Synthesis and Biological Evaluation of Bacterial DNA Gyrase Inhibitors

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MANOJ C

ID No 2011PHXF026H

Under the supervision of

D. SRIRAM

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BIRLA INSTITUTE OF TECHNOLOGY & SCIENCE, PILANI

CERTIFICATE

This is to certify that the thesis entitled "**Synthesis and Biological Evaluation of Bacterial DNA Gyrase Inhibitors**" and submitted by **MANOJ C** ID No. **2011PHXF026H** for award of Ph.D. of the Institute embodies original work done by him under my supervision.

Signature of the supervisor:

Name in capital letters : **D. SRIRAM**

Designation : **Professor**

Date:

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Date: ***Manoj C***

Abstract

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

In the present study we focused on achieving promising anti-tubercular compounds by molecular design, synthesis and anti-mycobacterial evaluation of compounds based on reported anti-tubercular agents. To explore possible targets for action we subjected the synthesized compounds for *Mycobacterium tuberculosis* enzyme assay against *Mycobacterium tuberculosis* Gyrase.

In the present work, five series of compounds (total 188 compounds) were synthesized by simple and commercially feasible methods. Out of these, 28 molecules showed gyrase IC_{50} less than 5 µM. Among all the compounds **BP_24** and **IB_38** showed promising results with gyrase inhibitory potential at 0.41 and 0.72 µM and good correlating supercoiling IC_{50} of 0.72 and 0.26 µM respectively. The compounds when tested against drug sensitive strains of *Mycobacterium tuberculosis* exhibited MICs 48.31 and 1.38 µM respectively.

Among the 188 molecules, 81 molecules were found to show supercoiling IC_{50} less than 5 µM. With respect to supercoiling assay **BZ_18** and **IB_38** with IC_{50s} of 0.51 and 0.26 µM were identified as potent molecules. These compounds when tested against drug sensitive strains of *Mycobacterium tuberculosis* showed MICs 4.41 and 1.38 µM respectively.

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

We believe that the present class of inhibitors reported as potent, selective and no cytotoxicity with few compounds could emerge as valid leads for further chemical optimization as novel potential anti-tubercular agents.

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List of Abbreviations

µg	:	Microgram
µM	:	Micromolar
^{13}C NMR	:	Carbon Nuclear Magnetic Resonance
^{1}H NMR	:	Proton Nuclear Magnetic Resonance
ATP	:	Adenosine Triphosphate
d	:	Doublet
DCM	:	Dichloromethane
DMF	:	*N*,*N*-Dimethylformamide
DMSO-d_6	:	Dimethyl sulphoxide deuterated
DNA	:	Deoxyribonucleic acid
DOTS	:	Directly Observed Treatment, Short course
DSF	:	Differential Scanning Fluorimeter
EMA	:	European Medicines Agency
HIV	:	Human Immunodeficiency Virus
HTS	:	High throughput screening
IC_{50}	:	Half Maximal Inhibitory Concentration
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
PDB	:	Protein Data Bank
ppm	:	Parts per million
RNA	:	Ribonucleic acid
rRNA	:	Ribosomal Ribonucleic acid
s	:	Singlet
SAR	:	Structure Activity Relationship
t	:	Triplet
TB	:	Tuberculosis
TDR-TB	:	Totally Drug-Resistant *Mycobacterium tuberculosis*
TEA	:	Triethylamine
TFA	:	Trifluoroacetic acid
THF	:	Tetrahydrofuran
TLC	:	Thin-layer chromatography
T_m	:	Melting temperature
TMS	:	Tetramethylsilane
US	:	United States
WHO	:	World Health Organisation
XDR-TB	:	Extensively Drug-Resistant *Mycobacterium tuberculosis*
XP	:	Extra Precision
δ	:	Chemical shift

Introduction

Chapter 1

Koch's disease or tuberculosis (TB) caused by *Mycobacterium tuberculosis*, a facultative intracellular organism discovered by Robert Koch in 1882 is an ancient contagious disease that currently presents an immense global health challenge. The fate of the initial exposure to *Mycobacterium tuberculosis* varies from immediate elimination of the organism by the host's innate immune response to infected individuals developing active primary TB [Flynn J.L., *et al*., 2001]. In immunocompetent individuals the initial acute infection is controlled by the immune system, and living bacteria are confined in a peculiar localized pulmonary structure called granuloma. There the bacteria endures indefinitely in a latent non-virulent form, and gets reactivated whenever an immunosuppressive condition occurs [Ferraris D.M., *et al*., 2011]. However, these patients have the risk of 5-10 % to develop active form during their life even with the absence of any cause of immunosuppression (**Figure 1.1**) [Clark-Curtiss., *et al*., 2003]. The increasing emergence of drug resistant TB and HIV co-infection, which compromises host defence and allows latent infection to reactivate or render individuals more susceptible to TB, pose as further challenge for effective control of the disease [Corbett E.L., *et al*., 2003].

The World Health Organization (WHO) estimates that in 2014, about 9 million people developed TB and 1.5 million died from the disease (3,60,000 of whom were HIV-positive), with the overwhelming majority of these from developing parts of the world [WHO Global Tuberculosis Report 2014]. An estimated 1.1 million (13%) of the 9 million people who developed TB in 2013 were HIV-positive. In 2013, an estimated 5,10,000 women died as a result of TB. From 2000 to 2013, 37 million lives were saved through effective diagnosis and treatment. Out of 9 million people who developed TB in 2013, more than half (56%) were in the South-East Asia and Western Pacific regions. India and China alone accounted for 24% and 11% of total cases, respectively. This situation highlights the relative shortcomings of the current treatment strategies for TB and the limited effectiveness of public health systems; particularly in resource-poor countries where the main TB burden lies.

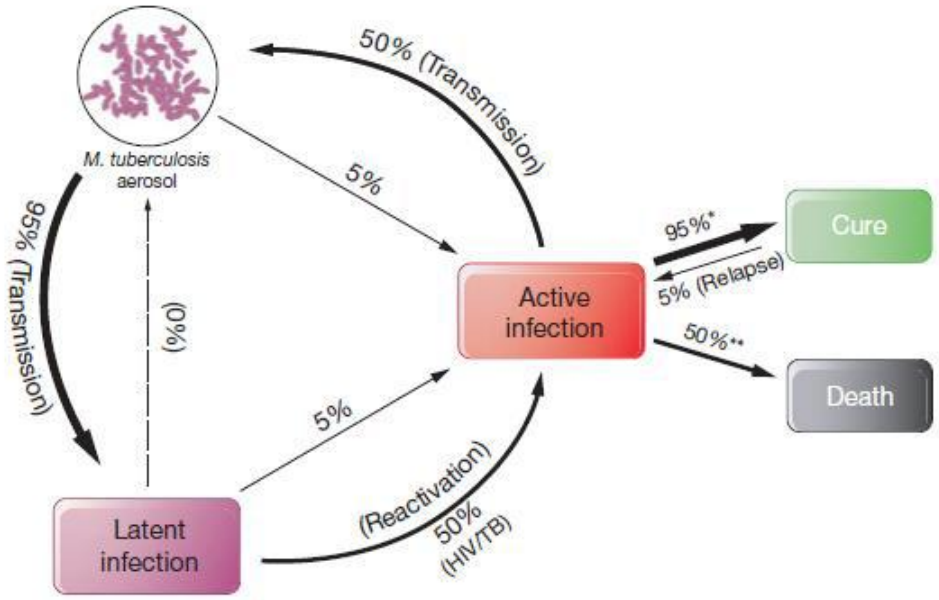


Figure 1.1: Stages of *Mycobacterium tuberculosis* infection [Koul A., *et al*., 2011] *Mycobacterium tuberculosis* aerosol can transmit and progress into infectious TB or can remain non-infectious (latent) disease depending upon person's immune system. A sizeable pool of latently infected people may relapse into active TB, years after their first exposure to the bacterium. Latent TB is commonly activated by immune suppression, as in the case of HIV. In cases of drug-susceptible-TB (denoted by an asterisk), 95% of patients recover upon treatment, whereas 5% relapse. If untreated (denoted by two asterisks), high mortality results [Koul A., *et al*., 2011].

1.1. *Mycobacterium tuberculosis* - the etiological agent of TB

Mycobacterium tuberculosis is a causative agent for TB which is found in one third of world's population. It has a complex cell wall which is responsible for survival in host for many years in dormant form. The cell wall is composed of peptidoglycans and lipids mainly mycolic acids which are a significant determinant of its virulence [Rivers E.C., *et al*., 2008; Barrera L., *et al*., 2007; Godreuil S., *et al*., 2007]. This contributes to the chronic nature of the disease, imposing lengthy treatment regimens and represents a formidable obstacle for researchers [Cole S.T., *et al.*, 1998].

1.2. History of the current TB drug chemotherapy

During the period from 1950's to 60's, most of the first-line TB drugs were discovered [Villemagne D., *et al*., 2012]. The first effective anti-tubercular agent streptomycin, was

discovered in 1943. This aminoglycoside blocks protein biosynthesis through an interaction with the small 30S subunit of the ribosome [Carter A.P. *et al.*, 2000; Jones D., *et al*., 1944; Schatz A., *et al*., 1944]. Later in the year 1946, para-aminosalicylic acid was discovered. One of the most active anti-tubercular drug isoniazid, a mycolic acid biosynthesis inhibitor was also discovered in the same year [Timmins G.S. and Deretic V., 2006; Fox W., *et al*., 1999; Bernstein J., *et al*., 1952]. A significant reduction in the TB treatment period from 9 to 6 months was accomplished by the invention of pyrazinamide in 1952 [Palomino J.C., *et al.*, 2014; Malone L., *et al*., 1952]. Later ethionamide and prothionamide arised as anti-tubercular drugs because of the further studies carried out on pyrazinamide. Inspired from polyamines and diamines, a series of diamine analogues were synthesized and investigated for anti-tubercular activity studies which lead to the discovery of ethambutol in 1961 [Thomas J.P., *et al*., 1961]. The last member of the present first line anti-tubercular drug, rifampin appeared in 1971 was found to be effective against replicating and non-replicating mycobacteria and this class of drugs inhibits the RNA synthesis by binding to the β-subunit of the DNA-dependent polymerase [Wehrli W., 1983; McClure W.R and Cech C.L., 1978; Binda G., *et al*., 1971]. Inspite the discovery of first line drugs for treatment TB, soon the mycobacterium has developed resistance towards monotherapy initiating the need of combination therapy. The WHO-recommended DOTS (directly observed treatment, short course) anti-tubercular therapy involving a 6-month chemotherapy regimen using a combination of 4 drugs (rifampicin, isoniazid, ethambutol, and pyrazinamide for 2 months, followed by rifampicin and isoniazid for 4 months) with cure rates of approximately 90% in human immunodeficiency virus (HIV)–negative patients is the globally accepted standard treatment of drug-susceptible, active TB [Haydel S.E., 2010]. In this active bacilli in all stages were killed in two months and dormant forms were killed by continuation therapy with rifampicin and isoniazid for four months [Bhowruth V., *et al*., 2007; Raviglione M.C., *et al*., 2007; Bayer R., *et al*., 1995].

Development of resistance to first line drugs initiated the urge of second line drugs [Dorman S.E., *et al*., 2007]. However, second-line agents exhibit lower potency and/or greater toxicity. The fluoroquinolone FQ, aminoglycoside, and capreomycin antibiotics target DNA replication and protein synthesis, and offer the greatest effectiveness of the second-line anti-tubercular drugs [Mukherjee J.S., *et al.*, 2004]. The remaining antibiotics such as kanamycin, amikacin exhibit bacteriostatic activity and are considerably less potent, more toxic, and more expensive [Dorman S.E., *et al*., 2007].

1.3. The emergence of drug resistance and the challenges in TB treatment

Drawbacks in TB treatment such as long duration of therapy (6 months), use of multiple drugs and side effects leading to non-adherence of patient to therapy which resulted in multidrug resistant tuberculosis and extremely drug resistant tuberculosis forms. Mycolic acid rich waxy cell envelope, efflux pumps and chromosomally encoded resistance genes resulted *Mycobacterium tuberculosis* intrinsically resistant to many antibiotics. The major mechanisms of acquired drug resistance in *Mycobacterium tuberculosis* are categorised into: 1) Mutations in drug target: developed resistance to rifampicin, ethambutol by altering FQ binding sites of their targets, namely β-subunit of RNA polymerase [Campbell E.A., *et al.*, 2001], a glycosyltransferase [Telenti A., *et al.*, 1997], and DNA gyrase [Takiff H.E., *et al.*, 1994].

2) Inability of prodrug activation: structural mutations that lower or abolish the enzymatic activity of KatG [Hazbon M.H., *et al.*, 2006; Ramaswamy S.V., *et al.*, 2003], EthA [Morlock G.P., *et al.*, 2003; Baulard A.R., *et al.*, 2000; Debarber A.E., *et al.*, 2000], and PncA [Rajendran V., *et al*., 2013; Stoffels K., *et al.*, 2012] involved in activation of prodrugs were found to lead to isoniazid, ethionamide and pyrazinamide resistance, respectively.

3) Enzymatic inactivation of drug: in *Mycobacterium tuberculosis* β-lactamase, BlaC, provides natural resistance to pencillin [Hugonnet J.E., *et al.*, 2007]. Simillarly aminoglycosides modifying enzymes can be attributed for acquired resistance to kanamycin and amikacin [Labby K.J., *et al*., 2013; Ramirez M.S., *et al*., 2010].

1.3.1. Multi drug resistant-TB (MDR-TB)

Inappropriate use of antibiotics for TB treatment led to development of drug resistant-TB. *Mycobacterium tuberculosis* strains resistant to first line drugs mainly isoniazid and rifampicin are called as MDR-TB. Globally in 2013, 3.5% of new and 20.5% of previously treated TB cases was estimated to have MDR-TB (**Figure 1.2**). This translates into an estimated 4,80,000 people having developed MDR-TB in 2013 [WHO Global Tuberculosis Report 2014]. In 2013, a total of 97,000 patients were started treatment for MDR-TB, a three-fold increase compared with 2009. If all notified TB patients (6.1 million new and previously treated) had been tested for drug resistance in 2013, an estimated 3,00,000 cases of MDR-TB would have been detected, more than half of these in three countries alone: India, China and the Russian Federation. MDR treatment involves the use of expensive, toxic, less potent

injectable second line drugs under DOTS plus programme for 18-24 months with chances of cure about 50-60%. The WHO currently recommends the use of a regimen including amikacin, ethionamide, a FQ (such as moxifoxacin), and pyrazinamide to treat MDR-TB [Veziris N., *et al.*, 2003].

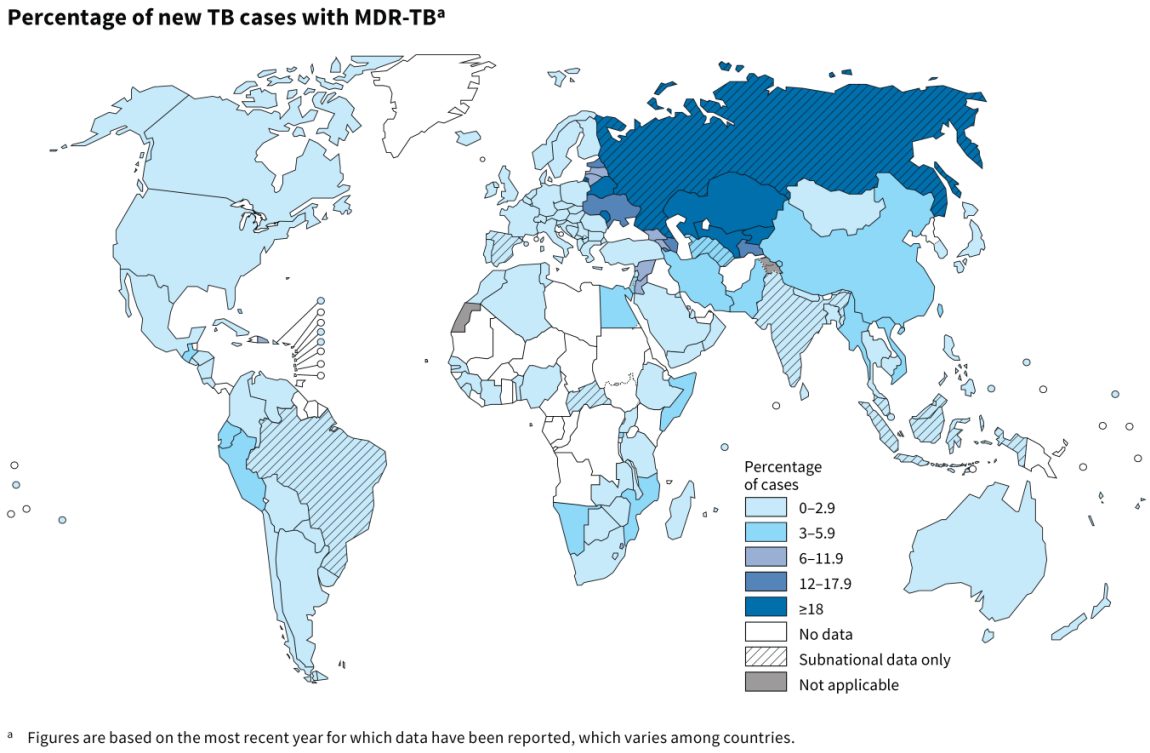


Figure 1.2: Percentage of new TB cases with MDR-TB [WHO Global Tuberculosis Report 2014].

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

Shah and co-workers observed the resistance of *Mycobacterium tuberculosis* to second line drugs. MDR-TB resistant to any FQs and at least one of injectable second line antibiotic (capreomycin, kanamycin and amikacin) can be termed as XDR-TB [Shah N.S., *et al.*, 2007]. In 2013, on an average around 9% of patients with MDR-TB had XDR-TB, and it has been extensively reported in 100 countries. Globally, 3232 XDR-TB cases were enrolled on treatment, up from 1852 cases in 2012 and reflecting increase in enrolments in 17 high MDR-TB burden countries. Most of the cases in 2013 were found from Ukraine (1006), South Africa (612), India (364) and Kazakhstan (305) [WHO Global Tuberculosis Report 2014]. New drugs and more effective regimens are urgently needed to improve the outcomes for patients with XDR-TB [Hopewell P.C., *et al.*, 2006].

1.3.3. Totally drug resistant-TB (TDR-TB)

TDR-TB refers to XDR-TB resistant to all the available second line drugs. Though the prevalence of TDR-TB is less; few cases were reported in Italy, Iran and India [Udwadia Z.F., *et al.*, 2012; Velayati A.A., *et al.*, 2009; Migliori G.B., *et al.*, 2007]. Emergence of TDR-TB initiates the need of development of novel anti-tubercular drugs [Parida S.K., *et al.*, 2014]. Recently, three new drugs namely, bedaquiline (TMC-207), delamanid (OPC-67683) and linezolid, approved by the US-Food and Drug Administration and the European Medicines Agency (EMA).

1.4 The current TB drug development pipeline

Substantial progress has been made in development of new drugs during the past decade, for the first time in 40 years; a portfolio of promising new compounds is on the horizon. Some of these are expected to resolve problems of current TB treatment regimens.

Researchers in anti-tubercular drugs showed much success in repurposing drugs like rifapentin and FQs (moxifloxacin and gatifloxacin) which are under phase-3 trials (**Figure 1.3**) [Lienhardt C., *et al*., 2012; Sterling T.R., *et al*., 2011; Nunn A.J., *et al*., 2008; Rustomjee R., *et al*., 2008]. With success of FQs, attempts were made to modify existing antibacterial agents. Some of the scaffolds showing promising results can be categorised into nitroimidazoles (delamanid, PA-824 and TBA-354), 1,2-ethylenediamine (SQ109), oxazolidinone derivatives (sutezolid, AZD-5847, radezolid and tedizolid) [Lechartier B., *et al.*, 2014; Sotgiu G., *et al.*, 2012].

Other classes of drugs under development are imidazopyridine and benzothiazinones. Q203 of imidazopyridine class has similar target as bedaquiline, but more efficacious as it blocks ATP synthesis in aerobic and hypoxic conditions as well as resistant strains of TB [Zumla A.I., *et al.*, 2014; Pethe K., *et al.*, 2013]. BTZ-043 and PBTZ-169 belonging to class of benzothiazinones targets decaprenylphosphoryl-β-D-ribose 2´epimerase (DprE1), essential for bacterial survival and are in late stage of clinical development [Zhang D., *et al.*, 2012; Makarov V., *et al.*, 2009].

Combinations of these new drugs with existing anti-tubercular therapies can lead to shorter and better tolerated regimens, and have lesser drug-drug interactions compared with existing regimens. Since anti-tubercular drugs need to be given in combination to prevent drug

resistance, trials are underway with companion drugs to simplify, improve, or shorten treatment regimens for drug-sensitive and resistant TB-strains. Further exploration is needed in areas like biology of persistence, factors involved in tissue liquefaction, cavity formation and host immune mechanisms that control latent infection to find out newer targets for drug discovery.

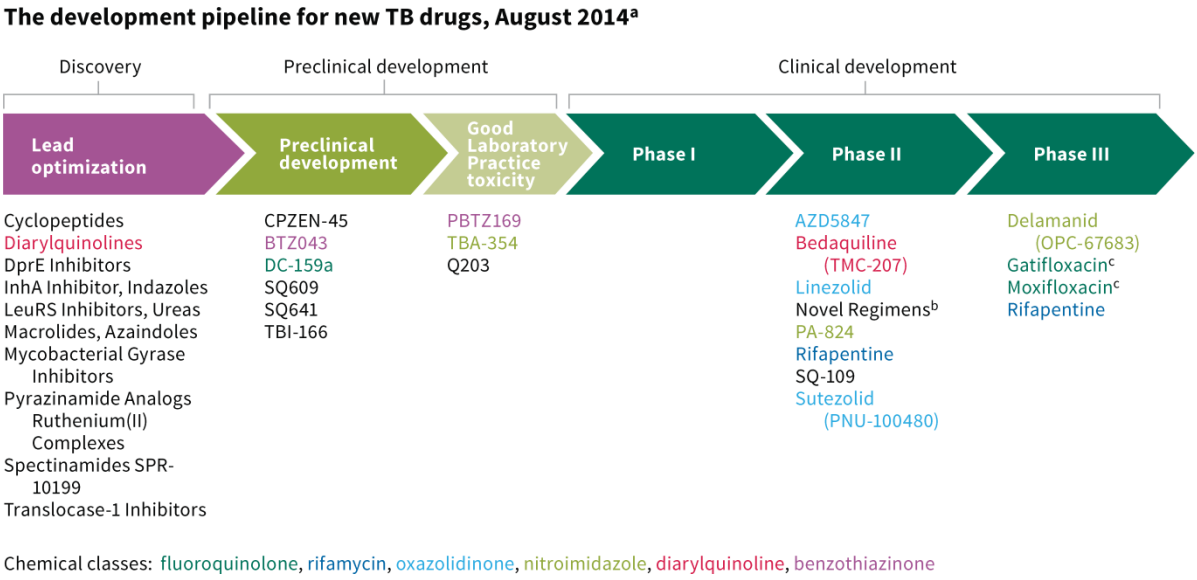


Figure 1.3: The development pipeline for new TB drugs [WHO Global Tuberculosis Report 2014].

Literature review

Chapter 2

Topoisomerases are a ubiquitous class of enzymes that are conserved and catalyze the interconversion between different topological isomers of DNA. Among these enzymes, the most prominent one is the DNA gyrase, as it is the only enzyme that has the ability to negatively supercoil DNA [Novak S.M., *et al.*, 1993]. Apart from inducing negative supercoils, the gyrase can also relax the positively supercoiled DNA and catalyze catenation/decatenation as well as knotting/unknotting reactions. Thus DNA gyrase is an important enzyme for maintaining the topology of the DNA. Bacterial DNA gyrase is an important and novel target for many antibacterial agents. DNA gyrase is a type II topoisomerase with A2B2 heterotetramer complex. The A subunit mediates the breakage and reunion of the reaction at the active site while the B subunit is involved in the promotion of the ATP hydrolysis [Palomino J.C., *et al*., 1999]. These DNA topoisomerases are ubiquitous enzymes that control and coordinate the topology of DNA through replication, transcription and recombination. DNA gyrase mainly makes double stranded breakage passing one of the single strands of the DNA through the nick and reseals the break, thereby changing the linking number of the molecule by one. The basic mechanism involved in this cleavage is ATP –independent that involves a covalent linkage between the tyrosine residue at the active site of the N-terminal domain of the enzyme and the 5' phosphoste group of the cleaved DNA strand [Ahmed S.A., *et al*., 1994]. This reaction is metal dependent and requires magnesium ion, which is targeted by the drugs aimed at the GyrA and is much explored from past three decades. The other part of the protein GyrB subunit is less explored and current research is concentrated on this subunit, as bacteria have gained intrinsic drug resistance over the currently available drugs including the FQs [Barton J., *et al*., 2008] resulting in disease causing effects. Therefore one attractive strategy to control the disease is to inhibit DNA gyrase, necessary for bacterial cell growth and division. DNA gyrase as the biological target has aroused a general interest in developing inhibitors as potential antibacterial drugs for several reasons as such as:

1) These are essential components of all bacteria.

2) These proteins are essential for bacterial viability involved in bacterial DNA replication and cell division as well.

3) There are many distinct structural differences when compared to the mammalian enzyme counterparts to allow for bacterial specificity.

4) Multiple target sites have been identified within the enzymes, insisting a broad range for the drug action with the enzyme.

5) Inhibition of their function in many bacteria usually leads to a bactericidal event, revealing the importance of the enzyme.

6) The structural similarity between the different topoisomerases allows ligands to inhibit related enzymes.

While the DNA gyrase is an enzyme with A2B2 heterologous component, with A and B subunits in it, the related topoisomerase IV (TopoIV) is also a heterologous structure with Par C and Par E. The function is complementary to that of the DNA gyrase. The main function of the Topo IV is to decatenate interlinked DNA molecules before cell division. The separated molecules are called catenenes. Many inhibitors for GyrA have been reported which includes the quinolones like the moxifloxacin, ciprofloxacin and norfloxacin [Hiasa H., *et al*., 2003]. These inhibitors are very effective and have very low IC_{50} and MIC values. While this class of antibacterials has gained a strong position in the treatment and therapy of bacterial infections, its use, however, is hampered by resistance developed by the organisms and undesired side effects. The GyrA-DNA-Drug forms a ternary complex after the DNA is nicked, thus a stabilized and low energy ternary complex is formed [Sherer B., A *et al*., 2011]. The GyrB inhibitors mainly novobiocin, coumermycin and cyclothialidines are highly effective drugs, which target the ATPase activity of the enzyme, by competitive inhibition with the ATP thus abolishing the energy-dependent reactions catalyzed by the DNA gyrase. Poor tolerability and solubility issues has restricted or refrained their use clinically. Although the first coumarin GyrB inhibitors were discovered in 1950's novobiocin (IC_{50} = 3nM) approved for clinical use in 1960's was withdrawn from the market due to its hazardous side effects and toxicity, thus creating a gap again in the drug discovery field. Thus the focus is placed on the GyrB subunit which has high scope for the molecular inhibition studies and drug discovery. Very recently GSK, Vertex pharmaceuticals, AstraZeneca and few others have reported some novel inhibitors of DNA GyrB with potential antibacterial activity both structurally and mechanistically distinct from FQs [Paul S.C., *et al*., 2009]. These new drugs do not form a stabilized ternery complex but rather form a pre-formed cleavage complex as

seen in the *Staphylococcus aureus* [Peter A., *et al*., 2004]. For eg. GSK299423, an NBTI (Novel Bacterial Topoisomerase Inhibitor) is derived from a chemical series originating from an unbiased antibacterial screening. GSK299423 shows potent inhibition of supercoiling by DNA gyrase with an IC_{50} value of 4 nM [Paul S.C., *et al*., 2010].

2.1 Fluoroquinolones (FQs)

FQs are the most successful antibacterials targeted to DNA gyrase. FQs were derived from quinine. Nalidixic acid, the first quinolone derivative was introduced in 1962 by George Lesher *et al* discovered as a by-product of chloroquine synthesis [Marriner G.A., *et al*., 2011; Lesher G.Y., *et al*., 1962]. Most FQs are being evaluated as potential anti-TB drugs, also for their potential to shorten TB treatment duration, one of the major strategies for TB control. They are the class of antibiotics that have potent antimicrobial activity against a wide range of gram positve and gram negative organisms. Several members of the FQs class of drugs are currently already used as second line TB drugs for the treatment of MDR-TB. They have high bioavailability in the range of 70-90%, even when given orally.

2.1.1. Different classes of FQs

All FQs have a basic 4-quinolone structure, with a fluorine atom at C-6 position as shown in **Figure 2.1**.

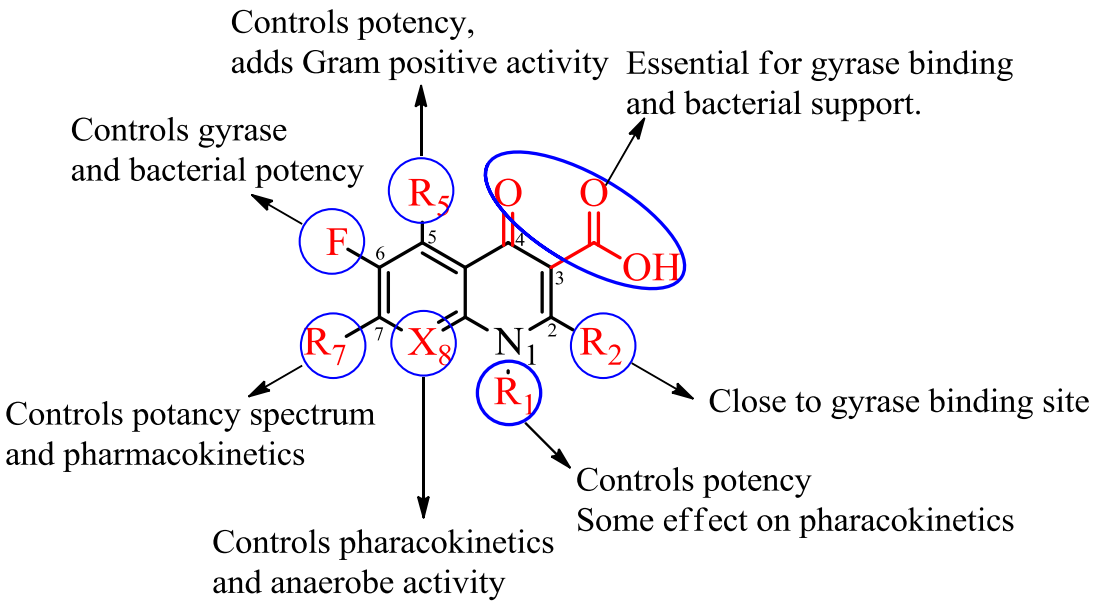


Figure 2.1: SAR of quinolone.

The C-2 position lies near the DNA gyrase binding site, and thus a sterically undemanding hydrogen atom at R_2 is optimal [Gootz T.D., *et al*., 1996]. The dicarbonyl moiety is required for binding to DNA gyrase and thus is critical for activity. Modifications at C-5 control in-vitro potency with the most active groups being small electron-rich groups such as -NH_2, -OH, and -CH_3 [Domagala J.M., *et al*., 1994]. Additionally, C-5 modifications affect activity against both gram-negative and gram-positive organisms. The fluorine atom at C-6 (for which the class is named) enhances DNA gyrase inhibition [Mitscher L.A., *et al*., 2005; Gootz T.D., *et al*., 1996] and can increase the MIC of the compound 100-fold over that of other substitutions [Domagala J.M., *et al*., 1994]. The most active substituents at C-7 have been five- and six-membered nitrogen heterocycles, with pyrrolidines increasing activity against gram-negative bacteria and piperazines affecting potency against gram-positive organisms. The C-8 position controls absorption and half-life, and optimal modifications for in-vivo efficacy include groups that create an electron deficient pi system [Bolon M.K., *et al*., 2009]. Several modifications that create an N-1 to C-8 bridge have also been successful, *i.e.*, ofloxacin and levofloxacin, which both display significant gyrase inhibition [Gootz T.D., *et al*., 1996].

2.1.2. Moxifloxacin and gatifloxacin

Gatifloxacin was discovered by Bristol-Myers Squibb (BMS) in 1999 for the treatment of respiratory tract infections whereas moxifloxacin developed by Bayer AG, was marketed worldwide under the brand names of Avelox. In 1999, moxifloxacin hydrochloride (Avelox) was approved by USFDA for use in US.

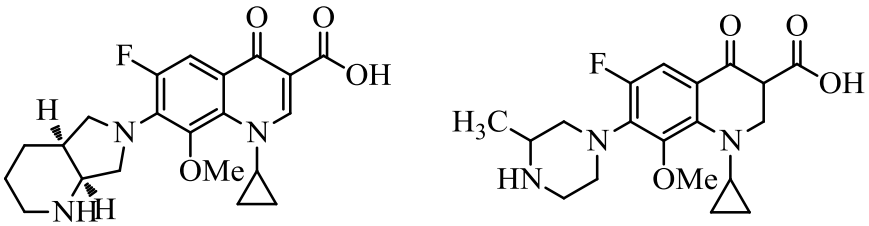


Figure 2.2: Structures of moxifloxacin and gatifloxacin**.**

Moxifloxacin and gatifloxacin, the fourth-generation FQs inhibit the bacterial enzymes DNA gyrase and topoisomerase IV (**Figure 2.2**). But in *Mycobacterium tuberculosis* it is assumed that they target solely DNA gyrase since there is no evidence of topoisomerase IV present in *Mycobacterium tuberculosis*. Other quinolones, such as ciprofloxacin and ofloxacin, have

been used as second-line anti-tubercular drugs, but moxifloxacin and gatifloxacin are more potent in-vitro than these older quinolones. Moxifloxacin and gatifloxacin has an extended spectrum of antibacterial activity and provides better coverage of gram-positive organisms and favourable pharmacokinetic properties. Moxifloxacin and gatifloxacin inhibited *Mycobacterium tuberculosis* DNA gyrase with an IC_{50} of 4.5 and 3 µg/mL respectively, whereas *Mycobacterium tuberculosis* MIC was found to be 0.05 and 0.12 µg/mL respectively [Villemagne B., *et al.*, 2012].

They are attached with a methoxy group at position 8 and a bulky side chain at position 7. The C-8 methoxy group has potent activity against DNA gyrase (topoisomerase II) and topoisomerase IV, a capability that allows both these agents to kill resting bacterial cells as well as those that are actively multiplying. That leads to the prevention of the emergence of bacterial resistance to the quinolones. A cyclopropyl group at N-1 and fluorine at C-6 brings an enhanced antibacterial activity.

The bulky side-chain and bicyclic side chain at position C-7 of moxifloxacin and gatifloxacin respectively reduces the ability of the bacterial cells eflux pump to flush out the antibiotic, resulting to the increased drug stay in the bacterial cell improves the enhanced activity, expanded spectrum of activity and additional defense against resistance.

Moxifloxacin and gatifloxacin are currently in phase III clinical trials. The potential adverse effects that have been reported for these drugs are dysglycemia with gatifloxacin and QT prolongation with moxifloxacin [Alvirez-Freites E.J., *et al.*, 2002].

2.2. Aminocoumarins

Aminocoumarin is a class of antibiotics that act by an inhibition of the DNA gyrase enzyme involved in the cell division in bacteria. Aminocoumarins has 3-amino-4,7-dihydroxycoumarin ring as the basic skelton. In comparison with FQs; aminocoumarins are regarded as the 'Cinderellas' of the gyrase inhibitors. Novobiocin (**Figure 2.3)**, clorobiocin and coumermycin A_1 are the class of aminocoumarins isolated from *Streptomyces* species; have many derivatives made by genetic manipulation, metabolic engineering and mutasynthesisand by chemical synthesis. Coumermycin A_1 has coumarin rings attached to either side of a pyrrole group; a substituted sugar is attached to each of the coumarin rings, and a pyrrole group is attached to each noviose sugar. Structurally clorobiocin and novobiocin are differing in the substitution of the methyl group at the 8′ position of the

coumarin ring with a chlorine atom, and a 5-methyl-pyrrole-2-carboxyl group substitutes the carbamoyl group at the 3′ position of the noviose.

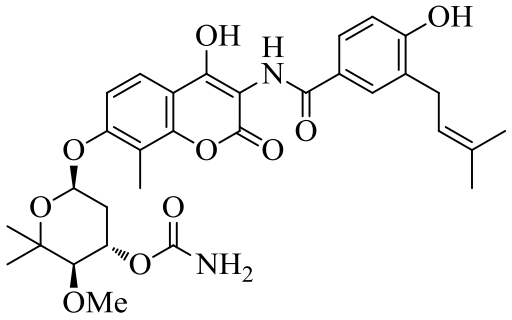


Figure 2.3: Structure of novobiocin**.**

Novobiocin exhibited an MIC of 4 mg/mL against drug sensitive *Mycobacterium tuberculosis* H37Rv strain and MIC's in th range of 0.62 mg/mL – 8 mg/mL against various drug resistant strains of *Mycobacterium tuberculosis* [Chopra S., *et al*., 2012].

Simocyclinones are another variety of aminocoumarin moety with an additional angucyclinone polyketide group. Both aminocoumarins and simocyclinones are two different non-quinolone, gyrase-targeted antibacterials that bind at different subunits and are evolutionarily related also.

Though aminocoumarins are potent inhibitors of DNA gyrase in-vitro, their poor activity against gram-negative bacteria, cytotoxicity and poor solubility prevent them from being clinically successful drugs. So their further structural exploration is still needed.

2.3. Pyrrolamides

The pyrrolamide class of compounds was first identified at AstraZeneca as novel DNA gyrase inhibitors through *Mycobacterium tuberculosis* screening and structure-guided design, and were reported to have antibacterial activity (**Figure 2.4**). It was found that the pyrrolamides target DNA gyrase, an essential enzyme across bacterial species and inhibition results in the disruption of DNA synthesis and subsequently to cell death. The optimization of biochemical activity and other drug-like properties through substitutions to the pyrrole, piperidine, and heterocycle portions of the molecule resulted in pyrrolamides with improved cellular activity and in-vivo efficacy. The antibacterial activity, spectrum and mode of action of these compounds underscore the promise of the pyrrolamide series as attractive candidates

for the treatment of several clinical indications, including respiratory and soft tissue infections [Maria U.N., *et al.*, 2013].

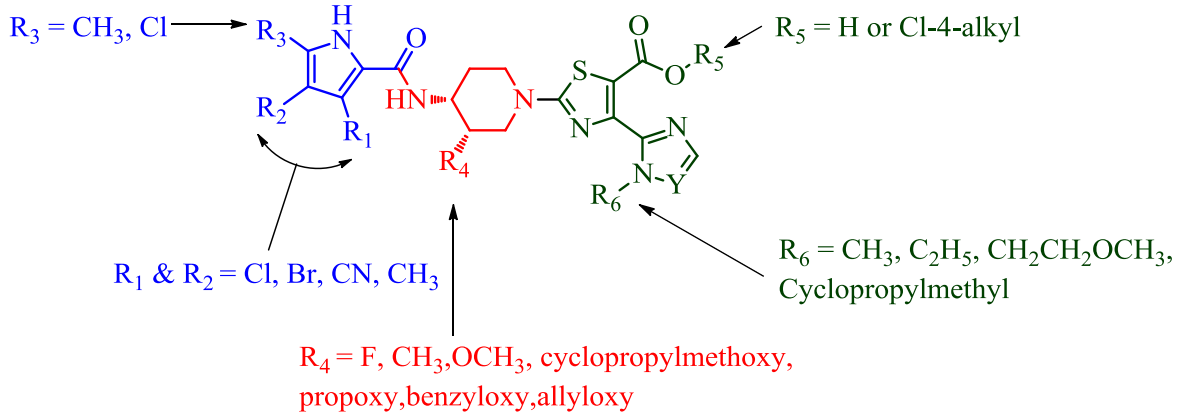


Figure 2.4: Pyrrolamide based mycobacterial gyrase inhibitors.

In 2014, Gregory S.B., *et al* has developed AZP5099 (**Figure 2.5**), belonging to pyrrolamide class based on fragment based approach. This compound targets ATP binding site type II topoisomerase and has successfully entered phase I trials for treatment of resistant bacterial infection [Gregory S.B., *et al.*, 2014].

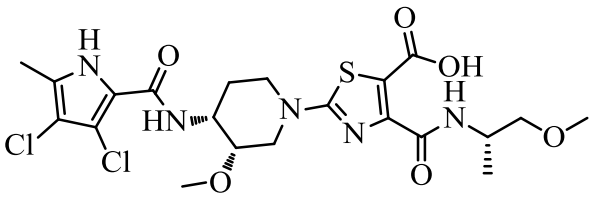


Figure 2.5: Structure of AZP5099.

2.4. Aminopyrazinamides

In 2012, Shirude P.S. *et al* reported a novel series of aminopyrazinamides identified from a high throughput screen (HTS) of the AstraZeneca compound collection against *Mycobacterium smegmatis* DNA gyrase which displayed chemical tracability, robust structure activity relationship (SAR), and potent anti-tubercular activity. Aminopyrazinamides are highly bactericidal against both replicating and non-replicating *Mycobacterium tuberculosis* and have potent intracellular activity against *Mycobacterium tuberculosis* residing within macrophages.

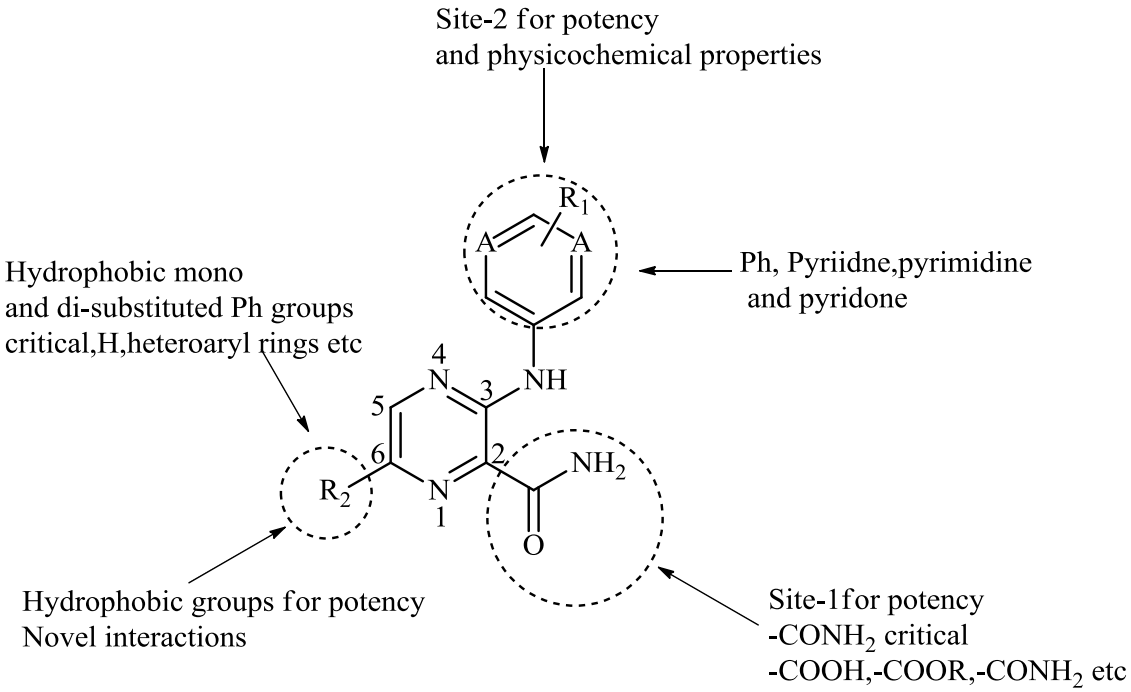
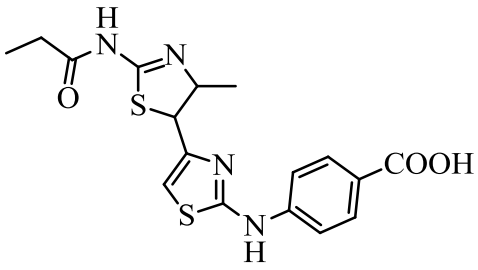


Figure 2.6: Aminopyrazinamide based mycobacterial gyrase inhibitors.

The primary amide group at site-1 is needed to retainthe bactericidal activity. Replacement by a carboxylic acid or an ester moiety led to a complete loss of activity. Presence of an aryl or heteroaryl group at site-2 resulted in more activity than an aliphatic chain. The presence of the di-substituted phenyl ring (hydrophobic groups) at the C-6 position was very important for activity and the replacement with other groups led to a completely inactive molecule (**Figure 2.6**). The pharmacokinetic profiling of this class was not so promising, though the aminopyrazinamide class has the capability for further stabilisation to attain good pharmacokineticproperties with the use of medicinal chemistry [Pravin S.S., *et al.*, 2012].

2.5. Bithiazoles

Brvar M. *et al* in 2012, reported a novel series of 4'-methyl-N^2-phenyl-[4,5'-bithiazole]-2,2'-diamine inhibitors of DNA gyrase with a low micromolar inhibitory activity by implementing a two step structure-based design procedure. They investigated the series extensively by using various techniques and revealed the binding mode of one of the potent inhibitor by X-ray crystallography. The most active compound **18** (**Figure 2.7**) from the series showed DNA gyrase IC_{50} of 1.1±0.2 μM [Brvar M., *et al.*, 2012]



Compound **18**

DNA Gyrase IC_{50} = 1.1±0.2 µM

Figure 2.7: Structure of bithiazole derivative (compound 18).

Furthermore, this class of 4, 5-bithiazole compounds displayed a collection of promising lead compounds for further optimization and development to yield novel drugs aimed to combat ever-present bacterial infections.

2.6. Thiazolopyridines

In 2014, Kale R.R. *et al* synthesised a series of thiazolopyridone ureas with potent anti-tubercular activity acting through inhibition of DNA gyrase activity. Structural diversity was introduced, by extension of substituents from the thiazolopyridone N-4 position, to access hydrophobic interactions in the ribose pocket of the ATP binding region of GyrB. Further optimization of hydrogen bond interactions with arginines in site-2 of GyrB active site pocket led to potent inhibition of the enzyme (IC_{50} = 2 nM) along with potent cellular activity (MIC = 0.1 µM) against *Mycobacterium tuberculosis*. Efficacy was demonstrated in an acute mouse model of TB on oral administration [Manoj G.K., *et al*., 2013]

2.7. Benzothiazinones

In 2009, Makarov *et al* discoverd 1,3-benzothiazin-4-ones (BTZs) as promising new agents for the treatment of TB (Makarov M., *et al*., 2009). BTZ043a nitroaromatic compound, was reported to be active against MDR-clinical isolates of *Mycobacterium tuberculosis tuberculosis* [Pasca., *et al*., 2010; Makarov M., *et al*., 2009] and targets the essential flavoprotein subunit, DprE1, of decaprenylphosphoryl-beta-D-ribose 2-epimerase (**Figure 2.8**). This enzyme produces the sole source of the D-arabinose required for biosynthesis of the key cell wall components arabinogalactan and lipoarabinomannan. BTZ043 serves as a suicide substrate for the reduced form of DprE1 undergoing nitro reduction to yield a nitroso species that specifically attacks the thiol side chain of the active site cysteine residue,

Cys387, thereby forming a covalent adduct and irreversibly inactivating the enzyme [Neres *et al*., 2012; Trefzer *et al.*, 2012].

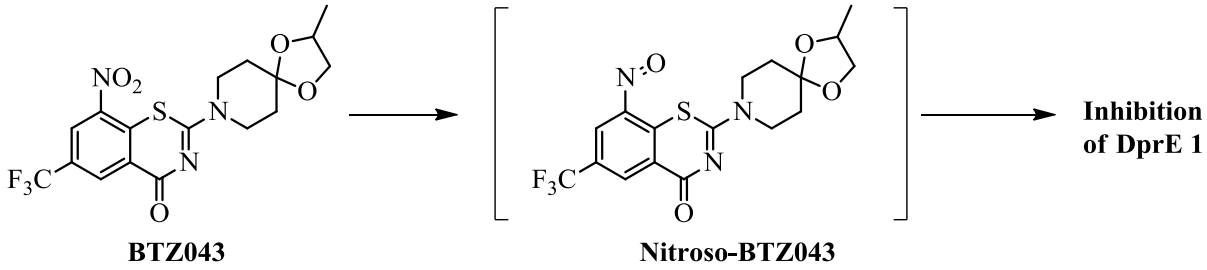
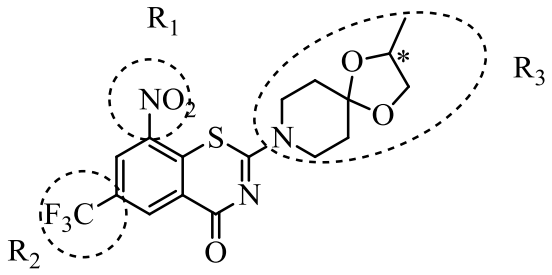


Figure 2.8: Structure and mechanism of action of BTZ043 [Tiwari *et al*., 2013].

In murine models of acute and chronic TB, BTZ043 showed efficacy which was extraordinary relative to existing drugs, such as isoniazid and ethionamide and also active against drug-susceptible MDR and XDR clinical isolates of *Mycobacterium tuberculosis*. The MIC of BTZ043 was found to be 1 ng/mL against *Mycobacterium tuberculosis* [Makarov V. *et al*., 2014]. No antagonism was found when BTZ043 was used in combination with other anti-tubercular drug candidates rifampicin, isoniazid, ethionamide, bedaquiline, PA-824, moxifloxacin and meropenem. Almost all of the interactions elicited additive effects, but synergy was observed when BTZ043 was combined with bedaquiline [Lechartier B., *et al.*, 2012].

By maintaining the groups at positions R_1 and R_2 (NO_2 and CF_3 respectively), Karoli T. *et al* made considerable variation in the structure of the R_3 group (**Figure 2.9**). At position R_3, the region around the spiroketal of **2** was modified by the nucleophilic addition of different amines (selection based on ligand and solubility). Like other benzothiazinones, poor aqueous solubility had been identified, and the most highly active compounds exhibited a low solubility. Also, the drug candidates revealed the necessity to improve the bioavailability and ADME/T properties also [Karoli T., *et al.*, 2012].



(RS) 1 (BTZ)-10526038
(S) 2 (BTZ)-10526043

Figure 2.9: Lead benzothiazinone structures. R_1 acts as site of covalent addition to DprE1 after enzymatic activation, and an electron withdrawing group at R_2 is required. Variation is permitted in R_3. Both enantiomers (chiral center highlighted by $*$) are equipotent in-vitro [Karoli T. *et al.*, 2012].

The discovery of BTZs, especially BTZ043 as potent agent for the treatment of TB, prompted intensive research related to development of potential anti-tubercular agents based on electron deficient nitro aromatic scaffolds. Tiwari R. *et al* in 2013, reported the syntheses, computational and NMR studies and anti-tubercular activity of oxidation products, 1,3-benzothiazinone sulfoxide (BTZ-SO) and 1,3-benzothiazinone sulfone (BTZ-SO_2) derived from BTZ043 (**Figure 2.10**). The combined computational and NMR work revealed differences in the total charge densities and molecular shapes of the oxidation products. While docking studies suggested similar interactions and binding patterns for both products with the target DprE1 enzyme, anti-tubercular assays indicated remarkable differences in their activity. Interestingly, BTZ-SO possesses potent activity against non-pathogenic and pathogenic mycobacterial strains, but BTZ-SO_2 is only weakly active [Tiwari R., *et al.*, 2013].

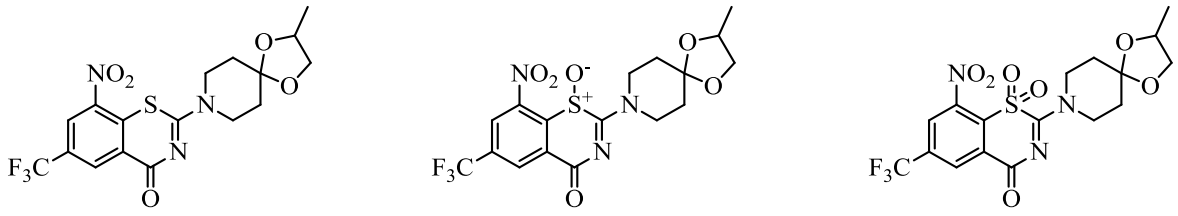
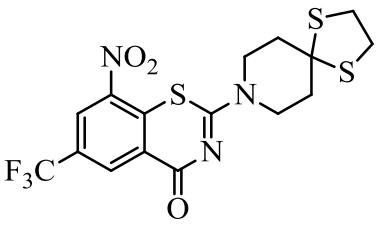


Figure 2.10: Oxidation products, 1,3-benzothiazinone sulfoxide (BTZ-SO) and 1,3-benzothiazinone sulfone (BTZ-SO_2) derived from BTZ043 [Tiwari R., *et al.*, 2013].

In 2013, Gao C. *et al* reported synthesis and anti-mycobacterial activities of *N*-Alkyl and heterocycle substituted BTZs derivatives. It was found that an extended or branched alkyl chain analogue could enhance the potency, and activities of *N*-alkyl substituted BTZs were not affected by either nitro or trifluoromethyl at 6-position. Trifluoromethyl group played an important role in maintaining anti-tubercular activity in the piperazine or piperidine analogues. Compound **8o**, which contains an azaspirodithiolane group, showed a MIC of 0.1 nM against *Mycobacterium tuberculosis* H37Rv, 20-fold more potent than BTZ043 racemate. These results suggested that the volume and lipophilicity of the substituent were important in maintaining activity (**Figure 2.11**). In addition, compound **8o** was non-toxic to vero cells and orally bioavailable in a preliminary pharmacokinetics study.

Impressively **8o** also showed an acceptable oral bioavailability and the peak concentration (Cmax) was noted far above the MIC against *Mycobacterium tuberculosis* (more than 10,000-fold). These results also suggested that the introduction of heterocycles and alkylamine as substituents of the BTZs could enhance and retain anti-microbial activities, making these compounds interesting starting points for further optimization [Gao C., *et al*., 2013].



8o

Figure 2.11: BTZ043 analogue [Gao C., *et al.*, 2013].

In 2014, Tiwari R. *et al* explored BTZ043 to the effect of functional groups (scaffold simplification strategy) such as sulfonamides, reverse-amides and esters that were attached to the nitroaromatic rings on their anti-tubercular activity (**Figure 2.12**). The importance of electron deficient aromatic ring was the central theme that was confirmed from the in-vitro activity studies. Nitroaromatic sulphonamides and nitrobenzoic acid esters with two nitro substituents were most active compounds evaluated against the H37Rv strain of *Mycobacterium tuberculosis*.

SAR pattern displayed by this class of compounds was similar to both sulfonamides and reverseamides. In brief, the *m*-dinitrobenzoate esters displayed more potent activity than the corresponding *m*-trifluoromethyl-*m*-nitro benzoates. Sulfonamides and nitrobenzoic acid ester analogues with dinitro substituents were more active. *m*-dinitrobenzoate esters displayed more potent activity than the corresponding *m*-trifluoromethyl-*m*-nitro benzoates. The studies suggested that simple sulfonamides and nitrobenzoic acid ester analogues with dinitro substituents were more active but less potent than BTZ043. The reverse amide functionality drastically affected the in-vitro anti-tubercular activity of the studied nitroaromatic scaffold [Tiwari R., *et al.*, 2014].

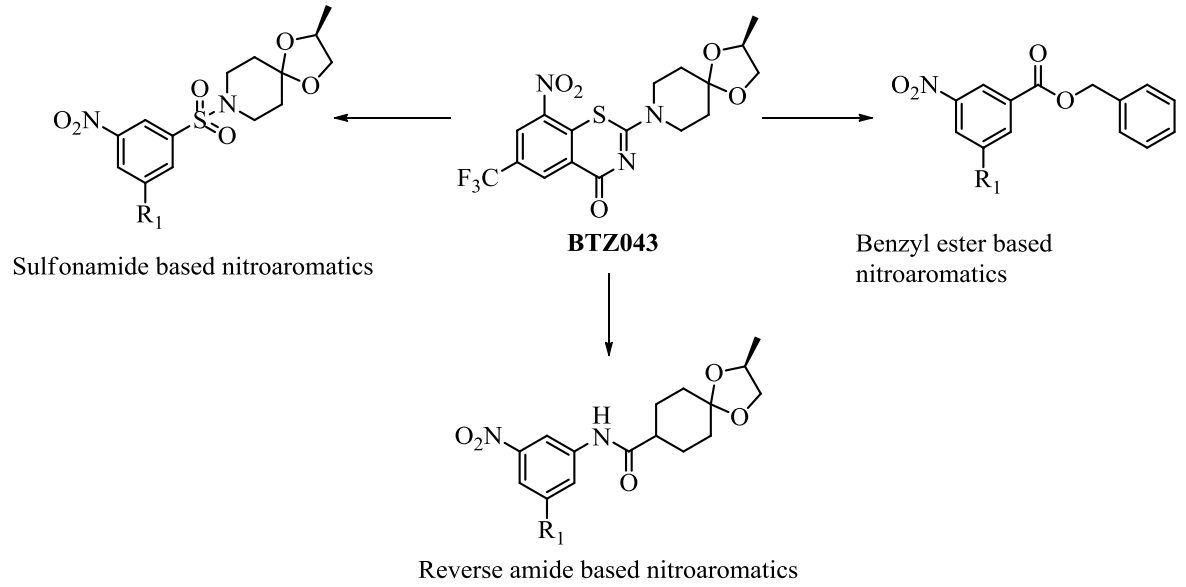


Figure 2.12: BTZ043 inspired analogues [Tiwari R., *et al.*, 2014].

2.8. Benzimidazoles

Vertex pharmaceuticals by high throughput screening (HTS) developed benzimidazole ureas, a novel class of antibacterial targetting both GyrB and topoisomerase IV. In 2012, Chopra S. *et al* evaluated benzimidazole ureas for GyrB inhibition potency and found that they exhibit IC_{50} in nanomolar range and also active against FQ resistant-TB strains and murine models of TB. Various substitutions were attempted on the benzimidazole nucleus for generating SAR (**Figure 2.13**). A smaller alkyl chain was favoured at the R_1 position as it allowed the core to penetrate deeper into the active-site pocket, leading to tighter binding. It was observed that the pocket occupied by the ribose of ATP was accessed by the ring attached to the benzimidazole R_2 position. This interaction was considered important for the optimal binding

of the ligand in the ATP-binding pocket of GyrB. The pyridyl ring was optimized to be the ideal substituent at this position. With respect to the substituents on the pyridyl ring at the R_3 position, a variety of substituents were well tolerated with respect to gyrase activity, but the presence of fluoro group enhanced the antibacterial potency almost 6 to 18 times. It was observed that co-planarity at the R_3 position provided optimal enzyme inhibitory potency. One compound that met the criteria for co-planarity was the 3-fluoropyridin-2-yl compound and was optimized to be the ideal substituent at this position.

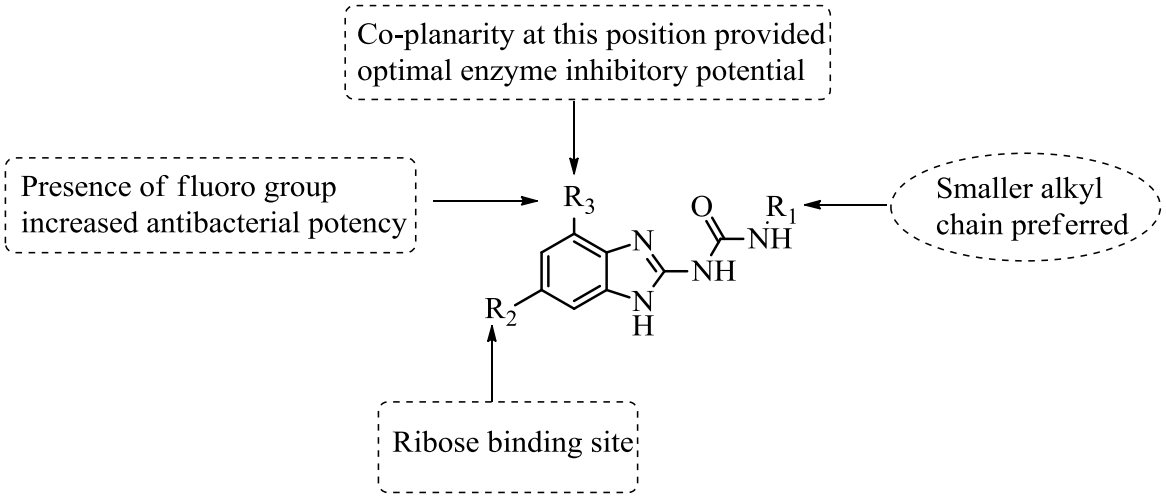


Figure 2.13: SAR of benzimidazole based analogues.

Objectives and Plan of work

Chapter 3

3.1. Objectives

The main objectives of the proposed work were:

1. To design novel *Mycobacterium tuberculosis* DNA gyrase inhibitors based on reported anti-tubercular leads.
2. To synthesize and characterize designed compounds.
3. To evaluate the inhibitory potency of the synthesized compounds by in-vitro *Mycobacterium tuberculosis* DNA GyrB and supercoiling assay.
4. To undertake in-vitro antimycobacterial screening of the synthesized compounds against *Mycobacterium tuberculosis* and
5. To perform the in-vitro cytotoxicity studies of the synthesized compounds.

3.2. Plan of work

The plan of work was classified into the following categories:

3.2.1. Design of anti-tubercular agents

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

1. *Molecular hybridization strategy*

2. *Molecular derivatization strategy*

3.2.2. Synthesis and characterization

The designed molecules were further synthesized in our laboratory utilizing previously reported methodology available in literature for structurally related molecules. All reactions were monitored using thin layer chromatography and LCMS. The synthesized compounds were fully characterized using modern analytical techniques. LCMS, ^{1}H NMR and ^{13}C NMR

were recorded and analysed to confirm the structure of the compounds. Purity of the compounds was evaluated by elemental analysis.

3.2.3. In-vitro enzyme inhibitory potency

The synthesized compounds were evaluated in-vitro for their *Mycobacterium smegmatis* DNA GyrB inhibitory potency and *Mycobacterium tuberculosis* DNA Gyrase supercoiling assay.

3.2.4. In-vitro *Mycobacterium tuberculosis* activity studies

All the synthesized compounds were further screened for their in-vitro antimycobacterial activity against *Mycobacterium tuberculosis* H37Rv (ATCC27294) by using micro plate alamar blue assay (MABA) method.

3.2.5. In-vitro cytotoxicity screening

The synthesized compounds were also screened for their in-vitro cytotoxicity against RAW 264.7 cell line (mouse leukemic monocyte macrophage) using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.

Materials and Methods

Chapter 4

4.1. Design of the molecules

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

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

2. *Molecular derivatization strategy*: Novel compounds were 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 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 column chromatography. Temperatures were reported in degrees celsius and are uncorrected. Compounds were analysed for C, H, N using Elementar and the analytical results obtained were found within ±0.4% of the calculated values for the formula shown. Molecular weights of the synthesized compounds were checked by Shimadzu, LCMS-2020 and the method used was electron spray ionisation (ESI-MS) method.

4.2.1. Synthesis of the designed molecules

Scheme-1: *Synthesis of 2-((4-aminophenyl)amino)-4H-benzo[e][1,3]thiazin-4-one derivatives as novel mycobacterium DNA Gyrase inhibitors*

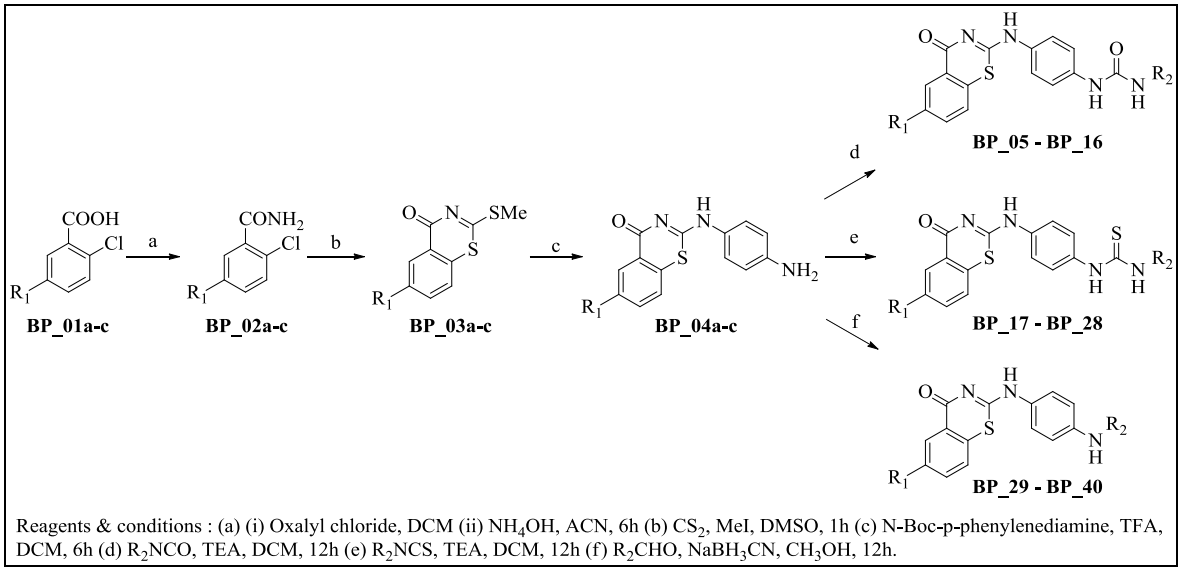


Figure 4.1: Synthetic protocol utilized for the synthesis of compounds **BP_05 – BP_40.**

Scheme-2: *Synthesis of 2-(4-aminocyclohexyl)-4H-benzo[e][1,3]thiazin-4-one derivatives as novel mycobacterium DNA Gyrase inhibitors*

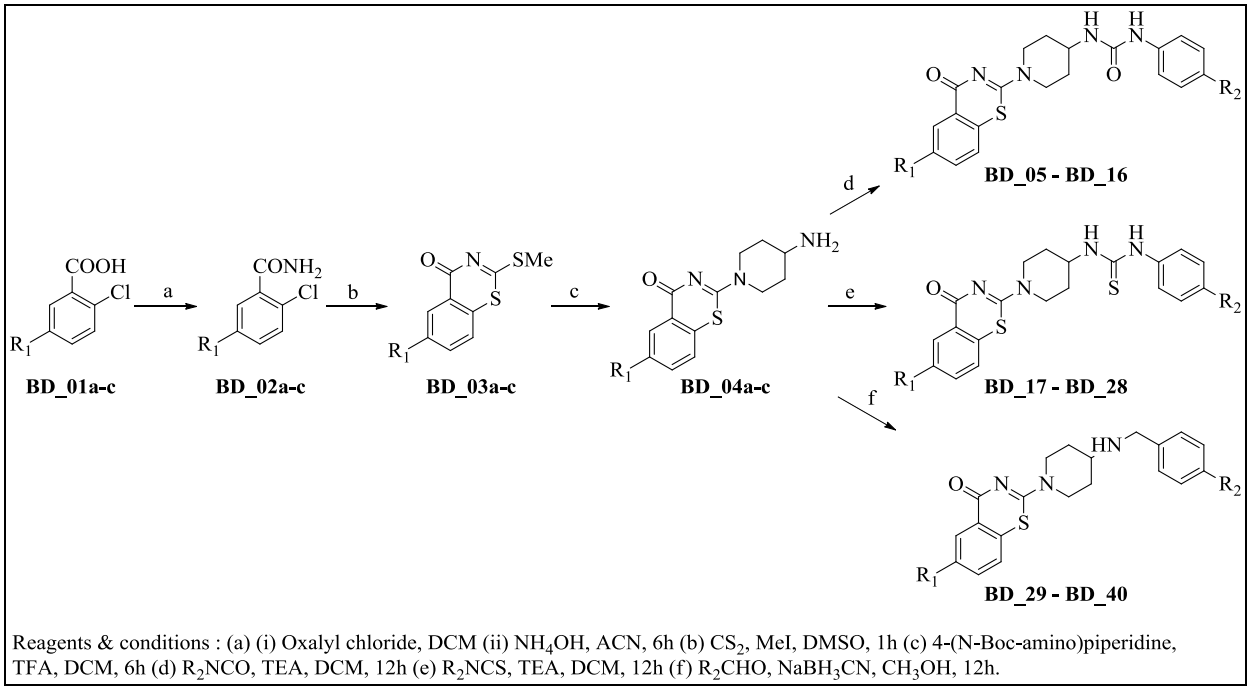


Figure 4.2: Synthetic protocol utilized for the synthesis of compounds **BD_05 – BD_40.**

Scheme-3: *Synthesis of 2-(piperazin-1-yl)-4H-benzo[e][1,3]thiazin-4-one derivatives as novel mycobacterium DNA Gyrase inhibitors*

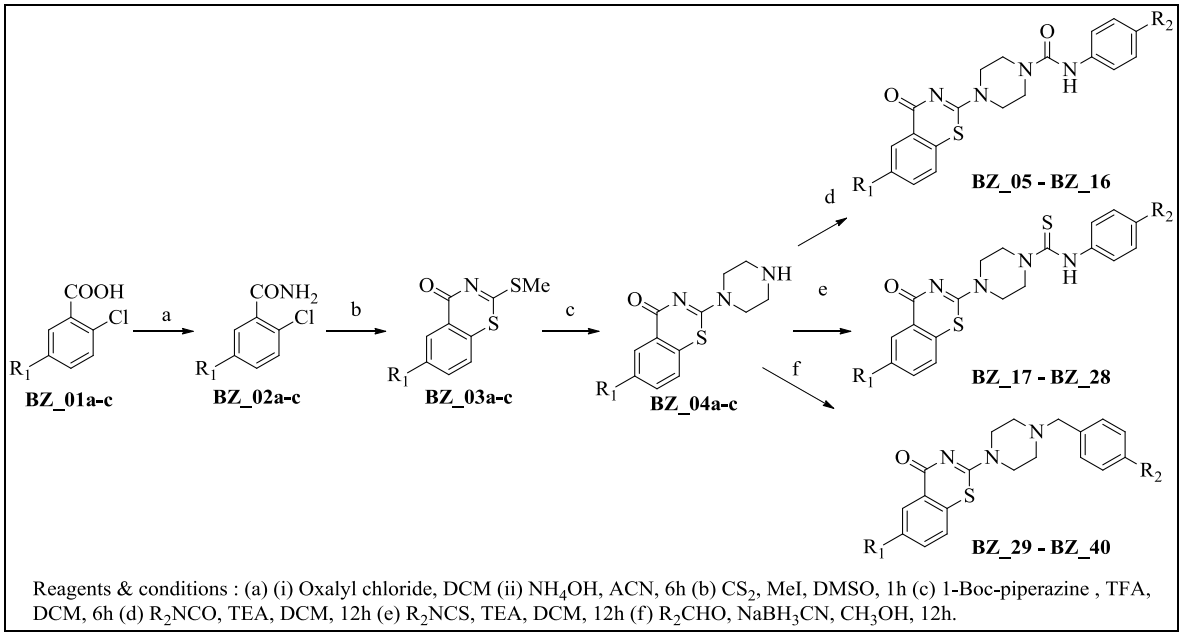


Figure 4.3: Synthetic protocol utilized for the synthesis of compounds **BZ_05 – BZ_40.**

Scheme-4: *Synthesis of 4-(1H-benzo[d]imidazol-2-yl)aniline derivatives as novel mycobacterium DNA Gyrase inhibitors*

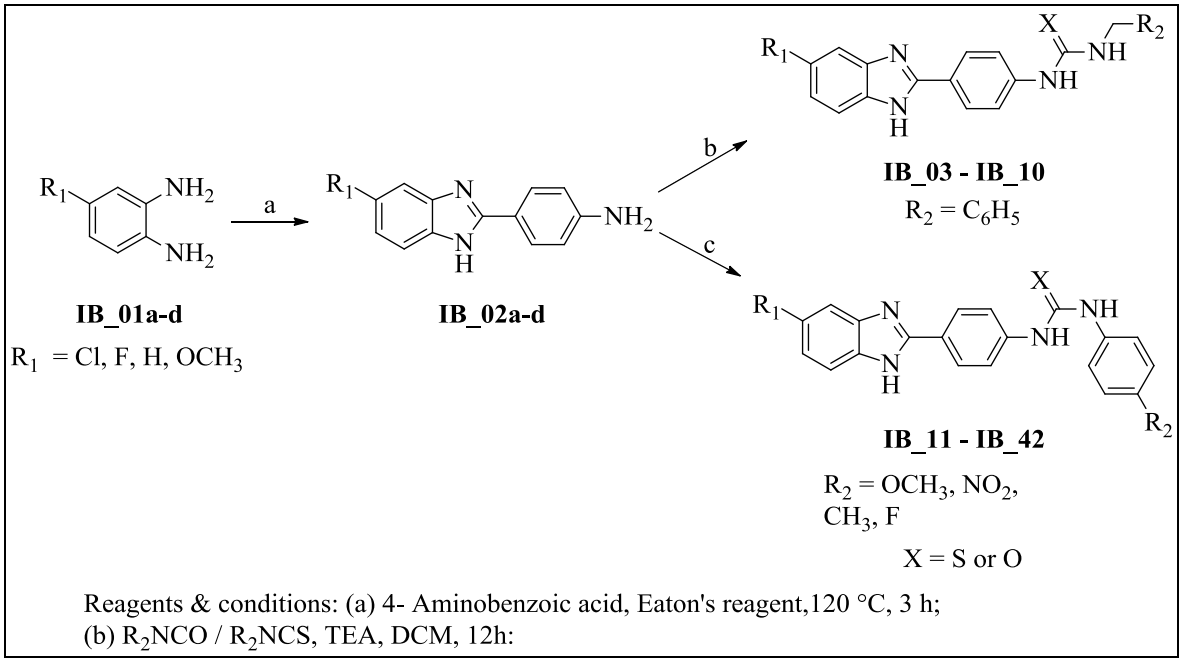


Figure 4.4: Synthetic protocol utilized for the synthesis of compounds **IB_03 – IB_42.**

Scheme-5: *Synthesis of 2-(piperidin-4-yl)-1H-benzo[d]imidazole derivatives as novel mycobacterium DNA Gyrase inhibitors*

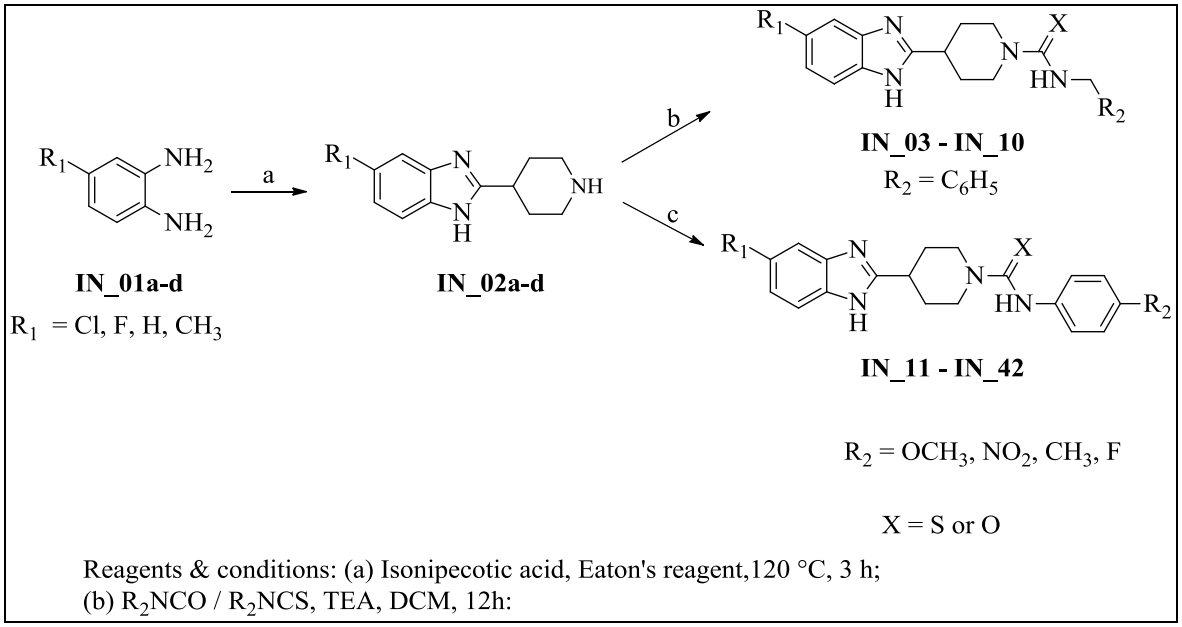


Figure 4.5: Synthetic protocol utilized for the synthesis of compounds **IN_03 – IN_42.**

4.3. Biological screening

4.3.1. *Mycobacterium smegmatis* DNA GyrB cloning, protein expression and purification

The vectors used for cloning and expression were from Qiagen, while the primers were from Sigma-aldrich and all the enzymes unless otherwise mentioned were from New England Biolabs. Cloning of *Mycobacterium smegmatis* DNA GyrB was performed by amplifying the gene from mc^2155 host strains genomic DNA using the specific forward and reverse primers and the desired restriction enzymes 5' CACCCATATGGTGGCTGCCCAGAAGAACAA 3' (NdeI), and 5' AGCTAAGCTTTTAAACATCCAGGAAGCGAA 3' (Hind III) respectively for about 35 cycler reaction [Jeankumar V.U., *et al.*, 2013]. The digested products were ligated at the same site of the pQE2 vector, downstream of the T5 promoter with an *N*-terminal His tag, the clone was later authenticated by sequencing using a DNA sequencer. Final clones after confirmation were transformed into BL21 (DE3) pLysS cells of *E. coli* as they possess a better compatibility. Transformants were grown in Luria Bertani (LB) broth (Himedia) at 37 ^{o}C shaking (rpm 140), in the presence of an antibiotic ampicillin (100 µg/mL) (Sigma-aldrich) until the starting optical density of 0.1 reached the value of 0.4−0.6 when measured in a cuvette. The protein expression was induced with 0.2 mM IPTG

(Himedia) in the growing culture and further grown overnight for induction of the protein, at 18 °C. Further, on the following day cells were harvested by centrifugation at 5500 rpm (4 °C, 15 minutes) and suspended in lysis buffer containing 20 mM Tris-HCl (pH 7.4), 0.1 M NaCl, 2 mM KCl, 1.3 mM K_2HPO_4,10 mM Na_2HPO_4, 5% Glycerol, 1 mM DTT, 1:200 µL protease inhibitor cocktail (Sigma-aldrich). The mixture was subjected to sonication (amplitude 35%, 1 s on 2 s off for 6 minutes) and was centrifuged at 12000 rpm (4 °C, 20 minutes). To the supernatant, pre-equilibrated Ni-NTA beads (GE) were mixed and swirled for an hour in cold room, centrifuged at 500 rpm for 5 minutes at 4 ^oC twice, later the pellets were redissolved in lysis buffer and loaded onto the Bio-Rad column, each loaded fraction was washed with 50 mL Tris-HCl (pH ~ 7.4), 500mM NaCl, 2mM KCl, 1.3mM K_2HPO_4, 10mM Na_2HPO_4, 5% Glycerol, 1mM DTT. Protein was eluted with 25mM Tris-HCl (pH 8), 140mM NaCl, 5% Glycerol, 1mM DTT, and 1mM PMSF. Initial wash was done with elution buffer without imidazole. Subsequently, elution was carried out with various imidazole concentration gradients from 5 mM to 500 mM. Samples were collected in autoclaved 2 mL eppendorf tubes. Dialysis was performed 4 times overnight, against (25mM Tris-HCl pH ~ 7.4, 140mM NaCl, 2mM dithiothreitol, 15% glycerol, 1mM EDTA), and dialyzed protein was concentrated at (4,500 rpm, 4^oC) to a final concentration of 2 mg/mL. The purity of the protein was analysed by SDS-PAGE. A 25 µL volume of the dialyzed protein was applied on the polyacrylamide gel (1mm, 10%), and 10 µL of a commercially available pre-stained multi-coloured protein molecular weight marker (Genetix) was added. The electrophoresis was run in 1X TBE buffer (Tris-HCl pH ~ 7.5, 1mM boric acid, 1mM EDTA) for a period of 90 minutes at a constant voltage. Later the gel was transferred to a solution of coomassie Brilliant Blue dye mixed with 20% acetic acid. After 20 minutes of shaking in an orbital shaker, it was destained several times with 10% acetic acid in 30% methanol and 60% of water until the staining is lost and transparency of the gel was achieved. Subsequently, the purity of the protein was determined to be >85% as only single bands corresponding to its molecular weight 72 kDa was observed.

4.3.2. In-vitro GyrB assay for the determination of IC_{50}

The in-vitro ATPase assay was performed by *Mycobacterium smegmatis* DNA GyrB subunit. As the assay does not involve any substrate it is called as DNA independent assay. GyrB being a catalytic domain undergoes the ATPase assay, resulting in hydrolysis of ATP and in energy generation. This assay was performed similar to previously reported method. It was

performed in 30 µL reaction volume for 100 minutes at 25°C in reaction buffer containing 60mM HEPES-KOH (pH ~ 7.7), 200mM KCl, 250mM potassium glutamate, 2mM $MgCl_2$, 1 mM DTT, 2% Glycerol, 4% DMSO, 0.001% BriJ-35, 0.65mM ATP, 40nM GyrB. The protein undergoes the ATPase assay, resulting in hydrolysis of ATP. The assay was performed in 96 well flat-bottomed plates (polystyrene untreated). Desired drug concentrations of the compounds were aliquoted in the assay well, followed by 6 µL of 5X assay buffer mixed with substrate along with 1 µL of enzyme and the reaction volume was made to 30 µL. The contents were added and incubated in the above sequential order as it was of importance for the binding and interaction of the protein consequently the enzyme reaction was initiated by adding 14 µL of $MgCl_2$ solution. The reaction was allowed to proceed for about 100 minutes at room temperature without shaking. Subsequently, the reaction was quenched by adding 20 µL malachite green reagent (Bioassay systems, USA). Inorganic phosphates (Pi) released during the reaction were read at 620 nm after 20 minutes incubation.

4.3.3. In-vitro supercoiling assay

DNA supercoiling assay was performed using gyrase of *Mycobacterium tuberculosis* DNA gyrase. The assay was performed using the commercially available kit (DNA gyrase supercoiling assay kit: MTS001) from Inspiralis limited, Norwich, UK. The assay was carried out in a 1.5 mL eppendorf tubes at room temperature [Jeankumar V.U., *et al.*, 2013]. Usually 1U of *Mycobacterium tuberculosis* DNA gyrase was incubated with 0.5 µg of relaxed pBR 322 DNA (substrate) in 30 µL reaction volume at 37 °C, 30 minutes in 40 mM HEPES. KOH (pH ~ 7.6), 10mM magnesium acetate, 10mM DTT, 2mM ATP, 500mM potassium glutamate, 0.05 mg/mL albumin (BSA). Novobiocin was used as a positive control and 4% DMSO was considered for negative control. DNA gyrase supercoils the relaxed pBR 322 effectively resulting in a denser supercoiled DNA. Subsequently, each reaction was stopped by addition of 30 µL of stop dye [40% sucrose, 100mM Tris-HCl (pH ~ 7.5), 1mM EDTA and 0.5 mg/mL bromophenol blue], followed by a brisk centrifugation for 45 sec and was run in 1% agarose gel in 1X TAE buffer (40mM Tris acetate, 2mM EDTA) [Jeankumar V.U., *et al.*, 2014]. Furthermore, concentration of the range of compounds that inhibits 50% of supercoiling activity IC_{50} of the enzyme was determined using densitometry and NIH image through Bio-Rad GelDoc image viewer.

4.3.4. *Mycobacterium tuberculosis* MABA assay for MIC determination

MABA assay was performed to check the MIC of the *Mycobacterium tuberculosis*bacteria.In brief, the inoculum was prepared from fresh LJ medium resuspended in 7H9 medium (7H9 broth, 0.1% casitone, 0.5% glycerol, supplemented oleic acid, albumin, dextrose, and catalase - OADC), subsequently, adjusted to a McFarland tube No.1, and diluted 1:20; 100 mL was used as inoculum. Each drug stock solution was thawedand diluted in 7H9-S at four-fold the final highest concentration tested [Jeankumar V.U., *et al*., 2013; Jeankumar V.U., *et al*., 2014]. Serial two-fold dilutions of each drug were prepared directly in a sterile 96-well microtiter plate using 100 mL 7H9-S. Agrowth control containing no antibiotic and a sterile control were also prepared on each plate. Sterile water was added to all perimeter 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 of 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 (oxidized 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.

4.3.5. Cell cytotoxic studies by MTT assay

As the *Mycobacterium tuberculosis* organism targets the macrophage cell lines, the toxicity studies were performed in the RAW cell lines. Cytotoxic safety profiling of all the test compounds was done on RAW 264.7 mouse leukemic monocyte macrophage cell line from ATCC [Jeankumar V.U., *et al*., 2014]. Briefly, RAW 264.7 cells were seeded at 6000 cells per well in a 96-well microtiter plate (NEST) in Roswell Park Memorial Institute (RPMI-1640) media. After 24 h incubation, the cells were washed with PBS and 2-fold dilutions of the drug was made in 200 µL of standard culture media (RPMI + 5% FBS + 1% penicillin and streptomycin) was added, while the final DMSO concentration of the culture was limited to 0.5%. Furthermore, the cells were incubated with a drug concentration of 100 µM at 37 °C in 5% CO_2/95% air for 72 h to analyse the toxicity levels. Untreated cells with 0.5% DMSO were included as controls. The viability of the cells were assessed on the basis of cellular conversion of the dye MTT (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) into formazan crystals using Perkin Elmer Victor X3 Titre 96 plate reader at 570 nm. Ciprofloxacin (3% inhibition) and novobiocin (9.8% inhibition) were used as a standards in this assay.

Results and Discussion

Chapter 5

5.1. Benzothiazinone derivatives

Recently, 1,3-benzothiazin-4-ones has generated lot of interest among researchers owing to their potent activity against *Mycobacterium tuberculosis*. In present study we have developed three novel benzothiazinone derivatives namely: novel benzothiazinone- *p*-phenylenediamine linked analogues, benzothiazinone-aminopiperidine hybrid analogues, and benzothiazinone-piperazine derivatives as *Mycobacterium tuberculosis* DNA Gyrase inhibitors.

5.1a. Development of novel benzothiazinone - *p*-phenylenediamine linked analogues as *Mycobacterium tuberculosis* DNA GyrB inhibitors

A relatively well-known molecular hybridization strategy was employed to design novel inhibitors wherein molecular fusion/hybridization between two or more pharmacophoric subunits from the molecular structures of previously reported ligands/prototypes possessing an inhibitory profile/potency against the targeted protein/disease was explored [Maia R., *et al*., 2010; Fraga C.A.M., *et al*., 2009; Lazar C., *et al*., 2004]. Subsequently, the new lead molecule formed with two or more potent moieties resulted in having improved affinity/ specificity towards the target protein, along with increased efficacy than the parent compounds. In this study we utilized chemical structures of previously reported anti-tubercular benzothiazinone bearing (left hand side-LHS) derivative BTZ043 and *Mycobacterium tuberculosis* DNA gyrase inhibitor bearing aryl (thio)urea right hand side (RHS) chain resulted in designed hybrid scaffold through molecular hybridization with the linker as shown in **Figure 5.1**.

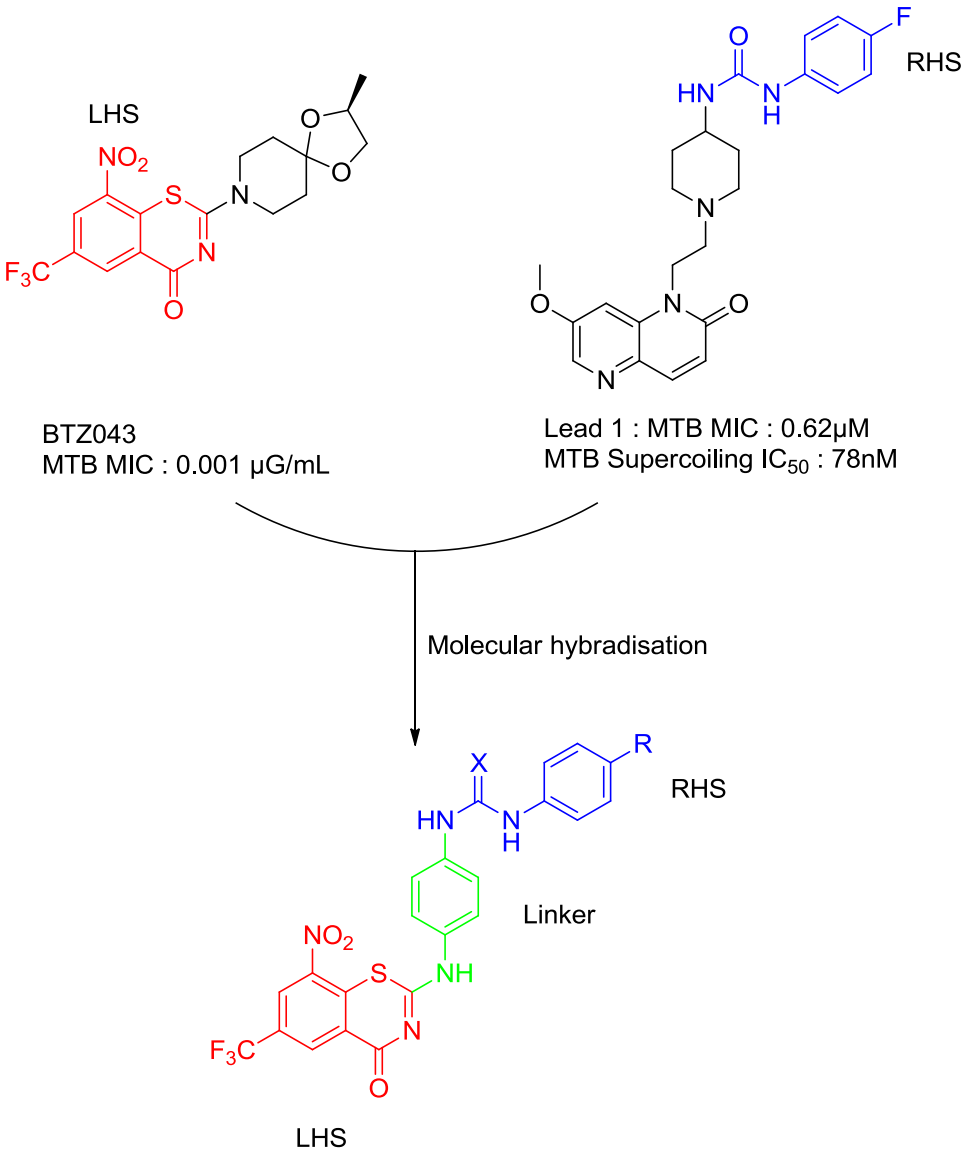


Figure 5.1: Strategy employed for designing the lead. Chemical structure of previously reported anti-tubercular benzothiazinone bearing (LHS) derivative BTZ043 and *Mycobacterium tuberculosis* DNA gyrase inhibitor bearing aryl (thio) urea RHS chain and the inhibitor designed through molecular hybridization with linker.

The constructed ligand thus had three parts: the linker sandwiched between the benzothiazinone on the LHS and aryl urea/thiourea on the RHS. The presence of a benzothiazinone nucleus showed very less *Mycobacterium tuberculosis* MIC's in many of the previously reported inhibitors which made us to retain this scaffold as left hand core. The selection of the right hand substituent as aryl thiourea was purely based on the previously reported inhibitors against DNA gyrase. Thus fusion of both the enzyme level inhibitor with an effective *Mycobacterium tuberculosis* MIC possessing moiety would result in a lead with

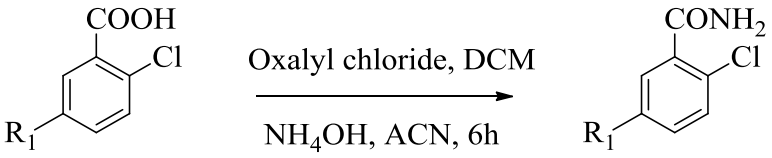
embedded properties of the two parents. Moreover, the presence of hydrophobic pocket in the *Mycobacterium smegmatis* GyrB domain as evidenced by the recent crystal structure encouraged us to attempt a more hydrophobic group as one of the right hand substituent. Further phenyl substituents were also incorporated to trace the SAR and lead optimization.

5.1a.1. Chemical synthesis

The synthetic pathway used to achieve the target compounds has been delineated in **Figure 4.1**. Synthesis of the target compounds started with conversion of commercially available substituted 2-chlorobenzoic acids (**BP_01a-c**) into corresponding 2-chlorobenzoyl chlorides using DMF-catalyzed treatment in presence of oxalyl chloride in dichloromethane. The obtained 2-chlorobenzoyl chlorides were converted into corresponding carboxamide intermediates (**BP_02a-c**) by the addition of 25% aqueous ammonia drop wise at -20 °C. The carboxamide intermediates were further treated with carbon disulphide, methyl iodide and sodium hydroxide in DMSO to afford the thio-alkylated products (**BP_03a-c**), which upon treatment with *N*-Boc-*p*-phenylenediamine in ethanol followed by deprotection using trifluoroacetic acid gave the scaffolds (**BP_04a-c**) in good yield. The final library of compounds was then assembled by treating the scaffolds **BP_04a-c** with the desired isocyanates/isothiocyantes and aldehyde to afford compounds **BP_05 – BP_40** in excellent yields.

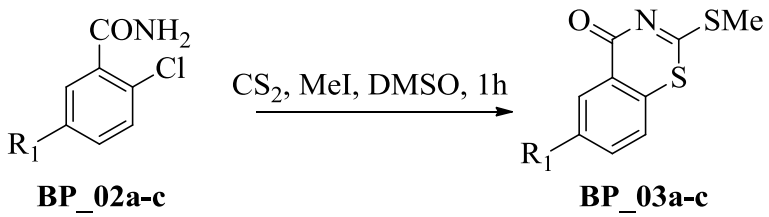
5.1a.2. Experimental protocol utilised for synthesis

General procedure for the synthesis of 5-substituted-2-chlorbenzamides (BP_02a-c).To a stirred solution of the corresponding acid (**BP_01a-c**) (1.0 mmol) in dichloromethane (15 mL) at -10°C was added oxalyl chloride (2.5 mmol). The solution was refluxed for about 6 hours (monitored by TLC & LCMS for completion), and solvent evaporated under reduced pressure. The residue was further diluted with acetonitrile (30 mL), cooled to -20°C and added ammonium hydroxide solution dropwise and allowed to stir for 30 minutes. The resulting solid was filtered out to afford the corresponding amide (**BP_02a-c**) in good yield.



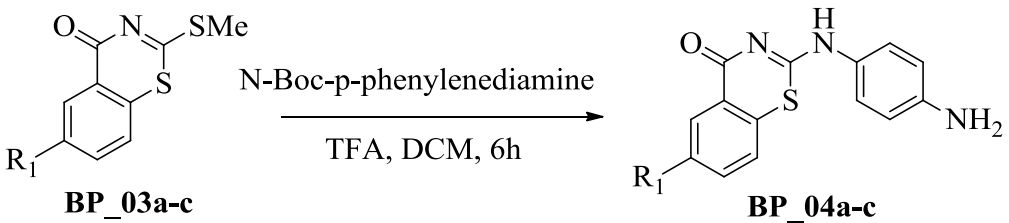
BP_01a-c **BP_02a-c**

General procedure for the synthesis of 5-substituted-2-(methylthio)-4H-benzo[e][1,3] thiazin-4-one (BP_03a-c). To a stirred solution of the corresponding benzamide (**BP_02a-c**) (1.0 mmol) in DMSO (15 mL) at 10 °C was added carbon disulphide (3.0 mmol), sodium hydroxide (2.0 mmol), and the mixture was allowed to stand for 15 minutes. Subsequently methyl iodide (1.2 mmol) was added. The reaction mixture was allowed to stand for another 30 minutes, and 50 mL of water was added. The resulting white solid separated by filtration to afford the corresponding benzothiazinone (**BP_03a-c**) in good yield.



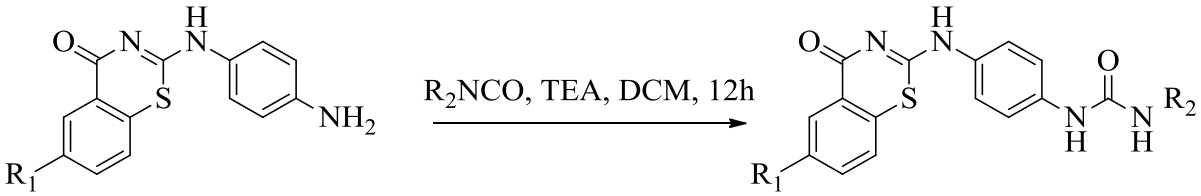
BP_02a-c **BP_03a-c**

General procedure for the synthesis of substituted 2-((4-aminophenyl)amino)-4H-benzo[e][1,3]thiazin-4-ones (BP_04a-c). To a stirred solution of the corresponding benzothiazinone (**BP_03a-c**) (1.0 mmol) in ethanol (15 mL) at room temperature was added *N*-Boc-*p*-phenylenediamine (1.0 mmol). The solution was refluxed for about 12 hours and solvent evaporated under reduced pressure. The residue was further diluted with water (30 mL) and ethyl acetate (50 mL) and the layers were separated. The organic layer was dried over anhydrous sodium sulphate and evaporated under reduced pressure. The residue was purified by silica gel column chromatography using hexane: ethylacetate as eluent to give the corresponding *N*-boc protected 4-aminophenyl benzothiazinones in good yield; which was taken in dichloromethance (60 mL) was cooled to 0 °C was added trifluoroacetic acid (8 mL) and stirred the reaction at room temperature for 1 hour. After completion of the reaction by TLC, the reaction mixture was cooled to 0 °C and basified to pH ~ 8. using saturated aqueous $NaHCO_3$ solution. The organic layer was separated, washed with water (2 × 20 mL) and brine (1 × 20 mL) and dried over anhydrous sodium sulphate. The organic layer was concentrated under vacuum afforded the free amine as pale brown oil. The crude material was used for final reactions without purification.



BP_03a-c **BP_04a-c**

General procedure for the synthesis of 1-(4-((6-substituted-4-oxo-4Hbenzo[e][1,3]thiazin-2-yl)amino)phenyl)-3-arylurea derivatives (BP_05 – BP_16). To a cooled solution of 2-((4-aminophenyl)amino)-6-substituted-4*H*-benzo[*e*][1,3]thiazin-4-one (1.0 mmol) in anhydrous DCM (2 mL) was added corresponding isocyanate (1.0 mmol), triethylamine (10 mmol) and stirred the reaction mixture at room temperature for 12 hours and solvent evaporated under reduced pressure. The residue was further diluted with water (30 mL) and ethyl acetate (50 mL) and the layers were separated. The organic layer was dried over anhydrous sodium sulphate and evaporated under reduced pressure. The residue was purified by silica gel column chromatography using ethylacetate/hexane as eluent to give the corresponding urea derivatives (**BP_05 – BP_16**) in good yields.

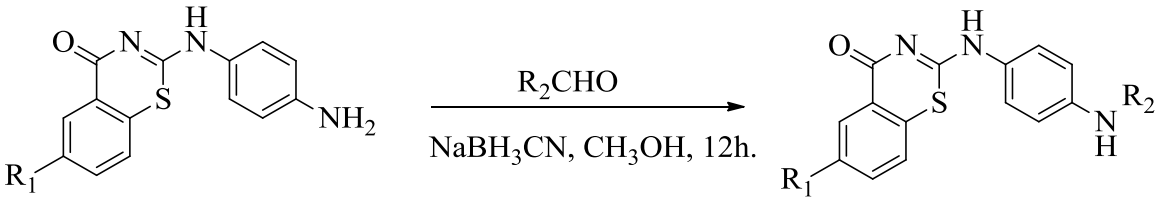


BP_04a-c **BP_05 - BP_16**

General procedure for the synthesis of 1-(4-((6-substituted-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)amino)phenyl)-3-arylthiourea derivatives (BP_17 – BP_28). To a cooled solution of 2-((4-aminophenyl)amino)-6-substituted-4*H*-benzo[*e*][1,3]thiazin-4-one (1 mmol) in anhydrous DCM (2 mL) was added corresponding isothiocyanate (1.0 mmol), triethylamine (1.0 mmol) and stirred the reaction mixture at room temperature for 12 hours (monitored by TLC & LCMS for completion) and solvent evaporated under reduced pressure. The residue was further diluted with water (30 mL) and ethyl acetate (50 mL) and the layers were separated. The organic layer was dried over anhydrous sodium sulphate and evaporated under reduced pressure. The residue was purified by silica gel column chromatography using ethylacetate/hexane as eluent to give the corresponding thiourea derivative (**BP_17 – BP_28**) in good yield.

BP_04a-c **BP_17 - BP_28**

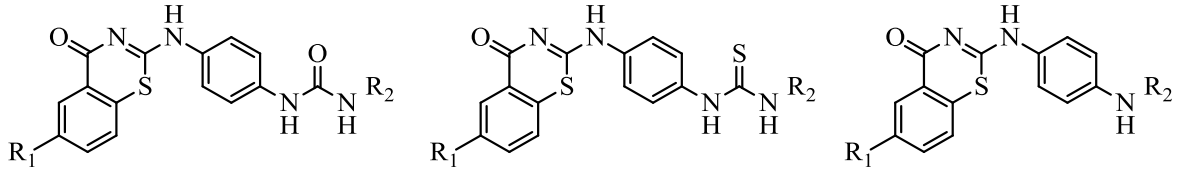
General procedure for the synthesis of2-((4-(benzylamino)phenyl)amino)-6-substituted-4H-benzo[e][1,3]thiazin-4-one derivatives (BP_29 – BP_40). To a cooled solution of 2-((4-aminophenyl)amino)-6-substituted-4H-benzo[*e*][1,3]thiazin-4-one (1.0 mmol) in methanol (2 mL) was added aldehyde and sodium cyanoborohydride (1.0 mmol) and stirred the reaction mixture at room temperature for 12 hours and solvent evaporated under reduced pressure. The residue was further diluted with water (30 mL) and ethyl acetate (50 mL) and the layers were separated. The organic layer was dried over anhydrous sodium sulphate and evaporated under reduced pressure. The residue was purified by silica gel column chromatography using ethylacetate/hexane as eluent to give the corresponding final derivatives (**BP_29 – BP_40**) in good yields.



BP_04a-c **BP_29 - BP_40**

The physicochemical properties of synthesized derivatives are shown in **Table 5.1**.

Table 5.1: Physiochemical properties of synthesized compounds **BP_05 – BP_40**



BP_05 - BP_16 **BP_17 - BP_28** **BP_29 - BP_40**

Compd	R_1	R_2	Yield (%)	M.P. (°C)	Molecular formula	Molecular weight
BP_05	Nitro	Phenyl	51	211-213	$C_{21}H_{15}N_5O_4S$	433.44
BP_06	Nitro	4-Chlorophenyl	57	203-205	$C_{21}H_{14}ClN_5O_4S$	467.88
BP_07	Nitro	4-Methylphenyl	39	208-210	$C_{22}H_{17}N_5O_4S$	447.47
BP_08	Nitro	4-Nitrophenyl	15	226-228	$C_{21}H_{14}N_6O_6S$	478.44
BP_09	Trifluoromethyl	Phenyl	54	213-215	$C_{22}H_{15}F_3N_4O_2S$	449.51
BP_10	Trifluoromethyl	4-Chlorophenyl	36	211-213	$C_{22}H_{14}ClF_3N_4O_2S$	483.95

Contd.

Compd	R_1	R_2	Yield (%)	M.P. (°C)	Molecular formula	Molecular weight
BP_11	Trifluoromethyl	4-Methylphenyl	36	216-218	$C_{23}H_{17}F_3N_4O_2S$	463.53
BP_12	Trifluoromethyl	4-Nitrophenyl	26	201-203	$C_{22}H_{14}F_3N_4O_2S$	494.5
BP_13	Chloro	Phenyl	38	217-219	$C_{21}H_{15}ClN_4O_2S$	404.44
BP_14	Chloro	4-Chlorophenyl	38	222-224	$C_{21}H_{14}Cl_2N_4O_2S$	438.89
BP_15	Chloro	4-Methylphenyl	46	197-199	$C_{22}H_{17}ClN_4O_2S$	418.47
BP_16	Chloro	4-Nitrophenyl	46	215-217	$C_{21}H_{14}ClN_5O_4S$	449.44
BP_17	Nitro	Phenyl	48	221-223	$C_{21}H_{15}N_5O_3S_2$	456.44
BP_18	Nitro	4-Chlorophenyl	47	189-191	$C_{21}H_{14}ClN_5O_3S_2$	490.89
BP_19	Nitro	4-Methylphenyl	49	186-188	$C_{22}H_{17}N_5O_3S_2$	470.47
BP_20	Nitro	4-Nitrophenyl	27	192-194	$C_{21}H_{14}N_6O_5S_2$	501.44
BP_21	Trifluoromethyl	Phenyl	41	179-181	$C_{22}H_{15}F_3N_4OS_2$	472.51
BP_22	Trifluoromethyl	4-Chlorophenyl	41	193-195	$C_{22}H_{14}ClF_3N_4OS_2$	506.95
BP_23	Trifluoromethyl	4-Methylphenyl	46	183-185	$C_{23}H_{17}F_3N_4OS_2$	486.53
BP_24	Trifluoromethyl	4-Nitrophenyl	32	192-194	$C_{22}H_{14}F_3N_5O_3S_2$	517.5
BP_25	Chloro	Phenyl	35	199-201	$C_{21}H_{15}ClN_4OS_2$	427.44
BP_26	Chloro	4-Chlorophenyl	15	206-208	$C_{21}H_{14}Cl_2N_4OS_2$	461.89
BP_27	Chloro	4-Methylphenyl	49	221-223	$C_{21}H_{14}Cl_2N_4OS_2$	441.47
BP_28	Chloro	4-Nitrophenyl	33	227-229	$C_{22}H_{17}ClN_4OS_2$	472.44
BP_29	Nitro	Benzyl	36	189-191	$C_{21}H_{14}ClN_5O_3S_2$	422.89
BP_30	Nitro	4-Chlorobenzyl	47	204-206	$C_{21}H_{15}ClN_4O_3S$	457.33
BP_31	Nitro	4-Methylbenzyl	40	217-219	$C_{22}H_{18}N_4O_3S$	436.91
BP_32	Nitro	4-Nitrobenzyl	40	201-203	$C_{21}H_{15}N_5O_5S$	467.88
BP_33	Trifluoromethyl	Benzyl	52	186-188	$C_{22}H_{16}F_3N_3OS$	438.95
BP_34	Trifluoromethyl	4-Chlorobenzyl	64	182-184	$C_{22}H_{15}ClF_3N_3OS$	473.4
BP_35	Trifluoromethyl	4-Methylbenzyl	49	193-195	$C_{23}H_{18}F_3N_3OS$	452.98
BP_36	Trifluoromethyl	4-Nitrobenzyl	49	183-185	$C_{22}H_{15}F_3N_4O_3S$	483.95
BP_37	Chloro	Benzyl	55	197-199	$C_{21}H_{16}ClN_3OS$	393.89
BP_38	Chloro	4-Chlorobenzyl	52	205-207	$C_{21}H_{15}Cl_2N_3OS$	428.33

Contd.

Compd	R_1	R_2	Yield (%)	M.P. (°C)	Molecular formula	Molecular weight
BP_39	Chloro	4-Methylbenzyl	36	211-213	$C_{22}H_{18}ClN_3OS$	407.92
BP_40	Chloro	4-Nitrobenzyl	40	215-217	$C_{21}H_{15}ClN_4O_3S$	438.89

5.1a.3. Characterization of synthesized compounds

A series of 36 derivatives were prepared using the above method and both analytical and spectral data (^{1}H NMR, ^{13}C NMR and mass spectra) of all the synthesized compounds were in full agreement with the proposed structures.

2-Chloro-5-nitrobenzamide (BP_02a).The compound was synthesized according to the general procedure using 2-chloro-5-nitrobenzoic acid (**BP_01a**) (5.0 g, 0.02 mmol), oxalyl chloride (5.3 mL, 0.05 mmol) and aqueous ammonium hydroxide (50 mL) to afford **BP_02a** (3.9 g, 78.4 %) as yellow solid. M.p: 185-187 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.28-7.83 (m, 3H), 7.52 (b, 2H). ^{13}C NMR (DMSO-d_6): δ_C 168.2, 146.2, 140.3, 133.3, 130.7, 128.5, 122.1. ESI-MS *m/z* 201 $[M+H]^+$. Anal calcd for $C_7H_5ClN_2O_3$: C, 41.92; H, 2.51; N, 13.97; Found: C, 41.89; H, 2.54; N, 13.99.

2-Chloro-5-(trifluoromethyl)benzamide (BP_02b). The compound was synthesized according to the general procedure using 2-chloro-5-(trifluoromethyl)benzoic acid (**BP_01b**) (4.0 g, 0.01 mmol), oxalyl chloride (3.8 mL, 0.04 mmol) and aqueous ammonium hydroxide (40 mL) to afford **BP_02b** (1.84 g, 46.2 %) as white solid. M.p: 176-178 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.02-7.64 (m, 3H), 7.54 (b, 2H). ^{13}C NMR (DMSO-d_6): δ_C 168.4, 137.7, 132.3, 129.7, 129.5, 128.1, 125.3. 123.5. ESI-MS *m/z* 224 $[M+H]^+$. Anal.Calcd.For $C_8H_5ClF_3NO$: C, 42.98; H, 2.25; N, 6.26; Found: C, 41.89; H, 2.54; N, 13.99.

2,5-Dichlorobenzamide (BP_02c). The compound was synthesized according to the general procedure using 2,5-dichlorobenzoic acid (**BP_01c**) (4.0 g, 0.02 mmol), oxalyl chloride (4.5 mL, 0.05 mmol) and aqueous ammonium hydroxide (40 mL) to afford **BP_02c** (2.12 g, 53.4 %) as white solid. M.p: 181-183 °C. ^{1}H NMR (DMSO-d_6): δ_H 7.89-7.63 (m, 3H), 7.52 (b, 2H). ^{13}C NMR (DMSO-d_6): δ_C 168.6, 133.5, 132.5, 132.3, 132.1, 129.4, 128.6. ESI-MS *m/z* 192 $[M+H]^+$. Anal.Calcd.For $C_7H_5Cl_2NO$: C, 44.24; H, 2.65; N, 7.37; Found: C, 44.26; H, 2.64; N, 7.39.

2-(Methylthio)-6-nitro-4H-benzo[e][1,3]thiazin-4-one (BP_03a). The compound was synthesized according to the general procedure using 2-chloro-5-nitrobenzamide (**BP_02a**) (2.0 g, 9.97 mmol), carbon disulphide (1.8 mL, 29.9 mmol), sodim hydroxide (0.79g, 19.9 mmol) and methyl iodide (0.73 mL, 11.9 mmol) to afford **BP_03a** (1.82 g, 71.9 %) as yellow solid. M.p: 211-213 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.36-7.82 (m, 3H), 2.72 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 167.6, 162.4, 145.3, 143.6, 138.4, 130.5, 128.4, 123.6, 14.2. ESI-MS *m/z* 255 $[M+H]^+$. Anal.Calcd.For $C_9H_6N_2O_3S_2$: C, 42.51; H, 2.38; N, 11.02; Found: C, 42.54; H, 2.36; N, 11.05.

2-(Methylthio)-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (BP_03b).The compound was synthesized according to the general procedure using 2-chloro-5-(trifluoromethyl)benzamide (**BP_02b**) (1.5 g, 6.71 mmol), carbon disulphide (1.2 mL, 20.1 mmol), sodim hydroxide (0.53g, 13.4 mmol) and methyl iodide (0.49 mL, 8.1 mmol) to afford **BP_03b** (1.63 g, 88.1 %) as white solid. M.p: 208-210 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.35-7.78 (m, 3H), 2.72 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 167.4, 162.5, 140.3, 137.6, 131.4, 130.6, 128.4, 126.6, 123.5, 14.3. ESI-MS *m/z* 278 $[M+H]^+$. Anal.Calcd.For $C_{10}H_6F_3NOS_2$: C, 43.32; H, 2.18; N, 5.05; Found: C, 43.33; H, 2.16; N, 5.03.

6-Chloro-2-(methylthio)-4H-benzo[e][1,3]thiazin-4-one (BP_03c). The compound was synthesized according to the general procedure using 2,5-dichlorobenzamide (**BP_02c**) (2.0 g, 10.52 mmol), carbon disulphide (1.9 mL, 31. mmol), sodim hydroxide (0.84 g, 21.1 mmol) and methyl iodide (0.78 mL, 12.6 mmol) to afford **BP_03c** (1.57 g, 61.3 %) as white solid. M.p: 219-221 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.36-7.74 (m, 3H), 2.72 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 167.8, 162.5, 138.3, 135.6, 134.4, 131.6, 131.4, 130.6, 14.3. ESI-MS *m/z* 244 $[M+H]^+$. Anal.Calcd.For $C_9H_6ClNOS_2$: C, 44.35; H, 2.48; N, 5.75; Found: C, 44.33; H, 2.46; N, 5.73.

2-((4-Aminophenyl)amino)-6-nitro-4H-benzo[e][1,3]thiazin-4-one (BP_04a). The compound was synthesized according to the general procedure using 2-(methylthio)-6-nitro-4*H*-benzo[*e*][1,3]thiazin-4-one (**BP_03a**) (1.8 g, 7.09 mmol), *N*-Boc-*p*-phenylenediamine (1.47 g, 7.09 mmol) and trifluoroacetic acid (2 mL) to afford **BP_04a** (1.57 g, 70.7 %) as yellow solid. M.p: 212-214 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.33-6.24 (m, 7H), 6.25 (b, 2H), 4.2 (s, 1H). ^{13}C NMR (DMSO-d_6): δ_C 167.5, 159.4, 145.3, 143.6, 138.4, 138.3, 130.4, 129.6, 128.7, 123.5, 117.2 (4C). ESI-MS *m/z* 315 $[M+H]^+$. Anal.Calcd.For $C_{14}H_{10}N_4O_3S$: C, 53.50; H, 3.21; N, 17.82; Found: C, 53.52; H, 3.25; N, 17.79.

2-((4-Aminophenyl)amino)-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (BP_04b). The compound was synthesized according to the general procedure using 2-(methylthio)-6-(trifluoromethyl)-4*H*-benzo[*e*][1,3]thiazin-4-one (**BP_03b**) (1.6 g, 6.31 mmol), *N*-Boc-*p*-phenylenediamine (1.31 g, 6.31 mmol) and trifluoroacetic acid (2 mL) to afford **BP_04b** (1.43 g, 73.7 %) as white solid. M.p: 221-223 °C. ^{1}H NMR (DMSO-d_6): δ_H 7.87-6.28 (m, 7H), 6.24 (b, 2H), 4.1 (s, 1H). ^{13}C NMR (DMSO-d_6): δ_C 167.4, 159.1, 140.3, 138.6, 137.4, 131.3, 130.5, 129.8, 128.3, 126.5, 123.2, 117.2 (4C). ESI-MS *m/z* 338 $[M+H]^+$. Anal.Calcd.For $C_{15}H_{10}F_3N_3OS$: C, 53.41; H, 2.99; N, 12.46; Found: C, 53.44; H, 2.97; N, 12.42.

2-((4-Aminophenyl)amino)-6-chloro-4H-benzo[e][1,3]thiazin-4-one (BP_04c). The compound was synthesized according to the general procedure using 6-chloro-2-(methylthio) -4*H*-benzo[*e*][1,3]thiazin-4-one (**BP_03c**) (1.5 g, 6.15 mmol), *N*-Boc-*p*-phenylenediamine (1.27 g, 6.15 mmol) and trifluoroacetic acid (2 mL) to afford **BP_04c** (1.38 g, 73.1 %) as white solid. M.p: 217-219 °C. ^{1}H NMR (DMSO-d_6): δ_H 7.67-6.31 (m, 7H), 6.22 (b, 2H), 4.2 (s, 1H). ^{13}C NMR (DMSO-d_6): δ_C 167.5, 159.2, 138.3, 138.1, 135.4, 134.3, 131.5, 131.2, 130.3, 129.5, 117.3 (4C). ESI-MS *m/z* 304 $[M+H]^+$. Anal.Calcd.For $C_{14}H_{10}ClN_3OS$: C, 55.35; H, 3.32; N, 13.83; Found: C, 55.36; H, 3.36; N, 13.85.

1-(4-((6-Nitro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)amino)phenyl)-3-phenylurea (BP_05). The compound was synthesized according to the general procedure using 2-((4-aminophenyl)amino)-6-nitro-4*H*-benzo[*e*][1,3]thiazin-4-one (**BP_04a**) (0.1 g, 0.32 mmol) and Phenyl isocyanate (0.04 g, 0.32 mmol) to afford **BP_05** (0.07 g, 50.72 %) as yellow solid. M.p: 211-213 °C. ^{1}H NMR (DMSO-d_6): δ_H 10.32 (s, 1H), 10.21 (s, 1H), 9.47 (s, 1H), 8.71-6.83 (m, 12H). ^{13}C NMR (DMSO-d_6): δ_C 167.5, 159.5, 152.7, 145.3, 143.6, 139.1, 138.2, 135, 130.7, 129.1, 128.5 (3C), 128, 123.7, 121.3 (2C), 119 (2C), 116.3 (2C). ESI-MS *m/z* 434 $[M+H]^+$. Anal.Calcd.For $C_{21}H_{15}N_5O_4S$: C, 58.19; H, 3.49; N, 16.16; Found: C, 58.21; H, 3.45; N, 16.18.

1-(4-Chlorophenyl)-3-(4-((6-nitro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)amino)phenyl)urea (BP_06). The compound was synthesized according to the general procedure using 2-((4-aminophenyl)amino)-6-nitro-4*H*-benzo[*e*][1,3]thiazin-4-one (**BP_04a**) (0.1 g, 0.32 mmol) and 4-chlorophenyl isocyanate (0.05 g, 0.32 mmol) to afford **BP_06** (0.08 g, 57.05 %) as yellow solid. M.p: 203-205 °C. ^{1}H NMR (DMSO-d_6): δ_H 10.29 (s, 1H), 10.22 (s, 1H), 9.36 (s, 1H), 8.65-6.77 (m, 11H). ^{13}C NMR (DMSO-d_6): δ_C 167.4, 159.2, 152.5, 145.5, 143.4, 138.2,

137.4, 135, 133.1, 130.5, 129.5, 129 (2C), 128.7, 123.5, 120.6 (2C), 119.0 (2C), 116.4 (2C). ESI-MS *m/z* 468 $[M+H]^+$. Anal.Calcd.For $C_{21}H_{14}ClN_5O_4S$: C, 53.91; H, 3.02; N, 14.97; Found: C, 53.89; H, 3.05; N, 14.95.

1-(4-((6-Nitro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)amino)phenyl)-3-(p-tolyl)urea (BP_07). The compound was synthesized according to the general procedure using 2-((4-aminophenyl)amino)-6-nitro-4*H*-benzo[*e*][1,3]thiazin-4-one (**BP_04a**) (0.1 g, 0.32 mmol) and *p*-tolylisocyanate (0.04 g, 0.32 mmol) to afford **BP_07** (0.05 g, 39.30 %) as yellow solid. M.p: 208-210 °C. ^{1}H NMR (DMSO-d_6): δ_H 10.31 (s, 1H), 10.25 (s, 1H), 9.52 (s, 1H), 8.68-6.81 (m, 11H), 2.36 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 167.6, 159.3, 152.5, 145.4, 143.7, 138.1, 136.4, 136.2, 135, 130.7, 129.5, 129.2 (2C), 128.7, 123.3, 121.4 (2C), 119 (2C), 116.5 (2C), 21.4. ESI-MS *m/z* 448 $[M+H]^+$. Anal.Calcd.For $C_{22}H_{17}N_5O_4S$: C, 59.05; H, 3.83; N, 15.65; Found: C, 59.07; H, 3.81; N, 15.67.

1-(4-((6-Nitro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)amino)phenyl)-3-(4-nitrophenyl)urea (BP_08). The compound was synthesized according to the general procedure using 2-((4-aminophenyl)amino)-6-nitro-4*H*-benzo[*e*][1,3]thiazin-4-one (**BP_04a**) (0.1 g, 0.32 mmol) and 4-nitrophenyl isocyanate (0.05 g, 0.32 mmol) to afford **BP_08** (0.02 g, 15.4 %) as yellow solid. M.p: 226-228 °C. ^{1}H NMR (DMSO-d_6): δ_H 10.25 (s, 1H), 10.18 (s, 1H), 9.52 (s, 1H), 8.83-6.67 (m, 11H). ^{13}C NMR (DMSO-d_6): δ_C 167.5, 159.2, 152.7, 145.4, 145.2, 143.5, 143.2, 138.2, 135, 130.7, 129.5, 128.7, 124.2 (2C), 123.5, 119.5 (2C), 119.0 (2C), 116.5 (2C). ESI-MS *m/z* 479 $[M+H]^+$. Anal.Calcd.For $C_{21}H_{14}N_6O_6S$: C, 52.72; H, 2.95; N, 17.57; Found: C, 52.75; H, 2.92; N, 17.55.

1-(4-((4-Oxo-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-2-yl)amino)phenyl)-3-phenyl urea (BP_09). The compound was synthesized according to the general procedure using 2-((4-aminophenyl)amino)-6-(trifluoromethyl)-4*H*-benzo[*e*][1,3]thiazin-4-one (**BP_04b**) (0.1 g, 0.29 mmol) and phenyl isocyanate (0.03 g, 0.29 mmol) to afford **BP_09** (0.07 g, 53.9 %) as white solid. M.p: 213-215 °C. ^{1}H NMR (DMSO-d_6): δ_H 10.15 (s, 1H), 10.06 (s, 1H), 9.29 (s, 1H), 8.12-6.82 (m, 12H). ^{13}C NMR (DMSO-d_6): δ_C 167.5, 159.2, 152.6, 140.5, 139.2, 137.9, 135, 131.3, 130.5, 129.5, 128.7 (2C), 128.5, 128, 126.4, 123.5, 121.5 (2C), 119.0 (2C), 116.4 (2C). ESI-MS *m/z* 457 $[M+H]^+$. Anal.Calcd.For $C_{22}H_{15}F_3N_4O_2S$: C, 57.89; H, 3.31; N, 12.27; Found: C, 57.86; H, 3.29; N, 12.29.

1-(4-Chlorophenyl)-3-(4-((4-oxo-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-2-yl)amino) phenyl)urea (BP_10). The compound was synthesized according to the general procedure using 2-((4-aminophenyl)amino)-6-(trifluoromethyl)-4*H*-benzo[*e*][1,3]thiazin-4-one (**BP_04b**) (0.1 g, 0.29 mmol) and 4-chlorophenyl isocyanate (0.04 g, 0.29 mmol) to afford **BP_10** (0.05 g, 35.7 %) as white solid. M.p: 211-213 °C. ^{1}H NMR (DMSO-d_6): δ_H 10.18 (s, 1H), 10.09 (s, 1H), 9.31 (s, 1H), 8.11-6.83 (m, 11H). ^{13}C NMR (DMSO-d_6): δ_C 167.5, 159.2, 152.7, 146.3, 137.5, 137.2, 135, 133.1, 131.2, 130.3, 129.5, 129 (2C), 128.4, 126.4, 123.6, 120.7 (2C), 119.0 (2C), 116.3 (2C). ESI-MS *m/z* 491 $[M+H]^+$. Anal.Calcd.For $C_{22}H_{14}ClF_3N_4O_2S$: C, 53.83; H, 2.87; N, 11.41; Found: C, 53.79; H, 2.89; N, 11.44.

1-(4-((4-Oxo-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-2-yl)amino)phenyl)-3-(p tolyl)urea (BP_11). The compound was synthesized according to the general procedure using 2-((4-aminophenyl)amino)-6-(trifluoromethyl)-4*H*-benzo[*e*][1,3]thiazin-4-one (**BP_04b**) (0.1 g, 0.29 mmol) and *p*-tolylisocyanate (0.04 g, 0.29 mmol) to afford **BP_11** (0.04 g, 36.0 %) as white solid. M.p: 216-218 °C. ^{1}H NMR (DMSO-d_6): δ_H 10.21 (s, 1H), 10.14 (s, 1H), 9.22 (s, 1H), 8.16-6.75 (m, 11H), 2.36 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 167.8, 159.4, 152.8, 140.5, 137.8, 137.5, 136.4, 135, 131.1, 130.3, 129.5, 129.0 (2C), 128.4, 126.6, 123.6, 121.7 (2C), 119.0 (2C), 116.5 (2C), 21.2. ESI-MS *m/z* 471 $[M+H]^+$. Anal.Calcd.For $C_{23}H_{17}F_3N_4O_2S$: C, 58.72; H, 3.64; N, 11.91; Found: C, 58.69; H, 3.66; N, 11.89.

1-(4-Nitrophenyl)-3-(4-((4-oxo-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-2-yl) amino)phenyl)urea (BP_12). The compound was synthesized according to the general procedure using 2-((4-aminophenyl)amino)-6-(trifluoromethyl)-4*H*-benzo[*e*][1,3]thiazin-4-one (**BP_04b**) (0.1 g, 0.29 mmol) and 4-nitrophenyl isocyanate (0.05 g, 0.29 mmol) to afford **BP_12** (0.03 g, 26.2 %) as white solid. M.p: 201-203 °C. ^{1}H NMR (DMSO-d_6): δ_H 10.08 (s, 1H), 9.95 (s, 1H), 9.18 (s, 1H), 8.15-6.77 (m, 11H). ^{13}C NMR (DMSO-d_6): δ_C 167.5, 159.2, 152.6, 145.6, 143.6, 140.5, 137.7, 135, 131.1, 130.3, 129.5, 128.2, 126.4, 124.2 (2C), 123.5, 119.8 (2C), 119.0 (2C), 116.4 (2C). ESI-MS *m/z* 502 $[M+H]^+$. Anal.Calcd.For $C_{22}H_{14}F_3N_4O_2S$: C, 52.70; H, 2.81; N, 13.97; Found: C, 52.73; H, 2.84; N, 13.95.

1-(4-((6-Chloro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)amino)phenyl)-3-phenylurea (BP_13). The compound was synthesized according to the general procedure using 2-((4-aminophenyl)amino)-6-chloro-4*H*-benzo[*e*][1,3]thiazin-4-one (**BP_04c**) (0.1 g, 0.33 mmol) and phenyl isocyanate (0.04 g, 0.33 mmol) to afford **BP_13** (0.05 g, 37.9 %) as white solid. M.p: 217-219 °C. ^{1}H NMR (DMSO-d_6): δ_H 10.16 (s, 1H), 10.08 (s, 1H), 9.26 (s, 1H), 7.87-

6.81 (m, 12H). ^{13}C NMR (DMSO-d_6): δ_C 167.6, 159.4, 152.8, 139.3, 138.7, 135.5, 135, 134.6, 131.5, 131.5, 130.4, 129.3, 128.8 (2C), 128, 121.5 (2C), 119.0 (2C), 116.6 (2C). ESI-MS *m/z* 423 $[M+H]^+$. Anal.Calcd.For $C_{21}H_{15}ClN_4O_2S$: C, 59.64; H, 3.58; N, 13.25; Found: C, 59.61; H, 3.61; N, 13.22.

1-(4-((6-Chloro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)amino)phenyl)-3-(4-chlorophenyl)urea (BP_14). The compound was synthesized according to the general procedure using 2-((4-aminophenyl)amino)-6-chloro-4*H*-benzo[*e*][1,3]thiazin-4-one (**BP_04c**) (0.1 g, 0.33 mmol) and 4-chlorophenyl isocyanate (0.05 g, 0.33 mmol) to afford **BP_14** (0.05 g, 37.7 %) as white solid. M.p: 222-224 °C. ^{1}H NMR (DMSO-d_6): δ_H 10.21 (s, 1H), 10.14 (s, 1H), 9.19 (s, 1H), 7.92-6.75 (m, 11H). ^{13}C NMR (DMSO-d_6): δ_C 167.5, 159.2, 152.8, 138.6, 137.4, 135.5, 135, 134.8, 133.4, 131.3, 131.1, 130.3, 129.5, 129.0 (2C), 120.7 (2C), 119.0 (2C), 116.4 (2C). ESI-MS *m/z* 458 $[M+H]^+$. Anal.Calcd.For $C_{21}H_{14}Cl_2N_4O_2S$: C, 55.15; H, 3.09; N, 12.25; Found: C, 55.18; H, 3.12; N, 12.21.

1-(4-((6-Chloro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)amino)phenyl)-3-(p-tolyl)urea (BP_15). The compound was synthesized according to the general procedure using 2-((4-aminophenyl)amino)-6-chloro-4*H*-benzo[*e*][1,3]thiazin-4-one (**BP_04c**) (0.1 g, 0.33 mmol) and *p*-tolylisocyanate (0.04 g, 0.33 mmol) to afford **BP_15** (0.06 g, 45.7 %) as white solid. M.p: 197-199 °C. ^{1}H NMR (DMSO-d_6): δ_H 10.11 (s, 1H), 10.06 (s, 1H), 9.22 (s, 1H), 7.88-6.71 (m, 11H), 2.36 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 167.5, 159.4, 152.6, 138.5, 136.7, 136.4, 135.3, 135, 134.7, 131.7, 131.2, 130.3, 129.5, 129.1 (2C), 121.4 (2C), 119.0 (2C), 116.4 (2C), 21.3. ESI-MS *m/z* 437 $[M+H]^+$. Anal.Calcd.For $C_{22}H_{17}ClN_4O_2S$: C, 60.48; H, 3.92; N, 12.82; Found: C, 60.45; H, 3.89; N, 12.85.

1-(4-((6-Chloro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)amino)phenyl)-3-(4nitrophenyl)urea (BP_16). The compound was synthesized according to the general procedure using 2-((4-aminophenyl)amino)-6-chloro-4*H*-benzo[*e*][1,3]thiazin-4-one (**BP_04c**) (0.1 g, 0.33 mmol) and 4-nitrophenyl isocyanate (0.05 g, 0.33 mmol) to afford **BP_16** (0.07 g, 45.9 %) as white solid. M.p: 215-217 °C. ^{1}H NMR (DMSO-d_6): δ_H 10.08 (s, 1H), 10.02 (s, 1H), 9.23 (s, 1H), 7.79-6.63 (m, 11H). ^{13}C NMR (DMSO-d_6): δ_C 167.6, 159.4, 152.8, 145.3, 143.7, 138.5, 135.3, 135, 134.6, 131.5, 131.4, 130.4, 129.4, 124.2 (2C), 119.6 (2C), 119.0 (2C), 116.4 (2C). ESI-MS *m/z* 468 $[M+H]^+$. Anal.Calcd.For $C_{21}H_{14}ClN_5O_4S$: C, 53.91; H, 3.02; N, 14.97; Found: C, 53.93; H, 3.04; N, 14.99.

1-(4-((6-Nitro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)amino)phenyl)-3-phenylthiourea (BP_17). The compound was synthesized according to the general procedure using 2-((4-aminophenyl)amino)-6-nitro-4*H*-benzo[*e*][1,3]thiazin-4-one (**BP_04a**) (0.1 g, 0.31 mmol) and phenyl isothiocyanate (0.04 g, 0.31 mmol) to afford **BP_17** (0.06 g, 47.5 %) as yellow solid. M.p: 221-223 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.62 (s, 1H), 9.46 (s, 1H), 9.21 (s, 1H), 8.78-6.74 (m, 12H). ^{13}C NMR (DMSO-d_6): δ_C 179.8, 167.5, 159.2, 145.1, 143.5, 138.6, 138.4, 135.6, 130.7, 129.0 (2C), 128.8, 128.6, 128.4, 127.4 (2C), 126.5 (2C), 123.6, 116.6 (2C). ESI-MS *m/z* 450 $[M+H]^+$. Anal.Calcd.For $C_{21}H_{15}N_5O_3S_2$: C, 56.11; H, 3.36; N, 15.58; Found: C, 56.14; H, 3.32; N, 15.54.

1-(4-Chlorophenyl)-3-(4-((6-nitro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)amino)phenyl)thiourea (BP_18). The compound was synthesized according to the general procedure using 2-((4-aminophenyl)amino)-6-nitro-4*H*-benzo[*e*][1,3]thiazin-4-one (**BP_04a**) (0.1 g, 0.31 mmol) and 4-Chlorophenyl isothiocyanate (0.05 g, 0.31 mmol) to afford **BP_18** (0.07 g, 46.7 %) as yellow solid. M.p: 189-191 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.58 (s, 1H), 9.44 (s, 1H), 9.19 (s, 1H), 8.76-6.82 (m, 11H). ^{13}C NMR (DMSO-d_6): δ_C 179.9, 167.5, 159.4, 145.2, 143.5, 138.4, 136.5, 135.6, 133.6, 131.2 (2C), 130.8, 129.1 (2C), 128.8, 128.5, 127.2 (2C), 123.7, 116.5 (2C). ESI-MS *m/z* 484 $[M+H]^+$. Anal.Calcd.For $C_{21}H_{14}ClN_5O_3S_2$: C, 52.12; H, 2.92; N, 14.47; Found: C, 52.14; H, 2.89; N, 14.45.

1-(4-((6-Nitro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)amino)phenyl)-3-(p-tolyl)thiourea (BP_19). The compound was synthesized according to the general procedure using 2-((4-aminophenyl)amino)-6-nitro-4*H*-benzo[*e*][1,3]thiazin-4-one (**BP_04a**) (0.1 g, 0.31 mmol) and *p*-tolylisothiocyanate (0.05 g, 0.31 mmol) to afford **BP_19** (0.07 g, 49.4 %) as yellow solid. M.p: 186-188 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.64 (s, 1H), 9.45 (s, 1H), 9.24 (s, 1H), 8.81-6.77 (m, 11H), 2.36 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 179.8, 167.6, 159.2, 145.4, 143.5, 138.2, 137.2, 135.6, 135.3, 130.7, 129.4 (2C), 128.8, 128.5, 127.4 (2C), 126.5 (2C), 123.7, 116.6 (2C), 21.4. ESI-MS *m/z* 464 $[M+H]^+$. Anal.Calcd.For $C_{22}H_{17}N_5O_3S_2$: C, 57; H, 3.70; N, 15.11; Found: C, 57.02; H, 3.68; N, 15.13.

1-(4-((6-Nitro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)amino)phenyl)-3-(4-nitrophenyl)thiourea (BP_20). The compound was synthesized according to the general procedure using 2-((4-aminophenyl)amino)-6-nitro-4*H*-benzo[*e*][1,3]thiazin-4-one (**BP_04a**) (0.1 g, 0.31 mmol) and 4-nitrophenyl isothiocyanate (0.06 g, 0.31 mmol) to afford **BP_20** (0.04 g, 27.3 %) as yellow solid. M.p: 192-194 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.59 (s, 1H),

9.52 (s, 1H), 9.25 (s, 1H), 8.83-6.79 (m, 11H). ^{13}C NMR (DMSO-d_6): δ_C 179.9, 167.6, 159.2, 145.1, 144.5, 143.7, 143.4, 138.5, 135.3, 130.7, 128.7, 128.5, 127.4 (2C), 124.6 (2C), 124.3 (2C), 123.7, 116.5 (2C). ESI-MS *m/z* 495 $[M+H]^+$. Anal.Calcd.For $C_{21}H_{14}N_6O_5S_2$: C, 51.01; H, 2.85; N, 16.99; Found: C, 51.04; H, 2.81; N, 16.96.

1-(4-((4-Oxo-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-2-yl)amino)phenyl)-3-phenylthiourea (BP_21). The compound was synthesized according to the general procedure using 2-((4-aminophenyl)amino)-6-(trifluoromethyl)-4*H*-benzo[*e*][1,3]thiazin-4-one (**BP_04b**) (0.1 g, 0.29 mmol) and phenyl isothiocyanate (0.04 g, 0.29 mmol) to afford **BP_21** (0.05 g, 41.3 %) as white solid. M.p: 179-181 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.55 (s, 1H), 9.39 (s, 1H), 9.18 (s, 1H), 8.21-6.82 (m, 12H). ^{13}C NMR (DMSO-d_6): δ_C 179.8, 167.5, 159.2, 140.6, 138.7, 137.7, 135.4, 131.3, 130.3, 129.0 (2C), 128.6, 128.3, 128.1, 127.5 (2C), 126.4 (3C), 123.6, 116.5 (2C). ESI-MS *m/z* 473 $[M+H]^+$. Anal.Calcd.For $C_{22}H_{15}F_3N_4OS_2$: C, 55.92; H, 3.20; N, 11.86; Found: C, 55.95; H, 3.18; N, 11.87.

1-(4-Chlorophenyl)-3-(4-((4-oxo-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-2-yl)amino)phenyl)thiourea (BP_22). The compound was synthesized according to the general procedure using 2-((4-aminophenyl)amino)-6-(trifluoromethyl)-4*H*-benzo[*e*][1,3]thiazin-4-one (**BP_04b**) (0.1 g, 0.29 mmol) and 4-chlorophenyl isothiocyanate (0.05 g, 0.29 mmol) to afford **BP_22** (0.06 g, 41.2 %) as white solid. M.p: 193-195 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.58 (s, 1H), 9.37 (s, 1H), 9.20 (s, 1H), 8.24-6.77 (m, 11H). ^{13}C NMR (DMSO-d_6): δ_C 179.8, 167.6, 159.4, 140.5, 137.4, 136.5, 135.6, 133.7, 131.4, 131.1 (2C), 130.1, 129.3 (2C), 128.4, 128.2, 127.2 (2C), 126.4, 123.6, 116.6 (2C). ESI-MS *m/z* 507 $[M+H]^+$. Anal.Calcd.For $C_{22}H_{14}ClF_3N_4OS_2$: C, 52.12; H, 2.78; N, 11.05; Found: C, 52.09; H, 2.81; N, 11.02.

1-(4-((4-Oxo-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-2-yl)amino)phenyl)-3-(p-tolyl)thiourea (BP_23). The compound was synthesized according to the general procedure using 2-((4-aminophenyl)amino)-6-(trifluoromethyl)-4*H*-benzo[*e*][1,3]thiazin-4-one (**BP_04b**) (0.1 g, 0.29 mmol) and *p*-tolylisothiocyanate (0.04 g, 0.29 mmol) to afford **BP_23** (0.06 g, 46.4 %) as white solid. M.p: 183-185 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.53 (s, 1H), 9.40 (s, 1H), 9.21 (s, 1H), 8.14-6.71 (m, 11H), 2.36 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 179.8, 167.6, 159.2, 140.5, 137.5, 137.2, 135.4, 135.1, 131.2, 130.3, 129.4 (2C), 128.6, 128.4, 127.5 (2C), 126.4, 126.2 (2C), 123.6, 116.6 (2C), 21.4. ESI-MS *m/z* 487 $[M+H]^+$. Anal.Calcd.For $C_{23}H_{17}F_3N_4OS_2$: C, 56.78; H, 3.52; N, 11.52; Found: C, 56.77; H, 3.49; N, 11.55.

1-(4-Nitrophenyl)-3-(4-((4-oxo-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-2-yl)amino)phenyl)thiourea (BP_24). The compound was synthesized according to the general procedure using 2-((4-aminophenyl)amino)-6-(trifluoromethyl)-4*H*-benzo[*e*][1,3]thiazin-4-one (**BP_04b**) (0.1 g, 0.29 mmol) and 4-nitrophenyl isothiocyanate (0.05 g, 0.29 mmol) to afford **BP_24** (0.04 g, 31.9 %) as white solid. M.p: 192-194 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.59 (s, 1H), 9.41 (s, 1H), 9.17 (s, 1H), 8.29-6.68 (m, 11H). ^{13}C NMR (DMSO-d_6): δ_C 179.8, 167.6, 159.4, 144.5, 143.8, 140.5, 137.6, 135.3, 131.4, 130.3, 128.4, 128.2, 127.3 (2C), 126.5, 124.7 (2C), 124.3 (2C), 123.6, 116.9 (2C). ESI-MS *m/z* 518 $[M+H]^+$. Anal.Calcd.For $C_{22}H_{14}F_3N_5O_3S_2$: C, 51.06; H, 2.73; N, 13.53; Found: C, 51.09; H, 2.70; N, 13.55.

1-(4-((6-Chloro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)amino)phenyl)-3-phenylthiourea (BP_25). The compound was synthesized according to the general procedure using 2-((4-aminophenyl)amino)-6-chloro-4*H*-benzo[*e*][1,3]thiazin-4-one (**BP_04c**) (0.1 g, 0.33 mmol) and phenyl isothiocyanate (0.04 g, 0.33 mmol) to afford **BP_25** (0.05 g, 35.2 %) as white solid. M.p: 199-201 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.48 (s, 1H), 9.39 (s, 1H), 9.19 (s, 1H), 7.88-6.79 (m, 12H). ^{13}C NMR (DMSO-d_6): δ_C 179.8, 167.6, 159.2, 138.6, 138.4, 135.3 (2C), 134.7, 131.4, 131.3, 130.1, 129.0 (2C), 128.6, 128.3, 127.2 (2C), 126.4 (2C), 116.4 (2C). ESI-MS *m/z* 439 $[M+H]^+$. Anal.Calcd.For $C_{21}H_{15}ClN_4OS_2$: C, 57.46; H, 3.44; N, 12.76; Found: C, 57.44; H, 3.46; N, 12.75.

1-(4-((6-Chloro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)amino)phenyl)-3-(4 chlorophenyl)thiourea (BP_26). The compound was synthesized according to the general procedure using 2-((4-aminophenyl)amino)-6-chloro-4*H*-benzo[*e*][1,3]thiazin-4-one (**BP_04c**) (0.1 g, 0.33 mmol) and 4-chlorophenyl isothiocyanate (0.05 g, 0.33 mmol) to afford **BP_26** (0.02 g, 14.7 %) as white solid. M.p: 206-208 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.51 (s, 1H), 9.40 (s, 1H), 9.26 (s, 1H), 7.92-6.77 (m, 11H). ^{13}C NMR (DMSO-d_6): δ_C 179.7, 167.5, 159.4, 138.5, 136.4, 135.2 (2C), 134.8, 133.6, 131.5, 131.4, 131.1 (2C), 130.2, 129.2 (2C), 128.4, 127.4 (2C), 116.5 (2C). ESI-MS *m/z* 474 $[M+H]^+$. Anal.Calcd.For $C_{21}H_{14}Cl_2N_4OS_2$: C, 53.28; H, 2.98; N, 11.84; Found: C, 53.24; H, 2.97; N, 11.81.

1-(4-((6-Chloro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)amino)phenyl)-3-(p-tolyl)thiourea (BP_27). The compound was synthesized according to the general procedure using 2-((4-aminophenyl)amino)-6-chloro-4*H*-benzo[*e*][1,3]thiazin-4-one (**BP_04c**) (0.1 g, 0.33 mmol) and *p*-tolylisothiocyanate (0.05 g, 0.33 mmol) to afford **BP_27** (0.07 g, 48.8 %) as white solid. M.p: 221-223 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.45 (s, 1H), 9.37 (s, 1H), 9.20 (s, 1H),

7.87-6.83 (m, 11H), 2.36 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 179.8, 167.6, 159.4, 138.6, 137.3, 135.4, 135.2 (2C), 134.7, 131.7, 131.4, 130.5, 129.4 (2C), 128.3, 127.1 (2C), 126.5 (2C), 116.5 (2C), 21.4. ESI-MS *m/z* 453 $[M+H]^+$. Anal.Calcd.For $C_{22}H_{17}ClN_4OS_2$: C, 58.33; H, 3.78; N, 12.37; Found: C, 58.31; H, 3.75; N, 12.39.

1-(4-((6-Chloro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)amino)phenyl)-3-(4-nitrophenyl)thiourea (BP_28). The compound was synthesized according to the general procedure using 2-((4-aminophenyl)amino)-6-chloro-4*H*-benzo[*e*][1,3]thiazin-4-one (**BP_04c**) (0.1 g, 0.29 mmol) and 4-nitrophenyl isothiocyanate (0.06 g, 0.33 mmol) to afford **BP_28** (0.05 g, 32.5 %) as white solid. M.p: 227-229 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.49 (s, 1H), 9.35 (s, 1H), 9.25 (s, 1H), 7.76-6. (m, 11H). ^{13}C NMR (DMSO-d_6): δ_C 179.8, 168.5, 159.2, 144.5, 143.6, 138.4, 135.2 (2C), 134.7, 131.5, 131.2, 130.5, 128.6, 127.5 (2C), 124.6 (2C), 124.3 (2C), 116.4 (2C). ESI-MS *m/z* 484 $[M+H]^+$. Anal.Calcd.For $C_{21}H_{14}ClN_5O_3S_2$: C, 52.12; H, 2.92; N, 14.47; Found: C, 52.09; H, 2.95; N, 14.49.

2-((4-(Benzylamino)phenyl)amino)-6-nitro-4H-benzo[e][1,3]thiazin-4-one (BP_29). The compound was synthesized according to the general procedure using 2-((4-aminophenyl)amino)-6-nitro-4*H*-benzo[*e*][1,3]thiazin-4-one (**BP_04a**) (0.1 g, 0.31 mmol), benzaldehyde (0.03 g, 0.31 mmol) and sodium cyanoborohydride (0.02 g, 0.31 mmol) to afford **BP_29** (0.04 g, 35.7 %) as yellow solid. M.p: 189-191 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.24 (s, 1H), 8.96 (s, 1H), 8.75-6.81 (m, 12H), 4.36 (s, 2H). ^{13}C NMR (DMSO-d_6): δ_C 167.5, 159.2, 145.3, 144, 143.2, 139.7, 138.2, 130.5, 128.6, 128.4 (2C), 127.5, 126.4 (2C), 126.1, 123.4, 118.6 (2C), 117.2(2C), 48.0. ESI-MS *m/z* 405 $[M+H]^+$. Anal.Calcd.For $C_{21}H_{16}N_4O_3S$: C, 62.36; H, 3.99; N, 13.85; Found: C, 62.33; H, 3.95; N, 13.88.

2-((4-((4-Chlorobenzyl)amino)phenyl)amino)-6-nitro-4H-benzo[e][1,3]thiazin-4-one (BP_30). The compound was synthesized according to the general procedure using 2-((4-aminophenyl)amino)-6-nitro-4*H*-benzo[*e*][1,3]thiazin-4-one (**BP_04a**) (0.1 g, 0.31 mmol) 4-chlorobenzaldehyde (0.04 g, 0.31 mmol) and sodium cyanoborohydride (0.02 g, 0.31 mmol) to afford **BP_30** (0.06 g, 47.2 %) as yellow solid. M.p: 204-206 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.22 (s, 1H), 8.95 (s, 1H), 8.73-6.84 (m, 11H), 4.35 (s, 2H). ^{13}C NMR (DMSO-d_6): δ_C 167.5, 159.2, 145.3, 144, 143.6, 138.2, 138, 132.4, 130.7, 129.4 (2C), 128.6, 128.4 (2C), 127.6, 123.7, 118.4 (2C), 117.2 (2C), 48.0. ESI-MS *m/z* 439 $[M+H]^+$. Anal.Calcd.For $C_{21}H_{15}ClN_4O_3S$: C, 57.47; H, 3.44; N, 12.77; Found: C, 57.44; H, 3.47; N, 12.74.

2-((4-((4-Methylbenzyl)amino)phenyl)amino)-6-nitro-4H-benzo[e][1,3]thiazin-4-one (BP_31). The compound was synthesized according to the general procedure using 2-((4-aminophenyl)amino)-6-nitro-4*H*-benzo[*e*][1,3]thiazin-4-one (**BP_04a**) (0.1 g, 0.31 mmol), 4-tolualdehyde (0.04 g, 0.31 mmol) and sodium cyanoborohydride (0.02 g, 0.31 mmol) to afford **BP_31** (0.05 g, 39.7 %) as yellow solid. M.p: 217-219 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.21 (s, 1H), 8.92 (s, 1H), 8.79-6.78 (m, 11H), 4.33 (s, 2H), 2.36 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 167.5, 159.4, 145.2, 144.0, 143.3, 138.4, 136.7, 136.2, 130.5, 128.7, 128.5 (2C), 128.1 (2C), 127.5, 123.7, 118.4 (2C), 117.2 (2C), 48.0, 21.2. ESI-MS *m/z* 419 $[M+H]^+$. Anal.Calcd.For $C_{22}H_{18}N_4O_3S$: C, 63.14; H, 4.34; N, 13.39; Found: C, 63.11; H, 4.36; N, 13.42.

6-Nitro-2-((4-((4-nitrobenzyl)amino)phenyl)amino)-4H-benzo[e][1,3]thiazin-4-one (BP_32). The compound was synthesized according to the general procedure using 2-((4-aminophenyl)amino)-6-nitro-4*H*-benzo[*e*][1,3]thiazin-4-one (**BP_04a**) (0.1 g, 0.31 mmol), 4-Nitro benzaldehyde (0.05 g, 0.31 mmol) and sodium cyanoborohydride (0.02 g, 0.31 mmol) to afford **BP_32** (0.05 g, 39.8 %) as yellow solid. M.p: 201-203 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.20 (s, 1H), 8.94 (s, 1H), 8.81-6.77 (m, 11H), 4.34 (s, 2H). ^{13}C NMR (DMSO-d_6): δ_C 167.5, 159.2, 146, 145.6, 145.1, 144.0, 143.2, 138.4, 130.6, 128.7, 127.5 (3C), 123.5, 123.4 (2C), 118.4 (2C), 117.2 (2C), 48.0. ESI-MS *m/z* 450 $[M+H]^+$. Anal.Calcd.For $C_{21}H_{15}N_5O_5S$: C, 56.12; H, 3.36; N, 15.58; Found: C, 56.15; H, 3.31; N, 15.61.

2-((4-(Benzylamino)phenyl)amino)-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (BP_33). The compound was synthesized according to the general procedure using 2-((4-aminophenyl)amino)-6-(trifluoromethyl)-4*H*-benzo[*e*][1,3]thiazin-4-one (**BP_04b**) (0.1 g, 0.29 mmol), benzaldehyde (0.03 g, 0.29 mmol) and sodium cyanoborohydride (0.02 g, 0.29 mmol) to afford **BP_33** (0.06 g, 52.1 %) as white solid. M.p: 186-188 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.25 (s, 1H), 8.86 (s, 1H), 8.65-6.63 (m, 12H), 4.35 (s, 2H). ^{13}C NMR (DMSO-d_6): δ_C 167.5, 159.4, 144.0, 140.5, 139.7, 137.5, 131.1, 130.4, 128.4 (2C), 128.2, 127.5, 126.6 (2C), 126.3, 126.1, 123.5, 118.4 (2C), 117.2 (2C), 48.0. ESI-MS *m/z* 428 $[M+H]^+$. Anal.Calcd.For $C_{22}H_{16}F_3N_3OS$: C, 61.82; H, 3.77; N, 9.83; Found: C, 61.78; H, 3.79; N, 9.81.

2-((4-((4-Chlorobenzyl)amino)phenyl)amino)-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (BP_34). The compound was synthesized according to the general procedure using 2-((4-aminophenyl)amino)-6-(trifluoromethyl)-4*H*-benzo[*e*][1,3]thiazin-4-one (**BP_04b**) (0.1 g, 0.29 mmol), 4-chloro benzaldehyde (0.04 g, 0.29 mmol) and sodium cyanoborohydride (0.02

g, 0.29 mmol) to afford **BP_34** (0.08 g, 64.2 %) as white solid. M.p: 182-184 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.22 (s, 1H), 8.82 (s, 1H), 8.69-6.67 (m, 11H), 4.33 (s, 2H). ^{13}C NMR (DMSO-d_6): δ_C 167.5, 159.2, 144.0, 140.3, 138.0, 137.5, 132.4, 131.2, 130.3, 129.4 (2C), 128.5 (2C), 128.3, 127.7, 126.4, 123.5, 118.6 (2C), 117.2 (2C), 48.0. ESI-MS *m/z* 462 $[M+H]^+$. Anal.Calcd.For $C_{22}H_{15}ClF_3N_3OS$: C, 57.21; H, 3.27; N, 9.10; Found: C, 57.19; H, 3.29; N, 9.12.

2-((4-((4-Methylbenzyl)amino)phenyl)amino)-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (BP_35). The compound was synthesized according to the general procedure using 2-((4-aminophenyl)amino)-6-(trifluoromethyl)-4*H*-benzo[*e*][1,3]thiazin-4-one (**BP_04b**) (0.1 g, 0.29 mmol), 4-tolualdehyde (0.03 g, 0.29 mmol) and sodium cyanoborohydride (0.02 g, 0.29 mmol) to afford **BP_35** (0.06 g, 48.8 %) as white solid. M.p: 193-195 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.20 (s, 1H), 8.88 (s, 1H), 8.62-6.70 (m, 11H), 4.34 (s, 2H), 2.36 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 167.5, 159.4, 144.0, 140.5, 137.6, 136.8, 136.5, 131.3, 130.4, 128.7 (2C), 128.3, 128.1 (2C), 127.9, 126.6, 123.7, 118.5 (2C), 117.2 (2C), 48.0, 21.2 ESI-MS *m/z* 442 $[M+H]^+$. Anal.Calcd.For $C_{23}H_{18}F_3N_3OS$: C, 62.57; H, 4.11; N, 9.52; Found: C, 62.55; H, 4.15; N, 9.49.

2-((4-((4-Nitrobenzyl)amino)phenyl)amino)-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (BP_36). The compound was synthesized according to the general procedure using 2-((4-aminophenyl)amino)-6-(trifluoromethyl)-4*H*-benzo[*e*][1,3]thiazin-4-one (**BP_04b**) (0.1 g, 0.29 mmol), 4-nitrobenzaldehyde (0.04 g, 0.29 mmol) and sodium cyanoborohydride (0.02 g, 0.29 mmol) to afford **BP_36** (0.06 g, 48.5 %) as white solid. M.p: 183-185 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.23 (s, 1H), 8.84 (s, 1H), 8.61-6.64 (m, 11H), 4.35 (s, 2H). ^{13}C NMR (DMSO-d_6): δ_C 167.5, 159.2, 146.0, 145.8, 144.0, 140.7, 137.5, 131.3, 130.3, 128.4, 127.6 (3C), 126.4, 123.5 (3C), 118.4 (2C), 117.2 (2C), 48.0. ESI-MS *m/z* 473 $[M+H]^+$. Anal.Calcd.For $C_{22}H_{15}F_3N_4O_3S$: C, 55.93; H, 3.20; N, 11.86; Found: C, 55.95; H, 3.18; N, 11.85.

2-((4-(Benzylamino)phenyl)amino)-6-chloro-4H-benzo[e][1,3]thiazin-4-one (BP_37). The compound was synthesized according to the general procedure using 2-((4-aminophenyl)amino)-6-chloro-4*H*-benzo[*e*][1,3]thiazin-4-one (**BP_04c**) (0.1 g, 0.33 mmol), benzaldehyde (0.03 g, 0.33 mmol) and sodium cyanoborohydride (0.02 g, 0.33 mmol) to afford **BP_37** (0.07 g, 55.3 %) as white solid. M.p: 197-199 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.22 (s, 1H), 8.86 (s, 1H), 8.59-6.58 (m, 12H), 4.36 (s, 2H). ^{13}C NMR (DMSO-d_6): δ_C 167.5,

159.2, 144.0, 139.7, 138.8, 135.3, 134.8, 131.5, 131.4, 130.1, 128.4 (2C), 127.6, 126.9 (2C), 126.6, 118.5 (2C), 117.2 (2C), 48.0. ESI-MS *m/z* 394 $[M+H]^+$. Anal.Calcd.For $C_{21}H_{16}ClN_3OS$: C, 64.03; H, 4.09; N, 10.67; Found: C, 64.05; H, 4.12; N, 10.66.

6-Chloro-2-((4-((4-chlorobenzyl)amino)phenyl)amino)-4H-benzo[e][1,3]thiazin-4-one (BP_38). The compound was synthesized according to the general procedure using 2-((4-aminophenyl)amino)-6-chloro-4*H*-benzo[*e*][1,3]thiazin-4-one (**BP_04c**) (0.1 g, 0.33 mmol), 4-chlorobenzaldehyde (0.05 g, 0.33 mmol) and sodium cyanoborohydride (0.02 g, 0.33 mmol) to afford **BP_38** (0.07 g, 51.7 %) as white solid. M.p: 205-207 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.21 (s, 1H), 8.84 (s, 1H), 8.56-6.61 (m, 11H), 4.33 (s, 2H). ^{13}C NMR (DMSO-d_6): δ_C 167.5, 159.3, 144.0, 138.6, 138.2, 135.2, 134.8, 132.4, 131.7, 131.4, 130.3, 129.6 (2C), 128.4 (2C), 127.8, 118.4 (2C), 117.2 (2C), 48.0. ESI-MS *m/z* 428 $[M+H]^+$. Anal.Calcd.For $C_{21}H_{15}Cl_2N_3OS$: C, 58.89; H, 3.53; N, 9.81; Found: C, 58.85; H, 3.51; N, 9.85.

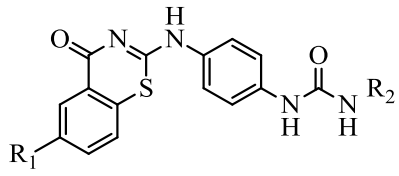
6-Chloro-2-((4-((4-methylbenzyl)amino)phenyl)amino)-4H-benzo[e][1,3]thiazin-4-one (BP_39). The compound was synthesized according to the general procedure using 2-((4-aminophenyl)amino)-6-chloro-4*H*-benzo[*e*][1,3]thiazin-4-one (**BP_04c**) (0.1 g, 0.33 mmol), 4-tolualdehyde (0.04 g, 0.33 mmol) and sodium cyanoborohydride (0.02 g, 0.33 mmol) to afford **BP_39** (0.04 g, 35.6 %) as white solid. M.p: 211-213 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.23 (s, 1H), 8.83 (s, 1H), 8.57-6.56 (m, 11H), 4.35 (s, 2H), 2.36 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 167.5, 159.2, 144.0, 138.7, 136.8, 136.2, 135.3, 134.7, 131.5, 131.5, 130.3, 128.7 (2C), 128.2 (2C), 127.6, 118.4 (2C), 117.2 (2C), 48.0, 21.2. ESI-MS *m/z* 408 $[M+H]^+$. Anal.Calcd.For $C_{22}H_{18}ClN_3OS$: C, 64.78; H, 4.45; N, 10.30; Found: C, 64.75; H, 4.44; N, 10.28.

6-Chloro-2-((4-((4-nitrobenzyl)amino)phenyl)amino)-4H-benzo[e][1,3]thiazin-4-one (BP_40). The compound was synthesized according to the general procedure using 2-((4-aminophenyl)amino)-6-chloro-4*H*-benzo[*e*][1,3]thiazin-4-one (**BP_04c**) (0.1 g, 0.33 mmol), 4-nitrobenzaldehyde (0.05 g, 0.33 mmol) and sodium cyanoborohydride (0.02 g, 0.33 mmol) to afford **BP_40** (0.05 g, 40.1 %) as white solid. M.p: 215-217 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.20 (s, 1H), 8.82 (s, 1H), 8.63-6.52 (m, 11H), 4.36 (s, 2H). ^{13}C NMR (DMSO-d_6): δ_C 167.5, 159.2, 146.0, 145.8, 144.0, 138.7, 135.2, 134.6, 131.4, 131.1, 130.3, 127.6 (3C), 123.5 (2C), 118.4 (2C), 117.2 (2C), 48.0. ESI-MS *m/z* 439 $[M+H]^+$. Anal.Calcd.For $C_{21}H_{15}ClN_4O_3S$: C, 57.47; H, 3.44; N, 12.77; Found: C, 57.45; H, 3.46; N, 12.73.

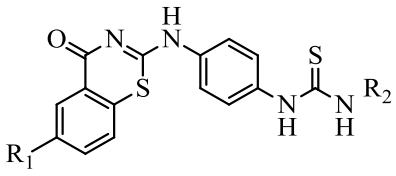
5.1a.4. In-vitro *Mycobacterium* GyrB assay, supercoiling assay, antimycobacterial potency and cytotoxicity studies of the synthesized molecules

All the synthesized derivatives were first evaluated for their in-vitro *Mycobacterium* GyrB assay and supercoiling assay as steps towards the derivation of SAR and hit optimization. The compounds were further subjected to a whole cell screening against *Mycobacterium tuberculosis* H37Rv strain to understand their bactericidal potency using the MABA assay and later the safety profile of these molecules were evaluated by checking the in-vitro cytotoxicity against RAW 264.7 cell line (mouse macrophage) by MTT assay, and the results are tabulated in **Table 5.2**.

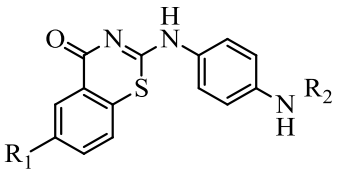
Table 5.2: In-vitro biological evaluation of the synthesized derivatives **BP_05 – BP_40**



BP_05 - BP_16



BP_17 - BP_28



BP_29 - BP_40

Compd	R_1	R_2	MS GyrB assay (IC_{50}) µM	MTB supercoiling assay (IC_{50}) µM	MTB MIC µM	Cytotoxicity[a] % inhibition
BP_05	Nitro	Phenyl	0.72±0.54	0.43±0.35	7.21	6.89
BP_06	Nitro	4-Chlorophenyl	11.81±1.32	4.72±0.34	53.43	7.22
BP_07	Nitro	4-Methylphenyl	19.44±0.82	6.89±0.71	13.97	2.63
BP_08	Nitro	4-Nitrophenyl	3.15±0.77	2.63±0.15	52.25	11.36
BP_09	Trifluoromethyl	Phenyl	35.73±0.61	11.65±0.66	1.71	8.12
BP_10	Trifluoromethyl	4-Chlorophenyl	12.61±0.43	13.89±0.43	6.37	7.22
BP_11	Trifluoromethyl	4-Methylphenyl	17.23±0.85	3.92±0.39	53.14	11.27
BP_12	Trifluoromethyl	4-Nitrophenyl	6.78±0.91	2.89±0.41	24.93	5.67
BP_13	Chloro	Phenyl	0.93±0.42	0.78±0.17	14.78	8.12
BP_14	Chloro	4-Chlorophenyl	8.44±0.91	9.57±0.51	6.83	9.33
BP_15	Chloro	4-Methylphenyl	14.28±1.33	4.14±0.22	28.61	16.28
BP_16	Chloro	4-Nitrophenyl	13.42±0.62	5.62±0.42	1.67	14.22

Contd.

Compd	R_1	R_2	MS GyrB assay (IC_{50}) µM	MTB supercoiling assay (IC_{50}) µM	MTB MIC µM	Cytotoxicity[a] % inhibition
BP_17	Nitro	Phenyl	8.22±0.71	3.18±0.19	13.90	6.93
BP_18	Nitro	4-Chlorophenyl	10.52±1.61	2.98±0.32	6.46	8.11
BP_19	Nitro	4-Methylphenyl	4.82±0.69	1.85±0.37	26.97	15.39
BP_20	Nitro	4-Nitrophenyl	21.82±1.89	2.94±0.25	25.28	18.23
BP_21	Trifluoromethyl	Phenyl	27.33±2.33	10.86±0.79	52.91	7.92
BP_22	Trifluoromethyl	4-Chlorophenyl	13.84±1.67	4.53±0.62	49.31	8.21
BP_23	Trifluoromethyl	4-Methylphenyl	8.52±0.97	2.67±0.34	25.69	2.99
BP_24	Trifluoromethyl	4-Nitrophenyl	0.41±0.55	0.72±0.78	48.31	14.23
BP_25	Chloro	Phenyl	42.53±2.37	18.43±0.88	56.95	9.33
BP_26	Chloro	4-Chlorophenyl	9.45±0.92	5.33±0.28	6.60	8.34
BP_27	Chloro	4-Methylphenyl	18.32±0.66	5.88±0.33	27.60	6.98
BP_28	Chloro	4-Nitrophenyl	13.62±1.56	4.03±0.38	3.22	5.33
BP_29	Nitro	Benzyl	12.42±0.27	13.78±0.29	15.45	14.95
BP_30	Nitro	4-Chlorobenzyl	22.42±2.71	7.33±0.27	28.48	16.92
BP_31	Nitro	4-Methylbenzyl	19.55±0.67	4.16±0.21	14.94	12.54
BP_32	Nitro	4-Nitrobenzyl	38.91±2.88	17.42±0.44	6.95	17.22
BP_33	Trifluoromethyl	Benzyl	2.96±0.48	2.47±0.36	58.49	8.34
BP_34	Trifluoromethyl	4-Chlorobenzyl	1.92±0.53	0.81±0.14	54.13	5.33
BP_35	Trifluoromethyl	4-Methylbenzyl	3.88±0.72	1.33±0.17	56.63	12.92
BP_36	Trifluoromethyl	4-Nitrobenzyl	15.72±0.69	4.25±0.25	26.46	11.98
BP_37	Chloro	Benzyl	47.21±2.93	28.44±0.61	3.96	6.93
BP_38	Chloro	4-Chlorobenzyl	26.42±0.89	6.28±0.37	14.59	14.93
BP_39	Chloro	4-Methylbenzyl	18.55±0.81	4.07±0.22	61.29	7.33
BP_40	Chloro	4-Nitrobenzyl	28.43±2.78	10.77±0.38	28.48	16.34
Novobiocin			0.273±0.28	0.068±0.31	>200	9.36

MS=*Mycobactrium smegmatis*, MTB=*Mycobacterium tuberculosis*, [a] at 50 µM against RAW 264.7 cells, ND indicates not determined.

5.1a.5. Discussion

Thirty six compounds synthesized were subjected to preliminary biological screenings. Initially, DNA GyrB ATPase assay was performed to evaluate their effectiveness on the DNA GryB enzyme. *Mycobacterium smegmatis* DNA Gry B was used to perform the ATPase assay as the ATPase activity of *Mycobacterium tuberculosis* GyrB protein was found to be very low when compared to *Mycobacterium smegmatis* GyrB owing to its slow growing mechanism [Shirude P.S., *et al*., 2013]. As the growth mechanism was slow so did the protein production. Seemingly, the *Mycobacterium tuberculosis* and *Mycobacterium smegmatis* DNA gyrase protein showed almost 87% identity in sequence and thus ATP binding pocket of the two organisms were conserved. Hence, *Mycobacterium smegmatis* DNA GyrB subunit was used as a surrogate enzyme to perform ATPase assays [Sriram D., *et al*., 2005]. Gry B gene from mc^2155 strain genomic DNA of *Mycobacterium smegmatis* was cloned into an expression vector pQE2, transformed and expressed in BL21 (DE3) *pLysS* competent cells, and further induction of the expressed protein was carried by the addition of 0.2mM IPTG (Isopropyl-b-D thiogalactopyranoside) to the log phase culture for further protein synthesis. The desired protein was purified by Ni-NTA column and the eluent was loaded on to SDS-PAGE. Assays were performed initially at 250 µM, 62.5 µM, 15.62 µM, and 3.90 µM. Compounds with more than 70% inhibitions were further assayed at 125 µM, 31.25 µM, 7.81 M and 1.95 µM. Compounds with similar inhibition rates were regarded as potential hits and were carried forward for subsequent lower concentrations. At 0.95 and 0.47 µM concentrations, only three compounds (**BP_01**, **BP_24** and **BP_29**) showed more than 50% inhibition. Compound **BP_24,** 1-(4-nitrophenyl)-3-(4-((4-oxo-6-(trifluoromethyl)-4H-benzo[*e*][1,3]thiazin-2 yl)amino)phenyl)thioureawas found to be the most potent molecule with an IC_{50} of 0.41±0.12 µM. Whereas, compounds **BP_05** and **BP_29** showed IC_{50}s of 0.72±0.18 and 0.93±0.15 µM respectively. Novobiocin was employed as standard for this assay with an IC_{50} of 0.27±0.046 µM. All the thirty six compounds showed good inhibitory profile with an IC_{50} values in the range of 0.41 µM to 42.53 µM in the DNA GyrB assay. Novobiocin a well-known potent inhibitor of DNA GyrB domain failed in the pharmacokinetic and toxicity related parameter hence was recalled in 1960's [Garry M.W., *et al*., 1958; Oblak M., *et al*., 2007]. When compared to thio substituted compounds (**BP_17-BP_28**), most of the oxygen substituted molecules (**BP_05 - BP_16**) exhibited better inhibitory profile. Substitution of R_1 with nitro and R_2 with 4-chlorophenyl, 4-methylphenyl and 4-nitrophenyl resulted in moderate activity, whereas substitution of R_2 with phenyl group

resulted in an IC_{50} of 0.72±0.54 µM. All the oxy and thio derivatized compounds with R_1 as trifluoro substitution and different R_2 groups like phenyl, 4-chlorophenyl and 4-methylphenyl showed moderate activities within a range of 6-35 µM except trifluoro substituted nitrophenyl with an IC_{50} of 0.41±0.55 µM emerging as the most active compound **BP_24**, as shown in **Figure 5.2.** The phenyl group was found to increase hydrophobicity of the ligand, while the electrophilic nitro group protruded away from the active site pocket. Secondly, the compounds from **BP_29 - BP_40** showed less inhibitory profiles up to a range of 47 µM, and only the R_1 trifluoro groups with benzyl, 4-chlorobenzyl and 4- methylbenzyl showed good inhibitory activities against DNA GyrB protein. Furthermore, this series of molecules selectively inhibited mycobacterial DNA GyrB enzyme and were found to inactive against *Staphylococcus aureus* and *Escherichia coli* DNA gyrase proteins.

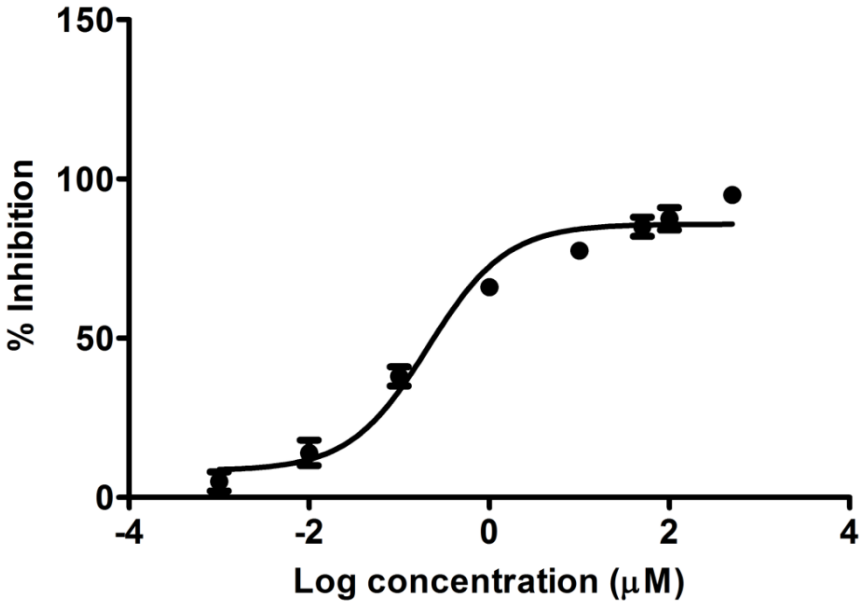


Figure 5.2: Dose-response curve of most active compound **BP_24**.

Subsequently, to analyze the interaction profile of the ligands reported in the DNA GyrB assay, compounds were docked to the GyrB ATPase domain of the available MS protein retrieved from protein data bank (PDB ID: 4B6C) using extra precision mode (XP) of Glide module. The protein had a co-crystallized ligand 6-(3,4-dimethylphenyl)-3-[[4-[3-(4-methylpiperazin-1-yl)propoxy]phenyl]amino]pyrazine-2-carboxamide with a docking score of -7.9 kcal/mol, well fit within the hydrophobic pocket of the GyrB protein. Closer analysis of the binding pattern revealed the ligand to interact with two prominent H-bonds crucial for

the GyrB activity, a bond between the amino group of the carboxamide moiety and the oxygen atom of Asp79 while the other H-bond observed between the nitrogen atom of piperazine ring and hydrogen atom present on the guanidine moiety of Arg82. The hydrophobic pocket was occupied by 3,4-dimethyl phenyl moiety of the crystal ligand stabilized by non-polar interactions with Ile84, Val99, Val123 and Val128. It was reported that these hydrophobic interactions were crucial in bringing selectivity observed at the enzyme level [Shirude P.S., *et al*., 2013]. Presently, the most potent ligand **BP_24** showed similar orientation pattern in the DNA GyrB protein active site as that of the reference crystal ligand with a docking score of -8.4 kcal/mol and an RMSD value of 0.18 Å indicating similar alignment and orientation [Jeankumar V.U., *et al*., 2013] as shown in **Figure 5.3**. The 6-(trifluoromethyl)-2H-benzo[e][1,3]thiazin-4(3H)-one moiety of the compound **BP_24** was found to be fitting into the hydrophobic pocket with trifluoromethyl group imparting more hydrophobicity for the stabilization of the complex as shown in **Figure 5.4**. Further the nitrogen atom of 1,3-thiazinan-4-one moiety interacted through hydrogen bond with the oxygen atom of Asp79 residue, while the nitrogen atom of the 2-phenylamino group interacted with the guanidine moiety of Arg82, thus retaining the crucial bonding with the amino acid residues as shown in the **Figure 5.5**.

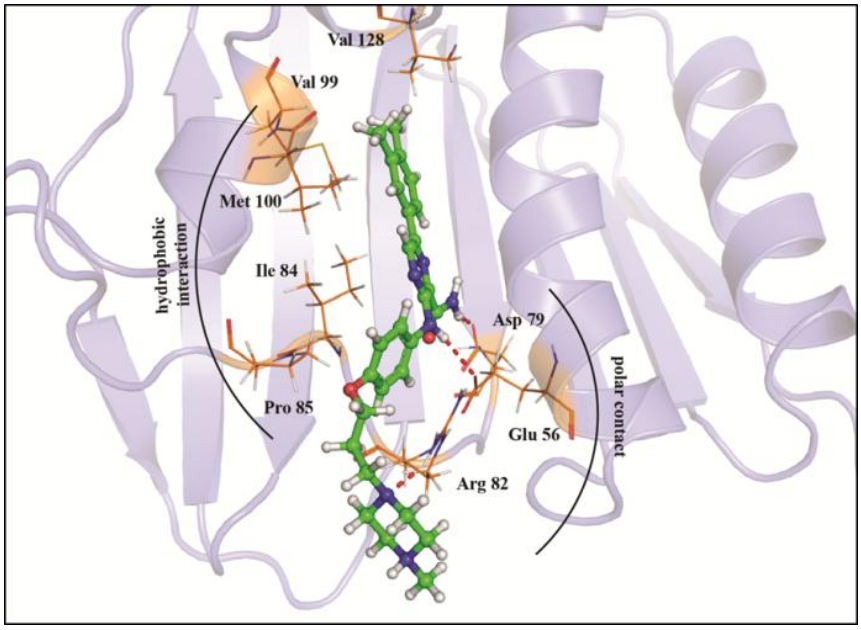


Figure 5.3: 2D-picture depicting aminopyrazinamide derivative with GyrB ATPase domain of *M smegmatis.* While the polar contacts are represented with red dots, the hydrophobic pocket is highlighted with the residues interacting with the crystal ligand [Jeankumar V.U., *et al*., 2013].

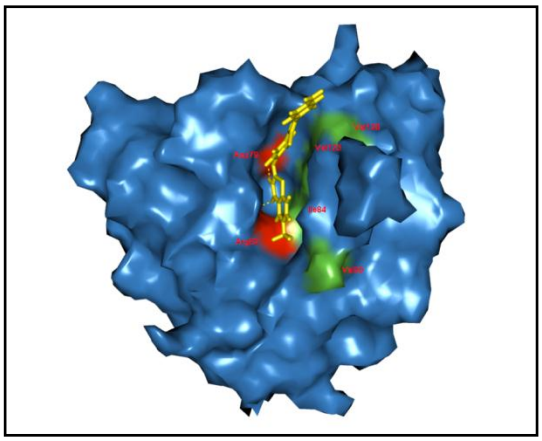


Figure 5.4: Surface interaction picture of ligand **BP_24** with interacting Asp79 and Arg82 (red colour) amino acids and a within the hydrophobic pocket (green colour) with the GyrB ATPase domain of *Mycobacterium smegmatis.*

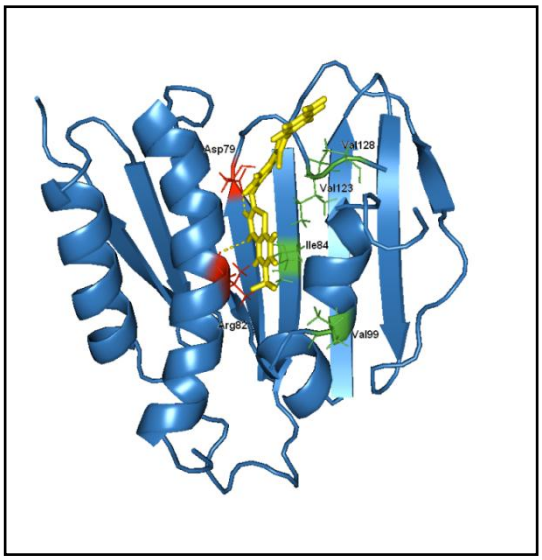


Figure 5.5: Compound **BP_24** interaction picture, with the polar contacts (Asp79) (red), cation-p interaction (Arg82)(red) and the hydrophobic interaction with Ile84, Val99, Val123 and Val128 (Green colour).

The hallmark of the DNA gyrase enzyme was the supercoiling action it performed on a relaxed substrate in the presence of ATP. As the entire series of compounds had a good GyrB inhibitory profile, the compounds were also checked for their *Mycobacterium tuberculosis* DNA supercoiling activity studies using the kit from Inspiralis Pvt. Limited, (Norwich) Jeankumar V.U., *et al*., 2014]. Each of the compounds tested showed dose-dependent inhibition of the mycobacterial DNA gyrase enzyme. Assays were performed at an initial concentration of 50 µM, and almost all the compounds showed more than 60% inhibition as

depicted in **Table 5.2**. Subsequently, the compounds were tested at lower concentrations to analyze the inhibition rates and to obtain IC_{50} values. Almost twenty nine compounds showed IC_{50}s less than 10 µM, revealing the importance of benzothiazinone moiety in gyrase inhibition. Compound **BP_24** showed greater inhibitory profile on DNA gyrase with an IC_{50} of 0.72 µM that was well correlated with a good DNA GyrB inhibition profile. While the standard compound novobiocin showed 100% inhibition at 50 and 25 µM concentrations with an IC_{50} of 46±10 nM. To analyze the compound specificity, all of them were subjected to DNA supercoiling assays of different organisms like *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aureginosa* (Inspiralis, Norwich), as the percentage of inhibitions observed were not more than 15%, to conclude that the compounds were *Mycobacterium tuberculosis* target specific.

Biological screenings were further continued with in-vitro antimycobacterial studies against *M. tuberculosis* H37Rv strain [Jeankumar V.U., *et al*., 2014]. The assay was performed by microplate alamar blue method for all the thirty six synthesized compounds. Among them, twenty five compounds showed MIC values < 20 µM. Fifteen compounds (**BP_05**, **BP_08**, **BP_11**, **BP_12**, **BP_24**, **BP_25**, **BP_26**, **BP_27**, **BP_29**, **BP_30**, **BP_32**, **BP_34**, **BP_36**, **BP_37** and **BP_38**) showed better MICs than the first-line TB drugs ethambutol (MIC: 15.31 µM). Though the standard drug isoniazid (MIC: 0.66 µM) and moxifloxacin (MIC: 1.2 µM) were best among the ones used, compound **BP_05**, **BP_29, BP_32**, **BP_36** and **BP_37** showed greater MIC values than the most potent enzyme inhibitor **BP_24** from this series indicating the possibility of multi-enzyme target inhibitions by these drugs like DprE1 enzyme as reported in the literature [Batt S.M., *et al*., 2012].

Eukaryotic mammalian cell cytotoxicity was the main concern when synthesizing a drug and analyzing for its safety profile, as most of the drugs failed in the clinical trials because of the toxicity issues. In accordance with this, safety profile of all the compounds targeting the *Mycobacterium tuberculosis* DNA gyrase were observed by testing in-vitro cytotoxicity against mouse macrophage RAW 264.7 cell line at 100 µM concentration, employing MTT assay [Jeankumar V.U., *et al*., 2014]. The most promising analogue **BP_24** displayed a good safety profile showing only 14.23% inhibition. Assay was performed in duplicates, thrice to prevent occurrence of any artifacts. Blank was set initially to nullify the background noise and control had 0.2% DMSO, the solvent in which drugs were diluted. Percentage inhibitions by the compounds are reported in **Table 5.2**.

Biologically, the stability of the ligand with the protein complex could be analyzed in real-time by running a differential scanning fluorimetry experiment [Jeankumar V.U., *et al*., 2014]. The interaction profile of compound **BP_24** to stabilize the *Mycobacterium smegmatis* DNA GyrB protein was evaluated by measuring the fluorescence of the SYPRO orange dye with native protein and its protein-ligand complex. It was accounted that, fluorescence was maximum when the protein was denatured completely; as a result the native Gry B protein T_m was observed to be 44.2 °C whereas the T_m of the protein in complex with ligand **BP_24** was found to be 47.1 °C as shown in the **Figure 5.6**. A higher or positive shift towards right side of T_m curve signified better stabilization of the protein-ligand complex compared to the native protein alone which further re-ascertained for the interaction of the compound with DNA GyrB protein.

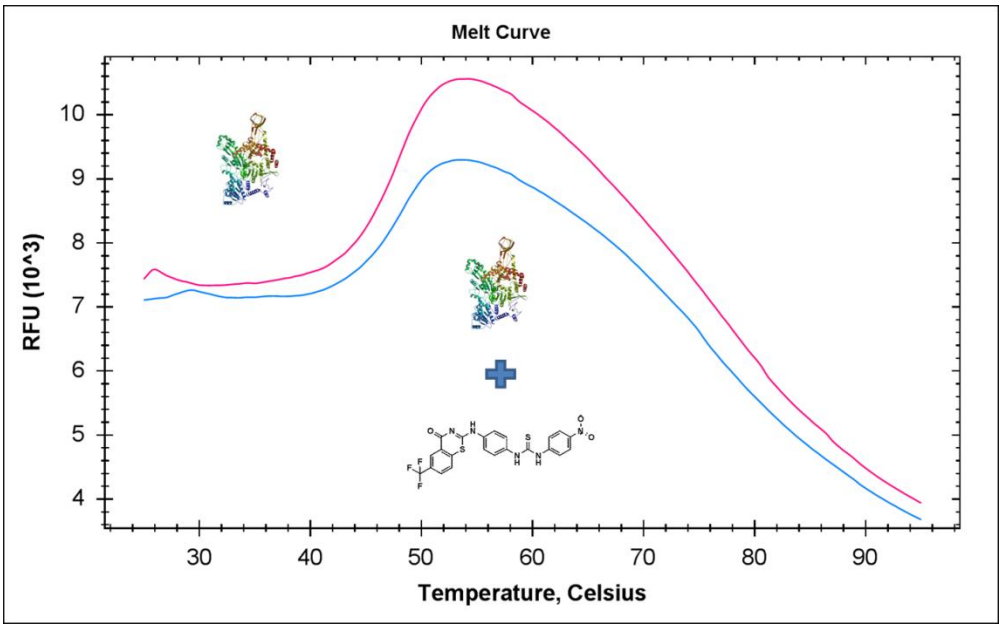
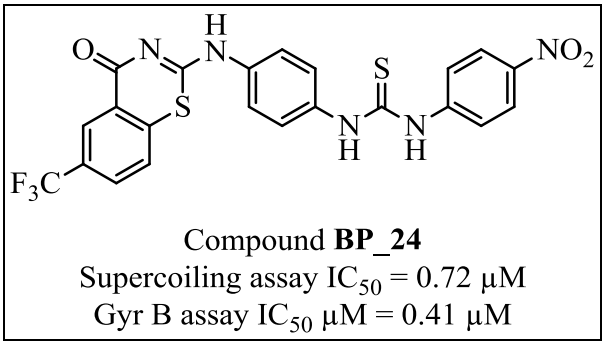


Figure 5.6: DSF picture for compound **BP_24** depicting an increase in thermal stability between the native *Mycobacterium smegmatis* protein (pink) and *Mycobacterium smegmatis* protein-ligand **BP_24** complex (blue) with a positive Tm of 2.7 °C.

5.1a.6. Highlights of the study

In this study, a series of thirty six compounds were prepared by following the strategy of molecular hybridization and evaluating the process by various biological assays concerned with DNA gyrase enzyme. Out of synthesised 36 molecules compound **BP_24** exhibited IC_{50} of 0.41 ± 0.55 µM also well correlating supercoiling IC_{50} of 0.72 ± 0.78 µM and MIC of 48.31 µM (**Figure 5.7**). The lead compound was also found to be devoid of cytotoxicity with

percentage inhibition of 14.23 against RAW cell lines. Here we succeeded in re-engineering few previously reported antibacterial leads and thus experimentally characterized a novel class of DNA GyrB inhibitors. Overall, the inhibitors had good synthetic accessibility combined with excellent in-vitro enzyme inhibitory profile, traceable SAR, along with anti-tubercular activity and least cytotoxicity. Thus, the present class of DNA GyrB inhibitors provided an interesting potential for further optimization and has greater scope to combat increasing mycobacterial infections in near future.



Compound **BP_24**
Supercoiling assay IC_{50} = 0.72 µM
Gyr B assay IC_{50} µM = 0.41 µM

Figure 5.7: Chemical structure and biological activity of most active compound in DNA Gyrase **BP_24.**

5.1b. Development of benzothiazinone-aminopiperidine hybrid analogues as efficient *Mycobacterium tuberculosis* DNA Gyrase inhibitors

Research efforts from both industry and academia on the development of novel anti-bacterial DNA gyrase inhibitors has resulted in identification of many potent inhibitors but none has reached the market. Hence, we laid our efforts on design and synthesis of chemical class of benzothiazinone-aminopiperidine inhibitors to develop them as therapeutics against TB.

Successful implementation of molecular hybridization approach and designing new inhibitors was achieved by our group previously [Jeankumar V.U., *et al*., 2014]. This is an emerging structural tool involving in adequate fusion of the two or more molecular pharmacophoric units derived from previously reported successful leads/drugs to design a new hybrid that maintains preselected characteristics of the original template along with an improved efficiency with least cytotoxicity and drug like properties. Re-engineering the previously reported (*S*)-2-(2-methyl-1,4-dioxa-8-azaspiro[4.5]decan-8-yl)-8-nitro-6-(trifluoromethyl)-4*H*-benzo[*e*][1,3]thiazin-4-one with 1-(4-fluorophenyl)-3-(1-(2-(7-methoxy-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-yl)urea thus could deliver a new scaffold of benzothiazinone-aminopiperidine lead with better antimycobacterial activity via inhibition of the gyrase domain. (**Figure 5.8**) depicts thedesign strategy utilized for developing the inhibitor. It was decided to retain the 8-nitro-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one of BTZ043 and 1-(4-fluorophenyl)-3-(piperidin-4-yl)urea moiety of second parent in our initial SAR exploration, as it was understood to be an important requisite in retaining the gyrase inhibitory potential.

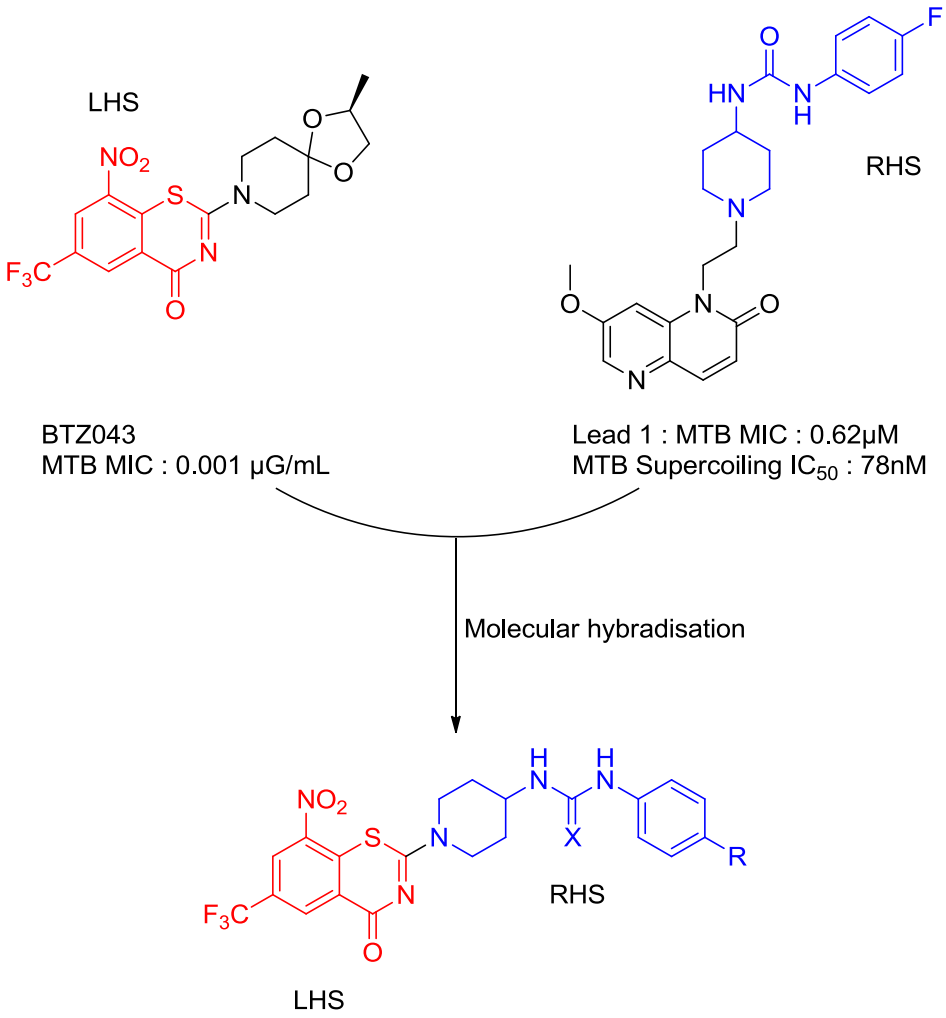


Figure 5.8: Strategy employed for designing the lead. Chemical structure of previously reported antitubercular benzothiazinone bearing (left hand side) derivative BTZ043 and *Mycobacterium tuberculosis* DNA gyrase inhibitor bearing aryl (thio) urea right hand side chain and the inhibitor designed through molecular hybridization.

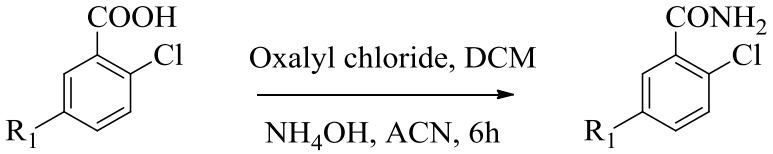
5.1b.1. Chemical synthesis

Synthesis of the compounds started with conversion of commercially available substituted 2-chlorobenzoic acid (**1a-c**) into corresponding 2-chlorobenzoyl chlorides by DMF-catalyzed treatment in presence of oxalyl chloride in dichloromethane. The obtained 2-chlorobenzoyl chlorides were converted into corresponding carboxamides (**BD_02a-c**) intermediate by drop-wise addition of 25% aqueous ammonia at -20 °C.The amide intermediates were further treated with carbon disulphide, methyl iodide and sodium hydroxide in DMSO to afford the thio-alkylated products (**BD_03a-c**), which upon treatment with 4-(*N*-Boc-amino) piperidine in ethanol followed by deprotection using trifluoroacetic acid gave the scaffolds (**BD_04a-c**)

in good yield. The design strategy and the steps involved to obtain the final product is sketched in (**Figure 4.2**). The final library was then assembled by treating the obtained scaffolds with the desired isocyanates/isothiocyantes and aldehyde to afford compounds **BD_05 – BD_40** in excellent yields.

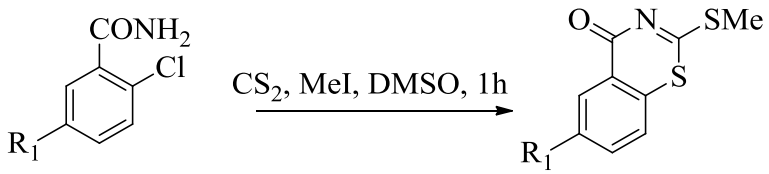
5.1b.2. Experimental protocol utilized for synthesis

5-Substituted-2-chlorbenzamides (*BD_0*2a-c). To a stirred solution of the corresponding acid (**BD_01a-c**) (1.0 mmol) in dichloromethane (15 mL) at -10°C was added oxalyl chloride (2.5 mmol). The solution was refluxed for about 6 hours and solvent evaporated under reduced pressure. The residue was further diluted with acetonitrile (30 mL), cooled to -20 °C and added ammonium hydroxide solution dropwise and allowed to stirred for 30 minutes. The resulting solid was filtered out to afford the corresponding amide (**BD_02a-c**) in good yield.



BD_01a-c **BD_02a-c**

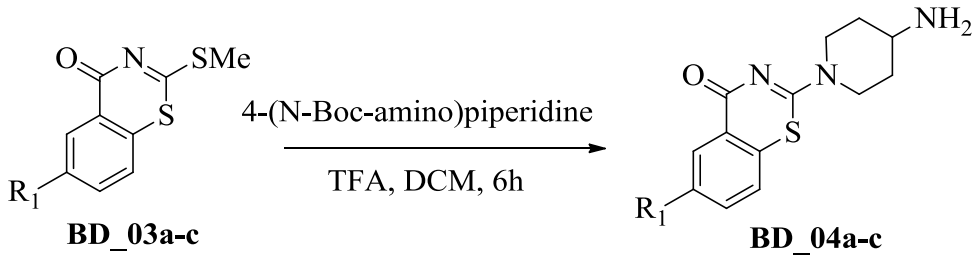
General procedure for the synthesis of 5-substituted-2-(methylthio)-4H-benzo[e][1,3] thiazin-4-one (*BD_0*3a-c). To a stirred solution of the corresponding benzamide (**BD_02a-c**) (1.0 mmol) in DMSO (15 mL) at 10 °C was added carbon disulphide (3 mmol), sodium hydroxide (2.0 mmol), and the mixture was allowed to stand for 15 minutes. Subsequently methyl iodide (1.2 mmol) was added. The reaction mixture was allowed to stand for another 30 minutes, and 50 mL of water was added. The resulting white solid separated by filtration to afford the corresponding benzothiazinone (**BD_03a-c**) in good yield.



BD_02a-c **BD_03a-c**

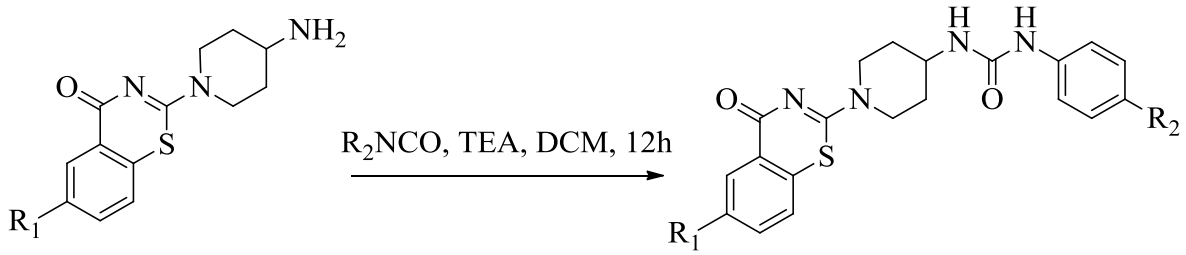
General procedure for the synthesis of 6-substituted 2-(4-aminopiperidin-1-yl)-4H-benzo[e][1,3]thiazin-4-ones (BD_04a-c). To a stirred solution of the corresponding benzothiazinone (**BD_03a**) (1 mmol) in ethanol (15 mL) at room temperature was added 4-

(*N*-Boc-amino)piperidine (1.0 mmol). The solution was refluxed for about 12 hours (monitored by TLC & LCMS for completion), and solvent evaporated under reduced pressure. The residue was further diluted with water (30 mL) and ethyl acetate (50 mL) and the layers were separated. The organic layer was dried over anhydrous sodium sulphate and evaporated under reduced pressure. The residue was purified by silica gel column chromatography using hexane: ethylacetate as eluent to give the corresponding *N*-boc protected 4-aminopiperidine benzothiazinones in good yield; which was taken in dichloromethance (60 mL) was cooled to 0^{o}C was added trifluoroacetic acid (8 mL) and stirred the reaction at room temperature for 1hour. After completion of the reaction by TLC, the reaction mixture was cooled to 0 ^{o}C and basified to pH ~8.0 using saturated aqueous $NaHCO_3$ solution. The organic layer was separated, washed with water (2 x 20 mL) and brine (1 x 20 mL) and dried over anhydrous sodium sulfate. The organic layer was concentrated under vacuum afforded the free amine as pale brown oil. The crude material was used for final reactions without purification.



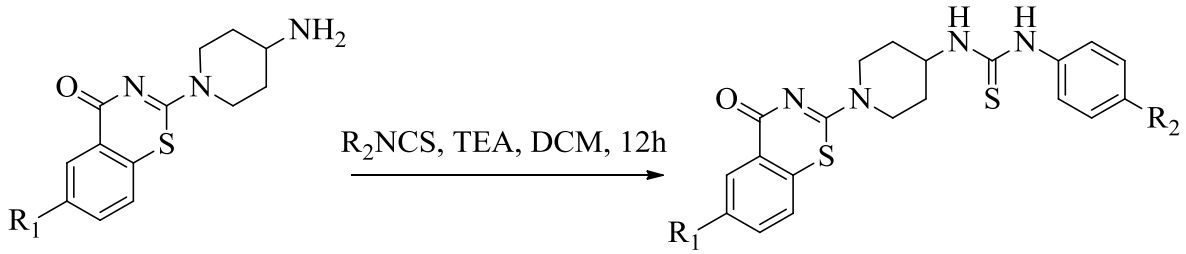
BD_03a-c **BD_04a-c**

1-(1-(6-substituted-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)piperidin-4-yl)-3-arylurea derivatives (BD_05 – BD_16). To a cooled solution of 2-(4-aminopiperidin-1-yl)-6-substituted-4*H*-benzo[*e*][1,3]thiazin-4-one (1 mmol) in anhydrous DCM (2 mL) was added corresponding isocyanate (1 mmol), triethylamine (1mmol) and stirred the reaction mixture at room temperature for 12 hours (monitored by TLC & LCMS for completion), and solvent evaporated under reduced pressure. The residue was further diluted with water (30 mL) and ethyl acetate (50 mL) and the layers were separated. The organic layer was dried over anhydrous sodium sulphate and evaporated under reduced pressure. The residue was purified by silica gel column chromatography using hexane: ethylacetate as eluent to give the corresponding urea derivative (**BD_05 – BD_16**) in good yield.



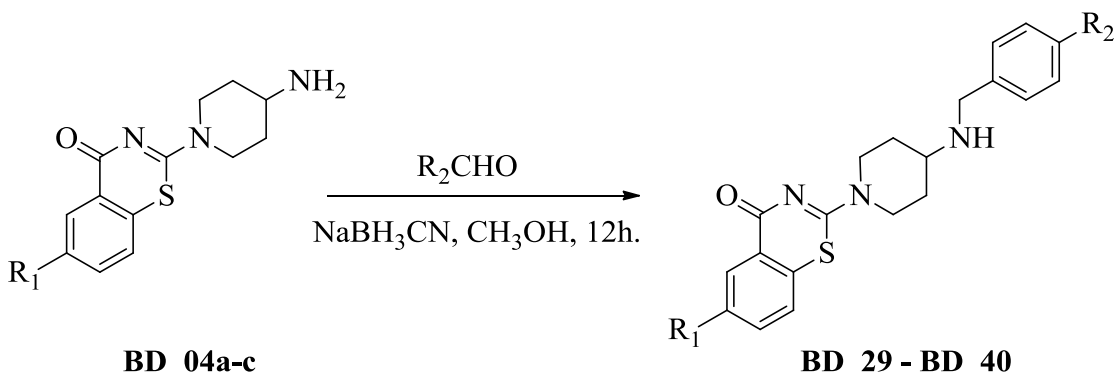
BD_04a-c **BD_05 - BD_16**

1-(1-(6-substituted-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)piperidin-4-yl)-3-arylthiourea derivatives (BD_17 – BD_28). To a cooled solution of 2-(4-aminopiperidin-1-yl)-6-substituted-4H-benzo[e][1,3]thiazin-4-one (1 mmol) in anhydrous DCM (2 mL) was added corresponding isothiocyanate (1 mmol), triethylamine (1 mmol) and stirred the reaction mixture at room temperature for 12 hours (monitored by TLC & LCMS for completion), and solvent evaporated under reduced pressure. The residue was further diluted with water (30 mL) and ethyl acetate (50 mL) and the layers were separated. The organic layer was dried over anhydrous sodium sulphate and evaporated under reduced pressure. The residue was purified by silica gel column chromatography using hexane: ethylacetate as eluent to give the corresponding thiourea derivative (**BD_17 – BD_28**) in good yield.



BD_04a-c **BD_17 - BD_28**

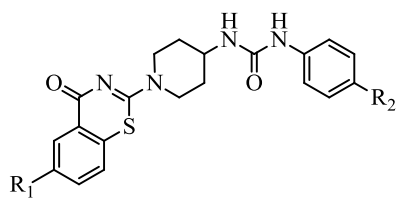
2-(4-(Benzylamino)piperidin-1-yl)-6-nitro-4H-benzo[e][1,3]thiazin-4-one derivatives (BD_29 – BD_40). To a cooled solution of 2-(4-aminopiperidin-1-yl)-6-substituted-4H-benzo[e][1,3]thiazin-4-one (1 mmol) in methanol (2 mL) was added aldehyde and sodium cyanoborohydride (1 mmol) and stirred the reaction mixture at room temperature for 12 hours (monitored by TLC & LCMS for completion), and solvent evaporated under reduced pressure. The residue was further diluted with water (30 mL) and ethyl acetate (50 mL) and the layers were separated. The organic layer was dried over anhydrous sodium sulphate and evaporated under reduced pressure. The residue was purified by silica gel column chromatography using hexane: ethylacetate as eluent to give the corresponding final derivative (**BD_29 – BD_40**) in good yield.



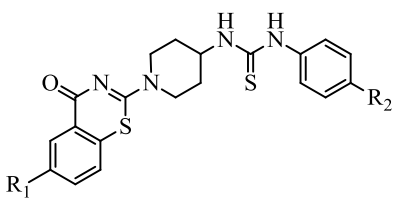
BD_04a-c **BD_29 - BD_40**

The physicochemical properties of synthesized derivatives are shown in **Table 5.3**.

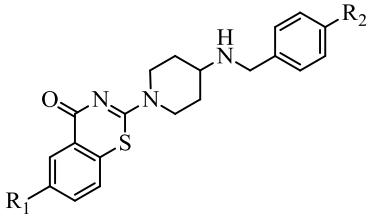
Table 5.3: Physicochemical properties of synthesized compounds **BD_05 – BD_40**.



BD_05 - BD_16



BD_17 - BD_28



BD_29 - BD_40

Compd	R_1	R_2	Yield (%)	M.P. (°C)	Molecular formula	Molecular weight
BD_05	NO_2	H	50	189-191	$C_{20}H_{19}N_5O_4S$	425.46
BD_06	NO_2	Cl	57	208-210	$C_{20}H_{18}ClN_5O_4S$	459.91
BD_07	NO_2	CH_3	39	226-228	$C_{21}H_{21}N_5O_4S$	439.49
BD_08	NO_2	NO_2	15	213-215	$C_{20}H_{18}N_6O_6S$	470.46
BD_09	CF_3	H	54	211-213	$C_{21}H_{19}F_3N_4O_2S$	448.46
BD_10	CF_3	Cl	35	186-188	$C_{21}H_{18}ClF_3N_4O_2S$	482.91
BD_11	CF_3	CH_3	33	192-194	$C_{22}H_{21}F_3N_4O_2S$	462.49
BD_12	CF_3	NO_2	26	203-205	$C_{21}H_{18}F_3N_4O_2S$	493.46
BD_13	Cl	H	38	201-203	$C_{20}H_{19}ClN_4O_2S$	414.91
BD_14	Cl	Cl	38	186-188	$C_{20}H_{18}Cl_2N_4O_2S$	449.35
BD_15	Cl	CH_3	46	182-184	$C_{21}H_{21}ClN_4O_2S$	428.94
BD_16	Cl	NO_2	46	193-195	$C_{20}H_{18}ClN_5O_4S$	459.91
BD_17	NO_2	H	47	197-199	$C_{20}H_{19}N_5O_3S_2$	441.53

Contd.

Compd	R_1	R_2	Yield (%)	M.P. (°C)	Molecular formula	Molecular weight
BD_18	NO_2	Cl	46	215-217	$C_{20}H_{18}ClN_5O_3S_2$	475.97
BD_19	NO_2	CH_3	49	211-213	$C_{21}H_{21}N_5O_3S_2$	455.55
BD_20	NO_2	NO_2	27	211-213	$C_{20}H_{18}N_6O_5S_2$	486.52
BD_21	CF_3	H	41	215-217	$C_{21}H_{19}F_3N_4OS_2$	464.53
BD_22	CF_3	Cl	41	183-185	$C_{21}H_{18}ClF_3N_4OS_2$	498.97
BD_23	CF_3	CH_3	46	217-219	$C_{22}H_{21}F_3N_4OS_2$	478.55
BD_24	CF_3	NO_2	32	221-223	$C_{21}H_{18}F_3N_5O_3S_2$	509.52
BD_25	Cl	H	35	217-219	$C_{20}H_{19}ClN_4OS_2$	430.97
BD_26	Cl	Cl	15	222-224	$C_{20}H_{18}Cl_2N_4OS_2$	465.42
BD_27	Cl	CH_3	49	192-194	$C_{21}H_{21}ClN_4OS_2$	445
BD_28	Cl	NO_2	32	199-201	$C_{20}H_{18}ClN_5O_3S_2$	475.97
BD_29	NO_2	H	36	206-208	$C_{20}H_{20}N_4O_3S$	396.46
BD_30	NO_2	Cl	47	221-223	$C_{20}H_{19}ClN_4O_3S$	430.91
BD_31	NO_2	CH_3	40	197-199	$C_{21}H_{22}N_4O_3S$	410.49
BD_32	NO_2	NO_2	40	205-207	$C_{20}H_{19}N_5O_5S$	441.46
BD_33	CF_3	H	52	179-181	$C_{21}H_{20}F_3N_3OS$	419.46
BD_34	CF_3	Cl	64	193-195	$C_{21}H_{19}ClF_3N_3OS$	453.91
BD_35	CF_3	CH_3	49	183-185	$C_{22}H_{22}F_3N_3OS$	433.49
BD_36	CF_3	NO_2	48	227-229	$C_{21}H_{19}F_3N_4O_3S$	464.46
BD_37	Cl	H	55	189-191	$C_{20}H_{20}ClN_3OS$	385.91
BD_38	Cl	Cl	51	204-206	$C_{20}H_{19}Cl_2N_3OS$	420.36
BD_39	Cl	CH_3	36	216-218	$C_{21}H_{22}ClN_3OS$	399.94
BD_40	Cl	NO_2	40	201-203	$C_{20}H_{19}ClN_4O_3S$	430.91

5.1b.3. Characterization of synthesized compounds

A series of 36 derivatives wereprepared using the above method and both analytical and spectral data (^{1}H NMR, ^{13}C NMR, and mass spectra) of all the synthesized compounds were in full agreement with the proposed structures.

2-Chloro-5-nitrobenzamide (BD_02a). The compound was synthesized according to the general procedure using 2-chloro-5-nitrobenzoic acid (**BD_01a**) (5.0 g, 0.02 mmol), oxalyl chloride (5.3 mL, 0.05 mmol) and aqueous ammonium hydroxide (50 mL) to afford **BD_02a** (3.9 g, 78.4 %) as yellow solid. M.p: 185-187 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.28-7.83 (m, 3H), 7.52 (b, 2H). ^{13}C NMR (DMSO-d_6): δ_C 168.2, 146.2, 140.3, 133.2, 130.7, 128.5, 122.1. ESI-MS *m/z* 201 $[M+H]^+$. Anal.Calcd.For $C_7H_5ClN_2O_3$: C, 41.92; H, 2.51; N, 13.97; Found: C, 41.89; H, 2.54; N, 13.99.

2-Chloro-5-(trifluoromethyl)benzamide (BD_02b). The compound was synthesized according to the general procedure using 2-chloro-5-(trifluoromethyl)benzoic acid (**BD_01b**) (4.0 g, 0.01 mmol), oxalyl chloride (3.8 mL, 0.04 mmol) and aqueous ammonium hydroxide (40 mL) to afford **BD_02b** (1.84 g, 46.2 %) as white solid. M.p: 176-178 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.02-7.64 (m, 3H), 7.54 (b, 2H). ^{13}C NMR (DMSO-d_6): δ_C 168.4, 137.7, 132.3, 129.7, 129.5, 128.1, 125.3. 123.5. ESI-MS *m/z* 224 $[M+H]^+$. Anal.Calcd.For $C_8H_5ClF_3NO$: C, 42.98; H, 2.25; N, 6.26; Found: C, 41.89; H, 2.54; N, 13.99.

2,5-Dichlorobenzamide (BD_02c). The compound was synthesized according to the general procedure using 2, 5-dichlorobenzoic acid (**BD_01c**) (4.0 g, 0.02 mmol), oxalyl chloride (4.5 mL, 0.05 mmol) and aqueous ammonium hydroxide (40 mL) to afford **BD_02c** (2.12 g, 53.4 %) as white solid. M.p: 181-183 °C. ^{1}H NMR (DMSO-d_6): δ_H 7.89-7.63 (m, 3H), 7.52 (b, 2H). ^{13}C NMR (DMSO-d_6): δ_C 168.6, 133.5, 132.5, 132.3, 132.1, 129.4, 128.6. ESI-MS *m/z* 192 $[M+H]^+$. Anal.Calcd.For $C_7H_5Cl_2NO$: C, 44.24; H, 2.65; N, 7.37; Found: C, 44.26; H, 2.64; N, 7.39.

2-(Methylthio)-6-nitro-4H-benzo[e][1,3]thiazin-4-one (BD_03a). The compound was synthesized according to the general procedure using 2-chloro-5-nitrobenzamide (**BD_02a**) (2.0 g, 9.97 mmol), carbon disulphide (1.8 mL, 29.9 mmol), sodim hydroxide (0.79g, 19.9 mmol) and methyl iodide (0.73 mL, 11.9 mmol) to afford **BD_03a** (1.82 g, 71.9 %) as yellow solid. M.p: 211-213 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.36-7.82 (m, 3H), 2.72 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 167.6, 162.4, 145.3, 143.6, 138.4, 130.5, 128.4, 123.6, 14.2. ESI-MS *m/z* 255 $[M+H]^+$. Anal.Calcd.For $C_9H_6N_2O_3S_2$: C, 42.51; H, 2.38; N, 11.02; Found: C, 42.54; H, 2.36; N, 11.05.

2-(Methylthio)-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (BD_03b). The compound was synthesized according to the general procedure using 2-chloro-5-

(trifluoromethyl)benzamide (**BD_02b**) (1.5 g, 6.71 mmol), carbon disulphide (1.2 mL, 20.1 mmol), sodim hydroxide (0.53g, 13.4 mmol) and methyl iodide (0.49 mL, 8.1 mmol) to afford **BD_03b** (1.63 g, 88.1 %) as white solid. M.p: 208-210 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.35-7.78 (m, 3H), 2.72 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 167.4, 162.5, 140.3, 137.6, 131.4, 130.6, 128.4, 126.6, 123.5, 14.3. ESI-MS *m/z* 278 $[M+H]^+$. Anal.Calcd.For $C_{10}H_6F_3NOS_2$: C, 43.32; H, 2.18; N, 5.05; Found: C, 43.33; H, 2.16; N, 5.03.

6-Chloro-2-(methylthio)-4H-benzo[e][1,3]thiazin-4-one (BD_03c). The compound was synthesized according to the general procedure using 2, 5-dichlorobenzamide (**BD_02c**) (2.0 g, 10.52 mmol), carbon disulphide (1.9 mL, 31. mmol), sodim hydroxide (0.84 g, 21.1 mmol) and methyl iodide (0.78 mL, 12.6 mmol) to afford **BD_03c** (1.57 g, 61.3 %) as white solid. M.p: 219-221 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.36-7.74 (m, 3H), 2.72 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 167.8, 162.5, 138.3, 135.6, 134.4, 131.6, 131.4, 130.6, 14.3. ESI-MS *m/z* 244 $[M+H]^+$. Anal.Calcd.For $C_9H_6ClNOS_2$: C, 44.35; H, 2.48; N, 5.75; Found: C, 44.33; H, 2.46; N, 5.73.

2-(4-Aminopiperidin-1-yl)-6-nitro-4H-benzo[e][1,3]thiazin-4-one (BD_04a). The compound was synthesized according to the general procedure using 2-(methylthio)-6-nitro-4*H*-benzo[*e*][1,3]thiazin-4-one (**BD_03a**) (1.8 g, 7.08 mmol), 4-(*N*-Boc-amino)piperidine (1.41 g, 7.08 mmol) and trifluoroacetic acid (2 mL) to afford **BD_04a** (1.51 g, 69.9 %) as yellow solid. M.p: 208-210 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.33-7.24 (m, 3H), 5.25 (b, 2H), 3.12-1.88 (m, 9H). ^{13}C NMR (DMSO-d_6): δ_C 167.5, 159.3, 145.6, 143.6, 138.4, 130.3, 128.4, 123.6, 46.7, 41.5 (2C), 32.2 (2C). ESI-MS *m/z* 307 $[M+H]^+$. Anal.Calcd.For $C_{13}H_{14}N_4O_3S$: C, 50.97; H, 4.61; N, 18.29; Found: C, 50.96; H, 4.65; N, 18.31.

2-(4-Aminopiperidin-1-yl)-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (BD_04b). The compound was synthesized according to the general procedure using 2-(methylthio)-6-(trifluoromethyl)-4*H*-benzo[*e*][1,3]thiazin-4-one (**BD_03b**) (1.6 g, 5.57 mmol), 4-(*N*-Boc-amino)piperidine (1.15 g, 5.57 mmol) and trifluoroacetic acid (2 mL) to afford **BD_04b** (1.42 g, 71.7 %) as white solid. M.p: 221-223 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.32-7.27 (m, 3H), 5.26 (b, 2H), 3.14-1.85 (m, 9H). ^{13}C NMR (DMSO-d_6): δ_C 167.2, 159.4, 140.7, 137.6, 131.4, 130.3, 128.5, 126.8, 123.3, 46.7, 41.4 (2C), 32.5 (2C). ESI-MS *m/z* 330 $[M+H]^+$. Anal.Calcd.For $C_{14}H_{14}F_3N_3OS$: C, 51.06; H, 4.28; N, 12.76; Found: C, 51.08; H, 4.27; N, 12.73.

2-(4-Aminopiperidin-1-yl)-6-chloro-4H-benzo[e][1,3]thiazin-4-one (BD_04c). The compound was synthesized according to the general procedure using 6-chloro-2-(methylthio)-4*H*-benzo[*e*][1,3]thiazin-4-one (**BD_03c**) (1.5 g, 6.15 mmol), 4-(*N*-Boc-amino)piperidine (1.23 g, 6.15 mmol) and trifluoroacetic acid (2 mL) to afford **BD_04c** (1.55 g, 85.6 %) as white solid. M.p: 217-219 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.33-7.28 (m, 3H), 5.24 (b, 2H), 3.12-1.88 (m, 9H). ^{13}C NMR (DMSO-d_6): δ_C 167.4, 159.5, 138.6, 135.1, 134.4, 131.3, 131.1, 130.2, 46.8, 41.4 (2C), 32.2 (2C). ESI-MS *m/z* 296 $[M+H]^+$. Anal.Calcd.For $C_{13}H_{14}ClN_3OS$: C, 52.79; H, 4.77; N, 14.21; Found: C, 52.82; H, 4.76; N, 14.25.

1-(1-(6-Nitro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)piperidin-4-yl)-3-phenylurea (BD_05). The compound was synthesized according to the general procedure using 2-(4-aminopiperidin-1-yl)-6-nitro-4*H*-benzo[*e*][1,3]thiazin-4-one (**BD_04a**) (0.1 g, 0.32 mmol) and Phenyl isocyanate (0.04 g, 0.32 mmol) to afford **BD_05** (0.07 g, 50.41 %) as yellow solid. M.p: 189-191 °C. ^{1}H NMR (DMSO-d_6): δ_H 10.59 (b, 1H), 10.42 (b, 1H), 8.56-7.48 (m, 8H), 3.12-1.88 (m, 9H). ^{13}C NMR (DMSO-d_6): δ_C 167.5, 159.4, 154.7, 145.3, 143.6, 139.1, 138.2, 130.2, 128.7 (3C), 128.1, 123.5, 121 (2C), 49.6, 40.7 (2C), 29.3 (2C). ESI-MS *m/z* 426 $[M+H]^+$. Anal.Calcd.For $C_{20}H_{19}N_5O_4S$: C, 56.46; H, 4.50; N, 16.46; Found: C, 56.44; H, 4.52.45; N, 16.48.

1-(4-Chlorophenyl)-3-(1-(6-nitro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)piperidin-4-yl)urea (BD_06). The compound was synthesized according to the general procedure using 2-(4-aminopiperidin-1-yl)-6-nitro-4*H*-benzo[*e*][1,3]thiazin-4-one (**BD_04a**) (0.1 g, 0.32 mmol) and 4-chlorophenyl isocyanate (0.05 g, 0.32 mmol) to afford **BD_06** (0.08 g, 56.62 %) as yellow solid. M.p: 208-210 °C. ^{1}H NMR (DMSO-d_6): δ_H 10.58 (b, 1H), 10.41 (b, 1H), 8.54-7.43 (m, 7H), 3.12-1.88 (m, 9H). ^{13}C NMR (DMSO-d_6): δ_C 167.4, 159.2, 154.4, 145.1, 143.8, 138.1, 137.2, 133.2, 130.7, 129.1 (2C), 128.5, 123.6, 120.4 (2C), 49.5, 40.6 (2C), 29.4 (2C). ESI-MS *m/z* 460 $[M+H]^+$. Anal.Calcd.For $C_{20}H_{18}ClN_5O_4S$: C, 52.23; H, 3.94; N, 15.23; Found: C, 52.19; H, 3.92; N, 15.97.

1-(1-(6-Nitro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)piperidin-4-yl)-3-(p-tolyl)urea (BD_07). The compound was synthesized according to the general procedure using 2-(4-aminopiperidin-1-yl)-6-nitro-4*H*-benzo[*e*][1,3]thiazin-4-one (**BD_04a**) (0.1 g, 0.32 mmol) and *p*-tolylisocyanate (0.04 g, 0.32 mmol) to afford **BD_07** (0.05 g, 39.04 %) as yellow solid. M.p: 226-228 °C. ^{1}H NMR (DMSO-d_6): δ_H 10.56 (b, 1H), 10.43 (b, 1H), 8.57-7.46 (m, 7H), 3.11-1.87 (m, 9H), 2.36 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 167.6, 159.4, 154.7, 145.3, 143.6,

138.1, 136.6, 136.2, 130.7, 129.1 (2C), 128.5, 123.5, 121.4 (2C), 49.3, 40.5 (2C), 29.2 (2C), 21.4. ESI-MS *m/z* 440 $[M+H]^+$. Anal.Calcd.For $C_{21}H_{21}N_5O_4S$: C, 57.39; H, 4.82; N, 15.94; Found: C, 57.42; H, 4.81; N, 15.90.

1-(1-(6-Nitro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)piperidin-4-yl)-3-(4-nitrophenyl)urea (BD_08). The compound was synthesized according to the general procedure using 2-(4-aminopiperidin-1-yl)-6-nitro-4*H*-benzo[*e*][1,3]thiazin-4-one (**BD_04a**) (0.1 g, 0.32 mmol) and 4-nitrophenyl isocyanate (0.05 g, 0.32 mmol) to afford **BD_08** (0.02 g, 14.98 %) as yellow solid. M.p: 213-215 °C. ^{1}H NMR (DMSO-d_6): δ_H 10.56 (b, 1H), 10.44 (b, 1H), 8.58-7.48 (m, 7H), 3.14-1.89 (m, 9H). ^{13}C NMR (DMSO-d_6): δ_C 167.4, 159.3, 154.4, 145.7, 145.2, 143.5, 143.3, 138.2, 130.7, 128.1, 124.5 (2C), 123.6, 119.4 (2C), 49.5, 40.6 (2C), 29.3 (2C). ESI-MS *m/z* 471 $[M+H]^+$. Anal.Calcd.For $C_{20}H_{18}N_6O_6S$: C, 51.06; H, 3.86; N, 17.86; Found: C, 51.08; H, 3.84; N, 17.87.

1-(1-(4-Oxo-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-2-yl)piperidin-4-yl)-3-phenylurea (BD_09). The compound was synthesized according to the general procedure using 2-(4-aminopiperidin-1-yl)-6-(trifluoromethyl)-4*H*-benzo[*e*][1,3]thiazin-4-one (**BD_04b**) (0.1 g, 0.30 mmol) and phenyl isocyanate (0.03 g, 0.30 mmol) to afford **BD_09** (0.07 g, 53.62 %) as white solid. M.p: 211-213 °C. ^{1}H NMR (DMSO-d_6): δ_H 10.52 (b, 1H), 10.39 (b, 1H), 8.51-7.40 (m, 8H), 3.12-1.84 (m, 9H). ^{13}C NMR (DMSO-d_6): δ_C 167.2, 159.5, 154.6, 140.3, 139.6, 137.1, 131.2, 130.2, 128.7 (2C), 128.3, 128.1, 126.4, 123.5, 121.4 (2C), 49.2, 40.5 (2C), 29.1 (2C). ESI-MS *m/z* 449 $[M+H]^+$. Anal.Calcd.For $C_{21}H_{19}F_3N_4O_2S$: C, 56.24; H, 4.27; N, 12.49; Found: C, 56.22; H, 4.30; N, 12.51.

1-(4-Chlorophenyl)-3-(1-(4-oxo-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-2-yl)piperidin-4-yl)urea (BD_10). The compound was synthesized according to the general procedure using 2-(4-aminopiperidin-1-yl)-6-(trifluoromethyl)-4*H*-benzo[*e*][1,3]thiazin-4-one (**BD_04b**) (0.1 g, 0.30 mmol) and 4-chlorophenyl isocyanate (0.04 g, 0.30 mmol) to afford **BD_10** (0.05 g, 35.47 %) as white solid. M.p: 186-188 °C. ^{1}H NMR (DMSO-d_6): δ_H 10.51 (b, 1H), 10.42 (b, 1H), 8.50-7.38 (m, 7H), 3.14-1.82 (m, 9H). ^{13}C NMR (DMSO-d_6): δ_C 167.6, 159.1, 154.4, 140.1, 137.8, 137.6, 133.1, 131.2, 130.2, 129.4 (2C), 128.1, 126.5, 123.6, 120.6 (2C), 49.4, 40.5 (2C), 29.3 (2C). ESI-MS *m/z* 483 $[M+H]^+$. Anal.Calcd.For $C_{21}H_{18}ClF_3N_4O_2S$: C, 52.23; H, 3.76; N, 11.60; Found: C, 52.27; H, 3.74; N, 11.62.

1-(1-(4-Oxo-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-2-yl)piperidin-4-yl)-3-(p-tolyl)urea (BD_11). The compound was synthesized according to the general procedure using 2-(4-aminopiperidin-1-yl)-6-(trifluoromethyl)-4*H*-benzo[*e*][1,3]thiazin-4-one (**BD_04b**) (0.1 g, 0.30 mmol) and *p*-tolylisocyanate (0.04 g, 0.30 mmol) to afford **BD_11** (0.04 g, 32.76 %) as white solid. M.p: 192-194 °C. ^{1}H NMR (DMSO-d_6): δ_H 10.54 (b, 1H), 10.43 (b, 1H), 8.51-7.41 (m, 7H), 3.13-1.83 (m, 9H), 2.38 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 167.5, 159.4, 154.6, 140.8, 137.2, 136.6, 136.2, 131.2, 130.4, 129.3 (2C), 128.4, 126.6, 123.6, 121.4 (2C), 49.2, 40.1 (2C), 29.3 (2C), 21.4. ESI-MS *m/z* 463 $[M+H]^+$. Anal.Calcd.For $C_{22}H_{21}F_3N_4O_2S$: C, 57.13; H, 4.58; N, 12.11; Found: C, 57.15; H, 4.61; N, 12.08.

1-(4-Nitrophenyl)-3-(1-(4-oxo-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-2-yl)piperidin-4-yl)urea (BD_12). The compound was synthesized according to the general procedure using 2-(4-aminopiperidin-1-yl)-6-(trifluoromethyl)-4*H*-benzo[*e*][1,3]thiazin-4-one (**BD_04b**) (0.1 g, 0.30 mmol) and 4-nitrophenyl isocyanate (0.05 g, 0.30 mmol) to afford **BD_012** (0.03 g, 26.03 %) as white solid. M.p: 203-205 °C. ^{1}H NMR (DMSO-d_6): δ_H 10.55 (b, 1H), 10.44 (b, 1H), 8.55-7.46 (m, 7H), 3.15-1.85 (m, 9H). ^{13}C NMR (DMSO-d_6): δ_C 167.7, 159.2, 154.1, 145.8, 143.2, 140.6, 137.2, 131.3, 130.2, 128.4, 126.3, 124.4 (2C), 123.6, 119.6 (2C), 49.5, 40.8 (2C), 29.4 (2C). ESI-MS *m/z* 494 $[M+H]^+$. Anal.Calcd.For$C_{21}H_{18}F_3N_4O_2S$: C, 51.11; H, 3.68; N, 14.19; Found: C, 51.14; H, 3.66; N, 14.22.

1-(1-(6-Chloro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)piperidin-4-yl)-3-phenylurea (BD_13). The compound was synthesized according to the general procedure using 2-(4-aminopiperidin-1-yl)-6-chloro-4*H*-benzo[*e*][1,3]thiazin-4-one (**BD_04c**) (0.1 g, 0.33 mmol) and phenyl isocyanate (0.04 g, 0.33 mmol) to afford **BD_13** (0.05 g, 37.79 %) as white solid. M.p: 201-203 °C. ^{1}H NMR (DMSO-d_6): δ_H 10.50 (b, 1H), 10.39 (b, 1H), 8.54-7.39 (m, 8H), 3.16-1.89 (m, 9H). ^{13}C NMR (DMSO-d_6): δ_C 167.4, 159.5, 154.2, 139.8, 138.2, 135.6, 134.2, 131.3, 131.2, 130.4, 128.7 (2C), 128.4, 121.6 (2C), 49.6, 40.4 (2C), 29.2 (2C). ESI-MS *m/z* 415 $[M+H]^+$. Anal.Calcd.For $C_{20}H_{19}ClN_4O_2S$: C, 57.90; H, 4.62; N, 13.50; Found: C, 57.88; H, 4.59; N, 13.53.

1-(1-(6-Chloro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)piperidin-4-yl)-3-(4-chlorophenyl)urea (BD_14). The compound was synthesized according to the general procedure using 2-(4-aminopiperidin-1-yl)-6-chloro-4*H*-benzo[*e*][1,3]thiazin-4-one (**BD_04c**) (0.1 g, 0.33 mmol) and 4-chlorophenyl isocyanate (0.05 g, 0.33 mmol) to afford **BD_14** (0.05 g, 37.53 %) as white solid. M.p: 186-188 °C. ^{1}H NMR (DMSO-d_6): δ_H 10.51 (b, 1H), 10.41 (b, 1H), 8.56-

7.42 (m, 7H), 3.14-1.86 (m, 9H). ^{13}C NMR (DMSO-d_6): δ_C 167.6, 159.2, 154.4, 138.8, 137.2, 135.5, 134.2, 133.5, 131.4, 131.1, 130.5, 129.7 (2C), 120.6 (2C), 49.4, 40.2 (2C), 29.1 (2C). ESI-MS *m/z* 450 $[M+H]^+$. Anal.Calcd.For $C_{20}H_{18}Cl_2N_4O_2S$: C, 53.46; H, 4.04; N, 12.47; Found: C, 53.42; H, 4.05; N, 12.49.

1-(1-(6-Chloro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)piperidin-4-yl)-3-(p-tolyl)urea (BD_15). The compound was synthesized according to the general procedure using 2-(4-aminopiperidin-1-yl)-6-chloro-4*H*-benzo[*e*][1,3]thiazin-4-one (**BD_04c**) (0.1 g, 0.33 mmol) and *p*-tolylisocyanate (0.04 g, 0.33 mmol) to afford **BD_15** (0.06 g, 45.52 %) as white solid. M.p: 182-184 °C. ^{1}H NMR (DMSO-d_6):δ_H 10.49 (b, 1H), 10.44 (b, 1H), 8.51-7.42 (m, 7H), 3.14-1.84 (m, 9H), 2.34 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 167.4, 159.7, 154.1, 138.7, 136.6, 136.2, 135.5, 134.3, 131.5, 131.3, 130.1, 129.5 (2C), 121.7 (2C), 49.5, 40.4 (2C), 29.3 (2C), 21.4. ESI-MS *m/z* 429 $[M+H]^+$. Anal.Calcd.For $C_{21}H_{21}ClN_4O_2S$: C, 58.80; H, 4.93; N, 13.06; Found: C, 58.82; H, 4.90; N, 13.05.

1-(1-(6-Chloro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)piperidin-4-yl)-3-(4-nitrophenyl)urea (BD_16). The compound was synthesized according to the general procedure using 2-(4-aminopiperidin-1-yl)-6-chloro-4*H*-benzo[*e*][1,3]thiazin-4-one (**BD_04c**) (0.1 g, 0.33 mmol) and 4-nitrophenyl isocyanate (0.05 g, 0.33 mmol) to afford **BD_16** (0.07 g, 45.67 %) as white solid. M.p: 193-195 °C. ^{1}H NMR (DMSO-d_6): δ_H 10.52 (b, 1H), 10.48 (b, 1H), 8.53-7.47 (m, 7H), 3.15-1.81 (m, 9H). ^{13}C NMR (DMSO-d_6): δ_C 167.7, 159.3, 154.2, 145.7, 143.2, 138.5, 135.3, 134.5, 131.3, 131.1, 130.5, 124.3 (2C), 119.8 (2C), 49.5, 40.6 (2C), 29.5 (2C). ESI-MS *m/z* 460 $[M+H]^+$. Anal.Calcd.For $C_{20}H_{18}ClN_5O_4S$: C, 52.23; H, 3.94; N, 15.23; Found: C, 52.19; H, 3.95; N, 15.25.

1-(1-(6-Nitro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)piperidin-4-yl)-3-phenylthiourea (BD_17). The compound was synthesized according to the general procedure using 2-(4-aminopiperidin-1-yl)-6-nitro-4*H*-benzo[*e*][1,3]thiazin-4-one (**BD_04a**) (0.1 g, 0.32 mmol) and phenyl isothiocyanate (0.04 g, 0.32 mmol) to afford **BD_17** (0.06 g, 47.18 %) as yellow solid. M.p: 197-199 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.97(b, 1H), 8.52-7.71 (m, 8H), 6.85 (b, 1H), 3.06-1.84 (m, 9H). ^{13}C NMR (DMSO-d_6): δ_C 177.6, 167.5, 159.2, 145.2, 143.7, 138.2, 138.1, 130.3, 129.3 (2C), 128.4, 128.1, 126.5 (2C), 123.3, 54.7, 41.5 (2C), 29.6 (2C). ESI-MS *m/z* 442 $[M+H]^+$. Anal.Calcd.For $C_{20}H_{19}N_5O_3S_2$: C, 54.41; H, 4.34; N, 15.86; Found: C, 54.39; H, 4.32; N, 15.88.

1-(4-Chlorophenyl)-3-(1-(6-nitro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)piperidin-4-yl)thiourea (BD_18). The compound was synthesized according to the general procedure using 2-(4-aminopiperidin-1-yl)-6-nitro-4*H*-benzo[*e*][1,3]thiazin-4-one (**BD_4a**) (0.1 g, 0.32 mmol) and 4-Chlorophenyl isothiocyanate (0.05 g, 0.32 mmol) to afford **BD_18** (0.07 g, 46.34 %) as yellow solid. M.p: 215-217 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.95(b, 1H), 8.55-7.73 (m, 7H), 6.82 (b, 1H), 3.04-1.86 (m, 9H). ^{13}C NMR (DMSO-d_6): δ_C 177.4, 167.6, 159.4, 145.7, 143.2, 138.4, 136.1, 133.3, 131.3 (2C), 130.4, 129.1 (2C), 128.5, 123.4, 54.6, 41.8 (2C), 29.5 (2C). ESI-MS *m/z* 476 $[M+H]^+$. Anal.Calcd.For $C_{20}H_{18}ClN_5O_3S_2$: C, 50.47; H, 3.81; N, 14.71; Found: C, 50.49; H, 3.83; N, 14.69.

1-(1-(6-Nitro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)piperidin-4-yl)-3-(p-tolyl)thiourea (BD_19). The compound was synthesized according to the general procedure using 2-(4-aminopiperidin-1-yl)-6-nitro-4*H*-benzo[*e*][1,3]thiazin-4-one (**BD_04a**) (0.1 g, 0.32 mmol) and *p*-tolylisothiocyanate (0.05 g, 0.32 mmol) to afford **BD_19** (0.07 g, 49.09 %) as yellow solid. M.p: 211-213 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.93(b, 1H), 8.58-7.81 (m, 7H), 6.81 (b, 1H), 3.07-1.88 (m, 9H), 2.36 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 177.3, 167.5, 159.8, 145.6, 143.1, 138.4, 137.1, 135.3, 130.3, 129.4 (2C), 128.1, 126.5 (2C), 123.5, 54.8, 41.3 (2C), 29.2 (2C), 21.4. ESI-MS *m/z* 456 $[M+H]^+$. Anal.Calcd.For $C_{21}H_{21}N_5O_3S_2$: C, 55.37; H, 4.65; N, 15.37; Found: C, 55.36; H, 4.64; N, 15.39.

1-(1-(6-Nitro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)piperidin-4-yl)-3-(4-nitrophenyl)thiourea (BD_20). The compound was synthesized according to the general procedure using 2-(4-aminopiperidin-1-yl)-6-nitro-4*H*-benzo[*e*][1,3]thiazin-4-one (**BD_04a**) (0.1 g, 0.32 mmol) and 4-nitrophenyl isothiocyanate (0.06 g, 0.32 mmol) to afford **BD_20** (0.04 g, 27.08 %) as yellow solid. M.p: 211-213 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.95 (b, 1H), 8.61-7.42 (m, 7H), 6.86 (b, 1H), 3.08-1.81 (m, 9H). ^{13}C NMR (DMSO-d_6): δ_C 177.5, 167.3, 159.5, 145.8, 144.6, 143.4, 143.1, 138.3, 130.2, 128.4, 124.5 (2C), 124.2 (2C), 123.7, 54.6, 41.5 (2C), 29.4 (2C). ESI-MS *m/z* 487 $[M+H]^+$. Anal.Calcd.For $C_{20}H_{18}N_6O_5S_2$: C, 49.37; H, 3.73; N, 17.27; Found: C, 49.35; H, 3.75; N, 17.24.

1-(1-(4-Oxo-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-2-yl)piperidin-4-yl)-3 phenylthiourea (BD_21).The compound was synthesized according to the general procedure using 2-(4-aminopiperidin-1-yl)-6-(trifluoromethyl)-4*H*-benzo[*e*][1,3]thiazin-4-one (**BD_04b**) (0.1 g, 0.30 mmol) and phenyl isothiocyanate (0.04 g, 0.30 mmol) to afford **BD_21** (0.05 g, 41.13 %) as white solid. M.p: 215-217 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.97 (b,

1H), 8.62-7.45 (m, 8H), 6.81 (b, 1H), 3.10-1.85 (m, 9H). ^{13}C NMR (DMSO-d_6): δ_C 177.1, 167.9, 159.6, 140.8, 138.6, 137.4, 131.6, 130.3, 129.2 (2C), 128.4, 128.2, 126.2 (3C), 123.5, 54.7, 41.2 (2C), 29.6 (2C). ESI-MS *m/z* 465 $[M+H]^+$. Anal.Calcd.For $C_{21}H_{19}F_3N_4OS_2$: C, 54.30; H, 4.12; N, 12.06; Found: C, 54.28; H, 4.11; N, 12.09.

1-(4-Chlorophenyl)-3-(1-(4-oxo-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-2-yl)piperidin-4-yl)thiourea (BD_22). The compound was synthesized according to the general procedure using 2-(4-aminopiperidin-1-yl)-6-(trifluoromethyl)-4*H*-benzo[*e*][1,3]thiazin-4-one (**BD_04b**) (0.1 g, 0.30 mmol) and 4-chlorophenyl isothiocyanate (0.05 g, 0.30 mmol) to afford **BD_22** (0.06 g, 40.93 %) as white solid. M.p: 183-185 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.94 (b, 1H), 8.63-7.41 (m, 7H), 6.84 (b, 1H), 3.12-1.88 (m, 9H). ^{13}C NMR (DMSO-d_6): δ_C 177.8, 167.7, 159.4, 140.5, 137.6, 136.4, 133.6, 131.3, 131.2 (2C), 130.4, 129.2 (2C), 128.2, 126.5, 123.6, 54.8, 41.6 (2C), 29.4 (2C). ESI-MS *m/z* 499 $[M+H]^+$. Anal.Calcd.For $C_{21}H_{18}ClF_3N_4OS_2$: C, 50.55; H, 3.64; N, 11.23; Found: C, 50.53; H, 3.66; N, 11.20.

1-(1-(4-Oxo-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-2-yl)piperidin-4-yl)-3-(p-tolyl)thiourea (BD_23). The compound was synthesized according to the general procedure using 2-(4-aminopiperidin-1-yl)-6-(trifluoromethyl)-4*H*-benzo[*e*][1,3]thiazin-4-one (**BD_04b**) (0.1 g, 0.30 mmol) and *p*-tolylisothiocyanate (0.04 g, 0.30 mmol) to afford **BD_23** (0.06 g, 46.12 %) as white solid. M.p: 217-219 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.91 (b, 1H), 8.59-7.63 (m, 7H), 6.79 (b, 1H), 3.15-1.85 (m, 9H), 2.35 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 177.5, 167.1, 159.9, 140.2, 137.4, 137.2, 135.4, 131.6, 130.3, 129.2 (2C), 128.4, 126.5, 126.2 (2C), 123.5, 54.4, 41.2 (2C), 29.1 (2C), 21.4. ESI-MS *m/z* 479 $[M+H]^+$. Anal.Calcd.For $C_{22}H_{21}F_3N_4OS_2$: C, 55.22; H, 4.42; N, 11.71; Found: C, 55.21; H, 4.46; N, 11.69.

1-(4-Nitrophenyl)-3-(1-(4-oxo-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-2-yl)piperidin-4-yl)thiourea (BD_24). The compound was synthesized according to the general procedure using 2-(4-aminopiperidin-1-yl)-6-(trifluoromethyl)-4*H*-benzo[*e*][1,3]thiazin-4-one (**BD_04b**) (0.1 g, 0.30 mmol) and 4-nitrophenyl isothiocyanate (0.05 g, 0.30 mmol) to afford **BD_24** (0.04 g, 31.68 %) as white solid. M.p: 221-223 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.98 (b, 1H), 8.62-7.61 (m, 7H), 6.88 (b, 1H), 3.11-1.82 (m, 9H). ^{13}C NMR (DMSO-d_6): δ_C 177.1, 167.3, 159.4, 144.2, 143.4, 140.2, 137.4, 131.6, 130.3, 128.2, 126.4, 124.5 (2C), 124.2 (2C), 123.7, 54.3, 41.7 (2C), 29.5 (2C). ESI-MS *m/z* 510 $[M+H]^+$. Anal.Calcd.For $C_{21}H_{18}F_3N_5O_3S_2$: C, 49.50; H, 3.56; N, 13.74; Found: C, 49.53; H, 3.54; N, 13.75.

1-(1-(6-Chloro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)piperidin-4-yl)-3-phenylthiourea (BD_25). The compound was synthesized according to the general procedure using 2-(4-aminopiperidin-1-yl)-6-chloro-4*H*-benzo[*e*][1,3]thiazin-4-one (**BD_04c**) (0.1 g, 0.33 mmol) and phenyl isothiocyanate (0.04 g, 0.33 mmol) to afford **BD_25** (0.05 g, 35.401 %) as white solid. M.p: 217-219 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.92 (b, 1H), 8.58-7.66 (m, 8H), 6.81 (b, 1H), 3.14-1.85 (m, 9H). ^{13}C NMR (DMSO-d_6): δ_C 177.5, 167.5, 159.4, 138.6, 138.4, 135.2, 134.4, 131.6, 131.2, 130.2, 129.4 (2C), 128.5, 126.6 (2C), 54.3, 41.6 (2C), 29.4 (2C). ESI-MS *m/z* 431 $[M+H]^+$. Anal.Calcd.For $C_{20}H_{19}ClN_4OS_2$: C, 55.74; H, 4.44; N, 13.00; Found: C, 55.72; H, 4.46; N, 13.03.

1-(1-(6-Chloro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)piperidin-4-yl)-3-(4 chlorophenyl)thiourea (BD_26). The compound was synthesized according to the general procedure using 2-(4-aminopiperidin-1-yl)-6-chloro-4*H*-benzo[*e*][1,3]thiazin-4-one (**BD_04c**) (0.1 g, 0.33 mmol) and 4-chlorophenyl isothiocyanate (0.05 g, 0.33 mmol) to afford **BD_26** (0.02 g, 14.62 %) as white solid. M.p: 222-224 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.89 (b, 1H), 8.53-7.74 (m, 7H), 6.81 (b, 1H), 3.09-1.89 (m, 9H). ^{13}C NMR (DMSO-d_6): δ_C 177.1, 167.4, 159.8, 138.9, 136.4, 135.5, 134.7, 133.6, 131.4, 131.2, 131.1 (2C), 130.4, 129.5 (2C), 54.5, 41.5 (2C), 29.7 (2C). ESI-MS *m/z* 466 $[M+H]^+$. Anal.Calcd.For $C_{20}H_{18}Cl_2N_4OS_2$: C, 51.61; H, 3.90; N, 12.04; Found: C, 51.59; H, 3.88; N, 12.07.

1-(1-(6-Chloro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)piperidin-4-yl)-3-(p-tolyl)thiourea (BD_27). The compound was synthesized according to the general procedure using 2-(4-aminopiperidin-1-yl)-6-chloro-4*H*-benzo[*e*][1,3]thiazin-4-one (**BD_04c**) (0.1 g, 0.33 mmol) and *p*-tolylisothiocyanate (0.05 g, 0.33 mmol) to afford **BD_27** (0.07 g, 48.53 %) as white solid. M.p: 192-194 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.91 (b, 1H), 8.54-7.71 (m, 7H), 6.87 (b, 1H), 3.05-1.92 (m, 9H), 2.36 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 177.7, 167.5, 159.1, 138.2, 137.4, 135.5, 135.3, 134.6, 131.4, 131.2, 130.3, 129.4 (2C), 126.5 (2C), 54.3, 41.9 (2C), 29.5 (2C), 21.4. ESI-MS *m/z* 446 $[M+H]^+$. Anal.Calcd.For $C_{21}H_{21}ClN_4OS_2$: C, 56.68; H, 4.76; N, 12.59; Found: C, 56.70; H, 4.77; N, 12.55.

1-(1-(6-Chloro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)piperidin-4-yl)-3-(4-nitrophenyl)thiourea (BD_28). The compound was synthesized according to the general procedure using 2-(4-aminopiperidin-1-yl)-6-chloro-4*H*-benzo[*e*][1,3]thiazin-4-one (**BD_04c**) (0.1 g, 0.33 mmol) and 4-nitrophenyl isothiocyanate (0.06 g, 0.33 mmol) to afford **BD_28** (0.05 g, 32.32 %) as white solid. M.p: 199-201 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.91 (b,

1H), 8.61-7.74 (m, 7H), 6.82 (b, 1H), 3.08-1.91 (m, 9H), 2.36. ^{13}C NMR (DMSO-d_6): δ_C 177.8, 167.1, 159.2, 144.2, 143.4, 138.5, 135.7, 134.4, 131.4, 131.3, 130.5, 124.4 (2C), 124.1 (2C), 54.5, 41.7 (2C), 29.5 (2C). ESI-MS *m/z* 476 $[M+H]^+$. Anal.Calcd.For $C_{20}H_{18}ClN_5O_3S_2$: C, 50.47; H, 3.81; N, 14.71; Found: C, 50.49; H, 3.84; N, 14.73.

2-(4-(Benzylamino)piperidin-1-yl)-6-nitro-4H-benzo[e][1,3]thiazin-4-one (BD_29). The compound was synthesized according to the general procedure using 2-(4-aminopiperidin-1-yl)-6-nitro-4*H*-benzo[*e*][1,3]thiazin-4-one (**BD_04a**) (0.1 g, 0.32 mmol), benzaldehyde (0.03 g, 0.32 mmol) and sodium cyanoborohydride (0.02 g, 0.32 mmol) to afford **BD_29** (0.04 g, 35.55 %) as yellow solid. M.p: 206-208 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.52-7.49 (m, 8H), 6.22 (b, 1H), 3.51 (s, 2H), 3.12-1.78 (m, 9H). ^{13}C NMR (DMSO-d_6): δ_C 167.4, 159.4, 145.2, 143.8, 140.5, 138.7, 130.4, 128.4, 128.2 (2C), 127.5 (2C), 127.2, 123.1, 58.3, 52.5, 41.6 (2C), 30.3 (2C). ESI-MS *m/z* 397 $[M+H]^+$. Anal.Calcd.For $C_{20}H_{20}N_4O_3S$: C, 60.59; H, 5.08; N, 14.13; Found: C, 60.57; H, 5.05; N, 14.12.

2-(4-((4-Chlorobenzyl)amino)piperidin-1-yl)-6-nitro-4H-benzo[e][1,3]thiazin-4-one (BD_30). The compound was synthesized according to the general procedure using 2-(4-aminopiperidin-1-yl)-6-nitro-4*H*-benzo[*e*][1,3]thiazin-4-one (**BD_04a**) (0.1 g, 0.32 mmol) 4-chlorobenzaldehyde (0.04 g, 0.32 mmol) and sodium cyanoborohydride (0.02 g, 0.32 mmol) to afford **BD_30** (0.06 g, 46.93 %) as yellow solid. M.p: 221-223 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.53-7.47 (m, 7H), 6.23 (b, 1H), 3.54 (s, 2H), 3.14-1.80 (m, 9H). ^{13}C NMR (DMSO-d_6): δ_C 167.5, 159.6, 145.4, 143.7, 138.5 (2C), 132.7, 130.6, 130.4 (2C), 128.6, 128.4 (2C), 123.2, 58.4, 52.7, 41.5 (2C), 30.4 (2C). ESI-MS *m/z* 431 $[M+H]^+$. Anal.Calcd.For $C_{20}H_{19}ClN_4O_3S$: C, 55.75; H, 4.44; N, 13.00; Found: C, 55.73; H, 4.46; N, 13.02.

2-(4-((4-Methylbenzyl)amino)piperidin-1-yl)-6-nitro-4H-benzo[e][1,3]thiazin-4-one (BD_31). The compound was synthesized according to the general procedure using 2-(4-aminopiperidin-1-yl)-6-nitro-4*H*-benzo[*e*][1,3]thiazin-4-one (**BD_04a**) (0.1 g, 0.32 mmol), 4-tolualdehyde (0.04 g, 0.32 mmol) and sodium cyanoborohydride (0.02 g, 0.32 mmol) to afford **BD_31** (0.05 g, 39.56 %) as yellow solid. M.p: 197-199 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.55-7.44 (m, 7H), 6.21 (b, 1H), 3.54 (s, 2H), 3.12-1.78 (m, 9H), 2.98 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 167.5, 159.4, 145.5, 143.6, 138.5, 137.7, 136.6, 130.4, 129.6 (2C), 128.4, 128.2 (2C), 123.7, 58.5, 52.3, 41.6 (2C), 30.3 (2C), 21.4. ESI-MS *m/z* 411 $[M+H]^+$. Anal.Calcd.For $C_{21}H_{22}N_4O_3S$: C, 61.44; H, 5.40; N, 13.65; Found: C, 61.46; H, 5.37; N, 13.66.

6-Nitro-2-(4-((4-nitrobenzyl)amino)piperidin-1-yl)-4H-benzo[e][1,3]thiazin-4-one (BD_32). The compound was synthesized according to the general procedure using 2-(4-aminopiperidin-1-yl)-6-nitro-4*H*-benzo[*e*][1,3]thiazin-4-one (**BD_04a**) (0.1 g, 0.32 mmol), 4-Nitro benzaldehyde (0.05 g, 0.32 mmol) and sodium cyanoborohydride (0.02 g, 0.32 mmol) to afford **BD_32** (0.05 g, 39.56 %) as yellow solid. M.p: 205-207 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.58-7.47 (m, 7H), 6.22 (b, 1H), 3.56 (s, 2H), 3.11-1.79 (m, 9H). ^{13}C NMR (DMSO-d_6): δ_C 167.4, 159.3, 146.5, 146.4, 145.5, 143.7, 138.6, 130.4, 128.6, 128.4 (2C), 123.4, 123.2 (2C), 58.6, 52.2, 41.6 (2C), 30.4 (2C). ESI-MS *m/z* 442 $[M+H]^+$. Anal.Calcd.For $C_{20}H_{19}N_5O_5S$: C, 54.41; H, 4.34; N, 15.86; Found: C, 54.39; H, 4.31; N, 15.88.

2-(4-(Benzylamino)piperidin-1-yl)-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (BD_33). The compound was synthesized according to the general procedure using 2-(4-aminopiperidin-1-yl)-6-(trifluoromethyl)-4*H*-benzo[*e*][1,3]thiazin-4-one (**BD_04b**) (0.1 g, 0.30 mmol), benzaldehyde (0.03 g, 0.30 mmol) and sodium cyanoborohydride (0.02 g, 0.30 mmol) to afford **BD_33** (0.06 g, 51.83 %) as white solid. M.p: 179-181 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.60-7.52 (m, 8H), 6.23 (b, 1H), 3.54 (s, 2H), 3.14-1.82 (m, 9H). ^{13}C NMR (DMSO-d_6): δ_C 167.2, 159.5, 140.5, 140.4, 137.5, 131.7, 130.6, 128.4 (2C), 128.1, 127.4 (2C), 127.2, 126.2, 123.5, 58.8, 52.4, 41.7 (2C), 30.2 (2C). ESI-MS *m/z* 420 $[M+H]^+$. Anal.Calcd.For $C_{21}H_{20}F_3N_3OS$: C, 60.13; H, 4.81; N, 10.02; Found: C, 60.09; H, 4.83; N, 10.01.

2-(4-((4-Chlorobenzyl)amino)piperidin-1-yl)-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (BD_34). The compound was synthesized according to the general procedure using 2-(4-aminopiperidin-1-yl)-6-(trifluoromethyl)-4*H*-benzo[*e*][1,3]thiazin-4-one (**BD_04b**) (0.1 g, 0.30 mmol), 4-chloro benzaldehyde (0.04 g, 0.30 mmol) and sodium cyanoborohydride (0.02 g, 0.30 mmol) to afford **BD_34** (0.08 g, 63.86 %) as white solid. M.p: 193-195 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.59-7.51 (m, 7H), 6.24 (b, 1H), 3.55 (s, 2H), 3.16-1.80 (m, 9H). ^{13}C NMR (DMSO-d_6): δ_C 167.5, 159.5, 140.5, 138.4, 137.5, 132.7, 131.6, 130.4 (2C), 130.1, 128.4 (2C), 128.2, 126.2, 123.4, 58.7, 52.2, 41.6 (2C), 30.3 (2C). ESI-MS *m/z* 454 $[M+H]^+$. Anal.Calcd.For $C_{21}H_{19}ClF_3N_3OS$: C, 55.57; H, 4.22; N, 9.26; Found: C, 55.59; H, 4.19; N, 9.25.

2-(4-((4-Methylbenzyl)amino)piperidin-1-yl)-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (BD_35). The compound was synthesized according to the general procedure using 2-(4-aminopiperidin-1-yl)-6-(trifluoromethyl)-4*H*-benzo[*e*][1,3]thiazin-4-one (**BD_04b**) (0.1 g,

0.30 mmol), 4-tolualdehyde (0.03 g, 0.30 mmol) and sodium cyanoborohydride (0.02 g, 0.30 mmol) to afford **BD_35** (0.06 g, 48.63 %) as white solid. M.p: 183-185 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.61-7.54 (m, 7H), 6.22 (b, 1H), 3.52 (s, 2H), 3.14-1.83 (m, 9H), 2.98 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 167.5, 159.3, 140.4, 137.4, 137.1, 136.7, 131.5, 130.4, 129.1 (2C), 128.7 (2C), 128.2, 126.4, 123.7, 58.6, 52.3, 41.6 (2C), 30.1 (2C), 21.4. ESI-MS *m/z* 434 $[M+H]^+$. Anal.Calcd.For $C_{22}H_{22}F_3N_3OS$: C, 60.96; H, 5.12; N, 9.69; Found: C, 60.99; H, 5.15; N, 9.66.

2-(4-((4-Nitrobenzyl)amino)piperidin-1-yl)-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (BD_36). The compound was synthesized according to the general procedure using 2-(4-aminopiperidin-1-yl)-6-(trifluoromethyl)-4*H*-benzo[*e*][1,3]thiazin-4-one (**BD_04b**) (0.1 g, 0.30 mmol), 4-nitrobenzaldehyde (0.04 g, 0.30 mmol) and sodium cyanoborohydride (0.02 g, 0.30 mmol) to afford **BD_36** (0.06 g, 48.22 %) as white solid. M.p: 227-229 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.58-7.52 (m, 7H), 6.20 (b, 1H), 3.54 (s, 2H), 3.14-1.83 (m, 9H). ^{13}C NMR (DMSO-d_6): δ_C 167.7, 159.3, 146.4, 146.2, 140.1, 137.7, 131.5, 130.2, 128.7 (2C), 128.4, 126.2, 123.4 (3C), 58.5, 52.4, 41.8 (2C), 30.1 (2C). ESI-MS *m/z* 465 $[M+H]^+$. Anal.Calcd.For $C_{21}H_{19}F_3N_4O_3S$: C, 54.30; H, 4.12; N, 12.06; Found: C, 54.28; H, 4.16; N, 12.05.

2-(4-(Benzylamino)piperidin-1-yl)-6-chloro-4H-benzo[e][1,3]thiazin-4-one (BD_37). The compound was synthesized according to the general procedure using 2-(4-aminopiperidin-1-yl)-6-chloro-4*H*-benzo[*e*][1,3]thiazin-4-one (**BD_04c**) (0.1 g, 0.33 mmol), benzaldehyde (0.03 g, 0.33 mmol) and sodium cyanoborohydride (0.02 g, 0.33 mmol) to afford **BD_37** (0.07 g, 55.20 %) as white solid. M.p: 189-191 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.63-7.55 (m, 8H), 6.24 (b, 1H), 3.56 (s, 2H), 3.12-1.81 (m, 9H). ^{13}C NMR (DMSO-d_6): δ_C 167.5, 159.2, 140.4, 138.2, 135.1, 134.7, 131.5, 131.2, 130.7, 128.4 (2C), 127.2 (2C), 127.4, 58.4, 52.6, 41.7 (2C), 30.3 (2C). ESI-MS *m/z* 386 $[M+H]^+$. Anal.Calcd.For $C_{20}H_{20}ClN_3OS$: C, 62.25; H, 5.22; N, 10.89; Found: C, 62.23; H, 5.23; N, 10.88.

6-Chloro-2-(4-((4-chlorobenzyl)amino)piperidin-1-yl)-4H-benzo[e][1,3]thiazin-4-one (BD_38). The compound was synthesized according to the general procedure using 2-(4-aminopiperidin-1-yl)-6-chloro-4*H*-benzo[*e*][1,3]thiazin-4-one (**BD_04c**) (0.1 g, 0.33 mmol), 4-chlorobenzaldehyde (0.05 g, 0.33 mmol) and sodium cyanoborohydride (0.02 g, 0.33 mmol) to afford **BD_38** (0.07 g, 51.38 %) as white solid. M.p: 204-206 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.60-7.54 (m, 7H), 6.24 (b, 1H), 3.55 (s, 2H), 3.11-1.82 (m, 9H). ^{13}C NMR (DMSO-d_6): δ_C 167.6, 159.4, 138.4, 138.2, 135.4, 134.6, 132.5, 131.3, 131.1, 130.4 (2C),

130.2, 128.4 (2C), 58.5, 52.3, 41.7 (2C), 30.2 (2C). ESI-MS *m/z* 421 $[M+H]^+$. Anal.Calcd.For $C_{20}H_{19}Cl_2N_3OS$: C, 57.15; H, 4.56; N, 10.00; Found: C, 57.17; H, 4.55; N, 10.03.

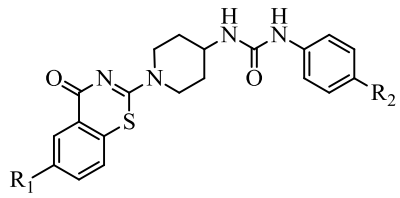
6-Chloro-2-(4-((4-methylbenzyl)amino)piperidin-1-yl)-4H-benzo[e][1,3]thiazin-4-one (BD_39). The compound was synthesized according to the general procedure using 2-(4-aminopiperidin-1-yl)-6-chloro-4*H*-benzo[*e*][1,3]thiazin-4-one (**BD_04c**) (0.1 g, 0.33 mmol), 4-tolualdehyde (0.04 g, 0.33 mmol) and sodium cyanoborohydride (0.02 g, 0.33 mmol) to afford **BD_39** (0.04 g, 35.51 %) as white solid. M.p: 216-218 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.62-7.52 (m, 7H), 6.22 (b, 1H), 3.52 (s, 2H), 3.12-1.81 (m, 9H), 2.98 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 167.5, 159.2, 138.5, 137.2, 136.4, 135.6, 134.5, 131.4, 131.2, 130.1, 129.4 (2C), 128.2 (2C), 58.4, 52.6, 41.6 (2C), 30.1 (2C), 21.3. ESI-MS *m/z* 400 $[M+H]^+$. Anal.Calcd.For $C_{21}H_{22}ClN_3OS$: C, 63.07; H, 5.54; N, 10.51; Found: C, 63.08; H, 5.53; N, 10.49.

6-Chloro-2-(4-((4-nitrobenzyl)amino)piperidin-1-yl)-4H-benzo[e][1,3]thiazin-4-one (BD_40). The compound was synthesized according to the general procedure using 2-(4-aminopiperidin-1-yl)-6-chloro-4*H*-benzo[*e*][1,3]thiazin-4-one (**BD_04c**) (0.1 g, 0.33 mmol), 4-nitrobenzaldehyde (0.05 g, 0.33 mmol) and sodium cyanoborohydride (0.02 g, 0.33 mmol) to afford **BD_40** (0.05 g, 39.82 %) as white solid. M.p: 201-203 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.64-7.56 (m, 7H), 6.23 (b, 1H), 3.56 (s, 2H), 3.12-1.81 (m, 9H). ^{13}C NMR (DMSO-d_6): δ_C 167.4, 159.3, 146.5, 146.2, 138.4, 135.4, 134.9, 131.6, 131.1, 130.4, 128.8 (2C), 123.7 (2C), 58.4, 52.2, 41.4 (2C), 30.2 (2C). ESI-MS *m/z* 431 $[M+H]^+$. Anal. Calcd.For $C_{20}H_{19}ClN_4O_3S$: C, 55.75; H, 4.44; N, 13.00; Found: C, 55.77; H, 4.43; N, 13.01.

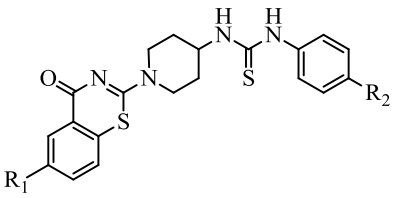
5.1b.4. In-vitro *Mycobacterium tuberculosis* supercoiling assay, antimycobacterial potency and cytotoxicity studies of the synthesized molecules

All the synthesized derivatives were first evaluated for their in-vitro *Mycobacterium tuberculosis* supercoiling assay as steps towards the derivation of SAR and hit optimization. The compounds were further subjected to a whole cell screening against *Mycobacterium tuberculosis* H37Rv strain to understand their bactericidal potency using the MABA assay and later the safety profile of these molecules were evaluated by checking the in-vitro cytotoxicity against RAW 264.7 cell line (mouse macrophage) by MTT assay, and the results are tabulated in **Table 5.4**.

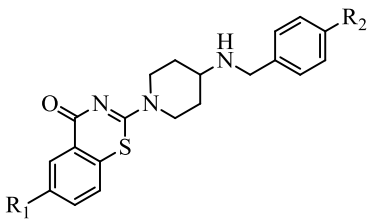
Table 5.4: In-vitro biological evaluation of the synthesized derivatives **BD_05 - BD_40.**



BD_05 - BD_16



BD_17 - BD_28



BD_29 - BD_40

Comp	R_1	R_2	MTB Supercoiling assay (IC_{50}) µM	MTB MIC µM	Cytotoxicity[a] % inhibition
BD_05	NO_2	H	12.16±0.38	24.37	ND
BD_06	NO_2	Cl	3.12±0.27	6.75	18.12
BD_07	NO_2	CH_3	3.95±0.22	7.12	16.16
BD_08	NO_2	NO_2	5.82±0.31	6.60	2.56
BD_09	CF_3	H	10.72±0.43	27.87	ND
BD_10	CF_3	Cl	6.88±0.33	6.47	13.56
BD_11	CF_3	CH_3	5.47±0.19	6.47	25.46
BD_12	CF_3	NO_2	18.36±0.72	18.99	15.68
BD_13	Cl	H	11.57±0.68	15.06	28.65
BD_14	Cl	Cl	9.25±0.37	6.94	12.56
BD_15	Cl	CH_3	23.96±0.71	58.28	ND
BD_16	Cl	NO_2	10.95±0.72	27.17	ND
BD_17	NO_2	H	3.12±0.44	14.15	11.56
BD_18	NO_2	Cl	5.76±0.24	6.56	12.56
BD_19	NO_2	CH_3	9.87±0.37	27.43	ND
BD_20	NO_2	NO_2	4.95±0.42	3.20	7.89
BD_21	CF_3	H	8.77±0.29	26.90	ND
BD_22	CF_3	Cl	8.92±0.22	25.05	ND
BD_23	CF_3	CH_3	11.54±0.38	13.06	4.56
BD_24	CF_3	NO_2	4.27±0.32	12.26	5.23

Contd.

Comp	R_1	R_2	MTB Supercoiling assay (IC_{50}) µM	MTB MIC µM	Cytotoxicity[a] % inhibition
BD_25	Cl	H	12.97±0.56	14.44	25.63
BD_26	Cl	Cl	5.53±0.36	26.65	ND
BD_27	Cl	CH_3	6.60±0.44	28.08	ND
BD_28	Cl	NO_2	13.38±0.69	13.12	26.58
BD_29	NO_2	H	10.84±0.71	31.52	ND
BD_30	NO_2	Cl	6.13±0.66	14.50	28.65
BD_31	NO_2	CH_3	10.54±0.37	15.20	29.65
BD_32	NO_2	NO_2	4.65±0.31	14.15	3.25
BD_33	CF_3	H	2.91±0.22	14.90	2.56
BD_34	CF_3	Cl	2.85±0.26	27.53	5.93
BD_35	CF_3	CH_3	0.86±0.13	2.88	4.33
BD_36	CF_3	NO_2	2.93±0.19	6.70	6.58
BD_37	Cl	H	0.91±0.12	2.41	8.95
BD_38	Cl	Cl	2.45±0.14	3.71	18.95
BD_39	Cl	CH_3	2.66±0.22	5.20	6.35
BD_40	Cl	NO_2	2.19±0.24	1.61	28.56
Novobiocin			0.068±0.04	>200	9.36

MTB=*Mycobacterium tuberculosis*, [a] at 50 µM against RAW 264.7 cells, ND indicates not determined.

5.1b.5. Discussion

DNA Gyrase assay was performed using *Mycobacterium tuberculosis* DNA gyrase kit from Inspiralis, Norwich. P. [Shirude S., *et al.*, 2012]. The assay was performed for all the thirty six synthesized compounds preliminary at an inhibitor concentrations of 500, 125, 31.3, 7.8, 1.95 µM respectively, subsequently the active ones were further tested at 250, 62.5, 15.6, 3.9 and 0.97 µM concentrations, the final molecules showing more than 60% activity at 0.97 µM were further screened at lower concentrations of 0.48 to ensure the activity profile of the best compounds. Among the entire series of fourteen compounds **BD_06**, **BD_07**, **BD_17**, **BD_20**, **BD_24**, **BD_32**, **BD_33**, **BD_34**, **BD_35**, **BD_36**, **BD_37**, **BD_38**, **BD_39** and **BD_40** showed an IC_{50} of less than 5 µM. While the most active compound **BD_35** had an

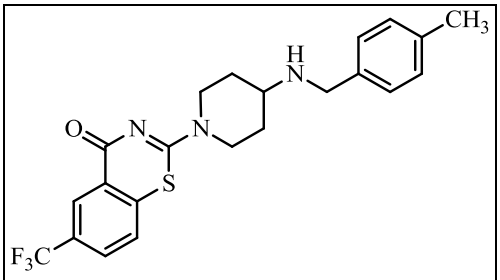
IC_{50} of 0.86±0.13 µM with an electronegative imparting trifluromethyl group in the R_1 position and a methyl group at the R_2 position. Closer analysis shows that compounds from **BD_33 - BD_40** have a better in-vitro enzymes inhibition profiles associated with the in-vitro MIC inhibitions. Substitution of 2-(4-(benzylamino)piperidin-1-yl)-4H-benzo[e][1,3]thiazin-4-one at R1 position with trifluoromethyl or chlorine groups and on subsequent R2 position with hydrogen, chloro, methyl and nitro groups favors the DNA gyrase inhibition and mycobacterial inhibition too. All through the assay novobicin and moxifloxacin were considered as positive controls as they are potent inhibitors of DNA supercoiling of *Mycobacterium tuberculosis* DNA gyrase. Except for few of the compounds like **BD_05, BD_12, BD_13, BD_15, BD_23, BD_25** and **BD_28** others exhibited much better enzyme supercoiling inhibitions when compared to standard moxifloxacin drug whose IC_{50} was 11.2±0.61 µM, furthermore novobiocin had an IC_{50} of 0.068±0.04 µM considered as one of the potent DNA gyrase inhibitor till date, but has a limitation of cytotoxic to eukaryotic cells. A dose dependent inhibitory profile of the most active compound **BD_35** at different inhibitor concentrations along with standard novobiocin was performed.

All the synthesized benzothiazinone derivatives were further screened for their in-vitro antimycobacterial activity against *Mycobacterium tuberculosis* H37Rv strain by microplate Alamar blue MABA assay [Batt S. M., *et al*., 2012]. Furthermore, as the synthesized compounds were mostly hydrophilic, the in-vitro antimycobacterial inhibitions too were in commendable range of MIC between 1-59 µM. Throughout the assay ethambutol (MIC: 15.31 µM), isoniazid (MIC: 0.66 mM), moxifloxacin (MIC: 1.2 µM) and novobiocin (MIC: >200 µM) were considered as standards. Many compounds had a better inhibitory profile than ethambutol while only compound **BD_40** showed a relative inhibitory potential as that of moxifloxacin [Shirude P. S., *et al*., 2013]. The potent compound **BD_35** had an MIC of 2.88 µM. Two other compounds **BD_37** and **BD_40** exhibited greater antimycobacterial inhibitory profiles than that of the potent analogue **BD_35**, attributing these drug inhibitions towards the DprE1 enzyme [Batt S. M., *et al*., 2012] too thus concluding these as multitarget enzyme inhibitors. Compared to first line antitubercular drugs like ethambutol compound **BD_40** showed better inhibitory activity and was relatively comparable to the standard isoniazid.These compounds can be promising antitubercular leads with best in-vitro DNA gyrase enzyme inhibitory profile too.

Few of the synthesized compounds with better MIC values were subsequently tested for their eukaryotic cell safety profile by in-vitro MTT assay in RAW 264.7 cell lines (Mouse leukemic monocyte macrophage cell line) at 50 µM concentration [Shirude P. S., *et al*., 2013; Ferrari M., *et al*., 1990; Jeankumar U. V., *et al*., 2014]. The assay was performed byusing MTT dye. The compounds showed less cytotoxicity within a range of 2-30 % inhibitions as shown in (**Table 5.4)**. Compound **BD_31** showed highest toxicity of 29.65% among the determined drugs whereas **BD_08** and **BD_33** showed least cytotoxicity of 2.56%. The most promising anti-TB compound **BD_35** had 4.3% cytotoxicity which is within the safety profile limit. Novobiocin was used as standard with 9.36% inhibition.

5.1b.6. Highlights of the study

Combining molecular hybridization strategy, 36 compounds synthesized were evaluated for their biological studies, therefore we successfully re-engineered few previously reported antibacterial leads along with experimental characterization to develop a new class of mycobacterial DNA gyrase inhibitors possessing certain attributes like synthetic feasibility, traceable structure activity relationship, excellent in-vitro biological enzyme inhibitions and anti-tubercular activity in H37Rv strain. Because of ever-increasing urge for newer antibiotics and with urgent requirement of new anti-TB agents consequently, we believe that the present benzothiazinone class of DNA gyrase inhibitors reported, provides an interesting potential for further optimization. Among the synthesised compounds lead compound **BD_35** shows promising results with IC_{50} of 0.86 µM in supercoiling assay and also good IC_{50} of 2.88 µM in MABA assay on sensitive strains of *Mycobacterium tuberculosis* (**Figure 5.9**). The lead was also found to be nontoxic as the percentage inhibition in RAW cells was found to be 4.33.



Compound **BD_35**
MTB MIC = 2.88 µM
Supercoiling assay IC_{50} = 0.86 µM

Figure 5.9: Structure and activity of most active compound **BD_35**.

5.1c. Development of benzothiazinone-piperazine derivatives as efficient *Mycobacterium tuberculosis* DNA Gyrase Inhibitors

In the present work we initiated drug discovery program through molecular hybridization targeting DNA gyrase of *Mycobacterium tuberculosis* and here we describe the design and development of benzothiazinone hybrids as novel compounds displaying unique activity against *Mycobacterium tuberculosis* DNA gyrase with promising anti-tubercular activity in-vitro.

Molecular hybridization was used for drug design and development where fusion/hybridization of two or more pharmacophoric subunits occurs from the molecular structure of ligands/prototypes previously reported to have an inhibitory effect against the targeted disease. This newly designed hybrid can lead to compounds with improved affinity and efficacy with low side effects than the parent template compounds, while retaining the desired characteristics of the original template (**Figure 5.10**). Encouraged by our previous successful research efforts in this regard [Sacksteder, K. A., *et al*., 2012; Leach, K. L., *et al*., 2011], it was decided to further extend the above methodology to identify novel starting points to design inhibitors for ATPase domain of mycobacterial gyrase.

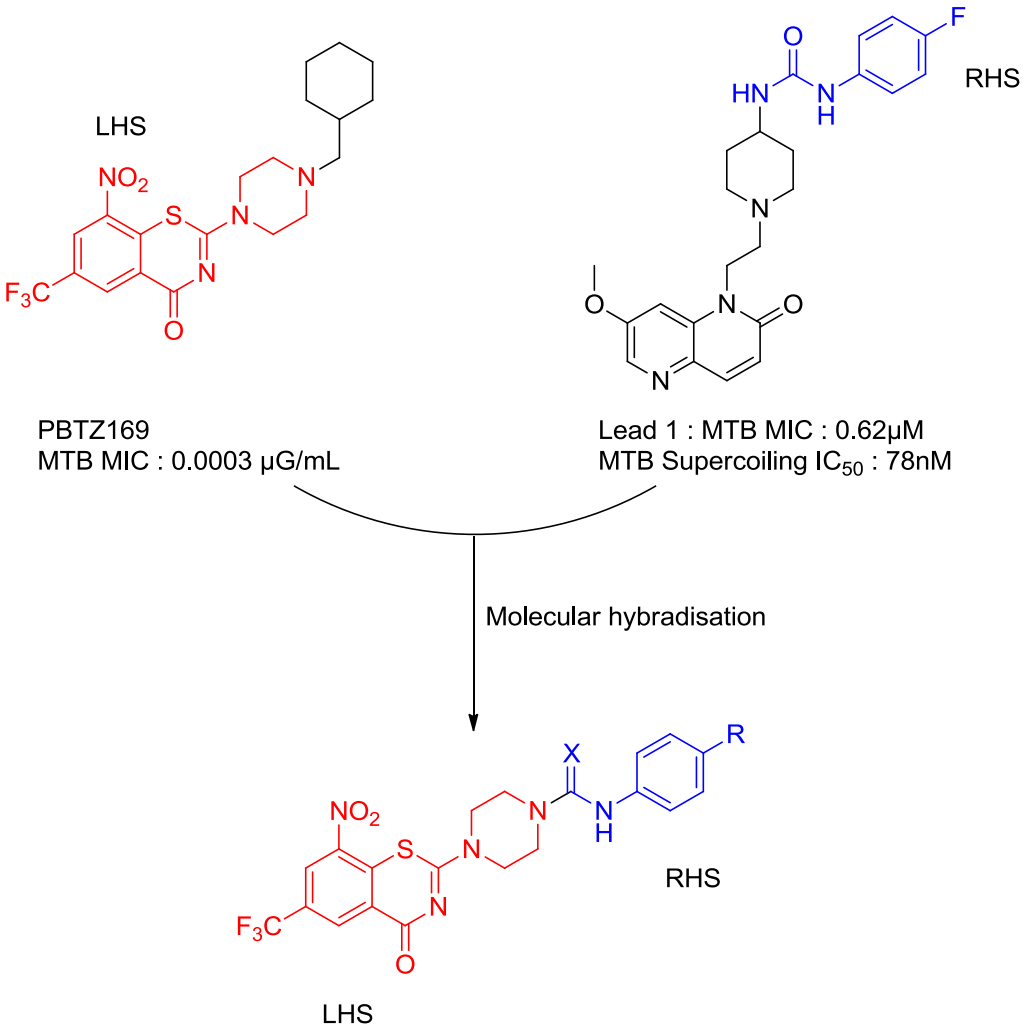


Figure 5.10: Strategy employed for designing the lead. Chemical structure of previously reported anti-tubercular benzothiazinone bearing (left hand side) derivative PBTZ169 and *Mycobacterium tuberculosis* DNA gyrase inhibitor bearing aryl (thio) urea right hand side chain and the inhibitor designed through molecular hybridization.

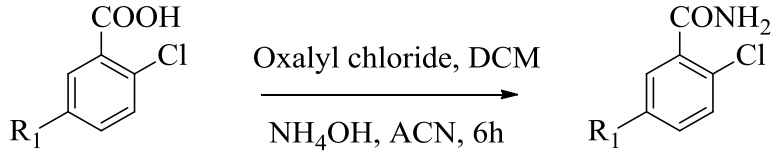
5.1c.1. Chemical synthesis

The synthetic pathway used to achieve the target compounds has been delineated in **Figure 4.3**. Synthesis of the compounds started with conversion of commercially available substituted 2-chlorobenzoic acids into corresponding 2-chlorobenzoyl chlorides by DMF-catalyzed treatment in presence of oxalyl chloride in dichloromethane. The obtained 2-chlorobenzoyl chlorides were converted into corresponding amide intermediate by dropwise addition of 25% aqueous ammonia at -20° C.The amide intermediates were further treated with carbon disulphide, methyl iodide and sodium hydroxide in DMSO to afford the thio-alkylated products, which upon treatment with 1-Boc-piperazine in ethanol followed by

deprotection using trifluoroacetic acid gave the scaffolds in good yield. The final library was then assembled by treating the obtained scaffolds with the desired isocyanates/isothiocyantes and aldehydes to afford compound **BZ_05 - BZ_40** in excellent yields. A series of 36 derivatives were synthesized using the above method in excellent yields.

5.1c.2. Experimental protocol utilized for synthesis

General procedure for the synthesis of 5-substituted-2-chlorbenzamides **(BZ_02a-c).**To a stirred solution of the corresponding acid (**BZ_01a-c**) (1 mmol) in dichloromethane (15 mL) at -10°C was added oxalyl chloride (2.5 mmol). The solution was refluxed for about 6 hours (monitored by TLC & LCMS for completion), and solvent evaporated under reduced pressure. The residue was further diluted with acetonitrile (30 mL), cooled to -20°C and added ammonium hydroxide solution dropwise and allowed to stirred for 30 minutes. The resulting solid was filtered out to afford the corresponding amide (**BZ_02a-c**) in good yield.



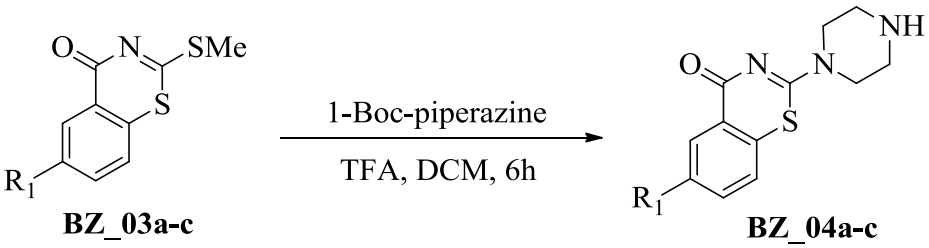
BZ_01a-c **BZ_02a-c**

General procedure for the synthesis of 5-substituted-2-(methylthio)-4H-benzo[e][1,3]thiazin-4-one **(BZ_03a-c).** To a stirred solution of the corresponding benzamide (**BZ_02a-c**) (1 mmol) in DMSO (15 mL) at 10°C was added carbon disulphide (3 mmol), sodium hydroxide (2 mmol), and the mixture was allowed to stand for 15 minutes. Subsequently methyl iodide (1.2 mmol) was added. The reaction mixture was allowed to stand for another 30 minutes, and 50 mL of water was added. The resulting white solid separated by filtration to afford the corresponding benzothiazinone (**BZ_03a-c**) in good yield.

BZ_02a-c **BZ_03a-c**

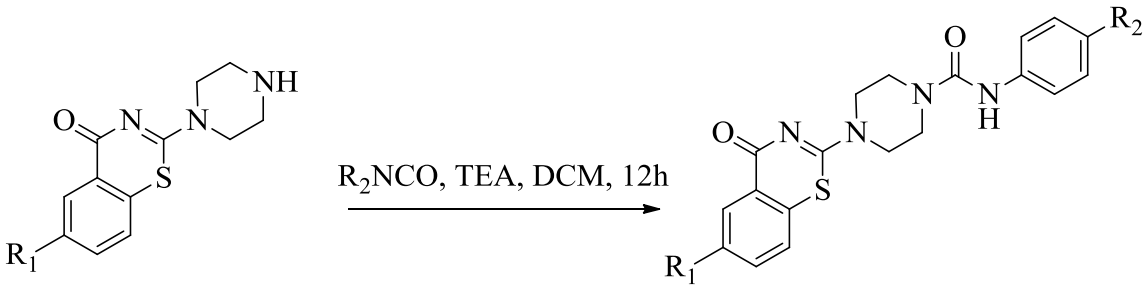
General procedure for the synthesis of 6-substitutedo-2-(piperazin-1-yl)-4H-benzo[e][1,3]thiazin-4-ones (BZ_04a-c). To a stirred solution of the corresponding benzothiazinone (**BZ_03a**) (1 mmol) in ethanol (15 mL) at room temperature was added 1-

Boc-piperazine (1 mmol). The solution was refluxed for about 12 hours (monitored by TLC & LCMS for completion), and solvent evaporated under reduced pressure. The residue was further diluted with water (30 mL) and ethyl acetate (50 mL) and the layers were separated. The organic layer was dried over anhydrous sodium sulphate and evaporated under reduced pressure. The residue was purified by silica gel column chromatography using hexane: ethylacetate as eluent to give the corresponding *N*-boc protected piperazine benzothiazinones in good yield; which was taken in dichloromethance (60 mL) was cooled to 0 ^{o}C was added trifluoroacetic acid (8 mL) and stirred the reaction at room temperature for 1 hour. After completion of the reaction by TLC, the reaction mixture was cooled to 0 ^{o}C and basified to pH ~8.0 using saturated aqueous $NaHCO_3$ solution. The organic layer was separated, washed with water (2 x 20 mL) and brine (1 x 20 mL) and dried over anhydrous sodium sulfate. The organic layer was concentrated under vacuum afforded the free amine as pale brown oil. The crude material was used for final reactions without purification.



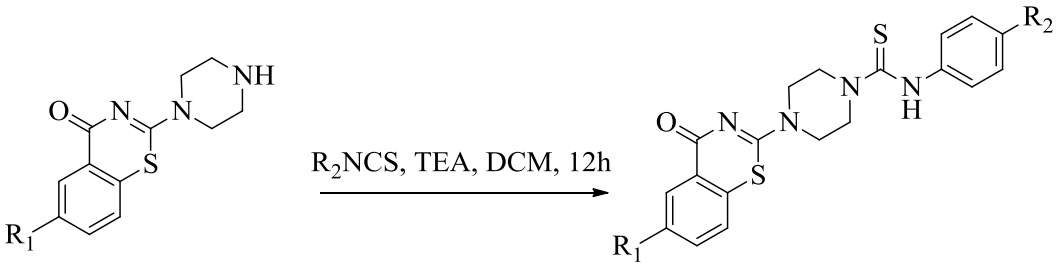
BZ_03a-c **BZ_04a-c**

General procedure for the synthesis of 4-(6-substituted-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)-N-arylpiperazine-1-carboxamide derivatives (BZ_05 - BZ_16). To a cooled solution of 6-substituted-2-(piperazin-1-yl)-4H-benzo[e][1,3]thiazin-4-one (1 mmol) in anhydrous DCM (2 mL) was added corresponding isocyanate (1 mmol), triethylamine (1mmol) and stirred the reaction mixture at room temperature for 12 hours (monitored by TLC & LCMS for completion), and solvent evaporated under reduced pressure. The residue was further diluted with water (30 mL) and ethyl acetate (50 mL) and the layers were separated. The organic layer was dried over anhydrous sodium sulphate and evaporated under reduced pressure. The residue was purified by silica gel column chromatography using hexane: ethylacetate as eluent to give the corresponding urea derivative (**BZ_05 - BZ_16**) in good yield.



BZ_04a-c **BZ_05 - BZ_16**

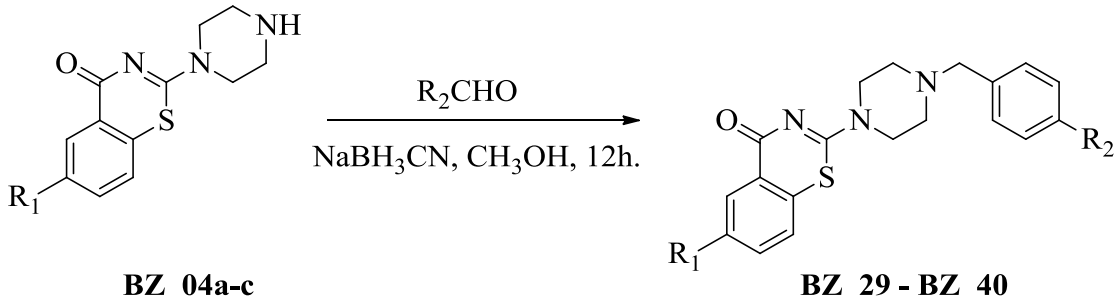
General procedure for the synthesis of 4-(6-substituted-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)-N-arylpiperazine-1-carbothioamide derivatives (BZ_17 - BZ_28). To a cooled solution of 6-substituted-2-(piperazin-1-yl)-4H-benzo[e][1,3]thiazin-4-one (1 mmol) in anhydrous DCM (2 mL) was added corresponding isothiocyanate (1 mmol), triethylamine (1 mmol) and stirred the reaction mixture at room temperature for 12 hours (monitored by TLC & LCMS for completion), and solvent evaporated under reduced pressure. The residue was further diluted with water (30 mL) and ethyl acetate (50 mL) and the layers were separated. The organic layer was dried over anhydrous sodium sulphate and evaporated under reduced pressure. The residue was purified by silica gel column chromatography using hexane: ethylacetate as eluent to give the corresponding thiourea derivative (**BZ_17 - BZ_28**) in good yield.



BZ_04a-c **BZ_17 - BZ_28**

General procedure for the synthesis of 2-(4-benzylpiperazin-1-yl)-6-substituted-4H-benzo[e][1,3]thiazin-4-one derivatives (BZ_29 - BZ_40). To a cooled solution of 6-substituted-2-(piperazin-1-yl)-4H-benzo[e][1,3]thiazin-4-one (1 mmol) in methanol (2 mL) was added aldehyde (1 mmol) and sodium cyanoborohydride (1 mmol) and stirred the reaction mixture at room temperature for 12 hours (monitored by TLC & LCMS for completion), and solvent evaporated under reduced pressure. The residue was further diluted with water (30 mL) and ethyl acetate (50 mL) and the layers were separated. The organic layer was dried over anhydrous sodium sulphate and evaporated under reduced pressure. The

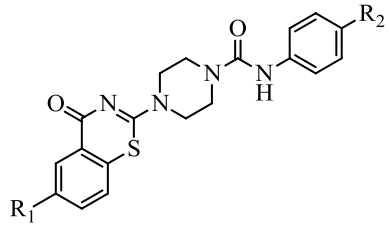
residue was purified by silica gel column chromatography using hexane: ethylacetate as eluent to give the corresponding final derivative (**BZ_29 - BZ_40**) in good yield.



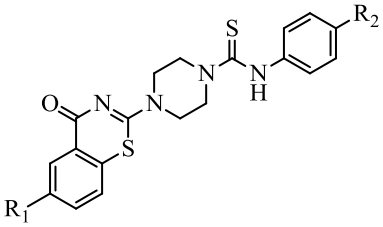
BZ_04a-c **BZ_29 - BZ_40**

The physicochemical properties of synthesized derivatives are shown in **Table 5.1**.

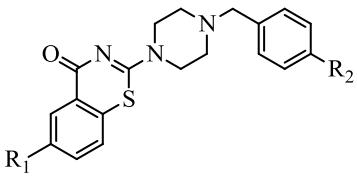
Table 5.5: Physicochemical properties of synthesized compounds **BZ_05 - BZ_40**.



BZ_05 - BZ_16



BZ_17 - BZ_28



BZ_29 - BZ_40

Compd	R_1	R_2	Yield (%)	M.P. (°C)	Molecular formula	Molecular weight
BZ_05	NO_2	H	46	192-194	$C_{19}H_{17}N_5O_4S$	411.43
BZ_06	NO_2	Cl	53	203-205	$C_{19}H_{16}ClN_5O_4S$	445.88
BZ_07	NO_2	CH_3	36	201-203	$C_{20}H_{19}N_5O_4S$	425.46
BZ_08	NO_2	NO_2	15	186-188	$C_{19}H_{16}N_6O_6S$	456.43
BZ_09	CF_3	H	62	182-184	$C_{20}H_{17}F_3N_4O_2S$	434.43
BZ_10	CF_3	Cl	35	193-195	$C_{20}H_{16}ClF_3N_4O_2S$	468.88
BZ_11	CF_3	CH_3	32	197-199	$C_{21}H_{19}F_3N_4O_2S$	448.46
BZ_12	CF_3	NO_2	26	215-217	$C_{20}H_{16}F_3N_5O_4S$	479.43
BZ_13	Cl	H	34	199-201	$C_{19}H_{17}ClN_4O_2S$	400.88
BZ_14	Cl	Cl	37	206-208	$C_{19}H_{16}Cl_2N_4O_2S$	435.33
BZ_15	Cl	CH_3	45	221-223	$C_{20}H_{19}ClN_4O_2S$	414.91
BZ_16	Cl	NO_2	52	197-199	$C_{19}H_{16}ClN_5O_4S$	445.88

Contd.

Compd	R_1	R_2	Yield (%)	M.P. (°C)	Molecular formula	Molecular weight
BZ_17	NO_2	H	47	205-207	$C_{19}H_{17}N_5O_3S_2$	427.5
BZ_18	NO_2	Cl	46	179-181	$C_{19}H_{16}ClN_5O_3S_2$	461.95
BZ_19	NO_2	CH_3	48	183-185	$C_{20}H_{19}N_5O_3S_2$	441.53
BZ_20	NO_2	NO_2	27	217-219	$C_{19}H_{16}N_6O_5S_2$	472.5
BZ_21	CF_3	H	41	221-223	$C_{20}H_{17}F_3N_4OS_2$	450.5
BZ_22	CF_3	Cl	40	193-195	$C_{20}H_{16}ClF_3N_4OS_2$	484.95
BZ_23	CF_3	CH_3	45	183-185	$C_{21}H_{19}F_3N_4OS_2$	464.53
BZ_24	CF_3	NO_2	52	227-229	$C_{20}H_{16}F_3N_5O_3S_2$	495.5
BZ_25	Cl	H	34	189-191	$C_{19}H_{17}ClN_4OS_2$	416.95
BZ_26	Cl	Cl	24	201-203	$C_{19}H_{16}Cl_2N_4OS_2$	451.39
BZ_27	Cl	CH_3	48	217-219	$C_{20}H_{19}ClN_4OS_2$	430.97
BZ_28	Cl	NO_2	32	189-191	$C_{19}H_{16}ClN_5O_3S_2$	461.95
BZ_29	NO_2	H	35	208-210	$C_{19}H_{18}N_4O_3S$	382.44
BZ_30	NO_2	Cl	46	204-206	$C_{19}H_{17}ClN_4O_3S$	416.88
BZ_31	NO_2	CH_3	39	216-218	$C_{20}H_{20}N_4O_3S$	396.46
BZ_32	NO_2	NO_2	39	226-228	$C_{19}H_{17}N_5O_5S$	427.43
BZ_33	CF_3	H	65	213-215	$C_{20}H_{18}F_3N_3OS$	405.44
BZ_34	CF_3	Cl	63	211-213	$C_{20}H_{17}ClF_3N_3OS$	439.88
BZ_35	CF_3	CH_3	48	186-188	$C_{21}H_{20}F_3N_3OS$	419.46
BZ_36	CF_3	NO_2	59	211-213	$C_{20}H_{17}F_3N_4O_3S$	450.43
BZ_37	Cl	H	55	211-213	$C_{19}H_{18}ClN_3OS$	371.88
BZ_38	Cl	Cl	51	215-217	$C_{19}H_{17}Cl_2N_3OS$	406.33
BZ_39	Cl	CH_3	50	222-224	$C_{20}H_{20}ClN_3OS$	385.91
BZ_40	Cl	NO_2	53	192-194	$C_{19}H_{17}ClN_4O_3S$	416.88

5.1c.3. Characterization of synthesized compounds

A series of 36 derivatives werepreparedusing the above method and both analytical and spectral data (^{1}H NMR, ^{13}C NMR, and mass spectra) of all the synthesized compounds were in full agreement with the proposed structures.

2-Chloro-5-nitrobenzamide (BZ_02a).The compound was synthesized according to the general procedure using 2-chloro-5-nitrobenzoic acid (**BZ_01a**) (5.0 g, 0.02 mmol), oxalyl chloride (5.3 mL, 0.05 mmol) and aqueous ammonium hydroxide (50 mL) to afford **BZ_02a** (3.9 g, 78.4 %) as yellow solid. M.p: 185-187°C. ^{1}H NMR (DMSO-d_6): δ_H 8.28-7.83 (m, 3H), 7.52 (b, 2H). ^{13}C NMR (DMSO-d_6): δ_C 168.2, 146.2, 140.3, 133.2, 130.7, 128.5, 122.1. ESI-MS *m/z* 201 $[M+H]^+$. Anal.Calcd.For $C_7H_5ClN_2O_3$: C, 41.92; H, 2.51; N, 13.97; Found: C, 41.89; H, 2.54; N, 13.99.

2-Chloro-5-(trifluoromethyl)benzamide (BZ_02b). The compound was synthesized according to the general procedure using 2-chloro-5-(trifluoromethyl)benzoic acid (**BZ_01b**) (4.0 g, 0.01 mmol), oxalyl chloride (3.8 mL, 0.04 mmol) and aqueous ammonium hydroxide (40 mL) to afford **BZ_02b** (1.84 g, 46.2 %) as white solid. M.p: 176-178 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.02-7.64 (m, 3H), 7.54 (b, 2H). ^{13}C NMR (DMSO-d_6): δ_C 168.4, 137.7, 132.3, 129.7, 129.5, 128.1, 125.3. 123.5. ESI-MS *m/z* 224 $[M+H]^+$. Anal.Calcd.For $C_8H_5ClF_3NO$: C, 42.98; H, 2.25; N, 6.26; Found: C, 41.89; H, 2.54; N, 13.99.

2,5-Dichlorobenzamide (BZ_02c). The compound was synthesized according to the general procedure using 2, 5-dichlorobenzoic acid (**BZ_01c**) (4.0 g, 0.02 mmol), oxalyl chloride (4.5 mL, 0.05 mmol) and aqueous ammonium hydroxide (40 mL) to afford **BZ_02c** (2.12 g, 53.4 %) as white solid. M.p: 181-183 °C. ^{1}H NMR (DMSO-d_6): δ_H 7.89-7.63 (m, 3H), 7.52 (b, 2H). ^{13}C NMR (DMSO-d_6): δ_C 168.6, 133.5, 132.5, 132.3, 132.1, 129.4, 128.6. ESI-MS *m/z* 192 $[M+H]^+$. Anal.Calcd.For $C_7H_5Cl_2NO$: C, 44.24; H, 2.65; N, 7.37; Found: C, 44.26; H, 2.64; N, 7.39.

2-(Methylthio)-6-nitro-4H-benzo[e][1,3]thiazin-4-one (BZ_03a). The compound was synthesized according to the general procedure using 2-chloro-5-nitrobenzamide (**BZ_02a**) (2.0 g, 9.97 mmol), carbon disulphide (1.8 mL, 29.9 mmol), sodim hydroxide (0.79g, 19.9 mmol) and methyl iodide (0.73 mL, 11.9 mmol) to afford **BZ_03a** (1.82 g, 71.9 %) as yellow solid. M.p: 211-213 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.36-7.82 (m, 3H), 2.72 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 167.6, 162.4, 145.3, 143.6, 138.4, 130.5, 128.4, 123.6, 14.2. ESI-MS *m/z* 255 $[M+H]^+$. Anal.Calcd.For $C_9H_6N_2O_3S_2$: C, 42.51; H, 2.38; N, 11.02; Found: C, 42.54; H, 2.36; N, 11.05.

2-(Methylthio)-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (BZ_03b). The compound was synthesized according to the general procedure using 2-chloro-5-

(trifluoromethyl)benzamide (**BZ_02b**) (1.5 g, 6.71 mmol), carbon disulphide (1.2 mL, 20.1 mmol), sodim hydroxide (0.53g, 13.4 mmol) and methyl iodide (0.49 mL, 8.1 mmol) to afford **BZ_03b** (1.63 g, 88.1 %) as white solid. M.p: 208-210 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.35-7.78 (m, 3H), 2.72 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 167.4, 162.5, 140.3, 137.6, 131.4, 130.6, 128.4, 126.6, 123.5, 14.3. ESI-MS *m/z* 278 $[M+H]^+$. Anal.Calcd.For $C_{10}H_6F_3NOS_2$: C, 43.32; H, 2.18; N, 5.05; Found: C, 43.33; H, 2.16; N, 5.03.

6-Chloro-2-(methylthio)-4H-benzo[e][1,3]thiazin-4-one (BZ_03c). The compound was synthesized according to the general procedure using 2, 5-dichlorobenzamide (**BZ_02c**) (2.0 g, 10.52 mmol), carbon disulphide (1.9 mL, 31. mmol), sodim hydroxide (0.84 g, 21.1 mmol) and methyl iodide (0.78 mL, 12.6 mmol) to afford **BZ_03c** (1.57 g, 61.3 %) as white solid. M.p: 219-221 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.36-7.74 (m, 3H), 2.72 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 167.8, 162.5, 138.3, 135.6, 134.4, 131.6, 131.4, 130.6, 14.3. ESI-MS *m/z* 244 $[M+H]^+$. Anal.Calcd.For $C_9H_6ClNOS_2$: C, 44.35; H, 2.48; N, 5.75; Found: C, 44.33; H, 2.46; N, 5.73.

6-Nitro-2-(piperazin-1-yl)-4H-benzo[e][1,3]thiazin-4-one (BZ_04a). The compound was synthesized according to the general procedure using 2-(methylthio)-6-nitro-4*H*-benzo[*e*][1,3]thiazin-4-one (**BZ_03a**) (1.8 g, 7.08 mmol), 1-Boc-piperazine (1.31 g, 7.08 mmol) and trifluoroacetic acid (2 mL) to afford **BZ_04a** (1.33 g, 64.5 %) as yellow solid. M.p: 208-210 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.33-7.24 (m, 3H), 5.25 (b, 2H), 3.12-1.88 (m, 9H). ^{13}C NMR (DMSO-d_6): δ_C 167.5, 159.3, 145.6, 143.6, 138.4, 130.3, 128.4, 123.6, 46.7, 41.5 (2C), 32.2 (2C). ESI-MS *m/z* 307 $[M+H]^+$. Anal.Calcd.For $C_{13}H_{14}N_4O_3S$: C, 50.97; H, 4.61; N, 18.29; Found: C, 50.96; H, 4.65; N, 18.31.

2-(Piperazin-1-yl)-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (BZ_04b).The compound was synthesized according to the general procedure using 2-(methylthio)-6-(trifluoromethyl)-4*H*-benzo[*e*][1,3]thiazin-4-one (**BZ_03b**) (1.6 g, 5.77 mmol), 1-Boc-piperazine (1.07 g, 5.77 mmol) and trifluoroacetic acid (2 mL) to afford **BZ_04b** (1.28 g, 70.7 %) as white solid. M.p: 221-223 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.32-7.27 (m, 3H), 5.26 (b, 2H), 3.14-1.85 (m, 9H). ^{13}C NMR (DMSO-d_6): δ_C 167.2, 159.4, 140.7, 137.6, 131.4, 130.3, 128.5, 126.8, 123.3, 46.7, 41.4 (2C), 32.5 (2C). ESI-MS *m/z* 330 $[M+H]^+$. Anal.Calcd.For $C_{14}H_{14}F_3N_3OS$: C, 51.06; H, 4.28; N, 12.76; Found: C, 51.08; H, 4.27; N, 12.73.

6-Chloro-2-(piperazin-1-yl)-4H-benzo[e][1,3]thiazin-4-one (BZ_04c). The compound was synthesized according to the general procedure using 6-chloro-2-(methylthio)-4*H*-benzo[*e*][1,3]thiazin-4-one (**BZ_03c**) (1.5 g, 6.15 mmol), 1-Boc-piperazine (1.14 g, 6.15 mmol) and trifluoroacetic acid (2 mL) to afford **BZ_04c** (1.44 g, 83.2 %) as white solid. M.p: 217-219°C. ^{1}H NMR (DMSO-d_6): δ_H 8.33-7.28 (m, 3H), 5.24 (b, 2H), 3.12-1.88 (m, 9H). ^{13}C NMR (DMSO-d_6): δ_C 167.4, 159.5, 138.6, 135.1, 134.4, 131.3, 131.1, 130.2, 46.8, 41.4 (2C), 32.2 (2C). ESI-MS *m/z* 296 $[M+H]^+$. Anal.Calcd.For $C_{13}H_{14}ClN_3OS$: C, 52.79; H, 4.77; N, 14.21; Found: C, 52.82; H, 4.76; N, 14.25.

4-(6-Nitro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)-N-phenylpiperazine-1-carboxamide (BZ_05). The compound was synthesized according to the general procedure using 6-nitro-2-(piperazin-1-yl)-4*H*-benzo[*e*][1,3]thiazin-4-one (**BZ_04a**) (0.1 g, 0.34 mmol) and Phenyl isocyanate (0.04 g, 0.34 mmol) to afford **BZ_05** (0.06 g, 46.18 %) as yellow solid. M.p: 192-194 °C. ^{1}H NMR (DMSO-d_6): δ_H 10.59 (b, 1H), 8.56-7.48 (m, 8H), 3.12-1.88 (m, 8H). ^{13}C NMR (DMSO-d_6): δ_C 167.4, 159.2, 155.7, 145.3, 143.6, 139.1, 138.2, 130.2, 128.7 (3C), 128.1, 123.5, 121.5 (2C), 51.6 (2C), 48.5 (2C). ESI-MS *m/z* 412 $[M+H]^+$. Anal.Calcd.For $C_{19}H_{17}N_5O_4S$: C, 55.47; H, 4.16; N, 17.02; Found: C, 55.44; H, 4.52.15; N, 17.06.

N-(4-Chlorophenyl)-4-(6-nitro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)piperazine-1-carboxamide (BZ_06). The compound was synthesized according to the general procedure using 6-nitro-2-(piperazin-1-yl)-4*H*-benzo[*e*][1,3]thiazin-4-one (**BZ_04a**) (0.1 g, 0.34 mmol) and 4-chlorophenyl isocyanate (0.05 g, 0.34 mmol) to afford **BZ_06** (0.08 g, 53.10 %) as yellow solid. M.p: 203-205 °C. ^{1}H NMR (DMSO-d_6): δ_H 10.58 (b, 1H), 8.54-7.43 (m, 7H), 3.12-1.88 (m, 8H). ^{13}C NMR (DMSO-d_6): δ_C 167.5, 159.3, 155.5, 145.7, 143.4, 138.2, 137.2, 133.7, 130.1, 129.5 (2C), 128.5, 123.7, 120.5 (2C), 51.6 (2C), 48.4 (2C). ESI-MS *m/z* 446 $[M+H]^+$. Anal.Calcd.For $C_{19}H_{16}ClN_5O_4S$: C, 51.18; H, 3.62; N, 15.71; Found: C, 51.21; H, 3.64; N, 15.69.

4-(6-Nitro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)-N-(p-tolyl)piperazine-1-carboxamide (BZ_07). The compound was synthesized according to the general procedure using 6-nitro-2-(piperazin-1-yl)-4*H*-benzo[*e*][1,3]thiazin-4-one (**BZ_04a**) (0.1 g, 0.34 mmol) and *p*-tolylisocyanate (0.04 g, 0.34 mmol) to afford **BZ_07** (0.05 g, 35.73 %) as yellow solid. M.p: 201-203 °C. ^{1}H NMR (DMSO-d_6): δ_H 10.56 (b, 1H), 8.57-7.46 (m, 7H), 3.11-1.87 (m, 8H), 2.36 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 167.4, 159.2, 155.7, 145.3, 143.2, 138.6, 136.8, 136.3, 130.5, 129.5 (2C), 128.8, 123.6, 121.3 (2C), 51.3 (2C), 48.4 (2C), 21.2. ESI-MS *m/z* 426

$[M+H]^+$. Anal.Calcd.For $C_{20}H_{19}N_5O_4S$: C, 56.46; H, 4.50; N, 16.46; Found: C, 56.43; H, 4.54; N, 16.43.

4-(6-Nitro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)-N-(4-nitrophenyl)piperazine-1-carboxamide (BZ_08). The compound was synthesized according to the general procedure using 6-nitro-2-(piperazin-1-yl)-4*H*-benzo[*e*][1,3]thiazin-4-one (**BZ_04a**) (0.1 g, 0.34 mmol) and 4-nitrophenyl isocyanate (0.05 g, 0.34 mmol) to afford **BZ_08** (0.02 g, 14.73 %) as yellow solid. M.p: 186-188 °C. ^{1}H NMR (DMSO-d_6): δ_H 10.56 (b, 1H), 8.58-7.48 (m, 7H), 3.14-1.89 (m, 8H). ^{13}C NMR (DMSO-d_6): δ_C 167.6, 159.4, 155.1, 145.7, 145.2, 143.6, 143.4, 138.5, 130.9, 128.7, 124.2 (2C), 123.6, 119.5 (2C), 51.5 (2C), 48.6 (2C). ESI-MS *m/z* 457 $[M+H]^+$. Anal.Calcd.For $C_{19}H_{16}N_6O_6S$: C, 50.00; H, 3.53; N, 18.41; Found: C, 50.02; H, 3.51; N, 18.38.

4-(4-Oxo-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-2-yl)-N-phenylpiperazine-carboxamide (BZ_09). The compound was synthesized according to the general procedure using 2-(piperazin-1-yl)-6-(trifluoromethyl)-4*H*-benzo[*e*][1,3]thiazin-4-one (**BZ_04b**) (0.1 g, 0.31 mmol) and phenyl isocyanate (0.03 g, 0.31 mmol) to afford **BZ_09** (0.08 g, 62.39 %) as white solid. M.p: 182-184 °C. ^{1}H NMR (DMSO-d_6): δ_H 10.52 (b, 1H), 8.51-7.40 (m, 8H), 3.12-1.84 (m, 8H). ^{13}C NMR (DMSO-d_6): δ_C 167.7, 159.4, 155.6, 140.7, 139.4, 137.6, 131.3, 130.6, 128.7 (2C), 128.2, 128.1, 126.1, 123.4, 121.5 (2C), 51.2 (2C), 48.4 (2C). ESI-MS *m/z* 435 $[M+H]^+$. Anal.Calcd.For $C_{20}H_{17}F_3N_4O_2S$: C, 55.29; H, 3.94; N, 12.90; Found: C, 55.26; H, 3.95; N, 12.88.

N-(4-Chlorophenyl)-4-(4-oxo-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-2-yl)piperazine-1-carboxamide (BZ_10). The compound was synthesized according to the general procedure using 2-(piperazin-1-yl)-6-(trifluoromethyl)-4*H*-benzo[*e*][1,3]thiazin-4-one (**BZ_04b**) (0.1 g, 0.31 mmol) and 4-chlorophenyl isocyanate (0.04 g, 0.31 mmol) to afford **BZ_10** (0.05 g, 34.95 %) as white solid. M.p: 193-195 °C. ^{1}H NMR (DMSO-d_6): δ_H 10.51 (b, 1H), 8.50-7.38 (m, 7H), 3.14-1.82 (m, 8H). ^{13}C NMR (DMSO-d_6): δ_C 167.4, 159.6, 155.7, 140.2, 137.7, 137.3, 133.2, 131.2, 130.4, 129.1 (2C), 128.5, 126.6, 123.6, 120.7 (2C), 51.5 (2C), 48.4 (2C). ESI-MS *m/z* 469 $[M+H]^+$. Anal.Calcd.For $C_{20}H_{16}ClF_3N_4O_2S$: C, 51.23; H, 3.44; N, 11.95; Found: C, 51.25; H, 3.45; N, 11.96.

4-(4-Oxo-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-2-yl)-N-(p-tolyl)piperazine-1-carboxamide (BZ_11). The compound was synthesized according to the general procedure

using 2-(piperazin-1-yl)-6-(trifluoromethyl)-4*H*-benzo[*e*][1,3]thiazin-4-one (**BZ_04b**) (0.1 g, 0.31 mmol) and *p*-tolylisocyanate (0.04 g, 0.31 mmol) to afford **BZ_11** (0.04 g, 32.33 %) as white solid. M.p: 197-199 °C. ^{1}H NMR (DMSO-d_6): δ_H 10.54 (b, 1H), 8.51-7.41 (m, 7H), 3.13-1.83 (m, 8H), 2.38 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 167.6, 159.5, 155.6, 140.7, 137.2, 136.6, 136.4, 131.2, 130.2, 129.3 (2C), 128.3, 126.6, 123.6, 121.4 (2C), 51.2 (2C), 48.1 (2C), 21.3. ESI-MS *m/z* 449 $[M+H]^+$. Anal.Calcd.For $C_{21}H_{19}F_3N_4O_2S$: C, 56.24; H, 4.27; N, 12.49; Found: C, 56.28; H, 4.24; N, 12.47.

N-(4-Nitrophenyl)-4-(4-oxo-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-2-yl)piperazine-1-carboxamide (BZ_12).The compound was synthesized according to the general procedure using 2-(piperazin-1-yl)-6-(trifluoromethyl)-4*H*-benzo[*e*][1,3]thiazin-4-one (**BZ_04b**) (0.1 g, 0.31 mmol) and 4-nitrophenyl isocyanate (0.05 g, 0.31 mmol) to afford **BZ_12** (0.03 g, 25.64 %) as white solid. M.p: 215-217 °C. ^{1}H NMR (DMSO-d_6): δ_H 10.55 (b, 1H), 8.55-7.46 (m, 7H), 3.15-1.85 (m, 8H). ^{13}C NMR (DMSO-d_6): δ_C 167.7, 159.2, 155.1, 145.8, 143.2, 140.6, 137.2, 131.3, 130.2, 128.4, 126.3, 124.4 (2C), 123.6, 119.6 (2C), 51.5 (2C), 48.8 (2C). ESI-MS *m/z* 480 $[M+H]^+$. Anal.Calcd.For $C_{20}H_{16}F_3N_5O_4S$: C, 50.10; H, 3.36; N, 14.61; Found: C, 50.13; H, 3.34; N, 14.58.

4-(6-Chloro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)-N-phenylpiperazine-1-carboxamide (BZ_13). The compound was synthesized according to the general procedure using 6-chloro-2-(piperazin-1-yl)-4*H*-benzo[*e*][1,3]thiazin-4-one (**BZ_04c**) (0.1 g, 0.35 mmol) and phenyl isocyanate (0.04 g, 0.35 mmol) to afford **BZ_13** (0.04 g, 34.44 %) as white solid. M.p: 199-201 °C. ^{1}H NMR (DMSO-d_6): δ_H 10.50 (b, 1H), 8.54-7.39 (m, 8H), 3.16-1.89 (m, 8H). ^{13}C NMR (DMSO-d_6): δ_C 167.4, 159.5, 155.2, 139.8, 138.2, 135.6, 134.2, 131.3, 131.2, 130.4, 128.7 (2C), 128.4, 121.6 (2C), 51.6 (2C), 48.4 (2C). ESI-MS *m/z* 401 $[M+H]^+$. Anal.Calcd.For $C_{19}H_{17}ClN_4O_2S$: C, 56.93; H, 4.27; N, 13.98; Found: C, 56.90; H, 4.29; N, 13.95.

4-(6-Chloro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)-N-(4-chlorophenyl)piperazine-1-carboxamide (BZ_14). The compound was synthesized according to the general procedure using 6-chloro-2-(piperazin-1-yl)-4*H*-benzo[*e*][1,3]thiazin-4-one (**BZ_04c**) (0.1 g, 0.35 mmol) and 4-chlorophenyl isocyanate (0.05 g, 0.35 mmol) to afford **BZ_14** (0.05 g, 36.89 %) as white solid. M.p: 206-208 °C. ^{1}H NMR (DMSO-d_6): δ_H 10.51 (b, 1H), 8.56-7.42 (m, 7H), 3.14-1.86 (m, 8H). ^{13}C NMR (DMSO-d_6): δ_C 167.6, 159.2, 155.4, 138.8, 137.2, 135.5, 134.2, 133.5, 131.4, 131.1, 130.5, 129.7 (2C), 120.6 (2C), 51.4 (2C), 48.2 (2C). ESI-MS *m/z* 436

$[M+H]^+$. Anal.Calcd.For $C_{19}H_{16}Cl_2N_4O_2S$: C, 52.42; H, 3.70; N, 12.87; Found: C, 52.43; H, 3.68; N, 12.89.

4-(6-Chloro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)-N-(p-tolyl)piperazine-1-carboxamide (BZ_15). The compound was synthesized according to the general procedure using 6-chloro-2-(piperazin-1-yl)-4*H*-benzo[*e*][1,3]thiazin-4-one (**BZ_04c**) (0.1 g, 0.35 mmol) and *p*-tolylisocyanate (0.04 g, 0.35 mmol) to afford **BZ_15** (0.06 g, 44.82 %) as white solid. M.p: 221-223 °C. ^{1}H NMR (DMSO-d_6): δ_H 10.49 (b, 1H), 8.51-7.42 (m, 7H), 3.14-1.84 (m, 8H), 2.34 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 167.4, 159.7, 155.1, 138.7, 136.6, 136.2, 135.5, 134.3, 131.5, 131.3, 130.1, 129.5 (2C), 121.7 (2C), 51.5 (2C), 48.4 (2C), 21.4. ESI-MS *m/z* 415 $[M+H]^+$. Anal.Calcd.For $C_{20}H_{19}ClN_4O_2S$: C, 57.90; H, 4.62; N, 13.50; Found: C, 57.89; H, 4.59; N, 13.52.

4-(6-Chloro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)-N-(4-nitrophenyl)piperazine-1-carboxamide (BZ_16). The compound was synthesized according to the general procedure using 6-chloro-2-(piperazin-1-yl)-4*H*-benzo[*e*][1,3]thiazin-4-one (**BZ_04c**) (0.1 g, 0.35 mmol) and 4-nitrophenyl isocyanate (0.05 g, 0.35 mmol) to afford **BZ_16** (0.08 g, 51.82 %) as white solid. M.p: 197-199 °C. ^{1}H NMR (DMSO-d_6): δ_H 10.52 (b, 1H), 8.53-7.47 (m, 7H), 3.15-1.81 (m, 8H). ^{13}C NMR (DMSO-d_6): δ_C 167.7, 159.3, 155.2, 145.7, 143.2, 138.5, 135.3, 134.5, 131.3, 131.1, 130.5, 124.3 (2C), 119.8 (2C), 51.5 (2C), 48.6 (2C). ESI-MS *m/z* 446 $[M+H]^+$. Anal.Calcd.For $C_{19}H_{16}ClN_5O_4S$: C, 51.18; H, 3.62; N, 15.71; Found: C, 51.19; H, 3.65; N, 15.74.

4-(6-Nitro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)-N-phenylpiperazine-1-carbothioamide (BZ_17). The compound was synthesized according to the general procedure using 6-nitro-2-(piperazin-1-yl)-4*H*-benzo[*e*][1,3]thiazin-4-one (**BZ_04a**) (0.1 g, 0.34 mmol) and phenyl isothiocyanate (0.04 g, 0.34 mmol) to afford **BZ_17** (0.06 g, 46.50 %) as yellow solid. M.p: 205-207 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.97(b, 1H), 8.52-7.71 (m, 8H), 3.06-1.84 (m, 8H). ^{13}C NMR (DMSO-d_6): δ_C 181.6, 167.5, 159.2, 145.2, 143.7, 138.2, 138.1, 130.3, 129.3 (2C), 128.4, 128.1, 126.5 (2C), 123.3, 56.7 (2C), 48.5 (2C). ESI-MS *m/z* 428 $[M+H]^+$. Anal.Calcd.For $C_{19}H_{17}N_5O_3S_2$: C, 53.38; H, 4.01; N, 16.38; Found: C, 53.40; H, 4.02; N, 16.37.

N-(4-Chlorophenyl)-4-(6-nitro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)piperazine-1-carbothioamide (BZ_18). The compound was synthesized according to the general procedure

using 6-nitro-2-(piperazin-1-yl)-4*H*-benzo[*e*][1,3]thiazin-4-one (**BZ_05 4a**) (0.1 g, 0.34 mmol) and 4-Chlorophenyl isothiocyanate (0.05 g, 0.34 mmol) to afford **BZ_18** (0.07 g, 45.56 %) as yellow solid. M.p: 179-181 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.95(b, 1H), 8.55-7.73 (m, 7H), 3.04-1.86 (m, 8H). ^{13}C NMR (DMSO-d_6): δ_C 181.4, 167.6, 159.4, 145.7, 143.2, 138.4, 136.1, 133.3, 131.3 (2C), 130.4, 129.1 (2C), 128.5, 123.4, 56.6 (2C), 48.8 (2C). ESI-MS *m/z* 462 $[M+H]^+$. Anal.Calcd.For $C_{19}H_{16}ClN_5O_3S_2$: C, 49.40; H, 3.49; N, 15.16; Found: C, 49.43; H, 3.51; N, 15.19.

4-(6-Nitro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)-N-(p-tolyl)piperazine-1-carbothioamide (BZ_19). The compound was synthesized according to the general procedure using 6-nitro-2-(piperazin-1-yl)-4*H*-benzo[*e*][1,3]thiazin-4-one (**BZ_04a**) (0.1 g, 0.34 mmol) and *p*-tolylisothiocyanate (0.05 g, 0.34 mmol) to afford **BZ_19** (0.07 g, 48.33 %) as yellow solid. M.p: 183-185 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.93(b, 1H), 8.58-7.81 (m, 7H), 3.07-1.88 (m, 8H), 2.36 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 181.3, 167.5, 159.8, 145.6, 143.1, 138.4, 137.1, 135.3, 130.3, 129.4 (2C), 128.1, 126.5 (2C), 123.5, 56.8 (2C), 48.3 (2C), 21.4. ESI-MS *m/z* 442 $[M+H]^+$. Anal.Calcd.For $C_{20}H_{19}N_5O_3S_2$: C, 54.41; H, 4.34; N, 15.86; Found: C, 54.39; H, 4.35; N, 15.89.

4-(6-Nitro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)-N-(4-nitrophenyl)piperazine-1 carbothioamide (BZ_20). The compound was synthesized according to the general procedure using 6-nitro-2-(piperazin-1-yl)-4*H*-benzo[*e*][1,3]thiazin-4-one (**BZ_04a**) (0.1 g, 0.34 mmol) and 4-nitrophenyl isothiocyanate (0.06 g, 0.34 mmol) to afford **BZ_20** (0.04 g, 26.60 %) as yellow solid. M.p: 217-219 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.95 (b, 1H), 8.61-7.42 (m, 7H), 3.08-1.81 (m, 8H). ^{13}C NMR (DMSO-d_6): δ_C 181.5, 167.3, 159.5, 145.8, 144.6, 143.4, 143.1, 138.3, 130.2, 128.4, 124.5 (2C), 124.2 (2C), 123.7, 56.6 (2C), 48.5 (2C). ESI-MS *m/z* 473 $[M+H]^+$. Anal.Calcd.For $C_{19}H_{16}N_6O_5S_2$: C, 48.30; H, 3.41; N, 17.79; Found: C, 48.33; H, 3.45; N, 17.80.

4-(4-Oxo-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-2-yl)-N-phenylpiperazine-1-carbothioamide (BZ_21). The compound was synthesized according to the general procedure using 2-(piperazin-1-yl)-6-(trifluoromethyl)-4*H*-benzo[*e*][1,3]thiazin-4-one (**BZ_04b**) (0.1 g, 0.31 mmol) and phenyl isothiocyanate (0.04 g, 0.31 mmol) to afford **BZ_21** (0.05 g, 40.58 %) as white solid. M.p: 221-223 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.97 (b, 1H), 8.62-7.45 (m, 8H), 3.10-1.85 (m, 8H). ^{13}C NMR (DMSO-d_6): δ_C 181.1, 167.9, 159.6, 140.8, 138.6, 137.4, 131.6, 130.3, 129.2 (2C), 128.4, 128.2, 126.2 (3C), 123.5, 56.7 (2C), 48.2 (2C). ESI-MS *m/z*

451 $[M+H]^+$. Anal.Calcd.For $C_{20}H_{17}F_3N_4OS_2$: C, 53.32; H, 3.80; N, 12.44; Found: C, 55.29; H, 3.91; N, 12.42.

N-(4-Chlorophenyl)-4-(4-oxo-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-2-yl)piperazine-1-carbothioamide (BZ_22). The compound was synthesized according to the general procedure using 2-(piperazin-1-yl)-6-(trifluoromethyl)-4*H*-benzo[*e*][1,3]thiazin-4-one (**BZ_04b**) (0.1 g, 0.31 mmol) and 4-chlorophenyl isothiocyanate (0.05 g, 0.31 mmol) to afford **BZ_22** (0.06 g, 40.29 %) as white solid. M.p: 193-195 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.94 (b, 1H), 8.63-7.41 (m, 7H), 3.12-1.88 (m, 8H). ^{13}C NMR (DMSO-d_6): δ_C 181.8, 167.7, 159.4, 140.5, 137.6, 136.4, 133.6, 131.3, 131.2 (2C), 130.4, 129.2 (2C), 128.2, 126.5, 123.6, 56.8 (2C), 48.6 (2C). ESI-MS *m/z* 485 $[M+H]^+$. Anal.Calcd.For $C_{20}H_{16}ClF_3N_4OS_2$: C, 49.53; H, 3.33; N, 11.55; Found: C, 49.51; H, 3.35; N, 11.54.

4-(4-Oxo-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-2-yl)-N-(p-tolyl)piperazine-1-carbothioamide (BZ_23). The compound was synthesized according to the general procedure using 2-(piperazin-1-yl)-6-(trifluoromethyl)-4*H*-benzo[*e*][1,3]thiazin-4-one (**BZ_04b**) (0.1 g, 0.31 mmol) and *p*-tolylisothiocyanate (0.04 g, 0.31 mmol) to afford **BZ_23** (0.06 g, 45.46 %) as white solid. M.p: 183-185 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.91 (b, 1H), 8.59-7.63 (m, 7H), 3.15-1.85 (m, 8H), 2.35 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 181.5, 167.1, 159.9, 140.2, 137.4, 137.2, 135.4, 131.6, 130.3, 129.2 (2C), 128.4, 126.5, 126.2 (2C), 123.5, 56.4 (2C), 48.2 (2C), 21.4. ESI-MS *m/z* 465 $[M+H]^+$. Anal.Calcd.For $C_{21}H_{19}F_3N_4OS_2$: C, 54.30; H, 4.12; N, 12.06; Found: C, 54.32; H, 4.16; N, 12.07.

N-(4-Nitrophenyl)-4-(4-oxo-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-2-yl)piperazine-1-carbothioamide (BZ_24). The compound was synthesized according to the general procedure using 2-(piperazin-1-yl)-6-(trifluoromethyl)-4*H*-benzo[*e*][1,3]thiazin-4-one (**BZ_04b**) (0.1 g, 0.31 mmol) and 4-nitrophenyl isothiocyanate (0.05 g, 0.31 mmol) to afford **BZ_24** (0.08 g, 52.16 %) as white solid. M.p: 227-229 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.98 (b, 1H), 8.62-7.61 (m, 7H), 3.11-1.82 (m, 8H). ^{13}C NMR (DMSO-d_6): δ_C 181.1, 167.3, 159.4, 144.2, 143.4, 140.2, 137.4, 131.6, 130.3, 128.2, 126.4, 124.5 (2C), 124.2 (2C), 123.7, 56.3 (2C), 48.7 (2C). ESI-MS *m/z* 496 $[M+H]^+$. Anal.Calcd.For $C_{20}H_{16}F_3N_5O_3S_2$: C, 48.48; H, 3.25; N, 14.13; Found: C, 48.49; H, 3.22; N, 14.17.

4-(6-Chloro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)-N-phenylpiperazine-1-carbothioamide (BZ_25). The compound was synthesized according to the general procedure using 6-chloro-

2-(piperazin-1-yl)-4*H*-benzo[*e*][1,3]thiazin-4-one (**BZ_04c**) (0.1 g, 0.35 mmol) and phenyl isothiocyanate (0.04 g, 0.35 mmol) to afford **BZ_25** (0.05 g, 34.47 %) as white solid. M.p: 189-191 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.92 (b, 1H), 8.58-7.66 (m, 8H), 3.14-1.85 (m, 8H). ^{13}C NMR (DMSO-d_6): δ_C 181.5, 167.5, 159.4, 138.6, 138.4, 135.2, 134.4, 131.6, 131.2, 130.2, 129.4 (2C), 128.5, 126.6 (2C), 56.3 (2C), 48.6 (2C). ESI-MS *m/z* 417 $[M+H]^+$. Anal.Calcd.For $C_{19}H_{17}ClN_4OS_2$: C, 54.73; H, 4.11; N, 13.44; Found: C, 54.75; H, 4.14; N, 13.42.

4-(6-Chloro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)-N-(4-chlorophenyl)piperazine-1-carbothioamide (BZ_26). The compound was synthesized according to the general procedure using 6-chloro-2-(piperazin-1-yl)-4*H*-benzo[*e*][1,3]thiazin-4-one (**BZ_04c**) (0.1 g, 0.35 mmol) and 4-chlorophenyl isothiocyanate (0.05 g, 0.35 mmol) to afford **BZ_26** (0.03 g, 24.34 %) as white solid. M.p: 201-203 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.89 (b, 1H), 8.53-7.74 (m, 7H), 3.09-1.89 (m, 8H). ^{13}C NMR (DMSO-d_6): δ_C 181.1, 167.4, 159.8, 138.9, 136.4, 135.5, 134.7, 133.6, 131.4, 131.2, 131.1 (2C), 130.4, 129.5 (2C), 56.5 (2C), 48.5 (2C). ESI-MS *m/z* 452 $[M+H]^+$. Anal.Calcd.For $C_{19}H_{16}Cl_2N_4OS_2$: C, 50.56; H, 3.57; N, 12.41; Found: C, 50.57; H, 3.59; N, 12.39.

4-(6-Chloro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)-N-(p-tolyl)piperazine-1-carbothioamide (BZ_27). The compound was synthesized according to the general procedure using 6-chloro-2-(piperazin-1-yl)-4*H*-benzo[*e*][1,3]thiazin-4-one (**BZ_04c**) (0.1 g, 0.35 mmol) and *p*-tolylisothiocyanate (0.05 g, 0.35 mmol) to afford **BZ_27** (0.07 g, 47.73 %) as white solid. M.p: 217-219 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.91 (b, 1H), 8.54-7.71 (m, 7H), 3.05-1.92 (m, 8H), 2.36 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 181.7, 167.5, 159.1, 138.2, 137.4, 135.5, 135.3, 134.6, 131.4, 131.2, 130.3, 129.4 (2C), 126.5 (2C), 56.3 (2C), 48.9 (2C), 21.4. ESI-MS *m/z* 431 $[M+H]^+$. Anal.Calcd.For $C_{20}H_{19}ClN_4OS_2$: C, 55.74; H, 4.44; N, 13.00; Found: C, 55.73; H, 4.45; N, 13.03.

4-(6-Chloro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)-N-(4-nitrophenyl)piperazine-1-carbothioamide (BZ_28). The compound was synthesized according to the general procedure using 6-chloro-2-(piperazin-1-yl)-4*H*-benzo[*e*][1,3]thiazin-4-one (**BZ_04c**) (0.1 g, 0.35 mmol) and 4-nitrophenyl isothiocyanate (0.06 g, 0.35 mmol) to afford **BZ_28** (0.05 g, 31.72 %) as white solid. M.p: 189-191 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.91 (b, 1H), 8.61-7.74 (m, 7H), 3.08-1.91 (m, 8H). ^{13}C NMR (DMSO-d_6): δ_C 181.8, 167.1, 159.2, 144.2, 143.4, 138.5, 135.7, 134.4, 131.4, 131.3, 130.5, 124.4 (2C), 124.1 (2C), 56.5 (2C), 48.7 (2C). ESI-MS *m/z*

462 $[M+H]^+$. Anal.Calcd.For $C_{19}H_{16}ClN_5O_3S_2$: C, 49.40; H, 3.49; N, 15.16; Found: C, 49.44; H, 3.52; N, 15.13.

2-(4-Benzylpiperazin-1-yl)-6-nitro-4H-benzo[e][1,3]thiazin-4-one (BZ_29). The compound was synthesized according to the general procedure using 6-nitro-2-(piperazin-1-yl)-4*H*-benzo[*e*][1,3]thiazin-4-one (**BZ_04a**) (0.1 g, 0.34 mmol), benzaldehyde (0.03 g, 0.34 mmol) and sodium cyanoborohydride (0.02 g, 0.34 mmol) to afford **BZ_29** (0.04 g, 35.16 %) as yellow solid. M.p: 208-210 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.52-7.49 (m, 8H), 3.51 (s, 2H), 3.12-1.78 (m, 8H). ^{13}C NMR (DMSO-d_6): δ_C 167.4, 159.4, 145.2, 143.8, 138.5, 138.3, 130.4, 128.6, 128.4 (2C), 128.2 (2C), 127.5, 123.1, 64.5, 54.3 (2C), 49.6 (2C). ESI-MS *m/z* 383 $[M+H]^+$. Anal.Calcd.For $C_{19}H_{18}N_4O_3S$: C, 59.67; H, 4.74; N, 14.65; Found: C, 59.66; H, 4.71; N, 14.67.

2-(4-(4-Chlorobenzyl)piperazin-1-yl)-6-nitro-4H-benzo[e][1,3]thiazin-4-one (BZ_30). The compound was synthesized according to the general procedure using 6-nitro-2-(piperazin-1-yl)-4*H*-benzo[*e*][1,3]thiazin-4-one (**BZ_04a**) (0.1 g, 0.34 mmol) 4-chlorobenzaldehyde (0.04 g, 0.34 mmol) and sodium cyanoborohydride (0.02 g, 0.34 mmol) to afford **BZ_30** (0.06 g, 46.28 %) as yellow solid. M.p: 204-206 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.53-7.47 (m, 7H), 3.54 (s, 2H), 3.14-1.80 (m, 8H). ^{13}C NMR (DMSO-d_6): δ_C 167.5, 159.6, 145.4, 143.7, 138.5, 136.7, 132.6, 131.4 (2C), 130.6, 128.4, 128.2 (2C), 123.7, 64.5, 54.4 (2C), 49.5 (2C). ESI-MS *m/z* 417 $[M+H]^+$. Anal.Calcd.For $C_{19}H_{17}ClN_4O_3S$: C, 54.74; H, 4.11; N, 13.44; Found: C, 54.72; H, 4.15; N, 13.47.

2-(4-(4-Methylbenzyl)piperazin-1-yl)-6-nitro-4H-benzo[e][1,3]thiazin-4-oneone (BZ_31). The compound was synthesized according to the general procedure using 6-nitro-2-(piperazin-1-yl)-4*H*-benzo[*e*][1,3]thiazin-4-one (**BZ_04a**) (0.1 g, 0.34 mmol), 4-tolualdehyde (0.04 g, 0.34 mmol) and sodium cyanoborohydride (0.02 g, 0.34 mmol) to afford **BZ_31** (0.05 g, 39.08 %) as yellow solid. M.p: 216-218 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.55-7.44 (m, 7H), 3.54 (s, 2H), 3.12-1.78 (m, 8H), 2.98 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 167.5, 159.6, 145.4, 143.7, 138.5, 136.7, 135.6, 130.6, 130.4 (2C), 128.6, 128.5 (2C), 123.2, 64.5, 54.4 (2C), 49.5 (2C), 21.4. ESI-MS *m/z* 397 $[M+H]^+$. Anal.Calcd.For $C_{20}H_{20}N_4O_3S$: C, 60.59; H, 5.08; N, 14.13; Found: C, 60.62; H, 5.10; N, 14.11.

6-Nitro-2-(4-(4-nitrobenzyl)piperazin-1-yl)-4H-benzo[e][1,3]thiazin-4-one (BZ_32). The compound was synthesized according to the general procedure using 6-nitro-2-(piperazin-1-

yl)-4*H*-benzo[*e*][1,3]thiazin-4-one (**BZ_04a**) (0.1 g, 0.34 mmol), 4-Nitro benzaldehyde (0.05 g, 0.34 mmol) and sodium cyanoborohydride (0.02 g, 0.34 mmol) to afford **BZ_32** (0.05 g, 39.98 %) as yellow solid. M.p: 226-228 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.58-7.47 (m, 7H), 3.56 (s, 2H), 3.11-1.79 (m, 8H). ^{13}C NMR (DMSO-d_6): δ_C 167.4, 159.3, 146.5, 145.5, 144.7, 143.6, 138.4, 130.6, 129.4 (2C), 128.4, 123.5, 123.2 (2C), 64.6, 54.2 (2C), 49.6 (2C). ESI-MS *m/z* 428 $[M+H]^+$. Anal.Calcd.For $C_{19}H_{17}N_5O_5S$: C, 53.39; H, 4.01; N, 16.38; Found: C, 53.37; H, 4.02; N, 16.36.

2-(4-Benzylpiperazin-1-yl)-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (BZ_33). The compound was synthesized according to the general procedure using 2-(piperazin-1-yl)-6-(trifluoromethyl)-4*H*-benzo[*e*][1,3]thiazin-4-one (**BZ_04b**) (0.1 g, 0.31 mmol), benzaldehyde (0.03 g, 0.31 mmol) and sodium cyanoborohydride (0.02 g, 0.31 mmol) to afford **BZ_33** (0.08 g, 64.52 %) as white solid. M.p: 213-215 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.60-7.52 (m, 8H), 3.54 (s, 2H), 3.14-1.82 (m, 8H). ^{13}C NMR (DMSO-d_6): δ_C 167.2, 159.5, 140.5, 138.4, 137.5, 131.7, 130.6, 128.7 (2C), 128.4 (2C), 128.2, 127.4, 126.2, 123.2, 64.4, 54.8 (2C), 49.7 (2C). ESI-MS *m/z* 406 $[M+H]^+$. Anal.Calcd.For $C_{20}H_{18}F_3N_3OS$: C, 59.25; H, 4.47; N, 10.36; Found: C, 59.24; H, 4.49; N, 10.35.

2-(4-(4-Chlorobenzyl)piperazin-1-yl)-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (BZ_34). The compound was synthesized according to the general procedure using 2-(piperazin-1-yl)-6-(trifluoromethyl)-4*H*-benzo[*e*][1,3]thiazin-4-one (**BZ_04b**) (0.1 g, 0.31 mmol), 4-chloro benzaldehyde (0.04 g, 0.31 mmol) and sodium cyanoborohydride (0.02 g, 0.31 mmol) to afford **BZ_34** (0.08 g, 63.05 %) as white solid. M.p: 211-213 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.59-7.51 (m, 7H), 3.55 (s, 2H), 3.16-1.80 (m, 8H). ^{13}C NMR (DMSO-d_6): δ_C 167.5, 159.5, 140.5, 137.4, 136.5, 132.7, 131.6 (3C), 130.4, 128.5 (2C), 128.2, 126.2, 123.2, 64.5, 54.7 (2C), 49.6 (2C). ESI-MS *m/z* 440 $[M+H]^+$. Anal.Calcd.For $C_{20}H_{17}ClF_3N_3OS$: C, 54.61; H, 3.90; N, 9.55; Found: C, 54.60; H, 3.88; N, 9.56.

2-(4-(4-Methylbenzyl)piperazin-1-yl)-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (BZ_35). The compound was synthesized according to the general procedure using 2-(piperazin-1-yl)-6-(trifluoromethyl)-4*H*-benzo[*e*][1,3]thiazin-4-one (**BZ_04b**) (0.1 g, 0.31 mmol), 4-tolualdehyde (0.03 g, 0.31 mmol) and sodium cyanoborohydride (0.02 g, 0.31 mmol) to afford **BZ_35** (0.06 g, 48.09 %) as white solid. M.p: 186-188 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.61-7.54 (m, 7H), 3.52 (s, 2H), 3.14-1.83 (m, 8H), 2.98 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 167.5, 159.3, 140.4, 137.4, 136.1, 135.7, 131.5, 130.4, 130.1 (2C), 128.7

(2C), 128.2, 126.4, 123.7, 64.6, 54.3 (2C), 49.6 (2C), 21.1. ESI-MS *m/z* 420 $[M+H]^+$. Anal.Calcd.For $C_{21}H_{20}F_3N_3OS$: C, 60.13; H, 4.81; N, 10.02; Found: C, 60.15; H, 4.79; N, 10.01.

2-(4-(4-Nitrobenzyl)piperazin-1-yl)-6-(trifluoromethyl)-4H-benzo[e][1,3]thiazin-4-one (BZ_36). The compound was synthesized according to the general procedure using 2-(piperazin-1-yl)-6-(trifluoromethyl)-4*H*-benzo[*e*][1,3]thiazin-4-one (**BZ_04b**) (0.1 g, 0.31 mmol), 4-nitrobenzaldehyde (0.04 g, 0.31 mmol) and sodium cyanoborohydride (0.02 g, 0.31 mmol) to afford **BZ_36** (0.08 g, 58.77 %) as white solid. M.p: 211-213 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.58-7.52 (m, 7H), 3.54 (s, 2H), 3.14-1.83 (m, 8H). ^{13}C NMR (DMSO-d_6): δ_C 167.7, 159.3, 146.4, 144.2, 140.1, 137.7, 131.5, 130.2, 129.7 (2C), 128.4, 126.2, 123.4, 123.2 (2C), 64.5, 54.4 (2C), 49.8 (2C). ESI-MS *m/z* 451 $[M+H]^+$. Anal.Calcd.For $C_{20}H_{17}F_3N_4O_3S$: C, 53.33; H, 3.80; N, 12.44; Found: C, 53.29; H, 3.81; N, 12.43.

2-(4-Benzylpiperazin-1-yl)-6-chloro-4H-benzo[e][1,3]thiazin-4-one (BZ_37). The compound was synthesized according to the general procedure using 6-chloro-2-(piperazin-1-yl)-4*H*-benzo[*e*][1,3]thiazin-4-one (**BZ_04c**) (0.1 g, 0.35 mmol), benzaldehyde (0.03 g, 0.35 mmol) and sodium cyanoborohydride (0.02 g, 0.35 mmol) to afford **BZ_37** (0.07 g, 54.55 %) as white solid. M.p: 211-213 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.63-7.55 (m, 8H), 3.56 (s, 2H), 3.12-1.81 (m, 8H). ^{13}C NMR (DMSO-d_6): δ_C 167.5, 159.2, 138.4, 138.2, 135.1, 134.7, 131.5, 131.2, 130.7, 128.4 (2C), 128.2 (2C), 127.4, 64.4, 54.6 (2C), 49.7 (2C). ESI-MS *m/z* 372 $[M+H]^+$. Anal.Calcd.For $C_{19}H_{18}ClN_3OS$: C, 61.36; H, 4.88; N, 11.30; Found: C, 61.33; H, 4.89; N, 11.31.

6-Chloro-2-(4-(4-chlorobenzyl)piperazin-1-yl)-4H-benzo[e][1,3]thiazin-4-oneone (BZ_38). The compound was synthesized according to the general procedure using 6-chloro-2-(piperazin-1-yl)-4*H*-benzo[*e*][1,3]thiazin-4-one (**BZ_04c**) (0.1 g, 0.35 mmol), 4-chlorobenzaldehyde (0.05 g, 0.35 mmol) and sodium cyanoborohydride (0.02 g, 0.35 mmol) to afford **BZ_38** (0.07 g, 50.62 %) as white solid. M.p: 215-217 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.60-7.54 (m, 7H), 3.55 (s, 2H), 3.11-1.82 (m, 8H). ^{13}C NMR (DMSO-d_6): δ_C 167.6, 159.4, 138.4, 136.2, 135.4, 134.6, 132.5, 131.6, 131.3, 131.1 (2C), 130.4, 128.4 (2C), 64.5, 54.3 (2C), 49.7 (2C). ESI-MS *m/z* 407 $[M+H]^+$. Anal.Calcd.For $C_{19}H_{17}Cl_2N_3OS$: C, 56.16; H, 4.22; N, 10.34; Found: C, 56.17; H, 4.25; N, 10.33.

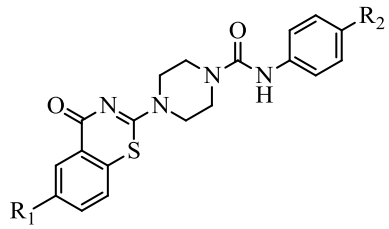
6-Chloro-2-(4-(4-methylbenzyl)piperazin-1-yl)-4H-benzo[e][1,3]thiazin-4-one (BZ_39). The compound was synthesized according to the general procedure using 6-chloro-2-(piperazin-1-yl)-4*H*-benzo[*e*][1,3]thiazin-4-one (**BZ_04c**) (0.1 g, 0.35 mmol), 4-tolualdehyde (0.04 g, 0.35 mmol) and sodium cyanoborohydride (0.02 g, 0.35 mmol) to afford **BZ_39** (0.06 g, 50.38 %) as white solid. M.p: 222-224 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.62-7.52 (m, 7H), 3.52 (s, 2H), 3.12-1.81 (m, 8H), 2.98 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 167.5, 159.2, 138.5, 136.2, 135.4, 135.2, 134.5, 131.2, 131.1, 130.4, 130.1 (2C), 128.6 (2C), 64.4, 54.6 (2C), 49.6 (2C), 21.1. ESI-MS *m/z* 386 $[M+H]^+$. Anal.Calcd.For $C_{20}H_{20}ClN_3OS$: C, 62.25; H, 5.22; N, 10.89; Found: C, 62.25; H, 5.24; N, 10.88.

6-Chloro-2-(4-(4-nitrobenzyl)piperazin-1-yl)-4H-benzo[e][1,3]thiazin-4-one (BZ_40). The compound was synthesized according to the general procedure using 6-chloro-2-(piperazin-1-yl)-4*H*-benzo[*e*][1,3]thiazin-4-one (**BZ_04c**) (0.1 g, 0.35 mmol), 4-nitrobenzaldehyde (0.05 g, 0.35 mmol) and sodium cyanoborohydride (0.02 g, 0.35 mmol) to afford **BZ_40** (0.07 g, 52.72 %) as white solid. M.p: 192-194 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.64-7.56 (m, 7H), 3.56 (s, 2H), 3.12-1.81 (m, 8H). ^{13}C NMR (DMSO-d_6): δ_C 167.4, 159.3, 146.5, 144.2, 138.4, 135.4, 134.9, 131.6, 131.1, 130.4, 129.8 (2C), 123.7 (2C), 64.4, 54.2 (2C), 49.4 (2C). ESI-MS *m/z* 417 $[M+H]^+$. Anal.Calcd.For $C_{19}H_{17}ClN_4O_3S$: C, 54.74; H, 4.11; N, 13.44; Found: C, 54.76; H, 4.13; N, 13.42.

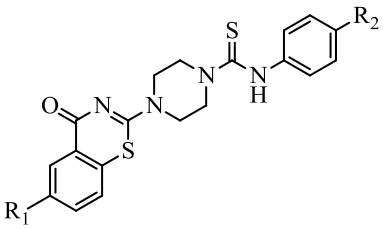
5.1c.4. In-vitro *Mycobacterium tuberculosis* supercoiling assay, antimycobacterial potency and cytotoxicity studies of the synthesized molecules

All the synthesized derivatives were first evaluated for their in-vitro *Mycobacterium tuberculosis* supercoiling assay as steps towards the derivation of SAR and hit optimization. The compounds were further subjected to a whole cell screening against *Mycobacterium tuberculosis* H37Rv strain to understand their bactericidal potency using the MABA assay and later the safety profile of these molecules were evaluated by checking the in-vitro cytotoxicity against RAW 264.7 cell line (mouse macrophage) by MTT assay, and the results are tabulated in **Table 5.6**.

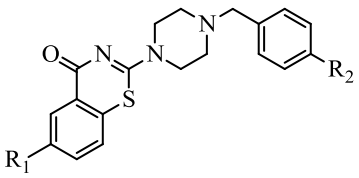
Table 5.6: In-vitro biological evaluation of the synthesized derivatives **BZ_05 - BZ_40**.



BZ_05 - BZ_16



BZ_17 - BZ_28



BZ_29 - BZ_40

Compd	R_1	R_2	MTB Supercoiling assay (IC_{50}) µM	MTB MIC µM	Cytotoxicity[a] % inhibition
BZ_05	NO_2	H	16.84±0.27	30.38	ND
BZ_06	NO_2	Cl	23.71±0.21	28.03	ND
BZ_07	NO_2	CH_3	9.62±0.17	14.68	16.24
BZ_08	NO_2	NO_2	9.11±0.34	13.67	15.23
BZ_09	CF_3	H	10.95±0.22	19.18	ND
BZ_10	CF_3	Cl	10.52±0.26	13.32	10.24
BZ_11	CF_3	CH_3	5.26±0.35	11.54	7.56
BZ_12	CF_3	NO_2	26.45±0.31	52.14	ND
BZ_13	Cl	H	17.51±0.28	15.59	16.52
BZ_14	Cl	Cl	10.46±0.24	17.91	2.56
BZ_15	Cl	CH_3	2.62±0.13	6.02	5.14
BZ_16	Cl	NO_2	4.61±0.18	5.24	3.56
BZ_17	NO_2	H	0.77±0.06	1.82	3.25
BZ_18	NO_2	Cl	0.51±0.16	4.41	1.81
BZ_19	NO_2	CH_3	0.82±0.03	2.83	4.22
BZ_20	NO_2	NO_2	2.59±0.14	5.29	2.21
BZ_21	CF_3	H	0.83±0.18	4.62	6.48
BZ_22	CF_3	Cl	4.23±0.33	8.59	3.15
BZ_23	CF_3	CH_3	4.51±0.26	9.54	16.45
BZ_24	CF_3	NO_2	2.16±0.11	5.04	5.48

Contd.

Compd	R_1	R_2	MTB Supercoiling assay (IC_{50}) µM	MTB MIC µM	Cytotoxicity[a] % inhibition
BZ_25	Cl	H	10.93±0.34	11.22	2.35
BZ_26	Cl	Cl	6.86±0.24	6.92	25.64
BZ_27	Cl	CH_3	11.56±0.18	14.5	2.48
BZ_28	Cl	NO_2	6.23±0.15	13.52	1.45
BZ_29	NO_2	H	3.21±0.32	4.08	5.41
BZ_30	NO_2	Cl	3.68±0.19	5.99	1.56
BZ_31	NO_2	CH_3	8.93±0.24	15.76	26.54
BZ_32	NO_2	NO_2	4.21±0.33	7.31	21.35
BZ_33	CF_3	H	11.52±0.17	15.41	ND
BZ_34	CF_3	Cl	3.86±0.13	6.31	8.45
BZ_35	CF_3	CH_3	11.63±0.98	16.73	18.56
BZ_36	CF_3	NO_2	4.55±0.31	5.19	14.56
BZ_37	Cl	H	17.64±0.66	33.61	ND
BZ_38	Cl	Cl	12.11±0.42	15.38	5.36
BZ_39	Cl	CH_3	21.54±0.59	24.25	2.36
BZ_40	Cl	NO_2	7.33±0.36	14.99	5.48
Novobiocin			0.068±0.07	>200	9.36

MTB=*Mycobacterium tuberculosis*, [a] at 50 µM against RAW 264.7 cells, ND indicates not determined.

5.1c.5. Discussion

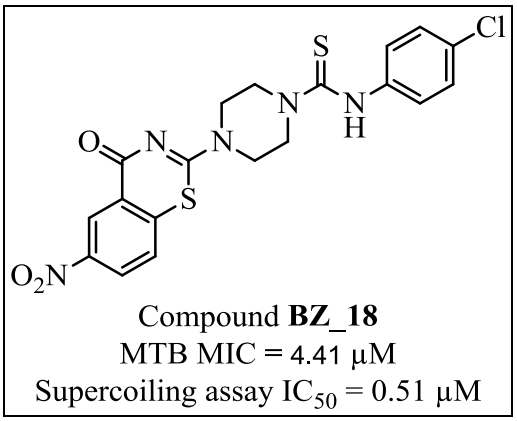
The supercoiling of the DNA is required to maintain the bacteria in viable state. All the thirty six inhibitors synthesized were screened for their effectivity to inhibit the supercoiling activity of the DNA gyrase. Among the tested compounds, (**18**) emerged at submicromolar inhibitory profile. The nitro group at the R_1 position and the chloro group at the R_2 positions were favourable for the effective inhibition of the benzothiazinone derivative with an IC_{50} of 0.51±0.16 µM. Compounds synthesized had various strong electron withdrawing substitutions like nitro, trifluoro and weak chloro groups, at the substituted R_1 position while the R2 was substituted with hydrogen, chloro, methyl and nitro groups. Among the twelve R_1substituted nitro groups, ten molecules showed an IC_{50} of less than 10 µM, only compound

BZ_05 and **BZ_06** had lower inhibition rates highlighting the importance of the nitro group substitution at this position. All the enzyme studies were carried out in the presence of standard drug novobiocin with an IC_{50} was 68 nM. Among the 36 compounds subjected to inhibitory assays, compound **BZ_18,** N-(4-chlorophenyl)-4-(6-nitro-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)piperazine-1-carbothioamide was the most potent analogue. About four compounds **BZ_17, BZ_18, BZ_19** and **BZ_21** showed nanomolar range inhibitions in the supercoiling assay as shown in Table 1. Furthermore, all the compounds were then subjected to a number of tertiary screens in order to assess their in-vitro anti-tubercular potency and cell cytotoxicity insights. Moreover, one of the major hurdles in target based drug discovery is the lack of translation of the drug potency and selectivity observed at the enzyme level to that of mycobacterial cidality. In order to re-ascertain the compounds efficiencies, molecules were first subjected to whole cell screening against *Mycobacterium tuberculosis* H37Rv strain using broth dilution method [Franzbalu G. S., *et al*., 1998] with compound concentrations ranging from 50 µg/mL to 1.56 µg/mL in triplicates. Isoniazid, rifampicin and ofloxacin were used as reference compounds for comparison of the synthesized drugs. Fifteen compounds showed an MIC of less than 10 µM concentration highlighting the importance of this series in the bactericidal activity. Compounds **BZ_17, BZ_19** and **BZ_21** had MIC less than 5 µM. The most active compound **BZ_18** showed 4.41 µM MIC probably due to the cell efflux pumps, while the compound **BZ_17** had a better MIC than **BZ_18** owing to its neutral R_2 positions H group. Further, all the synthesized compounds were further screened for their in-vitro cytotoxicity's in the RAW 264.7 mouse macrophage cell lines [Ferrari M., *et al*., 1990], since the predominant host cells for *Mycobacterium tuberculosis* are the lung macrophages.The concentration was set at 100 µM and the assay was done by the (4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. Compounds with an MIC of less than 15 µM alone were tested for the cytotoxicity studies. All those compounds tested showed below 25% cell toxicities. The most active compound **BZ_18** had 1.81% inhibition comparatively very less than the standard novobiocin. All the results are represented in **Table 5.6**.

5.1c.6. Highlights of the study

In our continuous efforts to discover novel antimicrobial compounds with anti-gyrase activity, we have described the discovery ofbenzothiazinone as gyrase inhibitors with potent *Mycobacterium tuberculosis* MIC and inhibitory profiles of the gyrase enzyme with well

correlatedStructural activity relationship and less cytotoxic effect. Among 36 compounds, compound **BZ_18** shows supercoiling IC_{50} of 0.51 µM and well correlating MIC of 4.41 µM against *Mycobacterium tuberculosis*. The compound was also found to devoid of cytotoxicity against RAW cell lines. Furthermore, we believe that this class of compounds has potential further to be developed as anti-TB drug candidate.



Compound **BZ_18**
MTB MIC = 4.41 µM
Supercoiling assay IC_{50} = 0.51 µM

Figure 5.11: Structure and activity of most active compound **BZ_18**.

5.2. Benzimidazole derivatives

Investigation of benzimidazole pharmacophore has recently generated significant interest from a medicinal chemistry point of view and their synthesis has been well explored in the literature.

Recently, our research group explored the e-pharmacophore approach, that efficiently utilizes the aspects of energy based and the ligand based techniques to identify putative ligands that bind to the active site of mycobacterial ATPase domain using the crystal structure of the pyrrolamides (PDB ID 4BAE) bound to *Mycobacterium smegmatis* GyrB ATPase as template [Communicated to ACS]. The pharmacophore hypotheses based on mapping of the energetic terms from the extra precision Glide scoring function (Glide XP) (Glide, version 5.7, Schrödinger, LLC, New York, NY) onto atom centers was employed to derive pharmacophore sites. These were based on the structural and energy information between the protein and the ligand using phase (Phase, v3.3, Schrödinger, LLC, New York, NY). The docking result (Xpdes) was then imported to find the structure-based pharmacophoric features, which would help in finding the best featured functional groups. In structure based design the energy contribution for binding of ligand to the protein plays a key factor in deriving pharmacophoric features. On the basis of their energy scores, a four (2R, 1N and 1D) and three (2R and 1N) feature pharmacophore was generated. These four point and three point pharmacophore were then utilized for the virtual screening of a commercial chemical database (**Asinex**, with a compound collection of 500,000 compounds) using a protocol summarized in **Figure 5.12**.

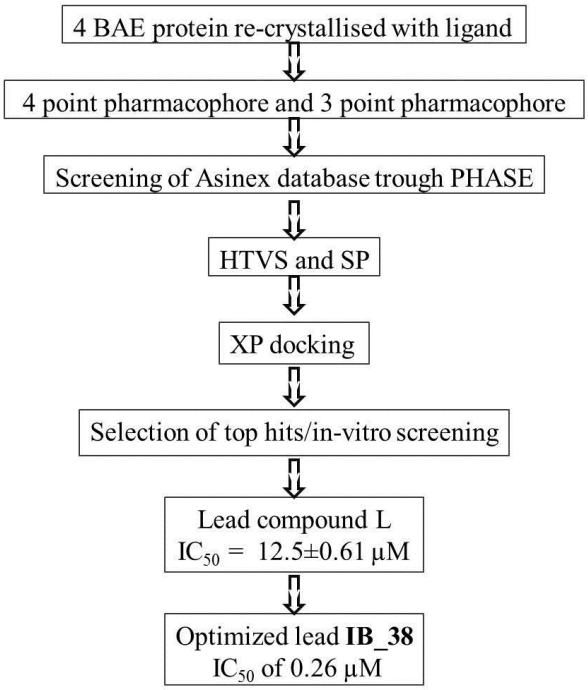
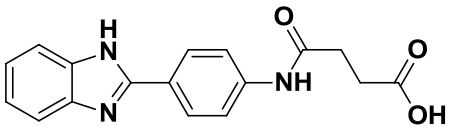


Figure 5.12: Workflow utilized for lead identification and optimization of putative ligands as mycobacterial DNA GyrB inhibitors.

Compounds retrieved by the e-pharmacophore model using phase with a fit value above 1.0 were regarded as potential hits and were carried forward for high-throughput virtual screening. Top compounds from this screen resulting in a score of ≥ -5.0 kcal mol^{-1} and docked with one or more hydrogen bonds were subjected to another round of docking by Glide XP. The Glide XP combines accurate, physics-based scoring terms and thorough sampling, and the results gave scores ranging from -9.15 to -6.50 kcal mol^{-1}. Final short listing of possible hit compounds was based on visual inspection of the important amino acid residues in the active site cleft involved in binding that included hydrogen bonds to Asp79, Arg141, and Arg82. The top 12 compounds retrieved from Asinex database in the Glide XP docking study were procured and experimentally screened for the in-vitro GyrB inhibitory potential using a malachite green assay adapted to a 96 well plate format as described in literature (**Figure 5.12**).



Lead L
IC_{50} = 12.5 ± 0.61 µM

Figure 5.13: Chemical structure and GyrB IC_{50} of initial hit compound L identified in this study from Asinex database.

One of the lead 4-((4-(1*H*-benzo[*d*]imidazol-2-yl)phenyl)amino)-4-oxobutanoic acid (**Lead L**) displaying GyrB IC_{50} of 12.5±0.61 µM was utilized as a structural framework for further optimization in order to improve its GyrB inhibition potency [Communicated to ACS].

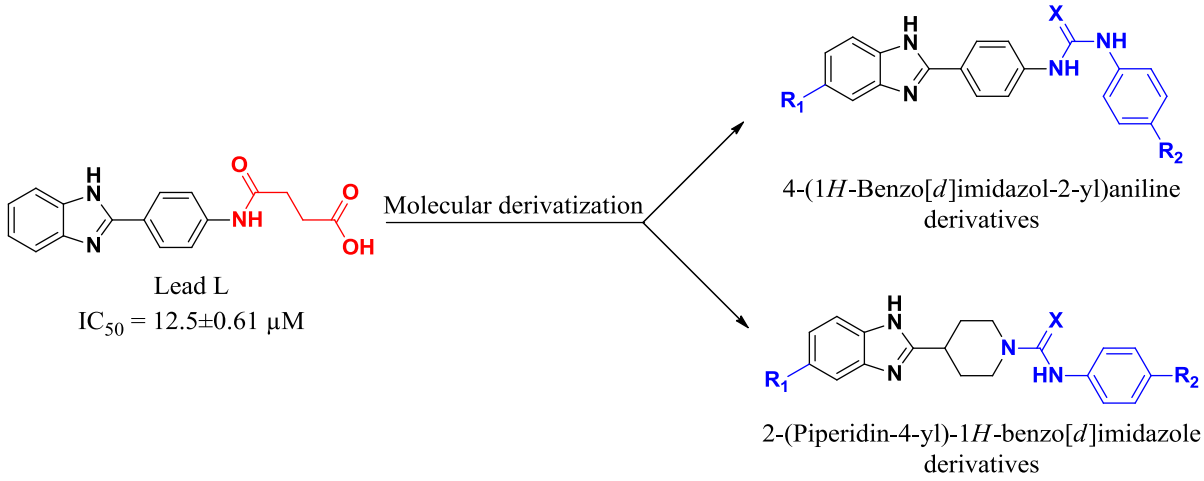


Figure 5.14: Molecular derivatization strategy.

Based on this and our prior experience in TB research, further modifications (and combinations thereof) were explored in a ligand expansion step through molecular derivatization for developing a strong SAR profile and also to understand the ideal site for introducing chemical diversity (**Figure 5.14**). The synthetic pathway used to achieve the lead modifications is delineated in **Figure 4.4** and **Figure 4.5**. Thus two different series of 4-(1*H*-benzo[*d*]imidazol-2-yl)aniline derivatives and 2-(piperidin-4-yl)-1*H*-benzo[*d*]imidazole derivatives was synthesized and evaluated for their ability to inhibit GyrB enzyme as step towards the derivation of SAR and hit optimization.

5.2a. Development of novel benzimidazole derivatives as *Mycobacterium tuberculosis* DNA GyrB inhibitors

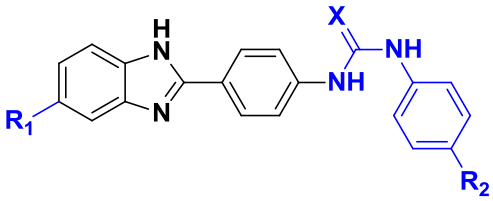


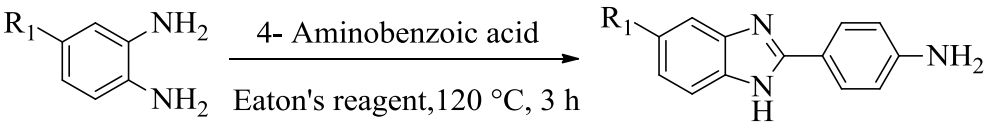
Figure 5.15: General structure of 4-(1*H*-benzo[*d*]imidazol-2-yl)aniline derivatives obtained from molecular derivatization strategy.

5.2a.1. Chemical synthesis

The synthetic pathway used to achieve the target compounds has been delineated in **Figure 4.4**. Synthesis of the compounds started with conversion of commercially available various 1,2-phenylenediamines and 4-amino benzoic acid in Eaton's reagent was heated at 130 °C for 5–6 h resulted in the formation of substituted benzimidazoles. The final library was then assembled by treating the obtained scaffolds with the desired isocyanates/isothiocyantes to afford compound **IB_03 - IB_42** in excellent yields. A series of 40 derivatives was synthesized using the above method in excellent yields.

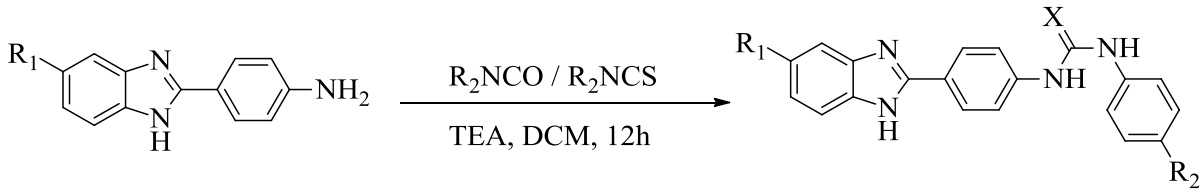
5.2a.2. Experimental protocol utilised for synthesis IB_03 – IB_42

General procedure for the synthesis of 4-(5-substituted-1H-benzo[d]imidazol-2-yl) aniline (IB_02a-d). Eaton's reagent (10 vol) was added drop wise to a well pulverised mixture of the corresponding 1, 2-phenylenediamine (1 equiv) and 4-amino benzoic acid (1 equiv) at 0°C. The reaction mixture was then heated at 130 °C for 5–6 h (monitored by TLC and LCMS for completion). The reaction mixture was cooled and neutralised with 10% sodium hydroxide solution to pH of 6–7, the precipitate formed was filtered and washed repeatedly with water and dried. The solid obtained was recrystallized from ethanol to afford the desired product (**IB_02a-d**) in good yield.



IB_01a-d **IB_02a-d**

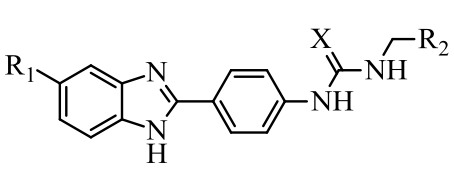
General procedure for the synthesis of 4-(6-substituted-4-oxo-4H-benzo[e][1,3]thiazin-2-yl)-N-arylpiperazine-1-carboxamide derivatives (IB_03 – IB_42). To a cooled solution of 6-substituted-2-(piperazin-1-yl)-4H-benzo[e][1,3]thiazin-4-one (1 mmol) in anhydrous DCM (2 mL) was added corresponding isocyanate (1 mmol), triethylamine (1mmol) and stirred the reaction mixture at room temperature for 12 hours (monitored by TLC & LCMS for completion), and solvent evaporated under reduced pressure. The residue was further diluted with water (30 mL) and ethyl acetate (50 mL) and the layers were separated. The organic layer was dried over anhydrous sodium sulphate and evaporated under reduced pressure. The residue was purified by silica gel column chromatography using hexane: ethylacetate as eluent to give the corresponding urea derivative **(IB_03 – IB_42)** in good yield.



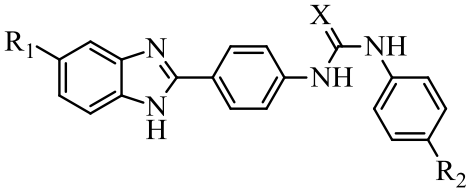
IB_02a-d **IB_03 - IB_42**

The physicochemical properties of synthesized derivatives are shown in **Table 5.7**.

Table 5.7: Physicochemical properties of synthesized compounds **(IB_03 – IB_42)**.



IB_03 - IB_10



IB_11 - IB_42

Compd	R_1	R_2	X	Yield (%)	M.P. (°C)	Molecular formula	Molecular weight
IB_03	-Cl	$-C_6H_5$	O	41	244-246	$C_{21}H_{17}ClN_4O$	376.84
IB_04	-F	$-C_6H_5$	O	43	289-291	$C_{21}H_{17}FN_4O$	360.38
IB_05	-H	$-C_6H_5$	O	52	231-233	$C_{21}H_{18}N_4O$	342.39
IB_06	$-OCH_3$	$-C_6H_5$	O	57	179-181	$C_{22}H_{20}N_4O_2$	372.42
IB_07	-Cl	$-C_6H_5$	S	68	259-261	$C_{21}H_{17}ClN_4S$	392.9

Contd.

Compd	R_1	R_2	X	Yield (%)	M.P. (°C)	Molecular formula	Molecular weight
IB_08	-F	$-C_6H_5$	S	66	289-291	$C_{21}H_{17}FN_4S$	376.45
IB_09	-H	$-C_6H_5$	S	72	278-280	$C_{21}H_{18}N_4S$	358.46
IB_10	$-OCH_3$	$-C_6H_5$	S	69	231-233	$C_{22}H_{20}N_4OS$	388.49
IB_11	-Cl	$-OCH_3$	O	74	277-279	$C_{21}H_{17}ClN_4O_2$	392.84
IB_12	-F	$-OCH_3$	O	46	213-215	$C_{21}H_{17}FN_4O_2$	376.38
IB_13	-H	$-OCH_3$	O	76	277-279	$C_{21}H_{18}N_4O_2$	358.39
IB_14	$-OCH_3$	$-OCH_3$	O	55	241-243	$C_{22}H_{20}N_4O_3$	388.42
IB_15	-Cl	$-OCH_3$	S	56	212-214	$C_{21}H_{17}ClN_4OS$	408.9
IB_16	-F	$-OCH_3$	S	81	232-234	$C_{21}H_{17}FN_4OS$	392.45
IB_17	-H	$-OCH_3$	S	68	290-292	$C_{21}H_{18}N_4OS$	374.46
IB_18	$-OCH_3$	$-OCH_3$	S	38	254-256	$C_{22}H_{20}N_4O_2S$	404.48
IB_19	-Cl	$-NO_2$	O	59	244-246	$C_{20}H_{14}ClN_5O_3$	407.81
IB_20	-F	$-NO_2$	O	48	296-298	$C_{20}H_{14}FN_5O_3$	391.36
IB_21	-H	$-NO_2$	O	67	187-189	$C_{20}H_{15}N_5O_3$	373.36
IB_22	$-OCH_3$	$-NO_2$	O	74	238-240	$C_{21}H_{17}N_5O_4$	403.39
IB_23	-Cl	$-NO_2$	S	56	279-281	$C_{20}H_{14}ClN_5O_2S$	423.88
IB_24	-F	$-NO_2$	S	84	275-277	$C_{20}H_{14}FN_5O_2S$	407.42
IB_25	-H	$-NO_2$	S	87	221-223	$C_{20}H_{15}N_5O_2S$	389.43
IB_26	$-OCH_3$	$-NO_2$	S	85	251-253	$C_{21}H_{17}N_5O_3S$	419.46
IB_27	-Cl	$-CH_3$	O	66	287-289	$C_{21}H_{17}ClN_4O$	376.84
IB_28	-F	$-CH_3$	O	53	229-231	$C_{21}H_{17}FN_4O$	360.38
IB_29	-H	$-CH_3$	O	55	211-213	$C_{21}H_{18}N_4O$	342.39
IB_30	$-OCH_3$	$-CH_3$	O	59	281-283	$C_{22}H_{20}N_4O_2$	372.42
IB_31	-Cl	$-CH_3$	S	84	241-243	$C_{21}H_{17}ClN_4S$	392.9
IB_32	-F	$-CH_3$	S	83	221-223	$C_{21}H_{17}FN_4S$	376.45
IB_33	-H	$-CH_3$	S	61	293-295	$C_{21}H_{18}N_4S$	358.46
IB_34	$-OCH_3$	$-CH_3$	S	68	263-265	$C_{22}H_{20}N_4OS$	388.49
IB_35	-Cl	-F	O	67	213-215	$C_{20}H_{14}ClFN_4O$	380.8

Contd.

Compd	R_1	R_2	X	Yield (%)	M.P. (°C)	Molecular formula	Molecular weight
IB_36	-F	-F	O	69	229-231	$C_{20}H_{14}F_2N_4O$	364.35
IB_37	-H	-F	O	54	198-200	$C_{20}H_{15}FN_4O$	346.36
IB_38	-OCH_3	-F	O	52	240-242	$C_{21}H_{17}FN_4O_2$	376.38
IB_39	-Cl	-F	S	72	261-263	$C_{20}H_{14}ClFN_4S$	396.87
IB_40	-F	-F	S	58	189-191	$C_{20}H_{14}F_2N_4S$	380.41
IB_41	-H	-F	S	63	221-223	$C_{20}H_{15}FN_4S$	362.42
IB_42	-OCH_3	-F	S	71	275-277	$C_{21}H_{17}FN_4OS$	392.45

5.2a.3. Characterization of synthesized compounds

A series of 40 derivatives were prepared using the above method and both analytical and spectral data (^{1}H NMR, ^{13}C NMR, and mass spectra) of all the synthesized compounds were in full agreement with the proposed structures.

4-(5-Chloro-1H-benzo[d]imidazol-2-yl)aniline (IB_02a).The compound was synthesized according to the general procedure using 4-chloro-1, 2-phenylenediamine (**IB_01a**) (1 g, 7.02 mmol), 4-amino benzoic acid (0.96 g, 7.02 mmol) and Eaton's reagent (10 mL) to afford **IB_02a** (1.1 g, 64% yield) as buff coloured solid. Mp: 291-293 °C. ^{1}H NMR (DMSO- d_6): δ_H 8.95 (s, 1H), 8.39–7.08 (m, 7H), 6.45 (s, 2H). ^{13}C NMR (DMSO- d_6): δ_C 153.1, 145.7, 133.2, 131.2, 129.6, 128.4 (2C), 124.3, 116.7, 116.3, 115.9 (2C), 115.2. ESI-MS m/z: 244 $[M+H]^+$. Anal. Calcd for $C_{13}H_{10}ClN_3$: C, 64.07; H, 4.14; N, 17.24. Found C, 64.11; H, 4.16; N, 17.19.

4-(5-Fluoro-1H-benzo[d]imidazol-2-yl)aniline (IB_02b). The compound was synthesized according to the general procedure using 4-fluoro-1, 2-phenylenediamine (**IB_01b**) (1 g, 7.93 mmol), 4-amino benzoic acid (1.09 g, 7.93 mmol) and Eaton's reagent (10 mL) to afford **IB_02b** (1.02 g, 56% yield) as brown solid. Mp: 242-244 °C. 1H NMR (DMSO- d_6): δ_H 8.95 (s, 1H), 8.09–7.01 (m, 7H), 6.33 (s, 2H). 13C NMR (DMSO- d_6): δ_C 156.6, 153.3, 145.6, 139.1, 137.8, 128.2 (2C), 116.8, 115.6 (2C), 110.3, 101.9. ESI-MS m/z: 228 $[M+H]^+$. Anal. Calcd for $C_{13}H_{10}FN_3$: C, 68.71; H, 4.44; N, 18.49. Found: C, 68.73; H, 4.42; N, 18.47.

4-(1H-Benzo[d]imidazol-2-yl)aniline (IB_02c). The compound was synthesized according to the general procedure using 1, 2-phenylenediamine (**IB_01c**) (1 g, 9.25 mmol), 4-amino benzoic acid (1.27 g, 9.25 mmol) and Eaton's reagent (10 mL) to afford **IB_02c** (1.42 g, 74%

yield) as pale brown solid. M.p: 259-261 °C. ^{1}H NMR (DMSO- d_6): δ_H 8.95 (s, 1H), 7.98–6.82 (m, 8H), 6.42 (s, 2H). ^{13}C NMR (DMSO- d_6): δ_C 152.8, 145.4, 141.5 (2C), 128.7 (2C), 123.5 (2C), 116.7, 115.5 (2C), 115.2 (2C). ESI-MS m/z: 210 $[M+H]^{+\cdot}$Anal. Calcd for $C_{13}H_{11}N_3$: C, 74.62; H, 5.30; N, 20.08. Found: C, 74.64; H, 5.33; N, 20.12.

4-(5-Methoxy-1H-benzo[d]imidazol-2-yl)aniline (IB_02d). The compound was synthesized according to the general procedure using 4-methoxy-1, 2-phenylenediamine (**IB_01d**) (1 g, 7.24 mmol), 4-amino benzoic acid (0.99 g, 7.24 mmol) and Eaton's reagent (10 mL) to afford **IB_02d** (0.91 g, 53%) as reddish brown solid. MP: 162-165 °C. ^{1}H NMR (DMSO- d_6): δ_H 8.95 (s, 1H), 8.13–7.04 (m, 7H), 6.51 (s, 2H), 3.85 (s, 3H). ^{13}C NMR (DMSO- d_6): δ_C 156.7, 153.4, 145.7, 139.5, 134.5, 128.4 (2C), 116.8, 115.3 (2C), 112.1, 111.2, 100.6, 56.1. ESI-MS m/z: 240 $[M+H]^+$. Anal. Calcd for $C_{14}H_{13}N_3O$: C, 70.28; H, 5.48; N, 17.56. Found: C, 70.31; H, 5.47; N, 17.58.

1-Benzyl-3-(4-(5-chloro-1H-benzo[d]imidazol-2-yl)phenyl)urea (IB_03). The compound was synthesized according to the general procedure using 4-(5-chloro-1*H*-benzo[*d*]imidazol-2-yl) aniline (**IB_02a**) (0.1 g, 0.34 mmol) and Benzyl isocyanate (0.04 g, 0.34 mmol) to afford **IB_03** (0.06 g, 41.47 %) as yellow solid. M.p: 244-246 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.95 (s, 1H), 8.83 (s, 1H), 8.08-7.19 (m, 13H), 4.25 (s, 2H). ^{13}C NMR (DMSO-d_6): δ_C 154.3, 152.9, 139.4, 137.9, 132.8, 130.9, 129.2, 128.5 (2C), 127.7 (2C), 126.9 (2C), 126.7, 124.1, 121.8, 119.7 (2C), 116.6, 115.8, 44.4. ESI-MS *m/z* 377 $[M+H]^+$. Anal.Calcd.For $C_{21}H_{17}ClN_4O$: C, 66.93; H, 4.55; N, 14.87; Found: C, 66.91; H, 4.52.15; N, 14.86.

1-Benzyl-3-(4-(5-fluoro-1H-benzo[d]imidazol-2-yl)phenyl)urea (IB_04). The compound was synthesized according to the general procedure using4-(5-fluoro-1*H*-benzo[*d*]imidazol-2-yl) aniline (**IB_02b**) (0.1 g, 0.34 mmol) and Benzyl isocyanate (0.05 g, 0.34 mmol) to afford **IB_04** (0.07 g, 43.21 %) as yellow solid. M.p: 289-291 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.92 (s, 1H), 8.85 (s, 1H), 8.06-7.16 (m, 13H), 4.24 (s, 2H).^{13}C NMR (DMSO-d_6): δ_C 156.5, 154.3, 152.9, 140.5, 139.4, 137.9, 137.3, 128.5 (2C), 127.7 (2C), 126.9 (2C), 126.7, 121.9, 119.7 (2C), 116.6, 109.9, 102.4, 44.4. ESI-MS *m/z* 361 $[M+H]^+$. Anal.Calcd.For $C_{21}H_{17}FN_4O$: C, 69.99; H, 4.75; N, 15.55; Found: C, 69.98; H, 4.74; N, 15.59.

1-(4-(1H-Benzo[d]imidazol-2-yl)phenyl)-3-benzylurea (IB_05). The compound was synthesized according to the general procedure using 4-(1*H*-benzo[*d*]imidazol-2-yl) aniline (**IB_02c**) (0.1 g, 0.34 mmol) and Benzyl isocyanate (0.04 g, 0.34 mmol) to afford **IB_05**

(0.06 g, 52.43 %) as yellow solid. M.p: 231-233 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.97 (s, 1H), 8.84 (s, 1H), 8.11-7.12 (m, 14H), 4.25 (s, 2H). ^{13}C NMR (DMSO-d_6): δ_C 154.3, 152.9, 141.7 (2C), 139.4, 137.9, 128.5 (2C), 127.7 (2C), 126.9 (2C), 126.7, 123(2C), 121.9, 119.1 (2C), 115.2 (2C), 44.4. ESI-MS *m/z* 343 $[M+H]^+$. Anal.Calcd.For $C_{21}H_{18}N_4O$: C, 73.67; H, 5.30; N, 16.36; Found: C, 73.69; H, 5.34; N, 16.33.

1-Benzyl-3-(4-(5-methoxy-1H-benzo[d]imidazol-2-yl)phenyl)urea (IB_06). The compound was synthesized according to the general procedure using 4-(5-methoxy-1*H*-benzo[*d*]imidazol-2-yl) aniline (**IB_02d**) (0.1 g, 0.34 mmol) and Benzyl isocyanate (0.05 g, 0.34 mmol) to afford **IB_06** (0.05 g, 57.89 %) as yellow solid. M.p: 179-181 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.98 (s, 1H), 8.87 (s, 1H), 8.09-7.14 (m, 13H), 4.25 (s, 2H), 3.89 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 156.1, 152.9, 154.3, 139.9, 139.4, 137.9, 134, 128.5 (2C), 127.7 (2C), 126.9 (2C), 126.7, 121.9, 119.7 (2C), 116.6, 115.8, 100.8, 55.8, 44.4. ESI-MS *m/z* 373 $[M+H]^+$. Anal.Calcd.For $C_{22}H_{20}N_4O_2$: C, 70.95; H, 5.41; N, 15.04; Found: C, 70.92; H, 5.44; N, 15.06.

1-Benzyl-3-(4-(5-chloro-1H-benzo[d]imidazol-2-yl)phenyl)thiourea (IB_07). The compound was synthesized according to the general procedure using 4-(5-chloro-1*H*-benzo[*d*]imidazol-2-yl) aniline (**IB_02a**) (0.1 g, 0.31 mmol) and Benzyl isothiocyanate (0.03 g, 0.31 mmol) to afford **IB_07** (0.08 g, 68.41 %) as white solid. M.p: 259-261 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.87 (s, 1H), 8.79 (s, 1H), 8.07-7.18 (m, 13H), 4.26 (s, 2H). ^{13}C NMR (DMSO-d_6): δ_C 179.5, 152.9, 138.5, 137.9, 132.8, 130.9, 129.2, 128.5 (2C), 127.8 (2C), 126.9 (2C), 126.7, 124.6 (2C), 124.1, 122.3, 116.6, 115.8, 50.8. ESI-MS *m/z* 393 $[M+H]^+$. Anal.Calcd.For $C_{21}H_{17}ClN_4S$: C, 64.19; H, 4.36; N, 14.26; Found: C, 64.16; H, 4.35; N, 14.28.

1-Benzyl-3-(4-(5-fluoro-1H-benzo[d]imidazol-2-yl)phenyl)thiourea (IB_08). The compound was synthesized according to the general procedure using 4-(5-fluoro-1*H*-benzo[*d*]imidazol-2-yl) aniline (**IB_02b**) (0.1 g, 0.31 mmol) and Benzyl isothiocyanate (0.04 g, 0.31 mmol) to afford **IB_08** (0.06 g, 66.15 %) as white solid. M.p: 289-291 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.86 (s, 1H), 8.78 (s, 1H), 8.07-7.16 (m, 13H), 4.26 (s, 2H). ^{13}C NMR (DMSO-d_6): δ_C 179.5, 156.5, 152.9, 140.5, 138.5, 137.9, 137.3, 128.5 (2C), 127.8 (2C), 126.9 (2C), 126.7, 124.6 (2C), 122.3, 116.8, 109.9, 102.4, 50.8. ESI-MS *m/z* 377 $[M+H]^+$. Anal.Calcd.For $C_{21}H_{17}FN_4S$: C, 67.00; H, 4.55; N, 14.88; Found: C, 67.02; H, 4.53; N, 14.87.

1-(4-(1H-Benzo[d]imidazol-2-yl)phenyl)-3-benzylthiourea (IB_09). The compound was synthesized according to the general procedure using 4-(1*H*-benzo[*d*]imidazol-2-yl) aniline (**IB_02c**) (0.1 g, 0.31 mmol) and Benzyl isothiocyanate (0.04 g, 0.31 mmol) to afford **IB_09** (0.07 g, 72.52 %) as white solid. M.p: 278-279 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.98 (s, 1H), 8.83 (s, 1H), 8.11-7.13 (m, 14H), 4.26 (s, 2H). ^{13}C NMR (DMSO-d_6): δ_C 179.5, 152.9, 141.7 (2C), 138.5, 137.9, 128.5 (2C), 127.8 (2C), 126.9 (2C), 126.7, 124.6 (2C), 123 (2C), 122.3, 115.2 (2C), 50.8. ESI-MS *m/z* 359 $[M+H]^+$. Anal.Calcd.For $C_{21}H_{18}N_4S$: C, 70.36; H, 5.06; N, 15.63; Found: C, 70.38; H, 5.04; N, 15.65.

1-Benzyl-3-(4-(5-methoxy-1H-benzo[d]imidazol-2-yl)phenyl)thiourea (IB_10). The compound was synthesized according to the general procedure using 4-(5-methoxy-1*H*-benzo[*d*]imidazol-2-yl) aniline (**IB_02d**) (0.1 g, 0.31 mmol) and Benzyl isothiocyanate (0.05 g, 0.31 mmol) to afford **IB_10** (0.07 g, 69.43 %) as white solid. M.p: 231-233 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.97 (s, 1H), 8.84 (s, 1H), 8.18-7.17 (m, 13H), 4.26 (s, 2H), 3.82 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 179.5, 156.1, 152.9, 139.9, 138.5, 137.9, 134, 128.5 (2C), 127.8 (2C), 126.9 (2C), 126.7, 124.6 (2C), 122.3, 116.2, 111.5, 100.8, 55.8, 50.8. ESI-MS *m/z* 389 $[M+H]^+$. Anal.Calcd.For $C_{22}H_{20}N_4OS$: C, 68.02; H, 5.19; N, 14.42; Found: C, 68.03; H, 5.16; N, 14.44.

1-(4-(5-Chloro-1H-benzo[d]imidazol-2-yl)phenyl)-3-(4-methoxyphenyl)urea (IB_11). The compound was synthesized according to the general procedure using 4-(5-chloro-1*H*-benzo[*d*]imidazol-2-yl) aniline (**IB_02a**) (0.1 g, 0.35 mmol) and 4-methoxyphenyl isocyanate (0.04 g, 0.35 mmol) to afford **IB_11** (0.06 g, 74.41 %) as white solid. M.p: 277-279 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.92 (s, 1H), 8.83 (s, 1H), 8.07-7.21 (m, 12H), 3.87 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 158.9, 152.9 (2C), 139.4, 132.5, 131.7, 130.9, 129.2, 127.7 (2C), 124.1, 121.9, 119.8 (2C), 119.7 (2C), 116.6, 115.8, 114.5 (2C), 55.8. ESI-MS *m/z* 393 $[M+H]^+$. Anal.Calcd.For $C_{21}H_{17}ClN_4O_2$: C, 64.21; H, 4.36; N, 14.26; Found: C, 64.19; H, 4.38; N, 14.25.

1-(4-(5-Fluoro-1H-benzo[d]imidazol-2-yl)phenyl)-3-(4-methoxyphenyl)urea **(IB_12).** The compound was synthesized according to the general procedure using 4-(5-fluoro-1*H*-benzo[*d*]imidazol-2-yl) aniline (**IB_02b**) (0.1 g, 0.35 mmol) and 4-methoxyphenyl isocyanate (0.05 g, 0.35 mmol) to afford **IB_12** (0.05 g, 46.82 %) as white solid. M.p: 213-215 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.94 (s, 1H), 8.83 (s, 1H), 8.08-7.23 (m, 12H), 3.87 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 158.9, 156.5, 152.9 (2C), 140.5, 139.4, 137.3, 131.7, 127.7 (2C), 121.9, 119.8

(2C), 119.7 (2C), 116.8, 114.5 (2C), 109.9, 102.4, 55.8. ESI-MS *m/z* 377 $[M+H]^+$. Anal.Calcd.For $C_{21}H_{17}FN_4O_2$: C, 67.01; H, 4.55; N, 14.89; Found: C, 67.03; H, 4.58; N, 14.85.

1-(4-(1H-Benzo[d]imidazol-2-yl)phenyl)-3-(4-methoxyphenyl)urea (IB_13). The compound was synthesized according to the general procedure using 4-(1*H*-benzo[*d*]imidazol-2-yl) aniline (**IB_02c**) (0.1 g, 0.35 mmol) and 4-methoxyphenyl isocyanate (0.04 g, 0.35 mmol) to afford **IB_13** (0.08 g, 76.84 %) as white solid. M.p: 277-279 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.95 (s, 1H), 8.83 (s, 1H), 8.09-7.16 (m, 13H), 3.87 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 158.9, 152.9 (2C), 141.7 (2C), 139.4, 131.7, 127.7 (2C), 123 (2C), 121.9, 119.8 (2C), 119.7 (2C), 115.2 (2C), 114.5 (2C), 55.8. ESI-MS *m/z* 359 $[M+H]^+$. Anal.Calcd.For $C_{21}H_{18}N_4O_2$: C, 70.38; H, 5.06; N, 15.63; Found: C, 70.35; H, 5.09; N, 15.60.

1-(4-(5-Methoxy-1H-benzo[d]imidazol-2-yl)phenyl)-3-(4-methoxyphenyl)urea (IB_14). The compound was synthesized according to the general procedure using 4-(5-methoxy-1*H*-benzo[*d*]imidazol-2-yl) aniline (**IB_02d**) (0.1 g, 0.35 mmol) and 4-methoxyphenyl isocyanate (0.05 g, 0.35 mmol) to afford **IB_14** (0.08 g, 55.82 %) as white solid. M.p: 241-242 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.94 (s, 1H), 8.82 (s, 1H), 8.08-7.15 (m, 12H), 3.87 (s, 6H). ^{13}C NMR (DMSO-d_6): δ_C 158.9, 156.1, 152.9 (2C), 139.9, 139.4, 134, 131.7, 127.7 (2C), 121.9, 119.8 (2C), 119.7 (2C), 116.2, 114.5 (2C), 111.5, 100.8, 55.8 (2C). ESI-MS *m/z* 389 $[M+H]^+$. Anal.Calcd.For $C_{22}H_{20}N_4O_3$: C, 68.03; H, 5.19; N, 14.42; Found: C, 68.02; H, 5.15; N, 14.44.

1-(4-(5-Chloro-1H-benzo[d]imidazol-2-yl)phenyl)-3-(4-methoxyphenyl)thiourea (IB_15). The compound was synthesized according to the general procedure using 4-(5-chloro-1*H*-benzo[*d*]imidazol-2-yl) aniline (**IB_2a**) (0.1 g, 0.34 mmol) and 4-methoxyphenyl isothiocyanate (0.04 g, 0.34 mmol) to afford **IB_15** (0.07 g, 56.50 %) as yellow solid. M.p: 212-214 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.92 (s, 1H), 8.81 (s, 1H), 8.09-7.19 (m, 12H), 3.86 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 179.9, 159.3, 152.9, 138.5, 132.8, 130.9, 130.8, 129.2, 127.8 (2C), 127.5 (2C), 124.6 (2C), 124.1, 122.3, 116.6, 115.8, 114.6 (2C), 55.8. ESI-MS *m/z* 409 $[M+H]^+$. Anal.Calcd.For $C_{21}H_{17}ClN_4OS$: C, 61.68; H, 4.19; N, 13.70; Found: C, 61.64; H, 4.17; N, 13.68.

1-(4-(5-Fluoro-1H-benzo[d]imidazol-2-yl)phenyl)-3-(4-methoxyphenyl)thiourea (IB_16). The compound was synthesized according to the general procedure using 4-(5-fluoro-1*H*-benzo[*d*]imidazol-2-yl) aniline (**IB_02b**) (0.1 g, 0.34 mmol) and 4-methoxyphenyl

isothiocyanate (0.05 g, 0.34 mmol) to afford **IB_16** (0.08 g, 81.22 %) as yellow solid. M.p: 232-234 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.92 (s, 1H), 8.82 (s, 1H), 8.10-7.18 (m, 12H), 3.86 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 179.9, 159.3, 156.5, 152.9, 140.5, 138.5, 137.3, 130.8, 127.8 (2C), 127.5 (2C), 124.6 (2C), 122.3, 116.8, 114.6 (2C), 109.9, 102.4, 55.8. ESI-MS *m/z* 393 $[M+H]^+$. Anal.Calcd.For $C_{21}H_{17}FN_4OS$: C, 64.27; H, 4.37; N, 14.28; Found: C, 64.31; H, 4.35; N, 14.26.

1-(4-(1H-Benzo[d]imidazol-2-yl)phenyl)-3-(4-methoxyphenyl)thiourea (IB_17). The compound was synthesized according to the general procedure using 4-(1*H*-benzo[*d*]imidazol-2-yl) aniline (**IB_02c**) (0.1 g, 0.34 mmol) and 4-methoxyphenyl isothiocyanate (0.05 g, 0.34 mmol) to afford **IB_17** (0.07 g, 68.34 %) as yellow solid. M.p: 290-292 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.96 (s, 1H), 8.83 (s, 1H), 8.12-7.17 (m, 13H), 3.86 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 179.9, 159.3, 152.9, 141.7 (2C), 138.5, 130.8, 127.8 (2C), 127.5 (2C), 124.6 (2C), 123 (2C), 122.3, 115.2 (2C), 114.6 (2C), 55.8. ESI-MS *m/z* 375 $[M+H]^+$. Anal.Calcd.For $C_{21}H_{18}N_4OS$: C, 67.36; H, 4.85; N, 14.96; Found: C, 67.39; H, 4.83; N, 14.95.

1-(4-(5-Methoxy-1H-benzo[d]imidazol-2-yl)phenyl)-3-(4-methoxyphenyl) thiourea (IB_18). The compound was synthesized according to the general procedure using 4-(5-methoxy-1*H*-benzo[*d*]imidazol-2-yl) aniline (**IB_2d**) (0.1 g, 0.34 mmol) and 4-methoxyphenyl isothiocyanate (0.06 g, 0.34 mmol) to afford **IB_18** (0.05 g, 38.62 %) as yellow solid. M.p: 254-256 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.94 (s, 1H), 8.85 (s, 1H), 8.13-7.19 (m, 12H), 3.86 (s, 6H). ^{13}C NMR (DMSO-d_6): δ_C 179.9, 159.3, 156.1, 152.9, 139.9, 138.5, 134, 130.8, 127.8 (2C), 127.5 (2C), 122.3, 124.6 (2C), 116.2, 114.6 (2C), 111.5, 100.8, 55.8 (2C). ESI-MS *m/z* 405 $[M+H]^+$. Anal.Calcd.For $C_{22}H_{20}N_4O_2S$: C, 65.33; H, 4.98; N, 13.85; Found: C, 65.35; H, 4.95; N, 13.84.

1-(4-(5-Chloro-1H-benzo[d]imidazol-2-yl)phenyl)-3-(4-nitrophenyl)urea (IB_19). The compound was synthesized according to the general procedure using 4-(5-chloro-1*H*-benzo[*d*]imidazol-2-yl) aniline (**IB_02a**) (0.1 g, 0.31 mmol) and 4-nitrophenyl isocyanate (0.04 g, 0.31 mmol) to afford **IB_19** (0.07 g, 59.57 %) as white solid. M.p: 244-246 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.89 (s, 1H), 8.79 (s, 1H), 8.07-7.21 (m, 12H). ^{13}C NMR (DMSO-d_6): δ_C 152.9 (2C), 145.5, 143.5, 139.4, 132.8, 130.9, 129.2, 127.7 (2C), 124.1 (3C), 121.9, 119.9 (2C), 119.7 (2C), 116.6, 115.8. ESI-MS *m/z* 408 $[M+H]^+$. Anal.Calcd.For $C_{20}H_{14}ClN_5O_3$: C, 58.90; H, 3.46; N, 17.17; Found: C, 58.89; H, 3.42; N, 17.19.

1-(4-(5-Fluoro-1H-benzo[d]imidazol-2-yl)phenyl)-3-(4-nitrophenyl)urea (IB_20). The compound was synthesized according to the general procedure using 4-(5-fluoro-1*H*-benzo[*d*]imidazol-2-yl) aniline (**IB_02b**) (0.1 g, 0.31 mmol) and 4-nitrophenyl isocyanate (0.05 g, 0.31 mmol) to afford **IB_20** (0.06 g, 48.82 %) as white solid. M.p: 296-298 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.88 (s, 1H), 8.79 (s, 1H), 8.08-7.20 (m, 12H). ^{13}C NMR (DMSO-d_6): δ_C 156.5, 152.9 (2C), 145.5, 143.5, 140.5, 139.4, 137.3, 127.7 (2C), 124.1 (2C), 121.9, 119.9 (2C), 119.7 (2C), 116.8, 109.9, 102.4. ESI-MS *m/z* 392 $[M+H]^+$. Anal.Calcd.For $C_{20}H_{14}FN_5O_3$: C, 61.38; H, 3.61; N, 17.90; Found: C, 61.41; H, 3.58; N, 17.92.

1-(4-(1H-Benzo[d]imidazol-2-yl)phenyl)-3-(4-nitrophenyl)urea (IB_21). The compound was synthesized according to the general procedure using 4-(1*H*-benzo[*d*]imidazol-2-yl) aniline (**IB_02c**) (0.1 g, 0.31 mmol) and 4-nitrophenyl isocyanate (0.04 g, 0.31 mmol) to afford **IB_21** (0.06 g, 67.29 %) as white solid. M.p: 187-189 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.92 (s, 1H), 8.80 (s, 1H), 8.09-7.18 (m, 13H). ^{13}C NMR (DMSO-d_6): δ_C 152.9 (2C), 145.5, 143.5, 141.7 (2C), 139.4, 127.7 (2C), 124.1 (2C), 123 (2C), 121.9, 119.9 (2C), 119.7 (2C), 115.2 (2C). ESI-MS *m/z* 374 $[M+H]^+$. Anal.Calcd.For $C_{20}H_{15}N_5O_3$: C, 64.34; H, 4.05; N, 18.76; Found: C, 64.32; H, 4.06; N, 18.77.

1-(4-(5-Methoxy-1H-benzo[d]imidazol-2-yl)phenyl)-3-(4-nitrophenyl)urea (IB_22). The compound was synthesized according to the general procedure using 4-(5-methoxy-1*H*-benzo[*d*]imidazol-2-yl) aniline (**IB_02d**) (0.1 g, 0.31 mmol) and 4-nitrophenyl isocyanate (0.05 g, 0.31 mmol) to afford **IB_22** (0.08 g, 74.16 %) as white solid. M.p: 238-240 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.91 (s, 1H), 8.81 (s, 1H), 8.09-7.19 (m, 12H), 3.86 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 156.1, 152.9 (2C), 145.5, 143.5, 139.9, 139.4, 134, 127.7 (2C), 124.1 (2C), 121.9, 119.9 (2C), 119.7 (2C), 116.2, 111.5, 100.8, 55.8. ESI-MS *m/z* 404 $[M+H]^+$. Anal.Calcd.For $C_{21}H_{17}N_5O_4$: C, 62.53; H, 4.25; N, 13.36; Found: C, 62.50; H, 4.22; N, 13.37.

1-(4-(5-Chloro-1H-benzo[d]imidazol-2-yl)phenyl)-3-(4-nitrophenyl)thiourea (IB_23). The compound was synthesized according to the general procedure using 4-(5-chloro-1*H*-benzo[*d*]imidazol-2-yl) aniline (**IB_02a**) (0.1 g, 0.35 mmol) and 4-nitrophenyl isothiocyanate (0.04 g, 0.35 mmol) to afford **IB_23** (0.05 g, 56.47 %) as white solid. M.p: 279-281 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.93 (s, 1H), 8.84 (s, 1H), 8.11-7.23 (m, 12H). ^{13}C NMR (DMSO-d_6): δ_C 179.9, 152.9, 144.6, 143.9, 138.5, 132.8, 130.9, 129.2, 127.8 (2C), 124.8 (2C), 124.6 (2C), 124.2 (2C), 124.1, 122.3, 116.6, 115.8. ESI-MS *m/z* 424 $[M+H]^+$. Anal.Calcd.For $C_{20}H_{14}ClN_5O_2S$: C, 56.67; H, 3.33; N, 16.52; Found: C, 56.65; H, 3.34; N, 16.54.

1-(4-(5-Fluoro-1H-benzo[d]imidazol-2-yl)phenyl)-3-(4-nitrophenyl)thiourea (IB_24). The compound was synthesized according to the general procedure using 4-(5-fluoro-1*H*-benzo[*d*]imidazol-2-yl) aniline (**IB_02b**) (0.1 g, 0.35 mmol) and 4-nitrophenyl isothiocyanate (0.05 g, 0.35 mmol) to afford **IB_24** (0.08 g, 84.34 %) as white solid. M.p: 275-277 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.92 (s, 1H), 8.83 (s, 1H), 8.12-7.22 (m, 12H). ^{13}C NMR (DMSO-d_6): δ_C 179.9, 156.5, 152.9, 144.6, 143.9, 140.5, 138.5, 137.3, 127.8 (2C), 124.8 (2C), 124.6 (2C), 124.2 (2C), 122.3, 116.8, 109.9, 102.4. ESI-MS *m/z* 408 $[M+H]^+$. Anal.Calcd.For $C_{20}H_{14}FN_5O_2S$: C, 58.96; H, 3.46; N, 17.19; Found: C, 58.97; H, 3.49; N, 17.21.

1-(4-(1H-Benzo[d]imidazol-2-yl)phenyl)-3-(4-nitrophenyl)thiourea (IB_25). The compound was synthesized according to the general procedure using 4-(1*H*-benzo[*d*]imidazol-2-yl) aniline (**IB_02c**) (0.1 g, 0.35 mmol) and 4-nitrophenyl isothiocyanate (0.05 g, 0.35 mmol) to afford **IB_25** (0.08 g, 87.73 %) as white solid. M.p: 221-223 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.94 (s, 1H), 8.84 (s, 1H), 8.11-7.21 (m, 13H). ^{13}C NMR (DMSO-d_6): δ_C 179.9, 152.9, 144.6, 143.9, 141.7 (2C), 138.5, 127.8 (2C), 124.8 (2C), 124.6 (2C), 124.2 (2C), 123 (2C), 122.3, 115.2 (2C). ESI-MS *m/z* 390 $[M+H]^+$. Anal.Calcd.For $C_{20}H_{15}N_5O_2S$: C, 61.68; H, 3.88; N, 17.98; Found: C, 61.71; H, 3.85; N, 17.97.

1-(4-(5-Methoxy-1H-benzo[d]imidazol-2-yl)phenyl)-3-(4-nitrophenyl)thiourea (IB_26). The compound was synthesized according to the general procedure using 4-(5-methoxy-1*H*-benzo[*d*]imidazol-2-yl) aniline (**IB_02d**) (0.1 g, 0.35 mmol) and 4-nitrophenyl isothiocyanate (0.06 g, 0.35 mmol) to afford **IB_26** (0.85 g, 85.45 %) as white solid. M.p: 251-253 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.93 (s, 1H), 8.85 (s, 1H), 8.10-7.21 (m, 12H), 3.86 (s, 3H). ^{13}C NMR δ_C 179.9, 156.1, 152.9, 144.6, 143.9, 139.9, 138.5, 134, 127.8 (2C), 124.8 (2C), 124.6 (2C), 124.2 (2C), 122.3, 116.2, 111.5, 100.8, 55.8. ESI-MS *m/z* 420 $[M+H]^+$. Anal.Calcd.For $C_{21}H_{17}N_5O_3S$: C, 60.13; H, 4.09; N, 16.70; Found: C, 60.14; H, 4.11; N, 16.68.

1-(4-(5-Chloro-1H-benzo[d]imidazol-2-yl)phenyl)-3-(p-tolyl)urea (IB_27). The compound was synthesized according to the general procedure using 4-(5-chloro-1*H*-benzo[*d*]imidazol-2-yl) aniline (**IB_02a**) (0.1 g, 0.34 mmol), and *p*-tolyl isocyanate (0.02 g, 0.34 mmol) to afford **IB_27** (0.06 g, 66.36 %) as yellow solid. M.p: 287-289 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.92 (s, 1H), 8.85 (s, 1H), 8.13-7.25 (m, 12H), 2.52 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 152.9 (2C), 139.4, 136.8, 136.4, 132.8, 130.9, 129.2 (3C), 127.7 (2C), 124.1, 121.9, 121.5 (2C), 119.7 (2C), 116.6, 115.8, 21.3. ESI-MS *m/z* 377 $[M+H]^+$. Anal.Calcd.For $C_{21}H_{17}ClN_4O$: C, 66.93; H, 4.55; N, 14.87; Found: C, 66.96; H, 4.51; N, 14.86.

1-(4-(5-Fluoro-1H-benzo[d]imidazol-2-yl)phenyl)-3-(p-tolyl)urea (IB_28). The compound was synthesized according to the general procedure using 4-(5-fluoro-1*H*-benzo[*d*]imidazol-2-yl) aniline (**IB_02b**) (0.1 g, 0.34 mmol) and *p*-tolyl isocyanate (0.02 g, 0.34 mmol) to afford **IB_28** (0.06 g, 53.22 %) as yellow solid. M.p: 229-231 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.93 (s, 1H), 8.84 (s, 1H), 8.12-7.26 (m, 12H), 2.52 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 156.5, 152.9 (2C), 140.5, 139.4, 137.3, 136.8, 136.4, 129.2 (2C), 127.7 (2C), 121.9, 121.5 (2C), 119.7 (2C), 116.8, 109.9, 102.4, 21.3. ESI-MS *m/z* 361 $[M+H]^+$. Anal.Calcd.For $C_{21}H_{17}FN_4O$: C, 69.99; H, 4.75; N, 15.55; Found: C, 69.96; H, 4.78; N, 15.53.

1-(4-(1H-Benzo[d]imidazol-2-yl)phenyl)-3-(p-tolyl)urea (IB_29). The compound was synthesized according to the general procedure using 4-(1*H*-benzo[*d*]imidazol-2-yl) aniline (**IB_02c**) (0.1 g, 0.34 mmol), and *p*-tolyl isocyanate (0.02 g, 0.34 mmol) to afford **IB_29** (0.05 g, 55.08 %) as yellow solid. M.p: 211-213 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.93 (s, 1H), 8.83 (s, 1H), 8.13-7.27 (m, 13H), 2.86 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 152.9 (2C), 141.7 (2C), 139.4, 136.8, 136.4, 129.2 (2C), 127.7 (2C), 123 (2C), 121.9, 121.5 (2C), 119.7 (2C), 115.2 (2C), 21.3. ESI-MS *m/z* 343 $[M+H]^+$. Anal.Calcd.For $C_{21}H_{18}N_4O$: C, 73.67; H, 5.30; N, 16.36; Found: C, 73.64; H, 5.33; N, 16.35.

1-(4-(5-Methoxy-1H-benzo[d]imidazol-2-yl)phenyl)-3-(p-tolyl)urea (IB_30). The compound was synthesized according to the general procedure using 4-(5-methoxy-1*H*-benzo[*d*]imidazol-2-yl) aniline (**IB_02d**) (0.1 g, 0.34 mmol), and *p*-tolyl isocyanate (0.02 g, 0.34 mmol) to afford **IB_30** (0.05 g, 59.08 %) as yellow solid. M.p: 281-283 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.92 (s, 1H), 8.82 (s, 1H), 8.12-7.22 (m, 12H), 3.86 (s, 3H), 2.52 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 156.1, 152.9 (2C), 139.9, 139.4, 136.8, 136.4, 134, 129.2 (2C), 127.7 (2C), 121.9, 121.5 (2C), 119.7 (2C), 116.6, 111.5, 100.8, 55.8, 21.3. ESI-MS *m/z* 373 $[M+H]^+$. Anal.Calcd.For $C_{22}H_{20}N_4O_2$: C, 70.95; H, 5.41; N, 15.04; Found: C, 70.92; H, 5.39; N, 15.02.

1-(4-(5-Chloro-1H-benzo[d]imidazol-2-yl)phenyl)-3-(p-tolyl)thiourea (IB_31). The compound was synthesized according to the general procedure using 4-(5-chloro-1*H*-benzo[*d*]imidazol-2-yl) aniline (**IB_02a**) (0.1 g, 0.31 mmol), and *p*-tolyl isothiocyanate (0.02 g, 0.31 mmol) to afford **IB_31** (0.08 g, 84.57 %) as white solid. M.p: 241-243 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.93 (s, 1H), 8.81 (s, 1H), 8.10-7.23 (m, 12H), 2.52 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 179.9, 152.9, 138.5, 137.2, 135.5, 132.8, 130.9, 129.3 (2C), 129.2, 127.8 (2C), 126.4 (2C), 124.6 (2C), 124.1, 122.3, 116.6, 115.8, 21.3. ESI-MS *m/z* 393 $[M+H]^+$.

Anal.Calcd.For $C_{21}H_{17}ClN_4S$: C, 64.19; H, 4.36; N, 14.26; Found: C, 64.22; H, 4.39; N, 14.25.

1-(4-(5-Fluoro-1H-benzo[d]imidazol-2-yl)phenyl)-3-(p-tolyl)thiourea (IB_32). The compound was synthesized according to the general procedure using 4-(5-fluoro-1*H*-benzo[*d*]imidazol-2-yl) aniline (**IB_02b**) (0.1 g, 0.31 mmol), and *p*-tolyl isothiocyanate (0.02 g, 0.31 mmol) to afford **IB_32** (0.08 g, 83.15 %) as white solid. M.p: 221-223 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.91 (s, 1H), 8.82 (s, 1H), 8.11-7.24 (m, 12H), 2.52 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 179.9, 156.5, 152.9, 140.5, 138.5, 137.3, 137.2, 135.5, 129.3 (2C), 127.8 (2C), 126.4 (2C), 124.6 (2C), 122.3, 116.8, 109.9, 102.4, 21.3. ESI-MS *m/z* 377 $[M+H]^+$. Anal.Calcd.For $C_{21}H_{17}FN_4S$: C, 67.00; H, 4.55; N, 14.88; Found: C, 67.02; H, 4.58; N, 14.85.

1-(4-(1H-Benzo[d]imidazol-2-yl) phenyl)-3-(p-tolyl)thiourea **(IB_33).** The compound was synthesized according to the general procedure using 4-(1*H*-benzo[*d*]imidazol-2-yl) aniline (**IB_02c**) (0.1 g, 0.31 mmol), and *p*-tolyl isothiocyanate (0.02 g, 0.31 mmol) to afford **IB_33** (0.06 g, 61.18 %) as white solid. M.p: 293-295 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.93 (s, 1H), 8.83 (s, 1H), 8.13-7.26 (m, 13H), 2.52 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 179.9, 152.9, 141.7 (2C), 138.5, 137.2, 135.5, 129.3 (2C), 127.8 (2C), 126.4 (2C), 124.6 (2C), 123 (2C), 122.3, 115.2 (2C), 21.3. ESI-MS *m/z* 359 $[M+H]^+$. Anal.Calcd.For $C_{21}H_{18}N_4S$: C, 70.36; H, 5.06; N, 15.63; Found: C, 70.35; H, 5.09; N, 15.61.

1-(4-(5-Methoxy-1H-benzo[d]imidazol-2-yl)phenyl)-3-(p-tolyl)thiourea (IB_34). The compound was synthesized according to the general procedure using 4-(5-methoxy-1*H*-benzo[*d*]imidazol-2-yl) aniline (**IB_02d**) (0.1 g, 0.31 mmol), and *p*-tolyl isothiocyanate (0.02 g, 0.31 mmol) to afford **IB_34** (0.08 g, 68.56 %) as white solid. M.p: 263-265 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.92 (s, 1H), 8.81 (s, 1H), 8.14-7.24 (m, 12H), 3.86 (s, 3H), 2.52 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 179.9, 156.1, 152.9, 139.9, 138.5, 137.2, 135.5, 134, 129.3 (2C), 127.8 (2C), 126.4 (2C), 124.6 (2C), 122.3, 116.2, 115.5, 100.8, 55.8, 21.3. ESI-MS *m/z* 389 $[M+H]^+$. Anal.Calcd.For $C_{22}H_{20}N_4OS$: C, 68.02; H, 5.19; N, 14.42; Found: C, 68.05; H, 5.22; N, 14.43.

1-(4-(5-Chloro-1H-benzo[d]imidazol-2-yl)phenyl)-3-(4-fluorophenyl)urea (IB_35). The compound was synthesized according to the general procedure using 4-(5-chloro-1*H*-benzo[*d*]imidazol-2-yl) aniline (**IB_02a**) (0.1 g, 0.35 mmol), and 4-fluorophenyl isocyanate (0.02 g, 0.35 mmol) to afford **IB_35** (0.07 g, 67.81 %) as white solid. M.p: 213-215 °C. ^{1}H

NMR (DMSO-d_6): δ_H 8.88 (s, 1H), 8.79 (s, 1H), 8.08-7.14 (m, 12H). ^{13}C NMR (DMSO-d_6): δ_C 162.9, 152.9 (2C), 139.4, 135, 132.8, 130.9, 129.2, 127.7 (2C), 124.1, 121.9, 119.7 (2C), 119.3 (2C), 116.6, 115.8, 115.7 (2C). ESI-MS *m/z* 381 $[M+H]^+$. Anal.Calcd.For $C_{20}H_{14}ClFN_4O$: C, 63.08; H, 3.71; N, 14.71; Found: C, 63.06; H, 3.69; N, 14.73.

1-(4-(5-Fluoro-1H-benzo[d]imidazol-2-yl)phenyl)-3-(4-fluorophenyl)urea (IB_36). The compound was synthesized according to the general procedure using 4-(5-fluoro-1*H*-benzo[*d*]imidazol-2-yl) aniline (**IB_02b**) (0.1 g, 0.35 mmol), and 4-fluorophenyl isocyanate (0.02 g, 0.35 mmol) to afford **IB_36** (0.07 g, 69.87 %) as white solid. M.p: 229-231 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.87 (s, 1H), 8.78 (s, 1H), 8.09-7.16 (m, 12H). ^{13}C NMR (DMSO-d_6): δ_C 162.9, 156.5, 152.9 (2C), 140.5, 139.4, 137.3, 135, 127.7 (2C), 121.9, 119.7 (2C), 119.3 (2C), 116.8, 115.7 (2C), 109.9, 102.4. ESI-MS *m/z* 365 $[M+H]^+$. Anal.Calcd.For $C_{20}H_{14}F_2N_4O$: C, 65.93; H, 3.87; N, 15.38; Found: C, 65.91; H, 3.89; N, 15.35.

1-(4-(1H-Benzo[d]imidazol-2-yl) phenyl)-3-(4-fluorophenyl)urea (IB_37). The compound was synthesized according to the general procedure using 4-(1*H*-benzo[*d*]imidazol-2-yl) aniline (**IB_02c**) (0.1 g, 0.35 mmol), and 4-fluorophenyl isocyanate (0.02 g, 0.35 mmol) to afford **IB_37** (0.06 g, 54.75 %) as white solid. M.p: 198-200 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.89 (s, 1H), 8.77 (s, 1H), 8.09-7.14 (m, 13H). ^{13}C NMR (DMSO-d_6): δ_C 162.9, 152.9 (2C), 141.7 (2C), 139.4, 135, 127.7 (2C), 123 (2C), 121.9, 119.7 (2C), 119.3 (2C), 115.7 (2C), 115.2 (2C). ESI-MS *m/z* 347 $[M+H]^+$. Anal.Calcd.For $C_{20}H_{15}FN_4O$: C, 69.35; H, 4.37; N, 16.18; Found: C, 69.32; H, 4.34; N, 16.20.

1-(4-Fluorophenyl)-3-(4-(5-methoxy-1H-benzo[d]imidazol-2-yl)phenyl)urea (IB_38). The compound was synthesized according to the general procedure using 4-(5-methoxy-1*H*-benzo[*d*]imidazol-2-yl) aniline (**IB_2d**) (0.1 g, 0.35 mmol), and 4-fluorophenyl isocyanate (0.02 g, 0.35 mmol) to afford **IB_38** (0.07 g, 52.62 %) as white solid. M.p: 240-242 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.88 (s, 1H), 8.77 (s, 1H), 8.08-7.16 (m, 12H), 3.86 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 162.9, 156.1, 152.9 (2C), 139.9, 139.4, 135, 134, 127.7 (2C), 121.9, 119.7 (2C), 119.3 (2C), 116.2, 115.7 (2C), 111.5, 100.8, 55.8. ESI-MS *m/z* 377 $[M+H]^+$. Anal.Calcd.For $C_{21}H_{17}FN_4O_2$: C, 67.01; H, 4.55; N, 14.89; Found: C, 67.04; H, 4.53; N, 14.91.

1-(4-(5-Chloro-1H-benzo[d]imidazol-2-yl)phenyl)-3-(4-fluorophenyl)thiourea (IB_39). The compound was synthesized according to the general procedure using 4-(5-chloro-1*H*-

benzo[*d*]imidazol-2-yl) aniline (**IB_02a**) (0.1 g, 0.35 mmol), and 4-fluorophenyl isothiocyanate (0.02 g, 0.35 mmol) to afford **IB_39** (0.07 g, 72.73 %) as white solid. M.p: 261-263 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.87 (s, 1H), 8.78 (s, 1H), 8.07-7.12 (m, 12H). ^{13}C NMR (DMSO-d_6): δ_C 179.9, 163.3, 152.9, 138.5, 134.1, 132.8, 131 (2C), 130.9, 129.2, 127.8 (2C), 124.6 (2C), 124.1, 122.3, 116.6, 115.8 (3C). ESI-MS *m/z* 397 $[M+H]^+$. Anal.Calcd.For $C_{20}H_{14}ClFN_4S$: C, 60.53; H, 3.56; N, 14.12; Found: C, 60.55; H, 3.53; N, 14.14.

1-(4-(5-Fluoro-1H-benzo[d]imidazol-2-yl)phenyl)-3-(4-fluorophenyl)thiourea (IB_40). The compound was synthesized according to the general procedure using 4-(5-fluoro-1*H*-benzo[*d*]imidazol-2-yl) aniline (**IB_02b**) (0.1 g, 0.35 mmol), and 4-fluorophenyl isothiocyanate (0.02 g, 0.35 mmol) to afford **IB_40** (0.07 g, 58.16 %) as white solid. M.p: 189-191 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.88 (s, 1H), 8.79 (s, 1H), 8.08-7.13 (m, 12H). ^{13}C NMR (DMSO-d_6): δ_C 179.9, 163.3, 156.5, 152.9, 140.5, 138.5, 137.3, 134.1, 131 (2C), 127.8 (2C), 124.6 (2C), 122.3, 116.8, 115.8 (2C), 109.9, 102.4. ESI-MS *m/z* 381 $[M+H]^+$. Anal.Calcd.For $C_{20}H_{14}F_2N_4S$: C, 63.15; H, 3.71; N, 14.73; Found: C, 63.16; H, 3.169 N, 14.72.

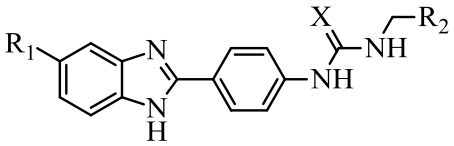
1-(4-(1H-Benzo[d]imidazol-2-yl)phenyl)-3-(4-fluorophenyl)thiourea (IB_41). The compound was synthesized according to the general procedure using 4-(1*H*-benzo[*d*]imidazol-2-yl) aniline (**IB_02c**) (0.1 g, 0.35 mmol), and 4-fluorophenyl isothiocyanate (0.02 g, 0.35 mmol) to afford **IB_41** (0.07 g, 63.94 %) as white solid. M.p: 221-223 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.89 (s, 1H), 8.80 (s, 1H), 8.09-7.15 (m, 13H). ^{13}C NMR (DMSO-d_6): δ_C 179.9, 163.3, 152.9, 141.7 (2C), 138.5, 134.1, 131 (2C), 127.8 (2C), 124.6 (2C), 123 (2C), 122.3, 115.8 (2C), 115.2 (2C). ESI-MS *m/z* 363 $[M+H]^+$. Anal.Calcd.For $C_{20}H_{15}FN_4S$: C, 66.28; H, 4.17; N, 15.46; Found: C, 66.26; H, 4.13; N, 15.42.

1-(4-Fluorophenyl)-3-(4-(5-methoxy-1H-benzo[d]imidazol-2-yl)phenyl)thiourea (IB_42). The compound was synthesized according to the general procedure using 4-(5-methoxy-1*H*-benzo[*d*]imidazol-2-yl) aniline (**IB_02d**) (0.1 g, 0.35 mmol), and 4-fluorophenyl isothiocyanate (0.02 g, 0.35 mmol) to afford **IB_42** (0.07 g, 71.16 %) as white solid. M.p: 275-277 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.88 (s, 1H), 8.78 (s, 1H), 8.08-7.13 (m, 12H), 3.86 (s, 3H). ^{13}C NMR (DMSO-d_6): δ_C 179.9, 163.3, 156.1, 152.9, 139.9, 138.5, 134.1, 134, 131 (2C), 127.8 (2C), 124.6 (2C), 122.3, 116.2, 115.8 (2C), 111.5, 100.8, 55.8. ESI-MS *m/z* 393 $[M+H]^+$. Anal.Calcd.For $C_{21}H_{17}FN_4OS$: C, 64.27; H, 4.37; N, 14.28; Found: C, 64.26; H, 4.33; N, 14.26.

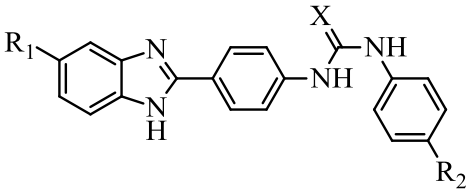
5.2a.4. In-vitro *Mycobacterium tuberculosis* GyrB assay, *Mycobacterium tuberculosis* supercoiling assay and antimycobacterial potency of the synthesized molecules

All the synthesized derivatives were first evaluated for their in-vitro *Mycobacterium tuberculosis* GyrB assay and *Mycobacterium tuberculosis* supercoiling assay as steps towards the derivation of SAR and hit optimization. The compounds were further subjected to a whole cell screening against *Mycobacterium tuberculosis* H37Rv strain to understand their bactericidal potency using the MABA assay, and the results are tabulated in **Table 5.8.**

Table 5.8: In-vitro biological evaluation of the synthesized derivatives **IB_03 - IB_42**.



IB_03 - IB_10



IB_11 - IB_42

Compd	R_1	R_2	X	MS GyraseB assay (IC_{50}) µM	MTB Supercoiling assay (IC_{50}) µM	MTB MIC µM	Cytotoxicity[a] % inhibition
IB_03	-Cl	$-C_6H_5$	O	22.54	15.22	16.58	56.56
IB_04	-F	$-C_6H_5$	O	>50	>50	ND	32.88
IB_05	-H	$-C_6H_5$	O	1.96	1.32	3.65	80.65
IB_06	$-OCH_3$	$-C_6H_5$	O	62.33	48.46	ND	76.47
IB_07	-Cl	$-C_6H_5$	S	6.93	3.565	7.95	68.38
IB_08	-F	$-C_6H_5$	S	17.22	11.62	22.19	15.58
IB_09	-H	$-C_6H_5$	S	8.91	5.11	8.72	67.13
IB_10	$-OCH_3$	$-C_6H_5$	S	4.36	2.81	6.43	27.78
IB_11	-Cl	$-OCH_3$	O	64.83	>50	ND	50.02
IB_12	-F	$-OCH_3$	O	15.11	7.14	16.65	56.45
IB_13	-H	$-OCH_3$	O	0.82	0.37	4.62	36.29
IB_14	$-OCH_3$	$-OCH_3$	O	8.51	3.66	16.09	33.38

Contd.

Compd	R_1	R_2	X	MS GyraseB assay (IC_{50}) µM	MTB Supercoiling assay (IC_{50}) µM	MTB MIC µM	Cytotoxicity[a] % inhibition
IB_15	-Cl	$-OCH_3$	S	67.47	>50	ND	43.24
IB_16	-F	$-OCH_3$	S	3.86	2.11	3.18	12.04
IB_17	-H	$-OCH_3$	S	44.97	32.19	33.38	68.97
IB_18	$-OCH_3$	$-OCH_3$	S	0.97	0.48	1.23	37.69
IB_19	-Cl	$-NO_2$	O	>100	>50	ND	46.53
IB_20	-F	$-NO_2$	O	>100	>50	ND	33.69
IB_21	-H	$-NO_2$	O	17.82	9.66	16.74	9.11
IB_22	$-OCH_3$	$-NO_2$	O	1.15	0.67	1.54	1.88
IB_23	-Cl	$-NO_2$	S	74.31	>50	ND	43.83
IB_24	-F	$-NO_2$	S	69.53	49.32	ND	56.51
IB_25	-H	$-NO_2$	S	18.51	12.21	16.05	59.26
IB_26	$-OCH_3$	$-NO_2$	S	12.99	7.38	14.90	0.08
IB_27	-Cl	$-CH_3$	O	79.62	>50	ND	62.67
IB_28	-F	$-CH_3$	O	10.71	4.60	6.93	36.92
IB_29	-H	$-CH_3$	O	11.33	5.35	18.25	53.34
IB_30	$-OCH_3$	$-CH_3$	O	2.93	1.13	4.18	26.10
IB_31	-Cl	$-CH_3$	S	66.47	>50	ND	66.36
IB_32	-F	$-CH_3$	S	81.21	>50	ND	29.11
IB_33	-H	$-CH_3$	S	23.55	11.21	34.87	16.69
IB_34	$-OCH_3$	$-CH_3$	S	7.81	3.37	8.04	71.67
IB_35	-Cl	-F	O	38.72	20.71	16.41	67.01
IB_36	-F	-F	O	0.96	0.51	2.14	35.33
IB_37	-H	-F	O	17.44	9.82	18.04	54.16
IB_38	$-OCH_3$	-F	O	0.72	0.26	1.38	16.52
IB_39	-Cl	-F	S	21.74	10.25	15.75	43.26
IB_40	-F	-F	S	1.89	0.58	1.64	71.29
IB_41	-H	-F	S	4.43	1.97	4.31	63.11

Contd.

Compd	R_1	R_2	X	MS GyraseB assay (IC_{50}) µM	MTB Supercoiling assay (IC_{50}) µM	MTB MIC µM	Cytotoxicity[a] % inhibition
IB_42	$-OCH_3$	-F	S	3.82	0.71	6.37	33.12
	Novobiocin			0.273±0.28	0.068±0.31	>200	9.36

MS= *Mycobacterium smegmatis,* MTB=*Mycobacterium tuberculosis,* [a] at 50 µM against RAW 264.7 cells, ND indicates not determined.

5.2a.5. Discussion

A series of benzimidazol-2-yl phenyl urea and thiourea derivatives were synthesized and were evaluated for their *Mycobacterium tuberculosis* gyrB inhibition ability employing gyrB ATPase assay, supercoiling assay and their IC_{50} values were determined. The compounds were also evaluated for their in-vitro antimycobacterial activity against *Mycobacterium tuberculosis* H37RV strain and also for the safety profile by in-vitro MTT assay in RAW 264.7 cell lines. Attempt was done to correlate the activity of these compounds with respect to their structure in order to study their SAR. The basic structure of these compounds includes a benzimidazole core with a phenyl group at its 2nd position and substitutions like fluoro, chloro and methoxy at 5th position. The phenyl group at 2nd position was substituted by urea and thiourea at its *para* position. The major substitutions were made at two positions: **R_1** - left hand side at 5th position of benzimidazole with chloro, fluoro and methoxy groups and **R_2** - right hand side on free amino group of urea/thiourea with benzyl and various *p*-substituted phenyl derivatives. The total series of 40 compounds can be divided into 5 subsets based on their substitutions at their **R_2** position – (i) compounds **IB_03 - IB_10** with benzyl group (ii) compounds **IB_11 - IB_18** with *p*-methoxy phenyl group, (iii) compounds **IB_19 - IB_26** with *p*-nitro phenyl group, (iv) compounds **IB_27 - IB_34** with *p*-methyl phenyl group and (v) compounds **IB_35 - IB_42** with *p*-fluoro phenyl group.

Compounds **IB_03 - IB_10** were found with benzyl group on right hand side yielding four compounds with their GyrB activity below 10 µM. Compounds **IB_05** and **IB_10** were found to be active with IC_{50} 1.96 µM and 4.36 µM respectively in GyrB assay and IC_{50} 1.32 and 2.81 µM respectively in supercoiling assay. Also these compounds exhibited a decent anti-tubercular activity with MIC values 3.65 µM and 6.43 µM respectively. **IB_10** was found to be devoid of cytotoxicity as it shows percentage inhibition of 27.78 against RAW cell lines.

Substitution of *p*-methoxy phenyl moiety at R_2 position (**IB_11 - IB_18**) yielded compounds with wide range of GyrB inhibitory activity falling between 0.82 – 67.47 µM. Of these, four compounds were found to be active below 10 µM with compounds **IB_13** and **IB_18** being top active compounds below 1 µM. Presence of *p*-methoxy phenyl group in place of benzyl group in compounds **IB_05** and **IB_10** yielded in compounds **IB_13** and **IB_18** with 2 fold and 4 fold enhanced gyrB inhibitory activity respectively.

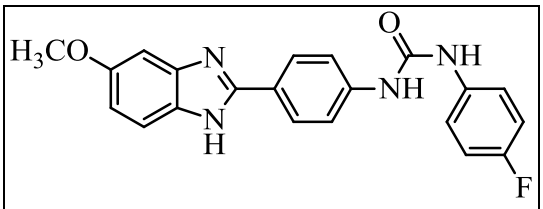
Substitution of compounds with *p*-nitro phenyl (**IB_19 - IB_26**) and *p*-methyl phenyl group (**IB_27 - IB_34**) resulted in compounds with decreased GyrB inhibitory ability with a few exceptions of compound **IB_22** and **IB_30**. Both the compounds are similar with respect to their structure with methoxy at 5^{th} position, urea attached to the phenyl ring but with nitro and methyl groups on their R_2 position exhibiting GyrB inhibitory activity of 1.15 µM and 2.93 µM respectively and supercoiling inhibitory activity of 0.67 and 1.13 µM respectively. These compounds exhibit a well correlating MIC of 1.54 and 4.18 µM respectively. They were also found to be devoid of cytotoxicity as the percentage inhibitions are 1.88 and 26.10 respectively against RAW cell lines.Replacement of nitro group with methyl resulted in decrease of activity by 2.5 fold implicating the importance of nitro group on the phenyl ring for the activity. This might be probably accounted for the high electron affinity nature of oxygen which might readily takes part in hydrogen bonding at the active site of GyrB.

Of all the substitutions, *p*-fluoro phenyl group at R_2 position was observed to be the desirable one yielding five compounds out of eight with IC_{50} below 5 µM. Of these, compounds **IB_38** with methoxy and **IB_36** with fluoro at R_1 positions were found to be the top active compounds of the series proving the importance of fluoro group over the compounds for *Mycobacterium tuberculosis* gyrB inhibitory activity.

5.2a.6. Highlights of the study

Among the series of compounds synthesised compound **IB_38** was found to be active with gyrase IC_{50} of 0.72 µM and supercoiling assay IC_{50} of 0.26 µM. The compound was also found to be active against Mycobacterium tuberculosis in MABA assay with MIC of 1.38 µM. The compound was found to devoid of cytotoxicity as the percentage inhibiton was 16.52 against RAW cell lines. In a nutshell, benzimidazole derivatives act as suitable inhibitors against gyrB ATPase domain as observed from the gyrB IC_{50} values and anti-tubercular MIC results from **Table 5.8**. Substitution on right hand side with hydrophobic

moieties such as fluoro (as observed in compounds **IB_36**, **IB_38**, **IB_40**, **IB_41** and **IB_42**) and methoxy (as in compounds **IB_13**, **IB_16** and **IB_18)** moieties yielded in inhibitors with good activity. Substitutions like nitro, methyl and also some of benzyl were found to be highly unfavourable for gyrB activity. Simple benzimidazole or hydrophobic substitutions like fluoro and methoxy groups can be highly recommended for improving inhibitory ability of the compounds. Considering the activity profiles of the compounds, urea derivatives are more likely to be better gyrB inhibitors when compared to that of thiourea ones. Compounds with these specifications can be suitable and active inhibitors of gyrB which can be further optimized and evaluated in the process of anti-tubercular drug discovery.



Compound **IB_38**
MTB MIC = 1.38 μM
GyraseB assay IC_{50} = 0.72 μM
Supercoiling assay IC_{50} = 0.26μM

Figure 5.16: Structure and activity of most active compound **IB_38.**

5.2b Development of novel benzimidazol-2-yl piperidine-1-carboxamide/carbothioamide derivatives as *Mycobacterium tuberculosis* DNA GyrB inhibitors

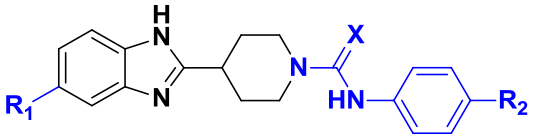


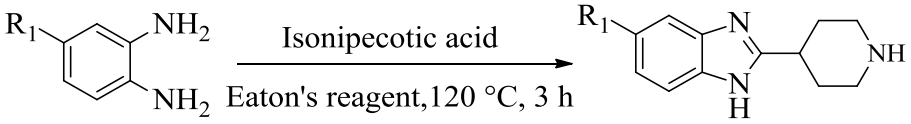
Figure 5.17: General structure of 2-(piperidin-4-yl)-1*H*-benzo[*d*]imidazole derivatives obtained through molecular derivatization.

5.2b.1. Chemical synthesis

The synthetic pathway used to achieve the target compounds has been delineated in **Figure 4.5**. Synthesis of the compounds started with conversion of commercially available various 1,2-phenylenediamines and isonipecotic acid in Eaton's reagent was heated at 130 °C for 5–6 h resulted in the formation of substituted benzimidazoles. The final library was then assembled by treating the obtained scaffolds with the desired isocyanates/isothiocyantes to afford compound **IN_03 - IN_42** in excellent yields. A series of 40 derivatives was synthesized using the above method in excellent yields.

5.2b.2. Experimental protocol utilised for synthesis

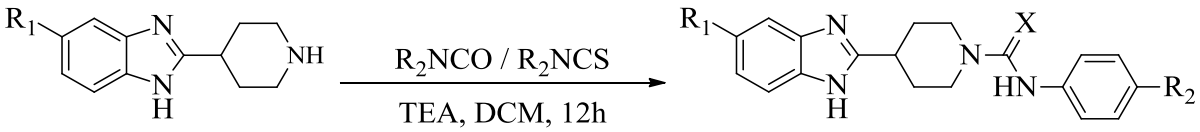
General procedure for the synthesis of 5-substituted-2-(piperidin-4-yl)-1H-benzo[d]imidazole (**IN_02a-d**). Eaton's reagent (10 vol) was added drop wise to a wellpulverised mixture of the corresponding 1,2-phenylenediamine(1 equiv) and isonipecotic acid (1 equiv) at 0°C. The reaction mixture was then heated at 130°C for 5–6 h (monitored by TLC and LCMS for completion).The reaction mixture was cooled and neutralised with 10% sodiumhydroxide solution to pH of 6–7, the precipitate formed was filteredand washed repeatedly with water and dried. The solidobtained was recrystallized from ethanol to afford the desiredproduct(**IN_02a-d**) in good yield.



IN_01a-d **IN_02a-d**

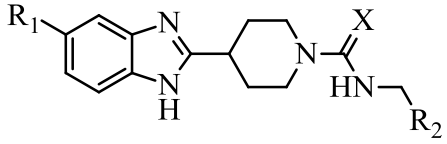
General procedure for the synthesis of4-(5-substituted-1H-benzo[d]imidazol-2-yl)-N-phenylpiperidine-1-carboxamide/carbothioamide (IN_03 - IN_42). To a cooled solution of5-substituted-2-(piperidin-4-yl)-1H-benzo[d]imidazole(1 mmol) in anhydrous DCM (2 mL) was added corresponding isocyanate/isothiocyanate (1 mmol), triethylamine (1mmol) and stirred the reaction mixture at room temperature for 12 hours (monitored by TLC & LCMS for completion), and solvent evaporated under reduced pressure. The residue was further diluted with water (30 mL) and ethyl acetate (50 mL) and the layers were separated. The organic layer was dried over anhydrous sodium sulphate and evaporated under reduced pressure. The residue was purified by silica gel column chromatography using hexane: ethylacetate as eluent to give the corresponding urea/thiourea derivatives **(IN_03 - IN_42)** in good yield.

The physicochemical properties of synthesized derivatives are shown in **Table 5.9**.

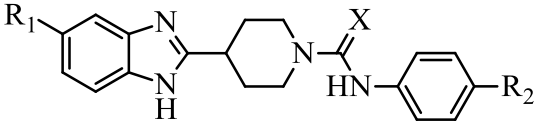


IN_02a-d **IN_03 - IN_42**

Table 5.9: Physicochemical properties of synthesized compounds **(IN_03 - IN_42)**.



IN_03 - IN_10



IN_11 - IN_42

Compd	R_1	R_2	X	Yield (%)	M.P. (°C)	Molecular formula	Molecular weight
IN_03	-H	$-C_6H_5$	O	52	258-260	$C_{20}H_{22}N_4O$	334.41
IN_04	$-CH_3$	$-C_6H_5$	O	74	226-228	$C_{21}H_{24}N_4O$	348.44
IN_05	-Cl	$-C_6H_5$	O	41	211-213	$C_{20}H_{21}ClN_4O$	368.86
IN_06	-F	$-C_6H_5$	O	44	197-199	$C_{20}H_{21}FN_4O_2$	352.41
IN_07	-H	$-C_6H_5$	S	71	243-245	$C_{20}H_{22}N_4S$	350.48
IN_08	$-CH_3$	$-C_6H_5$	S	53	248-250	$C_{21}H_{24}N_4S$	364.51

Contd.

Compd	R_1	R_2	X	Yield (%)	M.P. (°C)	Molecular formula	Molecular weight
IN_09	-Cl	$-C_6H_5$	S	37	259-261	$C_{20}H_{21}ClN_4S$	384.93
IN_10	-F	$-C_6H_5$	S	39	241-243	$C_{22}H_{21}FN_4S$	368.47
IN_11	-H	$-OCH_3$	O	46	264-266	$C_{20}H_{22}N_4O_2$	350.41
IN_12	$-CH_3$	$-OCH_3$	O	56	261-263	$C_{21}H_{24}N_4O_2$	364.44
IN_13	-Cl	$-OCH_3$	O	68	219-221	$C_{20}H_{21}ClN_4O_2$	384.86
IN_14	-F	$-OCH_3$	O	76	231-233	$C_{20}H_{21}FN_4O_2$	368.40
IN_15	-H	$-OCH_3$	S	59	208-210	$C_{20}H_{22}N_4OS$	366.48
IN_16	$-CH_3$	$-OCH_3$	S	65	271-273	$C_{21}H_{24}N_4OS$	380.51
IN_17	-Cl	$-OCH_3$	S	67	276-278	$C_{20}H_{21}ClN_4OS$	400.92
IN_18	-F	$-OCH_3$	S	46	249-251	$C_{20}H_{21}FN_4OS$	384.47
IN_19	-H	$-NO_2$	O	50	228-230	$C_{19}H_{19}N_5O_3$	365.39
IN_20	$-CH_3$	$-NO_2$	O	48	289-291	$C_{20}H_{21}N_5O_3$	379.41
IN_21	-Cl	$-NO_2$	O	51	219-221	$C_{19}H_{18}ClN_5O_3$	399.83
IN_22	-F	$-NO_2$	O	72	239-241	$C_{19}H_{18}FN_5O_3$	383.38
IN_23	-H	$-NO_2$	S	53	268-270	$C_{19}H_{19}N_5O_2S$	381.45
IN_24	$-CH_3$	$-NO_2$	S	66	249-251	$C_{20}H_{21}N_5O_2S$	395.48
IN_25	-Cl	$-NO_2$	S	59	211-213	$C_{19}H_{18}ClN_5OS$	415.90
IN_26	-F	$-NO_2$	S	49	255-254	$C_{19}H_{18}FN_5O_2S$	399.44
IN_27	-H	$-CH_3$	O	86	280-282	$C_{20}H_{22}N_4O$	334.41
IN_28	$-CH_3$	$-CH_3$	O	75	242-244	$C_{21}H_{24}N_4O$	348.44
IN_29	-Cl	$-CH_3$	O	64	229-231	$C_{20}H_{21}ClN_4O$	368.86
IN_30	-F	$-CH_3$	O	43	273-275	$C_{20}H_{21}FN_4O$	352.41
IN_31	-H	$-CH_3$	S	74	229-231	$C_{20}H_{22}N_4S$	350.48
IN_32	$-CH_3$	$-CH_3$	S	73	222-224	$C_{21}H_{24}N_4S$	364.51
IN_33	-Cl	$-CH_3$	S	49	288-290	$C_{20}H_{21}ClN_4S$	384.93
IN_34	-F	$-CH_3$	S	78	258-260	$C_{20}H_{21}FN_4S$	368.47
IN_35	-H	-F	O	65	247-249	$C_{19}H_{19}FN_4O$	338.38
IN_36	$-CH_3$	-F	O	58	218-220	$C_{20}H_{21}FN_4O$	352.41

Contd.

Compd	R_1	R_2	X	Yield (%)	M.P. (°C)	Molecular formula	Molecular weight
IN_37	-Cl	-F	O	57	241-243	$C_{19}H_{18}ClFN_4O$	372.82
IN_38	-F	-F	O	68	253-255	$C_{19}H_{18}F_2N_4O$	356.37
IN_39	-H	-F	S	62	277-279	$C_{19}H_{19}FN_4S$	354.44
IN_40	$-CH_3$	-F	S	69	197-199	$C_{20}H_{21}FN_4S$	368.47
IN_41	-Cl	-F	S	62	226-228	$C_{19}H_{18}ClFN_4S$	388.89
IN_42	-F	-F	S	71	263-265	$C_{19}H_{18}F_2N_4S$	372.43

5.2b.3. Characterization of synthesized compounds

A series of 40 derivatives wereprepared using the above method and both analytical and spectral data (^{1}H NMR, ^{13}C NMR, and mass spectra) of all the synthesized compounds were in full agreement with the proposed structures.

2-(Piperidin-4-yl)-1H-benzo[d]imidazol (IN_02a).The compound was synthesized according to the general procedure using 1, 2-phenylenediamine (**IN_01a**) (1 g, 7.02 mmol), isonipecotic acid (0.96 g, 7.02 mmol) and eatons reagent (10 mL) to afford **IN_02a** (1.1 g, 64% yield) as buff coloured solid. Mp: 291-293 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.95 (s, 1H), 8.22–7.64 (m, 4H), 5.4 (s, 1H), 3.21-1.84 (m, 9H). ^{13}C NMR (DMSO-d_6): δ_C 141.4, 138.7 (2C), 123.2 (2C), 115.2 (2C), 40.6 (2C), 35.4, 31.3 (2C). ESI-MS m/z: 202 $[M+H]^+$. Anal.Calcd for $C_{12}H_{15}N_3$: C, 71.61; H, 7.51; N, 20.88. Found: C, 71.63; H, 7.56; N, 20.86.

5-Methyl-2-(piperidin-4-yl)-1H-benzo[d]imidazole (IN_02b).The compound was synthesized according to the general procedure using 4-methyl-1,2-phenylenediamine (**IN_01b**) (1 g, 7.93 mmol), isonipecotic acid (1.09 g, 7.93 mmol) and eatons reagent(10 mL) to afford **IN_02b** (1.02 g, 56% yield) as brown solid. Mp: 242-244 °C. 1H NMR (DMSO-d_6): δ_H 8.95 (s, 1H), 7.93–7.64 (m, 3H), 5.4 (s, 1H), 3.21-1.84 (m, 12H). 13C NMR (DMSO-d_6): δ_C 141.4, 138.7, 135.6, 132.5, 125.8, 115.2, 115.1, 40.6 (2C), 35.3, 31.9 (2C), 21.2. ESI-MS m/z: 216 $[M+H]^+$. Anal. Calcd for $C_{13}H_{17}N_3$: C, 72.52; H, 7.96; N, 19.52. Found: C, 72.53; H, 7.92; N, 19.56.

5-Chloro-2-(piperidin-4-yl)-1H-benzo[d]imidazole (IN_02c).The compound was synthesized according to the general procedure using 4-chloro-1,2-phenylenediamine (**IN_01c**) (1 g, 9.25 mmol), isonipecotic acid(1.27 g, 9.25 mmol) and eatons reagent (10 mL) toafford **IN_02c**

(1.42 g, 74% yield) as pale brown solid. M.p: 259-261 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.95 (s, 1H), 7.91–7.66 (m, 3H), 5.4 (s, 1H), 3.21-1.84 (m, 9H). ^{13}C NMR (DMSO-d_6): δ_C 141.4, 140.2, 137.5, 129.7, 124.5, 116.7, 115.5, 40.7 (2C), 35.2, 31.8 (2C). ESI-MS m/z: 236 $[M+H]^+$. Anal. Calcd for $C_{12}H_{14}ClN_3$: C, 61.15; H, 5.99; N, 17.83. Found: C, 61.14; H, 5.97; N, 17.85.

5-Fluoro-2-(piperidin-4-yl)-1H-benzo[d]imidazole (IN_02d).The compound was synthesized according to the general procedure using4-fluoro-1,2-phenylenediamine (**IN_01d**) (1 g,7.24 mmol), isonipecotic acid(0.99 g, 7.24 mmol) and eatonsreagent (10 mL) to afford **IN_02d** (0.91 g, 53%) as reddish brown solid.MP: 162-165 °C. ^{1}H NMR (DMSO-d_6): δ_H 8.95 (s, 1H), 7.98–7.73 (m, 3H), 5.4 (s, 1H), 3.24-1.86 (m, 9H). ^{13}C NMR (DMSO-d_6): δ_C 156.7, 141.4,140.7, 134.5, 116.5, 109.4, 102.8, 40.3 (2C), 35.1, 31.6 (2C). ESI-MS m/z: 220 $[M+H]^+$. Anal. Calcd for $C_{12}H_{14}FN_3$: C, 65.73; H, 6.44; N, 19.16. Found: C, 65.74; H, 6.47; N, 19.18.

4-(1H-Benzo[d]imidazol-2-yl)-N-benzylpiperidine-1-carboxamide (IN_03).The compound was synthesized according to the general procedure using 2-(piperidin-4-yl)-1*H*-benzo[*d*]imidazole (**IN_02a**) (0.1 g, 0.49 mmol) and Benzyl isocyanate (0.06 g, 0.49 mmol) to afford **IN_03** (0.06 g, 52.27 %) as yellow solid. M.p: 258-260 °C. ^{1}H NMR (DMSO-d_6): δ_H 12.30 (b, 1H), 8.34 (s, 1H), 7.42-6.88 (m, 9H), 4.18-1.76 (m, 11H). ^{13}C NMR (DMSO-d_6): δ_C 155.7, 141.5, 138.9 (2C), 137.9, 128.5 (2C), 126.9 (2C), 126.7, 123.1 (2C), 115.2 (2C), 46.7 (2C), 44.7, 34.9, 28.7 (2C). ESI-MS *m/z* 335 $[M+H]^+$. Anal.Calcd.For $C_{20}H_{22}N_4O$: C, 71.83; H, 6.63; N, 16.75; Found: C, 71.81; H, 6.62.15; N, 16.78.

N-Benzyl-4-(5-methyl-1H-benzo[d]imidazol-2-yl)piperidine-1-carboxamide (IN_04). The compound was synthesized according to the general procedure using5-methyl-2-(piperidin-4-yl)-1*H*-benzo[*d*]imidazole (**IN_02b**) (0.1 g, 0.46 mmol) and Benzyl isocyanate (0.06 g, 0.46 mmol) to afford **IN_04** (0.08 g, 74.12 %) as yellow solid. M.p: 226-228 °C. ^{1}H NMR (DMSO-d_6): δ_H 12.30 (b, 1H), 8.34 (s, 1H), 7.44-6.86 (m, 8H), 4.18-1.74 (m, 14H). ^{13}C NMR (DMSO-d_6): δ_C 155.7, 141.5, 138.8, 137.9, 135.9, 132.7, 128.5 (2C), 126.9 (2C), 126.7, 125.8, 115.3, 115.1, 46.7 (2C), 44.7, 34.9, 28.7 (2C), 21.3. ESI-MS *m/z* 349 $[M+H]^+$. Anal.Calcd.For $C_{21}H_{24}N_4O$: C, 72.39; H, 6.44; N, 16.08; Found: C, 72.42; H, 6.47; N, 16.11.

N-Benzyl-4-(5-chloro-1H-benzo[d]imidazol-2-yl)piperidine-1-carboxamide (IN_05).The compound was synthesized according to the general procedure using 5-chloro-2-(piperidin-4-

yl)-1*H*-benzo[*d*]imidazole (**IN_02c**) (0.1 g, 0.42 mmol) and Benzyl isocyanate (0.05 g, 0.42 mmol) to afford **IN_05** (0.05 g, 41.74 %) as yellow solid. M.p: 211-213 °C. ^{1}H NMR (DMSO-d_6): δ_H 12.30 (b, 1H), 8.34 (s, 1H), 7.43-6.84 (m, 8H), 4.16-1.73 (m, 11H). ^{13}C NMR (DMSO-d_6): δ_C 155.7, 141.5, 140.3, 137.9, 137.2, 129.2, 128.5 (2C), 126.9 (2C), 126.7, 124.1, 116.6, 115.8, 46.7 (2C), 44.7, 34.9, 28.7 (2C). ESI-MS *m/z* 369 $[M+H]^+$. Anal.Calcd.For $C_{20}H_{21}ClN_4O$: C, 65.12; H, 5.74; N, 15.19; Found: C, 65.14; H, 5.73; N, 15.16.

N-Benzyl-4-(5-fluoro-1H-benzo[d]imidazol-2-yl)piperidine-1-carboxamide (IN_06). The compound was synthesized according to the general procedure using 5-fluoro-2-(piperidin-4-yl)-1*H*-benzo[*d*]imidazole (**IN_02d**) (0.1 g, 0.45 mmol) and Benzyl isocyanate (0.06 g, 0.45 mmol) to afford **IN_06** (0.06 g, 44.13 %) as yellow solid. M.p: 197-199 °C. ^{1}H NMR (DMSO-d_6): δ_H 12.30 (b, 1H), 8.34 (s, 1H), 7.44-6.88 (m, 8H), 4.18-1.72 (m, 11H). ^{13}C NMR (DMSO-d_6): δ_C 156.5, 155.7, 141.5, 140.5, 137.9, 134.5, 128.5 (2C), 126.9 (2C), 126.7, 116.8, 109.9, 102.4, 46.7 (2C), 44.7, 34.9, 28.7 (2C). ESI-MS *m/z* 353 $[M+H]^+$. Anal.Calcd.For $C_{20}H_{21}FN_4O_2$: C, 68.16; H, 6.01; N, 15.90; Found: C, 68.12; H, 6.04; N, 15.94.

4-(1H-Benzo[d]imidazol-2-yl)-N-benzylpiperidine-1-carbothioamide (IN_07). The compound was synthesized according to the general procedure using2-(piperidin-4-yl)-1*H*-benzo[*d*]imidazole (**IN_02a**) (0.1 g, 0.49 mmol) and Benzyl isothiocyanate (0.07 g, 0.49 mmol) to afford **IN_07** (0.08 g, 71.29 %) as white solid. M.p: 243-245 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.47 (b, 1H), 8.34 (s, 1H), 7.48-6.72 (m, 9H), 4.16-1.74 (m, 11H). ^{13}C NMR (DMSO-d_6): δ_C 186.4, 141.5, 138.9 (2C), 137.9, 128.5 (2C), 126.9 (2C), 126.7, 123.2 (2C), 115.2 (2C), 51.8 (2C), 51.1, 35.9, 29.4 (2C). ESI-MS *m/z* 351 $[M+H]^+$. Anal.Calcd.For $C_{20}H_{22}N_4S$: C, 68.54; H, 6.33; N, 15.99; Found: C, 68.56; H, 6.35; N, 15.98.

N-Benzyl-4-(5-methyl-1H-benzo[d]imidazol-2-yl)piperidine-1-carbothioamide (IN_08). The compound was synthesized according to the general procedure using 5-methyl-2-(piperidin-4-yl)-1*H*-benzo[*d*]imidazole (**IN_02b**) (0.1 g, 0.46 mmol) and Benzyl isothiocyanate (0.06 g, 0.46 mmol) to afford **IN_08** (0.05 g, 53.91 %) as white solid. M.p: 248-250 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.47 (b, 1H), 8.34 (s, 1H), 7.46-6.70 (m, 8H), 4.17-1.75 (m, 14H). ^{13}C NMR (DMSO-d_6): δ_C 186.4, 141.5, 138.8, 137.9, 135.9, 132.7, 128.5 (2C), 126.9 (2C), 126.7, 125.8, 115.3, 115.1, 51.8 (2C), 51.1, 35.9, 29.4 (2C), 21.3. ESI-MS *m/z* 365 $[M+H]^+$. Anal.Calcd.For $C_{21}H_{24}N_4S$: C, 69.20; H, 6.64; N, 15.37; Found: C, 69.18; H, 6.63; N, 15.38.

N-Benzyl-4-(5-chloro-1H-benzo[d]imidazol-2-yl)piperidine-1-carbothioamide (IN_09). The compound was synthesized according to the general procedure using 5-chloro-2-(piperidin-4-yl)-1*H*-benzo[*d*]imidazole (**IN_02c**) (0.1 g, 0.42 mmol) and Benzyl isothiocyanate(0.06 g, 0.42 mmol) to afford **IN_09** (0.04 g, 37.31 %) as white solid. M.p: 259-261 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.47 (b, 1H), 8.34 (s, 1H), 7.45-6.71 (m, 8H), 4.16-1.74 (m, 11H). ^{13}C NMR (DMSO-d_6): δ_C 186.4, 141.5, 140.3, 137.9, 137.1, 129.2, 128.5 (2C), 126.9 (2C), 126.7, 124.2, 116.6, 115.8, 51.8 (2C), 51.1, 35.9, 29.4 (2C). ESI-MS *m/z* 385 $[M+H]^+$. Anal.Calcd.For $C_{20}H_{21}ClN_4S$: C, 62.41; H, 5.50; N, 14.56; Found: C, 62.38; H, 5.54; N, 14.51.

N-Benzyl-4-(5-fluoro-1H-benzo[d]imidazol-2-yl)piperidine-1-carbothioamide (IN_10). The compound was synthesized according to the general procedure using 5-fluoro-2-(piperidin-4-yl)-1*H*-benzo[*d*]imidazole (**IN_02d**) (0.1 g, 0.45 mmol) and Benzyl isothiocyanate(0.06 g, 0.45 mmol) to afford **IN_10** (0.04 g, 39.54 %) as white solid. M.p: 241-243 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.47 (b, 1H), 8.34 (s, 1H), 7.46-6.73 (m, 8H), 4.15-1.72 (m, 11H). ^{13}C NMR (DMSO-d_6): δ_C 186.4, 156.5, 141.5, 140.5, 137.9, 134.5, 128.5 (2C), 126.9 (2C), 126.7, 116.8, 109.9, 102.4, 51.8 (2C), 51.1, 35.9, 29.4 (2C). ESI-MS *m/z* 369 $[M+H]^+$. Anal.Calcd.For $C_{20}H_{21}FN_4S$: C, 65.19; H, 5.74; N, 15.21; Found: C, 65.15; H, 5.76; N, 15.24.

4-(1H-Benzo[d]imidazol-2-yl)-N-(4-methoxyphenyl)piperidine-1-carboxamide (IN_11). The compound was synthesized according to the general procedure using 2-(piperidin-4-yl)-1*H*-benzo[*d*]imidazole (**IN_02a**) (0.1 g, 0.49 mmol) and 4-methoxyphenyl isocyanate (0.07 g, 0.49 mmol) to afford **IN_11** (0.05 g, 46.94 %) as white solid. M.p: 264-266 °C. ^{1}H NMR (DMSO-d_6): δ_H 12.30 (b, 1H), 8.36 (s, 1H), 7.47-6.82 (m, 8H), 4.18-1.77 (m, 12H). ^{13}C NMR (DMSO-d_6): δ_C 158.9, 153.1, 141.5, 138.9 (2C), 131.7, 123.2 (2C), 119.8 (2C), 115.2 (2C), 114.5 (2C), 55.8, 46.7 (2C), 34.9, 28.7 (2C). ESI-MS *m/z* 351 $[M+H]^+$. Anal.Calcd.For $C_{20}H_{22}N_4O_2$: C, 68.55; H, 6.33; N, 15.99; Found: C, 68.59; H, 6.37; N, 15.95.

N-(4-Methoxyphenyl)-4-(5-methyl-1H-benzo[d]imidazol-2-yl)piperidine-1-carboxamide (IN_12). The compound was synthesized according to the general procedure using 5-methyl-2-(piperidin-4-yl)-1*H*-benzo[*d*]imidazole (**IN_02b**) (0.1 g, 0.46 mmol) and 4-methoxyphenyl isocyanate (0.06 g, 0.46 mmol) to afford **IN_012** (0.06 g, 56.19 %) as white solid. M.p: 261-263 °C. ^{1}H NMR (DMSO-d_6): δ_H 12.30 (b, 1H), 8.36 (s, 1H), 7.46-6.81 (m, 7H), 4.20-1.74 (m, 15H). ^{13}C NMR (DMSO-d_6): δ_C 158.9, 153.1, 141.5, 138.8, 135.9, 132.7, 131.7, 125.8, 119.8 (2C), 115.3, 115.1, 114.5 (2C), 55.8, 46.7 (2C), 34.9, 28.7 (2C), 21.3. ESI-MS *m/z* 365

$[M+H]^+$. Anal.Calcd.For $C_{21}H_{24}N_4O_2$: C, 69.21; H, 6.64; N, 15.37; Found: C, 69.19; H, 6.68; N, 15.35.

4-(5-Chloro-1H-benzo[d]imidazol-2-yl)-N-(4-methoxyphenyl)piperidine-1-carboxamide (IN_13). The compound was synthesized according to the general procedure using 5-chloro-2-(piperidin-4-yl)-1*H*-benzo[*d*]imidazole (**IN_02c**) (0.1 g, 0.42 mmol) and 4-methoxyphenyl isocyanate (0.06 g, 0.42 mmol) to afford **IN_13** (0.07 g, 68.89 %) as white solid. M.p: 219-221 °C. ^{1}H NMR (DMSO-d_6): δ_H 12.30 (b, 1H), 8.36 (s, 1H), 7.44-6.84 (m, 7H), 4.17-1.75 (m, 12H). ^{13}C NMR (DMSO-d_6): δ_C 158.9, 153.1, 141.5, 140.3, 137.2, 131.7, 129.2, 124.3, 119.8 (2C), 116.6, 115.8, 114.5 (2C), 55.8, 46.7 (2C), 34.9, 28.7 (2C). ESI-MS *m/z* 385 $[M+H]^+$. Anal.Calcd.For $C_{20}H_{21}ClN_4O_2$: C, 62.42; H, 5.50; N, 14.56; Found: C, 62.39; H, 5.53; N, 14.60.

4-(5-Fluoro-1H-benzo[d]imidazol-2-yl)-N-(4-methoxyphenyl)piperidine-1-carboxamide (IN_14). The compound was synthesized according to the general procedure using 5-fluoro-2-(piperidin-4-yl)-1*H*-benzo[*d*]imidazole (**IN_02d**) (0.1 g, 0.45 mmol) and 4-methoxyphenyl isocyanate (0.06 g, 0.45 mmol) to afford **IN_14** (0.08 g, 76.82 %) as white solid. M.p: 231-233 °C. ^{1}H NMR (DMSO-d_6): δ_H 12.30 (b, 1H), 8.36 (s, 1H), 7.46-6.83 (m, 7H), 4.18-1.74 (m, 12H). ^{13}C NMR (DMSO-d_6): δ_C 158.9, 156.5, 153.1, 141.5, 140.5, 134.5, 131.7, 119.8 (2C), 116.8, 114.5 (2C), 109.9, 102.4, 55.8, 46.7 (2C), 34.9, 28.7 (2C). ESI-MS *m/z* 369 $[M+H]^+$. Anal.Calcd.For $C_{20}H_{21}FN_4O_2$: C, 65.20; H, 5.75; N, 15.21; Found: C, 65.23; H, 5.71; N, 15.24.

4-(1H-Benzo[d]imidazol-2-yl)-N-(4-methoxyphenyl)piperidine-1-carbothioamide (IN_15). The compound was synthesized according to the general procedure using 2-(piperidin-4-yl)-1*H*-benzo[*d*]imidazole (**IN_02a**) (0.1 g, 0.49 mmol) and 4-methoxyphenyl isothiocyanate (0.08 g, 0.49 mmol) to afford **IN_15** (0.06 g, 59.53 %) as yellow solid. M.p: 208-210 °C. ^{1}H NMR (DMSO-d_6): δ_H 10.92 (b, 1H), 8.36 (s, 1H), 7.38-6.78 (m, 8H), 4.14-1.78 (m, 12H). ^{13}C NMR (DMSO-d_6): δ_C 187.2, 159.3, 141.5, 138.9 (2C), 127.5 (2C), 123.1 (2C), 115.2 (2C), 114.6 (2C), 113.3, 55.8, 51.8 (2C), 35.9, 29.4 (2C). ESI-MS *m/z* 367 $[M+H]^+$. Anal.Calcd.For $C_{20}H_{22}N_4OS$: C, 65.55; H, 6.05; N, 15.29; Found: C, 65.57; H, 6.04; N, 15.31.

N-(4-Methoxyphenyl)-4-(5-methyl-1H-benzo[d]imidazol-2-yl)piperidine-1-carbothioamide (IN_16). The compound was synthesized according to the general procedure using 5-methyl-2-(piperidin-4-yl)-1*H*-benzo[*d*]imidazole (**IN_02b**) (0.1 g, 0.46 mmol) and 4-methoxyphenyl

isothiocyanate (0.07 g, 0.46 mmol) to afford **IN_16** (0.07 g, 65.96 %) as yellow solid. M.p: 271-273 °C. ^{1}H NMR (DMSO-d_6): δ_H 10.92 (b, 1H), 8.36 (s, 1H), 7.38-6.77 (m, 7H), 4.15-1.78 (m, 15H). ^{13}C NMR (DMSO-d_6): δ_C 187.2, 159.3, 141.5, 138.8, 135.9, 132.7, 127.5 (2C), 125.8, 115.3, 115.1, 114.6 (2C), 113.3, 55.8, 51.8 (2C), 35.9, 29.4 (2C), 21.3. ESI-MS *m/z* 381 $[M+H]^+$. Anal.Calcd.For $C_{21}H_{24}N_4OS$: C, 66.29; H, 6.36; N, 14.72; Found: C, 66.31; H, 6.35; N, 14.76.

4-(5-Chloro-1H-benzo[d]imidazol-2-yl)-N-(4-methoxyphenyl)piperidine-1-carbothioamide (IN_17). The compound was synthesized according to the general procedure using 5-chloro-2-(piperidin-4-yl)-1*H*-benzo[*d*]imidazole (**IN_02c**) (0.1 g, 0.42 mmol) and 4-methoxyphenyl isothiocyanate (0.07 g, 0.42 mmol) to afford **IN_17** (0.07 g, 67.38 %) as yellow solid. M.p: 276-278 °C. ^{1}H NMR (DMSO-d_6): δ_H 10.92 (b, 1H), 8.36 (s, 1H), 7.39-6.75 (m, 7H), 4.18-1.74 (m, 12H). ^{13}C NMR (DMSO-d_6): δ_C 187.2, 159.3, 141.5, 140.3, 137.1, 129.2, 127.5 (2C), 124.1, 116.6, 115.8, 114.6 (2C), 113.3, 55.8, 51.8 (2C), 35.9, 29.4 (2C). ESI-MS *m/z* 401 $[M+H]^+$. Anal.Calcd.For $C_{20}H_{21}ClN_4OS$: C, 59.91; H, 5.28; N, 13.97; Found: C, 59.88; H, 5.30; N, 13.95.

4-(5-Fluoro-1H-benzo[d]imidazol-2-yl)-N-(4-methoxyphenyl)piperidine-1-carbothioamide (IN_18). The compound was synthesized according to the general procedure using 5-fluoro-2-(piperidin-4-yl)-1*H*-benzo[*d*]imidazole (**IN_02d**) (0.1 g, 0.45 mmol) and 4-methoxyphenyl isothiocyanate (0.07 g, 0.45 mmol) to afford **IN_18** (0.05 g, 46.65 %) as yellow solid. M.p: 249-251 °C. ^{1}H NMR (DMSO-d_6): δ_H 10.92 (b, 1H), 8.36 (s, 1H), 7.38-6.76 (m, 7H), 4.17-1.77 (m, 12H). ^{13}C NMR (DMSO-d_6): δ_C 187.2, 159.3, 156.5, 141.5, 140.5, 134.5, 127.5 (2C), 116.8, 114.6 (2C), 113.3, 109.9, 102.4, 55.8, 51.8 (2C), 35.9, 29.4 (2C). ESI-MS *m/z* 385 $[M+H]^+$. Anal.Calcd.For $C_{20}H_{21}FN_4OS$: C, 62.48; H, 5.51; N, 14.57; Found: C, 62.45; H, 5.55; N, 14.54.

4-(1H-Benzo[d]imidazol-2-yl)-N-(4-nitrophenyl)piperidine-1-carboxamide (IN_19). The compound was synthesized according to the general procedure using 2-(piperidin-4-yl)-1*H*-benzo[*d*]imidazole (**IN_02a**) (0.1 g, 0.49 mmol) and 4-nitrophenyl isocyanate (0.08 g, 0.49 mmol) to afford **IN_19** (0.06 g, 50.18 %) as white solid. M.p: 228-230 °C. ^{1}H NMR (DMSO-d_6): δ_H 12.30 (b, 1H), 8.31 (s, 1H), 7.64-6.85 (m, 8H), 4.18-1.74 (m, 9H). ^{13}C NMR (DMSO-d_6): δ_C 153.1, 145.5, 143.5, 141.5, 138.9 (2C), 124.1 (2C), 123.2 (2C), 119.9 (2C), 115.2 (2C), 46.7 (2C), 34.9, 28.7 (2C). ESI-MS *m/z* 366 $[M+H]^+$. Anal.Calcd.For $C_{19}H_{19}N_5O_3$: C, 62.46; H, 5.24; N, 19.17; Found: C, 62.49; H, 5.22; N, 19.19.

4-(5-Methyl-1H-benzo[d]imidazol-2-yl)-N-(4-nitrophenyl)piperidine-1-carboxamide (IN_20). The compound was synthesized according to the general procedure using 5-methyl-2-(piperidin-4-yl)-1*H*-benzo[*d*]imidazole (**IN_02b**) (0.1 g, 0.46 mmol) and 4-nitrophenyl isocyanate (0.07 g, 0.46 mmol) to afford **IN_20** (0.06 g, 48.19 %) as white solid. M.p: 289-291 °C. ^{1}H NMR (DMSO-d_6): δ_H 12.30 (b, 1H), 8.31 (s, 1H), 7.66-6.88 (m, 7H), 4.17-1.75 (m, 12H). ^{13}C NMR (DMSO-d_6): δ_C 153.1, 145.5, 143.5, 141.5, 138.8, 135.9, 132.7, 125.8, 124.1 (2C), 119.9 (2C), 115.3, 115.1, 46.7 (2C), 34.9, 28.7 (2C), 21.3. ESI-MS *m/z* 380 $[M+H]^+$. Anal.Calcd.For $C_{20}H_{21}N_5O_3$: C, 63.31; H, 5.58; N, 18.46; Found: C, 63.33; H, 5.55; N, 18.42.

4-(5-Chloro-1H-benzo[d]imidazol-2-yl)-N-(4-nitrophenyl)piperidine-1-carboxamide (IN_21). The compound was synthesized according to the general procedure using 5-chloro-2-(piperidin-4-yl)-1*H*-benzo[*d*]imidazole (**IN_02c**) (0.1 g, 0.42 mmol) and 4-nitrophenyl isocyanate (0.06 g, 0.42 mmol) to afford **IN_21** (0.06 g, 51.482 %) as white solid. M.p: 219-221 °C. ^{1}H NMR (DMSO-d_6): δ_H 12.30 (b, 1H), 8.31 (s, 1H), 7.66-6.86 (m, 7H), 4.18-1.72 (m, 9H). ^{13}C NMR (DMSO-d_6): δ_C 153.1, 145.5, 143.5, 141.5, 140.3, 137.2, 129.2, 124.1 (3C), 119.9 (2C), 116.6, 115.8, 46.7 (2C), 34.9, 28.7 (2C). ESI-MS *m/z* 400 $[M+H]^+$. Anal.Calcd.For $C_{19}H_{18}ClN_5O_3$: C, 57.07; H, 4.54; N, 17.52; Found: C, 57.09; H, 4.56; N, 17.48.

4-(5-Fluoro-1H-benzo[d]imidazol-2-yl)-N-(4-nitrophenyl)piperidine-1-carboxamide (IN_22). The compound was synthesized according to the general procedure using 5-fluoro-2-(piperidin-4-yl)-1*H*-benzo[*d*]imidazole (**IN_02d**) (0.1 g, 0.45 mmol) and 4-nitrophenyl isocyanate (0.07 g, 0.45 mmol) to afford **IN_22** (0.08 g, 72.26 %) as white solid. M.p: 239-241 °C. ^{1}H NMR (DMSO-d_6): δ_H 12.30 (b, 1H), 8.31 (s, 1H), 7.63-6.89 (m, 7H), 4.17-1.74 (m, 9H). ^{13}C NMR (DMSO-d_6): δ_C 156.5, 153.1, 145.5, 143.5, 141.5, 140.5, 134.5, 124.1 (2C), 119.9 (2C), 116.8, 109.9, 102.4, 46.7 (2C), 34.9, 28.7 (2C). ESI-MS *m/z* 384 $[M+H]^+$. Anal.Calcd.For $C_{19}H_{18}FN_5O_3$: C, 59.52; H, 4.73; N, 18.27; Found: C, 59.50; H, 4.72; N, 18.31.

4-(1H-Benzo[d]imidazol-2-yl)-N-(4-nitrophenyl)piperidine-1-carbothioamide (IN_23). The compound was synthesized according to the general procedure using 2-(piperidin-4-yl)-1*H*-benzo[*d*]imidazole (**IN_02a**) (0.1 g, 0.49 mmol) and 4-nitrophenyl isothiocyanate (0.08 g, 0.49 mmol) to afford **IN_23** (0.05 g, 53.47 %) as white solid. M.p: 268-270 °C. ^{1}H NMR (DMSO-d_6): δ_H 10.84 (b, 1H), 8.35 (s, 1H), 7.38-6.76 (m, 8H), 4.16-1.74 (m, 9H). ^{13}C NMR

(DMSO-d_6): δ_C 187.2, 144.6, 143.5, 141.5, 138.9 (2C), 124.8 (2C), 124.2 (2C), 123.3 (2C), 115.2 (2C), 51.8 (2C), 35.9, 29.4 (2C). ESI-MS *m/z* 382 $[M+H]^+$. Anal.Calcd.For $C_{19}H_{19}N_5O_2S$: C, 59.82; H, 5.02; N, 18.36; Found: C, 59.85; H, 5.04; N, 18.34.

4-(5-Methyl-1H-benzo[d]imidazol-2-yl)-N-(4-nitrophenyl)piperidine-1-carbothioamide (IN_24). The compound was synthesized according to the general procedure using 5-methyl-2-(piperidin-4-yl)-1*H*-benzo[*d*]imidazole (**IN_02b**) (0.1 g, 0.46 mmol) and 4-nitrophenyl isothiocyanate (0.08 g, 0.46 mmol) to afford **IN_24** (0.06 g, 66.14 %) as white solid. M.p: 249-251 °C. ^{1}H NMR (DMSO-d_6): δ_H 10.84 (b, 1H), 8.35 (s, 1H), 7.36-6.74 (m, 7H), 4.18-1.75 (m, 12H). ^{13}C NMR (DMSO-d_6): δ_C 187.2, 144.6, 143.9, 141.5, 138.8, 135.9, 132.7, 125.8, 124.8 (2C), 124.2 (2C), 115.3, 115.1, 51.8 (2C), 35.9, 29.4 (2C), 21.3. ESI-MS *m/z* 396 $[M+H]^+$. Anal.Calcd.For $C_{20}H_{21}N_5O_2S$: C, 60.74; H, 5.35; N, 17.71; Found: C, 60.77; H, 5.38; N, 17.68.

4-(5-Chloro-1H-benzo[d]imidazol-2-yl)-N-(4-nitrophenyl)piperidine-1-carbothioamide (IN_25). The compound was synthesized according to the general procedure using 5-chloro-2-(piperidin-4-yl)-1*H*-benzo[*d*]imidazole (**IN_02c**) (0.1 g, 0.42 mmol) and 4-nitrophenyl isothiocyanate (0.07 g, 0.42 mmol) to afford **IN_25** (0.07 g, 59.72 %) as white solid. M.p: 211-213 °C. ^{1}H NMR (DMSO-d_6): δ_H 10.84 (b, 1H), 8.35 (s, 1H), 7.34-6.77 (m, 7H), 4.17-1.77 (m, 9H). ^{13}C NMR (DMSO-d_6): δ_C 187.2, 144.6, 143.5, 141.5, 140.3, 137.1, 129.2, 124.8 (2C), 124.2 (2C), 124.1, 116.6, 115.8, 51.8 (2C), 35.9, 29.4 (2C). ESI-MS *m/z* 416 $[M+H]^+$. Anal.Calcd.For $C_{19}H_{18}ClN_5O_2S$: C, 54.87; H, 4.36; N, 16.84; Found: C, 54.85; H, 4.35; N, 16.87.

4-(5-Fluoro-1H-benzo[d]imidazol-2-yl)-N-(4-nitrophenyl)piperidine-1-carbothioamide (IN_26). The compound was synthesized according to the general procedure using 5-fluoro-2-(piperidin-4-yl)-1*H*-benzo[*d*]imidazole (**IN_02d**) (0.1 g, 0.45 mmol) and 4-nitrophenyl isothiocyanate (0.08 g, 0.45 mmol) to afford **IN_26** (0.05 g, 49.78 %) as white solid. M.p: 255-257 °C. ^{1}H NMR (DMSO-d_6): δ_H 10.84 (b, 1H), 8.35 (s, 1H), 7.35-6.76 (m, 7H), 4.16-1.76 (m, 9H). ^{13}C NMR (DMSO-d_6): δ_C 187.2, 156.5, 144.6, 143.9, 141.5, 140.5, 134.5, 124.8 (2C), 124.2 (2C), 116.8, 109.9, 102.4, 51.8 (2C), 35.9, 29.4 (2C). ESI-MS *m/z* 400 $[M+H]^+$. Anal.Calcd.For $C_{19}H_{18}FN_5O_2S$: C, 57.13; H, 4.54; N, 17.53; Found: C, 57.14; H, 4.51; N, 17.58.

4-(1H-Benzo[d]imidazol-2-yl)-N-(p-tolyl)piperidine-1-carboxamide (IN_27). The compound was synthesized according to the general procedure using 2-(piperidin-4-yl)-1*H*-benzo[*d*]imidazole (**IN_02a**) (0.1 g, 0.49 mmol), and *p*-tolyl isocyanate (0.06 g, 0.49 mmol) to afford **IN_27** (0.08 g, 86.11 %) as yellow solid. M.p: 280-282 °C. ^{1}H NMR (DMSO-d_6): δ_H 12.28 (b, 1H), 8.36 (s, 1H), 7.44-6.86 (m, 8H), 4.18-1.74 (m, 12H). ^{13}C NMR (DMSO-d_6): δ_C 153.1, 141.5, 138.9 (2C), 136.8, 136.4, 129.2 (2C), 123.4 (2C), 121.5 (2C), 115.2 (2C), 46.7 (2C), 34.9, 28.7 (2C), 21.3. ESI-MS *m/z* 335 $[M+H]^+$. Anal.Calcd.For $C_{20}H_{22}N_4O$: C, 71.83; H, 6.63; N, 16.75; Found: C, 71.86; H, 6.61; N, 16.76.

4-(5-Methyl-1H-benzo[d]imidazol-2-yl)-N-(p-tolyl)piperidine-1-carboxamide (IN_28). The compound was synthesized according to the general procedure using 5-methyl-2-(piperidin-4-yl)-1*H*-benzo[*d*]imidazole (**IN_02b**) (0.1 g, 0.46 mmol) and *p*-tolyl isocyanate (0.06 g, 0.46 mmol) to afford **IN_28** (0.07 g, 75.24 %) as yellow solid. M.p: 242-244 °C. ^{1}H NMR (DMSO-d_6): δ_H 12.28 (b, 1H), 8.36 (s, 1H), 7.46-6.84 (m, 7H), 4.16-1.72 (m, 15H). ^{13}C NMR (DMSO-d_6): δ_C 153.1, 141.5, 138.8, 136.8, 136.4, 135.4, 132.5, 129.2 (2C), 125.8, 121.5 (2C), 115.3, 115.1, 46.7 (2C), 34.9, 28.7 (2C), 21.3 (2C). ESI-MS *m/z* 349 $[M+H]^+$. Anal.Calcd.For $C_{21}H_{24}N_4O$: C, 72.39; H, 6.94; N, 16.08; Found: C, 72.36; H, 6.98; N, 16.04.

4-(5-Chloro-1H-benzo[d]imidazol-2-yl)-N-(p-tolyl)piperidine-1-carboxamide (IN_29). The compound was synthesized according to the general procedure using 5-chloro-2-(piperidin-4-yl)-1*H*-benzo[*d*]imidazole (**IN_02c**) (0.1 g, 0.42 mmol), and *p*-tolyl isocyanate (0.05 g, 0.42 mmol) to afford **IN_29** (0.06 g, 64.04 %) as yellow solid. M.p: 229-231 °C. ^{1}H NMR (DMSO-d_6): δ_H 12.28 (b, 1H), 8.36 (s, 1H), 7.45-6.83 (m, 7H), 4.15-1.74 (m, 12H). ^{13}C NMR (DMSO-d_6): δ_C 153.1, 141.5, 140.3, 137.1, 136.8, 136.4, 129.2 (3C), 124.1, 121.5 (2C), 116.6, 115.8, 46.7 (2C), 34.9, 28.7 (2C), 21.3. ESI-MS *m/z* 369 $[M+H]^+$. Anal.Calcd.For $C_{20}H_{21}ClN_4O$: C, 65.12; H, 5.74; N, 15.19; Found: C, 65.14; H, 5.73; N, 15.15.

4-(5-Fluoro-1H-benzo[d]imidazol-2-yl)-N-(p-tolyl)piperidine-1-carboxamide (IN_30). The compound was synthesized according to the general procedure using5-fluoro-2-(piperidin-4-yl)-1*H*-benzo[*d*]imidazole (**IN_02d**) (0.1 g, 0.45 mmol), and *p*-tolyl isocyanate (0.06 g, 0.45 mmol) to afford **IN_30** (0.05 g, 43.82 %) as yellow solid. M.p: 273-275 °C. ^{1}H NMR (DMSO-d_6): δ_H 12.28 (b, 1H), 8.36 (s, 1H), 7.47-6.85 (m, 7H), 4.18-1.71 (m, 12H). ^{13}C NMR (DMSO-d_6): δ_C 156.5, 153.1, 141.5, 140.5, 136.8, 136.4, 134.5, 129.2 (2C), 121.5 (2C), 116.8, 109.9, 102.4, 46.7 (2C), 34.9, 28.7 (2C), 21.3. ESI-MS *m/z* 353[$(M+H]^+$.

Anal.Calcd.For $C_{20}H_{21}FN_4O$: C, 68.16; H, 6.01; N, 15.90; Found: C, 68.12; H, 6.03; N, 15.88.

4-(1H-Benzo[d]imidazol-2-yl)-N-(p-tolyl)piperidine-1-carbothioamide (IN_31). The compound was synthesized according to the general procedure using 2-(piperidin-4-yl)-1*H*-benzo[*d*]imidazole (**IN_02a**) (0.1 g, 0.49 mmol), and *p*-tolyl isothiocyanate (0.07 g, 0.49 mmol) to afford **IN_31** (0.08 g, 74.55 %) as white solid. M.p: 229-231 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.56 (b, 1H), 8.36 (s, 1H), 7.48-6.72 (m, 8H), 4.16-1.74 (m, 12H). ^{13}C NMR (DMSO-d_6): δ_C 187.2, 141.5, 138.9 (2C), 137.2, 135.5, 129.3 (2C), 126.4 (2C), 123.2 (2C), 115.2 (2C), 51.8 (2C), 35.9, 29.4 (2C), 21.3. ESI-MS *m/z* 351 $[M+H]^+$. Anal.Calcd.For $C_{20}H_{22}N_4S$: C, 68.54; H, 6.33; N, 15.99; Found: C, 68.52; H, 6.35; N, 15.95.

4-(5-Methyl-1H-benzo[d]imidazol-2-yl)-N-(p-tolyl)piperidine-1-carbothioamide (IN_32). The compound was synthesized according to the general procedure using5-methyl-2-(piperidin-4-yl)-1*H*-benzo[*d*]imidazole (**IN_02b**) (0.1 g, 0.46 mmol), and *p*-tolyl isothiocyanate (0.06 g, 0.46 mmol) to afford **IN_32** (0.08 g, 73.15 %) as white solid. M.p: 222-224 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.56 (b, 1H), 8.36 (s, 1H), 7.44-6.76 (m, 7H), 4.15-1.75 (m, 15H). ^{13}C NMR (DMSO-d_6): δ_C 187.2, 141.5, 138.8, 137.2, 135.9, 135.5, 132.7, 129.3 (2C), 126.4 (2C), 125.8, 115.3, 115.1, 51.8 (2C), 35.9, 29.4 (2C), 21.3 (2C). ESI-MS *m/z* 365 $[M+H]^+$. Anal.Calcd.For $C_{21}H_{24}N_4S$: C, 69.20; H, 6.64; N, 15.37; Found: C, 69.24; H, 6.68; N, 15.35.

4-(5-Chloro-1H-benzo[d]imidazol-2-yl)-N-(p-tolyl)piperidine-1-carbothioamide (IN_33). The compound was synthesized according to the general procedure using 5-chloro-2-(piperidin-4-yl)-1*H*-benzo[*d*]imidazole (**IN_02c**) (0.1 g, 0.42 mmol), and *p*-tolyl isothiocyanate (0.06 g, 0.42 mmol) to afford **IN_33** (0.06 g, 49.21 %) as white solid. M.p: 288-290 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.56 (b, 1H), 8.36 (s, 1H), 7.45-6.78 (m, 7H), 4.17-1.77 (m, 12H). ^{13}C NMR (DMSO-d_6): δ_C 187.2, 141.5, 140.2, 137.2, 137.1, 135.5, 129.3 (2C), 129.2, 126.4 (2C), 124.1, 116.6, 115.8, 51.8 (2C), 35.9, 29.4 (2C), 21.3. ESI-MS *m/z* 385 $[M+H]^+$. Anal.Calcd.For $C_{20}H_{21}ClN_4S$: C, 62.41; H, 5.50; N, 14.56; Found: C, 62.39; H, 5.52; N, 14.60.

4-(5-Fluoro-1H-benzo[d]imidazol-2-yl)-N-(p-tolyl)piperidine-1-carbothioamide (IN_34). The compound was synthesized according to the general procedure using 5-fluoro-2-(piperidin-4-yl)-1*H*-benzo[*d*]imidazole (**IN_02d**) (0.1 g, 0.45 mmol), and *p*-tolyl

isothiocyanate (0.06 g, 0.45 mmol) to afford **IN_34** (0.08 g, 78.76 %) as white solid. M.p: 258-260 °C. ^{1}H NMR (DMSO-d_6): δ_H 9.56 (b, 1H), 8.36 (s, 1H), 7.46-6.76 (m, 7H), 4.16-1.75 (m, 12H). ^{13}C NMR (DMSO-d_6): δ_C 187.2, 156.5, 141.5, 140.5, 137.2, 135.5, 134.5, 129.3 (2C), 126.4 (2C), 116.8, 109.9, 102.4, 51.8 (2C), 35.9, 29.4 (2C), 21.3. ESI-MS *m/z* 369 $[M+H]^+$. Anal.Calcd.For $C_{20}H_{21}FN_4S$: C, 65.19; H, 5.74; N, 15.21; Found: C, 65.15; H, 5.72; N, 15.23.

4-(1H-Benzo[d]imidazol-2-yl)-N-(4-fluorophenyl)piperidine-1-carboxamide (IN_35). The compound was synthesized according to the general procedure using 2-(piperidin-4-yl)-1*H*-benzo[*d*]imidazole (**IN_02a**) (0.1 g, 0.49 mmol), and 4-fluorophenyl isocyanate (0.06 g, 0.49 mmol) to afford **IN_35** (0.07 g, 65.54 %) as white solid. M.p: 247-249 °C. ^{1}H NMR (DMSO-d_6): δ_H 12.23 (b, 1H), 8.34 (s, 1H), 7.48-6.71 (m, 8H), 4.17-1.74 (m, 9H). ^{13}C NMR (DMSO-d_6): δ_C 162.9, 153.1, 141.5, 138.9 (2C), 135.1, 123.2 (2C), 119.3 (2C), 115.7 (2C), 115.2 (2C), 46.7 (2C), 34.9, 28.7 (2C). ESI-MS *m/z* 339 $[M+H]^+$. Anal.Calcd.For $C_{19}H_{19}FN_4O$: C, 67.44; H, 5.66; N, 16.56; Found: C, 67.46; H, 5.69; N, 16.53.

N-(4-Fluorophenyl)-4-(5-methyl-1H-benzo[d]imidazol-2-yl)piperidine-1-carboxamide (IN_36). The compound was synthesized according to the general procedure using5-methyl-2-(piperidin-4-yl)-1*H*-benzo[*d*]imidazole (**IN_02b**) (0.1 g, 0.46 mmol), and 4-fluorophenyl isocyanate (0.06 g, 0.46 mmol) to afford **IN_36** (0.07 g, 58.61 %) as white solid. M.p: 218-220 °C. ^{1}H NMR (DMSO-d_6): δ_H 12.23 (b, 1H), 8.34 (s, 1H), 7.46-6.69 (m, 7H), 4.15-1.77 (m, 12H). ^{13}C NMR (DMSO-d_6): δ_C 162.9, 153.1, 141.5, 138.8, 135.9, 135.2, 132.7, 125.8, 119.3 (2C), 115.7 (2C), 115.3, 115.1, 46.7 (2C), 34.9, 28.7 (2C), 21.3. ESI-MS *m/z* 353 $[M+H]^+$. Anal.Calcd.For $C_{20}H_{21}FN_4O$: C, 68.16; H, 6.01; N, 15.90; Found: C, 68.15; H, 6.04; N, 15.93.

4-(5-Chloro-1H-benzo[d]imidazol-2-yl)-N-(4-fluorophenyl)piperidine-1-carboxamide (IN_37). The compound was synthesized according to the general procedure using 5-chloro-2-(piperidin-4-yl)-1*H*-benzo[*d*]imidazole (**IN_02c**) (0.1 g, 0.42 mmol), and 4-fluorophenyl isocyanate (0.05 g, 0.42 mmol) to afford **IN_37** (0.06 g, 57.19 %) as white solid. M.p: 241-243 °C. ^{1}H NMR (DMSO-d_6): δ_H 12.23 (b, 1H), 8.34 (s, 1H), 7.45-6.68 (m, 7H), 4.17-1.78 (m, 9H). ^{13}C NMR (DMSO-d_6): δ_C 162.9, 153.1, 141.5, 140.3, 137.3, 135.1, 129.2, 124.1, 119.3 (2C), 116.6, 115.8, 115.7 (2C), 46.7 (2C), 34.9, 28.7 (2C). ESI-MS *m/z* 373 $[M+H]^+$. Anal.Calcd.For $C_{19}H_{18}ClFN_4O$: C, 61.21; H, 4.87; N, 15.03; Found: C, 61.24; H, 4.84; N, 15.06.

4-(5-Fluoro-1H-benzo[d]imidazol-2-yl)-N-(4-fluorophenyl)piperidine-1-carboxamide (IN_38). The compound was synthesized according to the general procedure using 5-fluoro-2-(piperidin-4-yl)-1*H*-benzo[*d*]imidazole (**IN_02d**) (0.1 g, 0.45 mmol), and 4-fluorophenyl isocyanate (0.06 g, 0.45 mmol) to afford **IN_38** (0.07 g, 68.81 %) as white solid. M.p: 253-255 °C. ^{1}H NMR (DMSO-d_6): δ_H 12.23 (b, 1H), 8.34 (s, 1H), 7.44-6.70 (m, 7H), 4.18-1.76 (m, 9H). ^{13}C NMR (DMSO-d_6): δ_C 162.9, 156.5, 153.1, 141.5, 140.5, 135.1, 134.5, 119.3 (2C), 116.8, 115.7 (2C), 109.9, 102.4, 46.7 (2C), 34.9, 28.7 (2C). ESI-MS *m/z*357 $[M+H]^+$. Anal.Calcd.For $C_{19}H_{18}F_2N_4O$: C, 64.04; H, 5.09; N, 15.72; Found: C, 64.05; H, 5.11; N, 15.68.

4-(1H-Benzo[d]imidazol-2-yl)-N-(4-fluorophenyl)piperidine-1-carbothioamide (IN_39). The compound was synthesized according to the general procedure using 2-(piperidin-4-yl)-1*H*-benzo[*d*]imidazole (**IN_02a**) (0.1 g, 0.49 mmol), and 4-fluorophenyl isothiocyanate (0.07 g, 0.49 mmol) to afford **IN_39** (0.07 g, 62.12 %) as white solid. M.p: 277-279 °C. ^{1}H NMR (DMSO-d_6): δ_H 10.13 (b, 1H), 8.32 (s, 1H), 7.48-6.66 (m, 8H), 4.16-1.74 (m, 9H). ^{13}C NMR (DMSO-d_6): δ_C 187.2, 163.2, 141.5, 138.9 (2C), 134.1, 131.2 (2C), 123.1 (2C), 115.8 (2C), 115.2 (2C), 51.8 (2C), 35.9, 29.4 (2C). ESI-MS *m/z*355 $[M+H]^+$. Anal.Calcd.For $C_{19}H_{19}FN_4S$: C, 64.38; H, 5.40; N, 15.81; Found: C, 64.35; H, 5.43; N, 15.84.

N-(4-Fluorophenyl)-4-(5-methyl-1H-benzo[d]imidazol-2-yl)piperidine-1-carbothioamide (IN_40). The compound was synthesized according to the general procedure using 5-methyl-2-(piperidin-4-yl)-1*H*-benzo[*d*]imidazole (**IN_02b**) (0.1 g, 0.46 mmol), and 4-fluorophenyl isothiocyanate (0.07 g, 0.46 mmol) to afford **IN_40** (0.07 g, 69.77 %) as white solid. M.p: 197-199 °C. ^{1}H NMR (DMSO-d_6): δ_H 10.13 (b, 1H), 8.32 (s, 1H), 7.46-6.63 (m, 7H), 4.17-1.76 (m, 12H). ^{13}C NMR (DMSO-d_6): δ_C 187.2, 163.2, 141.5, 138.8, 135.9, 134.1, 132.7, 131.2 (2C), 125.8, 115.8 (2C), 115.3, 115.1, 51.8 (2C), 35.9, 29.4 (2C), 21.3. ESI-MS *m/z* 369 $[M+H]^+$. Anal.Calcd.For $C_{20}H_{21}FN_4S$: C, 65.19; H, 5.74; N, 15.21; Found: C, 65.16; H, 5.77 N, 15.22.

4-(5-Chloro-1H-benzo[d]imidazol-2-yl)-N-(4-fluorophenyl)piperidine-1-carbothioamide (IN_41). The compound was synthesized according to the general procedure using 5-chloro-2-(piperidin-4-yl)-1*H*-benzo[*d*]imidazole (**IN_02c**) (0.1 g, 0.42 mmol), and 4-fluorophenyl isothiocyanate (0.06 g, 0.42 mmol) to afford **IN_41** (0.07 g, 62.22 %) as white solid. M.p: 226-228 °C. ^{1}H NMR (DMSO-d_6): δ_H 10.13 (b, 1H), 8.32 (s, 1H), 7.44-6.64 (m, 7H), 4.16-1.75 (m, 9H). ^{13}C NMR (DMSO-d_6): δ_C 187.2, 163.2, 141.5, 140.3, 137.2, 134.1, 131.2 (2C),

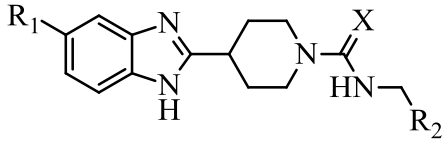
129.2, 124.1, 116.6, 115.8 (3C), 51.8 (2C), 35.9, 29.4 (2C). ESI-MS *m/z* 389 $[M+H]^+$. Anal.Calcd.For $C_{19}H_{18}ClFN_4S$: C, 58.68; H, 4.67; N, 14.41; Found: C, 58.66; H, 4.68; N, 14.45.

4-(5-Fluoro-1H-benzo[d]imidazol-2-yl)-N-(4-fluorophenyl)piperidine-1-carbothioamide (IN_42). The compound was synthesized according to the general procedure using 5-fluoro-2-(piperidin-4-yl)-1*H*-benzo[*d*]imidazole (**IN_02d**) (0.1 g, 0.45 mmol), and 4-fluorophenyl isothiocyanate (0.06 g, 0.45 mmol) to afford **IN_42** (0.07 g, 71.82 %) as white solid. M.p: 263-265 °C. ^{1}H NMR (DMSO-d_6): δ_H 10.13 (b, 1H), 8.32 (s, 1H), 7.45-6.65 (m, 7H), 4.15-1.74 (m, 9H). ^{13}C NMR (DMSO-d_6): δ_C 187.2, 163.2, 156.5, 141.5, 140.5, 134.5, 134.1, 131.2 (2C), 116.8, 115.8 (2C), 109.9, 102.4, 51.8 (2C), 35.9, 29.4 (2C). ESI-MS *m/z* 373 $[M+H]^+$. Anal.Calcd.For $C_{19}H_{18}F_2N_4S$: C, 61.27; H, 4.87; N, 15.04; Found: C, 61.26; H, 4.83; N, 15.06.

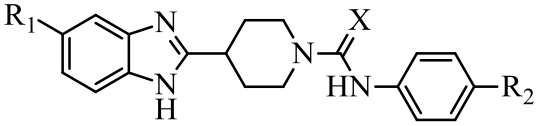
5.2b.4. In-vitro *Mycobacterium smegmatis* GyrB assay, *Mycobacterium tuberculosis* supercoiling assay, antimycobacterial potency and cytotoxicity studies of the synthesized molecules

All the synthesized derivatives were first evaluated for their in-vitro *Mycobacterium tuberculosis* GyrB assay and *Mycobacterium tuberculosis* supercoiling assay as steps towards the derivation of SAR and hit optimization. The compounds were further subjected to a whole cell screening against *Mycobacterium tuberculosis* H37Rv strain to understand their bactericidal potency using the MABA assay and later the safety profile of these molecules were evaluated by checking the in-vitro cytotoxicity against RAW 264.7 cell line (mouse macrophage) by MTT assay, and the results are tabulated in **Table 5.10**.

Table 5.10: In-vitro biological evaluation of the synthesized derivatives **IN_03 - IN_42**.



IN_03 - IN_10



IN_11 - IN_42

Compd	R_1	R_2	X	MS GyraseB assay (IC_{50}) µM	MTB Supercoiling assay (IC_{50}) µM	MTB MIC µM	Cytotoxicity[a] % inhibition
IN_03	-H	$-C_6H_5$	O	11.81	5.13	37.38	40.24
IN_04	$-CH_3$	$-C_6H_5$	O	21.33	10.86	71.75	41.25
IN_05	-Cl	$-C_6H_5$	O	10.77	4.86	33.89	54.14
IN_06	-F	$-C_6H_5$	O	9.81	4.16	141.88	28.94
IN_07	-H	$-C_6H_5$	S	18.26	7.55	71.33	13.64
IN_08	$-CH_3$	$-C_6H_5$	S	15.28	8.46	137.17	18.99
IN_09	-Cl	$-C_6H_5$	S	18.31	9.56	32.47	14.49
IN_10	-F	$-C_6H_5$	S	27.31	13.98	67.85	11.45
IN_11	-H	$-OCH_3$	O	16.81	11.88	71.34	15.91
IN_12	$-CH_3$	$-OCH_3$	O	28.11	15.98	34.30	34.46
IN_13	-Cl	$-OCH_3$	O	11.21	6.44	64.96	21.90
IN_14	-F	$-OCH_3$	O	15.17	10.56	67.86	46.73
IN_15	-H	$-OCH_3$	S	19.55	12.89	136.43	18.12
IN_16	$-CH_3$	$-OCH_3$	S	19.27	8.82	65.70	45.78
IN_17	-Cl	$-OCH_3$	S	26.54	11.89	31.18	6.94
IN_18	-F	$-OCH_3$	S	21.22	19.66	32.51	2.22
IN_19	-H	$-NO_2$	O	6.12	4.16	8.55	18.31
IN_20	$-CH_3$	$-NO_2$	O	8.64	6.61	32.95	8.90
IN_21	-Cl	$-NO_2$	O	3.12	2.19	125.05	10.92
IN_22	-F	$-NO_2$	O	6.98	3.96	130.42	24.89
IN_23	-H	$-NO_2$	S	11.34	4.88	131.08	32.17

Contd.

Compd	R_1	R_2	X	MS GyraseB assay (IC_{50}) µM	MTB Supercoiling assay (IC_{50}) µM	MTB MIC µM	Cytotoxicity[a] % inhibition
IN_24	$-CH_3$	$-NO_2$	S	16.54	5.91	7.90	25.56
IN_25	-Cl	$-NO_2$	S	9.88	5.66	60.11	31.32
IN_26	-F	$-NO_2$	S	8.16	3.12	125.18	40.87
IN_27	-H	$-CH_3$	O	9.81	4.66	18.69	18.93
IN_28	$-CH_3$	$-CH_3$	O	15.31	10.94	143.50	18.48
IN_29	-Cl	$-CH_3$	O	12.65	11.94	67.78	8.08
IN_30	-F	$-CH_3$	O	11.57	9.31	17.74	45.59
IN_31	-H	$-CH_3$	S	5.78	3.44	71.33	7.54
IN_32	$-CH_3$	$-CH_3$	S	15.54	13.61	17.15	26.52
IN_33	-Cl	$-CH_3$	S	11.21	6.34	32.47	30.16
IN_34	-F	$-CH_3$	S	7.13	5.13	67.85	15.62
IN_35	-H	-F	O	5.64	3.12	36.94	54.11
IN_36	$-CH_3$	-F	O	9.81	4.33	70.94	46.46
IN_37	-Cl	-F	O	2.12	1.61	33.53	1.76
IN_38	-F	-F	O	2.89	1.44	35.08	20.43
IN_39	-H	-F	S	3.24	1.88	17.63	15.19
IN_40	$-CH_3$	-F	S	15.34	9.87	16.96	44.71
IN_41	-Cl	-F	S	19.55	15.48	8.04	16.86
IN_42	-F	-F	S	5.79	3.12	8.39	42.61
Novobiocin				0.273±0.28	0.068±0.31	>200	9.36

MS= *Mycobacterium smegmatis,* MTB=*Mycobacterium tuberculosis,* [a] at 50 µM against RAW 264.7 cells, ND indicates not determined.

5.2b.5. Discussion

A series of benzimidazol-2-yl piperidine-1-carboxamide and benzimidazole-2-yl piperidine-1-carbothioamide derivatives were synthesized and were evaluated for their *Mycobacterium tuberculosis* GyrB inhibition ability employing GyrB ATPase assay, supercoiling assay and their IC_{50} values were determined. The compounds were also evaluated for their in-vitro antimycobacterial activity against *Mycobacterium tuberculosis* H37RV strain and also for the

safety profile by in-vitro MTT assay in RAW 264.7 cell lines. An attempt was done to correlate the activity of these compounds with respect to their structure in order to study their structure activity relationship (SAR). The basic structure of these compounds includes a benzimidazole core with a piperidine group at its 2nd position and substitutions like fluoro, chloro and methyl at 5th position. The piperidine group at 1st position (on nitrogen) was linked to carboxamide and carbothioamide. The major substitutions were made at two positions: **R_1** - left hand side at 5th position of benzimidazole with methyl, chloro and fluoro groups and **R_2** - right hand side on free amino group of carboxamide/carbothioamide with benzyl and various *p*-substituted phenyl derivatives. The total series of 40 compounds can be divided into 5 subsets based on their substitutions at their **R_2** position – (i) compounds **IN_03 - IN_10** with benzyl group (ii) compounds **IN_11 - IN_18** with *p*-methoxy phenyl group, (iii) compounds **IN_19 - IN_26** with *p*-nitro phenyl group, (iv) compounds **IN_27 - IN_34** with *p*-methyl phenyl group and (v) compounds **IN_35 - IN_42** with *p*-fluoro phenyl group.

Compounds **IN_03 - IN_10** were found with benzyl group on right hand side yielding one compound with its GyrB activity below 10 µM. Compound **6** was found to be active with GyrB IC_{50} of 9.81 µM and DNA supercoiling inhibitory activity of 4.16 µM. This compound shows a MIC of 141.88 µM and was found to be devoid of cytotoxicity with a percentage inhibition of 28.94 against RAW cell lines. The remaining compounds of this subgroup with benzyl moiety were found to be inactive suggesting its unsuitability for GyrB activity.

Substitution of methoxy group at R_2 position (**IN_11 - IN_18**) resulted in compounds with complete loss of GyrB inhibitory activity with their IC_{50} ranging between 11.21-28.11 µM. The DNA supercoiling activity for these compounds was also found to be less with IC_{50} ranging from 6.44-19.66 µM. The MIC activity for these compounds was found to be in the range of 31.18-136.43 µM. These activity profiles of the compounds infer the unfavourability of *p*-methoxy phenyl moiety for GyrB activity.

Substitution of nitro (**IN_19 - IN_26**) group at R_2 position resulted in six compounds, out of eight, with GyrB inhibitory activity below 10 µM IC_{50}. The DNA supercoiling activity of the compounds was found in the range of 2.19-6.61 µM with all compounds below 10 µM. Of these, compound **IN_21** was found to be active with GyrB activity of 3.12 µM and DNA supercoiling activity of 2.19 µM. This compound exhibits an MIC of 125.05 µM and percentage inhibition of 10.92against RAW cell lines. Unlike the above two substitutions

(benzyl and methoxy), *p*-nitro phenyl group yielded the compounds with effective GyrB activity displaying the suitability of nitro group for activity.

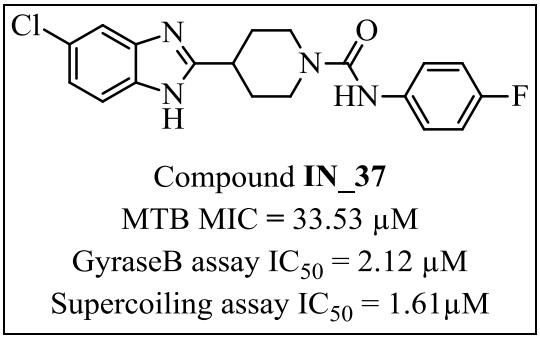
Substitution at R_2 position with methyl group (**IN_27 - IN_34**) resulted in compounds with decreased GyrB inhibitory ability, when compared to that of nitro substitution, with a few exceptions of compound **IN_31, IN_34** and **IN_27** with IC_{50} below 10 µM. Compounds **IN_31** and **IN_34** shows GyrB activity of 5.78 µM and 7.13 µM, and supercoiling activity of 3.44 and 5.13 µM. These compounds shows MIC of 71.33 and 67.85 µM and are found to be devoid of cytotoxicity as the percentage inhibition is 7.54 and 15.62 respectively. Both the compounds are similar with respect to their structure with an exception of fluoro group over benzimidazole ring in compound **IN_34** and both the compounds differ in the presence of carboxamide (**IN_31**) and carbothioamide (**IN_34**). Replacement of nitro group (**IN_21**) with methyl (**IN_29**) resulted in decrease of activity by 4 fold implicating the importance of nitro group on the phenyl ring for the activity. This might be probably accounted for the high electron affinity nature of oxygen which might readily take part in hydrogen bonding with the amino acids at the GyrB active site.

Of all the substitutions, fluoro group at R_2 position was observed to be the desirable one yielding six compounds out of eight with IC_{50} below 10 µM and almost 4 compounds below 6 µM. Of these, compounds **IN_37** and **IN_38** were found to be the top active compounds of the series, with GyrB activity of 2.12 µM and 2.89 µM and DNA supercoiling activity of 1.61 µM and 1.44 µM, MIC of 33.53 and 35.08 µM, cytotoxicity of 1.76 and 20.43 respectively proving the importance of fluoro group over the compounds for GyrB inhibitory activity. Compound **IN_37**, when compared to its counter parts compounds **IN_13** and **IN_29**, was found to be active by 5 and 6 folds respectively. The high electronegative nature of fluorine atom might be of importance for GyrB inhibitory activity of these compounds.

5.2b.6. Highlights of the study

Out of 40 compounds synthesised compound **IN_37** was found to be active with gyrase IC_{50} of 2.12 µM and it also shows correlating supercoiling IC_{50} of 1.61 µM, MIC of 12.5 µM in MABA assay (**Figure 5.18**). The compound was found to devoid of cytotoxicity as the percentage inhibiton was 1.76. against RAW cell lines. In a nutshell, benzimidazole derivatives act as suitable inhibitors against GyrB ATPase domain as observed from the GyrB IC_{50} values and anti-tubercular MIC results from **Table 5.10**. Substitution on right hand

side with hydrophobic moieties such as fluoro (as observed in compounds **IN_35**, **IN_37**, **IN_38**, **IN_39** and **IN_42**), methyl (as observed in compounds **IN_27**, **IN_31** and **IN_34**) and nitro (as in compounds **IN_19**, **IN_20**, **IN_21**, **IN_22**, **IN_25** and **IN_26)** moieties resulted in inhibitors with superior activity. Substitutions like benzyl, methoxy and some of methyl were found to be highly unfavorable for GyrB activity. Simple benzimidazole or hydrophobic substitutions like fluoro and chloro groups can be highly recommended for improving inhibitory ability of the compounds. Considering the activity profiles of the compounds, carboxamide derivatives are more likely to be better GyrB inhibitors when compared to that of carbothioamide ones. Compounds with these specifications can be suitable and active inhibitors of GyrB which can be further optimized and evaluated in the process of anti-tubercular drug discovery.



Compound **IN_37**
MTB MIC = 33.53 μM
GyraseB assay IC_{50} = 2.12 μM
Supercoiling assay IC_{50} = 1.61μM

Figure 5.18: Structure and activity of most active compound **IN_37**.

Summary and Conclusion

Chapter 6

TB is the major cause of death in developing countries especially in places where HIV is prevalent. Development of resistance to existing antibiotics initiates the need for development of newer drugs which can combat resistant strains as well as shorten duration of therapy with limited side effects. Researchers all over the world are trying to develop safe and efficacious drugs for TB.

Among the different targets in tuberculosis, DNA gyrase is a well validated target. Fluoroquinolones were successful in market as they target gyrase and inhibit DNA supercoiling. But emergence of resistance to this class of antibiotics makes researchers to develop newer antibiotics which can inhibit DNA gyrase A subunit or target both A and B subunits of gyrase.

In this work we explored benzothiazinone and benzimidazole nucleus which were known to possess antitubercular activity. By using molecular hybridization approach benzothiazinone scaffold was coupled with different linkers and series of compounds was synthesised and evaluated to develop SAR. The compounds were evaluated for potency for gyrase, supercoiling and MABA assay. The safety of these compounds was also evaluated by measuring the percentage inhibition against RAW 264.7 cell lines.

Among the derivatives of 2-((4-aminophenyl)amino)-4*H*-benzo[*e*][1,3]thiazin-4-ones, compound **BP_24** was found to be the most active compound with gyrase IC_{50} of 0.41 µM , supercoiling IC_{50} of 0.72 µM and MIC of 48.31 µM against *Mycobacterium tuberculosis*. This showed that it was a potent inhibitor of gyrase.

Among the derivatives of 2-(4-aminocyclohexyl)-4*H*-benzo[*e*][1,3]thiazin-4-ones, compound **BD_35** was found to be the most active compound with supercoiling IC_{50} of 0.86 µM and this compound exhibits MIC of 2.88 µM against *Mycobacterium tuberculosis*. This showed that it was a potent inhibitor of gyrase.

Among the derivatives of 2-(piperazin-1-yl)-4*H*-benzo[*e*][1,3]thiazin-4-ones, compound **BZ_18** was found to be the most active compound with supercoiling IC_{50} of 0.51 µM. The compound was further evaluated for MIC by MABA assay using *Mycobacterium*

tuberculosis and it shows well correlating results of 4.41 µM. These results suggested that it was a potent inhibitor of gyrase.

Among the derivatives of 4-(1*H*-benzo[*d*]imidazol-2-yl)aniline, compound **IB_38** was found to be the most active compound with gyrase IC_{50} of 0.72 µM and supercoiling IC_{50} of 0.26 µM. The compound was further evaluated for MIC by MABA assay using *Mycobacterium tuberculosis* and it shows well correlating results of 1.38 µM. Theses results suggested that it was a potent inhibitor of gyrase.

Among the derivatives of 2-(piperidin-4-yl)-1*H*-benzo[*d*]imidazole, compound **IN_37** was found to be the most active compound with gyrase IC_{50} of 2.12 µM and supercoiling IC_{50} of 1.61 µM. The compound was further evaluated by MABA assay using *Mycobacterium tuberculosis* and it exhibits MIC of 33.53 µM. These results suggested that it could emerge as promising inhibitor of gyrase.

Out of 188 molecules synthesised 28 molecules showed gyrase IC_{50s} less than 5 µM. Among the compounds **BP_24** and **IB_38** exhibited promising results with gyrase inhibitory potential of 0.41 and 0.72 µM and good correlating supercoiling IC_{50} of 0.72 and 0.26 µM respectively. The compounds when tested against drug sensitive strains of *Mycobacterium tuberculosis* showed MICs of 48.31 and 1.38 µM, respectively.

Among 188 molecules, 81 molecules showed supercoiling IC_{50s} less than 5 µM. With respect to supercoiling assay **BZ_18** and **IB_38** with IC_{50s} of 0.51 and 0.26 µM respectively were identified as potent molecules. These compounds when tested against drug sensitive strains of *Mycobacterium tuberculosis* exhibited MICs of 4.41 and 1.38 µM respectively. These compounds thus could be taken as leads and could be further optimised for development of newer drugs by exploring their pharmacokinetic and pharmacodynamic properties.

Future perspectives

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

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

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

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

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

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

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

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Appendix

List of Publications

1. **Manoj Chandran**, Janupally Renuka, Jonnalagadda Padma Sridevi, Perumal Yogeeswari, Dharmarajan Sriram. "Benzothiazinone-piperazine derivatives as efficient *Mycobacterium tuberculosis* DNA Gyrase Inhibitors". *International Journal of Mycobacteriology*, doi:10.1016/j.ijmyco.2015.02.002.

2. Jeankumar Variam Ullas, **Manoj Chandran**, Ganesh Samala, Mallika Alvala, Pulla Venkat Koushik, Perumal Yogeeswari, Elena G. Salina, Dharmarajan Sriram. "Development of 5-nitrothiazole derivatives: Identification of leads against both replicative and latent *Mycobacterium tuberculosis*". *Bioorganic & Medicinal Chemistry Letters* 22, no. 24 (2012): 7414-7417.

3. **Manoj Chandran**, Janupally Renuka, Jonnalagadda Padma Sridevi, Perumal Yogeeswari, Dharmarajan Sriram. "Synthesis and biological evaluation of novel benzothiazinone analogues as *Mycobacterial tuberculosis* DNA GyrB inhibitors". (Communicated to *Bioorganic & Medicinal Chemistry Letters*)

4. **Manoj Chandran**, Janupally Renuka, Jonnalagadda Padma Sridevi, Perumal Yogeeswari, Dharmarajan Sriram. "Benzothiazinone-aminopiperidine hybrid analogues as efficient *Mycobacterium tuberculosis* DNA Gyrase inhibitors". (Communicated to *International Journal of Cellular Biotechnology*)

BIOGRAPHY OF MANOJ C

Manoj C completed his Bachelor of Science and Master of Science (Analytical chemistry) from Mahatma Gandhi University Kottayam, Kerala. He has about 7 and half years of industrial experience. He has worked at Syngene International Limited (A Biocon company) – Bangalore as Senior Research Associate. He was appointed as ICMR Senior Research Fellow from Aug 2011 – May 2014, at Birla Institute of Technology & Science, Pilani, Hyderabad campus under the supervision of Prof. D. Sriram. He has published two scientific papers in well-renowned international journals.

BIOGRAPHY OF Professor D. SRIRAM

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