Design, Synthesis and Biological Evaluation of Novel *Mycobacterium tuberculosis* PknB Inhibitors

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CERTIFICATE

This is to certify that the thesis entitled "**Design, Synthesis and Biological Evaluation of Novel *Mycobacterium tuberculosis* PknB Inhibitors**" and submitted by **N. RADHIKA** ID No. **2012PHXF515H** for award of Ph.D. of the Institute embodies original work done by her 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. The majority of TB cases were found in the age group of 20-40 and it is the one of the top five killers of women worldwide. Although the existing drugs possess immense value in controlling drug sensitive TB, but they have several shortcomings such as lengthy therapy, development of drug resistance and poor economic value. The TB bacterium has developed resistance to all the approved drugs which underlines the need of novel drugs which act through different mechanism of action.

M. tuberculosis PknB is an essential mycobacterial enzyme necessary for bacterial cell survival. *M. tuberculosis* PknB is a novel and attractive target for the anti-tubercular drug development which is not much explored in literature. In this present study we focused on achieving potential anti-tubercular compounds by adequate design, synthesis, anti-mycobacterial and PknB inhibition studies based on reported promising anti-tubercular agents.

In the present work, seven libraries of compounds (total 250 compounds) were designed and synthesized utilizing economically cheaper starting materials and bulk scale feasible methods. Compound **TZ_09** (5-(4-methoxybenzylidene)-2-(phenylimino)thiazolidin-4-one) and compound **BO_45** (5-nitro-*N*-(3-(5-nitrobenzo[*d*]oxazol-2-yl)phenyl)furan-2-carboxamide) emerged as the most active in inhibiting *M. tuberculosis* PknB enzyme with IC_{50} of 0.52 µM and 0.63 µM respectively.

The safety profile of synthesized libraries was evaluated by conducting *in vitro* cytotoxicity studies against RAW 264.7 cell line (mouse leukemic monocyte macrophage) by MTT assay and the anti-tuberbuar screening against H37Rv strain by MABA assay.

The excellent *M. tuberculosis* PknB inhibitory activity of few molecules, their *in vitro M. tuberculosis* potency and no cytotoxicity make us to anticipate their emergence as valid leads for further chemical optimization as novel potential anti-tubercular agents.

List of Tables

Table No.	Description	Page No.
Table 1.1	WHO recommended regimen for drug-susceptible TB	5
Table 1.2	Order of the drugs to be chosen on sensitivities for MDR-TB treatment	7
Table 2.1	Group name and mechanism of action of first and second line anti-TB agents	19
Table 2.2	Phases in clinical trials	21
Table 2.3	New anti-TB drugs, stage of development and their targets	30
Table 5.1	Physiochemical properties of the synthesized compounds **TD_04** – **TD_39**	74
Table 5.2	*In vitro* biological evaluation of synthesized compounds **TD_04** – **TD_39**	84
Table 5.3	Physiochemical properties of the synthesized compounds **PT_07 – PT_26** and **OT_04 – OT_13**	95
Table 5.4	Biological activities of synthesized compounds **PT_07 – PT_26** and **OT_04 – OT_13**	103
Table 5.5	Physiochemical properties of the synthesized compounds **TZ_04** – **TZ_56**	114
Table 5.6	*In vitro* biological evaluation of synthesized derivatives **TZ_04** – **TZ_56**	128
Table 5.7	Physiochemical properties of the synthesized compounds **BO_04 – BO_47**	137
Table 5.8	*In vitro* biological evaluation of synthesized compounds **BO_04** – **BO_47**	149
Table 5.9	Physiochemical properties of the synthesized compounds **BT_04 – BT_36**	157
Table 5.10	*In vitro* biological evaluation of synthesized compounds **BT_04** – **BT_36**	167
Table 5.11	Physiochemical properties of the synthesized compounds **TA_05 – TA_19**	176

Table No.	Description	Page No.
Table 5.12	*In vitro* biological evaluation of synthesized compounds **TA_05** – **TA_19**	181
Table 5.13	Physiochemical properties of the synthesized compounds **IS_04** – **IS_42**	187
Table 5.14	*In vitro* biological evaluation of synthesized compounds **IS_04** – **IS_42**	198

Table of contents

Contents	Page No.
Certificate	*i*
Acknowledgements	*ii*
Abstract	*iv*
List of Tables	*v*
List of Figures	*vii*
Abbreviations	*ix*
Chapter 1 – Introduction	1-10
1.1 Global burden of TB	2
1.2 Etiology of TB	3
1.3 Risk of developing TB disease over a life time	4
1.4 Drug-susceptible TB/Drug-sensitive TB	5
1.5 Drug Resistance in TB	5
1.5.1 Primary and secondary drug resistant TB	6
1.5.2 Multidrug-resistant TB (MDR-TB)	6
1.5.3 Extensively drug-resistant TB (XDR-TB)	9
1.5.4 RR-TB, XXDR-TB and TDR-TB	10
Chapter 2 - Literature review	11-57
2.1 Classification of current anti-TB drugs	11
2.1.1 First line anti-TB drugs	11
2.1.2 Second line injectable anti-TB drugs	14
2.1.3 Second line oral bacteriostatic anti-TB drugs	17
2.2 Anti-TB drug discovery-pipeline	19
2.2.1 Current drugs in phase III clinical trials	21
2.2.1.1 Bedaquiline	21
2.2.1.2 Delamanid	23
2.2.1.3 Rifapentine	24
2.2.1.4 Pretomanid-Moxifloxacin-Pyrazinamide regimen	24

Contents	Page No.
2.2.2 Current drugs in phase II clinical trials	27
2.2.2.1 SQ 109	27
2.2.2.2 Oxazolidinones (Sutezolid and AZD 5847)	28
2.2.3 Current drugs in phase I clinical trials	29
2.2.3.1 TBA-354	29
2.3 Promising heterocyclic classes of compounds as anti-TB drugs	30
2.3.1 Literature survey on 1,3,4-thiadiazoles as anti-tubercular agents	31
2.3.2 Literature survey on 2-Aminothiophene-3-carbaxamide derivatives as anti-tubercular agents	34
2.3.3 Literature survey on thiazolidine-4-one derivatives as anti-tubercular agents	36
2.3.4 Literature survey on benzoxazoles as anti-tubercular agents	39
2.3.5 Literature survey on benzothiazoles as anti-tubercular agents	42
2.3.6 Literature survey on 2-aminothiazoles as anti-tubercular agents	48
2.3.7 Literature survey on isatin/1*H*-indole-2,3-diones as anti-tubercular agents	52
2.4 "*M. tuberculosis* PknB" – A novel and interesting target for anti-TB drug discovery	55
Chapter 3 - Objectives and Plan of work	58-59
3.1 Objectives	58
3.2 Plan of work	58
3.2.1 Designing of novel *M. tuberculosis* PknB inhibitors	58
3.2.2 Synthesis and characterization of designed molecules	59
3.2.3 *In vitro* anti-mycobacterial activity against *M. tuberculosis* H37Rv	59
3.2.4 Enzymatic evaluation of synthesized compounds	59
3.2.5 *In vitro* cytotoxicity screening	59
Chapter 4 - Materials and Methods	60-67
4.1 Designing of the molecules	60
4.2 Chemistry and methodology	60

Contents	Page No.
4.2.1 Synthesis of the designed molecules	61
4.3 Biological screening	65
4.3.1 *In vitro* anti-mycobacterial screening	65
4.3.2 Cytotoxicity	66
4.3.3 *M. tuberculosis* PknB assay	66
Chapter 5 - Results and Discussion	68-200
5.1 Development of 5-heteroarylsubstituted 2-amino 1,3,4-thiadiazoles as novel *M. tuberculosis* PknB inhibitors	68
5.1.1 Design of the molecules	68
5.1.2 Experimental procedures utilized for the synthesis of TD_04 – TD_39	69
5.1.3 Characterization of the synthesized molecules	75
5.1.4 *In vitro M. tuberculosis* screening, *M. tuberculosis* PknB enzyme inhibition assay and cytotoxicity studies of the synthesized molecules	83
5.1.5 SAR and discussion	85
5.1.6 Highlights of the study	87
5.2 Development of tetrahydrothieno[2,3-*c*]pyridine-3-carboxamides and from hexahydrocycloocta[*b*]thiophene-3-carboxamides as novel antimycobacterial known antimycobacterial lead	88
5.2.1 Design of lead molecule	88
5.2.2 Experimental procedures utilized for the synthesis of PT_07 – PT_26 and OT_04 – OT_13	89
5.2.3 Characterization of the synthesized molecules	96
5.2.4 *In vitro M. tuberculosis* screening and cytotoxicity studies of the synthesized molecules	103
5.2.5 SAR and discussion	105
5.2.6 Highlights of the study	107
5.3 Design and synthesis of 2-iminothiazolidin-4-one derivatives as novel *M.tuberculosis* PknB agents	108

Contents	Page No.
5.3.1 Design of the molecules	108
5.3.2 Experimental procedures utilized for the synthesis of TZ_04 – TZ_56	109
5.3.3 Characterization of the synthesized molecules	116
5.3.4 *In vitro M. tuberculosis* screening, *M. tuberculosis* PknB enzyme inhibition assay and cytotoxicity studies of the synthesized molecules	128
5.3.5 SAR and discussion	131
5.3.6 Highlights of the study	132
5.4 Design and synthesis of 3-(benzo[*d*]oxazol-2-yl)aniline derivatives as novel *M. tuberculosis* PknB agents	133
5.4.1 Design of the molecules	133
5.4.2 Experimental procedures utilized for the synthesis of BO_04 – BO_47	134
5.4.3 Characterization of the synthesized molecules	139
5.4.4 *In vitro M. tuberculosis* screening, *M. tuberculosis* PknB enzyme inhibition assay and cytotoxicity studies of the synthesized molecules	149
5.4.5 SAR and Discussion	151
5.4.6 Highlights of the study	152
5.5 Development of 2-(3-aminophenyl)-benzothiazole derivatives as potential *M. tuberculosis* PknB inhibitors	154
5.5.1 Design of the molecules	154
5.5.2 Experimental procedures utilized for the synthesis of BT_04 – BT_36	155
5.5.3 Characterization of the synthesized molecules	159
5.5.4 *In vitro M. tuberculosis* screening, *M. tuberculosis* PknB enzyme inhibition assay and cytotoxicity studies of the synthesized molecules	167
5.5.5 SAR and Discussion	169
5.5.6 Highlights of the study	170

Contents	Page No.
5.6 Study of potentiality of *N,N*-di-substituted-2-aminothiazole derivatives in inhibition of *M. tuberculosis* PknB enzyme	171
5.6.1 Design of the molecules	171
5.6.2 Experimental procedures utilized for the synthesis of TA_05 – TA_19	172
5.6.3 Characterization of the synthesized molecules	177
5.6.4 *In vitro M. tuberculosis* screening, *M. tuberculosis* PknB enzyme inhibition assay and cytotoxicity studies of the synthesized molecules	181
5.6.5 SAR and discussion	182
5.6.6 Highlights of the study	183
5.7 Design, synthesis and biological evaluation of 1,2,4-triazino[5,6-*b*]indole derivatives as potent *M. tuberculosis* PknB inhibitors	184
5.7.1 Design of the molecules	184
5.7.2 Experimental procedures utilized for the synthesis of IS_04 – IS_42	185
5.7.3 Characterization of the synthesized molecules	188
5.7.4 *In vitro M. tuberculosis* screening, *M. tuberculosis* PknB enzyme inhibition assay and cytotoxicity studies of the synthesized molecules	198
5.7.5 SAR and discussion	200
5.7.6 Highlights of the study	201
Chapter 6 - Summary and Conclusion	202-204
Future perspectives	205
References	206-228
Appendix	229-232
List of publications and presentations	229
Biography of the supervisor	231
Biography of the candidate	232

List of Figures

Figure No.	Description	Page No.
Figure 1.1	Worldwide majority of TB cases	3
Figure 1.2	The development and spread of drug-sensitive and MDR-TB	7
Figure 2.1	New TB drugs in development pipeline	20
Figure 2.2	Available drugs containing 1,3,4-thiadiazole ring moiety	31
Figure 2.3	2-Aminothiophene derivatives in clinical (1 & 2) or in preclinical development (3-6)	34
Figure 2.4	Marketed drugs containing thiazolidin-4-ones nucleus	36
Figure 2.5	Marketed drugs of benzothiazole	43
Figure 2.6	Drugs containing aminothizole nucleus	49
Figure 2.7	potent aminothiazole derivatives against *M. tuberculosis*	51
Figure 2.8	Drugs containing isatin/indole-2,3-dione	53
Figure 4.1	Synthetic protocol utilized for the synthesis of compounds **TD_04 - TD_39**	61
Figure 4.2	Synthetic protocol utilized for the synthesis of compounds **PT_07 - PT_26** and **OT_04 – OT_13**	62
Figure 4.3	Synthetic protocol utilized for the synthesis of compounds **TZ_04 - TZ_56**	63
Figure 4.4	Synthetic protocol utilized for the synthesis of compounds **BO_04 – BO_47**	63
Figure 4.5	Synthetic protocol utilized for the synthesis of compounds **BT_04 – BT_36**	64
Figure 4.6	Synthetic protocol utilized for the synthesis of compounds **TA_05 – TA_19**	64
Figure 4.7	Synthetic protocol utilized for the synthesis of compounds **IS_04 – IS_42**	65
Figure 5.1	Design of lead molecule by molecular hybridization approach	69

Figure No.	Description	Page No.
Figure 5.2	Mechanism of conversion of compound **TD_02a-b** to **TD_03a-b**	70
Figure 5.3	Chemical structure and biological activity of the most active compound **TD_09**	87
Figure 5.4	Structural modification of lead compound	89
Figure 5.5	Structure and biological activity of most active compound **OT_09**	107
Figure 5.6	Reported 4-thiazolidionones as anti-tubercular compounds	108
Figure 5.7	Designing of lead molecule based on previous work	109
Figure 5.8	Mechanism of conversion of compound **TZ_02a-e** to **TZ_03a-e**	110
Figure 5.9	Structure and biological activity of most active compound **TZ_09**	132
Figure 5.10	Lead molecule (**8a**) for the synthesis of compounds **BO_04 – BO_47**	134
Figure 5.11	Mechanism of conversion of compound **BO_1a-d** to **BO_3a-d**	135
Figure 5.12	Structure and biological activity of most active compound **BO_45**	153
Figure 5.13	Structure of lead molecule for the generation of compounds **BT_04 – BT_36**	154
Figure 5.14	Structure and biological activity of most active compound **BT_12**	170
Figure 5.15	Design of molecules by molecular modification and H-bond switching approach	172
Figure 5.16	Mechanism of conversion of compound **TA_02a-e** to **TZ_04a-e**	173
Figure 5.17	Chemical structure and biological activity of the most active compound **TA_06**	183
Figure 5.18	Structure of lead molecule for the generation of compounds **IS_04** – **IS_42**	184
Figure 5.19	Chemical structure and biological activity of the most active compound **IS_11**	201

List of Abbreviations

µg	:	Microgram
µM	:	Micromolar
^{13}C NMR	:	Carbon Nuclear Magnetic Resonance
^{1}H NMR	:	Proton Nuclear Magnetic Resonance
ATP	:	Adenosine Triphosphate
CAP	:	Capreomycin
$CDCl_3$	:	Chloroform deuterated
d	:	Doublet
DCM	:	Dichloromethane
DMF	:	*N*,*N*-Dimethylformamide
DMSO-d_6	:	Dimethyl sulphoxide deuterated
DNA	:	Deoxyribonucleic acid
DOTS	:	Directly Observed Treatment, Short course
EDCI	:	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
EMB	:	Ethambutol
EU	:	European Union
FDA	:	Food and Drug Administration
HIV	:	Human Immuno Deficiency Virus
HOBt	:	Hydroxybenzotriazole
IC_{50}	:	Half Maximal Inhibitory Concentration
INH	:	Isoniazid
J	:	Coupling constant
KM	:	Kanamycin
LCMS	:	Liquid chromatography–Mass Spectrometry
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
nM	:	Nanomolar
NMM	:	N-methylmorpholine
ppm	:	Parts per million
PZA	:	Pyrazinamide
RIF	:	Rifampicin
RNA	:	Ribonucleic acid
RNTCP		Revised National Tuberculosis Control Program
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
TMS	:	Tetramethylsilane
WHO	:	World Health Organisation
XDR-TB	:	Extensively Drug-Resistant *Mycobacterium tuberculosis*
δ	:	Chemical shift

Introduction

Chapter 1

Tuberculosis (TB) is one of the most influential diseases in the history of mankind due to its disastrous effect on health and its high mortality rate across the world. Although tuberculosis has been studied for centuries, it is still accountable for more human deaths than any other single contagious disease, and World Health Organization (WHO) stated TB as the global public health emergency in 1993 [Moller M., *et al*., 2009]. It has been figured that one tierce of the world's population is contaminated with *Mycobacterium tuberculosis* (*M. tuberculosis*) with documented cases in nearly every country [Barry CE., *et al*., 2009]. WHO calculated a prevalence of 9.6 million TB cases and 1.5 million deaths in 2014 making TB the second leading cause of mortality due to infectious disease after HIV worldwide [WHO Global Tuberculosis report - 2015].

The proportion of people who become sick with TB each year is stable or diminishing worldwide but, owing to the population growth, the absolute number of new cases arising each year is still increasing, posing a continued health and financial burden in various parts of the world especially in Asia and Africa [Sharma S., *et al*., 2011]. Despite the fact that many other diseases like cholera, plague and smallpox have destructed the lives of lakhs of people, they demised in period of short time, but TB has been ever present [Sharma SK., *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 TM., *et al*., 2006].

TB is the top five causes of death for women approximately one million young (aged 15-44) women per year are victimized with tuberculosis in the developing world [Tripathi RP., *et al*., 2005]. In high-prevalence countries, most of TB patients are in the age of 20- 40, resulting in the enormous socioeconomic loss as this is the most fruitful generation [Lange C, Mori T., *et al*., 2010]. 95% of new TB cases occur in the developing countries every year [Farmer P., *et al*., 1998; Tripathi RP., *et al*., 2005]. India having 2% of the land area and 15% of the total population of the world accounts high 30% of the TB burden. In India, TB kills 14 times more people than all tropical disease. Approximately 50% of India's population is reported to have positive tuberculin test [Dhingra VK., *et al*., 2002], and one person dies from TB for every

minute [Singh MM., *et al*., 2003]. It was observed that low and middle income countries account for greater than 95% of TB deaths.

1.1. Global burden of TB

TB is caused by *M. tuberculosis*, an atavistic disease that has plagued human civilization since its emergence. Highly contagious via air transmission, *M. tuberculosis* persists in living conditions that are crowded and among the malnourished, thus becoming a disease of poverty and making the greatest impact in developing countries and centers of urban decay in the industrialized world. TB occurs in every part of the world. Despite the low decline in the incidence of TB in recent years, the global burden of TB is quite large [Ahmad S., *et al*., 2014]. Though the development and improvement of efficient antibiotics, socioeconomic circumstances in the 19^{th} and 20^{th} century, helped reduce mortality rates in developed countries but TB continues to be a major scourge specially in the developing world [Moller M., *et al*., 2010]. The greatest burden of TB incidence and fatality rate noticed in the age group of 15-49 [Van Soolingen D., *et al*., 2001]. Therefore the economic cost of TB, in terms of lost production, is considerable [Murray CJ., *et al*., 1990].

In 2014, an estimated 9.6 million people developed TB which includes 5.4 million men, 3.2 million women and 1.0 million children. Globally about 1.5 million deaths (among them 0.4 million HIV-positive and 1.1 million HIV-negative people). Although most (around 60%) TB cases and deaths take place among men, the burden of disease among women is also high. In 2014 an approximated 480 000 women, 890 000 men and140 000 children died because of TB [WHO Global Tuberculosis report-2015].

Globally in 2015 the rate of TB mortality was brought down by 47% and rate of prevalence reduced by 42% than in 1990. Between 2000 and 2014, 35 million HIV-negative people lives were saved by the treatment alone. TB treatment and antiretroviral therapy saved 8.0 million HIV-positive people additionally. The dispersion of disease is not consistent across the globe [Sharma S., *et al*., 2011]. In 2014, of the total estimated TB cases, more than half were in South East Asia (58%), African Region (28%) and smaller proportions in Eastern Mediterranean Region (8%), the European Region (3%), and Region of Americas (3%).

India, Indonesia and China possess the highest number of TB cases account for 23%, 10% and 10% of the total global cases respectively (**Figure 1.1**). In 2014, 22 high burden countries explicate 83% of all approximated incident cases worldwide. The six countries having highest number of incident cases in 2014 were India, China, Nigeria, Pakistan, Indonesia and South Africa. Altogether, 32% of TB cases were guessed to be coinfected with HIV in African Region which explicate 74% of TB cases among HIV-positive people globally. In Parts of South Africa, greater than 50% of TB cases were co-infected with HIV.

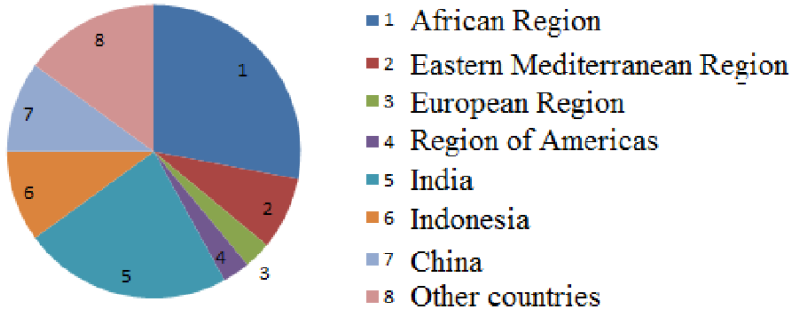


Figure 1.1: Worldwide majority of TB cases [WHO Global Tuberculosis report - 2015]

1.2. Etiology of TB

M. tuberculosis is the principle TB etiological agent in humans; it is a weak Gram-positive rod shaped bacterium. The causative agent first isolated by the German scientist professor Robert Koch in 1882, is a fungus like bacterium called *M. tuberculosis* or tubercle bacillus [Lange C., *et al*., 2010], *M. tuberculosis* exhibits many unique clinical properties such as it divides for every 16 to 20 hours, an extremely slow rate than other bacteria [Cox RA., *et al*., 2004] due to the presence of high lipid content. *M. tuberculosis* has cell wall but lacks a phospholipid outer membrane.

The chemical composition of cell wall includes peptidoglycans and complex lipids, especially mycolic acids, which are significant determinants of virulence [Rivers EC., *et al*., 2008; Barrera L., *et al*., 2007; Godreuil S., *et al*., 2007]. The unique structure of the cell of *M. tuberuclosis* permits the bacteria to lie dormant for many years a latent infection, particularly as it can grow readily inside the macrophages, hiding it from the host's immune system. Its impermeability for nutrients causes the slow growth of these organisms [Rivers EC., *et al*., 2008]. *M.* tuberculosis

can withstand weak disinfectants and survive in dry state for weeks. This is leading to the major obstacle to the global control of TB since the existing drugs target only the actively growing bacterial cell processes such as cell wall biogenesis and chromosome replication. Patients who carry latent infection are at risk of reactivation of the disease [Smith CV., *et al*., 2004].

1.3. Risk of developing TB disease over a life time

Practically the only source of *M. tuberculosis* that leads to spread infection is the patient with pulmonary TB. In most of exposure cases the immune system clears the bacteria, but when bacterial multiplication surpasses the immune response mounted to control bacterial growth results in the reactivation of latent TB infection (LTBI) to active TB [Barry CE., *et al*., 2009; Zuniga J., *et al*., 2012]. About 5-10% of LTBI are at risk to develop active TB. [Friedon T., *et al*., 2003].

The risk of developing TB is much higher in certain high risk groups like HIV-positive people has a greater risk of 50-110 times than a person with no known risk factors [Menzies D., *et al*., 2014] and the probability of developing primary disease after infection is also much higher in immune deficient individuals. Young children (less than five years), who are close contact of people with pulmonary TB, those with silicosis, diabetes mellitus, and severe malnutrition are at moderately high risk. Prisioners, health care workers are also at greater risk of acquiring LTBI (and active TB), than the normal personals. The risk of developing active TB can be decreased by treating the latently infected poeple.

Although the same drugs are used for the treatment of TB and LTBI but the principles of treatment are different. People with active TB require a combination of four drugs for long duration and treatment with a single drug is not advocated to treat active TB due to the risk of developing resistance. In contrast, people with LTBI can be treated in shorter durations with standard therapy of single or combination of two or more drugs [Sharma SK., *et al*., 2013]. Without treatment, around 5% of persons infected with *M. tuberculosis* will develop disease in the first two years, and another 5% will develop diseases after sometime in life. Thus no treatment, about 10% of persons infected with *M. tuberculosis* with normal immune systems acquire TB disease at some point in their lives. A person with active TB can infect on an average of 10 to 15 people every year.

1.4. Drug-susceptible TB/ Drug-sensitive TB

Newly diagnosed patients with TB are known to have drug-susceptible disease. The current standard regimen for drug-susceptible TB recommended by the WHO includes an intensive phase of 2 months of a four drug combination "isoniazid (INH), rifampin (RIF), pyrazinamide (PZA) and ethambutol (EMB), followed by a continuation phase of 4 months of INH and RIF [Wong EB., *et al*., 2013]. All these four are orally administered drugs, their daily dose and adverse effects were presented in **Table 1.1**. This regimen is often highly effective if it is used correctly but it is also lengthy the patient has to take it for six months, complex and significantly toxic. These problems with standard anti-tuberculat treament lead to drug resistance and emerged as a deadly problem for the past 20 years.

To get over this situation, the WHO has promoted a program known as "directly observed treatment-short course (DOTS)" [Pieroni M., *et al*., 2014; Chan B., *et al*., 2013]. In this type of treatment there is a direct observation by trained personnel on patient 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 1.1: WHO recommended regimen for drug-susceptible TB [Prasad R., *et al*., 2013; Chan ED., *et al*., 2012]

Drug	Daily dose	Adverse effects
INH	5 mg/kg (maximum of daily dose 300 mg)	Hepatitis, peripheral neuropathy, lupus-like syndrome
RIF	10 mg/kg (maximum of daily dose 600 mg)	Orange discoloration of secretions, hepatitis, gastrointestinal upset, fever
PZA	25 mg/kg	Hepatitis, arthritis, hyperuricemia
EMB	15 mg/kg	Optic neuritis

1.5. Drug Resistance in TB

Drug-resistant is not a recent phenomenon. *M. tuberculosis* strains that were resistant to streptomycin seemed soon after the introduction of the drug for the treatment of TB in 1944 [Gupta A., *et al*., 2015]. Genetic resistance to an antiTB drug is because of spontaneous chromosomal mutations at a low frequency (10^{-6} to 10^{-8}) of mycobacterial replications,

amplification of genetic mutations due to human errors result in arisinng clinically drug resistant tuberculosis [Zhang Y., *et al*., 2009; Yew WW., *et al*., 2008]. However several factors including inadequate treatment, patient noncompliance and the non-availability of drugs during this period have led to the failure to achieve a cure, making the emergence of resistance an event practically unavoidable in many resource limited countries [Jassal MS., *et al*., 2010].

This treatment will cure approximately 85-90% of drug sensitive TB patients if the treatment regimen is strictly followed [Cooper CB., *et al*., 2013]. Each of these drugs, however, exhibit side effects, such as gastrointestinal inflammation or pain, liver toxicity and neurological or behavioral manifestations [Dheda K., *et al*., 2012]. In many circumstances the requirement to remain on this regimen for a long period of time is wholly unacceptable and the patients give up their treatment before sterilization is fully complete. This then leads to the emergence of various forms of drug resistant tuberculosis including multidrug resistant, extremely drug resistant and recently diagnosed totally drug resistant TB [Migliori GB., *et al*., 2013]. Treatment for these forms of TB are even more limited, with much longer treatment periods 12-18 months or longer requiring second line, injectable agents which can only be administered in the hospital or clinic settings[Cooper CB., *et al*., 2013].

1.5.1. Primary and secondary drug resistant TB

Drug-resistant TB disease can develop in two different ways, called primary and secondary resistance [Conaty S., *et al*., 2004]. Primary resistance occurs in person to person transmission of drug resistant organisms. Acquired or secondary resistance, develops during TB therapy, either because the patient was treated with an inadequate regimen, did not take the prescribed regimen appropriately [Khalilzadeh S., *et al*., 2006], or because of other conditions such as drug- drug interactions that led to low serum levels (**Figure 1.2**).

1.5.2. Multidrug-resistant TB (MDR-TB)

According to WHO, over 400,000 people develop MDR-TB each year, and cases have been reported across all regions of the world [Lilienkampf A., *et al*., 2010]. MDR-TB is defined as resistance to atleast INH and RIF which are the most effective first line antiTB drugs [Onajole OK., *et al*., 2013; Poce G., *et al*., 2014; Babu GR., *et al*., 2012]. For MDR-TB, the idiom

'prevention is better than cure' meaningfully applies. Currently, it is preferentially clear that MDR-TB cannot be cured as efficiently as its swift creation through the erratic TB management [Yew WW., *et al*., 2008]. Difficult to cure MDR-TB usually 18-24 months of treatment is required with a regimen of 4-5 active drugs [Orenstein EW., *et al*., 2009]. The regimen should contain 4-5 active drugs including any first-line drugs for which resistance has not developed in addition to an injectable, a fluoroquinolone and other second-line drugs required to form the 4 or 5 drug regimen (**Table 1.2**). Depending upon the sensitivities 5 drugs has to be selected for the treatment of MDR-TB, once after establishing the isolated strain as multidrug resistant one [Bhunia SK., *et al*., 2015].

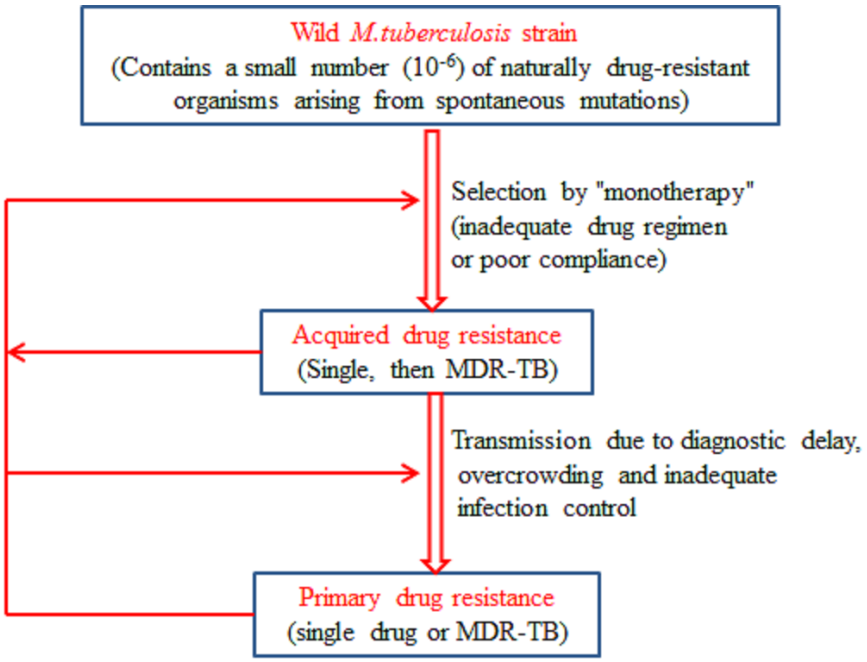


Figure 1.2: The development and spread of drug-sensitive and MDR-TB

Table 1.2: Order of the drugs to be chosen on sensitivities for MDR-TB treatment

Order	Drugs to be chosen
1	An amino glycoside (amikacin, kanamycin) or polypeptide antibiotic (capreomycin)
2	PZA
3	EMB
4	A fluoroquinolones: moxifloxacin is preferred
5	Rifabutin
6	CS

Order	Drugs to be chosen
7	ETH
8	*P*-amino salicylic acid
9	A macrolide (clarithromycin)
10	Linezolid
11	High-dose INH (if low-level resistance)
12	Interferon-γ
13	Thioridazine
14	Meropenem and clavulanic acid[

In order to strengthen and improve TB control activities, the Government of India launched the RNTCP. The standard treatment regimen under RNTCP program consists of 6 drugs (kanamycin,levofloxacin, ETH, PZA, EMB, and CS) during 6-9 months of intensive phase and 4 drugs (levofloxacin, ETH, EMB, and CS) during 18 months of the continuation phase for the treatment of MDR-TB cases, in this regimen PAS is added as a substitute for any of mycobactericidal drugs (levofloxacin, PZA, ETH or kanamycin) or any two other cidal drugs (EMB, CS). Treatment for MDR-TB is complicated by the lack of evidence based guidelines limitations of second-line drug in terms of limited efficacy, high costs and frequent toxicities and lengthy treatment durations [Falzon D., *et al*., 2013,]. Additional issue includes HIV coinfection, lack of facilities for resistance testing and isolation of cases and irregular access to second-line durgs. MDR-TB treatment using currently available second-line drugs may cure only 65% to 75% of patients. Hence the new drugs are needed to strengthen the existing drugs.

Globally an estimated 3.3% of new cases and 20% of previously treated cases have MDR-TB. In 2014 there were approximately 480 000 (ranges 360 000-600 000) new cases of MDR-TB, and approximately 190 000 (range 120 000-260 000) deaths from MDR-TB worldwide. Among patients with pulmonary TB who were notified in 2014 as estimated 300 000 (range 220 000-370 000) had MDR-TB. More than half of these patients were in India, China and Russian Federation [WHO Global Tuberculosis report 2015].

MDR-TB has emerged as a new challenge especially in developing countries. Because of insufficient fundng to support the treatment of MDR-TB with second line anti-TB drugs. The countries with greatest amount of funding available for MDR-TB in 2013 were India, China, Kazakistan, Russian Federation, South Africa and Ukraine [Mishra R., *et al*., 2015]. The cost of

just the drugs treating MDR-TB in patient can be 50 to 200 times higher than that of drugs susceptible TB patients that is leading to the global burden and health related crisis [Sterling TR., *et al*., 2003].

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

The term 'exclusively drug resistant' (XDR) TB was first proposed by the Centre for Disease Control and Prevention (CDC) and WHO in march 2006 the definition was revised in October 2006 [Jassal M., *et al*., 2009; Mitrzyk BM., *et al*., 2008]. XDR-TB is a form of MDR-TB. Mismanagement of MDR-TB with erratic use of second-line drugs may develop more severe drug resistance tuberculosis known as extensively drug resistance TB.

Extensively drug resistant TB is defined as the resistance to at least INH and RIF and any fluoroquinolone and one of the three second-line injectable drugs, kanamycin, amikacin or capreomycin [Jain A., *et al*., 2012; Caminero J., *et al*., 2006]. XDR-TB was first described in two Italian women who died after 422 and 625 days spent in hospital, and 60 months of treatment respectively [Dheda K., *et al*., 2014]. More than 400,000 cases of MDR-TB and 25,000 cases of XDR-TB cases emerge across the world every year as a result of poor management of drug sensitive as well as drug resistant TB [Prasad R., *et al*., 2007]. It represents a progression from the multidrug-resistant (MDR) TB. In the presence of such resistance, treatment options are severely restricted hence the mortality rates are extremely high. XDR-TB is emerging as a global public health threat [Prasad R., *et al*., 2013].

The treatment of XDR-TB is much expensive, difficult, and the outcomes much severe hence the identification of risk factors for XDR-TB are important to design effective TB control strategies [Flor de Lima B., *et al*., 2014]. The emergence of XDR-TB is mainly a manmade phenomenon being an indicator of failing TB programs. Pre-XDR-TB is another worrying international phenomenon. Pre-XDR-TB is defined as disease caused by a strain resistant to INH and RIF and either fluoroquinolone or second-line injectable drug but not both [Banerjee R., *et al*., 2008]. High rates of quinolone resistance and pre-XDR-TB have been reported from California, Philippines and China [Zhao M., *et al*., 2009]. The treatment of XDR-TB is costly and challenging. Ideally treatment should be done under the supervision of an expert doctor who is experienced with dealing such cases, since this treatment represents the patients last chance of a

cure [Prasad R., *et al*., 2013]. In 2014 extensively drug resistant TB (XDR-TB) has been reported by 105 countries, it is estimated that 9.7% of total 480,000 people with MDR-TB have developed XDR-TB globally [WHO Global Tuberculosis report 2015].

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

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 GB., *et al.,* 2007]. XXDR-TB can be defined as the isolates of *M. tuberculosis* resistant to all first-line and second-line available anti-TB drugs in addition to other drugs rifabutin, thiacetazone, clofazamine, dapsone and clarithromycin [Sharma SK., *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 all first line and second line licenced anti-tubercular drugs is defined as totally drug resistant TB (TDR-TB) [Sharma SK., *et al*., 2013]. The clinical isolates of TDR-TB were observed in Italy for the first time in 2003 [Migliori GB., *et al.*, 2007], next in Iran [Velayati AA., *et al.*, 2009] and now it has been reported from India that there were TB patients who do not respond to any of the anti- tubercular regimens [Udwadia Z., *et al.*, 2013; Udwadia ZF., *et al.*, 2012]. Though it is not yet prevalent, chances are bright to through a challenge of TDR-TB to researchers across the globe.

It is clear that, TB is a devastating disease and cannot be eradicated completely by the current available drugs, the need of novel drugs possessing novel mechanism of action are absolutely necessary. For the discovery of novel anti-TB agents, we have in depth reviewed the literature of current treatment regimens and their limitations.

Literature Review

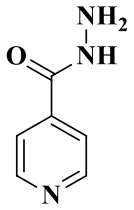
Chapter 2

2.1. Classification of current anti-TB drugs

The WHO approved current available anti-TB drugs can be classified as a) First line anti-TB drugs –INH, RIF, EMB and PZA. These four drugs constituent the standard drug regimen recommended by WHO for the start of treatment of TB. b) Second line injectable anti-TB drugs – Streptomycin, Kanamycin/Amikacin and Capreomycin. These drugs along with other second line drugs constitute a regimen for the treatment of MDR-TB. c) Second line fluoroquinolones – Ciprofloxacin, Gatifloxacin, Moxifloxacin, Ofloxacin, Levofloxacin and Sparfloxacin. d) Second line oral bacteriostatic anti-TB drugs – Ethionamide, its analogue Prothionamide, Cycloserine and *p*-Aminosalicylic acid (**Table 2.1**). The success rate using the above drugs in drug susceptible TB is very high (94-98%), but once the bacterium acquires the resistance then the above said drugs fail to combat the drug resistant bacterium. The potentiality of the above drugs, their MIC values, mechanism of action and their limitations will be discussed in following paragraphs.

2.1.1. First line anti-TB drugs

INH

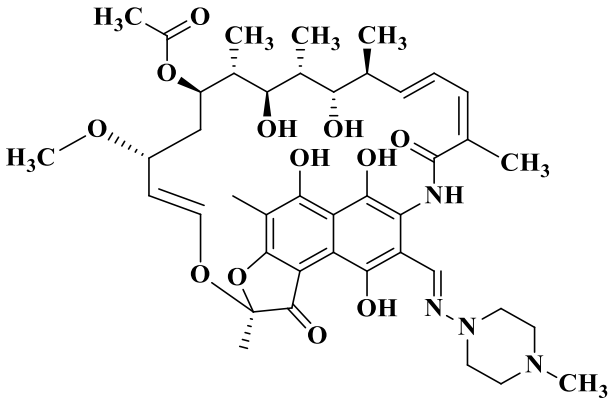


INH or pyridine-4-carboxylicacidhydrazide is also known as isonicotinylhydrazide a highly potent drug for the treatment of tuberculosis [Thapliyal N., *et al*., 2015]. INH was discovered in 1952 by three pharmaceutical companies BAYER (Leverkusen, Germany); Hoffmann LaRoche (Nutley, NJ, USA); and ER Squibb and Sons (Princeton, NJ, USA) it has cured many patients hence it was defined as a 'magic drug'. It is a widely used first line anti-tubercular orally active drug [Vilcheze C., *et al*., 2011] and has very low MICs 0.02-0.06ug/ml. INH is also available in tablets, syrup and injectable forms. INH is a prodrug that is activated by MTB catalase-

peroxidase katG enzyme to form an INH-NAD complex which inhibits the nicotinamide adenine dinucleotide (NADH)-dependent enoyl-ACP reductase (known as InhA) which is involved in the mycolic acid synthesis [Matsumoto M., *et al*., 2007; Murillo A., *et al*., 2007; Nguta JM., *et al*., 2015]. The inhibition of enoyl-ACP reductase causes an accumulation of long chain fatty acids and cell death and depletion of mycolic acids results in the bacterial killing [Nguta JM., *et al*., 2015].

The high level resistance to INH is due to mutations within the *KatG* gene, while mutations in the InhA regulatory region confer low level resistance to INH, mutations in other genes and induction of efflux pumps are also involved in INH resistance but have a minor role [Almeida Da Silva PE., *et al*., 2011]. Because of its wide use, resistance to INH has been seen more frequently among clinical isolates of *M. tuberculosis* infected patients. Resistance to INH reduces the ability of catalase peroxidase to activate the INH prodrug. Hepatitis, lupus-like syndrome, peripheral neuropathy and drug-drug interactions are major adverse reactions of INH [Vilcheze C., *et al*., 2011].

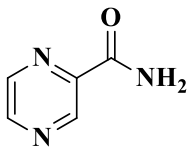
RIF



RIF an antibacterial drug belonging to the rifamycin group of semisynthetic bactericidal antibiotics isolated from Streptomyces mediterrani [Riccardi G., *et al*., 2014]. Introduction of RIF into clinical use, the treatment of active TB was reduced from 9-12 months to 6 months, and the treatment duration of latent TB was reduced from 9 months to 3 months [Riccardi G., *et al*., 2009]. RIFs are among the few drugs which kills the dormant (non-replicating) strains of MTB. RIF inhibits *M. tuberculosis* at concentrations ranging from 0.1-0.2 µg/ml. It is characterized by chromophoric napthohydroquinone group spanned by a long aliphatic bridge and are potent

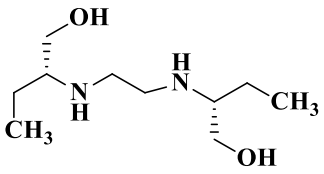
inhibitors of prokaryotic DNA-dependent RNA polymerase, an enzyme required for RNA synthesis. RIF acts by inhibiting DNA-dependent RNA polymerase in bacterial cells by binding its β-subunit to form a stable complex leading to suppression of transcription to RNA and subsequent translation to proteins. More specifically, the β-subunit of this complex enzyme is the site of action of the drug, although RIF binds only to the holoenzyme. Resistance to rifampin is a result of mutations in the *rpoB* gene, which encodes the β-subunit of RNA polymerase. To avoid rapid development of bacterial resistance RIF is recommended in combination with other first line agents either INH or EMB. However, combination of INH and RIF may increase risk of hepotoxicity.

PZA



PZA an analog of nicotinamide is a first-line drug of short course TB therapy prodrug that requires activation to its active form pyrazinoicacid, the active anti-TB molecule by the enzyme pyrazinamidase/nicotinamidase [Almeida Da Silva PE., *et al*., 2011]. Its activity is highly specific to *M. tuberculosis*. PZA is active against semi dormant bacilli not affected by any other drug and it is never used on its own it should be used in combination with other drugs such as INH and RIF to reduce the treatment to 6 months. PZA in combination with RIF is a preferred treatment for latent tuberculosis. The PZA resistance in *M. tuberculosis* is due to mutations in the PncA, which is responsible for the production of pyrazinamidase. Some pyazinoic acid esters also reported to possess good anti-tubercular activities [Tripathi RP., *et al*., 2005]. Hyperuricemia, gouty arthritis and rarely nephritis are major adverse reactions observed with PZA [Jureen P., *et al*., 2008].

EMB

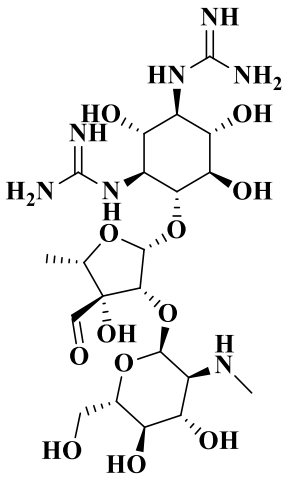


EMB (ethylene diamino-di-1-butanol) is a first line anti-tubercular drug, together with INH, RIF and PZA constitutes short course for the treatment of drug sensitive TB. It interferes with the biosynthesis of cell wall of *M. tuberculosis*. EMB inhibits the enzyme arabinoyltransferase which is needed for the synthesis of arabinogalactan, the inhibition of synthesis of arabinogalactan (the chief constituent of bacterial cell wall) leads to increased permeability of the bacterial cell wall. S, S (dextro) form of EMB is 600 times more active than R, R-isomer [Yendapally R., *et al*., 2008; Tripathi RP., *et al*., 2012].

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

2.1.2. Second line injectable anti-TB drugs

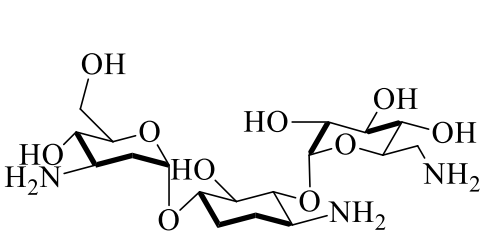
Streptomycin



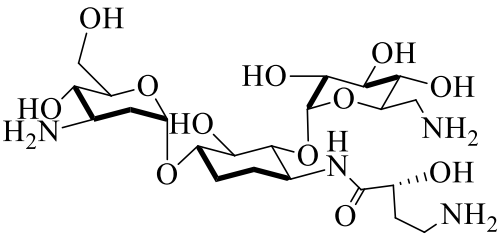
Streptomycin was first effective TB drug, derived from Streptomyces griseus by Albert Schatz and Selman Waksman in 1944. It is an aminoglycoside antibiotic made up of three components streptidine, streptose and N-methyl-L-glucosamine. Streptomycin kills actively growing tubercle

bacilli but it is inactive against non-growing or intracellular bacilli [Zhang Y., *et al*., 2009; Yew WW., *et al*., 2011]. 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 RP., *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. Resistance to streptomycin is due to mutations in S12 protein encoded by *rpsL* gene and 16S rRNA encoded by rrs gene. Streptomycin is not a popular choice of drug for treating tuberculosis because its toxic effects are mainly manifested on peripheral, central nervous system at higher dosage and hypersensitivity reaction.

Kanamycin/amikacin



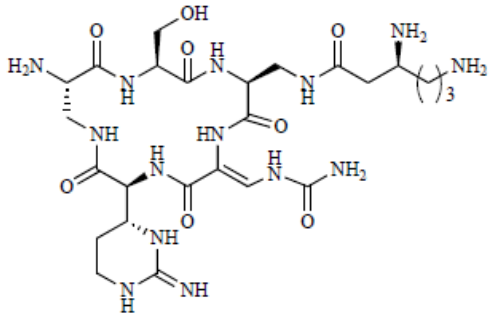
Kanamycin



Amikacin

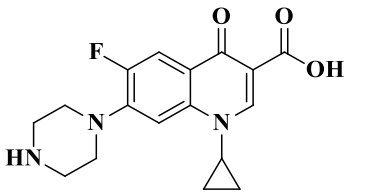
Kanamycin (KM) and its derivative amikacin are bactericidal injectable drugs belonging to the family of aminoglycoside and are inhibitors of protein synthesis. These drugs target the 30S subunit of ribosome [Alangaden GJ., *et al*., 1998; Suzuki Y., *et al*., 1998] in *M. tuberculosis* strain. KM modifies the ribosomal structure at 16S RNA there by inhibits protein synthesis [Maus CE., *et al*., 2005; Johansen SK., *et al*., 2006]. In *M. tuberculosis* resistant to kanamycin is due to the mutations at 16S RNA in the *rrs* gene encoding [Alangaden G.J., *et al*., 1998].

Capreomycin

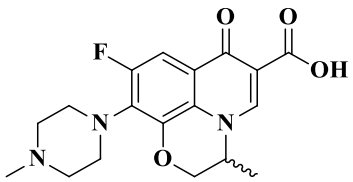


Capreomycin (CPM) is a second line injectable anti-tubercular agent belonging to the class of polypeptide antibiotics. It possesses the bactericidal activity and is a protein synthesis inhibitor. Mutations in the gene called tlyA encoding rRNA methyltransferase confer the resistance to CPM.

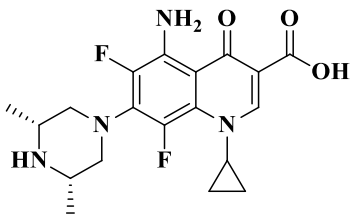
Second line Fluoroquinolones:



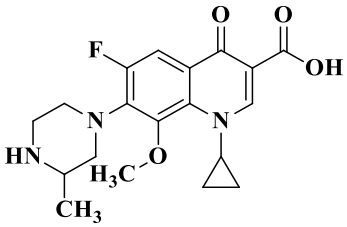
Ciprofloxacin



Ofloxacin (R/S)
S: Levofloxacin



Sparfloxacin



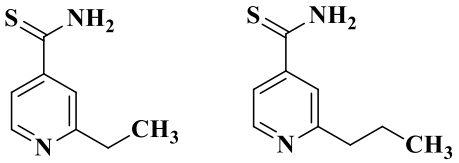
Gatifloxacin

Fluoroquinolones (FQs) are Synthetic derivatives of nalidixic acid. FQs have attained a prominent position in regimens or the treatment of drug-resistant tuberculosis [Pham D., *et al*., 2015]. FQs are the broad spectrum antibiotics and are effective against extracellular multiplying bacteria as well as intracellular latent bacteria hence FQs are useful in treating MDR-TB [Garcia-Tapia A., *et al*., 2004; Cole ST., *et al*., 2011]. Mechanism of action of FQs involves the inhibition of DNA gyrase (topoisomeraseII and topoisomeraseIV) [Takei M., *et al*., 2001; Kato

J., *et al*., 1990], which consists of two subunits A and B encoded by the gyrA and gyrB genes respectively, and cause cell death owing to DNA replication and repair. A conserved region, the quinolone-resistance-determining region (QRDR) of gyrA and gyrB, has found to be the most important area involved in the exhibition of FQ resistance in *M. tuberculosis* [Zhang Y., *et al*., 2009]. Mutations in gyrA and gyrB genes or changes in drug efflux pumps confer resistance to FQs in *M. tuberculosis* [Rieder HL., *et al*., 2009].

2.1.3. Second line oral bacteriostatic anti-TB drugs:

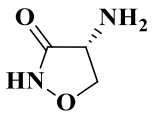
Ethionamide and prothionamide



Ethionamide **Prothionamide**

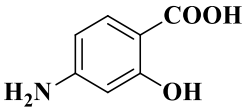
Ethionamide (2-ethylpyridine-4-carbothioamide or 2-ethylisothionicotinamide) is a derivative of isonicotinic acid structurally related to INH and is bacteriostatic in nature [Ahmad S., *et al*., 2014]. Ethionamide is used as a part of five drug regimen for the treatment of drug-resistant (MDR and XDR) tuberculosis, but it causes frequent toxic side effects such as anorexia, vomiting, dysgeusia, neurological reactions and reversible hepatitis. Like INH, Ethionamide is also a prodrug requiring activation by the monooxygenase EthA/EtaA [Vannelli TA., *et al*., 2002; DeBarber AE., *et al*., 2000]. EtaA/EthA is a flavin adenosine dinucleotide (FAD) containing enzyme that oxidizes Ethionamide to the corresponding S-oxide which is further oxidized to 2-ethyl-4-amidopyridine via the unstable sulfinicacid intermediate. Similar to INH, Ethionamide inhibits mycolic acid synthesis by binding to the enzyme InhA. Protionamide is almost similar to Ethionamide in structure and activity [Yew WW., *et al*., 2011]. Resistance to Ethionamide is because of mutations in genes *ethA* or *inhA* [Vilcheze C., *et al*., 2008; Baulard AR., *et al*., 2000].

Cycloserine



D-Cycloserine, is a structural analog of the amino acid D-alanine, chemically defined as D-4-amino-3-isooxazolidinone. It blocks the peptidoglycan biosynthesis by the inhibition of D-alanine racemase and D-alanine: D-alanine ligase [Strych U., *et al*., 2001]. Cycloserine has activity against a wide range of bacteria [Otten H., *et al.*, 1998], and inhibits *M. tuberculosis* with MIC of 5-20 μg/mL [David HL., *et al*., 1969]. It is well absorbed and distributed throughout the body following the oral administration. Cycloserine produces side effects in the central nervous system that can also generate psychotic states with suicidal tendencies and epileptic convulsion [Cacers NE., *et al*., 1997]. Resistance to cycloserine is due to overexpression of AlrA and Dd1 [Feng Z., *et al*., 2003].

***p*-Aminosalicylic acid (PAS)**



4-Aminosalicylic acid, commonly known as PAS was used as an oral TB therapy reported in 1946, although it was synthesized long before. It is a prodrug available in the form of sodium and calcium salt. *p*-aminosalicylic acid acts as an inhibitor of *M. tuberculosis* by impairing folate synthesis [Zheng J., *et al*., 2013]. This drug is less potent and more costly than the five first-line drugs but it is useful in the treatment of MDR-TB. The mode of action of this drug is unclear yet but it is suggested that it interferes with the salicylate-dependent biosynthesis of iron chelating mycobactins involved in iron assimilation. It is thought to act through NF-kB (nuclear factor-kappa B) inhibition and free radical scavenging.

Table 2.1: Group name and mechanism of action of first and second line anti-TB agents

Name of the group	Drug*	Mechanism of action
First line anti-TB drugs	**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	**Streptomycin**	Protein Synthesis Inhibitor
	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
	***para*-Aminosalicylic acid**	Inhibition of folic acid and Iron metabolism

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

Along with current available WHO approved anti-TB drugs, we have studied the literature of current anti-TB drug pipeline.

2.2. Anti-TB drug discovery-pipeline

The set of anti-TB agents currently in research and development is usually referred as TB drug-pipeline [Laurenzi M., *et al*., 2007]. After the decades of dormancy in the field of TB drug development with the effort of various groups recently a promising pipeline has generated, with

several promising drugs targeting various *M. tuberculosis* terminating sites are in different stages of development (**Figure 2.1**). There are few drug candidates in various stages of development are also in market (e.g., Deltyba, Sirturo) 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.

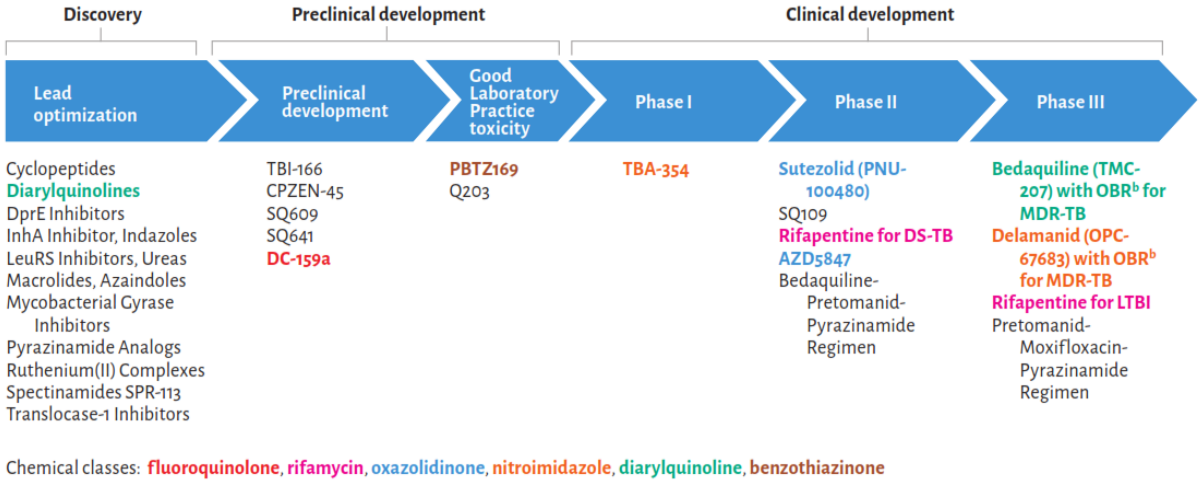


Figure 2.1: New TB drugs in development pipeline [WHO Global Tuberculosis report-2015]

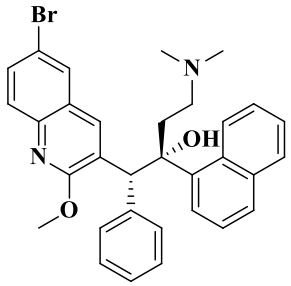
According to WHO Global Tuberculosis report, currently there are eight drugs in phase I, Phase II and Phase III clinical trials, and many new individual drug candidates and class of molecules are in lead optimization and preclinical development.

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
Phase IIb	Addresses risks and efficacy with large number of participants
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. Bedaquiline



Bedaquiline (also known as TMC207, R207910, Sirturo or 'J' compound) belonging to a new class of drugs diarylquinoline was discovered by Janssen pharmaceutica. During a mass

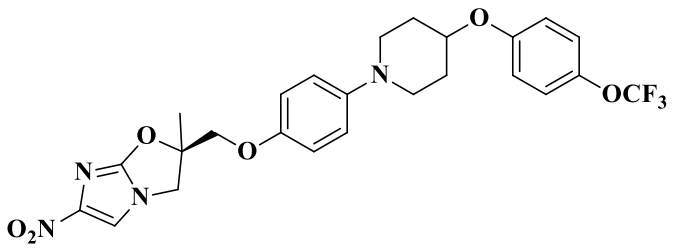
screening program of over 70,0000 compounds with inhibition against *Mycobacterium smegmatis* [Kakkar AK., *et al*., 2014]. Bedaquiline is the first drug approved by U.S. Food and Drug Administration (US-FDA) on 28th December 2012 after 40 years and is also the first agent approved specifically for the treatment of MDR-TB.

Bedaquiline has promising efficacy against both the drug-sensitive and drug-resistant TB. Since an attractive feature of bedaquiline is it has potent activity against both replicating and non-replicating (dormant) *M. tuberculosis* bacilli [Koul A., *et al*., 2008; Khan SR., *et al*., 2013; Koul A., *et al*., 2007] it may have potency to shorten the treatment duration of drug-susceptible pulmonary TB and MDR-TB [Ibekwe NN., *et al*., 2014; Balganesh T., *et al*., 2007]. Another remarkable feature of bedaquiline is it possesses long half-life, a desirable feature for inclusion of this drug in intermittent regimen [Veziris N., *et al*., 2009]. Though ATP-synthase down-regulation during dormancy, it showed more activity on dormant compared to actively replicating bacilli of *M. tuberculosis* [Koul A., *et al*., 2008].

TMC207 acts by inhibiting mycobacterium membrane-bound ATP synthase and decreases the intracellular ATP levels. Bedaquiline assumed to act by interfering with the proton translocation step necessary for ATP production. This unique mechanism of action offers great potential as there is little similarity between the mycobacterial and human proteins encoded by the atpE gene that codes for the c subunit of ATP synthase (The human mitochondrial ATP synthase is 20,000-fold less sensitive to bedaquiline than its mycobacterial counterpart), which has been identified as the specific target of TMC207 [Zumla A., *et al*., 2013]. Bedaquiline has high penetration capacity into the tissues such as lung and spleen [Poce G., *et al*., 2014].

Bedaquiline inhibits both actively replicating and non-replicating wild type and multidrug-resistant *M. tuberculosis*. Bedaquiline accumulates in tissues hence care should be taken to avert carry-over effects when measuring its activity [Lounis N., *et al*., 2008].The common side effects of bedaquiline are nausea, joint and chest pain, and headache [Singh S., *et al*., 2015].

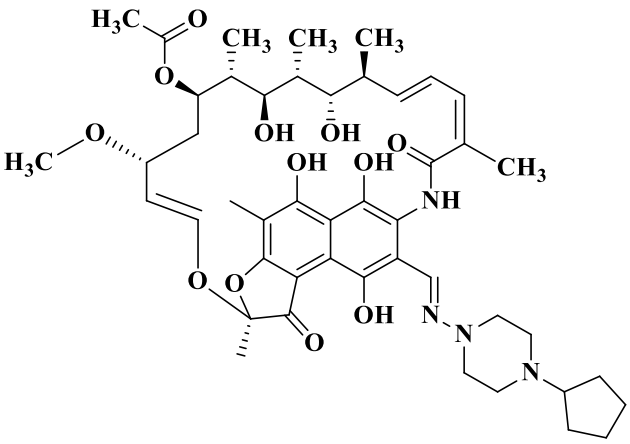
2.2.1.2. Delamanid



OPC-67683 also known as delamanid, is a nitrodihydro-imidaxooxazole derivative, is another metronidazole derivative. Delamanid has completed its phase II clinical studies and showed excellent *in vitro* anti-bacterial activity against both drug sensitive and drug resistant strains of *M. tuberculosis* with observed MIC of an extremely lower range of 0.006-0.024 µg/mL, and has been shown to be effective with acceptable toxicity when combined with other first-line anti-TB drugs in an MDR-TB regimen [Zhang Q., *et al*., 2013].

Delamanid is currently in Phase III clinical evaluation and is approved by EMA with the name of Deltyba, for the treatment of MDR-TB in the European Union (EU) on April 2014. Like INH and PZA, delamanid also a prodrug which is activated by the enzyme deazaflavin dependent nitroreductase (Rv3547), Like PA-824 delamanid needs nitroreduction by Ddn for activation [Matsumoto M., *et al*., 2006; Manjunatha UH., *et al*., 2006]. OPC-67683 acts by inhibiting mycolic acid biosynthesis and disrupts the mycobacterial cell wall [Wong EB., *et al*., 2013]. Due to their potential to cause arrhythmia, it is recommended that bedaquiline and Delbyta are used only in patients for whom other treatments are failed [Marriner GA., *et al*., 2011; Cohen J., *et al*., 2013].

2.2.1.3. Rifapentine



Rifapentine, is a durable semisynthetic cyclopentyl rigfamycin which was approved by FDA in 1998 at a dosage of 10 mg/kg (oral administration) once or twice weekly for the therapy of active and latent TB [Nguta JM., *et al*., 2015]. Phase II clinical trials are in progress, in which RIF is substituted by high dose rifapentine, to evaluate its efficacy to abridge the treatment duration of drug-susceptible TB. Also being evaluated in Phase III clinical trials for the treatment of LTBI. It demonstrates bactericidal activity to intracellular and extracellular TB bacilli and showed to be more active than RIF both *in vitro* and *in vivo* and displayed better pharmacokinetic profile in mice.

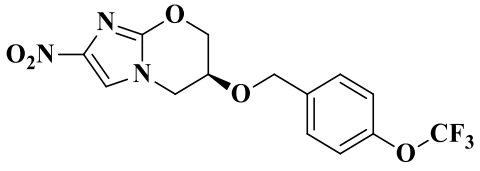
But Rifapentine exhibited cross-resistance with RIF but because of its good pharmacokinetic properties it could be useful in repressing treatment and reducing cost of therapy. Indeed the, half-life and protein binding (13-14 hours and 97%) of rifapentine is much longer than RIF (2-3 hours and 85%) [Chan JGY., *et al*., 2014]. The mechanism of action is similar to RIF it also acts by binding beta-subunit of RNA polymerase in *M. tuberculosis*. The enzyme human arylacetamide deacetylase (AADAC) metabolizes rifapentine into its major metabolite 25-O-deacetylrifapentine and it retains activity. Rifapentine is marketed under the brand name PRIFTIN for oral administration by Sanofi-Aventis Pharmaceuticals.

2.2.1.4. Pretomanid-Moxifloxacin-Pyrazinamide regimen

There is novel regimen consisting of three drugs pretomanid (new generic name of PA-824), moxifloxacin and pyrazinamide is in phase III clinical trial. This regimen has shown promising

activity towards the sensitive as well as resistant TB. Among this trio drugs, pyrazinamide is a first line standard drug, moxifloxacin is a second line fluoroquinolone and pretomanid is a newly developed drug. The following paragraphs discuss briefly about structural and activity feature of PA-824 and moxifloxacin.

PA-824



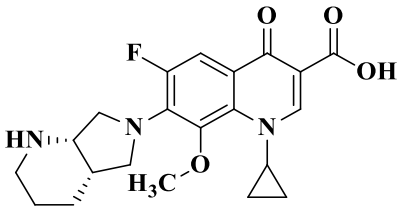
PA-824 is a nitroimidazo-oxazine and a metronidazole derivative belonging to the class of nitroimidazopyrans [Stover CK., *et al*., 2000], is being developed by Global Alliance for TB Drug Development (TB Alliance). PA-824 is currently under Phase II clinical trials for the treatment of drug-susceptible as well as drug-resistant (MDR/XDR) TB [Zumla A., *et al*., 2013; Laurenzi M., *et al*., 2007]. MDR strains exhibited comparable susceptibility to PA-824 suggesting that no cross-resistance is present with current anti-TB drugs.

PA-824 is active against both replicating and non-replicating *M. tuberculosis* cells with MIC of 0.015 to 0.25 µg/mL. PA-824 is also a prodrug like INH. PA-824 requires intracellular activation of aromatic nitro group by an F420-deazaflavin-dependent nitroreductase (Ddn) which in turn generates reactive nitrogen species, such as NO, these are the major effectors of its anaerobic activity [Yew WW., *et al*., 2011; Singh R., *et al*., 2008]. Mechanism of action of PA-824 is involved by two-fold as it inhibits synthesis of both protein and cell wall lipid but does not affect nucleic acid biosynthesis. It undergoes nitro reduction producing highly reactive intermediates which then reacts with multiple targets inside the bacterial cell.

PA-824 has been observed to kill bacteria in two different 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 (similar mechanism to cyanide) [Singh R., *et al*., 2008], these two mechanisms explain both its aerobic activity against replicating bacteria (cell wall effect) and anaerobic bacteria against non-replicating bacteria (nitric oxide effect) [Jia L., *et al*., 2005]. Mutations in the gene the F420 enzyme are responsible

for few instances of drug resistance identified *in vitro* [Manjunatha UH., *et al*., 2006]. As this drug has many attractive characteristics such as activity against all drug-resistant strains, its novel mechanism of action, no cross-resistance, and its activity as both potential bactericidal as well as sterilizing agent in mice, and it has no significant activity against other Gram-positive and Gram-negative bacteria this drug could be the potential corner stone of future TB and drug-resistant TB treatment regimens [www.tballiane.org].

Moxifloxacin

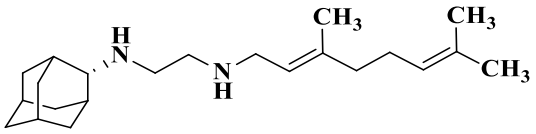


Moxifloxacin (BA12-8039), a fourth generation synthetic fluoroquinolone developed by Bayer AG [**Table 2.3**], was marketed globally (as hydrochloride) under the brand names of Avelox and Avelon for oral treatment [www.Avelox.com; Meena J., *et al*., 2015]. It was found that moxifloxacin had greatest bactericidal activity as compared to INH. Another study suggested that moxifloxacin also had potent sterilizing activity [Kwon YS., *et al*., 2014].

In the standard four-drug regimen the use of moxifloxacin thought to be shorten the treatment duration of drug-susceptible TB to test this hypothesis moxifloxacin was used in two different combination regimens (i) ReMox trial in which moxifloxacin replaces either EMB or INH (ii) Rifaquin trial in which moxifloxacin replaces INH in the intensive phase of treatment and in continuation phase rifapentine was used, but the results are not encouraging. This drug is currently in Phase III clinical trials and is used in combined treatment regimen for the treatment of MDR-TB.

2.2.2. Current drugs in phase II clinical trials

2.2.2.1. SQ 109



SQ109 or *N*-geranyl-*N*'-(2-adamantyl)ethane-1,2-diamine) is an ethylenediamine derivative primitively synthesized as EMB analogue but with entirely dissimilar mode of action hence it is active against EMB-resistant strains [Protopopova M., *et al*., 2005]. SQ109 acts by inhibition of mycobacterial growth by inhibiting the assembly of mycolicacid into the mycobacterial cell wall [Tahlan K., *et al*., 2012]. SQ109 aiming MmpL3 an essential membrane trans-porter belonging to family of resistance, nodulation and division (RND), the primary function of MmpL3 in *M. tuberculosis* is to transport the trehalose monomycolate into the envelope hence interfering with mycolicacid synthesis in the mycobacterial cell. MmpL3 also aids with iron acquisition for mycobacteria survival [Owens CP., *et al*., 2013].

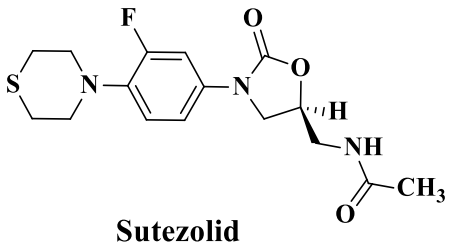
SQ109 displayed activity against both drug-susceptible and drug-resistant strains of *M. tuberculosis*, and excellent *in vitro* activity against *M. tuberculosis*, including EMB, INH and RIF resistant strains [Protopopova M., *et al*., 2005]. The combined administration of SQ109 and RIF or INH demonstrates synergistic *in vitro* activity with no antagonistic interaction in combination with other first-line therapies [Chen P., *et al*., 2006]. This synergy arises from the activation of SQ109 by RIF-induced *M. tuberculosis* cytochrome P450 (CYP), giving rise active oxidized metabolites. Experiments with mice have also proven *in vivo* synergy when SQ109 is used with INH, RIF and PZA.

In Phase I studies it was found that SQ109 was safe and well tolerated in single dose up to 300 mg [Sacksteder KA., *et al*., 2012; Nuermberger EL., *et al*., 2010], and has no serious side effects. The substitution of EMB with SQ 109 in standard regimen enhanced the efficacy in mouse model [Nikonenko BV., *et al*., 2007]. It has MIC of 0.16-0.64 µg/mL and did not show cross-resistance with EMB. SQ109 holds synergetic effects with TMC-207 and prosperous interactions with sutezolid *in vitro* [Reddy VM., *et al*., 2010; Reddy VM., *et al*., 2012]. It has

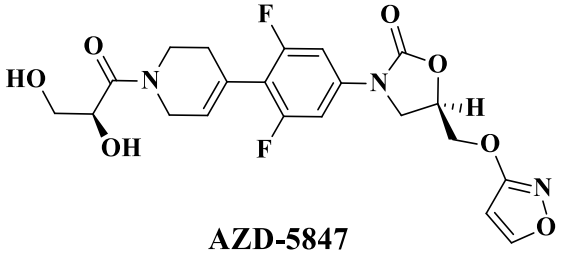
been shown that SQ109 has synergistic effects with bedaquiline because it weakens the mycobacterium cell wall, thereby furnishing bedaquiline to reach its target enzyme ATP synthase more efficiently [Reddy VM., *et al*., 2010].

Hence it is presumed that SQ109 is a promising new drug that may bring down the duration of anti-tubercular regimens. SQ109 exhibits excellent *in vitro* activity against *M. tuberculosis*, including EMB, INH and RIF resistant strains [Rivers., *et al*., 2008]. An interesting characteristic feature of SQ109 is it has very low mutation rate which offers it a desirable profile for inclusion in treatment of patients with relapsing TB. SQ109 is in phase II clinical trials for the treatment of drug-susceptible TB [Migliori GB., *et al*., 2013].

2.2.2.2. Oxazolidinones (Sutezolid and AZD 5847)



Sutezolid



AZD-5847

Sutezolid (PNU-100480 [U-480]), is a new oxazolidinone developed by Pfizer is a thiomorpholinyl analog of linezolid with superior efficacy against *M. tuberculosis* along with improved safety profile [Shaw KJ., *et al*., 2011]. Anti-TB activity of sutezolid was first reported in 1996 [Stover CK et al 2000]. Sutezolid is a novel compound currently in phase II clinical trials for the treatment of MDR-/XDR-TB [Alffenaar JW., *et al*., 2011].

Anti-TB activity of sutezolid was first reported in 1996 [Lai BS., *et al*., 2012]. Sutezolid is more active than linezolid both *in vitro* and *in vivo*, demonstrated with lower MICs than linezolid towards clinical isolates of MDR-TB. Pharmacokinetic data shows that sutezolid converts into sulfone and sulfoxide metabolites, the sulfoxide metabolite is more active and reaches four fold higher in concentration than parent compound [Barbachyn MR., *et al*., 1996], the addition of sutezolid to TMC-207, PA-824 and clofazimine found to be more effective than other first-line drugs (RIF-INH-PZA) [Williams K., *et al*., 2012] in shortening the duration of tuberculosis treatment in rats.

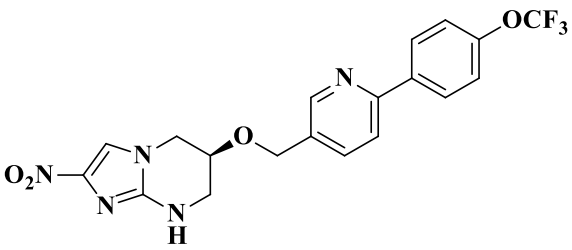
Sutezolid also showed additive effect *in vitro* with SQ-109 and with first-line drugs in murine model for TB infection. Studies on healthy volunteers showed that sutezolid is well absorbed and distributed, well tolerated at the dosage of 1000 mg [Wallis RS., *et al*., 2010]. PNU-100480 did not exhibit cross-resistance with any of the first-line drugs. It has improved the bactericidal activity of existing first-line drugs and also experimental drugs such as bedaquiline and SQ109 [Reddy VM., *et al*., 2012; Williams KN., *et al*., 2009; Williams K., *et al*., 2012]. It also gave better results when used in combination with moxifloxacin and PZA. These results suggest that sutezolid has the potential to reduce the treatment duration in both drug-susceptible and drug-resistant TB. [Balasubramanian V., *et al*., 2014].

AZD-5847 (Posizolid) is a novel oxazolidinone drug in pipeline developed by Astrazeneca for the treatment of pulmonary TB [Wookey A., *et al*., 2012]. AZD-5847 demonstrated enhanced *in vitro* bactericidal activity against intracellular as well as extracellular *M. tuberculosis* compared to that of linezolid. It binds to 50S ribosomal subunit and blocks initiation of protein synthesis and exhibited activity against drug-susceptible, MDR-TB and XDR-TB isolates [Kwon YS., *et al*., 2014, Balasubramanian V., *et al.*, 2014].

AZD 5847 is not antagonistic with other TB drugs and the efficiency of AZD-5847 was additive when tested in combination with other existing TB agents suggesting that AZD-5847 could function well in combination therapies [Zumla A., *et al*., 2013]. In toxicological testing AZD-5847 was well tolerated and safe when administered in healthy volunteers (2400 mg maximum dose for 14 days) but on higher exposure 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 EB., *et al*., 2013; Meena J., *et al*., 2015].

2.2.3. Current drugs in phase I clinical trials

2.2.3.1. TBA-354



TBA-354 is a potent next-generation nitroimidazole derivative TBA-354 is narrow spectrum bactericidal drug active against both replicating and non-replicating *M. tuberculosis* with potency equal to that of delamanid and higher than that of PA-824 [Upton AM et al 2015]. TBA-354 is the first drug candidate entered Phase I clinical trials since 2009, it is developed by the Global Alliance for TB drug Development.

In pre-clinical studies TBA-354 demonstrated higher potency and sterilizing activity than that of pretomanid [www.tballiance.org]. In the murine models the monotherapy studies demonstrated that TBA-354 is 5-10 times more potent than PA-824. The combination studies showed that TBA-354 is 2-4 times more potent than PA-824 when used in combination with bedaquiline, when administered at a dose equivalent to that of PA-824 [Tasneen R., et al., 2015].

Table 2.3: New anti-TB drugs, stage of development and their targets [Yew WW., *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 (RIC)
TMC-207	Diarylquinoline	Tibotec/Janssen	II	ATP synthase
PNU-100480	Oxazolidinone	Pfizer	II	RIC
AZD-5847	Oxazolidinone	Astrazeneca	II	Unknown
SQ-109	Diethylamine	Sequella	II	Unknown

2.3. Promising heterocyclic classes of compounds as anti-TB drugs

After thoroughly reviewing the literature of current available anti-TB drugs and pro-drugs which are under various clinical phases, we have also studied the literature of other potential drugs

which have been reported since last two decades. After literature search we investigated that, 80% of all anti-TB drugs belongs to heterocyclic compounds family. The below mentioned heterocyclic family of drugs were proven as promising anti-TB drugs.

2.3.1. Literature survey on 1,3,4-thiadiazoles as anti-tubercular agents

Thiadiazole is a five membered heterocycle containing one sulphur and two nitrogen atoms. 1,3,4-thiadiazoles are the most important among the isomeric 1,2,3-thiadiazoles, 1,2,4-thiadiazoles and 1,2,5-thiadiazoles. 1,3,4-thiadiazole's importance owing to its presence as core structural component in broad spectrum antibiotics, *viz* antimircrobial, anti-inflammatory, analgesic, antiepileptic, antiviral and anti-tubercular agents.

In literature 1,3,4-thiadiazoles and its dihydro-derivatives were much explored and more work has been carried out on the 1,3,4-thiadiazole than all remaining isomers combined [Jain AK., *et al*., 2013]. Thiadiazole moiety acts as "two-electron donor system" as well as "hydrogen binding domain". Many drugs possessing 1,3,4-thiadiazole pharmacophore are available in the market (**Figure 2.2**) [Ramprasad J., *et al*., 2015].

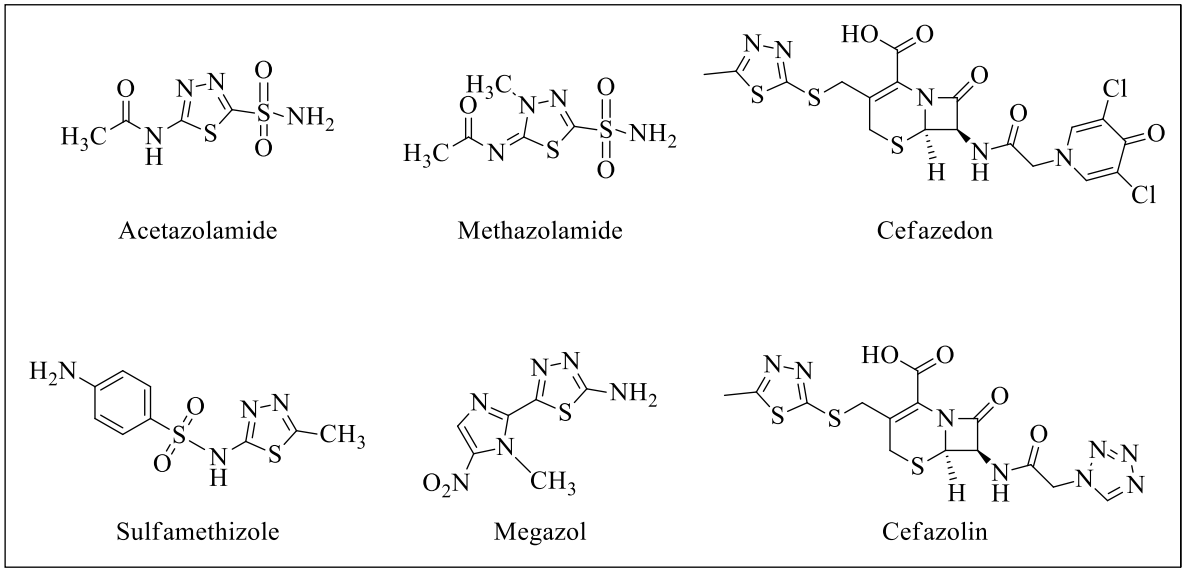
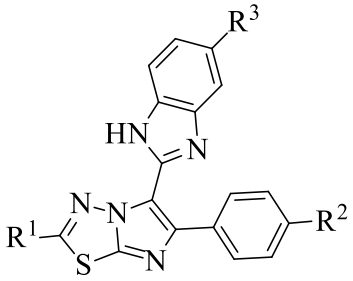


Figure 2.2: Available drugs containing 1,3,4-thiadiazole ring moiety

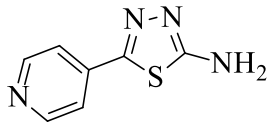
Recently, Ramprasad and co-workers have reported synthesis and anti-mycobacterial evolution of 2-substituted-6-arylimidazo[2,1-*b*][1.3.4]thiadiazole derivatives. They started synthesis from substituted aromatic carboxylic acids and thiosemicarbazide. The reaction of thiosemicarbazide with carboxylicacids in $POCl_3$ at 75 °C produced 5-substituted-2-aminothiadiazole, in next step they treated with phenacyl bromide in ethanol to yield bicylic imidazo-thiazole ring. Then derivatization carried out on imidazole ring. Out of synthesized twenty nine derivatives, seven compounds found to be active against *M. tuberculosis* H37Rv at 3.125 µg/mL concentration [Ramprasad J., *et al*., 2015].



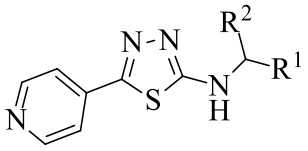
R^1, R^2 and R^3 : various substituents

N.S. Mahajan and co-workers recently reported synthesis of 1,3,4-thiadiazole derivatives from easy and cheaper starting materials in support of cost effective drug discovery. The synthetic route as follows, in first step ethylester of isonicotinic acid was chemtransformed into hydrazide using hydrazine hydrate. In second step, the amino group of isonicotinohydrazide was treated with base followed by carbon disulfide to produce potassium salt of 2-isonicotinoylhydrazinecarbodithioic acid, in next step cyclisation was carried out using sulphuric acid to produce parent 2-aminothiadiazole ring. In last step, parent "pyridinyl-thiadiazole" was reacted with various chloro-substituted compounds for analog synthesis [Mahajan NS., *et al*., 2015].

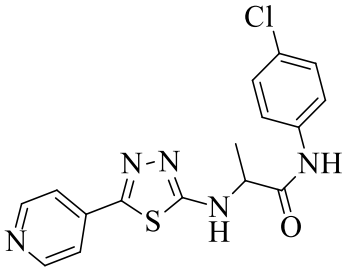
The synthesized molecules were subjected to *M. tuberculosis* MIC determination; among thirty six derivatives the most active molecule (**6f**) showed MIC of 0.12 ± 0.46 µM.



Parent pyridino-thiadiazole for analog synthesis

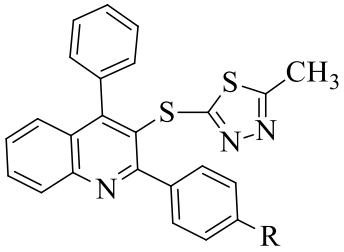


R^1, R^2: various substituents



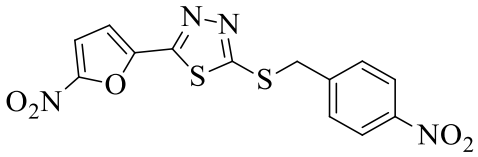
6f, MIC_{50} = 0.12 ± 0.46 µM

Chitra *et al*. synthesized 3-heteroarylthioquinoline derivatives of 1,3,4-thiadiazole and screened their *in vitro* antimycobacterial activity against *M. tuberculosis* H37Rv using agar dilution method. Activity was considerably effected by substituents like 2-methyl-1,3,4-thiadiazole, benzothiazole and 2-phenyl-2*H*-tetrazole on the 3-position of quinolone ring and it was further supported by the fact that compounds with no substitution did not show any considerable activity. Compounds 78 and 79 with chloro and bromo-substituted aromatic ring found to be more active (MIC = 3.2–3.5 µg/mL) [Chitra S., *et al*., 2011].



78: R = Cl, **79**: R = Br

Foroumadi and team synthesized 2-(5-nitro-2-furyl)- and 2- (1-methyl-5-nitro-l*H*-imidazol-2-yl)-1,3,4-thiadiazole derivatives starting from (5-nitrofuran-2-yl)methylene) hydrazinecarbothioamide in four steps. Synthesized compounds screened for their *in vitro* antimycobacterial activity against *M. tuberculosis* H37Rv. Compounds with 5-nitro-2-furyl and 4-nitrobenzyl substitution (**8e**) showed the highest activity against *M. tuberculosis* (MIC = 3.13 µg/mL) [Foroumadi A., *et al*, 2004].



8e, MTB MIC 3.13 µg/mL

2.3.2. Literature survey on 2-Aminothiophene-3-carbaxamide derivatives as anti-tubercular agents

Substituted-2-aminothiophenes attained an ultimate position in the field of drug design and synthesis of pharmaceuticals due to their advantageous properties- the thiophene ring is bioisosteric replacement of phenyl group broadly present in various active drugs, the thiophene ring found in many natural and synthetic pharamceuticals. Multisubstituted 2-aminothiophene scaffolds derived from the Gewald reaction has attracted considerable attention to design various biologically active molecues such as antipsychotic agents, anti-inflammatory agents, anti-tubercular agents, allosteric enhancers of adenosine A_1 receptors.

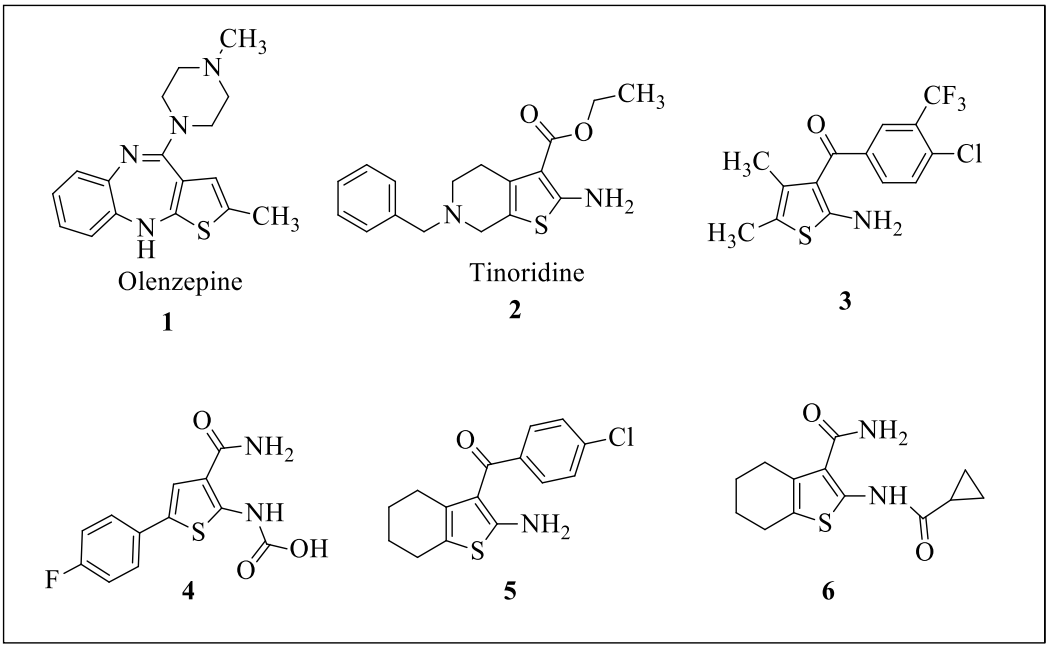
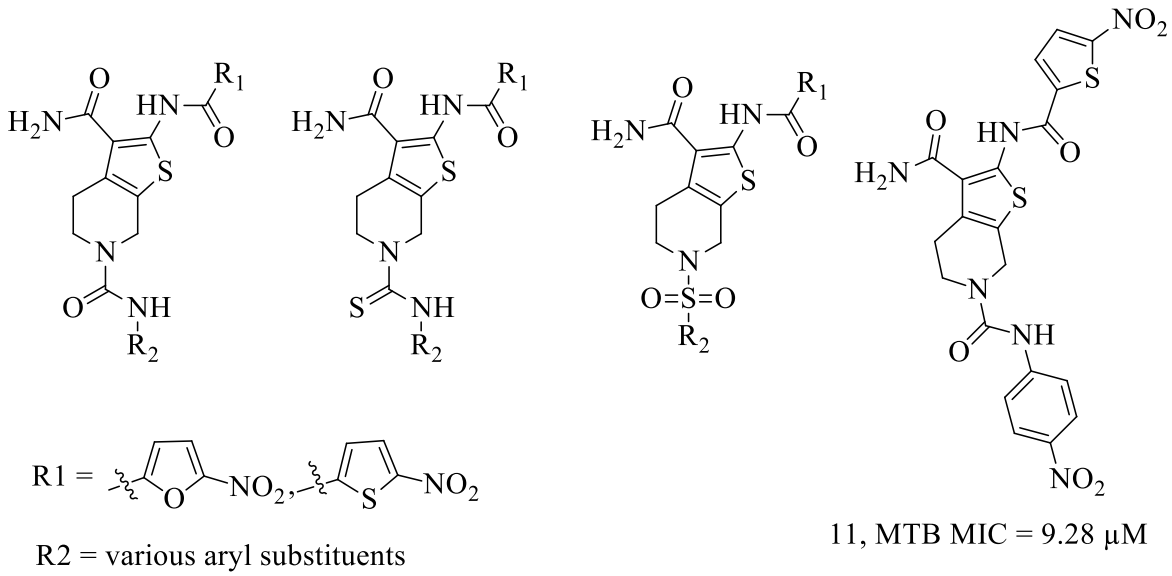


Figure 2.3: 2-Aminothiophene derivatives in clinical (1 & 2) or in preclinical development (3-6)

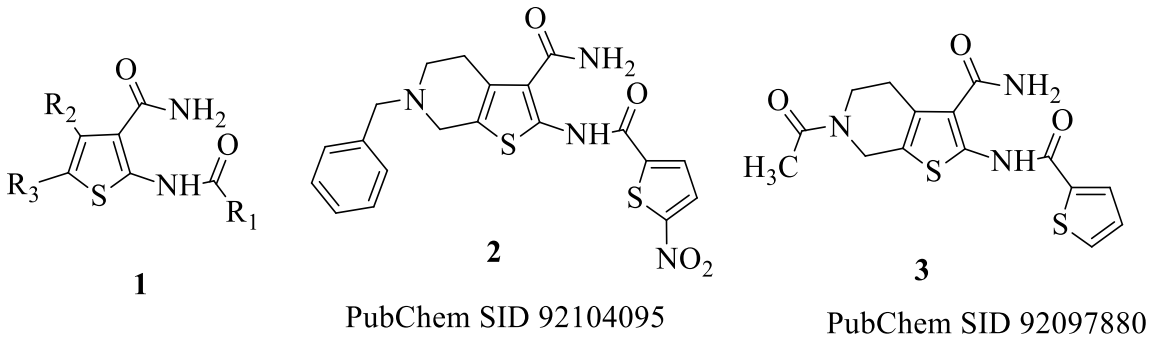
G.Samala *et al* designed a series of 2,6-disubstituted 4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine-3-carboxamide derivatives and synthesized by using commercially available piperidine-4-one following a five step synthetic protocol. the Boc-protected piperidine-4-one was treated with cyanoacetamide under Gewald reaction conditions to afford 2-aminothiophene derivative. 2-aminothiophene derivative was further subjected to amidation with two different carboxylic acids to get the corresponding amide derivatives. In the next step of the reaction the Boc deprotection was carried out with CF_3COOH and treated with various arylisocyanates,

arylisothiocyanates and sulphonylchlorides to afford the desired urea, thiourea, sulphonamide derivatives respectively.

Then the synthesized compounds were evaluated against *M. tuberculosis* H37Rv *in vitro* using MABA method. The compounds have shown the significant activity against *M. tuberculosis* H37Rv with MIC values ranging from 9.28 µM to 53.19 µM. The compound 6-(4-nitrophneylsulfonyl)-2-(5-nitrothiophene-2-carboxamido)-4,5,,6,7-tetrahydrothieno[2,3-*c*]pyridine-3-carboxamide (**11**) was found to be the most active compound against *M. tuberculosis* H37Rv with an MIC of 9.28 µM and was non-cytotoxic toward RAW 264.7 cell lines at 50 µM [Samala G., *et al*., 2014].



Reynolds R.C *et al* reported anti-TB compounds **SID 92104095(** 6-benzyl-2-(5-nitrothiophene-2-carboxamido)-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine-3-carboxamide), **SID 92097880** (6-acetyl-2-(thiophene-2-carboxamido)-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine-3-carboxamide) [Reynolds RC., *et al*., 2012] with *M. tuberculosis* MIC of 9.15 µM.



2.3.3. Literature survey on thiazolidine-4-one derivatives as anti-tubercular agents

Thiazolidine-4-ones are an important building blocks in various pharmaceutical and biologically active products. Thiazolidine-4-one derivatives demonstrated to possess diverse biological activities in the area of medicine such as anti-inflamatory, anticonvulsant, antiviral, antidiabetic, antiproliferative, anti-hyperlipidemic, cardiovascular, anti-tubercular, antitumor, antifungal and antibacterial. Some drugs are already in the market based on this pharmacophore.

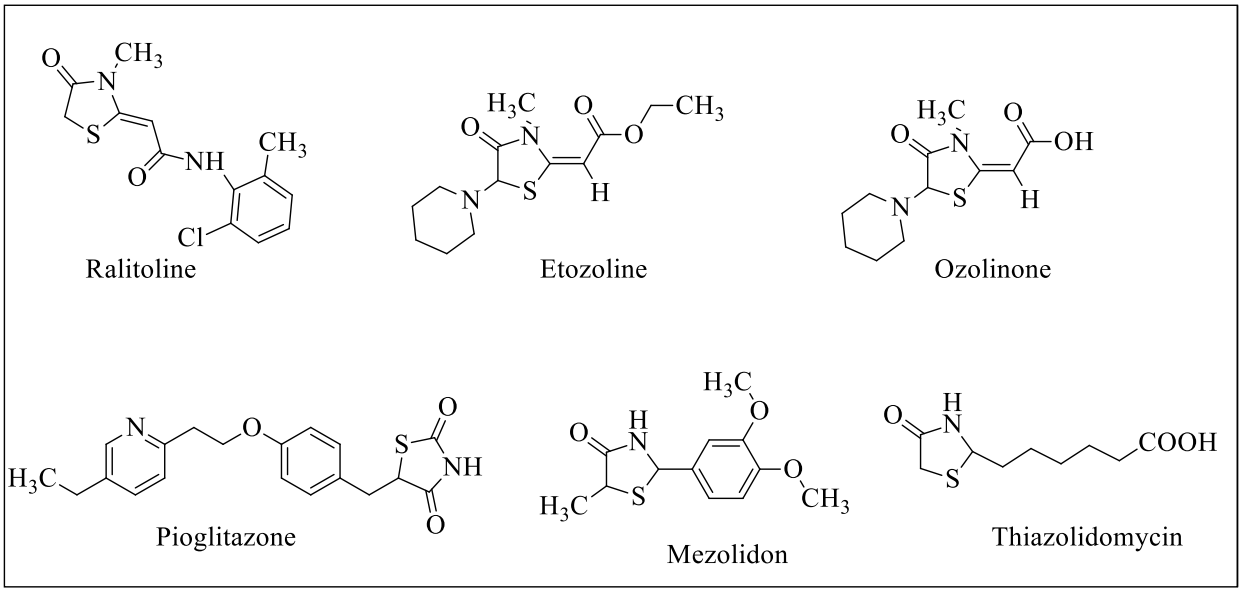
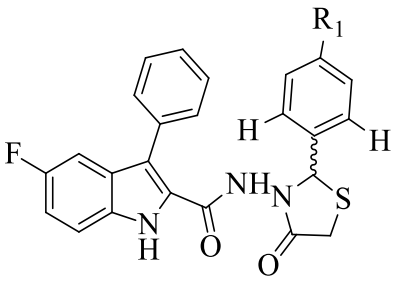


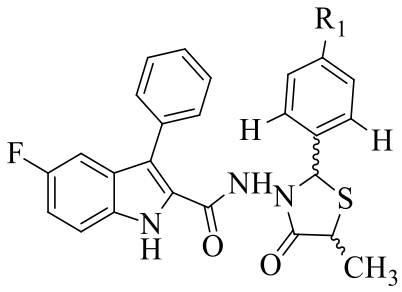
Figure 2.4: Marketed drugs containing thiazolidin-4-one nucleus

To develop the effective and selective anti-tubercular agents Cihan-Ustuindag G *et al* synthesized a novel thiazolidinone derivatives of 5-fluoro-3-phenyl-1*H*-indole. All the synthesized molecules were tested for their *in vitro* anti-tubercular activity against *M. tuberculosis* H37Rv using BACTEC 460TB system. Few of synthesized 4-thazolidinone

derivatives showed appreciable anti-tubercular activity with 99% inhibition at MIC ranging from 6.25-25.0 µg/mL with low cytotoxicity against mammalian cell lines. The compounds **7g,7h,7i,8h** and **8j** showed anti-tubercular activity at concentrations 10-fold lower than those cytotoxic for mammalian cells. [Cihan-Ustundag G., *et al.*, 2015].

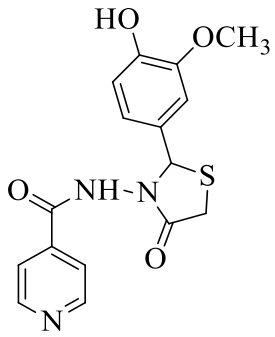


7g: R_1 = CN; MIC = 6.25 µg/mL
7h: R_1 = NO_2; MIC = 6.25 µg/mL
7i: R_1 = NO_2; MIC = 12.5 µg/mL



8g: R_1 = CN; MIC = 12.5 µg/mL
8h: R_1 = NO_2; MIC = 12.5 µg/mL

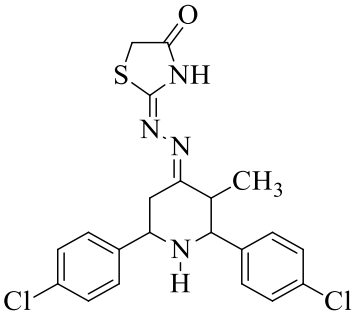
A series of isonicotinylhydrazide derivatives were synthesized and evaluated for their *in vitro* anti-tubercular activity against *M. tuberculosis* H37Rv using alamar blue assay method. It was identified that the anti-tubercular activity was affected by the substitution on the aromatic ring of 4-thiazolidinone and it was found that the compounds without substitution on aromatic ring did not show significant activity. The compound (**71**) with OH, OCH_3 substitution on aromatic ring found to be the most active compound with MIC 0.31 µg/mL [Jain AK., *et al*., 2012].



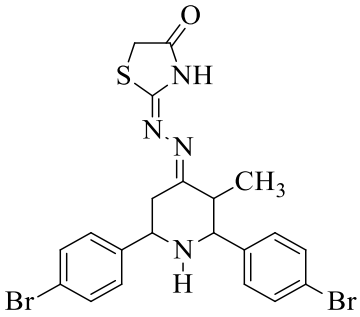
71, MTB MIC = 0.31µg/mL

Aridoss G *et al* synthesized some stereospecific thiazolidinones and evaluated for their anti-tubercular activity against *M. tuberculosis* H37Rv. Few compounds [**19, 20, 24**] have displayed

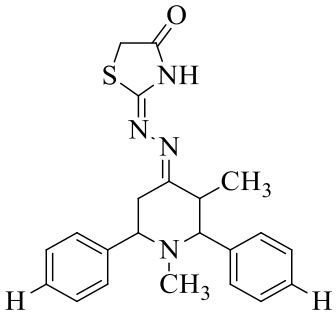
two-fold elevated potency than RIF with MIC of 16 µg/mL [Aridoss G., *et al*., 2009].



19, MTB MIC = 16 µg/mL

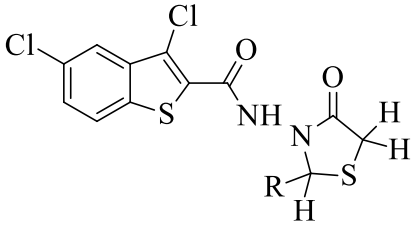


20, MTB MIC = 16 µg/mL



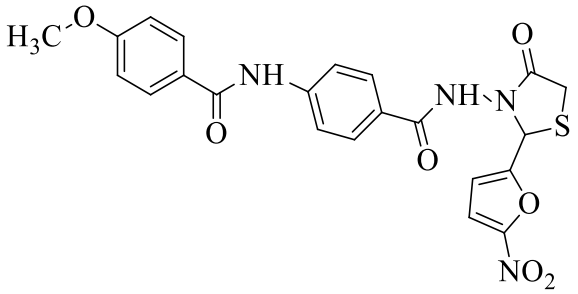
24, MTB MIC = 16 µg/mL

Narute A *et al* studied the anti-tubercular activity of (substituted 1,2-dihydro)-4-thiazolidinones by taking various physico chemical descriptors into consideration [Narute A., *et al*., 2008].

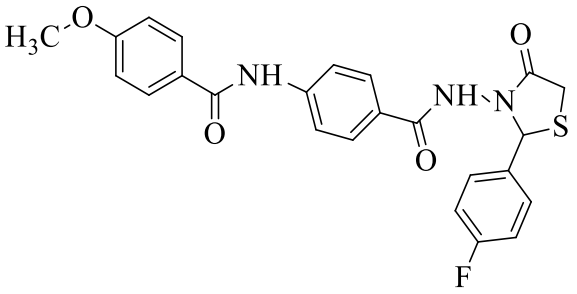


2-Aryl-5*H*-3-(3',5'-dichloro-2'-benzo(b)thiophenylamino)-4-thiazolidinones

Kucukguzel SG *et al* reported the anti-tubercular activity of a novel series of thiazolidine-4-one derivatives. The desired compounds were synthesized by using a multistep-reaction protocol and evaluated for their anti-tubercular activity against *M. tuberculosis* H37RV strain. The primary screening was carried out at 6.25 µg/mL in BACTEC 12B medium using BACTEC 460 radiometric system. Compounds with at least 90% of inhibition were tested further at lower concentrations but none of the compounds have shown activity at less than 6.25 µg/mL of concentration. The compounds **7a** and **7b** found as the most active compounds demonstrating 90 and 98% inhibition of mycobacterial growth at MIC of 6.25 µg/mL respectively [Kucukguzel SG., *et al* 2002].



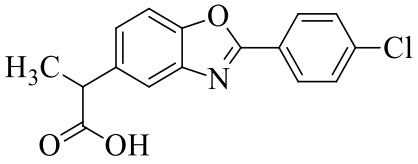
7a, MTB MIC = 6.25 µg/mL



7b, MTB MIC = 6.25 µg/mL

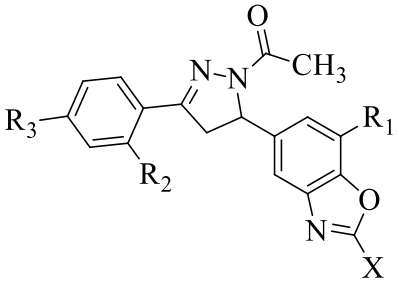
2.3.4. Literature survey on benzoxazoles as anti-tubercular agents

Benzoxazole is an aromatic compounnd consists of benzene ring fused with oxazole ring. It is used in the research as a starting material for the synthesis of larger, usually bioactive structures that possess interesting biological activities such as antimicrobial, anti-tubercular, anticancer, antifungal and analgesics. Benzoxazoles can be considered as the bioisosteres of naturally occurring nucleic bases such as adenine and guanine, which allow them to interact easly with biopolymers of a living system.



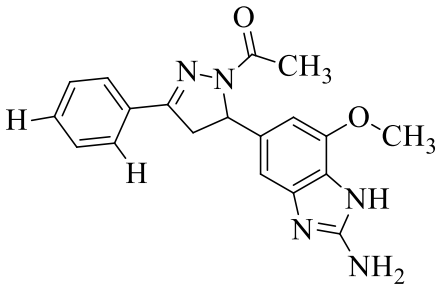
Benazoprofen
Marketed drug of benzoxazole derivative

Rana DN and co-workers designed a series of benzoxazole-based pyrazoline derivatives and the synthesis was carried out by aldol condensation of 4-hydroxy-3-nitro-5-substituted-benzaldehyde with substituted acetophenones using NaOH to afford the corresponding chalcone derivatives. The cyclization reaction of these chalcone derivatives with hydrazine hydrate in presence of glacial acetic acid produced the corresponding pyrazoline derivatives. The reduction of NO_2 group was carried by sodium dithionite to afford respective *o*-aminophenol derivatives, furthur cyclization of these aminophenols was carried out in two different ways (a) treatment with cyanogen bromide in MeOH-H_2O mixture at room temperature afforded the corresponding 2-aminobenzoxazole derivatives and (b) treatment with CS_2 and KOH in EtOH under reflux conditions produced 2-mercaptobenzoxazole derivatives.



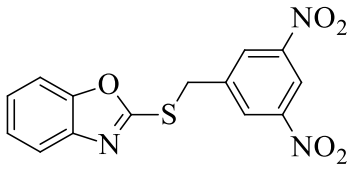
X = NH_2, SH
R_1 = H,Cl,OCH_3
R_2 = H, F, Cl
R_3 = H, Cl, Br

The synthesized compounds were evaluated against *M.* tuberculosis H37Rv and MDR-TB strains. The MTB screening was carried by using Lowenstein-Jensen medium (L.J. medium). It was found that most of the compounds showed potent activity (MICs ranging from 0.625-25.0 µg/mL) and few compounds found to be more potent than INH against MDR-TB. 1-(5-(2-amino-7-methoxy-1*H*-benzo[*d*]imidazol-5-yl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone (**5a**) found to be the most active compound against *M. tuberculosis* H37Rv with MIC of 0.625 µg/mL and it showed MIC 6.25 µg/mL against MDR-TB [Rana DN., *et al*., 2014].

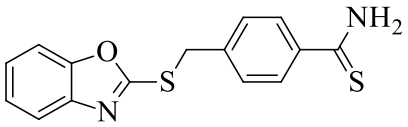


5a, MIC (MTB) = 0.625 µg/mL
MIC (MDR-TB) = 6.25 µg/mL

To facilitate drug design of benzoxazole as potential anti-tubercular agent Klimesova V *et al* synthesized a series of 2-benzylsulfanyl derivatives of benzoxazole and tested for their *in vitro* antimycobacterial activity against *M. tuberculosis*, non-tuberculosis mycobacteria and multi-drug *M. tuberculosis*. the dinitro derivatives have displayed a significant activity against both sensitive as well as resistant strains of *M. tuberculosis*. It was found that the 2-((3,5-dinitrobenzyl)thio)benzo[*d*]oxazole (**3t**) and 4-((benzo[*d*]oxazol-2-ylthio)methyl)benzothioamide (**4a**) were found to exhibit significant activity against both drug-sensitive and drug-resistant strains of tuberculosis [Klimesova V., *et al*., 2009].



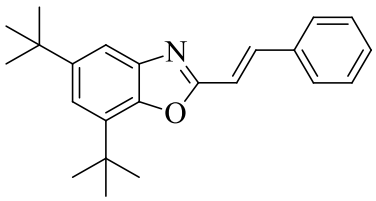
3t, MIC (MTB) = 8μmol/L)
MIC (MDR-TB) = 2-4 μmol/L)



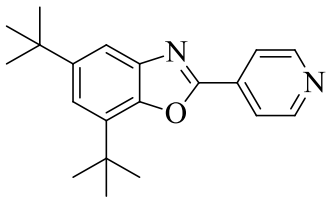
4a, MIC (MTB) = 8-16 μmol/L)
MIC (MDR-TB) = 8-32 μmol/L)

Vinsova J *et al* synthesized a series of 30 novel 2-substituted 5,7-di-*tert*-butylbenzoxazoles using various synthetic pathways and evaluated for their *in vitro* antimycobacterial activity using MABA assay method. Several compounds exhibited interesting anti-tubercular activity equal or higher than INH. Cytotoxicity of the compounds was tested with MTT assay by using INH as the standard reference drug.

Out of the tested compounds 5,7-di-*tert*-butyl-2-(pyridin-4-yl)benzoxazole (**8e**) found to be the efficient compound with 100% inhibition of *M. tuberculosis* growth with MIC of 6.25 μg/mL and especially 5,7-di-*tert*-butyl-2-styrylbenzoxazole (**8a**) was found to be the most active compound with MIC of 3.13 μg/mL with lowest toxicity to human intestinal cells HCT-8 (IC_{50} = 9.02.2 μg/mL) [Vinsova J., *et al*., 2006].

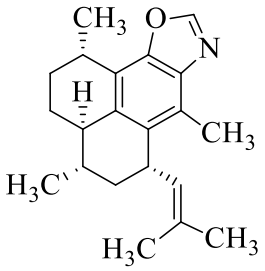


8a, MTB MIC = 3.13μg/mL



8e, MTB MIC = 6.25 μg/mL

Okunade AL *et al* reported that the naturally occurring pure compounds as well as extracts of plants, microorganisms and marine organisms found to possess an inhibitory activity against *M. tuberculosis* is wide spread in nature. The benzoxazole alkaloids **7-9** are marine metabolites found to be the strong inhibitors of *M. tuberculosis* (MIC of 12.5 μg/mL) [Okunade AL., *et al*., 2004].



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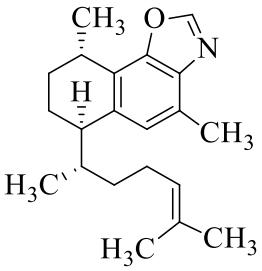

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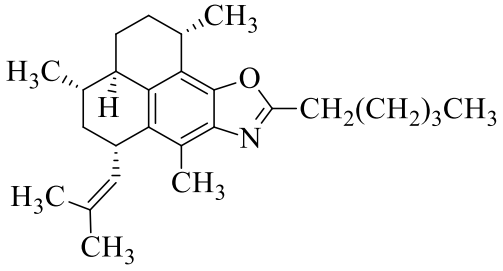
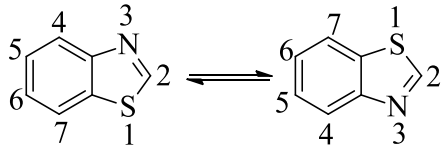


9

2.3.5. Literature survey on benzothiazoles as anti-tubercular agents

Chemistry of heterocycles lies at the heart of drug discovery. During the recent years heterocyclic compounds analogues and derivatives have attracted strongly owing to their useful biological and pharmacological properties. Benzothiazole belongs to the class of bicyclic heterocyclic compounds consists of a five membered 1,3-thiazole ring (containing nitrogen and sulphur) fused to benzene ring. It exists in two tautomeric forms. Benzothiazoles present in many marine or terrestrial natural compounds which have useful biological activities.

Since benzothiazole is a heterocyclic compound is useful in research as a starting material for the synthesis of larger, usually bioactive structures. Because of its aromaticity it is relatively stable, although, as a heterocycle, it has reactive sites, which allow for functionalization hence a large number of therapeutic agents can be synthesized with the help of benzothiazole nucleus. Benzothiazole is a highly important scaffold for drug development in the field of medicinal chemistry because of its remarkable pharmacological potentials. Benzothiazole nucleus found to possess a wide spectrum of pharmacological activities such as anti-tubercular, anticancer, anti-inflammatory, antimicrobial, antiviral, antidiabetic, and anticonvulsant.



Numbering and tautomeric forms in benzothiazole

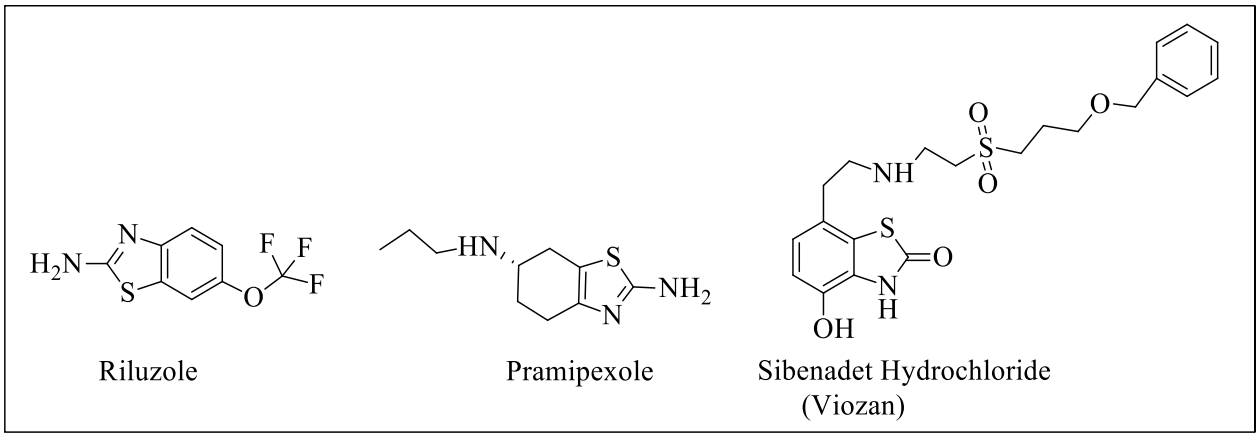
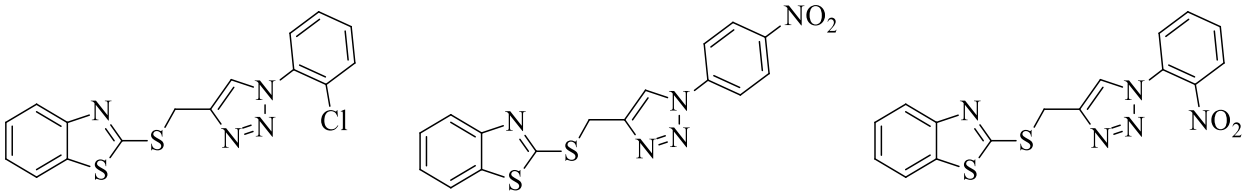


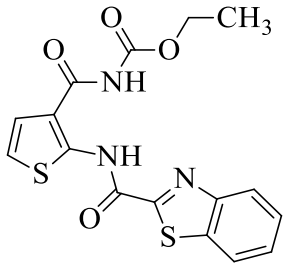
Figure 2.5: Marketed drugs of benzothiazole [Gill RK., *et al*., 2015].

2-Mercaptobenzothiazole and triazole conjugates were synthesized and evaluated for their anti-TB activity against *M. tuberculosis* H37Rv strain. Three compounds (**8f, 8g, 8i**) found to inhibit the growth of H37Rv strain at 8 µg/mL of concentrations [Mir F., *et al*., 2014].



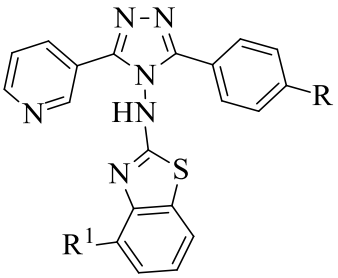
8f **8g** **8i**

TCA1 is a small molecule discovered in a cell-based high-throughput screen which exhibits the activity against both replicating as well as non-replicating *M. tuberculosis*. It is also efficacious in acute and chronic rodent models of TB alone or combined with front-line TB drugs. TCA1 functions with a unique mechanism of action, inhibits the enzymes involved in cell and molybdenum cofactor biosynthesis. The discovery of TCA1 lead to the significance advance in the search for the new anti-TB agents for the treatment of drug-susceptible and drug-resistant TB [Wang F., *et al*., 2013].

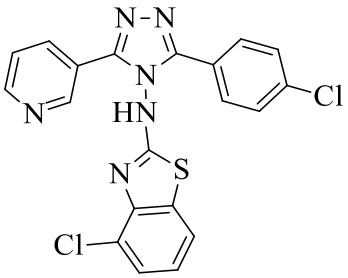


TCA1: MIC_{50}= 0.01µg/mL

Navin B. Patel reported the synthesis and antimycobacterial actvity of a series of 3-(3-pyridyl)-5-(4-substituted phenyl)-4-(*N*-substituted-1,3-benzothiazol-2-amino)-4*H*-1,2,4-triazole derivatives. The synthesis started from the ethylester of nicotinic acid (prepared from nicotinic acid) on reaction with hydrazinehydrate produced nicotinoylhydrazide which on inter molecular cyclisation with various 4-substitued benzoic acids in presence of $POCl_3$ yielded the oxadiazole derivative which on furthur condensation with 2-hydrazino-1,3-benzothiazoles in presence of dry pyridine produced the desired 3-(3-pyridyl)-5-(4-substituted phenyl)-4-(*N*-substituted-1,3-benzothiazol-2-amino)-4*H*-1,2,4-triazole derivatives. All the sunthesized compounds were tested for antimycobacterial actvity using Lowenstein-Jensen agar method out of the tested compounds three compounds (**9a,9d,9g**) showed good activity (MIC values 50-62.5 µg/mL) and 4-chloro-*N*-(3-(4-chlorophenyl)-5-(pyridin-3-yl)-4*H*-1,2,4-triazol-4-yl)benzo[*d*]thiazol-2-amine (**9j)** was found to be the most active compound with MIC of 12.5 µg/mL (98% of inhibition) [Patel NB., *et al*., 2013].

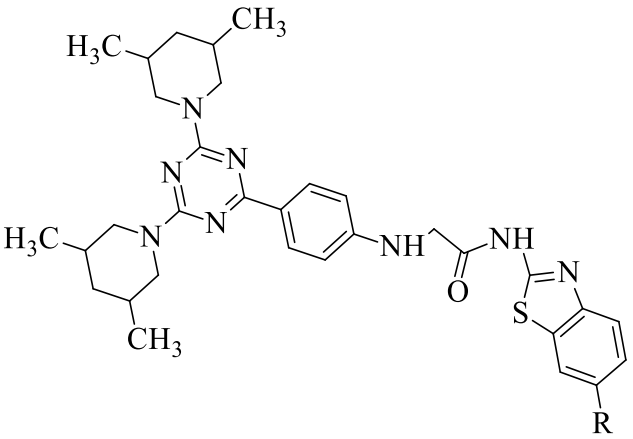


R= NO_2, Cl,
R^1= various substituents



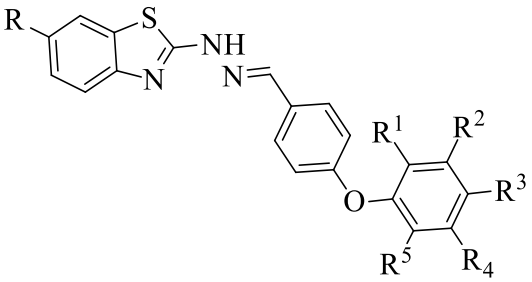
9j, MTB MIC =12.5 µg/mL (98% of inhibition)

Amit B. Patel reported the synthesis and anti-tubercular activity of bis(3,5-dimethylpiperidinyl)-1,3,5-trazinyl-*N*-(benzothiazolyl)-acetamide derivaties. Two compounds **9d, 9j** displayed highest inhibition at an MIC values of 3.12 and 6.25 µg/mL respectively [Patel AB., et al., 2013].



9d: R=F; MTB MIC =3.12 µg/mL'
% inhibition (MtbH37Rv)= 99%
9j: R=OEt; MTB MIC=6.25 µg/mL'
% inhibition (MtbH37Rv)= 99%

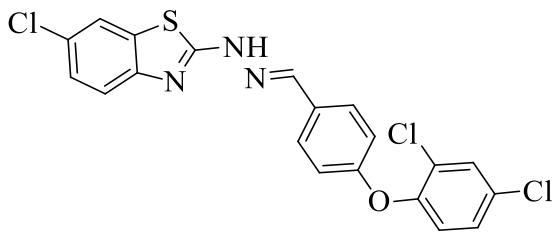
A series of novel substituted 2-(2-(4-aryloxybenzylidene) hydrazinyl)benzothiazole derivatives synthesized and evaluated for their activity against *M. tuberculosis* H37Rv by Telvekar VN.



R, R^1, R^2, R^3, R^4, R^5 = various substituents

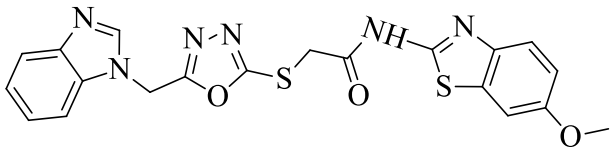
general structure of 2-(2-(4-aryloxybenzylidene) hydrazinyl)benzothiazole

All the synthesized molecules were screened against *M. tuberculosis* H37Rv and the MIC values were determined using Resazurin microtiter assay (REMA). All the synthesized molecules found to exhibit promising activity with MIC values ranging from 5.1-29.0 µg/mL. Five of the tested molecules were found to exhibit good activity with MIC <3.0 µg/mL. Compound (*E*)-6-chloro-2-(2-(4-(2,4-dichlorophenoxy)benzylidene)hydrazinyl)benzothiazole (**10i)** was found to be the most active compound with MIC of 1.5 µg/mL. Hence this compound could be the potential lead for the development of new anti-tubercular agents [Telvekar VN., et al., 2012].



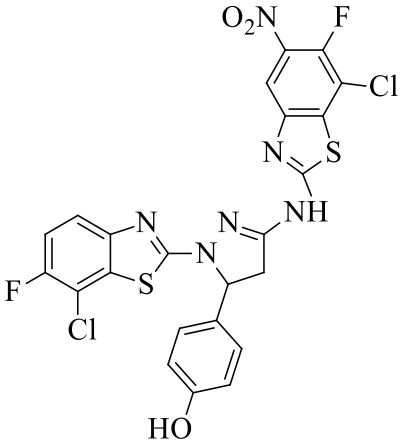
10i, MTB MIC =1.5 µg/mL

Series of benzimidazole based 1,3,4-oxadiazoles were synthesized and screened against eight bacteria including *M. tuberculosis* H37Rv. Majority of the compounds were found to exhibit significant anti-tubercular activity (6.25-25 µg/mL of MIC) using Lowenstein-Jensen agar (L.J) method. 2-((5-((1*H*-benzo[*d*]imidazol-1-yl)methyl)-1,3,4-oxadiazol-2-yl)thio)-*N*-(6-methoxybenzo[*d*]thiazol-2-yl)acetamide (**6i**) found to be the most active compound with 6.25 µg/mL of MIC and 99% of inhibition [Patel RV., et al., 2012].



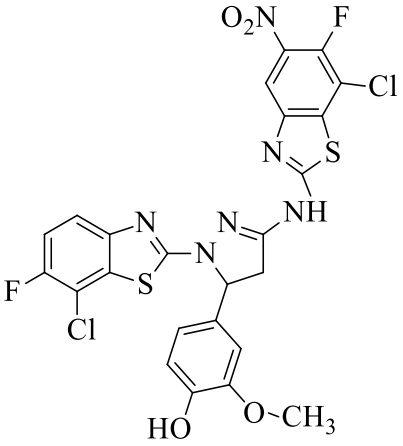
6i, MTB MIC = 6.25 µg/mL
% inhibition (Mtb H37Rv) = 99%

K. Hazra and co-workers synthesized a series of fluoronitrobenzothiazolopyrazoline regio isomers and screened for their anti-tubercular activity against *M. tuberculosis* H37Rv strain using Middlebrook7H-9 broth. The introduction of nitro group at position 5 of benzothiazole ring enhanced the activity than the unsubstituted benzothiazole ring, the introduction of nitro group at position 4 of benzothiazole ring reduced the activity. The compounds 12b, 12c and 12d found to exhibit better activity [Hazra K., *et al*., 2012].



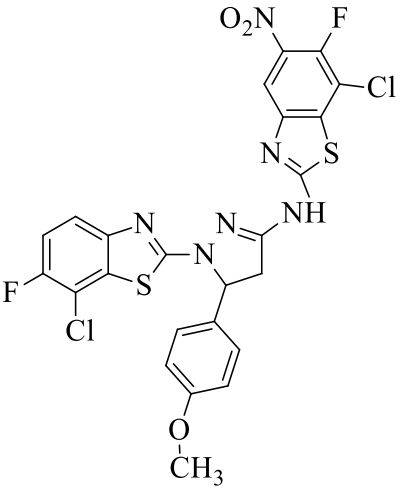
12b

MTB MIC = 0.042 mM



12c

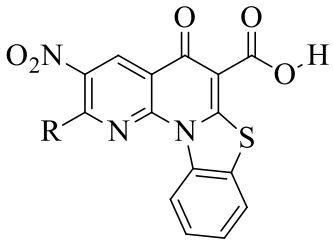
MTB MIC = 0.040 mM



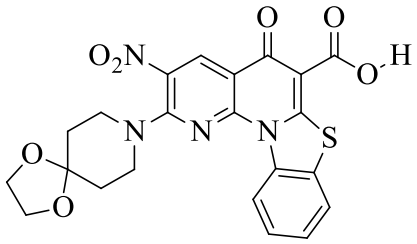
12d

MTB MIC = 0.041 mM

Dinakaran M *et al* synthesized a series of sixteen novel 3-nitro-2-(sub)-5,12-dihydro-5-oxobenzothiazolo[3,2-*a*]-1,8-napthyridine-6-carboxylic acids starting from the commercially available 2,6-dimethoxynicotinic acid and 2-aminothiophenol and evaluated for their anti-tubercular activities *in vitro* and *in vivo* against *M. tuberculosis* $H_{37}Rv$ and MDR-TB. Out of the sixteen compounds synthesized, 3-nitro-5-oxo-2-(1,4-dioxa-8-azaspiro[4.5]decan-8-yl)-5*H*-benzo[4,5]thiazolo[3,2-*a*][1,8]naphthyridine-6-carboxylic acid (**10n**) was found to be most active compound *in vitro* with 0.19 µM against *M. tuberculosis* (which is more active than INH, MIC 0.36 µM) and 0.04 µM against MDR-TB [Dinakaran M., *et al*., 2009].



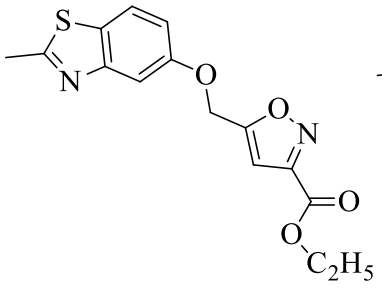
10a-p



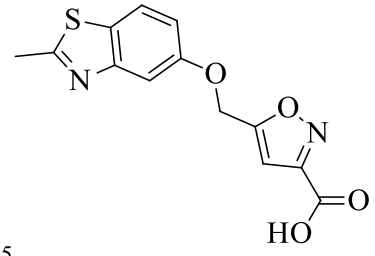
10n: MIC (MTB) = 0 .19 µM
MIC (MDR-TB) = 0.04 µM

Haung *et al* synthesized a series of potent 5-(2-methylbenzothiazol-5-yloxy-methyl)isoxazole-3-carboxamide derivatives starting from the commercially available benzothiazole on reaction with 5-bromomethylisoxazole-3-carboxylic acid ethyl ester in presence of K_2CO_3 and catalytic amount of TBAI obtained compound **5**, the basic hydrolysis of **5** in MeOH gave the

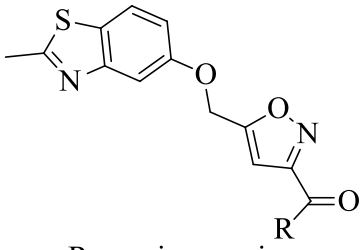
corresponding carboxylic acid **6** which was coupled with various amines using the amide coupling agents to generate the desired compounds **7a-w**.



5



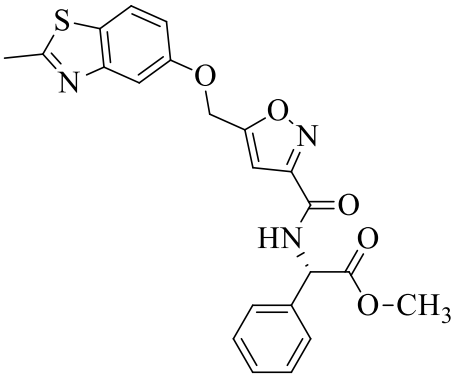
6



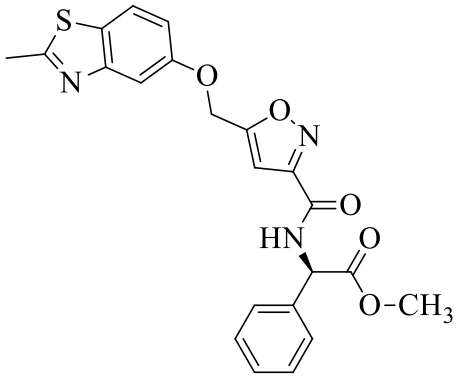
R= various amine groups

7a-w

All the synthesized compounds were then evaluated for their antimycobacterial activity against *M. tuberculosis* H37Rv using the microplate Alamar blue (MABA) assay method. All the synthesized compounds had shown the MICs ranging from 1.4-128 µM. Among the synthesized compounds **7j** and **7s** were found to be the most active compounds with MIC values 1.4 and 1.9 µM respectively. All the active compounds were tested for the cytotoxicity with Vero cells and found to be nontoxic toward Vero cells (IC_{50} >128 µM) [Huang Q., *et al*., 2009].



7j, MTB MIC =1.4 µM



7s, MTB MIC =1.9 µM

2.3.6. Literature survey on 2-aminothiazoles as anti-tubercular agents

Thiazolamine is one of the most important scaffolds in heterocyclic chemistry, drug design and discovery and is widely found in various pharmacologically active products and in some naturally occurring compounds. 2-Aminothiazol scaffold has structural similarity with thiolactomycin (TLM), a naturally occurring and synthetically challenging antibiotic. 2-

Aminothiazol and its derivatives have been used as precursors for the synthesis of various biologically active compounds. 2-Aminothiazol scaffold plays a vital role in various drug structures shown in **Figure 2.6**.

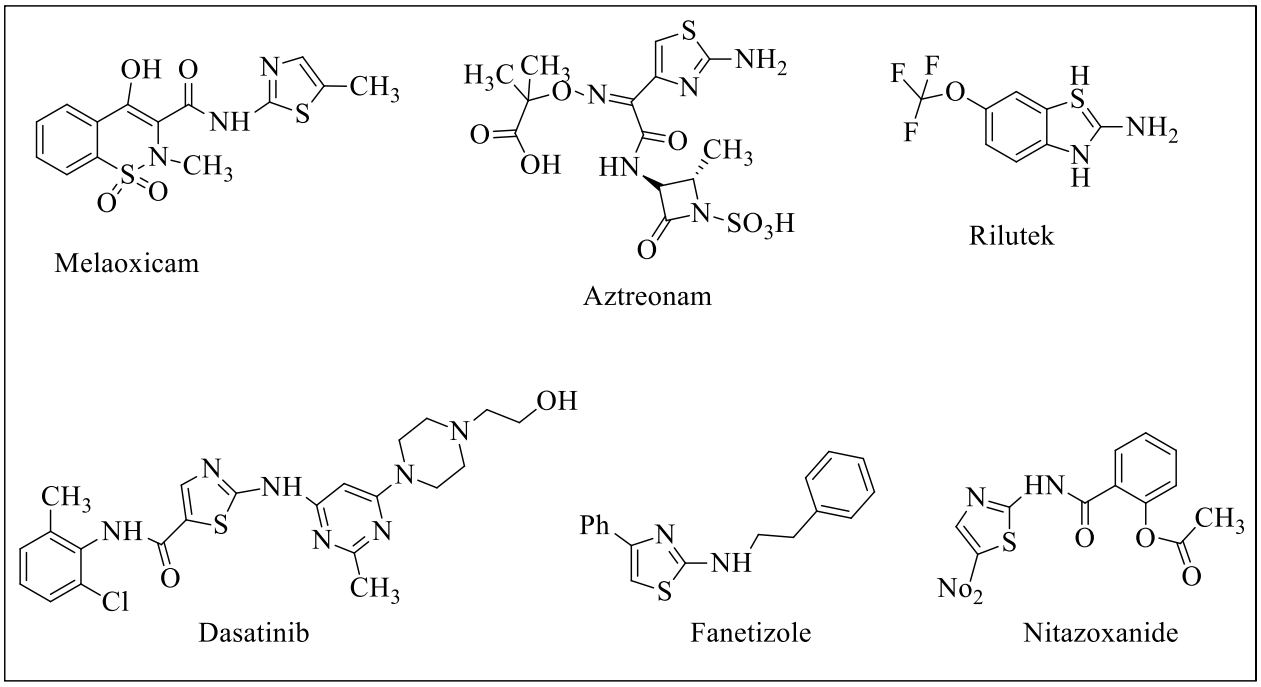
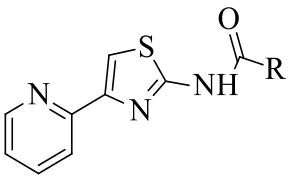
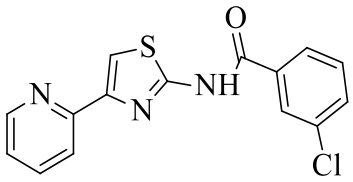


Figure 2.6. Drugs containing aminothizole nucleus

In order to develop new anti-tubercular agents Meissner A *et al* synthesized a series of 2-aminothiazoles and evaluated for their anti-tubercular activity against *M. tuberculosis* H37Rv. It was found that almost all the synthesized molecules displayed significant anti-tubercular activity and one of the compounds 3-chloro-*N*-(4-(pyridin-2-yl)thiazol-2-yl)benzamide (**55**) found as the most promising analogues with MIC of 0.024 µM or 0.008 µg/mL in 7H9 media and therapeutic index of **55** is about 300 and was rapidly metabolized by human liver microsomes with $t_{1/2}$ = 28 min [Meissner A., *et al*., 2013].

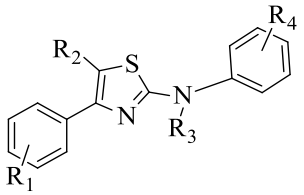


R= various substituents

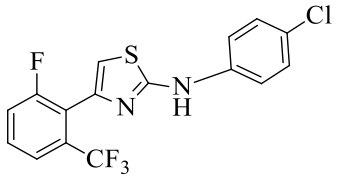


55, MTB MIC = 0.024 µM or 0.008µg/mL

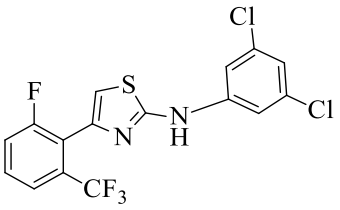
Pieroni M *et al* synthesized a series containing 38 compounds of aminothiazole derivtives and evaluated for their anti-tubercular activity against both the resistant as well as persistant(H37Rv) strains of *M. tuberculosis* using MABA method. The compounds showing an encouraging results were furthur tested in low oxygen recovery assay (LORA) it is an *in vitro* model for the preliminary assessment of activty against the persistant *M. tuberculosis* phentype. The two compounds **4d** and **4h** found to be the most active compounds with MIC values 19.2 µM (MABA) or 31.5 µM (LORA) and 15.0 µM (MABA) or 15.2 µM (LORA) respectively [Pieroni M., *et al*., 2013].



R_1, R_2, R_3, R_4 = various substituents

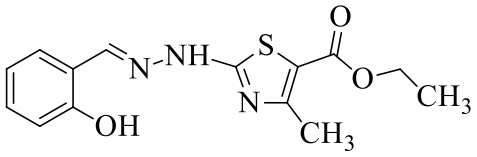


4d, MTB MIC = 19.2 µM

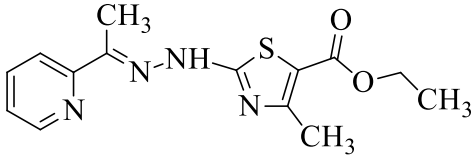


4h, MTB MIC = 31.5 µM

In order to discover new potent inhibitors for *M. tuberculosis* a series of 2-(2-hydrazinyl)thiazole derivatives were designed and synthesized by Makam P *et al*. All the synthesized compounds were evaluated for their inhibitory potential against *M. tuberculosis* H37Rv. Among the tested compounds (*E*)-ethyl 2-(2-(2-hydroxybenzylidene)hydrazinyl)-4-methylthiazole-5-carboxylate (**2i**) and (*E*)-ethyl 4-methyl-2-(2-(1-(pyridin-2-yl)ethylidene)hydrazinyl)thiazole-5-carboxylate (**4d**) were found to be potential molecules against *M. tuberculosis* H37Rv with MIC values 25 µM and 12.5 µM respectively [Makam P., *et al.,* 2013].



2i, MTB MIC = 25.0 µM



4d, MTB MIC = 12.5 µM

Some more compounds reported as potent inhibitors of *M. tuberculosis* are shown in **Figure 2.7** [Makam P., *et aI.,*2013].

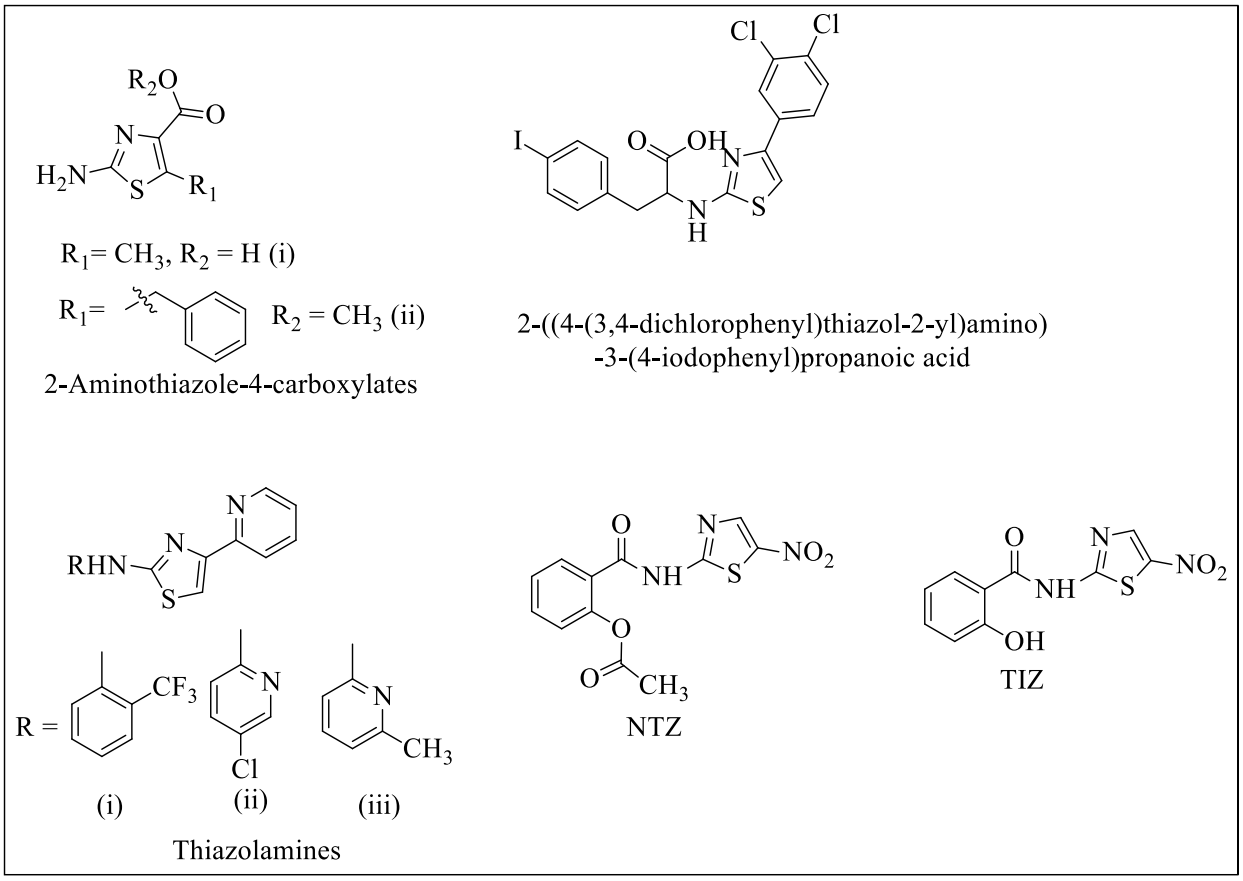
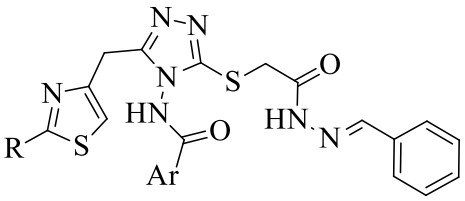
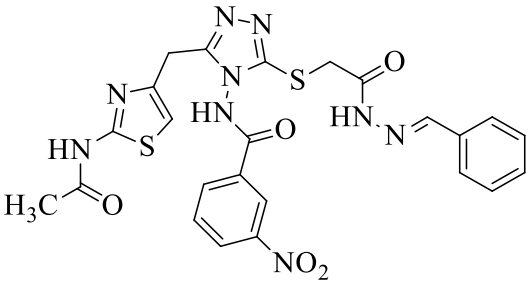


Figure 2.7: Potent aminothiazole derivatives against *M. tuberculosis*

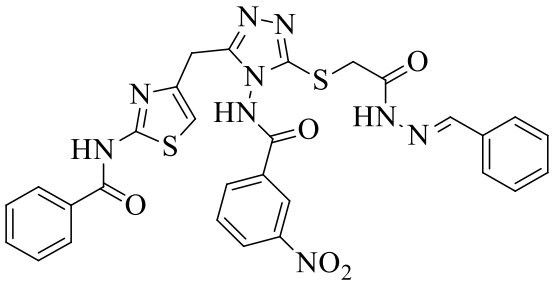
N-{4-[(4-amino-5-sulfanyl-4*H*-1,2,4-triazol-3-yl)methyl]-1,3-thiazol-2-yl}-2-substituted amide derivatives were synthesized and evaluated for their anti-tubercular activity against *M. tuberculosis* H37Rv strain using microdilution assay. Almost all the compounds displayed a significant anti-tubercular activity and two compounds **8f** and **8g** emerged as the most active compounds with MIC values 0.39 and 0.78 µM respectively [Shiradkar MR., *et al*., 2007].



R, Ar = various substituens



8f, MTB MIC = 0.78 μM



8g, MTB MIC = 0.39 μM

2.3.7. Literature survey on isatin/1*H*-indole-2,3-diones as anti-tubercular agents

Isatin/ 1*H*-indole-2,3-dione and its derivatives are biologically active and have signiicant importance in the medicinal chemistry. Isatin is an endogenous compound found in humans, and its effect has been studied in a variety of systems. Biological properties of isatin include a range of actions in brain and protect against certain types of infections. In recent years isatin derivatives reported to exhibit broad spectrum biological activities such as antiviral, anti-tubercular, antifungal and antibacterial.

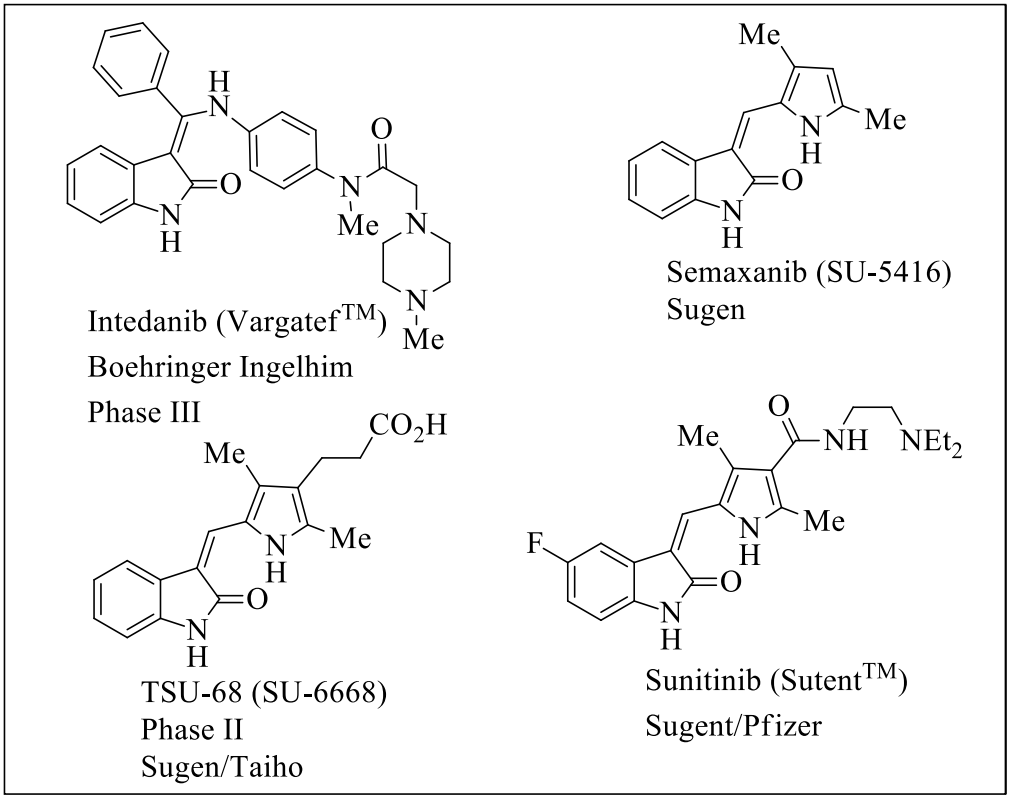
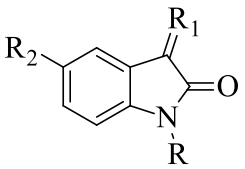
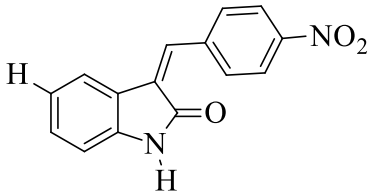


Figure 2.8: Drugs containing isatin/indole-2,3-dione

Jeankumar and co-workers synthesized a series of isatin derivatives and studied their *in vitro* ability to inhibit the Chorismate Mutase enzyme (MTB CM) and *in vitro* anti-tubercular activity against *M. tuberculosis* H37Rv. The *in vito* anti-tubercular activity was carried out by using MABA method and the cytotoxicity of the active compounds was tested against RAW 264.7 cells with MTT assay and it was found that the compounds were little or non-toxic. 3-(4-nitrobenzylidene)indolin-2-one (**24**) emerged as the most active compound with an MIC of 23.5 µM and was found to be non-toxic [Jeankumar VU., *et al*., 2014].



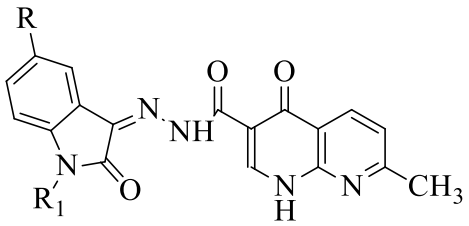
R,R1,R^2 = various substituents



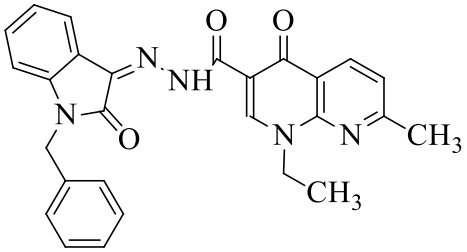
24, MTB MIC = 23.5 µM

Aboul-Fadl T *et al* designed and synthesized a series of Schiff bases of nalidixic acid carbohydrazide and isatin derivatives and screened against four mycobacterium strains :

Mycobacterium intercellulari, *Mycobacterium xenopi*, *Mycobacteriumchleneo*, *Mycobacterium smegmatis*. The compound **5f** found to be the potent anti-TB agent with MIC 0.625 µg/mL which is 20 times more potent than the reference drug INH, (MIC = 12.5 µg/mL) [Aboul-Fadl T., *et al*., 2010].

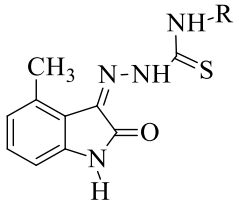


R = H, Br
R_1 = various substituents

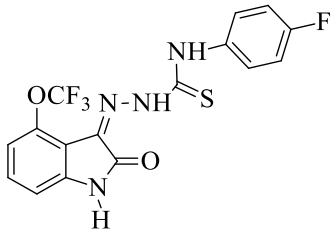


5f, MTB MIC = 0.625 µg/mL

Guzel O *et al* reported the anti-tubercular activity of a series of 5-methyl/trifluoromethoxy-1*H*-indole-2,3-dione-3-thiosemicarbazones, 1-methyl-5-methyl/trifluoromethoxy-1*H*-indole-2,3-dione-3-thiosemicarbazones and 5-trifluoromethoxy-1-morpholinomethyl -1*H*-indole-2,3-dione-3-thiosemicarbazones and screened for their *in vitro* anti-tubercular activity against *M. tuberculosis* H37Rv in BACTEC 12B medium using a broth microdilution assay, MABA method and the cytotoxicity of the compounds were tested toward VERO cells using MTT assay. It was found that some of the compounds were found to be the potent inhibitors of *M. tuberculosis* growth with the IC_{90} values ranging from 0.795 µg/mL to 1.587 µg/mL. *N*-cyclohexyl-2-(4-methyl-2-oxoindolin-3-ylidene)hydrazinecarbothioamide (**6c**) was found to be the most active compond with an IC_{90} of 0.795 µg/mL [Guzel O., *et al*., 2008].



3b; R = C_4H_9, IC_{90} (MTB) = 0.911 µg/mL
3d; R = 4-FC_6H_4, IC_{90} (MTB) = 1.587 µg/mL
3f; R = 3-BrC_6H_4, IC_{90} (MTB) = 1.489 µg/mL
6c; R = cycl-C_6H_{11}, IC_{90} (MTB) = 0.795 µg/mL
6d; R = C_6H%, IC_{90} (MTB) = 1.433



3q; R = 4-FC6H4, IC90 (MTB) = 1.546 µg/mL
3r; R = 4-ClC6H4, IC90 (MTB) = 1.521 µg/mL
3s; R = 4-BrC6H4, IC90 (MTB) = 1.530 µg/mL

The thorough search of literature of existing drugs, drugs which are in pipeline and other promising reported pro-drugs revealed that,

1. There are many standard drugs for the treatment of drug-susceptible TB, but the cleverest mycobacterium has developed the resistance to all the existing drugs.

2. There are many novel classes of heterocyclic compounds possessing promising anti-TB activity, but their mechanism of action is not much explored.

3. The re-design and SAR studies of reported anti-TB compounds, study of their mechanism of action will be fruitful in the discovery of novel anti-TB drugs to act with novel mechanism of action.

2.4. "*M. tuberculosis* PknB" – A novel and interesting target for anti-TB drug discovery

Protein kinase B (PknB) is a serine/threonine protein kinase and in *M. tuberculosis* serine/threonine protein kinases have been estimated to phosphorylate several hundred proteins, and over 250 serine/threonine protein kinases have been identified recently [Chao J., *et al*., 2010]. PknB is essential for *M. tuberculosis* growth as it phosphorylates diverse substrates including proteins involved in the peptidoglycan synthesis, cell division, the stress response, transcription, metabolic control, and mycolic acid layer synthesis [Parikh A., *et al*., 2009; O'Hare HM., *et al*., 2008].

The *M. tuberculosis* genome has 11 putative eukaryotic type serine/threonine kinases PknA to PknL and three phosphoproteinphosphatases. While two of the 11 serine/threonine protein kinases PknG and PknH are soluble kinases, nine are predicted transmembrane "receptor-like" proteins with an N-Terminal eukaryotic like kinase domain linked through a single transmembrane helix to an extracellular sensor domain [Parikh A., *et al*., 2009]. Protein kinase A (PknA) and PknB contain putative transmembrane domains and hence are predicted to localize to the cell membrane. PknA and PKnB are activated upon the auto phosphorylation of serine/threonine residues in the activation loop and are inactivated by the sole protein threonine phosphatase [Park ST., *et al*., 2008].

Adenosine triphosphate (ATP) is an important component of cellular processes. Many ATP enzymes especially protein kinases have been recently investigated. Protein kinases are the enzymes that catalyze the transfer of the γ-phosphate of ATP to the hydroxyl group on serine, threonine or tyrosine residue in the target protein varying their activity as a result. This process is called phosphorylation and is reversed by the action of phosphatases, which remove phosphoryl moieties from target proteins [Duran R., *et al*., 2005]. Protein kinases are a diverse class of proteins that have been shown to play a critical role in regulating cellular processes by transmitting extracellular cues/intracellular signals to their downstream substrates by phosphorylation of serine, threonine, or tyrosine residues on the substrates. [Tiwari D., *et al*., 2009]. Based on the amino acids they phosphorylate, the two main classes of kinases are tyrosine kinases which phosphorylate tyrosine, and serine-threonine protein kinases (STPKs) which phosphorylate serine or threonine.

An optimal anti-bacterial drug should kill as many bacteria as possible and it should be safe for normal human microbiota such an activity profile is not possible but efforts should be made to achieve this ideal situation. This goal can be achieved by selective targeting of STPKs with novel drug. The ATP binding site is widely used as target for small molecular weight modulators of protein kinase activity [Zakharevich NV., *et al*., 2012; Noble ME., *et al*., 2004].

M. tuberculosis has a rigid cell wall that forbids the passage of nutrients into and excreted from the cell. The cell envelop comprises of a peptide layer and free lipids also a complex structure of fatty acid layer such as mycolic acid. The rigidity of the cell wall is owing to the presence of mycolic acid layer [Chakraborti PK., *et al*., 2011]. PknB regulates major metabolic pathways directly via the phosphorylation of various protein substrates, such as protein regulator GarA that shuts down the TCA cycle [O'Hare HM., *et al*., 2008], a number of proteins required for the synthesis of mycolic acid a key component of the *M. tuberculosis* cell wall, these proteins include malanoyl-CoA:ACp transacylase FabD; the β-ketoacyl ACP synthase, FabH, KasA and KasB; enoyl ACP reductase,

InhA, MabA, MmaA4/Hma, KasA, InhA, MabA [Veyron-Churlet R., et al., 2009]. and Wag31 proteins required for cell division and morphology [Kang CM., *et al*., 2005], PknB also known to play a role in regulating SigH, the stress-response sigma factor, via phosphorylation of the anti-sigma factor RshA [Greenstein AE., *et al*., 2007] and cell wall biosynthetic enzyme GlmU or

PBPA9 [Park ST., *et al*., 2008; Parikh A., *et al*., 2009], PknB modulates the peptidoglycan synthesis by regulating the acetyl transferase activity of GlmU, so the GlmU is a novel target of PknB [Park ST., *et al*., 2008]. Recently it has been proposed that PknB might be involved in latency exit.

Crystal structure analysis of PknB kinase domain found to possess two lobes N- and C-teminal lobes. The N-terminal lobe found to have the ATP binding site, where as the C-terminal is involved in rendering an active state and in stabilizing interactions with the substrate [Lombana TN., *et al*., 2010]. The extra cellular domain of PknB is presaged to have four conserved PASTA (pencillin-binding protein and serine/threonine kinase-associated) domains ([Young TA., *et al*., 2003; Yeats C., *et al*., 2002]. This domain has been proposed to play a role in the identification of D-alanyl-D-alanine dipeptides used to develop the peptidoglycan layer [Chawla Y., *et al*., 2014].

PknB has been regarded as an attractive target for the development of anti-TB agents because this enzyme is essential for growth of bacteria and survival of the pathogen within the host [Fernandez P., *et al*., 2006, Wehenkel A., *et al*., 2006]. Moreover, PknB possesses low sequence identity of 21% as compared to human kinase, which provides anticipating selectivity for the mycobacterial kinase [Young TA., *et al*., 2003]. Inhibitors of PknB are capable to target PknB from exterior of *M. tuberculosis* cell, avoiding the problem of poor cell wall permeability [Lougheed KE., *et al*., 2011]. Because of poor cell wall permeability many anti-TB agents with anticipating enzyme potency exhibit weak or no cellular activity against *M. tuberculosis* [Freundlich JS., *et al*., 2009; Boyne ME., *et al*., 2007; Sullivan TJ., *et al*., 2006]. With a remarkable inhibition mechanism, PknB inhibitors would be strong candidates for future, effective anti-TB drugs [Punkvang A., et al., 2015].

We have selected the much unexplored and novel target PknB, as our target for the synthesis of novel anti-TB compounds.

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 *M. tuberculosis* PknB inhibitors as anti-tubercular agents having superior qualities over the existing in terms of potency against drug resistant bacteria.

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 M. tuberculosis* PknB enzyme inhibition assays.

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

3.2. Plan of work

The plan of work was classified into the following categories:

3.2.1. Designing of novel *M. tuberculosis* PknB inhibitors

For designing the novel *M. tuberculosis* PknB inhibitors we have 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 approaches will be taken up for synthesis in our laboratory using previously reported methodologies available in literature for structurally related molecules. Wherever possible we will carry out reactions using microwave assisted methods for less exposure of hazardous chemicals/vapours to the environment. Most of the synthesized molecules will be purified by trituration, recrystallization techniques and flash chromatography with lesser amount of solvents for eco-friendly conditions.

Characterization: characterization of the synthesized compounds will be 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 will be carried out against *M. tuberculosis* H37Rv bacteria. This test will be performed using micro plate alamar blue assay (MABA) method.

3.2.4. Enzymatic evaluation of synthesized compounds

The synthesized compounds will be subjected to *M.tubberculosis* PknB enzyme inhibition studies.

3.2.5. *In vitro* cytotoxicity screening

The synthesized compounds will be 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.

Materials and Methods

Chapter 4

4.1. Designing of the molecules

For designing the new anti-TB molecules we have 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 these sub-units, leads to the design of new hybrid architectures that maintain 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 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 1,3,4-thiadiazole derivatives as novel M. tuberculosis PknB inhibitors

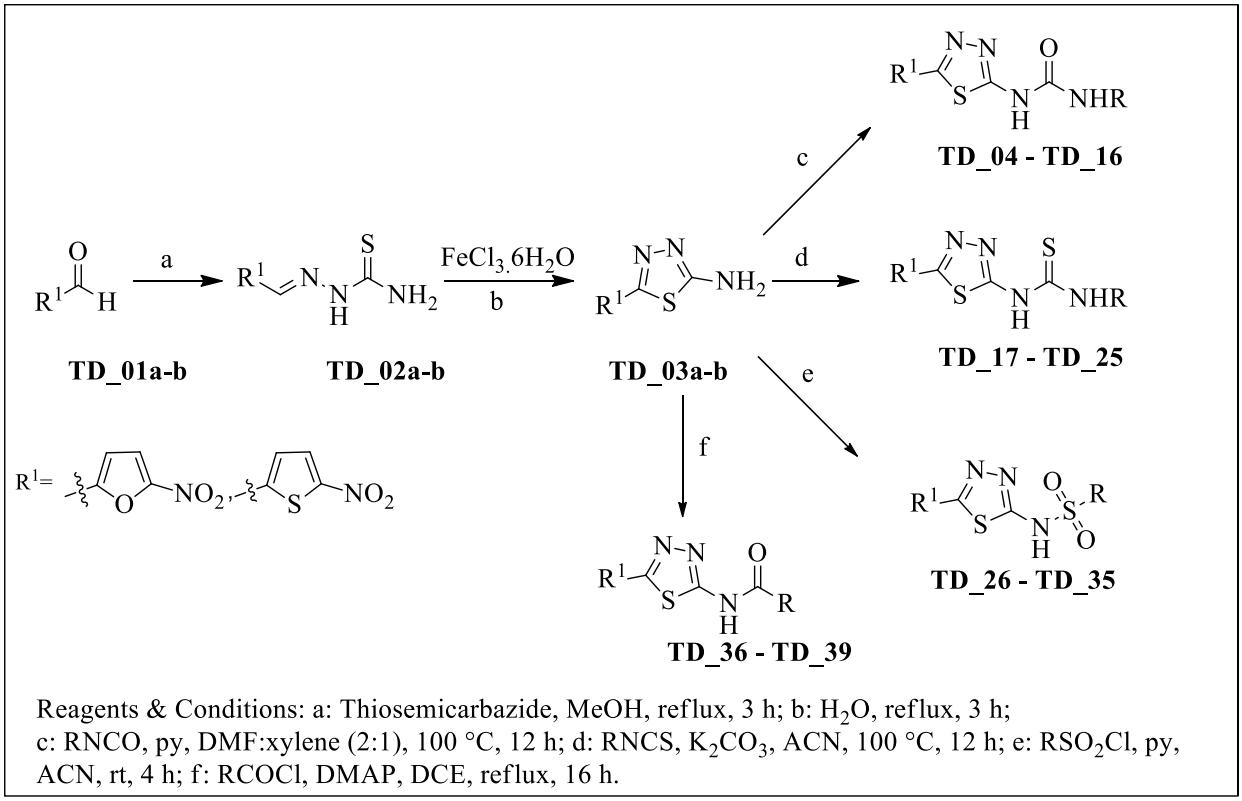
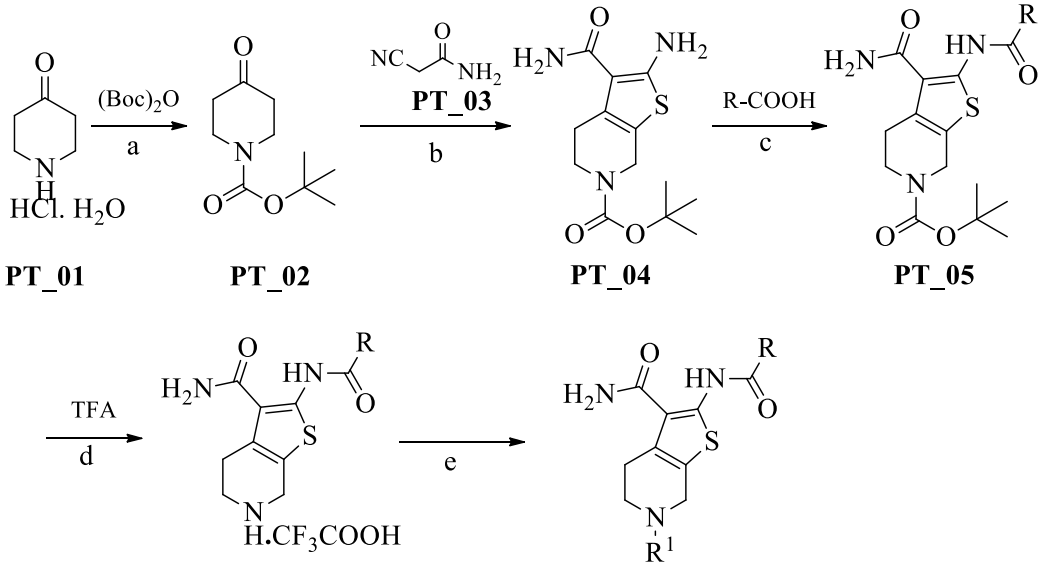


Figure 4.1: Synthetic protocol utilized for the synthesis of compounds **TD_04 – TD_39**

Scheme – 2: Synthesis of tetrahydrothieno[2,3-c]pyridine-3-carboxamides and hexahydrocycloocta[b]thiophene-3-carboxamides as novel M. tuberculosis PknB inhibitors

2a: Synthesis of tetrahydrothieno[2,3-c]pyridine-3-carboxamides



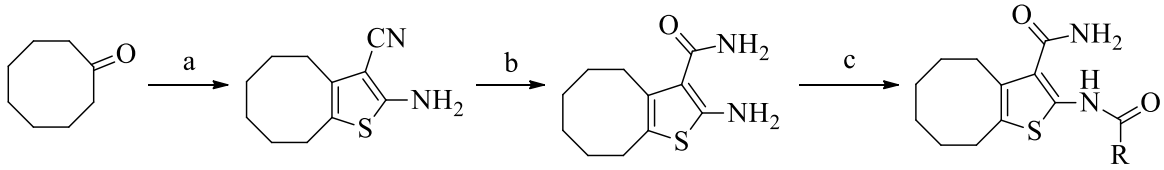
PT_01 **PT_02** **PT_04** **PT_05**

PT_06

R^1 = Ac: **PT_07 - PT_16;**
R^1 = CH_3: **PT_17 - PT_26:**

Reagents & Conditions: a: $(Boc)_2O$, Et_3N, DCM, MeOH, rt; b: S_8, Morpholine, EtOH, rt, 6h;
c: EDCI, HOBt, Et_3N, DCM, rt, 9h; d: TFA, DCM, rt, 2h; e: R^1 = Acetyl; Acetylchloride, Et_3N, CH_2Cl_2 and R^1 = Methyl, HCHO, $Na(OAc)_3BH$, MeOH, H_2O, rt, 2h.

2b: Synthesis of hexahydrocycloocta[b]thiophene-3-carboxamides



OT_01 **OT_02** **OT_03** **OT_04 - OT_13**

Reagents & Conditions: a: Malanonitrile, S_8, Et_3N, DMF, rt; b: Conc H_2SO_4, rt, 6 h; c: RCOOH, EDCI, HOBt, Et_3N, DCM, rt, 9 h.

Figure 4.2: Synthetic protocol utilized for the synthesis of compounds **PT_07 – PT_26** and **OT_04 – OT_13**

Scheme – 3: Synthesis of 2-(phenylimino) thiazolidin-4-one derivatives as M. tuberculosis PknB inhibitors

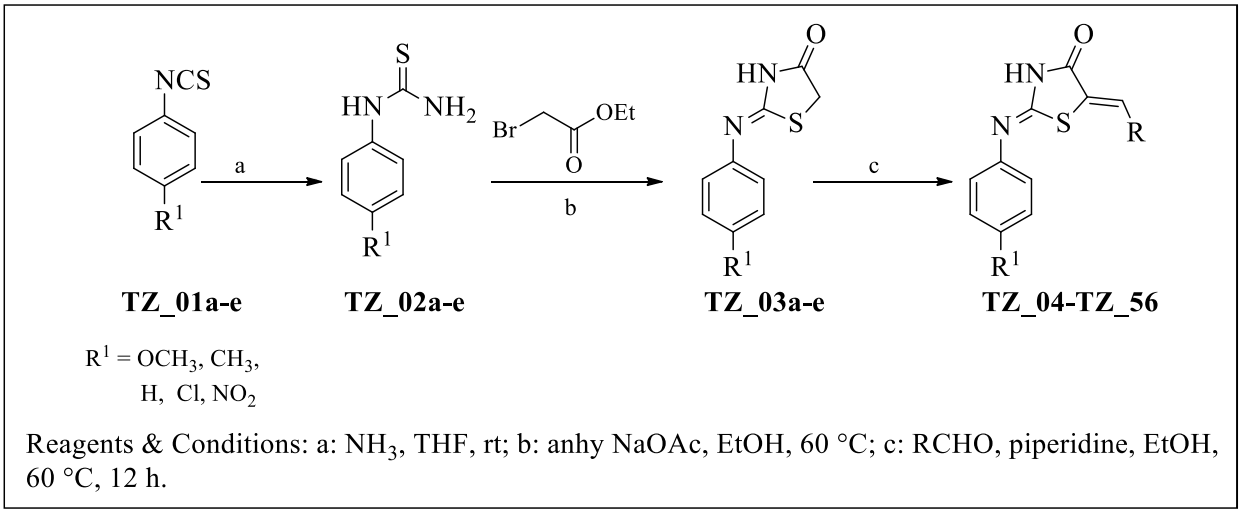


Figure 4.3: Synthetic protocol utilized for the synthesis of compounds **TZ_04 – TZ_56**

Scheme – 4: Synthesis of 2-phenylbenzo[d]oxazole derivatives as novel M. tuberculosis PknB inhibitors

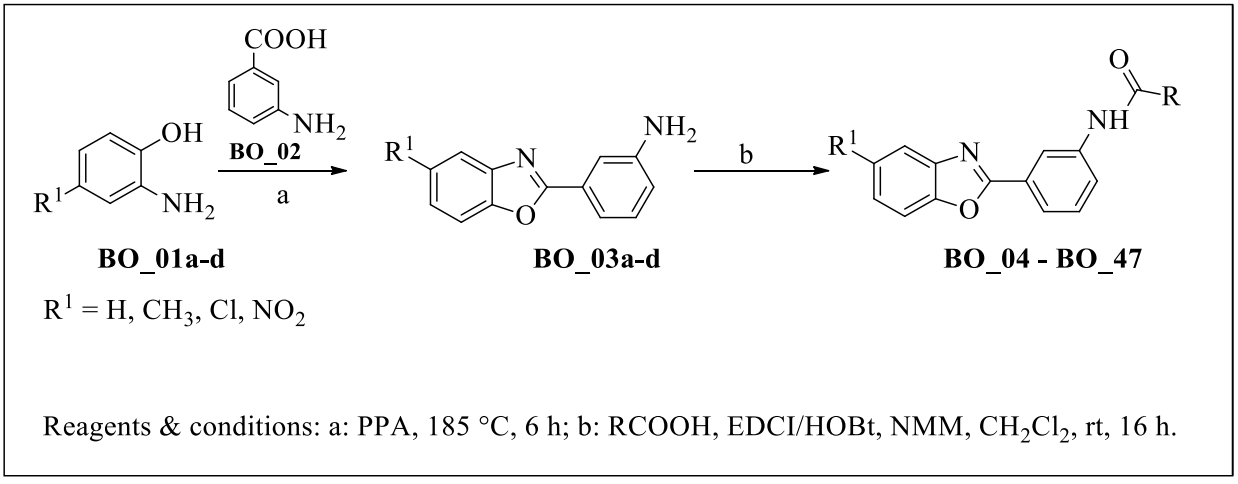


Figure 4.4: Synthetic protocol utilized for the synthesis of compounds **BO_04 – BO_47**

Scheme – 5: Synthesis of 2-phenylbenzo[d]thiazole derivatives as novel M. tuberculosis PknB inhibitors

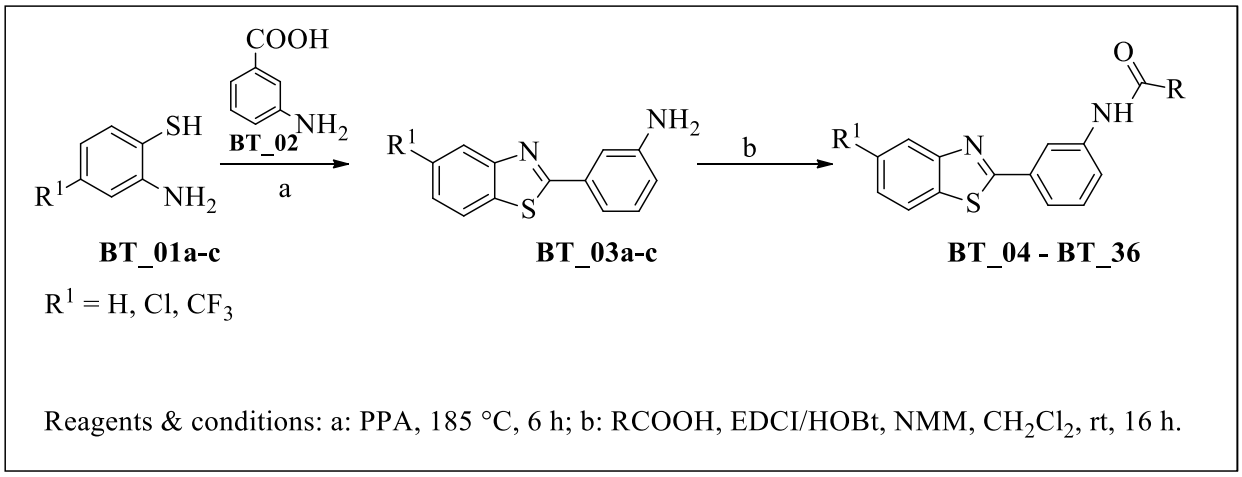


Figure 4.5: Synthetic protocol utilized for the synthesis of compounds **BT_04 – BT_36**

Scheme – 6: Synthesis of N,N-di-substituted-2-aminothiazole derivatives as novel M. tuberculosis PknB inhibitors

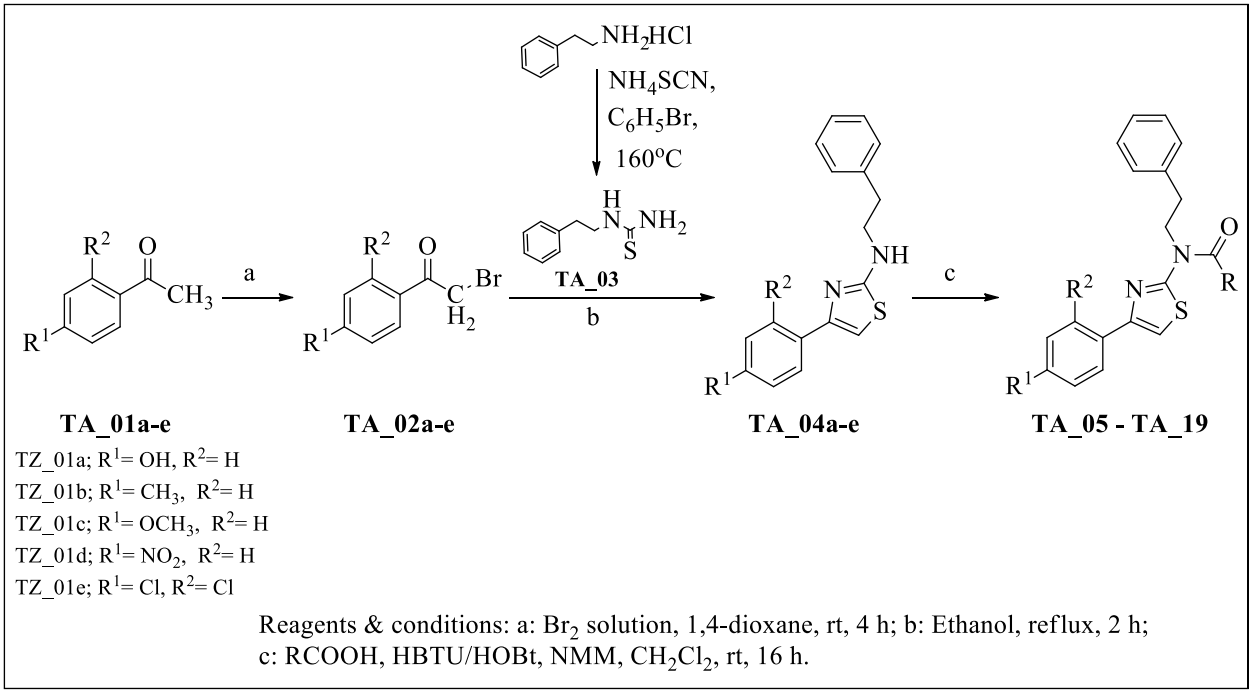


Figure 4.6: Synthetic protocol utilized for the synthesis of compounds **TA_05 – TA_19**

Scheme – 7: Synthesis of 3-hydrazinyl-5H-[1,2,4]triazino[5,6-b]indole derivatives as M. tuberculosis PknB inhibitors

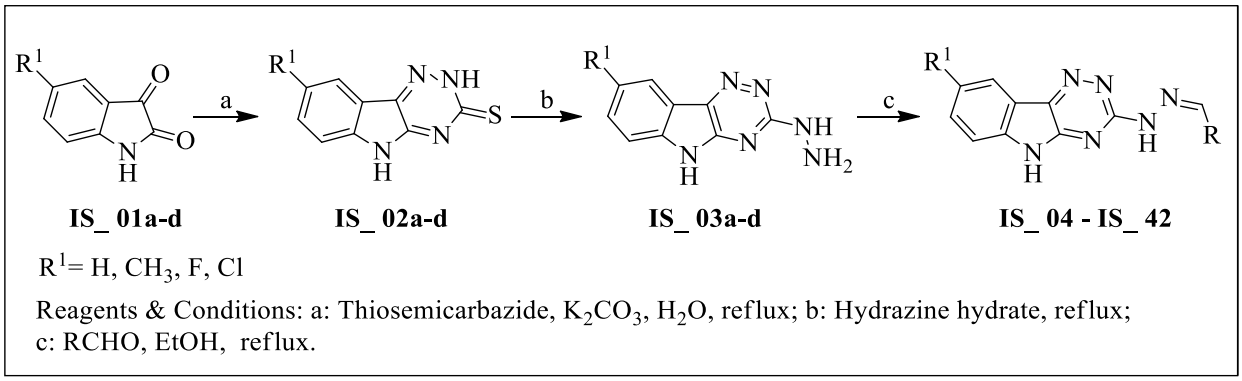


Figure 4.7: Synthetic protocol utilized for the synthesis of compounds **IS_04 – IS_42**

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 48 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* PknB assay

All the synthesized compounds were evaluated for i*n vitro* enzymatic inhibition studies against PknB according to the previously reported procedure [Chawla Y., *et al*., 2014; Tiwari D., *et al*., 2009]. *In vitro* PknB assay was performed in 40 µl reaction containing 25 mM HEPES-NaOH, pH 7.4, 20 mM magnesium acetate, 20 µM $MnCl_2$, 1 mM DTT, 200 µM sodium orthovanadate, 100 µM cold ATP, 10 µCi of [$_{\gamma}$-^{32}P]ATP, and 100 pmol of GarA or other substrates such as myelin basic protein (Mbp), with or without 2.5 pmol of PknB for 10 min at 30 °C. The reaction

was stopped by adding 15 µl of 4× SDS sample buffer followed by heating at 95 °C for 5 min. Reaction was resolved on 12% SDS-PAGE gels, and either transferred to nitrocellulose membrane or stained with Coomassie/silver and exposed to x-ray films for autoradiography. For quantitation, the desired bands were cut from the gels, soaked in scintillation mixture W (Spectrochem), and counts were taken using a liquid scintillation counter (Packard analyzer Tri-carb 2100 TR).

Results and Discussion

Chapter 5

5.1. Development of 5-heteroarylsubstituted 2-amino 1,3,4-thiadiazoles as novel *M. tuberculosis* PknB inhibitors

'The most fruitful basis for the discovery of a new drug is to start with an old drug.'

– Sir James Whyte Black, (1988 Nobel Prize in medicine)

Among the five membered heterocycles with three heteroatoms, thiadiazoles are particularly important in view of medicinal chemistry. In literature molecules containing thiadiazole pharmacophore possess various biological activities *viz*. antibacterial, anticancer, anti-tubercular, antiviral, antifungal, anti-helicobacter pylori, anticonvulsant activity, anti-inflammatory and anti-leishmanial activity [Jain AK., *et al*., 2013; Padmavathi V., *et al*., 2009], especially their potency against cancer and tuberculosis have been much studied [Padmavathi V., *et al*., 2008]. Out of four isomeric forms of thiadiazoles (1,2,3-thiadiazole, 1,2,5-thiadiazole, 1,2,4-thiadiazole and 1,3,4-thiadiazole) 1,3,4-thiadiazole version had spurred lot of interest among the medicinal chemists owing to its broad spectrum of activity and chemical feasibility. On the other hand, five membered heterocycles with one heteroatom containing electron withdrawing nitro substitution such as 5-nitro furan and 5-nitro thiophene ring systems were found to an add on for the activity, found in many biological potent molecules which are mainly antibacterial, anti-microbial and anti-tubercular [Jazayeri S., *et al*., 2008; Foroumadi A., *et al*., 2003].

5.1.1. Design of the molecules

In this work, we have chosen the most active anti-tubercular reported 1,3,4-thiadiazoles (**5b** and **6a**) [Foroumadi A., *et al*., 2002] and the most active molecule (**BITS-NR-79**) from our in-house library for design of novel molecules by subjecting to molecular hybridization method, to exhibit enhanced activity (**Figure 5.1**). The 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 leads to the design of new hybrid architectures that maintain pre-selected characteristics of the original templates.

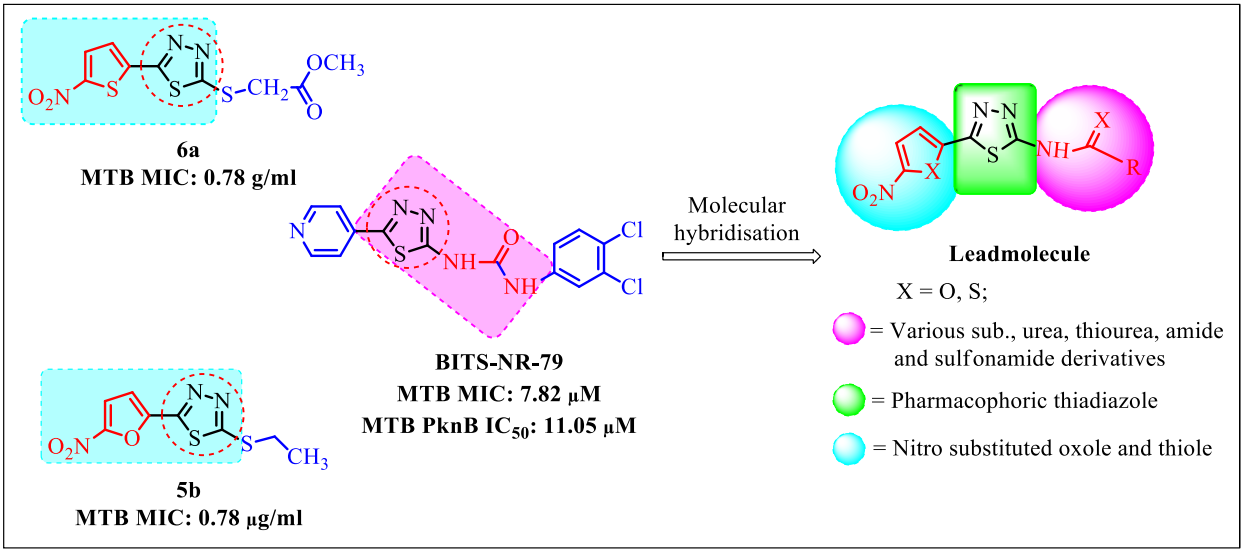


Figure 5.1: Design of lead molecule by molecular hybridization approach

5.1.2. Experimental procedures utilized for the synthesis of TD_04 – TD_39

The target molecules were synthesized by following a three step synthetic protocol (**Figure 4.1**), wherein the first step of the reaction was of 5-nitrofuran-2-carbaldehyde/5-nitrothiophene-2-carbaldehyde **(TD_01a-b)** with thiosemicarbazide in methanol under reflux conditions to yield the corresponding thiosemicarbazone **(TD_02a-b)** in good yield. In the next step, the oxidative cyclisation of **(TD_02a-b)** was carried out using an oxidizing agent $FeCl_3.6H_2O$ in water under reflux conditions. The separated solid was filtered and washed with excess of water, cold ethanol and diethyl ether and dried to afford the **(TD_03a-b)** as yellow solids mechanism of cyclization is shown in **Figure 5.2**. Further **(TD_03a-b)** on reaction with various substituted aryl/hetero aryl isocyanates in DMF/xylene (2:1) in presence of pyridine at 100 °C, isothiocyanates in CH_3CN in presence of K_2CO_3 at 100 °C, sulfonyl chlorides in CH_3CN presence of pyridine at room temperature and acid chlorides in DCE in presence of DMAP under reflux conditions produced the corresponding urea derivatives **(TD_04 - TD_16)**, thiourea derivatives **(TD_17 - TD_25)**, sulfonamide derivatives **(TD_26 - TD_35)** and amide derivatives **(TD_36 - TD_39)**.

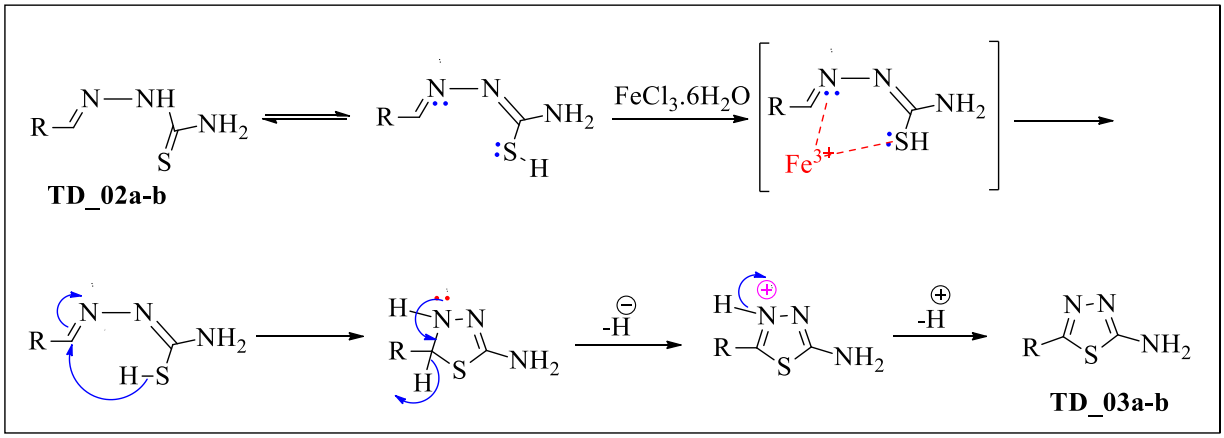
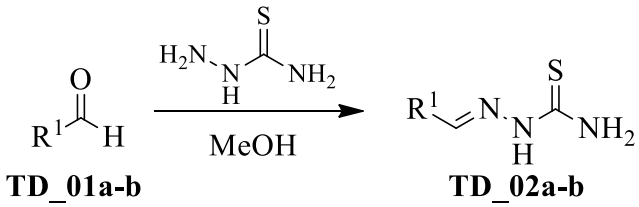
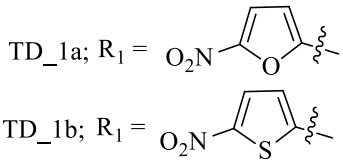


Figure 5.2: Mechanism of conversion of compound **TD_02a-b** to **TD_03a-b**

General procedure for the preparation of TD_02a-b





Thiosemicarbazide (1.0 equiv) was added to the stirred solution of corresponding hetero aromatic aldehydes (**TD_01a-b)** (1.0 equiv) in methanol and allowed to reflux for 3 hours. Then the reaction mixture was cooled to room temperature and the solids formed in the reaction mixture was filtered, washed with hexanes and dried to afford the compounds **TD_02a-b** as white solid.

2-((5-Nitrofuran-2-yl)methylene)hydrazinecarbothioamide (TD_02a)

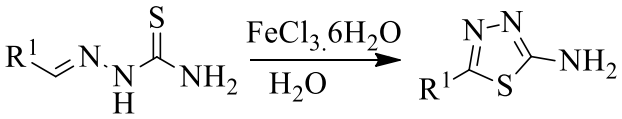
Following the general procedure the product was synthesized from 5-nitrofuran-2-carbaldehyde **TD_01a** (7.5 g, 35.46 mmol), thiosemicarbazide (3.23 g, 35.46 mmol) in methanol and resulting solid washed with hexanes to produce 2-((5-nitrofuran-2-yl)methylene)hydrazinecarbothioamide

(TD_02a) (6.31 g, 98%) as white solid. ESI-MS showed 215 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.84 (s, 1H), 8.52 (s, 1H), 8.03 (s, 1H), 7.97 (s, 1H), 7.79 (d, *J* = 3.9 Hz, 1H), 7.37 (d, *J* = 4.2 Hz, 1H).

2-((5-Nitrofuran-2-yl)methylene)hydrazinecarbothioamide (TD_02b)

White solid (Yield: 98%). ESI-MS showed 231 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.82 (s, 1H), 8.50 (s, 1H), 8.00 (s, 1H), 7.96 (s, 1H), 7.72 (d, *J* = 3.8 Hz, 1H), 7.28 (d, *J* = 4.0 Hz, 1H).

General procedure for the preparation of TD_03a-b



TD_02a-b **TD_03a-b**

Compounds **TD_02a-b** (1.0 equiv) was added to the dissolved solution of $FeCl_3.6H_2O$ (5.0 equiv) in water (20 vol) and allowed to reflux for 12 h. The solids obtained were filtered washed with excess of water, ethanol and diethyl ether and dried to afford the respective compounds **TD_03a-b** as yellow solids and used for the next step.

5-(5-Aminofuran-2-yl)-1,3,4-thiadiazol-2-amine (TD_03a)

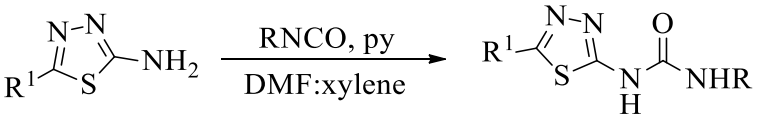
Following the general procedure the product was synthesized from 2-((5-nitrofuran-2-yl) methylene)hydrazinecarbothioamide **(TD_02a**) (7.50 g, 35.04 mmol), $FeCl_3.6H_2O$ (42.00 g, 175.23 mmol) in water. The compound 5-(5-aminofuran-2-yl)-1,3,4-thiadiazol-2-amine **(TD_03a**) (5.20 g, 96%) produced as yellow solid. ESI-MS showed 183 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.91–7.85 (m, 3H), 7.33 (s, 1H).

5-(5-Aminofuran-2-yl)-1,3,4-thiadiazol-2-amine (TD_03b)

Following the general procedure the product was synthesized from (2-((5-nitrothiophen-2-yl)methylene)hydrazinecarbothioamide **(TD_02b**) (3.3 g, 14.34 mmol), $FeCl_3.6H_2O$ (20.00 g, 71.73 mmol) in water. The compound 5-(5-aminothiophen-2-yl)-1,3,4-thiadiazol-2-amine

(TD_03b) (3.00 g, 93%) produced as yellow solid. ESI-MS showed 183 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.14 (d, J = 4.5 Hz, 1H), 7.88 (s, 2H), 7.54 (d, J = 4.5 Hz, 1H).

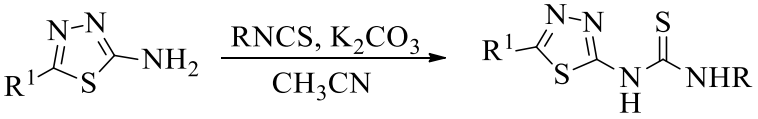
General procedure for the synthesis of compounds TD_04 - TD_16



TD_03a-b **TD_04 - TD_16**

A drop of pyridine was added to a stirred solution of compounds **TD_03a-b** (1.0 equiv) in DMF/xylene (2:1) and the appropriate aryl RNCO (1.30 equiv), allowed the reaction mixture to stir at 100 °C for 12 hours. The reaction mixture was cooled to room temperature and poured into the crushed ice, the resulting precipitate was filtered, washed with dilute HCl, ethanol followed by diethyl ether and dried to afford the desired compounds **TD_04 - TD_16** in pure form.

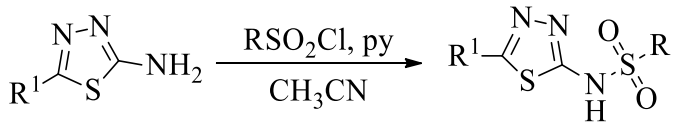
General procedure for the synthesis of compounds TD_17 - TD_25



TD_03a-b **TD_17 - TD_25**

K_2CO_3 (3.0 equiv) was added to the stirred solution of compounds **TD_03a-b** (1.0 equiv) in CH_3CN, then aryl RNCS (2.0 equiv) was added and allowed the reaction mixture to stir at 100 °C for 12 hours. Upon completion of the reaction the solvent was evaporated under reduced pressure, then re-dissolved the reaction mixture in CH_2Cl_2 and washed with water and brine solution. The separated organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated the solvent under reduced pressure, the crude compound obtained was purified by column chromatography using CH_2Cl_2/CH_3OH as an eluent to afford the desired compounds **TD_17 - TD_25** in pure form.

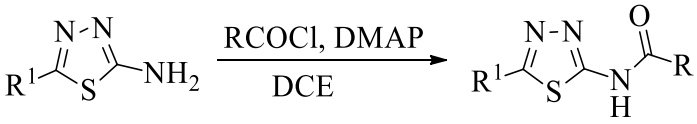
General procedure for the synthesis of compounds TD_26 - TD_35



TD_03a-b **TD_26 - TD_35**

Pyridine (1.1 equiv) was added to the stirred solution of compounds **TD_03a-b** (1.0 equiv) in CH_3CN, then the corresponding RSO_2Cl (3.0 equiv) was added and allowed the reaction mixture to stir at room temperature for 16 hours. Upon completion of the reaction the reaction mixture was quenched with 2M HCl and the solvent evaporated under reduced pressure, then re-dissolved the reaction mixture in EtOAc and washed with water and brine solution. The separated organic layer dried over anhydrous Na_2SO_4, filtered and evaporated the solvent under reduced pressure, the crude compound obtained was purified by column chromatography using EtOAc/hexane as an eluent to afford the desired compounds **TD_26 - TD_35** in pure form.

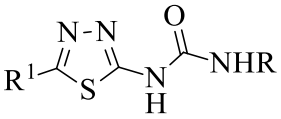
General procedure for the synthesis of compounds TD_36 - TD_39



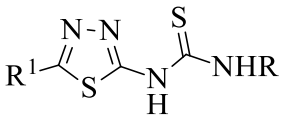
TD_03a-b **TD_36 - TD_39**

DMAP (2.0 equiv) was added to the stirred solution of compounds **TD_03a-b** (1.0 equiv) in dichloroethane (DCE), then the corresponding RCOCl (3.0 equiv) was added and allowed the reaction mixture to reflux for 16 hours. Upon completion of the reaction, solvent was evaporated under reduced pressure, then re-dissolved the reaction mixture in EtOAc and washed with water and brine solution. The separated organic layer dried over anhydrous Na_2SO_4, filtered and evaporated the solvent under reduced pressure; the crude compound obtained was purified by column chromatography using EtOAc/hexane as an eluent to afford the desired compounds **TD_36 - TD_39** in pure form (**Table 5.1**).

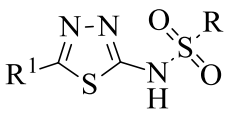
Table 5.1: Physiochemical properties of the synthesized compounds **TD_04 - TD_39**



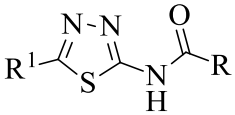
TD_04 - TD_16



TD_17- TD_25



TD_26 - TD_35



TD_36 - TD_39

Compd	R^1	R	Yield (%)	M.P. (°C)	Molecular formula	Molecular Weight
TD_04	5-nitrofuran-2-yl	Isopropyl	60	201-202	$C_{10}H_{11}N_5O_4S$	297.29
TD_05	5-nitrofuran-2-yl	Benzyl	91	252-253	$C_{14}H_{11}N_5O_4S$	345.33
TD_06	5-nitrofuran-2-yl	4-Tolyl	82	251-252	$C_{14}H_{11}N_5O_4S$	345.33
TD_07	5-nitrofuran-2-yl	4-Ethoxyphenyl	81	247-248	$C_{15}H_{13}N_5O_5S$	375.36
TD_08	5-nitrofuran-2-yl	4-Bromophenyl	79	220-221	$C_{13}H_8BrN_5O_4S$	410.20
TD_09	5-nitrofuran-2-yl	4-Nitrophenyl	88	270-271	$C_{13}H_8N_6O_6S$	376.30
TD_10	5-nitrothiophen-2-yl	Isopropyl	69	251-252	$C_{10}H_{11}N_5O_3S_2$	313.36
TD_11	5-nitrothiophen-2-yl	Phenyl	76	189-190	$C_{13}H_9N_5O_3S_2$	347.37
TD_12	5-nitrothiophen-2-yl	Benzyl	80	162-163	$C_{14}H_{11}N_5O_3S_2$	361.40
TD_13	5-nitrothiophen-2-yl	4-Tolyl	87	142-143	$C_{14}H_{11}N_5O_3S_2$	361.40
TD_14	5-nitrothiophen-2-yl	4-EthoxyPhenyl	74	184-185	$C_{15}H_{13}N_5O_4S_2$	391.42
TD_15	5-nitrothiophen-2-yl	4-Bromophenyl	69	274-275	$C_{13}H_8BrN_5O_3S_2$	426.27
TD_16	5-nitrothiophen-2-yl	4-Nitrophenyl	89	250-251	$C_{13}H_8N_6O_5S_2$	392.37
TD_17	5-nitrofuran-2-yl	Phenyl	84	223-224	$C_{13}H_9N_5O_3S_2$	347.37
TD_18	5-nitrofuran-2-yl	Benzyl	90	268-269	$C_{14}H_{11}N_5O_3S_2$	361.40
TD_19	5-nitrofuran-2-yl	4-Methoxyphenyl	88	277-278	$C_{14}H_{11}N_5O_4S_2$	377.40
TD_20	5-nitrofuran-2-yl	4-Fluorophenyl	76	201-202	$C_{13}H_8FN_5O_3S_2$	365.36
TD_21	5-nitrofuran-2-yl	4-chlorophenyl	78	172-173	$C_{13}H_8ClN_5O_3S_2$	381.82
TD_22	5-nitrothiophen-2-yl	Phenyl	87	206-207	$C_{13}H_9N_5O_2S_3$	363.44
TD_23	5-nitrothiophen-2-yl	Benzyl	93	238-239	$C_{14}H_{11}N_5O_2S_3$	377.46
TD_24	5-nitrothiophen-2-yl	4-Fluorophenyl	79	184-185	$C_{13}H_8FN_5O_2S_3$	381.43
TD_25	5-nitrothiophen-2-yl	4-chlorophenyl	84	194-195	$C_{13}H_8ClN_5O_2S_3$	397.88

Contd

Compd	R^1	R	Yield (%)	M.P. (°C)	Molecular formula	Molecular Weight
TD_26	5-nitrofuran-2-yl	4-Fluorophenyl	72	186-187	$C_{12}H_7FN_4O_5S_2$	370.34
TD_27	5-nitrofuran-2-yl	4-Bromophenyl	68	175-176	$C_{12}H_7BrN_4O_5S_2$	431.24
TD_28	5-nitrofuran-2-yl	4-Nitrophenyl	62	274-275	$C_{12}H_7N_5O_7S_2$	397.34
TD_29	5-nitrofuran-2-yl	4-Acetylphenyl	60	141-142	$C_{14}H_{10}N_4O_6S_2$	394.38
TD_30	5-nitrofuran-2-yl	2-Thiophenyl	63	155-156	$C_{10}H_6N_4O_5S_3$	358.37
TD_31	5-nitrothiophen-2-yl	4-Fluorophenyl	69	191-192	$C_{12}H_7FN_4O_4S_3$	386.40
TD_32	5-nitrothiophen-2-yl	4-Bromophenyl	63	174-175	$C_{12}H_7BrN_4O_4S_3$	447.31
TD_33	5-nitrothiophen-2-yl	4-Nitrophenyl	81	256-257	$C_{12}H_7N_5O_6S_3$	413.41
TD_34	5-nitrothiophen-2-yl	4-Acetylphenyl	83	214-215	$C_{14}H_{10}N_4O_5S_3$	410.45
TD_35	5-nitrothiophen-2-yl	2-Thiophenyl	72	185-186	$C_{10}H_6N_4O_4S_4$	374.44
TD_36	5-nitrofuran-2-yl	Phenyl	52	215-216	$C_{13}H_8N_4O_4S$	316.29
TD_37	5-nitrofuran-2-yl	2-Napthyl	55	224-225	$C_{17}H_{10}N_4O_4S$	366.35
TD_38	5-nitrothiophen-2-yl	Phenyl	51	227-228	$C_{13}H_8N_4O_3S_2$	332.36
TD_39	5-nitrothiophen-2-yl	2-Napthyl	54	235-236	$C_{17}H_{10}N_4O_3S_2$	382.42

5.1.3. Characterization of the synthesized molecules

1-Isopropyl-3-(5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-yl)urea (TD_04): Yield: 60%; m.p. 201–202 °C; MS(ESI) *m/z* 298 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.09 (s, 1H), 8.17(d, *J* = 4.2 Hz, 1H), 7.75 (d, *J* = 4.5 Hz, 1H), 6.59 (d, *J* = 5.4 Hz, 1H), 3.88–3.78 (m, 1H), 1.14 (d, *J* = 6.6 Hz, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 175.1, 167.3, 161.5, 153.5, 141.6, 139.1, 136.1, 15.6, 10.6 (2C). Anal. calcd for: $C_{10}H_{11}N_5O_4S$: C, 40.40; H, 3.73; N, 23.56 % Found C, 40.45; H,3.79; N, 23.61%.

1-Benzyl-3-(5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-yl)urea (TD_05): Yield: 91%; m.p. 252–253 °C; MS(ESI) *m/z* 346 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.08 (t, *J* = 7.5 Hz, 1H), 7.88(d, *J* = 3.9 Hz, 1H), 7.54 (d, *J* = 4.2 Hz, 1H), 7.34–7.28 (m, 5H), 4.78 (d, *J* = 5.7 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 183.4, 168.1, 165.3, 143.1, 137.8, 136.8, 136.2(2C), 134.5, 134.1, 129.4(2C), 118.3, 61.5. Anal. calcd for: $C_{14}H_{11}N_5O_4S$: C, 48.69; H, 3.21; N, 20.28 % Found C, 48.74; H,3.28; N, 20.36%.

1-(5-(5-Nitrofuran-2-yl)-1,3,4-thiadiazol-2-yl)-3-(*p*-tolyl)urea (TD_06): Yield: 82%; m.p. 251–252 °C; MS(ESI) *m/z* 346 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.08 (t, *J* = 7.5 Hz, 1H), 7.88(d, *J* = 3.9 Hz, 1H), 7.54 (d, *J* = 4.2 Hz, 1H), 7.34–7.28 (m, 5H), 4.78 (d, *J* = 5.7 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.5, 164.1, 157.2, 150.7, 141.9, 138.3, 133.1(2C), 131.8(2C), 126.1, 123.2, 119.1, 34.2. Anal. calcd for: $C_{14}H_{11}N_5O_4S$: C, 48.69; H, 3.21; N, 20.28 % Found C, 48.72; H,3.24; N, 20.34%.

1-(4-Ethoxyphenyl)-3-(5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-yl)urea (TD_07):

Yield: 81%; m.p. 247–248 °C MS(ESI) *m/z* 376 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.41 (s, 1H), 9.00 (s, 1H), 7.88 (d, *J* = 3.9 Hz, 1H), 7.53 (d, *J* = 3.9 Hz, 1H), 7.39 (d, *J* = 8.7 Hz,2H), 6.90 (d, *J* = 9.0 Hz, 2H), 3.99 (q, *J* = 6.9 Hz, 2H), 1.31 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 183.9, 164.1, 158.9, 150.1, 144.1, 138.9, 136.9, 134.1(2C), 133.6(2C), 123.9, 119.6, 61.5, 12.9. Anal. calcd for: $C_{15}H_{13}N_5O_5S$: C, 48.00; H, 3.49; N, 18.66 % Found C, 48.09; H, 3.54; N, 18.72%.

1-(4-Bromophenyl)-3-(5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-yl)urea (TD_08):

Yield: 79%; m.p. 220–221 °C; MS(ESI) *m/z* 411 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.95 (s, 1H), 8.18 (d, *J* = 4.2 Hz, 1H), 7.85 (d, *J* = 3.9 Hz, 1H), 7.56 (d, *J* = 8.4 Hz, 2H), 7.38 (d, *J* = 8.7 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 181.1, 169.7, 162.6, 153.8, 144.1, 139.5, 136.1, 134.2(2C), 130.5, 126.7(2C), 118.9. Anal. calcd for: $C_{13}H_8BrN_5O_4S$: C, 38.06; H, 1.97; N, 17.07 % Found C, 38.09; H, 1.99; N, 17.10%.

1-(5-(5-Nitrofuran-2-yl)-1,3,4-thiadiazol-2-yl)-3-(4-nitrophenyl)urea (TD_09):

Yield: 88%; m.p. 270–271 °C; MS(ESI) *m/z* 377 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.19 (s, 1H), 8.28–8.12 (m, 3H), 7.82–7.71 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 181.3, 163.7, 158.1, 153.2, 144.1, 138.5, 135.5(2C), 133.9(2C), 126.3, 121.2, 119.5. Anal. calcd for: $C_{13}H_8N_6O_6S$: C, 41.49; H, 2.14; N, 22.33 % Found C, 41.54; H, 2.18; N, 22.37%.

1-Isopropyl-3-(5-(5-nitrothiophen-2-yl)-1,3,4-thiadiazol-2-yl)urea (TD_10):

Yield: 69%; m.p. 251–252 °C; MS(ESI) *m/z* 314 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.12 (s, 1H), 7.87(d, *J* = 3.9 Hz, 1H), 7.49 (d, *J* = 3.9 Hz, 1H), 6.46 (d, *J* = 5.7 Hz,1H), 3.89–

3.77 (m, 1H), 1.14 (d, *J* = 6.6 Hz, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 174.9, 167.0, 162.5, 154.3, 140.1, 138.9, 135.8, 15.4, 10.5 (2C). Anal. calcd for: $C_{10}H_{11}N_5O_3S_2$: C, 38.33; H, 3.54; N, 22.35 % Found C, 38.41; H, 3.61; N, 22.39%.

1-(5-(5-Nitrothiophen-2-yl)-1,3,4-thiadiazol-2-yl)-3-phenylurea (TD_11):

Yield: 76%; m.p. 189–190 °C; MS(ESI) *m/z* 348 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.76 (s, 1H), 10.03 (s, 1H), 8.28–8.11 (m, 5H), 7.85–7.77 (m, 1H), 7.75–7.67 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 178.3, 164.4, 162.0, 152.9, 141.2, 137.8, 134.1(2C), 131.1(2C), 127.9, 126.9, 116.6. Anal. calcd for: $C_{13}H_9N_5O_3S_2$: C, 44.95; H, 2.61; N, 20.16 % Found C, 44.99; H, 2.67; N, 20.25%.

1-Benzyl-3-(5-(5-nitrothiophen-2-yl)-1,3,4-thiadiazol-2-yl)urea (TD_12):

Yield: 80%; m.p. 162–163 °C; MS(ESI) *m/z* 362 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.55 (s, 1H), 8.17(d, *J* = 4.5 Hz, 1H), 7.75 (d, *J* = 4.5 Hz, 1H), 7.37–7.23 (m,5H), 6.40 (s, 1H), 4.23 (d, *J* = 5.4 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.8, 167.9, 164.9, 142.9, 137.2, 136.6(2C), 134.0, 133.7, 132.6, 128.6(2C), 117.7, 55.2. Anal. calcd for: $C_{14}H_{11}N_5O_3S_2$: C, 46.53; H, 3.07; N, 19.38 % Found C, 46.56; H, 3.09; N, 19.41%.

1-(5-(5-Nitrothiophen-2-yl)-1,3,4-thiadiazol-2-yl)-3-(*p*-tolyl)urea (TD_13):

Yield: 87%; m.p. 142–143 °C; MS(ESI) *m/z* 362 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.36 (s, 1H), 9.00 (s, 1H), 8.19 (d, *J* = 4.5 Hz, 1H), 7.80 (d, *J* = 4.2 Hz, 1H), 7.37 (d, *J* = 8.4 Hz, 2H), 7.15 (d, *J* = 8.4 Hz, 2H), 2.26 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.7, 162.9, 158.1, 150.1, 141.2, 137.5, 132.6(2C), 130.1(2C), 125.3, 121.9, 118.2, 34.1. Anal. calcd for: $C_{14}H_{11}N_5O_3S_2$: C, 46.53; H, 3.07; N, 19.38 % Found C, 46.59; H, 3.09; N, 19.44%.

1-(4-Ethoxyphenyl)-3-(5-(5-nitrothiophen-2-yl)-1,3,4-thiadiazol-2-yl)urea (TD_14):

Yield: 74%; m.p. 184–185 °C; MS(ESI) *m/z* 392 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.41 (s, 1H), 9.00 (s, 1H), 7.83 (d, *J* = 4.0 Hz, 1H), 7.48 (d, *J* = 4.0 Hz, 1H), 7.35 (d, *J* = 8.4 Hz, 2H), 6.87 (d, *J* = 9.0 Hz, 2H), 3.97 (q, *J* = 6.9 Hz, 2H), 1.30 (t, *J* = 6.9 Hz, 3H); δ 183.6, 163.7, 158.4, 149.6, 143.2, 138.2, 136.4, 133.3(2C), 132.9(2C), 123.4, 119.4, 61.2, 12.5. Anal. calcd for: $C_{15}H_{13}N_5O_4S_2$: C, 46.03; H, 3.35; N, 17.89 % Found C, C, 46.09; H, 3.39; N, 17.94 %.

1-(4-Bromophenyl)-3-(5-(5-nitrothiophen-2-yl)-1,3,4-thiadiazol-2-yl)urea (TD_15):

Yield: 69%; m.p. 274–275 °C; MS(ESI) *m/z* 427 $[M+H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.05 (s, 1H), 9.70 (s, 1H), 7.91 (d, *J* = 3.9 Hz, 1H), 7.72 (d, *J* = 4.2 Hz, 1H), 7.34 (d, *J* = 8.4 Hz, 2H), 7.02 (d, *J* = 8.2 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 180.2, 169.2, 161.9, 153.1, 142.9, 138.8, 135.7, 134.2(2C), 130.6, 126.5(2C), 119.7. Anal. calcd for: $C_{13}H_8BrN_5O_4S$: C, 36.63; H, 1.89; N, 16.43 % Found C, 36.68; H, 1.96; N, 16.49%.

1-(4-Nitrophenyl)-3-(5-(5-nitrothiophen-2-yl)-1,3,4-thiadiazol-2-yl)urea (TD_16):

Yield: 89%; m.p. 250–251 °C; MS(ESI) *m/z* 393 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.98 (s, 1H), 8.01 (d, *J* = 8.4 Hz, 2H), 7.98 (d, *J* = 4.2 Hz, 1H), 7.70 (d, *J* = 3.9 Hz, 1H), 7.68 (d, *J* = 8.7 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 180.5, 162.5, 160.1, 152.5, 140.4, 137.7, 135.1(2C), 132.7(2C), 125.8, 120.7, 119.8. Anal. calcd for: $C_{13}H_8N_6O_5S_2$: C, 39.79; H, 2.06; N, 21.42 % Found C, 39.81; H, 2.09; N, 21.45%.

1-(5-(5-Nitrofuran-2-yl)-1,3,4-thiadiazol-2-yl)-3-phenylthiourea (TD_17):

Yield: 84%; m.p. 223–224 °C; MS(ESI) *m/z* 348 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.65 (s, 1H), 7.89(d, *J* = 3.6 Hz, 1H), 7.67 (d, *J* = 3.9 Hz, 2H), 7.42(m, 1H) 7.34 (t, *J* = 5.8 Hz, 2H) 7.13(m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 178.4, 162.4, 160.9, 153.1, 141.5, 137.4, 133.3(2C), 130.3(2C), 127.2, 126.4, 116.1. Anal. calcd for: $C_{13}H_9N_5O_3S_2$: C, C, 44.95; H, 2.61; N, 20.16 % Found C, 45.05; H, 2.69; N, 20.25%.

1-Benzyl-3-(5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-yl)thiourea (TD_18):

Yield: 90%; m.p. 268–269 °C; MS(ESI) *m/z* 362 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.16 (s, 1H), 7.88(d, *J* = 3.9 Hz, 1H), 7.54 (d, *J* = 4.2 Hz, 1H), 7.53–7.28 (m, 6H), 4.78 (d, *J* = 5.7 Hz, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 182.6, 167.4, 154.6, 143.6, 138.3, 136.2, 135.8(2C), 134.1, 133.9, 128.9(2C), 117.9, 61.2. Anal. calcd for: $C_{14}H_{11}N_5O_3S_2$: C, 46.53; H, 3.07; N, 19.38 % Found C, 46.56; H, 3.09; N, 19.41%.

1-(4-Methoxyphenyl)-3-(5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-yl)thiourea (TD_19):

Yield: 88%; m.p. 277–278 °C; MS(ESI) *m/z* 378 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.57 (s, 1H), 7.87 (d, *J* = 3.0 Hz, 1H), 7.53–7.49 (m, 3H), 6.92 (d, *J* = 6.3 Hz, 2H), 3.75 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 179.2, 162.8, 156.3, 152.7, 142.5, 137.3, 134.8(2C), 133.2(2C), 125.6, 120.6, 118.8, 58.5. Anal. calcd for: $C_{14}H_{11}N_5O_4S_2$: C, 44.56; H, 2.94; N, 18.56% Found C, 44.63; H, 2.99; N, 18.63%.

1-(4-Fluorophenyl)-3-(5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-yl)thiourea (TD_20):

Yield: 76%; m.p. 201–202 °C; MS(ESI) *m/z* 366 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.68 (s, 1H), 7.89 (d, *J* = 3.9 Hz,1H), 7.65 (m, 3H), 7.54 (d, *J* = 4.2 Hz, 1H), 7.25–7.16 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.2, 162.8, 156.3, 152.7, 142.5, 137.3, 134.8(2C), 133.2(2C), 125.6, 120.6, 118.9. Anal. calcd for: $C_{13}H_8FN_5O_3S_2$: C, 42.74; H, 2.21; N, 19.17% Found C, 42.81; H, 2.27; N, 19.25%.

1-(4-Chlorophenyl)-3-(5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-yl)thiourea (TD_21):

Yield: 78%; m.p. 172–173 °C; MS(ESI) *m/z* 382 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.75 (s, 1H), 7.87 (d, *J* = 4.0 Hz, 1H), 7.72 (d, *J* = 8.4 Hz, 2H), 7.54 (d, *J* = 4.0 Hz, 1H), 7.39 (d, *J* = 8.8 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 181.1, 157.5, 149.4, 143.2, 139.6, 138.3, 136.5, 134.2(2C), 129.8(2C), 120.4, 117.4. Anal. calcd for: $C_{13}H_8ClN_5O_3S_2$: C, 40.89; H, 2.11; N, 18.34% Found C, 40.98; H, 2.18; N, 18.39%.

1-(5-(5-Nitrothiophen-2-yl)-1,3,4-thiadiazol-2-yl)-3-phenylthiourea (TD_22):

Yield: 87%; m.p. 206–207 °C; MS(ESI) *m/z* 364 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.62 (s, 1H), 7.87 (d, *J* = 3.6 Hz, 1H), 7.65 (d, *J* = 3.7 Hz, 2H), 7.40 (m, 1H) 7.34 (t, *J* = 5.8 Hz, 2H) 7.12(m, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 177.9, 165.2, 161.3, 152.5, 140.8, 136.8, 132.7(2C), 129.1(2C), 126.5, 125.1, 115.6. Anal. calcd for: $C_{13}H_9N_5O_2S_3$: C, 42.96; H, 2.50; N, 19.27 % Found C, 42.99; H, 2.58; N, 19.39%.

1-Benzyl-3-(5-(5-nitrothiophen-2-yl)-1,3,4-thiadiazol-2-yl)thiourea (TD_23):

Yield: 93%; m.p. 238–239 °C; MS(ESI) *m/z* 378 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.01 (s, 1H), 8.17(d, *J* = 4.5 Hz, 1H), 7.80 (d, *J* = 4.2 Hz, 1H), 7.34–7.19 (m, 6H), 4.78 (d, *J* = 5.4 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 181.2, 166.2, 165.9, 142.5, 139.6, 137.4,

136.2(2C), 135.0, 133.9, 129.6(2C), 119.7, 54.7. Anal. calcd for: $C_{14}H_{11}N_5O_2S_3$: C, 44.55; H, 2.94; N, 18.55 % Found C, 44.58; H, 2.99; N, 18.58%.

1-(4-Fluorophenyl)-3-(5-(5-nitrothiophen-2-yl)-1,3,4-thiadiazol-2-yl)thiourea (TD_24):

Yield: 79%; m.p. 184–185 °C; MS(ESI) *m/z* 382 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.60 (s, 1H), 8.17 (d, *J* = 4.2 Hz, 1H), 7.81 (d, *J* = 4.5 Hz, 1H), 7.67–7.63 (m, 2H), 7.54–7.50 (m, 2H) ; ^{13}C NMR (100 MHz, DMSO-d_6) δ 177.1, 169.2, 162.6, 153.5, 143.7, 139.5, 136.2, 13.6(2C), 129.4, 125.9(2C), 118.8. Anal. calcd for: $C_{13}H_8FN_5O_3S_2$: C, 42.74; H, 2.21; N, 19.17 % Found C, 42.79; H, 2.27; N, 19.27%.

1-(4-Chlorophenyl)-3-(5-(5-nitrothiophen-2-yl)-1,3,4-thiadiazol-2-yl)thiourea (TD_25):

Yield: 84%; m.p. 194–195 °C; MS(ESI) *m/z* 398 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.67 (s, 1H), 8.17 (d, *J* = 3.3 Hz, 1H), 7.79 (d, *J* = 3.3 Hz, 1H), 7.71 (d, *J* = 8.4 Hz, 2H), 7.39 (d, *J* = 8.4 Hz, 2H) ; ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.2, 159.2, 148.3, 142.8, 139.1, 137.6, 135.8, 133.9(2C), 128.9(2C), 120.1, 116.9. Anal. calcd for: $C_{13}H_8ClN_5O_2S_3$: C, 39.24; H, 2.03; N, 17.60 % Found C, 39.31; H, 2.09; N, 17.69%.

4-Fluoro-*N*-(5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-yl)benzenesulfonamide (TD_26):

Yield: 72%; m.p. 186–187 °C %; MS(ESI) *m/z* 371 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.43 (s, 1H), 8.19 (d, *J* = 4.5 Hz,1H), 7.91–7.88 (m, 2H), 7.80 (d, *J* = 4.6 Hz,1H), 7.37–7.41 (m, 2H) ; ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.7, 160.2, 155.9, 146.3, 140.2, 138.8, 138.6, 136.1(2C), 130.4, 124.8, 121.9. Anal. calcd for: $C_{12}H_7FN_4O_5S_2$: C, 38.92; H, 1.91; N, 15.13 % Found C, 38.99; H, 1.994; N, 15.21%.

4-Bromo-*N*-(5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-yl)benzenesulfonamide (TD_27):

Yield: 68%; m.p. 175–176 °C; MS(ESI) *m/z* 432 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.41 (s, 1H), 7.89 (d, *J* = 3.9 Hz, 1H), 7.83–7.73 (m, 4H), 7.60 (d, *J* = 3.9 Hz,1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.9, 163.3, 156.1, 146.3, 139.5, 138.8, 138.6, 135.4(2C), 129.9, 124.9, 120.5. Anal. calcd for: $C_{12}H_7BrN_4O_5S_2$: C, 33.42; H, 1.64; N, 12.99 % Found C, 33.47; H, 1.72; N, 13.09%.

4-Nitro-*N*-(5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-yl)benzenesulfonamide (TD_28):

Yield: 62%; m.p. 274–275 °C; MS(ESI) *m/z* 398 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.60 (s, 1H), 8.37 (d, *J* = 8.7 Hz, 2H), 8.09 (d, *J* = 8.7 Hz, 2H), 7.89 (d, *J* = 4.2 Hz, 1H), 7.59 (d, *J* = 3.9 Hz, 1H) ; ^{13}C NMR (75 MHz, DMSO-d_6) δ 171.1, 168.3, 155.2, 146.4, 139.9, 137.4, 137.2, 135.9, 135.2, 128.3, 124.7, 119.8. Anal. calcd for: $C_{12}H_7N_5O_7S_2$: C, 36.27; H, 1.78; N, 17.63 % Found C, 36.36; H, 1.86; N, 17.67%.

4-Acetyl-*N*-(5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-yl)benzenesulfonamide (TD_29):

Yield: 60%; m.p. 141–142 °C; MS(ESI) *m/z* 395 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.18 (d, *J* = 4.5 Hz, 1H), 8.09 (d, *J* = 8.4 Hz, 2H), 7.95 (d, *J* = 8.4 Hz, 2H), 7.81 (d, *J* = 4.5 Hz, 1H), 2.61 (s, 3H) ; ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.5, 168.1, 159.1, 152.6, 144.5, 139.2, 135.1(2C), 132.8(2C), 128.1, 125.3, 119.0, 34.4. Anal. calcd for: $C_{14}H_{10}N_4O_6S_2$: C, 42.64; H, 2.56; N, 14.21 % Found C, 42.68; H, 2.59; N, 14.25%.

***N*-(5-(5-Nitrofuran-2-yl)-1,3,4-thiadiazol-2-yl)thiophene-2-sulfonamide (TD_30):**

Yield: 63%; m.p. 155–156 °C; MS(ESI) *m/z* 359 $[M+H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.88 (d, *J* = 3.9 Hz, 2H), 7.62 (d, *J* = 2.7 Hz, 1H), 7.56 (d, *J* = 3.9 Hz, 1H), 7.13 (d, *J* = 4.5 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.2, 167.9, 155.1, 143.2, 139.4, 133.3, 130.8, 129.2, 127.4, 126.2. Anal. calcd for: $C_{10}H_6N_4O_5S_3$: C, 33.51; H, 1.69; N, 15.63 % Found C, 33.54; H, 1.73; N, 15.66%.

4-Fluoro-*N*-(5-(5-nitrothiophen-2-yl)-1,3,4-thiadiazol-2-yl)benzenesulfonamide (TD_31):

Yield: 69%; m.p. 192–193 °C; MS(ESI) *m/z* 387 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.40 (s, 1H), 7.92–7.88 (m, 3H), 7.58 (d, *J* = 4.0 Hz, 1H), 7.42–7.38 (m, 2H) ; ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.6, 167.4, 154.7, 145.7, 140.0, 138.4, 138.1, 135.8(2C), 130.0, 124.1, 120.6. Anal. calcd for: $C_{12}H_7FN_4O_4S_3$: C, 37.30; H, 1.83; N, 14.50 % Found C, 37.37; H, 1.89; N, 14.58%.

4-Bromo-*N*-(5-(5-nitrothiophen-2-yl)-1,3,4-thiadiazol-2-yl)benzenesulfonamide (TD_32):

Yield: 63%; m.p. 174–175 °C; MS(ESI) *m/z* 448 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.48 (s, 1H), 7.81 (d, *J* = 4.2 Hz, 1H), 7.75 (m, 4H), 7.58 (d, *J* = 3.9 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.6, 168.1, 155.7, 146.0, 139.2, 138.5, 138.1, 135.8(2C), 130.1, 124.3, 121.7 Anal. calcd for: $C_{12}H_7BrN_4O_4S_3$: C, 32.22; H, 1.58; N, 12.53 % Found C, 32.30; H, 1.64; N, 12.59%.

4-Nitro-*N*-(5-(5-nitrothiophen-2-yl)-1,3,4-thiadiazol-2-yl)benzenesulfonamide (TD_33):

Yield: 81%; m.p. 256–257 °C; MS(ESI) *m/z* 414 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.54 (s, 1H), 8.35 (d, *J* = 8.7 Hz, 2H), 8.17 (d, *J* = 4.5 Hz, 1H), 8.06 (d, *J* = 8.7 Hz, 2H), 7.75 (d, *J* = 4.2 Hz, 1H) ; ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.4, 169.1, 154.9, 146.1, 139.6, 137.1, 136.8, 134.9, 134.3, 127.9, 124.5, 119.5 Anal. calcd for: $C_{12}H_7N_5O_6S_3$: C, 34.86; H, 1.71; N, 16.94 % Found C, 34.94; H, 1.78; N, 16.99%.

4-Acetyl-*N*-(5-(5-nitrothiophen-2-yl)-1,3,4-thiadiazol-2-yl)benzenesulfonamide (TD_34):

Yield: 83%; m.p. 214–215 °C; MS(ESI) *m/z* 411 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.19 (d, *J* = 3.9 Hz, 1H), 8.10 (d, *J* = 8.1 Hz, 2H), 7.96 (d, *J* = 8.4 Hz, 2H), 7.82 (d, *J* = 4.2 Hz, 1H), 2.62 (s, 3H) ; ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.6, 167.8, 158.8, 152.4, 144.3, 138.8, 135.1(2C), 131.1(2C), 127.5, 125.1, 118.8, 34.1. Anal. calcd for: $C_{14}H_{10}N_4O_5S_3$: C, 40.97; H, 2.46; N, 13.65 % Found C, 41.07; H, 2.54; N, 13.72%.

***N*-(5-(5-Nitrothiophen-2-yl)-1,3,4-thiadiazol-2-yl)thiophene-2-sulfonamide (TD_35):**

Yield: 72%; m.p. 185–186 °C; MS(ESI) *m/z* 375 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.51 (s, 1H), 8.20 (d, *J* = 4.5 Hz, 1H), 7.93–7.91 (m, 1H), 7.88 (d, *J* = 4.5 Hz, 1H), 7.67–7.65 (m, 1H), 7.17–7.14 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.1, 167.4, 154.8, 142.8, 139.0, 132.8, 130.3, 128.9, 128.1, 126.1. Anal. calcd for: $C_{10}H_6N_4O_4S_4$: C, 32.08; H, 1.62; N, 14.96 % Found C, 32.16; H, 1.68; N, 14.99%.

***N*-(5-(5-Nitrofuran-2-yl)-1,3,4-thiadiazol-2-yl)benzamide (TD_36):**

Yield: 52%; m.p. 215–216 °C; MS(ESI) *m/z* 317 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.54 (s, 1H), 8.37 (d, *J* = 8.7 Hz, 2H), 8.37 (d, *J* = 8.7 Hz, 2H), 8.09 (d, *J* = 8.7 Hz, 2H), 7.89 (d, *J* = 4.2 Hz, 1H),; ^{3}C NMR (100 MHz, DMSO-d_6) δ 172.3, 168.1, 159.7, 151.4, 139.8, 135.1,

131.2(2C), 129.7(2C), 125.2, 124.1, 118.1. Anal. calcd for: $C_{13}H_8N_4O_4S$: C, 49.37; H, 2.55; N, 17.71 % Found C, 49.43; H, 2.65; N, 17.78%.

***N*-(5-(5-Nitrofuran-2-yl)-1,3,4-thiadiazol-2-yl)-2-naphthamide (TD_37):**

Yield: 55%; m.p. 224–225 °C; MS(ESI) *m/z* 367 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.71 (s, 1H), 8.49 (d, *J* = 7.5 Hz, 1H), 8.21 (d, *J* = 7.2 Hz, 1H), 8.14–7.90 (m, 3H), 7.70–7.58 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.1, 163.9, 154.3, 149.5, 146.7, 136.9, 136.2, 135.3, 134.5, 133.4, 132.7, 128.6, 127.2, 126.0, 124.8, 122.4, 119.4. Anal. calcd for: $C_{17}H_{10}N_4O_4S$: C, 55.73; H, 2.75; N, 15.29 % Found C, 55.77; H, 2.79; N, 15.34%.

***N*-(5-(5-Nitrofuran-2-yl)-1,3,4-thiadiazol-2-yl)benzamide (TD_38):**

Yield: 51%; m.p. 227–228 °C; MS(ESI) *m/z* 333 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.48 (s, 1H), 8.22 (d, *J* = 4.5 Hz, 1H), 8.15 (d, *J* = 7.2 Hz, 2H), 7.89 (d, *J* = 4.2 Hz, 1H), 7.72–7.56 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.1, 167.9, 159.5, 152.1, 139.4, 134.6, 130.8(2C), 129.4(2C), 125.0, 123.9, 117.8. Anal. calcd for: $C_{13}H_8N_4O_3S_2$: C, 46.98; H, 2.43; N, 16.86 % Found C, 47.01; H, 2.48; N, 16.95%.

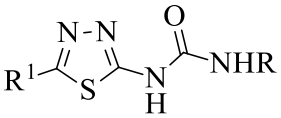
***N*-(5-(5-Nitrothiophen-2-yl)-1,3,4-thiadiazol-2-yl)-2-naphthamide (TD_39):**

Yield: 54%; m.p. 235–236 °C; MS(ESI) *m/z* 382 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.42 (s, 1H), 8.31–8.17 (m, 3H), 8.06 (d, *J* = 8.1 Hz, 1H), 8.02–7.92 (m, 2H), 7.68–7.63 (m, 3H); δ 171.9, 164.3, 155.2, 148.7, 146.3, 137.2, 136.0, 135.1, 134.6, 132.1, 130.4, 127.8, 127.4, 125.7, 124.6, 123.1, 118.2. Anal. calcd for: $C_{17}H_{10}N_4O_3S_2$: C, 53.39; H, 2.64; N, 14.65 % Found C, 53.46; H, 2.72; N, 14.74 %.

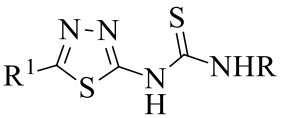
5.1.4. *In vitro M. tuberculosis* screening, *M. tuberculosis* PknB enzyme inhibition assay and cytotoxicity studies of the synthesized molecules

All the synthesized compounds were screened for their *in vitro* anti-tubercular activity against *M. tuberculosis* H37Rv using MABA method. INH and EMB were used as reference compounds for comparison. All the synthesized derivatives were also screened for their *in vitro M. tuberculosis* PknB 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 5.2**.

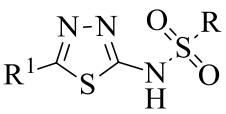
Table 5.2: *In vitro* biological evaluation of synthesized compounds **TD_04** – **TD_39**



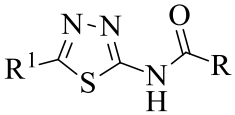
TD_04 - TD_16



TD_17- TD_25



TD_26 - TD_35



TD_36 - TD_39

Compd	R^1	R	MTB PKnB IC_{50} in µM	MTB MIC in µM[a]	Cytotoxicity[b] at 50 µM % inhibition
TD_04	5-nitrofuran-2-yl	Isopropyl	>25	21.02	32.10
TD_05	5-nitrofuran-2-yl	Benzyl	>25	72.39	NT
TD_06	5-nitrofuran-2-yl	4-Tolyl	14.13	4.51	25.48
TD_07	5-nitrofuran-2-yl	4-Ethoxyphenyl	>25	15.96	40..35
TD_08	5-nitrofuran-2-yl	4-Bromophenyl	16.21	3.80	28.37
TD_09	5-nitrofuran-2-yl	4-Nitrophenyl	0.81	2.07	20.50
TD_10	5-nitrothiophen-2-yl	Isopropyl	5.67	2.48	27.86
TD_11	5-nitrothiophen-2-yl	Phenyl	16.31	71.96	NT
TD_12	5-nitrothiophen-2-yl	Benzyl	15.62	69.17	NT
TD_13	5-nitrothiophen-2-yl	4-Tolyl	20	69.17	NT
TD_14	5-nitrothiophen-2-yl	4-EthoxyPhenyl	15	4.13	40.64
TD_15	5-nitrothiophen-2-yl	4-Bromophenyl	1.24	64.66	28.44
TD_16	5-nitrothiophen-2-yl	4-Nitrophenyl	1.01	3.97	24.37
TD_17	5-nitrofuran-2-yl	Phenyl	20	17.99	20.12
TD_18	5-nitrofuran-2-yl	Benzyl	>20	17.29	31.27
TD_19	5-nitrofuran-2-yl	4-Methoxyphenyl	12.13	2.15	29.38
TD_20	5-nitrofuran-2-yl	4-Fluorophenyl	1.06	2.13	18.69
TD_21	5-nitrofuran-2-yl	4-chlorophenyl	19.12	65.47	NT
TD_22	5-nitrothiophen-2-yl	Phenyl	17.11	17.99	35.67
TD_23	5-nitrothiophen-2-yl	Benzyl	16.13	17.29	20.94
TD_24	5-nitrothiophen-2-yl	4-Fluorophenyl	10.12	2.44	21.67

Contd

Compd	R^1	R	MTB PKnB IC_{50} in µM	MTB MIC in µM[a]	Cytotoxicity[b] at 50 µM % inhibition
TD_25	5-nitrothiophen-2-yl	4-chlorophenyl	9.12	7.85	19.47
TD_26	5-nitrofuran-2-yl	4-Fluorophenyl	>20	16.21	23.54
TD_27	5-nitrofuran-2-yl	4-Bromophenyl	>20	7.24	36.27
TD_28	5-nitrofuran-2-yl	4-Nitrophenyl	>20	3.92	25.64
TD_29	5-nitrofuran-2-yl	4-Acetylphenyl	9.47	15.26	32.08
TD_30	5-nitrofuran-2-yl	2-Thiophenyl	>20	4.17	29.31
TD_31	5-nitrothiophen-2-yl	4-Fluorophenyl	>20	62.28	NT
TD_32	5-nitrothiophen-2-yl	4-Bromophenyl	>20	27.95	36.49
TD_33	5-nitrothiophen-2-yl	4-Nitrophenyl	>20	60.47	NT
TD_34	5-nitrothiophen-2-yl	4-Acetylphenyl	>20	7.34	31.66
TD_35	5-nitrothiophen-2-yl	2-Thiophenyl	>20	66.77	NT
TD_36	5-nitrofuran-2-yl	Phenyl	>20	19.75	18.98
TD_37	5-nitrofuran-2-yl	2-Napthyl	11.25	34.11	40.58
TD_38	5-nitrothiophen-2-yl	Phenyl	18.37	72.17	NT
TD_39	5-nitrothiophen-2-yl	2-Napthyl	16.18	32.68	25.01
Isoniazid			NT	0.72	NT
Ethambutol			NT	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.

5.1.5. SAR and discussion

The selected lead molecule was taken for the start of chemistry and constructed a library of thirty six molecules for the study of SAR. We have utilised primary amino group at 2nd position of 1,3,4-thiadiazole pharmacophore and synthesized thirteen (**TD_04 – TD_16**) urea, nine (**TD_17 – TD_25**) thiourea, ten (**TD_26 – TD_35**) sulphonamide and four (**TD_36 – TD_39**) amide derivatives, by keeping 5-nitro-2-furanyl and 5-nitro-2-thiophenyl substituents fixed at 5th position.

Introduction of urea and thiourea substitutions in a bioactive molecule fetch two additional benefits; these substituents fulfil the Lipinski rule by certain extent by allowing hydrogen bond

donors as well as acceptors. Among the urea derivatives (**TD_04 – TD_16**) with respect to *M. tuberculosis* MIC, electron withdrawing substitution on phenyl ring (**TD_08** and **TD_09**) showed better activity than electron donating substitutions, in terms of potency against *M. tuberculosis* PknB the similar trend was observed. Compound **TD_09** which has nitro group on phenyl ring has showed top activity by inhibiting *M. tuberculosis* with MIC of 2.07 µM and *M. tuberculosis* PknB with IC_{50} of 0.81 µM. Among 5-nitro-2-thiophenyl substituted urea derivatives, isopropyl urea (**TD_10**) showed good activity.

Among 5-nitro-2-furyl substituted thiadiazolo-thioureas (**TD_17 – TD_21**), compound **TD_20** which has more electronegative fluorine atom on phenyl ring showed good activity against *M. tuberculosis* H37Rv with MIC of 2.13 µM and excellently inhibited *M. tuberculosis* PknB enzymatic action with IC_{50} of 1.06 µM. Whereas substitution of chloro in place of fluoro reduced the activity by several folds. Amongst thioureas of 5-nitro-2-thiophenyl substitution (**TD_22 – TD_25**) chloro substitution (**TD_25**) on phenyl ring showed slightly better activity than fluoro substitution (**TD_24**) than electron donating substitutions.

Introduction of sulphonamide group ($-SO_2NH_2$) is one of the most interesting and useful area of study in medicinal chemistry. Sulphonamides were discovered as anti-bacterial agents in early 1930's and have been studying owing to its pharmacophoric nature which is found in many biologically active molecules and enzyme inhibitors. In our present study, we have synthesized five molecules each of furanyl-sulphonamides (**TD_26 – TD_30**) and thiophenyl-sulphonamides (**TD_31 – TD_35**) to study their SAR. Out of ten sulphonamide derivatives, only one (**TD_29**) found to be exhibit *M. tuberculosis* PknB $IC_{50} < 10$ µM, whereas four sulphonamides (**TD_27**, **TD_28**, **TD_30** and **TD_34**) were found to be active against drug sensitive bacteria by possessing MIC < 10 µM. Which reveals that though thiadiazolo-sulphonamides were not attractive towards the enzyme *M. tuberculosis* PknB, they are excellent in inhibiting drug sensitive bacterium. Among amide "-NHCO-" derivatives (**TD_36** – **TD_39**) none were found to be active against drug sensitive bacterium as well *M. tuberculosis* PknB enzyme.

5.1.6. Highlights of the study

In summary, we identified a novel lead based on the molecular hybridization approach from known bioactive molecules (**5b**, **6a** and **BITS in-house molecule**) and synthesized a library of thirty six molecules. Eleven out of thirty six molecules exhibited *M. tuberculosis* MIC of 5 µM. Compound **TD_09 (**1-(5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-yl)-3-(4-nitrophenyl)urea) was found to be the most active compound with IC_{50} of 0.81 µM against *M. tuberculosis* PknB, inhibited drug sensitive *M. tuberculosis* with MIC of 2.07 µM and was non-cytotoxic at 50 µM (**Figure 5.3**). The results were fruitful for further studies and to transform them into potential drug candidates.

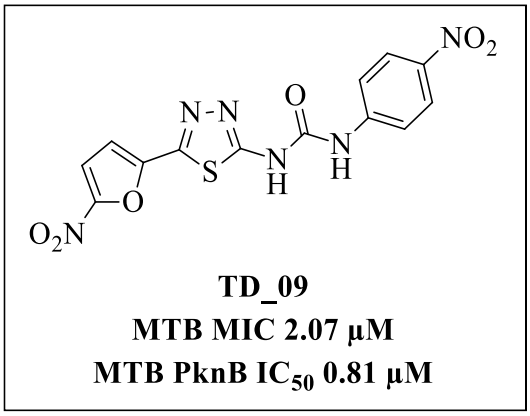


Figure 5.3: Chemical structure and biological activity of the most active compound **TD_09**

5.2. Development of tetrahydrothieno[2,3-*c*]pyridine-3-carboxamides and hexahydrocycloocta[*b*]thiophene-3-carboxamides as novel antimycobacterial from known antimycobacterial lead

In literature there were reports on 2,3,4-tri substituted thiophene derivatives and tetra substituted fused thiophene derivatives as antimycobacterial agents [Samala G., *et al*., 2014]. In our present study we have utilized one of the known antimycobacterial agent containing fused thiophene moiety (**SID 92097880**, *M. tuberculosis* MIC 9.15 µM) as lead molecule for further development. A library of twenty six molecules were designed with diverse substitution, synthesized and evaluated *in vitro* for their potency against *M. tuberculosis*. Among the test results, compound **OT_09** showed excellent potency against *M. tuberculosis* with MIC of 1.23 µM in the presence of an efflux pump inhibitor Verapamil, and showed no cytotoxicity at 50 µM.

5.2.1. Design of lead molecule

To invade the ever growing disease, there were many government agencies, non-profit organizations working together. One such organisation is "Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF)", which 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 antituberculosis drug discovery research. Recently TAACF reported anti-TB high-throughput results of large libraries of drug-like small molecules [Reynolds RC., *et al*., 2012; Ananthan S., et al., 2009; Maddry JA., *et al*., 2009]. One among them was the molecule **SID 92097880** 6-acetyl-2-(thiophene-2-carboxamido)-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine-3-carboxamide that showed activity against *M. tuberculosis* H37Rv MIC of 9.15 µM and selectivity index of 13 (**Figure 5.4**).

We chose **SID 92097880** as the starting point to design and synthesize various 2,6-disubstituted 4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine-3-carboxamides and 2-substituted 4,5,6,7,8,9-hexahydrocycloocta[*b*]thiophene-3-carboxamides to study extensively their SAR and to develop a more potent compound.

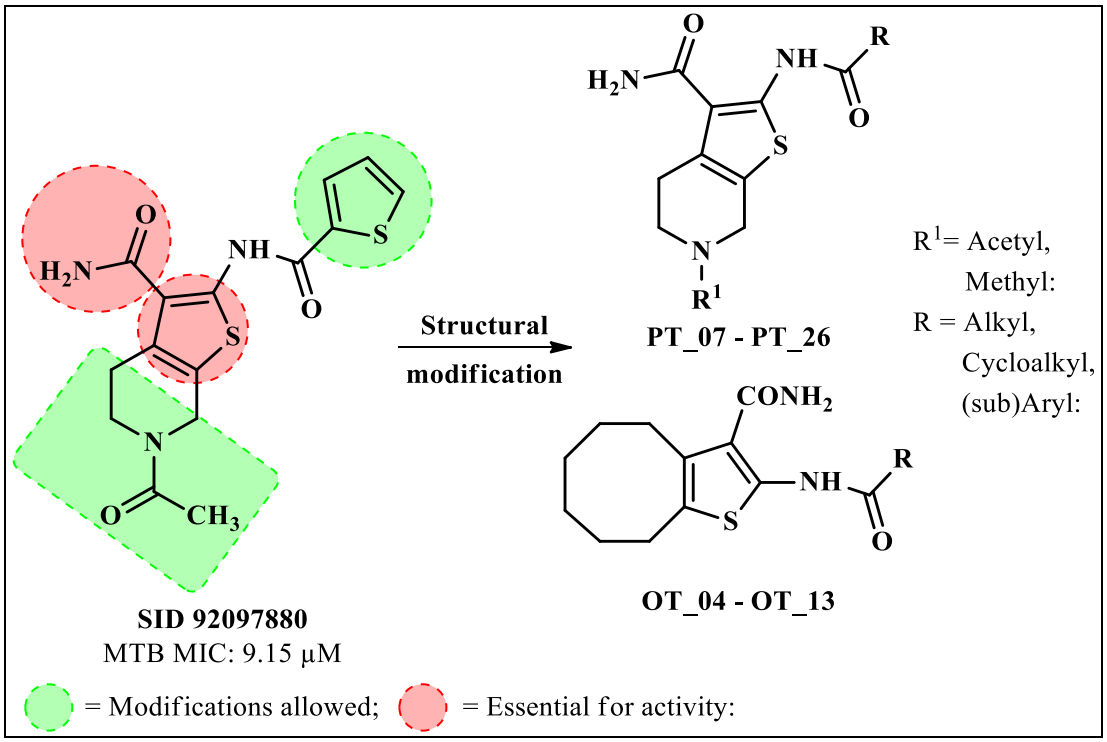


Figure 5.4: Structural modification of lead compound

5.2.2. Experimental procedures utilized for the synthesis of PT_07 –PT_26 and OT_04 – OT_13

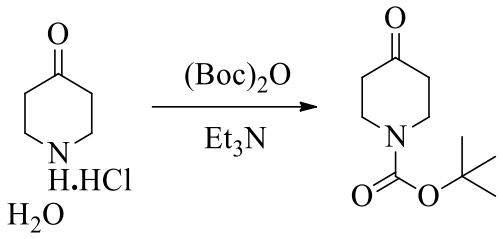
In scheme **2a** (**Figure 4.2**), we synthesized 2,6-disubstituted 4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxamides by following a five step synthetic protocol starting from the commercially available, less expensive 4-piperidone monohydrate hydrochloride (**PT_01**). In first step we protected the secondary amine group by Boc-protection to get '*tert*-butyl 4-oxopiperidine-1-carboxylate' (**PT_02**). In next step, by following Gewald reaction conditions using morpholine, cyanoacetamide (**PT_03**) and elemental sulphur achieved '*tert*-butyl 2-amino-3-carbamoyl-4,5-dihydrothieno[2,3-*c*]pyridine-6(7*H*)-carboxylate' (**PT_04**). This conversion was successful in both the conditions i.e., at room temperature and at 70 °C. But they differ in reaction time, purification process and yield. When reaction carried out at 70 °C we observed the complete conversion of starting material in less than 2 h and it has taken 6-9 hours to complete the reaction at room temperature. Compared to reaction at 70 °C, we noticed good yields (nearly 20% more) when the reaction was carried out at room temperature, and in addition also observed that there was a good amount of solids formed in the reaction mixture which eased the

purification process. The primary aromatic amine group of compound **PT_04** was coupled with various alkyl, cycloalkyl, (sub)aryl carboxylic acids by employing peptide coupling reagents 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI) and HOBt to produce compounds **PT_05a-j**. Due to the poor solubility of reaction products, we observed formation of desired product as solid in reaction mixture. Direct filtration of reaction mixture washing with distilled water, hexane produced pure products without any further purification. In next step deprotection of Boc-protection was carried out by using trifluoroacetic acid to get compounds **PT_06a-j**. The conversion of compounds **PT_06a-j** to target compounds **PT_07 – PT_16** was achieved by treating with acetyl chloride in dichloromethane using Et_3N as base.

In order to achieve the synthesis of compound **PT_17 – PT_26**, we tried direct methylation using CH_3I and sodium hydride conditions, but the reaction was unsuccessful. Then we employed reductive amination conditions using formaldehyde and $Na(OAc)_3BH$ in MeOH with catalytic amount of acetic acid to produce compounds **PT_17 – PT_26**. We also tried to synthesize the final compounds using 1-acetylpiperidin-4-one and 1-methylpiperidin-4-one respectively as the starting materials in order to achieve them in two steps. We succeeded in first step i.e, synthesis of 6-acetyl-2-amino-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine-3-carboxamide and 2-amino-6-methyl-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine-3-carboxamide. But in next step, due to very poor solubility, the coupling reaction was unsuccessful with various carboxylic acids.

In scheme **2b** (**Figure 4.2**) we used (2-substituted 4,5,6,7,8,9-hexahydrocycloocta[b]thiophene-3-carboxamides), for the preparation of intermediate compound **OT_03,** we used the same reaction conditions which are followed for the conversion **PT_02** to **PT_04** (morpholine, cyanoacetamide and elemental sulphur)**,** but the reaction was unsuccessful. Hence instead of cyanoacetamide we used malanonitrile under Gewald reaction conditions to get 2-amino-4,5,6,7,8,9-hexahydrocycloocta[*b*]thiophene-3-carbonitrile (**OT_03**). Compound **OT_03** was then treated with conc. H_2SO_4 to afford 2-amino-4,5,6,7,8,9-hexahydrocycloocta[*b*]thiophene-3-carboxamide (**OT_13**). The free amino group of compound **OT_03** on reaction with various carboxylic acids produced the target molecules **OT_04–OT_13**.

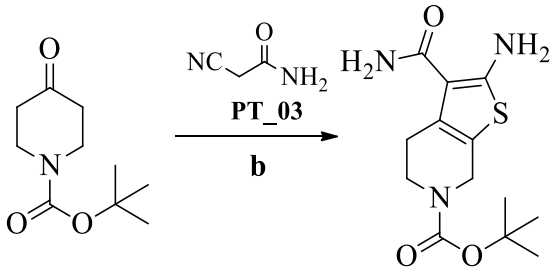
Preparation of tert-butyl 4-oxopiperidine-1-carboxylate (PT_02)



PT_01 **PT_02**

Et_3N (26.45 mL, 185.17 mmol) was added drop-wise to a stirred solution of compound (**PT_01**) (4.0 g, 29.60 mmol) in CH_2Cl_2 (20 mL) and MeOH (4 mL) at 0 °C, then $(Boc)_2O$ (7.74 mL, 35.52 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 (30 mL) and extracted with CH_2Cl_2 (3 × 60 mL). The separated organic layer was concentrated under reduced pressure and the crude residue was washed with hexanes to get compound **PT_02** (4.30 g, 72%) as an off-white solid. MS (ESI) 200 $[M+H]^+$. Anal. calcd for $C_{10}H_{17}NO_3$: C, 60.28; H, 8.60; N, 7.03 % Found C, 60.34; H, 8.64; N, 7.11 %.

Preparation of tert-butyl 2-amino-3-carbamoyl-4,5-dihydrothieno[2,3-c]pyridine-6(7H)-carboxylate (PT_04)

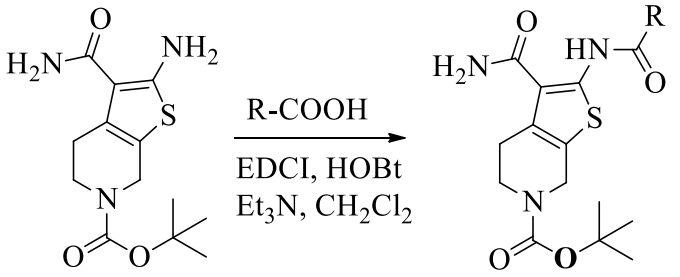


PT_02 **PT_04**

To the stirred solution of compound **PT_02** (6.0 g, 30.14 mmol), cyanoacetamide (3.01 g, 37.18 mmol), sulphur powder (2.13 g, 30.14 mmol) in ethanol (60 mL) was added morpholine (6.01 mL, 60.30 mmol) and stirred the reaction mixture at room temperature for 12 h. The reaction mixture was concentrated, and the crude solid was filtered, washed with H_2O and dried in vacuum oven for 2 h. The obtained dried compound was purified by column chromatography to

yield (3.67 g, 82%) as a light yellow solid. MS (ESI) 298 $[M+H]^+$. ^1H 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); Anal. calcd for $C_{13}H_{19}N_3O_3S$: C, 52.51; H, 6.44; N, 14.13 % Found C, 52.64; H, 6.55; N, 14.11 %.

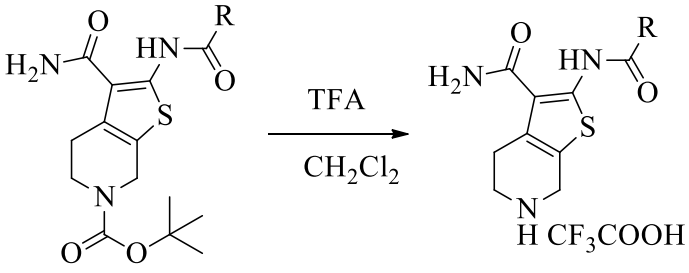
General Procedure for the Preparation of PT_05a–j



PT_04 **PT_05a-j**

To the stirred solution of R-COOH (1.20 equiv) in CH_2Cl_2 was added EDCI (1.30 equiv), HOBt (1.30 equiv) and Et_3N (2.50 equiv) allowed the reaction mixture to stir for few minutes, then added compound **PT_04** (1.00 equiv). The reaction mixture was stirred at room temperature for 3 h. Concentrated and triturated with H_2O to get solid compound. The solids were given cold Ethanol, diethyl ether and hexane washings to get pure products **PT_05a-j**.

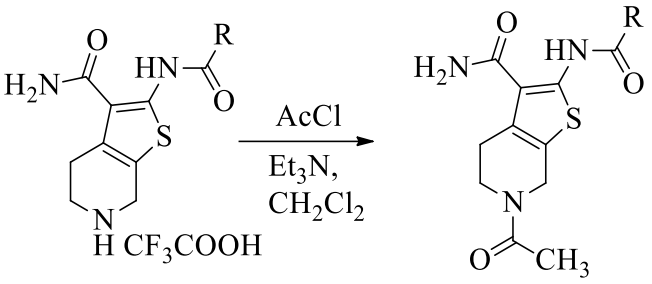
Preparation of PT_06a–j



PT_05a-j **PT_06a-j**

To the stirred solution of Compound **PT_05a–j** in CH_2Cl_2 at 0 °C under N_2 atm, was added TFA (2 volumes) and allowed to stir the reaction mixture at room temperature for 2 h. The reaction mixture was concentrated to dryness and the obtained solids were washed with hexanes to afford respective compounds **PT_06a–j**.

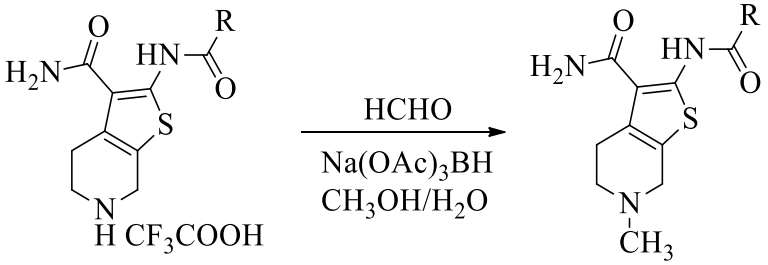
General Procedure for the Preparation of PT_07 – PT_16



PT_06a-j **PT_07 - PT_16**

To the stirred solution of compounds **PT_06a–j** in CH_2Cl_2 at 0 °C under N_2 atm, was added Et_3N (2.0 equiv), followed by acetyl chloride (1.2 equiv) and allowed to stir the reaction mixture at room temperature for 4 h. The reaction mixture was concentrated to dryness and the obtained solids were washed with excess water, cold ethanol and hexanes to afford respective final compounds **PT_07 – PT_16**.

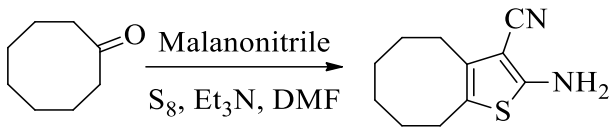
General Procedure for the Preparation of PT_17 – PT_26



PT_06a-j **PT_17 - PT_26**

40 % formaldehyde solution (6 vol) was added to the stirred solution of compound **PT_06a**–j in MeOH/H_2O (9:1) and stirred for 1 h, then $Na(OAc)_3BH$ (2.0 equiv) was added. The reaction mixture was stirred at room temperature for 6 h. The product was extracted with EtOAc and purified by triturating with CH_2Cl_2, diethyl ether and hexanes to get title compounds **PT_17 – PT_26**. The molecular formula, molecular weights and melting points of all the target molecules were shown in **Table 5.3**.

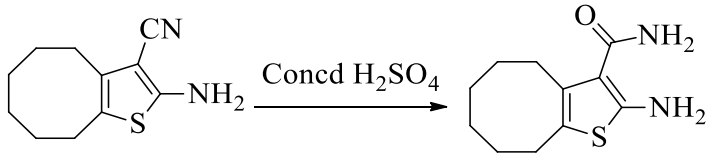
Preparation of 2-amino-4,5,6,7,8,9-hexahydrocycloocta[*b*]thiophene-3-carbonitrile (OT_02):



OT_01 **OT_02**

To the stirred solution of cyclooctanone (**OT_01**) (2.5 mL, 19.0 mmol), malanonitrile (1.38 g, 20.9 mmol) and elemental sulphur (670 mg, 20.9 mmol) in DMF (10 mL) was added Et_3N (4.0 mL, 28.5 mmol) and stirred at room temperature for 9 h. The reaction mixture was diluted with EtOAc (60 mL), the organic layer was washed with 1M HCl (2 × 20 mL) and brine (2 × 30 mL). The separated organic layer was dried over anhydrous Na_2SO_4 and concentrated under *vacuo* to get crude compound. The crude compound was triturated with CH_2Cl_2, diethyl ether and hexanes to get compound **OT_02** (2.70 g, 66%) as an off-white solid. ESI-MS showed 207 $[M+H]^+$. Anal. calcd for $C_{11}H_{14}N_2S$: C, 64.04; H, 6.84; N, 13.58 % Found C, 64.12; H, 6.89; N, 13.64%.

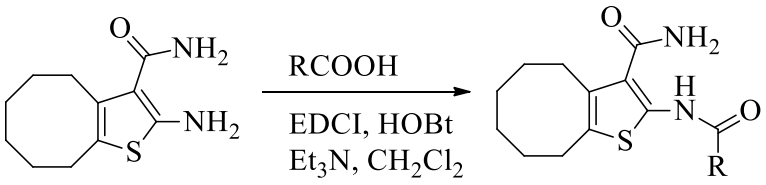
Preparation of 2-amino-4,5,6,7,8,9-hexahydrocycloocta[b]thiophene-3- carboxamide (OT_03)



OT_02 **OT_03**

Compound **OT_02** (2.70 g) was suspended in conc. H_2SO_4 (10 mL) and stirred for 12 h. The reaction mixture was quenched with ice, neutralised with solid Na_2CO_3, and the product was extracted with EtOAc, and the combined organic layer was concentrated under reduced pressure to get compound **OT_03** (1.6 g, 54%) as an off-white solid. MS (ESI) 225 $[M+H]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 6.55 (bs, 2H), 6.10 (bs, 2H), 2.77–2.62 (m, 4H), 1.70–1.35 (m, 8H); Anal. calcd for $C_{11}H_{16}N_2OS$: C, 58.90; H, 7.19; N, 12.49 % Found C, 58.94; H, 7.29; N, 12.60%.

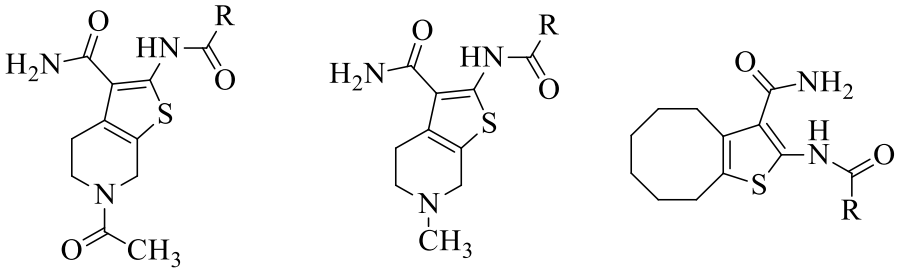
General Procedure for the Preparation of OT_04 – OT_13



OT_03 **OT_04 - OT_13**

To the stirred solution of R-COOH (1.20 equiv) in CH_2Cl_2 was added EDCI (1.30 equiv), HOBt (1.30 equiv) and Et_3N (2.50 equiv) while stirred the mixture for few minutes, then added compound **OT_03** (1.0 equiv). The reaction mixture was stirred at room temperature for 3 h. The reaction mixture was concentrated and triturated with H_2O to get solid compound. The solids were given cold ethanol, diethyl ether and hexane washings to get pure products.

Table 5.3: Physiochemical properties of the synthesized compounds **PT_07 – PT_26** and **OT_04 – OT_13**



PT_07 - PT_16 **PT_17 - PT_26** **OT_04 - OT_13**

Compound	R	Yield (%)	M.P. (°C)	Molecular formula	Molecular weight
PT_07	Methyl	81	223-224	$C_{12}H_{15}N_3O_3S$	281.33
PT_08	Phenyl	63	231-232	$C_{17}H_{17}N_3O_3S$	343.40
PT_09	2-Methoxyphenyl	70	202-204	$C_{18}H_{19}N_3O_4S$	373.43
PT_10	3-Nitrophenyl	84	251-252	$C_{17}H_{16}N_4O_5S$	388.40
PT_11	4-Tolyl	79	204-205	$C_{18}H_{19}N_3O_3S$	357.43
PT_12	4-Phenoxyphenyl	72	190-191	$C_{23}H_{21}N_3O_4S$	435.50

Contd

Compound	R	Yield (%)	M.P. (°C)	Molecular formula	Molecular weight
PT_13	1-Napthyl	61	270-271	$C_{21}H_{19}N_3O_3S$	393.46
PT_14	Cyclopropyl	74	204-205	$C_{14}H_{17}N_3O_3S$	307.37
PT_15	Cyclopentyl	64	280-281	$C_{16}H_{21}N_3O_3S$	335.42
PT_16	Cyclohexyl	78	208-209	$C_{17}H_{23}N_3O_3S$	349.45
PT_17	Methyl	58	222-223	$C_{11}H_{15}N_3O_2S$	253.32
PT_18	Phenyl	66	182-183	$C_{16}H_{17}N_3O_2S$	315.39
PT_19	2-Methoxyphenyl	62	205-206	$C_{17}H_{19}N_3O_3S$	345.42
PT_20	3-Nitrophenyl	81	224-225	$C_{16}H_{16}N_4O_4S$	360.39
PT_21	4-Tolyl	73	133-135	$C_{17}H_{19}N_3O_2S$	329.42
PT_22	4-Phenoxyphenyl	73	207-208	$C_{22}H_{21}N_3O_3S$	407.49
PT_23	1-Napthyl	56	180-181	$C_{20}H_{19}N_3O_2S$	365.45
PT_24	Cyclopropyl	72	231-232	$C_{13}H_{17}N_3O_2S$	279.36
PT_25	Cyclopentyl	62	260-261	$C_{15}H_{21}N_3O_2S$	307.49
PT_26	Cyclohexyl	72	206-207	$C_{16}H_{23}N_3O_2S$	321.44
OT_04	Methyl	79	186-187	$C_{13}H_{18}N_2O_2S$	266.36
OT_05	Phenyl	88	213-214	$C_{18}H_{20}N_2O_2S$	328.43
OT_06	2-Methoxyphenyl	60	243-244	$C_{19}H_{22}N_2O_3S$	358.45
OT_07	3-Nitrophenyl	69	228-229	$C_{18}H_{19}N_3O_4S$	373.43
OT_08	4-Tolyl	63	216-217	$C_{19}H_{22}N_2O_2S$	342.46
OT_09	4-Phenoxyphenyl	69	210-211	$C_{24}H_{24}N_2O_3S$	420.52
OT_10	1-Napthyl	70	190-191	$C_{22}H_{22}N_2O_2S$	378.49
OT_11	Cyclopropyl	66	145-146	$C_{15}H_{20}N_2O_2S$	292.40
OT_12	Cyclopentyl	54	175-176	$C_{17}H_{24}N_2O_2S$	320.45
OT_13	Cyclohexyl	79	223-224	$C_{18}H_{26}N_2O_2S$	334.48

5.2.3. Characterization of the synthesized molecules

2-Acetamido-6-acetyl-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine-3-carboxamide (PT_07):

MS (ESI) 282 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.42 (s, 2H), 7.90 (s, 1H), 4.61(s, 2H), 3.91(t, J = 7.6 Hz, 2H), 3.18 (t, J = 8.0 Hz, 2H), 2.19 (s, 3H), 2.10 (s, 3H); ^{13}C NMR (100

MHz, DMSO-d_6) δ 172.3, 169.8, 161.6, 133.1, 129.8, 128.5, 112.2, 51.9, 42.8, 26.3, 23.4, 22.5. Anal. calcd for $C_{12}H_{15}N_3O_3S$: C, 51.23; H, 5.37; N, 14.94 % Found C, 51.32; H, 5.39; N, 15.01%.

6-Acetyl-2-benzamido-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine-3-carboxamide (PT_08): MS (ESI) 344 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.17 (s, 2H), 7.81–7.63 (m, 6H), 4.50 (s, 2H), 3.81 (t, *J* = 7.6 Hz, 2H), 3.10 (t, *J* = 7.6 Hz, 2H), 2.20 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 178.3, 172.4, 167.9, 139.4, 133.8, 131.4(2C), 130.8, 128.4(2C), 126.3, 123.8, 118.9, 43.8, 39.7, 25.0, 22.5. Anal. calcd for $C_{17}H_{17}N_3O_3S$: C, 59.46; H, 4.99; N, 12.24 % Found C, 59.52; H, 5.12; N, 12.32%.

6-Acetyl-2-(2-methoxybenzamido)-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine-3-carboxamide (PT_09):

MS (ESI) 374 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.45 (s, 1H), 9.97 (s, 2H), 7.74–7.63 (m, 2H), 7.56 (d, *J* = 7.6 Hz, 1H), 7.49 (t, *J* = 8.0 Hz, 1H), 4.58 (s, 2H), 3.94 (s, 3H), 3.72 (t, *J* = 7.2 Hz, 2H), 3.15 (t, *J* = 7.6 Hz, 2H), 2.16 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 181.3, 176.3, 171.9, 148.9, 141.8, 139.4, 133.8, 132.9, 130.3, 128.7, 127.4, 122.4, 114.8, 55.8, 41.6, 38.9, 22.5, 21.6. Anal. calcd for $C_{18}H_{19}N_3O_4S$: C, 57.89; H, 5.13; N, 11.25 % Found C, 57.92; H, 5.22; N, 11.32%.

6-Acetyl-2-(3-nitrobenzamido)-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine-3-carboxamide (PT_10):

MS (ESI) 389 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.21 (s, 1H), 8.31 (s, 1H), 8.10 (s, 2H), 7.99–7.90 (m, 2H), 7.72 (t, *J* = 8.4 Hz, 1H), 4.59 (s, 2H), 3.79 (t, *J* = 7.6 Hz, 2H), 3.09 (t, *J* = 8.0 Hz, 2H), 2.23 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 177.9, 175.3, 168.4, 141.9, 139.8, 132.5, 130.5, 128.4, 126.9, 124.8, 122.4, 120.4, 116.3, 45.2, 38.4, 24.9, 22.5. Anal. calcd for $C_{17}H_{16}N_4O_5S$: C, 52.57; H, 4.15; N, 14.43 % Found C, 52.62; H, 4.22; N, 14.52%.

6-Acetyl-2-(4-methylbenzamido)-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine-3-carboxamide (PT_11):

MS (ESI) 358 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.27 (s, 2H), 7.81 (s, 1H), 7.90 (d, *J* = 8.0 Hz, 2H), 7.63 (d, *J* = 8.0 Hz, 2H), 4.60 (s, 2H), 3.81 (t, *J* = 7.6 Hz, 2H), 3.18 (t, *J* = 7.2 Hz, 2H), 2.41 (s, 3H), 2.18 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 178.7, 169.3, 166.2, 134.0, 132.9, 130.3(2C), 129.3(2C), 127.4, 123.4, 121.4, 116.3, 51.8, 42.6, 26.2, 24.5, 23.7. Anal. calcd for $C_{18}H_{19}N_3O_3S$: C, 60.49; H, 5.36; N, 11.76 % Found C, 60.52; H, 5.39; N, 11.91%.

6-Acetyl-2-(4-phenoxybenzamido)-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine-3-carboxamide (PT_12)

MS (ESI) 436 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.27 (s, 1H), 7.92 (s, 2H), 7.81 (d, *J* = 8.0 Hz, 2H), 7.72 (d, *J* = 8.4 Hz, 2H), 7.69–7.54 (m, 5H), 4.70 (s, 2H), 3.80 (t, *J* = 8.4 Hz, 2H), 3.13 (t, *J* = 8.0 Hz, 2H), 2.23 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.7, 172.7, 169.6, 146.3, 143.7, 132.3(2C), 129.0(2C), 128.4, 127.4(2C), 125.9, 125.1(2C), 122.6, 123.5, 121.8, 115.9, 46.8, 41.8, 23.6, 20.4. Anal. calcd for $C_{23}H_{21}N_3O_4S$: C, 63.43; H, 4.86; N, 9.65 % Found C, 63.52; H, 4.99; N, 9.72%.

2-(1-Naphthamido)-6-acetyl-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine-3-carboxamide (PT_13):

MS (ESI) 394 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.29 (s, 1H), 8.19–8.10 (m, 4H), 7.92–7.74 (m, 5H), 4.57 (s, 2H), 3.90 (t, *J* = 8.0 Hz, 2H), 3.14 (t, *J* = 7.2 Hz, 2H), 2.14 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 179.2, 176.2, 168.2, 141.3, 140.7, 138.4, 137.9, 136.2, 135.4, 132.9, 128.7, 126.0, 125.3, 123.3, 122.6, 121.8, 118.1, 44.4, 39.8, 26.2, 21.7. Anal. calcd for $C_{21}H_{19}N_3O_3S$: C, 64.10; H, 4.87; N, 10.68 % Found C, 64.12; H, 5.02; N, 10.72%.

6-Acetyl-2-(cyclopropanecarboxamido)-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine-3-carboxamide (PT_14):

MS (ESI) 308 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.97 (s, 2H), 6.93 (s, 1H), 4.66 (s, 2H), 3.97(t, *J* = 8.0 Hz, 2H), 3.21 (t, *J* = 8.0 Hz, 2H), 2.25 (s, 3H), 1.92–1.86 (m, 1H), 1.28–1.03 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 178.4, 172.9, 166.4, 133.3, 131.3, 129.4, 119.1, 43.4, 41.6, 27.4, 24.5, 22.3, 20.7(2C). Anal. calcd for $C_{14}H_{17}N_3O_3S$: C, 54.71; H, 5.57; N, 13.67 % Found C, 54.82; H, 5.64; N, 13.71%.

6-Acetyl-2-(cyclopentanecarboxamido)-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine-3-carboxamide (PT_15):

MS (ESI) 336 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.81 (s, 2H), 7.69 (s, 1H), 4.71 (s, 2H), 3.94 (t, *J* = 7.6 Hz, 2H), 3.16 (t, *J* = 7.2 Hz, 2H), 2.18 (s, 3H), 2.16–2.10 (m, 1H), 1.89–1.60 (m, 8H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.9, 167.8, 161.6, 133.2, 132.7, 124.5, 114.9, 47.9, 46.4, 41.2(2C), 36.9, 31.5(2C), 26.5, 23.4. Anal. calcd for $C_{16}H_{21}N_3O_3S$: C, 57.29; H, 6.31; N, 12.53 % Found C, 57.32; H, 6.42; N, 12.61%.

6-Acetyl-2-(cyclohexanecarboxamido)-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine-3-carboxamide (PT_16):

ESI-MS showed 350 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.97 (s, 2H), 7.72 (s, 1H), 4.66 (s, 2H), 3.97(t, *J* = 8.0 Hz, 2H), 3.21 (t, *J* = 8.0 Hz, 2H), 2.14–2.09 (m, 4H), 1.98–1.53 (m, 10H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 176.7, 171.8, 166.6, 136.1, 130.8, 122.5, 116.6, 49.9, 44.8, 38.6(2C), 30.4(2C), 26.3, 24.4, 23.5, 21.4. Anal. calcd for $C_{17}H_{23}N_3O_3S$: C, 58.43; H, 6.63; N, 12.02 % Found C, 58.52; H, 6.73; N, 12.13%.

2-Acetamido-6-methyl-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine-3-carboxamide (PT_17):

MS (ESI) 254 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.53 (s, 1H), 8.01 (s, 2H), 4.12 (s, 2H), 3.44 (t, *J* = 7.6 Hz, 2H), 3.15 (t, *J* = 8.0 Hz, 2H), 2.41 (s, 3H), 2.16 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 178.7, 167.4, 158.7, 133.4, 129.8, 127.6, 51.3, 49.8, 42.7, 25.2, 22.5. Anal. calcd for $C_{11}H_{15}N_3O_2S$: C, 52.15; H, 5.97; N, 16.59 % Found C, 52.22; H, 6.02; N, 16.72%.

2-Benzamido-6-methyl-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine-3-carboxamide (PT_18):

MS (ESI) 316 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.63 (s, 1H), 9.97 (s, 2H), 7.92–7.63 (m, 5H), 4.54 (s, 2H), 3.71 (t, *J* = 8.4 Hz, 2H), 3.13 (t, *J* = 7.6 Hz, 2H), 2.52 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 176.4, 170.5, 136.2, 129.5, 127.2(2C), 125.8(2C), 125.1, 124.4, 123.6, 115.4, 48.3, 42.6, 39.0, 21.8. Anal. calcd for $C_{16}H_{17}N_3O_2S$: C, 60.93; H, 5.43; N, 13.32 % Found C, 61.02; H, 5.52; N, 13.42%.

2-(2-Methoxybenzamido)-6-methyl-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine-3-carboxamide (PT_19):

MS (ESI) 346 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.45 (s, 2H), 7.81 (d, *J* = 7.6 Hz, 1H), 7.72–7.54 (m, 4H), 4.61 (s, 2H), 3.90 (s, 3H), 3.69 (t, *J* = 7.6 Hz, 2H), 3.21 (t, *J* = 8.0 Hz, 2H), 2.46 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 178.4, 172.3, 146.9, 142.8, 134.4, 133.9, 132.3, 131.4, 128.4, 126.9, 125.1, 120.0, 60.1, 56.6, 52.9, 39.9, 23.6. Anal. calcd for $C_{17}H_{19}N_3O_3S$: C, 59.11; H, 5.54; N, 12.17 % Found C, 59.22; H, 5.61; N, 12.16%.

6-Methyl-2-(3-nitrobenzamido)-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine-3-carboxamide (PT_20):

MS (ESI) 361 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.21 (s, 2H), 9.45 (s, 1H), 8.40 (s, 1H), 8.01 (d, *J* = 8.0 Hz, 1H), 7.81–7.72 (m, 2H), 4.61 (s, 2H), 3.81 (t, *J* = 8.0 Hz, 2H), 3.12 (t, *J* = 8.0 Hz, 2H), 2.34 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 181.8, 173.3, 149.8, 144.6, 139.4, 137.4, 136.4, 133.4, 129.9, 126.8, 124.4, 118.3, 51.2, 46.4, 38.9, 26.5. Anal. calcd for $C_{16}H_{16}N_4O_4S$: C, 53.32; H, 4.47; N, 15.55 % Found C, 53.42; H, 4.52; N, 15.64%.

6-Methyl-2-(4-methylbenzamido)-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine-3-carboxamide (PT_21):

MS (ESI) 330 $[M+H]^+$. ^1H NMR (400 MHz, DMSO-d_6): δ 9.47 (s, 1H), 7.84–7.72 (m, 4H), 7.54 (d, *J* = 8.0 Hz, 2H), 4.31 (s, 2H), 3.61 (t, *J* = 7.6 Hz, 2H), 3.04 (t, *J* = 7.6 Hz, 2H), 2.43 (s, 3H), 2.39 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.4, 168.3, 148.2, 146.5, 139.0, 136.5, 130.6(2C), 128.4(2C), 126.3, 118.8, 49.8, 43.6, 38.2, 24.5, 21.9. Anal. calcd for $C_{17}H_{19}N_3O_2S$: C, 61.98; H, 5.81; N, 12.76 % Found C, 62.02; H, 5.89; N, 12.81%.

6-Methyl-2-(4-phenoxybenzamido)-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine-3-carboxamide (PT_22):

MS (ESI) 408 $[M+H]^+$. ^1H NMR (400 MHz, DMSO-d_6): δ 11.97 (s, 2H), 7.83 (d, *J* = 8.4 Hz, 2H), 7.72–7.63 (m, 3H), 7.54–7.45 (m, 5H), 4.84 (s, 2H), 3.72 (t, *J* = 8.4 Hz, 2H), 3.21 (t, *J* = 8.4 Hz, 2H), 2.43 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 183.1, 178.7, 148.2, 146.9, 136.4(2C), 136.0, 134.3, 132.0(2C), 129.5, 128.2(2C), 126.9(2C), 125.3, 124.6, 119.9, 52.9, 46.8, 36.9, 24.1. Anal. calcd for $C_{22}H_{21}N_3O_3S$: C, 64.85; H, 5.19; N, 10.31 % Found C, 64.92; H, 5.21; N, 10.52%.

2-(1-Naphthamido)-6-methyl-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine-3-carboxamide (PT_23):

MS (ESI) 366 $[M+H]^+$. ^1H NMR (400 MHz, DMSO-d_6): δ 9.99 (s, 2H), 8.22–8.10 (m, 2H), 7.89–7.72 (m, 6H), 4.54 (s, 2H), 3.79 (t, *J* = 8.0 Hz, 2H), 3.13 (t, *J* = 7.6 Hz, 2H), 2.34 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.2, 168.4, 148.2, 139.3, 138.6, 138.0, 137.4, 135.8, 135.2, 134.5, 132.1, 129.8, 127.2, 126.3, 124.6, 121.1, 51.3, 44.8, 37.8, 22.7. Anal. calcd for $C_{20}H_{19}N_3O_2S$: C, 65.73; H, 5.24; N, 11.50 % Found C, 65.82; H, 5.32; N, 11.62%.

2-(Cyclopropanecarboxamido)-6-methyl-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine-3-carboxamide (PT_24):

MS (ESI) 280 $[M+H]^+$. ^1H NMR (300 MHz, DMSO-d_6): δ 10.11 (s, 1H), 8.22 (s, 2H), 4.45 (s, 2H), 3.81 (t, *J* = 8.4 Hz, 2H), 3.18 (t, *J* = 8.0 Hz, 2H), 2.45 (s, 3H), 2.14–2.10 (m, 1H), 1.54–1.12 (m, 4H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 176.4, 171.9, 144.4, 134.3, 132.3, 120.9, 48.4, 43.6, 38.4, 22.5, 19.3, 18.7(2C). Anal calcd for $C_{13}H_{17}N_3O_2S$: C, 55.89; H, 6.13; N, 15.04 % Found C, 55.92; H, 6.24; N, 15.11%.

2-(Cyclopentanecarboxamido)-6-methyl-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine-3-carboxamide (PT_25):

MS (ESI) 308 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.21 (s, 2H), 8.67 (s, 1H), 4.64 (s, 2H), 3.86 (t, *J* = 8.0 Hz, 2H), 3.12 (t, *J* = 7.6 Hz, 2H), 2.38 (s, 3H), 2.11–2.0 (m, 1H), 1.69–1.34 (m, 8H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 176.9, 169.8, 149.6, 132.2, 130.7, 122.5, 52.9, 49.4, 46.2, 39.9, 31.6(2C), 26.3(2C), 21.4. Anal. calcd for $C_{15}H_{21}N_3O_2S$: C, 58.61; H, 6.89; N, 13.67 % Found C, 58.72; H, 7.02; N, 13.71%.

2-(Cyclohexanecarboxamido)-6-methyl-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine-3-carboxamide (PT_26):

MS (ESI) 322 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.45 (s, 1H), 7.74 (s, 2H), 4.58 (s, 2H), 3.87 (t, *J* = 8.0 Hz, 2H), 3.19 (t, *J* = 8.0 Hz, 2H), 2.61 (s, 3H), 2.16–2.09 (m, 1H), 1.89–1.44 (m, 10H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.2, 169.9, 132.1, 129.8, 128.4, 119.7, 61.0, 56.5, 47.8, 43.5, 31.6(2C), 28.4, 23.3(2C), 22.4. Anal. calcd for $C_{16}H_{23}N_3O_2S$: C, 59.78; H, 7.21; N, 13.07 % Found C, 59.82; H, 7.29; N, 13.11%.

2-Acetamido-4,5,6,7,8,9-hexahydrocycloocta[*b*]thiophene-3-carboxamide (OT_04):

MS (ESI) 267 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.22 (s, 2H), 7.47 (s, 1H), 2.73–2.50 (m, 4H), 2.23 (s, 3H), 1.60–1.35 (m, 8H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.4, 166.9, 149.4, 135.3, 133.3, 123.4, 29.7, 27.3, 25.4, 24.8(2C), 23.6, 22.5. Anal. calcd for $C_{13}H_{18}N_2O_2S$: C, 58.62; H, 6.81; N, 10.52 % Found C, 58.69; H, 6.84; N, 10.61%.

2-Benzamido-4,5,6,7,8,9-hexahydrocycloocta[*b*]thiophene-3-carboxamide (OT_05)

MS (ESI) 329 $[M+H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.92 (s, 2H), 7.81–7.62 (m, 6H), 2.64–2.36 (m, 4H), 1.71–1.41 (m, 8H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.2, 169.9, 146.2, 139.7, 136.3(2C), 134.4(2C), 129.3, 127.6, 126.7, 120.8, 27.8, 26.9, 26.3, 24.5(2C), 22.9. Anal. calcd for $C_{18}H_{20}N_2O_2S$: C, 65.83; H, 6.14; N, 8.53 % Found C, 65.99; H, 6.24; N, 8.71%.

2-(2-Methoxybenzamido)-4,5,6,7,8,9-hexahydrocycloocta[*b*]thiophene-3-carboxamide (OT_06)

MS (ESI) 359 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.25 (s, 1H), 7.90 (d, *J* = 8.0 Hz, 1H), 7.81 (s, 1H), 7.72–7.63 (m, 4H), 3.81 (s, 3H), 2.81–2.49 (m, 4H), 1.62–1.39 (m, 8H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.2, 164.9, 152.2, 144.7, 138.3, 136.5, 134.0, 133.2, 130.5, 128.7, 126.9, 123.6, 54.9, 26.8, 25.4, 24.2(2C), 22.9, 22.3. Anal. calcd for $C_{19}H_{22}N_2O_3S$: C, 63.66; H, 6.19; N, 7.82 % Found C, 63.72; H, 6.21; N, 8.01%.

2-(3-Nitrobenzamido)-4,5,6,7,8,9-hexahydrocycloocta[*b*]thiophene-3-carboxamide (OT_07)

MS (ESI) 374 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.21 (s, 2H), 9.00 (s, 1H), 8.39 (s, 1H), 7.99 (d, *J* = 8.4 Hz, 1H), 7.83–7.69 (m, 2H), 2.81–2.39 (m, 4H), 1.59–1.19 (m, 8H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 183.8, 175.6, 148.6, 143.5, 138.8, 133.6, 132.6, 130.5, 128.6, 126.7, 125.3, 121.4, 27.9, 27.2, 25.6(2C), 24.3, 22.5. Anal. calcd for $C_{18}H_{19}N_3O_4S$: C, 57.89; H, 5.13; N, 11.25 % Found C, 57.92; H, 5.22; N, 11.34%.

2-(4-Methylbenzamido)-4,5,6,7,8,9-hexahydrocycloocta[*b*]thiophene-3-carboxamide (OT_08)

MS (ESI) 343 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.56 (s, 2H), 8.01 (d, *J* = 8.0 Hz, 2H), 7.84 (s, 1H), 7.72 (d, *J* = 8.0 Hz, 2H), 2.52 (s, 3H), 2.70–2.29 (m, 4H), 1.87–1.36 (m, 8H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 181.2, 173.9, 147.2, 138.4, 137.7(2C), 136.4, 135.3(2C), 127.3, 125.4, 121.3, 26.8, 25.2, 24.9, 24.3(2C), 22.5, 21.4. Anal. calcd for $C_{19}H_{22}N_2O_2S$: C, 66.64; H, 6.48; N, 8.18 % Found C, 66.72; H, 6.59; N, 8.31%.

2-(4-Phenoxybenzamido)-4,5,6,7,8,9-hexahydrocycloocta[*b*]thiophene-3-carboxamide (OT_09)

MS (ESI) 421 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.40 (s, 1H), 7.89 (d, *J* = 8.7 Hz, 2H), 7.48 (s, 2H), 7.44 (t, *J* = 7.8 Hz, 2H), 7.24 (t, *J* = 7.5 Hz, 1H), 7.13 (d, *J* = 8.4 Hz, 4H), 2.84–2.75 (m, 4H), 1.56–1.42 (m, 6H), 1.19–1.17 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 167.8, 162.0, 160.4, 155.2, 141.4, 131.9, 130.3(2C), 130.2(2C), 129.4, 127.1, 124.6, 119.8(2C), 117.9(2C), 117.7, 32.2, 29.8, 26.2, 25.6, 25.0, 24.3. Anal. calcd for $C_{24}H_{24}N_2O_3S$: C, 68.55; H, 5.75; N, 6.66 % Found C, 68.62; H, 5.81; N, 6.72%.

2-(1-Naphthamido)-4,5,6,7,8,9-hexahydrocycloocta[*b*]thiophene-3-carboxamide (OT_10)

MS (ESI) 379 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.34–8.19 (m, 4H), 7.92–7.81 (m, 4H), 7.72–7.63 (m, 2H), 2.79–2.38 (m, 4H), 1.53–1.20 (m, 8H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 179.2, 166.3, 146.3, 139.6, 138.2, 137.6, 137.2, 136.0, 135.3, 133.9, 133.2, 130.5, 129.4, 126.3, 122.6, 118.7, 28.9, 27.6, 26.2(2C), 24.2, 19.6. Anal calcd for $C_{22}H_{22}N_2O_2S$: C, 69.81; H, 5.86; N, 7.40 % Found C, 69.92; H, 5.94; N, 7.62%.

2-(Cyclopropanecarboxamido)-4,5,6,7,8,9-hexahydrocycloocta[*b*]thiophene-3-carboxamide (OT_11)

MS (ESI) 293 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.45 (s, 2H), 6.77 (s, 1H), 2.79–2.46 (m, 4H), 2.20–2.07 (m, 1H), 1.69–1.12 (m, 12H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 176.4,

171.9, 156.4, 136.3, 133.3, 121.1, 28.7, 27.2, 26.4(2C), 24.8, 22.3, 21.4, 18.7(2C). Anal. calcd for $C_{15}H_{20}N_2O_2S$: C, 61.62; H, 6.89; N, 9.58 % Found C, 61.69; H, 6.94; N, 9.71%.

2-(Cyclopentanecarboxamido)-4,5,6,7,8,9-hexahydrocycloocta[*b*]thiophene-3-carboxamide (OT_12)

MS (ESI) 321 $[M+H]^+$. ^1H NMR (300 MHz, $CDCl_3$): δ 8.21 (s, 2H), 7.27 (s, 1H), 2.73–2.49 (m, 4H), 2.01–1.93 (m, 1H), 1.80–1.33 (m, 16H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.2, 166.5, 152.1, 136.6, 128.7, 120.4, 44.8, 33.3(2C), 29.5, 27.2(2C), 25.2, 24.6(2C), 23.4, 22.5. Anal. calcd for $C_{17}H_{24}N_2O_2S$: C, 63.72; H, 7.55; N, 8.74 % Found C, 63.82; H, 7.72; N, 8.82%.

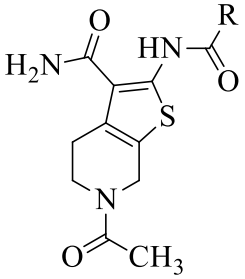
2-(Cyclohexanecarboxamido)-4,5,6,7,8,9-hexahydrocycloocta[*b*]thiophene-3-carboxamide (OT_13)

MS (ESI) 335 $[M+H]^+$. ^1H NMR (400 MHz, DMSO-d_6): δ 7.27 (s, 2H), 6.73 (s, 1H), 2.74–2.41 (m, 4H), 2.18–2.10 (m, 1H), 1.92–1.24 (m, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.3, 171.9, 149.2, 133.3, 129.6, 119.6, 48.9, 29.8, 27.4, 25.2, 24.9(2C), 22.9(2C), 22.0(2C), 21.6, 20.5. Anal. calcd for $C_{18}H_{26}N_2O_2S$: C, 64.64; H, 7.84; N, 8.38 % Found C, 64.72; H, 7.99; N, 8.52%.

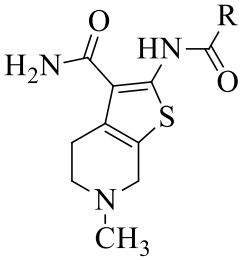
5.2.4. *In vitro M. tuberculosis* screening and cytotoxicity studies of the synthesized molecules

The MIC values of all the synthesized compounds were obtained by *in vitro* screening 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. EMB, INH, ciprofloxacin and reported lead molecule SID 92097880 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 50 μM concentration using MTT assay and results are tabulated as **Table 5.4**.

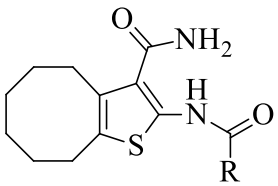
Table 5.4: Biological activities of synthesized compounds **PT_07 – PT_26** and **OT_04 – OT_13**



PT_07 - PT_16



PT_17 - PT_26



OT_04 - OT_13

Compound	R	MTB MIC in µM[a]	MTB MIC in µM [in presence of verapamil]	Cytotoxicity[b] at 50 µM (RAW 264.7 cells) % inhibition
PT_07	Methyl	88.6	NT	NT
PT_08	Phenyl	72.65	NT	NT
PT_09	2-Methoxyphenyl	66.84	NT	NT
PT_10	3-Nitrophenyl	64.26	NT	NT
PT_11	4-Tolyl	69.80	NT	NT
PT_12	4-Phenoxyphenyl	57.30	NT	NT
PT_13	1-Napthyl	63.45	NT	NT
PT_14	Cyclopropyl	81.15	NT	NT
PT_15	Cyclopentyl	74.40	NT	NT
PT_16	Cyclohexyl	71.40	NT	NT
PT_17	Methyl	39.43	NT	NT
PT_18	Phenyl	37.87	NT	NT
PT_19	2-Methoxyphenyl	36.12	NT	NT
PT_20	3-Nitrophenyl	34.62	NT	NT
PT_21	4-Tolyl	37.87	NT	NT
PT_22	4-Phenoxyphenyl	30.60	NT	NT
PT_23	1-Napthyl	17.07	8.53	28.42
PT_24	Cyclopropyl	5.57	2.78	22.16
PT_25	Cyclopentyl	5.06	5.06	18.16
PT_26	Cyclohexyl	7.76	2.58	18.28
OT_04	Methyl	18.72	9.36	24.56
OT_05	Phenyl	15.19	5.06	26.62
OT_06	2-Methoxyphenyl	13.92	6.96	24.80
OT_07	3-Nitrophenyl	16.71	16.71	36.14
OT_08	4-Tolyl	4.54	2.27	20.52
OT_09	4-Phenoxyphenyl	3.70	1.23	22.16

Contd

Compound	R	MTB MIC in µM[a]	MTB MIC in µM [in presence of verapamil]	Cytotoxicity[b] at 50 µM (RAW 264.7 cells) % inhibition
OT_10	1-Napthyl	13.19	3.29	28.32
OT_11	Cyclopropyl	17.06	8.53	16.86
OT_12	Cyclopentyl	9.73	9.73	18.34
OT_13	Cyclohexyl	9.33	3.11	16.64
Isoniazid		0.72	0.72	NT
Ethambutol		7.64	3.82	NT
Ciproflaxacin		9.41	9.41	NT
SID 92097880		9.15	4.57	NT

MTB, *Mycobacterium tuberculosis*; MIC, minimum inhibitory concentration;

[a]*In vitro* activity against MTB H37Rv; [b]Against RAW 264.7 cells; NT, Not tested.

5.2.5. SAR and discussion

All the synthesized compounds showed activity against *M. tuberculosis* with MIC ranging from 3.70 to 88.6 µM. Seven compounds (**PT_24 - PT_26, OT_08, OT_09, OT_12** and **OT_13**) inhibited MTB with MIC <10 µM. Compound **OT_09** (2-(4-phenoxybenzamido)-4,5,6,7,8,9-hexahydrocycloocta[b]thiophene-3-carboxamide) was found to be the most active compound *in vitro* with MIC of 3.70 µM against log-phase culture of *M. tuberculosis*. All the synthesized compounds were less potent than standard first line antitubercular compound INH. Three compounds were found to be more potent than EMB (MIC 7.64 µM). Five compounds were found to be more potent than lead compound SID 92097880 (MIC 9.15 µM).

With respect to SAR of 2,6-disubstituted 4,5,6,7-tetrahydrothieno[2,3-c]pyridine-3-carboxamides (**PT_07 - PT_16** and **PT_17 - PT_26**); we have prepared various alkyl, cycloalkyl and (sub)aryl amide at 2nd position and at N-6 position we have prepared acetyl (**PT_07 - PT_16**) and methyl (**PT_17 - PT_26**) derivatives. At N-6 position, compounds with N-methyl derivatives showed better activity (MIC ranging 5.06 to 39.43 µM) than N-acetyl derivatives (MIC ranging 57.30 to 88.60 µM). These results indicated that alkyl substituent favour bioactivity than the acyl derivative. Among compounds **PT_17 - PT_26**; hydrophobic

substituent at C-2 position increased the activity. Cycloalkyl derivatives (**PT_24 - PT_26**) showed promising activity with MIC less than 10 μM; followed by naphthyl derivative (**PT_23**) with MIC of 17.07 μM. When compared to phenyl derivative (**PT_18**), napththyl derivative showed two times more potency. Among (sub)phenyl derivatives (**PT_18 - PT_22**), not much difference were found between electron withdrawing and donating groups at phenyl ring. With respect to SAR of 4,5,6,7,8,9-hexahydrocycloocta[b]thiophene-3-carboxamides (**OT_04 - OT_13**), where we replaced N-acetyl tetrahydropyridine moiety of lead compound SID 92097880 with highly hydrophobic cyclooctyl moiety showed better activity with MIC ranging from 3.70 to 18.72 μM. In this series some of substituted phenyl derivatives at C-2 position (**OT_08** and **OT_09**) showed better activity (less than 5 μM) than cycloalkyl (**OT_11 – OT_13**) and naphthyl derivative (**OT_10**).

When compared to lead compound SID 92097880, many of the synthesized compounds showed less activity. It could be due to the involvement of wide array of efflux mechanisms mediated by several ABC (ATP-binding cassette) transporters and major facilitator superfamily (MFS) proteins, or antibiotic-modifying and-degrading enzymes, to name a few possibilities [Derossi E., *et al*., 2006]. Multiple drugs like verapamil, reserpine, phenothiazines such as thioridazine, and piperine have been shown to inhibit bacterial efflux pumps *in vitro* [Rodrigues L., *et al*., 2011]. In general, the mechanisms by which these agents act were poorly understood. Several models have been proposed [Pages J.M., *et al*., 2009], such as: (1) direct binding and inhibition of pump assembly or function; (2) disruption of the transmembrane gradients utilized by secondary transporters; (3) inhibitor binding to the antimicrobial compound; and (4) competition for efflux. We have tested some selected compounds [MIC of < 20 μM], in presence of reported efflux pump inhibitor verapamil; and in most of the cases MIC was decreased 2 to 3 fold when compared to absence of efflux pump inhibitor. Most active compound **OT_09** showed MIC of 1.23 μM in this study (**Figure 5.5**).

Compounds with MIC less than 20 μM were further examined for cytotoxicity in a RAW 264.7 cell line (mouse leukaemic monocyte macrophage) at single concentration of 50 μM. We have selected this macrophage cell line to test the toxicity as generally *M. tuberculosis* reside inside the macrophages and we expected the new molecules not to show any toxicity against

macrophages. After 72 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. Most of the tested compounds were not cytotoxic to RAW 264.7 cells based on their percentage growth inhibitions (less than 30%) as reported in Table 1. The most active anti-TB compound **OT_09** showed 22.6% cytotoxicity at 50 µM with selectivity index of > 10 for *M. tuberculosis*.

5.2.6. Highlights of the study

In this study we have designed, synthesized and studied SAR of various inhibitors of *M. tuberculosis* based on the lead compound SID 92097880 earlier reported by TAACF. Among the compounds, 2-(4-phenoxybenzamido)-4,5,6,7,8,9-hexahydrocycloocta[b]thiophene-3-carboxamide (**OT_09**) was found to be the most active compound *in vitro* with MIC of 3.70 µM and also non-toxic up to 50 µM. Compound **OT_09** was ~3 times more potent than lead compound SID: 92097880. Compound **OT_09** showed MIC of 1.23 µM in the presence of efflux pump inhibitor. Further structural optimization can be performed to get compounds with better potency than the lead compound and also to explore the possible mechanism of action against various MTB essential enzymes.

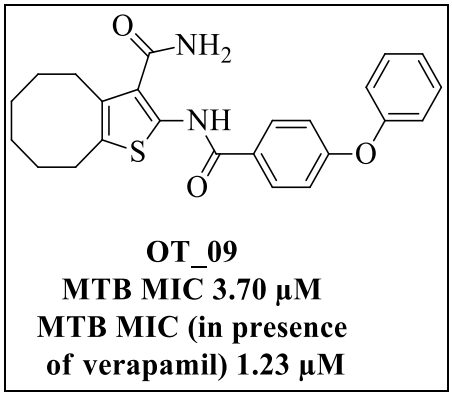


Figure 5.5: Structure and biological activity of most active compound **OT_09**

5.3. Design and synthesis of 2-iminothiazolidin-4-one derivatives as novel *M. tuberculosis* PknB agents

Thiazolidinones are synthetically feasible and important class of heterocycles possessing two hetero atoms nitrogen and sulphur in the ring and exocyclic oxygen atom. In literature thizolidinones were found to possess various biological activities *viz*. antibacterial, analgesic, CNS stimulant, hypnotics, anti-HIV, local and spiral anaesthetic [Gokce CU., *et al*., 2015; Patel RB., *et al*., 2006]. 4-thiazolidinone derivatives were well studied for their anti-tubercular activity, few of the reported anti-tubercular thiazolidin-4-ones were presented in **Figure 5.6** [Samala G., *et al*., 2014; Aridoss G., *et al*., 2009; Babaoglu K., *et al*., 2003; Kucukguzel SG., *et al*., 2002].

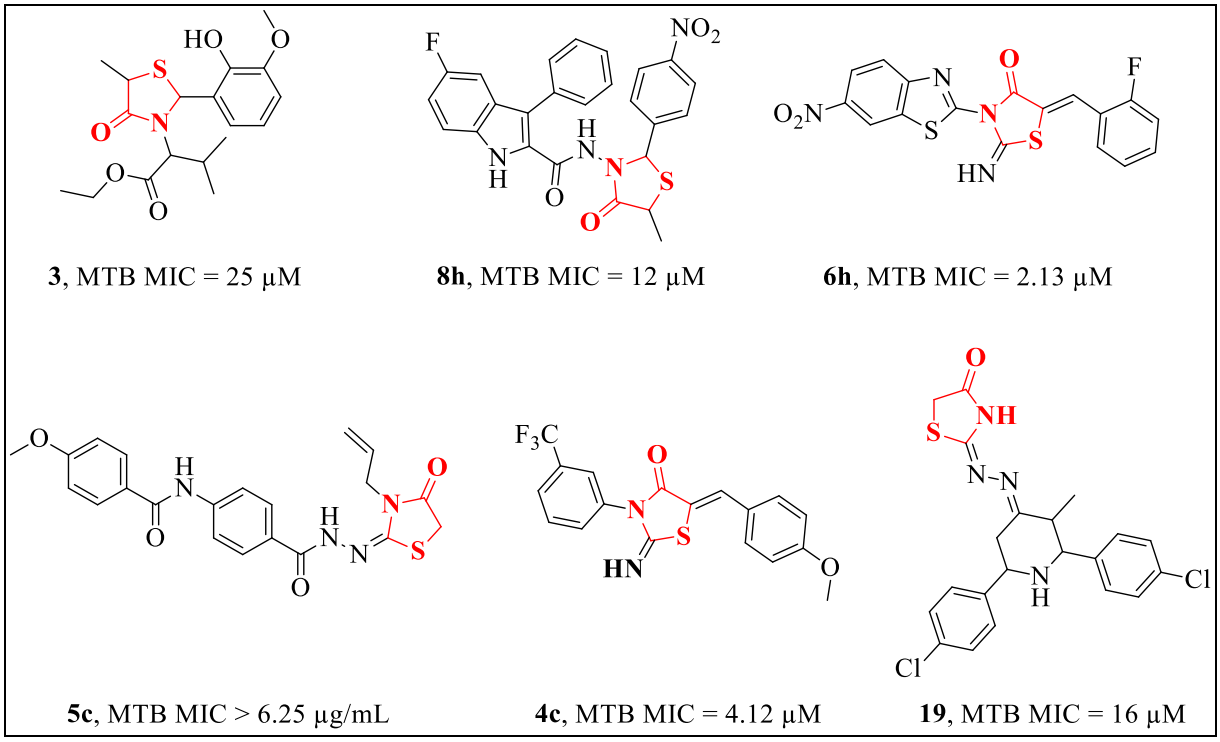


Figure 5.6: Reported 4-thiazolidionones as anti-tubercular compounds

5.3.1. Design of the molecules

We have reported the design and synthesis of 3-substituted-2-iminothiazolidin-4-ones as potential anti-tubercular agents, fourteen molecules of the thirty six compound library inhibited *M. tuberculosis* with MIC < 5 µM [Samala G., *et al*., 2014]. After thorough search of literature

of thiazolidinones and based on our knowledge in designing of novel anti-tubercular agents, we forebode the better activity of 2-iminothiazolidin-4-ones derivatization at 2nd position. We have synthesized two thiazolidine-4-ones (**TZ_L01** and **TZ_L02**) subjecting to derivatization at 2nd position (extending our previous work which had substitutions at 3rd position) anticipating better activity. The two molecules **TZ_L01** and **TZ_L02** showed good activity against *M. tuberculosis* and out of four tested enzymes, they showed better activities against *M. tuberculosis* PknB [**Figure 5.7**]. We have selected **TZ_01** and **TZ_02** as lead molecules for further development and to study structure activity relationship.

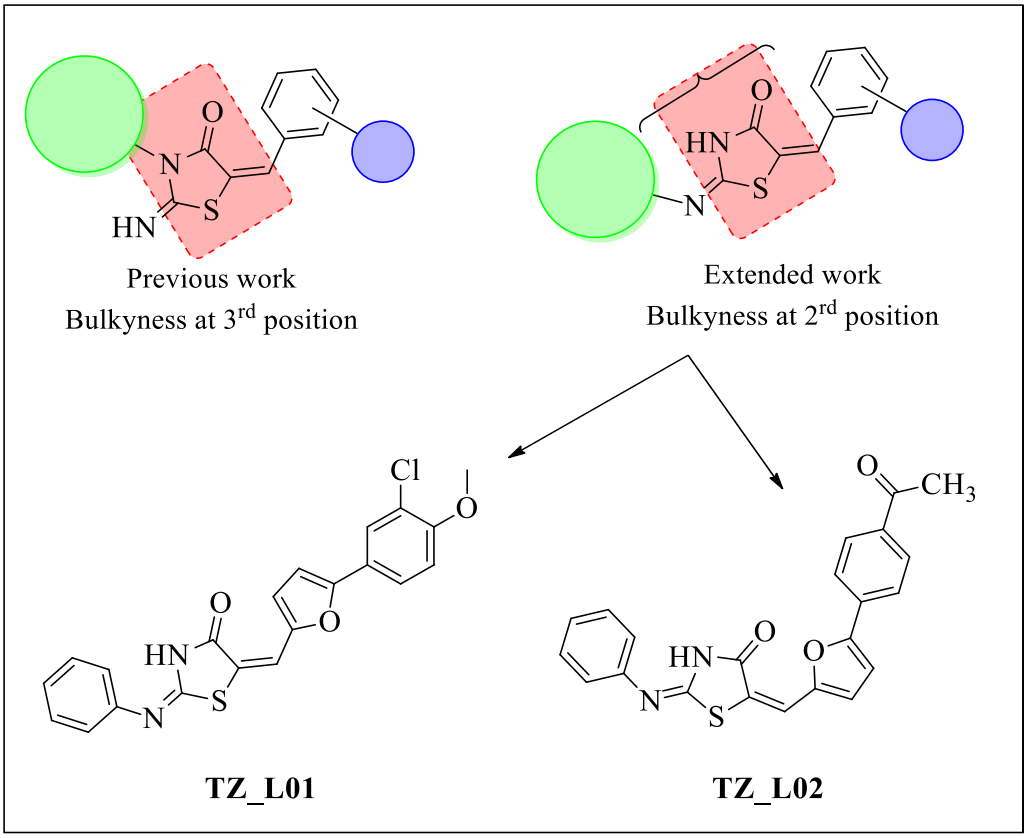


Figure 5.7: Designing of lead molecule based on previous work

5.3.2. Experimental procedures utilized for the synthesis of TZ_04 – TZ_56

The target molecules were synthesized by following the three step synthetic protocol (**Figure 4.3**). Synthesis started with the commercially available various substituted arylisothiocyanates on reaction with ammonia solution in THF and the resulting solid was filtered and washed with excess of water, cold ethanol and diethyl ether and dried to afford the corresponding substituted

arylthioureas (**TZ_02a-e**). Further the cyclization reaction was carried out by the reaction of (**TZ_02a-e**) with ethyl 2-bromoacetate and anhydrous NaOAc in absolute ethanol at 60 °C to afford the key intermediate 2-(substitutedaryimino)thiazolidin-4-one (**TZ_03a-e**) in good yields. The mechanism of formation of (**TZ_03a-e**) is illustrated in **Figure 5.8**. These reactions were also successfully carried out using ethyl-2-chloroacetate, anhydrous NaOAc in absolute ethanol at 60 °C but the former reaction conditions resulted in good yields. In final step we used Knoevenagal condensation of the compound **TZ_03a-e** with various substituted aldehydes using piperidine in absolute ethanol at 60 °C, upon complete consumption of the starting material the reaction mixture was filtered to remove the bromide salts and the filtrate was concentrated and obtained residue was re-dissolved in ethylacetate and washed with water and brine solution and purified to produce title compounds **TZ_04-TZ_56** (**Table 5.5**).

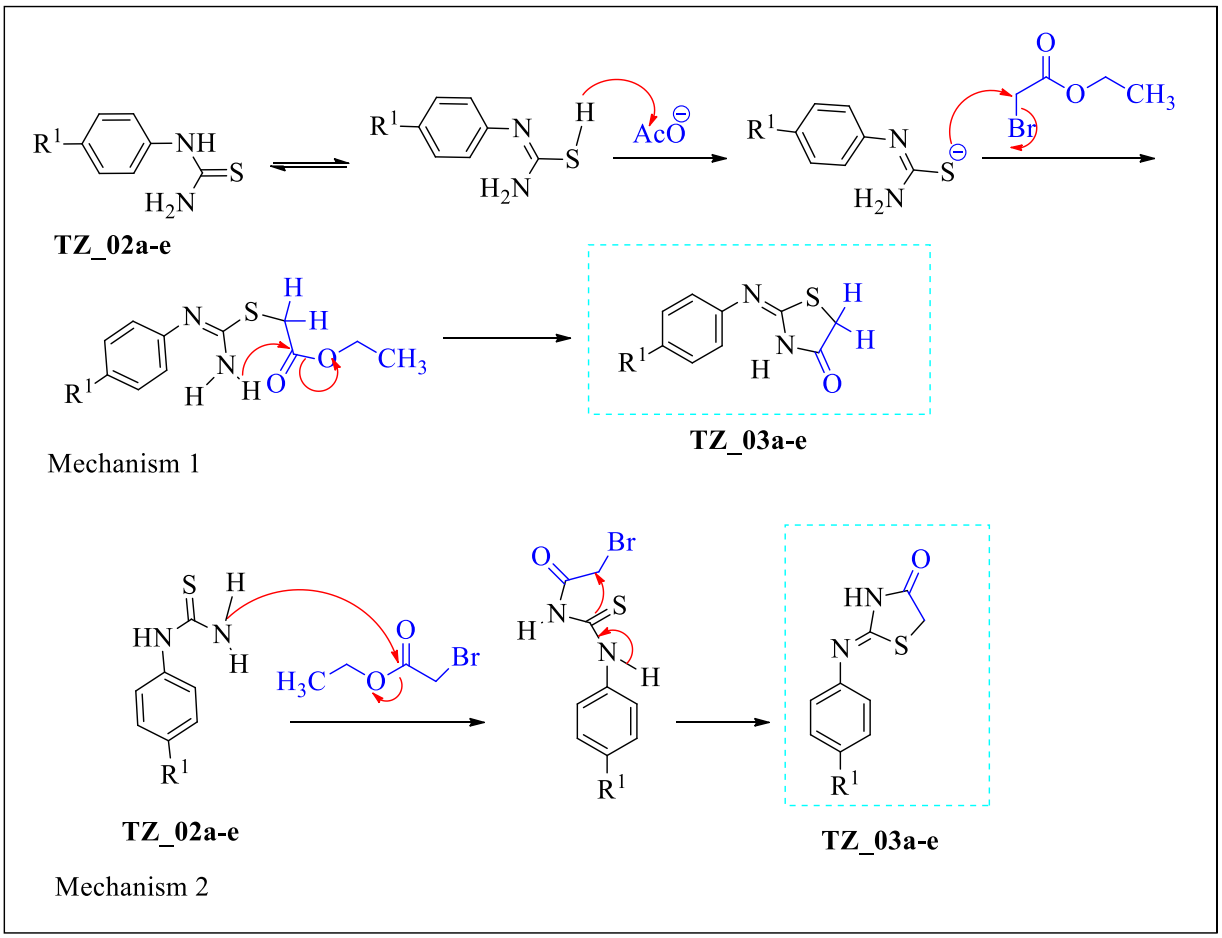
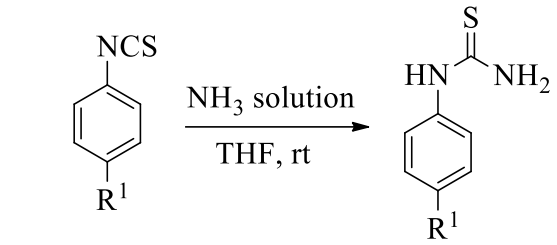


Figure 5.8: Mechanism of conversion of compound **TZ_02a-e** to **TZ_03a-e**

General procedure for the synthesis of (TZ_02a-e)



TZ_01a-e
R_1 = H, CH_3, OCH_3
Cl, NO_2

TZ_02a-e

To the starting material **TZ_01a-e** (1.0 equiv) in THF, was added NH_3 solution (10 vol) under cooling conditions and allowed the reaction mixture to stir at room temperature for 3 h, then the solids formed in the reaction mixture was filtered and washed with diethyl ether and dried to afford the pure product **(TZ_02a-e)** as white solid (yields 90-95%).

1-Phenylthiourea (TZ_02a)

Following the general procedure the product was synthesized from phenylisothiocyanate **TZ_01a** (3.00 g, 22.22 mmol), NH_3 solution (30 mL) produced 1-phenylthiourea **(TZ_02a**) (3.2 g, 98%) as white solid. ESI-MS showed 153 $[M+H]^+$ and carried to next step.

1-(*p*-Tolyl)thiourea (TZ_02b)

Following the general procedure the product was synthesized from p-tolylisothiocyanate **TZ_01b** (5.00 g, 33.55 mmol), NH_3 solution (50 mL) produced 1-(p-tolyl)thiourea **(TZ_02b**) (5.4 g, 98%) as white solid. ESI-MS showed 167 $[M+H]^+$ and carried to next step.

1-(4-Methoxyphenyl)thiourea (TZ_02c)

Following the general procedure the product was synthesized from 4-methoxyphenylisothio cyanate **TZ_01c** (5.00 g, 30.30 mmol), NH_3 solution (50 mL) produced 1-(4-methoxyphenyl)thiourea **(TZ_02c**) (5.4 g, 98%) as white solid. ESI-MS showed 183 $[M+H]^+$ and carried to next step.

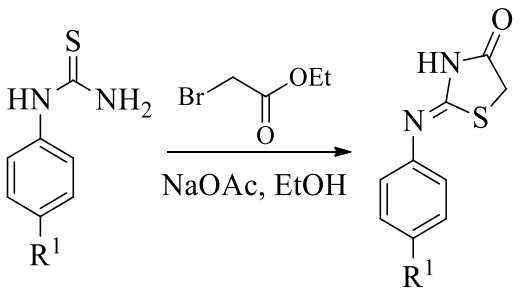
1-(4-Chlorophenyl)thiourea (TZ_02d)

Following the general procedure the product was synthesized from 4-chlorophenylisothio cyanate **TZ_01d** (5.00 g, 29.58 mmol), NH_3 solution (50 mL) produced 1-(4-chlorophenyl)thiourea **(TZ_02d**) (5.3 g, 96%) as white solid. ESI-MS showed 187 $[M+H]^+$ and carried to next step.

1-(4-Nitrophenyl)thiourea (TZ_02e)

Following the general procedure the product was synthesized from 4-nitrophenylisothio cyanate **TZ_01e** (5.00 g, 29.58 mmol), NH_3 solution (50 mL) produced 1-(4-nitrophenyl)thiourea **(TZ_02e**) (5.3 g, 98%) as white solid. ESI-MS showed 198 $[M+H]^+$ and carried to next step.

General procedure for the synthesis of (TZ_03a-e)



TZ_02a-e **TZ_03a-e**

To the stirred solution of **TZ_02a-e** (1.0 equiv) in EtOH (10 vol) was added anhydrous NaOAc (5.0 equiv) followed by ethylbromoacetate (2.0 equiv) and the reaction mixture was heated at 60 °C for 7 h. The solids formed in the reaction mixture were filtered and washed with EtOH, the filtrate was concentrated and the solid obtained was partitioned between ethyl acetate and water then washed with brine solution, the Ethyl acetate layer was dried over anhydrous Na_2SO_4 and concentrated under vacuo then the solid obtained was triturated with CH_2Cl_2/hexanes the resulting solid was filtered, washed with diethyl ether and dried to get **TZ_03a-e** in pure form (yields >80%) and used for the next step.

2-(Phenylimino)thiazolidin-4-one (TZ_03a)

Following the general procedure the product was synthesized from 1-phenylthiourea **TZ_02a** (3.20 g, 21.05 mmol), anhydrous NaOAc (8.63 g, 105.05 mmol) and Ethylbromoacetate (4.65 mL, 42.10 mmol) produced 2-(phenylimino)thiazolidin-4-one **(TZ_03a**) (3.6 g, 89%) as yellow

solid. ESI-MS showed 193 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.73 (s,0.5×1H), 11.15 (s,0.5×1H), 7.70 (m, 1H), 7.40–7.37 (m, 2H), 7.18–7.13 (m, 1H), 7.00 (m, 1H), 4.01 (s, 1H), 3.97 (s, 1H).

2-(p-Tolylimino)thiazolidin-4-one (TZ_03b)

Following the general procedure the product was synthesized from 1-(p-tolyl)thiourea **(TZ_02b**) (5.40 g, 32.53 mmol), anhydrous NaOAc (13.33 g, 162.65 mmol) and ethylbromoacetate (7.19 mL, 65.06 mmol) produced 2-(p-tolylimino)thiazolidin-4-one **(TZ_03b**) (5.8 g, 86%) as yellow solid. ESI-MS showed 207 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.33 (s, 1H), 7.56 (d, J = 7.4 Hz, 1H), 6.91 (d, J = 7.6 Hz, 1H), 3.98 (s, 1H), 3.91 (s, 1H), 2.28 (s, 3H).

2-((4-Methoxyphenyl)imino)thiazolidin-4-one (TZ_03c)

Following the general procedure the product was synthesized from 1-(4-methoxyphenyl)thiourea

TZ_02c (5.40 g, 29.67 mmol), anhydrous NaOAc (12.16 g, 148.35 mmol) and ethylbromoacetate (6.56 mL, 59.34 mmol) produced 2-((4-methoxyphenyl)imino)thiazolidin-4-one **(TZ_03c**) (5.6 g, 86%) as brown solid. ESI-MS showed 223 $[M+H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.95 (s, 1H), 7.42 (d, J = 7.4 Hz, 1H), 7.07–6.98 (m, 3H), 3.79 (s, 3H), 3.42 (s, 2H).

2-((4-Chlorophenyl)imino)thiazolidin-4-one (TZ_03d)

Following the general procedure the product was synthesized from 1-(4-chlorophenyl)thiourea

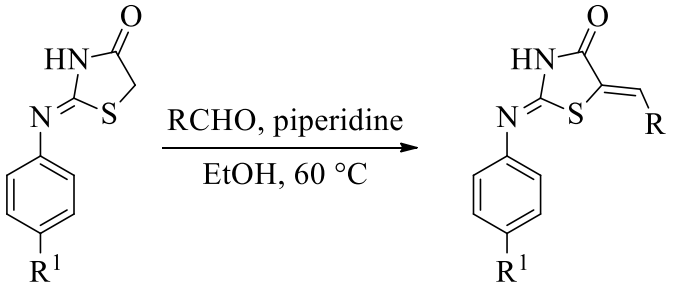
TZ_02d (5.30 g, 28.49 mmol), anhydrous NaOAc (11.68 g, 142.47 mmol) and ethylbromoacetate (6.30 mL, 56.98 mmol) produced 2-((4-chlorophenyl)imino)thiazolidin-4-one **(TZ_03d**) (5.4 g, 84%) as an brown solid. ESI-MS showed 227 $[M+H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.01 (s, 1H), 7.54–7.46 (bm, 4H), 3.54 (s, 2H).

2-((4-Nitrophenyl)imino)thiazolidin-4-one (TZ_03e)

Following the general procedure the product was synthesized from 1-(4-nitrophenyl)thiourea **TZ_02e** (5.30 g, 26.90 mmol), anhydrous NaOAc (11.02 g, 134.50 mmol) and ethylbromoacetate (5.95 mL, 53.80 mmol) produced 2-((4-nitrophenylimino)thiazolidin-4-one

(TZ_03d) (5.4 g, 84%) as yellow solid. ESI-MS showed 238 $[M+H]^+$. ^1H NMR (300 MHz, $CDCl_3$) δ 10.01 (s, 1H), 7.95 (d, *J* = 7.6 Hz, 1H), 7.59–48 (m, 3H), 3.98 (s, 2H).

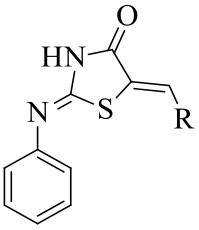
General procedure for the synthesis of compounds TZ_04 – TZ_56



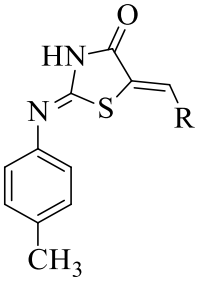
TZ_03a-e **TZ_04-TZ_56**

To the stirred solution of **TZ_03a-e** (1.0 equiv) in EtOH, was added piperidine (1.0 equiv) and RCHO (1.2 equiv) and heated at 60 °C for 12 h, then the solids formed in the reaction mixture were filtered and washed with EtOH, hexanes to afford the pure product in good yields.

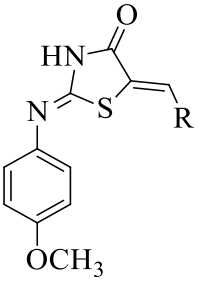
Table 5.5: Physiochemical properties of the synthesized compounds **TZ_04 – TZ_56**



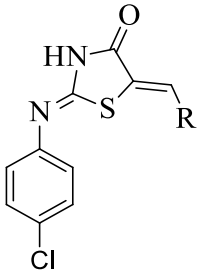
TZ_04-TZ_14



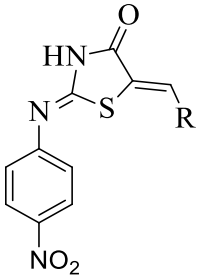
TZ_15-TZ_25



TZ_26-TZ_34



TZ_35-TZ_45



TZ_46-TZ_56

Compd	R	Yield (%)	M.P. (°C)	Molecular formula	Molecular weight
TZ_04	Phenyl	78	191-192	$C_{16}H_{12}N_2OS$	280.34
TZ_05	4-Fluorophenyl	71	227-228	$C_{16}H_{11}FN_2OS$	298.33
TZ_06	4-Tolyl	75	203-204	$C_{17}H_{14}N_2OS$	294.37
TZ_07	3-Trifluoromethylphenyl	82	189-190	$C_{17}H_{11}F_3N_2OS$	348.34
TZ_08	4-Hydroxyphenyl	77	241-242	$C_{16}H_{12}N_2O_2S$	296.34
TZ_09	4-Methoxyphenyl	79	240-241	$C_{17}H_{14}N_2O_2S$	310.37

Contd

Compd	R	Yield (%)	M.P. (°C)	Molecular formula	Molecular weight
TZ_10	4-Benzyloxyphenyl	84	199-200	$C_{23}H_{18}N_2O_2S$	386.47
TZ_11	3-Nitrophenyl	69	260-261	$C_{16}H_{11}N_3O_3S$	325.34
TZ_12	4-Dimethylaminophenyl	74	204-205	$C_{18}H_{17}N_3OS$	323.41
TZ_13	5-Nitro-2-furyl	65	241-242	$C_{14}H_9N_3O_4S$	315.30
TZ_14	5-Nitro-2-thiophenyl	62	187-188	$C_{14}H_9N_3O_3S_2$	331.37
TZ_15	Phenyl	81	178-179	$C_{17}H_{14}N_2OS$	294.37
TZ_16	4-Fluorophenyl	72	234-235	$C_{17}H_{13}FN_2OS$	312.36
TZ_17	4-Tolyl	81	245-246	$C_{18}H_{16}N_2OS$	308.40
TZ_18	3-Trifluoromethylphenyl	79	225-226	$C_{18}H_{13}F_3N_2OS$	362.37
TZ_19	4-Hydroxyphenyl	68	211-212	$C_{17}H_{14}N_2O_2S$	310.37
TZ_20	4-Methoxyphenyl	76	214-215	$C_{18}H_{16}N_2O_2S$	324.40
TZ_21	4-Benzyloxyphenyl	75	231-232	$C_{24}H_{20}N_2O_2S$	400.49
TZ_22	3-Nitrophenyl	69	241-242	$C_{17}H_{13}N_3O_3S$	339.37
TZ_23	4-Dimethylaminophenyl	67	247-248	$C_{19}H_{19}N_3OS$	337.44
TZ_24	5-Nitro-2-furyl	61	229-230	$C_{15}H_{11}N_3O_4S$	329.33
TZ_25	5-Nitro-2-thiophenyl	60	260-261	$C_{15}H_{11}N_3O_3S_2$	345.40
TZ_26	Phenyl	74	219-220	$C_{17}H_{14}N_2O_2S$	310.37
TZ_27	4-Fluorophenyl	78	222-223	$C_{17}H_{13}FN_2O_2S$	328.36
TZ_28	4-Tolyl	81	261-262	$C_{18}H_{16}N_2O_2S$	324.40
TZ_29	3-Trifluoromethylphenyl	68	238-239	$C_{18}H_{13}F_3N_2O_2S$	378.37
TZ_30	4-Hydroxyphenyl	77	217-218	$C_{17}H_{14}N_2O_3S$	326.37
TZ_31	4-Methoxyphenyl	81	223-224	$C_{18}H_{16}N_2O_3S$	340.40
TZ_32	4-Benzyloxyphenyl	84	235-236	$C_{24}H_{20}N_2O_3S$	416.49
TZ_33	3-Nitrophenyl	69	246-247	$C_{17}H_{13}N_3O_4S$	355.37
TZ_34	4-Dimethylaminophenyl	67	240-241	$C_{19}H_{19}N_3O_2S$	353.44
TZ_35	Phenyl	82	260-261	$C_{16}H_{11}ClN_2OS$	314.79
TZ_36	4-Fluorophenyl	75	191-192	$C_{16}H_{10}ClFN_2OS$	332.78

Contd

Compd	R	Yield (%)	M.P. (°C)	Molecular formula	Molecular weight
TZ_37	4-Tolyl	69	204-205	$C_{17}H_{13}ClN_2OS$	328.82
TZ_38	3-Trifluoromethylphenyl	72	241-242	$C_{17}H_{10}ClF_3N_2OS$	382.79
TZ_39	4-Hydroxyphenyl	63	187-188	$C_{16}H_{11}ClN_2O_2S$	330.79
TZ_40	4-Methoxyphenyl	67	178-179	$C_{17}H_{13}ClN_2O_2S$	344.82
TZ_41	4-Benzyloxyphenyl	75	234-235	$C_{23}H_{17}ClN_2O_2S$	420.91
TZ_42	3-Nitrophenyl	62	245-246	$C_{16}H_{10}ClN_3O_3S$	359.79
TZ_43	4-Dimethylaminophenyl	68	225-226	$C_{18}H_{16}ClN_3OS$	357.86
TZ_44	5-Nitro-2-furyl	61	211-212	$C_{14}H_8ClN_3O_4S$	349.75
TZ_45	5-Nitro-2-thiophenyl	64	214-215	$C_{14}H_8ClN_3O_3S_2$	365.81
TZ_46	Phenyl	87	253-254	$C_{16}H_{11}N_3O_3S$	325.79
TZ_47	4-Fluorophenyl	85	231-232	$C_{16}H_{10}FN_3O_3S$	343.33
TZ_48	4-Tolyl	83	241-242	$C_{17}H_{13}N_3O_3S$	339.37
TZ_49	3-Trifluoromethylphenyl	86	247-248	$C_{17}H_{10}F_3N_3O_3S$	393.34
TZ_50	4-Hydroxyphenyl	79	229-230	$C_{16}H_{11}N_3O_4S$	341.34
TZ_51	4-Methoxyphenyl	75	235-236	$C_{17}H_{13}N_3O_4S$	355.37
TZ_52	4-Benzyloxyphenyl	77	241-242	$C_{23}H_{17}N_3O_4S$	431.46
TZ_53	3-Nitrophenyl	73	260-261	$C_{16}H_{10}N_4O_5S$	370.34
TZ_54	4-Dimethylaminophenyl	81	250-251	$C_{18}H_{16}N_4O_3S$	368.41
TZ_55	5-Nitro-2-furyl	68	247-248	$C_{14}H_8N_4O_6S$	360.30
TZ_56	5-Nitro-2-thiophenyl	63	238-239	$C_{14}H_8N_4O_5S_2$	376.37

5.3.3. Characterization of the synthesized molecules

5-Benzylidene-2-(phenylimino)thiazolidin-4-one (TZ_04): Yield: 78%; m.p. 191–192 °C; MS(ESI) *m/z* 281 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.71 (s, 1H), 7.92–7.72 (m, 6H), 7.69–754 (m, 5H); ^{13}C NMR (100 MHz, DMSO-d_6) 176.3, 166.4, 152.3, 142.8, 133.9, 133.3(2C), 130.2(2C), 129.3, 127.0(2C), 126.9, 125.4(2C), 121.4. Anal calcd for $C_{16}H_{12}N_2OS$: C, 68.55; H, 4.31; N, 9.99% Found C, 68.62; H, 4.33; N, 10.15%.

5-(4-Fluorobenzylidene)-2-(phenylimino)thiazolidin-4-one (TZ_05): Yield: 71%; m.p. 227–228 °C; MS(ESI) *m/z* 299 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.62 (s, 1H), 7.81 (d, *J* = 7.6Hz, 2H), 7.78–7.69 (m, 7H), 7.49 (t, *J* = 7.2 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) 178.8, 167.2, 159.2, 144.3, 136.8, 134.1(2C), 132.3(2C), 128.0(2C), 126.3(2C), 124.4, 123.4, 122.4. Anal calcd for $C_{16}H_{11}FN_2OS$: C, 64.41; H, 3.72; N, 9.39% Found C, 64.52; H, 3.79; N, 9.45%.

5-(4-Methylbenzylidene)-2-(phenylimino)thiazolidin-4-one (TZ_06): Yield: 75%; m.p. 203–204 °C; MS(ESI) *m/z* 295 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.35 (s, 1H), 7.91–7.84 (m, 3H), 7.74 (d, *J* = 7.6Hz, 2H), 7.67–7.56 (m, 5H), 2.52 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) 177.5, 165.1, 156.3, 145.1, 135.9, 133.2(2C), 132.6, 131.3(2C), 128.3(2C), 126.0(2C), 125.4, 123.2, 22.3. Anal calcd for $C_{17}H_{14}N_2OS$: C, 69.36; H, 4.79; N, 9.52% Found C, 69.42; H, 4.83; N, 9.56%.

2-(Phenylimino)-5-(3-(trifluoromethyl)benzylidene)thiazolidin-4-one (TZ_07): Yield: 82%; m.p. 189–190 °C; MS(ESI) *m/z* 349 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.65 (s, 1H), 7.88–7.76 (m, 5H), 7.72–7.64 (m, 3H), 7.60–7.54 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) 179.3, 167.3, 158.0, 148.3, 136.4, 134.3(2C), 133.2, 131.4(2C), 129.3, 128.1, 127.3, 126.9, 125.9, 124.0, 122.4. Anal calcd for $C_{17}H_{11}F_3N_2OS$: C, 58.62; H, 3.18; N, 8.04% Found C, 69.42; H, 4.83; N, 9.56%.

5-(4-Hydroxybenzylidene)-2-(phenylimino)thiazolidin-4-one (TZ_08): Yield: 77%; m.p. 241–242 °C; MS(ESI) *m/z* 297 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.25 (s, 1H), 9.43 (s, 1H), 7.89 (d, *J* = 7.2Hz, 2H), 7.81 (s, 1H), 7.74–7.58 (m, 7H); ^{13}C NMR (100 MHz, DMSO-d_6) 180.2, 164.9, 160.2, 144.4, 135.8, 133.6(2C), 132.0(2C), 128.6, 127.7(2C), 126.4(2C), 126.0, 124.6. Anal calcd for $C_{16}H_{12}N_2O_2S$: C, 64.85; H, 4.08; N, 9.45% Found C, 64.92; H, 4.12; N, 9.52%.

5-(4-Methoxybenzylidene)-2-(phenylimino)thiazolidin-4-one (TZ_09): Yield: 79%; m.p. 240–241 °C; MS(ESI) *m/z* 311 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.11 (s, 1H), 7.93–7.88 (m, 3H), 7.81 (d, *J* = 7.6Hz, 2H), 7.72–7.67 (m, 5H), 3.96 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) 177.3, 166.4, 159.2, 145.1, 134.4, 133.3(2C), 131.8(2C), 129.4, 128.2(2C), 127.0(2C), 125.4, 122.1, 61.3. Anal calcd for $C_{17}H_{14}N_2O_2S$: C, 65.79; H, 4.55; N, 9.03% Found C, 65.81; H, 4.63; N, 9.06%.

5-(4-(Benzyloxy)benzylidene)-2-(phenylimino)thiazolidin-4-one (TZ_10): Yield: 84%; m.p. 199–200 °C; MS(ESI) *m/z* 387 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.01 (s, 1H), 7.88–7.76 (m, 6H), 7.81 (d, *J* = 7.6Hz, 2H), 7.76–7.60 (m, 7H), 5.26 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) 179.2, 165.6, 160.1, 146.3, 135.4, 134.0(2C), 132.3(2C), 130.6, 129.4(2C), 128.4, 127.7(2C), 126.2(2C), 125.3, 124.8(2C), 123.0, 122.8, 79.9. Anal calcd for $C_{23}H_{18}N_2O_2S$: C, 71.48; H, 4.69; N, 7.25% Found C, 71.51; H, 4.73; N, 7.36%.

5-(3-Nitrobenzylidene)-2-(phenylimino)thiazolidin-4-one (TZ_11): Yield: 69%; m.p. 260–261 °C; MS(ESI) *m/z* 326 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.25 (s, 1H), 8.31 (d, *J* = 7.6Hz, 1H), 7.92–7.81 (m, 4H), 7.72 (d, *J* = 7.2Hz, 2H), 7.67–7.53 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) 181.4, 164.3, 156.9, 143.3, 133.9, 133.1, 132.3, 130.8(2C), 129.4, 128.4, 127.4, 126.3(2C), 125.6, 123.2. Anal calcd for $C_{16}H_{11}N_3O_3S$: C, 59.07; H, 3.41; N, 12.92% Found C, 59.12; H, 3.52; N, 12.99%.

5-(4-(Dimethylamino)benzylidene)-2-(phenylimino)thiazolidin-4-one (TZ_12): Yield: 74%; m.p. 204–205 °C; MS(ESI) *m/z* 324 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.96 (s, 1H), 7.91 (d, *J* = 7.6Hz, 2H), 7.87 (s, 1H), 7.81–7.69 (m, 4H), 7.63–7.49 (m, 3H), 3.04 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) 174.2, 162.4, 157.3, 147.4, 134.1, 132.9(2C), 131.3(2C), 130.7, 127.6(2C), 126.5(2C), 126.0, 123.3, 43.9, 43.6. Anal calcd for $C_{18}H_{17}N_3OS$: C, 66.85; H, 5.30; N, 12.99% Found C, 66.92; H, 5.42; N, 13.05%.

5-((5-Nitrofuran-2-yl)methylene)-2-(phenylimino)thiazolidin-4-one (TZ_13): Yield: 65%; m.p. 241–242 °C; MS(ESI) *m/z* 316 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.93 (s, 1H), 8.31 (d, *J* = 8.0 Hz, 1H), 7.72–7.63 (m, 2H), 7.58 (s, 1H), 7.54 (d, *J* = 8.0Hz, 1H), 7.49–7.32 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 176.3, 164.2, 156.0, 146.2, 135.2, 133.1, 132.0, 130.7(2C), 127.2, 125.9(2C), 124.2, 119.3. Anal calcd for $C_{14}H_9N_3O_4S$: C, 53.33; H, 2.88; N, 13.33% Found C, 53.42; H, 2.92; N, 13.45%.

5-((5-Nitrothiophen-2-yl)methylene)-2-(phenylimino)thiazolidin-4-one (TZ_14): Yield: 62%; m.p. 187–188 °C; MS(ESI) *m/z* 332 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.45 (s, 1H), 8.23 (d, *J* = 8.4 Hz, 1H), 7.69–7.61 (m, 3H), 7.56 (d, *J* = 8.0Hz, 1H), 7.51–7.40 (m, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) 175.8, 163.8, 156.2, 145.1, 134.8, 132.4, 131.4, 130.0(2C), 127.9,

125.3(2C), 125.0, 117.4. Anal calcd for $C_{14}H_9N_3O_3S_2$: C, 50.74; H, 2.74; N, 12.68% Found C, 50.82; H, 2.82; N, 12.75%.

5-Benzylidene-2-(*p*-tolylimino)thiazolidin-4-one (TZ_15): Yield: 81%; m.p. 178–179 °C; MS(ESI) *m/z* 295 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 (s, 1H), 7.81 (d, *J* = 8.4 Hz, 2H) 7.78–7.72 (m, 3H), 7.63–7.45 (m, 5H), 2.41 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 172.3, 164.2, 157.4, 143.3, 135.2, 132.1(2C), 129.8(2C), 129.2, 128.5(2C), 127.3(2C), 125.2, 123.6, 22.5. Anal calcd for $C_{17}H_{14}N_2OS$: C, 69.36; H, 4.79; N, 9.52% Found C, 69.42; H, 4.83; N, 9.65%.

5-(4-Fluorobenzylidene)-2-(*p*-tolylimino)thiazolidin-4-one (TZ_16): Yield: 72%; m.p. 234–235 °C; MS(ESI) *m/z* 313 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.82 (d, *J* = 8.0 Hz, 2H), 7.69 (d, *J* = 8.4 Hz, 2H), 7.63–7.56 (m, 4H), 7.45 (d, *J* = 7.6 Hz, 2H), 2.46 (s, 3H) ; ^{13}C NMR (100 MHz, DMSO-d_6) 173.4, 165.2, 158.2, 144.3, 137.4, 132.5(2C), 130.3(2C), 129.7, 128.8(2C), 128.1(2C), 125.8, 123.8, 23.6 Anal calcd for $C_{17}H_{13}FN_2OS$: C, 65.37; H, 4.19; N, 8.97% Found C, 65.42; H, 4.27; N, 9.03%.

5-(4-Methylbenzylidene)-2-(*p*-tolylimino)thiazolidin-4-one (TZ_17): Yield: 81%; m.p. 245–246 °C; MS(ESI) *m/z* 309 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.35 (s, 1H), 7.83–7.71 (m, 5H), 7.64 (d, *J* = 8.0 Hz, 2H), 7.53 (m, 2H), 2.52 (s, 3H), 2.46 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) 177.1, 164.9, 155.8, 144.8, 135.6, 132.8(2C), 132.5, 131.1(2C), 127.8(2C), 125.8(2C), 125.4, 123.1, 22.3 (2C). Anal calcd for $C_{18}H_{16}N_2OS$: C, 70.10; H, 5.23; N, 9.08% Found C, 70.12; H, 5.33; N, 9.16%.

2-(p-Tolylimino)-5-(3-(trifluoromethyl)benzylidene)thiazolidin-4-one (TZ_18): Yield: 79%; m.p. 225–226 °C; MS(ESI) *m/z* 363 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (s, 1H), 7.77 (s, 1H), 7.68–7.61 (m, 2H), 7.56–7.45 (m, 4H), 7.36 (d, *J* = 8.4 Hz, 2H), 2.41(s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) 178.9, 167.1, 157.7, 148.1, 135.5, 133.7(2C), 131.6, 131.1(2C), 129.0, 127.7, 127.1, 126.2, 125.3, 123.8, 122.1, 22.2. Anal calcd for $C_{18}H_{13}F_3N_2OS$: C, 59.66; H, 3.62; N, 7.73% Found C, 59.74; H, 3.73; N, 7.86%.

5-(4-Hydroxybenzylidene)-2-(*p*-tolylimino)thiazolidin-4-one (TZ_19): Yield: 68%; m.p. 211–212 °C; MS(ESI) *m/z* 311 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.45 (s, 1H), 9.21 (s, 1H), 7.72 (d, *J* = 7.6 Hz, 2H), 7.67 (s, 1H), 7.64–7.53 (m, 4H), 7.42 (d, *J* = 8.0 Hz, 2H), 2.52 (s, 3H);

^{13}C NMR (75 MHz, DMSO-d_6) 179.8, 164.4, 160.0, 143.8, 135.2, 133.1(2C), 131.5(2C), 128.1, 127.1(2C), 126.2(2C), 125.6, 124.2, 22.4. Anal calcd for $C_{17}H_{14}N_2O_2S$: C, 65.79; H, 4.55; N, 9.03% Found C, 65.88; H, 4.71; N, 9.12%.

5-(4-Methoxybenzylidene)-2-(*p*-tolylimino)thiazolidin-4-one (TZ_20): Yield: 76%; m.p. 214–215 °C; MS(ESI) *m/z* 325 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.45 (s, 1H), 7.92 (d, *J* = 8.0 Hz, 2H), 7.87–7.78 (m, 3H), 7.72 (d, *J* = 7.6 Hz, 2H), 7.63–7.53 (m, 2H), 3.89 (s, 3H), 2.39 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 177.0, 166.1, 158.7, 144.7, 133.8, 132.9(2C), 131.5(2C), 129.1, 127.5(2C), 126.7(2C), 125.1, 121.8, 61.2, 23.1. Anal calcd for $C_{18}H_{16}N_2O_2S$: C, 66.64; H, 4.97; N, 8.64% Found C, 66.81; H, 5.03; N, 8.76%.

5-(4-(Benzyloxy)benzylidene)-2-(*p*-tolylimino)thiazolidin-4-one (TZ_21): Yield: 75%; m.p. 231–232 °C; MS(ESI) *m/z* 401 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.11 (s, 1H), 7.91–7.82 (m, 4H), 7.74 (d, *J* = 8.0 Hz, 2H), 7.69–7.57 (m, 4H), 7.54–7.48 (m, 4H), 5.19 (s, 2H), 2.54 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) 178.8, 165.2, 159.8, 146.1, 134.9, 133.8(2C), 132.1(2C), 130.2, 129.1(2C), 127.9, 127.4(2C), 125.6(2C), 125.1, 124.3(2C), 123.1, 122.5, 79.8, 23.2. Anal calcd for $C_{24}H_{20}N_2O_2S$: C, 71.98; H, 5.03; N, 6.99% Found C, 80.11; H, 5.13; N, 7.06%.

5-(3-Nitrobenzylidene)-2-(*p*-tolylimino)thiazolidin-4-one (TZ_22): Yield: 69%; m.p. 241–242 °C; MS(ESI) *m/z* 340 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.25 (s, 1H), 8.45 (s, 1H), 8.10 (d, *J* = 8.0 Hz, 1H), 7.92 (d, *J* = 8.4 Hz, 1H), 7.69–7.58 (m, 4H), 7.54–7.42 (m, 2H), 2.46 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 181.1, 164.0, 156.5, 143.1, 133.5, 133.1, 132.0, 130.5(2C), 128.8, 128.2, 127.2, 126.1(2C), 125.2, 122.8, 23.6 Anal calcd for $C_{17}H_{13}N_3O_3S$: C, 60.17; H, 3.86; N, 12.38% Found C, 60.32; H, 3.92; N, 12.46%.

5-(4-(Dimethylamino)benzylidene)-2-(*p*-tolylimino)thiazolidin-4-one (TZ_23): Yield: 67%; m.p. 247–248 °C; MS(ESI) *m/z* 338 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.06 (s, 1H), 7.87 (d, *J* = 8.0 Hz, 2H), 7.82–7.72 (m, 3H), 7.67 (d, *J* = 8.0 Hz, 2H), 7.62 (d, *J* = 8.4 Hz, 2H), 3.14 (s, 6H), 2.42 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) 173.8, 162.2, 157.1, 147.0, 133.6, 132.6(2C), 131.1(2C), 130.3, 127.3(2C), 126.1(2C), 125.8, 123.1, 43.8, 43.6, 23.3. Anal calcd for $C_{19}H_{19}N_3OS$: C, 67.63; H, 5.68; N, 12.45% Found C, 67.72; H, 5.76; N, 12.52%.

5-((5-Nitrofuran-2-yl)methylene)-2-(*p*-tolylimino)thiazolidin-4-one (TZ_24): Yield: 61%; m.p. 229–230 °C; MS(ESI) *m/z* 330 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.20 (s, 1H), 8.17 (d, *J* = 8.6 Hz, 1H), 7.70–7.61 (m, 4H), 7.55 (d, *J* = 8.0 Hz, 2H), 2.47 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) 176.2, 164.1, 155.9, 146.2, 135.2, 133.0, 132.1, 130.8(2C), 127.1, 125.7(2C), 124.1, 119.1, 25.2. Anal calcd for $C_{15}H_{11}N_3O_4S$: C, 54.71; H, 3.37; N, 12.76% Found C, 54.77; H, 3.42; N, 12.81%.

5-((5-Nitrothiophen-2-yl)methylene)-2-(*p*-tolylimino)thiazolidin-4-one (TZ_25): Yield: 60%; m.p. 260–261 °C; MS(ESI) *m/z* 346 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.21 (s, 1H), 8.19 (d, *J* = 8.8 Hz, 1H), 7.71–7.63 (m, 4H), 7.58 (d, *J* = 8.0 Hz, 2H), 2.48 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 175.7, 163.5, 156.0, 145.1, 134.6, 132.2, 131.1, 130.2(2C), 127.9, 125.4(2C), 125.1, 117.1, 24.8. Anal calcd for $C_{15}H_{11}N_3O_3S_2$: C, 52.16; H, 3.21; N, 12.17% Found C, 52.22; H, 3.32; N, 12.25%.

5-Benzylidene-2-(4-methoxyphenylimino)thiazolidin-4-one (TZ_26): Yield: 74%; m.p. 219–220 °C; MS(ESI) *m/z* 311 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.91 (s, 1H), 7.91 (d, *J* = 8.0 Hz, 2H), 7.72–7.67 (m, 3H), 7.60 (d, *J* = 8.0 Hz, 2H), 7.58–7.42 (m, 3H), 3.91 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 178.1, 169.2, 154.3, 144.5, 134.4, 133.8(2C), 130.4(2C), 129.9, 127.5(2C), 127.2, 125.8(2C), 121.7, 54.2. Anal calcd for $C_{17}H_{14}N_2O_2S$: C, 65.79; H, 4.55; N, 9.03% Found C, 65.82; H, 4.63; N, 9.09%.

5-(4-Fluorobenzylidene)-2-(4-methoxyphenylimino)thiazolidin-4-one (TZ_27): Yield: 78%; m.p. 222–223 °C; MS(ESI) *m/z* 329 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.88 (s, 1H), 8.12 (d, *J* = 8.0 Hz, 2H), 7.74 (d, *J* = 8.8 Hz, 2H), 7.69 (s, 1H), 7.50 (d, *J* = 8.0 Hz, 2H), 7.42 (d, *J* = 8.0 Hz, 2H), 3.96 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) 179.8, 169.1, 160.4, 145.1, 137.4, 134.6(2C), 133.0(2C), 128.6(2C), 126.9(2C), 124.7, 123.6, 122.9 54.5. Anal calcd for $C_{17}H_{13}FN_2O_2S$: C, 62.18; H, 3.99; N, 8.53% Found C, 62.22; H, 4.07; N, 8.63%.

2-(4-Methoxyphenylimino)-5-(4-methylbenzylidene)thiazolidin-4-one (TZ_28): Yield: 81%; m.p. 261–262 °C; MS(ESI) *m/z* 325 $[M+H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.44 (s, 1H), 7.94 (d, *J* = 8.4 Hz, 2H), 7.82 (d, *J* = 8.0 Hz, 2H), 7.78 (d, *J* = 8.0 Hz, 2H), 7.63–7.55 (m, 3H), 3.92 (s, 3H), 2.39 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) 179.5, 167.1, 157.3, 146.2, 136.2, 133.6(2C),

132.9, 131.6(2C), 129.1(2C), 126.3(2C), 125.7, 124.2, 54.1, 23.1. Anal calcd for $C_{18}H_{16}N_2O_2S$: C, 66.64; H, 4.97; N, 8.64% Found C, 66.72; H, 4.93; N, 8.76%.

2-(4-Methoxyphenylimino)-5-(3-(trifluoromethyl)benzylidene)thiazolidin-4-one (TZ_29): Yield: 68%; m.p. 238–239 °C; MS(ESI) *m/z* 379 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (d, *J* = 8.4 Hz, 2H), 7.77 (s, 1H), 7.71–7.63 (m, 3H), 7.58 (d, *J* = 8.0 Hz, 2H), 7.49–7.40 (m, 2H), 3.99 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) 180.1, 168.2, 159.1, 149.0, 136.8, 134.9(2C), 133.7, 131.8(2C), 129.7, 128.6, 127.9, 127.2, 126.4, 124.4, 122.8, 55.1. $C_{18}H_{13}F_3N_2O_2S$: C, 57.14; H, 3.46; N, 7.40% Found C, 57.24; H, 3.53; N, 7.46%.

5-(4-Hydroxybenzylidene)-2-(4-methoxyphenylimino)thiazolidin-4-one (TZ_30): Yield: 77%; m.p. 217–218 °C; MS(ESI) *m/z* 327 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.39 (s, 1H), 8.91 (s, 1H), 7.81 (d, *J* = 8.0 Hz, 2H), 7.78 (s, 1H), 7.72 (d, *J* = 8.0 Hz, 2H), 7.63 (d, *J* = 8.4 Hz, 2H), 7.48 (d, *J* = 8.0 Hz, 2H), 4.03 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) 182.2, 166.4, 162.1, 145.4, 136.7, 134.5(2C), 132.6(2C), 128.9, 128.1(2C), 127.5(2C), 126.9, 124.6, 56.4. Anal calcd for $C_{17}H_{14}N_2O_3S$: C, 62.56; H, 4.32; N, 8.58% Found C, 62.68; H, 4.41; N, 8.65%.

5-(4-Methoxybenzylidene)-2-(4-methoxyphenylimino)thiazolidin-4-one (TZ_31): Yield: 81%; m.p. 223–224 °C; MS(ESI) *m/z* 341 $[M+H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.86 (s, 1H), 7.92 (d, *J* = 8.0 Hz, 2H), 7.84–7.68 (m, 3H), 7.62 (d, *J* = 7.6 Hz, 2H), 7.58–7.47 (m, 2H), 3.96 (s, 3H), 2.50 (s, 3H), ^{13}C NMR (75 MHz, $CDCl_3$) 179.3, 168.4, 160.4, 146.5, 135.8, 133.9(2C), 132.7(2C), 130.3, 129.1(2C), 127.5(2C), 125.8, 122.8, 58.7, 61.8. Anal calcd for $C_{18}H_{16}N_2O_3S$: C, 63.51; H, 4.74; N, 8.23% Found C, 63.61; H, 4.83; N, 8.36%.

5-(4-(Benzyloxy)benzylidene)-2-(4-methoxyphenylimino)thiazolidin-4-one (TZ_32): Yield: 84%; m.p. 235–236 °C; MS(ESI) *m/z* 417 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.41 (s, 1H), 7.89–7.81 (m, 3H), 7.78–7.71 (m, 2H), 7.66 (d, *J* = 8.4 Hz, 2H), 7.60–7.54 (m, 4H), 7.49–7.33 (m, 3H), 5.23 (s, 2H), 3.88 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) 180.2, 167.4, 161.5, 148.1, 137.3, 135.6(2C), 133.4(2C), 131.5, 130.3(2C), 129.3, 128.6(2C), 127.1(2C), 126.3, 125.7(2C), 123.8, 123.2, 80.2, 56.8. Anal calcd for $C_{24}H_{20}N_2O_2S$: C, 69.21; H, 4.84; N, 6.73% Found C, 69.30; H, 4.95; N, 6.81%.

2-(4-Methoxyphenylimino)-5-(3-nitrobenzylidene)thiazolidin-4-one (TZ_33): Yield: 69%; m.p. 246–247 °C; MS(ESI) *m/z* 356 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.25 (s, 1H), 8.32 (s, 1H), 8.04 (d, *J* = 8.4 Hz, 1H), 7.90 (d, *J* = 8.8 Hz, 2H), 7.81 (d, *J* = 8.0 Hz, 1H), 7.72–7.63 (m, 2H), 7.54 (d, *J* = 8.4 Hz, 2H), 4.05 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 183.3, 165.6, 157.9, 144.5, 134.6, 133.7, 132.8, 131.5(2C), 130.2, 129.2, 128.3, 127.1(2C), 126.6, 124.2, 56.1Anal calcd for $C_{17}H_{13}N_3O_4S$: C, 57.46; H, 3.69; N, 11.82% Found C, 57.52; H, 3.82; N, 11.96%.

5-(4-(Dimethylamino)benzylidene)-2-(4-methoxyphenylimino)thiazolidin-4-one (TZ_34): Yield: 67%; m.p. 240–241 °C; MS(ESI) *m/z* 354 $[M+H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.06 (s, 1H), 7.92 (d, *J* = 8.0 Hz, 2H), 7.77 (s, 1H), 7.71–7.60 (m, 4H), 7.54 (d, *J* = 8.8 Hz, 2H), 3.89 (s, 3H), 3.18 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) 176.1, 164.5, 159.4, 148.4, 135.2, 133.5(2C), 131.8(2C), 131.2, 128.4(2C), 127.4(2C), 126.5, 124.1,55.4, 43.9, 43.6. Anal calcd for $C_{19}H_{19}N_3O_2S$: C, 64.57; H, 5.42; N, 11.89% Found C, 64.72; H, 5.56; N, 11.98%.

5-Benzylidene-2-((4-chlorophenyl)imino)thiazolidin-4-one (TZ_35): Yield: 82%; m.p. 260–261 °C; MS(ESI) *m/z* 315 $[M+H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.22 (s, 1H), 7.81 (d, *J* = 8.2 Hz, 2H) 7.80–7.76 (m, 4H), 7.60–7.51 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) 177.3, 168.2, 154.1, 143.5, 134.5, 133.8(2C), 131.7(2C), 130.8, 128.1(2C), 127.6, 126.5(2C), 122.5. Anal calcd for $C_{16}H_{11}ClN_2OS$: C, 61.05; H, 3.52; N, 8.90% Found C, 61.11; H, 3.57; N, 8.94 %.

2-((4-Chlorophenyl)imino)-5-(4-fluorobenzylidene)thiazolidin-4-one (TZ_36): Yield: 75%; m.p. 191–192 °C; MS(ESI) *m/z* 333 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.82 (d, *J* = 8.0 Hz, 2H), 7.75 (d, *J* = 8.4 Hz, 2H), 7.68–7.55 (m, 4H), 7.48 (d, *J* = 8.0 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) 179.7, 169.1, 160.4, 145.1, 137.2, 134.8(2C), 132.9(2C), 128.8(2C), 126.7(2C), 125.2, 124.1, 123.4. Anal calcd for $C_{16}H_{10}ClFN_2OS$: C, 57.75; H, 3.03; N, 8.42% Found C, 57.81; H, 3.09; N, 8.47%.

2-((4-Chlorophenyl)imino)-5-(4-methylbenzylidene)thiazolidin-4-one (TZ_37): Yield: 69%; m.p. 204–205 °C; MS(ESI) *m/z* 329 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.29 (s, 1H), 7.78–7.72 (m, 4H), 7.60 (d, *J* = 8.2 Hz, 2H), 7.57 (m, 3H), 2.42 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) 179.2, 167.0, 157.6, 146.2, 136.4, 134.3(2C), 133.3, 131.8(2C), 129.4(2C),

126.8(2C), 126.2, 123.6, 22.8. Anal calcd for $C_{17}H_{13}ClN_2OS$: C, 62.10; H, 3.98; N, 8.52% Found C, 62.16; H, 4.03; N, 8.57%.

2-((4-Chlorophenyl)imino)-5-(4-(trifluoromethyl)benzylidene)thiazolidin-4-one (TZ_38): Yield: 72%; m.p. 241–242 °C; MS(ESI) *m/z* 383 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (s, 1H), 7.66–7.61 (m, 4H), 7.55–7.47 (m, 3H), 7.40 (d, *J* = 8.2 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) 180.1, 168.2, 159.2, 149.2, 137.3, 135.1(2C), 134.3, 132.1(2C), 130.1, 129.2, 128.4, 127.5, 126.2, 124.6, 122.8. Anal calcd for $C_{17}H_{10}ClF_3N_2OS$: C, 53.34; H, 2.63; N, 7.32% Found C, 53.39; H, 2.70; N, 7.36%.

2-((4-Chlorophenyl)imino)-5-(4-hydroxybenzylidene)thiazolidin-4-one (TZ_39): Yield: 63%; m.p. 187–188 °C; MS(ESI) *m/z* 331 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.40 (s, 1H), 9.17 (s, 1H), 7.80 (d, *J* = 8.0 Hz, 2H), 7.67 (s, 1H), 7.66–7.54 (m, 4H), 7.39 (d, *J* = 8.2 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) 181.3, 165.8, 161.4, 145.6, 136.5, 134.3(2C), 132.5(2C), 129.4, 128.1(2C), 127.1(2C), 126.5, 124.9. Anal calcd for $C_{16}H_{11}ClN_2O_2S$: C, 58.09; H, 3.35; N, 8.47% Found C, 58.15; H, 3.39; N, 8.54%.

2-((4-Chlorophenyl)imino)-5-(4-methoxybenzylidene)thiazolidin-4-one (TZ_40): Yield: 67%; m.p. 178–179 °C; MS(ESI) *m/z* 345 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.45 (s, 1H), 7.92 (d, *J* = 8.6 Hz, 2H), 7.90–7.80 (m, 3H), 7.72 (d, *J* = 8.0 Hz, 2H), 7.65–7.55 (m, 2H), 3.91 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) 179.1, 167.1, 159.9, 146.2, 135.6, 133.7(2C), 132.3(2C), 130.1, 128.8(2C), 127.7(2C), 126.1, 123.1, 61.4. Anal calcd for $C_{17}H_{13}ClN_2O_2S$: C, 59.21; H, 3.80; N, 8.12% Found C, 59.27; H, 3.85; N, 8.16%.

5-(4-(Benzyloxy)benzylidene)-2-((4-chlorophenyl)imino)thiazolidin-4-one (TZ_41): Yield: 75%; m.p. 234–235 °C; MS(ESI) *m/z* 421 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.09 (s, 1H), 7.90–7.82 (m, 4H), 7.79 (d, *J* = 8.2 Hz, 2H), 7.70–7.58 (m, 4H), 7.56–7.50 (m, 4H), 5.21 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) 180.4, 167.1, 161.2, 147.4, 136.3, 134.5(2C), 132.9(2C), 131.3, 129.9(2C), 129.1, 128.5(2C), 127.1(2C), 126.1, 125.3(2C), 123.4, 123.5, 80.1.. Anal calcd for $C_{23}H_{17}ClN_2O_2S$: C, 65.63; H, 4.07; N, 6.66% Found C, 65.68; H, 4.13; N, 6.70%.

2-((4-Chlorophenyl)imino)-5-(4-nitrobenzylidene)thiazolidin-4-one (TZ_42): Yield: 62%; m.p. 245–246 °C; MS(ESI) *m/z* 360 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.25 (s, 1H),

8.40 (s, 1H), 8.10 (d, *J* = 8.0 Hz, 1H), 7.91 (d, *J* = 8.4 Hz, 1H), 7.69–7.62 (m, 4H), 7.55–7.46 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) 182.5, 165.4, 157.6, 144.5, 134.5, 133.6, 132.8, 131.3(2C), 130.2, 128.7, 127.9, 126.8(2C), 126.2, 123.6. Anal calcd for $C_{16}H_{10}ClN_3O_3S$: C, 53.41; H, 2.80; N, 11.68% Found C, 53.45; H, 2.87; N, 11.75%.

2-((4-Chlorophenyl)imino)-5-(4-(dimethylamino)benzylidene)thiazolidin-4-one (TZ_43): Yield: 68%; m.p. 225–226 °C; MS(ESI) *m/z* 358 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.07 (s, 1H), 7.90 (d, *J* = 8.6 Hz, 2H), 7.83–7.77 (m, 3H), 7.70 (d, *J* = 8.4 Hz, 2H), 7.64 (d, *J* = 8.4 Hz, 2H), 3.15 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) 176.3, 164.1, 158.4, 148.2, 134.8, 133.5(2C), 131.8(2C), 131.5, 128.5(2C), 126.9(2C), 126.2, 124.2, 44.1, 43.8. Anal calcd for $C_{18}H_{16}ClN_3OS$: C, 60.41; H, 4.51; N, 11.74% Found C, 60.45; H, 4.55; N, 11.79%.

2-((4-Chlorophenyl)imino)-5-((5-nitrofuran-2-yl)methylene)thiazolidin-4-one (TZ_44): Yield: 61%; m.p. 211–212 °C; MS(ESI) *m/z* 350 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.20 (s, 1H), 8.11 (d, *J* = 8.8 Hz, 1H), 7.73–7.62 (m, 4H), 7.58 (d, *J* = 8.0 Hz, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) 178.4, 166.3, 156.7, 146.9, 136.1, 134.0, 132.7, 131.6(2C), 129.1, 126.6(2C), 124.8, 119.8. Anal calcd for $C_{14}H_8ClN_3O_4S$: C, 48.08; H, 2.31; N, 12.01% Found C, 48.14; H, 2.38; N, 12.06%.

2-((4-Chlorophenyl)imino)-5-((5-nitrothiophen-2-yl)methylene)thiazolidin-4-one (TZ_45): Yield: 64%; m.p. 214–215 °C; MS(ESI) *m/z* 366 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.20 (s, 1H), 8.13 (d, *J* = 8.6 Hz, 1H), 7.72–7.64 (m, 4H), 7.55 (d, *J* = 8.2 Hz, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) 177.6, 165.2, 157.8, 146.5, 135.4, 133.3, 132.3, 131.1(2C), 128.6, 125.8(2C), 125.4, 118.3. Anal calcd for $C_{14}H_8ClN_3O_3S_2$: C, 45.97; H, 2.20; N, 11.49% Found C, 46.05; H, 2.25; N, 11.52%.

5-Benzylidene-2-((4-nitrophenyl)imino)thiazolidin-4-one (TZ_46): Yield: 87%; m.p. 253–254 °C; MS(ESI) *m/z* 326 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.43 (s, 1H), 7.88 (d, *J* = 8.4 Hz, 2H), 7.84–7.78 (m, 3H), 7.63–7.57 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) 179.3, 169.8, 156.2, 145.4, 135.3, 134.6(2C), 132.8(2C), 131.7, 129.1(2C), 128.5, 127.8(2C), 124.6. Anal calcd for $C_{16}H_{11}N_3O_3S$: C, 59.07; H, 3.41; N, 12.92% Found C, 59.13; H, 3.47; N, 12.96%.

5-(4-Fluorobenzylidene)-2-((4-nitrophenyl)imino)thiazolidin-4-one (TZ_47): Yield: 85%; m.p. 231–232 °C; MS(ESI) *m/z* 344 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.21 (d, *J* = 8.0 Hz, 2H), 7.77 (d, *J* = 8.4 Hz, 2H), 7.69–7.63 (m, 4H), 7.52 (d, *J* = 8.0 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) 181.6, 171.2, 162.3, 147.4, 138.1, 135.7(2C), 133.7(2C), 129.8(2C), 127.8(2C), 126.3, 125.3, 124.5. Anal calcd for $C_{16}H_{10}FN_3O_3S$: C, 55.97; H, 2.94; N, 12.24 % Found C, 56.04; H, 2.99; N, 12.27 %.

2-((4-Nitrophenyl)imino)-5-(4-methylbenzylidene)thiazolidin-4-one (TZ_48): Yield: 83%; m.p. 241–242 °C; MS(ESI) *m/z* 340 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.36 (s, 1H), 7.88–7.80 (m, 5H), 7.67 (d, *J* = 8.2 Hz, 2H), 7.59 (m, 2H), 2.46 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) 180.5, 168.8, 158.7, 147.4, 137.6, 135.6(2C), 134.5, 132.7(2C), 130.5(2C), 127.7(2C), 126.8, 124.4, 23.6. Anal calcd for $C_{17}H_{13}N_3O_3S$: C, 60.17; H, 3.86; N, 12.38% Found C, 60.24; H, 3.90; N, 12.42 %.

2-((4-Nitrophenyl)imino)-5-(3-(trifluoromethyl)benzylidene)thiazolidin-4-one (TZ_49): Yield: 86%; m.p. 247–248 °C; MS(ESI) *m/z* 394 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.18 (s, 1H), 7.71–7.65 (m, 4H), 7.62–7.54 (m, 3H), 7.43 (d, *J* = 8.4 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) 180.1, 168.2, 159.2, 149.2, 137.3, 135.1(2C), 134.3, 132.1(2C), 130.1, 129.2, 128.4, 127.5, 126.2, 124.6, 122.8. Anal calcd for $C_{17}H_{10}F_3N_3O_3S$: C, 51.91; H, 2.56; N, 10.68% Found C, 51.95; H, 2.62; N, 10.75%.

2-((4-Nitrophenyl)imino)-5-(3-hydroxybenzylidene)thiazolidin-4-one (TZ_50): Yield: 79%; m.p. 229–230 °C; MS(ESI) *m/z* 342 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.49 (s, 1H), 9.23 (s, 1H), 7.79 (d, *J* = 8.0 Hz, 2H), 7.69 (s, 1H), 7.67–7.59 (m, 4H), 7.46 (d, *J* = 8.4 Hz, 2H), ^{13}C NMR (75 MHz, DMSO-d_6) 183.5, 167.6, 163.5, 147.5, 138.4, 135.2(2C), 133.4(2C), 129.8, 128.8(2C), 127.7(2C), 127.2, 125.6. Anal calcd for $C_{16}H_{11}N_3O_4S$: C, 56.30; H, 3.25; N, 12.31% Found C, 56.36; H, 3.29; N, 12.36%.

2-((4-Nitrophenyl)imino)-5-(3-methoxybenzylidene)thiazolidin-4-one (TZ_51): Yield: 75%; m.p. 235–236 °C; MS(ESI) *m/z* 356 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.51 (s, 1H), 8.08 (d, *J* = 8.2 Hz, 2H), 7.91–7.82 (m, 3H), 7.79 (d, *J* = 7.8 Hz, 2H), 7.67–7.59 (m, 2H), 3.90 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) 182.2, 169.1, 160.7, 147.2, 136.3, 134.5(2C), 133.3(2C),

131.0, 129.7(2C), 128.6(2C), 126.7, 123.8, 61.6. Anal calcd for $C_{17}H_{13}N_3O_4S$: C, 57.46; H, 3.69; N, 11.82% Found C, 57.51; H, 3.75; N, 11.87%.

5-(3-(Benzyloxy)benzylidene)-2-((4-nitrophenyl)imino)thiazolidin-4-one (TZ_52): Yield: 77%; m.p. 241–242 °C; MS(ESI) *m/z* 432 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.21 (s, 1H), 8.10–7.95 (m, 4H), 7.79 (d, *J* = 8.4 Hz, 2H), 7.73–7.62 (m, 4H), 7.58–7.52 (m, 4H), 5.22 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) 182.5, 169.2, 163.2, 149.3, 138.3, 135.4(2C), 133.7(2C), 132.4, 130.8(2C), 129.6, 128.9(2C), 127.8(2C), 126.6, 125.7(2C), 123.9, 123.5, 80.7. Anal calcd for $C_{23}H_{17}N_3O_4S$: C, 64.03; H, 3.97; N, 9.74% Found C, 64.11; H, 4.03; N, 9.79%.

2-((4-Nitrophenyl)imino)-5-(3-nitrobenzylidene)thiazolidin-4-one (TZ_53): Yield: 73%; m.p. 260–261 °C; MS(ESI) *m/z* 371 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.33 (s, 1H), 8.47 (s, 1H), 8.15 (d, *J* = 8.4 Hz, 1H), 7.98 (d, *J* = 8.2 Hz, 1H), 7.73–7.65 (m, 4H), 7.60–7.51 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) 185.4, 168.6, 159.5, 146.4, 135.4, 134.3, 133.8, 132.4(2C), 131.1, 129.6, 128.9, 127.3(2C), 126.8, 124.7. Anal calcd for $C_{16}H_{10}N_4O_5S$: C, 51.89; H, 2.72; N, 15.13% Found C, 51.94; H, 2.77; N, 15.18%.

5-(4-(Dimethylamino)benzylidene)-2-((4-nitrophenyl)imino)thiazolidin-4-one (TZ_54): Yield: 81%; m.p. 250–251 °C; MS(ESI) *m/z* 369 $[M+H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.12 (s, 1H), 7.94 (d, *J* = 8.6 Hz, 2H), 7.88–7.76 (m, 3H), 7.74 (d, *J* = 8.4 Hz, 2H), 7.70 (d, *J* = 8.4 Hz, 2H), 3.16 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) 179.1, 166.3, 159.7, 149.4, 136.2, 134.6(2C), 132.7(2C), 132.1, 129.4(2C), 127.8(2C), 126.7, 125.3, 44.5, 44.1. Anal calcd for $C_{18}H_{16}N_4O_3S$: C, 58.68; H, 4.38; N, 15.21% Found C, 58.73; H, 4.42; N, 15.26%.

5-((5-Nitrofuran-2-yl)methylene)-2-((4-nitrophenyl)imino)thiazolidin-4-one (TZ_55): Yield: 68%; m.p. 247–248 °C; MS(ESI) *m/z* 361 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.29 (s, 1H), 8.21 (d, *J* = 8.4 Hz, 1H), 7.76–7.65 (m, 4H), 7.59 (d, *J* = 8.2 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) 180.2, 168.4, 158.6, 148.5, 138.2, 135.1, 133.6, 132.4(2C), 129.6, 127.4(2C), 125.8, 120.6. Anal calcd for $C_{14}H_8N_4O_6S$: C, C, 46.67; H, 2.24; N, 15.55% Found C, 46.71; H, 2.29; N, 15.63%.

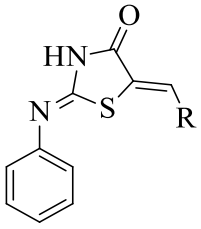
2-((4-Nitrophenyl)imino)-5-((5-nitrothiophen-2-yl)methylene)thiazolidin-4-one (TZ_56): Yield: 63%; m.p. 238–239 °C; MS(ESI) *m/z* 377 $[M+H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.26 (s,

1H), 8.18 (d, *J* = 8.4 Hz, 1H), 7.73–7.60 (m, 4H), 7.61 (d, *J* = 8.6 Hz, 2H), ^{13}C NMR (75 MHz, 300 MHz, $CDCl_3$) 179.8, 167.4, 158.8, 147.6, 136.5, 134.3, 133.2, 132.4(2C), 129.4, 126.5(2C), 125.8, 119.7. Anal calcd for $C_{14}H_8N_4O_5S_2$: C, 44.68; H, 2.14; N, 14.89% Found C, 44.73; H, 2.18; N, 14.95%.

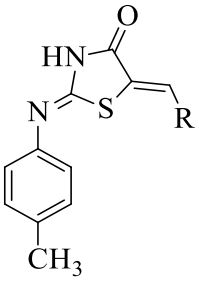
5.3.4. *In vitro M. tuberculosis* screening, *M. tuberculosis* PknB enzyme inhibition assay and cytotoxicity studies of the synthesized molecules

The compounds were screened for their *in vitro* antimycobacterial activity against *M. tuberculosis* H37Rv by MABA method for the determination of MIC in duplicate. The MIC is defined as the minimum concentration of compound required to give complete inhibition of bacterial growth. INH and EMB were used as a reference compounds for comparison. Synthesized derivatives were also screened for their *in vitro M. tuberculosis* PknB inhibitory potency as steps towards hit optimization. All the compounds 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 5.6**.

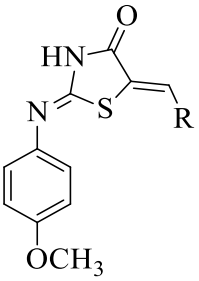
Table 5.6: *In vitro* biological evaluation of synthesized derivatives **TZ_04** – **TZ_56**



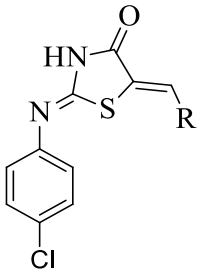
TZ_04-TZ_14



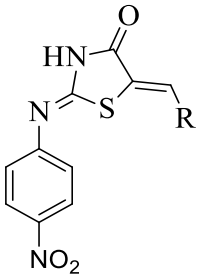
TZ_15-TZ_25



TZ_26-TZ_34



TZ_35-TZ_45



TZ_46-TZ_56

Compd	R	MTB PknB IC_{50} in µM	MTB MIC in µM[a]	Cytotoxicity[b] at 50 µM % inhibition
TZ_04	Phenyl	>20	89.26	NT
TZ_05	4-Fluorophenyl	11.15	20.96	25.02
TZ_06	4-Tolyl	16.24	21.25	32.15

Contd

Compd	R	MTB PknB IC_{50} in µM	MTB MIC in µM[a]	Cytotoxicity[b] at 50 µM % inhibition
TZ_07	3-Trifluoromethylphenyl	18.29	35.91	NT
TZ_08	4-Hydroxyphenyl	>20	84.44	NT
TZ_09	4-Methoxyphenyl	0.52	1.98	6.89
TZ_10	4-Benzyloxyphenyl	4.69	32.37	NT
TZ_11	3-Nitrophenyl	6.91	76.91	NT
TZ_12	4-Dimethylaminophenyl	5.20	19.34	25.36
TZ_13	5-Nitro-2-furyl	9.31	39.67	NT
TZ_14	5-Nitro-2-thiophenyl	16.12	37.76	NT
TZ_15	Phenyl	8.31	21.25	47.69
TZ_16	4-Fluorophenyl	2.64	10.01	15.67
TZ_17	4-Tolyl	10.24	40.57	32.15
TZ_18	3-Trifluoromethylphenyl	7.36	2.154	20.81
TZ_19	4-Hydroxyphenyl	4.21	10.07	33.24
TZ_20	4-Methoxyphenyl	5.42	19.28	50.73
TZ_21	4-Benzyloxyphenyl	1.02	3.90	19.87
TZ_22	3-Nitrophenyl	5.13	18.43	13.39
TZ_23	4-Dimethylaminophenyl	6.34	4.63	31.24
TZ_24	5-Nitro-2-furyl	3.28	9.49	20.15
TZ_25	5-Nitro-2-thiophenyl	5.61	9.05	22.07
TZ_26	Phenyl	5.34	40.31	NT
TZ_27	4-Fluorophenyl	5.71	38.10	NT
TZ_28	4-Tolyl	5.39	19.28	19.74
TZ_29	3-Trifluoromethylphenyl	4.91	33.06	NT
TZ_30	4-Hydroxyphenyl	4.67	38.33	NT
TZ_31	4-Methoxyphenyl	5.02	18.37	16.74
TZ_32	4-Benzyloxyphenyl	0.63	15.01	11.28
TZ_33	3-Nitrophenyl	4.97	8.80	22.34

Contd

Compd	R	MTB PknB IC_{50} in µM	MTB MIC in µM[a]	Cytotoxicity[b] at 50 µM % inhibition
TZ_34	4-Dimethylaminophenyl	4.31	35.39	NT
TZ_35	Phenyl	7.56	19.90	24.65
TZ_36	4-Fluorophenyl	4.38	37.64	NT
TZ_37	4-Tolyl	0.59	3.58	28.31
TZ_38	3-Trifluoromethylphenyl	0.61	16.36	23.64
TZ_39	4-Hydroxyphenyl	10.54	75.75	NT
TZ_40	4-Methoxyphenyl	1.10	10.28	20.76
TZ_41	4-Benzyloxyphenyl	1.35	15.67	19.54
TZ_42	3-Nitrophenyl	11.25	17.40	34.02
TZ_43	4-Dimethylaminophenyl	12.91	35.00	NT
TZ_44	5-Nitro-2-furyl	>20	71.63	NT
TZ_45	5-Nitro-2-thiophenyl	14.35	68.49	NT
TZ_46	Phenyl	16.21	76.91	NT
TZ_47	4-Fluorophenyl	>20	<2.28	14.98
TZ_48	4-Tolyl	>20	4.60	28.80
TZ_49	3-Trifluoromethylphenyl	15.37	1.98	23.05
TZ_50	4-Hydroxyphenyl	16.34	2.28	17.49
TZ_51	4-Methoxyphenyl	18.24	18.65	23.94
TZ_52	4-Benzyloxyphenyl	15.67	3.62	16.34
TZ_53	3-Nitrophenyl	17.35	8.44	13.51
TZ_54	4-Dimethylaminophenyl	12.34	2.11	22.37
TZ_55	5-Nitro-2-furyl	11.05	8.68	34.28
TZ_56	5-Nitro-2-thiophenyl	>20	8.31	39.87
Isoniazid			0.72	NT
Ethambutol			7.64	NT

MTB, *Mycobacterium tuberculosis*; MIC, minimum inhibitory concentration;

[a]*In vitro* activity against MTB H37Rv; [b]Against RAW 264.7 cells; NT indicates not tested.

5.3.5. SAR and discussion

To study the SAR we synthesized a library of fifty three molecules (**Table 5.6**) with variations at 2^{st} and 5^{th} positions of lead molecule. At N-2 (on exocyclic nitrogen atom) position we tried five modifications with electron donating (**TZ_15 – TZ_34**), electron withdrawing (**TZ_35 – TZ_56**) and neutral substitutions (**TZ_04 – TZ_14**) on phenyl ring. The phenyl ring bearing electron withdrawing nitro group (**TZ_46 – TZ_56**) at second position were found to be more active than phenyl ring bearing electron donating and neutral substituents.

The order of activity with respect to N-2 position were; 4-nitrophenyl > 4-chlorophenyl > 4-tolyl > phenyl > 4-methoxyphenyl. At the C-5 position we have prepared molecules with phenyl ring with both electron donating and electron with drawing groups and also with few heterocycles. Bulky substituents on phenyl ring at C-5 position (**TZ_09**, **TZ_10**, **TZ_12**, **TZ_20**, **TZ_21**, **TZ_23**, **TZ_31**, **TZ_32**, **TZ_34**, **TZ_40** and **TZ_41**) possess good inhibitory activity against the enzyme *M. tuberculosis* PknB.

The compounds showed MIC's ranging from 1.98 - 76.91 µM; and thirty-one compounds showed activity with MIC of less than 20 µM. Seventeen compounds (**TZ_09**, **TZ_18**, **TZ_21**, **TZ_23 - TZ_25**, **TZ_33**, **TZ_37**, **TZ_47 - TZ_50**, **TZ_52 - TZ_56**) showed promising activity with MIC of less than 10 µM. Out of fifty three compounds tested, eleven compounds (**TZ_09**, **TZ_18**, **TZ_21**, **TZ_23**, **TZ_37**, **TZ_47 - TZ_50**, **TZ_52**, **TZ_54**) possessed *M. tuberculosis* MIC of less than 5 µM making the study valuable. When compared to standard first line antitubercular drug EMB (MIC of 7.64 µM), eleven compounds were found to be more active and when compared to pyrazinamide (MIC of 50.77 µM), forty six compounds were more active. All the molecules were found be less active than INH (MIC of 0.72 µM) and RIF (MIC of 0.24 µM) but eleven compounds were more active than DNA gyrase inhibitor ciprofloxacin (MIC of 4.71 µM). Among the compounds, 5-(4-methoxybenzylidene)-2-(phenylimino)thiazolidin-4-one (**TZ_09**) was found to be the most active compound *in vitro* with MICs of 1.98 µM, which also inhibited the activity of PknB enzyme with IC_{50} of 0.52 µM making it potential inhibitor of the enzyme. The results were tabulated in **Table 5.6**.

5.3.6. Highlights of the study

In this study we have designed, synthesized various inhibitors of *M. tuberculosis* PknB based on the lead compound identified from in-house database. Among the library of fifty three compounds, 5-(4-methoxybenzylidene)-2-(phenylimino)thiazolidin-4-one (**TZ_09**) was found to be the most active compound *in vitro* with MICs of 1.98 µM against *M. tuberculosis*, PknB IC_{50} of 0.52 µM and also non-cytotoxic up to 100 µM (**Figure 5.9**). Further structural optimization required to get the compounds with better potency.

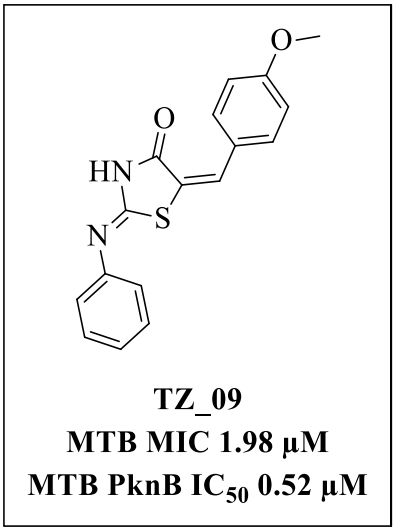


Figure 5.9: Structure and biological activity of most active compound **TZ_09**

5.4. Design and synthesis of 3-(benzo[*d*]oxazol-2-yl)aniline derivatives as novel *M. tuberculosis* PknB agents

Benzoxazole nucleus is distinguished for its biological activity. In literature benzoxazoles attained focus owing to its structural bio-isosterism with naturally occurring nucleotides namely adenine and guanine [Barghash RF., *et al*., 2014]. This structural bio-isosterism of benzoxazoles makes them easy to interact with biopolymers of living system. Benzoxazole derivatives possess a wide variety of biological activities viz. antibacterial, fungicidal, insecticidal, anticancer, anti-tubercular and HIV-1 reverse transcriptase inhibitors [Jauhari PK., *et al*., 2008]. Also its low toxicity in warm blooded animals makes them selective bactericidal. In present study, a library consisting of forty four derivatives of 2-phenylbenzo[*d*]oxazole were synthesized and evaluated for *in vitro* activities against *M. tuberculosis* PknB enzyme, *M. tuberculosis* H37Rv and cytotoxicity against RAW 264.7 cells.

5.4.1. Design of the molecules

Jarmila Vinsova *et al*., [Vinsova J., *et al*., 2006] synthesized a chemical library consisting of 2-substituted 5,7-di-*tert*-butylbenzoxazoles as new potential antimicrobial agents. They have synthesized library by varying substituents on phenyl ring at C-2 position. Out of all tested compounds, one molecule (**8a**) showed *M. tuberculosis* MIC < 10 µM. We have selected compound **8a** as our lead molecule for further development.

Based on our knowledge in designing of novel anti-tubercular agents, we anticipated that phenyl group (containing amide links) directly linked to C-2 position of benzoxazole ring will bind to enzyme better than the link by two carbon chain, which is present in lead molecule. Keeping the pharmacophoric fused ring system intact, derivatization made at 5^{th} position of benzoxazole by substituting with electron donating methyl group, electron withdrawing chloro, nitro groups apart from unsubstituted benzoxazole ring. We have also modified on C-2 phenyl; by preparing various substituted amide derivatives (**Figure 5.10**).

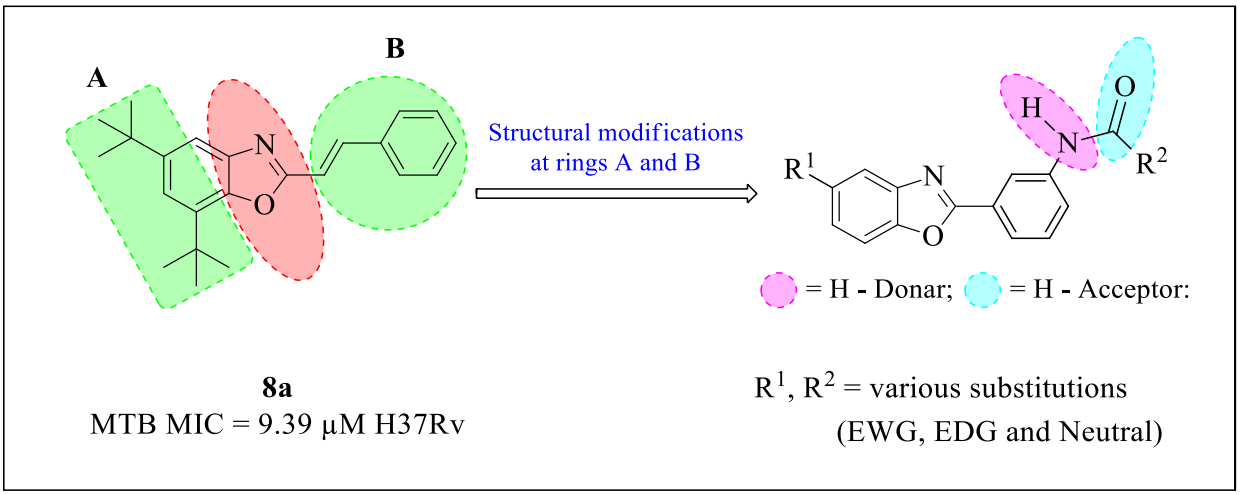
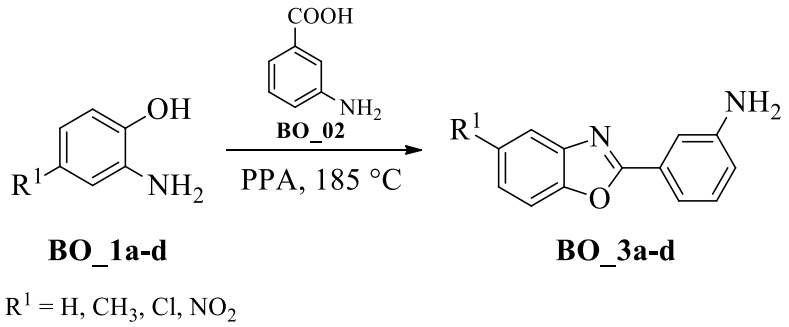


Figure 5.10: Lead molecule (**8a**) for the synthesis of compounds **BO_04 – BO_47**

5.4.2. Experimental procedures utilized for the synthesis of BO_04 – BO_47

The target molecules were synthesized by following two step synthetic protocol (**Figure 4.4**), starting with commercially available, less expensive, 2-aminophenol, substituted2-amino-phenols **(BO_1a-d)** and 3-aminobenzoic acid **(BO_02)**. Initially the cyclisation reaction for the preparation of substituted2-phenylbenzoxazoles **(BO_3a-d)** was carried out by coupling 3-aminobenzoic acid with **(BO_1a-d)** in the presence of polyphosphoric acid under reflux conditions, 3-aminobenzoic acid reacts with PPA to form mixed anhydride an *in situ* (**Figure 5.11**). Substituted 2- aminophenols **(BO_1a-d)** reacts with the activated carbonoyl mixed anhydride to produce an ester, as the first intermediate which undergoes rapid acyl migration to generate 2-hydroxyanilide then ring closure to form cyclized product substituted 2-phenylbenzoxazoles **(BO_3a-d).** Furthur the amidation was carried out by treating the **(BO_3a-d)** with various substituted aromatic/heteroaromatic/acyclic carboxylic acids in presence of coupling agents EDCI, and HOBt and few of the final molecules were prepared by using the acid chloride in presence of Et_3N and CH_2Cl_2 as solvent to get the final compounds **BO_04 - BO_47**.

General procedure for the preparation of BO_3a-d



To the mixture of compound **BO_1a-d** (1.1 equiv) and 3-aminobenzoic acid **BO_02** (1.0 equiv) was added PPA (7.0 volumes) and allowed the reaction mixture to stir at 185 °C for 6 hours. The reaction mixture was cooled to room temperature and added cold 6N NaOH solution drop wise at 0 °C. The precipitate obtained was filtered and dissolved in DMF and heated to 60 °C for 15 minutes, filtered and the filtrate was poured into crushed ice. The solids obtained was filtered and washed with excess of water, cold ethanol and diethyl ether to afford the compound **3a-d** respectively.

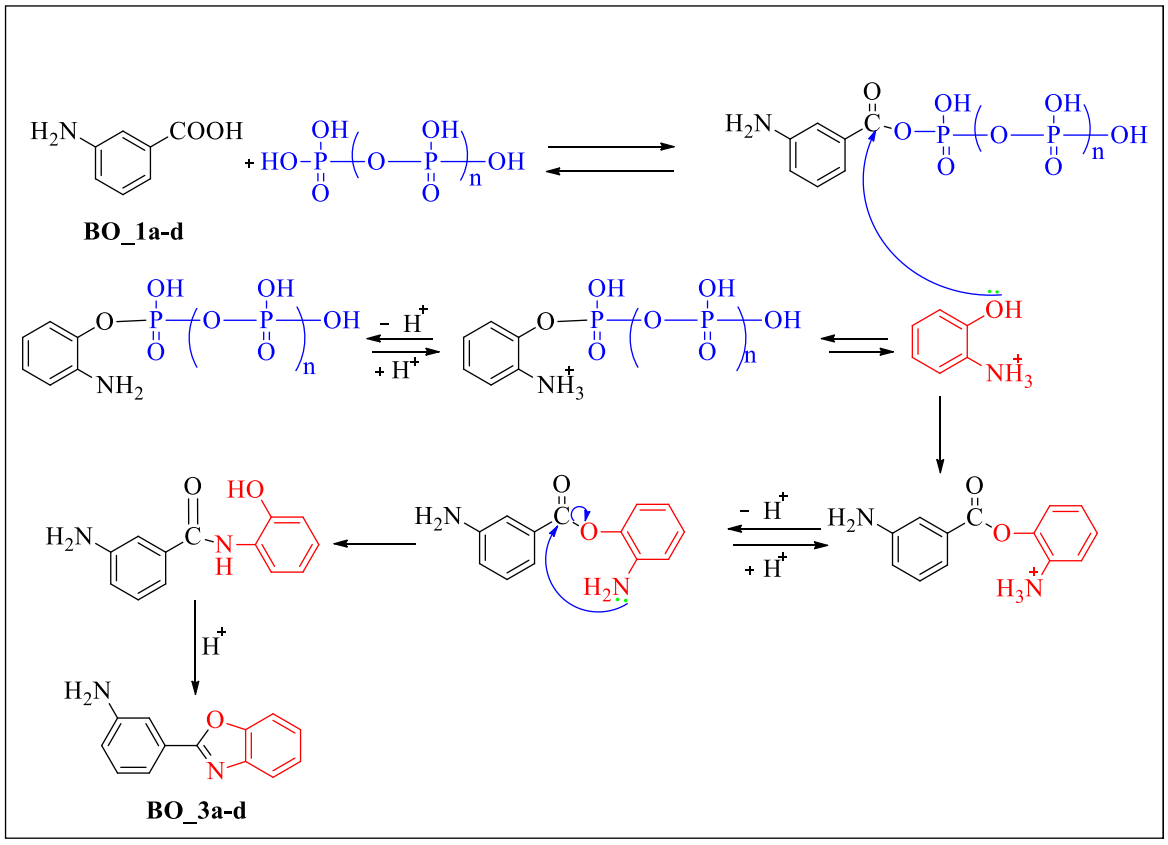


Figure 5.11: Mechanism of conversion of compound **BO_1a-d** to **BO_3a-d**

3-(Benzo[*d*]oxazol-2-yl)aniline (BO_3a)

Following the general procedure the product was synthesized from 2-aminophenol **BO_1a** (1.70 g, 16.05 mmol), 3-aminobenzoic acid (2.00 g, 14.59 mmol) and PPA (14.00 g) and recrystallization from ethanol produced 3-(benzo[*d*]oxazol-2-yl)benzoic acid **(BO_3a**) (2.50 g, 83%) as an off white solid. ESI-MS showed 211 $[M+H]^+$ and carried to next step.

3-(5-Methylbenzo[*d*]oxazol-2-yl)aniline (BO_3b)

Following the general procedure the product was synthesized from 2-amino-4-methylphenol **BO_1b** (4.94 g, 40.11 mmol), 3-aminobenzoic acid (5.00 g, 36.46 mmol) and PPA (35.00 g) and recrystallization from ethanol produced 3-(5-methylbenzo[*d*]oxazol-2-yl)benzoic acid **(BO_3b**) (6.30 g, 77%) as white solid. ESI-MS showed 225 $[M+H]^+$ and carried to next step.

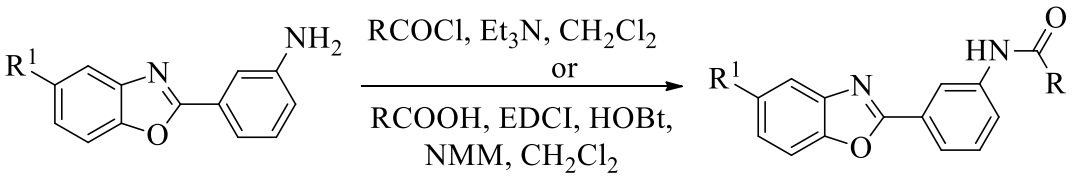
3-(5-Chlorobenzo[*d*]oxazol-2-yl)aniline (BO_3c)

Following the general procedure the product was synthesized from 2-amino-4-chlorophenol **BO_1c** (5.79 g, 40.11 mmol), 3-aminobenzoic acid (5.00 g, 36.46 mmol) and PPA (35.00 g) and compound was purified by column chromatography using EtOAc/hexane as eluent to afford 3-(5-chlorobenzo[*d*]oxazol-2-yl)benzoic acid **(BO_3c**) (7.02 g, 78%) as white solid. ESI-MS showed 245 $[M+H]^+$ and carried to next step.

3-(5-Nitrobenzo[*d*]oxazol-2-yl)aniline (BO_3d)

Following the general procedure the product was synthesized from 2-amino-4-nitrophenol **BO_1d** (6.10 g, 40.11 mmol), 3-aminobenzoic acid (5.00 g, 36.46 mmol) and PPA (35.00 g) and compound was purified by column chromatography using EtOAc/hexane as eluent to afford 3-(5-nitrobenzo[*d*]oxazol-2-yl)benzoic acid **(BO_3d**) (7.50 g, 78%) as yellow solid. ESI-MS showed 256 $[M+H]^+$ and carried to next step.

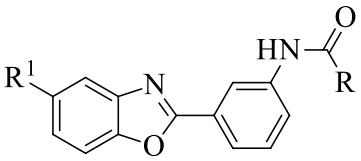
General procedure for the preparation of BO_04 - BO_47



BO_3a-d **BO_04 - BO_47**

To the stirred solution of carboxylic acid (1.0 equiv), EDCI (1.2 equiv), HOBt (1.2 equiv) and NMM (2.5 equiv) in CH_2Cl_2 at 0 °C, was added compound **BO_03a-d** (1.05 equiv) respectively and allowed the reaction mixture to stir at room temperature for 3 h. Upon completion of the reaction the reaction mixture was diluted with CH_2Cl_2 and washed with H_2O and brine solution and the separated organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure, purified by column chromatography using EtOAc/hexanes as eluent.

Table 5.7: Physiochemical properties of the synthesized compounds **BO_04 – BO_47**



BO_04 - BO_47

Compd	R^1	R	Yield (%)	M.P. (°C)	Molecular formula	Molecular weight
BO_04	H	Phenyl	81	218-219	$C_{20}H_{14}N_2O_2$	314.34
BO_05	H	4-Tolyl	72	225-226	$C_{21}H_{16}N_2O_2$	328.36
BO_06	H	2-Methoxypenyl	70	241-242	$C_{21}H_{16}N_2O_3$	344.36
BO_07	H	4-Phenyloxyphenyl	73	230-231	$C_{26}H_{18}N_2O_3$	406.43
BO_08	H	2-Trifluoromethylphenyl	76	222-223	$C_{21}H_{13}F_3N_2O_2$	382.34
BO_09	H	1-Napthyl	67	243-244	$C_{24}H_{16}N_2O_2$	364.40
BO_10	H	4-Pyridyl	65	213-214	$C_{19}H_{13}N_3O_2$	315.33
BO_11	H	2-Furyl	68	228-229	$C_{18}H_{12}N_2O_3$	304.30
BO_12	H	5-Nitro-2-furyl	71	246-247	$C_{18}H_{11}N_3O_5$	349.30

Contd

Compd	R^1	R	Yield (%)	M.P. (°C)	Molecular formula	Molecular weight
BO_13	H	5-Phenyl-2-furyl	74	205-206	$C_{24}H_{16}N_2O_3$	380.40
BO_14	H	5-Nitro-2-thiophenyl	78	244-245	$C_{18}H_{11}N_3O_4S$	365.36
BO_15	H	Cyclohexyl	67	236-237	$C_{20}H_{20}N_2O_2$	320.38
BO_16	CH_3	Phenyl	74	198-199	$C_{21}H_{16}N_2O_2$	328.36
BO_17	CH_3	4-Tolyl	72	180-181	$C_{22}H_{18}N_2O_2$	342.39
BO_18	CH_3	2-Methoxypenyl	77	218-219	$C_{22}H_{18}N_2O_3$	358.39
BO_19	CH_3	4-Phenyloxyphenyl	79	225-226	$C_{27}H_{20}N_2O_3$	420.46
BO_20	CH_3	2-Trifluoromethylphenyl	73	187-188	$C_{22}H_{15}F_3N_2O_2$	396.36
BO_21	CH_3	1-Napthyl	69	230-231	$C_{25}H_{18}N_2O_2$	378.42
BO_22	CH_3	4-Pyridyl	61	222-223	$C_{20}H_{15}N_3O_2$	329.35
BO_23	CH_3	5-Nitro-2-furyl	71	228-229	$C_{19}H_{13}N_3O_5$	363.32
BO_24	CH_3	5-Nitro-2-thiophenyl	75	243-244	$C_{19}H_{13}N_3O_4S$	379.39
BO_25	CH_3	Cyclohexyl	74	213-214	$C_{21}H_{22}N_2O_2$	334.41
BO_26	Cl	Phenyl	80	228-229	$C_{20}H_{13}ClN_2O_2$	348.78
BO_27	Cl	4-Tolyl	74	246-247	$C_{21}H_{15}ClN_2O_2$	362.81
BO_28	Cl	2-Methoxyphenyl	76	171-172	$C_{21}H_{15}ClN_2O_3$	378.81
BO_29	Cl	4-phenyloxyphenyl	79	189-190	$C_{26}H_{17}ClN_2O_3$	440.88
BO_30	Cl	2-Trifluoromethylphenyl	65	215-216	$C_{21}H_{12}ClF_3N_2O_2$	416.78
BO_31	Cl	1-Napthyl	74	236-237	$C_{24}H_{15}ClN_2O_2$	398.84
BO_32	Cl	4-Pyridyl	64	252-253	$C_{19}H_{12}ClN_3O_2$	349.77
BO_33	Cl	2-Furyl	62	180-181	$C_{18}H_{11}ClN_2O_3$	338.74
BO_34	Cl	5-Nitro-2-furyl	69	219-220	$C_{18}H_{10}ClN_3O_5$	383.03
BO_35	Cl	5-Nitro-2-thiophenyl	71	226-227	$C_{18}H_{10}ClN_3O_4S$	399.01
BO_36	Cl	Cyclohexyl	78	231-232	$C_{20}H_{19}ClN_2O_2$	354.83
BO_37	NO_2	Phenyl	84	245-246	$C_{20}H_{13}N_3O_4$	359.33
BO_38	NO_2	4-Tolyl	81	204-205	$C_{21}H_{15}N_3O_4$	373.36
BO_39	NO_2	2-Methoxyphenyl	79	171-172	$C_{21}H_{15}N_3O_5$	389.36

Contd

Compd	R^1	R	Yield (%)	M.P. (°C)	Molecular formula	Molecular weight
BO_40	NO_2	4-Phenyloxyphenyl	76	189-190	$C_{26}H_{17}N_3O_5$	451.43
BO_41	NO_2	1-Napthyl	78	232-233	$C_{24}H_{15}N_3O_4$	409.39
BO_42	NO_2	4-Pyridyl	69	208-209	$C_{19}H_{12}N_4O_4$	360.32
BO_43	NO_2	2-Furyl	63	249-250	$C_{18}H_{11}N_3O_5$	349.30
BO_44	NO_2	2-Thiophenyl	74	218-219	$C_{18}H_{11}N_3O_4S$	365.36
BO_45	NO_2	5-Nitro-2-furyl	71	210-211	$C_{18}H_{10}N_4O_7$	394.29
BO_46	NO_2	5-Nitro-2-thiophenyl	69	223-224	$C_{18}H_{10}N_4O_6S$	410.36
BO_47	NO_2	Cyclohexyl	75	201-202	$C_{20}H_{19}N_3O_4$	365.38

5.4.3. Characterization of the synthesized molecules

***N*-(3-(Benzo[*d*]oxazol-2-yl)phenyl)benzamide (BO_04):** Yield: 81%; m.p. 218–219 °C; MS(ESI) *m/z* 315 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.21 (s, 1H), 7.90 (s,1H), 7.84 (d, *J* = 8.0 Hz, 2H), 7.81–7.67 (m, 6H), 7.63–7.51 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.4, 169.4, 153.6, 142.6, 136.5, 134.6, 133.0, 132.3, 130.6, 128.6, 127.1(2C), 126.0 125.6(2C), 124.5, 124.1, 123.0, 121.9, 110.2. Anal. calcd for $C_{20}H_{14}N_2O_2$: C, 76.42; H, 4.49; N, 8.91% Found C, 76.51; H, 4.52; N, 8.96%.

***N*-(3-(Benzo[*d*]oxazol-2-yl)phenyl)-4-methylbenzamide (BO_05) :** Yield: 72%; m.p. 225–226 °C; MS(ESI) *m/z* 329 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.48 (s, 1H), 7.89 (s, 1H), 7.83 (d, *J* = 6.8 Hz, 1H), 7.74 (d, *J* = 8.0 Hz, 1H), 7.70–7.61 (m, 3H), 7.56–7.48 (m, 3H), 7.39 (d, *J* = 7.6 Hz, 1H), 7.40–7.36 (m, 2H) 2.43 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.3, 166.6, 152.4, 143.1, 135.9, 134.3, 133.4, 133.1, 129.8, 127.2, 126.9(2C), 126.3 125.2(2C), 124.3, 123.9, 123.4, 122.6, 114.3, 22.3. Anal. calcd for $C_{21}H_{16}N_2O_2$: C, 76.81; H, 4.91; N, 8.53% Found C, 76.91; H, 4.98; N, 8.58%.

***N*-(3-(Benzo[*d*]oxazol-2-yl)phenyl)-2-methoxybenzamide (BO_06) :** Yield: 70%; m.p. 241–242 °C; MS(ESI) *m/z* 345 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.50 (s, 1H), 7.81 (s, 1H), 7.85 (d, *J* =7.0 Hz, 1H), 7.72 (d, *J* = 8.2 Hz, 1H), 7.70–7.63 (m, 4H), 7.58–7.49 (m, 4H), 7.40 (d, *J* = 8.0 Hz, 1H), 3.92 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.8, 165.9, 151.4, 148.3,

136.8, 134.6, 133.2, 132.4, 130.8, 128.0, 127.1(2C), 126.4 124.9(2C), 124.1, 123.6, 123.0, 121.2, 111.8, 61.2. Anal. calcd for $C_{21}H_{16}N_2O_3$: C, 73.24; H, 4.68; N, 8.13% Found C, 73.29; H, 4.72; N, 8.18%.

***N*-(3-(Benzo[*d*]oxazol-2-yl)phenyl)-4-phenoxybenzamide (BO_07) :** Yield: 73%; m.p. 230–231 °C; MS(ESI) *m/z* 407 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.51 (s, 1H), 8.42 (s,1H), 7.85 (s,1H), 7.85–7.77 (m, 4H), 7.71 (d, *J* = 8.6 Hz, 2H), 7.70–7.63 (m, 4H), 7.60–7.46 (m, 5H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.2, 168.4, 153.2, 144.1, 142.8, 137.3, 135.3, 134.0, 133.4, 131.4(2C), 130.5, 129.3, 128.1, 127.6(2C), 127.1 126.0, 125.4(2C), 124.8(2C), 124.0, 122.8, 122.2, 109.3. Anal. calcd for $C_{26}H_{18}N_2O_3$: C, 76.83; H, 4.46; N, 6.89% Found C, 76.88; H, 4.52; N, 6.96%.

***N*-(3-(Benzo[*d*]oxazol-2-yl)phenyl)-2-(trifluoromethyl)benzamide (BO_08) :** Yield: 76%; m.p. 222–223 °C; MS(ESI) *m/z* 383 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.53 (s, 1H), 7.89 (s, 1H), 7.66–7.58 (m, 3H), 7.53–7.47 (m, 4H), 7.46–7.35 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.9, 162.4, 144.3, 140.3, 137.3, 136.3, 135.4, 133.9, 132.3, 131.4, 130.5, 129.3, 128.3 127.9, 127.4, 126.3, 125.6, 126.0, 124.3, 121.3, 118.3. Anal. calcd for $C_{21}H_{13}F_3N_2O_2$: C, 65.97; H, 3.43; F, 14.91; N, 7.33% Found C, 66.85; H, 3.52; F, 14.99; N, 7.45%.

***N*-(3-(Benzo[*d*]oxazol-2-yl)phenyl)-1-naphthamide (BO_09) :** Yield: 67%; m.p. 243–244 °C; MS(ESI) *m/z* 365 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.96 (s, 1H), 8.78 (d, *J* = 8.8 Hz, 1H), 8.18–7.72 (m, 6H), 7.66–7.56 (m, 3H), 7.51–7.36 (m, 5H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 169.3, 164.3, 147.3, 139.3, 138.1, 137.3, 136.6, 135.4, 136.1, 135.6, 134.4, 133.3, 132.3, 131.4, 129.6, 128.4, 127.6, 126.3, 125.7, 125.4, 126.5, 124.7, 124.3, 114.5. Anal. calcd for $C_{24}H_{16}N_2O_2$: C, 79.11; H, 4.43; N, 7.69% Found C, 79.18; H, 4.47; N, 7.72%.

***N*-(3-(Benzo[*d*]oxazol-2-yl)phenyl)isonicotinamide (BO_10) :** Yield: 65%; m.p. 213–214 °C; MS(ESI) *m/z* 316 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.68 (s, 1H), 8.21 (s,1H), 8.03 (d, *J* = 8.8 Hz, 2H), 7.94-7.86 (m, 4H), 7.78-7.69 (m, 3H), 7.64 (d, J = 9.2 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.6, 163.3, 143.8, 141.2, 138.3, 136.0, 134.5, 133.9, 133.0(2C), 132.0, 128.6, 127.3, 126.1, 125.2(2C), 122.6, 120.3, 115.0. Anal. calcd for $C_{19}H_{13}N_3O_2$: C, 72.37; H, 4.16; N, 13.33% Found C, 72.41; H, 4.18; N, 13.43%.

***N*-(3-(Benzo[*d*]oxazol-2-yl)phenyl)furan-2-carboxamide (BO_11) :** Yield: 68%; m.p. 228–229 °C; MS(ESI) *m/z* 305 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.97 (s, 1H), 8.13 (s, 1H), 8.01 (d, *J* = 8.4 Hz, 1H), 7.92–7.83 (m, 2H), 7.80–7.67 (m, 4H), 7.62–7.54 (m, 2H), 7.27 (t, *J* = 7.6 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.6, 156.4, 148.3, 138.3, 136.0, 134.7, 134.2, 133.8, 133.0, 131.4, 129.3, 128.0, 127.3, 126.3, 125.6, 123.2, 122.2, 113.4. Anal. calcd for $C_{18}H_{12}N_2O_3$: C, 71.05; H, 3.97; N, 9.21% Found C, 71.12; H, 3.42; N, 9.30%.

***N*-(3-(Benzo[*d*]oxazol-2-yl)phenyl)-5-nitrofuran-2-carboxamide (BO_12) :** Yield: 74%; m.p. 205–206 °C; MS(ESI) *m/z* 350 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.95 (s, 1H), 8.42 (s,1H), 7.61 (s,1H), 7.58 (d, *J* = 8.2 Hz, 1H), 7.56–7.46 (m, 4H), 7.45–7.33 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.3, 166.4, 156.8, 148.8, 140.2, 136.6, 135.2, 134.4, 133.7, 133.0, 132.6, 129.0, 128.9, 128.3, 126.9, 125.3, 124.7, 116.3. Anal. calcd for $C_{18}H_{11}N_3O_5$: C, 61.89; H, 3.17; N, 12.03% Found C, 61.72; H, 3.25; N, 12.09%.

***N*-(3-(Benzo[*d*]oxazol-2-yl)phenyl)-5-phenylfuran-2-carboxamide (BO_13) :** Yield: 71%; m.p. 246–247 °C; MS(ESI) *m/z* 381 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.01 (s, 1H), 8.15 (s, 1H), 8.03 (d, *J* = 8.4 Hz, 1H), 7.94–7.81 (m, 4H), 7.81–7.70 (m, 5H), 7.63–7.51 (m, 2H), 7.29–7.21 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.2, 168.4, 155.3, 147.3, 141.3, 137.3, 136.0, 135.3, 134.3, 133.3, 132.4, 130.6(2C), 129.3, 128.6, 128.2, 127.4, 127.0(2C), 126.5, 125.1, 124.1, 122.3, 114.3. Anal. calcd for $C_{24}H_{16}N_2O_3$: C, 75.78; H, 4.24; N, 7.36% Found C, 75.84; H, 4.29; N, 7.43%.

***N*-(3-(Benzo[*d*]oxazol-2-yl)phenyl)-5-nitrothiophene-2-carboxamide (BO_14) :** Yield: 78%; m.p. 244–245 °C; MS(ESI) *m/z* 366 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.33 (s, 1H), 7.90 (s, 1H), 7.63–7.54 (m, 4H), 7.49–7.36 (m, 5H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.2, 164.4, 160.3, 144.1, 140.2, 138.2, 136.3, 136.0, 135.1, 134.2, 133.0, 131.3, 128.4, 128.0, 127.1, 126.9, 123.4, 115.2. Anal. calcd for $C_{18}H_{11}N_3O_4S$: C, 59.17; H, 3.03; N, 11.50% Found C, 51.3o; H, 5.41; N, 15.05%.

***N*-(3-(Benzo[*d*]oxazol-2-yl)phenyl)cyclohexanecarboxamide (BO_15) :** Yield: 67%; m.p. 236–237 °C; MS(ESI) *m/z* 321$[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.97 (s, 1H), 7.87 (s, 1H), 7.81–7.67 (m, 4H), 7.66–7.53 (m, 3H), 2.47–2.38 (m, 1H), 1.93–1.65 (m, 5H), 1.50–1.16 (m, 5H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 176.2, 165.3, 156.3, 140.3, 138.0, 137.2, 136.3,

135.8, 133.9, 130.5, 127.4, 125.6, 124.2, 116.1, 44.3, 31.3(2C), 27.0, 26.1(2C). Anal. calcd for $C_{20}H_{20}N_2O_2$: C, 74.98; H, 6.29; N, 8.74% Found C, 75.02; H, 6.37; N, 8.93%.

***N*-(3-(5-Methylbenzo[*d*]oxazol-2-yl)phenyl)benzamide (BO_16) :** Yield: 74%; m.p. 198–199 °C; MS(ESI) *m/z* 329 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.71 (s, 1H), 7.83 (s,1H), 7.74 (s,1H), 7.72 (d, *J* = 7.6 Hz, 2H), 7.69–7.63 (m, 4H), 7.60–7.48 (m, 4H), 2.43 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.7, 164.2, 149.3, 141.2, 137.6, 136.9, 136.3, 135.2, 134.2, 133.8, 132.2, 133.0, 128.6(2C), 127.3, 126.5(2C), 125.1, 123.4, 112.8, 22.3. Anal. calcd for $C_{21}H_{16}N_2O_2$: C, 76.81; H, 4.91; N, 8.53% Found C, 76.95; H, 5.04; N, 8.67%.

4-Methyl-*N*-(3-(5-methylbenzo[*d*]oxazol-2-yl)phenyl)benzamide (BO_17) : Yield: 72%; m.p. 180–181 °C; MS(ESI) *m/z* 343 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 10.70 (s, 1H), 8.09 (s,1H), 7.83 (s,1H), 7.76 (d, *J* = 8.4 Hz, 2H), 7.72–7.63 (m, 4H), 7.56–7.43 (m, 3H), 2.47 (s, 3H), 2.43 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.3, 163.6, 147.4, 145.1, 139.9, 137.4, 136.3, 136.0, 135.4, 134.9, 134.2, 133.3, 129.3(2C), 128.0, 127.2(2C), 126.0, 124.2, 114.6, 22.7, 22.3. Anal. calcd for $C_{22}H_{18}N_2O_2$: C, 77.17; H, 5.30; N, 8.18% Found C, 77.25; H, 5.43; N, 8.27%.

2-Methoxy-*N*-(3-(5-methylbenzo[*d*]oxazol-2-yl)phenyl)benzamide (BO_18) : Yield: 77%; m.p. 218–219 °C; MS(ESI) *m/z* 359 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.34 (s, 1H), 8.12 (s,1H), 7.84 (s,1H), 7.78–7.66 (m, 5H), 7.63–7.54 (m, 4H), 3.94 (s, 3H), 2.43 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.6, 163.3, 146.8, 142.4, 137.7, 137.4, 136.6, 136.3, 135.9, 134.3, 133.7, 133.0, 132.4, 129.4, 129.1, 128.3, 127.6, 125.3, 126.2, 123.5, 61.2, 20.8. Anal. calcd for $C_{22}H_{18}N_2O_3$: C, 73.73; H, 5.06; N, 7.82% Found C, 73.79; H, 5.12; N, 7.93%.

***N*-(3-(5-Methylbenzo[*d*]oxazol-2-yl)phenyl)-4-phenoxybenzamide (BO_19) :** Yield: 79%; m.p. 225–226 °C; MS(ESI) *m/z* 421 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.53 (s, 1H), 8.47 (s,1H), 7.85 (s,1H), 7.85–7.78 (m, 4H), 7.72 (d, *J* = 8.6 Hz, 2H), 7.70–7.61 (m, 5H), 7.57–7.45 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.4, 165.8, 156.4, 152.4, 144.9, 140.4, 138.7, 136.9, 136.3, 135.2, 134.1, 133.9, 131.3, 129.2(2C), 128.0, 127.4(2C), 126.3, 125.0, 124.4(2C), 123.3(2C), 122.3, 113.4, 20.9. Anal. calcd for $C_{27}H_{20}N_2O_3$: C, 77.13; H, 4.79; N, 6.66% Found C, 77.16; H, 4.87; N, 6.72%.

***N*-(3-(5-Methylbenzo[*d*]oxazol-2-yl)phenyl)-2-(trifluoromethyl)benzamide (BO_20) :** Yield: 73%; m.p. 187–188 °C; MS(ESI) *m/z* 397 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.63 (s, 1H), 8.11 (s,1H), 7.78 (s,1H), 7.72 (d, *J* = 8.4 Hz, 1H), 7.68–7.56 (m, 5H), 7.53–7.39 (m, 3H), 2.43 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.2, 164.0, 144.6, 141.2, 138.3, 137.6, 137.3, 136.5, 136.8, 135.1, 134.5, 134.3, 133.9, 129.4, 128.5, 127.4, 126.2, 125.2, 124.2, 123.6, 116.3, 22.2. Anal. calcd for $C_{22}H_{15}F_3N_2O_2$: C, 66.67; H, 3.81; N, 7.07% Found C, 66.71; H, 3.43; N, 7.09%.

***N*-(3-(5-Methylbenzo[*d*]oxazol-2-yl)phenyl)-1-naphthamide (BO_21) :** Yield: 69%; m.p. 230–231 °C; MS(ESI) *m/z* 379 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.56 (s, 1H), 8.93 (d, *J* = 8.4 Hz, 1H), 7.84 (s,1H), 7.78–7.69 (m, 6H), 7.63–7.53 (m, 3H), 7.49–7.43 (m, 3H), 2.43 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.2, 166.4, 148.4, 142.4, 137.4, 137.1, 136.2, 135.7, 135.3, 134.8, 134.4, 134.0, 133.4, 132.9, 130.6, 129.9, 129.4, 128.4, 128.0, 127.5, 126.7, 125.6, 124.3, 116.3, 22.3. Anal. calcd for $C_{25}H_{18}N_2O_2$: C, 79.35; H, 4.79; N, 7.40% Found C, 79.40; H, 4.82; N, 7.52%.

***N*-(3-(5-Methylbenzo[*d*]oxazol-2-yl)phenyl)isonicotinamide (BO_22) :** Yield: 61%; m.p. 222–223 °C; MS(ESI) *m/z* 330 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.87 (s, 1H), 7.87 (s,1H), 7.72 (d, *J* = 8.0 Hz, 2H), 7.63 (s, 1H), 7.60 (d, *J* = 8.4 Hz, 2H), 7.56–7.43 (m, 5H), 2.43 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.7, 166.2, 149.8, 141.3, 137.6, 137.3, 136.7, 136.0, 134.6(2C), 133.9, 133.2, 132.3, 130.9, 129.4(2C), 127.7, 124.3, 118.4, 22.4. Anal. calcd for $C_{20}H_{15}N_3O_2$: C, 72.94; H, 4.59; N, 12.76% Found C, 73.01; H, 4.63; N, 12.81%.

***N*-(3-(5-Methylbenzo[*d*]oxazol-2-yl)phenyl)-5-nitrofuran-2-carboxamide (BO_23) :** Yield: 71%; m.p. 228–229 °C; MS(ESI) *m/z* 364 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.98 (s, 1H), 8.16 (s,1H), 7.76 (s,1H), 7.72–7.58 (m, 3H), 7.54–7.46 (m, 2H), 7.45–7.39 (m, 2H), 2.46 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.2, 167.3, 144.7, 143.5, 138.4, 137.3, 136.6, 135.3, 133.9, 133.2, 132.3, 130.5, 129.3, 127.3, 126.6, 126.2, 125.3, 124.7, 115.2, 22.2. Anal. calcd for $C_{19}H_{13}N_3O_5$: C, 62.81; H, 3.61; N, 11.57% Found C, 62.90; H, 3.70; N, 11.63%.

***N*-(3-(5-Methylbenzo[*d*]oxazol-2-yl)phenyl)-5-nitrothiophene-2-carboxamide (BO_24) :** Yield: 75%; m.p. 243–244 °C; MS(ESI) *m/z* 380 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.23 (s, 1H), 8.18 (s,1H), 7.81 (s,1H), 7.76 (d, *J* = 7.2 Hz, 1H), 7.68–7.58 (m, 4H), 7.55–7.47

(m, 2H), 2.47 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.3, 167.6, 160.4, 143.2, 139.6, 137.2, 136.4, 136.0, 135.4, 133.0, 132.6, 132.1, 129.3, 127.8, 126.1, 125.4, 123.6, 115.2, 22.5. Anal. calcd for $C_{19}H_{13}N_3O_4S$: C, 60.15; H, 3.45; N, 11.08% Found C, 60.18; H, 3.51; N, 11.16%.

***N*-(3-(5-Methylbenzo[*d*]oxazol-2-yl)phenyl)cyclohexanecarboxamide (BO_25) :** Yield: 74%; m.p. 213–214 °C; MS(ESI) *m/z* 335 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.78 (s, 1H), 8.16 (s,1H), 7.74 (s,1H), 7.70–7.56 (m, 6H), 7.51–7.44 (m, 2H), 2.42–2.38 (m, 1H), 1.92–1.71 (m, 4H), 1.62–1.13 (m, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.4, 163.6, 151.4, 138.2, 136.4, 135.9, 134.7, 133.0, 130.4, 128.5, 127.3, 126.1, 124.4, 118.3, 46.2, 31.2(2C), 23.8(2C), 23.1, 21.9. Anal. calcd for $C_{21}H_{22}N_2O_2$: C, 75.42; H, 6.63; N, 8.38% Found C, 75.54; H, 6.70; N, 8.42%.

***N*-(3-(5-Chlorobenzo[*d*]oxazol-2-yl)phenyl)benzamide (BO_26) :** Yield: 80%; m.p. 228–229 °C; MS(ESI) *m/z* 349 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.72 (s, 1H), 8.61 (s,1H), 7.69 (s,1H), 7.65–7.54 (m, 4H), 7.49–7.36 (m, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.8, 166.3, 148.3, 139.9, 138.3, 137.3, 136.9, 135.3, 133.9(2C), 133.3, 131.4, 129.2(2C), 128.0, 127.6, 126.4, 125.1, 124.5, 115.7. Anal. calcd for $C_{20}H_{13}ClN_2O_2$: C, 68.87; H, 3.76; N, 8.03% Found C, 68.93; H, 3.81; N, 8.09%.

***N*-(3-(5-Chlorobenzo[*d*]oxazol-2-yl)phenyl)-4-methylbenzamide (BO_27) :** Yield: 74%; m.p. 246–247 °C; MS(ESI) *m/z* 363 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.99 (s, 1H), 8.53 (s,1H), 7.76 (s,1H), 7.72–7.63 (m, 4H), 7.61–7.56 (m, 2H), 7.54–7.47 (m, 3H) 2.47 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.8, 165.3, 150.2, 140.4, 137.5, 136.1, 135.7, 134.8, 134.1(2C), 133.4, 132.6, 130.6, 128.7(2C), 128.3, 126.9, 126.3, 124.8, 116.3, 22.2. Anal. calcd for $C_{21}H_{15}ClN_2O_2$: C, 69.52; H, 4.17; N, 7.72% Found C, 70.01; H, 4.19; N, 7.90%.

***N*-(3-(5-Chlorobenzo[*d*]oxazol-2-yl)phenyl)-2-methoxybenzamide (BO_28) :** Yield: 76%; m.p. 171–172 °C; MS(ESI) *m/z* 379 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.07 (s, 1H), 8.58 (s,1H), 7.83 (s,1H), 7.81–7.69 (m, 5H), 7.66 (d, J = 6.8 Hz, 1H), 7.63–7.54 (m, 3H), 3.96 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 170.1, 166.0, 149.3, 139.4, 137.3, 136.6, 136.0, 135.3, 134.3, 133.9, 133.4, 131.6, 130.9, 129.5, 128.3, 127.4, 126.9, 126.2, 125.1, 118.6, 63.2. Anal. calcd for $C_{21}H_{15}ClN_2O_3$: C, 66.58; H, 3.99; N, 7.40% Found C, 66.63; H, 4.02; N, 7.45%.

***N*-(3-(5-Chlorobenzo[*d*]oxazol-2-yl)phenyl)-4-phenoxybenzamide (BO_29) :** Yield: 79%; m.p. 189–190 °C; MS(ESI) *m/z* 441 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.55 (s, 1H), 8.49 (s,1H), 7.89 (s,1H), 7.85–7.76 (m, 3H), 7.74 (d, *J* = 8.8 Hz, 2H), 7.71–7.62 (m, 4H), 7.58–7.44 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.4, 160.7, 153.4, 144.3, 138.0, 137.6, 137.3, 136.3, 135.1, 134.4, 133.4, 132.2(2C), 131.4, 130.5(2C), 129.3, 128.5, 127.7, 127.3, 126.8(2C), 125.3, 124.6(2C), 115.9. Anal. calcd for $C_{26}H_{17}ClN_2O_3$: C, 70.83; H, 3.89; N, 6.35% Found C, 70.94; H, 3.95; N, 6.44%.

***N*-(3-(5-Chlorobenzo[*d*]oxazol-2-yl)phenyl)-2-(trifluoromethyl)benzamide (BO_30) :** Yield: 65%; m.p. 215–216 °C; MS(ESI) *m/z* 417 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.62 (s, 1H), 8.18 (s,1H), 7.69 (s,1H), 7.66 (d, *J* = 7.6 Hz, 1H), 7.64–7.48 (m, 4H), 7.45–7.33 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.3, 162.6, 149.4, 146.1, 137.5, 136.8, 136.3, 135.3, 134.9, 134.3, 133.6, 132.4, 132.0, 131.5, 130.3, 129.2, 127.8, 126.6, 126.3, 124.3, 116.1. Anal. calcd for $C_{21}H_{12}ClF_3N_2O_2$: C, 60.52; H, 2.90; N, 6.72% Found C, 60.57; H, 2.94; N, 6.83%.

***N*-(3-(5-Chlorobenzo[*d*]oxazol-2-yl)phenyl)-1-naphthamide (BO_31) :** Yield: 74%; m.p. 236–237 °C; MS(ESI) *m/z* 399 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.60 (s, 1H), 8.61 (d, *J* = 8.4 Hz, 1H), 7.91 (s,1H), 7.84–7.76 (m, 4H), 7.72–7.58 (m, 6H), 7.53–7.46 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.2, 166.3, 148.3, 142.3, 138.2, 137.4, 136.7, 136.2, 135.4, 134.8, 133.9, 133.3, 132.6, 132.1, 130.7, 130.2, 129.5, 129.0, 128.4, 127.8, 126.7, 125.6, 124.3, 114.9. Anal. calcd for $C_{24}H_{15}ClN_2O_2$: C, 72.27; H, 3.79; N, 7.02% Found C, 72.90; H, 3.83; N, 7.07%.

***N*-(3-(5-Chlorobenzo[*d*]oxazol-2-yl)phenyl)isonicotinamide (BO_32) :** Yield: 64%; m.p. 252–253 °C; MS(ESI) *m/z* 350 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.27 (s, 1H), 8.64 (s,1H), 7.81 (s,1H), 7.78–7.72 (m, 2H), 7.69 (d, *J* = 8.4 Hz, 2H), 7.65–7.56 (m, 3H), 7.54–7.49 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.9, 164.3, 148.1, 138.3, 136.6(2C), 135.8, 135.0, 134.7, 134.2, 133.5, 132.7, 129.1, 128.5(2C), 127.4, 126.2, 124.7, 114.5. Anal. calcd for $C_{19}H_{12}ClN_3O_2$: C, 65.24; H, 3.46; N, 12.01% Found C, 65.28; H, 3.51; N, 12.06%.

***N*-(3-(5-Chlorobenzo[*d*]oxazol-2-yl)phenyl)furan-2-carboxamide (BO_33) :** Yield: 62%; m.p. 180–181 °C; MS(ESI) *m/z* 339 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.80 (s, 1H), 8.61 (s,1H), 7.83 (s,1H), 7.79–7.72 (m, 2H), 7.66 (d, *J* = 8.0 Hz, 1H), 7.58–7.47 (m, 3H), 7.44–7.38 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.3, 158.4, 144.3, 137.4, 135.3, 134.5, 134.3,

133.6, 132.6, 131.4, 130.5, 128.6, 128.2, 127.2, 125.8, 124.6, 122.4, 116.0. Anal. calcd for $C_{18}H_{11}ClN_2O_3$: C, 63.82; H, 3.27; N, 8.27% Found C, 63.87; H, 3.33; N, 8.34%.

***N*-(3-(5-Chlorobenzo[*d*]oxazol-2-yl)phenyl)-5-nitrofuran-2-carboxamide (BO_34) :** Yield: 69%; m.p. 219–220 °C; MS(ESI) *m/z* 384 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.98 (s, 1H), 8.49 (s,1H), 7.63 (s,1H), 7.61 (d, *J* = 8.4 Hz, 1H), 7.56–7.48 (m, 3H), 7.45–7.33 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.4, 160.4, 151.3, 143.7, 138.2, 137.4, 136.8, 135.1, 133.6, 130.6, 129.2, 127.4, 126.9, 125.7, 124.3, 123.8, 122.7, 115.9. Anal. calcd for $C_{18}H_{10}ClN_3O_5$: C, 56.34; H, 2.63; N, 10.95% Found C, 56.40; H, 2.69; N, 11.01%.

***N*-(3-(5-Chlorobenzo[*d*]oxazol-2-yl)phenyl)-5-nitrothiophene-2-carboxamide (BO_35) :** Yield: 71%; m.p. 226–227 °C; MS(ESI) *m/z* 400 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.01 (s, 1H), 8.53 (s,1H), 7.76 (s,1H), 7.73–7.67 (m, 2H), 7.62–7.54 (m, 3H), 7.51–7.44 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.6, 164.2, 153.6, 146.3, 139.5, 137.7, 136.5, 135.3, 134.3, 133.9, 130.6, 128.3, 127.6, 126.1, 125.5, 124.2, 123.9, 117.6. Anal. calcd for $C_{18}H_{10}ClN_3O_4S$: C, 54.07; H, 2.52; N, 10.51% Found C, 54.13; H, 2.58; N, 10.59%.

***N*-(3-(5-Chlorobenzo[*d*]oxazol-2-yl)phenyl)cyclohexanecarboxamide (BO_36) :** Yield: 78%; m.p. 231–232 °C; MS(ESI) 355 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.21 (s, 1H), 8.67 (s,1H), 7.94 (s,1H), 7.85–7.74 (m, 3H), 7.58–7.46 (m, 2H), 2.45–2.36 (m, 1H), 1.94–1.66 (m, 5H), 1.52–1.12 (m, 5H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.7, 163.8, 152.4, 147.8, 140.2, 137.4, 134.3, 133.9, 129.7, 128.5, 126.9, 125.2, 124.6, 114.9, 48.4, 31.5(2C), 27.5(2C), 26.8. Anal. calcd for $C_{20}H_{19}ClN_2O_2$: C, 67.70; H, 5.40; N, 7.89% Found C, 67.77; H, 5.45; N, 7.95%.

***N*-(3-(5-Nitrobenzo[*d*]oxazol-2-yl)phenyl)benzamide (BO_37) :** Yield: 84%; m.p. 245–246 °C; MS(ESI) *m/z* 360 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.21 (s, 1H), 8.67 (s,1H), 7.94 (s,1H), 7.85–7.74 (m, 2H), 7.58–7.46 (m, 2H), 2.45–2.36 (m, 1H), 1.52–1.12 (m, 5H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.4, 162.7, 153.6, 143.6, 139.5, 137.8, 136.4, 135.6, 133.4(2C), 131.6, 130.4, 129.4, 128.5, 127.6, 126.5, 125.8, 125.3, 124.6, 116.1. Anal. calcd for $C_{20}H_{13}N_3O_4$: C, 66.85; H, 3.65; N, 11.69% Found C, 67.16; H, 3.74; N, 11.77%.

4-Methyl-*N*-(3-(5-nitrobenzo[*d*]oxazol-2-yl)phenyl)benzamide (BO_38) : Yield: 81%; m.p. 204–205 °C; MS(ESI) *m/z* 374 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.89 (s, 1H), 8.68

(s,1H), 8.39 (s,1H), 7.98–7.87 (m, 3H), 7.81–7.74 (m, 2H), 7.69 (d, *J* = 7.6 Hz, 2H), 7.64–7.54 (m, 2H), 2.50 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.9, 163.5, 148.5, 144.7, 138.3, 136.9, 136.3, 135.4, 136.8, 134.2, 132.4, 130.8(2C), 128.8, 127.8, 126.1(2C), 124.9, 123.8, 116.1, 22.4. Anal. calcd for $C_{21}H_{15}N_3O_4$: C, 67.56; H, 4.05; N, 11.25% Found C, 67.64; H, 4.12; N, 11.31%.

2-Methoxy-*N*-(3-(5-nitrobenzo[*d*]oxazol-2-yl)phenyl)benzamide (BO_39) : Yield: 79%; m.p. 171–172 °C; MS(ESI) *m/z* 390 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.69 (s, 1H), 8.70 (s,1H), 8.45 (s,1H), 8.08–7.99 (m, 3H), 7.86–7.81 (m, 2H), 7.74 (d, *J* = 8.0 Hz, 1H), 7.72–7.56 (m, 3H), 3.94 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.3, 161.4, 147.5, 145.4, 137.9, 137.1, 136.4, 136.0, 134.8, 134.3, 133.6, 132.4, 130.5, 128.7, 127.6, 127.1, 126.0, 125.3, 124.9, 117.7, 63.6. Anal. calcd for $C_{21}H_{15}N_3O_5$: C, 64.78; H, 3.88; N, 10.79% Found C, C, 64.85; H, 3.96; N, 10.88%.

***N*-(3-(5-Nitrobenzo[*d*]oxazol-2-yl)phenyl)-4-phenoxybenzamide (BO_40) :** Yield: 76%; m.p. 189–190 °C; MS(ESI) *m/z* 452 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.21 (s, 1H), 9.02 (s,1H), 7.93 (s,1H), 7.89–7.77 (m, 3H), 7.75 (d, *J* = 8.6 Hz, 2H), 7.72–7.64 (m, 4H), 7.59–7.44 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.3, 166.3, 154.3, 149.9, 138.5, 137.4, 136.9, 136.6, 135.5, 134.6, 134.0, 133.9(2C), 132.8, 131.2(2C), 128.7, 127.8, 127.4, 127.1, 126.3(2C), 125.7, 125.2(2C), 116.3. Anal. calcd for $C_{26}H_{17}N_3O_5$: C, 69.18; H, 3.80; N, 9.31% Found C, 69.25; H, 3.85; N, 9.36%.

***N*-(3-(5-Nitrobenzo[*d*]oxazol-2-yl)phenyl)-1-naphthamide (BO_41) :** Yield: 78%; m.p. 232–233 °C; MS(ESI) *m/z* 410 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.13 (s, 1H), 8.63 (s,1H), 7.99 (s,1H), 7.92 (d, *J* = 8.8 Hz, 1H), 7.78–7.69 (m, 3H), 7.66–7.48 (m, 6H), 7.42–7.32 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) 170.7, 167.4, 149.7, 143.6, 137.7, 136.9, 136.3, 135.6, 134.7, 133.6, 133.0, 132.7, 132.3, 131.5, 130.4, 129.9, 129.4, 128.8, 128.3, 127.9, 127.3, 126.3, 125.7, 115.5. Anal. calcd for $C_{24}H_{15}N_3O_4$: C, 70.41; H, 3.69; N, 10.26 % Found C, 70.52; H, 3.72; N, 10.27%.

***N*-(3-(5-Nitrobenzo[*d*]oxazol-2-yl)phenyl)isonicotinamide (BO_42) :** Yield: 69%; m.p. 208–209 °C; MS(ESI) *m/z* 361 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.21 (s, 1H), 8.67 (s,1H), 7.88 (s,1H), 7.80–7.72 (m, 2H), 7.70 (d, *J* = 8.2 Hz, 2H), 7.69–7.61 (m, 3H), 7.59–7.53 (m, 2H);

^{13}C NMR (100 MHz, DMSO-d_6) δ 167.6, 165.2, 149.5, 139.5, 137.3, 136.3(2C), 136.9, 135.3, 133.9, 133.3, 132.6, 131.4, 129.4(2C), 128.3, 127.7, 125.8, 116.1. Anal. calcd for $C_{19}H_{12}N_4O_4$: C, 63.33; H, 3.36; N, 15.55% Found C, 63.39; H, 3.42; N, 15.59%.

***N*-(3-(5-Nitrobenzo[*d*]oxazol-2-yl)phenyl)furan-2-carboxamide (BO_43) :** Yield: 63%; m.p. 249–250 °C; MS(ESI) *m/z* 350 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.57 (s, 1H), 8.62 (s,1H), 7.94 (s,1H), 7.84–7.68 (m, 3H), 7.64–7.55 (m, 3H), 7.49–7.35 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.8, 164.3, 148.3, 138.6, 136.4, 135.3, 134.9, 133.3, 133.0, 132.6, 130.7, 129.3, 127.8, 127.3, 126.4, 125.2, 123.6, 115.3. Anal. calcd for $C_{18}H_{11}N_3O_5$: C, 61.89; H, 3.17; N, 12.03% Found C, 61.96; H, 3.24; N, 12.09%.

***N*-(3-(5-Nitrobenzo[*d*]oxazol-2-yl)phenyl)thiophene-2-carboxamide (BO_44) :** Yield: 74%; m.p. 218–219 °C; MS(ESI) *m/z* 366 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.53 (s, 1H), 8.64 (s,1H), 7.96 (s,1H), 7.84–7.63 (m, 4H), 7.60–7.53 (m, 2H), 7.47–7.33 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.6, 162.9, 146.1, 136.9, 135.3, 134.6, 133.9, 132.3, 131.6, 130.9, 130.4, 128.6, 128.3, 126.4, 125.9, 125.1, 124.3, 113.3. Anal. calcd for $C_{18}H_{11}N_3O_4S$: C, 59.17; H, 3.03; N, 11.50% Found C, 59.21; H, 3.10; N, 11.56%.

5-Nitro-*N*-(3-(5-nitrobenzo[*d*]oxazol-2-yl)phenyl)furan-2-carboxamide (BO_45) : Yield: 71%; m.p. 210–211 °C; MS(ESI) *m/z* 395 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.96 (s, 1H), 8.73 (s,1H), 8.71 (s,1H), 8.38 (d, *J* = 8.8 Hz, 1H), 7.84–7.63 (m, 2H), 7.60–7.53 (m, 2H), 7.47–7.33 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.3, 163.3, 149.3, 144.9, 139.4, 138.1, 137.3, 136.5, 134.9, 133.5, 130.6, 128.8, 127.5, 126.3, 125.9, 124.6, 123.4, 116.7. Anal. calcd for $C_{18}H_{10}N_4O_7$: C, 54.83; H, 2.56; N, 14.21% Found C, 54.98; H, 2.67; N, 14.29%.

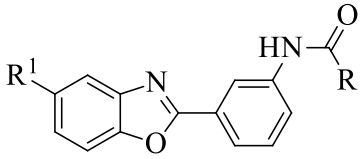
5-Nitro-*N*-(3-(5-nitrobenzo[*d*]oxazol-2-yl)phenyl)thiophene-2-carboxamide (BO_46) : Yield: 69%; m.p. 223–224 °C; MS(ESI) *m/z* 411 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.05 (s, 1H), 8.57 (s,1H), 7.98 (s,1H), 7.88–7.72 (m, 2H), 7.65–7.56 (m, 3H), 7.53–7.44 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 169.4, 162.9, 156.3, 149.6, 138.4, 137.5, 136.3, 135.5, 133.6, 133.0, 132.4, 129.8, 128.5, 127.3, 126.1, 125.2, 124.6, 118.3. Anal. calcd for $C_{18}H_{10}N_4O_6S$: C, 52.68; H, 2.46; N, 13.65% Found C, 52.72; H, 2.49; N, 13.71%.

***N*-(3-(5-Nitrobenzo[*d*]oxazol-2-yl)phenyl)cyclohexanecarboxamide (BO_47) :** Yield: 75%; m.p. 201–202 °C; MS(ESI) *m/z* 366 $[M+H]^+$. ^1H NMR (400 MHz, $CDCl_3$) δ 11.05 (s, 1H), 8.91 (s,1H), 7.99 (s,1H), 7.89–7.78 (m, 3H), 7.60–7.51 (m, 2H), 2.47–2.39 (m, 1H), 1.94–1.72 (m, 5H), 1.53–1.14 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) 174.9, 166.4, 148.3, 139.5, 137.6, 136.7, 136.0, 133.9, 129.3, 128.7, 127.3, 126.4, 125.7, 116.3, 49.6, 31.8(2C), 27.2, 26.9(2C). Anal. calcd for $C_{20}H_{19}N_3O_4$: C, 65.74; H, 5.24; N, 11.50% Found C, 65.79; H, 5.29; N, 11.57%.

5.4.4. *In vitro M. tuberculosis* screening, *M. tuberculosis* PknB 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. INH, EMB, were used as a reference compounds for comparison. All the synthesized derivatives were also screened for their *in vitro M. tuberculosis* PknB inhibitory potency as steps towards hit optimization. All the compounds 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 5.12**

Table 5.8: *In vitro* biological evaluation of synthesized compounds **BO_04** – **BO_47**



BO_04 - BO_47

Compd	R^1	R	MTB MIC[a] in μM	MTB PknB IC_{50} in μM	Cytotoxicity[b] at 50 μM % inhibition
BO_04	H	Phenyl	2.48	11.05	10.98
BO_05	H	4-Tolyl	19.05	>20	NT
BO_06	H	2-Methoxypenyl	36.33	15.42	NT
BO_07	H	4-Phenyloxyphenyl	15.39	7.20	9.54
BO_08	H	2-Trifluoromethylphenyl	65.44	>20	NT
BO_09	H	1-Napthyl	68.68	12.04	NT
BO_10	H	4-Pyridyl	2.47	>20	NT

Contd

Compd	R^1	R	MTB MIC[a] in µM	MTB PknB IC_{50} in µM	Cytotoxicity[b] at 50 µM % inhibition
BO_10	H	4-Pyridyl	2.47	>20	NT
BO_11	H	2-Furyl	5.13	18.01	16.54
BO_12	H	5-Nitro-2-furyl	4.47	1.09	23.01
BO_13	H	5-Phenyl-2-furyl	4.11	16.76	58.31
BO_14	H	5-Nitro-2-thiophenyl	17.12	4.91	32.01
BO_15	H	Cyclohexyl	20.42	>20	NT
BO_16	CH_3	Phenyl	76.21	6.72	NT
BO_17	CH_3	4-Tolyl	73.09	4.58	NT
BO_18	CH_3	2-Methoxypenyl	4.36	7.85	24.35
BO_19	CH_3	4-Phenyloxyphenyl	59.52	4.84	NT
BO_20	CH_3	2-Trifluoromethylphenyl	1.97	8.24	12.35
BO_21	CH_3	1-Napthyl	33.06	16.38	NT
BO_22	CH_3	4-Pyridyl	2.37	7.51	15.64
BO_23	CH_3	5-Nitro-2-furyl	34.43	12.03	NT
BO_24	CH_3	5-Nitro-2-thiophenyl	2.05	0.87	13.24
BO_25	CH_3	Cyclohexyl	2.33	5.02	18.64
BO_26	Cl	Phenyl	2.24	19.24	23.14
BO_27	Cl	4-Tolyl	34.53	3.04	26.21
BO_28	Cl	2-Methoxyphenyl	8.26	8.16	19.87
BO_29	Cl	4-phenyloxyphenyl	14.20	4.34	13.27
BO_30	Cl	2-Trifluoromethylphenyl	60.09	19.28	NT
BO_31	Cl	1-Napthyl	31.40	9.07	NT
BO_32	Cl	4-Pyridyl	71.63	>20	NT
BO_33	Cl	2-Furyl	18.49	18.26	26.97
BO_34	Cl	5-Nitro-2-furyl	4.07	4.63	35.64
BO_35	Cl	5-Nitro-2-thiophenyl	7.83	4.38	30.24
BO_36	Cl	Cyclohexyl	70.62	5.04	NT

Contd

Compd	R^1	R	MTB MIC[a] in µM	MTB PknB IC_{50} in µM	Cytotoxicity[b] at 50 µM % inhibition
BO_37	NO_2	Phenyl	69.63	5.22	NT
BO_38	NO_2	4-Tolyl	33.51	10.06	NT
BO_39	NO_2	2-Methoxyphenyl	64.26	15.24	NT
BO_40	NO_2	4-Phenyloxyphenyl	13.85	18.07	17.64
BO_41	NO_2	1-Napthyl	7.64	4.97	32.19
BO_42	NO_2	4-Pyridyl	4.34	16.08	42.31
BO_43	NO_2	2-Furyl	71.63	19.54	NT
BO_44	NO_2	2-Thiophenyl	68.49	9.71	NT
BO_45	NO_2	5-Nitro-2-furyl	1.97	0.63	12.94
BO_46	NO_2	5-Nitro-2-thiophenyl	3.96	5.31	19.82
BO_47	NO_2	Cyclohexyl	34.24	>20	NT
Isoniazid			0.72	NT	NT
Ethambutol			7.64	NT	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.

5.4.5. SAR and Discussion

As per our anticipation, the extension of chain on phenyl ring of C-2 position instead in between phenyl and benzoxazole rings showed better activities. The synthesized library of compounds exhibited activity against *M. tuberculosis* with MIC ranging from 1.97 to 76.21 µM. Twenty four compounds inhibited *M. tuberculosis* with MIC of <20 µM. When compared to lead molecule **8a**, eighteen compounds (**BO_04**, **BO_10 - BO_13**, **BO_18**, **BO_20**, **BO_22**, **BO_24 - BO_26**, **BO_28**, **BO_34 - BO_35**, **BO_41 - BO_42**, **BO_45 - BO_46**). Fifteen compounds found to be more active in inhibiting *M. tuberculosis* than standard first line antitubercular drug EMB. Fourteen compounds were found to be possess *M. tuberculosis* MIC of < 5 µM. Eight compounds (**BO_04**, **BO_10**, **BO_20**, **BO_22**, **BO_24 - BO_26**, **BO_45**) were found to be possess *M. tuberculosis* MIC of < 3 µM.

Compound **BO_45** (5-nitro-N-(3-(5-nitrobenzo[*d*]oxazol-2-yl)phenyl)furan-2-carboxamide) was found to be the most active compound *in vitro* with MIC of 1.97 μM against log-phase culture of *M. tuberculosis*. Compound **BO_45** also exhibited excellent activity against *M. tuberculosis* PknB enzyme with IC_{50} 0.63 μM. In general, the activity trend with respect to substitution on benzoxazole ring at 5^{th} positions follows as CH_3>H> NO_2>Cl. Five molecules (**BO_18**, **BO_20**, **BO_22**, **BO_24 - BO_25**) containing CH_3- substation at 5^{th} position showed *M. tuberculosis* MIC < 5 μM, there was slight decrease in activity when CH_3- group was replaced by H-atom at 5^{th} positon. Five molecules (**BO_04**, **BO_10 - BO_13)** which have no substitution at 5^{th} position of benzoxazole core showed MIC < 6 μM. Whereas, on substitution of electron withdrawing groups (Cl and NO_2) at 5^{th} position of benzoxazole core, the reduction in activity was observed.

Among the substitutions at 3^{rd} position of C-2 phenyl ring, the substitutes having heterocycles were found to be active than substituted aryl and alicyclic groups. Compound **BO_45** (5-nitro-N-(3-(5-nitrobenzo[*d*]oxazol-2-yl)phenyl)furan-2-carboxamide) which has two NO_2 groups, one at 5^{th} position of benzoxazole ring and another at 5^{th} position of furan ring which is bonded as amide derivative of C-2 phenyl ring at 3^{rd} position found to be most active.

5.4.6. Highlights of the study

In this work we designed 3-(benzo[*d*]oxazol-2-yl)aniline analogs as novel antitubercular agents based on reported anti-tubercular compound. Synthesized a library of forty four molecules and subjected to *M. tuberculosis* PknB inhibition studies, *M. tuberculosis* MIC determination and cytotoxicity studies. More than one third of library exhibited better activity than lead molecule. In conclusion, it has been demonstrated that the potency, selectivity, and low cytotoxicity of these compounds make them valid leads for further studies. Compound **BO_45** (5-nitro-N-(3-(5-nitrobenzo[*d*]oxazol-2-yl)phenyl)furan-2-carboxamide) was found to be the most active compound (**Figure 5.12**) with IC_{50} of 0.63 μM against *M. tuberculosis* PknB, inhibited drug sensitive *M. tuberculosis* with MIC of 1.97 μM and was non-cytotoxic at 50 μM.

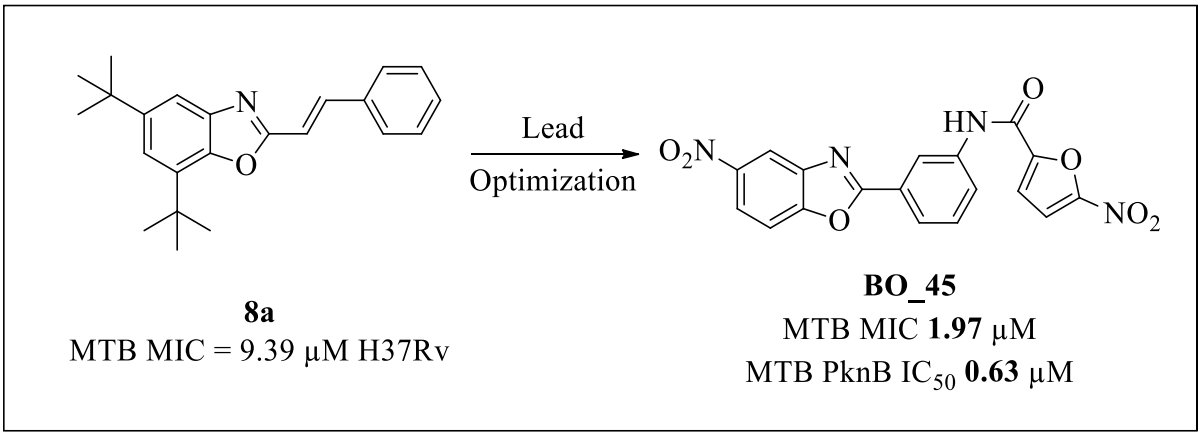


Figure 5.12: Structure and biological activity of most active compound **BO_45**

5.5. Development of 2-(3-aminophenyl)-benzothiazole derivatives as potential *M. tuberculosis* PknB inhibitors

5.5.1. Design of the molecules

In order to develop the potent molecules against *M. tuberculosis*, high-through put virtual screening of our in-house data base (BITS-pilani), *in vitro* anti-tubercular screening against *M. tuberculosis* H37Rv strain and enzymatic evaluation against various enzymes has carried out in our research group. **BITS- 115** is one of the potent molecules identified with the significant *in vitro* anti-tubercular activity against *M. tuberculosis* H37Rv with MIC of 15.1 µM and promising enzymatic activity with an IC_{50} of 5.02 µM against *M. tuberculosis* PknB with a good safety profile [unpublished data]. After the exhaustive literature survey on benzothiazoles as anti-tubercular agents and based on our knowledge in the design of anti-tubercular agents, we have taken **BITS-115** (**Figure 5.13**) as the lead molecule and subjected to the molecular derivatization technique to develop a series of analogues of the lead molecule as the novel inhibitors of *M. tuberculosis* PknB with enhanced potency, to establish the structure-activity relationship (SAR), to evaluate anti-tubercular activity and cytotoxicity.

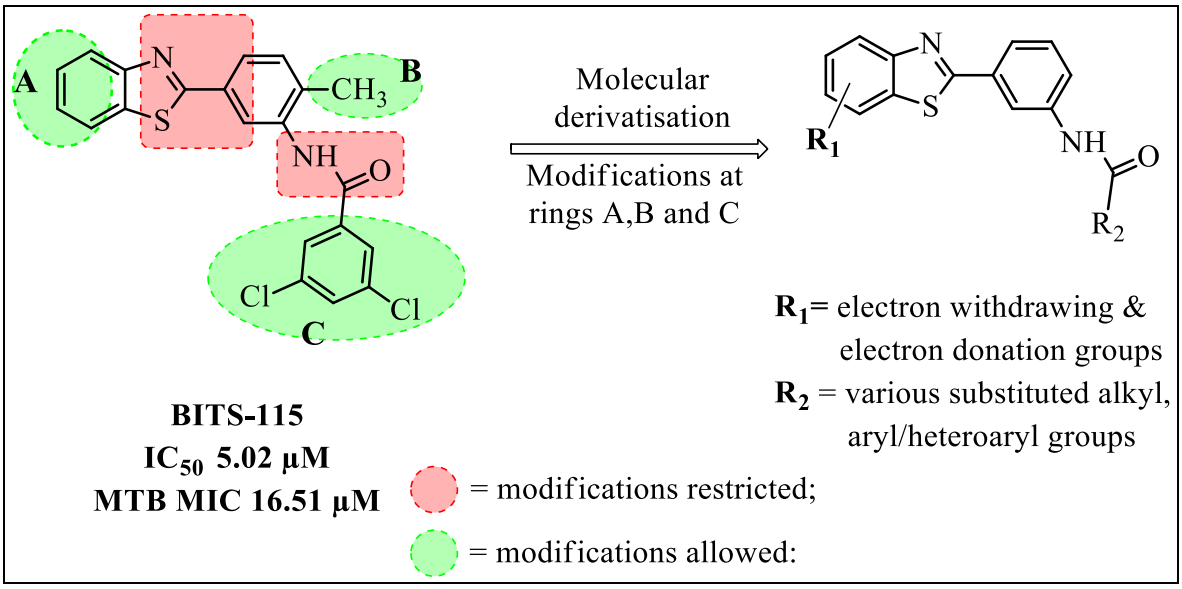
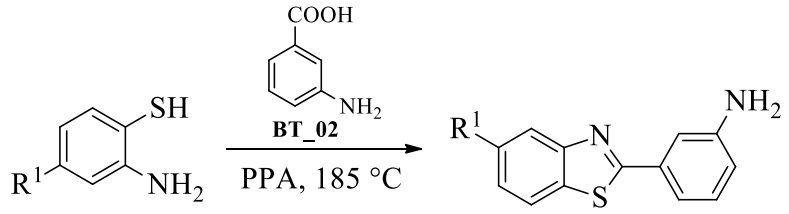


Figure 5.13: Structure of lead molecule for the generation of compounds **BT_04 – BT_36**

5.5.2. Experimental procedures utilized for the synthesis of BT_04 – BT_36

The target molecules were synthesized by following two step synthetic protocol (**Figure 4.5**), starting with commercially available, less expensive, 2-aminobenzenethiol, 2-amino-substitutedbenzenethiol **(BT_01a-c)** and 3-aminobenzoic acid **(BT_02)**. Initially the cyclisation reaction for the preparation of substituted 2-phenylbenzothiazoles **(BT_03a-c)** were carried out by coupling 3-aminobenzoic acid with **(BT_01a-c)** in the presence of polyphosphoric acid under reflux conditions, in this step of the reaction 2-amino-substitutedbenzenethiol **(BT_01a-c)** coupled with mixed anhydride of 3-aminobenzoic acid **(BT_02)** and PPA formed as *in situ* to give the cyclized product **(BT_03a-c)** (**Figure 5.14**)**.** In next step the amidation reaction was carried out by treating the **(BT_03a-c)** with various substituted aromatic/hetero aromatic/acyclic carboxylic acids in presence of coupling agents EDCI, and HOBt and few of the final molecules were prepared by using the acid chloride in presence of Et_3N and CH_2Cl_2 as solvent to get the final compounds **BT_04 - BT_36.**

General procedure for the preparation of BT_03a-c



BT_1a-c **BT_3a-c**

R^1 = H, Cl, CF_3

To the mixture of compound **BT_01a-c** (1.1 equiv) and 3-aminobenzoic acid **BT_02** (1.0 equiv) was added PPA (10 volumes) and allowed the reaction mixture to stir at 185 °C for 6 hours. The reaction mixture was cooled to room temperature and added cold 6N NaOH solution dropwise at 0 °C. The precipitate obtained was filtered and dissolved in DMF and heated to 60 °C for 15 minutes, filtered and the filtrate was poured into crushed ice. The solids obtained was filtered and washed with excess of water, cold ethanol and diethyl ether to afford the compound **3a**-c respectively.

3-(Benzo[*d*]thiazol-2-yl)aniline (BT_03a) :

To the mixture of 2-aminobenzenethiol **BT_01a** (5.0 g, 40.11 mmoles) and 3-aminobenzoic acid **BT_02** (5.0 g, 36.46 mmoles) was added PPA (35.0 g) and allowed the reaction mixture to stir at 185 °C for 6 hours. The reaction mixture was cooled to room temperature and added cold 6N NaOH solution drop wise until the solution became alkaline at 0 °C. The precipitate obtained was filtered and dissolved in DMF and heated to 60 °C for 15 minutes, filtered and the filtrate was poured into crushed ice. The solids obtained was filtered and washed with excess of water, cold ethanol and diethyl ether to afford 3-(benzo[*d*]thiazol-2-yl)aniline **BT_03a** (7.2 g, 79%) as an off- white solid. MS(ESI) *m/z* 227 $[M+H]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ_H 8.20–7.61(m, 4H), 7.20–6.52 (m, 4H), 4.01 (m, 2H).

3-(5-Chlorobenzo[*d*]thiazol-2-yl)aniline (BT_03b) :

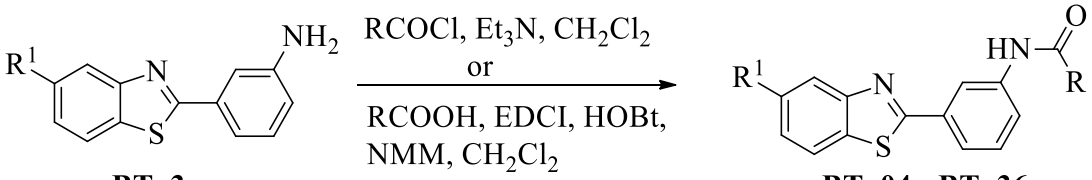
To the mixture of 2-amino-4-chlorobenzenethiol **BT_01b** (3.2 g, 20.05 mmoles) and 3-aminobenzoic acid **BT_02** (2.5 g, 18.23 mmoles) was added PPA (25.0 g) and allowed the reaction mixture to stir at 185 °C for 6 hours. The reaction mixture was cooled to room temperature and added cold 6N NaOH solution drop wise until the solution became alkaline at 0 °C. The precipitate obtained was filtered and dissolved in DMF and heated to 60 °C for 15 minutes, filtered and the filtrate was poured into crushed ice. The solids obtained was filtered and washed with excess of water, cold ethanol and diethyl ether to afford 3-(5-chlorobenzo[*d*]thiazol-2-yl)aniline **BT_03b** (4.2 g, 80%) as brown solid. MS(ESI) *m/z* 261 $[M+H]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ_H 8.22–7.64(m, 4H), 7.50–7.02 (m, 3H), 6.01 (m, 2H).

3-(5-(Trifluoromethyl)benzo[*d*]thiazol-2-yl)aniline (BT_03c) :

To the mixture of 2-amino-4-(trifluoromethyl)benzenethiol **BT_01c** (4.59 g, 20.07 mmoles) and 3-aminobenzoic acid **BT_02** (2.0 g, 18.24 mmoles) was added PPA (20.0 g) and allowed the reaction mixture to stir at 185 °C for 6 hours. The reaction mixture was cooled to room temperature and added cold 6N NaOH solution drop wise until the solution became alkaline at 0 °C. The precipitate obtained was filtered and dissolved in DMF and heated to 60 °C for 15 minutes, filtered and the filtrate was poured into crushed ice. The solids obtained was filtered and washed with excess of water, cold ethanol and diethyl ether to afford 3-(5-(trifluoromethyl)benzo[*d*]thiazol-2-yl)aniline **BT_03c** (5.4 g, 77%) as an brown solid. MS(ESI)

m/z 295 $[M+H]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ_H 8.25–7.81(m, 3H), 7.75–7.56 (m, 4H), 6.87 (m, 2H).

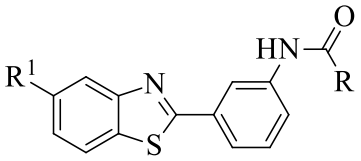
General procedure for the preparation of BT_04 - BT_36



BT_3a-c **BT_04 - BT_36**

To the stirred solution of carboxylic acid (1.0 equiv), EDCI (1.2 equiv), HOBt (1.2 equiv) and NMM (2.5 equiv) in CH_2Cl_2 at 0 °C, was added compound **BT_03a-c** (1.05 equiv) and allowed the reaction mixture to stir at room temperature for 3 h. Upon completion of the reaction the reaction mixture was diluted with CH_2Cl_2 and washed with H_2O and brine solution and the separated organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure, purified by column chromatography using EtOAc/hexanes as eluent to afford the final compounds **BT_04 - BT_36**.

Table 5.9: Physiochemical properties of the synthesized compounds **BT_04 – BT_36**



BT_04 - BT_36

Compd	R^1	R	Yield (%)	M.P. (°C)	Molecular formula	Molecular weight
BT_04	H	Phenyl[#]	87	184-185	$C_{20}H_{14}N_2OS$	330.40
BT_05	H	4-Tolyl[#]	81	215-216	$C_{21}H_{16}N_2OS$	344.43
BT_06	H	2-Methoxyphenyl	79	220-221	$C_{21}H_{16}N_2O_2S$	360.43
BT_07	H	4-Phenyloxyphenyl	83	209-210	$C_{26}H_{18}N_2O_2S$	422.50
BT_08	H	2-Trifluoromethylphenyl	84	205-206	$C_{21}H_{13}F_3N_2OS$	398.40
BT_09	H	1-Napthyl[#]	86	244-245	$C_{24}H_{16}N_2OS$	380.46

Contd

Compd	R^1	R	Yield (%)	M.P. (°C)	Molecular formula	Molecular weight
BT_10	H	4-Pyridyl	65	271-271	$C_{19}H_{13}N_3OS$	331.39
BT_11	H	2-Furyl	69	265-266	$C_{18}H_{12}N_2O_2S$	320.37
BT_12	H	5-Nitro-2-furyl	74	251-252	$C_{18}H_{11}N_3O_4S$	365.36
BT_13	H	5-Nitro-2-thiophenyl	71	236-237	$C_{18}H_{11}N_3O_3S_2$	381.43
BT_14	H	Cyclohexyl#	84	198-199	$C_{20}H_{20}N_2OS$	336.45
BT_15	Cl	Phenyl#	82	218-219	$C_{20}H_{13}ClN_2OS$	364.85
BT_16	Cl	4-Tolyl#	78	224-225	$C_{21}H_{15}ClN_2OS$	378.87
BT_17	Cl	2-Methoxyphenyl	73	235-236	$C_{21}H_{15}ClN_2O_2S$	394.87
BT_18	Cl	4-Phenyloxyphenyl	81	241-242	$C_{26}H_{17}ClN_2O_2S$	456.94
BT_19	Cl	2-Trifluoromethylphenyl	78	219-220	$C_{21}H_{12}ClF_3N_2OS$	432.85
BT_20	Cl	1-Napthyl#	76	230-231	$C_{24}H_{15}ClN_2OS$	414.91
BT_21	Cl	4-Pyridyl	67	246-247	$C_{19}H_{12}ClN_3OS$	365.84
BT_22	Cl	2-Furyl	72	218-219	$C_{18}H_{11}ClN_2O_2S$	354.81
BT_23	Cl	2-Thiophenyl	73	189-190	$C_{18}H_{11}ClN_2OS_2$	370.88
BT_24	Cl	5-Nitro-2-furyl	68	249-250	$C_{18}H_{10}ClN_3O_4S$	399.81
BT_25	Cl	5-Nitro-2-thiophenyl	72	231-232	$C_{18}H_{10}ClN_3O_3S_2$	415.87
BT_26	Cl	Cyclohexyl#	75	176-177	$C_{20}H_{19}ClN_2OS$	370.90
BT_27	CF_3	Phenyl #	71	218-219	$C_{21}H_{13}F_3N_2OS$	398.40
BT_28	CF_3	4-Tolyl#	68	244-245	$C_{22}H_{15}F_3N_2OS$	412.43
BT_29	CF_3	2-Trifluoromethylphenyl	64	252-253	$C_{22}H_{12}F_6N_2OS$	466.40
BT_30	CF_3	1-Napthyl#	67	192-193	$C_{25}H_{15}F_3N_2OS$	448.46
BT_31	CF_3	4-Pyridyl	62	280-281	$C_{20}H_{12}F_3N_3OS$	399.39
BT_32	CF_3	2-Furyl	67	245-246	$C_{19}H_{11}F_3N_2O_2S$	388.36
BT_33	CF_3	2-Thiophenyl	64	237-238	$C_{19}H_{11}F_3N_2OS_2$	404.43
BT_34	CF_3	5-Nitro-2-furyl	63	271-272	$C_{19}H_{10}F_3N_3O_4S$	433.36
BT_35	CF_3	5-Nitro-2-thiophenyl	66	262-263	$C_{19}H_{10}F_3N_3O_3S_2$	449.43
BT_36	CF_3	Cyclohexyl#	72	214-215	$C_{21}H_{19}F_3N_2OS$	404.45

Amidation was carried out by using acidchloride.

5.5.3. Characterization of the synthesized molecules

***N*-(3-(Benzo[*d*]thiazol-2-yl)phenyl)benzamide (BT_04) :**

Yield: 87%; m.p. 184–185 °C; MS(ESI) *m/z* 331 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.19 (s, 1H), 7.88 (s,1H), 7.82 (d, *J* = 8.2 Hz, 2H), 7.80–7.69 (m, 6H), 7.63–7.54 (m, 4H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 171.1, 169.1, 152.2, 140.6, 134.5, 132.7, 131.8, 130.1, 128.6, 126.3, 124.5(2C), 122.0 121.3(2C), 119.5, 118.5, 117.0, 168.9, 110.5. Anal. calcd for $C_{20}H_{14}N_2OS$: C, 72.70; H, 4.27; N, 8.48% Found C, 72.77; H, 4.34; N, 8.54%.

***N*-(3-(Benzo[*d*]thiazol-2-yl)phenyl)-4-methylbenzamide (BT_05) :**

Yield: 81%; m.p. 215–216 °C; MS(ESI) *m/z* 345 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.39 (s, 1H), 7.84 (s, 1H), 7.78 (d, *J* = 7.0 Hz, 1H), 7.69 (d, *J* = 8.0 Hz, 1H), 7.58–7.50 (m, 3H), 7.45–7.38 (m, 3H), 7.28 (d, *J* = 7.8 Hz, 1H), 7.22–7.19 (m, 2H) 2.42 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.4, 165.7, 150.4, 142.5, 133.7, 132.3, 131.6, 130.1, 129.4, 126.4, 125.4(2C), 124.3, 123.2(2C), 122.5, 122.0, 121.7, 121.0, 114.0, 22.1. Anal. calcd for $C_{21}H_{16}N_2OS$: C, 73.23; H, 4.68; N, 8.13 % Found C, 73.31; H, 4.72; N, 8.18 %.

***N*-(3-(Benzo[*d*]thiazol-2-yl)phenyl)-2-methoxybenzamide (BT_06) :**

Yield: 79%; m.p. 220–221 °C; MS(ESI) *m/z* 361 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.47 (s, 1H), 7.78 (s, 1H), 7.74 (d, *J* =7.0 Hz, 1H), 7.70 (d, *J* = 8.0 Hz, 1H), 7.68–7.63 (m, 4H), 7.55–7.44 (m, 4H), 7.38 (d, *J* = 8.0 Hz, 1H), 3.91 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.8, 162.4, 150.4, 147.2, 134.6, 130.9, 130.1, 129.8, 129.4, 128.2, 127.0(2C), 126.2, 124.9(2C), 124.1, 123.6, 123.0, 121.0, 111.6, 61.0. Anal. calcd for $C_{21}H_{16}N_2O_2S$: C, 69.98; H, 4.47; N, 7.77 % Found C, 70.16; H, 4.54; N, 7.81 %.

***N*-(3-(Benzo[*d*]thiazol-2-yl)phenyl)-4-phenoxybenzamide (BT_07) :**

Yield: 83%; m.p. 209–210 °C; MS(ESI) *m/z* 423 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.48 (s, 1H), 8.40 (s,1H), 7.81 (s,1H), 7.80–7.73 (m, 4H), 7.68 (d, *J* = 8.4 Hz, 2H), 7.62–7.58 (m, 4H), 7.56–7.52 (m, 5H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.1, 168.4, 153.0, 143.5, 141.9, 137.0, 134.3, 133.5, 133.0, 131.2(2C), 130.5, 129.1, 128.3, 127.2(2C), 126.4, 126.1,

125.1(2C), 124.2(2C), 123.2, 122.2, 121.4, 109.1.Anal. calcd for $C_{26}H_{18}N_2O_2S$: C, 73.91; H, 4.29; N, 6.63 % Found C, 73.98; H, 4.36; N, 6.72 %.

***N*-(3-(Benzo[*d*]thiazol-2-yl)phenyl)-2-(trifluoromethyl)benzamide (BT_08) :**

Yield: 84%; m.p. 205–206 °C; MS(ESI) *m/z* 399 $[M+H]^+$. ^1H NMR (300 MHz, DMSO-d_6) δ 10.51 (s, 1H), 7.85 (s, 1H), 7.63–7.58 (m, 3H), 7.50–7.45 (m, 4H), 7.42–7.34 (m, 4H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 166.2, 162.1, 143.1, 140.1, 136.4, 135.5, 134.6, 133.1, 131.5, 130.8, 130.1, 128.5, 128.0 127.2, 126.6, 125.2, 124.1, 123.6, 123.3, 121.3, 117.6. Anal. calcd for $C_{21}H_{13}F_3N_2OS$: C, 63.31; H, 3.29; N, 7.03 % Found C, 63.38; H, 3.36; N, 7.09 %.

***N*-(3-(Benzo[*d*]thiazol-2-yl)phenyl)-1-naphthamide (BT_09) :**

Yield: 86%; m.p. 244–245 °C; MS(ESI) *m/z* 381 $[M+H]^+$. ^1H NMR (300 MHz, DMSO-d_6): δ 9.92 (s, 1H), 8.76 (d, *J* = 8.8 Hz, 1H), 8.16–7.72 (m, 6H), 7.65–7.55 (m, 3H), 7.50–7.38 (m, 5H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 169.0, 163.4, 146.5, 138.5, 137.7, 136.8, 135.7, 135.0, 135.6, 135.1, 134.1, 132.6, 132.2, 131.1, 129.2, 128.2, 127.3, 126.1, 125.4, 125.0, 124.5, 124.2, 123.2, 114.1. Anal. calcd for $C_{24}H_{16}N_2OS$: C, 75.77; H, 4.24; N, 7.36 % Found C, 75.83; H, 4.31; N, 7.45 %.

***N*-(3-(Benzo[*d*]thiazol-2-yl)phenyl)isonicotinamide (BT_10) :**

Yield: 65%; m.p. 271–272 °C; MS(ESI) *m/z* 332 $[M+H]^+$. ^1H NMR (300 MHz, $CDCl_3$): δ 10.60 (s, 1H), 8.18 (s,1H), 8.00 (d, *J* = 8.4 Hz, 2H), 7.90-7.82 (m, 4H), 7.76-7.68 (m, 3H), 7.61 (d, J = 9.0 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.6, 162.3, 141.8, 141.2, 138.0, 136.1, 134.0, 133.4, 132.1(2C), 131.4, 128.1, 127.3, 126.5, 125.0(2C), 121.4, 120.1, 114.2. Anal. calcd for $C_{19}H_{13}N_3OS$: C, 68.86; H, 3.95; N, 12.68 % Found C, 68.92; H, 4.03; N, 12.72 %.

***N*-(3-(Benzo[*d*]thiazol-2-yl)phenyl)furan-2-carboxamide (BT_11) :**

Yield: 69%; m.p. 265–266 °C; MS(ESI) *m/z* 321 $[M+H]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 9.94 (s, 1H), 8.11 (s, 1H), 7.95 (d, *J* = 8.2 Hz, 1H), 7.88–7.82 (m, 2H), 7.80–7.69 (m, 4H), 7.60–7.55 (m, 2H), 7.24 (t, *J* = 7.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.6, 154.4, 146.1, 136.4, 135.2, 133.7, 133.1, 132.4, 132.0, 130.4, 127.6, 126.5, 124.4, 124.0, 123.6, 122.4, 121.3, 112.5. Anal. calcd for $C_{18}H_{12}N_2O_2S$: C, 67.48; H, 3.78; N, 8.74 % Found C, 67.54; H, 3.83; N, 8.79 %.

***N*-(3-(Benzo[*d*]thiazol-2-yl)phenyl)-5-nitrofuran-2-carboxamide (BT_12) :**

Yield: 74%; m.p. 251–252 °C; MS(ESI) *m/z* 366 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.93 (s, 1H), 8.40 (s,1H), 7.58 (s,1H), 7.55 (d, *J* = 8.0 Hz, 1H), 7.52–7.46 (m, 4H), 7.43–7.33 (m, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 171.8, 166.1, 154.8, 147.7, 139.4, 135.7, 134.5, 132.4, 131.7, 131.2, 130.6, 128.2, 127.7, 126.5, 126.1, 125.2, 124.1, 115.3. Anal. calcd for $C_{18}H_{11}N_3O_4S$: C, 59.17; H, 3.03; N, 11.50 % Found C, 59.25; H, 3.09; N, 11.58 %.

***N*-(3-(Benzo[*d*]thiazol-2-yl)phenyl)-5-nitrothiophene-2-carboxamide (BT_13) :**

Yield: 71%; m.p. 236–237 °C; MS(ESI) *m/z* 382 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.34 (s, 1H), 7.92 (s, 1H), 7.62–7.56 (m, 4H), 7.47–7.36 (m, 5H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.4, 164.3, 160.3, 143.9, 139.7, 137.5, 136.3, 136.0, 135.1, 133.5, 133.0, 130.8, 129.1, 128.2, 127.1, 125.9, 123.1, 115.2. Anal. calcd for $C_{18}H_{11}N_3O_3S_2$: C, 56.68; H, 2.91; N, 11.02 % Found C, 56.72; H, 2.97; N, 11.09%.

***N*-(3-(Benzo[*d*]thiazol-2-yl)phenyl)cyclohexanecarboxamide (BT_14) :**

Yield: 84%; m.p. 198–199 °C; MS(ESI) *m/z* 337 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.94 (s, 1H), 7.83 (s, 1H), 7.80–7.68 (m, 4H), 7.65–7.54 (m, 3H), 2.45–2.37 (m, 1H), 1.92–1.65 (m, 5H), 1.50–1.14 (m, 5H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 175.6, 164.7, 154.6, 140.1, 137.8, 137.1, 135.2, 132.6, 131.4, 130.5, 127.2, 125.1, 124.1, 115.1, 44.1, 31.1(2C), 27.1, 26.0(2C). Anal. calcd for $C_{20}H_{20}N_2OS$: C, 71.40; H, 5.99; N, 8.33 % Found C, 71.45; H, 6.09; N, 8.45 %.

***N*-(3-(5-Chlorobenzo[*d*]thiazol-2-yl)phenyl)benzamide (BT_15) :**

Yield: 82%; m.p. 218–219 °C; MS(ESI) *m/z* 365 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.73 (s, 1H), 8.60 (s,1H), 7.65 (s,1H), 7.63–7.54 (m, 4H), 7.48–7.35 (m, 6H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 168.5, 166.2, 148.3, 139.9, 138.3, 137.0, 136.9, 134.3, 133.4(2C), 133.1, 131.4, 129.2(2C), 127.4, 127.0, 126.4, 125.4, 124.5, 114.7. Anal. calcd for $C_{20}H_{13}ClN_2OS$: C, 65.84; H, 3.59; N, 7.68 % Found C, 65.93; H, 3.64; N, 7.72 %.

***N*-(3-(5-Chlorobenzo[*d*]thiazol-2-yl)phenyl)-4-methylbenzamide (BT_16) :**

Yield: 78%; m.p. 224–225 °C; MS(ESI) *m/z* 379 $[M+H]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 10.78 (s, 1H), 8.45 (s,1H), 7.72 (s,1H), 7.70–7.63 (m, 4H), 7.58–7.54 (m, 2H), 7.50–7.46 (m, 3H) 2.46 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.8, 165.1, 149.1, 140.0, 137.3, 135.8, 135.1, 134.2, 133.6(2C), 132.8, 131.4, 130.6, 128.2(2C), 127.6, 126.2, 125.6, 123.8, 115.1, 22.1.Anal. calcd for $C_{21}H_{15}ClN_2OS$: C, 66.57; H, 3.99; N, 7.39 % Found C, 66.63; H, 4.05; N, 7.45 %.

***N*-(3-(5-Chlorobenzo[*d*]thiazol-2-yl)phenyl)-2-methoxybenzamide (BT_17) :**

Yield: 73%; m.p. 235–236 °C; MS(ESI) *m/z* 395 $[M+H]^+$. ^1H NMR (400 MHz, DMSO-d_6): δ 11.01 (s, 1H), 8.55 (s,1H), 7.80 (s,1H), 7.78–7.69 (m, 5H), 7.62 (d, *J* = 7.0 Hz, 1H), 7.58–7.53 (m, 3H), 3.94 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.5, 165.4, 148.8, 139.1, 136.6, 135.8, 135.3, 134.2, 133.1, 132.4, 131.6, 131.1, 130.2, 129.1, 128.0, 127.4, 126.5, 126.0, 125.1, 118.2, 63.1. Anal. calcd for $C_{21}H_{15}ClN_2O_2S$: C, 63.87; H, 3.83; N, 7.09 % Found C, 63.94; H, 3.94; N, 7.18 %.

***N*-(3-(5-Chlorobenzo[*d*]thiazol-2-yl)phenyl)-4-phenoxybenzamide (BT_18) :**

Yield: 81%; m.p. 241–242 °C; MS(ESI) *m/z* 456 $[M+H]^+$. ^1H NMR (300 MHz, $CDCl_3$): δ 10.53 (s, 1H), 8.47 (s,1H), 7.86 (s,1H), 7.84–7.76 (m, 3H), 7.73 (d, *J* = 8.6 Hz, 2H), 7.71–7.64 (m, 4H), 7.57–7.42 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.9, 160.6, 153.4, 144.4, 138.1, 137.6, 137.3, 136.1, 134.5, 133.8, 133.1, 131.9(2C), 131.4, 130.3(2C), 128.1, 127.4, 126.5, 126.1, 125.8(2C), 125.1, 124.4(2C), 115.3. Anal. calcd for $C_{26}H_{17}ClN_2O_2S$: C, 68.34; H, 3.75; N, 6.13 % Found C, 68.43; H, 3.81; N, 6.18 %.

***N*-(3-(5-Chlorobenzo[*d*]thiazol-2-yl)phenyl)-2-(trifluoromethyl)benzamide (BT_19) :**

Yield: 78%; m.p. 219–220 °C; MS(ESI) *m/z* 433 $[M+H]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 10.58 (s, 1H), 8.15 (s,1H), 7.67 (s,1H), 7.64 (d, *J* = 7.4 Hz, 1H), 7.60–7.48 (m, 4H), 7.45–7.33 (m, 4H); ^{13}C NMR (100 MHz, CDl_3) δ 166.5, 162.8, 148.4, 145.7, 136.5, 136.1, 134.7, 134.4, 134.0, 133.6, 133.1, 132.4, 132.0, 131.5, 130.1, 128.6, 128.1, 126.8, 126.3, 125.1, 115.8. Anal. calcd for $C_{21}H_{12}ClF_3N_2OS$: C, 58.27; H, 2.79; N, 6.47% Found C, 58.36; H, 2.86; N, 6.54%.

***N*-(3-(5-Chlorobenzo[*d*]thiazol-2-yl)phenyl)-1-naphthamide (BT_20) :**

Yield: 76%; m.p. 230–231 °C; MS(ESI) *m/z* 415 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.56 (s, 1H), 8.60 (d, *J* = 8.0 Hz, 1H), 7.89 (s,1H), 7.84–7.76 (m, 4H), 7.70–7.56 (m, 6H), 7.51–7.45 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 169.1, 165.5, 147.4, 141.8, 137.6, 137.1, 136.2, 135.5, 134.7, 133.9, 133.2, 132.7, 132.1, 131.1, 130.6, 130.1, 129.1, 128.4, 128.0, 127.5, 126.2, 125.0, 124.1, 114.7. Anal. calcd for $C_{24}H_{15}ClN_2OS$: C, 69.48; H, 3.64; N, 6.75 % Found C, 69.54; H, 3.72; N, 6.81 %.

_N_-(3-(5-Chlorobenzo[_d_]thiazol-2-yl)phenyl)isonicotinamide (BT_21) :

Yield: 67%; m.p. 246–247 °C; MS(ESI) *m/z* 366 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.24 (s, 1H), 8.60 (s,1H), 7.78 (s,1H), 7.75–7.71 (m, 2H), 7.65 (d, *J* = 8.2 Hz, 2H), 7.62–7.56 (m, 3H), 7.52–7.44 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.9, 164.6, 147.4, 138.1, 135.9(2C), 134.8, 134.2, 133.7, 133.2, 132.6, 131.7, 128.6, 127.6(2C), 127.1, 126.0, 124.1, 114.7. Anal. calcd for $C_{19}H_{12}ClN_3OS$: C, 62.38; H, 3.31; N, 11.49 % Found C, 62.45; H, 3.37; N, 11.57 %.

_N_-(3-(5-Chlorobenzo[_d_]thiazol-2-yl)phenyl)furan-2-carboxamide (BT_22) :

Yield: 72%; m.p. 218–219 °C; MS(ESI) *m/z* 355 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.75 (s, 1H), 8.58 (s,1H), 7.83 (s,1H), 7.76–7.71 (m, 2H), 7.61 (d, *J* = 8.0 Hz, 1H), 7.55–7.45 (m, 3H), 7.42–7.37 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.3, 157.4, 144.2, 137.4, 135.2, 133.8, 133.2, 132.8, 132.1, 130.4, 130.0, 129.6, 128.5, 127.4, 125.8, 124.1, 121.4, 115.6. Anal. calcd for $C_{18}H_{11}ClN_2O_2S$: C, 60.93; H, 3.12; N, 7.90 % Found C, 60.99; H, 3.18; N, 7.97 %.

_N_-(3-(5-Chlorobenzo[_d_]thiazol-2-yl)phenyl)thiophene-2-carboxamide (BT_23) :

Yield: 73%; m.p. 189–190 °C; MS(ESI) *m/z* 371 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.71 (s, 1H), 8.55 (s,1H), 7.81 (s,1H), 7.74–7.70 (m, 2H), 7.60 (d, *J* = 8.0 Hz, 1H), 7.52–7.45 (m, 3H), 7.41–7.35 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 165.8, 157.8, 145.2, 138.1, 135.4, 134.2, 133.6, 132.9, 132.3, 130.8, 130.1, 129.8, 128.6, 127.1, 124.8, 123.9, 121.8, 115.9. Anal. calcd for $C_{18}H_{11}ClN_2OS_2$: C, 58.29; H, 2.99; N, 7.55% Found C, 58.36; H, 3.07; N, 7.63%.

_N_-(3-(5-Chlorobenzo[_d_]thiazol-2-yl)phenyl)-5-nitrofuran-2-carboxamide (BT_24) :

Yield: 68%; m.p. 249–250 °C; MS(ESI) *m/z* 400 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.98 (s, 1H), 8.51 (s,1H), 7.74 (s,1H), 7.72–7.65 (m, 2H), 7.60–7.54 (m, 3H), 7.50–7.43 (m,

2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 170.1, 165.1, 154.4, 146.1, 140.1, 137.2, 136.2, 134.9, 134.1, 133.5, 130.1, 127.8, 127.1, 126.3, 125.2, 124.2, 123.6, 116.8. Anal. calcd for $C_{18}H_{10}ClN_3O_4S$: C, 54.07; H, 2.52; N, 10.51 % Found C, 54.16; H, 2.59; N, 10.63 %.

***N*-(3-(5-Chlorobenzo[*d*]thiazol-2-yl)phenyl)-5-nitrothiophene-2-carboxamide (BT_25) :** Yield: 72%; m.p. 231–232 °C; MS(ESI) *m/z* 416 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.00 (s, 1H), 8.52 (s,1H), 7.73 (s,1H), 7.73–7.64 (m, 2H), 7.62–7.55 (m, 3H), 7.51–7.44 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.8, 165.2, 153.8, 145.9, 140.2, 137.1, 136.1, 134.7, 133.9, 133.4, 130.3, 127.7, 127.2, 126.4, 125.0, 123.5, 123.1, 116.9. Anal. calcd for $C_{18}H_{10}ClN_3O_3S_2$: C, 51.99; H, 2.42; N, 10.10% Found C, 52.07; H, 2.48; N, 10.18%.

***N*-(3-(5-Chlorobenzo[*d*]thiazol-2-yl)phenyl)cyclohexanecarboxamide (BT_26) :**

Yield: 75%; m.p. 176–177 °C; MS(ESI) 392 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.22 (s, 1H), 8.66 (s,1H), 7.92 (s,1H), 7.83–7.71 (m, 3H), 7.54–7.47 (m, 2H), 2.45–2.37 (m, 1H), 1.94–1.67 (m, 5H), 1.52–1.13 (m, 5H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 172.1, 162.8, 152.4, 148.1, 141.2, 137.1, 133.8, 133.2, 129.1, 127.5, 126.2, 125.0, 124.2, 115.1, 48.3, 31.5(2C), 27.4(2C), 26.7. Anal. calcd for $C_{20}H_{19}ClN_2OS$: C, 64.77; H, 5.16; N, 7.55 % Found C, 64.81; H, 5.27; N, 7.63 %.

***N*-(3-(5-(Trifluoromethyl)benzo[*d*]thiazol-2-yl)phenyl)benzamide (BT_27) :**

Yield: 71%; m.p. 218–219 °C; MS(ESI) *m/z* 399 $[M+H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.18 (s, 1H), 8.64 (s,1H), 7.92 (s,1H), 7.81–7.72 (m, 2H), 7.54–7.45 (m, 2H), 2.45–2.36 (m, 1H), 1.52–1.13 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.8, 163.8, 154.7, 143.7, 139.6, 136.8, 137.8, 136.5, 133.4(2C), 130.1, 129.4(2C), 128.6, 128.1, 127.0, 126.1, 125.2, 124.6, 123.0, 115.1. Anal. calcd for $C_{21}H_{13}F_3N_2OS$: C, 63.31; H, 3.29; N, 7.03 % Found C, 63.43; H, 3.36; N, 7.09 %.

4-Methyl-*N*-(3-(5-(trifluoromethyl)benzo[*d*]thiazol-2-yl)phenyl)benzamide (BT_28) :

Yield: 68%; m.p. 244–245 °C; MS(ESI) *m/z* 413 $[M+H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.85 (s, 1H), 8.65 (s,1H), 8.35 (s,1H), 7.95–7.86 (m, 3H), 7.79–7.74 (m, 2H), 7.65 (d, J = 7.8 Hz, 2H), 7.60–7.54 (m, 2H), 2.48 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.6, 161.5, 144.5, 143.7, 137.3,

136.5, 135.3, 134.5, 133.2, 132.3, 131.4, 130.1(2C), 129.1(2C), 127.8, 126.1(2C), 124.2, 122.5, 114.5, 22.5. Anal. calcd for $C_{22}H_{15}F_3N_2OS$: C, 64.07; H, 3.67; N, 6.79 % Found C, 64.16; H, 3.74; N, 6.87 %.

2-(Trifluoromethyl)-*N*-(3-(5-(trifluoromethyl)benzo[*d*]thiazol-2-yl)phenyl)benzamide (BT_28) :

Yield: 64%; m.p. 252–253 °C; MS(ESI) *m/z* 467 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.59 (s, 1H), 8.72 (s,1H), 8.46 (s,1H), 8.16–7.89 (m, 4H), 7.81–7.69 (m, 5H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.9, 160.5, 152.3, 142.4, 139.3, 138.5, 137.8, 136.2, 135.8, 135.4, 134.9, 134.3, 133.9, 132.4, 130.5, 129.3, 128.2, 127.8, 126.4, 125.6, 125.1, 124.0. Anal. calcd for $C_{22}H_{12}F_6N_2OS$: C, 56.65; H, 2.59; N, 6.01 % Found C, 56.72; H, 2.66; N, 6.09 %.

***N*-(3-(5-(Trifluoromethyl)benzo[*d*]thiazol-2-yl)phenyl)-1-naphthamide (BT_30) :**

Yield: 67%; m.p. 192–193 °C; MS(ESI) *m/z* 449 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.97 (s, 1H), 8.60 (s,1H), 7.94 (s,1H), 7.87 (d, *J* = 8.4 Hz, 1H), 7.72–7.66 (m, 3H), 7.62–7.48 (m, 6H), 7.40–7.32 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) 169.7, 166.4, 149.6, 143.5, 137.6, 136.7, 136.0, 135.6, 134.7, 133.6, 133.0, 132.6, 132.1, 131.5, 130.4, 129.9(2C), 129.0, 128.7, 128.1, 127.5, 127.1, 125.9, 125.7, 115.1. Anal. calcd for $C_{25}H_{15}F_3N_2OS$: C, 66.96; H, 3.37; N, 6.25 % Found C, 67.07; H, 3.44; N, 6.36 %.

***N*-(3-(5-(Trifluoromethyl)benzo[*d*]thiazol-2-yl)phenyl)isonicotinamide (BT_31) :**

Yield: 62%; m.p. 280–281 °C; MS(ESI) *m/z* 400 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.15 (s, 1H), 8.62 (s,1H), 7.84 (s,1H), 7.80–7.70 (m, 2H), 7.68 (d, *J* = 8.0 Hz, 2H), 7.64–7.60 (m, 3H), 7.54–7.48 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.0, 164.8, 149.1, 138.5, 137.0, 136.3(2C), 136.9, 135.1, 133.7, 133.1, 132.2, 131.5, 129.2(2C), 128.6(2C), 127.5, 124.6, 115.4. Anal. calcd for $C_{20}H_{12}F_3N_3OS$: C, 60.15; H, 3.03; N, 10.52 % Found C, 60.27; H, 3.18; N, 10.63 %.

***N*-(3-(5-(Trifluoromethyl)benzo[*d*]thiazol-2-yl)phenyl)furan-2-carboxamide (BT_32) :** Yield: 67%; m.p. 245–246 °C; MS(ESI) *m/z* 389 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.54 (s, 1H), 8.60 (s,1H), 7.91 (s,1H), 7.82–7.69 (m, 3H), 7.62–7.56 (m, 3H), 7.45–7.35 (m,

2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.1, 163.5, 147.3, 137.6, 135.4, 134.3, 133.9, 133.0, 132.0, 131.4, 130.1, 129.1(2C), 128.1, 127.3, 125.8, 125.0, 123.2, 115.1. Anal. calcd for $C_{19}H_{11}F_3N_2O_2S$: C, 58.76; H, 2.85; N, 7.21 % Found C, 58.76; H, 2.85; N, 7.21 %

***N*-(3-(5-(Trifluoromethyl)benzo[*d*]thiazol-2-yl)phenyl)thiophene-2-carboxamide (BT_33) :** Yield: 64%; m.p. 237–238 °C; MS(ESI) *m/z* 405 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.51 (s, 1H), 8.60 (s,1H), 7.91 (s,1H), 7.79–7.63 (m, 4H), 7.59–7.51 (m, 2H), 7.41–7.33 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 165.6, 161.6, 145.6, 135.9, 135.3, 134.6, 133.4, 132.1, 131.5, 130.4, 130.0, 128.9(2C), 127.7, 127.0, 124.9, 124.1, 123.8, 114.3. Anal. calcd for $C_{19}H_{11}F_3N_2OS_2$: C, 56.43; H, 2.74; N, 6.93 % Found C, 56.52; H, 2.83; N, 6.99%.

5-Nitro-*N*-(3-(5-(trifluoromethyl)benzo[*d*]thiazol-2-yl)phenyl)furan-2-carboxamide (BT_34) :

Yield: 63%; m.p. 271–272 °C; MS(ESI) *m/z* 434 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.91 (s, 1H), 8.70 (s,1H), 8.69 (s,1H), 8.39 (d, *J* = 8.4 Hz, 1H), 7.82–7.64 (m, 2H), 7.60–7.55 (m, 2H), 7.47–7.35 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 169.3, 164.3, 148.3, 144.2, 138.6, 138.1, 136.3, 135.5, 134.9, 133.5, 131.6, 129.8, 128.7, 127.4, 126.1, 125.6, 124.6, 123.1, 115.7. Anal. calcd for $C_{19}H_{10}F_3N_3O_4S$: C, 52.66; H, 2.33; N, 9.70 % Found C, 52.75; H, 2.43; N, 9.79 %.

5-Nitro-*N*-(3-(5-(trifluoromethyl)benzo[*d*]thiazol-2-yl)phenyl)thiophene-2-carboxamide (BT_35) :

Yield: 66%; m.p. 262–263 °C; MS(ESI) *m/z* 450 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.01 (s, 1H), 8.54 (s,1H), 7.95 (s,1H), 7.85–7.72 (m, 2H), 7.66–7.56 (m, 3H), 7.51–7.44 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.2, 161.9, 155.3, 148.6, 137.4, 136.5, 135.3, 134.5, 132.6, 132.1, 131.6, 129.8, 128.6. 128.0, 127.1, 126.5, 125.1, 124.1, 115.3. Anal. calcd for $C_{19}H_{10}F_3N_3O_3S_2$: C, 50.78; H, 2.24; N, 9.35% Found C, 50.83; H, 2.36; N, 9.45%.

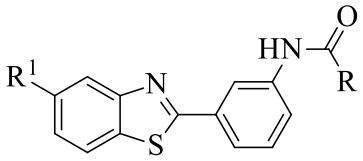
***N*-(3-(5-(Trifluoromethyl)benzo[*d*]thiazol-2-yl)phenyl)cyclohexanecarboxamide (BT_36) :** Yield: 72%; m.p. 214–215 °C; MS(ESI) *m/z* 405 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.97 (s, 1H), 8.87 (s,1H), 7.94 (s,1H), 7.87–7.76 (m, 3H), 7.60–7.53 (m, 2H), 2.46–2.38 (m, 1H), 1.94–1.73 (m, 5H), 1.53–1.13 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) 173.8, 166.5, 147.4, 139.5,

137.6, 136.7, 136.3, 133.6, 129.3(2C), 127.9, 127.3, 126.1, 125.2, 115.3, 49.4, 31.7(2C), 27.1, 26.9(2C). Anal. calcd for $C_{21}H_{19}F_3N_2OS$: C, 62.36; H, 4.74; N, 6.93 % Found C, 62.45; H, 4.81; N, 6.99 %.

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

The synthesized compounds were first screened for their *in vitro* anti-tubercular activity against *M. tuberculosis* H37Rv using MABA method. INH, EMB were used as a reference compounds for comparison. All the synthesized derivatives were also screened for their *in vitro M. tuberculosis* PknB inhibitory potency as steps towards hit optimization. Compounds showing *M. tuberculosis* MICs <30 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 synthesized compounds **BT_04** – **BT_36**



BT_04 - BT_36

Compd	R^1	R	MTB MIC[a] in µM	MTB PknB IC_{50} in µM	Cytotoxicity[b] at 50 µM % inhibition
BT_04	H	Phenyl[#]	37.87	6.99	NT
BT_05	H	4-Tolyl[#]	72.67	7.20	NT
BT_06	H	2-Methoxyphenyl	69.44	6.81	NT
BT_07	H	4-Phenyloxyphenyl	59.24	5.04	NT
BT_08	H	2-Trifluoromethylphenyl	62.81	>20	NT
BT_09	H	1-Napthyl[#]	65.78	>20	NT
BT_10	H	4-Pyridyl	75.52	>20	NT
BT_11	H	2-Furyl	39.06	16.12	NT
BT_12	H	5-Nitro-2-furyl	11.02	1.10	12.51

Contd

Compd	R^1	R	MTB MIC[a] in µM	MTB PknB IC_{50} in µM	Cytotoxicity[b] at 50 µM % inhibition
BT_13	H	5-Nitro-2-thiophenyl	16.40	1.18	38.27
BT_14	H	Cyclohexyl[#]	74.40	>20	NT
BT_15	Cl	Phenyl[#]	68.68	8.11	NT
BT_16	Cl	4-Tolyl[#]	>66.13	6.21	NT
B _17	Cl	2-Methoxyphenyl	63.45	4.33	NT
BT_18	Cl	4-Phenyloxyphenyl	54.82	3.81	NT
BT_19	Cl	2-Trifluoromethylphenyl	68.49	>20	NT
BT_20	Cl	1-Napthyl[#]	30.19	5.20	NT
BT_21	Cl	4-Pyridyl	34.24	12.07	NT
BT_22	Cl	2-Furyl	70.62	2.71	NT
BT_23	Cl	2-Thiophenyl	16.89	9.10	25.64
BT_24	Cl	5-Nitro-2-furyl	>62.65	3.04	NT
BT_25	Cl	5-Nitro-2-thiophenyl	60.38	3.10	NT
BT_26	Cl	Cyclohexyl[#]	67.56	>20	NT
BT_27	CF_3	Phenyl [#]	>62.81	20	NT
BT_28	CF_3	4-Tolyl[#]	60.67	11.08	NT
BT_29	CF_3	2-Trifluoromethylphenyl	26.82	20	36.21
BT_30	CF_3	1-Napthyl[#]	55.80	4.97	NT
BT_31	CF_3	4-Pyridyl	15.66	>20	28.91
BT_32	CF_3	2-Furyl	64.43	8.19	NT
BT_33	CF_3	2-Thiophenyl	30.94	15.14	NT
BT_34	CF_3	5-Nitro-2-furyl	28.86	2.92	25.48
BT_35	CF_3	5-Nitro-2-thiophenyl	55.67	6.54	NT
BT_36	CF_3	Cyclohexyl[#]	15.47	>20	19.37
Isoniazid			0.72	NT	NT
Ethambutol			7.64	NT	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

The synthesized compounds showed activity against *M. tuberculosis* with MIC ranging from 11.02 to 74.40 µM. Out of thirty three derivatives, five molecules inhibited (**BT_12 – BT_13, BT_23, BT_31** and **BT_36**) *M. tuberculosis* with MIC of <25 µM and only one molecule (**BT_12**) inhibited with MIC of <15 µM. When compared to standard first line drugs INH and EMB none of the synthesized molecules were more active, but when compared to lead molecule **BITS-115** (MIC 16.15 µM) three molecules (**BT_12, BT_31** and **BT_36**) were found to be more potent.

A good number of synthesized molecules showed better inhibitory activity against *M. tuberculosis* PknB enzyme, there were nineteen molecules out of thirty three inhibited *M. tuberculosis* PknB enzyme with IC_{50} less than 10 µM. And there were nine molecules (**BT_12 – BT_13**, **BT_17 – BT_18**, **BT_22**, **BT_24 – BT_25**, **BT_30** and **BT_34**) inhibited *M. tuberculosis* PknB with IC_{50} less than 5 µM. Compounds **BT_12** and **BT_13** were excellently inhibited the activity of *M. tuberculosis* PknB with IC_{50} of 1.10 µM and 1.03 µM respectively. Compound **BT_12** also inhibited *M. tuberculosis* with MIC of 11.02 µM and emerged as the most potent molecule. Compound **BT_12** was five times more active than the lead molecule against *M. tuberculosis* PknB enzyme.

With respect to SAR, overall the order of activity was 5-chloro substituted derivatives (**BT_15** – **BT_26**) showed better activity followed by no substitution at 5^{th} position of benzothiazole ring (**BT_04** – **BT_14**) and 5-trifluoromethyl substituted derivatives (**BT_27** – **BT_36**). Among the 5-chloro substituted derivatives, nine compounds were found to be possessing *M. tuberculosis* PknB IC_{50} less than 10 µM and five compounds (**BT_17**, **BT_18**, **BT_22**, **BT_24** and **BT_25**) were inhibited *M. tuberculosis* PknB IC_{50} less than 5 µM.

With respect to derivatization on the right hand side of the molecules, the amides generated from the heterocyclic carboxylic acids (**BT_12**, **BT_13**, **BT_22**, **BT_24**, **BT_25**, **BT_34** and **BT _35**) were excellently inhibited the action of *M. tuberculosis* PknB than the amides generated from substituted aryl carboxylic acids. The presence of bulky napthyl group favored the activity in molecules having 5-chloro and 5-trifluoromethyl substitution (**BT _20** and **BT _30**), where as in case of unsubstituted benzothiazole (**BT_09**), bulky napthyl group did favor activity.

5.5.6. Highlights of the study

In the present work we designed novel 3-(benzo[d]thiazol-2-yl)aniline derivatives by molecular derivatization approach using our in-house database anti-tubercular compound (**BITS-115**) and synthesized thirty three analogues. Many of the compounds showed better *M. tuberculosis* PknB inhibition and few showed *M. tuberculosis* MICs. In conclusion, it has been demonstrated that the potency of these compounds against *M. tuberculosis* PknB make them valid leads for further development. Overall, compound **BT_12** (N-(3-(benzo[*d*]thiazol-2-yl)phenyl)-5-nitrofuran-2-carboxamide) was found to be the most active compound (**Figure 5.14**) with IC_{50} of 1.10 µM against *M. tuberculosis* PknB, inhibited drug sensitive *M. tuberculosis* with MIC of 11.02 µM and was non-cytotoxic at 50 µM.

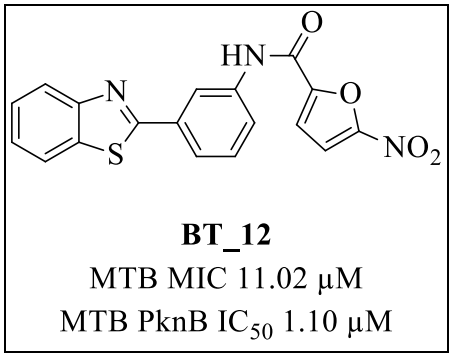


Figure 5.14: Structure and biological activity of most active compound **BT_12**

5.6. Study of potentiality of *N,N*-di-substituted-2-aminothiazole derivatives in inhibition of *M. tuberculosis* PknB enzyme

In the literature solid amount of research has been carried out on thiazole ring owing to its diverse biological activity. In last few years, a lot of research has been done on thiazole ring to evaluate its pharmacological activity *viz*. analgesic, anti-inflammatory, antioxidant, antifungal, antiviral, diuretic, anticonvulsant and anti-tubercular [Shiv Jee K., et al, 2012]. In present study we have added a little more work in thiazole's medicinal chemistry by studying the inhibitory activity of *N,N*-di-substituted-2-aminothiazoles against *M. tuberculosis* PknB enzyme.

5.6.1. Design of the molecules

Thaizole is a clear pale yellow coloured liquid; it is slightly soluble in water and has been a study of interest for its biological activity. Thiazole ring is naturally found in Vitamain B_1, a water soluble vitamin and thiazole is a key component in various chemical entities including sulphur drugs, fungicides, dyes and biocides [Shiv Jee K., et al, 2012]. There are few reports in literature on the anti-tubercular activity of *N*-substituted-2-aminothiazoles, but their mechanism of action was not much explored and also essentiality of the free –NH at C-2 was not studied. In our present work, we have studied the anti-tubercular effect of di-substitution on nitrogen atom of 2-aminothiazole and their potentiality in inhibition of *M. tuberculosis* PknB enzyme. For this purpose, we have selected a reported, promising '*N*-substituted-2-aminothiazole derivative' **7m** [Makam P., *et al*, 2014] (**Figure 5.15**) as lead molecule, and synthesized fifteen compounds by di-substitution at 2-amino group with appropriate substitutions.

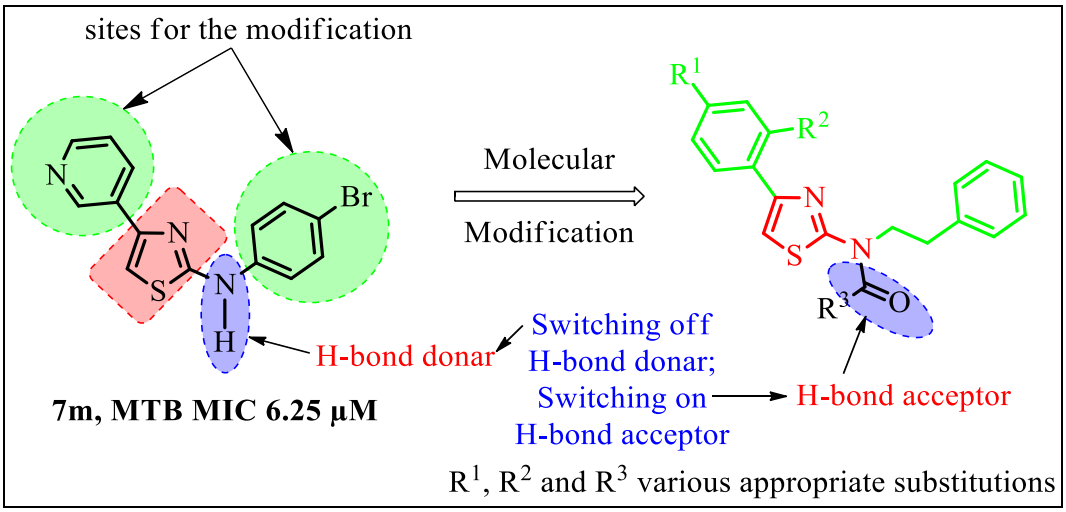


Figure 5.15: Design of molecules by molecular modification and H-bond switching approach

5.6.2. Experimental procedures utilized for the synthesis of TA_05 – TA_19

The target molecules were synthesized by following a three step synthetic protocol as shown in **Figure 4.6**. The first step of the synthetic sequence is the reaction was of substituted acetophenones with bromine in 1,4-dioxane solvent, under mild conditions to give '2-bromo-1-phenylethanone' derivatives (**TA_02a-e**). In the next step, the compounds **TA_02a-e** treated with 1-phenethylthiourea (**TA_03**) using ethanol as solvent under reflux conditions to afford *N*-phenethyl-4-phenylthiazol-2-amine derivatives (**TA_04a-e**). In final step, the secondary aryl amine treated with various substituted carboxylic acids using HBTU-HOBt conditions to produce final compounds (**TA_05 – TA_19**) in good yields.

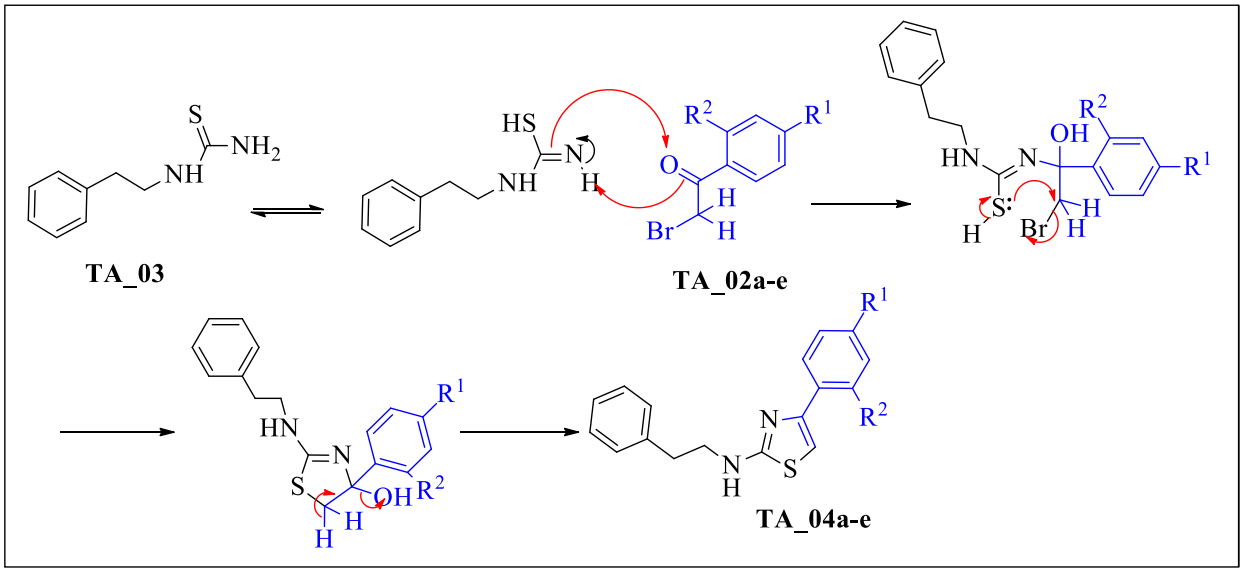
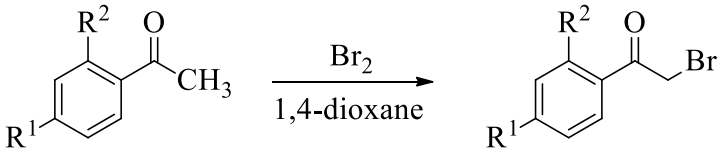


Figure 5.16: Mechanism of conversion of compound **TA_02a-e** to **TZ_04a-e**

General procedure for the preparation of TA_02a-e



TA_01a-e **TA_02a-e**

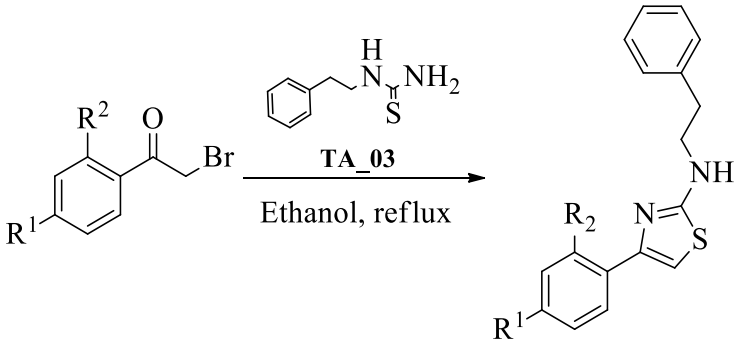
a. R^1= OH, R^2= H
b. R^1= OCH_3, R^2= H
c. R^1= CH_3, R^2= H
d.R^1= NO_2, R^2= H
e. R^1= Cl, R^2= Cl

Substituted acetophenone (1.0 equiv) was dissolved in 1,4-dioxane and added bromine solution (0.8 equiv) in 1,4-dioxane at 0 °C dropwise. The reaction was allowed to room temperature and stirred for 4 h (monitored by TLC), quenched by aqueous $NaHCO_3$ and extracted with EtOAc (2 × 60 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and evaporated in *vacuo*. Purified by column chromatography using EtOAc/hexanes as eluent.

2-Bromo-1-(4-methoxyphenyl)ethanone (TA_02b)

According to general procedure presented above, **TA_02b** was synthesized using 4-methoxyacetophenone as starting material. MS(ESI) *m/z* 215 $[M+H]^+$. Yield: 84%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.89 (d, *J* = 7.6 Hz, 2H), 6.88 (d, *J* = 7.6 Hz, 2H), 4.56 (s, 2H).

General procedure for the preparation TA_04a-e



TA_02a-e **TA_04a-e**

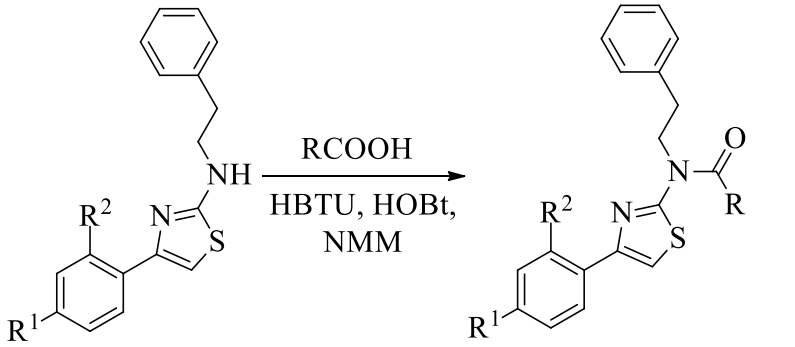
a. R^1= OH, R^2= H
b. R^1= OCH_3, R^2= H
c. R^1= CH_3, R^2= H
d.R^1= NO_2, R^2= H
e. R^1= Cl, R^2= Cl

To the mixture of compounds **TA_02a-e** and **TA_03** in round bottom flask was added ethanol and refluxed the mixture for 2 hours. The reaction mixture was evaporated and obtained solids were washed with saturated Na_2CO_3, water and small amount of cold ethanol. The obtained solid product was dried under vacuum to obtain the corresponding products **TA_04a-e**.

4-(4-Methoxyphenyl)-*N*-phenethylthiazol-2-amine (TA_04b)

According to general procedure presented above, **TA_04b** was synthesized using 2-bromo-1-(4-methoxyphenyl)ethanone (**TA_02b**) as starting material. MS(ESI) *m/z* 311 $[M+H]^+$. Yield: 89%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.72 (d, *J* = 7.6 Hz, 2H), 7.38–7.26 (m, 6H), 6.93 (d, *J* = 7.2 Hz, 2H), 6.72 (s, 1H), 3.61 (s, 3H), 3.57 (t, *J* = 6.8 Hz, 2H), 2.97 (t, *J* = 6.8 Hz, 2H).

General procedure for the preparation TA_05 – TA_19



TA_04a-e **TA_05- TA_19**

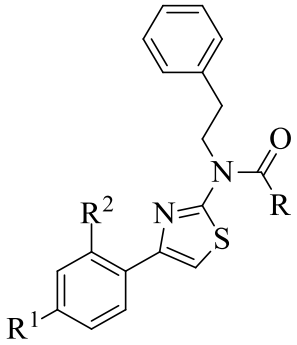
R_3 = aryl, hetero aryl groups

To the stirred solution of RCOOH in CH_2Cl_2 under nitrogen atmosphere was added HBTU (1.5 equiv), HOBt (1.5 equiv) and stirred for few minutes. Then NMM followed by compound **TA_04a-e** was added and stirred the reaction mixture at room temperature overnight. The solids formed in the reaction mixture were filtered and successfully washed with water to remove excess base, inorganic impurities and with cold ethanol to yield pure product without further purification.

P**reparation of TA_03**

Dry HCl gas was passed into a solution of phenethylamine (**3a**) (6.0 g, 49.5 mmol) in THF at -10 °C, the precipitated salt was filtered, washed to with THF and dried to yield phenethylamine hydrochloride salt (**3b**) (6.9 g, 88%). Compound **3b** (3.0 g, 19.03 mmol) and NH_4SCN (2.89 g, 38.06 mmol) were taken in bromobenzene and heated at 160 °C for 3 h. The reaction mixture was cooled to yield solid compound. The compound was filtered and dried to produce compound **3c** (2.79 g, 79%) as a white solid.

Table 5.11: Physiochemical properties of the synthesized compounds **TA_05 – TA_19**



TA_05 - TA_19

Compd	R^1	R^2	R	Yield (%)	M.P. (°C)	Molecular formula	Molecular Weight
TA_05	OH	H	furan-2-yl	88	148-149	$C_{22}H_{18}N_2O_3S$	390.45
TA_06	OH	H	5-nitrofuran-2-yl	79	202-203	$C_{22}H_{17}N_3O_5S$	435.45
TA_07	OH	H	5-nitrothiophen-2-yl	84	145-146	$C_{22}H_{17}N_3O_4S_2$	451.52
TA_08	OCH_3	H	5-nitrofuran-2-yl	80	113-114	$C_{23}H_{19}N_3O_5S$	449.48
TA_09	OCH_3	H	5-nitrothiophen-2-yl	73	154-155	$C_{23}H_{19}N_3O_4S_2$	465.54
TA_10	CH_3	H	5-nitrofuran-2-yl	76	171-172	$C_{23}H_{19}N_3O_4S$	433.48
TA_11	CH_3	H	5-nitrothiophen-2-yl	91	178-179	$C_{23}H_{19}N_3O_3S_2$	449.55
TA_12	NO_2	H	Furan-2-yl	70	170-171	$C_{22}H_{17}N_3O_4S$	419.45
TA_13	NO_2	H	5-nitrofuran-2-yl	78	177-178	$C_{22}H_{16}N_4O_6S$	464.45
TA_14	NO_2	H	5-nitrothiophen-2-yl	81	165-166	$C_{22}H_{16}N_4O_5S$	448.45
TA_15	Cl	Cl	Furan-2-yl	84	177-178	$C_{22}H_{16}Cl_2N_2O_2S$	443.35
TA_16	Cl	Cl	5-nitrofuran-2-yl	72	205-206	$C_{22}H_{15}Cl_2N_3O_4S$	488.34
TA_17	Cl	Cl	5-nitrothiophen-2-yl	79	150-151	$C_{22}H_{15}Cl_2N_3O_3S_2$	504.41
TA_18	Cl	Cl	2-Methoxyphenyl	81	155-156	$C_{25}H_{20}Cl_2N_2O_2S$	483.41
TA_19	Cl	Cl	2-Trifluoromethylphenyl	79	179-180	$C_{25}H_{17}Cl_2F_3N_2OS$	521.38

5.6.3. Characterization of the synthesized molecules

_N_-(4-(4-Hydroxyphenyl)thiazol-2-yl)-_N_-phenethylfuran-2-carboxamide (TA_05):

Yield: 88%; m.p. 148-149 °C; MS(ESI) *m/z* 391 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.25 (d, J = 7.6 Hz, 1H), 7.81 (d, J = 8.0 Hz, 2H), 7.76–7.64 (m, 3H), 7.54–7.45 (m, 4H), 7.36 (d, J = 6.8 Hz, 1H), 7.21 (d, J = 6.4 Hz, 2H), 5.54 (s, 1H), 4.76 (t, J = 7.2 Hz, 2H), 3.06 (t, J = 6.8 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 163.4, 161.4, 156.2, 143.8, 141.4, 139.6, 137.4, 134.1(2C), 133.0, 132.1(2C), 131.8, 130.5, 128.2(2C), 126.9(2C), 125.8, 123.8, 58.4, 36.9. Anal calcd for $C_{22}H_{18}N_2O_3S$: C, 67.67; H, 4.65; N, 7.17 % Found C, 67.78; H, 4.83; N, 7.25%.

_N_-(4-(4-Hydroxyphenyl)thiazol-2-yl)-5-nitro-_N_-phenethylfuran-2-carboxamide (TA_06):

Yield: 79%; m.p. 202-203 °C; MS(ESI) *m/z* 436 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.43 (d, J = 7.2 Hz, 1H), 7.90 (d, J = 7.6 Hz, 2H), 7.81 (d, J = 5.6 Hz, 1H), 7.74–7.48 (m, 4H), 7.38 (d, J = 6.8 Hz, 2H), 7.18 (d, J = 6.0 Hz, 2H), 5.61 (s, 1H), 4.79 (t, J = 7.6 Hz, 2H), 3.13 (t, J = 6.8 Hz, 2H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 164.7, 162.3, 158.6, 144.0, 140.8, 138.4, 137.9, 134.6(2C), 133.3, 132.4(2C), 131.2, 130.6, 129.4(2C), 127.6(2C), 126.3, 124.5, 60.1, 37.2. Anal calcd for $C_{22}H_{17}N_3O_5S$: C, 60.68; H, 3.93; N, 9.65 % Found C, 60.78; H, 3.99; N, 9.72%.

_N_-(4-(4-Hydroxyphenyl)thiazol-2-yl)-5-nitro-_N_-phenethylthiophene-2-carboxamide (TA_07):

Yield: 84%; m.p. 145-146 °C; MS(ESI) *m/z* 452 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.37 (d, J = 6.8 Hz, 1H), 7.86 (d, J = 7.2 Hz, 2H), 7.76 (d, J = 5.6 Hz, 1H), 7.74–7.42 (m, 4H), 7.33 (d, J = 6.4 Hz, 2H), 7.12 (d, J = 5.2 Hz, 2H), 5.44 (s, 1H), 4.70 (t, J = 7.6 Hz, 2H), 3.10 (t, J = 6.8 Hz, 2H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 164.0, 161.6, 156.9, 143.3, 142.4, 137.8, 137.3, 135.0(2C), 133.5, 131.8(2C), 130.2, 129.1, 127.9(2C), 127.2(2C), 125.2, 123.6, 59.4, 36.8. Anal calcd for $C_{22}H_{17}N_3O_4S_2$: C, 58.52; H, 3.79; N, 9.31% Found C, 58.56; H, 3.84; N, 9.38%.

_N_-(4-(4-Methoxyphenyl)thiazol-2-yl)-5-nitro-_N_-phenethylfuran-2-carboxamide (TA_08):

Yield: 80%; m.p. 113-114 °C; MS(ESI) *m/z* 450 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.38 (d, J = 6.4 Hz, 1H), 8.01 (d, J = 7.2 Hz, 1H), 7.78 (d, J = 6.4 Hz, 2H), 7.72 (s, 1H), 7.48 (d, J = 6.0 Hz, 2H), 7.35–7.28 (m, 3H), 7.13 (d, J = 6.8 Hz, 2H), 4.72 (t, J = 7.2 Hz, 2H), 4.03 (s,

3H), 3.21 (t, *J* = 6.8 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.3, 164.1, 159.4, 145.3, 142.4, 139.7, 138.3, 133.4(2C), 133.1, 132.6(2C), 132.5, 131.6, 130.7(2C), 128.7(2C), 127.3, 125.7, 63.5, 58.8, 38.3. Anal calcd for $C_{23}H_{19}N_3O_5S$: C, 61.46; H, 4.26; N, 9.35 % Found C, 61.58; H, 4.39; N, 9.42%.

_N_-(4-(4-Methoxyphenyl)thiazol-2-yl)-5-nitro-_N_-phenethylthiophene-2-carboxamide (TA_09):

Yield: 73%; m.p. 154-155 °C; MS(ESI) *m/z* 466 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.32 (d, *J* = 6.0 Hz, 1H), 7.94 (d, *J* = 6.4 Hz, 1H), 7.74 (d, *J* = 6.4 Hz, 2H), 7.69 (s, 1H), 7.45 (d, *J* = 6.4 Hz, 2H), 7.38–7.29 (m, 3H), 7.11 (d, *J* = 6.4 Hz, 2H), 4.68 (t, *J* = 7.2 Hz, 2H), 3.99 (s, 3H), 3.18 (t, *J* = 6.8 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.7, 164.3, 158.3, 146.0, 142.1, 138.3, 136.6, 134.1(2C), 132.4, 132.0(2C), 131.4, 129.3, 127.4(2C), 126.2(2C), 125.8, 123.4, 61.2, 58.3, 37.9. Anal calcd for $C_{23}H_{19}N_3O_4S_2$: C, 59.34; H, 4.11; N, 9.03 % Found C, 59.38; H, 4.19; N, 9.12%.

5-Nitro-_N_-phenethyl-_N_-(4-(p-tolyl)thiazol-2-yl)furan-2-carboxamide (TA_10):

Yield: 76%; m.p. 171-172 °C; MS(ESI) *m/z* 434 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.29 (d, *J* = 6.8 Hz, 1H), 8.12 (d, *J* = 7.6 Hz, 1H), 7.72 (d, *J* = 6.0 Hz, 2H), 7.67 (s, 1H), 7.45–7.36 (m, 3H), 7.28 (d, *J* = 6.4 Hz, 2H), 7.19 (d, *J* = 6.8 Hz, 2H), 4.69 (t, *J* = 7.6 Hz, 2H), 3.18 (t, *J* = 7.2 Hz, 2H), 2.43 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 163.4, 162.2, 158.9, 148.2, 144.6, 137.4, 136.0, 132.8(2C), 132.1, 130.7(2C), 129.5(2C), 128.6, 127.7, 126.3(2C), 125.2, 123.4, 58.2, 37.5, 22.7. Anal calcd for $C_{23}H_{19}N_3O_4S$: C, 63.73; H, 4.42; N, 9.69 % Found C, 63.78; H, 4.49; N, 9.72%.

5-Nitro-_N_-phenethyl-_N_-(4-(*p*-tolyl)thiazol-2-yl)thiophene-2-carboxamide (TA_11):

Yield: 91%; m.p. 178-179 °C; MS(ESI) *m/z* 450 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.24 (d, *J* = 6.8 Hz, 1H), 8.09 (d, *J* = 7.6 Hz, 1H), 7.69 (d, *J* = 6.0 Hz, 2H), 7.48–7.33 (m, 4H), 7.30 (d, *J* = 6.8 Hz, 2H), 7.12 (d, *J* = 7.2 Hz, 2H), 4.62 (t, *J* = 7.6 Hz, 2H), 3.13 (t, *J* = 7.6 Hz, 2H), 2.39 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 162.9, 162.3, 158.2, 147.7, 143.9, 137.1, 136.3, 133.0(2C), 131.9, 131.4(2C), 129.2, 128.3(2C), 126.9, 126.0(2C), 125.4, 124.1, 59.3,

38.1, 22.5. Anal calcd for $C_{23}H_{19}N_3O_3S_2$: C, 61.45; H, 4.26; N, 9.35 % Found C, 61.48; H, 4.39; N, 9.42%.

_N_-(4-(4-Nitrophenyl)thiazol-2-yl)-_N_-phenethylfuran-2-carboxamide (TA_12):

Yield: 70%; m.p. 170-171 °C; MS(ESI) *m/z* 420 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.46 (d, J = 7.6 Hz, 2H), 8.13 (d, J = 6.0 Hz, 1H), 7.81 (d, J = 7.2 Hz, 2H), 7.74–7.53 (m, 3H), 7.49–7.40 (m, 2H), 7.20 (d, J = 6.8 Hz, 2H), 6.93 (d, J = 6.4 Hz, 1H), 4.72 (t, J = 7.2 Hz, 2H), 3.15 (t, J = 6.8 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.3, 161.6, 158.3, 149.8, 146.4, 137.6, 137.0(2C), 136.1, 134.3, 133.1(2C), 131.6, 130.3, 128.3(2C), 126.4(2C), 125.3, 124.6, 60.3, 38.4. Anal calcd for $C_{22}H_{17}N_3O_4S$: C, 63.00; H, 4.09; N, 10.02 % Found C, 63.08; H, 4.13; N, 10.15%.

5-Nitro-_N_-(4-(4-nitrophenyl)thiazol-2-yl)-_N_-phenethylfuran-2-carboxamide (TA_13):

Yield: 78%; m.p. 177-178 °C; MS(ESI) *m/z* 465 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.48 (d, J = 6.9 Hz, 2H), 8.10 (d, J = 7.8 Hz, 1H), 7.84 (d, J = 7.2 Hz, 2H), 7.72–7.66 (m, 3H), 7.54–7.44 (m, 2H), 7.33 (d, J = 7.2 Hz, 2H), 4.76 (t, J = 8.1 Hz, 2H), 3.16 (t, J = 6.9 Hz, 2H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 163.7, 162.0, 158.2, 146.4, 142.8, 136.9, 136.3, 135.6(2C), 134.3, 133.4(2C), 132.4, 131.8, 129.6(2C), 128.6(2C), 127.5, 122.4, 61.0, 38.3. Anal calcd for $C_{22}H_{16}N_4O_6S$: C, 56.89; H, 3.47; N, 12.06 % Found C, 56.98; H, 3.54; N, 12.12%.

5-Nitro-_N_-(4-(4-nitrophenyl)thiazol-2-yl)-_N_-phenethylthiophene-2-carboxamide (TA_14):

Yield: 81%; m.p. 165-166 °C; MS(ESI) *m/z* 481 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.41 (d, J = 6.8 Hz, 2H), 7.96 (d, J = 7.2 Hz, 1H), 7.76–7.69 (m, 4H), 7.56–7.47 (m, 3H), 7.30 (d, J = 6.2 Hz, 2H), 4.71 (t, J = 7.2 Hz, 2H), 3.14 (t, J = 6.8 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 163.9, 161.3, 156.4, 144.0, 142.1, 136.3, 135.6, 134.0(2C), 133.2, 132.1(2C), 130.9, 129.3, 128.4(2C), 127.7(2C), 124.3, 119.6, 58.3, 37.6. Anal calcd for $C_{22}H_{16}N_4O_5S_2$: C, 54.99; H, 3.36; N, 11.66% Found C, 55.06; H, 3.44; N, 11.78%.

_N_-(4-(2,4-Dichlorophenyl)thiazol-2-yl)-_N_-phenethylfuran-2-carboxamide (TA_15):

Yield: 84%; m.p. 177-178 °C; MS(ESI) *m/z* 443 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.29 (d, J = 7.2 Hz, 1H), 8.01 (d, J = 7.8 Hz, 1H), 7.72–7.64 (m, 4H), 7.60 (d, J = 7.2 Hz, 1H),

7.56–7.47 (m, 3H), 7.36 (d, *J* = 6.9 Hz, 2H), 4.68 (t, *J* = 7.2 Hz, 2H), 3.13 (t, *J* = 6.8 Hz, 2H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 166.7, 163.4, 154.3, 148.5, 146.0, 139.3, 138.3, 136.1, 135.0, 134.3, 132.6, 131.4, 130.4, 128.6(2C), 127.0(2C), 124.7, 122.1, 118.4, 56.9, 37.8. Anal calcd for $C_{22}H_{16}Cl_2N_2O_2S$: C, 59.60; H, 3.64; N, 6.32 % Found C, 59.68; H, 3.73; N, 6.35%.

_N_-(4-(2,4-Dichlorophenyl)thiazol-2-yl)-5-nitro-_N_-phenethylfuran-2-carboxamide (TA_16):

Yield: 72%; m.p. 205-206 °C; MS(ESI) *m/z* 489 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.44 (d, *J* = 6.9 Hz, 1H), 8.32 (d, *J* = 7.8 Hz, 1H), 7.81 (d, *J* = 6.6 Hz, 1H), 7.76–7.69 (m, 3H), 7.58–7.49 (m, 2H), 7.36–7.28 (m, 3H), 4.72 (t, *J* = 7.2 Hz, 2H), 3.18 (t, *J* = 6.9 Hz, 2H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 164.4, 163.2, 159.6, 149.9, 143.0, 135.7, 134.2, 133.0, 132.6, 131.5, 130.4, 129.3, 128.4, 128.0, 127.2(2C), 126.3(2C), 124.2, 119.4, 61.2, 38.6. Anal calcd for $C_{22}H_{15}Cl_2N_3O_4S$: C, 54.11; H, 3.10; N, 8.60 % Found C, 54.18; H, 3.14; N, 8.72%.

_N_-(4-(2,4-Dichlorophenyl)thiazol-2-yl)-5-nitro-_N_-phenethylthiophene-2-carboxamide (TA_17):

Yield: 79%; m.p. 150-151 °C; MS(ESI) *m/z* 504 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.39 (d, *J* = 6.6 Hz, 1H), 8.28 (d, *J* = 7.2 Hz, 1H), 7.74 (d, *J* = 6.6 Hz, 1H), 7.69–7.60 (m, 2H), 7.56–7.47 (m, 3H), 7.33–7.24 (m, 3H), 4.70 (t, *J* = 7.2 Hz, 2H), 3.15 (t, *J* = 6.9 Hz, 2H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 165.3, 163.7, 158.3, 148.5, 143.3, 136.2, 134.0, 133.4, 132.9, 130.8, 129.7, 129.2, 128.7, 127.4, 126.9(2C), 126.2(2C), 125.1, 118.5, 61.0, 38.1. Anal calcd for $C_{22}H_{15}Cl_2N_3O_3S_2$: C, 52.39; H, 3.00; N, 8.33% Found C, 52.46; H, 3.12; N, 8.38%.

_N_-(4-(2,4-Dichlorophenyl)thiazol-2-yl)-2-methoxy-_N_-phenethylbenzamide (TA_18):

Yield: 81%; m.p. 155-156 °C; MS(ESI) *m/z* 484 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.23 (d, *J* = 8.0 Hz, 1H), 7.92 (d, *J* = 8.0 Hz, 1H), 7.84–7.68 (m, 5H), 7.63–7.49 (m, 4H), 7.26 (d, *J* = 8.0 Hz, 2H), 4.62 (t, *J* = 7.2 Hz, 2H), 3.96 (s, 3H), 3.06 (t, *J* = 7.2 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.6, 163.4, 159.4, 148.3, 138.4, 137.2, 135.6, 134.3, 133.9, 132.6, 131.6, 130.9(2C), 129.6, 128.4, 127.5, 126.4, 126.0(2C), 125.1, 123.4, 119.4, 59.6, 56.4, 37.3. Anal calcd for $C_{25}H_{20}Cl_2N_2O_2S$: C, 62.11; H, 4.17; N, 5.79% Found C, 62.22; H, 4.24; N, 5.85%.

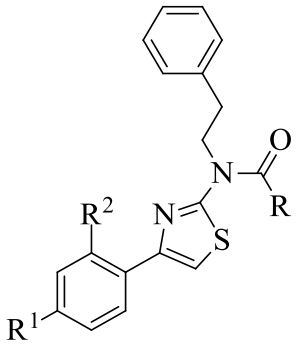
***N*-(4-(2,4-Dichlorophenyl)thiazol-2-yl)-*N*-phenethyl-2-(trifluoromethyl)benzamide (TA_19):**

Yield: 79%; m.p. 179-180 °C; MS(ESI) *m/z* 522 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.28 (d, *J* = 7.2 Hz, 1H), 7.89 (d, *J* = 8.0 Hz, 1H), 7.81–7.66 (m, 4H), 7.63 (s, 1H), 7.58–7.49 (m, 4H), 7.33–7.27 (m, 2H), 4.66 (t, *J* = 7.2 Hz, 2H), 3.15 (t, *J* = 7.2 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.4, 165.4, 160.4, 152.3, 141.4, 139.3, 134.3, 132.3, 132.0, 131.4, 130.3, 129.4(2C), 128.3, 127.1, 126.8, 126.4, 125.3(2C), 124.6, 122.8, 120.5, 118.3, 60.3, 38.2. Anal calcd for $C_{25}H_{17}Cl_2F_3N_2OS$: C, 57.59; H, 3.29; N, 5.37% Found C, 57.62; H, 3.34; N, 5.49%.

5.6.4. *In vitro M. tuberculosis* screening, *M. tuberculosis* PknB 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. INH and EMB were used as reference compounds for comparison. All the synthesized derivatives were also screened for their *in vitro M. tuberculosis* PknB 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 5.12**.

Table 5.12: *In vitro* biological evaluation of synthesized compounds **TA_05** – **TA_19**



TA_05 - TA_19

Compd	R^1	R^2	R	MTB MIC in µM	MTB PknB IC_{50} in µM	Cytotoxicity at 50 µM % inhibition
TA_05	OH	H	furan-2-yl	8.00	>25	22.16

Contd

Compd	R^1	R^2	R	MTB MIC in µM	MTB PknB IC_{50} in µM	Cytotoxicity at 50 µM % inhibition
TA_06	OH	H	5-nitrofuran-2-yl	1.79	>25	28.4
TA_07	OH	H	5-nitrothiophen-2-yl	27.68	>25	32.16
TA_08	OCH_3	H	5-nitrofuran-2-yl	27.81	>25	29.07
TA_09	OCH_3	H	5-nitrothiophen-2-yl	13.42	>25	30.69
TA_10	CH_3	H	5-nitrofuran-2-yl	28.83	>25	36.72
TA_11	CH_3	H	5-nitrothiophen-2-yl	3.47	>25	26.78
TA_12	NO_2	H	Furan-2-yl	29.80	>25	17.9
TA_13	NO_2	H	5-nitrofuran-2-yl	3.36	>25	29.86
TA_14	NO_2	H	5-nitrothiophen-2-yl	26.01	>25	36.54
TA_15	Cl	Cl	Furan-2-yl	3.82	>25	15.45
TA_16	Cl	Cl	5-nitrofuran-2-yl	13.76	>25	20.45
TA_17	Cl	Cl	5-nitrothiophen-2-yl	26.59	>25	40.07
TA_18	Cl	Cl	2-Methoxyphenyl	13.92	>25	23.12
TA_19	Cl	Cl	2-Trifluoromethylphenyl	3.20	>25	21.34
Isoniazid				0.72	NT	NT
Ethambutol				7.64	NT	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.

5.6.5. SAR and discussion

To study the effect of di-substitution on C-2 nitrogen of thiazole ring, we have synthesized a small library of fifteen molecules. Based on thorough literature search and our experience in design of novel TB drug candidates, we found that substitution of a bulky group separated by a linker of two carbons by one of two hydrogen atoms of C-2 amine group enhanced the activity by better binding to the active site of the enzyme. And the other hydrogen atom of C-2 amine group was substituted by peptide bonds by treating with various carboxylic acids. The idea here is to study the effect of free secondary amino group at C-2 by "switching the hydrogen donating nature of C-2 secondary amine to hydrogen acceptor nature".

The "H-bond switching approach" worked well and better results were obtained than the lead molecule. Out of fifteen tested compounds, six compounds (**TA_05**, **TA_06**, **TA_11**, **TA_13**, **TA_15** and **TA_19**) showed *M. tuberculosis* MIC < 10 µM and five compounds (**TA_06**, **TA_11**, **TA_13**, **TA_15** and **TA_19**) inhibited *M. tuberculosis* with MIC <5 µM.

All the synthesized compounds were screened for their inhibitory potency against the enzyme *M. tuberculosis* PknB. But none of the compounds showed good activity in inhibition of PknB, this indicates that the mechanism of action is not through the enzyme *M. tuberculosis* PknB, this means PknB is not a valid target for the synthesized compounds. All the final compounds (**TA_05 – TA19**) were also tested for their toxicity; most of the compounds were non-toxic at 50 µM concentration.

5.6.6. Highlights of the study

In summary, we synthesized a library of fifteen molecules to study the effect of switching of H-bond donating nature of at C-2 position of thiazole to H-bond accepting nature. Few of the molecules showed excellent activity and were not toxic. The synthesized molecules did not show any promising inhibitory potency towards the enzyme *M. tuberculosis* PknB, suggests that PknB is not an appropriate target. Compound **TA_06** (N-(4-(4-hydroxyphenyl)thiazol-2-yl)-5-nitro-N-phenethylfuran-2-carboxamide) was emerged as the most active compound with *M. tuberculosis* MIC of 1.79 µM (**Figure 5.17**).

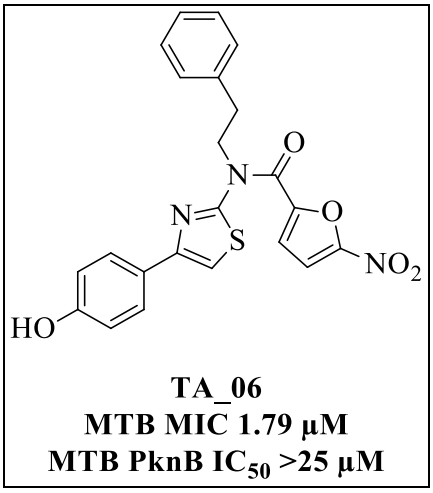


Figure 5.17: Chemical structure and biological activity of the most active compound **TA_06**

5.7. Design, synthesis and biological evaluation of 1,2,4-triazino[5,6-*b*]indole derivatives as potent *M. tuberculosis* PknB inhibitors

In literature there were many reports on indole and triazine derivatives, especially in last few years huge efforts were made to synthesize various substituted indole derivatives due to their diverse biological activities. In last decade there were many reports on broad spectrum pharmacological potency of indole derivatives, *viz* anticonvulsant, antimicrobial, anthelmintic, antitumor, anti-tubercular, anti-inflammatory, antipsychotic, antidiabetic and antifungal [Poojitha J., *et al*., 2015]. In our present study we designed and synthesized fused indolo-triazine derivatives for the inhibition of *M. tuberculosis* PknB enzyme.

5.7.1. Design of the molecules

Triazine is a promising scaffold for its versatile biological behavior and indoles are well known for their potency in inhibiting various bacterial enzymes. There was a report on fused 1,2,4-triazino-indole derivatives as excellent antiviral agents [Upadhyay K., *et al*., 2013]. Based on our previous research experience in PknB drug design, we anticipated fused indolo-triazines will best fit in active site of PknB enzyme. *M. tuberculosis* PknB is an essential enzyme that catalyzes the transfer of the γ-phosphate of ATP to the hydroxyl group on serine, threonine or tyrosine residue in the target protein. PknB is not well explored target for novel anti-tubercular drug discovery. To check the hit and trial we screened a library of fused 1,2,4-triazino-indole derivatives procured from commercial Asinex database (**Figure 5.18**).

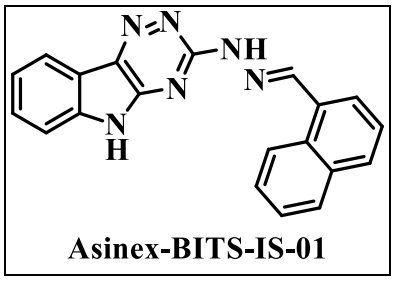


Figure 5.18: Structure of lead molecule for the generation of compounds **IS_04** – **IS_42**

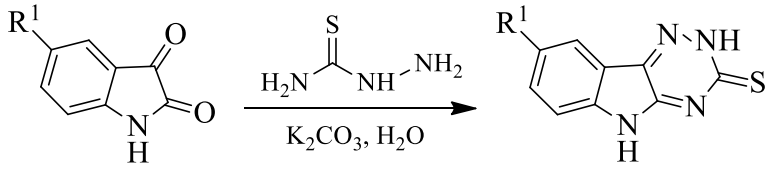
Among tested 1,2,4-triazino-indole derivatives, the molecule possessing hydrazine derivative at 3rd position of triazine ring showed better activity. To study the SAR, we have synthesized a

library of thirty nine compounds by various appropriate substitutions on phenyl ring of indole and phenyl ring of hydrazone.

5.7.2. Experimental procedures utilized for the synthesis of IS_04 – IS_42

The final compounds were synthesized by following three step synthetic protocol [**Figure 4.7**]. Wherein, the first step of synthetic sequence consists of reaction of commercially available substituted isatins (**IS_01a-d**) with thiosemicarbazide using K_2CO_3 as base and refluxing in water to afford corresponding tricyclic ring compounds '2*H*-[1,2,4]triazino[5,6-*b*]indole-3(5*H*)-thione' (**IS_02a-d**) in excellent yields. In second step, the sulphur atom of thiones were removed by treating with commercially available 35% hydrazine hydrate to afford corresponding hydrazines '3-hydrazinyl-5*H*-[1,2,4]triazino[5,6-*b*]indole' (**IS_03a-d**). In last step, the free amino group of hydrazine was treated with various substituted aldehydes under ethanol reflux conditions to produce '3-(2-benzylidenehydrazinyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole' (**IS_04 – IS_42**) derivatives respectively (**Table 5.13**).

General procedure for the preparation of IS_02a-d

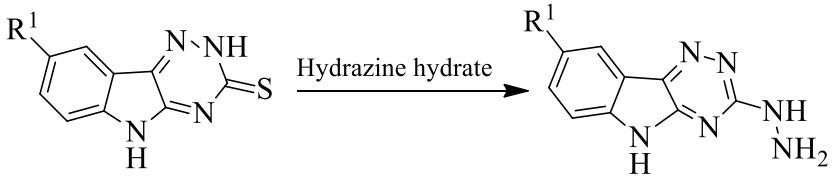


IS_ 01a-d **IS_ 02a-d**

R^1= H, CH_3, F, Cl

Thiosemicarbazide (1.0 equiv) was added to the stirred solution of corresponding isatin (**IS_01a-d)** (1.0 equiv) and K_2CO_3 (1.5 equiv) in water and allowed to reflux for 16 hours. Then the reaction mixture was cooled to room temperature and acidified with AcOH at 0 °C, solids formed in the reaction mixture was filtered, washed with water, EtOH followed by diethylether and dried in vacuum oven to afford the compounds **IS_02a-d** as solid in pure form.

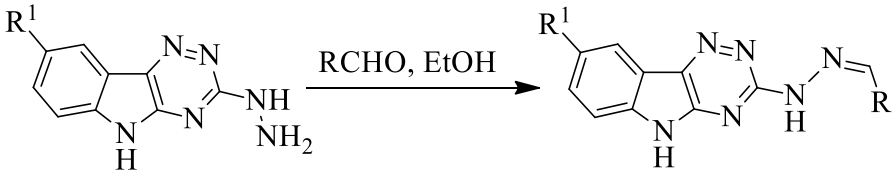
General procedure for the preparation of IS_03a-d



IS_ 02a-d **IS_ 03a-d**

Compound **IS_02a-d** (1.0 equiv) in 35% $N_2H_4.H_2O$ (30 vol) was refluxed for 12 h and cooled the reaction mixture to room temperature, the solids formed were filtered and washed with excess of H_2O, EtOH and dethylether and dried in vacuum oven to get respective compound **IS_03a-d** as solids and used for the next step.

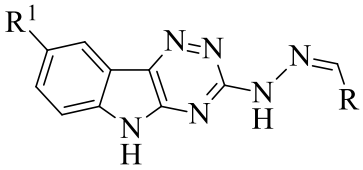
General procedure for the synthesis of compounds IS_04 - IS_42



IS_ 03a-d **IS_ 04 - IS_ 42**

Compound **IS_03a-d** (1.0 equiv) and the appropriate aromatic aldehyde (1.3 equiv) were taken in Ethanol (10 vol) and refluxed for 6h. The solids formed in the reaction mixture were filtered and washed with H_2O, cold Ethanol, Hexanes and Diethyl ether. The solid compound was dried in vacuum oven to get the desired compound (**IS_04- IS_42**) as solid.

Table 5.13: Physiochemical properties of the synthesized compounds **IS_04 - IS_42**



IS_ 04 - IS_ 42

Compd	R^1	R	Yield (%)	M.P. (°C)	Molecular Formula	Molecular Weight
IS_04	H	Phenyl	82	231–232	$C_{16}H_{12}N_6$	288.31
IS_05	H	4-Fluorophenyl	86	241–242	$C_{16}H_{11}FN_6$	306.30
IS_06	H	3-Tolyl	74	239–240	$C_{17}H_{14}N_6$	302.33
IS_07	H	4-isopropylphenyl	82	251–252	$C_{19}H_{18}N_6$	330.39
IS_08	H	2-Hydroxyphenyl	78	254–255	$C_{16}H_{12}N_6O$	304.31
IS_09	H	2-Methoxyphenyl	74	262–263	$C_{17}H_{14}N_6O$	318.33
IS_10	H	2-Benzyloxyphenyl	77	258–259	$C_{23}H_{18}N_6O$	394.43
IS_11	H	3-Nitrophenyl	84	271–272	$C_{16}H_{11}N_7O_2$	333.30
IS_12	H	4-Dimethylaminophenyl	81	253–254	$C_{18}H_{17}N_7$	331.37
IS_13	H	5-Nitro-2-furyl	71	247–248	$C_{14}H_9N_7O_3$	323.27
IS_14	H	5-Nitro-2-thiophenyl	69	245–246	$C_{14}H_9N_7O_2S$	339.33
IS_15	CH_3	4-Fluorophenyl	74	235–236	$C_{17}H_{13}FN_6$	320.32
IS_16	CH_3	3-Tolyl	75	264–265	$C_{18}H_{16}N_6$	316.36
IS_17	CH_3	2-Hydroxyphenyl	78	234–235	$C_{17}H_{14}N_6O$	318.33
IS_18	CH_3	2-Methoxyphenyl	83	269–270	$C_{18}H_{16}N_6O$	332.36
IS_19	CH_3	2-Benzyloxyphenyl	85	261–262	$C_{24}H_{20}N_6O$	408.46
IS_20	CH_3	3-Nitrophenyl	76	275–276	$C_{17}H_{13}N_7O_2$	347.33
IS_21	CH_3	4-Dimethylaminophenyl	81	264–265	$C_{19}H_{22}N_6$	334.42
IS_22	CH_3	5-Nitro-2-furyl	73	239–240	$C_{15}H_{11}N_7O_3$	337.29
IS_23	F	4-Fluorophenyl	85	214–215	$C_{16}H_{10}F_2N_6$	324.29
IS_24	F	3-Tolyl	86	274–275	$C_{17}H_{13}FN_6$	320.32

Contd

Compd	R^1	R	Yield (%)	M.P. (°C)	Molecular Formula	Molecular Weight
IS_25	F	2-Hydroxyphenyl	83	239–240	$C_{16}H_{11}FN_6O$	322.30
IS_26	F	2-Methoxyphenyl	84	214–215	$C_{17}H_{13}FN_6O$	336.32
IS_27	F	2-Benzyloxyphenyl	88	242–243	$C_{23}H_{17}FN_6O$	412.42
IS_28	F	3-Nitrophenyl	91	262–263	$C_{16}H_{10}FN_7O_2$	351.29
IS_29	F	4-Dimethylaminophenyl	84	246–247	$C_{18}H_{19}FN_6$	338.38
IS_30	F	5-Nitro-2-furyl	80	247–248	$C_{14}H_8FN_7O_3$	341.26
IS_31	F	5-Nitro-2-thiophenyl	75	229–230	$C_{14}H_8FN_7O_2S$	357.32
IS_32	Cl	Phenyl	84	251–252	$C_{16}H_{11}ClN_6$	322.75
IS_33	Cl	4-Fluorophenyl	90	232–233	$C_{16}H_{10}ClFN_6$	340.74
IS_34	Cl	3-Tolyl	86	229–230	$C_{17}H_{13}ClN_6$	336.78
IS_35	Cl	4-isopropylphenyl	78	224–225	$C_{19}H_{17}ClN_6$	364.83
IS_36	Cl	2-Hydroxyphenyl	79	235–236	$C_{16}H_{11}ClN_6O$	338.75
IS_37	Cl	2-Methoxyphenyl	86	239–240	$C_{17}H_{13}ClN_6O$	352.78
IS_38	Cl	2-Benzyloxyphenyl	92	247–248	$C_{23}H_{17}ClN_6O$	428.87
IS_39	Cl	3-Nitrophenyl	93	254–255	$C1_6H_{10}ClN_7O_2$	367.75
IS_40	Cl	4-Dimethylaminophenyl	89	249–250	$C_{18}H_{19}ClN_6$	354.84
IS_41	Cl	5-Nitro-2-furyl	811	246–247	$C_{14}H_8ClN_7O_3$	357.71
IS_42	Cl	5-Nitro-2-thiophenyl	78	238–239	$C_{14}H_8ClN_7O_2S$	373.78

5.7.3. Characterization of the synthesized molecules

3-(2-Benzylidenehydrazinyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (IS_04):

Yield: 82%; m.p. 231–232 °C; ESI-MS showed 289 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.70 (s, 1H), 9.49 (s, 1H), 7.90 (s, 1H), 7.63 (d, J = 7.2 Hz, 2H), 7.49–7.32 (m, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.4, 142.3, 140.6, 138.8, 136.4, 135.5, 133.9, 130.4(2C), 127.4(2C), 126.0, 125.4, 122.8, 119.3, 114.3. Anal calcd for $C_{16}H_{11}ClN_6$: C, 66.66; H, 4.20; N, 29.15 % Found C, 66.71; H, 4.27; N, 29.25%.

3-(2-(4-Fluorobenzylidene)hydrazinyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (IS_05):

Yield: 86%; m.p. 241–242 °C; ESI-MS showed 307 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.20 (s, 1H), 9.69 (s, 1H), 7.69 (s, 1H), 7.62 (d, *J* = 7.2 Hz, 2H), 7.49 (t, 1H), 7.41 (d, *J* = 7.2 Hz, 2H), 7.33 (t, 1H), 7.27 (d, *J* = 6.4 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.9, 152.3, 142.3, 138.6, 137.2, 135.5, 133.3, 131.3(2C), 130.4, 128.6(2C), 127.3, 124.1, 118.7, 116.2. Anal calcd for $C_{16}H_{11}FN_6$: C, 62.74; H, 3.62; N, 27.44 % Found C, 62.81; H, 3.67; N, 27.52 %.

3-(2-(3-Methylbenzylidene)hydrazinyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (IS_06):

Yield: 74%; m.p. 239–240 °C; ESI-MS showed 303 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.79 (s, 1H), 9.81 (s, 1H), 7.87 (s, 1H), 7.54–7.35 (m, 7H), 7.31 (s, 1H), 2.39 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.4, 146.2, 141.4, 137.6, 136.9, 134.3, 132.6, 132.0, 130.7, 129.4, 128.1, 127.7, 125.3, 120.7, 117.3, 114.9, 22.3. Anal calcd for $C_{17}H_{14}N_6$: C, 67.54; H, 4.67; N, 27.80 % Found C, 67.61; H, 4.72; N, 27.90%.

3-(2-(4-Isopropylbenzylidene)hydrazinyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (IS_07):

Yield: 82%; m.p. 251–252 °C; ESI-MS showed 331 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.71 (s, 1H), 9.93 (s, 1H), 7.92 (s, 1H), 7.74 (d, *J* = 6.8 Hz, 2H), 7.61–7.54 (m, 2H), 7.42 (t, 2H), 7.23(d, *J* = 7.2 Hz, 2H), 2.61 (m, 1H), 1.24 (d, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.3, 145.9, 143.1, 138.4, 135.1, 133.9, 131.3, 129.3(2C), 128.3, 127.2(2C), 126.1, 123.2, 119.3, 115.1, 34.3, 22.9(2C). Anal calcd for $C_{19}H_{18}N_6$: C, 69.07; H, 5.49; N, 25.44 % Found C, 69.12; H, 5.53; N, 25.49%.

2-((2-(5*H*-[1,2,4]Triazino[5,6-*b*]indol-3-yl)hydrazono)methyl)phenol (IS_08):

Yield: 78%; m.p. 254–255 °C; ESI-MS showed 305 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.97 (s, 1H), 10.92 (s, 1H), 9.97 (s, 1H), 7.81 (s, 1H), 7.72 (d, *J* = 7.6 Hz, 1H), 7.62–7.54 (m, 7H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.4, 156.1, 142.3, 138.6, 137.0, 134.9, 133.3, 132.4, 130.6, 129.4, 127.7, 126.3, 125.6, 123.4, 118.7, 116.9. Anal calcd for $C_{16}H_{12}N_6O$: C, 63.15; H, 3.97; N, 27.62 % Found C, 63.21; H, 3.99; N, 27.71 %.

3-(2-(2-Methoxybenzylidene)hydrazinyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (IS_09):

Yield: 74%; m.p. 262–263 °C; ESI-MS showed 319 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.10 (s, 1H), 10.24 (s, 1H), 7.92 (s, 1H), 7.84 (d, *J* = 6.8 Hz, 1H), 7.72–7.63 (m, 6H), 7.24 (t,

1H), 3.72 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.3, 154.5, 145.1, 139.3, 137.0, 135.4, 134.0, 133.4, 131.8, 129.6, 128.1, 126.9, 125.3, 124.7, 120.4, 117.4, 60.9. Anal calcd for $C_{17}H_{14}N_6O$: C, 64.14; H, 4.43; N, 26.40 % Found C, 64.21; H, 4.54; N, 26.44%.

3-(2-(2-(Benzyloxy)benzylidene)hydrazinyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (IS_10):

Yield: 77%; m.p. 258–259 °C; ESI-MS showed 395 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.97 (s, 1H), 9.72 (s, 1H), 8.19(s, 1H), 7.69–7.58 (m, 5H), 7.54 (t, 2H), 7.44 (d, *J* = 6.8 Hz, 2H), 7.39–7.31 (m, 4H), 5.29 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.4, 155.4, 146.3, 138.3, 136.3, 135.1, 132.5, 130.4(2C), 129.4, 128.4(2C), 128.0, 126.9(2C), 127.3, 125.8(2C), 123.4, 122.6, 118.8, 116.3, 72.0. Anal calcd for $C_{23}H_{18}N_6O$: C, 70.04; H, 4.60; N, 21.31 % Found C, 70.12; H, 4.66; N, 21.42%.

3-(2-(3-Nitrobenzylidene)hydrazinyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (IS_11):

Yield: 84%; m.p. 271–272 °C; ESI-MS showed 334 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.17 (s, 1H), 8.80 (s, 1H), 8.10 (s, 1H), 7.90–7.72 (m, 7H), 7.27 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.2, 152.2, 143.1, 137.4, 136.9, 133.8, 133.4, 132.6, 131.4, 128.5, 126.0, 125.1, 124.9, 124.2, 121.7, 117.9. Anal calcd for $C_{16}H_{11}N_7O_2$: C, 57.66; H, 3.33; N, 29.42 % Found C, 57.71; H, 3.39; N, 29.54 %.

4-((2-(5*H*-[1,2,4]Triazino[5,6-*b*]indol-3-yl)hydrazono)methyl)-*N*,*N*-dimethylaniline (IS_12): Yield: 81%; m.p. 253–254 °C; ESI-MS showed 332 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.92 (s, 1H), 9.91 (s, 1H), 7.84 (s, 1H), 7.72 (d, *J* = 7.2 Hz, 2H), 7.54–7.49 (m, 2H), 7.33 (t, 2H), 7.20 (d, *J* = 7.2 Hz, 2H), 3.06 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.9, 151.5, 143.2, 138.3, 136.9, 135.2, 134.4, 132.0(2C), 128.8, 127.5, 126.3(2C), 124.3, 121.3, 114.3, 43.9(2C). Anal calcd for $C_{18}H_{17}N_7$: C, 65.24; H, 5.17; N, 29.59% Found C, 65.31; H, 5.22; N, 29.68%.

3-(2-((5-Nitrofuran-2-yl)methylene)hydrazinyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (IS_13): Yield: 71%; m.p. 247–248 °C; ESI-MS showed 324 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.34 (s, 1H), 11.10 (s, 1H), 7.99 (s, 1H), 7.93 (d, *J* = 8.0 Hz, 1H), 7.72 (d, *J* = 7.6 Hz, 1H), 7.56–7.49 (m, 3H), 7.45 (t, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.6, 154.0, 148.3, 137.3, 136.9, 134.3, 132.4, 131.9, 129.1, 127.8, 126.4, 125.2, 119.3, 114.4. Anal calcd for $C_{14}H_9N_7O_3$: C, 52.02; H, 2.81; N, 30.33 % Found C, 52.11; H, 2.97; N, 30.42 %.

3-(2-((5-Nitrothiophen-2-yl)methylene)hydrazinyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (IS_14): Yield: 69%; m.p. 245–246 °C; ESI-MS showed 340 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.23 (s, 1H), 11.01 (s, 1H), 7.92 (s, 1H), 7.86 (d, *J* = 7.6 Hz, 1H), 7.69 (d, *J* = 7.2 Hz, 1H), 7.57 (t, 1H), 7.42–7.36 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.4, 152.9, 147.4, 136.7, 135.4, 134.8, 131.3, 129.9, 129.4, 128.0, 126.6, 124.3, 117.8, 115.0. Anal calcd for $C_{14}H_9N_7O_2S$: C, 49.55; H, 2.67; N, 28.89 % Found C, 49.66; H, 2.72; N, 28.96 %.

3-(2-(3-Fluorobenzylidene)hydrazinyl)-8-methyl-5*H*-[1,2,4]triazino[5,6-*b*]indole (IS_15): Yield: 74%; m.p. 235–236 °C; ESI-MS showed 321 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.02 (s, 1H), 9.74 (s, 1H), 8.10 (s, 1H), 7.77 (s, 1H), 7.65–7.54 (m, 5H), 7.45 (s, 1H), 2.52 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.4, 152.6, 144.7, 143.2, 139.6, 137.3, 135.3, 134.7, 133.0, 130.4, 129.4, 127.1, 126.0, 124.5, 123.2, 116.2, 22.9. Anal calcd for $C_{17}H_{13}FN_6$: C, 63.74; H, 4.09; N, 26.24 % Found C, 63.87; H, 4.12; N, 26.31 %.

8-Methyl-3-(2-(3-methylbenzylidene)hydrazinyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (IS_16): Yield: 75%; m.p. 264–265 °C; ESI-MS showed 317 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.29 (s, 1H), 9.92 (s, 1H), 8.01 (s, 1H), 7.72 (s, 1H), 7.81–7.64 (m, 6H), 2.36 (s, 3H), 2.31 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.3, 149.5, 143.7, 142.1, 138.4, 137.1, 134.6, 133.3, 132.8, 131.4, 128.3, 126.3, 125.2, 124.7, 123.3, 117.0, 22.9, 22.3. Anal calcd for $C_{18}H_{16}N_6$: C, 68.34; H, 5.10; N, 26.56% Found C, 68.46; H, 5.22; N, 26.72 %.

2-((2-(8-Methyl-5*H*-[1,2,4]triazino[5,6-*b*]indol-3-yl)hydrazono)methyl)phenol (IS_17): Yield: 78%; m.p. 234–235 °C; ESI-MS showed 319 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.98 (s, 1H), 9.72 (s, 1H), 9.81 (s, 1H), 7.92 (s, 1H), 7.81(s, 1H), 7.63–7.53 (m, 6H), 2.41 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.9, 159.5, 146.5, 142.6, 139.6, 138.3, 136.0, 134.8, 133.8, 132.4, 127.5, 125.6, 124.2, 123.8, 120.3, 117.5, 21.9. Anal calcd for $C_{17}H_{14}N_6O$: C, 64.14; H, 4.43; N, 26.40 % Found C, 64.21; H, 4.54; N, 26.53 %.

3-(2-(4-Methoxybenzylidene)hydrazinyl)-8-methyl-5*H*-[1,2,4]triazino[5,6-*b*]indole (IS_18): Yield: 83%; m.p. 269–270 °C; ESI-MS showed 333 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.21 (s, 1H), 9.79 (s, 1H), 7.99 (s, 1H), 7.72 (d, *J* = 8.0 Hz, 2H), 7.62 (s, 1H), 7.51 (d, *J* = 8.0 Hz, 2H), 7.48 (d, *J* = 8.0 Hz, 1H), 7.39 (d, *J* = 7.6 Hz, 1H), 3.76 (s, 3H), 2.38 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.4, 160.2, 146.3, 139.6, 136.4, 135.3, 133.4(2C), 132.2, 128.8(2C),

127.3, 126.2, 123.3, 119.9, 117.2, 61.2, 22.4. Anal calcd for $C_{18}H_{16}N_6O$: C, 65.05; H, 4.85; N, 25.29 % Found C, 65.12; H, 4.92; N, 25.36 %.

3-(2-(4-(Benzyloxy)benzylidene)hydrazinyl)-8-methyl-5*H*-[1,2,4]triazino[5,6-*b*]indole (IS_19):

Yield: 85%; m.p. 261–262 °C; ESI-MS showed 409 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.01 (s, 1H), 10.54 (s, 1H), 8.01 (s, 1H), 7.77 (d, *J* = 7.2 Hz, 2H), 7.72 (s, 1H), 7.63 (d, *J* = 7.6 Hz, 2H), 7.54–7.36 (m, 7H), 5.20 (s, 2H), 2.43 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.3, 159.3, 147.1, 139.0, 137.3, 135.4, 134.3, 133.4, 132.1, 129.2(2C), 128.6(2C), 127.8, 127.2(2C), 126.5, 125.9(2C), 124.4, 122.3, 117.7, 72.4, 23.1. Anal calcd for $C_{24}H_{20}N_6O$: C, 70.57; H, 4.94; N, 20.58 % Found C, 70.63; H, 4.99; N, 2.61 %.

8-Methyl-3-(2-(3-nitrobenzylidene)hydrazinyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (IS_20): Yield: 76%; m.p. 275–276 °C; ESI-MS showed 348 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.99 (s, 1H), 9.74 (s, 1H), 8.31 (s, 1H), 7.99 (s, 1H), 7.81–7.72 (m, 3H), 7.66 (s, 1H), 7.54 (d, *J* = 8.0 Hz, 1H), 7.45 (d, *J* = 7.6 Hz, 1H), 2.37 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.8, 152.5, 144.0, 142.3, 139.6, 136.5, 136.2, 135.9, 134.3, 133.0, 128.3, 126.4, 125.1, 124.0, 120.6, 116.9, 22.2. Anal calcd for $C_{17}H_{13}N_7O_2$: C, 58.79; H, 3.77; N, 28.23 % Found C, 58.89; H, 3.92; N, 28.31 %.

***N*,*N*-Dimethyl-4-((2-(8-methyl-5*H*-[1,2,4]triazino[5,6-*b*]indol-3-yl)hydrazono)methyl) aniline (IS_21):**

Yield: 81%; m.p. 264–265 °C; ESI-MS showed 346 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.33 (s, 1H), 9.54 (s, 1H), 8.19 (s, 1H), 7.74 (d, *J* = 7.6 Hz, 2H), 7.63 (s, 1H), 7.45–7.39 (m, 4H), 3.12 (s, 6H), 2.49 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.3, 154.8, 142.6, 140.3, 138.6, 133.2, 130.6, 129.9(2C), 128.6, 127.9, 126.4, 124.3(2C), 120.2, 115.1, 44.1(2C), 22.8. Anal calcd for $C_{19}H_{19}N_7$: C, 66.07; H, 5.54; N, 28.39 % Found C, 66.18; H, 5.71; N, 28.49 %.

8-Methyl-3-(2-((5-nitrofuran-2-yl)methylene)hydrazinyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (IS_22):

Yield: 73%; m.p. 239–240 °C; ESI-MS showed 338 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.18 (s, 1H), 9.97 (s, 1H), 8.20 (s, 1H), 7.82 (s, 1H), 7.74–7.59 (m, 3H), 7.49 (d, *J* = 7.6 Hz, 1H), 2.46 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.3, 156.3, 147.8, 138.6, 137.5, 136.6, 134.9, 133.6, 128.8, 128.3, 127.3, 126.1, 119.6, 115.2, 23.2. Anal calcd for $C_{15}H_{11}N_7O_3$: C, 53.41; H, 3.29; N, 29.07 % Found C, 53.55; H, 3.31; N, 29.23 %.

8-Fluoro-3-(2-(4-fluorobenzylidene)hydrazinyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (IS_23): Yield: 85%; m.p. 214–215 °C; ESI-MS showed 325 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.07 (s, 1H), 9.72 (s, 1H), 7.81 (s, 1H), 7.72 (d, *J* = 7.6 Hz, 2H), 7.54 (s, 1H), 7.48 (d, *J* = 7.2 Hz, 2H), 7.42 (d, *J* = 6.4 Hz, 1H), 7.27 (d, *J* = 6.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.6, 159.7, 148.4, 139.4, 138.6, 137.3, 133.9(2C), 129.9, 127.4, 125.3(2C), 124.4, 121.9, 119.7, 116.3. Anal calcd for $C_{16}H_{10}F_2N_6$: C, 59.26; H, 3.11; N, 25.92 % Found C, 59.32; H, 3.21; N, 25.99 %.

8-Fluoro-3-(2-(4-methylbenzylidene)hydrazinyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (IS_24): Yield: 86%; m.p. 274–275 °C; ESI-MS showed 321 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.20 (s, 1H), 9.81 (s, 1H), 7.89 (s, 1H), 7.72 (d, *J* = 6.8 Hz, 2H), 7.56 (d, *J* = 7.2 Hz, 2H), 7.49–7.43 (m, 3H), 2.41 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.6, 152.9, 144.3, 142.3, 138.9, 136.7, 132.1, 129.8(2C), 128.6, 127.9(2C), 126.3, 121.4, 118.9, 115.6, 22.7. Anal calcd for $C_{17}H_{13}FN_6$: C, 63.74; H, 4.09; N, 26.24 % Found C, 63.81; H, 4.14; N, 26.32 %.

2-((2-(8-Fluoro-5*H*-[1,2,4]triazino[5,6-*b*]indol-3-yl)hydrazono)methyl)phenol (IS_25): Yield: 83%; m.p. 239–240 °C; ESI-MS showed 323 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.67 (s, 1H), 9.92 (s, 1H), 8.94 (s, 1H), 8.12 (s, 1H), 7.75–7.63 (m, 6H), 6.72 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.2, 158.4, 144.0, 137.9, 137.3, 136.6, 134.5, 133.9, 131.3, 129.6, 127.8, 126.6, 126.0, 124.7, 120.6, 117.3. Anal calcd for $C_{16}H_{11}FN_6O$: C, 59.63; H, 3.44; N, 26.08 % Found C, 59.71; H, 3.51; N, 26.12 %.

8-Fluoro-3-(2-(2-methoxybenzylidene)hydrazinyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (IS_26): Yield: 84%; m.p. 214–215 °C; ESI-MS showed 337 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.29 (s, 1H), 10.22 (s, 1H), 8.19 (s, 1H), 7.77–7.69 (m, 4H), 7.24–7.14 (m, 3H), 3.70 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.3, 156.3, 147.3, 140.4, 139.8, 136.2, 135.1, 133.9, 131.6,

128.9, 127.7, 127.2, 126.2, 125.8, 123.6, 118.1, 61.1. Anal calcd for $C_{17}H_{13}FN_6O$: C, 60.71; H, 3.90; N, 24.99% Found C, 60.76; H, 3.94; N, 25.04%.

3-(2-(4-(Benzyloxy)benzylidene)hydrazinyl)-8-fluoro-5*H*-[1,2,4]triazino[5,6-*b*]indole (IS_27):

Yield: 88%; m.p. 242–243 °C; ESI-MS showed 413 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.33 (s, 1H), 9.99 (s, 1H), 8.19 (s, 1H), 7.81 (d, *J* = 8.0 Hz, 2H), 7.76 (S, 1H), 7.72–7.63 (m, 7H), 7.41 (d, *J* = 8.0 Hz, 2H), 5.49 (S, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.7, 158.3, 154.1, 140.4, 138.4, 137.3, 136.9, 132.3(2C), 130.6, 129.6(2C), 128.2, 127.8(2C), 126.0, 125.9(2C), 124.1, 122.7, 119.2, 115.4, 72.7. Anal calcd for $C_{23}H_{17}FN_6O$: C, 66.98; H, 4.15; N, 20.38% Found C, 66.81; H, 4.21; N, 20.45%.

8-Fluoro-3-(2-(3-nitrobenzylidene)hydrazinyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (IS_28): Yield: 91%; m.p. 262–263 °C; ESI-MS showed 352 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.00 (s, 1H), 10.10 (s, 1H), 8.21 (s, 1H), 7.83–7.66 (m, 6H), 7.31 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.4, 158.4, 146.6, 139.6, 137.3, 134.8, 133.9, 132.6, 130.7, 128.0, 127.3, 126.3, 125.3, 123.8, 122.4, 118.2. Anal calcd for $C_{16}H_{10}FN_7O_2$: C, 54.70; H, 2.87; N, 27.91% Found C, 54.79; H, 2.89; N, 27.99 %.

4-((2-(8-Fluoro-5*H*-[1,2,4]triazino[5,6-*b*]indol-3-yl)hydrazono)methyl)-*N*,*N*-dimethylaniline (IS_29):

Yield: 84%; m.p. 251–252 °C; ESI-MS showed 350 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.98 (s, 1H), 10.62 (s, 1H), 8.01 (s, 1H), 7.54–7.45 (m, 5H), 7.11 (d, *J* = 7.2 Hz, 2H), 2.88 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.6, 160.3, 153.6, 138.9, 137.9, 136.7, 133.4, 131.4(2C), 129.5, 128.4, 127.3(2C), 125.1, 123.8, 116.8, 43.7(2C). Anal calcd for $C_{18}H_{16}FN_7$: C, 61.88; H, 4.62; F, 5.44; N, 28.06% Found C, 59.31; H, 4.156; N, 26.90%.

8-Fluoro-3-(2-((5-nitrofuran-2-yl)methylene)hydrazinyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (IS_30): Yield: 80%; m.p. 247–248 °C; ESI-MS showed 342 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.32 (s, 1H), 10.29 (s, 1H), 8.10 (s, 1H), 7.96 (d, *J* = 8.0 Hz, 1H), 7.47–7.36 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.8, 162.7, 157.6, 142.6, 139.5, 137.4, 136.2, 132.4,

129.5, 128.9, 127.4, 126.6, 118.8, 116.5. Anal calcd for $C_{14}H_8FN_7O_3$: C, 49.27; H, 2.36; N, 28.73 % Found C, 49.31; H, 2.34; N, 28.91 %.

8-Fluoro-3-(2-((5-nitrothiophen-2-yl)methylene)hydrazinyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (IS_31):

Yield: 75%; m.p. 229–230 °C; ESI-MS showed 358 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.47 (s, 1H), 11.12 (s, 1H), 8.12 (s, 1H), 7.99 (d, *J* = 7.6 Hz, 1H), 7.50–7.38 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.2, 162.3, 156.3, 143.1, 138.3, 136.9, 136.3, 133.0, 129.6, 128.4, 127.9, 126.7, 119.3, 116.8. Anal calcd for $C_{14}H_8FN_7O_2S$: C, 47.06; H, 2.26; N, 27.44 % Found 47.11; H, 2.30; N, 27.49 %.

3-(2-Benzylidenehydrazinyl)-8-chloro-5*H*-[1,2,4]triazino[5,6-*b*]indole (IS_32):

Yield: 84%; m.p. 251–252 °C; ESI-MS showed 323 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.25 (s, 1H), 9.87 (s, 1H), 8.03 (s, 1H), 7.68 (s, 1H), 7.81–7.69 (m, 7H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.3, 151.4, 143.9, 140.2, 138.9, 137.7, 136.3, 134.8, 133.3, 131.4, 129.6, 127.4, 126.2, 125.3, 122.4, 115.9. Anal calcd for $C_{16}H_{11}ClN_6$: C, 59.54; H, 3.44; N, 26.04% Found C, 59.59; H, 3.49; N, 26.10 %.

8-Chloro-3-(2-(4-fluorobenzylidene)hydrazinyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (IS_33): Yield: 90%; m.p. 232–233 °C; ESI-MS showed 341 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.02 (s, 1H), 9.79 (s, 1H), 7.72 (s, 1H), 7.69 (d, *J* = 7.6 Hz, 2H), 7.58 (s, 1H), 7.47 (d, *J* = 7.2 Hz, 2H), 7.39 (d, *J* = 6.4 Hz, 1H), 7.27 (d, *J* = 6.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.4, 162.3, 146.4, 140.2, 138.6, 135.3, 134.6, 132.8(2C), 130.4, 129.2, 127.4(2C), 125.1, 123.2, 114.3. Anal calcd for $C_{16}H_{10}ClFN_6$: C, 56.40; H, 2.96; N, 24.66 % Found C, 56.54; H, 2.99; N, 24.81 %.

8-Chloro-3-(2-(4-methylbenzylidene)hydrazinyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (IS_34): Yield: 86%; m.p. 229–230 °C; ESI-MS showed 337 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.30 (s, 1H), 9.90 (s, 1H), 8.03 (s, 1H), 7.82 (s, 1H), 7.81–7.66 (m, 7H), 2.34 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.3, 150.4, 146.2, 140.3, 137.7, 135.9, 133.4, 129.6, 129.2(2C), 128.6, 127.9(2C), 123.4, 120.7, 116.1, 23.2. Anal calcd for $C_{17}H_{13}ClN_6$: C, 68.34; H, 5.10; N, 26.56% Found C, 68.46; H, 5.14; N, 26.62 %.

7-Chloro-3-(2-(4-isopropylbenzylidene)hydrazinyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (IS_35): Yield: 78%; m.p. 224–225 °C; ESI-MS showed 365 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.21 (s, 1H), 9.95 (s, 1H), 7.94 (s, 1H), 7.72 (s, 1H), 7.61–7.54 (m, 2H), 7.42 (t, 2H), 7.23(d, *J* = 7.2 Hz, 2H), 2.62 (m, 1H), 1.26 (d, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.9, 148.3, 144.1, 139.8, 136.6, 134.5, 132.0, 129.7(2C), 128.4, 128.1, 127.6(2C), 125.3, 120.4, 116.3, 35.1, 23.4(2C). Anal calcd for $C_{19}H_{17}ClN_6$: C, 62.55; H, 4.70; N, 23.04 % Found C, 62.59; H, 4.76; N, 23.09 %.

2-((2-(8-Chloro-5*H*-[1,2,4]triazino[5,6-*b*]indol-3-yl)hydrazono)methyl)phenol (IS-36):

Yield: 79%; m.p. 235–236 °C; ESI-MS showed 339 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.97 (s, 1H), 9.72 (s, 1H), 7.92 (s, 1H), 7.72–7.54 (m, 7H), 6.55 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.2, 163.4, 148.1, 140.3, 138.3, 136.2, 134.7, 133.9, 131.6, 129.2, 128.3, 127.5, 126.9, 124.0, 118.3, 115.8. Anal calcd for $C_{16}H_{11}ClN_6O$: C, 56.73; H, 3.27; N, 24.81 % Found C, 56.37; H, 3.34; N, 24.56 %.

8-Chloro-3-(2-(2-methoxybenzylidene)hydrazinyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (IS_37): Yield: 86%; m.p. 239–240 °C; ESI-MS showed 353 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.27 (s, 1H), 10.21 (s, 1H), 8.19 (s, 1H), 7.78–7.67 (m, 4H), 7.24–7.12 (m, 3H), 3.69 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.4, 158.4, 148.7, 143.2, 138.6, 137.8, 136.0, 135.3, 133.2, 130.3, 129.6, 128.0, 126.9, 125.3, 124.2, 119.3, 60.9. Anal calcd for $C_{17}H_{13}ClN_6O$: C, 57.88; H, 3.71; N, 23.82 % Found C, 57.76; H, 3.89; N, 23.87%.

3-(2-(2-(Benzyloxy)benzylidene)hydrazinyl)-8-chloro-5*H*-[1,2,4]triazino[5,6-*b*]indole (IS_38): Yield: 92%; m.p. 247–248 °C; ESI-MS showed 429 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.93 (s, 1H), 9.81 (s, 1H), 8.28 (s, 1H), 7.81–7.77 (m, 8H), 7.54–7.47 (m, 4H), 5.29 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.4, 163.4, 156.5, 143.2, 139.9, 138.2, 137.4, 134.0(2C), 132.7, 130.4(2C), 129.4, 129.0, 128.3(2C), 126.4(2C), 125.2, 123.1, 118.8, 116.6, 73.1. Anal calcd for $C_{23}H_{17}ClN_6O$: C, 64.41; H, 4.00; N, 19.60 % Found C, 64.76; H, 4.12; N, 19.71%.

8-Chloro-3-(2-(3-nitrobenzylidene)hydrazinyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (IS_39): Yield: 93%; m.p. 254–255 °C; ESI-MS showed 368 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ

10.17 (s, 1H), 8.80 (s, 1H), 8.10 (s, 1H), 7.90–7.72 (m, 6H), 7.27 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.6, 153.6, 147.3, 140.2, 138.6, 136.3, 134.3, 133.0, 131.9, 129.4, 128.4, 126.8, 125.9, 124.3, 123.6, 119.3. Anal calcd for $C_{16}H_{10}ClN_7O_2$: C, 52.26; H, 2.74; N, 26.66 % Found C, 52.33; H, 2.89; N, 26.81 %.

4-((2-(8-Chloro-5*H*-[1,2,4]triazino[5,6-*b*]indol-3-yl)hydrazono)methyl)-*N*,*N*-dimethylaniline (IS_40):

Yield: 89%; m.p. 249–250 °C; ESI-MS showed 366 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.33 (s, 1H), 10.71 (s, 1H), 7.97 (s, 1H), 7.47–7.36 (m, 5H), 7.09 (d, *J* = 6.4 Hz, 2H), 2.97 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.3, 162.3, 154.9, 140.3, 138.7, 137.4, 134.6, 132.6(2C), 130.3, 129.8(2C), 128.2, 126.9, 124.5, 117.3, 43.5(2C). Anal calcd for $C_{18}H_{16}ClN_7$: C, 59.10; H, 4.41; N, 26.80% Found C, 59.31; H, 4.156; N, 26.90%.

8-Chloro-3-(2-((5-nitrofuran-2-yl)methylene)hydrazinyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (IS_41):

Yield: 81%; m.p. 246–247 °C; ESI-MS showed 358 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.52 (s, 1H), 10.89 (s, 1H), 8.12 (s, 1H), 7.92 (d, *J* = 8.0 Hz, 1H), 7.63 (d, *J* = 7.6 Hz, 1H), 7.54–7.45 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.4, 162.4, 157.2, 141.4, 140.3, 137.0, 136.6, 133.1, 128.7, 128.3, 127.2, 125.8, 117.6, 114.8. Anal calcd for $C_{14}H_8ClN_7O_3$: C, 47.01; H, 2.25; N, 27.41 % Found C, 47.10; H, 2.29; N, 27.45 %.

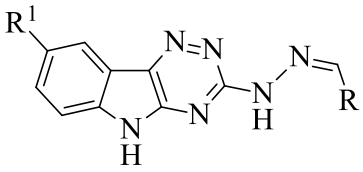
8-Chloro-3-(2-((5-nitrothiophen-2-yl)methylene)hydrazinyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole (IS_42):

Yield: 78%; m.p. 238–239 °C; ESI-MS showed 374 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.45 (s, 1H), 11.14 (s, 1H), 8.10 (s, 1H), 7.97 (d, *J* = 7.6 Hz, 1H), 7.45–7.36 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.8, 161.9, 157.3, 140.7, 141.1, 137.3, 136.2, 132.6, 127.9, 126.8, 126.2, 124.2, 116.8, 114.4. Anal calcd for $C_{14}H_8ClN_7O_2S$: C, 44.99; H, 2.16; N, 26.23 % Found C, 45.10; H, 2.23; N, 26.34 %.

5.7.4. *In vitro M. tuberculosis* screening, *M. tuberculosis* PknB 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. INH, EMB were used as reference compounds for comparison. All the compounds were also screened *in vitro* for their *M. tuberculosis* PknB 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 5.14**.

Table 5.14: *In vitro* biological evaluation of synthesized compounds **IS_04** – **IS_42**



IS_ 04 - IS_ 42

Compd	R^1	R	MTB PKnB IC_{50} in µM	MTB MIC in µM[a]	Cytotoxicity[b] at 50 µM % inhibition
IS_04	H	Phenyl	5.29	2.70	18.02
IS_05	H	4-Fluorophenyl	15.13	81.67	NT
IS_06	H	3-Tolyl	12.40	82.69	NT
IS_07	H	4-isopropylphenyl	16.74	37.87	NT
IS_08	H	2-Hydroxyphenyl	18.32	82.23	NT
IS_09	H	2-Methoxyphenyl	>20	19.65	28.34
IS_10	H	2-Benzyloxyphenyl	15.67	31.69	NT
IS_11	H	3-Nitrophenyl	5.02	2.34	19.51
IS_12	H	4-Dimethylaminophenyl	8.64	18.85	39.10
IS_13	H	5-Nitro-2-furyl	7.23	4.83	24.12
IS_14	H	5-Nitro-2-thiophenyl	8.17	9.21	25.48

Contd

Compd	R^1	R	MTB PKnB IC_{50} in µM	MTB MIC in µM[a]	Cytotoxicity[b] at 50 µM % inhibition
IS_15	CH_3	4-Fluorophenyl	7.28	19.53	35.21
IS_16	CH_3	3-Tolyl	6.78	19.75	26.34
IS_17	CH_3	2-Hydroxyphenyl	6.12	19.65	17.34
IS_18	CH_3	2-Methoxyphenyl	8.27	18.80	39.19
IS_19	CH_3	2-Benzyloxyphenyl	17.13	61.19	NT
IS_20	CH_3	3-Nitrophenyl	6.37	>71.98	NT
IS_21	CH_3	4-Dimethylaminophenyl	6.21	36.18	NT
IS_22	CH_3	5-Nitro-2-furyl	7.34	36.05	NT
IS_23	F	4-Fluorophenyl	12.39	9.63	38.49
IS_24	F	3-Tolyl	6.03	39.02	NT
IS_25	F	2-Hydroxyphenyl	19.37	39.30	NT
IS_26	F	2-Methoxyphenyl	6.38	77.56	NT
IS_27	F	2-Benzyloxyphenyl	13.54	7.57	29.37
IS_28	F	3-Nitrophenyl	6.98	71.16	NT
IS_29	F	4-Dimethylaminophenyl	5.98	17.88	58.37
IS_30	F	5-Nitro-2-furyl	5.27	36.62	NT
IS_31	F	5-Nitro-2-thiophenyl	5.67	17.49	21.89
IS_32	Cl	Phenyl	16.34	4.84	20.14
IS_33	Cl	4-Fluorophenyl	15.34	19.11	35.64
IS_34	Cl	3-Tolyl	6.72	37.11	NT
IS_35	Cl	4-isopropylphenyl	11.92	25.04	22.38
IS_36	Cl	2-Hydroxyphenyl	7.64	41.07	NT
IS_37	Cl	2-Methoxyphenyl	12.40	71.02	NT
IS_38	Cl	2-Benzyloxyphenyl	14.37	58.28	NT
IS_39	Cl	3-Nitrophenyl	11.08	21.52	24.97
IS_40	Cl	4-Dimethylaminophenyl	>20	68.34	NT

Contd

Compd	R^1	R	MTB PKnB IC_{50} in µM	MTB MIC in µM[a]	Cytotoxicity[b] at 50 µM % inhibition
IS_41	Cl	5-Nitro-2-furyl	18.37	4.36	39.46
IS_42	Cl	5-Nitro-2-thiophenyl	7.20	8.36	36.12
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.

5.7.5. SAR and discussion

All the synthesized compounds showed activity against *M. tuberculosis* with MIC ranging from 2.34 to 82.69 µM. Out of thirty nine library molecules, eighteen molecules inhibited *M. tuberculosis* with MIC of <20 µM, nine molecules (**IS_04**, **IS_11, IS_13** – **IS_14**, **IS_23, IS_27**, **IS_32, IS_41** and **IS_42**) inhibited with MIC of <10 µM and five molecules (**IS_04**, **IS_11, IS_13**, **IS_32** and **IS_41**) inhibited with MIC of <5 µM. All the synthesized compounds were less potent than standard antitubercular compound INH, but six (**IS_04**, **IS_11, IS_13**, **IS_27, IS_32** and **IS_41**) compounds were found to be more active than first line standard drug EMB. Compound **IS_11** was found to be the most active compound *in vitro* with MIC of 2.34 µM; also Compound **IS_11** was four times more potent than EMB (MIC 7.64 µM) and two times than second line fluoroquinolone ciprofloxacin. Compound **IS_11** was also the most active among the library in inhibiting *M. tuberculosis* PknB enzyme, which inhibited PknB with IC_{50} 5.02 µM.

Many of the library molecules showed better inhibitory activity against *M. tuberculosis* PknB enzyme, there were twenty one molecules out of thirty nine inhibited *M. tuberculosis* PknB enzyme with IC_{50} less than 10 µM. And there were five molecules inhibited *M. tuberculosis* PknB with IC_{50} less than 6 µM. With respect to SAR, overall the order of activity was 8-fluoro substituted derivatives (**IS_23** – **IS_31**) showed better activity followed by 8-methyl substituted derivatives (**IS_15** – **IS_22**), unsubstituted derivatives (**IS_04** – **IS_14**) and 8-chloro substituted derivatives (**IS_32** – **IS_42**). The most activity for 8-fluoro and 8-methyl group might be to best fitting of these groups in active site of enzyme.

With respect to substitution pattern on hydrazine at 3rd position of triazine ring, phenyl ring bearing electron withdrawing group (-NO_2) (**IS_11**, **IS_20**, **IS_28**) and heteroaryl ring (**IS_13** – **IS_14**, **IS_22** and **IS_42**) showed slight better activity than neutral and electron donating groups. Apart from the antimycobacterial evaluation and enzymatic assay, the molecules were also tested for their toxicity using MTT assay method. Many of the molecules were found to be non-toxic at 50 µM concentration.

5.7.6. Highlights of the study

In summary, we identified a novel lead from the screening results of Asinex molecules, and synthesized thirty nine novel derivatives for the inhibition of *M. tuberculosis* PknB enzyme. Twenty one compounds showed *M. tuberculosis* PknB inhibition with IC_{50} <10 µM and many of them showed good *M. tubercular* MICs. Compound **IS_11** (3-(2-(3-nitrobenzylidene)hydrazinyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole) was found to be the most active compound with *M. tuberculosis* PknB IC_{50} of 5.02 µM and inhibited drug sensitive *M. tuberculosis* with MIC of 2.34 µM (**Figure 5.19**).

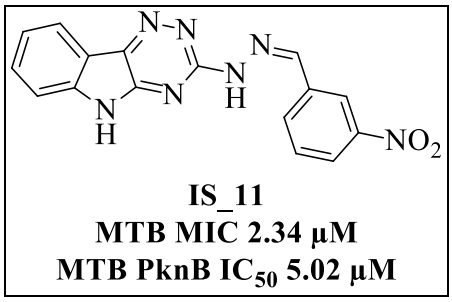


Figure 5.19: Chemical structure and biological activity of the most active compound **IS_11**

Summary and Conclusion

Chapter 6

TB, the ancient enemy of humanity and has been considered as the leading cause of death since the start of last century. The discovery of standard first line drugs in the middle decades of last century made TB as a curable disease, but very soon the cleverest mycobacterium developed resistnace to these drugs. There has been a considerable gap in the TB drug discovery for last few decades, which is responsible for two million deaths globally per annum.

In order to invade the massive distruction of TB, there are many organisations working togethere, they are publishing the screening data of their library molecules to allow the global researchers to access the high quality screeing data for further development. In the literature, there were many chemical entities possessing *M. tuberculosis* with MIC of <1 µM, but they are not turning into potent drug candidates due to many other side reactions.

We have chosen potent molecules form commercial database and 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 lead molecules were taken up for library generation, a total of 250 molecules from seven different series were synthesized and biologically evaluated in our laboratory.

Among the molecules of 5-heteroarylsubstituted 2-amino 1,3,4-thiadiazole series, compound **TD_09** [1-(5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-yl)-3-(4-nitrophenyl)urea] emerged as the most active molecule with promising inhibitory activity against *M. tuberculosis* PknB enzyme with an IC_{50} of 0.81 µM, it also displayed good activity against *M. tuberculosis* H37RV with an MIC of 2.07 µM.

Among the tetrahydrothieno[2,3-*c*]pyridine-3-carboxamides and hexahydrocycloocta [*b*]thiophene-3-carboxamide derivatives, the compound **OT_09** [2-(4-phenoxybenzamido)-4,5,6,7,8,9-hexahydrocycloocta[*b*]thiophene-3-carboxamide] emerged as the most active compound exhibiting remarkable inhibition against *M. tuberculosis* with an MIC of 3.70 µM and it also exhibited MIC of 1.23 µM in presence of efflux pump inhibitor verapamil and was found to be non-cytotoxic at 50 µM.

Among the 2-iminothiazolidin-4-one derivatives, compound **TZ_09** [5-(4-methoxybenzylidene)-2-(phenylimino)thiazolidin-4-one] was found to be the most active compound *in vitro* with MICs of 1.98 µM against *M. tuberculosis* and exhibited potent activity against the enzyme *M. tuberculosis* PknB with IC_{50} of 0.52 µM.

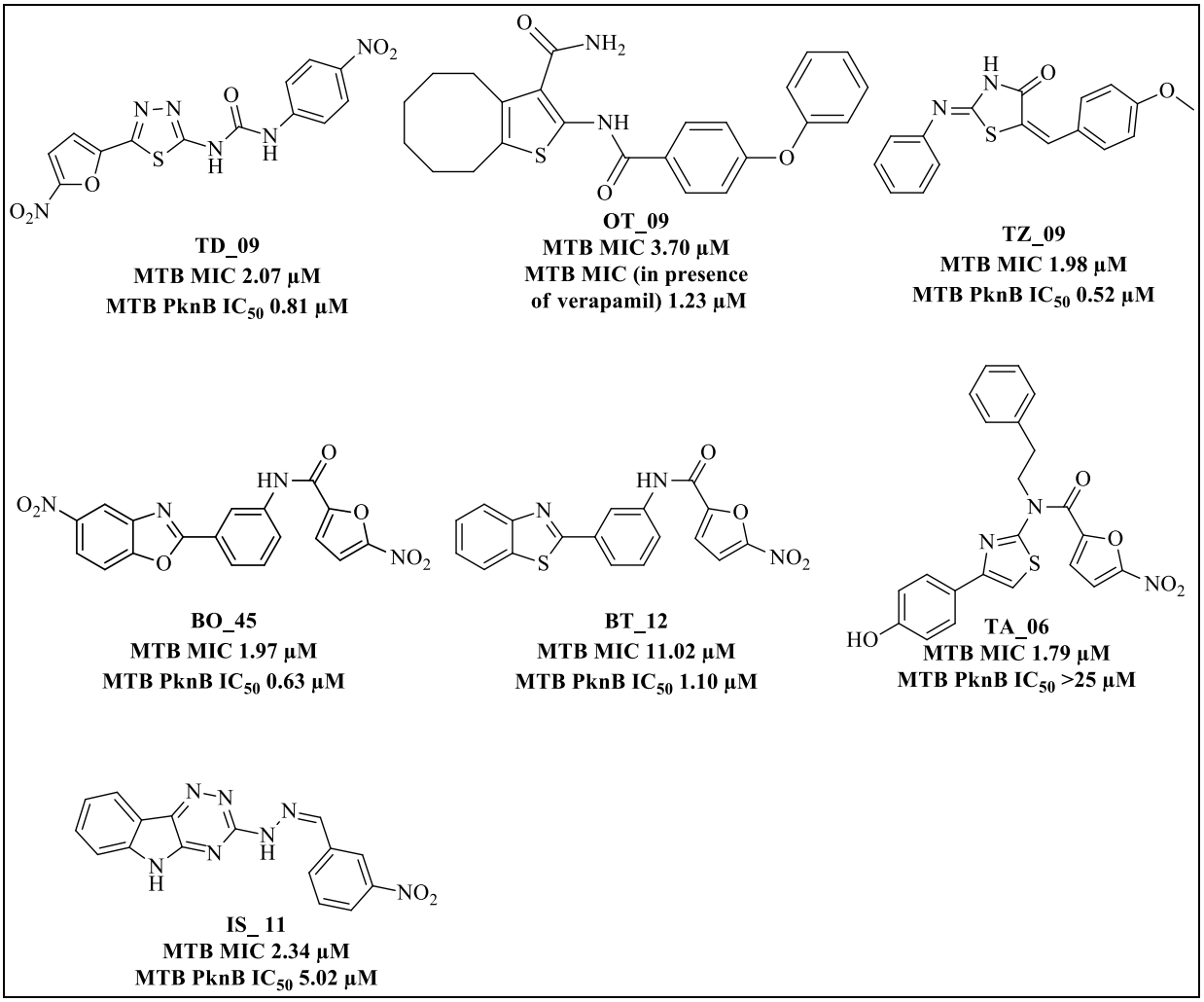
Among the derivatives of 3-(benzo[*d*]oxazol-2-yl)anilines, compound **BO_45 [**5-nitro-*N*-(3-(5-nitrobenzo[*d*]oxazol-2-yl)phenyl)furan-2-carboxamide] emerged as the most active against *M. tuberculosis* H37Rv with MIC of 1.97 µM, it also demonstrated good activity against the enzyme *M. tuberculosis* PknB with an IC_{50} of 0.63 µM.

Among the molecules of 2-(3-aminophenyl)-benzothiazole series, compound **BT_12** [*N*-(3-(benzo[*d*]thiazol-2-yl)phenyl)-5-nitrofuran-2-carboxamide] found to be the most active molecule exhibiting the inhibition of *M. tuberculosis* with MIC of 11.02 µM, which also showed *M. tuberculosis* PKnB enzyme IC_{50} = 1.10 µM.

Among the *N,N*-di-substituted-2-aminothiazoles derivatives, compound **TA_06** [*N*-(4-(4-hydroxyphenyl)thiazol-2-yl)-5-nitro-*N*-phenethylfuran-2-carboxamide] egressed as the most active molecule exhibiting the inhibition of *M. tuberculosis* with MIC of 1.79 µM, but it was found to be inactive against *M. tuberculosis* PKnB enzyme.

Among the molecules of 1,2,4-triazino[5,6-*b*]indole derivatives, compound **IS_11 [**3-(2-(3-nitrobenzylidene)hydrazinyl)-5*H*-[1,2,4]triazino[5,6-*b*]indole] was found to be the most active with *M. tuberculosis* MIC of 2.34 µM and also exhibited *M. tuberculosis* PKnB IC_{50} of 5.02 µM.

Structures of most active molecules from each series:



TD_09
MTB MIC 2.07 µM
MTB PknB IC_{50} 0.81 µM

OT_09
MTB MIC 3.70 µM
MTB MIC (in presence of verapamil) 1.23 µM

TZ_09
MTB MIC 1.98 µM
MTB PknB IC_{50} 0.52 µM

BO_45
MTB MIC 1.97 µM
MTB PknB IC_{50} 0.63 µM

BT_12
MTB MIC 11.02 µM
MTB PknB IC_{50} 1.10 µM

TA_06
MTB MIC 1.79 µM
MTB PknB IC_{50} >25 µM

IS_ 11
MTB MIC 2.34 µM
MTB PknB IC_{50} 5.02 µM

Future perspectives

The present thesis delineates the design, synthesis, biological assay and SAR of seven chemically diverse series of molecules as promising anti-tubercular agents. The molecules reported in the thesis displayed appreciable *in vitro* enzyme inhibition and potency against *M. tuberculosis* H37Rv strain. Out of synthesized 250 compounds, there are many molecules inhibiting *M. tuberculosis* with MIC < 3µM and there are few molecules inhibited *M. tuberculosis* PknB with IC_{50} < 1µM. Although the *in vitro* results are encouraging, in order to mould them into potential drug candidates further development is still needed.

- The side effect profile of all the synthesized compounds may be studied extensively.
- Sub-acute and acute toxicological screening of novel chemical entities has to be carried out.
- Pharmacodynamic and pharmacokinetic studies of the non-toxic compounds have to be undertaken in higher animal models.
- The furtherance of any of the molecules presented in this thesis along a drug development track will need a substantial investment in medicinal chemistry, preclinical and clinical studies.
- After having all the clinical data, the synthetic feasibility and cost effectiveness synthetic procedures for bulk scale preparation needs to be mapped.

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Appendix

List of Publications

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

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, **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.

4. 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.

5. Samala G, Shruti S Kakan, **Nallangi R**, Devi PB, 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.*

6. 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.*

Papers presented at Conferences

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

2. CTPS 2012 (Current Trends in Pharmaceutical Sciences)-The 2nd Annual National Symposium of BITS Pilani, Hyderabad Campus – November 2012.

3. 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 Chair 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 16 years. He has 300 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 13 Ph.D. students and 9 students are pursuing Ph.D. currently. His research is funded by agencies like the UGC, CSIR, ICMR, DBT and DST.

BIOGRAPHY OF N. RADHIKA

Ms. N. Radhika completed her Bachelor of Science and Master of Science (Organic chemistry) from Osmania University Hyderabad, Telangana. She has about 2 years of industrial experience, worked at Albany Molecular Research Inc. (AMRI) – Hyderabad Research Centre as Research Scientist. She has awarded UGC-JRF in June 2011. She has been appointed as UGC Junior Research Fellow from Apr 2012 – Apr 2014 and UGC Senior Research Fellow from May 2014 – Mar 2016 at Birla Institute of Technology & Science, Pilani, Hyderabad campus under the supervision of Prof. D. Sriram. She has published six scientific papers in well-renowned international journals and also presented papers at national and international conferences.