal calcd for: $C_{20}H_{16}N_6O_6S_3$: C, 45.10; H, 3.03; N, 15.78 % Found C, 45.21; H, 3.09; N, 15.81%.

6-(Benzylcarbamothioyl)-2-(5-nitrothiophene-2-carboxamido)-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine-3-carboxamide (TP_16): Yield: 67%; m.p. 163–164 °C; MS(ESI) *m/z* 502 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.24 (s, 1H), 10.06 (s, 1H), 8.52 (t, *J* = 6.4 Hz, 1H), 8.01 (d, *J* = 7.2 Hz, 1H), 7.62–7.45 (m, 7H), 5.19 (s, 2H), 4.81 (s, 2H), 4.12 (t, *J* = 7.2 Hz, 2H), 3.01 (t, *J* = 7.2 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 180.7, 171.9, 161.1, 160.9, 149.3, 139.4, 138.6, 136.9, 134.6, 133.9 (2C), 130.7 (2C), 127.4, 126.4, 116.6, 112.6, 49.2, 43.8, 22.5, 20.5. Anal calcd for: $C_{21}H_{19}N_5O_4S_3$: C, 50.28; H, 3.82; N, 13.96 % Found C, 50.31; H, 3.87; N, 14.04%.

General procedure for the synthesis of final compounds TP_17 – TP_21, TP_30 – TP_34



TP_08a-b **TP_17 - TP_21** **TP_30 - TP_34**

To the stirred solution of compound **TP_08a** (for **TP_17 – TP_21**)/ **TP_08b** (for **TP_30 – TP_34**) in DMF at 0 °C under N_2 atmosphere, was added Et_3N (2.0 equiv) followed by addition of arylsulphonyl chloride (1.2 equiv) and allowed to stir at room temperature for 4 h. The reaction mixture was diluted with EtOAc and washed with brine solution and H_2O. The separated organic layer was dried over anhydrous Na_2SO_4 and evaporated *in vacuo*, the obtained product was purified by column chromatography using EtOAc/Hexanes as eluant.

6-(4-Fluorophenylsulfonyl)-2-(5-nitrothiophene-2-carboxamido)-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine-3-carboxamide (TP_17): Yield: 67%; m.p. 230–231 °C; MS(ESI) *m/z* 511 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.03 (s, 1H), 8.22 (d, *J* = 7.6 Hz, 1H), 7.99 (d, *J* = 7.2 Hz, 1H), 7.92 (d, *J* = 8.0 Hz, 2H), 7.81–7.69 (m, 4H), 4.61 (s, 2H), 3.99 (t, *J* = 6.8 Hz, 2H), 3.01 (t, *J* = 6.8 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.1, 171.7, 166.3, 164.1, 162.6, 146.2, 145.3, 142.5, 139.4(2C), 136.3, 133.2(2C), 130.5, 126.0, 119.4, 48.4, 44.3, 22.2. Anal calcd for: $C_{19}H_{15}FN_4O_6S_3$: C, 44.70; H, 2.96; N, 10.97 % Found C, 44.73; H, 3.03; N, 10.98%.

6-(4-Bromophenylsulfonyl)-2-(5-nitrothiophene-2-carboxamido)-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine-3-carboxamide (TP_18): Yield: 74%; m.p. 222–223 °C; MS(ESI) *m/z* 571 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.82 (s, 1H), 8.19 (d, *J* = 7.2 Hz, 1H), 7.92 (d, *J* = 7.2 Hz, 1H), 7.87 (d, *J* = 8.0 Hz, 2H), 7.83–7.72 (m, 4H), 4.59 (s, 2H), 4.12 (t, *J* = 6.8 Hz, 2H), 3.04 (t, *J* = 6.8 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 178.1, 170.7, 166.3, 163.4, 157.6, 146.5, 142.3, 138.6, 137.2(2C), 135.1(2C), 132.3, 128.5, 124.4, 120.4, 49.3, 46.2, 21.6. Anal calcd for: $C_{19}H_{15}BrN_4O_6S_3$: C, 39.93; H, 2.65; N, 9.80 % Found C, 39.99; H, 2.71; N, 9.88%.

6-(4-Nitrophenylsulfonyl)-2-(5-nitrothiophene-2-carboxamido)-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine-3-carboxamide (TP_19): Yield: 78%; m.p. 228–229 °C; MS(ESI) *m/z* 538 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.22 (s, 1H), 8.31 (d, *J* = 7.6 Hz, 1H), 8.03 (d, *J* = 7.6 Hz, 1H), 7.97 (d, *J* = 8.0 Hz, 2H), 7.89 (d, *J* = 8.0 Hz, 2H), 7.74 (s, 2H), 4.41 (s, 2H), 4.03 (t, *J* = 7.2 Hz, 2H), 3.10 (t, *J* = 6.8 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.5, 174.7, 163.4, 161.1, 159.6, 156.2, 150.2, 142.2, 137.2(2C), 135.3, 132.7(2C), 129.4, 124.8, 120.2, 47.4, 43.3, 21.2. Anal calcd for: $C_{19}H_{15}N_5O_8S_3$: C, 42.45; H, 2.81; N, 13.03 % Found C, 42.51; H, 2.88; N, 13.06%.

6-(4-Acetylphenylsulfonyl)-2-(5-nitrothiophene-2-carboxamido)-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine-3-carboxamide (TP_20): Yield: 81%; m.p. 243–244 °C; MS(ESI) *m/z* 534 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.91 (s, 1H), 8.01 (d, *J* = 7.2 Hz, 1H), 7.89 (d, *J* = 7.2 Hz, 1H), 7.81–7.72 (m, 6H), 4.32 (s, 2H), 4.03 (t, *J* = 6.8 Hz, 2H), 2.99 (t, *J* = 6.8 Hz, 2H), 2.23 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 189.9, 178.9, 171.4, 166.9, 160.3, 144.4, 144.0, 142.9, 141.6, 138.9 (2C), 136.2, 131.4(2C), 125.2, 121.2, 114.6, 48.2, 44.4, 24.2, 21.4. Anal calcd for: $C_{21}H_{18}N_4O_7S_3$: C, 47.18; H, 3.39; N, 10.48 % Found C, 47.21; H, 3.44; N, 10.53%.

6-(4-Methoxyphenylsulfonyl)-2-(5-nitrothiophene-2-carboxamido)-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine-3-carboxamide (TP_21): Yield: 63%; m.p. 213–214 °C; MS(ESI) *m/z* 523 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.56 (s, 1H), 8.31 (d, *J* = 7.2 Hz, 1H), 7.92 (d, *J* = 7.2 Hz, 1H), 7.81 (d, *J* = 7.6 Hz, 2H), 7.74 (s, 2H), 7.69 (d, *J* = 8.0 Hz, 2H), 4.63 (s, 2H), 4.12 (t, *J* = 6.8 Hz, 2H), 3.89 (s, 3H), 3.12 (t, *J* = 6.8 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 181.1, 176.0, 172.2, 164.2, 161.9, 152.2, 148.2, 141.4, 134.4, 133.2(2C), 132.1, 129.9(2C), 124.4, 119.2, 62.1, 49.3, 45.6, 24.0. Anal calcd for: $C_{20}H_{18}N_4O_7S_3$: C, 45.97; H, 3.47; N, 10.72 % Found C, 45.99; H, 3.51; N, 10.79%.

6-(4-Chlorophenylcarbamoyl)-2-(5-nitrofuran-2-carboxamido)-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine-3-carboxamide (TP_22): Yield: 71%; m.p. 228–229 °C; MS(ESI) *m/z* 490 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.32 (s, 1H), 10.44 (s, 1H), 8.19 (d, *J* = 7.6 Hz, 1H), 8.01 (d, *J* = 7.2 Hz, 1H), 7.81 (d, *J* = 8.0 Hz, 2H), 7.72 (d, *J* = 8.0 Hz, 2H), 7.63 (s, 2H), 4.45 (s, 2H), 3.94 (t, *J* = 7.2 Hz, 2H), 3.04 (t, *J* = 6.8 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 183.2, 172.5, 166.4, 154.2, 151.2, 149.5, 142.5, 139.5, 136.8, 132.6(2C), 130.7, 128.5(2C), 126.4, 123.3, 114.4, 46.7, 45.9, 21.4. Anal calcd for: $C_{20}H_{16}ClN_5O_6S$: C, 49.03; H, 3.29; N, 14.30 % Found C, 49.16; H, 3.34; N, 14.41%.

6-(4-Nitrophenylcarbamoyl)-2-(5-nitrofuran-2-carboxamido)-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine-3-carboxamide (TP_23): Yield: 66%; m.p. 246–247 °C; MS(ESI) *m/z* 501 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.23 (s, 1H), 11.11 (s, 1H), 8.31 (d, *J* = 7.2 Hz, 1H), 8.19 (d, *J* = 7.2 Hz, 1H), 7.92 (d, *J* = 7.6 Hz, 2H) 7.83–7.67 (m, 4H), 4.74 (s, 2H), 4.12 (t, *J* = 7.2 Hz, 2H), 3.21 (t, *J* = 6.8 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 180.7, 166.5, 162.3, 155.4, 152.1, 148.2, 145.4, 142.1, 136.2, 133.2, 130.4(2C), 127.8, 126.5(2C), 124.5, 115.4, 47.4, 45.4, 21.4. Anal calcd for: $C_{20}H_{16}N_6O_8S$: C, 48.00; H, 3.22; N, 16.79 % Found C, 48.03; H, 3.24; N, 16.82%.

6-(Benzylcarbamoyl)-2-(5-nitrofuran-2-carboxamido)-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine-3-carboxamide (TP_24): Yield: 67%; m.p. 171–172 °C; MS(ESI) *m/z* 470 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.25 (s, 1H), 11.31 (s, 1H), 8.41 (d, *J* = 7.2 Hz, 1H), 8.08 (d, *J* = 7.2 Hz, 1H), 7.87–7.72 (m, 7H), 5.22 (s, 2H), 4.52 (s, 2H), 4.11 (t, *J* = 6.8 Hz, 2H), 3.04 (t, *J* = 6.8 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.0, 169.2, 162.3, 157.5, 150.4, 148.3, 143.4, 137.2, 133.4, 133.0(2C), 132.6, 130.3(2C), 127.8, 126.9, 112.7, 48.1, 46.4, 43.6, 19.9. Anal calcd for: $C_{21}H_{19}N_5O_6S$: C, 53.73; H, 4.08; N, 14.92 % Found C, 53.81; H, 4.16; N, 14.96%.

6-(4-Methoxyphenylcarbamoyl)-2-(5-nitrofuran-2-carboxamido)-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine-3-carboxamide (TP_25): Yield: 74%; m.p. 189–190 °C; MS(ESI) *m/z* 486 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.34 (s, 1H), 11.76 (s, 1H), 8.32 (d, *J* = 7.2 Hz, 1H), 8.21 (d, *J* = 7.2 Hz, 1H), 7.92 (d, *J* = 7.6 Hz, 2H), 7.81 (s, 2H), 7.72 (d, *J* = 7.6 Hz, 2H), 4.48 (s, 2H), 4.08 (t, *J* = 6.8 Hz, 2H), 3.94 (s, 3H), 3.13 (t, *J* = 6.8 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 181.7, 168.9, 164.3, 153.9, 151.0, 148.6, 144.8, 141.6, 137.6, 136.4, 133.6(2C), 130.5(2C), 126.9, 124.6, 114.4, 61.2, 49.0, 47.6, 20.7. Anal calcd for: $C_{21}H_{19}N_5O_7S$: C, 51.95; H, 3.94; N, 14.43 % Found C, 52.03; H, 3.92; N, 14.58%.

6-(4-Fluorophenylcarbamothioyl)-2-(5-nitrofuran-2-carboxamido)-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine-3-carboxamide (TP_26): Yield: 70%; m.p. 215–216 °C; MS(ESI) *m/z* 490 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.23 (s, 1H), 10.24 (s, 1H), 8.26 (d, *J* = 7.2 Hz, 1H), 7.98 (d, *J* = 7.2 Hz, 1H), 7.91 (d, *J* = 8.0 Hz, 2H), 7.84 (d, *J* = 8.0 Hz, 2H), 7.78 (s, 2H), 4.69 (s, 2H), 3.93 (t, *J* = 7.2 Hz, 2H), 3.00 (t, *J* = 7.2 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 179.1, 176.4, 164.2, 158.4, 152.2, 150.6, 147.4, 145.3, 141.4, 139.4, 134.4, 133.2(2C), 130.4, 126.6(2C), 114.3, 47.3, 46.1, 19.9. Anal calcd for: $C_{20}H_{16}FN_5O_5S_2$: C, 49.07; H, 3.29; N, 14.31 % Found C, 49.11; H, 3.31; N, 14.40%.

6-(4-Chlorophenylcarbamothioyl)-2-(5-nitrofuran-2-carboxamido)-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine-3-carboxamide (TP_27): Yield: 62%; m.p. 210–211 °C; MS(ESI) *m/z* 506 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.26 (s, 1H), 11.11 (s, 1H), 8.22 (d, *J* = 7.6 Hz, 1H), 8.10 (d, *J* = 7.2 Hz, 1H), 7.92 (d, *J* = 8.0 Hz, 2H), 7.78–7.69 (m, 4H), 4.49 (s, 2H), 4.03 (t, *J* = 6.8 Hz, 2H), 3.03 (t, *J* = 6.8 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.1, 179.2, 166.9, 164.8, 158.8, 144.9, 142.8, 138.5, 137.3, 133.9, 132.6, 127.3(2C), 126.6(2C), 123.9, 116.1, 47.5, 46.7, 19.2. Anal calcd for: $C_{20}H_{16}ClN_5O_5S_2$: C, 47.48; H, 3.19; N, 13.84 % Found C, 47.52; H, 3.24; N, 13.89%.

2-(5-Nitrofuran-2-carboxamido)-6-(4-nitrophenylcarbamothioyl)-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine-3-carboxamide (TP_28): Yield: 69%; m.p. 252–253 °C; MS(ESI) *m/z* 517 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.37 (s, 1H), 11.18 (s, 1H), 8.34 (d, *J* = 7.2 Hz, 1H), 8.10 (d, *J* = 7.2 Hz, 1H), 7.81–7.72 (m, 6H), 4.66 (s, 2H), 4.12 (t, *J* = 6.8 Hz, 2H), 3.22 (t, *J* = 6.8 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 180.2, 171.9, 167.8, 158.4, 156.7, 144.8, 141.6, 135.7, 134.2, 133.3(2C), 132.7(2C), 130.5, 126.3, 124.2, 112.4, 48.1, 46.6, 19.8. Anal calcd for: $C_{20}H_{16}N6O_7S_2$: C, 46.51; H, 3.12; N, 16.27 % Found C, 46.60; H, 3.18; N, 16.31%.

6-(Benzylcarbamothioyl)-2-(5-nitrofuran-2-carboxamido)-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine-3-carboxamide (TP_29): Yield: 66%; m.p. 180–181 °C; MS(ESI) *m/z* 486 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.19 (s, 1H), 10.74 (s, 1H), 8.29 (d, *J* = 7.6 Hz, 1H), 8.13 (d, *J* = 7.2 Hz, 1H), 7.81 (d, *J* = 7.6 Hz, 2H), 7.74–7.63 (m, 5H), 5.11 (s, 2H), 4.39 (s, 2H), 4.13 (t, *J* = 6.8 Hz, 2H), 2.9 (t, *J* = 6.8 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 178.3, 172.6, 166.2, 160.9, 155.4, 154.1, 145.7, 144.9, 143.4, 137.4, 135.5, 132.6(2C), 129.4, 127.4(2C), 117.7, 63.0, 49.2, 46.7, 20.6. Anal calcd for: $C_{21}H_{19}N_5O_5S_2$: C, 51.95; H, 3.94; N, 14.42 % Found C, 52.06; H, 3.91; N, 14.56%.

6-(4-Fluorophenylsulfonyl)-2-(5-nitrofuran-2-carboxamido)-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine-3-carboxamide (TP_30): Yield: 74%; m.p. 219–220 °C; MS(ESI) *m/z* 495 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.13 (s, 1H), 8.31 (d, *J* = 7.6Hz, 1H), 8.09 (d, *J* = 7.2 Hz, 1H), 7.89 (d, *J* = 8.0 Hz, 2H), 7.81 (d, *J* = 7.6 Hz, 2H), 7.69 (s, 2H), 4.51 (s, 2H), 4.09 (t, *J* = 7.2 Hz, 2H), 3.04 (t, *J* = 6.8 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 183.6, 169.9, 168.3, 163.6, 149.9, 149.2, 141.4, 138.6, 136.5, 132.0(2C), 129.4, 127.6(2C), 123.2, 111.9, 48.3, 47.3, 21.4. Anal calcd for: $C_{19}H_{15}FN_4O_7S_2$: C, 46.15; H, 3.06; N, 11.33 % Found C, 46.23; H, 3.13; N, 11.38%.

6-(4-Bromophenylsulfonyl)-2-(5-nitrofuran-2-carboxamido)-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine-3-carboxamide (TP_31): Yield: 76%; m.p. 226–227 °C; MS(ESI) *m/z* 555 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.22 (s, 1H), 8.21 (d, *J* = 7.2 Hz, 1H), 8.02 (d, *J* = 7.2 Hz, 1H), 7.92 (d, *J* = 8.0 Hz, 2H), 7.81–7.69 (m, 4H), 4.47 (s, 2H), 4.04 (t, *J* = 6.8 Hz, 2H), 2.89 (t, *J* = 6.8 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 180.7, 176.6, 172.7, 162.6, 160.2, 147.2, 144.6, 133.2(2C), 132.6, 132.0(2C), 127.3, 126.9, 124.2, 117.2, 48.4, 47.3, 21.6. Anal calcd for: $C_{19}H_{15}BrN_4O_7S_2$: C, 41.09; H, 2.72; N, 10.09 % Found C, 41.17; H, 2.81; N, 10.17%.

2-(5-Nitrofuran-2-carboxamido)-6-(4-nitrophenylsulfonyl)-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine-3-carboxamide (TP_32): Yield: 79%; m.p. 231–232 °C; MS(ESI) *m/z* 522 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.18 (s, 1H), 8.24 (d, *J* = 7.6 Hz, 1H), 7.93 (d, *J* = 7.6 Hz, 1H), 7.74 (d, *J* = 8.0 Hz, 2H), 7.69 (s, 2H), 7.63 (d, *J* = 8.0 Hz, 2H), 4.32 (s, 2H), 3.93 (t, *J* = 7.2 Hz, 2H), 2.89 (t, *J* = 6.8 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.1, 174.2, 171.9, 154.3, 152.8, 150.9, 142.3, 139.6, 133.6, 130.6, 128.6(2C), 126.1(2C), 124.9, 118.4, 48.4, 47.3, 19.6. Anal calcd for: $C_{19}H_{15}N_5O_9S_2$: C, 43.76; H, 2.90; N, 13.43 % Found C, 43.81; H, 2.93; N, 13.50%.

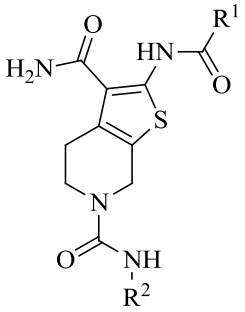
6-(4-Acetylphenylsulfonyl)-2-(5-nitrofuran-2-carboxamido)-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine-3-carboxamide (TP_33): Yield: 72%; m.p. 245–246 °C; MS(ESI) *m/z* 519 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.21 (s, 1H), 8.11 (d, *J* = 7.2 Hz, 1H), 7.99 (d, *J* = 7.2 Hz, 1H), 7.92 (d, *J* = 7.6 Hz, 2H), 7.81–7.72 (m, 4H), 4.26 (s, 2H), 4.07 (t, *J* = 6.8 Hz, 2H), 3.09 (t, *J* = 6.8 Hz, 2H), 2.20 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 188.1, 178.6, 174.8, 160.4, 146.7, 144.2, 142.4, 139.5, 136.8, 134.2(2C), 132.4(2C), 128.4, 125.3, 123.6, 114.3, 47.5, 46.9, 22.6, 20.7. Anal calcd for: $C_{21}H_{18}N_4O_8S_2$: C, 48.64; H, 3.50; N, 10.81 % Found C, 48.71; H, 3.57; N, 10.89%.

6-(4-Methoxyphenylsulfonyl)-2-(5-nitrofuran-2-carboxamido)-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine-3-carboxamide (TP_34): Yield: 74%; m.p. 204–205 °C; MS(ESI) *m/z* 507 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.06 (s, 1H), 8.21 (d, *J* = 7.2 Hz, 1H), 7.99 (d, *J* = 7.2 Hz, 1H), 7.80 (d, *J* = 7.6 Hz, 2H), 7.72–7.58 (m, 4H), 4.53 (s, 2H), 4.04 (t, *J* = 6.8 Hz, 2H), 3.90 (s, 3H), 3.04 (t, *J* = 6.8 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 177.1, 172.6, 169.9, 164.3, 163.8, 144.9, 138.3, 135.4, 132.4, 129.4, 125.1(2C), 124.4(2C), 123.5, 116.3, 60.5, 47.4, 45.9, 22.0. Anal calcd for: $C_{20}H_{18}N_4O_8S_2$: C, 47.43; H, 3.58; N, 11.06 % Found C, 47.49; H, 3.60; N, 11.09%.

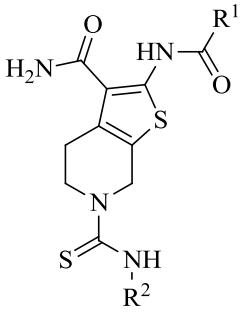
5.4.4. *In vitro M. tuberculosis* screening, *M. tuberculosis* PS enzyme inhibition assay and cytotoxicity studies of the synthesized molecules

All the synthesized compounds were first screened for their *in vitro* anti-tubercular activity against *M. tuberculosis* H37Rv using MABA method. Isoniazid, ethambutol, **SID 92097880** and compound **PP_09** were used as a reference compounds for comparison. All the synthesized derivatives were also screened for their *in vitro M. tuberculosis* PS inhibitory potency as steps towards hit optimization. Compounds showing *M. tuberculosis* MICs <25 µM were also tested for *in vitro* cytotoxicity against RAW 264.7 cells at 50 µM concentration using MTT assay and results are tabulated as **Table 5.10**.

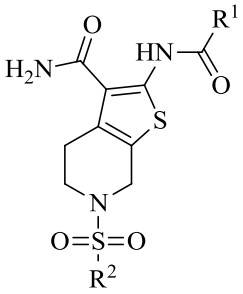
Table 5.10: *In vitro* biological evaluation of the synthesized compounds **TP_09** – **TP_34**



TP_09 - TP_12
TP_22 - TP_25



TP_13 - TP_16
TP_26 - TP_29



TP_17 - TP_21
TP_30 - TP_34

Compd	R^1	R^2	MTB MIC in µM	MTB PS IC_{50} µM	Cytotoxicity[a] at 50 µM % inhibition
TP_09	5-Nitrothiophen-2-yl	4-Chlorophenyl	12.32	10.22±0.8	24.42
TP_10	5-Nitrothiophen-2-yl	4-Nitrophenyl	12.09	9.68±0.28	33.63
TP_11	5-Nitrothiophen-2-yl	Benzyl	25.72	18.16±0.80	26.12
TP_12	5-Nitrothiophen-2-yl	4-Methoxyphenyl	24.90	14.50±0.66	20.86
TP_13	5-Nitrothiophen-2-yl	4-Fluorophenyl	12.34	10.16±0.51	26.76
TP_14	5-Nitrothiophen-2-yl	4-Chlorophenyl	11.95	6.01±0.32	21.42
TP_15	5-Nitrothiophen-2-yl	4-Nitrophenyl	11.72	9.11±0.43	28.62
TP_16	5-Nitrothiophen-2-yl	Benzyl	19.92	13.24±1.02	22.16
TP_17	5-Nitrothiophen-2-yl	4-Fluorophenyl	12.23	11.72±0.06	30.16
TP_18	5-Nitrothiophen-2-yl	4-Bromophenyl	21.89	14.22±0.09	26.82
TP_19	5-Nitrothiophen-2-yl	4-Nitrophenyl	9.28	5.77±0.12	29.16
TP_20	5-Nitrothiophen-2-yl	4-Acetylphenyl	46.73	>25	NT
TP_21	5-Nitrothiophen-2-yl	4-Methoxyphenyl	23.90	19.05±0.86	24.50
TP_22	5-Nitrofuran-2-yl	4-Chlorophenyl	51.0	>25	NT
TP_23	5-Nitrofuran-2-yl	4-Nitrophenyl	24.95	19.79±1.01	36.12
TP_24	5-Nitrofuran-2-yl	Benzyl	26.60	14.08±0.67	24.31
TP_25	5-Nitrofuran-2-yl	4-Methoxyphenyl	53.19	15.06±0.53	NT
TP_26	5-Nitrofuran-2-yl	4-Fluorophenyl	25.51	20.86±1.25	28.12

Contd

Compd	R^1	R^2	MTB MIC in µM	MTB PS IC_{50} µM	Cytotoxicity[a] at 50 µM % inhibition
TP_27	5-Nitrofuran-2-yl	4-Chlorophenyl	24.70	19.16±0.96	24.69
TP_28	5-Nitrofuran-2-yl	4-Nitrophenyl	24.18	18.72±0.64	28.21
TP_29	5-Nitrofuran-2-yl	Benzyl	26.48	17.02±0.73	18.12
TP_30	5-Nitrofuran-2-yl	4-Fluorophenyl	25.25	17.56±0.94	22.06
TP_31	5-Nitrofuran-2-yl	4-Bromophenyl	45.04	>25	NT
TP_32	5-Nitrofuran-2-yl	4-Nitrophenyl	47.89	>25	NT
TP_33	5-Nitrofuran-2-yl	4-Acetylphenyl	48.16	>25	NT
TP_34	5-Nitrofuran-2-yl	4-Methoxyphenyl	24.65	20.33±1.4	19.43
Isoniazid			0.72	>25	NT
Ethambutol			7.64	>25	NT
SID 92097880			9.15	>25	NT
Compound PP_09			26.7	21.8±0.8	0

IC_{50}, 50% inhibitory concentration; MTB, *Mycobacterium tuberculosis*; MIC, minimum inhibitory concentration; [a]Against RAW 264.7 cells; ND, Not tested.

5.4.5. SAR and Discussion

The synthesized compounds showed activity against *M. tuberculosis* with MIC ranging from 9.28 to 51 µM. Eight compounds (**TP_09-TP_10**, **TP_13-TP_17** and **TP_19**) inhibited *M. tuberculosis* with MIC of <20 µM. Compound **TP_19** (6-(4-nitrophenylsulfonyl)-2-(5-nitrothiophene-2-carboxamido)-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine-3-carboxamide) was found to be the most active compound *in vitro* with MIC of 9.28 µM against log-phase culture of *M. tuberculosis*. All the synthesized compounds were less potent than standard anti-tubercular compounds like isoniazid and ethambutol. Only compound **TP_19** showed equipotent as one of the lead compound **SID 92097880** (MIC 9.15 µM). When compared to another lead compound **PP_09** (MIC 21.8 µM), eight compounds were found to be more active.

In the mechanistic studies, the synthesized compounds showed *M. tuberculosis* PS enzyme inhibition in the range of 18.0-82.6% during initial screening at 25 µM. Twenty one compounds showed >50% inhibition against *M. tuberculosis* PS and were further selected for IC_{50} estimation and compounds showed IC_{50} in the range from 5.77±0.12 µM to 20.86±1.25

µM. All the twenty one compounds were found to be more potent than our earlier reported lead compound **PP_09** (IC_{50} of 21.8 µM). Compound **TP_19**, which showed good MIC in the *in vitro M. tuberculosis* screening was found to be most potent *M. tuberculosis* PS inhibitor with IC_{50} of 5.77±0.12 µM.

The most active compound **TP_19** was further taken for docking studies using crystal structure of *M. tuberculosis* PS co-crystallized with 2-(2-(benzofuran-2-ylsulfonylcarbamoyl)-5-methoxy-1*H*-indol-1-yl) acetic acid [**PDB: 3IVX**]. Analysis of the crystal structure of 3IVX revealed two key hydrogen-bonding interactions between the sulfone oxygen atom and both the backbone amide group of Met40 and the side-chain nitrogen atom of His47. It was also found to have hydrogen bonding interactions with Val187, Ser196, Ser197 and His44.

TP_19 made six hydrogen bonding interactions with the relevant amino acid residues such as Val187, Gln72, HIE44, Ser196, Ser197 and Lys160. In addition to hydrogen bonding interaction, the compound was further stabilized by π-π interaction with Hie44 and His135 amino acid residues. The NO_2 group on thiophene ring interacted with the side chain of Val187. His44 interacted with carbonyl oxygen of the amide and showed a π-π interaction with the thiophene ring, as well. Docking score of -9.22 kcal/mole, suggested that this compound was well placed in the active site and thus showed a good *M. tuberculosis* PS inhibitory activity. The binding pattern of the most active compound **TP_19** is represented in **Figure 5.16**.

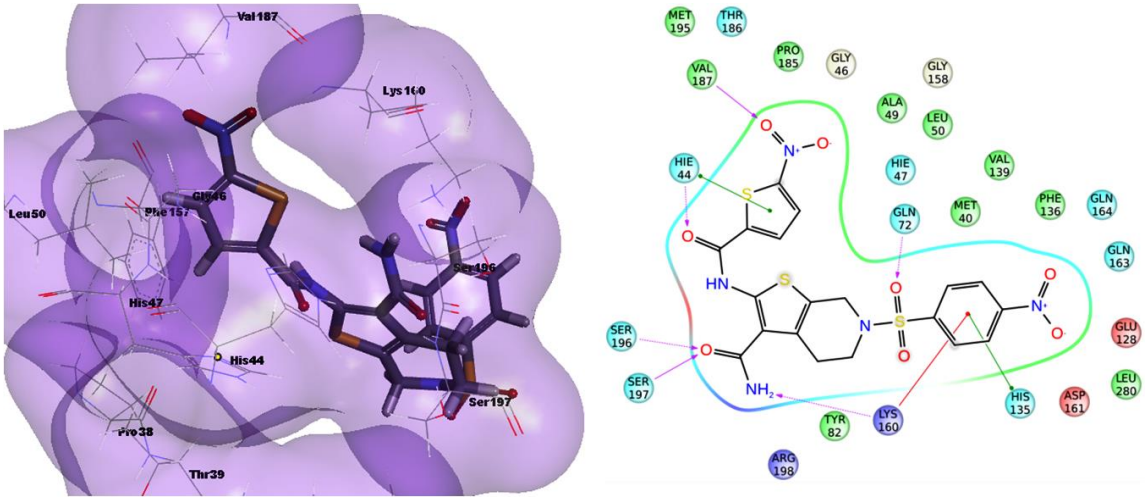


Figure 5.16: Binding pose and interaction pattern of most active compound **TP_19** with the protein

5.4.6. Highlights of the study

In this work we designed novel 2,6-disubstituted 4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine-3-carboxamide derivatives by molecular hybridization approach using reported anti-tubercular compounds and synthesized twenty six analogues. Many of the compounds showed better *M. tuberculosis* PS inhibition and *M. tuberculosis* MICs. In conclusion, it has been demonstrated that the potency, selectivity, and low cytotoxicity of these compounds make them valid leads for synthesizing new compounds that possess better activity. Compound **TP_19 (**6-((4-nitrophenyl)sulfonyl)-2-(5-nitrothiophene-2-carboxamido)-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine-3-carboxamide) was found to be the most active compound (**Figure 5.17**) with IC_{50} of 5.77±0.12 µM against *M. tuberculosis* PS, inhibited drug sensitive *M. tuberculosis* with MIC of 9.28 µM and was non-cytotoxic at 50 µM .

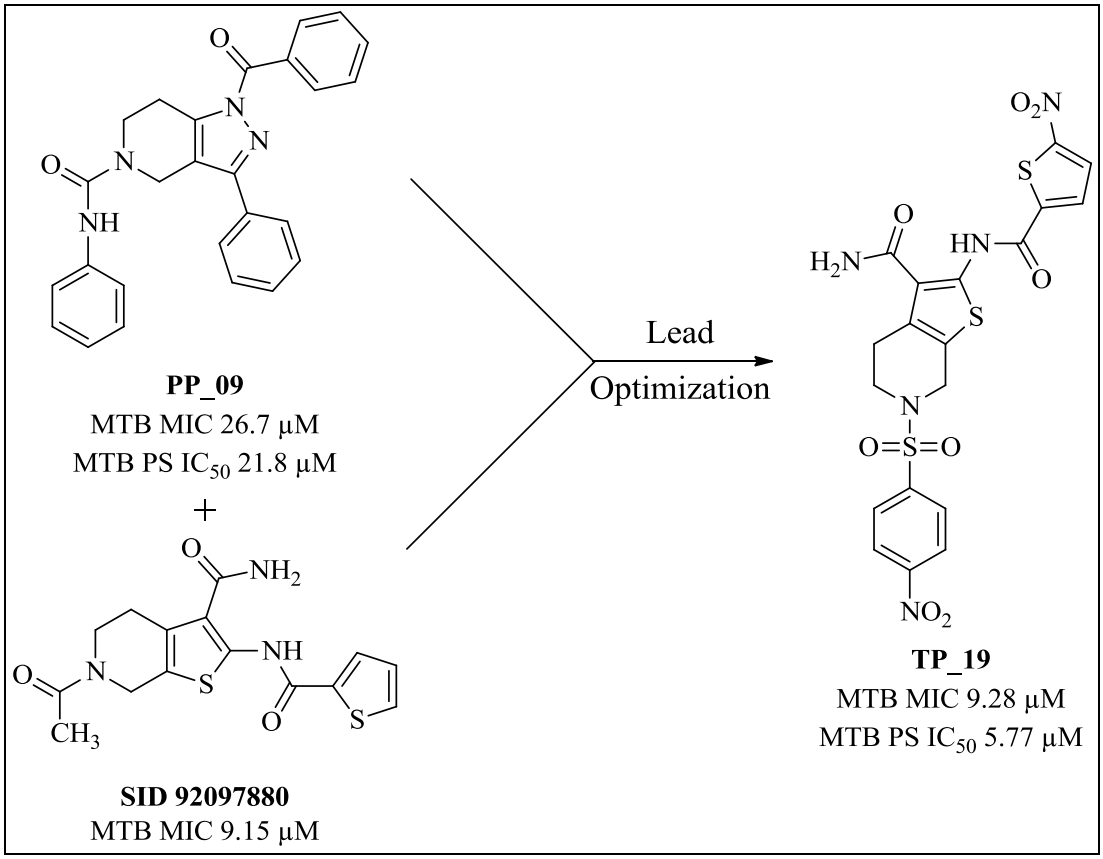


Figure 5.17: Structure and biological activity of most active compound **TP_19**

5.5. Development of pyrazolidine-3,5-dione derivatives as novel anti-tubercular agents

Thirty analogues of reported anti-tubercular 1-(4-chlorophenyl)-4-(4-hydroxy-3-methoxy-5-nitrobenzylidene)pyrazolidine-3,5-dione (**CD59**) were prepared and evaluated for *in vitro* activities against *M. tuberculosis* and cytotoxicity against RAW 264.7 cells. To explore possible target, most active compounds were also screened against *M. tuberculosis* PS, LAT and ADH enzymes.

5.5.1. Design of the molecules

Vilcheze Catherine *et al*., [Vilcheze C., *et al*., 2011] used a subset of a chemical library, composed of 300 compounds inhibiting *Plasmodium falciparum* enoyl reductase, and were tested against *M. tuberculosis*. Among them one of the molecule **CD59** (1-(4-chlorophenyl)-4-(4-hydroxy-3-methoxy-5-nitrobenzylidene)pyrazolidine-3,5-dione) showed good activity against *M. tuberculosis* with MIC of 1.5 µM (**Figure 5.18**). We considered **CD59** as the starting point to design more analogues by keeping pyrazolidine-3,5-dione nucleus intact and modified 1st and 4th positions with various aryl and heteroaryl moiety to investigate the SAR of the lead compound.

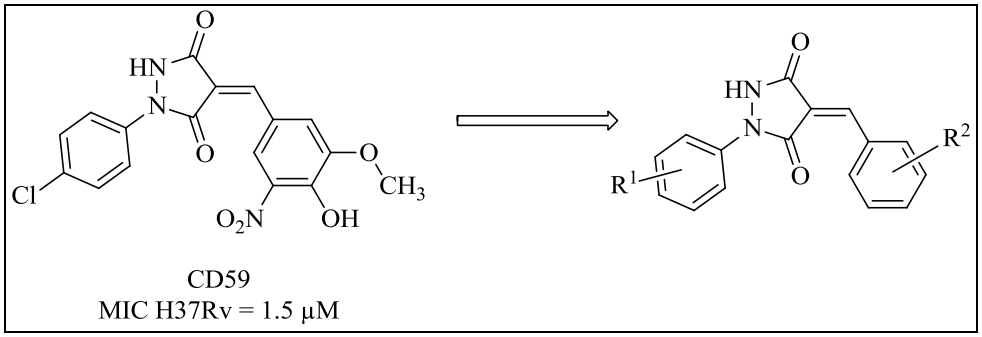


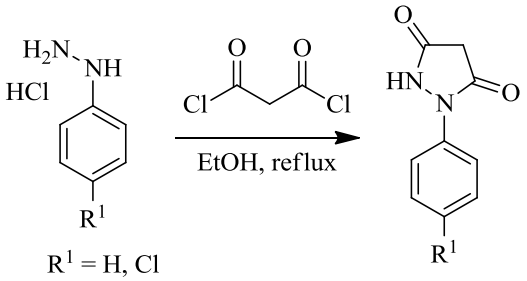
Figure 5.18: Lead molecule (CD59) for the synthesis of compounds **PZ_03 – PZ_32**

5.5.2. Experimental procedures utilized for the synthesis of PZ_03 – PZ_32

The target molecules were synthesized by a two-step synthetic protocol (**Figure 4.6**). In first step we initially treated the (sub)phenylhydrazine with malonic acid using methanol as solvent under reflux conditions for 2 h. These reactions were also carried with malonyl chloride instead of malonic acid in ethanol as solvent under reflux conditions for 3 h, we obtained better yields when the reactions were carried using malonyl chloride. The commercially available 4-chlorophenylhydrazine was a hydrochloride salt; here we first

converted the hydrochloride salt into free amine by taking compound in saturated $NaHCO_3$ solution, stirred for few minutes and then extracted the free amine with dichloromethane, the evaporation of solvent produced the free amine. The obtained free amine compound was treated with malonyl chloride using ethanol reflux conditions. The reaction mixture was concentrated and triturated with water to obtain solid compound and the solids were washed with water, cold ethanol, hexanes and dried *in vacuo* to get compound **PZ_02a-b**. In the next step we condensed the active methylene group of compound **PZ_02a-b** (1-substituted-pyrazolidine-3,5-dione) with various substituted aldehydes to produce target molecules (**PZ_03 – PZ_32**) [Kumar., *et al*., 2013]. Here we refluxed the compound **PZ_02a/PZ_02b** and substituted phenyl/heteroaryl aldehydes (**Table 5.11**) in acetic acid for 4 h. We observed the formation of solids in the reaction mixture direct filtration of reaction mixture and washing of the residue with excess water removed the traces of acetic acid and dried in vacuum oven to get pure products with ease of purification and with good yields.

General procedure for the preparation of compound PZ_02a-b



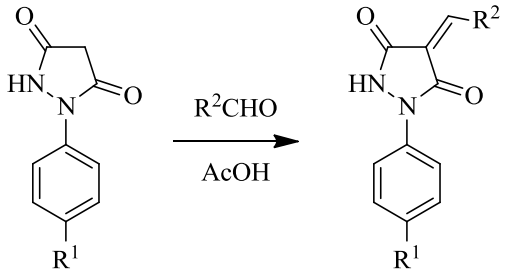
To the stirred solution of (sub)phenylhydrazine (**PZ_01a-b**) (1.00 equiv) in ethanol under N_2 atmosphere added malonyl chloride (1.05 equiv) and the reaction mixture was stirred under reflux conditions for 3 h. The reaction mixture was concentrated to get crude compound. The crude compound was purified by column chromatography (EtOAc/Hexanes as eluant) to get pure compound **PZ_02a-b**.

Preparation of 1-phenylpyrazolidine-3,5-dione (PZ_02a)

To the stirred solution of phenyl hydrazine (3.0 g, 27.77 mmol) in ethanol under N_2 atmosphere added malonyl chloride (2.81 mL, 29.16 mmol) and the reaction mixture was stirred under reflux conditions for 3 h. After completion of the reaction by checking TLC, reaction mixture was concentrated to get crude compound. The crude compound was directly

purified by column chromatography (EtOAc/Hexanes as eluant) to get 1-phenylpyrazolidine-3,5-dione (3.60 g, 74%) as an off-white solid. ESI-MS showed 177 $[M+H]^+$ and was carried to next step.

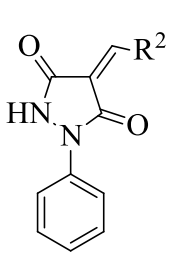
General procedure for the preparation of compounds PZ_03 – PZ_32



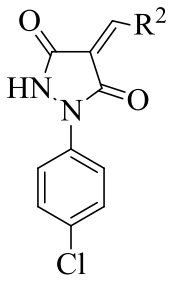
PZ_02a-b **PZ_03 - PZ_32**

Compound **PZ_02a/PZ_02b** (1.0 equiv) and aldehyde (1.0 equiv) were taken in acetic acid and heated at 100 °C for 4 h. The solids formed in the reaction mixture was filtered and washed with H_2O, cold EtOH and hexanes to get compounds **PZ_03 – PZ_32**.

Table 5.11: Physiochemical properties of the synthesized compounds **PZ_03 – PZ_32**



PZ_03 - PZ_17



PZ_18 - PZ_32

Compd	R^2	Yield (%)	M.P. (°C)	Molecular formula	Molecular weight
PZ_03	Phenyl	78	235-236	$C_{16}H_{12}N_2O_2$	264.28
PZ_04	4-Hydroxyphenyl	81	210-211	$C_{16}H_{12}N_2O_3$	280.28
PZ_05	4-Methoxyphenyl	65	198-199	$C_{17}H_{14}N_2O_3$	294.30
PZ_06	4-Benzyloxyphenyl	71	205-206	$C_{23}H_{18}N_2O_3$	370.40
PZ_07	2-Benzyloxyphenyl	69	213-214	$C_{23}H_{18}N_2O_3$	370.40

Contd

Compd	R^2	Yield (%)	M.P. (°C)	Molecular formula	Molecular weight
PZ_08	4-Methylphenyl	63	240-241	$C_{17}H_{14}N_2O_2$	278.31
PZ_09	3,4,5-Trimethoxyphenyl	79	260-261	$C_{19}H_{18}N_2O_5$	354.36
PZ_10	4-Fluorophenyl	54	191-192	$C_{16}H_{11}FN_2O_2$	282.27
PZ_11	2-Chlorophenyl	61	204-205	$C_{16}H_{11}ClN_2O_2$	298.72
PZ_12	4-Chlorophenyl	72	241-242	$C_{16}H_{11}ClN_2O_2$	298.72
PZ_13	2-Bromophenyl	65	187-188	$C_{16}H_{11}BrN_2O_2$	343.17
PZ_14	4-Bromophenyl	70	178-179	$C_{16}H_{11}BrN_2O_2$	343.17
PZ_15	3-Nitrophenyl	63	234-235	$C_{16}H_{11}N_3O_4$	309.28
PZ_16	Furan-2-yl	57	245-246	$C_{14}H_{10}N_2O_3$	254.24
PZ_17	5-Nitrofuran-2-yl	76	225-226	$C_{14}H_9N_3O_5$	299.24
PZ_18	Phenyl	66	211-213	$C_{16}H_{11}ClN_2O_2$	298.72
PZ_19	4-Hydroxyphenyl	52	214-215	$C_{16}H_{11}ClN_2O_3$	314.72
PZ_20	4-Methoxyphenyl	76	231-232	$C_{17}H_{13}ClN_2O_3$	328.75
PZ_21	4-Benzyloxyphenyl	81	241-242	$C_{23}H_{17}ClN_2O_3$	404.85
PZ_22	2-Benzyloxyphenyl	69	247-248	$C_{23}H_{17}ClN_2O_3$	404.85
PZ_23	4-Methylphenyl	74	229-231	$C_{17}H_{13}ClN_2O_2$	312.75
PZ_24	3,4,5-Trimethoxyphenyl	69	260-261	$C_{19}H_{17}ClN_2O_5$	388.80
PZ_25	4-Fluorophenyl	54	219-220	$C_{16}H_{10}ClFN_2O_2$	316.71
PZ_26	2-Chlorophenyl	64	222-223	$C_{16}H_{10}Cl_2N_2O_2$	333.17
PZ_27	4-Chlorophenyl	67	261-262	$C_{16}H_{10}Cl_2N_2O_2$	333.17
PZ_28	2-Bromophenyl	72	238-239	$C_{16}H_{10}BrClN_2O_2$	377.62
PZ_29	4-Bromophenyl	60	217-218	$C_{16}H_{10}BrClN_2O_2$	377.62
PZ_30	3-Nitrophenyl	63	223-224	$C_{16}H_{10}ClN_3O_4$	343.72
PZ_31	Furan-2-yl	58	235-236	$C_{14}H_9ClN_2O_3$	288.62
PZ_32	5-Nitrofuran-2-yl	62	246-247	$C_{14}H_8ClN_3O_5$	333.68

5.5.3. Characterization of the synthesized molecules

4-Benzylidene-1-phenylpyrazolidine-3,5-dione (PZ_03): 1-Phenylpyrazolidine-3,5-dione (0.6 g, 3.41 mmol) and benzaldehyde (0.34 mL, 3.41 mmol) were taken in acetic acid and

heated at 100 °C for 4 h. The solids formed in the reaction mixture was filtered and washed with water, cold ethanol and hexanes to get 4-benzylidene-1-phenylpyrazolidine-3,5-dione (704 mg, 78%) as an off-white solid. Yield: 78%; m.p. 235–236 °C; MS(ESI) *m/z* 265 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.41 (bs, 1H), 8.49 (d, *J* = 7.2 Hz, 2H), 7.99–7.40 (m, 8H), 7.19 (t, *J* = 7.6 Hz, 1H). Anal calcd for $C_{16}H_{12}N_2O_2$: C, 72.72; H, 4.58; N, 10.60% Found C, 72.81; H, 4.61; N, 10.71%.

4-(4-Hydroxybenzylidene)-1-phenylpyrazolidine-3,5-dione (PZ_04): Yield: 81%; m.p. 210–211 °C; MS(ESI) *m/z* 281 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.23 (s, 1H), 9.54 (s, 1H), 7.92–7.81 (m, 4H), 7.72 (s, 1H), 7.69(d, *J* = 7.2 Hz, 2H), 7.63–7.56 (m, 3H). Anal calcd for $C_{16}H_{12}N_2O_3$: C, 68.56; H, 4.32; N, 9.99% Found C, 68.61; H, 4.41; N, 10.03%.

4-(4-Methoxybenzylidene)-1-phenylpyrazolidine-3,5-dione (**PZ_05**)**:** Yield: 65%; m.p. 198–199 °C; MS(ESI) *m/z* 295 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.10 (s, 1H), 8.08 (d, *J* = 7.2 Hz, 2H), 7.90 (d, *J* = 7.6 Hz, 2H), 7.81 (s, 1H), 7.77–7.63 (m, 3H), 7.54 (d, *J* = 8.0 Hz, 2H), 4.03 (s, 3H). Anal calcd for $C_{17}H_{14}N_2O_3$: C, 69.38; H, 4.79; N, 9.52% Found C, 69.41; H, 4.91; N, 9.58%.

4-(4-(Benzyloxy)benzylidene)-1-phenylpyrazolidine-3,5-dione (PZ_06): Yield: 71%; m.p. 205–206 °C; MS(ESI) *m/z* 371 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.22 (s, 1H), 7.92 (d, *J* = 7.6 Hz, 2H), 7.81–7.77 (m, 6H), 7.63 (d, *J* = 7.6 Hz, 2H), 7.58–7.49 (m, 5H), 5.20 (s, 2H). Anal calcd for $C_{23}H_{18}N_2O_3$: C, 74.58; H, 4.90; N, 7.56% Found C, 74.61; H, 4.99; N, 7.63%.

4-(2-(Benzyloxy)benzylidene)-1-phenylpyrazolidine-3,5-dione (PZ_07): Yield: 69%; m.p. 213–214 °C; MS(ESI) *m/z* 371 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.28 (s, 1H), 7.92–7.81 (m, 4H), 7.77 (s, 1H), 7.72–7.58 (m, 6H), 7.54–7.45 (m, 4H), 5.10 (s, 2H). Anal calcd for $C_{23}H_{18}N_2O_3$: C, 74.58; H, 4.90; N, 7.56% Found C, 74.63; H, 4.93; N, 7.68%.

4-(4-Methylbenzylidene)-1-phenylpyrazolidine-3,5-dione (PZ_08): Yield: 63%; m.p. 240–241 °C; MS(ESI) *m/z* 279 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.91 (s, 1H), 7.99 (d, *J* = 7.2 Hz, 2H), 7.81 (d, *J* = 7.2 Hz, 2H), 7.78 (s, 1H), 7.72–7.58 (m, 3H), 7.48 (d, *J* = 8.0 Hz, 2H), 2.63 (s, 3H). Anal calcd for $C_{17}H_{14}N_2O_2$: C, 73.37; H, 5.07; N, 10.07% Found C, 73.41; H, 5.11; N, 10.15%.

1-Phenyl-4-(3,4,5-trimethoxybenzylidene)pyrazolidine-3,5-dione (PZ_09): Yield: 79%; m.p. 260–261 °C; MS(ESI) *m/z* 355 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.01 (s, 1H), 7.92 (d, *J* = 7.6 Hz, 2H), 7.81 (t, *J* = 6.8 Hz, 2H), 7.63 (t, *J* = 6.8 Hz, 1H), 7.54 (s, 1H), 7.36 (s, 2H), 3.94 (s, 9H). Anal calcd for $C_{19}H_{18}N_2O_5$: C, 64.40; H, 5.12; N, 7.91% Found C, 64.47; H, 5.21; N, 7.99%.

4-(4-Fluorobenzylidene)-1-phenylpyrazolidine-3,5-dione (PZ_10): Yield: 54%; m.p. 191–192 °C; MS(ESI) *m/z* showed 283 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.61 (s, 1H), 8.10 (s, 1H), 7.81(d, *J* = 7.2 Hz, 2H), 7.72–7.54 (m, 5H), 7.49 (d, *J* = 7.6 Hz, 2H). Anal calcd for $C_{16}H_{11}FN_2O_2$: C, 68.08; H, 3.93; N, 9.92% Found C, 68.11; H, 3.99; N, 9.97%.

4-(2-Chlorobenzylidene)-1-phenylpyrazolidine-3,5-dione (PZ_11): Yield: 61%; m.p. 204–205 °C; MS(ESI) *m/z* 299 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.31 (s, 1H), 7.99 (s, 1H), 7.81 (d, *J* = 7.6 Hz, 1H), 7.78–7.60 (m, 7H), 7.49 (t, 1H). Anal calcd for $C_{16}H_{11}ClN_2O_2$: C, 64.33; H, 3.71; N, 9.38% Found C, 64.36; H, 3.76; N, 9.43%.

4-(4-Chlorobenzylidene)-1-phenylpyrazolidine-3,5-dione (PZ_12): Yield: 72%; m.p. 241–242 °C; MS(ESI) *m/z* 299 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.70 (s, 1H), 8.13 (s, 1H), 7.89 (d, *J* = 7.2 Hz, 2H), 7.76–7.49 (m, 7H). Anal calcd for $C_{16}H_{11}ClN_2O_2$: C, 64.33; H, 3.71; N, 9.38% Found C, 64.36; H, 3.79; N, 9.43%.

4-(2-Bromobenzylidene)-1-phenylpyrazolidine-3,5-dione (PZ_13): Yield: 65%; m.p. 187–188 °C; MS(ESI) *m/z* 343 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.23 (s, 1H), 8.01 (s, 1H), 7.89 (d, *J* = 7.2 Hz, 1H), 7.81–7.63 (m, 7H), 7.54 (t, 1H). Anal calcd for $C_{16}H_{11}BrN_2O_2$: C, 56.00; H, 3.23; N, 8.16% Found C, 56.10; H, 3.31; N, 8.21%.

4-(4-Bromobenzylidene)-1-phenylpyrazolidine-3,5-dione (PZ_14): Yield: 70%; m.p. 178–179 °C; MS(ESI) *m/z* 343 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.41 (s, 1H), 7.91(d, *J* = 7.2 Hz, 2H), 7.82 (d, *J* = 7.6 Hz, 2H), 7.78 (s, 1H), 7.72–7.63 (m, 3H), 7.49 (d, *J* = 7.2 Hz, 2H). Anal calcd for $C_{16}H_{11}BrN_2O_2$: C, 56.00; H, 3.23; N, 8.16% Found C, 56.12; H, 3.33; N, 8.22%.

4-(3-Nitrobenzylidene)-1-phenylpyrazolidine-3,5-dione (PZ_15): Yield: 63%; m.p. 234–235 °C; MS(ESI) *m/z* 310 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.43 (s, 1H), 8.45 (s, 1H), 7.99 (d, *J* = 6.8 Hz, 1H), 7.92 (d, *J* = 7.2 Hz, 1H), 7.86 (s, 1H), 7.81 (d, *J* = 7.6 Hz, 2H),

7.72 (t, *J* = 6.8 Hz, 1H), 7.68–7.54 (m, 3H). Anal calcd for $C_{16}H_{11}N_3O_4$: C, 62.14; H, 3.58; N, 13.59% Found C, 62.21; H, 3.60; N, 13.63%.

4-(Furan-2-ylmethylene)-1-phenylpyrazolidine-3,5-dione (PZ_16): Yield: 57%; m.p. 245–246 °C; MS(ESI) *m/z* 255 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.32 (s, 1H), 8.01 (d, *J* = 7.2 Hz, 1H), 7.92 (s, 1H), 7.81–7.74 (m, 3H), 7.63 (t, *J* = 7.2 Hz, 1H), 7.58–7.45 (m, 3H). Anal calcd for $C_{14}H_{10}N_2O_3$: C, 66.14; H, 3.96; N, 11.02% Found C, 66.26; H, 4.03; N, 11.07%.

4-((5-Nitrofuran-2-yl)methylene)-1-phenylpyrazolidine-3,5-dione (PZ_17): Yield: 76%; m.p. 225–226 °C; MS(ESI) *m/z* 300 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.24 (s, 1H), 8.12 (d, *J* = 7.2 Hz, 1H), 7.89 (s, 1H), 7.78 (d, *J* = 7.2 Hz, 2H), 7.72 (t, *J* = 6.8 Hz, 1H), 7.62–7.49 (m, 3H). Anal calcd for $C_{14}H_9N_3O_5$: C, 56.19; H, 3.03; N, 14.04% Found C, 56.26; H, 3.13; N, 14.08%.

4-Benzylidene-1-(4-chlorophenyl)pyrazolidine-3,5-dione (PZ_18): Yield: 66%; m.p. 211–212 °C; MS(ESI) *m/z* 299 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.22 (bs, 1H), 8.35 (d, *J* = 7.2 Hz, 2H), 8.10–7.81 (m, 4H), 7.72 (d, *J* = 6.8 Hz, 2H), 7.63–7.54 (m, 2H). Anal calcd for $C_{16}H_{11}ClN_2O_2$: C, 64.33; H, 3.71; N, 9.38% Found C, 64.41; H, 3.76; N, 9.43%.

1-(4-Chlorophenyl)-4-(4-hydroxybenzylidene)pyrazolidine-3,5-dione (PZ_19): Yield: 52%; m.p. 214–215 °C; MS(ESI) *m/z* 315 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.10 (s, 1H), 9.63 (s, 1H), 8.01 (d, *J* = 7.6 Hz, 2H), 7.92–7.77 (m, 5H), 7.63 (d, *J* = 7.2 Hz, 2H). Anal calcd for $C_{16}H_{11}ClN_2O_3$: C, 61.06; H, 3.52; N, 8.90% Found C, 61.12; H, 3.56; N, 8.98%.

1-(4-Chlorophenyl)-4-(4-methoxybenzylidene)pyrazolidine-3,5-dione (PZ_20): Yield: 76%; m.p. 231–232 °C; MS(ESI) *m/z* 329 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.32 (s, 1H), 7.92 (d, *J* = 7.6 Hz, 2H), 7.81 (d, *J* = 7.6 Hz, 2H), 7.76 (s, 1H), 7.65 (d, *J* = 7.6 Hz, 2H), 7.54 (d, *J* = 7.6 Hz, 2H), 4.12 (s, 3H). Anal calcd for $C_{17}H_{13}ClN_2O_3$: C, 62.11; H, 3.99; N, 8.52% Found C, 62.21; H, 4.05; N, 8.64%.

4-(4-(Benzyloxy)benzylidene)-1-(4-chlorophenyl)pyrazolidine-3,5-dione (PZ_21): Yield: 81%; m.p. 241–242 °C; MS(ESI) *m/z* 405 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.45 (s, 1H), 8.02 (d, *J* = 7.2 Hz, 2H), 7.92 (d, *J* = 7.6 Hz, 2H), 7.87–7.72 (m, 4H), 7.63–7.58 (m,

4H), 7.45 (d, *J* = 8.0 Hz, 2H), 5.13 (s, 2H). Anal calcd for $C_{23}H_{17}ClN_2O_3$: C, 68.23; H, 4.23; N, 6.92% Found C, 68.31; H, 4.29; N, 6.99%.

4-(2-(Benzyloxy)benzylidene)-1-(4-chlorophenyl)pyrazolidine-3,5-dione (PZ_22): Yield: 69%; m.p. 247–248 °C; MS(ESI) *m/z* 405 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.43 (s, 1H), 8.12–7.81 (m, 6H), 7.77–7.63 (m, 6H), 7.58–7.49 (m, 2H), 5.22 (s, 2H). Anal calcd for $C_{23}H_{17}ClN_2O_3$: C, 68.23; H, 4.23; N, 6.92% Found C, 68.35; H, 4.26; N, 7.02%.

1-(4-Chlorophenyl)-4-(4-methylbenzylidene)pyrazolidine-3,5-dione (PZ_23): Yield: 74%; m.p. 229–230 °C; MS(ESI) *m/z* 313 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.31 (s, 1H), 7.92 (d, *J* = 7.2 Hz, 2H), 7.84 (d, *J* = 7.2 Hz, 2H), 7.78 (s, 1H), 7.71 (d, *J* = 7.6 Hz, 2H), 7.63 (d, *J* = 7.6 Hz, 2H), 2.58 (s, 3H). Anal calcd for $C_{17}H_{13}ClN_2O_2$: C, 65.29; H, 4.19; N, 8.96% Found C, 65.41; H, 4.21; N, 9.07%.

1-(4-Chlorophenyl)-4-(3,4,5-trimethoxybenzylidene)pyrazolidine-3,5-dione (PZ_24): Yield: 69%; m.p. 260–261 °C; MS(ESI) *m/z* 389 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.24 (s, 1H), 7.99(d, *J* = 8.0 Hz, 2H), 7.83 (d, *J* = 7.6 Hz, 2H), 7.72 (s, 1H), 7.58 (s, 2H), 3.96 (s, 9H). Anal calcd for $C_{19}H_{17}ClN_2O_5$: C, 58.69; H, 4.41; N, 7.21% Found C, 58.72; H, 4.49; N, 7.30%.

1-(4-Chlorophenyl)-4-(4-fluorobenzylidene)pyrazolidine-3,5-dione (PZ_25): Yield: 54%; m.p. 219–220 °C; MS(ESI) *m/z* 317 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.61 (s, 1H), 7.99 (d, *J* = 7.2 Hz, 2H), 7.81 (d, *J* = 7.2 Hz, 2H), 7.72 (d, *J* = 7.2 Hz, 2H), 7.65 (s, 1H), 7.49 (d, *J* = 7.2 Hz, 2H). Anal calcd for $C_{16}H_{10}ClFN_2O_2$: C, 60.68; H, 3.18; N, 8.85% Found C, 60.72; H, 3.21; N, 8.91%.

4-(2-Chlorobenzylidene)-1-(4-chlorophenyl)pyrazolidine-3,5-dione (PZ_26): Yield: 64%; m.p. 222–223 °C; MS(ESI) *m/z* 333 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.31 (s, 1H), 7.89 (d, *J* = 7.2 Hz, 2H), 7.81 (s, 1H), 7.76–7.58 (m, 6H). Anal calcd for $C_{16}H_{10}Cl_2N_2O_2$: C, 57.68; H, 3.03; N, 8.41% Found C, 57.70; H, 3.07; N, 8.54%.

4-(4-Chlorobenzylidene)-1-(4-chlorophenyl)pyrazolidine-3,5-dione (PZ_27): Yield: 67%; m.p. 261–262 °C; MS(ESI) *m/z* 333 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.70 (s, 1H), 8.01 (d, *J* = 7.2 Hz, 2H), 7.92 (d, *J* = 7.2 Hz, 2H), 7.72 (d, *J* = 7.2 Hz, 2H), 7.63 (s, 1H), 7.54 (d, *J* = 7.2 Hz, 2H). Anal calcd for $C_{16}H_{10}Cl_2N_2O_2$: C, 57.68; H, 3.03; N, 8.41% Found C, 57.72; H, 3.12; N, 8.53%.

4-(2-Bromobenzylidene)-1-(4-chlorophenyl)pyrazolidine-3,5-dione (PZ_28): Yield: 72%; m.p. 238–239 °C; MS(ESI) *m/z* 377 $[M+H]^+$; ^1H NMR (400 MHz, DMSO-d_6) δ 11.22 (s, 1H), 7.81 (d, *J* = 7.2 Hz, 1H), 7.77 (s, 1H), 7.72–7.58 (m, 6H), 7.47 (d, *J* = 7.2 Hz, 1H). Anal calcd for $C_{16}H_{10}BrClN_2O_2$: C, 50.89; H, 2.67; N, 7.42% Found C, 50.94; H, 2.74; N, 7.47%.

4-(4-Bromobenzylidene)-1-(4-chlorophenyl)pyrazolidine-3,5-dione (PZ_29): Yield: 60%; m.p. 217–218 °C; MS(ESI) *m/z* 377 $[M+H]^+$; ^1H NMR (400 MHz, DMSO-d_6) δ 11.43 (s, 1H), 8.04 (d, *J* = 7.2 Hz, 2H), 7.92 (d, *J* = 7.6 Hz, 2H), 7.69 (d, *J* = 8.0 Hz, 2H), 7.58 (s, 1H), 7.45 (d, *J* = 7.6 Hz, 2H). Anal calcd for $C_{16}H_{10}BrClN_2O_2$: C, 50.89; H, 2.67; N, 7.42% Found C, 50.94; H, 2.72; N, 7.54%.

1-(4-Chlorophenyl)-4-(3-nitrobenzylidene)pyrazolidine-3,5-dione (PZ_30): Yield: 63%; m.p. 223–224 °C; MS(ESI) *m/z* 344 $[M+H]^+$; ^1H NMR (400 MHz, DMSO-d_6) δ 11.34 (s, 1H), 8.49 (s, 1H), 8.08–7.94 (m, 3H), 7.83 (d, *J* = 7.6 Hz, 2H), 7.76 (t, *J* = 6.8 Hz, 1H), 7.72 (d, *J* = 7.2 Hz, 2H). Anal calcd for $C_{16}H_{10}ClN_3O_4$: C, 55.91; H, 2.93; N, 12.23% Found C, 55.97; H, 2.98; N, 12.34%.

1-(4-Chlorophenyl)-4-(furan-2-ylmethylene)pyrazolidine-3,5-dione (PZ_31): Yield: 58%; m.p. 235–236 °C; MS(ESI) *m/z* 289 $[M+H]^+$; ^1H NMR (400 MHz, DMSO-d_6) δ 11.43 (s, 1H), 8.12 (d, *J* = 7.2 Hz, 1H), 7.96 (d, *J* = 7.6 Hz, 1H), 7.89–7.79 (m, 3H), 7.76 (t, *J* = 6.8 Hz, 1H) 7.72 (d, *J* = 7.2 Hz, 2H). Anal calcd for $C_{14}H_9ClN_2O_3$: C, 58.25; H, 3.14; N, 9.70% Found C, 58.31; H, 3.21; N, 9.81%.

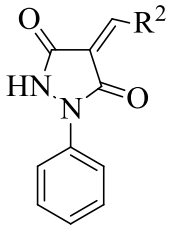
1-(4-Chlorophenyl)-4-((5-nitrofuran-2-yl)methylene)pyrazolidine-3,5-dione (PZ_32): Yield: 62%; m.p. 246–247 °C; MS(ESI) *m/z* 334 $[M+H]^+$; ^1H NMR (400 MHz, DMSO-d_6) δ 11.49 (s, 1H), 8.28 (d, *J* = 7.2 Hz, 1H), 8.01 (d, *J* = 7.6 Hz, 1H), 7.89 (d, *J* = 8.0 Hz, 2H), 7.81 (s, 1H), 7.74 (d, *J* = 7.6 Hz, 2H). Anal calcd for $C_{14}H_8ClN_3O_5$: C, 50.39; H, 2.42; N, 12.59% Found C, 50.45; H, 2.49; N, 12.71%.

5.5.4. *In vitro M. tuberculosis* screening, *M. tuberculosis* PS, ADH and LAT enzymes inhibition assay and cytotoxicity studies of the synthesized molecules

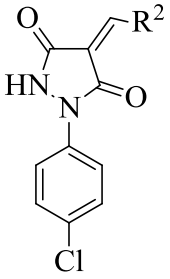
All the synthesized compounds were screened for their *in vitro* anti-tubercular activity against *M. tuberculosis* H37Rv using MABA method. Isoniazid, ethambutol, rifampicin, pyrazinamide, ciprofloxacin were used as reference compounds for comparison. All the synthesized derivatives were also screened for their *in vitro M. tuberculosis* PS, *in vitro M.*

tuberculosis ADH and *in vitro M. tuberculosis* LAT inhibitory potency as steps towards mechanistic evaluation. Compounds showing *M. tuberculosis* MICs <50 µM were also tested for *in vitro* cytotoxicity against RAW 264.7 cells at 50 µM concentration using MTT assay and all the results were presented in **Table 5.12**.

Table 5.12: *In vitro* biological evaluation of the synthesized derivatives **PZ_03 – PZ_32**



PZ_03 - PZ_17



PZ_18 - PZ_32

Compd	R^2	MTB MIC in µM[a]	MTB PS IC_{50} in µM	MTB ADH IC_{50} in µM	MTB LAT IC_{50} in µM	Cytotoxicity[b] at 50 µM % inhibition
PZ_03	Phenyl	188.6	NT	NT	NT	NT
PZ_04	4-Hydroxyphenyl	111.2	NT	NT	NT	NT
PZ_05	4-Methoxyphenyl	84.74	NT	NT	NT	NT
PZ_06	4-Benzyloxyphenyl	67.38	NT	NT	NT	NT
PZ_07	2-Benzyloxyphenyl	134.77	NT	NT	NT	NT
PZ_08	4-Methylphenyl	179.21	NT	NT	NT	NT
PZ_09	3,4,5-Trimethoxyphenyl	140.84	NT	NT	NT	NT
PZ_10	4-Fluorophenyl	5.53	5.47±0.24	>50	50.00±0.12	22.16
PZ_11	2-Chlorophenyl	5.21	>50	>50	11.45±0.11	18.12
PZ_12	4-Chlorophenyl	5.21	>50	>50	29.92±0.16	20.86
PZ_13	2-Bromophenyl	4.54	>50	10.63±0.10	>50	18.42
PZ_14	4-Bromophenyl	18.22	>50	>50	21.40±0.16	24.62
PZ_15	3-Nitrophenyl	10.08	50.00±1.32	>50	40.64±0.22	30.16
PZ_16	Furan-2-yl	24.5	>50	8.14±0.09	>50	26.52
PZ_17	5-Nitrofuran-2-yl	10.41	6.98±0.12	>50	40.43±2.1	28.32

Contd

Compd	R^2	MTB MIC in µM[a]	MTB PS IC_{50} in µM	MTB ADH IC_{50} in µM	MTB LAT IC_{50} in µM	Cytotoxicity [b] at 50 µM % inhibition
PZ_18	Phenyl	20.84	12.51±0.08	22.39±0.30	50.00±2.68	16.12
PZ_19	4-Hydroxyphenyl	15.87	4.18±0.16	>50	>50	12.28
PZ_20	4-Methoxyphenyl	37.99	NT	NT	NT	NT
PZ_21	4-Benzyloxyphenyl	7.71	>50	8.97±0.34	13.60±0.90	14.56
PZ_22	2-Benzyloxyphenyl	41.15	NT	NT	NT	NT
PZ_23	4-Methylphenyl	53.31	NT	NT	NT	NT
PZ_24	3,4,5-Trimethoxyphenyl	16.06	3.73±0.11	15.84±0.4	>50	11.86
PZ_25	4-Fluorophenyl	7.88	6.14±0.33	>50	>50	NT
PZ_26	2-Chlorophenyl	4.68	12.98±0.30	30.02±0.60	19.94±0.31	27.42
PZ_27	4-Chlorophenyl	7.50	50±2.08	50.01±2.07	19.92±0.11	23.14
PZ_28	2-Bromophenyl	4.13	9.68±0.26	19.47±0.10	50.04±0.41	26.12
PZ_29	4-Bromophenyl	13.26	>50	50.34±1.68	32.16±1.60	18.12
PZ_30	3-Nitrophenyl	18.16	>50	>50	30.42±1.70	29.19
PZ_31	Furan-2-yl	17.30	>50	12.46±1.00	>50	16.18
PZ_32	5-Nitrofuran-2-yl	7.48	10.16±0.09	>50	>50	24.53
Isoniazid		0.72	>50	>50	>50	NT
Rifampicin		0.24	>50	>50	>50	NT
Ethambutol		7.64	>50	>50	>50	NT
Pyrazinamide		50.77	>50	>50	>50	NT
Ciprofloxacin		4.71	>50	>50	>50	NT

IC_{50}, 50% inhibitory concentration; MTB, *Mycobacterium tuberculosis*; MIC, minimum inhibitory concentration; [a]*In vitro* activity against MTB H37Rv; [b]Against RAW 264.7 cells, NT, not tested.

5.5.5. SAR and discussion

To study the SAR we prepared the compounds with variations at 1st and 4th positions of lead molecule. At N-1 position we tried two modifications, one with phenyl group (**PZ_03 – PZ_17**) and the other with 4-chlorophenyl group (**PZ_18** – **PZ_32**). In the C-4 position we had prepared molecules with phenyl ring with both electron donating and electron with drawing groups and also with few heterocycles. The order of anti-tubercular activity with

respect to N-1 position was 4-chlorophenyl (**PZ_18** – **PZ_32**) compounds were found to be more potent than phenyl ring containing compounds (**PZ_03** – **PZ_17**). Presence of phenyl ring at N-1 position and phenyl ring with electron donating groups at C-3 position (**PZ_04** – **PZ_09**) resulted in higher inhibition than that of phenyl ring with electron withdrawing groups at C-3 position (**PZ_10** – **PZ_15**). Compound **PZ_03** without any substituent on either of the phenyl rings, displayed an MIC of 188.6 µM and served as the standard for potency comparison. The activity of **PZ_04** improved 1.6 times (111.2 µM) when a hydroxyl group was introduced at the 4^{th} position of phenyl ring at C-3. Replacement of hydroxyl group with methoxy group (**PZ_05**) further enhanced the potency with MIC of 84.74 and it further increased with MIC of 67.38 µM when introduced bulky benzyloxy group at 4^{th} position (**PZ_06**). Whereas shifting benzyloxy group (**PZ_07**) from 4^{th} position to 2^{nd} position reduced activity two times with MIC of 134.77 µM. Introduction of methyl group at 4^{th} position (**PZ_08)** did not results in much difference in activity when compared to compound **PZ_03**. Similarly introduction of three methoxy groups (**PZ_09**) also did not showed promising activity (140.84 µM). Introduction of electron withdrawing group at 2^{nd}, 3^{rd}, and 4^{th} position (**PZ_10** – **PZ_15**) enhanced potency ten or more times with MICs ranging from 4.54-18.22 µM. Introduction of furan ring (**PZ_16**) improved activity 7.6 times (24.5 µM) than compound **PZ_03**, whereas introduction of nitro group at 5^{th} position of furan ring (**PZ_17**) further enhanced the activity (10.41 µM). In case of the second set of compounds (**PZ_18 – PZ_32**) with 4-chloro phenyl ring at N-1 position, introduction of phenyl ring at C-3 position (**PZ_18**) yielded nine times more potency than compound **PZ_03**. Introduction of electron donating groups like hydroxyl, methoxy, methyl did not alter activity except 4-benzyloxy group (**PZ_21**) which was found to be thrice more potent than compound **PZ_18**. Compounds with electron withdrawing substituents (**PZ_25 – PZ_30**) were equal or less active than their counterpart in the first set (**PZ_10 – PZ_15**). Nine compounds showed better activity when compared to standard drug ethambutol.

Compounds **PZ_03** to **PZ_09** and few compounds (**PZ_20**, **PZ_22** – **PZ_23**) were not tested further in the enzyme study as they were either not very promising as anti-mycobacterial or due to being inactive at an initial concentration of 50 µM tested.

Twelve compounds (**PZ_10**, **PZ_15**, **PZ_17** – **PZ_18**, **PZ_21** and **PZ_24** – **PZ_28**) were found to inhibit more than one mycobacterial enzymes, among which four compounds (**PZ_18**, **PZ_25** – **PZ_27**) inhibited all the three enzymes.

Overall, **PZ_26** seem to be more promising as the compound inhibited all the mycobacterial enzymes PS, ADH and LAT and exhibited promising antimycobacterial MIC of 4.68 which was better than standard drugs ethambutol and ciprofloxacin.

To explore the possible target of action, most active compounds were screened for three *M. tuberculosis* enzymes namely, *M. tuberculosis* PS, ADH and LAT. The most active compounds for each of the above three *M. tuberculosis* enzymes were docked in the active site cavity of enzymes in order to know the possible binding interactions to support the activity, docking interactions are presented in **Fig 5.19 – 5.21**.

The IC_{50} of the screened compounds are reported in **Table 5.12**. Ten compounds inhibited *M. tuberculosis* PS with $IC_{50} \leq 50$ µM. Five compounds (**PZ_10**, **PZ_17**, **PZ_19**, **PZ_24** and **PZ_28**) inhibited with $IC_{50} < 10$ µM, and compound 1-(4-chlorophenyl)-4-(3,4,5-trimethoxybenzylidene)pyrazolidine-3,5-dione (**PZ_24**) emerged as the most potent one with IC_{50} of 3.73±0.11 µM, which inhibited *M. tuberculosis* with MIC of 16.06 µM.

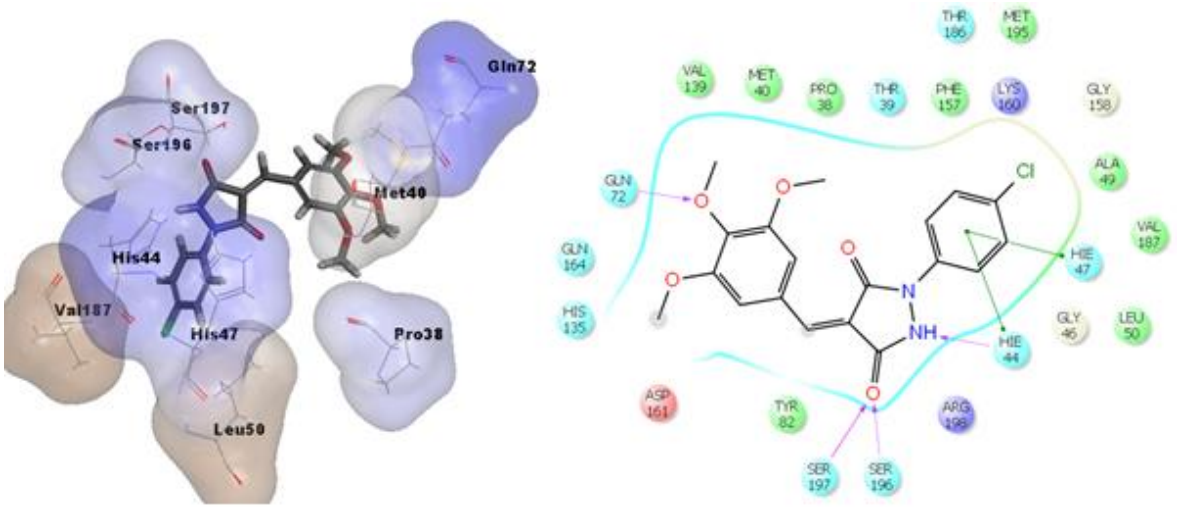


Figure 5.19: Binding pose and interaction pattern of the most active compound **PZ_24** with *M. tuberculosis* PS enzyme

Ten compounds inhibited *M. tuberculosis* ADH with $IC_{50} \leq 50$ µM. Four compounds (**PZ_13**, **PZ_16**, **PZ_21**, and **PZ-31**) inhibited with $IC_{50} < 15$ µM, and 4-(furan-2-ylmethylene)-1-phenylpyrazolidine-3,5-dione (**PZ_16**) was found to be the most potent compound with IC_{50} of 8.14±0.09 µM, which inhibited the *M. tuberculosis* with MIC of 24.5 µM.

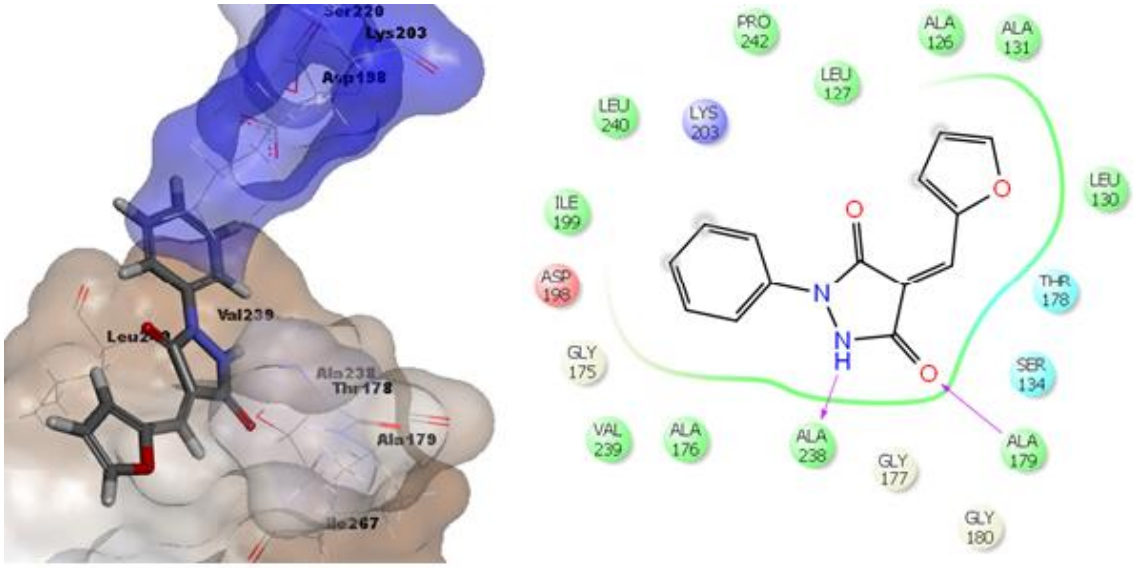


Figure 5.20: Binding pose and interaction pattern of the most active compound **PZ_16** with *M. tuberculosis* ADH enzyme

Thirteen compounds inhibited *M. tuberculosis* LAT with $IC_{50} \leq 50$ µM. Four compounds (**PZ_11**, **PZ_21**, **PZ_26** and **PZ_27**) inhibited LAT with $IC_{50} < 20$ µM, and 4-(2-chlorobenzylidene)-1-phenylpyrazolidine-3,5-dione (**PZ_11**) was found to be the most potent compound with IC_{50} of 11.45±0.16 µM, which inhibited the *M. tuberculosis* with MIC of 5.21 µM.

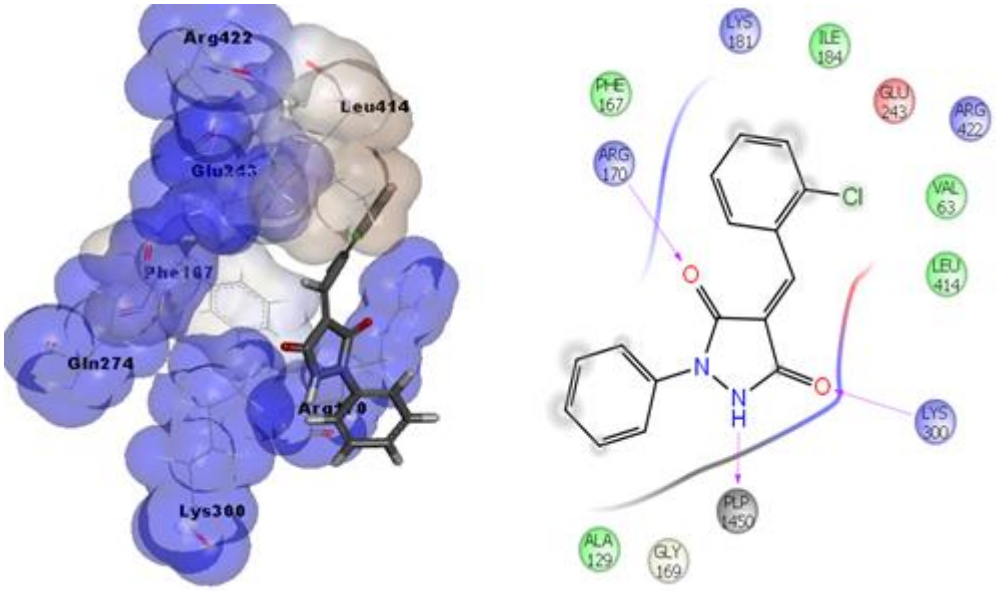


Figure 5.21: Binding pose and interaction pattern of the most active compound **PZ_11** with *M. tuberculosis* LAT enzyme

5.5.6. Highlights of the study

In present study, we report design, synthesis and biological evaluation of thirty pyrazolidine-3,5-dione derivatives against *M. tuberculosis* as well as explored the possible enzyme target for most active compounds against three *M. tuberculosis* enzymes. The synthesized compounds showed MICs ranging from 4.13-188.6 µM; and eleven compounds showed promising activity with MIC less than 10 µM. When compared to original lead compound **CD59**; all the compounds were found to be less active. When compared to standard first line anti-tubercular drug ethambutol (MIC of 7.64 µM), nine compounds were found to be more active and when compared to pyrazinamide (MIC of 50.77 µM), twenty two compounds were more active. All the molecules were found be less active than isoniazid (MIC of 0.72 µM) and rifampicin (MIC of 0.24 µM) but three compounds were more/equally active than the DNA gyrase inhibitor ciprofloxacin (MIC of 4.71 µM). Among the synthesized compounds, 4-(2-bromobenzylidene)-1-(4-chlorophenyl)pyrazolidine-3,5-dione (**PZ_28**) was found to be the most active compound with *in vitro* MICs of 4.13 µM. A diagrammatic depiction has been presented in **Figure 5.22** of the most promising compounds.

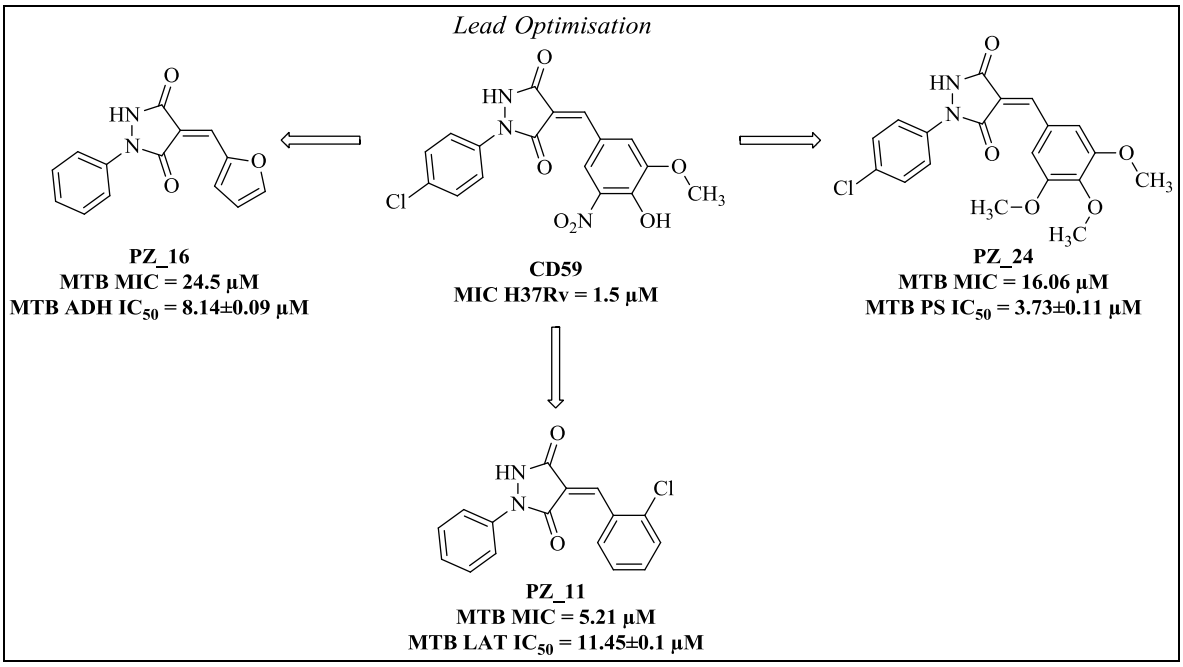


Figure 5.22: Structures and biological activities of most active compounds **PZ_11**, **PZ_16** and **PZ_24**

5.6. Development of 2-iminothiazolidine-4-one derivatives as novel anti-tubercular agents

5.6.1. Design of the molecules

The Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) was established by the National Institute of Allergy and Infectious Diseases (NIAID) in 1994 to allow researchers access to high quality screening services in order to encourage anti-tubercular drug discovery research. Recently TAACF reported their anti-tubercular high-throughput screening results of large libraries of drug like small molecules, [Reynolds R.C., *et al*., 2012; Ananthan S., *et al*., 2009; Maddry J.A., *et al*., 2009] and among them one of the molecule **SID: 24823007** 5-(Furan-2-ylmethylene)-2-imino-3-(thiazol-2-yl)thiazolidin-4-one) showed good activity against *M. tuberculosis* H37Rv with MIC of <0.2 µM and selectivity index of >115 (**Fig 5.23**). We have taken **SID: 24823007** as the starting point to design more analogues by keeping 2-iminothiazolidine-4-one nucleus intact and modified 3rd and 5th position with various aryl and heteroaryl moiety to study the SAR of the lead compound.

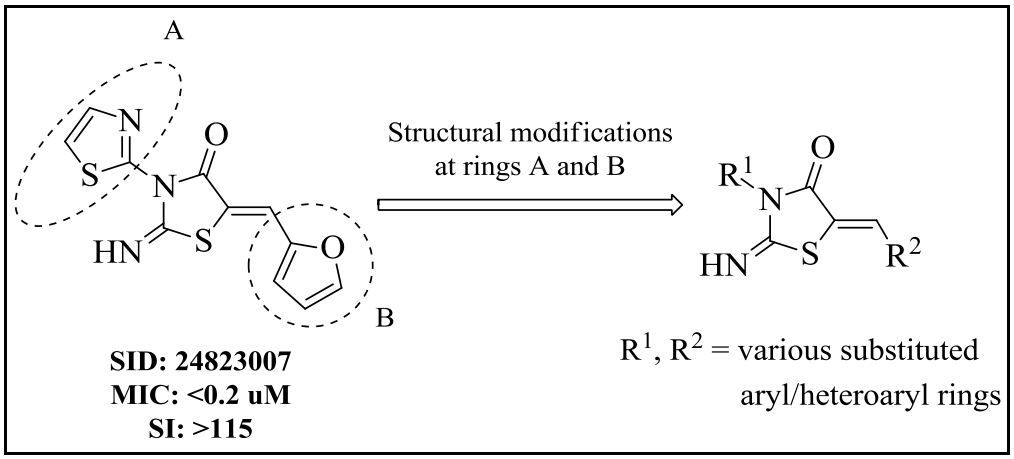


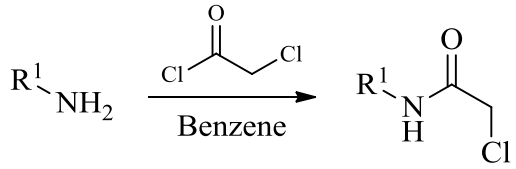
Figure 5.23: Structure of lead molecule **SID 24823007**

5.6.2. Experimental procedures utilized for the synthesis of TZ_04 – TZ_39

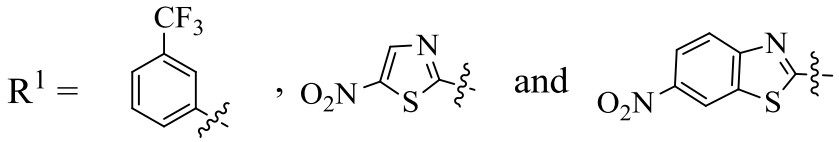
The target molecules were synthesized by following a three step synthetic protocol (**Figure 4.7**). In the first step we treated the amines (**TZ_01a-c**) like 3-trifluromethyaniline, 2-amino-5-nitrothiazole and 2-amino-6-nitrobenzothiazole with chloroacetyl chloride without using any base under thermal conditions to get corresponding chloroacetamide derivatives (**TZ_02a-c**). Refluxing of chloroacetamide derivatives (**TZ_02a-c**) and potassium thiocyanate (KSCN) in acetone for 3 h yielded the corresponding cyclised 3-substituted-2-

iminothiazolidine-4-ones (**TZ_03a-c**). In the final step we used Knoevenagal condensation of the compounds **TZ_03a-c** with various substituted aldehydes using NaOAc/acetic acid at 100 °C, to produce titled compounds (**TZ_04 - TZ_39**). These reactions were also successfully carried out using piperidine/ethanol at 90 °C, but the former reaction conditions favoured an easy purification, due to the lower solubility of formed products in acetic acid. Direct filtration of reaction mixture and washing of residue with excess water, cold ethanol, ether and hexanes produced the final compounds with good purity and in excellent yields.

General procedure for the synthesis of *N*-substituted chloroacetamides (TZ_02a-c)



TZ_01a-c **TZ_02a-c**



Compound **TZ_01a-c** (1.0 equiv) and chloroacetyl chloride (1.0 equiv) were taken in benzene and the reaction mixture was refluxed for 6 h. After completion of the reaction monitored by TLC, and was diluted with EtOAc and washed with sat $NaHCO_3$, H_2O and brine. The combined organic layer was dried over anhydrous Na_2SO_4, and evaporated to get compounds **TZ_02a-c**.

2-Chloro-*N*-(3-(trifluoromethyl)phenyl)acetamide (TZ_02a)

3-(Trifluoromethyl)aniline (3.0 g, 18.63 mmol) and chloroacetyl chloride (1.48 mL, 18.63 mmol) were taken in benzene (25 mL) and the reaction mixture was refluxed for 6 h. The reaction mixture was diluted with EtOAc and washed with sat $NaHCO_3$ (3 × 30 mL), H_2O (2 × 30 mL) and brine (2 × 20 mL). The combined organic layer was dried over anhydrous Na_2SO_4, evaporated under vacuum to get solid product. The solids were washed with hexanes to get 2-chloro-*N*-(3-(trifluoromethyl)phenyl)acetamide (3.60 g, 81%) as an off-white solid. MS(ESI) *m/z* 238 $[M+H]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 8.21 (s, 1H), 7.77 (s, 1H), 7.54 (d, *J* = 8.4 Hz, 1H), 7.49 (d, *J* = 7.6 Hz, 1H), 7.36 (t, *J* = 7.6 Hz, 1H), 4.23 (s, 2H).

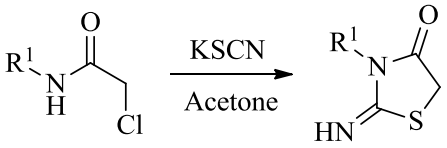
2-Chloro-*N*-(5-nitrothiazol-2-yl)acetamide (TZ_02b)

5-Nitrothiazol-2-amine (3.0 g, 20.80 mmol) and chloroacetyl chloride (1.64 mL, 20.80 mmol) were taken in benzene (25 mL) and the reaction mixture was refluxed for 6 h. The reaction mixture was diluted with EtOAc and washed with sat $NaHCO_3$ (3 × 30 mL), H_2O (2 × 30 mL) and brine (2 × 20 mL). The combined organic layer was dried over anhydrous Na_2SO_4, evaporated under vacuum to get solid product, the solids were washed with hexanes to get 2-chloro-*N*-(5-nitrothiazol-2-yl)acetamide (3.40 g, 74%) as an off-white solid. MS(ESI) *m/z* 222 $[M+H]^+$; ^1H NMR (400 MHz, DMSO-d_6) δ 9.43 (s, 1H), 8.64 (s, 1H), 4.24 (s, 2H).

2-Chloro-*N*-(6-nitrobenzo[d]thiazol-2-yl)acetamide (TZ_02c)

6-Nitrobenzo[*d*]thiazol-2-amine (3.0 g, 15.38 mmol) and chloroacetyl chloride (1.21 mL, 15.38 mmol) were taken in benzene (25 mL) and the reaction mixture was refluxed for 6 h. The reaction mixture was diluted with EtOAc and washed with sat $NaHCO_3$ (3 × 30 mL), H_2O (2 × 30 mL) and brine (2 × 20 mL). The combined organic layer was dried over anhydrous Na_2SO_4, evaporated under vacuum to get solid product, the solids were washed with hexanes to get 2-chloro-*N*-(6-nitrobenzo[d]thiazol-2-yl)acetamide (2.97 g, 71%) as an off-white solid. MS(ESI) *m/z* 272 $[M+H]^+$; ^1H NMR (400 MHz, DMSO-d_6) δ 10.03 (s, 1H), 8.49 (s, 1H), 8.32 (d, *J* = 8.4 Hz, 1H), 8.22 (d, *J* = 8.0 Hz, 1H), 4.26 (s, 2H).

General procedure for the synthesis of 3-substituted-2-iminothiazolidine-4-one (TZ_03a-c)



TZ_02a-c **TZ_03a-c**

Compound (**TZ_02a-c**) (1.0 equiv) and KSCN (1.6 equiv) were taken in dry acetone and refluxed for 2 h. The reaction mixture was concentrated and the obtained solid was washed with H_2O and dried in vacuum oven to get compound (**TZ_03a-c**)**.**

2-Imino-3-(3-(trifluoromethyl)phenyl)thiazolidin-4-one (TZ_03a)

2-Chloro-N-(3-(trifluoromethyl)phenyl)acetamide (3.60 g, 15.18 mmoles) and KSCN (2.36 g, 24.30 mmoles) were taken in dry acetone and refluxed for 2 h. The reaction mixture was

concentrated and the obtained solid was washed with H_2O and dried in vacuum oven to get compound 2-imino-3-(3-(trifluoromethyl)phenyl)thiazolidin-4-one (3.30 g, 83%) as an off-white solid. MS(ESI) *m/z* 261 $[M+H]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 9.12 (s, 1H), 7.78 (s, 1H), 7.64 (d, *J* = 8.0 Hz, 1H), 7.54 (d, *J* = 7.6 Hz, 1H), 7.45 (t, *J* = 7.6 Hz, 1H), .4.32 (s, 2H).

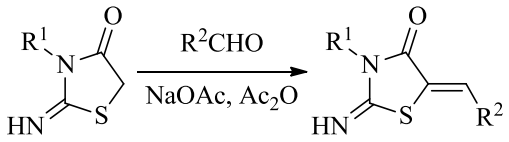
2-Imino-3-(5-nitrothiazol-2-yl)thiazolidin-4-one (TZ_03b)

2-Chloro-N-(5-nitrothiazol-2-yl)acetamide (3.40 g, 15.38 mmoles) and KSCN (2.40 g, 24.61 mmoles) were taken in dry acetone and refluxed for 2 h. The reaction mixture was concentrated and obtained solid was washed with H_2O and dried in vacuum oven to get compound 2-imino-3-(5-nitrothiazol-2-yl)thiazolidin-4-one (3.15 g, 84%) as an off-white solid. MS(ESI) *m/z* 245 $[M+H]^+$. ^1H NMR (400 MHz, DMSO-d_6) δ 9.97 (s, 1H), 8.81 (s, 1H), 4.27 (s, 2H).

2-Imino-3-(6-nitrobenzo[*d*]thiazol-2-yl)thiazolidin-4-one(TZ_03c)

2-Chloro-*N*-(6-nitrobenzo[*d*]thiazol-2-yl)acetamide (2.97 g, 10.95 mmoles) and KSCN (1.71 g, 17.53 mmoles) were taken in dry acetone and refluxed for 2 h. The reaction mixture was concentrated and obtained solid was washed with H_2O and dried in vacuum oven to get compound 2-imino-3-(6-nitrobenzo[*d*]thiazol-2-yl)thiazolidin-4-one (2.34 g, 72%) as an off-white solid. MS(ESI) *m/z* 295 $[M+H]^+$; ^1H NMR (400 MHz, DMSO-d_6) δ 9.72 (s, 1H), 8.53 (s, 1H), 8.34 (d, *J* = 8.4 Hz, 1H), 8.17 (d, *J* = 8.0 Hz, 1H), 4.20 (s, 2H).

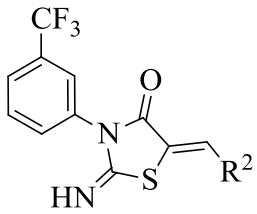
General procedure for the synthesis of compounds TZ_04 – TZ_39



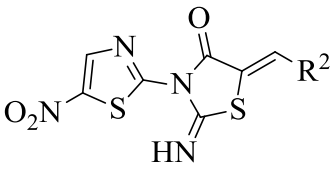
TZ_03a-c **TZ_04 - TZ_39**

Compound **TZ_03a-c** (1.0 equiv), NaOAc (2.0 equiv) and various substituted aldehydes (**Table 5.13**) (1.2 equiv) were taken in acetic acid and heated at 100 °C for 3 h, the solids formed in the reaction mixture were filtered and washed with water, cold ethanol and hexanes to afford titled compounds **TZ_04** – **TZ_39**.

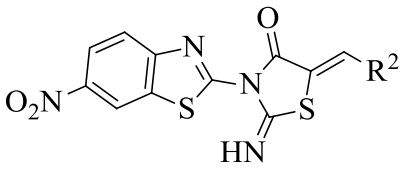
Table 5.13: Physiochemical properties of the synthesized compounds **TZ_04** – **TZ_39**



TZ_04 - TZ_15



TZ_16 - TZ_27



TZ_28 - TZ_39

Compd	R^2	Yield (%)	M.P. (°C)	Molecular formula	Molecular weight
TZ_04	2-Hydroxyphenyl	90	208-209	$C_{17}H_{11}F_3N_2O_2S$	364.34
TZ_05	4-Hydroxyphenyl	88	221-222	$C_{17}H_{11}F_3N_2O_2S$	364.34
TZ_06	4-Methoxyphenyl	79	189-190	$C_{18}H_{13}F_3N_2O_2S$	378.37
TZ_07	4-Benzyloxyphenyl	93	218-220	$C_{24}H_{17}F_3N_2O_2S$	454.46
TZ_08	4-Methylphenyl	81	205-207	$C_{18}H_{13}F_3N_2OS$	362.37
TZ_09	4-(Dimethylamino)phenyl	84	234-235	$C_{19}H_{16}F_3N_3OS$	391.41
TZ_10	3,4,5-Trimethoxyphenyl	89	208-209	$C_{20}H_{17}F_3N_2O_4S$	438.42
TZ_11	2-Fluorophenyl	80	201-203	$C_{17}H_{10}F_4N_2OS$	366.33
TZ_12	4-Chlorophenyl	79	209-211	$C_{17}H_{10}ClF_3N_2OS$	382.79
TZ_13	3-Nitrophenyl	76	180-181	$C_{17}H_{10}F_3N_3O_3S$	393.34
TZ_14	5-Nitrofuran-2-yl	92	231-232	$C_{15}H_8F_3N_3O_4S$	383.30
TZ_15	5-Nitrothiophen-2-yl	72	241-243	$C_{15}H_8F_3N_3O_3S_2$	399.37
TZ_16	2-Hydroxyphenyl	88	218-220	$C_{13}H_8N_4O_4S_2$	348.36
TZ_17	4-Hydroxyphenyl	79	213-214	$C_{13}H_8N_4O_4S_2$	348.36
TZ_18	4-Methoxyphenyl	84	204-205	$C_{14}H_{10}N_4O_4S_2$	362.38
TZ_19	4-Benzyloxyphenyl	93	241-243	$C_{20}H_{14}N_4O_4S_2$	438.48
TZ_20	4-Methylphenyl	89	260-262	$C_{14}H_{10}N_4O_3S_2$	346.38
TZ_21	4-(Dimethylamino)phenyl	76	290-291	$C_{15}H_{13}N_5O_3S_2$	375.43
TZ_22	3,4,5-Trimethoxyphenyl	88	207-208	$C_{16}H_{14}N_4O_6S_2$	422.44
TZ_23	2-Fluorophenyl	78	236-238	$C_{13}H_7FN_4O_3S_2$	350.33
TZ_24	4-Chlorphenyl	81	222-223	$C_{13}H_7ClN_4O_3S_2$	366.80

Contd

Compd	R^2	Yield (%)	M.P. (°C)	Molecular formula	Molecular weight
TZ_25	3-Nitrophenyl	88	196-198	$C_{13}H_7N_5O_5S_2$	377.36
TZ_26	5-Nitrofuran-2-yl	69	232-233	$C_{11}H_5N_5O_6S_2$	367.32
TZ_27	5-Nitrothiophen-2-yl	76	241-242	$C_{11}H_5N_5O_5S_3$	383.38
TZ_28	2-Hydroxyphenyl	83	218-220	$C_{17}H_{10}N_4O_4S_2$	398.42
TZ_29	4-Hydroxyphenyl	81	227-228	$C_{17}H_{10}N_4O_4S_2$	398.42
TZ_30	4-Methoxyphenyl	84	232-233	$C_{18}H_{12}N_4O_4S_2$	413.42
TZ_31	4-Benzyloxyphenyl	91	208-209	$C_{24}H_{16}N_4O_4S_2$	489.23
TZ_32	4-Methylphenyl	88	249-250	$C_{18}H_{12}N_4O_3S_2$	397.65
TZ_33	4-(Dimethylamino)phenyl	72	281-282	$C_{19}H_{15}N_5O_3S_2$	426.34
TZ_34	3,4,5-Trimethoxyphenyl	83	210-211	$C_{20}H_{16}N_4O_6S_2$	473.12
TZ_35	2-Fluorophenyl	79	223-224	$C_{17}H_9FN_4O_3S_2$	401.72
TZ_36	4-Chlorphenyl	72	243-244	$C_{17}H_9ClN_4O_3S_2$	415.98
TZ_37	3-Nitrophenyl	84	204-205	$C_{17}H_9N_5O_5S_2$	428.28
TZ_38	5-Nitrofuran-2-yl	70	254-255	$C_{15}H_7N_5O_6S_2$	418.81
TZ_39	5-Nitrothiophen-2-yl	72	241-242	$C_{15}H_7N_5O_5S_3$	434.90

5.6.3. Characterization of the synthesized molecules

5-(2-Hydroxybenzylidene)-2-imino-3-(3-(trifluoromethyl)phenyl)thiazolidin-4-one (TZ_04): 2-Imino-3-(3-(trifluoromethyl)phenyl)thiazolidin-4-one (0.25 g, 0.96 mmol), NaOAc (0.15 g, 1.92 mmoles) and 2-hydroxybenzaldehyde (0.14 g, 1.15 mmol) were taken in acetic acid (2.0 mL) and heated at 100 °C for 3 h, the solids formed in the reaction mixture were filtered and washed with water, little amount of ethanol and hexanes to afford title compound **TZ_04** (0.38 g, 90%) as an off-white solid. m.p. 208–209 °C; MS(ESI) *m/z* 365 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.51 (s, 1H), 10.02 (s, 1H), 8.30 (s, 1H), 7.81 (d, *J* = 8.0 Hz, 1H), 7.78–7.60 (m, 4H), 7.54–7.36 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.0, 153.4, 152.3, 144.9, 136.5, 133.2, 131.5, 128.4, 127.3, 127.0, 126.4, 125.7, 124.4, 123.6, 122.4, 121.3, 119.4. Anal. calcd. for $C_{17}H_{11}F_3N_2O_2S$: C, 56.04; H, 3.04; N, 7.69% Found C, 56.12; H, 3.10; N, 7.72%

5-(4-Hydroxybenzylidene)-2-imino-3-(3-(trifluoromethyl)phenyl)thiazolidin-4-one (TZ_05): Yield: 88%; m.p. 221–222 °C; MS(ESI) *m/z* 365 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.91 (s, 1H), 9.42 (s, 1H), 8.34 (s, 1H), 7.99 (d, *J* = 7.6Hz, 2H), 7.72–7.63 (m, 4H), 7.27 (d, *J* = 8.0 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.8, 160.6, 152.4, 149.4, 134.5, 133.4, 130.5(2C), 129.8, 129.1, 127.4, 126.3, 125.4, 124.7, 123.4(2C), 119.7. Anal. calcd. for $C_{17}H_{11}F_3N_2O_2S$: C, 56.04; H, 3.04; N, 7.69% Found C, 56.14; H, 3.10; N, 7.77%.

2-Imino-5-(4-methoxybenzylidene)-3-(3-(trifluoromethyl)phenyl)thiazolidin-4-one (TZ_06): Yield: 79%; m.p. 189–190 °C; MS(ESI) *m/z* 379 $[M+H]^+$; ^{1}H NMR (300 MHz, DMSO-d_6) 12.40 (s, 1H), 8.78–7.20 (m, 3H), 7.92–7.77 (m, 4H), 7.69–7.54 (m, 2H), 4.05 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 163.5, 156.4, 149.4, 144.5, 136.4, 135.3, 133.4(2C), 132.6, 130.5, 129.5, 127.6, 126.0, 125.4, 124.6, 123.3(2C), 120.6. Anal calcd for $C_{18}H_{13}F_3N_2O_2S$: C, 57.14; H, 3.46; N, 7.40 % Found C, 57.21; H, 3.48; N, 7.43 %.

5-(4-(Benzyloxy)benzylidene)-2-imino-3-(3-(trifluoromethyl)phenyl)thiazolidin-4-one (TZ_07): Yield: 93%; m.p. 218–219 °C; MS(ESI) *m/z* 455 $[M+H]^+$; ^{1}H NMR (300 MHz, $CDCl_3$) 12.45 (s, 1H), 8.56 (s, 1H), 8.12 (d, *J* = 7.6 Hz, 2H), 7.99–7.72 (m, 6H), 7.63–7.51 (m, 5H), 4.99 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) 167.3, 161.1, 154.3, 148.7, 139.5, 138.4, 137.4, 136.3(2C), 134.5, 133.2(2C), 131.4, 130.4(2C), 129.6, 128.4, 127.4, 126.3(2C), 125.2, 123.4, 120.5, 69.3. Anal calcd for $C_{24}H_{17}F_3N_2O_2S$: C, 63.43; H, 3.77; N, 6.16% Found C, 63.52; H, 3.87; N, 6.25%.

2-Imino-5-(4-methylbenzylidene)-3-(3-(trifluoromethyl)phenyl)thiazolidin-4-one (TZ_08): Yield: 81%; m.p. 205–206 °C; MS(ESI) *m/z* 363 $[M+H]^+$; ^{1}H NMR (300 MHz, DMSO-d_6) 12.24 (s, 1H), 8.34 (s, 1H), 7.89 (d, *J* = 7.6 Hz, 2H), 7.81–7.72 (m, 6H), 2.52 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) 164.3, 159.4, 155.3, 152.4, 141.3, 139.5, 138.4, 136.4, 135.4(2C), 133.2, 130.4(2C), 127.4, 126.4, 125.3, 122.4, 22.5. Anal calcd for $C_{18}H_{13}F_3N_2OS$: C, 59.66; H, 3.62; N, 7.73% Found C, 59.73; H, 3.67; N, 7.87%.

5-(4-(Dimethylamino)benzylidene)-2-imino-3-(3-(trifluoromethyl)phenyl)thiazolidin-4-one (TZ_09): Yield: 84%; m.p. 234–235 °C; MS(ESI) *m/z* 393 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) 9.99 (s, 1H), 8.28 (s, 1H), 7.97 (d, *J* = 7.6 Hz, 2H), 7.82–7.74 (m, 2H), 7.63–7.45 (m, 4H), 3.15 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) 164.4, 154.4, 152.6, 143.4, 136.4, 134.5, 133.6(2C), 132.9, 132.1, 131.3(2C), 127.5, 126.4, 124.6, 123.4, 120.4, 43.2(2C). Anal calcd for $C_{19}H_{16}F_3N_3OS$: C, 58.30; H, 4.12; N, 10.74% Found C, 58.41; H, 4.18; N, 10.83%.

2-Imino-3-(3-(trifluoromethyl)phenyl)-5-(3,4,5-trimethoxybenzylidene)thiazolidin-4-one (TZ_10): Yield: 89%; m.p. 208–209 °C; MS(ESI) *m/z* 439 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) 12.60 (s, 1H), 8.49 (s, 1H), 8.10 (d, *J* = 7.6 Hz, 2H), 7.99 (t, 1H), 7.63 (d, *J* = 7.2 Hz, 1H), 7.49 (s, 2H), 4.12 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 168.3, 162.4(2C), 161.1, 152.4, 149.6, 142.3, 138.5, 137.4, 135.9, 133.0, 131.4, 128.4, 126.2, 124.2, 123.4(2C), 63.4, 61.2(2C). Anal. calcd. for $C_{20}H_{17}F_3N_2O_4S$: C, 54.79; H, 3.91; N, 6.39% Found C, 54.82; H, 3.98; N, 6.36%.

5-(2-Fluorobenzylidene)-2-imino-3-(3-(trifluoromethyl)phenyl)thiazolidin-4-one (TZ_11): Yield: 80%; m.p. 201–203 °C; MS(ESI) *m/z* 367 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.42 (s, 1H), 7.99 (s, 1H), 7.92 (s, 1H), 7.81–7.74 (m, 2H), 7.63 (d, *J* = 6.8 Hz, 1H), 7.58–7.49 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) 167.2, 162.2, 154.3, 149.6, 135.3, 134.6, 133.2, 129.2, 126.3, 126.0, 125.2, 123.7, 123.2, 122.6, 121.4, 120.3, 119.9. Anal. calcd. for $C_{17}H_{10}F_4N_2OS$: C, 55.74; H, 2.75; N, 7.65% Found C, 55.81; H, 2.81; N, 7.74%

5-(4-Chlorobenzylidene)-2-imino-3-(3-(trifluoromethyl)phenyl)thiazolidin-4-one (TZ_12): Yield: 79%; m.p. 209–211 °C; MS(ESI) *m/z* 383 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.88 (s, 1H), 8.21 (s, 1H), 7.81 (d, *J* = 7.2 Hz, 2H), 7.77 (d, *J* = 6.8 Hz, 1H), 7.72–7.68 (m, 3H), 7.35 (d, *J* = 8.0 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) 167.2, 163.3, 155.2, 148.9, 136.8, 136.2, 135.3(2C), 133.8, 130.4, 129.4, 127.2, 126.4, 124.7(2C), 123.1, 120.6. Anal. calcd. for $C_{17}H_{10}ClF_3N_2OS$: C, 53.34; H, 2.63; N, 7.32% Found C, 53.37; H, 2.72; N, 7.41%

2-Imino-5-(3-nitrobenzylidene)-3-(3-(trifluoromethyl)phenyl)thiazolidin-4-one (TZ_13): Yield: 76%; m.p. 180–181 °C; MS(ESI) *m/z* 394 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.94 (s, 1H), 8.34 (s, 1H), 8.21 (s, 1H), 7.98 (d, *J* = 7.6 Hz, 1H), 7.81–7.74 (m, 3H), 7.63–7.54 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) 164.6, 162.2, 151.2, 146.4, 137.8, 136.3, 134.2, 133.8, 132.6, 131.6, 130.4, 128.2, 126.7, 125.1, 124.2, 123.2, 121.5. Anal. calcd. for $C_{17}H_{10}F_3N_3O_3S$: C, 51.91; H, 2.56; N, 10.68% Found C, 51.94; H, 2.62; N, 10.73%

2-Imino-5-((5-nitrofuran-2-yl)methylene)-3-(3-(trifluoromethyl)phenyl)thiazolidin-4-one (TZ_14): Yield: 92%; m.p. 231–232 °C; MS(ESI) *m/z* 384 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.63 (s, 1H), 8.31 (d, *J* = 7.2 Hz, 1H), 8.21 (s, 1H), 7.92 (d, *J* = 7.2 Hz, 1H), 7.81–7.72 (m, 2H), 7.58 (d, *J* = 7.2 Hz, 1H), 7.54 (t, *J* = 7.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) 168.1, 160.2, 153.2, 152.1, 149.3, 136.3, 135.3, 134.9, 133.9, 131.4, 127.2, 126.4, 124.2,

122.1, 120.5. Anal. calcd. for $C_{15}H_8F_3N_3O_4S$: C, 47.00; H, 2.10; N, 10.96% Found C, 47.11; H, 2.13; N, 10.98%.

2-Imino-5-((5-nitrothiophen-2-yl)methylene)-3-(3-(trifluoromethyl)phenyl)thiazolidin-4-one (TZ_15): Yield: 72%; m.p. 241–242 °C; MS(ESI) *m/z* 400 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.51 (s, 1H), 8.29 (d, *J* = 7.2 Hz, 1H), 8.19 (s, 1H), 7.89 (d, *J* = 7.2 Hz, 1H), 7.79–7.69 (m, 2H), 7.63 (d, *J* = 7.2 Hz, 1H), 7.58 (t, *J* = 7.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) 166.9, 161.1, 152.3, 150.3, 148.2, 135.5, 134.1, 133.4, 132.9, 130.2, 128.4, 126.2, 122.3, 120.3, 118.8. Anal. calcd. for $C_{15}H_8F_3N_3O_3S_2$: C, 45.11; H, 2.02; N, 10.52% Found C, 45.19; H, 2.10; N, 10.59%.

5-(2-Hydroxybenzylidene)-2-imino-3-(5-nitrothiazol-2-yl)thiazolidin-4-one (TZ_16): Yield: 88%; m.p. 218–219 °C; MS(ESI) *m/z* 349 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.42 (s, 1H), 9.70 (s, 1H), 8.31 (s, 1H), 7.92 (d, *J* = 7.2 Hz, 1H), 7.81 (s, 1H), 7.72–7.69 (m, 2H), 7.63 (t, *J* = 7.6 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) 172.6, 163.4, 156.4, 149.4, 137.4, 131.4, 128.5, 126.4, 125.2, 123.8, 121.4, 120.4, 119.3. Anal. calcd. for $C_{13}H_8N_4O_4S_2$: C, 44.82; H, 2.31; N, 16.08% Found C, 44.92; H, 2.41; N, 16.12%

5-(4-Hydroxybenzylidene)-2-imino-3-(5-nitrothiazol-2-yl)thiazolidin-4-one (TZ_17): Yield: 79%; m.p. 213–214 °C; MS(ESI) *m/z* 349 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.78 (s, 1H), 9.21 (s, 1H), 8.31 (s, 1H), 7.92 (s, 1H), 7.63 (d, *J* = 7.6 Hz, 2H), 7.32 (d, *J* = 8.0 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) 169.9, 163.6, 154.9, 148.5, 134.5, 132.4, 129.5(2C), 127.8, 124.9, 123.1(2C), 121.5. Anal. calcd. for $C_{13}H_8N_4O_4S_2$: C, 44.82; H, 2.31; N, 16.08% Found C, 44.94; H, 2.34; N, 16.18%

2-Imino-5-(4-methoxybenzylidene)-3-(5-nitrothiazol-2-yl)thiazolidin-4-one (TZ_18): Yield: 84%; m.p. 204–205 °C; MS(ESI) *m/z* 363 $[M+H]^+$; ^{1}H NMR (300 MHz, DMSO-d_6, δ) δ 13.12 (s, 1H), 8.31 (s, 1H), 7.69 (s, 1H), 7.65 (d, *J* = 7.5 Hz, 2H), 7.29 (d, *J* = 7.2 Hz, 2H), 3.99 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) 171.9, 166.6, 156.2, 150.5, 141.5, 136.2, 132.5(2C), 128.3, 126.3, 122.4(2C), 120.6, 60.3. Anal. calcd. for $C_{14}H_{10}N_4O_4S_2$: C, 46.40; H, 2.78; N, 15.46% Found C, 46.51; H, 2.81; N, 15.52%.

5-(4-(Benzyloxy)benzylidene)-2-imino-3-(5-nitrothiazol-2-yl)thiazolidin-4-one (TZ_19): Yield: 93%; m.p. 241–242 °C; MS(ESI) *m/z* 439 $[M+H]^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 13.12 (s, 1H), 8.53 (s, 1H), 7.81 (s, 1H), 7.72 (d, *J* = 7.2 Hz, 2H), 7.67–7.54 (m, 7H), 5.22 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) 169.6, 162.6, 154.7, 144.9, 139.6, 136.6, 134.2, 133.2(2C),

132.5, 132.0(2C), 130.5, 128.2(2C), 126.2, 124.8(2C), 120.6, 70.2. Anal. calcd. for $C_{20}H_{14}N_4O_4S_2$: C, 54.78; H, 3.22; N, 12.78% Found C, 54.85; H, 3.26; N, 12.81%.

2-Imino-5-(4-methylbenzylidene)-3-(5-nitrothiazol-2-yl)thiazolidin-4-one (TZ_20): Yield: 89%; m.p. 260–261 °C; MS(ESI) *m/z* 347 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.54 (s, 1H), 8.41 (s, 1H), 7.71 (d, *J* = 7.2 Hz, 2H), 7.67 (s, 1H), 7.58 (d, *J* = 7.2 Hz, 2H), 2.49 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 172.1, 166.4, 152.5, 149.4, 138.4, 136.3, 135.2(2C), 134.2, 131.4(2C), 126.2, 122.1, 22.3. Anal. calcd. for $C_{14}H_{10}N_4O_3S_2$: C, 48.54; H, 2.91; N, 16.17% Found C, 48.61; H, 2.93; N, 16.21%

5-(4-(Dimethylamino)benzylidene)-2-imino-3-(5-nitrothiazol-2-yl)thiazolidin-4-one (TZ_21): Yield: 76%; m.p. 290–291 °C; MS(ESI) *m/z* 376 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.87 (s, 1H), 8.35 (s, 1H), 7.72 (s, 1H), 7.63 (d, *J* = 7.2 Hz, 2H), 7.54 (d, *J* = 8.0 Hz, 2H), 3.15 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) 169.9, 164.1, 153.3, 149.4, 141.4, 136.4, 133.2(2C), 130.9, 128.8, 126.3(2C), 125.5, 44.4(2C). Anal. calcd. for $C_{15}H_{13}N_5O_3S_2$: C, 47.99; H, 3.49; N, 18.65% Found C, 47.97; H, 3.58; N, 18.71%

2-Imino-3-(5-nitrothiazol-2-yl)-5-(3,4,5-trimethoxybenzylidene)thiazolidin-4-one (TZ_22): Yield: 88%; m.p. 207–208 °C; MS(ESI) *m/z* 424 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.23 (s, 1H), 8.45 (s, 1H), 7.72 (s, 1H), 7.11 (s, 2H), 3.90 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6) 171.7, 166.2, 152.1(2C), 148.2, 146.3, 142.3, 137.2, 135.9, 132.6, 130.3, 126.4(2C), 61.3, 60.6(2C). Anal. calcd. for $C_{16}H_{14}N_4O_6S_2$: C, 45.49; H, 3.34; N, 13.26% Found C, 45.52; H, 3.41; N, 13.32%.

5-(2-Fluorobenzylidene)-2-imino-3-(5-nitrothiazol-2-yl)thiazolidin-4-one (TZ_23): Yield: 78%; m.p. 236–237 °C; MS(ESI) *m/z* 350 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.91 (s, 1H), 8.31 (s, 1H), 8.18 (s, 1H), 7.94 (d, *J* = 7.6 Hz, 1H), 7.81–7.63 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) 168.3, 163.5, 156.1, 146.2, 134.8, 134.2, 133.5, 132.9, 128.4, 126.9, 124.4, 120.3, 119.4. Anal. calcd. for $C_{13}H_7FN_4O_3S_2$: C, 44.57; H, 2.01; N, 15.99% Found C, 44.61; H, 1.98; N, 16.01%

5-(4-Chlorobenzylidene)-2-imino-3-(5-nitrothiazol-2-yl)thiazolidin-4-one (TZ-24): Yield: 81%; m.p. 222–223 °C; MS(ESI) *m/z* 367 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.02 (s, 1H), 8.41 (s, 1H), 7.81 (s, 1H), 7.72 (d, *J* = 8.0 Hz, 2H), 7.56 (d, *J* = 8.0 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) 166.9, 160.2, 152.7, 146.8, 137.4, 134.8, 133.3(2C), 132.3,

128.4(2C), 126.2, 121.5. Anal. calcd. for $C_{13}H_7ClN_4O_3S_2$: C, 42.57; H, 1.92; N, 15.27% Found C, 42.63; H, 1.99; N, 15.32%

2-Imino-5-(3-nitrobenzylidene)-3-(5-nitrothiazol-2-yl)thiazolidin-4-one (TZ_25): Yield: 88%; m.p. 196–197 °C; MS(ESI) *m/z* 378 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.11 (s, 1H), 8.32 (s, 1H), 8.27 (s, 1H), 7.94–7.76 (m, 3H), 7.62 (t, *J* = 7.6 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) 170.1, 163.2, 152.2, 147.3, 138.1, 136.2, 135.0, 133.3, 132.4, 127.3, 125.9, 123.3, 121.6. Anal. calcd. for $C_{13}H_7N_5O_5S_2$: C, 41.38; H, 1.87; N, 18.56% Found C, 41.41; H, 1.96; N, 18.61%

2-Imino-5-((5-nitrofuran-2-yl)methylene)-3-(5-nitrothiazol-2-yl)thiazolidin-4-one (TZ_26): Yield: 69%; m.p. 232–234 °C; MS(ESI) *m/z* 368 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.21 (s, 1H), 8.49 (s, 1H), 8.39 (d, *J* = 6.8 Hz, 1H), 8.21 (d, *J* = 7.2 Hz, 1H), 7.99 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) 172.1, 163.3, 152.8, 150.8, 143.2, 142.4, 136.3, 133.9, 126.8, 124.2, 123.6. Anal. calcd. for $C_{11}H_5N_5O_6S_2$: C, 35.97; H, 1.37; N, 19.07% Found C, 36.02; H, 1.39; N, 19.19%.

2-Imino-3-(5-nitrothiazol-2-yl)-5-((5-nitrothiophen-2-yl)methylene)thiazolidin-4-one (TZ_27): Yield: 76%; m.p. 241–242 °C; MS(ESI) *m/z* 384 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.14 (s, 1H), 8.53 (s, 1H), 8.34 (d, *J* = 6.8 Hz, 1H), 8.17 (d, *J* = 7.2 Hz, 1H), 7.82 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) 169.4, 161.3, 151.4, 150.3, 142.8, 141.3, 135.2, 132.7, 125.9, 123.9, 122.4. Anal calcd for $C_{11}H_5N_5O_5S_3$: C, 34.46; H, 1.31; N, 18.27% Found C, 34.52; H, 1.32; N, 18.29%.

5-(2-Hydroxybenzylidene)-2-imino-3-(6-nitrobenzo[*d*]thiazol-2-yl)thiazolidin-4-one (TZ_28): Yield: 83%; m.p. 218–219 °C; MS(ESI) *m/z* 399 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.60 (s, 1H), 9.49 (s, 1H), 8.31 (d, *J* = 7.2 Hz, 1H), 8.12 (s, 1H), 8.01 (d, *J* = 7.2 Hz, 1H), 7.81–7.74 (m, 4H), 7.63 (d, *J* = 8.0Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) 169.6, 161.1, 155.3, 148.8, 138.0, 136.1, 132.2, 130.5, 128.1, 127.4, 126.3, 125.8, 125.0, 124.0, 122.1, 120.6, 120.0. Anal. calcd. for $C_{17}H_{10}N_4O_4S_2$: C, 51.25; H, 2.53; N, 14.06% Found C, 51.30; H, 2.65; N, 14.16%.

5-(4-Hydroxybenzylidene)-2-imino-3-(6-nitrobenzo[*d*]thiazol-2-yl)thiazolidin-4-one (TZ_29): Yield: 81%; m.p. 227–228 °C; MS(ESI) *m/z* 399 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.05 (s, 1H), 9.36 (s, 1H), 8.21 (d, *J* = 7.2 Hz, 1H), 8.18 (s, 1H), 8.01 (d, *J* = 6.8 Hz, 1H), 7.92 (s, 1H), 7.77 (d, *J* = 7.6 Hz, 2H), 7.54 (d, *J* = 8.0 Hz, 2H); ^{13}C NMR (100

MHz, DMSO-d_6) 166.4, 162.6, 157.6, 153.7, 148.4, 143.4, 137.5, 133.4(2C), 130.6, 129.3, 127.2, 125.6, 124.4(2C), 122.4, 120.6. Anal. calcd. for $C_{17}H_{10}N_4O_4S_2$: C, 51.25; H, 2.53; N, 14.06% Found C, 51.29; H, 2.62; N, 14.12%.

2-Imino-5-(4-methoxybenzylidene)-3-(6-nitrobenzo[*d*]thiazol-2-yl)thiazolidin-4-one (TZ_30): Yield: 84%; m.p. 232–233 °C; MS(ESI) *m/z* 413 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.98 (s, 1H), 8.47 (s, 1H), 8.39 (d, *J* = 6.8 Hz, 1H), 8.29 (d, *J* = 7.2 Hz, 1H), 7.87 (d, *J* = 7.6 Hz, 2H), 7.72 (d, *J* = 7.2 Hz, 2H), 7.63 (s, 1H), 3.94 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) 165.3, 160.6, 156.5, 152.4, 144.3, 142.4, 139.3, 132.9(2C), 130.4, 128.6, 126.9, 126.0, 123.9(2C), 122.4, 120.3, 60.1. Anal. calcd. for $C_{18}H_{12}N_4O_4S_2$: C, 52.42; H, 2.93; N, 13.58% Found C, 52.49; H, 3.00; N, 13.67%.

5-(4-(Benzyloxy)benzylidene)-2-imino-3-(6-nitrobenzo[*d*]thiazol-2-yl)thiazolidin-4-one (TZ_31): Yield: 91%; m.p. 208–209 °C; MS(ESI) *m/z* 489 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.42 (s, 1H), 8.61 (s, 1H), 8.32 (d, *J* = 7.2 Hz, 1H), 8.10 (d, *J* = 7.2 Hz, 1H), 7.99 (d, *J* = 7.2 Hz, 2H), 7.87–7.69 (m, 6H), 7.63–7.58 (m, 2H), 5.15 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) 168.7, 163.0, 155.4, 145.4, 141.6, 139.2, 138.3, 136.4, 135.3, 134.2, 133.8(2C), 132.6, 131.8(2C), 131.2, 130.7, 127.4(2C), 125.9, 125.2(2C), 121.5, 71.1. Anal. calcd. for $C_{24}H_{16}N_4O_4S_2$: C, 59.00; H, 3.30; N, 11.47% Found C, 59.12; H, 3.34; N, 11.52%.

2-Imino-5-(4-methylbenzylidene)-3-(6-nitrobenzo[*d*]thiazol-2-yl)thiazolidin-4-one (TZ_32): Yield: 88%; m.p. 249–250 °C; MS(ESI) *m/z* 397 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) 12.33 (s, 1H), 8.32 (s, 1H), 8.21 (d, *J* = 7.2 Hz, 1H), 8.01 (d, *J* = 7.6 Hz, 1H), 7.72 (s, 1H), 7.63 (d, *J* = 7.2 Hz, 2H), 7.56 (s, 2H), 2.43 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 166.3, 161.8, 153.3, 149.6, 141.3, 139.2, 135.6(2C), 134.1(2C), 133.2, 131.5, 128.6, 127.2, 126.2, 124.6, 123.4, 21.9. Anal. calcd. for $C_{18}H_{12}N_4O_3S_2$: C, 54.53; H, 3.05; N, 14.13% Found C, 54.61; H, 3.09; N, 14.21%.

5-(4-(Dimethylamino)benzylidene)-2-imino-3-(6-nitrobenzo[*d*]thiazol-2-yl)thiazolidin-4-one (TZ_33): Yield: 72%; m.p. 281–282 °C; MS(ESI) *m/z* 426 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) 12.40 (s, 1H), 8.34 (s, 1H), 8.19 (d, *J* = 7.2 Hz, 1H), 8.01 (d, *J* = 7.2 Hz, 1H), 7.72 (s, 1H), 7.69–7.63 (m, 2H), 7.54 (d, *J* = 8.0 Hz, 2H), 3.12 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) 168.7, 163.2, 156.4, 149.4, 144.5, 143.4, 138.6, 136.2, 134.0(2C), 133.4, 130.4, 127.9, 126.8(2C), 125.1, 123.4, 44.1(2C). Anal. calcd. for $C_{19}H_{15}N_5O_3S_2$: C, 53.63; H, 3.55; N, 16.46% Found C, 53.69; H, 3.62; N, 16.51%.

2-Imino-3-(6-nitrobenzo[*d*]thiazol-2-yl)-5-(3,4,5-trimethoxybenzylidene)thiazolidin-4-one (TZ_34): Yield: 83%; m.p. 210–211 °C; MS(ESI) *m/z* 473 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) 13.03 (s, 1H), 8.55 (s, 1H), 8.44 (d, *J* = 7.2 Hz, 1H), 8.26 (d, *J* = 7.6 Hz, 1H), 7.72 (s, 1H), 7.54 (s, 2H), 3.96 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6) 168.4, 162.3, 154.8, 154.2(2C), 146.2, 145.6, 138.7, 136.9, 135.6, 134.5, 133.2, 132.6, 131.3, 130.6, 126.2(2C), 61.6, 61.2(2C). Anal. calcd. for $C_{20}H_{16}N_4O_6S_2$: C, 50.84; H, 3.41; N, 11.86% Found C, 50.92; H, 3.45; N, 11.92%.

5-(2-Fluorobenzylidene)-2-imino-3-(6-nitrobenzo[*d*]thiazol-2-yl)thiazolidin-4-one (TZ_35): Yield: 79%; m.p. 223–224 °C; MS(ESI) *m/z* 401 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.43 (s, 1H), 8.44 (s, 1H), 8.31 (d, *J* = 7.2 Hz, 1H), 8.22 (d, *J* = 6.8 Hz, 1H), 7.89 (t, *J* = 7.2 Hz, 1H), 7.81–7.69 (m, 3H), 7.65 (d, *J* = 7.6 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) 166.5, 160.6, 153.3, 147.4, 137.4, 136.4, 134.9, 132.5, 131.3, 129.6, 127.4, 126.7, 124.6, 123.3, 122.4, 121.3, 120.6. Anal calcd for $C_{17}H_9FN_4O_3S_2$: C, 50.99; H, 2.27; N, 13.99% Found C, 51.10; H, 2.29; N, 14.06%.

5-(4-Chlorobenzylidene)-2-imino-3-(6-nitrobenzo[*d*]thiazol-2-yl)thiazolidin-4-one (TZ_36): Yield: 72%; m.p. 243–244 °C; MS(ESI) *m/z* 417 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.79 (s, 1H), 8.24 (d, *J* = 7.2Hz, 1H), 8.12 (d, *J* = 6.8Hz, 1H), 7.99 (s, 1H), 7.81 (d, *J* = 7.6Hz, 2H), 7.79 (s, 1H), 7.77 (d, *J* = 8.0Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) 165.3, 158.8, 154.4, 146.2, 139.3, 136.9, 135.1, 133.4, 132.8(2C), 129.1(2C), 127.4, 125.1, 123.7, 121.4, 120.0. Anal. calcd. for $C_{17}H_9ClN_4O_3S_2$: C, 48.98; H, 2.18; N, 13.44% Found C, 49.03; H, 2.23; N, 13.53%.

2-Imino-3-(6-nitrobenzo[*d*]thiazol-2-yl)-5-(3-nitrobenzylidene)thiazolidin-4-one (TZ_37): Yield: 84%; m.p. 204–205 °C; MS(ESI) *m/z* 399 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.75 (s, 1H), 8.31 (s, 1H), 8.16 (d, *J* = 7.2 Hz, 1H), 8.11 (s, 1H), 7.92 (d, *J* = 7.2 Hz, 1H), 7.82 (s, 1H), 7.72–7.62 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) 167.1, 161.4, 156.2, 147.2, 142.4, 139.4, 138.1, 136.2, 134.5, 133.0, 132.4, 128.0, 126.0, 124.1, 123.6, 121.6, 120.4. Anal. calcd. for $C_{17}H_9N_5O_5S_2$: C, 47.77; H, 2.12; N, 16.39% Found C, 47.81; H, 2.16; N, 16.44%.

2-Imino-3-(6-nitrobenzo[*d*]thiazol-2-yl)-5-((5-nitrofuran-2-yl)methylene)thiazolidin-4-one (TZ_38): Yield: 70%; m.p. 254–255 °C; MS(ESI) *m/z* 418 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.33 (s, 1H), 8.31 (d, *J* = 7.2 Hz, 1H), 8.21 (d, *J* = 7.6 Hz, 1H), 8.08 (d, *J* = 7.2

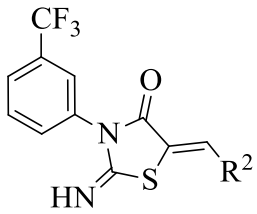
Hz, 1H), 7.99 (s, 1H), 7.91 (d, *J* = 7.6 Hz, 1H), 7.80 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) 170.2, 161.1, 156.2, 152.4, 144.6, 140.4, 138.3, 136.5, 134.8, 133.3, 129.4, 126.4, 124.6, 123.6, 121.5. Anal. calcd. for $C_{15}H_7N_5O_6S_2$: C, 43.17; H, 1.69; N, 16.78% Found C, 43.22; H, 1.72; N, 16.82%.

2-Imino-3-(6-nitrobenzo[*d*]thiazol-2-yl)-5-((5-nitrothiophen-2-yl)methylene)thiazolidin-4-one (TZ_39): Yield: 72%; m.p. 241–242 °C; MS(ESI) *m/z* 434 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.44 (s, 1H), 8.29 (d, *J* = 7.2 Hz, 1H), 8.17 (d, *J* = 7.6 Hz, 1H), 8.01 (d, *J* = 7.2 Hz, 1H), 7.99 (s, 1H), 7.89 (d, *J* = 7.6 Hz, 1H), 7.72 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) 171.3, 160.4, 156.6, 153.0, 145.3, 142.0, 139.1, 137.2, 134.7, 133.3, 128.9, 127.1, 125.2, 124.2, 121.9. Anal calcd for $C_{15}H_7N_5O_5S_3$: C, 41.57; H, 1.63; N, 16.16% Found C, 41.62; H, 1.69; N, 16.24%.

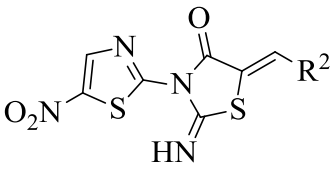
5.6.4. *In vitro M. tuberculosis* screening and cytotoxicity studies of the synthesized molecules

All the synthesized compounds were screened for their *in vitro* anti-tubercular activity against *M. tuberculosis* H37Rv using MABA method. All the compounds were also tested for their *in vitro M. tubercular* activity in presence of efflux pump inhibitor verapamil. Ethambutol, pyrazinamide and ciprofloxacin were used as reference compounds for comparison. Compounds showing *M. tuberculosis* MICs <50 μM were also tested for *in vitro* cytotoxicity against RAW 264.7cells at 100 μM concentration using MTT assay and results are tabulated as **Table 5.14**.

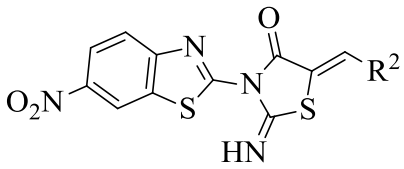
Table 5.14: *In vitro* biological evaluation of synthesized derivatives **TZ_04** – **TZ_39**



TZ_04 - TZ_15



TZ_16 - TZ_27



TZ_28 - TZ_39

Compd	R^2	MTB MIC in µM[a]	MTB MIC in µM (in presence of verapamil)	Cytotoxicity[b] at 100 µM % inhibition
TZ_04	2-Hydroxyphenyl	65.24	NT	NT
TZ_05	4-Hydroxyphenyl	16.31	NT	22.60
TZ_06	4-Methoxyphenyl	4.12	2.06	17.82
TZ_07	4-Benzyloxyphenyl	6.88	6.88	19.04
TZ_08	4-Methylphenyl	138.12	NT	NT
TZ_09	*N*,*N*-Dimethylaminophenyl	110.13	NT	NT
TZ_10	3,4,5-Trimethoxyphenyl	7.11	1.77	21.64
TZ_11	2-Fluorophenyl	4.25	1.06	22.80
TZ_12	4-Chlorophenyl	130.54	NT	NT
TZ_13	3-Nitrophenyl	63.45	NT	NT
TZ_14	5-Nitrofuran-2-yl	4.07	4.07	26.72
TZ_15	5-Nitrothiophen-2-yl	12.53	NT	24.62
TZ_16	2-Hydroxyphenyl	117.3	NT	12.86
TZ_17	4-Hydroxyphenyl	39.1	NT	18.98
TZ_18	4-Methoxyphenyl	3.78	1.89	14.56
TZ_19	4-Benzyloxyphenyl	6.40	1.60	16.40
TZ_20	4-Methylphenyl	63.12	NT	NT
TZ_21	*N*,*N*-Dimethylaminophenyl	118.48	NT	NT
TZ_22	3,4,5-Trimethoxyphenyl	3.31	0.82	12.40
TZ_23	2-Fluorophenyl	3.90	3.90	16.86

Contd

Compd	R^2	MTB MIC in µM[a]	MTB MIC in µM (in presence of verapamil)	Cytotoxicity[b] at 100 µM % inhibition
TZ_24	4-Chlorphenyl	59.95	NT	NT
TZ_25	3-Nitrophenyl	14.63	NT	20.62
TZ_26	5-Nitrofuran-2-yl	7.49	3.74	24.62
TZ_27	5-Nitrothiophen-2-yl	7.21	3.60	18.72
TZ_28	2-Hydroxyphenyl	44.85	NT	28.72
TZ_29	4-Hydroxyphenyl	22.42	NT	16.32
TZ_30	4-Methoxyphenyl	17.22	NT	22.30
TZ_31	4-Benzyloxyphenyl	15.26	NT	20.63
TZ_32	4-Methylphenyl	54.18	NT	NT
TZ_33	*N*,*N*-Dimethylaminophenyl	132.62	NT	NT
TZ_34	3,4,5-Trimethoxyphenyl	7.40	3.7	14.80
TZ_35	2-Fluorophenyl	4.26	2.13	20.62
TZ_36	4-Chlorphenyl	85.30	NT	NT
TZ_37	3-Nitrophenyl	66.31	NT	NT
TZ_38	5-Nitrofuran-2-yl	6.81	1.70	18.92
TZ_39	5-Nitrothiophen-2-yl	6.52	1.63	23.26
Isoniazid		0.72	0.72	NT
Rifampicin		0.24	0.24	NT
Ethambutol		7.64	3.82	NT
Pyrazinamide		50.77	NT	NT
Ciprofloxacin		4.71	4.71	NT

MTB, *Mycobacterium tuberculosis*; MIC, minimum inhibitory concentration;
[a]*In vitro* activity against MTB H37Rv; [b]Against RAW 264.7 cells; NT, Not tested.

5.6.5. SAR and discussion

The compounds showed MIC's ranging from 3.31-138.12 µM; and fifteen compounds showed promising activity with MIC of less than 10 µM. When compared to original lead compound **SID: 24823007** all the compounds were found to be less active. When compared to standard first line anti-tubercular drug ethambutol (MIC of 7.64 µM), fifteen compounds

were found to be more active and when compared to pyrazinamide (MIC of 50.77 µM), twenty five compounds were more active. All the molecules were found be less active than isoniazid (MIC of 0.72 µM) and rifampicin (MIC of 0.24 µM) but seven compounds were more active than the DNA gyrase inhibitor ciprofloxacin (MIC of 4.71 µM). Among the compounds, 2-imino-3-(5-nitrothiazol-2-yl)-5-(3,4,5-trimethoxybenzylidene)thiazolidin-4-one (**TP_11**) was found to be the most active compound *in vitro* with MIC of 3.31 µM. To study the SAR we prepared the compounds with variations in N-3 and C-5 positions. In N-3 position we tried with 3-trifluoromethyphenyl (**TZ_04 – TZ_15**), 5-nitrothiazol-2-yl (**TZ-16 – TZ_27**) and 6-nitrobenzothiazol-2-yl (**TZ_28 – TZ_39**) groups. The order of activity with respect to N-3 position was 6-nitrobenzothiazol-2-yl > 3-trifluoromethyphenyl > 5-nitrothiazol-2-yl group. In the C-5 position we prepared molecules with phenyl ring with both electron donating and electron with drawing groups and also with few heterocycles. In the case of phenyl ring at position C-5; 4-hydroxyl substituent (**TZ_05**, **TZ_17** and **TZ_29**) was found to be two to four times more potent than 2-hydroxyl substituent (**TZ_04**, **TZ_16** and **TZ_28**). Replacement of 4-hydroxyl group with 4-methoxyl (**TZ_06**, **TZ_18** and **TZ_30**) and 4-benzyloxy group (**TZ_07**, **TZ_19** and **TZ_31**) increased the activity; whereas methyl group (**TZ_08**, **TZ_20** and **TZ_32**) and dimethylamino group (**TZ_09**, **TZ_21** and **TZ_32**) decreased the activity drastically. Tri-substitution with 3,4,5-trimethoxyl group (**TZ_10**, **TZ_22** and **TZ_33**) showed good potency with MIC of <8 µM. In the case of electron withdrawing groups; substitution with 2-fluoro group (**TZ_11**, **TZ_23** and **TZ_34**) showed good activity with MIC of <5 µM; whereas substituents like 4-chloro (**TZ_12**, **TZ_24** and **TZ_35**) and 3-nitro groups (**TZ_13**, **TZ_25** and **TZ_36**) were detrimental for activity. With respect to heterocyclic substituents; we prepared 5-nitrofuran-2-yl and 5-nitrothiophen-2-yl compounds and both of them provided good activity.

When compared to lead compound **SID: 24823007**, all the synthesized compounds showed less activity. We tested some selected compounds [MIC of < 10 µM], in the presence of reported efflux pump inhibitor verapamil; and most of the cases MIC decreased 2 to 4 fold when compared to absence of efflux pump. Most active compound **TZ_22** showed MIC of 0.82 µM.

The synthesized compounds were not active against any of the enzymes *M. tuberculosis* PS, LAT and ADH, which implies that there would be other targets involved in its bioactivity.

5.6.6. Highlights of the study

In this study we designed and synthesized various inhibitors of *M. tuberculosis* based on the lead compound **SID: 24823007** reported by TAACF. Among the compounds, 2-imino-3-(5-nitrothiazol-2-yl)-5-(3,4,5-trimethoxybenzylidene)thiazolidin-4-one (**TZ_22**) was found to be the most active *in vitro* with MIC of 3.31 µM against log-phase culture of *M. tuberculosis* and also non-cytotoxic up to 100 µM, but less potent than lead compound **SID: 24823007**. Compound **TZ_22** showed *M. tuberculosis* MIC of 0.82 µM in presence of efflux pump inhibitor verapamil. Further structural optimization could be required to get the compounds with better potency than the lead compound and also to explore the possible mechanism of action against various *M. tuberculosis* essential enzymes. In addition, efflux could be the rate-limiting step in the discovery of novel anti-tubercular compounds, as already been recognized in the discovery of drugs for gram-negative bacterial infections. The discovery of new pumps with multiple specificities in *M. tuberculosis* and the impact of these pumps on anti-tubercular therapy by conferring resistance to many of the new molecules discovered necessitate the study of efflux mechanisms as an important therapeutic target.

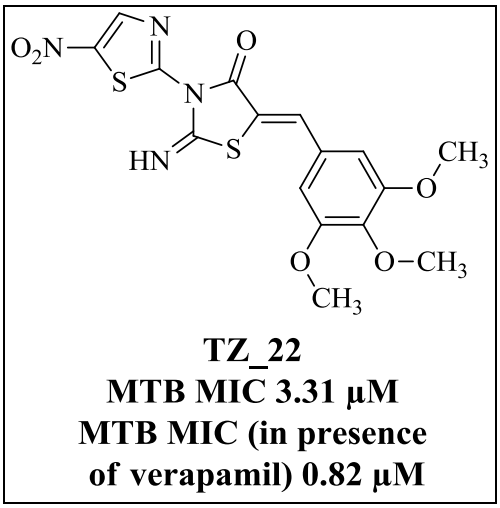


Figure 5.24: Structure and biological activity of most active compound **TZ_22**

Summary and Conclusion

Chapter 6

Throughout the known human history, TB changed its names to phthisis, white plague, king's evil, wasting disease, pott's disease but it never changed its character of consumption. It has never respected anybody, treated the king and the soldier, the rich and the poor, the American and the African alike with equal scorn.

Form literature search we found that there are many good chemical moieties which were inhibiting *M. tuberculosis* with MIC of <1 µM, but they were not turning into potent drug candidates due to many other side reactions. We had chosen reported anti-tubercular compounds with good MIC's as lead molecules and redesigned to get more drug like properties by maintaining its core structure for the activity. These leads were taken up for hit expansion by chemical synthesis and a total of 225 molecules from seven different series were synthesized and biologically evaluated in our laboratory.

Amid the molecules of 2-methylimidazo[1,2-*a*]pyridine-3-carboxylic acid series, compound **IP_06** (*N'*-(1-naphthoyl)-2-methylimidazo[1,2-*a*]pyridine-3-carbohydrazide) egressed as the most active molecule exhibiting inhibition of *M. tuberculosis* with MIC of 4.53 µM, which also showed *M. tuberculosis* PS enzyme IC_{50} of 1.90±0.12 µM. This suggests that compound **IP_06** was good inhibitor for the enzyme *M. tuberculosis* PS.

Among the molecules of 6-methylimidazo[2,1-*b*]thiazole-5-carboxylic acid, compound **IT_25** (*N*-(4-bromophenyl)-6-methylimidazo[2,1-*b*]thiazole-5-carboxamide) emerged as the most active molecule exhibiting inhibition of *M. tuberculosis* with MIC of 2.32 µM, which also showed *M. tuberculosis* PS enzyme IC_{50} = 0.69±0.12 µM. This suggested that compound **IT_25** was a good inhibitor for the enzyme *M. tuberculosis* PS.

Among the molecules of 5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-amine derivatives, compound **PR_31** (*N*-(2,5-dimethylphenyl)-2-phenyl-5,6,7,8-tetrahydro benzo[4,5]thieno[2,3-*d*]pyrimidin-4-amine) was found to be the most active with *M. tuberculosis* MIC of 2.02 µM and also exhibited *M. tuberculosis* PS IC_{50} of 5.82 µM.

In 3-phenyl-4,5,6,7-tetrahydro-1*H*-pyrazolo[4,3-*c*]pyridine series, compound **PP_09** (1-benzoyl-*N*-(4-nitrophenyl)-3-phenyl-6,7-dihydro-1H-pyrazolo[4,3-*c*]pyridine-5(4*H*)-carboxamide) inhibited the activity of *M. tuberculosis* PS enzyme with 95.7% (IC_{50} = 21.8±0.8 µM) and showed *M. tuberculosis* MIC of 26.7 µM.

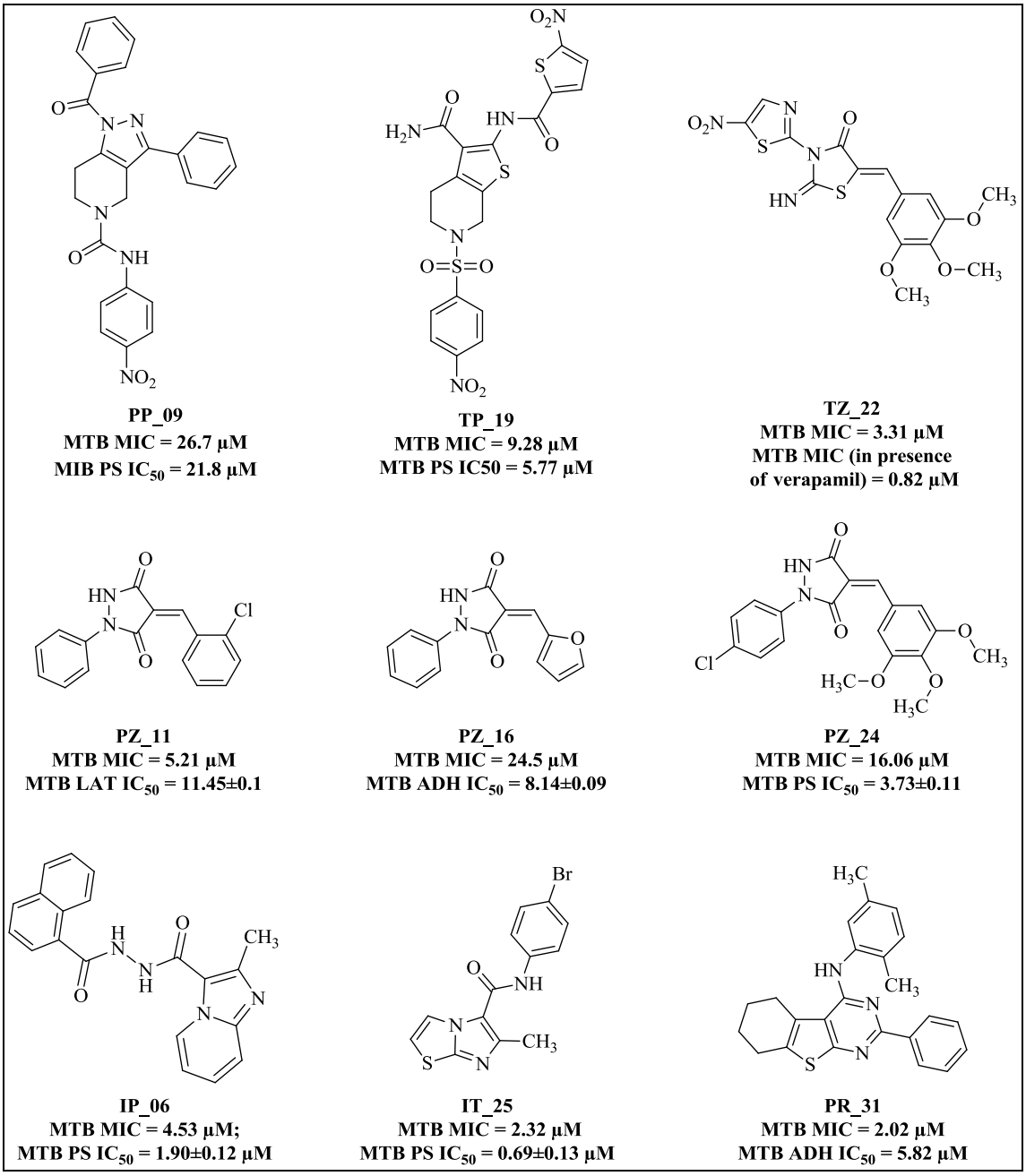
Among the molecules of tetrahydrothieno[2,3-*c*]pyridine-3-carboxamide series, compound **TP_19** (6-((4-nitrophenyl)sulfonyl)-2-(5-nitrothiophene-2-carboxamido)-4,5,6,7-tetrahydro thieno[2,3-*c*]pyridine-3-carboxamide) emerged as the most active compound exhibiting inhibitory activity against *M. tuberculosis* with MIC 9.28 µM and *M. tuberculosis* PS IC_{50} of 5.87±0.12 µM. It was non-cytotoxic at 50 µM.

In pyrazolidine-3,5-dione series, compound **PZ_28** (4-(2-bromobenzylidene)-1-(4-chlorophenyl)pyrazolidine-3,5-dione) was found to be the most active compound *in vitro* with MIC of 4.13 µM against *M. tuberculosis* and also non-cytotoxic up to 50 µM. Twelve compounds were found to inhibit more than one mycobacterial enzymes, among which four compounds (**PZ_18** and **PZ_25** – **PZ_27**) inhibited all the three enzymes. The results were that: i) Compound **PZ_24** exhibited good activity against *M. tuberculosis* PS with IC_{50} of 3.73±0.11 µM, ii) Compound **PZ_16** showed good activity against the enzyme *M. tuberculosis* ADH with IC_{50} of 8.14±0.09 µM, iii) Compound **PZ_11** exposed good activity against *M. tuberculosis* LAT with IC_{50} of 11.45±0.1 µM.

Among the 36 derivatives of 2-iminothiazolidin-4-ones, compound **TZ_22** emerged as the most active against *M. tuberculosis* H37Rv with MIC of 3.31 µM and it also exhibited MIC of 0.82 µM in presence of efflux pump inhibitor verapamil. Compound **TZ_11** emerged as the second most active compound with *M. tuberculosis* MIC of 4.25 µM and 1.06 µM in presence of verapamil. The compounds were not active against *M. tuberculosis* PS, ADH and LAT there could be other targets involved in its bioactivity.

Overall, thirty compounds were found to be more active in inhibiting *M. tuberculosis* compared to standard first line anti-TB drug ethambutol, whereas fifteen compounds possessed better *M. tuberculosis* MIC than ciprofloxacin. Amongst all the synthesized compounds, **IT_25** (MTB MIC = 2.32 µM) and **PR_31** (MTB MIC = 2.02 µM) were emerged as the most promising anti-tubercular drug candidates. Compound **IT_25** egressed as the most potent compound to inhibit the activity of *M. tuberculosis* PS with IC_{50} of 0.69±0.12 µM.

Structures of most potent compounds from each series:



PP_09
MTB MIC = 26.7 µM
MIB PS IC_{50} = 21.8 µM

TP_19
MTB MIC = 9.28 µM
MTB PS IC50 = 5.77 µM

TZ_22
MTB MIC = 3.31 µM
MTB MIC (in presence of verapamil) = 0.82 µM

PZ_11
MTB MIC = 5.21 µM
MTB LAT IC_{50} = 11.45±0.1

PZ_16
MTB MIC = 24.5 µM
MTB ADH IC_{50} = 8.14±0.09

PZ_24
MTB MIC = 16.06 µM
MTB PS IC_{50} = 3.73±0.11

IP_06
MTB MIC = 4.53 µM;
MTB PS IC_{50} = 1.90±0.12 µM

IT_25
MTB MIC = 2.32 µM
MTB PS IC_{50} = 0.69±0.13 µM

PR_31
MTB MIC = 2.02 µM
MTB ADH IC_{50} = 5.82 µM

In conclusion, the class of compounds depicted here besets a collection of promising lead compounds for further drug optimization and development to yield best novel drugs aimed to combat ever-present and everywhere-present mycobacterial infections. The study also provides the basis for further chemical optimization of these potent inhibitors as potential anti-tubercular agents.

Future perspectives

The present thesis described the development of seven chemically diverse series of molecules as potential anti-tubercular agents. The molecules reported here displayed considerable *in vitro* enzyme inhibition and potency against *M. tuberculosis* H37Rv strain. Although these results are encouraging, lead optimization is still needed.

The advancement of any of the candidate molecules presented in this thesis along a drug development track would require a substantial investment in medicinal chemistry, preclinical and clinical studies.

Extensive side effect profile of all the synthesized compounds may be studied.

Sub-acute and acute toxicological screening of novel chemical entities has to be carried out.

Extensive pharmacodynamic and pharmacokinetic studies of the safer compounds have to be undertaken in higher animal models.

Based on the pharmacophore model proposed, various substituents which lead to activity proposed could be incorporated into the compounds synthesized and study further in various animal models.

Further, the feasibility, cost effectiveness and reproducibility of synthesizing these compounds in bulk have to be tried.

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Appendix

List of Publications

From thesis work:

1. **Samala G,** Nallangi R, Devi PB, Saxena S, Yadav R, Sridevi JP, Yogeeswari P, Sriram D. "Identification and development of 2-methylimidazo[1,2-*a*]pyridine-3-carboxamides as *Mycobacterium tuberculosis* pantothenate synthetase inhibitors". *Bioorganic & Medicinal Chemistry* 2014; 22: 4223-4232.

2. **Samala G**, Devi PB, Nallangi R, Sridevi JP, Saxena S, Yogeeswari P, Sriram D. "Development of novel tetrahydrothieno[2,3-*c*]pyridine-3-carboxamide based *Mycobacterium tuberculosis* pantothenate synthetase inhibitors: molecular hybridization from known antimycobacterial leads". *Bioorganic & Medicinal Chemistry* 2014; 22: 1938-1947.

3. **Samala G**, Chunduri M, Sridevi JP, Nallangi R, Yogeeswari P, Sriram D. "Synthesis and antitubercular evaluation of 2-iminothiazolidine-4-ones". *European Journal of Chemistry* 2014; 5: 550-556.

4. **Samala G**, Shruti S Kakan, Nallangi R, Devi PB, Sridevi JP, Saxena S, Yogeeswari P, Sriram D. "Investigating structure–activity relationship and mechanism of action of antitubercular 1-(4-chlorophenyl)-4-(4-hydroxy-3-methoxy-5-nitrobenzylidene) pyrazolidine-3,5-dione [CD59]". *International Journal of Mycobacteriology* 2014; 3: 117-126.

5. **Samala G**, Devi PB, Nallangi R, Yogeeswari P, Sriram D. "Development of 3-phenyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridine derivatives as novel *Mycobacterium tuberculosis* pantothenate synthetase inhibitors". *European Journal of Medicinal Chemistry* 2013; 69: 356-364.

Other publications:

6. Saxena S, **Samala G**, Sridevi JP, Devi PB, Yogeeswari P, Sriram D. "Design and development of novel *Mycobacterium tuberculosis* L-alanine dehydrogenase inhibitors". *European Journal of Medicinal Chemistry* 2015; 92: 401-414.

7. Devi PB, **Samala G**, Sridevi JP, Saxena S, Alvala M, Salina EG, Sriram D, Yogeeswari P. "Structure-Guided Design of Thiazolidine Derivatives as *Mycobacterium tuberculosis* Pantothenate Synthetase inhibitors". *Chem Med Chem* 2014; 9: 2538-2547.

8. Nallangi R, **Samala G**, Sridevi JP, Yogeeswari P, Sriram D. "Development of antimycobacterial tetrahydrothieno[2,3-*c*]pyridine-3-carboxamides and hexahydro cycloocta[*b*]thiophene-3-carboxamides: Molecular modification from known antimycobacterial lead". *European Journal of Medicinal Chemistry* 2014; 76: 110-117.

9. Yogeeswari P, Sharma M, **Samala G**, Gangadhar M, Karthick S, Mallipeddi S, Semwal A, Sriram D. "Discovery of novel tetrahydro-pyrazolo [4,3-*c*] pyridines for the treatment of neuropathic pain: synthesis and neuropharmacology". *European Journal of Medicinal Chemistry* 2013; 66: 211-220.

10. Jeankumar VU, Chandran M, **Samala G**, Alvala M, Koushik PV, Yogeeswari P, Salina EG, Sriram D. "Development of 5-nitrothiazole derivatives: identification of leads against both replicative and latent *Mycobacterium tuberculosis*". *Bioorganic & Medicinal Chemistry Letters* 2012; 22: 7414-7417.

11. Saxena S, **Samala G**, Renuka J, Sridevi JP, Yogeeswari P, Sriram D. "Development of 2-amino-5-phenylthiophene-3-carboxamide derivatives as novel and potent inhibitors of *Mycobacterium tuberculosis* DNA GyrB domain". *Bioorganic & Medicinal Chemistry.* (Under Review)

Papers presented at Conferences

1. 13th Eurasia Conference on Chemical Sciences – 14th to 18th December 2014, Indian Institute of Sciences, Bangalore, India.

2. 15th Tetrahedron Symposium – Asia Edition: 28th to 31st October 2014, Singapore Expo, International convention and exhibition centre, Singapore.

3. International Conference on Drugs for the Future: Infectious Diseases – 26th to 27th March 2014, National Institute of Pharmaceutical Education and Research, Hyderabad.

4. CTPS 2011 (Current Trends in Pharmaceutical Sciences)-The 1st Annual National Symposium of BITS Pilani, Hyderabad Campus – 11th November 2011.

BIOGRAPHY OF Professor D. SRIRAM

D. Sriram is presently working in the capacity of Professor at Department of Pharmacy, Birla Institute of Technology & Science, Pilani, Hyderabad campus. He received his Ph.D. in 2000 from Banaras Hindu University (IIT-Varanasi), Varanasi. He has been involved in teaching and research for last 15 years. He has 250 peer-reviewed research publications to his credit. He has collaborations with various national and international organizations such as Karolinska Institute, Sweden; Institute of Science and Technology for Tuberculosis, Porto Allegre, Brazil; National Institute of Immunology, New Delhi etc. He was awarded the Young Pharmacy Teacher of the year award of 2006 by the Association of Pharmacy Teachers of India. He received ICMR Centenary year award in 2011. He has guided 8 Ph.D. students and 12 students are pursuing Ph.D. currently. His research is funded by agencies like the UGC, CSIR, ICMR, DBT and DST.

BIOGRAPHY OF S. GANESH

Mr. S. Ganesh completed his Bachelor of Science and Master of Science (Organic chemistry) from Osmania University Hyderabad, Telangana. He has about 3 years of industrial experience. He has worked at Albany Molecular Research Inc. (AMRI) – Hyderabad Research Centre as Research Scientist. He has been appointed as DST Junior Research Fellow from Apr 2011 – June 2012, CSIR Junior Research Fellow from July 2012 – June 2014 and CSIR Senior Research Fellow from July 2014 – Mar 2015 at Birla Institute of Technology & Science, Pilani, Hyderabad campus under the supervision of Prof. D. Sriram. He has published ten scientific papers in well-renowned international journals and also presented papers at national and international conferences.